SYNTHESIS OF UNUSUAL α-AMINO ACIDS AND STUDY OF THE EFFECT OF THEIR INCORPORATION ANTIMICROBIAL PEPTIDES. TOTAL SYNTHESIS OF BIOACTIVE MARINE NATURAL PRODUCTS AND ANALOGUES THEREOF

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Total synthesis of bioactive marine natural products and analogues thereof

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La doctora Montserrat Heras Corominas, professora titular de l'Àrea de Química Orgánica de la Universitat de Girona, i la catedràtica Janine Cossy, directora del *Laboratoire de Chimie Organique* de l' *École Superieur de Physique et Chimie Industrielles de la ville de Paris*.

CERTIFIQUEM:

Que aquest treball titulat "*Synthesis of unusual α-amino acids and study of the effect of their incorporation into antimicrobial peptides. Total synthesis of bioactive marine natural products and analogues thereof*", que presenta el Sr. Abdellatif El Marrouni El Ghzaoui per a l'obtenció del títol de Doctor, ha estat realitzat sota la nostra direcció i que compleix els requeriments per poder optar a Menció Europea.

Signatura

Dra. Montserrat Heras Corominas Prof. Janine Cossy

Girona, 3 de Febrer de 2012.

Dedicated
to
my beloved family
and my lovely wife

If we knew what it was we were doing, it would not be called research, would it?

(A. Einstein)

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Abstract

The principle theme of this thesis is the synthesis of bioactive compounds. The development of products with interesting biological activities is assessed, on one hand, by the rational design of new synthetic compounds and/or modification of existing drugs. On the other hand, marine natural products proved to be a rich source of novel therapeutics to protect against and combat diseases, as well as serve as lead compounds in crop protection. Hence, this thesis was divided into two main projects.

The first project (Chapter I) was carried out in the Department of Chemistry of the University of Girona under the supervision of Dr Montserrat Heras, concerned the design and the synthesis of new unnatural amino acids bearing a pyrimidine ring within their side chain for incorporation into the antimicrobial peptide **BP100** and an evaluation of their activity. Non-proteinogenic amino acids, especially unnatural synthetic α-amino acids, have played a significant role in drug development. Their intrinsic biological properties and structural diversity make them both valuable pharmaceuticals and useful building blocks in peptide chemistry. Consequently, synthetic unnatural α-amino acids have emerged as important synthetic targets and a variety of stereoselective methods have recently been developed for their preparation. As part of our research aimed at synthesizing new antimicrobial peptides by incorporating unnatural α-amino acids, and following an ongoing research group project involving pyrimidine chemistry, the synthesis of a collection of pyrimidines substituted at C2- and C4-positions with an α-amino acid residue was investigated.

In particular, a coupling reaction between the electron-rich 2-morpholino-4(3*H*)-pyrimidinone and the nucleophilic side chains of several natural N^{α}-Boc protected α-amino esters promoted by phosphonium salt was applied in order to incorporate the α-amino acid at the C4-position of the pyrimidine ring. After a thorough optimization study, several N^{α}-Boc pyrimidin-4-yl amino esters were obtained successfully without loss of optical integrity using a two-step approach through the easily available benzotriazol-1-yloxy pyrimidine intermediate. On the other hand, the incorporation of an amino acid at the C2-position of the pyrimidine ring was achieved by a nucleophilic *ipso*-substitution reaction between various 2-benzylsulfonyl-4-isopropoxypyrimidines

and several natural N^{α}-Boc protected α-amino esters. The reaction conditions were optimized for each α-amino ester individually in order to achieve the N^{α}-Boc pyrimidin-2-yl α-amino esters in good yields (40-88%) and without racemization. This optimized method was extended to the synthesis of pyrimidin-2-one α-amino esters by using a more acid-labile than isopropoxy group at the C4-position of the starting 2-benzylsulfonylpyrimidine. The N^{α}-Boc pyrimidinyl α-amino esters were easily converted into the target N^{α}-Fmoc pyrimidinyl amino acids in good yields (40-93%) using standard procedures of deprotection and protection of functional groups. With these useful building blocks in hand for solid-phase peptide synthesis, the last part of this project involved the rational incorporation of these pyrimidinyl amino acids into the antimicrobial peptide, known as **BP100**, previously developed in the laboratory. A library of 14 peptides was thus synthesized and tested against plant as well as human pathogens and evaluated for their hemolytic activity. The **BP100** analogues that incorporated hydrophilic pyrimidinyl amino acids, showed the best activity profile. All biological assays were carried out at the Centre for Innovation and Development in Plant Health (CIDSAV).

The second project (Chapters II and III), on the other hand, was carried out in the *Laboratoire de Chimie Organique* (ESPCI-ParisTech) under the joint supervision of Pr Janine Cossy and Dr Stellios Arseniyadis.

Hence, Chapter II focuses on the total synthesis of two macrolides of marine origin: acremolide B and lyngbouilloside. First, a simple strategy towards the total synthesis of the lipodepsipeptide acremolide B, which structure was not confirmed, was developed relying on four key steps: two stereoselective allylations/crotylations, a cross-metathesis to introduce the fatty-acid side chain, an esterification to link the dipeptide unit to the polypropionate fragment, and a macrolactamization to build the 12-membered ring. This synthetic strategy gave rise to a stereoisomer of the targeted natural product in 16 steps and 7.6% overall yield starting from (*S*)-Roche ester. Next, we focused on the total synthesis of an antitumor metabolite, lyngbouilloside. Consequently, a convergent and straightforward strategy was developed relying on three key steps – a Sonogashira coupling to build the side-chain, a hydrosilylation/protodesilylation sequence to generate the diene, and a thermal macrolactonization to build the 14-membered ring.

This route allowed to isolate nominal lyngbouilloside aglycon in 21 steps and 2.1% overall yield starting from commercially available 3-methylbut-3-enol.

Finally, Chapter III describes the total synthesis of (–)-bitungolide F, a marine product bearing a β-ethyl α,β-unsaturated δ-lactone. The synthesis of this dual-specificity phosphatase inhibitor was realized using two highly convergent and enantioselective routes. The first strategy counted on an asymmetric Evans alkylation, a stereoselective pentenylation to introduce the ethyl side chain, a ring-closing metathesis to build the lactone ring, and a chiral boron-mediated aldol/1,3-*anti* reduction end game. This strategy allowed to complete the synthesis of (–)-bitungolide F in 11 steps and 14.6% overall yield starting from levulinic acid. The second strategy led to the total synthesis of (–)-bitungolide F in only nine steps and 11.4% overall yield starting from simple propanal and 3-buten-2-one, employing a slightly more flexible approach which incorporated a key enantioselective organo-catalyzed Michael addition instead of an Evans alkylation. This second approach is particularly appealing as it is highly flexible, it does not involve the use of any protecting group, and is therefore amenable to a wide variety of potentially useful synthetic analogues.

Resum

El tema principal d'aquesta tesi és la síntesi de compostos bioactius. El desenvolupament de productes amb activitats biològiques d'interés es basa, per un costat, en el disseny racional de nous compostos i/o en la modificació de fàrmacs ja existens. D'altra banda, els productes naturals marins han esdevingut també una font molt rica de nous agents terapèutics, a més, també s'utilitzen com a compostos "lead" en el disseny de nous fàrmacs. Dins d'aquest context, aquesta tesi doctoral està dividida en dos projectes principals.

El primer projecte (Capítol I) es va portar a terme en el Departament de Química de la Universitat de Girona sota la direcció de la Dra. Montserrat Heras. Es va centrar en el disseny i síntesi de nous α-aminoàcids no naturals que continguin un anell de pirimidina en la seva cadena lateral, amb l'objectiu d'incorporar-los dins la seqüència del pèptid antimicrobià ***BP100****, recentment desenvolupat en el grup de recerca, per estudiar-ne les propietats biològiques. Els aminoàcids no proteïnogènics, especialment els α-aminoàcids no naturals sintètics, han esdevingut elements clau en el desenvolupament de nous medicaments. Les seves propietats biològiques i diversitat estructural els han convertit tant en fàrmacs eficients com en peces útils en el camp de la química de pèptids. Conseqüentment, els α-aminoàcids no naturals s'han convertit en importants dianes sintètiques i recentment, s'han desenvolupat una gran diversitat de mètodes estereoselectius per a la seva preparació. Atés que aquest treball té com objectiu la síntesi de nous pèptids antimicrobians modificats per incorporació d'α-aminoàcids no naturals, i seguint un projecte del grup de recerca sobre la química de pirimidines, es va investigar la síntesi d'una col·lecció de pirimidines substituïdes en la posició C2 o C4 amb un residu d'α-aminoàcid.*

*Concretament, es va portar a terme una reacció d'acoblament assistida per sals de fosfoni entre la 2-morfolino-4(3*H*)-pirimidinona i la cadena lateral nucleòfila de diferents α-aminoesters naturals N^{α}-Boc protegits. D'aquesta manera, i després d'un intens estudi d'optimització, es van aconseguir els N^{α}-Boc pirimidin-4-il aminoesters en bons rendiments i sense pèrdua d'activitat òptica mitjançant una estratègia en dues etapes a través de l'intermedi benzotriazol-1-iloxipirimidina. D'altra banda, es van*

*preparar nous compostos amb residus d'α-aminoàcids units per la seva cadena lateral a la posició C2 d'un anell de pirimidina. En aquest cas, es va realitzar una reacció d'ipso-substitució nucleòfila entre diverses 2-benzilsulfonil-4-isopropoxipirimidines i diferents α-aminoesters naturals N^{α}-Boc protegits. Les condicions de reacció es van optimitzar per a cada α-aminoester de manera individual per tal d'obtenir els corresponents N^{α}-Boc pirimidin-2-il aminoesters en rendiments acceptables (40-88%) i sense racemització. A més, aquest mètode es va estendre a la preparació de N^{α}-Boc pyrimidin-2-ona α-aminoesters emprant un grup més àcid làbil que l'isopropoxi en la posició C4 de la 2-benzilsulfonilpirimidina de partida. Els N^{α}-Boc pirimidinil aminoesters es van poder transformar en els corresponents N^{α}-Fmoc pirimidinil aminoàcids en bons rendiments (40-93%), utilitzant procediments estàndards de protecció i desprotecció de grups funcionals. Finalment, la col·lecció de nous α-aminoàcids pirimidínics obtinguts es va emprar en el disseny i síntesi de derivats del pèptid **BP100**. Es van sintetizar un total de 14 pèptids en els quals es va reemplaçar algun dels α-aminoàcids de la seqüència peptídica pels preparats en aquest treball. Seguidament, es va testar l'activitat antimicrobiana dels 14 anàlegs de **BP100**, tant contra patògens vegetals com patògens animals, i també es va avaluar la seva activitat hemolítica. Les seqüències peptídiques que contenien aminoàcids pirimidínics de naturalesa hidrofílica són els que van mostrar millors perfils biològics. Tots els assajos biològics es varen realitzar al Centre d'Innovació i Desenvolupament en Sanitat Vegetal (CIDSAV).*

El segon projecte (Capítols II i III) es va desenvolupar en el Laboratoire de Chimie Organique (ESPCI-ParisTech) sota la direcció conjunta de la professora Janine Cossy i el doctor Stellios Arseniyadis.

El Capítol II es va centrar en la síntesi total de dos productes d'origen marí amb estructura macrocíclica, l'acremolide B i la lyngbouilloside. En primer lloc, es va desenvolupar una estràtegia simple per a l'obtenció del lipodepsipeptide acremolide B, l'estructura de la qual no estava confirmada. Aquesta estratègia es va assentar en 4 reaccions clau: dues alil·lacions/crotil·lacions estereoselectives, una reacció de metàtesi creuada per introduir l'àcid gras de la cadena lateral, una esterificació per annexar la unitat dipeptídica al fragment polipropionat, i finalment una macrolactamització per construir l'anell de 12 baules. Aquesta estratègia sintètica va

conduir a la preparació d'un estereoisòmer del producte natural en setze etapes i amb un rendiment global del 7.6% partint de l'éster de (S)-Roche. Posteriorment, es va investigar la síntesi total de la lynboguilloside, un metabòlit antitumoral. Se'n va desenvolupar una estratègia senzilla i convergent basada en tres etapes clau – un acoblament de Sonogashira seguit d'una seqüència d'hidrosililació/deprotosililació per introduir la cadena lateral diennica, i una macrolactonització tèrmica per construir l'anell de 14 baules. Aquesta tàctica va permetre aïllar l'estructura proposada per la lyngbouilloside sense el monosacàrid en vint-i-una etapes amb un rendiment global del 2.1% començant amb el producte comercial 3-metilbut-3-enol.

En el Capítol III descriu la síntesi total de la (–)-bitungolide F, un producte d'origen marí que conté en la seva estructura una δ-lactona α,β-insaturada amb un substituent etil a la posició β de l'anell de δ-lactona. La síntesi d'aquest inhibidor de la fosfatasa de doble especificitat es va realitzar utilitzant dues rutes sintètiques molt convergents i enantioselectives. La primera estratégia es va fonamentar en una alquilació asimètrica d'Evans, una pentenil·lació estereoselectiva per introduir el grup etil de la cadena lateral, una reacció de metàtesi de tancament d'anell per construir la δ-lactona, i una seqüència final d'aldolització asimètrica mitjançant un reactiu de bor quiral seguida d'una reducció 1,3-anti. Aquesta maniobra va permetre completar la síntesi de la (–)-bitungolide F en onze etapes amb un rendiment global del 14.6% començant amb l'àcid levulínic. La segona estratègia va permetre completar la síntesi del producte natural en nou etapes i un 11.4% de rendiment global partint de dos compostos molt senzills, el propanal i la 3-buten-2-ona. En aquest cas es va emprar una reacció d'addició de Michael organocatalitzada en lloc de l'alquilació d'Evans convertint el procés molt més flexible. Aquesta darrera ruta sintètica és interessant degut a la seva versatibilitat i perqué no necessita l'ús de grups protectors per tant es podria emprar en la preparació d'una gran varietat d'anàlegs de la (–)-bitungolide F potencialment bioactius.

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List of Abbreviations/Symbols

Ac	Acetyl
AMPs	Antimicrobial peptides
Aq.	Aqueous
Boc	*tert*-Butoxycarbonyl
BOP	Benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate
Bn	Benzyl
c	Concentration
CAL-B	*Candida antarctica* lipase B
CAN	Cerium ammonium nitrate
cat.	Catalytic
Cbz	Benzyloxycarbonyl
CM	Cross-metathesis
m-CPBA	*meta*-Chloroperbenzoic acid
d	Day(s)
DBU	1,8-Diazabicylo[5.4.0]undec-7-ene
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutyl aluminum hydride
DIEA	Diisopropylethylamine / Hunig's base
DMAP	4-Dimethylaminopyridine
DMP	Dess-Martin periodinane
EDCI	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimde
ee	Enantiomeric excess
er	Enantiomeric ratio
Eq	Equation
equiv	Equivalent(s)
ESI	Electrospray ionization
ESI-MS or MS(ESI)	Mass spectroscopy by electrospray ionization
Fmoc	9-Fluorenylmethoxycarbonyl

Fmoc-OSu	*N*-(9-Fluorenylmethoxycarbonyloxy) succinimide
h	Hour(s)
HATU	*O*-(7-Aza-1*H*-benzotriazole-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
HBTU	*O*-Benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectroscopy
HMPA	Hexamethylphosphoramide
HOBt	1-Hydroxybenzotriazole
HOPt	1-Hydroxypyridotriazole
IC_{50}	Molar concentration at which 50% inhibition is observed
imid	Imidazole
IR	Infrared spectroscopy
J	Coupling constant
LDA	Lithium diisopropyl amine
LIPPSO	Innovation Laboratory in Organic Processes and Products
M	Molar
MBHA	4-Methylbenzhydrylamine
MIC	Minimum inhibitory concentration
min	Minute(s)
mL	Milliliter(s)
mol	Mole(s)
Mp	Melting point
Ms	Methane sulfonyl (mesyl)
MS	Molecular sieves, mass spectroscopy
MW	Microwave irradiation
m/z	Mass/charge ratio
NaHMDS	Sodium bis(trimethylsilyl)amide
nd	Not determined
NMO	*N*-methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance

NOESY	Nuclear Overhauser Effect Spectroscopy
Nu	Nucleophile
[O]	Oxidation
OTf	Trifluoromethane sulfonyl
PCC	Pyrimidinium chlorochromate
PEG	Polyethylene glycol
Piv	Pivaloyl
PMB	*para*-Methoxybenzyl
PLE	Pig liver esterase
PMP	*para*-Methoxyphenyl
i-Pr	Isopropyl
PPTS	Pyridinium *para*-toluenesulfonate
Pr	Propyl
PS	Polystyrene
PyAOP	(7-Azabenzotriazol-1-yloxy)tris(pyrrolidino) phosphonium hexafluorophosphate
PyBOP	Benzotriazole-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
Py	Pyridine
quant.	Quantitative
RCM	Ring closing metathesis
R_f	Retention factor
rt	Room temperature
S_NAr	Aromatic nucleophilic substitution
SPPS	Solid-phase peptide synthesis
TABH	Tetramethylammonium triacetoxyborohydride
(*R*,*R*)-TADDOL	(4*R*)-*trans*-2,2-dimethyl-tetraphenyl-1,3-dioxolane-4,5-dimethanol
TBAF	Tetra-*n*-butylammonium fluoride
TBS	*tert*-Butyldimethylsilyl
TCBC	2,4,6-Trichlorobenzoyl chloride
TES	Triethylsilyl
Tf	Triflate
TFA	Trifluoroacetic acid

TIPS or TIS	Triisopropylsilyl
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TPAP	Tetra-*n*-propylammonium perruthenate
t_R	Retention time
Ts	*para*-Toluenesulfonyl
UV	Ultra-violet
Z	Benzylcarboxylcarbonyl

GENERAL INTRODUCTION

The development of potential drugs relies on both the rational design of new synthetic compounds and/or the modification of existing drugs. Natural products, which are present in all life kingdoms, are also a rich source of novel therapeutic agents and, as such, represent a great source of inspiration.

Among the incredible structural diversity displayed by natural products, we chose to focus the present PhD study on the synthesis of both non-proteinogenic amino acids and marine natural products. Indeed, non-proteinogenic amino acids exhibit particularly valuable biological properties and serve as building blocks for surrogates of proteinogenic amino acids in known peptidic entities thus allowing to modulate their biological behaviour. Marine natural products, on the other hand, provide useful pharmaceuticals but also serve as lead compounds in the design of new drugs.

This thesis, which is structured around these two main topics, contains three Chapters. Chapter I concerns the design and the synthesis of unusual α-amino acids and their use as building blocks in the development of new antimicrobial peptides. The next two Chapters focus on the straightforward and flexible total synthesis of three marine natural products which exhibit promising biological properties: acremolide B, lyngbouilloside and (–)-bitungolide F.

CHAPTER I

Synthesis of unusual α-amino acids and study of the effect of their incorporation into antimicrobial peptides

I.1. INTRODUCTION

I.1.1. NATURALLY OCCURING AMINO ACIDS

I.1.1.1. Proteinogenic amino acids

Natural proteins and peptides are linear polyamides, which are ribosomally synthesized under nucleic acid control from the 20 so-called DNA-encoded or proteinogenic α-amino acids. Proteins display a wide variety of chemical, physical and physiological properties in living organisms. As a matter of fact, enzymes as well as several hormones that catalyze and regulate reactions necessary for life are proteins. The properties of proteins are dependent on the amino acid sequence as well as on their three-dimensional structure. Peptides are simply smaller versions of proteins. The main difference between peptides and proteins is essentially the size or length of the α-amino acids backbone. The three-dimensional structures of peptides tend to be less well defined, but many, such as the peptide-hormones vasopressin, oxytocin, and calcitonin, the neuroactive peptides found in the brain, or the toxins of certain animals and bacteria, are biologically important. There are currently several peptides or peptide-based drugs in widespread clinical use. In addition, peptide molecules show much promise as potential therapeutic agents against infectious disorders.[1]

[1] Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Chemical approaches to the synthesis of peptides and proteins*, Florida, CRC Press, **1997**.

On the other hand, the DNA-encoded α-amino acids by themselves play a central role in chemistry and biology. Their availability is critical in basic research applications, as well as in industry. About 66% of amino acids produced are used in the food industry as flavour enhancers, 31% as feed additives and the remaining 3% in medicine and cosmetics and as starting materials in the chemical industry.[2]

In 1820, glycine was isolated from gelatin hydrolyzates. In the following decades, the remaining nineteen proteinogenic α-amino acids were identified (Figure I.1). All except proline have the same basic structure, which incorporates a primary amino group and differs only in the nature of the side chain. Proline, on the other hand, is unique in having a cyclic structure with a secondary amine.

[2] Bhat, S. V.; Nagasampagi, B. A.; Sivakumar, M. *Chemistry of Natural Products*, Berlin; New York, Springer, **2005**.

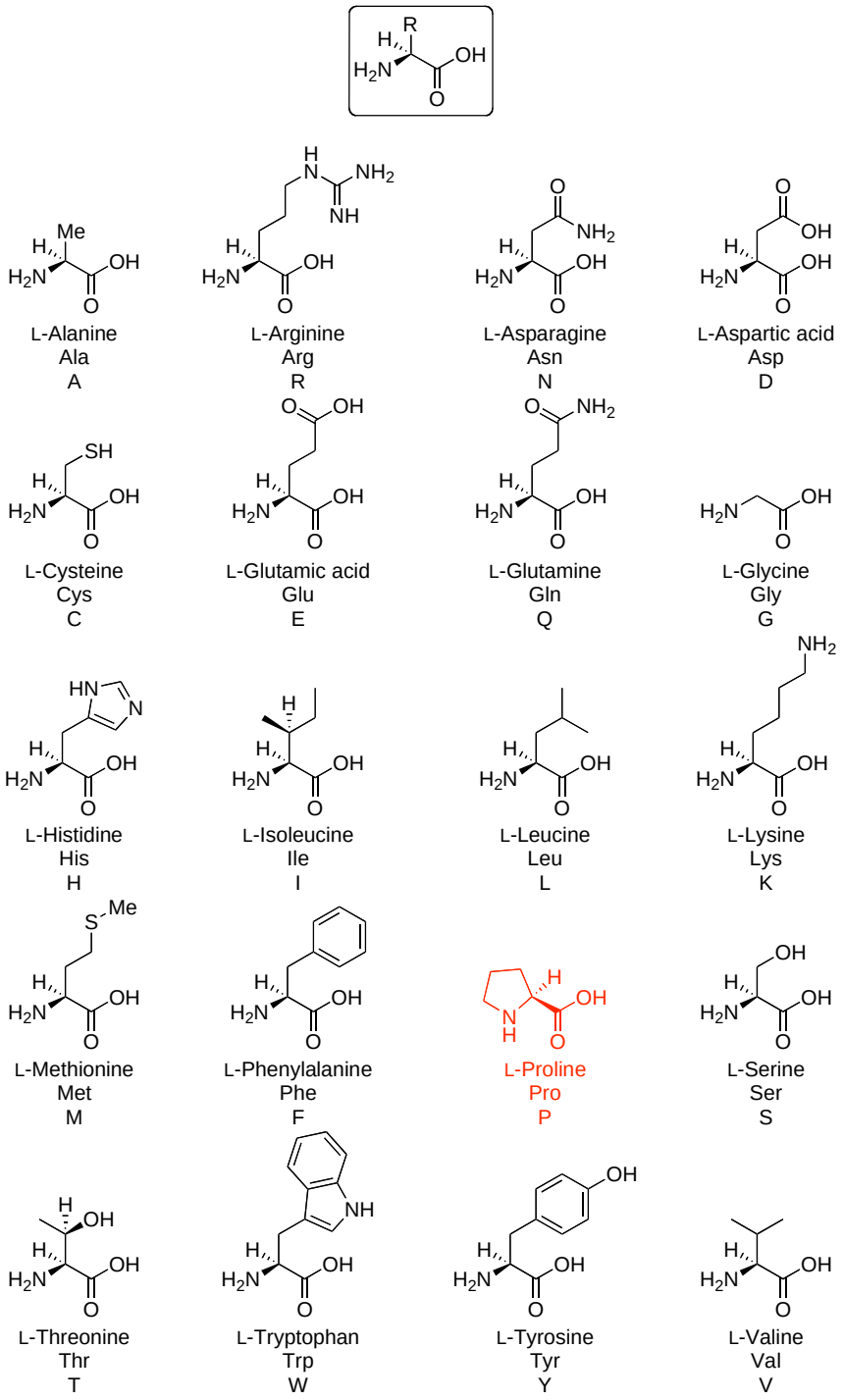


Figure I.1.[3] The proteinogenic α-amino acids

With the exception of glycine, all are chiral, due to the presence of at least one stereogenic carbon atom, and belong to the L-stereochemical series. The chiral α-amino acids all have the (*S*) configuration at the α-carbon atom, except cysteine in which it is (*R*) as a consequence of the manner in which the Cahn-Ingold-Prelog convention

[3] The three-letter and one-letter codes for each amino acid are given below the chemical name.

functions.[4] Two amino acids, threonine and isoleucine, have a second stereogenic center at the β-carbon atom, giving rise to four possible diastereoisomers for each one. In the case of L-threonine this second stereogenic center has the stereochemistry (*R*), while in L-isoleucine, the β-carbon atom has the (*S*) configuration.

I.1.1.2. Non-proteinogenic amino acids

Non-proteinogenic amino acids, also referred to as non-ribosomal or non-coded amino acids, are building blocks that are not incorporated normally into proteins and peptides by the ribosome. Thus, they do not include the twenty DNA-encoded α-amino acids. There are thousands of non-proteinogenic amino acids present in protein and peptide sequences, which also play various important biological roles. Certain non-coded amino acid residues are sometimes found in ribosomally synthesized peptides and proteins as a consequence of some post-translational enzymatic modification.[5] In addition, in several types of lower organisms, such as algae, sponges, yeast and fungi, peptides are often biosynthesized enzymatically[6] rather ribosomally. Apart from the proteinogenic amino acids, these peptides may contain other modified amino acids of which many hundreds are not yet known.[7]

The majority of the non-proteinogenic amino acids known today were discovered during the search for new antibiotics in the culture media of microorganisms, or as components of the antibiotics in fungi, seeds, in numerous plants and fruits, and in the body fluids of animals.[7]

The non-proteinogenic amino acids may be divided into several broad classes of which some may simply be enantiomers of the proteinogenic α-amino acids, such as D-alanine found in the cell walls of bacteria and also in higher plants. Others may be methylated derivatives either at the amino group or at the α-carbon atom, such as *N*-methylleucine or aminoisobutyric acid, respectively (Figure I.2).[1] Some are amino acids that have the amino group at a position other than the α-carbon; for example, β-amino acids are key components of a variety of biologically active molecules

[4] Cahn, R. S.; Ingold, C.; Prelog, V. *Angew. Chem. Int. Ed. Engl.* **1966**, *5*, 385-415.
[5] Hughes, A. B. *Amino Acids, Peptides and Proteins in Organic Chemistry*. Vol. 1-*Origins and Synthesis of Amino Acids*, Weinheim, WILEY-VCH Verlag GmbH & Co. KGaA, **2009**.
[6] (a) Lipmann, F. *Science* **1971**, *173*, 875-884. (b) Kleinkauf, H.; Von Döhren, *Eur. J. Biochem.* **1996**, *236*, 335-351.
[7] Wagner, I.; Musso, H. *Angew. Chem. Int. Ed. Engl*. **1983**, *22*, 816-828.

including the antitumor agent Taxol®.[8] Likewise, γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). Others γ-amino acids[9] have an important role in the structure of natural products with antitumor activity such as hapalosin[10] or dolastatin 10[11] (Figure I.2).

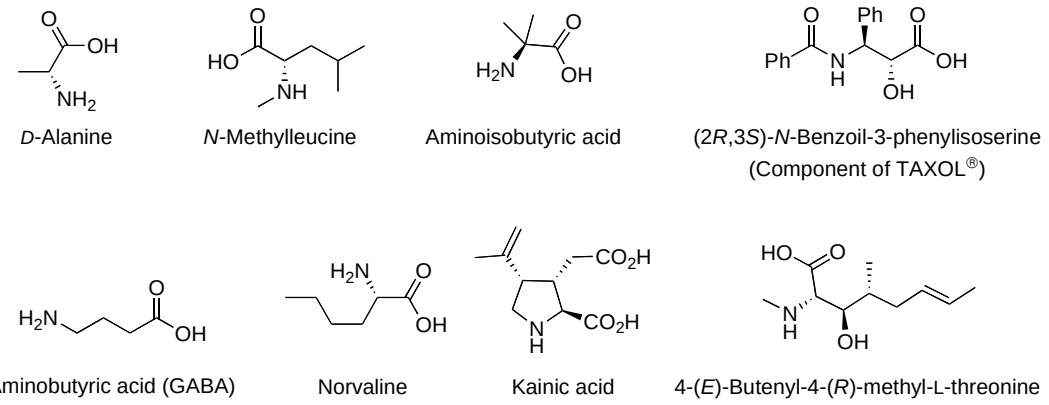


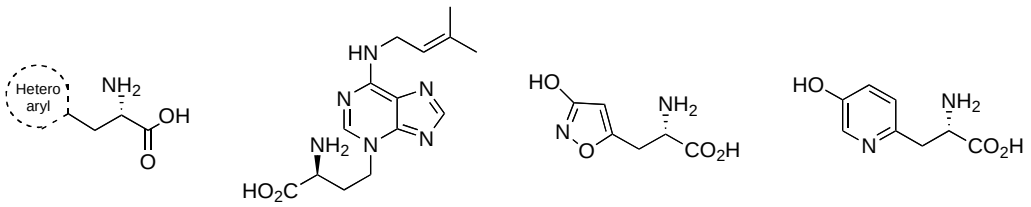
Figure I.2. Natural occurring non-proteinogenic amino acids

α-Amino acids with modified side chains constitute another wide range of non-proteinogenic amino acids. This category includes the important groups of arylglycines,[12] α,α-disubstituted amino acids,[13] proline derivatives,[14] biaryl amino acids,[15] aliphatic amino acids,[7] unsaturated amino acids,[7] heterocyclic amino acids,[7] cyclic and bicyclic amino acids,[16] etc... Some of them may be quite simple as in the case of norvaline, an aliphatic α-amino acid isomeric with valine which is a component of an antifungal peptide of *Bacillus subtilis*, or more complex as kainic acid, a proline derivative, which was isolated from the alga *Digenea simplex* and used as a research tool in neuropharmacology.[17] Several of these structural features are often combined in

[8] Kingston, D. G. I. *J. Nat. Prod*. **2000**, *63*, 726-734.
[9] Ordoñez, M.; Ctiviela, C. *Tetrahedron: Asymmetry* **2007**, *18*, 3-99.
[10] Stratmann, K.; Burgoyne, D. L.; Moore, R. E.; Patterson, G. M. L. *J. Org. Chem.* **1994**, *59*, 7219-7226.
[11] Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. *J. Am. Chem. Soc.* **1987**, *109*, 6883-6885.
[12] (a) Willians, R. M.; Hendrix, J. A. *Chem. Rev*. **1992**, *92*, 889-917. (b) Beenen, M. A.; Weix, D. J.; Ellman, J. A. *J. Am. Chem. Soc.* **2006**, *128*, 6304-6305. (c) Shang, G.; Yang, Q.; Zhang, X. *Angew. Chem. Int. Ed*. **2006**, *45*, 6360-6362.
[13] Vogt, H.; Bräse, S. *Org. Biomol. Chem.* **2007**, *5*, 406-430.
[14] (a) Van Esseveldt, B. C. J.; Vervoort, P. W. H.; Van Delft, F. L.; Rutjes, F. P. J. T. *J. Org. Chem.* **2005**, *70*, 1791-1795. (b) Onomura, O.; Kirira, P. G.; Tanaka, T.; Tsukada, S.; Matsumura, Y.; Demizu, Y. *Tetrahedron* **2008**, *64*, 7498-7503.
[15] Feliu, L.; Planas, M. *Int. J. Pept. Res. Ther.* **2005**, *11*, 53-97.
[16] (a) Meyer, U.; Bisel, P.; Bräuner-Osborne, H.; Madsen, U.; Höfner, G.; Wanner, K. TH.; Frahm, A. W. *Chirality* **2005**, *17*, 99-107. (b) Trabocchi, A.; Scarpi, D.; Guarna, A. *Amino Acids* **2008**, *34*, 1-24.
[17] Takita, S.; Yokoshima, S.; Fukuyama, T. *Org. Lett.* **2011**, *13*, 2068-2070.

one amino acid such as 4-(*E*)-butenyl-4-(*R*)-methyl-L-threonine, a component of cyclosporine,[18] which can be classified as a *N*-methylated amino acid and also as an unsaturated amino acid (Figure I.2).

Our research group within the Laboratory of Innovation in Organic Products and Processes (LIPPSO) of the University of Girona has also been interested in the design and in the synthesis of non-coded amino acids, focusing on heterocyclic amino acids of type **I**, a class of non-proteinogenic α-amino acid whose chain carries a heterocyclic ring[19] (Figure I.3). In fact, the majority of natural non-coded amino acids reported in literature are members of this class.[7] Several examples include L-azatyrosine,[20] a naturally occurring amino acid isolated from *Streptomyces chibanesis*, which displays antibacterial and anticancer activities, discadenine,[21] a spore germination inhibitor of the slime mould *Dictyostelium discoideum* or ibotenic acid,[22] an active constituent of the psychotropic fly agaric mushroom *Amanita muscaria* that acts as a glutamate receptor agonist (Figure I.3).



Heteroarylalanines **Type I** | Discadenine | Iboteic acid | L-Azatyrosine

Figure I.3. Natural heterocyclic α-amino acids

Some natural heterocyclic α-amino acids such as willardiine[23] and L-lathyrine[24] present a pyrimidine ring in their structure (Figure I.4). Willardiine, which was isolated

[18] Cyclosporin is an immunosuppressant drug widely used in post-allogeneic organ transplant.
[19] Kolar, P.; Petric, A.; Tisler, M. *J. Heterocyclic Chem.* **1997**, *34*, 1067-1098.
[20] (a) Ye, B.; Burke, T. R. *J. Org. Chem.* **1995**, *60*, 2640-2641. (b) Adamczyk, M.; Akireddy, S. R.; Reddy, R. E. *Org. Lett.* **2001**, *3*, 3157-3159. (c) Wang, H. P.; Hwang, T. L.; Lee, O.; Tseng, Y. J.; Shu, C. Y.; Lee, S. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4272-4274.
[21] (a) Nomura, T.; Tanaka, Y.; Abe, H.; Uchiyama, M. *Phytochemistry* **1977**, *16*, 1819-1820. (b) Son, J. K.; Ramaligam, K.; Woodard, R. W. *Synthesis* **1988**, 240-242.
[22] (a) Li, C.; Oberlines, N. H. *Life Sci.* **2005**, *78*, 532-538. (b) Jørgensen, C. G.; Clausen, P. R.; Hansen, K. B.; Bräuner-Osborne, H.; Nielsen, B; Metzler, B.; Kehler, J.; Krogsgaard-Larsen, P.; Madsen, U. *Org. Biomol. Chem.* **2007**, *5*, 463-471.
[23] (a) Jane, D.; Hoo, K.; Kamboj, R.; Deverill, M.; Bleakman, D.; Mandelzys, A. *J. Med. Chem.* **1997**, *40*, 3645-3650. (b) Dolman, N. P.; More, J. C. A.; Alt, A.; Knauss, J. L.; Pentikäinen, T. O.; Glasser, R. C.; Bleakman, D.; Mayer, M. L.; Collingridge, G. L.; Jane, D. E. *J. Med. Chem.* **2007**, *50*, 1558-1570.

from the seeds of *Acacia willardiana*. Recently, and N^3-substituted willardiine analogs were shown to be potent and selective kainate receptor antagonists.[23b] L-Lathyrine, on the other hand, was isolated from the seeds of *Lathyrus tingitanus* and displays a diverse range of bioactivity such as growth inhibition, antitumor properties, and a hypoglycaemic activity.

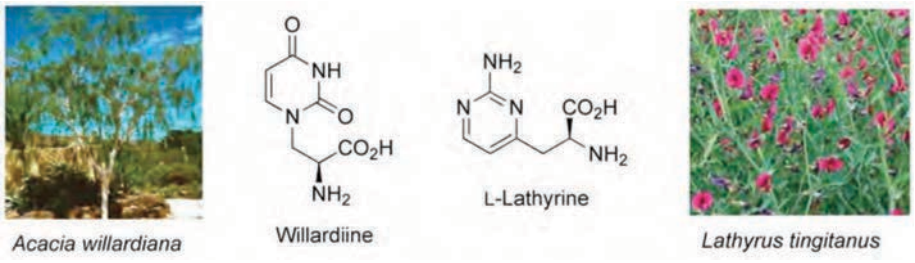


Figure I.4. Pyrimidine containing natural amino acids

I.1.2. UNNATURAL AMINO ACIDS

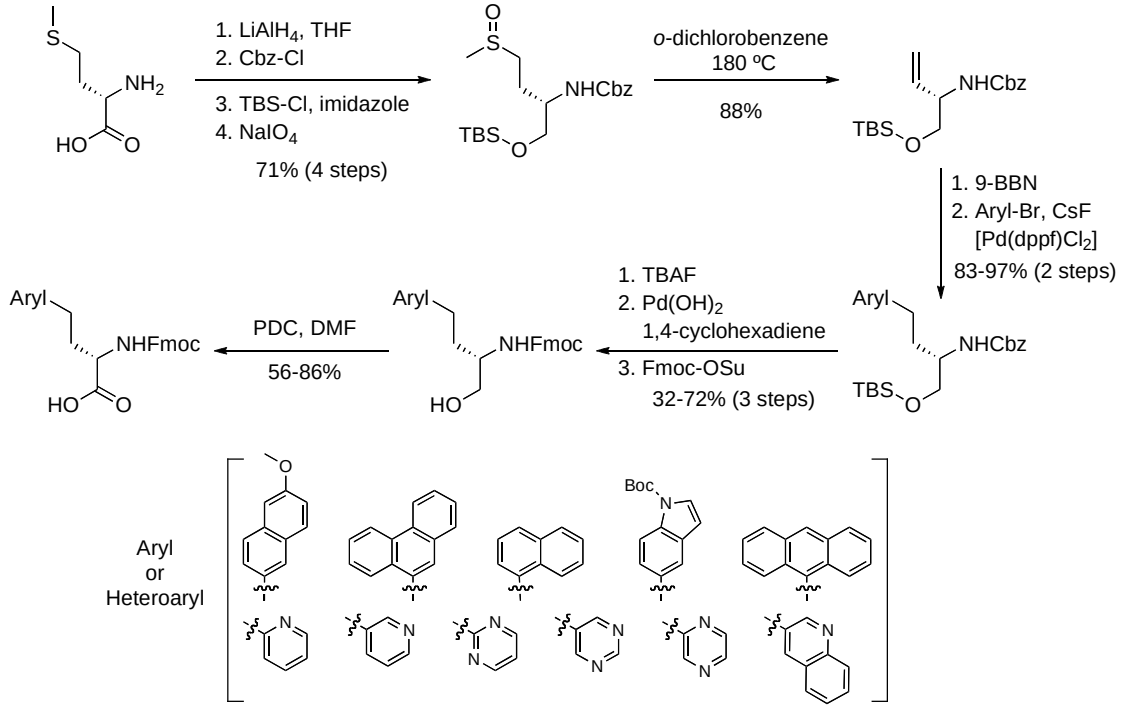
The non-proteinogenic α-amino acids found in nature provide us with some of the most powerful molecular tools for biology and medicine known today.[5] Antibiotics, immunosuppressant, anticancer, antivirals, anti-inflammatory are a few of the diverse biological properties of non-proteinogenic amino acids. Consequently, synthetic non-proteinogenic amino acids, also called unnatural amino acids, have emerged as important synthetic targets.[25] Many of these unnatural amino acids are critical components in pharmaceuticals and developmental drugs. Furthermore, their incorporation in protein and peptide sequences has allowed to develop new mechanistic probes or to improve the activity, stability, bioavailability and selectivity of peptide-containing therapeutic agents.

On the other hand, the chirality of α-amino acids is critically essential for molecular recognition and biological activity. Thus, in recent years many stereoselective

[24] (a) Brown, E. G.; Turan, Y. *Phytochemistry* **1996**, *43*, 1029-1031. (b) Adlington, R. M.; Baldwin, J. E.; Catterick, D.; Pritchard, G. J. *Chem. Commun.* **1997**, 1757-1758.
[25] (a) Kaiser, J.; Kinderman, S. S.; Van Esseveldt, B. C. J.; Van Delft, F. L.; Schoemaker, H. E.; Blaauw, R. H.; Rutjes, F. P. *Org. Biomol, Chem.* **2005**, *3*, 3435-3467. (b) Scott, W. L; Alsina, J.; Audu, C. O.; Babaev, E.; Cook, L.; Dage, J. L.; Goodwin, L. A.; Martynow, J. G.; Matosiuk, D.; Royo, M.; Smith, J. G.; Strong, A. T.; Wickizer, K.; Woerly, E. M.; Zhou, Z.; O'Donnell, M. J. *J. Comb. Chem.* **2009**, *11*, 14-23.

methods[26] to access new synthetic enantiopure unnatural α-amino acids have been reported. These methods are mainly based on the enantioselective transformation of prochiral starting materials or by modifying natural α-amino acids.[5]

In this sense, standard chemical modifications of amino acids with functionalities in the side chains, such as serine, cysteine, tyrosine, lysine, aspartic or glutamic acid,[27] allow a straightforward access to a wide variety of unnatural amino acids including heterocyclic α-amino acids. For example, Göbel *et al.* reported an efficient synthesis of a collection of unnatural aryl and heteroaryl amino acids starting from methionine through Suzuki couplings with various aryl halides (Scheme I.1).[28]

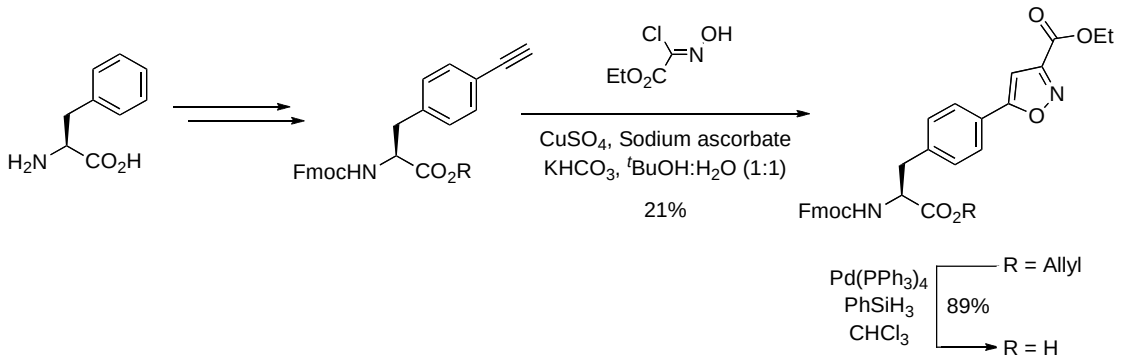


Scheme I.1. Synthesis of aryl and heteroaryl unnatural amino acids

More recently, Yao and co-workers reported the synthesis of a novel unnatural α-amino acid with an isoxazole ring in its side chain that mimics phosphotyrosine.[29] The final Fmoc-protected form of this unnatural α-amino acid was synthesized in

[26] (a) Duthaler, R. O. *Tetrahedron* **1994**, *50*, 1539-1650. (b) Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung, D. *J. Am. Chem. Soc.* **1997**, *119*, 656-673. (c) Hruby, V. J.; Soloshonok, V. A. *Asymmetric synthesis of novel sterically constrained amino acids. Symposia-in-print; Tetrahedron*; Eds.; **2001**, *57*, 6329-6650. (d) Perdih, A.; Sollner, M. D. *Curr. Org. Chem.* **2007**, *11*, 801-832. (e) Nájera, C.; Sansano, J. M. *Chem. Rev.* **2007**, *107*, 4584-4671. (f) Haldar, D. *Curr. Org. Synth.* **2008**, *5*, 61-80.
[27] Blayo, A.-L.; Brunel, F.; Martinez, J.; Fehrentz, J.-A. *Eur. J. Org. Chem.* **2011**, 4293-4297.
[28] Krebs, A.; Ludwig, V.; Pfizer, J.; Dürner, G.; Göbel, M. W. *Chem. Eur. J.* **2004**, *10*, 544-553.
[29] Ge, J.; Wu, H.; Yao, S. Q. *Chem. Commun.* **2010**, *46*, 2980-2982.

several steps starting from the phenylalanine. The key step in this sequence was the isoxazole ring formation *via* a 1,3-dipolar cycloaddition reaction (Scheme I.2).



Scheme I.2. Synthesis of a Fmoc-protected unnatural phenylalanine derivative

I.1.3. NATURAL AND SYNTHETIC PYRIMIDINES

The structural feature common to the most of the bioactive non-proteinogenic α-amino acids is the presence of a heterocycle moiety in the side chain, and of special interest is the presence of a pyrimidine or pyrimidine derivative as the main pharmacophore group.

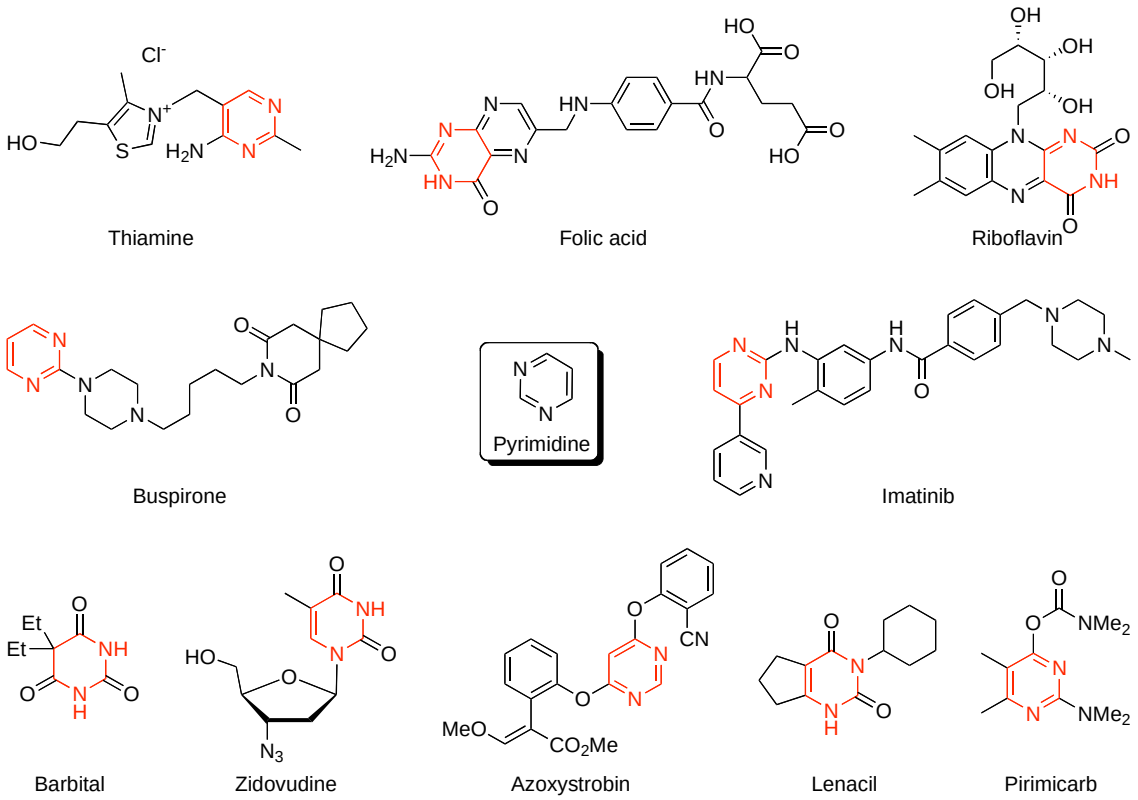


Figure I.5. Natural and pharmaceuticals pyrimidine-based products

Pyrimidine and its various oxo forms are crucial in biological systems due to their presence in all naturally occurring nucleobases. Since the discovery of the first pyrimidine derivative in 1818, there has been great interest in this type of heterocycle.[30] Apart from nucleobases, the pyrimidine ring is present in some of the most important biologically active compounds such as thiamine, folic acid or riboflavin (Figure I.5).[31] In addition, synthetic pyrimidine derivatives have important medicinal[32] and agrochemical[33] properties. One of the earliest series of pyrimidine pharmaceuticals is represented by the hypnotic barbiturates such as barbital (Figure I.5). Other examples of pyrimidine-based pharmaceuticals include buspirone, used to treat anxiety disorders, the antiviral agent zidovudine, and imatinib (Gleevec®), a tyrosine kinase inhibitor used in the treatment of certain types of cancers. Azoxystrobin, lenacil and pirimicarb are

[30] (a) Von Angerer, S. *Science of Synthesis. Six-Membered Hetarenes with Two Identical Heteroatoms: Pyrimidines*, Thieme, Stuttgart, **2003**, *16*, 379-572. (b) Rewcastle, G. W. *Comprehensive Heterocyclic Chemistry III: Pyrimidines and their Benzo Derivatives*, Elsevier, **2008**, *8*, 117-272.
[31] Lagoja, I. M. *Chemistry & Biodiversity* **2005**, *2*, 1-50.
[32] Jain, K. S.; Chitre, T. S.; Miniyar, P. B.; Kathiravan, M. K.; Bendre, V. S.; Veer, V. S.; Shahane, S. R.; Shishoo, C. J. *Curr. Sci.* **2006,** *90*, 793-803.
[33] Laimberth, C. *Heterocycles* **2006**, *68*, 561-603.

examples of commercially available pyrimidine-based fungicides, herbicides and insecticides, respectively (Figure I.5).

I.1.3.1. Tautomeric equilibrium in pyrimidine ring

Tautomerism generally occurs in pyrimidines when substituted by hydroxyl, thiol or amino groups. Both 2- and 4-hydroxypyrimidine can exist as their hydroxyl or keto tautomeric form, and the proportion of each is highly dependent on the state of the molecule. In the gas phase, for instance, 2-hydroxypyrimidine exists primarily in the hydroxyl form (Figure I.6, Eq 1) whereas the 4-isomer exists predominantly in the oxo form (Figure I.6, Eq 2). Finally, solvation tends to shift the equilibrium toward the oxo form for both isomers.

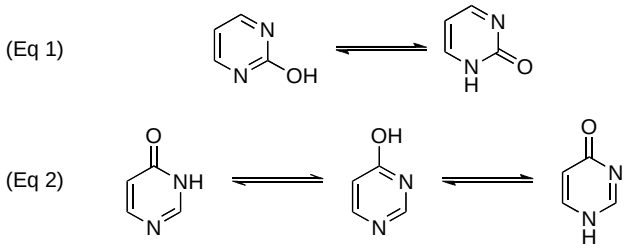


Figure I.6. Tautomeric equilibrium of 2- and 4-hydroxypyrimidine

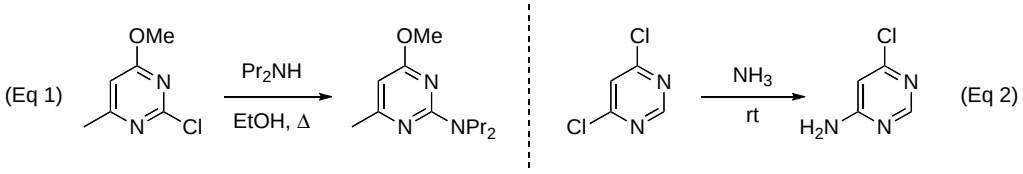
The stability of the thiol tautomers of mercaptopyrimidines compared to the corresponding thione forms is considerably higher than for hydroxypyrimidines, although the thione is still dominant in solution. However, 2- and 4-aminopyrimidines exist predominantly in the amino form rather than the imino form.[34] Consequently, these equilibriums convert pyrimidines with tautomerizable substituents into tri or tetradentate nucleophiles under alkylation or acylation conditions.[30b]

I.1.3.2. Nucleophilic substitution reaction in pyrimidine ring

A heteroaromatic nucleophilic *ipso*-substitution reaction is commonly used to introduce molecular diversity in many electron-deficient heterocycles such as pyrimidines. Therefore, halopyrimidines and especially chloropyrimidines are important intermediates in the synthesis of pyrimidine derivatives.

[34] (a) Stanovnik, B.; Tisler, M.; Katritzky, A. R.; Denisko, O. V. *Advances in Heterocyclic Chemistry*, San Diego, Elsevier, **2006**. (b) Freeman, F.; Po, H. N. *J. Phys. Chem. A* **2006**, *110*, 7904-7912.

As mentioned previously, pyrimidine is a π-deficient heterocycle. The two nitrogen atoms of the pyrimidine ring exert a strong electron-withdrawing effect that is comparable to the nitro groups in 1,3-dinitrobenzene. This effect particularly reduces electron densities at the 2-, 4-, and 6-positions making these positions susceptible to a nucleophilic attack, whereas electrophilic reactions can take place at the 5-position or on the nitrogen atoms.[35] Consequently, halogens in all ring positions can undergo nucleophilic substitution in the presence of a wide variety of nucleophiles (alcohols, amines, thiols, alkoxys, etc...), but C2, C4 and C6 are more favoured than C5. The reaction conditions strongly depend on the substituents attached on the pyrimidine ring. Electron-donating substituents decrease the nucleophilic displacement rate (Scheme I.3, Eq 1), whereas electron-withdrawing substituents have the opposite effect (Scheme I.3, Eq 2).



Scheme I.3. Nucleophilic substitution of substituted-chloropyrimidine

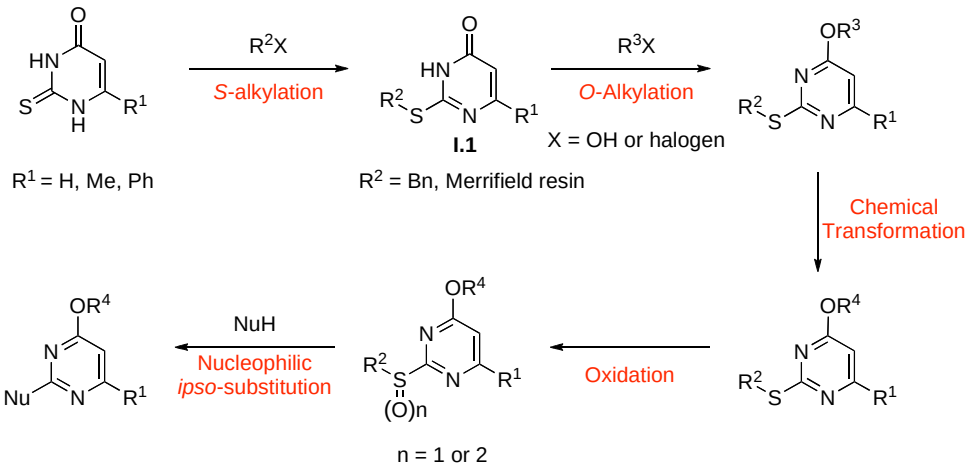
I.1.3.3. Background to the research group

During the last few years, our laboratory has been developing efficient methods for the preparation of libraries of pyrimidinyl compounds with a high degree of molecular diversity through solution or solid-phase synthesis. These new synthetic strategies are based either on the selective *O*-alkylation of 2-(alkylsulfanyl)-4(3*H*)-pyrimidinones (**I.1**) with alkyl halides under basic conditions or on a Mitsunobu reaction, followed by subsequent chemical transformations at position 4 of the pyrimidine ring and, a final nucleophilic *ipso*-substitution step of the oxidized sulfur with a variety of nucleophiles (Scheme I.4).[36] The starting compound **I.1** was easily obtained by selective *S*-alkylation of the corresponding 2-thiouracil derivatives. Indeed, it is well known that alkylation of

[35] Eicher, T.; Hauptmann, S. *The chemistry of heterocycles*, Weinheim, Wiley-VCH, **2003**.
[36] (a) Font, D.; Heras, M.; Villalgordo, J. M. *Synthesis* **2002**, 1833-1842. (b) Font, D.; Heras, M.; Villalgordo, J. M. *J. Comb. Chem.* **2003**, *5*, 311-321. (c) Font, D.; Linden, A.; Heras, M.; Villalgordo, J. M. *Tetrahedron* **2006**, *62*, 1433-1443. (d) Font, D.; Heras, M.; Villalgordo, J. M. *Tetrahedron* **2008**, *64*, 5226-5235.

tautomeric thiones invariably gives the *S*-alkyl derivative as the *N*-, *O*-, and *C*-alkylation are less rapid.

The two key steps in this synthetic strategy are the selective *O*-alkylation of 4(3*H*)-pyrimidinones **I.1** and the nucleophilic *ipso*-substitution reaction of the oxidized sulfur.

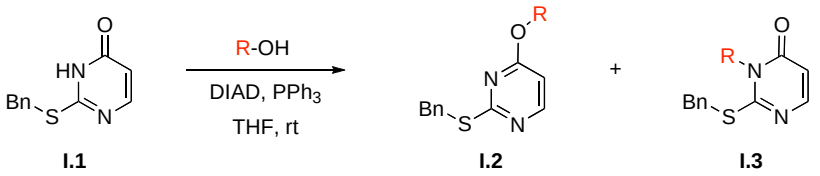


Scheme I.4. Synthetic strategy for the preparation of pyrimidinyl compounds

I.1.3.3.1. Selective O-alkylation of 4(3H)-pyrimidinones

It is well accepted that the chemoselectivity in the alkylation of 4(3*H*)-pyrimidinones is largely governed by the nature of the alkylating agent, the reaction conditions (solvent, base, etc...) and the stereoelectronic effects induced by the adjacent substituents.[37] However, *N*-alkylation is sterically more demanding than *O*-alkylation. The reaction rate of nitrogen decreases relatively to that of oxygen as the bulk of the alkylating reagent increases. In good agreement with these facts, the selective *O*-alkylation of 2-(alkylsulfanyl)-4(3*H*)-pyrimidinones **I.1** can be achieved using bulky alkyl halides or bulky alcohols when the reaction are carried out under basic or Mitsunobu conditions, respectively (Scheme I.5).[36]

[37] (a) Jonak, J. P.; Hopkins, G. C.; Minnemeyer, H. J.; Tieckelmann, H. *J. Org. Chem.* **1970**, *35*, 2512-2516. (b) Gambacorta, A.; Farah, M. E.; Tofani, D. *Tetrahedron* **1999**, *55*, 12615-12628.



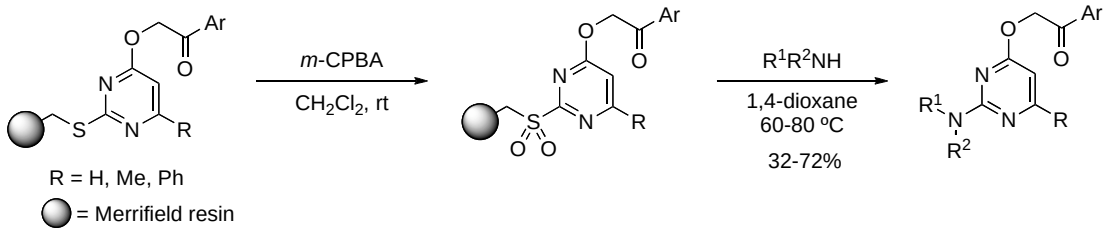
I.1 I.2 I.3

R	Ratio (*O*-alkylated / *N*-alkylated)	Yield (%)
$-CH_3$	25:75	86
$-CH_2C_6H_5$	60:40	96
$-CH(CH_3)_2$	95:5	92
$-C(CH_3)_3$	100:0	51

Scheme I.5. Alkylation of 4(3*H*)-pyrimidinones **I.1** under Mitsunobu conditions

I.1.3.3.2. Nucleophilic ipso-substitution of 2-alkylsulphonylpyrimidines

Pyrimidines bearing an alkylsulfinyl-, an arylsulfinyl-, an alkylsulfonyl- or an arylsulfonyl substituent were shown to be equally or more reactive than the corresponding halogen-substituted pyrimidines (Cl or Br).[38] In the synthetic method developed in our laboratory (Scheme I.4), the nucleophilic *ipso*-substitution reaction was used not only to introduce molecular diversity at the C2-position of the pyrimidine ring but also as a cleavage step when the reactions were carried out on solid support (Scheme I.6).[36b]



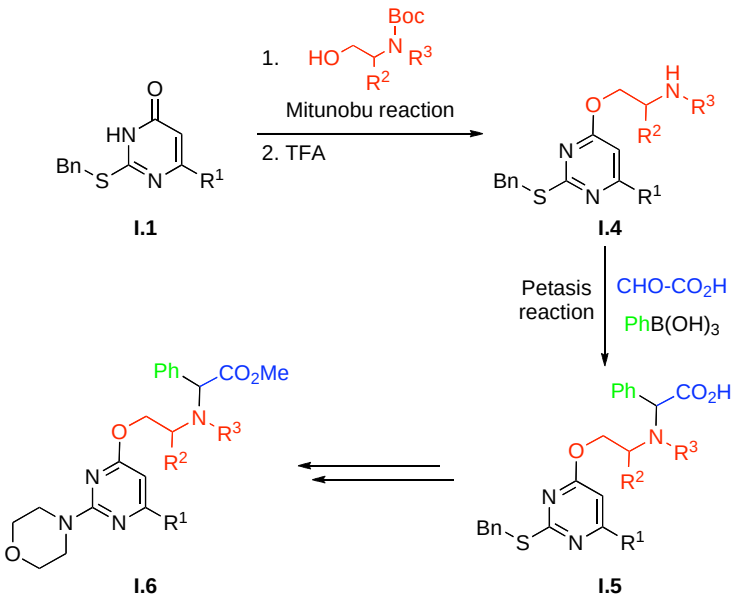
Scheme I.6. Solid-phase synthesis of C4 substituted 2-aminopyrimidines

I.1.3.3.3. Synthesis of pyrimidinyl α-amino acids

Following this method, the synthesis of novel *N*-pyrimidinyl arylglycines of type **I.6** was recently reported by our laboratory. Thus, when the readily available 2-benzylsulfanyl-4(3*H*)-pyrimidinones **I.1** were treated with different primary and

[38] (a) Barlin, G. B.; Brown, W. V. *J. Chem. Soc. (B)* **1967**, 648-650. (b) Barlin, G. B.; Brown, W. V. *J. Chem. Soc. (B)* **1967**, 736-740.

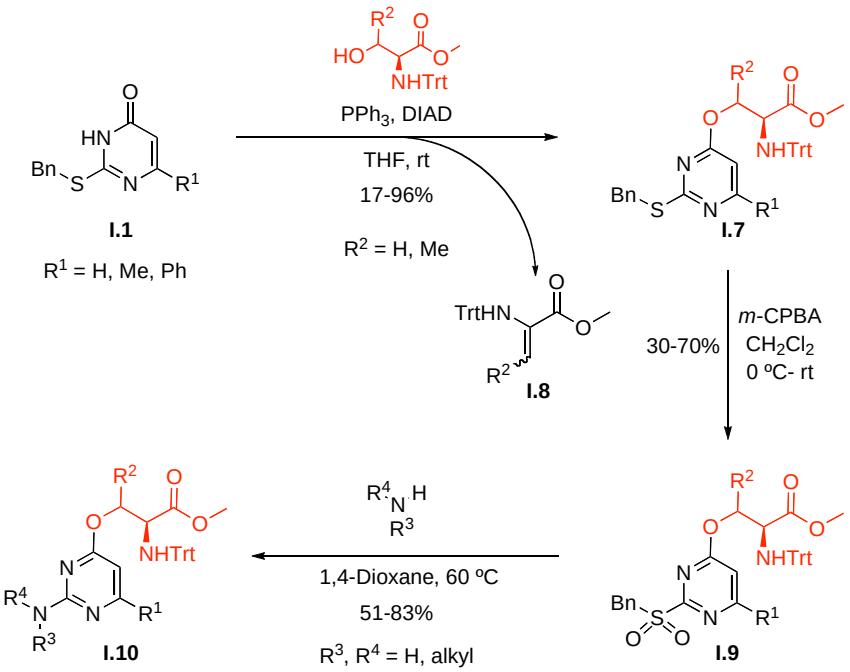
secondary *N*-Boc aminoalcohols under Mitsunobu conditions, 4-alkoxypyrimidines **I.3** were obtained in good yields. Removal of the *N*-Boc protecting group afforded the corresponding amines **I.4**, which were engaged in a Petasis reaction with glyoxylic acid and phenylboronic acid. Nucleophilic *ipso*-substitution of the activated sulfur with morpholine eventually furnished the corresponding *N*-pyrimidinyl arylglycines **I.6** (Scheme I.7).[36d]



Scheme I.7. Synthesis of *N*-pyrimidinyl arylglycines **I.6**

Apart from the methods used to obtain willardiine and lathyrine analogues (Figure I.4), there are few useful synthetic methods that have been reported in the literature for the synthesis of new unnatural α-amino acids carrying a pyrimidine ring in their side chain. In this sense, our laboratory has begun research to synthesize novel pyrimidin-4-yl α-amino acids following the method previously described. The incorporation of an amino acid residue at the C4-position of the pyrimidine ring was achieved *via* a Mitsunobu reaction between 4(3*H*)-pyrimidinones **I.1** and the side chain of *N*-trityl methyl serinate or *N*-trityl methyl threoninate. As shown below, Mitsunobu reactions between pyrimidinones **I.1** and bulky alcohols were completely regioselective in favour of *O*-alkylated derivatives. Subsequent oxidation of the resulting compounds **I.7** to the corresponding sulfone derivatives with *m*-CPBA followed by treatment with primary and secondary amines gave the corresponding target pyrimidines **I.10** (Scheme I.8).

However, this method was limited by the Mitsunobu reaction to α-amino acids bearing a hydroxyl group on the side chain (serine and threonine). Moreover, under Mitsunobu conditions serine and threonine amino acids could give the well-documented β-elimination reaction to the dehydroalanine derivatives **I.8**.[39] In a later study, Cherney *et al*. demonstrated that *N*-phenylfluorenyl or *N*-trityl-protected serine could be engaged in a Mitsunobu reaction whereas *N*-Boc-protected serine methyl ester yielded a large amount of dehydroalanine **I.8** under otherwise identical conditions. In good agreement with the literature,[40] this side reaction could be circumvented using the *N*-trityl serine methyl ester instead of the *N*-Boc derivative and the expected compounds **I.7** were isolated in high yields as the sole reaction products. However, in the case of *N*-trityl threonine methyl ester the desired compound **I.7** was obtained in only a 17% yield along with a large amount of the dehydrolalanine **I.8** (Scheme I.8).



Scheme I.8. Synthesis of pyrimidin-4-yl α-amino acids **I.10**

[39] Mitsunobu, O. *Synthesis* **1981**, 1-28.
[40] Cherney, R. J.; Wang, L. *J. Org. Chem.* **1996**, *61*, 2544-2546.

I.1.4. ANTIMICROBIAL PEPTIDES

Plant diseases caused by pathogenic microorganisms are currently one of the major factors limiting worldwide crop production.[41] Their control requires the continued use of pesticides based mainly on copper compounds and antibiotics.[42] Although antibiotics are highly efficient, they are not authorized in several countries as they accumulate in soils and water and affect the environment and public health.[43] In addition, it has been reported that these compounds evoke resistance in some plant pathogens.[44] Therefore, there is a need for new compounds with low environmental impact, broad-spectrum activity, reasonable bacterial selectivity and low eukaryotic cytotoxicity. Antimicrobial peptides (AMPs) fulfil these requirements, therefore a great deal of scientific effort has been invested in studying their application in human, veterinary and plant disease control.[45]

Antimicrobial peptides, also called host-defence peptides, have evolved in almost every class of living organism as a defence mechanism against invading microorganisms. AMPs are components of the innate system and have been found in virtually all forms of life ranging from microorganisms to plants,[46] invertebrates,[47] and vertebrates including mammals.[48]

In general, AMPs are small (between 6 and 59 amino acid residues), cationic and have the ability to adopt an amphipathic conformation in which positively charged and hydrophobic groups segregate onto opposing faces of an α-helix, a β-sheet, or other secondary structures.[48] Examples of AMPs with an α-helix structure are cecropins, isolated from the giant silk moth *Hyalophora cecropia*, melitin found in the venom of the honeybee *Apis mellifera* and magainins isolated from the skin secretions of the frog *Xenopus laevi*. An important family of AMPs with a β-sheet structure are the

[41] Agrios, G. N. *Plant Pathology*, 5th Ed., London: Elseiver Academic Press; **2005**.
[42] (a) McManus, P. S.; Stockwell, V. O.; Sundin, V. O.; Jones, A. L. *Annu. Rev. Phytopathol.* **2002**, *40*, 443-465. (b) Vidaver, A. K. *Clin. Infect. Dis.* **2002**, *34*, S107-110.
[43] Karabelas, A. J.; Plakas, K. V.; Solomou, E. S.; Drossou, V. O.; Sarigiannis, D. A. *Environ. Int.* **2009**, *35*, 1096-1107.
[44] Loper, J. E.; Henkels, M. *Plant. Dis.* **1991**, *75*, 287-290.
[45] (a) Hancock, R. E. W.; Sahl, H. G. *Nat. Biotechnol.* **2006**, *24*, 1551-1557. (b) Montesinos, E. *FEMS Microbiol. Lett.* **2007**, *270*, 1-11.
[46] García-Olmedo, F.; Molina, A.; Alamillo, J. M.; Rodríguez-Plalenzuela, P. *Biopolymers (Peptide Science)* **1998**, *47*, 479-491.
[47] Hancock, R. E. W.; Sahl, K. L.; Mookherjee, N. *Immunobiology* **2006**, *211*, 315-322.
[48] Jenssen, H.; Hamill, P.; Hancock, R. E. W. *Clin. Microbiol. Rev.* **2006**, *19*, 491-511.

defensins[49] such as α-defensins and β-defensins, which are distributed in mammals (Table I.1).

Table I.1. Selected examples of natural antimicrobial peptides

Structure	Peptide	Peptide sequence[a]	Source
α-Helical	Cecropin A	KWKLFKKIEKVGQNIRDGIKAGPAVAVVGQATQIAK-NH_2	Silk moth
	Melitin	GIGAVLKVLTTGLPALISWIKRKRQQ-NH_2	Bee
	Magainins	GIGKFLHSAKKFGKAFVGEIMNS	Frog
β-Sheet	α-Defensin (HNP3)	$DC_1YC_2RIPAC_3IAGERRYGTC_2IYQGRLWAFC_3C_1$	Human
	β-Defensin (TAP)	$NPVSC_1VRNKGIC_2VPIRC_3PGSMKQIGTC_2VGRAVKC_1C_3RKK$	Bovine

[a] Subscript numbers represent amino acids joined by disulfide bridges.

The biological activity of AMPs has been largely associated with their interaction with membranes. Although the exact mechanism of action has not been clearly unveiled, there is general agreement that amphipathicity of AMPs is essential for their membrane permeabilization involved in cell death.[50] Particularly, four mechanisms are proposed in the literature (Figure I.6). In general, all these models propose that positively charged basic amino acids of AMPs first interact with the negatively charged acidic phospholipids of the cell membrane followed by insertion of the hydrophobic side chains of the AMPs into the hydrophobic core of the membrane. In the first case, the membrane is permeabilized by the formation of the transmembrane pores composed of the bundle of amphipathic helices according to the "barrel-stave model". In the second case, called "carpet mechanism", peptides act as a detergent and disrupt the membrane. In the third case, lipids are inserted between helices to form a mixed pore according to the "toroidal pore model". The last model, called "disordered toroidal pore

[49] Ganz, T. *Nat. Rev. Immunol.* **2003**, *3*, 710-720.
[50] (a) Yeaman, M. R.; Yount, N. Y. *Pharmacol. Rev.* **2003**, *55* (1), 27-55. (b) Brogden, K. A. *Nat. Rev. Microbiol.* **2005**, *3*, 238-250.

model", is a recent modification of the toroidal pore model in which peptides adopt a less-rigid conformation and orientation (Figure I.7).[51]

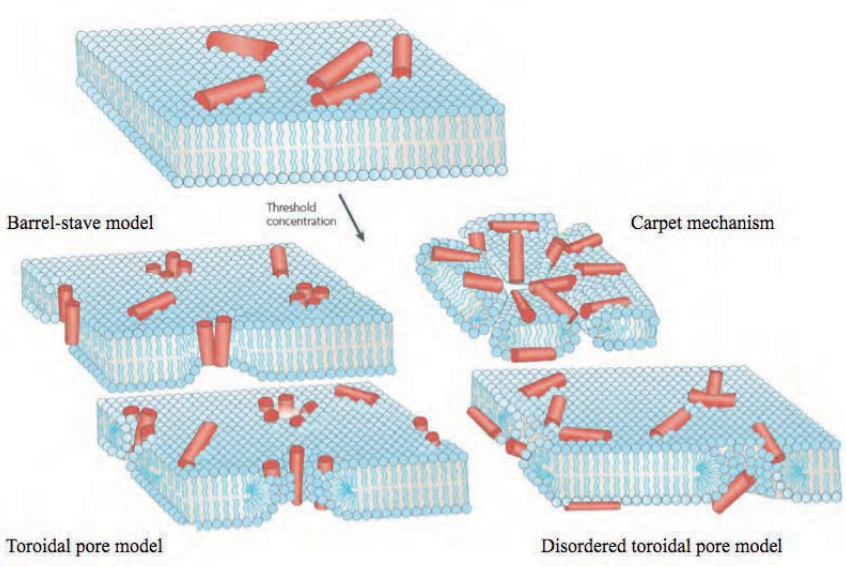


Figure I.7. Mechanisms of AMP-mediated membrane disruption
(Taken from *Nat. Rev. Microbiol.* **2009**, *7*, 245-250)

Nevertheless, whatever the mechanism involved, the first step is the peptide adsorption at the membrane surface. Consequently, it seems difficult for bacteria to develop resistance to AMPs because this would require dramatic changes in the phospholipid membrane composition and/or organization.[52]

Another interesting property of AMPs is their selectivity for prokaryotic membranes over mammalian and plant membranes. On the basis of the above theory, the selectivity for bacterial in front of mammalian cells is attributed to both AMP characteristics due to their amphipathicity, and membrane features. Concerning the membrane, two factors have been described as being involved in AMP selectivity. First, the presence of membrane-stabilizing sterols protects cells from the disruptive effect of AMPs. Therefore, mammalian membranes, which are enriched with sterols, are less susceptible to AMPs than are bacterial membranes, which do not incorporate sterols. Second, bacterial cells contain a high percentage of negatively charged phospholipids, while

[51] Melo, M. N.; Ferre, R.; Castanho, M. A. R. B. *Nat. Rev. Microbiol.* **2009**, *7*, 245-250.
[52] Zaloff, M. *Nature* **2002**, *415*, 389-395.

mammalian cells contain a much higher concentration of zwitterionic phospholipids (Figure I.8).[53]

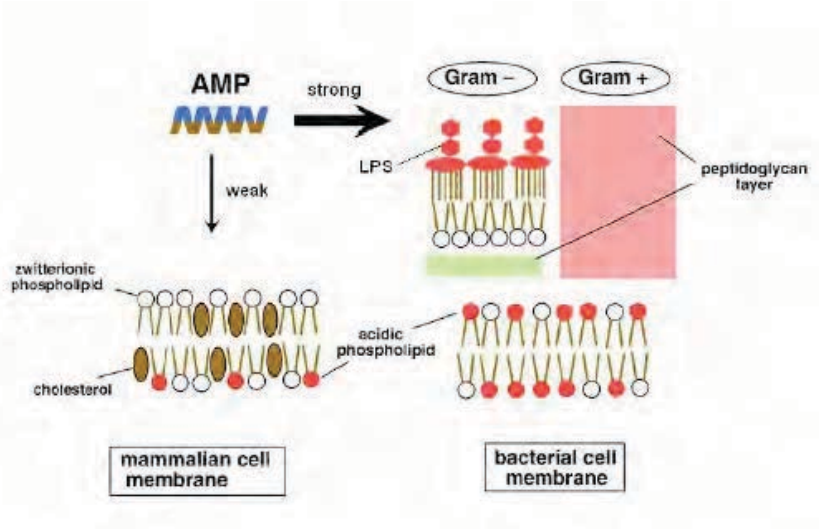


Figure I.8. Molecular basis of AMP cell selectivity
(Adapted from *Biochim. Biophys. Acta* **2009**, *1788*, 1687-1692)

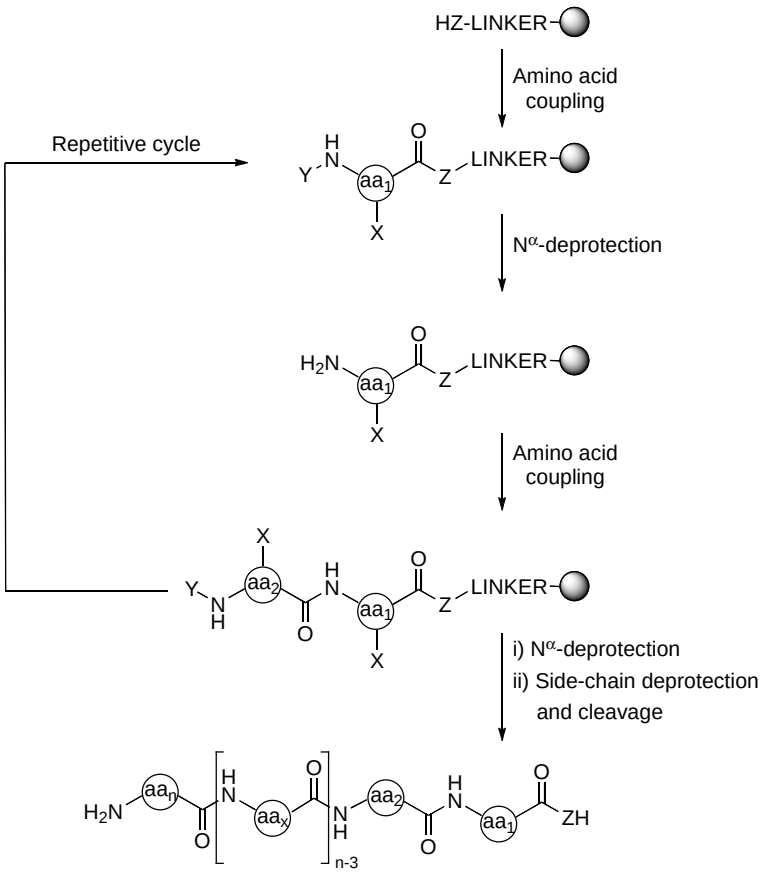
I.1.4.1. Solid-phase peptide synthesis

Since its introduction by Merrifield in 1963,[54] solid-phase peptide synthesis (SPPS) has been used in the production of many peptides containing up to 50 amino acids in acceptable yields and purities. Nowadays, solid-phase synthesis is the most common method for the preparation of peptides but also small proteins, but also of oligosaccharides, oligonucleotides and a huge number of diverse organic compounds.

SPPS essentially consists of the covalent attachment of the *C*-terminal growing peptide chain onto an insoluble solid support. SPPS offers several advantages over the traditionally solution-phase procedures, such as high efficiency and straightforward purification steps. Briefly, the main steps of SPPS consist of: i) attachment of the first conveniently-protected amino acid onto the solid support which incorporates a linker or a spacer to facilitate the final release of the peptide; ii) selective N^{α}-deprotection of the previously incorporated amino acid; iii) cycles of coupling and N^{α}-deprotection steps of the corresponding protected amino acids until the desired peptide is achieved; iv) final

[53] Matsuzaki, K. *Biochim. Biophys. Acta* **2009**, *1788*, 1687-1692.
[54] Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.

deprotection and release of the peptide from the solide support to obtain the desired peptide (Scheme I.9).



Scheme I.9. General strategy of SPPS. X: amino acid side-chain protecting group; Y: *N*$^{\alpha}$-protecting group; Z: O, NH.

I.1.4.1.1. The solid support

The solid support consists of an insoluble and therefore filterable polymer, which should be mechanically robust, inert to all reagents and reaction conditions, and allow fast solvent and reagent diffusion and access to all reactive sites. In addition, the solid support must contain a functionality that enables efficient anchoring of the linker or the first amino acid. Nowadays, the most widely used solid supports are resin beads made from cross-linked polystyrene (PS), polyacrylamide, and polyethylene glycol (PEG) grafted onto a cross-linked polystyrene.[55]

[55] Kate, S. A.; Albericio, F. *Solid-phase synthesis a practical guide,* New York, Marcel Dekker, Inc. **2000**.

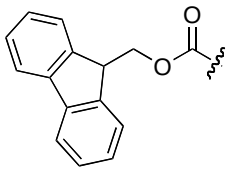
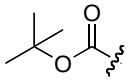
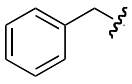
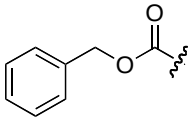
I.1.4.1.2. The linker

The linker is a bifunctional molecule that is bound to both the solid support and the first amino acid of the peptide sequence. One side of the linker is irreversibly anchored to the solid support while the other side serves as the attachment of the first amino acid and behaves as a cleavable protecting group for the final release of the peptide. They are commonly categorized according to their cleavage conditions and their resulting *C*-terminus functionality.

I.1.4.1.3. Protecting groups

In order to control the formation of the desired peptide amide bond, protecting groups are essential for masking the functional groups that are not involved in the reaction. Temporary protecting groups are required for the protection of N^{α}-amino and permanent protecting groups for functional groups within the amino acid side chains. The latter must be stable to cleaving conditions of the temporary groups and are usually safely removed at the end of the synthesis during the peptide cleavage step.

Table I.2. Some representative protecting groups and removal conditions

Structure	Name	Removal
	9-Fluorenylmethoxycarbonyl (**Fmoc**)	Piperidine or DBU
	*tert*-Butyloxycarbonyl (**Boc**)	Concentrated TFA
	*tert*-Butyl (***t*-Bu**)	Concentrated TFA
	Benzyl (**Bn**)	HF or H_2
	Benzyloxycarbonyl (**Z**)	HF or H_2

The most common strategies for SPPS are the *tert*-butyloxycarbonyl (Boc)/benzyl (Bn)[54] and the 9-fluorenylmethoxycarbonyl (Fmoc)/*tert*-butyl (*t*-Bu).[56] Unlike the Boc/Bn strategy, which uses a regime of graduated acidolysis to achieve selective removal of the temporary and permanent protection, the Fmoc/*t*-Bu method is based on an orthogonal protecting group strategy, involving the base-labile Fmoc group for the protection of the amine residue and the acid-labile *t*-Bu and its derivatives for the side-chain functional groups (Table I.2).

I.1.4.1.4. Coupling reagents

Coupling reagents are essential for the formation of the peptide amide bond under mild conditions as they activate the carboxyl group of the amino acid to be incorporated thus facilitating its reaction with the N^{α}-amino group of the residue anchored onto the solid support. Nowadays, the most common coupling reagents for SPPS are carbodiimides, aminium/uronium and phosphonium salts (Figure I.9).[57]

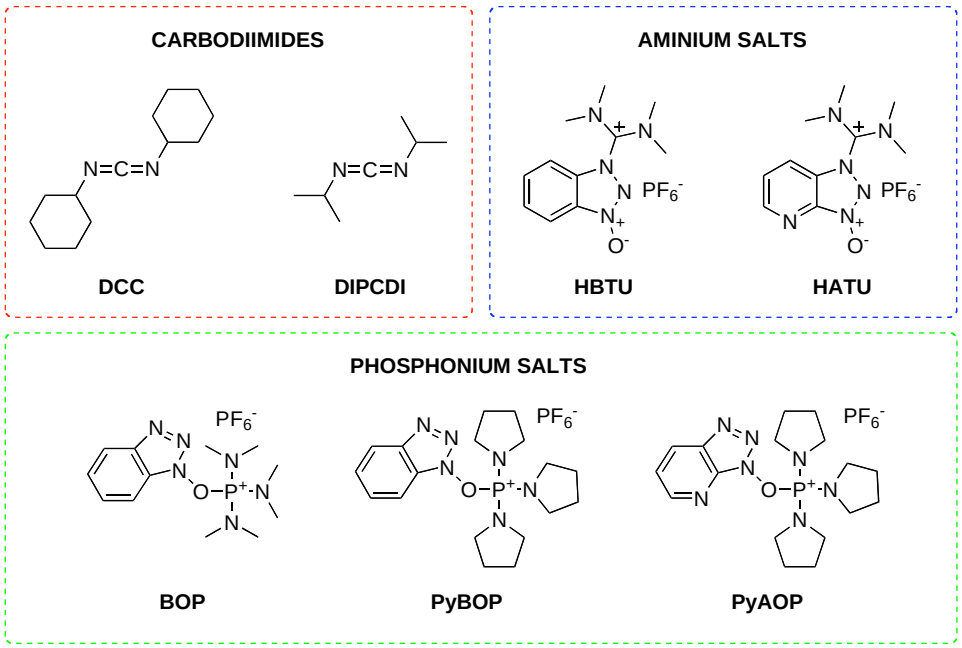


Figure I.9. Some representative coupling reagents

[56] Carpino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748-5749.
[57] (a) Valeur, E.; Bradley, M.; *Chem. Soc. Rev.* **2009**, *38*, 606-631. (b) Han, S-Y.; Kim, Y-A. *Tetrahedron* **2004**, *60*, 2447-2467.

I.1.4.2. Background to the research group

The excellent properties displayed by AMPs, such as a wide spectrum of activity, selectivity towards microbial targets, and a low frequency in developing microbial resistance, have prompted their use in plant protection.[58] In this sense, our research group LIPPSO in collaboration with the Laboratory of Plant Pathology, Institute of Food and Agricultural Technology (CIDSAV-CerTA) of the University of Girona has been involved in a research project designed to identify short synthetic peptides with specific activity against economically important plant pathogenic bacteria. Their efforts are mainly focused on *Erwinia amylovora*, a gram-negative bacterium which is the causal agent of fire blight, a devastating bacterial disease that affects several plant species, mainly members of the rosaceous family, e.g. fruit trees such as pear and apple, and ornamental plants.[59] Moreover, they are also interested in developing new control methods against *Xanthomonas vesicatoria*, which is a gram-negative bacterium responsible for bacterial spot of tomato and pepper,[60] and *Pseudomonas syringae*, which is also a gram-negative bacterium that infects a wide range of deciduous fruit trees, such as pear, cherry, peach and plumb as well as other woody plant species.[61] Until now, an effective method to treat these plant diseases has not been described.

Our laboratory has prepared a 125-member library of synthetic linear undecapeptides, which are cecropin A-melitin hybrids.[62] As noted above, these peptides are naturally occurring AMPs that present an α-helix structure. Cecropin A displays a powerful antibacterial activity against mainly all gram-negative bacteria and some gram-positive bacteria, and does not have cytotoxic effects against human erythrocytes (hemolytic activity) and other eukaryotic cells, but is not stable in plant extracts due to protease degradation. Melitin also displays a powerful, broad-spectrum antimicrobial activity, but is highly hemolytic (Table I.1).

The synthetic peptides were designed using a combinatorial chemistry approach by incorporating amino acids possessing various degrees of hydrophobicity and

[58] Marco, J. F.; Muñoz, A.; Perez-Payá, E.; Misra, S.; López-Garcia, B. *Annuv. Rev. Phytopathol.* **2008**, *46*, 273-301.
[59] (a) Vanneste, J. *Fire blight: the disease and its causative agent Erwinia amylovora*. New York: CABI Publishing; **2000**. (b) Cabrefiga, J.; Montesinos, E. *Phytopathology* **2005**, *95*, 1430-1437.
[60] Leyns, F.; De Cleene, M.; Swings, J.; De Ley, J. *Bot. Rev.* **1984**, *50*, 305-355.
[61] Moragrega, C.; Llorente, I.; Manceau, C.; Montesinos, E. *Eur. J. Plant. Pathol.* **2003**, *109*, 319-326.
[62] (a) Ferre, R.; Badosa, E.; Feliu, L.; Planas, M.; Montesinos, E.; Bardají, E. *Appl. Environ. Microbiol*. **2006**, *72*, 3302-3308. (b) Badosa, E.; Ferre, R.; Planas, M.; Feliu, L.; Besalú, E.; Cabrefiga, J.; Bardají, E.; Montesinos, E. *Peptides* **2007**, *28*, 2276-2285.

hydrophilicity at positions 1 and 10 and by varying the *N*-terminus. The general structure of the synthesized undecapeptides is **R-X^{1}KLFKKILKX^{10}L-NH$_2$** where **R** = H, Ac, Bn, Bz, Ts; **X^1** = Lys, Leu, Trp, Tyr, Phe and **X^{10}** = Lys, Val, Trp, Tyr, Phe (Table I.3). This peptide library was screened for *in vitro* growth inhibition of *E. amylovora*, *X. vesicatoria* and *P. syringae*. Hemolytic activity and stability towards protease degradation of the most active peptides were also examined. Eight peptides with a good balance between antibacterial and hemolytic activities were identified (Table I.3). The most promising peptides were then tested *ex vivo* by evaluating their preventive effect of inhibition of *E. amylovora* infection in detached apple and pear flowers. The peptide H-KKLFKKILKYL-NH$_2$ (**BP100**) showed an efficacy *ex vivo* comparable to that of streptomycin, an antibiotic currently used for fire blight control.

Table I.3. Linear synthetic peptides

Peptide		MIC[a] (µM)			Hemolysis (%)[b]	
Code	Sequence	Ps[c]	Xv[c]	Ea[c]	50 µM	150 µM
BP76	KKLFKKILKFL-NH$_2$	2.5-5.0	2.5-5.0	2.5-5.0	3±1.0	34±2.1
BP66	FKLFKKILKFL-NH$_2$	5.0-7.5	2.5-5.0	2.5-5.0	9±2.2	63±5.9
BP77	Ac-KKLFKKILKFL-NH$_2$	5.0-7.5	<2.5	5.0-7.5	6±0.6	40±3.8
BP81	LKLFKKILKFL-NH$_2$	2.5-5.0	<2.5	2.5-5.0	10±1.3	65±1.5
BP100	**KKLFKKILKYL-NH$_2$**	**2.5-5.0**	**5.0-7.5**	**2.5-5.0**	**3±0.1**	**22±2.8**
BP105	LKLFKKILKYL-NH$_2$	5.0-7.5	2.5-5.0	5.0-7.5	14±1.6	91±6.2
BP125	Ts-YKLFKKILKKL-NH$_2$	2.5-5.0	2.5-5.0	>7.5	2±0.7	8±1.6
BP126	Bz-KKLFKKILKKL-NH$_2$	2.5-5.0	2.5-5.0	5.0-7.5	2±0.4	14±2.9

[a] MIC, minimum inhibitory concentration. [b] α = 0.05. [c] Ps, *Pseudomonas syringae*; Xv, *Xanthomonas vesicatoria*; Ea, *Erwinia amylovora*.

Although this *lead* sequence with high bioactivity *in vitro* was identified, it does not guarantee a good activity *in vivo* due to the fact that, generally, bioactive peptides and,

especially, AMPs have limitations such as their poor stability toward protease degradation and their low bioavailability. One of the most prominent approaches devised to overcome these limitations is the synthesis of bioactive peptides by modification of known natural sequences. These modifications include cyclization, chirality changes, or *N*- or *C*-terminus modifications. Another simple modification is the introduction or replacement of one or more proteinogenic amino acid residues by non-proteinogenic amino acids.[63] The introduction of non-coded amino acid, which generates modifications in the secondary and tertiary structures of a peptide, is widely used to further enhance the stability and activity of bioactive peptide sequences.[64]

For example, Pritz *et al.* reported a study on the biological effect of the replacement of tryptophan in the antimicrobial peptide Ac-RRWWRF-NH_2 by an unnatural amino acid bearing a bicyclo[1.1.1]pentane moiety in its side chain (Figure I.10). The new linear peptide sequences obtained enhanced both the antimicrobial and bilayer-permeabilizing activity.[65]

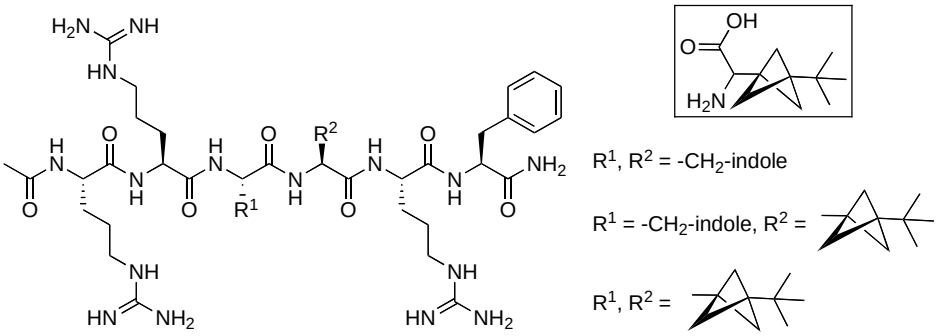


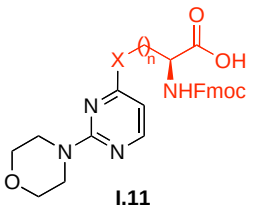
Figure I.10. Small library of peptides incorporating an unnatural amino acid

[63] Adessi, C.; Soto, C. *Curr. Med. Chem.* **2002**, *9*, 963-978.
[64] (a) Hicks, R. P.; Bhnsle, J. B.; Venugopal, D.; Koser, B. W.; Magill, A. L. *J. Med. Chem.* **2007**, *50*, 3026-3036. (b) Jones, M. A.; Notta, J. K.; Cobbold, M.; Palendira, M.; Hislop, A. D.; Wilkie, J.; Snaith, J. S. *J. Pept. Sci.* **2008**, *14*, 313-320. (c) Karstad, R.; Isaksen, G.; Brandsdal, B-O.; Svendsen, J. S.; Svenson, J. *J. Med. Chem.* **2010**, *53*, 5558-5566.
[65] Pritz, S.; Pätzel, M.; Szeimies, G.; Dathe, M.; Bienert, M. *Org. Biomol. Chem.* **2007**, *5*, 1789-1794.

I.1.5. OBJECTIVES

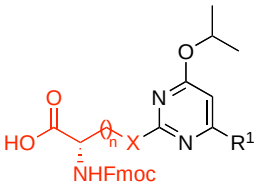
Taking into account the interest in non-proteinogenic amino acids and their utility as building blocks in the design of more useful antimicrobial peptides, the aims of the first chapter of this thesis are:

1. Synthesis of N^{α}-Fmoc-pyrimidin-4-yl amino acids **I.11**.

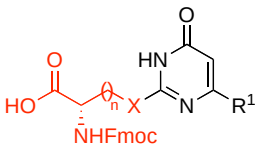


I.11

2. Synthesis of N^{α}-Fmoc-pyrimidin-2-yl amino acids **I.12** and N^{α}-Fmoc-pyrimidin-2-one amino acids **I.13**.



I.12



I.13

3. Synthesis of new antimicrobial **BP100**-derivative peptides containing pyrimidinyl amino acids **I.11**, **I.12** and/or **I.13**.

4. Evaluation of the biological activity of a library of **BP100**-derivative peptides incorporating pyrimidinyl amino acids.

I.2. RESULTS & DISCUSSION

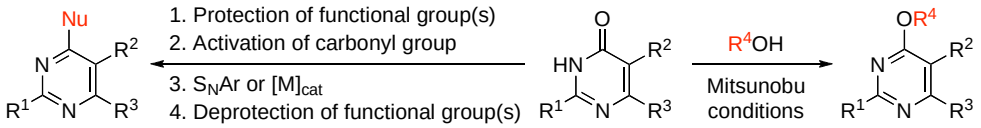
I.2.1. SYNTHESIS OF N^{α}-FMOC PYRIMIDIN-4-YL AMINO ACIDS

I.2.1.1. Synthesis of C4 functionalized pyrimidines

As described in Section I.1.3, pyrimidine derivatives have received a considerable amount of attention due to the interesting pharmacological properties associated with this heterocyclic scaffold. Despite the wide variety of synthetic approaches described for the construction of the pyrimidine nucleus,[30,66] few available methods exist for the efficient synthesis of C4-substituted pyrimidines. The most convergent and perhaps the most widely used synthesis of highly functionalized pyrimidines involves a cyclocondensation reaction between a bidentate nucleophilic fragment (e.g. ureas, thioureas, guanidines or amidines) and a 1,3-dicarbonyl derivative.[67] However, this method is somewhat limited due to the unavailability of the corresponding bidentate fragments. A complementary approach for the synthesis of substituted pyrimidines involves the functionalization of a preformed pyrimidine scaffold. This method allows to access pyrimidine derivatives which would otherwise be difficult to obtain.

[66] Radi, M.; Schenoneb, S.; Botta, M. *Org. Biomol. Chem.* **2009**, *7*, 2841-2847.
[67] Hill, M. D.; Movassaghi, M. *Chem. Eur. J.* **2008**, *14*, 6836-6844.

In this sense, the synthesis of pyrimidines modified at the C4-position is generally performed starting from a pyrimidin-4(3*H*)-one derivative by activation of the carbonyl group followed by coupling with a carefully chosen nucleophiles. This is probably the most useful approach because it complements the existing convergent synthetic methods.[68] In general, the carbonyl group is activated by halogenation often under relatively harsh and acidic conditions with reagents such as thionyl chloride, phosphoryl chloride and phosphorus pentachloride. Consequently, this strategy often involves four synthetic steps including i) the protection of the functional groups; ii) the activation of the carbonyl group, generally *via* halogenation; iii) the functionalization with either nucleophiles *via* S_NAr displacement or organometallics through transition-metal-catalysed cross coupling;[69] and iv) the final deprotection of the functional groups (Scheme I.10). Selective *O*-alkylation of pyrimidin-4(3*H*)-one derivatives with bulky alcohols by Mitsunobu reaction is an alternative way to functionalize the C4-position of the pyrimidine ring under mild conditions. However, this approach is limited to the synthesis of 4-alkoxypyrimidines. Indeed, the chemoselectivity of the Mitsunobu reaction in this kind of substrate is rarely exclusive in favour of the *O*-alkylation product and, usually, a significant amount of the *N*-alkylated regioisomer is obtained decreasing the reaction yield (Scheme I.10).

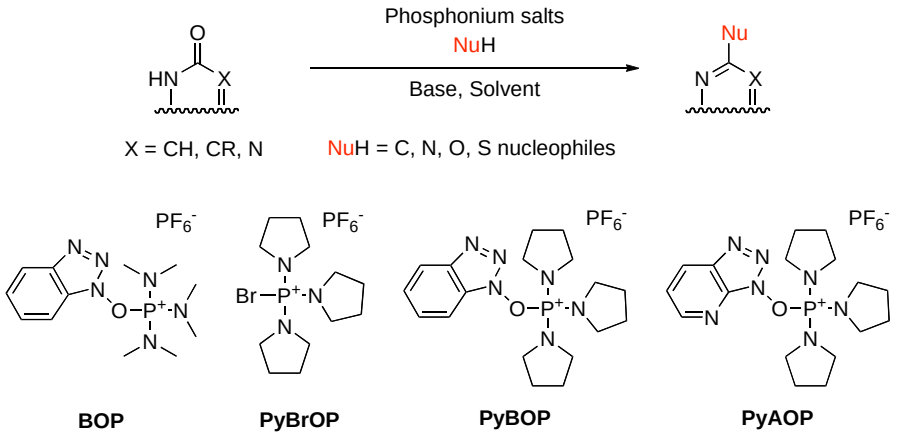


Scheme I.10. Synthesis of the C4-functionalizated pyrimidines from pyrimdin-4(3*H*)-ones

As reported in the previous Section I.1.3.3.3, our laboratory studied the synthesis of pyridimin-4-yl amino esters **I.10** using a Mitsunobu reaction as a key step. However, this method was limited by the Mitsunobu reaction to α-amino acids bearing a hydroxyl group on the side chain. Therefore, a method was sought to allow the incorporation of a range of amino acid residues at the C4-position of the pyrimidine ring.

[68] (a) Maruenda, H.; Chenna, A.; Liem, L.-K.; Singer, B. *J. Org. Chem.* **1998**, *63*, 4385-4389. (b) Van Brocklin, H. F.; Lim, J. K.; Coffing, S. L.; Hom, D. L.; Negash, K.; Ono, M. Y.; Gilmore, J. L.; Bryant, I.; Riese, D. J. I. I. *J. Med. Chem.* **2005**, *48*, 7445-7456. (c) Al-Abdullah, E. S.; Al-Obaid, A-R. M.; Al-Deeb, O. A.; Habib, E. E.; El-Emam, A. A. *Eur. J. Med. Chem.* **2011,** *46*, 4642-4647.
[69] Capek, P.; Pohl, R.; Hocek, M. *J. Org. Chem.* **2005**, *70*, 8001-8008.

Phosphonium coupling has recently emerged as a mild, efficient, and versatile method for the direct C-C, C-N, C-O and C-S bond formation of tautomerizable heterocycles. It proceeds *via* C-OH bond activation of a tautomerizable heterocycle with a phosphonium salt, and the subsequent functionalization with a nucleophile through S_NAr displacement (Scheme I.11).[70]

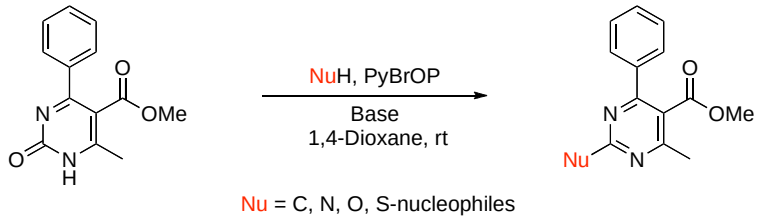


Scheme I.11. Phosphonium coupling reaction of tautomerizable heterocycles

Alkyl and aryl amines have been widely used as nucleophiles in this transformation, while phenols, alcohols, thiophenols, malonates, sulfonamides and nitrogen-containing heterocyclic nucleophiles have been used less. Only a few examples of the direct amination of tautomerizable heterocycles with indoles or imidazoles *via* phosphonium coupling have been reported in the literature.[71]

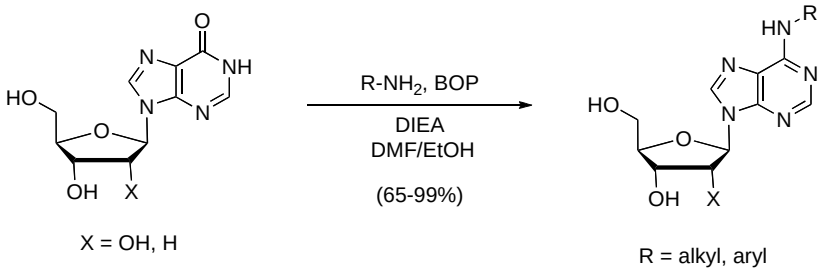
Kang and co-workers reported the first example of a phosphonium-mediated carbon-nucleophile bond formation in the synthesis of C2 substituted pyrimidines from pyrimidin-2(1*H*)-one. The optimal conditions for this mild *in situ* activation coupling were achieved using bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBrOP) as the phosphonium reagent in 1,4-dioxane at rt (Scheme I.12).[71]

[70] For recent reviews on phosphonium coupling, see: (a) Kang, F-A.; Sui, Z.; Murray, W. V. *Eur. J. Org. Chem.* **2009**, 461-479. (b) Mansour, T. S.; Bardhan, S.; Wan, Z-K. *Synlett* **2010**, 1143-1169.
[71] Kang, F-A.; Kodah, J.; Guan, Q.; Li, X.; Murray, W. V. *J. Org. Chem.* **2005**, *70*, 1957-1960.



Scheme I.12. Synthesis of C2-substituted pyrimidines *via* phosphonium-mediated coupling

This method was quickly extended to the synthesis of nucleosides, purines and other heterocycles containing an amide linkage as part of the cyclic system. For example, Wan *et al.* applied this method to the synthesis of N^6-adenosine and N^6-2'-deoxyadenosine derivatives. In this example, treatment of inosine or 2'-deoxyinosine with alkyl or arylamines without protecting the sugar hydroxyl groups in the presence of benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP) and *N*,*N*-diisopropylethylamine (DIEA) in DMF led to the formation of nucleoside derivatives in good to excellent yields (Scheme I.13).[72]

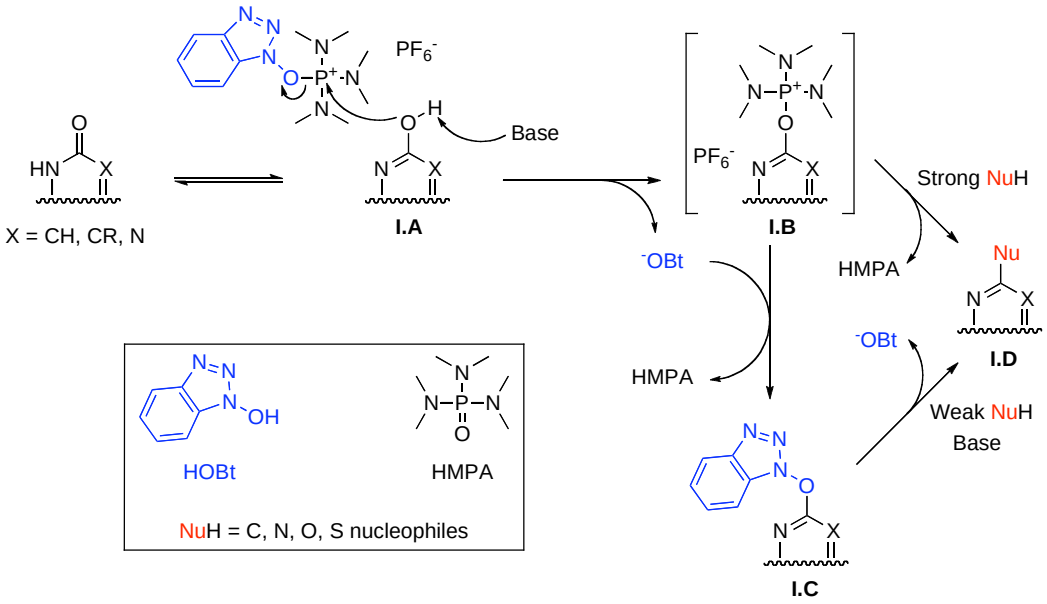


Scheme I.13. Synthesis of nucleoside derivatives through phosphonium coupling reaction

The mechanistic aspects of the phosphonium-mediated reactions have been thoroughly discussed in the literature.[73] Phosphonium salt **I.B** is presumably readily formed when cyclic amides or ureas are treated with phosphonium reagents in the presence of a base. These phosphonium intermediates **I.B** have been isolated and characterized by ^{1}H and ^{31}P NMR spectroscopy. The S_NAr reaction of phosphonium intermediates **I.B** with reactive nucleophiles leads to the formation of the desired products **I.D**. Benzotriazolyloxy- or pyridotriazolyloxy-containing phosphonium reagents (BOP, PyBOP and PyAOP) sometimes conduct to the formation of

[72] Wan, Z. K.; Binnun, F.; Wilson, D. P.; Lee, Y. *Org. Lett.* **2005**, *7*, 5877-5880.
[73] (a) Lakshman, M. K.; Frank, J. *Org. Biomol. Chem.* **2009**, *7*, 2933-2940. (b) Wan, Z-K.; Wacharansindhu, S.; Levins, C. G.; Lin, M.; Tabei, K.; Mansour, T. S. *J. Org. Chem.* **2007**, *72*, 10194-10210.

N-hydroxybenzotriazole (HOBt) or *N*-hydroxypyridotriazole (HOPt) adducts, e.g. compound **I.C**, when less reactive or more hindered nucleophiles are used, usually when its nucleophilicity follows the order Nu < OBt or OPt. In such cases, a second nucleophilic substitution, which would need an additional base, leads to the formation of the expected product **I.D** (Scheme I.14).



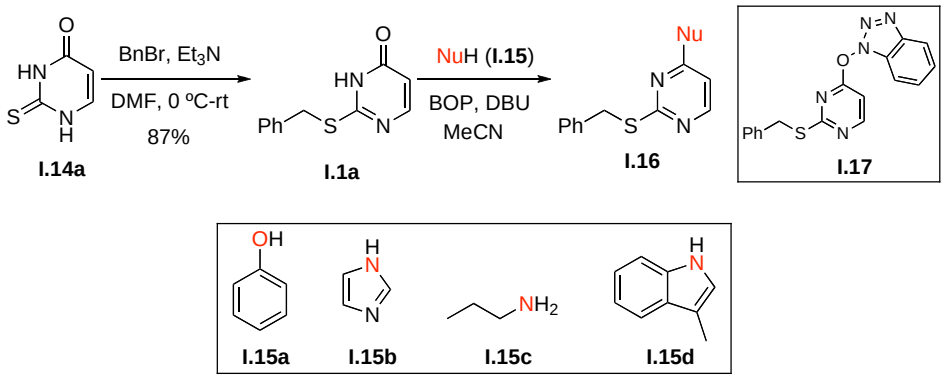
Scheme I.14. Mechanism of BOP-mediated nucleophilic substitutions

I.2.1.2. Preliminar study of phosphonium-mediated synthesis of C4 substituted pyrimidines

Following this phosphonium-mediated method, our laboratory started to study the phosphonium coupling reaction for the synthesis of C4 substituted pyrimidines starting from 2-benzylsulfanyl-4(3*H*)-pyrimidinone **I.1a**, easily obtained by selective *S*-alkylation of commercially available thiouracil **I.14a** with benzyl bromide (Table I.14). First of all, a screening of the reaction conditions on pyrimidinone **I.1a** was performed to identify the most effective reagent combination. In this manner, compound **I.1a** was treated with imidazole as a nucleophile in the presence of different coupling reagents (PyBrOP, PyBOP and BOP), bases (Et_3N and DBU) and solvents (acetonitrile and 1,4-dioxane). BOP in conjunction with 1,8-diazabicyclo[5.4.0]undecene-7 (DBU) in acetonitrile gave the best results for this transformation. These optimized conditions were used to couple pyrimidinone **I.1a** with various nucleophiles **I.15** such as phenol,

imidazole, *n*-propylamine and 3-methyl-1*H*-indole as models of amino acid side chains of tyrosine, histidine, lysine and tryptophan, respectively. All reactions led to the expected compounds **I.16** in moderate to good yields, except for 3-methyl-1*H*-indole **I.15d**. The coupling reaction with strong nucleophiles such as *n*-propylamine and phenol proceeded efficiently at rt in short reaction times (Table I.4, entries 1 and 3).[74] However, the coupling reaction with weak nucleophile sucha as imidazole needed to be heated at 60 ºC to reach completion and compound **I.16b** was obtained in a moderate yield (Table I.4, entry 2). The phosphonium-mediated reaction with 3-methyl-1*H*-indole **I.16d** failed as only the OBt adduct **I.17** was isolated from the crude reaction mixture (Table I.4, entry 4).

Table I.4. Synthesis of C4 substituted pyrimidines **I.16** promoted by BOP

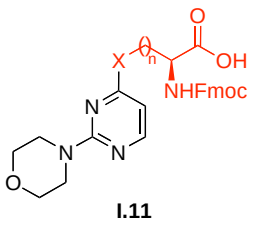


Entry	NuH (**I.15**)	Conditions[a]	Time (h)	T (ºC)	Product (**I.16**)	Yield (%)[b]
1	**I.15a**	B	2	rt	**I.16a**	61
2	**I.15b**	B	24	60	**I.16b**	60
3	**I.15c**	A	12	rt	**I.16c**	70
4	**I.15d**	B	24	60	**I.16d**	-[c]

[a] Conditions **A**: **I.1a** (1 equiv), BOP (1.3 equiv), DBU (1.5 equiv); **I.15** (1.3 equiv).
Conditions **B**: **I.1a** (1 equiv), BOP (1.3 equiv), DBU (1.5 equiv); **I.15** (1.3 equiv), DBU (1.5 equiv).
[b] Isolated yields.
[c] Only OBt-adduct **I.17** was isolated in 60% yield.

[74] Planellas, M.; Díaz. E.; Pascual, M.; Bardají, E.; Heras, M "Synthesis of new non-proteinogenic α-amino acids" (poster presented at the Third West Mediterranean Chemistry Meeting, La Grande Motte, France, June 14–15, **2007**).

Based on these results, we decided to focus on the synthesis of the target amino acids **I.11** bearing a C4-substituted pyrimidine ring on the side chain (Figure I.11).

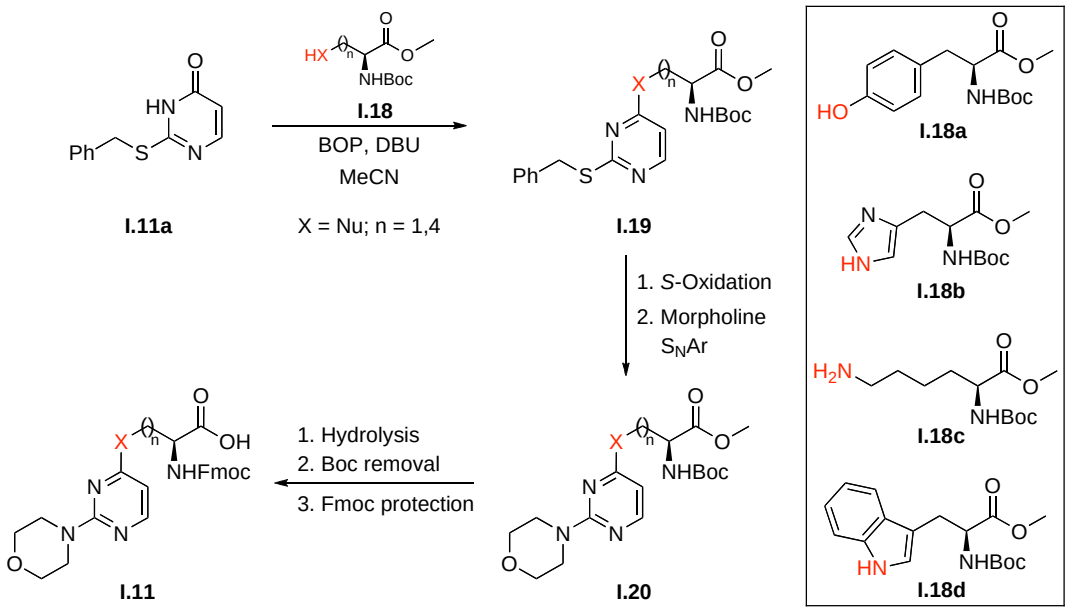


I.11

Figure I.11. Pyrimidin-4-yl amino acids **I.11**

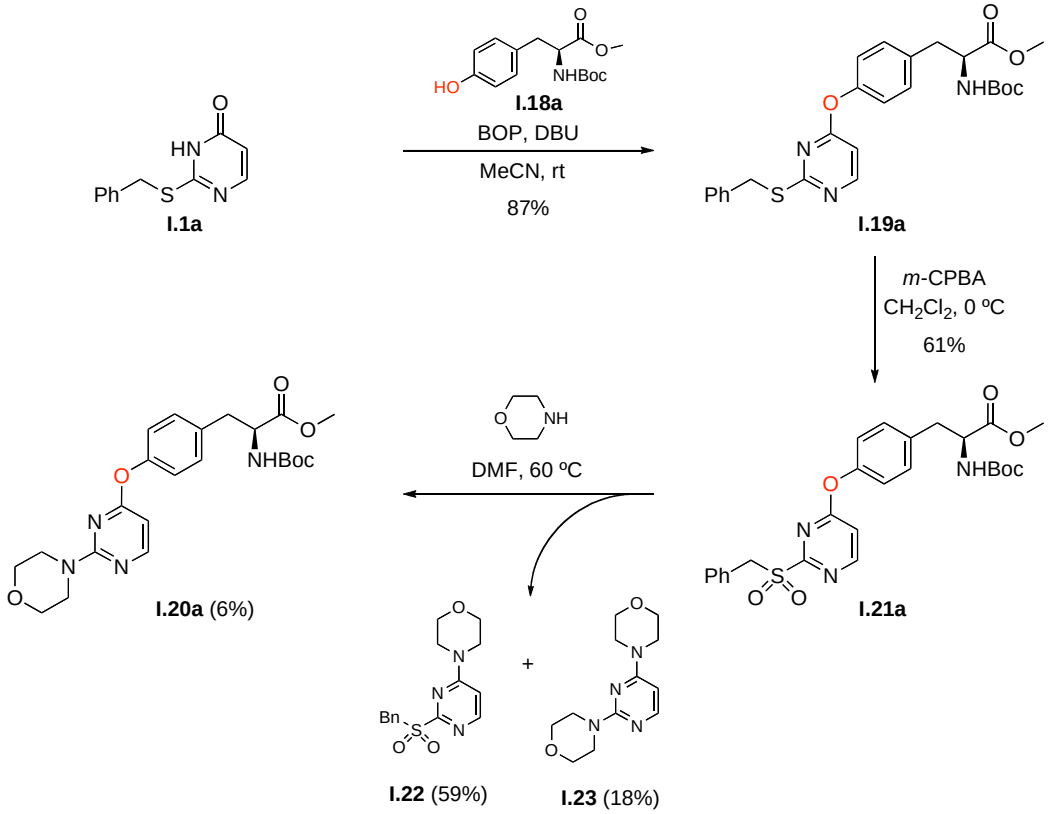
I.2.1.3. First strategy to synthesize pyrimidin-4-yl amino acids

Consistent with this goal, the synthesis of pyrimidin-4-yl amino acids **I.11** was envisioned following the synthetic strategy depicted in Scheme I.15. First, pyrimidinone **I.1a** could be engaged in a phosphonium coupling reaction with several N^{α}-Boc-protected amino esters **I.18** to get the corresponding pyrimidin-4-yl amino esters **I.19**. Next, the functionalization of **I.19** at the C2-position of the pyrimidine ring by nucleophilic displacement of the activated benzylsulfonyl group with morpholine could afford compounds **I.20**. Taking into account that one of the objectives of this thesis was to design and synthesize new antimicrobial peptides incorporating non-proteinogenic α-amino acids in their sequences, N^{α}-Boc-amino esters **I.20** were not useful building blocks for the solid-phase peptide synthesis following a Fmoc/*t*-Bu strategy. Consequently, compounds **I.20** was converted to the target N^{α}-Fmoc-pyrimidin-4-yl amino acids **I.11**. This was achieved in a three-step sequence including an ester hydrolysis followed by the removal of the Boc protecting group and final reprotection of the primary amine with the Fmoc group (Scheme I.15).



Scheme I.15. First strategy for the synthesis of pyrimidin-4-yl amino acids **I.11**

The phosphonium coupling reaction of 4(3*H*)-pyrimidinone **I.1a** was first carried out with N^{α}-Boc methyl tyrosinate **I.18a** in the presence of BOP and DBU in acetonitrile at rt to afford the pyrimidin-4-yl amino ester analogue **I.19a** in 87% yield and without appreciable racemization. Next, compound **I.19a** was treated with an excess of *m*-CPBA to obtain the corresponding sulfone **I.21a** in 61% yield. Unexpectedly, the subsequent nucleophilic substitution reaction of compound **I.21a** with morpholine failed. The purification of the crude mixture only afforded a 6% yield of the expected compound **I.20a** along with a large amount of side products **I.22** and **I.23** (Scheme I.16). These results clearly indicated that the tyrosine residue in the C4-position underwent aminolysis faster than the benzylsulfonyl group at the C2-position of the pyrimidine ring.

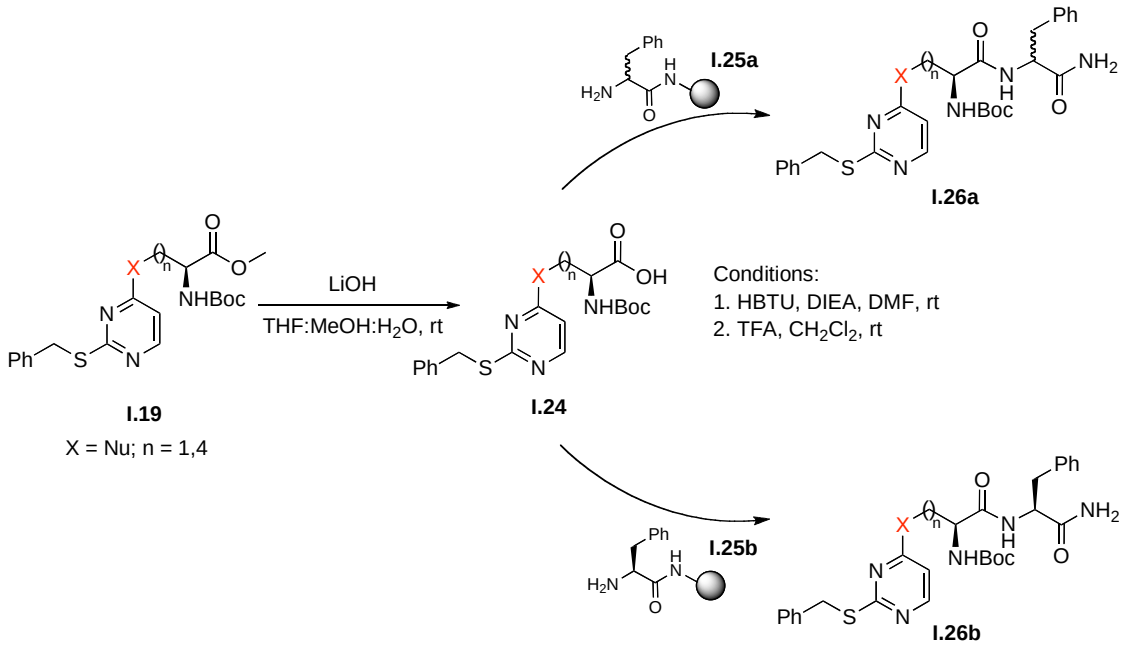


Scheme I.16. Synthesis of N^{α}-Boc-pyrimidin-4-yl tyrosine methyl ester **I.20a**

I.2.1.4. Determination of optical purity of pyrimidin-4-yl amino acids

A chromatography method was established to determine the possible racemization of the compounds **I.18** caused by the basic conditions of the phosphonium coupling reaction. Thus, the optical purity of compounds **I.19** was verified by coupling the pyrimidinyl α-amino acids **I.24** obtained after saponification, with both racemic phenylalanine resin **I.25a** and L-phenylalanine resin **I.25b** in order to measure the degree of racemization by high-performance liquid chromatography (HPLC). Particularly, a sample of N^{α}-Boc-amino esters **I.19a** was hydrolysed using LiOH to give the free N^{α}-Boc-amino acids **I.24** in quantitative yield. The latter was first coupled to racemic phenylalanine resin **I.25a** using standard protocols for solid-phase peptide synthesis following a Fmoc/*t*-Bu strategy. After cleavage from the resin by acid treatment with trifluoroacetic acid (TFA), the HPLC analysis of the resulting dipeptide **I.26a** showed the formation of two diastereoisomers. Analogously, dipeptide **I.26b** was then synthesized by coupling N^{α}-Boc-amino acids **I.24** to L-phenylalanine resin **I.25b**. When no reasonable racemization occurred during the synthesis of **I.19**, HPLC analysis

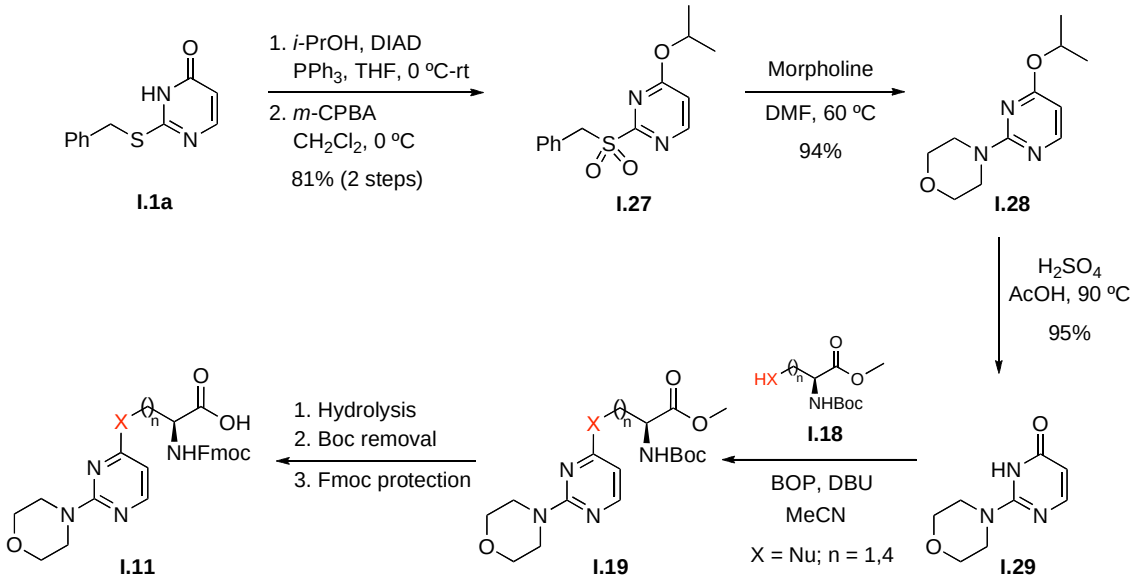
of **I.26b** showed only one peak corresponding to the formation of one single diastereoisomer (Scheme I.17).



Scheme I.17. Determination of optical purity of compounds **I.19**

I.2.1.5. Second strategy to synthesize pyrimidin-4-yl amino acids

To circumvent the previous problem, we decided to incorporate the amino acid residue during the last step. Consequently, the functionalization of the C2-position of the pyrimidine ring with morpholine needed to be performed first. This transformation could be easily achieved by converting pyrimidinone **I.1a** to the corresponding 2-morpholino-4(3*H*)-pyrimidinone **I.29** by means of the synthetic method previously developed in the laboratory.[36a] Then, the latter could be engaged in a coupling reaction with protected amino esters **I.18** to obtain N^{α}-Boc-pyrimidin-4-yl amino esters **I.20**. A final series of protecting group transformations would lead to the target N^{α}-Fmoc-pyrimidin-4-yl amino acids **I.11** (Scheme I.18).



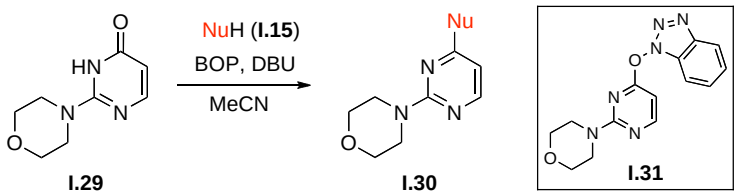
Scheme I.18. Second strategy for the synthesis of N^{α}-Fmoc pyrimidin-4-yl amino acids **I.11**

Therefore, pyrimidinone **I.1a** was selectively *O*-alkylated with 2-propanol under Mitsunobu conditions and, the resulting 2-benzylsulfanyl-4-isopropoxypyrimidine was oxidized with *m*-CPBA to provide sulfone **I.27** in good yield. Next, compound **I.27** was treated with morpholine to afford pyrimidine **I.28** which was subsequently treated with a mixture of sulphuric acid and acetic acid at 90 ºC for 15 min in order to remove the isopropyl group and thus afforde the desired pyrimidinone **I.29** (Scheme I.18).

It has been reported that the electronic properties of the substrate likely dictate the outcome of the phosphonium-mediated couplings. Indeed, this transformation is much less efficient with electron-rich tautomerizable heterocycles.[70] Knowing this, we initally explored the phosphonium coupling between 2-morpholino-4(3*H*)-pyrimidinone **I.29** and the model nucleophiles **I.15** (Table I.5). Compared to the previous study with substrate **I.1a** (Table I.4), the phosphonium coupling proceeded more slowly and under harsher conditions. In all cases, the reactions were run at 60 ºC for at least 24 h and the addition of an extra base was required to promote the S_NAr displacement, even in the case of *n*-propylamine **I.15c**. The expected products **I.30** were obtained in moderate to good yield, with the exception of the indole derivative **I.30d,** which showed no product formation even after 40 h of heating (Table I.5, entries 1-4). In this latter case, the OBt-adduct **I.31** was the sole compound isolated from the crude reaction mixture. When compound **I.31** was subjected again to a S_NAr with 3-methyl-1*H*-indole **I.15d** and

potassium carbonate, the target N^{α}-Boc-pyrimidin-4-yl indole **I.30d** was isolated in 47% yield (Table I.5, entry 5).

Table I.5. Synthesis of C4 substituted pyrimidines **I.30** promoted by BOP



Entry	NuH	Conditions[a]	Time (h)	T (ºC)	Product	Yield (%)[b]
1	**I.15a**	A	24	60	**I.30a**	68
2	**I.15b**	A	24	60	**I.30b**	55
3	**I.15c**	A	24	60	**I.30c**	82
4	**I.15d**	A	40	60	**I.30d**	–[c]
5	**I.15d**	B	15	60	**I.30d**	47

[a] Conditions **A**: **I.29** (1 equiv), BOP (1.5 equiv), DBU (1.5 equiv); **I.15** (1.5 equiv), DBU (1.5 equiv).
Conditions **B**: **I.31** (1 equiv), K_2CO_3 (4.0 equiv); **I.15** (1.3 equiv).

[b] Isolated yields.

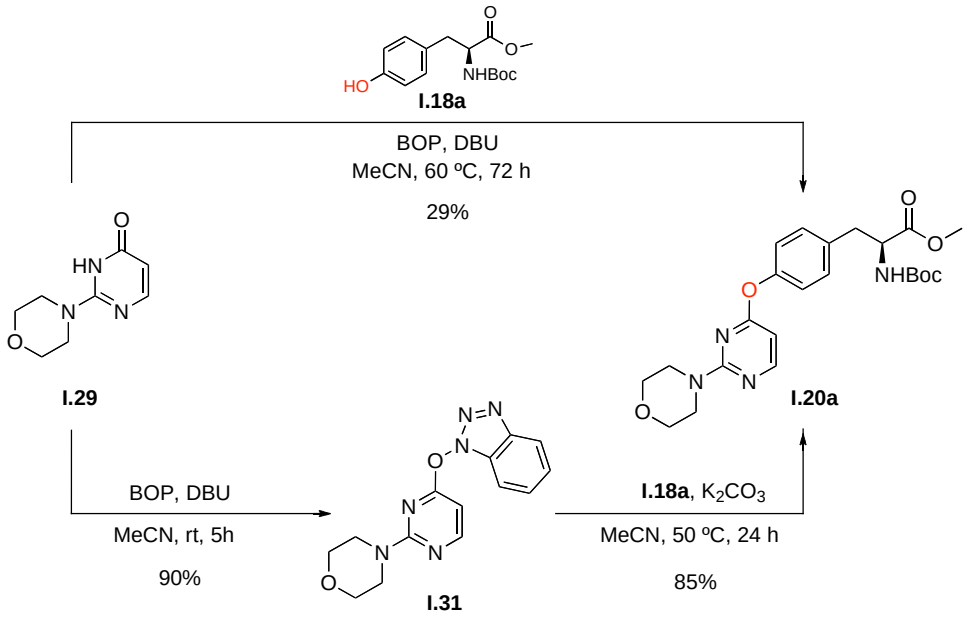
[c] Only OBt-adduct **I.31** was isolated.

I.2.1.6. Synthesis of pyrimidin-4-yl tyrosine derivatives

The extension of the phosphonium coupling reaction of pyrimidinone **I.29** to amino esters **I.18** started with N^{α}-Boc methyl tyrosinate **I.18a**. Under the set of conditions previously optimized, the synthesis of N^{α}-Boc-pyrimidin-4-yl tyrosine methyl ester **I.20a** through a BOP-mediated reaction in the presence of DBU at 60 ºC for 72 h resulted in a poor yield of 29%. In addition, compound **I.20a** presented a partial racemization[75] probably due to the long reaction time under basic conditions. As the major compound isolated in this reaction was the OBt-adduct **I.31**, we therefore investigated the synthesis of **I.20** using a two-step approach through the OBt-adduct **I.31**. Thus, pyrimidinone **I.29** was treated with BOP and DBU at rt for 5 h without the presence of nucleophiles in order to get compound **I.31** in a good yield. The latter was

[75] The determination of the optical purity was assessed by the HPLC method described in Section I.2.1.4.

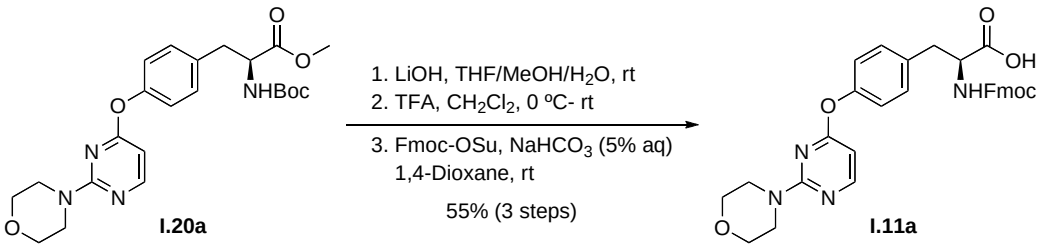
subsequently engaged in a S_NAr reaction with N^{α}-Boc-methyl tyrosinate **I.18a** in the presence of a weak base, potassium carbonate, at 50 ºC for 24 h. Under these conditions the expected product **I.20a** could be obtained in high yield (85%) and without significant racemization (Scheme I.19).



Scheme I.19. Synthesis of N^{α}-Boc-pyrimidin-4-yl tyrosine methyl ester **I.20a**

Once the N^{α}-Boc-pyrimidin-4-yl tyrosine methyl ester **I.20a** in hand, it was converted into the desired N^{α}-Fmoc-pyrimidin-4-yl tyrosine **I.11a** following the proposed synthetic strategy. First, the methyl ester of compound **I.20a** was saponified using an excess of LiOH in a mixture of tetrahydrofuran, methanol and water at rt. After an acid work-up to neutralize the excess of base, the crude residue was engaged directly in the next step without further purification. The N^{α}-Boc protecting group was thus removed by TFA treatment to afford the free amino acid, which was subsequently protected with *N*-(9-fluorenylmethoxycarbonyloxy) succinimide (Fmoc-OSu) in a mixture of aqueous sodium bicarbonate and 1,4-dioxane at rt for 24 h.[76] Finally, the crude reaction mixture was purified by flash chromatography to furnish the expected product **I.11a** in 55% yield over three steps (Scheme I.20).

[76] Lapatsanis, L.; Milias, G.; Froussios, K.; Kolovos, M. *Synthesis* **1998**, 671-673.



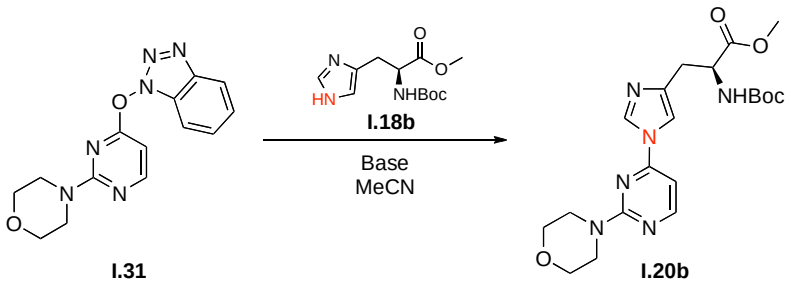
Scheme I.20. Synthesis of N^{α}-Fmoc-pyrimidin-4-yl tyrosine **I.11a**

I.2.1.7. Synthesis of pyrimidin-4-yl histidine derivatives

Following the results obtained with the tyrosine derivative, we decided to synthesize the remaining pyrimidin-4-yl amino esters **I.20** using the same strategy.

Therefore, OBt-adduct **I.31** was treated with the N^{α}-Boc methyl histidinate **I.18b** using DBU as a base to obtain the desired amino ester **I.20b** in moderate yields, but with considerable racemization, even when the reaction was performed at rt (Table I.6, entries 1 and 2). Although the racemization of compound **I.20b** could be completely avoided using an excess of potassium carbonate at 50 ºC for 48 h, the reaction yield was lower due to the incomplete conversion of the starting material (Table I.6, entry 3). Hence, we planned to perform the reaction using histidine **I.18b** as the limiting reactant and an excess of OBt-adduct **I.31** in the presence of potassium carbonate. This modification on the limiting reactant caused a significant improvement in both the yield and the reaction time. Indeed, the reaction was completed in only 19 h and furnished the optically pure compound **I.20b** in a good yield of 72% (Table I.6, entry 4). In addition, the excess amount of OBt-adduct **I.31** was easily recovered during the purification step by flash chromatography and could be reused.

Table I.6. Synthesis of N^{α}-Boc-pyrimidin-4-yl histidine methyl ester **I.20b**



Entry	**I.31** (equiv)	**I.18b** (equiv)	Base (equiv)	Time (h)	T (ºC)	Yield[a] (%)
1	1.0	1.5	DBU (1.5)	32	50	60[b]
2	1.0	1.5	DBU (1.5)	40	rt	70[b]
3	1.0	1.5	K_2CO_3 (4.0)	48	50	20
4	2.2	1.0	K_2CO_3 (4.0)	19	50	72[c]

[a] Isolated yields.

[b] Partial racemization was observed.

[c] The excess amount of **I.31** was recovered by flash chromatography.

The two nitrogen atoms of the imidazole ring of histidine are not equivalent. As such, the imidazole ring can exist in two tautomeric forms. Therefore, the nucleophilic attack could, in principle, take place by $N(\pi)$ or $N(\tau)$ atoms and consequently two regioisomers can be obtained (Figure I.12).

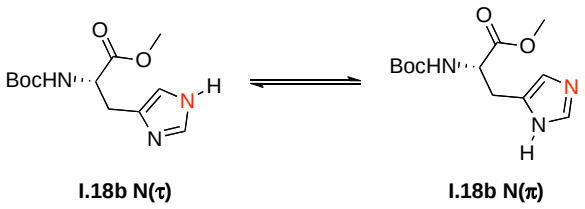


Figure I.12. Tautomeric equilibrium of histidine **I.18b**

Although the $N(\tau)$ derivative is usually the major product due to steric factors, it is rarely exclusive.[77] Interestingly however, all the reactions tested were completely regioselective in favour of the $N(\tau)$ derivative **I.20b** according to a NOESY NMR

[77] (a) Bambal, R.; Hanzlik, R. P. *J. Org. Chem.* **1994**, *59*, 729-732. (b) Jain, R.; Cohen, L. A. *Tetrahedron* **1996**, *52*, 5363-5370.

experiment. Indeed, a strong NOESY correlation between the H5 proton of the pyrimidine ring and the H5' and H2' protons of the imidazole ring was observed. This result was consistent only with the *N*(τ) regioisomer, which had both protons H2' and H5' of the imidazole ring close in space with the H5 proton of the pyrimidine ring (Figure I.13).

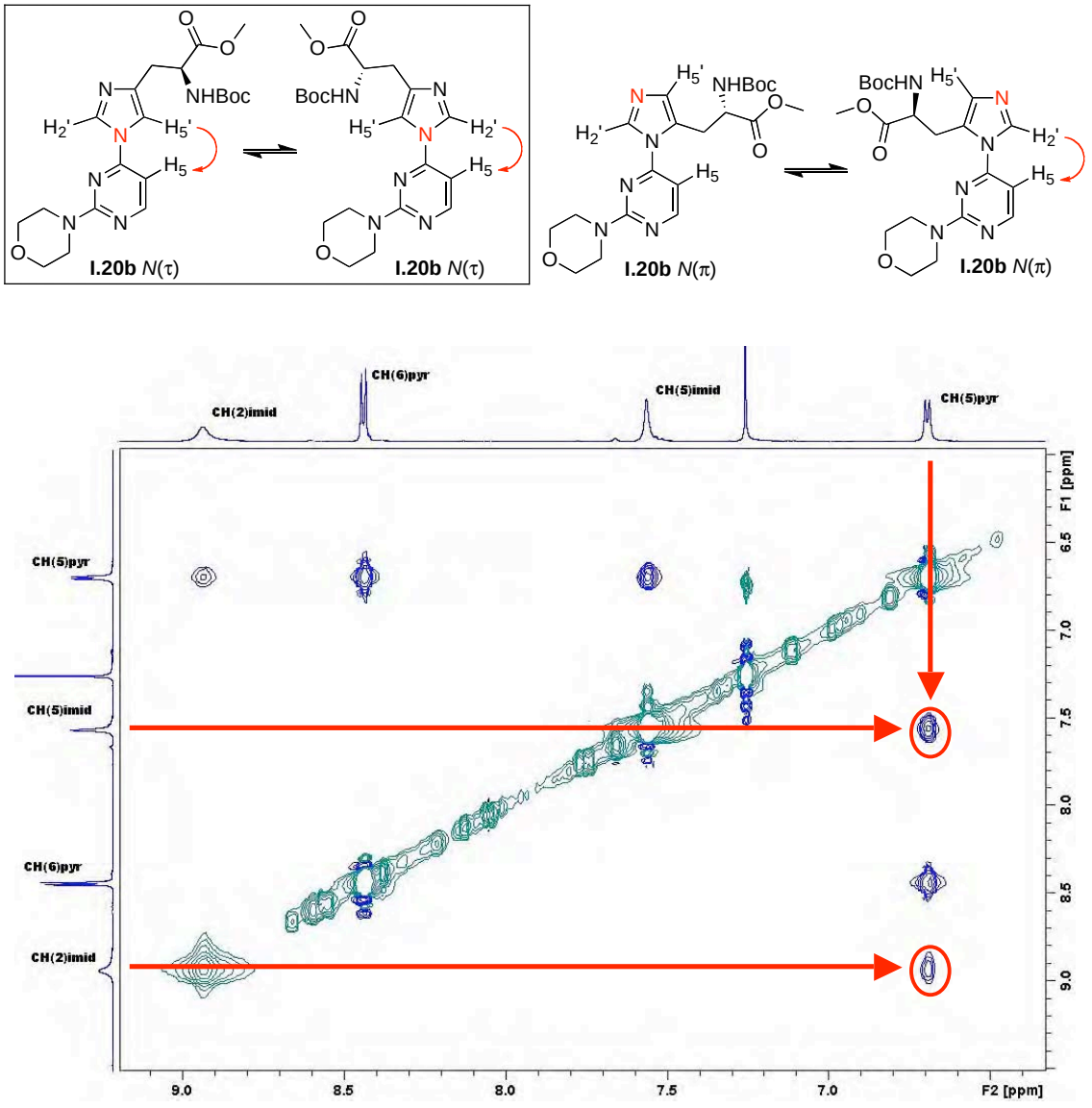
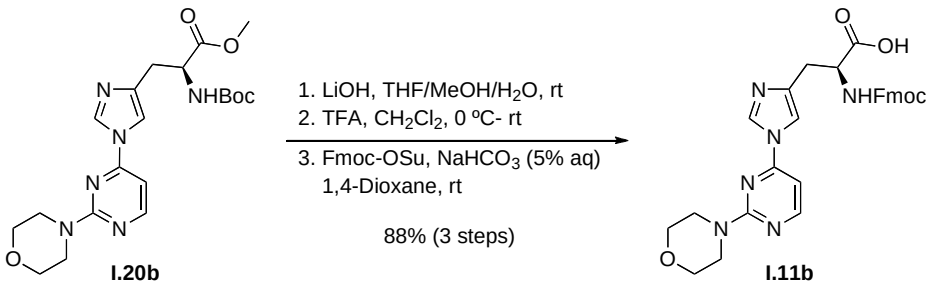


Figure I.13. NOESY spectra (region 9.2-6.3 ppm) of N^{α}-Boc-pyrimidin-4-yl histidine methyl ester **I.20b** recorded at 400 MHz in d_6-DMSO

Analogously to the previous section, the N^{α}-Boc pyrimidin-4-yl methyl histidinate **I.20b** was then converted to the target N^{α}-Fmoc-pyrimidin-4-yl histidine **I.11b** by applying the standard three-step procedure: methyl ester saponification, Boc protecting group removal and reprotection of the amino functionality with the Fmoc group. In this

case, the desired product **I.11b** was obtained in an excellent yield of 88% over three steps (Scheme I.21).

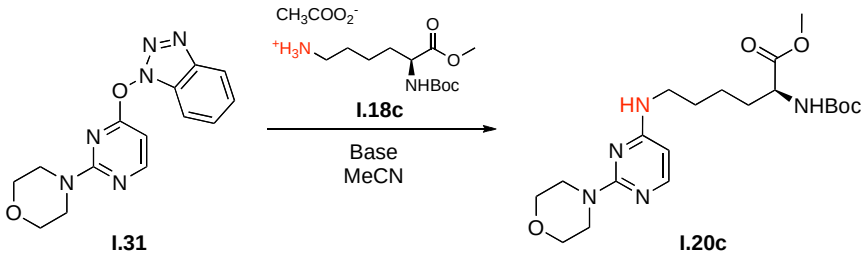


Scheme I.21. Synthesis of N^{α}-Fmoc-pyrimidin-4-yl histidine **I.11b**

I.2.1.8. Synthesis of pyrimidin-4-yl lysine derivatives

The synthesis of the N^{α}-Boc-pyrimidin-4-yl lysine methyl ester **I.20c** began by treating pyrimidinyl OBt-adduct **I.31** with the commercially available N^{α}-Boc methyl lysinate acetate salt **I.18c** using potassium carbonate as a base at 50 ºC for 48 h. Under these conditions, the expected product **I.20c** was isolated in 67% yield but with a significant degree of racemization (Table I.7, entry 1). In order to avoid the racemization, the reaction was performed at rt. In this case, compound **I.20c** was obtained without detectable optical degradation; however, the reaction time was extended to 72 h and the yield was lower (Table I.7, entry 2). In a new test, the reaction was effected under a controlled heating below 40 ºC. After 42 h, compound **I.20c** was isolated in 66% yield and without appreciable racemization (Table I.7, entry 3). This last result could be improved using an excess of the starting OBt-adduct **I.31** at 40 ºC. In only 24 h, this reaction furnished compound **I.20c** as a sole enantiomer in 84% yield (Table I.7, entry 4). Analogously to the previous Section the excess amount of the OBt-adduct **I.31** could be recovered and reused.

Table I.7. Synthesis of N^{α}-Boc-pyrimidin-4-yl lysine methyl ester **I.20c**



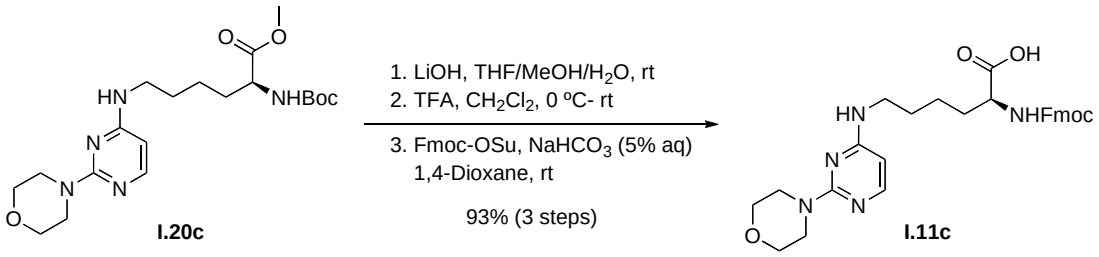
Entry	**I.31** (equiv)	**I.18c** (equiv)	Base (equiv)	Time (h)	T (ºC)	Yield[a] (%)
1	1.0	1.5	K_2CO_3 (4.0)	48	50	67[b]
2	1.0	1.5	K_2CO_3 (4.0)	76	rt	43
3	1.0	1.5	K_2CO_3 (4.0)	42	40	66
4	2.2	1.0	K_2CO_3 (4.0)	24	40	84[c]

[a] Isolated yields.

[b] Partial racemization was observed.

[c] The excess amount of **I.31** was recovered by flash chromatography.

Subsequently, the target N^{α}-Fmoc pyrimidin-4-yl lysine **I.11c** was obtained in 93% yield after subjecting the N^{α}-Boc pyrimidin-4-yl methyl lysinate **I.20c** to the three-step sequence previously used (Scheme I.22).



Scheme I.22. Synthesis of N^{α}-Fmoc-pyrimidin-4-yl lysine **I.11c**

I.2.1.9. Synthesis of pyrimidin-4-yl tryptophan derivatives

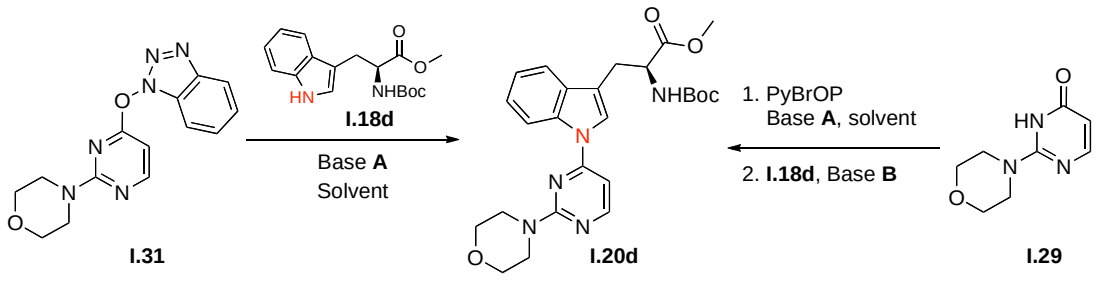
The synthesis of the N^{α}-Boc-pyrimidin-4-yl tryptophan methyl ester **I.20d** was problematic due to the low nucleophilicity of the indole nitrogen atom. Consequently, a large number of experiments were run to reach the target compound **I.20d** with

reasonable efficiency (Table I.8). First, the reaction was carried out with N^{α}-Boc methyl tryptophanate **I.18d** and potassium carbonate as a base in acetonitrile at 50 ºC for 24 h, which afforded the product **I.20d** in 22% yield (Table I.8, entry 1). In order to improve the reaction yield, an excess of OBt-adduct **I.31** was used; however, after 6 days at 50 ºC compound **I.20d** was isolated in only 11% yield (Table I.8, entry 2). The use of a more polar solvent such as DMF instead of acetonitrile did not produce any significant change (Table I.8, entry 3). The main cause of this low yield could be attributed to a poor reaction conversion. In all cases, the reaction conversion was around 25% after 24 h according to the monitoring of the progress of the reaction by HPLC. Besides, HPLC-monitoring showed that increasing the reaction time beyond 24 h did not improve the conversion. Thereafter, the heating method was changed to improve the conversion and enhance the yield. Thus, two experiments were performed using the above reaction conditions at different temperatures under microwave heating.[78] However, microwave heating did not ameliorate the results obtained by conventional heating (Table I.8, entries 4 and 5).

According to the literature, Kang *et al.*[70] achieved the phosphonium coupling between an electron-deficient pyrimidin-2(1*H*)-one and an indole using PyBrOP as the activating agent. Hence, the direct phosphonium coupling reaction between the electron-rich 2-morpholino-4(3*H*)-pyrimidinone **I.29** and N^{α}-Boc methyl tryptophan **I.18d** was tested using the same reaction conditions as the ones reported by Kang and co-workers. However, no reaction took place under these conditions and only the starting material **I.29** was isolated after 45 h stirring (Table I.8, entry 6). Thereafter, the direct coupling reaction between pyrimidinone **I.29** and amino ester **I.18d** promoted by PyBrOP was carried out under different reaction conditions. Unfortunately, all reactions failed, even using different combinations of bases and solvents under conventional or microwave heating (Table I.8, entries 7-9). In general, the starting substrate **I.29** was totally recovered and only a 10% yield of the expected product **I.20d** was obtained when Et_3N and K_2CO_3 were used as bases in acetonitrile at 80 ºC under microwave irradiation (Table I.8, entry 7).

[78] Ashton, T. D.; Scammells, P. J. *Aust. J. Chem.* **2008**, *61*, 49-58.

Table I.8. Synthesis of N^{α}-Boc-pyrimidin-4-yl tryptophan methyl ester **I.20d**



Entry	**I.29** or **I.31** (equiv)	**I.18d** (equiv)	Base **A** (equiv)	Base **B** (equiv)	Solvent	Time (h)	T (ºC)	Yield[a] (%)
1	**I.31** (1.0)	1.5	K_2CO_3 (4.0)	-	MeCN	24	50	22
2	**I.31** (2.2)	1.0	K_2CO_3 (4.0)	-	MeCN	6 days	50	11[b]
3	**I.31** (1.0)	2.0	K_2CO_3 (4.0)	-	DMF	24	50	16
4	**I.31** (1.0)	1.5	K_2CO_3 (4.0)	-	MeCN	1	80[c]	-
5	**I.31** (1.0)	1.5	K_2CO_3 (4.0)	-	MeCN	1	110[c]	< 5[d]
6	**I.29** (1.0)	1.3	Et_3N (2.5)	KO*t*-Bu (1.3)	1,4-dioxane	45	rt	-
7	**I.29** (1.0)	1.3	Et_3N (2.5)	K_2CO_3 (4.0)	1,4-dioxane	42	50	-
8	**I.29** (1.0)	1.3	Et_3N (2.5)	K_2CO_3 (4.0)	MeCN	1	80[c]	10
9	**I.29** (1.0)	2.0	DBU (2.5)	DBU (2.0)	MeCN	1	80[c]	-

[a] Isolated yields.
[b] The excess amount of **I.31** was recovered by flash chromatography.
[c] Microwave heating.
[d] Yield based on HPLC of crude material.

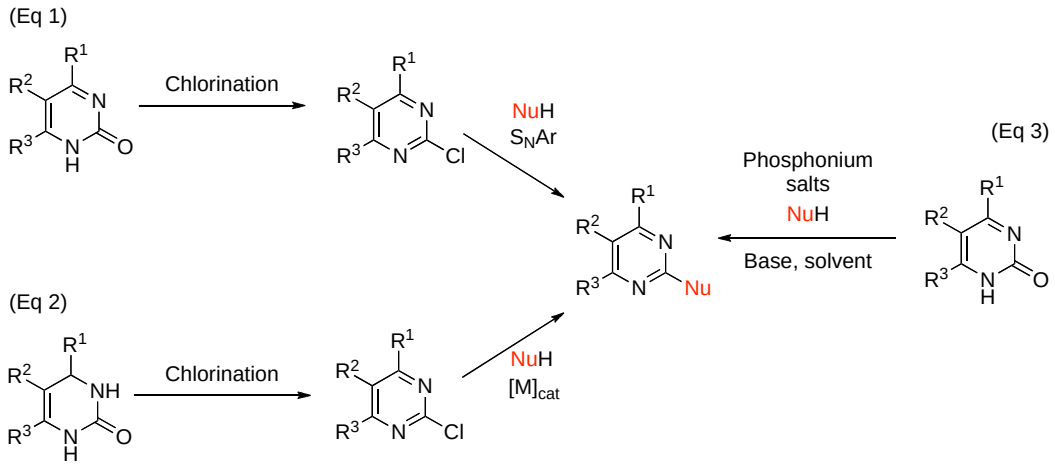
In summary, with the exception of the tryptophan derivative **I.20b**, the incorporation of an amino acid residue at the C4-position of the electron-rich 2-morpholino-4(3*H*)-pyrimidinone **I.29** was achieved in high yields using a two-step approach *via* the OBt-adduct **I.31**. We demonstrated that the base, the reaction temperature and the number of reagent equivalents played a crucial role in obtaining these compounds in good yield and without loss of optical integrity. Moreover, the N^{α}-Boc-pyrimidin-4-yl

amino esters **I.20** were successfully converted to the corresponding N^{α}-Boc-pyrimidin-4-yl amino acids **I.11** useful as building blocks for the solid-phase peptide synthesis.

I.2.2. SYNTHESIS OF N^{α}-FMOC PYRIMIDIN-2-YL AMINO ACIDS

I.2.2.1. Synthesis of C2 functionalized pyrimidines

Analogously to C4-substituted pyrimidines, the synthesis of pyrimidines modified at the C2-position is generally performed from pyimidin-2(1*H*)-one derivatives by activation of the carbonyl group, followed by coupling with nucleophiles as a complementary approach to convergent synthetic methods of C2-substituted pyrimidines. Chlorination is the most common method used to activate the carbonyl function and both nucleophilic displacement and metal-catalyzed cross coupling reactions[79] are usually employed to functionalize the C2-position (Scheme I.23, Eq 1 and Eq 2). Alternatively, the phosphonium coupling reaction between pyrimidin-2(1*H*)-one derivatives and nucleophiles is an efficient way to synthesize C2-substituted pyrimidines (Scheme I.23, Eq 3).[70] This method has been largely discussed in the previous Section I.2.1.1. However, some limitations still exist, such as the harsh reaction conditions, the use of expensive catalysts and/or the long reaction times.



Scheme I.23. Synthesis of the C2-functionalizated pyrimidines

[79] Gholap, A. R.; Toti, K. S.; Shirazi, F.; Deshpande, M. V.; Srinivasan, K. V. *Tetrahedron* **2008**, *64*, 10214-10223.

As mentioned in Section I.1.3.3, our laboratory has been developing efficient methods for the preparation of pyrimidinyl compounds with a high degree of molecular diversity.[36] These approaches include a method to functionalize the C2-position of the pyrimidine ring based on the *ipso*-substitution reaction of 2-alkylsulfonylpyrimidines with a variety of nucleophiles under mild reaction conditions (Scheme I.4). While procedure was applied in a previous work to achieve the synthesis of *N*-pyrimidinyl arylglycines **I.6,**[36d] we applied it for the synthesis of N^{α}-Fmoc pyrimidin-4-yl α-amino acids **I.11** (Figure I.14).

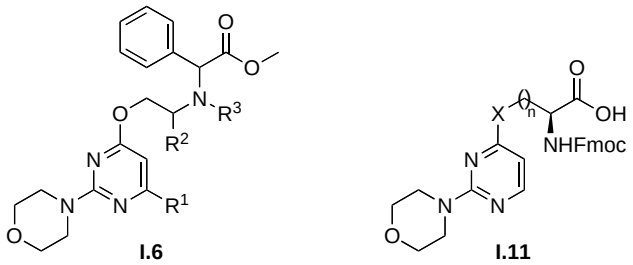


Figure I.14. *N*-pyrimidinyl arylglycines **I.6** and N^{α}-Fmoc pyrimidin-4-yl α-amino acids **I.11**

Based upon these results, we envisioned to apply the *ipso*-substitution reaction of 2-alkylsulfonylpyrimidines method to access new pyrimidin-2-yl amino acids **I.12** and pyrimidin-2-one amino acids **I.13** (Figure I.15).

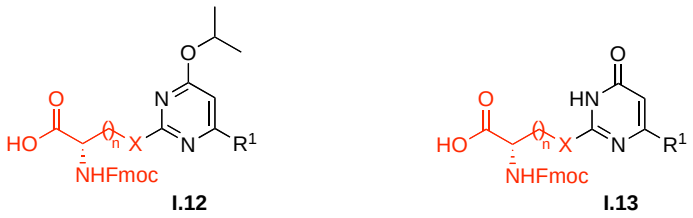


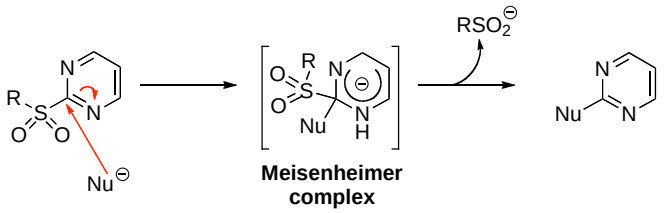
Figure I.15. N^{α}-Fmoc pyrimidin-2-yl amino acids **I.12** and N^{α}-Fmoc pyrimidin-2-one amino acids **I.13**

I.2.2.2. Synthetic strategy to pyrimidin-2-yl amino acids

The synthesis of pyrimidin-2-yl amino acids **I.12** was envisioned *via* a key S_NAr reaction of an alkylsulfonyl group. As described in Section I.1.3.3.2, this reaction works well with electron-deficient heterocycles such as a pyrimidine ring.[80] Besides, alkyl and arylsulfonyl groups proved to be better leaving groups than the corresponding halides in

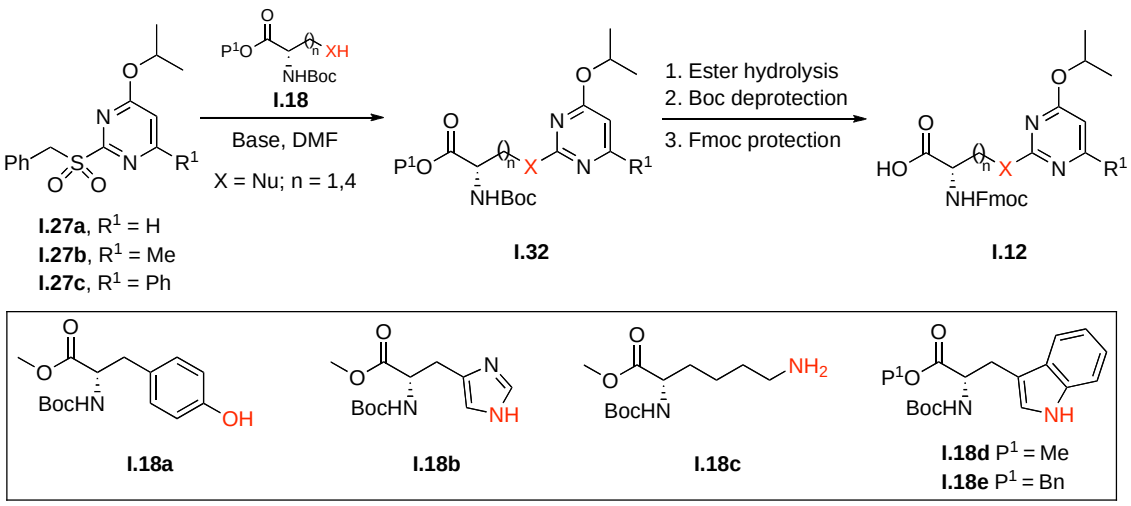
[80] Sheperd, R. G.; Fedrick, J. *Adv. Heterocycl. Chem.* **1965**, *4*, 145-423.

the S_NAr reaction on the pyrimidine ring.[38] The overall mechanism of this reaction is an addition-elimination process through a Meisenheimer complex with the release of an alkyl- or an arylsulfonyl group in the last step (Scheme I.24).



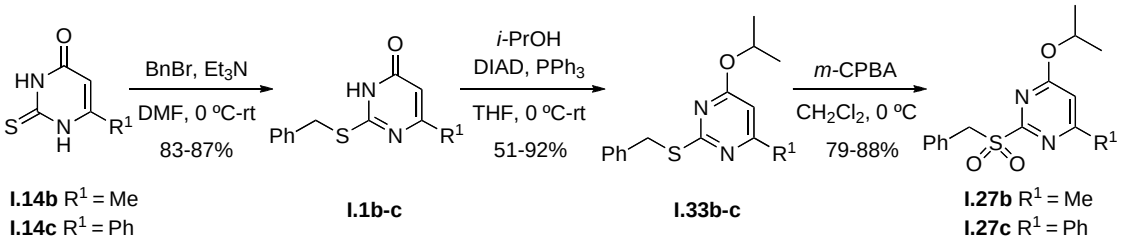
Scheme I.24. Mechanism of S_NAr reaction on alkyl or arylsulfonylpyrimidines

Consistent with this goal, the incorporation of an α-amino acid residue at the C2-position of the pyrimidine ring could be achieved by an S_NAr reaction between 2-benzylsulfonyl-4-isopropoxypyrimidines **I.27a-c** and the nucleophilic side chain of several natural α-amino acids **I.18**. Consequently, the amine and carboxyl functional groups must be suitably protected (Scheme I.25). In addition, the S_NAr reaction should be performed in the presence of a base to reinforce the nucleophilicity of the side chains. Therefore, the sulfones **I.27a-c** would be treated under basic conditions with N^{α}-Boc-amino esters **I.18** in order to obtain the corresponding pyrimidin-2-yl amino esters **I.32**. Once again, a series of protecting group transformations should lead to the target N^{α}-Fmoc pyrimidin-2-yl amino acids **I.12** (Scheme I.25).



Scheme I.25. Synthetic strategy for the synthesis of pyrimidin-2-yl amino acids **I.12**

The starting compounds **I.27b** and **I.27c** were obtained in good yield as depicted in Scheme I.26 following the procedure previously used to prepare **I.27a**.[36a] First, thiouracil derivatives **I.14b** and **I.14c** were selectively *S*-alkylated with benzyl bromide under basic conditions to afford the corresponding 2-benzylsulfanylpyrimidin-4(3*H*)-ones **I.1b** and **I.1c**. These latter compounds were then *O*-alkylated with 2-propanol under Mitsunobu conditions and the resulting 2-benzylsulfanyl-4-isopropoxypyrimidines **I.33b** and **I.33c** were oxidized with *m*-CPBA to give rise to the desired sulfones **I.27b** and **I.27c** (Scheme I.26).



Scheme I.26. Synthesis of 2-benzylsulfonyl-4-isopropoxypyrimidines **I.27b** and **I.27c**

I.2.2.2.1. Synthesis of pyrimidin-2-yl tyrosine derivatives

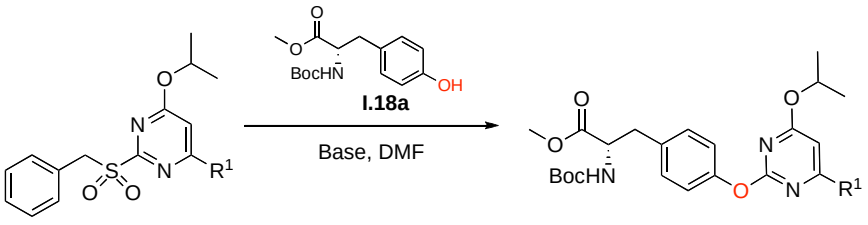
In the initial experiments with the N^{α}-Boc-tyrosine methyl ester **I.18a**, we studied the nucleophilic displacement starting from 2-benzylsulfonylpyrimidine **I.27a** and employing several bases in anhydrous DMF (Table I.9). This polar aprotic solvent was chosen because it favours nucleophilic substitution reactions and allows a good solubility of the starting materials. The first attempt was carried out with potassium *tert*-butoxide as a base at 80 ºC. Under these conditions, no reaction took place and only the starting material **I.27a** was recovered after 24 h (Table I.9, entry 1). Better results were obtained using both sodium hydride and potassium carbonate affording the desired compound **I.32aa** without appreciable racemization (Table I.9, entries 2 and 3).[81] While the reaction with sodium hydride occurred at rt, in the case of potassium carbonate the reaction needed to be heated to 50 ºC to reach completion but the yield was higher than with NaH.

These last reaction conditions were extended to 2-benzylsulfonylpyrimidines **I.27b** and **I.27c**, which afforded the corresponding pyrimidinyl amino esters **I.32ab** and

[81] The determination of the optical purity was assessed by the HPLC method described in Section I.2.1.4.

I.32ac in good yields (Table I.9, entries 4 and 5). Unfortunately, compound **I.32ac** was obtained with a high degree of racemization (er = 60:40). To avoid the loss of optical integrity of this compound, the reaction was tested at lower temperatures. A substantial reduction in the racemization (er = 87:13) was noticed when the reaction was carried out at 40 ºC, whereas at rt the expected product **I.32ac** was isolated without any appreciable racemization but in a considerably lower yield (Table I.9, entries 6 and 7). However, it was possible to obtain an X-ray structure of this compound **I.32ac**, which showed the tyrosine residue linked to the C2-position of the pyrimidine ring by the phenoxy group of its side chain (Figure I.16).

Table I.9. Synthesis of N^{α}-Boc-pyrimidin-2-yl tyrosine methyl ester **I.32a**



I.27a R^1 = H
I.27b R^1 = Me
I.27c R^1 = Ph

I.32aa R^1 = H
I.32ab R^1 = Me
I.32ac R^1 = Ph

Entry	Sulfone	Base (equiv)	Time (h)	T (ºC)	Product	Yield[a] (%)	er[c]
1	**I.27a**	KO*t*-Bu (1.1)	24	80	**I.32aa**	-	
2	**I.27a**	NaH (1.2)	18	rt	**I.32aa**	60	
3	**I.27a**	**K_2CO_3 (2.4)**	**24**	**50**	**I.32aa**	**79**	
4	**I.27b**	K_2CO_3 (2.4)	24	50	**I.32ab**	60	
5	**I.27c**	K_2CO_3 (2.4)	24	50	**I.32ac**	90[b]	60:40
6	**I.27c**	K_2CO_3 (2.4)	24	40	**I.32ac**	58[b]	87:13
7	**I.27c**	K_2CO_3 (2.4)	24	rt	**I.32ac**	40	

[a] Isolated yields.

[b] Partial racemisation observed.

[c] er: enantiomeric ratio determined by HPLC of dipeptide derivative **I.26** (Section I.2.1.4).

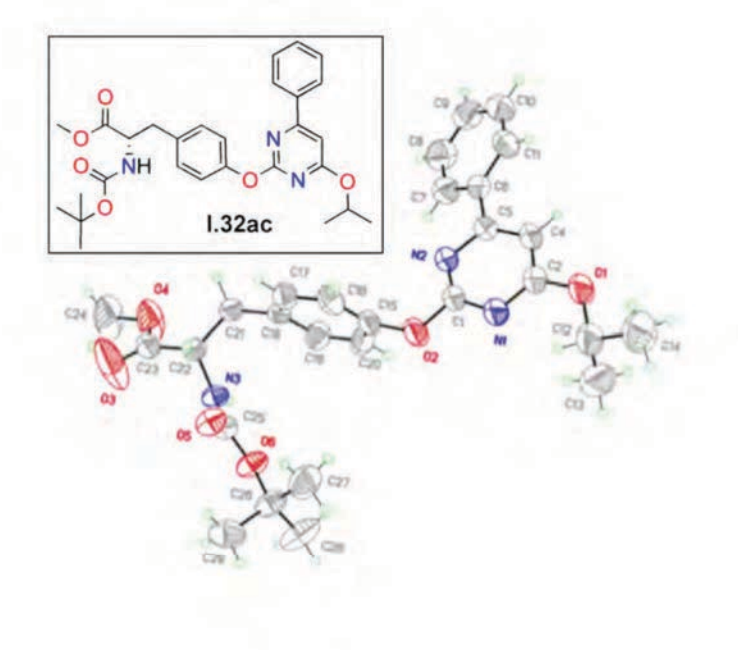
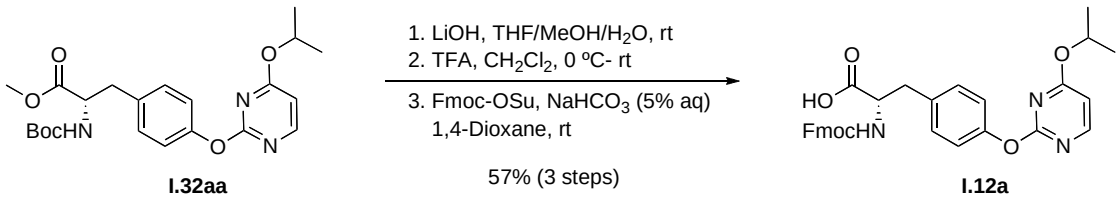


Figure I.16. ORTEP representation of compound **I.32ac**

We therefore selected the N^{α}-Boc-pyrimidin-2-yl tyrosine methyl ester **I.32aa**, which afforded best results, to be converted to the target N^{α}-Fmoc-pyrimidin-2-yl tyrosine **I.12a**. First, the methyl ester of compound **I.32aa** was saponified using LiOH, and the resulting acid was directly engaged in the next reaction without further purification. The N^{α}-Boc protecting group was then removed by treatment with TFA to release the free primary amine, which was subsequently protected using Fmoc-OSu. This three-step sequence furnished the expected product **I.12a** in 57% overall yield after purification by flash chromatography (Scheme I.27).



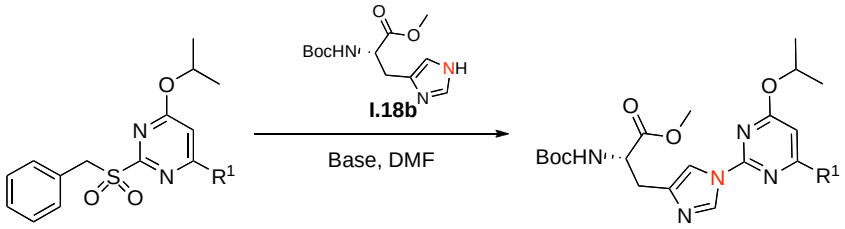
Scheme I.27. Synthesis of N^{α}-Fmoc-pyrimidin-2-yl tyrosine **I.12a**

I.2.2.2.2. Synthesis of pyrimidin-2-yl histidine derivatives

Analogously to the previous Section, the N^{α}-Boc-histidine methyl ester **I.18b** was first treated with sulfone **I.27a** using potassium *tert*-butoxide as a base in DMF at 50 ºC

for 15 h. Under these conditions, the expected N^{α}-Boc-pyrimidin-2-yl histidine methyl ester **I.32ba** was obtained in a low yield of 37% (Table I.10, entry 1). The use of DBU as a base gave better results (Table I.10, entries 2-4). Hence, the reaction between sulfones **I.27a-c** with protected histidine **I.18b** and DBU at 50 ºC allowed the isolation of the desired pyrimidin-2-yl amino esters **I.32ba-c** in very good yield and without appreciable racemization except for compound **I.32bc** bearing a phenyl group at the C6-position of the pyrimidine ring showed a substantial degree of racemization (Table I.10, entry 4). It proved possible to avoid racemization of compound **I.32bc** carrying out the reaction at rt; however, the yield was lower (Table I.10, entry 5).

Table I.10. Synthesis of N^{α}-Boc-pyrimidin-2-yl histidine methyl ester **I.32b**



I.27a R^1 = H
I.27b R^1 = Me
I.27c R^1 = Ph

I.32ba R^1 = H
I.32bb R^1 = Me
I.32bc R^1 = Ph

Entry	Sulfone	Base (equiv)	Time (h)	T (ºC)	Product	Yield[a] (%)	er[c]
1	**I.27a**	KO*t*-Bu (1.1)	15	50	**I.32ba**	37	
2	**I.27a**	**DBU (1.2)**	**8**	**50**	**I.32ba**	**80**	
3	**I.27b**	DBU (1.2)	8	50	**I.32bb**	79	
4	**I.27c**	DBU (1.2)	24	50	**I.32bc**	79[b]	70:30
5	**I.27c**	DBU (1.2)	24	rt	**I.32bc**	58	

[a] Isolated yields.
[b] Partial racemisation observed.
[c] er: enantiomeric ratio determined by HPLC of dipeptide derivative **I.26** (Section I.2.1.4).

As in the case of the synthesis of N^{α}-Boc-pyrimidin-4-yl histidine methyl ester **I.20b**, this reaction could afford two regioisomers because the two nitrogen atoms of the imidazole ring of histidine are not equivalent and the nucleophilic attack could, in principle, take place by $N(\pi)$ or $N(\tau)$ atoms. However, in all cases the reaction was

completely regioselective in favour of the *N*(τ) derivative. The unambiguous assignment of the structures **I.32ba-c** was secured by X-ray analysis of the compound **I.32bc**. As evidenced in Figure I.17, the histidine residue is attached to the C2-position of the pyrimidine ring by the *N*(τ) atom of the imidazole ring.

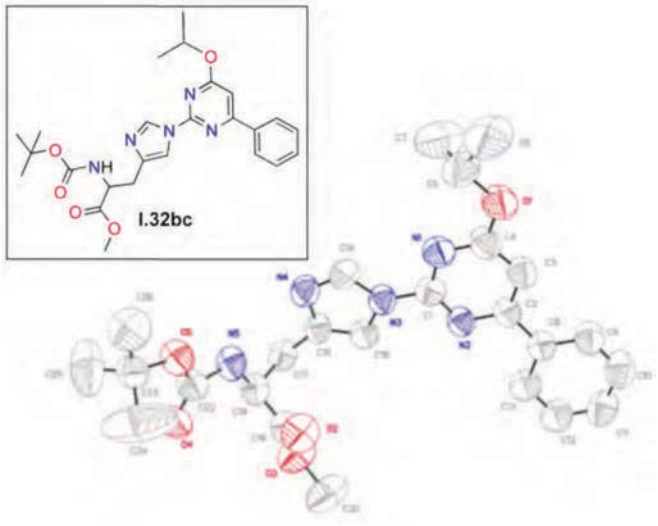
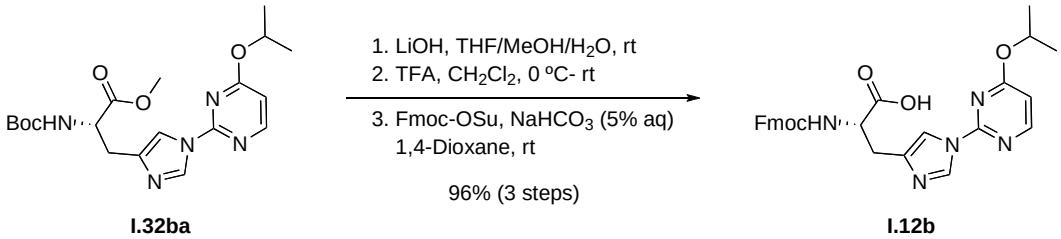


Figure I.17. ORTEP representation of compound **I.32bc**

N^{α}-Boc-pyrimidin-2-yl histidine methyl ester **I.32ba** was then converted to the corresponding N^{α}-Fmoc-pyrimidin-2-yl histidine **I.12b**. Thus, the standard three-step procedure – methyl ester hydrolysis, Boc removal and reprotection of the amino group with Fmoc protecting group – was applied to compound **I.32ba**. In this case, the desired product **I.12b** was isolated in a near quantitative yield (Scheme I.28).



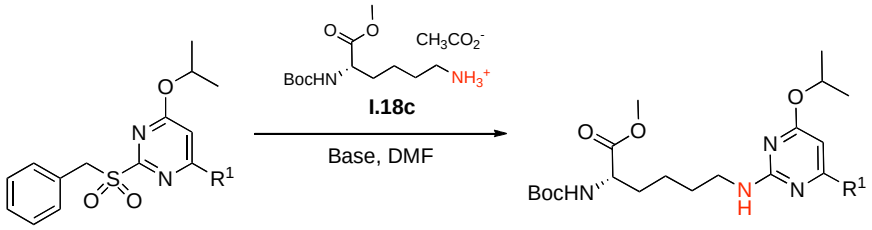
Scheme I.28. Synthesis of N^{α}-Fmoc-pyrimidin-2-yl histidine **I.12b**

I.2.2.2.3. Synthesis of pyrimidin-2-yl lysine derivatives

In the case of the N^{α}-Boc-lysine methyl ester, we used the commercially available N^{α}-Boc-lysine methyl ester acetate salt **I.18c**. Thus, even if the lysine has an aliphatic primary amine on its side chain, which is a good nucleophile, the nucleophilic *ipso*-substitution reaction between sulfones **I.27a-c** and amino ester **I.18c** also required basic conditions.

First of all, the starting sulfone **I.27a** was treated with the protected amino ester **I.18c** and a base (triethylamine and DIEA). In both cases, the reaction was not complete even after several days of heating at 50 ºC, and consequently, the expected product **I.32ca** was isolated in poor yield (Table I.11, entries 1 and 2). The yield could be improved and the reaction time reduced by using a stronger base such as DBU at 50 ºC. Under these reaction conditions, compounds **I.32cb** and **I.32cc** were obtained in moderate yields and without appreciable racemization (Table I.11, entries 5 and 6). Compound **I.32ca**, on the other hand, was isolated with a noticeable loss of optical purity (Table I.11, entry 3). This could be circumvented by using K_2CO_3 and heating at 40 ºC for 24 h. In addition, under this set of conditions the reaction yield was substantially increased and the pyrimidin-2-yl lysine methyl ester **I.32ca** could be obtained in an excellent yield of 88% (Table I.11, entry 4).

Table I.11. Synthesis of N^{α}-Boc-pyrimidin-2-yl lysine methyl esters **I.32c**



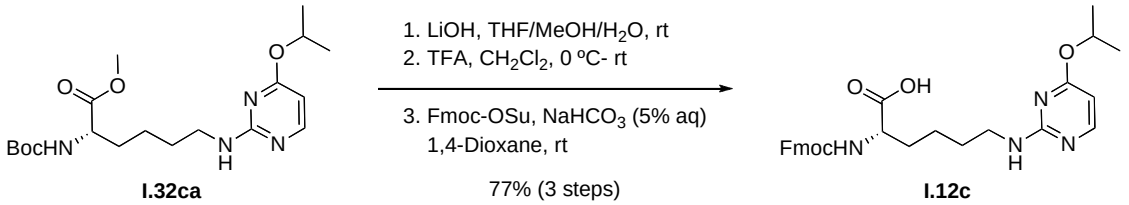
I.27a R^1 = H
I.27b R^1 = Me
I.27c R^1 = Ph

I.32ca R^1 = H
I.32cb R^1 = Me
I.32cc R^1 = Ph

Entry	Sulfone	Base (equiv)	Time (h)	T (ºC)	Product	Yield[a] (%)	er[c]
1	**I.27a**	Et_3N (2.0)	96	50	**I.32ca**	14	
2	**I.27a**	DIEA (2.0)	48	50	**I.32ca**	16	
3	**I.27a**	DBU (1.2)	28	50	**I.32ca**	60[b]	89:11
4	**I.27a**	**K_2CO_3 (4.0)**	**24**	**40**	**I.32ca**	**88**	
5	**I.27b**	DBU (1.2)	24	50	**I.32cb**	43	
6	**I.27c**	DBU (1.2)	24	50	**I.32cc**	42	

[a] Isolated yields.
[b] Partial racemisation observed.
[c] er: enantiomeric ratio determined by HPLC of dipeptide derivative **I.26** (Section I.2.1.4).

Finally, the N^{α}-Boc-pyrimidin-2-yl lysine methyl ester **I.32ca** was converted to the target N^{α}-Fmoc-pyrimidin-2-yl lysine **I.12c** following the three-step procedure used in the previous Sections. Methyl ester was first saponified using LiOH, and treated with TFA to cleave the N^{α}-Boc protecting group. The resulting free amine was then reprotected as a Fmoc carbamate leading to the expected N^{α}-Fmoc-pyrimidin-2-yl lysine **I.12c** with a global yield of 77% (Scheme I.29).



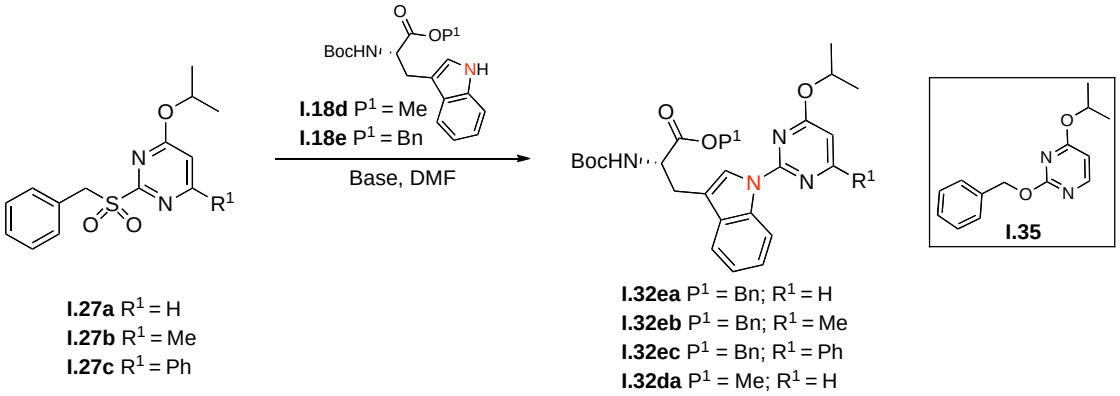
Scheme I.29. Synthesis of N^{α}-Fmoc-pyrimidin-2-yl lysine **I.12c**

An important issue in this three-step sequence was the work-up, as compounds such as **I.12c** contained a cyclic guanidine moiety which could be protonated.

I.2.2.2.4. Synthesis of pyrimidin-2-yl tryptophan derivatives

Initially, the nucleophilic *ipso*-substitution reaction between sulfones **I.27** and N^{α}-Boc-tryptophan was studied using the commercially available benzyl ester derivative **I.18e**. First, the reaction was carried out at 50 ºC with sulfone **I.27a** using potassium *tert*-butoxide as a base. Although the reaction was completed in 1 h, the desired pyrimidinyl amino ester **I.32ea** was obtained in only 5% yield. Because the major compounds isolated from the crude mixture were the transesterification product N^{α}-Boc-tryptophan *tert*-butyl ester **I.34** together with the 53% yield of 2-benzyloxy-4-isopropoxypyrimidine **I.35** (Table I.12, entry 1). Apparently, a transesterification reaction occurred between amino ester **I.18e** and potassium *tert*-butoxide affording a benzyloxy anion, which reacted as a nucleophile with the sulfone **I.27a** to form the side product **I.35**. To circumvent this issue, NaH and DBU, two strong bases with poor nucleophilicity, were tested at different temperatures. In all cases, the corresponding pyrimidinyl amino esters **I.32ea-c** were obtained in moderate yields, ranging from 34% to 57%, and with a complete loss of optical integrity. Even at rt, the reaction between starting sulfone **I.27a** and protected tryptophan **I.18e** in the presence of DBU or NaH resulted in a complete racemization of the product **I.32ea** (Table I.12, entries 2-4, 7 and 8). The degree of racemization could be substantially reduced when sulfone **I.27a** and tryptophan **I.18e** were treated with an excess of the weak base, potassium carbonate, at 50 ºC (Table I.12, entry 5), while at rt pyrimidinyl amino ester **I.32ea** was obtained without appreciable racemization but in a poor yield of 19% (Table I.12, entry 6).

Table I.12. Synthesis of N^{α}-Boc-pyrimidin-2-yl tryptophan esters **I.32d** and **I.32e**



I.27a R^1 = H
I.27b R^1 = Me
I.27c R^1 = Ph

I.32ea P^1 = Bn; R^1 = H
I.32eb P^1 = Bn; R^1 = Me
I.32ec P^1 = Bn; R^1 = Ph
I.32da P^1 = Me; R^1 = H

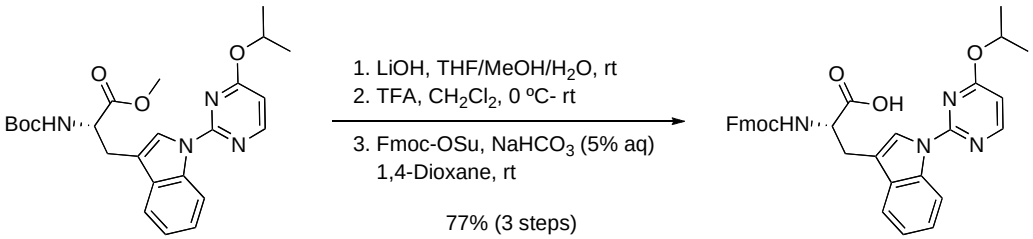
Entry	P^1	Sulfone	Base (equiv)	Time (h)	T (ºC)	Product	Yield[a] (%)	er[b]
1	Bn	**I.27a**	KO*t*-Bu (1.2)	1	50	**I.32ea**	5[c]	
2	Bn	**I.27a**	NaH (1.2)	24	rt	**I.32ea**	34[d]	50:50
3	Bn	**I.27a**	DBU (1.2)	24	50	**I.32ea**	50[d]	50:50
4	Bn	**I.27a**	DBU (1.2)	24	rt	**I.32ea**	50[d]	50:50
5	Bn	**I.27a**	K_2CO_3 (2.4)	24	50	**I.32ea**	37[e,f]	75:25
6	Bn	**I.27a**	K_2CO_3 (2.4)	24	rt	**I.32ea**	19[f]	
7	Bn	**I.27b**	DBU (1.2)	24	50	**I.32eb**	40[d]	50:50
8	Bn	**I.27c**	DBU (1.2)	24	50	**I.32ec**	57[d]	50:50
9	**Me**	**I.27a**	**K_2CO_3 (4.0)**	**24**	**40**	**I.32da**	**40[f]**	
10	Me	**I.27a**	K_2CO_3 (4.0)	48	40	**I.32da**	40[f]	
11	Me	**I.27a**	K_2CO_3 (4.0)	7 days	rt	**I.32da**	35[f]	

[a] Isolated yields.
[b] er: enantiomeric ratio determined by HPLC of dipeptide derivative **I.26** (Section I.2.1.4).
[c] Compounds **I.34** and **I.35** were isolated as a major product reaction.
[d] Complete racemisation observed.
[e] Partial racemisation observed.
[f] About 50% of starting sulfone **I.27a** recovered.

At this point, the study was continued using the more readily available N^{α}-Boc-tryptophan methyl ester **I.18d** instead of the benzyl ester derivative **I.18e**. Hence,

treatment of sulfone **I.27a** with amino ester **I.18d** in the presence of an excess of potassium carbonate at 40 ºC gave the pyrimidinyl amino ester **I.32da** in 40% yield and without significant racemization (Table I.12, entry 9). These results clearly indicated that the *ipso*-substitution reaction could be performed without any racemization by using potassium carbonate as the base and by running the reaction at temperatures below 40 ºC. Moreover, the low yields obtained for compounds **I.32ea** and **I.32da** could be attributed to the low level of conversion. In general, HPLC-monitoring of the progress of the reaction showed that the conversion was about 50% after 24 h and that longer reaction times did not improve the conversion. Nevertheless, the starting sulfone **I.27a** could be recovered and reused (Table I.12, entries 10 and 11).

The N^{α}-Boc-pyrimidin-2-yl tryptophan methyl ester **I.32da** that afforded the best results was therefore converted to the target N^{α}-Fmoc-pyrimidin-2-yl tryptophan **I.12d** following the three-step procedure previously used. First, the methyl ester of compounds **I.32da** was saponified using lithium hydroxide and the resulting acid was directly engaged to the cleavage of the N^{α}-Boc protecting group by treatment with TFA. Finally, the free amino group was reprotected with Fmoc-Osu leading to the expected N^{α}-Fmoc-pyrimidin-2-yl amino acid **I.12d** with a global yield of 77% (Scheme I.30).



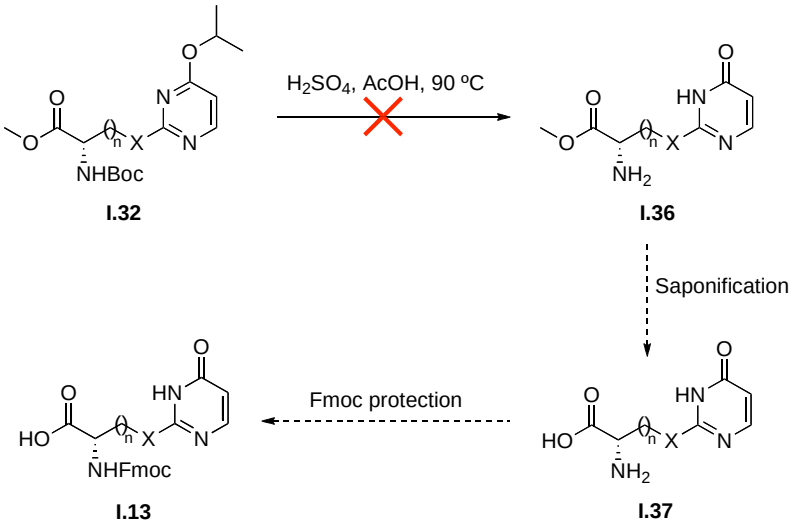
I.32da **I.12d**

Scheme I.30. Synthesis of N^{α}-Fmoc-pyrimidin-2-yl tryptophan **I.12d**

In summary, the synthesis of pyrimidinyl amino esters **I.32** could be achieved *via* a nucleophilic aromatic substitution reaction between sulfones **I.27** and the nucleophilic side chain of tyrosine, histidine, lysine and tryptophan. In general, the best reaction conditions were potassium carbonate as base and a reaction temperature ranging from rt to 50 ºC. Pyrimidinyl amino esters **I.32** could be converted in good yields to the corresponding Fmoc derivatives **I.12**, which would be used as useful building blocks for the solid-phase peptide synthesis.

I.2.2.3. Synthesis of pyrimidin-2-one amino esters

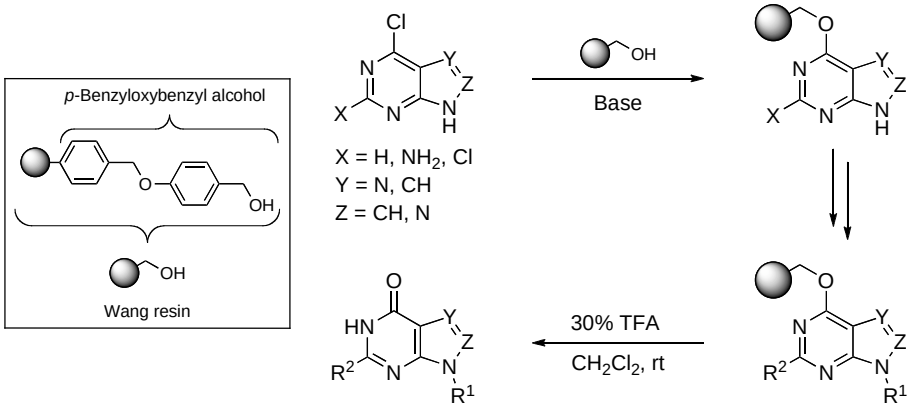
Initially, the preparation of pyrimidin-2-one amino acids **I.13** was envisioned by a simply cleaving the isopropoxy group of the pyrimidin-2-yl amino esters **I.32** under acidic conditions as previously described in Section I.2.1.5 (Scheme I.31).[36a] Thus, the target compounds **I.13** could be achieved from the simultaneous cleavage of both isopropoxy and Boc groups after acid treatment of pyrimidin-2-yl amino esters **I.32** followed by a subsequent ester saponification and Fmoc protection of the free amino acid (Scheme I.31). Disappointingly, the strong acidic conditions required to remove the isopropoxy group caused the decomposition of the starting compounds **I.32**.



Scheme I.31. Attempted synthesis of N^{α}-Fmoc pyrimidin-2-one amino acids **I.13**

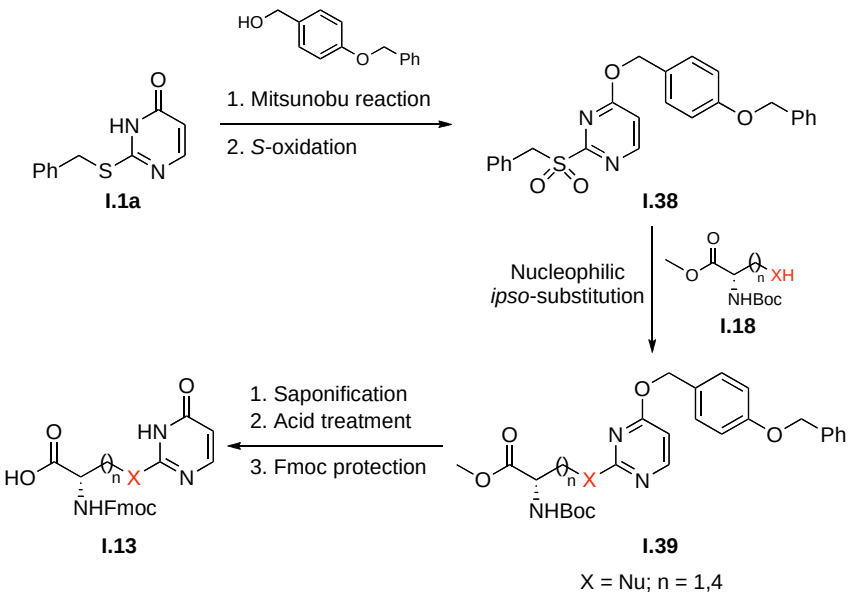
As a result of a literature search, we decided that the *p*-benzyloxybenzyloxy group should be used instead of the isopropoxy. The *p*-benzyloxybenzyl ether is an acid labile group, which can be cleaved under mild acidic conditions, generally, with TFA at rt. This group is an analogue to the linker of the Wang resin, widely used in solid-phase organic chemistry.[82] For example, Lam *et al.* reported the solid-phase synthesis of a library of purine analogues where the final compounds were efficiently cleaved from the Wang resin with 30% TFA in CH_2Cl_2 at rt (Scheme I.32).[83]

[82] (a) Wang, S.-S. *J. Am. Chem. Soc.* **1973**, *95*, 1328-1333. (b) James, I. W. *Tetrahedron* **1999**, *55*, 4855-4946.
[83] Chin-Tan, T. M.; Yang, F.; Fu, H.; Raghavendra, M. S.; Lam, Y. *J. Comb. Chem.* **2007**, *9*, 210-218.



Scheme I.32. Solid-phase synthesis of purine analogues library

We planned to prepare the target compounds **I.13** following the strategy depicted in Scheme I.33. First, a selective *O*-alkylation of 2-benzylsulfonyl-4(3*H*)-pyrimidinone **I.1a** with *p*-benzyloxybenzyl alcohol under Mitsunobu conditions followed by oxidation of the resulting pyrimidine would furnish the sulfone **I.38**. This compound **I.38** would then be treated with the N^{α}-Boc amino esters **I.18** under basic conditions to afford the corresponding N^{α}-Boc-pyrimidin-2-yl amino esters **I.39**. Finally, compounds **I.39** could be converted to the target N^{α}-Fmoc-pyrimidin-2-one amino acids **I.13** through the three-step sequence used in the previous sections: i) ester saponification, ii) acid treatment to remove simultaneously both the Boc and the *p*-benzyloxybenzyloxy groups, and iii) Fmoc protection of the free amino acid (Scheme I.33).

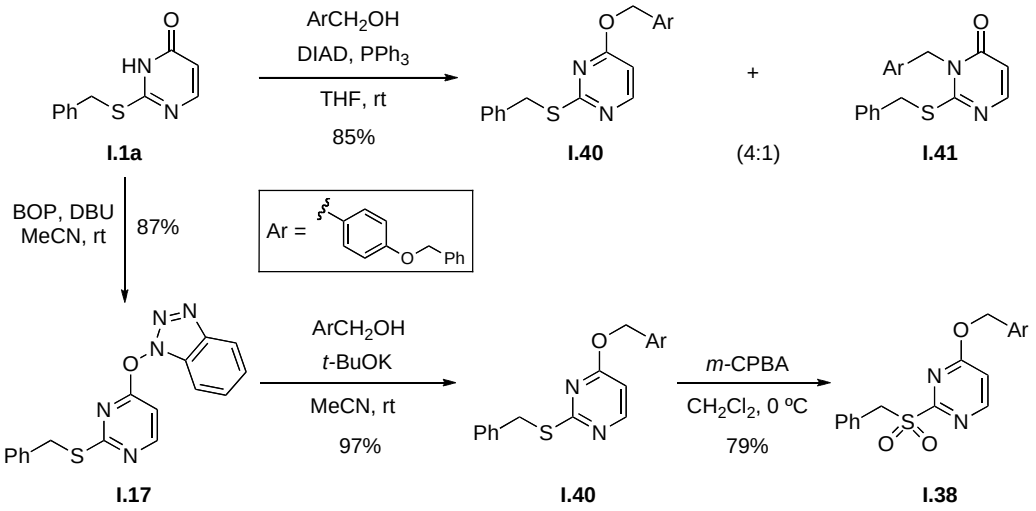


Scheme I.33. Synthetic strategy of pyrimidin-2-one amino acids **I.13**

I.2.2.3.1. Synthesis of 4-(4-benzyloxybenzyloxy)-2-benzylsulfonylpyrimidine (I.38)

The Mitsunobu reaction between 2-benzylsulfonyl-4(3*H*)-pyrimidinone **I.1a** and *p*-benzyloxybenzyl alcohol at rt yielded a mixture of regioisomers **I.40** and **I.41** in a 4:1 ratio.[84] In this case, the Mitsunobu reaction was not completely selective in favour of the *O*-alkylated product **I.40** as a significant amount of *N*-alkylated product **I.41** was also obtained (Scheme I.34). Although the desired compound **I.40** was obtained as the major regioisomer in a good yield, the separation of these isomers **I.40** and **I.41** by flash column chromatography proved to be problematic. For this reason, the phosphonium coupling reaction previously described was also tested as an alternative synthesis of compound **I.38**. Based on the anterior results, the two-step method mediated by the OBt-adduct **I.17** was applied. Thus, pyrimidinone **I.1a** was first treated with the phosphonium reagent BOP and DBU as a base in acetonitrile to afford the OBt-adduct **I.17** in 87% yield. The latter was then subjected to a S_NAr with *p*-benzyloxybenzyl alcohol in the presence of potassium *tert*-butoxide in acetonitrile to provide the desired product **I.40** in an excellent yield. Finally, pyrimidine **I.40** was oxidized using *m*-CPBA to afford the sulfone **I.38** in 79% yield (Scheme I.34).

[84] Regioisomer ratio determined by HPLC analysis of the crude reaction mixture.



Scheme I.34. Synthesis of sulfone **I.38**

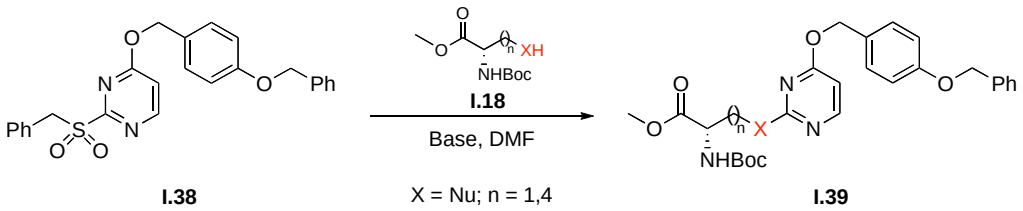
In order to test the deprotection conditions of the *p*-benzyloxybenzyloxy group, a sample of pyrimidine **I.40** was subjected to 30% TFA in CH_2Cl_2 at rt. After 2 h, compound **I.40** was completely converted to the starting 2-benzylsulfanyl-4(3*H*)-pyrimidinone **I.1a**.

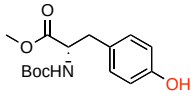
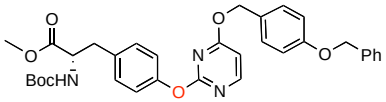
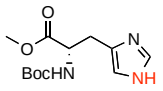
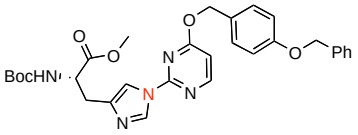
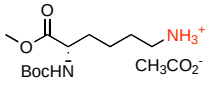
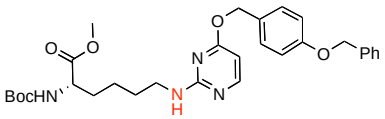
I.2.2.3.2. Synthesis of pyrimidin-2-one amino acids

The *ipso*-substitution reaction between pyrimidinyl sulfone **I.38** and the suitably protected amino esters **I.18** – tyrosine, histidine and lysine – was carried out using potassium carbonate as the base in DMF at a maximum of 50 ºC to prevent the racemization of the final products. Under this set of conditions, the corresponding pyrimidin-2-yl amino esters **I.39** were isolated in excellent yields and without appreciable racemization[85] (Table I.13, entries 1-3).

[85] The optical purity of compounds **I.39** was verified mediated the HPLC method described in Section I.2.1.4.

Table I.13. Synthesis of N^{α}-Boc-pyrimidin-2-yl amino esters **I.39**

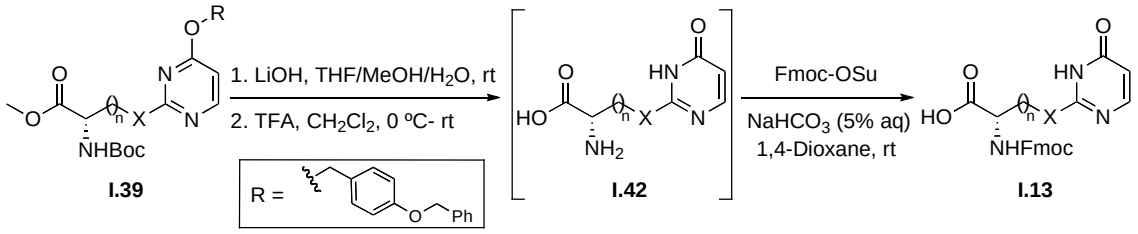


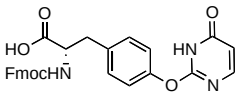
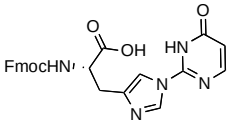
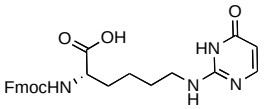
Entry	**I.18**	Base (equiv)	T (ºC)	Product	Yield[a] (%)
1	 **I.18a**	K_2CO_3 (1.2)	50	 **I.39a**	89
2	 **I.18b**	K_2CO_3 (1.2)	50	 **I.39b**	94
3	 **I.18c**	K_2CO_3 (2.4)	45	 **I.39c**	84

[a] Isolated yields.

The N^{α}-Boc-pyrimidin-2-yl amino esters **I.39** were then converted to the target N^{α}-Fmoc-pyrimidin-2-one amino acids **I.13**. Initially, methyl ester was saponified successfully and, as expected, the treatment with TFA removed both the Boc and the *p*-benzyloxybenzylxoy group. The resulting 4(3*H*)-pyrimidin-2-one **I.42** carrying an amino acid moiety at the C2-position was immediately protected as a Fmoc carbamate to achieve the target N^{α}-Fmoc-pyrimidin-2-one amino acids **I.13**. In all cases, the global yield was moderate, ranging from 40% to 55% (Table I.14). The main cause of these moderate yields was the high polarity of the final compound **I.13** that made its purification by flash chromatography difficult.

Table I.14. Synthesis of N^{α}-Fmoc-pyrimidin-2-one amino acids **I.13**



Entry	**I.39**	Product	Yield[a] (%)
1	**I.39a**	 **I.13a**	55
2	**I.39b**	 **I.13b**	40
3	**I.39c**	 **I.13c**	53

[a.] Isolated yields.

In the case of N^{α}-Fmoc-pyrimidin-2-yl lysine **I.13c**, the NMR spectra showed a dynamic process probably due to interchangeable protons on the guanidine, which makes its characterization difficult. At rt the H5 and H6 protons of the pyrimidinone ring and their corresponding carbons exhibited broad signals. Hence, a variable-temperature ^{1}H NMR study was undertaken. As evidenced in Figure I.18, at rt, proton H6 appeared as a broad doublet compared to the doublet of H5. As the temperature increased, the resolution of this signal also improved and, finally, both protons became similar in intensity at 70 ºC.

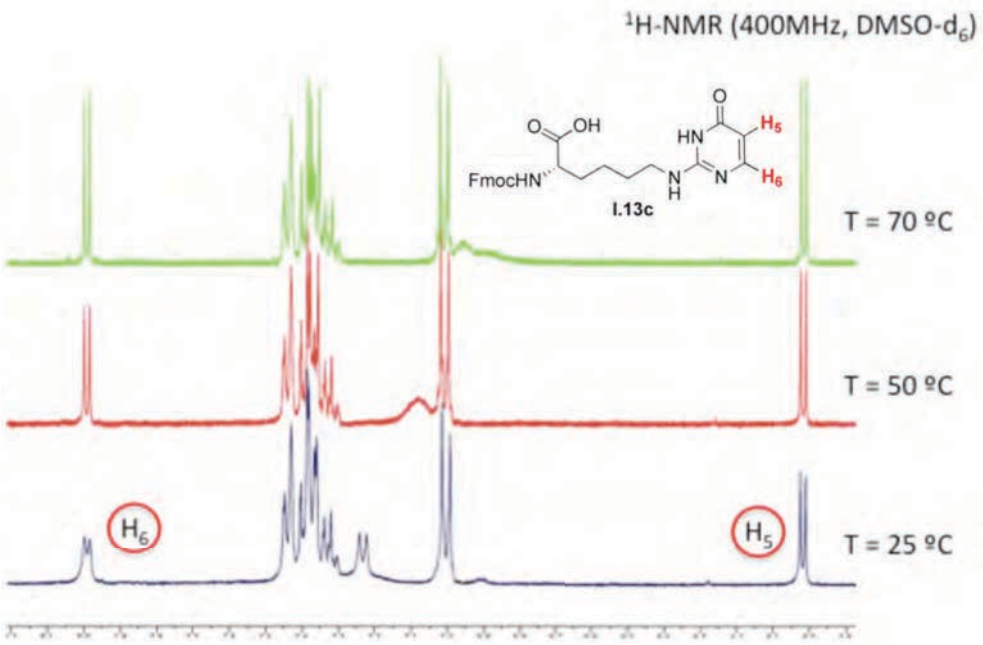


Figure I.18. ^{1}H NMR spectra (region 8.2-5.9 ppm) of compound **I.13c** recorded at 400 MHz in d_6-DMSO from 25 to 70 ºC

I.2.3. SYNTHESIS AND BIOLOGICAL EVALUATION OF BP100 DERIVATIVES

I.2.3.1. Design and synthesis of BP100-derivatives

Among all the pyrimidinyl amino acids synthesized in the previous sections, we chose compounds **I.11c**, **I.12a**, **I.12b** and **I.12c** to study the effect of their incorporation into the antimicrobial peptide **BP100**. The synthesis of **BP100**-derivatives was designed based on the ideal α-helical wheel diagram[86] of H-K^{1}KLFKKILKY^{10}L-NH_2 (**BP100**) where black background stands for hydrophilic amino acids and white background for hydrophobic amino acids (Figure I.19).

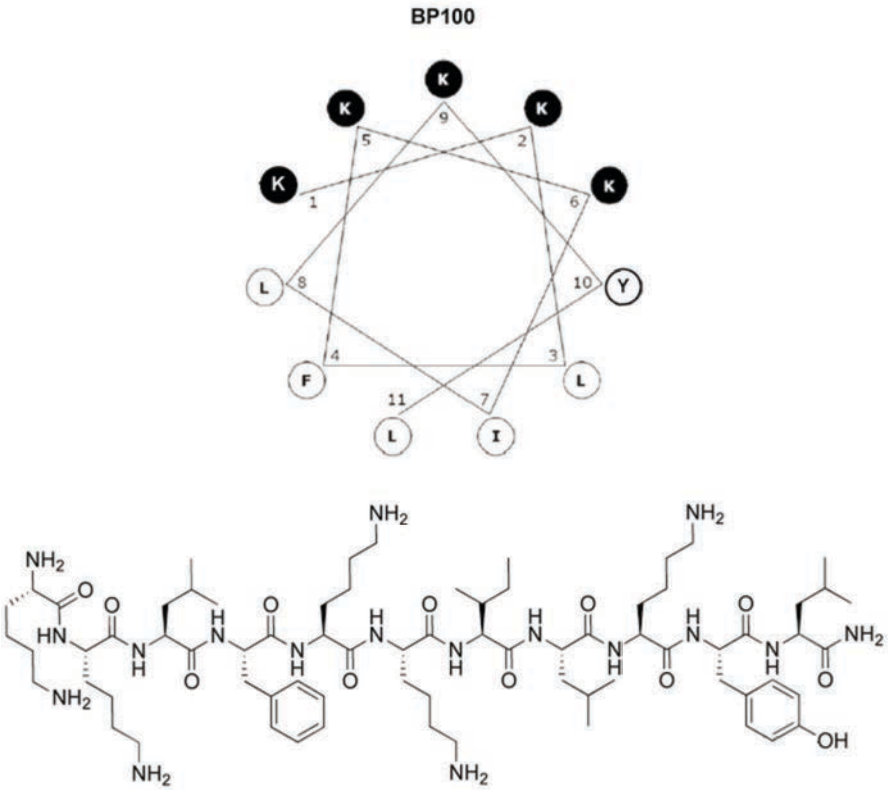


Figure I.19. Edmunson wheel projection of the peptide **BP100**

It has been reported that amino acid substitution in AMPs may considerably alter some fundamental parameters such as the overall hydrophobicity and amphipathicity, which are essential for their antimicrobial activity. Therefore, the analogues derived

[86] Shiffer, M.; Edmunson, A. B. *Biophysical J.* **1967**, *7*, 121-135.

from peptide **BP100** incorporating an unnatural pyrimidinyl amino acid were designed by taking into account the different nature of the selected pyrimidinyl amino acids: compounds **I.12a** [**Tyr(Py)**] and **I.12b** [**His(Py)**] with an aromatic side chain could be considered as hydrophobic amino acids, while **I.11c** [**Lys(Mor)**] and **I.12c** [**Lys(Py)**] had a more hydrophilic character. In this sense, we designed the **BP100** analogues with the aim of studying two effects: i) the change of a proteinogenic amino acid residue by a pyrimidinyl amino acid keeping the same hydrophobic or hydrophilic character, and ii) the replacement of a proteinogenic amino acid residue in the hydrophobic-hydrophilic interface by a pyrimidinyl amino acid with the opposite nature in order to analyse the effect of extending the hydrophilic or hydrophobic character of the peptide.

Consequently, hydrophobic pyrimidinyl amino acids **I.12a** and **I.12b** would replace both aromatic amino acid residues, phenylalanine and tyrosine, located at the positions 4 and 10, respectively, in the **BP100** sequence. And, on the other side, these synthetic amino acids would substitute hydrophilic lysine at position 1 (Figure I.20).

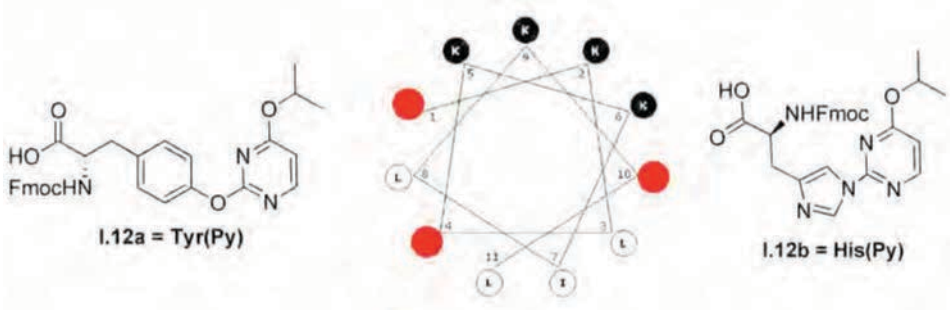


Figure I.20. Pyrimidinyl amino acids **I.12a** and **I.12b** were incorporated into bioactive peptide **BP100** at positions indicated in red circles

Analogously, hydrophilic pyrimidinyl amino acids **I.11c** and **I.12c** would take the place of lysines at the positions 1 and 2 in the **BP100** sequence. In addition, tyrosine at position 10 would also be replaced by both pyrimidinyl lysines **I.11c** and **I.12c** (Figure I.21). In order to not alter the original amphipathicity character of **BP100**, lysine at position 2 would not be substituted by amino acids **I.12a** and **I.12b**, and phenylalanine at position 4 would not be replaced by amino acids **I.11c** and **I.12c**.

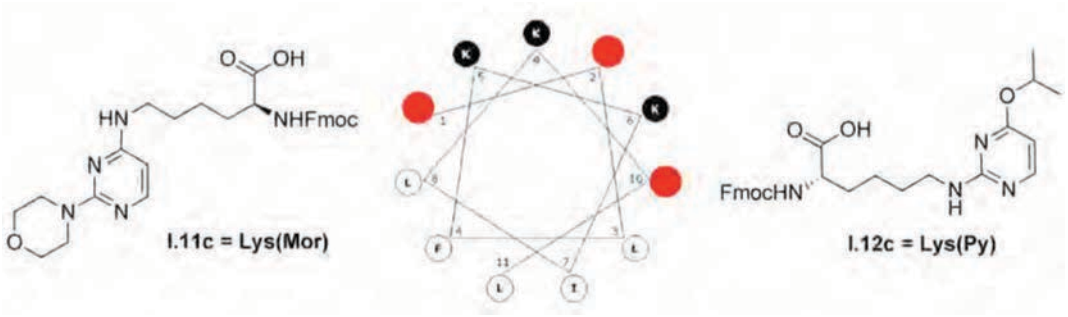


Figure I.21. Pyrimidinyl amino acids **I.11c** and **I.12c** were incorporated into bioactive peptide **BP100** at positions indicated in red circles

All **BP100** analogues were synthesized by solid-phase methods using a Fmoc/*t*-Bu strategy and prepared as *C*-terminal amide. Particularly, 4-methylbenzylhydrylamine (MBHA) resin coupled to Rink-amide linker was used for the synthesis of the peptides. This resin made of polystyrene cross-linked with 1% of divinylbenzene shows excellent swelling properties with aprotic polar solvents, such as DMF. The linker Fmoc-Rink-amide, or *p*-{(*R*,*S*)-α-[1-(9*H*-fluoren-9-yl)-methoxycarbonylamino]-2,4-dimethyoxybenzyl}-phenoxyacetic acid, is an acid-labile handle which affords the peptide amide after cleavage (Figure I.22).

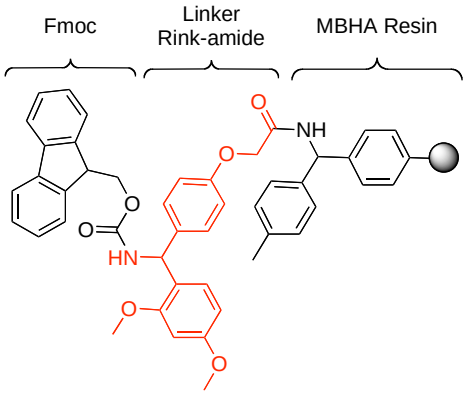
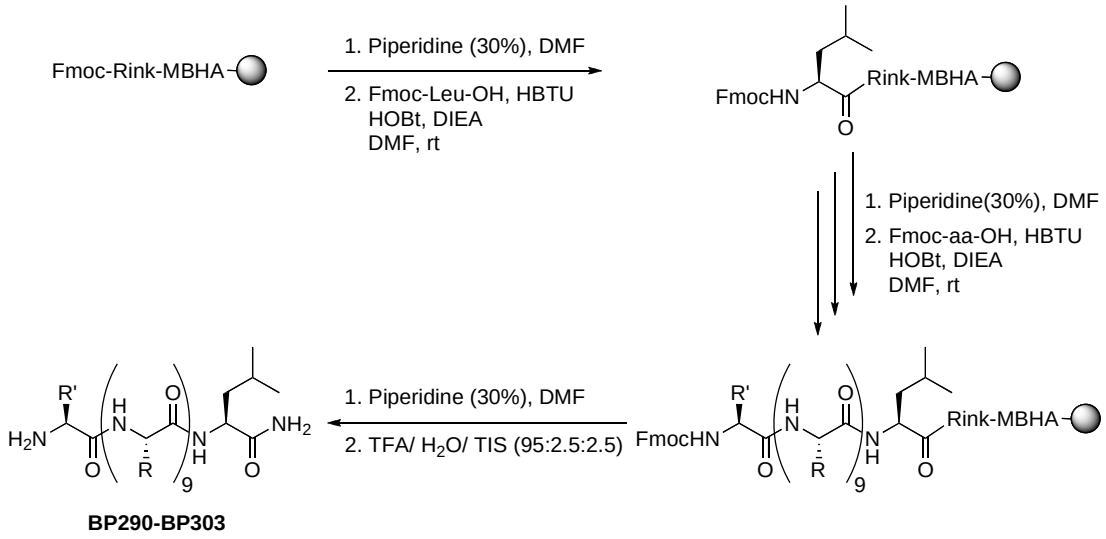


Figure I.22. MBHA resin and linker Fmoc-Rink-amide

According to the synthetic pathway depicted in the following scheme, a collection of fourteen peptides **BP290-BP303** was synthesized successfully (Scheme I.35). The N^{α}-Fmoc amine deprotection was carried out by treatment with piperidine in DMF, and the amide bond was formed using *O*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU) as the coupling reagent, DIEA as the base and in the presence of HOBt as an additive in order to prevent any racemization. Side-chain

deprotection and release of the peptides from the solid support was promoted by treatment with TFA in the presence of small amounts of water and triisopropylsilane (TIS), which both act as carbocation scavengers.



Scheme I.35. Synthesis of pyrimidinyl peptides **BP290-303**

The identities of the resulting peptides **BP290-303** were confirmed by mass spectrometry. For all peptides, the molecular masses determined experimentally were consistent with the masses calculated from the proposed structures. The HPLC analysis of the synthesized sequences showed a purity range from 60% to 99% (Table I.15).

Table I.15. Analyses of pyrimidinyl **BP100** analogues **BP290-303**

Peptide	Sequence	t_R (min)[a]	Purity (%)[b]	MS[c]
BP100	H-KKLFKKILKYL-NH_2	6.23	99	1421.10
BP290	H-**Tyr(Py)**-KLFKKILKYL-NH_2	6.82	83	1592.90
BP291	H-KKLFKKILK-**Tyr(Py)**-L-NH_2	6.69	89	1557.00
BP292	H-KKL-**Tyr(Py)**-KKILKYL-NH_2	6.24	68	1573.00
BP293	H-**Tyr(Py)**-KLFKKILK-**Tyr(Py)**-L-NH_2	6.81	70	1709.00
BP294	H-**His(Py)**-KLFKKILKYL-NH_2	6.85	73	1565.90
BP295	H-KKLFKKILK-**His(Py)**-L-NH_2	6.40	99	1531.00
BP296	H-KKL-**His(Py)**-KKILKYL-NH_2	6.23	99	1547.00
BP297	H-**His(Py)**-KLFKKILK-**His(Py)**-L-NH_2	6.45	99	1657.00
BP298	H-K-**Lys(Py)**-LFKKILKYL-NH_2	6.65	92	1578.88[d]
BP299	H-K-**Lys(Mor)**-LFKKILKYL-NH_2	6.41	70	1584.09
BP300	H-**Lys(Py)**-KLFKKILKYL-NH_2	6.72	71	1556.97
BP301	H-**Lys(Mor)**-KLFKKILKYL-NH_2	6.49	80	1583.99
BP302	H-KKLFKKILK-**Lys(Py)**-L-NH_2	6.46	67	1523.20
BP303	H-KKLFKKILK-**Lys(Mor)**-L-NH_2	6.27	60	1549.20

[a] t_R: retention time in HPLC. [b] Determined by HPLC at 220 nm.
[c] Mass correspondent to $[M+H]^+$. [d] Mass correspondent to $[M+Na]^+$.

I.2.3.2. Evaluation of biological activity

The synthesized peptide library **BP290-303** was evaluated for antimicrobial as well as hemolytic activities in the Laboratory of Plant Pathology of the Institute of Food and Agricultural Technology CIDSAV-CerTA.

I.2.3.2.1. Evaluation of antimicrobial activity

The antimicrobial activity of the synthesized pyrimidinyl conjugated peptides was tested against the bacterial strains *Pseudomonas syringae*, *Xanthomonas vesicatoria* and *Erwinia amylovora* as plant pathogens, and *Escherillia coli*, *Listeria* and *Salmonella* as animal pathogens. The antimicrobial activity was assessed as a minimal inhibitory concentration (MIC), which is the lowest concentration of a peptide that completely inhibits the growth of a microorganism. Indeed, the tests were performed at different peptide concentrations: 2.5, 5, 10 and 15 μM.

In general, the effects of the incorporation of a pyrimidinyl amino acid into the **BP100** sequence depended on the nature of the substituted amino acid. On one hand, the replacement of the hydrophilic lysine at position 1 of the **BP100** sequence by the hydrophobic pyrimidinyl amino acids **I.12a** and **I.12b** caused a slight decrease of the antimicrobial activity against the tested pathogens, with the exception of *Xanthomonas vesicatoria* (sequences **BP290** and **BP294**). This could be attributed to the reduction of the peptide cationic charge. While the substitution of the tyrosine residue at position 10 for these pyrimidinyl amino acids **I.12a** and **I.12b** resulted in an improvement of the general antimicrobial activity (sequences **BP291** and **BP295**). The replacement of the phenylalanine residue at position 4 by these unnatural amino acids produced a significant loss of the antimicrobial activity against all the tested pathogens (sequences **BP292** and **BP296**). Apparently, modifications in the hydrophobic part of the **BP100** sequence were problematic even with the replacement by unnatural amino acids with similar character. The sequences **BP293** and **BP297** with a double-residue substitution with amino acids **I.12a** and **I.12b** led to an important increase of the MIC values compared to control peptide **BP100**. Probably, a double-residue substitution considerably altered its α-helical structure, which is essential for the antimicrobial activity.

Table I.16. Antimicrobial activity of pyrimidinyl **BP100** analogues **BP290-303**

Peptide	MIC[a] (μM)					
	Ps[b]	*Ea*[b]	*Xv*[b]	*Ec*[b]	*Listeria*	*Salmonella*
BP100	5	5	5	5	>15	10
BP290	10	15	5	5	10	10
BP291	**5**	**5**	**2.5**	**2.5**	**10**	**5**
BP292	10	10-15	10	5-10	>15	>15
BP293	10-15	15	5-10	10	>15	15
BP294	10-15	15	5	5-10	10-15	15
BP295	**5**	**5-10**	**2.5-5**	**2.5**	**15**	**10-15**
BP296	15	>15	15	15	>15	>15
BP297	15	>15	10	15	>15	>15
BP298	**2.5-5**	**2.5-5**	**nd[c]**	**15**	**2.5-5**	**>15**
BP299	2.5-5	<2.5	nd	>15	5-10	10-15
BP300	**2.5-5**	**<2.5**	**nd**	**>15**	**2.5-5**	**10-15**
BP301	>15	<2.5	nd	>15	5-10	15
BP302	2.5-5	<2.5	nd	>15	5-10	>15
BP303	2.5-5	<2.5	nd	>15	10-15	>15

[a] MIC: minimum inhibitory concentration. [b] Ps, *Pseudomonas syringae*; Ea, *Erwinia amylovora*; Xv, *Xanthomonas vesicatoria*; Ec, *Escherillia coli*. [c] nd: not determined.

On the other hand, the peptides **BP298-BP303** that incorporated modified lysines **I.11c** and **I.12c** in positions 1, 2 and 10 presented a considerable antimicrobial improvement, especially against *Pseudomonas syringae*, *Erwinia amylovora* and *Listeria*, even when hydrophobic tyrosine residue was replaced. These results indicated that an increase in the cationic charge of **BP100** analogues correlated with an improvement of the antimicrobial activity. In addition, modifications in the hydrophilic part of **BP100** instead of its hydrophobic part by replacement with unnatural amino

acids **I.11c** and **I.12c** with similar characters of lysine improved the antimicrobial activity.

To sum up, the increase of hydrophilic amino acids in the **BP100** sequence significantly enhanced the antimicrobial activity. However, the extension of hydrophobic residues into the peptides resulted in a slight loss of antimicrobial activity.

I.2.3.2.2. Evaluation of hemolytic activity

The ability of the peptides to disrupt the membrane integrity of mammalian cells was interrogated by a hemolytic assay at different peptide concentrations: 50, 150 and 250 μM (Table I.17). Basically, the sequences incorporating at least one modified amino acid resulted in higher hemolytic activity. However, among the synthesized peptides in this library, only sequence **BP296** showed a lower hemolytic activity than the peptide pattern **BP100**.

The candidates that showed an increase in antimicrobial activity, **BP295**, **BP299** and **BP303**, presented acceptable hemolytic values at 50 μM taking into account their MIC in front of some bacteria, which were ten times lower.

It has previously been demonstrated that increasing hydrophobicity correlates with hemolytic activity.[87] Accordingly, the hydrophobicity of the peptides was evaluated based on their retention times on reversed phase HPLC. In general, the introduction of a pyrimidinyl α-amino acid resulted in an increase of hydrophobicity compared to the parent peptide **BP100**. Consequently, they showed a significant increase in the ability to disrupt mammalian cells.

Peptide **BP296** showed the lowest hemolytic activity and the lowest retention time, which means the lowest hydrophobicity. On the other hand, peptides such as **BP293** or **BP294**, which are the most hydrophobic candidates, presented the highest hemolysis values at 150 μM.

[87] Tachi, T.; Epand, R. F.; Epand, R. M.; Matsuzaki, K. *Biochemistry* **2002**, *41*, 10723-10731.

Table I.17. Hemolytic activity of pyrimidinyl **BP100** analogues **BP290-303**

Peptide	Hemolysis (%)[a]			t_R (min)[c]
	50 µM	150 µM	250 µM	
BP100	3 ± 0.1	22 ± 2.8	nd	6.23
BP290	73.0 ± 6.6	93.5 ± 1.5	94.1 ± 1.8	6.82
BP291	79.0 ± 3.3	89.0 ± 2.2	92.4 ± 1.6	6.69
BP292	12.8 ± 2.9	55.5 ± 8.5	84.2 ± 4.7	6.24
BP293	46.6 ± 3.4	101 ± 9.2	95.4 ± 3.7	6.81
BP294	53.4 ± 6.7	96.0 ± 13.7	95.2 ± 3.8	6.85
BP295	30.3 ± 3.9	82.0 ± 3.8	88.4 ± 4.1	6.40
BP296	**1.5 ± 0.3**	**6.9 ± 1.3**	**19.8 ± 0.7**	6.23
BP297	nd[b]	nd	nd	6.45
BP298	88.0 ± 5.2	93.4 ± 1.5	95.6 ± 2.8	6.65
BP299	47.7 ± 9.4	90.0 ± 0.4	97.9 ± 3.4	6.41
BP300	94.5 ± 13.5	87.8 ± 1.1	93.7 ± 3.3	6.72
BP301	84.5 ± 4.7	93.5 ± 2.9	97.5 ± 8.9	6.49
BP302	77.9 ± 5.2	93.4 ± 2.6	94.8 ± 2.3	6.46
BP303	16.7 ± 5.9	69.1 ± 2.0	81.3 ± 1.4	6.27

[a] $\alpha = 0.05$. [b] nd: not determined. [c] t_R: retention time in HPLC.

Overall, these results were consistent with previous studies that showed that increasing hydrophobicity results in a higher hemolytic tendency, indicating that a fine

balance between hydrophobicity and charge as in the case of magainin-like peptides is crucial for redesigning with pyrimidine analogues.[88]

[88] Meng, H.; Kumar, K. *J. Am. Chem. Soc.* **2007**, *129*, 15615-15622.

I.3. CONCLUSIONS

From the first part of this thesis, the following conclusions can be drawn:

- The synthesis of N^{α}-Boc-pyrimidin-4-yl α-amino esters **I.20a-c** has been achieved by phosphonium coupling reaction between 2-morpholinopyrimdin-4(3*H*)-one **I.29** and the nucleophilic side-chain of the protected amino acids – tyrosine **I.18a**, histidine **I.18b** and lysine **I.18c** – using a two-step approach through OBt-adduct **I.31**. It has been demonstrated that the base, the reaction temperature and the reagent equivalents played a key role in the preparation of these compounds **I.20a-c** in good yields (72-82%) and without loss of optical integrity. In general, the best reaction conditions were potassium carbonate as base, an excess of OBt-adduct **I.31** and heating at 40 or 50 ºC.

- N^{α}-Boc-pyrimidin-4-yl tryptophan derivative **I.20d** was obtained in only 22% yield using the above two-step approach. All reaction conditions attempted, including microwave heating or the direct coupling reaction between pyrimidinone **I.29** and tryptophan **I.18d** assisted by PyBrOP, failed to improve this result. This unfruitful outcome has been attributed to the low nucleophilicity of the indole nitrogen atom together with the poor reaction conversion.

- The synthesis of N^{α}-Boc-pyrimidin-2-yl α-amino esters **I.32** and **I.39** has been achieved by nucleophilic aromatic substitution of 4-alkoxy-2-

benzylsulfonylpyrimidine **I.27a-c** and **I.38**, respectively, using the side chain of proteinogenic amino esters **I.18a-e** as nucleophiles. The reaction conditions (base, temperature and reaction time) have been optimized for each α-amino ester **I.18** individually in order to afford the target compounds in good yields (40-88%) and without degradation of the optical integrity. In general, the best reaction conditions were the use of potassium carbonate as a base and a reaction temperature ranging from rt to 50 ºC.

- All the nucleophilic aromatic substitutions carried out with N^{α}-Boc histidine methyl ester **I.18b** were completely regioselective in favour of regioisomer $N(\tau)$ as demonstrated by NOESY experiment and X-ray analysis of compounds **I.20b** and **I.32bc**, respectively.

- N^{α}-Boc-pyrimidinyl α-amino esters **I.20**, **I.32** and **I.39** can be converted into the corresponding N^{α}-Fmoc-pyrimidinyl α-amino acids **I.11** and **I.12**, and N^{α}-Fmoc-pyrimidin-2-one α-amino esters **I.13**, respectively, through standard procedures of deprotection and protection of functional groups. Essentially, methyl ester was saponified using LiOH, N^{α}-Boc protecting group was removed by TFA treatment and the resulting free amino acid were subsequently protected with Fmoc-OSu. The overall yields of this three-step sequence were generally very good: **I.11a-c** (55-93%), **I.12a-d** (57-96%) and **I.13a-c** (40-50%).

- It has been demonstrated that *p*-benzyloxybenzyloxy group at the C4-position of the N^{α}-Boc-pyrimidin-2-yl α-amino esters **I.39** can be removed under mild acid conditions to recover the pyrimidinone ring. Particularly, the hydrolysis of this group can be performed simultaneously with N^{α}-Boc removal by TFA treatment at rt.

- A rational design and synthesis of a small library of peptides analogues of **BP100** has been undertaken, replacing one or two proteinogenic amino acids of the **BP100** sequence with pyrimidinyl amino acids **I.11c**, **I.12a**, **I.12b** and **I.12c**. **BP290-303** peptides have been obtained in purities ranging from 60 to 99% and characterized by mass spectrometry.

- The modified peptides **BP290-303** have been tested for antimicrobial activity against plant pathogens *Pseudomonas syringae*, *Xanthomonas vesicatoria* and *Erwinia amylovora*, as well as animal pathogens *Escherillia coli*, *Salmonella* and *Listeria*. Indeed, they have been evaluated for hemolytic activity.

- In general, the replacement of a proteinogenic amino acid residue at positions 1, 4 and/or 10 of the **BP100** sequence by the hydrophobic pyrimidinyl amino acids **I.12a** and **I.12b** produced a decrease of the antimicrobial activity against plant as well as animal pathogens. Concluding that the reduction of the cationic charge or modifications in the hydrophobic part of **BP100** considerably alter the structural features of **BP100** essential for their antimicrobial activity.

- Peptides **BP298-303** that incorporated hydrophilic pyrimidinyl amino acids **I.11c** and **I.12c** at positions 1, 2 or 10 of the **BP100** sequence enhanced the antimicrobial activity against most of the tested pathogens, especially against *Erwinia amylovora* (MIC < 2.5 μM). Demonstrating that an increase in the cationic charge or modifications in the hydrophilic part of **BP100** improve the antimicrobial activity.

- As a rule, **BP100** analogues exhibited hemolytic values higher than **BP100** in agreement with the observed hydrophobicity according to the retention time on HPLC. Therefore, **BP295**, **BP299** and **BP303** presented a good balance between antimicrobial and hemolytic activities, as the hemolytic values at 50 μM are acceptable (12-47%) taking into account that MIC are one order lower.

I.4. EXPERIMENTAL PART OF CHAPTER I

I.4.1. MATERIALS AND METHODS

I.4.1.1. General reagents

General reagents were purchased from Aldrich, Fluka, Bachem SA, Panreac, Novabiochem or Iris Biotech and were used throughout without purification, except for PyBroP (Novabiochem), which was purified by crystallization with CH_2Cl_2-hexane.[89]

I.4.1.2. Solvents

H_2O was deionized and filtered using a COT Millipore Q-gradient (COT < 3 ppb) system with a resistivity of 18 MΩ cm^{-1}. The most common used organic solvents were synthesis grade, except for DMF and MeCN, which were multisolvent HPLC grade. They were purchased from Aldrich (1,4-dioxane), Sharlau (DMF, MeCN), SDS (CH_2Cl_2) or VWR International (hexane, ether, EtOAc, THF, MeOH). DMF and 1,4-dioxane were dried over activated 4 Å molecular sieves. Dry THF and MeCN were purified and dried by passing through an activated alumina purification system (MBraun SPS-800).

I.4.1.3. General instruments

Melting points (capillary tube) were measured with an Electrothermal digital melting point apparatus IA 91000 and were uncorrected.

IR spectra were recorded on a Mattson-Galaxy Satellite FT-IR using a MKII Golden Gate using a single reflection ATR system (Spacec) as a sampling accessory. Absorption band position is registered in cm^{-1}.

NMR spectra were recorded on a Bruker 200, 300 or 400 MHz NMR Advance spectrometer. ^{1}H NMR spectra were recorded at 400, 300 or 200 MHz and ^{13}C NMR spectra were recorded at 100, 75 or 50 MHz. Spectra recorded in $CDCl_3$ were referenced to residual $CHCl_3$ at 7.26 ppm for ^{1}H or 77.0 ppm for ^{13}C. Spectra recorded in d_6-DMSO were referenced to residual DMSO at 2.49 ppm for ^{1}H or 39.5 ppm for ^{13}C. Coupling constants (*J*) were given in Hertz (Hz). Abbreviations used in the

[89] Alsina, J.; Barany, G.; Albericio, F.; Kates, S. A. *Lett. Peptide Sci*. **1999**, *6*, 243-245.

description of resonance were as follows: singlet: s, doublet: d, triplet: t, quartet: q, sextet: sext, septet: sept, multiplet: m, double of doublet: dd, broad: br, apparent: app.

X-ray diffraction all measurements were conducted on a single X-Ray Bruker SMART Apex CCD diffractometer using graphite-monochromated Mo K_α radiation (λ = 0.71073 Å) from an X-Ray tube, CCD area detector and X-Ray capillary collimator (Monocap).

Optical rotations ($[\alpha]_D^T$ (*c* g/100 mL)) were measured on a Perkin-Elmer polarimeter (Model 343 Plus), using the sodium D line. Specific rotations $[\alpha]_D^T$ are given in 10^{-1} cm^2 g^{-1}, and concentration (*c*) is expressed in g per 100 mL.

Elemental analyses were performed on an EA1110-CHNS apparatus from Thermo-Finnigan/CE Instruments.

The microwave-assisted synthesis was performed using an Ethos SEL labstation from Milestone equipped with a dual magnetron system (1600W). The experiment time, temperature and power were controlled with the EasyControl software package. The temperature was monitored through the ATC-400FO Automatic Fiber Optic Temperature Control System immersed into a standard Milestone reference vessel.

Electrospray ionization (ESI) mass spectra were acquired using a Navigator quadrupole mass spectrometer (Finnigan AQA thermoQuest) equipped with an electrospray ion source. The instrument was operated in the positive ESI (+) ion mode at a probe tip voltage of 3 kV. The other ESI-MS analyses were recorded on an esquire 6000 ESI ion Trap LC/MS (Bruker Daltonics) equipped with an electrospray ion source. The instrument was operated in the positive ESI (+). Samples were introduced into the mass spectrometer ion source directly through a HPLC autosampler with a 5 µL sample. The mobile phase flow (100 $\mu L \cdot min^{-1}$ of 80:20 vol/vol MeCN/H_2O) was delivered by an 1100 Series HPLC pump (Agilent). Nitrogen was employed as both a drying and nebulising gas.

Matrix-assisted laser desorption/ionization-Time-of-flight (MALDI-TOF) mass spectra were recorded on a Bruker ULTRAflex TOF mass spectrometer equipped with a nitrogen laser.

High-resolution mass spectra (HRMS) were determined under conditions of ESI on a Bruker Micro-Q-Tof instrument using a hybrid quadrupole time-of-flight mass spectrometer (University of Zaragoza). Samples were introduced into the mass spectrometer ion source directly through an 1100 Series Agilent HPLC autosampler and were external calibrated using sodium formiate. The instrument was operated in the positive ESI (+) ion mode.

I.4.1.4. Chromatography

Thin layer chromatography (TLC) was performed on precoated TLC plates, silica gel 60 F_{254} (Merck). The spots on the TLC plates were visualized with UV/visible light (254 nm) and/or stained with a solution of potassium permanganate (1.5 g/100 mL H_2O).

Flash chromatography purifications were performed on silica gel 60 (230-400 mesh, Merck).

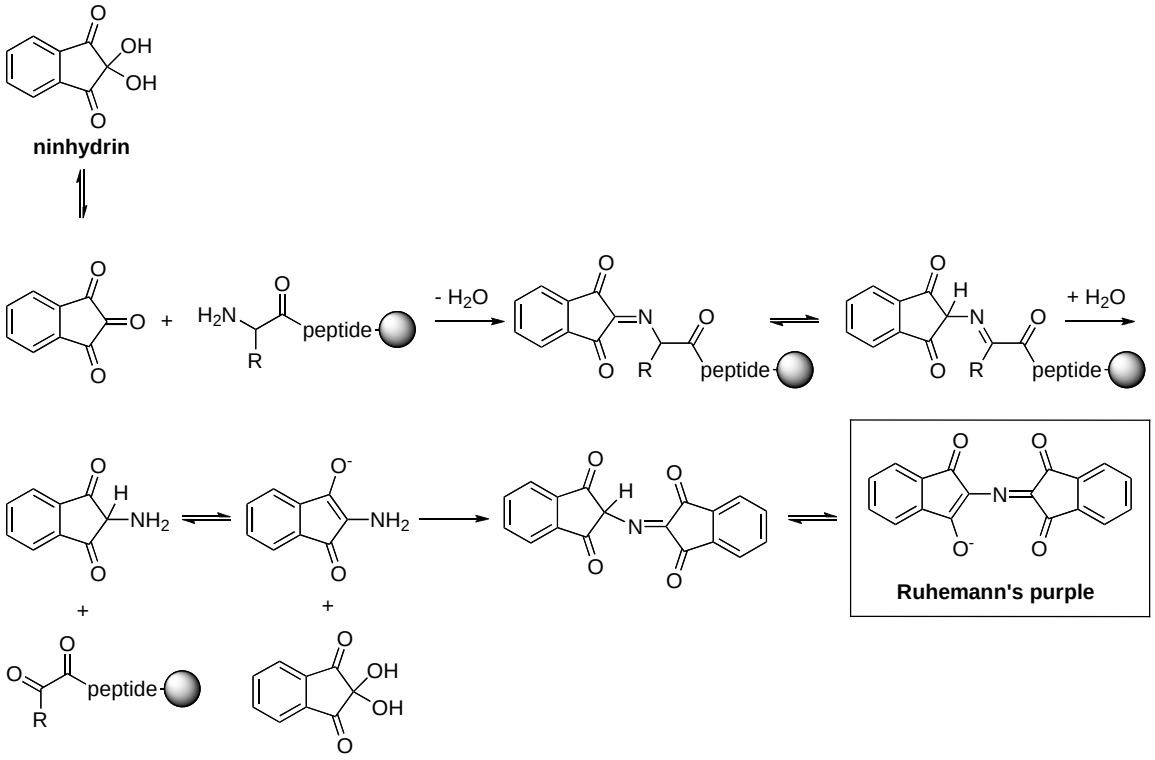
High performance liquid chromatography (HPLC) was performed on a Summit Dionex instruments composed of P680 binary pump, UVD 170U 4-Channel UV-Vis Detector, ASI-100 Autosampler and the Chromaleon 6.60 software from Dionex, on a C_{18} Kromasil reverse-phase column (4.6 x 40 mm; 3.5 μm particle size). The conditions used were a linear gradient of (2-100%) solvent B at a flow rate of 1.0 mL/min over 17 min; solvent A: H_2O/0.1% TFA, solvent B: MeCN/0.1% TFA. Purity estimates are based upon area percent of the peaks detected at 220 nm.

I.4.1.5. Kaiser test for monitoring solid-phase peptide synthesis

The ninhydrin or Kaiser test[90] is a qualitative colour test to detect the presence or absence of free primary amino groups, constituting a useful indication about the completeness of the amino acid coupling in Fmoc solid-phase peptide synthesis. The

[90] Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

test is based on the reaction of ninhydrin with the free primary amines, which gives an intensive dark blue colour. In particular, ninhydrin (triketohydrindene hydrate) is a strong oxidizing agent, which causes the oxidative deamination of the α-amino function as depicted in Scheme I.36. The product of the reaction is the hydrindatin, a reduced derivative of ninhydrin, which reacts with another molecule of ninhydrin to yield a purple chromophore product (Ruhemann's purple).



Scheme I.36. Mechanism of Kaiser test

Ninhydrin test was performed as follows: a few resin beads from the reaction vessel were transferred into a small test tube and 3 drops of each solution of the test kit were added. Next, the suspension was mixed well and heated at 100 ºC for 3 min. The resin beads and the solution turn dark blue when free primary amines are present. The resin beads remain their colour and the solution stays yellow when no free primary amines are present. The ninhydrin test kit contains three solutions:

Solution A: phenol (40 g) in absolute ethanol (10 mL).

Solution B: 2 mL of a solution of KCN (65 g) in H_2O (100 mL) are added to pyridine (100 mL).

Solution C: ninhydrin (2.5 g) in absolute ethanol (50 mL).

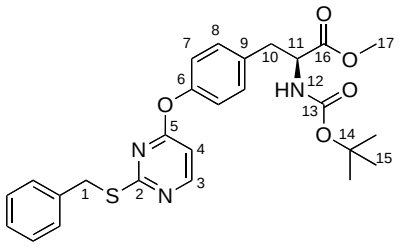
I.4.2. EXPERIMENTAL PROCEDURES

I.4.2.1. Synthesis of pyrimidin-4-yl amino acids

I.4.2.1.1. Synthesis of 2-(benzylsulfanyl)-4-isopropoxypyrimidines I.1 and I.27

The synthesis of these compounds was described in reference 36a.

*I.4.2.1.2. Synthesis of methyl (2**S**)-**N**-Boc-2-amino-3-[4-(2-benzylsulfanylpyrimidin-4-yloxy)phenyl]propanoate (I.19a)*



MW (g/mol): 495.59 **Molecular formula:** $C_{26}H_{29}N_3O_5S$

To a solution of 2-(benzylsulfanyl)pyrimidin-4(3*H*)-one **I.1a** (100 mg, 0.46 mmol) and DBU (0.11 mL, 0.69 mmol) in acetonitrile (1.5 mL) was added BOP (265 mg, 0.6 mmol) and the solution was stirred at rt for 15 min. Then, N^{α}-Boc-(*S*)-tyrosine methyl ester (204 mg, 0.69 mmol) and DBU (0.11 mL, 0.69 mmol) were added as an acetonitrile solution (0.5 mL). The resulting mixture was stirred at rt. Upon completion of the reaction (TLC monitoring, 12 h), the solvent was removed under reduced pressure and the crude material was purified by flash chromatography (*n*-hexane/EtOAc, from 15:1 to 1:1) to afford compound 198 mg (87%) of compound **I.19a** as a colourless oil.

$\boldsymbol{R_f}$ (*n*-hexane/EtOAc, 1:1): 0.68.

$\mathbf{[\alpha]_D^{25}}$ +3.92 (*c* 1.95, $CHCl_3$).

IR (neat): 3357, 3038, 2978, 1736, 1698, 1557, 1443 cm^{-1}.

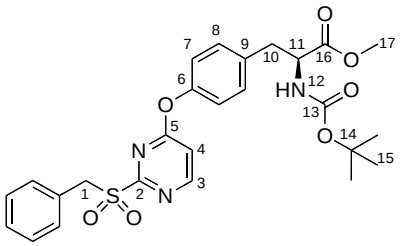
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, J = 5.8 Hz, 1H, H_3), 7.23-7.15 (m, 7H, H_{aryl}), 7.07 (d, J = 8.8 Hz, 2H, H_7), 6.50 (d, J = 5.8 Hz, 1H, H_4), 5.00 (d, J = 7.6 Hz, 1H, H_{12}),

4.58 (m, 1H, H_{11}), 4.16 (s, 2H, H_1), 3.68 (s, 3H, H_{17}), 3.15 (dd, *J* = 13.9, 5.6 Hz, 1H, H_{10}), 3.05 (dd, *J* = 13.9, 5.9 Hz, 1H, H_{10}), 1.42 (s, 9H, H_{15}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0 (s, C_{16}), 171.8 (s, C_5), 168.7 (s, C_2), 158.7 (d, C_3), 154.9 (s, C_{13}), 151.2 (s, C_{aryl}), 137.5 (s, C_{aryl}), 133.7 (s, C_7), 130.4 (d, 2C, C_{aryl}), 128.9 (d, 2C, C_{aryl}), 128.2 (d, 2C, C_{aryl}), 127.0 (d, C_{aryl}), 121.7 (d, 2C, C_{aryl}), 103.3 (d, C_4), 80.0 (s, C_{14}), 54.4 (d, C_{11}), 52.2 (q, C_{17}), 37.8 (t, C_{10}), 35.0 (t, C_1), 28.2 (q, 3C, C_{15}).

HRMS (ESI) *m/z*: calculated for $C_{26}H_{29}KN_3O_5S$ $[M+K]^+$ 534.1460, found 534.1460; calculated for $C_{26}H_{29}NaN_3O_5S$ $[M+Na]^+$ 518.1720, found 518.1724; calculated for $C_{26}H_{30}N_3O_5S$ $[M+H]^+$ 496.1901, found 496.1910.

*I.4.2.1.3. Synthesis of methyl (2S)-*N*-Boc-2-amino-3-[4-(2-benzylsulfonylpyrimidin-4-uloxy)phenyl]propanoate (I.21a)*



MW (g/mol): 527.5894 **Molecular formula:** $C_{26}H_{29}N_3O_7S$

Compound **I.19a** (120 mg, 0.24 mmol) was dissolved in CH_2Cl_2 (2.5 mL). The solution was cooled in an ice bath and *m*-CPBA (118 mg, 5.23 mmol) was added in small portions. The resulting mixture was warmed to rt and stirred during 3h. Upon completion of the reaction (TLC monitoring), the solvent was removed under reduced pressure and the residue was dissolved in EtOAc (10 mL), washed with saturated $NaHCO_3$ solution (2 x 5 mL) and brine (2 x 5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (*n*-hexane/EtOAc, from 10:1 to 1:1) to afford 78 mg (61%) of compound **I.21a** as a colourless solid.

$\boldsymbol{R_f}$ (*n*-hexane/EtOAc, 1:1): 0.40.

$\boldsymbol{[\alpha]_D^{20}}$ +10.28 (*c* 0.7, $CHCl_3$).

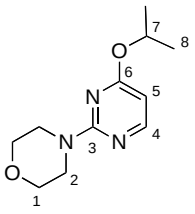
IR (neat): 3380, 3040, 2972, 1741, 1711, 1570, 1543, 1434, 1310 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.67 (d, J = 5.8 Hz, 1H, H_3), 7.30-7.22 (m, 7H, H_{aryl}), 7.11 (d, J = 8.5 Hz, 2H, H_{aryl}), 6.98 (d, J = 5.8 Hz, 1H, H_4), 5.03 (d, J = 7.8 Hz, 1H, H_{12}), 4.59 (br s, 3H, H_1, H_{11}), 3.70 (s, 3H, H_{17}), 3.17 (dd, J = 13.8, 5.7 Hz, 1H, H_{10}), 3.06 (dd, J = 13.8, 6.5 Hz, 1H, H_{10}), 1.42 (s, 9H, H_{15}).

^{13}C NMR (50 MHz, $CDCl_3$) δ 172.0 (s, C_{16}), 169.9 (s, C_5), 164.9 (s, C_2), 159.6 (d, C_3), 155.0 (s, C_{13}), 150.7 (s, C_{aryl}), 134.8 (s, C_{aryl}), 131.3 (d, 2C, C_{aryl}), 130.9 (d, 2C, C_{aryl}), 128.8 (d, C_{aryl}), 128.6 (d, 2C, C_{aryl}), 126.7 (s, C_{aryl}), 121.3 (d, 2C, C_7), 110.8 (d, C_4), 80.1 (s, C_{14}), 57.1 (t, C_1), 54.5 (d, C_{11}), 52.3 (q, C_{17}), 38.0 (t, C_{10}), 28.3 (q, 3C, C_{15}).

HRMS (ESI) *m/z*: calculated for calculated for $C_{26}H_{29}NaN_3O_7S$ $[M+Na]^+$ 550.1618, found 550.1621.

I.4.2.1.4. Synthesis of 4-isopropoxy-2-morpholinopyrimidine (I.28)



MW (g/mol): 223.2715 **Molecular formula:** $C_{11}H_{17}N_3O_2$

Morpholine (3.75 mL, 42.8 mmol) was added to a solution of pyrimidinyl sulfone **I.27a** (5 g, 17.12 mmol) in dry 1,4-dioxane (50 mL) and the resulting solution was stirred at 60 ºC under a nitrogen atmosphere until completion of the reaction (TLC monitoring). Then, the solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane/EtOAc, 1:1) to afford 3.44 g (90%) of pyrimidine **I.28** as a colourless oil.

R_f (*n*-hexane/EtOAc, 4:1): 0.58.

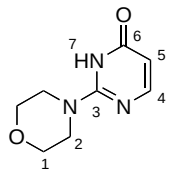
IR (neat): 2977, 2852, 1578, 1559, 1493, 1438, 1335, 1299, 1267, 1250, 1234, 1113, 1089, 1014, 977, 945, 799 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.06 (d, J = 5.8 Hz, 1H, H_4), 5.97 (d, J = 5.8 Hz, 1H, H_5), 5.29 (sept, J = 6.2 Hz, 1H, H_7), 3.77 (s, 8H, H_1, H_2), 1.35 (d, J = 6.2 Hz, 6H, H_8).

^{13}C NMR (50 MHz, $CDCl_3$) δ 168.1 (s, C_6), 160.8 (s, C_3), 156.8 (d, C_4), 96.7 (d, C_5), 67.3 (d, C_7), 65.8 (t, 2C, C_1), 43.3 (t, 2C, C_2), 20.8 (q, 2C, C_8).

MS (ESI) *m/z*: 224.1 $[M+H]^+$.

I.4.2.1.5. Synthesis of 2-morpholinopyrimidin-4(3H)-one (I.29)



MW (g/mol): 181.1918 **Molecular formula:** $C_8H_{11}N_3O_2$

Pyrimidine **I.28** (3.3 g, 14.8 mmol) was dissolved in glacial acetic acid (14.8 mL) and concentrated H_2SO_4 (14.8 mL). The resulting mixture was stirred at 90 ºC for 15 min (TLC monitoring). After this time, upon cooling, the crude mixture was neutralized with a 5M NaOH solution. Next, the aqueous solution was extracted with CH_2Cl_2 (3 x 25 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$ and concentrated under reduced pressure affording 2.14 g (80%) of pyrimidinone **I.29** as a colourless solid.

Mp: 171-174 ºC.

R_f ($MeOH/CH_2Cl_2/NH_3$, 1:10:0.1): 0.30.

IR (neat): 3015, 2920, 1629, 1552, 1485, 1303, 1274, 1257, 1115, 998, 969, 891, 835 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.80 (d, J = 6.2 Hz, 1H, H_4), 5.79 (d, J = 6.2 Hz, 1H, H_5), 3.85-3.75 (s, 8H, H_1, H_2).

^{13}C NMR (50 MHz, $CDCl_3$) δ 165.0 (s, C_6), 155.9 (d, C_4), 136.0 (s, C_3), 101.8 (d, C_5), 65.4 (t, 2C, C_1), 43.9 (t, 2C, C_2).

MS (ESI) *m/z*: 182.1 $[M+H]^+$; 204.1 $[M+Na]^+$.

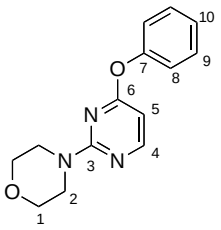
I.4.2.1.6. Synthesis of C4 substituted 2-morpholinopyrimidines I.30

I.4.2.1.6.1. General procedure

A mixture of pyrimidinone **I.29** (1.0 equiv), DBU (1.5 equiv) and BOP (1.5 equiv) in MeCN (0.4M) was stirred at rt for 15 min. Next, the corresponding nucleophile **I.15** (1.5 equiv) and DBU (1.5 equiv) were subsequently added to this mixture and the

resulting solution was stirred at 60 ºC for 24 h. Upon completion of the reaction (TLC monitoring), the solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane/EtOAc, from 9:1 to 1:1) to afford compounds **I.30**.

I.4.2.1.6.2. 2-Morpholino-4-phenoxypyrimidine (I.30a)



MW (g/mol): 257.2878 **Molecular formula:** $C_{14}H_{15}N_3O_2$

Synthesized according to general procedure from pyrimidinone **I.29** (100 mg, 0.55 mmol), BOP (367 mg, 0.83 mmol), DBU (0.25 mL, 1.65 mmol) and phenol **I.15a** (78 mg, 0.83 mmol), 96 mg (68%) of compound **I.30a** was obtained as a colourless solid.

Mp: 99-103 ºC.

***R*$_f$** (CH_2Cl_2/MeOH, 20:1): 0.80.

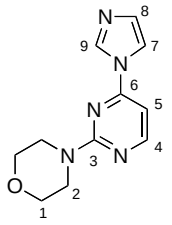
IR (neat): 2956, 2921, 2857, 1583, 1551, 1499, 1462, 1434, 1329, 1240, 1109, 1023, 970, 949 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 5.6 Hz, 1H, H_4), 7.37-7.41 (m, 2H, H_9), 7.23 (m, 1H, H_{10}), 7.14-7.12 (m, 2H, H_8), 6.03 (d, J = 5.6 Hz, 1H, H_5), 3.73-3.63 (m, 8H, H_1, H_2).

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0 (s, C_6), 161.2 (s, C_3), 158.7 (d, C_4), 152.5 (s, C_7), 129.5 (d, 2C, C_9), 125.4 (d, C_{10}), 121.6 (d, 2C, C_8), 96.2 (d, C_5), 66.6 (t, 2C, C_1), 44.2 (t, 2C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{14}H_{16}N_3O_2$ $[M+H]^+$ 258.1237, found 258.1248.

I.4.2.1.6.3. 4-(1*H*-Imidazol-1-yl)-2-morpholinopyrimidine (I.30b)



MW (g/mol): 231.2538 **Molecular formula:** $C_{11}H_{13}N_5O$

Synthesized according to general procedure from pyrimidinone **I.29** (100 mg, 0.55 mmol), BOP (367 mg, 0.83 mmol), DBU (0.25 mL, 1.65 mmol) and imidazole **I.15b** (56 mg, 0.83 mmol), 70 mg (55%) of compound **I.30b** was obtained as a colourless solid.

Mp: 114-116 ºC.

$\boldsymbol{R_f}$ (CH_2Cl_2/MeOH, 10:1): 0.48.

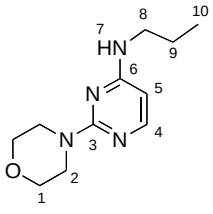
IR (neat): 3012, 2959, 2927, 2852, 1626, 1607, 1582, 1538, 1498, 1427, 1362, 1296, 1259, 1224, 1088, 1052, 1033 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 5.2 Hz, 1H, H_4), 8.35 (s, 1H, H_9), 7.58 (d, J = 0.8 Hz, 1H, H_7), 7.17 (s, 1H, H_8), 6.53 (d, J = 5.2 Hz, 1H, H_5), 3.86-3.83 (m, 4H, H_1), 3.79-3.76 (m, 4H, H_2).

^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7 (s, C_3 or C_6), 160.4 (d, C_4), 155.5 (s, C_3 or C_6), 135.2 (d, C_9), 131.2 (d, C_8), 115.9 (d, C_7), 97.2 (d, C_5), 66.9 (t, 2C, C_1), 44.4 (t, 2C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{11}H_{14}N_5O$ $[M+H]^+$ 232.1193, found 232.1183.

I.4.2.1.6.4. 2-Morpholino-*N*-propylpyrimidin-4-amine (I.30c)



MW (g/mol): 222.2868 **Molecular formula:** $C_{11}H_{18}N_4O$

Synthesized according to general procedure from pyrimidone **I.28** (100 mg, 0.55 mmol), BOP (367 mg, 0.83 mmol), DBU (0.25 mL, 1.65 mmol) and propylamine

I.15c (68 μL, 0.83 mmol), 99 mg (82%) of compound **I.30c** was obtained as a colourless oil.

***R*$_f$** (CH_2Cl_2/MeOH, 9:1): 0.48.

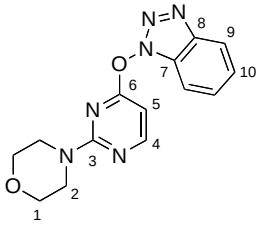
IR (neat): 3347, 2958, 2924, 1579, 1478, 1432, 1339, 1267, 1246, 1229, 1110, 1067 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 5.6 Hz, 1H, H_4), 5.73 (d, J = 5.6 Hz, 1H, H_5), 4.92 (br s, 1H, H_7), 3.79-3.68 (m, 8H, H_1, H_2), 3.26-3.24 (m, 2H, H_8), 1.60 (sext, J = 7.3 Hz, 2H, H_9), 0.97 (t, J = 7.3 Hz, 3H, H_{10}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 162.8 (s, C_3 or C_6), 160.8 (s, C_3 or C_6), 154.5 (d, C_4), 94.4 (d, C_5), 66.8 (t, 2C, C_1), 44.3 (t, 2C, C_2), 43.0 (t, C_8), 22.7 (t, C_9), 11.5 (q, C_{10}).

HRMS (ESI) *m/z*: calculated for $C_{11}H_{19}N_4O$ $[M+H]^+$ 223.1553, found 223.1554.

*I.4.2.1.7. Synthesis of 1-(2-(morpholinopyrimidin-4-yloxy)-1*H*-benzo[*d*]-[1,2,3]-benzotriazole (I.31)*



MW (g/mol): 298.2999 **Molecular formula:** $C_{14}H_{14}N_6O_2$

To a suspension of pyrimidinone **I.29** (1.4 g, 7.73 mmol) and BOP (4.1 g, 9.27 mmol) in MeCN (26 mL), DBU (1.8 mL, 11.59 mmol) was added. The resulting solution was stirred at rt for 5 h (TLC monitoring). After this time, the solvent was removed under reduced pressure and the crude material was purified by flash chromatography (*n*-hexane/EtOAc, from 20:1 to 15:1) to afford 2.3 g (90%) of OBt-adduct **I.31** as a colourless solid.

Mp: 105-106 ºC.

***R*$_f$** (*n*-hexane/EtOAc, 1:1): 0.38.

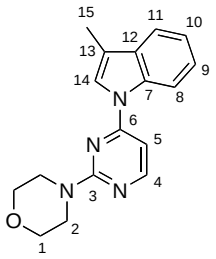
IR (neat): 3073, 2982, 1619, 1539, 1511, 1436, 1337, 1275, 1220, 1078, 1001, 881, 777 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J = 5.5 Hz, 1H, H_4), 8.06 (d, J = 8.4 Hz, 1H, H_{aryl}), 7.50 (m, 1H, H_{aryl}), 7.44-7.38 (m, 2H, H_{aryl}), 6.27 (d, J = 5.5 Hz, 1H, H_5), 3.49-3.33 (m, 8H, H_1, H_2).

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8 (s, C_6), 161.3 (s, C_3), 160.9 (d, C_4), 143.5 (s, C_{aryl}), 129.2 (s, C_{aryl}), 128.7 (d, C_{aryl}), 124.9 (d, C_{aryl}), 120.6 (d, C_{aryl}), 109.1 (d, C_{aryl}), 93.1 (d, C_5), 66.6 (t, 2C, C_1), 44.1 (t, 2C, C_2).

HRMS (ESI) *m/z:* calculated for $C_{14}H_{15}N_6O_2$ $[M+H]^+$ 299.1251, found: 299.1243; calculated for $C_{14}H_{14}N_6NaO_2$ $[M+Na]^+$ 321.1070, found 321.1072.

*I.4.2.1.8. Synthesis of 3-methyl-1-(2-morpholinopyrimidin-4-yl)-1*H*-indole (I.30d)*



MW (g/mol): 294.3510 **Molecular formula:** $C_{17}H_{18}N_4O$

3-Methyl-1*H*-indole **I.15d** (65 mg, 0.5 mmol) and K_2CO_3 (91 mg, 0.66 mmol) were dissolved in acetonitrile (1 mL) and the resulting mixture was stirred at rt for 15 min. Then, OBt-adduct **I.31** (100 mg, 0.33 mmol) was added as an acetonitrile solution (0.5 mL) and the reaction mixture was stirred at 60 ºC. Upon completion of the reaction (TLC monitoring, 15 h), the solvent was removed under reduced pressure and the crude material was purified by flash chromatography (*n*-hexane/EtOAc, 20:1 to 10:1) to afford 46 mg (47%) of compound **I.30d** as a colourless solid.

MP: 122-124 ºC

$\boldsymbol{R_f}$ (*n*-Hexane/EtOAc, 1:1): 0.44.

IR (neat): 3035, 2971, 1599, 1553, 1550, 1478, 1459 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (m, 1H, H_{11}), 8.31 (d, J = 5.6 Hz, 1H, H_4), 7.57 (m, 1H, H_8), 7.47 (d, ^4J = 1.2 Hz, 1H, H_{14}), 7.33 (m, 1H, H_9), 7.25 (m, 1H, H_{10}), 6.63 (d, J = 5.6 Hz, 1H, H_5), 3.92-3.89 (m, 4H, H_1), 3.84-3.82 (m, 4H, H_2), 2.34 (t, ^4J = 1.2 Hz, 3H, H_{15}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 161.8 (s, C_6), 158.6 (d, C_3), 158.5 (d, C_4), 135.3 (s, C_{12}), 131.7 (s, C_7), 123.7 (d, C_{14}), 122.1 (d, C_9), 121.7 (d, C_{11}), 119.2 (d, C_{10}), 116.4 (s, C_{13}), 114.8 (d, C_8), 98.3 (d, C_5), 66.9 (t, 2C, C_1), 44.5 (t, 2C, C_2), 9.7 (q, C_{15}).

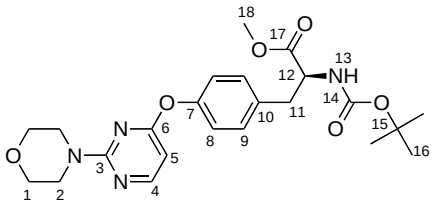
HRMS (ESI) *m/z*: calculated for $C_{17}H_{19}N_4O$ $[M+H]^+$ 295.1553, found 295.1559.

I.4.2.1.9. Synthesis of N^{α}-Boc pyrimidin-4-yl amino esters I.20

I.4.2.1.9.1. General procedure

K_2CO_3 (4 equiv) was added to a solution of the corresponding N^{α}-Boc-amino ester **I.18a-d** (1.1-1.5 equiv) in MeCN (0.4M) and the resulting mixture was stirred at rt for 15 min. After this time, OBt-adduct **I.31** (1.0-2.2 equiv) was added, as a MeCN solution (1M). The resulting mixture was stirred at the temperature specified for each compound. Upon completion of the reaction (TLC monitoring), the solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane/EtOAc, from 15:1 to 1:1) to afford N^{α}-Boc-pyimidin-4-yl amino esters **I.20**.

I.4.2.1.9.2. Methyl (2*S*)-*N*-Boc-2-amino-3-[4-(2-morpholinopyrimidin-4-yloxy)phenyl]propanoate (I.20a)



MW (g/mol): 458.5075 **Molecular formula:** $C_{23}H_{30}N_4O_6$

Synthesized according to general procedure from OBt-adduct **I.31** (299 mg, 1.0 mmol), N^{α}-Boc-(*S*)-tyrosine methyl ester **I.18a** (438 mg, 1.5 mmol) and K_2CO_3 (275 mg, 2 mmol) at 50 ºC for 24 h. Before flash chromatography, the crude material was diluted in EtOAc (20 mL) and washed with a 1M NaOH solution (2 x 10 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure.[91] The resulting

[91] Compound **I.20a** and amino ester **I.18a** have the same R_f in TLC. Without this work-up the purification of compound **I.20a** proved to be problematic.

residue was purified by flash chromatography (*n*-hexane/EtOAc, 7:3), 417.7 mg (85%) of product **I.20a** was obtained as a colourless solid.

Mp: 118-119 ºC.

$\boldsymbol{R_f}$ (*n*-hexane/EtOAc, 1:1): 0.41.

$\boldsymbol{[\alpha]_D^{25}}$ +25.48 (*c* 0.11, MeOH).

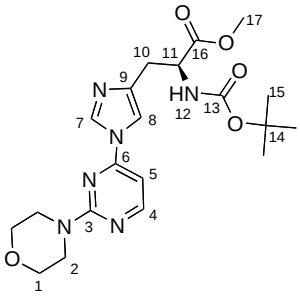
IR (neat): 3340, 1736, 1683, 1589, 1553, 1500, 1439, 1337, 1290, 1231, 1163, 1111, 1007 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 5.6 Hz, 1H, H_4), 7.13 (d, *J* = 8.5 Hz, 2H, H_8), 7.04 (d, *J* = 8.5 Hz, 2H, H_9), 5.99 (d, *J* = 5.6 Hz, 1H, H_5), 5.02 (d, *J* = 8.0 Hz, 1H, H_{13}), 4.57 (m, 1H, H_{12}), 3.70 (s, 3H, H_{18}), 3.69-3.66 (m, 8H, H_1, H_2), 3.12 (dd, *J* = 14.0, 5.5 Hz, 1H, H_{11}), 3.03 (dd, *J* = 14.0, 6.3 Hz, 1H, H_{11}), 1.41 (s, 9H, H_{16}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7 (s, C_{17}), 170.5 (s, C_6), 162.3 (s, C_3), 160.0 (d, C_4), 155.6 (s, C_{14}), 152.3 (s, C_7), 133.8 (s, C_{10}), 131.0 (d, C_9), 130.9 (d, C_9), 122.4 (d, 2C, C_8), 96.7 (d, C_5), 80.6 (s, C_{15}), 67.3 (t, 2C, C_1), 55.0 (d, C_{12}), 52.8 (q, C_{18}), 44.7 (t, 2C, C_2), 38.5 (t, C_{11}), 28.9 (q, 3C, C_{16}).

HRMS (ESI) *m/z*: calculated for $C_{23}H_{31}N_4O_6$ $[M+H]^+$ 459.2238, found 459.2240; calculated for $C_{23}H_{30}N_4NaO_6$ $[M+Na]^+$ 481.2058, found 481.2053.

I.4.2.1.9.3. Methyl (2*S*)-*N*-Boc-2-amino-3-[4-(2-morpholinopyrimidin-4-yloxy)-1*H*-imidazol-4-yl]propanoate (I.20b)



MW (g/mol): 432.4735 **Molecular formula:** $C_{20}H_{28}N_6O_5$

Synthesized according to general procedure from OBt-adduct **I.31** (610 mg, 2.05 mmol), N^{α}-Boc-(*S*)-histidine methyl ester **I.18b** (250 mg, 0.93 mmol) and K_2CO_3

(257.1 mg, 1.86 mmol) at 50 ºC for 19 h, 254.1 mg (72%) of compound **I.20b** was obtained as a colourless solid.

Mp: 60-62 ºC.

$\boldsymbol{R_f}$ (EtOAc/*n*-hexane, 1:1): 0.47.

$\mathbf{[\alpha]_D^{25}}$ +10.65 (*c* 0.17, MeOH).

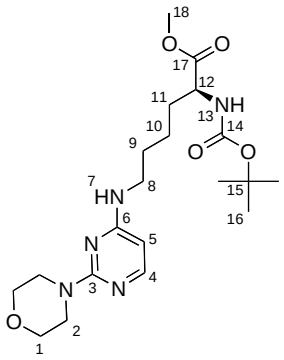
IR (neat): 3692, 1741, 1707, 1595, 1553, 1506, 1479, 1451, 1442, 1390, 1345, 1249, 1230, 1161, 1111, 1055, 951 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, J = 5.4 Hz, 1H, H_4), 8.28 (d, ^4J = 1.1 Hz, H_7), 7.41 (d, ^4J = 1.1 Hz, 1H, H_8), 6.50 (d, J = 5.4 Hz, 1H, H_5), 5.81 (d, J = 5.4 Hz, 1H, H_{12}), 4.64 (m, 1H, H_{11}), 3.89-3.74 (m, 8H, H_1, H_2), 3.70 (s, 3H, H_{17}), 3.17-3.09 (m, 2H, H_{10}), 1.46 (s, 9H, H_{15}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5 (s, C_{16}), 161.5 (s, C_6), 160.3 (d, C_4), 155.6 (s, C_3), 155.1 (s, C_{13}), 139.4 (s, C_9), 134.8 (d, C_7), 113.3 (d, C_8), 96.8 (d, C_5), 79.8 (s, C_{14}), 66.8 (t, 2C, C_1), 53.3 (d, C_{11}), 52.4 (q, C_{17}), 44.3 (t, 2C, C_2), 30.5 (t, C_{10}), 28.3 (q, 3C, C_{15}).

HRMS (ESI) *m/z*: calculated for $C_{20}H_{29}N_6O_5$ $[M+H]^+$ 433.2194, found 433.2208; calculated for $C_{20}H_{28}N_6NaO_5$ $[M+Na]^+$ 455.2056, found 455.2038.

I.4.2.1.9.4. Methyl (2*S*)-*N*-Boc-2-amino-6-[*N*-(2-morpholinopyrimidin-4-yl)amino]hexanoate (I.20c)



MW (g/mol): 423.5065 **Molecular formula:** $C_{20}H_{33}N_5O_5$

Synthesized according to general procedure from OBt-adduct **I.31** (1.75 g, 5.87 mmol), N^{α}-Boc-(*S*)-lysine methyl ester **I.18c** (855.4 mg, 2.67 mmol) and K_2CO_3 (737.9 mg,

5.34 mmol) at 40 ºC for 24 h, 1.78 mg (84%) of compound **I.20c** was obtained as a colourless oil.

$\boldsymbol{R_f}$ (*n*-hexane/EtOAc, 4:1): 0.40.

$\boldsymbol{[\alpha]_D^{25}}$ –6.07 (*c* 0.28, MeOH).

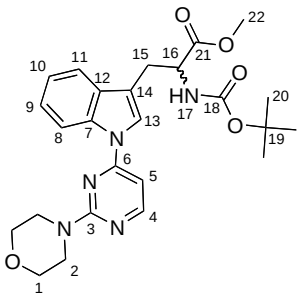
IR (neat): 3364, 2982, 1740, 1708, 1583, 1480, 1459, 1435, 1364, 1342, 1244, 1169, 1113 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 6.4 Hz, 1H, H_4), 5.66 (d, J = 6.4 Hz, 1H, H_5), 5.06 (d, J = 7.6 Hz, 1H, H_{13}), 4.73 (s, 1H, H_7), 4.28 (m, 1H, H_{12}), 3.72-3.71 (m, 11H, H_1, H_2, H_{18}), 3.28-3.19 (m, 2H, H_8), 1.85-1.52 (m, 4H, H_9, H_{11}), 1.46-1.38 (m, 2H, H_{10}), 1.41 (s, 9H, H_{16}).

^{13}C NMR (400 MHz, $CDCl_3$) δ 173.1 (s, C_{17}), 162.7 (s, C_6), 161.6 (s, C_3), 155.4 (s, C_{14}), 155.1 (d, C_4), 94.2 (d, C_5), 79.9 (s, C_{15}), 66.8 (t, 2C, C_1), 53.1 (d, C_{12}), 52.2 (q, C_{18}), 44.2 (t, 2C, C_2), 40.7 (t, C_8), 32.6 (t, C_{11}), 28.9 (t, C_9), 28.2 (q, 3C, C_{16}), 22.7 (d, C_{10}).

HRMS (ESI) *m/z*: calculated for $C_{20}H_{34}N_5O_5$ $[M+H]^+$ 424.2554, found 424.2571.

I.4.2.1.9.5. Methyl *N*-Boc-2-amino-3-[1-(2-(morpholinopyrimidin-4-yl)-1*H*-indol-3-yl]propanoate (I.20d)



MW (g/mol): 481.5441 **Molecular formula:** $C_{25}H_{31}N_5O_5$

Synthesized according to general procedure from OBt-adduct **I.31** (184.8 mg, 0.62 mmol), N^{α}-Boc-(*S*)-tryptophan methyl ester **I.18d** (250 mg, 0.93 mmol) and K_2CO_3 (171.4 mg, 1.24 mmol) at 50 ºC for 24 h, 254.1 mg (22%) of compound **I.20d** was obtained as a colourless solid.

$\boldsymbol{R_f}$ (*n*-hexane/EtOAc, 1:1): 0.35.

IR (neat): 3364, 1740, 1708, 1583, 1480, 1459, 1435, 1364, 1342, 1245, 1160, 1113 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, J = 5.6 Hz, 1H, H_4), 8.30 (d, J = 8.8 Hz, 1H, H_{11}), 7.56-7.54 (m, 2H, H_8, H_{13}), 7.32 (m, 1H, H_9), 7.24 (m, 1H, H_{10}), 6.64 (d, J = 5.6 Hz, 1H, H_5), 5.12 (d, J = 8.0 Hz, 1H, H_{17}), 4.69 (m, 1H, H_{16}), 3.91-3.81 (m, 8H, H_1, H_2), 3.69 (s, 3H, H_{22}), 3.32 (dd, J = 14.8, 5.5 Hz, 1H, H_{15}), 3.24 (dd, J = 14.8, 5.8 Hz, 1H, H_{15}), 1.43 (s, 9H, H_{20}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5 (s, C_{21}), 161.8 (s, C_6), 159.0 (d, C_4), 158.4 (s, C_3), 155.1 (s, C_{18}), 135.2 (d, C_{11}), 130.9 (s, C_{12}), 123.9 (d, C_{13}), 123.4 (s, C_7), 121.9 (d, C_8), 119.1 (d, C_9), 115.0 (s, C_{14}), 114.7 (d, C_{10}), 98.6 (d, C_5), 80.0 (s, C_{19}), 66.8 (t, 2C, C_1), 53.7 (d, C_{16}), 52.3 (q, C_{22}), 44.5 (t, 2C, C_2), 29.6 (t, C_{15}), 28.3 (q, 3C, C_{20}).

HRMS (ESI) *m/z*: calculated for $C_{25}H_{31}N_5O_5$ $[M+H]^+$ 482.2430, found 482.2421; calculated for $C_{25}H_{30}N_5NaO_5$ $[M+Na]^+$ 504.2217, found 504.2214.

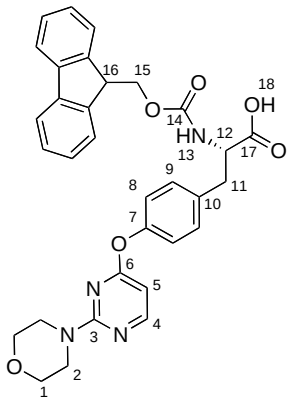
I.4.2.1.10. Synthesis of N$^{\alpha}$*-Fmoc pyrimidin-4-yl amino acids I.11*

I.4.2.1.10.1. General procedure

LiOH monohydrate (2.5 equiv) was added to a solution of appropriate N^{α}-Boc-amino methyl esters **I.20** (1 equiv) in THF/MeOH/H_2O (1:2:2; 0.12M), and the reaction mixture was stirred at rt for 3-4 h. Upon completion of the reaction (TLC monitoring), the organic solvents were removed under reduced pressure. The pH of the resulting aqueous solution was then adjusted to pH 4 with glacial acetic acid, and the solution was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Next, the free N^{α}-Boc-amino acid was dissolved in CH_2Cl_2 (0.7M) and the solution was cooled in an ice bath. TFA (0.7M) was added dropwise and the resulting mixture was stirred at 0 ºC for 1-2 h. Upon completion of the reaction (TLC monitoring), the solvent was removed under reduced pressure. The crude material obtained was dissolved in 1,4-dioxane (0.3M) and the resulting solution was adjusted with 5% $NaHCO_3$ aqueous solution to 7. Fmoc-Osu (1.02-1.05 equiv) was added slowly. During this addition, the pH was readjusted with 5% $NaHCO_3$ aqueous solution to pH 7. The resulting mixture was stirred at rt for 8-12 h upon completion of the reaction (TLC monitoring). The solvent was removed under

reduced pressure and the resulting residue was diluted in water (10 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were then extracted with saturated $NaHCO_3$ solution (3 x 5 mL). The combined basic aqueous layers were then acidified to pH 1-2 with 1% HCl aqueous solution, and extracted with EtOAc (3 x 5 mL). These combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude material obtained was purified by flash chromatography (*n*-hexane/EtOAc/AcOH, from 4:1:0 to 0:20:1) to afford N^{α}-Fmoc pyrimidin-4-yl amino acids **I.11**.

I.4.2.1.10.2. (2*S*)-*N*-Fmoc-2-amino-3-[4-(2-morpholinopyrimidin-4-yloxy) phenyl]propanoic acid (I.11a)



MW (g/mol): 566.6038 **Molecular formula:** $C_{32}H_{30}N_4O_6$

Synthesized according to general procedure from N^{α}-Boc-pyrimidin-4-yl amino ester **I.20a** (417 mg, 0.91 mmol), 283 mg (55%) of compound **I.11a** was obtained as a colourless solid.

Mp: 88-89 ºC.

R_f (EtOAc/MeOH/AcOH, 10:2:0.1): 0.49

$[\alpha]_D^{25}$ +5.02 (*c* 0.22, MeOH).

IR (neat): 2957, 2919, 2850, 1714, 1586, 1549, 1502, 1438, 1338, 1235, 1200, 1105, 1021, 1006, 739 cm^{-1}.

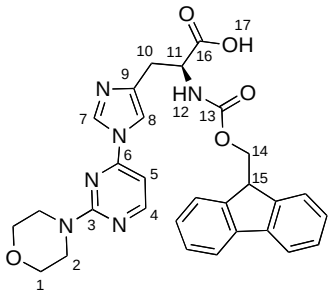
^{1}H NMR (400 MHz, d_6-DMSO) δ 12.86 (br s, 1H, H_{18}), 8.17 (d, J = 5.7 Hz, 1H, H_4), 7.87 (d, J = 7.4 Hz, 2H, H_{Fmoc}), 7.64 (t, J = 6.5 Hz, 2H, H_{Fmoc}), 7.42-7.37 (m, 2H, H_{Fmoc}), 7.32-7.26 (m, 4H, $H_{9,}$ H_{Fmoc}), 7.07 (d, J = 8.2 Hz, 2H, H_8), 6.06 (d, J = 5.7 Hz,

1H, H_5), 4.22-4.17 (m, 4H, H_{12}, H_{15}, H_{16}), 3.89 (dd, *J* = 13.6, 10.2 Hz, 1H, H_{11}), 3.65-3.57 (m, 8H, H_1, H_2), 3.18 (dd, *J* = 13.6, 3.9 Hz, 1H, H_{11}), 2.89 (dd, *J* = 13.6, 10.2 Hz, 1H, H_{11}).

^{13}C NMR (100 MHz, d_6-DMSO) δ 173.5 (s, C_{17}), 170.0 (s, C_6), 161.9 (s, C_{14}), 160.5 (d, C_4), 144.4 (s, C_3), 143.2 (s, C_7), 141.3 (s, C_{10}), 140.1 (s, 2C, C_{Fmoc}), 138.1 (s, 2C, C_{Fmoc}), 129.4 (d, 2C, C_{Fmoc}), 127.7 (d, 4C, C_{Fmoc}), 126.0 (d, 2C, C_{aryl}), 121.8 (d, 2C, C_{Fmoc}), 120.4 (d, 2C, C_{aryl}), 96.5 (d, C_5), 66.3 (t, 2C, C_1), 65.7 (t, C_{15}), 55.5 (d, C_{16}), 47.3 (d, C_{12}), 44.3 (t, 2C, C_2), 25.6 (t, C_{11}).

HRMS (ESI) *m/z*: calculated for $C_{32}H_{31}N_4O_6$ $[M+H]^+$ 567.2254, found 567.2238.

I.4.2.1.10.3. (2*S*)-*N*-Fmoc-2-amino-3-[1-(2-morpholinopyrimidin-4-yl)-1*H*-imidazol-4-yl]propanoic acid (I.11b)



MW (g/mol): 540.5698 **Molecular formula:** $C_{29}H_{28}N_6O_5$

Synthesized according to general procedure from N^{α}-Boc-pyrimidin-4-yl amino ester **I.20b** (220 mg, 0.51 mmol), 242 mg (88%) of compound **I.11b** was obtained as a colourless solid.

Mp: 139-140 ºC.

R_f (EtOAc/MeOH/AcOH, 10:3:0.2): 0.62.

$[\alpha]_D^{25}$ +7.06 (*c* 0.32, MeOH).

IR (neat): 3410, 1682, 1598, 1553, 1518, 1484, 1443, 1346, 1253, 1206, 1179, 1132, 952, 738 cm^{-1}.

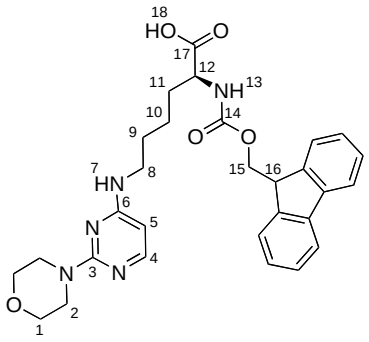
^{1}H NMR (400 MHz, d_6-DMSO) δ 12.79 (br s, 1H, H_{17}), 8.64 (s, 1H, H_7), 8.43 (d, *J* = 5.4 Hz, 1H, H_4), 7.83 (d, *J* = 6.8 Hz, 2H, H_{Fmoc}), 7.77 (s, 1H, H_8), 7.70 (d, *J* = 8.2 Hz, 1H, H_{12}), 7.63 (t, *J* = 6.9 Hz, 2H, H_{Fmoc}), 7.39-7.34 (m, 2H, H_{Fmoc}), 7.27-

7.21 (m, 2H, H_{Fmoc}), 6.97 (d, J = 5.4 Hz, 1H, H_5), 4.35 (m, 1H, H_{11}), 4.23-4.19 (m, 3H, H_{14}, H_{15}), 3.72-3.70 (m, 4H, H_1 or H_2), 3.63-3.61 (m, 4H, H_1 or H_2), 3.03 (dd, J = 14.6, 4.1 Hz, 1H, H_{10}), 2.94 (dd, J = 14.6, 9.7 Hz, 1H, H_{10}).

^{13}C NMR (400 MHz, d_6-DMSO) δ 173.2 (s, C_{16}), 160.9 (s, C_6), 160.6 (d, C_4), 155.9 (s, C_3), 154.7 (s, C_{13}), 143.7 (s, 2C, C_{Fmoc}), 140.6 (s, 2C, C_{Fmoc}), 139.3 (s, C_9), 135.2 (d, C_7), 127.6 (d, 2C, C_{Fmoc}), 127.0 (d, 2C, C_{Fmoc}), 125.2 (d, 2C, C_{Fmoc}), 120.1 (d, 2C, C_{Fmoc}), 113.5 (d, C_8), 97.1 (d, C_5), 65.8 (t, 2C, C_1), 65.6 (t, C_{14}), 53.5 (d, C_{11}), 46.5 (d, C_{15}), 43.8 (t, 2C, C_2), 29.6 (t, C_{10}).

HRMS (ESI) *m/z*: calculated for $C_{29}H_{29}N_6O_5$ $[M+H]^+$ 541.2194, found 541.2183.

I.4.2.1.10.4. (2*S*)-*N*-Fmoc-2-amino-6-[*N*-(2-morpholinopyrimidin-4-yl)amino] hexanoic acid (I.11c)



MW (g/mol): 531.6028 **Molecular formula:** $C_{29}H_{33}N_5O_5$

Synthesized according to general procedure from N^{α}-Boc-pyrimidin-4-yl amino ester **I.20c** (380 mg, 0.89 mmol), 440 mg (93%) of compound **I.11c** was obtained as a colourless solid.

Mp: 96-98 ºC.

***R*$_f$** (EtOAc/MeOH/AcOH, 2:3:0.3): 0.57.

[α]$_D^{25}$ –2.99 (*c* 0.30, MeOH).

IR (neat): 3270, 1688, 1656, 1621, 1583, 1198, 1176, 1125, 1052, 1025, 1004 cm^{-1}.

^{1}H NMR (400 MHz, d_6-DMSO) δ 12.56 (br s, 1H, H_{18}), 7.88 (d, J = 7.4 Hz, 2H, H_{Fmoc}), 7.73-7.71 (m, 3H, H_{Fmoc}), 7.54 (br s, 1H, H_{13}), 7.43-7.39 (m, 2H, H_{Fmoc}), 7.34-7.30 (m, 2H, H_{Fmoc}), 7.05 (br s, 1H, H_7), 5.76 (d, J = 5.8 Hz, 1H, H_5), 4.29-4.20 (m, 3H, H_{15},

H_{16}), 3.91 (dd, *J* = 8.8, 4.8 Hz, 1H, H_{12}), 3.58 (s, 8H, H_1, H_2), 3.22 (br s, 2H, H_8), 1.70 (m, 1H, H_{11}), 1.62 (m, 1H, H_{11}), 1.52-1.50 (m, 2H, H_9), 1.47-1.41 (m, 2H, H_{10}).

^{13}C NMR (400 MHz, d_6-DMSO) δ 174.3 (s, C_{17}), 162.8 (s, C_6), 161.6 (s, C_{14}), 156.4 (s, C_3), 154.2 (d, C_4), 143.8 (s, 2C, C_{Fmoc}), 140.73 (s, C_{Fmoc}), 140.71 (s, C_{Fmoc}), 127.6 (d, 2C, C_{Fmoc}), 127.1 (d, 2C, C_{Fmoc}), 125.29 (d, C_{Fmoc}), 125.26 (d, C_{Fmoc}), 120.1 (d, 2C, C_{Fmoc}), 96.2 (d, C_5), 66.1 (t, 2C, C_1), 65.5 (t, C_{15}), 54.0 (d, C_{12}), 46.7 (d, C_{16}), 43.9 (t, 2C, C_2), 30.7 (t, C_8), 28.4 (t, C_{11}), 25.2 (t, C_9), 23.2 (t, C_{10}).

HRMS (ESI) *m/z*: calculated for $C_{29}H_{34}N_5O_5$ $[M+H]^+$ 532.2554, found 532.2570.

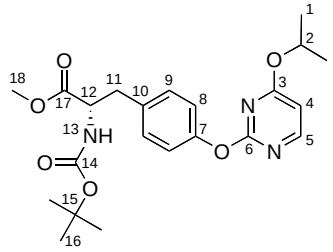
I.4.2.2. Synthesis of pyrimidin-2-yl amino acids

I.4.2.2.1. Synthesis of N^{α}-Boc pyrimidin-2-yl amino esters I.32

I.4.2.2.1.1. General procedure

The appropriate N^{α}-Boc-amino ester **I.18a-e** (1.1-1.2 equiv) was dissolved in dry DMF (0.4M) under a nitrogen atmosphere. The corresponding base (1.2-4.0 equiv) was added and the resulting mixture was stirred at rt for 15 min. After this time, pyrimidinyl sulfone **I.27a-c** (1 equiv) was added to this mixture as a DMF solution (1M). The resulting solution was stirred under nitrogen at the temperature specified for each compound. Upon completion of the reaction (TLC monitoring), the solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane/EtOAc) to afford N^{α}-Boc-pyrimidinyl amino esters **I.32**.

I.4.2.2.1.2. Methyl (2*S*)-*N*-Boc-2-amino-3-{4-[4-(propan-2-yloxy)pyrimidin-2-yloxy]phenyl}propanoate (I.32aa)



MW (g/mol): 431.4822 **Molecular formula:** $C_{22}H_{29}N_3O_6$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27a** (292.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-tyrosine methyl ester **I.18a** (354.4 mg, 1.2 mmol) and K_2CO_3 (136.7 mg, 1.2 mmol) at 50 ºC for 24 h, 340 mg (79%) of compound **I.32aa** was obtained as a colourless solid.

Mp: 114-115 ºC.

$\boldsymbol{R_f}$ (*n*-hexane/EtOAc, 1:1): 0.27.

$\mathbf{[\alpha]_D^{25}}$ –2.80 (*c* 0.14, MeOH).

IR (neat): 3360, 2978, 1739, 1699, 1517, 1508, 1450, 1383, 1366, 1321, 1272, 1252, 1224, 1202, 1171, 1150, 1110, 1041, 1017, 839, 786 cm^{-1}.

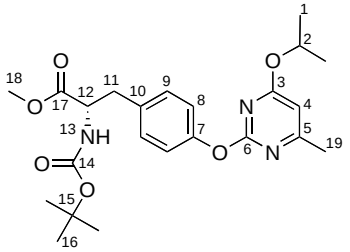
^{1}H NMR (200 MHz, $CDCl_3$) δ 8.18 (d, J = 5.6 Hz, 1H, H_5), 7.29-7.16 (m, 4H, H_8, H_9), 6.38 (d, J = 5.6 Hz, 1H, H_4), 5.27 (sept, J = 6.2 Hz, 1H, H_2), 5.01 (br s, 1H, H_{13}), 4.62 (m, 1H, H_{12}), 3.74 (s, 3H, H_{18}), 3.16-3.12 (m, 2H, H_{11}), 1.45 (s, 9H, H_{16}), 1.34 (d, J = 6.2 Hz, 6H, H_1).

^{13}C NMR (50 MHz, $CDCl_3$) δ 172.9 (s, C_{17}), 171.6 (s, C_3), 165.7 (s, C_6), 159.3 (d, C_4), 155.4 (s, C_7), 152.7 (s, C_{14}), 133.6 (s, C_{10}), 130.9 (d, 2C, C_9), 122.5 (d, 2C, C_8), 104.5 (d, C_5), 80.7 (s, C_{15}), 70.6 (d, C_2), 55.1 (d, C_{12}), 52.9 (q, C_{18}), 38.5 (t, C_{11}), 28.9 (q, 3C, C_{16}), 22.4 (q, 2C, C_1).

MS (ESI) *m/z*: 431.9 $[M+H]^+$.

Elemental analyses: calculated for $C_{22}H_{29}N_3O_6$: C, 61.24; H, 6.77; N, 9.74; found: C, 61.39; H, 6.88; N, 9.90.

I.4.2.2.1.3. Methyl (2*S*)-*N*-Boc-2-amino-3-{4-[4-methyl-6-(propan-2-yloxy)pyrimidin-2-yloxy]phenyl}propanoate (I.32ab)



MW (g/mol): 445.5087 **Molecular formula:** $C_{23}H_{31}N_3O_6$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27b** (306.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-tyrosine methyl ester **I.18a** (354.4 mg, 1.2 mmol) and K_2CO_3 (136.7 mg, 1.2 mmol) at 50 ºC for 24 h, 266 mg (60%) of compound **I.32ab** was obtained as a colourless oil.

$\boldsymbol{R_f}$ (*n*-hexane/EtOAc, 1:1): 0.49.

$\boldsymbol{[\alpha]_D^{25}}$ –28.6 (*c* 0.3, MeOH).

IR (neat): 3355, 2979, 1744, 1712, 1596, 1561, 1533, 1505, 1452, 1354, 1321, 1247, 1211, 1165, 1101, 1075, 1017, 731 cm^{-1}.

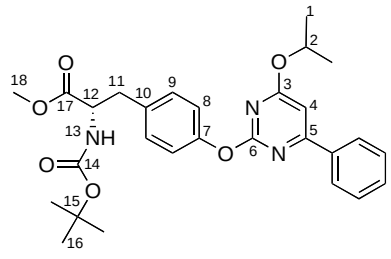
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.13 (s, 4H, H_8, H_9), 6.22 (s, 1H, H_4), 5.10 (sept, J = 6.2 Hz, 1H, H_2), 5.01 (br s, 1H, H_{13}), 4.61 (br s, 1H, H_{12}), 3.71 (s, 3H, H_{18}), 3.12-3.09 (m, 2H, H_{11}), 2.34 (s, 3H, H_{19}), 1.43 (s, 9H, H_{16}), 1.24 (d, J = 6.2 Hz, 6H, H_1).

^{13}C NMR (50 MHz, $CDCl_3$) δ 172.2 (s, C_{17}), 171.1 (s, C_3), 169.6 (s, C_6), 164.4 (s, C_5), 155.0 (s, C_7), 152.2 (s, C_{14}), 132.4 (s, C_{10}), 130.3 (d, 2C, C_9), 121.7 (d, 2C, C_8), 101.8 (d, C_4), 79.9 (s, C_{15}), 70.1 (d, C_2), 54.4 (d, C_{12}), 52.7 (q, C_{18}), 37.7 (t, C_{11}), 28.9 (q, 3C, C_{16}), 23.7 (q, C_{19}), 22.0 (q, 2C, C_1).

MS (ESI) *m/z*: 446 $[M+H]^+$.

Elemental analyses: calculated for $C_{23}H_{31}N_3O_6$: C, 62.01; H, 7.01; N, 9.43; found: C, 62.19; H, 7.19; N, 9.57.

I.4.2.2.1.4. Methyl (2*S*)-*N*-Boc-2-amino-3-{4-[4-phenyl-6-(propan-2-yloxy)pyrimidin-2-yloxy]phenyl}propanoate (I.32ac)



MW (g/mol): 507.5781 **Molecular formula:** $C_{28}H_{33}N_3O_6$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27c** (368.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-tyrosine methyl ester **I.18a** (354.4 mg, 1.2 mmol) and K_2CO_3

(136.7 mg, 1.2 mmol) at rt for 24 h, 202 mg (40%) of compound **I.32ac** was obtained as a colourless oil.

$\boldsymbol{R_f}$ (*n*-hexane/EtOAc, 1:1): 0.52.

$\boldsymbol{[\alpha]_D^{25}}$ –6.23 (*c* 0.26, MeOH).

IR (neat): 3442, 2978, 2928, 1744, 1713, 1580, 1549, 1503, 1453, 1431, 1356, 1324, 1250, 1212, 1164, 1095, 1065, 1018, 939, 770, 731, 693 cm^{-1}.

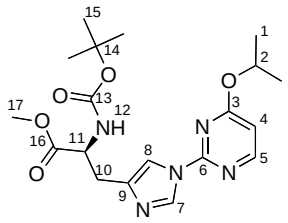
^{1}H NMR (200 MHz, $CDCl_3$) δ 8.01-7.94 (m, 2H, H_{aryl}), 7.50-7.43 (m, 3H, H_{aryl}), 7.26-7.21 (m, 4H, H_8, H_9), 6.81 (s, 1H, H_4), 5.27 (sept, J = 6.2 Hz, 1H, H_2), 5.03 (br s, 1H, H_{13}), 4.65 (m, 1H, H_{12}), 4.61 (d, J = 8.2 Hz, 1H, H_{13}), 3.73 (s, 3H, H_{18}), 3.16 (d, J = 5.4 Hz, 2H, H_{11}), 1.47 (s, 9H, H_{16}), 1.35 (d, J = 6.2 Hz, 6H, H_1).

^{13}C NMR (50 MHz, $CDCl_3$) δ 172.9 (s, C_{17}), 172.7 (s, C_3), 167.1 (s, C_5), 165.7 (s, C_6), 155.8 (s, C_7), 153.0 (s, C_{14}), 137.1 (s, C_{aryl}), 133.2 (s, C_{10}), 131.4 (d, C_{aryl}), 130.6 (d, 2C, C_9), 129.4 (d, 2C, C_{aryl}), 127.7 (d, 2C, C_{aryl}), 122.7 (d, 2C, C_8), 99.3 (d, C_4), 82.1 (s, C_{15}), 70.7 (d, C_2), 55.2 (d, C_{12}), 52.8 (q, C_{18}), 38.6 (t, C_{11}), 29.0 (q, 3C, C_{16}), 22.5 (q, 2C, C_1).

MS (ESI) *m/z*: 508 $[M+H]^+$.

Elemental analyses: calculated for $C_{28}H_{33}N_3O_6$: C, 66.26; H, 6.55; N, 8.28; found: C, 66.17; H, 6.41; N, 8.37.

I.4.2.2.1.5. Methyl (2*S*)-*N*-Boc-2-amino-3-{1-[4-(propan-2-yloxy)pyrimidin-2-yl]-1*H*-imidazol-4-yl}propanoate (I.32ba)



MW (g/mol): 405.4482 **Molecular formula:** $C_{19}H_{27}N_5O_5$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27a** (292.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-histidine methyl ester **I.18b** (296.2 mg, 1.1 mmol) and DBU (1.52 mL, 1.2 mmol) at 50 ºC for 8 h, 324 mg (80%) of compound **I.32ba** was obtained as a colourless solid.

Mp: 100-101 ºC.

$\boldsymbol{R_f}$ (*n*-hexane/EtOAc, 1:1): 0.19.

$\boldsymbol{[\alpha]_D^{25}}$ +8.19 (*c* 0.5, MeOH).

IR (neat): 3264, 2980, 1744, 1700, 1586, 1562, 1484, 1438, 1366, 1349, 1315, 1276, 1249, 1215, 1174, 1092, 1057, 1022, 949, 823 cm^{-1}.

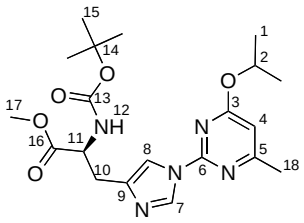
^{1}H NMR (200 MHz, $CDCl_3$) δ 8.46 (d, ^4J = 1.2 Hz, 1H, H_7), 8.32 (d, J = 5.8 Hz, 1H, H_5), 7.61 (d, ^4J = 1.2 Hz, 1H, H_8), 6.54 (d, J = 5.8 Hz, 1H, H_4), 5.86 (d, J = 8.0 Hz, 1H, H_{12}), 5.42 (sept, J = 6.2 Hz, 1H, H_2), 4.62 (m, 1H, H_{11}), 3.74 (s, 3H, H_{17}), 3.14-3.11 (m, 2H, H_{10}), 1.46 (s, 9H, H_{15}), 1.43 (d, J = 6.2 Hz, 6H, H_1).

^{13}C NMR (50 MHz, d_6-DMSO) δ 172.4 (s, C_{16}), 169.5 (s, C_3), 159.1 (d, C_5), 155.3 (s, C_6), 153.4 (s, C_{13}), 139.1 (s, C_9), 135.3 (d, C_7), 113.9 (d, C_8), 106.2 (d, C_4), 78.3 (s, C_{14}), 70.2 (d, C_2), 53.4 (d, C_{11}), 51.8 (q, C_{17}), 29.6 (t, C_{10}), 28.0 (q, 3C, C_{15}), 21.4 (q, 2C, C_1).

MS (ESI) *m/z*: 405.9 $[M+H]^+$.

Elemental analyses: calculated for $C_{19}H_{27}N_5O_5$: C, 56.28; H, 6.71; N, 17.27; found: C, 56.35; H, 6.58; N, 17.42.

I.4.2.2.1.6. Methyl (2*S*)-*N*-Boc-2-amino-3-{1-[4-methyl-6-(propan-2-yloxy)pyrimidin-2-yl]-1*H*-imidazol-4-yl}propanoate (I.32bb)



MW (g/mol): 419.4748 **Molecular formula:** $C_{20}H_{29}N_5O_5$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27b** (306.0 mg, 1.0 mmol), N^{α}-Boc-(*S*)-histidine methyl ester **I.18b** (296.2 mg, 1.1 mmol) and DBU (1.52 mL, 1.2 mmol) at 50 ºC for 8 h, 330 mg (79%) of compound **I.32bb** was obtained as a colourless solid.

Mp: 110-111 ºC.

***R*$_f$** (*n*-hexane/EtOAc, 1:1): 0.29.

[α]$_D^{25}$ +10.23 (*c* 0.17, MeOH).

IR (neat): 3344, 2978, 1711, 1600, 1554, 1481, 1450, 1401, 1366, 1311, 1164, 1102, 1042, 1014, 866 cm^{-1}.

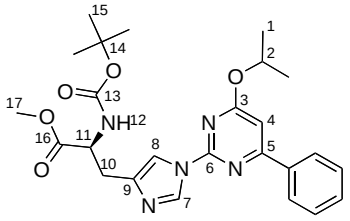
^{1}H NMR (200 MHz, $CDCl_3$) δ 8.48 (s, 1H, H_7), 7.63 (s, 1H, H_8), 6.38 (s, 1H, H_4), 5.86 (br s, 1H, H_{12}), 5.39 (sept, *J* = 6.2 Hz, 1H, H_2), 4.63-4.59 (m, 1H, H_{11}), 3.75 (s, 3H, H_{17}), 3.13-3.11 (m, 2H, H_{10}), 2.43 (s, 3H, H_{18}), 1.46 (s, 9H, H_{15}), 1.41 (d, *J* = 6.2 Hz, 6H, H_1).

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.2 (s, C_{16}), 171.0 (s, C_3), 169.7 (s, C_5), 156.2 (s, C_6), 154.2 (s, C_{13}), 139.1 (s, C_9), 136.6 (d, C_7), 114.8 (d, C_8), 105.3 (d, C_4), 80.3 (s, C_{14}), 70.7 (d, C_2), 54.1 (d, C_{11}), 52.9 (q, C_{17}), 31.0 (t, C_{10}), 29.0 (q, 3C, C_{15}), 24.4 (q, C_{18}), 22.4 (q, 2C, C_1).

MS (ESI) *m/z*: 419.9 $[M+H]^+$.

Elemental analyses: calculated for $C_{20}H_{29}N_5O_5$: C, 57.17; H, 6.97; N, 16.70; found: C, 57.35; H, 7.09; N, 16.82.

I.4.2.2.1.7. Methyl (2*S*)-*N*-Boc-2-amino-3-{1-[4-phenyl-6-(propan-2-yloxy)pyrimidin-2-yl]-1*H*-imidazol-4-yl}propanoate (I.32bc)



MW (g/mol): 481.5441 **Molecular formula:** $C_{25}H_{31}N_5O_5$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27c** (368.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-histidine methyl ester **I.18b** (296.2 mg, 1.1 mmol) and DBU (1.52 mL, 1.2 mmol) at rt for 24 h, 278 mg (58%) of compound **I.32bc** was obtained as a colourless solid.

Mp: 133-134 ºC.

***R*$_f$** (*n*-hexane/EtOAc, 1:1): 0.35.

[α]$_D^{25}$ +23.28 (*c* 0.35, MeOH).

IR (neat): 3383, 1748, 1716, 1596, 1552, 1480, 1454, 1399, 1369, 1309, 1226, 1209, 1160, 1031, 964 cm^{-1}.

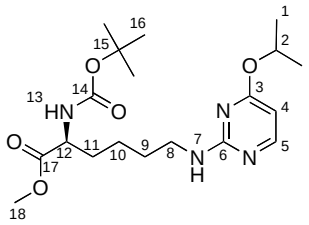
^{1}H NMR (200 MHz, d_6-DMSO) δ 8.77 (s, 1H, H_7), 8.38-8.36 (m, 2H, H_{aryl}), 7.91 (s, 1H, H_8), 7.66-7.45 (m, 3H, H_{aryl}), 7.32 (s, 1H, H_4), 7.01 (br s, 1H, H_{12}), 5.59 (sept, *J* = 6.2 Hz, 1H, H_2), 4.43 (m, 1H, H_{11}), 3.74 (s, 3H, H_{17}), 3.05-3.02 (m, 2H, H_{10}), 1.49 (d, *J* = 6.2 Hz, 6H, H_1), 1.45 (s, 9H, H_{15}).

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.2 (s, C_{16}), 171.7 (s, C_3), 166.6 (s, C_5), 156.0 (s, C_6), 154.5 (s, C_{13}), 139.3 (s, C_{aryl}), 136.7 (d, C_7), 131.7 (d, C_{aryl}), 129.5 (d, 2C, C_{aryl}), 129.4 (s, C_9), 127.6 (d, 2C, C_{aryl}), 114.9 (d, C_8), 101.9 (d, C_4), 80.3 (s, C_{14}), 71.2 (d, C_2), 54.2 (d, C_{11}), 52.9 (q, C_{17}), 31.1 (t, C_{10}), 29.0 (q, 3C, C_{15}), 22.5 (q, 2C, C_1).

MS (ESI) *m/z*: 480.9 $[M+H]^+$.

Elemental analyses: calculated for $C_{25}H_{31}N_5O_5$: C, 62.36; H, 6.49; N, 14.54; found: C, 62.45; H, 6.61; N, 14.72.

I.4.2.2.1.8. Methyl (2*S*)-*N*-Boc-2-amino-6-{*N*-[4-(propan-2-yloxy)pyrimidin-2-yl]amino}hexanoate (I.32ca)



MW (g/mol): 396.4812 **Molecular formula:** $C_{19}H_{32}N_4O_5$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27a** (292.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-lysine methyl ester **I.18c** (384.4 mg, 1.2 mmol) and K_2CO_3 (275.8 mg, 2.0 mmol) at 40 ºC for 24 h, 348.4 mg (88 %) of compound **I.32ca** was obtained as a colourless oil.

R$_f$ (*n*-hexane/EtOAc, 1:1): 0.27.

[α]$_D^{25}$ –5.60 (*c* 0.19, MeOH).

IR (neat): 3397, 3316, 2978, 1708, 1668, 1579, 1525, 1458, 1422, 1366, 1303, 1233, 1164 cm^{-1}.

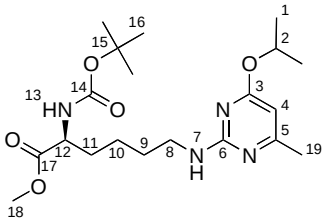
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.98 (d, J = 5.6 Hz, 1H, H_5), 6.36 (br s, 1H, H_7), 5.93 (d, J = 5.6 Hz, 1H, H_4), 5.28 (sept, J = 6.2 Hz, 1H, H_2), 5.14 (br s, 1H, H_{13}), 4.31 (m, 1H, H_{12}), 3.75 (s, 3H, H_{18}), 3.38 (q_{app}, J = 6.4 Hz, 2H, H_8), 1.88-1.53 (m, 6H, H_9, H_{10}, H_{11}), 1.46 (s, 9H, H_{16}), 1.34 (d, J = 6.2 Hz, 6H, H_1).

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.9 (s, C_{17}), 170.1 (s, C_3), 163.1 (s, C_6), 158.6 (d, C_5), 156.0 (s, C_{14}), 98.3 (d, C_4), 80.5 (s, C_{15}), 68.9 (d, C_2), 53.9 (d, C_{12}), 52.8 (q, C_{18}), 41.7 (t, C_8), 33.2 (t, C_{11}), 30.3 (t, C_9), 29.6 (q, 3C, C_{16}), 23.4 (t, C_{10}), 22.5 (q, 2C, C_1).

MS (ESI) *m/z*: 396.9 $[M+H]^+$.

Elemental analyses: calculated for $C_{19}H_{32}N_4O_5$: C, 57.56; H, 8.14; N, 14.13; found: C, 57.64; H, 8.23; N, 14.17.

I.4.2.2.1.9. Methyl (2*S*)-*N*-Boc-2-amino-6-{*N*-[4-methyl-6-(propan-2-yloxy)pyrimidin-2-yl]amino}hexanoate (I.32cb)



MW (g/mol): 410.5078 **Molecular formula:** $C_{20}H_{34}N_4O_5$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27b** (306.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-lysine methyl ester **I.18c** (384.4 mg, 1.2 mmol) and DBU (1.2 mL, 1.2 mmol) at 50 ºC for 24 h, 176.0 mg (43 %) of compound **I.32ca** was obtained as a colourless oil.

***R*$_f$** (*n*-hexane/EtOAc, 2:1): 0.46.

[α]$_D^{25}$ –12.38 (*c* 0.1, MeOH).

IR (neat): 3397, 3317, 2964, 2930, 1717, 1687, 1657, 1477, 1435, 1364, 1335, 1259, 1221, 1164, 1098, 1075, 1051, 1018, 798 cm^{-1}.

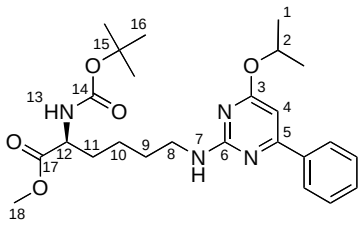
^{1}H NMR (200 MHz, $CDCl_3$) δ 5.83 (s, 1H, H_4), 5.26 (sept, J = 6.2 Hz, 1H, H_2), 4.97 (br s, 2H, H_7, H_{13}), 4.32 (m, 1H, H_{12}), 3.76 (s, 3H, H_{18}), 3.38 (q_{app}, J = 6.4 Hz, 2H, H_8), 2.29 (s, 3H, H_{19}), 2.07-1.63 (m, 6H, H_9, H_{10}, H_{11}), 1.47 (s, 9H, H_{16}), 1.34 (d, J = 6.2 Hz, 6H, H_1).

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.3 (s, C_{17}), 170.0 (s, C_3), 167.8 (s, C_5), 162.3 (s, C_6), 155.3 (s, C_{14}), 96.1 (d, C_4), 79.8 (s, C_{15}), 68.1 (d, C_2), 53.3 (d, C_{12}), 52.2 (q, C_{18}), 41.0 (t, C_8), 32.5 (t, C_{11}), 29.4 (t, C_9), 28.1 (q, 3C, C_{16}), 23.7 (q, C_{19}), 22.7 (t, C_{10}), 21.9 (q, 2C, C_1).

MS (ESI) *m/z*: 411 $[M+H]^+$.

Elemental analyses: calculated for $C_{20}H_{34}N_4O_5$: C, 58.52; H, 8.35; N, 13.65; found: C, 58.65; H, 8.51; N, 13.71.

I.4.2.2.1.10. Methyl (2*S*)-*N*-Boc-2-amino-6-{*N*-[4-phenyl-6-(propan-2-yloxy)pyrimidin-2-yl]amino}hexanoate (I.32cc):



MW (g/mol): 472.5771 **Molecular formula:** $C_{25}H_{36}N_4O_5$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27c** (368.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-lysine methyl ester **I.18c** (384.4 mg, 1.2 mmol) and DBU (1.2 mL, 1.2 mmol) at 50 ºC for 24 h, 198.0 mg (42 %) of compound **I.32ca** was obtained as a colourless oil.

***R**$_f$* (*n*-hexane/EtOAc, 2:1): 0.50.

[α]$_D^{25}$ –18.55 (*c* 0.15, MeOH).

IR (neat): 3367, 3315, 2977, 2930, 1739, 1709, 1578, 1559, 1497, 1459, 1391, 1366, 1319, 1210, 1164, 909, 771, 733 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.00-7.95 (m, 2H, H_{aryl}), 7.47-7.38 (m, 3H, H_{aryl}), 6.41 (s, 1H, H_4), 5.37 (sept, J = 6.2 Hz, 1H, H_2), 5.15 (br s, 2H, H_7, H_{13}), 4.30 (m, 1H, H_{12}),

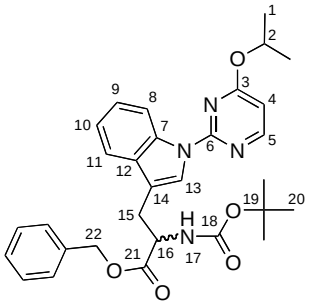
3.76 (s, 3H, H_{18}), 3.49 (q_{app}, J = 6.4 Hz, 2H, H_8), 2.07-1.66 (m, 6H, H_9, H_{10}, H_{11}), 1.47 (s, 9H, H_{16}), 1.39 (d, J = 6.2 Hz, 6H, H_1).

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.4 (s, C_{17}), 170.9 (s, C_3), 165.6 (s, C_5), 162.7 (s, C_6), 155.4 (s, C_{14}), 138.1 (s, C_{aryl}), 131.0 (d, C_{aryl}), 128.9 (d, 2C, C_{aryl}), 126.9 (d, 2C, C_{aryl}), 93.6 (d, C_4), 80.0 (s, C_{15}), 68.4 (d, C_2), 53.4 (d, C_{12}), 52.3 (q, C_{18}), 41.3 (t, C_8), 32.7 (t, C_{11}), 30.5 (t, C_9), 29.0 (q, 3C, C_{16}), 23.1 (t, C_{10}), 22.1 (q, 2C, C_1).

MS (ESI) *m/z*: 473 $[M+H]^+$.

Elemental analyses: calculated for $C_{25}H_{36}N_4O_5$: C, 63.54; H, 7.68; N, 11.86; found: C, 63.67; H, 7.71; N, 11.81.

I.4.2.2.1.11. Benzyl (±) *N*-Boc-2-amino-3-{1-[4-(propan-2-yloxy)pyrimidin-2-yl]-1*H*-indol-3-yl}propanoate (I.32ea)



MW (g/mol): 530.6148 **Molecular formula:** $C_{30}H_{34}N_4O_5$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27a** (292.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-tryptophan benzyl ester **I.18e** (433.8 mg, 1.1 mmol) and DBU (1.52 mL, 1.2 mmol) at 50 ºC for 24 h, 265 mg (50%) of compound **I.32ea** was obtained as a colourless solid.

Mp: 103-104 ºC.

***R*$_f$** (*n*-hexane/EtOAc, 2:1): 0.71.

IR (neat): 3366, 2973, 1735, 1696, 1565, 1506, 1471, 1447, 1434, 1372, 1293, 1167, 1140, 1107, 1018, 743 cm^{-1}.

^{1}H NMR (200 MHz, d_6-DMSO) δ 8.77 (d, J = 8.0 Hz, 1H, H_{11}), 8.60 (d, J = 5.6 Hz, 1H, H_5), 8.24 (s, 1H, H_{13}), 7.70 (d, J = 7.6 Hz, 1H, H_8), 7.55-7.30 (m, 8H, H_9, H_{10}, H_{17}, H_{aryl}), 6.75 (d, J = 5.6 Hz, 1H, H_4), 5.52 (sept, J = 6.0 Hz, 1H, H_2), 5.20 (s, 2H, H_{22}),

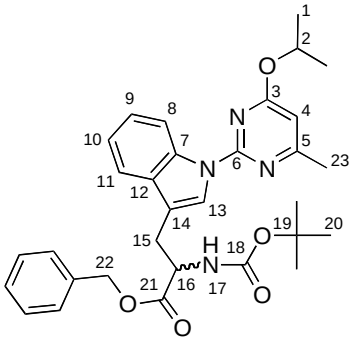
4.47 (m, 1H, H_{16}), 3.26-3.21 (m, 2H, H_{15}), 1.51 (d, J = 6.0 Hz, 6H, H_1), 1.41 (s, 9H, H_{20}).

^{13}C NMR (50 MHz, d_6-DMSO) δ 172.0 (s, C_{21}), 169.0 (s, C_3), 158.8 (d, C_5), 156.3 (s, C_6), 155.4 (s, C_{18}), 135.8 (s, C_7), 134.8 (s, C_{12}), 130.4 (s, C_{aryl}), 128.2 (d, 2C, C_{aryl}), 127.9 (d, C_{aryl}), 127.6 (d, 2C, C_{aryl}), 124.2 (d, C_{13}), 123.7 (d, C_8), 121.8 (d, C_9), 118.6 (d, C_{10}), 115.8 (s, C_{14}), 115.3 (d, C_{11}), 103.6 (d, C_4), 78.3 (s, C_{19}), 69.8 (d, C_2), 65.8 (t, C_{22}), 54.0 (d, C_{16}), 28.0 (q, 3C, C_{20}), 26.4 (t, C_{15}), 21.5 (q, 2C, C_1).

MS (ESI) *m/z*: 531.0 $[M+H]^+$.

Elemental analyses: calculated for $C_{30}H_{34}N_4O_5$: C, 67.91; H, 6.46; N, 10.56; found: C, 67.84; H, 6.58; N, 10.62.

I.4.2.2.1.12. Benzyl (±) *N*-Boc-2-amino-3-{1-[4-methyl-6-(propan-2-yloxy)pyrimidin-2-yl]-1*H*-indol-3-yl}propanoate (I.32eb)



MW (g/mol): 544.6413 **Molecular formula:** $C_{31}H_{36}N_4O_5$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27b** (306.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-tryptophan benzyl ester **I.18e** (433.8 mg, 1.1 mmol) and DBU (1.52 mL, 1.2 mmol) at 50 ºC for 24 h, 217 mg (40%) of compound **I.32eb** was obtained as a colourless oil.

***R**$_f$* (*n*-hexane/EtOAc, 2:1): 0.59.

IR (neat): 3438, 2976, 1709, 1588, 1458, 1390, 1323, 1162, 1102, 1014, 977, 884, 741, 703 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.80 (d, J = 8.4 Hz, 1H, H_{11}), 8.11 (s, 1H, H_{13}), 7.59 (d, J = 7.8 Hz, 1H, H_8), 7.55-7.30 (m, 7H, H_9, H_{10}, H_{aryl}), 6.32 (s, 1H, H_4), 5.51 (sept,

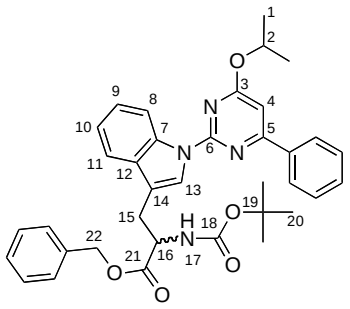
J = 6.0 Hz, 1H, H_2), 5.32 (br s, 1H, H_{17}), 5.22 (s, 2H, H_{22}), 4.77-4.73 (m, 1H, H_{16}), 3.36-3.34 (m, 2H, H_{15}), 2.52 (s, 3H, H_{23}), 1.49 (d, J = 6.0 Hz, 6H, H_1), 1.47 (s, 9H, H_{20}).

^{13}C NMR (50 MHz, $CDCl_3$) δ 172.6 (s, C_{21}), 170.6 (s, C_3), 168.9 (s, C_5), 157.8 (s, C_6), 156.4 (s, C_{18}), 136.2 (s, C_7), 135.8 (s, C_{12}), 131.4 (s, C_{aryl}), 129.1 (d, 2C, C_{aryl}), 128.9 (d, C_{aryl}), 128.8 (d, 2C, C_{aryl}), 125.2 (d, C_{13}), 124.3 (d, C_8), 122.3 (d, C_9), 119.3 (d, C_{10}), 116.8 (s, C_{14}), 114.3 (d, C_{11}), 102.6 (d, C_4), 80.1 (s, C_{19}), 70.1 (d, C_2), 67.7 (t, C_{22}), 55.0 (d, C_{16}), 28.9 (q, 3C, C_{20}), 28.8 (t, C_{15}), 24.6 (q, C_{23}), 22.5 (q, 2C, C_1).

MS (ESI) *m/z*: 545.0 $[M+H]^+$.

Elemental analyses: calculated for $C_{31}H_{36}N_4O_5$: C, 68.36; H, 6.66; N, 10.29; found: C, 68.18; H, 6.90; N, 10.23.

I.4.2.2.1.13. Benzyl (±) *N*-Boc-2-amino-3-{1-[4-phenyl-6-(propan-2-yloxy)pyrimidin-2-yl]-1*H*-indol-3-yl}propanoate (I.32ec)



MW (g/mol): 606.7107 **Molecular formula:** $C_{36}H_{38}N_4O_5$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27c** (368.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-tryptophan benzyl ester **I.18e** (433.8 mg, 1.1 mmol) and DBU (1.52 mL, 1.2 mmol) at 50 ºC for 24 h, 310 mg (57%) of compound **I.18ec** was obtained as a colourless solid.

Mp: 140-141 ºC.

***R*$_f$** (*n*-hexane/EtOAc, 2:1): 0.53.

IR (neat): 3378, 2977, 1731, 1682, 1582, 1552, 1518, 1465, 1389, 1369, 1321, 1286, 1245, 1210, 1158, 1101, 1014, 958, 766, 738, 691 cm^{-1}.

^{1}H NMR (200 MHz, d_6-DMSO) δ 8.85 (d, J = 8.2 Hz, 1H, H_{11}), 8.39-8.23 (m, 3H, H_{13}, $2H_{aryl}$), 7.55-7.30 (m, 13H, H_4, H_9, H_{10}, H_8, H_{17}, $8H_{aryl}$), 5.61 (sept, J = 6.0 Hz, 1H, H_2),

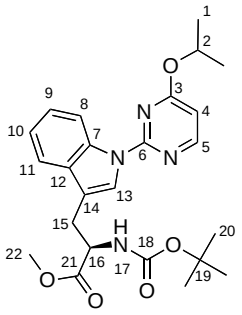
5.21 (s, 2H, H_{22}), 4.53-4.49 (m, 1H, H_{16}), 3.30-3.25 (m, 2H, H_{15}), 1.55 (d, J = 6.0 Hz, 6H, H_1), 1.41 (s, 9H, H_{20}).

^{13}C NMR (50 MHz, d_6-DMSO) δ 172.0 (s, C_{21}), 170.4 (s, C_3), 165.1 (s, C_5), 156.3 (s, C_6), 155.4 (s, C_{18}), 136.1 (s, C_7), 135.8 (s, C_{12}), 134.9 (s, C_{aryl}), 131.1 (d, C_{aryl}), 130.5 (s, C_{aryl}), 128.9 (d, 2C, C_{aryl}), 128.5 (d, 2C, C_{aryl}), 128.2 (d, C_{aryl}), 127.8 (d, 2C, C_{aryl}), 127.6 (d, 2C, C_{aryl}), 124.4 (d, C_{13}), 123.7 (d, C_8), 121.8 (d, C_9), 118.7 (d, C_{10}), 115.7 (d, C_{11}), 115.3 (s, C_{14}), 98.5 (d, C_4), 78.3 (s, C_{19}), 69.9 (d, C_2), 65.9 (t, C_{22}), 54.0 (d, C_{16}), 28.0 (q, 3C, C_{20}), 26.4 (t, C_{15}), 21.6 (q, 2C, C_1).

MS (ESI) *m/z*: 607.0 $[M+H]^+$.

Elemental analyses: calculated for $C_{36}H_{38}N_4O_5$: C, 71.27; H, 6.31; N, 9.23; found: C, 70.99; H, 6.56; N, 9.31.

I.4.2.2.1.14. Methyl (2*S*)-*N*-Boc-2-amino-3-{1-[4-(propan-2-yloxy)pyrimidin-2-yl]-1*H*-indol-3-yl}propanoate (I.32da)



MW (g/mol): 454.5188 **Molecular formula:** $C_{24}H_{30}N_4O_5$

Synthesized according to general procedure from pyrimidinyl sulfone **I.27a** (292.4 mg, 1.0 mmol), N^{α}-Boc-(*S*)-tryptophan methyl ester **I.18d** (350 mg, 1.1 mmol) and K_2CO_3 (276 mg, 2.0 mmol) at 40 ºC for 24 h, 182 mg (40%) of compound **I.32da** was obtained as a colourless solid.

Mp: 88-89 ºC.

R_f (*n*-hexane/EtOAc, 2:1): 0.61.

$[\alpha]_D^{25}$ +9.90 (*c* 0.11, MeOH).

IR (neat): 3645, 3372, 2978, 1735, 1690, 1573, 1518, 1465, 1425, 1361, 1304, 1271, 1247, 1159, 1136, 1107, 1092, 1076, 1006 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (d, J = 7.6 Hz, 1H, H_{11}), 8.36 (d, J = 5.7 Hz, 1H, H_5), 8.05 (s, 1H, H_{13}), 7.55 (d, J = 7.7 Hz, 1H, H_8), 7.25 (m, 1H, H_9), 7.16 (m, 1H, H_{10}), 6.32 (d, J = 5.7 Hz, 1H, H_4), 5.51 (sept, J = 6.2 Hz, 1H, H_2), 5.15 (d, J = 7.8 Hz, 1H, H_{17}), 4.70 (m, 1H, H_{16}), 3.70 (s, 3H, H_{22}), 3.31 (m, 2H, H_{15}), 1.47 (s, 9H, H_{20}), 1.44 (d, J = 6.2 Hz, 6H, H_1).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5 (s, C_{21}), 168.5 (s, C_3), 156.9 (d, C_5), 156.0 (s, C_{18}), 154.2 (s, C_{19}), 134.6 (s, C_7), 130.1 (s, C_{12}), 123.3 (d, C_{11}), 122.8 (d, C_{13}), 120.8 (d, C_8), 117.8 (d, C_9), 115.1 (d, C_{10}), 113.3 (s, C_{14}), 102.7 (d, C_4), 78.8 (s, C_{19}), 68.8 (d, C_2), 52.9 (d, C_{16}), 51.2 (q, C_{22}), 28.0 (q, 2C, C_1), 27.0 (t, C_{15}), 20.8 (q, 3C, C_{20}).

HRMS (ESI) *m/z*: calculated for $C_{24}H_{30}N_4NaO_5$ $[M+Na]^+$ 477.2108, found 477.2121; calculated for $C_{24}H_{31}N_4O_5$ $[M+H]^+$ 455.2289, found 455.2292.

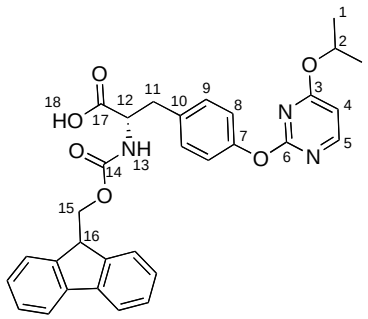
I.4.2.2.2. Synthesis of N^{α}-Fmoc pyrimidin-2-yl amino acids I.12

I.4.2.2.2.1. General procedure

LiOH monohydrate (2.5 equiv) was added to a solution of appropriate N^{α}-Boc-amino methyl esters **I.32** (1 equiv) in THF/MeOH/H_2O (1:2:2; 0.12M), and the reaction mixture was stirred at rt for 3-4 h. Upon completion of the reaction (TLC monitoring), the organic solvents were removed under reduced pressure. The pH of the resulting aqueous solution was then adjusted to 4 with glacial acetic acid, and the solution was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Next, the free N^{α}-Boc-amino acid was dissolved in CH_2Cl_2 (0.7M) and the solution was cooled in an ice bath. TFA (0.7M) was added dropwise and the resulting mixture was stirred at 0 ºC for 1-2 h. Upon completion of the reaction (TLC monitoring), the solvent was removed under reduced pressure. The crude material was dissolved in 1,4-dioxane (0.3M) and the resulting solution was adjusted with 5% $NaHCO_3$ aqueous solution to pH 7. Fmoc-Osu (1.02-1.05 equiv) was then added slowly. During this addition, pH was readjusting with 5% $NaHCO_3$ aqueous solution to 7. The resulting mixture was stirred at rt for 8-12 h upon completion of the reaction (TLC monitoring). After this time, the solvent was removed under reduced pressure and the resulting residue was diluted in water (10 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were then extracted

with saturated $NaHCO_3$ solution (3 x 5 mL). The combined basic aqueous layers were then acidified to pH 1-2 with 1% HCl aqueous solution, and extracted with EtOAc (3 x 5 mL). These combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting crude material was purified by flash chromatography (*n*-hexane/EtOAc/AcOH, from 4:1:0 to 0:20:1) to afford N^{α}-Fmoc pyrimidin-2-yl amino acids **I.12**.

I.4.2.2.2.2. (2*S*)-*N*-Fmoc-2-amino-3-{4-[4-(propan-2-yloxy)pyrimidin-2-yloxy]phenyl}propanoic acid (I.12a)



MW (g/mol): 539.5785 **Molecular formula:** $C_{31}H_{29}N_3O_6$

Synthesized according to general procedure from N^{α}-Boc-pyrimidin-2-yl amino ester **I.32aa** (172 mg, 0.40 mmol), 123 mg (57%) of compound **I.12a** was obtained as a colourless solid.

Mp: 72-74 ºC.

$\boldsymbol{R_f}$ (EtOAc/MeOH/AcOH, 10:3:0.2): 0.71.

$\boldsymbol{[\alpha]_D^{25}}$ +5.92 (*c* 0.32, MeOH).

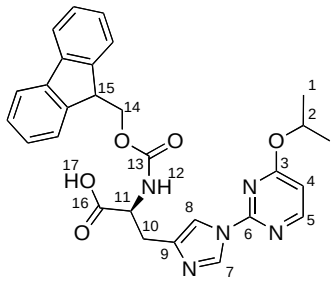
IR (neat): 3745, 3709, 3474, 3414, 3355, 3327, 2977, 1711, 1586, 1563, 1506, 1451, 1384, 1324, 1275, 1245, 1200, 1143, 1105, 1046, 836, 759, 739, 539, 515 cm^{-1}.

^{1}H NMR (200 MHz, d_6-DMSO) δ 8.20 (d, J = 5.6 Hz, 1H, H_5), 7.80-7.76 (m, 2H, H_{Fmoc}), 7.62-7.59 (m, 2H, H_{Fmoc}), 7.41-7.28 (m, 4H, H_{Fmoc}), 7.22-7.06 (m, 4H, H_8, H_9), 6.41 (d, J = 5.6 Hz, 1H, H_4), 5.54 (br s, 1H, H_{13}), 5.20 (sept, J = 6.0 Hz, 1H, H_2), 4.75 (m, 1H, H_{12}), 4.55-4.42 (m, 2H, H_{15}), 4.25 (m, 1H, H_{16}), 3.22-3.20 (m, 2H, H_{11}), 1.33 (d, J = 6.0 Hz, 6H, H_1).

^{13}C NMR (50 MHz, $CDCl_3$) δ 174.6 (s, C_{17}), 171.6 (s, C_3), 165.0 (s, C_6), 158.0 (d, C_5), 156.3 (s, C_{14}), 152.2 (s, C_7), 144.3 (s, C_{Fmoc}), 144.1 (s, C_{Fmoc}), 141.7 (s, 2C, C_{Fmoc}), 133.5 (s, C_{10}), 131.0 (d, 2C, C_{Fmoc}), 128.2 (d, 2C, C_9), 127.5 (d, 2C, C_{Fmoc}), 125.5 (d, 2C, C_{Fmoc}), 122.2 (d, 2C, C_8), 120.4 (d, 2C, C_{Fmoc}), 104.3 (d, C_4), 70.9 (d, C_2), 67.4 (t, C_{15}), 55.1 (d, C_{12}), 47.6 (d, C_{16}), 37.7 (t, C_{11}), 22.0 (q, 2C, C_1).

HRMS (ESI) *m/z*: calculated for $C_{31}H_{30}N_3O_6$ $[M+H]^+$ 540.2129, found 540.2131.

I.4.2.2.2.3. (2*S*)-*N*-Fmoc-2-amino-3-{1-[4-(propan-2-yloxy)pyrimidin-2-yl]-1*H*-imidazol-4-yl}propanoic acid (I.12b)



MW (g/mol): 513.5445 **Molecular formula:** $C_{28}H_{27}N_5O_5$

Synthesized according to general procedure from N^{α}-Boc-pyrimidin-2-yl amino ester **I.32ba** (162 mg, 0.40 mmol), 197 mg (96%) of compound **I.12b** was obtained as a colourless solid.

Mp: 80-83 ºC.

***R*$_f$** (EtOAc/MeOH/AcOH, 5:3:0.2): 0.50.

[α]$_D^{25}$ +5.50 (*c* 0.23, MeOH).

IR (neat): 3363, 1710, 1592, 1562, 1445, 1381, 1311, 1214, 1097, 1026, 947, 738 cm^{-1}.

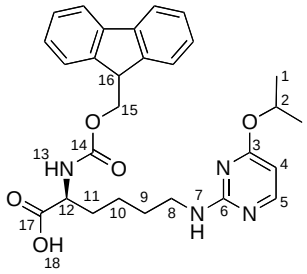
^{1}H NMR (300 MHz, d_6-DMSO) δ 8.48 (s, 1H, H_7), 8.44 (d, J = 5.8 Hz, 1H, H_5), 7.85 (d, J = 7.5 Hz, 2H, H_{Fmoc}), 7.68 (s, 1H, H_8), 7.64-7.60 (m, 3H, H_{12}, H_{Fmoc}), 7.38-7.32 (m, 2H, H_{Fmoc}), 7.26-7.18 (m, 2H, H_{Fmoc}), 6.76 (d, J = 5.8 Hz, 1H, H_4), 5.34 (sept, J = 6.2 Hz, 1H, H_2), 4.29-4.17 (m, 4H, H_{11}, H_{14}, H_{15}), 3.03 (dd, J = 14.5, 3.7 Hz, 1H, H_{10}), 2.90 (dd, J = 14.5, 9.4 Hz, 1H, H_{10}), 1.31 (d, J = 6.2 Hz, 3H, H_1), 1.28 (d, J = 6.2 Hz, 3H, H_1).

^{13}C NMR (100 MHz, d_6-DMSO) δ 173.1 (s, C_{16}), 169.2 (s, C_3), 158.8 (d, C_5), 155.6 (s, C_6), 153.1 (s, C_{13}), 143.4 (s, 2C, C_{Fmoc}), 140.4 (s, 2C, C_{Fmoc}), 139.3 (s, C_9), 135.0 (d,

C_7), 127.3 (d, 2C, C_{Fmoc}), 126.7 (d, 2C, C_{Fmoc}), 125.0 (d, 2C, C_{Fmoc}), 119.8 (d, 2C, C_{Fmoc}), 113.6 (d, C_8), 105.9 (d, C_4), 69.9 (d, C_2), 65.4 (t, C_{14}), 53.4 (d, C_{11}), 46.3 (d, C_{15}), 29.6 (t, C_{10}), 21.2 (q, 2C, C_1).

HRMS (ESI) *m/z*: calculated for $C_{28}H_{28}N_5O_5$ $[M+H]^+$ 514.2085, found 514.2071.

I.4.2.2.2.4. (2*S*)-*N*-Fmoc-2-amino-6-{*N*-[4-(propan-2-yloxy)pyrimidin-2-yl]amino}hexanoic acid (I.12c)



MW (g/mol): 504.5775 **Molecular formula:** $C_{28}H_{32}N_4O_5$

Synthesized according to general procedure from N^{α}-Boc-pyrimidin-2-yl amino ester **I.32ca** (158 mg, 0.40 mmol), 155 mg (77%) of compound **12c** was obtained as a colourless solid.

Mp: 54-57 ºC.

$\boldsymbol{R_f}$ (EtOAc/MeOH/AcOH, 10:3:0.2): 0.57.

$\boldsymbol{[\alpha]_D^{25}}$ –5.91 (*c* 0.10, MeOH).

IR (neat): 3385, 3343, 3316, 1674, 1640, 1474, 1461, 1400, 1323, 1201, 1180, 1130, 841, 799 cm^{-1}.

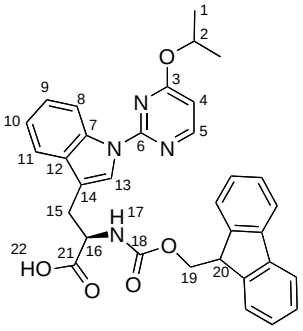
^{1}H NMR (400 MHz, d_6-DMSO) δ 12.63 (br s, 1H, H_{18}), 7.99 (d, J = 5.6 Hz, 1H, H_5), 7.93 (d, J = 7.6 Hz, 2H, H_{Fmoc}), 7.77 (d, J = 7.2 Hz, 2H, H_{Fmoc}), 7.67 (d, J = 8.0 Hz, 1H, H_7), 7.46 (t, J = 7.2 Hz, 2H, H_{Fmoc}), 7.37 (t, J = 7.6 Hz, 2H, H_{Fmoc}), 5.94 (d, J = 5.6 Hz, 1H, H_4), 5.27 (sept, J = 6.0 Hz, 1H, H_2), 4.33-4.25 (m, 3H, H_{15}, H_{16}), 3.98 (m, 1H, H_{12}), 3.29-3.25 (m, 2H, H_8), 1.75-1.73 (m, 2H, H_{11}), 1.70-1.69 (m, 2H, H_9 or H_{10}), 1.68-1.66 (m, 2H, H_9 or H_{10}), 1.30 (d, J = 6.0 Hz, 6H, H_1).

^{13}C NMR (100 MHz, d_6-DMSO) δ 172.3 (s, C_{17}), 162.5 (s, C_3), 157.7 (d, C_5), 157.2 (s, C_{14}), 147.4 (s, C_6), 145.3 (s, C_{Fmoc}), 145.1 (s, C_{Fmoc}), 142.1 (s, 2C, C_{Fmoc}), 128.4 (d, 2C, C_{Fmoc}), 128.2 (d, 2C, C_{Fmoc}), 126.3 (d, 2C, C_{Fmoc}), 121.0 (d, 2C, C_{Fmoc}), 99.4 (d, C_4),

73.3 (d, C_2), 67.5 (t, C_{15}), 55.8 (d, C_{12}), 48.1 (d, C_{16}), 41.9 (t, C_8), 32.0 (t, C_{11}), 29.1 (t, C_9 or C_{10}), 23.6 (t, C_9 or C_{10}), 21.8 (q, 2C, C_1).

HRMS (ESI) *m/z*: calculated for $C_{28}H_{32}N_4NaO_5$ $[M+Na]^+$ 527.2265, found 527.2244; calculated for $C_{28}H_{33}N_4O_5$ $[M+H]^+$ 505.2445, found 505.2435.

I.4.2.2.2.5. (2*S*)-*N*-Fmoc-2-amino-3-{1-[4-(propan-2-yloxy)pyrimidin-2-yl]-1*H*-indol-3-yl}propanoic acid (I.12d)



MW (g/mol): 562.6151 **Molecular formula:** $C_{33}H_{30}N_4O_5$

Synthesized according to general procedure from N^{α}-Boc-pyrimidin-2-yl amino ester **I.32da** (182 mg, 0.40 mmol), 173 mg (77%) of compound **I.12d** was obtained as a colourless solid.

Mp: 174-176 ºC.

R_f (EtOAc/MeOH/AcOH, 10:3:0.2): 0.77.

$[\alpha]_D^{25}$ +4.38 (*c* 0.14, MeOH).

IR (neat): 3342, 1731, 1686, 1559, 1537, 1469, 1430, 1305, 1235, 1223, 1087, 1032, 756 cm^{-1}.

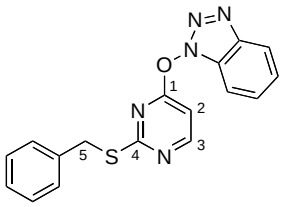
^{1}H NMR (400 MHz, d_6-DMSO) δ 12.84 (br s, 1H, H_{22}), 8.66 (d, J = 8.4 Hz, 1H, H_{11}), 8.46 (d, J = 5.6 Hz, 1H, H_5), 8.18 (s, 1H, H_{13}), 7.87-7.82 (m, 3H, H_{Fmoc}), 7.67 (d, J = 7.6 Hz, 1H, H_{Fmoc}), 7.61 (d, J = 7.6 Hz, 1H, H_{17}), 7.56 (d, J = 7.6 Hz, 1H, H_8), 7.42-7.35 (m, 3H, H_{Fmoc}, H_9), 7.30-7.23 (m, 2H, H_{Fmoc}), 7.14 (t, J = 7.6 Hz, 1H, H_{10}), 6.61 (d, J = 5.6 Hz, 1H, H_4), 5.40 (sept, J = 6.0 Hz, 1H, H_2), 4.40 (m, 1H, H_{16}), 4.20-4.12 (m, 3H, H_{19}, H_{20}), 3.26 (dd, J = 14.8, 4.4 Hz, 1H, H_{15}), 3.11 (dd, J = 14.8, 10.4 Hz, 1H, H_{15}), 1.42 (d, J = 6.0 Hz, 3H, H_1), 1.40 (d, J = 6.0 Hz, 3H, H_1).

^{13}C NMR (75 MHz, d_6-DMSO) δ 173.4 (s, C_{21}), 169.0 (s, C_3), 158.7 (d, C_5), 156.3 (s, C_6), 155.9 (s, C_{18}), 143.7 (s, 2C, C_{Fmoc}), 143.6 (s, 2C, C_{Fmoc}), 140.6 (s, C_7), 134.8 (s, C_{12}), 130.5 (s, C_{14}), 127.5 (d, C_{11}), 127.4 (d, C_{13}), 126.9 (d, C_{Fmoc}), 126.8 (d, C_{Fmoc}), 125.2 (d, C_{Fmoc}), 125.1 (d, C_{Fmoc}), 124.1 (d, C_{Fmoc}), 123.6 (d, C_{Fmoc}), 121.8 (d, C_8), 119.9 (d, 2C, C_{Fmoc}), 118.8 (d, C_9), 115.7 (d, C_{10}), 103.5 (d, C_4), 69.7 (d, C_2), 65.7 (t, C_{19}), 53.9 (d, C_{16}), 46.5 (d, C_{20}), 26.5 (t, C_{15}), 21.5 (q, C_1), 21.5 (q, C_1).

HRMS (ESI) *m/z*: calculated for $C_{33}H_{30}N_4NaO_5$ $[M+Na]^+$ 585.2108, found 585.2107; calculated for $C_{33}H_{31}N_4O_5$ $[M+H]^+$ 563.2294, found 563.2293.

I.4.2.3. Synthesis of pyrimidin-2-one amino acids

*I.4.2.3.1. Synthesis of 1-{[2-(benzylsulfanyl)pyrimidin-4-yl]oxy}-1*H*-1,2,3-benzotriazole] (I.17)*



MW (g/mol): 335.3830 **Molecular formula:** $C_{17}H_{13}N_5OS$

DBU (2 mL, 13.75 mmol) and BOP (6 g, 13.75 mmol) were subsequently added to a suspension of pyrimidinone **I.1a** (2 g, 9.17 mmol) in MECN (0.3M), and the resulting solution was stirred at rt for 3 h. After this time, the solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane/EtOAc, 7:3) to afford OBt-adduct **I.17** as a white solid (2.66 g, 87%).

Mp: 131-132 ºC.

R_f (*n*-hexane/EtOAc, 1:1): 0.59.

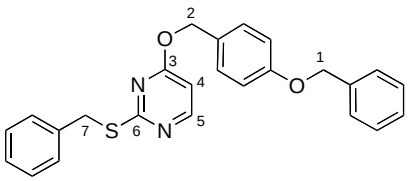
IR (neat): 3062, 3028, 1581, 1542, 1424, 1336, 1258, 1236, 1213, 1081, 929, 910, 742, 712 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, J = 5.6 Hz, 1H, H_3), 8.09 (dd, J = 8.1, 1.2 Hz, 1H, H_{OBt}), 7.55 (t_{app}, J = 7.2 Hz, 1H, H_{OBt}), 7.43 (t_{app}, J = 6.9 Hz, 2H, H_{OBt}), 7.15-6.94 (m, 3H, H_{aryl}), 6.93-6.91 (m, 2H, H_{aryl}), 6.79 (d, J = 5.6 Hz, 1H, H_2), 3.79 (s, 2H, H_5).

^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1 (s, C_1), 168.8 (s, C_4), 160.1 (d, C_3), 143.4 (s, C_{OBt}), 136.4 (s, C_{OBt}), 128.9 (d, C_{OBt}), 128.8 (s, C_{aryl}), 128.6 (d, 2C, C_{aryl}), 128.4 (d, 2C, C_{aryl}), 127.2 (d, C_{aryl}), 125.0 (d, C_{OBt}), 120.6 (d, C_{OBt}), 108.7 (d, C_2), 100.4 (d, C_{OBt}), 35.1 (t, C_5).

HRMS (ESI) *m/z*: calculated for $C_{17}H_{14}N_5OS$ $[M+H]^+$ 336.0914, found 336.0900; calculated for $C_{17}H_{13}N_5NaOS$ $[M+Na]^+$ 358.0733, found 358.0717.

I.4.2.3.2. Synthesis of 4-[4-(benzyloxy)benzyloxy]-2-(benzylsulfanyl)pyrimidine (I.40)



MW (g/mol): 414.5193 **Molecular formula:** $C_{25}H_{22}N_2O_2S$

To a stirring solution of OBt-adduct **17** (3.0 g, 9.0 mmol) in MECN (0.3 M), *t*-BuOK (2.0 g, 18 mmol) and 4-benzyloxybenzyl alcohol (2.9 g, 13.5 mmol) were added and the resulting mixture was stirred at rt for 90 min. After this time, the solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane/EtOAc, 85:15) to afford compound **I.40** as a white solid (3.62 g, 97%).

Mp: 65-66 ºC.

Rf (*n*-hexane/EtOAc, 1:1): 0.72.

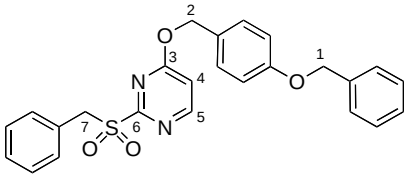
IR (neat): 3060, 3030, 1610, 1553, 1511, 1437, 1307, 1225, 1173, 983, 819, 696 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, J = 5.7 Hz, 1H, H_5), 7.46-7.28 (m, 12H, H_{aryl}), 6.98 (d, J = 8.7 Hz, 2H, H_{aryl}), 6.42 (d, J = 5.7 Hz, 1H, H_4), 5.33 (s, 2H, H_1 or H_2), 5.08 (s, 2H, H_1 or H_2), 4.44 (s, 2H, H_7).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2 (s, C_3), 168.5 (s, C_6), 158.9 (s, C_{aryl}), 157.4 (d, C_5), 137.6 (s, C_{aryl}), 136.9 (s, C_{aryl}), 130.1 (d, 2C, C_{aryl}), 128.9 (d, 2C, C_{aryl}), 128.7 (d, 2C, C_{aryl}), 128.6 (d, 2C, C_{aryl}), 128.4 (s, C_{aryl}), 128.0 (d, C_{aryl}), 127.5 (d, 2C, C_{aryl}), 127.2 (d, C_{aryl}), 115.0 (d, 2C, C_{aryl}), 104.2 (d, C_4), 70.1 (t, C_1 or C_2), 68.1 (t, C_1 or C_2), 35.4 (t, C_7).

HRMS (ESI) *m/z*: calculated for $C_{25}H_{23}N_2O_2S$ $[M+H]^+$ 415.1475, found 415.1478; calculated for $C_{25}H_{22}N_2NaO_2S$ $[M+Na]^+$ 437.1294, found 437.1301.

I.4.2.3.3. Synthesis of 4-[4-(benzyloxy)benzyloxy]-2-(benzylsulfonyl)pyrimidine (I.38)



MW (g/mol): 446.5182 **Molecular formula:** $C_{25}H_{22}N_2O_4S$

To a cooled solution (0 ºC) of benzylsulfanylpyrimidine **I.40** (3.5 g, 8.4 mmol) in CH_2Cl_2 (0.2M), *m*-CPBA (3.6 g, 21.1 mmol) was added in small portions. The resulting mixture was warmed up to rt and stirred for 1 h. Next, the solvent was removed under reduced pressure and the residue was dissolved in EtOAc (100 mL), washed with saturated $NaHCO_3$ solution (2 x 20 mL) and brine (1 x 20 mL). The organic layer was dried over $MgSO_4$, filtered, concentrated under reduced pressure and the resulting crude purified by flash chromatography (*n*-hexane/EtOAc, 6:4) to afford sulfone **I.38** as a colourless oil (2.89 g, 79%).

R_f (*n*-hexane/EtOAc, 1:1): 0.41.

IR (neat): 3062, 3032, 1578, 1535, 1511, 1467, 1449, 1318, 1239, 1174, 1123, 982, 734, 695 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, J = 5.7 Hz, 1H, H_5), 7.42-7.31 (m, 12H, H_{aryl}), 6.99 (d, J = 8.7 Hz, 2H, H_{aryl}), 6.85 (d, J = 5.7 Hz, 1H, H_4), 5.42 (s, 2H, H_1 or H_2), 5.06 (s, 2H, H_1 or H_2), 4.71 (s, 2H, H_7).

^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9 (s, C_3), 164.3 (s, C_6), 159.2 (s, C_{aryl}), 157.7 (d, C_5), 136.7 (s, C_{aryl}), 131.2 (d, 2C, C_{aryl}), 130.6 (d, 2C, C_{aryl}), 128.8 (d, C_{aryl}), 128.7 (d, 2C, C_{aryl}), 128.6 (d, 2C, C_{aryl}), 128.0 (d, C_{aryl}), 127.4 (d, 2C, C_{aryl}), 127.3 (s, C_{aryl}), 126.8 (s, C_{aryl}), 115.0 (d, 2C, C_{aryl}), 111.6 (d, C_4), 70.0 (t, C_1 or C_2), 69.6 (t, C_1 or C_2), 57.6 (t, C_7).

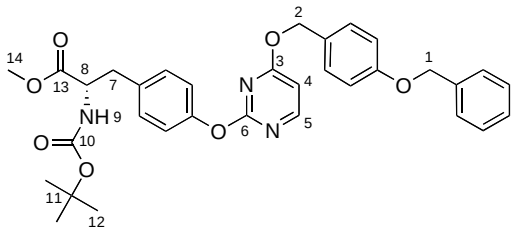
HRMS (ESI) *m/z*: calculated for $C_{25}H_2N_2NaO_4S$ $[M+Na]^+$ 469.1192, found 469.1177.

I.4.2.3.4. Synthesis of N$^{\alpha}$*-Boc pyrimidin-2-yl amino esters I.39*

I.4.2.3.4.1. General procedure

K_2CO_3 (1.2-2.4 equiv) was added to a solution of the corresponding N^{α}-Boc-amino ester **I.18a-d** (1.1 equiv) in dry DMF (0.4M) and the resulting mixture was stirred at rt for 15 min. After this time, pyrimidinyl sulfone **I.38** (1.0 equiv) was added, as a DMF solution (1M). The resulting mixture was stirred at the temperature specified for each compound. Upon completion of the reaction (TLC monitoring), the solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane/EtOAc, from 15:1 to 1:1) to afford N^{α}-Boc-pyrimidin-2-yl amino esters **I.39**.

I.4.2.3.4.2. Methyl (2*S*)-*N*-Boc-2-amino-3-{4-[4-(4-(benzyloxy)benzyloxy) pyrimidin-2-yloxy]phenyl}propanoate (I.39a)



MW (g/mol): 585.6469 **Molecular formula:** $C_{33}H_{35}N_3O_7$

Synthesized according to the general procedure from pyridiminyl sufone **I.38** (250 mg, 0.56 mmol), N^{α}-Boc-(*S*)-tyrosine methyl ester **I.18a** (182 mg, 0.61 mmol) and K_2CO_3 (93 mg, 0.67 mmol) at 50 ºC for 24 h, 291 mg (89%) of compound **I.39a** was obtained as a colourless oil.

***R**$_f$* (*n*-hexane/EtOAc, 3:2): 0.25.

[α]$_D^{20}$ +22.9 (*c* 1.36, $CHCl_3$).

IR (neat): 3433, 1741, 1709, 1568, 1508, 1380, 1334, 1271, 1240, 1213, 1166, 1046, 1016, 730 cm^{-1}.

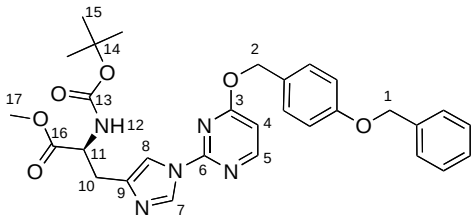
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, J = 5.6 Hz, 1H, H_5), 7.45-7.30 (m, 5H, H_{aryl}), 7.25 (m, 2H, H_{aryl}), 7.20 (d, J = 8.6 Hz, 2H, H_{aryl}), 7.13 (d, J = 8.6 Hz, 2H, H_{aryl}), 6.95 (d, J = 8.6 Hz, 2H, H_{aryl}), 6.45 (d, J = 5.6 Hz, 1H, H_4), 5.26 (s, 2H, H_1 or H_2), 5.08 (s,

2H, H_1 or H_2), 4.61 (m, 1H, H_8), 3.71 (s, 3H, H_{14}), 3.18-3.08 (m, 2H, H_7), 1.42 (s, 9H, H_{12}).

^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3 (s, C_{13}), 171.2 (s, C_3), 164.9 (s, C_6), 159.0 (s, C_{aryl}), 158.9 (d, C_5), 152.0 (s, C_{10}), 136.8 (s, C_{aryl}), 133.1 (s, 2C, C_{aryl}), 130.4 (d, 2C, C_{aryl}), 130.3 (d, 2C, C_{aryl}), 128.6 (d, 2C, C_{aryl}), 128.1 (s, C_{aryl}), 128.0 (d, C_{aryl}), 127.5 (d, 2C, C_{aryl}), 122.0 (d, 2C, C_{aryl}), 114.9 (d, 2C, C_{aryl}), 103.6 (d, C_4), 80.2 (s, C_{11}), 70.1 (t, C_1 or C_2), 68.2 (t, C_1 or C_2), 54.5 (d, C_8), 52.3 (q, C_{14}), 37.8 (t, C_7), 28.4 (q, 3C, C_{12}).

HRMS (ESI) *m/z*: calculated for $C_{33}H_{36}N_3O_7$ $[M+H]^+$ 586.2548, found 586.2555; calculated for $C_{33}H_{35}N_3NaO_7$ $[M+Na]^+$ 608.2367, found 608.2384.

I.4.2.3.4.3. Methyl (2*S*)-*N*-Boc-2-amino-3-{1-[4-(4-(benzyloxy)benzyloxy) pyrimidin-2-yl]-1*H*-imidazol-4-yl}propanoate (I.39b)



MW (g/mol): 552.6129 **Molecular formula:** $C_{30}H_{33}N_5O_6$

Synthesized according to the general procedure from pyridiminyl sufone **I.38** (250 mg, 0.56 mmol), N^{α}-Boc-(*S*)-histidine methyl ester **I.18b** (164 mg, 0.61 mmol) and K_2CO_3 (93 mg, 0.67 mmol) at 50 ºC for 24 h, 293 mg (94%) of compound was obtained as a colourless oil.

***R*$_f$** (*n*-hexane/EtOAc, 3:2): 0.25.

$[\alpha]_D^{20}$ +17.2 (*c* 1.5, $CHCl_3$)

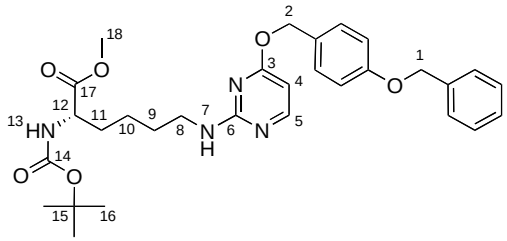
IR (neat): 2961, 2919, 1745, 1710, 1590, 1565, 1512, 1486, 1446, 1361, 1298, 1247, 1170, 1027 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (s, 1H, H_7), 8.33 (d, *J* = 5.7 Hz, 1H, H_5), 7.64 (s, 1H, H_8), 7.44-7.32 (m, 7H, H_{aryl}), 6.99 (d, *J* = 8.7 Hz, 2H, H_{aryl}), 6.61 (d, *J* = 5.7 Hz, 1H, H_4), 5.83 (d, *J* = 7.6 Hz, 1H, H_{12}), 5.41 (s, 2H, H_1 or H_2), 5.08 (s, 2H, H_1 or H_2), 4.62 (m, 1H, H_{11}), 3.73 (s, 3H, H_{17}), 3.16-3.12 (m, 2H, H_{10}), 1.44 (s, 9H, H_{15}).

^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5 (s, C_{16}), 170.3 (s, C_6), 159.0 (s, C_3), 158.5 (d, C_5), 155.3 (s, C_{13}), 136.9 (s, C_{aryl}), 135.9 (s, C_9), 131.7 (d, C_7), 130.2 (d, 2C, C_{aryl}), 128.7 (d, 2C, C_{aryl}), 128.1 (d, C_{aryl}), 127.9 (s, 2C, C_{aryl}), 127.5 (d, 2C, C_{aryl}), 115.2 (d, 2C, C_{aryl}), 114.3 (d, C_8), 106.4 (d, C_4), 79.6 (s, C_{14}), 70.2 (t, C_1 or C_2), 68.7 (t, C_1 or C_2), 53.5 (d, C_{11}), 52.3 (q, C_{17}), 29.8 (t, C_{10}), 28.4 (q, 3C, C_{15}).

HRMS (ESI) *m/z*: calculated for $C_{30}H_{34}N_5O_6$ $[M+H]^+$ 560.2504, found 560.2511; calculated for $C_{30}H_{33}N_5NaO_6$ $[M+Na]^+$ 582.2323, found 582.2294.

I.4.2.3.4.4. Methyl (2*S*)-*N*-Boc-2-amino-6-{*N*-[4-(4-(benzyloxy)benzyloxy) pyrimidin-2-yl]amino}hexanoate (I.39c)



MW (g/mol): 550.6459 **Molecular formula:** $C_{30}H_{38}N_4O_6$

Synthesized according to the general procedure from pyridiminyl sufone **I.38** (250 mg, 0.56 mmol), N^{α}-Boc-(*S*)-lysine methyl ester **I.18c** (200 mg, 0.61 mmol) and K_2CO_3 (155 mg, 1.22 mmol) at 45 ºC for 24 h, 280 mg (84%) of compound was obtained as a colourless oil.

R_f (*n*-hexane/EtOAc, 3:7): 0.51.

$[\alpha]_D^{20}$ +7.8 (*c* 1.0, $CHCl_3$)

IR (neat): 3365, 3255, 2935, 1739, 1685, 1584, 1528, 1511, 1453, 1427, 1282, 1243, 1225, 1171, 1159, 1010, 733 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 5.7 Hz, 1H, H_5), 7.44-7.31 (m, 7H, H_{aryl}), 6.98 (d, J = 8.7 Hz, 2H, H_{aryl}), 6.01 (d, J = 5.7 Hz, 1H, H_4), 5.27 (s, 2H, H_1 or H_2), 5.13 (br s, 1H, H_{13}), 5.07 (s, 2H, H_1 or H_2), 4.30 (br s, 1H, H_{12}), 3.71 (s, 3H, H_{18}), 3.40 (q, J = 6.7 Hz, 2H, H_8), 1.83 (m, 1H, H_{11}), 1.73-1.56 (m, 3H, H_9, H_{11}), 1.43 (m, 2H, H_{10}), 1.43 (s, 9H, H_{16}).

^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5 (s, C_{17}), 170.1 (s, C_3), 161.9 (s, C_6), 159.0 (s, C_{aryl}), 156.8 (d, C_5), 155.6 (s, C_{14}), 137.1 (s, C_{aryl}), 130.1 (d, 2C, C_{aryl}), 129.0 (s, C_{aryl}), 128.8

(d, 2C, C_{aryl}), 128.2 (d, C_{aryl}), 127.6 (d, 2C, C_{aryl}), 115.1 (d, 2C, C_{aryl}), 97.7 (d, C_4), 80.1 (s, C_{15}), 70.3 (t, C_1 or C_2), 67.6 (t, C_1 or C_2), 53.6 (d, C_{12}), 52.4 (q, C_{18}), 41.3 (t, C_8), 32.7 (t, C_{11}), 29.4 (t, C_9), 28.5 (q, 3C, C_{16}), 23.0 (t, C_{10}).

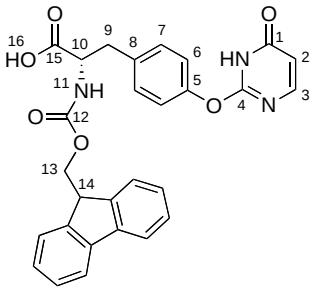
HRMS (ESI) *m/z*: calculated for $C_{30}H_{39}N_4O_6$ $[M+H]^+$ 551.2864, found 551.2884.

I.4.2.3.5. Synthesis of N^{α}-Fmoc pyrimidin-2-one amino acids I.13

I.4.2.3.5.1. General procedure

LiOH monohydrate (2.5 equiv) was added to a solution of appropriate N^{α}-Boc-amino methyl esters **I.39** (1 equiv) in THF/MeOH/H_2O (1:2:2; 0.12M), and the reaction mixture was stirred at rt for 3-4 h. Upon completion of the reaction (TLC monitoring), the organic solvents were removed under reduced pressure. The pH of the resulting aqueous solution was then adjusted to 4 with glacial acetic acid, and the solution was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Next, the free N^{α}-Boc-amino acid was dissolved in CH_2Cl_2 (0.7M) and the solution was cooled in an ice bath. TFA (0.3M) was added dropwise and the resulting mixture was stirred at 0 ºC for 5 h. Upon completion of the reaction (TLC monitoring), the solvent was removed under reduced pressure. The crude material was dissolved in 1,4-dioxane (0.3M) and the resulting solution was adjusted with 5% $NaHCO_3$ aqueous solution to pH 7. Fmoc-Osu (1.02-1.05 equiv) was then added slowly. During this addition, pH was readjusting with 5% $NaHCO_3$ aqueous solution to 7. The resulting mixture was stirred at rt for 8-12 h upon completion of the reaction (TLC monitoring). After this time, the solvent was removed under reduced pressure and the resulting residue was diluted in water (10 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were back extracted with saturated $NaHCO_3$ solution (3 x 5 mL). Then, the combined basic aqueous layers were then acidified to pH 1-2 with 1% HCl aqueous solution, and extracted with EtOAc (3 x 5 mL). These combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (*n*-hexane/EtOAc/AcOH, from 4:1:0 to 0:20:1) to afford N^{α}-Fmoc pyrimidin-2-one amino acids **I.13**.

I.4.2.3.5.2. (2*S*)-*N*-Fmoc-2-amino-3-[4-(6-oxo-1,6-dihydropyrimidin-2-yloxy)phenyl]propanoic acid (I.13a)



MW (g/mol): 497.4987 **Molecular formula:** $C_{28}H_{23}N_3O_6$

Synthesized according to the general procedure from N^{α}-Boc-pyrimidin-2-yl amino ester **I.39a** (140 mg, 0.25 mmol), 65 mg (55%) of compound **I.13a** was obtained as a colourless solid.

Mp: 117-118 ºC.

$\boldsymbol{R_f}$ (EtOAc/MeOH/AcOH, 10:2:0.1): 0.45.

$\mathbf{[\alpha]_D^{20}}$ –1.3 (*c* 0.4, DMF).

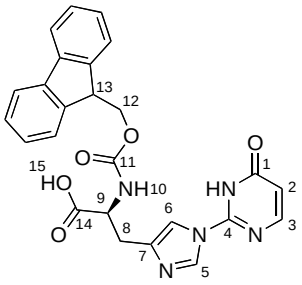
IR (neat): 3318, 1663, 1592, 1551, 1502, 1306, 1193, 1137, 842, 760, 738 cm^{-1}.

^{1}H NMR (300 MHz, d_6-DMSO) δ 7.89 (d, J = 7.4 Hz, 2H, H_{Fmoc}), 7.70-7.66 (m, 2H, H_{Fmoc}), 7.58 (br s, 1H, H_3), 7.42 (t, J = 7.4 Hz, 2H, H_{Fmoc}), 7.36-7.30 (m, 4H, H_{aryl}), 7.13 (d, J = 8.4 Hz, 2H, H_{Fmoc}), 6.09 (d, J = 6.4 Hz, 1H, H_2), 4.27-4.18 (m, 4H, H_{10}, H_{13}, H_{14}), 3.13 (dd, J = 14.1, 3.9 Hz, 1H, H_9), 2.91 (dd, J = 14.1, 10.2 Hz, 1H, H_9).

^{13}C NMR (75 MHz, d_6-DMSO) δ 173.5 (s, C_{15}), 164.2 (s, C_4), 159.6 (s, C_1), 155.9 (s, C_{12}), 153.9 (d, C_3), 150.1 (s, C_5), 143.8 (s, 2C, C_{Fmoc}), 140.6 (s, 2C, C_{Fmoc}), 135.7 (s, C_8), 130.2 (d, 2C, C_{Fmoc}), 127.6 (d, 2C, C_{Fmoc}), 127.0 (d, 2C, C_7), 125.2 (d, C_{Fmoc}), 125.1 (d, C_{Fmoc}), 121.3 (d, 2C, C_6), 120.0 (d, 2C, C_{Fmoc}), 108.4 (d, C_2), 65.5 (t, C_{13}), 55.7 (d, C_{10}), 46.6 (d, C_{14}), 35.9 (t, C_9).

HRMS (ESI) *m/z*: calculated for $C_{28}H_{24}N_3O_6$ $[M+H]^+$ 498.1660, found 498.1683; calculated for $C_{28}H_{23}N_3NaO_6$ $[M+Na]^+$ 520.1479, found 520.1493.

I.4.2.3.5.3. (2*S*)-*N*-Fmoc-2-amino-3-[1-(6-oxo-1,6-dihydropyrimidin-2-yl)-1*H*-imidazol-4-yl]propanoic acid (I.13b)



MW (g/mol): 471.4647 **Molecular formula:** $C_{25}H_{21}N_5O_5$

Synthesized according to the general procedure from N^{α}-Boc-pyrimidin-2-yl amino ester **I.39b** (150 mg, 0.25 mmol), 43 mg (40%) of compound **I.13b** was obtained as a colourless solid.

Mp: 163-164 ºC.

R_f (EtOAc/MeOH/AcOH, 10:2:0.1): 0.25.

$[\alpha]_D^{20}$ +17.4 (*c* 0.3, DMF).

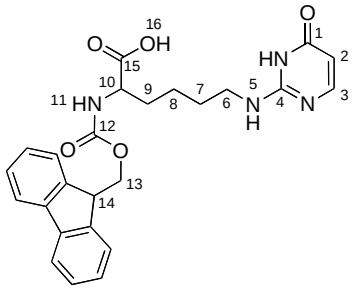
IR (neat): 3135, 1685, 1598, 1526, 1494, 1445, 1389, 1206, 1142, 1045, 758, 738 cm^{-1}.

^{1}H NMR (300 MHz, d_6-DMSO) δ 8.37 (s, 1H, H_5), 8.24 (d, J = 5.7 Hz, 1H, H_3), 7.85 (d, J = 7.4 Hz, 2H, H_{Fmoc}), 7.65-7.58 (m, 4H, H_6, H_{10}, H_{Fmoc}), 7.37 (t, J = 7.4 Hz, 2H, H_{Fmoc}), 7.26 (m, 2H, H_{Fmoc}), 6.48 (d, J = 5.7 Hz, 1H, H_2), 4.29-4.20 (m, 4H, H_9, H_{12}, H_{13}), 3.02 (dd, J = 14.7, 4.4 Hz, 1H, H_8), 2.90 (dd, J = 14.7, 9.4 Hz, 1H, H_8).

^{13}C NMR (75 MHz, d_6-DMSO) δ 173.4 (s, C_{14}), 171.2 (s, C_4), 157.8 (d, C_3), 155.9 (s, C_{11}), 153.8 (s, C_1), 143.7 (s, 2C, C_{Fmoc}), 140.6 (s, 2C, C_{Fmoc}), 139.0 (s, C_7), 134.9 (d, C_5), 127.5 (d, 2C, C_{Fmoc}), 127.0 (d, 2C, C_{Fmoc}), 125.2 (d, C_{Fmoc}), 125.1 (d, C_{Fmoc}), 120.0 (d, 2C, C_{Fmoc}), 113.8 (d, C_6), 106.4 (d, C_3), 65.6 (t, C_{12}), 53.7 (d, C_9), 46.5 (d, C_{13}), 29.8 (t, C_8).

HRMS (ESI) *m/z*: calculated for $C_{25}H_{22}N_5O_5$ $[M+H]^+$ 472.1615, found 472.1615.

I.4.2.3.5.4. (2*S*)-*N*-Fmoc-2-amino-6-[*N*-(4-oxo-3*H*-pyrimidin-2-yl)amino]hexanoic acid (I.13c)



MW (g/mol): 462.4977 **Molecular formula:** $C_{25}H_{26}N_4O_5$

Synthesized according to the general procedure from N^{α}-Boc-pyrimidin-2-yl amino ester **I.39c** (150 mg, 0.27 mmol), 65 mg (53%) of compound **I.13c** was obtained as a colourless solid.

Mp: 121-122 ºC.

***R*$_f$** (EtOAc/MeOH/AcOH, 10:2:0.1): 0.69.

[α]$_D^{20}$ +6.6 (*c* 1.0, DMF)

IR (neat): 2944, 1686, 1645, 1534, 1446, 1193, 1132, 735, 536 cm^{-1}.

^{1}H NMR (400 MHz, d_6-DMSO) δ 12.62 (br s, 1H, H_{16}), 7.89 (d, J = 7.5 Hz, 2H, H_{Fmoc}), 7.72 (d, J = 7.1 Hz, 2H, H_{Fmoc}), 7.63 (dd, J = 7.9, 5.3 Hz, 2H, H_{Fmoc}), 7.41 (t, J = 7.4 Hz, 2H, H_{Fmoc}), 7.33 (t, J = 7.4 Hz, 2H, H_{Fmoc}), 5.68 (d, J = 6.6 Hz, 1H, H_2), 4.30-4.20 (m, 3H, H_{13}, H_{14}), 3.93 (m, 1H, H_{10}), 3.27-3.25 (m, 2H, H_6), 1.72 (m, 1H, H_9), 1.63 (m, 1H, H_9), 1.55-1.47 (m, 2H, H_7), 1.41-1.31 (m, 2H, H_8).

^{13}C NMR (100 MHz, d_6-DMSO) δ 173.9 (s, C_{15}), 162.5 (s, C_4), 162.3 (s, C_1), 156.2 (s, C_{12}), 153.8 (d, C_2), 143.8 (s, 2C, C_{Fmoc}), 140.7 (s, 2C, C_{Fmoc}), 127.6 (d, 2C, C_{Fmoc}), 127.1 (d, 2C, C_{Fmoc}), 125.3 (d, 2C, C_{Fmoc}), 120.1 (d, 2C, C_{Fmoc}), 102.9 (d, C_2), 65.6 (t, C_{13}), 53.8 (d, C_{10}), 46.7 (d, C_{14}), 40.4 (t, C_6), 30.4 (t, C_9), 28.1 (t, C_7), 22.9 (t, C_8).

HRMS (ESI) *m/z*: calculated for $C_{25}H_{27}N_4O_5$ $[M+H]^+$ 463.1976, found 463.1984.

I.4.2.4. Analysis of optical purity of pyrimidinyl amino esters I.19, I.20, I.32 and I.39

As described in section I.2.1.4, the optical purity of these compounds was verified by coupling the pyrimidinyl α-amino acids, obtained after saponification reaction of **I.19**, **I.20**, **I.32** or **I.39** with both racemic phenylalanyl resin **I.25a** and L-phenylalanyl resin **25b** in order to measure the degree of racemization by HPLC.

I.4.2.4.1. Saponification reaction of pyrimidinyl amino esters I.19, I.20, I.32 and I.39. General procedure.

LiOH monohydrate (2.5 equiv) was added to a solution of appropriate N^{α}-Boc-amino methyl esters **I.19, I.20, I.32** or **I.39** (1 equiv) in THF/MeOH/H_2O (1:2:2; 0.12M), and the reaction mixture was stirred at rt for 3-4 h. Upon completion of the reaction (TLC monitoring), the organic solvents were removed under reduced pressure. The pH of the resulting aqueous solution was then adjusted to 4 with glacial acetic acid, and the solution was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting N^{α}-Boc-amino acids were used to couple with phenylalanyl resin **25a** and **25b** without further purification.

I.4.2.4.2. General protocol of solid-phase synthesis of dipeptides I.26

Dipeptides **I.26** were prepared manually by solid-phase method using Fmoc-Rink-MBHA resin (0.64 mmol/g) as solid support following standard Fmoc-strategy. Coupling of amino acids were mediated by HBTU (3 equiv) and DIEA (3 equiv) in DMF at rt for 3 h. The completion of the reactions was checked by the Kaiser test. Fmoc group was removed by treating the resin with 30% of piperidine in DMF (v/v) for 2 and 8 min. After each coupling and deprotection step, the resin was washed with DMF (6 x 1 min) and CH_2Cl_2 (3 x 1 min). The Fmoc-Rink-MBHA resin (10 mg) was placed into a plastic syringe fitted with a polypropylene frit and was swollen with CH_2Cl_2 (1 x 20 min) and DMF (1 x 20 min). Fmoc group was removed and subsequently couple with Fmoc-Phe-OH. After Fmoc group removal, resin was treated with the corresponding pyrimidinyl amino acid under coupling conditions. The resulting dipeptides were deprotected and cleaved from resin by treatment with TFA/CH_2Cl_2/TIS

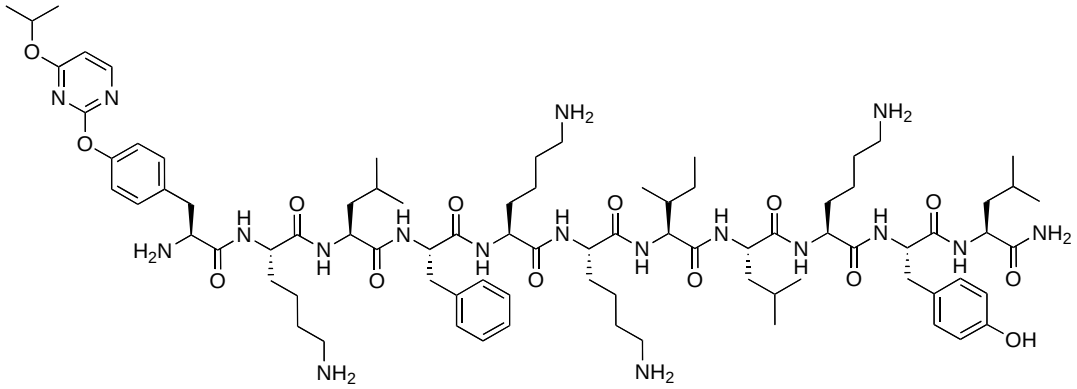
(95:2.5:2.5) for 2 h. The solvents were evaporated to dryness and the crude dipeptides were dissolved in H_2O, lyophilized and tested for purity on HPLC. Electrospray ionitzation mass spectrometry was used to confirm peptide identity.

I.4.2.5. Synthesis of peptides BP290-303

I.4.2.5.1. General protocol for the synthesis of BP290-303

All peptides **BP290-303** were synthesized manually by the solid-phase method using standard Fmoc chemistry. Fmoc-Rink-MBHA resin (200-400 mesh) with a 0.56 mmol/g functionalization was used as solid support. The Fmoc-Rink-MBHA resin (50 mg) was placed into a plastic syringe fitted with a polypropylene frit and was swollen with CH_2Cl_2 (1 x 20 min) and DMF (1 x 20 min). Fmoc group was removed by treating the resin twice with 30% of piperidine in DMF (v/v) for 2 and 8 min. Couplings of commercial Fmoc amino acids were carried out as follows: Fmoc-aa-OH (4 equiv) was dissolved in DMF and preactivated for 5 min with HBTU (3.8 equiv), HOBt (4 equiv) and DIEA (7.8 equiv). The mixture was added to the resin and shaken for 1 h at rt. Couplings of N^{α}-Fmoc pyrimidinyl amino acids **I.11** or **I.12** were performed using HBTU (3 equiv) and DIEA (3 equiv) in DMF under stirring for 3 h at rt. The completion of the reactions was checked by the Kaiser test. After each coupling and deprotection step, the resin was washed with DMF (6 x 1 min) and CH_2Cl_2 (3 x 2 min). The resulting peptides were cleaved from the resin with TFA/TIS/H_2O (95/5/5) under stirring for 2 h at rt. Following TFA evaporation and diethyl ether extraction, the crude peptides were dissolved in H_2O, lyophilized and tested for purity on HPLC. ESI-MS or MALDI-TOF mass spectrometry was used to confirm peptide identity.

I.4.2.5.1.1. H-Tyr(Py)-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Tyr-Leu-NH_2 (BP290)



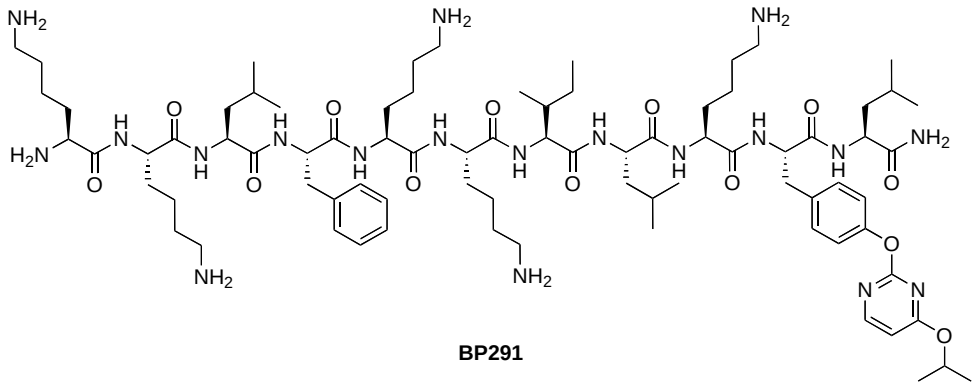
BP290

MW (g/mol): 1591.0218 **Molecular formula:** $C_{82}H_{130}N_{18}O_4$

HPLC (λ = 220 nm): t_R = 6.82 min (83%).

MS (MALDI-TOF) *m/z*: 1592.90 $[M+H]^+$.

I.4.2.5.1.2. H-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Tyr(Py)-Leu-NH_2 (BP291)



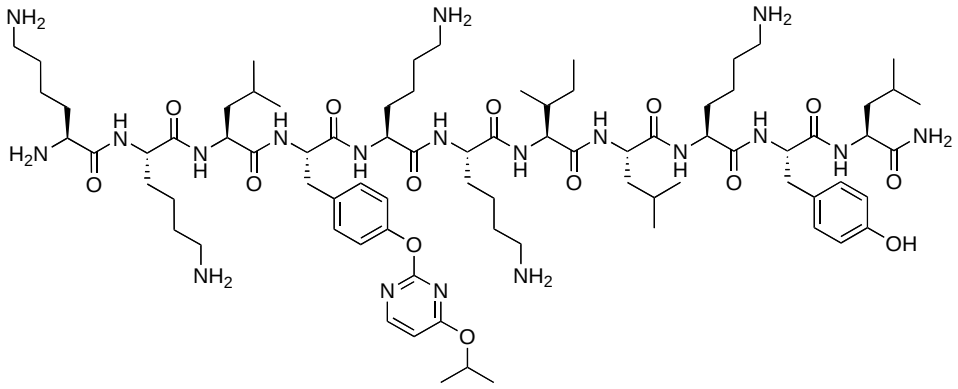
BP291

MW (g/mol): 1557.0208 **Molecular formula:** $C_{79}H_{138}N_{19}O_{13}$

HPLC (λ = 220 nm): t_R = 6.69 min (89%).

MS (MALDI-TOF) *m/z*: 1557.00 $[M+H]^+$.

I.4.2.5.1.3. H-Lys-Lys-Leu-Tyr(Py)-Lys-Lys-Ile-Leu-Lys-Tyr-Leu-NH_2 (BP292)



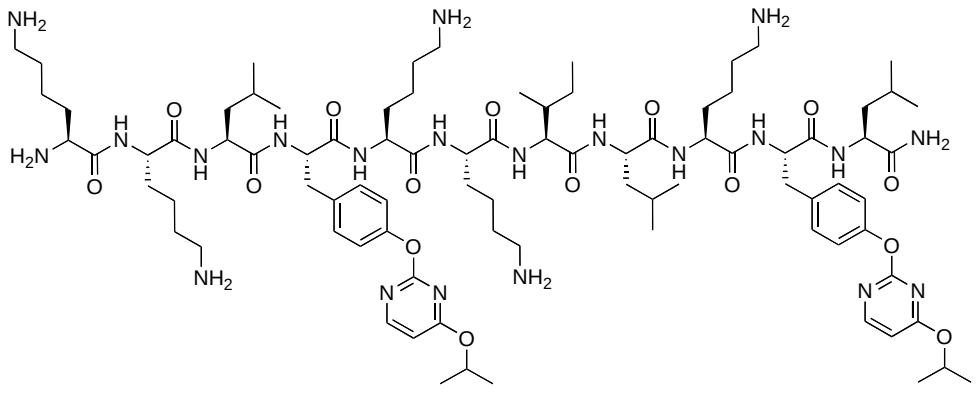
BP292

MW (g/mol): 1573.0202 **Molecular formula:** $C_{79}H_{133}N_{19}O_{14}$

HPLC (λ = 220 nm): t_R = 6.24 min (68%).

MS (MALDI-TOF) *m/z*: 1573.00 $[M+H]^+$.

I.4.2.5.1.4. H-Lys-Lys-Leu-Tyr(Py)-Lys-Lys-Ile-Leu-Lys-Tyr(Py)-Leu-NH_2 (BP293)



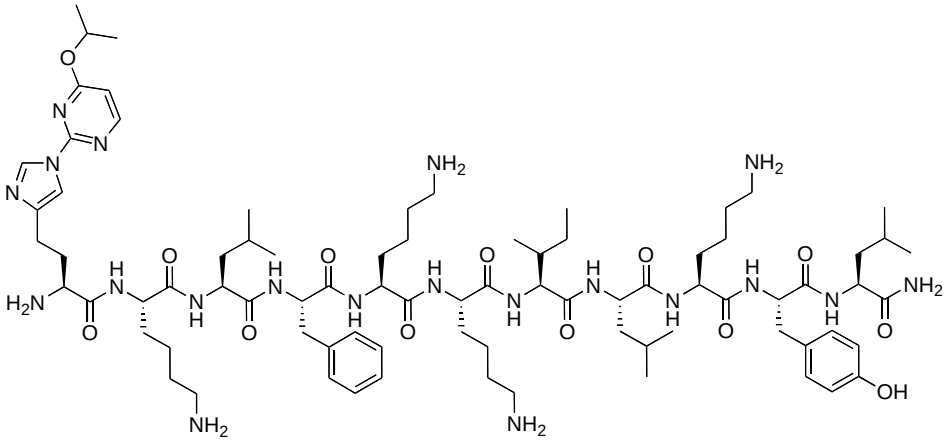
BP293

MW (g/mol): 1709.1714 **Molecular formula:** $C_{68}H_{141}N_{21}O_{15}$

HPLC (λ = 220 nm): t_R = 6.81 min (70%).

MS (MALDI) *m/z*: 1709.00 $[M+H]^+$.

I.4.2.5.1.5. H-His(Py)-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Tyr-Leu-NH_2 (BP294)



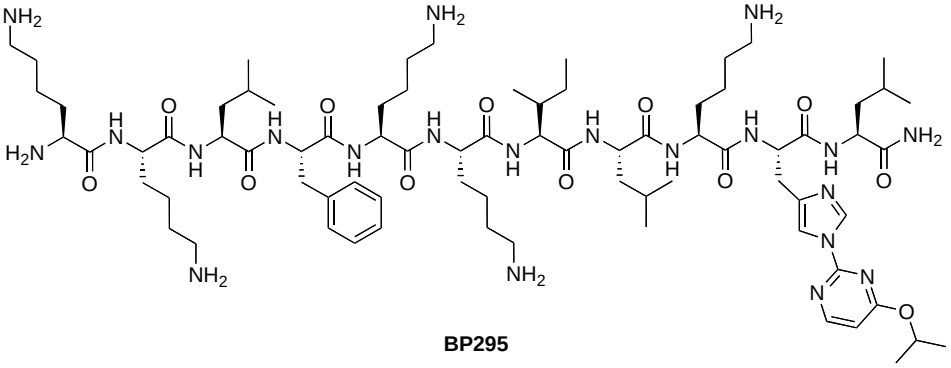
BP294

MW (g/mol): 1580.0144 **Molecular formula:** $C_{80}H_{130}N_{20}O_{13}$

HPLC (λ = 220 nm): t_R = 6.85 min (73%).

MS (MALDI-TOF) *m/z*: 1565.90 $[M+H]^+$.

I.4.2.5.1.6. H-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-His(Py)-Leu-NH_2 (BP295)



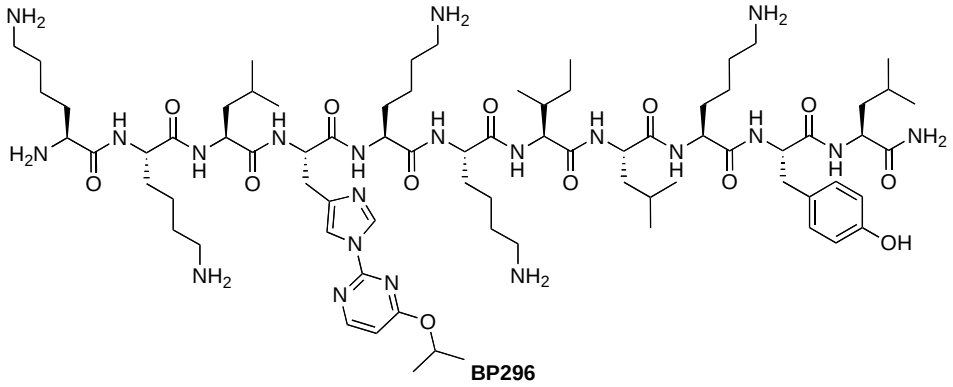
BP295

MW (g/mol): 1530.9868 **Molecular formula:** $C_{76}H_{131}N_{21}O_{12}$

HPLC (λ = 220 nm): t_R = 6.40 min (99%).

MS (MALDI-TOF) *m/z*: 1531.00 $[M+H]^+$.

I.4.2.5.1.7. H-Lys-Lys-Leu-His(Py)-Lys-Lys-Ile-Leu-Lys-Tyr-Leu-NH_2 (BP296)

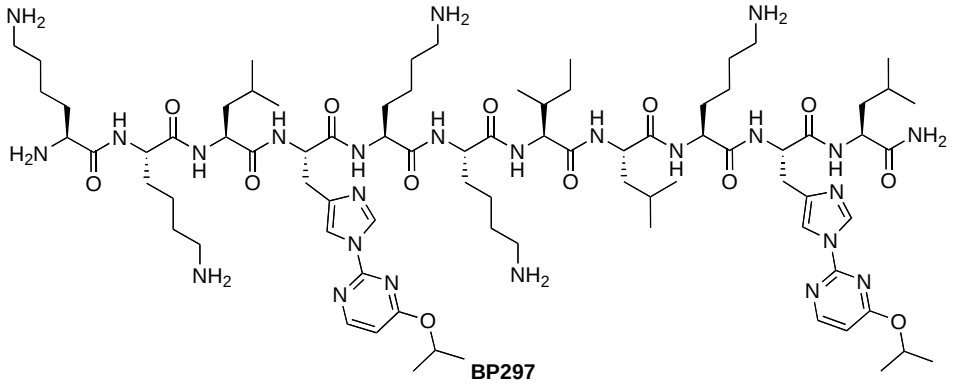


MW (g/mol): 1546.9862 **Molecular formula:** $C_{76}H_{131}N_{21}O_{13}$

HPLC (λ = 220 nm): t_R = 6.23 min (99%).

MS (MALDI-TOF) *m/z*: 1547.00 $[M+H]^+$.

I.4.2.5.1.8. H-Lys-Lys-Leu-His(Py)-Lys-Lys-Ile-Leu-Lys-His(Py)-Leu-NH_2 (BP297)

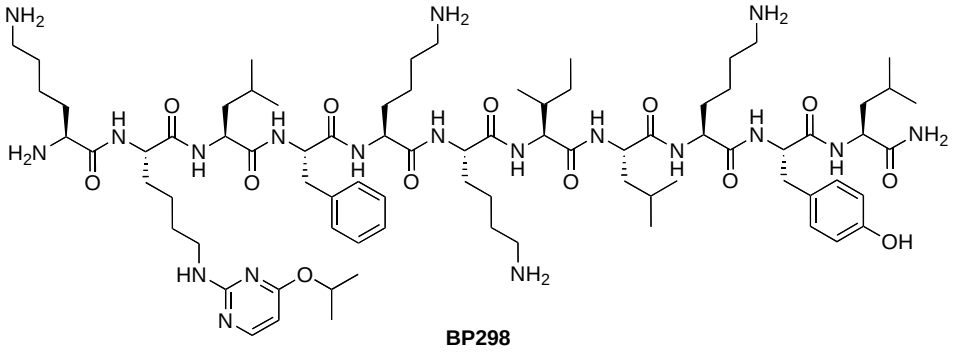


MW (g/mol): 1657.1034 **Molecular formula:** $C_{80}H_{137}N_{25}O_{13}$

HPLC (λ = 220 nm): t_R = 6.45 min (99%).

MS (MALDI-TOF) *m/z*: 1657.00 $[M+H]^+$.

I.4.2.5.1.9. H-Lys-Lys(Py)-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Y-Leu-NH_2 (BP298)



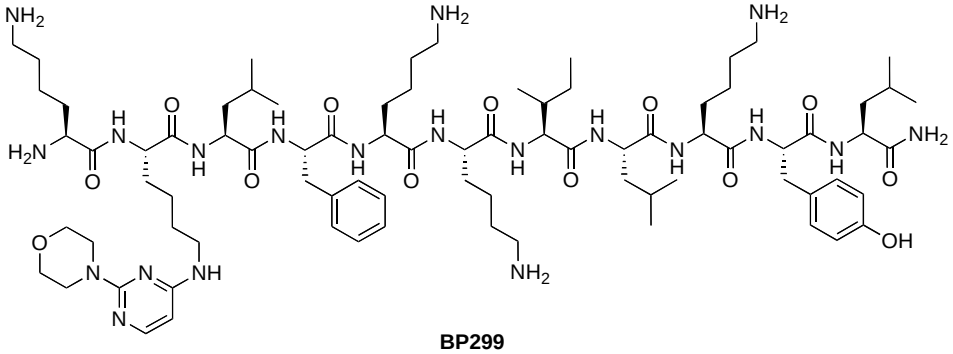
BP298

MW (g/mol): 1557.0208 **Molecular formula:** $C_{79}H_{133}N_{19}O_{13}$

HPLC (λ = 220 nm): t_R = 6.65 min (92%).

MS (MALDI-TOF) *m/z*: 1578.88 $[M+Na]^+$, 1594.87 $[M+K]^+$.

I.4.2.5.1.10. H-Lys-Lys(Mor)-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Y-Leu-NH_2 (BP299)



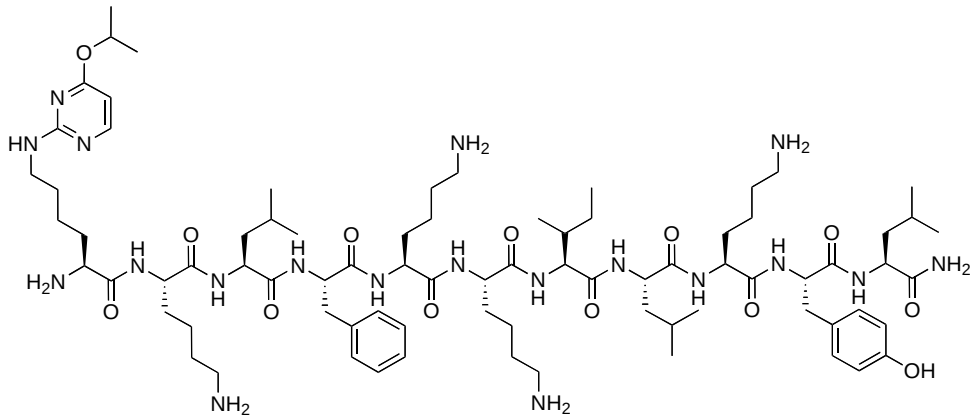
BP299

MW (g/mol): 1584.0461 **Molecular formula:** $C_{80}H_{134}N_{20}O_{13}$

HPLC (λ = 220 nm): t_R = 6.41 min (70%).

MS (MALDI-TOF) *m/z*: 1584.09 $[M+H]^+$, 1606.06 $[M+Na]^+$, 1623.05 $[M+K]^+$.

I.4.2.5.1.11. H-Lys(Py)-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Y-Leu-NH_2 (BP300)



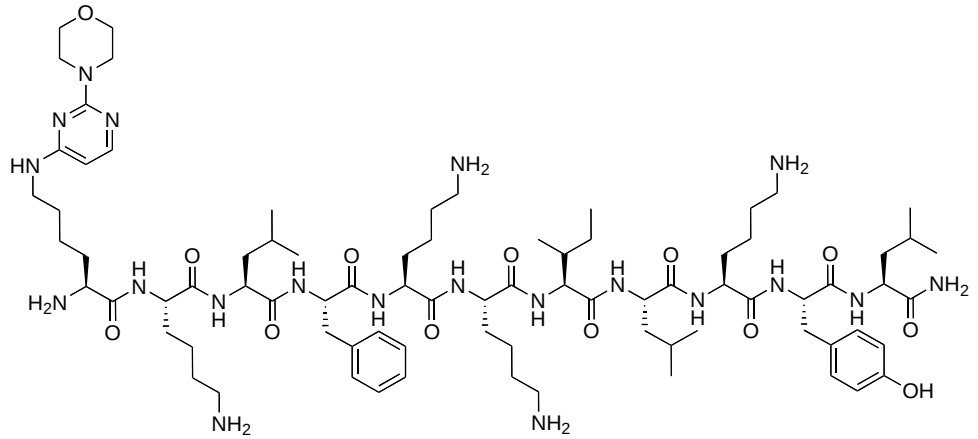
BP300

MW (g/mol): 1557.0208 **Molecular formula:** $C_{79}H_{133}N_{19}O_{13}$

HPLC (λ = 220 nm): t_R = 6.72 min (71%).

MS (MALDI-TOF) *m/z*: 1556.98 $[M+H]^+$, 1578.99 $[M+Na]^+$, 1594.98 $[M+K]^+$.

I.4.2.5.1.12. H-Lys(Mor)-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Y-Leu-NH_2 (BP301)



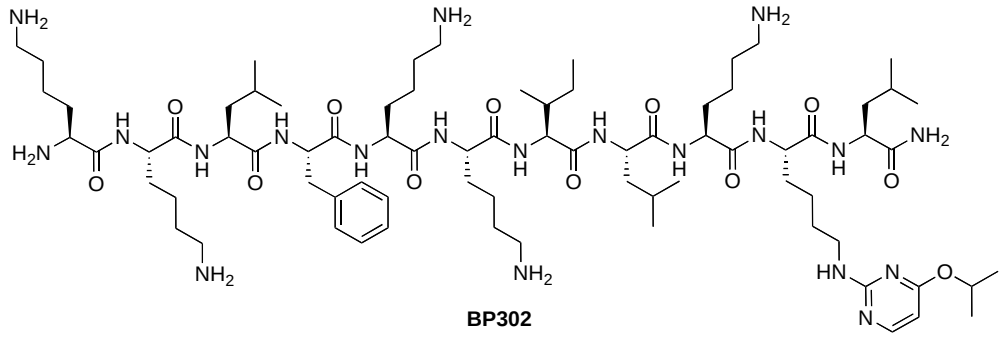
BP301

MW (g/mol): 1584.0461 **Molecular formula:** $C_{80}H_{134}N_{20}O_{13}$

HPLC (λ = 220 nm): t_R = 6.49 min (80%).

MS (MALDI-TOF) *m/z*: 1583.99 $[M+H]^+$, 1606.00 $[M+Na]^+$, 1621.99 $[M+K]^+$.

I.4.2.5.1.13. H-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Lys(Py)-Leu-NH_2 (BP302)



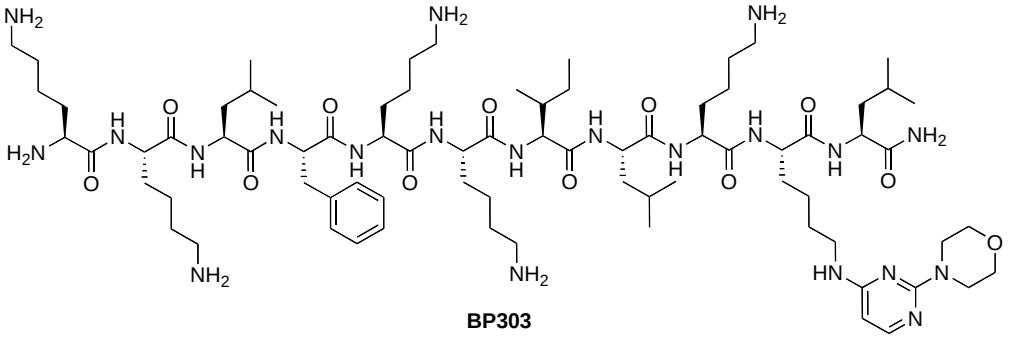
BP302

MW (g/mol): 1521.0646 **Molecular formula:** $C_{76}H_{136}N_{20}O_{12}$

HPLC (λ = 220 nm): t_R = 6.46 min (67%).

MS (ESI+) *m/z*: 1523.20 $[M+H]^+$, 761.6 $[M+2H]^{2+}$.

I.4.2.5.1.14. H-Lys-Lys-Leu-His(Py)-Lys-Lys-Ile-Leu-Lys-Lys(Mor)-Leu-NH_2 (BP303)



BP303

MW (g/mol): 1549.0451 **Molecular formula:** $C_{77}H_{137}N_{21}O_{12}$

HPLC (λ = 220 nm): t_R = 6.27 min (60%).

MS (ESI+) *m/z*: 1549.20 $[M+H]^+$, 775.5 $[M+2H]^{2+}$.

CHAPTER II

Total synthesis of bioactive marine natural products and analogues thereof

II. MARINE NATURAL PRODUCT CHEMISTRY

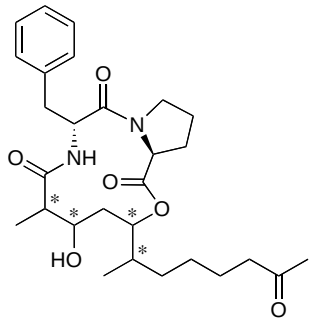
In the last 50 years, there has been an increasing interest for marine natural products since tremendous chemical and biological diversity are found among metabolites from marine species. Since the identification of the first marine natural product, chemists have been fascinated by the immense structural diversity and complexity of the metabolites isolated from organisms ranging from marine plants and invertebrates to microbes. The wide-spectrum of metabolites isolated from these organisms,[92,93] as well as the potent biological activities exhibited by many of these marine natural products,[94,95] led to consider the ocean as a great source of potential drugs.

Marine natural products are usually small to medium molecular weight compounds that have stimulated remarkable interdisciplinary studies by both chemists and biologists. From marine toxins that impact public health concerns to the search for new drugs, the study of the biologically active marine natural products has profoundly influenced the course of discovery in fields ranging from organic chemistry to cancer medicine.

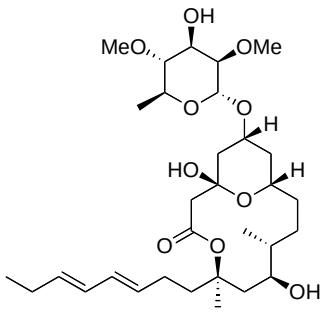
Natural products and synthetic chemistry have traditionally been strong allies. In this relationship, natural product chemists discover and present evidence for new natural products, which often possess an exotic array of carbon skeletons, heterocycles, and functionalities. For their part, synthetic chemists use these novel structures as platforms to develop new and efficient syntheses, which allow to confirm or revise the assigned structures. This alliance is all the more significant when the target natural product is in limited supply, has exciting and potentially very valuable properties, and/or when the structure assignment is incomplete or in doubt. The wealth of structurally diverse marine metabolites continues to provide synthetic chemists with an endless supply of inspiring structures.

[92] Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165-237.
[93] Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 1-6.
[94] (a) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216-1238. (b) Molinski, T. F.; Dalisay, S. L.; Lievens, S. L.; Saludes, J. P. *Nat. Rev. Drug Discovery* **2009**, *8*, 69-85. (c) Mayer, A. M. S.; Glaser, K. B.; Cuevas, C.; Jacobs, R. S.; Kem, W.; Little, R. D.; McInstosh, J. M.; Newman, D. J.; Potts, B. C.; Shuster, D. E. *Trends Pharmacol. Sci.* **2010**, *31*, 255-265.
[95] Bhakuni, D. S.; Dawar, D. S. *Bioactive marine natural products* New York, Springer, **2005**.

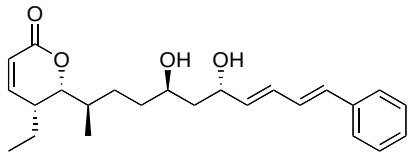
As mentionned previously, one of the fundamental goals of this thesis was the synthesis of biologically active compounds bearing a peptidic moiety. In this context, Chapter I focuses on the rational preparation of modified antimicrobial peptides incorporating synthetic pyrimidinyl α-amino acids. The next Chapter, on the other hand, concerns the total synthesis of two biologically active marine natural products bearing a macrocyclic lactone moiety. The first part of Chapter II will thus illustrate our efforts toward the first total synthesis of acremolide B, a natural 12-membered cyclic depsipeptide whose structure was unconfirmed, while the second part of Chapter II will focus on the development of the first efficient synthetic strategy toward lyngbouilloside, a 14-membered macrolide that attracted our attention due to its promising biological profile as well as its appealing structural features (Figure II.1). Finally, the last Chapter of this thesis will focus on our endeavour toward the expedient synthesis of the dual-specificity phosphatase inhibitor bitungolide F (Figure II.1).



Acremolide B



Lyngbouilloside



(–)-Bitungolide F

Figure II.1. Structure of acremolide B, lyngbouilloside and (–)-bitungolide F

PART 1

Total synthesis of a stereoisomer of Acremolide B

II.1.1. INTRODUCTION

II.1.1.1. ISOLATION, STRUCTURE AND BIOLOGICAL PROPERTIES OF ACREMOLIDES A–D

II.1.1.1.1. Isolation

In 2008, Capon *et al.* reported the isolation of a family of novel lipodepsipeptides named acremolides A–D from an Australian estuarine isolate of an *Acremonium* sp. (MST-MF588a) obtained from a sediment sample collected in the Huon River, near Franklin, Tasmania (Figure II.2).[96]

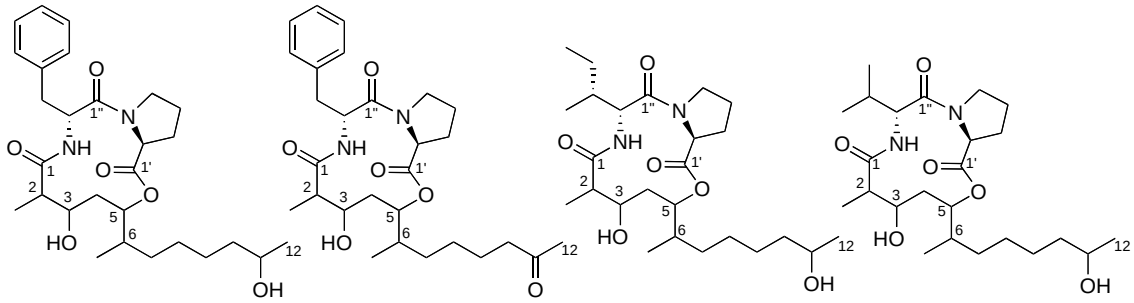


Figure II.2. Structures of acremolides A–D

[96] Ratnayake, R.; Fremlin, L. J.; Lacey, E.; Gill, J. H.; Capon, R. J. *J. Nat. Prod.* **2008**, *71*, 403-408.

Subsequent fractionation studies on the same *Acremonium* extract also identified the known mycotoxins 19-*O*-acetylchaetoglobosin B and D,[97] together with the known small-molecule aromatic compound RKB 3564S (Figure II.3).[98]

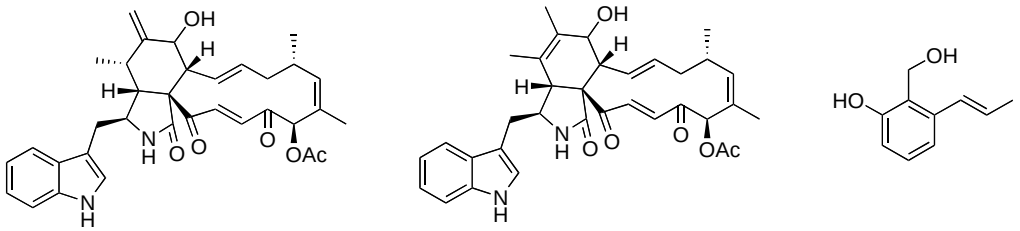


Figure II.3. Structures of compounds isolated from *Acremonium* sp.

II.1.1.1.2. Structural assignment for acremolide B

In the initial report by Capon *et al.*, the gross structure of the various acremolides was determined through a combination of chemical degradation, NMR spectroscopic methods and HRMS analysis. Although the structures of acremolides A–D were proposed to display a 12-membered-ring lactam constituted of a C1-C12 polypropionate unit linked to a dipeptide, the three-dimensional structure of the acremolides still remains undetermined.

While preliminary analysis of the ^{1}H NMR (d_6-DMSO) data of acremolide A revealed resonance doubling (ratio 2:1) that coalesced at elevated temperature thus suggesting the presence of equilibrating isomers, the HRMS revealed a pseudomolecular ion [M + Na] corresponding to a molecular formula $C_{28}H_{42}O_6N_2$ requiring nine double bond equivalents. Careful analysis of the NMR data allowed tabulation of the resonances attributable to both major and minor isomers and indicated the presence of phenylalanine and proline residues. The amino acid content in acremolide A was eventually confirmed by hydrolysis and C3 Marfey's analysis[99] as

[97] Probst, A.; Tamm, C. *Helv. Chim. Acta* **1981**, *64*, 2056-2064.

[98] Osada, H.; Kakeya, H.; Konno, H.; Kanazawa, S. Japanese Patent 051804, **2003**.

[99] Marfey's analysis relies on the hydrolysis of a peptide- or amino acid-containing substance followed by *in situ* conversion of the resulting amino acids using a chiral derivatizing agent such as 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (L-FDDA) to form the corresponding 2,4-dinitrophenyl-5-L-alanine amide (DNP) derivatives. The C18 HPLC retention times of the resulting DNP derivatives monitored by UV at 340 nm are then compared to DNP derivatives obtained from authentic amino acid standards allowing a diagnostic for both a given amino acid and a given stereochemistry, see: Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K. A. *Anal. Chem.* **1997**, *69*, 5146-5151.

L-Pro and D-Phe, while a key Heteronuclear Multiple Bond Correlation (HMBC) between these residues confirmed the amide linkage.[100]

A set of COSY and HMBC correlations together with ^{13}C NMR data identified the remaining structural fragment as a substituted fatty acid attached to D-Phe *via* an amide bond. In addition, these experiments allowed the identification and the positioning of C2- and C6-Me, and the C3- and C5-oxy substituents. In addition, the deshielded nature of the ^{13}C NMR chemical shift of C11 combined with the COSY correlation from an exchangeable OH resonance to H11 confirmed the presence of a hydroxyl group.

Unfortunately, while a pyridinium chlorochromate (PCC)-mediated oxidation of acremolide A afforded the corresponding diketone **II.1**, whose NMR data reasserted the assigned structure (Figure II.4), Mosher's analysis[101] proved impossible as neither if the hydroxyl moieties reacted with (*S*)-Mosher's reagent, returning unreacted starting material. In this context, neither the relative nor the absolute stereochemistry about the fatty acid substructure of acremolide A remains unassigned.

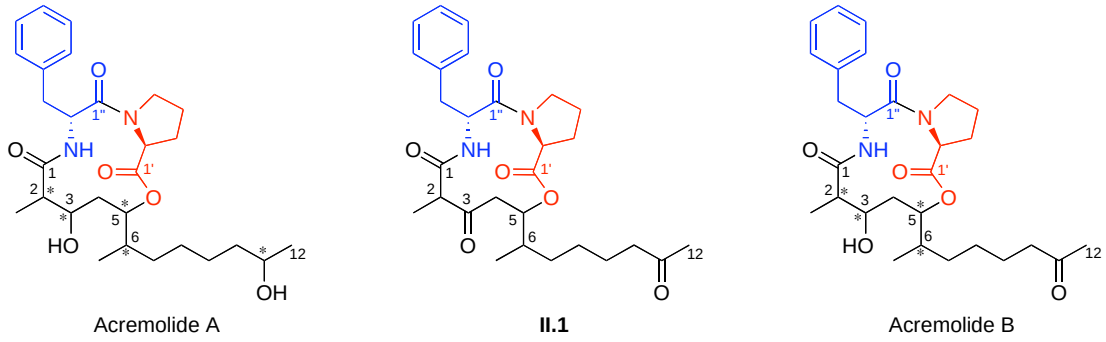


Figure II.4. Proposed structure of acremolide A, **II.1** and acremolide B

Acremolide B, on the other hand, was initially suggested to be a didehydro analogue of acremolide A. Indeed, analytical scale acid hydrolysis followed by C3 Marfey's analysis[99] confirmed the presence of D-Phe and L-Pro residues, while the NMR data were almost identical, revealing a 2:1 ratio of major *cis* versus minor *trans* rotamers. As might be expected, the major rotamer in acremolide B matched that found in

[100] Amides attached through proline nitrogen are known to be capable of existing as equilibrating rotamers, with literature empirical rules establishing that ^{13}C NMR chemical shift differences between proline β and γ carbon resonances are characteristic of *cis* (Δβγ ~ 8-12 ppm) vs *trans* (Δβγ ~ 2-6 ppm) prolinyl amide rotamers, respectively. See: Siemion, I. Z.; Wieland, T.; Pook, K. H. *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 702-703.

[101] (a) Dale, J. A.; Dull, D. H.; Mosher, H. S. *J. Org. Chem.* **1969,** *34*, 2543-2549. (b) Dale, J. A.; Dull, D. H.; Mosher, H. S. *J. Am. Chem Soc.* **1973,** *95*, 512-519.

acremolide A. More detailed analysis of these NMR data confirmed the presence of a ketone at C11, which justified the notable differences compared to acremolide A.

II.1.1.1.3. Biological properties of the acremolides A–D

The methanol extract from a solid phase culture of the fungus *Acremonium* sp. displayed significant cytotoxic activity against NS-1 cells. Subsequent bioassay and fractionation studies afforded 19-*O*-acetylchaetoglobosin B (LD_{99} 1.6 μg/mL) and 19-*O*-acetylchaetoglobosin D (LD_{99} 0.8 μg/mL), together with the co-metabolite RKB 3564S[98,102] and acremolides A-D all of which exhibited no cytotoxicity. Considering the estimated yields of the chaetoglobosins in the crude EtOAc extract (0.16 and 0.17%, respectively) and the modest cytotoxicity potency displayed by the chaetoglobosins compared to the overall cytotoxicity of the crude *Acremonium* extract, the authors suspected a possible synergistic effect. The acremolides were therefore tested in combination with each of the chaetoglobosins in order to establish if the former could synergize the cytotoxic properties of the latter against NS-1 cells and thereby account for the anomalous cytotoxicity of the crude *Acremonium* extract. Unfortunately, this study did not reveal any significant synergistic effect.

In addition, the acremolides displayed no antibacterial against (*Bacillus subtilis*) and antifungal against (*Candida albicans*) properties while sharing a similar structural scaffold with the known histone deacetylase (HDAC) inhibitors such as FR235222, apicidin A, and trapoxin (Figure II.5).

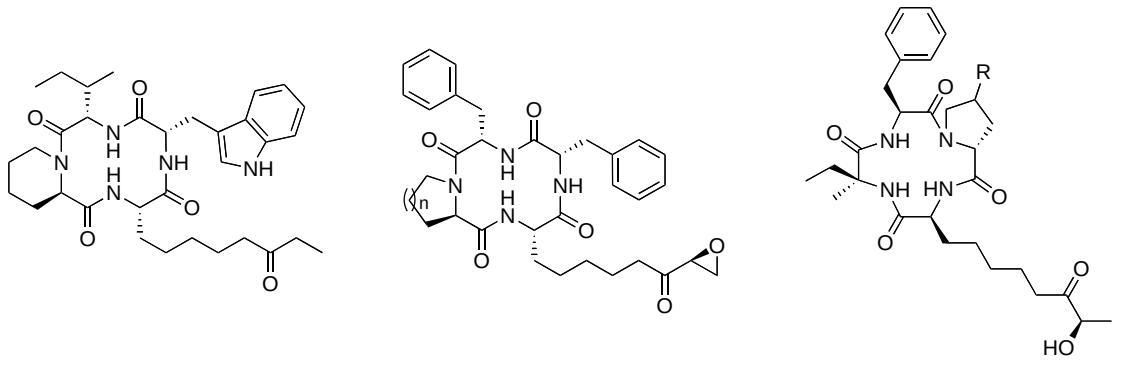


Figure II.5. Structures of apicidin A, trapoxins A and B, AS1387392 and FR235222

[102] Osada, H.; Kakeya, H.; Hayashi, Y.; Shoji, M.; Uchida, S. P. Japanese Patent 101483, **2004**.

Therefore, it remains unclear whether the acremolides' structural diversity or the associated prolinyl amide bond conformer bias adjusts the potency and/or the selectivity of the acremolides' biological/ecological response, particularly given that the ecological role of these molecules remains unknown.

II.1.1.2. STEREOSELECTIVE METHODS FOR POLYPROPIONATE SYNTHESIS

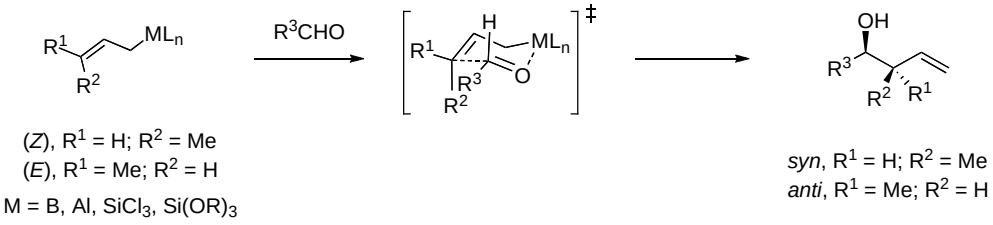
As mentioned above, the acremolides are a family of lipodepsipeptides bearing a polypropionate framework. This key structural feature, which is characterized by a sequence of methyl- and hydroxyl-bearing stereogenic centers, is present in a wide variety of biologically active natural products, and constitutes therefore a challenge for synthetic organic chemists. This has resulted in the development of a number of particularly useful allylation and crotylation methods, which will be presented hereafter.[103]

II.1.1.2.1. Asymmetric allylation and crotylation of aldehydes

The asymmetric allylation and crotylation of aldehydes have been extensively studied during the past two decades resulting in particularly predictable and efficient methods for the stereocontrolled synthesis of polypropionates. These methods, which involve the addition of an allylmetal usually derived from boron, tin, indium, titanium or silicon,[104] are of three types depending on their mechanism.

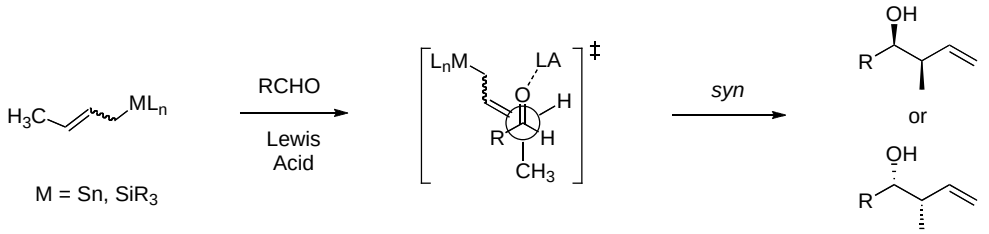
- Type I reagents, which mainly involve boranes, aluminum-derived reagents, and either trihalo- or trialkoxysilanes, proceed *via* a rigid chair-like transition state through the coordination of the carbonyl to the metal atom. Consequently, the *Z*/*E* ratio of the starting alkene dictates the *syn*/*anti* diastereoselectivity of the product while the enantioselectivity induced by the chiral auxilliary can be explained by a Zimmerman-Traxler model (Scheme II.1).[105]

[103] Li, J.; Menche, D. *Synthesis* **2009**, 2293-2315.
[104] Yamamoto, Y.; Asao, N. *Chem. Rev.* **1993**, *93*, 2207-2293.
[105] (a) Li, Y.; Houk, K. N. *J. Am. Chem. Soc.* **1989**, *111*, 1236-1240. (b) Vulpetti, A.; Gardner, M.; Gennari, C.; Bernardi, A.; Goodman, J. M.; Paterson, I. *J. Org. Chem.* **1993**, *58*, 1711-1718. (c) Gennari, C.; Fioravanzo, E.; Bernardi, A.; Vulpetti, A. *Tetrahedron* **1994**, *50*, 8815-8826. (d) Omoto, K.;



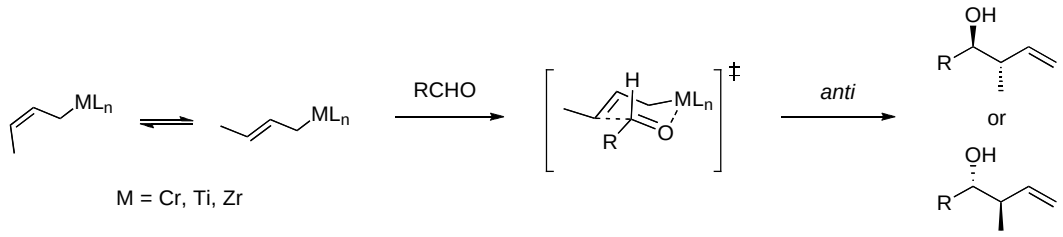
Scheme II.1. Mechanism of Type I allyl/crotylmetals onto aldehyde

- Type II reagents, exemplified by trialkylsilanes and stannanes, require the activation of the carbonyl substrate with an external Lewis acid and proceed usually *via* an open transition state. The product is predominantely *syn* independently of the geometry of the starting alkene (Scheme II.2).[106]



Scheme II.2. Mechanism of Type II allyl/crotylmetals onto aldehyde

- Type III reagents, which include chromium-, titanium- and zirconium-derived reagents, also proceed through a cyclic six-membered transition state as Type I reagents, however, they undergo metallotropic rearrangement faster than they actually react with the aldehyde. Thus, the diastereoselectivity does not depend on the geometry of the double bond and the product is exclusively *anti* (Scheme II.3).



Scheme II.3. Mechanism of Type III allyl/crotylmetals onto aldehyde

Fujimoto, H. *J. Org. Chem.* **1998**, *63*, 8331-8336. (e) Gajewski, J. J.; Bocian, W.; Brichford, N. L.; Henderson, J. L. *J. Org. Chem.* **2002**, *67*, 4236-4240.

[106] (a) Denmark, S. E.; Fu, J. *J. Am. Chem. Soc.* **2001**, *123*, 9488-9489. (b) Denmark, S. E.; Fu, J. *Chem. Commun.* **2003**, 167-170.

It is worth pointing out that a number of catalytic processes have also been developed for the allylation and crotylation of aldehydes,[107] however, they are not as general in terms of efficacy yet and will therefore not be presented here.

II.1.1.2.1.a. Boron allylation/crotylation of aldehydes

Pioneering work by Brown[108] and Roush[109] on the asymmetric allylation and crotylation of aldehydes has led to the development of particularly efficient chiral borane reagents, which are commonly used today. However, it is worth noting that others groups have also reported boron-mediated allyl- and crotylations such as Hoffmann,[110] Masamune,[111] Corey,[112] Soderquist[113] and Hall[114] (Figure II.6).

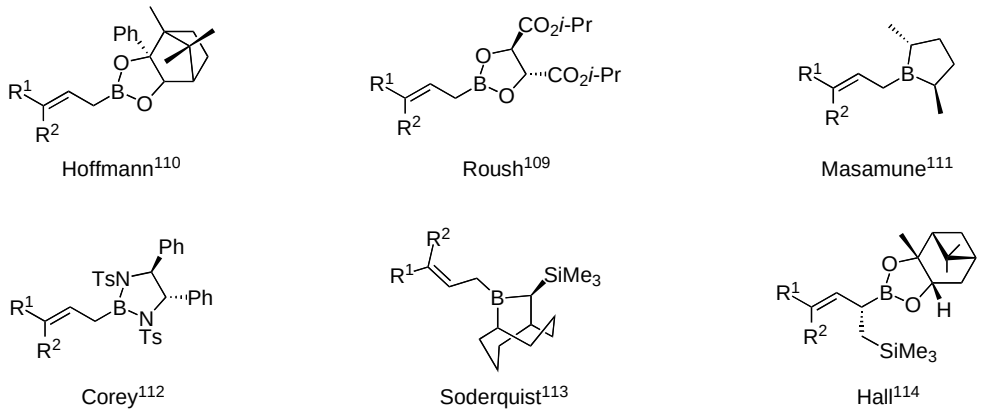


Figure II.6. Chiral boron reagents for asymmetric allyl/crotylations

Despite certain disadvantages such as being non catalytic and non storable, the allyl- and the crotyldiisopinocampheylboranes developed by Brown *et al.* are probably the

[107] (a) Masse, C. E.; Panek, J. C. *Chem. Rev.* **1995**, *95*, 1293-1316. (b) *Comprehensive Asymmetric Catalysis;* Jacobsen, E. N.; Pfaltz, A.; Yamamoto, H., Eds.; Springer: Berlin, **1999**, 965-982. (c) *Principles and Applications of Asymmetric Synthesis;* Lin, G.-Q.; Li, Y.-M.; Chan, A. S. C., Eds.; John Wiley & Sons: New York, **2001**, 167-178. (d) Denmark, S. E.; Fu, J. *Chem. Rev.* **2003**, *103*, 2763-2794. For a review on Lewis and Brønsted acid catalyzed allylborations of carbonyl compounds, see: Hall, D. G. *Synlett* **2007,** 1644-1655.

[108] (a) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 293-294. (b) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 5919-5923.

[109] (a) Roush, W. R.; Ando, K.; Powers, D. B.; Palkowitz, A. D.; Halterman, R. L. *J. Am. Chem. Soc.* **1990,** *112*, 6339-6348. (b) Roush, W. R.; Palkowitz, A. D.; Ando, K. *J. Am. Chem. Soc.* **1990,** *112*, 6348-6359. (c) Scheidt, K. A.; Tasaka, A.; Bannister, T. D.; Wendt, M. D.; Roush, W. R. *Angew. Chem. Int. Ed.* **1999,** *38*, 1652-1655.

[110] Herold, T.; Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 768-769.

[111] Garcia, J.; Kim, B.; Masamune, S. *J. Org. Chem.* **1987**, *52*, 4831-4832.

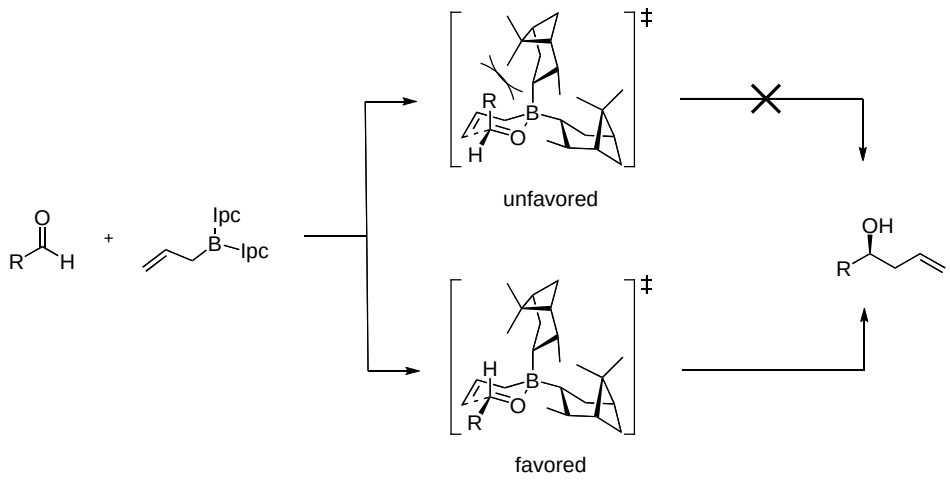
[112] Corey, E. J.; Yu, C.-M.; Kim, S. S. *J. Am. Chem. Soc.* **1989**, *111*, 5495-5496.

[113] Burgos, C. H.; Canales, E.; Matos, K.; Soderquist, J. A. *J. Am. Chem. Soc.* **2005**, *127*, 8044-8049.

[114] Peng, F.; Hall, D. G. *J. Am. Chem. Soc.* **2007**, *129*, 3070-3071.

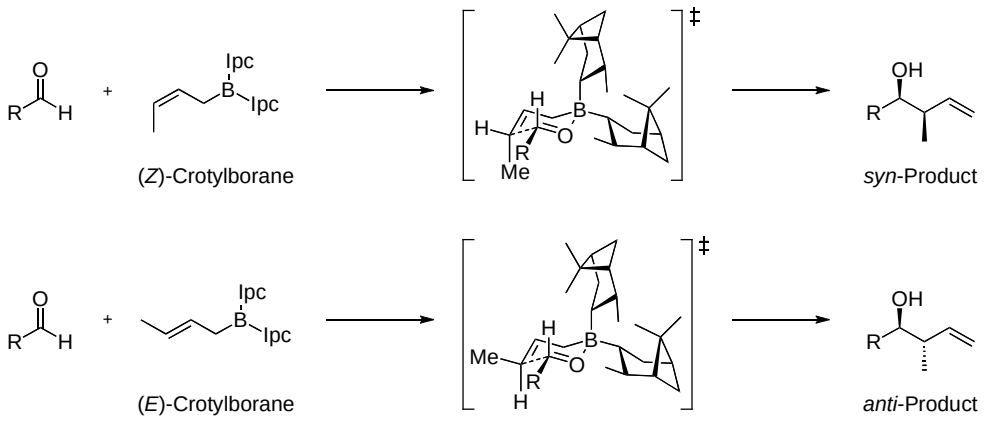
most commonly used among all the chiral boron reagents developed so far, due to their demonstrated good performance and to the commercial availability of the chiral auxiliary. In contrast, although Roush's chiral boranes are derived from tartrate analogues which are commercially available, much more stable and therefore easier to handle, they usually exhibit slightly lower face selectivity.

The high stereoselectivity induced by the diisopinocampheyl-derived boranes can be explained by a closed chair-like transition state where the boron is coordinated to the carbonyl oxygen. The aldehyde is oriented in such a manner that the R group is placed in a *pseudo*-equatorial position in order to minimize steric interactions between the chiral auxiliary and the allyl unit (Scheme II.4).



Scheme II.4. Brown's allylboration of aldehydes

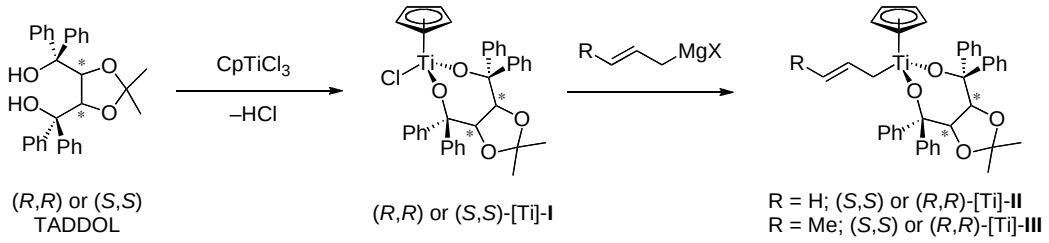
The crotyl variant behaves the same way except for the fact that two contiguous stereogenic centers are formed. While the absolute stereochemistry is controlled by the chiral auxiliary, the relative stereochemistry between the alcohol and the methyl is induced by the geometry of the alkene. Thus, the reaction of (*E*)- or (*Z*)-crotylboronates with aldehydes results in the formation of the *anti*- or *syn*-β-methyl homoallylic alcohols, respectively (Scheme II.5).



Scheme II.5. Brown's crotylboration of aldehydes

II.1.1.2.1.b. Allyl-/crotyltitanation of aldehydes

Duthaler *et al.*[115] introduced chiral titanium complexes elaborated from readily available nontoxic materials. Most importantly, the chiral ligand which is derived from tartaric acid, TADDOL,[116] is available in both enantiomeric forms. Unlike the boron reagents, these titanium complexes can be prepared by transmetalation with a variety of allyl- or crotyl-Grignard reagents and their isolation or purification is not necessary, rendering their manipulation easier (Scheme II.6).

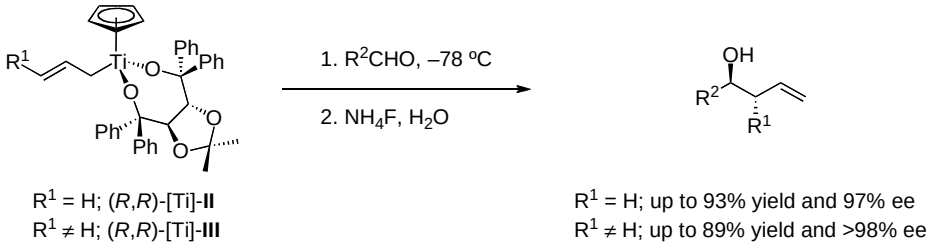


Scheme II.6. Preparation of the allyl- or crotyltitanium reagents

These allyl- and crotyltitanium reagents react with both aromatic and aliphatic aldehydes with a high degree of facial discrimination to afford the corresponding homoallylic alcohols in an enantio- and diastereoselective fashion regardless of the aldehyde. As a general trend, the (*R*,*R*)-[Ti]-**II** complex attacks preferentially the *Si* face of the aldehyde whereas the (*S*,*S*)-[Ti]-**II** complex attacks the opposite *Re* face, thus

[115] (a) Duthaler, R. O.; Hafner, A.; Riediker, M. *Pure Appl. Chem.* **1990**, *62*, 631-642. (b) Hafner, A.; Duthaler, R. O.; Marti, R.; Rihs, J.; Rothe-Streit, P.; Schwarzenbach, F. J. *J. Am. Chem. Soc.* **1992**, *114*, 2321-2336. (c) Duthaler, R. O.; Hafner, A. *Chem. Rev.* **1992**, *92*, 807-832.
[116] Seebach, D.; Beck, A. K.; Heckel, A. *Angew. Chem., Int. Ed.* **2001**, *20*, 92-138.

providing the (*S*)- and the (*R*)- homoallylic alcohols, respectively. In the case of the crotylation, the major product is exclusively the *anti*-diastereoisomer since the *E*-crotyl titanium species is favoured due to a rapid 1,3-migration of the titanium to the unsubstituted terminus of the crotyl group (Scheme II.7). Finally, the addition of a chiral titanium reagent to an aldehyde bearing an α-or a β-stereogenic center remains highly diastereoselective independently of its configuration, while the presence of a free hydroxyl group or a free amine does not affect either the reactivity of the reagent nor its selectivity which is a clear bonus in front of all the other allyl- and crotylmetal reagents.[117]

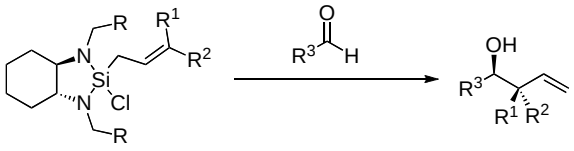


Scheme II.7. Allyl-/crotyltitanation of aldehydes

II.1.1.2.1.c. Silicon allylation/crotylation of aldehydes

In 2002, Leighton *et al*. developed a new method for the asymmetric allyl- and crotylation of carbonyl compounds featuring a highly reactive species derived from silicon.[118] These cristalline solid silacycles consisting of an allyl- or a crotylsilane and a chiral 1,2-diamine, are storable and may be prepared in bulk amounts, though their synthesis requires a few steps. A thorough survey of the performance of these reagents that act as chiral silicon-based Lewis acids was carried on a variety of aromatic, aliphatic and α,β-unsaturated aldehydes. In every single case, high levels of selectivity were observed and the chiral diamine could be recovered (Scheme II.8).[119]

[117] (a) BouzBouz, S.; Popkin, M. E.; Cossy, J. *Org. Lett.* **2000**, *2*, 3449-3451. (b) BouzBouz, S.; Cossy, J. *Org. Lett.* **2001,** *3*, 3995-3998.
[118] *Review:* Leighton, J. L. *Aldrichimica Acta* **2010**, *31*, 3-12.
[119] Kinnaird, J. W. A.; Ng, P. Y.; Kubota, K.; Wang, X., Leighton, J. L. *J. Am. Chem. Soc.* **2002**, *124*, 7920-7921.



R = 4-BrC_6H_4

Allylation:
$R^1 = R^2 = H$
Crotylation:
(*E*): $R^1 = H$, $R^2 = Me$; gives *anti*-product
(*Z*): $R^1 = Me$, $R^2 = H$; gives *syn*-product

Scheme II.8. Reactivity of Leighton's silicon reagents

In the course of this thesis, the construction of the polypropionate subunits in both acremolide B and lyngbouilloside were secured using the chiral titanium complexes developed by Duthaler *et al*. Hence, asymmetric allylations and *anti*-crotylations were performed using reagents such as [Ti]-**II** and [Ti]-**III**, respectively.

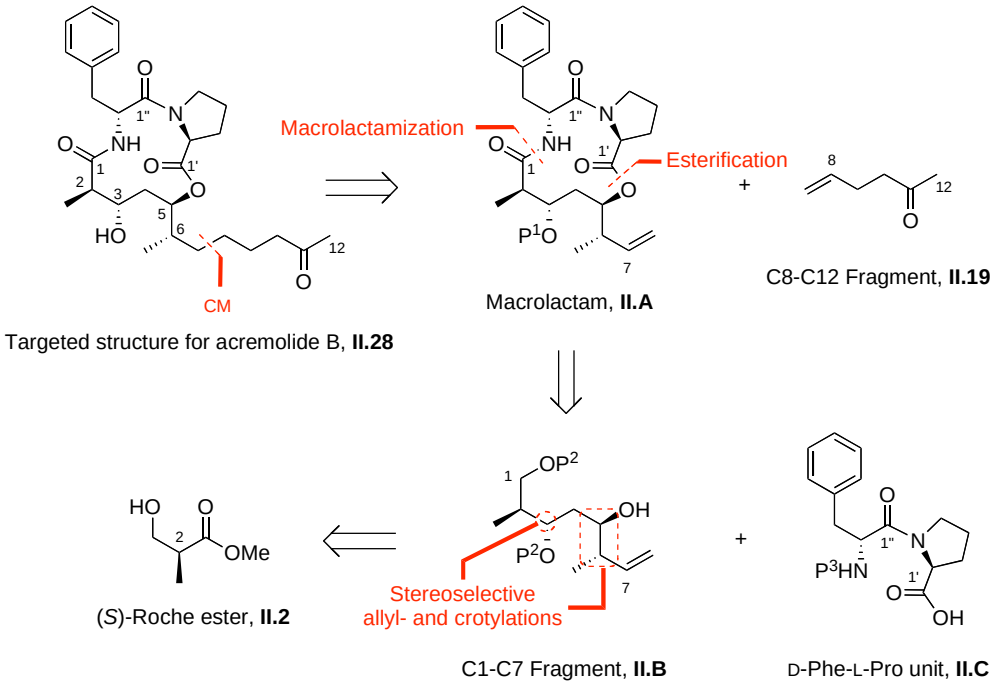
II.1.2. RESULTS & DISCUSSION

II.1.2.1. TOTAL SYNTHESIS OF A STEREOISOMER OF ACREMOLIDE B

Due to the biological potential of acremolides and with the goal to finally ascertain both their relative and absolute stereochemistry, we felt that it would be particularly interesting to develop a concise and flexible synthesis that would allow a straightforward access to these natural products and to various analogues thereof. After close examination of the NMR data, it was decided to first undertake the synthesis of the (2*R*,3*S*,5*R*,6*S*)-isomer of acremolide B.

II.1.2.1.1. Initial retrosynthetic analysis

The synthesis of acremolide B, which had been originally started by Aya Fukuda in the group prior to my arrival, featured a key cross-metathesis (CM) to introduce the fatty-acid side chain, an esterification to link the dipeptide unit to the C1-C9 polypropionate fragment, a macrolactamization to build the 12-membered ring, and two stereoselective allylations/crotylations to control the three stereogenic centers at C3, C5, and C6. Overall, these disconnections would enable a high level of versatility through the entire synthesis (Scheme II.9).

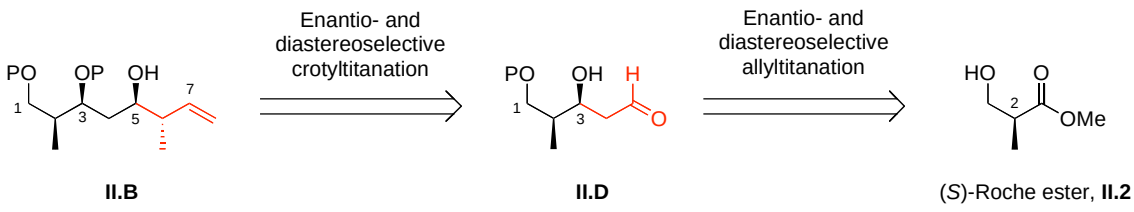


Scheme II.9. Initial retrosynthetic analysis

II.1.2.1.2. Synthesis of the C1-C7 fragment

II.1.2.1.2.a. Retrosynthetic analysis of the C1-C7 fragment

The synthesis of the C1-C7 fragment **II.B** featured two highly enantio- and diastereoselective titanium-mediated allylations and crotylations as the key steps in order to set the stereogenic centers with the 3*S*, 5*S* and 6*S* configurations. **II.B** could be synthesized from aldehyde **II.D** which would be obtained by transformation of the Roche ester, **II.2** (Scheme II.10).



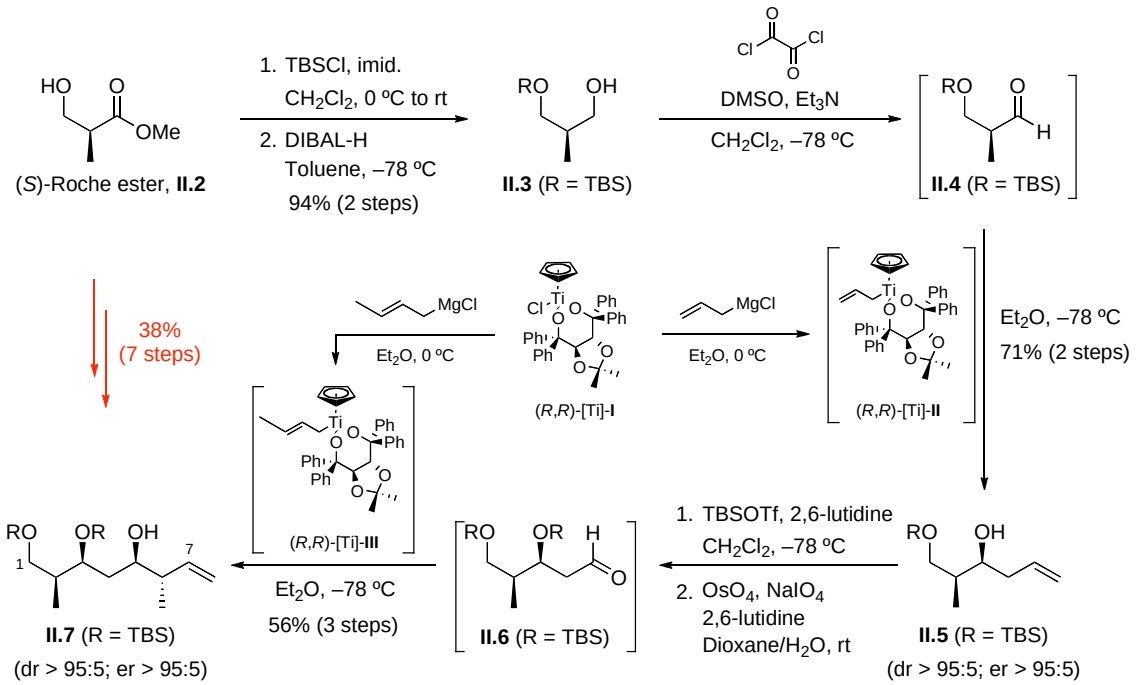
Scheme II.10. Retrosynthetic analysis of the C1-C7 fragment

II.1.2.1.2.b. Synthesis of the C1-C7 fragment

The synthesis of the C1-C7 subunit commenced by protecting the (*S*)-Roche ester **II.2** as a *tert*-butyldimethylsilyl (TBS) ether (TBSCl, CH_2Cl_2, 0 °C to rt) followed by a DIBAL-H-mediated reduction of the ester moiety (toluene, –78 °C) to the corresponding alcohol **II.3** in 94% yield over two steps. The latter was then oxidized to aldehyde **II.4** under standard Swern[120] conditions [$(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, –78 °C] and subsequently treated with the *in situ* generated (*R*,*R*)-TADDOL-derived allyltitanium complex,[115] (*R*,*R*)-[Ti]-**II**, thus setting the (3*S*) stereogenic center. Under this set of conditions, alcohol **II.5** was isolated as a single stereoisomer (dr > 95:5; er > 95:5) in 71% yield overall yield (Scheme II.11). The diastereomeric ratio was determined by ^{1}H NMR analysis of the crude reaction mixture, while the absolute configuration of the C3 stereogenic centre was secured as (3*S*) by comparing the optical rotation of the newly synthesized compound with the one reported in the literature {$[\alpha]^{25}_D$ –5.5 (*c* 1.08, $CHCl_3$); lit.[121] $[\alpha]^{22}_D$ –6.4 (*c* 0.33, $CHCl_3$)}.

The homoallylic alcohol **II.5** was then protected as a TBS ether (TBSOTf, 2,6-lutidine, CH_2Cl_2, –78 °C) and engaged in an OsO_4-catalyzed oxidative cleavage[122] in the presence of $NaIO_4$ and 2,6-lutidine in a 3:1 dioxane/H_2O mixture. The resulting aldehyde **II.6** was then directly treated with the (*R*,*R*)-TADDOL-derived crotyltitanium complex, (*R*,*R*)-[Ti]-**III**, in order to afford the desired homoallylic alcohol **II.7** (56% yield over three steps) and thus complete the synthesis of the C1-C7 fragment of acremolide B (Scheme II.11).

[120] Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651-1660.
[121] Roush, W. R.; Hoong, L. K.; Palmer, M. A.; Straub, J. A.; Palkowitz, A. D. *J. Org. Chem.* **1990**, *55*, 4117-4126.
[122] Yu, W.; Mei, Y.; Kang, Y.; Hua, Z.; Jin, Z. *Org. Lett.* **2004**, *6*, 3217-3219.



Scheme II.11. Synthesis of the C1-C7 fragment

A rational for the observed stereoselectivities is attributed to the fact that (*R*,*R*)-[Ti]-**II** and (*R*,*R*)-[Ti]-**III** react predominantly on the *Si*-face of aldehydes **II.4** and **II.6**, respectively. As noted previously, these allyl- and crotyltitanium species are well known to afford a very high degree of stereo-facial discrimination on both chiral and achiral aldehydes.[115,117]

II.1.2.1.3. Synthesis of macrolactam II.A

II.1.2.1.3.a. Retrosynthetic analysis

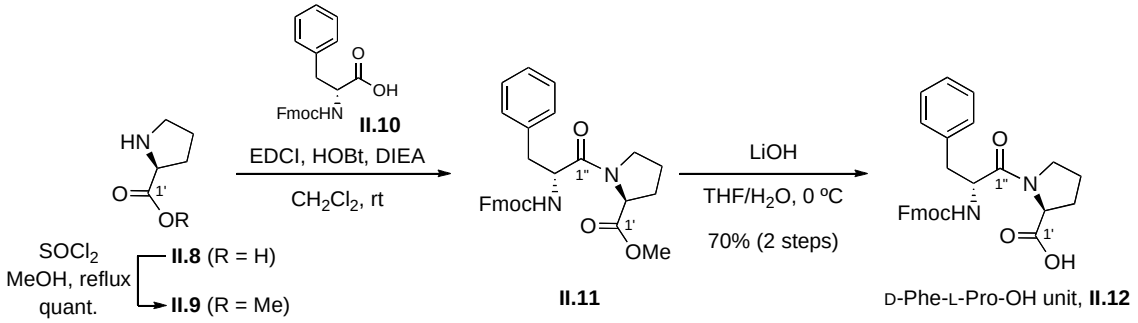
The devised route to macrolactam **II.A** featured an esterification between the C1-C7 fragment **II.B** and the dipeptide D-Phe-L-Pro-OH unit **II.C**, and a macrolactamization as the two key steps (Scheme II.9).

II.1.2.1.3.b. Synthesis of the dipeptide D-Phe-L-Pro-OH unit

The dipeptide Fmoc-D-Phe-L-Pro-OH unit **II.12** was prepared in three steps and 70% overall yield following a reported procedure[123] (Scheme II.12). The latter involved the

[123] Stüber, W.; Kosina, H.; Heimburger, N. *Int. J. Pept. Protein Res.* **1988**, *31*, 63-70.

conversion of proline **II.8** to the corresponding methyl ester ($SOCl_2$, MeOH, reflux) followed by a peptide coupling with Fmoc-D-Phe-OH **II.10** (EDCI, HOBt, DIEA, CH_2Cl_2, rt), and a selective saponification of the methyl ester moiety (LiOH, THF/H_2O, 0 °C).



Scheme II.12. Synthesis of dipeptide unit **II.12**

II.1.2.1.3.c. Synthesis of macrolactam II.19

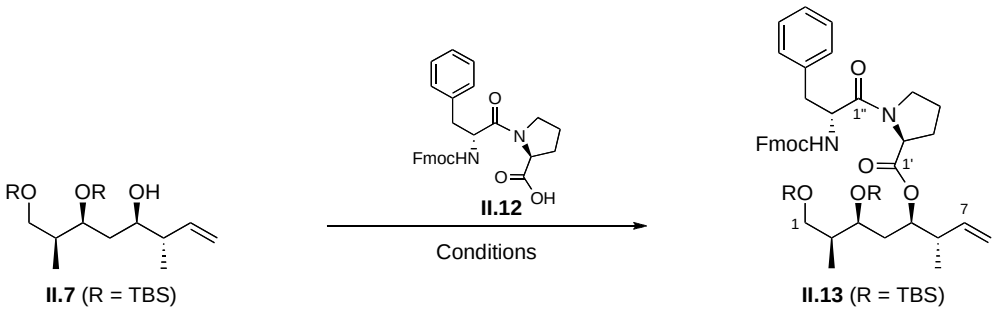
II.1.2.1.3.c.1. Esterification step

With the two partners **II.7** and **II.12** in hand, the stage was set for the key esterification. Interestingly, while our first attempt using the Steglich conditions[124] with *N*,*N*'-dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-dimethylaminopyridine (DMAP) at –20 °C led to the complete recovery of the starting material **II.7** (Table II.1, entry 1), increasing the temperature allowed to isolate the desired product **II.13** in 20% yield (Table II.1, entry 2). The reaction using a phosphonium-mediated esterification (BOP, HOBt, DIEA, CH_2Cl_2, rt), largely used in Solid Phase Peptide Synthesis (SPPS), gave a mixture of products which identification remained difficult (Table II.1, entry 3). On the other hand, whereas no reaction took place when combining 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and HOBt in the presence of DIEA (Table II.1, entry 4), the use of DMAP instead of HOBt afforded a moderate 45% yield (Table II.1, entry 5). Finally, the use of the Yamaguchi esterification conditions[125] (2,4,6-trichlorobenzoyl chloride, DMAP, DIEA, toluene, rt)

[124] Neises, B.; Steglich, W. *Angew. Chem. Int. Ed.*, **1978**, *17*, 522-524.
[125] (a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989-1993. (b) Kawanami, Y.; Dainobu, Y.; Inanaga, J.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 943-944.

afforded the best results, as the desired ester **II.13** was isolated in 85% yield (Table II.1, entry 6).

Table II.1. Esterification step between coupling partners **II.7** and **II.12**



II.7 (R = TBS) **II.13** (R = TBS)

Entry	Reagents (equiv)	T (ºC)	Time (h)	Solvent	Yield (%)[a]
1	**II.7** (3.0) DCC (3.0) + DMAP (0.6)	–20	24	CH_2Cl_2	–[b]
2	**II.7** (3.0) DCC (3.0) + DMAP (0.6)	rt	24	CH_2Cl_2	20[b]
3	**II.7** (1.5) BOP (1.5) + HOBt (1.5) DIEA (3.5)	rt	16	DMF/CH_2Cl_2 (5:1)	–[c]
4	**II.7** (1.5) EDCI (1.5) + HOBt (1.5) DIEA (3.5)	rt	40	CH_2Cl_2	–[b]
5	**II.7** (1.5) EDCI (1.5)+DMAP (0.15)	rt	40	CH_2Cl_2	45[b]
6	**II.7** (1.5) 2,4,6-Cl_3PhCOCl (3.0) DMAP (2.0)+DIEA (3.7)	rt	5	Toluene	85

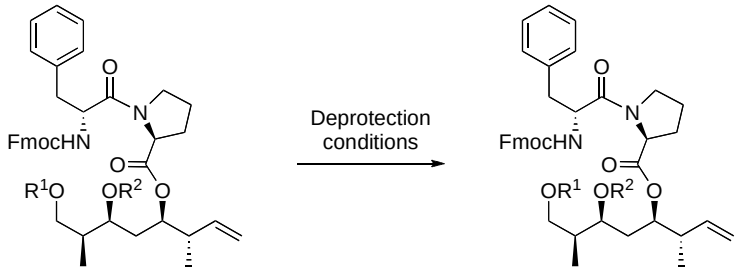
[a] Isolated yield. [b] Starting material **II.7** was recovered. [c] Mixture of unidentified by-products.

II.1.2.1.3.c.2. Synthesis of macrolactam II.19

The synthesis of macrolactam **II.19** began with the selective cleavage of the primary TBS ether. Interestingly however, while treatment with excess ammonium fluoride in

refluxing methanol[126] left the starting material **II.13** unreacted (Table II.2, entry 1), the use of a large excess of acetic acid in a THF/H_2O mixture led to a mixture of mono-deprotected and fully deprotected substrates in 50% and 35% yield, respectively (Table II.2, entry 2). After a detailed analysis of the former, ^{1}H NMR revealed the existence of two constitutional isomers resulting from an intramolecular silyl migration.[127] Our next attempt using camphorsulfonic acid (CSA) in a MeOH/CH_2Cl_2 mixture afforded the targeted product **II.14** in 46% yield along with a significant amount of the fully deprotected product (Table II.2, entry 3). Finally, treatment of **II.13** with $ZnBr_2$ (CH_2Cl_2, rt)[128] allowed the isolation of the required primary alcohol **II.14** in 94% yield (Table II.2, entry 4).

Table II.2. Selective cleavage of the primary TBS ether **II.13**



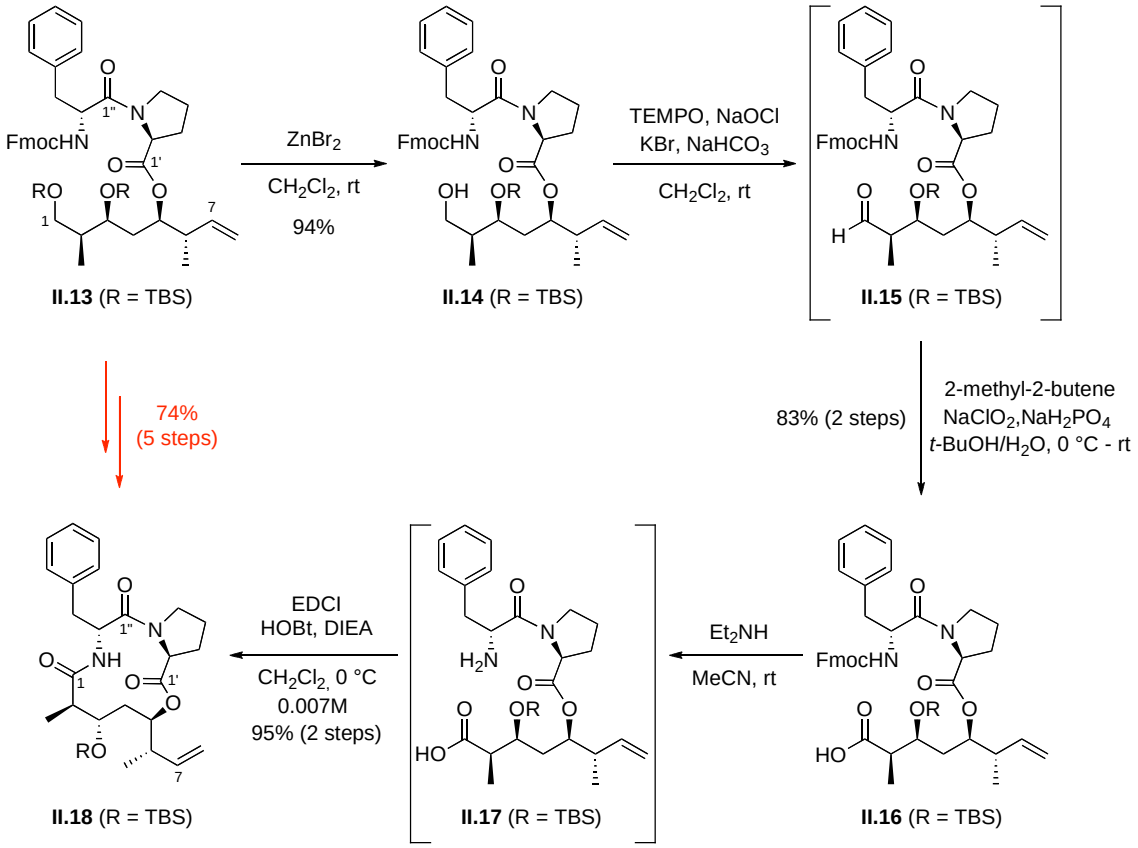
II.13 ($R^1 = R^2 = TBS$) **II.14** ($R^1 = H$, $R^2 = TBS$)

Entry	Reagents (equiv)	T (ºC)	Time (h)	Solvent	Products (Yield, %)[a]
1	NH_4F (10)	rt-reflux	5	MeOH	–[b]
2	AcOH (10)	rt	4	THF/H_2O (1:1)	R^1 = H/TBS, R^2 = TBS/H (50)[c] R^1, R^2 = H (35)
3	CSA (0.9)	0 ºC-rt	3	MeOH/CH_2Cl_2 (1:1)	R^1 = H, R^2 = TBS (46) R^1, R^2 = H (35)
4	$ZnBr_2$ (5.6)	rt	4	CH_2Cl_2	R^1 = H, R^2 = TBS (94)

[a] Isolated yield. [b] Starting material **II.13** was recovered. [c] Unseparable mixture.

[126] Zhang, W.; Robbins, M. J. *Tetrahedron Lett.* **1992**, *33*, 1177-1180.
[127] Wuts, P. G. M.; Bigelow, S. S. *J. Org. Chem.* **1988**, *53*, 5023-5034.
[128] Crouch, R. D.; Polizzi, J. M.; Cleiman, R. A.; Yi, J.; Romany, C. A. *Tetrahedron Lett.* **2002**, *43*, 7151-7153.

The resulting alcohol **II.14** was subsequently oxidized, first to aldehyde **II.15** using TEMPO and NaOCl as standard Swern conditions [$(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, –78 °C] failed dramatically. Then, the aldehyde was oxidized to the corresponding carboxylic acid **II.16** using 2-methyl-2-butene, $NaClO_2$, NaH_2PO_4, in a *t*-BuOH/H_2O mixture (83% yield over two steps).[129] The Fmoc protecting group was removed (Et_2NH, MeCN) to provide amino acid **II.17**, which was directly engaged in the key macrolactamization using standard peptide coupling conditions under higher dilution (*c* = 0.007M) in order to favour the intramolecular amide bond formation (EDCI, HOBt, DIEA, CH_2Cl_2, 0 °C). Under these conditions, the desired 12-membered ring macrolactam **II.18** was obtained in 95% yield (Scheme II.13).



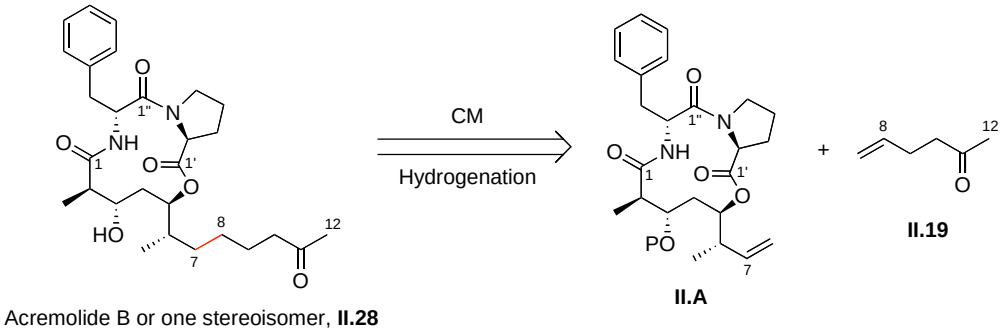
Scheme II.13. Synthesis of macrolactam **II.18**

[129] Kurosawa, K.; Matsuura, K.; Nagase, T.; Chida, N. *Bull. Chem. Soc. Jpn.* **2006**, *6*, 921-937.

II.1.2.1.4. Attempted synthesis of acremolide B

II.1.2.1.4.a. Retrosynthetic analysis of acremolide B

In order to introduce the side chain and complete the synthesis of acremolide B, a three-step sequence involving a CM with commercially available 5-hexen-2-one **II.29**, a hydrogenation of the newly formed C7-C8 double bond, and a final cleavage of the protecting group was envisioned (Scheme II.14).



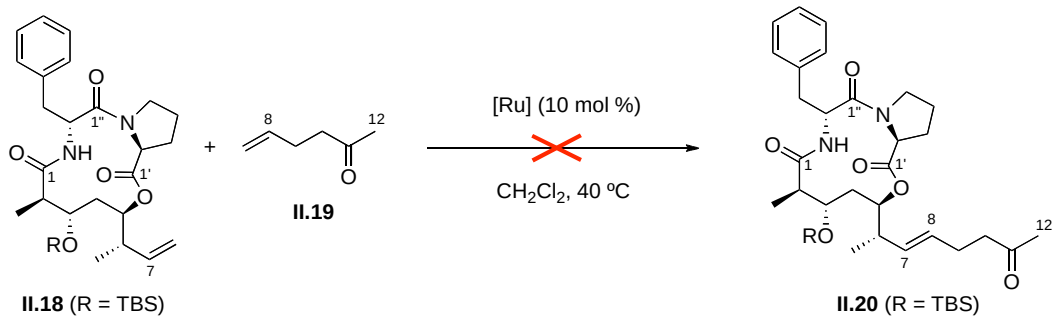
Scheme II.14. Retrosynthetic analysis of acremolide B, **II.28**

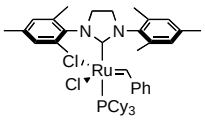
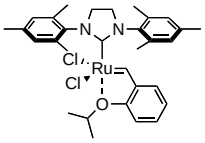
II.1.2.1.4.b. Attempted coupling of the C1-C7 and C8-C12 subunits by olefin cross-metathesis

The two coupling partners – macrolactone olefin **II.18** and 5-hexen-2-one (**II.19**) **–** were therefore engaged in a ruthenium-catalyzed CM to afford the acremolide precursor **II.20**. Unfortunately, none of the conditions tested using either the Grubbs second-generation catalyst ([Ru]-**I**)[130] or the Hoveyda-Grubbs second-generation catalyst ([Ru]-**II**)[131] produced the desired cross-coupled product (Table II.3, entries 1 and 2).

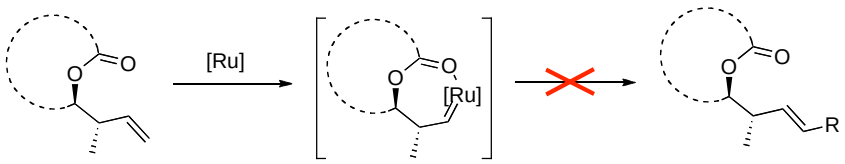
[130] Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953-956.
[131] (a) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 8168; (b) Gessler, S.; Randl, S.; Blechert, S. *Tetrahedron Lett.* **2000**, *41*, 9973-9976.

Table II.3. Cross-coupling metathesis between alkenes **II.18** and **II.19**



Entry	[Ru]	**II.19** (equiv)	Time (h)	Yield (%)
1	 [Ru]-**I**	2.0	15	–
2	 [Ru]-**II**	2.0	12	–

This unfortunate outcome may be the result of a counter-productive chelation between the ruthenium carbene species and the carbonyl present in its vicinity (Scheme II.15).[132]



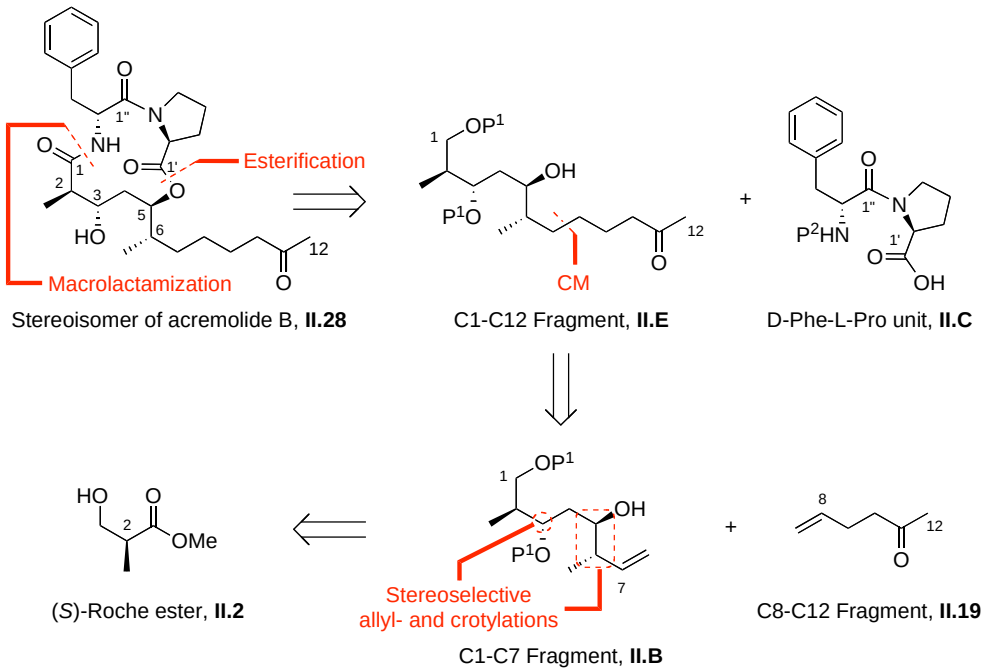
Scheme II.15. Proposed structure for the chelation of the [Ru] by the carbonyl group

[132] (a) Fürstner, A.; Langemann, K. *Synthesis* **1997**, 792-803. (b) Fürstner, A. *Top. Organomet. Chem.* **1998**, *1*, 37-72.

II.1.2.1.5. Second synthetic approach to acremolide B

II.1.2.1.5.a. Retrosynthetic analysis of acremolide B

In order to circumvent this particularly dramatic issue, we decided to slightly modify our route to acremolide B. Hence, the second strategy relied on the same four disconnections as previously, but implied the introduction of the side chain **II.19** prior to the dipeptide unit (Scheme II.16). Consequently, the C1-C7 fragment **II.B** could be obtained from (*S*)-Roche ester **II.2** *via* two stereoselective allyl- and crotyltitanations, while a CM with coupling partner **II.19** would enable the construction of the C7-C8 bond and thus afford the entire C1-C12 fragment. Esterification of the latter with the dipeptide unit **II.C** followed by a macrolactamization would eventually lead to acremolide B or to one of its stereoisomer.

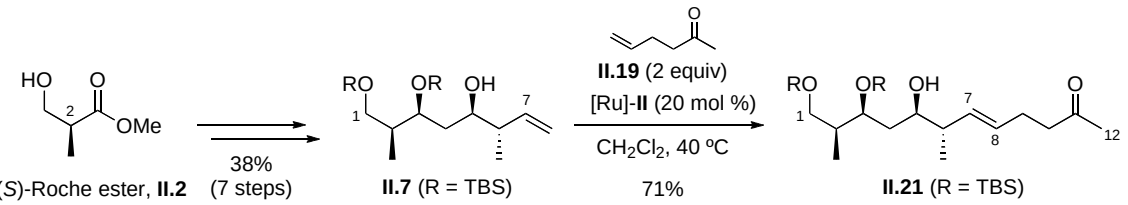


Scheme II.16. Second retrosynthetic analysis of acremolide B, **II.28**

II.1.2.1.5.b. Synthesis of the C1-C12 fragment

The C1-C7 fragment **II.7**, previously prepared in seven steps and 38% overall yield starting from (*S*)-Roche ester **II.2**, was directly engaged in a CM with 5-hexen-2-one (**II.19**) (2 equiv) using 20 mol % of the Hoveyda-Grubbs catalyst [Ru]-**II** in refluxing

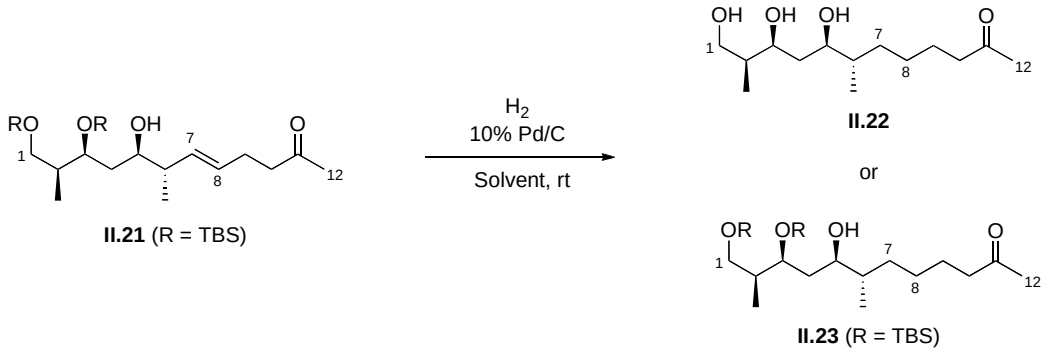
CH_2Cl_2. To our delight, the desired disubstituted olefin was obtained in 71% yield (Scheme II.17).



Scheme II.17. Synthesis of olefin **II.21**

In order to reduce the double bond, olefin **II.21** was engaged in a hydrogenation reaction using 10% Pd/C (MeOH, rt). Unfortunately, under these reaction conditions only the fully TBS-deprotected product **II.22** was isolated, albeit in high yield (Table II.4, entry 1). The cleavage of the silyl protecting groups under hydrogenation conditions being susceptible to significant solvent effects,[133] the hydrogenation was carried out in EtOAc under otherwise identical conditions. Fortunately, this allowed the isolation of the desired compound **II.23** in 90% yield (Table II.4, entry 2).

Table II.4. Hydrogenation of olefin **II.21**



Entry	Solvent	t (h)	Product	Yield (%)[a]
1	MeOH	3	**II.22**	85
2	EtOAc	4	**II.23**	90

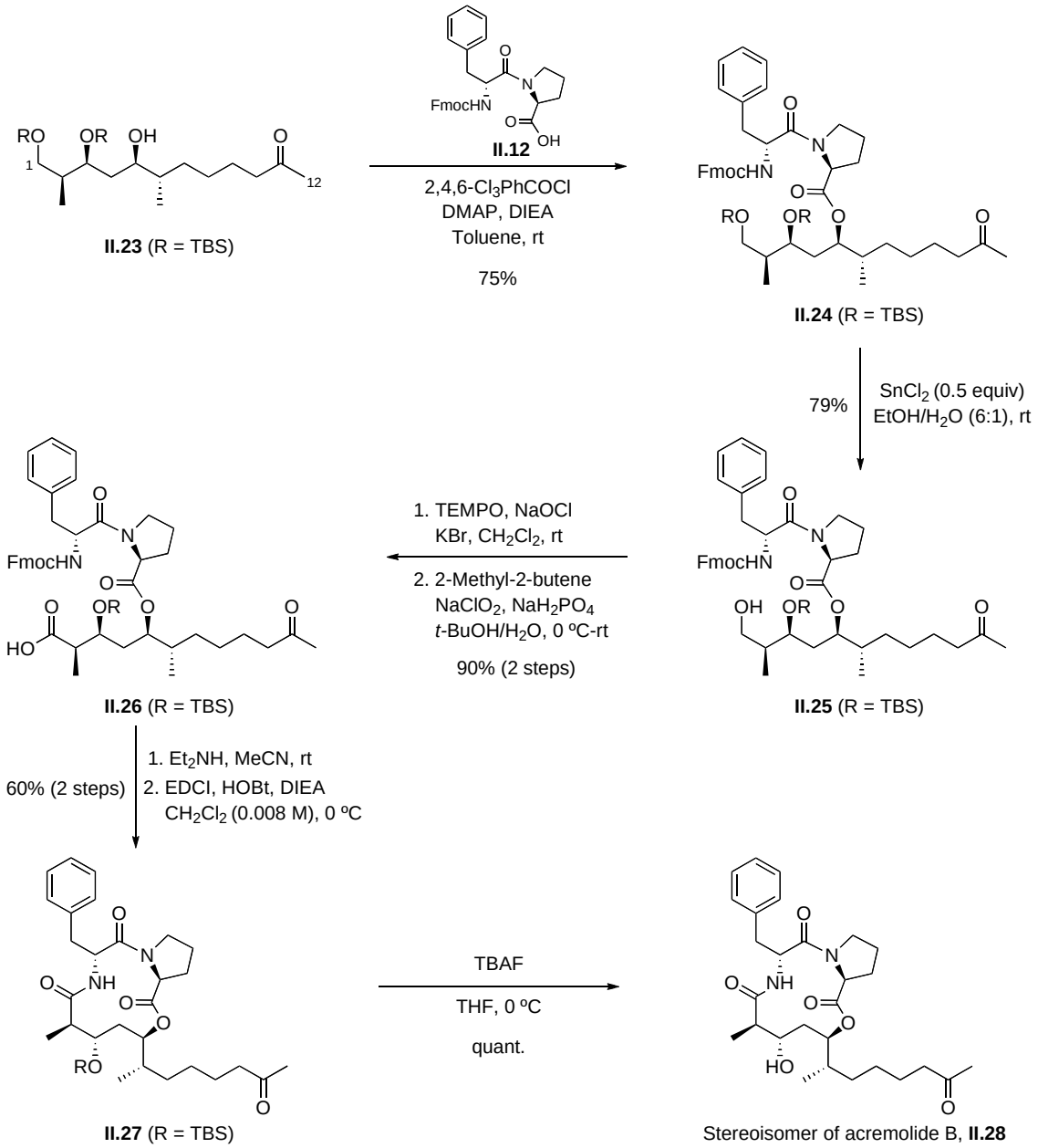
[a] Isolated yield.

[133] Ikawa, T.; Hattori, K.; Sajiki, H.; Hirota, K. *Tetrahedron* **2004**, *60*, 6901-6911.

II.1.2.1.5.c. Completion of the synthesis

The introduction of the dipeptide unit **II.12** using the same Yamaguchi esterification conditions (2,4,6-trichlorobenzoyl chloride, DMAP, DIEA, toluene, rt) as previously, afforded the desired coupled product **II.24** in 75% yield (Scheme II.18). The primary TBS ether was then selectively cleaved using 0.5 equiv of $SnCl_2$ in a 6:1 mixture of $EtOH/H_2O$[134] (79% yield) as $ZnBr_2$ induced a slightly lower yield (60%). The resulting alcohol **II.25** was subsequently oxidized first to the corresponding aldehyde using TEMPO, NaOCl, KBr in CH_2Cl_2, then to the carboxylic acid derivative **II.26** using 2-methyl-2-butene, $NaClO_2$, NaH_2PO_4, in a *t*-$BuOH/H_2O$ mixture (90% yield over two steps). The latter was then treated with Et_2NH in order to cleave the Fmoc protecting group, and the resulting amino acid was directly engaged in the key macrolactamization (EDCI, HOBt, DIEA, CH_2Cl_2, rt) at high dilution (c = 0.008M) which afforded the expected 12-membered-ring lactam **II.27** in 60% overall yield. The (2*R*,3*S*,5*R*,6*S*)-macrolactone **II.28** was finally isolated after removal of the TBS protecting group using TBAF (THF, 0 ºC). The latter was thus synthesized in 16 steps and 7.6% overall yield starting from (*S*)-Roche ester **II.2**.

[134] Hua, J.; Jiang, Z. Y.; Wang, Y. G. *Chin. Chem. Lett.* **2004**, *15*, 1430-1432.



Scheme II.18. Synthesis of *epi*-acremolide B, **II.28**

Careful analysis of the spectroscopic and physical data of **II.28** and comparison with the ones reported in the literature for the natural product {$[\alpha]^{20}_{D}$ –65.2 (*c* 0.02, MeOH); lit.[96] $[\alpha]_{D}$ –98 (*c* 0.02, MeOH)} confirmed the fact that we had synthesized one of the 16 possible stereoisomers of acremolide B. Due to the macrocyclic structure of the product, it is particularly difficult to speculate on the relative and absolute configuration of the C2, C3, C5 and C6 stereogenic centers of acremolide B since a slight modification of one of them could cause a significant change on the global shape of the molecule and consequently on all the chemical shifts.

In an attempt to revise the structure of acremolide B according to the information extracted from the NMR comparison using a graphic of the difference between the ^{13}C chemical shifts of the natural product and the synthetic one is presented.[135] Consequently, taking into account that the differences for C7, C14, C21 and C23 atoms are higher than the rest, their configurations should be different from the synthesized stereoisomer. Mainly, a proposed structure based on these results should include surprisingly a revision for the D-Phe stereogenic center, which was already determined by Capon *et al.*, as well as the *anti* relationship between the substituents at C7 and C8, which could give three different combinations: *anti*-(7*S*,8*R*), *syn*-(7*R*,8*R*) or *syn*-(7*S*,8*S*) (Figure II.7).

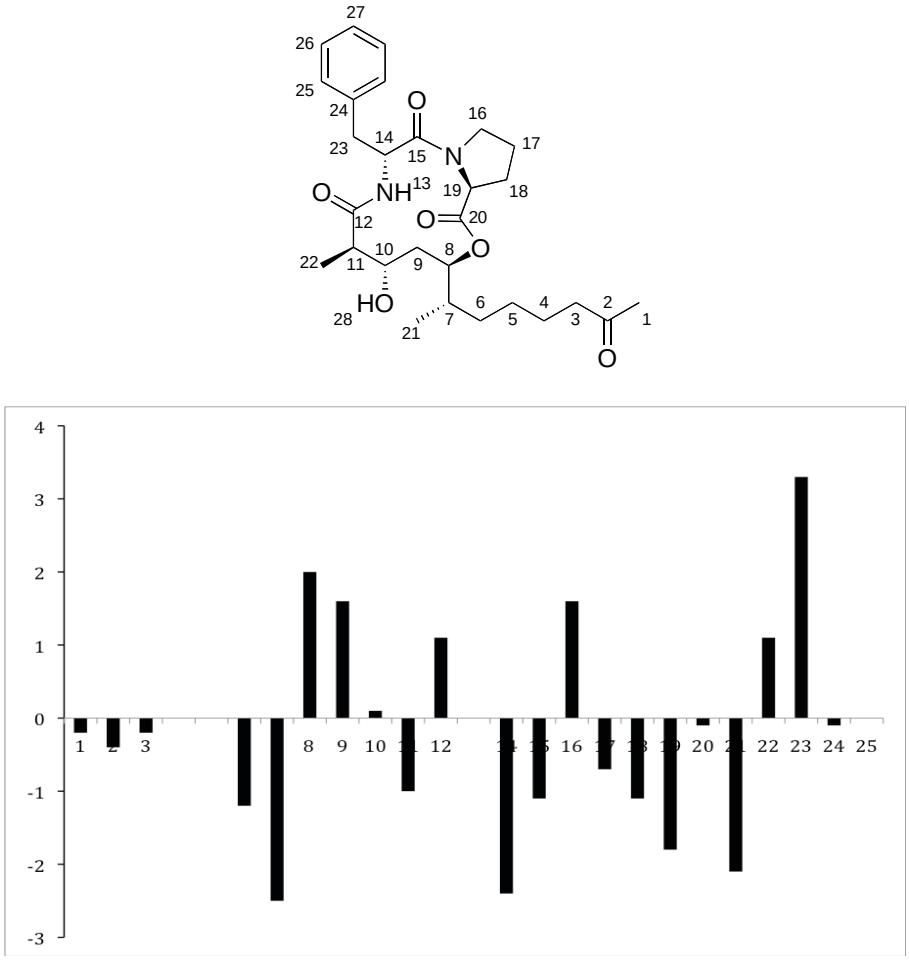


Figure II.7. Graphically depicted ^{13}C chemical shift differences (Δδ, ppm) for each carbon of acremolide B natural product and the synthetic stereoisomer, **II.28**

[135] The numbering of the atoms has been changed for an easier comparison with the reported data. In addition, C4, C5, C26 and C27 were not included as their corresponding ^{13}C chemical shift were not described for the natural product.

PART 2

Total synthesis of the nominal lyngbouilloside aglycon

II.2.1. INTRODUCTION

II.2.1.1. ISOLATION, STRUCTURE AND BIOLOGICAL PROPERTIES OF LYNGBOUILLOSIDE

II.2.1.1.1. Isolation

Lyngbouilloside is a glycosidic macrolide isolated by Gerwick *et al*. from the marine cyanobacterium *Lyngbya bouillonii* collected off the North Coast of Papua New Guinea (Figure II.8).[136] This particularly intriguing subtidal and filamentous species of *Lyngbya* was later identified as *Lyngbya bouillonii* Hoffman and Demoulin,[137] a species mainly found in coral reefs where it forms dark reddish, non-gelatinous, and tenacious plant masses.

L. bouillonii has quickly emerged as an exceptional source of new natural products including the linear tetrapeptide, lyngbyapeptin,[138] the three macrolides: laingolide, laingolide A and madangolide,[139] and various structural homologues of lyngbouilloside;

---

[136] Tan, L. T.; Márquez, B. L.; Gerwick, W. H. *J. Nat. Prod.* **2002**, *65*, 925-928.
[137] Hoffmann, L.; Demoulin, V. *Belg. J. Bot.* **1991**, *124*, 82-88.
[138] Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Castillo, G.; Demoulin, V. *Tetrahedron Lett.* **1999**, *40*, 695-696.
[139] (a) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Demoulin, V. *Tetrahedron Lett.* **1996**, *37*, 7519-7520. (b) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Castillo, G.; Demoulin, V. *J. Nat. Prod.* **1999**, *62*, 934-936.

lyngbyaloside,[140] lyngbyaloside B[141] and the recently isolated lyngbyaloside C (Figure II.8).[142]

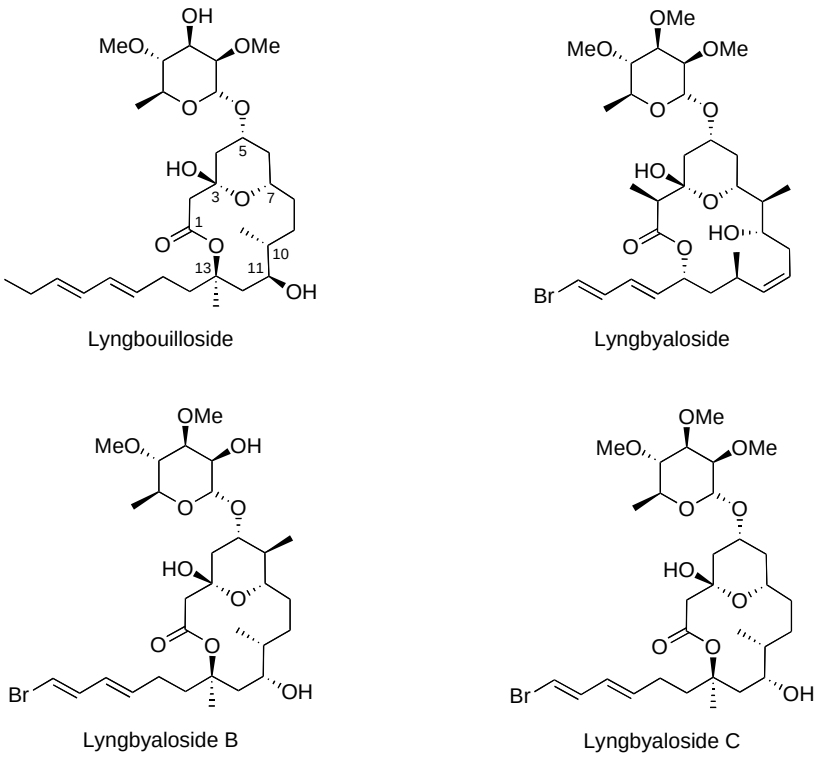


Figure II.8. Structures of lynbouilloside, lyngbyaloside, lyngbyalosides B and C

II.2.1.1.2. Structural assignment of lyngbouilloside

Despite the relative and absolute configuration of both the sugar and the aglycon units which still remain unknown, the gross structure of lyngbouilloside was elucidated after exhaustive spectroscopic analysis and chemical derivatizations.[136] HRMS analysis, for instance, indicated a molecular formula of $C_{31}H_{52}O_{10}$ suggesting the presence of six degrees of unsaturation. Three were assigned by ^{13}C NMR to two olefins, which correspond to a conjugated diene system according to the UV spectrum, and one carbonyl. This observation was eventually comforted by the IR data, which, in addition to the carbonyl moiety, also revealed the presence of hydroxyl groups. The remaining three unsaturations were therefore attributed to a tricycle structure. Interestingly,

[140] Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Demoulin, V. *J. Nat. Prod.* **1997**, *60*, 1057-1059.
[141] Luesch, H.; Yoshida, W. Y.; Harrigan, G. G.; Doom, J. P.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 1945-1948.
[142] Matthew, S.; Salvador, L.; Schupp, P.J.; Paul, V.J.; Luesch, H. *J. Nat. Prod.* **2010**, *73*, 1544-1552.

NMR analysis of the corresponding acetylated lyngbouilloside also helped in the determination of the structure of the backbone core. Indeed, while the planar structure of the natural product was established by 1D and 2D NMR analysis, the nature of the sugar moiety was secured by comparing the NMR data with the ones pertaining to the sugar moiety in the *Dolabella auricularia* metabolite auriside A (Figure II.9).[143] Consequently, the relative stereochemistry of the sugar ring was assigned arbitrarily as (1'*R*,2'*R*,3'*R*,4'*S*,5'*S*). Additional NMR experiments (ROESY correlations between H-5 and H-7, and H-7 and H-10) allowed to establish the relative stereochemistry of the aglycon portion of lyngbouilloside as (3*S*,5*R*,7*S*,10*R*,11*S*,13*R*).

As depicted in Figure II.8, lyngbouilloside was finally assigned as a 14-membered macrolide that contains a 2,4-di-*O*-methylrhamnopyranoside unit and a hemiketalpyran. Interestingly, although lyngbouilloside is the second example after acutiphycin of a macrolide glycoside isolated directly from a marine cyanobacterium, several structurally related compounds such as auriside A, callipeltoside A, phorbaside A or dolastatin 19 were isolated from marine invertebrates suggesting a common cyanobacterial origin either through sequestration in the diet (e.g., molluscs) or through symbiotic associations (e.g., sponges) (Figure II.9).[143, 144]

[143] Sone, H.; Kigoshi, H.; Yamada, K. *J. Org. Chem.* **1996**, *61*, 8956-8960.
[144] (a) Zampella, A.; Dauria, M. V.; Minale, L.; Debitus, C.; Roussakis, C. *J. Am. Chem. Soc.* **1996**, *118*, 11085-11088. (b) Pettit, G. R.; Xu, J. P.; Doubek, D. L.; Chapuis, J. C.; Schmidt, J. M. *J. Nat. Prod.* **2004**, *67*, 1252-1255.

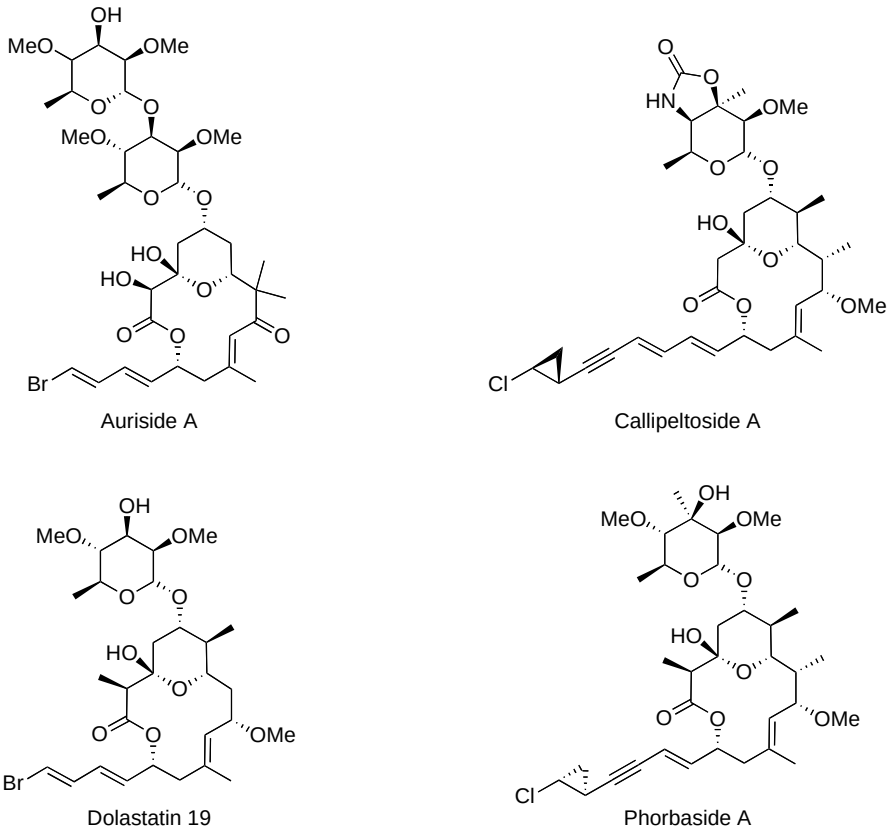


Figure II.9. Structure of auriside A, callipeltoside A, dolastatin 19 and phorbaside A

II.2.1.1.3. Biological properties of lyngbouilloside

The extreme scarcity of lyngbouilloside has hampered the efforts to establish a complete biological profile of the natural product. However, Gerwick *et al.* were able to show that it was moderately cytotoxic against neuro-2a neuroblastoma cells with an IC_{50} = 17 μM. Besides, the significant cytoxicity displayed by the structurally related 14-membered macrolides, callipeltosides, phorbaside A, lyngbyaloside, auriside A and dolastatin 19, suggests a promising cytotoxicity pattern.

II.2.1.2. REPORTED EFFORTS TOWARDS THE TOTAL SYNTHESIS OF LYNGBOUILLOSIDE

The encouraging cytotoxic activity of lyngbouilloside combined with its challenging molecular architecture and its natural scarcity attracted the attention of various groups

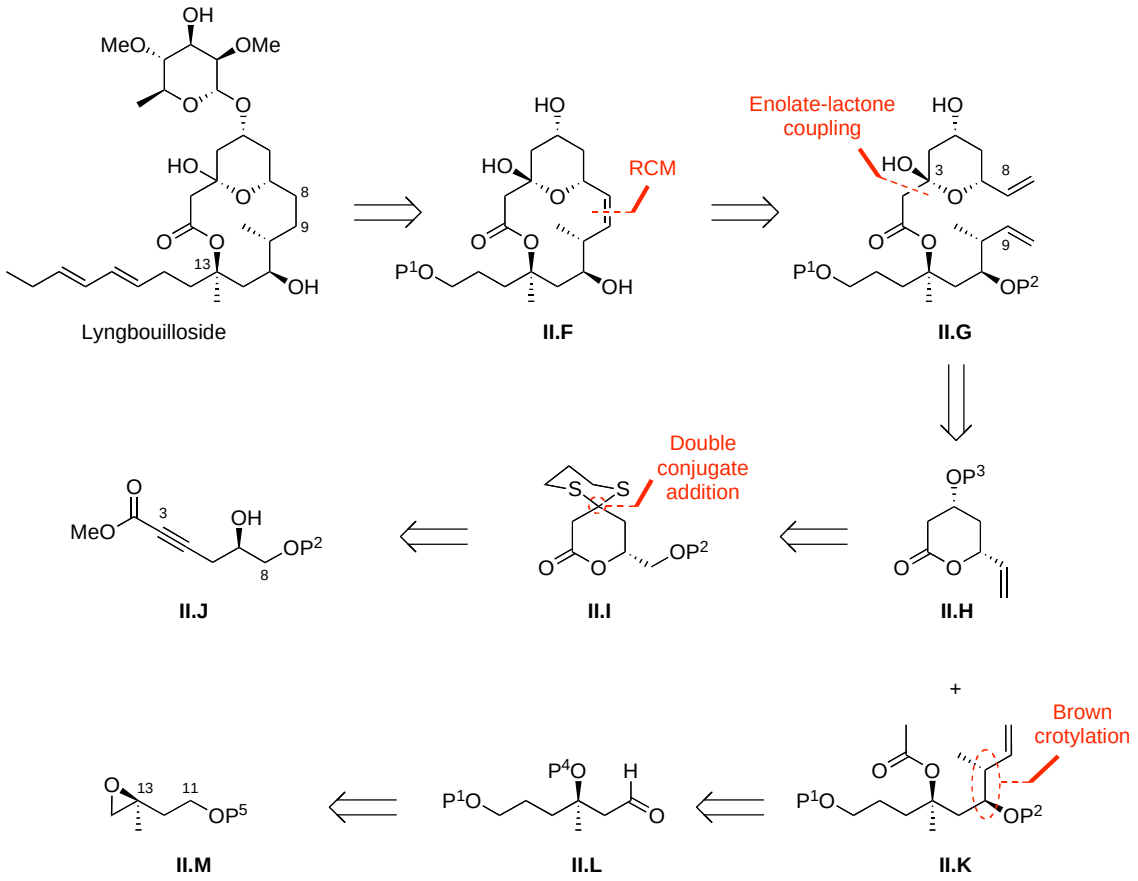
around the world. Although, no total synthesis has been reported so far, Ley *et al.*[145] and Cossy *et al.*[146] recently reported their efforts towards the synthesis of the lyngbouilloside framework.

II.2.1.2.1. Synthesis of the macrolactone core of lyngbouilloside by Ley *et al.*

II.2.1.2.1.a. Key steps

In 2009, Ley *et al.*[145] reported their initial efforts towards the synthesis of lyngbouilloside describing a synthetic route to the macrocyclic core of the natural product. The key steps of their strategy included a RCM to build the macrolactone[147] through the C8-C9 bond (Scheme II.19), an enolate-lactone coupling between **II.H** and **II.K** to install the C3 substituent on the pyran ring, and a double conjugate addition of propane-1,3-dithiol onto the conjugated ester **II.J** followed by an *in situ* cyclization to generate the desired lactone **II.I**.[148] Finally, the control of the C10 and C11 stereogenic centers was secured by a stereoselective Brown crotylation of aldehyde **II.L** (Scheme II.19).[108b]

[145] Webb, D.; Van den Heuvel, A.; Kögl, M.; Ley, S. V. *Synlett*, **2009**, 2320-2324.
[146] Gebauer, J.; Arseniyadis, S.; Cossy, J. *Synlett* **2008**, 712-714.
[147] Hong, S. H.; Sanders, D. P.; Lee, C. W.; Grubbs, R. H. *J. Am. Chem. Soc.* **2005**, *127*, 17160-17161.
[148] (a) Sneddon, H. F.; Gaunt, M. J.; Ley, S. V. *Org. Lett.* **2003**, *5*, 1147-1150. (b) Gaunt, M. J.; Sneddon, H. F.; Hewitt, P. R.; Orsini, P.; Hook, D. F.; Ley, S. V. *Org. Biomol. Chem.* **2003**, *1*, 15-16. (c) Sneddon, H. F.; Van den Heuvel, A.; Hirsch, A. K. H.; Booth, R. A.; Shaw, D. M.; Gaunt, M. J.; Ley, S. V. *J. Org. Chem.* **2006**, *71*, 2715-2725.



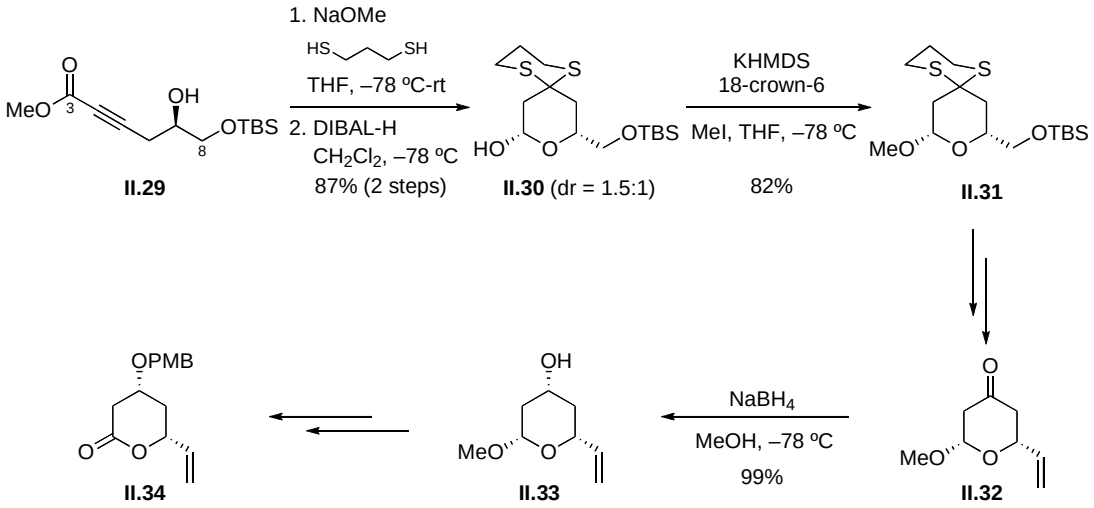
Scheme II.19. Ley's retrosynthetic analysis of the lyngbouilloside macrolactone core

II.2.1.2.1.b. Synthesis of the C3-C8 fragment II.35

The synthesis of the C3-C8 fragment of lyngbouilloside began from the known ynone **II.29**, which was prepared in two steps starting from (*S*)-glycidol.[149] Hence, ynone **II.29** was first engaged in a double conjugate addition with propane-1,3-dithiol to afford the corresponding hydroxyester which underwent simultaneous *in situ* cyclization to furnish the corresponding lactone. The latter was then reduced with DIBAL-H to the analogous hemiketal **II.30** which was obtained as a mixture of diastereoisomers (dr = 1.5:1) in 87% overall yield. The resulting δ-lactol was eventually deprotonated with KHMDS in the presence of 18-crown-6[150] to afford the corresponding alkoxide which underwent a highly diastereoselective methylation to the corresponding ketal **II.31**. A few subsequent steps enabled to set the terminal olefin and convert the dithiol moiety to the corresponding ketone **II.32**, while reduction with sodium borohydride

[149] Maguire, R. J.; Munt, S. P.; Thomas, E. J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2853-2864.
[150] Adderley, N. J.; Buchanan, D. J.; Dixon, D. J.; Laine, D. I. *Angew. Chem. Int. Ed.* **2003**, *42*, 4241-4244.

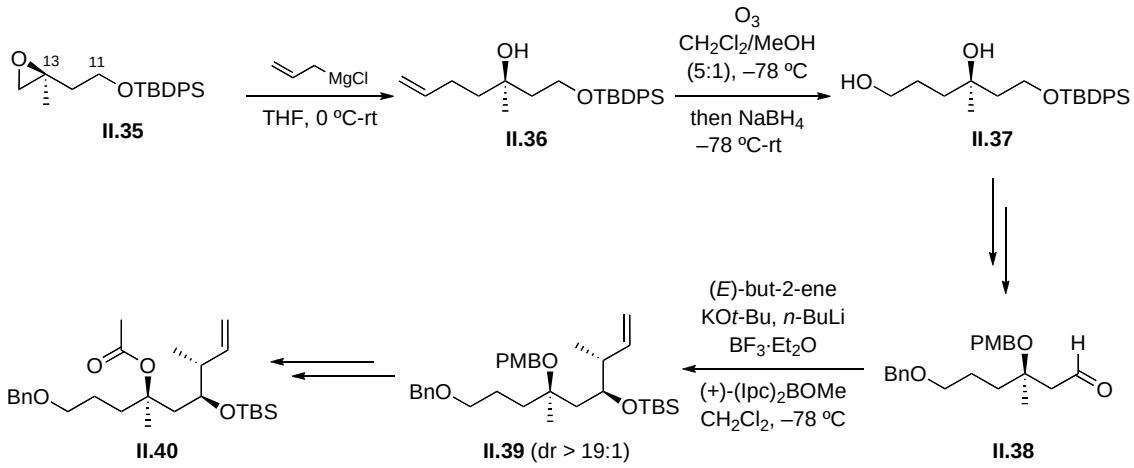
introduced the C5 stereogenic center and generated **II.33** as a single diastereoisomer in 99% yield. A final PMB-protection/hydrolysis/oxidation sequence ultimately provided the desired lactone **II.34** in 46% yield (Scheme II.20).



Scheme II.20. Ley's synthesis of the C3-C8 fragment **II.34**

II.2.1.2.1.c. Synthesis of the C2-C9 fragment II.40

The first step in the preparation of fragment **II.40** was the regioselective opening of the optically active epoxide **II.35** with allyl magnesium chloride. The ring-opening proceeded cleanly to generate the mono-protected 1,3-diol **II.36** which was converted to the corresponding primary alcohol **II.37** upon ozonolysis with a reductive work-up. The latter was then converted to aldehyde **II.38** after a few trivial transformations and subjected to a Brown crotylboration to afford the corresponding *anti*-homoallylic alcohol **II.39** with a high stereocontrol (dr > 19:1). Several protecting group manipulations ultimately gave the desired fragment **II.40** in 33.1% overall yield (Scheme II.21).

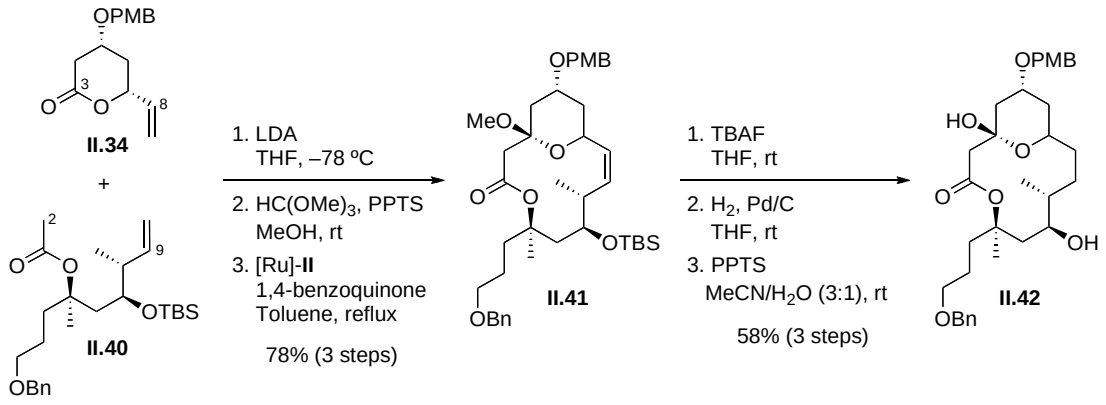


Scheme II.21. Ley's synthesis of the fragment **II.40**

II.2.1.2.1.d. Synthesis of the macrolactone core of lyngbouilloside

With effective routes to fragments **II.34** and **II.40** in place, attention was next turned to their successful coupling (Scheme II.22). To that effect, lactone **II.34** was added to 2 equiv of the lithium enolate derived from **II.40** to afford the corresponding lactol in quantitative yield. The latter was then converted to the acetal [$HC(OMe)_3$, PPTS] and ring-closed using the Hoveyda-Grubbs second-generation catalyst in the presence of 1,4-benzoquinone,[147] and the resulting macrocycle **II.41** was treated with TBAF to remove the TBS protecting group. Finally, hydrogenation followed by mild acid hydrolysis provided the desired macrolactone **II.42** in 46% yield over four steps (Scheme II.22).

This strategy afforded an advanced macrolactone intermediate **II.42** in 17 steps and 12.4% overall yield starting from homopropargylic alcohol **II.29** and terminal epoxide **II.35**. Most importantly, spectroscopical analysis and DFT chemical shift calculations of their lyngbouilloside precursor provided evidence of a possible structure misassigned.



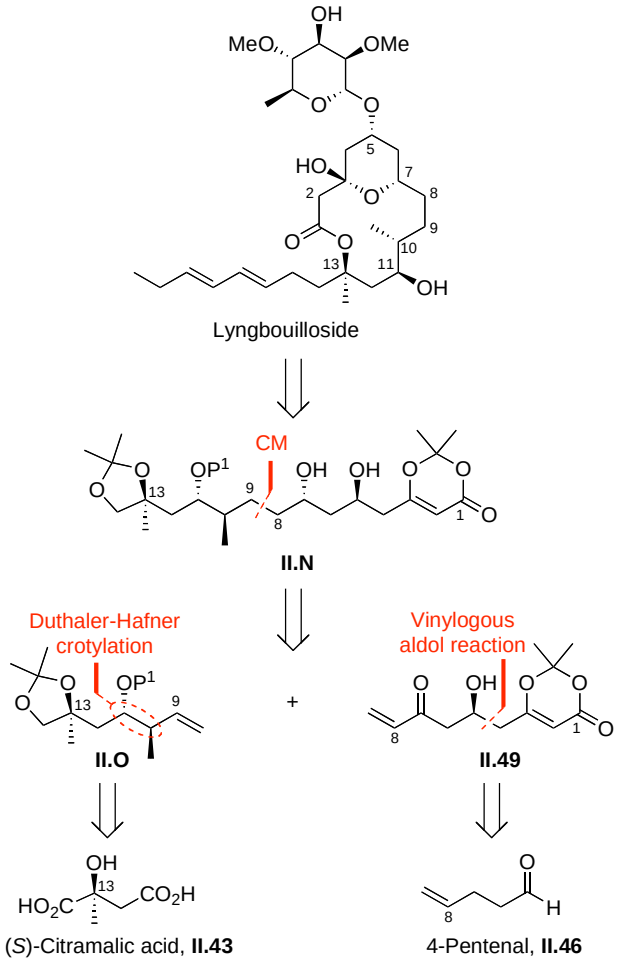
Scheme II.22. Ley's synthesis of the lyngbouilloside macrolactone core

II.2.1.2.2. Synthesis of the C1–C13 fragment of lyngbouilloside by Cossy *et al.*

II.2.1.2.2.a. Key steps

Cossy *et al.*[146] also got involved in the total synthesis of lyngbouilloside roughly at the same time as Ley and co-workers. Their approach involved a selective CM between the fully functionalized C1-C8 and C9-C13 fragments **II.49** and **II.O** to afford the macrolactone precursor **II.N**, while a highly enantio- and diastereoselective crotyltitanation and a vinylogous aldol[151] combined to a preparative chiral HPLC separation controlled the C5, C10 and C11 stereogenic centers (Scheme II.23).

[151] Bach, T.; Kirsch, S. *Synlett* **2001**, 1474-1476.



Scheme II.23. Retrosynthetic analysis of C1-C13 fragment by Cossy *et al.*

II.2.1.2.2.a. Synthesis of the C1-C13 fragment

The convergent strategy used for the synthesis of the C1-C13 carbon backbone of lyngbouilloside started with the preparation of the two subunits **II.49** and **II.45**. The former was synthesized using a vinylogous Mukaiyama aldol reaction between 4-pentenal (**II.46**) and the silyl dienol ether derived from 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (**II.47**)[152] to afford the corresponding alcohol **II.48** as a racemic mixture of enantiomers, which were separated by chiral preparative HPLC.[153]

The required (*R*)-enantiomer, which absolute configuration was secured by hydrogenation of the terminal double bond and comparison of its optical rotation

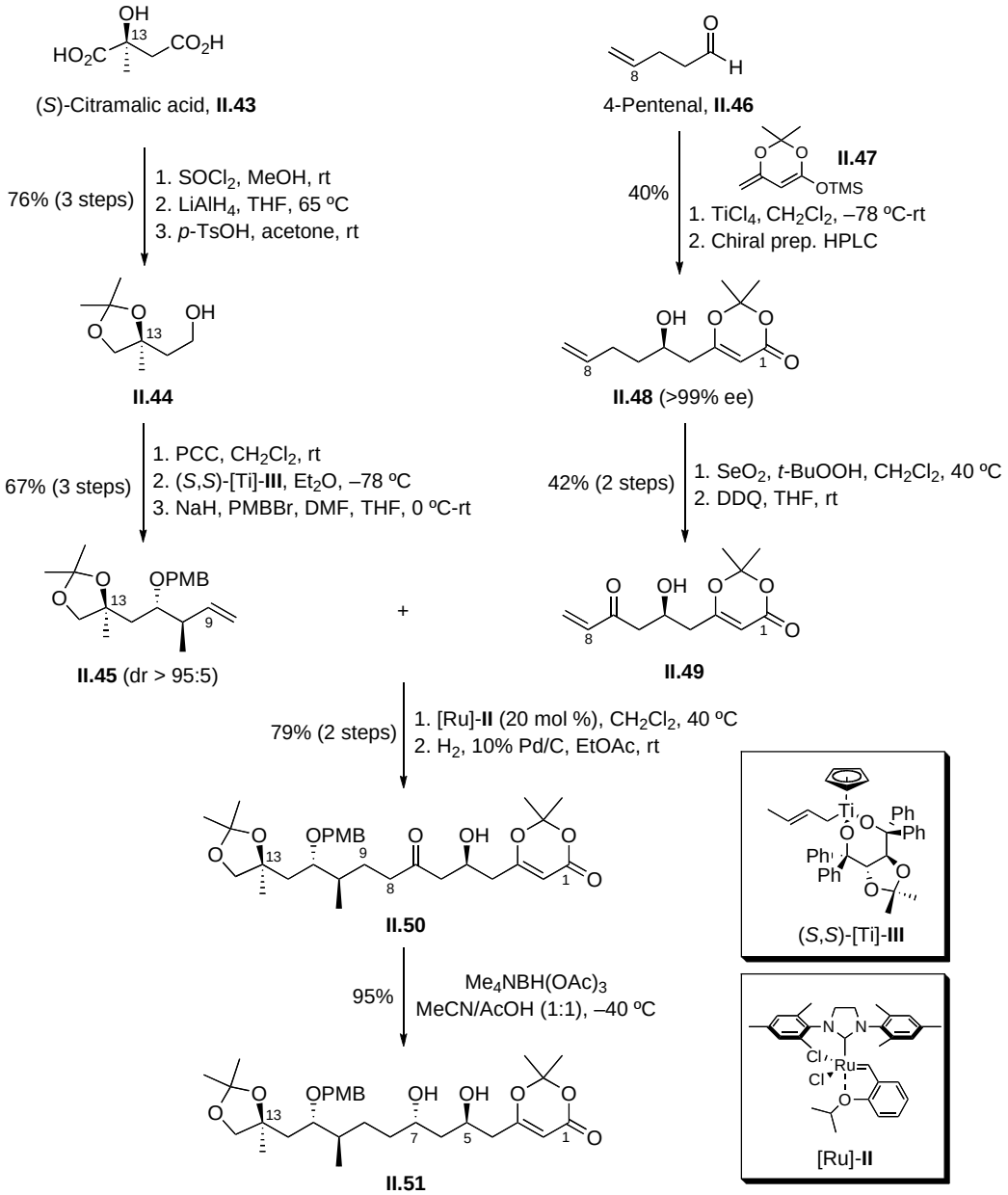
[152] (a) Grunwell, J. R.; Karipides, A.; Wigal, C. T.; Heinzman, S. W.; Parlow, J.; Surso, J. A.; Clayton, L.; Fleitz, F. J.; Daffner, M.; Stevens, J. E. *J. Org. Chem.* **1991**, *56*, 91-95. (b) Sugita, Y.; Sakaki, J.-I.; Sato, M.; Kaneko, C. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2855-2861.
[153] CHIRALPACK AD-H (SFC), MeOH/CO_2 = 8:92.

{$[\alpha]_D^{20}$ –21.0 (*c* 0.1, $CHCl_3$)} with the one reported in the literature[154] {$[\alpha]_D^{20}$ +19.0 ($CHCl_3$)}, was engaged in two concomitant allylic oxidations (*t*-BuOOH, SeO_2, CH_2Cl_2, 40 °C, then DDQ, THF, rt) to obtain the targeted enone **II.49** in 42% yield over two steps. On the other hand, the synthesis of compound **II.45** involved the esterification of (*S*)-citramalic acid ($SOCl_2$, MeOH, rt) followed by a $LiAlH_4$-mediated reduction and the protection of the resulting triol (*p*-TsOH, acetone) as a cyclic acetal. The remaining free primary alcohol **II.46** was then oxidized to the corresponding aldehyde (PCC, CH_2Cl_2, rt) and subjected to a highly enantio- and diastereoseletive crotyltitanation. The resulting homoallylic alcohol was finally protected as a PMB ether (NaH, PMBBr, DMF/THF) in order to complete the synthesis.

The two subunits were then coupled together using a second-generation Hoveyda-Grubbs catalyst (20 mol %)-promoted CM, and the resulting enone was directly reduced to the corresponding ketone **II.50** *via* a catalytic hydrogenation. In order to complete the synthesis of the C1-C13 fragment of lyngbouilloside, the latter was finally engaged in a 1,3-*anti* reduction using tetramethylammonium triacetoxyborohydride (TABH) to afford the desired *anti*-diol in quasi-quantitative yield (Scheme II.24).

This strategy resulted in the straightforward synthesis of the fully functionalized C1-C13 fragment **II.51** in nine steps (longest linear sequence) starting from commercially available (*S*)-citramalic acid **II.43** and 4-pentenal (**II.46**).

[154] Sato, M.; Sunami, S.; Sugita, Y.; Kaneko, C. *Heterocycles* **1995**, *41*, 1435-1444.



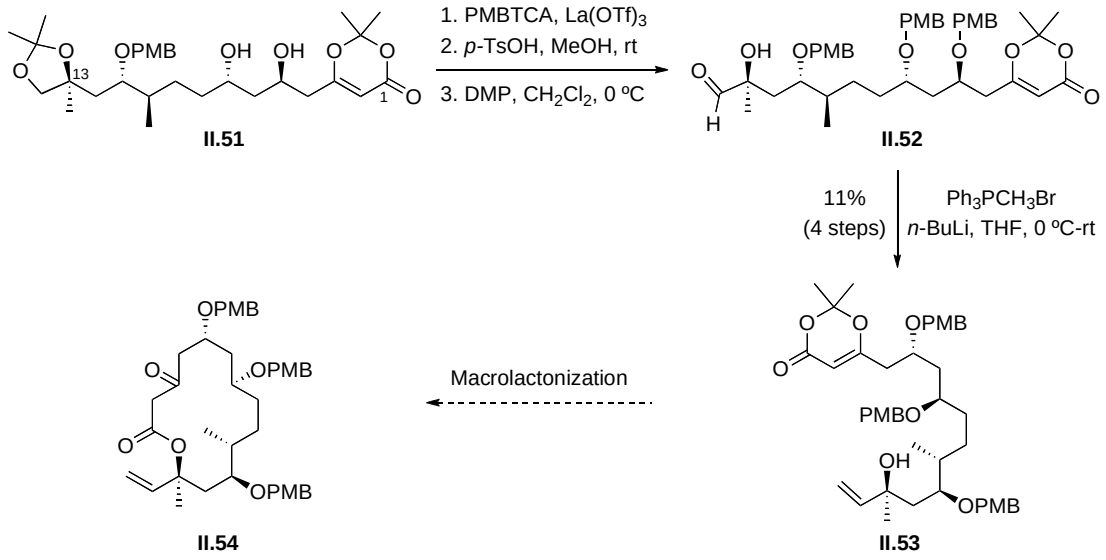
Scheme II.24. Synthesis of lyngbouilloside C1-C13 fragment

II.2.1.2.2.b. Synthesis of the macrolactone core of lyngbouilloside

Once the synthesis of the C1-C13 fragment **II.51** was secured, the next step involved the construction of the macrolactone core and the introduction of the side chain (Scheme II.25). In this context, diol **II.51** was protected as a bis-PMB ether [PMBTCA, $La(OTf)_3$],[155] while the acetal was removed under mild acidic conditions (*p*-TsOH, MeOH, rt). Oxidation of the newly released primary alcohol (DMP, CH_2Cl_2, 0 °C) and

[155] PMBTCA: *p*-(methoxybenzyl)trichloroacetimidate, see: Nakajima, N.; Horita, K.; Abe, R.; Yonemitsu, O. *Tetrahedron Lett.* **1988**, *29*, 4139–4142.

subsequent olefination under Wittig conditions (Ph_3PCH_3Br, *n*-BuLi, THF, 0 ºC-rt) eventually afforded the required terminal olefin **II.53**.[156] Unfortunately, the Wittig olefination appeared to be very low yielding resulting in only small amounts of the macrolactone precursor.[157] Nonetheless, the macrolactonization under the intramolecular acylketene-trapping conditions (refluxing in toluene under high dilution) was tempted, resulting to our delight in the isolation of the desired macrolactone **II.54**. However, do to the particularly low yields obtained in the Wittig olefination step, this approach had to be abandoned.



Scheme II.25. Attempted approach towards the lyngbouilloside aglycon

II.2.1.3. MACROLACTONIZATION METHODS

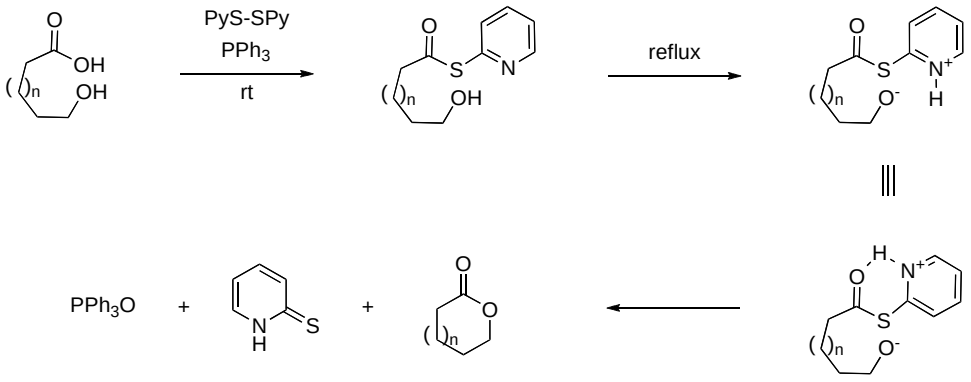
The macrocyclic lactone motif is present in a wide variety of natural products with a large spectrum of interesting biological properties. Hence, in the previous Section, we described the synthesis of an isomer of the 13-membered macrolactone acremolide B featuring an intramolecular peptide coupling to generate the macrocyclic core. In the next section, we will describe our efforts toward the first synthesis of lyngbouilloside aglycon, a 14-membered macrolactone with an unusual tertiary methyl carbinol, which features a macrolactonization *via* an intramolecular acylketene-trapping to generate the

[156] (a) Reber, K. P.; Tilley, S. D.; Sorensen, E. J. *Chem. Soc. Rev*. **2009**, *38*, 3022-3034. (b) Gebauer, J.; Blechert, S. *J. Org. Chem.* **2006**, *71*, 2021-2025. (c) Hoye, T. R.; Danielson, M. E.; May, A. E.; Zhao, H. *Angew. Chem. Int. Ed*. **2008**, *47*, 9743-9746.
[157] Unpublished results.

macrocycle. Since macrolactonizations are often used as key steps in natural product total synthesis, A brief overview of some of the existing methods used to prepare macrocyclic esters will be presented. Indeed, despite the use of high dilution or slow addition of the substrate, which tend to favour the intra- over the intermolecular process, or the immobilisation of the substrate on zeolites or in microemulsions that are not always applicable, there are a few methods that have been developed and that are commonly used today. All of these methods are based either on the activation of the acid moiety or on the activation of the alcohol, however, only the former strategy will be described here.[158]

II.2.1.3.1. Acid activation through thioester

This method, which was first described by Corey and Nicolaou,[159] proceeds through a one-pot double-activation procedure: a first activation of the acid *via* its conversion to the corresponding thioester derivative at rt, followed by a thermal activation to trigger the lactonization (Scheme II.26).

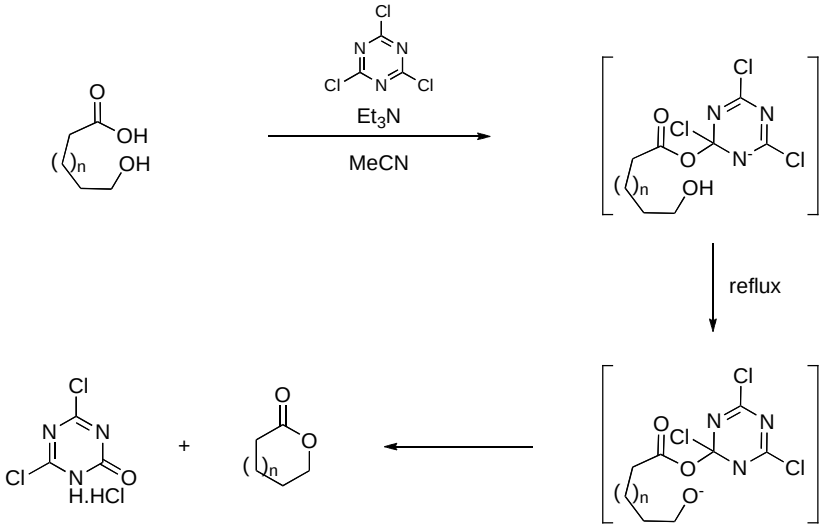


Scheme II.26. Macrolactonization through thioester-mediated activation

Among the various variants of this transformation, which mainly involve the preparation of other thioester derivatives or the addition of a metal to induce a chelation between the sulfur and the alcoholate,[160] Gerlach's modification is probably the most

[158] For a review, see: Parenty, A.; Moreau, X.; Campagne, J.-M. *Chem. Rev.* **2006**, *106*, 911-.
[159] (a) Corey, E. J.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1974**, *96*, 5614-5616. (b) Mukaiyama, T. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 707-721.
[160] (a) Nimitz, J. S.; Wollenberg, R. H. *Tetrahedron Lett.* **1978**, *19*, 3523-3526. (b) Schmidt, U.; Heermann, D. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 308-309. (c) Corey, E. J.; Clark, D. A. *Tetrahedron Lett.* **1979**, 2875-2878. (d) Hillis, L. R.; Ronald, R. C. *J. Org. Chem.* **1985**, *50*, 470-473.

accepted one.[161] It requires the use of silver salts such as $AgClO_4$, $AgBF_4$ and AgOTf and generally allows to carry out the reactions at rt. Another variation was introduced by Masamune *et al.*[162] The latter consisted in the preparation and the isolation of a thioester derivative which was then engaged in the actual macrolactonization in the presence of a thiophilic metal salt such as $Ag(CO_2CF_3)$, CuOTf or $Hg(CO_2CF_3)_2$. Nonetheless, despite the good results obtained using both approaches, the macrolactonizations still remain highly substrate-dependant. Finally, in addition to the methods introduced by Gerlach *et al.* and Masamune *et al.*, we can also include the cyanuric chloride-mediated acid activation method[163] developed by Venkataraman and Wagle which mechanism is closely related to Corey's and Nicolaou's lactonization (Scheme II.27).



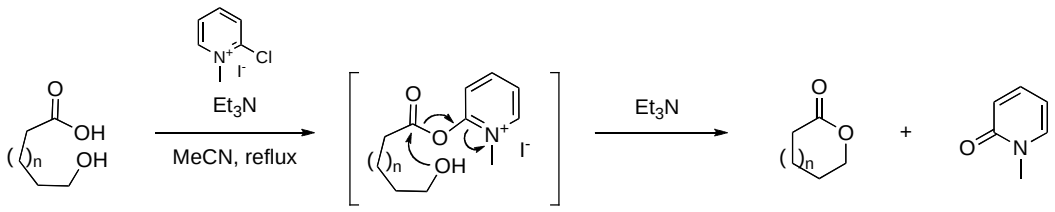
Scheme II.27. Macrolactonization through cyanuric chloride

II.2.1.3.2. Acid activation through Mukaiyama's salt

In 1976, Mukaiyama and co-workers[164] reported the first example of a macrolactonization using 1-methyl-2-chloropyridinium iodide as an acid activator in the presence of Et_3N. This reaction involves chloride substitution by the carboxylate ion to

[161] Gerlach, H.; Thalmann, A. *Helv. Chim. Acta* **1974**, *57*, 2661-2663.
[162] Masamune, S.; Bates, G. S.; Corcoran, J. W. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 585-607.
[163] (a) Venkataraman, K.; Wagle, D. R. *Tetrahedron Lett.* **1979**, *20*, 3037-3040. (b) Venkataraman, K.; Wagle, D. R. *Tetrahedron Lett.* **1980**, *21*, 1893-1896.
[164] Mukaiyama, T.; Usui, M.; Saigo, K. *Chem. Lett.* **1976**, 49-50.

give a highly activated acyloxypyridinium species which then undergoes macrolactonization (Scheme II.28).



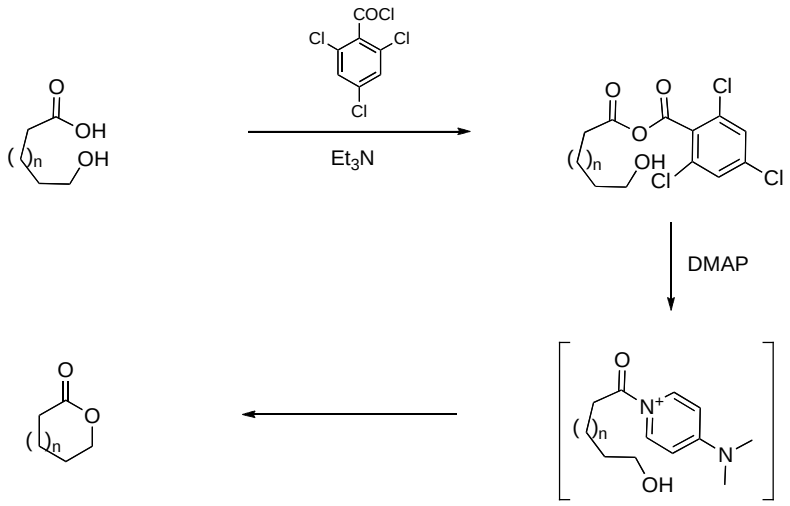
Scheme II.28. Mukaiyama's salt-mediated macrolactonization

This procedure has been successfully used in the total synthesis of various analogues of both paclitaxel[165] (Taxol®) and jatrophone.[166]

II.2.1.3.3. Acid activation through mixed anhydride

Despite the number of macrolactonization methods involving the formation of a mixed anhydride intermediate,[167] the one developed by Yamaguchi *et al.* is probably the most popular. It requires the use of 2,4,6-trichlorobenzoyl chloride and a stoichiometric amount of DMAP[168] (Scheme II.29). The mixed anhydride intermediate is highly reactive and thermally unstable, and leads to different results depending on the temperature and/or the order of addition of the reactants. One of the most useful modifications of this method was the development of a one-pot version which allows the regioselective synthesis of highly functionalized macrolactones.[169]

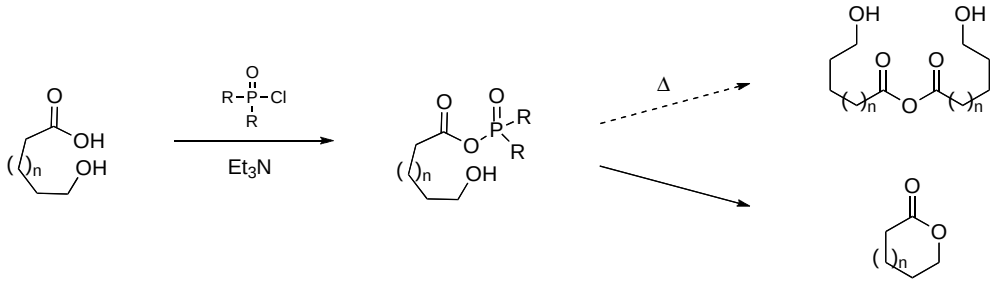
[165] Yuan, H.; Kingston, D. G. I.; Sackett, D. L.; Hamel, E. *Tetrahedron* **1999**, *55*, 9707-9716.
[166] Smith, A. B.; Malamas, M. S. *J. Org. Chem.* **1982**, *47*, 3442-3447.
[167] (a) Shiina, I.; Kubota, M.; Ibuka, R. *Tetrahedron Lett.* **2002**, *43*, 7535-7539. (b) Shiina, I.; Oshiumi, H.; Hashizume, M.; Yamai, Y.-s.; Ibuka, R.*Tetrahedron Lett.* **2004**, *45*, 543-547. (c) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822-1830. (d) White, J. D.; Johnson, A. T. *J. Org. Chem.* **1994**, *59*, 3347-3358. (e) Mukaiyama, T.; Izumi, J.; Miyashita, M.; Shiina, I. *Chem. Lett.* **1993**, 907-910. (f) Mukaiyama, T.; Izumi, J.; Shiina, I. *Chem. Lett.* **1997**, 187-188. (g) Ishihara, K.; Kubota, M.; Kurihara, H.; Yamamoto, H. *J. Org. Chem.* **1996**, *61*, 4560-4567.
[168] Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989-1993.
[169] (a) Hikota, M.; Tone, H.; Horita, K.; Yonemitsu, O. *J. Org. Chem.* **1990**, *55*, 7-9. (b) Evans, D. A.; Ng, H. P.; Rieger, D. L. *J. Am. Chem. Soc.* **1993**, *115*, 11446-11459.



Scheme II.29. Yamaguchi macrolactonization

II.2.1.3.4. Acid activation through phosphorus-based reagents

Masamune *et al.*[170] and Corey *et al.*[171] were the first to exploit the potential of the mixed carbon-phosphorus anhydrides[172] as acid-activating agents in macrolactonizations. Most importantly, this type of macrolactonization needs to be carried out at temperatures below 80 ºC in order to avoid the formation of symmetric anhydrides (Scheme II.30). This strategy was successfully used by Schreiber *et al.* and later by Roush and co-workers in the total synthesis of dynemicin A[173] and chlorothricolide,[174] respectively.



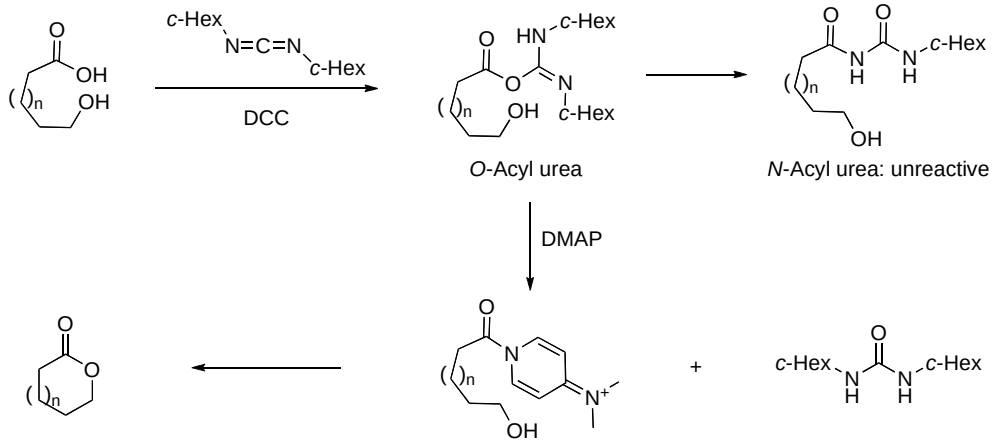
Scheme II.30. Macrolactonization through phosphorus-based reagents

[170] Kaiho, T.; Masamune, S.; Toyoda, T. *J. Org. Chem*. **1982**, *47*, 1612-1614.
[171] Corey, E. J.; Hua, D. H.; Pan, B. C.; Seitz, S. P. *J. Am. Chem. Soc.* **1982**, *104*, 6818-6820.
[172] Coste, J.; Frerot, E.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1991**, *32*, 1967-1970.
[173] Wood, J. L.; Porco, J. A., Jr.; Taunton, J.; Lee, A. Y.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 5898-5900.
[174] Roush, W. R.; Sciotti, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 7411-7419.

II.2.1.3.5. Acid activation through carbodiimides

The first macrolactonizations involving the use of carbodiimides as activating agents were based on the combination of DCC and DMAP. Also referred as Steglich protocol,[175] these conditions were barely applied due to the *N*-acyl urea by-product formed during the reaction (Scheme II.31). In contrast, the addition of DMAP·HCl provides higher yields and suppresses the formation of the *N*-acyl urea by-product by prolonging the lifetime of the activated acyl intermediate.

The formation of medium- and large-ring lactones from hydroxy acids using a combination of a dialkyl carbodiimide, an amine hydrochloride and an amine base is known as Keck macrolactonization.[176] This transformation requires the dissolution of the substrate in an aprotic solvent and subsequent addition to a refluxing solution of the reagents over several hours under high-dilution (c < 0.03 M). The activating agent is a *N*,*N'*-dialkyl carbodiimide such as DCC or EDCI and the corresponding activated acyl derivative is generated *in situ*. Hence, the procedure is inherently self-drying and cannot be compromised by serendipitous moisture. It is worth pointing out however that the main disadvantage of this method is the need to use large amounts of carbodiimide in order to achieve optimal yields.



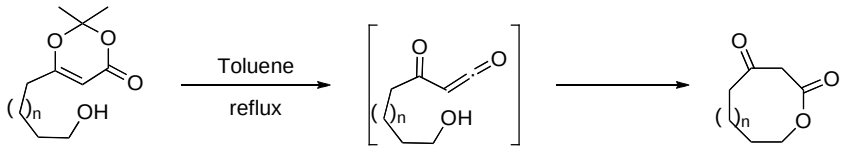
Scheme II.31. Carbodiimide-mediated macrolactonization

[175] Steglich, W.; Höfle, G. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 981.
[176] Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, 50, 2394–2395.

This type of macrolactonization was successfully applied in the total synthesis of various complex natural products such as colletodiol,[177] colletol,[178] dihydroerythronolide A,[179] calyculin,[180] epothilone B[181] and swinholide.[182]

II.2.1.3.6. Boeckman's macrolactonization

In 1989, Boeckman *et al.*[183] and Paquette *et al.*[184] reported the first total synthesis of (+)-ikuarungamycin at the same time demonstrating the use of acylketenes as intermediates for macrocyclization.[156,185] Dioxinone thermolysis is a particularly attractive alternative to the typical acid activation strategies previously described due to the ease of use as only heating is required to obtain the reactive β-acetylketene intermediate, which can be trapped intramolecularly to give the corresponding lactone (Scheme II.32). This method was actually chosen for our synthesis of lyngbouilloside.



Scheme II.32. Boeckman's macrolactonization

[177] Keck, G. E.; Boden, E. P.; Wiley, M. R. *J. Org. Chem.* **1989**, 54, 896–906.
[178] Keck, G. E.; Murry, J. A. *J. Org. Chem.* **1991**, 56, 6606–6611.
[179] Stork, G.; Rychnovsky, S. D. *J. Am. Chem. Soc.* **1986**, 109, 1565-1567.
[180] Evans, D. A.; Gage, J.; Leighton, J. L. *J. Am. Chem. Soc.* **1992**, 114, 9434-9453.
[181] Danishefsky, S. J.; Su, D. S.; Meng, D. F.; Bertinato, P.; Balog, A.; Sorensen, E. J.; Zheng, Y. H.; Chou, T. C.; He, L. F.; Horwitz, S. B. *Angew. Chem., Int. Ed.* **1997**, 36, 757-759.
[182] Patterson, I.; Yeung, K. S.; Ward, R. A.; Smith, J. D.; Cumming, G.; Lamboley, S. *Tetrahedron* **1995**, 51, 6497-9486.
[183] (a) Boeckman, R. K., Jr.; Weidner, C. H.; Perni, R. B.; Napier, J. J. *J. Am. Chem. Soc.* **1989**, *111*, 8036-8037.
[184] Paquette, L. A.; Macdonald, D.; Anderson, L. G.; Wright, J. *J. Am. Chem. Soc.* **1989**, *111*, 8037-8039.
[185] Boeckman, R. K., Jr.; Pruitt, J. R. *J. Am. Chem. Soc.* **1989**, *111*, 8286-8288.

II.2.2. RESULTS & DISCUSSION

II.2.2.1. FIRST STRATEGY TOWARDS THE SYNTHESIS OF LYNGBOUILLOSIDE AGLYCON

In this part, we describe our continued endeavour towards the total synthesis of the glycosidic macrolide lyngbouilloside. The main goal behind this project was to develop an efficient, highly straightforward and flexible route that would allow 1) to definitely secure the structure of the natural product, 2) to assign both its relative and absolute configuration, 3) to synthesize various structural analogues and 4) to establish a complete biological profile of the natural product and provide an insight into structure-activity relationships (SAR).

In front of the unsatisfying results obtained so far with the previous route and the supply problems of the necessary starting material (*S*)-citramalic acid, a new strategy based on more readily available starting materials was explored avoiding, when possible, the low-yielding reaction steps encountered previously.

II.2.2.1.1. First retrosynthetic analysis

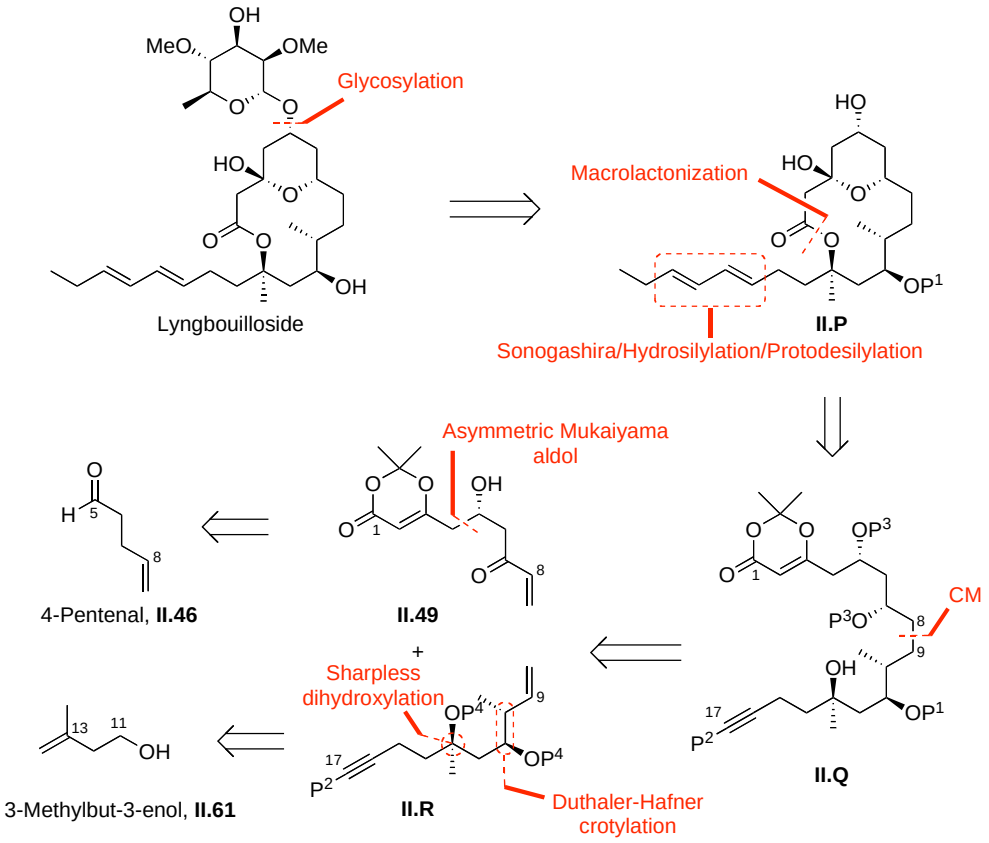
After considering the successes and downfalls of the previous approach, we decided to base our new strategy upon the following key steps (Scheme II.33):

- a glycosylation to introduce the hexapyranoside moiety,

- a Sonogashira coupling followed by a hydrosilylation/protodesilylation sequence to introduce the (*E*,*E*)-diene side chain,

-an intramolecular acylketene trapping macrolactonization to build the cyclic ester linkage on to the fully substituted C13 stereogenic center.

The linear C1-C17 fragment **II.Q** would in turn be obtained through a selective cross-metathesis between enone **II.49** and the olefinic coupling partner **II.R**, while an asymmetric version of the previous vinylogous Mukaiyama aldol would allow the installation of the C5 stereogenic center and introduce the dioxinone moiety. On the other hand, the C10 and C11 stereogenic centers would be controlled by a Duthaler-Hafner crotyltitanation, while the quaternary stereogenic center at C13 would be controlled by a Sharpless dihydroxylation of commercially available 3-methylbut-3-one (**II.61**).

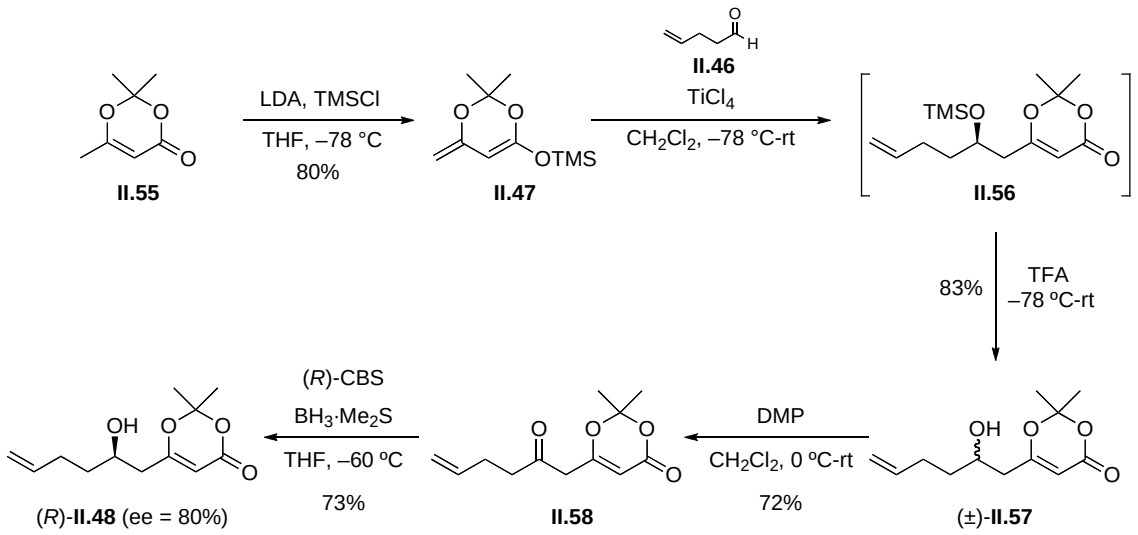


Scheme II.33. First retrosynthetic analysis of lyngbouilloside

II.2.2.1.2. Synthesis of C1-C8 fragment

As mentioned previously, we decided not to drastically modify our initial strategy towards the synthesis of lyngbouilloside, especially for the construction of the macrolactone core. However, among the few changes that were considered, we were particularly interested in developing a stereoselective synthesis of the C1-C8 fragment **II.49** in order to prevent the unnecessary waste of half of the material.

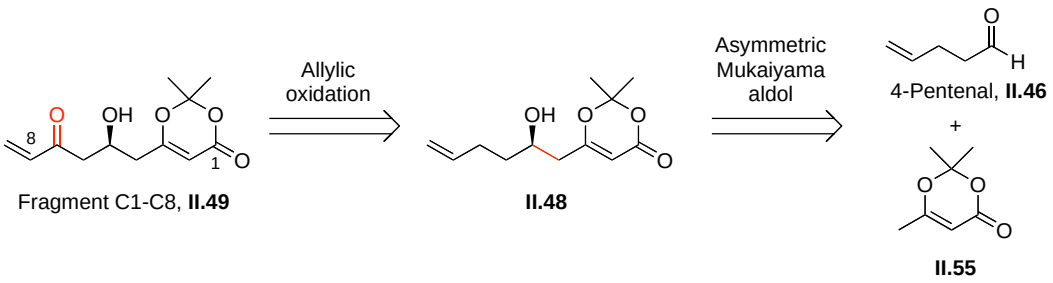
In this context, we planned to control the configuration of the alcohol at C5 by performing an enantioselective reduction of ketone **II.58** (Scheme II.34).[186] The latter, obtained by treating alcohol **II.57** with DMP (CH_2Cl_2, 0 °C-rt), was therefore subjected to the standard Corey-Bakshi-Shibata (CBS) reduction conditions ($BH_3{\cdot}Me_2S$, (*R*)-CBS, THF, –60 °C) to afford the desired enantio-enriched alcohol (*R*)-**II.48** in 53% yield and 80% ee. Unfortunately, despite the good level of enantioselectivty, this approach had to be abandoned due to persisting reproducibility issues.



Scheme II.34. CBS strategy towards the synthesis of alcohol (*R*)-**II.48**

In order to access the C1-C8 fragment whilst avoiding any unnecessary steps (oxidation, reduction…), we next considered the asymmetric Mukaiyama aldol between 4-pentenal (**II.46**) and the silyl dienol ether derived from 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (**II.55**) (Scheme II.35).

[186] (a) Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C. P.; Singh, V. K. *J. Am. Chem. Soc.* **1987**, *109*, 7925-7926. (b) Deloux, L.; Srebnik, M. *Chem. Rev.* **1993,** *93*, 763-784.



Scheme II.35. Retrosynthetic analysis of the C1-C8 fragment

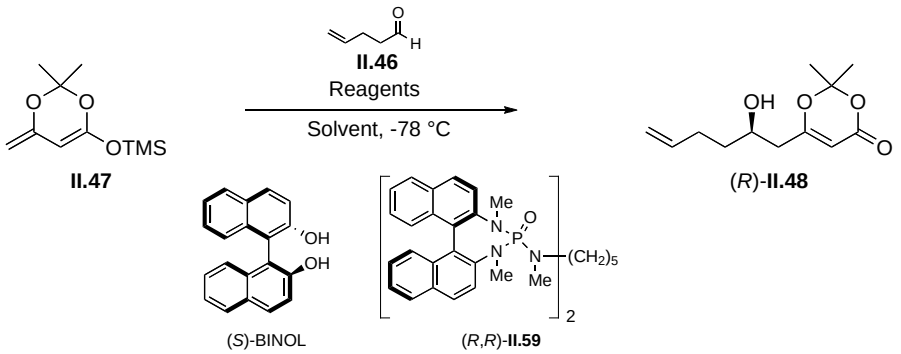
Among the various enantioselective catalytic processes developed so far in the field of asymmetric Mukaiyama aldol,[187] the ones reported by Denmark *et al.*[188] involving the combination of a catalytic amount of chiral bis-phosphoramide (*R*,*R*)-**II.59** and silicon tetrachloride to promote a highly enantio- and diastereoselective addition of silyl ketene acetals to aldehydes appeared particularly attractive. Unfortunately, the application of these conditions [$SiCl_4$, (*R*,*R*)-**II.59**, CH_2Cl_2, –78 °C] to our system afforded the desired product in 62% yield and in a moderate 65% ee as determined by chiral preparative HPLC[153] (Table II.5, entry 1). The conditions reported by Sato and co-workers,[154] which involve the use of a Ti(IV) complex in the presence of chiral (*S*)-BINOL (20 mol %) were also tested, however, the resulting product was obtained in only 22% yield and 30% ee (Table II.5, entry 2). More recently, Scettri *et al.*[189] developed a new protocol for the asymmetric Mukaiyama aldol between dienolates and aliphatic aldehydes in the presence of the same chiral Ti(O*i*-Pr)$_4$/BINOL catalytic system, showing the influence of the number of equivalents on the enantioselectivity. Interestingly, by employing their conditions [Ti(O*i*-Pr)$_4$ (8 mol %), (*S*)-BINOL (8 mol %), THF, –78 ºC], the expected aldol product (*R*)-**II.48** was isolated in 40% yield and up to 86% ee (Table II.5, entry 3). These conditions were therefore selected, and the mixture of the two enantiomers was used in the next step without further purification.

[187] (a) Evans, D. A.; Murry, J. A.; Kozlowski, M. C. *J. Am. Chem. Soc.* **1996**, *118*, 5814-5815. (b) Krüger, J.; Carreira, E. M. *J. Am. Chem. Soc.* **1998**, *120*, 669-685. (c) Pagenkopf, B. L.; Krüger, J.; Stojanovic, A.; Carreira, E. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 3124-3126. (d) Evans, D. A.; Kozlowski, M. C.; Murry, J. A.; Burgey, C. S.; Campos, K. R.; Connell, B. T.; Staples, R. J. *J. Am. Chem. Soc.* **1999**, *121*, 837-838. (e) Bluet, G.; Campagne, J.-M. *Tetrahedron Lett.* **1999**, *40*, 5507-5509. (f) Bluet, G.; Campagne, J.-M. *J. Org. Chem.* **2001**, *66*, 4293-4298.

[188] (a) Denmark, S. E.; Wynn, T.; Beutner, G. L. *J. Am. Chem. Soc.* **2002**, *124*, 13405-13407. (b) Denmark, S. E.; Beutner, G. L. *J. Am. Chem. Soc.* **2003**, *125*, 7800-7801. (c) Denmark, S. E.; Beutner, G. L.; Wynn, T.; Eastgate, M. D. *J. Am. Chem. Soc.* **2005**, *125*, 3774-3789.

[189] De Rosa, M.; Acocella, M. R.; Villano, R.; Soriente, A.; Scettri, A. *Tetrahedron: Asymmetry* **2003**, *14*, 2499-2502.

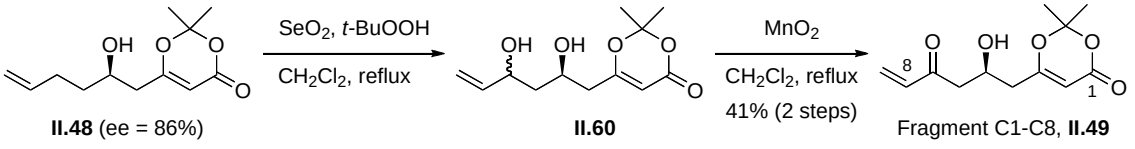
Table II.5. Asymmetric vinylogous aldol reaction



Entry	Reagents (equiv)	Solvent	Yield (%)[b]	ee (%)[a]
1	**II.47** (1.2) $SiCl_4$ (1.1) DIEA (5 mol %) + (*R*,*R*)-**II.59** (5 mol %)	CH_2Cl_2	62	65
2	**II.47** (1.4) Ti(*i*-PrO)$_4$ (20 mol %) (*S*)-BINOL (20 mol %)	THF	22	30
3	**II.47** (2.0) Ti(*i*-PrO)$_4$ (8 mol %) (*S*)-BINOL (8 mol %)	THF	40	86

[a] Enantiomeric excess determined by chiral SFC. [b] Isolated yield.

In order to complete the synthesis of the C1-C8 fragment of lyngbouilloside, alcohol **II.48** was then treated with SeO_2 and *t*-BuOOH (CH_2Cl_2, rt) to afford the corresponding diol **II.60** which was subsequently engaged in a MnO_2-mediated oxidation to provide the desired enone **II.49** in 41% yield (Scheme II.36). The C1-C8 fragment was thus obtained in five steps and 13% overall yield starting from commercially available 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (**II.55**).



Scheme II.36. Synthesis of the C1-C8 fragment

II.2.2.1.3. Synthesis of the C9-C17 fragment

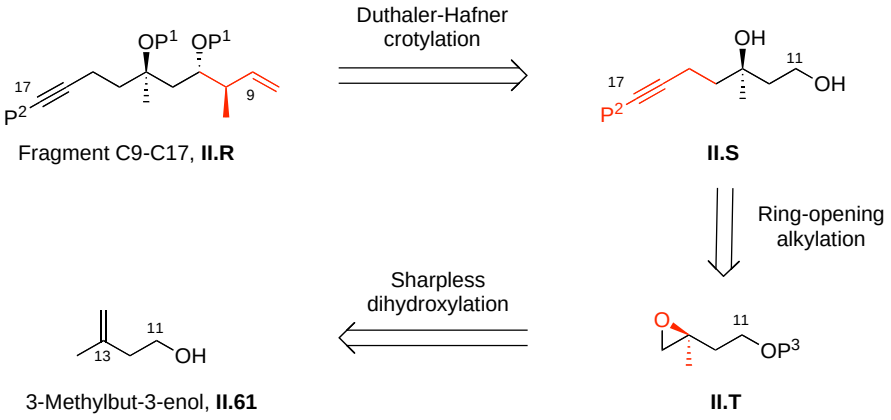
II.2.2.1.3.a. Retrosynthetic analysis

The synthesis of the C9-C17 fragment of lyngbouilloside was devised around three key steps (Scheme II.37):

- a Duthaler-Hafner crotyltitanation to control the C10 and C11 stereogenic centers,

- an epoxide ring-opening to install the propargyl substituent,

- an asymmetric Sharpless dihydroxylation to control the fully substituted C13 stereogenic center.



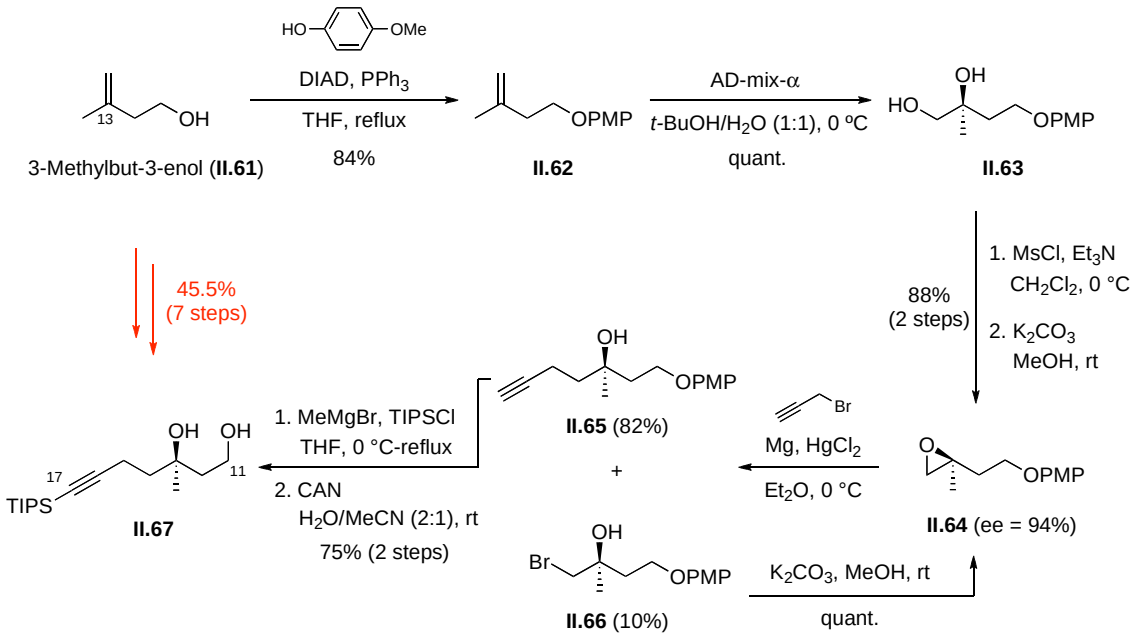
Scheme II.37. Retrosynthetic analysis of the C9-C17 fragment

II.2.2.1.3.b. Synthesis of the C9-C17 fragment

The synthesis of the C9-C17 fragment began with the protection of 3-methylbut-3-enol (**II.61**) as a *para*-methoxyphenyl (PMP) ether using diisopropylazodicarboxylate (DIAD) and triphenylphosphine (PPh_3) in refluxing THF.[39,190] The latter was then engaged in a Sharpless dihydroxylation[191] with AD-mix α (*t*-BuOH/H_2O, 0 ºC) to afford diol **II.63** in quantitative yield and 94% ee (Scheme II.38).[192] The primary alcohol was

[190] Mitsunobu, O.; Yamada, Y. *Bull. Chem. Soc. Japan* **1967**, *40*, 2380-2382.
[191] (a) Jacobsen, E. N.; Marko, I.; Mungall, W. S.; Schroeder, G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 1968-1970. (b) Kolb, H. C.; Van Nieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483-2547.
[192] The selectivity of the Sharpless dihydroxylation was determined by chiral SFC analysis of the corresponding epoxide **II.64** (CHIRALCEL OD-H, 100 bar, 5 mL.min^{-1}, 5% MeOH). This result was in absolute accordance with the studies performed by Tietze *et al.* who demonstrated that the nature of the

later converted to the corresponding mesylate (MsCl, Et_3N, 0 °C) which was subsequently treated with K_2CO_3 in MeOH to afford epoxide **II.64**[193] in 88% yield over two steps. Ring-opening of the epoxide with allenyl magnesium bromide[194] afforded an inseparable mixture of alcohol **II.65** and bromohydrin **II.66**, which was treated with K_2CO_3 (MeOH, rt) in order to convert the bromohydrin back to the epoxide. Flash column chromatography over silica gel eventually afforded the desired product **II.65** in 82% yield as well as 10% of the starting epoxide **II.64**. Compound **II.65** bearing a terminal alkyne was then selectively protected as a triisopropylsilylacetylene after treatment with methyl magnesium bromide and triisopropylsilyl chloride (TIPSCl), and the PMP protecting group was removed using cerium ammonium nitrate (CAN) in a water/acetonitrile mixture at rt to afford diol **II.67** in 75% yield over two steps. Diol **II.67** was obtained in seven steps starting from **II.61** with an overall yield of 45.5%.



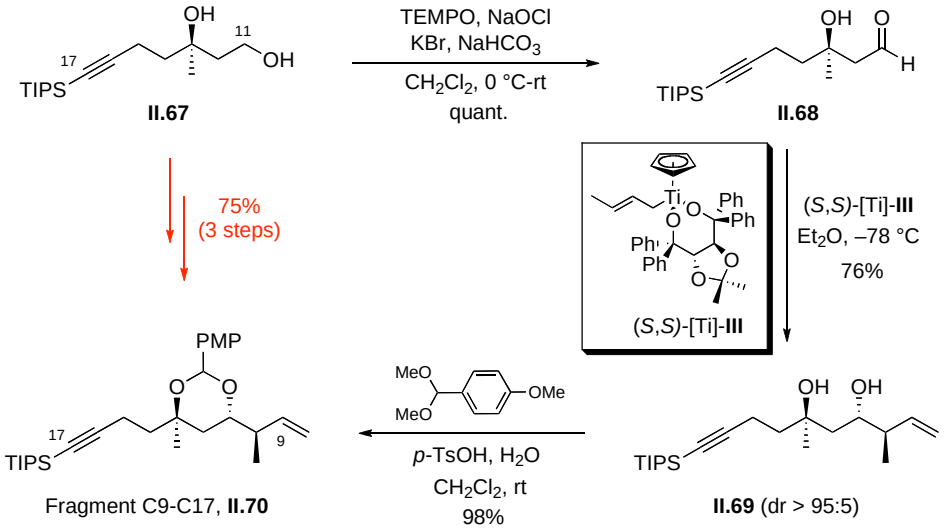
Scheme II.38. Synthesis of the C11-C17 fragment

protecting group had a strong influence on the enantioselectivity of the dihydroxylation, see : Tietze, L. F.; Görlitzer, J. *Synthesis* **1998**, 873-878.

[193] (a) Anikin, A.; Maslov, M.; Siele, J.; Blaurock, S.; Baldamus, J.; Hennig, L.; Findeisen, M.; Reinhardt, G.; Oehme, R.; Welzel, P. *Tetrahedron* **2003**, *59*, 5295-5306. (b) Sparks, S. M.; Chen, C.-L.; Martin, S. F. *Tetrahedron* **2007**, *63*, 8619-8635.

[194] Allenylmagnesium bromide was generated *in situ* from propargyl bromide and metallic magnesium in the presence of a catalytic amount of $HgCl_2$, see: (a) Nakayama, Y.; Kumar, G. B.; Kobayashi, Y. *J. Org. Chem.* **2000**, *65*, 707-715. (b) Dimitrieva, L. L.; Nikitina, L. P.; Albanov, A. I.; Nedolya, N. A. *Russian J. Org. Chem.* **2005**, *41*, 1583-1593.

In order to complete the synthesis of the C9-C17 fragment of lyngbouilloside, diol **II.67** was engaged in a TEMPO-mediated oxidation of the primary alcohol to provide aldehyde **II.68** in quantitative yield (Scheme II.39).[129] This aldehyde was then crotylated in a highly stereoselective fashion using the chiral titanium reagent (*S*,*S*)-[Ti]-**III** to afford the corresponding homoallylic alcohol **II.69** as a single diastereoisomer[195] in 76% yield. Finally, protection of diol **II.69** as a *p*-methoxybenzylidene acetal enabled the isolation of the C9-C17 fragment **II.70** in a quasi-quantitative yield. Three steps were therefore necessary to transform **II.67** into **II.70** with a high overall yield (75%) and an excellent diastereoselectivity.



Scheme II.39. Synthesis of the C9-C17 fragment

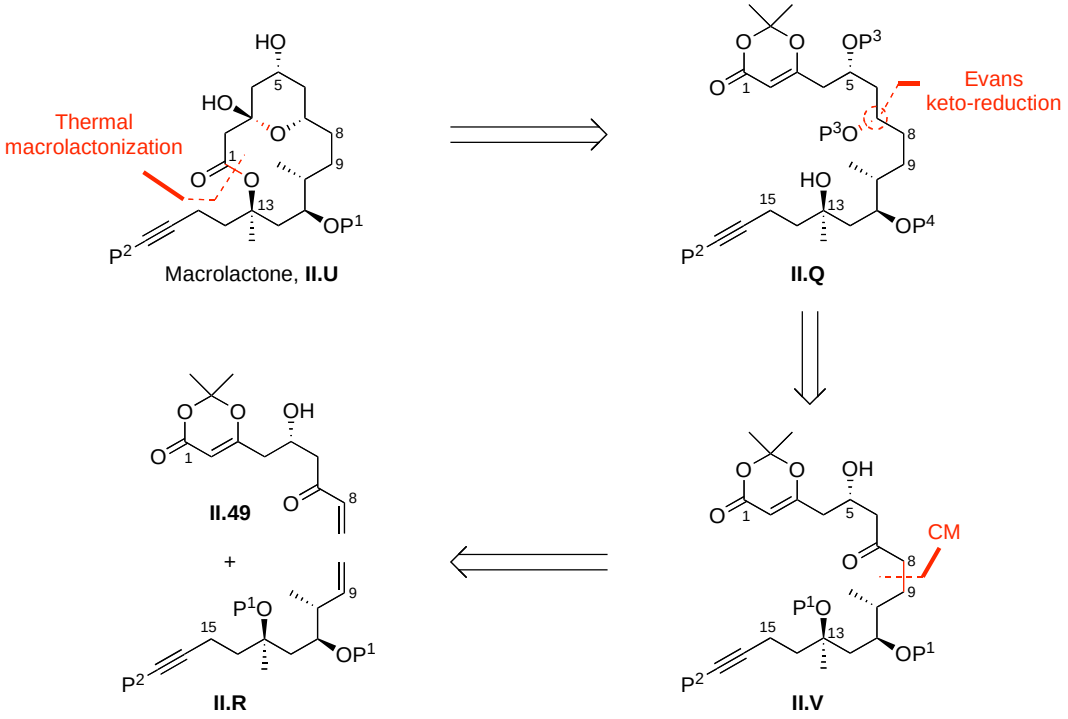
II.2.2.1.4. Synthesis of the macrolide core of lyngbouilloside

II.2.2.1.4.a. Retrosynthetic analysis

As depicted in Scheme II.40, the macrolactone core of lyngbouilloside, **II.U**, would be obtained *via* a thermal macrolactonization of the fully functionalized C1-C17 fragment **II.Q**. The latter would in turn be obtained through a cross-metathesis between enone **II.49** and the olefinic coupling partner **II.R** while the C7 stereogenic center

[195] The diastereoisomeric ration was determined by ^{1}H NMR analysis of the crude reaction mixture.

would be controlled by a tetramethylammonium triacetoxyborohydride (TABH)-mediated stereoselective β-hydroxy ketone reduction.[196,226]



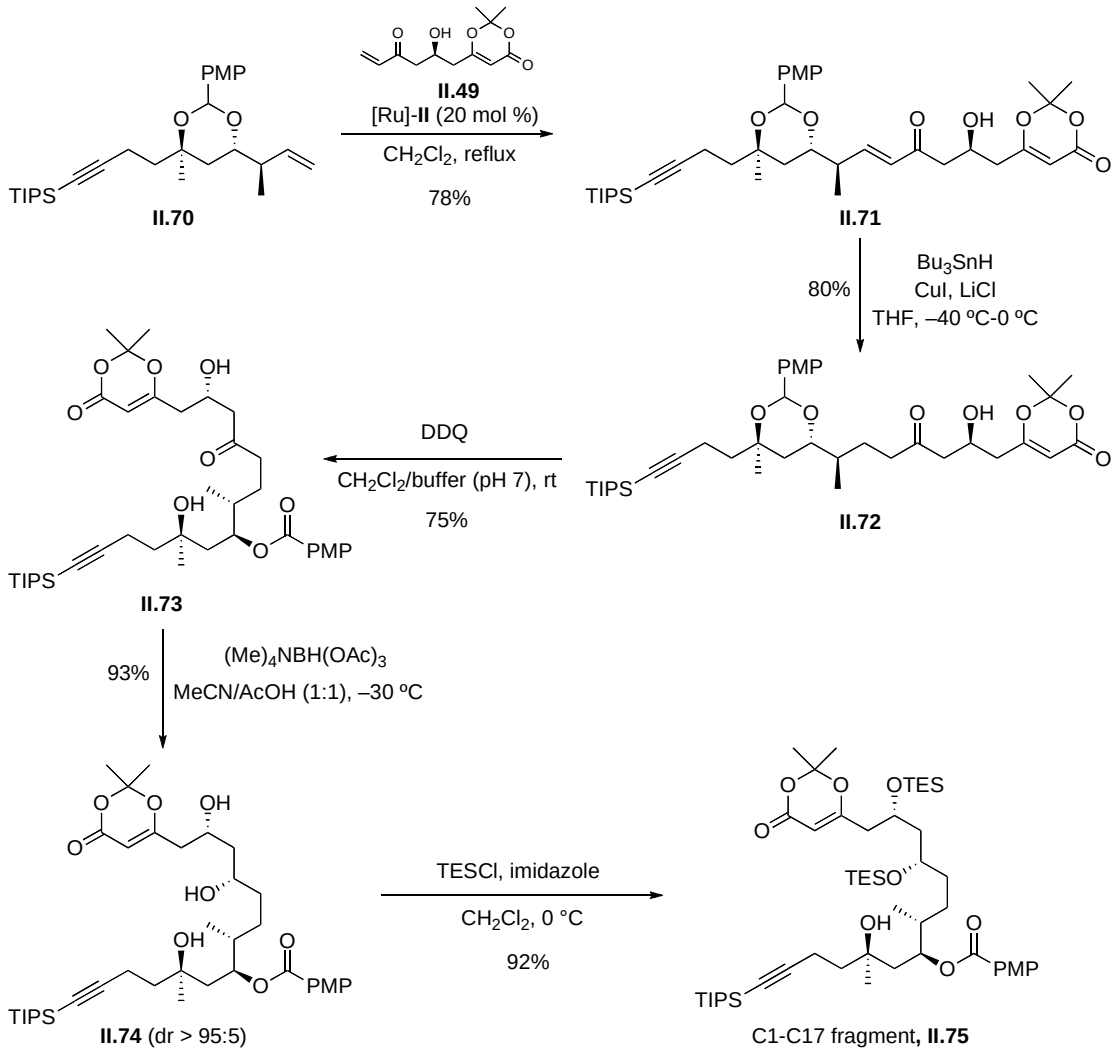
Scheme II.40. Retrosynthetic analysis of macrolactone **II.U**

II.2.2.1.4.b. Synthesis of the C1-C17 fragment

With the syntheses of the C1-C8 **II.49** and C9-C17 **II.70** subunits secured, the next step was the construction of the macrolactone precursor. The two olefinic coupling partners **II.49** and **II.70** were thus subjected to a second generation Hoveyda-Grubbs catalyst (20 mol %)-mediated cross-metathesis in refluxing CH_2Cl_2 to furnish enone **II.71** in 78% yield (Scheme II.41). However, it is worth pointing out that the hydroxyl group at C13 needed to be protected prior to the CM in order to prevent any concomitant oxo-Michael addition that could occur in the presence of the free hydroxyl group.[197] The α,β-unsaturated ketone **II.71** was then reduced chemoselectively using an *in situ* generated hydridocuprate (LiCl, CuI, Bu_3SnH, THF, –40 ºC)[198] to afford the saturated ketone **II.72** which was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in a CH_2Cl_2/phosphate buffer (pH = 7.2) mixture in order to

[196] Evans, D. A.; Chapman, K. T. *Tetrahedron Letters* **1986**, *27*, 5939-5942.
[197] Fuwa, H.; Noto, K.; Sasaki, M. *Org. Lett.* **2010**, *12*, 1636-1639.
[198] Lipshutz, B. H.; Ung, C. S.; Sengupta, S. *Synlett* **1989**, 64-66.

oxidatively open the cyclic PMP acetal in a regioselective fashion.[199] The resulting β-hydroxy ketone **II.73** was then diastereoselectively reduced using Evans protocol[196] ($Me_4NBH(OAc)_3$, MeCN/AcOH, –40 ºC) to afford the corresponding triol **II.74** in 70% overall yield as a single diastereoisomer (dr > 95:5) as confirmed by ^1H NMR analysis of the crude reaction mixture. Compound **II.74** was finally protected as a bis-triethylsilyl (TES) ether (TESCl, imidazole, CH_2Cl_2, 0 °C) in order to complete the synthesis of the macrolactone precursor **II.75**, which was isolated in 92% yield.



Scheme II.41. Synthesis of the C1-C17 fragment

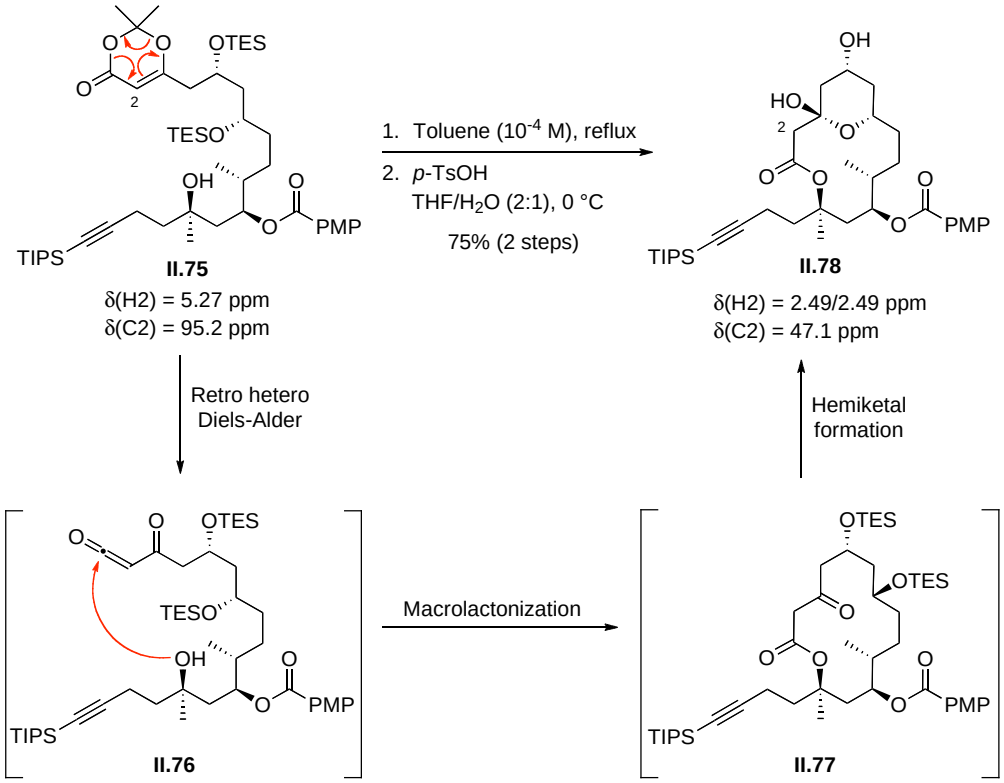
[199] Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 889-892.

II.2.2.1.4.c. Macrolide core formation

The obtained C1-C17 linear fragment **II.75** was ultimately subjected to a sequential acylketene macrolactonization/pyran hemiketal formation in order to build up the macrolactone core of lyngbouilloside **II.78**.[156,200]

Hence, the thermolysis of dioxinone **II.75** (δ_{H2} = 5.27 ppm, δ_{C2} = 95.2 ppm) in refluxing toluene under diluted conditions ($c = 10^{-4}$ M) promoted a retro-hetero Diels-Alder reaction that generated the highly reactive acylketene intermediate **II.76** which was instantaneously trapped intramolecularly by the tertiary alcohol to form the corresponding macrocyclic β-keto lactone **II.77** along with trace amounts of the corresponding methyl ketone derivative resulting from keto-acid decarboxylation (Scheme II.42).[201] The crude reaction mixture was finally treated with *p*-toluenesulfonic acid in a THF/H_2O mixture in order to concomitantly remove the TES protecting groups and generate the desired hemiketal **II.78** (δ_{H2} = 2.59/2.49 ppm, δ_{C2} = 47.1 ppm) in 75% yield over two steps.

[200] For a recent application of the acylketene dual macrolactonization/pyran hemiketal formation reaction, see: (f) Trost, B. M.; Gunzner, J. L. *J. Am. Chem. Soc.* **2001**, *123*, 9449-9450. (g) Trost, B. M.; Gunzner, J. L.; Dirat, O.; Rhee, Y. H. *J. Am. Chem. Soc.* **2002**, *124*, 10396-10415.
[201] Wentrup, C.; Heilmayer, W.; Kollenz, G. *Synthesis* **1994**, 1219-1248.



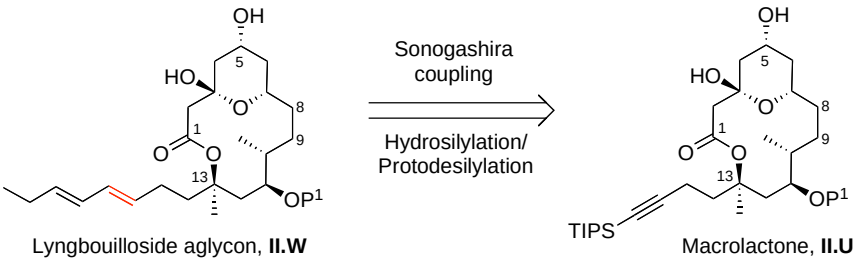
Scheme II.42. Synthesis of macrolactone **II.78** *via* thermal macrolactonization

II.2.2.1.5. Final efforts towards the synthesis of lyngbouilloside aglycon

II.2.2.1.5.a. Retrosynthetic analysis of the lyngbouilloside aglycon

With the synthesis of the macrolactone core secured, we next turned our attention to the construction the (*E*,*E*)-dienic side chain which was envisioned through a three-step sequence featuring a Sonogashira coupling[202] followed by a one-pot hydrosilylation/protodesilylation (Scheme II.43).[203]

[202] (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467-4470. (b) Chinchilla, R.; Nájera, C. *Chem Rev.* **2007**, *107*, 874-922.
[203] (a) Trost, B. M.; Ball, Z. T.; Jöge, T. *J. Am. Chem. Soc.* **2002**, *124*, 7922-7923. (b) Lacombe, F.; Radkowski, K.; Seidel, G.; Fürstner, A. *Tetrahedron* **2004**, *60*, 7315-7324. (c) Kleinbeck, F.; Carreira, E. M. *Angew. Chem. Int. Ed.* **2009**, *48*, 578-581.



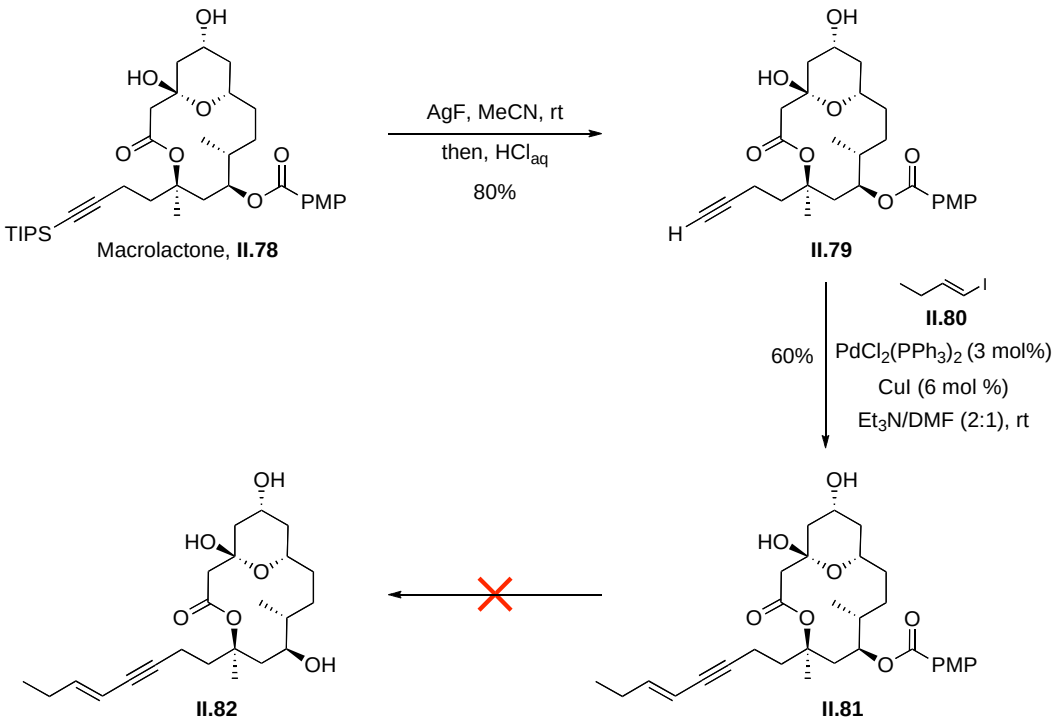
Scheme II.43. Retrosynthetic analysis of the lyngbouilloside aglycon

II.2.2.1.5.b. Attempted synthesis of the lyngbouilloside aglycon

II.2.2.1.5.b.1. Introduction of the lyngbouilloside side chain

The introduction of the side chain thus began with the removal of the TIPS protecting group using silver(I) fluoride (MeCN, rt) followed by a mild acidic work-up (Scheme II.44).[204] The corresponding terminal alkyne **II.79**, which was obtained in 80% yield, was subsequently engaged in a $PdCl_2(PPh_3)_2$/CuI-catalyzed Sonogashira coupling with (*E*)-1-iodobut-1-ene (**II.80**)[205] to afford the desired Sonogashira product **II.81** in a moderate 60% yield. At this time, instead of reducing the alkyne moiety to the corresponding (*E*)-olefin, we decided to explore the cleavage of the *p*-methoxybenzoate.

[204] Kim, S.; Kim, B.; In, J. *Synthesis* **2009**, *12*, 1963-1968.
[205] Prepared according to a reported procedure starting from but-1-yne, see: Zneifel, G.; Lewis, W. *J. Org. Chem.* **1978**, *43*, 2739-2744.



Scheme II.44. Introduction of the lyngbouilloside side chain and attempted cleavage of the *p*-methoxybenzoate

II.2.2.1.5.b.2. Attempted cleavage of the *p*-methoxybenzoate

Unfortunately, the cleavage of the *p*-methoxybenzoate appeared particularly troublesome as none of the conditions tested (Table II.6, entries 1-5)[206] resulted in the isolation of the fully deprotected lyngbouilloside precursor most likely due the inherent reactivity of the *p*-methoxybenzoate combined to the steric congestion in this region of the macrolide core.

Table II.6. Attempted cleavage conditions

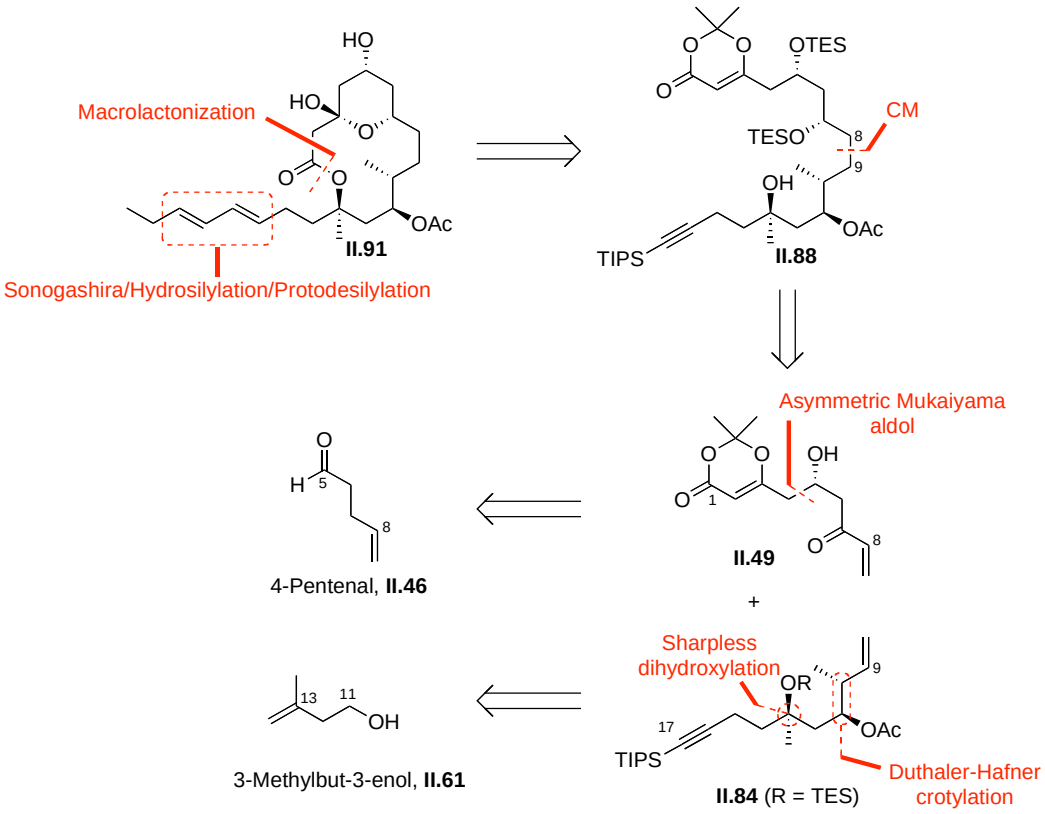
Entry	Reagents (equiv)	Solvent	T (ºC)	t (h)	Yield (%)
1	K_2CO_3 (4.0)	MeOH	rt	5	–[a]
2	K_2CO_3 (10.0)	MeOH	rt-40	4	–[a]
3	LiOH (3)	THF/H_2O (10:1)	0 ºC-rt	5	–[a]
4	NaOH (10)	THF/H_2O (10:1)	rt	9	–[a]
5	NaOMe (0.5)	MeOH	0 ºC	2	–[b]

[a] Starting material **II.81** was recovered . [b] Decomposition of the substrate.

[206] (a) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd edition, J. Wiley & Sons, **1991**. (b) Kocienski, P. J. *Protecting Groups*, Georg Thieme Verlag, **1994**.

II.2.2.2. SECOND STRATEGY TOWARDS THE SYNTHESIS OF LYNGBOUILLOSIDE

In front of the experienced difficulties to cleave the *p*-methoxybenzoate, we decided to slightly modify the protecting group strategy by replacing the *p*-methoxybenzoate by an acetate (Scheme II.45).[206]

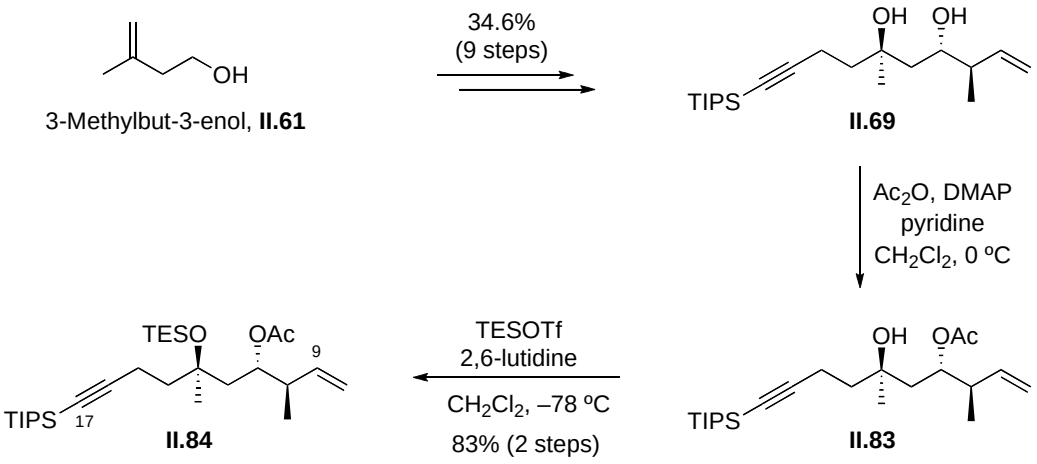


Scheme II.45. Second synthetic strategy

II.2.2.2.a. Synthesis of the C9-C17 fragment

The synthesis of the C9-C17 fragment thus began with the suitable protection of diol **II.69**, which was previously obtained in nine steps and 34.6% overall yield starting from commercially available 3-methyl-3-enol (**II.61**) (Scheme II.46). In this context, the secondary alcohol was treated with acetic anhydride (DMAP, Py, CH_2Cl_2, 0 °C) to afford the corresponding ester derivative **II.83**, which was immediately subjected to TESOTf in the presence of 2,6-lutidine to convert the tertiary alcohol into a TES ether.

The fully protected compound **II.84** was eventually isolated in 83% yield over two steps.

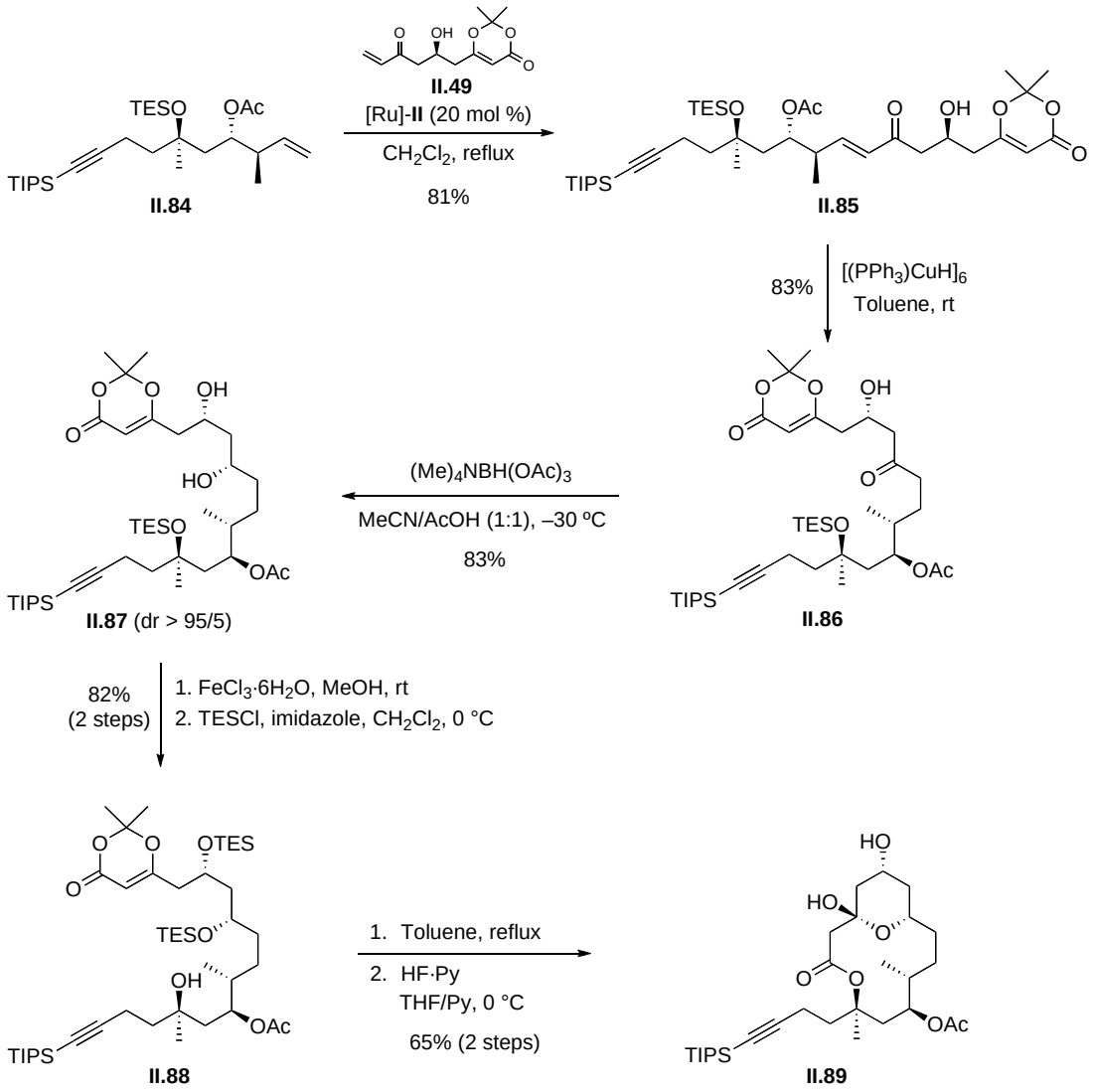


Scheme II.46. Synthesis of the C9-C17 fragment

II.2.2.2.b. Synthesis of the macrolide core of lyngbouilloside

The next step in the synthesis was to carry out the key CM between enone **II.49** and the olefinic coupling partner **II.84** to provide the linear C1-C17 fragment **II.85** (Scheme II.47). This was best achieved using the second generation Hoveyda-Grubbs catalyst in refluxing CH_2Cl_2 as the desired coupled product was obtained in 81% yield. The resulting enone was then selectively reduced to the corresponding β-hydroxy ketone using Stryker's reagent [$(PPh_3)CuH_6$, toluene, rt][207] and subsequently subjected to the approved Evans TABH-mediated 1,3-*anti* reduction ($Me_4NBH(OAc)_3$, MeCN/AcOH, –30 ºC) to afford the desired 1,3-*anti* diol **II.87** as a single diastereoisomer[208] in 69% overall yield. Removal of the TES protecting group from the tertiary alcohol using $FeCl_3 \cdot 6H_2O$ (MeOH, rt)[209] and treatment of the resulting triol with TESCl (imidazole, CH_2Cl_2, 0 °C) allowed to selectively protect the two secondary alcohol while keeping the tertiary alcohol free and thus complete the synthesis of the macrolactone precursor **II.88** (82% over two steps). The latter was finally engaged in the sequential thermal macrolactonization/pyran hemiketal formation to afford macrolactone **II.89** in 65% yield.

[207] (a) Mahoney, W. S.; Brestensky, D. M.; Stryker, J. M. *J. Am. Chem. Soc* **1988**, *110*, 291-293. (b) Paterson, I.; Blakey, S. B.; Cowden, C. J. *Tetrahedron Lett.* **2002**, *43*, 6005-6008.
[208] The diastereoisomeric ratio was determined by ^{1}H NMR of the crude reaction mixture.
[209] Yang, Y.-Q.; Cui, J.-R.; Zhu, L.-G.; Sun, Y.-P.; Wu, Y. *Synlett* **2006**, 1260-1262.

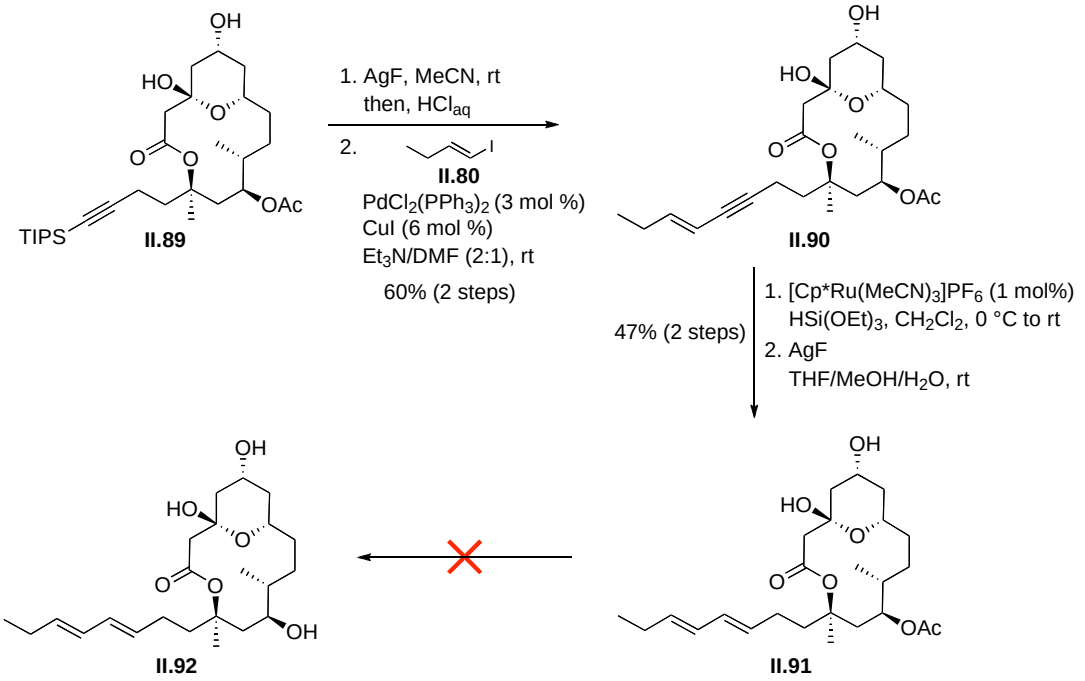


Scheme II.47. Synthesis of the C1-C17 fragment

II.2.2.2.c. Synthesis of lyngbouilloside aglycon

With compound **II.89** in hand, the stage was finally set for the construction of the (*E*,*E*)-octadienyl side chain and the final deprotection which would provide the much-coveted lyngbouilloside aglycon. The TIPS protecting group was therefore removed using AgF (MeCN, rt) followed by a mild acidic work-up, and the resulting terminal alkyne was subsequently engaged in the key $PdCl_2(PPh_3)_2$/CuI-catalyzed Sonogashira coupling with (*E*)-1-iodobut-1-ene (**II.80**) to afford the corresponding Sonogashira product **II.90** in a moderate 60% yield (Scheme II.48). The latter was then

subjected to the one-pot hydrosilylation/protodehydrosilylation[210] sequence developed by Trost *et al*. to afford the lyngbouilloside aglycon precursor **II.91** in 47% yield over two steps.



Scheme II.48. Towards the synthesis of lyngbouilloside aglycon

At this stage, we next focused on the crucial deprotection step, which would ultimately provide the lyngbouilloside aglycon. Unfortunately, despite the plethora of reaction conditions that were tested (K_2CO_3, NaOH, LiOH or even Na/MeOH), the cleavage of the acetate appeared once again particularly troublesome. The use of enzymes such as *Candida antarctica* B lipase (CAL-B), PLE, *Amano AK*, *Novozym* or *Candida Rugosa* under hydrolytic conditions or the use of stronger reagents such as DIBAL-H also resulted in no success as the starting material was either recovered or underwent total decomposition.

The unexpected lack of reactivity of both the acetate and the *p*-methoxybenzoate protecting groups led us to believe that electronic effects were most probably not responsible. As a matter of fact, molecular models of the two compounds **II.81** and

[210] (a) Trost, B. M.; Ball, Z. T. *J. Am. Chem. Soc.* **2001**, *123*, 12726-12727. (b) Trost, B. M.; Machacek, M. R.; Ball, Z. T. *Org. Lett.* **2003**, *5*, 1895-1898. (c) Chung, L. W.; Yu, Y.-D.; Trost, B. M.; Ball, Z. T. *J. Am. Chem. Soc.* **2003**, *125*, 11578-11582. (d) Bressy, C.; Bargiggia, F.; Guyonnet, M.; Arseniyadis, S.; Cossy, J. *Synlett* **2008**, *4*, 565-568. (d) Bressy, C.; Vors, J.-P.; Hillebrand, S.; Arseniyadis, S.; Cossy, J. *Angew. Chem, Int. Ed.* **2008**, *47*, 10137-10140.

II.91 showed a possible restricted conformation of each macrolide stabilized by an intramolecular hydrogen bond between the carbonyl of the acetate/*p*-methoxybenzoate and the hemiketal, rendering the former inaccessible and therefore the deprotection impossible.

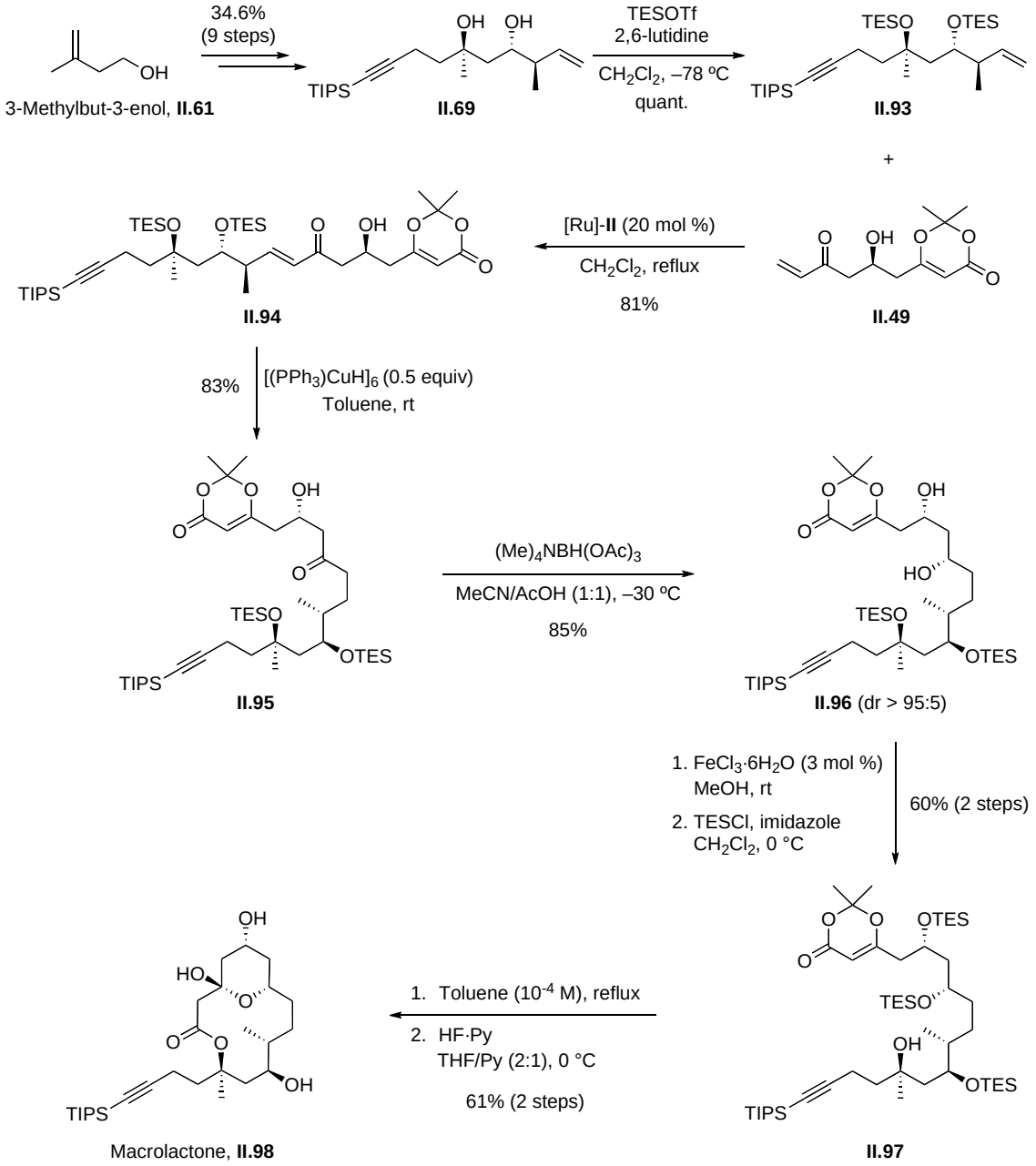
II.2.2.3. FINAL STRATEGY

After considering the rather promising results obtained so far in the previous routes, we decided to try an ultimate approach based on a silyl protecting group strategy that would avoid any internal hydrogen bond to occur and thus render the C11 hydroxyl much more accessible. Consequently, we opted for a TES protecting group, which would assure a relatively easy deprotection step without significantly altering the synthesis. In addition, we were fully aware that the TES groups would be removed simultaneously after the macrolactonization step, however, we were confident that a selective glycosylation of the less hindered C5 hydroxyl would be favoured in the final steps of the synthesis.

II.2.2.3.1. Synthesis of the macrolactone core

The synthesis of the macrolactone core of lyngbouilloside thus began with the suitable protection of diol **II.69** (Scheme II.49). The latter was therefore treated with an excess of TESOTf (2,6-lutidine, CH_2Cl_2, –78 °C) to quantitatively afford the bis-TES ether **II.93** which was going to be engaged in the exact same reaction sequence as previously. Hence, compound **II.93** was first subjected to the usual second generation Hoveyda-Grubbs catalyst-mediated CM with enone **II.49** to afford the desired coupled product **II.94** in 81% yield. The resulting enone was then reduced to the corresponding saturated ketone using Stryker's reagent [$(PPh_3)CuH_6$, toluene, rt] while a highly diastereoselective *anti*-reduction of the β-hydroxy ketone **II.95** under Evans' conditions ($Me_4NBH(OAc)_3$, MeCN/AcOH, –30 ºC) afforded the desired 1,3-*anti* diol **II.96** as a unique diastereoisomer as determined by ^1H NMR analysis of the crude reaction mixture. The TES protecting groups were removed under mild conditions using a catalytic amount of $FeCl_3 \cdot 6H_2O$ in methanol,[209] and the resulting secondary alcohols were re-protected as TES ethers (TESCl, imidazole, CH_2Cl_2, 0 °C) to afford the key macrolide precursor **II.97** in 60% yield over two steps.

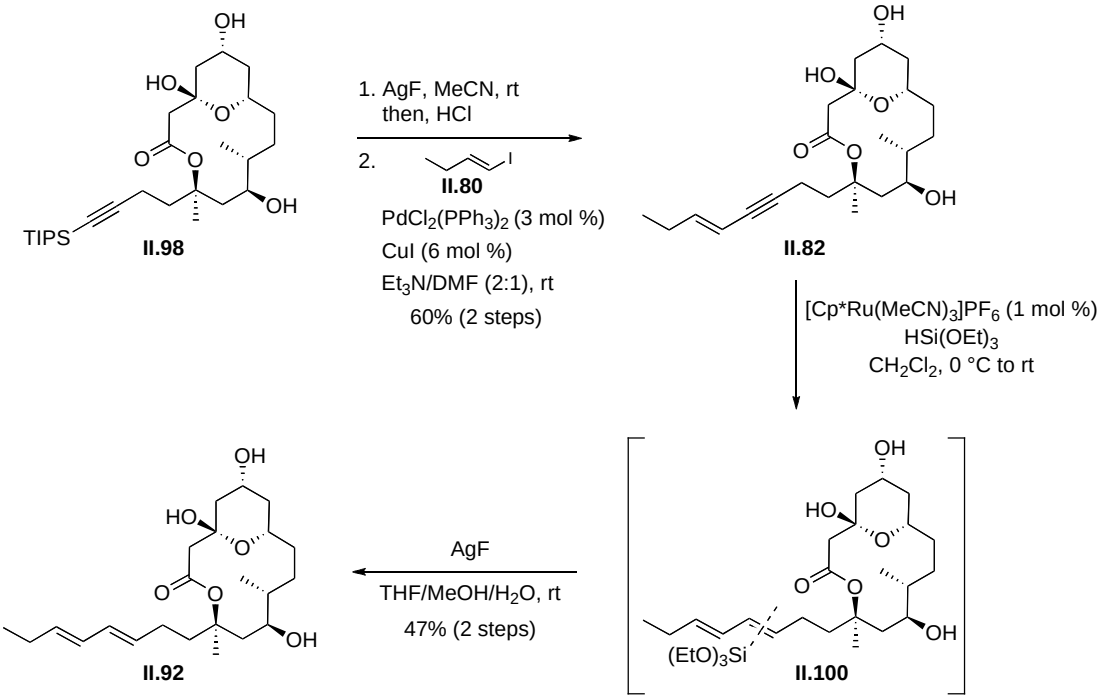
With dioxinone **II.97** in hand, the stage was set for the sequential thermal macrolactonization/pyran hemiketal formation which would ultimately afford the lyngbouilloside aglycon precursor. To our delight, thermolysis of dioxinone **II.97** in rigorously anhydrous refluxing toluene (c = 10^{-4} M) resulted in the formation of the macrolactone core, while treatment with HF·Py in a THF/Py mixture finally afforded the desired fully TES-deprotected macrolactone **II.98** in 61% overall yield.



Scheme II.49. Synthesis of macrolactone **II.98**

II.2.2.3.2. End-game

In order to complete the synthesis, macrolactone **II.98** was treated with AgF (MeCN, rt) to afford the corresponding terminal alkyne which was engaged in the same $PdCl_2(PPh_3)_2$/CuI-catalyzed Sonogashira coupling as previously (Scheme II.50). The resulting enyne was then subjected to the approved one-pot hydrosilylation/protodesilylation sequence,[203a,210] which resulted in the isolation of lyngbouilloside aglycon **II.92** in 47% yield over two steps.



Scheme II.50. Synthesis of lyngbouilloside aglycon

In summary, the proposed structure of lyngbouilloside aglycon **II.92** was synthesized in 21 steps and 2.1% overall yield starting from commercially available 3-methylbut-3-enol (**II.61**) featuring an acylketene macrolactonization and a late stage side chain introduction as the key steps.

II.2.2.3.3. Analysis of NMR data

Interestingly, comparison of the NMR chemical shifts of our synthetic aglycon with the ones reported for the natural lyngbouilloside (Table II.7, Figure II.2, top), particularly in the C9-C13 region, strongly suggests that the structure of the natural

product may have been originally misassigned. This observation, which was also made by Ley and co-workers after spectroscopic analysis and DFT chemical shift calculations of a closely related PMB-protected macrolactone,[145] is also comforted by the fact that the ^{13}C NMR data of both lyngbouilloside **II.101** and the more recently reported lyngbyaloside C **II.102** (Figure II.12)[142] are virtually identical within the region of the macrocycle (Figure II.12, bottom) while the proposed structures of the two natural products differ at C11.

Table II.7. C1 to C13 Chemical shifts (δ, ppm) for lyngbouilloside **II.101**, lyngbyaloside C **II.102** and our synthetic aglycon **II.92**, and the differences (Δδ, ppm) with lyngbouilloside **II.101**.[a]

Carbon	δ for (**II.101**)[a]	δ for (**II.92**)[a] (Δδ)	δ for (**II.102**)[b] (Δδ)
1	172.9	172.3 (+0.6)	172.3 (+0.6)
2	47.5	47.1 (+0.4)	46.9 (+0.6)
3	97.2	96.3 (+0.9)	96.6 (+0.6)
4	41.9	43.4 (-1.5)	41.5 (+0.4)
5	69.8	64.9 (+4.9)	69.2 (+0.6)
6	38.4	40.7 (-2.3)	37.7 (+0.7)
7	70.2	68.9 (+1.3)	69.8 (+0.4)
8	31.9	33.5 (-1.6)	31.4 (+0.5)
9	33.0	31.5 (+1.5)	32.4 (+0.6)
10	37.5	37.6 (-0.1)	37.0 (+0.5)
11	66.0	67.4 (-1.4)	65.5 (+0.5)
12	44.7	45.8 (-1.1)	44.1 (+0.6)
13	86.9	86.4 (+0.5)	86.2 (+0.7)

[a] 150 MHz. [b] 100 MHz.

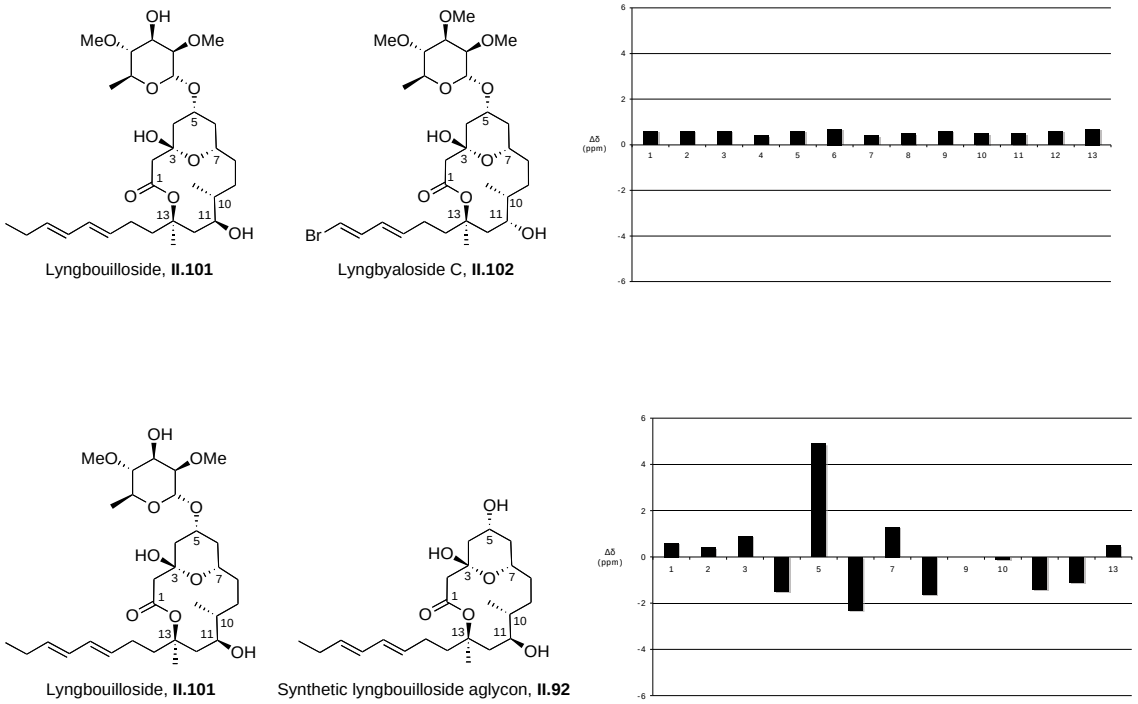
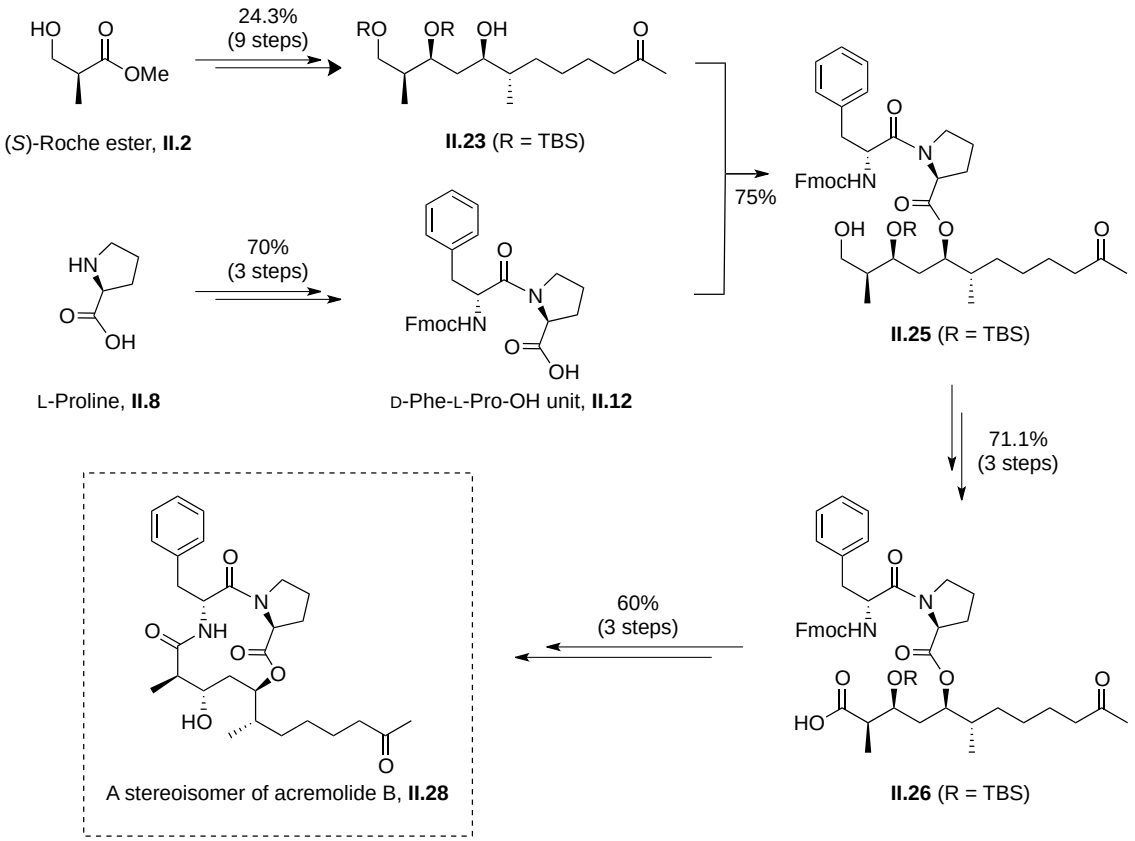


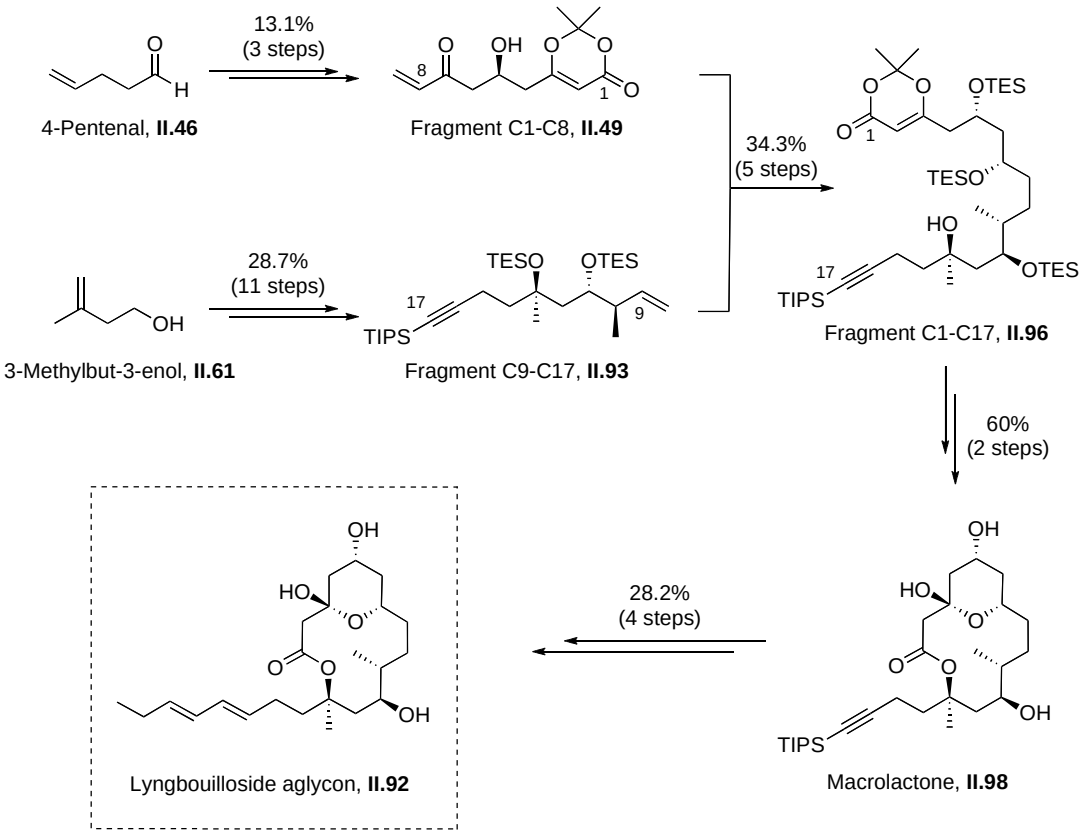
Figure II.10. (Δδ, ppm) for each carbon between C1 and C13 of lyngbouilloside **II.101** and our synthetic aglycon **II.92** (top), and lyngbouilloside **II.101** and lyngbyaloside C **II.102** (bottom).

II.3. CONCLUSIONS

In summary, we developed a straightforward and flexible synthesis of an isomer of acremolide B based on two stereoselective allylations/crotylations to control all four stereogenic centers of the C1-C12 polypropionate segment, a cross-metathesis to introduce the fatty acid side chain, an esterification to introduce the dipeptide unit and a macrolactamization to build the macrolide core. Hence, the (2*R*,3*S*,5*R*,6*S*)-isomer of acremolide B was synthesized in 16 steps and 7.6% overall yield starting from (*S*)-Roche ester **II.2**.



Following this work, we also completed the first synthesis of the proposed structure of lyngbouilloside aglycon. The latter was obtained in 21 steps and 2.1% overall yield starting from commercially available 4-pentenal and 3-methylbut-3-enol and featuring a Sonogashira coupling followed by a hydrosilylation/deprotosilylation sequence to build the (*E*,*E*)-diene side chain, and an acylketene macrolactonization to build the macrolide core. In addition, by comparing the NMR chemical shifts of our synthetic aglycon with the ones reported for the natural lyngbouilloside, we proposed a possible stereochemical reassignment.



II.4. EXPERIMENTAL PART OF CHAPTER II

II.4.1. MATERIALS AND METHODS

II.4.1.1. Reagents and solvents

All commercially available chemicals were used as purchased without further purification. Dichloromethane and toluene were distilled from calcium hydride. THF and Et_2O were distilled from sodium/benzophenone. DMF was distilled under vacuum over $MgSO_4$, and pyridine was distilled and stored over KOH pellets.

II.4.1.2. General instruments

Melting points were performed on a Kofler bench calibrated with an analytically pure standard.

Infrared spectra (**IR**) were recorded on a Bruker TENSOR^{TM} 27 (IRTF) and wave numbers are indicated in cm^{-1}.

NMR spectra were recorded on a Bruker AVANCE 400 spectrometer. ^1H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra were recorded at 100 MHz. Spectra recorded in $CDCl_3$ were referenced to residual $CHCl_3$ at 7.26 ppm for ^1H or 77.0 ppm ^{13}C. Spectra recorded in d_6-DMSO were referenced to residual DMSO at 2.49 ppm for ^1H or 39.5 ppm for ^{13}C. Coupling constants (*J*) were given in Hertz (Hz). Multiplicity with respect to carbon deduced from DEPT experiments. Abbreviations used in the description of resonance were as follows: singlet: s, doublet: d, triplet: t, quartet: q, septet: sept, multiplet: m, double of doublet: dd, broad: br, apparent: app.

Optical rotations ($[\alpha]_D^T$ (*c* g/100 mL)) were measured on a Perkin-Elmer polarimeter (Model 343 Plus), using the sodium D line.

High-resolution mass spectra (HRMS) were performed by "Groupe de Spectrométrie de Masse de l'Université Pierre et Marie Curie" (Paris, FRANCE).

II.4.1.3. Chromatography

Thin layer chromatography (TLC) was performed on precoated TLC plates, silica gel 60 F_{254} (Merck). The spots on the TLC plates were visualized with UV lamp (254 nm) and/or stained by using solutions of *p*-anisaldehyde/sulfuric acid/acetic acid in EtOH, phosphomolybdic acid in EtOH or $KMnO_4/K_2CO_3$/AcOH in water followed by heating.

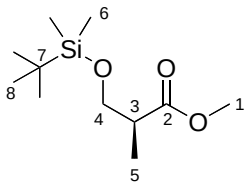
Flash chromatography was performed on silica gel 60 (230-400 mesh, Merck).

Supercritical fluid chromatography (SFC) was performed on a Minigram Berger Mettler-Toledo apparatus.

II.4.2. EXPERIMENTAL PROCEDURES

II.4.2.1. Synthesis of *epi*-acremolide

II.4.2.1.1. Synthesis of (S)-3-(tert-butyldimethylsilanyloxy)-2-methyl propionic acid methyl ester[211]



MW (g/mol): 202.2491 **Molecular formula:** $C_{13}H_{14}O_2$

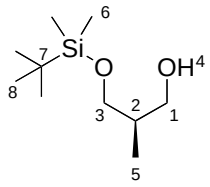
To a solution of (*S*)-3-hydroxy-2-methylpropionic acid methyl ester (**II.2**) (5 g, 42.3 mmol) in CH_2Cl_2 (170 mL) at 0 °C was added imidazole (3.5 g, 50.8 mmol) followed by *tert*-butyldimethylsilyl chloride (7.5 g, 46.6 mmol), and the reaction mixture was then stirred at rt for 22 h until complete conversion of the starting material (reaction monitored by TLC analysis). The reaction mixture was then filtered, concentrated under reduced pressure, and the crude residue was purified by flash chromatography (PE/Et_2O, 99:1) to afford 3-(*tert*-butyldimethylsilanyloxy)-2-methylpropionic acid methyl ester (9.8 g, 99%) as a colourless oil. Its spectroscopic and physical data matched the ones reported in the literature.[211]

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (dd, J = 9.7, 6.8 Hz, 1H, H_4), 3.64 (s, 3H, H_1), 3.61 (dd, J = 9.7, 6.0 Hz, 1H, H_4), 2.61 ($sext_{app}$, J = 7.0 Hz, 1H, H_3), 1.10 (d, J = 7.0 Hz, 3H, H_5), 0.84 (s, 9H, H_8), 0.01 (s, 3H, H_6), 0.00 (s, 3H, H_6).

^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5 (s, C_2), 65.2 (t, C_4), 51.5 (q, C_1), 42.5 (d, C_3), 25.8 (q, 3C, C_8), 18.2 (s, C_7), 13.5 (q, C_5), -5.5 (q, 2C, C_6).

[211] Marshall, J. A.; Blough, B. E. *J. Org. Chem.* **1990**, *55*, 1540–1547.

II.4.2.1.2. Synthesis of (R)-3-(tert-butyldimethylsilanyloxy)-2-methyl propan-1-ol (II.3)[212]



MW (g/mol): 204.3819 **Molecular formula:** $C_{10}H_{24}O_2Si$

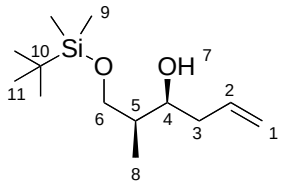
To a solution of 3-(*tert*-butyldimethylsilanyloxy)-2-methylpropionic acid methyl ester (9.5 g, 40.8 mmol) in toluene (150 mL) at –78 ºC, was slowly added DIBAL-H (98.5 mL of a 1M solution in toluene, 98.5 mmol). The resulting reaction mixture was stirred for 20 min, time after which it was quenched with a 1:1 mixture of EtOAc/saturated aqueous solution of sodium potassium tartrate (200 mL). Stirring was continued at rt overnight before the organic layer was separated. The aqueous layer was then extracted with EtOAc (2 x 100 mL), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (PE/EtOAc, 99:1) to afford (*R*)-3-(*tert*-butyldimethylsilanyloxy)-2-methylpropan-1-ol (**II.3**) as colorless oil (7.9 g, 95%). The spectroscopic and physical data of **II.3** matched the ones reported in the literature.[212]

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 (dd, J = 9.8, 4.8 Hz, 1H, H_1), 3.53 (d, J = 5.8 Hz, 2H, H_3), 3.48 (dd, J = 9.8, 7.5 Hz, 1H, H_1), 3.11 (br s, 1H, H_4), 1.84 ($sext_{app}$, J = 6.8 Hz, 1H, H_2), 0.83 (s, 9H, H_8), 0.78 (d, J = 7.0 Hz, 3H, H_5), 0.00 (s, 6H, H_6)

^{13}C NMR (100 MHz, $CDCl_3$) δ 68.7 (t, C_1), 68.2 (t, C_3), 37.0 (d, C_2), 25.8 (q, 3C, C_8), 18.2 (s, C_7), 13.1 (q, C_5), -5.5 (q, C_6), -5.6 (q, C_6).

[212] Roush, W. R.; Palkowitz, A. D.; Palmer, M. A. *J. Org. Chem.* **1987**, *52*, 316–318.

II.4.2.1.3. Synthesis of (2S,3S)-1-(tert-butyldimethylsilanyloxy)-2-methyl hex-5-en-3-ol (II.5)[212]



MW (g/mol): 244.4457 **Molecular formula:** $C_{13}H_{28}O_2Si$

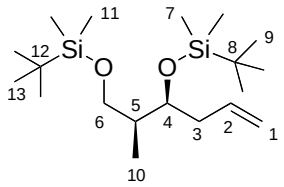
To a solution of oxalyl chloride (2.9 mL, 33.2 mmol) in CH_2Cl_2 (110 mL) at –78 ºC was slowly added DMSO (5.1 mL, 66.4 mmol), and the reaction mixture was stirred for 30 min. (*R*)-3-(*tert*-butyldimethylsilanyloxy)-2-methylpropan-1-ol (**II.3**) (3.36 g, 16.1 mmol) was then added dropwise and stirring was continued for an additional 30 min at the same temperature. Et_3N (13.9 mL, 99.6 mmol) was then added and the reaction mixture was warmed up to rt and quenched with a saturated aqueous NH_4Cl solution (50 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Hexane was then added and the precipitate was filtered over Celite©. The solvent was removed under reduced pressure and the resulting crude aldehyde was used in the next step without further purification.

To a solution of the (*R*,*R*)-[Ti]-**II** complex (13.2 g, 21.9 mmol) in Et_2O (166 mL) at –78 ºC was added allylmagnesium chloride (9.9 mL of a 2M solution in THF, 19.9 mmol), and the reaction mixture was stirred for 2 h at 0 ºC. The solution was then cooled to –78 ºC and the crude aldehyde (16.1 mmol) was added dropwise. The resulting reaction mixture was stirred for 4 h at the same temperature until complete conversion of the starting material (reaction monitored by TLC analysis), quenched with water (80 mL), and stirred overnight at rt. The reaction mixture was then filtered over Celite©, and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The resulting crude residue was purified by flash chromatography (PE/EtOAc, 97:3) to afford (2*S*,3*S*)-1-(*tert*-butyldimethylsilanyloxy)-2-methylhex-5-en-3-ol (**II.5**) (3.6 g, 71%) as a single stereoisomer, and as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (m, 1H, H_2), 5.14-4.94 (m, 2H, H_1), 3.80 (m, 1H, H_4), 3.69 (dd, J = 10.0, 6.0 Hz, 1H, H_6), 3.60 (dd, J = 9.8, 5.0 Hz, 1H, H_6), 2.92 (d, J = 3.0 Hz, 1H, H_7), 2.29-2.05 (m, 2H, H_3), 1.66 (m, 1H, H_5), 0.87 (d, J = 7.0 Hz, 3H, H_8), 0.83 (s, 9H, H_{11}), 0.00 (s, 6H, H_9).

^{13}C NMR (100 MHz, $CDCl_3$) δ 135.7 (d, C_2), 116.9 (t, C_1), 73.9 (d, C_4), 68.2 (t, C_6), 39.0 (d, C_5), 38.4 (t, C_3), 25.8 (q, 3C, C_{11}), 18.2 (s, C_{10}), 10.1 (q, C_8), -5.6 (q, C_9), -5.7 (q, C_9).

II.4.2.1.4. Synthesis of (4S,5S)-4,6-bis-(tert-butyldimethylsilanyloxy)-5-methylhex-1-ene



MW (g/mol): 358.7066 **Molecular formula:** $C_{19}H_{42}O_2Si_2$

To a solution of (2*S*,3*S*)-1-(*tert*-butyldimethylsilanyloxy)-2-methylhex-5-en-3-ol (**II.5**) (3.6 g, 14.7 mmol) in CH_2Cl_2 (75 mL) at -78 ºC was added 2,6-lutidine (3.4 mL, 29.5 mmol) and TBSOTf (5.8 g, 22.1 mmol). The resulting reaction mixture was stirred for 90 min at the same temperature before a saturated aqueous $NaHCO_3$ solution (40 mL) was added. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude was purified by flash chromatography (PE/Et_2O, 99:1) to afford (4*S*,5*S*)-4,6-bis-(*tert*-butyldimethylsilanyloxy)-5-methylhex-1-ene (4.2 g, 80%) as a colourless oil.

***R**$_f$* (PE/Et_2O, 9.9:0.1): 0.87.

[α]$_D^{20}$ +7.55 (*c* 1.1, $CHCl_3$).

IR (neat): 2929, 2858, 1472, 1253, 1095, 1039, 916 cm^{-1}.

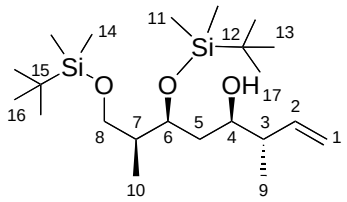
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (m, 1H, H_2), 5.08-4.94 (m, 2H, H_1), 3.81 (m, 1H, H_4), 3.50 (dd, J = 9.8, 6.8 Hz, 1H, H_6), 3.37 (dd, J = 9.8, 6.5 Hz, 1H, H_6), 2.29-2.12 (m, 2H, H_3), 1.66 (m, 1H, H_5), 0.86 (s, 9H, H_9 or H_{13}), 0.85 (s, 9H, H_9 or H_{13}), 0.80 (d,

J = 6.8 Hz, 3H, H_{10}), 0.02 (s, 3H, H_7 or H_{11}), 0.01 (s, 6H, H_7 or H_{11}), 0.00 (s, 3H, H_7 or H_{11}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 135.6 (d, C_1), 116.4 (t, C_2), 71.5 (d, C_4), 65.4 (t, C_6), 39.8 (d, C_5), 39.7 (t, C_3), 26.0 (q, 3C, C_9 or C_{13}), 25.9 (q, 3C, C_9 or C_{13}), 18.2 (s, C_8 or C_{12}), 18.1 (s, C_8 or C_{12}), 10.2 (q, C_{10}), -4.1 (q, C_7 or C_{11}), -4.7 (q, C_7 or C_{11}), -5.3 (q, C_7 or C_{11}), -5.4 (q, C_7 or C_{11}).

HRMS (ESI) *m/z*: calculated for $C_{19}H_{42}NaO_2Si_2$ $[M+Na]^+$ 381.2621, found 381.2620.

II.4.2.1.5. Synthesis of (3S,4R,6S,7S)-6,8-bis-(tert-butyldimethylsilanyloxy)-3,7-dimethyloct-1-en-4-ol (II.7)



MW (g/mol): 416.7857 **Molecular formula:** $C_{22}H_{48}O_3Si_2$

To a solution of (4*S*,5*S*)-4,6-bis-(*tert*-butyldimethylsilanyloxy)-5-methylhex-1-ene (2 g, 5.6 mmol) in a 3:1 dioxane/water mixture (56 mL) at 0 ºC, were added 2,6-lutidine (1.6 mL, 13.4 mmol), OsO_4 (3.5 mL of a 2.5% solution in water, 0.28 mmol) and $NaIO_4$ (4.75 g, 22.3 mmol). The resulting reaction mixture was stirred for 3.5 h at rt until complete conversion of the starting material (reaction monitored by TLC analysis). The reaction mixture was then quenched with a saturated aqueous $Na_2S_2O_3$ solution (20 mL) and stirred for 20 min. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude residue was finally filtered over a small plug of silica eluting with a PE/Et_2O (99:1) mixture. The solvent was removed under reduced pressure and the resulting crude aldehyde was used in the next step without further purification.

To a solution of the (*R*,*R*)-[Ti]-**III** complex (5.5 g, 8.9 mmol) in Et_2O (56 mL) at –78 ºC was added 2-butenylmagnesium chloride (16.6 mL of a 2M solution in THF, 8.3 mmol), and the resulting reaction mixture was stirred for 2 h at 0 ºC. The solution was then cooled to –78 ºC and the crude aldehyde **II.6** (5.6 mmol) was added dropwise. The

resulting reaction mixture was stirred for 4 h at the same temperature until complete conversion of the starting material (reaction monitored by TLC analysis), quenched with water (80 mL), and stirred overnight at rt. The reaction mixture was then filtered over Celite©, and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The resulting crude residue was purified by flash chromatography (PE/Et_2O, 95:5) to afford (3*S*,4*R*,6*S*,7*S*)-6,8-bis-(*tert*-butyldimethylsilanyloxy)-3,7-dimethyloct-1-en-4-ol (**II.7**) (1.6 g, 70%) as a colourless oil.

***R*$_f$** (PE/Et_2O, 9.5:0.5): 0.45.

[α]$_D^{20}$ –6.37 (c 0.97, $CHCl_3$).

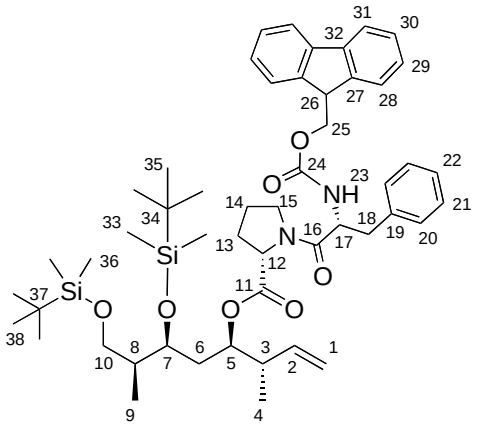
IR (neat): 2929, 2858, 1472, 1389, 1255, 1096, 1047, 1005, 914, 836, 775 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (m, 1H, H_2), 5.09-5.00 (m, 2H, H_1), 3.93 (td_{app}, *J* = 6.8, 3.0 Hz, 1H, H_4), 3.57 (dd, *J* = 9.5, 5.8 Hz, 1H, H_8), 3.48 (m, 1H, H_6), 3.41 (dd, *J* = 9.5, 7.0 Hz, 1H, H_8), 2.39 (br s, 1H, H_{17}), 2.16 (m, 1H, H_3), 1.79 (m, 1H, H_7), 1.60 (m, 1H, H_5), 1.45 (m, 1H, H_5), 0.99 (d, *J* = 6.8 Hz, 3H, H_9), 0.85 (s, 9H, H_{13} or H_{16}), 0.84 (s, 9H, H_{13} or H_{16}), 0.80 (d, *J* = 7.0 Hz, 3H, H_{10}), 0.05 (s, 3H, H_{11} or H_{14}), 0.03 (s, 3H, H_{11} or H_{14}), 0.00 (s, 3H, H_{11} or H_{14}), -0.01 (s, 3H, H_{11} or H_{14}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 140.4 (d, C_2), 115.6 (t, C_1), 73.2 (d, C_4), 72.7 (d, C_6), 64.8 (t, C_8), 44.2 (d, C_3), 40.5 (d, C_7), 37.3 (t, C_5), 26.0 (q, 3C, C_{13} or C_{16}), 25.9 (q, 3C, C_{13} or C_{16}), 18.3 (s, C_{12} or C_{15}), 18.0 (s, C_{12} or C_{15}), 15.7 (q, C_9), 11.6 (q, C_{10}), -4.3 (q, C_{11} or C_{14}), -4.5 (q, C_{11} or C_{14}), -5.3 (q, C_{11} or C_{14}), -5.4 (q, C_{11} or C_{14}).

HRMS (ESI) *m/z*: calculated for $C_{22}H_{48}O_3NaSi_2$ $[M+Na]^+$: 439.3034, found: 439.3031.

*II.4.2.1.6. Synthesis of (**R**)-1-[(**R**)-2-(9H-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1**R**,3**S**,4**S**)-3,5-bis-(tert-butyldimethylsilanyloxy)-4-methyl-1-((**S**)-1-methylallyl)-pentyl ester (II.13)*



MW (g/mol): 906.3032 **Molecular formula:** $C_{51}H_{74}N_2O_7$

To a solution of (3*S*,4*R*,6*S*,7*S*)-6,8-bis-(*tert*-butyldimethylsilanyloxy)-3,7-dimethyloct-1-en-4-ol (**II.7**) (68 mg, 0.16 mmol) and L-Pro-D-Phe **II.12** (87 mg, 0.18 mmol) in toluene (16 mL), was added DMAP (39 mg, 0.32 mmol). The reaction mixture was then cooled to –78 °C before DIEA (98 mL, 0.60 mmol) was added followed by 2,4,6-trichlorobenzoyl chloride (74 mL, 0.48 mmol). The resulting slurry was slowly warmed to rt over 2 h, stirred for an additional 6 h at the same temperature, and quenched with a saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash chromatography (PE/EtOH, 97:3) to afford (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*S*)-3,5-bis-(*tert*-butyldimethylsilanyloxy)-4-methyl-1-((*S*)-1-methylallyl)-pentyl ester (**II.13**) (120 mg, 85%) as a viscous oil. *Mixture of rotamers:*

$\boldsymbol{R_f}$ (CH_2Cl_2/CH_3OH, 9.9:0.1): 0.67.

$\boldsymbol{[\alpha]_D^{20}}$ –16.4 (*c* 0.45, $CHCl_3$).

IR (neat): 2954, 2929, 2857, 1727, 1644, 1449, 1251, 1187, 1098, 1042, 836, 775 cm^{-1}.

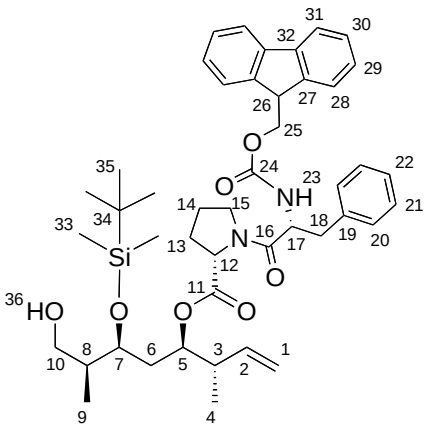
^{1}H NMR (400 MHz, $CDCl_3$) *Major rotamer:* δ 7.76 (d_{app}, *J* = 7.5 Hz, 2H, H_{28} or H_{31}), 7.60 (t_{app}, *J* = 8.2 Hz, 2H, H_{29} or H_{30}), 7.40 (t_{app}, *J* = 7.9 Hz, 2H, H_{29} or H_{30}), 7.36-7.29 (m, 2H, H_{28} or H_{31}), 7.28-7.16 (m, 5H, H_{20}, H_{21}, H_{22}), 5.81-5.64 (m, 2H, H_2, H_{23}), 5.12-

4.98 (m, 2H, H_1), 4.88 (m, 1H, H_5), 4.69 (m, 1H, H_{17}), 4.38 (dd, *J* = 10.2, 7.9 Hz, 1H, H_{25}), 4.37-4.24 (m, 2H, H_{12}, H_{25}), 4.21 (t_{app}, *J* = 7.5 Hz, 1H, H_{26}), 3.78 (m, 1H, H_7), 3.54-3.43 (m, 2H, H_{10}, H_{15}), 3.37 (dd, *J* = 9.4, 6.7 Hz, 1H, H_{10}), 3.11 (dd, *J* = 13.0, 5.5 Hz, 1H, H_{18}), 2.98 (dd, *J* = 13.0, 9.4 Hz, 1H, H_{18}), 2.63 (m, 1H, H_{15}), 2.51 (m, 1H, H_8), 1.96-1.76 (m, 3H, H_{13}, H_{14}), 1.75-1.59 (m, 3H, H_3, H_6), 1.50 (m, 1H, H_{14}), 1.08 (d, *J* = 6.7 Hz, 3H, H_4), 0.87 (s, 9H, H_{35} or H_{38}), 0.86 (s, 9H, H_{35} or H_{38}), 0.77 (d, *J* = 6.7 Hz, 3H, H_9), 0.05 (s, 3H, H_{33} or H_{36}), 0.02 (s, 3H, H_{33} or H_{36}), 0.01 (s, 3H, H_{33} or H_{36}), 0.00 (s, 3H, H_{33} or H_{36}).

^{13}C NMR (100 MHz, $CDCl_3$) *Major rotamer:* *δ* 171.1 (s, C_{16}), 169.6 (s, C_{11}), 155.5 (s, C_{24}), 144.0 (s, C_{27}), 143.8 (s, C_{27}), 141.3 (s, 2C, C_{32}), 138.8 (d, C_2), 136.3 (s, C_{19}), 129.6 (d, 2C, C_{21}), 128.5 (d, 2C, C_{20}), 127.7 (d, 2C, C_{28}), 127.1 (d, 2C, C_{31}), 127.0 (d, C_{22}), 125.3 (d, 2C, C_{29}), 120.0 (d, 2C, C_{30}), 116.0 (t, C_1), 75.0 (d, C_5), 68.5 (d, C_7), 67.0 (t, C_{25}), 65.5 (t, C_{10}), 58.8 (d, C_{12}), 54.1 (d, C_{17}), 47.2 (d, C_{26}), 46.8 (t, C_{15}), 41.1 (d, C_8), 40.4 (t, C_{18}), 39.6 (d, C_3), 35.9 (t, C_6), 31.0 (t, C_{13}), 26.0 (q, 3C, C_{35} or C_{38}), 25.9 (q, 3C, C_{35} or C_{38}), 24.3 (t, C_{14}), 18.3 (s, C_{34} or C_{37}), 18.1 (s, C_{34} or C_{37}), 15.4 (q, C_4), 9.8 (q, C_9), -4.1 (q, C_{33} or C_{36}), -4.7 (q, C_{33} or C_{36}), -5.3 (q, C_{33} or C_{36}), -5.4 (q, C_{33} or C_{36}).

HRMS (ESI) *m/z*: calculated for $C_{51}H_{74}N_2O_7NaSi_2$ $[M+Na]^+$: 905.4932, found: 905.4934.

*II.4.2.1.7. Synthesis of (**R**)-1-[(**R**)-2-(9**H**-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1**R**,3**S**,4**S**)-3-(tert-butyldimethylsilanyloxy)-5-hydroxy-4-methyl-1-((S)-1-methylallyl)-pentyl ester (II.14)*



MW (g/mol): 769.0526 **Molecular formula:** $C_{45}H_{60}N_2O_7Si$

To a solution of $ZnBr_2$ (110 mg, 0.73 mmol) in CH_2Cl_2 (8 mL) at rt was added a solution of (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*S*)-3,5-bis-(*tert*-butyldimethylsilanyloxy)-4-methyl-1-((*S*)-1-methylallyl)-pentyl ester (**II.13**) (110 mg, 0.13 mmol) in CH_2Cl_2 (16 mL). The resulting reaction mixture was then stirred for 4 h at the same temperature until complete conversion of the starting material (reaction monitored by TLC analysis), and quenched with a saturated aqueous $NaHCO_3$ solution (15 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL), and the combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash chromatography eluting with $CHCl_3$ to afford (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*S*)-3-(*tert*-butyldimethylsilanyloxy)-5-hydroxy-4-methyl-1-((*S*)-1-methylallyl)-pentyl ester (**II.14**) (83 mg, 83%) as a viscous oil. *Mixture of rotamers:*

$\boldsymbol{R_f}$ (CH_2Cl_2/CH_3OH, 9.9:0.1): 0.21.

$\boldsymbol{[\alpha]_D^{20}}$ –12.65 (*c* 0.63, $CHCl_3$).

IR (neat): 2956, 2928, 2856, 1722, 1639, 1524, 1450, 1251, 1188, 1086, 1041, 837, 775 cm^{-1}.

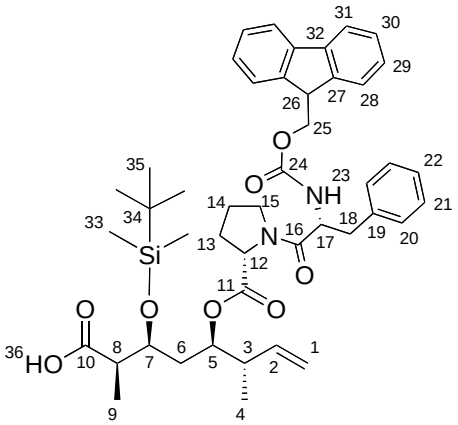
^{1}H NMR (400 MHz, $CDCl_3$) *Major rotamer:* δ 7.75 (d_{app}, *J* = 7.5 Hz, 2H, H_{28} or H_{31}), 7.59 (dd, *J* = 11.5, 7.5 Hz, 2H, H_{29} or H_{30}), 7.38 (t_{app}, *J* = 7.5 Hz, 2H, H_{28} or H_{31}), 7.30 (t_{app}, *J* = 7.2 Hz, 2H, H_{29} or H_{30}), 7.27-7.14 (m, 5H, H_{20}, H_{21}, H_{22}), 5.86 (d, *J* = 8.2 Hz, 1H, H_{23}), 5.72 (m, 1H, H_2), 5.10-4.98 (m, 2H, H_1), 4.99 (m, 1H, H_5), 4.70 (m, 1H, H_{17}), 4.41 (dd, *J* = 10.4, 7.2 Hz, 1H, H_{25}), 4.35-4.22 (m, 2H, H_{12}, H_{25}), 4.19 (t_{app}, *J* = 7.2 Hz, 1H, H_{26}), 3.80 (td, *J* = 7.6, 1.6 Hz, 1H, H_7), 3.55-3.36 (m, 3H, H_{10}, H_{15}), 3.10 (dd, *J* = 12.9, 5.4 Hz, 1H, H_{18}), 2.95 (dd, *J* = 12.9, 9.3 Hz, 1H, H_{18}), 2.63 (m, 1H, H_{15}), 2.41 (m, 1H, H_8), 1.98-1.74 (m, 4H, H_{13}, H_{14}, H_{36}), 1.72-1.62 (m, 3H, H_3, H_6), 1.49 (m, 1H, H_{14}), 1.04 (d, *J* = 6.8 Hz, 3H, H_4), 0.84 (s, 9H, H_{35}), 0.73 (d, *J* = 6.8 Hz, 3H, H_9), 0.02 (s, 6H, H_{33}).

^{13}C NMR (100 MHz, $CDCl_3$) *Major rotamer:* δ 171.4 (s, C_{16}), 169.9 (s, C_{11}), 155.6 (s, C_{24}), 144.0 (s, C_{27}), 143.8 (s, C_{27}), 141.3 (s, 2C, C_{32}), 138.9 (d, C_2), 136.2 (s, C_{19}), 129.5 (d, 2C, C_{21}), 128.5 (d, 2C, C_{20}), 127.7 (d, 2C, C_{28}), 127.1 (d, 3C, C_{22}, C_{31}), 125.3 (d, 2C,

C_{29}), 120.0 (d, 2C, C_{30}), 116.1 (t, C_1), 74.8 (d, C_5), 68.6 (d, C_7), 67.0 (t, C_{25}), 65.4 (t, C_{10}), 59.1 (d, C_{12}), 54.2 (d, C_{17}), 47.2 (d, C_{26}), 46.9 (t, C_{15}), 41.9 (d, C_8), 40.4 (t, C_{18}), 38.4 (d, C_3), 35.7 (t, C_6), 29.0 (t, C_{13}), 25.8 (q, 3C, C_{35}), 24.3 (t, C_{14}), 18.0 (s, C_{34}), 15.8 (q, C_4), 9.8 (q, C_9), -4.3 (q, C_{33}), -4.8 (q, C_{33}).

HRMS (ESI) *m/z*: calculated for $C_{45}H_{60}O_7N_2NaSi$ $[M+Na]^+$: 791.4062, found: 791.4057.

*II.4.2.1.8. Synthesis of (***R***)-1-[(***R***)-2-(9***H***-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1***R***,3***S***,4***R***)-3-(tert-butyldimethylsilanyloxy)-4-carboxy-1-(***S***)-1-methylallyl)-pentyl ester (II.16)*



MW (g/mol): 783.0361 **Molecular formula:** $C_{45}H_{58}N_2O_8$

To a solution of (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*S*)-3-(*tert*-butyldimethylsilanyloxy)-5-hydroxy-4-methyl-1-((*S*)-1-methylallyl)-pentyl ester, **II.15** (77 mg, 0.1 mmol) in CH_2Cl_2 (1 mL) at 0 ºC were added TEMPO (22 mg, 0.14 mmol), KBr (50 mL of a 0.2 M solution in water, 0.01 mmol) and NaOCl (52 mL of a 13% solution in water, 0.1 mmol). After stirring for 30 min at the same temperature, the organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude aldehyde **II.15** was used in the next step without further purification.

The crude aldehyde **II.15** (0.1 mmol), *t*-BuOH (5 mL), 2-methyl-2-butene (0.74 mL, 7.0 mmol), water (1 mL), $NaClO_2$ (68 mg, 0.6 mmol) and NaH_2PO_4 (36 mg, 0.3 mmol) were mixed at 0 ºC and the reaction mixture was stirred at rt for 30 min. *t*-BuOH was then removed under reduced pressure and EtOAc (15 mL) was added. The organic layer

was separated and the aqueous layer was extracted with EtOAc (2 x 15 mL). The combined organic layers were combined and dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash chromatography (CH_2Cl_2/MeOH, 99:1) to afford (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*R*)-3-(*tert*-butyldimethylsilanyloxy)-4-carboxy-1-((*S*)-1-methylallyl)-pentyl ester (**II.16**) (73 mg, 93%) as a viscous oil. *Mixture of rotamers:*

$\boldsymbol{R_f}$ (CH_2Cl_2/MeOH, 9.8:0.2): 0.22.

$\boldsymbol{[\alpha]_D^{20}}$ –24.0 (*c* 0.87, $CHCl_3$).

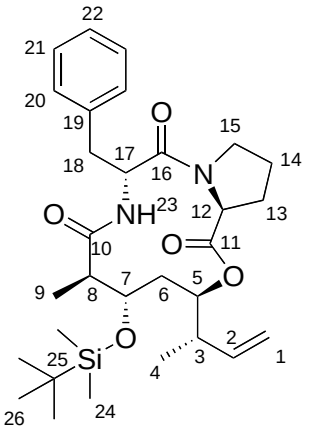
IR (neat): 2928, 2856, 1713, 1618, 1451, 1251, 1186, 1095, 1033, 837, 776, 759, 741 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) *Major rotamer:* δ 7.74 (d, *J* = 7.4 Hz, 2H, H_{28} or H_{31}), 7.58 (dd, *J* = 9.6, 7.6 Hz, 2H, H_{29} or H_{30}), 7.38 (t_{app}, *J* = 7.6 Hz, 2H, H_{29} or H_{30}), 7.29 (dd, *J* = 7.6, 0.8 Hz, 2H, H_{28} or H_{31}), 7.26-7.14 (m, 5H, H_{20}, H_{21}, H_{22}), 5.80 (d, *J* = 8.4 Hz, 1H, H_{23}), 5.72 (m, 1H, H_2), 5.11-4.96 (m, 2H, H_1), 4.91 (m, 1H, H_5), 4.71 (m, 1H, H_{17}), 4.40-4.32 (m, 2H, H_{12}, H_{25}), 4.29 (dd, *J* = 10.8, 7.2 Hz, 1H, H_{25}), 4.19 (t_{app}, *J* = 7.3 Hz, 1H, H_{26}), 4.09 (m, 1H, H_7), 3.51 (m, 1H, H_{15}), 3.08 (dd, *J* = 12.8, 5.6 Hz, 1H, H_{18}), 2.96 (dd, *J* = 12.8, 9.2 Hz, 1H, H_{18}), 2.68-2.52 (m, 2H, H_8, H_{15}), 2.43 (m, 1H, H_3), 1.96-1.72 (m, 3H, H_{13}, H_{14}), 1.71-1.62 (m, 2H, H_6), 1.49 (m, 1H, H_{14}), 1.10-0.99 (d, *J* = 7.0 Hz, 6H, H_4, H_9) 0.83 (s, 9H, H_{35}), 0.03 (s, 3H, H_{33}), 0.02 (s, 3H, H_{33}).

^{13}C NMR (100 MHz, $CDCl_3$) *Major rotamer:* δ 178.8 (s, C_{10}), 171.3 (s, C_{16}), 170.0 (s, C_{11}), 155.6 (s, C_{24}), 144.0 (s, C_{27}), 143.8 (s, C_{27}), 141.3 (s, 2C, C_{32}), 138.6 (d, C_2), 136.2 (s, C_{19}), 129.5 (d, 2C, C_{21}), 128.5 (d, 2C, C_{20}), 127.7 (d, 2C, C_{28}), 127.1 (d, 3C, C_{22}, C_{31}), 125.3 (d, 2C, C_{29}), 120.0 (d, 2C, C_{30}), 116.3 (t, C_1), 74.1 (d, C_5), 69.8 (d, C_7), 67.1 (t, C_{25}), 58.8 (d, C_{12}), 54.1 (d, C_{17}), 47.1 (d, C_{26}), 46.9 (t, C_{15}), 43.2 (d, C_8), 41.8 (d, C_3), 40.3 (t, C_{18}), 35.9 (t, C_6), 29.0 (t, C_{13}), 25.7 (q, 3C, C_{35}), 24.2 (t, C_{14}), 17.9 (s, C_{34}), 15.4 (q, C_4), 9.3 (q, C_9), -4.3 (q, C_{33}), -4.9 (q, C_{33}).

HRMS (ESI) *m/z*: calculated for $C_{45}H_{58}O_8N_2NaSi$ $[M+Na]^+$: 805.3855, found: 805.3859.

II.4.2.1.9. Synthesis of (5R,8R,9S,11R,13aR)-5-benzyl-9-(tert-butyldimethylsilanyloxy)-8-methyl-11-((S)-1-methylallyl)-decahydro-12-oxa-3a,6-diaza-cyclopentacyclododecene-4,7,13-trione (II.18)



MW (g/mol): 542.7821 **Molecular formula:** $C_{30}H_{46}N_2O_5$

To a solution of (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*R*)-3-(*tert*-butyldimethylsilanyloxy)-4-carboxy-1-((*S*)-1-methylallyl)-pentyl ester (**II.16**) (70 mg, 0.09 mmol) in MeCN (3.2 mL) at rt, was added Et_2NH (1.6 mL), and the reaction mixture was stirred at rt until complete conversion of the starting material (reaction monitored by TLC analysis). The solvent was then removed under reduced pressure and the resulting crude amino acid **II.17** was used in the next step without further purification.

To a solution of amino acid (0.09 mmol) in CH_2Cl_2 (13 mL) at 0 ºC were added EDCl (33 mg, 0.17 mmol), HOBt (23 mg, 0.17 mmol) and DIEA (64 mL, 0.39 mmol), and the resulting reaction mixture was stirred for 3 h at rt. The reaction was then quenched with a saturated aqueous NH_4Cl solution (10 mL), and the organic phase was separated. The aqueous layer was then extracted with CH_2Cl_2 (2 x 15 mL), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash chromatography eluting with $CHCl_3$ to afford (5*R*,8*R*,9*S*,11*R*,13a*R*)-5-benzyl-9-(*tert*-butyldimethylsilanyloxy)-8-methyl-11-((*S*)-1-methylallyl)-decahydro-12-oxa-3a,6-diazacyclopenta cyclododecene-4,7,13-trione (**II.18**) (46 mg, 95%) as an amorphous solid. *Mixture of rotamers:*

$\boldsymbol{R_f}$ (CH_2Cl_2/CH_3OH, 9.5:0.5): 0.66.

$\boldsymbol{[\alpha]_D^{20}}$ –12.7 (*c* 0.3, $CHCl_3$).

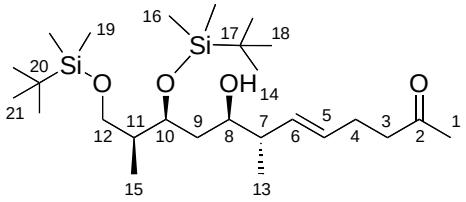
IR (neat): 3675, 2987, 2972, 2901, 1733, 1665, 1621, 1542, 1452, 1406, 1394, 1382, 1252, 1229, 1075, 1066 cm^{-1}.

^{1}H NMR (400 MHz, acetone-d_6) *Major rotamer:* δ 7.32-7.12 (m, 6H, H_{20}, H_{21}, H_{22}, H_{23}), 5.67 (ddd, J = 17.3, 10.3, 7.3 Hz, 1H, H_2), 5.21 (m, 1H, H_{17}), 5.08-4.96 (m, 2H, H_1), 4.72-4.62 (m, 2H, H_7, H_{12}), 3.76 (td, J = 9.4, 2.9 Hz, 1H, H_5), 3.56 (m, 1H, H_{15}), 3.33 (m, 1H, H_{15}), 3.16-2.98 (m, 2H, H_{13}), 2.30 (m, 1H, H_8), 2.26-2.02 (m, 3H, H_3, H_{18}), 1.85 (m, 1H, H_{14}), 1.69 (m, 1H, H_{14}), 1.57 (dd, J = 15.6, 3.3 Hz, 2H, H_6), 0.97 (d, J = 7.0 Hz, 3H, H_4), 0.91 (d, J = 7.0 Hz, 3H, H_9), 0.85 (s, 9H, H_{26}), 0.03 (s, 3H, H_{24}), 0.01 (s, 3H, H_{24}).

^{13}C NMR (100 MHz, acetone-d_6) *Major rotamer:* δ 179.9 (s, C_{10}), 177.4 (s, C_{16}), 175.6 (s, C_{11}), 144.6 (d, C_2), 143.5 (s, C_{19}), 134.5 (d, 2C, C_{21}), 133.8 (d, 2C, C_{20}), 132.1 (d, C_{22}), 121.0 (t, C_1), 77.4 (d, C_5), 65.4 (d, C_7), 62.7 (d, C_{12}), 52.9 (t, C_{15}), 49.5 (d, C_{17}), 48.8 (d, C_8), 41.6 (t, C_{18}), 41.0 (t, C_6), 38.2 (t, C_{13}), 35.0 (d, C_3), 30.9 (q, 3C, C_{26}), 26.7 (t, C_{14}), 23.1 (s, C_{25}), 22.6 (q, C_4), 20.0 (q, C_9), 0.9 (q, C_{24}), 0.0 (q, C_{24}).

HRMS (ESI) *m/z*: calculated for $C_{30}H_{46}O_5N_2NaSi$ $[M+Na]^+$: 565.3068, found: 565.3060.

*II.4.2.1.10. Synthesis of (*E*)-(7*S*,8*R*,10*S*,11*S*)-10,12-bis-(tert-butyldimethylsilanyloxy)-8-hydroxy-7,11-dimethyldodec-5-en-2-one (II.21)*



MW (g/mol): 486.8756 **Molecular formula:** $C_{26}H_{54}O_4Si_2$

To a stirred solution of (3*S*,4*R*,6*S*,7*S*)-6,8-bis-(*tert*-butyldimethylsilanyloxy)-3,7-dimethyloct-1-en-4-ol (**II.7**) (1.6 g, 3.84 mmol) and 5-hexene-2-one (**48**) (753 mg, 7.68 mmol) in CH_2Cl_2 (40 mL) was added the Hoveyda-Grubbs catalyst (480 mg, 0.77 mmol), and the resulting reaction mixture was refluxed for 24 h until complete conversion of the starting material (reaction monitored by TLC analysis). The solvent was then removed under reduced pressure and the crude residue was purified by flash column chromatography (PE/Et_2O, 90:10) to afford (*E*)-(7*S*,8*R*,10*S*,11*S*)-10,12-bis-

(*tert*-butyldimethylsilanyloxy)-8-hydroxy-7,11-dimethyldodec-5-en-2-one (1.3 g, 71%) as a colourless oil. *Mixture of isomers:*

R_f (PE/Et_2O, 7:3): 0.54.

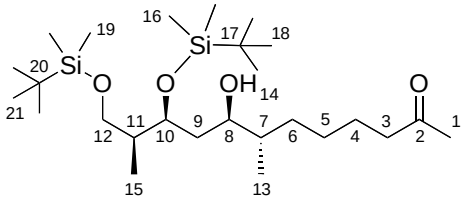
IR (neat): 3494, 2956, 2929, 2857, 1717, 1472, 1463, 1361, 1254, 1095, 1046, 836, 775 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) *Major rotamer:* δ 5.58-5.29 (m, 2H, H_5, H_6), 3.93 (m, 1H, H_8), 3.57 (dd, *J* = 9.5, 5.8 Hz, 1H, H_{12}), 3.48-3.35 (m, 2H, H_{10}, H_{12}), 2.51-2.43 (m, 2H, H_3), 2.32-2.21 (m, 2H, H_4), 2.10 (m, 1H, H_7), 2.09 (s, 3H, H_1), 1.78 (m, 1H, H_{11}), 1.59 (m, 1H, H_9), 1.45 (m, 1H, H_9), 0.95 (d, *J* = 7.0 Hz, 3H, H_{13}), 0.85 (*s*, 18H, H_{18}, H_{21}), 0.80 (d, *J* = 6.9 Hz, 3H, H_{15}), 0.04 (s, 3H, H_{16} or H_{19}), 0.03 (s, 3H, H_{16} or H_{19}), 0.00 (s, 3H, H_{16} or H_{19}), -0.01 (s, 3H, H_{16} or H_{19}).

^{13}C NMR (100 MHz, $CDCl_3$) *Major rotamer:* δ 208.2/207.0 (s, C_2), 136.7 and 132.9 (d, C_6), 129.9/123.2 (d, C_5), 73.2 (d, C_8), 72.2 (d, C_{10}), 65.1 (t, C_{12}), 43.4 (d, C_7), 43.2 (t, C_3), 40.3 (d, C_{11}), 37.7 (t, C_4), 29.9 (q, C_1), 26.9 (t, C_9), 26.0 (q, 3C, C_{18}), 25.9 (q, 3C, C_{17} or C_{21}), 18.3 (s, C_{17} or C_{21}), 18.0 (s, C_{20}), 16.3 (q, C_{13}), 11.3 (q, C_{15}), -4.3 (q, C_{16} or C_{19}), -4.5 (q, C_{16} or C_{19}), -5.4 (q, 2C, C_{16} or C_{19}).

HRMS (ESI) *m/z*: calculated for $C_{26}H_{54}O_4NaSi_2$ $[M+Na]^+$: 509.3453, found: 509.3444.

II.4.2.1.11. Synthesis of (7S,8R,10S,11S)-10,12-bis-(tert-butyldimethylsilanyloxy)-8-hydroxy-7,11-dimethyldodecan-2-one (II.23)



MW (g/mol): 488.8914 **Molecular formula:** $C_{26}H_{56}O_4Si_2$

To a stirred solution of (*E*)-(7*S*,8*R*,10*S*,11*S*)-10,12-bis-(*tert*-butyldimethylsilanyloxy)-8-hydroxy-7,11-dimethyldodec-5-en-2-one (**II.21**) (1.2 g, 2.46 mmol) in EtOAc (10 mL) at rt under an argon atmosphere was added 10% Pd/C (120 mg). The resulting reaction mixture was stirred under a hydrogen atmosphere (1 atm) at rt until complete conversion of the starting material (reaction monitored by TLC analysis). The crude

reaction mixture was then filtered over Celite©, the solvent was removed under reduced pressure, and the residue was finally purified by column chromatography (PE/Et_2O, 80:20) to afford (7*S*,8*R*,10*S*,11*S*)-10,12-bis-(*tert*-butyldimethylsilanyloxy)-8-hydroxy-7,11-dimethyldodecan-2-one (**II.68**) (1.08 g, 90%) as a colourless oil.

$\boldsymbol{R_f}$ (PE/Et_2O, 8:2): 0.28.

$\boldsymbol{[\alpha]_D^{20}}$ –4.73 (*c* 1.1, $CHCl_3$).

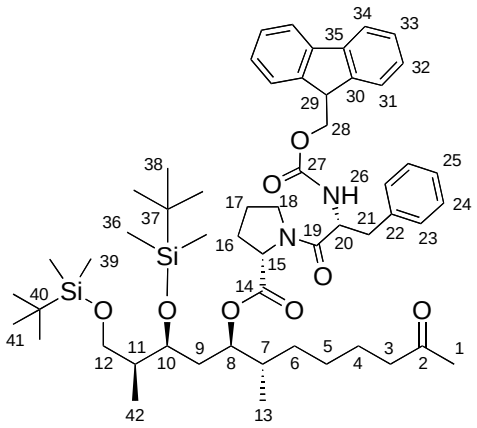
IR (neat): 3481, 2955, 2928, 2857, 1716, 1463, 1361, 1253, 1094, 1045, 835, 775 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) *Major rotamer:* δ 3.89 (m, 1H, H_8), 3.60 (dd, J = 9.5, 5.5 Hz, 1H, H_{12}), 3.52-3.38 (m, 2H, H_{10}, H_{12}), 2.38 (t, J = 7.3 Hz, 2H, H_3), 2.09 (s, 3H, H_1), 1.81 (m, 1H, H_7), 1.64-1.32 (m, 8H, H_4, H_5, H_9, H_{11}, H_{14}), 1.14-0.98 (m, 2H, H_6), 0.86 (s, 9H, H_{18} or H_{21}), 0.85-0.79 (m, 15H, H_{13}, H_{15}, H_{18} or H_{21}), 0.06 (s, 3H, H_{16} or H_{19}), 0.04 (s, 3H, H_{16} or H_{19}), 0.00 (s, 3H, H_{16} or H_{19}), -0.01 (s, 3H, H_{16} or H_{19}).

^{13}C NMR (100 MHz, $CDCl_3$) *Major rotamer:* δ 209.3 (s, C_2), 74.6 (d, C_8), 74.1 (d, C_{10}), 64.4 (t, C_{12}), 43.7 (t, C_3), 40.9 (d, C_{11}), 38.9 (d, C_7), 35.9 (t, C_9), 31.8 (t, C_6), 29.9 (q, C_1), 26.9 (t, C_5), 26.0 (q, 3C, C_{18} or C_{21}), 25.9 (q, 3C, C_{18} or C_{21}), 24.1 (t, C_4), 18.3 (s, C_{17} or C_{20}), 18.0 (s, C_{17} or C_{20}), 15.0 (q, C_{13}), 12.2 (q, C_{15}), -4.3 (q, C_{16} or C_{19}), -4.4 (q, C_{16} or C_{19}), -5.3 (q, C_{16} or C_{19}), -5.4 (q, C_{16} or C_{19}).

HRMS (ESI) *m/z*: calculated for $C_{26}H_{56}O_4NaSi_2$ $[M+Na]^+$: 511.3609, found: 511.3599.

*II.4.2.1.12. Synthesis of (***R***)-1-[(***R***)-2-(9***H***-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1***R***,2***S***)-1-[(2***S***,3***S***)-2,4-bis-(tert-butyldimethylsilanyloxy)-3-methylbutyl]-2-methyl-7-oxooctyl ester (II.24)***



MW (g/mol): 955.4192 **Molecular formula:** $C_{55}H_{82}N_2O_8Si_2$

To a solution of (7*S*,8*R*,10*S*,11*S*)-10,12-bis-(*tert*-butyldimethylsilanyloxy)-8-hydroxy-7,11-dimethyldodecan-2-one (**II.23**) (1.0 g, 2.0 mmol) and L-Pro-D-Phe **II.12** (1.09 g, 2.2 mmol) in toluene (40 mL) at rt was added DMAP (498 mg, 4.1 mmol). The reaction mixture was then cooled to –78 °C before DIEA (1.2 mL, 7.4 mmol) was added followed by 2,4,6-trichlorobenzoyl chloride (0.94 mL, 6.1 mmol). The resulting slurry was slowly warmed to rt over 2 h, stirred for an additional 6 h at the same temperature, and quenched with a saturated aqueous $NaHCO_3$ solution (30 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash chromatography (PE/EtOAc, 80:20) to afford (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*S*)-2,4-bis-(*tert*-butyldimethylsilanyloxy)-3-methylbutyl]-2-methyl-7-oxooctyl ester (**II.24**) (1.32 g, 75%) as a viscous oil. *Mixture of rotamers:*

$\boldsymbol{R_f}$ (PE/EtOAc, 8:2): 0.33.

$\boldsymbol{[\alpha]_D^{20}}$ –12.9 (*c* 0.83, $CHCl_3$).

IR (neat): 3294, 2954, 2927, 2856, 1716, 1642, 1449, 1250, 1187, 1099, 1040, 835, 774, 739 cm^{-1}.

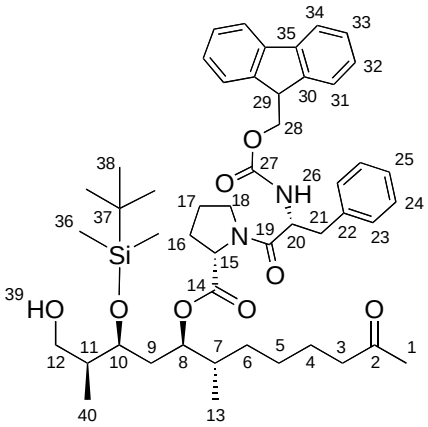
^{1}H NMR (400 MHz, $CDCl_3$) *Major rotamer:* δ 7.81-7.71 (d, J = 7.5 Hz, 2H, H_{34}), 7.64-7.56 (t_{app}, J = 8.0 Hz, 2H, H_{31}), 7.44-7.36 (m, 2H, H_{33}), 7.35-7.26 (m, 2H, H_{32}), 7.25-7.14 (m, 5H, H_{23}, H_{24}, H_{25}), 5.77 (m, 1H, H_{26}), 4.78 (m, 1H, H_8), 4.70 (m, 1H, H_{20}), 4.39 (m, 1H, H_{28}), 4.35-4.25 (m, 2H, H_{15}, H_{28}), 4.21 (m, 1H, H_{29}), 3.75 (m, 1H, H_{10}), 3.54-3.42 (m, 2H, H_{12}, H_{18}), 3.37 (m, 1H, H_{12}), 3.10 (dd, J = 12.5, 5.0 Hz, 1H, H_{21}), 2.96 (m, 1H, H_{21}), 2.54 (m, 1H, H_{18}), 2.46-2.30 (m, 2H, H_3), 2.09 (s, 3H, H_1), 1.99-1.62 (m, 8H, H_4, H_7, H_9, H_{11}, H_{16}), 1.60-1.47 (m, 2H, H_{17}), 1.43-1.08 (m, 4H, H_5, H_6), 0.92 (d, J = 7.3 Hz, 3H, H_{42}), 0.86 (s, 9H, H_{38} or H_{41}), 0.85 (s, 9H, H_{38} or H_{41}), 0.76 (d, J = 6.5 Hz, 3H, H_{13}), 0.04 (s, 3H, H_{36} or H_{39}), 0.01 (s, 3H, H_{36} or H_{39}), 0.00 (s, 3H, H_{36} or H_{39}), -0.01 (s, 3H, H_{36} or H_{39}).

^{13}C NMR (100 MHz, $CDCl_3$) *Major rotamer:* δ 209.2 (s, C_2), 171.2 (s, C_{14}), 169.6 (s, C_{19}), 155.5 (s, C_{27}), 144.0 (s, C_{30}), 143.8 (s, C_{30}), 141.3 (s, 2C, C_{35}), 136.3 (s, C_{22}), 129.6 (d, 2C, C_{23}), 128.4 (d, 2C, C_{24}), 127.7 (d, C_{25}), 127.1 (d, 2C, C_{32}), 127.0 (d, 2C,

C_{33}), 125.2 (d, 2C, C_{31}), 119.9 (d, 2C, C_{34}), 75.6 (d, C_8), 68.2 (d, C_{10}), 67.0 (t, C_{28}), 65.8 (t, C_{12}), 58.7 (d, C_{15}), 54.1 (d, C_{20}), 47.2 (d, C_{11}), 46.8 (t, C_{18}), 43.7 (t, C_3), 40.4 (t, C_{21}), 38.9 (d, C_7), 36.3 (d, C_{29}), 34.9 (t, C_9), 31.8 (t, C_{16}), 29.9 (q, C_1), 29.0 (t, C_{17}), 26.7 (t, C_5), 26.0 (q, 3C, C_{38} or C_{41}), 25.9 (q, 3C, C_{38} or C_{41}), 24.4 (t, C_6), 24.1 (t, C_4), 18.4 (s, C_{37} or C_{40}), 18.1 (s, C_{37} or C_{40}), 14.7 (q, C_{42}), 9.4 (q, C_{13}), -4.0 (q, C_{36} or C_{39}), -4.8 (q, C_{36} or C_{39}), -5.3 (q, C_{36} or C_{39}), -5.4 (q, C_{36} or C_{39}).

HRMS (ESI) *m/z*: calculated for $C_{55}H_{82}O_8N_2NaSi_2$ $[M+Na]^+$: 977.5502, found: 977.5500.

*II.4.2.1.13. Synthesis of (**R**)-1-[(**R**)-2-(9**H**-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1**R**,2**S**)-1-[(2**S**,3**S**)-2-(tert-butyldimethylsilanyloxy)-4-hydroxy-3-methylbutyl]-2-methyl-7-oxooctyl ester (II.25)*



MW (g/mol): 841.1582 **Molecular formula:** $C_{49}H_{68}N_2O_8Si$

$SnCl_2$ (60 mg, 0.3 mmol) was added to a 6:1 EtOH/water (7 mL) mixture at rt. Once the reaction mixture became homogenous, (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*S*)-2,4-bis-(*tert*-butyldimethylsilanyloxy)-3-methylbutyl]-2-methyl-7-oxooctyl ester (**II.24**) (600 mg, 0.6 mmol) was added, and the resulting reaction mixture was stirred for 2 h at rt. CH_2Cl_2 (10 mL) and water (4 mL) were then added, and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 x 10 mL), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash chromatography

(PE/EtOAc, 80:20) to afford (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*S*)-2-(*tert*-butyldimethylsilanyloxy)-4-hydroxy-3-methylbutyl]-2-methyl-7-oxooctyl ester (**II.25**) (418 mg, 79%). *Mixture of rotamers:*

$\boldsymbol{R_f}$ (PE/EtOAc, 7:3): 0.27.

$\boldsymbol{[\alpha]_D^{20}}$ –14.91 (*c* 1.63, $CHCl_3$).

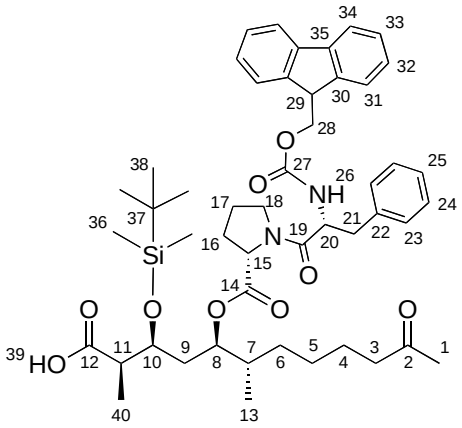
IR (neat): 3430, 2955, 2926, 2855, 1716, 1643, 1450, 1250, 1189, 1094, 1042, 837 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) *Major rotamer:* δ 7.79-7.68 (d, J = 7.6 Hz, 2H, H_{31} or H_{34}), 7.61-7.52 (dd, J = 7.6 Hz, 2H, H_{32} or H_{33}), 7.39-7.31 (t_{app}, J = 7.6 Hz, 2H, H_{32} or H_{33}), 7.30-7.24 (t_{app}, J = 7.3 Hz, 2H, H_{31} or H_{34}), 7.23-7.10 (m, 5H, H_{23}, H_{24}, H_{25}), 5.87 (m, 1H, H_{26}), 4.79 (m, 1H, H_8), 4.68 (m, 1H, H_{20}), 4.39 (m, 1H, H_{28}), 4.30-4.20 (m, 2H, H_{15}, H_{28}), 4.15 (m, 1H, H_{29}), 3.80 (t_{app}, J = 8.0 Hz, 2H, H_{10}), 3.52-3.44 (m, 2H, H_{12}, H_{18}), 3.41 (d_{app}, J = 6.8 Hz, 1H, H_{12}), 3.07 (dd, J = 12.7, 5.1 Hz, 1H, H_{21}), 2.93 (dd, J = 12.7, 9.6 Hz, 1H, H_{21}), 2.65 (m, 1H, H_{18}), 2.41-2.32 (m, 2H, H_3), 2.08 (s, 3H, H_1), 1.93-1.62 (m, 8H, H_4, H_7, H_9, H_{11}, H_{16}), 1.61-1.43 (m, 2H, H_{17}), 1.39-1.08 (m, 4H, H_5, H_6), 0.92-0.73 (m, 12H, H_{38}, H_{40}), 0.72 (d, J = 7.1 Hz, 3H, H_{13}), 0.01 (s, 3H, H_{36}), 0.00 (s, 3H, H_{36}).

^{13}C NMR (100 MHz, $CDCl_3$) *Major rotamer:* δ 209.0 (s, C_2), 171.6 (s, C_{14}), 169.8 (s, C_{19}), 155.6 (s, C_{27}), 144.0 (s, C_{30}), 143.8 (s, C_{30}), 141.3 (s, 2C, C_{35}), 136.3 (s, C_{22}), 129.5 (d, 2C, C_{23}), 128.4 (d, 2C, C_{24}), 127.7 (d, C_{25}), 127.1 (d, 2C, C_{32}), 127.0 (d, 2C, C_{33}), 125.2 (d, 2C, C_{31}), 119.9 (d, 2C, C_{34}), 75.5 (d, C_8), 68.1 (d, C_{10}), 67.0 (t, C_{28}), 65.4 (t, C_{12}), 59.1 (d, C_{15}), 54.2 (d, C_{20}), 47.2 (d, C_{11}), 46.9 (t, C_{18}), 43.6 (t, C_3), 40.3 (t, C_{21}), 38.2 (d, C_7), 36.6 (d, C_{29}), 34.7 (t, C_9), 31.9 (t, C_{16}), 29.6 (q, C_1), 29.0 (t, C_{17}), 26.6 (t, C_5), 25.8 (q, 3C, C_{38}), 24.3 (t, C_6), 23.9 (t, C_4), 18.0 (s, C_{37}), 14.7 (q, C_{40}), 9.2 (q, C_{13}), -4.2 (q, C_{36}), -4.9 (q, C_{36}).

HRMS (ESI) *m/z*: calculated for $C_{49}H_{68}O_8N_2NaSi$ $[M+Na]^+$: 863.4637, found: 863.4623.

II.4.2.1.14. Synthesis of (R)-1-[(R)-2-(9H-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1R,2S)-1-[(2S,3R)-2-(tert-butyldimethylsilanyloxy)-3-carboxybutyl]-2-methyl-7-oxooctyl ester (II.26)



MW (g/mol): 855.1418 **Molecular formula:** $C_{49}H_{66}N_2O_9Si$

To a solution of (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*S*)-2-(*tert*-butyldimethylsilanyloxy)-4-hydroxy-3-methylbutyl]-2-methyl-7-oxooctyl ester (**II.25**) (420 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) at 0 ºC were added TEMPO (117 mg, 0.7 mmol), KBr (0.25 mL of a 0.2M solution in water, 0.05 mmol) and NaOCl (0.26 mL of a 13% solution in water, 0.5 mmol). After stirring for 30 min at the same temperature, the organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude aldehyde was used in the next step without further purification.

The crude aldehyde (0.5 mmol), *t*-BuOH (25 mL), 2-methyl-2-butene (3.7 mL, 35 mmol), water (5 mL), $NaClO_2$ (337 mg, 3 mmol) and NaH_2PO_4 (180 mg, 1.5 mmol) were combined at 0 ºC and the reaction mixture was stirred at rt for 30 min. *t*-BuOH was then removed under reduced pressure and EtOAc (35 mL) was added. The organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 15 mL). The combined organic layers were combined and dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash chromatography (PE/acetone, 90:10) to afford (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*R*)-2-(*tert*-butyldimethylsilanyloxy)-3-carboxybutyl]-2-methyl-7-oxooctyl ester (**II.27**) (375 mg, 90%) as a viscous oil. *Mixture of rotamers:*

$\boldsymbol{R_f}$ (PE/acetone, 7:3): 0.5.

$\mathbf{[\alpha]_D^{20}}$ –25.2 (*c* 1.2, $CHCl_3$).

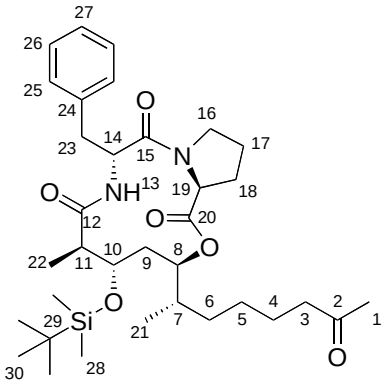
IR (neat): 3292, 2930, 1712, 1650, 1616, 1450, 1250, 1186, 1094, 837 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) *Major rotamer:* δ 7.79-7.73 (d, J = 7.5 Hz, 2H, H_{31} or H_{34}), 7.64-7.56 (t_{app}, J = 7.5 Hz, 2H, H_{32} or H_{33}), 7.44-7.35 (m, 2H, H_{32} or H_{33}), 7.34-7.28 (m, 2H, H_{32}), 7.27-7.16 (m, 5H, H_{23}, H_{24}, H_{25}), 5.84 (m, 1H, H_{26}), 4.81 (m, 1H, H_8), 4.73 (m, 1H, H_{20}), 4.46-4.27 (m, 3H, H_{15}, H_{28}), 4.26-4.05 (m, 2H, H_{11}, H_{29}), 3.55 (m, 1H, H_{10}), 3.10 (dd, J = 12.7, 5.3 Hz, 1H, H_{21}), 2.99 (m, 1H, H_{21}), 2.75-2.59 (m, 2H, H_{18}), 2.46-2.37 (m, 2H, H_3), 2.10 (s, 3H, H_1), 2.01-1.65 (m, 6H, H_9, H_{17}, H_{16}), 1.63-1.49 (m, 3H, H_4, H_7), 1.45-1.23 (m, 4H, H_5, H_6), 1.09 (d, J = 7.0 Hz, 3H, H_{40}), 1.00-0.88 (m, 3H, H_{13}), 0.86 (s, 9H, H_{38}), 0.06 (s, 3H, H_{36}), 0.00 (s, 3H, H_{36}).

^{13}C NMR (100 MHz, $CDCl_3$) *Major rotamer:* δ 209.2 (s, C_2), 179.2 (s, C_{12}), 171.4 (s, C_{14}), 169.8 (s, C_{19}), 155.6 (s, C_{27}), 144.0 (s, C_{30}), 143.8 (s, C_{30}), 141.3 (s, 2C, C_{35}), 136.3 (s, C_{22}), 129.5 (d, 2C, C_{23}), 128.4 (d, 3C, C_{24}, C_{25}), 127.7 (d, 2C, C_{31}), 127.1 (d, 2C, C_{32}), 125.2 (d, 2C, C_{33}), 119.9 (d, 2C, C_{34}), 74.9 (d, C_8), 69.7 (d, C_{10}), 67.0 (t, C_{28}), 58.9 (d, C_{15}), 54.1 (d, C_{20}), 47.2 (d, C_{11}), 46.8 (t, C_{18}), 43.6 (t, C_3), 42.8 (t, C_7), 40.2 (d, C_{21}), 36.7 (d, C_{29}), 35.1 (t, C_9), 31.8 (t, C_{16}), 29.8 (q, C_1), 28.9 (t, C_{17}), 26.6 (t, C_5), 25.7 (q, 3C, C_{38}), 24.2 (t, C_6), 23.9 (t, C_4), 17.9 (s, C_{37}), 14.7 (q, C_{40}), 8.8 (q, C_{13}), -4.2 (q, C_{36}), -5.0 (q, C_{36}).

HRMS (ESI) *m/z*: calculated for $C_{49}H_{66}O_9N_2NaSi$ $[M+Na]^+$: 877.4430, found: 877.4418.

II.4.2.1.15. Synthesis of (5R,8R,9S,11R,13aR)-5-benzyl-9-(tert-butyldimethylsilanyloxy)-8-methyl-11-((S)-1-methyl-6-oxoheptyl)-decahydro-12-oxa-3a,6-diazacyclopentacyclododecene-4,7,13-trione (II.27)



MW (g/mol): 614.8879 **Molecular formula:** $C_{34}H_{54}N_2O_6Si$

To a solution of (*R*)-1-[(*R*)-2-(9*H*-fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*R*)-2-(*tert*-butyldimethylsilanyloxy)-3-carboxybutyl]-2-methyl-7-oxooctyl ester (**II.26**) (360 mg, 0.4 mmol) in MeCN (15 mL) at rt, was added Et_2NH (7.6 mL), and the reaction mixture was stirred at rt until complete conversion of the starting material (reaction monitored by TLC analysis). The solvent was then removed under reduced pressure and the resulting crude amino acid was used in the next step without further purification.

To a solution of amino acid **(**0.4 mmol) in CH_2Cl_2 (50 mL) at 0 ºC were added EDCl (153 mg, 0.8 mmol), HOBt (108 mg, 0.8 mmol) and DIEA (0.3 mL, 1.8 mmol), and the resulting reaction mixture was stirred for 3 h at rt. The reaction was then quenched with a saturated aqueous NH_4Cl solution (30 mL), and the organic phase was separated. The aqueous layer was then extracted with CH_2Cl_2 (2 x 30 mL), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash chromatography (PE/acetone, 90:10) to afford (5*R*,8*R*,9*S*,11*R*,13a*R*)-5-benzyl-9-(*tert*-butyldimethylsilanyloxy)-8-methyl-11-((*S*)-1-methyl-6-oxoheptyl)-decahydro-12-oxa-3a,6-diazacyclopentacyclo dodecene-4,7,13-trione (**II.27**) (150 mg, 60%). *Mixture of rotamers:*

$\boldsymbol{R_f}$ (PE/acetone, 7:3): 0.7.

$\boldsymbol{[\alpha]_D^{20}}$ –28.9 (*c* 1.25, $CHCl_3$).

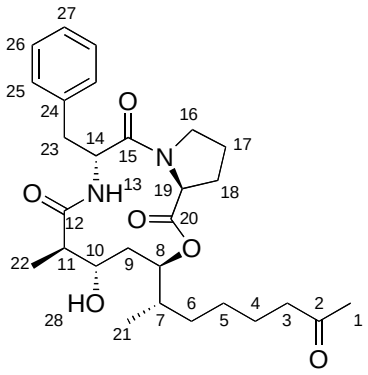
IR (neat): 3263, 2931, 1721, 1661, 1622, 1541, 1439, 1256, 1080, 1052, 835 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) *Major rotamer:* δ 7.34-7.14 (m, 5H, H_{25}, H_{26}, H_{27}), 5.36 (m, 1H, H_{13}), 5.23 (dd, J = 8.5, 3.5 Hz, 1H, H_{14}), 4.89 (m, 1H, H_{19}), 4.04 (d_{app}, J = 8.0 Hz, 1H, H_8), 3.86 (m, 1H, H_{10}), 3.65 (m, 1H, H_{16}), 3.54 (m, 1H, H_{16}), 3.21 (dd, J = 14.5, 4.8 Hz, 1H, H_{23}), 2.95 (dd, J = 14.5, 10.5 Hz, 1H, H_{23}), 2.43-2.34 (m, 2H, H_3), 2.21 (m, 1H, H_{18}), 2.14-2.02 (m, 6H, H_1, H_7, H_{11}, H_{18}), 1.91 (m, 1H, H_{17}), 1.70 (m, 1H, H_{17}), 1.59-1.42 (m, 4H, H_4, H_9), 1.39-1.12 (m, 4H, H_5, H_6), 0.96 (d, J = 7.0 Hz, 3H, H_{21}), 0.85 (s, 9H, H_{30}), 0.79 (d, J = 6.8 Hz, 3H, H_{22}), 0.00 (s, 6H, H_{28}).

^{13}C NMR (100 MHz, $CDCl_3$) *Major rotamer:* δ 208.7 (s, C_2), 174.8 (s, C_{12}), 172.3 (s, C_{15}), 170.5 (s, C_{20}), 135.9 (s, C_{24}), 129.0 (d, 2C, C_{26}), 128.7 (d, 2C, C_{25}), 127.4 (d, C_{27}), 74.6 (d, C_8), 71.3 (d, C_{10}), 60.3 (d, C_{19}), 55.9 (d, C_{14}), 48.0 (t, C_{16}), 43.9 (d, C_{11}), 43.5 (t, C_3), 38.7 (d, C_8), 36.2 (t, C_{23}), 35.3 (t, C_9), 33.1 (t, C_7), 31.8 (t, C_5), 29.8 (q, C_1), 26.8 (t, C_6), 25.8 (q, 3C, C_{30}), 23.8 (t, C_4), 21.5 (t, C_{17}), 17.9 (s, C_{29}), 17.4 (q, C_{21}), 14.4 (q, C_{22}), -3.8 (q, C_{28}), -4.8 (q, C_{28}).

HRMS (ESI) *m/z*: calculated for $C_{34}H_{54}O_6N_2SiNa$ $[M+Na]^+$: 637.3643, found: 637.3627.

*II.4.2.1.16. Synthesis of (5**R**,8**R**,9**S**,11**R**,13a**R**)-5-benzyl-9-hydroxy-8-methyl-11-((**S**)-1-methyl-6-oxo-heptyl)-decahydro-12-oxa-3a,6-diazacyclopenta cyclododecene-4,7,13-trione - epi-acremolide B (II.28)*



MW (g/mol): 500.6270 **Molecular formula:** $C_{28}H_{40}N_2O_6$

To a solution of (5*R*,8*R*,9*S*,11*R*,13a*R*)-5-benzyl-9-(*tert*-butyldimethylsilanyloxy)-8-methyl-11-((*S*)-1-methyl-6-oxoheptyl)-decahydro-12-oxa-3a,6-diazacyclopentacyclodo decene-4,7,13-trione (**II.27**) (75 mg, 0.12 mmol) in THF (4.7 mL) at 0 ºC was added

TBAF (0.1 mL, 0.4 mmol), and the resulting reaction mixture was stirred at the same temperature until complete conversion of the starting material (reaction monitored by TLC analysis). The solvent was then removed under reduced pressure and the resulting crude residue was purified by flash chromatography (PE/acetone, 70:30) to afford *epi*-acremolide B (**II.28**) as a colorless solid (46 mg, 75%). *Mixture of rotamers:*

$\boldsymbol{R_f}$ (PE/acetone, 7:3): 0.4.

$\boldsymbol{[\alpha]_D^{20}}$ –65.2 (*c* 0.02, MeOH).

IR (neat): 3307, 2933, 1714, 1659, 1622, 1524, 1454, 1426, 1272, 733, 700 cm^{-1}.

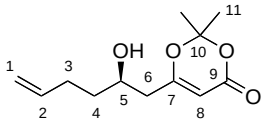
^{1}H NMR (400 MHz, d_6-DMSO) *Major rotamer:* δ 8.20 (d, J = 7.0 Hz, 1H, H_{13}), 7.35-7.15 (m, 5H, H_{25}, H_{26}, H_{27}), 5.11 (m, 1H, H_{19}), 4.81 (m, 1H, H_8), 4.72 (m, 1H, H_{28}), 4.35 (m, 1H, H_{14}), 3.58-3.41 (m, 2H, H_{10}, H_{16}), 3.23 (m, 1H, H_{16}), 2.97 (dd, J =14.0, 10.3 Hz, 1H, H_{23}), 2.85 (dd, J = 14.0, 5.3 Hz, 1H, H_{23}), 2.41 (t_{app}, J = 7.3 Hz, 2H, H_3), 2.21-2.12 (m, 2H, H_9), 2.07 (s, 3H, H_1), 2.03-1.94 (m, 2H, H_7, H_{18}), 1.89 (m, 1H, H_{18}), 1.58-1.38 (m, 5H, H_4, H_{11}, H_{17}), 1.32-1.14 (m, 4H, H_5, H_6), 1.00 (d, J = 7.0 Hz, 3H, H_{21}), 0.73 (d, J = 6.8 Hz, 3H, H_{22}).

^{13}C NMR (100 MHz, d_6-DMSO) *Major rotamer:* δ 208.4 (s, C_2), 174.6 (s, C_{12}), 171.3 (s, C_{20}), 169.9 (s, C_{15}), 138.0 (s, C_{24}), 128.9 (d, 2C, C_{25}), 128.0 (d, 2C, C_{26}), 126.3 (d, C_{27}), 73.3 (d, C_8), 69.1 (d, C_{10}), 59.4 (d, C_{19}), 57.3 (d, C_{14}), 46.9 (t, C_{16}), 42.7 (d, C_{11}), 42.6 (t, C_3), 37.2 (d, C_7), 35.2 (t, C_{23}), 33.5 (t, C_9), 32.4 (t, C_{18}), 31.6 (t, C_6), 29.6 (q, C_1), 26.0 (t, C_4), 23.3 (t, C_5), 20.9 (t, C_{17}), 17.1 (q, C_{21}), 14.0 (q, C_{22}).

HRMS (ESI) *m/z*: calculated for $C_{28}H_{40}O_6N_2Na$ $[M+Na]^+$: 523.2779, found: 523.2758.

II.4.2.2. Synthesis of lyngbouilloside aglycon

*II.4.2.2.1. Synthesis of 6-[(2**R**)-2-hydroxyhex-5-en-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.48)*



MW (g/mol): 226.2689 **Molecular formula:** $C_{12}H_{18}O_4$

A mixture of (*S*)-BINOL (410 mg, 1.4 mmol), Ti(O*i*-Pr)$_4$ (420 µL, 1.4 mmol) and 4 Å molecular sieves (6 g) in anhydrous THF (10 mL) was stirred vigorously at rt for 1 h. The resulting heterogeneous orange solution was then cooled to –78 °C before 1.5 g of aldehyde **II.46** (17.8 mmol) dissolved in 60 mL of THF was added by cannula. After stirring at the same temperature for 30 min, the enol silane (6.9 g, 32.2 mmol) was added drop-wise and the resulting reaction mixture was stirred vigorously for an additional 2 h. The cold bath was then removed and stirring was continued overnight. After cooling back the reaction to −78 °C, TFA (3 mL) was added and the mixture was allowed to warm to rt under continuous stirring. The reaction mixture was diluted with EtOAc (30 mL) and a saturated aqueous solution of $NaHCO_3$ (50 mL) was added drop-wise until gas evolution ceased. The organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/EtOAc, 85:15) to afford **II.48** as a colourless oil (1.16 g, 31%, 86% ee).

$\boldsymbol{R_f}$ (Et_2O/PE: 3:7) 0.45.

$\boldsymbol{[\alpha]_D^{20}}$ –19.5 (*c* 1.0, $CHCl_3$).

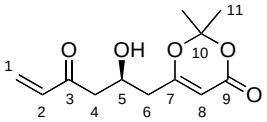
IR (neat): 3435, 2999, 1708, 1632, 1390, 1376, 1274, 1256, 1202, 1012, 904, 805 cm^{-1}.

^{1}H NMR (400 MHz, $CHCl_3$) δ 5.81-5.71 (m, 1H, H_2), 5.25 (s, 1H, H_8), 5.03-4.93 (m, 2H, H_1), 3.87 (m, 1H, H_5), 2.37-2.25 (m, 2H, H_3), 2.20-2.07 (m, 2H, H_6, OH), 1.63 (s, 6H, H_{11}), 1.57-1.52 (m, 3H, H_4, H_6).

^{13}C NMR (100 MHz, $CHCl_3$) δ 169.1 (s, C_7), 161.0 (s, C_9), 137.8 (d, C_2), 115.5 (t, C_1), 106.6 (s, C_{10}), 95.1 (d, C_8), 68.4 (d, C_5), 41.7 (t, C_6), 36.3 (t, C_4), 29.8 (t, C_3), 25.3 (q, C_{11}), 24.9 (q, C_{11}).

HRMS (ESI) *m/z*: calculated for $C_{12}H_{18}NaO_4$ $[M+Na]^+$: 249.1097, found: 249.1098.

II.4.2.2.2. Synthesis of 6-[(2S)-2-hydroxy-4-oxohex-5-en-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.49)



MW (g/mol): 240.2524 **Molecular formula:** $C_{12}H_{16}O_5$

To a solution of **II.48** (1.1 g, 5.1 mmol) in CH_2Cl_2 (17 mL) at rt was added SeO_2 (1.14 g, 10.2 mmol) followed by *t*-BuOOH (6.5 ml of a 5.5 M solution in decane, 35.7 mmol) and the mixture was stirred for 24 h at reflux. After addition of H_2O (10 mL) and vigorous stirring for 30 min, the aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were dried over anhydrous Na_2SO_4. Evaporation of the solvent left a residue, which was dissolved in CH_2Cl_2 and filtered through a short pad of silica. Elution with Et_2O and evaporation of the solvent under reduced pressure gave 700 mg of the crude diol, which were dissolved in CH_2Cl_2 (55 ml) and treated with MnO_2 (1.2 g, 14 mmol) for 2 h at reflux. A second portion of MnO_2 (1.2 g, 14 mmol) was then added and the resulting reaction mixture was stirred for two additional hours at the same temperature. The reaction mixture was then diluted with CH_2Cl_2 (30 mL) and filtered through Celite©. Evaporation of the solvent and purification of the residue by flash column chromatography (CH_2Cl_2/acetone, 95:5) afforded **II.49** (380 mg, 56%) as a yellowish viscous oil.

$\boldsymbol{R_f}$ (acetone/CH_2Cl_2, 1:4): 0.60.

$\boldsymbol{[\alpha]_D^{20}}$ +19.0 (*c* 1.0, $CHCl_3$).

IR (neat): 3436, 2999, 1714, 1633, 1391, 1377, 1274, 1256, 1202, 1013, 904, 805 cm^{-1}.

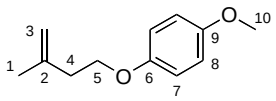
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.30 (dd, J = 17.8, 10.3 Hz, 1H, H_2), 6.19 (d, J = 17.8 Hz, 1H, H_1), 5.88 (d, J = 10.3 Hz, 1H, H_2), 5.29 (s, 1H, H_8), 4.34 (m, 1H, H_5),

2.80-2.67 (m, 2H, H_4), 2.44-2.29 (m, 2H, H_6), 1.64 (s, 3H, H_{11}), 1.63 (s, 3H, H_{11}), 1.50 (br s, 1H, OH).

^{13}C NMR (100 MHz, $CDCl_3$) δ 200.3 (s, C_3), 168.3 (s, C_7), 161.0 (s, C_9), 136.4 (d, C_2), 129.8 (t, C_1), 106.7 (s, C_{10}), 95.5 (d, C_8), 64.7 (d, C_5), 45.1 (t, C_4), 40.5 (t, C_6), 25.5 (q, C_{11}), 24.6 (q, C_{11}).

HRMS (ESI) *m/z*: calculated for $C_{12}H_{16}NaO_5$ $[M + Na]^+$: 263.0890, found: 263.0885.

II.4.2.2.3. Synthesis of 1-methoxy-4-[(3-methylbut-3-en-1-yl)oxy]benzene (II.62)[213]



MW (g/mol): 192.2500 **Molecular formula:** $C_{12}H_{16}O_2$

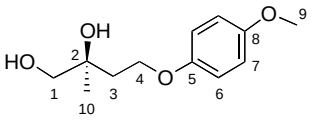
To a solution of 3-methylbut-3-en-1-ol (**II.61**) (12 mL, 116 mmol), 4-methoxyphenol (21.6 g, 174 mmol) and triphenylphosphine (39.5 g, 150 mmol) in anhydrous THF (400 mL) at 0 °C was added DIAD (29.3 mL, 150 mmol) drop-wise, and the resulting mixture was stirred at reflux for 3 h. The solvent was then evaporated under reduced pressure and the salts were precipitated by addition of Et_2O (200 mL) and filtered through a plug of Celite©. The Et_2O was evaporated under reduced pressure and the crude residue was purified by flash column chromatography (Et_2O/PE, 10:90) to afford 1-methoxy-4-[(3-methylbut-3-en-1-yl)oxy]benzene (**II.62**) (18.8 g, 84%) which spectroscopic and physical data matched the ones reported in the literature.[213]

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 (m, 4H, H_7, H_8), 4.87 (s, lH, H_1), 4.83 (s, lH, H_1), 4.05 (t, J = 6.8 Hz, 2H H_4), 3.78 **(**s, 3H, H_{10}), 2.51 (t, J = 6.8 Hz, 2H, H_5), 1.83 **(**s, 3H, H_3).

^{13}C NMR (400 MHz, $CDCl_3$) δ 153.8 (s, C_9), 153.1 (s, C_6), 142.3 (s, C_2), 115.6 (d, C_7), 114.6 (d, C_8), 111.9 (t, C_1), 67.1 (t, C_5), 55.6 (q, C_{10}), 37.3 (t, C_4), 22.8 (q, C_3).

[213] Corey, E. J.; Guzman-Perez, A.; Noe, M. *J. Am. Chem. Soc.*, **1995**, *117*, 10805-10816.

II.4.2.2.4. Synthesis of (2S)-4-(4-Methoxyphenoxy)-2-methylbutane-1,2-diol (II.63)[213]



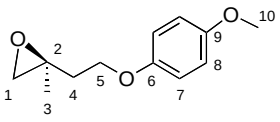
MW (g/mol): 226.27 **Molecular formula:** $C_{12}H_{18}O_4$

To a solution of **II.62** (7.95 g, 41.3 mmol) in *t*-BuOH (205 mL) at 0 °C was added a solution of AD-mix α (57.8 g, 1.4 g/mmol) and $(DHQ)_2PHAL$ (150 mg, 0.19 mmol) in water (205 mL). After stirring for 24 h at the same temperature, $Na_2S_2O_3$ (2.0 g) was added and the mixture was stirred for an additional 20 min. The crude residue was then extracted three times with an EtOAc/hexanes (2:1) mixture (3 x 100 mL) and the combined organic phases were washed with brine (100 mL). The organic layer was finally dried over anhydrous Na_2SO_4, filtered, and evaporatedunder reduced pressure, and the residue was purified by flash column chromatography (EtOAc, 100%) to afford (*S*)-4-(4-methoxyphenoxy)-2-methylbutane-1,2-diol (**II.63**) (9.30 g, 100%) as a white solid which spectroscopic and physical data matched the ones reported in the literature.[213]

^{1}H NMR (400 MHz, $CDC1_3$) δ 6.82 (m, 4H, H_7, H_8), 4.13 (ddd, J = 9.7, 7.5, 4.9 Hz, lH, H_5), 4.06 (ddd, J = 9.6, 6.4, 5.3 Hz, lH, H_5), 3.74 (s, 3H, H_{10}), 3.51 (dd, J = 11.1, 4.9 Hz, lH, H_1), 3.45 (dd, J = 11.1, 5.3 Hz, lH, H_1), 3.19 (br s, lH, OH), 3.05 (br s, lH, OH), 2.06 (ddd, J = 14.7, 7.5, 5.2 Hz, lH, H_4), 1.89 (ddd, J = 14.7, 6.4, 5.1 Hz, lH, H_4), 1.23 (s, 3H, H_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.0 (s, C_9), 152.4 s, C_6), 115.5 (d, C_7), 114.6 (d, C_8), 72.4 (t, C_1), 69.9 (t, C_2), 65.2(t, C_5), 55.6 (q, C_{10}), 37.4 (t, C_4), 23.9 (q, C_3).

II.4.2.2.4. Synthesis of (2S)-2-[2-(4-methoxyphenoxy)ethyl]-2-methyloxirane (II.64)



MW (g/mol): 208.2536 **Molecular formula:** $C_{12}H_{16}O_3$

To a solution of diol **II.63** (13.5 g, 59.6 mmol) and triethylamine (10.0 mL, 71.5 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added MsCl (4.9 mL, 62.6 mmol) drop-wise. After stirring for 45 min at 0 °C, most of the CH_2Cl_2 was evaporated under reduced pressure (T_{bath} < 20 °C). The salts were precipitated by addition of Et_2O (200 mL) and the organic fraction was washed with water (3 x 50 mL) and brine (50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated. The crude residue was then dissolved in MeOH (100 mL) before K_2CO_3 (24.7 g, 179 mmol) was added in one portion at 0 °C, and the reaction mixture was allowed to stir at rt for 30 min. Most of the MeOH was then evaporated (T_{bath} < 20 °C), and the residue was extracted with Et_2O (300 mL). The organic phase was eventually washed with water (3 x 100 mL) and brine (100 mL), dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was finally purified by flash column chromatography (EtOAc/PE, 20:80) to afford **II.64** (10.9 g, 88%) as a white solid.

Mp: 51-53 °C.

***R*$_f$** (PE/EtOAc, 7:3): 0.62.

Chiral SFC: Conditions: OD-H column, 100 bar, 5 mL/min, 5% MeOH; t_1: 1.7 min (3%); t_2: 2.0 min (97%).

$[\alpha]_D^{20}$ +13.2 (*c* 2.0, $CHCl_3$, 94% ee).

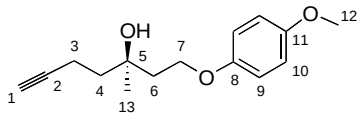
IR (neat): 1507, 1219, 1034, 872, 824, 797, 730 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (s, 4H, H_7, H_8), 4.09-3.91 (m, 2H, H_5), 3.76 (s, 3H, H_{10}), 2.73 (d, *J* = 4.8 Hz, 1H, H_1), 2.63 (d, *J* = 4.8 Hz, 1H, H_1), 2.13-1.94 (m, 2H, H_4), 1.40 (s, 3H, H_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8 (s, C_9), 152.7 (s, C_6), 115.3 (d, 2C, C_7), 114.6 (d, 2C, C_8), 64.8 (t, C_5), 55.6 (q, C_{10}), 55.2 (s, C_2), 53.9 (t, C_1), 36.1 (t, C_4), 21.5 (q, C_3).

HRMS (ESI) *m/z*: calculated for $C_{12}H_{16}NaO_3$ $[M + Na]^+$: 231.0992, found: 231.0989.

II.4.2.2.5. Synthesis of (3R)-1-(4-methoxyphenoxy)-3-methylhept-6-yn-3-ol (II.65)



MW (g/mol): 248.3175 **Molecular formula:** $C_{15}H_{20}O_3$

In a three necked flask equipped with a dropping funnel and a thermometer was added magnesium (3.9 g, 158.4 mmol), $HgCl_2$ (15 mg, 0.053 mmol) and Et_2O (10 mL). Grignard formation was initiated by adding a few drops of propargyl bromide (80 %wt solution in toluene) and gentle heating. As soon as the exotherm was observed, the media was cooled to 0 °C, and the rest of the Et_2O (110 mL) was added followed by propargyl bromide (11 mL of a 80 wt% solution in toluene, 176 mmol) at a rate such that the internal temperature did not exceed 10 °C. The epoxide **II.64** (11 g, 53 mmol) was then added at 0 °C as a solution in Et_2O (45 mL) and the resulting reaction mixture was stirred for 30 min at the same temperature plus an additional 30 min at rt. The mixture was then poured into a cold 2M aqueous HCl solution, extracted with with Et_2O (3 x 50 mL). The combined organic fractions were washed with water (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. To facilitate the isolation of the product from the side side product (corresponding bromohydrin), the crude residue was dissolved in MeOH (100 mL) and K_2CO_3 (2 g) was added in one portion. The resulting mixture was stirred at rt for 30 min before the solvent was removed and a 1:1 Et_2O/H_2O (200 mL) mixture was added. The organic layer was separated and the aqueous phase was extracted with Et_2O (100 mL) twice. The combined organic layers were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/PE, 5:95) to afford **II.65** (10.8 g, 82%) as colourless oil.

$\boldsymbol{R_f}$ (PE/EtOAc, 7:3): 0.48.

$\boldsymbol{[\alpha]_D^{20}}$ –6.45 (*c* 1.01, $CHCl_3$, 94% ee).

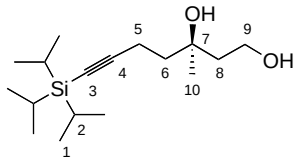
IR (neat): 3450, 3291, 2933, 1507, 1225, 1036, 825, 735, 634 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (s, 4H, H_9, H_{10}), 4.13 (t_{app}, J = 6.1 Hz, 2H, H_7), 3.76 (s, 3H, H_{12}), 2.67 (s, 1H, OH), 2.41-2.26 (m, 2H, H_3), 2.05-1.89 (m, 2H, H_6), 1.97 (t, J = 2.8 Hz, 1H, H_1), 1.90-1.72 (m, 2H, H_4), 1.26 (s, 3H, H_{13}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1 (s, C_{11}), 152.5 (s, C_8), 115.4 (d, 2C, C_9), 114.6 (d, 2C, C_{10}), 84.7 (s, C_2), 71.8 (s, C_5), 68.5 (d, C_1), 65.5 (t, C_7), 55.7 (q, C_{12}), 40.7 (t, C_4), 39.9 (t, C_6), 26.5 (q, C_{13}), 13.2 (t, C_3).

HRMS (ESI) *m/z*: calculated for $C_{15}H_{20}NaO_3$ $[M + Na]^+$: 271.1305, found: 271.1306.

*II.4.2.2.6. Synthesis of (3**R**)-3-methyl-7-[tris(propan-2-yl)silyl]hept-6-yne-1,3-diol (II.67)*



MW (g/mol): 298.5361 **Molecular formula:** $C_{17}H_{34}O_2Si$

In a round-bottom flask equipped with a condenser and a gas outlet was dissolved alkyne **II.65** (10.0 g, 41.0 mmol) in 150 mL of anhydrous THF. The solution was then cooled to 0 °C and 36 mL of MeMgBr (3M solution in Et_2O, 105 mmol) were gently added. After complete addition of the MeMgBr, the reaction mixture was stirred for 6 h at 70 °C, cooled to 0 °C and treated with TIPSCl (14 mL, 65.6 mmol). The reaction mixture was then stirred at 75 °C for an additional 5 h, time after which it was cooled and poured into a cold 2M aqueous HCl solution. The product was then extracted with Et_2O (3 x 100 mL), and the combined organic layers were evaporated under reduced pressure.

The crude residue was dissolved in acetonitrile (100 mL), cooled to 0 °C and treated with a solution of cerium ammonium nitrate (47 g, 86.1 mmol) in water (100 mL). After stirring for 30 min at rt, the product was extracted with Et_2O (3 x 100 mL) and the combined organic layers were washed twice with a 1M aqueous solution of NaOH (2 x 100 mL, in the absence of light) and once with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue

was finally purified by flash column chromatography (PE/Et_2O, 25:75) to afford **II.67** as a pale yellow oil (8.95 g, 75%).

$\boldsymbol{R_f}$ (Et_2O): 0.50.

$\boldsymbol{[\alpha]_D^{20}}$ –3.14 (*c* 0.88, $CHCl_3$, 94% ee).

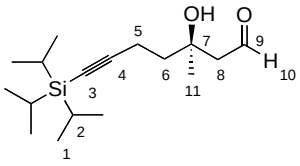
IR (neat): 3345, 2942, 2865, 2171, 1462, 1017, 996, 882, 660 cm^{-1}.

^{1}H NMR (400 MHz, $CHCl_3$) δ 3.95-3.79 (m, 2H, H_9), 3.38 (br s, 1H, OH), 3.25 (br s, 1H, OH), 2.42-2.34 (m, 2H, H_5), 1.89-1.59 (m, 4H, H_6, H_8), 1.25 (s, 3H, H_{10}), 1.06-0.98 (m, 21H, H_1, H_2).

^{13}C NMR (100 MHz, $CHCl_3$) δ 108.0 (s, C_3), 81.3 (s, C_4), 73.6 (s, C_7), 59.5 (t, C_9), 41.7 (t, C_8), 40.6 (t, C_6), 26.2 (q, C_{10}), 18.5 (q, 6C, C_1), 14.7 (t, C_5), 11.2 (d, 3C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{17}H_{34}NaO_2Si$ $[M + Na]^+$: 321.2220, found: 321.2211.

*II.4.2.2.7. Synthesis of (3**R**)-3-hydroxy-3-methyl-7-[tris(propan-2-yl)silyl] hept-6-ynal (II.68)*



MW (g/mol): 298.5361 **Molecular formula:** $C_{17}H_{34}O_2Si$

To a solution of alcohol **II.67** (3.0 g, 10.0 mmol) in CH_2Cl_2 (100 mL) at 0 ºC were added TEMPO (2 g, 12.8 mmol), KBr (5 mL of a 0.2 M solution in water, 1.0 mmol) and NaOCl (5 mL of a 13% solution in water, 11.0 mmol). After stirring for 30 min at the same temperature, brine was added and the organic layer was separated. The aqueous phase was extracted twice with EtOAc (50 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/Et_2O, 70:30) to afford aldehyde **II.68** (3.0 g, quant.) as a colourless oil.

$\boldsymbol{R_f}$ (PE/Et_2O, 1:1): 0.53.

$\boldsymbol{[\alpha]_D^{20}}$ –8.19 (*c* 0.57, $CHCl_3$, 94% ee).

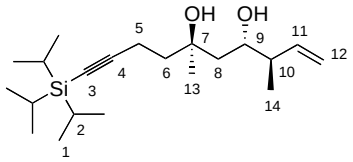
IR (neat): 3434, 2942, 2865, 2171, 1719, 1463, 883, 676, 661 cm^{-1}.

^{1}H NMR (400 MHz, $CHCl_3$) δ 9.82 (t, J = 2.0 Hz, 1H, H_{10}), 3.14 (br s, 1H, OH), 2.65 (dd, J = 16.3, 1.8 Hz, 1H, H_8), 2.53 (dd, J = 16.3, 2.3 Hz, 1H, H_8), 2.42-2.30 (m, 2H, H_5), 1.85-1.70 (m, 2H, H_6), 1.28 (s, 3H, H_{11}), 1.03-0.93 (m, 21H, H_1, H_2).

^{13}C NMR (100 MHz, $CHCl_3$) δ 202.8 (d, C_9), 108.3 (s, C_3), 81.6 (s, C_4), 71.6 (s, C_7), 53.9 (t, C_8), 40.4 (t, C_6), 27.0 (q, C_{11}), 18.5 (q, 6C, C_1), 14.5 (t, C_5), 11.1 (d, 3C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{17}H_{32}NaO_2Si$ $[M + Na]^+$: 319.2064, found: 319.2066.

II.4.2.2.8. Synthesis of (3R,4S,6R)-3,6-Dimethyl-10-[tris(propan-2-yl)silyl]dec-1-en-9-yne-4,6-diol (II.69)



MW (g/mol): 352.6266 **Molecular formula:** $C_{21}H_{40}O_2Si$

To a stirred solution of cyclopentadienyl[(4*R*,*trans*)-2,2-dimethyl-α,α,α',α'-tetraphenyl-1,3-dioxolane-4,5-dimethanolato-*O*,*O*']titanium chloride (8.0 g, 13.4 mmol) in anhydrous Et_2O (130 mL) at –78 °C, was added 2-butenylmagnesium chloride (25 mL of a 0.5 M solution in THF, 12.6 mmol) drop-wise. After stirring 2 h at 0 °C, the dark-orange solution was cooled back to –78 °C at which temperature a solution of aldehyde **II.68** (2.4 g, 8.0 mmol) in Et_2O (15 mL) was added dropwise. After 5 h stirring at –78 °C, the reaction was quenched by addition of H_2O (30 mL) and stirring was continued over-night at rt. The reaction mixture was then filtered over Celite© and the layers were separated. The aqueous phase was eventually extracted with Et_2O (3 × 100 mL) and the combined organic layers were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography (EtOAc/toluene, 2:98) provided the desired homoallylic alcohol **II.69** (2.2 g, 78%) as colourless oil.

R_f (PE/Et_2O, 1:1): 0.36.

$[\alpha]_D^{20}$ –3.34 (*c* 0.95, $CHCl_3$).

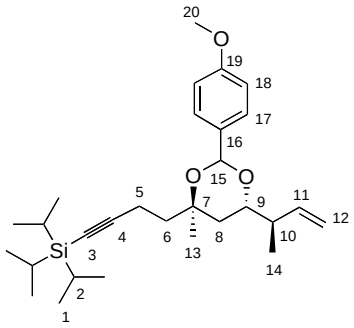
IR (neat): 3337, 2942, 2865, 2171, 1462, 997, 915, 882, 660 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (ddd, J = 18.5, 10.5, 8.0 Hz, 1H, H_{11}), 5.14-5.04 (m, 2H, H_{12}), 3.81 (m, 1H, H_9), 3.60 (br s, 1H, OH), 3.13 (br s, 1H, OH), 2.46-2.29 (m, 2H, H_5), 2.19 (m, 1H, H_{10}), 2.00 (m, 1H, H_8), 1.76-1.51 (m, 3H, H_6, H_8), 1.23 (s, 3H, H_{13}), 1.07-0.97 (m, 24H, H_1, H_2, H_{14}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 140.1 (d, C_{11}), 116.3 (t, C_{12}), 108.9 (s, C_3), 81.3 (s, C_4), 73.0 (s, C_7), 71.8 (d, C_9), 44.8 (d, C_{10}), 44.0 (t, C_6), 38.6 (t, C_8), 28.1 (q, C_{13}), 18.6 (q, 6C, C_1), 15.6 (q, C_{14}), 15.1 (t, C_5), 11.2 (d, 3C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{21}H_{40}NaO_2Si$ $[M + Na]^+$: 375.2690, found: 375.2692.

*II.4.2.2.9. Synthesis of {4-[(4**R**,6**S**)-6-[(2**R**)-but-3-en-2-yl]-2-(4-methoxyphenyl)-4-methyl-1,3-dioxan-4-yl]but-1-yn-1-yl}tris(propan-2-yl)silane (II.70)*



MW (g/mol): 470.7592 **Molecular formula:** $C_{29}H_{46}O_3Si$

To a solution of diol **II.69** (1.9 mg, 5.4 mmol) in CH_2Cl_2 (50 mL) was added *p*-anisaldehyde dimethylacetal (1.3 mL, 7.6 mmol) followed by *p*-TSOH (51.3 mg, 0.27 mmol). The resulting pink solution was stirred for 2 h at rt and filtered over alumina. The solvent was then removed under reduced pressure and the resulting crude residue was purified flash column chromatography (PE/Et_2O, 95:5) to afford the desired acetal **II.70** (2.56 g, 98%) as a colourless oil.

R_f (PE/Et_2O, 9:1): 0.59.

$[\alpha]_D^{20}$ –43.0 (*c* 1.0, $CHCl_3$).

IR (neat): 2943, 2865, 2171, 1517, 1463, 1248, 1075, 1037, 1004, 677, 663 cm^{-1}.

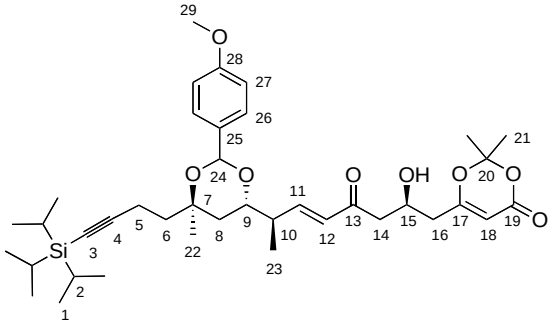
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 8.5 Hz, 2H, H_{17}), 6.88 (d, J = 8.5 Hz, 2H, H_{18}), 5.92 (m, 1H, H_{11}), 5.61 (s, 1H, H_{15}), 5.10-5.04 (m, 2H, H_{12}), 3.86 (m, 1H, H_9),

3.80 (s, 3H, H_{20}), 2.47 (m, 1H, H_8), 2.38-2.33 (m, 3H, H_5, H_{10}), 1.73-1.60 (m, 2H, H_6, H_8), 1.45 (dd, J = 13.6, 2.5 Hz, 1H, H_6), 1.28 (s, 3H, H_{13}), 1.10-1.01 (m, 24H, H_1, H_2, H_{14}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 159.7 (s, C_{19}), 140.2 (d, C_{11}), 131.8 (s, C_{16}), 127.3 (d, 2C, C_{17}), 114.8 (t, C_{12}), 113.5 (d, 2C, C_{18}), 108.8 (s, C_3), 94.3 (d, C_{15}), 80.2 (s, C_4), 75.7 (d, C_9), 73.4 (s, C_7), 55.2 (q, C_{20}), 42.5 (d, C_{10}), 38.0 (t, C_6), 33.7 (t, C_8), 28.3 (q, C_{13}), 18.6 (q, 6C, C_1), 15.4 (q, C_{14}), 14.0 (t, C_5), 11.3 (d, 3C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{29}H_{46}NaO_2Si$ $[M + Na]^+$: 493.3108, found: 493.3102.

II.4.2.2.10. Synthesis of 6-[(2S,5E,7R)-2-hydroxy-7-[(4S,6R)-2-(4-methoxyphenyl)-6-methyl-6-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-1,3-dioxan-4-yl]-4-oxooct-5-en-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.71)



MW (g/mol): 682.9585 **Molecular formula:** $C_{39}H_{58}O_8Si$

To a solution of olefin **II.70** (900 mg, 1.77 mmol) and enone **II.49** (600 mg, 2.47 mmol) in CH_2Cl_2 (10 mL) was added Hoveyda-Grubbs 2nd Generation catalyst (220 mg, 0.35 mmol) and the resulting mixture was stirred for 3 days at reflux. The solvent was then removed under reduced pressure and the crude residue was purified by flash column chromatography (EtOAc/PE, 20:80) to afford the corresponding enone **II.71** (1.0 g, 78%) as colourless oil.

***R*$_f$** (PE/EtOAc, 1:1): 0.48.

$[\alpha]_D^{20}$ –10.5 (*c* 0.2, $CHCl_3$).

IR (neat): 3468, 2942, 2864, 2170, 1726, 1376, 1248, 1012, 731, 676, 662 cm^{-1}.

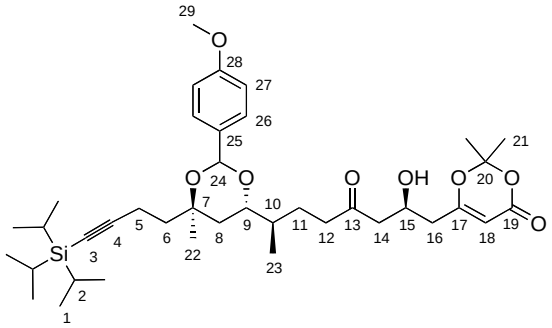
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 8.8 Hz, 2H, H_{26}), 6.95 (dd, J = 16.3, 7.9 Hz, 1H, H_{11}), 6.88 (d, J = 8.8 Hz, 2H, H_{27}), 6.12 (d, J = 16.3 Hz, 1H, H_{12}), 5.61 (s, 1H, H_{24}),

5.34 (s, 1H, H_{18}), 4.37 (m, 1H, H_{15}), 3.93 (m, 1H, H_9), 3.80 (s, 3H, H_{29}), 3.31 (br s, 1H, OH), 2.84-2.68 (m, 2H, H_{14}), 2.53-2.40 (m, 3H, H_{10}, H_{16}), 2.39-2.32 (m, 3H, H_5, H_8), 1.69 (m, 1H, H_8), 1.68 (s, 3H, H_{21}), 1.67 (s, 3H, H_{21}), 1.60-1.51 (m, 2H, H_6), 1.28 (s, 3H, H_{22}), 1.16 (d, J = 6.8 Hz, 3H, H_{23}), 1.08-1.00 (m, 21H, H_1, H_2).

^{13}C NMR (100 MHz, $CDCl_3$) δ 200.0 (s, C_{13}), 168.3 (s, C_{17}), 160.9 (s, C_{19}), 159.9 (s, C_{28}), 150.4 (d, C_{11}), 131.2 (s, C_{25}), 130.6 (d, C_{12}), 127.2 (d, 2C, C_{26}), 113.6 (d, 2C, C_{27}), 108.5 (s, C_3), 106.6 (s, C_{20}), 95.4 (d, C_{18}), 94.4 (d, C_{24}), 80.5 (s, C_4), 75.2 (d, C_9), 73.4 (s, C_7), 64.8 (d, C_{15}), 55.3 (q, C_{29}), 45.3 (t, C_{14}), 42.0 (d, C_{10}), 40.5 (t, C_{16}), 39.0 (t, C_6), 33.6 (t, C_8), 28.3 (q, C_{22}), 25.5 (q, C_{21}), 24.5 (q, C_{21}), 18.6 (q, 6C, C_1), 15.6 (q, C_{23}), 14.0 (t, C_5), 11.3 (d, 3C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{39}H_{58}NaO_8Si$ $[M + Na]^+$: 705.3793, found: 705.3801.

II.4.2.2.11. Synthesis of 6-[(2S,7R)-2-hydroxy-7-[(4S,6R)-2-(4-methoxyphenyl)-6-methyl-6-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-1,3-dioxan-4-yl]-4-oxooctyl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.72)



MW (g/mol): 684.9744 **Molecular formula:** $C_{39}H_{60}O_8Si$

In a two-neck round-bottom flask was added lithium chloride (811 mg, 19.1 mmol, flame-dried under vacuum), copper iodide (932 mg, 4.8 mmol) and THF (7 mL). After complete dissolution of the solids, the reaction mixture was cooled to –40 °C and *n*-Bu_3SnH (1.6 mL, 5.8 mmol) was added drop-wise followed by a solution of enone **II.71** (650 mg, 0.95 mmol) in anhydrous THF (7 mL). The orange-red solution was then stirred for 4 h, time during which the temperature was allowed to reaching 0 °C. The reaction was then quenched with a saturated aqueous solution of NH_4Cl (10 mL) and the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic layers were then dried over anhydrous Na_2SO_4, filtered and concentrated under reduced

pressure, and the crude residue was finally purified by flash column chromatography (PE/EtOAc, 70:30) to afford ketone **II.72** (521 mg, 80%) as pale yellow oil.

R_f (PE/EtOAc, 1:1): 0.53.

$[\alpha]_D^{20}$ –12.4 (*c* 0.5, $CHCl_3$).

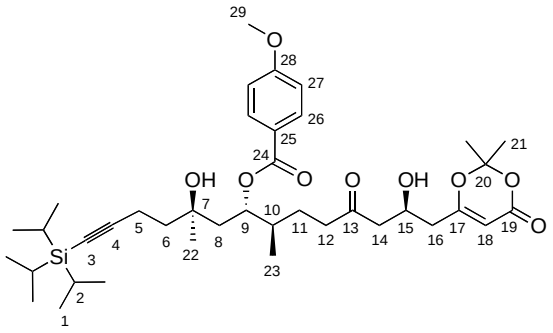
IR (neat): 3467, 2943, 2865, 2171, 1727, 1391, 1377, 1249, 1014 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.8 Hz, 2H, H_{26}), 6.87 (d, *J* = 8.8 Hz, 2H, H_{27}), 5.56 (s, 1H, H_{24}), 5.31 (s, 1H, H_{18}), 4.28 (m, 1H, H_{15}), 3.78 (s, 3H, H_{29}), 3.70 (q_{app}, *J* = 7.0 Hz, 1H, H_9), 3.17 (br s, 1H, OH), 2.58-2.40 (m, 6H, H_5, H_8, H_{12}, H_{14}), 2.39-2.30 (m, 3H, H_5, H_8, H_{16}), 2.24 (dd, *J* = 14.5, 4.5 Hz, 1H, H_{16}), 1.95 (m, 1H, H_{11}), 1.68 (s, 3H, H_{21}), 1.67 (s, 3H, H_{21}), 1.59 (m, 1H, H_{10}), 1.54(d_{app}, *J* = 7.2 Hz, 2H, H_6), 1.47 (m, 1H, H_{11}), 1.27 (s, 3H, H_{22}), 1.08-1.00 (m, 21H, H_1, H_2), 0.90 (d, *J* = 6.8 Hz, 3H, H_{23}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 211.4 (s, C_{13}), 168.3 (s, C_{17}), 160.9 (s, C_{19}), 159.8 (s, C_{28}), 131.6 (s, C_{25}), 127.3 (d, 2C, C_{26}), 113.6 (d, 2C, C_{27}), 108.7 (s, C_3), 106.6 (s, C_{20}), 95.3 (d, C_{18}), 94.5 (d, C_{24}), 80.3 (s, C_4), 76.6 (d, C_9), 73.4 (s, C_7), 64.6 (d, C_{15}), 55.3 (q, C_{29}), 48.0 (t, C_{14}), 41.4 (t, C_{12}), 40.3 (t, C_{16}), 38.5 (t, C_6), 37.5 (d, C_{10}), 33.6 (t, C_8), 28.4 (q, C_{22}), 26.4 (t, C_{11}), 25.5 (q, C_{21}), 24.5 (q, C_{21}), 18.6 (q, 6C, C_1), 15.1 (q, C_{23}), 13.9 (t, C_5), 11.2 (d, 3C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{39}H_{60}NaO_8Si$ $[M + Na]^+$: 707.3950, found: 707.3956.

II.4.2.2.12. Synthesis of (5R,7S,8R,13S)-14-(2,2-dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5,13-dihydroxy-5,8-dimethyl-11-oxo-1-[tris(propan-2-yl)silyl]tetradec-1-yn-7-yl 4-methoxybenzoate (II.73)



MW (g/mol): 700.9738 **Molecular formula:** $C_{39}H_{60}O_9Si$

In a round-bottom flask containing acetal **II.72** (510 mg, 0.74 mmol), CH_2Cl_2 (7 mL) and a pH 7.2 phosphate buffered solution (0.5 mL) was added DDQ (252 mg, 1.11 mmol). After 1 h stirring, a second equivalent of DDQ was added and the reaction mixture was stirred for an additional hour. The solvent was then removed under reduced pressure and the crude residue was purified by column chromatography (PE/EtOAc, 50:50) to afford hydroxyester **II.73** (391 mg, 75%) as colourless oil.

***R*$_f$** (PE/EtOAc, 1:1): 0.36.

[α]$_D^{20}$ +5.8 (*c* 0.5, $CHCl_3$).

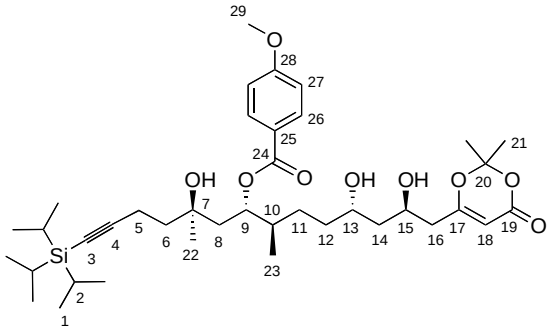
IR (neat): 3468, 2941, 2864, 2170, 1707, 1606, 1378, 1256, 1167, 1101, 1016 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.8 Hz, 2H, H_{26}), 6.90 (d, J = 8.8 Hz, 2H, H_{27}), 5.34 (s, 1H, H_{18}), 5.27 (m, 1H, H_9), 4.36 (m, 1H, H_{15}), 3.84 (s, 3H; H_{29}), 3.37 (br s, 1H, OH), 2.69-2.56 (m, 3H, H_{12}, H_{14}), 2.51 (m, 1H, H_{12}), 2.49-2.42 (m, 4H, H_5, H_{16}), 1.92-1.83 (m, 2H, H_8, H_{10}), 1.76-1.64 (m, 4H, H_6, H_8, H_{11}), 1.69 (s, 3H, H_{21}), 1.68 (s, 3H, H_{21}), 1.53 (m, 1H, H_{11}), 1.16 (s, 3H, H_{22}), 1.06-0.97 (m, 21H, H_1, H_2), 0.93 (d, J = 6.8 Hz, 3H, H_{23}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 210.8 (s, C_{13}), 168.3 (s, C_{17}), 166.1 (s, C_{24}), 163.5 (s, C_{28}), 161.0 (s, C_{19}), 131.5 (d, 2C, C_{26}), 122.5 (s, C_{25}), 113.7 (d, 2C, C_{27}), 108.8 (s, C_3), 106.6 (s, C_{20}), 95.4 (d, C_{18}), 81.1 (s, C_4), 73.6 (d, C_9), 71.6 (s, C_7), 64.8 (d, C_{15}), 55.4 (q, C_{29}), 48.6 (t, C_{14}), 41.5 (t, C_{12}), 41.1 (t, C_{16}), 40.7 (t, C_6), 40.5 (t, C_8), 36.7 (d, C_{10}), 26.2 (q, C_{22}), 25.6 (t, C_{11}), 25.5 (q, C_{21}), 24.5 (q, C_{21}), 18.5 (q, 6C, C_1), 14.8 (q, C_{23}), 14.7 (t, C_5), 11.2 (d, 3C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{39}H_{60}NaO_9Si$ $[M + Na]^+$: 723.3899, found: 723.3910.

II.4.2.2.13. Synthesis of (5R,7S,8R,11S,13R)-14-(2,2-dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5,11,13-trihydroxy-5,8-dimethyl-1-[tris(propan-2-yl) silyl]tetradec-1-yn-7-yl 4-methoxybenzoate (II.74)



MW (g/mol): 702.9897 **Molecular formula:** $C_{39}H_{62}O_9Si$

To a solution of ketone **II.73** (350 mg, 0.50 mmol) in a 1:1 MeCN/AcOH mixture (8 mL) at –30 °C was added tetramethylammonium triacetoxyborohydride (1.3 g, 5.0 mmol) in one portion. After stirring at the same temperature for 20 h, the reaction mixture was poured in a saturated aqueous solution of $NaHCO_3$ (10 mL), stirred for 30 min, and extracted with EtOAc (3 x 15 mL). The combined organic fractions were washed twice with a saturated aqueous solution of $NaHCO_3$ (2 x 10 mL) and once with brine (10 mL), dried over anhydrous Na_2SO_4 and filtered. The solvents were then removed under reduced pressure and the resulting crude residue was purified by flash column chromatography (PE/EtOAc, 40:60) to afford the desired triol **II.74** (320 mg, 91%) as a colourless oil.

R_f (PE/EtOAc, 3:7): 0.20.

$[\alpha]_D^{20}$ –1.8 (*c* 0.5, $CHCl_3$).

IR (neat): 3401, 2940, 2864, 2170, 1707, 1606, 1378, 1257, 1167, 1102, 1015 cm^{-1}.

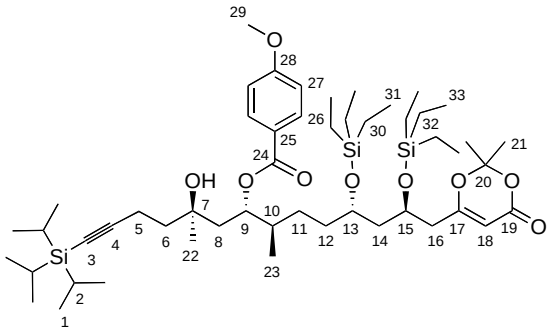
^{1}H NMR (400 MHz, $CHCl_3$) δ 7.94 (d, J = 8.8 Hz, 2H, H_{26}), 6.89 (d, J = 8.8 Hz, 2H, H_{27}), 5.33 (s, 1H, H_{18}), 5.30 (m, 1H, H_9), 4.23 (m, 1H, H_{15}), 3.91 (m, 1H, H_{13}), 3.83 (s, 3H, H_{29}), 3.23 (br s, 3H, OH), 2.46-2.30 (m, 4H, H_5, H_{16}), 1.95-1.81 (m, 2H, H_8, H_{10}), 1.77-1.48 (m, 9H, H_6, H_8, H_{11}, $H_{12,}$ H_{14}), 1.67 (s, 6H, H_{21}), 1.05 (s, 3H, H_{22}), 1.05-0.96 (m, 21H, H_1, H_2), 0.92 (d, J = 6.8 Hz, 3H, H_{23}).

^{13}C NMR (100 MHz, $CHCl_3$) δ 169.5 (s, C_{17}), 166.2 (s, C_{24}), 163.4 (s, C_{28}), 161.5 (s, C_{19}), 131.5 (d, 2C, C_{26}), 122.6 (s, C_{25}), 113.7 (d, 2C, C_{27}), 108.8 (s, C_3), 106.6 (s, C_{20}),

94.8 (d, C_{18}), 80.7 (s, C_4), 73.9 (d, C_9), 71.7 (s, C_7), 68.9 (d, C_{13}), 65.9 (d, C_{15}), 55.4 (q, C_{29}), 42.9 (t, C_{14}), 41.9 (t, C_{16}), 41.5 (t, C_6), 40.2 (t, C_8), 37.1 (d, C_{10}), 35.0 (t, C_{12}), 28.9 (t, C_{11}), 25.6 (q, C_{22}), 25.2 (q, C_{21}), 24.7 (q, C_{21}), 18.5 (q, 6C, C_1), 14.7 (t, C_5), 14.6 (q, C_{23}), 11.1 (d, 3C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{39}H_{62}NaO_9Si$ $[M + Na]^+$: 725.4055, found: 725.4062.

II.4.2.2.14. Synthesis of (5R,7S,8R,11S,13R)-14-(2,2-dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5-hydroxy-5,8-dimethyl-11,13-bis[(triethylsilyl)oxy]-1-[tris(propan-2-yl)silyl]tetradec-1-yn-7-yl 4-methoxybenzoate (II.75)



MW (g/mol): 931.5114 **Molecular formula:** $C_{51}H_{90}O_9Si_3$

To a solution of triol **II.74** (120 mg, 0.17 mmol) and imidazole (46 mg, 0.68 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added TESCl (0.06 mL, 0.36 mmol). After stirring for 30 min at rt, the solution was diluted with CH_2Cl_2, washed once with a saturated aqueous solution of $NaHCO_3$ (5 mL) and once with brine (5 mL), dried over anhydrous Na_2SO_4 and filtered. The solvent was then evaporated under reduced pressure and the crude residue was purified by flash column chromatography (PE/EtOAc, 85:15) to afford **II.75** (147 mg, 92%) as colourless oil.

R_f (PE/EtOAc, 8:2): 0.55.

$[\alpha]_D^{20}$ +2.4 (*c* 1.0, $CHCl_3$).

IR (neat): 3471, 2955, 2876, 2171, 1711, 1256, 1101, 1013, 741 cm^{-1}.

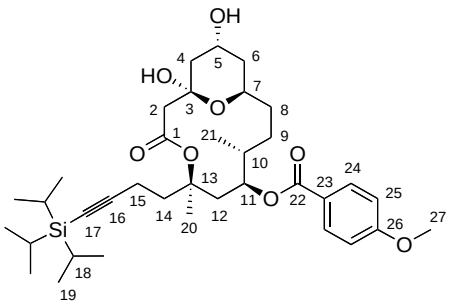
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.8 Hz, 2H, H_{26}), 6.90 (d, J = 8.8 Hz, 2H, H_{27}), 5.27 (s, 1H, H_{18}), 5.25 (m, 1H, H_9), 4.06 (quint$_{app}$, J = 6.0 Hz, 1H, H_{15}), 3.85 (s, 3H, H_{29}), 3.77 (m, 1H, H_{13}), 2.45-2.32 (m, 4H, H_5, H_{16}), 1.92 (dd, J = 15.1, 9.6 Hz, 1H, H_8), 1.83-1.64 (m, 4H, H_6, H_8, H_{10}), 1.67 (s, 3H, H_{21}), 1.66 (s, 3H, H_{21}), 1.62-1.53 (m,

3H, H_{12}, H_{14}), 1.52-1.40 (m, 2H, H_{11}, H_{12}), 1.21 (m, 1H, H_{11}), 1.19 (s, 3H, H_{22}), 1.06-0.96 (m, 21H, H_1, H_2), 0.94-0.92 (m, 21H, H_{23}, H_{31}, H_{33}), 0.59 (q, J = 8.0 Hz, 6H, H_{30}, H_{32}), 0.58 (q, J = 8.0 Hz, 6H, H_{30}, H_{32}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1 (s, C_{17}), 166.0 (s, C_{24}), 163.4 (s, C_{28}), 161.1 (s, C_{19}), 131.5 (d, 2C, C_{26}), 122.7 (s, C_{25}), 113.7 (d, 2C, C_{27}), 108.9 (s, C_3), 106.3 (s, C_{20}), 95.2 (d, C_{18}), 81.0 (s, C_4), 74.4 (d, C_9), 71.6 (s, C_7), 69.9 (d, C_{13}), 67.5 (d, C_{15}), 55.4 (q, C_{29}), 45.3 (t, C_{14}), 42.8 (t, C_{16}), 41.4 (t, C_6), 41.0 (t, C_8), 37.8 (d, C_{10}), 35.6 (t, C_{12}), 27.4 (t, C_{11}), 26.6 (q, C_{22}), 25.6 (q, C_{21}), 24.5 (q, C_{21}), 18.5 (q, 6C, C_1), 14.9 (q, C_{23}), 14.7 (t, C_5), 11.2 (d, 3C, C_2), 6.9 (q, 3C, C_{31}, C_{33}), 6.8 (q, 3C, C_{31}, C_{33}), 5.3 (t, 3C, C_{30}, C_{32}), 5.2 (t, 3C, C_{30}, C_{32}).

HRMS (ESI) *m/z*: calculated for $C_{51}H_{90}NaO_9Si_3$ $[M + Na]^+$: 953.5785, found: 953.5781.

II.4.2.2.15. Synthesis of (1R,5R,7S,8R,11R,13R)-1,13-dihydroxy-5,8-dimethyl-3-oxo-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo [9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.78)



MW (g/mol): 644.9105 **Molecular formula:** $C_{36}H_{56}O_8Si$

To a round-bottom flask containing a pre-heated (110 °C) solution of dry toluene (1 L) was added **II.75** (115 mg, 0.11 mmol in 4 mL of toluene) drop-wise. The reaction mixture was then stirred at reflux until complete consumption of the starting material (reaction monitored by TLC). The solvent was then removed under reduced pressure and the crude residue was used in the next step without further purification.

The crude residue was thus dissolved in a 2:1 THF/H_2O (6 mL) mixture. *p*-TsOH (24 mg, 0.11 mmol) was added and the resulting reaction mixture was stirred for 1 h at rt. A saturated aqueous solution of $NaHCO_3$ was then added with care to quench the

reaction, and the mixture was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (EtOAc/PE, 30:70) to afford **II.78** (69 mg, 75%) as colourless oil.

$\boldsymbol{R_f}$ (EtOAc/PE, 1:1): 0.5.

$\boldsymbol{[\alpha]_D^{20}}$ –6.8 (*c* 0.57, $CHCl_3$).

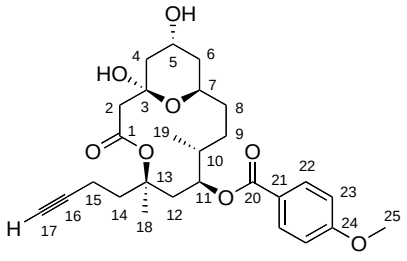
IR (neat): 3475, 2940, 2865, 2172, 1706, 1607, 1462, 1322, 1279, 1257, 1230, 1167, 1101, 1031 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 9.0 Hz, 2H, H_{24}), 6.91 (d, J = 9.0 Hz, 2H, H_{25}), 5.43 (d, J = 10.0 Hz, 1H, H_{11}), 4.56 (br s, 1H, OH), 4.14 (m, 1H, H_5), 3.85 (s, 3H, H_{27}), 3.65 (t, J = 10.9 Hz, 1H, H_7), 2.80-2.66 (m, 2H, H_4, H_{12}), 2.59 (d, J = 12.6 Hz, 1H, H_2), 2.49 (d, J = 12.6 Hz, 1H, H_2), 2.31 (m, 1H, H_4), 2.24-2.10 (m, 2H, H_4, H_{14}), 1.97 (m, 1H, H_{15}), 1.92 (m, 1H, H_6), 1.84-1.61 (m, 4H, H_8, H_{10}, H_{12}), 1.42 (m, 1H, H_9), 1.35-1.23 (m, 3H, H_6, H_9, H_{14}), 1.19 (br s, 3H, H_{20}), 1.06-1.01 (m, 21H, H_{18}, H_{19}), 0.88 (d, J = 7.2 Hz, 3H, H_{21}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4 (s, C_1), 165.7 (s, C_{22}), 163.3 (s, C_{26}), 131.4 (d, 2C, C_{24}), 123.2 (s, C_{23}), 113.6 (d, 2C, C_{25}), 108.0 (s, C_{17}), 96.3 (s, C_3), 84.8 (s, C_{13}), 80.4 (s, C_{16}), 71.9 (d, C_{11}), 69.2 (d, C_7), 64.9 (d, C_5), 55.4 (q, C_{27}), 47.1 (t, C_2), 43.4 (t, C_6), 40.5 (t, C_{14}), 38.8 (d, C_{10}), 34.2 (t, C_{12}), 32.3 (t, C_8), 28.8 (t, C_9), 24.0 (q, C_{20}), 18.6 (q, 6C, C_{19}), 14.6 (t, C_{15}), 11.2 (d, 3C, C_{18}). The C_4 (38.9, t) and C_{21} (15.3, q) chemical shifts have been determined by HMQC correlation.

HRMS (ESI) *m/z*: calculated for $C_{36}H_{56}NaO_8Si$ $[M + Na]^+$: 667.3642, found: 667.3639.

II.4.2.2.16. Synthesis of (1R,5R,7S,8R,11R,13R)-5-(but-3-yn-1-yl)-1,13-dihydroxy-5,8-dimethyl-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.79)



MW (g/mol): 488.5699 **Molecular formula:** $C_{27}H_{36}O_8$

To a stirred solution of **II.78** (50 mg, 0.07 mmol) in MECN (1.4 mL) at rt was added AgF (49 mg, 0.39 mmol) and the resulting reaction mixture was stirred in the absence of light for 5 h. A 1M aqueous solution of HCl (0.25 mL) was then added to the reaction, which was stirred for an additional 5 min and diluted with EtOAc (3 mL). The organic phase was then separated and the aqueous layer was extracted with EtOAc (3 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (EtOAc/PE, 40:60) to afford **II.79** (28 mg, 80%) as colourless oil.

$\boldsymbol{R_f}$ (EtOAc/PE, 1:1): 0.33.

$\boldsymbol{[\alpha]_D^{20}}$ –25.79 (*c* 1.3, $CHCl_3$).

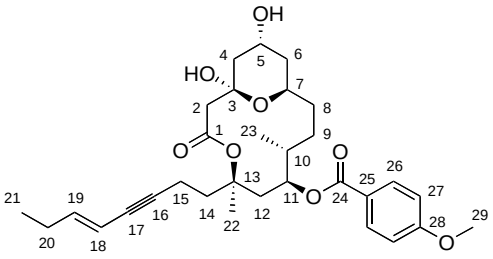
IR (neat): 3476, 3299, 2932, 1703, 1606, 1279, 1257, 1168, 1101, 1030, 773 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.8 Hz, 2H, H_{22}), 6.92 (d, *J* = 8.8 Hz, 2H, H_{23}), 5.44 (d, *J* = 9.3 Hz, 1H, H_{11}), 4.56 (br s, 1H, OH), 4.14 (m, 1H, H_5), 3.86 (s, 3H, H_{27}), 3.67 (m, 1H, H_7), 2.81-2.67 (m, 2H, H_4, H_{12}), 2.61 (d, *J* = 12.6 Hz, 1H, H_2), 2.50 (d, *J* = 12.6 Hz, 1H, H_2), 2.31-2.10 (m, 3H, H_6, H_{15}), 1.97-1.85 (m, 3H, H_4, H_{14}, H_{17}), 1.82-1.61 (m, 4H, H_8, H_{10}, H_{12}), 1.49-1.25 (m, 4H, H_6, H_9, H_{14}), 1.18 (br s, 3H, H_{18}), 0.89 (d, *J* = 6.8 Hz, 3H, H_{19}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5 (s, C_1), 165.7 (s, C_{20}), 163.3 (s, C_{24}), 131.4 (d, 2C, C_{22}), 123.2 (s, C_{21}), 113.7 (d, 2C, C_{23}), 96.4 (s, C_3), 84.7 (s, C_{13}), 83.7 (s, C_{16}), 77.2 (d, C_{17}), 69.3 (d, C_{11}), 68.5 (d, C_7), 65.0 (d, C_5), 55.4 (q, C_{27}), 47.1 (t, C_2), 43.4 (t, C_6), 40.6 (t, C_{14}), 34.3 (t, C_{12}), 32.4 (t, C_8), 29.7 (t, C_9), 23.9 (q, C_{18}), 18.6 (q, C_{19}), 13.1 (t, C_{15}). The C_4 (38.1, t) and C_{10} (38.8, d) chemical shifts have been determined by HMQC correlation.

HRMS (ESI) *m/z*: calculated for $C_{27}H_{36}NaO_8$ $[M + Na]^+$: 511.2302, found: 511.2298.

II.4.2.2.17. Synthesis of (1R,5R,7S,8R,11R,13R)-1,13-dihydroxy-5,8-dimethyl-5-[(5E)-oct-5-en-3-yn-1-yl]-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.81)



MW (g/mol): 542.6604 **Molecular formula:** $C_{31}H_{42}O_8$

To a solution of **II.79** (40 mg, 0.08 mmol) and vinyl iodide **II.80** (90 mg, 0.5 mmol) in a degassed Et_3N/DMF mixture (1:1, 3 mL) were added CuI (2 mg, 8.1 μmol) and $Pd(PPh_3)_2Cl_2$ (3 mg, 4.0 μmol). The resulting reaction mixture was stirred at rt for 2 h and diluted with EtOAc (3 mL). The organic layer was then separated and the aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (EtOAc/PE, 30:70) to afford **II.81** (28 mg, 63%) as colourless oil.

R_f (EtOAc/PE, 1:1): 0.41.

$[\alpha]_D^{20}$ –17.4 (*c* 0.4, $CHCl_3$).

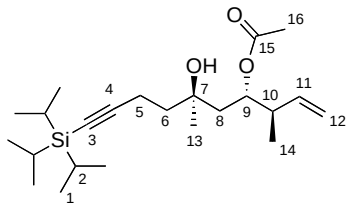
IR (neat): 3475, 2961, 2933, 1704, 1606, 1511, 1322, 1279, 1257, 1230, 1167, 1101, 1030, 772 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.8 Hz, 2H, H_{26}), 6.92 (d, J = 8.8 Hz, 2H, H_{27}), 6.08 (dt, J = 15.8, 6.8 Hz, 1H, H_{19}), 5.49-5.37 (m, 2H, H_{11}, H_{18}), 4.58 (br s, 1H, OH), 4.13 (m, 1H, H_5), 3.86 (s, 3H, H_{29}), 3.67 (m, 1H, H_7), 2.77-2.66 (m, 2H, H_4, H_{12}), 2.6 (d, J = 12.5 Hz, 1H, H_2), 2.49 (d, J = 12.5 Hz, 1H, H_2), 2.33 (m, 1H, H_{15}), 2.26 (m, 1H, H_{15}), 2.18 (dd, J = 12.0, 3.9 Hz, 1H, H_6), 2.09 (t, J = 8.0 Hz, 2H, H_{20}), 2.00-1.87 (m, 2H, H_4, H_{14}), 1.81-1.66 (m, 4H, H_8, H_{10}, H_{12}), 1.41(m, 1H, H_6), 1.37-1.22 (m, 3H, H_9, H_{14}), 1.19 (br s, 3H, H_{22}), 0.98 (t, J = 7.4 Hz, 3H, H_{21}), 0.88 (d, J = 6.8 Hz, 3H, H_{23}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5 (s, C_1), 165.7 (s, C_{24}), 163.3 (s, C_{28}), 145.1 (d, C_{19}), 131.4 (d, 2C, C_{26}), 123.2 (s, C_{25}), 113.7 (d, 2C, C_{27}), 108.7 (d, C_{18}), 96.4 (s, C_3), 87.6 (s, C_{17}), 84.8 (s, C_{13}), 79.6 (s, C_{16}), 71.5 (d, C_{11}), 69.2 (d, C_7), 65.0 (d, C_5), 55.4 (q, C_{29}), 47.1 (t, C_2), 43.4 (t, C_6), 40.6 (t, C_{14}), 38.8 (d, C_{10}), 38.4 (t, C_4), 34.3 (t, C_{12}), 32.4 (t, C_8), 29.7 (t, C_9), 26.0 (t, C_{20}), 23.9 (q, C_{22}), 15.3 (q, C_{23}), 14.1 (t, C_{15}), 13.0 (q, C_{21}).

HRMS (ESI) *m/z*: calculated for $C_{31}H_{42}NaO_8$ $[M + Na]^+$: 565.2772, found: 565.2758.

II.4.2.2.18. Synthesis of (3R,4S,6R)-6-hydroxy-3,6-dimethyl-10-[tris(propan-2-yl)silyl]dec-1-en-9-yn-4-yl acetate (II.83)



MW (g/mol): 394.6632 **Molecular formula:** $C_{23}H_{42}O_3Si$

A solution of diol **II.69** (1.7 g, 4.82 mmol) in CH_2Cl_2 (10 mL) and pyridine (8 mL), was treated with DMAP (24 mg, 0.2 mmol) and acetyl chloride (1.8 mL, 19.3 mmol) at 0 °C. The resulting mixture reaction was stirred for 1 h at rt, and then H_2O was added. The two layers were separated and the aqueous fraction was extracted with CH_2Cl_2 (25 mL x 2). The combined organic fractions were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The obtained residue was purified by flash chromatography (Et_2O/PE, from 15:85 to 30:70) to give pure product **II.83** as colourless oil (1.9 g, quant.).

R_f (PE/Et_2O, 7:3): 0.45.

$[\alpha]_D^{20}$ –4.43 (*c* 0.87, $CHCl_3$).

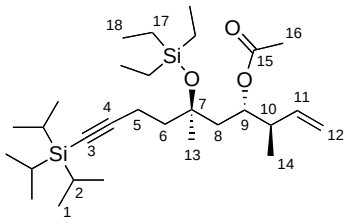
IR (neat): 3468, 2942, 2865, 2171, 1740, 1717, 1463, 1376, 1239, 1020, 883 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (m, 1H, H_{11}), 5.08-5.02 (m, 2H, H_9, H_{12}), 2.41 (dd, *J* = 12.2, 6.9 Hz, 1H, H_{10}), 2.35 (t, *J* = 7.6 Hz, 2H, H_5), 2.23 (br s, 1H, OH), 2.03 (s, 3H, H_{16}), 1.76-1.61 (m, 4H, H_6, H_8), 1.17 (s, 3H, H_{13}), 1.06-1.02 (m, 21H, H_1, H_2), 0.99 (d, *J* = 6.9 Hz, 3H, H_{14}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0 (s, C_{15}), 139.0 (d, C_{11}), 115.9 (t, C_{12}), 108.9 (s, C_3), 81.2 (s, C_4), 73.5 (d, C_9), 71.6 (s, C_7), 42.6 (d, C_{10}), 42.2 (t, C_8), 40.8 (t, C_6), 26.8 (q, C_{16}), 21.3 (q, C_{13}), 18.6 (q, 6C, C_1), 15.2 (q, C_{14}), 14.7 (t, C_5), 11.2 (q, 3C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{23}H_{42}NaO_3Si$ $[M + Na]^+$: 417.2795, found: 417.2790.

II.4.2.2.19. Synthesis of (3R,4S,6R)-3,6-dimethyl-6-[(triethylsilyl)oxy]-10-[tris(propan-2-yl)silyl]dec-1-en-9-yn-4-yl acetate (II.84)



MW (g/mol): 508.9241 **Molecular formula:** $C_{29}H_{56}O_3Si_2$

To a solution of acetate **II.83** (1.8 g, 4.56 mmol) in CH_2Cl_2 (15 mL), was added 2,6-lutidine (1.05 mL, 9.12 mmol). Then, the mixture was cooled down to –10 °C and TESOTf (1.55 mL, 6.84 mmol) was added dropwise. The resulting mixture was stirred for 1 h at –10 °C. A saturated aqueous solution of $NaHCO_3$ was added to quench the reaction. The two layers were separated and the aqueous fraction was extracted with Et_2O (2 x 25 mL). The combined organic fractions were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The obtained residue was purified by flash chromatography (Et_2O/PE, 2:98) to give pure product **II.84** as colourless oil (1.9 g, 83%).

R_f (PE/Et_2O, 19:1): 0.60.

$[\alpha]_D^{20}$ +0.91 (*c* 1.56, $CHCl_3$).

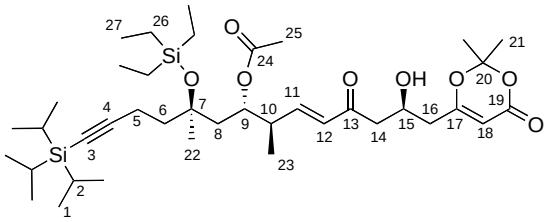
IR (neat): 3302, 2943, 2866, 2171, 1742, 1462, 1376, 1236, 1125, 1019, 1000, 883, 725 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.70 (m, 1H, H_{11}), 5.06 (s, 1H, H_9), 5.05-4.97 (m, 2H, H_{12}), 2.41 (dd, J = 11.8, 6.9 Hz, 1H, H_{10}), 2.32-2.26 (m, 2H, H_5), 2.02 (s, 3H, H_{16}), 1.71-1.58 (m, 4H, H_6, H_8), 1.20 (s, 3H, H_{13}), 1.07-1.02 (m, 21H, H_1, H_2), 0.98-0.91 (m, 12H, H_{14}, H_{18}), 0.57 (q, J = 7.8 Hz, 6H, H_{17}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (s, C_{15}), 139.4 (d, C_{11}), 115.6 (t, C_{12}), 109.5 (s, C_3), 79.6 (s, C_4), 74.3 (s, C_7), 73.6 (d, C_9), 42.7 (t, C_8), 42.5 (d, C_{10}), 41.9 (t, C_6), 27.4 (q, C_{16}), 21.5 (q, C_{13}), 18.6 (q, 6C, C_1), 14.8 (t, C_5), 14.7 (t, C_{14}), 11.3 (q, 3C, C_2), 7.1 (q, 3C, C_{18}), 6.8 (t, 3C, C_{17}).

HRMS (ESI) *m/z*: calculated for $C_{29}H_{56}NaO_3Si_2$ $[M + Na]^+$: 531.3660, found: 531.3660.

II.4.2.2.20. Synthesis of (5R,7S,8R,9E,13S)-14-(2,2-dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-13-hydroxy-5,8-dimethyl-11-oxo-5-[(triethylsilyl)oxy]-1-[tris(propan-2-yl)silyl] tetradec-9-en-1-yn-7-yl acetate (II.85)



MW (g/mol): 721.1234 **Molecular formula:** $C_{39}H_{68}O_8Si_2$

To a solution of olefin **II.84** (900 mg, 1.77 mmol) and enone **II.49** (600 mg, 2.47 mmol) in CH_2Cl_2 (9 mL), was added Hoveyda-Grubbs 2nd Generation catalyst (220 mg, 0.35 mmol). The resulting mixture was stirred for 3 days at reflux. Then, the reaction mixture was concentrated under reduced pressure and the obtained residue was purified by flash chromatography (EtOAc/PE, from 20:80 to 30:70) to give the pure product **II.85** as a colourless oil (1.03 g, 81%).

R_f (EtOAc/PE, 1:1): 0.55.

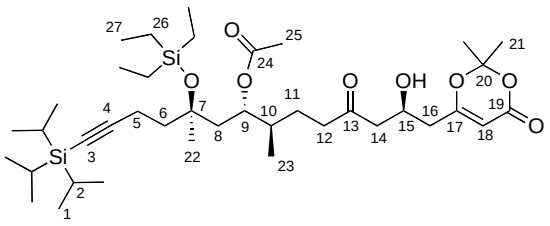
IR (neat): 3467, 2943, 2866, 2171, 1731, 1634, 1376, 1237, 1013, 725 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72 (dd, J = 15.9, 7.5 Hz, 1H, H_{11}), 6.06 (d, J = 15.9 Hz, 1H, H_{12}), 5.33 (s, 1H, H_{18}), 5.06 (m, 1H, H_9), 4.36 (oct, J = 4.0 Hz, 1H, H_{15}), 3.34 (d, J = 3.4 Hz, 1H, H_{28}), 2.79-2.66 (m, 2H, H_{14}), 2.59 (m, 1H, H_{10}), 2.42 (dd, J = 14.8, 8.8 Hz, 1H, H_{16}), 2.34 (dd, J = 14.8, 4.6 Hz, 1H, H_{16}), 2.28-2.22 (m, 2H, H_5), 2.00 (s, 3H, H_{25}), 1.68 (s, 3H, H_{21}), 1.67 (s, 3H, H_{21}), 1.68-1.57 (m, 4H, H_6, H_8), 1.18 (s, 3H, H_{22}), 1.05-0.96 (m, 24H, H_1, H_2, H_{23}), 0.91 (t, J = 7.9 Hz, 9H, H_{27}), 0.55 (q, J = 7.9 Hz, 6H, H_{26}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 199.6 (s, C_{13}), 170.5 (s, C_{24}), 168.3 (s, C_{17}), 160.9 (s, C_{19}), 149.1 (d, C_{11}), 130.8 (d, C_{12}), 108.9 (s, C_3), 106.6 (s, C_{20}), 95.3 (d, C_{18}), 80.0 (s, C_4), 74.1 (d, C_9), 72.8 (s, C_7), 64.6 (d, C_{15}), 45.3 (t, C_{14}), 42.9 (t, C_8), 42.5 (t, C_6), 41.9 (d, C_{10}), 40.4 (t, C_{16}), 27.1 (q, C_{22}), 25.4 (q, C_{21}), 24.4 (q, C_{21}), 21.1 (q, C_{25}), 18.5 (q, 6C, C_1), 14.9 (q, C_{23}), 14.7 (t, C_5), 11.1 (d, 3C, C_2), 7.0 (q, 3C, C_{27}), 6.6 (t, 3C, C_{26}).

HRMS (ESI) *m/z*: calculated for $C_{39}H_{68}NaO_8Si_2$ $[M + Na]^+$: 743.4345, found: 743.4364.

II.4.2.2.21. Synthesis of (5R,7S,8R,13S)-14-(2,2-dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-13-hydroxy-5,8-dimethyl-11-oxo-5-[(triethylsilyl)oxy]-1-[tris (propan-2-yl)silyl]tetradec-1-yn-7-yl acetate (II.86)



MW (g/mol): 723.1393 **Molecular formula:** $C_{39}H_{70}O_8Si_2$

A solution of enone **II.85** (620 mg, 0.86 mmol) in degassed toluene (10 mL) was transferred via syringe to a flask containing the Stryker's reagent (1 g, 0.51 mmol) in toluene (30 mL). The resulting mixture was stirred for 30 min at rt. Then, the reaction mixture was exposed to air and hexane was added. The stirring was continued for 20 min. The solvent was removed under reduced pressure and the obtained residue was purified by flash chromatography (EtOAc/PE, from 25:85 to 35:75) to give the pure product **II.86** as a colourless oil (516 mg, 83%).

R_f (EtOAc/PE, 2:3): 0.44.

$[\alpha]_D^{20}$ +4.11 (*c* 0.72, $CHCl_3$, 86% ee).

IR (neat): 3468, 2956, 2866, 2171, 1730, 1635, 1462, 1377, 1242, 1016, 725 cm^{-1}.

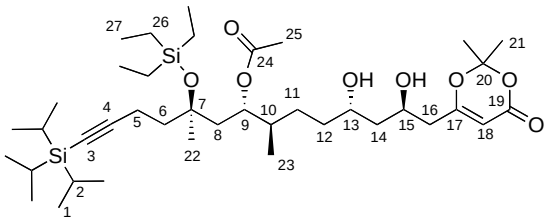
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.29 (s, 1H, H_{18}), 4.89 (dd, J = 8.6, 3.7 Hz, 1H, H_9), 4.30 (sept, *J* = 4.0 Hz, 1H, H_{15}), 3.38 (br s, 1H, OH), 2.64-2.51 (m, 3H, H_{14}, H_{12}), 2.43-2.24 (m, 5H, H_5, H_{14}, H_{16}), 2.00 (s, 3H, H_{25}), 1.65 (s, 3H, H_{21}), 1.64 (s, 3H, H_{21}), 1.69-1.55 (m, 4H, H_6, H_8), 1.53-1.38 (m, 3H, H_{10}, H_{11}), 1.15 (s, 3H, H_{22}), 1.01-0.99 (m, 21H, H_1,

H_2), 0.90 (t, *J* = 8.0 Hz, 9H, H_{27}), 0.80 (d, *J* = 6.7 Hz, 3H, H_{23}), 0.54 (q, *J* = 8.0 Hz, 6H, H_{26}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 210.6 (s, C_{13}), 170.7 (s, C_{24}), 168.2 (s, C_{17}), 160.9 (s, C_{19}), 109.2 (s, C_3), 106.6 (s, C_{20}), 95.4 (d, C_{18}), 79.9 (s, C_4), 74.2 (d, C_9), 73.5 (s, C_7), 64.8 (d, C_{15}), 48.5 (t, C_{14}), 42.9 (t, C_8), 41.0 (t, C_6), 40.5 (t, C_{12}), 40.4 (t, C_{16}), 36.5 (d, C_{10}), 27.8 (q, C_{22}), 25.8 (t, C_{11}), 25.5 (q, C_{21}), 24.5 (q, C_{21}), 21.4 (q, C_{25}), 18.6 (q, 6C, C_1), 14.8 (t, C_5), 14.3 (q, C_{23}), 11.2 (d, 3C, C_2), 7.1 (q, 3C, C_{27}), 6.8 (t, 3C, C_{26}).

HRMS (ESI) *m/z*: calculated for $C_{39}H_{70}NaO_8Si_2$ $[M + Na]^+$: 745.4501, found: 745.4519.

II.4.2.2.22. Synthesis of (5R,7S,8R,11S,13R)-14-(2,2-dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-11,13-dihydroxy-5,8-dimethyl-5-[(triethylsilyl)oxy]-1-[tris(propan-2-yl)silyl] tetradec-1-yn-7-yl acetate (II.87)



MW (g/mol): 725.1551 **Molecular formula:** $C_{39}H_{72}O_8Si_2$

To a solution of ketone **II.86** (350 mg, 0.48 mmol) in MeCN (3.7 mL) and AcOH (3.7 mL) at –30 °C, was added $Me_4NBH(OAc)_3$ (1.2 g, 4.8 mmol) in one portion. Then, the solution was diluted with EtOAc (5 mL) and poured in a $NaHCO_3$ solution under stirring. The organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The obtained residue was purified by flash chromatography (EtOAc/PE, 30:70) to afford the product **II.87** (290 mg, 84%) as a colourless oil.

Rf (EtOAc/PE, 1:1): 0.42.

$[\alpha]_D^{20}$ –3.5 (*c* 0.72, $CHCl_3$, 86% ee).

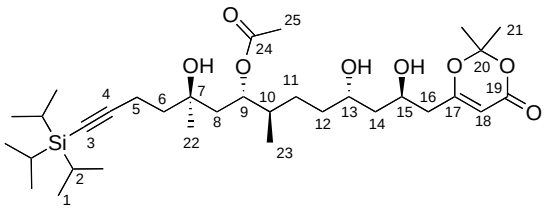
IR (neat): 3437, 2943, 2866, 2171, 1733, 1716, 1634, 1377, 1243, 1016 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (s, 1H, H_{18}), 4.91 (m, 1H, H_9), 4.23 (m, 1H, H_{15}), 3.94 (m, 1H, H_{13}), 2.49-2.36 (m, 2H, H_{16}), 2.35-2.26 (m, 2H, H_5), 2.03 (s, 3H, H_{25}), 1.80-1.38 (m, 9H, H_6, H_8, H_{10}, H_{12}, H_{14}), 1.69 (s, 6H, H_{21}), 1.36-1.28 (m, 2H, H_{11}), 1.19 (s, 3H, H_{22}), 1.07-0.98 (m, 21H, H_1, H_2), 0.94 (t, J = 8.0 Hz, 9H, H_{27}), 0.85 (d, J = 6.9 Hz, 3H, H_{23}), 0.57 (q, J = 8.0 Hz, 6H, H_{26}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8 (s, C_{24}), 169.1 (s, C_{17}), 161.1 (s, C_{19}), 109.3 (s, C_3), 106.6 (s, C_{20}), 95.1 (d, C_{18}), 79.8 (s, C_4), 74.3 (d, C_9), 74.1 (s, C_7), 69.0 (d, C_{13}), 66.2 (d, C_{15}), 42.9 (t, C_8), 42.8 (t, C_{16}), 41.7 (t, C_6), 40.4 (t, C_{14}), 36.9 (d, C_{10}), 34.9 (t, C_{12}), 28.5 (t, C_{11}), 27.4 (q, C_{25}), 25.3 (q, C_{21}), 24.7 (q, C_{21}), 21.4 (q, C_{22}), 18.6 (q, 6C, C_1), 14.8 (t, C_5), 14.4 (q, C_{23}), 11.2 (d, 3C, C_2), 7.1 (q, 3C, C_{27}), 6.8 (t, 3C, C_{26}).

HRMS (ESI) *m/z*: calculated for $C_{39}H_{72}NaO_8Si_2$ $[M + Na]^+$: 747.4658, found: 747.4668.

II.4.2.2.23. Synthesis of (5R,7S,8R,11S,13R)-14-(2,2-dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5,11,13-trihydroxy-5,8-dimethyl-1-[tris(propan-2-yl) silyl]tetradec-1-yn-7-yl acetate



MW (g/mol): 610.8943 **Molecular formula:** $C_{33}H_{58}O_8Si$

A solution of TES ether **II.87** (280 mg, 0.38 mmol) in MeOH (1.3 mL), was added $FeCl_3{\cdot}(H_2O)_6$ (4 mg, 0.015 mmol). The resulting mixture was stirred overnight at rt. The solvent was removed under reduced pressure and the obtained residue was purified by flash chromatography (EtOAc/PE, from 50:50 to 70:30) to give pure product as colourless oil (215 mg, 92%).

R_f (EtOAc/PE, 1:1): 0.15.

$[\alpha]_D^{20}$ –3.06 (*c* 0.70, $CHCl_3$).

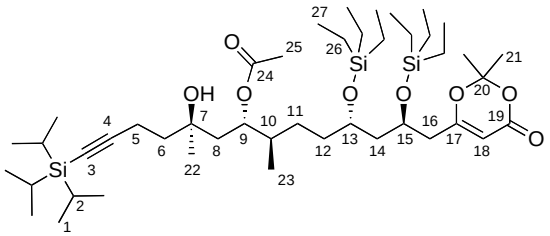
IR (neat): 3412, 2942, 2865, 2170, 1712, 1633, 1377, 1248, 1204, 1016 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.29 (s, 1H, H_{18}), 5.02 (d, J = 6.4 Hz, 1H, H_9), 4.15 (m, 1H, H_{15}), 3.89 (br s, 1H, OH), 3.82 (m, 1H, H_{13}), 3.60 (br s, 1H, OH), 3.06 (br s, 1H, OH), 2.44-2.24 (m, 4H, H_5, H_{16}), 1.98 (s, 3H, H_{25}), 1.73-1.59 (m, 4H, H_8, H_{14}), 1.63 (s, 6H, H_{21}), 1.57-1.37 (m, 5H, H_6, H_{10}, H_{12}), 1.17-1.10 (m, 2H, H_{11}), 1.08 (s, 3H, H_{22}), 1.04-0.90 (m, 21H, H_1, H_2), 0.82 (d, J = 6.4 Hz, 3H, H_{23}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0 (s, C_{24}), 169.6 (s, C_{17}), 161.6 (s, C_{19}), 108.8 (s, C_3), 106.5 (s, C_{20}), 94.7 (d, C_{18}), 80.5 (s, C_4), 73.6 (d, C_9), 71.5 (s, C_7), 68.6 (d, C_{13}), 65.7 (d, C_{15}), 42.9 (t, C_8), 41.8 (t, C_{16}), 41.5 (t, C_6), 39.9 (t, C_{14}), 36.8 (d, C_{10}), 34.9 (t, C_{12}), 28.7 (t, C_{11}), 25.5 (q, C_{22}), 25.1 (q, C_{21}), 24.6 (q, C_{21}), 21.3 (q, C_{25}), 18.5 (q, 6C, C_1), 14.5 (t, C_5), 14.3 (q, C_{23}), 11.1 (d, 3C, C_2).

HRMS (ESI) *m/z*: calculated for $C_{33}H_{58}NaO_8Si$ $[M + Na]^+$: 633.3793, found: 633.3795.

II.4.2.2.24. Synthesis of (5R,7S,8R,11S,13R)-14-(2,2-dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5-hydroxy-5,8-dimethyl-11,13-bis[(triethylsilyl)oxy]-1-[tris(propan-2-yl)silyl] tetradec-1-yn-7-yl acetate (II.88)



MW (g/mol): 839.4160 **Molecular formula:** $C_{45}H_{86}O_8Si_3$

A solution of triol (120 mg, 0.19 mmol) in CH_2Cl_2 (3.8 mL), was added imidazole (53 mg, 0.78 mmol) followed by TESCl (72 µl, 0.43 mmol) at 0 ºC. The resulting mixture was stirred for 30 min at 0 ºC. $NaHCO_3$ (5 mL) was added to quench the reaction. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL). The combined organic layers were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The obtained residue was purified by flash chromatography (EtOAc/PE, 10:90) to give pure product **II.88** as colourless oil (149 mg, 90%).

R_f (EtOAc/PE, 1:9): 0.3.

$[\alpha]_D^{20}$ –2.3 (*c* 1.01, $CHCl_3$).

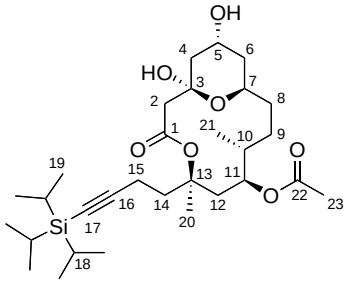
IR (neat): 3469, 2955, 2875, 2171, 1733, 1635, 1376, 1243, 1015, 741 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.26 (s, 1H, H_{18}), 4.99 (dd, J = 8.0, 3.7 Hz, 1H, H_9), 4.04 (quint, J = 5.9 Hz, 1H, H_{15}), 3.74 (quint, J = 5.9 Hz, 1H, H_{13}), 2.43-2.32 (m, 4H, H_5, H_{16}), 2.23 (br s, 1H, H_{28}), 2.01 (s, 3H, H_{25}), 1.78-1.62 (m, 4H, H_8, H_{14}), 1.66 (s, 3H, H_{21}), 1.65 (s, 3H, H_{21}), 1.59-1.28 (m, 7H, H_6, H_{10}, H_{11}, H_{12}), 1.16 (s, 3H, H_{22}), 1.04-0.99 (m, 21H, H_1, H_2), 0.93 (t, J = 8.0 Hz, 9H, H_{26}), 0.92 (t, J = 8.0 Hz, 9H, H_{26}), 0.85 (d, J = 7.0 Hz, 3H, H_{23}), 0.56 (q, J = 8.0 Hz, 12H, H_{27}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8 (s, C_{24}), 169.1 (s, C_{17}), 161.1 (s, C_{19}), 108.8 (s, C_3), 106.3 (s, C_{20}), 95.2 (d, C_{18}), 80.9 (s, C_4), 74.1 (d, C_9), 71.6 (s, C_7), 69.8 (d, C_{13}), 67.4 (d, C_{15}), 45.2 (t, C_8), 42.8 (t, C_{16}), 40.9 (t, C_6), 40.8 (t, C_{14}), 37.5 (d, C_{10}), 35.5 (t, C_{12}), 29.6 (t, C_{11}), 26.7 (q, C_{25}), 25.5 (q, C_{21}), 24.4 (q, C_{21}), 21.3 (q, C_{25}), 18.5 (q, 6C, C_1), 14.6 (t, C_5), 14.5 (q, C_{23}), 11.1 (d, 3C, C_2), 6.9 (q, 3C, C_{27}), 6.8 (q, 3C, C_{27}), 5.3 (t, 3C, C_{26}), 5.1 (t, 3C, C_{26}).

HRMS (ESI) *m/z*: calculated for $C_{45}H_{86}NaO_8Si_3$ $[M + Na]^+$: 861.5523, found: 861.5542.

II.4.2.2.25. Synthesis of (1R,5R,7S,8R,11R,13R)-1,13-dihydroxy-5,8-dimethyl-3-oxo-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo [9.3.1]pentadecan-7-yl acetate (II.89)



MW (g/mol): 552.8152 **Molecular formula:** $C_{30}H_{52}O_7Si$

A solution of TES ether **II.88** (130 mg, 0.15 mmol) in toluene (5 mL) was added to preheated dry toluene (800 mL) dropwise. The resulting mixture was stirred 2 h at reflux. The solvent was removed under reduced pressure and the obtained residue was placed in a plastic culture tube in THF (2 mL). A solution of HF·Py (0.5 mL) in THF/pyridine (3:2; 3.2 mL) was added dropwise at 0 °C. The resulting mixture was

stirred for 30 min at 0 °C. $NaHCO_3$ solution was added with care to quench the reaction. The mixture was extracted with EtOAc (2 x 5 mL), and the combined organic layers were washed with NaCl and concentrated *in vacuo*. The obtained residue was purified by flash chromatography (EtOAc/PE, from 50:50 to 70:30) to give pure product **II.89** as colourless oil (55 mg, 65%).

R_f (EtOAc/PE, 1:1): 0.5.

$[\alpha]_D^{20}$ –19.97 (*c* 1.1, $CHCl_3$).

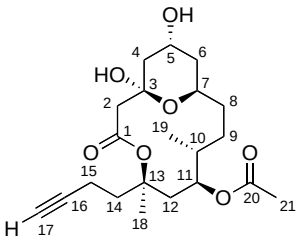
IR (neat): 3479, 2941, 2865, 2173, 1731, 1707, 1365, 1244, 1086, 1012, 883, 677 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.18 (d, J = 9.5 Hz, 1H, H_{11}), 4.51 (br s, 1H, OH), 4.11 (sept, J = 4.5 Hz, 1H, H_5), 3.61 (t, J = 10.9 Hz, 1H, H_7), 2.69-2.59 (m, 2H, H_4, H_{12}), 2.57 (d, J = 12.7 Hz, 1H, H_2), 2.48 (d, J = 12.7 Hz, 1H, H_2), 2.30 (m, 1H, H_{15}), 2.21-2.12 (m, 2H, H_6, H_{15}), 2.00 (s, 3H, H_{23}), 1.94 (m, 1H, H_4), 1.87 (m, 1H, H_{14}), 1.75-1.48 (m, 6H, H_8, H_9, H_{10}, H_{12}), 1.32-1.24 (m, 2H, H_6, H_{14}), 1.20 (s, 3H, H_{20}), 1.08-1.02 (m, 21H, H_{18}, H_{19}), 0.82 (d, J = 7.2 Hz, 3H, H_{21}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3 (s, C_1), 170.5 (s, C_{22}), 108.0 (s, C_{17}), 96.3 (s, C_3), 84.8 (s, C_{13}), 80.5 (s, C_{16}), 69.2 (d, C_7), 65.0 (d, C_5), 47.1 (t, C_2), 43.4 (t, C_6), 40.5 (t, C_{14}), 38.7 (t, C_4), 38.5 (d, C_{10}), 33.9 (t, C_{12}), 32.2 (t, C_8), 28.7 (t, C_9), 23.8 (q, C_{20}), 21.6 (q, C_{23}), 18.6 (q, 6C, C_{19}), 15.1 (q, C_{21}), 14.6 (t, C_{15}), 11.2 (d, 3C, C_{18}). C_{11} (71.5, d) was determined by HMQC.

HRMS (ESI) *m/z*: calculated for $C_{30}H_{52}NaO_7Si$ $[M + Na]^+$: 575.3374, found: 575.3363.

II.4.2.2.26. Synthesis of (1R,5R,7S,8R,11R,13R)-5-(but-3-yn-1-yl)-1,13-dihydroxy-5,8-dimethyl-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate



MW (g/mol): 396.4746 **Molecular formula:** $C_{21}H_{32}O_7$

To a stirred solution of TIPS alkyne **II.89** (50 mg, 0.09 mmol) in MeCN (1.8 mL), was added AgF (57 mg, 0.45 mmol) in the dark. The resulting mixture was stirred at rt for 5 h. Then, 1M HCl solution (0.3 mL) was added. The resulting mixture was stirred for 5 min, and then, diluted with EtOAc. The organic phase was separated and the aqueous phase extracted with EtOAc (2 x 5 mL), and the combined organic layers were washed with NaCl, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The obtained residue was purified by flash chromatography (EtOAc/PE, from 25:75 to 40:60) to give pure product as colourless oil (30 mg, 85%).

$\boldsymbol{R_f}$ (EtOAc/PE, 1:1): 0.33.

$\boldsymbol{[\alpha]_D^{20}}$ –16.3 (*c* 0.9, $CHCl_3$).

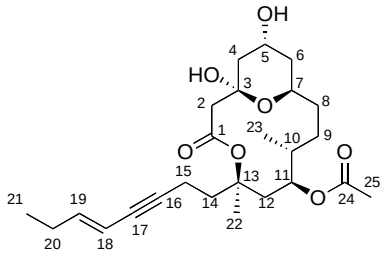
IR (neat): 3470, 3296, 2928, 1728, 1706, 1366, 1245, 1231, 1087, 1022, 913, 744 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.19 (d, *J* = 9.8 Hz, 1H, H_{11}), 4.52 (br s, 1H, OH), 4.11 (sept, *J* = 4.9 Hz, 1H, H_5), 3.61 (t, *J* = 11.4 Hz, 1H, H_7), 2.75-2.68 (m, 1H, H_4), 2.63 (d_{app}, *J* = 15.2 Hz, 1H, H_{12}), 2.57 (d, *J* = 12.5 Hz, 1H, H_2), 2.48 (d, *J* = 12.4 Hz, 1H, H_2), 2.25 (m, 1H, H_{15}), 2.19-2.06 (m, 2H, H_6, H_{15}), 2.00 (s, 3H, H_{21}), 1.95 (t, *J* = 2.6 Hz, 1H, H_{17}), 1.92-1.84 (m, 2H, H_4, H_{14}), 1.72-1.47 (m, 4H, H_8, H_{10}, H_{12}), 1.32-1.22 (m, 4H, H_6, H_9, H_{14}), 1.18 (s, 3H, H_{18}), 0.81 (d, *J* = 6.7 Hz, 3H, H_{19}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4 (s, C_1), 170.5 (s, C_{20}), 96.3 (s, C_3), 84.6 (s, C_{13}), 83.7 (s, C_{16}), 69.2 (d, C_7), 68.5 (d, C_{17}), 64.9 (d, C_5), 47.1 (t, C_2), 43.4 (t, C_6), 40.5 (t, C_{14}), 38.1 (t, C_4), 32.2 (t, C_8), 29.7 (t, C_9), 23.6 (q, C_{18}), 21.6 (q, C_{21}), 15.1 (q, C_{19}), 13.1 (t, C_{15}). C_{10} (38.1, d), C_{11} (71.3, d) and C_{12} (34.1, t) were determined by HMQC.

HRMS (ESI) *m/z*: calculated for $C_{21}H_{32}NaO_7$ $[M + Na]^+$: 419.2040, found: 419.2029.

*II.4.2.2.27. Synthesis of (1R,5R,7S,8R,11R,13R)-1,13-dihydroxy-5,8-dimethyl-5-[(5**E**)-oct-5-en-3-yn-1-yl]-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate (II.90)*



MW (g/mol): 450.5650 **Molecular formula:** $C_{25}H_{38}O_7$

To a solution of alkyne (27 mg, 0.07 mmol) and vinyl iodide **II.80** (74 mg, 0.4 mmol) in degassed Et_3N/DMF (1:1, 2.3 mL) were added CuI (1.3 mg, 7 µmol) and $Pd(PPh_3)_2Cl_2$ (2.4 mg, 3.5 µmol). The resulting mixture was stirred at rt for 2 h. H_2O and EtOAc was added. The organic phase was separated and the aqueous phase extracted with EtOAc (2 x 5 mL), and the combined organic layers were washed with NaCl, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The obtained residue was purified by flash chromatography (EtOAc/PE, 25:75) to give pure product **II.90** as colourless oil (21 mg, 68%).

$\boldsymbol{R_f}$ (EtOAc/PE, 1:1): 0.41.

$\boldsymbol{[\alpha]_D^{20}}$ –27.46 (*c* 0.5, $CHCl_3$).

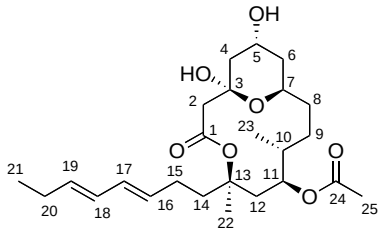
IR (neat): 3470, 2962, 2933, 1730, 1700, 1382, 1244, 1197, 1163, 1011, 960 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09 (dt, *J* = 16.0, 6.8 Hz, 1H, H_{19}), 5.42 (d, *J* = 16.0 Hz, 1H, H_{18}), 5.20 (d, *J* = 9.9 Hz, 1H, H_{11}), 4.54 (br s, 1H, OH), 4.11 (m, 1H, H_5), 3.61 (t, *J* = 11.1 Hz, 1H, H_7), 2.70-2.58 (m, 2H, H_4, H_{12}), 2.57 (d, *J* = 12.6 Hz, 1H, H_2), 2.47 (d, *J* = 12.6 Hz, 1H, H_2), 2.35 (m, 1H, H_{15}), 2.24 (m, 1H, H_{15}), 2.16 (dd, *J* = 12.0, 4.6 Hz, 1H, H_6), 2.09 (t, *J* = 7.1 Hz, 2H, H_{20}), 2.00 (s, 3H, H_{25}), 1.94-1.84 (m, 2H, H_4, H_{14}), 1.70-1.49 (m, 4H, H_8, H_{10}, H_{12}), 1.32-1.14 (m, 4H, H_6, H_9, H_{14}), 1.18 (s, 3H, H_{22}), 0.98 (t, *J* = 7.4 Hz, 3H, H_{21}), 0.81 (d, *J* = 7.1 Hz, 3H, H_{23}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4 (s, C_1), 170.5 (s, C_{24}), 145.1 (d, C_{19}), 108.7 (d, C_{18}), 96.3 (s, C_3), 87.5 (s, C_{17}), 84.8 (s, C_{13}), 79.5 (s, C_{16}), 69.2 (d, C_7), 65.0 (d, C_5), 47.1 (t, C_2), 43.4 (t, C_6), 40.5 (t, C_{14}), 38.3 (t, C_4), 34.1 (t, C_{12}), 32.2 (t, C_8), 28.8 (t, C_9), 26.0 (t, C_{20}), 23.6 (q, C_{22}), 21.7 (q, C_{25}), 14.1 (t, C_{15}), 13.0 (q, C_{21}). C_{10} (38.4, d), C_{11} (71.4, d) and C_{23} (15.0, q) were determined by HMQC.

HRMS (ESI) *m/z*: calculated for $C_{25}H_{38}NaO_7$ $[M + Na]^+$: 473.2510, found: 473.2501.

II.4.2.2.28. Synthesis of (1R,5R,7S,8R,11R,13R)-1,13-dihydroxy-5,8-dimethyl-5-[(3E,5E)-octa-3,5-dien-1-yl]-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate (II.91)



MW (g/mol): 452.5809 **Molecular formula:** $C_{25}H_{40}O_7$

To a solution of alkyne **II.91** (12 mg, 26 µmol) in CH_2Cl_2 (0.3 mL)) at 0 °C was added $(EtO)_3SiH$ (5 µL, 53 µmol) followed by Cp*Ru(MeCN)$_3$PF$_6$ (0.1 mg, 0.26 µmol). The flask was immediately allowed to warm to rt and stirred for 1 h. Then, the solvent was removed and a mixture of THF/MeOH/H_2O (2:1:1, 4 mL) was added followed by AgF (16 mg, 130 µmol). The resulting mixture was stirred under dark for 1 h. After that, the reaction mixture was filtered through Celite©, the solvent was removed and the obtained residue was purified by flash chromatography (EtOAc/PE, 25:75) to give the pure product **II.91** as a colourless oil (5.2 mg, 45%).

R_f (EtOAc/PE, 50/50): 0.57.

$[\alpha]_D^{20}$ –25.1 (*c* 0.4, $CHCl_3$).

IR (neat): 3469, 2960, 2930, 1730, 1704, 1381, 1244, 1198, 1164, 1011 cm^{-1}.

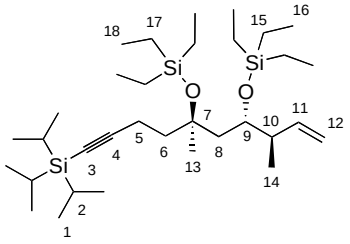
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09-5.91 (m, 2H, H_{17}, H_{18}), 5.62 (m, 1H, H_{19}), 5.53 (m, 1H, H_{16}), 5.38 (br s, 1H, OH), 5.20 (d, *J* = 9.4 Hz, 1H, H_{11}), 4.57 (br s, 1H, OH), 4.11 (m, 1H, H_5), 3.62 (t, *J* = 11.4 Hz, 1H, H_7), 2.59 (m, 1H, H_{12}), 2.57 (d, *J* = 12.5 Hz, 1H, H_2), 2.47 (d, *J* = 12.5 Hz, 1H, H_2), 2.45 (m, 1H, H_4), 2.20-2.15 (m, 2H, H_6, H_{15}), 2.11-2.01 (m, 2H, H_{20}), 2.00 (s, 3H, H_{25}), 1.90-1.84 (m, 2H, H_4, H_{14}), 1.77-1.44 (m, 5H, H_8, H_{10}, H_{12}, H_{15}), 1.38-1.12 (m, 4H, H_6, H_9, H_{14}), 1.17 (s, 3H, H_{22}), 0.97 (dt, *J* = 7.3, 2.0 Hz, 3H, H_{21}), 0.82 (d, *J* = 6.8 Hz, 3H, H_{23}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4 (s, C_1), 170.6 (s, C_{24}), 134.5 (d, C_{19}), 130.9 (d, C_{16}), 130.8 (d, C_{17}), 129.1 (d, C_{18}), 96.3 (s, C_3), 85.3 (s, C_{13}), 69.2 (d, C_7), 65.0 (d, C_5), 47.1 (t, C_2), 43.4 (t, C_4), 40.6 (t, C_{14}), 39.1 (t, C_{20}), 38.5 (d, C_{10}), 34.1 (t, C_{12}), 32.2 (t,

C_8), 29.7 (t, C_9), 26.6 (t, C_{20}), 25.6 (t, C_{15}), 23.4 (q, C_{22}), 21.7 (q, C_{25}), 15.0 (q, C_{23}), 13.6 (q, C_{21}). C_{11} (71.6, d) was determined by HMQC.

HRMS (ESI) *m/z*: calculated for $C_{25}H_{40}NaO_7$ $[M + Na]^+$: 475.2666, found: 475.2661.

II.4.2.2.29. Synthesis of (5R,7S)-7-[(2R)-but-3-en-2-yl]-3,3,9,9-tetraethyl-5-methyl-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,8-dioxa-3,9-disilaundecane (II.93)



MW (g/mol): 565.0735 **Molecular formula:** $C_{34}H_{68}O_2Si_2$

To a stirred solution of diol **II.69** (1.5 g, 4.26 mmol) in CH_2Cl_2 (50 mL) at –78 °C was added 2,6-lutidine (4 mL, 34 mmol) followed by TESOTf (3.8 mL, 17 mmol). The resulting reaction mixture was stirred for 30 min at the same temperature, time after which a saturated aqueous solution of $NaHCO_3$ was added. The two layers were separated and the aqueous fraction was extracted with Et_2O (2 x 50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (PE, 100%) to afford **II.93** (2.3 g, quant.) as a colourless oil.

R_f (PE, 100%): 0.43.

$[\alpha]_D^{20}$ –4.3 (*c* 0.46, $CHCl_3$).

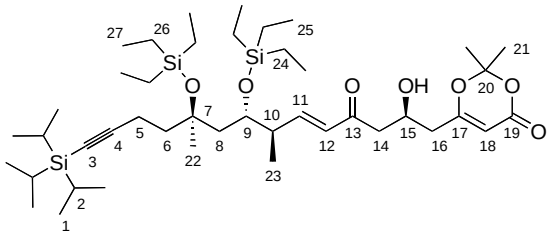
IR (neat): 2956, 2876, 2172, 1121, 1050, 1003, 723, 675 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (m, 1H, H_{11}), 5.06-4.99 (m, 2H, H_{12}), 3.84 (m, 1H, H_9), 2.41 (m, 1H, H_{10}), 2.33-2.29 (m, 2H, H_5), 1.83-1.64 (m, 2H, H_6), 1.54 (dd, *J* = 14.1, 3.6 Hz, 1H, H_8), 1.41 (dd, *J* = 14.1, 7.3 Hz, 1H, H_8), 1.20 (s, 3H, H_{13}), 1.09-1.01 (m, 21H, H_1, H_2), 0.99 (d, *J* = 6.6 Hz, 3H, H_{14}), 0.97 (t, *J* = 7.7 Hz, 9H, H_{16} or H_{18}), 0.94 (t, *J* = 7.7 Hz, H_{16} or H_{18}), 0.64 (q, *J* = 7.7 Hz, 6H, H_{15} or H_{17}), 0.56 (q, *J* = 7.7 Hz, 6H, H_{15} or H_{17}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 140.7 (d, C_{11}), 114.7 (t, C_{12}), 110.0 (s, C_3), 79.2 (s, C_4), 74.8 (s, C_7), 72.7 (d, C_9), 44.2 (d, C_{10}), 43.7 (t, C_8), 42.9 (t, C_6), 27.7 (q, C_{13}), 18.6 (q, 6C, C_1), 14.8 (t, C_5), 12.8 (q, C_{14}), 11.3 (d, 3C, C_2), 7.1 (q, 3C, C_{16} or C_{18}), 7.0 (q, 3C, C_{16} or C_{18}), 6.8 (t, 3C, C_{15} or C_{17}), 5.4 (t, 3C, C_{15} or C_{17}).

HRMS (ESI) *m/z*: calculated for $C_{33}H_{68}NaO_2Si_3$ $[M + Na]^+$: 603.4419, found: 603.4415.

II.4.2.2.30. Synthesis of 6-[(2S,5E,7R,8S,10R)-2-hydroxy-7,10-dimethyl-4-oxo-8,10-bis[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-5-en-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.94)



MW (g/mol): 793.3476 **Molecular formula:** $C_{43}H_{80}O_7Si_3$

To a solution of olefin **II.93** (1.5 g, 2.56 mmol) and enone **II.49** (800 mg, 3.32 mmol) in CH_2Cl_2 (10 mL) was added Hoveyda-Grubbs 2nd Generation catalyst (320 mg, 0.35 mmol) and the resulting mixture was stirred for 3 days at reflux. The solvent was then removed under reduced pressure and the crude residue was purified by flash column chromatography (EtOAc/CH_2Cl_2, 2:98) to afford the corresponding enone **II.94** (1.6 g, 81%) as colourless oil.

R_f (EtOAc/CH_2Cl_2, 1:9): 0.6.

IR (neat): 3468, 2955, 2875, 2171, 1730, 1634, 1376, 1204, 1010, 724, 675 cm^{-1}.

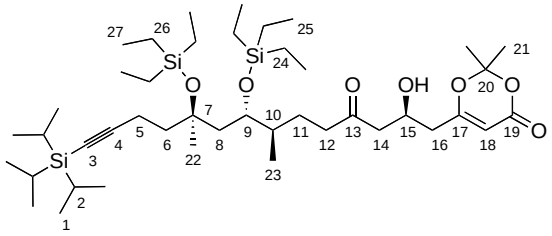
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (dd, J = 16.5, 7.7 Hz, 1H, H_{11}), 6.07 (dd, J = 16.5, 1.4 Hz, 1H, H_{12}), 5.34 (s, 1H, H_{18}), 4.37 (m, 1H, H_{15}), 3.93 (m, 1H, H_9), 3.41 (m, 1H, OH), 2.78 (dd, J = 17.8, 3.2 Hz, 1H, H_{14}), 2.68 (dd, J = 17.8, 8.4 Hz, 1H, H_{14}), 2.63 (m, 1H, H_{10}), 2.43 (dd, J = 14.6, 8.4 Hz, 1H, H_{16}), 2.34 (dd, J = 14.6, 4.5 Hz, 1H, H_{16}), 2.26-2.22 (m, 2H, H_5), 1.76-1.70 (m, 2H, H_6), 1.68 (s, 3H, H_{21}), 1.67 (s, 3H, H_{21}), 1.49 (dd, J = 14.1, 5.3 Hz, 1H, H_8), 1.38 (dd, J = 14.1, 5.3 Hz, 1H, H_8), 1.22 (s, 3H, H_{22}), 1.07-1.00 (m, 24H, H_1, H_2, H_{23}), 0.95 (t, J = 7.9 Hz, 9H, H_{25} or H_{27}), 0.91 (t, J = 7.9 Hz,

9H, H_{25} or H_{27}), 0.61 (q, *J* = 7.9 Hz, 6H, H_{24} or H_{26}), 0.55 (q, *J* = 7.9 Hz, 6H, H_{24} or H_{26}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 199.8 (s, C_{13}), 168.3 (s, C_{17}), 160.9 (s, C_{19}), 151.0 (d, C_{11}), 130.3 (d, C_{12}), 109.1 (s, C_3), 106.6 (s, C_{20}), 95.3 (d, C_{18}), 79.8 (s, C_4), 74.5 (s, C_7), 72.2 (d, C_9), 64.7 (d, C_{15}), 45.2 (t, C_{14}), 45.1 (t, C_8), 43.4 (t, C_6), 43.2 (d, C_{10}), 40.4 (t, C_{16}), 27.5 (q, C_{22}), 25.5 (q, C_{21}), 24.4 (q, C_{21}), 18.5 (q, 6C, C_1), 14.9 (t, C_5), 14.5 (q, C_{23}), 11.2 (d, 3C, C_2), 7.1 (q, 3C, C_{25} or C_{27}), 6.9 (q, 3C, C_{25} or C_{27}), 6.7 (t, 3C, C_{24} or C_{26}), 5.2 (t, 3C, C_{24} or C_{26}).

HRMS (ESI) *m/z*: calculated for $C_{43}H_{80}NaO_7Si_3$ $[M + Na]^+$: 815.5104, found: 815.5113.

II.4.2.2.31. Synthesis of 6-[(2S,7R,8S,10R)-2-hydroxy-7,10-dimethyl-4-oxo-8,10-bis[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.95)



MW (g/mol): 795.3635 **Molecular formula:** $C_{43}H_{82}O_7Si_3$

A solution of enone **II.94** (1.1 g, 1.38 mmol) in degassed toluene (85 mL) was added to a solution of Stryker's reagent (2.1 g, 0.71 mmol) in toluene (35 mL) at rt. The resulting reaction mixture was stirred for 30 min at the same temperature until complete conversion of the starting material (reaction monitored by TLC). The solution was the exposed to air and diluted with hexane (50 mL). Stirring was then continued for an additional 20 min, time after which the solvent was removed under reduced pressure. The crude residue was finally purified by flash column chromatography (EtOAc/PE, 20:80) to afford **II.95** (893 mg, 83%) as colourless oil.

R_f (EtOAc/PE, 1:3): 0.43.

$[\alpha]_D^{20}$ –1.32 (*c* 0.6, $CHCl_3$, 86% ee).

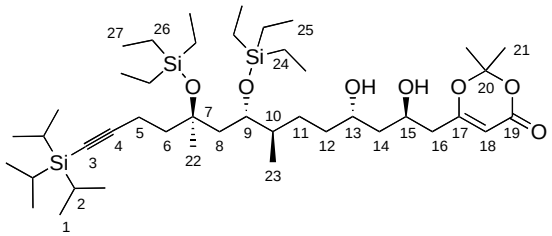
IR (neat): 3436, 2955, 2875, 2171, 1715, 1636, 1461, 1376, 1048, 1011, 723 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (s, 1H, H_{18}), 4.33 ($sept_{app}$, J = 4.1 Hz, 1H, H_{15}), 3.77 (m, 1H, H_9), 3.24 (br s, 1H, OH), 2.67-2.57 (m, 2H, H_{12}, H_{14}), 2.50-2.37 (m, 3H, H_{12}, $H_{14,}$ H_{16}), 2.35-2.27 (m, 3H, H_5, H_{16}), 1.85-1.65 (m, 2H, H_6), 1.69 (s, 3H, H_{21}), 1.68 (s, 3H, H_{21}), 1.62-1.48 (m, 2H, H_8), 1.44-1.40 (m, 2H, H_{10}, H_{11}), 1.32 (m, 1H, H_{11}), 1.22 (s, 3H, H_{22}), 1.05-1.00 (m, 21H, H_1, H_2), 0.94 (t, J = 7.9 Hz, 9H, H_{25} or H_{27}), 0.93 (t, J = 7.9 Hz, 9H, H_{25} or H_{27}), 0.84 (d, J = 6.6 Hz, 3H, H_{23}), 0.62-0.53 (m, 12H, H_{24}, H_{26}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 210.9 (s, C_{13}), 168.2 (s, C_{17}), 160.9 (s, C_{19}), 109.7 (s, C_3), 106.6 (s, C_{20}), 95.4 (d, C_{18}), 79.4 (s, C_4), 74.7 (s, C_7), 72.6 (d, C_9), 64.6 (d, C_{15}), 48.2 (t, C_{14}), 43.2 (t, C_8), 43.1 (t, C_6), 41.7 (t, C_{12}), 40.4 (t, C_{16}), 39.4 (d, C_{10}), 27.7 (q, C_{22}), 26.4 (t, C_{11}), 25.5 (q, C_{21}), 24.5 (q, C_{21}), 18.8 (q, 6C, C_1), 14.8 (t, C_5), 13.4 (q, C_{23}), 11.2 (d, 3C, C_2), 7.1 (q, 3C, C_{25} or C_{27}), 7.0 (q, 3C, C_{25} or C_{27}), 6.8 (t, 3C, C_{24} or C_{26}), 5.4 (t, 3C, C_{24} or C_{26}).

HRMS (ESI) *m/z*: calculated for $C_{43}H_{82}NaO_7Si_3$ $[M + Na]^+$: 817.5261, found: 817.5261.

*II.4.2.2.32. Synthesis of 6-[(2**R**,4**S**,7**R**,8**S**,10**R**)-2,4-dihydroxy-7,10-dimethyl-8,10-bis[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.96)*



MW (g/mol): 797.3794 **Molecular formula:** $C_{43}H_{84}O_7Si_3$

To a solution of ketone **II.95** (650 mg, 0.81 mmol) in a 1:1 MeCN/AcOH mixture (12 mL) at –30 °C was added tetramethylammonium triacetoxyborohydride (2.1 g, 8.1 mmol) in one portion. After stirring at the same temperature for 20 h, the reaction mixture was poured in a saturated aqueous solution of $NaHCO_3$ (10 mL), stirred for 30 min, and extracted with EtOAc (3 x 15 mL). The combined organic fractions were washed twice with a saturated aqueous solution of $NaHCO_3$ (2 x 10 mL) and once with brine (10 mL), dried over anhydrous Na_2SO_4 and filtered. The solvents were then

removed under reduced pressure and the resulting crude residue was purified by flash column chromatography (PE/EtOAc, 30:70) to afford the desired diol **II.96** (550 mg, 85%) as a colourless oil.

Rf (EtOAc/PE, 1:3): 0.25.

$[\alpha]_D^{20}$ –3.78 (*c* 3.0, $CHCl_3$, 86% ee).

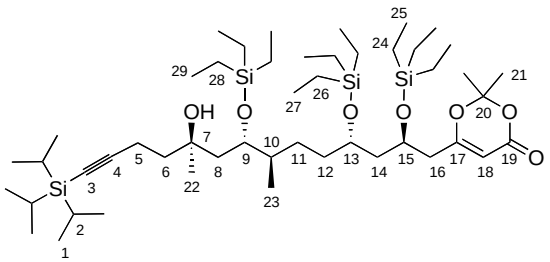
IR (neat): 3434, 2955, 2939, 2875, 2171, 1714, 1634, 1377, 1046, 1014, 724 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (s, 1H, H_{18}), 4.24 (m, 1H, H_{15}), 3.91 (m, 1H, H_{13}), 3.77 (m, 1H, H_9), 3.00 (br s, 1H, H_{OH}), 2.44 (dd, *J* = 14.6, 8.6 Hz, 1H, H_{16}), 2.39-2.25 (m, 3H, H_5, H_{16}), 1.85-1.67 (m, 2H, H_{14}), 1.69 (br s, 6H, H_{21}), 1.65-1.57 (m, 3H, H_6, H_{12}), 1.52 (m, 1H, H_6), 1.43 (d, J = 5.3 Hz, 2H, H_8), 1.39 (m, 1H, H_{10}), 1.28-1.17 (m, 2H, H_{11}), 1.21 (s, 3H, H_{22}), 1.08-1.00 (m, 21H, H_1, H_2), 0.94 (t, *J* = 8.0 Hz, 9H, H_{25} or H_{27}), 0.93 (t, *J* = 8.0 Hz, 9H, H_{25} or H_{27}), 0.86 (d, *J* = 6.8 Hz, 3H, H_{23}), 0.63-0.52 (m, 12H, H_{24}, H_{26}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1 (s, C_{17}), 161.2 (s, C_{19}), 109.9 (s, C_3), 106.6 (s, C_{20}), 95.0 (d, C_{18}), 79.3 (s, C_4), 74.8 (s, C_7), 72.7 (d, C_9), 69.5 (d, C_{13}), 66.2 (d, C_{15}), 43.1 (t, C_8), 43.0 (t, C_{16}), 42.4 (t, C_6), 41.7 (t, C_{14}), 40.2 (d, C_{10}), 35.8 (t, C_{12}), 29.7 (t, C_{11}), 27.7 (q, C_{22}), 25.2 (q, C_{21}), 24.8 (q, C_{21}), 18.6 (q, 6C, C_1), 14.8 (t, C_5), 13.6 (q, C_{23}), 11.3 (d, 3C, C_2), 7.2 (q, 3C, C_{25} or C_{27}), 7.1 (q, 3C, C_{25} or C_{27}), 6.8 (t, 3C, C_{24} or C_{26}), 5.4 (t, 3C, C_{24} or C_{26}).

HRMS (ESI) *m/z*: calculated for $C_{43}H_{84}NaO_7Si_3$ $[M + Na]^+$: 819.5417, found: 819.5418.

II.4.2.2.33. Synthesis of 6-[(2R,4S,7R,8S,10R)-10-hydroxy-7,10-dimethyl-2,4,8-tris[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.97)



MW (g/mol): 911.6402 **Molecular formula:** $C_{49}H_{98}O_7Si_4$

To a solution of **II.96** (400 mg, 1.0 mmol) in MeOH (3 mL) at rt was added $FeCl_3 \cdot 6H_2O$ (8 mg, 0.03 mmol) and the resulting reaction mixture was stirred overnight at the same temperature. The solvent was then removed under reduced pressure and the crude residue was subsequently dissolved with EtOAc and filtered through a pad of Celite©. The solvent was removed once again and the crude residue was used in the next step without further purification.

The latter was thus diluted in CH_2Cl_2 (20 mL) and cooled to 0 °C. Imidazole (408 mg, 6.0 mmol) was then added followed by TESCl (570 µL, 3.4 mmol) and the resulting reaction mixture was stirred for 30 min at the same temperature. A saturated aqueous solution of $NaHCO_3$ was then added, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL) and the combined organic layers were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash chromatography (Et_2O/PE, 10:90) to afford **II.97** (268 mg, 60%) as colourless oil.

$\boldsymbol{R_f}$ (Et_2O/PE, 3:9): 0.47.

$\boldsymbol{[\alpha]_D^{20}}$ –8.5 (*c* 0.65, $CHCl_3$).

IR (neat): 3505, 2955, 2915, 2877, 2171, 1736, 1637, 1461, 1376, 1102, 1049, 1013, 741 cm^{-1}.

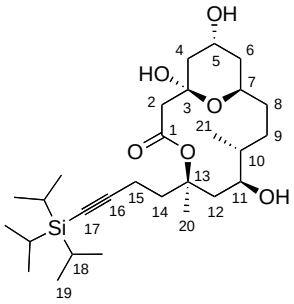
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.28 (s, 1H, H_{18}), 4.12-4.03 (m, 2H, H_9, H_{15}), 3.99 (s, 1H, OH), 3.73 (m, 1H, H_{13}), 2.46-2.38 (m, 3H, H_5, H_{16}), 2.27 (m, 1H, H_{16}), 1.93 (m, 1H, H_{14}), 1.70 (m, 1H, H_{14}), 1.68 (s, 3H, H_{21}), 1.67 (s, 3H, H_{21}), 1.63-1.58 (m, 4H, H_6, H_8), 1.52 (m, 1H, H_{12}), 1.40 (m, 1H, H_{12}), 1.33-1.14 (m, 3H, H_{10}, H_{11}), 1.12 (s, 3H, H_{22}), 1.07-1.03 (m, 21H, H_1, H_2), 0.99-0.93 (m, 27H, H_{25}, H_{27}, H_{29}), 0.90 (d, J = 6.6 Hz, 3H, H_{23}), 0.71-0.54 (m, 18H, H_{24}, H_{26}, H_{28}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9 (s, C_{17}), 161.0 (s, C_{19}), 109.4 (s, C_3), 106.4 (s, C_{20}), 95.3 (d, C_{18}), 79.7 (s, C_4), 73.8 (d, C_9), 71.4 (s, C_7), 70.1 (d, C_{13}), 67.5 (d, C_{15}), 45.4 (t, C_8), 42.9 (t, C_{16}), 40.1 (d, C_{10}), 39.7 (t, C_6), 39.0 (t, C_{14}), 36.5 (t, C_{12}), 29.2 (t, C_{11}), 27.6 (q, C_{22}), 25.6 (q, C_{21}), 24.6 (q, C_{21}), 18.6 (q, 6C, C_1), 14.9 (t, C_5), 12.9 (q, C_{23}), 11.2 (d, 3C, C_2), 7.0 (q, 3C, C_{25} or C_{27} or C_{29}), 6.9 (q, 3C, C_{25} or C_{27} or C_{29}), 6.8

(q, 3C, C_{25} or C_{27} or C_{29}), 5.4 (t, 3C, C_{24} or C_{26} or C_{28}), 5.3 (t, 3C, C_{24} or C_{26} or C_{28}), 5.2 (t, 3C, C_{24} or C_{26} or C_{28}).

HRMS (ESI) *m/z*: calculated for $C_{49}H_{98}NaO_7Si_4$ $[M + Na]^+$: 933.6282, found: 933.6263.

II.4.2.2.34. Synthesis of (1R,5R,7S,8R,11R,13R)-1,7,13-trihydroxy-5,8-dimethyl-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo[9.3.1] pentadecan-3-one (II.98)



MW (g/mol): 510.7785 **Molecular formula:** $C_{28}H_{50}O_6Si$

To a round-bottom flask containing a pre-heated (110 °C) solution of dry toluene (500 mL) was added **II.97** (50 mg, 0.15 mmol in 2 mL of toluene) drop-wise and the reaction mixture was stirred at reflux until complete consumption of the starting material (reaction monitored by TLC). The solvent was then removed under reduced pressure and the crude residue was used in the next step without further purification.

The crude macrolactone was thus dissolved in THF (2 mL) and placed in a plastic culture tube. A solution of HF·Py (500 mL) in a 2:1 THF/pyridine (3 mL) mixture was then added dropwise at 0 °C and the resulting reaction mixture was stirred for 30 min at the same temperature. A saturated aqueous solution of $NaHCO_3$ was then added with care in order to quench the reaction and the mixture was extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (EtOAc/PE, 20:80) to afford **II.98** (18 mg, 62%) as colourless oil.

$\boldsymbol{R_f}$ (EtOAc/PE, 3:2): 0.45.

$\boldsymbol{[\alpha]_D^{20}}$ –16.84 (*c* 1.1, $CHCl_3$).

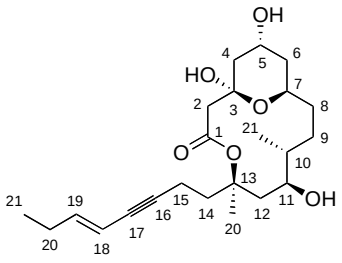
IR (neat): 3401, 2941, 2891, 2865, 2173, 1703, 1462, 1325, 1230, 1195, 1088, 1010, 883, 677 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (br s, 1H, H_{OH}), 4.18-4.07 (m, 2H, H_5, H_{11}), 3.64 (t, J = 11.4 Hz, 1H, H_7), 2.70 (m, 1H, H_4), 2.56 (d, J = 12.6 Hz, 1H, H_2), 2.55 (m, 1H, H_{12}), 2.50 (d, J = 12.6 Hz, 1H, H_2), 2.32 (m, 1H, H_{15}), 2.21-2.13 (m, 2H, H_6, H_{15}), 1.94-1.86 (m, 2H, H_4, H_{14}), 1.76 (m, 1H, H_8), 1.43 (s, 3H, H_{20}), 1.30-1.11 (m, 7H, H_6, H_8, H_9, H_{10}, H_{12}, H_{14}), 1.06-1.03 (m, 21H, H_{18}, H_{19}), 0.85 (d, J = 7.0 Hz, 3H, H_{21}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3 (s, C_1), 108.3 (s, C_{17}), 96.3 (s, C_3), 86.0 (s, C_{13}), 80.4 (s, C_{16}), 68.9 (d, C_7), 64.9 (d, C_{11}), 47.0 (t, C_2), 43.4 (t, C_6), 40.7 (t, C_{14}), 39.1 (t, C_4), 33.6 (t, C_8), 28.5 (t, C_9), 24.9 (q, C_{20}), 18.6 (q, 6C, C_{19}), 14.6 (t, C_{15}), 11.3 (d, 3C, C_{18}). The C_5 (67.5, d), C_{10} (35.3, d), C_{12} (37.3, t) and C_{21} (15.1, q) chemical shifts have been determined by HMQC correlation.

HRMS (ESI) *m/z*: calculated for $C_{28}H_{50}NaO_6Si$ $[M + Na]^+$: 533.3269, found: 533.3268.

II.4.2.2.35. Synthesis of (1R,5R,7S,8R,11R,13R)-1,7,13-trihydroxy-5,8-dimethyl-5-[(5E)-oct-5-en-3-yn-1-yl]-4,15-dioxabicyclo[9.3.1]pentadecan-3-one (II.82)



MW (g/mol): 408.5283 **Molecular formula:** $C_{23}H_{36}O_6$

To a stirred solution of **II.98** (25 mg, 0.05 mmol) in MECN (1 mL) at rt was added AgF (31.3 mg, 0.25 mmol) and the resulting reaction mixture was stirred in the absence of light for 5 h. A 1M aqueous solution of HCl (0.2 mL) was then added to the reaction which was stirred for an additional 5 min and diluted with EtOAc (3 mL). The organic phase was then separated and the aqueous layer was extracted with EtOAc (3 mL). The combined organic layers were then washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired terminal alkyne, which was used in the next step without further purification.

To a solution of the crude terminal alkyne (40 mg, 0.08 mmol) and vinyl iodide **II.80** (55 mg, 0.3 mmol) in a degassed Et_3N/DMF mixture (2:1, 3 mL) were added CuI (1.0 mg, 5 µmol) and $Pd(PPh_3)_2Cl_2$ (1.7 mg, 2.5 µmol). The resulting reaction mixture was stirred at rt for 2 h and diluted with EtOAc (3 mL). The organic layer was then separated and the aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (EtOAc/PE, 25:75) to afford **II.82** (12 mg, 60%) as colourless oil.

$\boldsymbol{R_f}$ (EtOAc/PE, 3:2): 0.31.

$\boldsymbol{[\alpha]_D^{20}}$ –16.2 (*c* 0.5, $CHCl_3$).

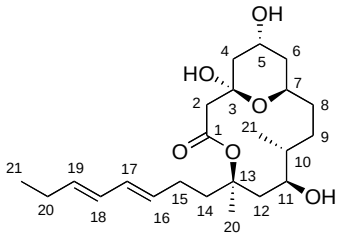
IR (neat): 3445, 2926, 2854, 1702, 1453, 1325, 1230, 1195, 1087, 1009, 958 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.08 (dt, *J* = 15.8, 6.5 Hz, 1H, H_{19}), 5.42 (d, *J* = 15.8 Hz, 1H, H_{18}), 4.68 (d, *J* = 2.2 Hz, 1H, H_{OH}), 4.18-4.08 (m, 2H, H_5, H_{11}), 3.64 (t, *J* = 11.3 Hz, 1H, H_7), 2.70 (m, 1H, H_4), 2.55 (d, *J* = 12.5 Hz, 1H, H_2), 2.53 (m, 1H, H_{12}), 2.49 (d, *J* = 12.5 Hz, 1H, H_2), 2.35 (m, 1H, H_{15}), 2.24 (m, 1H, H_{15}), 2.17 (m, 1H, H_6), 2.12-2.03 (m, 2H, H_{20}), 1.93-1.86 (m, 2H, H_4, H_{14}), 1.77 (m, 1H, H_8), 1.41 (s, 3H, H_{22}), 1.31-1.24 (m, 4H, H_6, H_{10}, H_{12}, H_{14}), 1.21 (m, 1H, H_8), 1.17-1.08 (m, 2H, H_9), 0.98 (t, *J* = 7.4 Hz, 3H, H_{21}), 0.85 (d, *J* = 7.1 Hz, 3H, H_{23}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3 (s, C_1), 145.0 (d, C_{19}), 108.8 (d, C_{18}), 96.3 (s, C_3), 87.7 (s, C_{17}), 85.8 (s, C_{13}), 79.4 (s, C_{16}), 68.9 (d, C_7), 64.9 (d, C_{11}), 47.0 (t, C_2), 43.4 (t, C_4), 40.7 (t, C_6), 38.6 (t, C_{14}), 37.4 (t, C_{12}), 33.5 (t, C_8), 29.7 (t, C_9), 26.0 (t, C_{20}), 24.6 (q, C_{22}), 14.1 (t, C_{15}), 13.1 (q, C_{21}). The C_5 (67.0, d) and C_{23} (14.7, q) chemical shifts have been determined by HMQC correlation. C_{10} could not be observed.

HRMS (ESI) *m/z*: calculated for $C_{23}H_{36}NaO_6$ $[M + Na]^+$: 431.2404, found: 431.2408.

*II.4.2.2.36. Synthesis of (1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,7,13-trihydroxy-5,8-dimethyl-5-[(3*E*,5*E*)-octa-3,5-dien-1-yl]-4,15-dioxabicyclo[9.3.1]pentadecan-3-one – lyngbouilloside aglycon (II.92)*



MW (g/mol): 410.5442 **Molecular formula:** $C_{23}H_{38}O_6$

To a solution of **II.82** (8 mg, 19 µmol) in CH_2Cl_2 (250 mL) at 0 °C was added $(EtO)_3SiH$ (8 µL, 38 µmol) followed by $Cp^*Ru(MeCN)_3PF_6$ (0.1 mg, 0.19 µmol). The flask was immediately allowed to warm to rt and the reaction mixture was stirred for 1 h. The solvent was then removed under reduced pressure and a THF/MeOH/H_2O mixture (1:2:2, 800 mL) was added followed by AgF (12 mg, 95 µmol). Stirring was continued in the absence of light for an additional hour, time after which the reaction mixture was filtered over Celite©. The solvent was then removed under reduced pressure and the crude residue was purified by flash column chromatography (EtOAc/PE, 30:70) to afford **II.92** (2.5 mg, 47%) as colourless oil.

R_f (EtOAc/PE, 1:1): 0.44.

$[\alpha]_D^{20}$ –31.2 (*c* 0.1, $CHCl_3$).

IR (neat): 3356, 2957, 2928, 2873, 2360, 2342, 1703, 1325, 1230, 1195, 1166, 1084, 1011 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.10-5.92 (m, 2H, H_{17}, H_{18}), 5.66-5.49 (m, 2H, H_{16}, H_{19}), 5.37 (m, 1H, OH), 4.71 (br s, 1H, OH), 4.19-4.05 (m, 2H, H_5, H_{11}), 3.64 (t, J = 11.6 Hz, 1H, H_7), 2.55 (d, J = 12.3 Hz, 1H, H_2), 2.52 (m, 1H, H_{12}), 2.49 (d, J = 12.3 Hz, 1H, H_2), 2.18 (m, 1H, H_4), 2.12-1.93 (m, 5H, H_{14}, H_{15}, H_{20}), 1.89 (m, 1H, H_6), 1.77 (m, 1H, H_8), 1.49 (m, 1H, H_{10}), 1.40 (s, 3H, H_{22}), 1.39 (m, 1H, H_{14}), 1.33-1.20 (m, 5H, H_4, H_8, H_9, H_{12}), 1.10 (m, 1H, H_6), 0.98 (t, J = 7.4 Hz, 3H, H_{21}), 0.85 (d, J = 6.8 Hz, 3H, H_{23}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3 (s, C_1), 134.4 (d, C_{19}), 131.1 (d, C_{16}), 130.7 (d, C_{17}), 129.1 (d, C_{18}), 96.3 (s, C_3), 86.4 (s, C_{13}), 68.9 (d, C_7), 67.4 (d, C_5), 64.9 (t, C_{11}),

47.1 (t, C_2), 43.6 (t, C_{12}), 43.4 (t, C_4), 40.7 (t, C_6), 39.4 (t, C_{14}), 37.6 (d, C_{10}), 33.5 (t, C_8), 26.7 (t, C_{20}), 25.6 (t, C_{15}), 14.6 (q, C_{23}), 13.6 (q, C_{21}), The C_{22} (24.9, q) chemical shifts has been determined by HMQC correlation. C_9 could not be observed.

HRMS (ESI) *m/z*: calculated for $C_{23}H_{38}NaO_6$ $[M + Na]^+$: 433.2561, found: 433.2561.

CHAPTER III

Total synthesis of (–)-bitungolide F

III.1. INTRODUCTION

In the course of our endeavour toward the synthesis of biologically active natural products, we became particularly interested in a new family of polyketides bearing an unusual γ-ethyl substituted α,β-unsaturated δ-lactone moiety which is only present in a few natural products, namely the leustroducsins, the phoslactomycins, and pironetin.

III.1.1. ISOLATION, STRUCTURE AND BIOLOGICAL PROPERTIES OF BITUNGOLIDES A-F

III.1.1.1. Isolation

Bitungolides A-F (Figure III.1) were isolated in 2002 by Tanaka and co-workers[214] from *Theonella* cf. *swinhoei*, a specimen of sponge collected in North Sulawesi in Indonesia, which produced a wide range of interesting biologically active compounds with fascinating structures.[215]

[214] Sirirath, S.; Tanaka, J.; Ohtani, I. I.; Ichiba, T.; Rachmat, R.; Ueda, K.; Usui, T.; Osada, H.; Higa, T. *J. Nat. Prod.* **2002**, *65*, 1820-1823.
[215] (a) Carmenly, S.; Kashman, Y. *Tetrahedron Lett.* **1985**, *26*, 511-514. (b) Kobayashi, M.; Tanaka, J.; Katori, T.; Matsuura, M.; Yamashira, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1990**, *38*, 2409-2418.

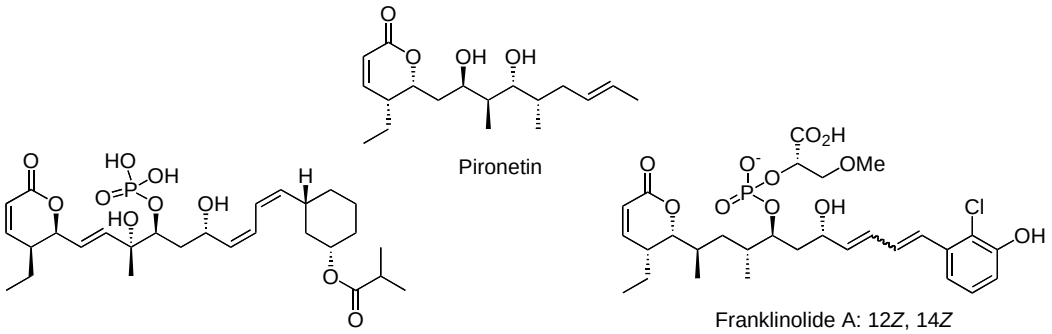
Bitungolide A: 12*Z*, 14*Z*
Bitungolide B: 12*E*, 14*E*
Bitungolide C: 12*Z*, 14*E*
Bitungolide D: 12*E*, 14*Z*

Bitungolide E: R = Me
Bitungolide F: R = H

Figure III.1. Structures of bitungolides A–F

III.1.1.2. Biological properties of the bitungolides A–F

The bitungolides are the first γ-ethyl-substituted α,β-unsaturated δ-lactone-containing polyketides isolated from a marine sponge. They share the same structural elements as the microbial metabolite pironetin, which is a known microtubule-targeting drug,[216] the phosphate inhibitor phoslactomycin A, which was isolated from a soil bacteria species,[217] and the franklinolides A–C, a new family of cytotoxic metabolites recently isolated from an Australian sponge (Figure III.2).[218]



Pironetin

Phoslactomycin A

Franklinolide A: 12*Z*, 14*Z*
Franklinolide B: 12*E*, 14*E*
Franklinolide C: 12*E*, 14*Z*

Figure III.2. Structure of pironetin, phoslactomycin A and franklinolides A–C

In biological assays with phosphatases, bitungolides showed weak activity against dual-specificity phosphatase (VHR),[219] while no activity was observed against

[216] Kondoh, M.; Usui, T.; Kobayashi, S.; Tsuchiya, K.; Nishikawa, K.; Nishikiori, T.; Mayumi, T.; Osada, H. *Cancer Lett.* **1998**, *126*, 29-32.
[217] Fushimi, S.; Nishikawa, S.; Shimazu, A.; Seto, H. *J. Antibiotics* **1989**, *42*, 1019-1025.
[218] Zhang, H.; Conte, M. M.; Capon, R. J. *Angew. Chem. Int. Ed.* **2010**, *49*, 9904-9906.
[219] Dual-specificity phosphatases are an emerging subclass of the protein tyrosine phosphatase (PTP) gene superfamily, which appears to be selective for dephosphorylating MAP kinases. Hence, natural products that inhibit these enzymes might serve as biologically validated starting points for the development of new and selective phosphatase inhibitors, see: (a) Camps, M.; Nichols, A.; Arkinstall, S.

serine/threonine phosphatase (PP1 and PP2A) or tyrosine phosphatase (PTP-S2). In addition, the bitungolides were shown to exhibit a cytotoxic effect against 3Y1 rat normal fibroblast cells (10 μg/mL). On the other hand, these compounds did not act on cytoskeletons (microtubules and actin) and no morphological change on nuclei was observed.[214] Considering the various structural elements present in the bitungolides such as the 5,6-dihydropyran-2-one unit, the *anti*-1,3-diol and two conjugated double bonds attached to a substituted arene, these natural products and their analogues could offer a wide range of applications, such as plant growth inhibitors, pheromones, antifeedal, antifungal, antibacterial, and antitumor agents.[220]

III.1.1.3. Bitungolides structural assignment

Among all the bitungolides, the structure of bitungolide A was the first one to be elucidated using spectroscopic data and X-ray diffraction analysis. Hence, while mass spectroscopy revealed pseudomolecular ions at *m/z* 447 and 449 (ratio 3:1) indicating the presence of a chlorine atom, IR and NMR spectra suggested the presence of an unsaturated lactone, two secondary hydroxyl groups, two secondary methyl groups and an ethyl together with 12 sp^2 carbons, which were assigned to a 1,2,3-trisubstitued benzene, a (*Z*,*Z*)-diene ($J_{12,13}$ = 11.5 Hz, $J_{14,15}$ = 11.3 Hz) and a double bond conjugated to the lactone. Finally, the gross structure of bitungolide A was secured by a single crystal X-ray diffraction study, which also allowed to confirm the absolute stereochemistry of the natural product.[221] Bitungolide B, on the other hand, was shown to have the same molecular formula as bitungolide A along with similar NMR signals except in the olefinic region where larger coupling constants were observed ($J_{12,13}$ = 15.3 Hz, $J_{14,15}$ = 15.5 Hz) indicating a diene of (*E*,*E*)-configuration. Similarly, bitungolides C and D differ from the two previous congeners only by the configuration of their diene moiety. Hence, bitungolide C bears a (*Z*,*E*)-diene ($J_{12,13}$ = 11.3 Hz, $J_{14,15}$ = 15.3 Hz) whereas bitungolide D displays a (*E*,*Z*)-diene ($J_{12,13}$ = 15.3 Hz, $J_{14,15}$ = 11.3 Hz). The structure of bitungolides E and F were also elucidated using HRMS, IR and exhaustive NMR analysis. Thus, both natural products were shown to lack the chlorine atom as well as one of the oxygens found in bitungolides A-D. In

FAEBS, **2000**, *14*, 6-16. (b) Brohm, D.; Metzger, S.; Bhargava, A.; Müller, O.; Lieb, F.; Waldman, H. *Angew. Chem. Int. Ed.* **2002**, *41*, 307-311.

[220] Davies-Coleman, M. T.; Rivett, D. E. A. *Fortschr. Chem. Org. Naturst.* **1989**, *55*, 1-35.

[221] Crystallographic data for the structure have been deposited at the Cambridge Crystallographic Data Center (Deposition number CCDC 191268).

addition, the NMR spectra showed the presence of a mono-substituted benzene instead of the 2,3-disubstituted phenol, confirming that the main structural differences between bitungolides A–D and bitungolides E–F were to be found on the aromatic ring. Finally, bitungolide F was shown to have two methyls and four methylenes instead of three methyls and three methylenes, with the demethylation occurring on carbon C8 according to NMR correlation studies (Figure III.1).

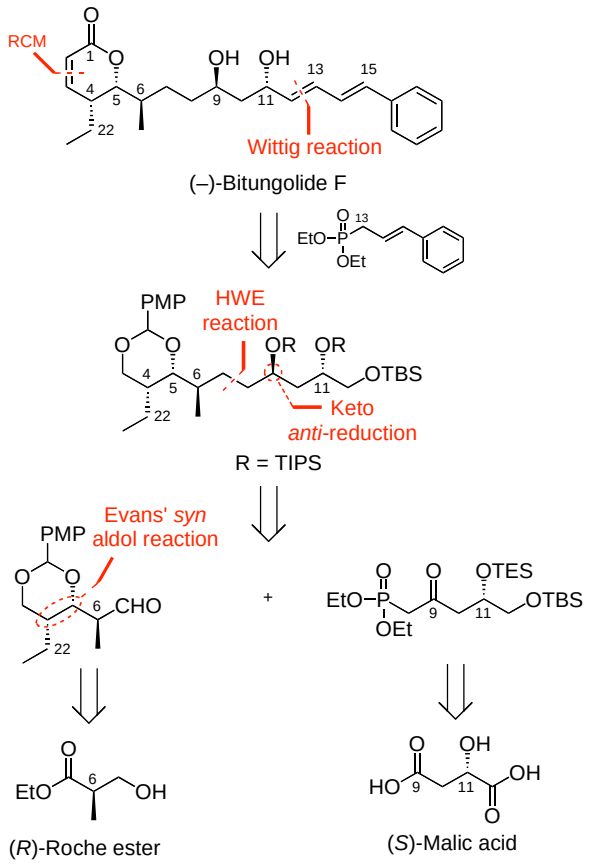
III.1.2. REPORTED TOTAL SYNTHESES OF (–)-BITUNGOLIDE F

Two substantial works directed toward the synthesis of bitungolide F have been documented from the research groups of Ghosh[222] and She.[223] The former was the first to report the total synthesis of the natural product and therefore to assign its absolute stereochemistry as (4*R*,5*R*,6*R*,9*R*,11*S*).

III.1.2.1. Total synthesis of (–)-bitungolide F by Ghosh *et al.*

Ghosh *et al*. reported the first total synthesis of bitungolide F in 22 steps and 4.7% overall yield starting from (*S*)-Roche ester and (*S*)-malic acid.[222] The key steps included a Wittig olefination to introduce the (*E*,*E*)-conjugated diene, a Horner-Wadsworth-Emmons (HWE) olefination[224] to construct the C7-C8 bond and a ring-closing metathesis (RCM)[225] to build the six-membered ring α,β-unsaturated δ-lactone. The stereochemistry at C9 was controlled by means of a hydroxy-directed reduction using Evans' protocol [$Me_4NBH(OAc)_3$, AcOH/MeOH, –20 ºC],[226] while a modified Evans' *syn*-aldol reaction using Crimmins' protocol[227] [*N*-propionyl oxazolidinone, $TiCl_4$ and (–)-sparteine] enabled the control of the C4 and C5 stereogenic centers (Scheme III.1).

[222] Ghosh, S.; Kumar, S. U.; Shashidhar, J. *J. Org. Chem.* **2008**, *73*, 1582-1585.
[223] Su, Y.; Xu, Y.; Han, J.; Zheng, J.; Qi, J.; Jiang, T.; Pan, X.; She, X. *J. Org. Chem.* **2009**, *74*, 2743-2749.
[224] Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183-2186.
[225] (a) Fürstner, A. *Angew. Chem. Int. Ed.* **2000**, *39*, 3012-3043. (b) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18-19. (c) Love, J. A. *Handbook of Metathesis*; Grubbs, R. H., Ed.; Wiley-VCH: Weinheim, Germany, **2003**; 296-322. (d) Grubbs, R. H. *Tetrahedron* **2004**, *60*, 7117-7140. (e) Deiters, A.; Martin, S. F. *Chem. Rev.* **2004**, *104*, 2199-2238. (f) Gradillas, A.; Pérez-Castells, J. *Angew. Chem. Int. Ed.* **2006**, *45*, 6086-6101.
[226] Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560-3578.
[227] Crimmins, M. T.; King, B. W.; Tabet, E. A.; Chaudhary, K. *J. Org. Chem.* **2001**, *66*, 894-902.



Scheme III.1. Ghosh's retrosynthetic analysis of (–)-bitungolide F

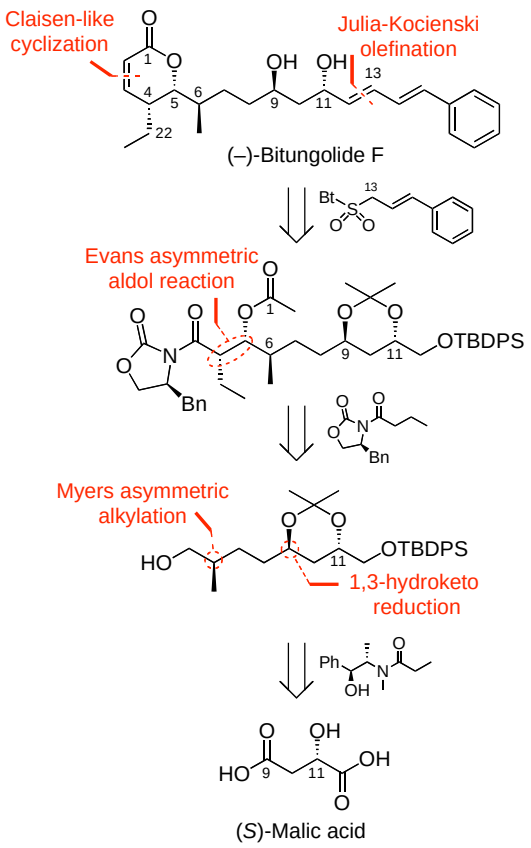
III.1.2.2. Total synthesis of (–)-bitungolide F by She *et al.*

More recently, She *et al.* completed the total synthesis of (–)-bitungolide F in 18 steps and 20.1% overall yield starting from commercially available (*S*)-malic acid.[223] The key steps included a Julia-Kocienski olefination to assemble the conjugated diene moiety and a Claisen-like cyclization[228] to construct the α,β-unsaturated δ-lactone. In addition, the ethyl side chain at C4 and the alcohol at C5 were installed in a *syn* fashion *via* an asymmetric aldol reaction[229] using Evans' chiral oxazolidinone, while a Myers

[228] (a) Brandänge, S.; Leijonmarck, H. *Tetrahedron Lett.* **1992**, *33*, 3025-3028. (b) Vanderwal, C. D.; Vosburg, D. A.; Weiler, S.; Sorensen, E. J. *Org. Lett.* **1999**, *1*, 645-648. (c) Hinterding, K.; Singhanat, S.; Oberer, L. *Tetrahedron Lett.* **2001**, *42*, 8463-8465. (d) Vanderwal, C. D.; Vosburg, D. A.; Weiler, S.; Sorensen, E. J. *J. Am. Chem. Soc.* **2003**, *125*, 5393-5407.

[229] (a) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127-2129. (b) Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 3099-3111. (c) Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem.* **1982**, *13*, 1-115. (d) Evans, D. A. *Aldrichimica Acta* **1982**, *15*, 23-32.

asymmetric alkylation[230] and a hydroxy-directed ketone reduction[226] allowed to control the stereogenic centers C6 and C9, respectively (Scheme III.2).



Scheme III.2. She's retrosynthetic analysis of (–)-bitungolide F

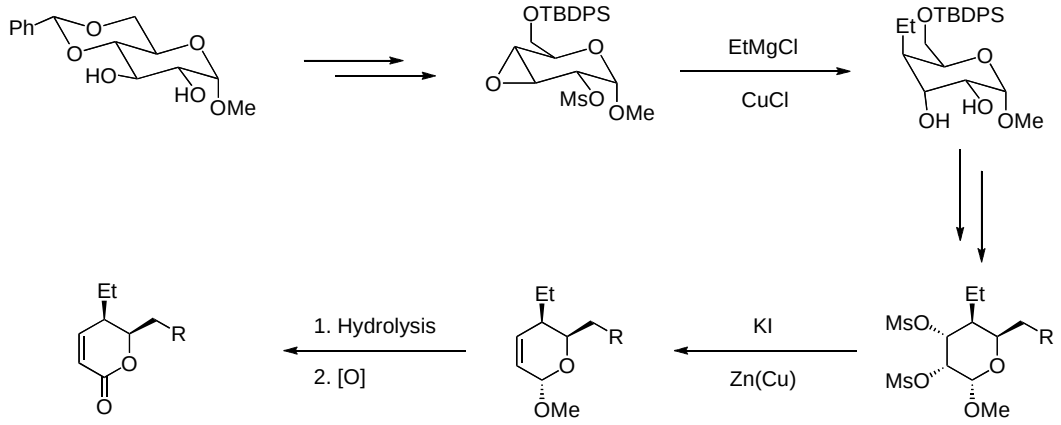
III.1.3. ASYMMETRIC METHODS TOWARDS ETHYL SUBSTITUTED α,β-UNSATURATED δ-LACTONES

The optically active 5-ethyl-5,6-dihydro-2*H*-pyran-2-one moiety is an important structural motif present in all the bitungolides as well as in a few other natural products with interesting biological properties, which renders its synthesis a challenge for synthetic organic chemists. Hence, prior to describing our synthesis of bitungolide F, we will give a brief overview of all the existing methods that have been developed to access this scaffold.

[230] (a) Myers, A. G.; Yang, B.; Chen, H.; Mckinstry, L.; Kopecky, D. J.; Gleason, J. L. *J. Am. Chem. Soc.* **1997**, *119*, 6496-6511. (b) Myers, A. G.; Yang, B. H.; Chen, H.; Kopecky, D. J. *Synlett* **1997**, 457-459.

III.1.3.1. Epoxide ring-opening strategy

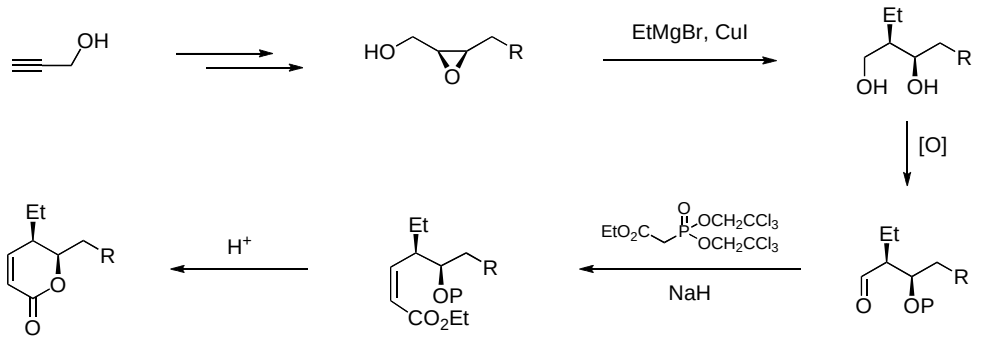
Kawada *et al.*[231] reported the first total synthesis of the natural product (–)-PA-48153C also referred to as pironetin, an immunosuppressive agent which contains the ethyl substituted α,β-unsaturated δ-lactone motif. Their strategy is based on a key copper(I) chloride-catalyzed ring-opening of a chiral epoxy mesylate with ethyl magnesium chloride to introduce the ethyl group onto the axial C5-position of a starting glycopyranoside derivative, followed by a double mesylate elimination,[232] a selective hydrolysis of the protected δ-lactol and a final oxidation to afford the corresponding δ-lactone (Scheme III.3).



Scheme III.3. Kawada's epoxide ring-opening strategy

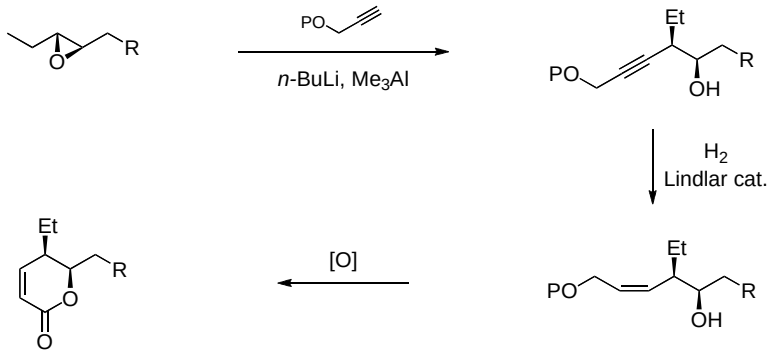
Later, Gurjar and co-workers[233] reported another route towards the total synthesis of pironetin which also involved a regioselective ring-opening alkylation of a chiral epoxide to introduce the ethyl side-chain. In contrast to the previous approach, the construction of the α,β-unsaturated δ-lactone ring involved a modified HWE to set the α,β-unsaturated ester followed by an acid-mediated lactonization to complete the synthesis (Scheme III.4).

[231] Yasui, K.; Tamura, Y.; Nakatani, T.; Kawada, K.; Ohtani, M. *J. Org. Chem.* **1995**, *60*, 7567-7574.
[232] Tipson, R. S.; Cohen, **A.** *Carbohydr. Res.* **1965**, *1*, 338.
[233] Gurjar, M.; Chakrabarti, A.; Rao, A. V. R. *Heterocycles* **1997**, *45*, 7-10.



Scheme III.4. Gurjar's epoxide ring-opening strategy

A similar strategy was also used by Kitahara *et al.* in their synthesis of pironetin[234] and, more recently, leustrodicsin B.[235] However, in contrast to Gurjar's approach, the epoxide ring-opening alkylation was used to introduce a protected propargylic alcohol moiety with the proper configuration. Lindlar reduction of the alkyne then furnished the corresponding (*Z*)-alkene, while a final oxidation step provided the α,β-unsaturated δ-lactone (Scheme III.5).



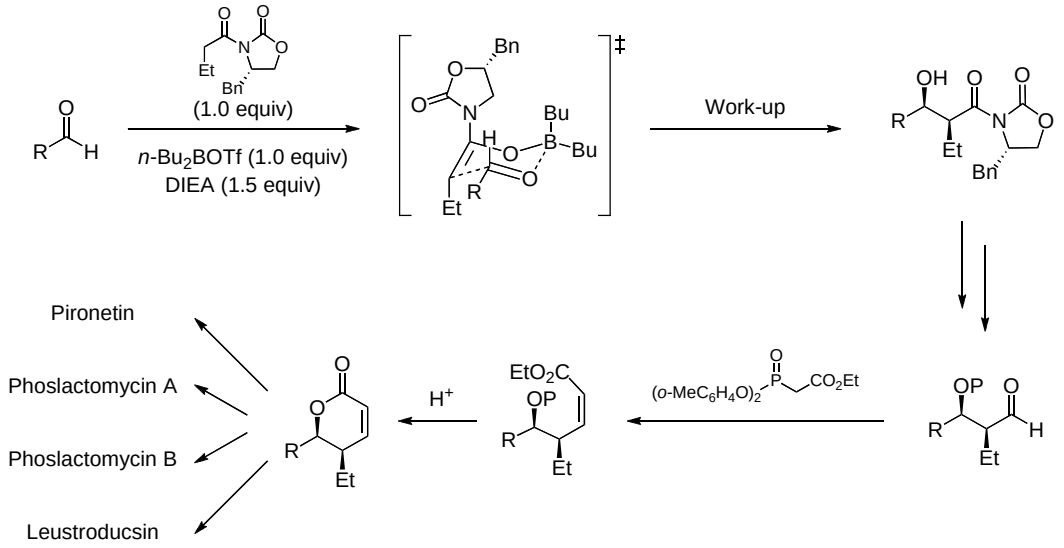
Scheme III.5. Kitahara's epoxide ring-opening strategy

III.1.3.2. Asymmetric aldol/olefination strategy

An other method used for the synthesis of the optically active 5-ethyl-5,6-dihydro-2*H*-pyran-2-one motif is based on the use of an asymmetric aldolization to install the ethyl side-chain, a HWE to introduce the (*Z*)-α,β-unsaturated carbonyl, and a lactonization to build the six-membered ring.

[234] Watanabe, H.; Watanabe, H.; Bando, M.; Kido, M.; Kitahara, T. *Tetrahedron* **1999**, *55*, 9755-9776.
[235] (a) Miyashita, K.; Tsunemi, T.; Hosokawa, T.; Ikejiri, M.; Imanishi, T. *Tetrahedron Lett.* **2007**, *48*, 3829-3833. (b) Miyashita, K.; Tsunemi, T.; Hosokawa, T.; Ikejiri, M.; Imanishi, T. *J. Org. Chem.* **2008**, *73*, 5360-5370.

In the example depicted in Scheme III.6, the aldol condensation takes place under Evans' protocol[236] using (*S*)-*N*-butanoyloxazolidinone in the presence of dibutylborontriflate (*n*-Bu_2BOTf, 1.0 equiv) to afford the *syn*-aldol product with a high level of diastereoselectivity, which is explained by a Zimmerman-Traxler transition state model. After a few trivial transformations, the aldehyde was then subjected to a modified HWE *cis*-olefination,[237] and the resulting conjugated ester was eventually hydrolyzed to afford the desired lactone. It is worth pointing out that this strategy was successfully used by various groups in the synthesis of pironetin,[238] phoslactomycin A,[239] phoslactomycin B[240] and leustroducsin B.[241]

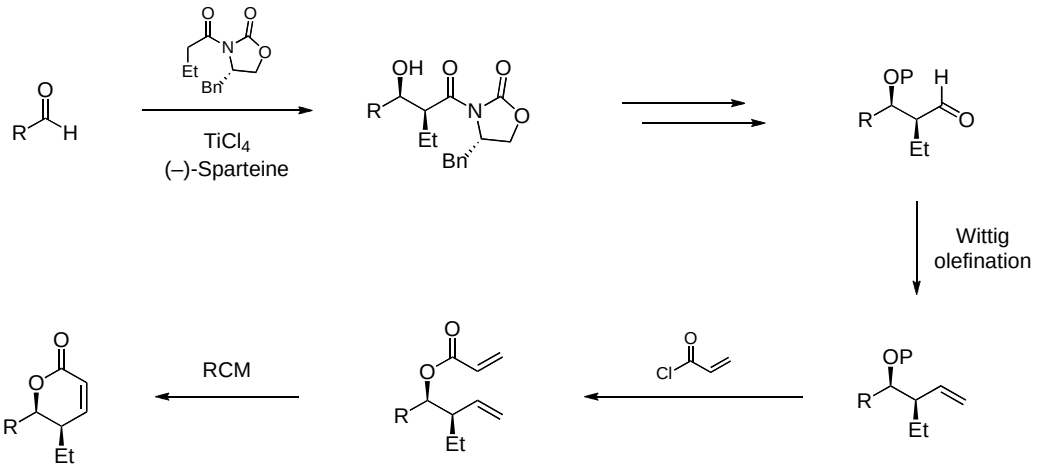


Scheme III.6. Asymmetric aldol/olefination strategy

A particularly interesting alternative to the previous strategy involves the use of a ring-closing metathesis to form the α,β-unsaturated δ-lactone. This approach, which was used by Ghosh *et al.* in their synthesis of bitungolide F[222] and, more recently, bitungolide E,[242] featured a modified Evans asymmetric aldol under Crimmins' protocol combined to a Wittig olefination to introduce the terminal alkene, and an acylation with

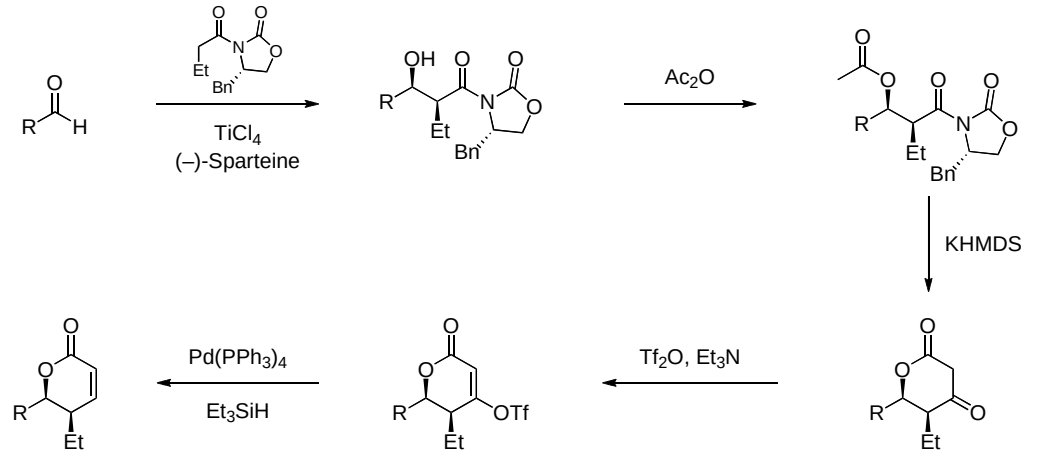
[236] Review: D. A. Evans *Aldrichim. Acta* **1982**, *15*, 23-32.
[237] (a) Reddy, M. A.; Gopal, R. J.; Rao, V. J. *Indian J. Chem.* **1996**, *35*B, 312. (b) Ando, K. *J. Org. Chem.* **1997**, *62*, 1934-1939.
[238] (a) Gurjar, M. K.; Henri Jr., J. T.; Bose, S. D.; Rao, A. V. R. *Tetrahedron Lett.* **1996**, *37*, 6615-6618. (b) Días, L. C.; Oliveira, L. G. Sousa, M. A. *Org. Lett.* **2003**, *5*, 265-268. (c) Días, L. C.; Oliveira, L. G. Sousa, M. A.; Ellensohn, R. M. *ARKIVOC* **2005**, *vi*, 62-87.
[239] Gebhardt, B.; König, C.; Schleth, C.; Dauber, M.; Koert, U. *Chem. Euro. J.* **2010**, *16*, 5934-5941.
[240] Wang, Y.-G.; Takeyama, R.; Kobayashi, Y. *Angew. Chem., Int. Ed.* **2006**, *45*, 3320-3323.
[241] Shimada, K.; Kaburagi, Y.; Fukuyama, T. *J. Am. Chem. Soc.* **2003**, *125*, 4048-4049.
[242] Shashidhar, J.; Reddy, K. M.; Ghosh, S. *Tetrahedron Lett.* **2011**, *52*, 3106-3109.

acryloyl chloride followed by a final RCM to complete the synthesis of the conjugated lactone (Scheme III.7).



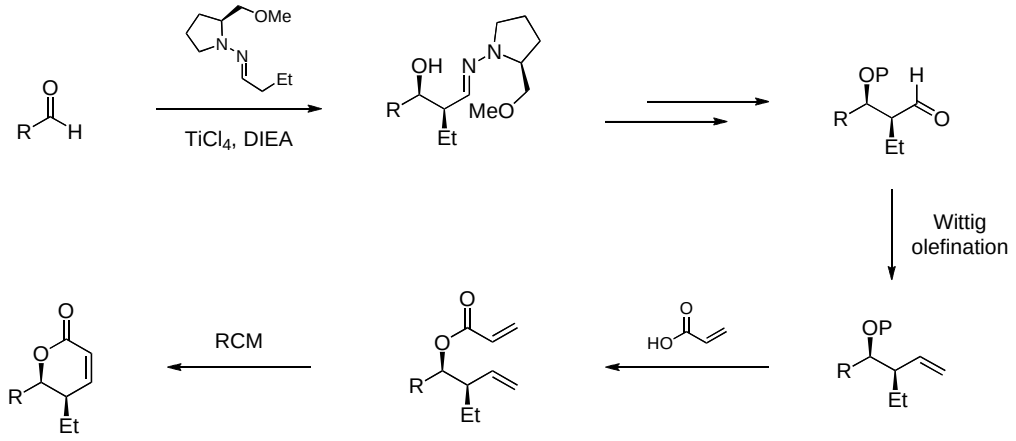
Scheme III.7. Asymmetric aldol/RCM strategy

In their synthesis of bitungolide F, She and co-workers[223] also exploited the modified asymmetric Evans aldolisation to set the ethyl side-chain, however, instead of using an olefination/lactonization or an acylation/RCM sequence to generate the α,β-unsaturated δ-lactone, they performed an uncommon potassium hexamethyldisilazide-mediated Claisen-like cyclization to afford a β-keto lactone which was subsequently converted first to the corresponding enol triflate then to the desired lactone in the presence of $Pd(PPh_3)_4$ and Et_3SiH (Scheme III.8).



Scheme III.8. Asymmetric aldol/Claisen-like cyclization strategy

Enders *et al.*[243] reported a modification of the aldol condensation in their synthesis of the optically active 5-ethyl-5,6-dihydro-2*H*-pyran-2-one motif of pironetin. Indeed, in order to control the C4 and C5 stereogenic centers of the lactone, they used their SAMP-/RAMP-hydrazone method. This synthetic tool, a *syn*-selective asymmetric aldol, involves a chelated titanium azaenolate derived from (*R*)- or (*S*)-1-amino-2-methoxymethylpyrrolidine hydrazone.[244] Hence, after cleavage of the chiral auxiliary, Wittig olefination of the resulting aldehyde, esterification with acrylic acid and final RCM, Enders *et al.* were able to isolate the desired lactone (Scheme III.9).



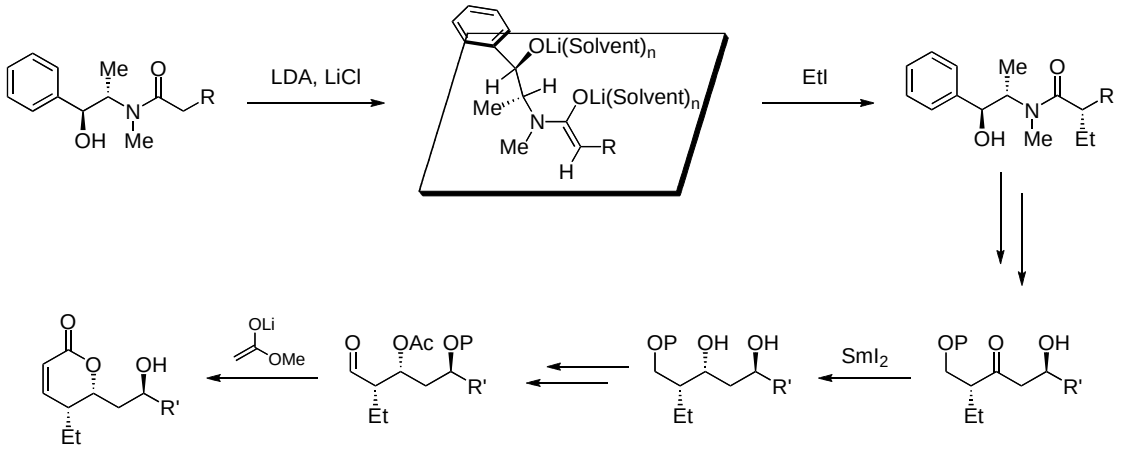
Scheme III.9. SAMP-/RAMP-hydrazone strategy

III.1.3.3. Pseudoephedrin-mediated alkylation/lactone annulation strategy

In 2001, Keck *et al.*[245] reported the total synthesis of pironetin employing a synthetic route, which featured a key Myers asymmetric alkylation[230a] to set the ethyl side-chain. The high diastereoselectivity observed for this kind of alkylation is explained by a proposed model in which one of the faces of the enolate is blocked by the solvent, allowing the electrophiles to approach from the opposite face. In order to complete the synthesis, a samarium-mediated *anti*-reduction[246] of a β-hydroxy ketone set the C5 stereogenic center of the lactone, while a lactone annulation procedure[247] involving an initial aldol condensation between the lithium enolate derived from methyl acetate and a

[243] Enders, D.; Dhulut, S.; Steinbusch, D.; Herrbach, A. *Chem. Eur. J.* **2007**, *13*, 3942-3949.
[244] Review: Job, A.; Janeck, C. F.; Bettray, W.; Peters, R.; Enders, D. *Tetrahedron* **2002**, *58*, 2253-2329.
[245] Keck, G. E.; Knutson, C. E.; Wiles, S. A. *Org. Lett.* **2009**, *3*, 707-710.
[246] Keck, G. E.; Wager, C. A.; Sell, T.; Wager, T. T. *J. Org. Chem*. **1999**, *64*, 2172-2173.
[247] Keck, G. E.; Li, X.-Y.; Knutson, C. E. *Org. Lett,* **1999**, *1*, 411-414.

β-acetoxy aldehyde with concomitant acyl migration, lactonization and a final β-elimination (Scheme III.10).

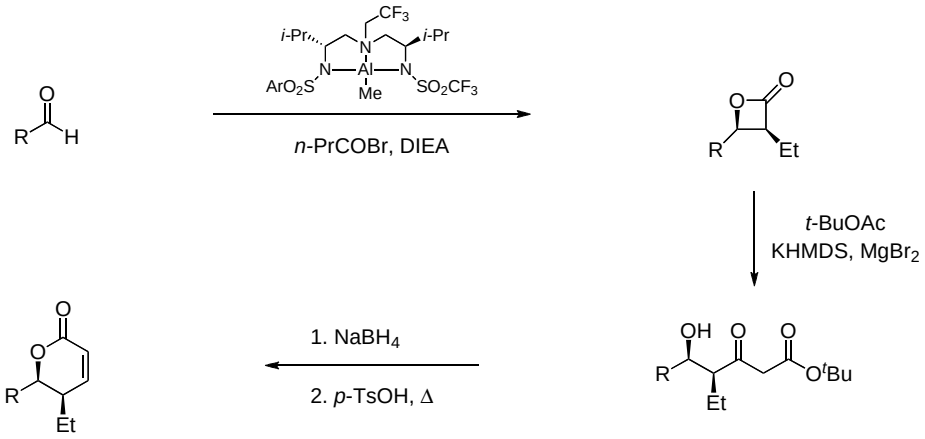


Scheme III.10. Pseudoephedrin-mediated alkylation/lactone annulation strategy

III.1.3.4. Acyl halide-aldehyde cyclocondensation strategy

Nelson and co-workers extended the use of the alkaloid-catalyzed acyl halide-aldehyde cyclocondensation (AAC)[248] to the asymmetric synthesis of the 5-ethyl-5,6-dihydro-2*H*-pyran-2-one motif present in pironetin.[249] Hence, the required aldehyde was engaged in a Lewis acid-catalyzed AAC homologation employing butyryl bromide as a butanoate enolate equivalent to afford the corresponding β-lactone with complete control of the newly formed stereogenic centers. Ring-opening with the magnesium enolate derived from *tert*-butylacetate, followed by ketone reduction and a subsequent thermal *p*-TsOH treatment induced the *tert*-butyl ester cleavage as well as the lactonization and the dehydration to generate the requisite 2-pyranone unit (Scheme III.11).

[248] Zhu, C.; Shen, X.; Nelson, S. G. *J. Am. Chem. Soc.* **2004**, *126*, 5352-5353.
[249] Shen, X.; Wasmuth, A. S.; Zhao, J.; Zhu, C.; Nelson, S. G. *J. Am. Chem. Soc.* **2006**, *128*, 7438-7439.

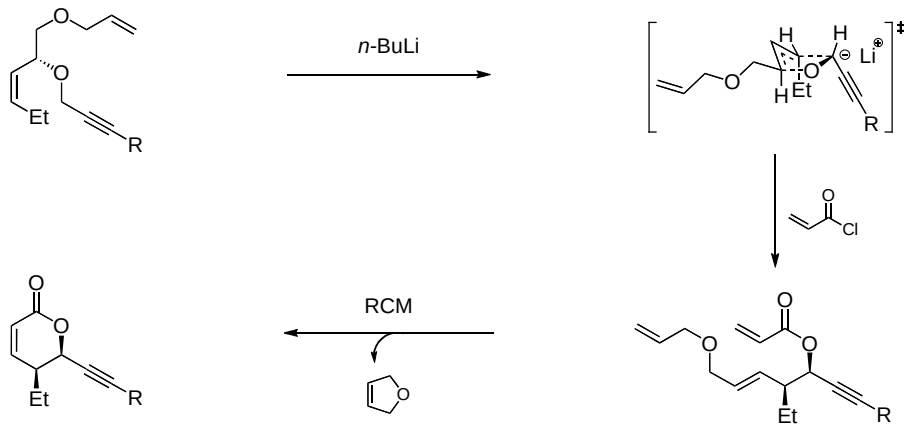


Scheme III.11. Acyl halide-aldehyde cyclocondensation strategy

III.1.3.5. Wittig rearrangement/RCM strategy

A different strategy was used by Cossy and Meyer and co-workers to construct the 5-ethyl-5,6-dihydro-2*H*-pyran-2-one moiety present in both the phoslactomycins and the leustroducsins.[250] It involved a highly diastereoselective [2,3]-Wittig rearrangement to control the C4 and C5 stereogenic centers, followed by a relay RCM to build the lactone ring. Hence, optically active propargylic ether was treated with *n*-BuLi to initiate the [2,3]-Wittig rearrangement through a five-membered-ring envelope conformation transition state wherein the allyloxymethyl chain preferentially occupies a pseudo-equatorial position, thus leading to a single diastereoisomer. The formation of the α,β-unsaturated δ-lactone was finally achieved by a relay ring-closing metathesis during which dihydrofuran is released (Scheme III.12).

[250] (a) Druais, V.; Hall, M. J.; Corsi, C.; Wenderborn, S. V.; Meyer, C.; Cossy, J. *Org. Lett.* **2009**, *11*, 935-938. (b) Druais, V.; Hall, M. J.; Corsi, C.; Wenderborn, S. V.; Meyer, C.; Cossy, J. *Tetrahedron* **2010**, *66*, 6358-6375.

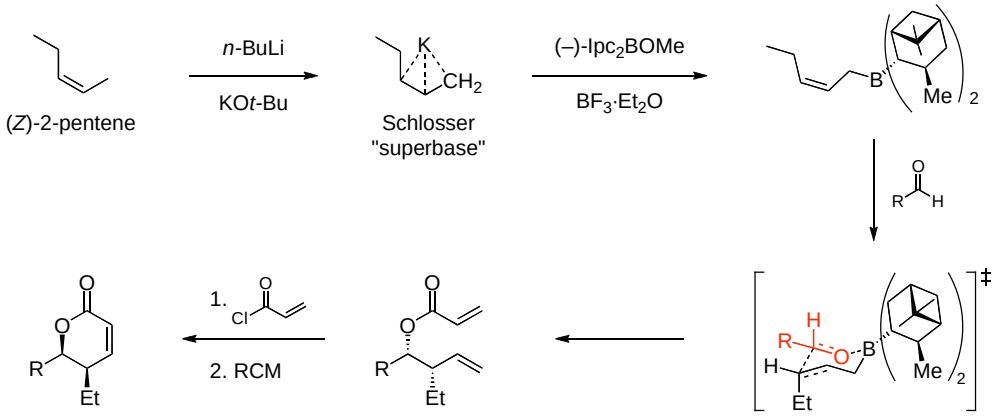


Scheme III.12. Wittig rearrangement/RCM strategy

III.1.3.6. Asymmetric pentenylation/RCM strategy

Inspired by the chiral crotylboron reagent developed by Brown *et al.*[251] in the early 80's, the boron-mediated pentenylation of aldehydes was developed quasi simultaneously by Hatekayama *et al.*[252] and Cossy *et al.*[253] This highly diastereo- and enantioselective transformation relies on the use of a chiral pentenylating agent derived from diisopinocampheylborane and either (*Z*)- or (*E*)-2-pentene, generated *in situ* following Brown's procedure.[251] Hence, in the presence of Schlosser's base,[254] the chiral reagent reacts with the aldehydes *via* a rigid chair-like Zimmerman-Traxler transition state, which ensures a high stereochemical transfer of the reagent's olefinic geometry thus allowing a direct access to both the *syn*- and the *anti*-ethyl-substituted homoallylic alcohols. In order to afford the α,β-unsaturated δ-lactone, the latter was eventually acylated with acryloyl chloride and subsequently engaged into a RCM (Scheme III.13).

[251] (a) Brown, H. C.; Jadhav, P. K. *J. Am. Chem. Soc.* **1983**, *105*, 2092-2093. (b) Brown, H. C.; Jadhav, P. K.; Bhat, K. S. *J. Am. Chem. Soc.* **1988**, *110*, 1535-1538.
[252] (a) Shibahara, S.; Fujino, M.; Tashiro, Y.; Takahashi, K.; Ishihara, J.; Hatakeyama, S. *Org. Lett.* **2008**, *10*, 2139-2142. (b) Shibahara, S.; Fujino, M.; Tashiro, Y.; Okamoto, N.; Esumi, T.; Takahashi, K.; Ishihara, J.; Hatakeyama, S. *Synthesis* **2009**, *17*, 2935-2953. (c) Sarkar, S. M.; Wanzala, E. N.; Shibahara, S.; Takahashi, K.; Ishihara, J.; Hatakeyama, S. *Chem. Commun.* **2009**, 5907-5909.
[253] Sonawane, R. P.; Joolakanti, S. R.; Arseniyadis, S.; Cossy, J. *Synlett* **2009**, 213-216.
[254] (a) Schlosser, A.; Hartmann, J. *J. Am. Chem. Soc.* **1976**, *98*, 4674-4676. (b) Roush, W.; Adam, M.; Walts, A.; Harris, D. *J. Am. Chem. Soc.* **1986**, *108*, 3422-3434. (c) Schlosser, A.; Despond, O.; Lehmann, R.; Moret, E.; Rauchschwalbe, G. *Tetrahedron* **1993**, *49*, 10175-10203.



Scheme III.13. Synthesis of *syn*-ethyl substituted homoallylic alcohol

This method was successfully applied in natural product synthesis, first by Hatakeyama *et al.* in their synthesis of phoslactomycin B,[252] then by Cossy *et al.* in their synthesis of pironetin[255] and leustroducsin B.[256]

[255] Bressy, C.; Vors, J.-P.; Hillebrand, S.; Arseniyadis, S.; Cossy, J. *Angew. Chem. Int. Ed.* **2008**, *47*, 10137-10140.
[256] Moïse, J.; Sonawane, R. P.; Corsi, C.; Wendeborn, S. V.; Arseniyadis, S.; Cossy, J. *Synlett*, **2008**, 2617-2620.

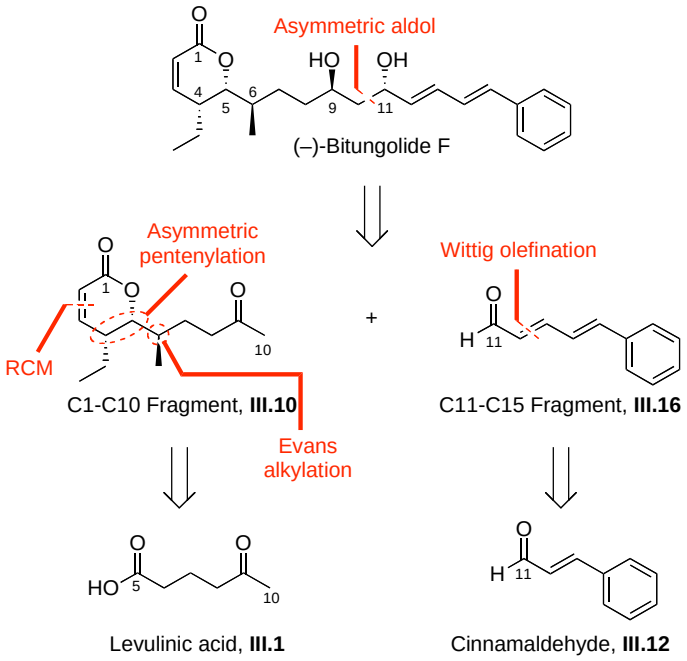
III.2. RESULTS & DISCUSSION

III.2.1. TOTAL SYNTHESIS OF (–)-BITUNGOLIDE F

The unique biological profile of the bitungolides combined with their challenging molecular architecture stimulated our interest. We therefore decided to develop a highly straightforward and flexible route that would not only allow an expedient access to the natural product, but also to various analogues thereof.

III.2.1.1. First retrosynthetic analysis

Our first strategy for the synthesis of (–)-bitungolide F relied on a few key steps which included a chiral boron-mediated aldol to link the two major subunits (**III.10** and **III.16**) together and control the configuration of the stereogenic center at C11, a stereoselective pentenylation to introduce the ethyl side chain at C4 and set the *syn* relationship between the substituents at C4 and C5, an asymmetric Evans type alkylation to control the stereogenic center at C6, and a RCM to introduce the α,β-unsaturated-δ-lactone moiety (Scheme III.14).

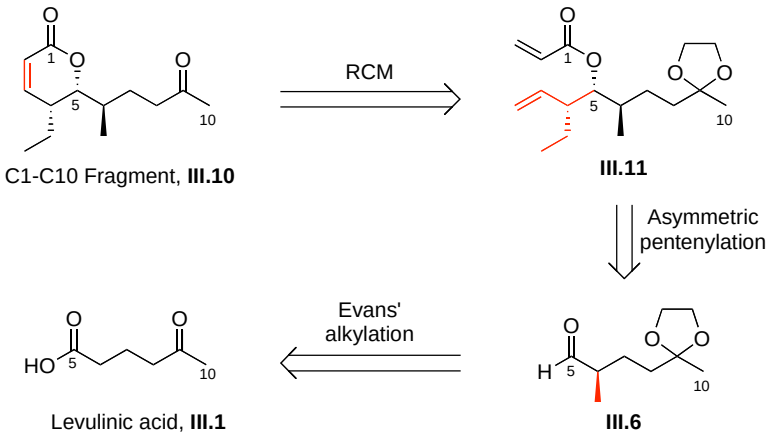


Scheme III.14. First retrosynthetic analysis

III.2.1.2. Synthesis of the C1-C10 fragment

III.2.1.2.1. Retrosynthetic analysis

The first strategy that was envisioned for the synthesis of the C1-C10 fragment **III.10** was based upon the implementation of a RCM applied to olefin **III.11**, which would arise from an asymmetric pentenylation previously developed in the Laboratory on aldehyde **III.6**. The latter would be obtained by subjecting commercially available levulinic acid **III.1** to an Evans asymmetric alkylation (Scheme III.15).

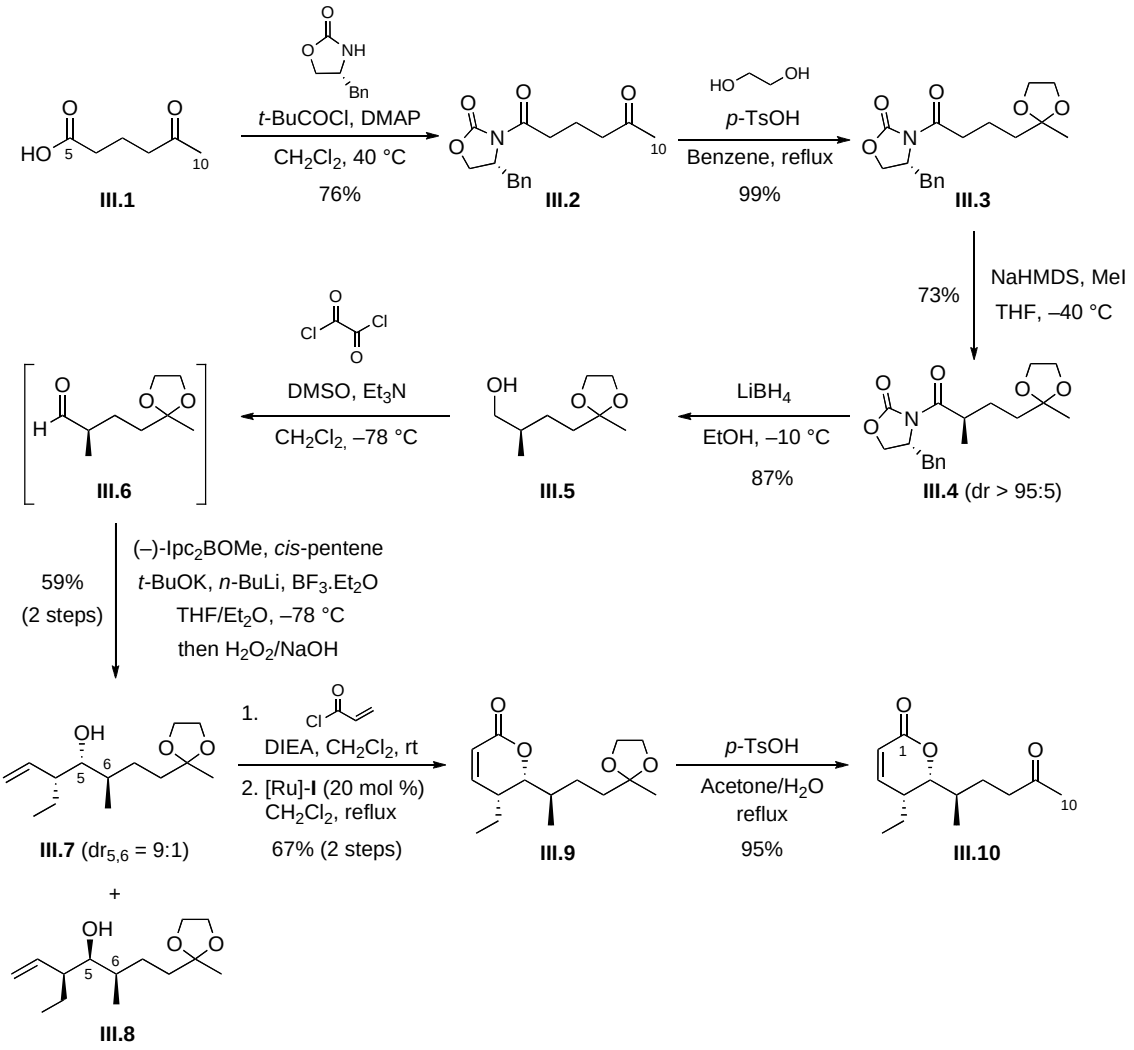


Scheme III.15. Retrosynthetic analysis of the C1-C10 fragment

III.2.1.2.1. Synthesis of the C1-C10 fragment

The synthesis of the C1-C10 fragment **III.10** of bitungolide F began by converting levulinic acid **III.1** to the corresponding chiral *N*-acyloxazolidinone **III.2** *via* the *in situ* formation of a mixed anhydride (Scheme III.16). The ketone moiety was then protected using ethylene glycol and *p*-toluenesulfonic acid (*p*-TsOH), and the resulting ketal **III.3** was subsequently engaged into a highly diastereoselective Evans alkylation[229d,257] to afford the corresponding C6 methylated product **III.4** (73% yield) as a unique diastereoisomer as confirmed by ^{1}H NMR analysis of the crude reaction mixture (dr > 95:5). The chiral auxiliary was then removed using a $LiBH_4$-mediated reduction[258] to afford the primary alcohol **III.5** (87% yield), which was subsequently oxidized under standard Swern conditions to afford the corresponding aldehyde **III.6**. The latter was next subjected to the boron-mediated asymmetric pentenylation discussed above. Hence, the chiral (*Z*)-pentenylborane reagent, which was generated *in situ* from (*Z*)-2-pentene, Schlosser's base and $BF_3{\cdot}Et_2O$, was added to aldehyde **III.6** to afford the corresponding homoallylic alcohol **III.7** in 59% yield over two steps. ^{1}H NMR analysis of the crude reaction mixture suggested the formation of two diastereoisomers in a 9:1 ratio (*anti*/*syn versus syn*/*syn*). The two diastereoisomers **III.7** and **III.8** were then separated by flash column chromatography over silica gel and the major isomer was engaged in a two-step sequence involving an acylation with acryloyl chloride followed by a Grubbs second generation catalyst-mediated RCM to afford the desired α,β-unsaturated δ-lactone **III.9** in 59% yield over two steps. The latter was finally treated with a catalytic amount of *p*-TsOH in an acetone/water mixture[259] in order to deprotect the ketone and thus complete the synthesis of the C1-C10 fragment **III.10**. Hence, fragment C1-C10 (**III.10**) was synthesized in six steps and 28.2% overall yield starting from levulinic acid.

[257] (a) Ager, D. J.; Prakash, I.; Schaad, D. R. *Aldrichimica Acta* **1997**, *30*, 3-12. (b) Evans, D. A.; Kim, A. S. *Handbook of Reagents for Organic Synthesis: Reagents, Auxiliaries, and Catalysts for C-C bond Formation*; Coates, R. M.; Denmark, S. E., Eds.; John Wiley and Sons: New York, **1999**; 91-101. (c) Smith, T. E.; Richardson, D. P.; Truran, G. A.; Belecki, K.; Onishi, M. *J. Chem. Ed.* **2008**, *85*, 695-697.
[258] Smith, A. B., III; Lee, D. *J. Am. Chem. Soc.* **2007**, *129*, 10957-10962.
[259] Meissner, R. S.; Perkins, J. J.; Duong, L. T.; Hartman, G. D.; Hoffman, W. F.; Huff, J. R.; Ihle, N. C.; Leu, C.-T.; Nagy, R. M.; Naylor-Olsen, A.; Rodan, G. A.; Whitman, D. B.; Wesolowski, G. A.; Duggan, M. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 25-29.

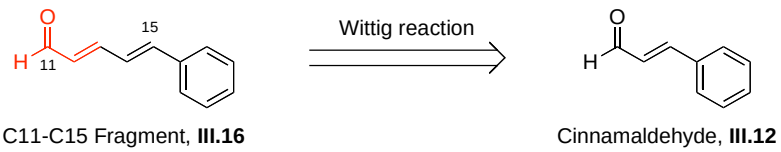


Scheme III.16. Synthesis of C1-C10 fragment

III.2.1.3. Synthesis of C11-C15 fragment

III.2.1.3.1. Retrosynthetic analysis

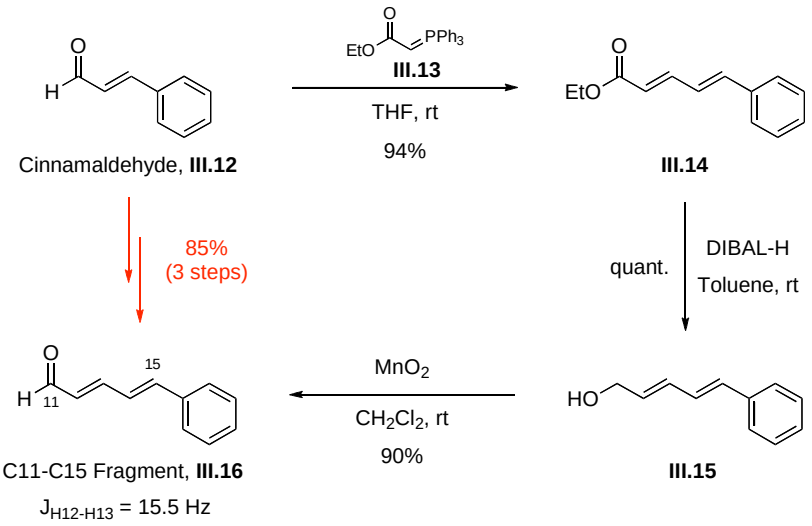
The synthesis of the C11-C15 fragment **III.16** bearing the (*E*,*E*)-diene moiety was envisioned through a key Wittig olefination between commercially available cinnamaldehyde **III.12** and a stabilized phosphorus ylide (Scheme III.17).



Scheme III.17. Retrosynthetic analysis of C11-C15 fragment

III.2.1.3.2. Synthesis of the C11-C15 fragment

The synthesis of the C11-C15 fragment started with a Wittig olefination between cinnamaldehyde **III.12** and ethyl (triphenylphosphoranylidene)acetate (**III.13**) which afforded the expected (*E*,*E*)-unsaturated ester **III.14** in 94% yield (Scheme III.18). The stereochemistry of the diene moiety was secured by the values of the coupling constant for H12 in agreement to an *E*-alkene (J = 15.5 Hz).[260] The latter was subsequently reduced to the corresponding alcohol **III.15** using DIBAL-H and oxidized to the α,β,γ,δ-unsaturated aldehyde **III.16** in the presence of MnO_2. The C11-C15 fragment of bitungolide F was thus obtained in three steps and 85% overall yield starting from commercially available cinnamaldehyde.[261]



Scheme III.18. Synthesis of C11-C15 fragment

III.2.1.4. Synthesis of (–)-bitungolide F

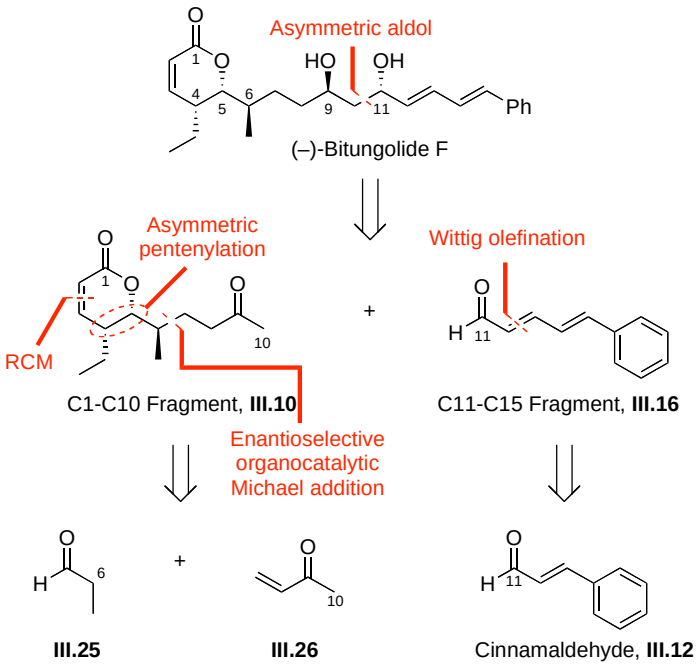
With the two subunits **III.10** and **III.16** in hand, the stage was set for the key asymmetric aldol that would allow a direct access to the entire carbon backbone of (–)-bitungolide F and control the C11 stereogenic center.

[260] The (*E*,*Z*) unsaturated ester was isolated in 6% yield.
[261] Cao, X.-P. *Tetrahedron* **2002**, *58*, 1301-1307.

As shown by Patterson *et al.*,[262] enol diisopinocampheylborinates, generated from achiral ketones in the presence of a tertiary amine base such as Et_3N or DIEA, induce a highly enantio- and diastereoselective aldolization when reacted with an aldehyde. Accordingly, ketone **III.10** was treated with (+)-$(Ipc)_2BCl$ in presence of Et_3N to afford the chiral boron enolate intermediate, which was then reacted with aldehyde **III.16**. In this case, the reaction proceeds through a boat-like transition state. The resulting β-hydroxy ketone **III.17**, whose crude ^1H NMR indicated a moderate diastereoisomeric ratio of 5:1, was directly engaged in a hydroxy-assisted ketone reduction using Evans' conditions[226] [$Me_4NBH(OAc)_3$ in an acetic acid/acetonitrile mixture at –20 °C] in order to prevent a partial elimination that could occur with such conjugated systems. Under these conditions, the corresponding 1,3-*anti* diol was isolated as a single diastereoisomer in 82% overall yield after purification by flash column chromatography over silica gel (Scheme III.19). The absolute and relative configuration of the newly formed C9 and C11 stereogenic centers were determined by comparison of the spectroscopic data of the synthetic product with those reported for the natural product.[214]

The spectroscopic and physical data of the synthetic bitungolide were identical with those reported for the natural product except for the optical rotation, which was of opposite sign: $[\alpha]^{20}_D$ –51.6 (*c* 1.56, $CHCl_3$) instead of $[\alpha]_{lit.}{}^{22}_D$ +43.0 (*c* 0.85, $CHCl_3$).[214]

[262] (a) Patterson, I.; Goodman, J. M. *Tetrahedron Lett.* **1989**, *30*, 997-1000. (b) Patterson, I.; Goodman, J. M.; Lister, M. A.; Schumann, R. C.; McClure, C. K.; Norcross, R. D. *Tetrahedron* **1990**, *46*, 4663-4684.



Scheme III.20. Second retrosynthetic analysis of (–)-bitungolide F

III.2.1.5.2. Introduction to enantioselective organo-catalyzed Michael addition

The first organo-catalyzed Michael reactions were developed using highly activated Michael acceptors such as nitroalkenes or alkylidenemalonates,[263] or highly activated nucleophiles such as malonate diesters[264] or nitroalkanes.[265] As a general trend, the Michael adducts were obtained in excellent yields and high enantiomeric excess. In 2003, Jørgensen *et al.*[266] were the first to develop an enantioselective organo-catalytic Michael addition of simple aldehydes to enones catalyzed by chiral proline-derived

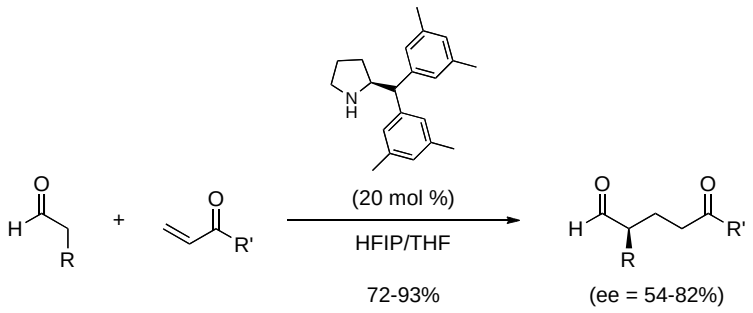
[263] (a) Betancort, J. M.; Barbas, C. F., III. *Org. Lett.* **2001**, *3*, 3737-3740. (b) Betancort, J. M.; Sakthivel, K.; Thayumanavan, R.; Barbas, C. F., III. *Tetrahedron Lett.* **2001**, *42*, 4441-4444. (c) Enders, D.; Seki, A. *Synlett* **2002**, 26-28. (d) Andrey, O.; Alexakis, A.; Bernardinelli, G. *Org. Lett.* **2003**, *5*, 2559-2561. (e) Mase, N.; Thayumanavan, R.; Tanaka, F.; Barbas, C. F., III. *Org. Lett.* **2004**, *6*, 2527-2530. (f) Betancort, J. M.; Sakthivel, K.; Thayumanavan, R.; Tanaka, F.; Barbas, C. F., III. *Synthesis* **2004**, 1509-1521. (g) Ishii, T.; Fujioka, S.; Sekiguchi, Y.; Kotsuki, H. *J. Am. Chem. Soc.* **2004**, *126*, 9558-9559. (h) Wang, W.; Wang, J.; Li, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 1369-1371.

[264] (a) Kawara, A.; Taguchi, T. *Tetrahedron Lett.* **1994**, *35*, 8805-8808. (b) Hanessian, S.; Pham, V. *Org. Lett.* **2000**, *2*, 2975-2978. (c) Halland, N.; Hansen, K. A.; Jørgensen, K. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 4955-4957. (d) Halland, N.; Aburel, P. S.; Jørgensen, K. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 661-665.

[265] (a) Yamaguchi, M.; Shiraishi, T.; Hirama, M. *J. Org. Chem.* **1996**, *61*, 3520-3530. (b) Corey, E. J.; Zhang, F.-Y. *Org. Lett.* **2000**, *2*, 4257-4259. (c) Halland, N.; Hazell, R. G.; Jørgensen, K. A. *J. Org. Chem.* **2002**, *67*, 8331-8338.

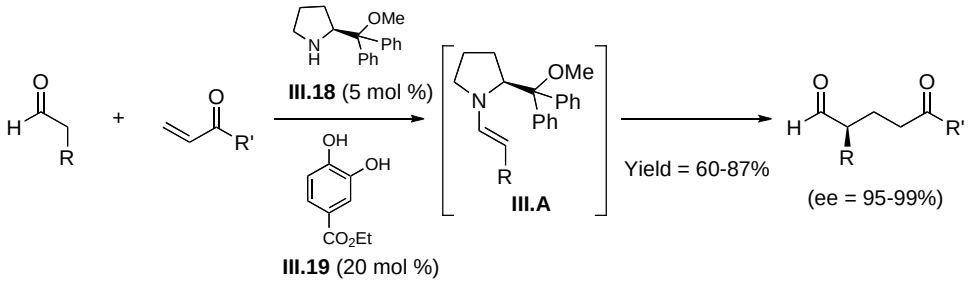
[266] Melchiorre, P.; Jørgensen, K. A. *J. Org. Chem.* **2003**, *68*, 4151-4152.

amines, affording the corresponding Michael adducts in high yields (up to 93%) and moderate to good enantioselectivities ranging from 53% to 82% (Scheme III.21).



Scheme III.21. Jørgensen's organo-catalyzed conjugated addition

More recently, Gellman *et al.* reported that diphenylprolinol methyl ether **III.18** in conjunction with catechol co-catalyst **III.19** could catalyze the enantioselective conjugate addition of aldehydes to simple enones with unprecedented levels of selectivity (Scheme III.22).[267] Concerning the mechanism, the authors suggest that the aldehyde is most probably converted to the corresponding enamine **III.A**, while the catechol electrophilically activates the enone by a hydrogen bonding with the carbonyl oxygen. It is worth pointing out, however, that other mechanisms of activation such as catechol catalysis of initial enamine formation cannot be ruled out at this time.[268]



Scheme III.22. Gellman's organo-catalyzed Michael addition

[267] Chi, Y.; Gellman, S. H. *Org. Lett.* **2005**, *19*, 4523-4256.
[268] Peelen, T. J.; Chi, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11598-11599.

III.2.1.6. Second synthesis of the C1-C10 fragment

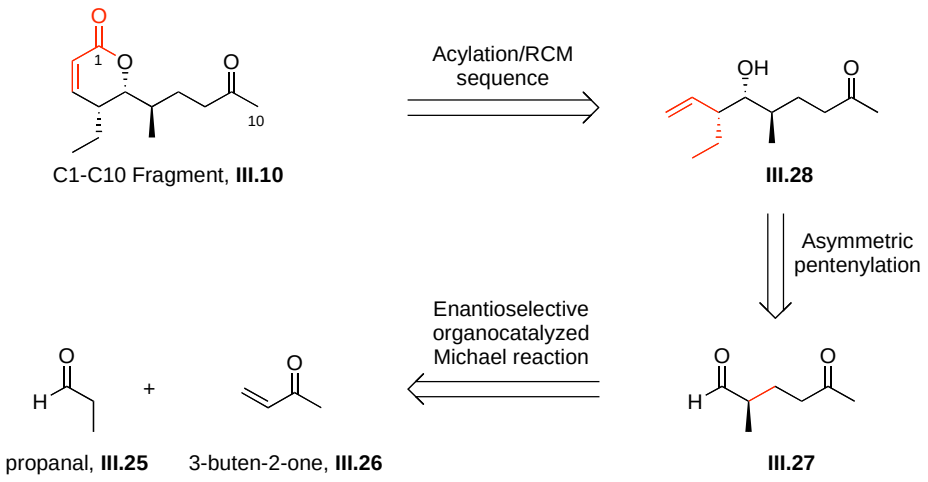
III.2.1.6.1. Second retrosynthetic analysis

As mentioned previously, the second strategy for the synthesis of the C1-C10 fragment of bitungolide F relies on three key transformations (Scheme III.23):

- an enantioselective diphenylprolinol methyl ether-catalyzed conjugate addition of propanal (**III.25**) to 3-buten-2-one (**III.26**) to form the required aldehyde **III.27**,

- an asymmetric chiral boron-mediated pentenylation to introduce the ethyl side chain and control the stereogenic centers at C4 and C5,

- an acylation/RCM sequence to construct the α,β-unsaturated δ-lactone.



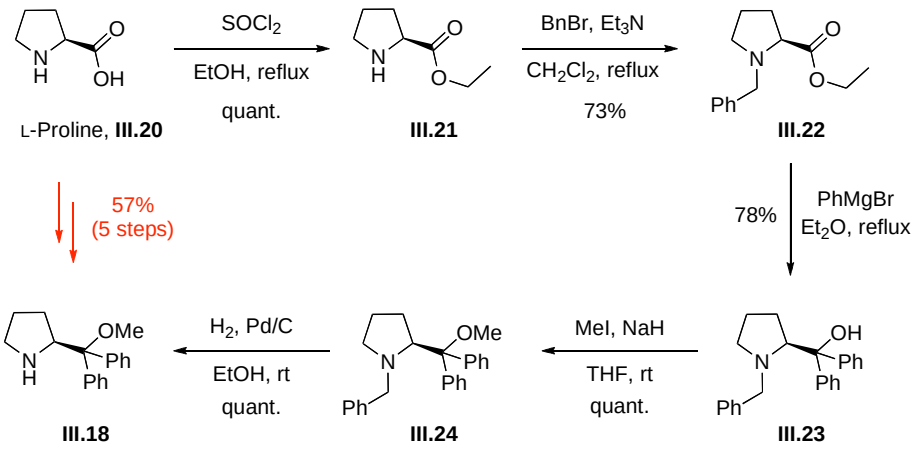
Scheme III.23. Second retrosynthetic analysis of the C1-C10 fragment

III.2.1.6.2. Synthesis of organocatalyst III.18

Diphenylpropinol methyl ether **III.18** was synthesized in five steps following the protocol reported by Enders *et al.*[269] starting from commercially available L-proline. L-Proline was thus subjected to an esterification with thionyl chloride in refluxing ethanol and the resulting amino ester **III.21** was alkylated using benzyl bromide to afford the corresponding *N*-benzyl protected proline **III.22** in 73% overall yield (Scheme III.24). The ester moiety was then converted to the corresponding methyl ether **III.23** by addition of phenylmagnesium bromide (2 equiv) and alkylation of the

[269] Enders, D.; Kipphardt, H.; Gerdes, P.; Brena-Valle, L. J. *Bull. Soc. Chim. Belg.* **1988**, *97*, 691-704.

resulting tertiary alcohol **III.23** with methyl iodide (NaH, THF, rt, 78% yield over two steps). Finally, the *N*-benzyl protected diphenylprolinol methyl ether was engaged in a hydrogenation over Pd/C to afford diphenylprolinol methyl ether **III.18** in five steps and 57% overall yield starting from L-proline **III.20**.

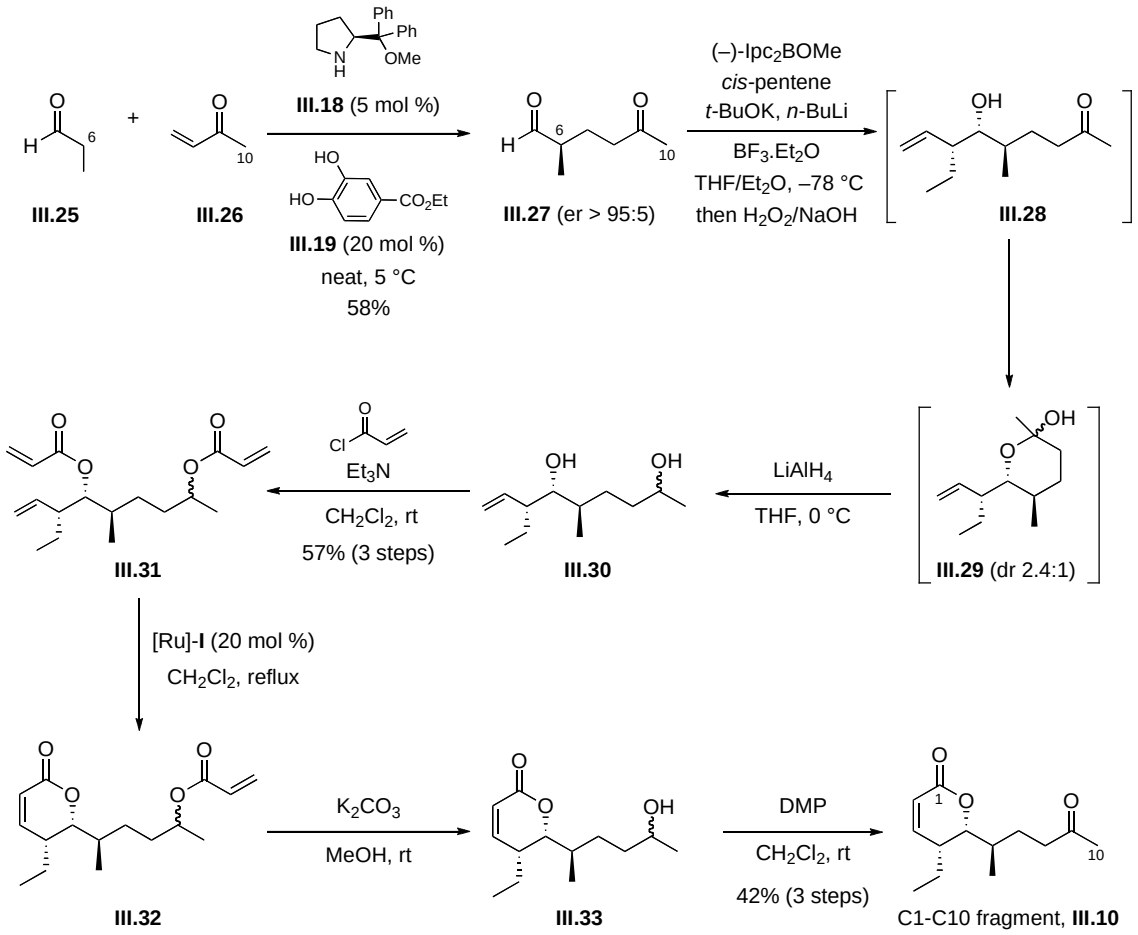


Scheme III.24. Synthesis of catalyst **III.18**

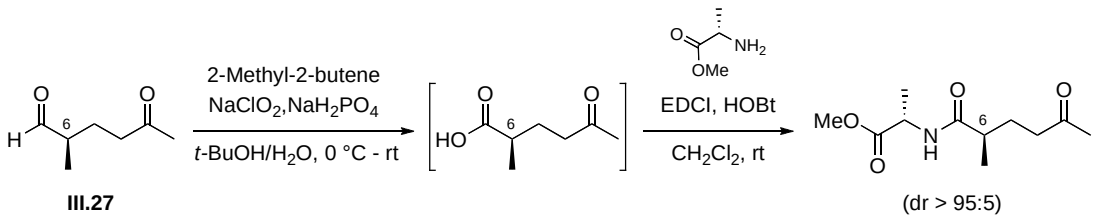
III.2.1.6.3. Second synthesis of the C1-C10 fragment

The synthesis of the C1-C10 fragment of bitungolide F started with the conjugate addition of commercially available propanal (**III.25**) to 3-buten-2-one (**III.26**) catalyzed by diphenylpropinol methyl ether **III.18** (5 mol %) and catechol **III.19** (20 mol %) according to the procedure reported by Gellman and Chi (Scheme III.25).[267] As pyrrolidine **III.18** could potentially catalyze both the conjugate addition[266] and the epimerization of the product *via* enamine formation,[270] we decided to separate the catalyst from the reaction mixture before reaching completion. Consequently, the reaction furnished aldehyde **III.27** in a slightly moderate yield of 58% however with an excellent enantioselectivity (er > 95:5). The absolute configuration of the resulting stereogenic center was confirmed by comparison of its optical rotation {$[\alpha]_D^{20}$ +1.77 (*c* 0.8, $CHCl_3$)} with the one reported in the literature[266] {$[\alpha]_D^{20}$ +5.4 (*c* 1.2, $CHCl_3$, 64% ee)} while the enantiomeric excess was determined by ^{1}H NMR analysis of a diastereisomeric ester derivative obtained after oxidation of the aldehyde to the corresponding acid followed by a peptide coupling with L-alanine methyl ester (Scheme III.26).

[270] List, B. *J. Am. Chem. Soc*. **2002**, *124*, 5656-5657.



Scheme III.25. Second synthesis of the C1-C10 fragment **III.10**



Scheme III.26. Synthesis of the diastereoisomeric ester derivative of **III.27**

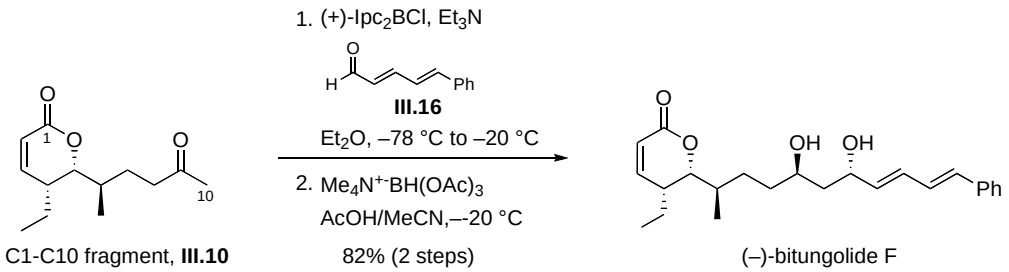
Aldehyde **III.27** was then engaged in the same asymmetric boron-mediated pentenylation as previously to afford the corresponding homoallylic alcohol **III.28** which underwent spontaneous hemiketalization.[271] Unfortunately, due to overlapping signals in the crude ^{1}H NMR, the determination of the selectivity pertaining to the boron-mediated pentenylation appeared impossible. In addition, although hemiketals are usually in equilibrium with their corresponding hydroxy ketones, all our attempts to

[271] The two diastereoisomeric hemiketals were obtained in 2.4:1 mixture according to ^{1}H NMR analysis.

selectively acylate the homoallylic alcohol failed. Therefore, we decided to directly reduce the crude reaction mixture with $LiAlH_4$ and bis-acylate the resulting diol with acryloyl chloride in presence of Et_3N (57% yield over three steps) to produce **III.31**. A RCM catalyzed by the Grubbs second generation catalyst followed by the saponification of the remaining acrylate under mild conditions (K_2CO_3, MeOH) and a final oxidation using Dess-Martin periodinane (DMP) provided the desired ketone **III.10** in 42% overall yield. The C1-C10 fragment **III.10** was thus synthesized in seven steps and 13.9% overall yield starting from simple and commercially available propanal (**III.25**) and 3-buten-2-one (**III.26**).

III.2.1.7. Second synthesis of (–)-bitungolide F

After completing the synthesis of the C1-C10 fragment, the latter was engaged in the same chiral boron-mediated aldol/*anti*-reduction sequence as previously to afford (–)-bitungolide F in 82% yield over the two-step sequence (Scheme III.27).

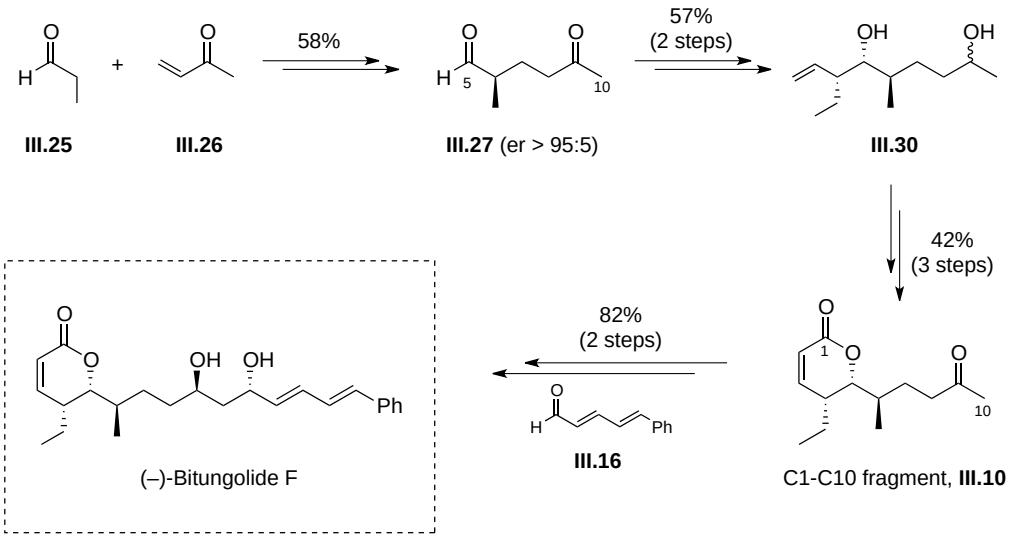


Scheme III.27. Second synthesis of (–)-bitungolide F

III.3. CONCLUSIONS

We have completed the synthesis of the dual-specificity phosphatase inhibitor (–)-bitungolide F using two highly convergent routes, which featured an asymmetric boron-mediated pentenylation, a stereoselective aldol and a hydroxyl-directed 1,3-*anti*-reduction to control all five stereogenic centers of the natural product. While the first strategy relied on an Evans type asymmetric alkylation and was achieved in 11 steps and 14.6% overall yield, the second strategy featured a key enantioselective organo-catalytic Michael addition and was completed in only nine steps and 11.4% overall yield. It is worth mentioning that while both syntheses are considerably shorter than the ones reported so far in the literature,[272] the second approach is particularly appealing as it is highly flexible, it does not involve the use of any protecting group, and is therefore amenable to a wide variety of potentially useful synthetic analogues.

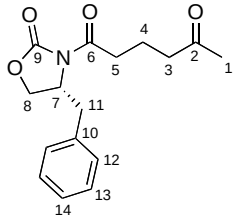
[272] She's synthesis of (–)-bitungolide F was achieved in 18 steps and 20.1% overall yield starting from (–)-malic acid, while Ghosh's synthesis was completed in 22 steps and 4.7% overall yield starting from the same starting material.


58%
57%
(2 steps)
42%
(3 steps)
82%
(2 steps)
III.25
III.26
III.27 (er > 95:5)
III.30
C1-C10 fragment, III.10
III.16
(–)-Bitungolide F

III.4. EXPERIMENTAL PART OF CHAPTER III

III.4.1. EXPERIMENTAL PROCEDURES

III.4.1.1. Synthesis of 1-((R)-4-benzyl-2-oxo-oxazolidin-3-yl)-hexane-1,5-dione (III.2)



MW (g/mol): 289.3264 **Molecular formula:** $C_{16}H_{19}NO_4$

To a solution of levulinic acid (**III.1**) (2.0 g, 15.3 mmol) in CH_2Cl_2 (150 mL) was added Et_3N (5.1 mL, 36.7 mmol) and pivaloyl chloride (2.2 mL, 18.4 mmol) at rt and the reaction mixture stirred for 3 h at the same temperature. (*S*)-4-Phenyloxazolidinone (2.3 g, 13.0 mmol) and DMAP (130.8 mg, 1.07 mmol) were then added and the solution was stirred at 45 °C for 7 h until complete conversion of the starting material (reaction monitored by TLC). After cooling to rt, water was added and the organic layer was separated. The aqueous layer was washed with CH_2Cl_2 (3 x 100 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/EtOAc, 75:25) to afford 1-((*R*)-4-benzyl-2-oxo-oxazolidin-3-yl)-hexane-1,5-dione (**III.2**) as an amorphous solid (3.2 g, 73%).

$\boldsymbol{R_f}$ (PE/EtOAc, 1:1): 0.5.

$\boldsymbol{[\alpha]_D^{20}}$ –51.4 (*c* 0.78, $CHCl_3$).

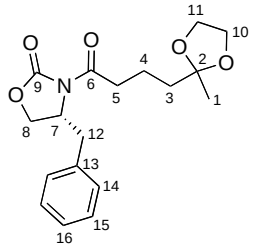
IR (neat): 2923, 1774, 1698, 1386, 1352, 1287, 1250, 1210, 1161, 1112, 1077, 1052, 995, 762, 751, 703 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.16 (m, 3H, H_{13}, H_{14}), 7.14-7.12 (m, 2H, H_{12}), 4.59 (m, 1H, H_7), 4.18-4.07 (m, 2H, H_8), 3.22 (dd, *J* = 13.5, 3.3 Hz, 1H, H_{11}), 2.87 (td, *J* = 7.1, 2.7 Hz, 2H, H_5), 2.69 (dd, *J* = 13.5, 9.7 Hz, 1H, H_{11}), 2.49 (t, *J* = 7.1 Hz, 2H, H_3), 2.09 (s, 3H, H_1), 1.90 (quintd, *J* = 7.1, 1.7 Hz, 2H, H_4).

^{13}C NMR (100 MHz, $CDCl_3$) δ 208.0 (s, C_2), 172.7 (s, C_5), 153.5 (s, C_9), 135.3 (s, C_{10}), 129.4 (d, 2C, C_{13}), 129.0 (d, 2C, C_{12}), 127.4 (d, C_{14}), 66.3 (t, C_8), 55.2 (d, C_7), 42.5 (t, C_{11}), 37.9 (t, C_5), 34.7 (t, C_3), 29.9 (q, C_1), 18.2 (t, C_4).

HRMS (ESI) *m/z*: calculated for $C_{16}H_{19}O_4NNa$ $[M+Na]^+$ 312.1206, found 312.1195.

III.4.1.2. Synthesis of (R)-4-benzyl-3-[4-(2-methyl-1,3-dioxolan-2-yl)-butyryl]-oxazolidin-2-one (III.3)



MW (g/mol): 333.3789 **Molecular formula:** $C_{18}H_{23}NO_5$

To a stirred solution of 1-((*R*)-4-benzyl-2-oxo-oxazolidin-3-yl)-hexane-1,5-dione (**III.2**) (2.5 g, 8.6 mmol) in benzene (90 mL) were added ethylene glycol (1.92 mL, 34.5 mmol) and *p*-TsOH (16.3 mg, 0.086 mmol), and the resulting reaction mixture was heated at reflux for 3 h using a Dean-Stark trap to remove water. The solution was then cooled to rt and washed with water (2 x 50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (PE/EtOAc, 75:25) to afford (*R*)-4-benzyl-3-[4-(2-methyl-1,3-dioxolan-2-yl)-butyryl]-oxazolidin-2-one (**III.3**) as a white solid (2.8 g, 99%).

R_f (PE/EtOAc, 1:1): 0.46.

$[\alpha]_D^{20}$ –45.1 (*c* 1.0, $CHCl_3$).

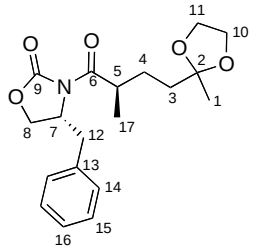
IR (neat): 2980, 2881, 1775, 1697, 1454, 1384, 1351, 1209, 1140, 1098, 1051, 948, 860, 762, 749, 702 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.17 (m, 3H, H_{15}, H_{16}), 7.16-7.11 (m, 2H, H_{14}), 4.59 (m, 1H, H_7), 4.21-4.00 (m, 2H, H_8), 3.90-3.82 (m, 4H, H_{10}, H_{11}), 3.22 (dd, *J* = 13.5, 3.2 Hz, 1H, H_{12}), 3.01-2.81 (m, 2H, H_5), 2.69 (dd, *J* = 13.5, 9.7 Hz, 1H, H_{12}), 1.81-1.71 (m, 4H, H_3, H_4), 1.27 (s, 3H, H_1).

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (s, C_6), 153.5 (s, C_9), 135.3 (s, C_{13}), 129.4 (d, 2C, C_{15}), 129.0 (d, 2C, C_{14}), 127.3 (d, C_{16}), 109.8 (s, C_2), 66.2 (t, C_8), 64.7 (t, 2C, C_{10}, C_{11}), 55.1 (d, C_7), 38.2 (t, C_3), 37.9 (t, C_{12}), 35.4 (t, C_5), 23.8 (q, C_1), 18.8 (t, C_4).

HRMS (ESI) *m/z*: calculated for $C_{18}H_{23}O_5NNa$ $[M+Na]^+$ 356.1468, found 356.1468.

*III.4.1.3. Synthesis of (**R**)-4-benzyl-3-[(**R**)-2-methyl-4-(2-methyl-1,3-dioxolan-2-yl)-butyryl]-oxazolidin-2-one (III.4)*



MW (g/mol): 347.4055 **Molecular formula:** $C_{19}H_{25}NO_5$

To a solution of (*R*)-4-benzyl-3-[4-(2-methyl-1,3-dioxolan-2-yl)-butyryl]-oxazolidin-2-one (**III.3**) (2.52 g, 7.5 mmol) in THF (75 mL) was added NaHMDS (15 mL of a 1M solution in THF), and the reaction mixture was stirred at –78 °C for 30 min. MeI (9.4 mL, 75 mmol) was then added and the stirring was continued for an extra 90 min at –40 °C. The reaction was quenched with H_2O and allowed to warm up to rt. The organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/EtOAc: 75:25) to afford (*R*)-4-benzyl-3-[(*R*)-2-methyl-4-(2-methyl-1,3-dioxolan-2-yl)-butyryl]-oxazolidin-2-one (**III.4**) as a viscous oil (1.88 g, 73%, dr > 95:5).

R_f (PE/EtOAc, 1:1): 0.5.

$[\alpha]_D^{20}$ –49.8 (*c* 0.81, $CHCl_3$).

IR (neat): 2980, 2879, 1778, 1696, 1455, 1386, 1350, 1210, 1102, 1070, 972, 949, 858, 762, 747, 703 cm^{-1}.

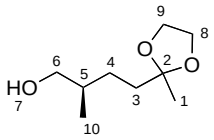
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24-7.13 (m, 3H, H_{15}, H_{16}), 7.12-7.07 (m, 2H, H_{14}), 4.56 (m, 1H, H_7), 4.12-4.02 (m, 2H, H_8), 3.89-3.73 (m, 4H, H_{10}, H_{11}), 3.59 (m, 1H, H_5),

3.14 (dd, J = 13.2, 2.8 Hz, 1H, H_{12}), 2.65 (dd, J = 13.2, 9.7 Hz, 1H, H_{12}), 1.74 (m, 1H, H_4), 1.58-1.50 (m, 2H, H_3), 1.43 (m, 1H, H_4), 1.20 (s, 3H, H_1), 1.11 (d, J = 6.7 Hz, 3H, H_{17}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0 (s, C_6), 153.0 (s, C_9), 135.3 (s, C_{13}), 129.5 (d, 2C, C_{15}), 128.9 (d, 2C, C_{14}), 127.3 (d, C_{16}), 109.8 (s, C_2), 66.0 (t, C_8), 64.7 (t, C_{11}), 64.6 (t, C_{10}), 55.3 (d, C_7), 37.9 (t, C_{12}), 37.6 (d, C_5), 36.4 (t, C_3), 27.6 (t, C_4), 23.8 (q, C_1), 17.6 (q, C_{17}).

HRMS (ESI) *m/z*: calculated for $C_{19}H_{26}O_5N$ $[M+H]^+$ 348.1806, found 348.1809; calculated for $C_{19}H_{25}O_5NNa$ $[M+Na]^+$ 370.1625, found 370.1625.

III.4.1.4. Synthesis of (R)-2-methyl-4-(2-methyl-1,3-dioxolan-2-yl)-butan-1-ol (III.5)



MW (g/mol): 174.2374 **Molecular formula:** $C_9H_{18}O_3$

To a solution of (*R*)-4-benzyl-3-[(*R*)-2-methyl-4-(2-methyl-1,3-dioxolan-2-yl)-butyryl]-oxazolidin-2-one (**III.4**) (1.8 g, 5.18 mmol) in Et_2O (90 mL) at –10 °C were added EtOH (0.36 mL, 6.2 mmol) and $LiBH_4$ (147 mg, 6.2 mmol). The solution was stirred at –10 °C for 1.5 h and quenched by addition of a 1M aqueous solution of NaOH (30 mL). After stirring for an additional 15 min at 0 °C, the reaction mixture was poured into a 1:1 Et_2O/brine solution (100 mL). The phases were separated, and the aqueous layer was extracted with Et_2O (3 x 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/EtOAc, 70;30) to afford (*R*)-2-methyl-4-(2-methyl-1,3-dioxolan-2-yl)-butan-1-ol (**III.5**) as a colourless oil (750 mg, 87%) .

R_f (PE/EtOAc: 7/3): 0.18.

$[\alpha]_D^{20}$ +7.6 (*c* 3.1, $CHCl_3$).

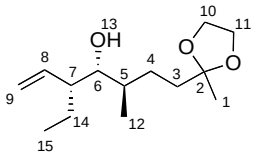
IR (neat): 3427, 2951, 2876, 1460, 1378, 1345, 1251, 1220, 1124, 1091, 1039, 988, 948, 858, 785 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89-3.75 (m, 4H, H_8, H_9), 3.36 (dd, J = 10.5, 5.9 Hz, 1H, H_6), 3.29 (dd, J =10.5, 5.9 Hz, 1H, H_6), 2.39 (m, 1H, H_7), 1.67-1.34 (m, 4H, H_3, H_4, H_5), 1.20 (s, 3H, H_1), 1.09 (m, 1H, H_4), 0.80 (d, J = 6.8 Hz, 3H, H_{10}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 110.2 (s, C_2), 67.7 (t, C_6), 64.5 (t, 2C, C_{10}), 36.3 (t, C_3), 35.7 (d, C_5), 27.1 (t, C_4), 23.7 (q, C_1), 16.6 (q, C_8).

HRMS (ESI) *m/z*: calculated for $C_9H_{18}O_3Na$ $[M+Na]^+$ 197.1148, found 197.1148.

III.4.1.5. Synthesis of (3R,4R,5R)-3-ethyl-5-methyl-7-(2-methyl-1,3-dioxolan-2-yl)-hept-1-en-4-ol (III.7)



MW (g/mol): 242.3544 **Molecular formula:** $C_{14}H_{26}O_3$

DMSO (1 mL, 12 mmol) was added slowly to an oxalyl chloride solution (0.53 mL, 6 mmol) in CH_2Cl_2 (20 mL) at –78 ºC and the reaction mixture was stirred for 30 min. (*R*)-2-methyl-4-(2-methyl-1,3-dioxolan-2-yl)-butan-1-ol (**III.5**) (520 mg, 3.0 mmol) was then added dropwise and the reaction was stirred for 30 min at the same temperature. Et_3N (2.5 mL, 18 mmol) was then added and the reaction mixture was allowed to stir at rt before the reaction was quenched with a saturated aqueous NH_4Cl solution (10 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Hexane was then added and the precipitate was filtered over Celite©. The solvent was removed under reduced pressure and the resulting crude aldehyde **III.6** was used in the next step without further purification.

To a stirred suspension of *t*-BuOK (400 mg, 3.3 mmol) and *cis*-butene (0.7 mL, 6.6 mmol) in THF (3.5 mL) at -78 °C was added *n*-BuLi (1.9 mL of a 2.5 M solution in hexane) dropwise. After complete addition, the reaction mixture was stirred for 5 min at

–50 °C. The resulting orange solution was then cooled to -78 °C and to it, was added (–)-Ipc$_2$BOMe (1.4 g, 4.2 mmol) in diethyl ether (3.2 mL). After stirring for 30 min at –78 °C, $BF_3{\cdot}Et_2O$ (0.6 mL, 4.5 mmol) was added followed by the aldehyde **III.6** (3.0 mmol) in THF (3.2 mL). The reaction mixture was then stirred overnight at the same temperature before it was treated with a 3M aqueous solution of NaOH and H_2O_2, and refluxed for 1 h. The reaction mixture was then extracted with EtOAc, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/EtOAc, 95:5) to afford (3*R*,4*R*,5*R*)-3-ethyl-5-methyl-7-(2-methyl-1,3-dioxolan-2-yl)-hept-1-en-4-ol (**III.7**) along with its minor diastereoisomer (dr = 91:9) in 59% yield (428 mg) as a colorless oil.

Major isomer:

***R*$_f$** (PE/EtOAc, 8:2): 0.33.

[α]$_D^{20}$ +10.4 (*c* 0.99, $CHCl_3$).

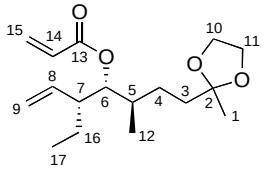
IR (neat): 3452, 3073, 2959, 2933, 2875, 1641, 1514, 1460, 1377, 1319, 1249, 1223, 1175, 1143, 1114, 1067, 1037, 997, 986, 946, 912 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60 (ddd, *J* = 17.1, 10.0, 9.3 Hz, 1H, H_8), 5.15-5.03 (m, 2H, H_9), 4.02-3.92 (m, 4H, H_{10}, H_{11}), 3.28 (dd, *J* = 10.5, 4.8 Hz, 1H, H_6), 2.10 (m, 1H, H_7), 1.82-1.58 (m, 5H, H_3, H_4, H_{13}, H_{14}), 1.51 (m, 1H, H_4), 1.32 (s, 3H, H_1), 1.29-1.12 (m, 2H, H_5, H_{14}), 0.93 (d, *J* = 7.0 Hz, 3H, H_{12}), 0.87 (t, *J* = 7.5 Hz, 3H, H_{15}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 139.8 (d, C_8), 116.1 (t, C_9), 110.3 (s, C_2), 78.9 (d, C_6), 64.6 (t, 2C, C_{10}, C_{11}), 49.4 (d, C_7), 36.6 (t, C_3), 35.7 (d, C_5), 24.3 (t, C_4), 23.7 (q, C_1), 21.8 (t, C_{14}), 16.8 (q, C_{12}), 11.8 (q, C_{15}).

HRMS (ESI) *m/z*: calculated for $C_{14}H_{26}O_3Na$ $[M+Na]^+$ 265.1774, found 265.1778.

*III.4.1.6. Synthesis of acrylic acid (1*R*,2*R*)-2-ethyl-1-[(*R*)-1-methyl-3-(2-methyl-1,3-dioxolan-2-yl)-propyl]-but-3-enyl ester*



MW (g/mol): 296.4018 **Molecular formula:** $C_{17}H_{28}O_4$

To a solution of (3*R*,4*R*,5*R*)-3-ethyl-5-methyl-7-(2-methyl-1,3-dioxolan-2-yl)-hept-1-en-4-ol (**III.7**) (290 mg, 1.2 mmol) in CH_2Cl_2 (6.5 mL) at 0 °C was added dropwise DIEA (1.2 mL, 7.2 mmol) followed by acryloyl chloride (0.3 mL, 3.6 mmol). The resulting reaction mixture was stirred at rt for 2 h and quenched with water (10 mL). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/EtOAc, 98:2) to afford acrylic acid (1*R*,2*R*)-2-ethyl-1-[(*R*)-1-methyl-3-(2-methyl-1,3-dioxolan-2-yl)-propyl]-but-3-enyl ester) along with its minor diastereoisomer (dr = 91:9) in 99% yield (355 mg) as a colorless oil.

Major isomer:

***R*$_f$** (PE/EtOAc, 1:1): 0.45.

[α]$_D^{20}$ –1.88 (*c* 1.54, $CHCl_3$).

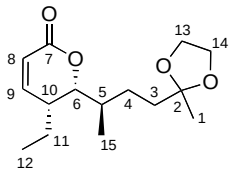
IR (neat): 3076, 2962, 2936, 2877, 1723, 1637, 1460, 1404, 1378, 1294, 1268, 1192, 1148, 1117, 1067, 1043, 984, 970, 917 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.40 (d_{app}, *J* = 17.3 Hz, 1H, H_{15}), 6.14 (dd_{app}, *J* = 17.3, 10.4 Hz, 1H, H_{14}), 5.83 (d_{app}, *J* = 10.4 Hz, 1H, H_{15}), 5.48 (td, *J* = 16.9, 9.5 Hz, 1H, H_8), 5.14-5.02 (m, 2H, H_9), 4.89 (dd, *J* = 8.6, 3.8 Hz, 1H, H_6), 4.01-3.86 (m, 4H, H_{10}, H_{11}), 2.28 (m, 1H, H_7), 1.78 (m, 1H, H_5), 1.73-1.56 (m, 2H, H_3), 1.54-1.40 (m, 2H, H_4, H_{16}), 1.30 (s, 3H, H_1), 1.24-1.11 (m, 2H, H_4, H_{16}), 0.87 (d, *J* = 6.9 Hz, 3H, H_{12}), 0.81 (t, *J* = 7.5 Hz, 3H, H_{17}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2 (s, C_{13}), 138.3 (d, C_8), 130.6 (t, C_{15}), 128.6 (d, C_{14}), 117.0 (t, C_9), 110.1 (s, C_2), 79.8 (d, C_6), 64.5 (t, 2C, C_{10}, C_{11}), 48.0 (d, C_7), 36.7 (t, C_3), 34.9 (d, C_5), 24.0 (t, C_4), 23.6 (q, C_1), 22.8 (t, C_{16}), 16.7 (q, C_{12}), 11.5 (q, C_{17}).

HRMS (ESI) *m/z*: calculated for $C_{17}H_{28}O_4Na$ $[M+Na]^+$ 319.1880, found 319.1875.

III.4.1.7. Synthesis of (5R,6R)-5-ethyl-6-[(R)-1-methyl-3-(2-methyl-1,3-dioxolan-2-yl)-propyl]-5,6-dihydro-pyran-2-one (III.9)



MW (g/mol): 268.3487 **Molecular formula:** $C_{15}H_{24}O_4$

To a stirred solution of acrylic acid (1*R*,2*R*)-2-ethyl-1-[(*R*)-1-methyl-3-(2-methyl-1,3-dioxolan-2-yl)-propyl]-but-3-enyl ester (200 mg, 0.67 mmol) in CH_2Cl_2 (10 mL) was added Grubbs second generation catalyst (57 mg, 0.067 mmol), and the resulting reaction mixture was refluxed for 24 h. The solvent was then removed under reduced pressure and the crude residue was purified by flash column chromatography (PE/EtOAc, 75:25) to afford (5*R*,6*R*)-5-ethyl-6-[(*R*)-1-methyl-3-(2-methyl-1,3-dioxolan-2-yl)-propyl]-5,6-dihydro-pyran-2-one (**III.9**) (117 mg, 67%) as a colorless oil.

R_f (PE/EtOAc, 7:3): 0.28.

[α]$_D^{20}$ –157.8 (*c* 0.68, $CHCl_3$).

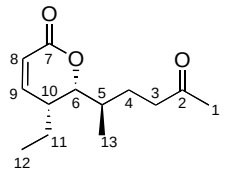
IR (neat): 2965, 2932, 2877, 1717, 1463, 1379, 1290, 1251, 1134, 1099, 1060, 1028, 984, 823 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (m, 1H, H_9), 6.03 (dd, J = 9.7, 2.4 Hz, 1H, H_8), 3.98 (dd, J = 10.4, 2.4 Hz, 1H, H_6), 3.94-3.88 (m, 4H, H_{13}, H_{14}), 2.32 (m, 1H, H_{10}), 2.02 (m, 1H, H_4), 1.88 (m, 1H, H_5), 1.82-1.53 (m, 3H, H_3, H_{11}), 1.47 (m, 1H, H_{11}), 1.31 (s, 3H, H_1), 1.18 (m, 1H, H_4), 0.95 (t, J = 7.5 Hz, 3H, H_{12}), 0.88 (d, J = 6.7 Hz, 3H, H_{15}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8 (s, C_7), 151.0 (d, C_9), 120.9 (d, C_8), 110.0 (s, C_2), 84.4 (d, C_6), 64.6 (t, C_{14}), 64.5 (t, C_{13}), 36.6 (d, C_{10}), 35.8 (t, C_3), 33.5 (d, C_5), 26.7 (t, C_4), 23.8 (q, C_1), 20.1 (t, C_{11}), 14.7 (q, C_{15}), 11.0 (q, C_{12}).

HRMS (ESI) *m/z*: calculated for $C_{15}H_{24}O_4Na$ $[M+Na]^+$ 291.1567, found 291.1558.

III.4.1.8. Synthesis of (5R,6R)-5-ethyl-6-((R)-1-methyl-4-oxo-pentyl)-5,6-dihydro-pyran-2-one (III.10)



MW (g/mol): 224.2961 **Molecular formula:** $C_{13}H_{20}O_3$

A stirred solution of (5*R*,6*R*)-5-ethyl-6-[(*R*)-1-methyl-3-(2-methyl-1,3-dioxolan-2-yl)-propyl]-5,6-dihydro-pyran-2-one (**III.9**) (110 mg, 0.4 mmol) and PPTS (41.2 mg, 0.16 mmol) in a 5:1 acetone/H_2O mixture (10 mL) was heated at reflux for 4 h. The reaction mixture was allowed to cool down to rt and was extracted with EtOAc. The combined organic layers were washed with H_20 (10 mL) and brine (10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/EtOAc, 75:25) to afford (5*R*,6*R*)-5-ethyl-6-((*R*)-1-methyl-4-oxo-pentyl)-5,6-dihydro-pyran-2-one (**III.10**) (85 mg, 95%) as a colourless oil.

R_f (PE/EtOAc, 1:1): 0.36.

$[\alpha]_D^{20}$ –228.2 (*c* 0.57, $CHCl_3$).

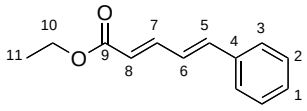
IR (neat): 2967, 2935, 2878, 1712, 1463, 1379, 1288, 1251, 1166, 1148, 1111, 1085, 1060, 1025, 983, 936, 864, 823 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (dd, *J* = 9.7, 6.5 Hz, 1H, H_9), 5.97 (d, *J* = 9.7 Hz, 1H, H_8), 3.95 (dd, *J* = 10.4, 3.0 Hz, 1H, H_6), 2.55-2.37 (m, 2H, H_3), 2.26 (m, 1H, H_{10}), 2.09 (s, 3H, H_1), 2.05 (m, 1H, H_4), 1.81 (m, 1H, H_5), 1.59 (m, 1H, H_{11}), 1.50-1.32 (m, 2H, H_4, H_{11}), 0.89 (t, *J* = 7.5 Hz, 3H, H_{12}), 0.83 (d, *J* = 6.8 Hz, 3H, H_{13}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 208.9 (s, C_2), 164.6 (s, C_7), 151.0 (d, C_9), 120.8 (d, C_8), 84.4 (d, C_6), 41.2 (t, C_3), 36.5 (d, C_{10}), 33.1 (d, C_5), 29.8 (q, C_1), 27.1 (t, C_4), 20.1 (t, C_{11}), 15.0 (q, C_{13}), 10.9 (q, C_{12}).

HRMS (ESI) *m/z*: calculated for $C_{13}H_{20}O_3Na$ $[M+Na]^+$ 247.1305, found 247.1299.

*III.4.1.9. Synthesis of ethyl 5-phenylpenta-(2**E**,4**E**)-dienoate (III.14)*[273]



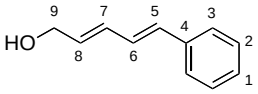
MW (g/mol): 202.2491 **Molecular formula:** $C_{13}H_{14}O_2$

To a stirred solution of ethyl (triphenylphosphoranylidene)acetate (9.6 g, 27.6 mmol) in anhydrous THF (60 mL) was added cinnamaldehyde (2.1 mL, 15.9 mmol). The reaction mixture was stirred at rt for 20 h (TLC monitoring). The reaction was quenched with H_2O (50 mL) and the organic phase was separated. The aqueous phase was extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/EtOAc, from 98:2 to 95:5) to afford compound **III.14** (3.0 g, 94%) as a colourless oil. Its spectroscopic and physical data matched the ones reported in the literature.[273]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.28 (m, 6H, H_1, H_2, H_3, H_7), 6.88 (m, 2H, H_5, H_6), 5.99 (d, J = 15.5 Hz, 1H, H_8), 4.24 (q, J = 7.1 Hz, 2H, H_{10}), 1.32 (t, J = 7.1 Hz, 3H, H_{11}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1 (s, C_9), 144.5 (d, C_7), 140.4 (d, C_5), 136.1 (s, C_4), 129.0 (d, 2C, C_2 or C_3), 128.8 (d, 2C, C_2 or C_3), 127.1 (d, C_1), 126.3 (d, C_6), 121.4 (d, C_8), 60.3 (t, C_{10}), 14.3 (q, C_{11}).

*III.4.1.10. Synthesis of (2**E**,4**E**)-5-phenyl-2,4-pentadiene-1-ol (III.15)*[261]



MW (g/mol): 160.2124 **Molecular formula:** $C_{11}H_{12}O$

To a stirred solution of **III.14** (1.81 g, 8.94 mmol) in anhydrous toluene (30 mL) was added a solution of DIBAL-H in toluene (1 M, 22.5 mL, 22.37 mmol) dropwise at 0 ºC. The reaction mixture was stirred at rt for 2 h (TLC monitoring). The reaction was quenched with saturated aqueous solution of Rochelle salt (50 mL) and the stirred

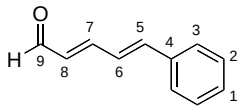
[273] Concellón, J. M.; Concellón, C.; Méjica, C. *J. Org. Chem.* **2005**, *70*, 6111-6113.

vigorously for 90 min. The organic phase was separated and the aqueous phase was extracted with EtOAc (50 mL). The combined organic layers were washed with brine (40 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/EtOAc, 85:15) to afford compound **88** (1.4 g, quant.) as a white solid. Its spectroscopic and physical data matched the ones reported in the literature.[261]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47-7.30 (m, 4H, H_2, H_3), 7.23 (m, 1H, H_1), 6.79 (dd, J = 15.7, 10.5 Hz, 1H, H_6), 6.56 (d, J = 15.7 Hz, 1H, H_5), 6.43 (dd_{app}, J = 15.2, 10.5 Hz, 1H, H_7), 5.95 (dt, J = 15.2, 5.9 Hz, 1H, H_8), 4.17 (dd, J = 5.9, 1.5 Hz, 2H, H_9), 1.49 (br s, 1H, OH).

^{13}C NMR (100 MHz, $CDCl_3$) δ 137.1 (s, C_4), 132.8 (d, C_5), 132.5 (d, C_8), 131.6 (d, C_6), 128.6 (d, 2C, C_2 or C_3), 128.2 (d, C_7), 127.7 (d, C_1), 126.4 (d, 2C, C_2 or C_3), 63.4 (t, C_9).

*III.4.1.11. Synthesis of (2*E*,4*E*)-5-phenyl-penta-2,4-dien-1-al (III.16)*[274]



MW (g/mol): 158.1965 **Molecular formula:** $C_{11}H_{10}O$

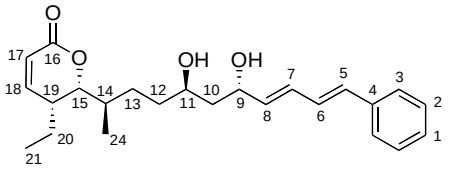
To a stirred solution of **III.15** (1.4 g, 8.4 mmol) in CH_2Cl_2 (50 mL) was added activated MnO_2 (5.9 g, 68 mmol). The reaction mixture was stirred at rt for 20 h (reaction monitored by TLC). The reaction mixture was filtered over Celite® and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (PE/EtOAc, from 90:10 to 85:15) to afford compound **76** (1.24 g, 90%) as a yellow solid. Its spectroscopic and physical data matched the ones reported in the literature.[274]

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (d, J = 8.0 Hz, 1H, H_9), 7.53 (m, 2H, H_3), 7.45-7.36 (m, 3H, H_1, H_2), 7.29 (m, 1H, H_7), 7.04 (m, 2H, H_5, H_6), 6.30 (dd, J = 15.2, 8.0 Hz, 1H, H_8).

[274] Macía, F.; García-Díaz, M.; Massanet, G. M.; Gómez-Madero, J.; Fronczek, F. R.; Galindo, J. C. G. *Tetrahedron* **2008**, *64*, 10996-11006.

^{13}C NMR (100 MHz, $CDCl_3$) δ 193.6 (d, C_9), 152.1 (d, C_7), 142.5 (d, C_5), 135.6 (s, C_4), 131.6 (d, C_8), 129.7 (d, 2C, C_2 or C_3), 129.0 (d, C_2 or C_3), 127.6 (d, C_1), 126.2 (d, C_6).

III.4.1.12. Synthesis of (5R,6R)-6-((7E,9E)-(1R,4R,6S)-4,6-Dihydroxy-1-methyl-10-phenyl-deca-7,9-dienyl)-5-ethyl-5,6-dihydro-pyran-2-one. (–)-Bitungolide F



MW (g/mol): 384.5085 **Molecular formula:** $C_{24}H_{32}O_4$

To a cooled (–78 °C) solution of (+)-Ipc_2BCl (70 mg, 0.22 mmol) in Et_2O (1.5 mL) were added a solution of Et_3N (0.1 mL, 0.44 mmol) afford (5*R*,6*R*)-5-ethyl-6-((*R*)-1-methyl-4-oxo-pentyl)-5,6-dihydro-pyran-2-one (**III.10**) (25 mg, 0.11 mmol) in Et_2O (1 mL) dropwise. The resulting reaction mixture was stirred at –78 °C for 30 min before the temperature was brought to –40 °C. After stirring at –40 °C for 1 h, the reaction mixture was cooled back to –78 °C and a solution of aldehyde **III.16** (45 mg, 0.28 mmol) in Et_2O (1 mL) was added *via* cannula. The reaction mixture was then stirred at –78 °C for 2 h and stored at -20 °C for 14 h. To the cooled reaction mixture (0 °C) was added a premixed solution of MeOH (3.9 mL) and pH 7 buffer (1.2 mL) and stirring was continued for 10 min. A premixed solution of pH 7 buffer (3 mL) and 30% H_2O_2 (1.5 mL) was then added dropwise and the resulting reaction mixture was stirred vigorously at 0 °C for 2.5 h before being diluted with H_2O (10 mL). Et_2O was then added and the layers were separated. The aqueous phase was extracted with Et_2O (20 mL) and the combined organic extracts were washed with $NaHCO_3$ (20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was used in the next step without further purification.

To a –40 °C precooled solution of the crude aldol product in acetonitrile (1 mL) were added tetramethylammonium triacetoxyborohydride (290 mg, 1.1 mmol) and glacial acetic acid (1 mL) dropwise. The resulting mixture was stirred at -20 °C for 10 h. A saturated aqueous solution of sodium potassium tartrate and EtOAc was added and the reaction mixture was stirred vigorously at rt for an additional 30 min. The mixture

was extracted with EtOAc and the combined organic layers was washed with water, $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography (PE/EtOAc, 1:1) to afford (–)-bitungolide F (35 mg, 82%) as a pale yellow solid.

***R*$_f$** (PE/EtOAc, 1:1): 0.32.

[α]$_D^{20}$ -51.5 (*c* 1.56, $CHCl_3$); (lit.[214] **[α]$_D^{22}$** +43.0 (*c* 0.85, $CHCl_3$).

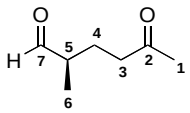
IR (neat): 3392, 3024, 2965, 2933, 2876, 1707, 1596, 1492, 1448, 1383, 1289, 1256, 1116, 1060, 1025, 988, 910, 823 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 7.3 Hz, 2H, H_3), 7.30 (t, *J* = 7.3 Hz, 2H, H_2), 7.21 (m, 1H, H_1), 7.07 (dd, *J* = 9.7, 6.5 Hz, 1H, H_{18}), 6.78 (dd, *J* = 15.6, 10.5 Hz, 1H, H_6), 6.53 (d, *J* = 15.6 Hz, 1H, H_5), 6.45 (dd, *J* = 15.2, 10.5 Hz, 1H, H_7), 6.03 (d, *J* = 9.7 Hz, 1H, H_{17}), 5.89 (dd, *J* = 15.2, 5.9 Hz, 1H, H_8), 4.59 (m, 1H, H_9), 3.99 (dd, *J* = 10.4, 3.0 Hz, 1H, H_{15}), 3.96 (m, 1H, H_{11}), 3.28-2.98 (2brs, 2H, OH), 2.32 (m, 1H, H_{19}), 2.01-1.82 (m, 2H, H_{13}, H_{14}), 1.80-1.72 (m, 2H, H_{10}), 1.71-1.52 (m, 2H, H_{12}, H_{20}), 1.51-1.33 (m, 2H, H_{12}, H_{20}), 1.29 (m, 1H, H_{13}), 0.94 (t, *J* = 7.5 Hz, 3H, H_{21}), 0.89 (d, *J* = 6.8 Hz, 3H, H_{24}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1 (s, C_{16}), 151.4 (d, C_{18}), 137.3 (s, C_4), 136.4 (d, C_8), 132.4 (d, C_5), 130.1 (d, C_7), 128.6 (d, 2C, C_2), 128.4 (d, C_6), 127.5 (d, C_1), 126.4 (d, 2C, C_3), 120.8 (d, C_{17}), 84.5 (d, C_{15}), 70.1 (d, C_9), 69.1 (d, C_{11}), 42.7 (t, C_{10}), 36.5 (d, C_{19}), 34.4 (t, C_{12}), 33.5 (d, C_{14}), 28.5 (t, C_{13}), 20.1 (t, C_{20}), 14.9 (q, C_{24}), 11.0 (q, C_{21}).

HRMS (ESI) *m/z*: calculated for $C_{24}H_{32}O_4Na$ $[M+Na]^+$ 407.2193, found 407.2194.

*III.4.1.13. Synthesis of (*R*)-2-methyl-5-oxo-hexanal (III.27)*[267]



MW (g/mol): 128.1690 **Molecular formula:** $C_7H_{12}O_2$

Propanal **III.25** (0.95 mL, 13.24 mmol) and 3-buten-2-one **III.26** (1.61 mL, 19.86 mmol) were added at 0 ºC to a mixture of diphenylprolinol methyl ether (**III.18**) (177 mg, 0.662 mmol) and ethyl 3,4-dihydroxybenzoate (**III.19**) (482 mg, 2.64 mmol) in a sealed vial. The reaction mixture was stirred at 4 °C for 36 h, time after which it

was filtered over a short plug of silica eluting with an 8:2 pentane/Et_2O mixture to afford (*R*)-2-methyl-5-oxo-hexanal (**III.27**) as a colorless oil (972 mg, 58%, er > 95:5). The spectroscopic and physical data of the product were identical with those reported in the literature.[267]

***R*$_f$** (Pentane/Et_2O, 8:2): 0.29.

[α]$_D^{20}$ +1.77 (*c* 1.8, $CHCl_3$).

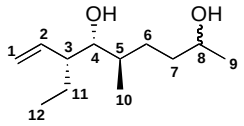
IR (neat): 2969, 2934, 1713, 1456, 1413, 1357, 1167, 972, 926 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.61 (d, J = 1.8 Hz, 1H, H_7), 2.49 (m, 2H, H_3), 2.38 (qd_{app}, J = 7.3, 1.8 Hz, 1H, H_5), 2.15 (s, 3H, H_1), 1.96 (hex_{app}, J = 7.3 Hz, 1H, H_4), 1.67 (m, 1H, H_4), 1.12 (d, J = 7.3 Hz, 3H, H_6).

^{13}C NMR (100 MHz, $CDCl_3$) δ 207.8 (s, C_2), 204.4 (d, C_7), 45.5 (d, C_5), 40.5 (t, C_3), 29.9 (q, C_1), 24.0 (t, C_4), 13.5 (q, C_6).

HRMS (ESI) *m/z*: calculated for $C_{24}H_{32}O_4Na$ $[M+Na]^+$ 407.2193, found 407.2194.

III.4.1.14. Synthesis of (5R,6R,7R)-7-ethyl-5-methyl-non-8-ene-2,6-diol (III.30)



MW (g/mol): 200.3178 **Molecular formula:** $C_{12}H_{24}O_2$

To a stirred suspension of *t*-BuOK (693 mg, 6.1 mmol) and *cis*-2-pentene (1.33 mL, 12.3 mmol) in THF (5.6 mL) at –78 °C was added *n*-BuLi (3.8 mL of a 1.6 M solution in hexane, 6.1 mmol) dropwise. After complete addition, the reaction mixture was stirred for 5 min at –50 °C and the resulting orange solution was cooled to –78 °C. To this reaction mixture was added a solution of (–)-Ipc_2BOMe (2.4 g, 7.5 mmol) in diethyl ether (5.9 mL). After stirring for 30 min at –78 °C, $BF_3{\cdot}Et_2O$ (1.1 mL, 8.4 mmol) was added followed by (*R*)-2-methyl-5-oxo-hexanal (**III.27**) (720 mg, 5.6 mmol) in THF (4.7 mL). The reaction mixture was then stirred overnight at the same temperature before it was treated with a 3M aqueous solution of NaOH (4.6 mL, 13.8 mmol) and H_2O_2 (1.9 mL of a 35% solution in water, 30.1 mmol) and refluxed for 1 h. The phases

were then separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure.

The crude residue was then dissolved in THF (18 mL) and cooled to 0 ºC before $LiAlH_4$ (533 mg, 11.23 mmol) was added in portions. The resulting reaction mixture was stirred at rt for 2 h until complete conversion of the starting material (reaction monitored by TLC) and quenched by adding EtOAc (20 mL) and water (20 mL). The reaction mixture was then filtered through Celite©, the organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (PE/EtOAc, 80:20) to afford (5*R*,6*R*,7*R*)-7-ethyl-5-methyl-non-8-ene-2,6-diol (**III.30**) along with its diastereoisomer in 57% yield (600 mg) as a colorless oil.

Mixture of two diastereoisomers:

***R*$_f$** (Pentane/EtOAc, 8:2): 0.07.

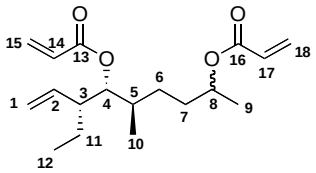
IR (neat): 3340, 2965, 2931, 2874, 2361, 2342, 1459, 1420, 1376, 1128, 998, 984, 912 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 (m, 1H, H_2), 5.09-5.03 (m, 2H, H_1), 3.71 (m, 1H, H_8), 3.22 (m, 1H, H_4), 2.01 (m, 1H, H_3), 1.75-1.16 (m, 9H, H_5, H_6, H_7, H_{11}, OH), 1.12/1.11 (d, J = 6.3 Hz, 3H, H_9), 0.87/0.86 (d, J = 6.8 Hz, 3H, H_{10}), 0.80 (t, J = 7.3 Hz, 3H, H_{12}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 139.9/139.8 (t, C_1), 116.2/116.1 (d, C_2), 78.9/78.8 (d, C_4), 68.6/67.9 (d, C_8), 49.4 (d, C_3), 36.9/36.5 (t, C_7), 35.7/35.2 (d, C_5), 26.2/26.0 (t, C_6), 23.5/23.4 (q, C_9), 21.8/21.7 (t, C_{11}), 16.9/16.8 (q, C_{10}), 11.8 (q, C_{12}).

HRMS (ESI) *m/z*: calculated for $C_{12}H_{24}O_2Na$ $[M+Na]^+$ 223.1668, found 223.1670.

*III.4.1.15. Synthesis of acrylic acid (1*R*,2*R*)-5-acryloyloxy-1-((*R*)-1-ethyl-allyl)-2-methyl-hexyl ester (III.31)*



MW (g/mol): 308.4125 **Molecular formula:** $C_{18}H_{28}O_4$

To a solution of (5*R*,6*R*,7*R*)-7-ethyl-5-methyl-non-8-ene-2,6-diol (**III.30**) (600 mg, 2.9 mmol) in CH_2Cl_2 (15 mL) at 0 ºC was added diisopropylethylamine (3 mL, 18.0 mmol) and acryloyl chloride (0.73 mL, 8.9 mmol) dropwise. The resulting reaction mixture was stirred at rt for 2 h until complete conversion of the starting material (reaction monitored by TLC) and quenched by addition of water (20 mL). The phases were separated, the aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL) and the combined organic layers were washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (PE/EtOAc, 98:2) to afford acrylic acid (1*R*,2*R*)-5-acryloyloxy-1-((*R*)-1-ethyl-allyl)-2-methyl-hexyl ester (**III.31**) along with its diastereoisomer quantitatively (922 mg) as a colorless oil.

Mixture of two diastereoisomers:

***R*$_f$** (PE/EtOAc, 8:2): 0.82.

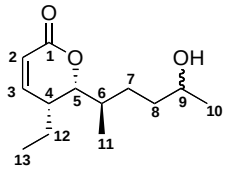
IR (neat) 2968, 1721, 1638, 1619, 1405, 1270, 1194, 1047, 985, 809 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.48-6.35 (m, 2H, H_{15} or H_{18}), 6.20-6.06 (m, 2H, H_{14} or H_{17}), 5.89-5.77 (m, 2H, H_{15} or H_{18}), 5.48 (m, 1H, H_2), 5.18-5.00 (m, 2H, H_1), 4.99-4.86 (m, 2H, H_4, H_8), 2.28 (m, 1H, H_3), 1.82 (m, 1H, H_5), 1.75-1.35 (m, 4H, H_6, H_7, H_{11}), 1.26/1.25 (d, J = 6.3 Hz, 3H, H_9), 1.23-1.06 (m, 2H, H_6, H_{11}), 0.90/0.89 (d, J = 6.8 Hz, 3H, H_{10}), 0.83 (t, J = 7.3 Hz, 3H, H_{12}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2/166.1 (q, C_{13} or C_{16}), 165.8 (s, C_{13} or C_{16}), 138.2 (d, C_2), 130.6/130.5 (t, C_{15} or C_{18}), 130.2/130.1 (t, C_{15} or C_{18}), 129.1 (d, C_{14} or C_{17}), 128.6/128.5 (d, C_{14} or C_{17}), 117.1 (t, C_1), 79.8/79.7 (d, C_4), 71.5/71.1 (d, C_8), 48.0 (d, C_3), 34.6/34.5 (d, C_5), 33.5/33.4 (t, C_7), 25.5/25.1 (t, C_6 or C_{11}), 22.9/22.8 (t, C_6 or C_{11}), 19.9/19.8 (q, C_9), 16.6 (q, C_{10}), 11.5 (q, C_{12}).

HRMS (ESI) *m/z*: calculated for $C_{18}H_{28}O_4Na$ $[M+Na]^+$ 331.1880, found 331.1880.

*III.4.1.16. Synthesis of (5**R**,6**R**)-5-ethyl-6-((**R**)-4-hydroxy-1-methyl-pentyl)-5,6-dihydro-pyran-2-one (III.33)*



MW (g/mol): 226.3120 **Molecular formula:** $C_{13}H_{22}O_3$

To a solution of acrylic acid (1*R*,2*R*)-5-acryloyloxy-1-((*R*)-1-ethyl-allyl)-2-methyl-hexyl ester (**III.31**) (370 mg, 1.2 mmol) in CH_2Cl_2 (15 mL) was added Grubbs second generation catalyst (101 mg, 0.2 mmol) and the resulting reaction mixture was refluxed for 24 h. The solvent was then removed under reduced pressure and the crude residue was filtered over a plug of silica.

The crude ester was then dissolved in MeOH (13 mL) and cooled to 0 ºC. K_2CO_3 (852 mg, 6.0 mmol) was then added and the resulting reaction mixture was stirred at rt for 2 h. EtOAc and water were added and the organic phase was separated. The aqueous layer was extracted with EtOAc (2 x 30 mL) and the combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was finally purified by flash column chromatography (PE/EtOAc, 60:40) to afford (5*R*,6*R*)-5-ethyl-6-((*R*)-4-hydroxy-1-methyl-pentyl)-5,6-dihydro-pyran-2-one (**III.33**) as a pale yellow oil (136 mg, 50%).

$\boldsymbol{R_f}$ (PE/EtOAc, 1:1): 0.20.

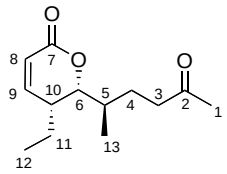
IR (neat): 3413, 2966, 2924, 1715, 1463, 1382, 1256, 1091, 1062, 1024, 910, 825, 731 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (dd, J = 9.5, 6.5 Hz, 1H, H_3), 5.97 (d, J = 9.8 Hz, 1H, H_2), 3.93 (dd, J = 10.5, 3.0 Hz, 1H, H_5), 3.75 (m, 1H, H_9), 2.25 (m, 1H, H_4), 1.96-1.70 (m, 2H, H_6, H_7), 1.69-1.25 (m, 6H, H_7, H_8, H_{12}, OH), 1.15/1.13 (d, J = 6.3 Hz, 3H, H_{10}), 0.89 (t, J = 7.5 Hz, 3H, H_{13}), 0.84 (d, J = 6.8 Hz, 3H, H_{11}).

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0 (s, C_1), 151.2 (d, C_3), 120.8 (d, C_2), 84.4/84.3 (d, C_5), 68.4/68.2 (d, C_9), 36.6/36.5 (d, C_4), 36.0/35.9 (t, C_8), 33.6 (d, C_6), 28.6/28.5 (t, C_7), 23.8/23.6 (q, C_{10}), 20.1 (t, C_{12}), 14.8 (q, C_{11}), 11.0 (q, C_{13}).

HRMS (ESI) *m/z*: calculated for $C_{13}H_{22}O_3Na$ $[M+Na]^+$ 249.1461, found 249.1463.

III.4.1.17. Synthesis of (5R,6R)-5-ethyl-6-((R)-1-methyl-4-oxo-pentyl)-5,6-dihydro-pyran-2-one (III.10)



To a solution of alcohol **III.33** (51 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) at 0 ºC was added Dess-Martin periodinane (143 mg, 0.3 mmol). The resulting reaction mixture was stirred at rt for 2 h and quenched by addition of a saturated aqueous $NaHCO_3$ solution (10 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (CH_2Cl_2/EtOAc, 98:2) to afford (5*R*,6*R*)-5-ethyl-6-((*R*)-1-methyl-4-oxo-pentyl)-5,6-dihydro-pyran-2-one (**III.10**) (42 mg, 84%) as a colourless oil. The spectroscopic and physical data of the product were identical with those reported previously.

GENERAL CONCLUSIONS

According to the main objective of this thesis which was the synthesis of biologically active compounds, we can conclude that:

- We have successfully synthesized three types of pyrimidinyl α-amino acids including N^{α}-Fmoc-pyrimidin-2-yl α-amino acids, N^{α}-Fmoc-pyrimidin-4-yl α-amino acids and N^{α}-Fmoc-pyrimidin-2-one α-amino acids *via* either a key phosphonium coupling or a nucleophilic aromatic substitution.

- We have demonstrated the use of these pyrimidinyl α-amino acids as building blocks in the preparation of antimicrobial **BP100**-analogues.

- We have found three peptide sequences, **BP295**, **BP299** and **BP303** that present a good balance between antimicrobial and hemolytic activities.

- We have synthesized the (2*R*,3*S*,5*R*,6*S*)-isomer of natural product acremolide B in 16 steps and 7.6% overall yield using a highly flexible and particularly straightforward strategy starting from commercially available (*S*)-Roche ester.

- We have finalized the first synthesis of the proposed structure of lyngbouilloside aglycon in 21 steps and 2.1% overall yield starting from 3-methylbut-3-enol and 4-pentenal.

- We have completed the synthesis of the dual-specificity phosphatase inhibitor (–)-bitungolide F using two very convergent routes. While the first synthesis was achieved in 11 steps and 14.6% overall yield starting from levulinic acid, the second one, entirely protecting group free, was accomplished in only nine steps and 11.4% overall yield starting from two simple and readily available compounds; such as propanal and 3-buten-2-one.

References

[1] Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Chemical approaches to the synthesis of peptides and proteins*, Florida, CRC Press, **1997**.

[2] Bhat, S. V.; Nagasampagi, B. A.; Sivakumar, M. *Chemistry of Natural Products*, Berlin; New York, Springer, **2005**.

[3] The three-letter and one-letter codes for each amino acid are given below the chemical name.

[4] Cahn, R. S.; Ingold, C.; Prelog, V. *Angew. Chem. Int. Ed. Engl.* **1966**, *5*, 385-415.

[5] Hughes, A. B. *Amino Acids, Peptides and Proteins in Organic Chemistry*. Vol. 1-*Origins and Synthesis of Amino Acids*, Weinheim, WILEY-VCH Verlag GmbH & Co. KGaA, **2009**.

[6] (a) Lipmann, F. *Science* **1971**, *173*, 875-884. (b) Kleinkauf, H.; Von Döhren, *Eur. J. Biochem.* **1996**, *236*, 335-351.

[7] Wagner, I.; Musso, H. *Angew. Chem. Int. Ed. Engl*. **1983**, *22*, 816-828.

[8] Kingston, D. G. I. *J. Nat. Prod*. **2000**, *63*, 726-734.

[9] Ordoñez, M.; Ctiviela, C. *Tetrahedron: Asymmetry* **2007**, *18*, 3-99.

[10] Stratmann, K.; Burgoyne, D. L.; Moore, R. E.; Patterson, G. M. L. *J. Org. Chem.* **1994**, *59*, 7219-7226.

[11] Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. *J. Am. Chem. Soc.* **1987**, *109*, 6883-6885.

[12] (a) Willians, R. M.; Hendrix, J. A. *Chem. Rev*. **1992**, *92*, 889-917. (b) Beenen, M. A.; Weix, D. J.; Ellman, J. A. *J. Am. Chem. Soc.* **2006**, *128*, 6304-6305. (c) Shang, G.; Yang, Q.; Zhang, X. *Angew. Chem. Int. Ed*. **2006**, *45*, 6360-6362.

[13] Vogt, H.; Bräse, S. *Org. Biomol. Chem.* **2007**, *5*, 406-430.

[14] (a) Van Esseveldt, B. C. J.; Vervoort, P. W. H.; Van Delft, F. L.; Rutjes, F. P. J. T. *J. Org. Chem.* **2005**, *70*, 1791-1795. (b) Onomura, O.; Kirira, P. G.; Tanaka, T.; Tsukada, S.; Matsumura, Y.; Demizu, Y. *Tetrahedron* **2008**, *64*, 7498-7503.

[15] Feliu, L.; Planas, M. *Int. J. Pept. Res. Ther*. **2005**, *11*, 53-97.

[16] (a) Meyer, U.; Bisel, P.; Bräuner-Osborne, H.; Madsen, U.; Höfner, G.; Wanner, K. TH.; Frahm, A. W. *Chirality* **2005**, *17*, 99-107. (b) Trabocchi, A.; Scarpi, D.; Guarna, A. *Amino Acids* **2008**, *34*, 1-24.

[17] Takita, S.; Yokoshima, S.; Fukuyama, T. *Org. Lett.* **2011**, *13*, 2068-2070.

[18] Cyclosporin is an immunosuppressant drug widely used in post-allogeneic organ transplant.

[19] Kolar, P.; Petric, A.; Tisler, M. *J. Heterocyclic Chem.* **1997**, *34*, 1067-1098.

[20] (a) Ye, B.; Burke, T. R. *J. Org. Chem.* **1995**, *60*, 2640-2641. (b) Adamczyk, M.; Akireddy, S. R.; Reddy, R. E. *Org. Lett.* **2001**, *3*, 3157-3159. (c) Wang, H. P.; Hwang, T. L.; Lee, O.; Tseng, Y. J.; Shu, C. Y.; Lee, S. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4272-4274.

[21] (a) Nomura, T.; Tanaka, Y.; Abe, H.; Uchiyama, M. *Phytochemistry* **1977**, *16*, 1819-1820. (b) Son, J. K.; Ramaligam, K.; Woodard, R. W. *Synthesis* **1988**, 240-242.

[22] (a) Li, C.; Oberlines, N. H. *Life Sci.* **2005**, *78*, 532-538. (b) Jørgensen, C. G.; Clausen, P. R.; Hansen, K. B.; Bräuner-Osborne, H.; Nielsen, B; Metzler, B.; Kehler, J.; Krogsgaard-Larsen, P.; Madsen, U. *Org. Biomol. Chem.* **2007**, *5*, 463-471.

[23] (a) Jane, D.; Hoo, K.; Kamboj, R.; Deverill, M.; Bleakman, D.; Mandelzys, A. *J. Med. Chem.* **1997**, *40*, 3645-3650. (b) Dolman, N. P.; More, J. C. A.; Alt, A.; Knauss, J. L.; Pentikäinen, T. O.; Glasser, R. C.; Bleakman, D.; Mayer, M. L.; Collingridge, G. L.; Jane, D. E. *J. Med. Chem.* **2007**, *50*, 1558-1570.

[24] (a) Brown, E. G.; Turan, Y. *Phytochemistry* **1996**, *43*, 1029-1031. (b) Adlington, R. M.; Baldwin, J. E.; Catterick, D.; Pritchard, G. J. *Chem. Commun.* **1997**, 1757-1758.

[25] (a) Kaiser, J.; Kinderman, S. S.; Van Esseveldt, B. C. J.; Van Delft, F. L.; Schoemaker, H. E.; Blaauw, R. H.; Rutjes, F. P. *Org. Biomol, Chem.* **2005**, *3*, 3435-3467. (b) Scott, W. L; Alsina, J.; Audu, C. O.; Babaev, E.; Cook, L.; Dage, J. L.; Goodwin, L. A.; Martynow, J. G.; Matosiuk, D.; Royo, M.; Smith, J. G.; Strong, A. T.; Wickizer, K.; Woerly, E. M.; Zhou, Z.; O'Donnell, M. J. *J. Comb. Chem.* **2009**, *11*, 14-23.

[26] (a) Duthaler, R. O. *Tetrahedron* **1994**, *50*, 1539-1650. (b) Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung, D. *J. Am. Chem. Soc.* **1997**, *119*, 656-673. (c) Hruby, V. J.; Soloshonok, V. A. *Asymmetric synthesis of novel sterically constrained amino acids. Symposia-in-print; Tetrahedron*; Eds.; **2001**, *57*, 6329-6650. (d) Perdih, A.; Sollner, M. D. *Curr. Org. Chem.* **2007**, *11*, 801-832. (e) Nájera, C.; Sansano, J. M. *Chem. Rev.* **2007**, *107*, 4584-4671. (f) Haldar, D. *Curr. Org. Synth.* **2008**, *5*, 61-80.

[27] Blayo, A.-L.; Brunel, F.; Martinez, J.; Fehrentz, J.-A. *Eur. J. Org. Chem.* **2011**, 4293-4297.

[28] Krebs, A.; Ludwig, V.; Pfizer, J.; Dürner, G.; Göbel, M. W. *Chem. Eur. J.* **2004**, *10*, 544-553.

[29] Ge, J.; Wu, H.; Yao, S. Q. *Chem. Commun.* **2010**, *46*, 2980-2982.

[30] (a) Von Angerer, S. *Science of Synthesis. Six-Membered Hetarenes with Two Identical Heteroatoms: Pyrimidines*, Thieme, Stuttgart, **2003**, *16*, 379-572. (b) Rewcastle, G. W. *Comprehensive Heterocyclic Chemistry III: Pyrimidines and their Benzo Derivatives*, Elsevier, **2008**, *8*, 117-272.

[31] Lagoja, I. M. *Chemistry & Biodiversity* **2005**, *2*, 1-50.

[32] Jain, K. S.; Chitre, T. S.; Miniyar, P. B.; Kathiravan, M. K.; Bendre, V. S.; Veer, V. S.; Shahane, S. R.; Shishoo, C. J. *Curr. Sci.* **2006,** *90*, 793-803.

[33] Laimberth, C. *Heterocycles* **2006**, *68*, 561-603.

[34] (a) Stanovnik, B.; Tisler, M.; Katritzky, A. R.; Denisko, O. V. *Advances in Heterocyclic Chemistry*, San Diego, Elsevier, **2006**. (b) Freeman, F.; Po, H. N. *J. Phys. Chem. A* **2006**, *110*, 7904-7912.

[35] Eicher, T.; Hauptmann, S. *The chemistry of heterocycles*, Weinheim, Wiley-VCH, **2003**.

[36] (a) Font, D.; Heras, M.; Villalgordo, J. M. *Synthesis* **2002**, 1833-1842. (b) Font, D.; Heras, M.; Villalgordo, J. M. *J. Comb. Chem.* **2003**, *5*, 311-321. (c) Font, D.; Linden, A.; Heras, M.; Villalgordo, J. M. *Tetrahedron* **2006**, *62*, 1433-1443. (d) Font, D.; Heras, M.; Villalgordo, J. M. *Tetrahedron* **2008**, *64*, 5226-5235.

[37] (a) Jonak, J. P.; Hopkins, G. C.; Minnemeyer, H. J.; Tieckelmann, H. *J. Org. Chem.* **1970**, *35*, 2512-2516. (b) Gambacorta, A.; Farah, M. E.; Tofani, D. *Tetrahedron* **1999**, *55*, 12615-12628.

[38] (a) Barlin, G. B.; Brown, W. V. *J. Chem. Soc. (B)* **1967**, 648-650. (b) Barlin, G. B.; Brown, W. V. *J. Chem. Soc. (B)* **1967**, 736-740.

[39] Mitsunobu, O. *Synthesis* **1981**, 1-28.

[40] Cherney, R. J.; Wang, L. *J. Org. Chem.* **1996**, *61*, 2544-2546.

[41] Agrios, G. N. *Plant Pathology*, 5th Ed., London: Elseiver Academic Press; **2005**.

[42] (a) McManus, P. S.; Stockwell, V. O.; Sundin, V. O.; Jones, A. L. *Annu. Rev. Phytopathol.* **2002**, *40*, 443-465. (b) Vidaver, A. K. *Clin. Infect. Dis.* **2002**, *34*, S107-110.

[43] Karabelas, A. J.; Plakas, K. V.; Solomou, E. S.; Drossou, V. O.; Sarigiannis, D. A. *Environ. Int.* **2009**, *35*, 1096-1107.

[44] Loper, J. E.; Henkels, M. *Plant. Dis.* **1991**, *75*, 287-290.

[45] (a) Hancock, R. E. W.; Sahl, H. G. *Nat. Biotechnol.* **2006**, *24*, 1551-1557. (b) Montesinos, E. *FEMS Microbiol. Lett.* **2007**, *270*, 1-11.

[46] García-Olmedo, F.; Molina, A.; Alamillo, J. M.; Rodríguez-Plalenzuela, P. *Biopolymers (Peptide Science)* **1998**, *47*, 479-491.

[47] Hancock, R. E. W.; Sahl, K. L.; Mookherjee, N. *Immunobiology* **2006**, *211*, 315-322.

[48] Jenssen, H.; Hamill, P.; Hancock, R. E. W. *Clin. Microbiol. Rev.* **2006**, *19*, 491-511.

[49] Ganz, T. *Nat. Rev. Immunol.* **2003**, *3*, 710-720.

[50] (a) Yeaman, M. R.; Yount, N. Y. *Pharmacol. Rev.* **2003**, *55* (1), 27-55. (b) Brogden, K. A. *Nat. Rev. Microbiol.* **2005**, *3*, 238-250.

[51] Melo, M. N.; Ferre, R.; Castanho, M. A. R. B. *Nat. Rev. Microbiol.* **2009**, *7*, 245-250.

[52] Zaloff, M. *Nature* **2002**, *415*, 389-395.

[53] Matsuzaki, K. *Biochim. Biophys. Acta* **2009**, *1788*, 1687-1692.

[54] Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.

[55] Kate, S. A.; Albericio, F. *Solid-phase synthesis a practical guide*, New York, Marcel Dekker, Inc. **2000**.

[56] Carpino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748-5749.

[57] (a) Valeur, E.; Bradley, M.; *Chem. Soc. Rev.* **2009**, *38*, 606-631. (b) Han, S-Y.; Kim, Y-A. *Tetrahedron* **2004**, *60*, 2447-2467.

[58] Marco, J. F.; Muñoz, A.; Perez-Payá, E.; Misra, S.; López-Garcia, B. *Annuv. Rev. Phytopathol.* **2008**, *46*, 273-301.

[59] (a) Vanneste, J. *Fire blight: the disease and its causative agent Erwinia amylovora*. New York: CABI Publishing; **2000**. (b) Cabrefiga, J.; Montesinos, E. *Phytopathology* **2005**, *95*, 1430-1437.

[60] Leyns, F.; De Cleene, M.; Swings, J.; De Ley, J. *Bot. Rev.* **1984**, *50*, 305-355.

[61] Moragrega, C.; Llorente, I.; Manceau, C.; Montesinos, E. *Eur. J. Plant. Pathol.* **2003**, *109*, 319-326.

[62] (a) Ferre, R.; Badosa, E.; Feliu, L.; Planas, M.; Montesinos, E.; Bardají, E. *Appl. Environ. Microbiol.* **2006**, *72*, 3302-3308. (b) Badosa, E.; Ferre, R.; Planas, M.; Feliu, L.; Besalú, E.; Cabrefiga, J.; Bardají, E.; Montesinos, E. *Peptides* **2007**, *28*, 2276-2285.

[63] Adessi, C.; Soto, C. *Curr. Med. Chem.* **2002**, *9*, 963-978.

[64] (a) Hicks, R. P.; Bhnsle, J. B.; Venugopal, D.; Koser, B. W.; Magill, A. L. *J. Med. Chem.* **2007**, *50*, 3026-3036. (b) Jones, M. A.; Notta, J. K.; Cobbold, M.; Palendira, M.; Hislop, A. D.; Wilkie, J.; Snaith, J. S. *J. Pept. Sci.* **2008**, *14*, 313-320. (c) Karstad, R.; Isaksen, G.; Brandsdal, B-O.; Svendsen, J. S.; Svenson, J. *J. Med. Chem.* **2010**, *53*, 5558-5566.

[65] Pritz, S.; Pätzel, M.; Szeimies, G.; Dathe, M.; Bienert, M. *Org. Biomol. Chem.* **2007**, *5*, 1789-1794.

[66] Radi, M.; Schenoneb, S.; Botta, M. *Org. Biomol. Chem.* **2009**, *7*, 2841-2847.

[67] Hill, M. D.; Movassaghi, M. *Chem. Eur. J.* **2008**, *14*, 6836-6844.

[68] (a) Maruenda, H.; Chenna, A.; Liem, L.-K.; Singer, B. *J. Org. Chem.* **1998**, *63*, 4385-4389. (b) Van Brocklin, H. F.; Lim, J. K.; Coffing, S. L.; Hom, D. L.; Negash, K.; Ono, M. Y.; Gilmore, J. L.; Bryant, I.; Riese, D. J. I. I. *J. Med. Chem.* **2005**, *48*, 7445-7456. (c) Al-Abdullah, E. S.; Al-Obaid, A-R. M.; Al-Deeb, O. A.; Habib, E. E.; El-Emam, A. A. *Eur. J. Med. Chem.* **2011,** *46*, 4642-4647.

[69] Capek, P.; Pohl, R.; Hocek, M. *J. Org. Chem.* **2005**, *70*, 8001-8008.

[70] For recent reviews on phosphonium coupling, see: (a) Kang, F-A.; Sui, Z.; Murray, W. V. *Eur. J. Org. Chem.* **2009**, 461-479. (b) Mansour, T. S.; Bardhan, S.; Wan, Z-K. *Synlett* **2010**, 1143-1169.

[71] Kang, F-A.; Kodah, J.; Guan, Q.; Li, X.; Murray, W. V. *J. Org. Chem.* **2005**, *70*, 1957-1960.

[72] Wan, Z. K.; Binnun, F.; Wilson, D. P.; Lee, Y. *Org. Lett.* **2005**, *7*, 5877-5880.

[73] (a) Lakshman, M. K.; Frank, J. *Org. Biomol. Chem.* **2009**, *7*, 2933-2940. (b) Wan, Z-K.; Wacharansindhu, S.; Levins, C. G.; Lin, M.; Tabei, K.; Mansour, T. S. *J. Org. Chem.* **2007**, *72*, 10194-10210.

[74] Planellas, M.; Díaz. E.; Pascual, M.; Bardají, E.; Heras, M "Synthesis of new non-proteinogenic α-amino acids" (poster presented at the Third West Mediterranean Chemistry Meeting, La Grande Motte, France, June 14–15, **2007**).

[75] The determination of the optical purity was assessed by the HPLC method described in Section I.2.1.4.

[76] Lapatsanis, L.; Milias, G.; Froussios, K.; Kolovos, M. *Synthesis* **1998**, 671-673.

[77] (a) Bambal, R.; Hanzlik, R. P. *J. Org. Chem.* **1994**, *59*, 729-732. (b) Jain, R.; Cohen, L. A. *Tetrahedron* **1996**, *52*, 5363-5370.

[78] Ashton, T. D.; Scammells, P. J. *Aust. J. Chem.* **2008**, *61*, 49-58.

[79] Gholap, A. R.; Toti, K. S.; Shirazi, F.; Deshpande, M. V.; Srinivasan, K. V. *Tetrahedron* **2008**, *64*, 10214-10223.

[80] Sheperd, R. G.; Fedrick, J. *Adv. Heterocycl. Chem.* **1965**, *4*, 145-423.

[81] The determination of the optical purity was assessed by the HPLC method described in Section I.2.1.4.

[82] (a) Wang, S.-S. *J. Am. Chem. Soc.* **1973**, *95*, 1328-1333. (b) James, I. W. *Tetrahedron* **1999**, *55*, 4855-4946.

[83] Chin-Tan, T. M.; Yang, F.; Fu, H.; Raghavendra, M. S.; Lam, Y. *J. Comb. Chem.* **2007**, *9*, 210-218.

[84] Regioisomer ratio determined by HPLC analysis of the crude reaction mixture.

[85] The optical purity of compounds **I.39** was verified mediated the HPLC method described in Section I.2.1.4.

[86] Shiffer, M.; Edmunson, A. B. *Biophysical J.* **1967**, *7*, 121-135.

[87] Tachi, T.; Epand, R. F.; Epand, R. M.; Matsuzaki, K. *Biochemistry* **2002**, *41*, 10723-10731.

[88] Meng, H.; Kumar, K. *J. Am. Chem. Soc.* **2007**, *129*, 15615-15622.

[89] Alsina, J.; Barany, G.; Albericio, F.; Kates, S. A. *Lett. Peptide Sci*. **1999**, *6*, 243-245.

[90] Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

[91] Compound **I.20a** and amino ester **I.18a** have the same R_f in TLC. Without this work-up the purification of compound **I.20a** proved to be problematic.

[92] Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165-237.

[93] Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 1-6.

[94] (a) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216-1238. (b) Molinski, T. F.; Dalisay, S. L.; Lievens, S. L.; Saludes, J. P. *Nat. Rev. Drug Discovery* **2009**, *8*, 69-85. (c) Mayer, A. M. S.; Glaser, K. B.; Cuevas, C.; Jacobs, R. S.; Kem, W.; Little, R. D.; McInstosh, J. M.; Newman, D. J.; Potts, B. C.; Shuster, D. E. *Trends Pharmacol. Sci.* **2010**, *31*, 255-265.

[95] Bhakuni, D. S.; Dawar, D. S. *Bioactive marine natural products* New York, Springer, **2005**.

[96] Ratnayake, R.; Fremlin, L. J.; Lacey, E.; Gill, J. H.; Capon, R. J. *J. Nat. Prod.* **2008**, *71*, 403-408.

[97] Probst, A.; Tamm, C. *Helv. Chim. Acta* **1981**, *64*, 2056-2064.

[98] Osada, H.; Kakeya, H.; Konno, H.; Kanazawa, S. Japanese Patent 051804, **2003**.

[99] Marfey's analysis relies on the hydrolysis of a peptide- or amino acid-containing substance followed by *in situ* conversion of the resulting amino acids using a chiral derivatizing agent such as 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (L-FDDA) to form the corresponding 2,4-dinitrophenyl-5-L-alanine amide (DNP) derivatives. The C18 HPLC retention times of the resulting DNP derivatives monitored by UV at 340 nm are then compared to DNP derivatives obtained from authentic amino acid standards allowing a diagnostic for both a given amino acid and a given stereochemistry, see: Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K. A. *Anal. Chem.* **1997**, *69*, 5146-5151.

[100] Amides attached through proline nitrogen are known to be capable of existing as equilibrating rotamers, with literature empirical rules establishing that ^{13}C NMR chemical shift differences between proline β and γ carbon resonances are characteristic of *cis* ($\Delta\beta\gamma$ ~ 8-12 ppm) vs *trans* ($\Delta\beta\gamma$ ~ 2-6 ppm) prolinyl amide rotamers, respectively. See: Siemion, I. Z.; Wieland, T.; Pook, K. H. *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 702-703.

[101] (a) Dale, J. A.; Dull, D. H.; Mosher, H. S. *J. Org. Chem.* **1969,** *34*, 2543-2549. (b) Dale, J. A.; Dull, D. H.; Mosher, H. S. *J. Am. Chem Soc.* **1973,** *95*, 512-519.

[102] Osada, H.; Kakeya, H.; Hayashi, Y.; Shoji, M.; Uchida, S. P. Japanese Patent 101483, **2004**.

[103] Li, J.; Menche, D. *Synthesis* **2009**, 2293-2315.

[104] Yamamoto, Y.; Asao, N. *Chem. Rev.* **1993**, *93*, 2207-2293.

[105] (a) Li, Y.; Houk, K. N. *J. Am. Chem. Soc.* **1989**, *111*, 1236-1240. (b) Vulpetti, A.; Gardner, M.; Gennari, C.; Bernardi, A.; Goodman, J. M.; Paterson, I. *J. Org. Chem.* **1993**, *58*, 1711-1718. (c) Gennari, C.; Fioravanzo, E.; Bernardi, A.; Vulpetti, A. *Tetrahedron* **1994**, *50*, 8815-8826. (d) Omoto, K.; Fujimoto, H. *J. Org. Chem.* **1998**, *63*, 8331-8336. (e) Gajewski, J. J.; Bocian, W.; Brichford, N. L.; Henderson, J. L. *J. Org. Chem.* **2002**, *67*, 4236-4240.

[106] (a) Denmark, S. E.; Fu, J. *J. Am. Chem. Soc.* **2001**, *123*, 9488-9489. (b) Denmark, S. E.; Fu, J. *Chem. Commun.* **2003**, 167-170.

[107] (a) Masse, C. E.; Panek, J. C. *Chem. Rev.* **1995**, *95*, 1293-1316. (b) *Comprehensive Asymmetric Catalysis;* Jacobsen, E. N.; Pfaltz, A.; Yamamoto, H., Eds.; Springer: Berlin, **1999**, 965-982. (c) *Principles and Applications of Asymmetric Synthesis;* Lin, G.-Q.; Li, Y.-M.; Chan, A. S. C., Eds.; John Wiley & Sons: New York, **2001**, 167-178. (d) Denmark, S. E.; Fu, J. *Chem. Rev.* **2003**, *103*, 2763-2794. For a review on Lewis and Brønsted acid catalyzed allylborations of carbonyl compounds, see: Hall, D. G. *Synlett* **2007,** 1644-1655.

[108] (a) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 293-294. (b) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 5919-5923.

[109] (a) Roush, W. R.; Ando, K.; Powers, D. B.; Palkowitz, A. D.; Halterman, R. L. *J. Am. Chem. Soc.* **1990,** *112*, 6339-6348. (b) Roush, W. R.; Palkowitz, A. D.; Ando, K. *J. Am. Chem. Soc.* **1990,** *112*, 6348-6359. (c) Scheidt, K. A.; Tasaka, A.; Bannister, T. D.; Wendt, M. D.; Roush, W. R. *Angew. Chem. Int. Ed.* **1999,** *38*, 1652-1655.

[110] Herold, T.; Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 768-769.

[111] Garcia, J.; Kim, B.; Masamune, S. *J. Org. Chem.* **1987**, *52*, 4831-4832.

[112] Corey, E. J.; Yu, C.-M.; Kim, S. S. *J. Am. Chem. Soc.* **1989**, *111*, 5495-5496.

[113] Burgos, C. H.; Canales, E.; Matos, K.; Soderquist, J. A. *J. Am. Chem. Soc.* **2005**, *127*, 8044-8049.

[114] Peng, F.; Hall, D. G. *J. Am. Chem. Soc.* **2007**, *129*, 3070-3071.

[115] (a) Duthaler, R. O.; Hafner, A.; Riediker, M. *Pure Appl. Chem.* **1990**, *62*, 631-642. (b) Hafner, A.; Duthaler, R. O.; Marti, R.; Rihs, J.; Rothe-Streit, P.; Schwarzenbach, F. J. *J. Am. Chem. Soc.* **1992**, *114*, 2321-2336. (c) Duthaler, R. O.; Hafner, A. *Chem. Rev.* **1992**, *92*, 807-832.

[116] Seebach, D.; Beck, A. K.; Heckel, A. *Angew. Chem., Int. Ed.* **2001**, *20*, 92-138.

[117] (a) BouzBouz, S.; Popkin, M. E.; Cossy, J. *Org. Lett.* **2000**, *2*, 3449-3451. (b) BouzBouz, S.; Cossy, J. *Org. Lett.* **2001,** *3*, 3995-3998.

[118] *Review:* Leighton, J. L. *Aldrichimica Acta* **2010**, *31*, 3-12.

[119] Kinnaird, J. W. A.; Ng, P. Y.; Kubota, K.; Wang, X., Leighton, J. L. *J. Am. Chem. Soc.* **2002**, *124*, 7920-7921.

[120] Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651-1660.

[121] Roush, W. R.; Hoong, L. K.; Palmer, M. A.; Straub, J. A.; Palkowitz, A. D. *J. Org. Chem.* **1990**, *55*, 4117-4126.

[122] Yu, W.; Mei, Y.; Kang, Y.; Hua, Z.; Jin, Z. *Org. Lett.* **2004**, *6*, 3217-3219.

[123] Stüber, W.; Kosina, H.; Heimburger, N. *Int. J. Pept. Protein Res.* **1988**, *31*, 63-70.

[124] Neises, B.; Steglich, W. *Angew. Chem. Int. Ed.*, **1978**, *17*, 522-524.

[125] (a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989-1993. (b) Kawanami, Y.; Dainobu, Y.; Inanaga, J.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 943-944.

[126] Zhang, W.; Robbins, M. J. *Tetrahedron Lett.* **1992**, *33*, 1177-1180.

[127] Wuts, P. G. M.; Bigelow, S. S. *J. Org. Chem.* **1988**, *53*, 5023-5034.

[128] Crouch, R. D.; Polizzi, J. M.; Cleiman, R. A.; Yi, J.; Romany, C. A. *Tetrahedron Lett.* **2002**, *43*, 7151-7153.

[129] Kurosawa, K.; Matsuura, K.; Nagase, T.; Chida, N. *Bull. Chem. Soc. Jpn.* **2006**, *6*, 921-937.

[130] Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953-956.

[131] (a) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 8168; (b) Gessler, S.; Randl, S.; Blechert, S. *Tetrahedron Lett.* **2000**, *41*, 9973-9976.

[132] (a) Fürstner, A.; Langemann, K. *Synthesis* **1997**, 792-803. (b) Fürstner, A. *Top. Organomet. Chem.* **1998**, *1*, 37-72.

[133] Ikawa, T.; Hattori, K.; Sajiki, H.; Hirota, K. *Tetrahedron* **2004**, *60*, 6901-6911.

[134] Hua, J.; Jiang, Z. Y.; Wang, Y. G. *Chin. Chem. Lett.* **2004**, *15*, 1430-1432.

[135] The numbering of the atoms has been changed for an easier comparison with the reported data. In addition, C4, C5, C26 and C27 were not included as their corresponding ^{13}C chemical shift were not described for the natural product.

[136] Tan, L. T.; Márquez, B. L.; Gerwick, W. H. *J. Nat. Prod.* **2002**, *65*, 925-928.

[137] Hoffmann, L.; Demoulin, V. *Belg. J. Bot.* **1991**, *124*, 82-88.

[138] Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Castillo, G.; Demoulin, V. *Tetrahedron Lett.* **1999**, *40*, 695-696.

[139] (a) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Demoulin, V. *Tetrahedron Lett.* **1996**, *37*, 7519-7520. (b) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Castillo, G.; Demoulin, V. *J. Nat. Prod.* **1999**, *62*, 934-936.

[140] Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Demoulin, V. *J. Nat. Prod.* **1997**, *60*, 1057-1059.

[141] Luesch, H.; Yoshida, W. Y.; Harrigan, G. G.; Doom, J. P.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 1945-1948.

[142] Matthew, S.; Salvador, L.; Schupp, P.J.; Paul, V.J.; Luesch, H. *J. Nat. Prod.* **2010**, *73*, 1544-1552.

[143] Sone, H.; Kigoshi, H.; Yamada, K. *J. Org. Chem.* **1996**, *61*, 8956-8960.

[144] (a) Zampella, A.; Dauria, M. V.; Minale, L.; Debitus, C.; Roussakis, C. *J. Am. Chem. Soc.* **1996**, *118*, 11085-11088. (b) Pettit, G. R.; Xu, J. P.; Doubek, D. L.; Chapuis, J. C.; Schmidt, J. M. *J. Nat. Prod.* **2004**, *67*, 1252-1255.

[145] Webb, D.; Van den Heuvel, A.; Kögl, M.; Ley, S. V. *Synlett*, **2009**, 2320-2324.

[146] Gebauer, J.; Arseniyadis, S.; Cossy, J. *Synlett* **2008**, 712-714.

[147] Hong, S. H.; Sanders, D. P.; Lee, C. W.; Grubbs, R. H. *J. Am. Chem. Soc.* **2005**, *127*, 17160-17161.

[148] (a) Sneddon, H. F.; Gaunt, M. J.; Ley, S. V. *Org. Lett.* **2003**, *5*, 1147-1150. (b) Gaunt, M. J.; Sneddon, H. F.; Hewitt, P. R.; Orsini, P.; Hook, D. F.; Ley, S. V. *Org. Biomol. Chem.* **2003**, *1*, 15-16. (c) Sneddon, H. F.; Van den Heuvel, A.; Hirsch, A. K. H.; Booth, R. A.; Shaw, D. M.; Gaunt, M. J.; Ley, S. V. *J. Org. Chem.* **2006**, *71*, 2715-2725.

[149] Maguire, R. J.; Munt, S. P.; Thomas, E. J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2853-2864.

[150] Adderley, N. J.; Buchanan, D. J.; Dixon, D. J.; Laine, D. I. *Angew. Chem. Int. Ed.* **2003**, *42*, 4241-4244.

[151] Bach, T.; Kirsch, S. *Synlett* **2001**, 1474-1476.

[152] (a) Grunwell, J. R.; Karipides, A.; Wigal, C. T.; Heinzman, S. W.; Parlow, J.; Surso, J. A.; Clayton, L.; Fleitz, F. J.; Daffner, M.; Stevens, J. E. *J. Org. Chem.* **1991**, *56*, 91-95. (b) Sugita, Y.; Sakaki, J.-I.; Sato, M.; Kaneko, C. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2855-2861.

[153] CHIRALPACK AD-H (SFC), MeOH/CO_2 = 8:92.

[154] Sato, M.; Sunami, S.; Sugita, Y.; Kaneko, C. *Heterocycles* **1995**, *41*, 1435-1444.

[155] PMBTCA: *p*-(methoxybenzyl)trichloroacetimidate, see: Nakajima, N.; Horita, K.; Abe, R.; Yonemitsu, O. *Tetrahedron Lett.* **1988**, *29*, 4139–4142.

[156] (a) Reber, K. P.; Tilley, S. D.; Sorensen, E. J. *Chem. Soc. Rev*. **2009**, *38*, 3022-3034. (b) Gebauer, J.; Blechert, S. *J. Org. Chem.* **2006**, *71*, 2021-2025. (c) Hoye, T. R.; Danielson, M. E.; May, A. E.; Zhao, H. *Angew. Chem. Int. Ed*. **2008**, *47*, 9743-9746.

[157] Unpublished results.

[158] For a review, see: Parenty, A.; Moreau, X.; Campagne, J.-M. *Chem. Rev*. **2006**, *106*, 911-.

[159] (a) Corey, E. J.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1974**, *96*, 5614-5616. (b) Mukaiyama, T. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 707-721.

[160] (a) Nimitz, J. S.; Wollenberg, R. H. *Tetrahedron Lett.* **1978**, *19*, 3523-3526. (b) Schmidt, U.; Heermann, D. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 308-309. (c) Corey, E. J.; Clark, D. A. *Tetrahedron Lett.* **1979**, 2875-2878. (d) Hillis, L. R.; Ronald, R. C. *J. Org. Chem.* **1985**, *50*, 470-473.

[161] Gerlach, H.; Thalmann, A. *Helv. Chim. Acta* **1974**, *57*, 2661-2663.

[162] Masamune, S.; Bates, G. S.; Corcoran, J. W. *Angew. Chem., Int. Ed. Engl*. **1977**, *16*, 585-607.

[163] (a) Venkataraman, K.; Wagle, D. R. *Tetrahedron Lett.* **1979**, *20*, 3037-3040. (b) Venkataraman, K.; Wagle, D. R. *Tetrahedron Lett*. **1980**, *21*, 1893-1896.

[164] Mukaiyama, T.; Usui, M.; Saigo, K. *Chem. Lett.* **1976**, 49-50.

[165] Yuan, H.; Kingston, D. G. I.; Sackett, D. L.; Hamel, E. *Tetrahedron* **1999**, *55*, 9707-9716.

[166] Smith, A. B.; Malamas, M. S. *J. Org. Chem.* **1982**, *47*, 3442-3447.

[167] (a) Shiina, I.; Kubota, M.; Ibuka, R. *Tetrahedron Lett.* **2002**, *43*, 7535-7539. (b) Shiina, I.; Oshiumi, H.; Hashizume, M.; Yamai, Y.-s.; Ibuka, R.*Tetrahedron Lett.* **2004**, *45*, 543-547. (c) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822-1830. (d) White, J. D.; Johnson, A. T. *J. Org. Chem.* **1994**, *59*, 3347-3358. (e) Mukaiyama, T.; Izumi, J.; Miyashita, M.; Shiina, I. *Chem. Lett*. **1993**, 907-910. (f) Mukaiyama, T.; Izumi, J.; Shiina, I. *Chem. Lett.* **1997**, 187-188. (g) Ishihara, K.; Kubota, M.; Kurihara, H.; Yamamoto, H. *J. Org. Chem.* **1996**, *61*, 4560-4567.

[168] Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989-1993.

[169] (a) Hikota, M.; Tone, H.; Horita, K.; Yonemitsu, O. *J. Org. Chem.* **1990**, *55*, 7-9. (b) Evans, D. A.; Ng, H. P.; Rieger, D. L. *J. Am. Chem. Soc.* **1993**, *115*, 11446-11459.

[170] Kaiho, T.; Masamune, S.; Toyoda, T. *J. Org. Chem*. **1982**, *47*, 1612-1614.

[171] Corey, E. J.; Hua, D. H.; Pan, B. C.; Seitz, S. P. *J. Am. Chem. Soc.* **1982**, *104*, 6818-6820.

[172] Coste, J.; Frerot, E.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1991**, *32*, 1967-1970.

[173] Wood, J. L.; Porco, J. A., Jr.; Taunton, J.; Lee, A. Y.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 5898-5900.

[174] Roush, W. R.; Sciotti, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 7411-7419.

[175] Steglich, W.; Höfle, G. *Angew. Chem., Int. Ed. Engl*. **1969**, *8*, 981.

[176] Boden, E. P.; Keck, G. E. *J. Org. Chem*. **1985**, 50, 2394–2395.

[177] Keck, G. E.; Boden, E. P.; Wiley, M. R. *J. Org. Chem*. **1989**, 54, 896–906.

[178] Keck, G. E.; Murry, J. A. *J. Org. Chem*. **1991**, 56, 6606–6611.

[179] Stork, G.; Rychnovsky, S. D. *J. Am. Chem. Soc*. **1986**, 109, 1565-1567.

[180] Evans, D. A.; Gage, J.; Leighton, J. L. *J. Am. Chem. Soc*. **1992**, 114, 9434-9453.

[181] Danishefsky, S. J.; Su, D. S.; Meng, D. F.; Bertinato, P.; Balog, A.; Sorensen, E. J.; Zheng, Y. H.; Chou, T. C.; He, L. F.; Horwitz, S. B. *Angew. Chem., Int. Ed*. **1997**, 36, 757-759.

[182] Patterson, I.; Yeung, K. S.; Ward, R. A.; Smith, J. D.; Cumming, G.; Lamboley, S. *Tetrahedron* **1995**, 51, 6497-9486.

[183] (a) Boeckman, R. K., Jr.; Weidner, C. H.; Perni, R. B.; Napier, J. J. *J. Am. Chem. Soc.* **1989**, *111*, 8036-8037.

[184] Paquette, L. A.; Macdonald, D.; Anderson, L. G.; Wright, J. *J. Am. Chem. Soc.* **1989**, *111*, 8037-8039.

[185] Boeckman, R. K., Jr.; Pruitt, J. R. *J. Am. Chem. Soc.* **1989**, *111*, 8286-8288.

[186] (a) Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C. P.; Singh, V. K. *J. Am. Chem. Soc.* **1987**, *109*, 7925-7926. (b) Deloux, L.; Srebnik, M. *Chem. Rev.* **1993,** *93*, 763-784.

[187] (a) Evans, D. A.; Murry, J. A.; Kozlowski, M. C. *J. Am. Chem. Soc.* **1996**, *118*, 5814-5815. (b) Krüger, J.; Carreira, E. M. *J. Am. Chem. Soc.* **1998**, *120*, 669-685. (c) Pagenkopf, B. L.; Krüger, J.; Stojanovic, A.; Carreira, E. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 3124-3126. (d) Evans, D. A.; Kozlowski, M. C.; Murry, J. A.; Burgey, C. S.; Campos, K. R.; Connell, B. T.; Staples, R. J. *J. Am. Chem. Soc.* **1999**, *121*, 837-838. (e) Bluet, G.; Campagne, J.-M. *Tetrahedron Lett.* **1999**, *40*, 5507-5509. (f) Bluet, G.; Campagne, J.-M. *J. Org. Chem.* **2001**, *66*, 4293-4298.

[188] (a) Denmark, S. E.; Wynn, T.; Beutner, G. L. *J. Am. Chem. Soc.* **2002**, *124*, 13405-13407. (b) Denmark, S. E.; Beutner, G. L. *J. Am. Chem. Soc.* **2003**, *125*, 7800-7801. (c) Denmark, S. E.; Beutner, G. L.; Wynn, T.; Eastgate, M. D. *J. Am. Chem. Soc.* **2005**, *125*, 3774-3789.

[189] De Rosa, M.; Acocella, M. R.; Villano, R.; Soriente, A.; Scettri, A. *Tetrahedron: Asymmetry* **2003**, *14*, 2499-2502.

[190] Mitsunobu, O.; Yamada, Y. *Bull. Chem. Soc. Japan* **1967**, *40*, 2380-2382.

[191] (a) Jacobsen, E. N.; Marko, I.; Mungall, W. S.; Schroeder, G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 1968-1970. (b) Kolb, H. C.; Van Nieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483-2547.

[192] The selectivity of the Sharpless dihydroxylation was determined by chiral SFC analysis of the corresponding epoxide **II.64** (CHIRALCEL OD-H, 100 bar, 5 $mL.min^{-1}$, 5% MeOH). This result was in absolute accordance with the studies performed by Tietze *et al.* who demonstrated that the nature of the protecting group had a strong influence on the enantioselectivity of the dihydroxylation, see : Tietze, L. F.; Görlitzer, J. *Synthesis* **1998**, 873-878.

[193] (a) Anikin, A.; Maslov, M.; Siele, J.; Blaurock, S.; Baldamus, J.; Hennig, L.; Findeisen, M.; Reinhardt, G.; Oehme, R.; Welzel, P. *Tetrahedron* **2003**, *59*, 5295-5306. (b) Sparks, S. M.; Chen, C.-L.; Martin, S. F. *Tetrahedron* **2007**, *63*, 8619-8635.

[194] Allenylmagnesium bromide was generated *in situ* from propargyl bromide and metallic magnesium in the presence of a catalytic amount of $HgCl_2$, see: (a) Nakayama, Y.; Kumar, G. B.; Kobayashi, Y. *J. Org. Chem.* **2000**, *65*, 707-715. (b) Dimitrieva, L. L.; Nikitina, L. P.; Albanov, A. I.; Nedolya, N. A. *Russian J. Org. Chem.* **2005**, *41*, 1583-1593.

[195] The diastereoisomeric ration was determined by ^1H NMR analysis of the crude reaction mixture.

[196] Evans, D. A.; Chapman, K. T. *Tetrahedron Letters* **1986**, *27*, 5939-5942.

[197] Fuwa, H.; Noto, K.; Sasaki, M. *Org. Lett.* **2010**, *12*, 1636-1639.

[198] Lipshutz, B. H.; Ung, C. S.; Sengupta, S. *Synlett* **1989**, 64-66.

[199] Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 889-892.

[200] For a recent application of the acylketene dual macrolactonization/pyran hemiketal formation reaction, see: (f) Trost, B. M.; Gunzner, J. L. *J. Am. Chem. Soc.* **2001**, *123*, 9449-9450. (g) Trost, B. M.; Gunzner, J. L.; Dirat, O.; Rhee, Y. H. *J. Am. Chem. Soc.* **2002**, *124*, 10396-10415.

[201] Wentrup, C.; Heilmayer, W.; Kollenz, G. *Synthesis* **1994**, 1219-1248.

[202] (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467-4470. (b) Chinchilla, R.; Nájera, C. *Chem Rev.* **2007**, *107*, 874-922.

[203] (a) Trost, B. M.; Ball, Z. T.; Jöge, T. *J. Am. Chem. Soc.* **2002**, *124*, 7922-7923. (b) Lacombe, F.; Radkowski, K.; Seidel, G.; Fürstner, A. *Tetrahedron* **2004**, *60*, 7315-7324. (c) Kleinbeck, F.; Carreira, E. M. *Angew. Chem. Int. Ed.* **2009**, *48*, 578-581.

[204] Kim, S.; Kim, B.; In, J. *Synthesis* **2009**, *12*, 1963-1968.

[205] Prepared according to a reported procedure starting from but-1-yne, see: Zneifel, G.; Lewis, W. *J. Org. Chem.* **1978**, *43*, 2739-2744.

[206] (a) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd edition, J. Wiley & Sons, **1991**. (b) Kocienski, P. J. *Protecting Groups*, Georg Thieme Verlag, **1994**.

[207] (a) Mahoney, W. S.; Brestensky, D. M.; Stryker, J. M. *J. Am. Chem. Soc* **1988**, *110*, 291-293. (b) Paterson, I.; Blakey, S. B.; Cowden, C. J. *Tetrahedron Lett.* **2002**, *43*, 6005-6008.

[208] The diastereoisomeric ratio was determined by ^{1}H NMR of the crude reaction mixture.

[209] Yang, Y.-Q.; Cui, J.-R.; Zhu, L.-G.; Sun, Y.-P.; Wu, Y. *Synlett* **2006**, 1260-1262.

[210] (a) Trost, B. M.; Ball, Z. T. *J. Am. Chem. Soc.* **2001**, *123*, 12726-12727. (b) Trost, B. M.; Machacek, M. R.; Ball, Z. T. *Org. Lett.* **2003**, *5*, 1895-1898. (c) Chung, L. W.; Yu, Y.-D.; Trost, B. M.; Ball, Z. T. *J. Am. Chem. Soc.* **2003**, *125*, 11578-11582. (d) Bressy, C.; Bargiggia, F.; Guyonnet, M.; Arseniyadis, S.; Cossy, J. *Synlett* **2008**, *4*, 565-568. (d) Bressy, C.; Vors, J.-P.; Hillebrand, S.; Arseniyadis, S.; Cossy, J. *Angew. Chem, Int. Ed.* **2008**, *47*, 10137-10140.

[211] Marshall, J. A.; Blough, B. E. *J. Org. Chem.* **1990**, *55*, 1540–1547.

[212] Roush, W. R.; Palkowitz, A. D.; Palmer, M. A. *J. Org. Chem.* **1987**, *52*, 316–318.

[213] Corey, E. J.; Guzman-Perez, A.; Noe, M. *J. Am. Chem. Soc.*, **1995**, *117*, 10805-10816.

[214] Sirirath, S.; Tanaka, J.; Ohtani, I. I.; Ichiba, T.; Rachmat, R.; Ueda, K.; Usui, T.; Osada, H.; Higa, T. *J. Nat. Prod.* **2002**, *65*, 1820-1823.

[215] (a) Carmenly, S.; Kashman, Y. *Tetrahedron Lett.* **1985**, *26*, 511-514. (b) Kobayashi, M.; Tanaka, J.; Katori, T.; Matsuura, M.; Yamashira, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1990**, *38*, 2409-2418.

[216] Kondoh, M.; Usui, T.; Kobayashi, S.; Tsuchiya, K.; Nishikawa, K.; Nishikiori, T.; Mayumi, T.; Osada, H. *Cancer Lett.* **1998**, *126*, 29-32.

[217] Fushimi, S.; Nishikawa, S.; Shimazu, A.; Seto, H. *J. Antibiotics* **1989**, *42*, 1019-1025.

[218] Zhang, H.; Conte, M. M.; Capon, R. J. *Angew. Chem. Int. Ed.* **2010**, *49*, 9904-9906.

[219] Dual-specificity phosphatases are an emerging subclass of the protein tyrosine phosphatase (PTP) gene superfamily, which appears to be selective for dephosphorylating MAP kinases. Hence, natural products that inhibit these enzymes might serve as biologically validated starting points for the development of new and selective phosphatase inhibitors, see: (a) Camps, M.; Nichols, A.; Arkinstall, S. *FAEBS*, **2000**, *14*, 6-16. (b) Brohm, D.; Metzger, S.; Bhargava, A.; Müller, O.; Lieb, F.; Waldman, H. *Angew. Chem. Int. Ed.* **2002**, *41*, 307-311.

[220] Davies-Coleman, M. T.; Rivett, D. E. A. *Fortschr. Chem. Org. Naturst.* **1989**, *55*, 1-35.

[221] Crystallographic data for the structure have been deposited at the Cambridge Crystallographic Data Center (Deposition number CCDC 191268).

[222] Ghosh, S.; Kumar, S. U.; Shashidhar, J. *J. Org. Chem.* **2008**, *73*, 1582-1585.

[223] Su, Y.; Xu, Y.; Han, J.; Zheng, J.; Qi, J.; Jiang, T.; Pan, X.; She, X. *J. Org. Chem.* **2009**, *74*, 2743-2749.

[224] Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183-2186.

[225] (a) Fürstner, A. *Angew. Chem. Int. Ed.* **2000**, *39*, 3012-3043. (b) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18-19. (c) Love, J. A. *Handbook of Metathesis*; Grubbs, R. H., Ed.; Wiley-VCH: Weinheim, Germany, **2003**; 296-322. (d) Grubbs, R. H. *Tetrahedron* **2004**, *60*, 7117-7140. (e) Deiters, A.; Martin, S. F. *Chem. Rev.* **2004**, *104*, 2199-2238. (f) Gradillas, A.; Pérez-Castells, J. *Angew. Chem. Int. Ed.* **2006**, *45*, 6086-6101.

[226] Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560-3578.

[227] Crimmins, M. T.; King, B. W.; Tabet, E. A.; Chaudhary, K. *J. Org. Chem.* **2001**, *66*, 894-902.

[228] (a) Brandänge, S.; Leijonmarck, H. *Tetrahedron Lett.* **1992**, *33*, 3025-3028. (b) Vanderwal, C. D.; Vosburg, D. A.; Weiler, S.; Sorensen, E. J. *Org. Lett.* **1999**, *1*, 645-648. (c) Hinterding, K.; Singhanat, S.; Oberer, L. *Tetrahedron Lett.* **2001**, *42*, 8463-8465. (d) Vanderwal, C. D.; Vosburg, D. A.; Weiler, S.; Sorensen, E. J. *J. Am. Chem. Soc.* **2003**, *125*, 5393-5407.

[229] (a) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127-2129. (b) Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 3099-3111. (c) Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem.* **1982**, *13*, 1-115. (d) Evans, D. A. *Aldrichimica Acta* **1982**, *15*, 23-32.

[230] (a) Myers, A. G.; Yang, B.; Chen, H.; Mckinstry, L.; Kopecky, D. J.; Gleason, J. L. *J. Am. Chem. Soc.* **1997**, *119*, 6496-6511. (b) Myers, A. G.; Yang, B. H.; Chen, H.; Kopecky, D. J. *Synlett* **1997**, 457-459.

[231] Yasui, K.; Tamura, Y.; Nakatani, T.; Kawada, K.; Ohtani, M. *J. Org. Chem.* **1995**, *60*, 7567-7574.

[232] Tipson, R. S.; Cohen, **A.** *Carbohydr. Res.* **1965**, *1*, 338.

[233] Gurjar, M.; Chakrabarti, A.; Rao, A. V. R. *Heterocycles* **1997**, *45*, 7-10.

[234] Watanabe, H.; Watanabe, H.; Bando, M.; Kido, M.; Kitahara, T. *Tetrahedron* **1999**, *55*, 9755-9776.

[235] (a) Miyashita, K.; Tsunemi, T.; Hosokawa, T.; Ikejiri, M.; Imanishi, T. *Tetrahedron Lett.* **2007**, *48*, 3829-3833. (b) Miyashita, K.; Tsunemi, T.; Hosokawa, T.; Ikejiri, M.; Imanishi, T. *J. Org. Chem.* **2008**, *73*, 5360-5370.

[236] Review: D. A. Evans *Aldrichim. Acta* **1982**, *15*, 23-32.

[237] (a) Reddy, M. A.; Gopal, R. J.; Rao, V. J. *Indian J. Chem.* **1996**, *35*B, 312. (b) Ando, K. *J. Org. Chem.* **1997**, *62*, 1934-1939.

[238] (a) Gurjar, M. K.; Henri Jr., J. T.; Bose, S. D.; Rao, A. V. R. *Tetrahedron Lett.* **1996**, *37*, 6615-6618. (b) Días, L. C.; Oliveira, L. G. Sousa, M. A. *Org. Lett.* **2003**, *5*, 265-268. (c) Días, L. C.; Oliveira, L. G. Sousa, M. A.; Ellensohn, R. M. *ARKIVOC* **2005**, *vi*, 62-87.

[239] Gebhardt, B.; König, C.; Schleth, C.; Dauber, M.; Koert, U. *Chem. Euro. J.* **2010**, *16*, 5934-5941.

[240] Wang, Y.-G.; Takeyama, R.; Kobayashi, Y. *Angew. Chem., Int. Ed.* **2006**, *45*, 3320-3323.

[241] Shimada, K.; Kaburagi, Y.; Fukuyama, T. *J. Am. Chem. Soc.* **2003**, *125*, 4048-4049.

[242] Shashidhar, J.; Reddy, K. M.; Ghosh, S. *Tetrahedron Lett.* **2011**, *52*, 3106-3109.

[243] Enders, D.; Dhulut, S.; Steinbusch, D.; Herrbach, A. *Chem. Eur. J.* **2007**, *13*, 3942-3949.

[244] Review: Job, A.; Janeck, C. F.; Bettray, W.; Peters, R.; Enders, D. *Tetrahedron* **2002**, *58*, 2253-2329.

[245] Keck, G. E.; Knutson, C. E.; Wiles, S. A. *Org. Lett.* **2009**, *3*, 707-710.

[246] Keck, G. E.; Wager, C. A.; Sell, T.; Wager, T. T. *J. Org. Chem.* **1999**, *64*, 2172-2173.

[247] Keck, G. E.; Li, X.-Y.; Knutson, C. E. *Org. Lett,* **1999**, *1*, 411-414.

[248] Zhu, C.; Shen, X.; Nelson, S. G. *J. Am. Chem. Soc.* **2004**, *126*, 5352-5353.

[249] Shen, X.; Wasmuth, A. S.; Zhao, J.; Zhu, C.; Nelson, S. G. *J. Am. Chem. Soc.* **2006**, *128*, 7438-7439.

[250] (a) Druais, V.; Hall, M. J.; Corsi, C.; Wenderborn, S. V.; Meyer, C.; Cossy, J. *Org. Lett.* **2009**, *11*, 935-938. (b) Druais, V.; Hall, M. J.; Corsi, C.; Wenderborn, S. V.; Meyer, C.; Cossy, J. *Tetrahedron* **2010**, *66*, 6358-6375.

[251] (a) Brown, H. C.; Jadhav, P. K. *J. Am. Chem. Soc.* **1983**, *105*, 2092-2093. (b) Brown, H. C.; Jadhav, P. K.; Bhat, K. S. *J. Am. Chem. Soc.* **1988**, *110*, 1535-1538.

[252] (a) Shibahara, S.; Fujino, M.; Tashiro, Y.; Takahashi, K.; Ishihara, J.; Hatakeyama, S. *Org. Lett.* **2008**, *10*, 2139-2142. (b) Shibahara, S.; Fujino, M.; Tashiro, Y.; Okamoto, N.; Esumi, T.; Takahashi, K.; Ishihara, J.; Hatakeyama, S. *Synthesis* **2009**, *17*, 2935-2953. (c) Sarkar, S. M.; Wanzala, E. N.; Shibahara, S.; Takahashi, K.; Ishihara, J.; Hatakeyama, S. *Chem. Commun.* **2009**, 5907-5909.

[253] Sonawane, R. P.; Joolakanti, S. R.; Arseniyadis, S.; Cossy, J. *Synlett* **2009**, 213-216.

[254] (a) Schlosser, A.; Hartmann, J. *J. Am. Chem. Soc.* **1976**, *98*, 4674-4676. (b) Roush, W.; Adam, M.; Walts, A.; Harris, D. *J. Am. Chem. Soc.* **1986**, *108*, 3422-3434. (c) Schlosser, A.; Despond, O.; Lehmann, R.; Moret, E.; Rauchschwalbe, G. *Tetrahedron* **1993**, *49*, 10175-10203.

[255] Bressy, C.; Vors, J.-P.; Hillebrand, S.; Arseniyadis, S.; Cossy, J. *Angew. Chem. Int. Ed.* **2008**, *47*, 10137-10140.

[256] Moïse, J.; Sonawane, R. P.; Corsi, C.; Wendeborn, S. V.; Arseniyadis, S.; Cossy, J. *Synlett*, **2008**, 2617-2620.

[257] (a) Ager, D. J.; Prakash, I.; Schaad, D. R. *Aldrichimica Acta* **1997**, *30*, 3-12. (b) Evans, D. A.; Kim, A. S. *Handbook of Reagents for Organic Synthesis: Reagents, Auxiliaries, and Catalysts for C-C bond Formation*; Coates, R. M.; Denmark, S. E., Eds.; John Wiley and Sons: New York, **1999**; 91-101. (c) Smith, T. E.; Richardson, D. P.; Truran, G. A.; Belecki, K.; Onishi, M. *J. Chem. Ed.* **2008**, *85*, 695-697.

[258] Smith, A. B., III; Lee, D. *J. Am. Chem. Soc.* **2007**, *129*, 10957-10962.

[259] Meissner, R. S.; Perkins, J. J.; Duong, L. T.; Hartman, G. D.; Hoffman, W. F.; Huff, J. R.; Ihle, N. C.; Leu, C.-T.; Nagy, R. M.; Naylor-Olsen, A.; Rodan, G. A.; Whitman, D. B.; Wesolowski, G. A.; Duggan, M. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 25-29.

[260] The (*E*,*Z*) unsaturated ester was isolated in 6% yield.

[261] Cao, X.-P. *Tetrahedron* **2002**, *58*, 1301-1307.

[262] (a) Patterson, I.; Goodman, J. M. *Tetrahedron Lett.* **1989**, *30*, 997-1000. (b) Patterson, I.; Goodman, J. M.; Lister, M. A.; Schumann, R. C.; McClure, C. K.; Norcross, R. D. *Tetrahedron* **1990**, *46*, 4663-4684.

[263] (a) Betancort, J. M.; Barbas, C. F., III. *Org. Lett.* **2001**, *3*, 3737-3740. (b) Betancort, J. M.; Sakthivel, K.; Thayumanavan, R.; Barbas, C. F., III. *Tetrahedron Lett.* **2001**, *42*, 4441-4444. (c) Enders, D.; Seki, A. *Synlett* **2002**, 26-28. (d) Andrey, O.; Alexakis, A.; Bernardinelli, G. *Org. Lett.* **2003**, *5*, 2559-2561. (e) Mase, N.; Thayumanavan, R.; Tanaka, F.; Barbas, C. F., III. *Org. Lett.* **2004**, *6*, 2527-2530. (f) Betancort, J. M.; Sakthivel, K.; Thayumanavan, R.; Tanaka, F.; Barbas, C. F., III. *Synthesis* **2004**, 1509-1521. (g) Ishii, T.; Fujioka, S.; Sekiguchi, Y.; Kotsuki, H. *J. Am. Chem. Soc.* **2004**, *126*, 9558-9559. (h) Wang, W.; Wang, J.; Li, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 1369-1371.

[264] (a) Kawara, A.; Taguchi, T. *Tetrahedron Lett.* **1994**, *35*, 8805-8808. (b) Hanessian, S.; Pham, V. *Org. Lett.* **2000**, *2*, 2975-2978. (c) Halland, N.; Hansen, K. A.; Jørgensen, K. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 4955-4957. (d) Halland, N.; Aburel, P. S.; Jørgensen, K. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 661-665.

[265] (a) Yamaguchi, M.; Shiraishi, T.; Hirama, M. *J. Org. Chem.* **1996**, *61*, 3520-3530. (b) Corey, E. J.; Zhang, F.-Y. *Org. Lett.* **2000**, *2*, 4257-4259. (c) Halland, N.; Hazell, R. G.; Jørgensen, K. A. *J. Org. Chem.* **2002**, *67*, 8331-8338.

[266] Melchiorre, P.; Jørgensen, K. A. *J. Org. Chem.* **2003**, *68*, 4151-4152.

[267] Chi, Y.; Gellman, S. H. *Org. Lett.* **2005**, *19*, 4523-4256.

[268] Peelen, T. J.; Chi, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11598-11599.

[269] Enders, D.; Kipphardt, H.; Gerdes, P.; Brena-Valle, L. J. *Bull. Soc. Chim. Belg.* **1988**, *97*, 691-704.

[270] List, B. *J. Am. Chem. Soc*. **2002**, *124*, 5656-5657.

[271] The two diastereoisomeric hemiketals were obtained in 2.4:1 mixture according to ^{1}H NMR analysis.

[272] She's synthesis of (–)-bitungolide F was achieved in 18 steps and 20.1% overall yield starting from (–)-malic acid, while Ghosh's synthesis was completed in 22 steps and 4.7% overall yield starting from the same starting material.

[273] Concellón, J. M.; Concellón, C.; Méjica, C. *J. Org. Chem.* **2005**, *70*, 6111-6113.

[274] Macía, F.; García-Díaz, M.; Massanet, G. M.; Gómez-Madero, J.; Fronczek, F. R.; Galindo, J. C. G. *Tetrahedron* **2008**, *64*, 10996-11006.

List of Publications

The following publications were completed as outputs from this research:

- *Design, synthesis and evaluation of antimicrobial peptides by incorporation of pyrimidinyl amino acids.* ElMarrouni, A; Badosa, E.; Bardají, E; Montesinos, E.; Heras, M. (*Manuscript in preparation*).

- *Total synthesis of the nominal Lyngbouilloside aglycon.* ElMarrouni, A.; Lebeuf, R.; Gebauer, J.; Heras, M.; Arseniyadis, S.; Cossy, J. *Org. Lett.* **2012**, *14*, 314–317.

- *Coupling reaction promoted by phosphonium salt between an electron-rich 4(3H)-pyrimidinone and α-amino acids.* ElMarrouni, A.; Fabrellas, J. M.; Heras, M. *Org. Biomol. Chem.* **2011**, *9*, 5967–5977.

- *Expedient synthesis of a stereoisomer of Acremolide B.* ElMarrouni, A.; Fukuda, A.; Heras, M.; Arseniyadis, S.; Cossy, J. *J. Org. Chem*. **2010**, *75*, 8478–8486.

- *Two concise total syntheses of* (–)-*Bitungolide F*. ElMarrouni, A.; Joolakanti, S. R.; Colon, A.; Heras, M.; Arseniyadis, S.; Cossy, J. *Org. Lett.* **2010**, *12*, 4074–4077.

- *A simple approach for the synthesis of new pyrimidinyl α-amino acids.* ElMarrouni, A.; Güell, M.; Collell, C.; Heras, M. *Tetrahedron* **2010**, *66*, 612–623.

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ANNEX I

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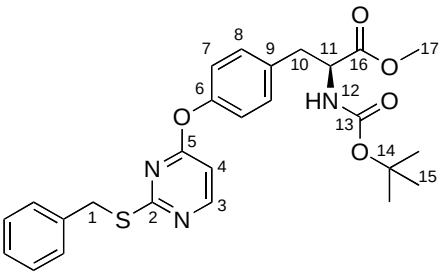
I. COPIES OF NMR SPECTRA

I.1. Pyrimidin-4-yl amino acids

Compound I.19a



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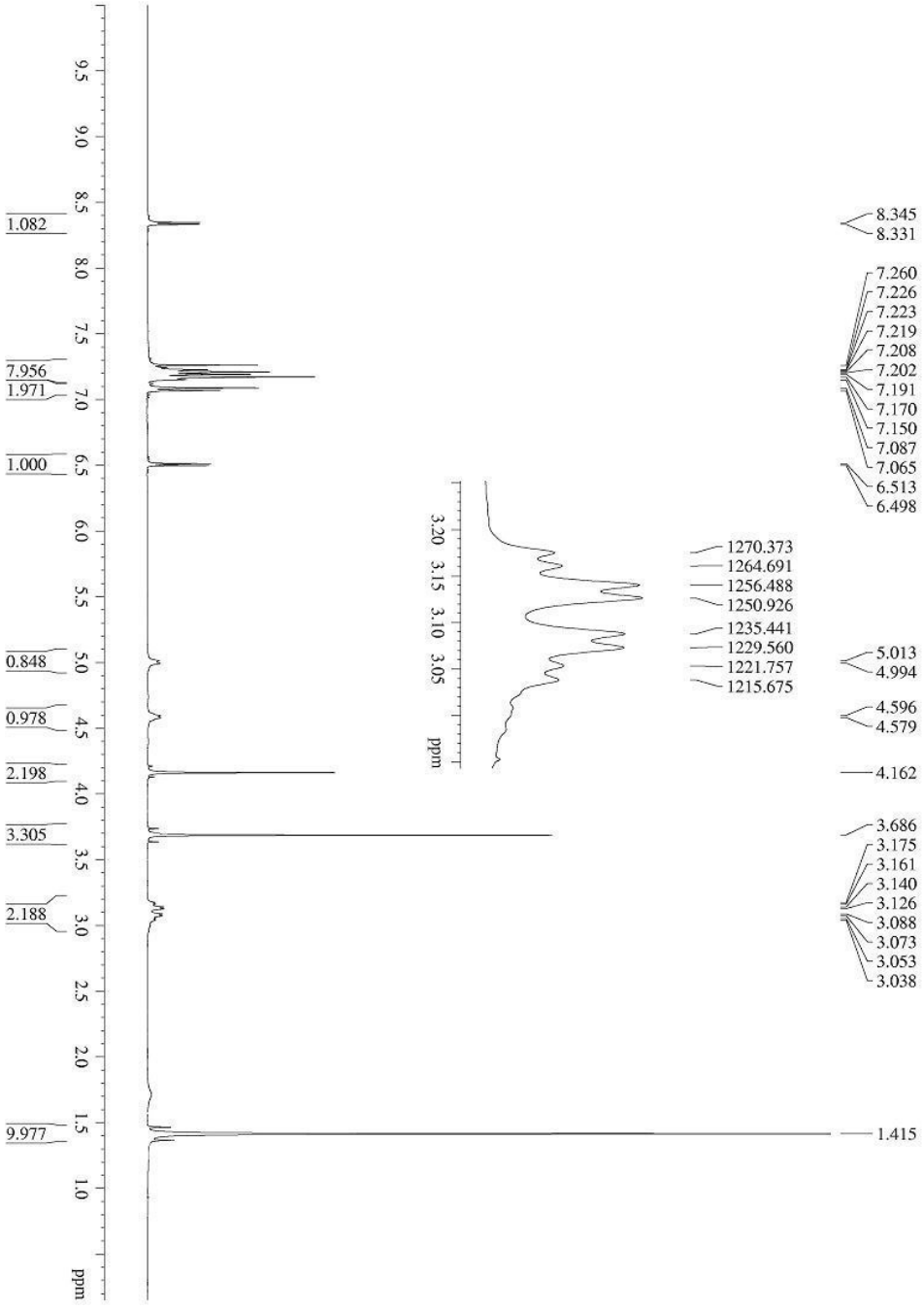

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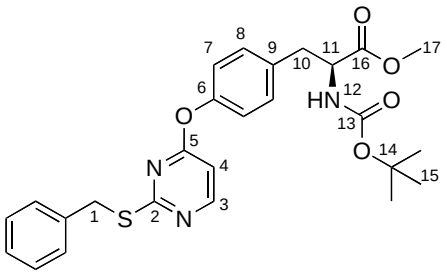

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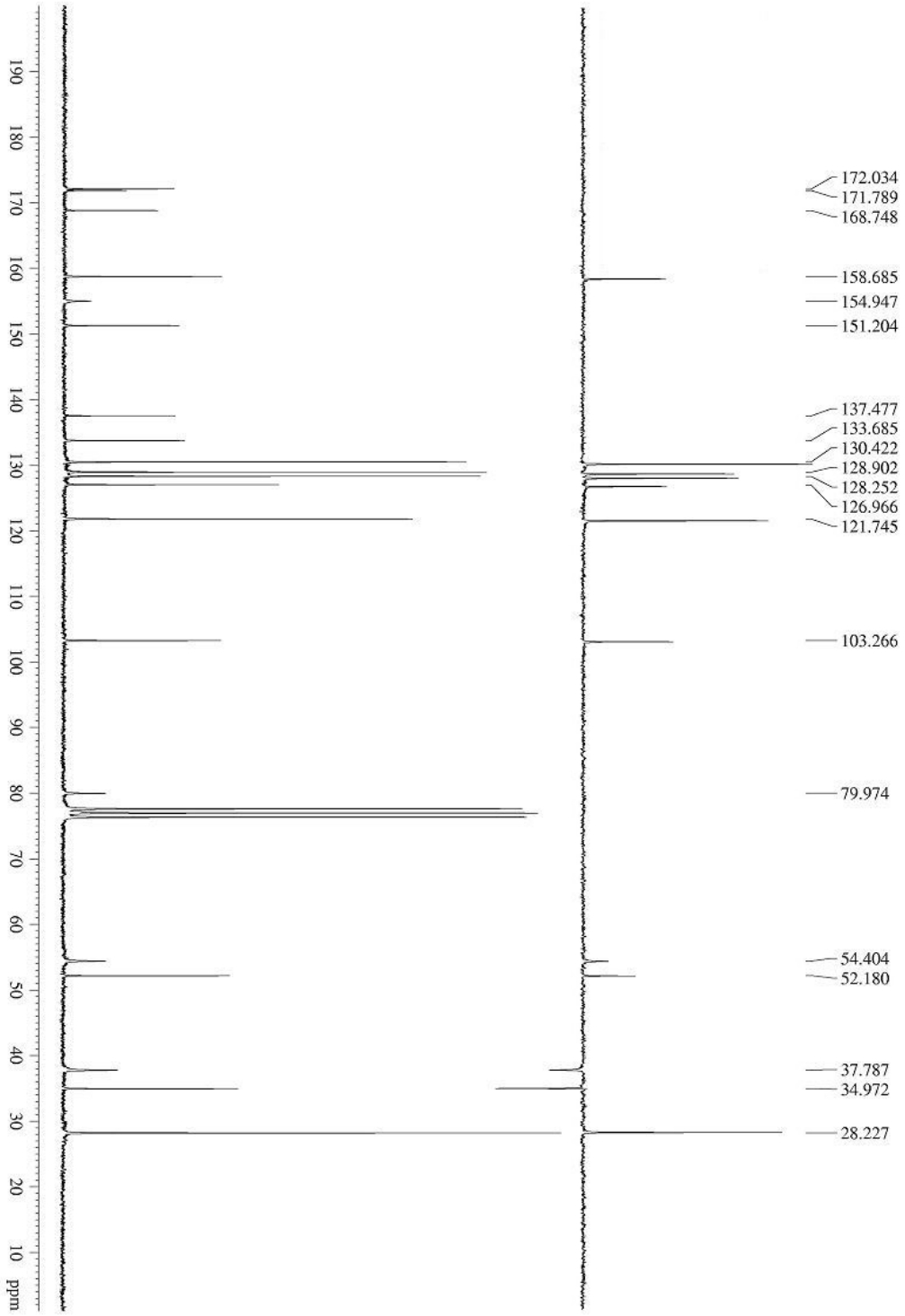

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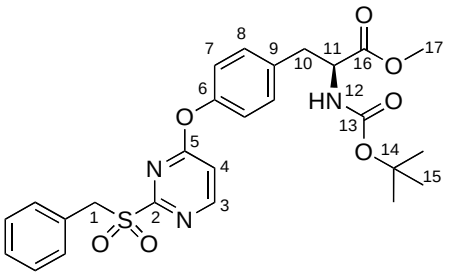

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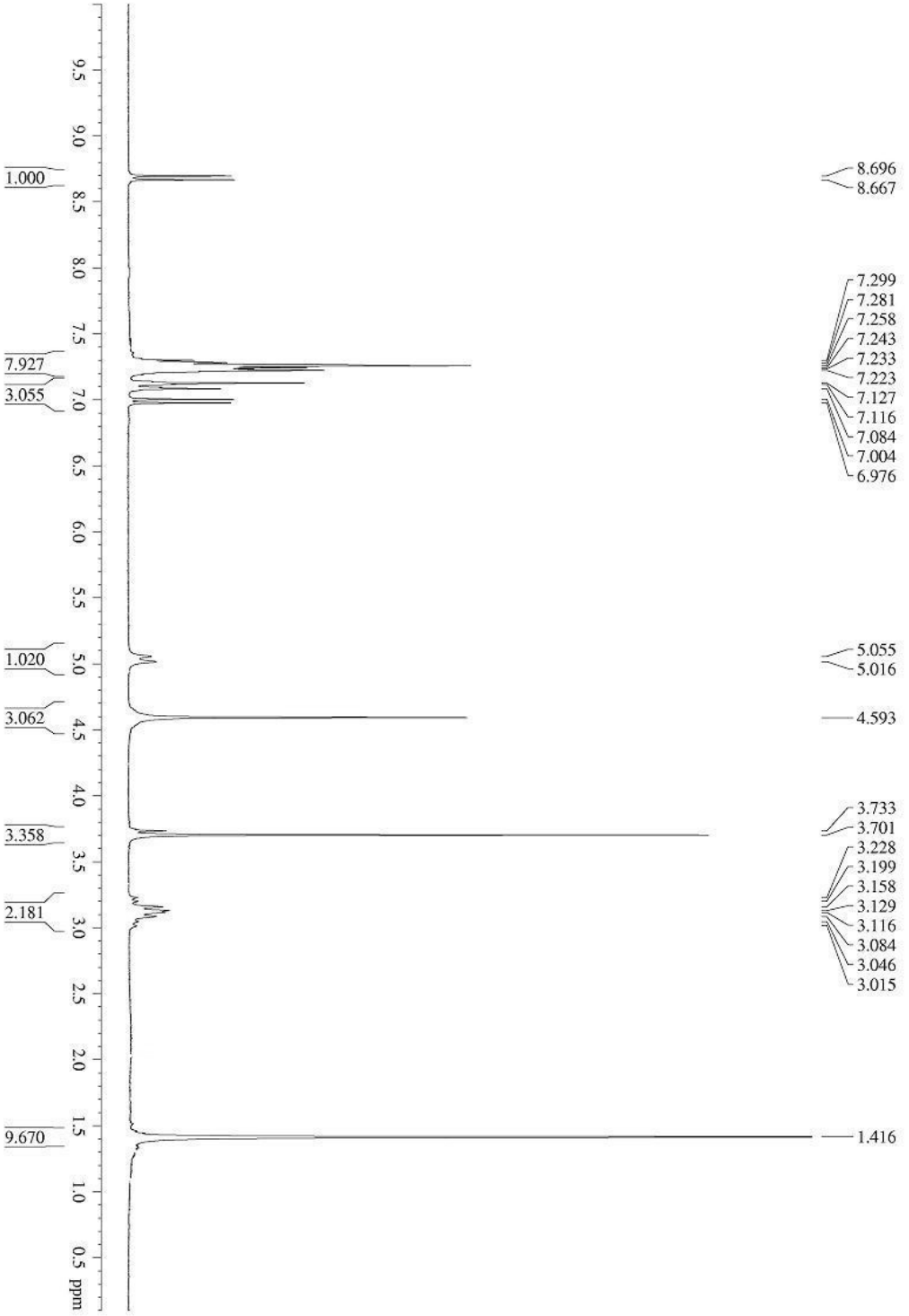

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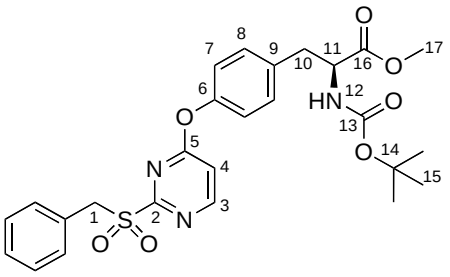

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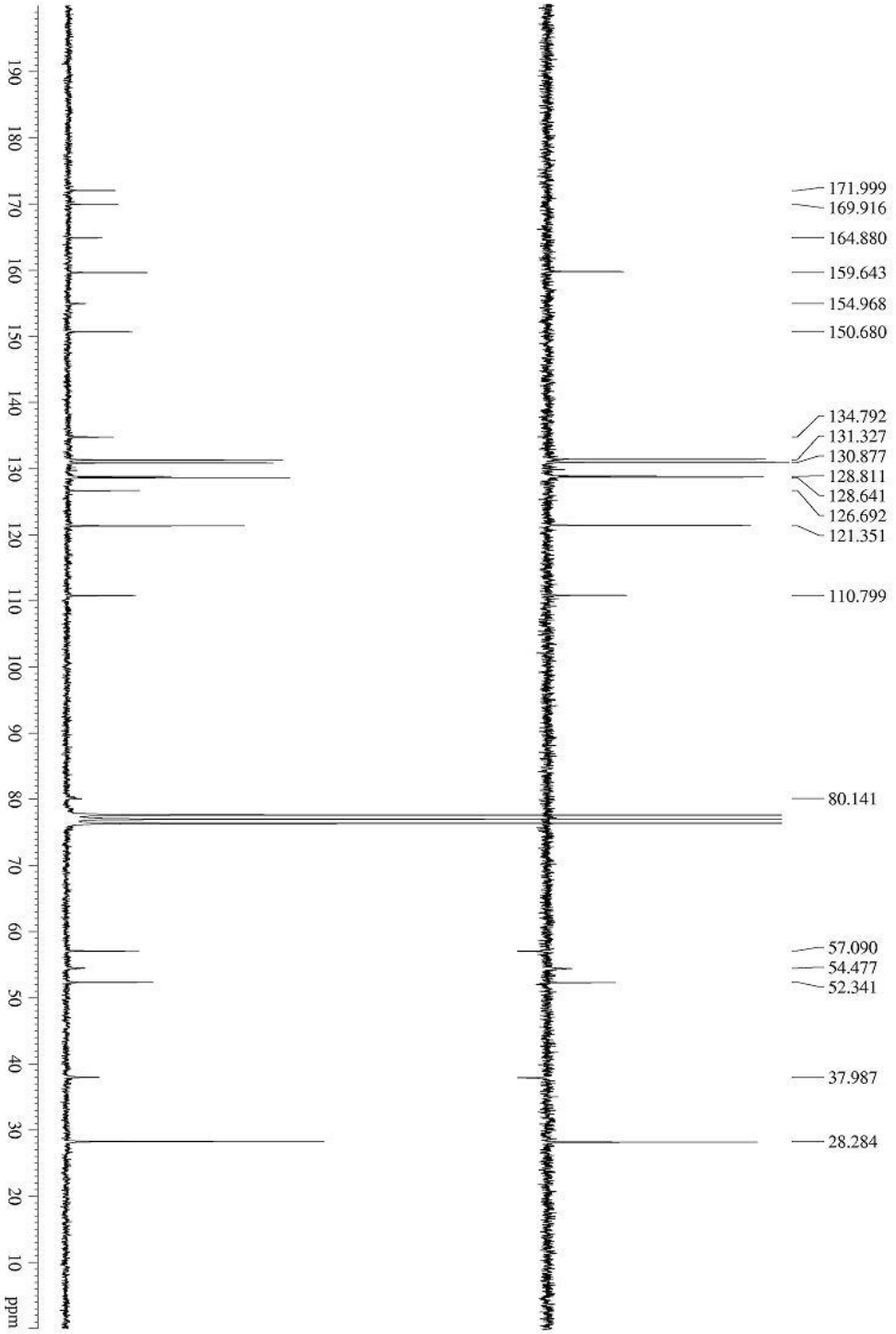

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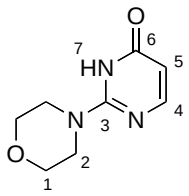

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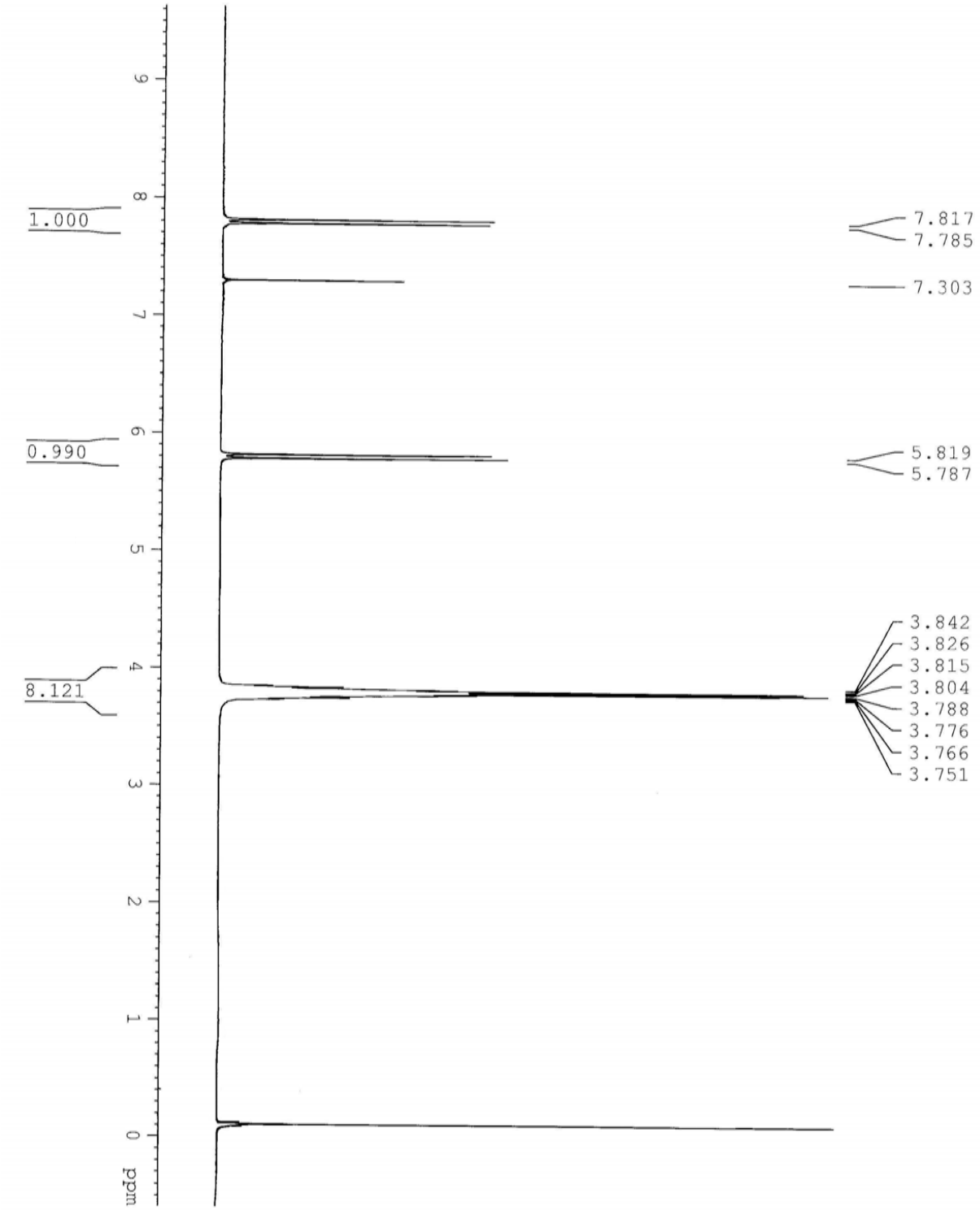

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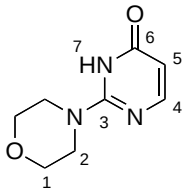

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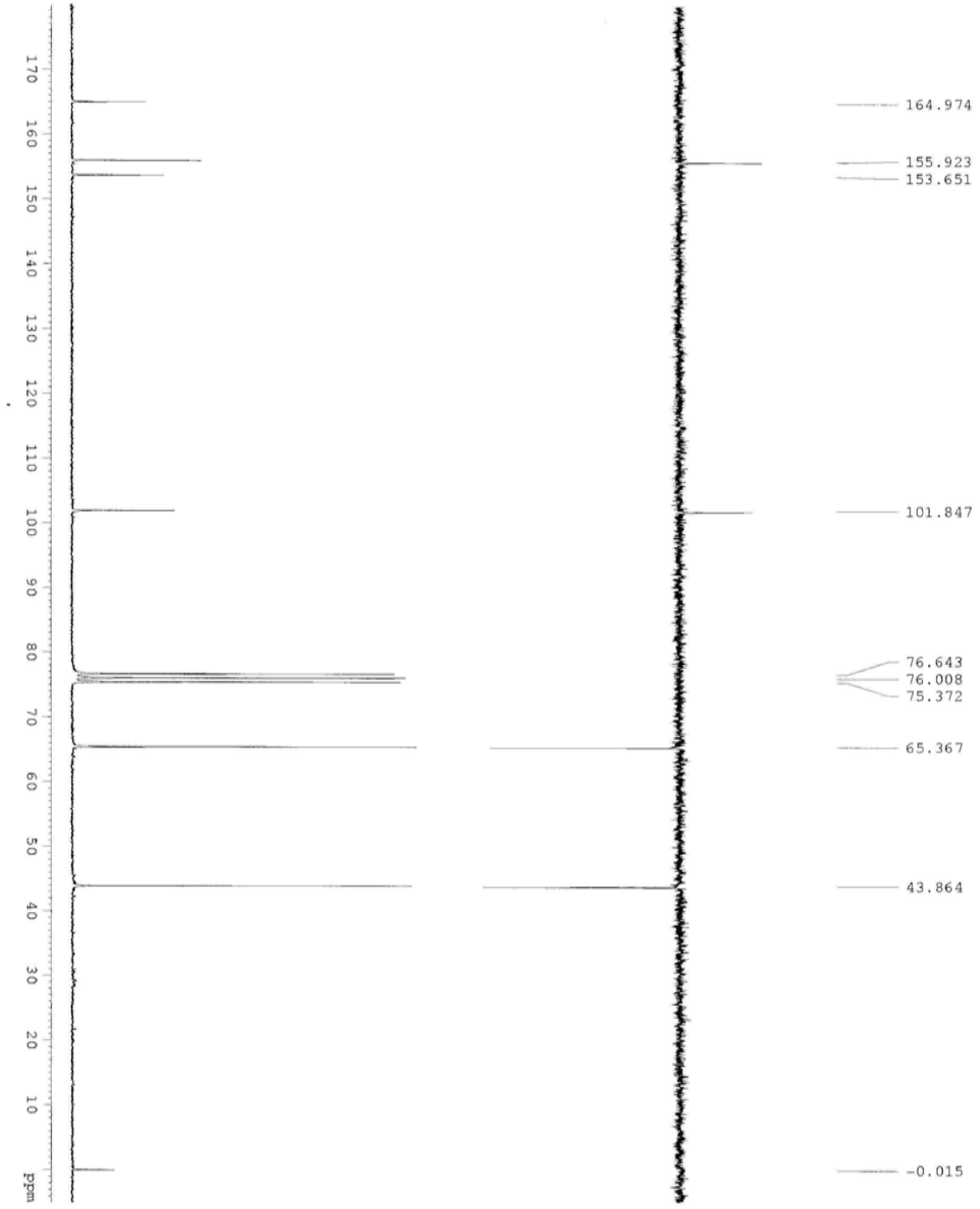

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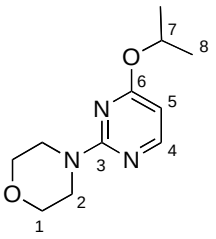

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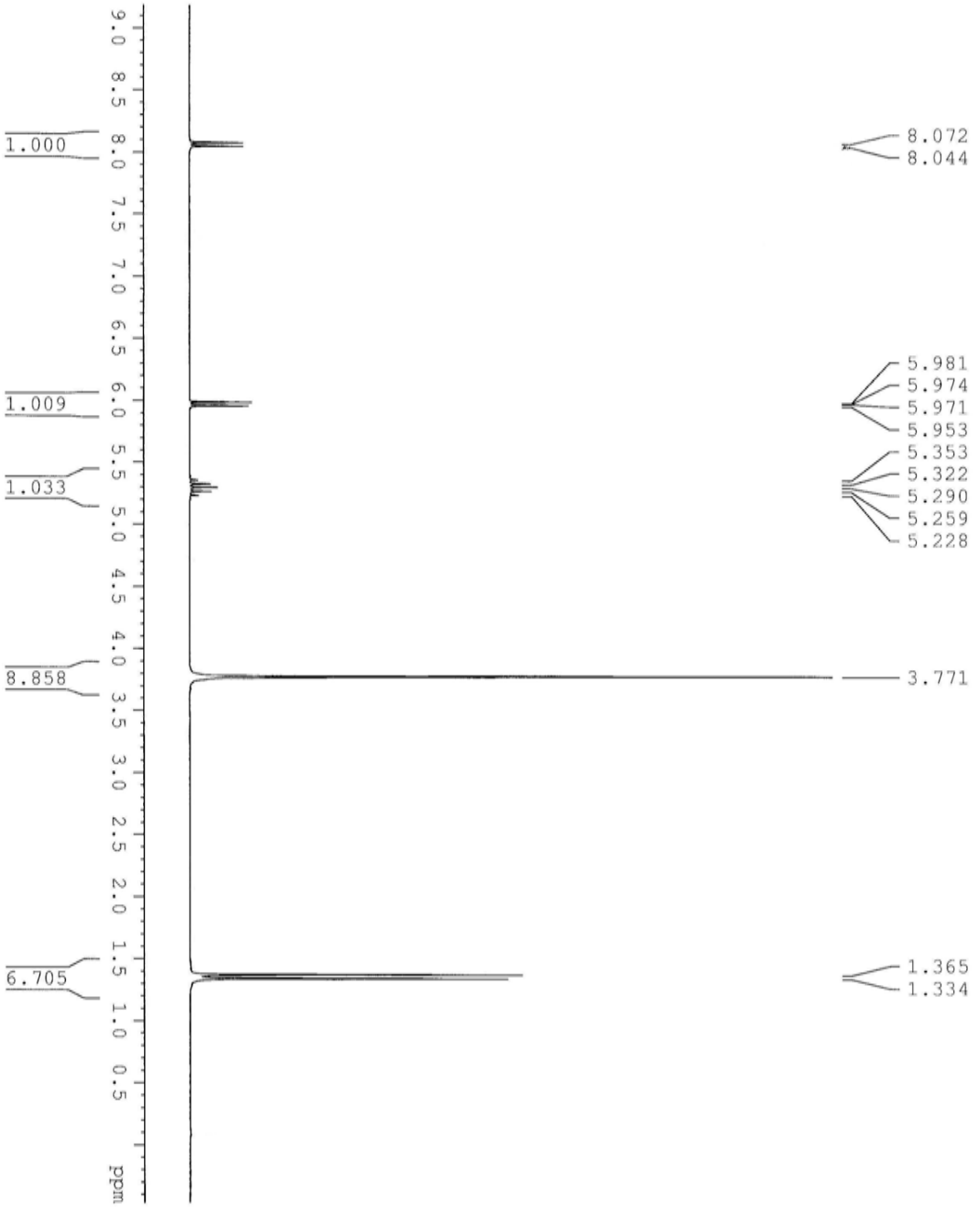

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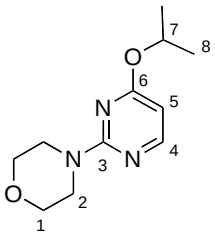

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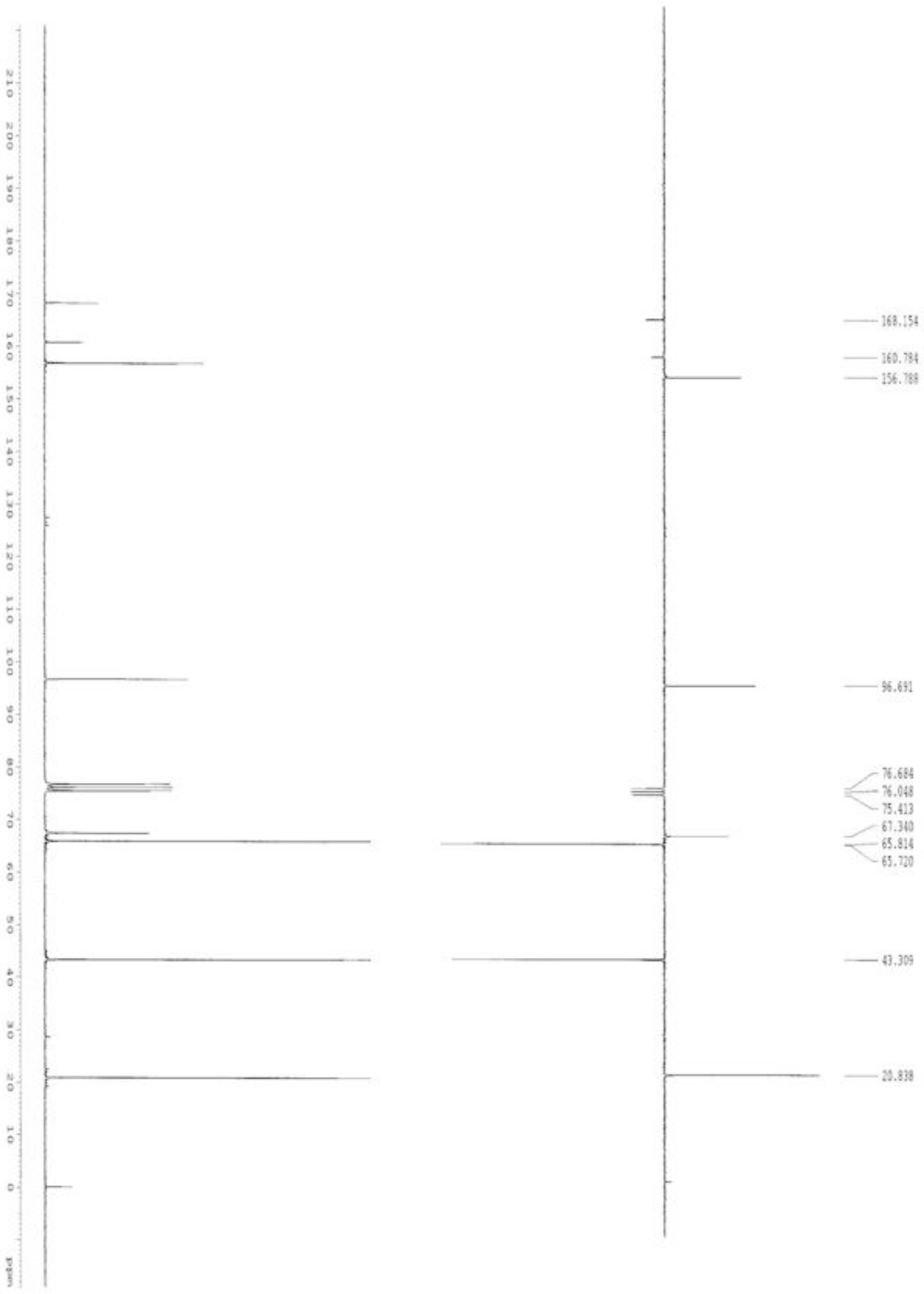
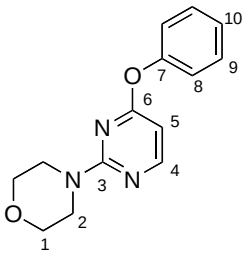
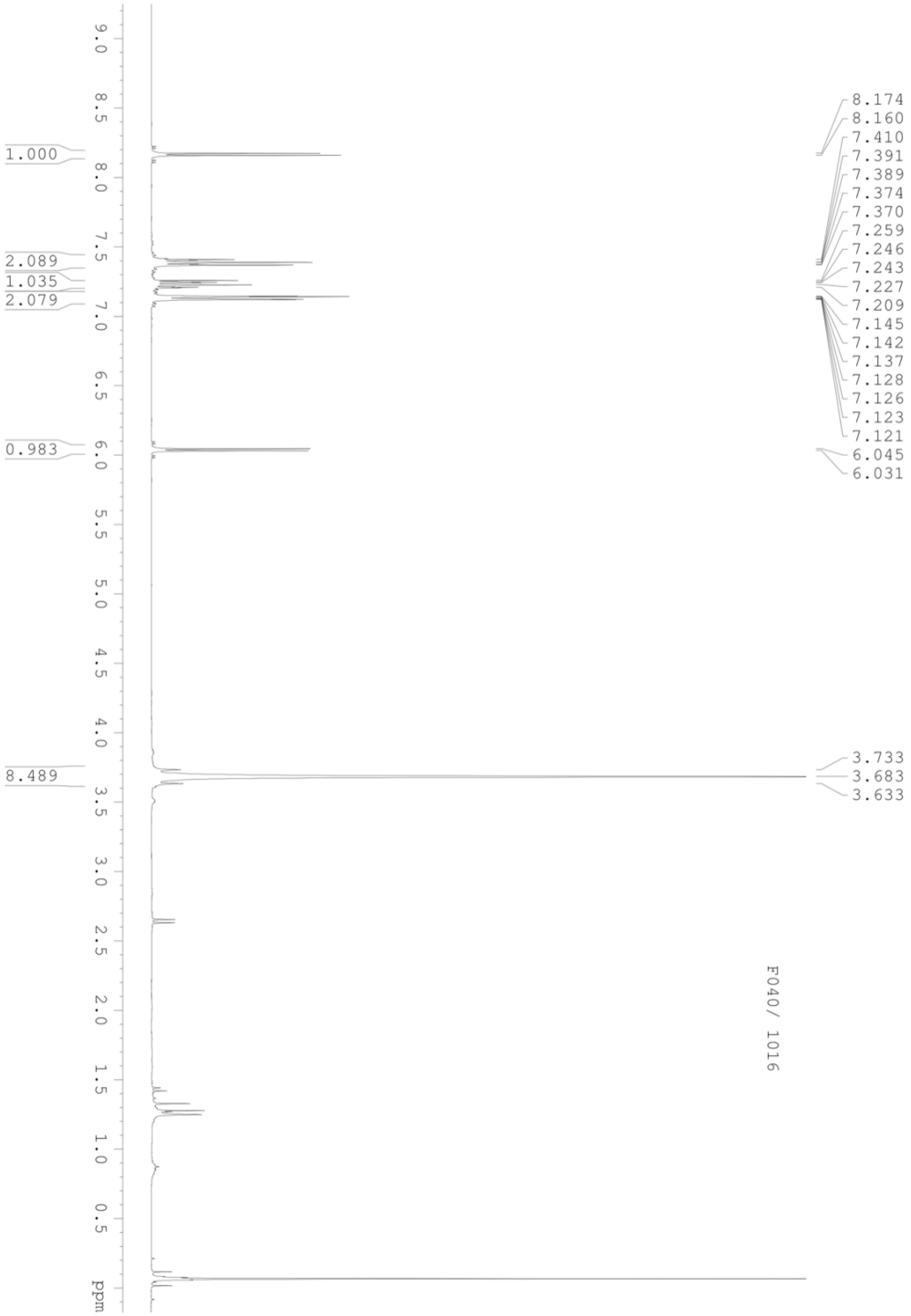


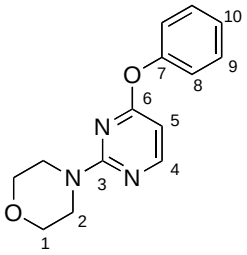
Compound I.30a



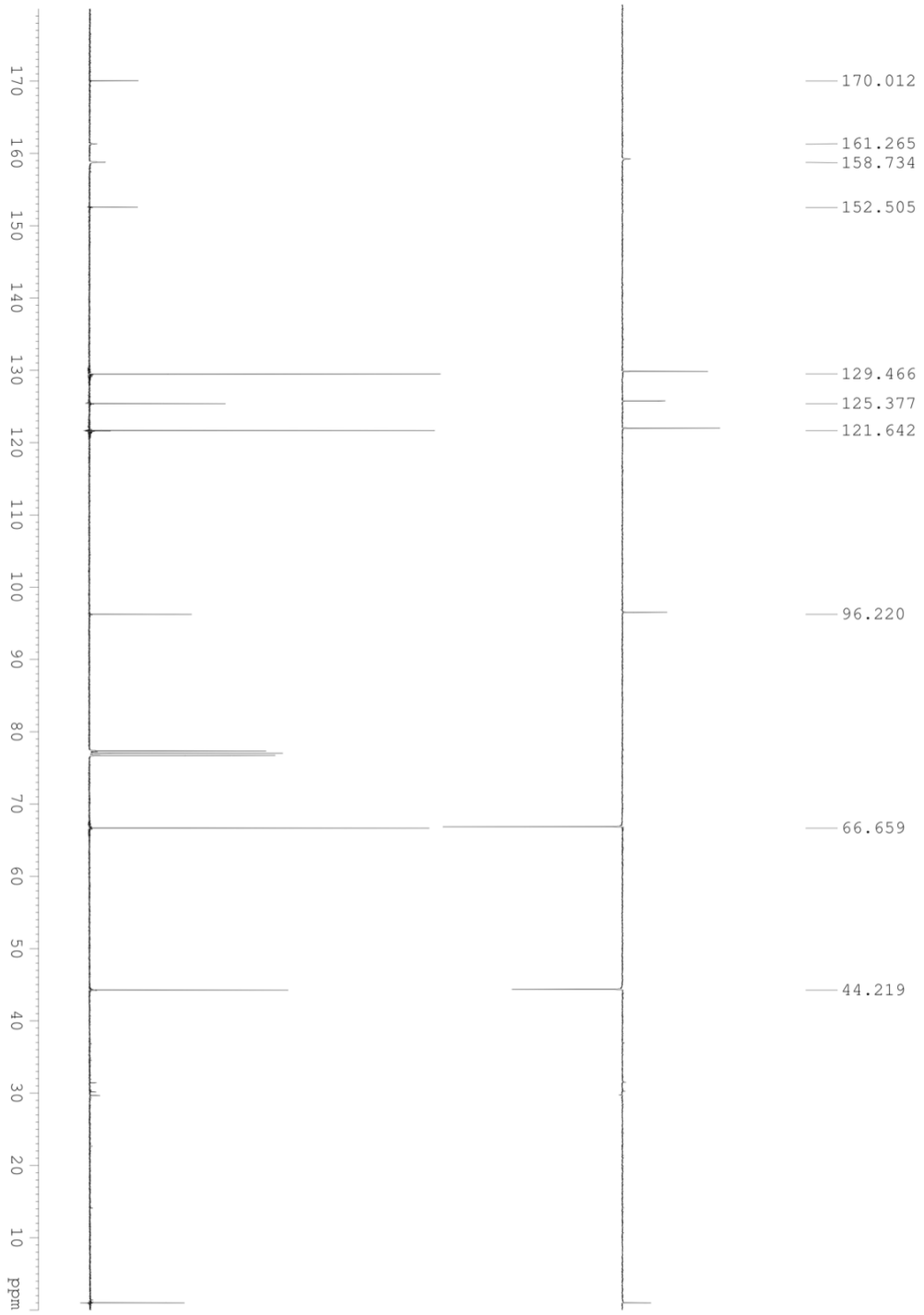
^{1}H NMR



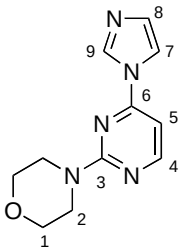
Compound I.30a



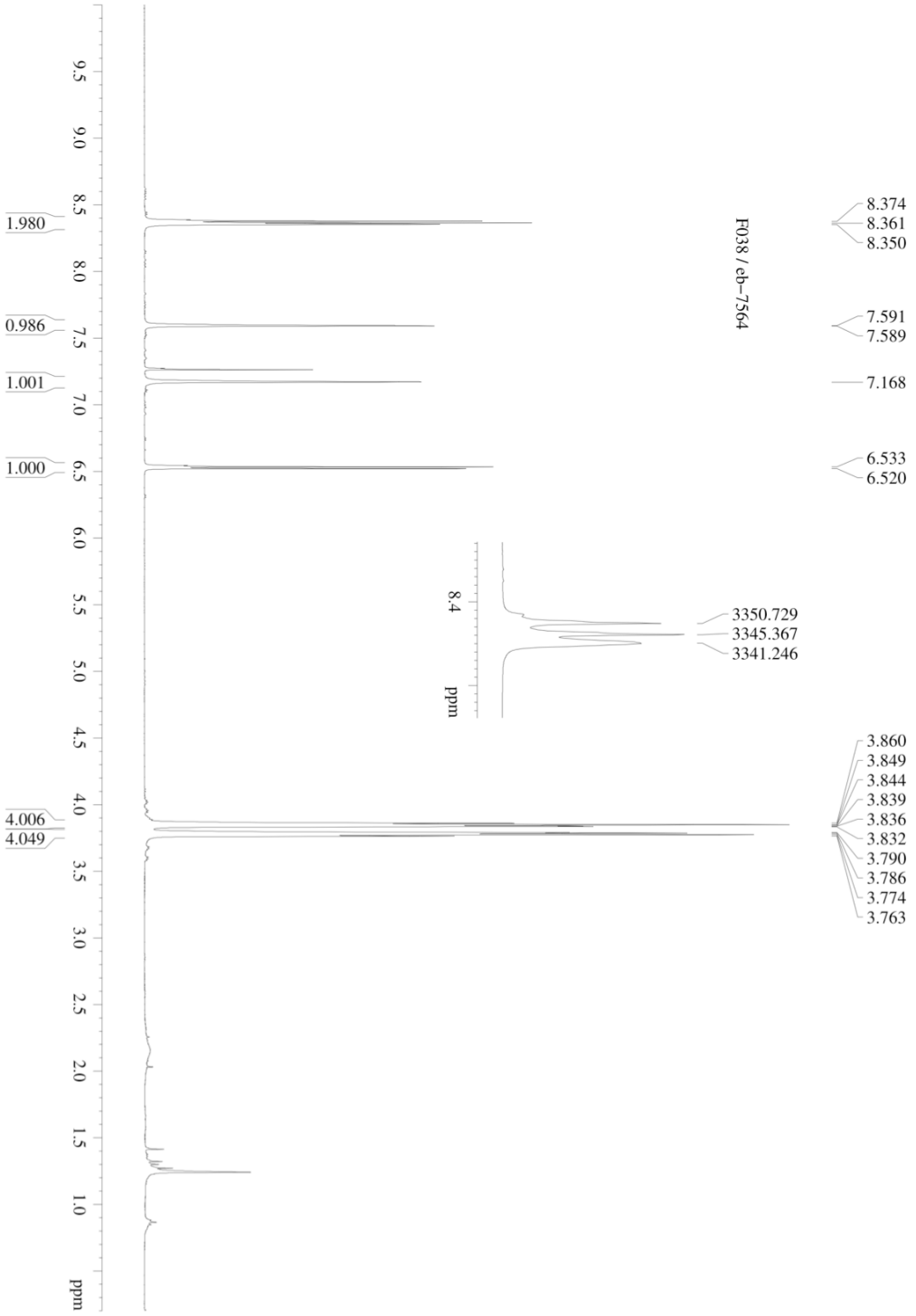
^{13}C / DEPT135 NMR



Compound I.30b



^{1}H NMR



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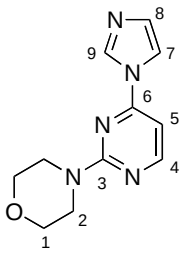
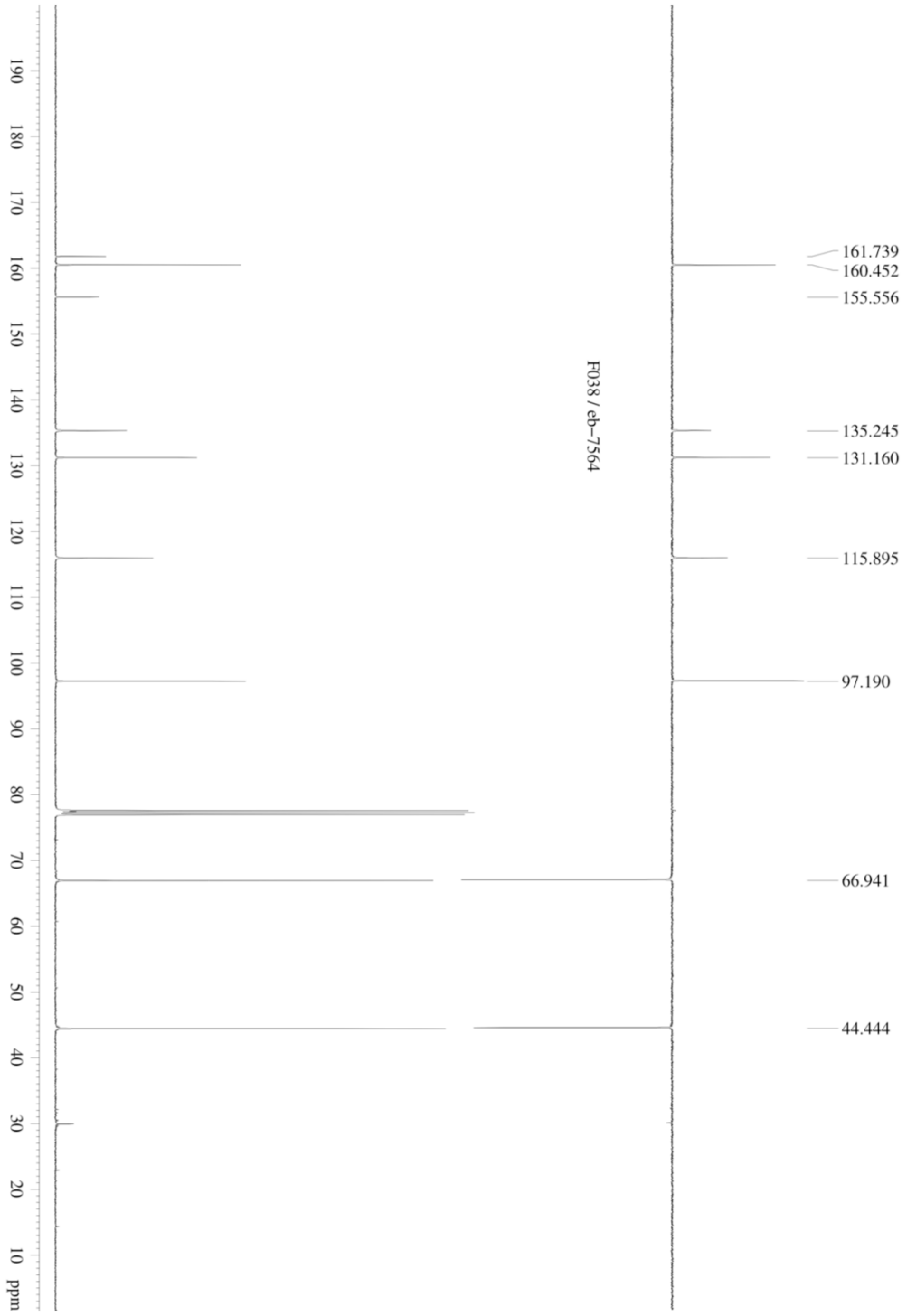
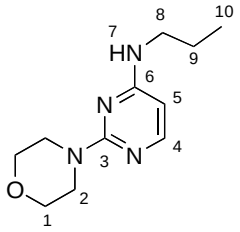


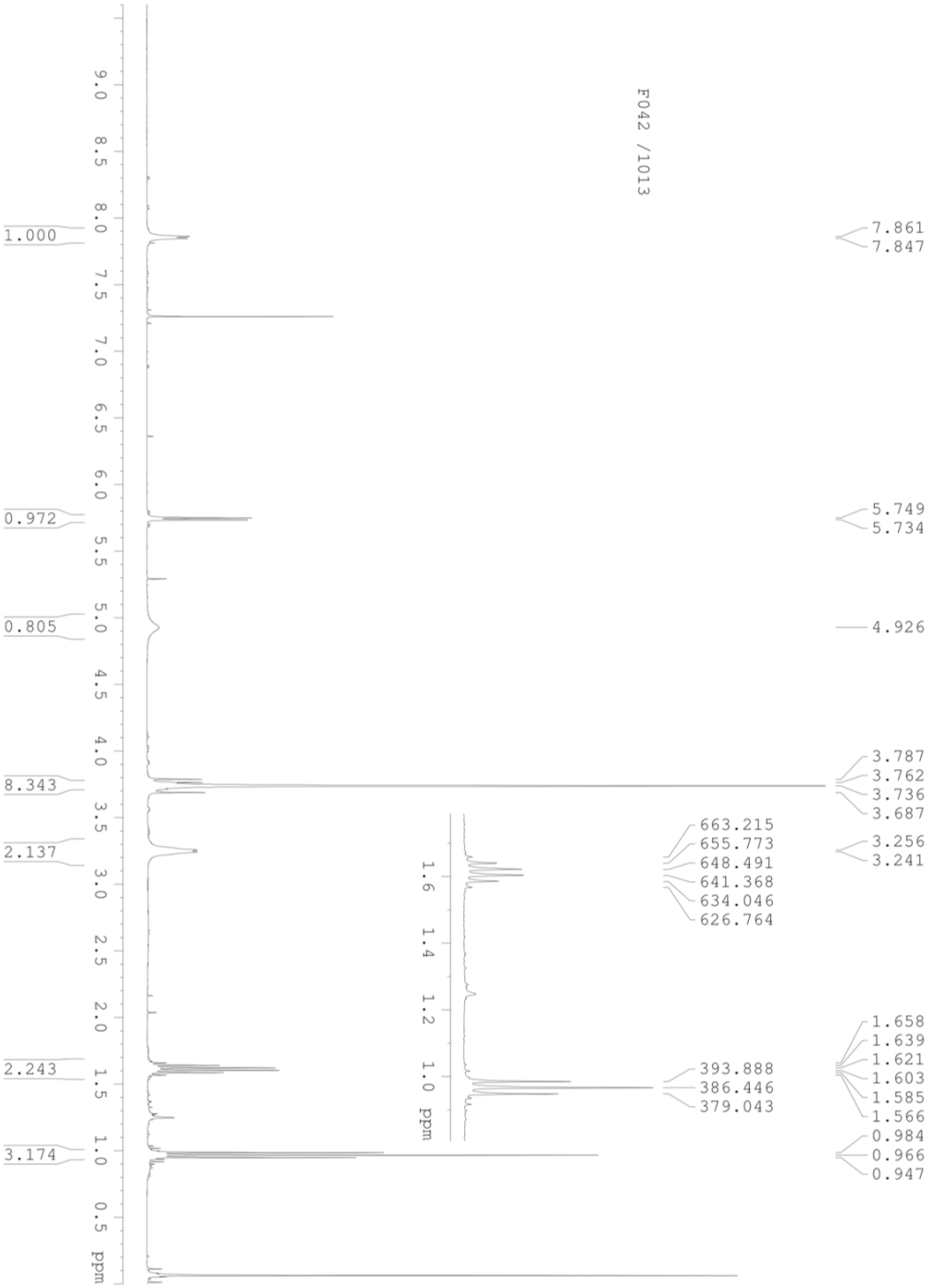
^{13}C / DEPT135 NMR



Compound I.30c



^{1}H NMR



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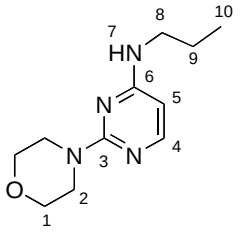
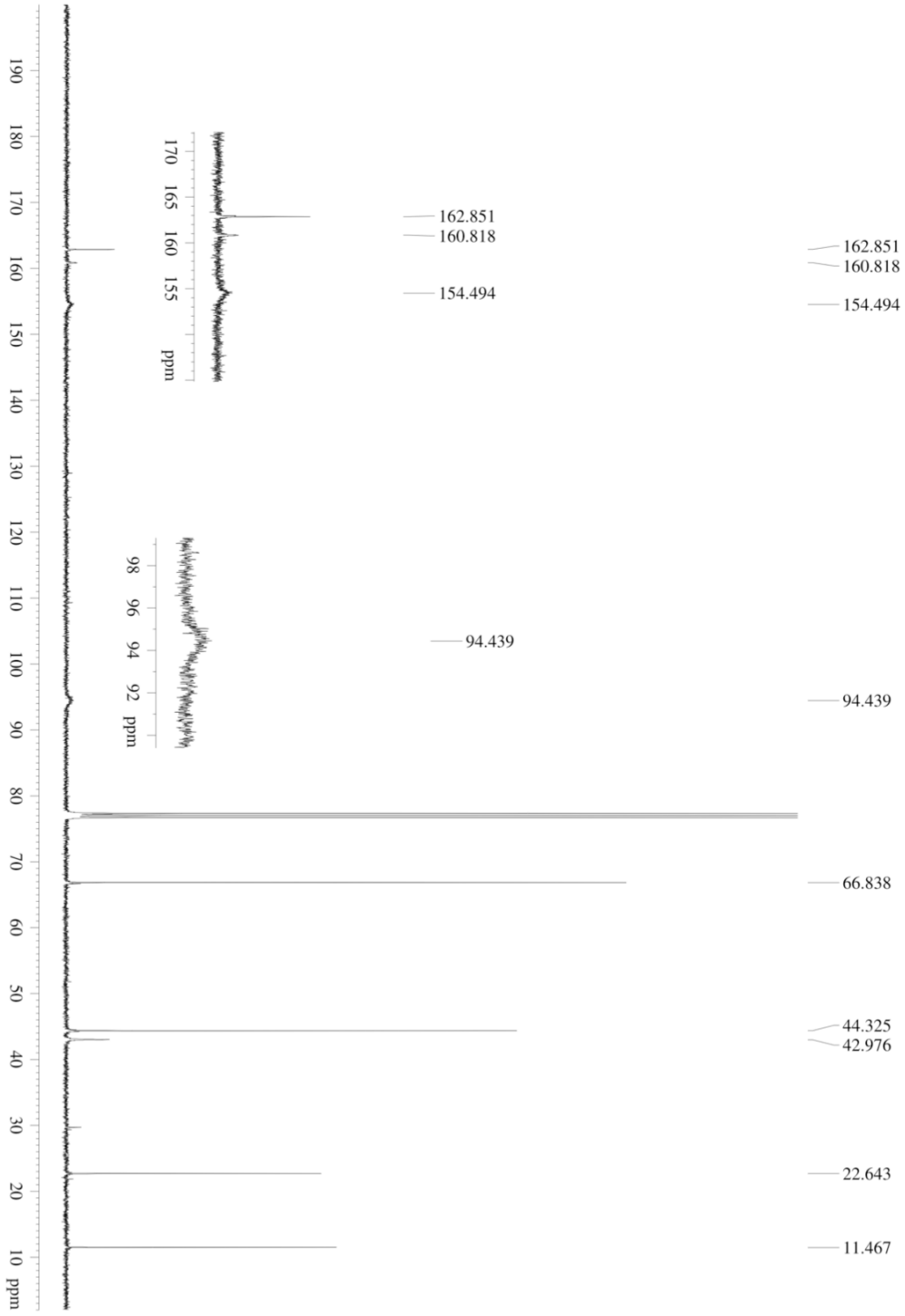
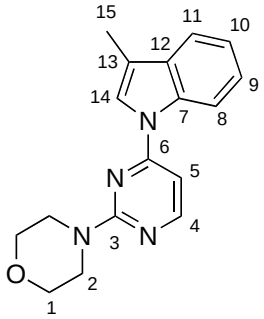


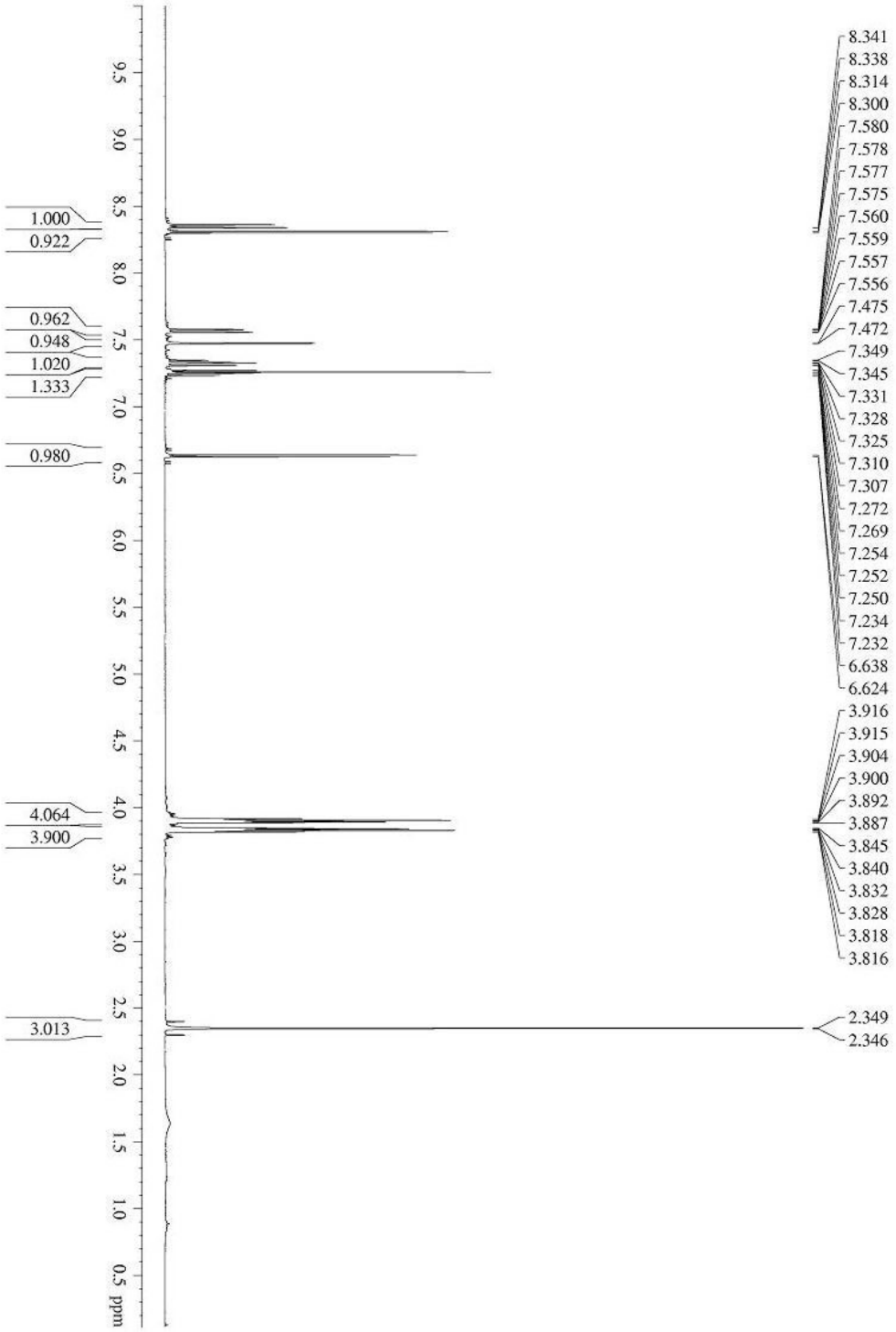
^{13}C / DEPT135 NMR



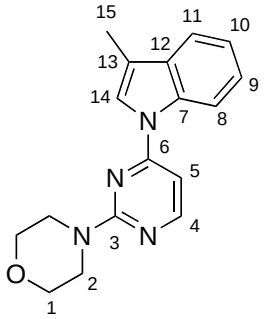
Compound I.30d



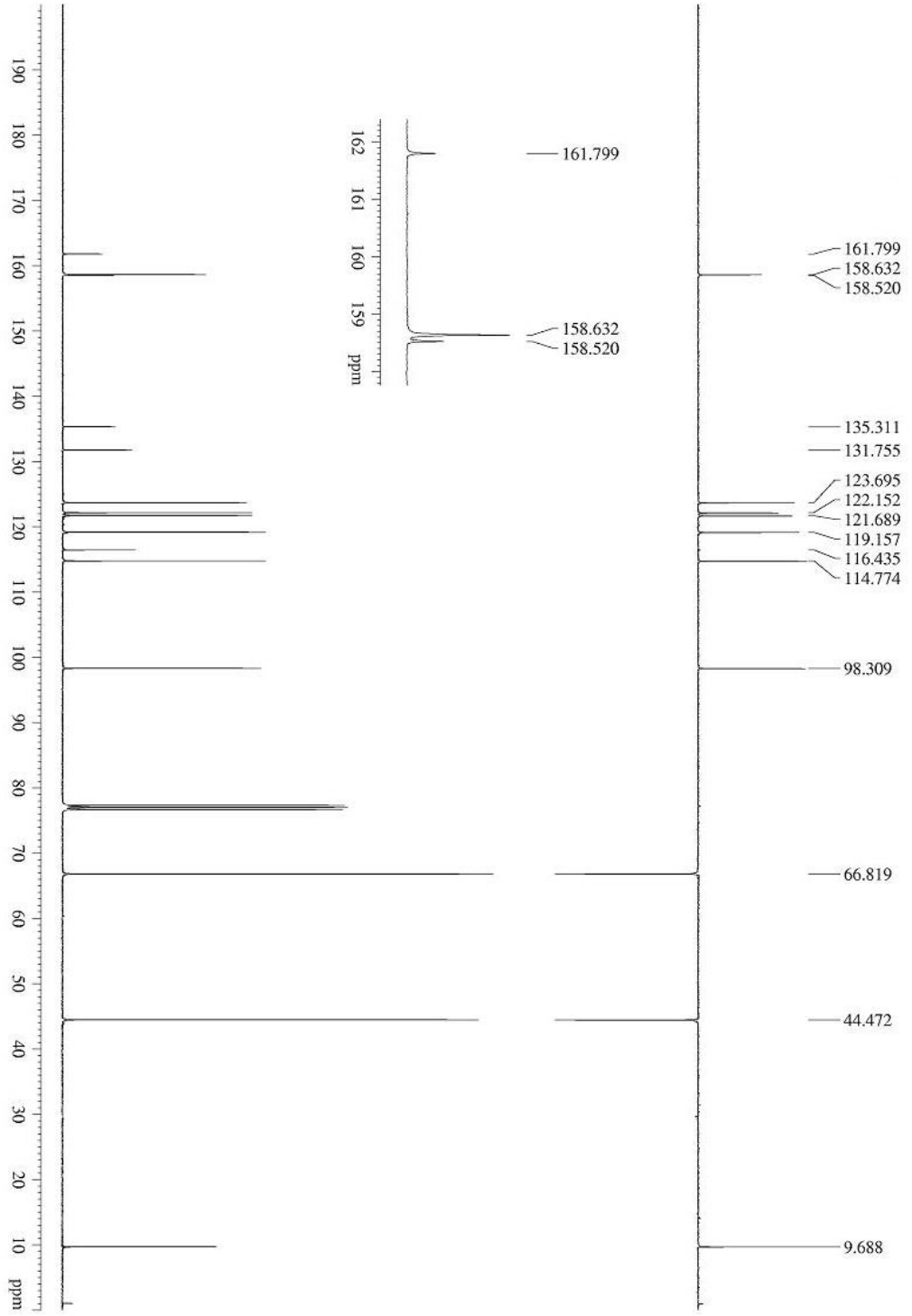
^{1}H NMR



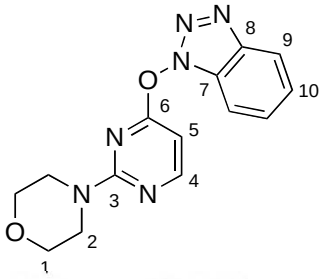
Compound I.30d



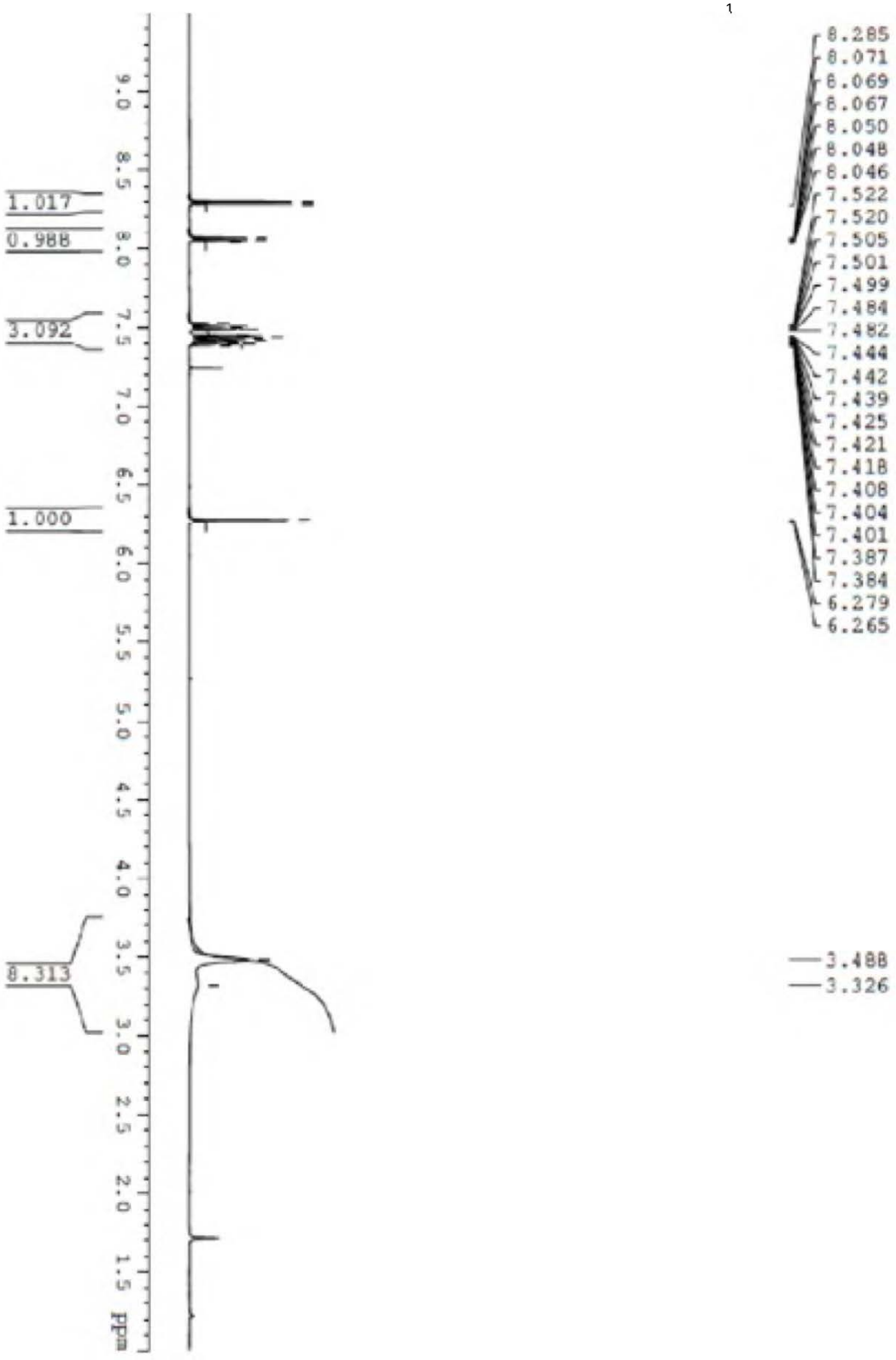
^{13}C NMR



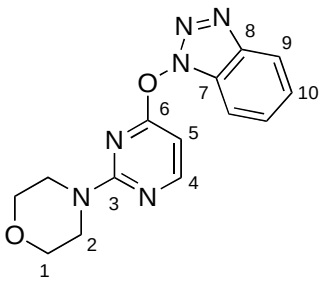
Compound I.31



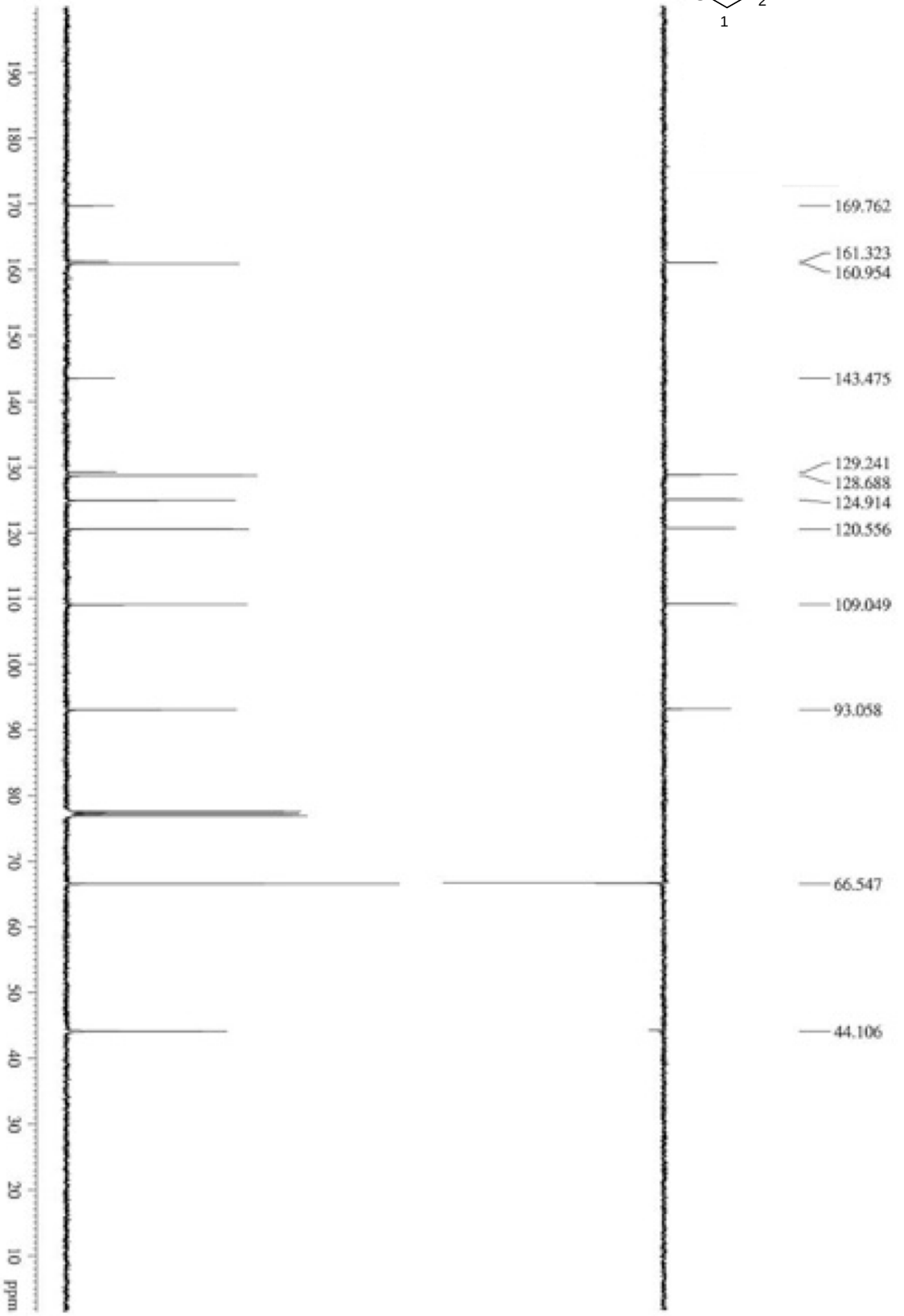
^{1}H NMR



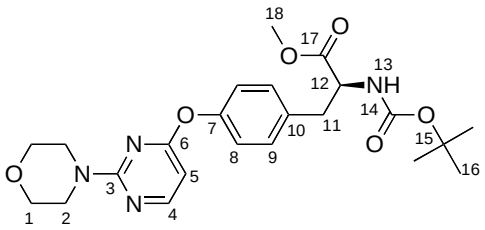
Compound I.31



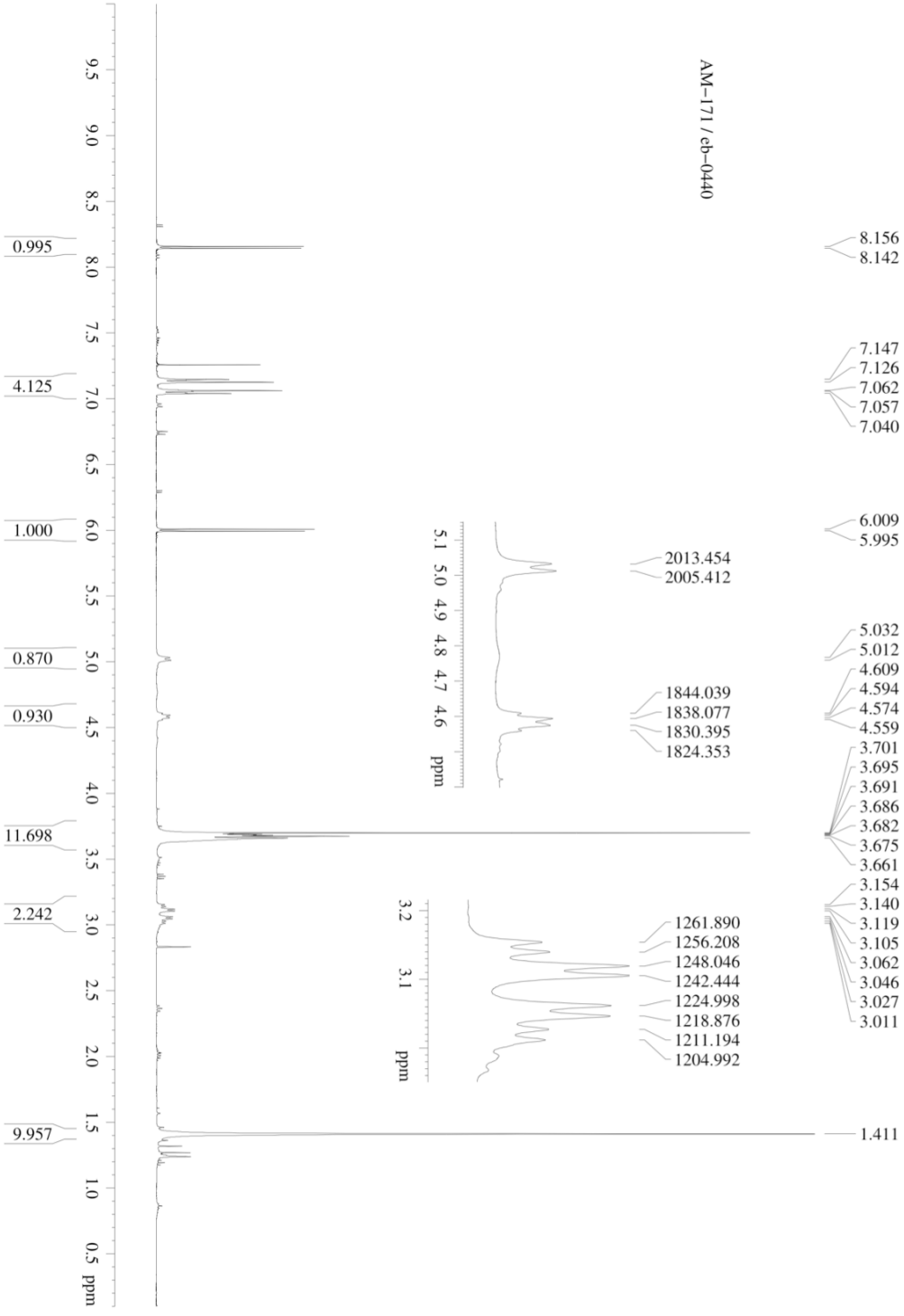
^{13}C / DEPT135 NMR



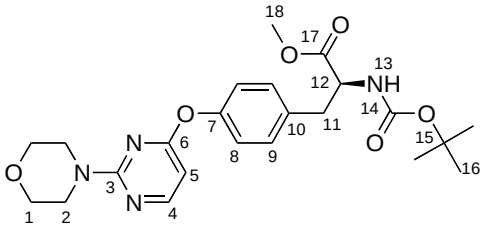
Compound I.20a



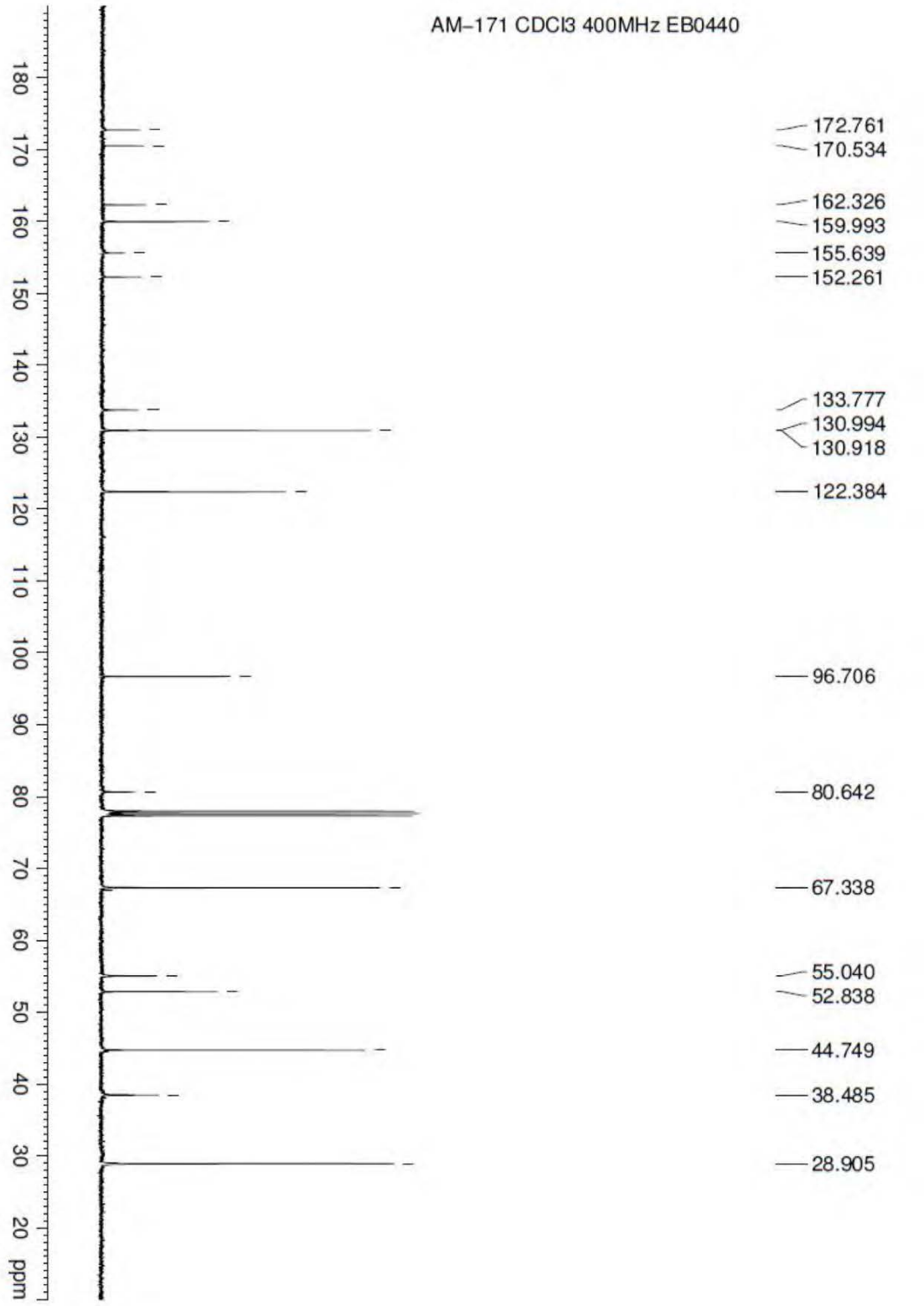
^{1}H NMR



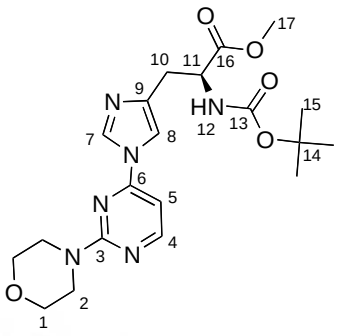
Compound I.20a



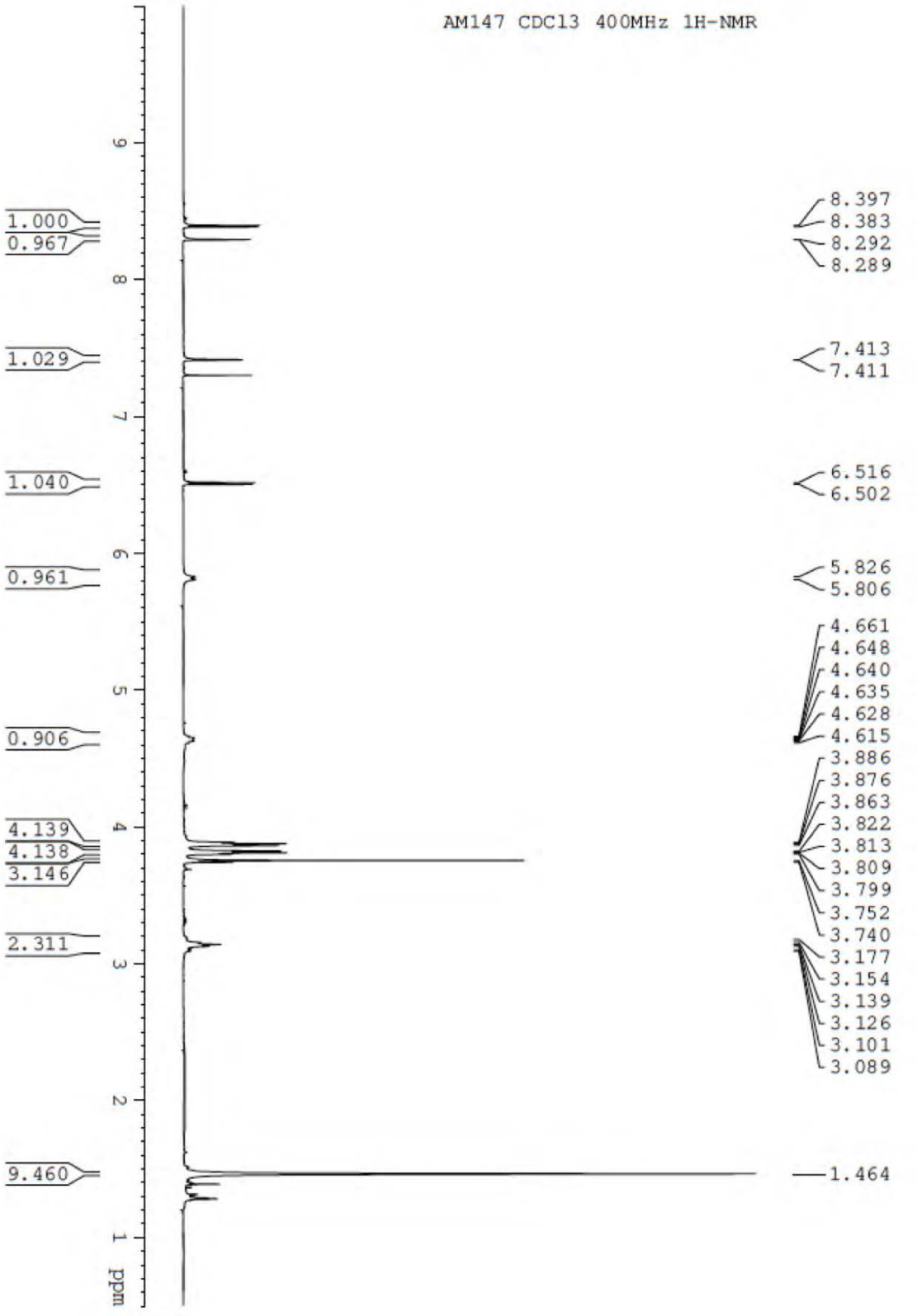
^{13}C NMR



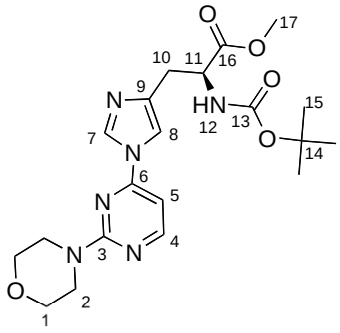
Compound I.20b



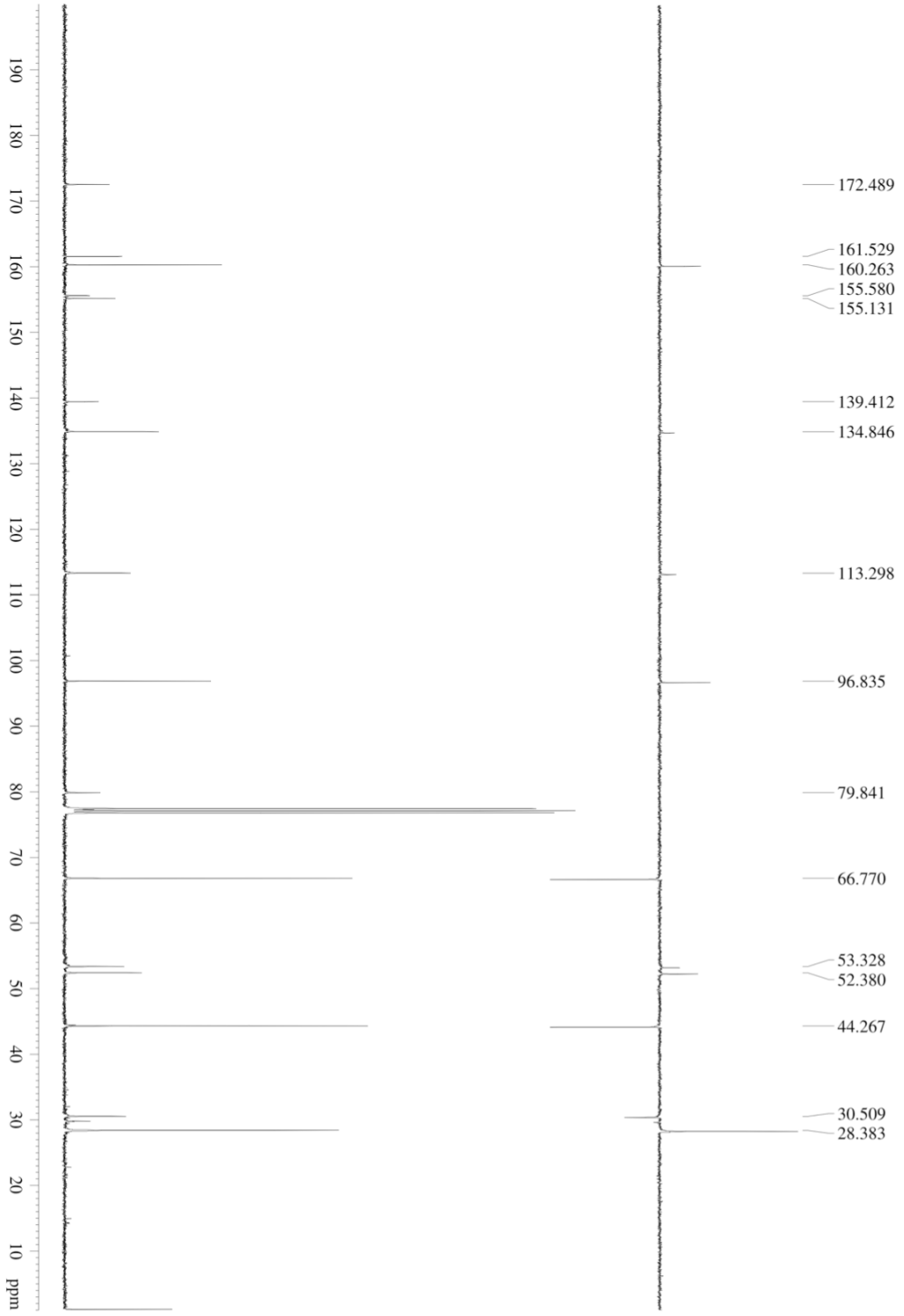
^{1}H NMR



Compound I.20b



^{13}C / DEPT135 NMR



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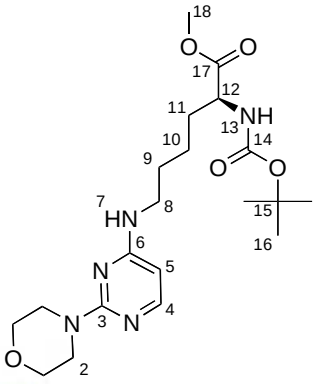
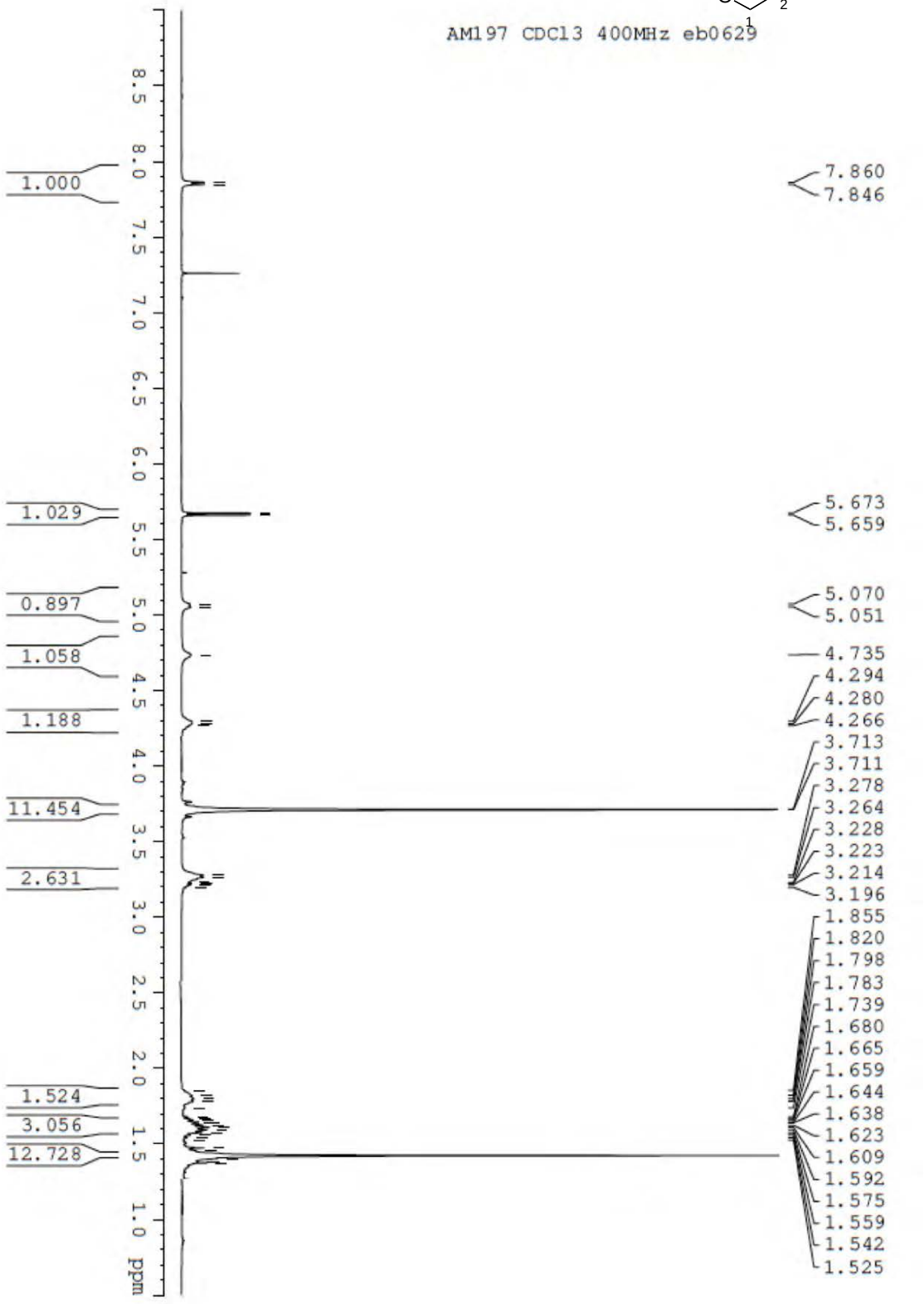
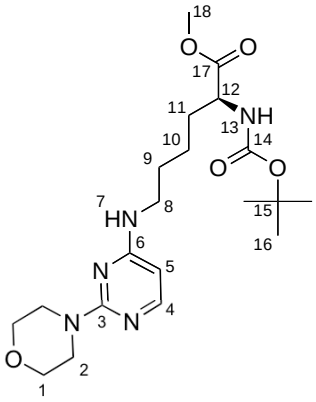


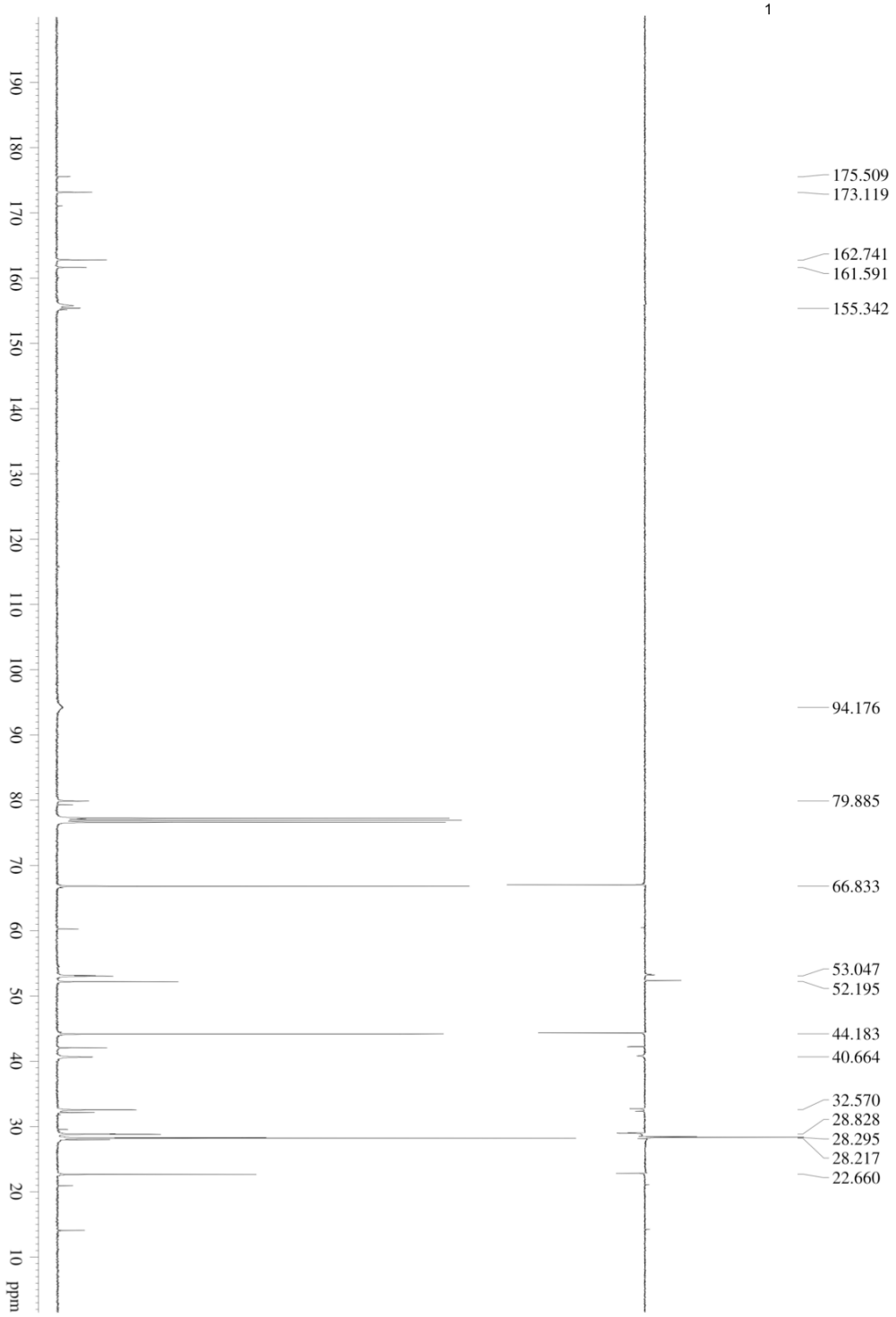
^{1}H NMR



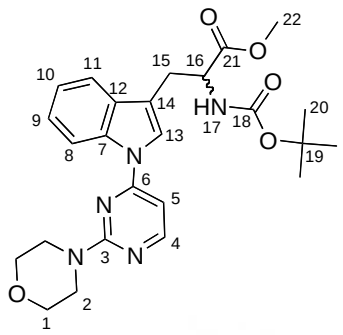
Compound I.20c



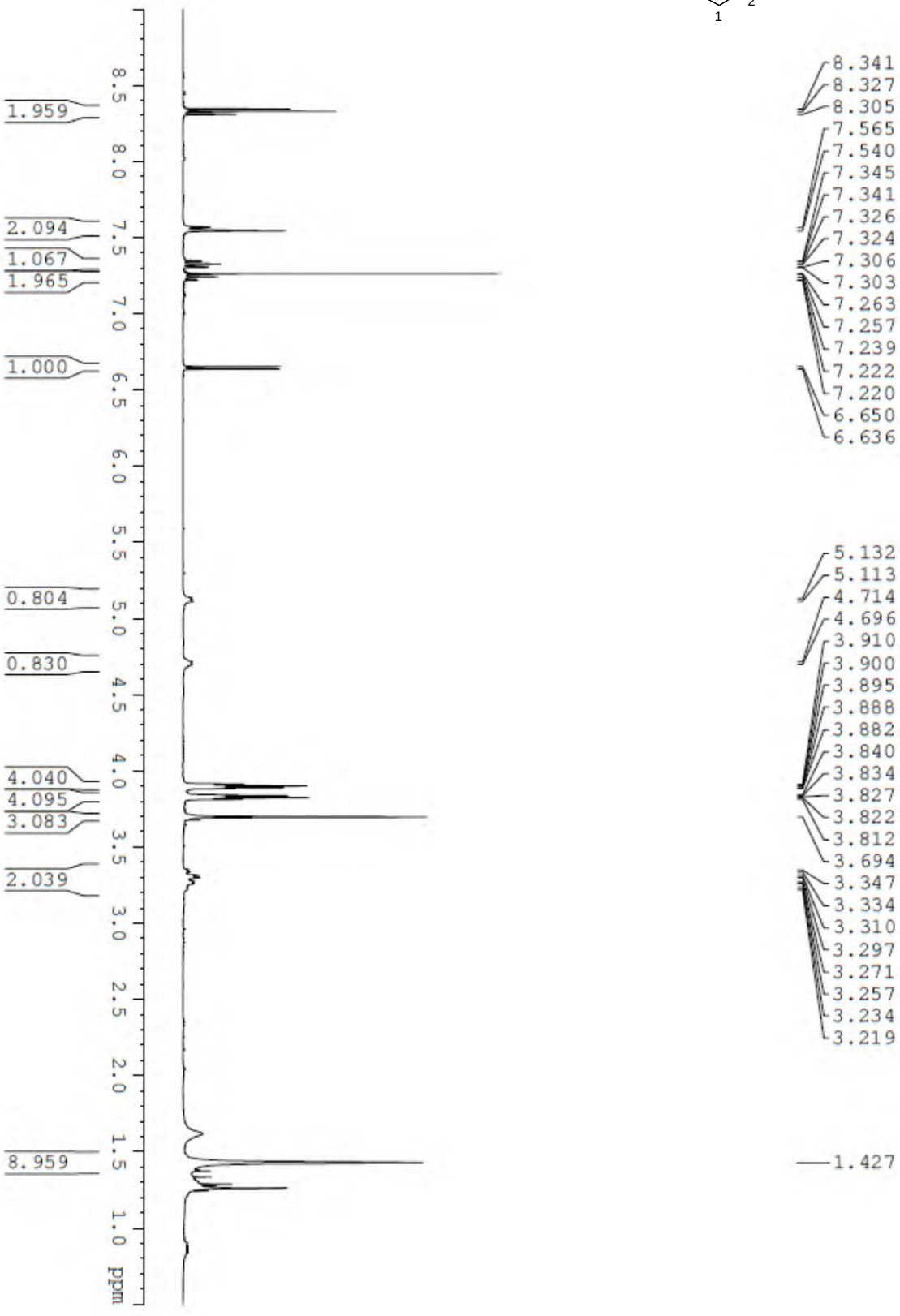
^{13}C / DEPT135 NMR



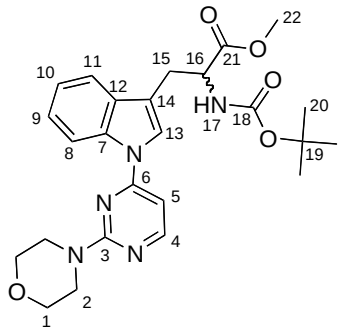
Compound I.20d



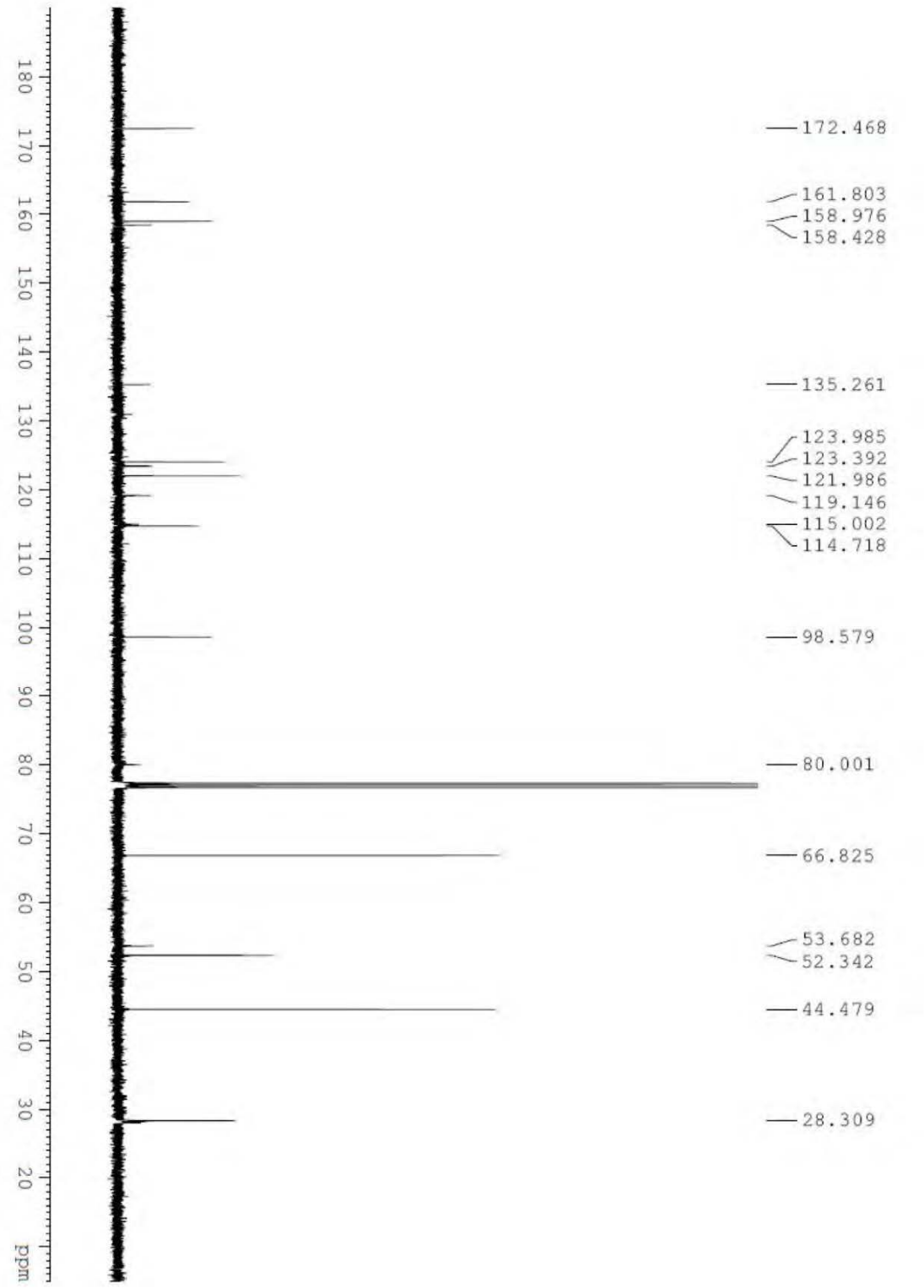
^{1}H NMR



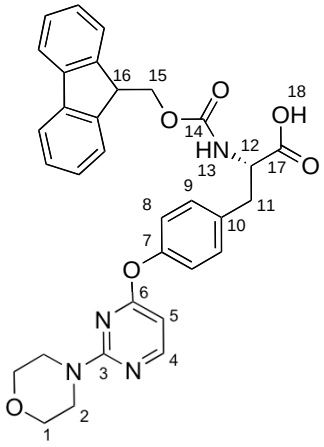
Compound I.20d



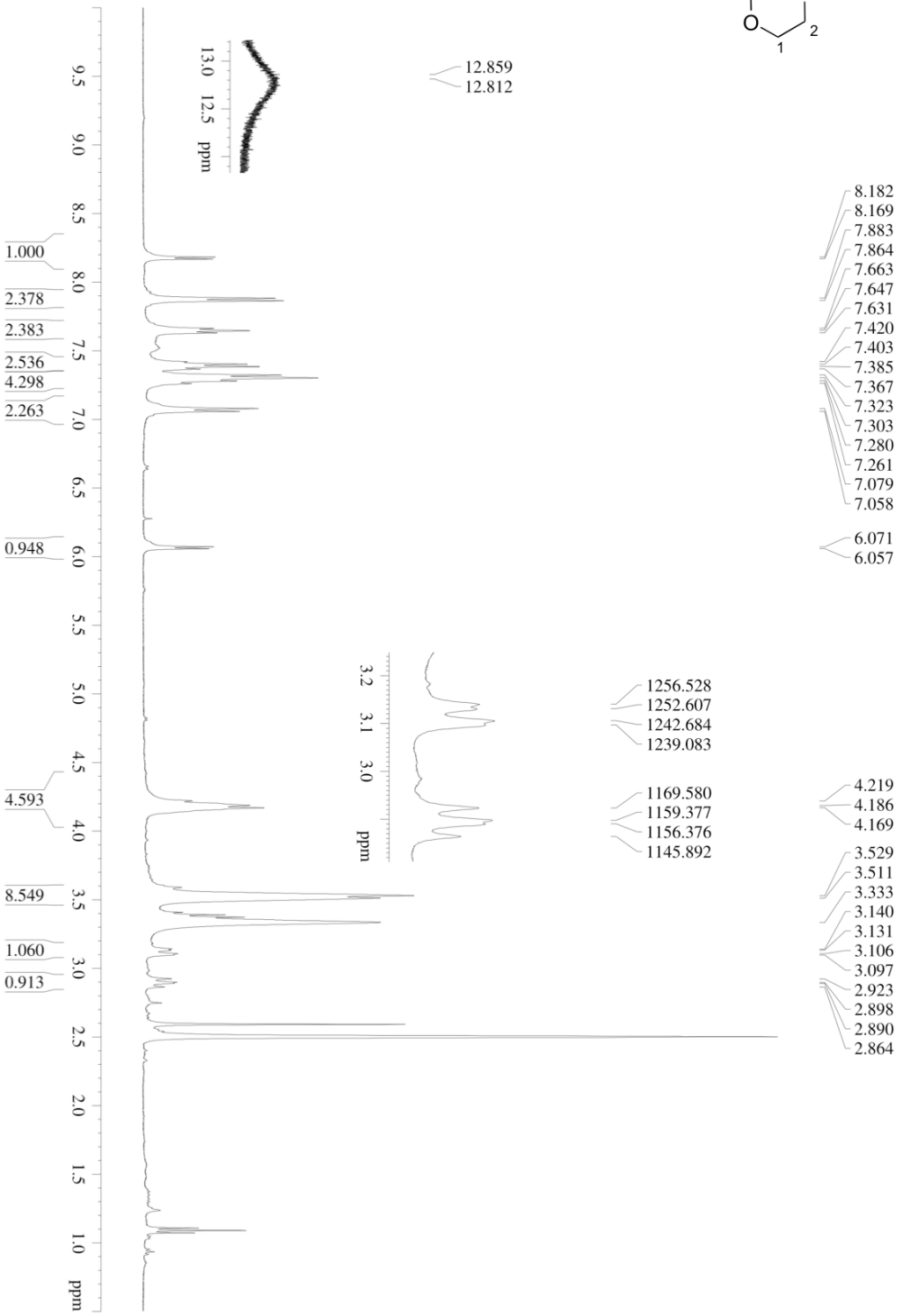
^{13}C NMR



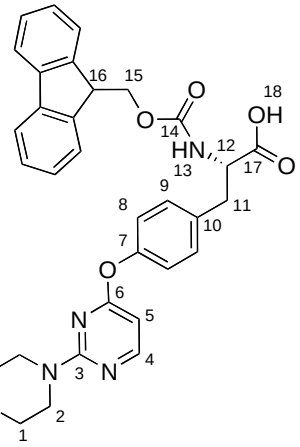
Compound I.11a



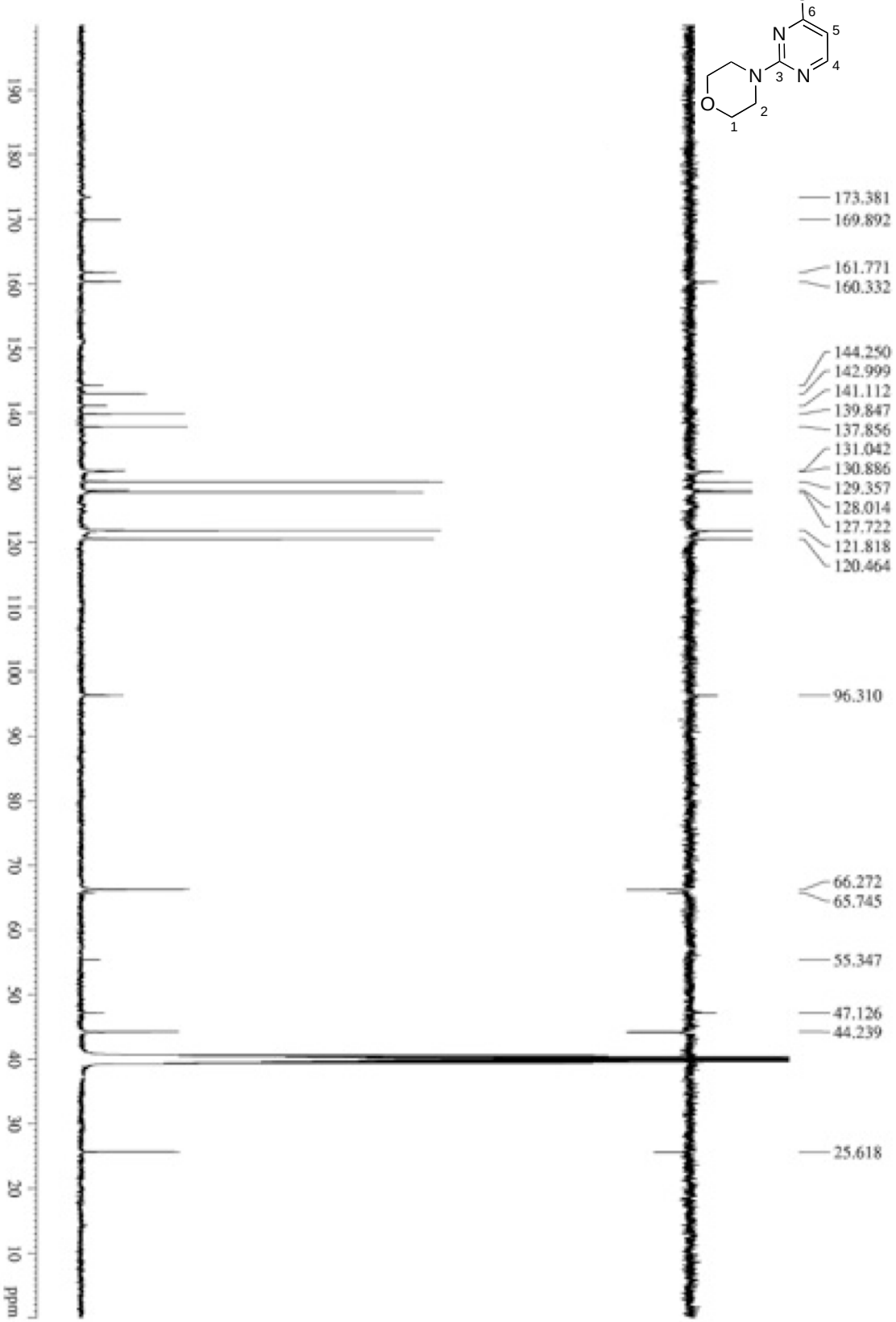
^{1}H NMR



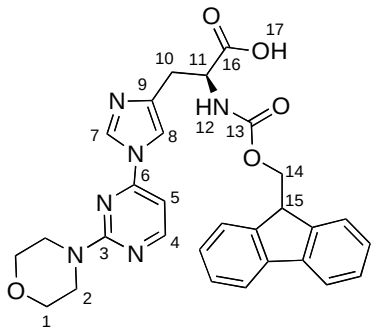
Compound I.11a



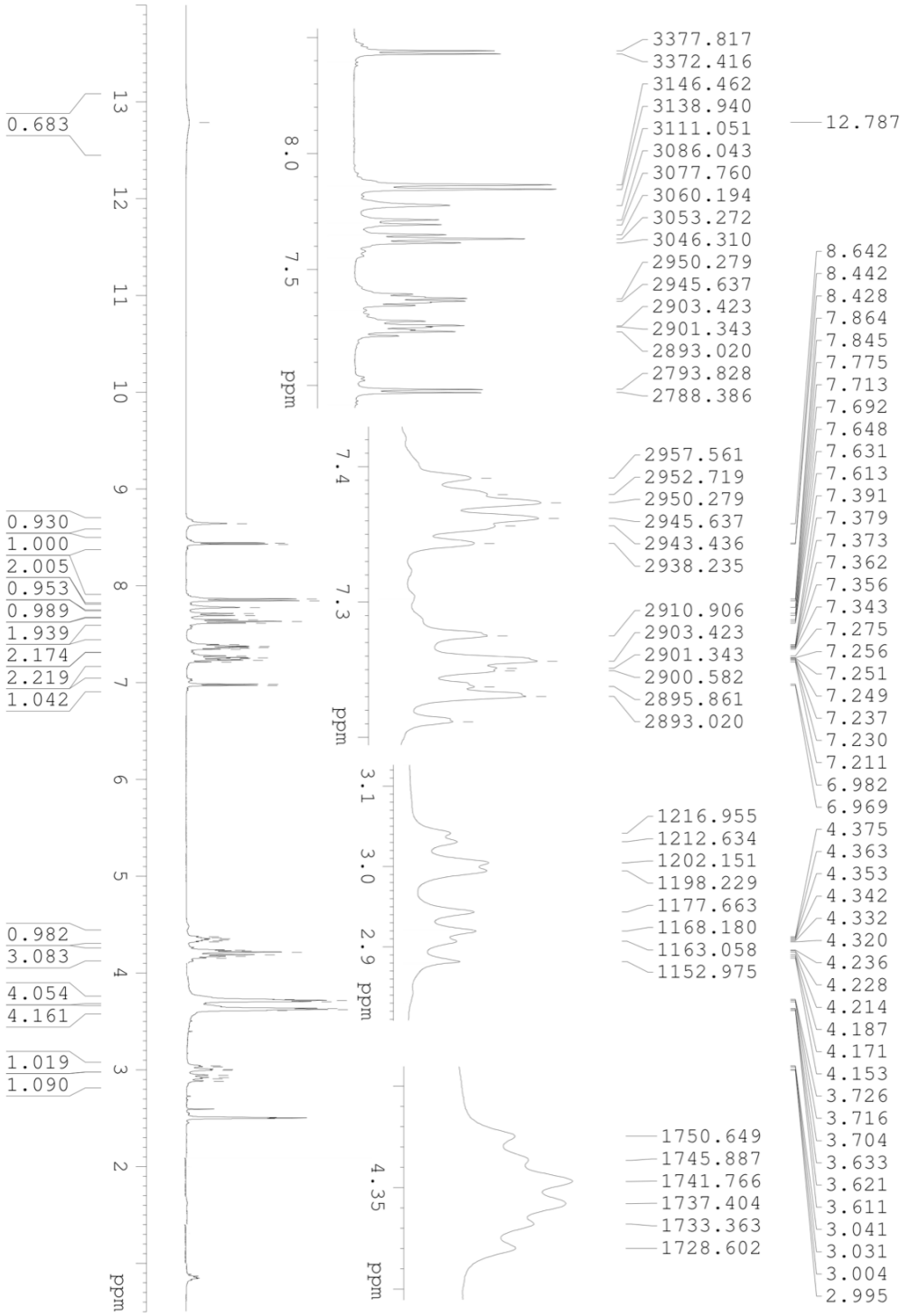
^{13}C / DEPT135 NMR



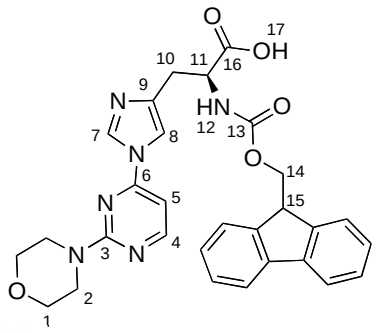
Compound I.11b



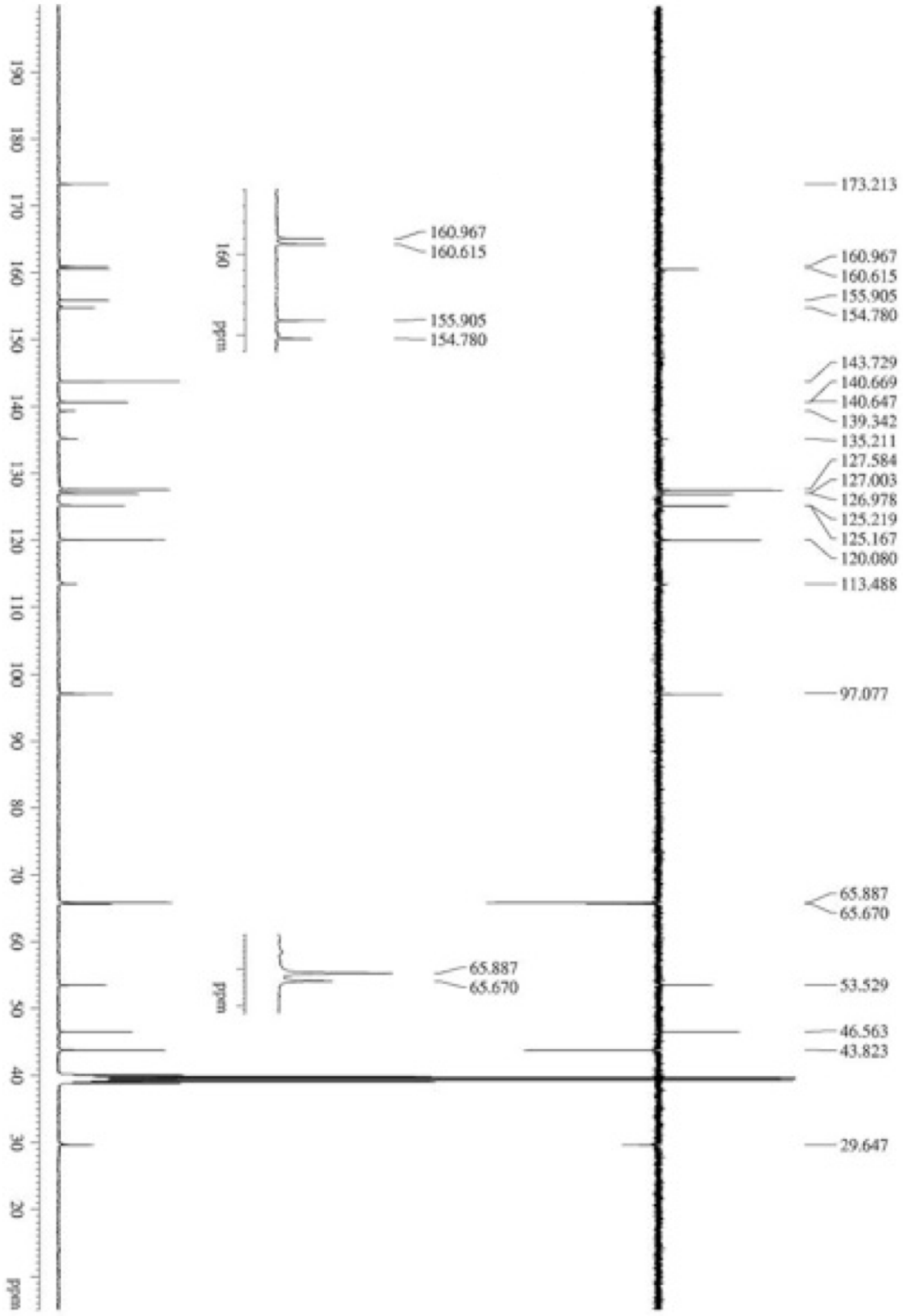
^{1}H NMR



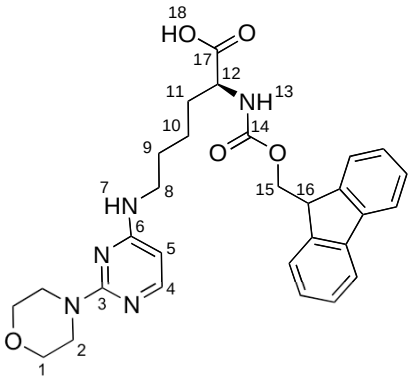
Compound I.11b



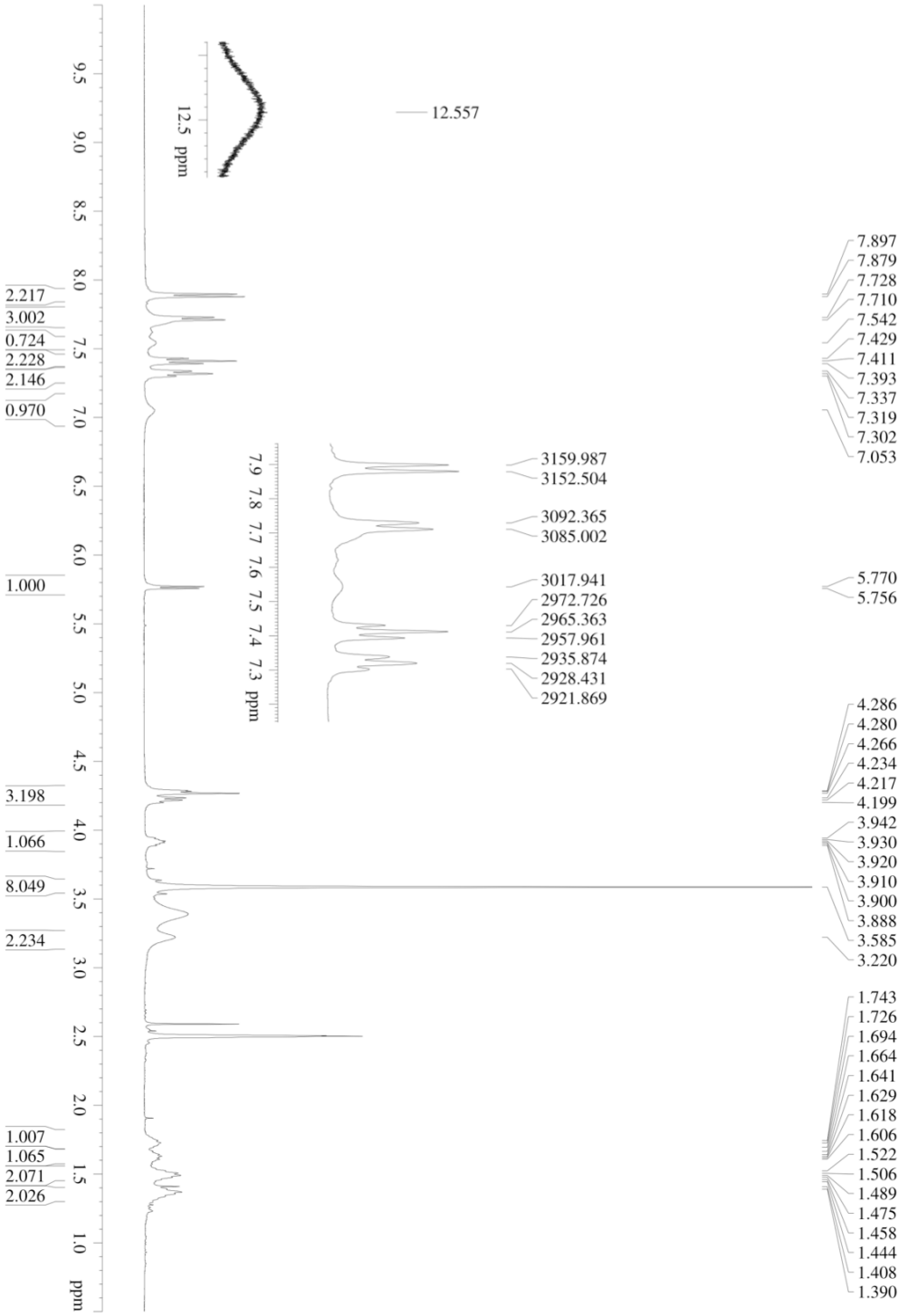
^{13}C / DEPT135 NMR



Compound I.11c

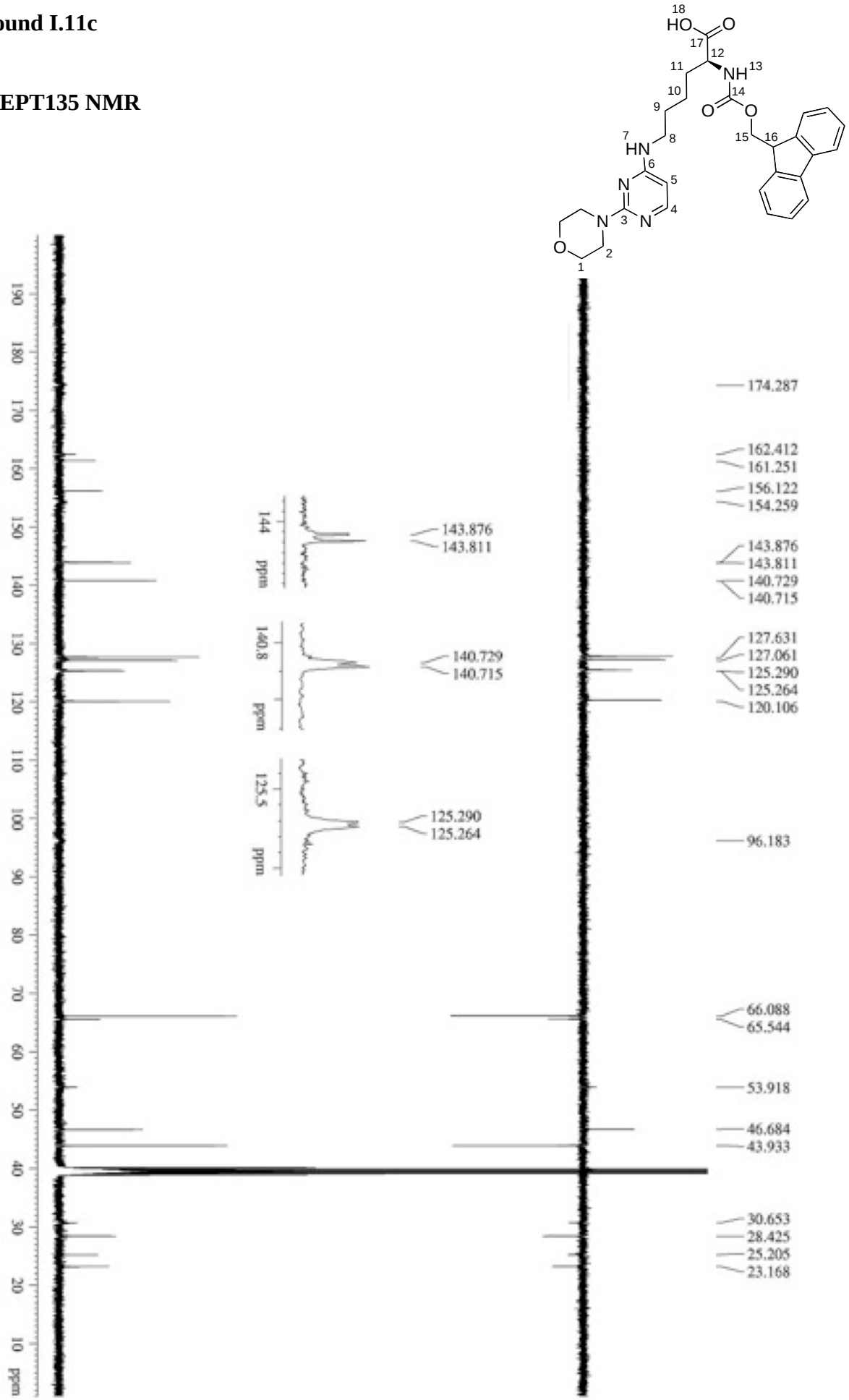


^{1}H NMR



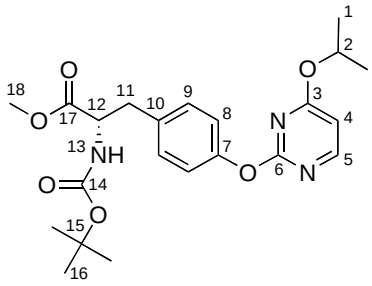
Compound I.11c

^{13}C / DEPT135 NMR

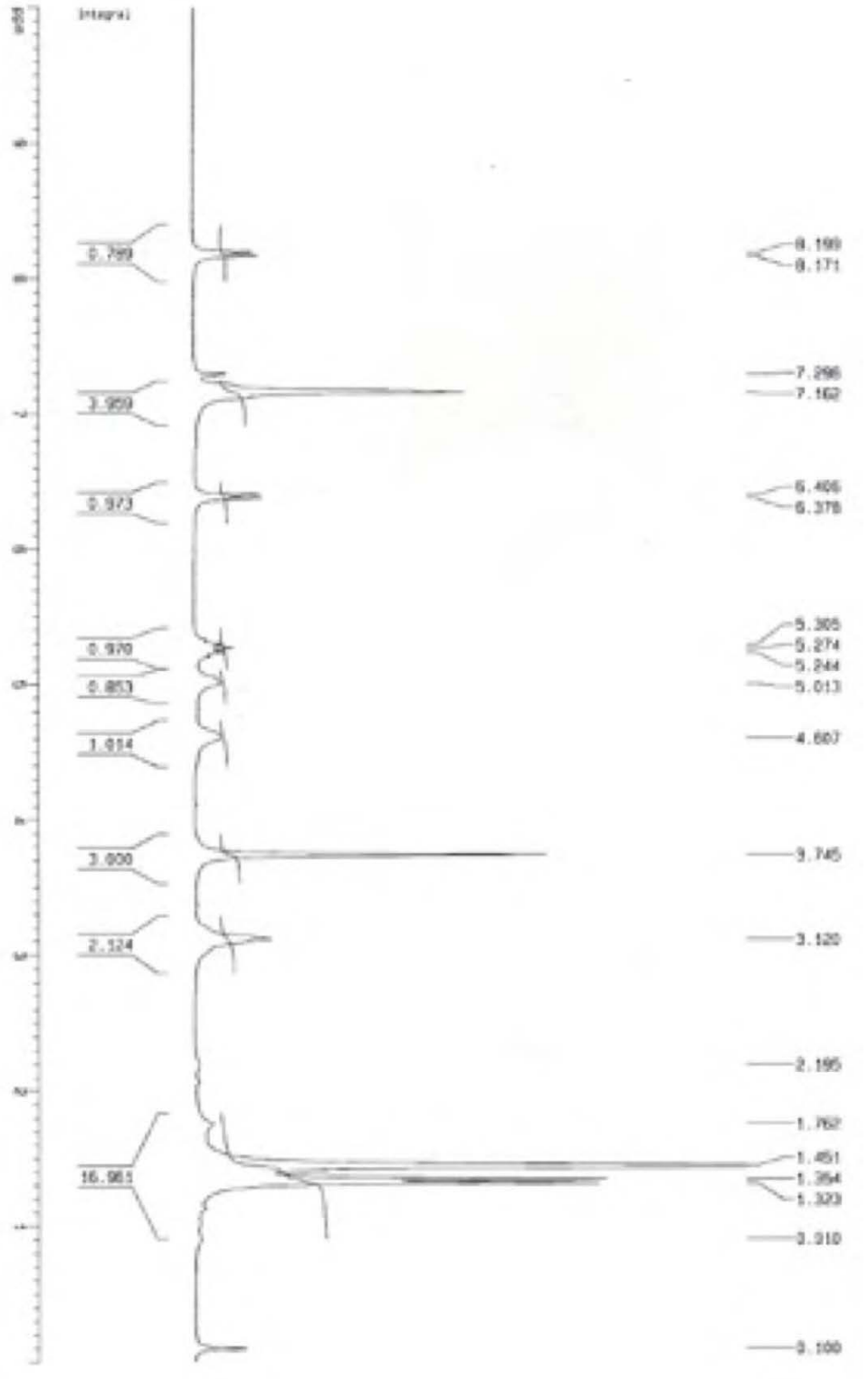


I.2. Pyrimidin-2-yl amino acids

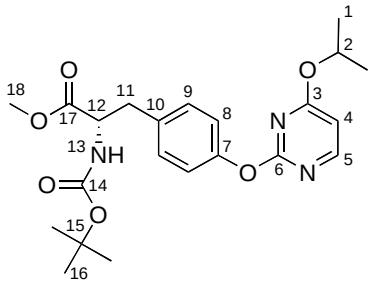
Compound I.32aa



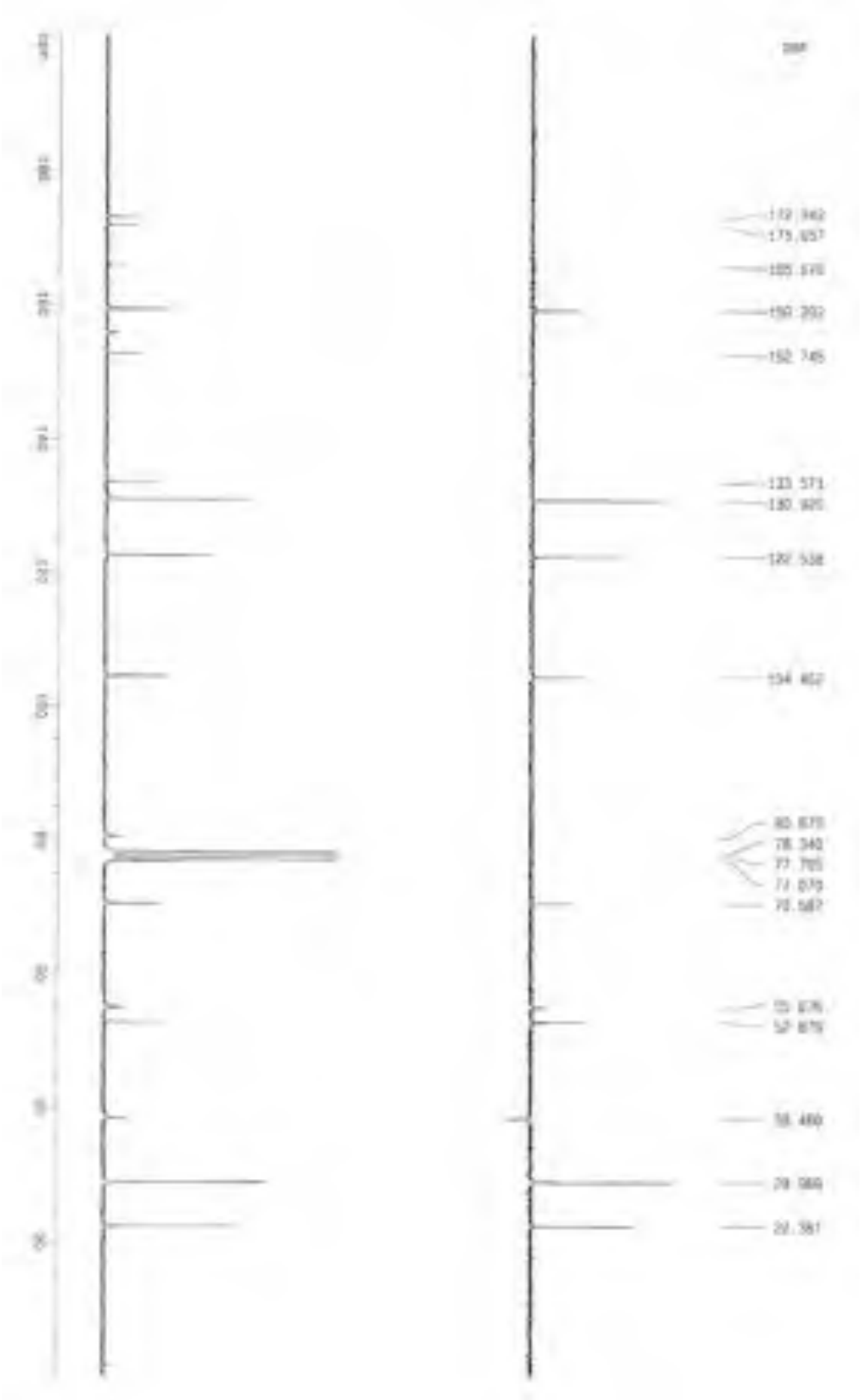
^{1}H NMR



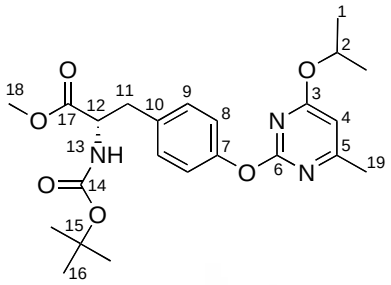
Compound I.32aa



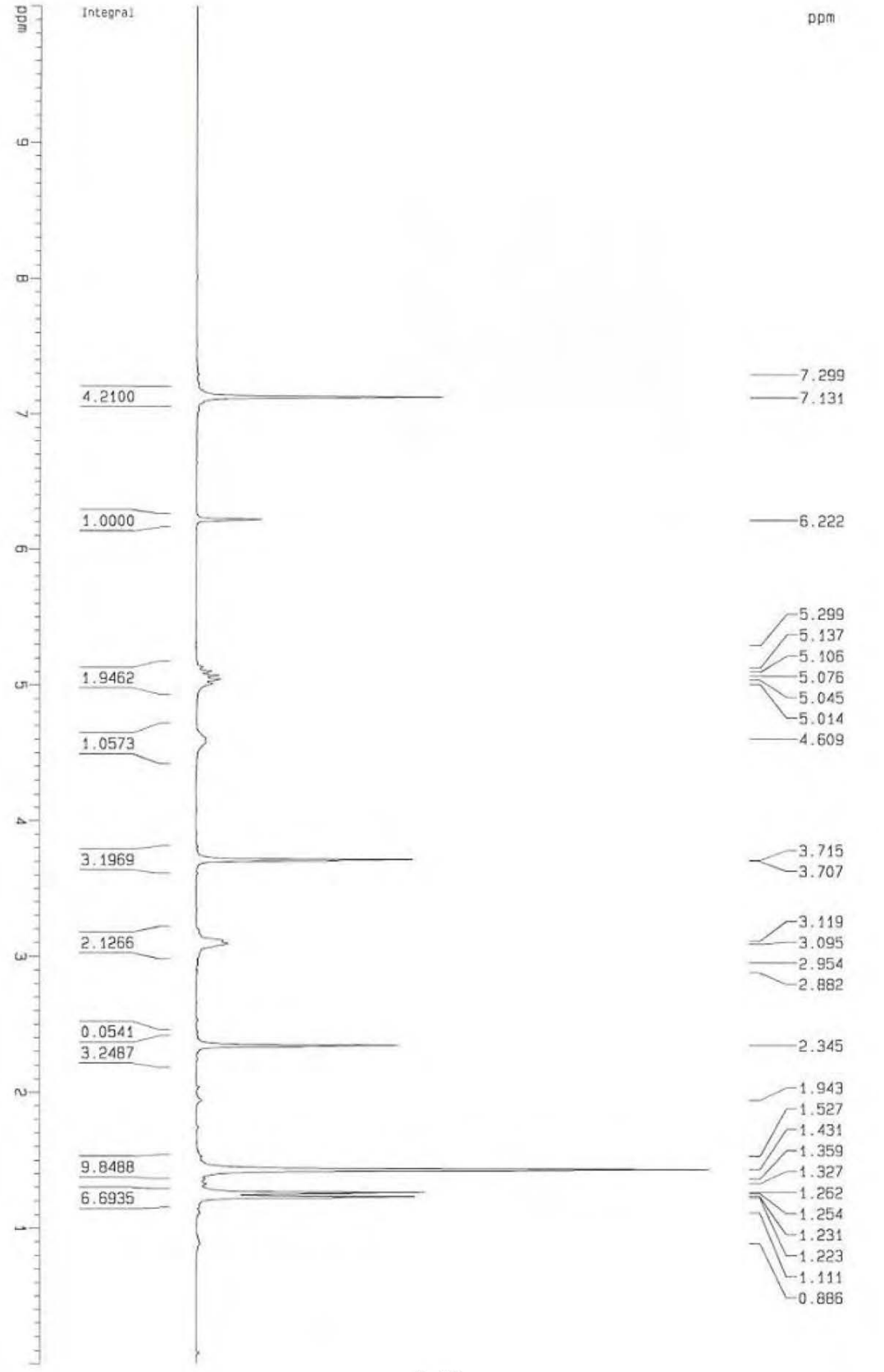
^{13}C / DEPT135 NMR



Compound I.32ab



^{1}H NMR



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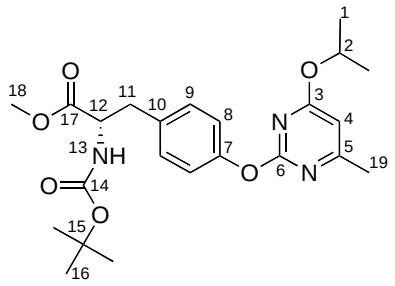
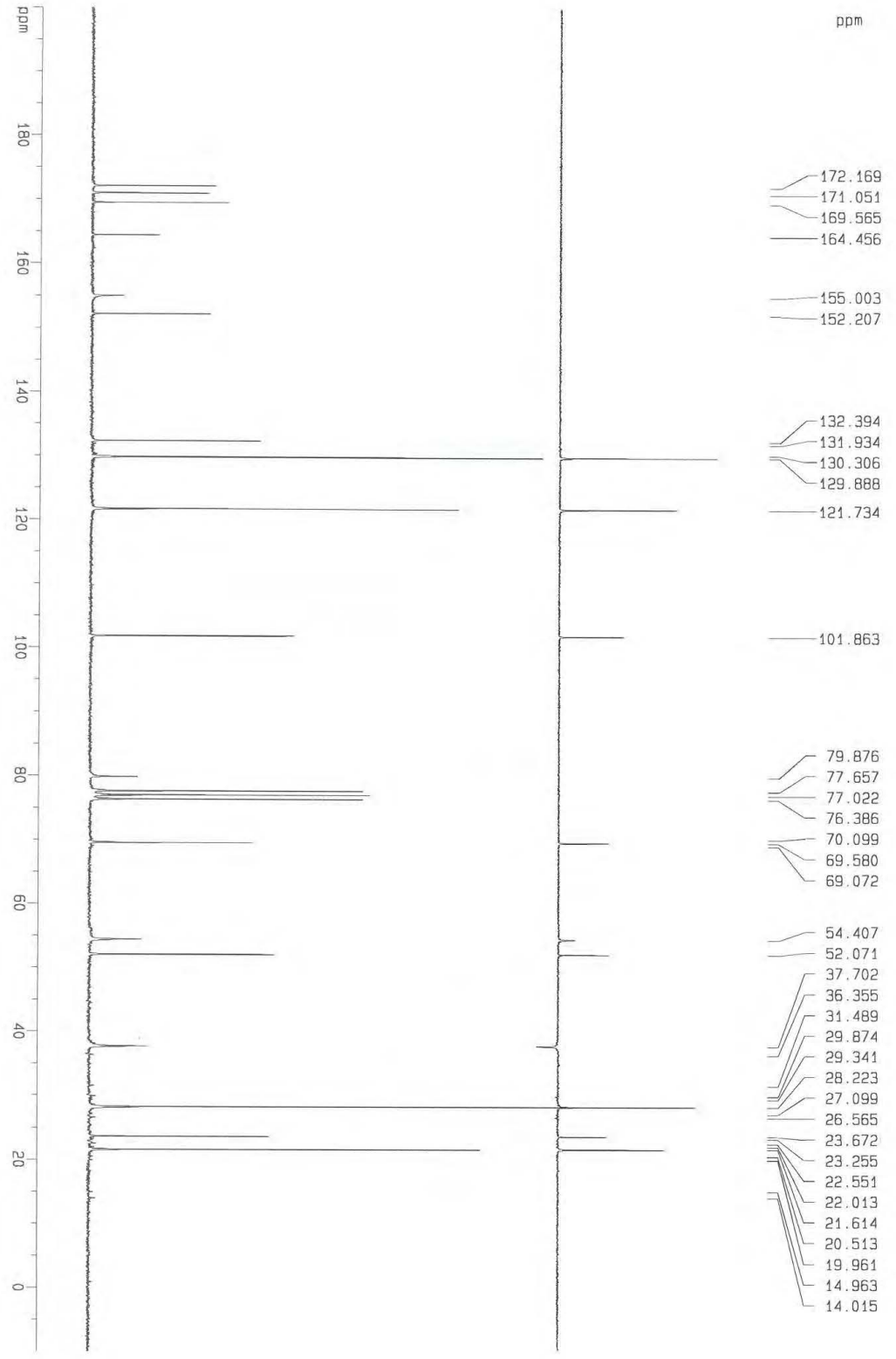
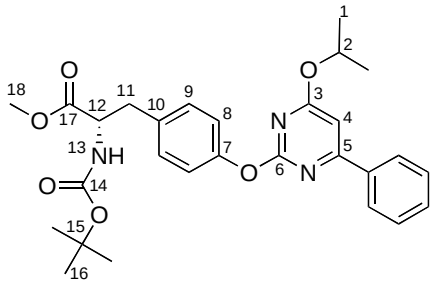


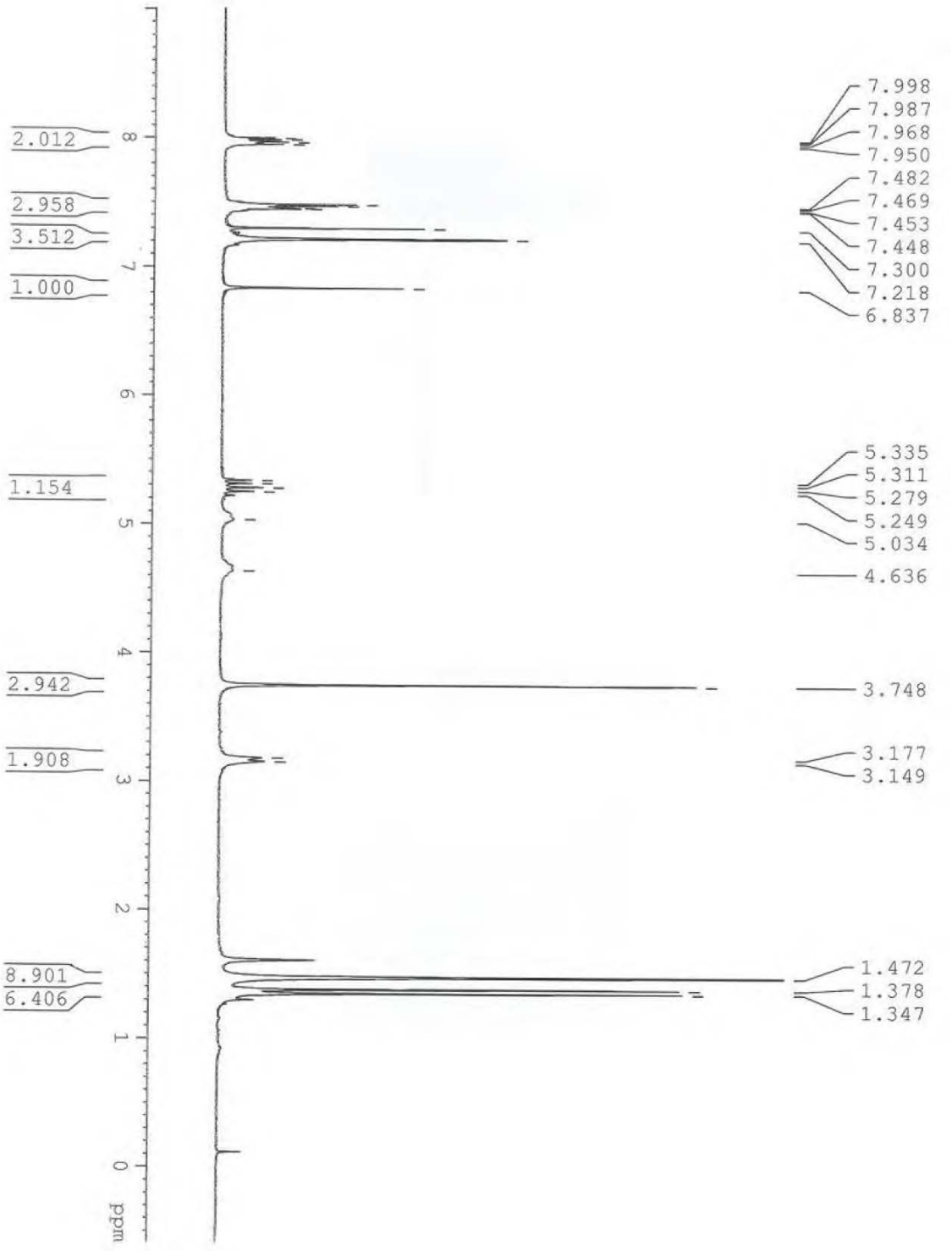
^{13}C / DEPT135 NMR



Compound I.32ac



^{1}H NMR



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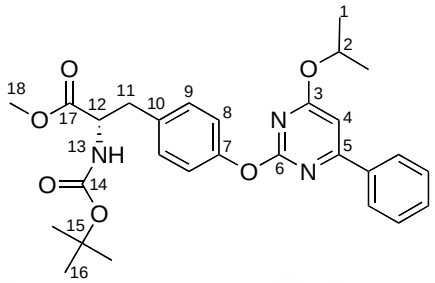
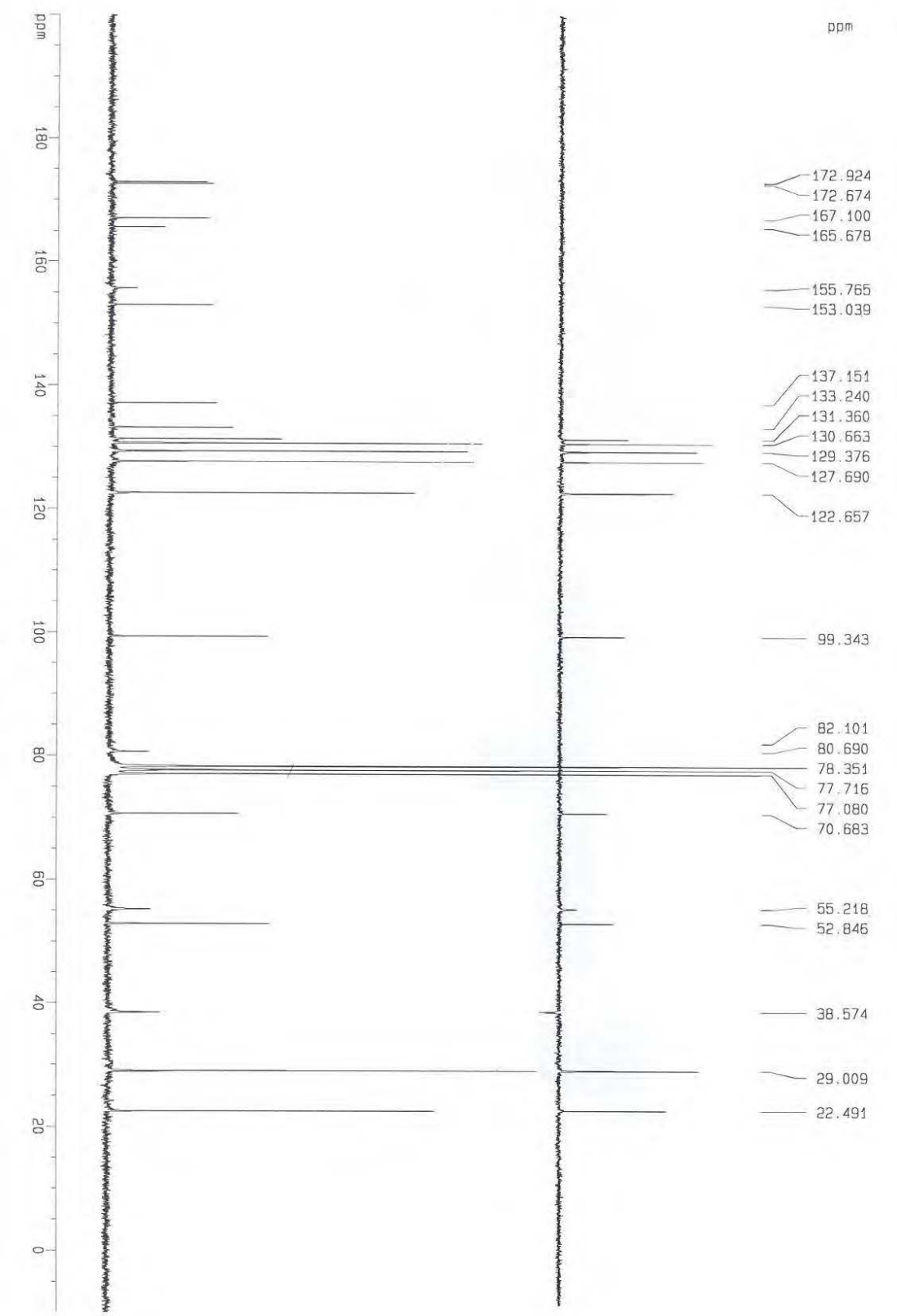
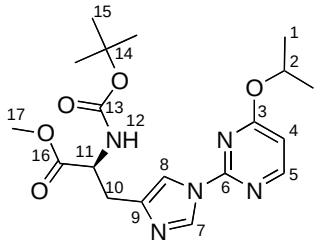


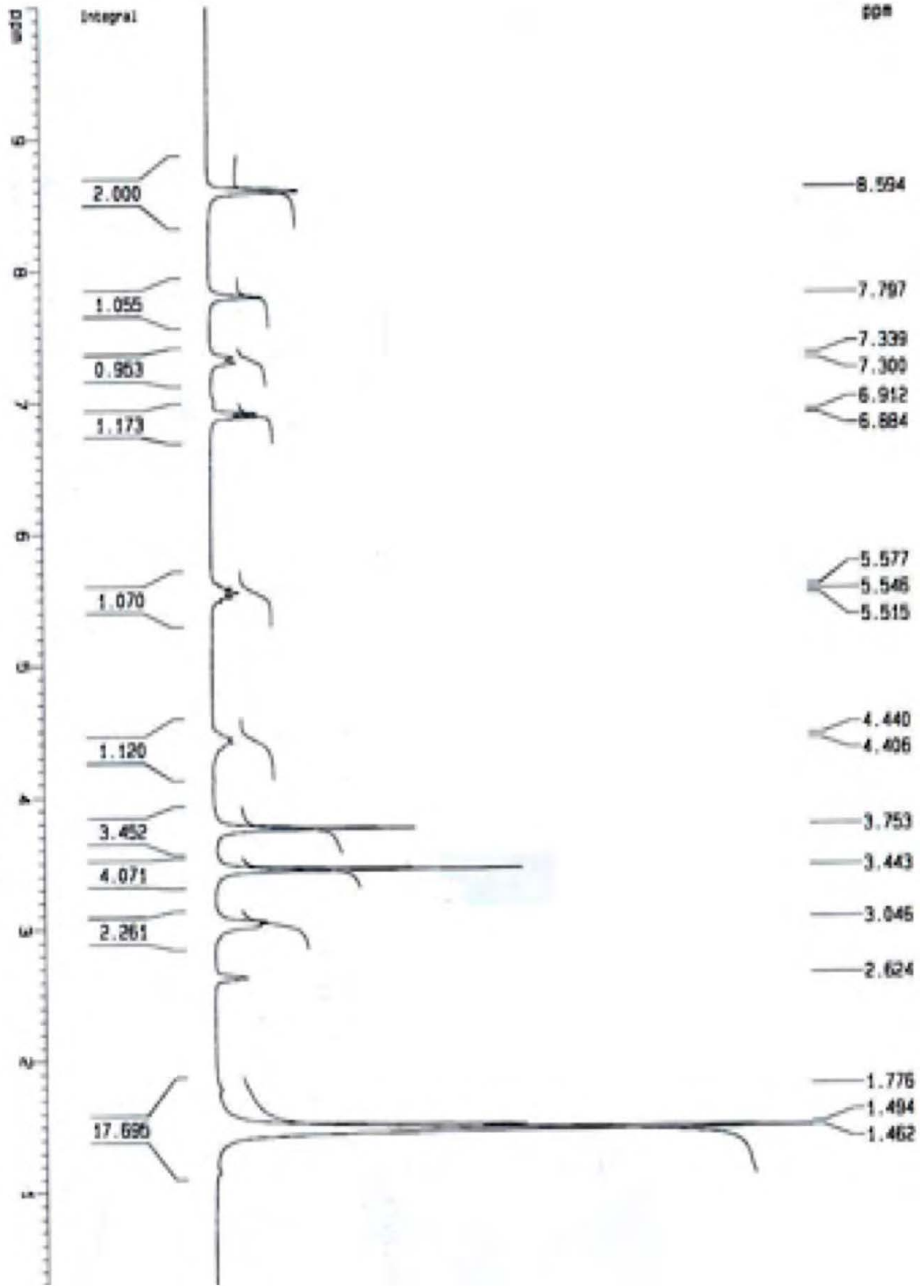
^{13}C /DEPT135 NMR



Compound I.32ba



^{1}H NMR



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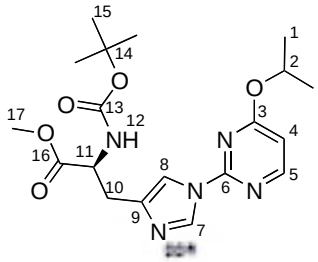
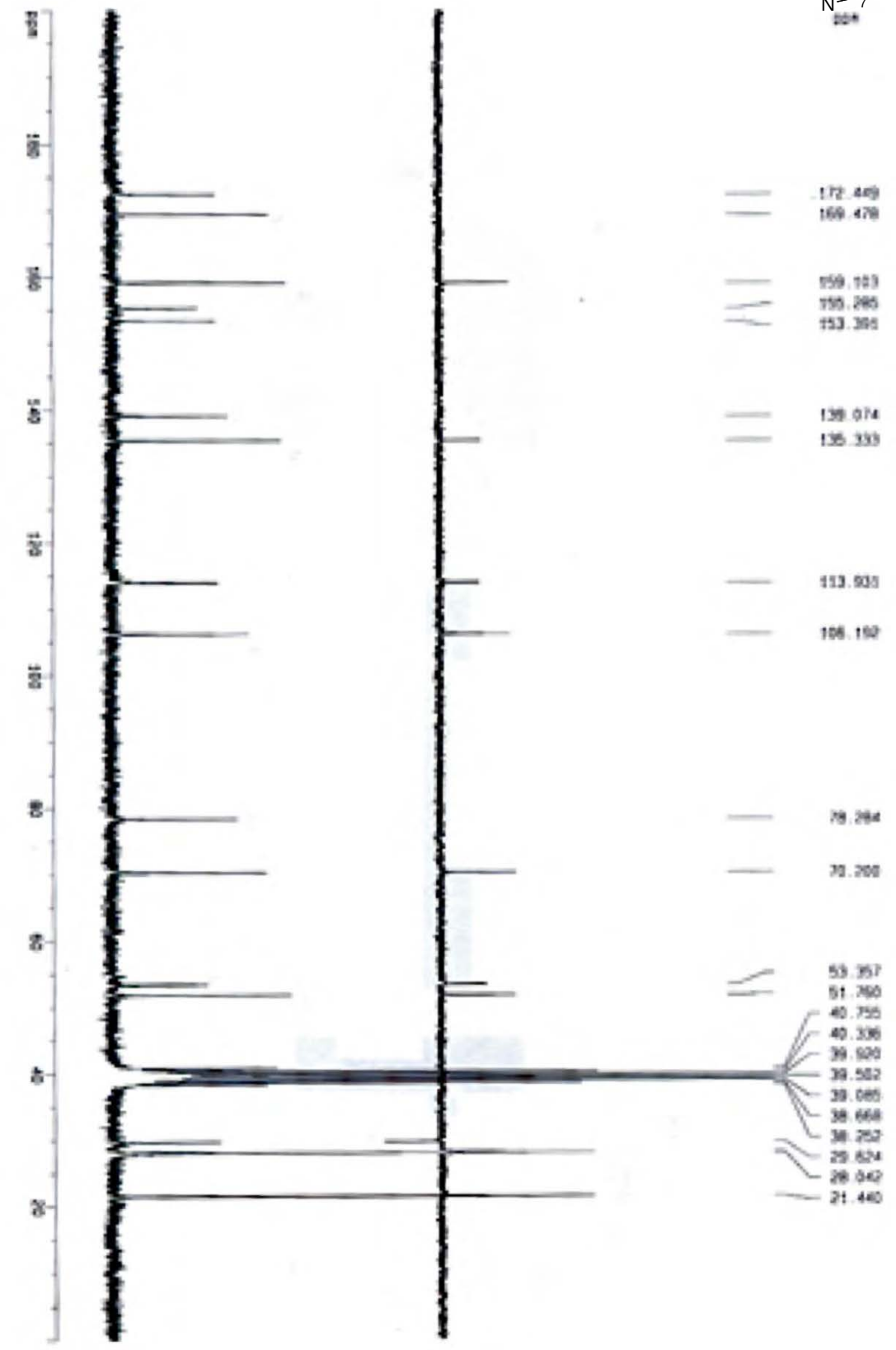
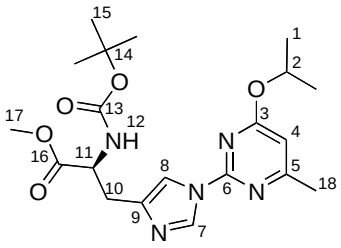


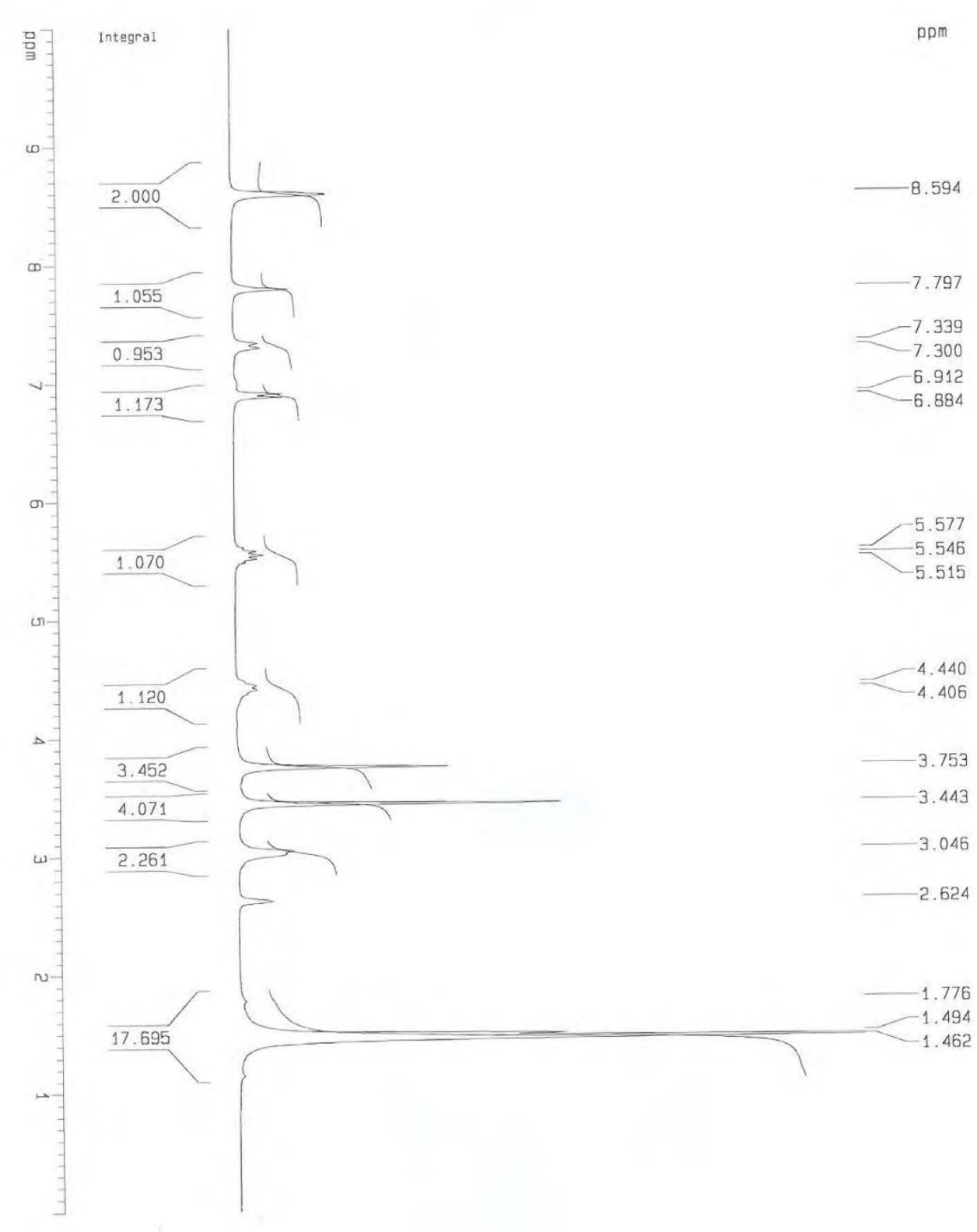
^{13}C / DEPT135 NMR



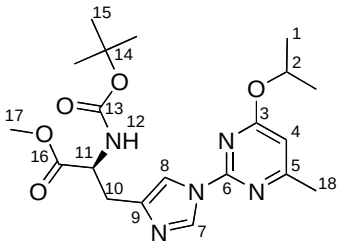
Compound I.32bb



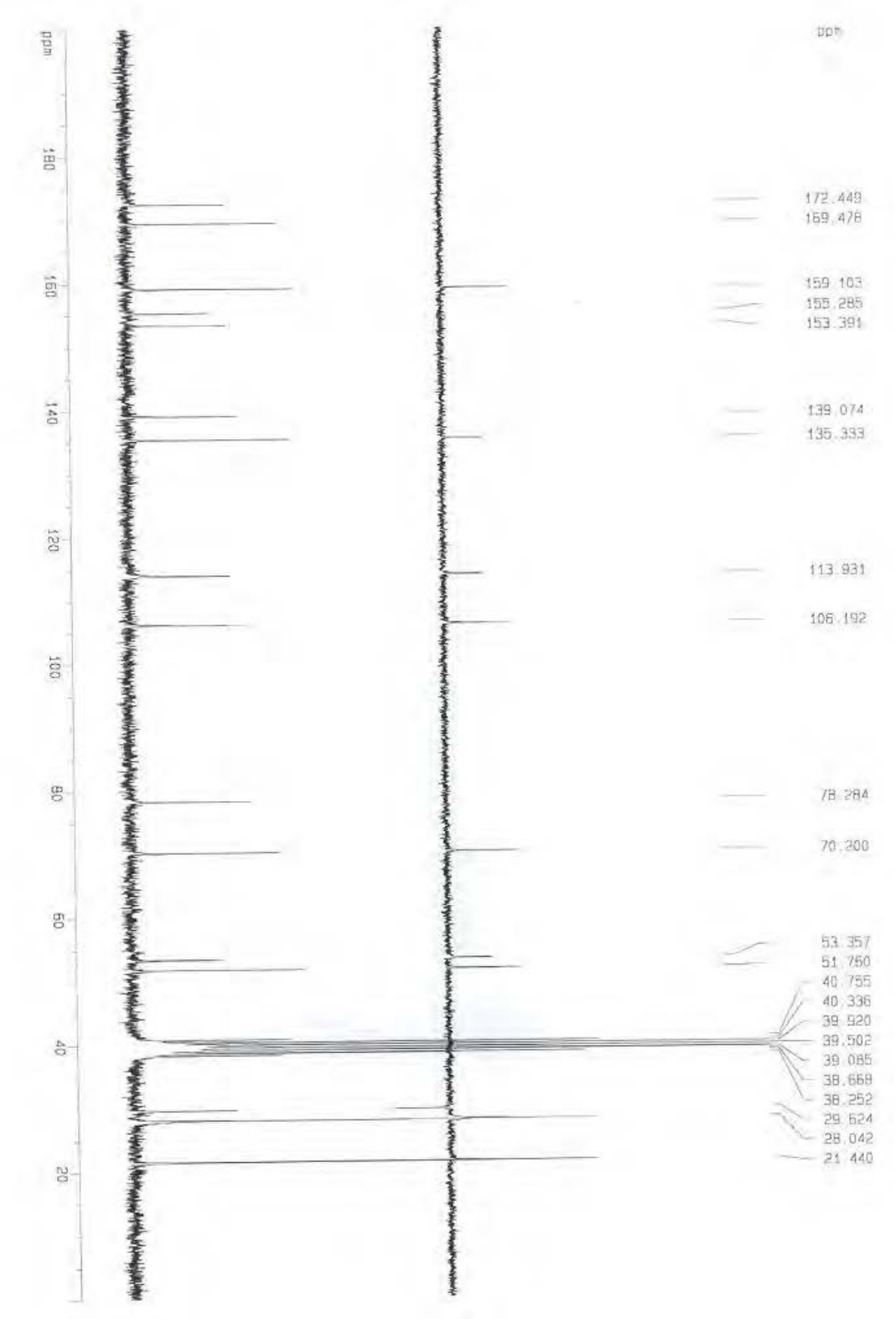
^{1}H NMR



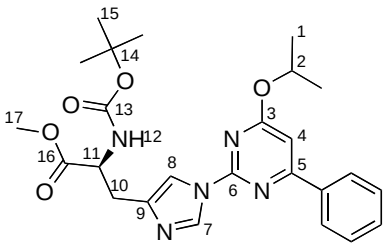
Compound I.32bb



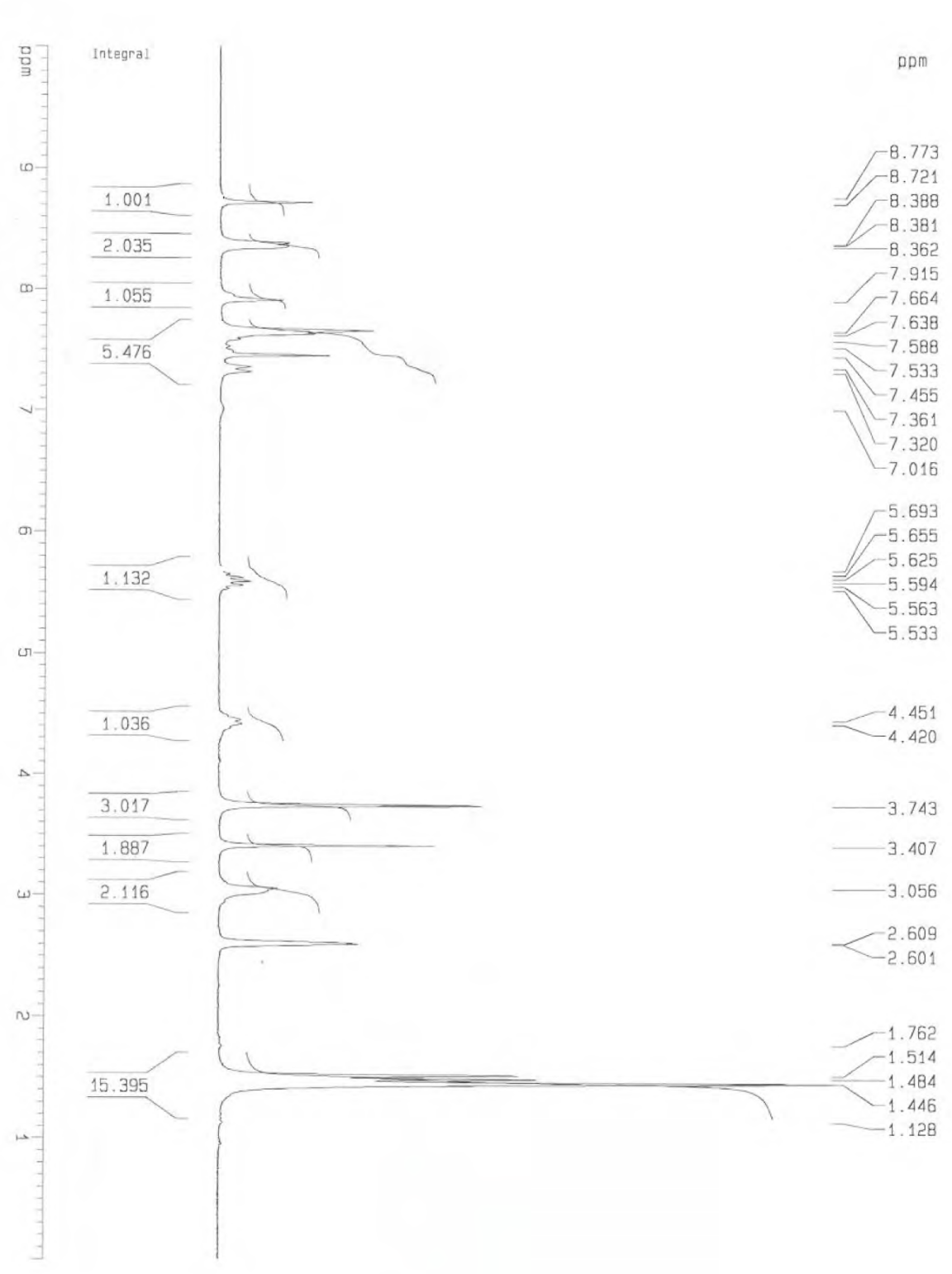
^{13}C / DEPT135 NMR



Compound I.32bc



^{1}H NMR



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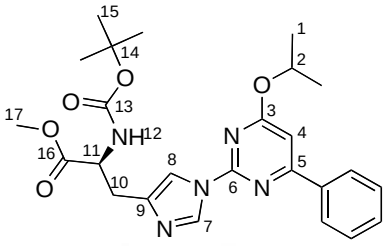
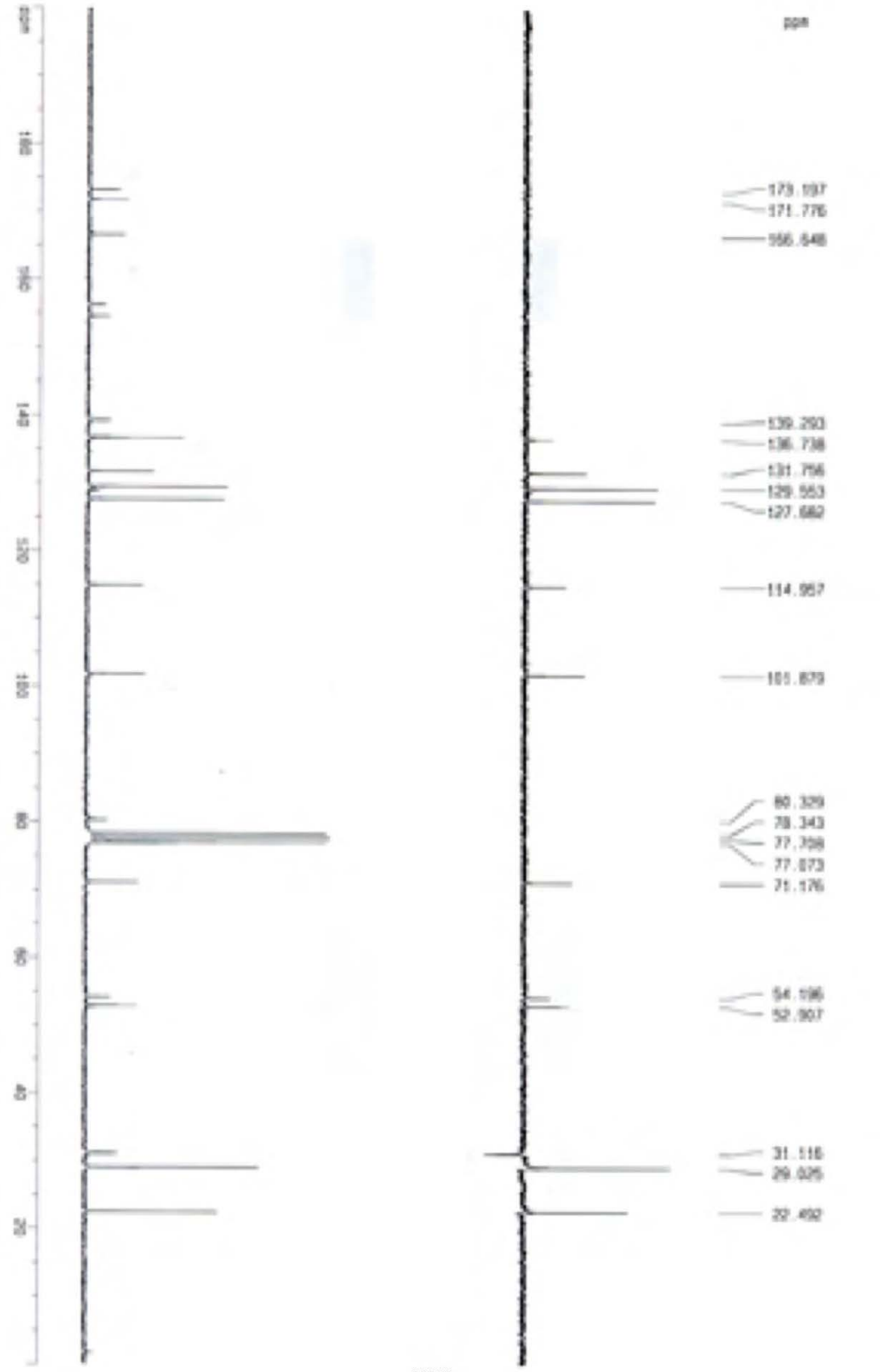
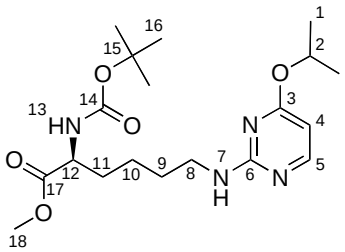


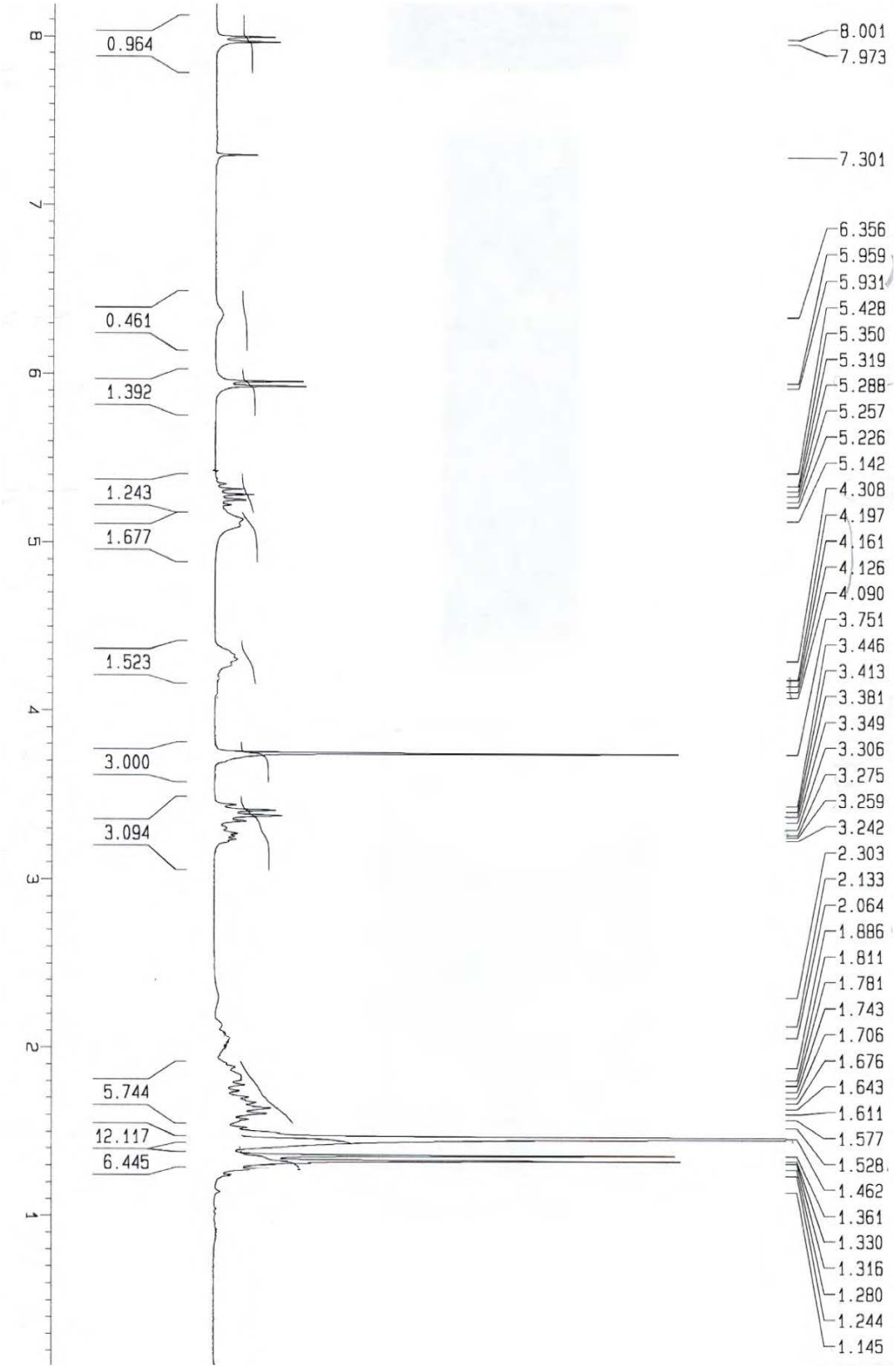
^{13}C / DEPT135 NMR



Compound I.32ca



^{1}H NMR



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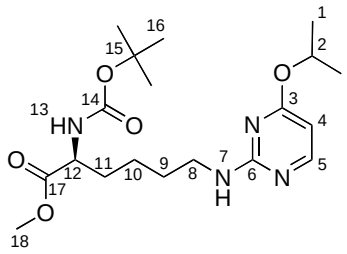
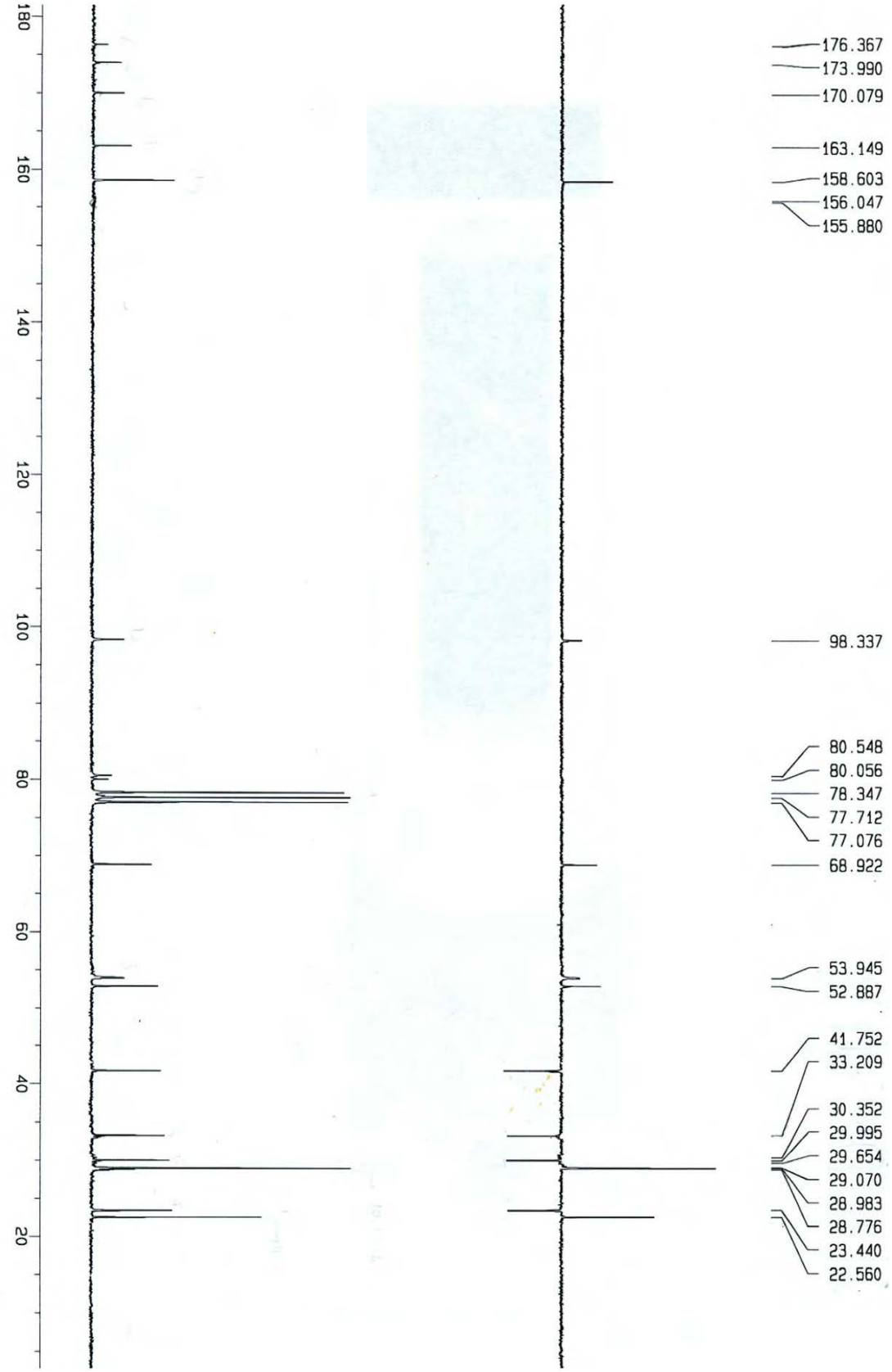


^{13}C / DEPT135 NMR



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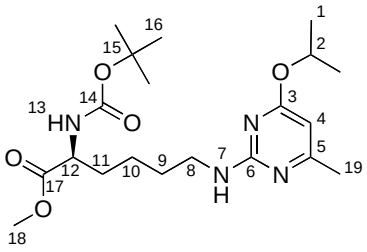
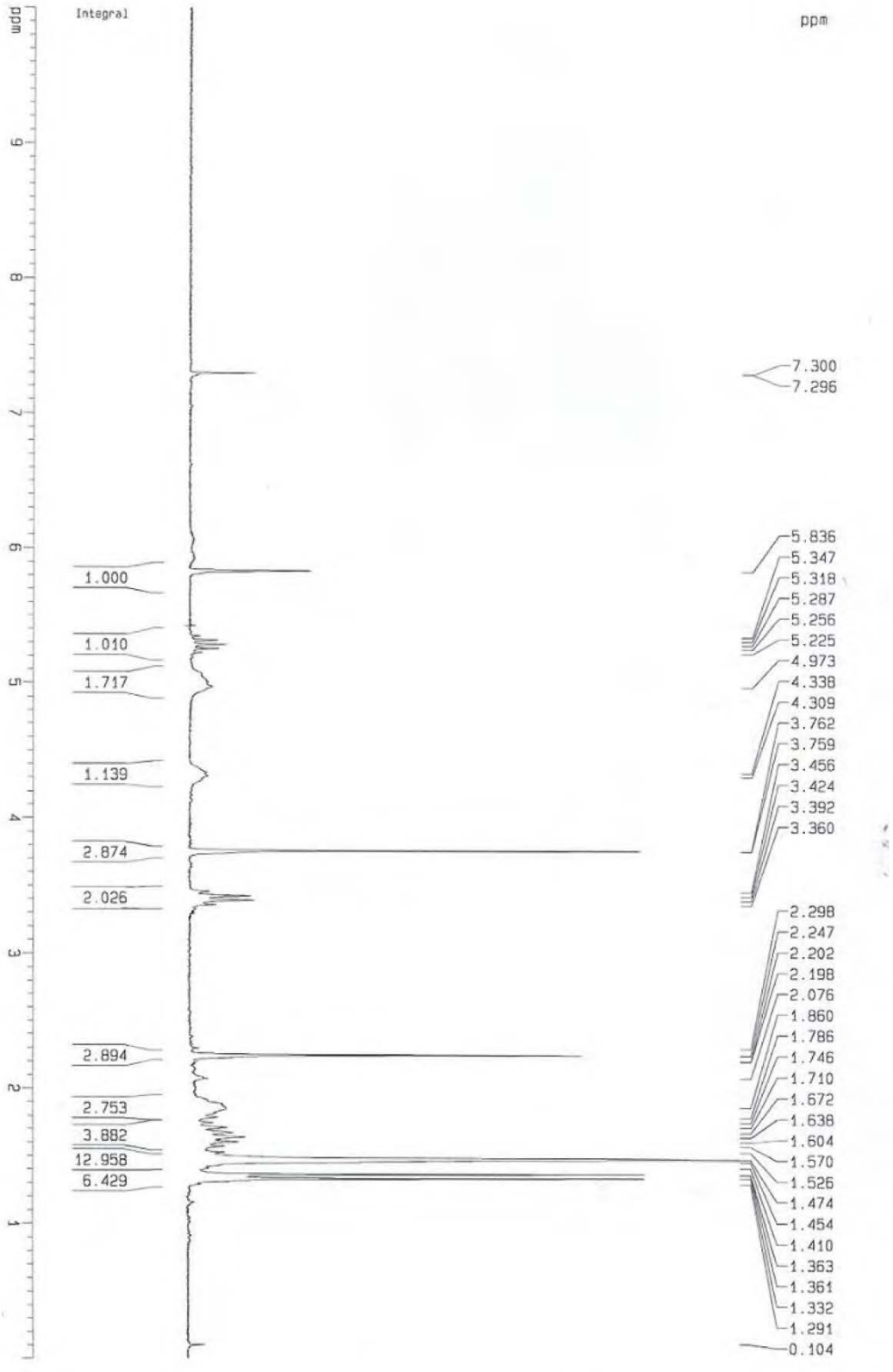


^{1}H NMR



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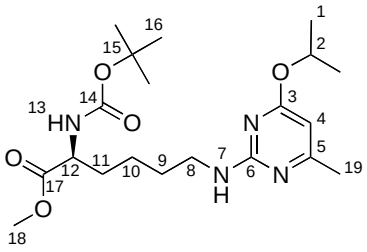
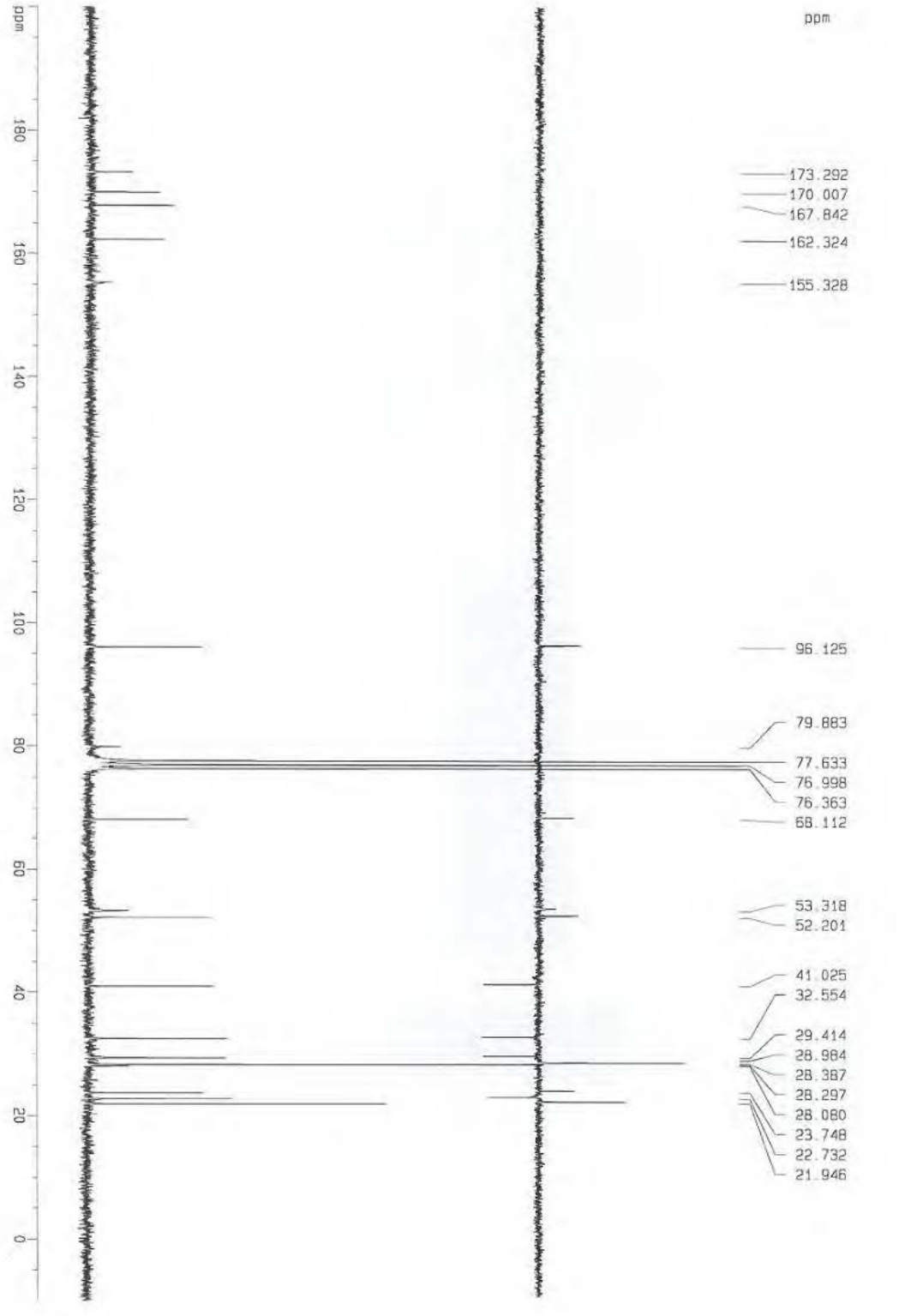


^{13}C / DEPT135 NMR



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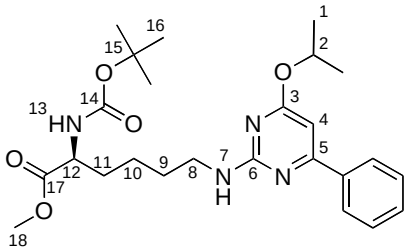
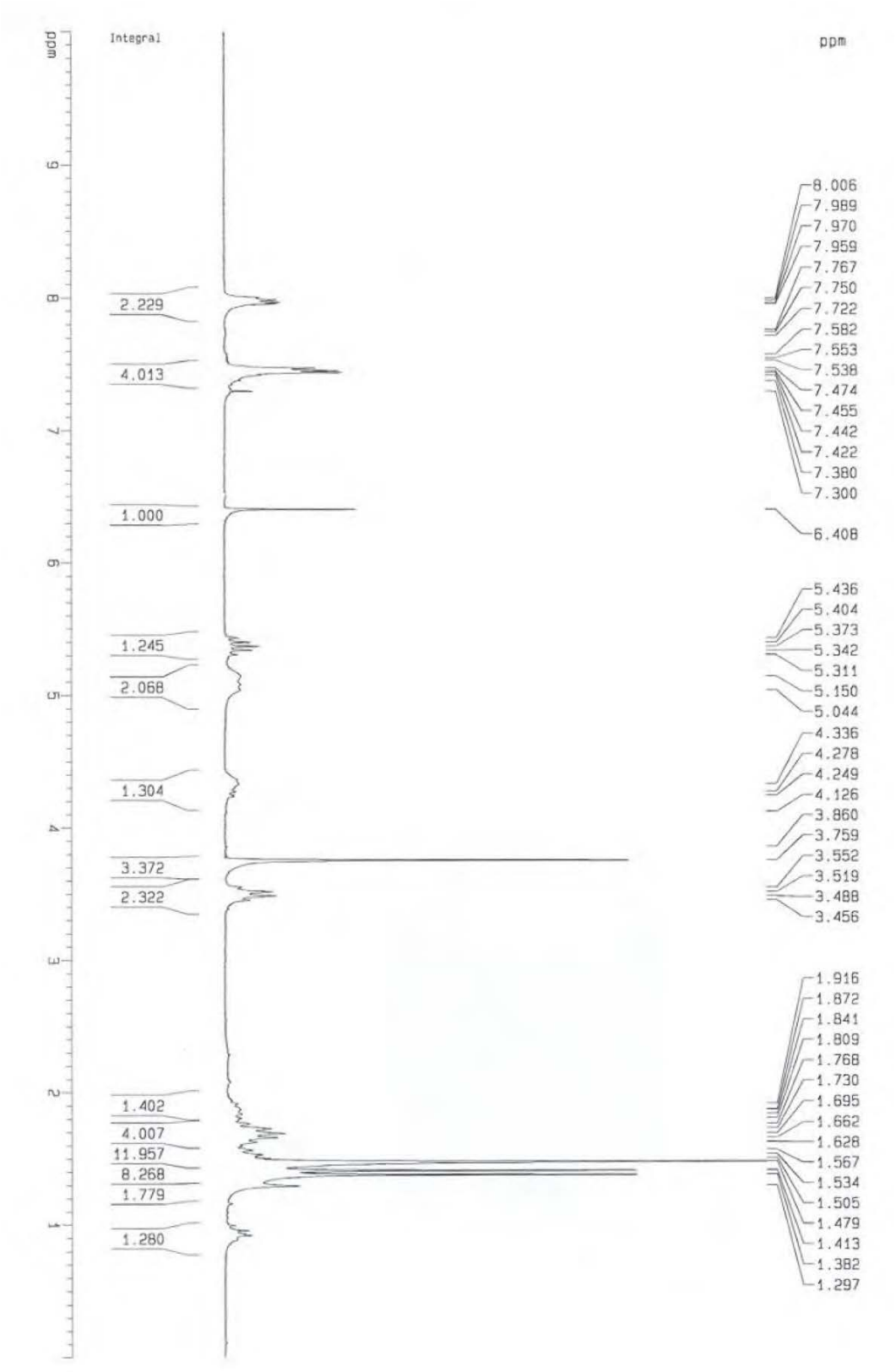


^{1}H NMR



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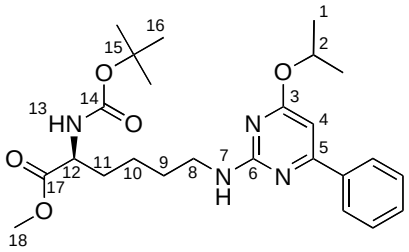
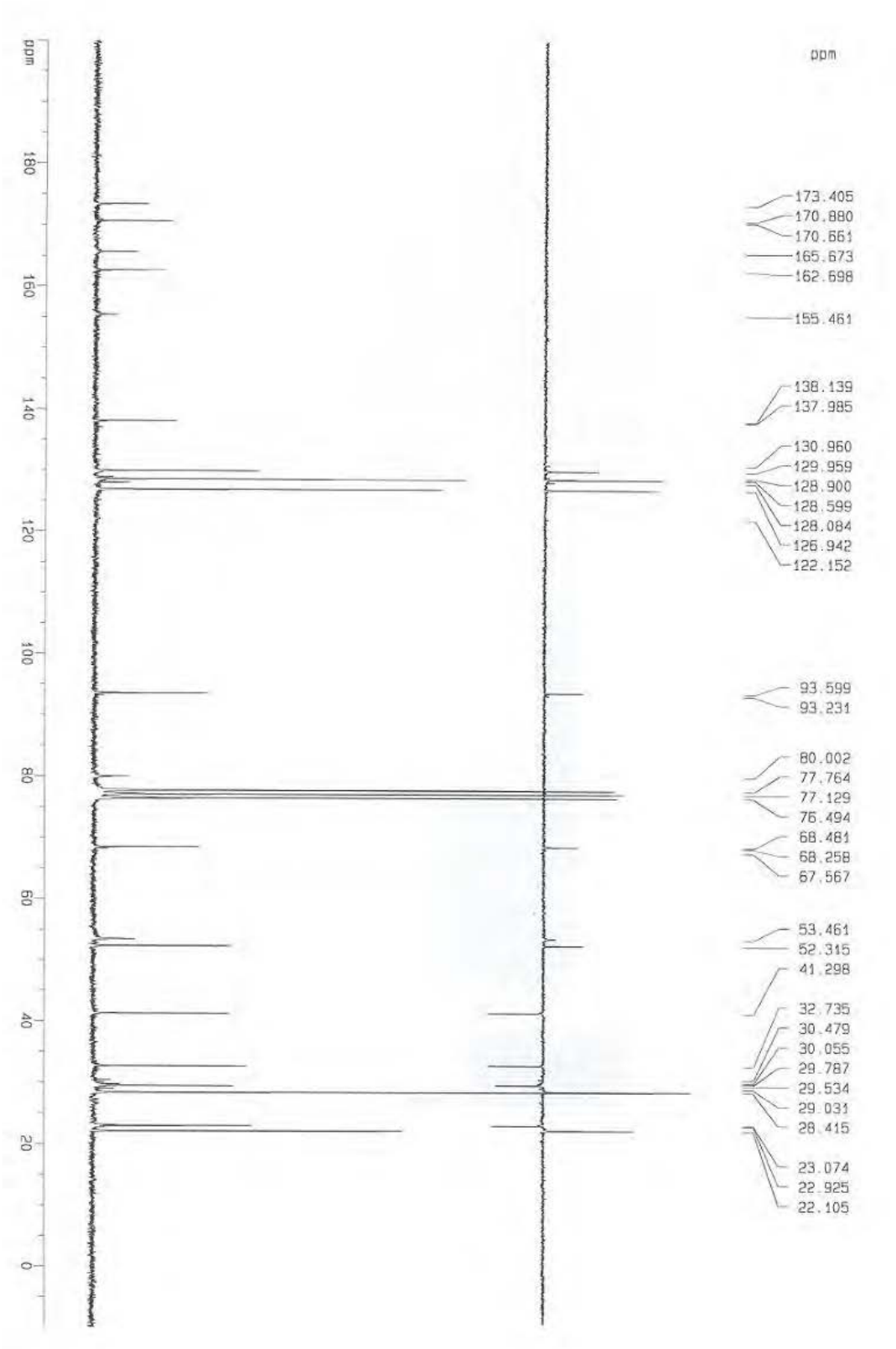


^{13}C / DEPT135 NMR



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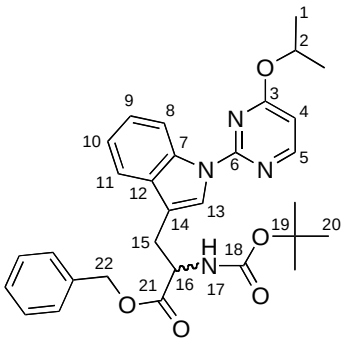
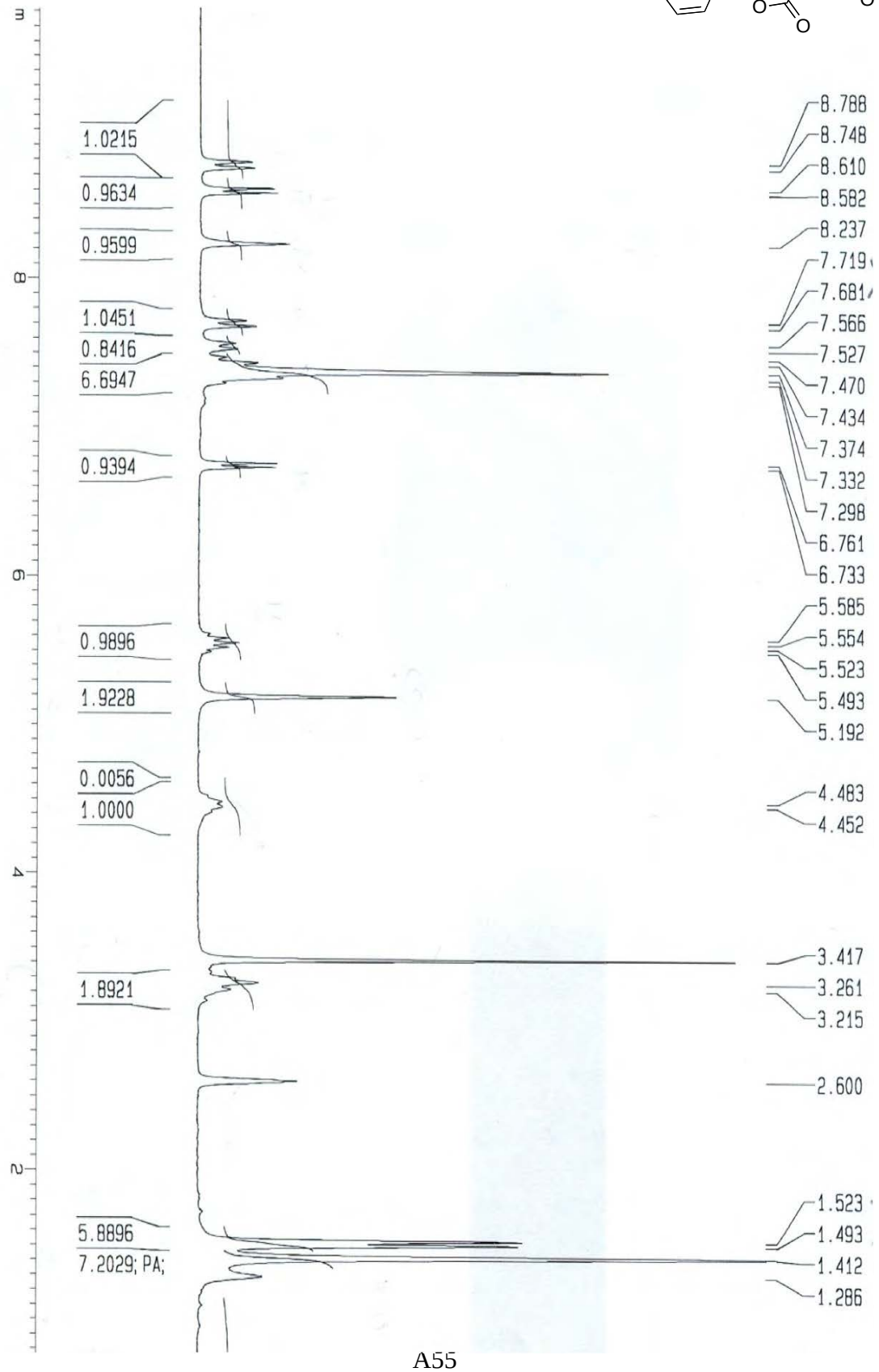


^{1}H NMR



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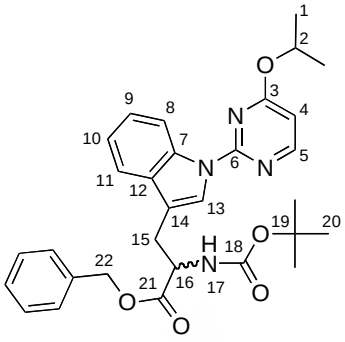
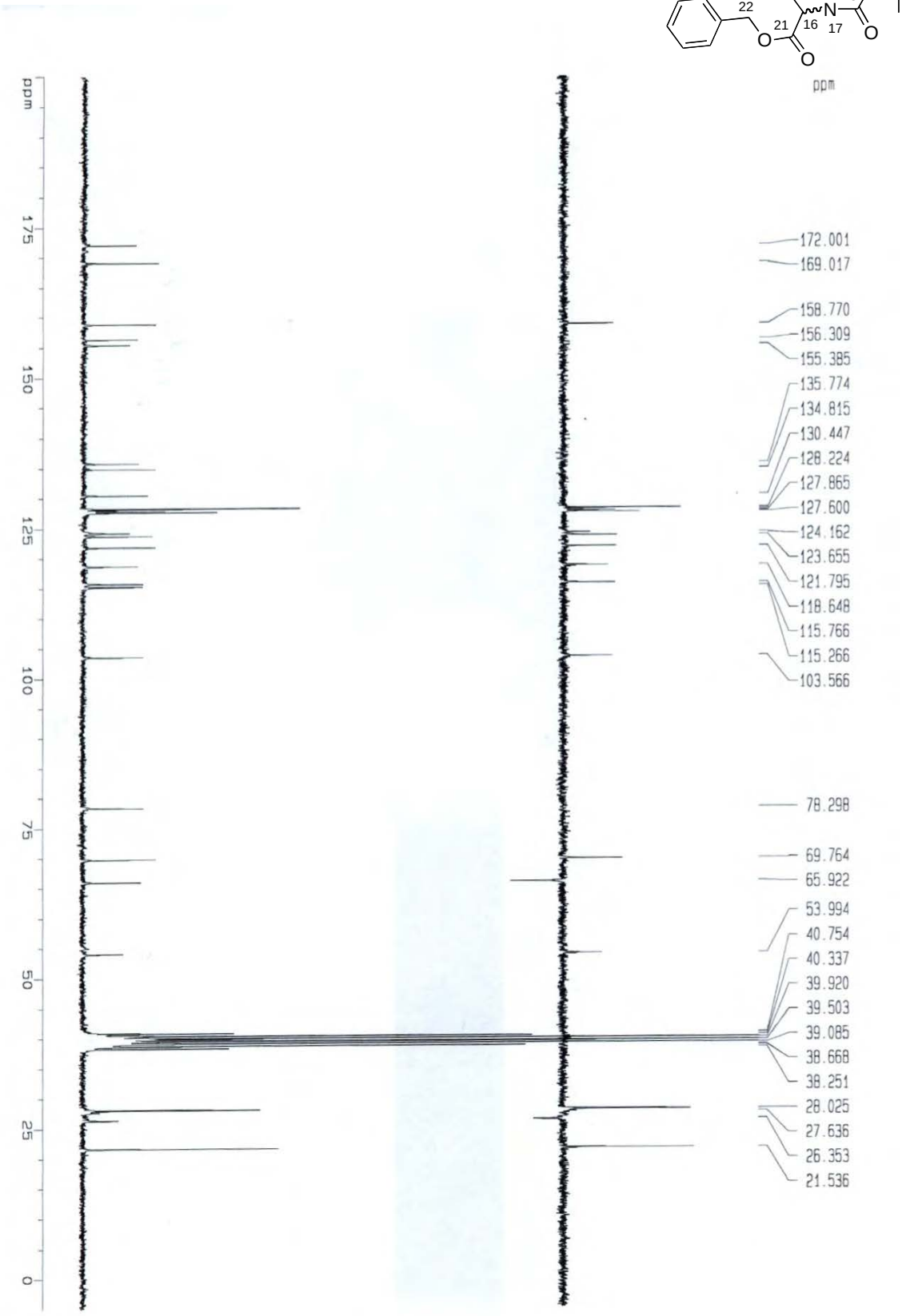


^{13}C / DEPT135 NMR



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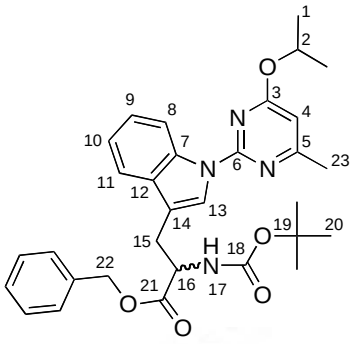
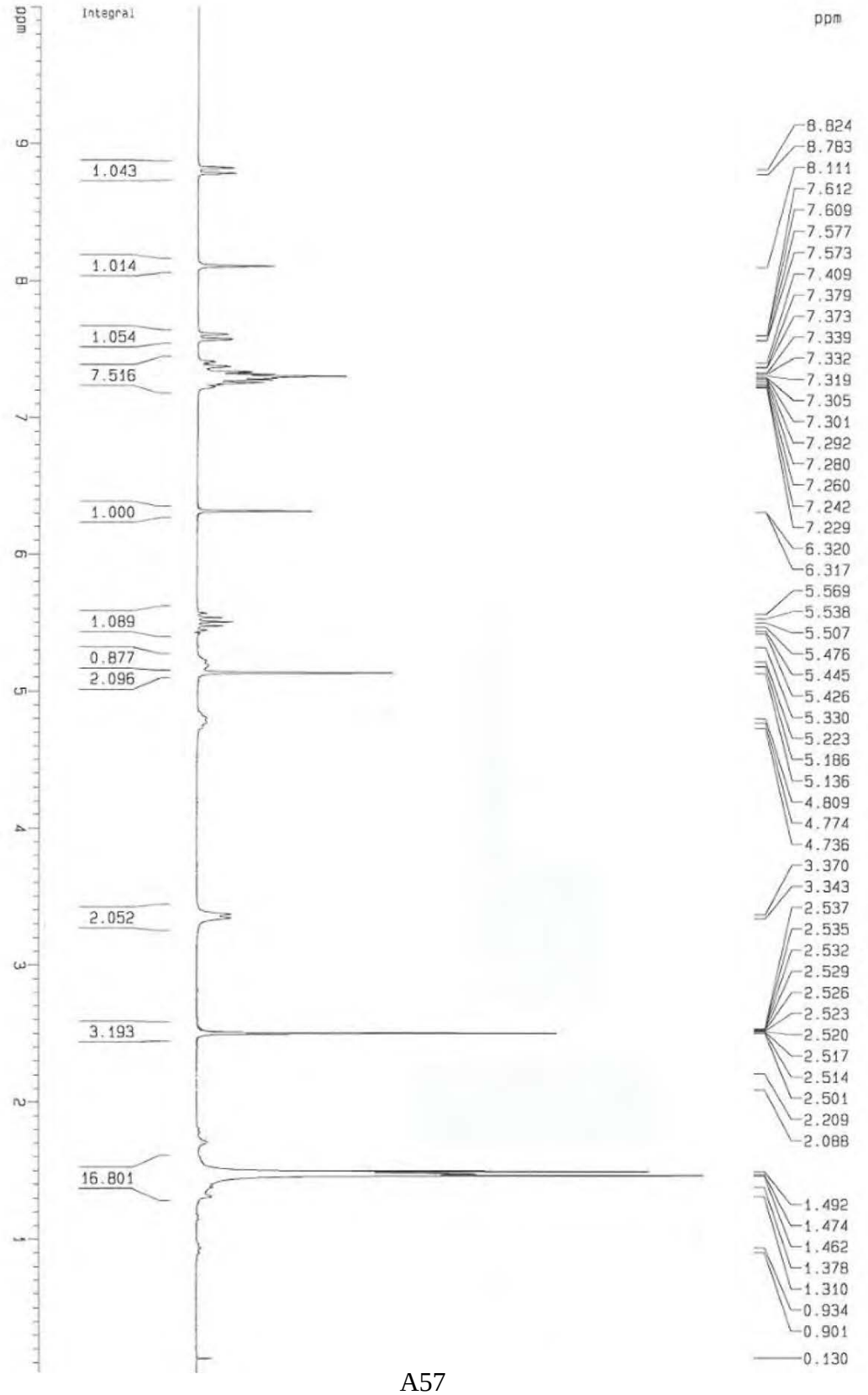


^{1}H NMR



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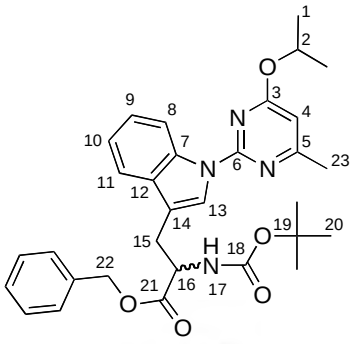
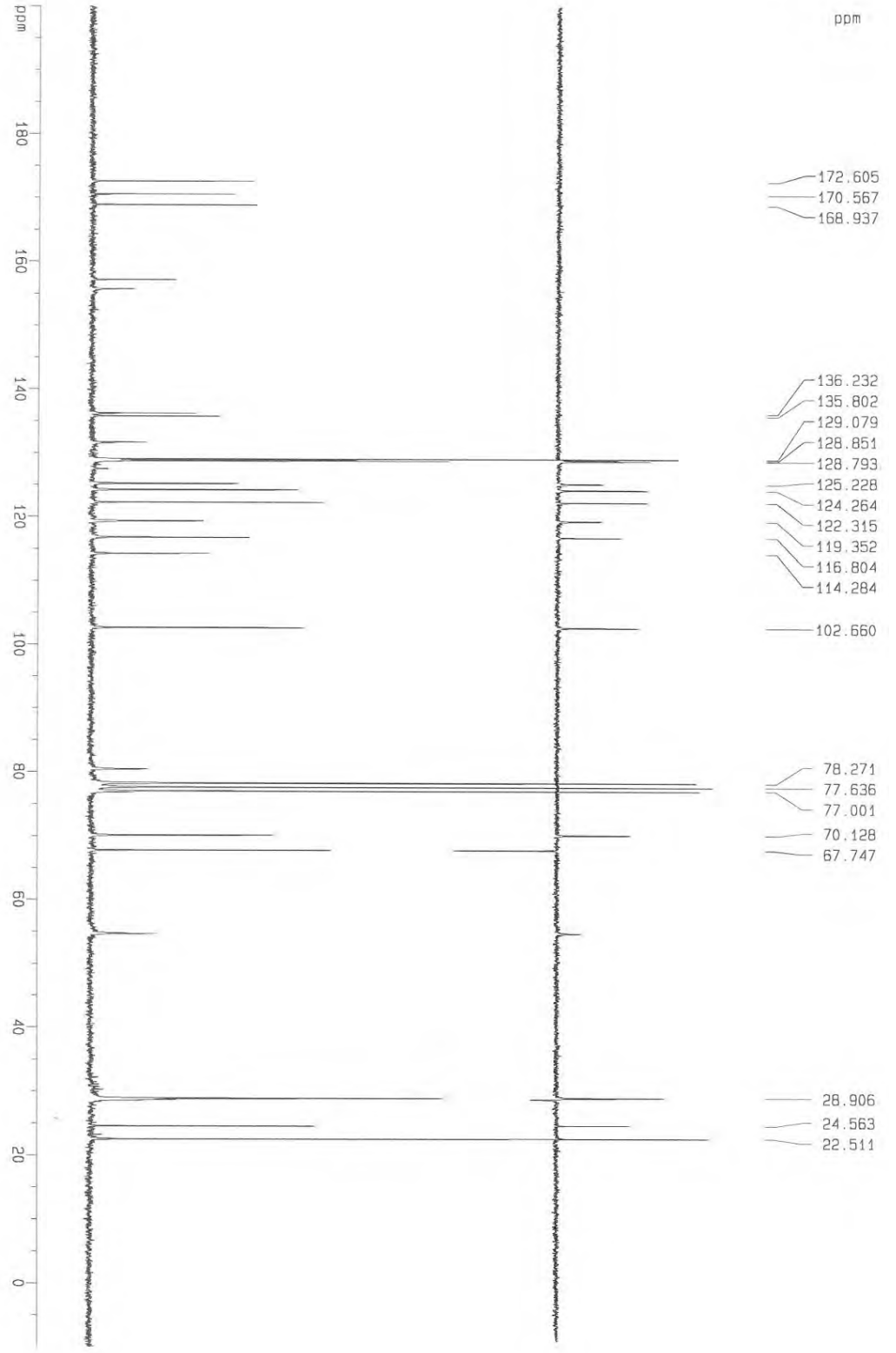
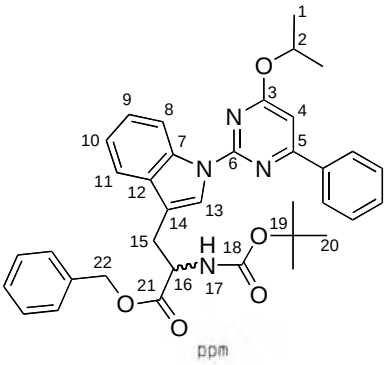


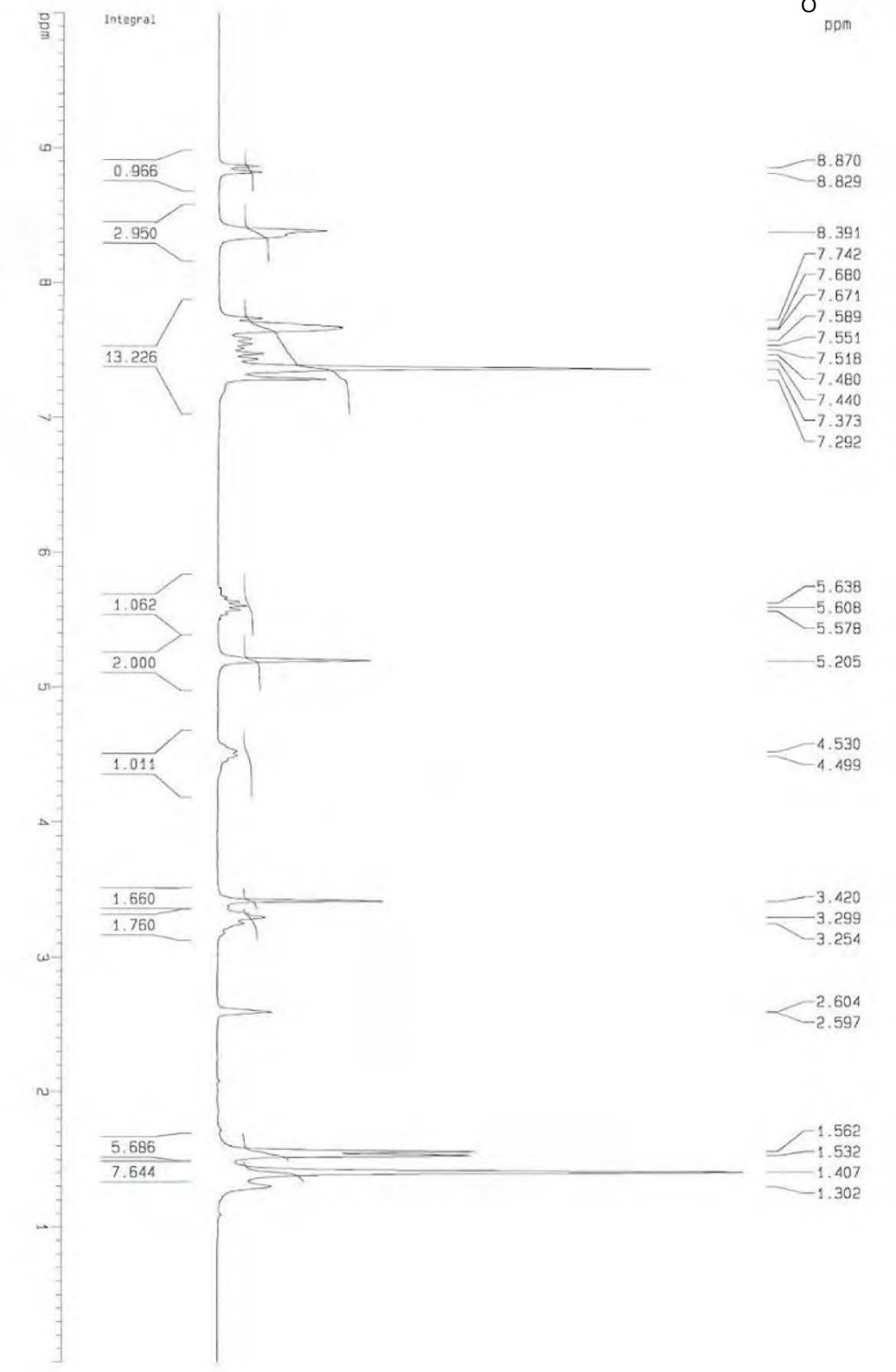
^{13}C / DEPT135 NMR



Compound I.32ec



^{1}H NMR



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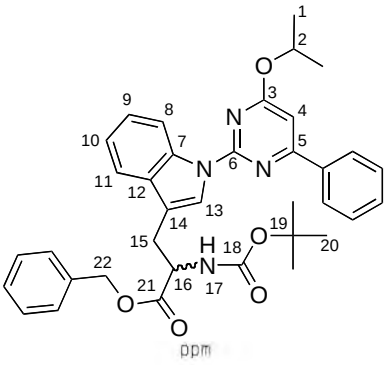
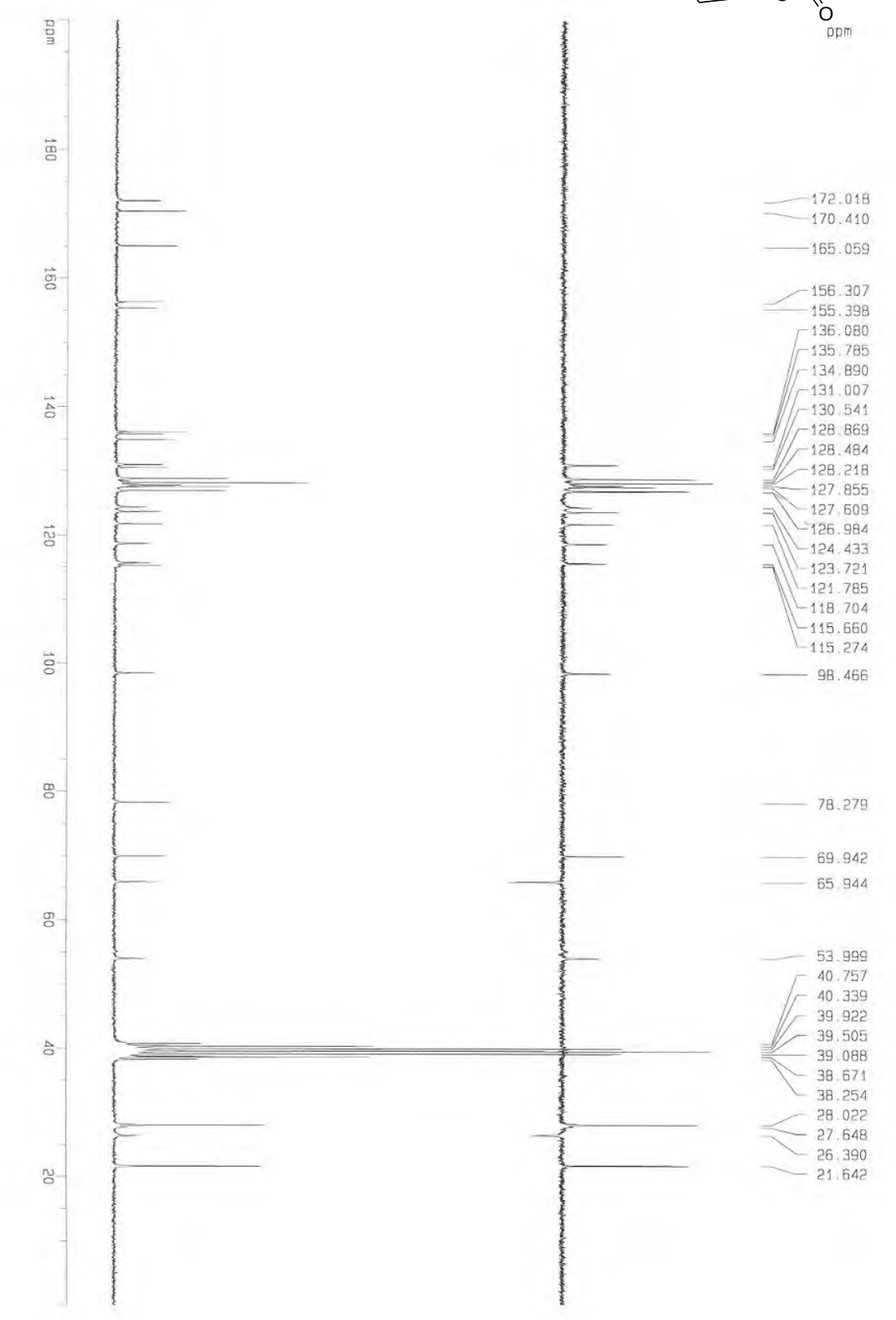
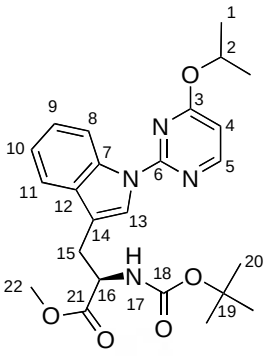


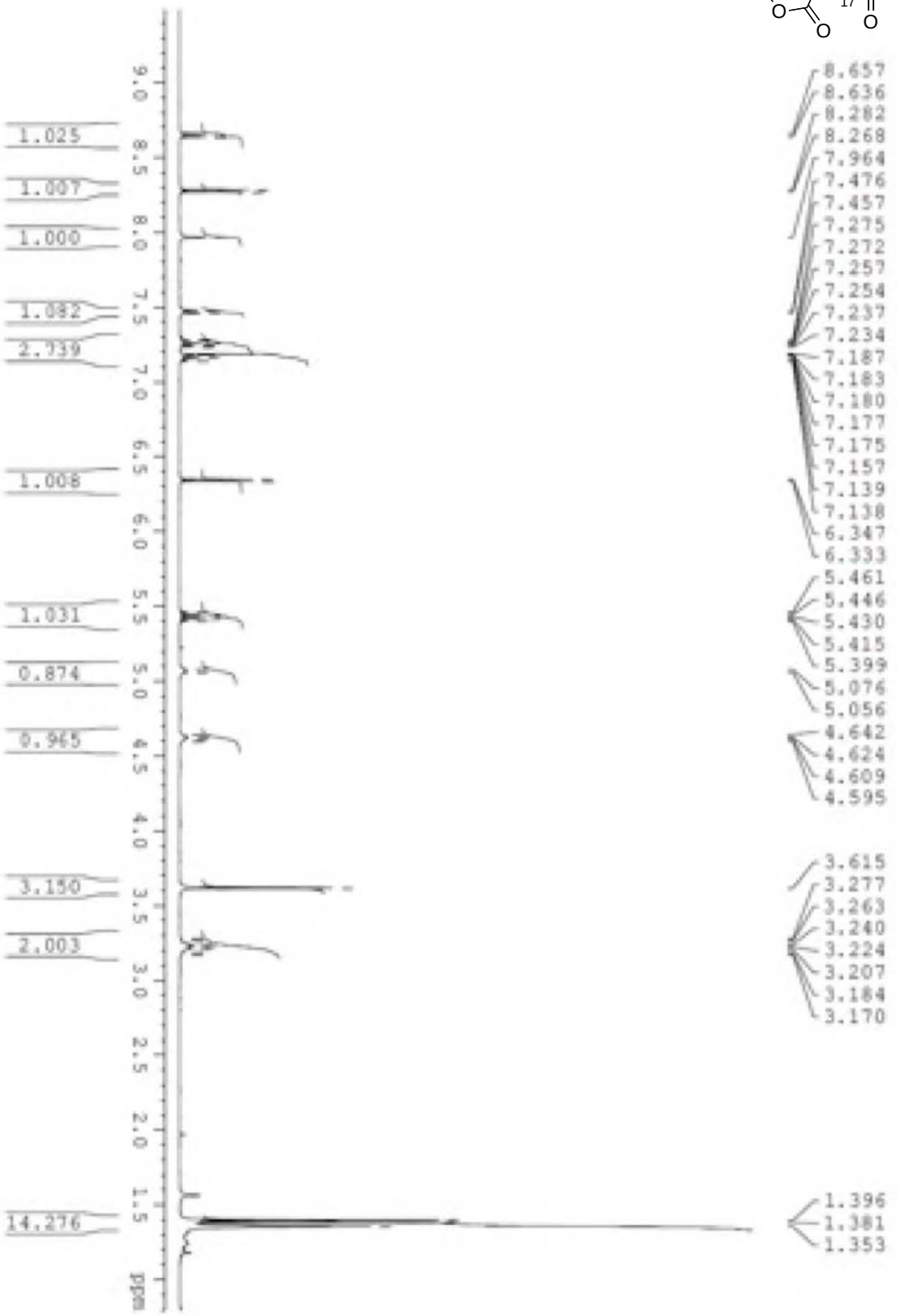
^{13}C / DEPT135 NMR



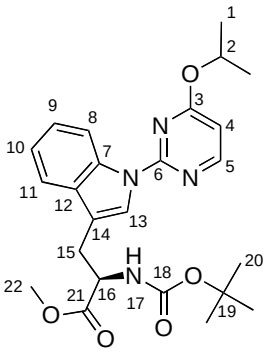
Compound I.32da



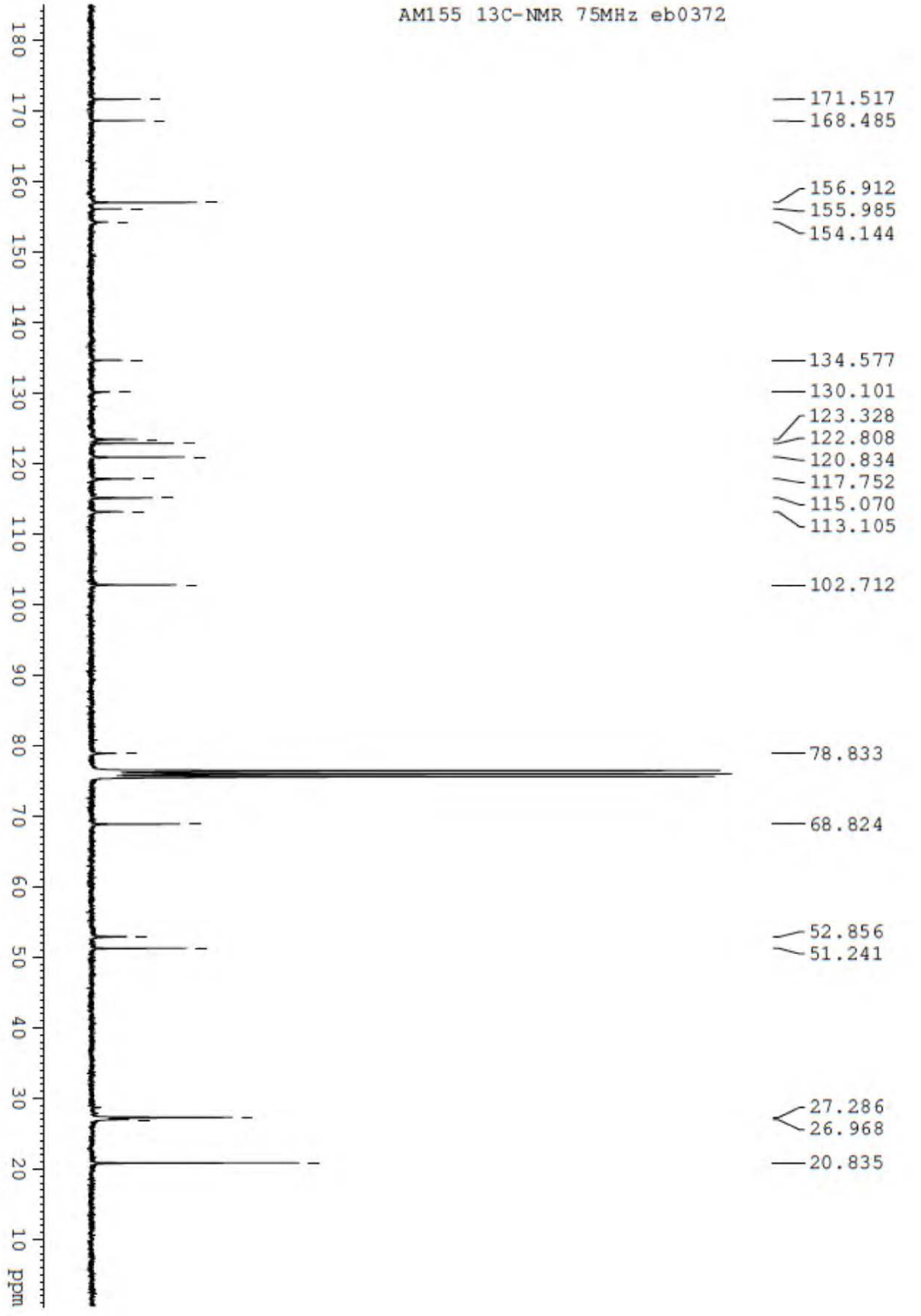
^{1}H NMR



Compound I.32da

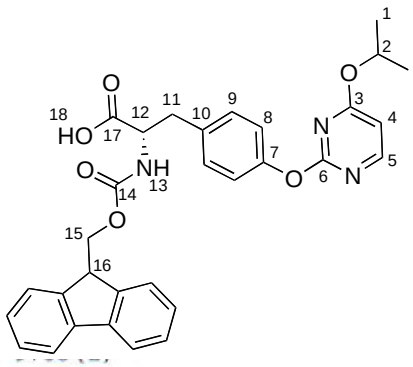


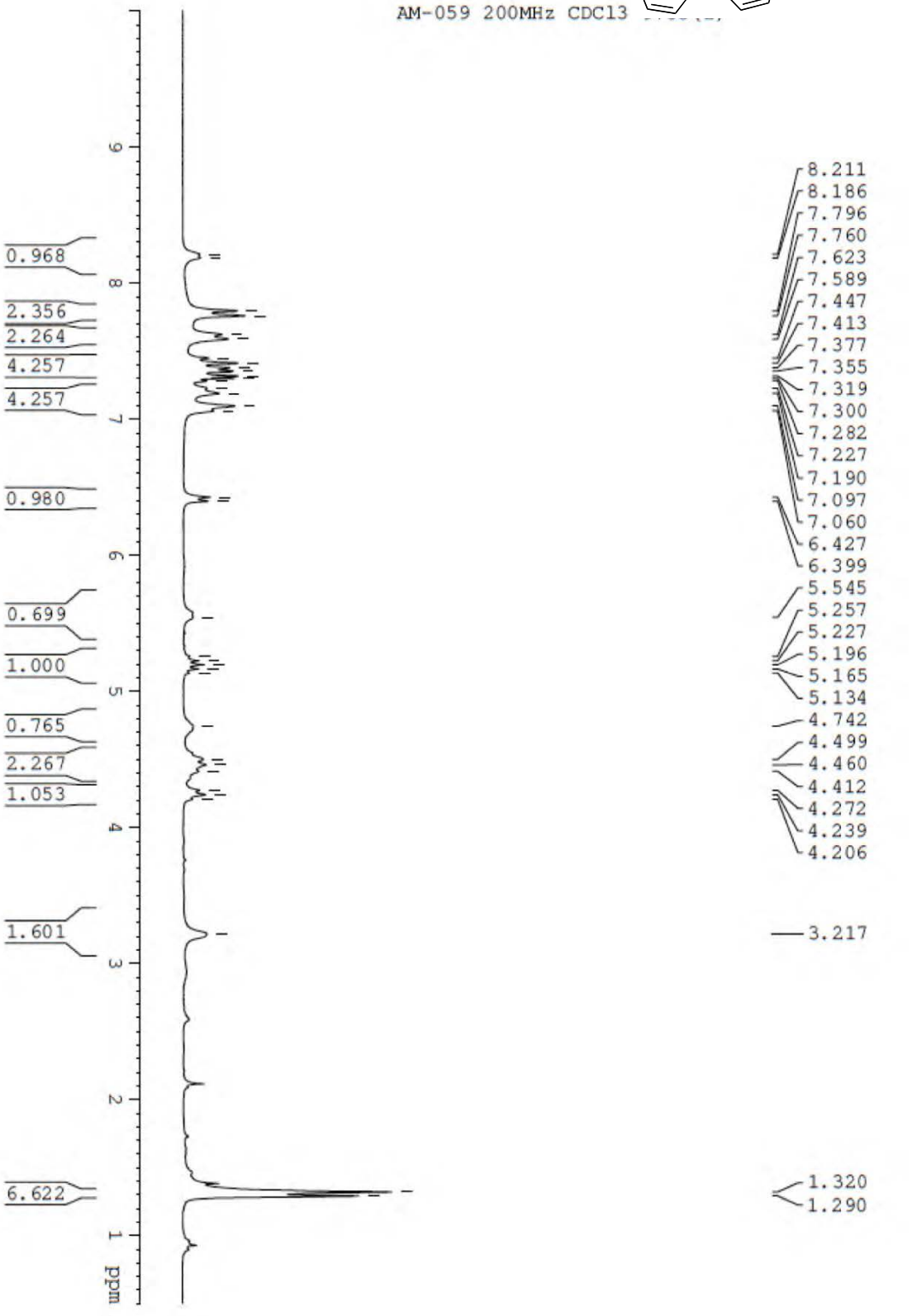
^{13}C NMR



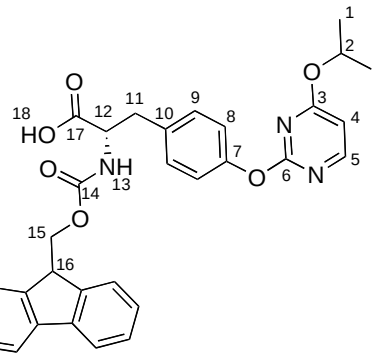
Compound I.12a

^{1}H NMR

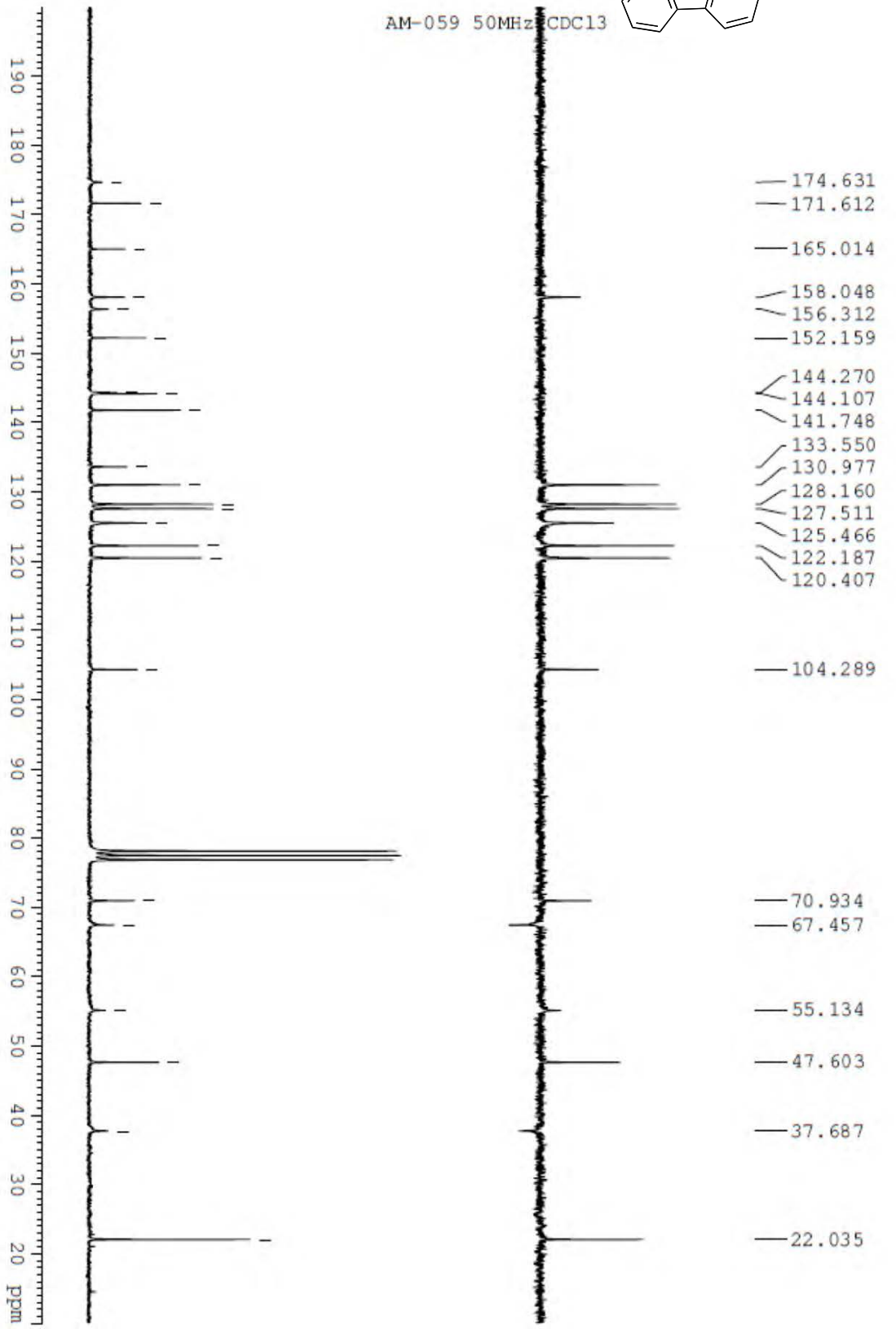




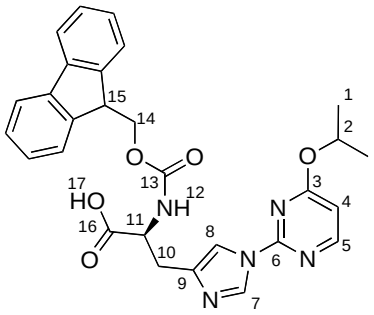
Compound I.12a



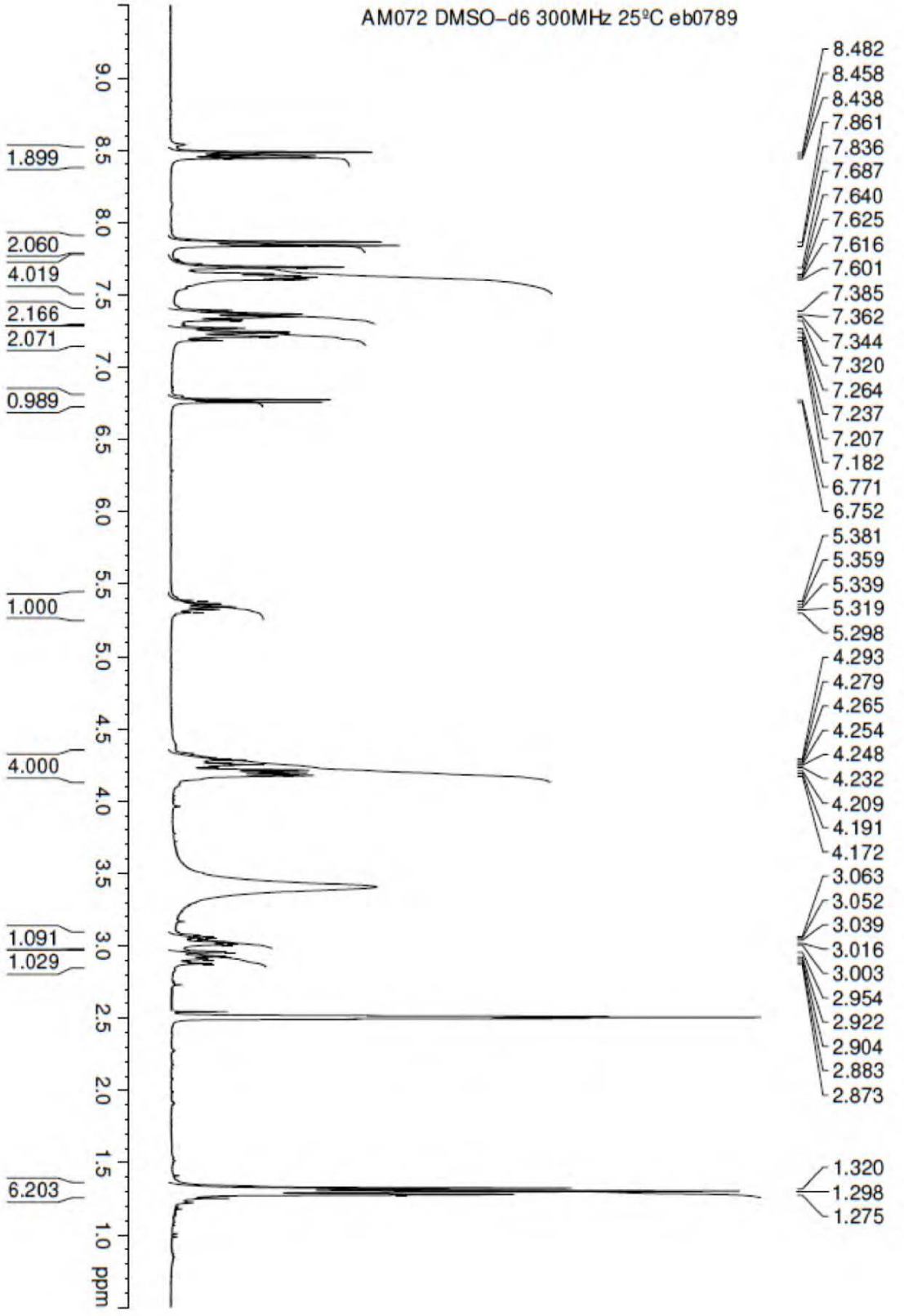
^{13}C / DEPT135 NMR



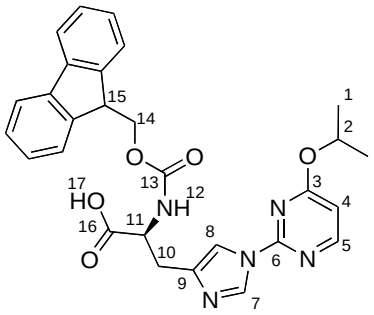
Compound I.12b



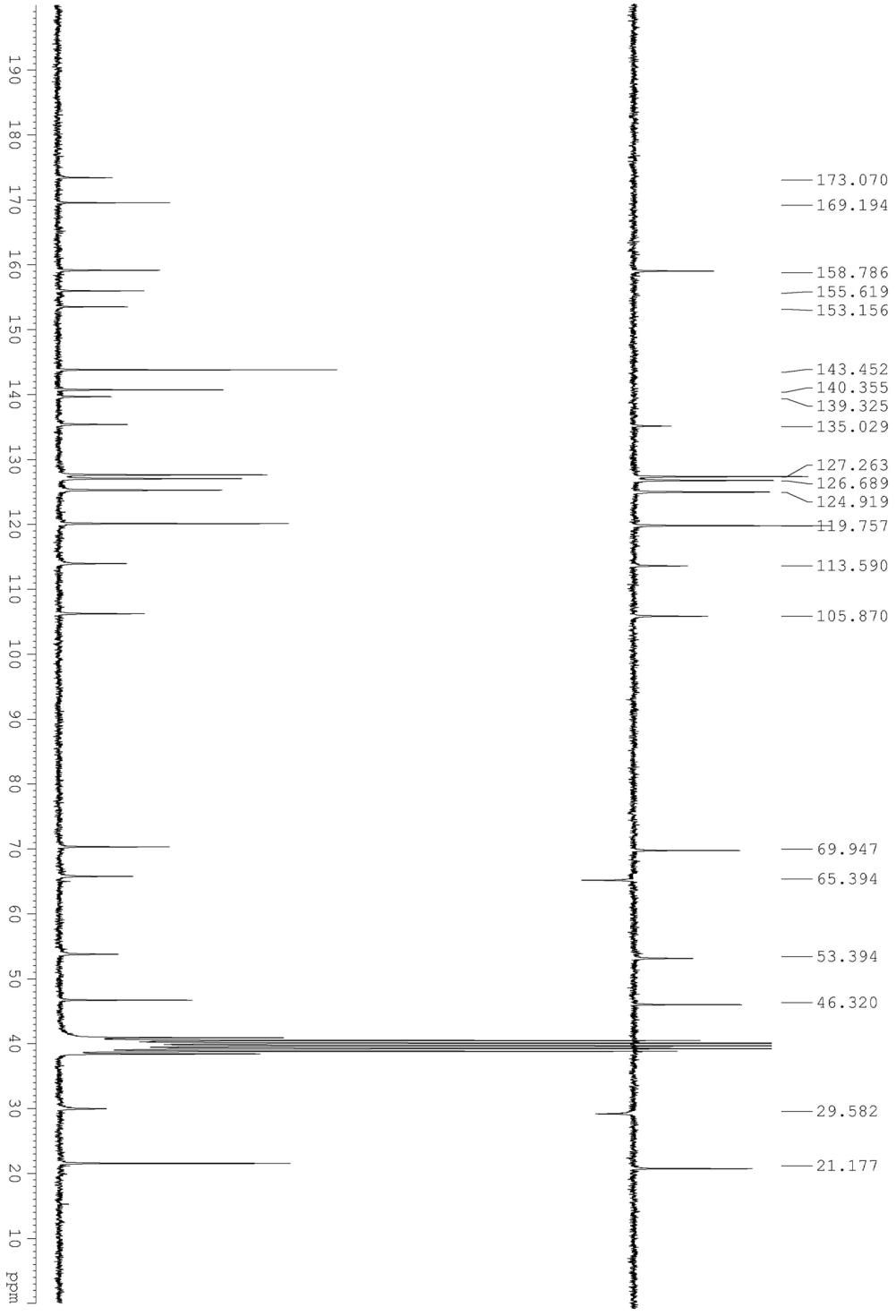
^{1}H NMR



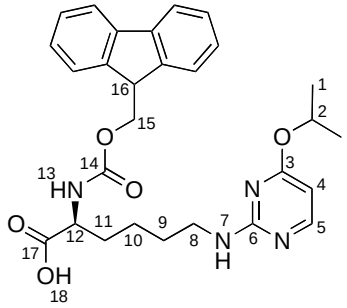
Compound I.12b



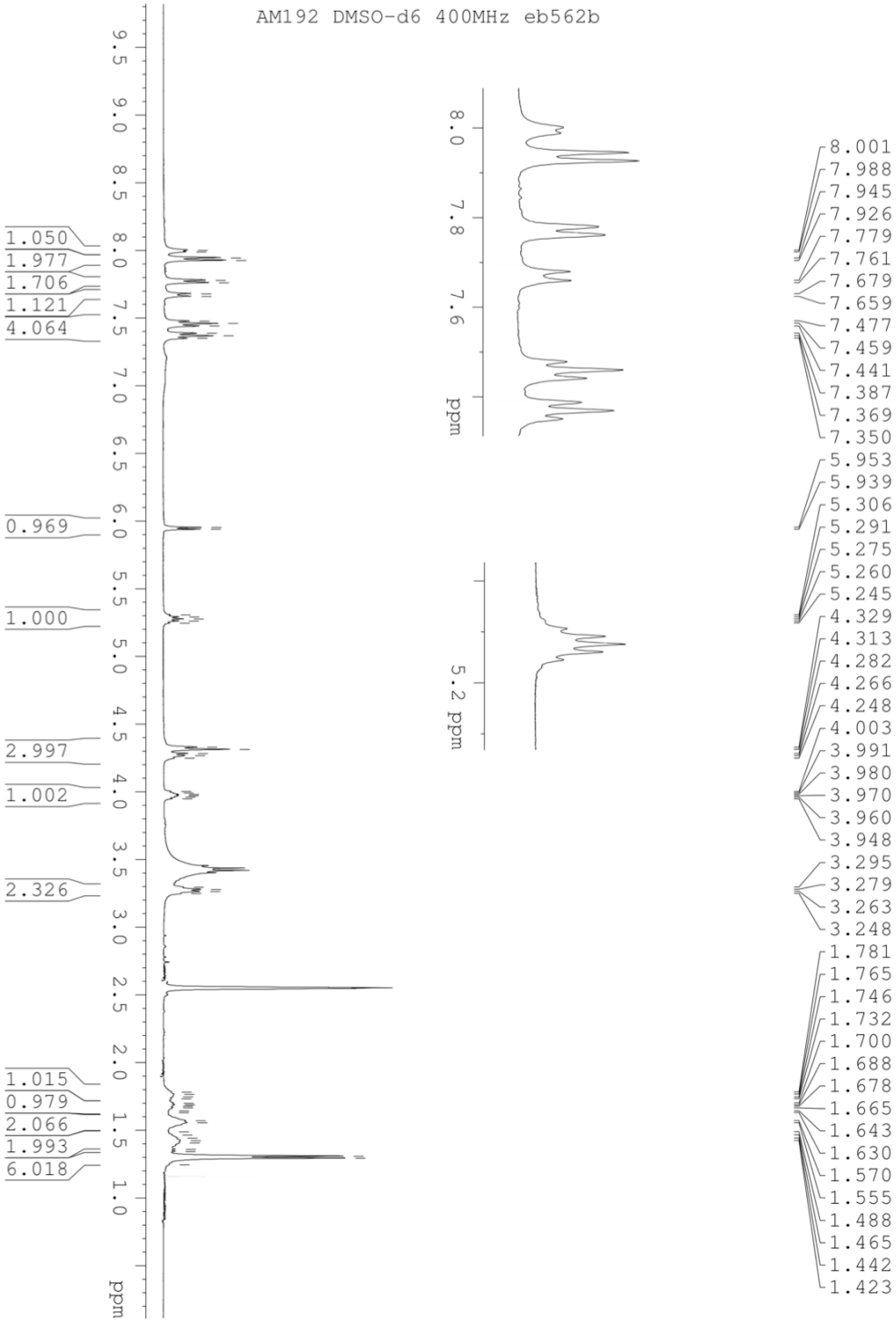
^{13}C NMR



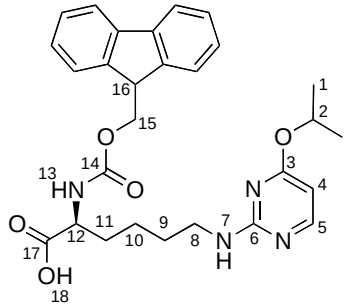
Compound I.12c



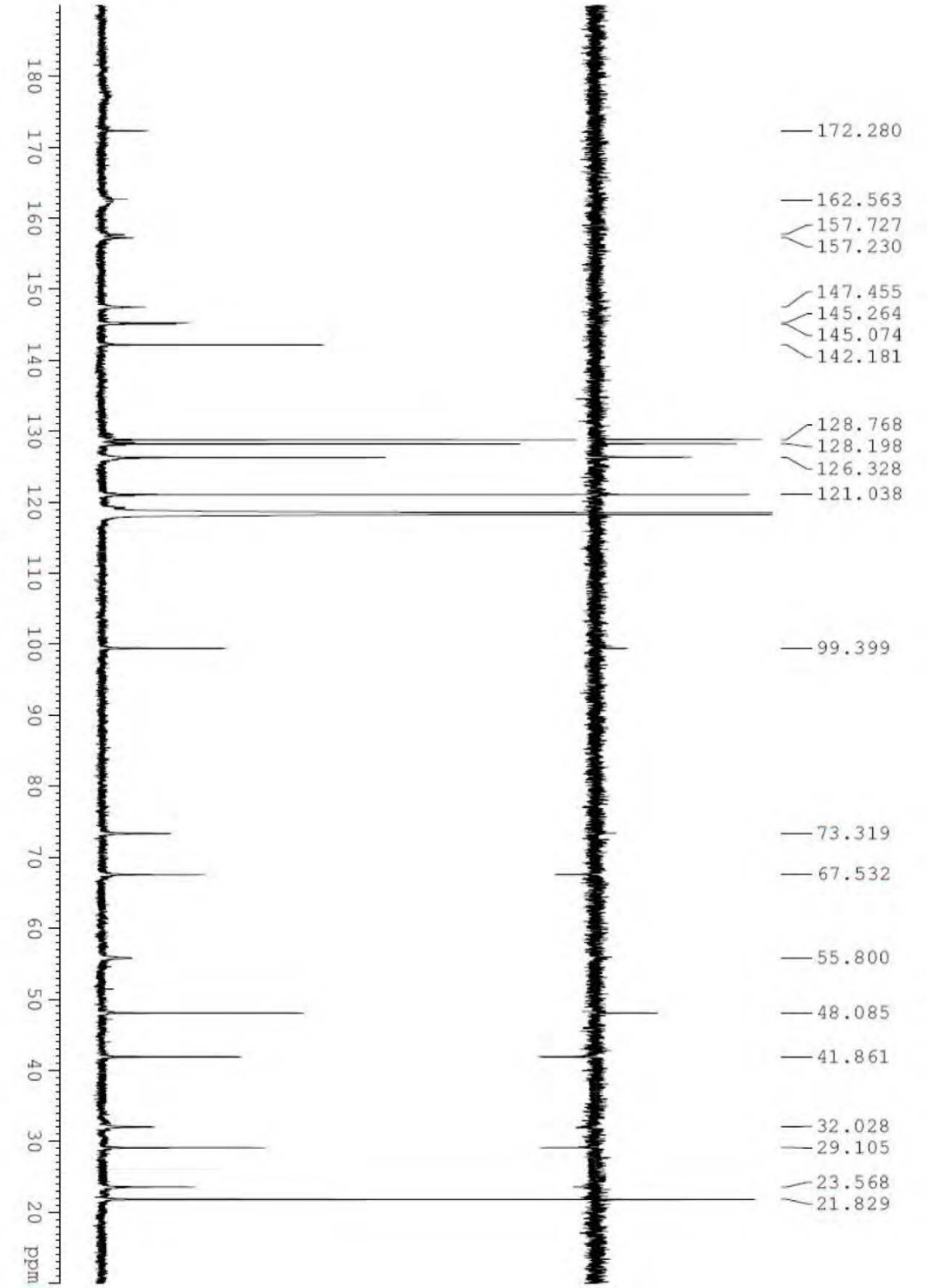
^{1}H NMR



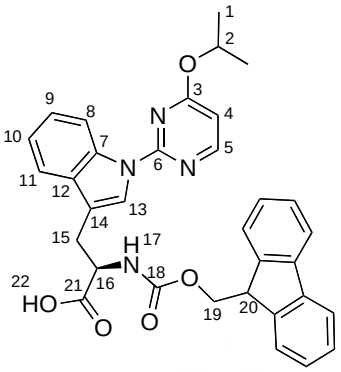
Compound I.12c



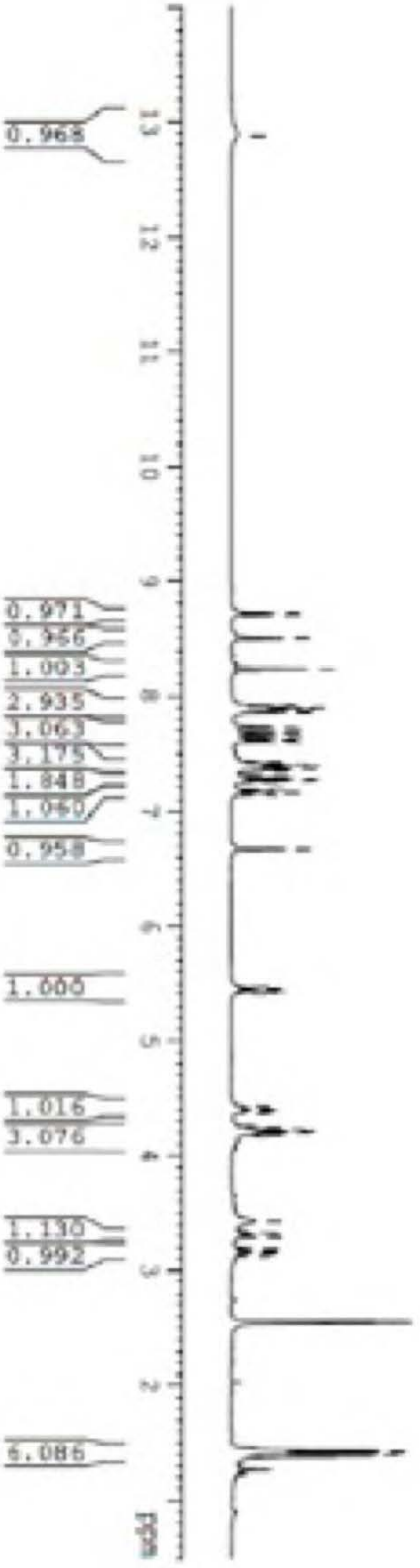
^{13}C / DEPT135 NMR

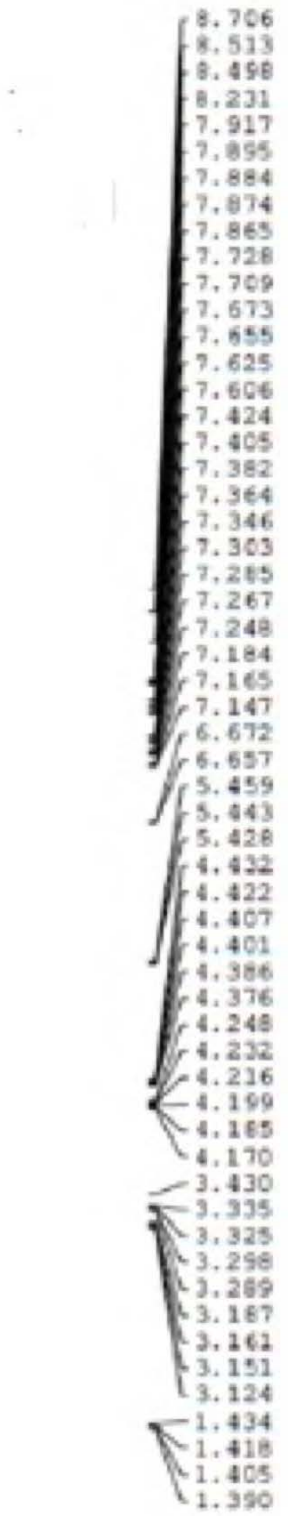


Compound I.12d

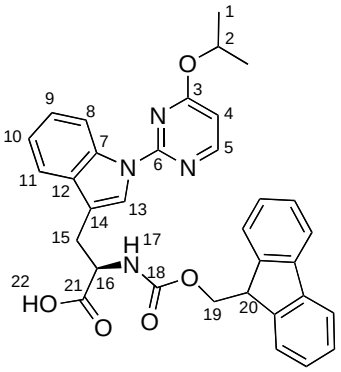


^{1}H NMR

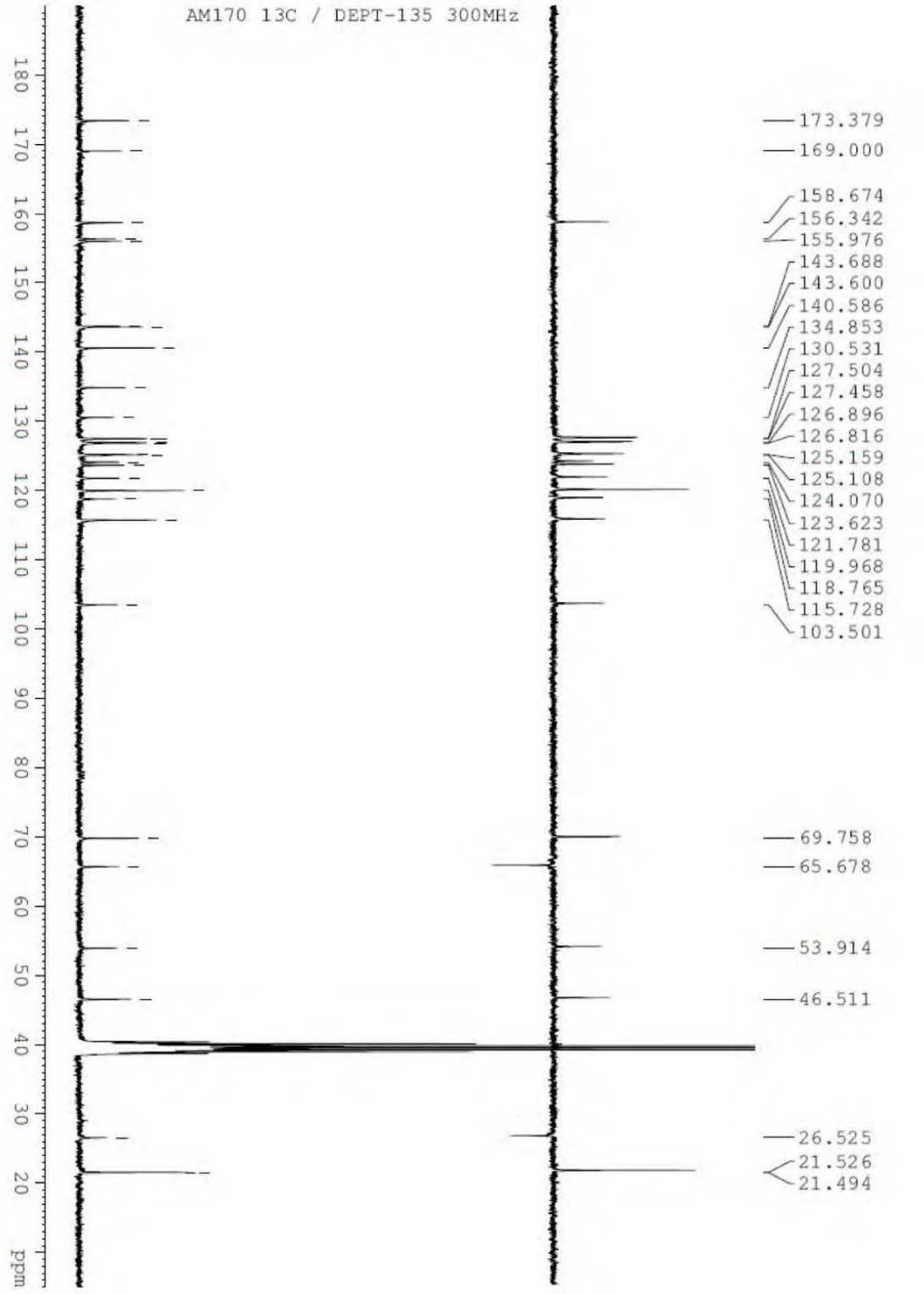




Compound I.12d

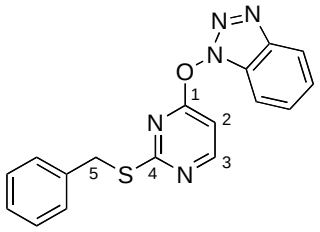


^{13}C NMR

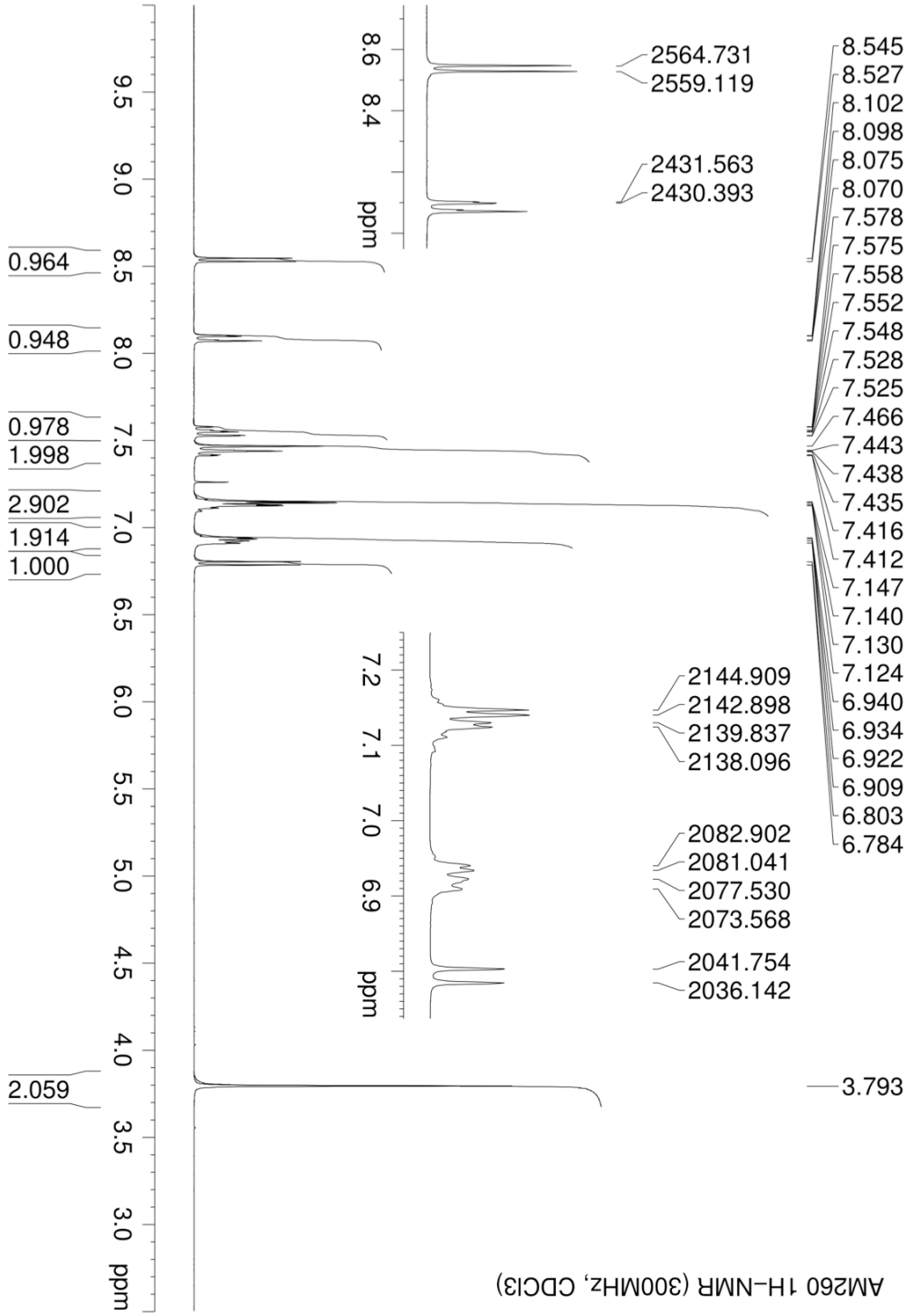


I.3. Pyrimidin-2-one amino acids

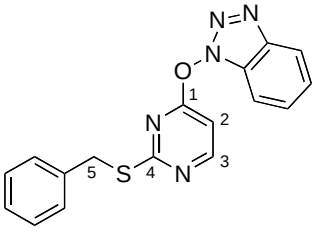
Compound I.17



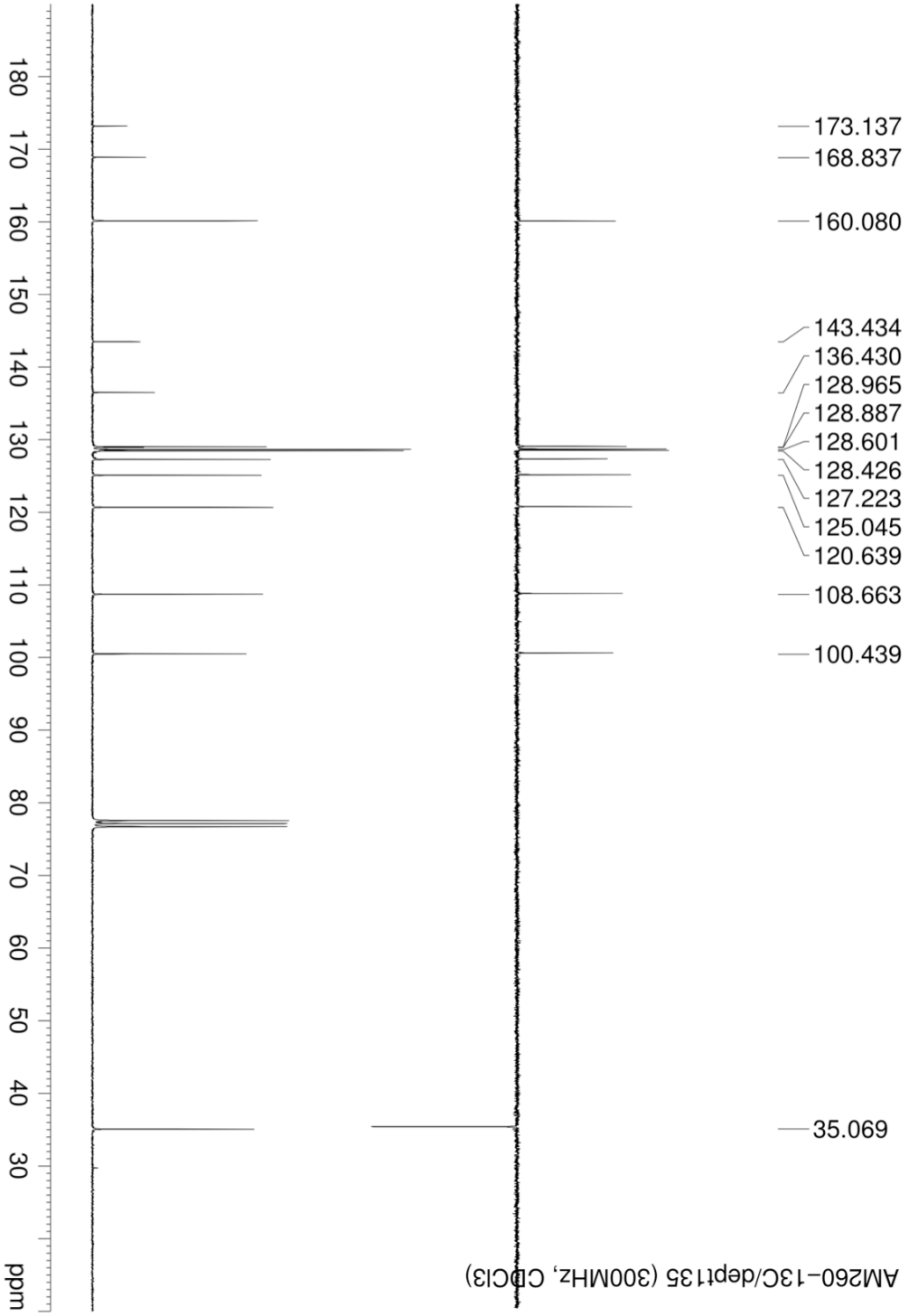
^{1}H NMR



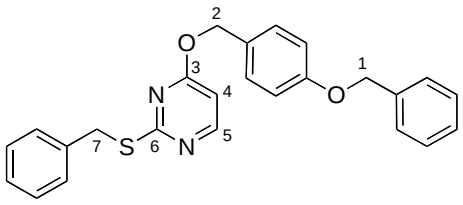
Compound I.17



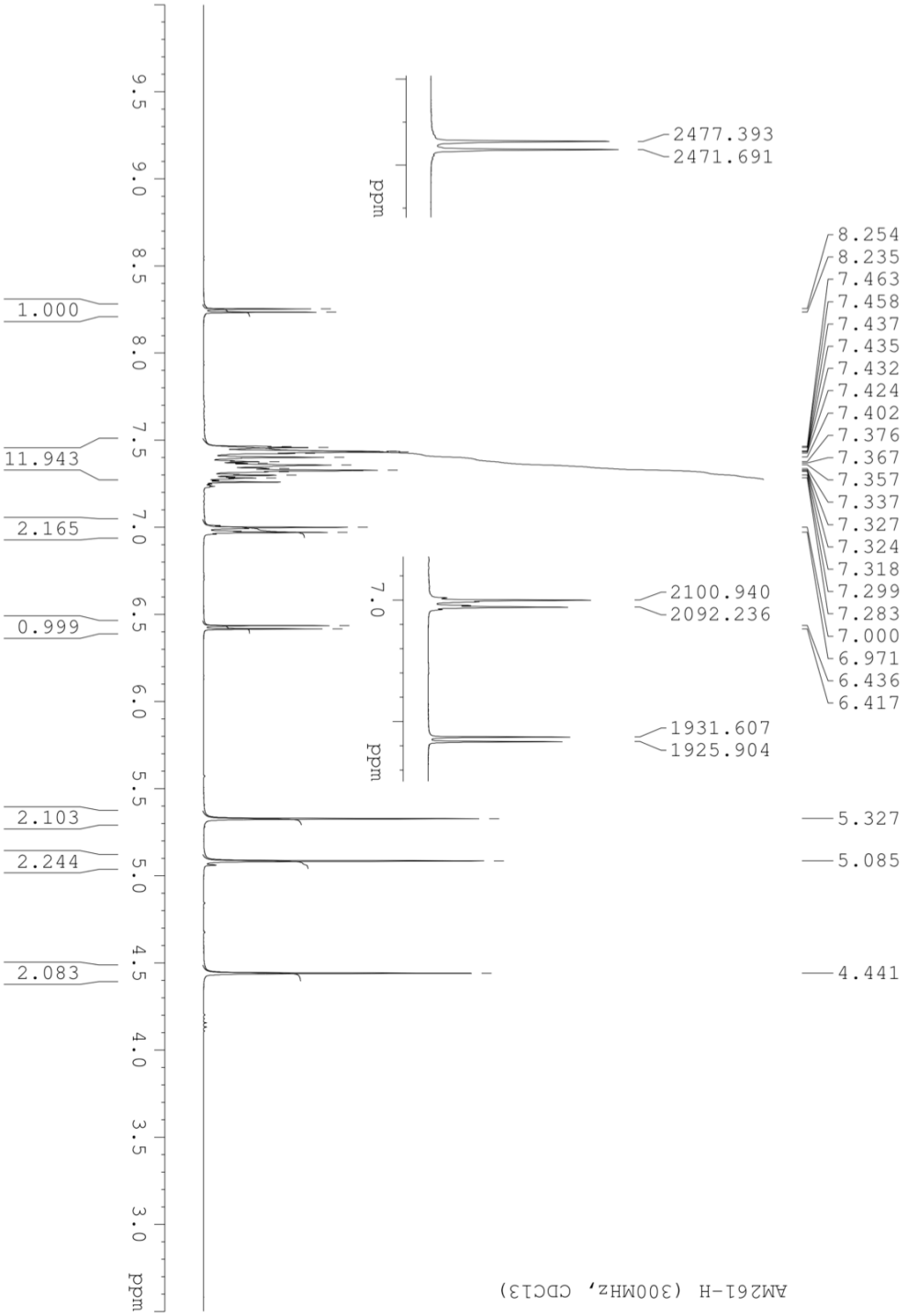
^{13}C NMR



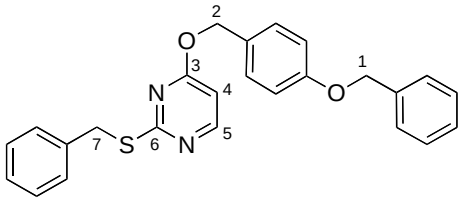
Compound I.40



^{1}H NMR



Compound I.40



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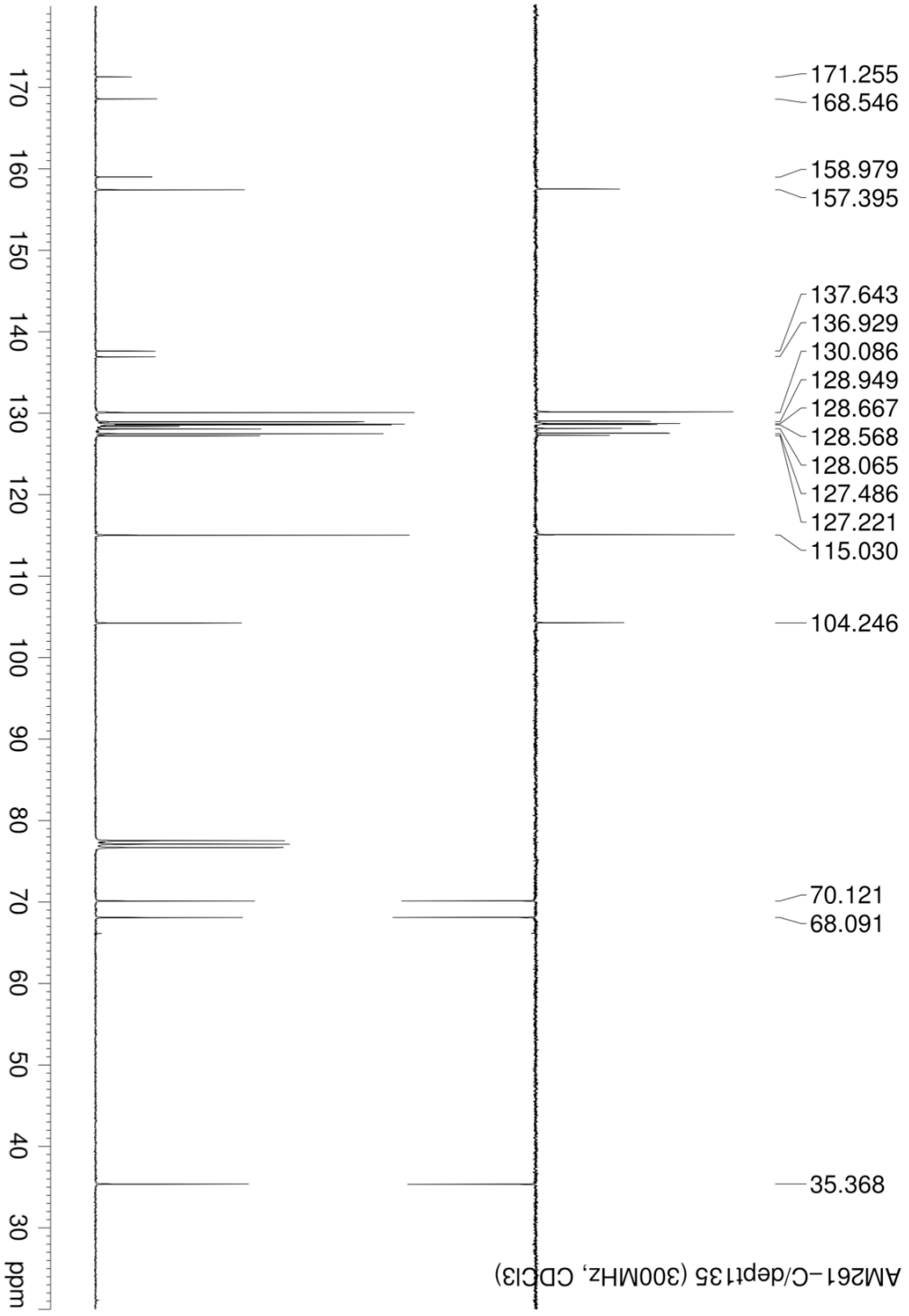
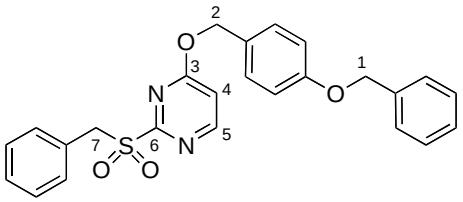
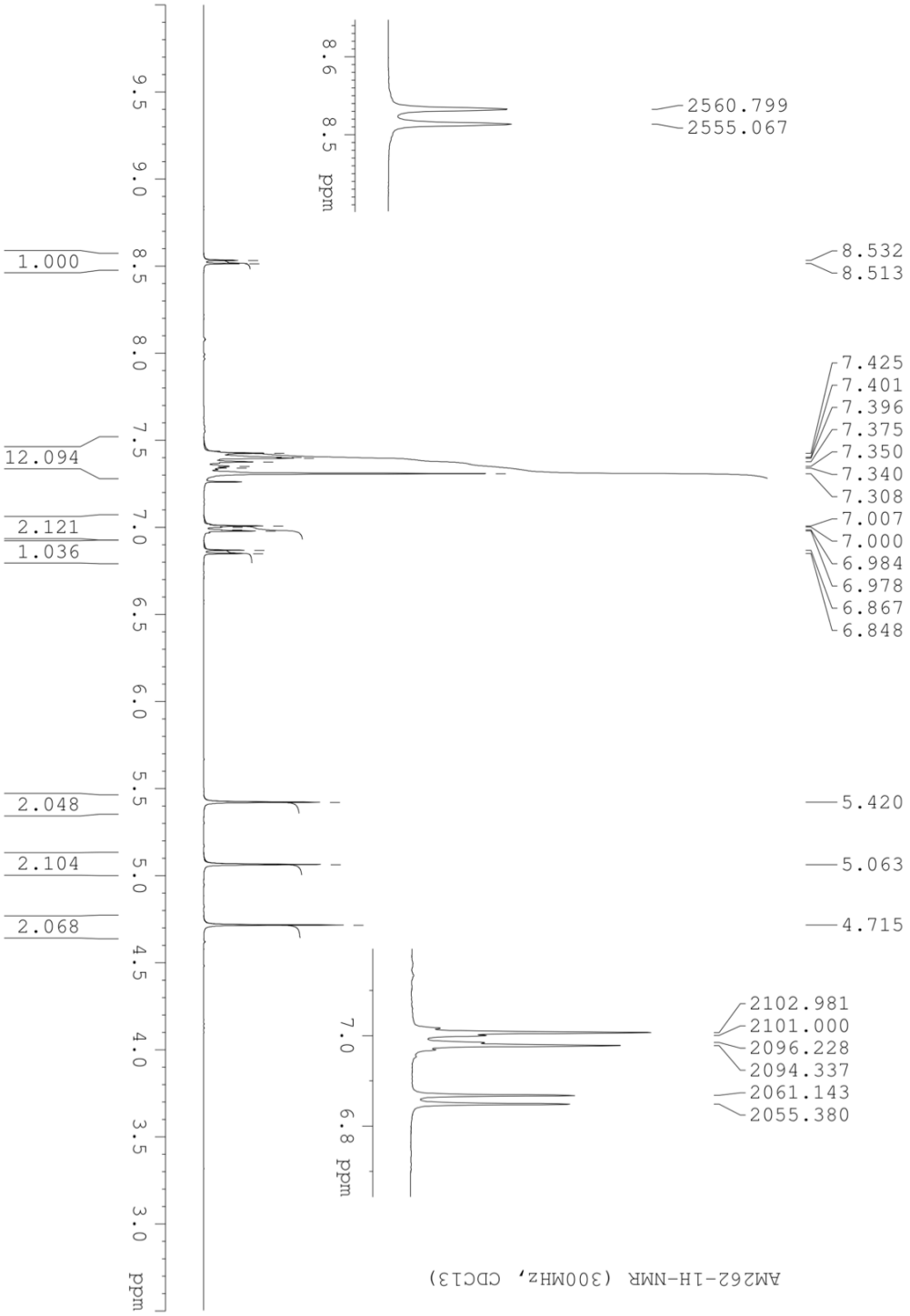


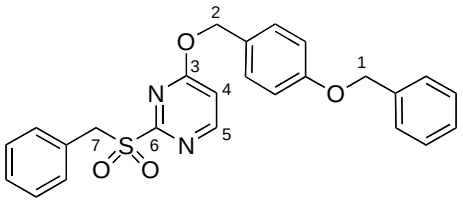
Compound I.38



^{1}H NMR



Compound I.38



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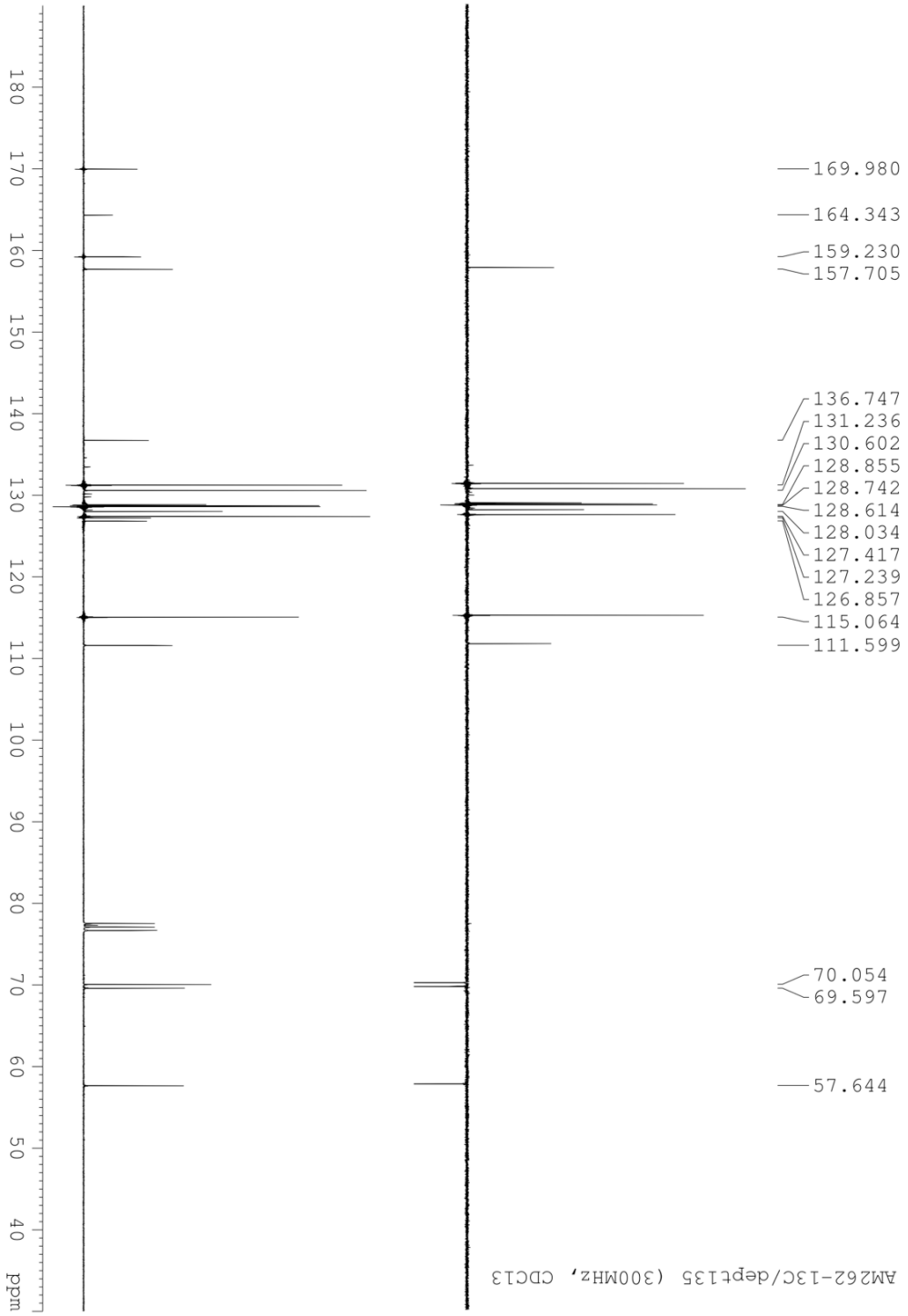
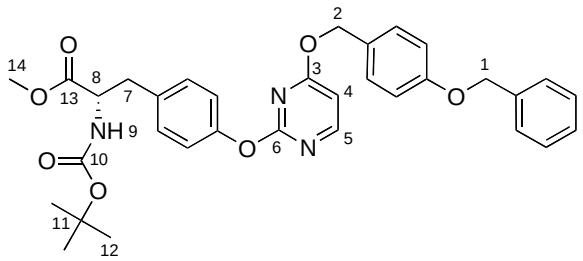
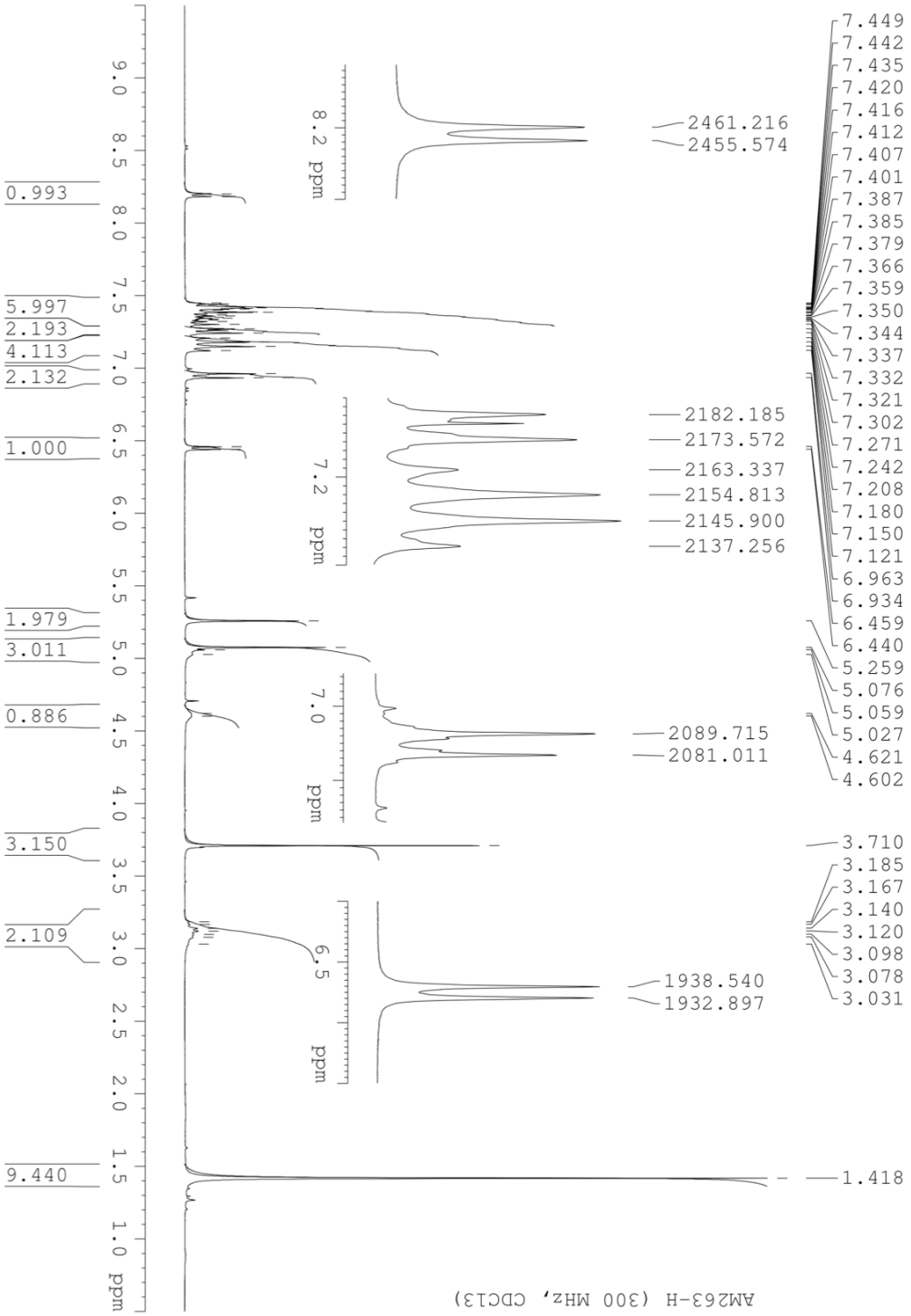


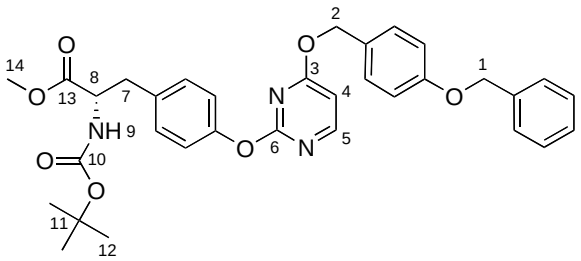
Compound I.39a



^{1}H NMR



Compound I.39a



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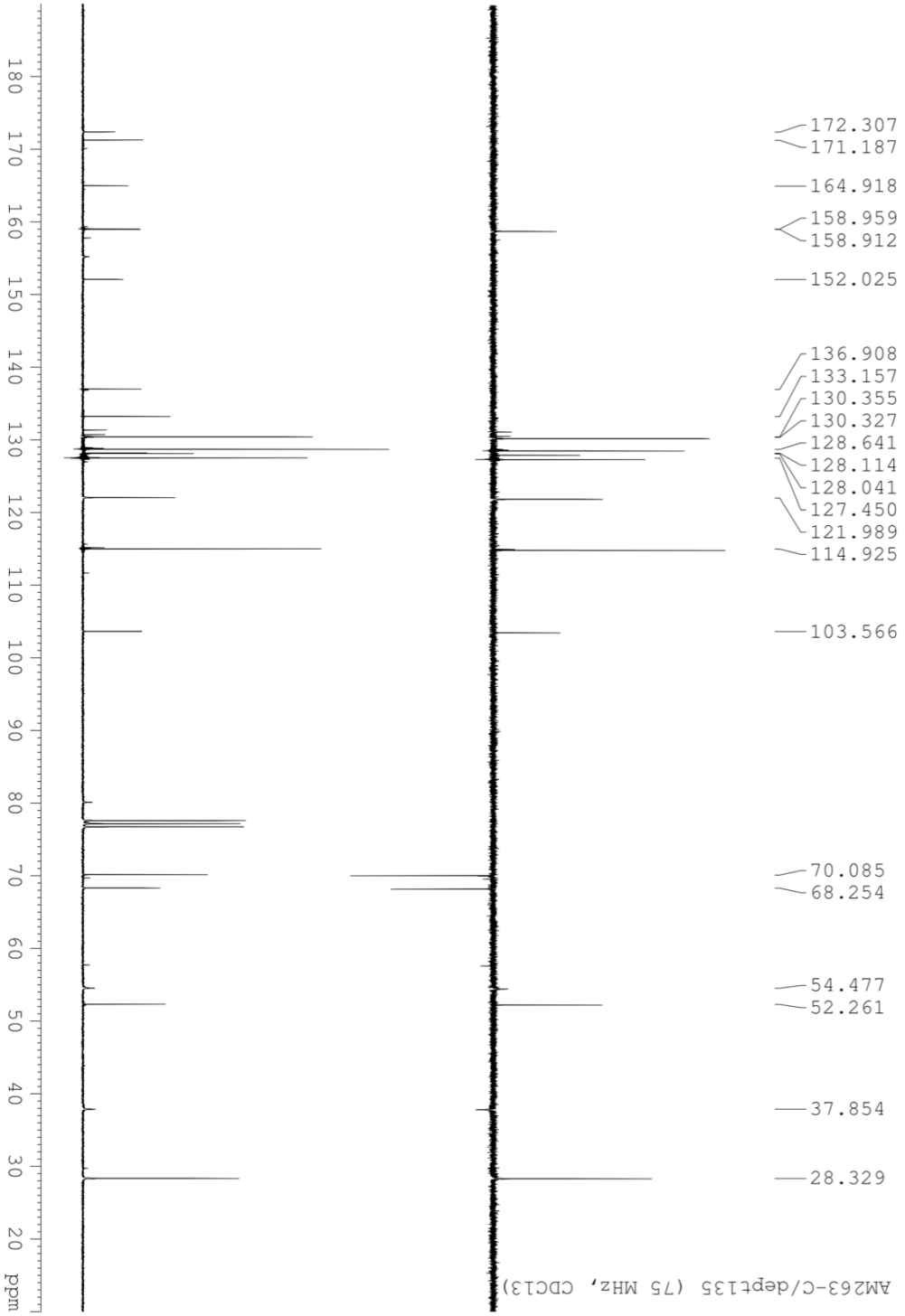
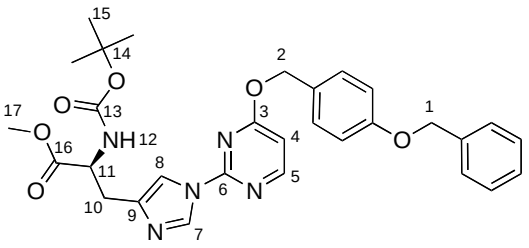
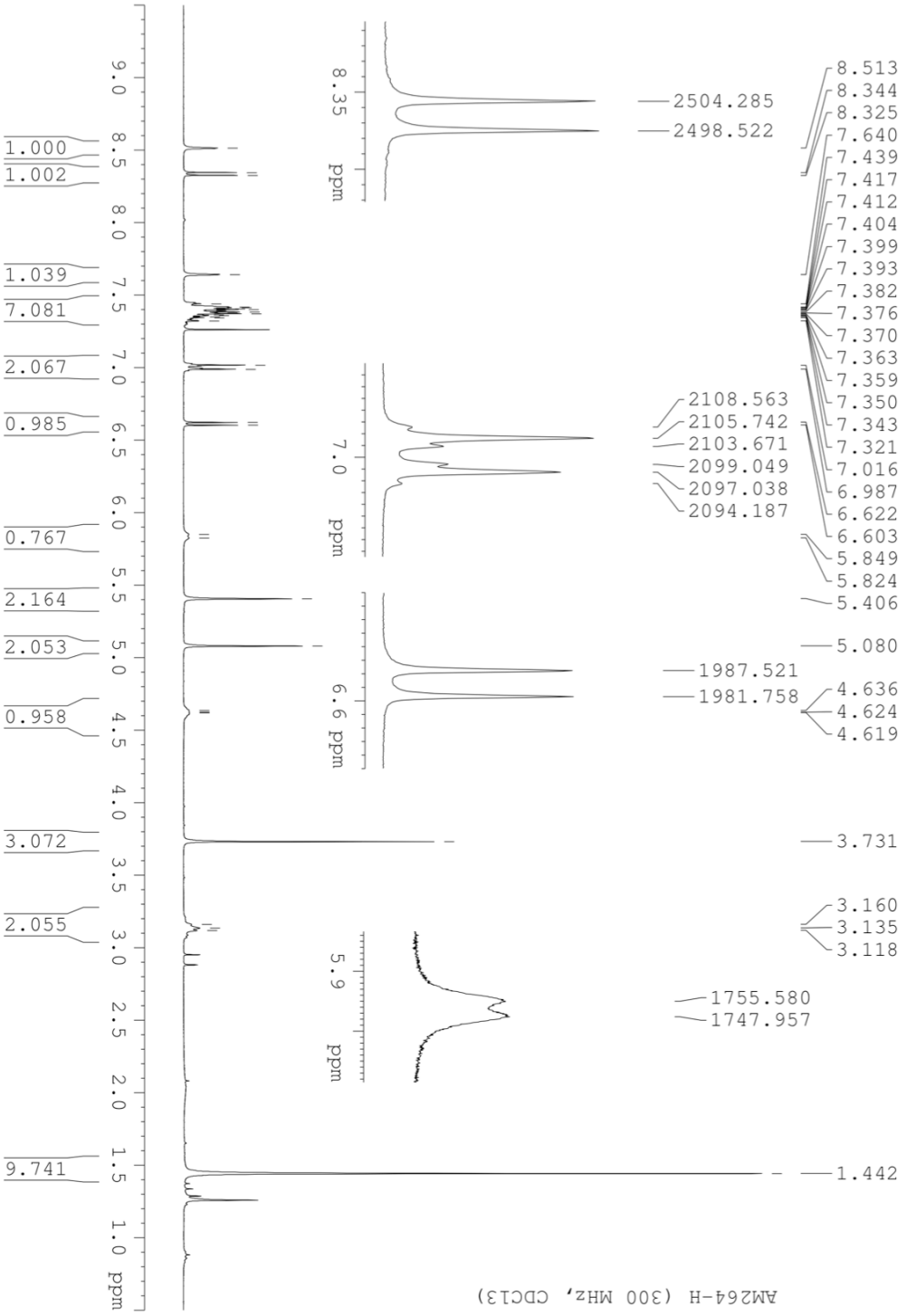


Compound I.39b

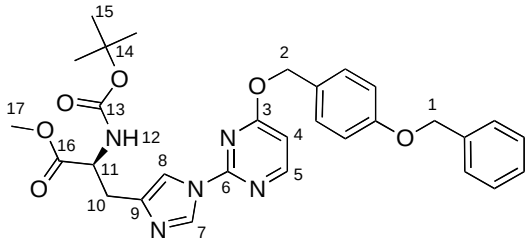


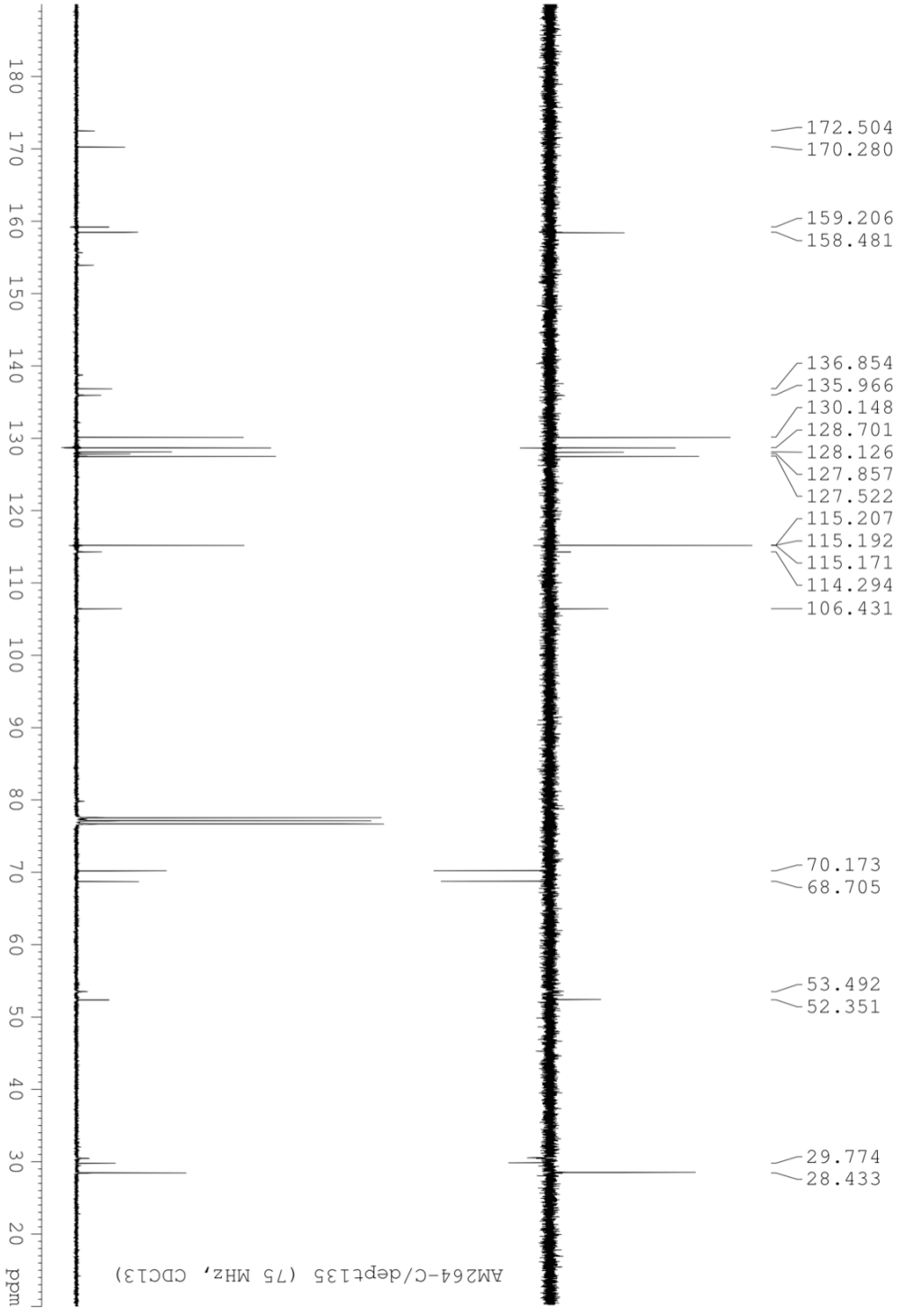
^{1}H NMR



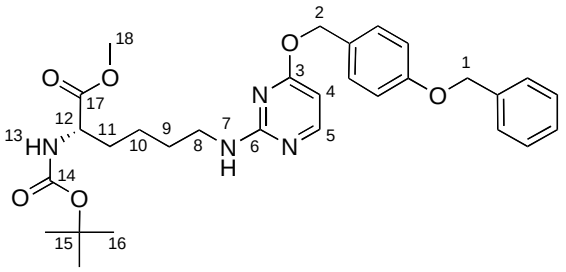
Compound I.39b

^{13}C NMR





Compound I.39c



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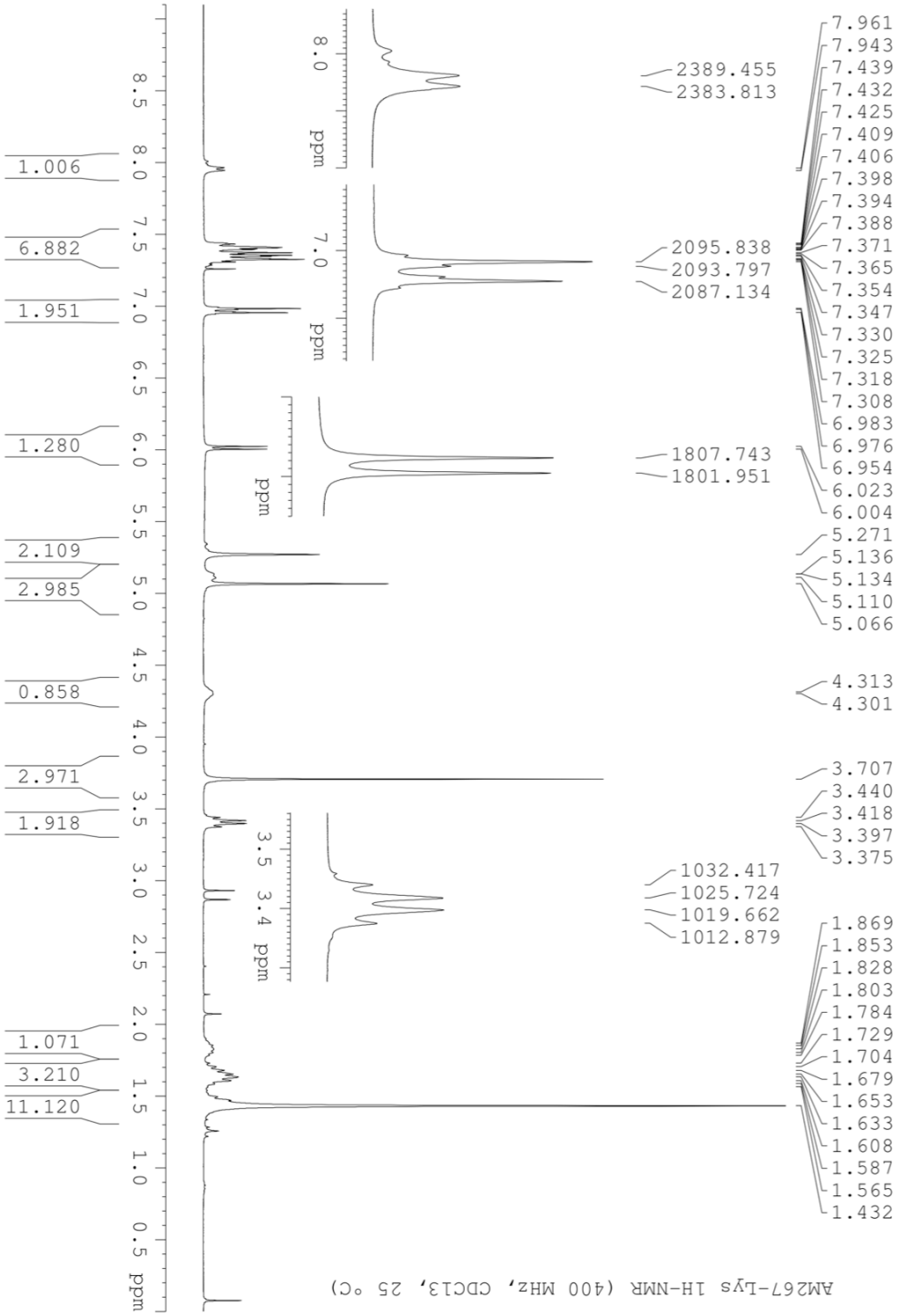
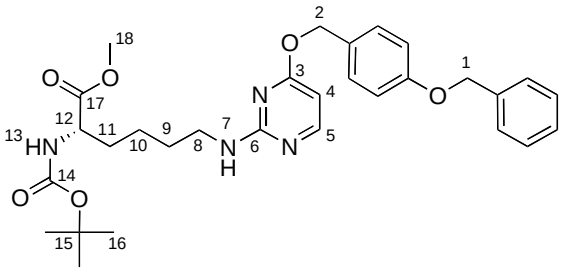


Compound I.39c



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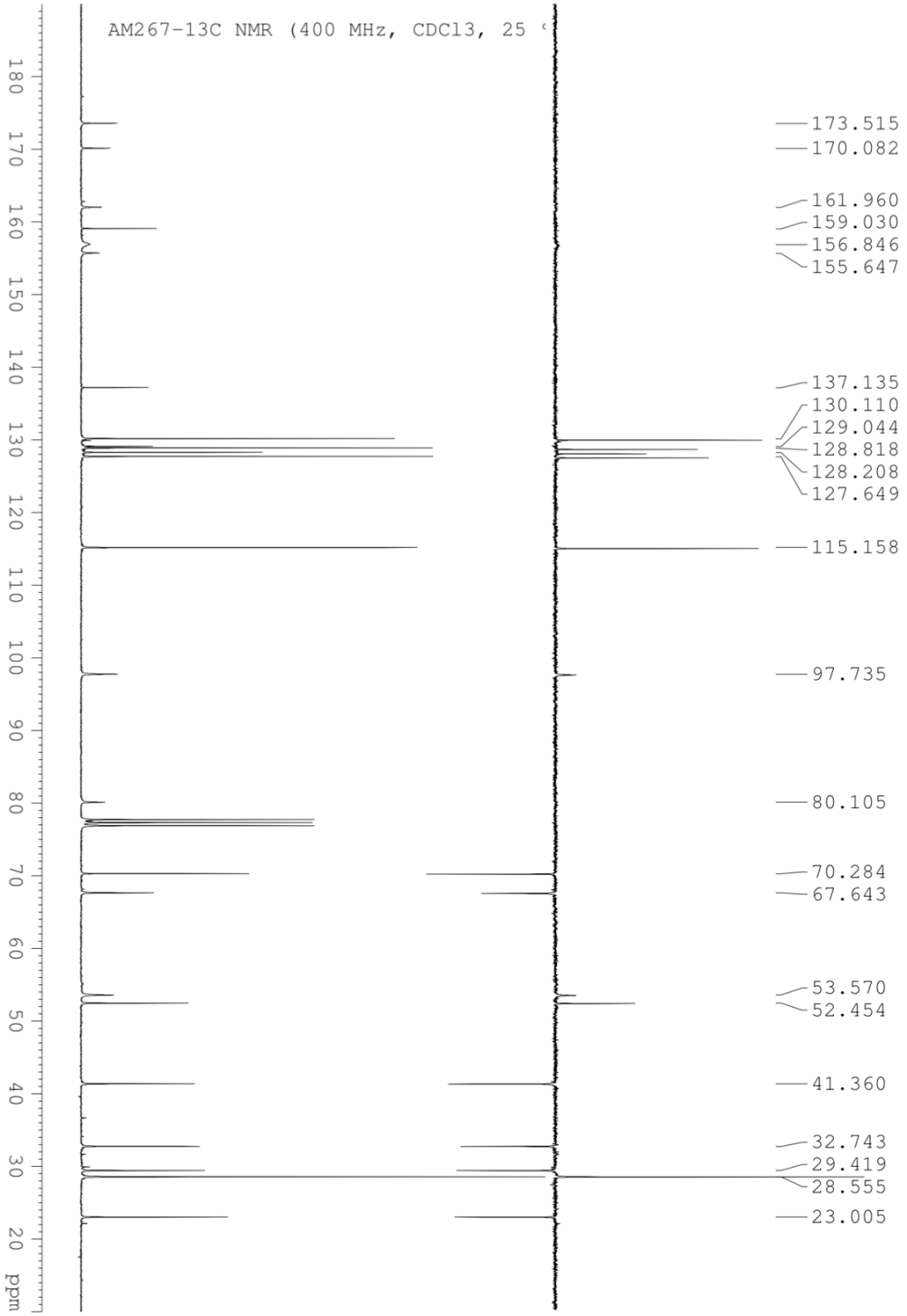
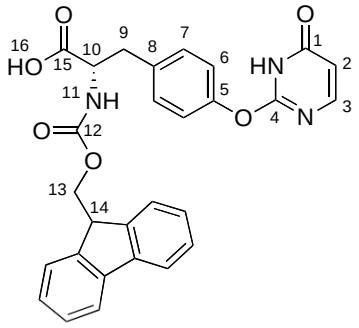
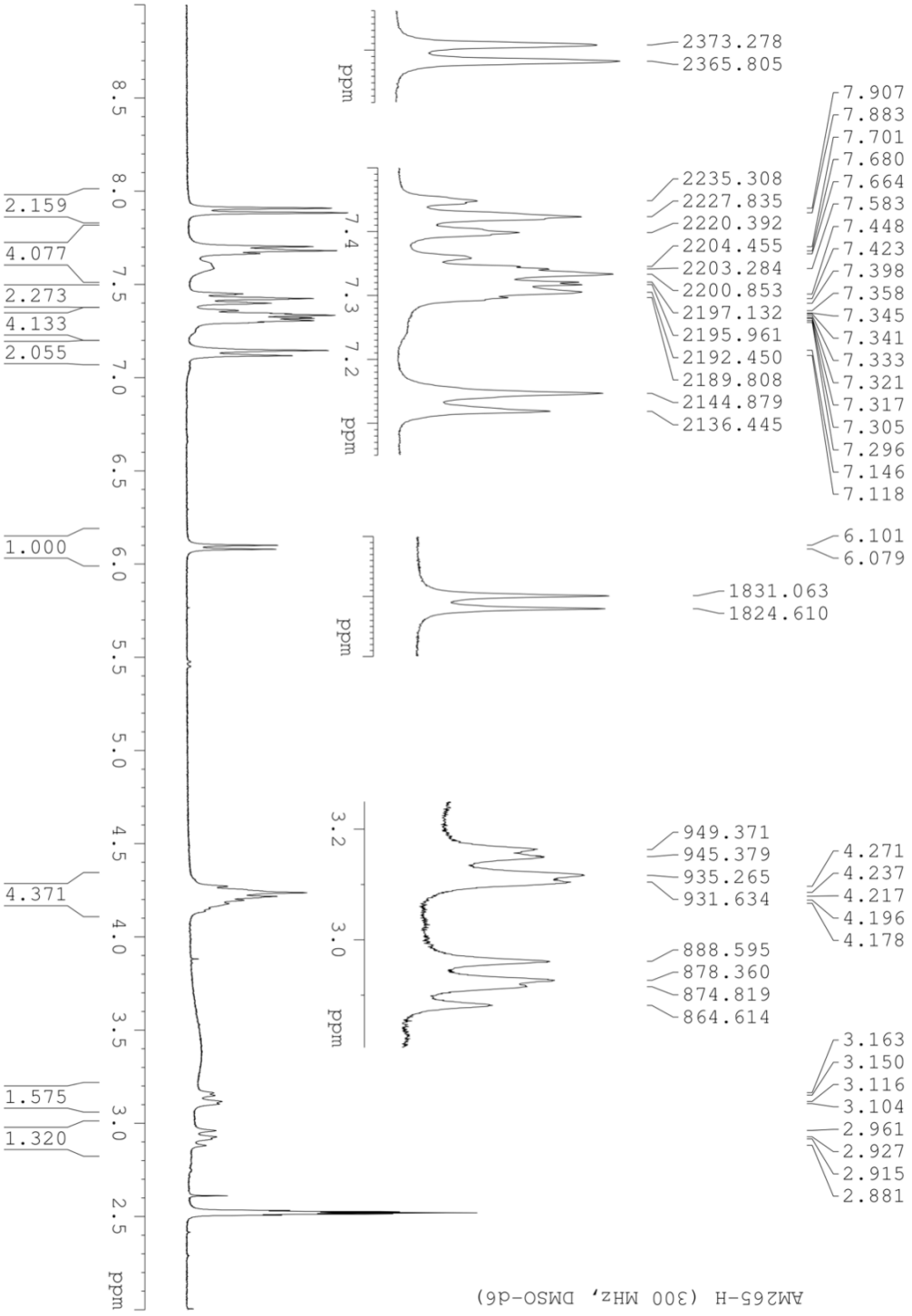


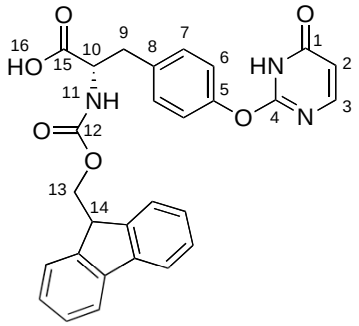
Compound I.13a



^{1}H NMR



Compound I.13a



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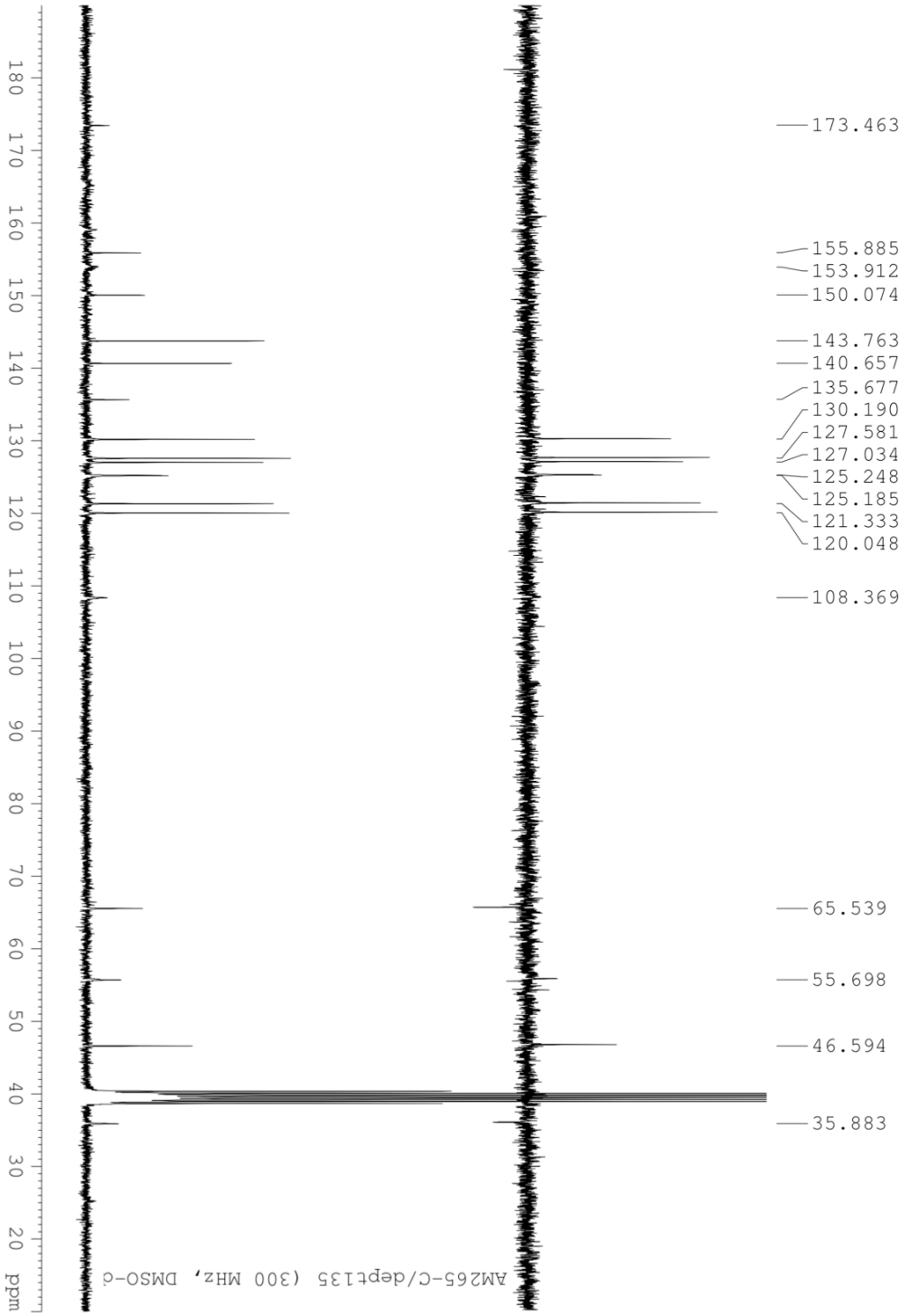
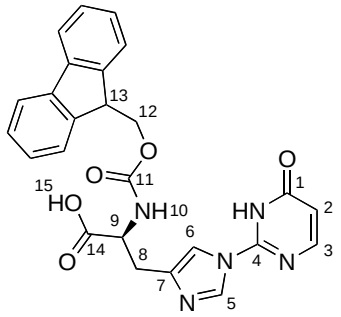
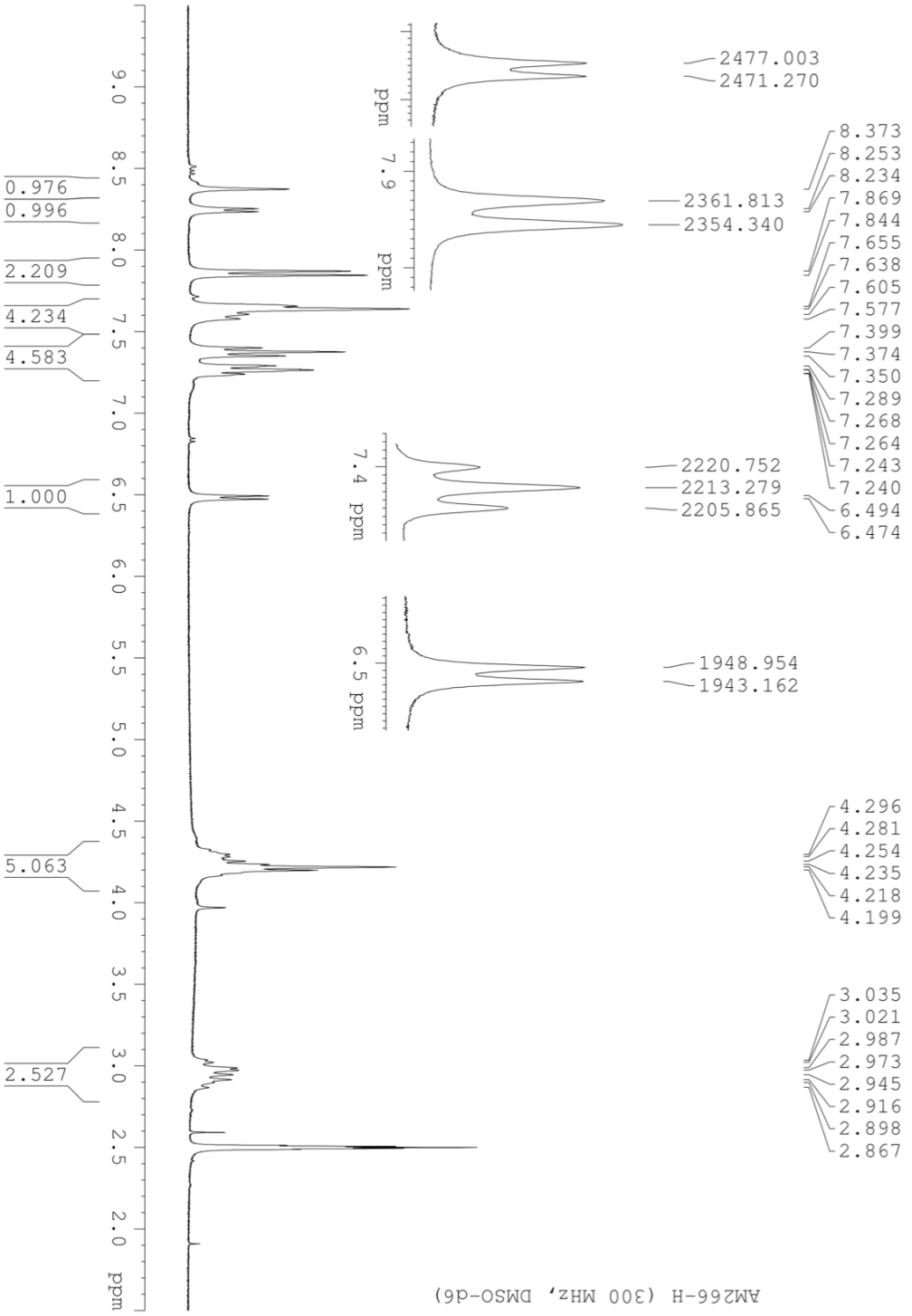


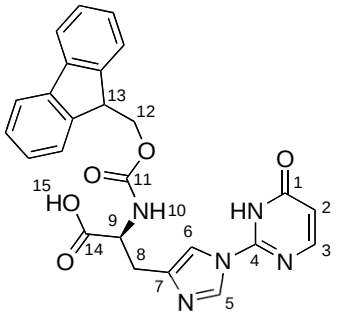
Compound I.13b



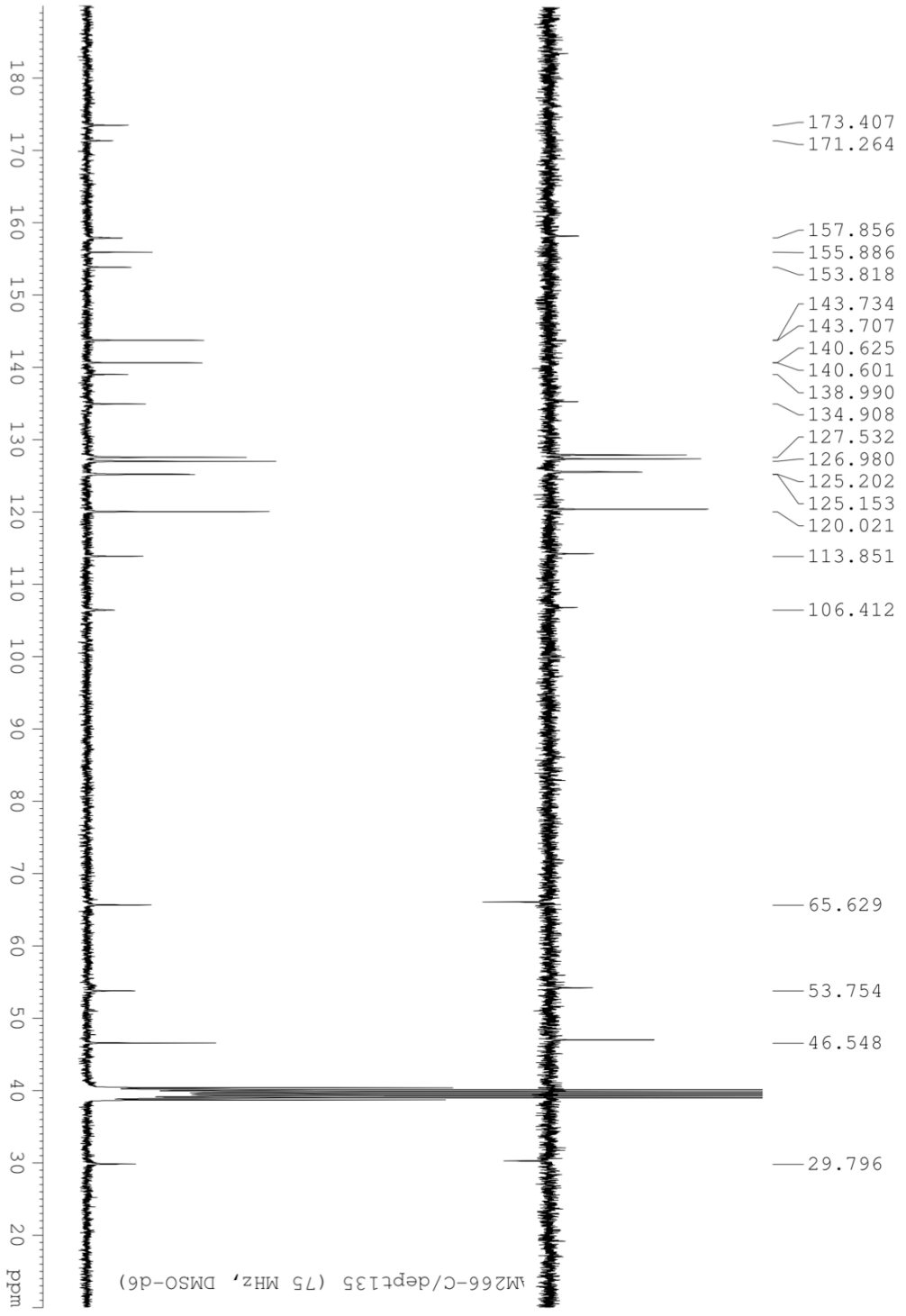
^{1}H NMR



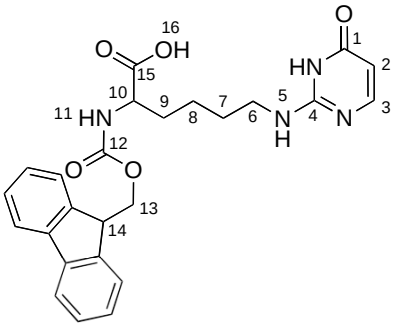
Compound I.13b



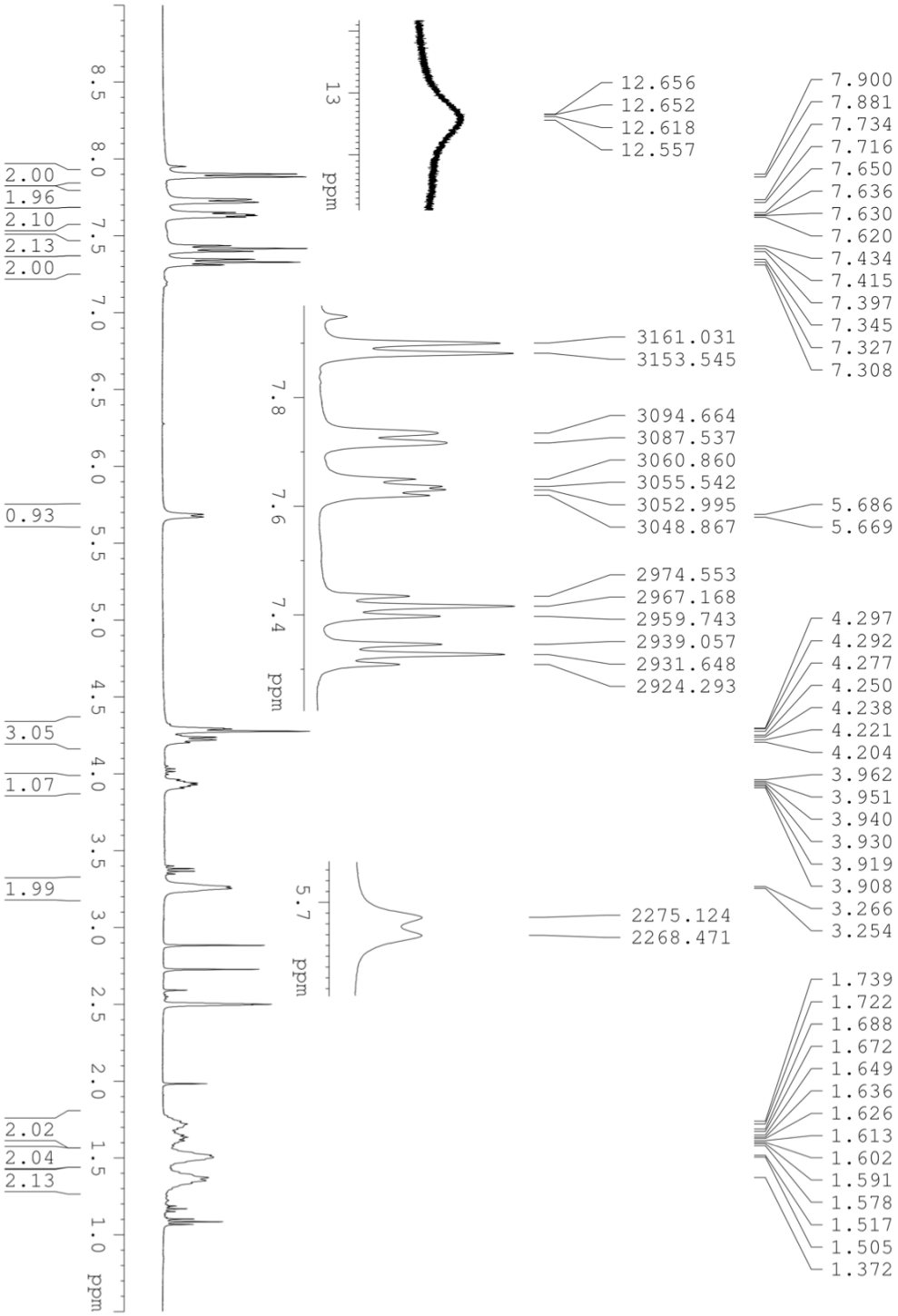
^{13}C NMR



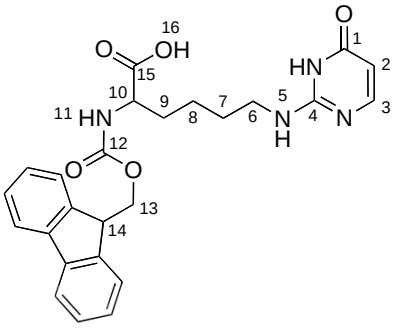
Compound I.13c



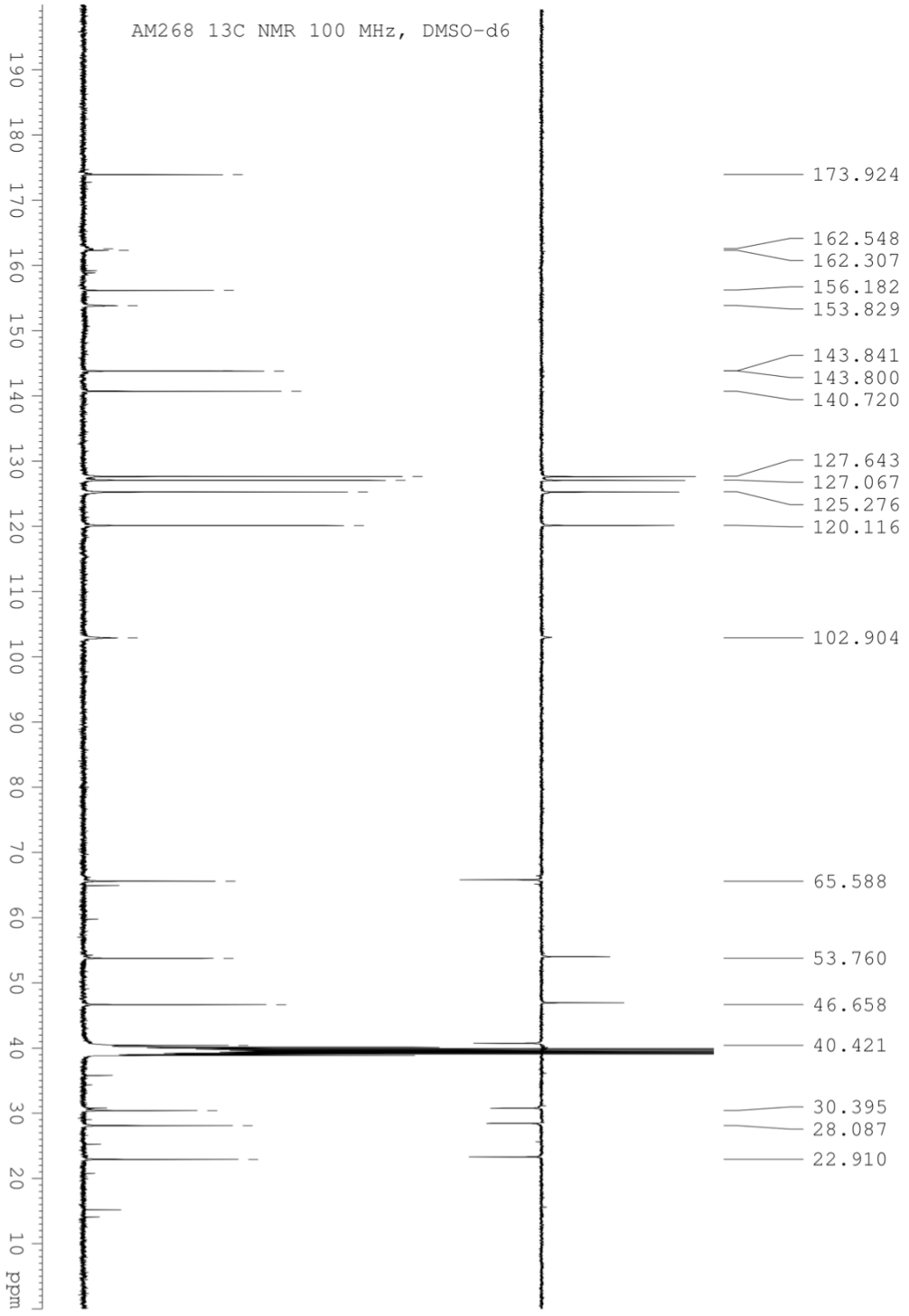
^{1}H NMR



Compound I.13c



^{13}C NMR

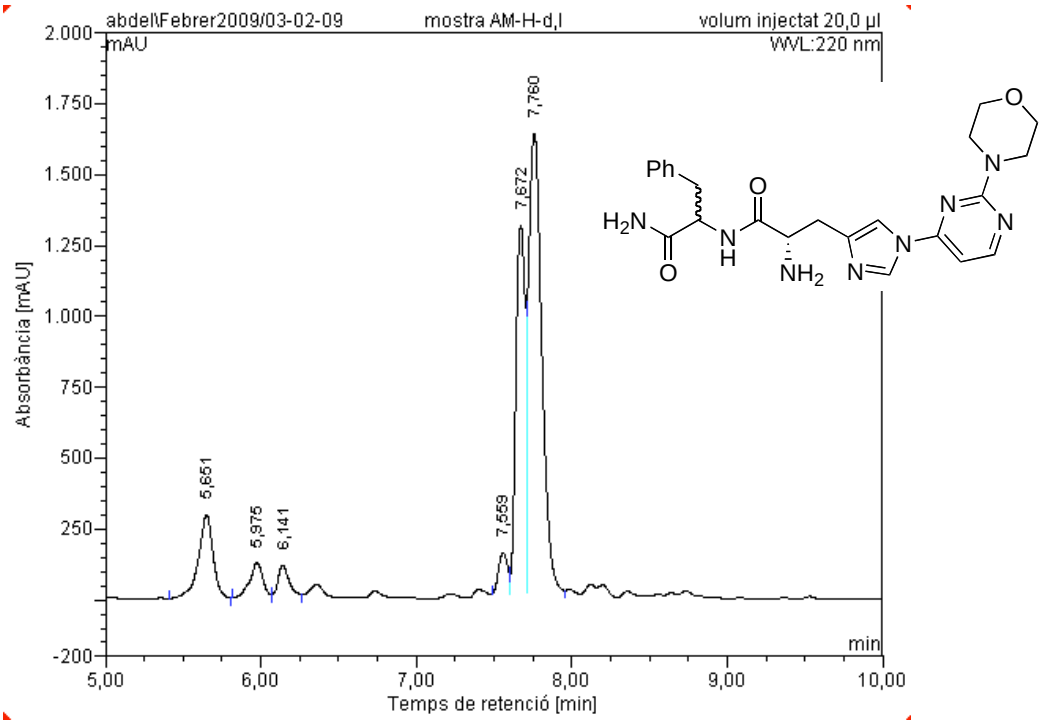


ANNEX II

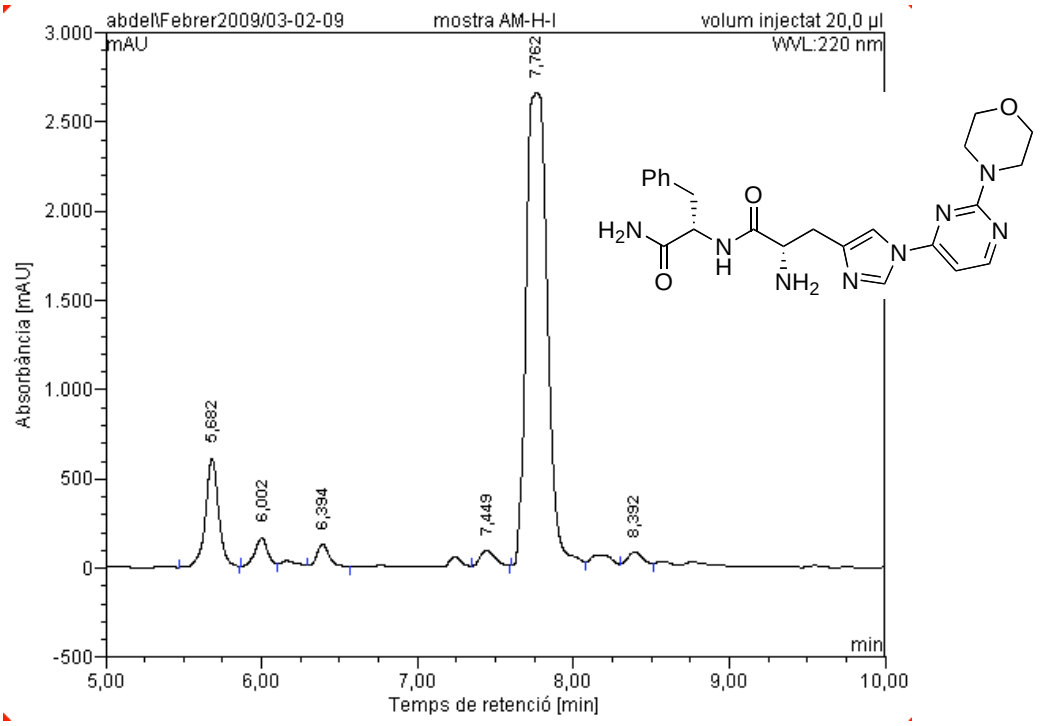
A.II.1. EXAMPLES OF DIPEPTIDES

A.II.1.1. Examples of dipeptides for the determination of the optical purity

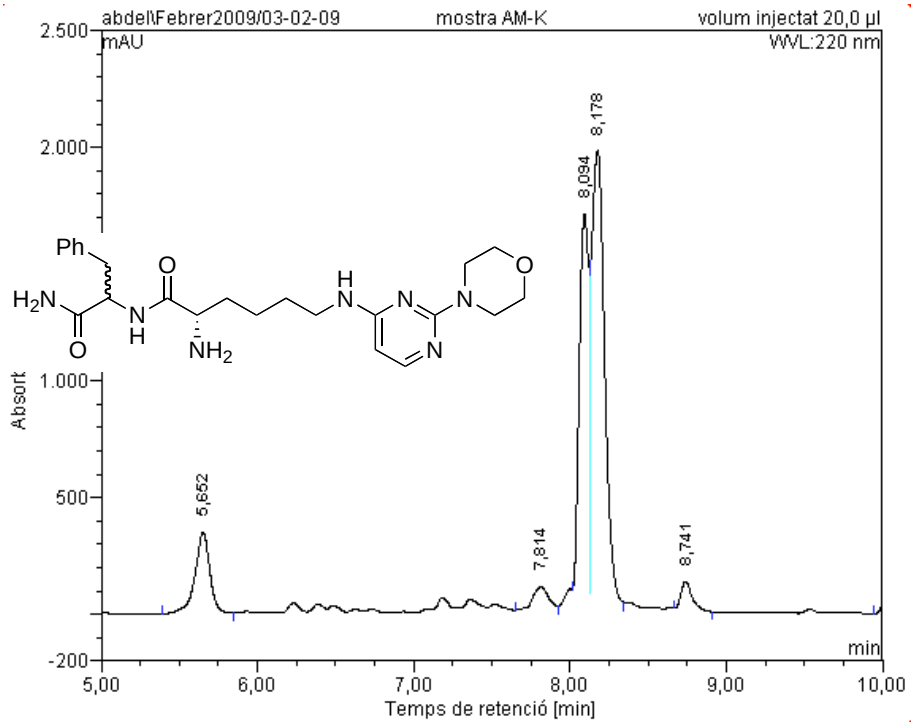
HPLC of dipeptide (I.20b)
Reactions described at Table I.6



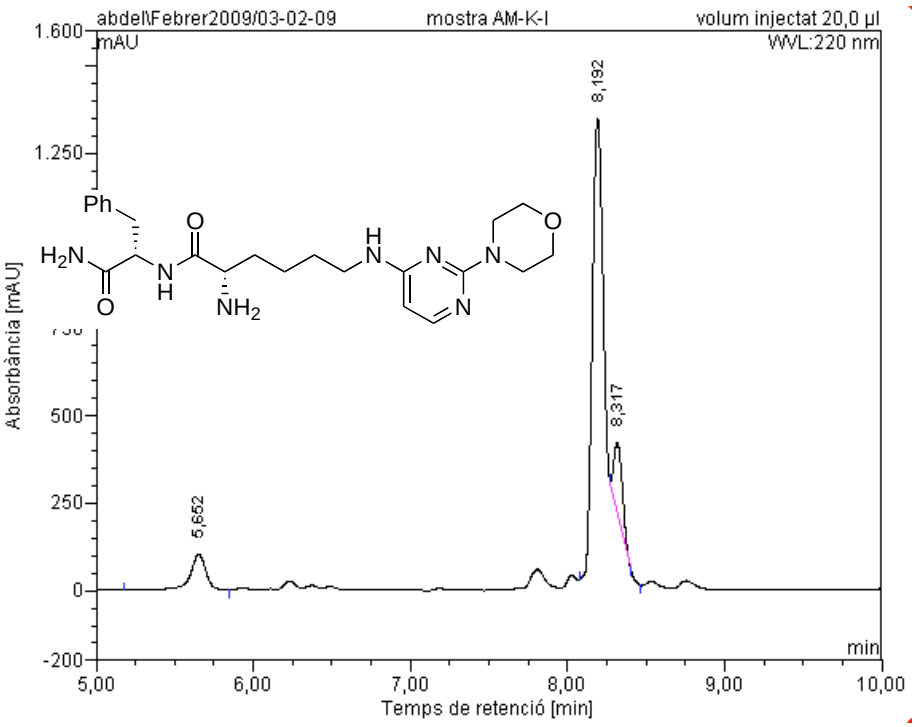
HPLC of dipeptide (I.20b)
Reaction performed with K_2CO_3 (entries 3 and 4,Table I.6)



HPLC of dipeptide (I.20c)
Reactions described at Table I.7

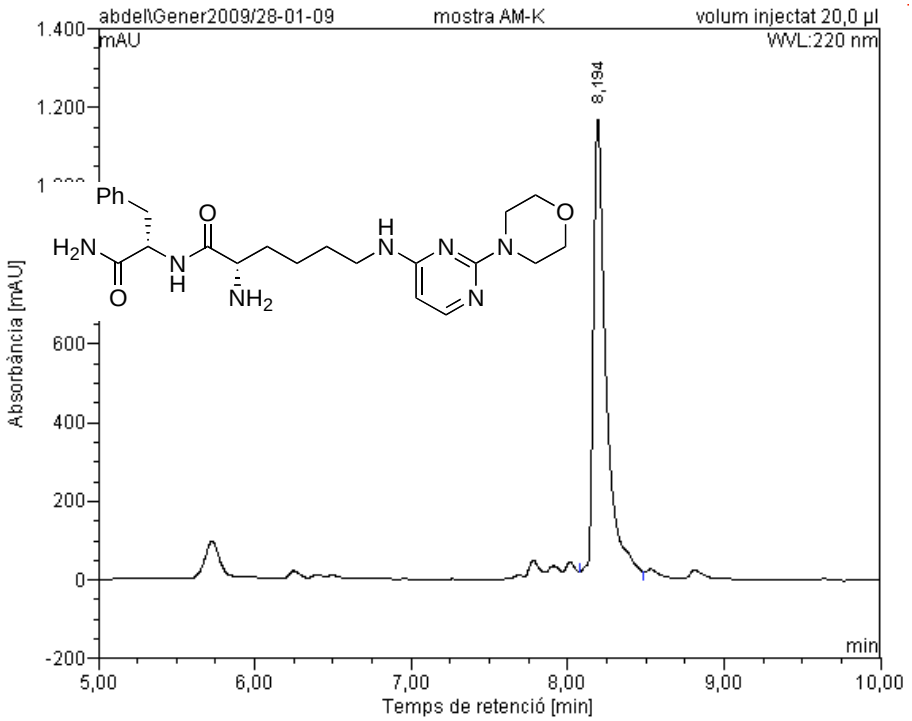


HPLC of dipeptide (I.20c)
Reaction performed with K_2CO_3 at 50ºC (entry 1, Table I.7)

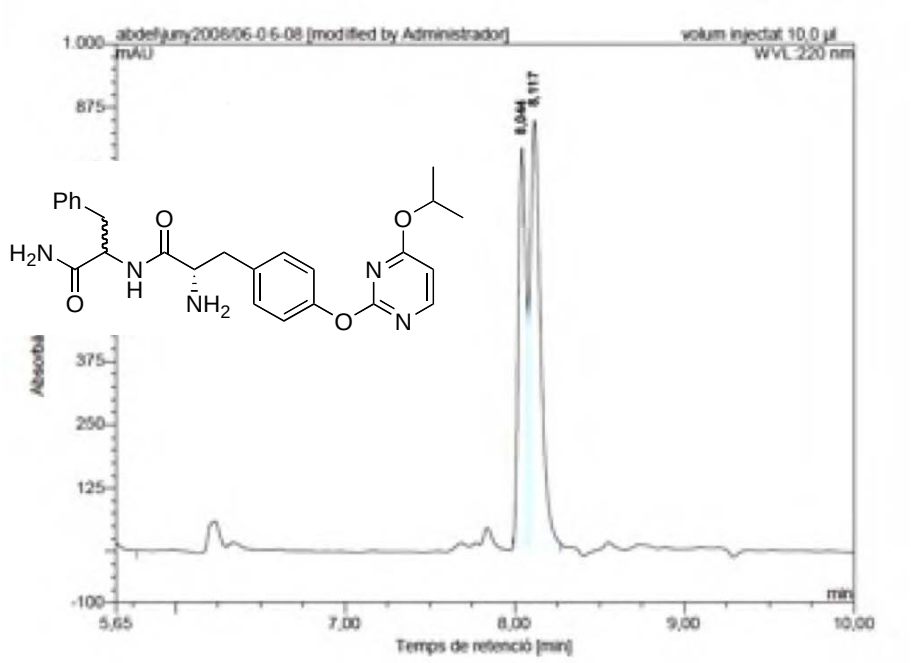


HPLC of dipeptide (I.20c)

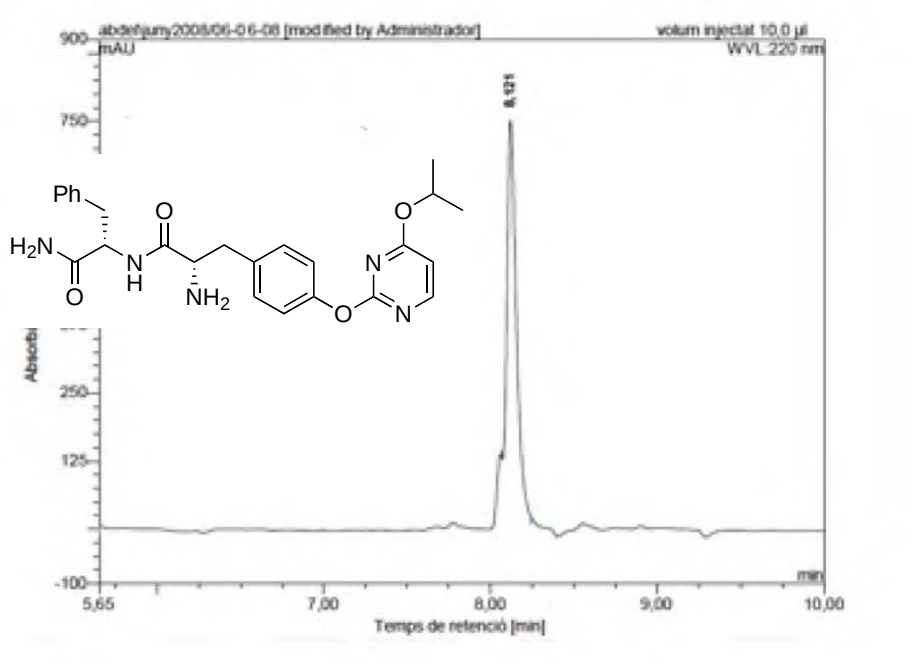
Reaction performed with K_2CO_3 at 40ºC (entry 4, Table I.7)



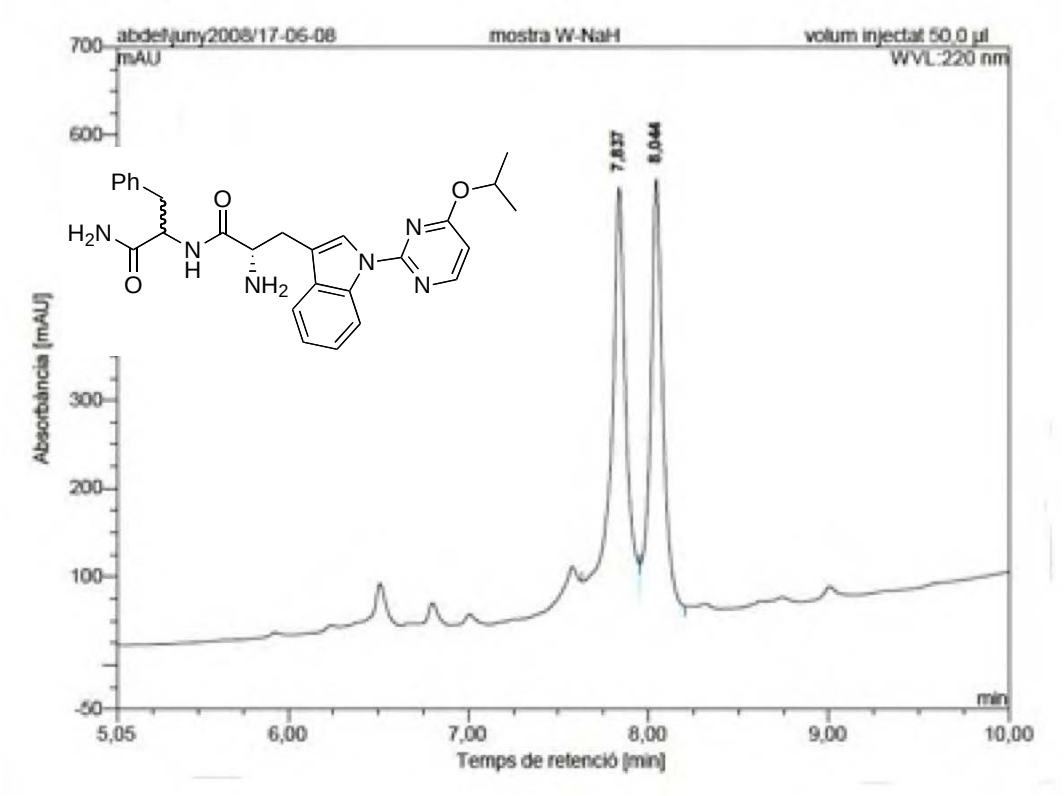
HPLC of dipeptide (I.32aa)
Reaction described at Table I.9



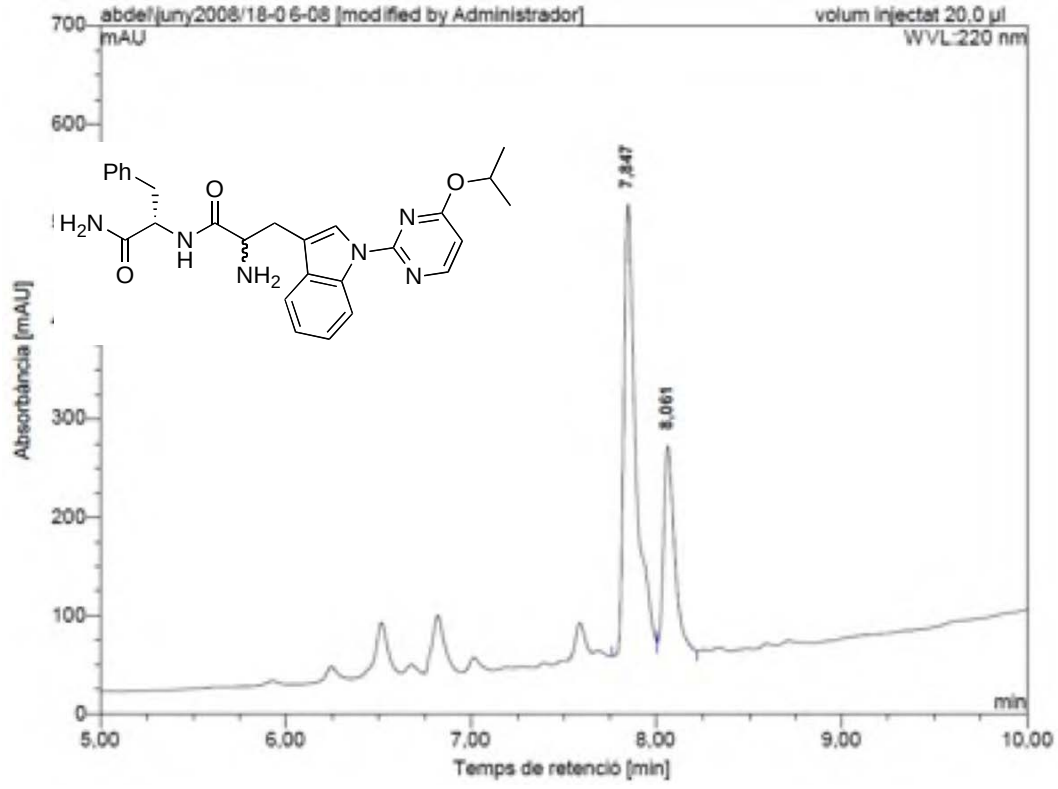
HPLC of dipeptide (I.32aa)
Reaction performed with K_2CO_3 at 50 ºC(entry 3, Table I.9)



HPLC of dipeptide (I.32ea)
Reaction described at Table I.12

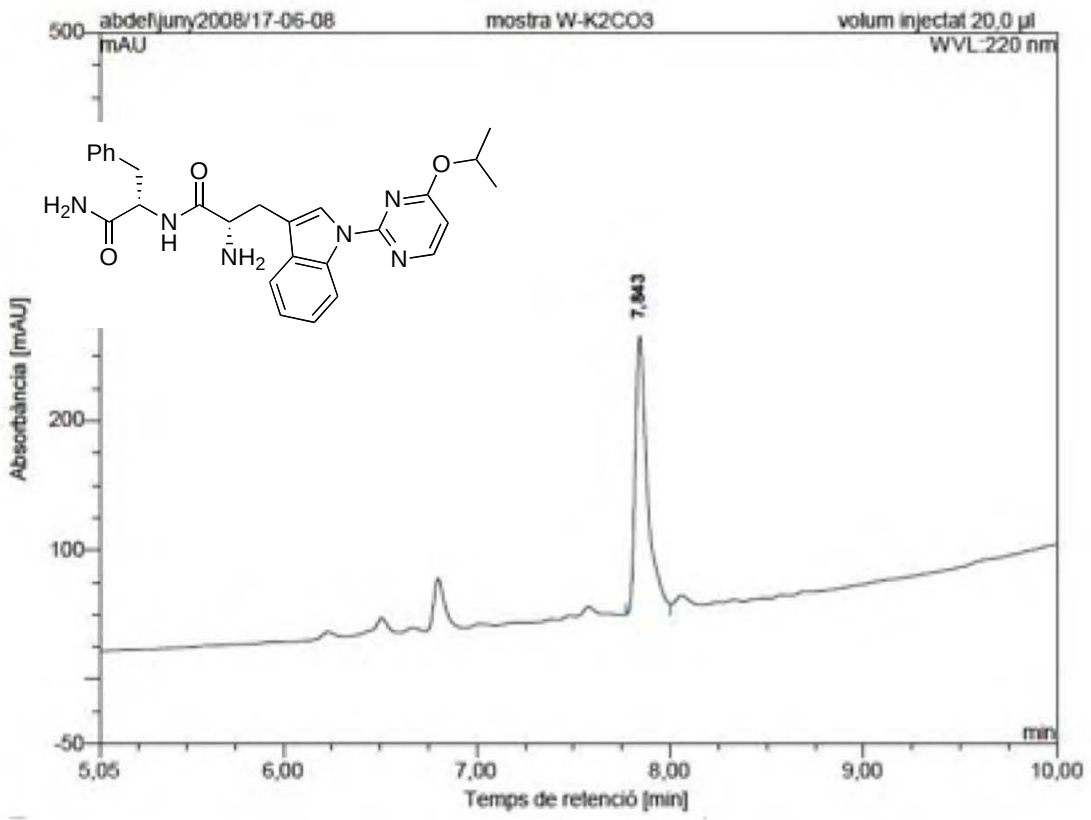


HPLC of dipeptide (I.32ea)
Reaction performed with K_2CO_3 at 50 ºC (entry 5, Table I.12)

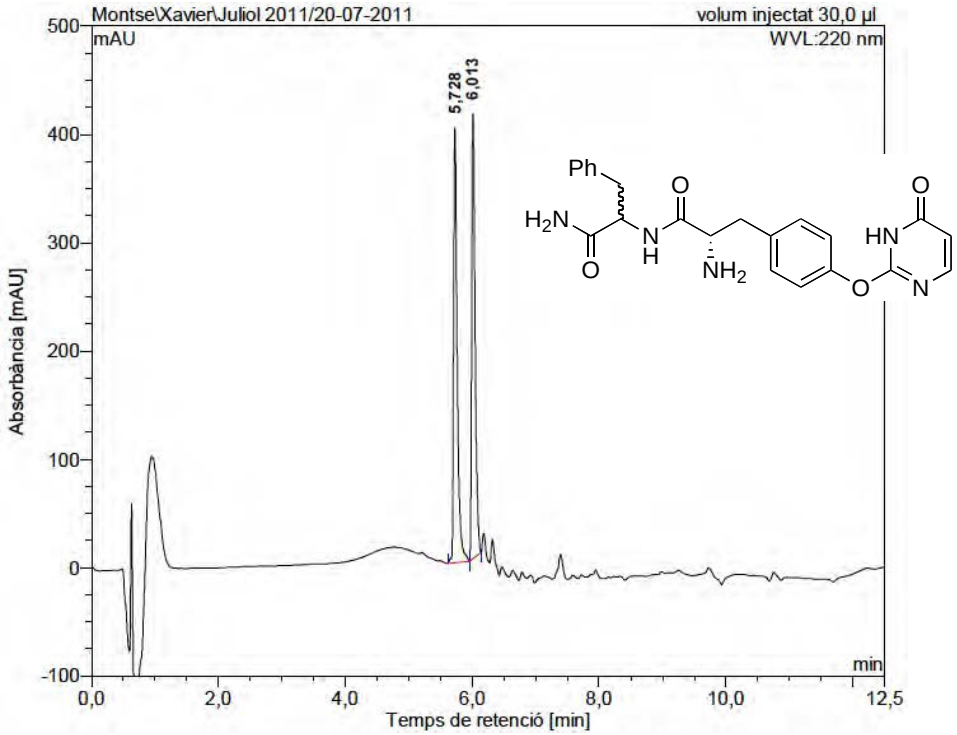


HPLC of dipeptide (I.32da)

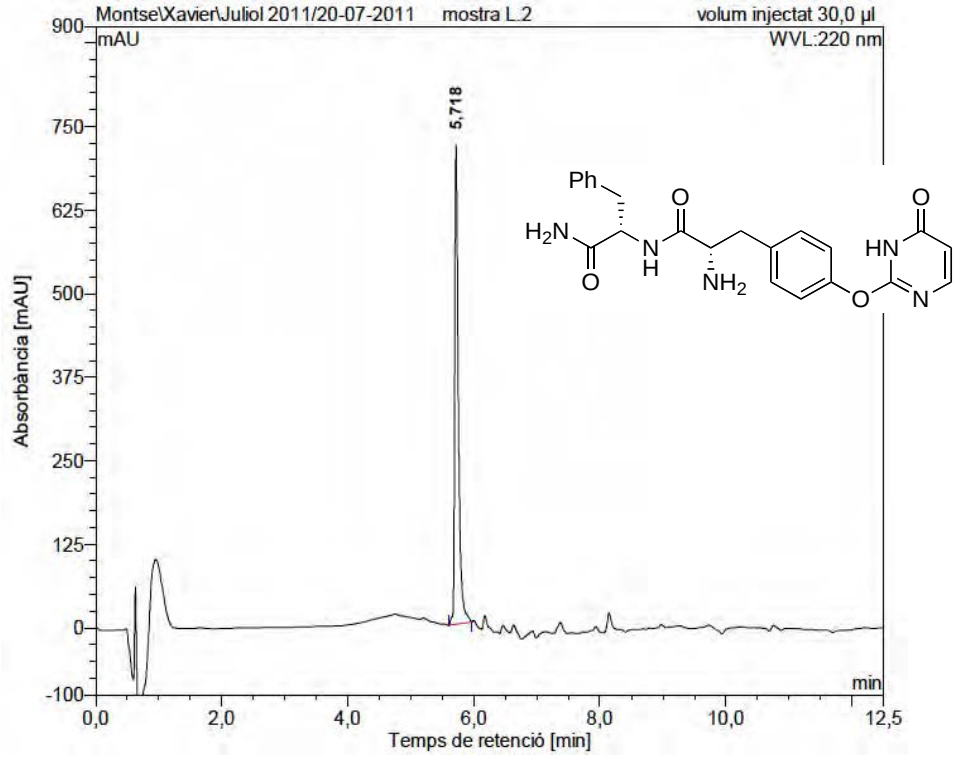
Reaction performed with K_2CO_3 at 40 ºC (entry 9, Table I.12)



HPLC of dipeptide (I.39a)
Reaction described at Table I.13

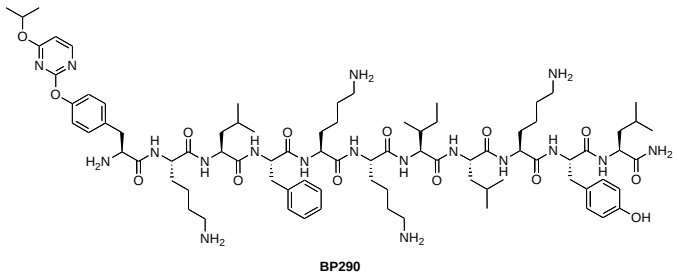


HPLC of dipeptide (I.39a)
Reaction performed with K_2CO_3 at 50 ºC (entry 1, Table I.13)



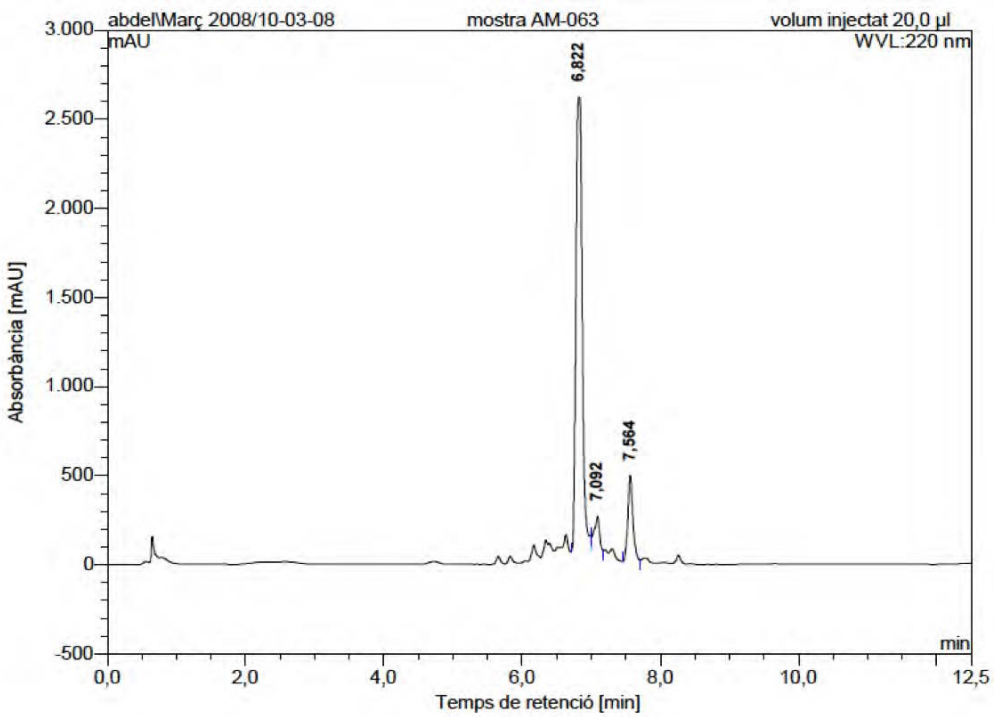
A.II.2. LIBRARY OF BP290-BP303

H-Tyr(Py)-K-L-F-K-K-I-L-K-Y-L-NH_2 (BP290)

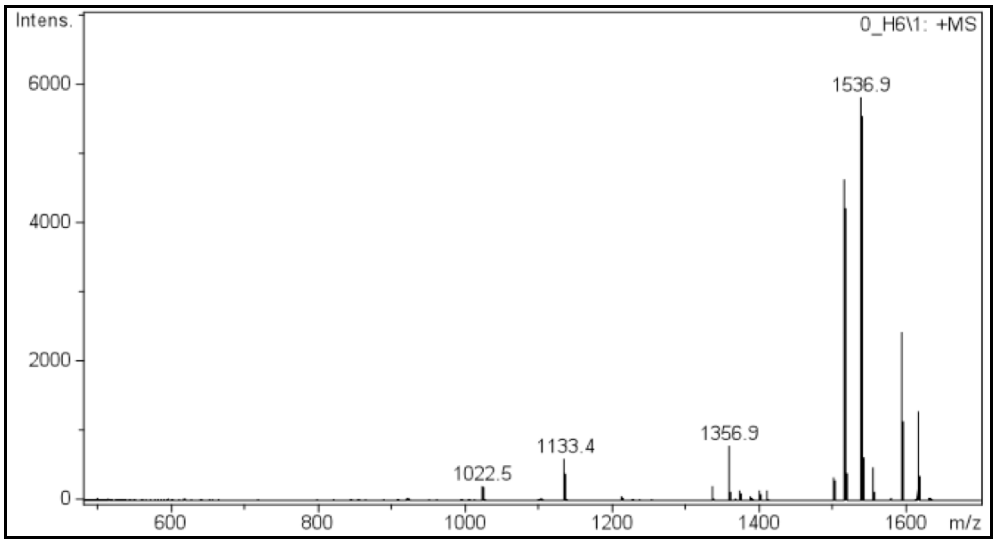


BP290

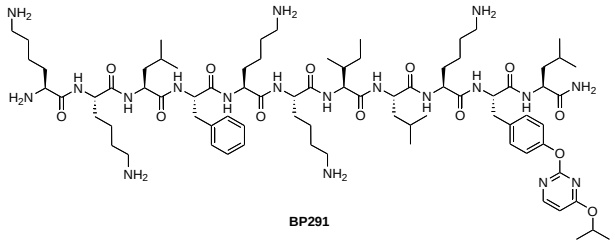
HPLC (λ = 220 nm)



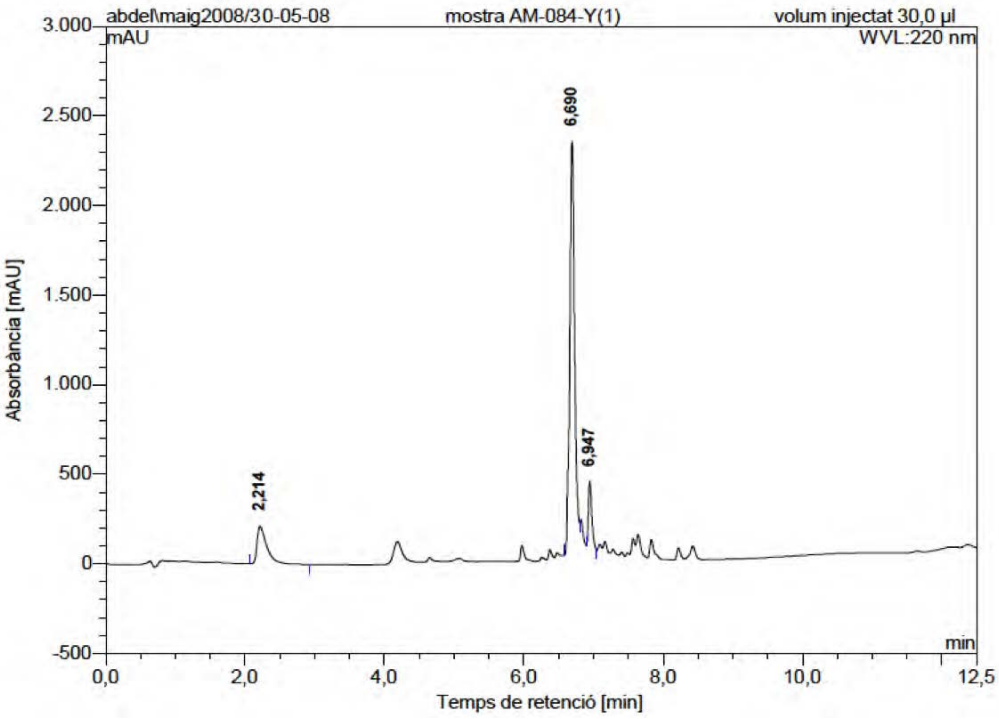
MS (MALDI-TOF)



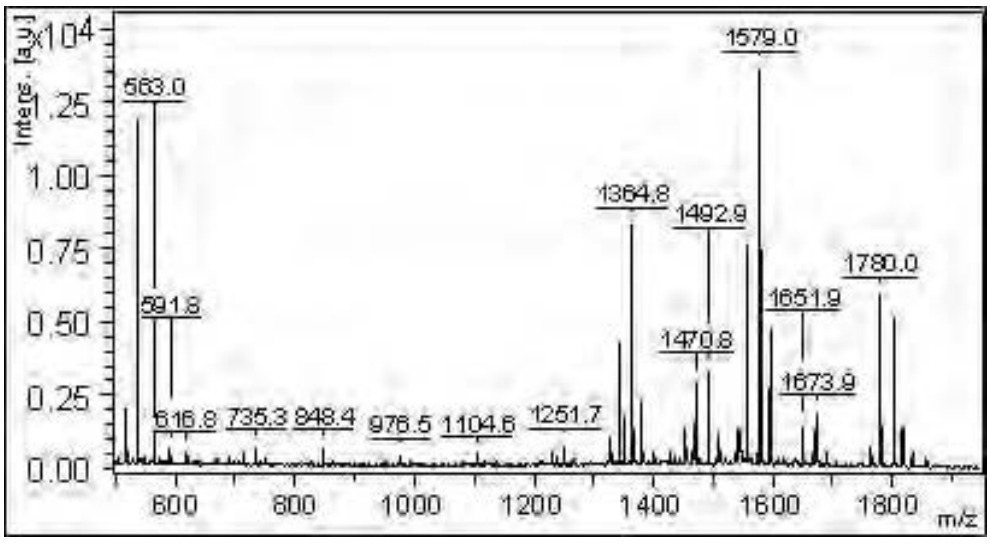
H-K-K-L-F-K-K-I-L-K-Tyr(Py)-L-NH_2 (BP291)



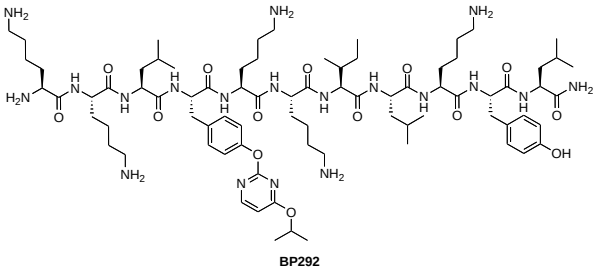
HPLC (λ = 220 nm)



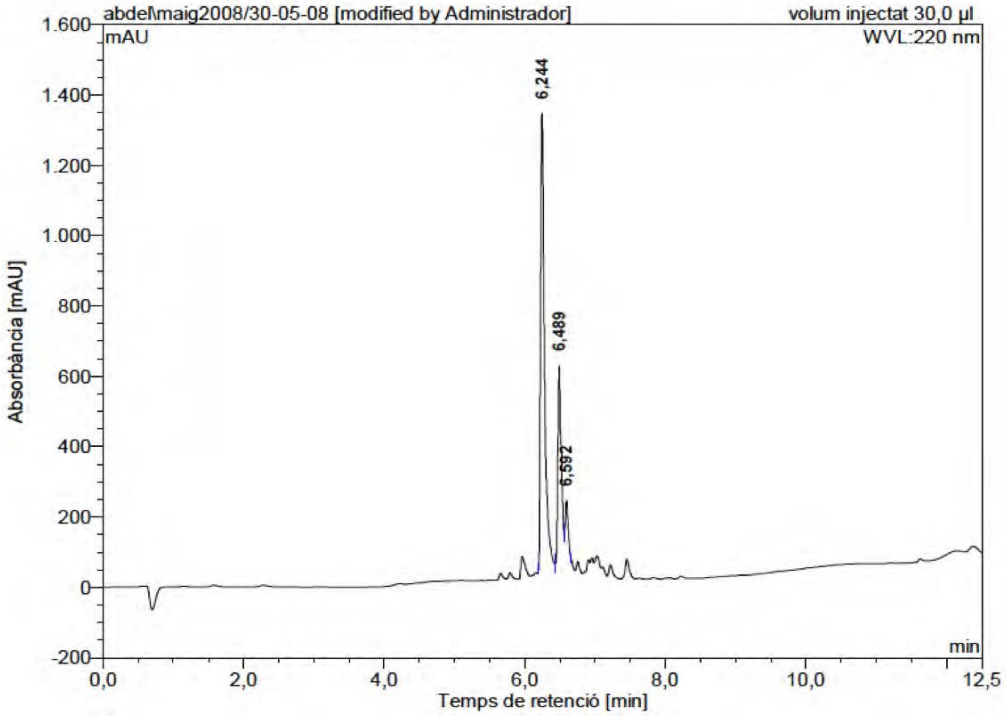
MS (MALDI-TOF)



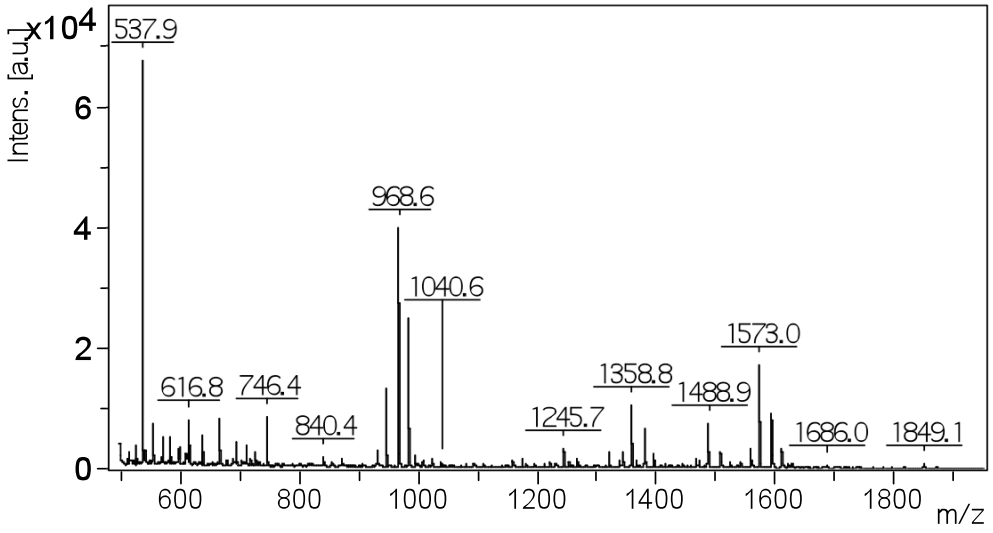
H-K-K-L-Tyr(Py)-K-K-I-L-K-Y-L-NH_2 (BP292)



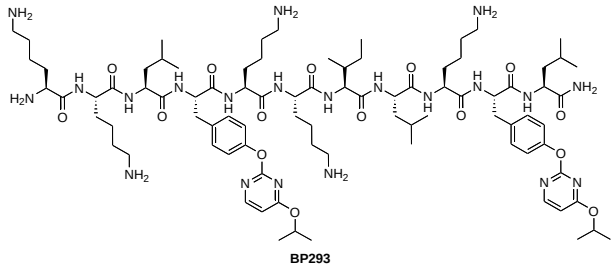
HPLC (λ = 220 nm)



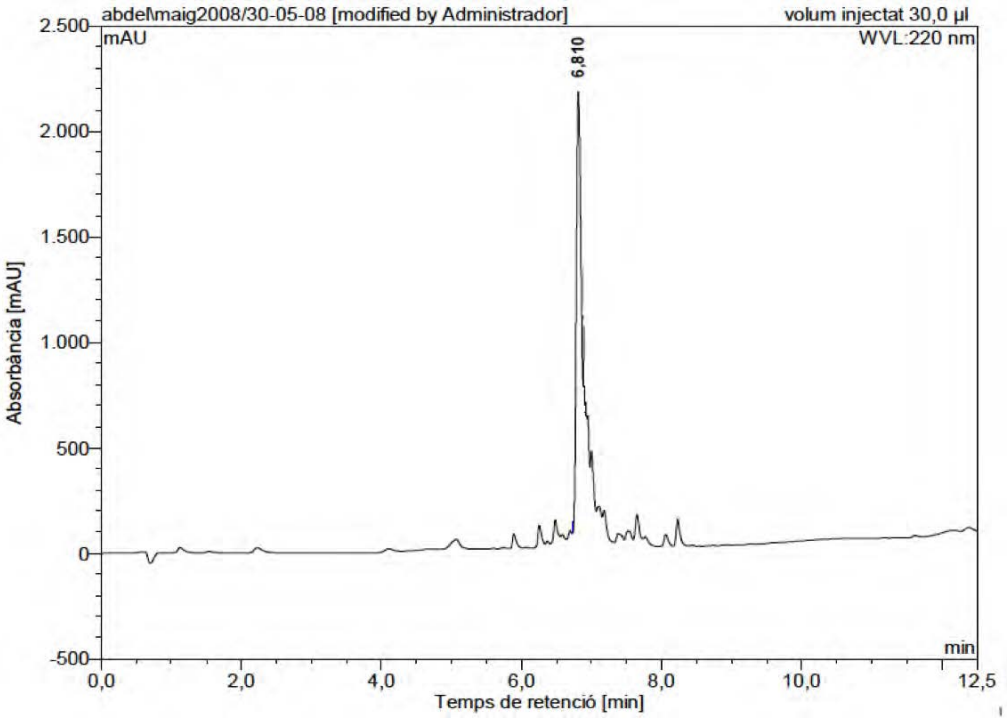
MS (MALDI-TOF)



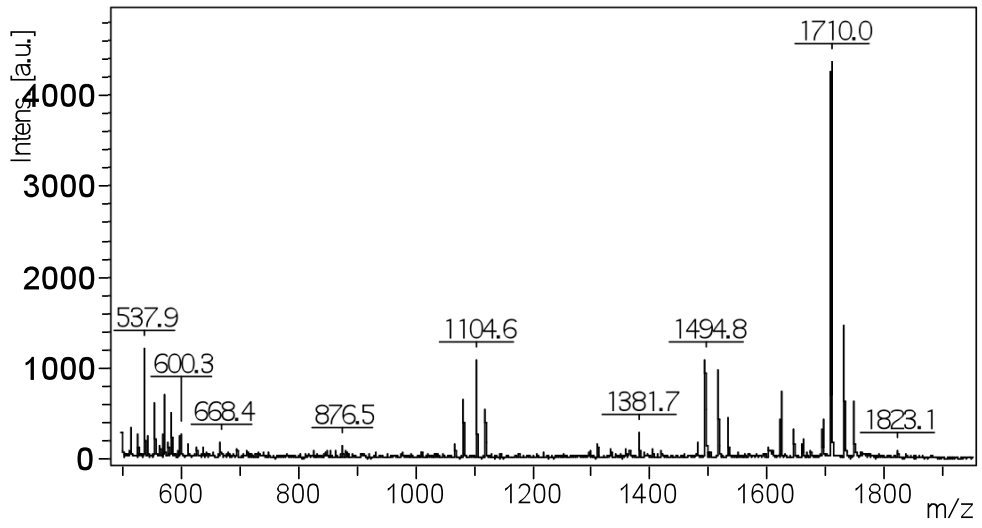
H-K-K-L-Tyr(Py)-K-K-I-L-K-Tyr(Py)-L-NH_2 (BP293)



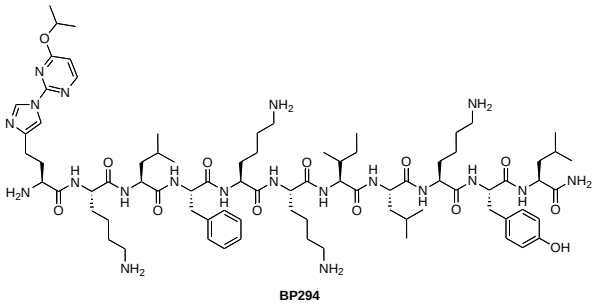
HPLC (λ = 220 nm)



MS (MALDI-TOF)

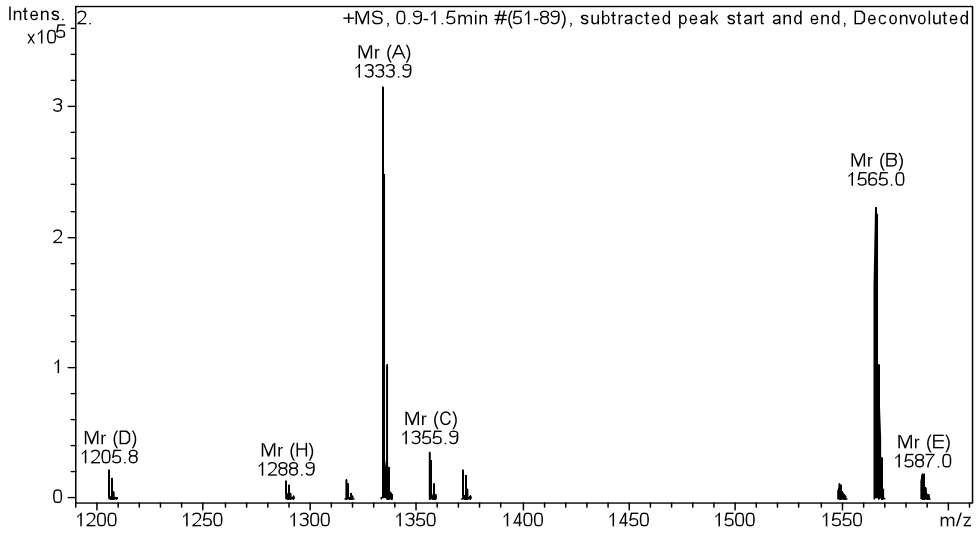


H-His(Py)-K-L-F-K-K-I-L-K-Y-L-NH_2 (BP294)

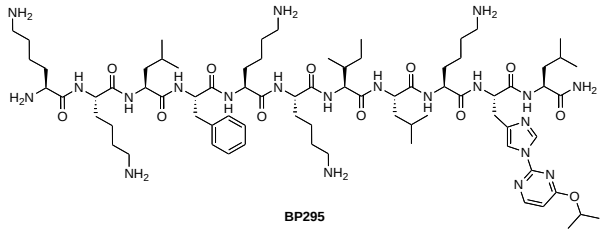


HPLC (λ = 220 nm): t_R = 6.85 min (73%).

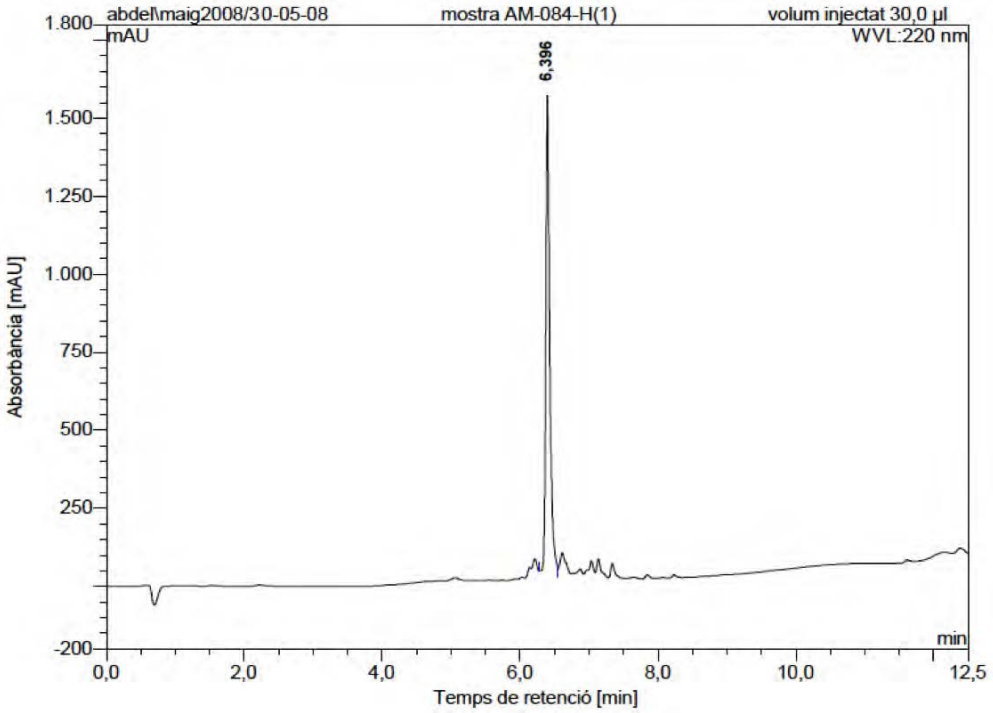
MS (ESI+)



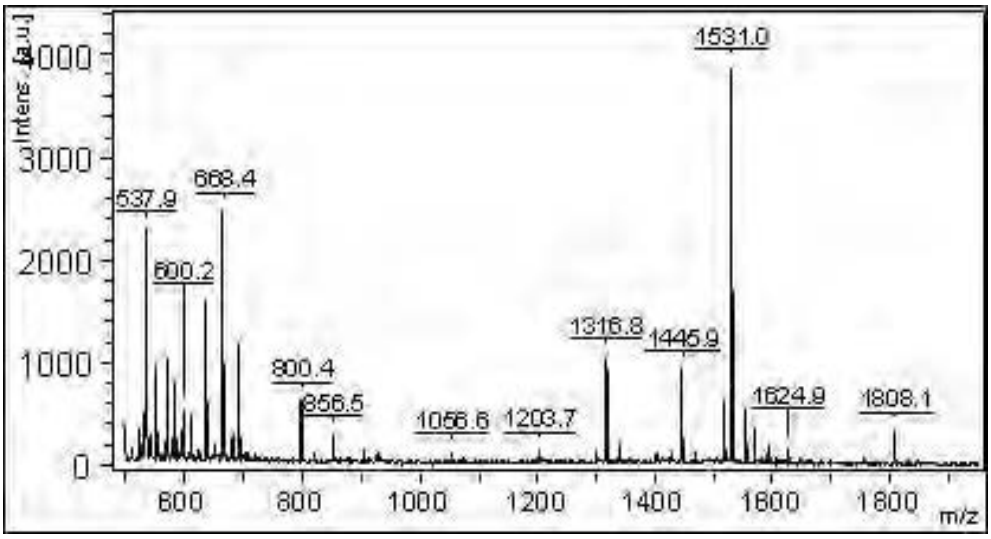
H-K-K-L-F-K-K-I-L-K-His(Py)-L-NH_2 (BP295)



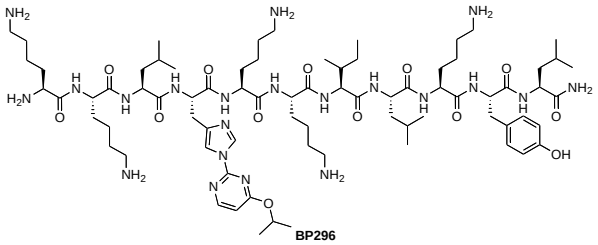
HPLC (λ = 220 nm)



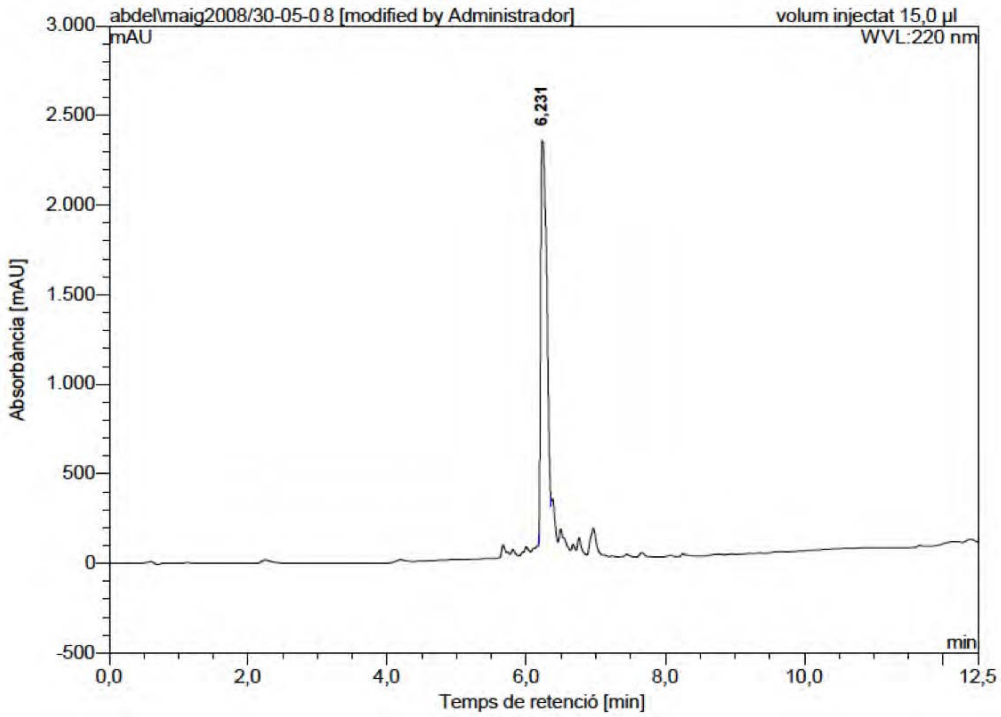
MS (ESI+)



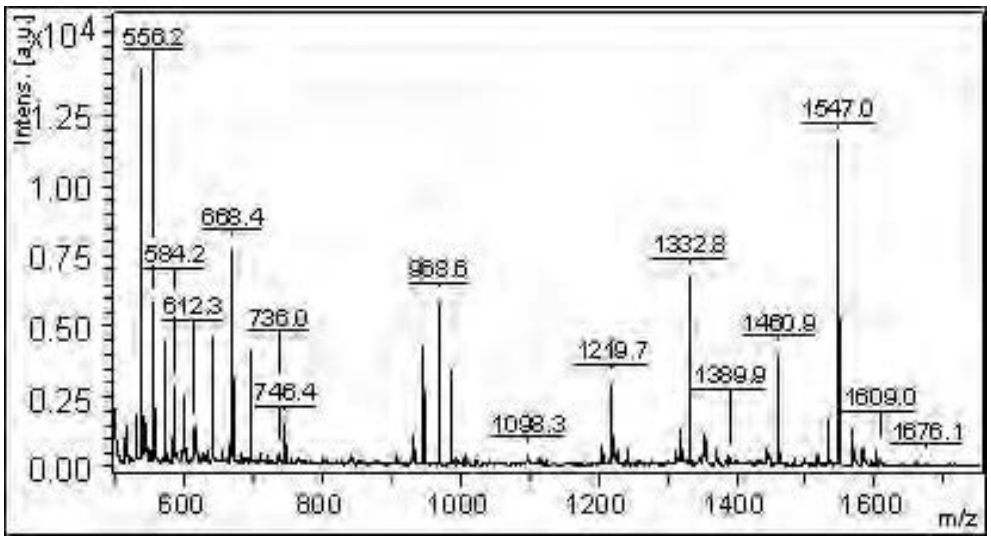
H-K-K-L-His(Py)-K-K-I-L-K-Y-L-NH_2 (BP296)



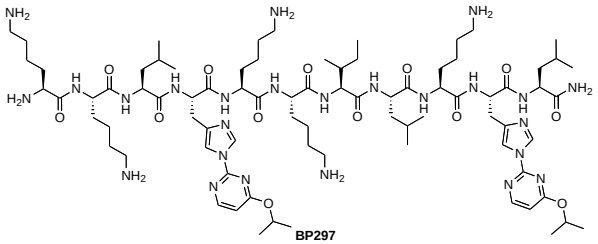
HPLC (λ = 220 nm)



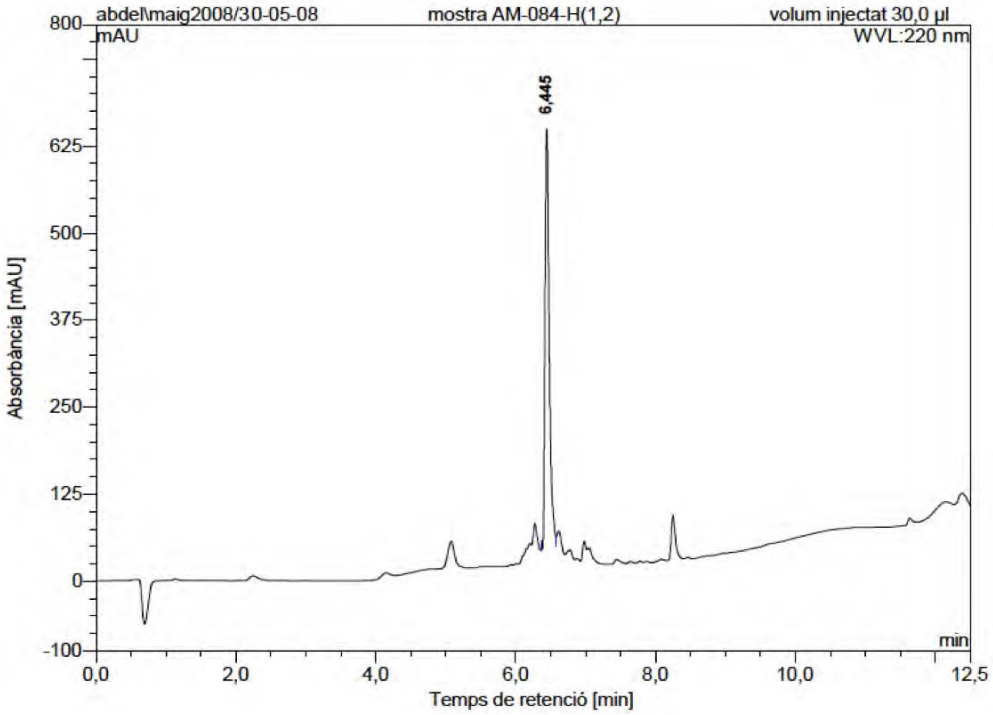
MS (ESI+)



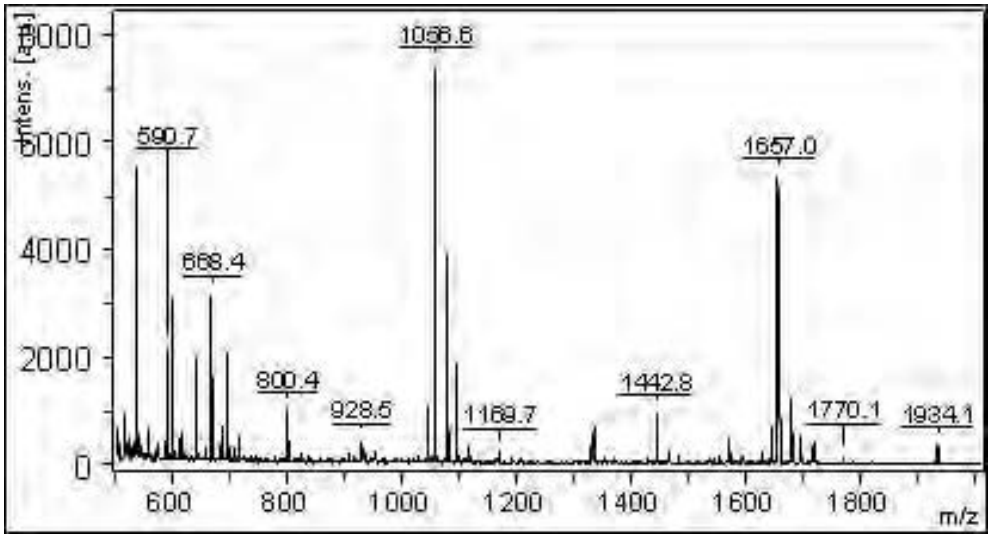
H-K-K-L-His(Py)-K-K-I-L-K-His(Py)-L-NH_2 (BP297)



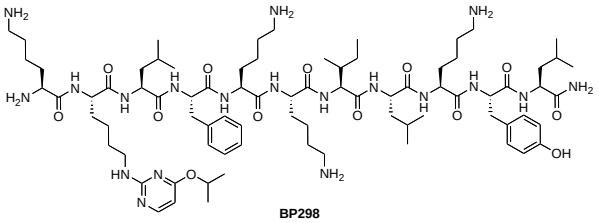
HPLC (λ = 220 nm)



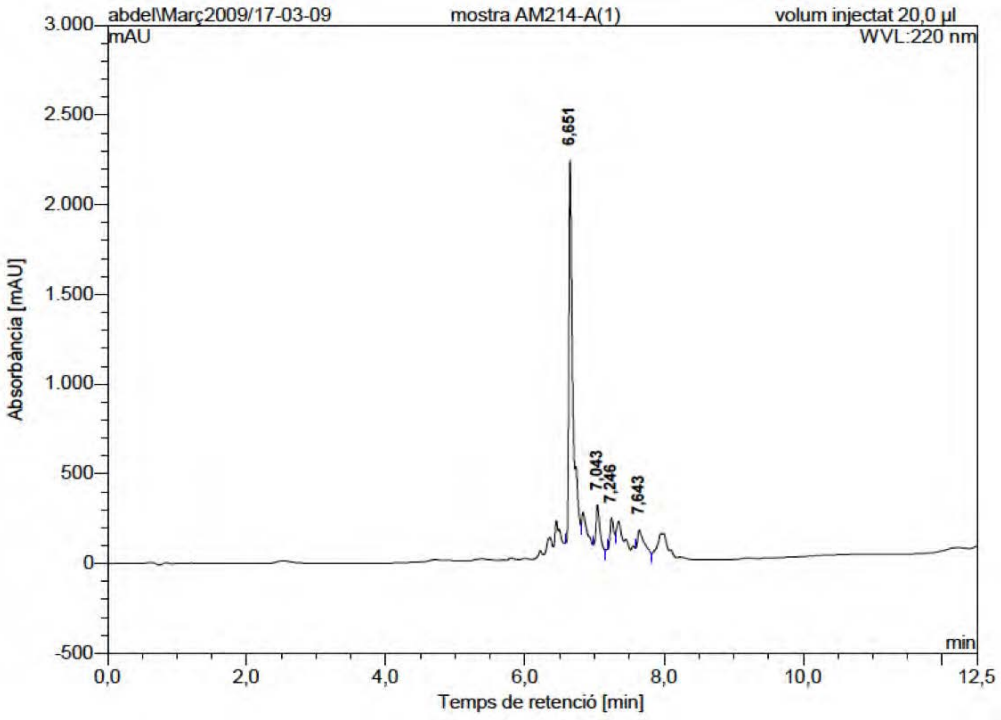
MS (ESI+)



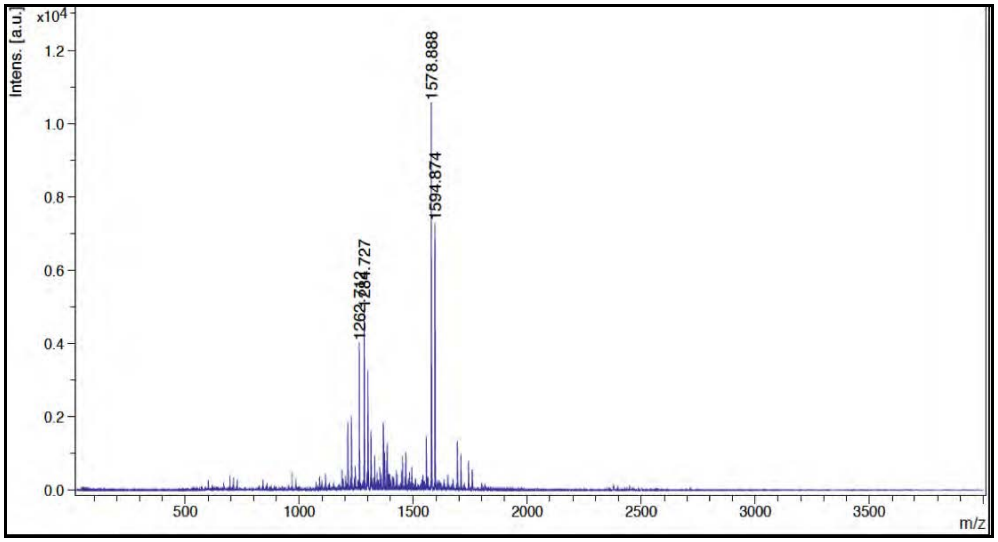
H-K-Lys(Py)-L-F-K-K-I-L-K-Y-L-NH_2 (BP298)



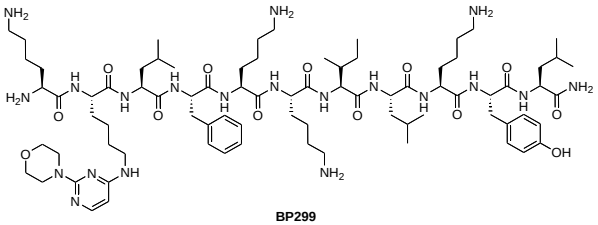
HPLC (λ = 220 nm)



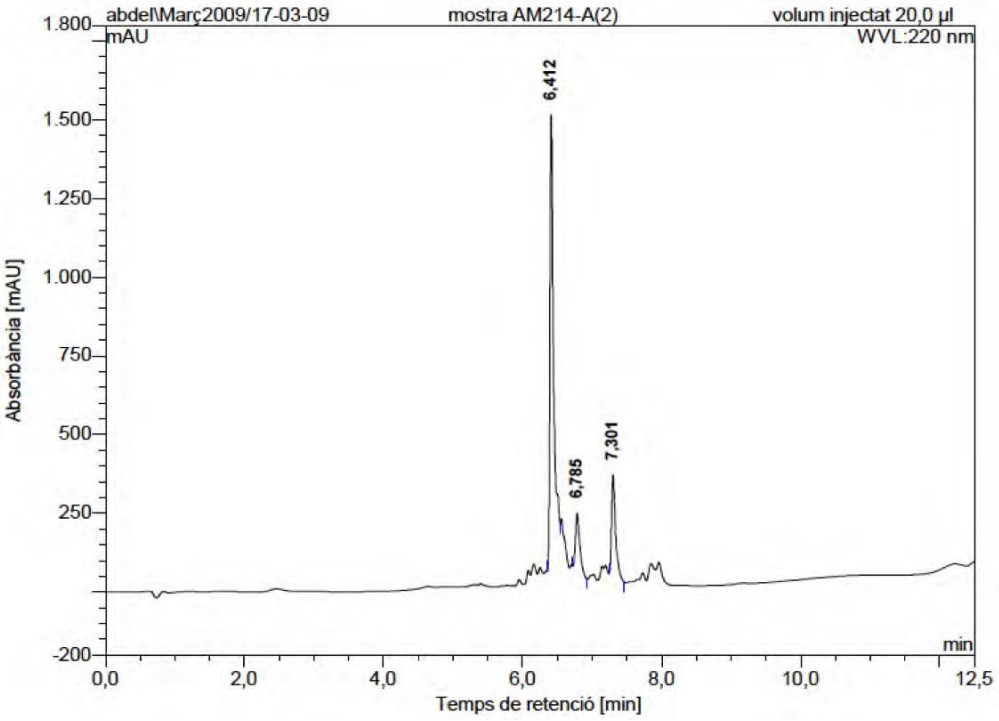
MS (MALDI-TOF)



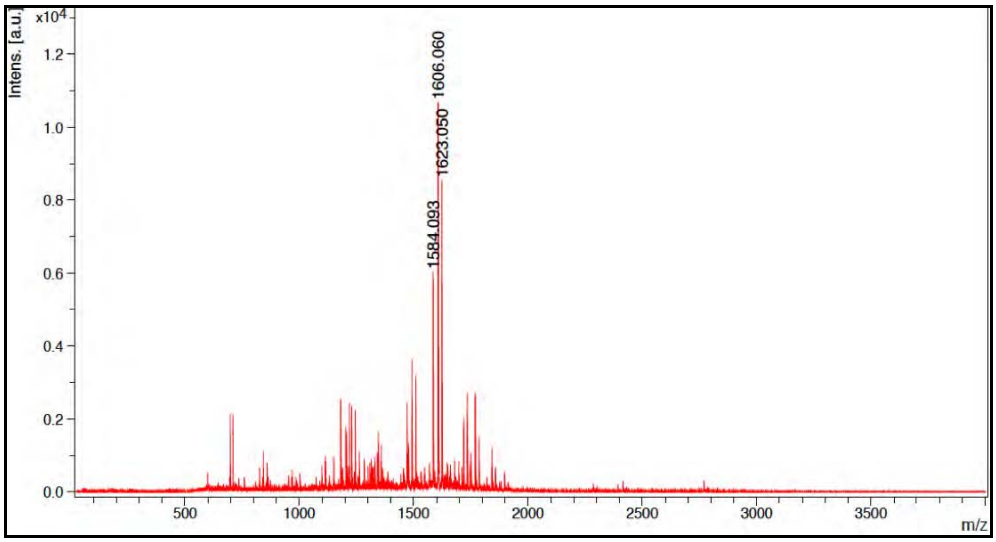
H-K-Lys(Mor)-L-F-K-K-I-L-K-Y-L-NH_2 (BP299)



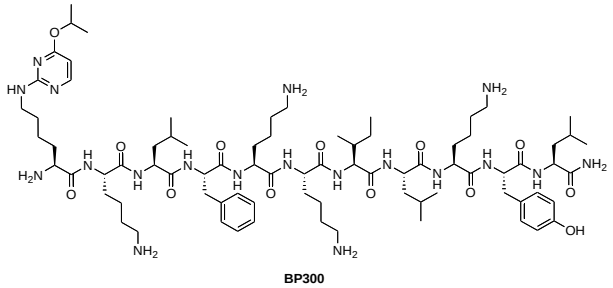
HPLC (λ = 220 nm)



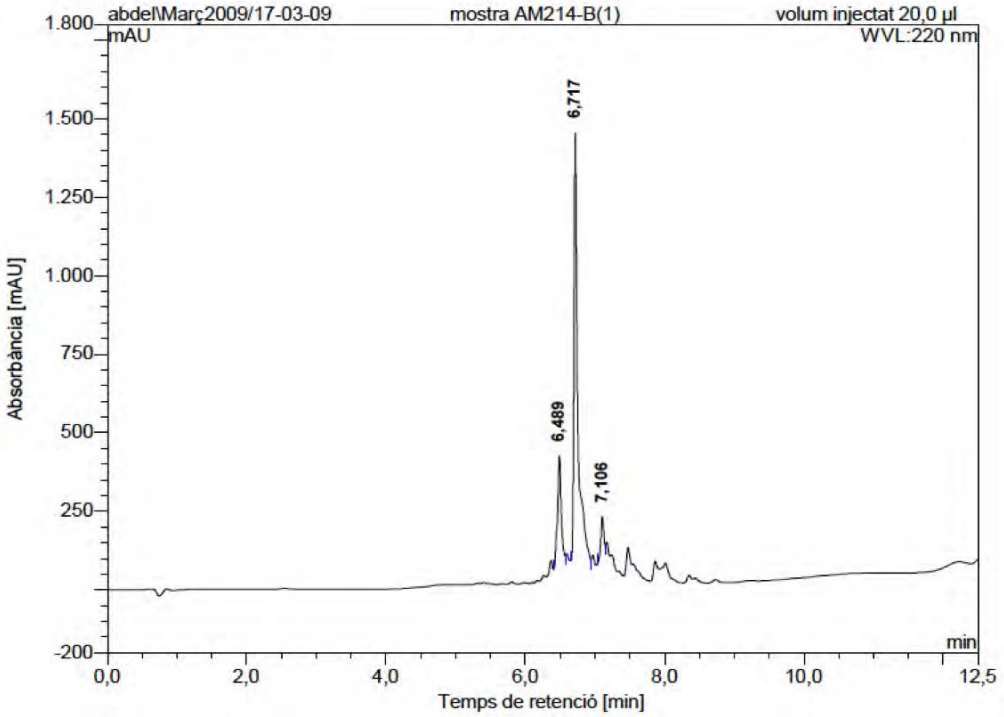
MS (MALDI-TOF)



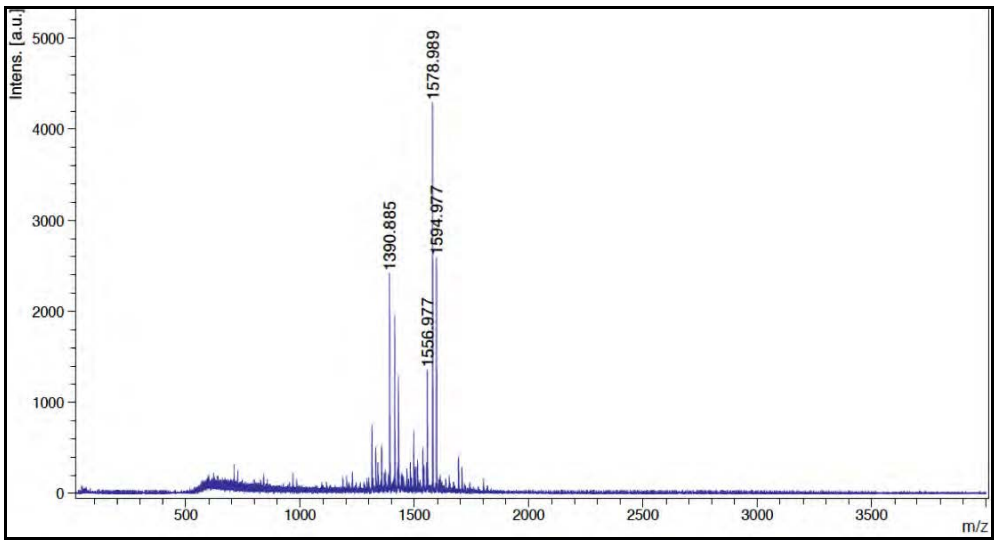
H-Lys(Py)-K-L-F-K-K-I-L-K-Y-L-NH_2 (BP300)



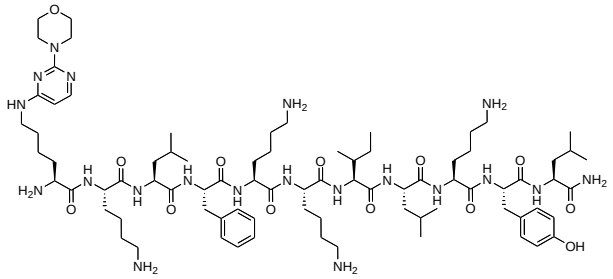
HPLC (λ = 220 nm)



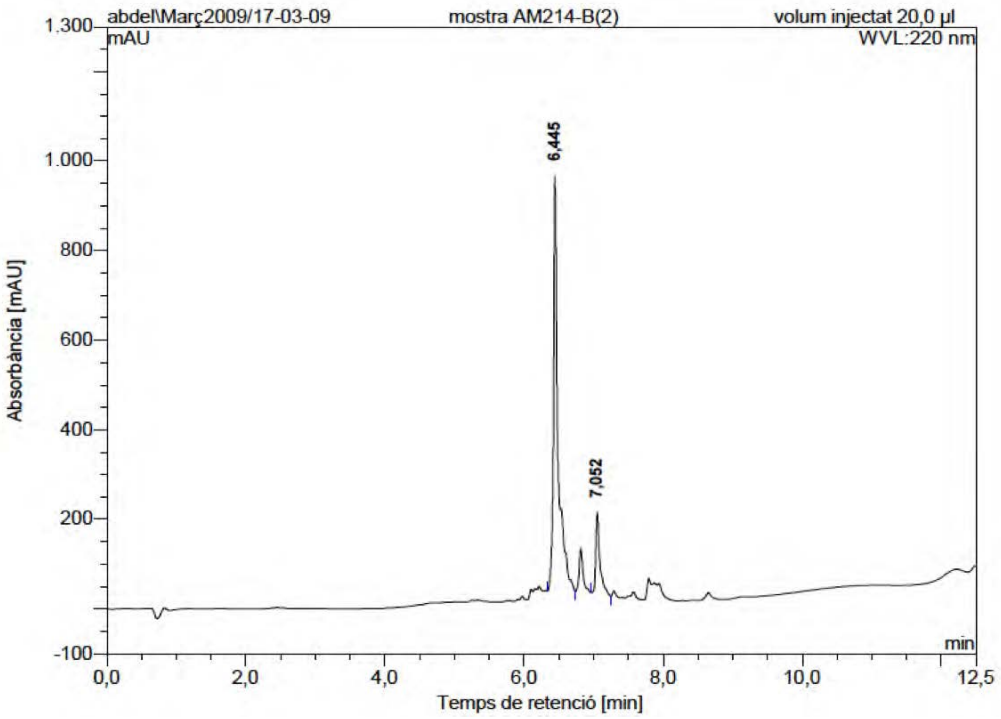
MS (MALDI-TOF)



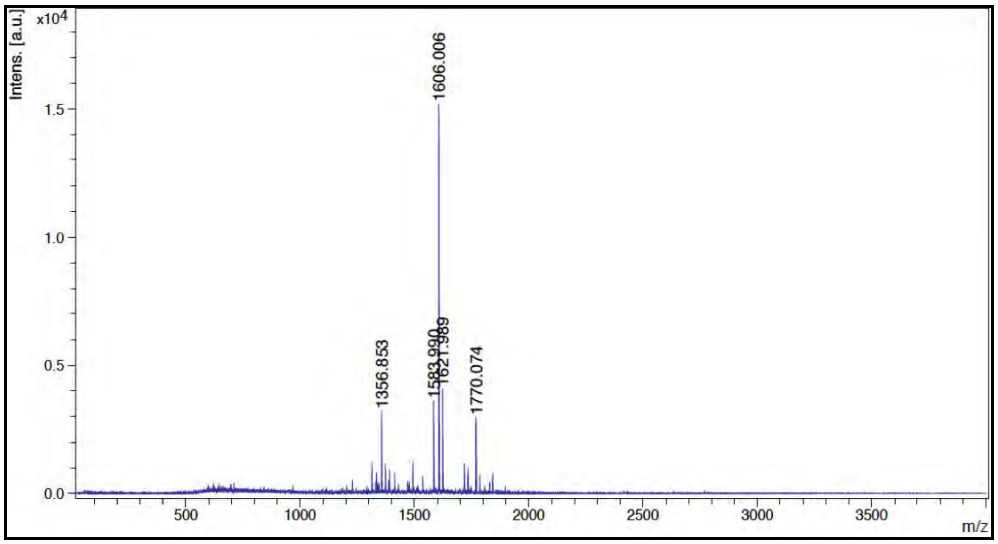
H-Lys(Mor)-K-L-F-K-K-I-L-K-Y-L-NH_2 (BP301)



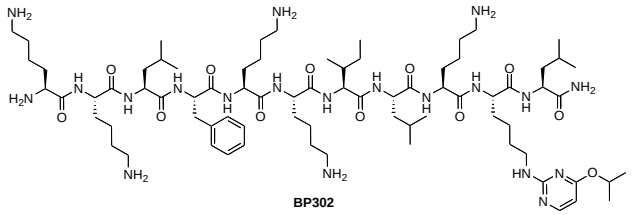
HPLC (λ = 220 nm)



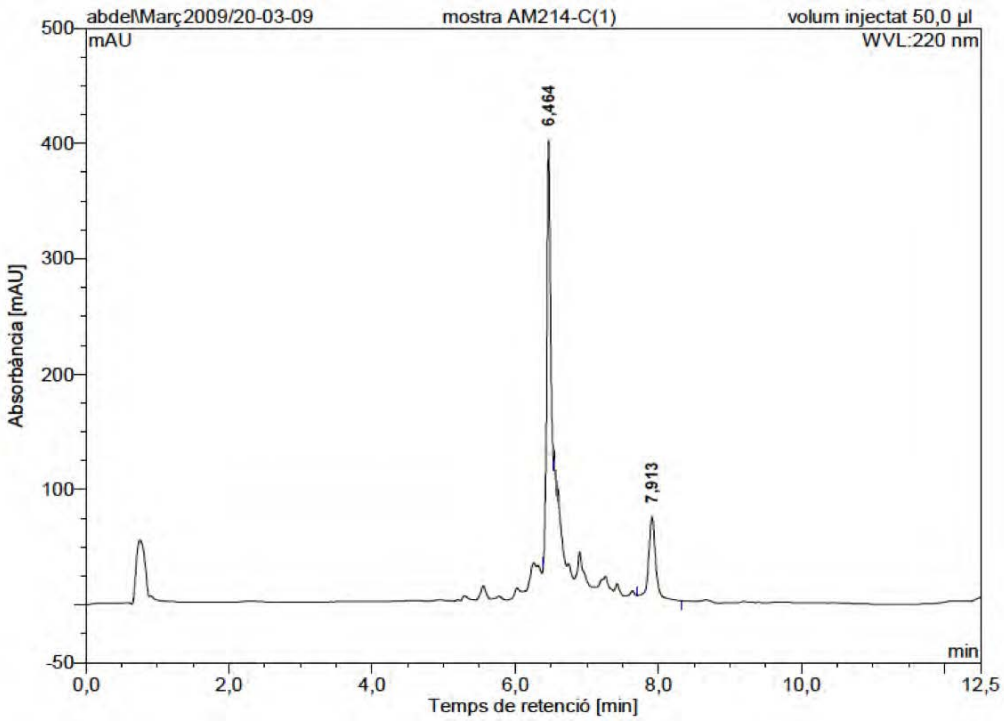
MS (MALDI-TOF)



H-K-K-L-F-K-K-I-L-K-Lys(Py)-L-NH_2 (BP302)

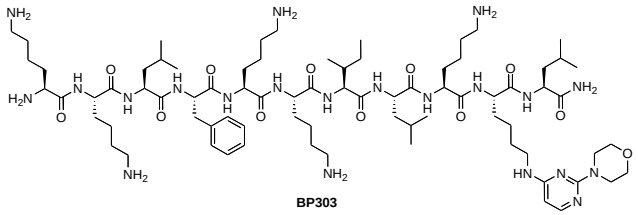


HPLC (λ = 220 nm)

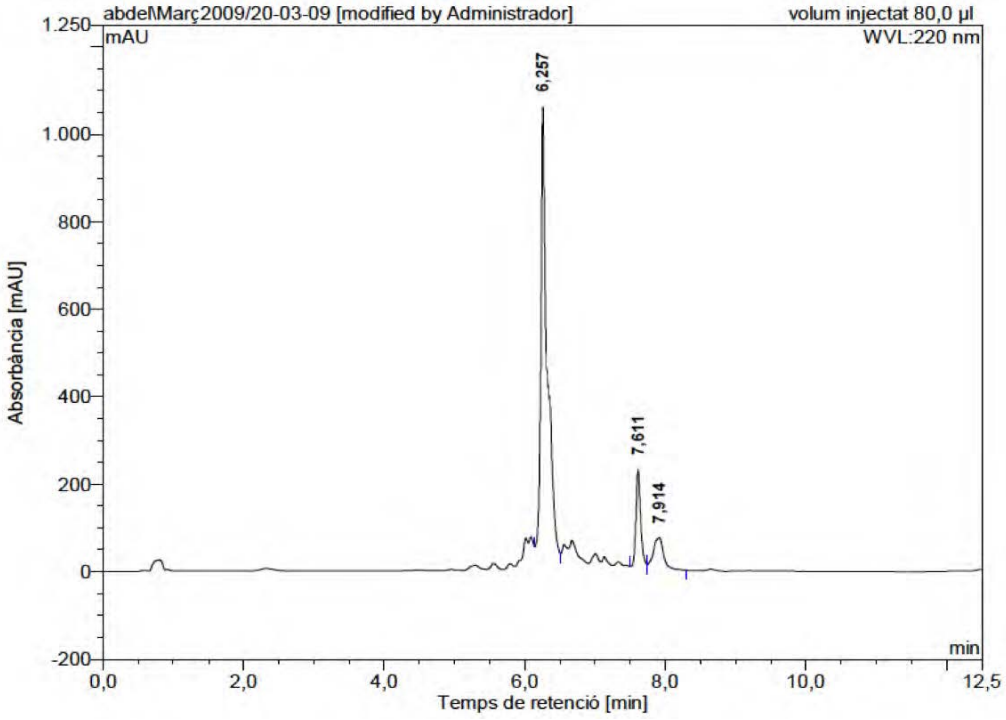


MS (ESI+) *m/z*: 1523.20 $[M+H]^+$, 761.6 $[M+2H]^{2+}$.

H-K-K-L-F-K-K-I-L-K-Lys(Mor)-L-NH_2 (BP303)



HPLC (λ = 220 nm)

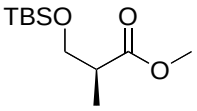


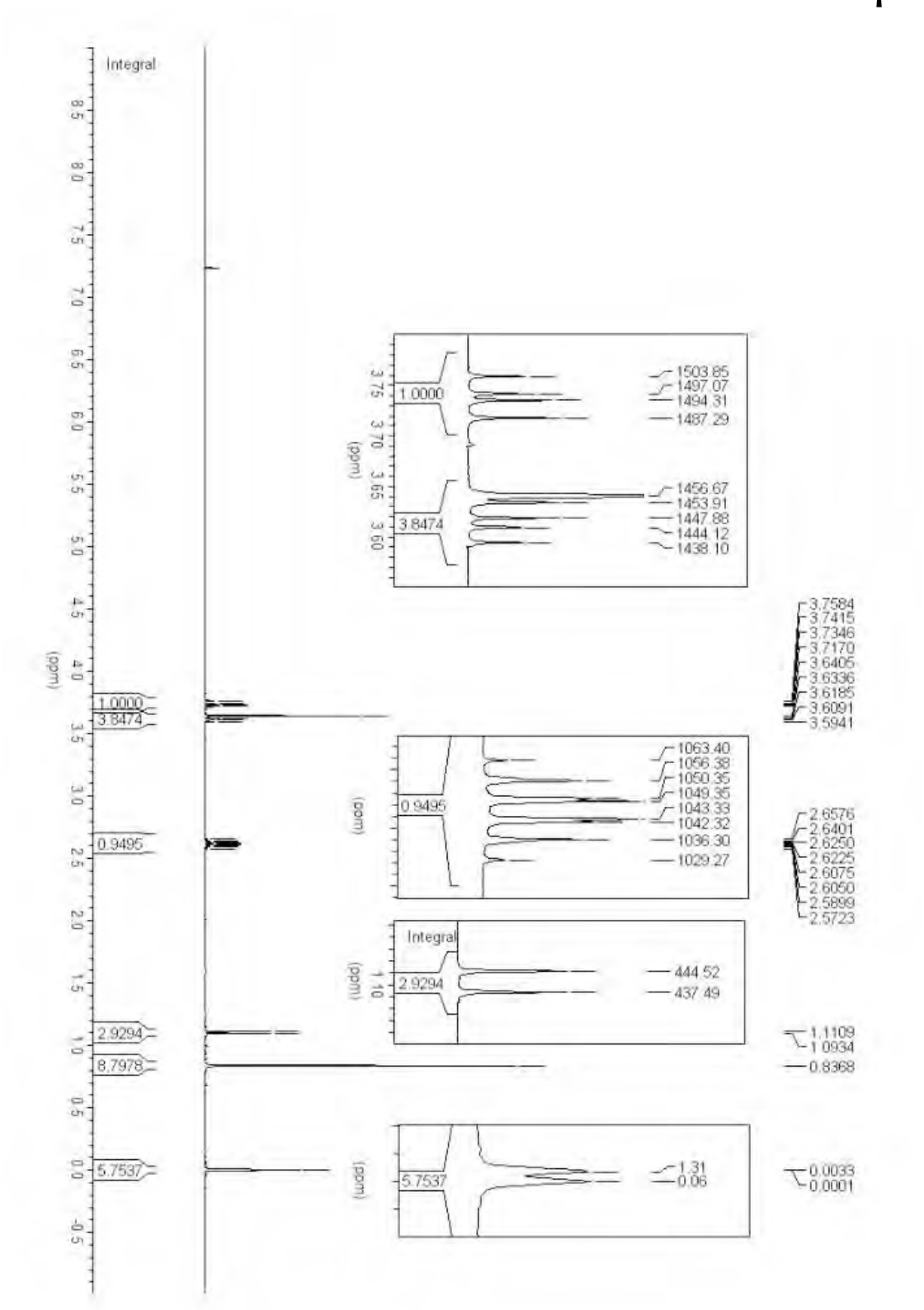
MS (ESI+) *m/z*: 1549.20 $[M+H]^+$, 775.5 $[M+2H]^{2+}$.

ANNEX III

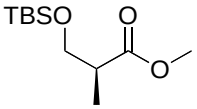
Copies of ^{1}H NMR & ^{13}C NMR

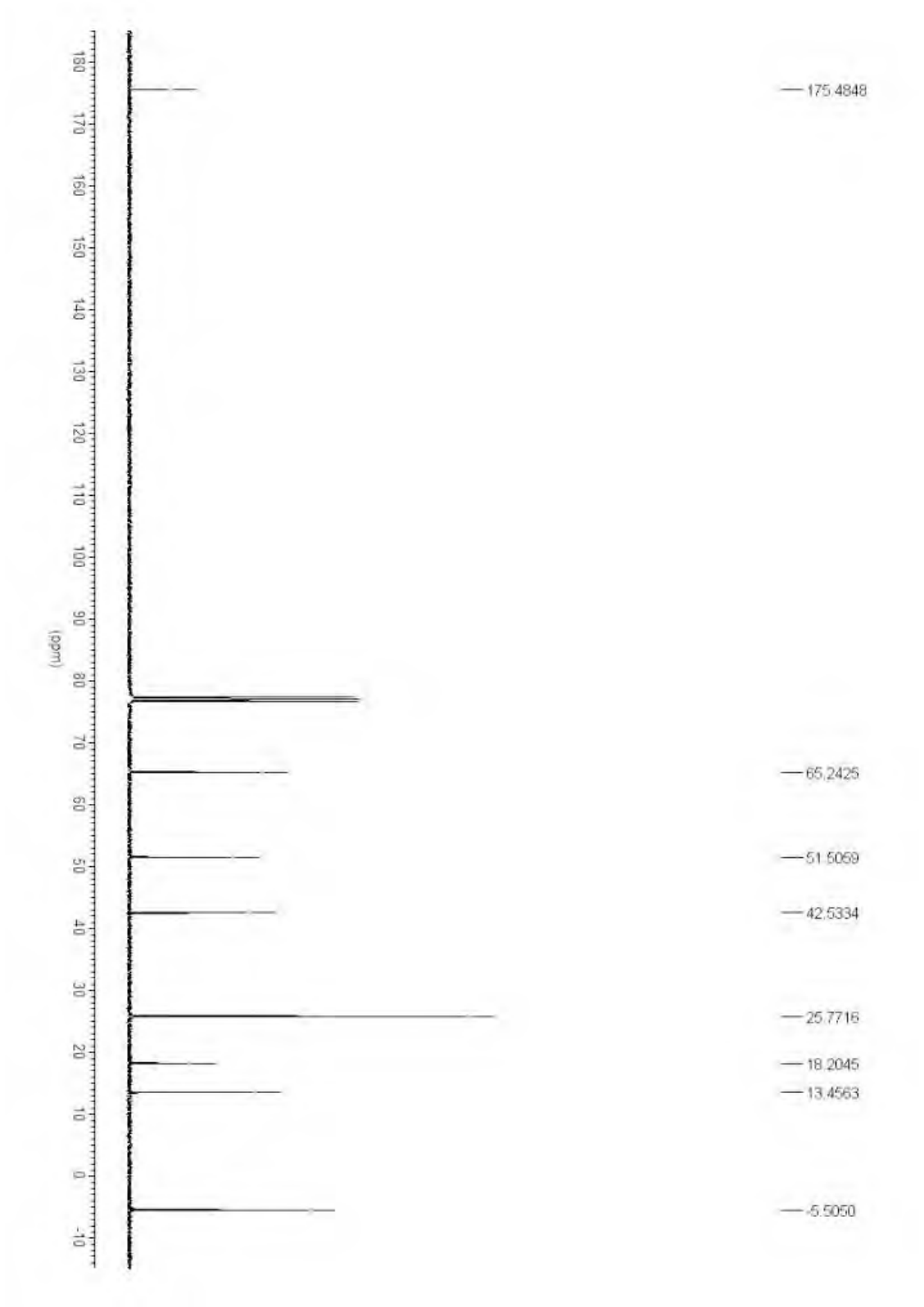
(*S*)-3-(*tert*-Butyldimethylsilanyloxy)-2-methylpropionic acid methyl ester



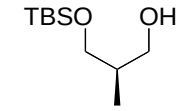


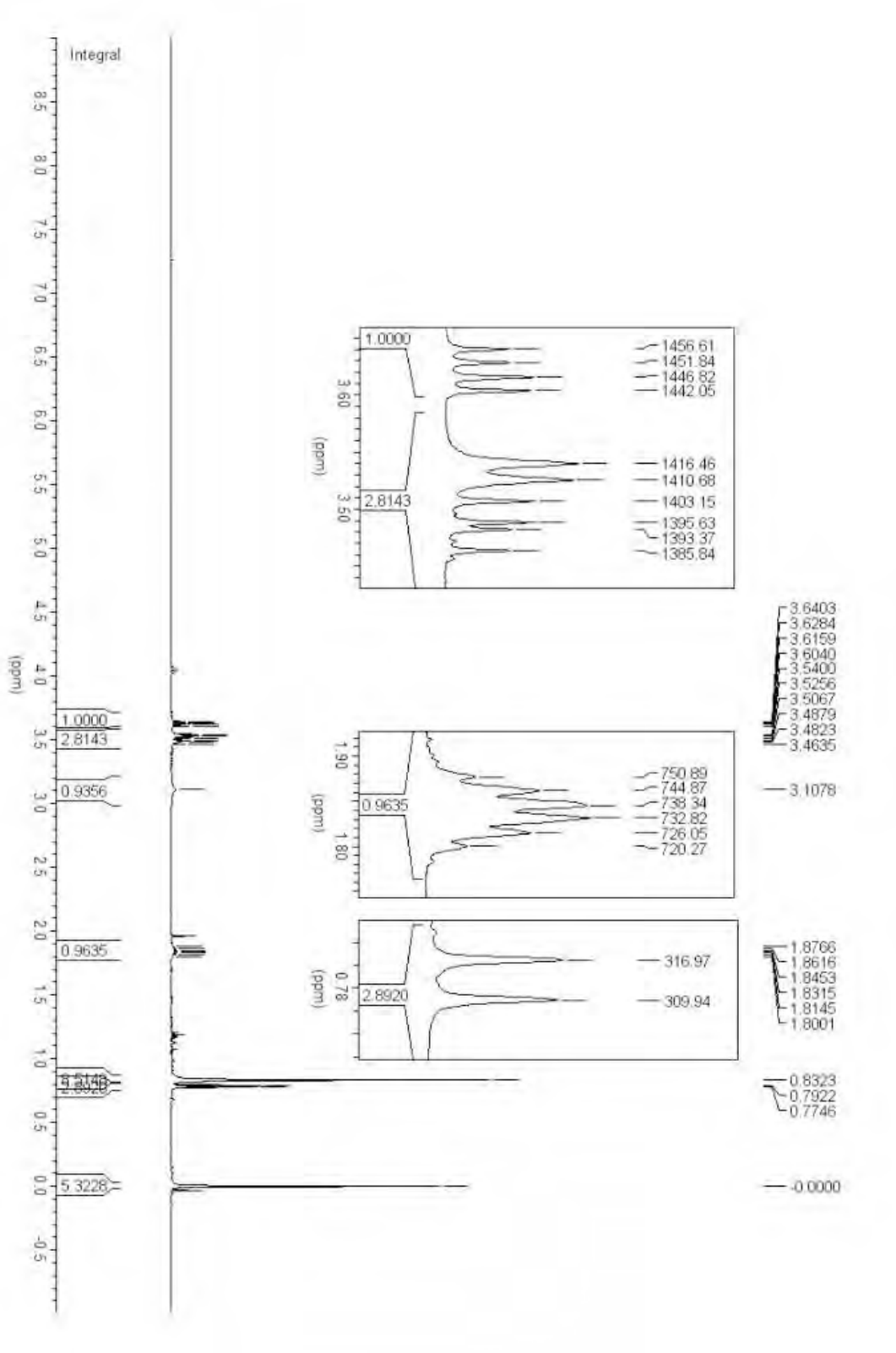
(*S*)-3-(*tert*-Butyldimethylsilanyloxy)-2-methylpropionic acid methyl ester



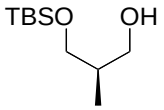


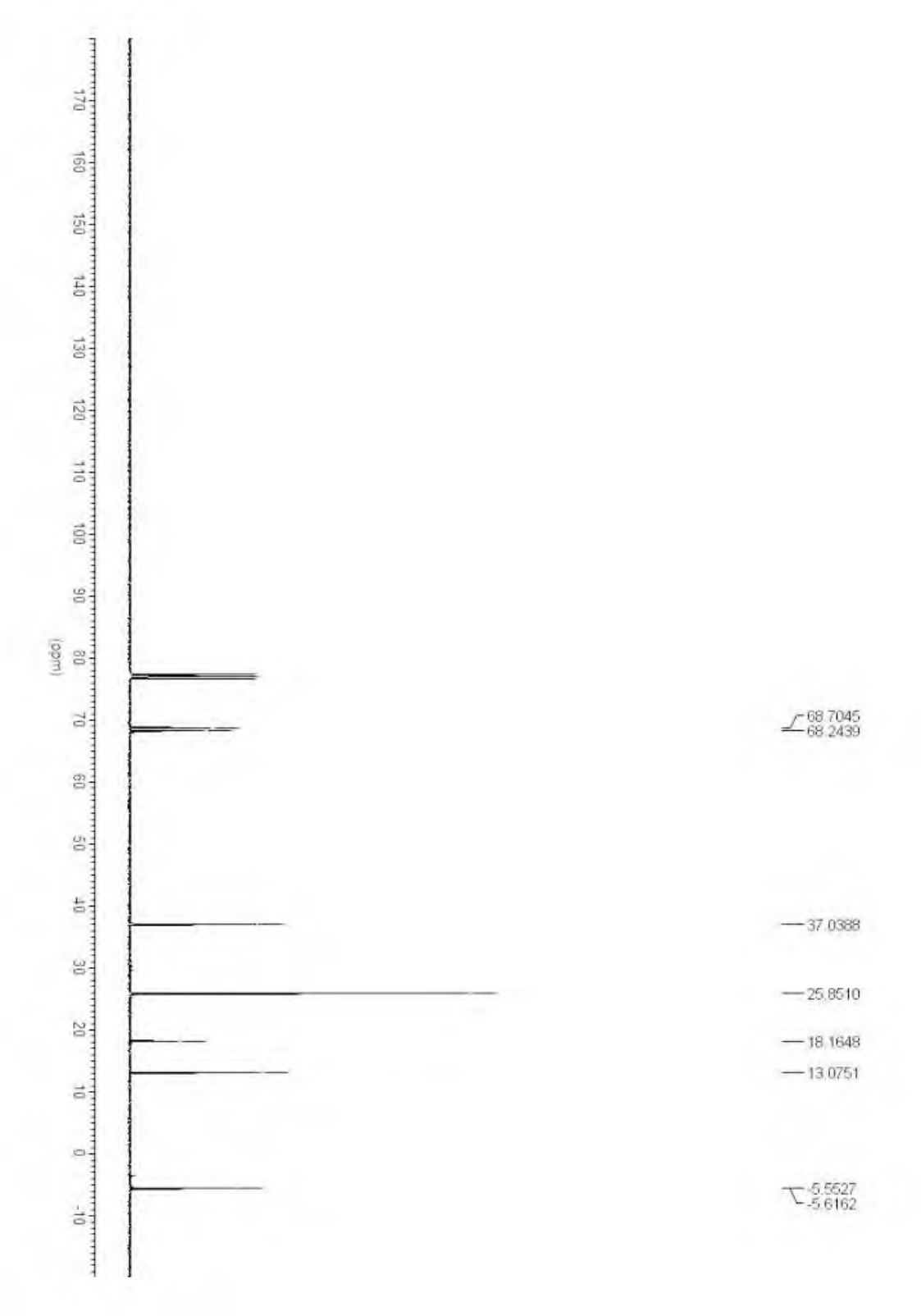
(*R*)-3-(*tert*-Butyldimethylsilanyloxy)-2-methylpropan-1-ol (II.3)



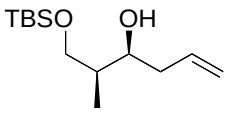


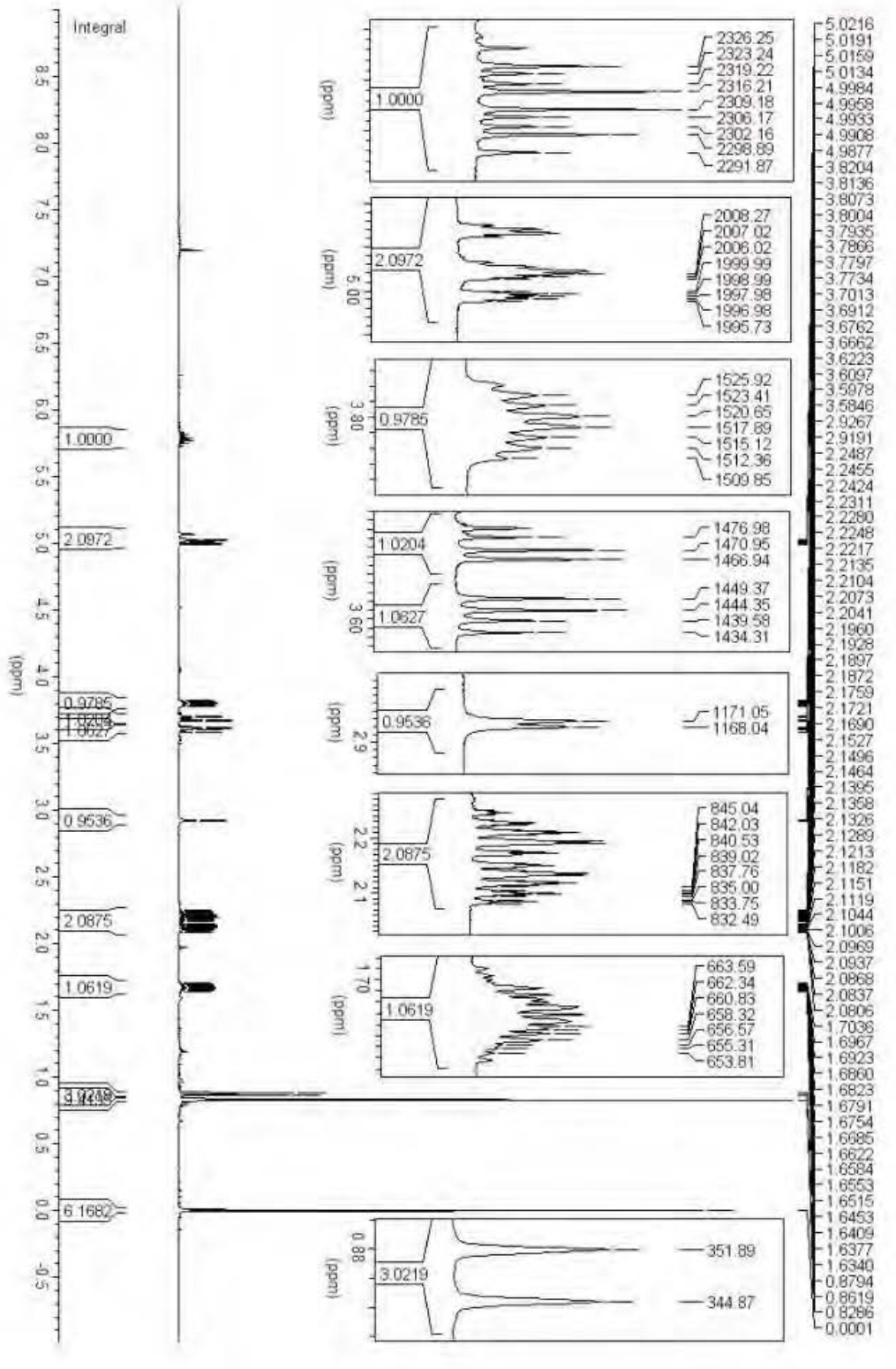
(*R*)-3-(*tert*-Butyldimethylsilanyloxy)-2-methylpropan-1-ol (II.3)



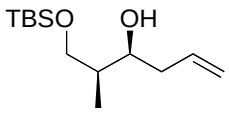


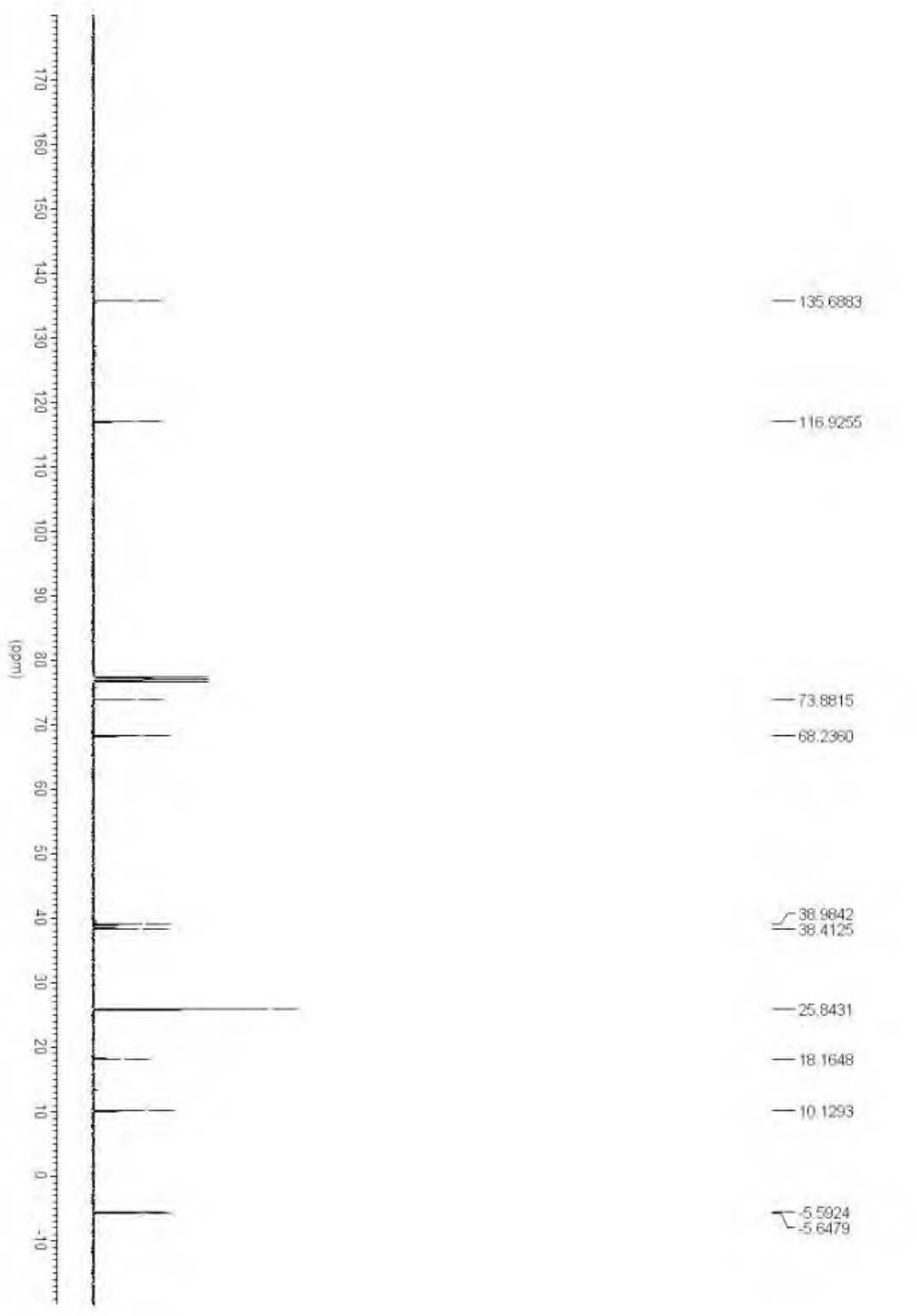
(2*S*,3*S*)-1-(*tert*-Butyldimethylsilanyloxy)-2-methylhex-5-en-3-ol (II.5)



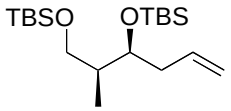


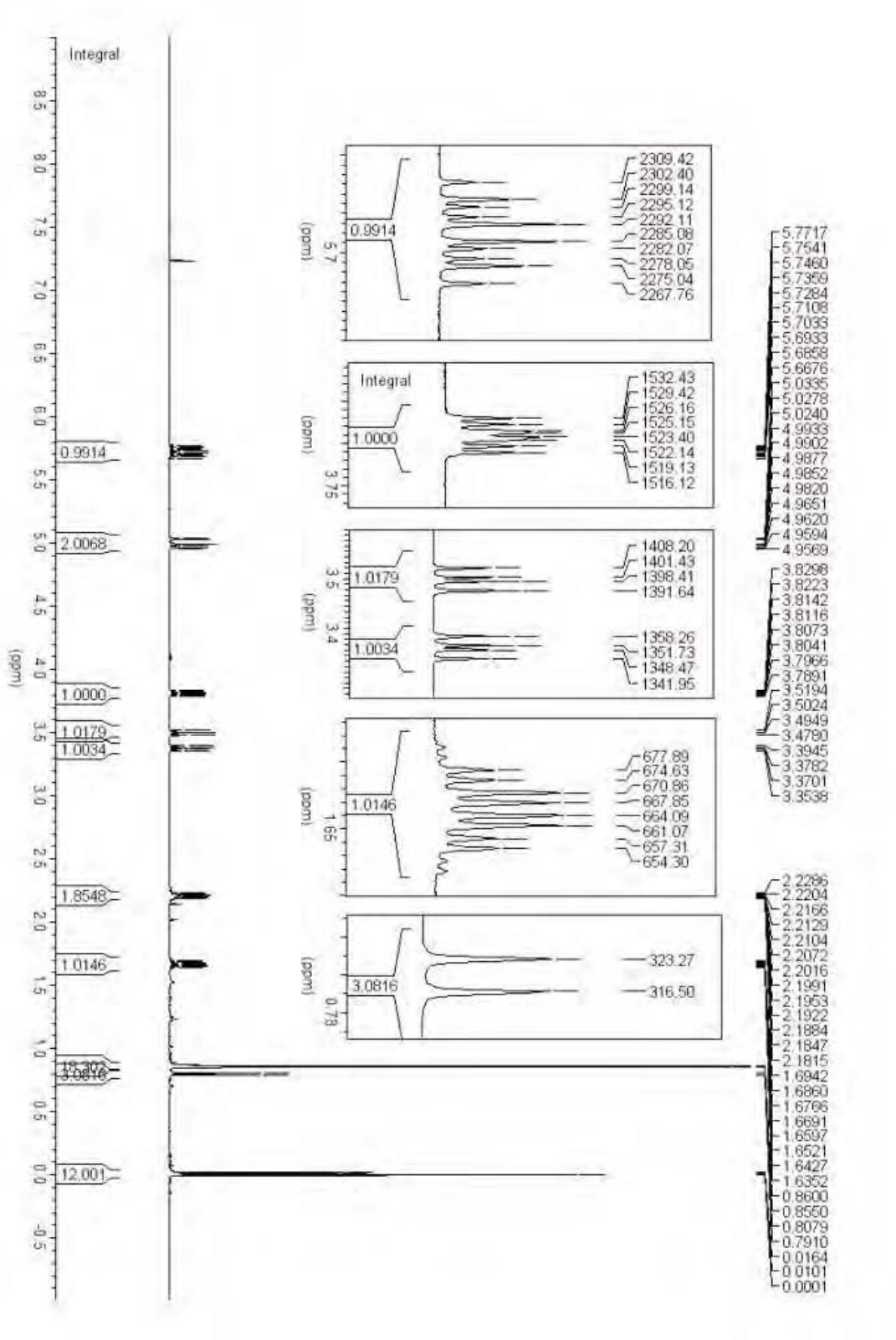
(2*S*,3*S*)-1-(*tert*-Butyldimethylsilanyloxy)-2-methylhex-5-en-3-ol (II.5)



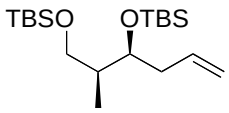


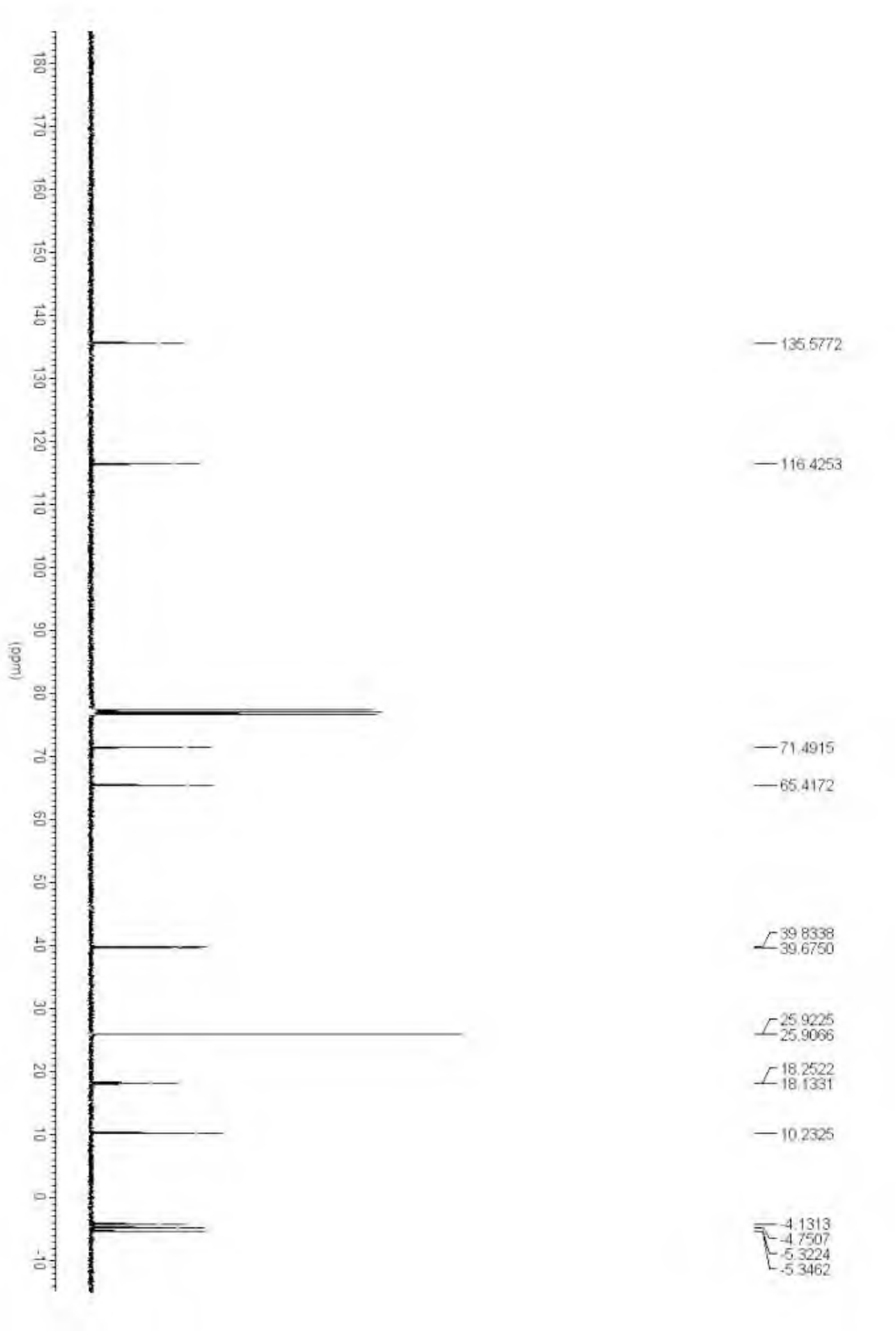
(4*S*,5*S*)-4,6-Bis-(*tert*-Butyldimethylsilanyloxy)-5-methylhex-1-ene



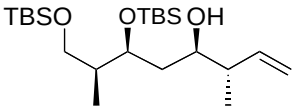


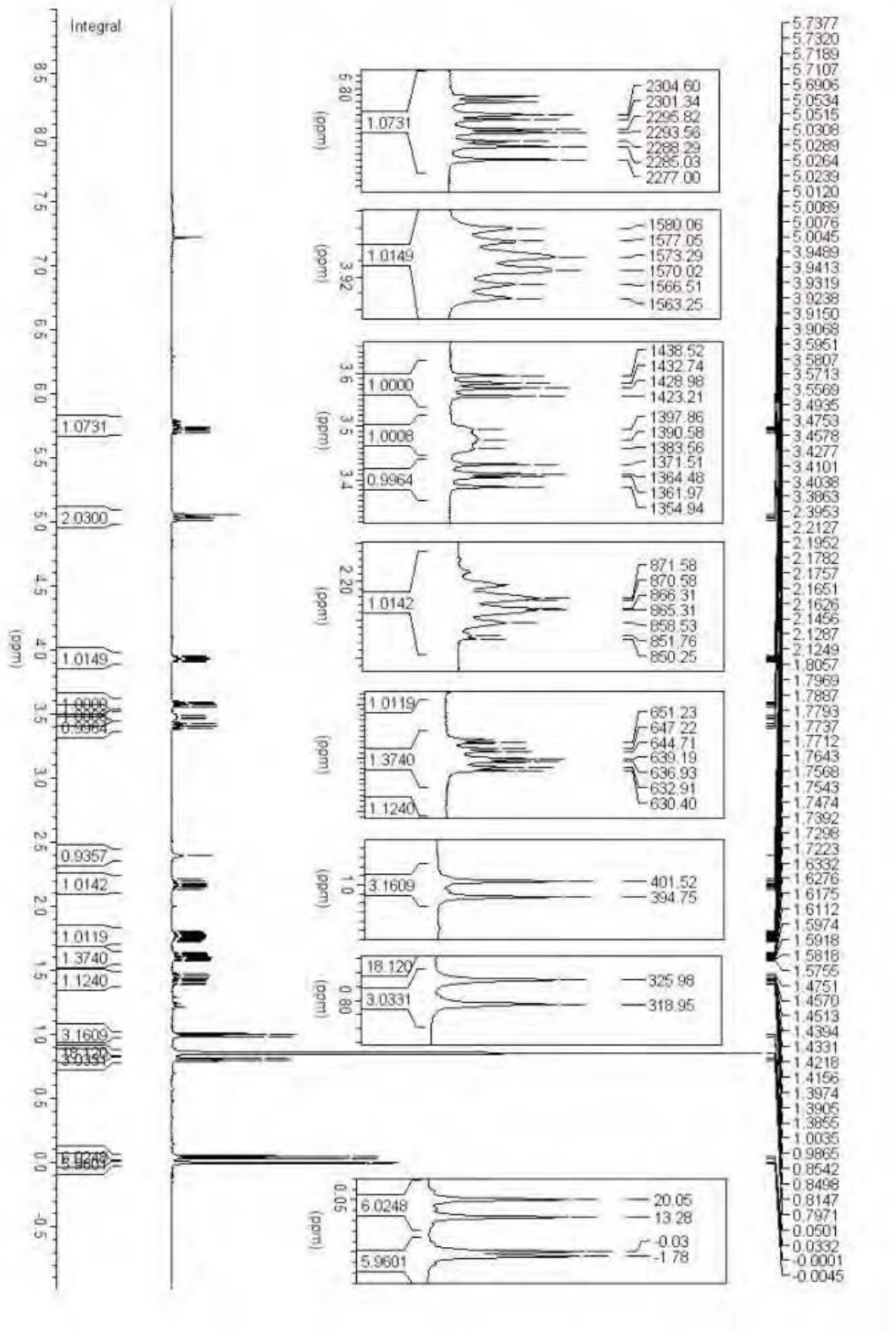
(4*S*,5*S*)-4,6-bis-(*tert*-Butyldimethylsilanyloxy)-5-methylhex-1-ene



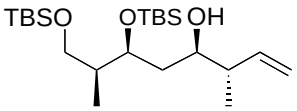


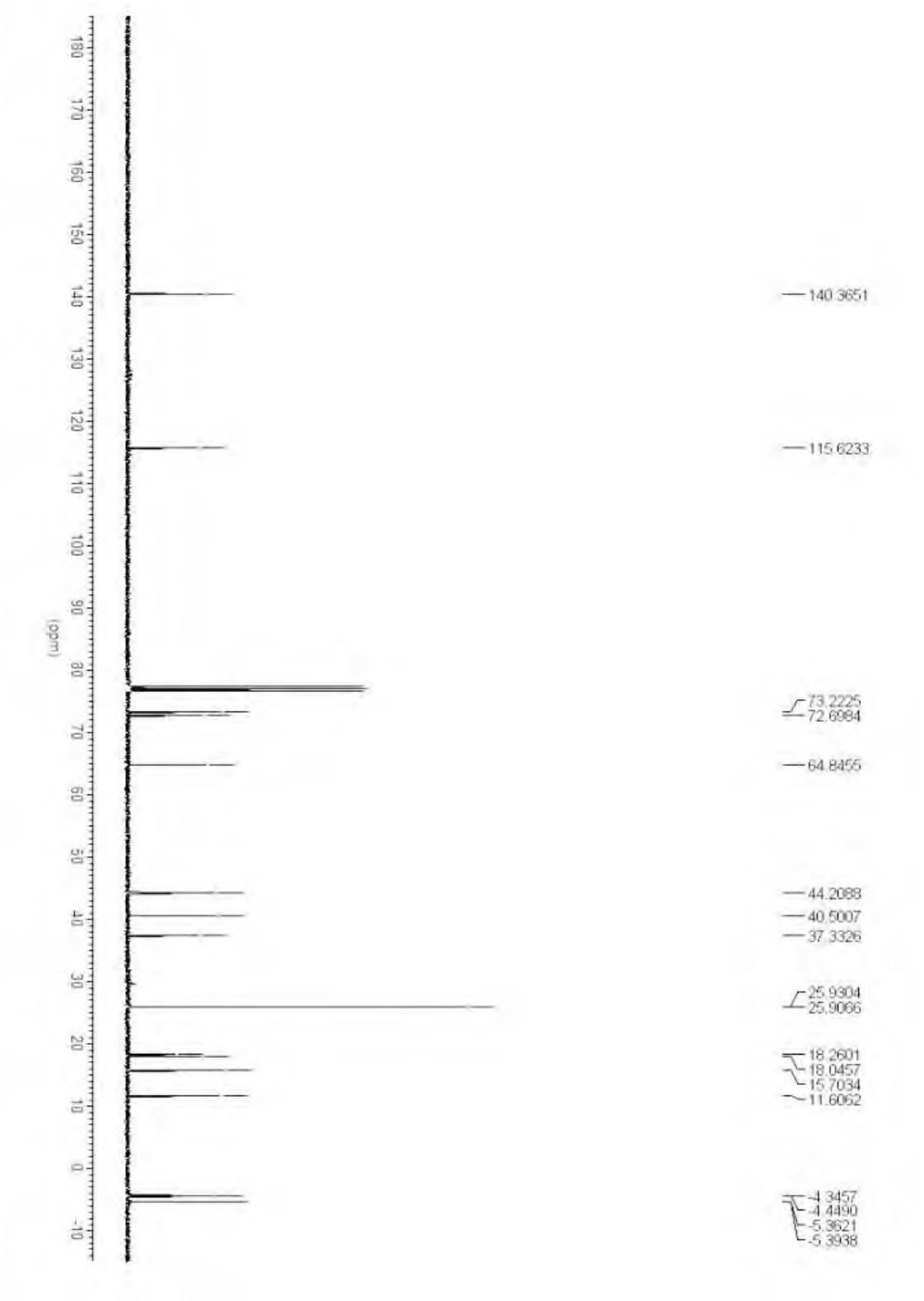
(3*S*,4*R*,6*S*,7*S*)-6,8-bis-(*tert*-Butyldimethylsilanyloxy)-3,7-dimethyl-oct-1-en-4-ol



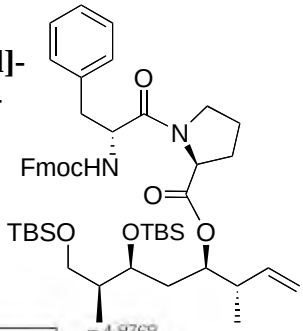


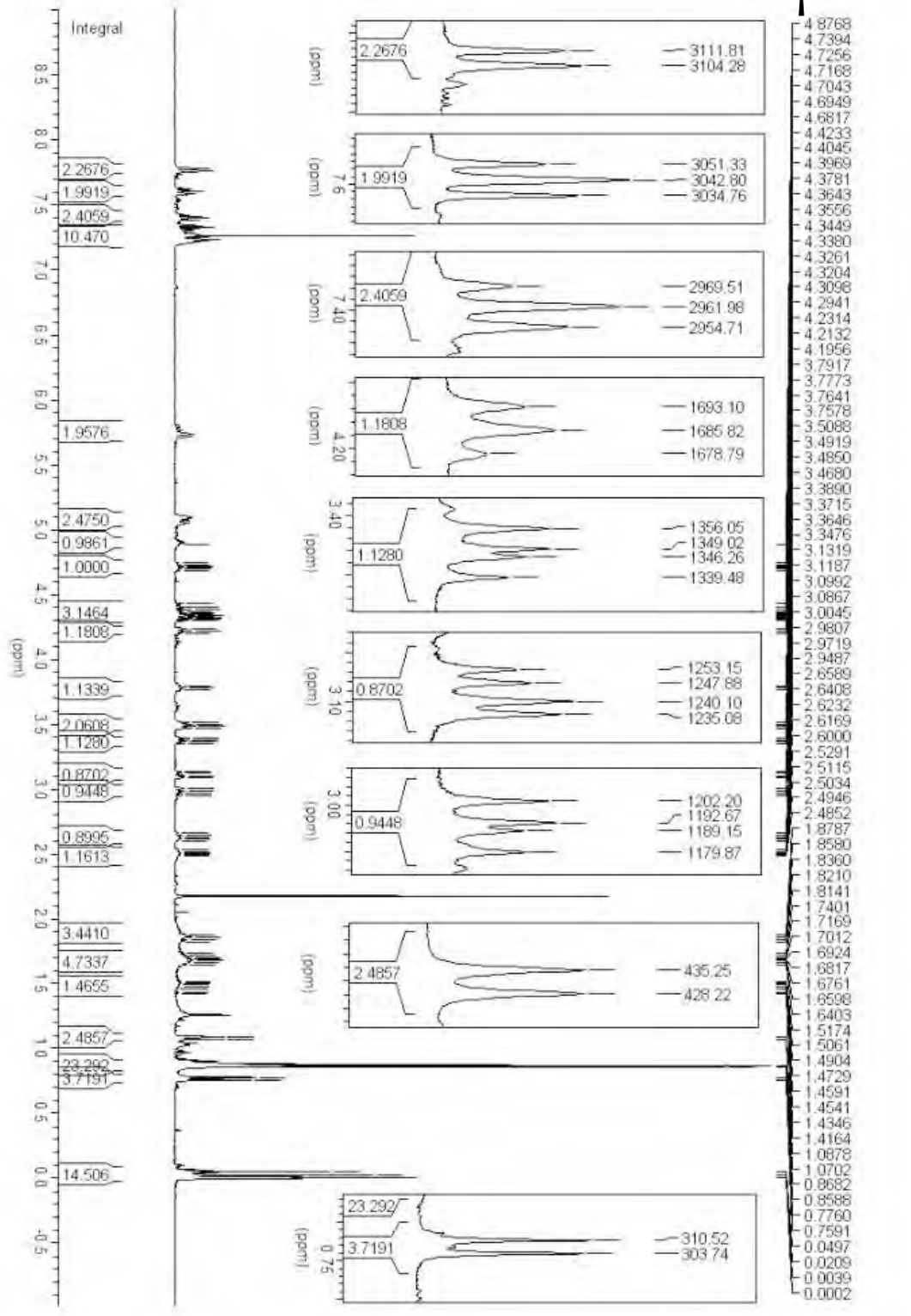
(3*S*,4*R*,6*S*,7*S*)-6,8-bis-(*tert*-Butyldimethylsilanyloxy)-3,7-dimethyl-oct-1-en-4-ol (II.7)



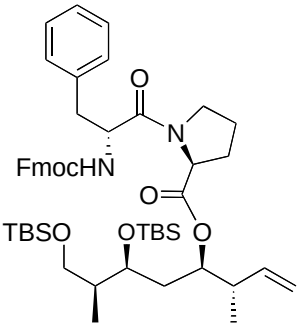


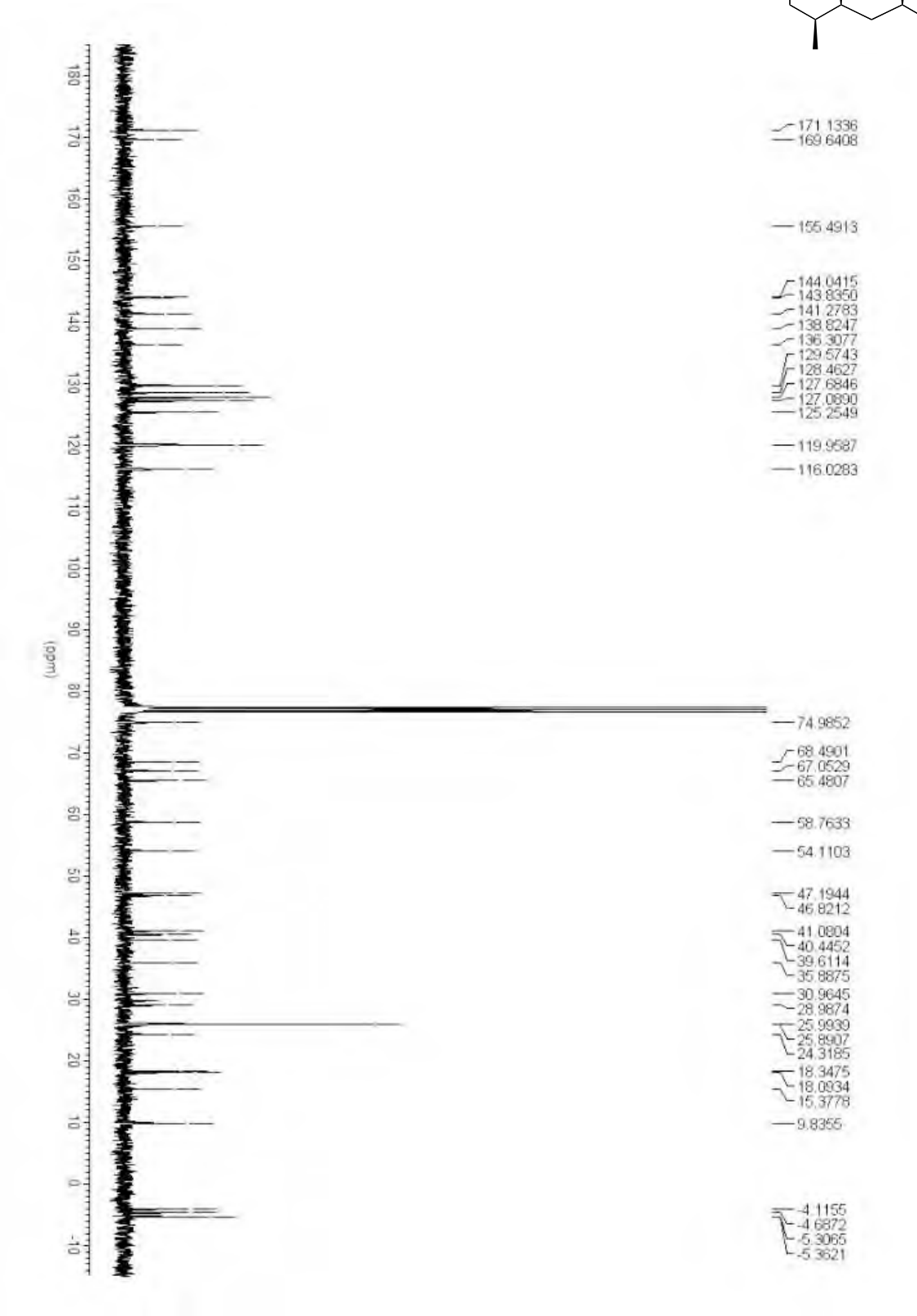
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*S*)-3,5-bis-(*tert*-butyldimethyl-silanyloxy)-4-methyl-1-((*S*)-1-methylallyl)-pentyl ester (II.7)



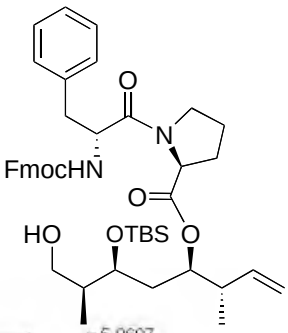


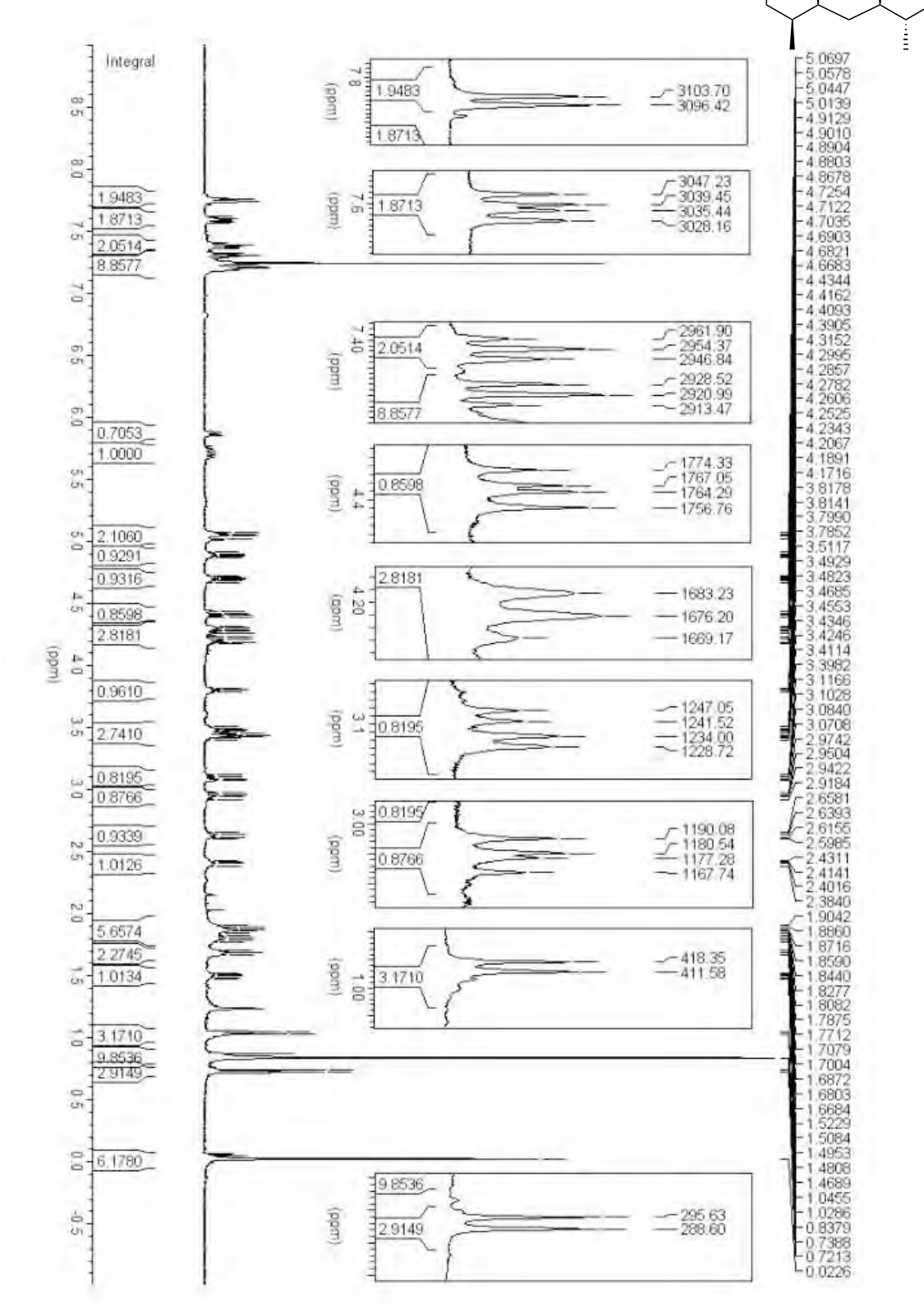
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenylpropionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*S*)-3,5-bis-(*tert*-butyldimethyl-silanyloxy)-4-methyl-1-((*S*)-1-methylallyl)-pentyl ester (II.7)



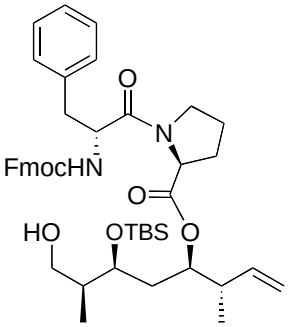


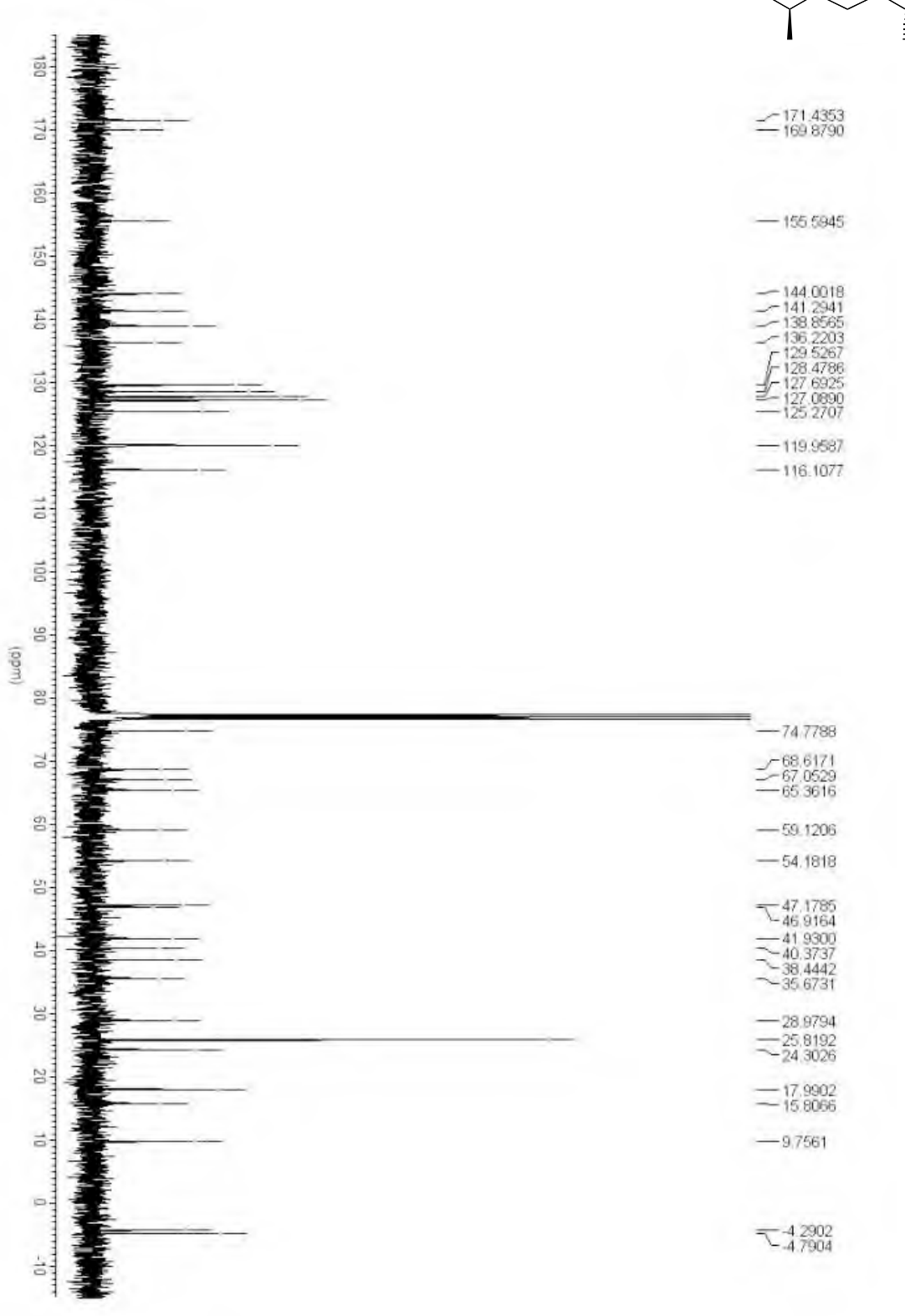
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenyl-propionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*S*)-3-(*tert*-butyldimethylsilanyloxy)-5-hydroxy-4-methyl-1-((*S*)-1-methyl-allyl)-pentyl ester (II.14)



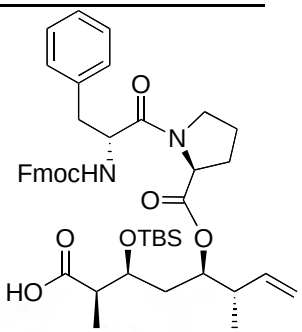


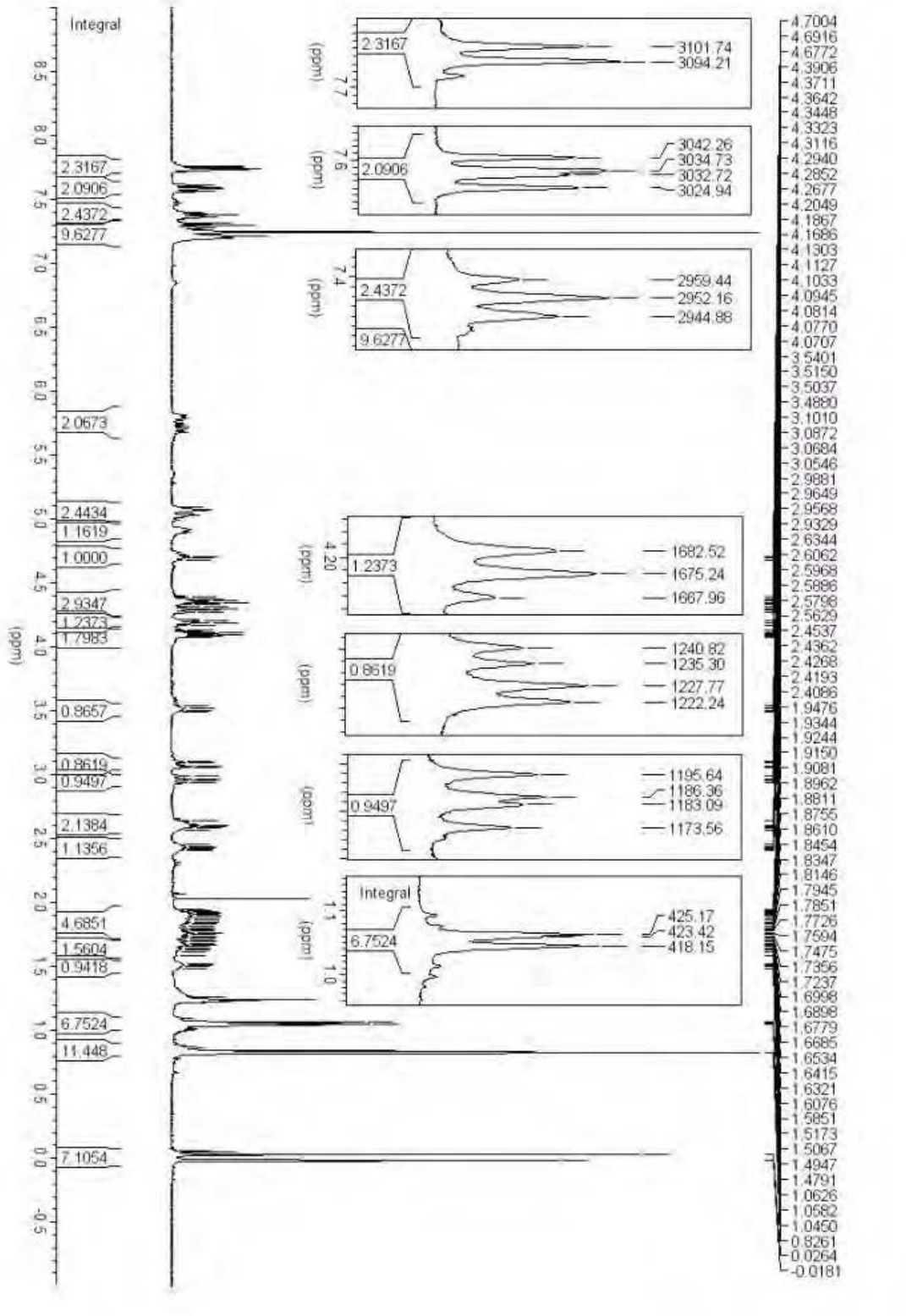
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenyl-propionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*S*)-3-(*tert*-butyldimethylsilanyloxy)-5-hydroxy-4-methyl-1-((*S*)-1-methyl-allyl)-pentyl ester (II.14)



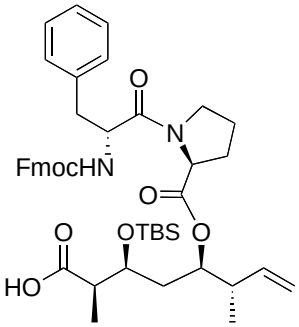


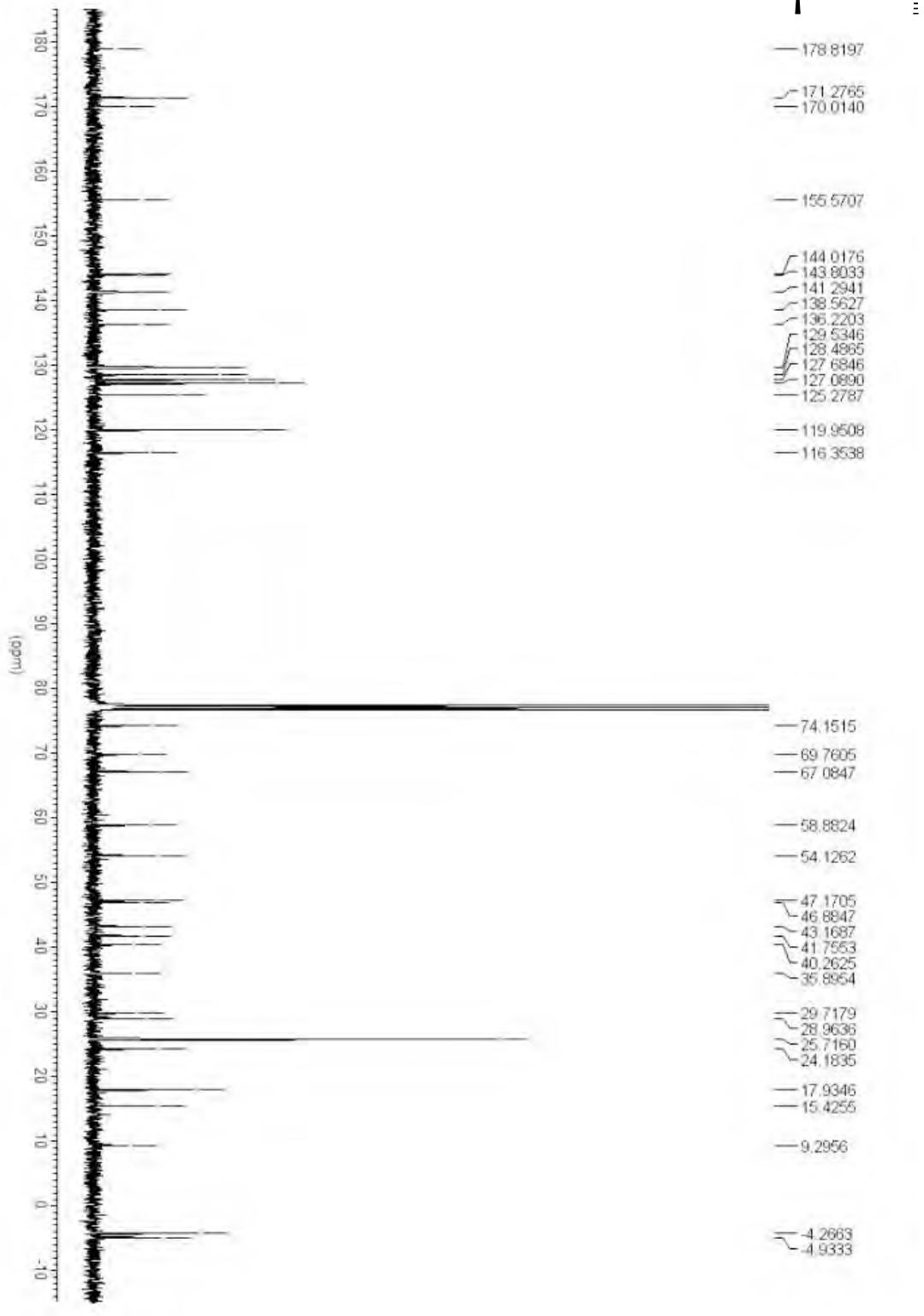
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenyl-propionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*R*)-3-(*tert*-butyldimethylsilanyloxy)-4-carboxy-1-((*S*)-1-methylallyl)-pentyl ester (II.16)



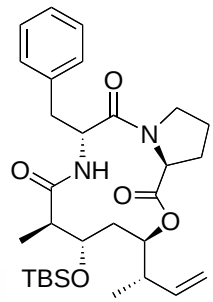


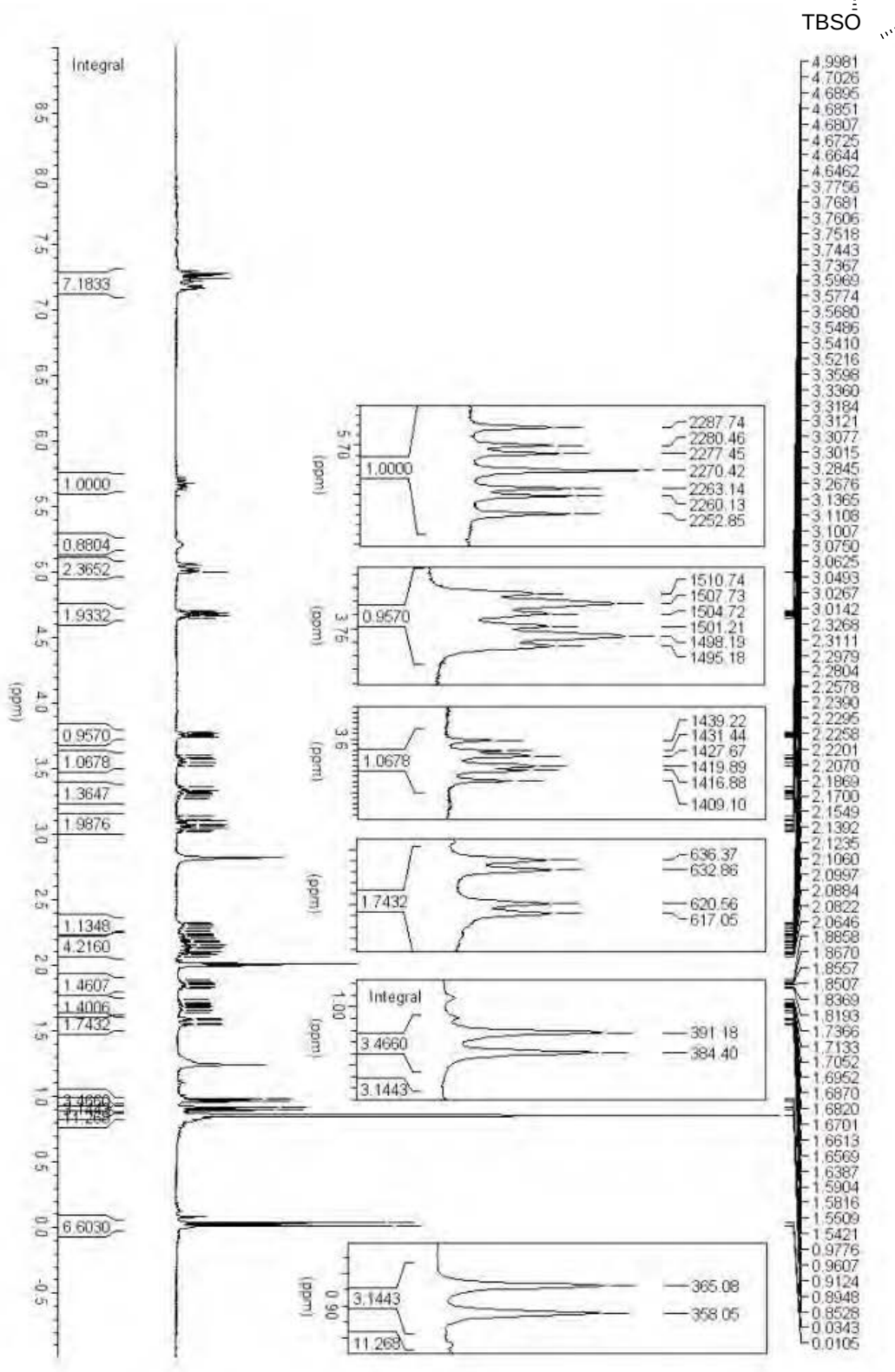
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenyl-propionyl]-pyrrolidine-2-carboxylic acid (1*R*,3*S*,4*R*)-3-(*tert*-butyldimethylsilanyloxy)-4-carboxy-1-((*S*)-1-methylallyl)-pentyl ester (II.16)



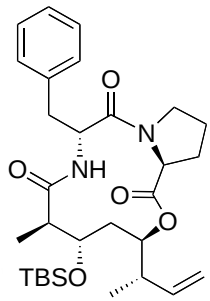


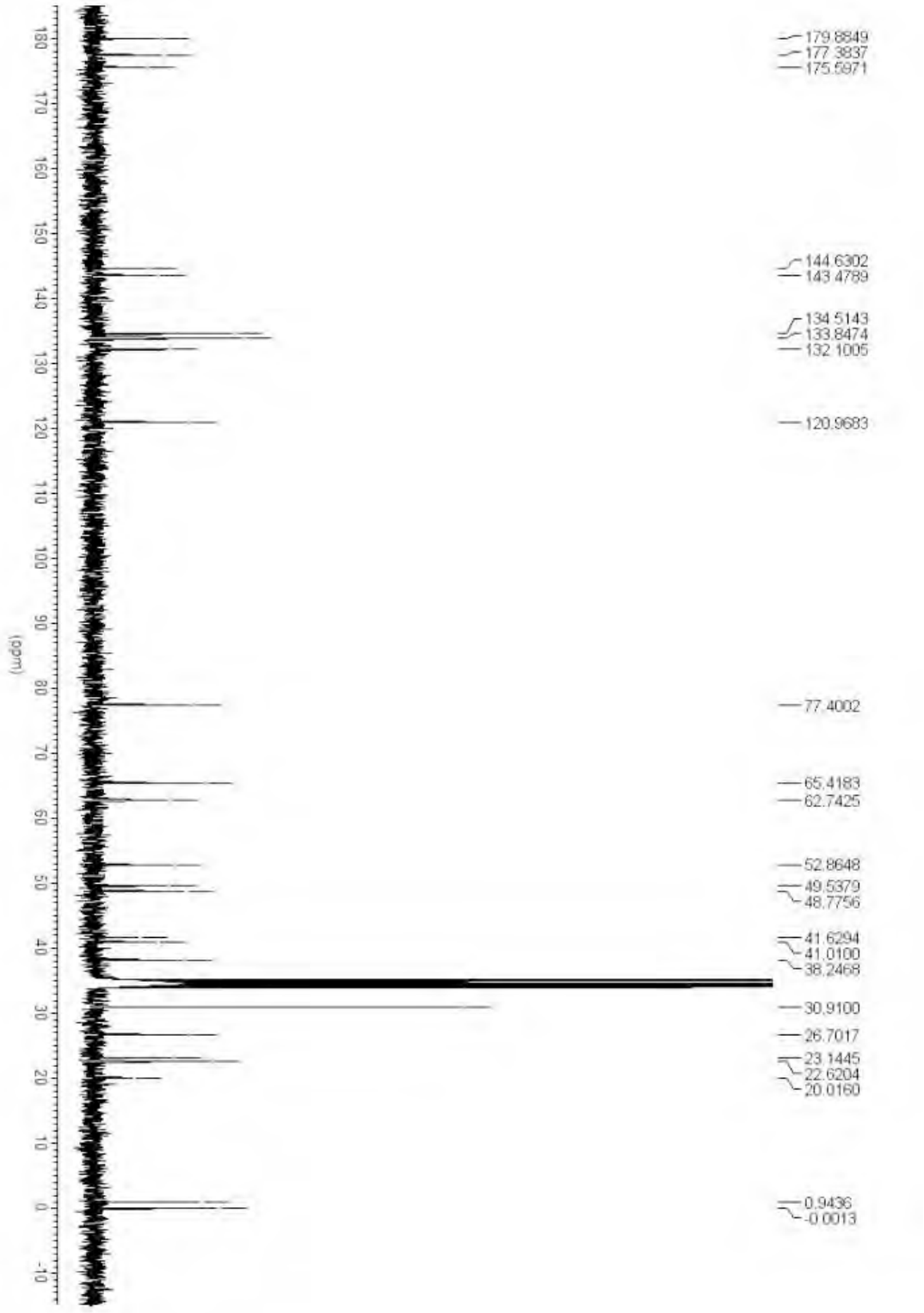
(5*R*,8*R*,9*S*,11*R*,13a*R*)-5-Benzyl-9-(*tert*-butyldimethylsilanyloxy)-8-methyl-11-((*S*)-1-methylallyl)-decahydro-12-oxa-3a,6-diaza-cyclopentacyclododecene-4,7,13-trione (II.18)



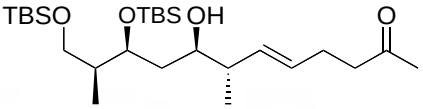


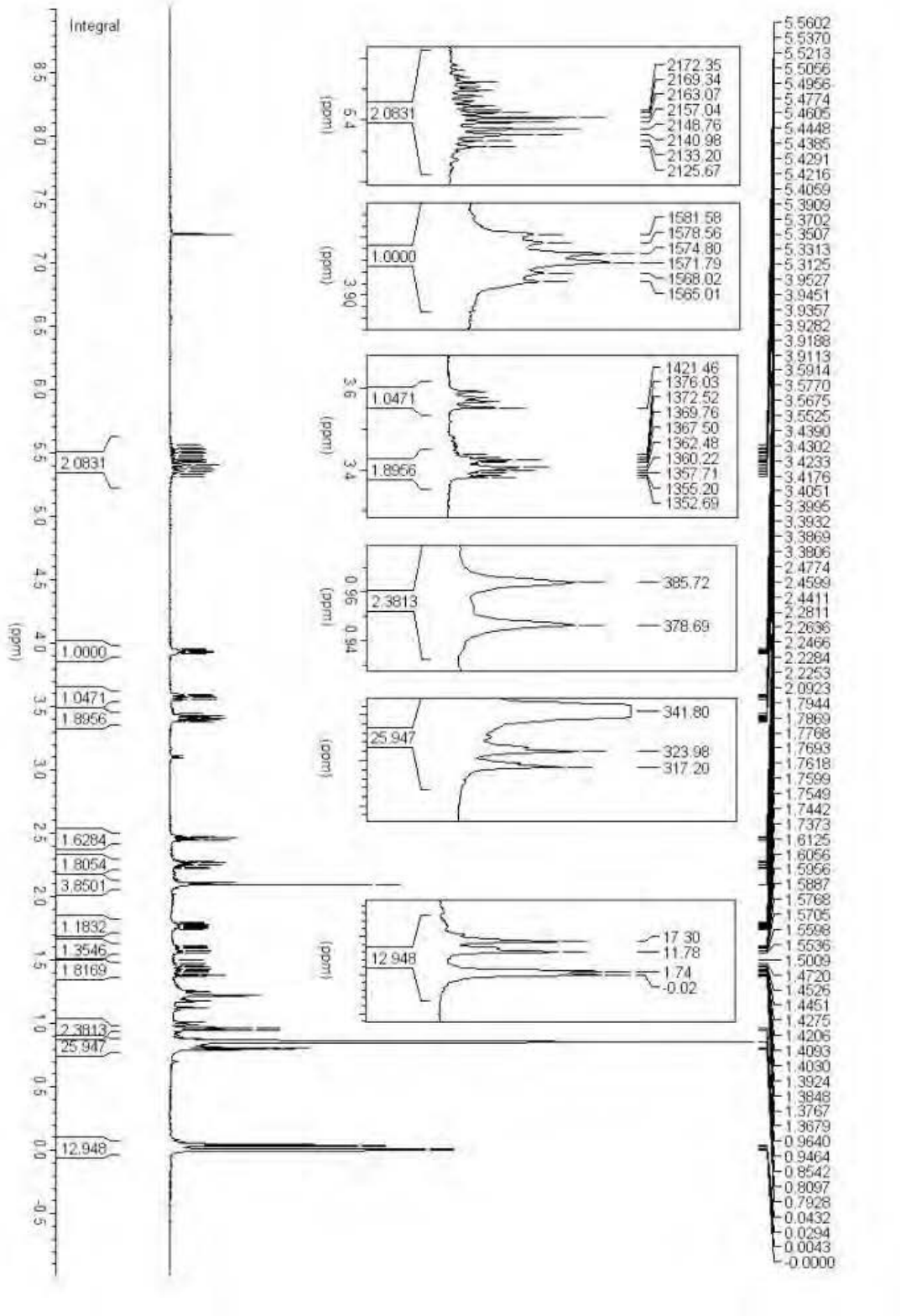
(5*R*,8*R*,9*S*,11*R*,13a*R*)-5-Benzyl-9-(*tert*-butyldimethylsilanyloxy)-8-methyl-11-((*S*)-1-methylallyl)-decahydro-12-oxa-3a,6-diaza-cyclopentacyclododecene-4,7,13-trione (II.18)



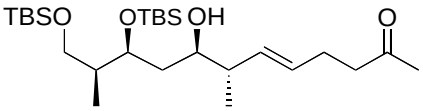


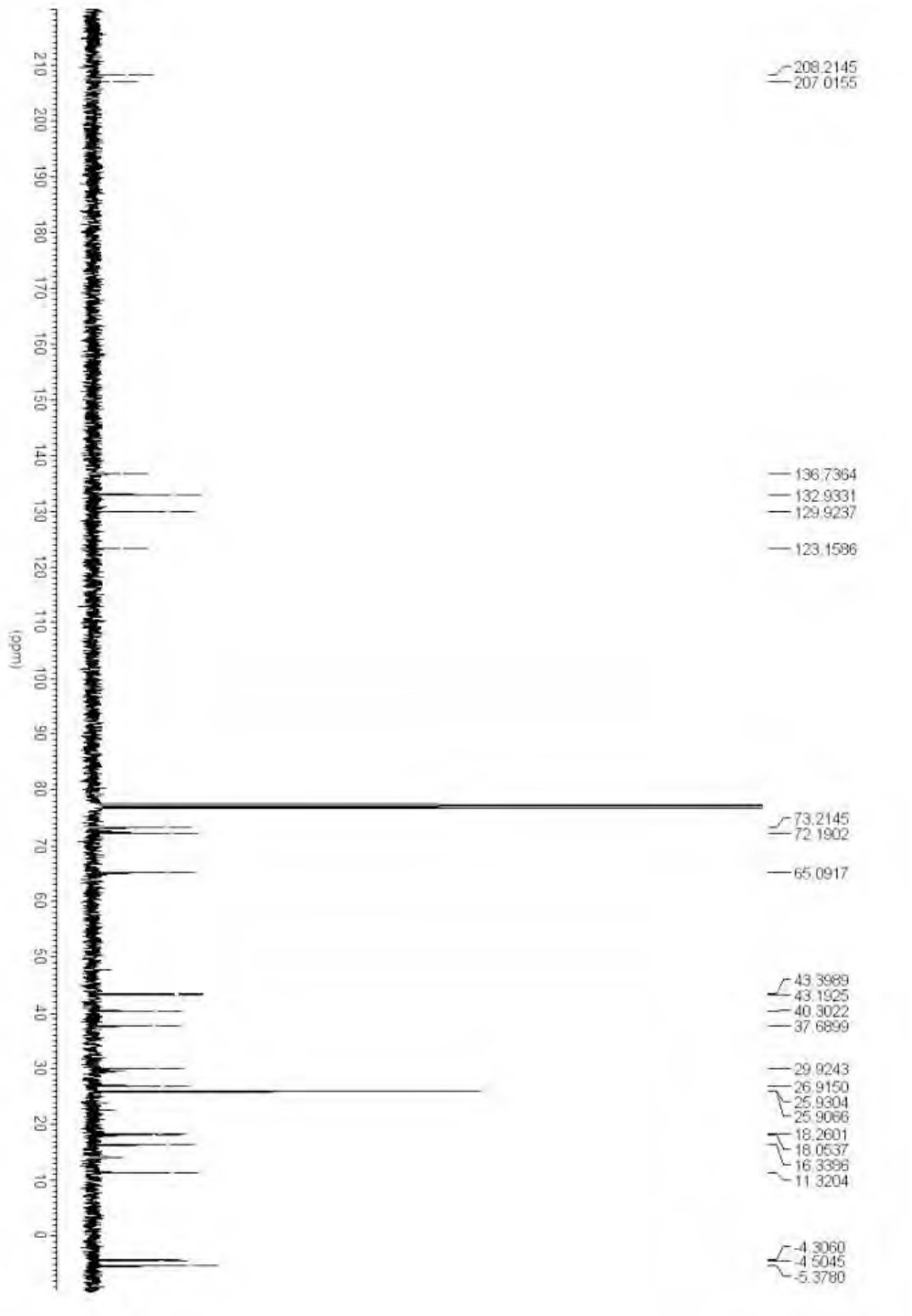
(*E*)-(7*S*,8*R*,10*S*,11*S*)-10,12-bis-(*tert*-Butyldimethylsilanyloxy)-8-hydroxy-7,11-dimethyldodec-5-en-2-one (II.21)



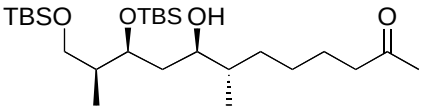


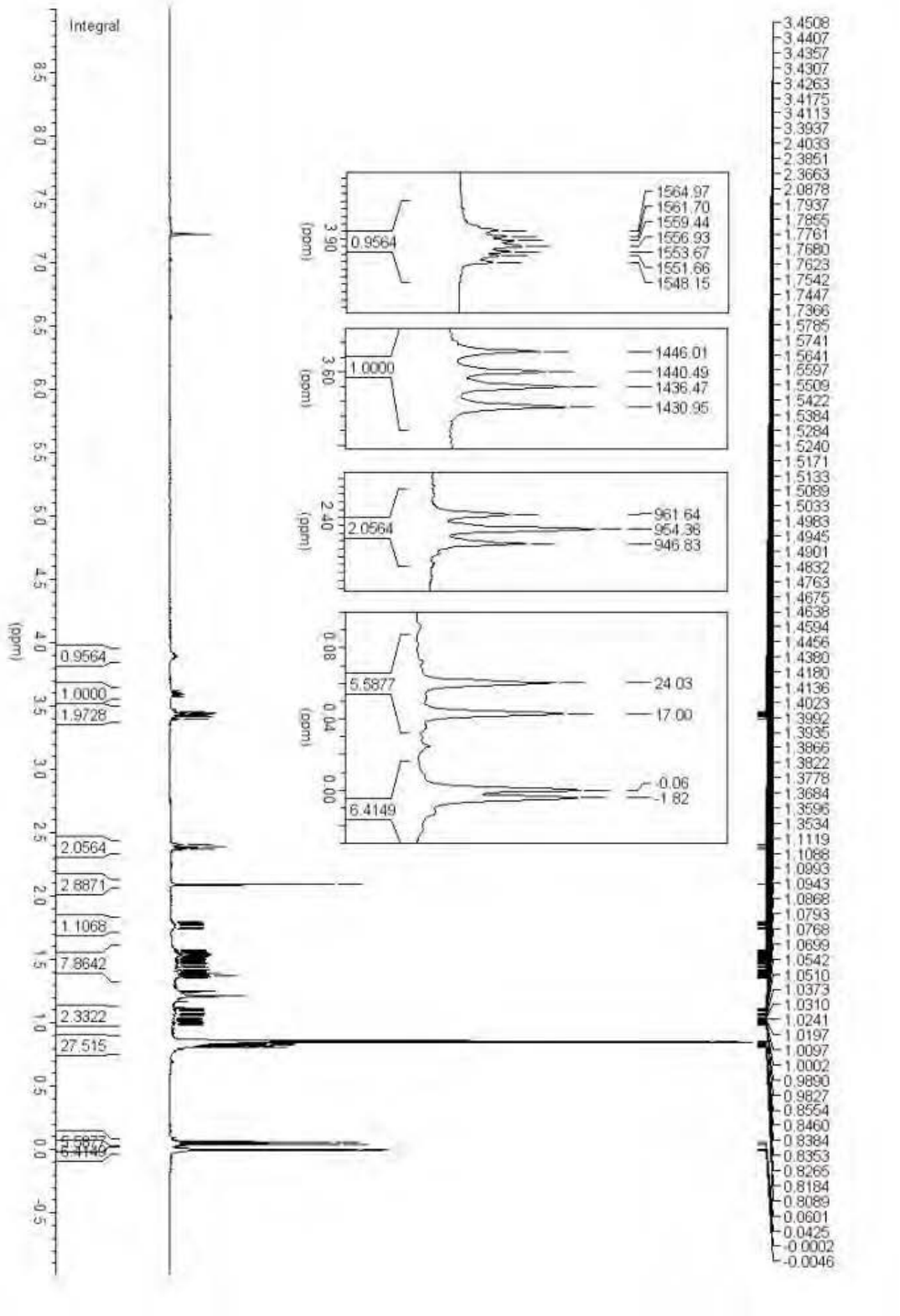
(*E*)-(7*S*,8*R*,10*S*,11*S*)-10,12-bis-(*tert*-Butyldimethylsilanyloxy)-8-hydroxy-7,11-dimethyldodec-5-en-2-one (II.21)



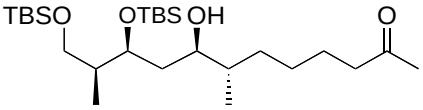


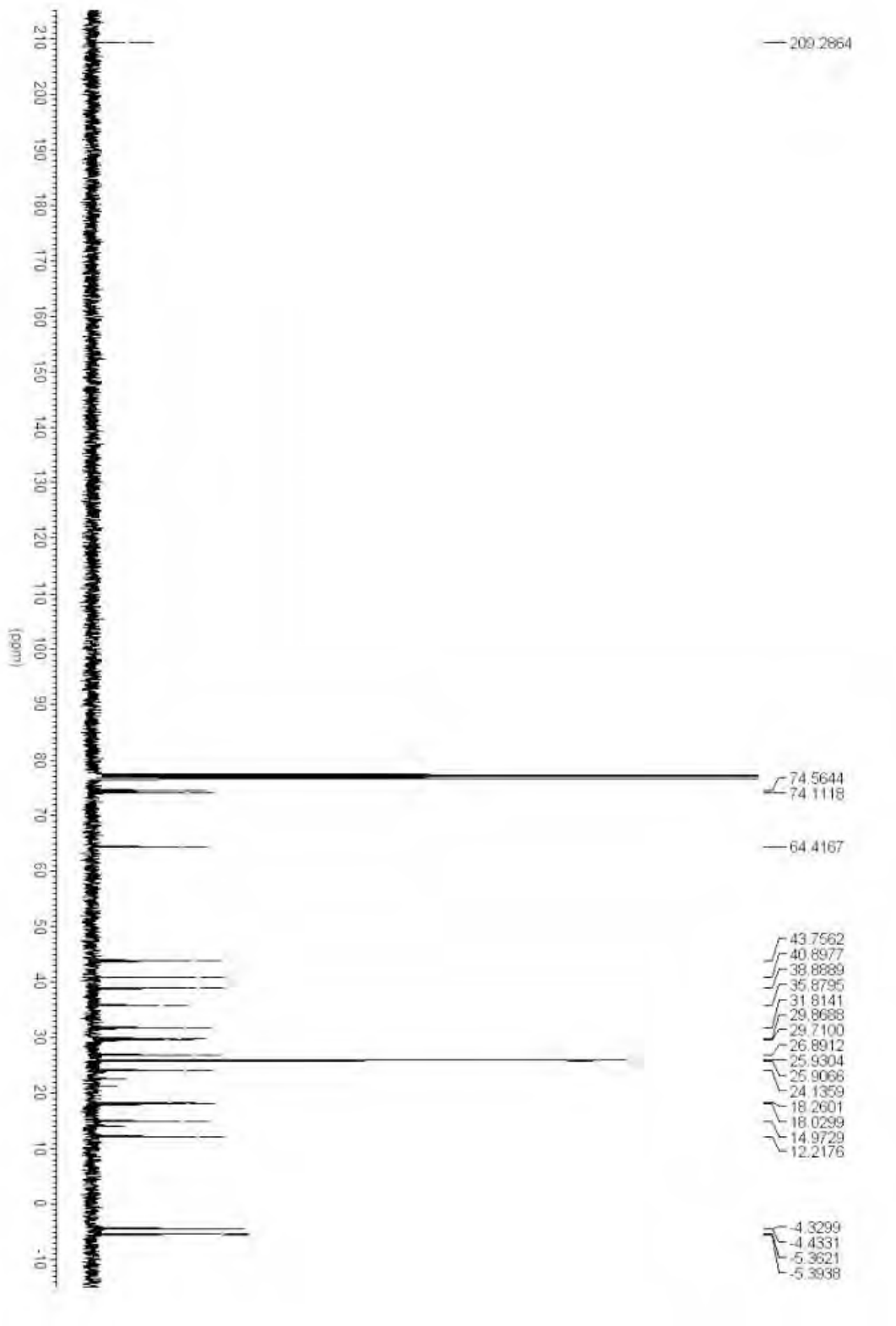
(7*S*,8*R*,10*S*,11*S*)-10,12-bis-(*tert*-Butyldimethylsilanyloxy)-8-hydroxy-7,11-dimethyldodecan-2-one (II.23)



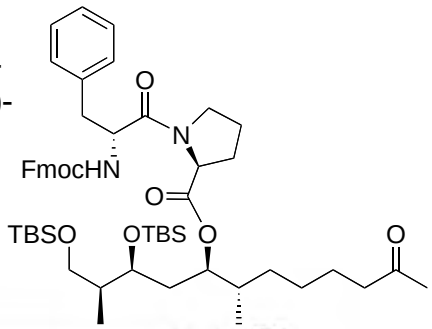


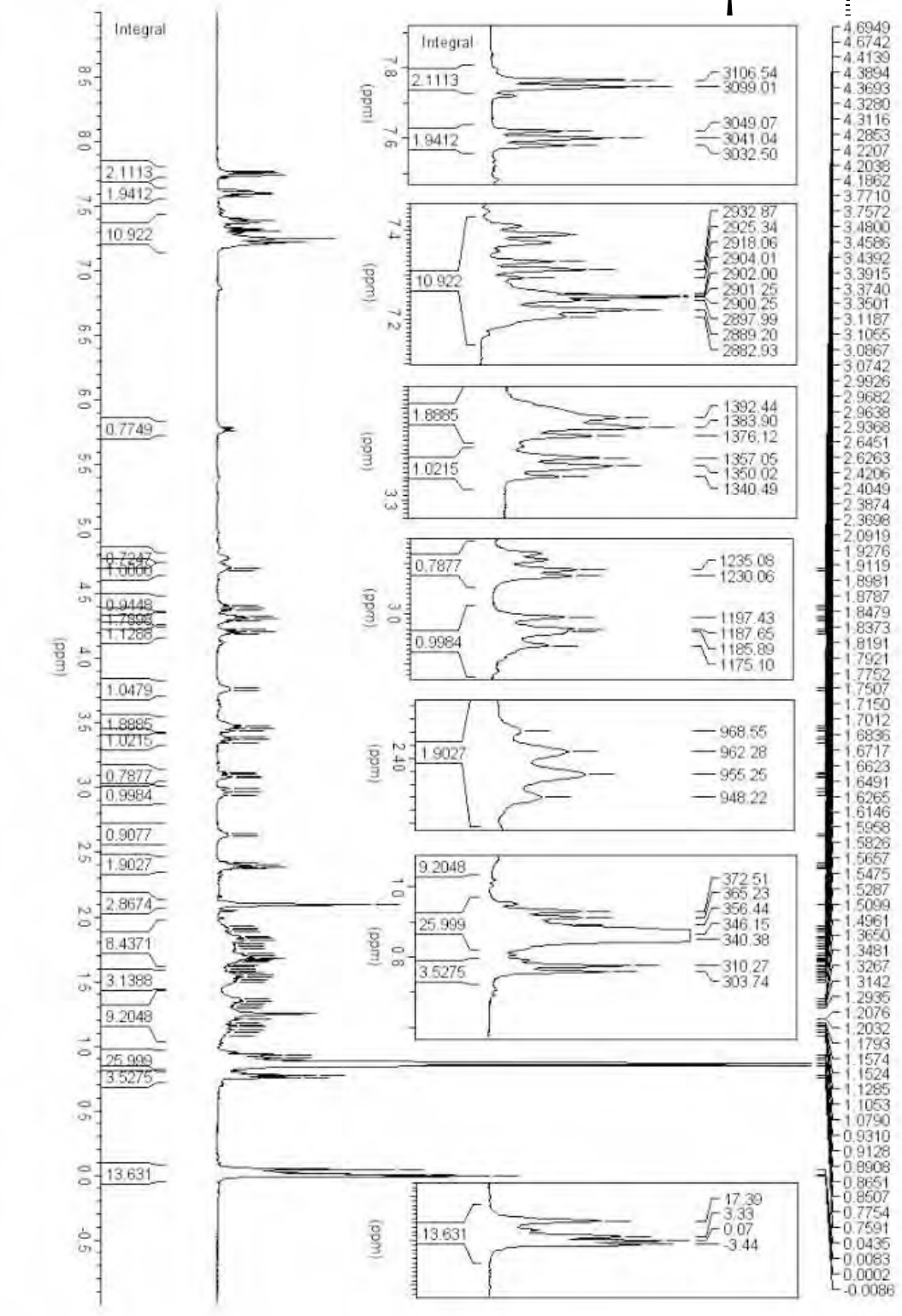
(7*S*,8*R*,10*S*,11*S*)-10,12-bis-(*tert*-Butyldimethylsilanyloxy)-8-hydroxy-7,11-dimethyldodecan-2-one (II.23)



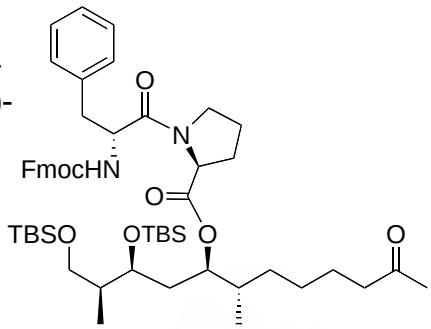


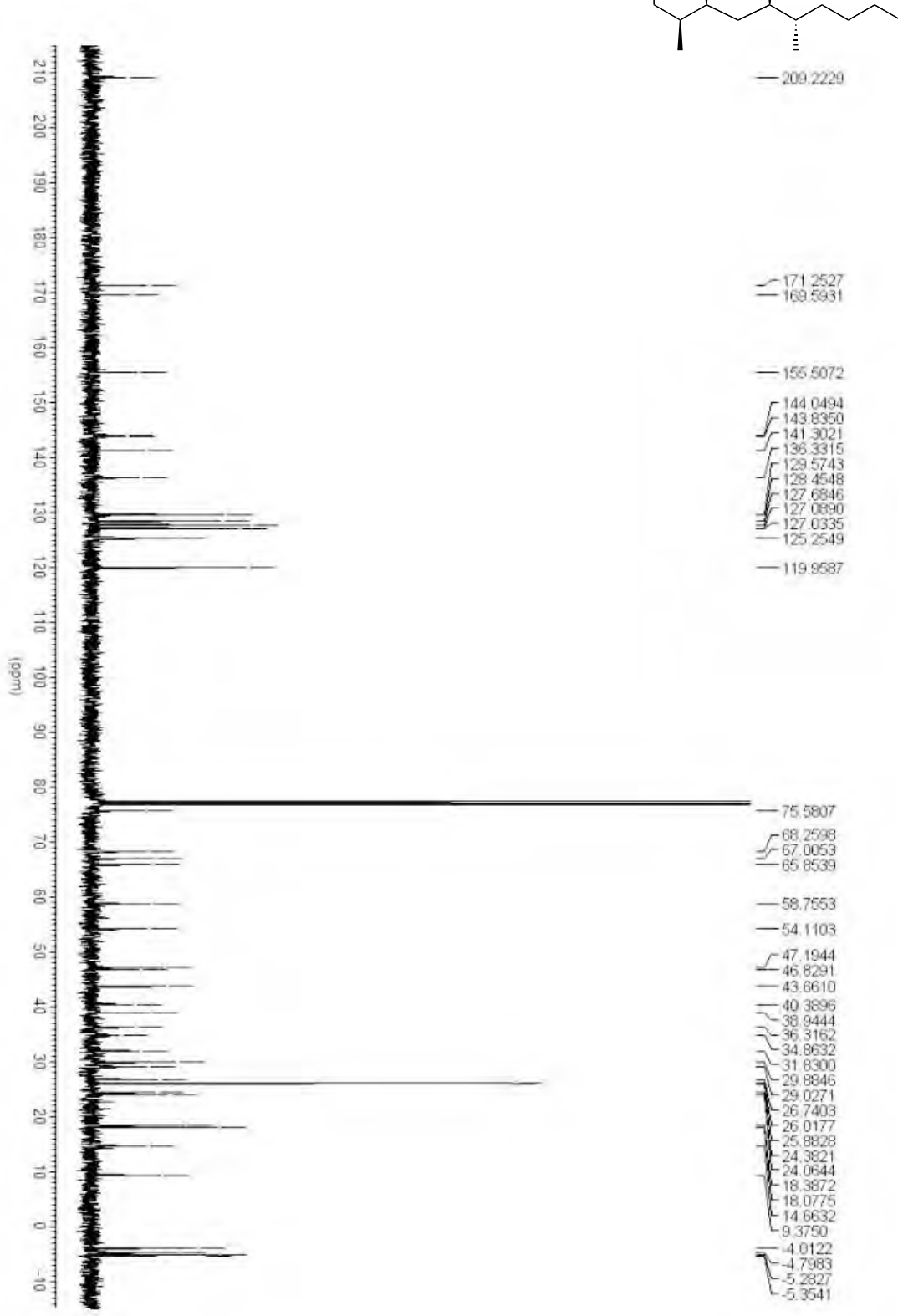
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenyl-propionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*S*)-2,4-bis-(*tert*-butyldimethylsilanyloxy)-3-methylbutyl]-2-methyl-7-oxooctyl ester (II.24)



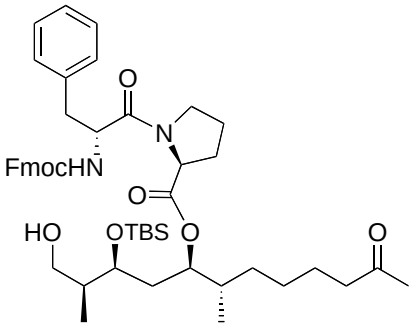


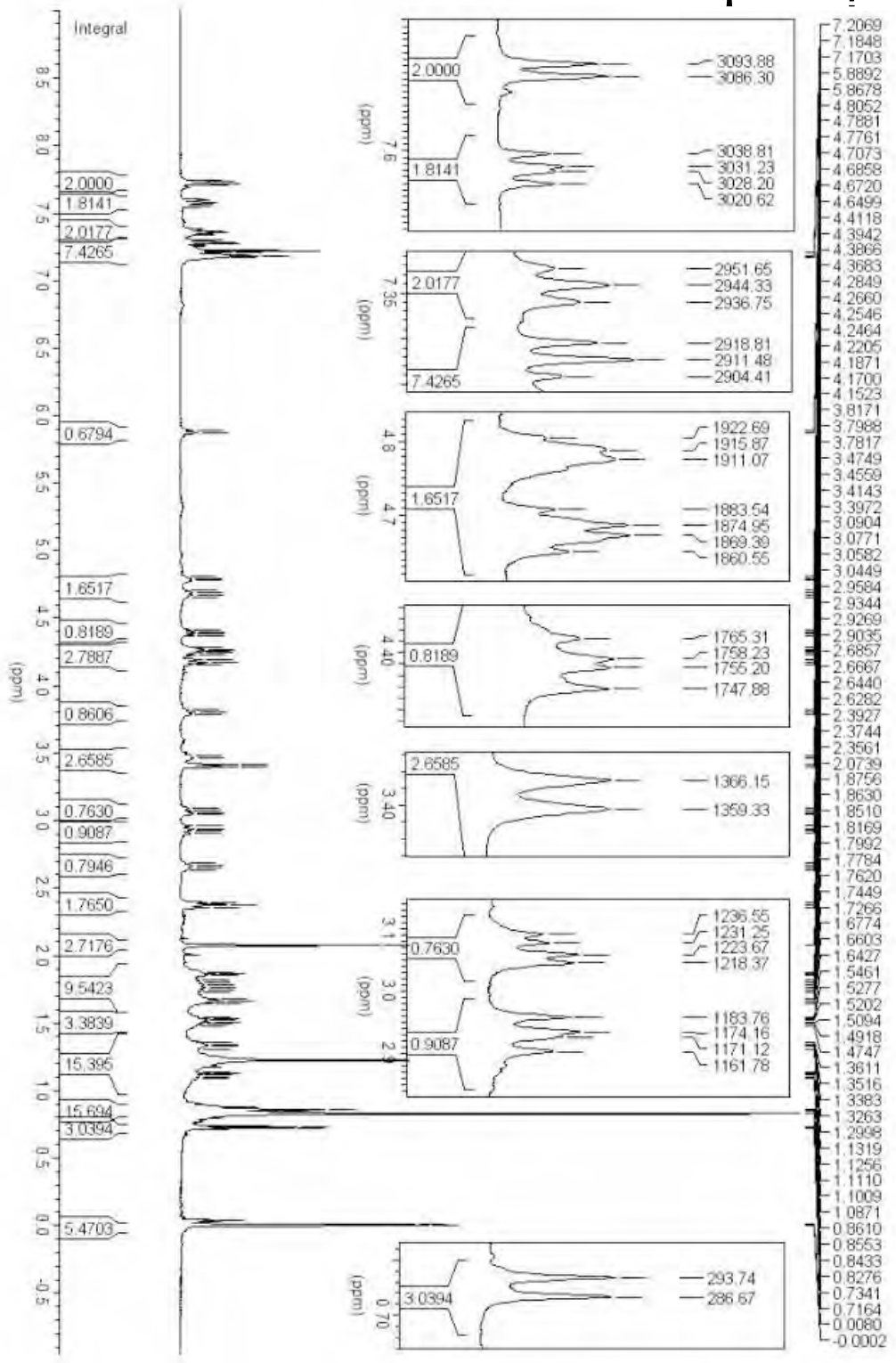
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenyl-propionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*S*)-2,4-bis-(*tert*-butyldimethylsilanyloxy)-3-methylbutyl]-2-methyl-7-oxooctyl ester (II.24)



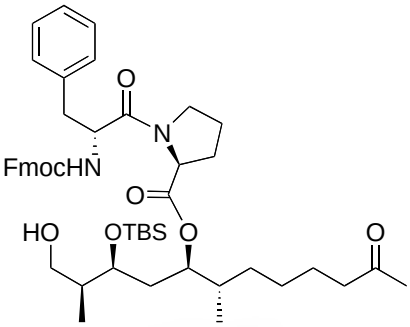


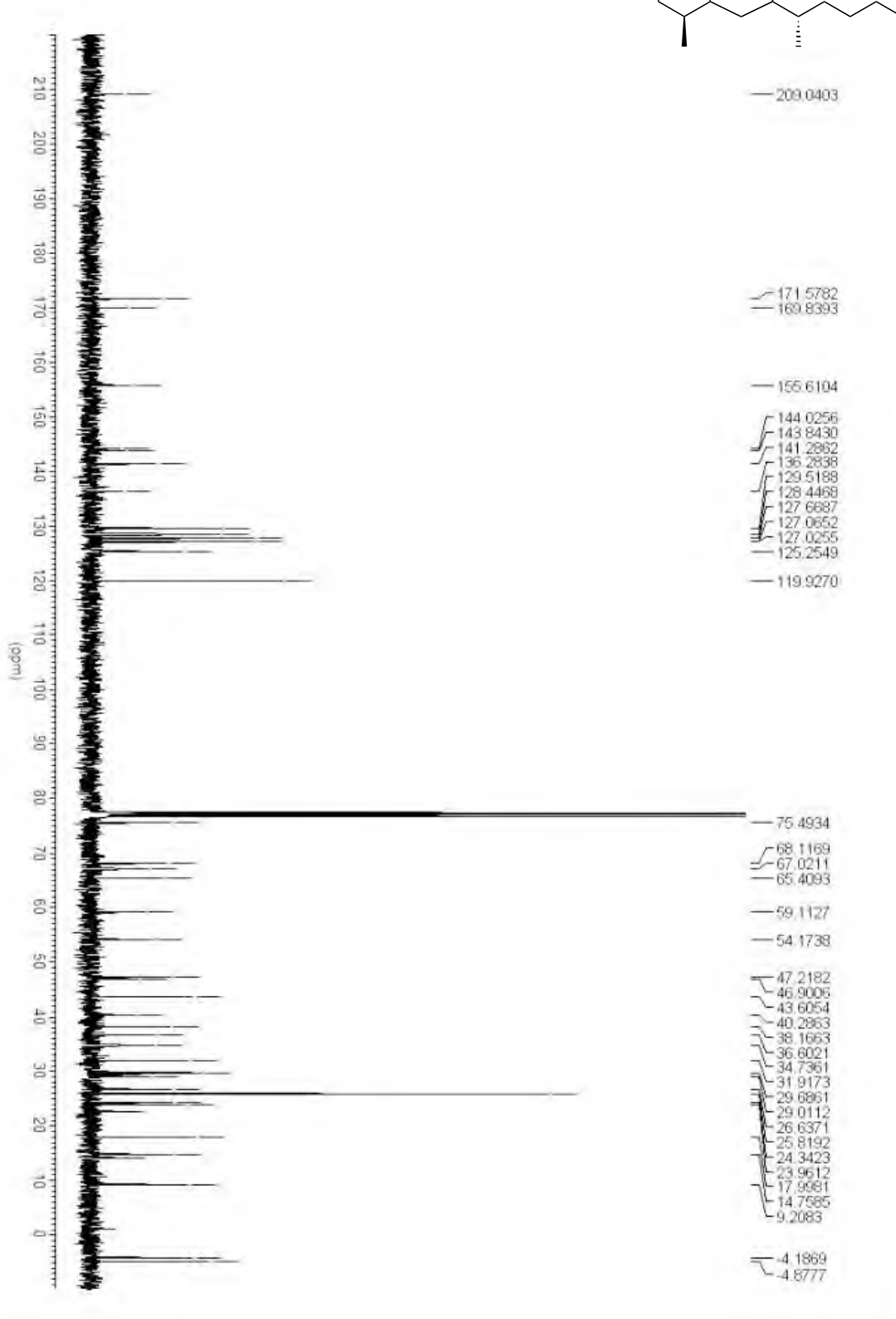
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenyl-propionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*S*)-2-(*tert*-butyldimethylsilanyloxy)-4-hydroxy-3-methylbutyl]-2-methyl-7-oxooctyl ester (II.25)



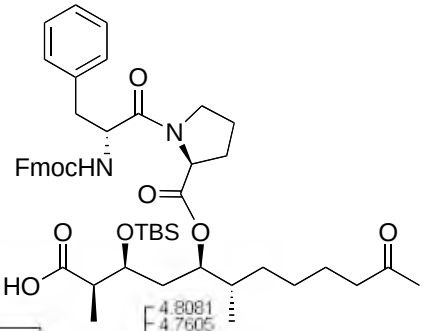


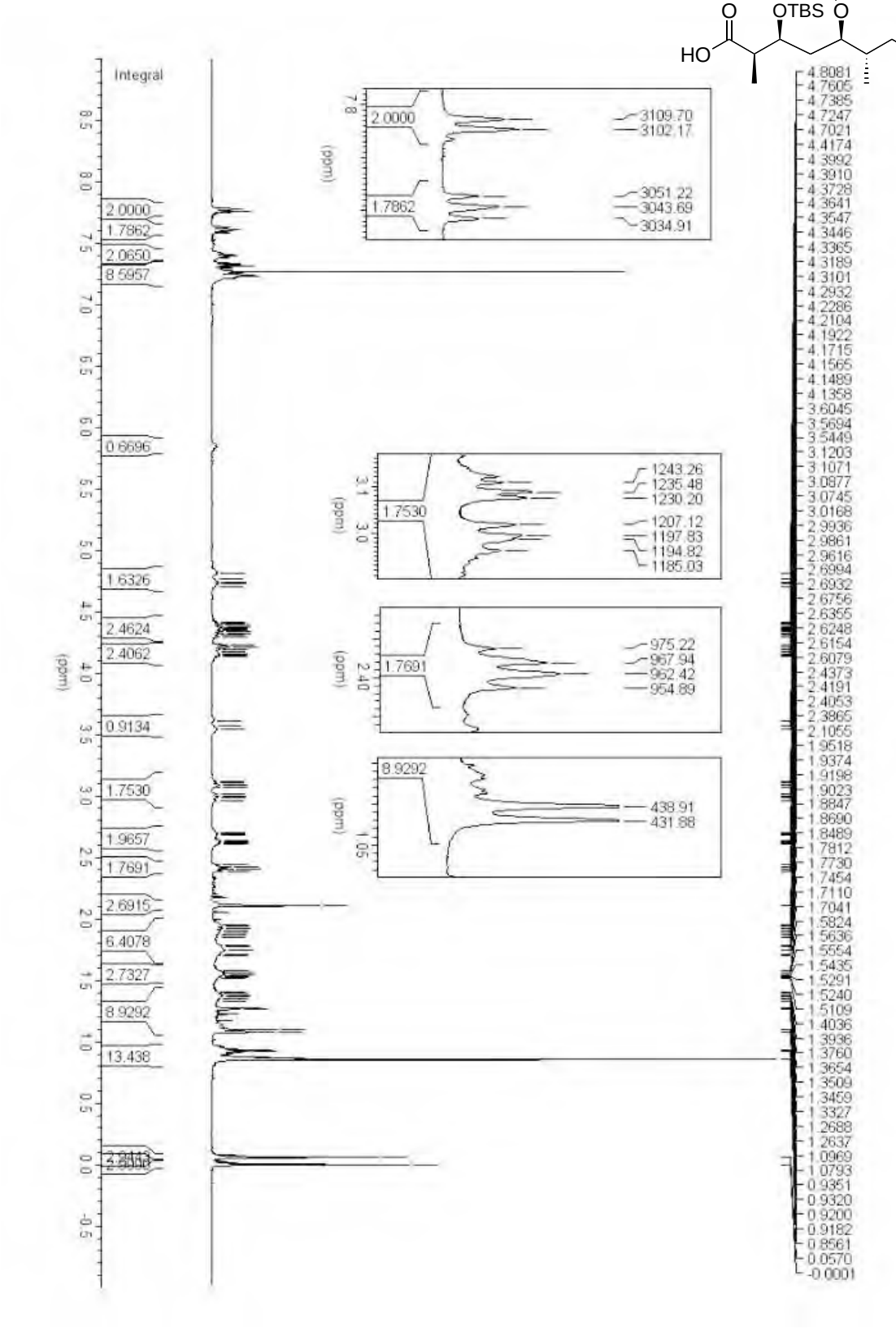
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenyl-propionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*S*)-2-(*tert*-butyldimethylsilanyloxy)-4-hydroxy-3-methylbutyl]-2-methyl-7-oxooctyl ester (II.25)



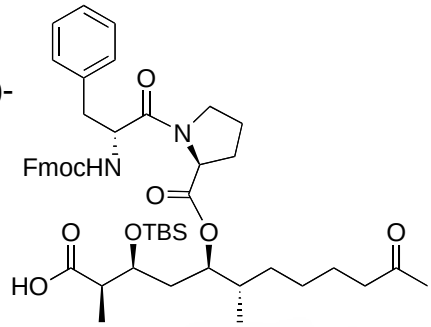


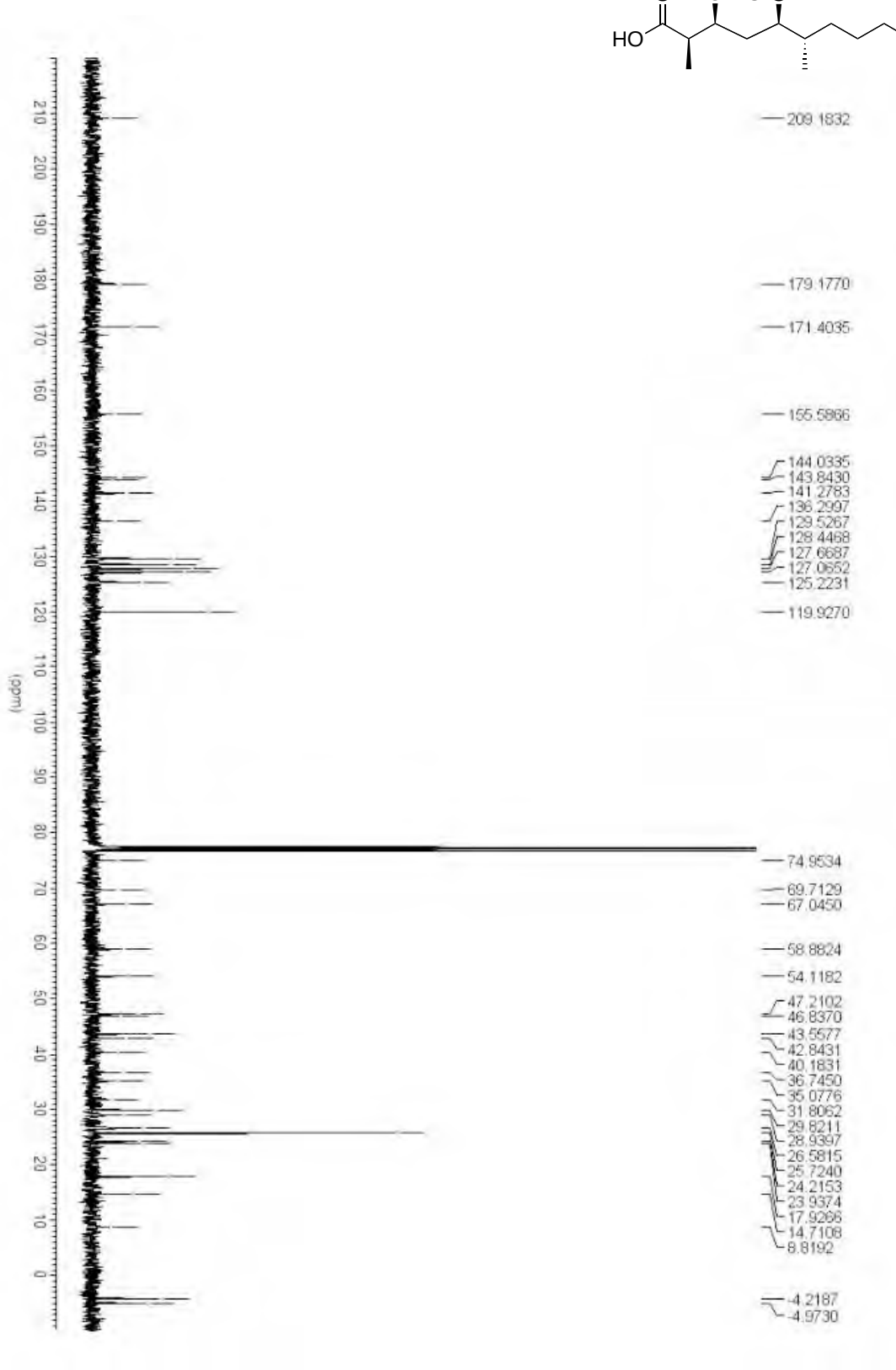
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenyl-propionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*R*)-2-(*tert*-butyldimethylsilanyloxy)-3-carboxybutyl]-2-methyl-7-oxooctyl ester (II.26)



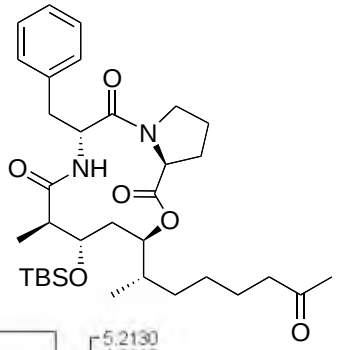


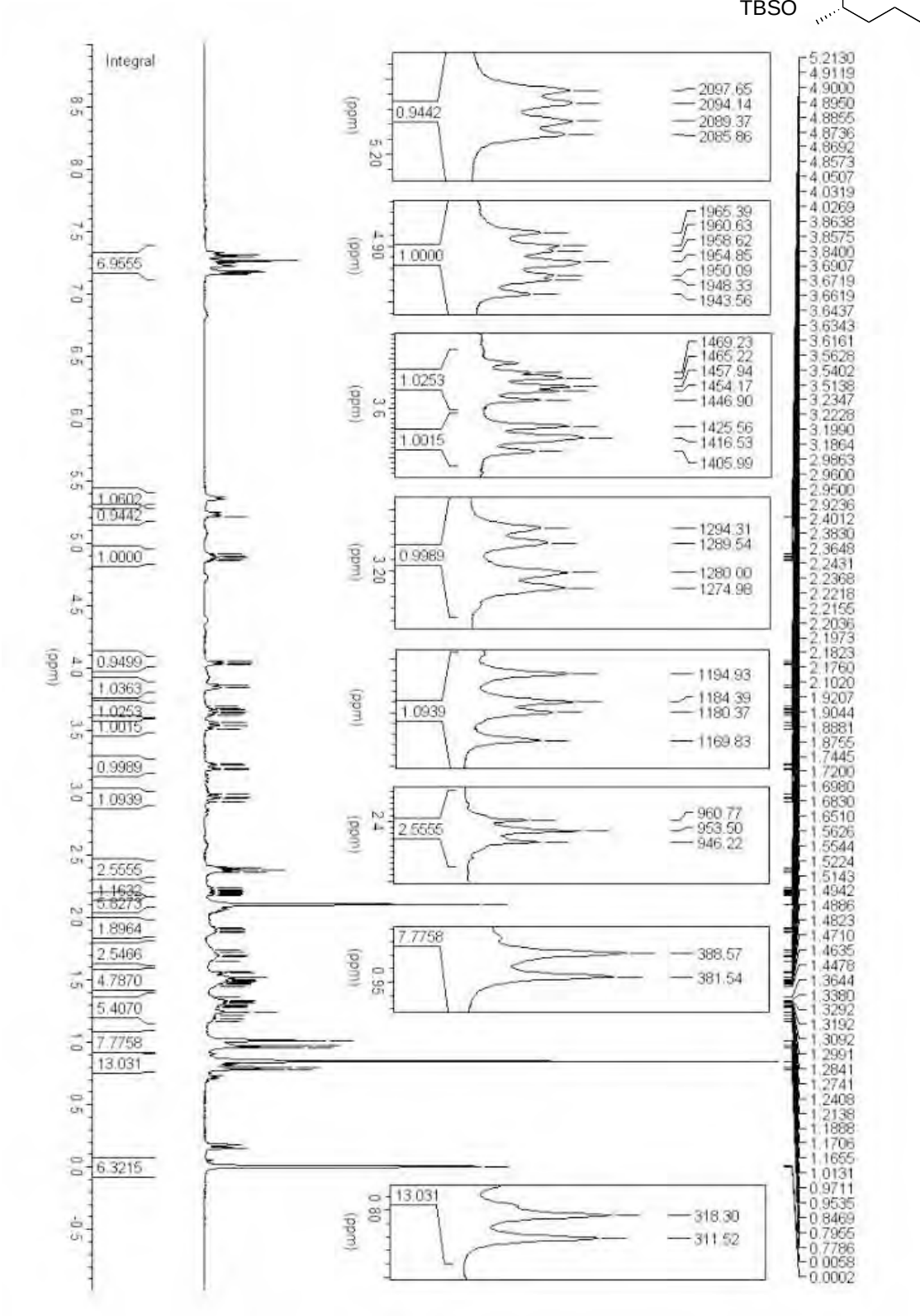
(*R*)-1-[(*R*)-2-(9*H*-Fluoren-9-yloxycarbonylamino)-3-phenyl-propionyl]-pyrrolidine-2-carboxylic acid (1*R*,2*S*)-1-[(2*S*,3*R*)-2-(*tert*-butyldimethylsilanyloxy)-3-carboxybutyl]-2-methyl-7-oxooctyl ester (II.26)



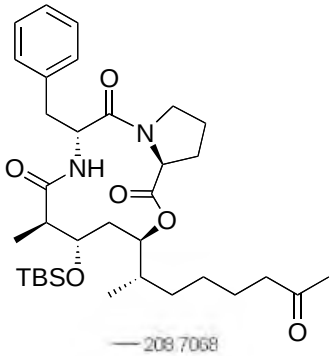


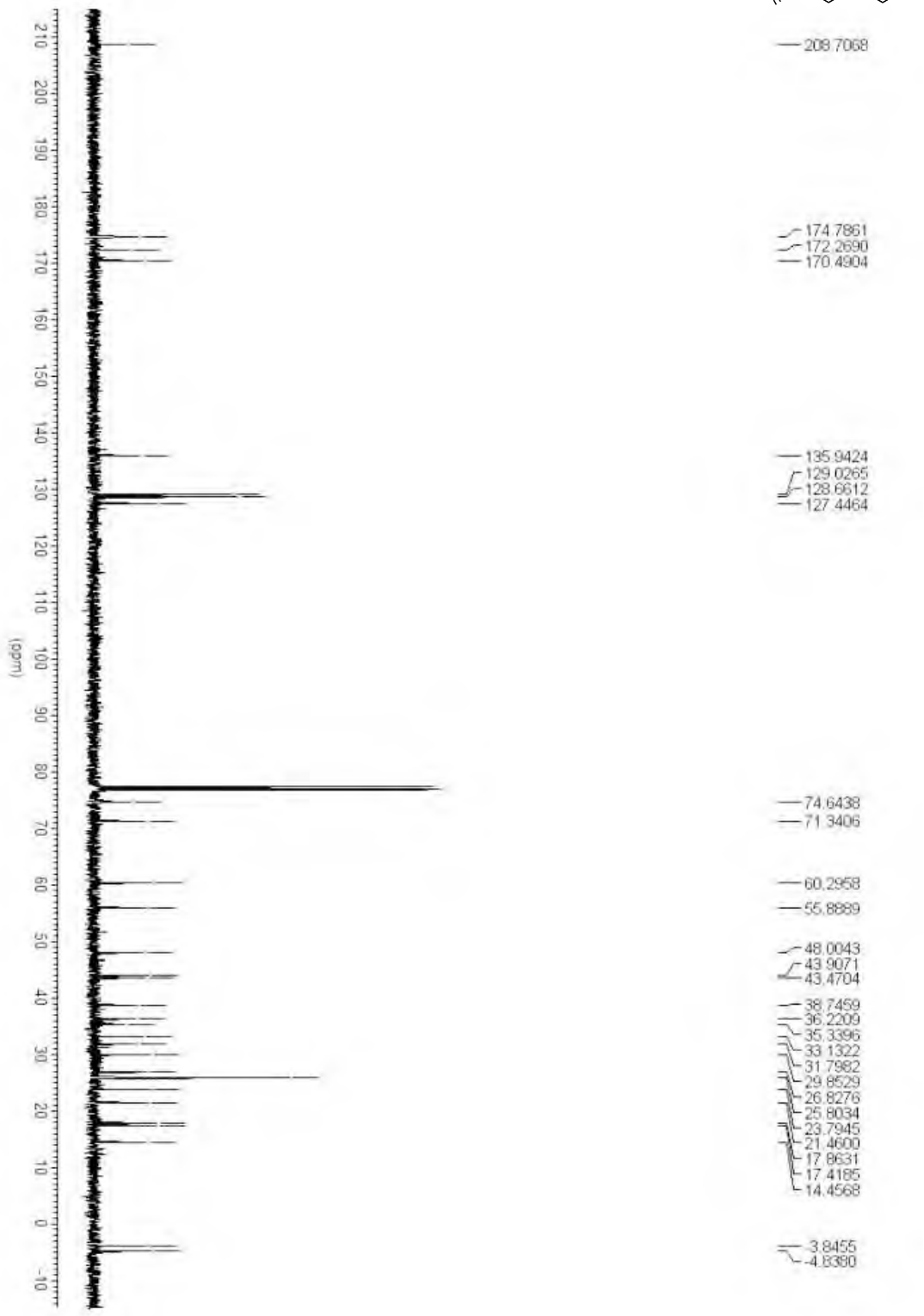
(5*R*,8*R*,9*S*,11*R*,13a*R*)-5-Benzyl-9-(*tert*-butyldimethylsilanyloxy)-8-methyl-11-((*S*)-1-methyl-6-oxoheptyl)-decahydro-12-oxa-3a,6-diazacyclopentacyclododecene-4,7,13-trione (II.27)



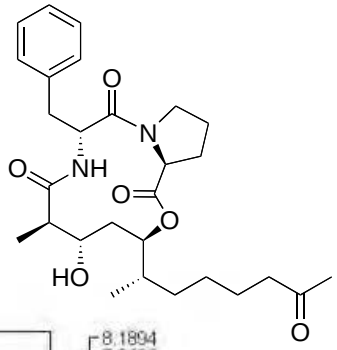


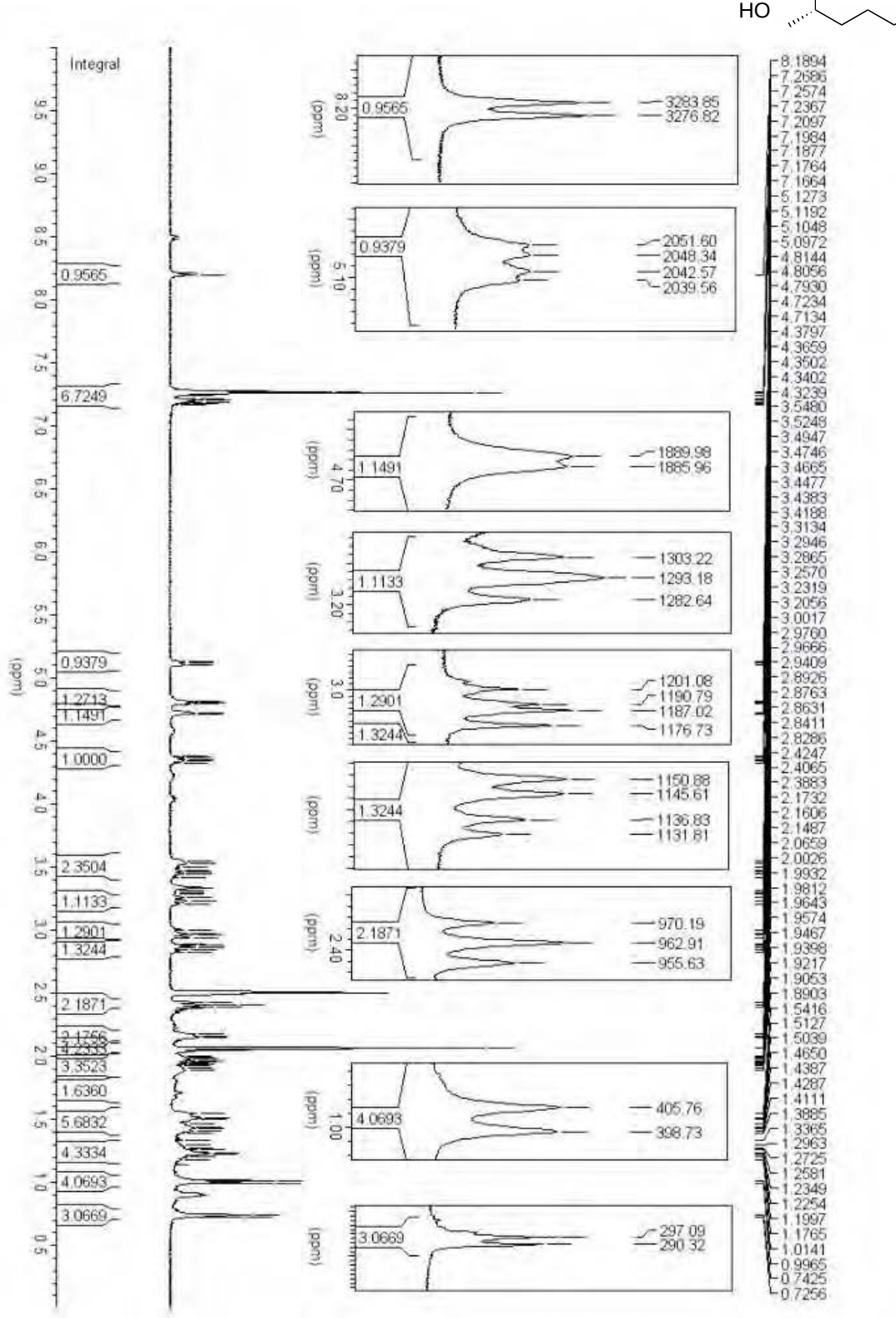
(5*R*,8*R*,9*S*,11*R*,13a*R*)-5-Benzyl-9-(*tert*-butyldimethylsilanyloxy)-8-methyl-11-((*S*)-1-methyl-6-oxoheptyl)-decahydro-12-oxa-3a,6-diazacyclopentacyclododecene-4,7,13-trione (II.27)



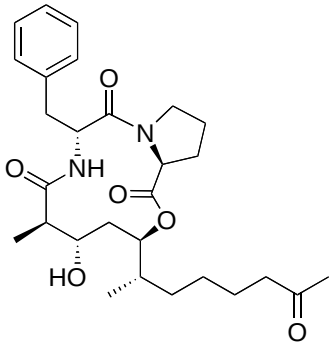


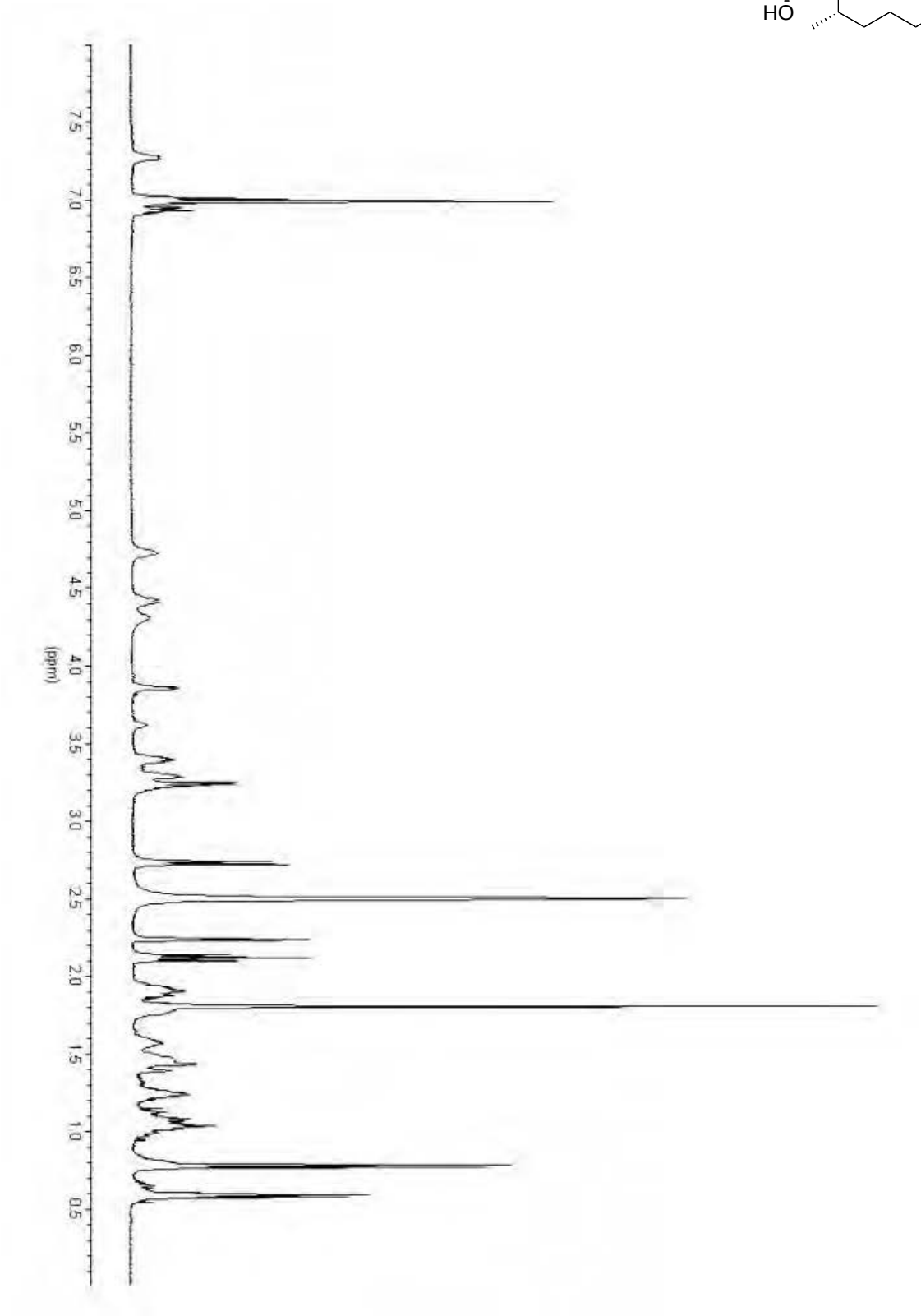
(5*R*,8*R*,9*S*,11*R*,13a*R*)-5-Benzyl-9-hydroxy-8-methyl-11-((*S*)-1-methyl-6-oxo-heptyl)-decahydro-12-oxa-3a,6-diazacyclopenta-cyclododecene-4,7,13-trione - *epi*-acremolide B (II.28)
(^{1}H NMR in d_6-DMSO at 25 °C)



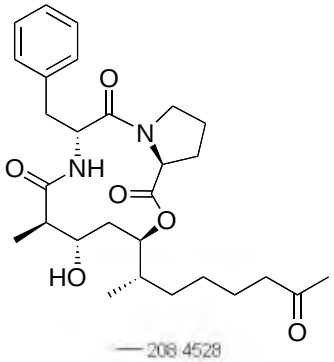


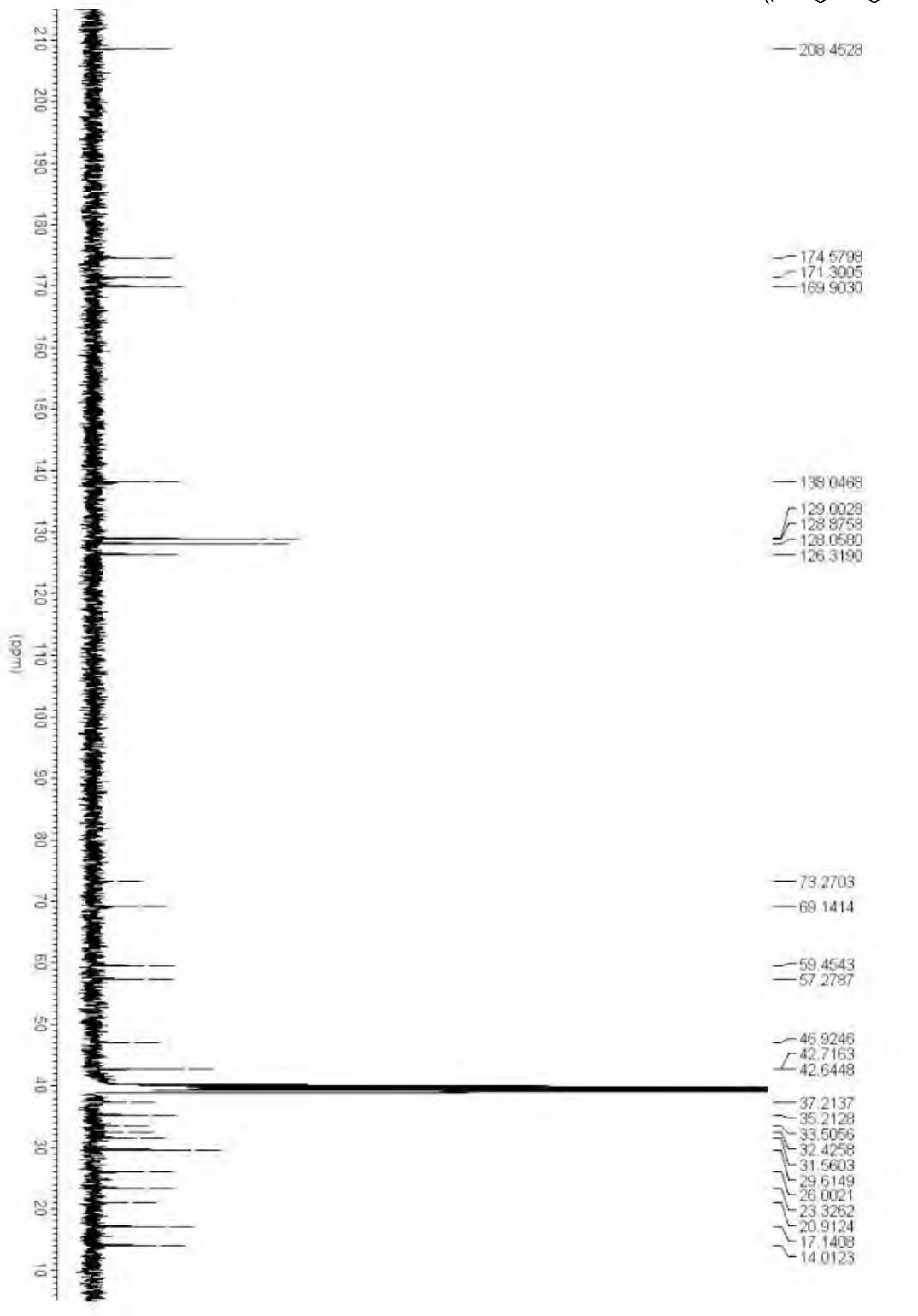
(5*R*,8*R*,9*S*,11*R*,13a*R*)-5-Benzyl-9-hydroxy-8-methyl-11-((*S*)-1-methyl-6-oxo-heptyl)-decahydro-12-oxa-3a,6-diazacyclopenta-cyclododecene-4,7,13-trione - *epi*-acremolide B (II.28)
(^{1}H NMR in d_6-DMSO at 140 °C)





(5*R*,8*R*,9*S*,11*R*,13a*R*)-5-Benzyl-9-hydroxy-8-methyl-11-((*S*)-1-methyl-6-oxo-heptyl)-decahydro-12-oxa-3a,6-diazacyclopenta-cyclododecene-4,7,13-trione - *epi*-acremolide B (II.28)
(^{13}C NMR in d_6-DMSO at 25 °C)

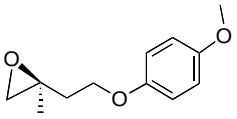


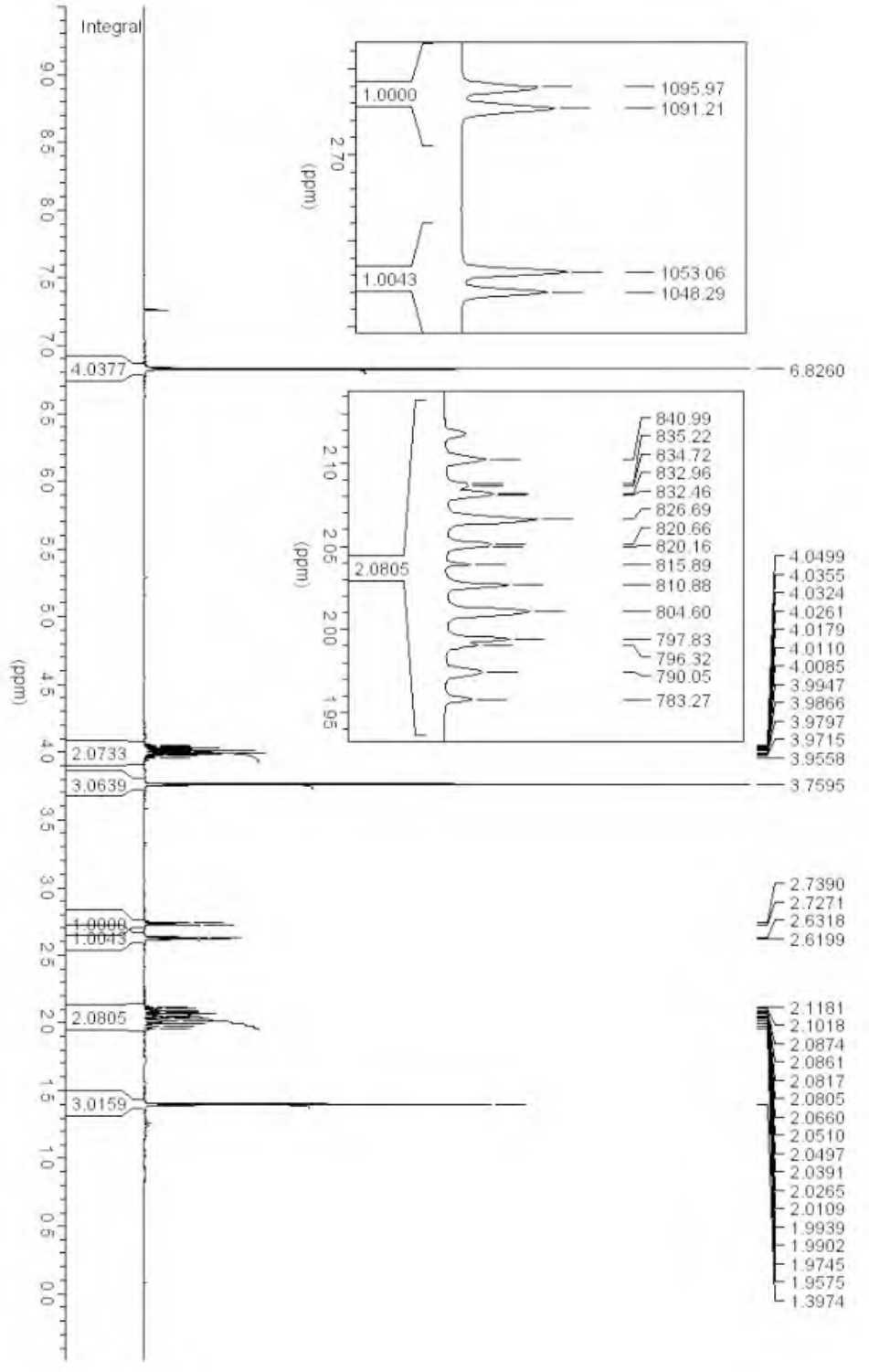


ANNEX IV

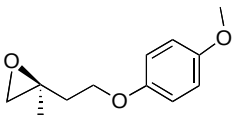
A.IV.1. Copies of ^{1}H NMR & ^{13}C NMR

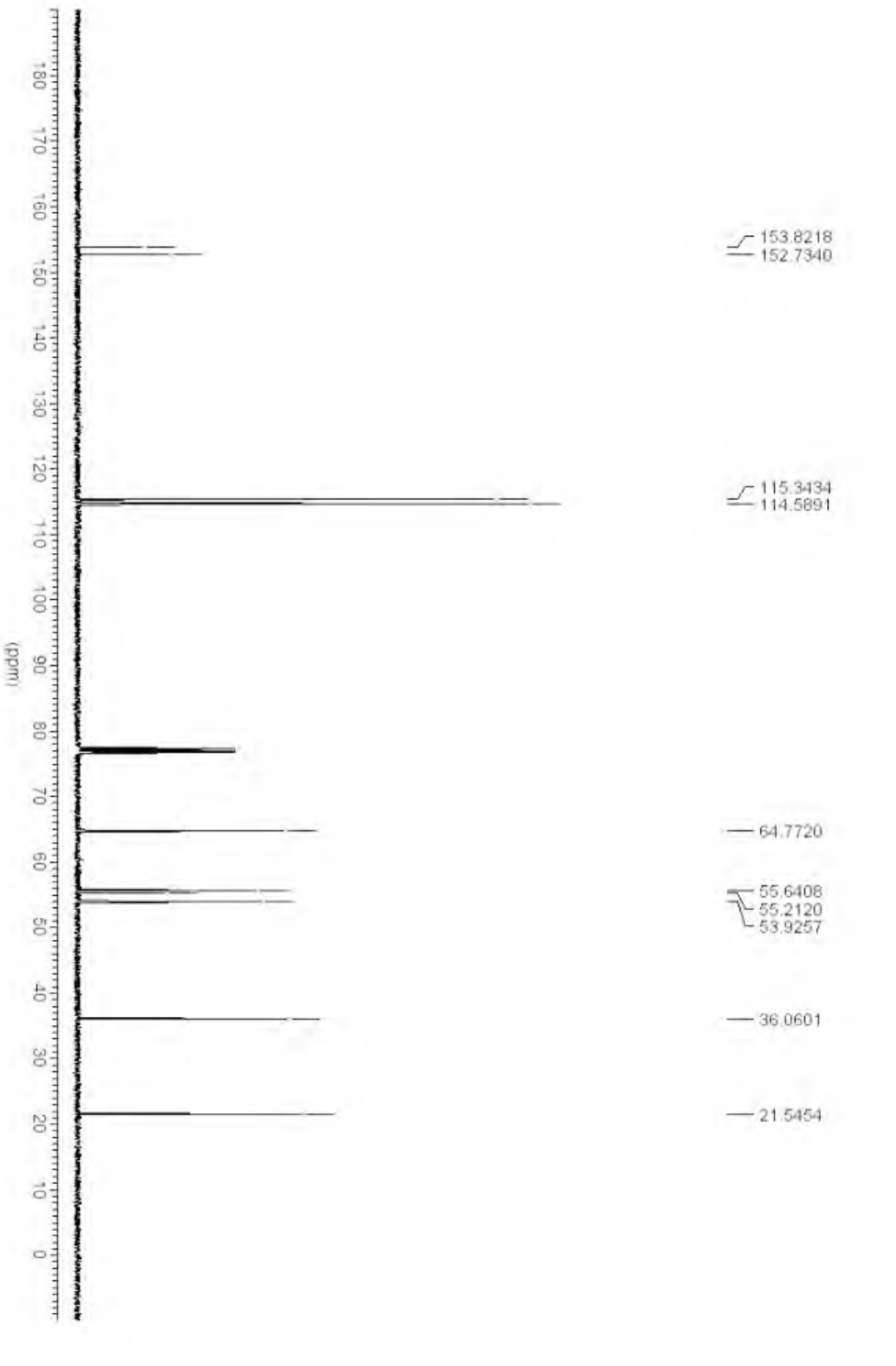
(2*S*)-2-[2-(4-Methoxyphenoxy)ethyl]-2-methyloxirane (II.64)



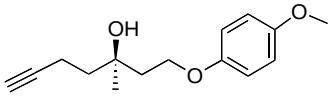


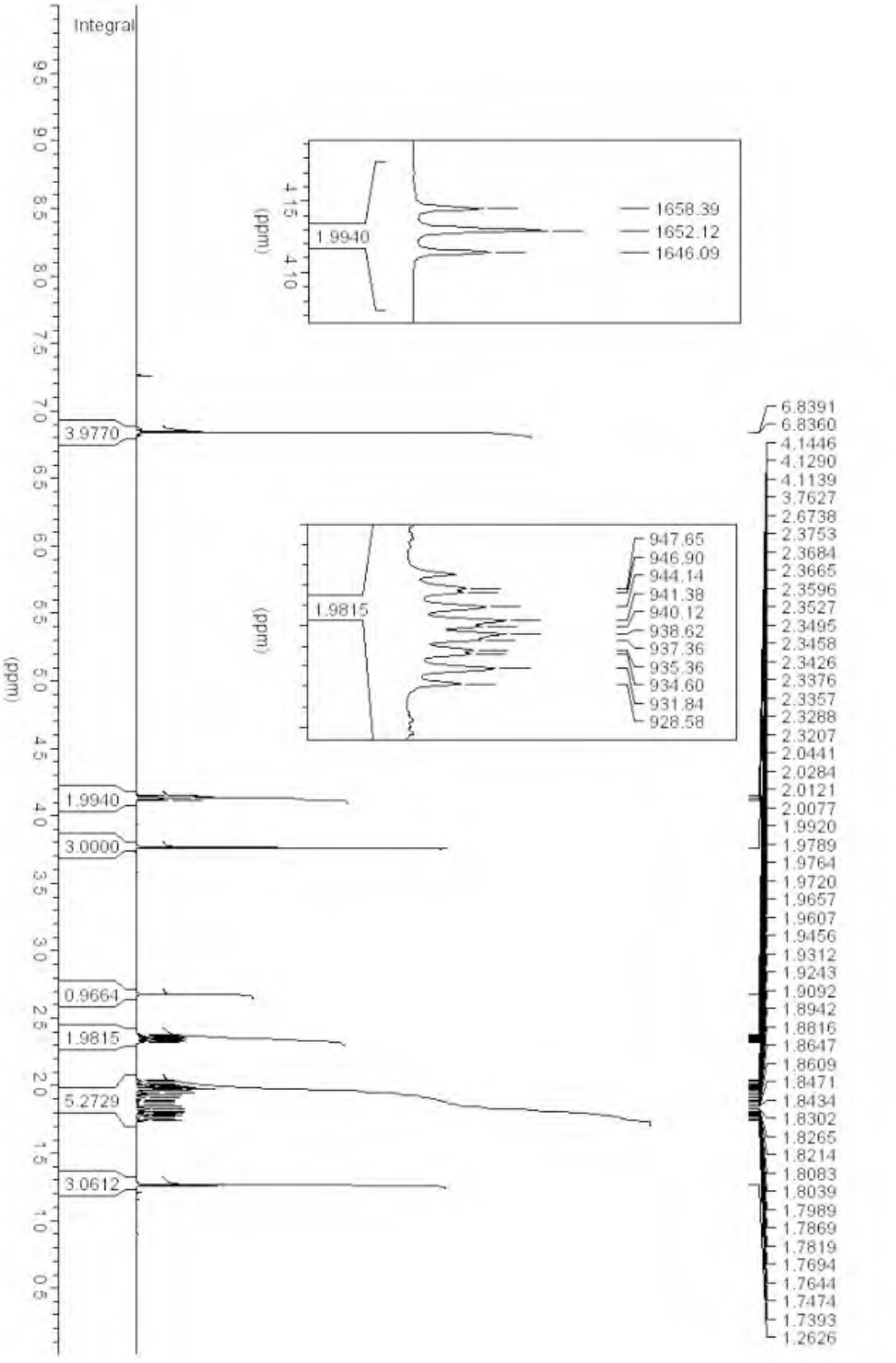
(2*S*)-2-[2-(4-Methoxyphenoxy)ethyl]-2-methyloxirane (II.64)



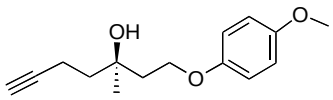


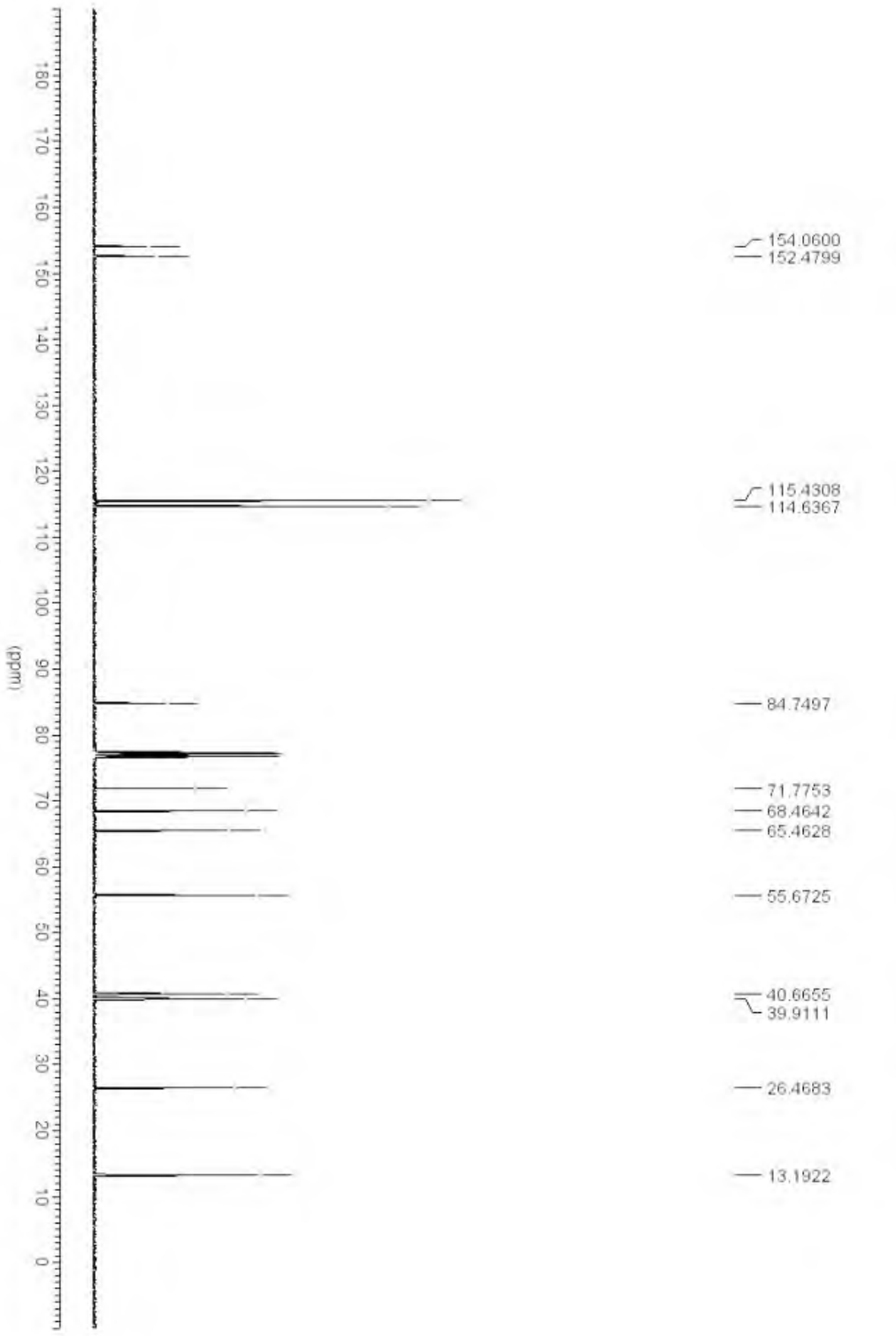
(3*R*)-1-(4-Methoxyphenoxy)-3-methylhept-6-yn-3-ol (II.65)



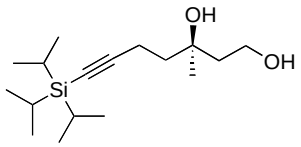


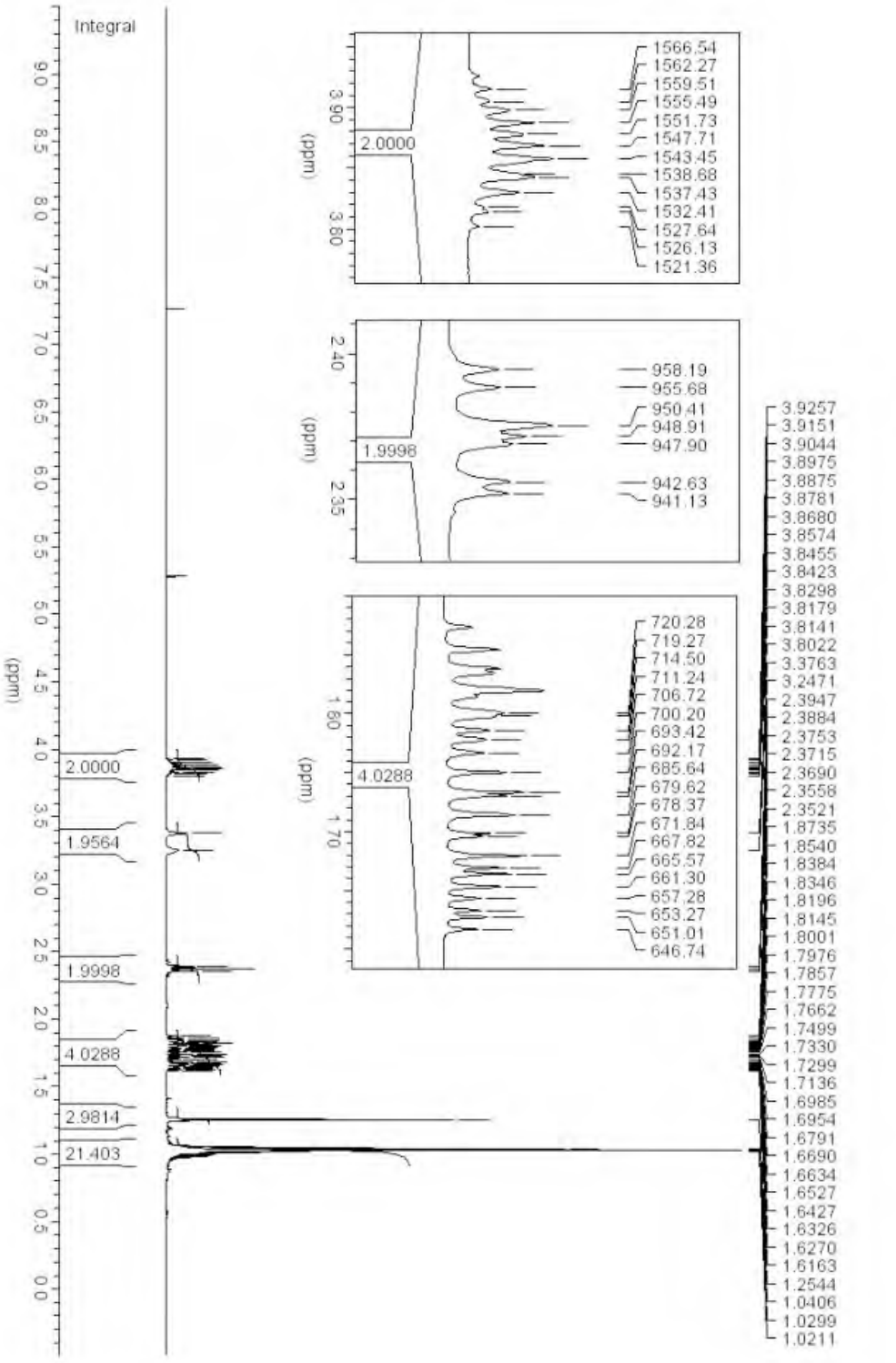
(3*R*)-1-(4-Methoxyphenoxy)-3-methylhept-6-yn-3-ol (II.65)



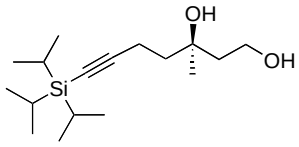


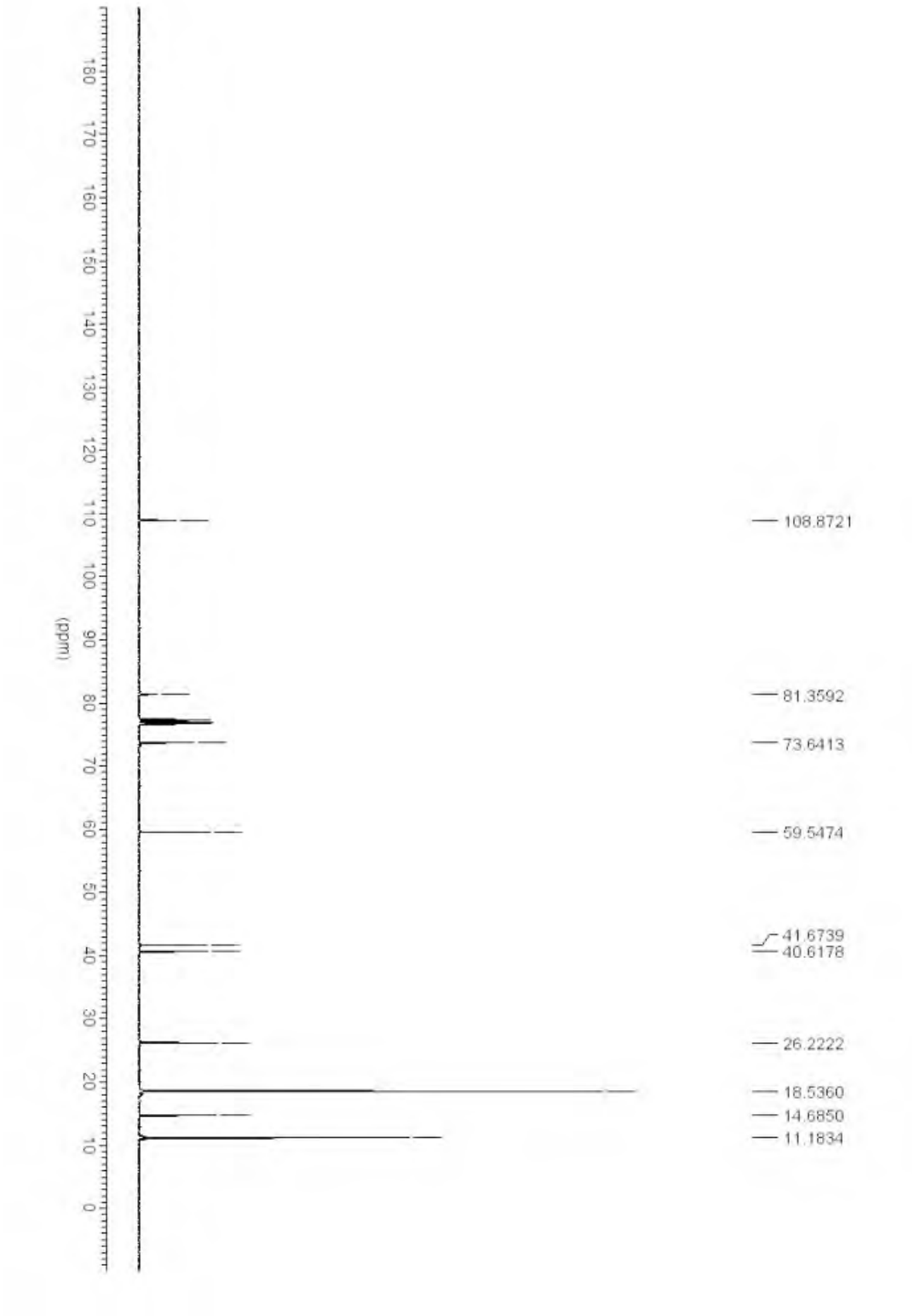
(3*R*)-3-Methyl-7-[tris(propan-2-yl)silyl]hept-6-yne-1,3-diol (II.67)



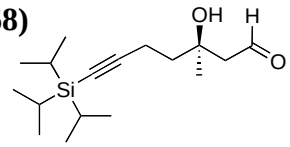


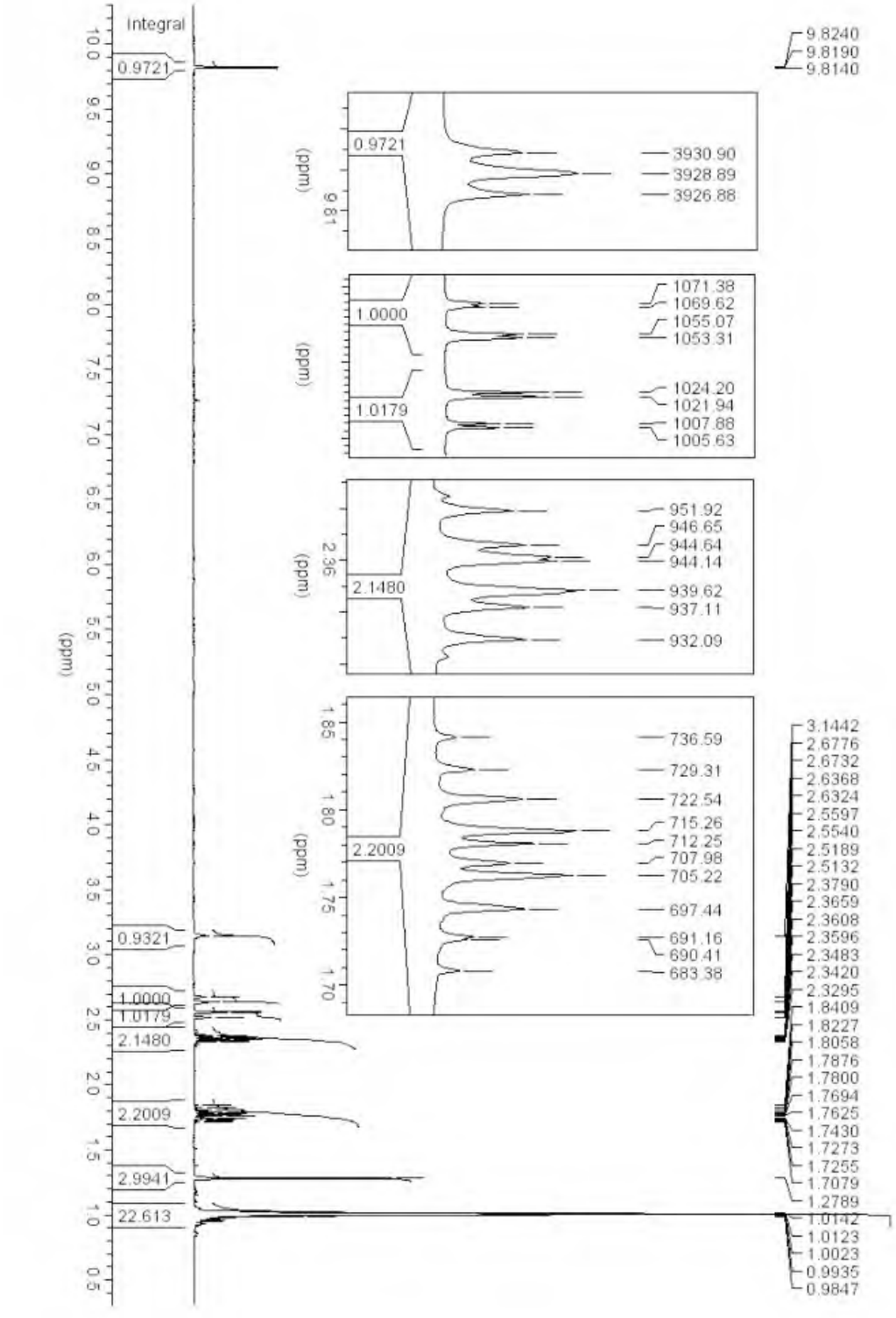
(3*R*)-3-Methyl-7-[tris(propan-2-yl)silyl]hept-6-yne-1,3-diol (II.67)



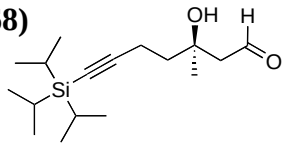


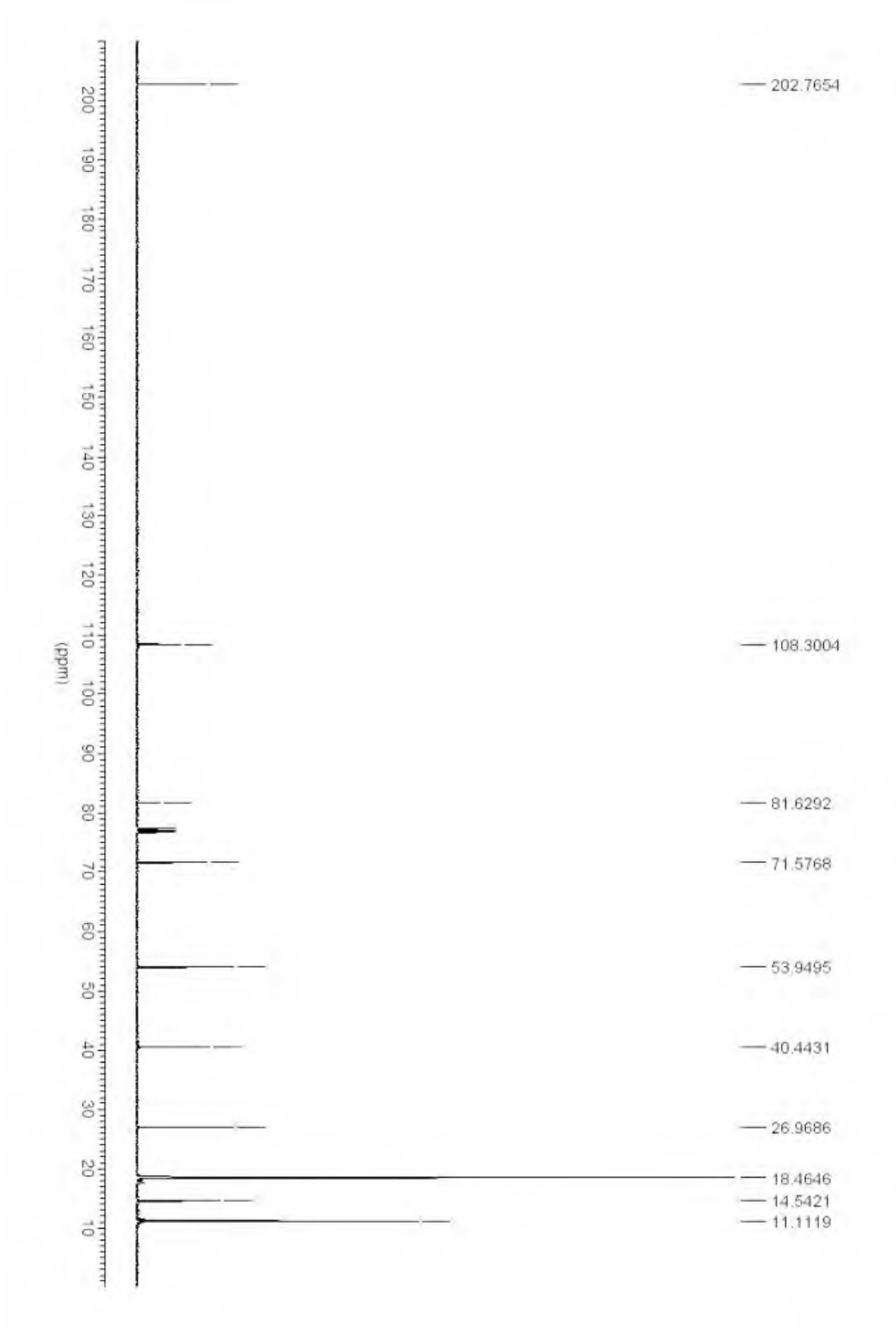
(3*R*)-3-Hydroxy-3-methyl-7-[tris(propan-2-yl)silyl]hept-6-ynal (II.68)



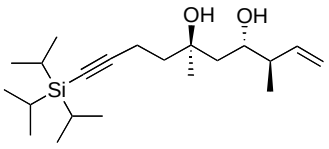


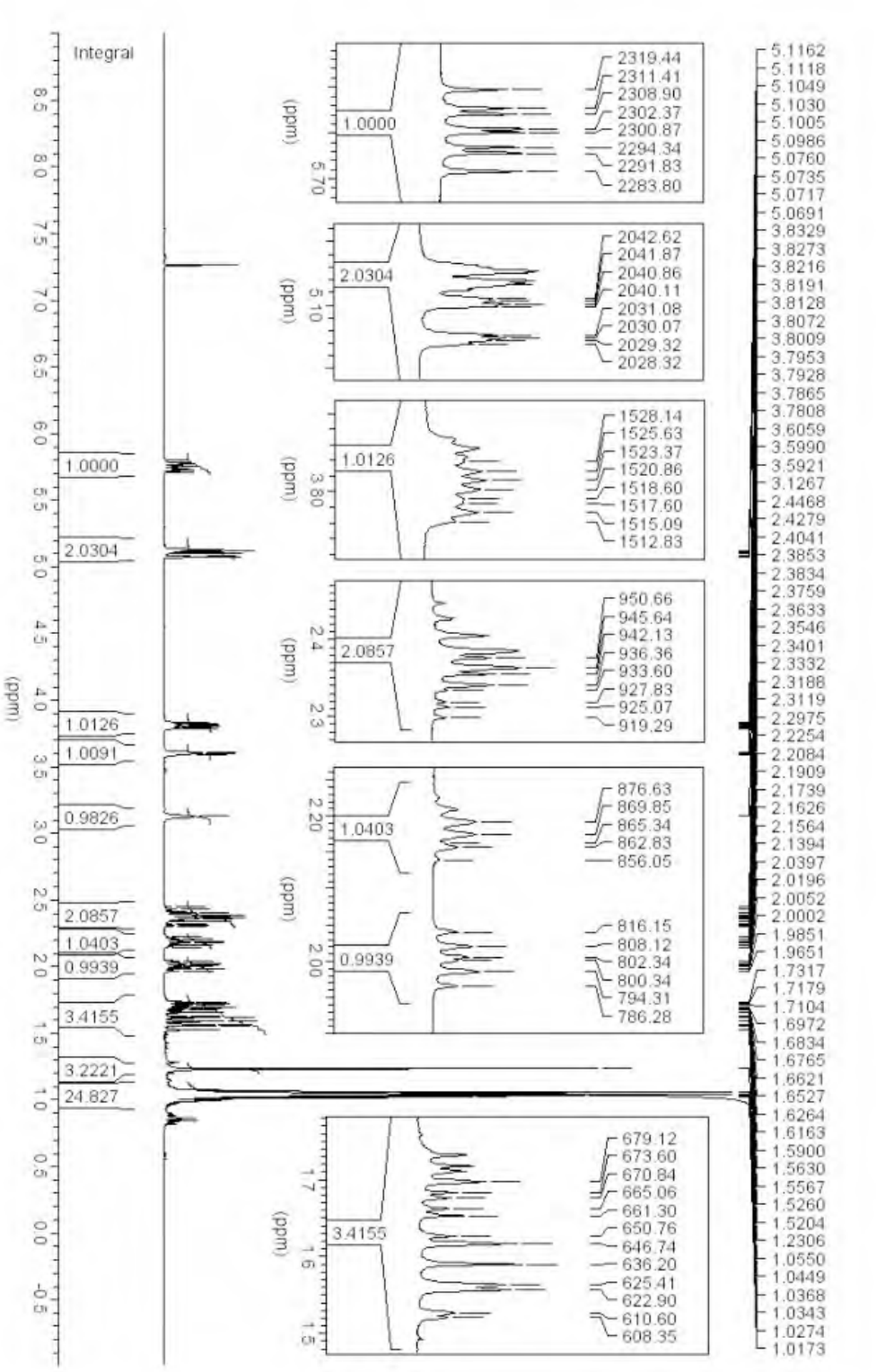
(3*R*)-3-Hydroxy-3-methyl-7-[tris(propan-2-yl)silyl]hept-6-ynal (II.68)



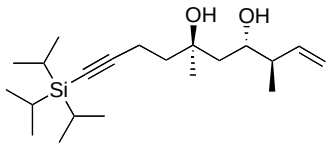


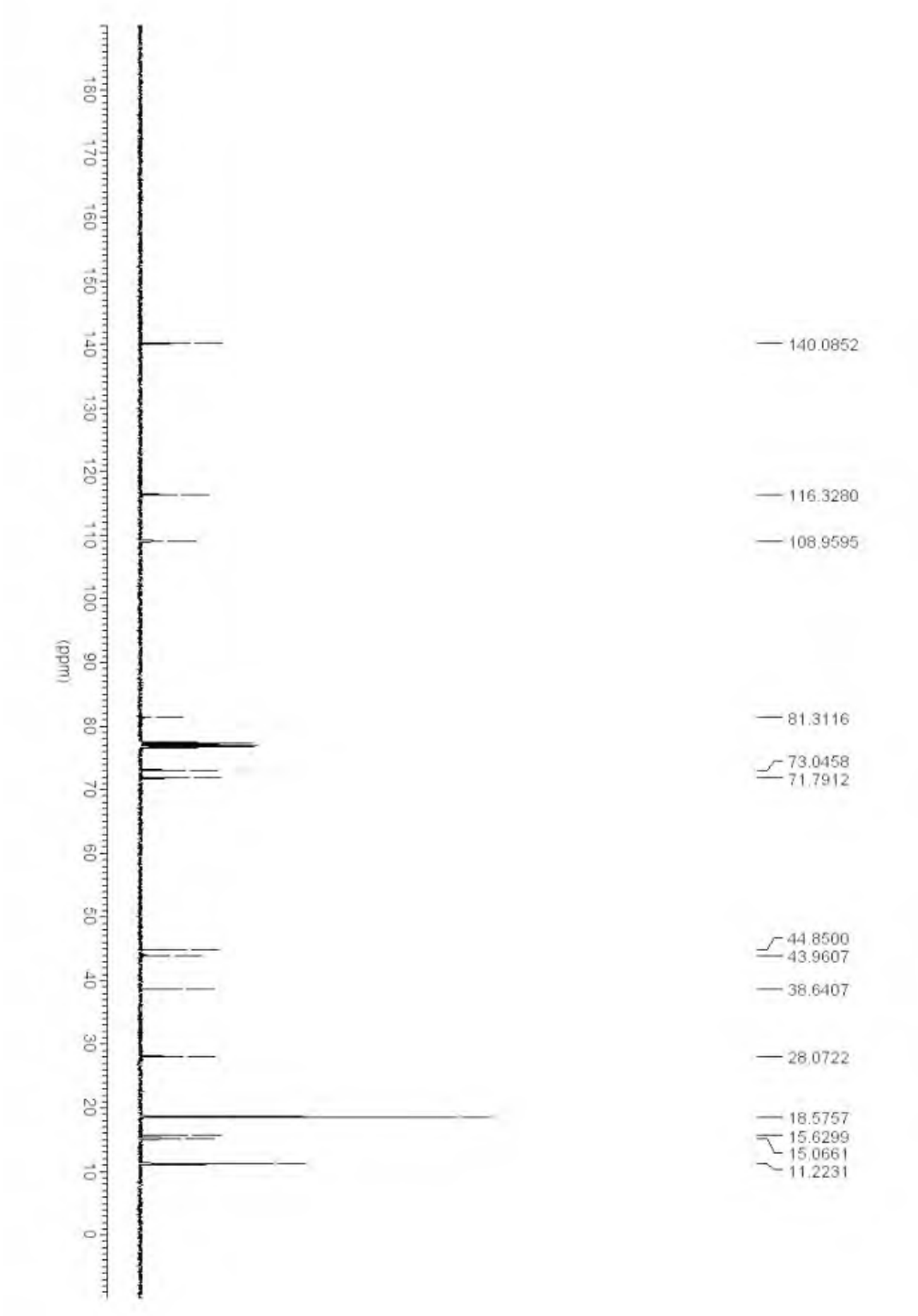
(3*R*,4*S*,6*R*)-3,6-Dimethyl-10-[tris(propan-2-yl)silyl]dec-1-en-9-yne-4,6-diol (II.69)



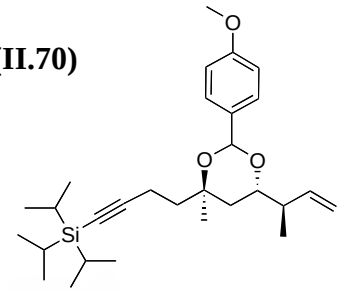


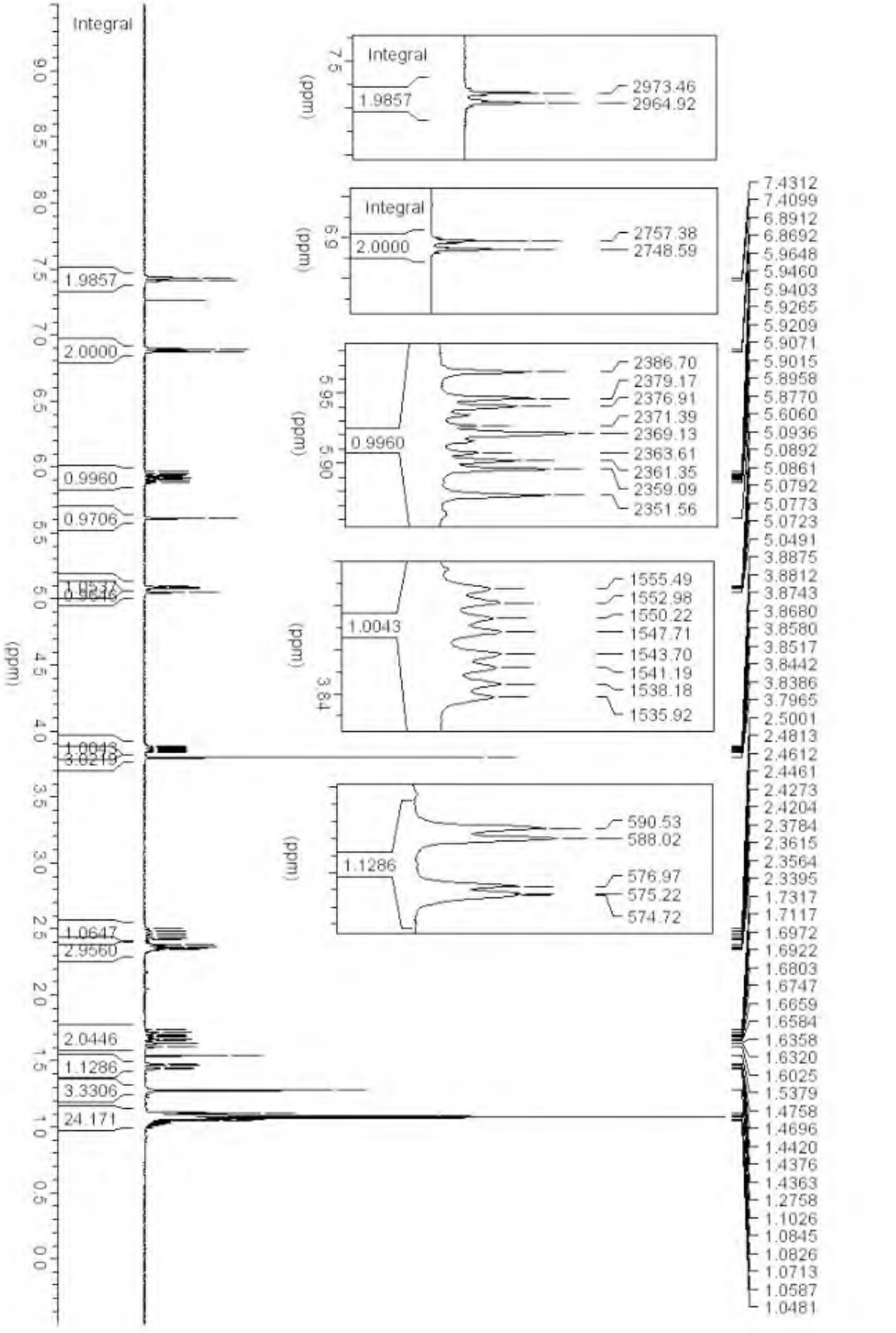
(3*R*,4*S*,6*R*)-3,6-Dimethyl-10-[tris(propan-2-yl)silyl]dec-1-en-9-yne-4,6-diol (II.69)



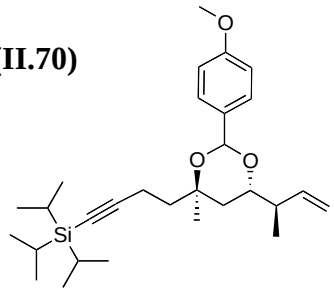


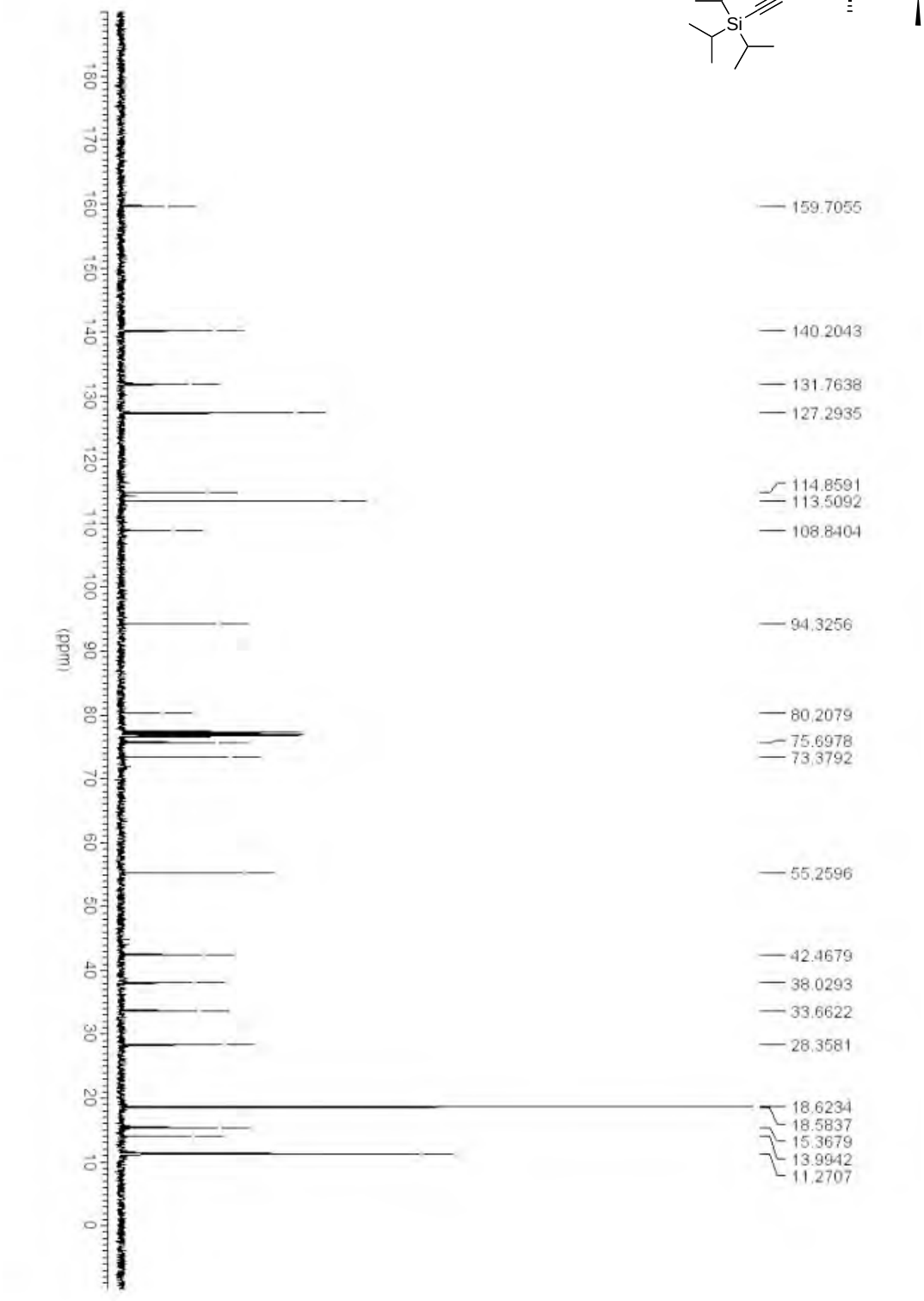
{4-[(4*R*,6*S*)-6-[(2*R*)-But-3-en-2-yl]-2-(4-methoxyphenyl)-4-methyl-1,3-dioxan-4-yl]but-1-yn-1-yl}tris(propan-2-yl)silane (II.70)



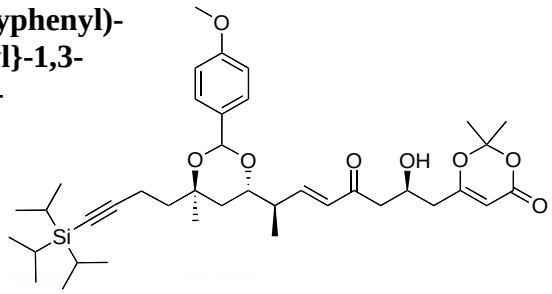


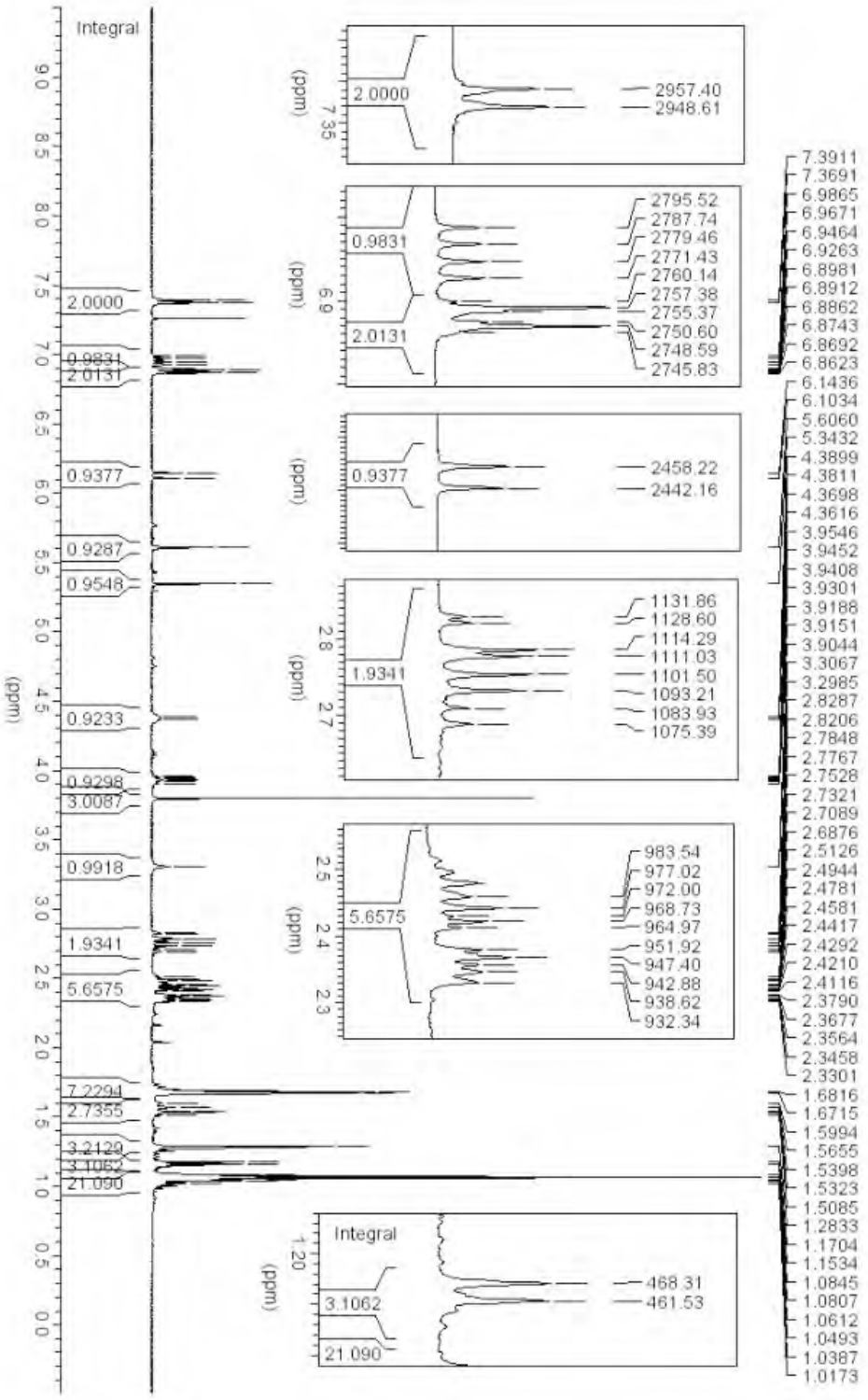
{4-[(4*R*,6*S*)-6-[(2*R*)-But-3-en-2-yl]-2-(4-methoxyphenyl)-4-methyl-1,3-dioxan-4-yl]but-1-yn-1-yl}tris(propan-2-yl)silane (II.70)



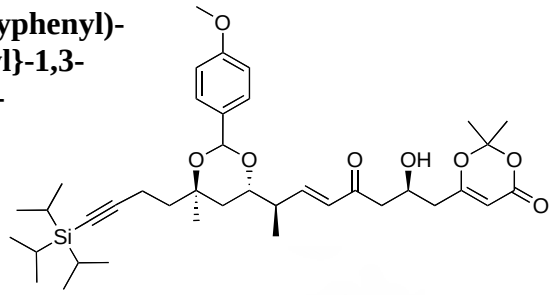


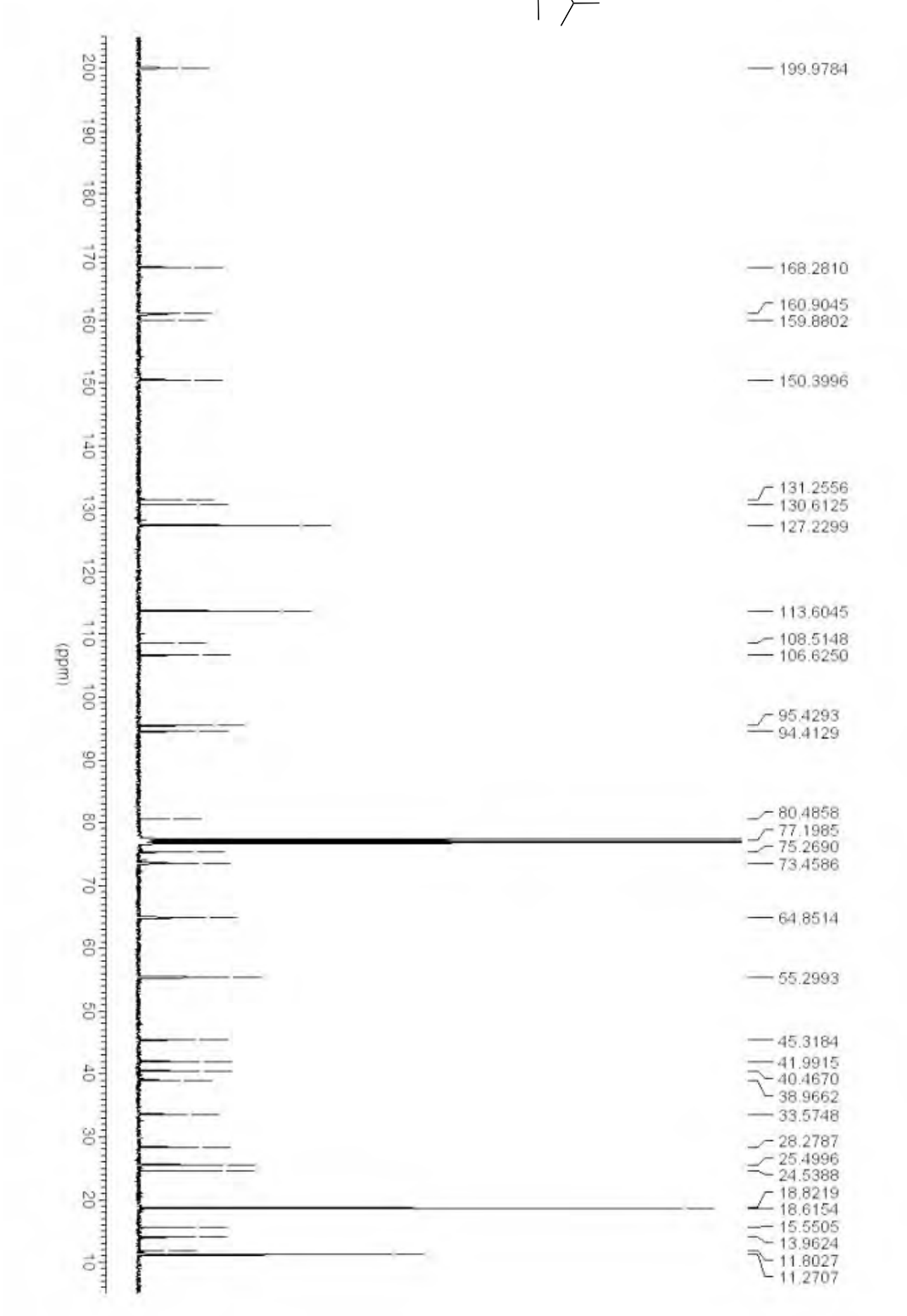
6-[(2*S*,5*E*,7*R*)-2-Hydroxy-7-[(4*S*,6*R*)-2-(4-methoxyphenyl)-6-methyl-6-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-1,3-dioxan-4-yl]-4-oxooct-5-en-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.71)



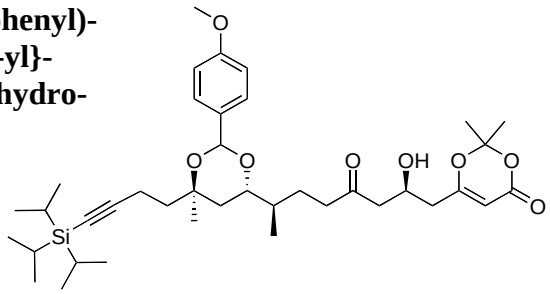


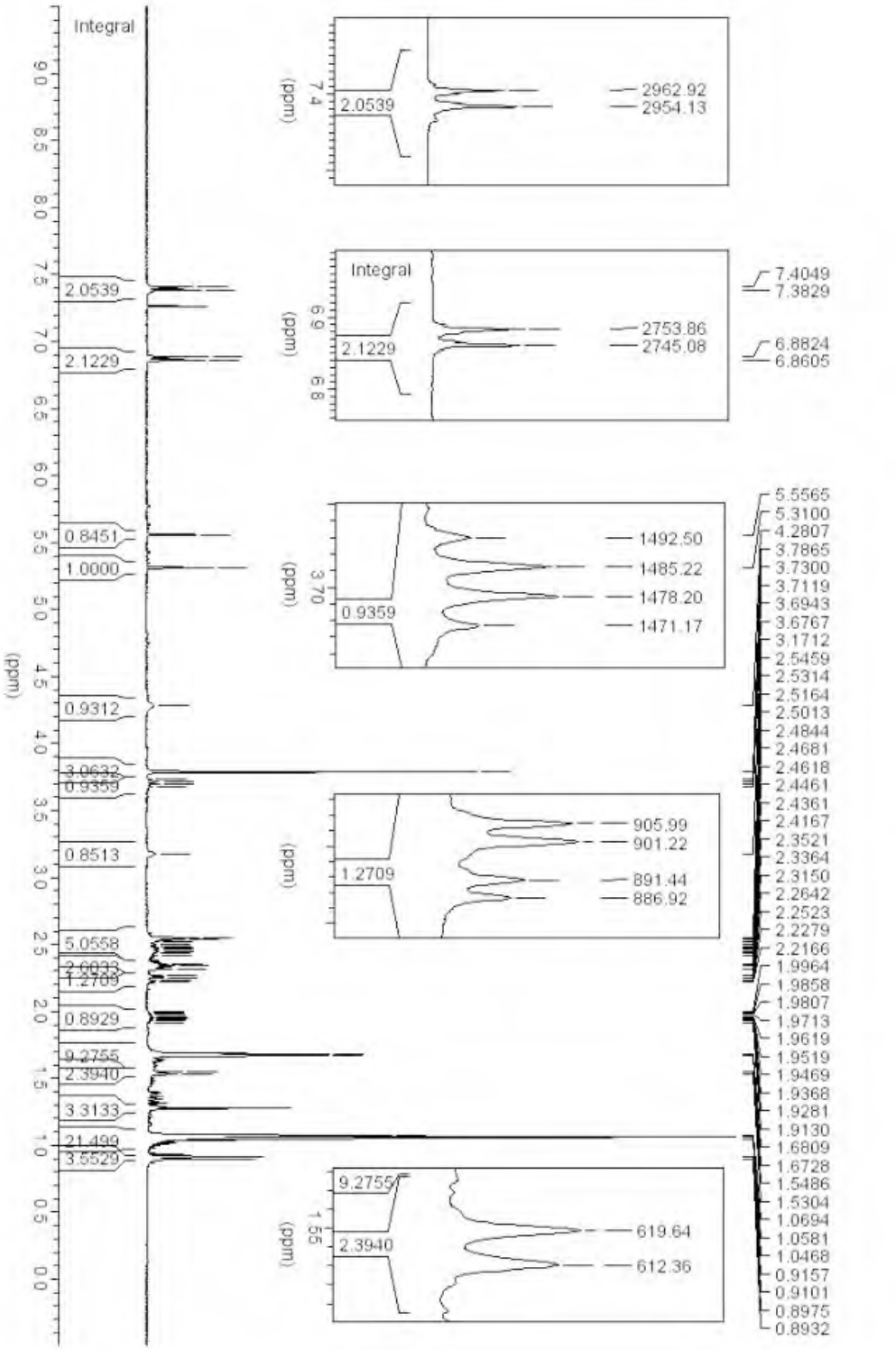
6-[(2*S*,5*E*,7*R*)-2-Hydroxy-7-[(4*S*,6*R*)-2-(4-methoxyphenyl)-6-methyl-6-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-1,3-dioxan-4-yl]-4-oxooct-5-en-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.71)



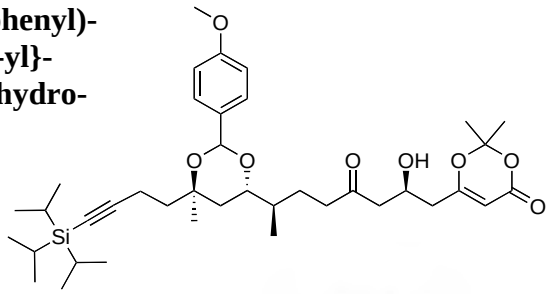


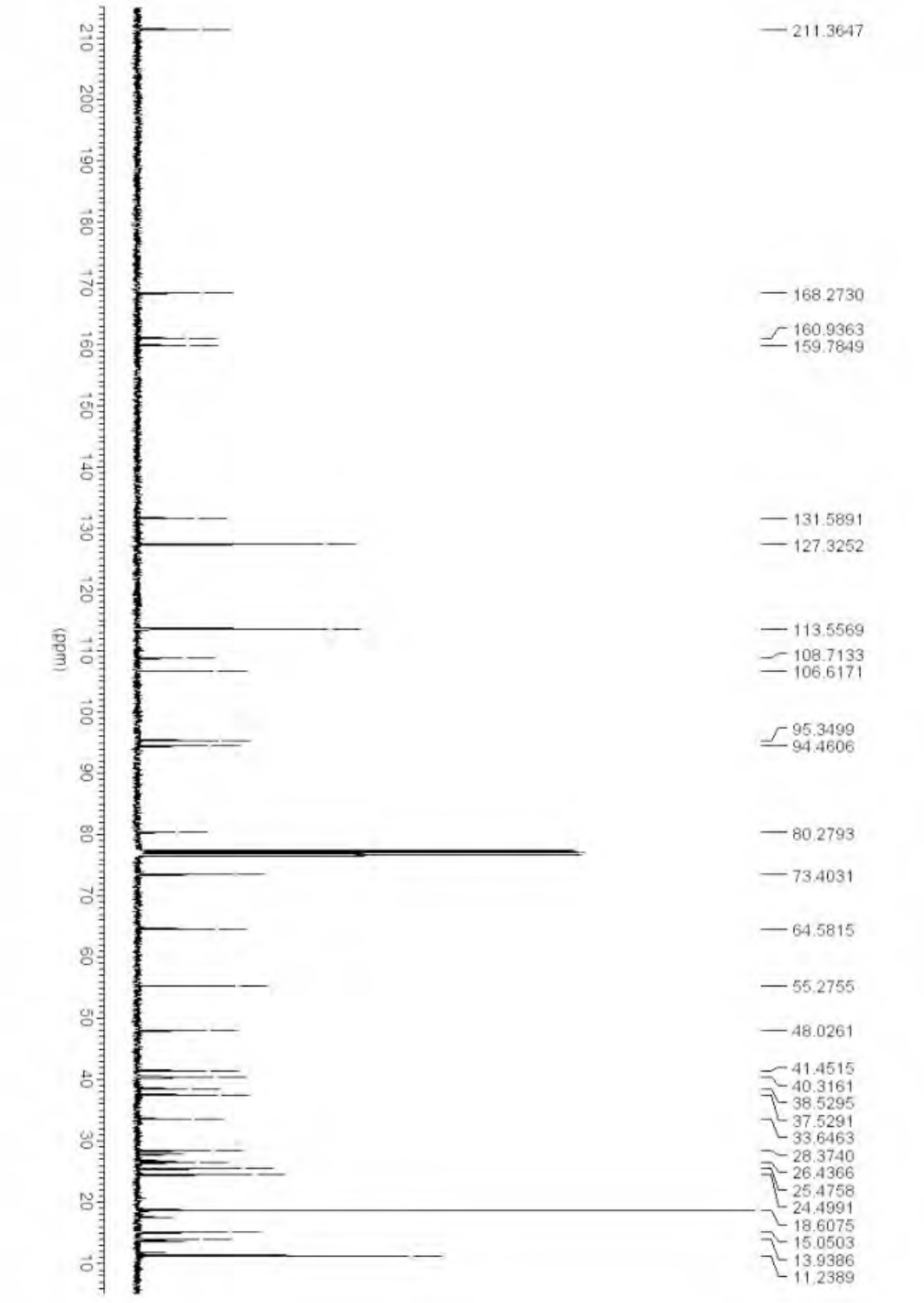
6-[(2*S*,7*R*)-2-Hydroxy-7-[(4*S*,6*R*)-2-(4-methoxyphenyl)-6-methyl-6-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-1,3-dioxan-4-yl]-4-oxooctyl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.72)



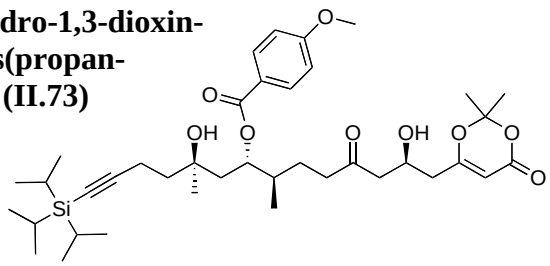


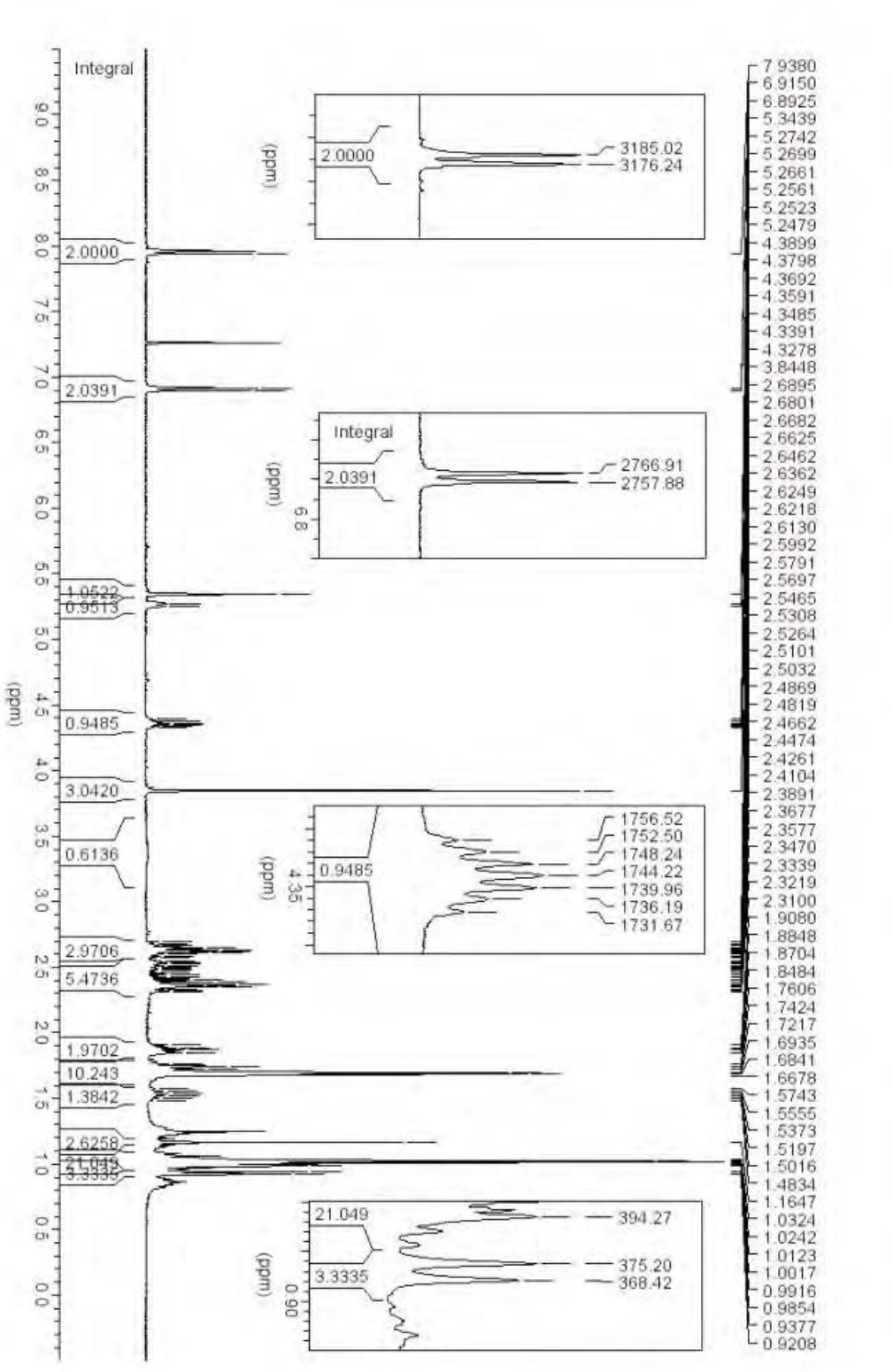
6-[(2*S*,7*R*)-2-Hydroxy-7-[(4*S*,6*R*)-2-(4-methoxyphenyl)-6-methyl-6-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-1,3-dioxan-4-yl]-4-oxooctyl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.72)



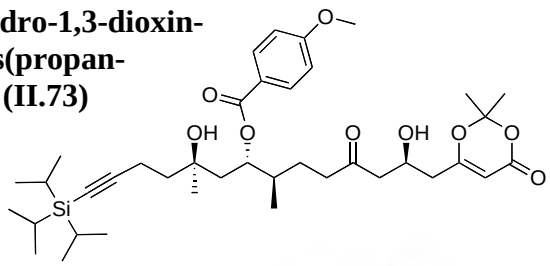


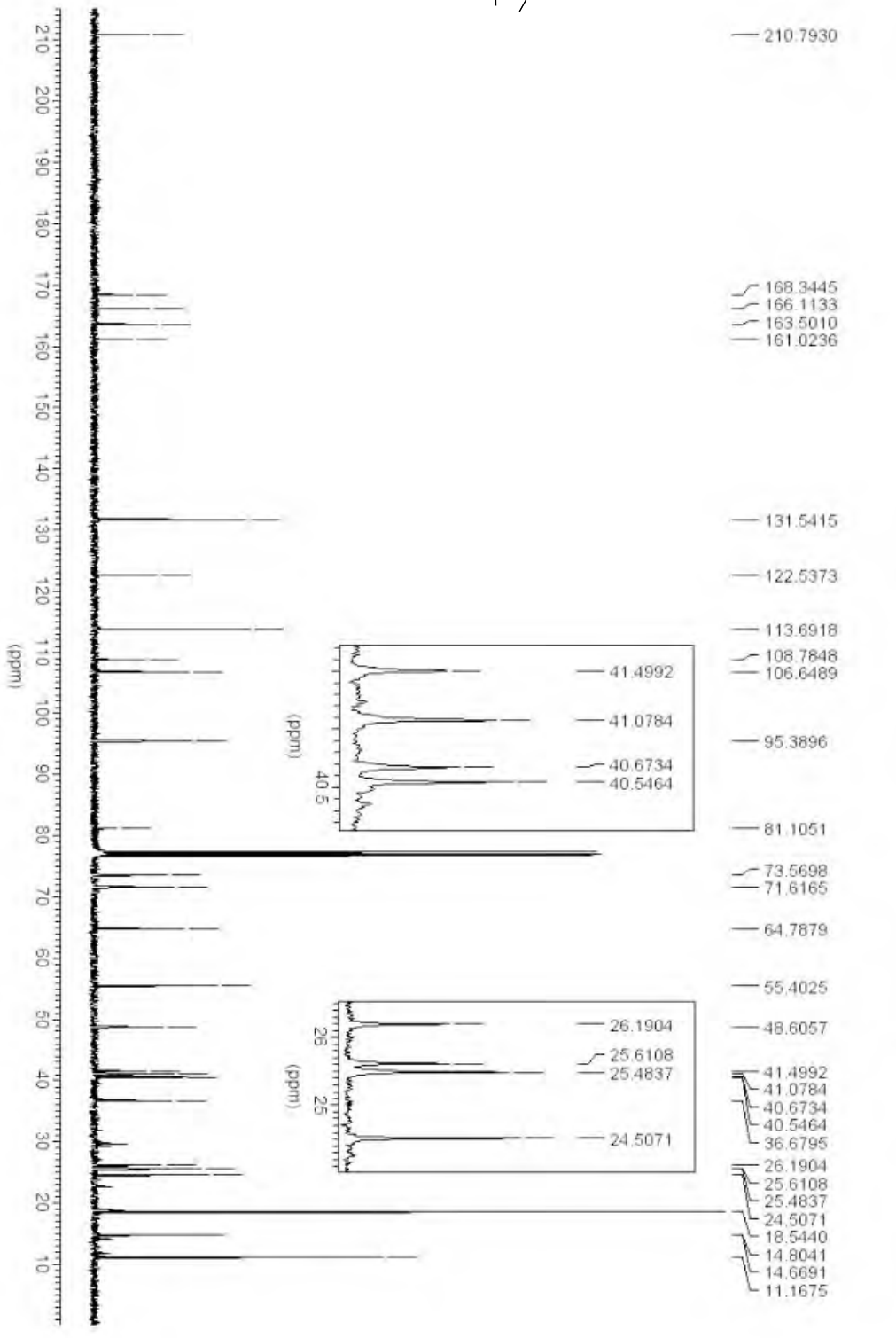
(5*R*,7*S*,8*R*,13*S*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5,13-dihydroxy-5,8-dimethyl-11-oxo-1-[tris(propan-2-yl)silyl]tetradec-1-yn-7-yl 4-methoxybenzoate (II.73)



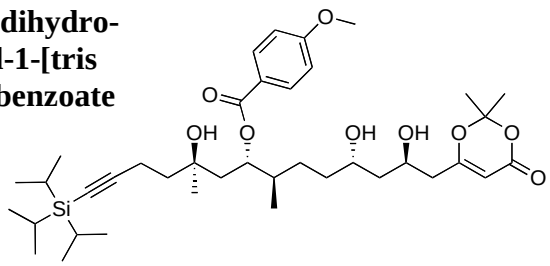


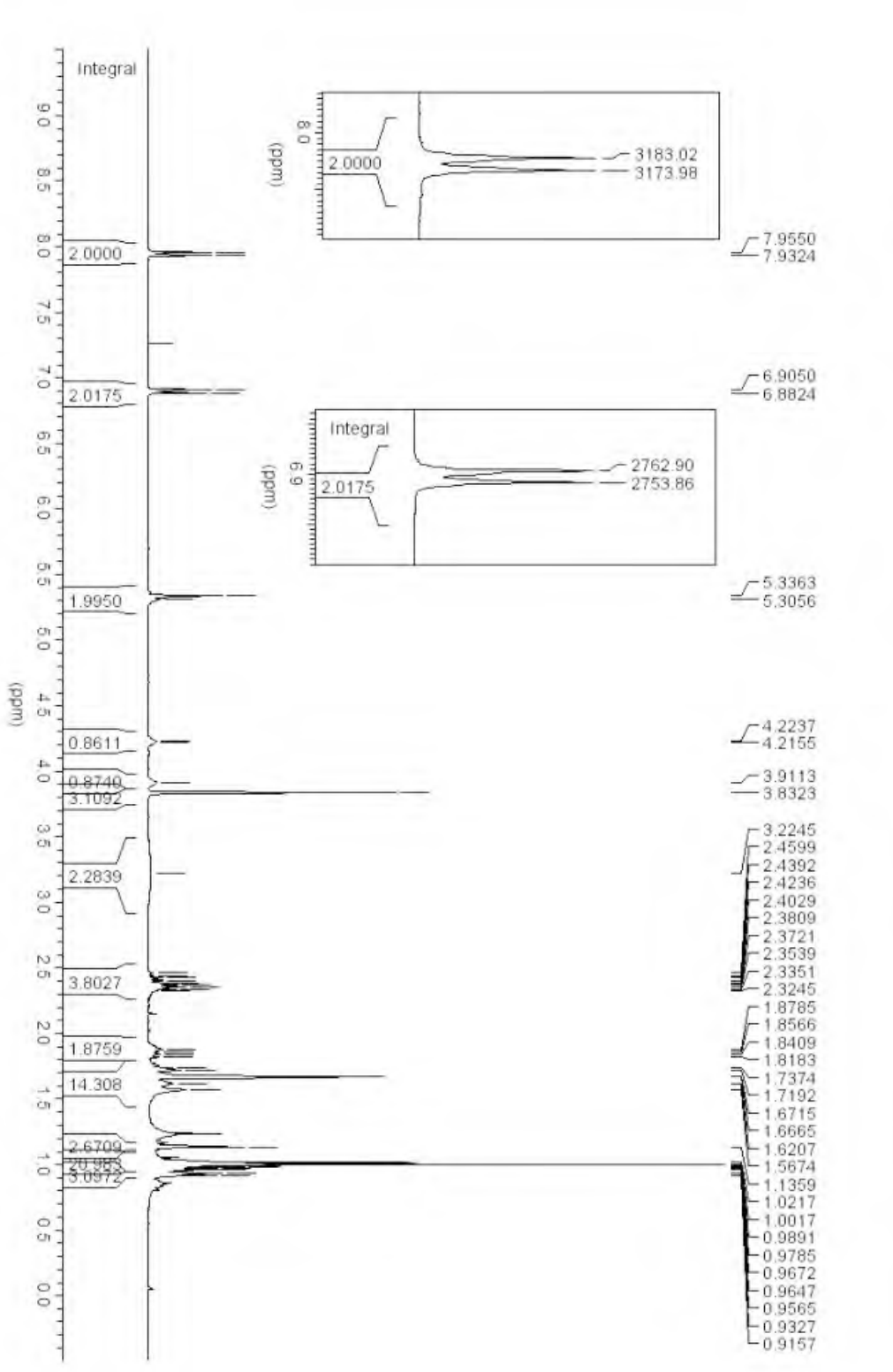
(5*R*,7*S*,8*R*,13*S*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5,13-dihydroxy-5,8-dimethyl-11-oxo-1-[tris(propan-2-yl)silyl]tetradec-1-yn-7-yl 4-methoxybenzoate (II.73)



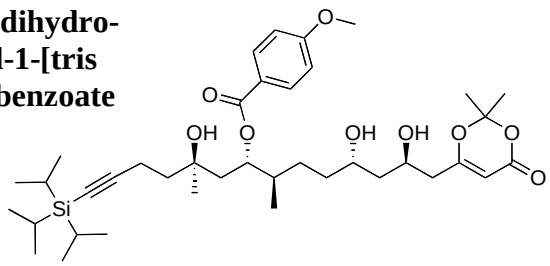


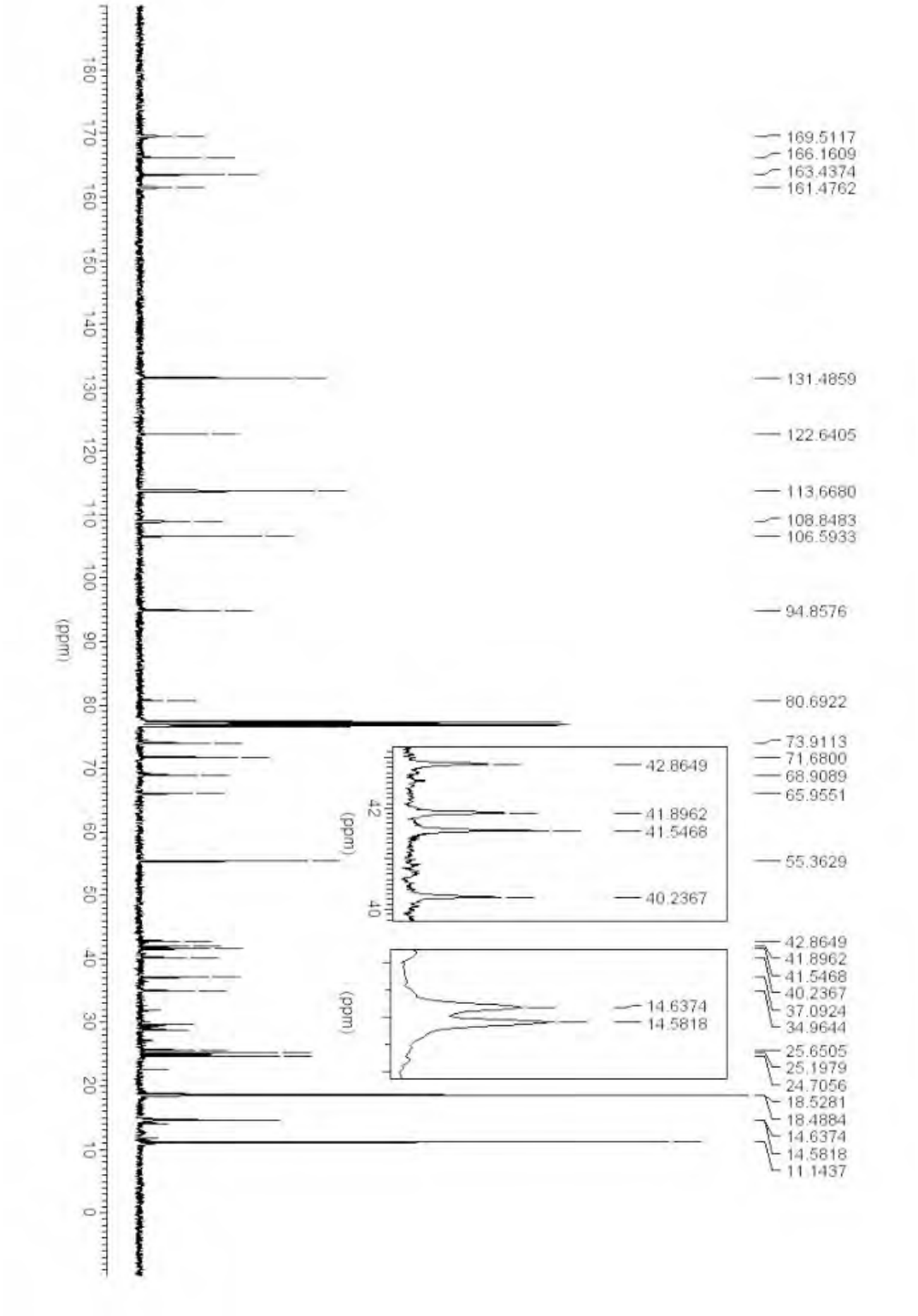
(5*R*,7*S*,8*R*,11*S*,13*R*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5,11,13-trihydroxy-5,8-dimethyl-1-[tris(propan-2-yl)silyl]tetradec-1-yn-7-yl 4-methoxybenzoate (II.74)



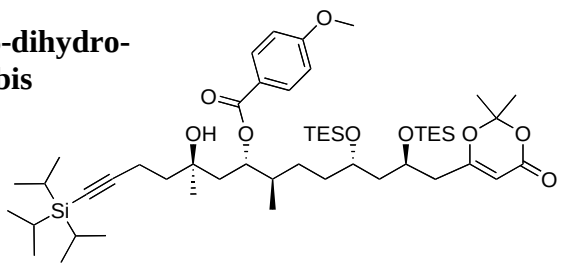


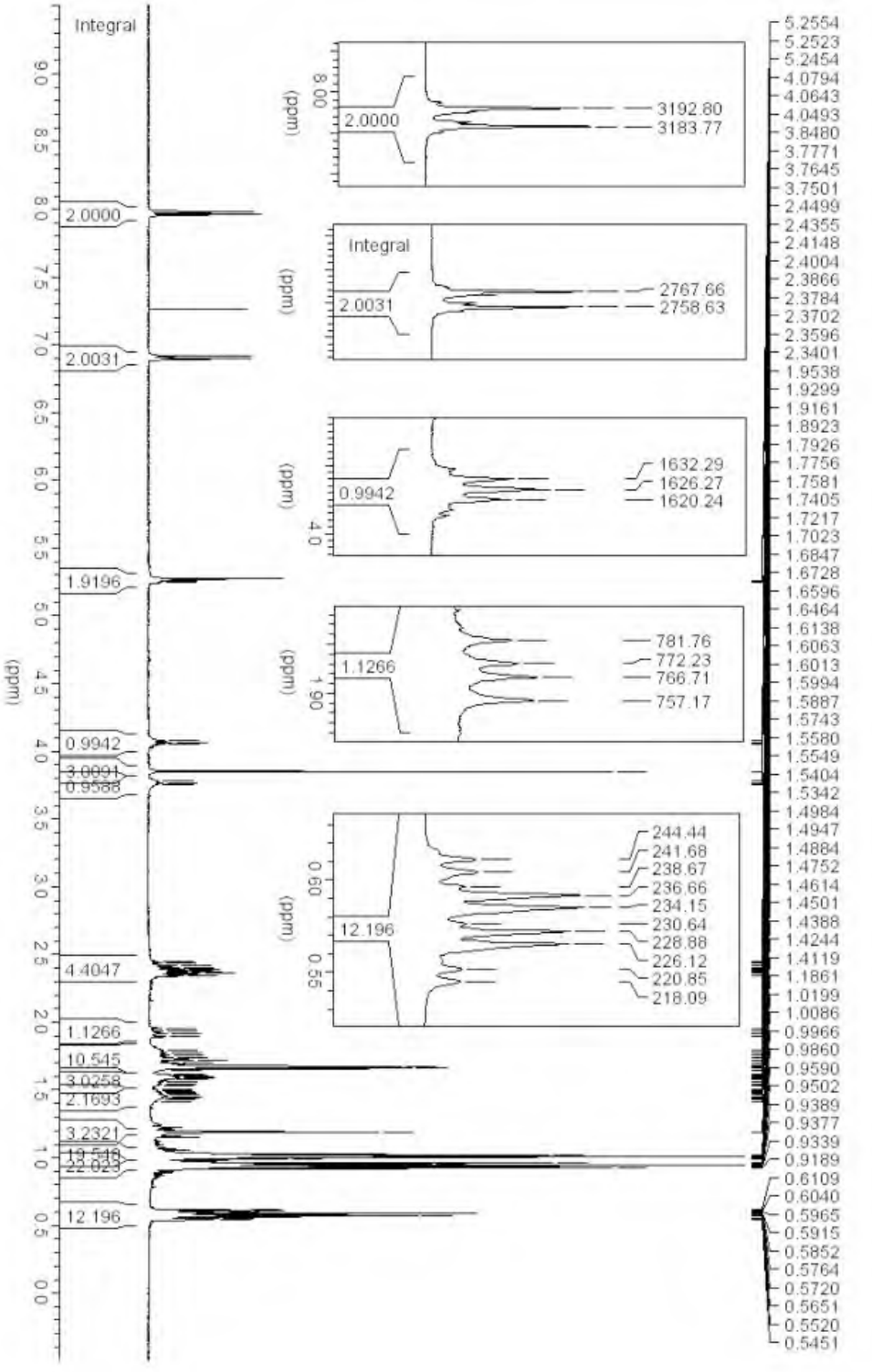
(5*R*,7*S*,8*R*,11*S*,13*R*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5,11,13-trihydroxy-5,8-dimethyl-1-[tris(propan-2-yl)silyl]tetradec-1-yn-7-yl 4-methoxybenzoate (II.74)



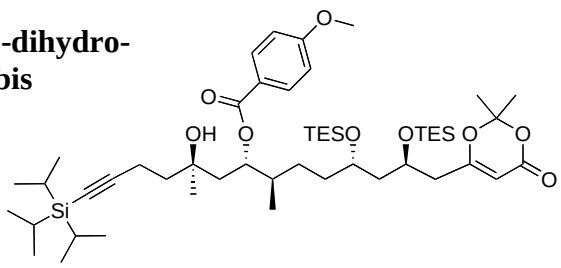


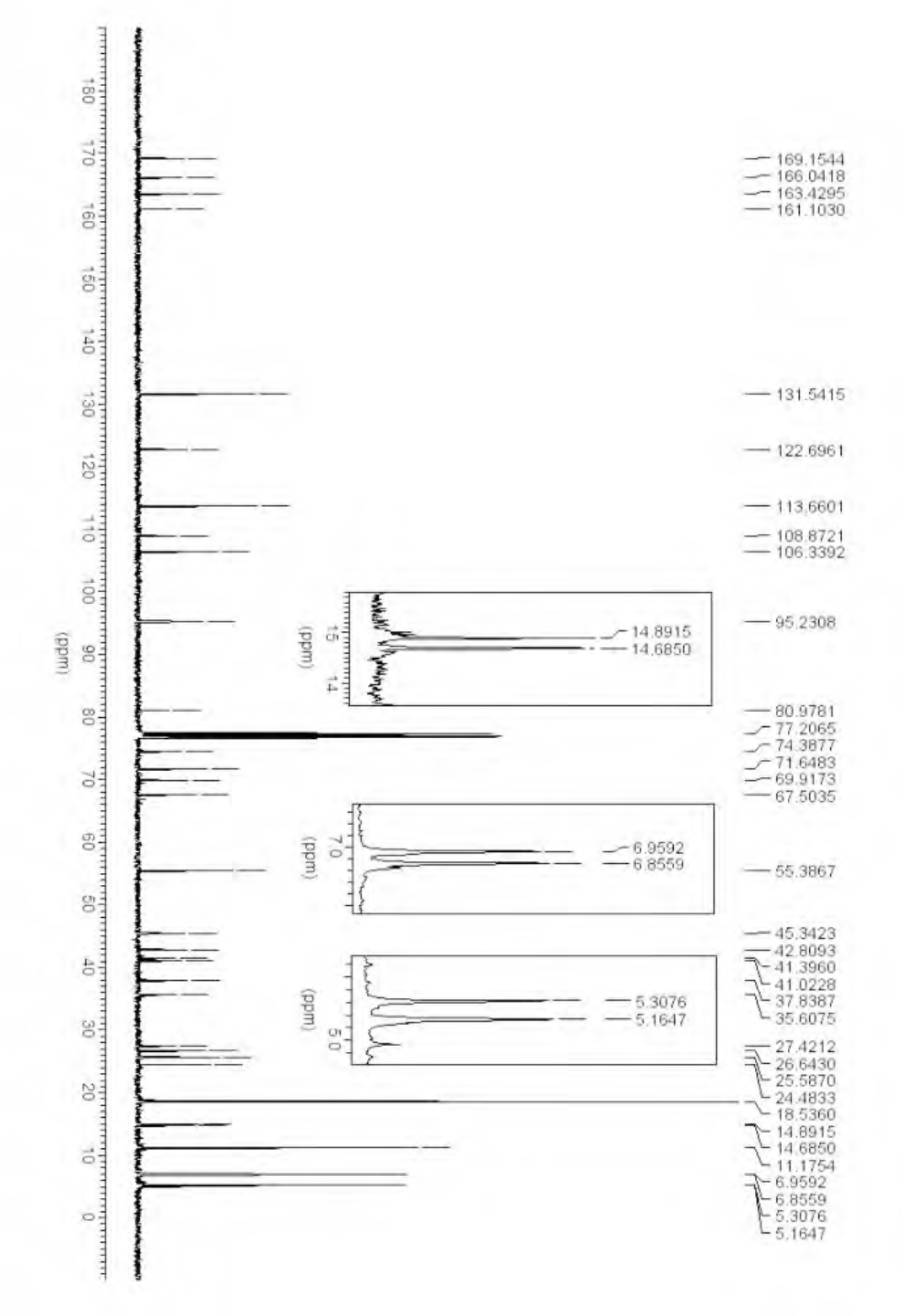
(5*R*,7*S*,8*R*,11*S*,13*R*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5-hydroxy-5,8-dimethyl-11,13-bis [(triethylsilyl)oxy]-1-[tris(propan-2-yl)silyl] tetradec-1-yn-7-yl 4-methoxybenzoate (II.75)



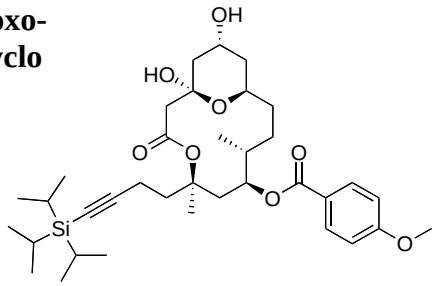


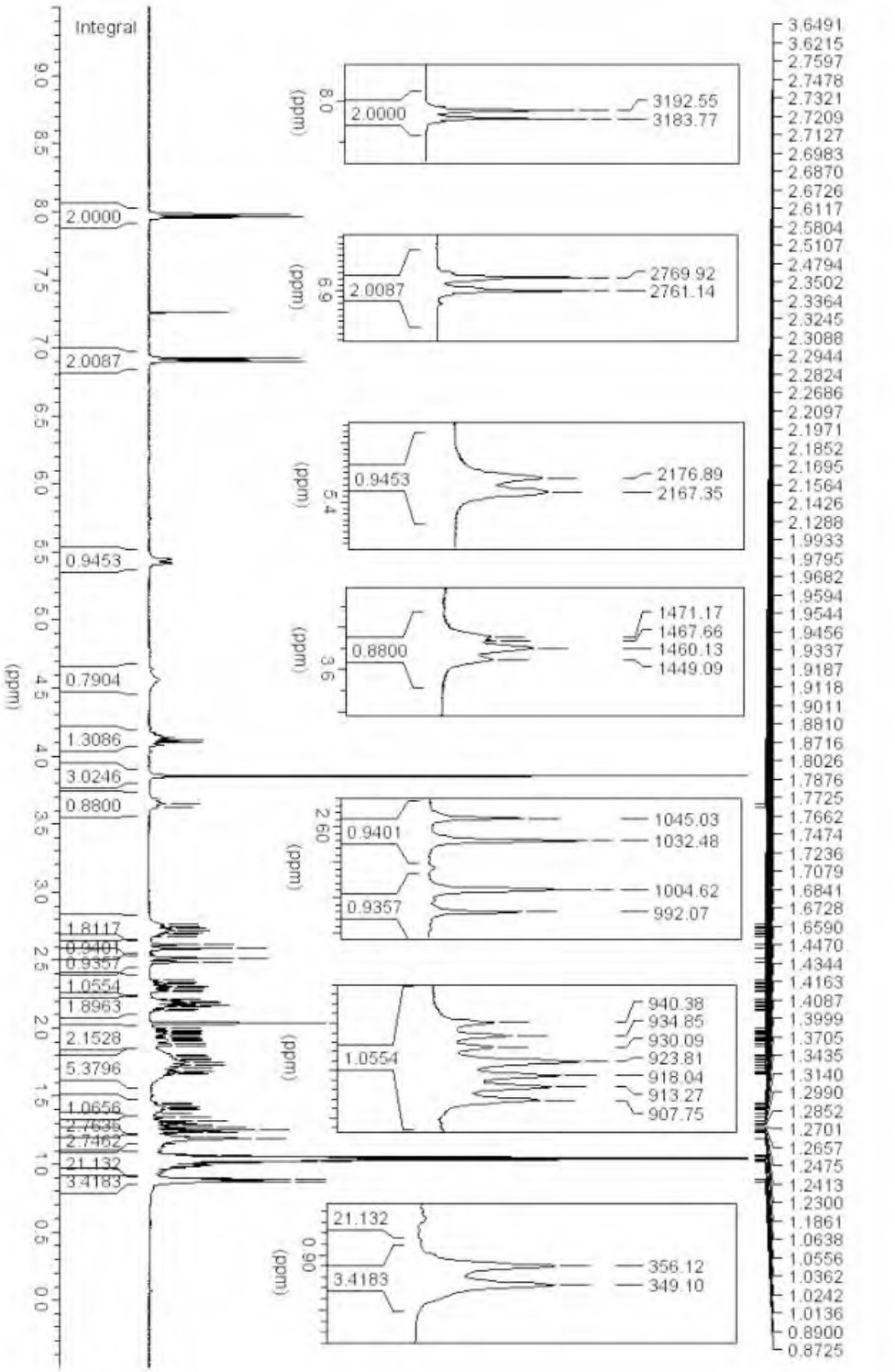
(5*R*,7*S*,8*R*,11*S*,13*R*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5-hydroxy-5,8-dimethyl-11,13-bis[(triethylsilyl)oxy]-1-[tris(propan-2-yl)silyl] tetradec-1-yn-7-yl 4-methoxybenzoate (II.75)



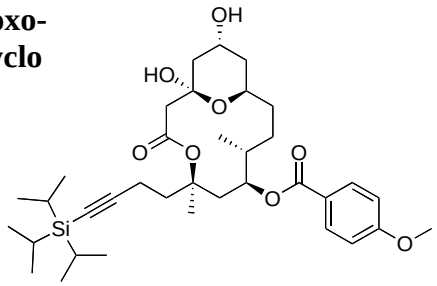


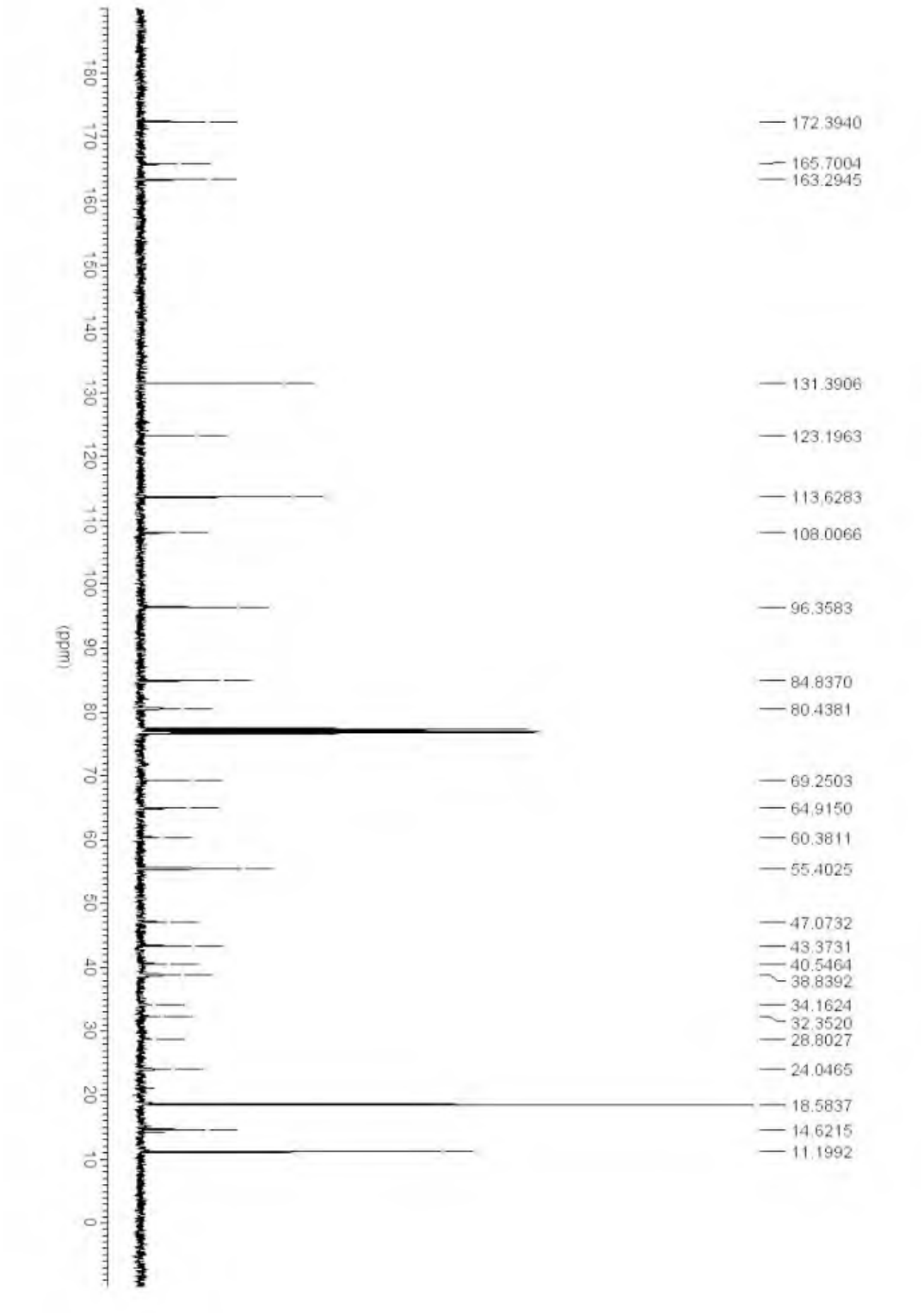
(1*R*,5*R*,7S,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-3-oxo-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo [9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.78)



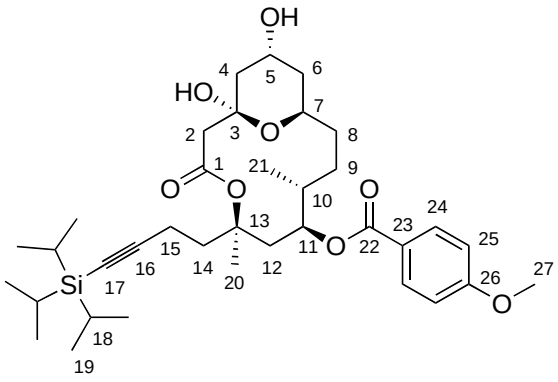


(1*R*,5*R*,7S,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-3-oxo-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo [9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.78)

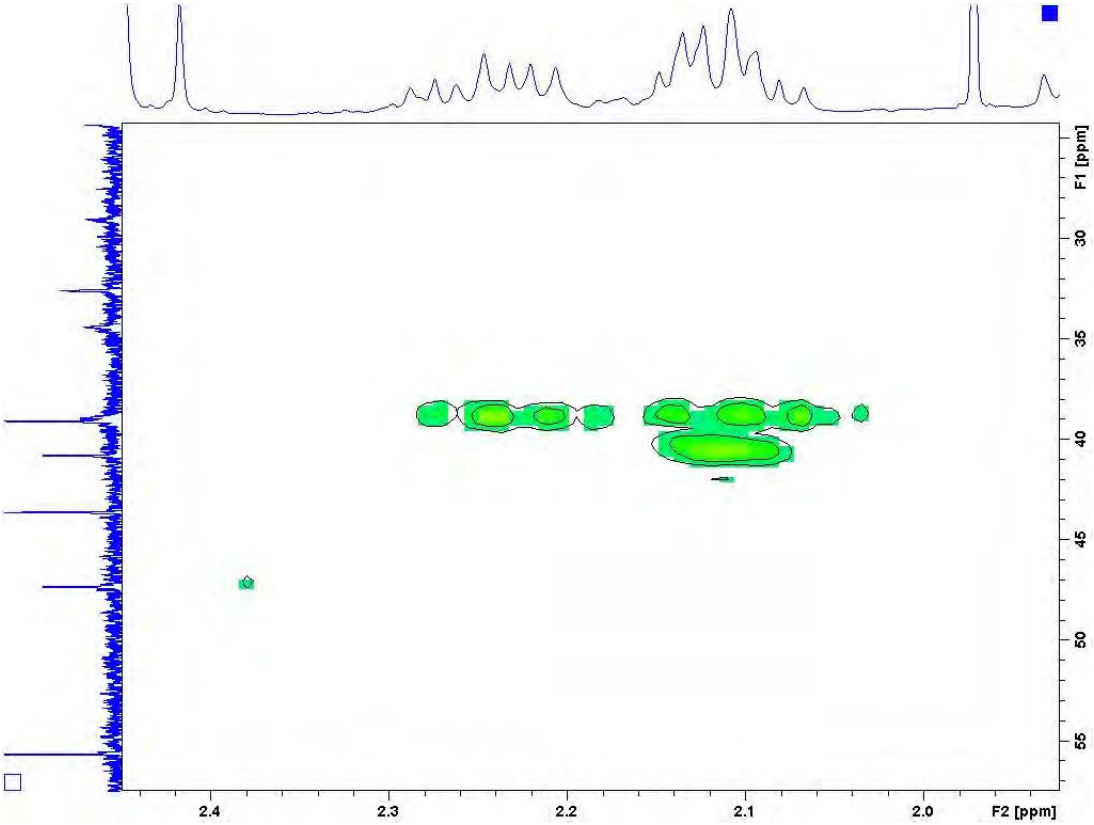




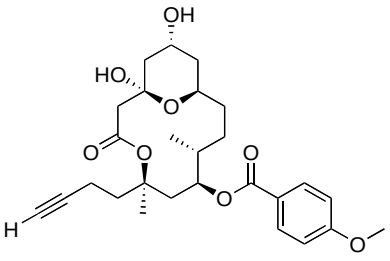
(1*R*,5*R*,7S,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-3-oxo-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.78)

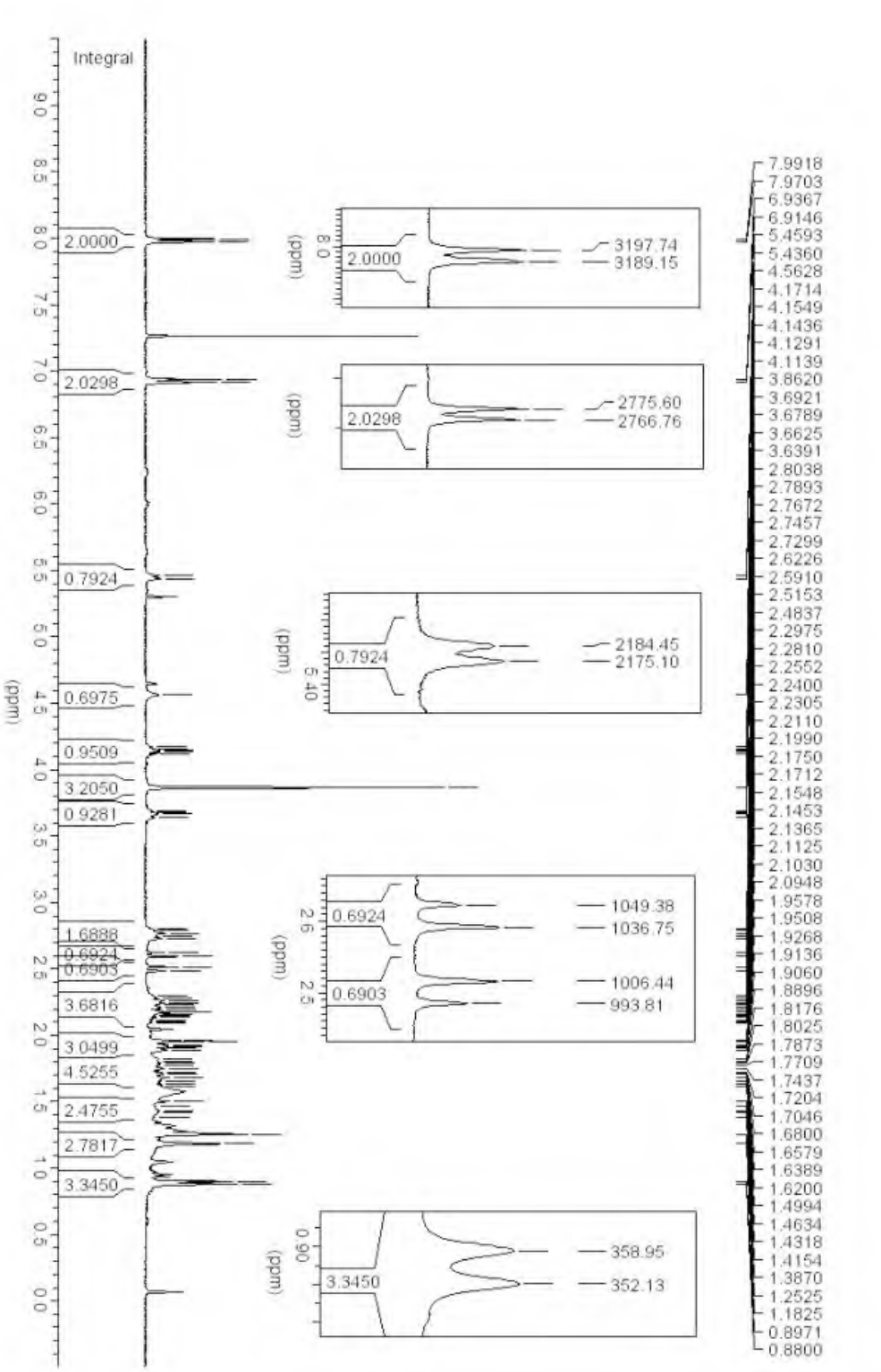


HMQC experiment: amplification of spectra for C4

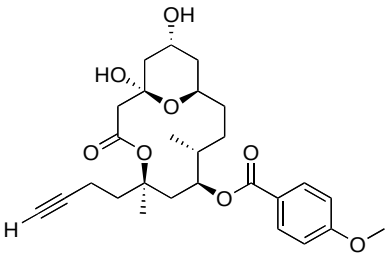


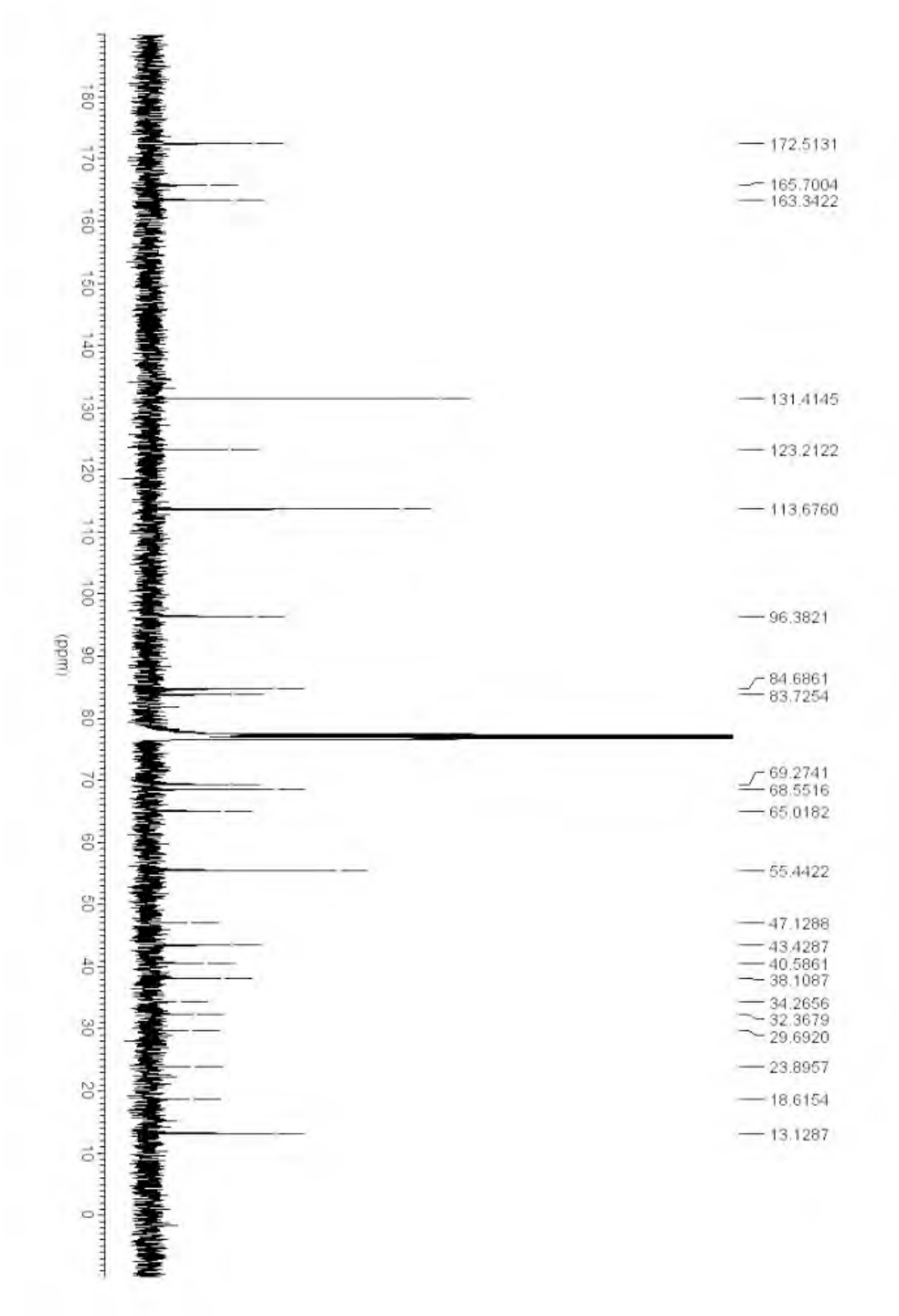
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-5-(But-3-yn-1-yl)-1,13-dihydroxy-5,8-dimethyl-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.79)



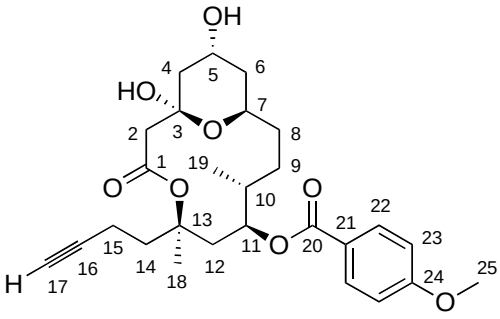


(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-5-(But-3-yn-1-yl)-1,13-dihydroxy-5,8-dimethyl-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.79)

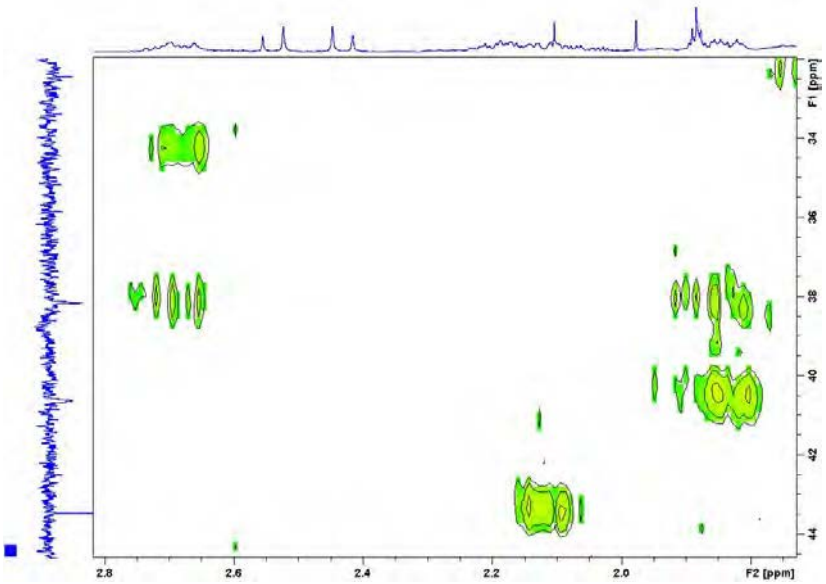




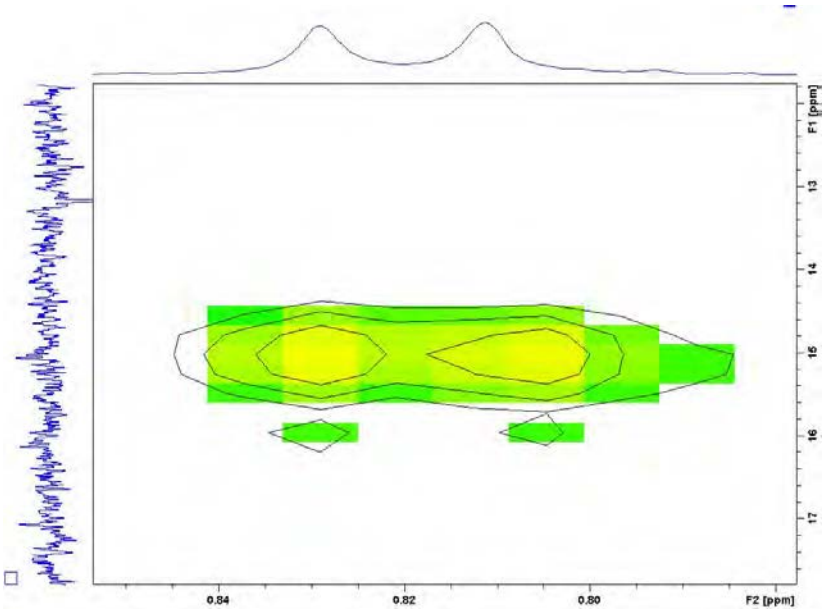
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-5-(But-3-yn-1-yl)-1,13-dihydroxy-5,8-dimethyl-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.79)



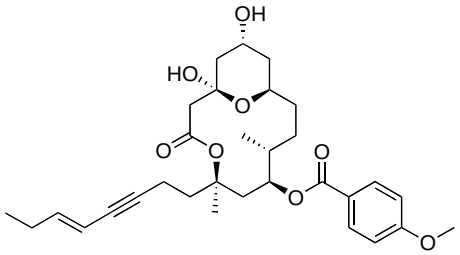
HMQC experiment: amplification of spectra for C4

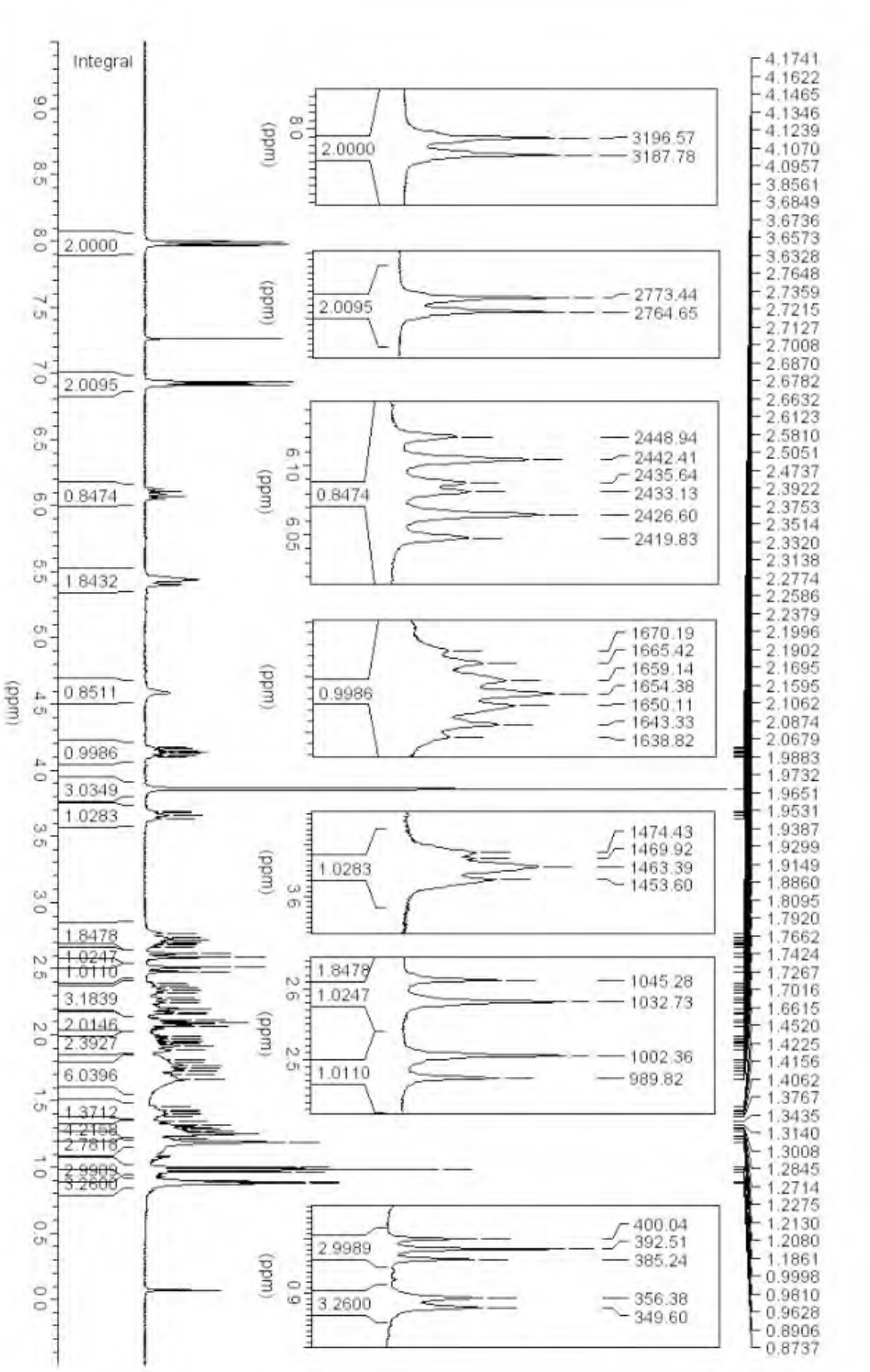


HMQC experiment: amplification of spectra for C19

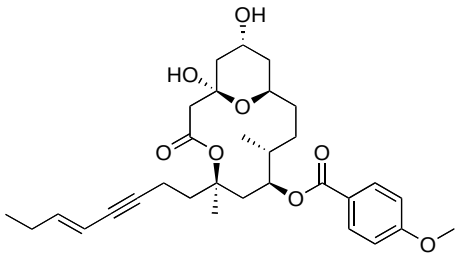


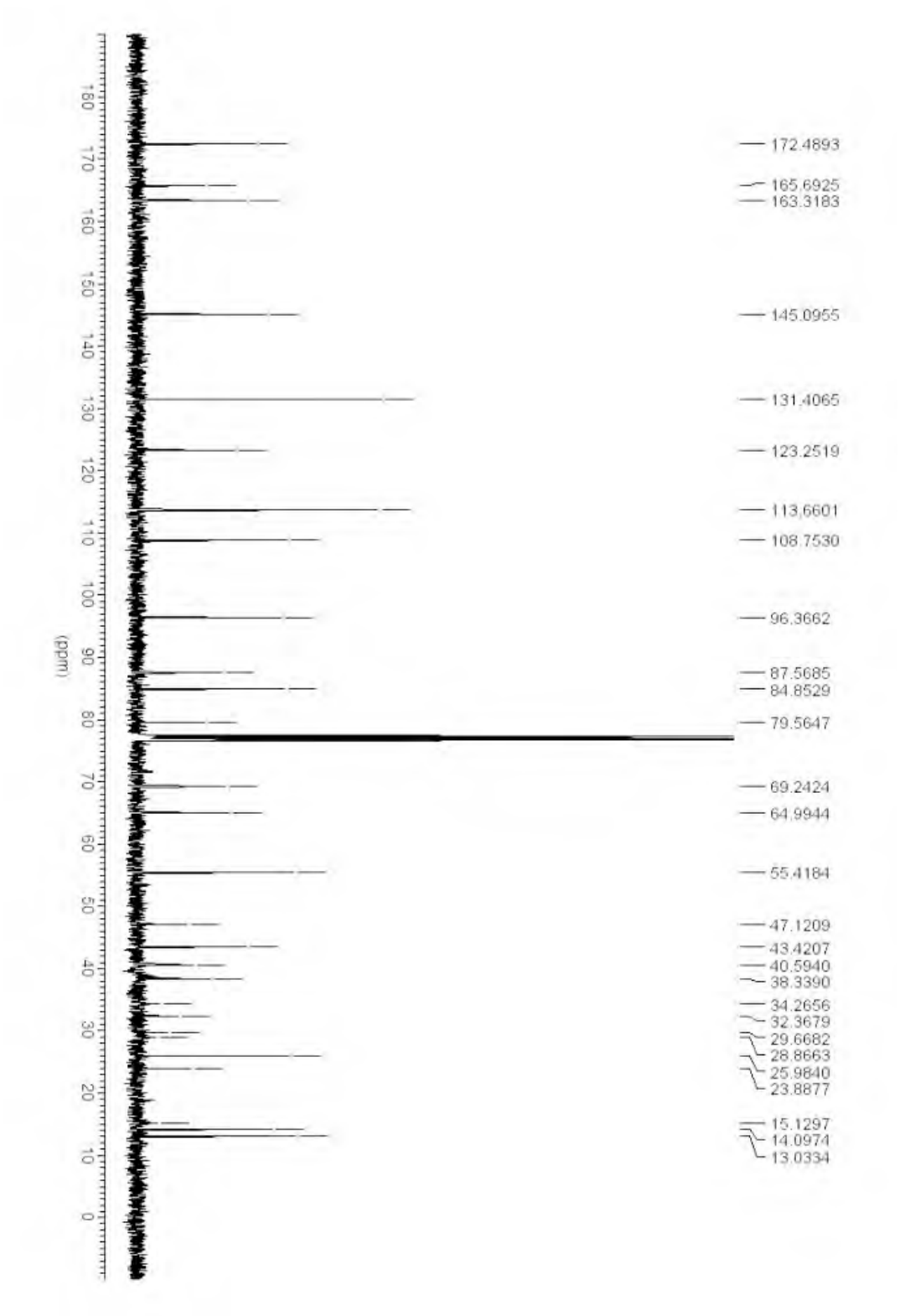
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-5-[(5*E*)-oct-5-en-3-yn-1-yl]-3-oxo-4,15-dioxabicyclo [9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.81)



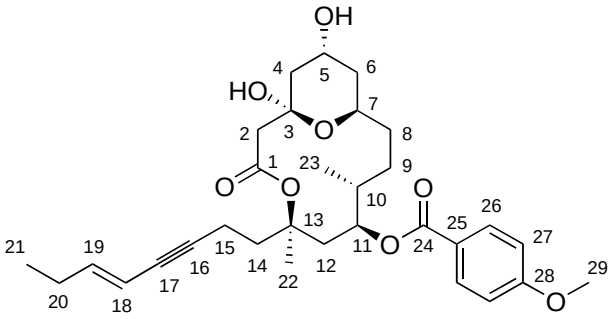


(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-5-[(5*E*)-oct-5-en-3-yn-1-yl]-3-oxo-4,15-dioxabicyclo [9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.81)

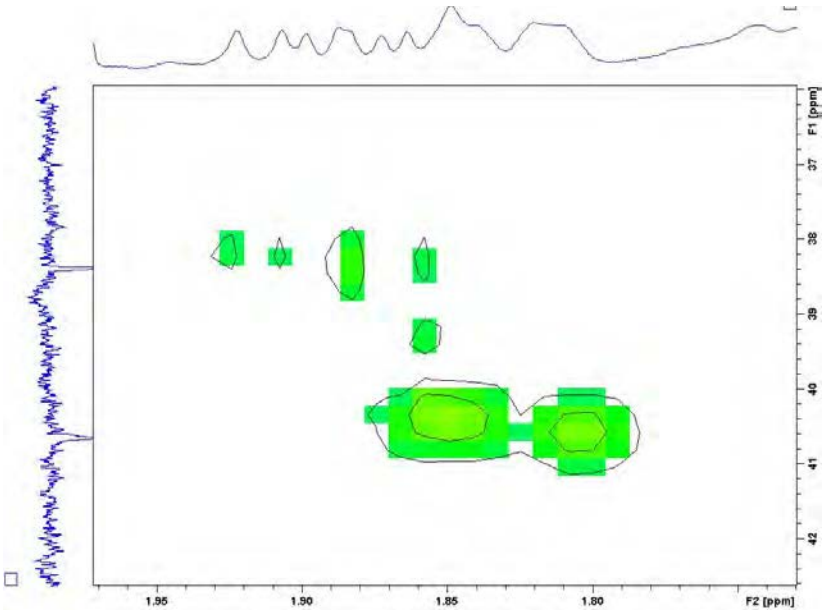




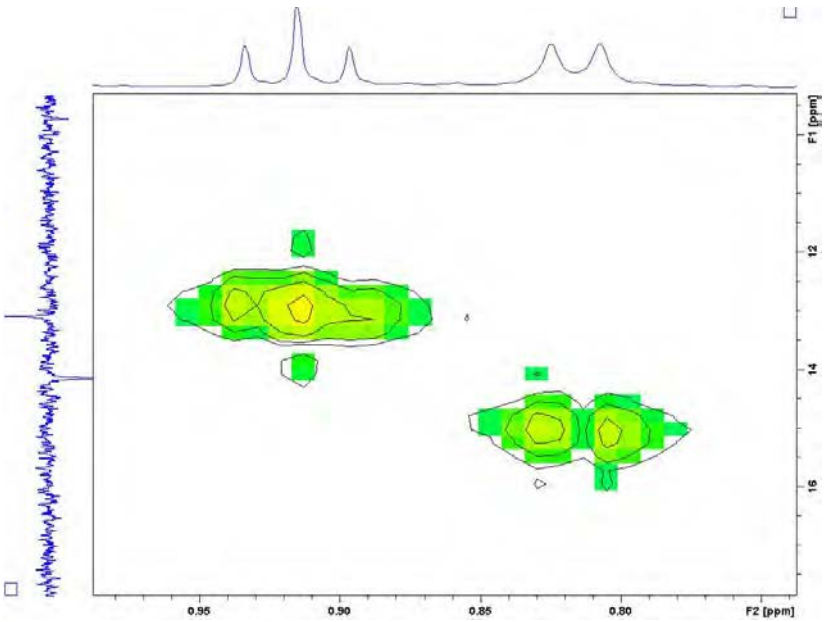
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-5-[(5*E*)-oct-5-en-3-yn-1-yl]-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl 4-methoxybenzoate (II.81)



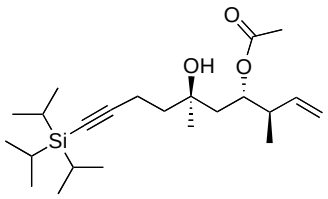
HMQC experiment: amplification of spectra for C10

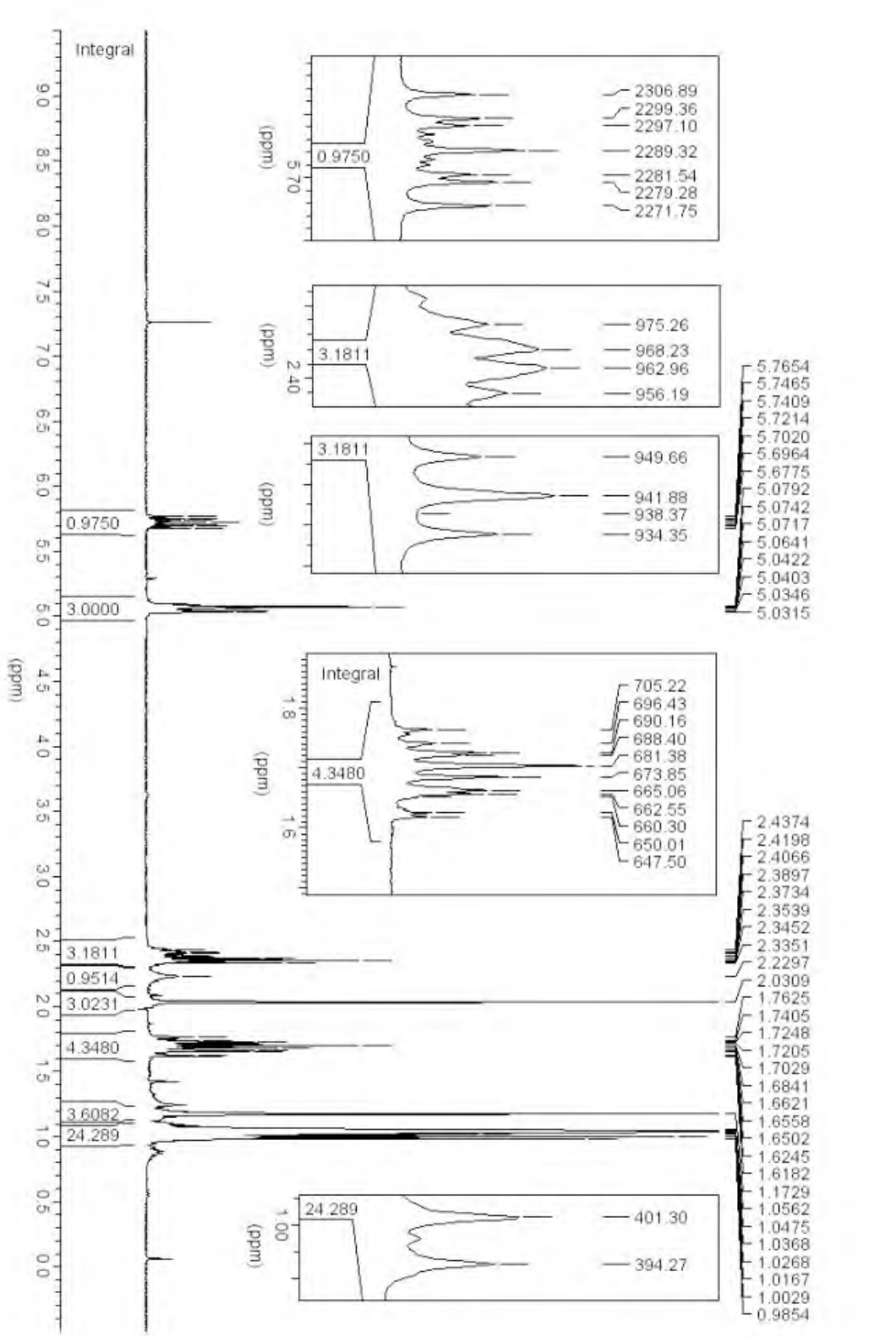


HMQC experiment: amplification of spectra for C23

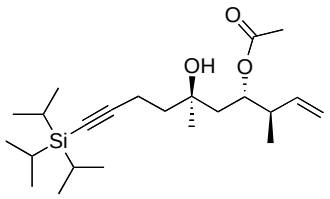


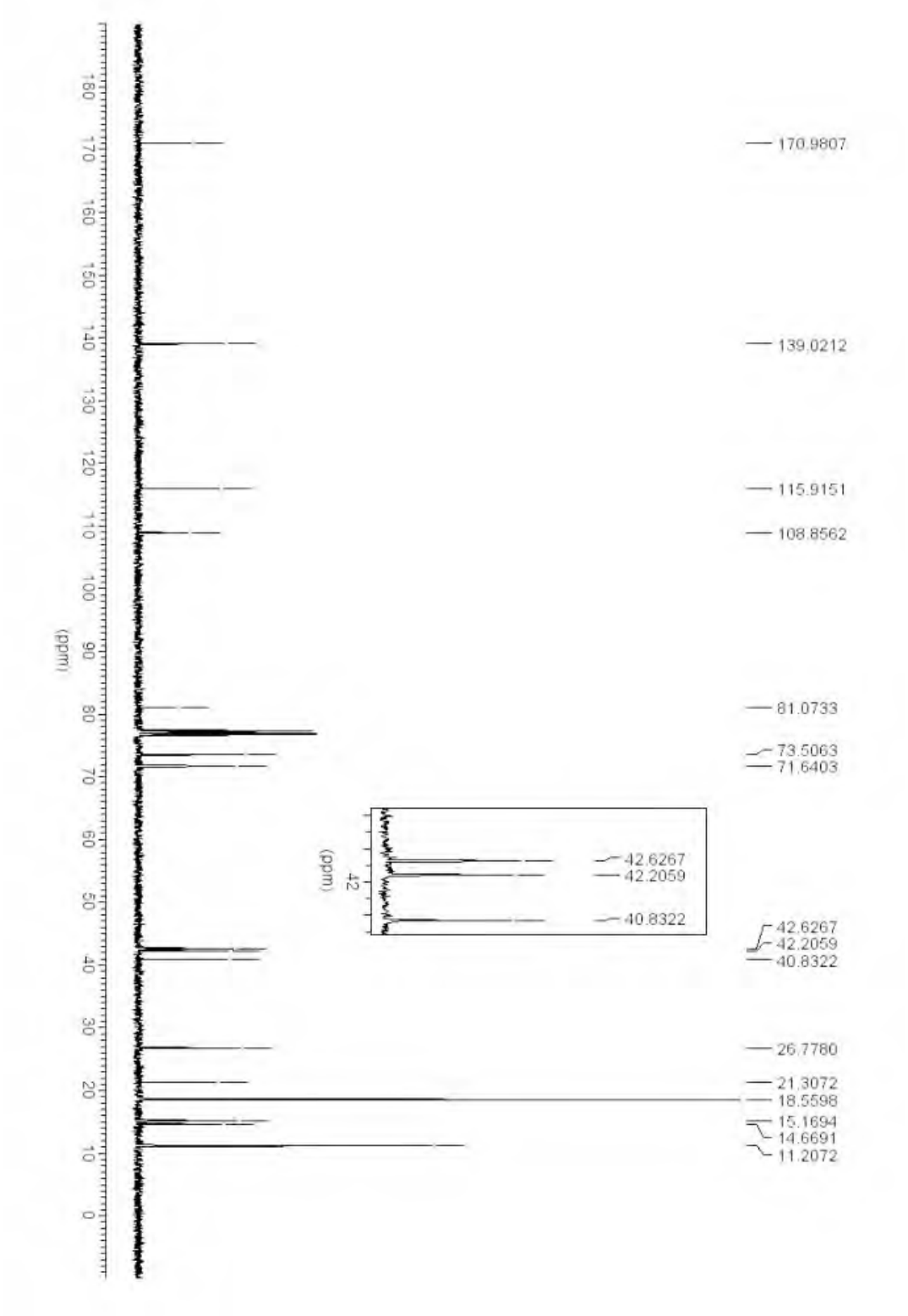
(3*R*,4*S*,6*R*)-6-Hydroxy-3,6-dimethyl-10-[tris(propan-2-yl) silyl]dec-1-en-9-yn-4-yl acetate (II.83)



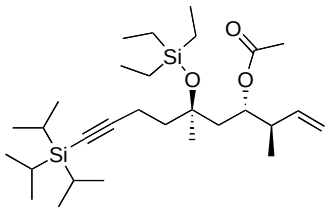


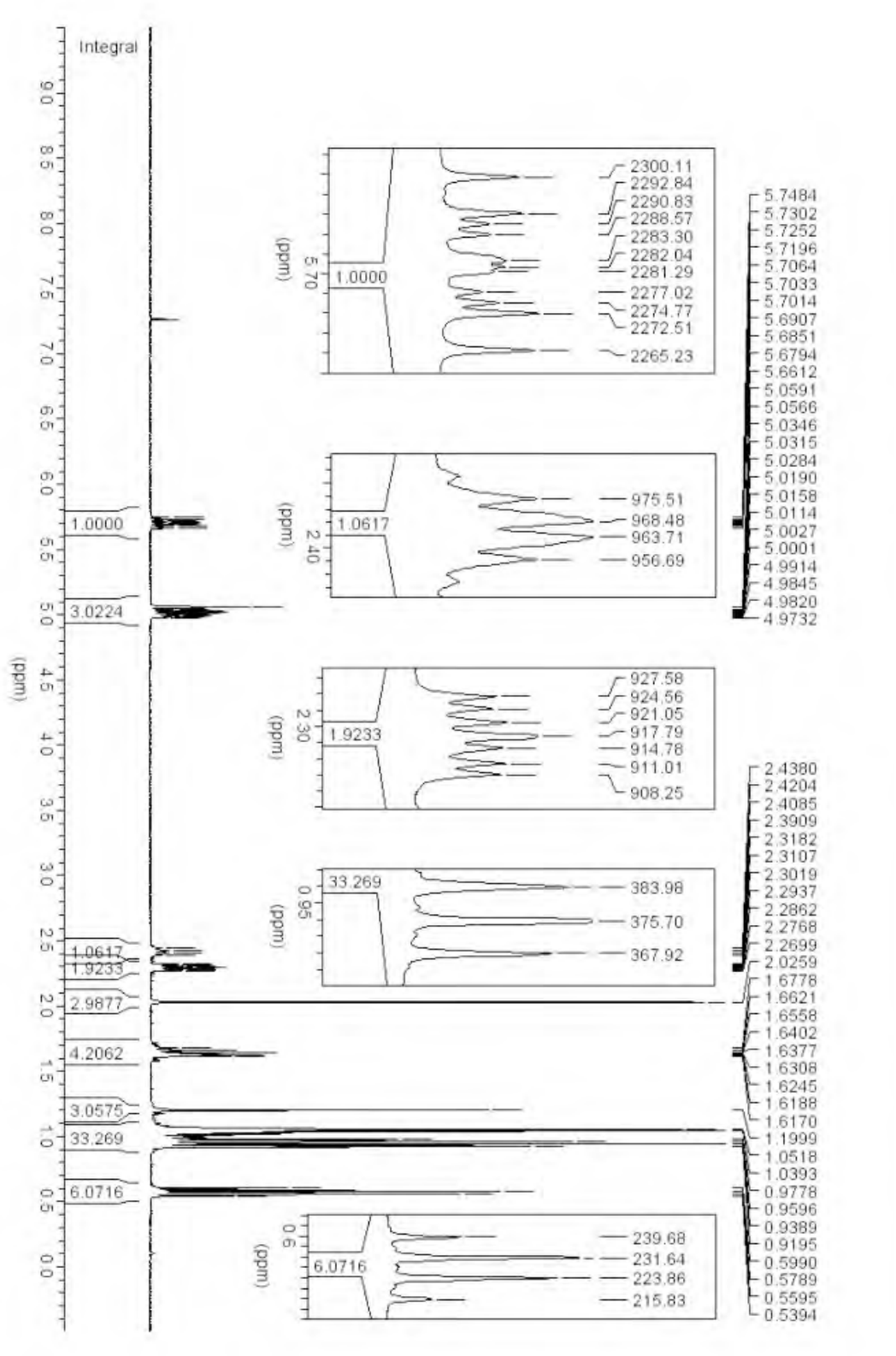
(3*R*,4*S*,6*R*)-6-Hydroxy-3,6-dimethyl-10-[tris(propan-2-yl) silyl]dec-1-en-9-yn-4-yl acetate (II.83)



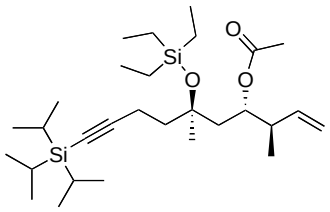


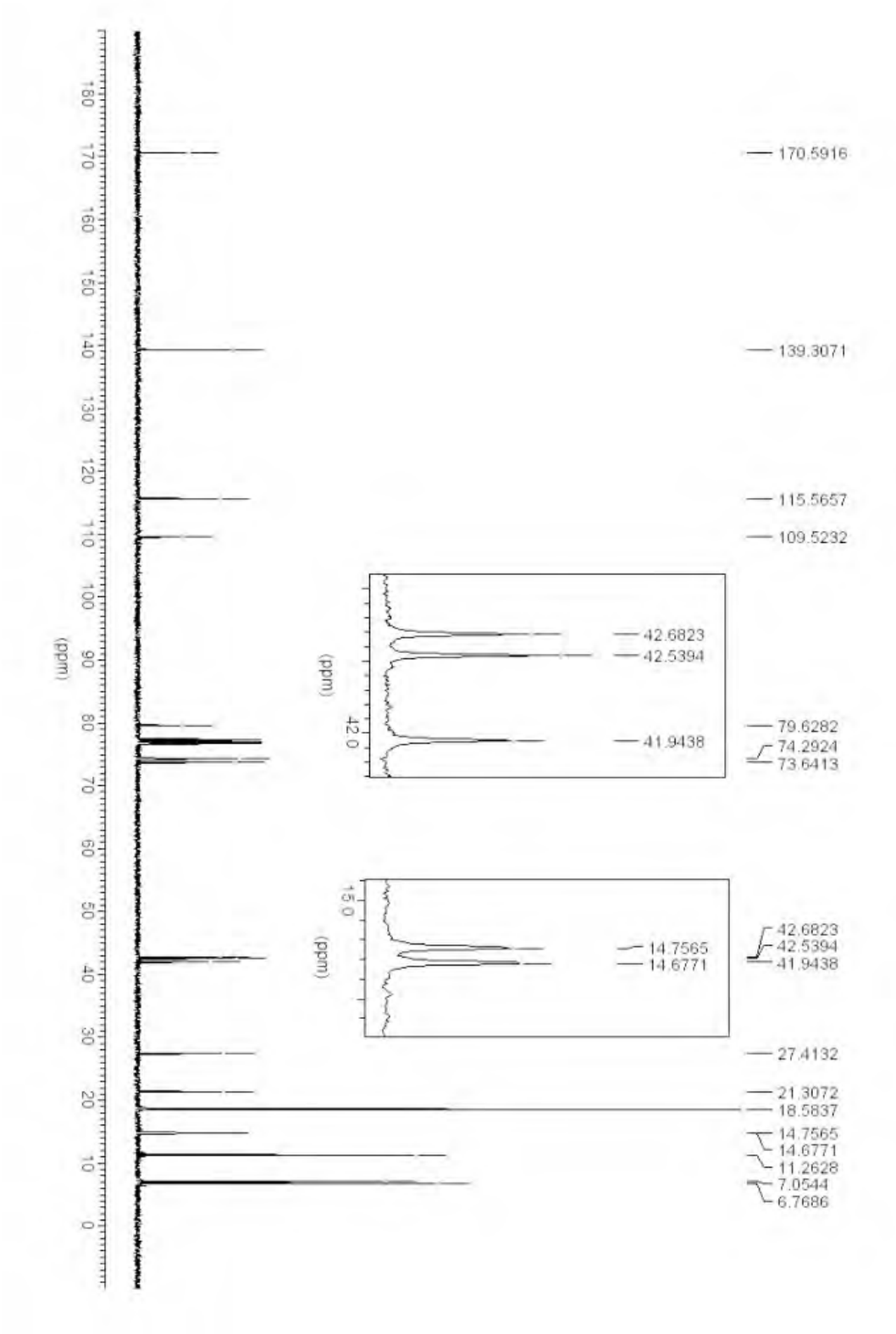
(3*R*,4*S*,6*R*)-3,6-Dimethyl-6-[(triethylsilyl)oxy]-10-[tris(propan-2-yl)silyl]dec-1-en-9-yn-4-yl acetate (II.84)



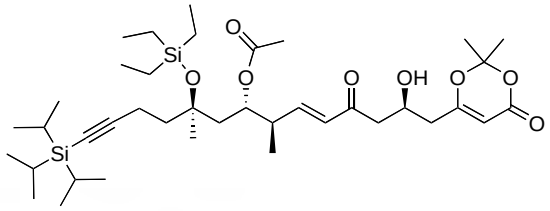


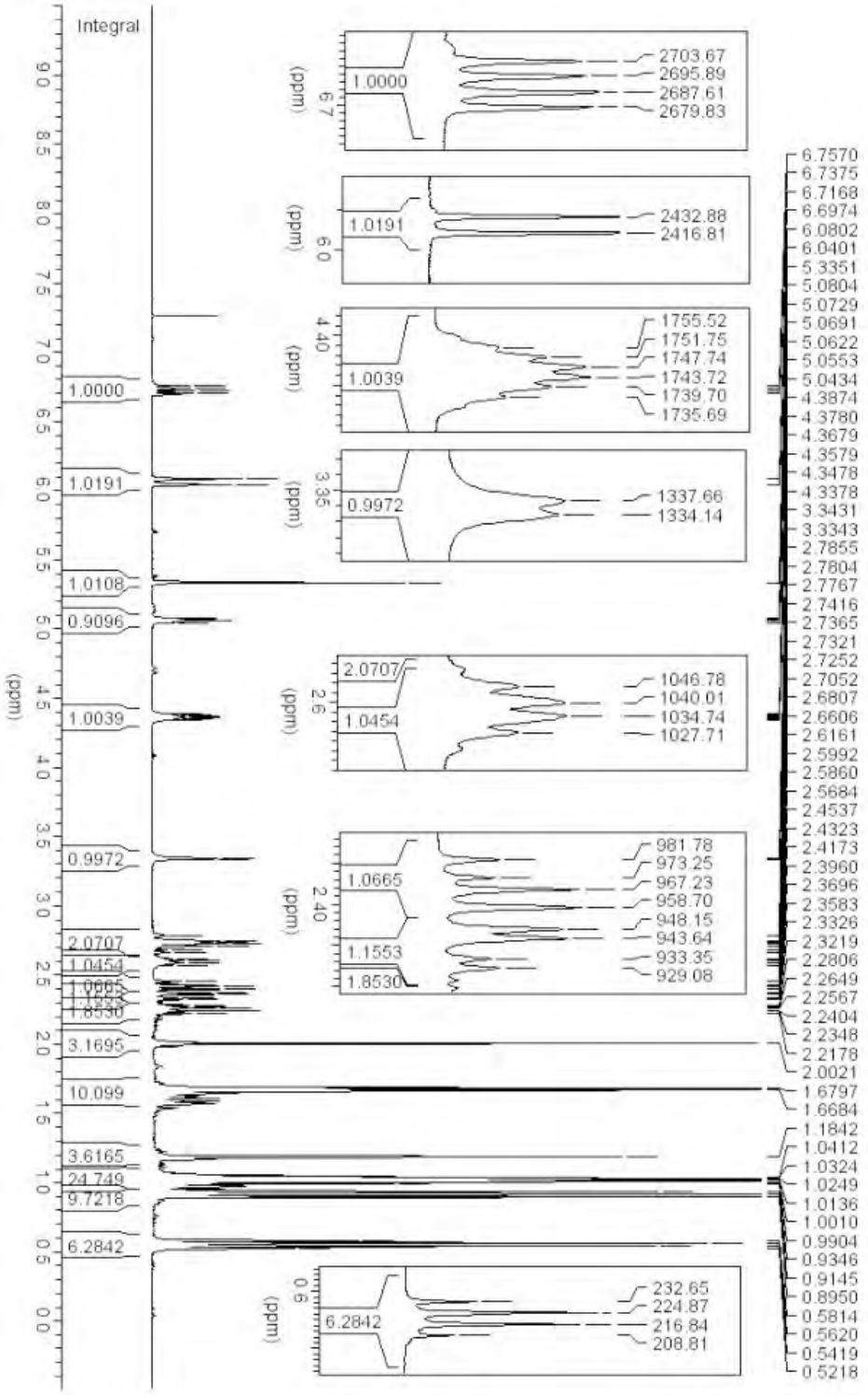
(3*R*,4*S*,6*R*)-3,6-Dimethyl-6-[(triethylsilyl)oxy]-10-[tris(propan-2-yl)silyl]dec-1-en-9-yn-4-yl acetate (II.84)



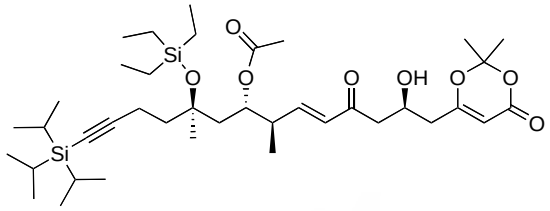


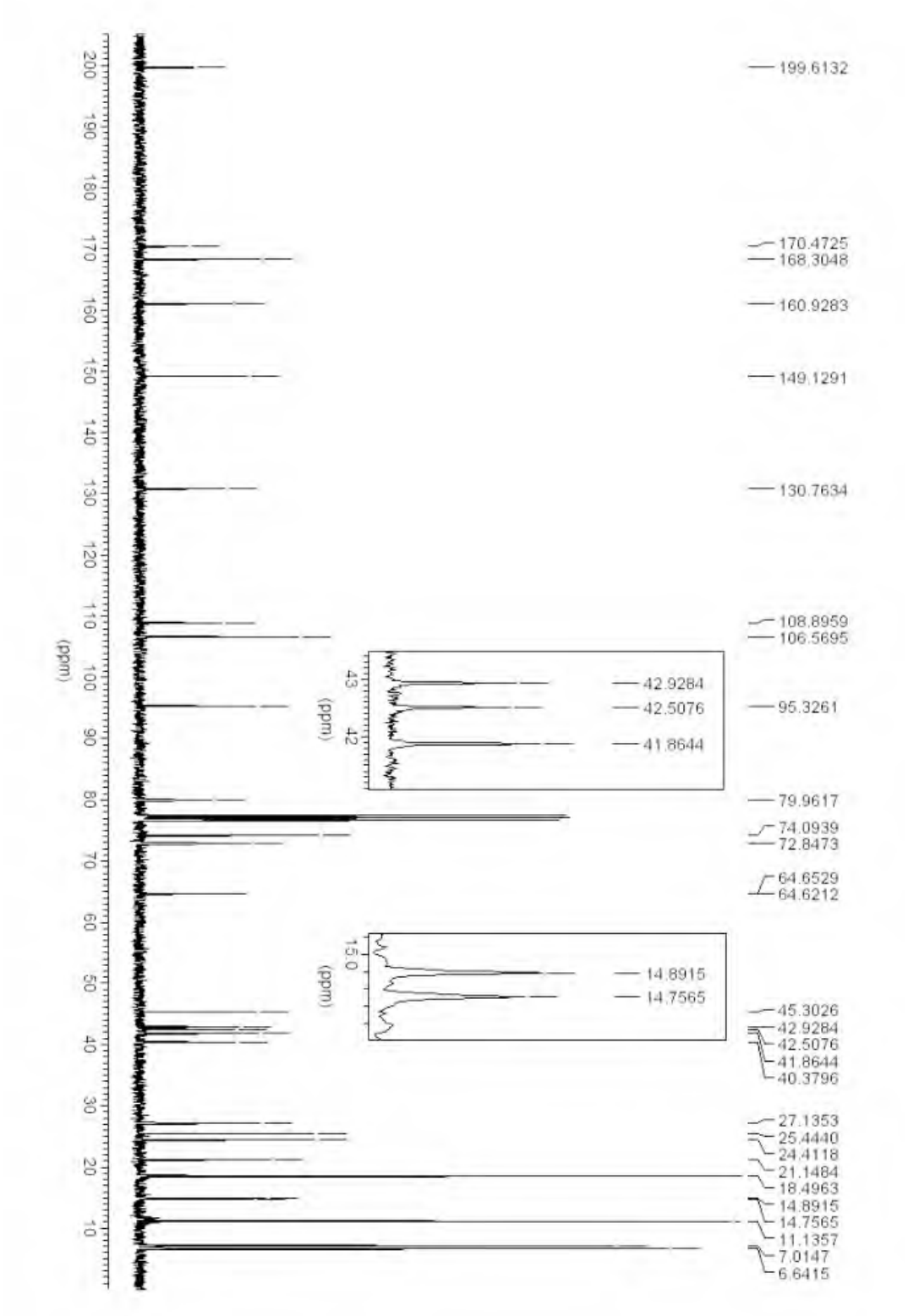
(5*R*,7*S*,8*R*,9*E*,13*S*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-13-hydroxy-5,8-dimethyl-11-oxo-5-[(triethylsilyl)oxy]-1-[tris(propan-2-yl)silyl] tetradec-9-en-1-yn-7-yl acetate (II.85)



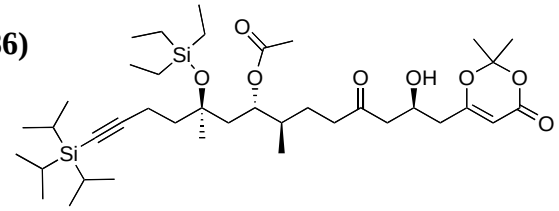


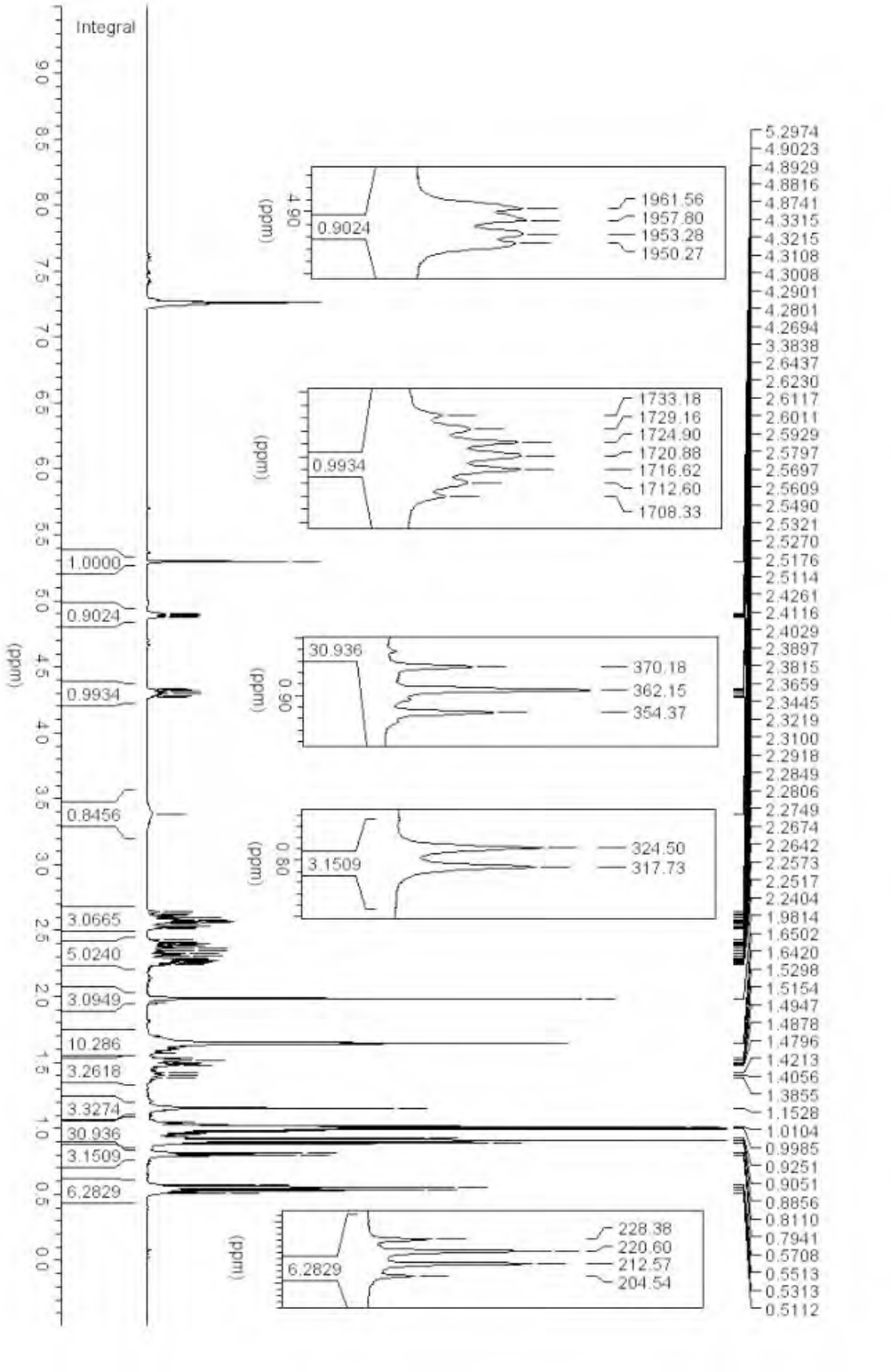
(5*R*,7*S*,8*R*,9*E*,13*S*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-13-hydroxy-5,8-dimethyl-11-oxo-5-[(triethylsilyl)oxy]-1-[tris (propan-2-yl)silyl] tetradec-9-en-1-yn-7-yl acetate (II.85)



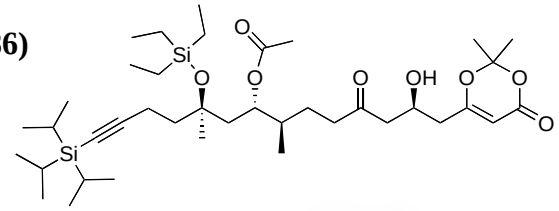


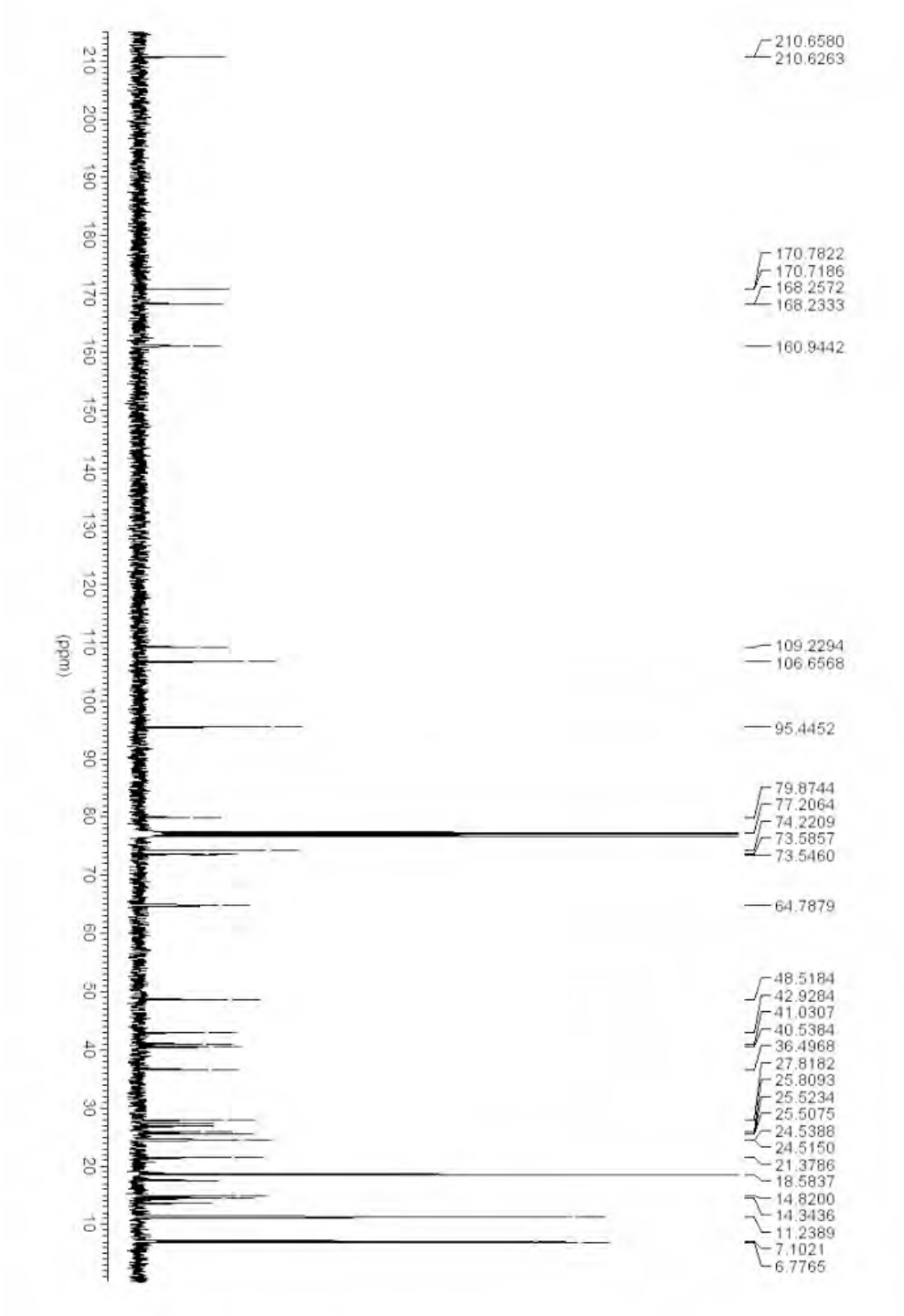
(5*R*,7*S*,8*R*,13*S*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-13-hydroxy-5,8-dimethyl-11-oxo-5-[(triethylsilyl)oxy]-1-[tris (propan-2-yl)silyl]tetradec-1-yn-7-yl acetate (II.86)



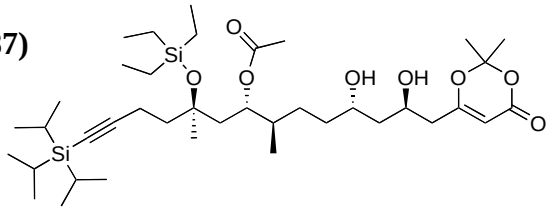


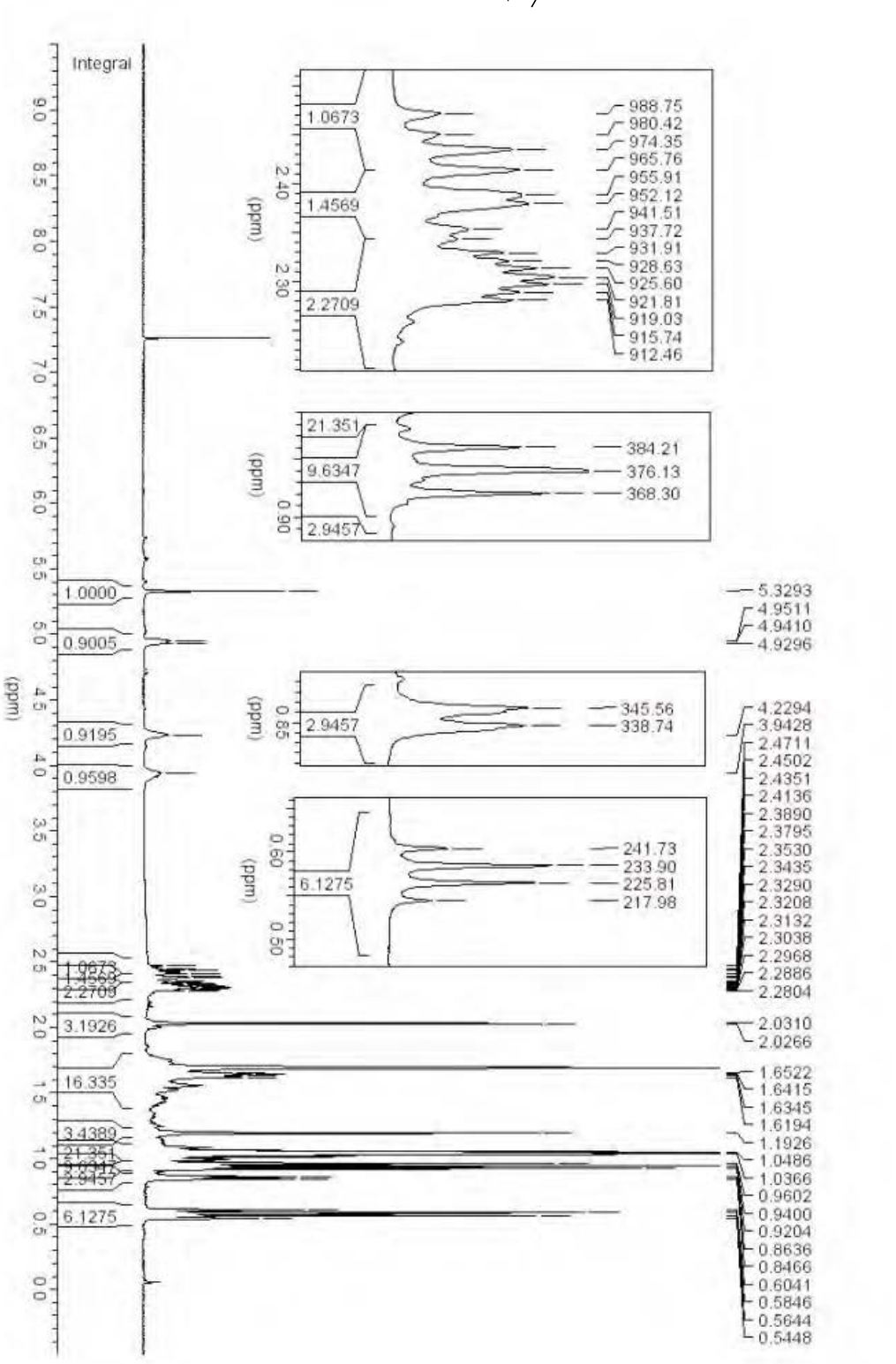
(5*R*,7*S*,8*R*,13*S*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-13-hydroxy-5,8-dimethyl-11-oxo-5-[(triethylsilyl)oxy]-1-[tris (propan-2-yl)silyl]tetradec-1-yn-7-yl acetate (II.86)



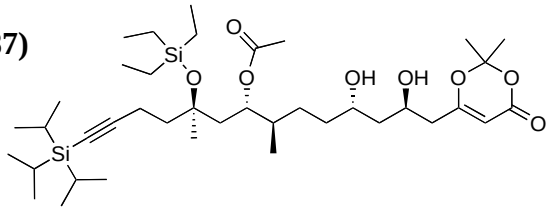


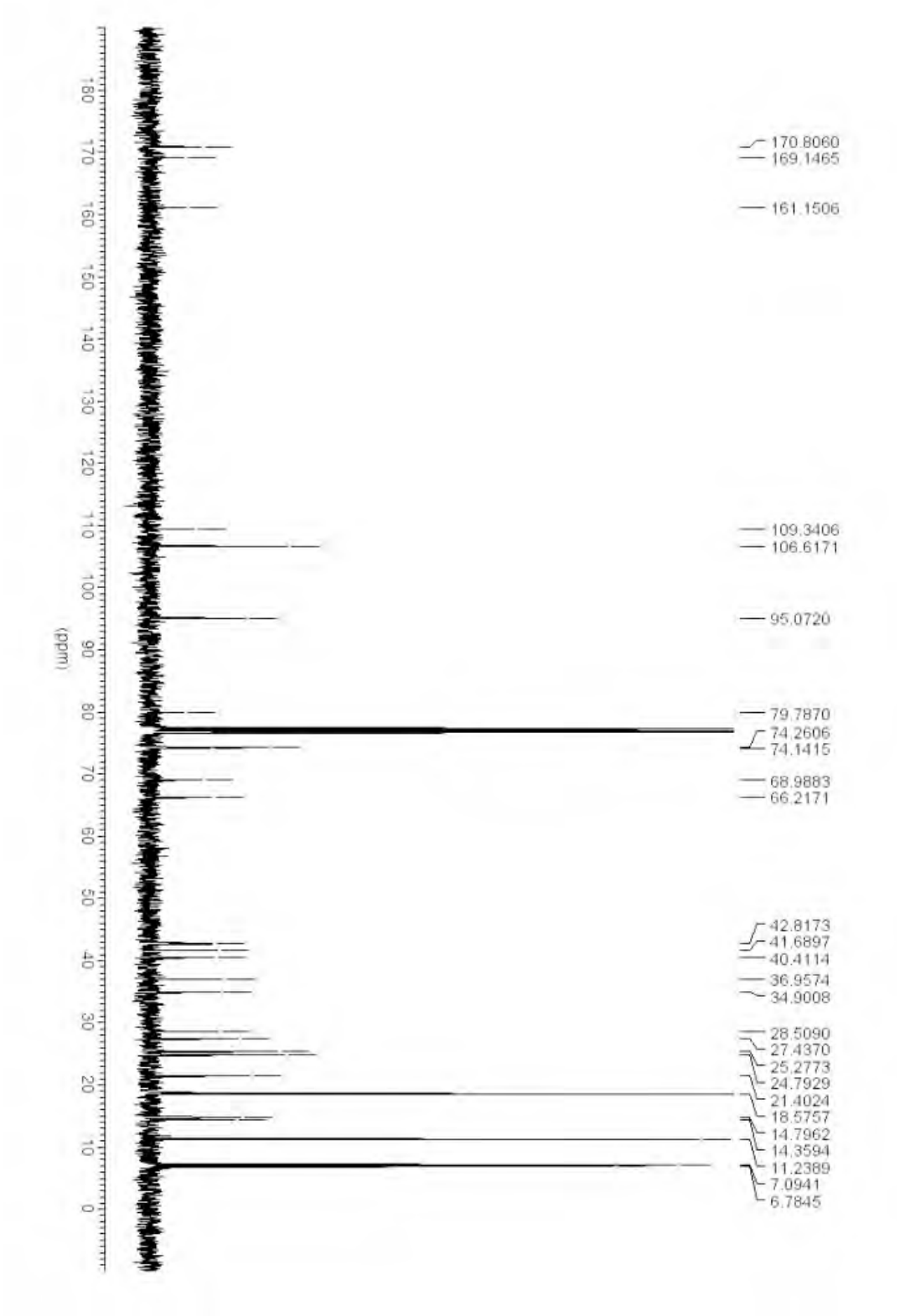
(5*R*,7*S*,8*R*,11*S*,13*R*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-11,13-dihydroxy-5,8-dimethyl-5-[(triethylsilyl)oxy]-1-[tris (propan-2-yl)silyl] tetradec-1-yn-7-yl acetate (II.87)



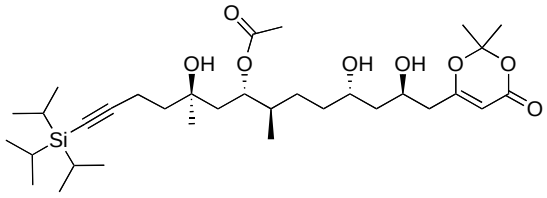


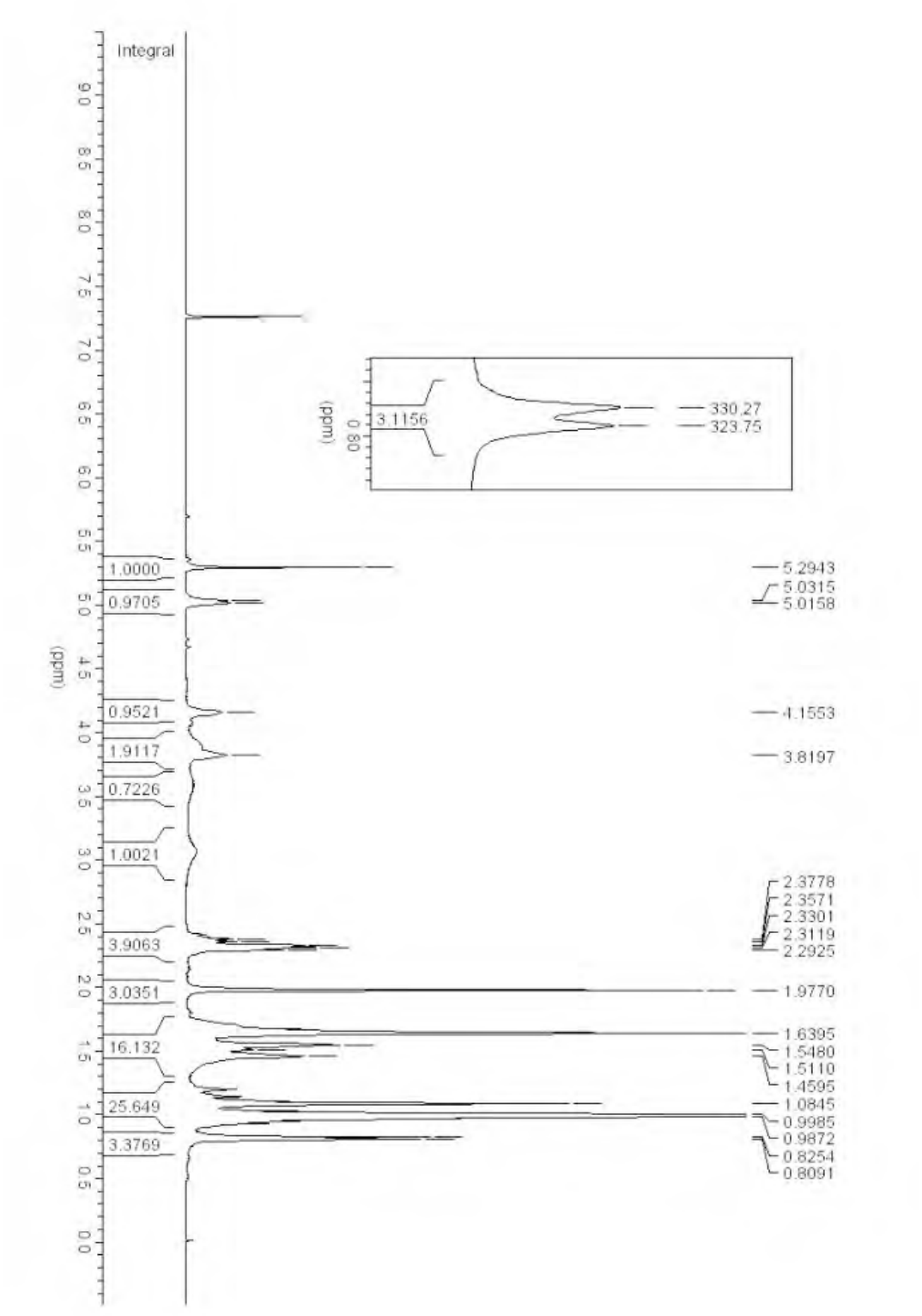
(5*R*,7*S*,8*R*,11*S*,13*R*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-11,13-dihydroxy-5,8-dimethyl-5-[(triethylsilyl)oxy]-1-[tris (propan-2-yl)silyl] tetradec-1-yn-7-yl acetate (II.87)



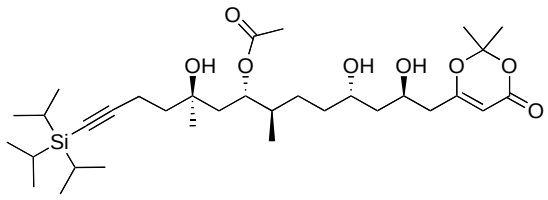


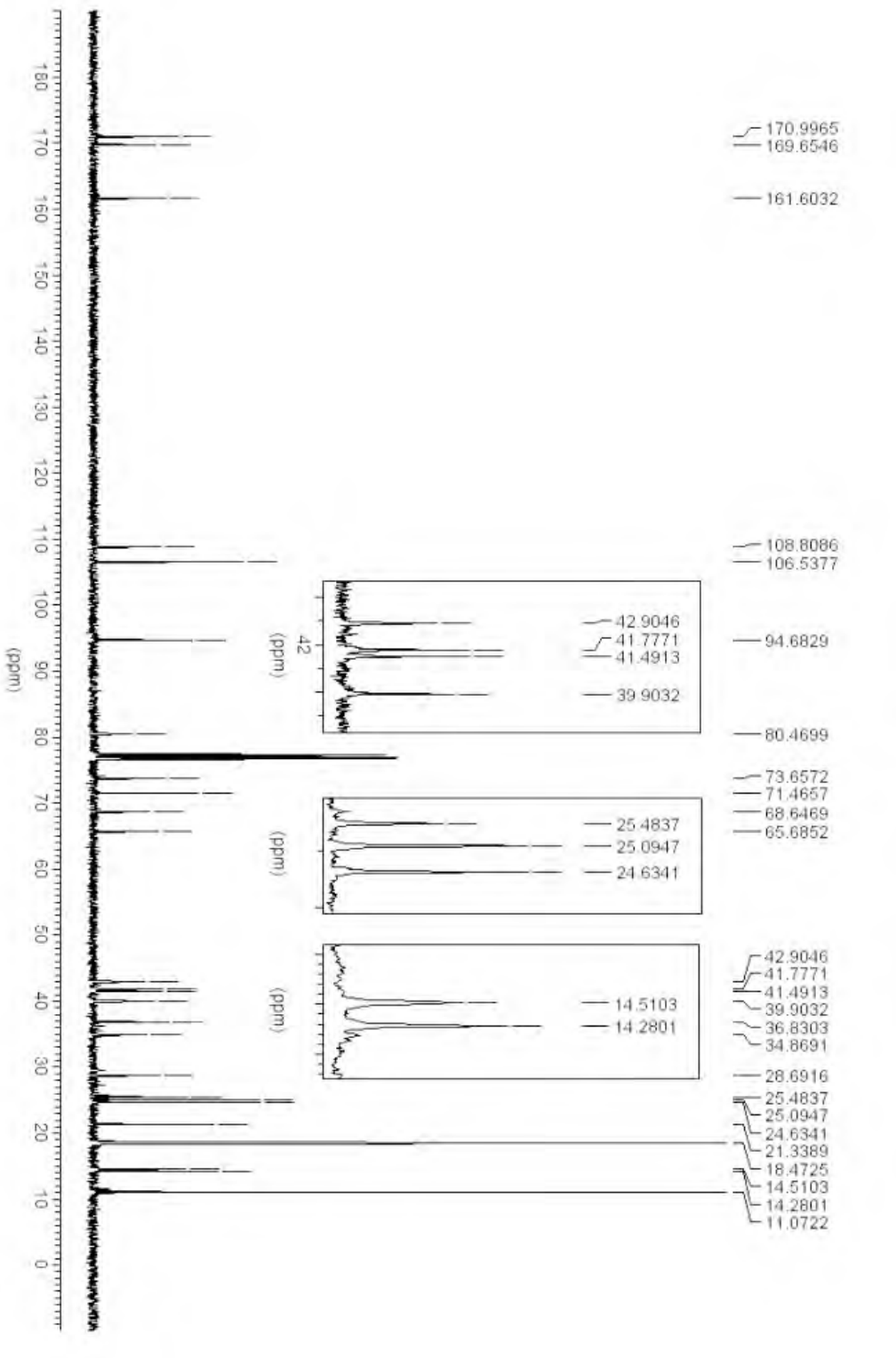
(5*R*,7*S*,8*R*,11*S*,13*R*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5,11,13-trihydroxy-5,8-dimethyl-1-[tris(propan-2-yl)silyl] tetradec-1-yn-7-yl acetate



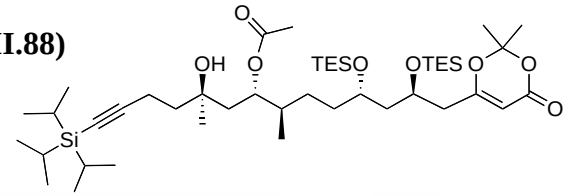


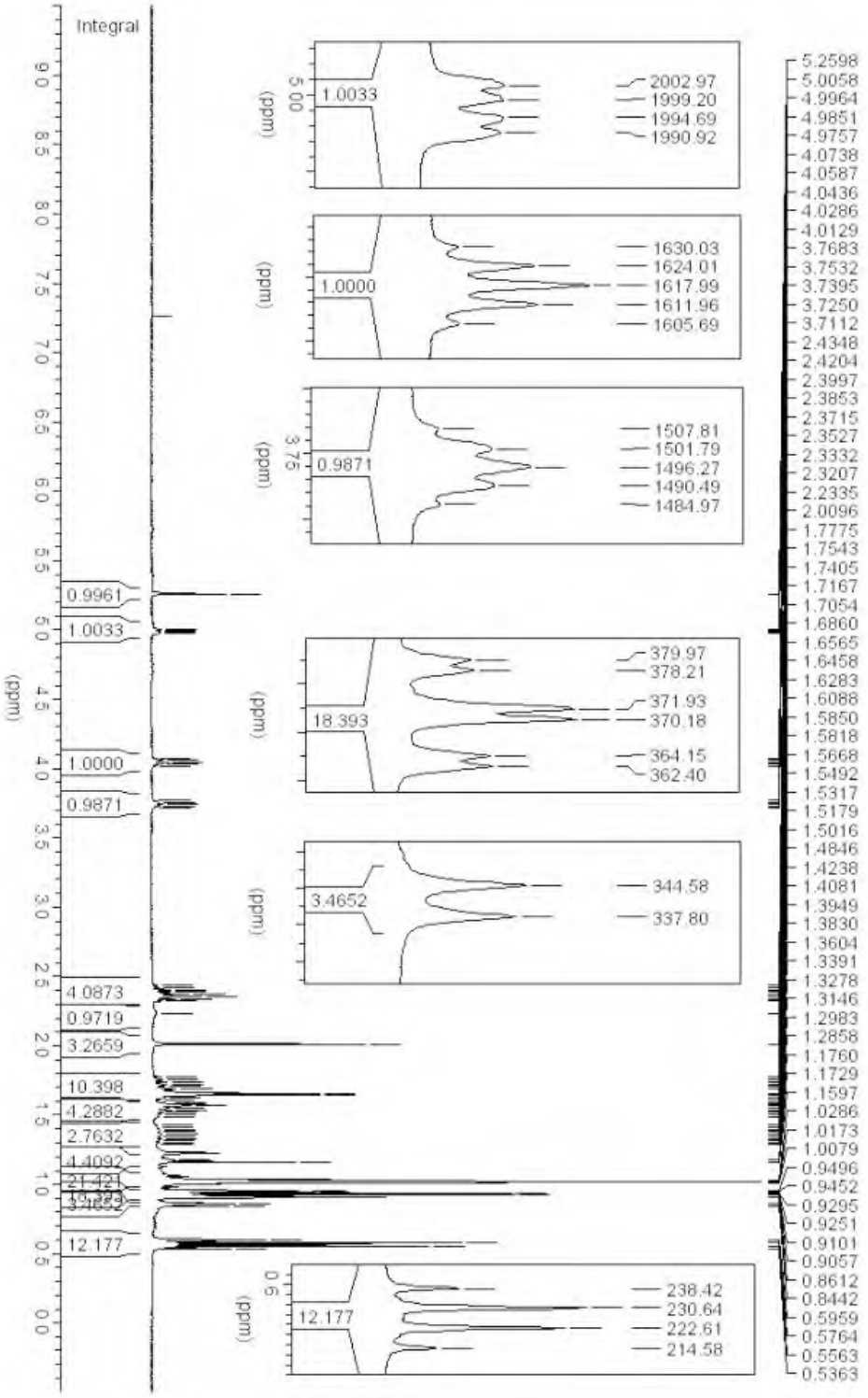
(5*R*,7*S*,8*R*,11*S*,13*R*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5,11,13-trihydroxy-5,8-dimethyl-1-[tris(propan-2-yl)silyl] tetradec-1-yn-7-yl acetate



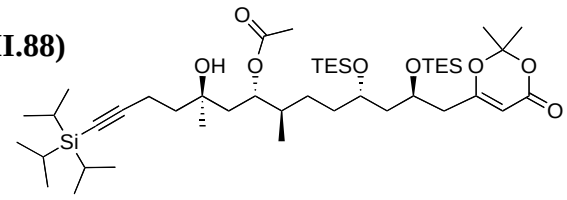


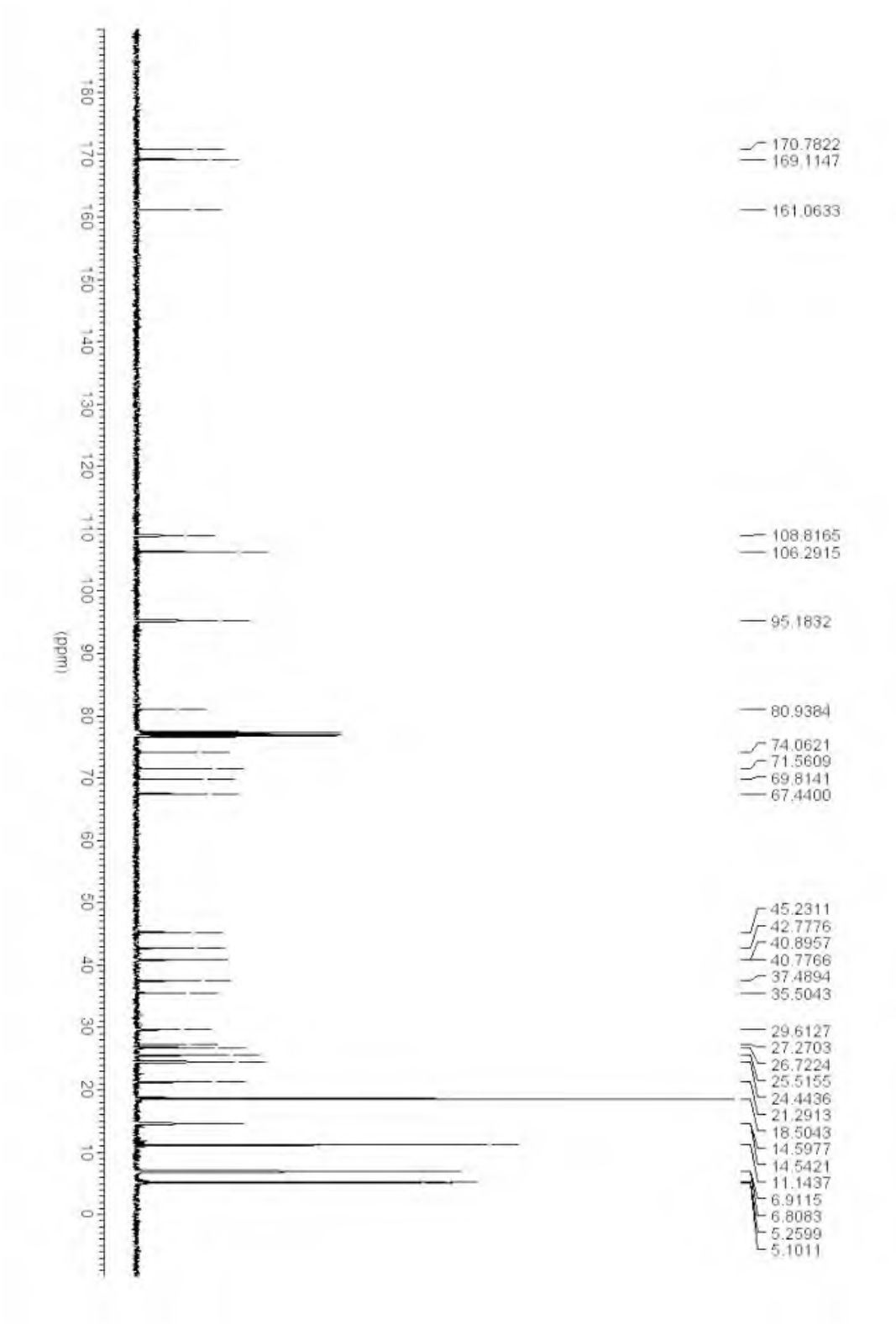
(5*R*,7*S*,8*R*,11*S*,13*R*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5-hydroxy-5,8-dimethyl-11,13-bis[(triethylsilyl)oxy]-1-[tris (propan-2-yl)silyl] tetradec-1-yn-7-yl acetate (II.88)



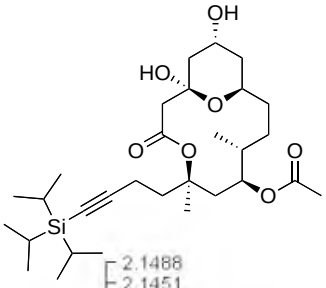


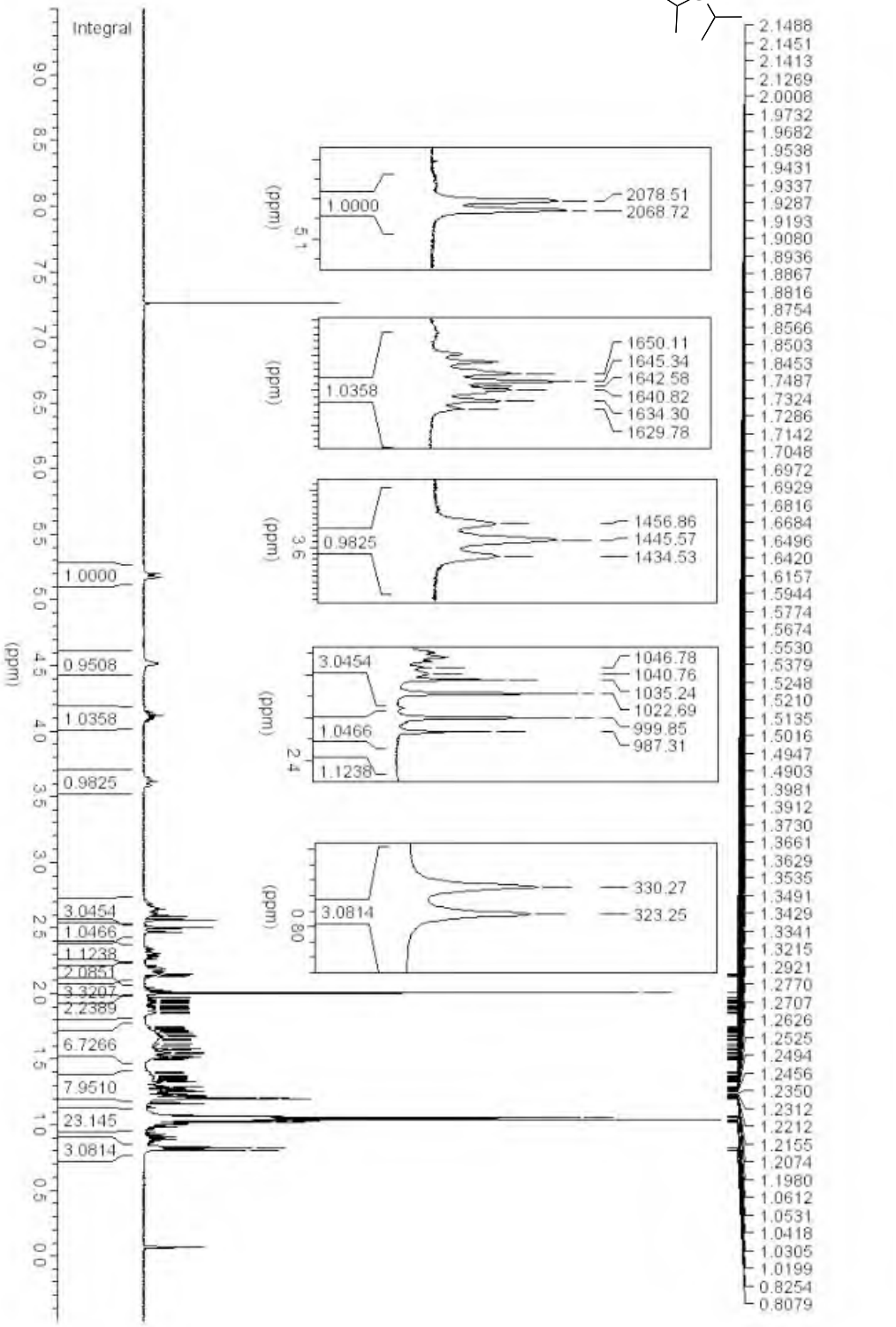
(5*R*,7*S*,8*R*,11*S*,13*R*)-14-(2,2-Dimethyl-6-oxo-2,6-dihydro-1,3-dioxin-4-yl)-5-hydroxy-5,8-dimethyl-11,13-bis[(triethylsilyl)oxy]-1-[tris (propan-2-yl)silyl] tetradec-1-yn-7-yl acetate (II.88)



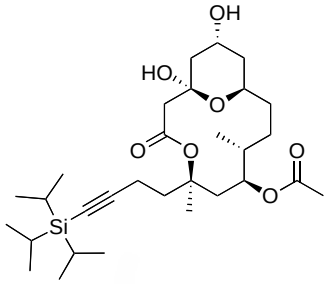


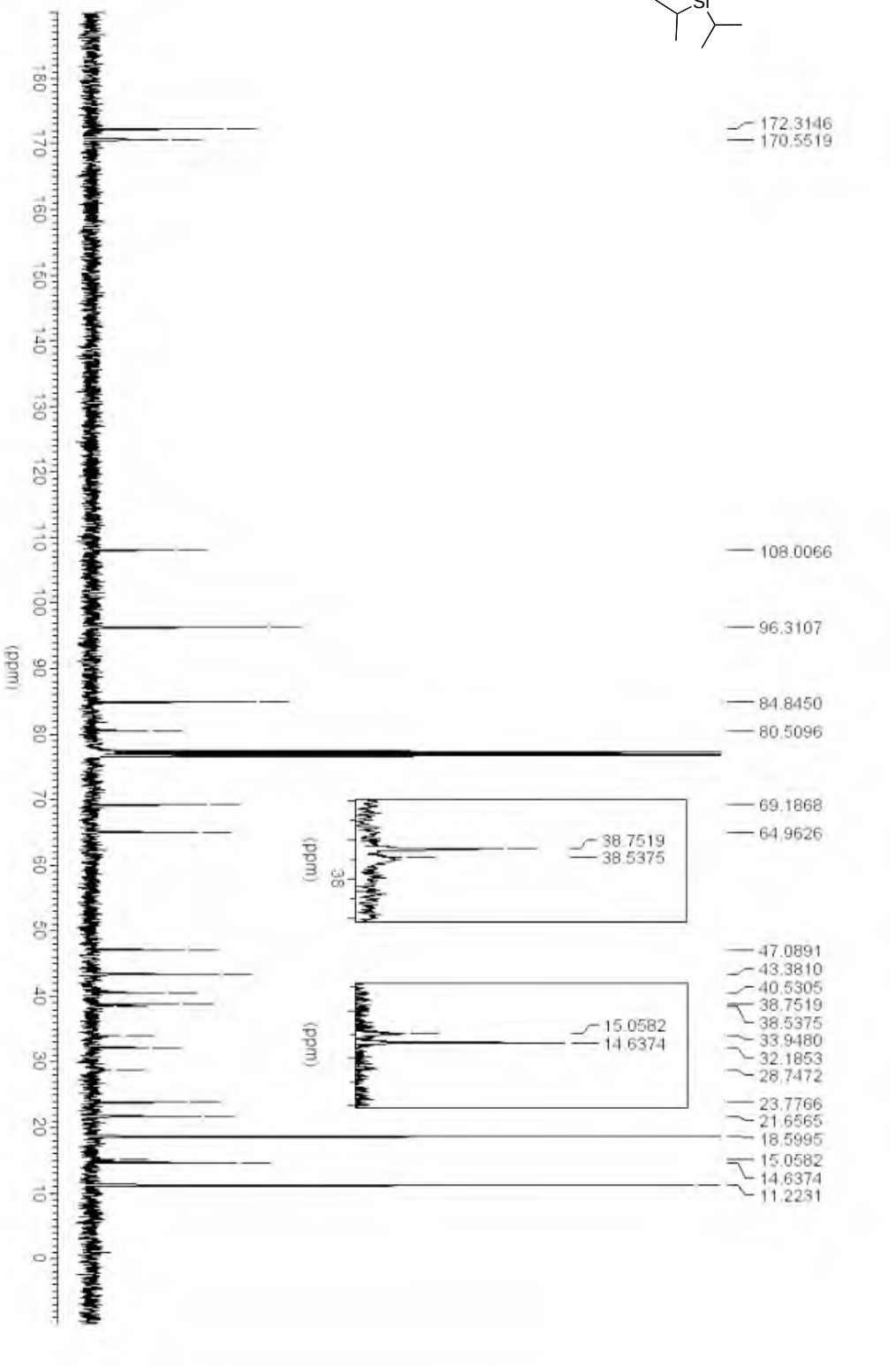
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-dihydroxy-5,8-dimethyl-3-oxo-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo[9.3.1] pentadecan-7-yl acetate (II.89)



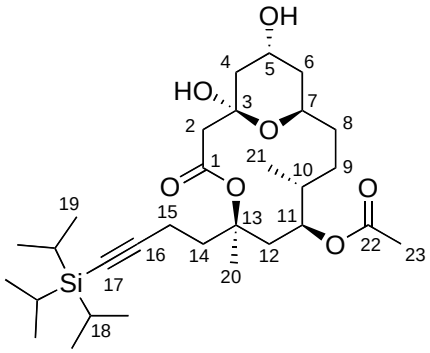


(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-dihydroxy-5,8-dimethyl-3-oxo-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo[9.3.1] pentadecan-7-yl acetate (II.89)

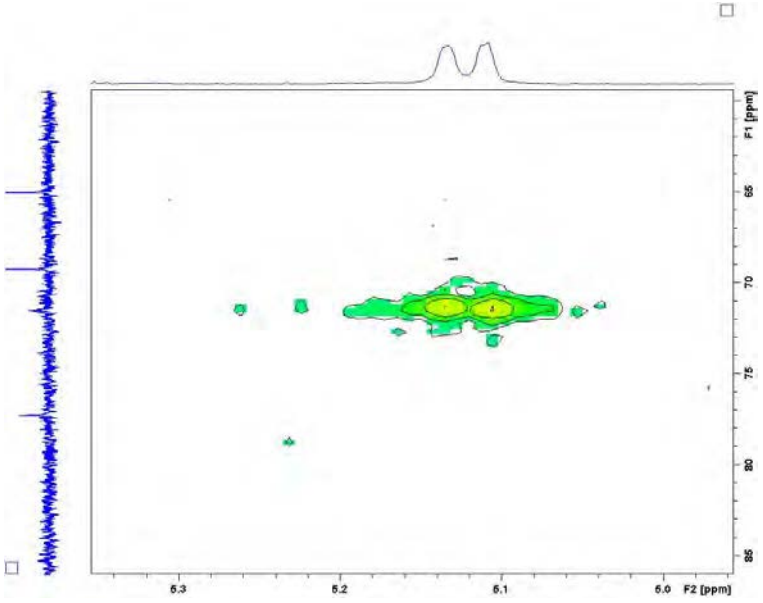




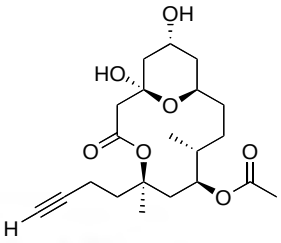
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-3-oxo-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate (II.89)

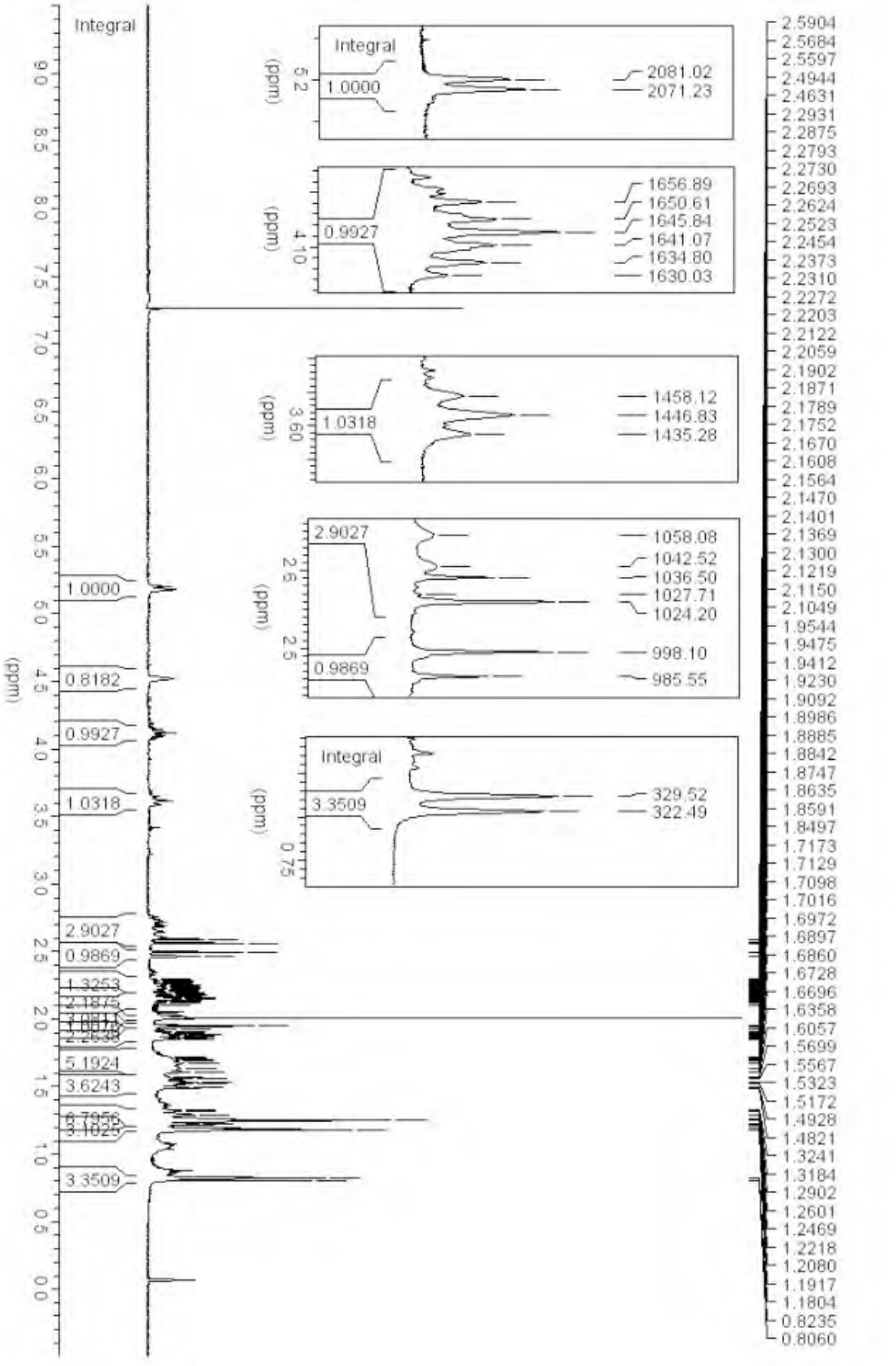


HMQC experiment: amplification of spectra for C11

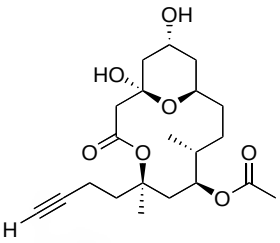


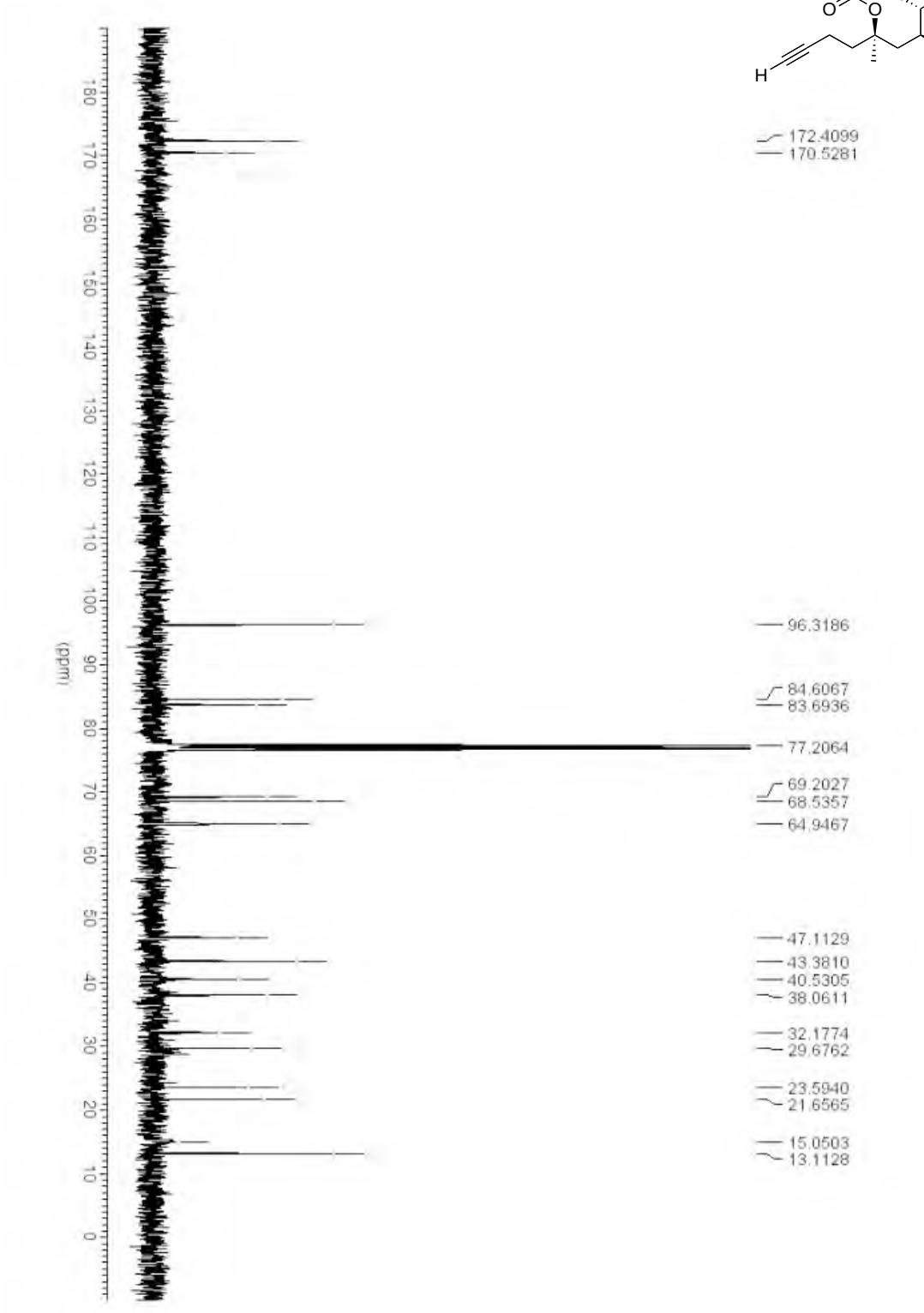
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-5-(But-3-yn-1-yl)-1,13-dihydroxy-5,8-dimethyl-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate



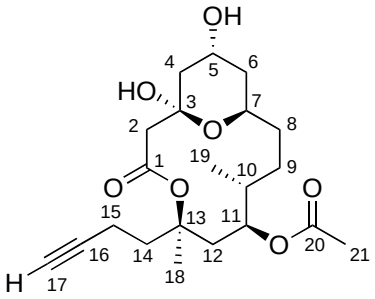


(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-5-(But-3-yn-1-yl)-1,13-dihydroxy-5,8-dimethyl-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate

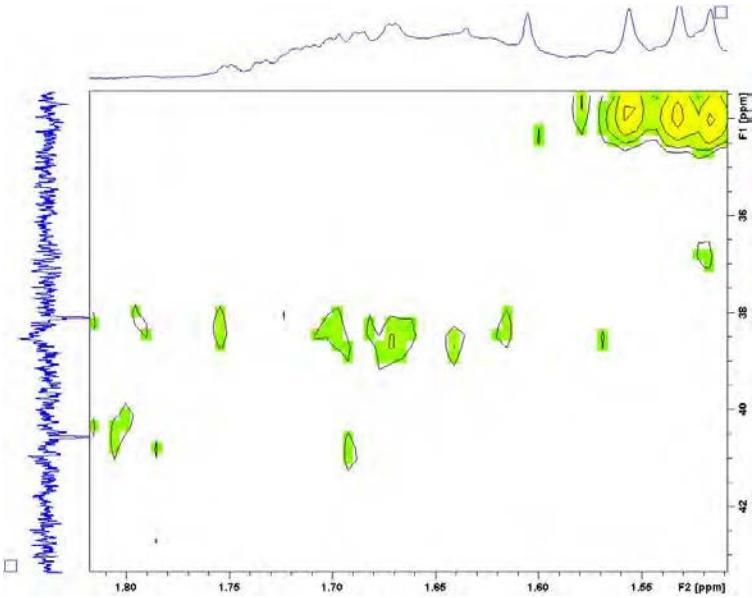




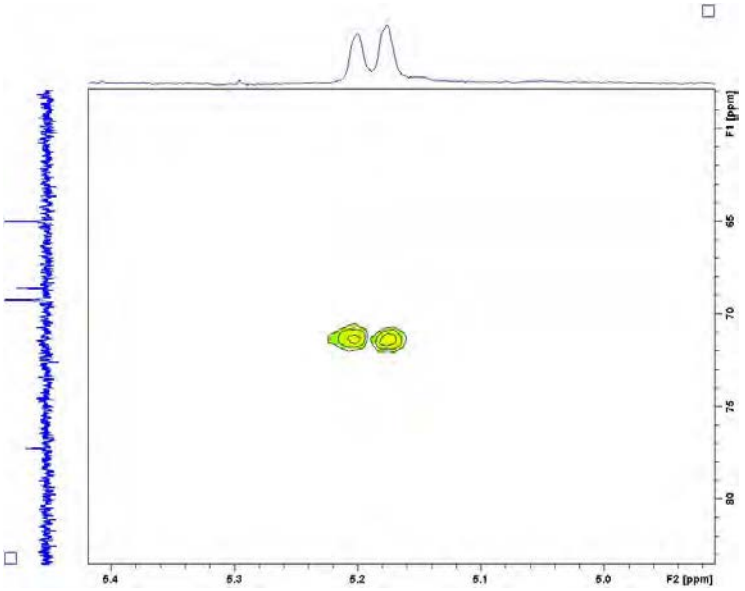
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-5-(but-3-yn-1-yl)-1,13-dihydroxy-5,8-dimethyl-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate



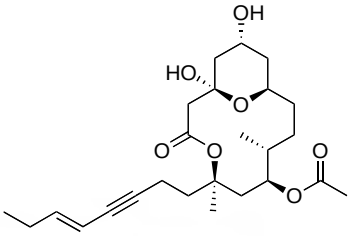
HMQC experiment: amplification of spectra for C10

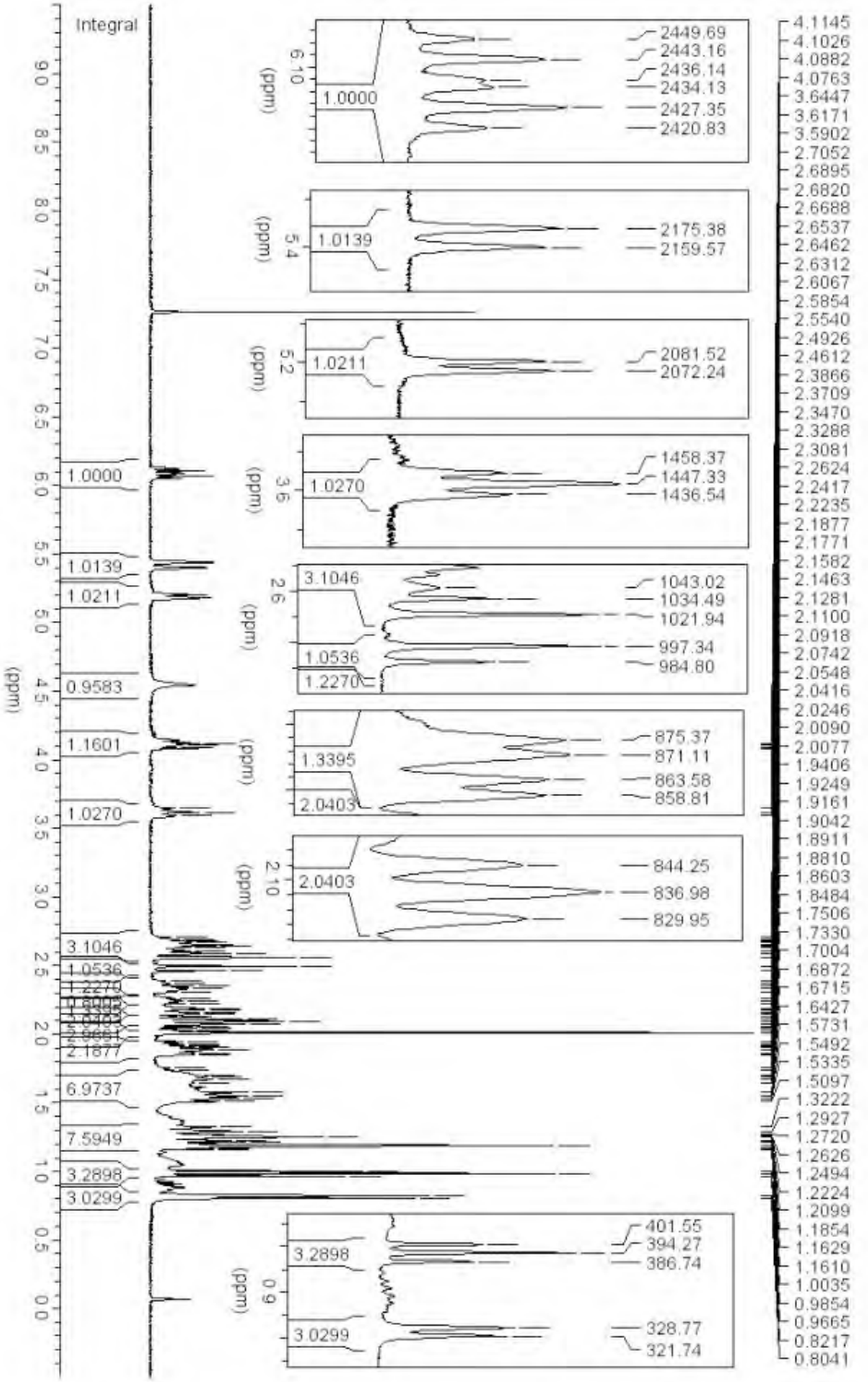


HMQC experiment: amplification of spectra for C11

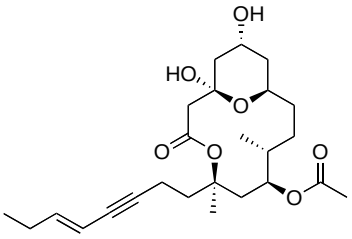


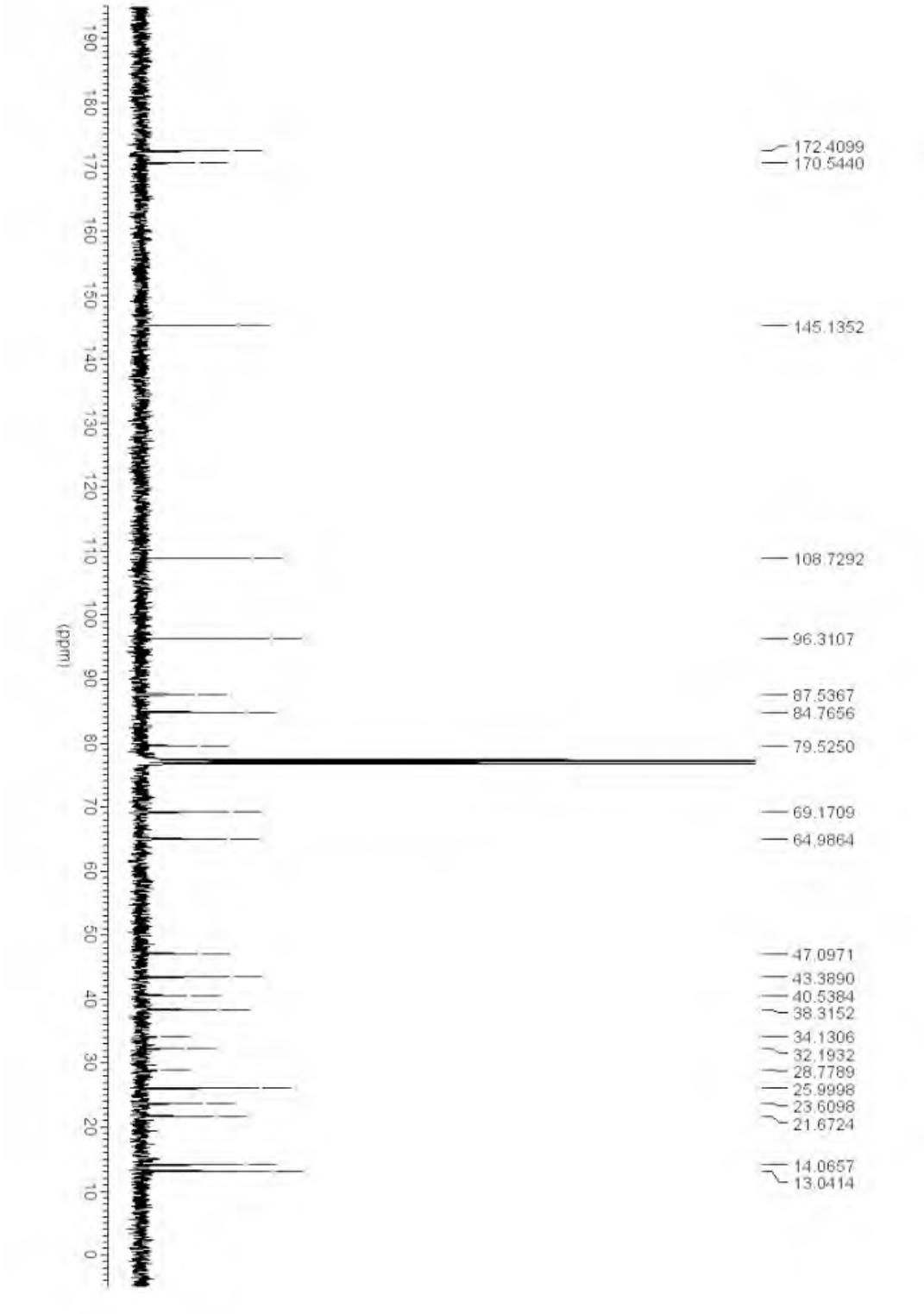
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-5-[(5*E*)-oct-5-en-3-yn-1-yl]-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate (II.90)



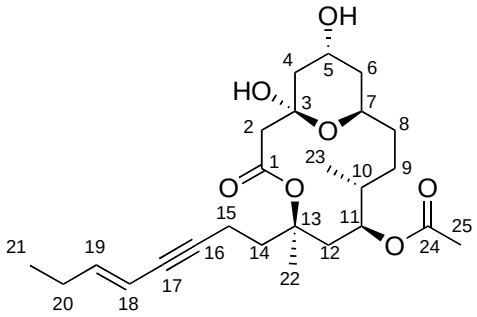


(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-5-[(5*E*)-oct-5-en-3-yn-1-yl]-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate (II.90)

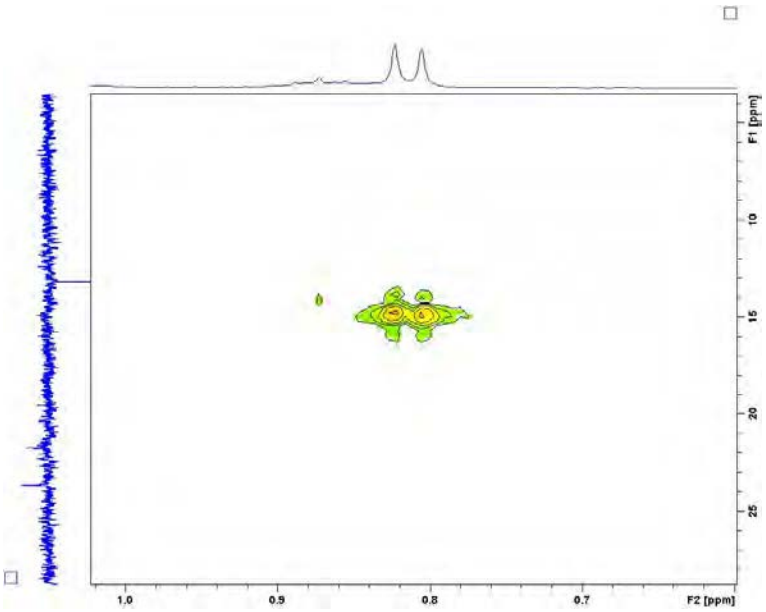




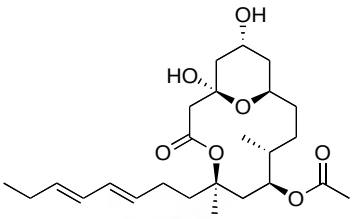
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-dihydroxy-5,8-dimethyl-5-[(5*E*)-oct-5-en-3-yn-1-yl]-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate (II.90)

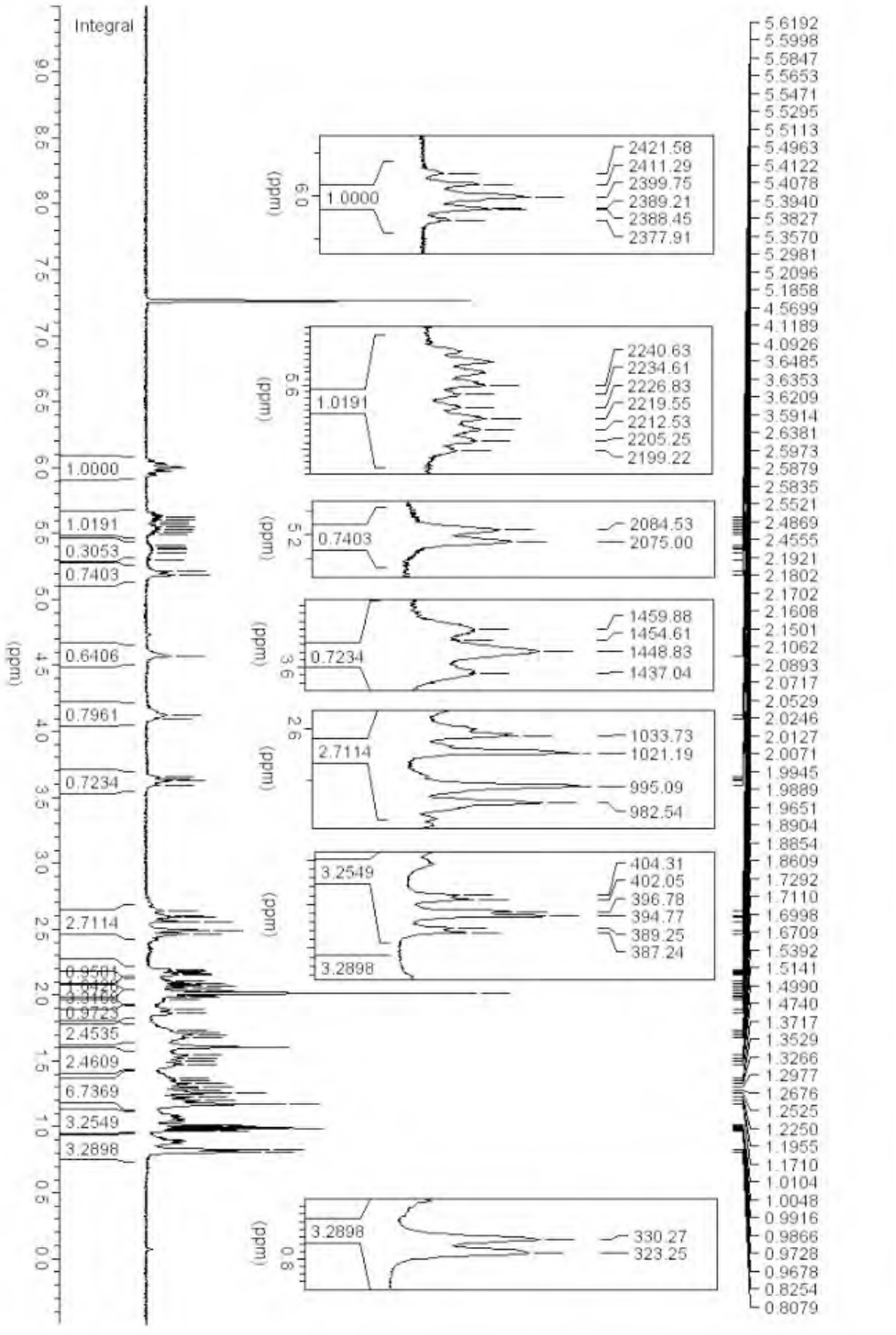


HMQC experiment: amplification of spectra for C23

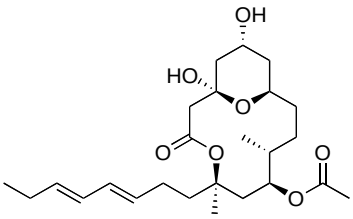


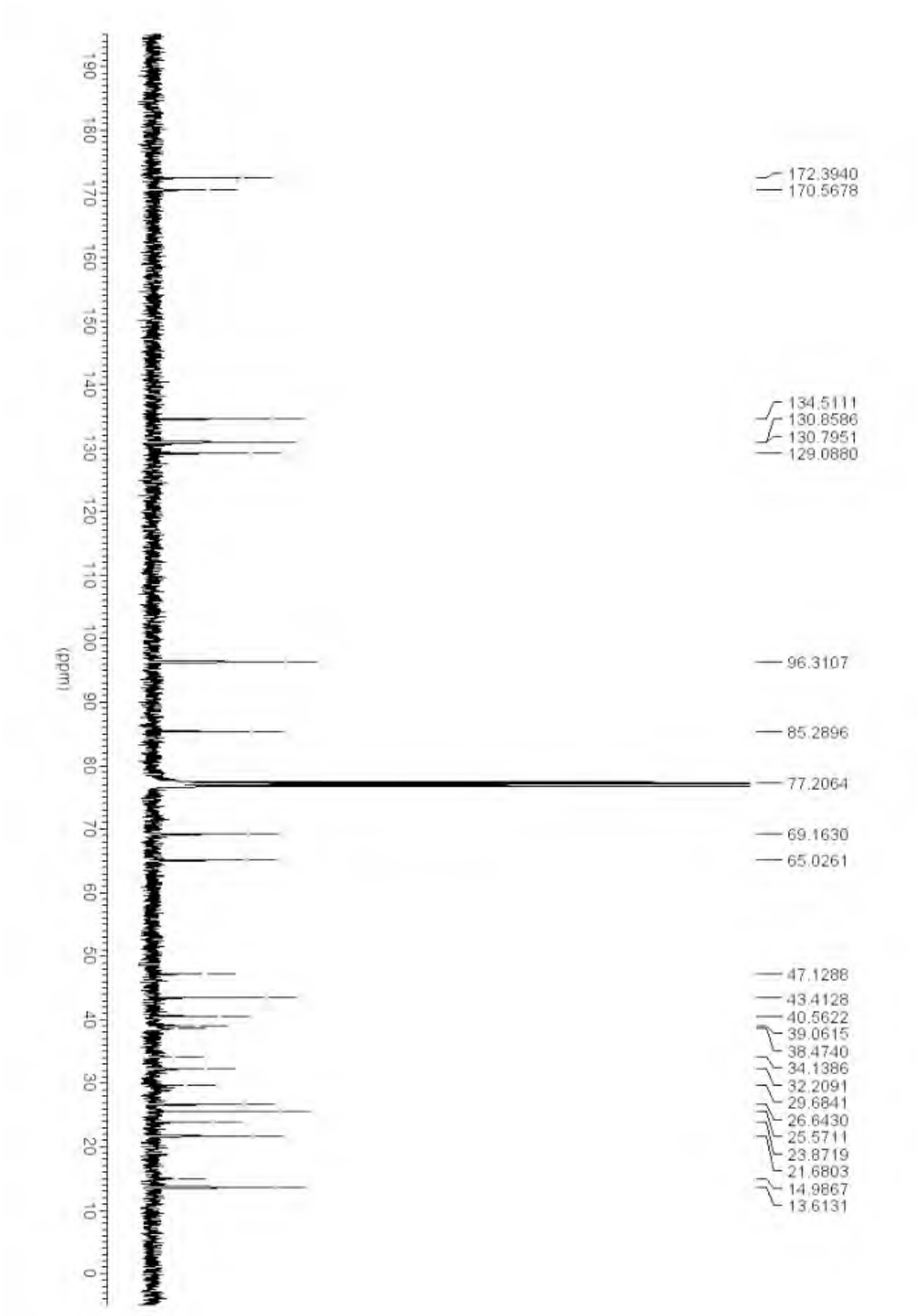
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-5-[(3*E*,5*E*)-octa-3,5-dien-1-yl]-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate (II.91)



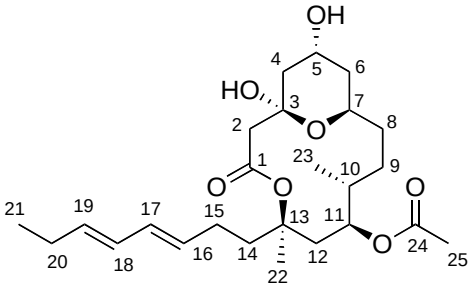


(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-5-[(3*E*,5*E*)-octa-3,5-dien-1-yl]-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate (II.91)

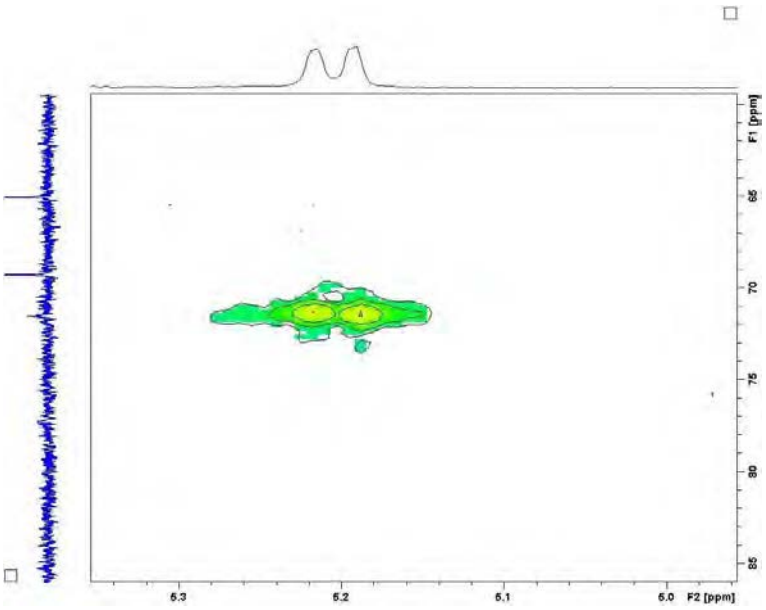




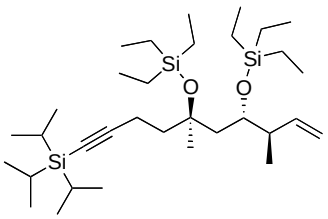
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,13-Dihydroxy-5,8-dimethyl-5-[(3*E*,5*E*)-octa-3,5-dien-1-yl]-3-oxo-4,15-dioxabicyclo[9.3.1]pentadecan-7-yl acetate (II.91)

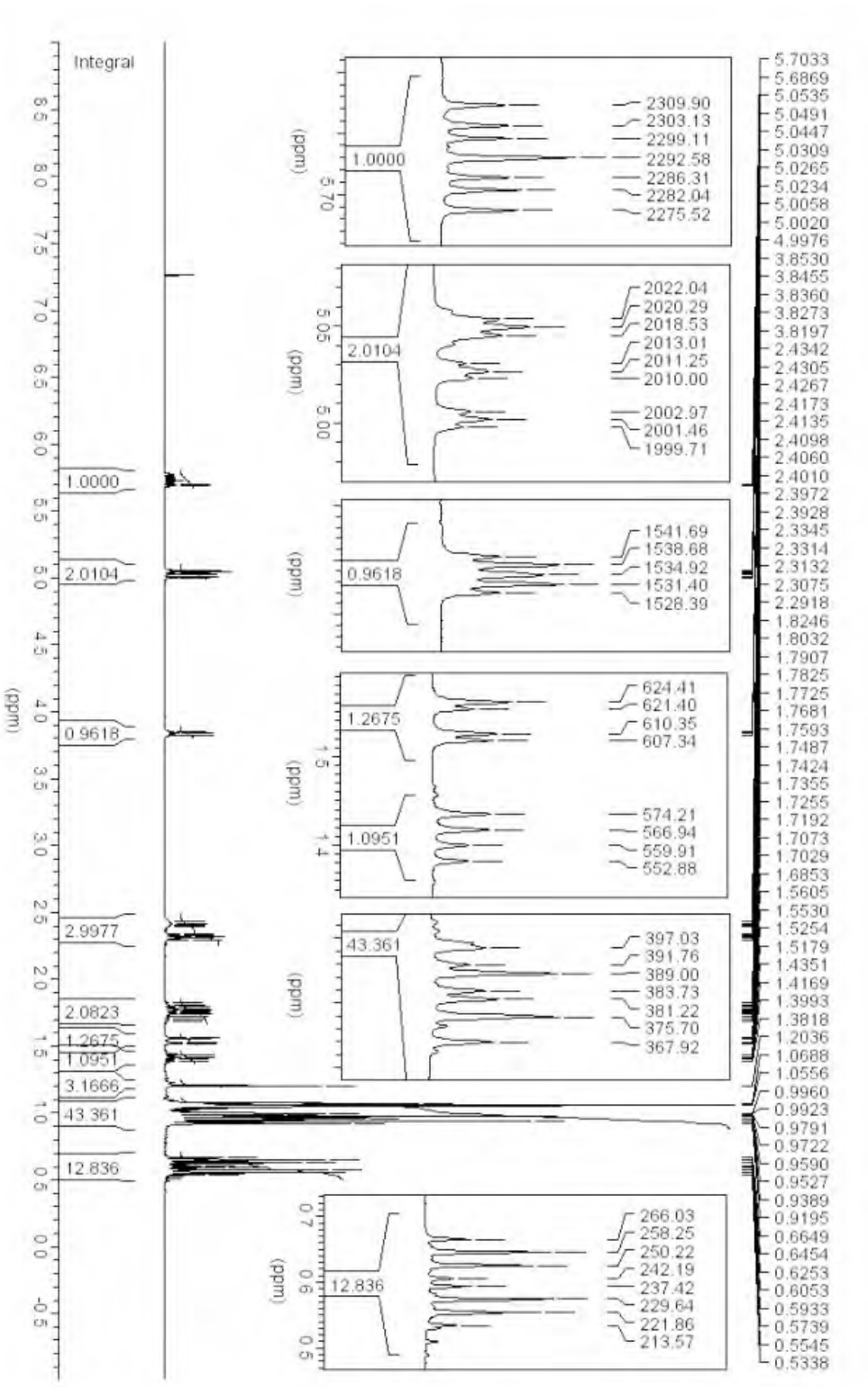


HMQC experiment: amplification of spectra for C11

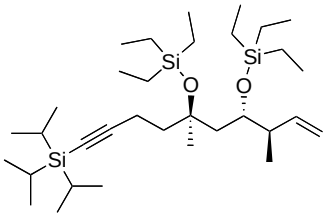


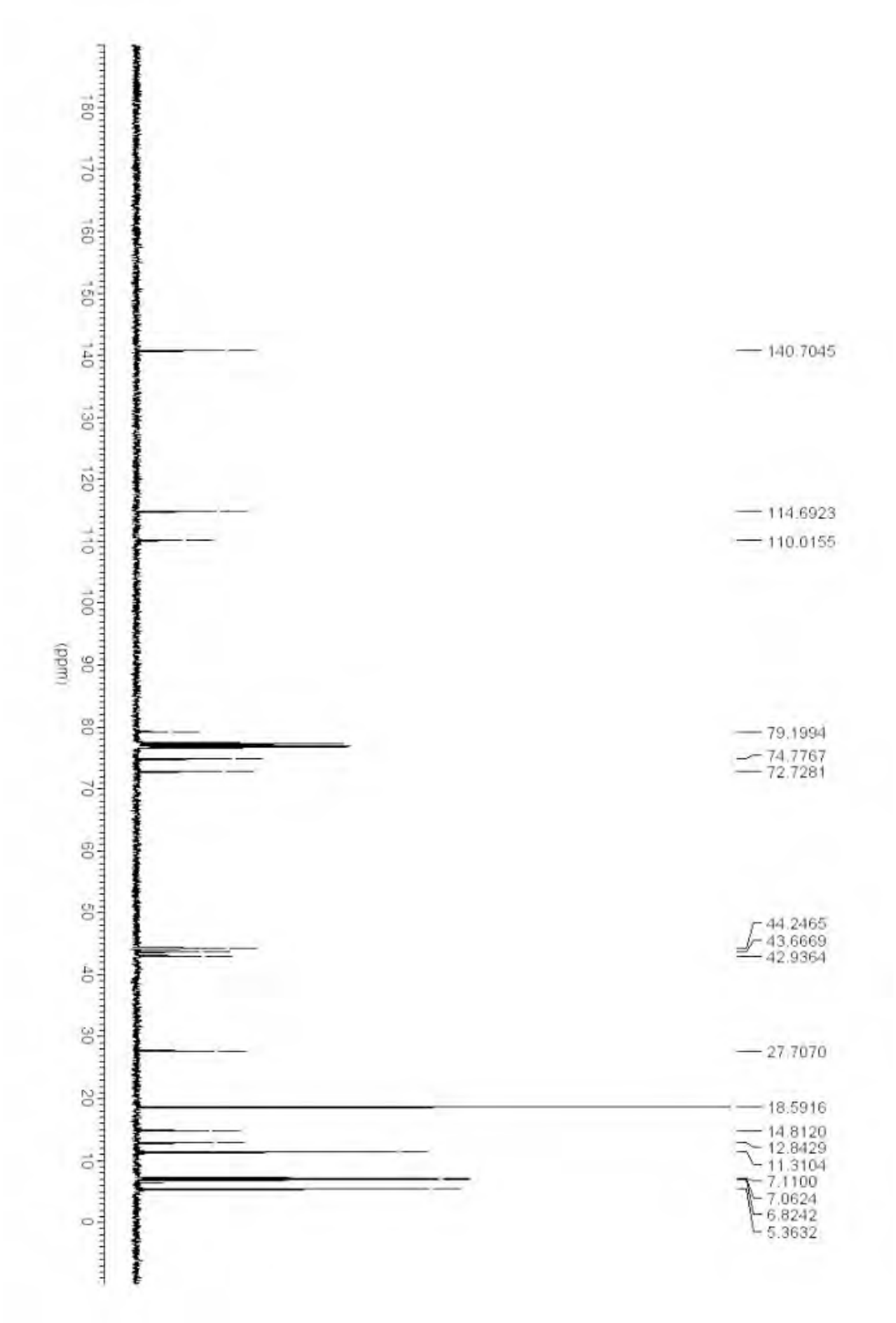
(5*R*,7*S*)-7-[(2*R*)-But-3-en-2-yl]-3,3,9,9-tetraethyl-5-methyl-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,8-dioxa-3,9-disilaundecane (II.93)



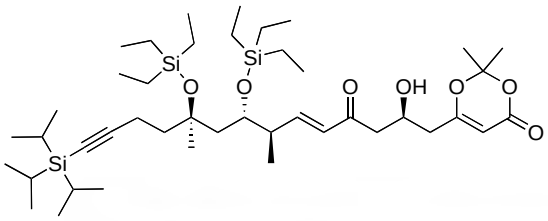


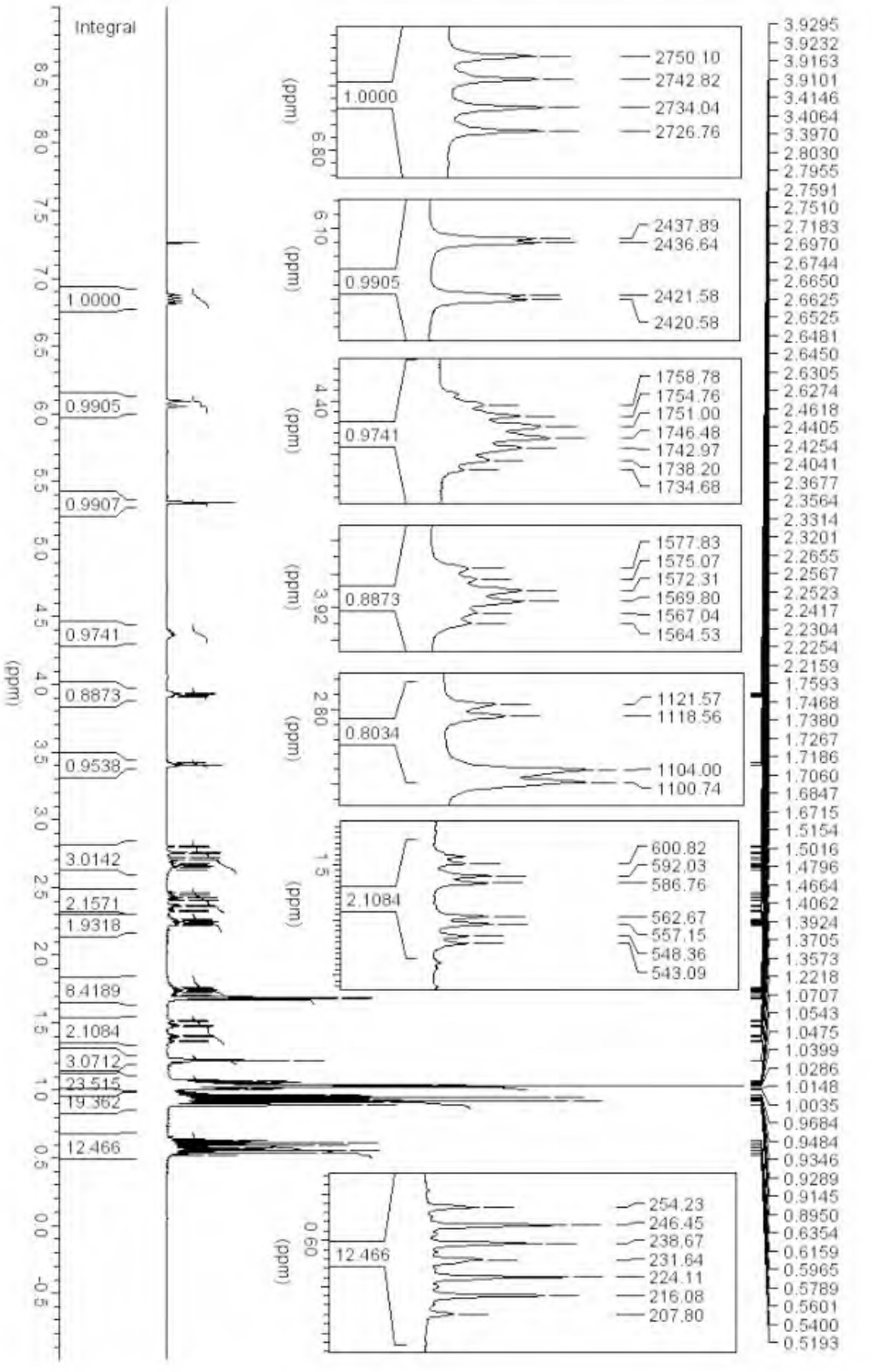
(5*R*,7*S*)-7-[(2*R*)-But-3-en-2-yl]-3,3,9,9-tetraethyl-5-methyl-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,8-dioxa-3,9-disilaundecane (II.93)



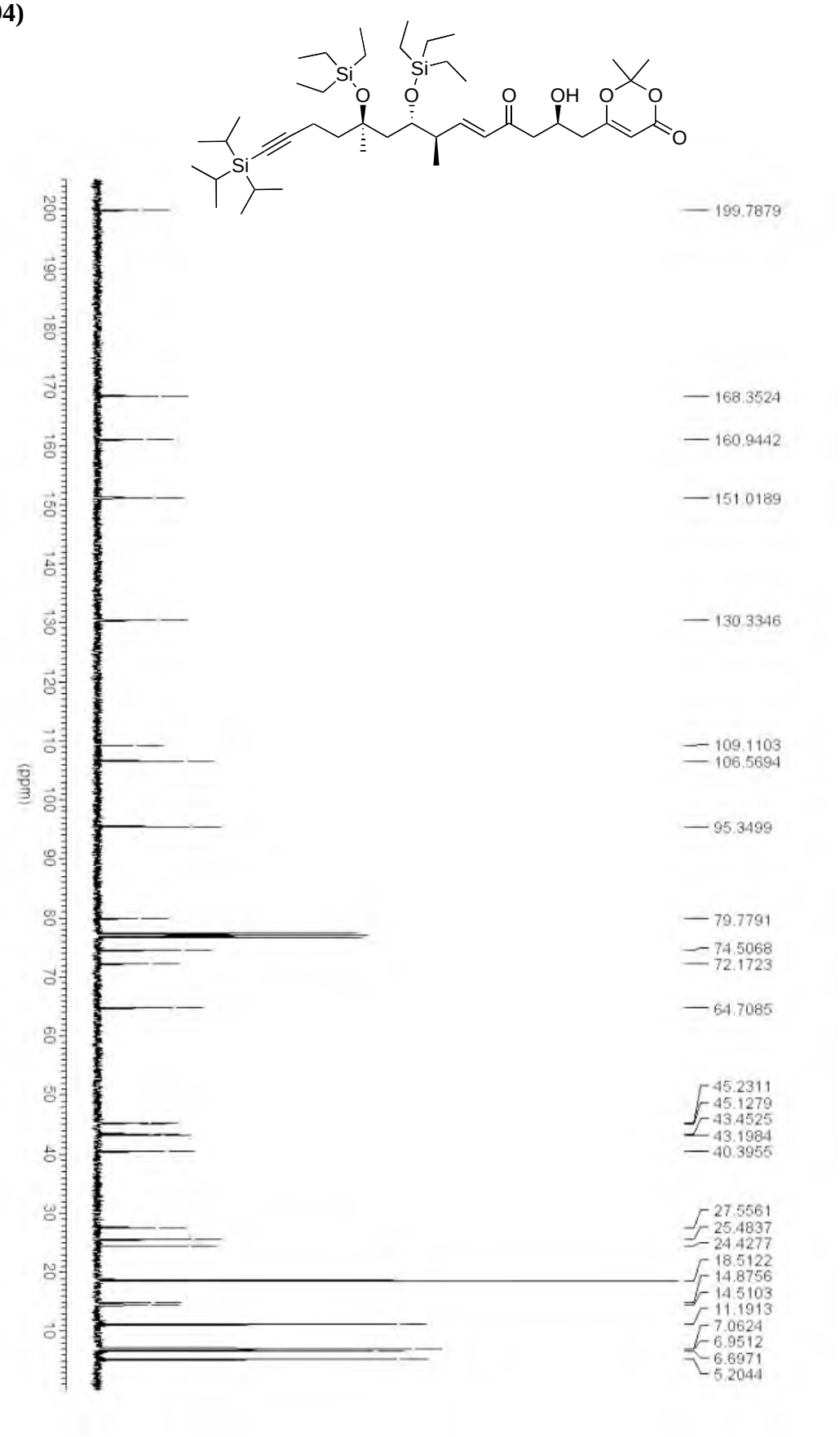


6-[(2*S*,5*E*,7*R*,8*S*,10*R*)-2-Hydroxy-7,10-dimethyl-4-oxo-8,10-bis[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-5-en-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.94)

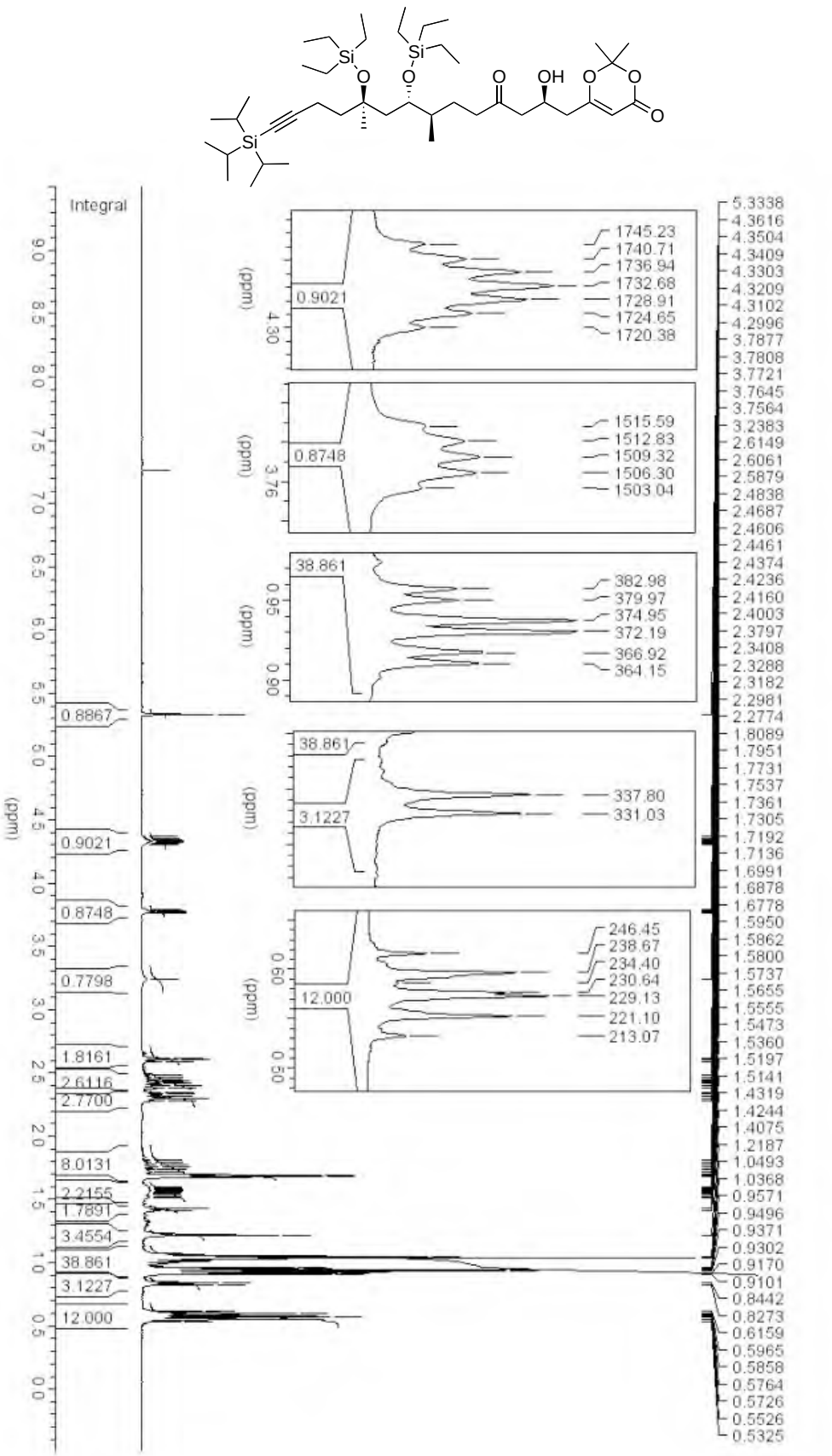




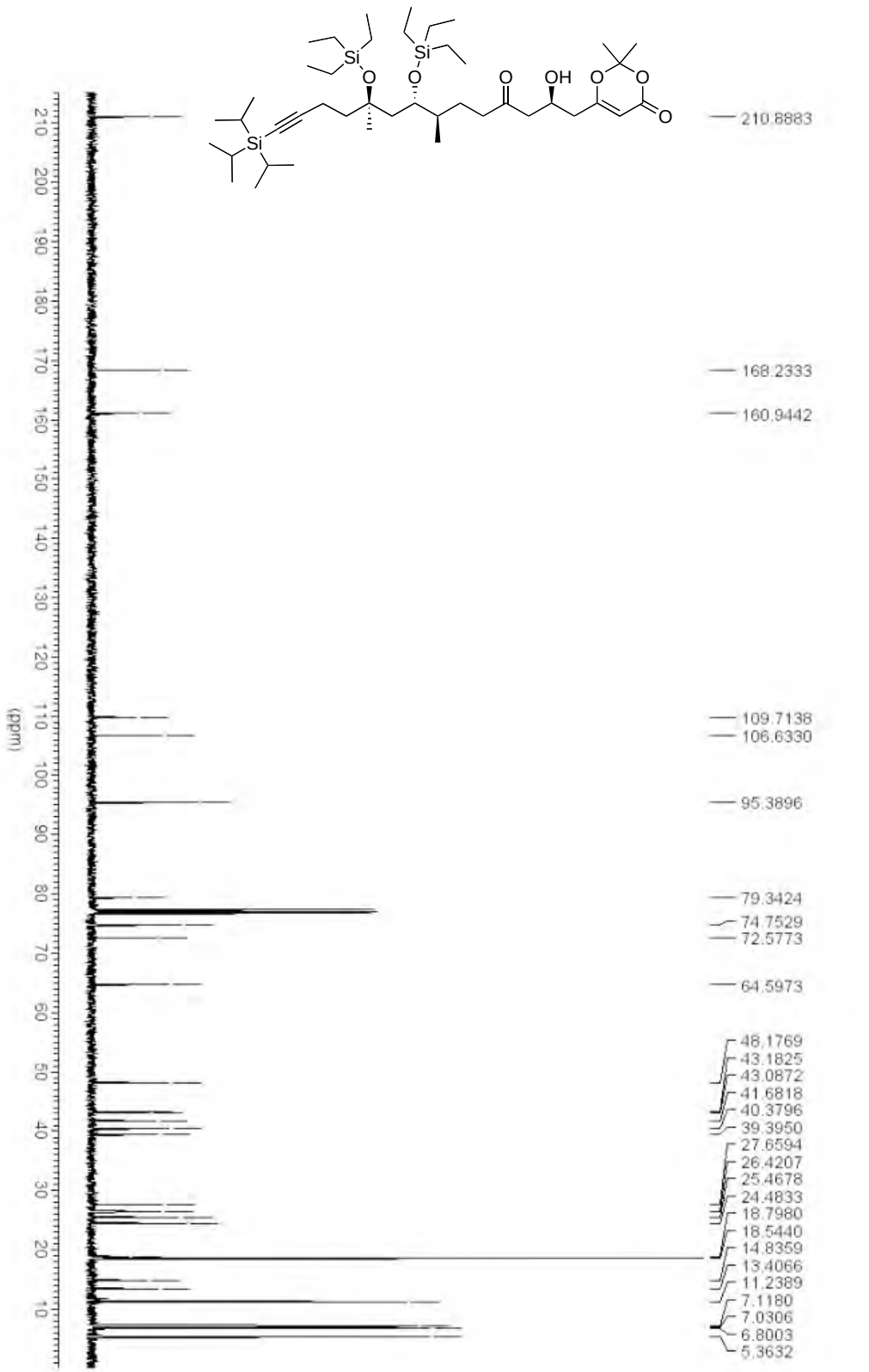
6-[(2*S*,5*E*,7*R*,8*S*,10*R*)-2-Hydroxy-7,10-dimethyl-4-oxo-8,10-bis[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-5-en-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.94)



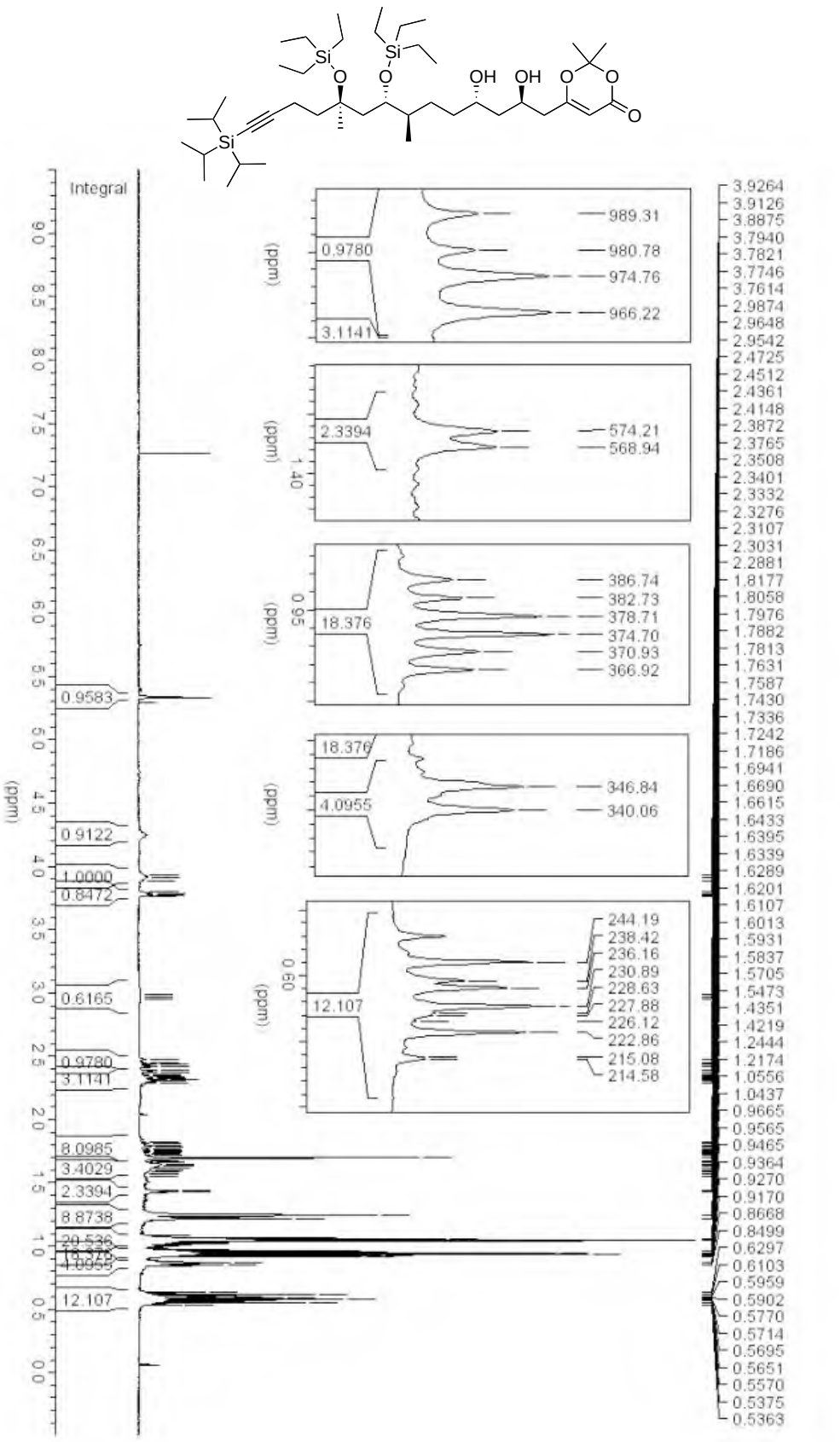
6-[(2*S*,7*R*,8*S*,10*R*)-2-Hydroxy-7,10-dimethyl-4-oxo-8,10-bis[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.95)



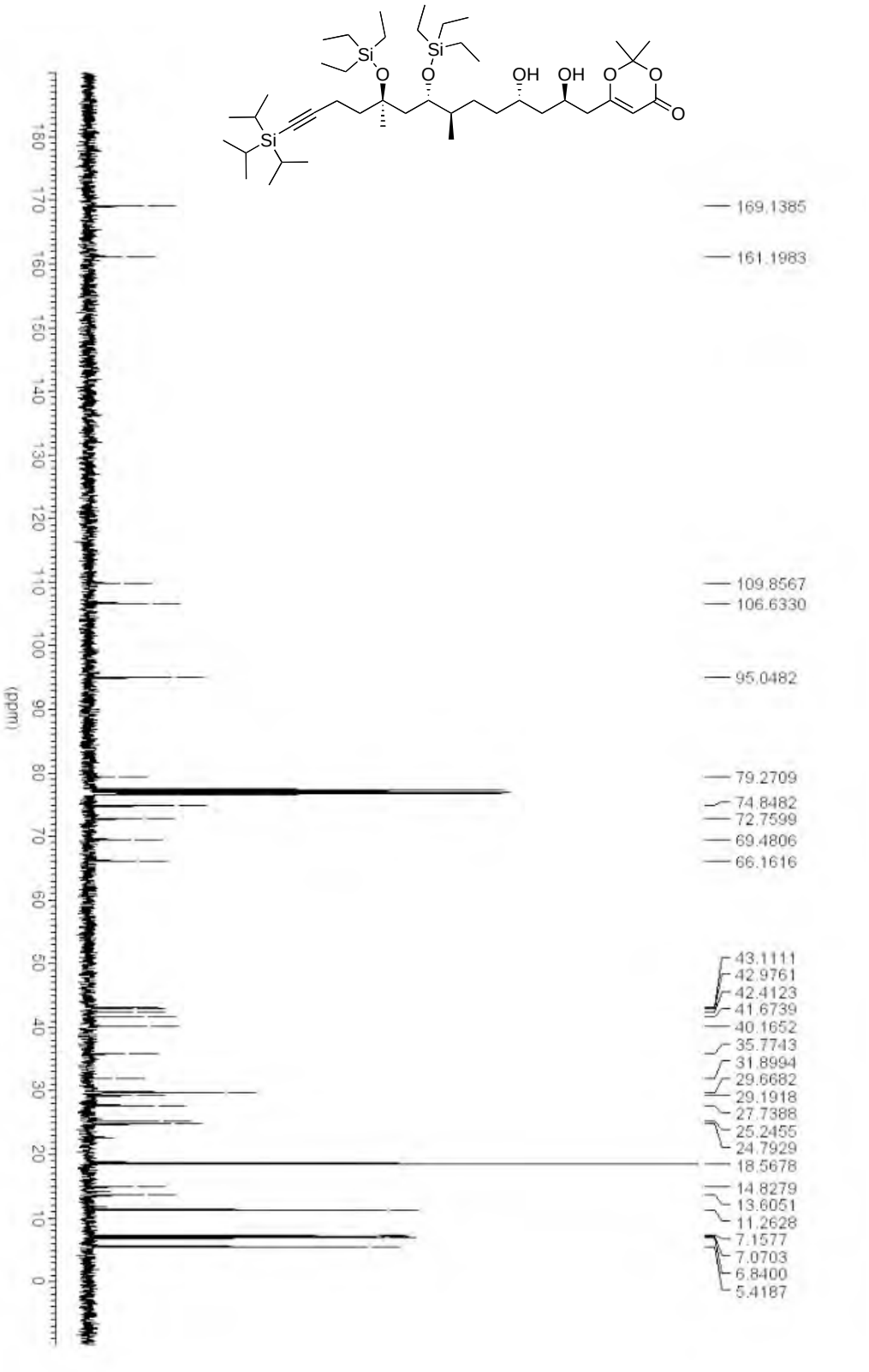
6-[(2*S*,7*R*,8*S*,10*R*)-2-Hydroxy-7,10-dimethyl-4-oxo-8,10-bis[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.95)



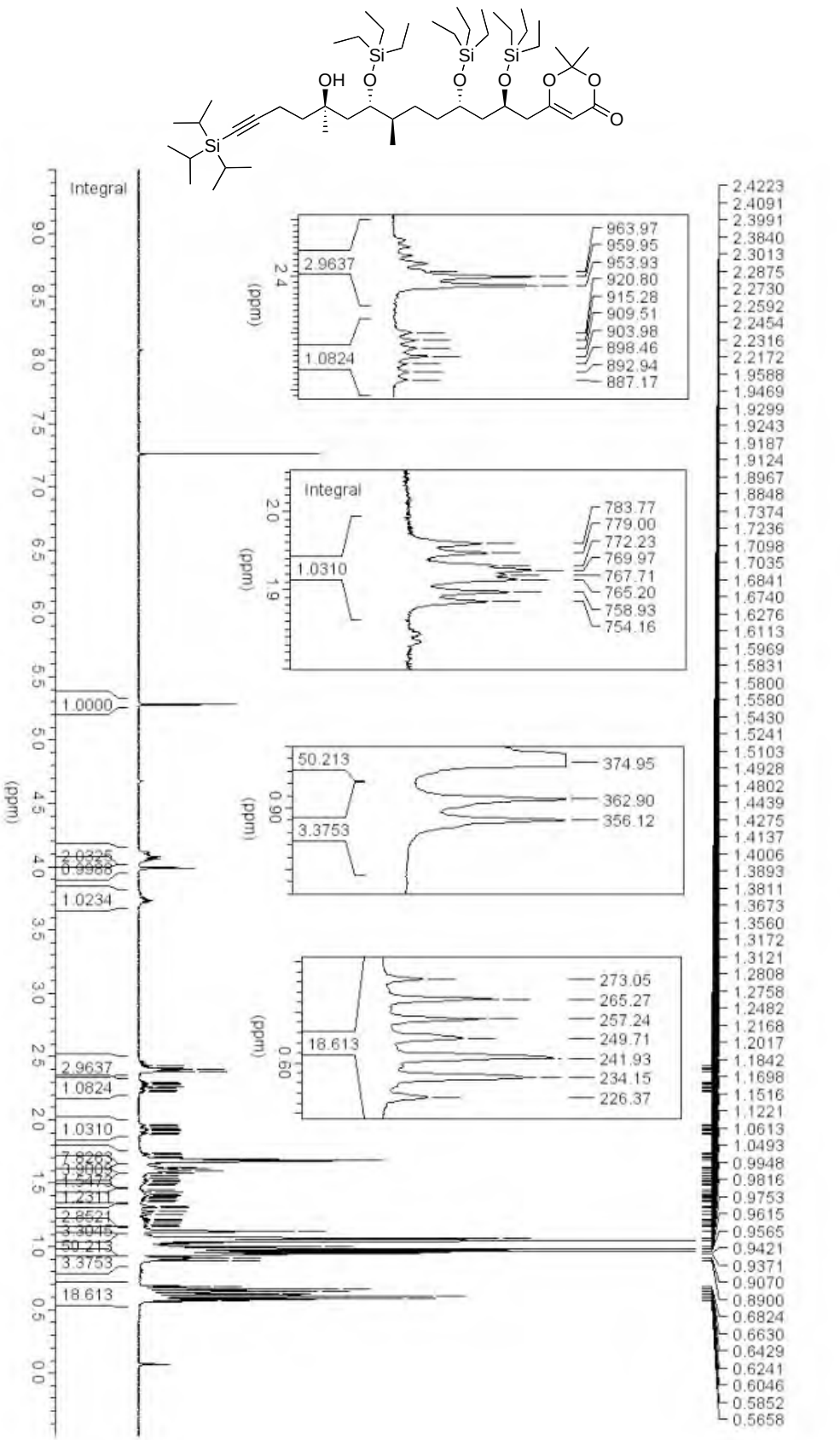
6-[(2*R*,4*S*,7*R*,8*S*,10*R*)-2,4-Dihydroxy-7,10-dimethyl-8,10-bis[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.96)



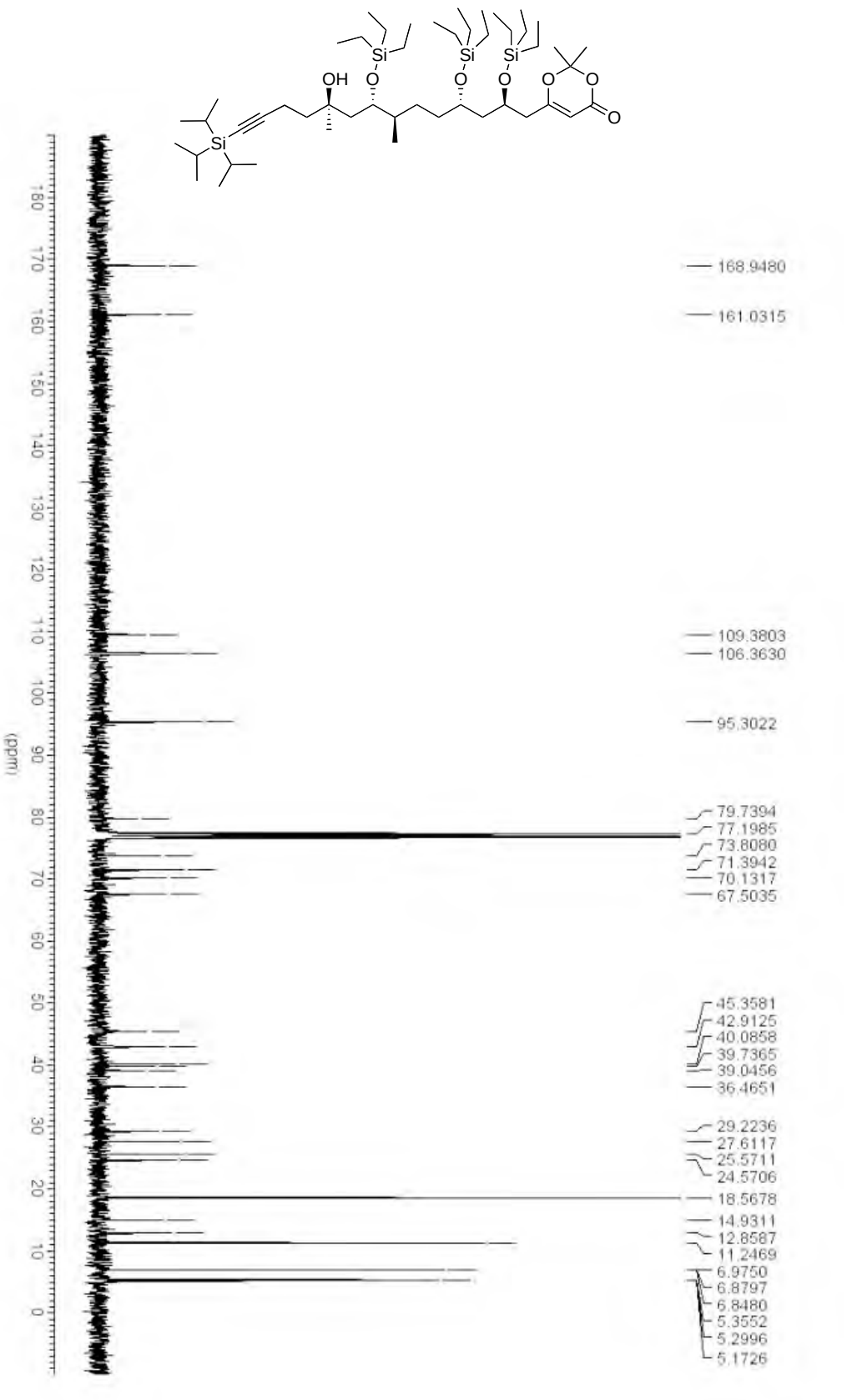
6-[(2*R*,4*S*,7*R*,8*S*,10*R*)-2,4-Dihydroxy-7,10-dimethyl-8,10-bis[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.96)



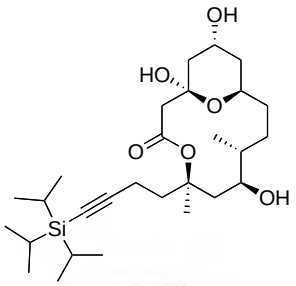
6-[(2*R*,4*S*,7*R*,8*S*,10*R*)-10-Hydroxy-7,10-dimethyl-2,4,8-tris[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.97)

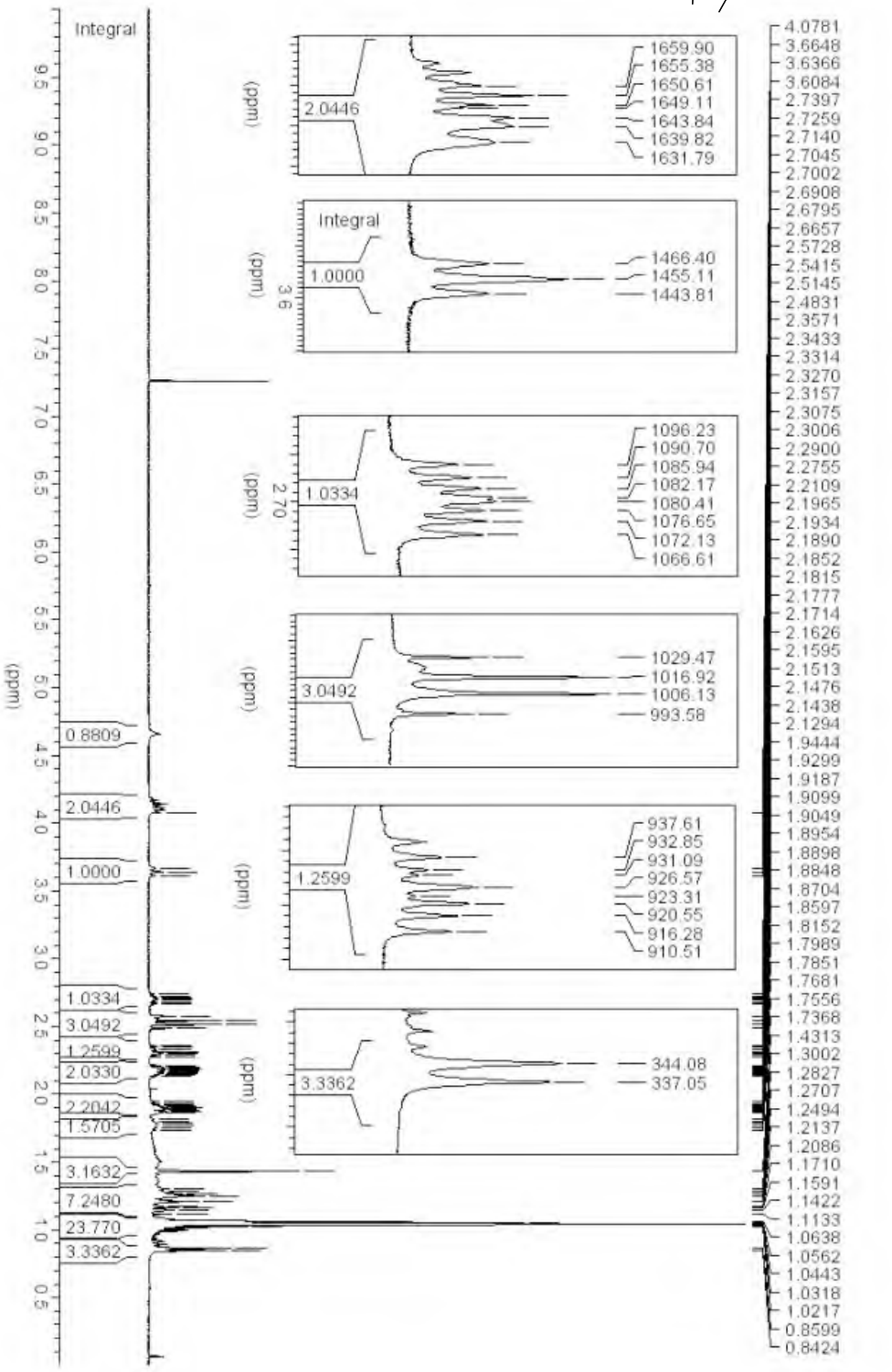


6-[(2*R*,4*S*,7*R*,8*S*,10*R*)-10-Hydroxy-7,10-dimethyl-2,4,8-tris[(triethylsilyl)oxy]-14-[tris(propan-2-yl)silyl]tetradec-13-yn-1-yl]-2,2-dimethyl-2,4-dihydro-1,3-dioxin-4-one (II.97)

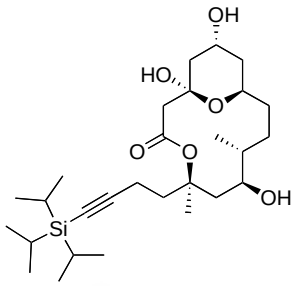


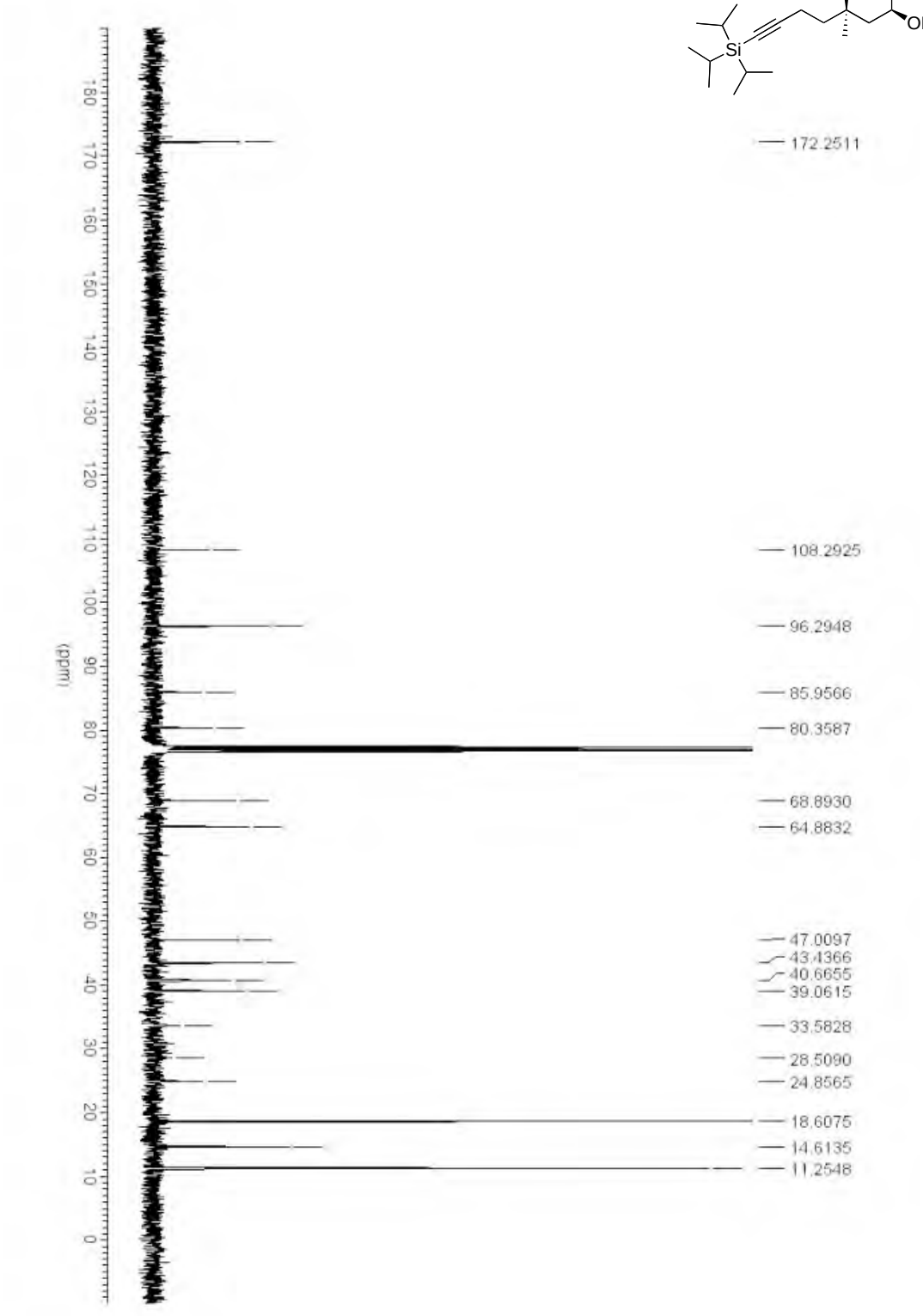
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,7,13-Trihydroxy-5,8-dimethyl-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo[9.3.1]pentadecan-3-one (II.98)



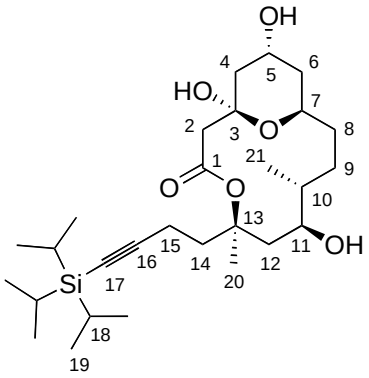


(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,7,13-Trihydroxy-5,8-dimethyl-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo[9.3.1]pentadecan-3-one (II.98)

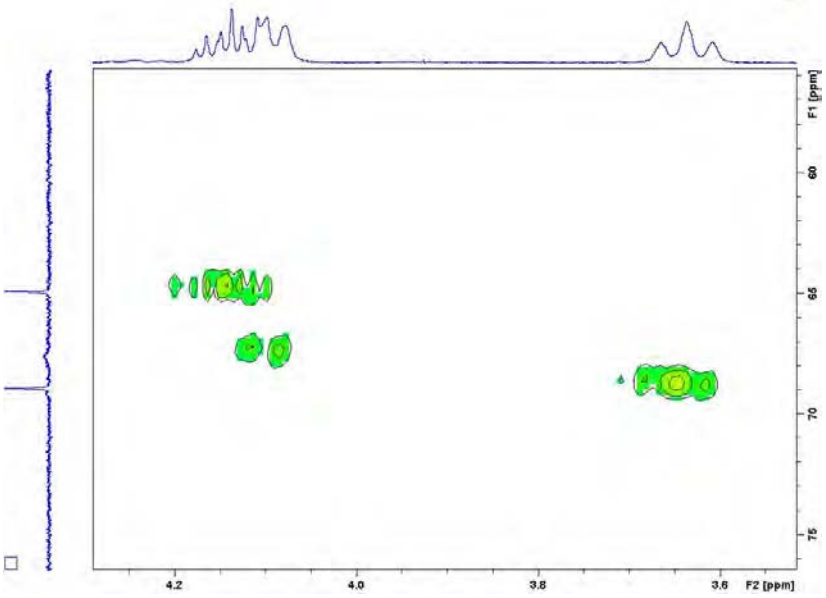




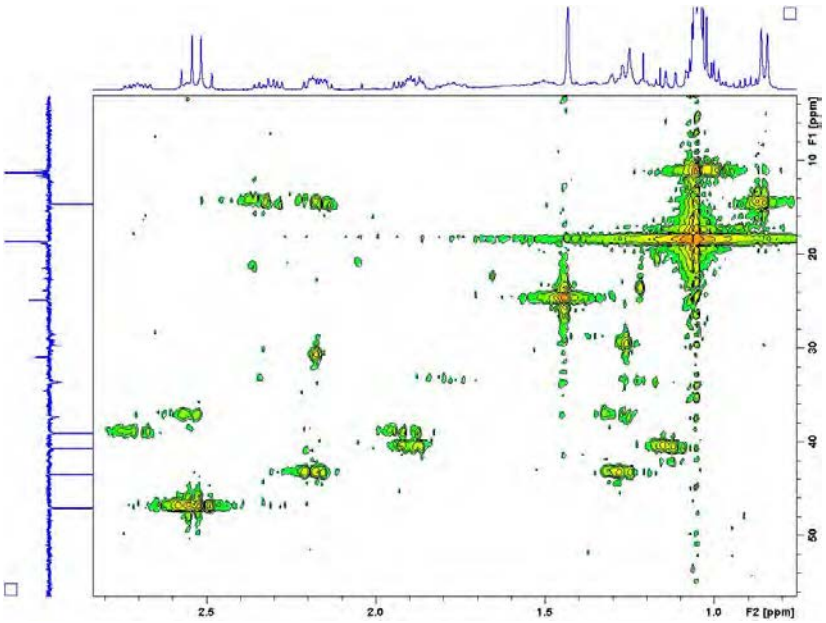
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,7,13-Trihydroxy-5,8-dimethyl-5-{4-[tris(propan-2-yl)silyl]but-3-yn-1-yl}-4,15-dioxabicyclo[9.3.1]pentadecan-3-one (II.98)



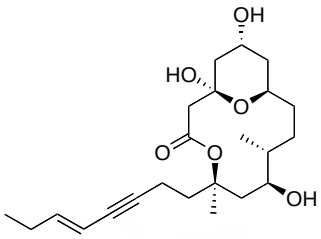
HMQC experiment: amplification of spectra for C5

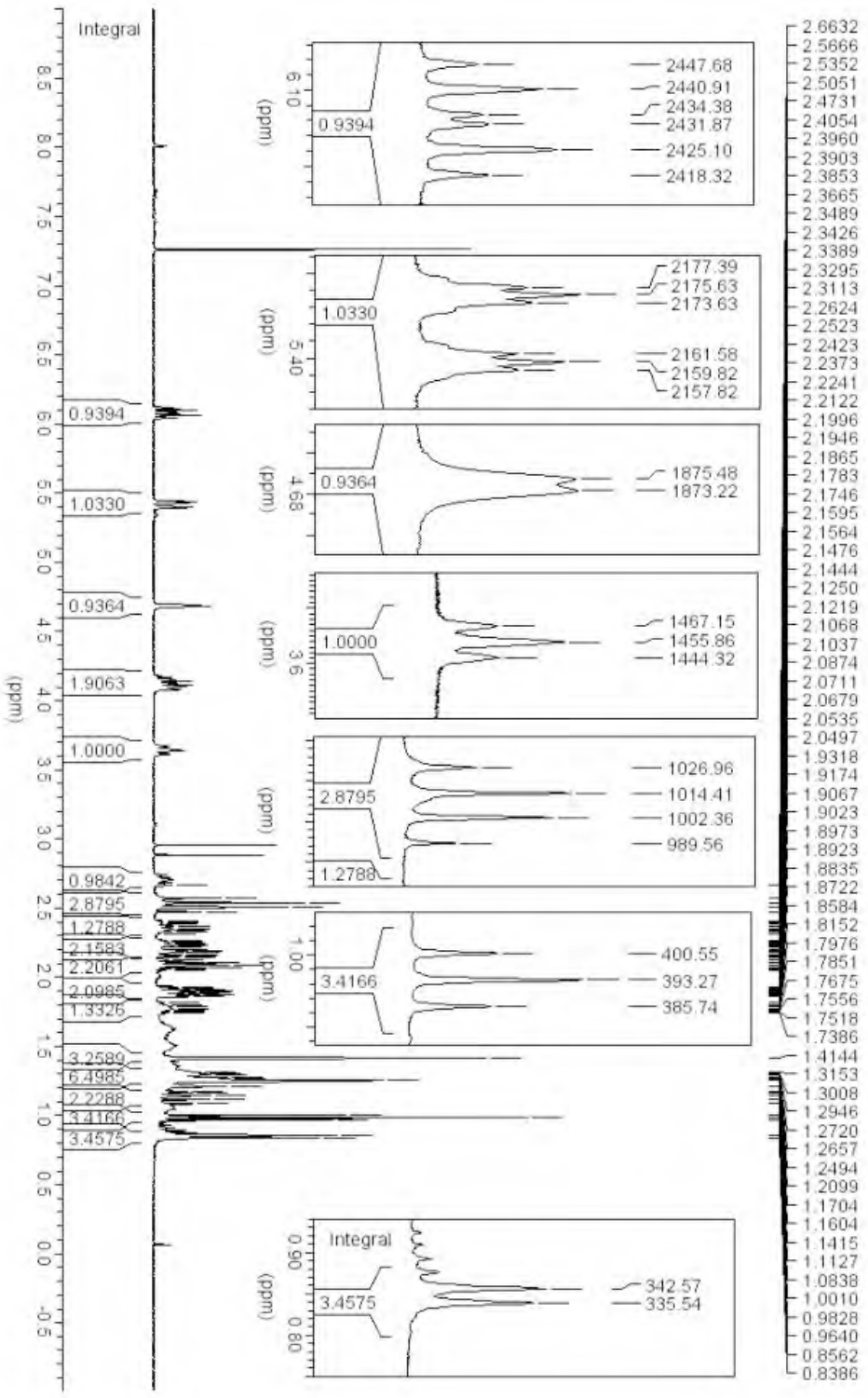


HMQC experiment: amplification of spectra for C10, C12 and C21

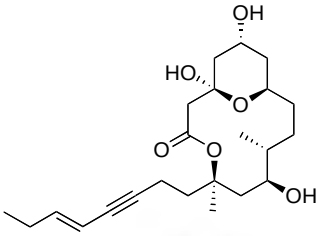


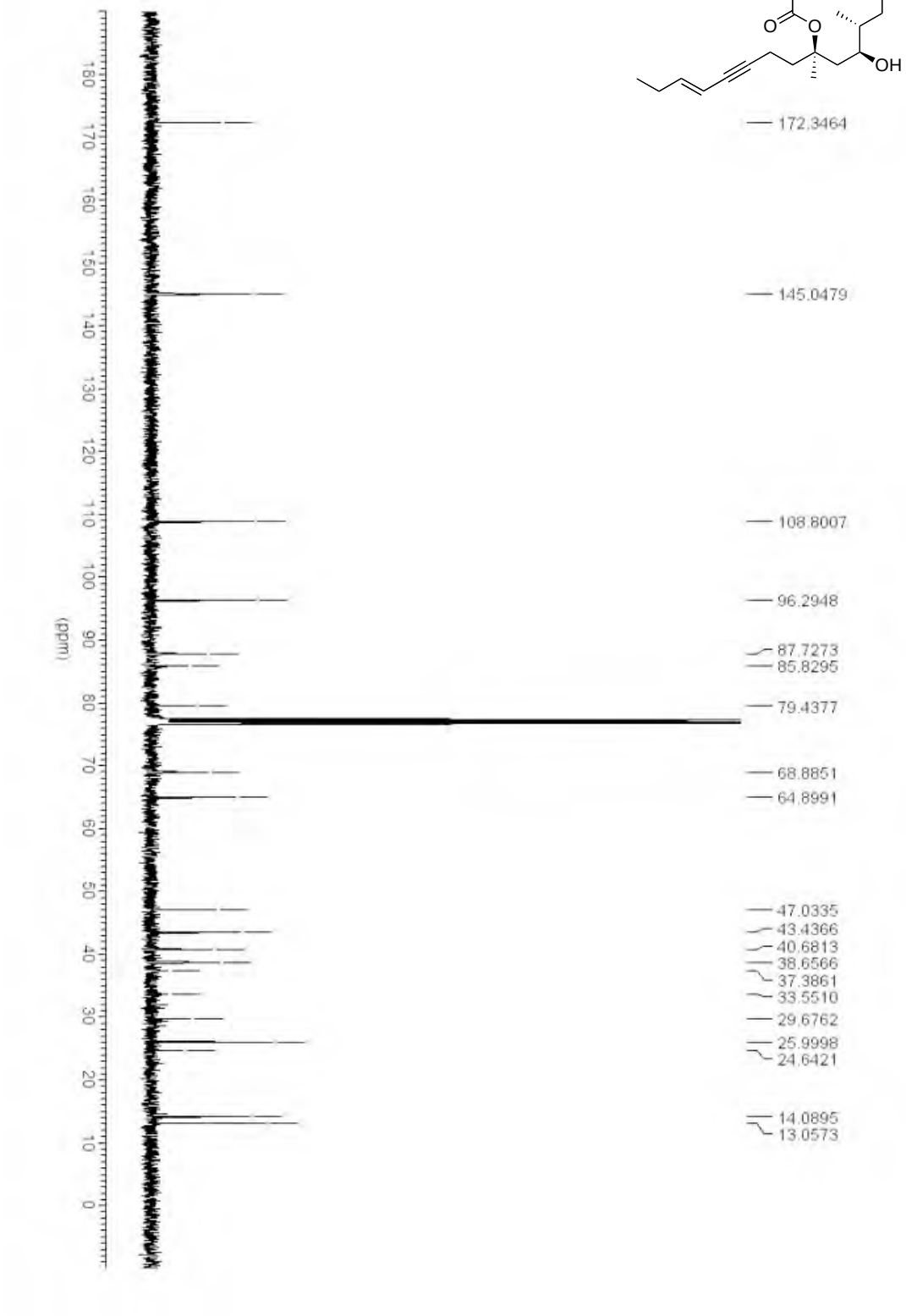
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,7,13-Trihydroxy-5,8-dimethyl-5-[(5*E*)-oct-5-en-3-yn-1-yl]-4,15-dioxabicyclo[9.3.1]pentadecan-3-one (II.82)



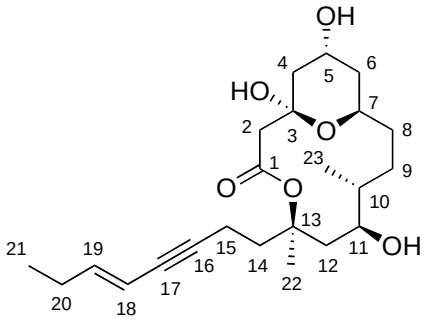


(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,7,13-Trihydroxy-5,8-dimethyl-5-[(5*E*)-oct-5-en-3-yn-1-yl]-4,15-dioxabicyclo[9.3.1]pentadecan-3-one (II.82)

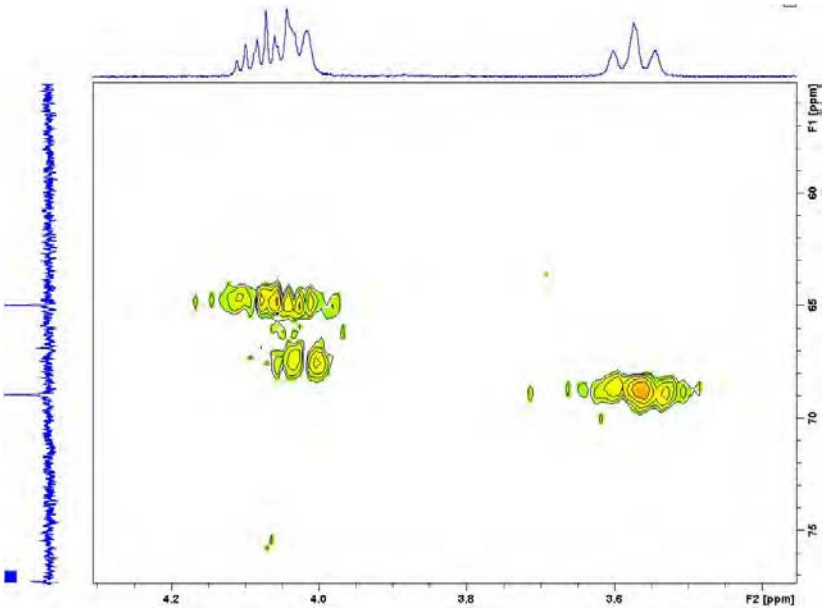




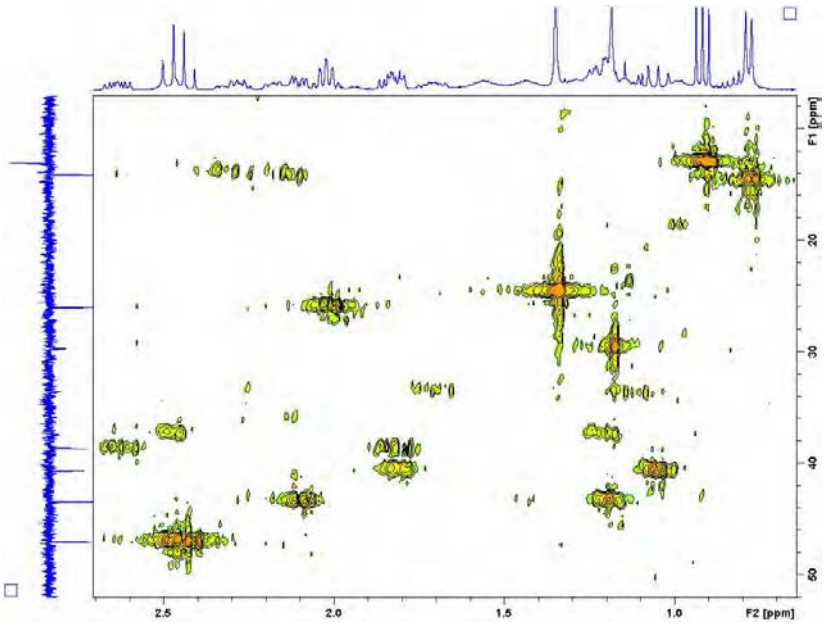
(1*R*,5*R*,7*S*,8*R*,11*R*,13*R*)-1,7,13-Trihydroxy-5,8-dimethyl-5-[(5*E*)-oct-5-en-3-yn-1-yl]-4,15-dioxabicyclo[9.3.1]pentadecan-3-one (II.82)



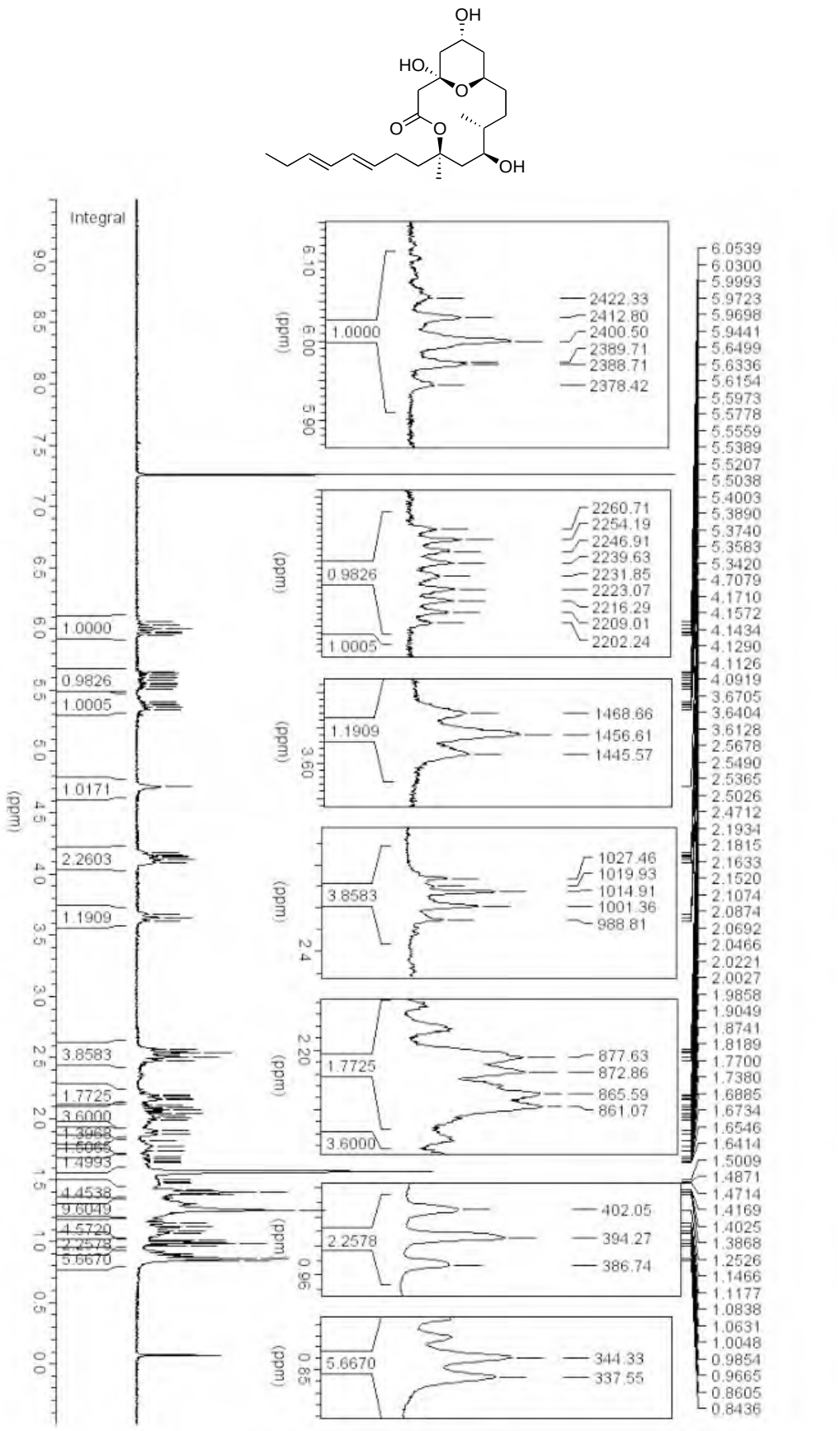
HMQC experiment: amplification of spectra for C5



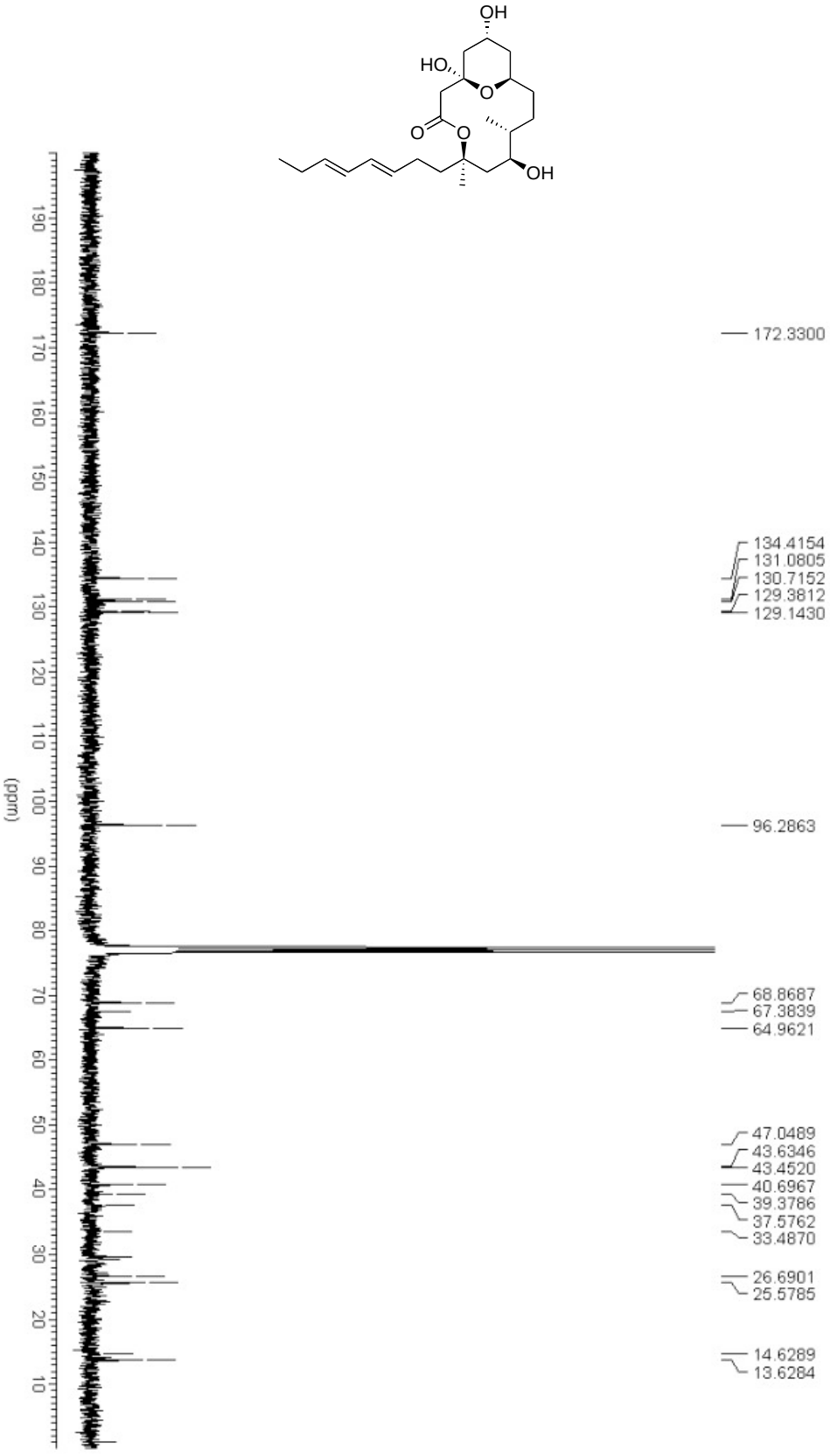
HMQC experiment: amplification of spectra for C23



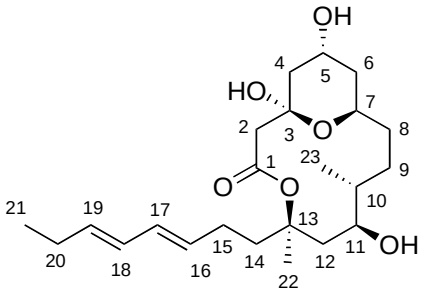
(1*R*,5*R*,7S,8*R*,11*R*,13*R*)-1,7,13-Trihydroxy-5,8-dimethyl-5-[(3*E*,5*E*)-octa-3,5-dien-1-yl]-4,15-dioxabicyclo[9.3.1]pentadecan-3-one – lyngbouilloside aglycon (II.92)



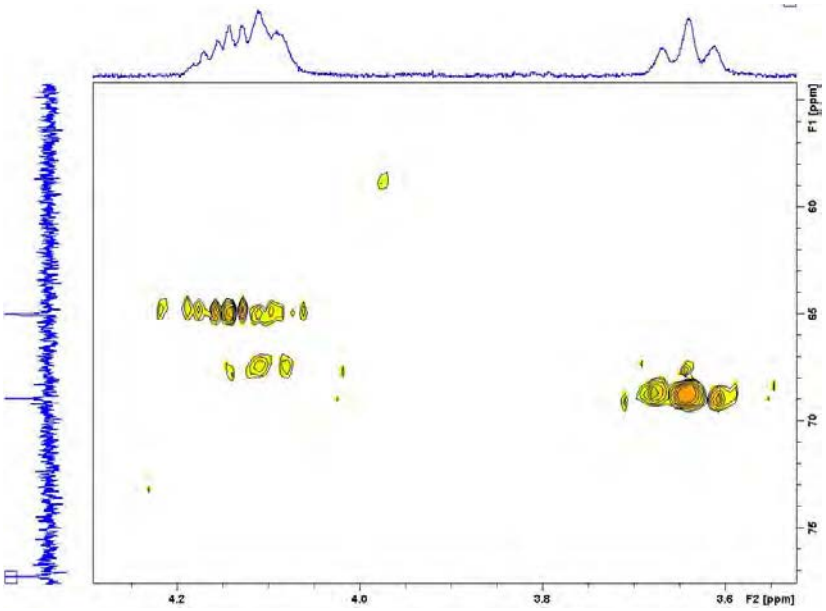
(1*R*,5*R*,7S,8*R*,11*R*,13*R*)-1,7,13-Trihydroxy-5,8-dimethyl-5-[(3*E*,5*E*)-octa-3,5-dien-1-yl]-4,15-dioxabicyclo[9.3.1]pentadecan-3-one – lyngbouilloside aglycon (II.92)



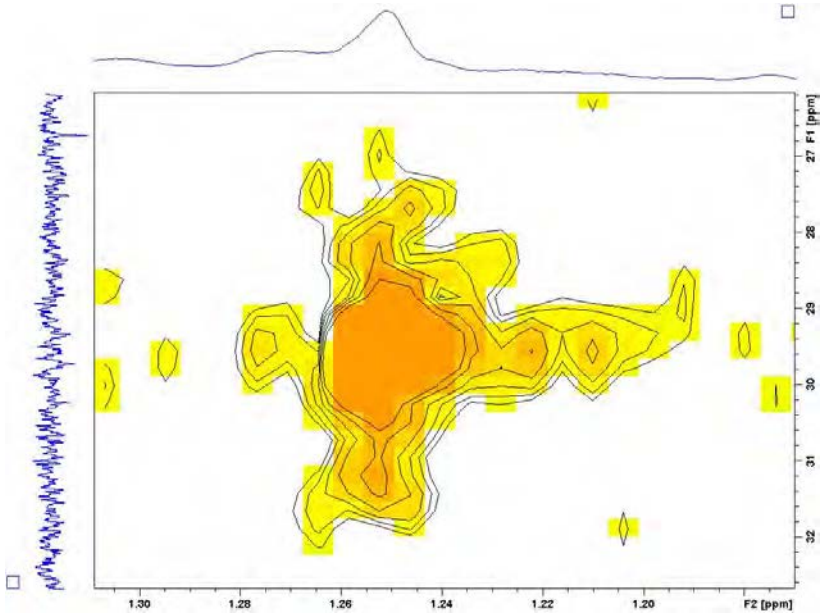
(1*R*,5*R*,7S,8*R*,11*R*,13*R*)-1,7,13-Trihydroxy-5,8-dimethyl-5-[(3*E*,5*E*)-octa-3,5-dien-1-yl]-4,15-dioxabicyclo[9.3.1]pentadecan-3-one – lyngbouilloside aglycon (II.92)



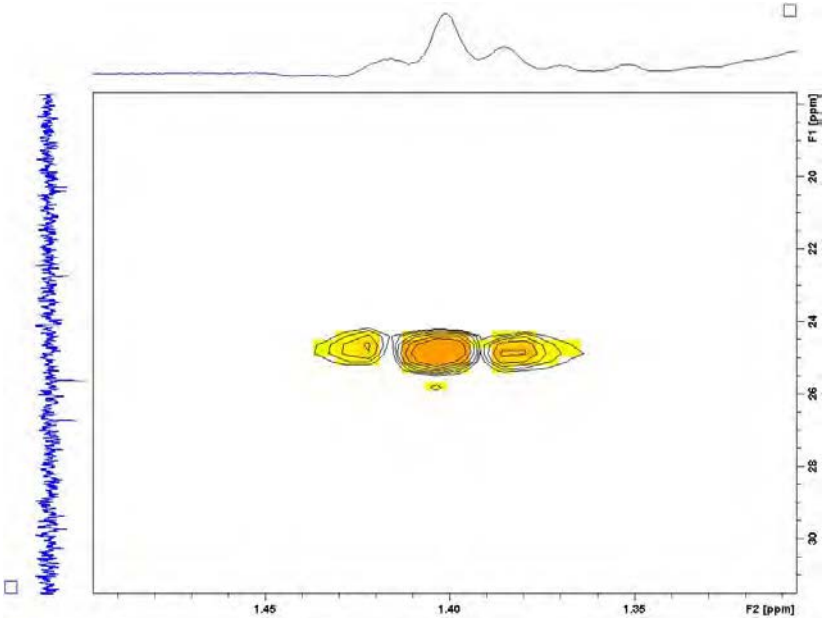
HMQC experiment: amplification of spectra for C5



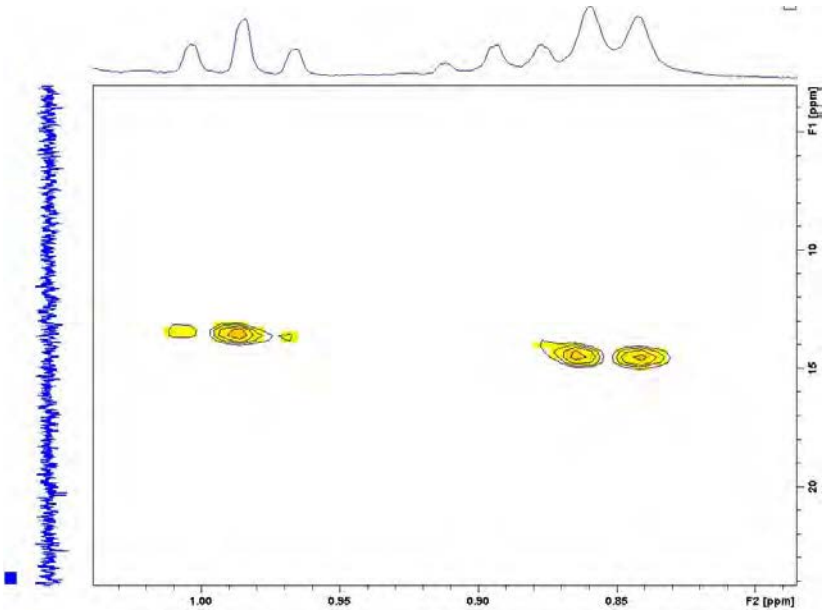
HMQC experiment: amplification of spectra for C9



HMQC experiment: amplification of spectra for C22



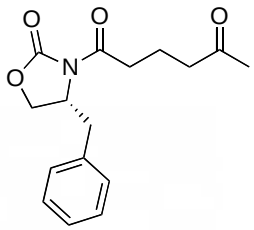
HMQC experiment: amplification of spectra for C21 and C23

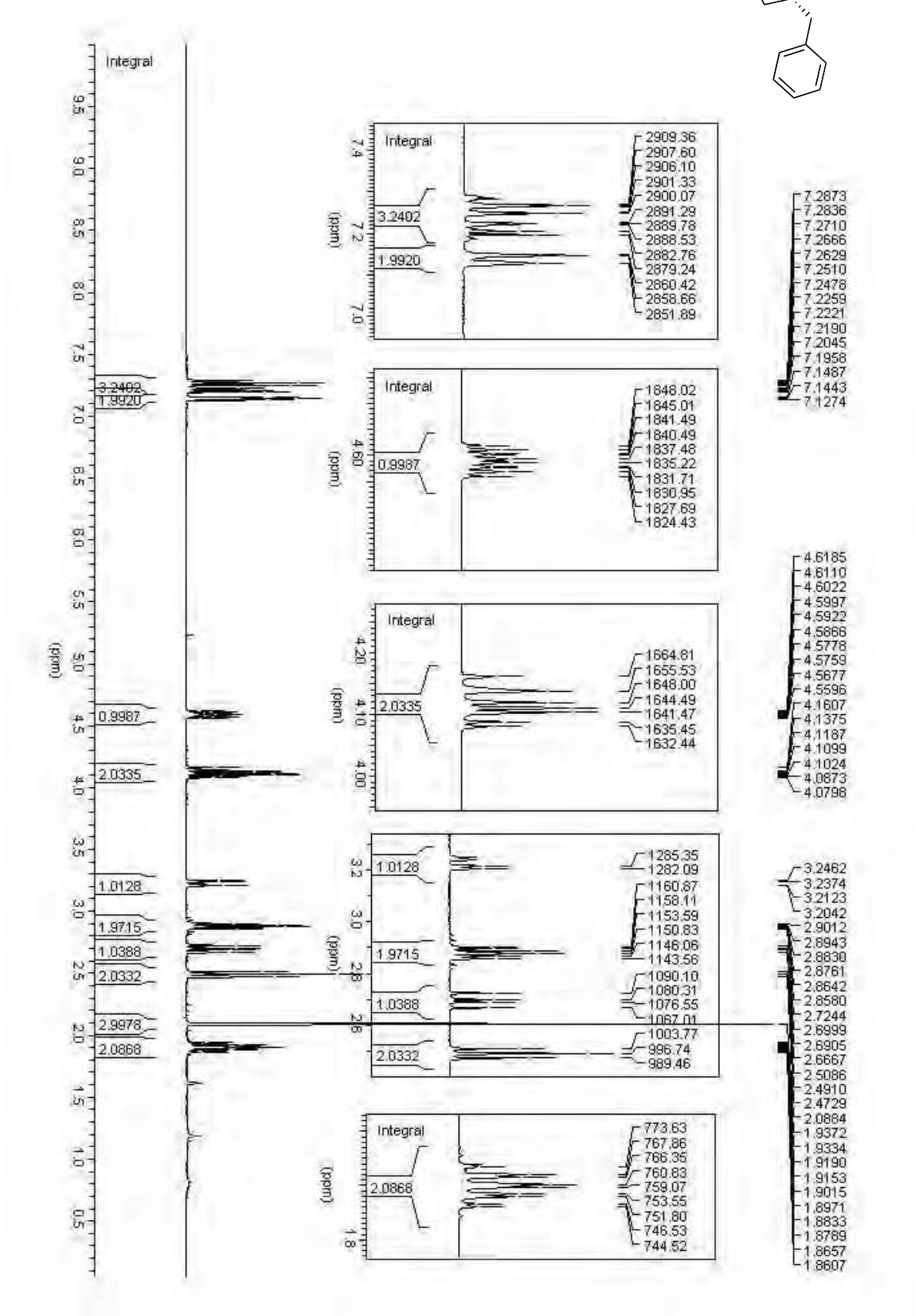


ANNEX V

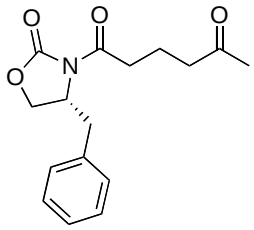
Copies of ^{1}H NMR & ^{13}C NMR

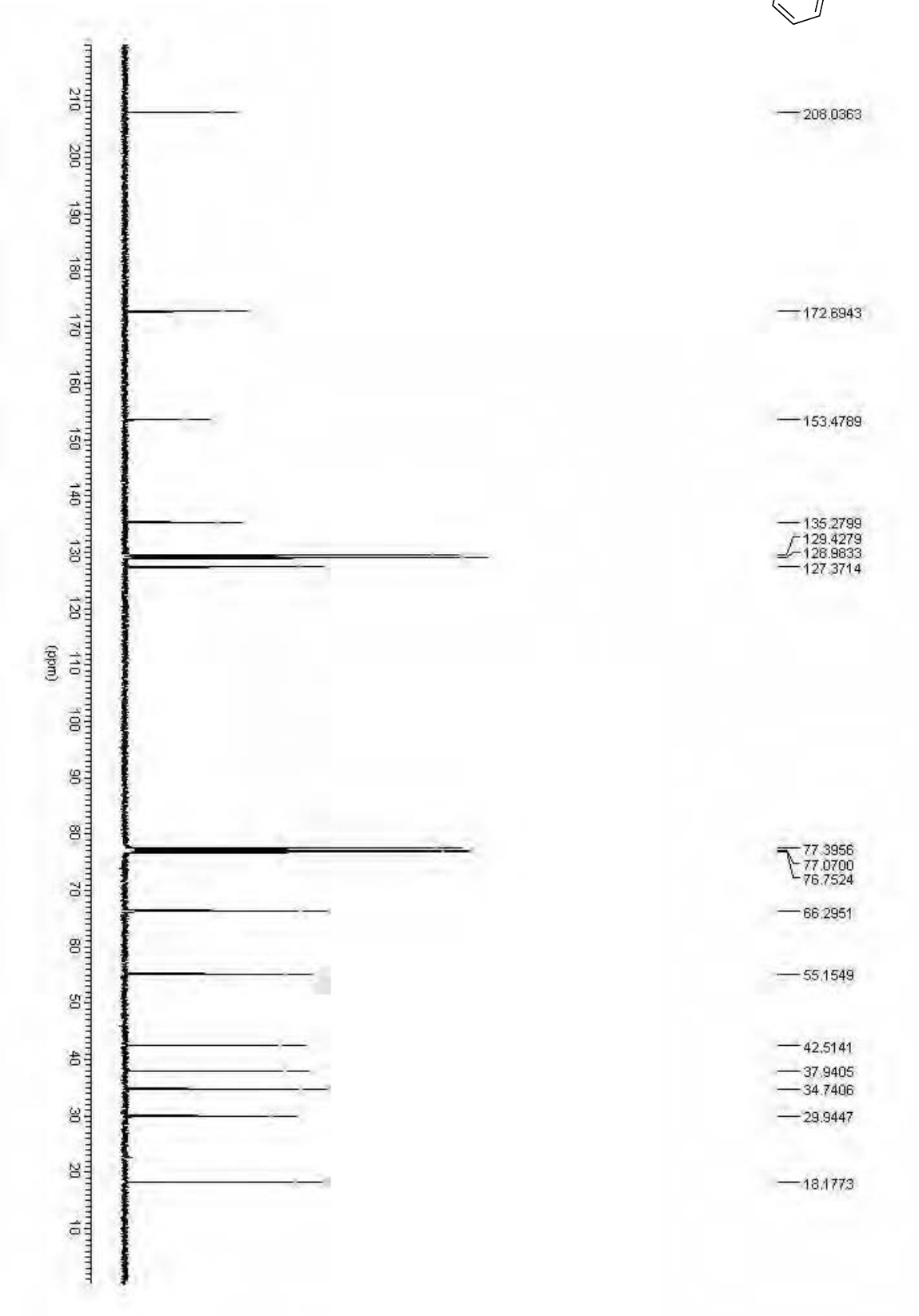
1-((*R*)-4-Benzyl-2-oxo-oxazolidin-3-yl)-hexane-1,5-dione (III.2)



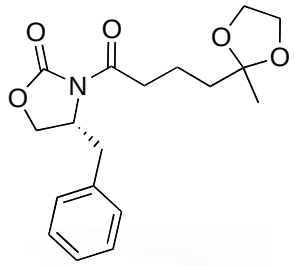


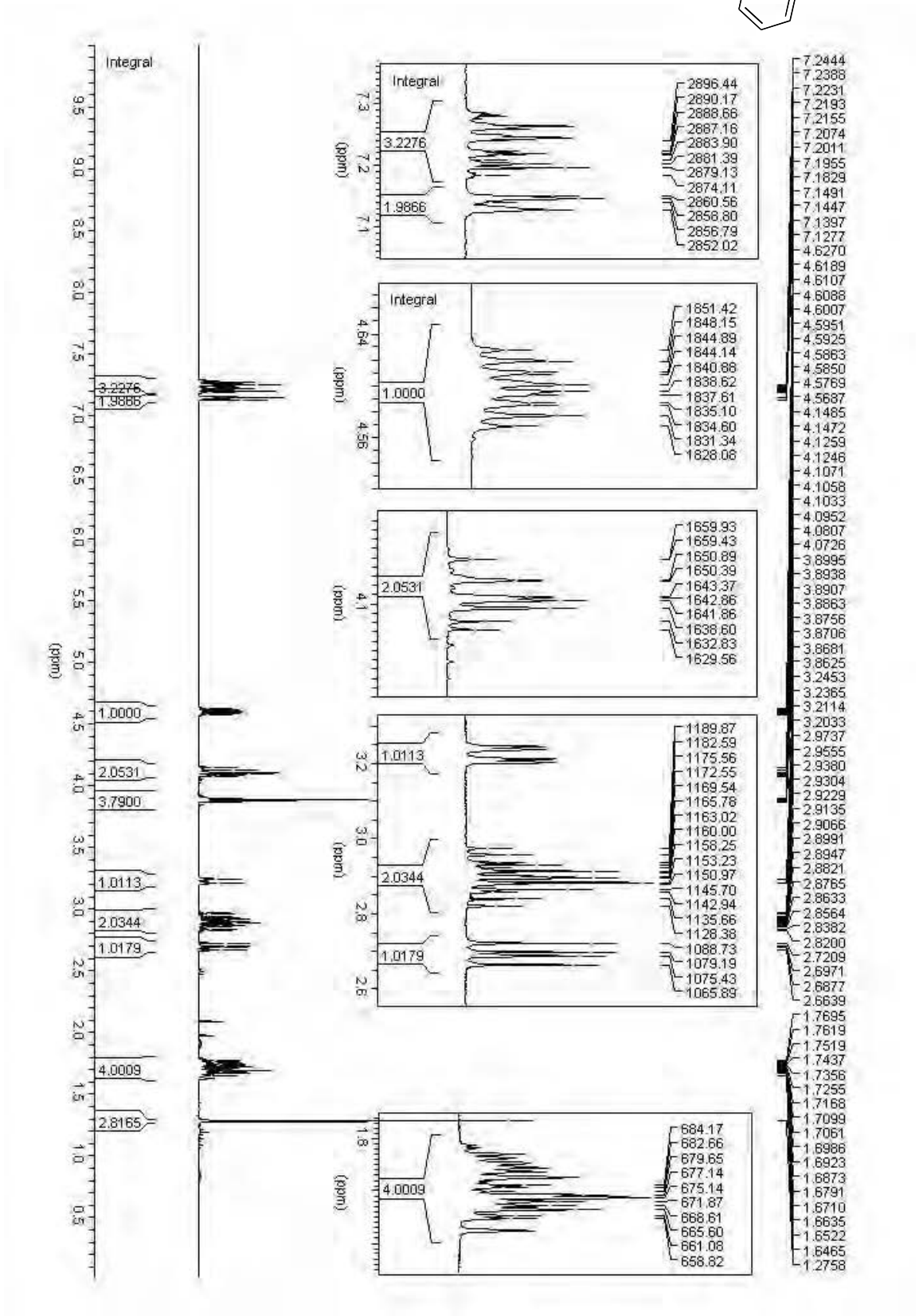
1-((*R*)-4-Benzyl-2-oxo-oxazolidin-3-yl)-hexane-1,5-dione (III.2)



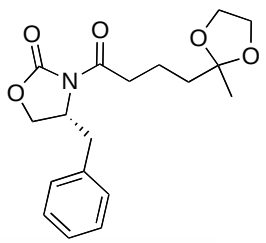


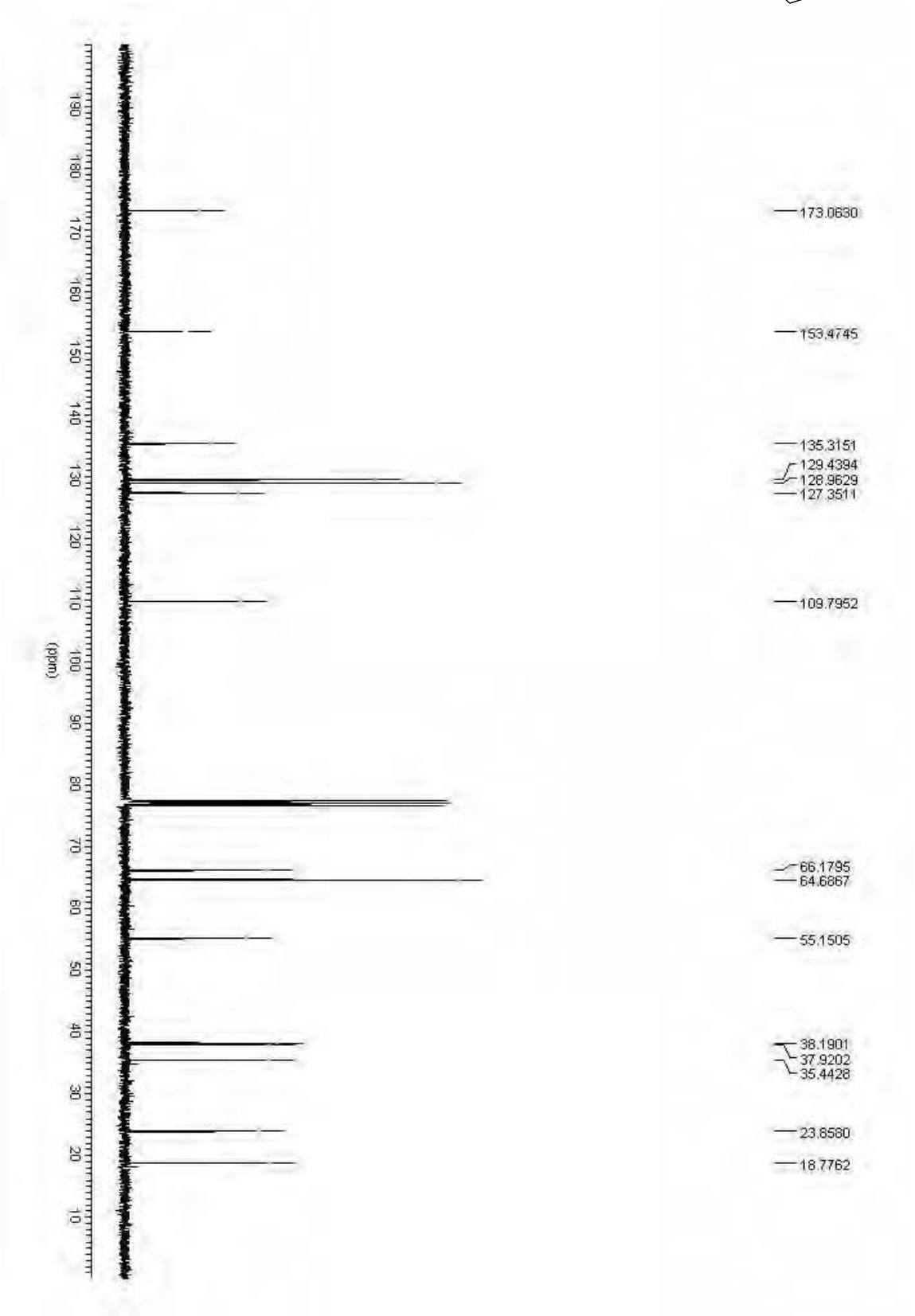
(*R*)-4-benzyl-3-(4-(2-methyl-1,3-dioxolan-2-yl)butanoyl) oxazolidin-2-one (III.3)



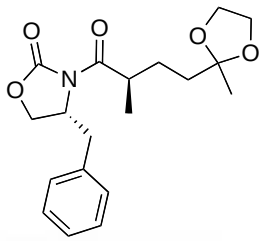


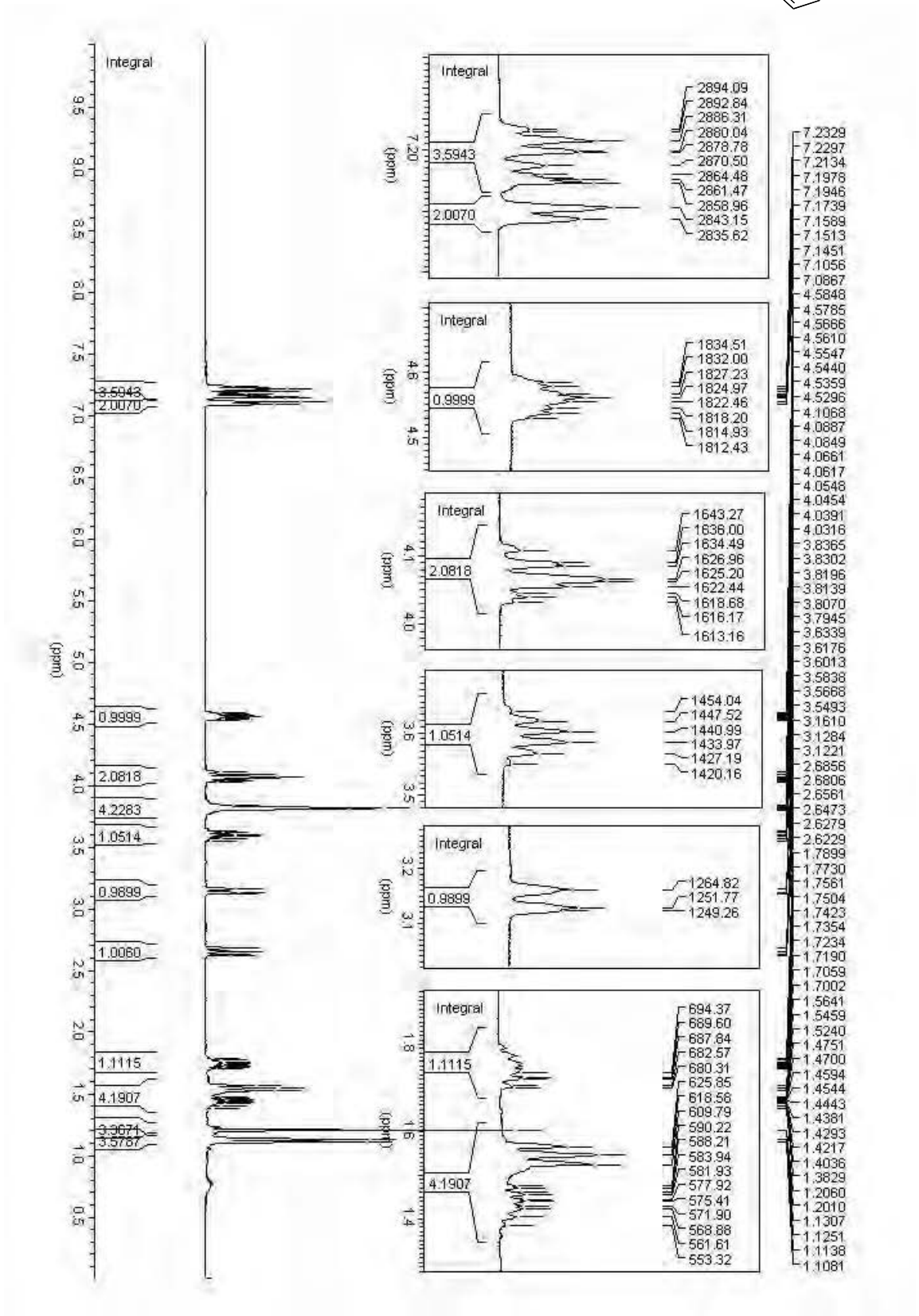
(*R*)-4-benzyl-3-(4-(2-methyl-1,3-dioxolan-2-yl)butanoyl) oxazolidin-2-one (III.3)



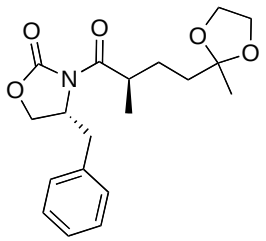


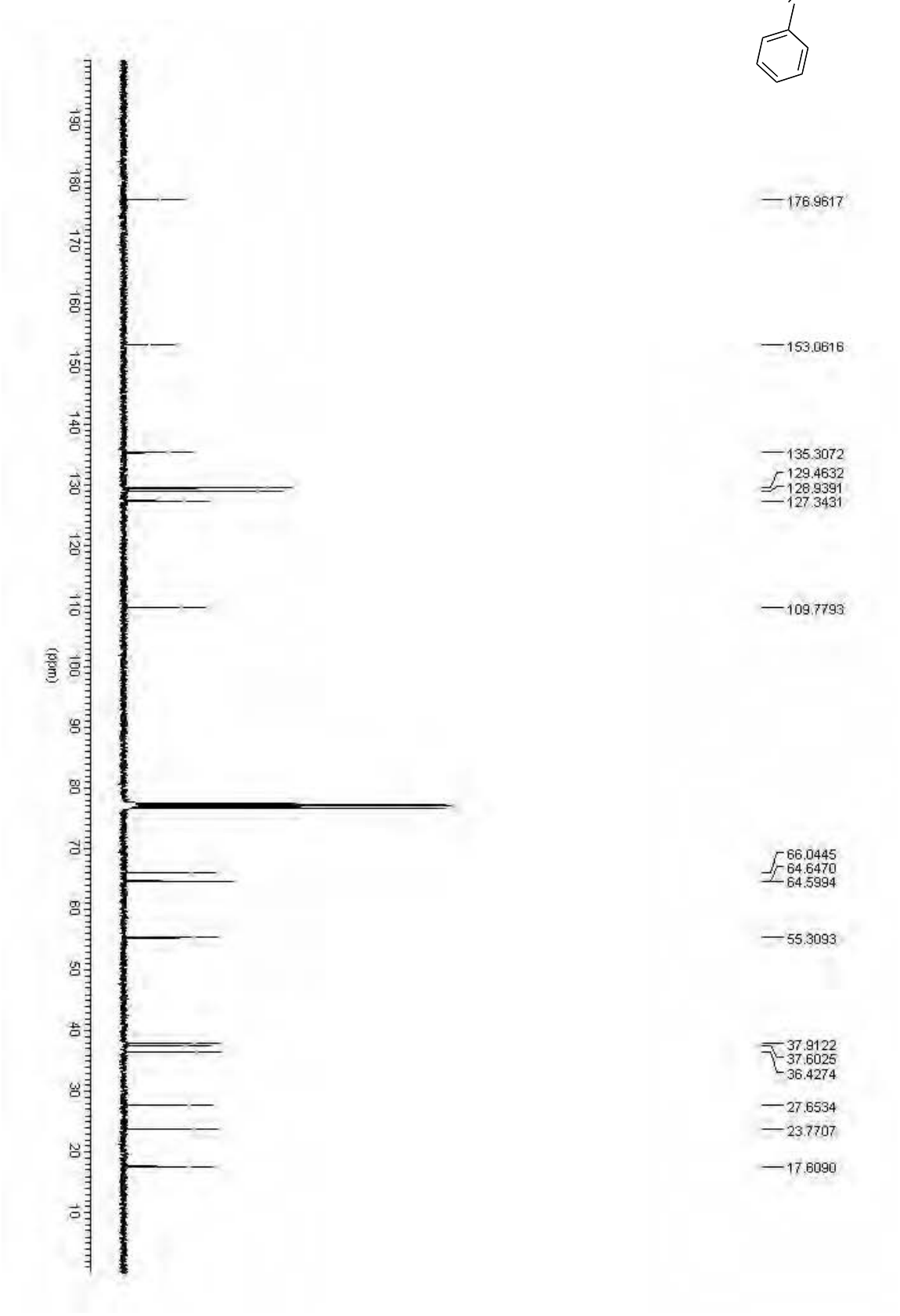
(*R*)-4-benzyl-3-((*R*)-2-methyl-4-(2-methyl-1,3-dioxolan-2-yl)butanoyl) oxazolidin-2-one (III.4)



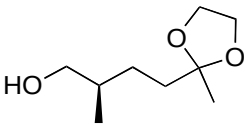


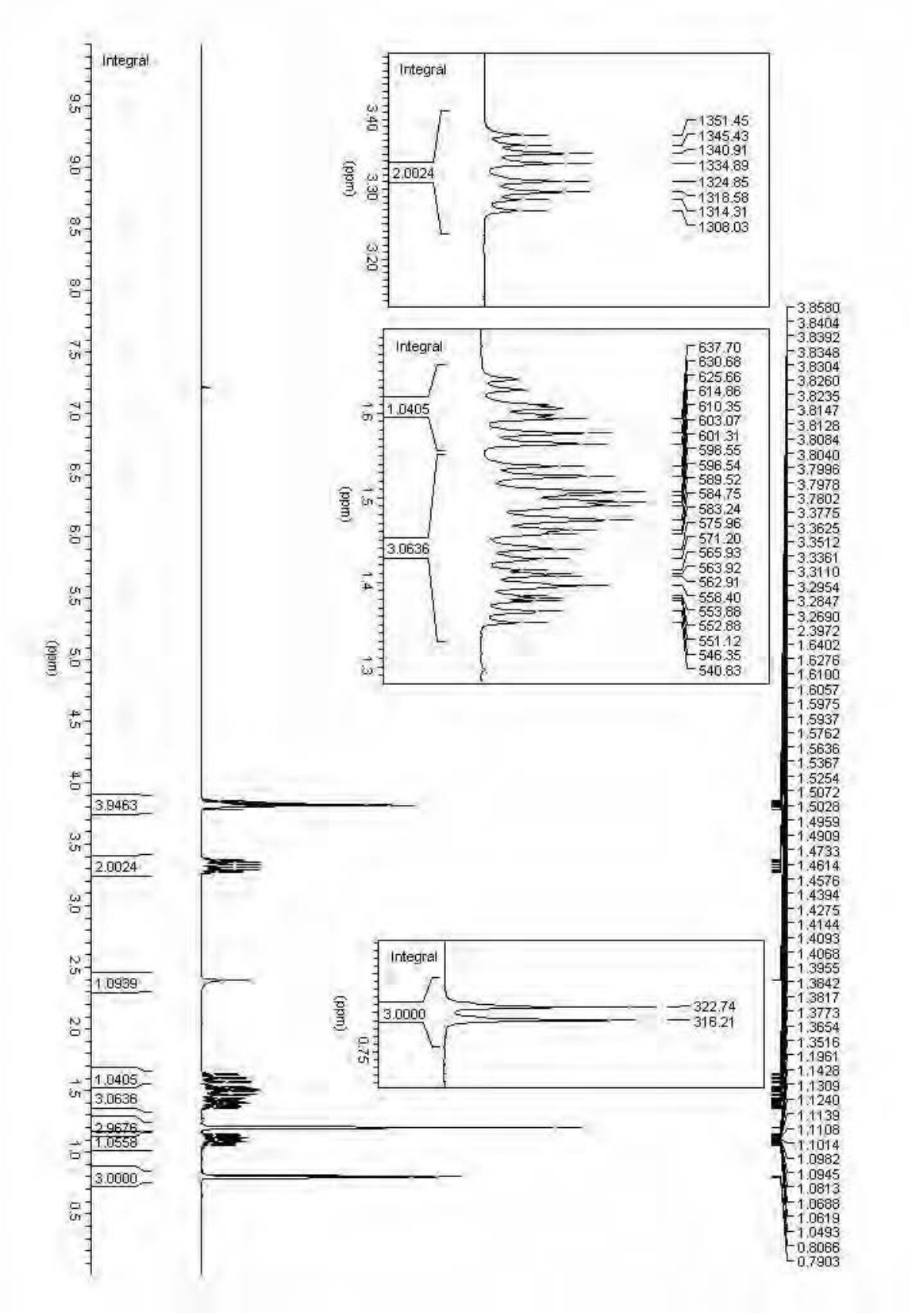
(*R*)-4-benzyl-3-((*R*)-2-methyl-4-(2-methyl-1,3-dioxolan-2-yl)butanoyl) oxazolidin-2-one (III.4)



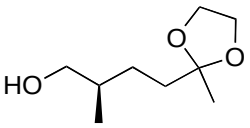


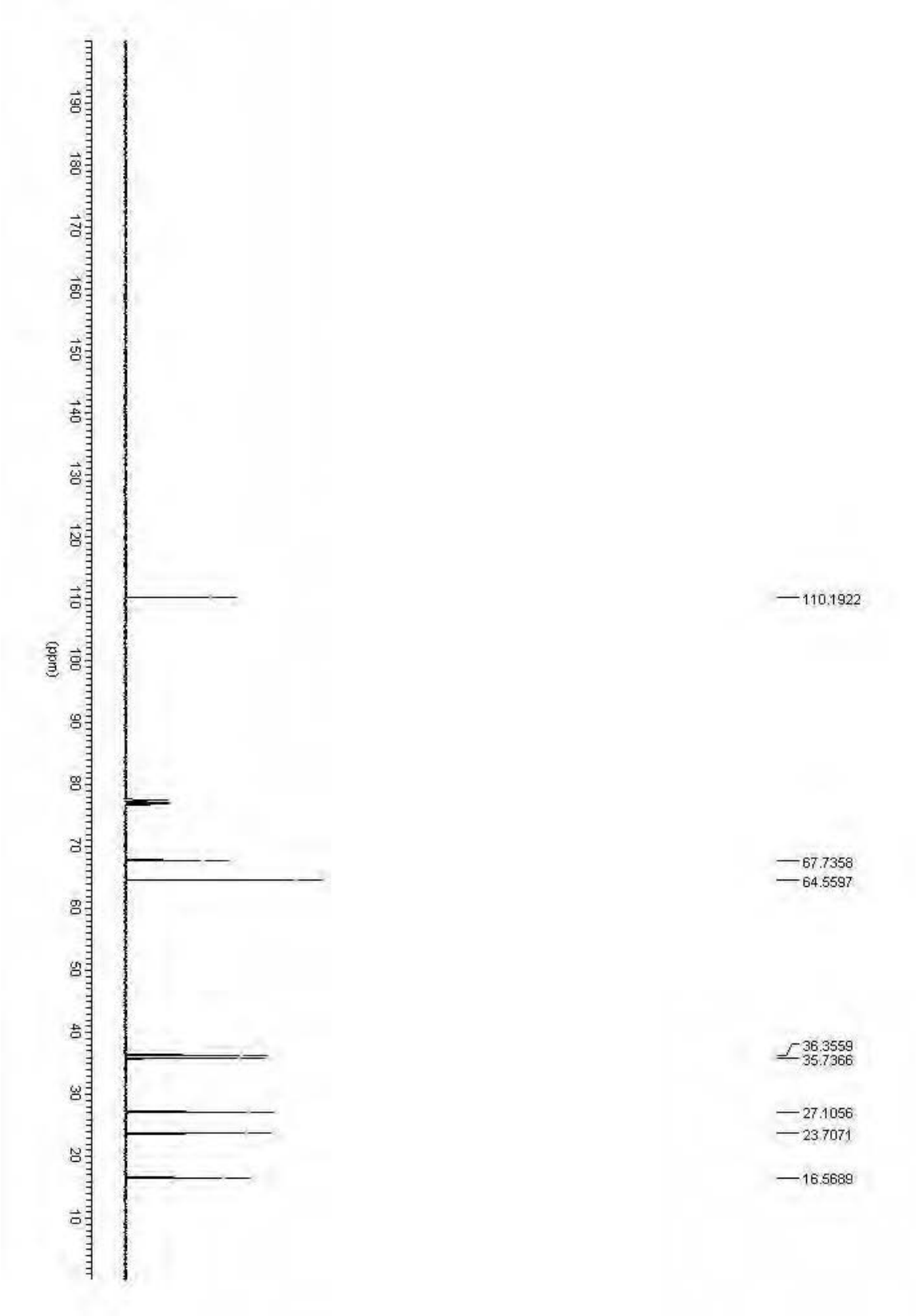
(*R*)-2-Methyl-4-(2-methyl-1,3-dioxolan-2-yl)butan-1-ol (III.5)



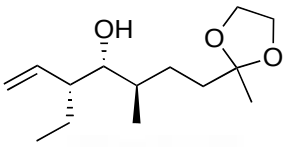


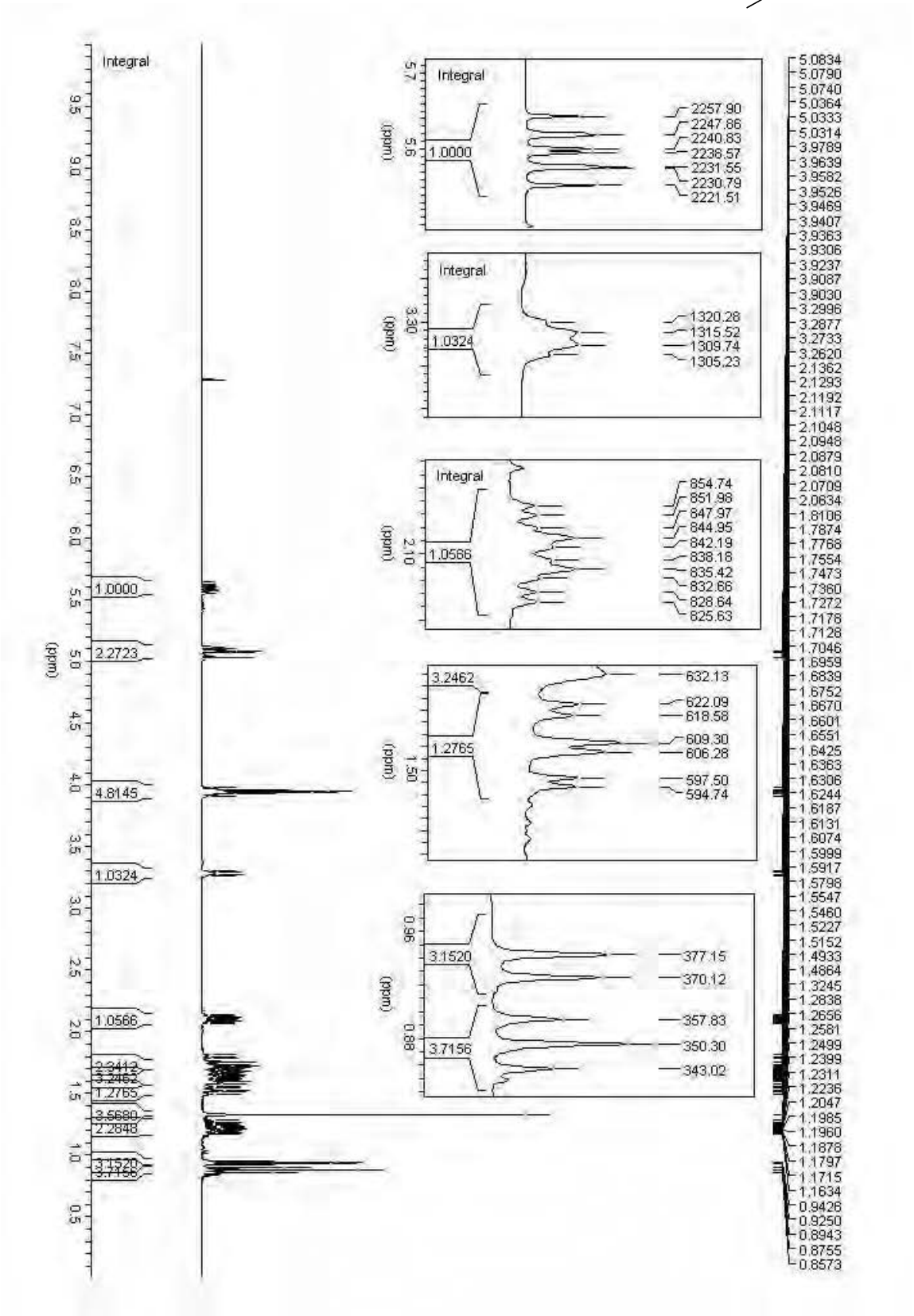
(*R*)-2-Methyl-4-(2-methyl-1,3-dioxolan-2-yl)butan-1-ol (III.5)



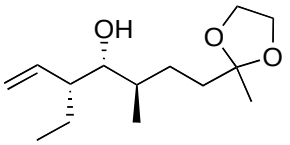


(3*R*,4*R*,5*R*)-3-Ethyl-5-methyl-7-(2-methyl-1,3-dioxolan-2-yl)hept-1-en-4-ol (III.7)

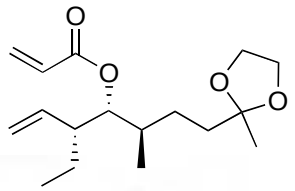


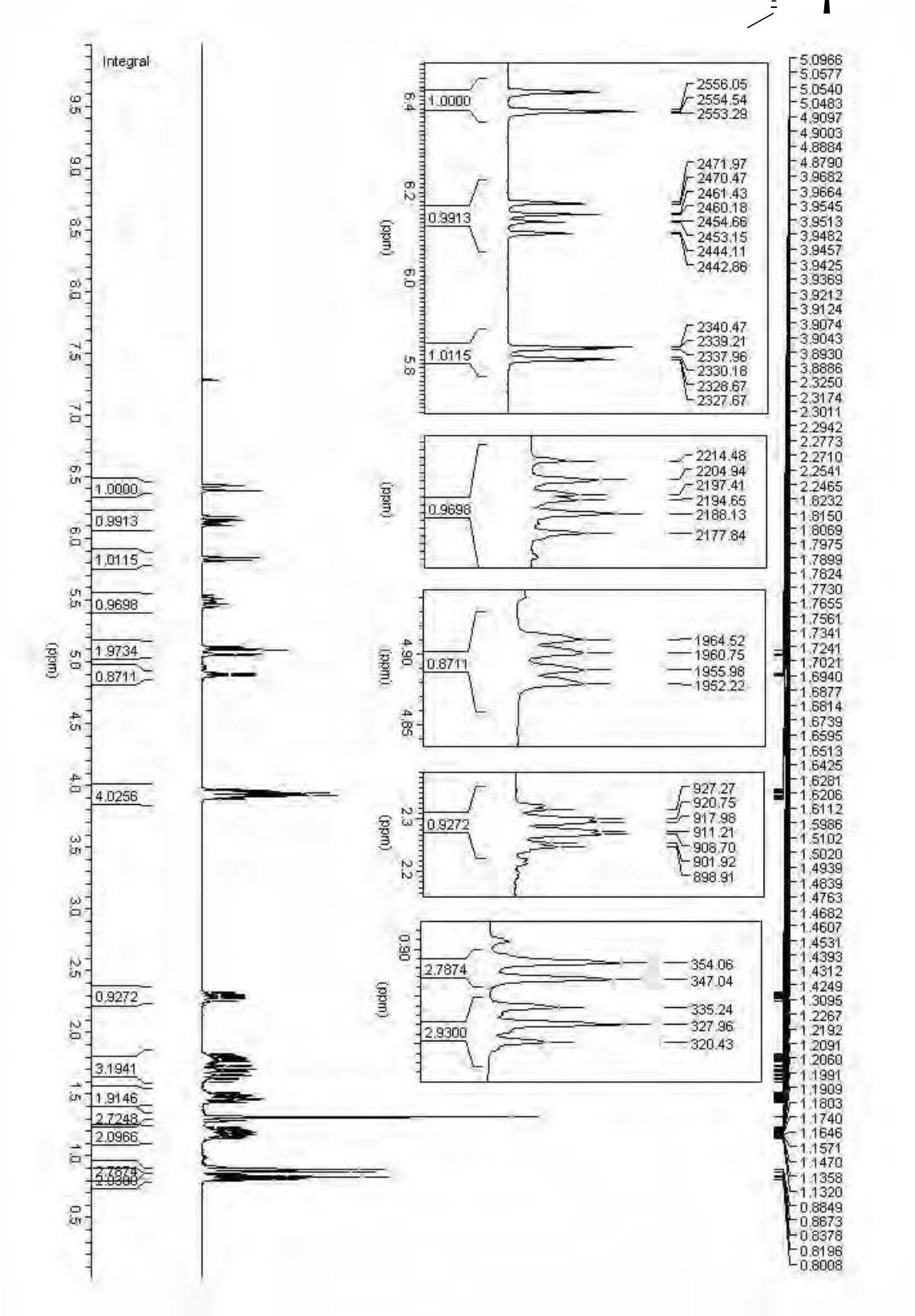


(3*R*,4*R*,5*R*)-3-Ethyl-5-methyl-7-(2-methyl-1,3-dioxolan-2-yl)hept-1-en-4-ol (III.7)

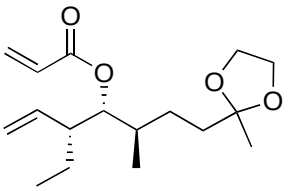


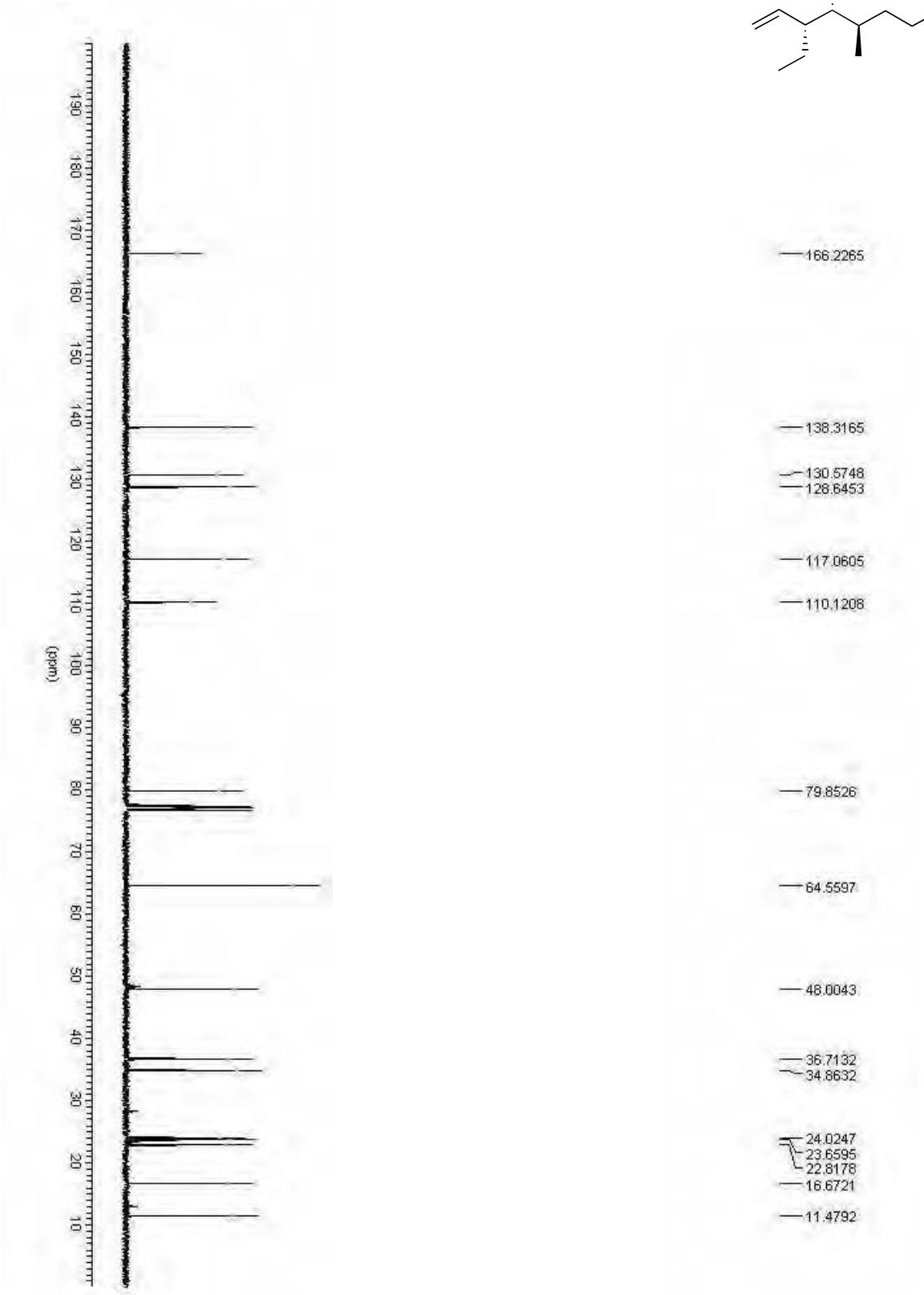
(3*R*,4*R*,5*R*)-3-Ethyl-5-methyl-7-(2-methyl-1,3-dioxolan-2-yl)hept-1-en-4-yl acrylate



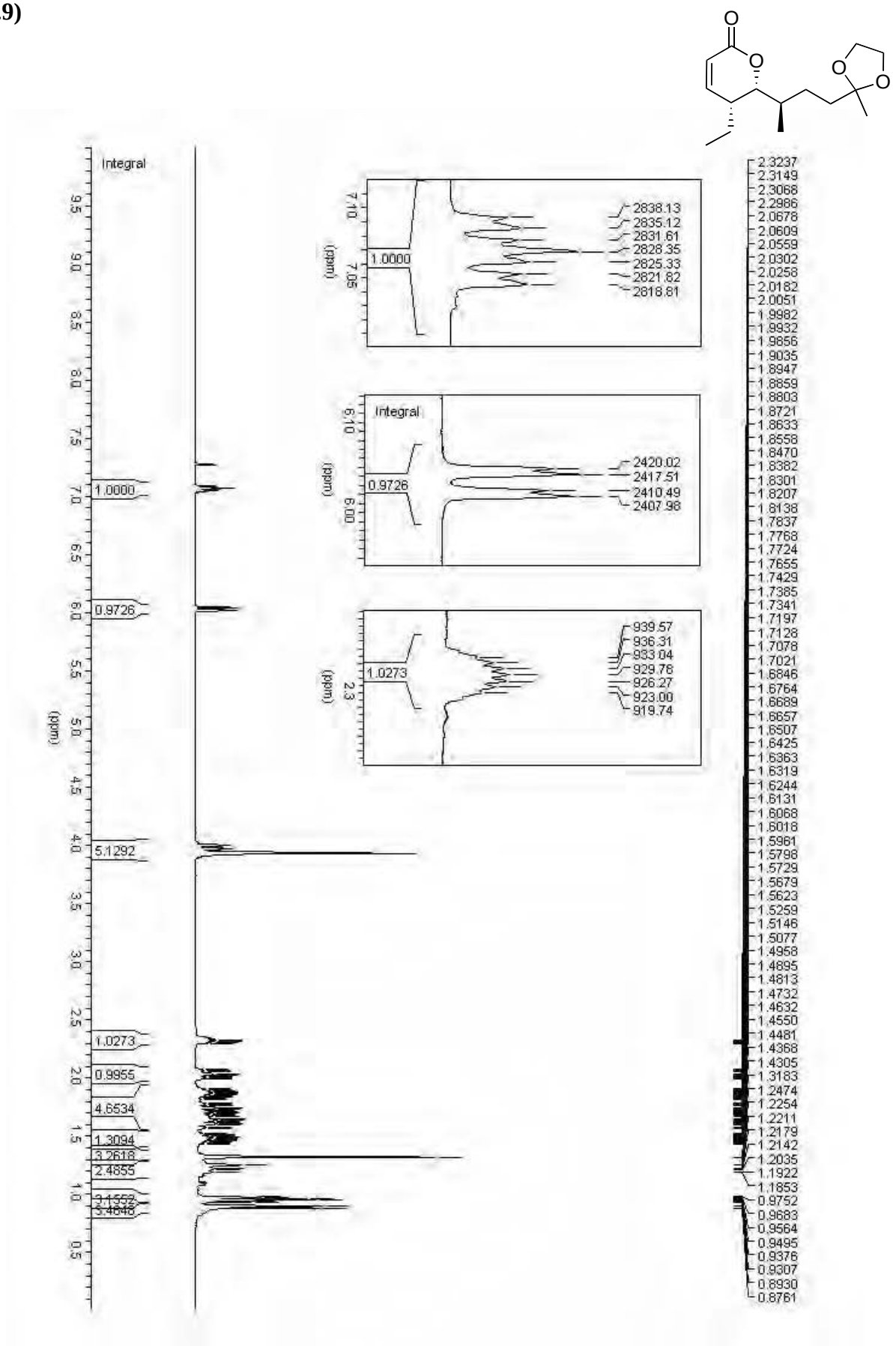


(3*R*,4*R*,5*R*)-3-Ethyl-5-methyl-7-(2-methyl-1,3-dioxolan-2-yl)hept-1-en-4-yl acrylate

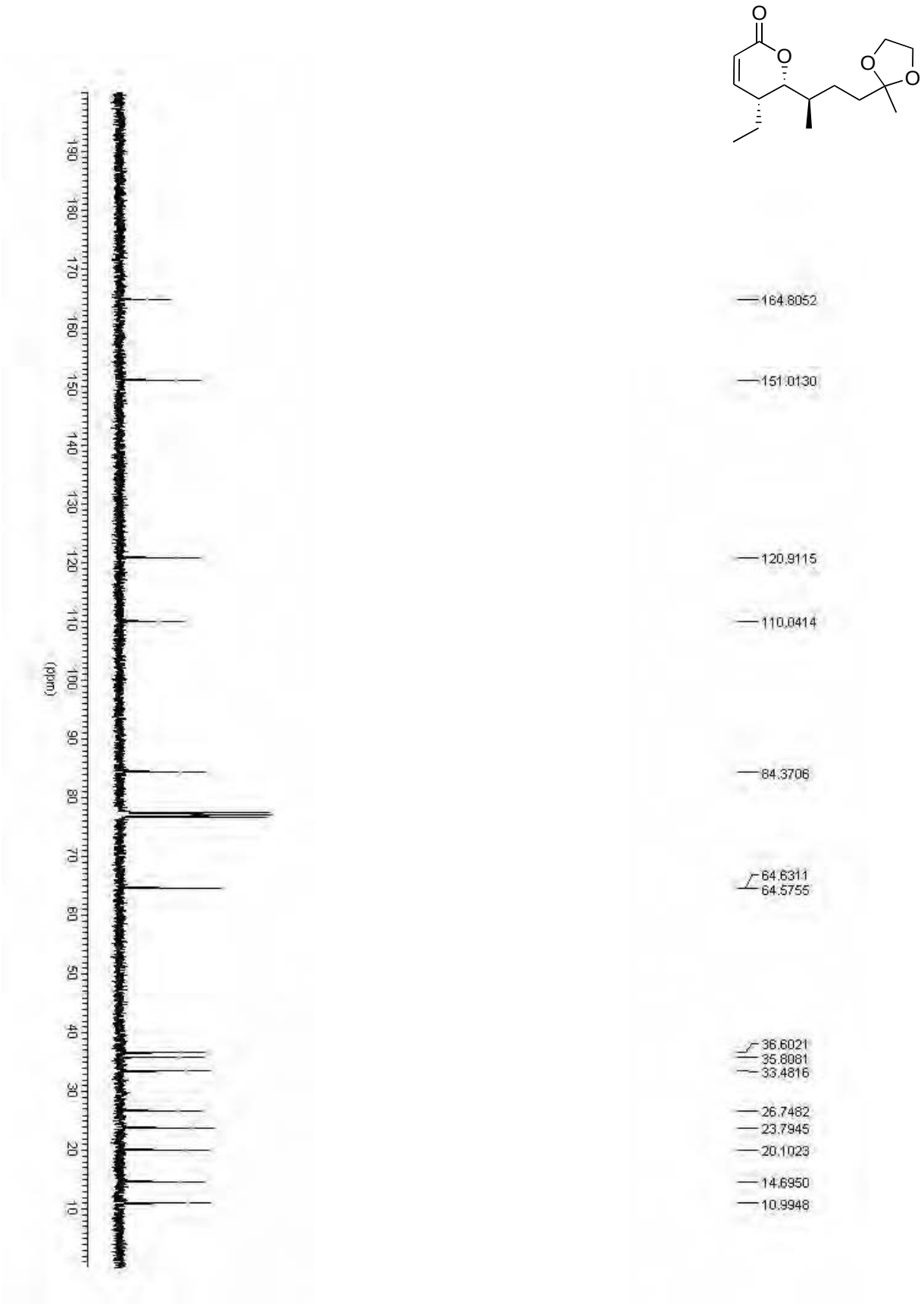




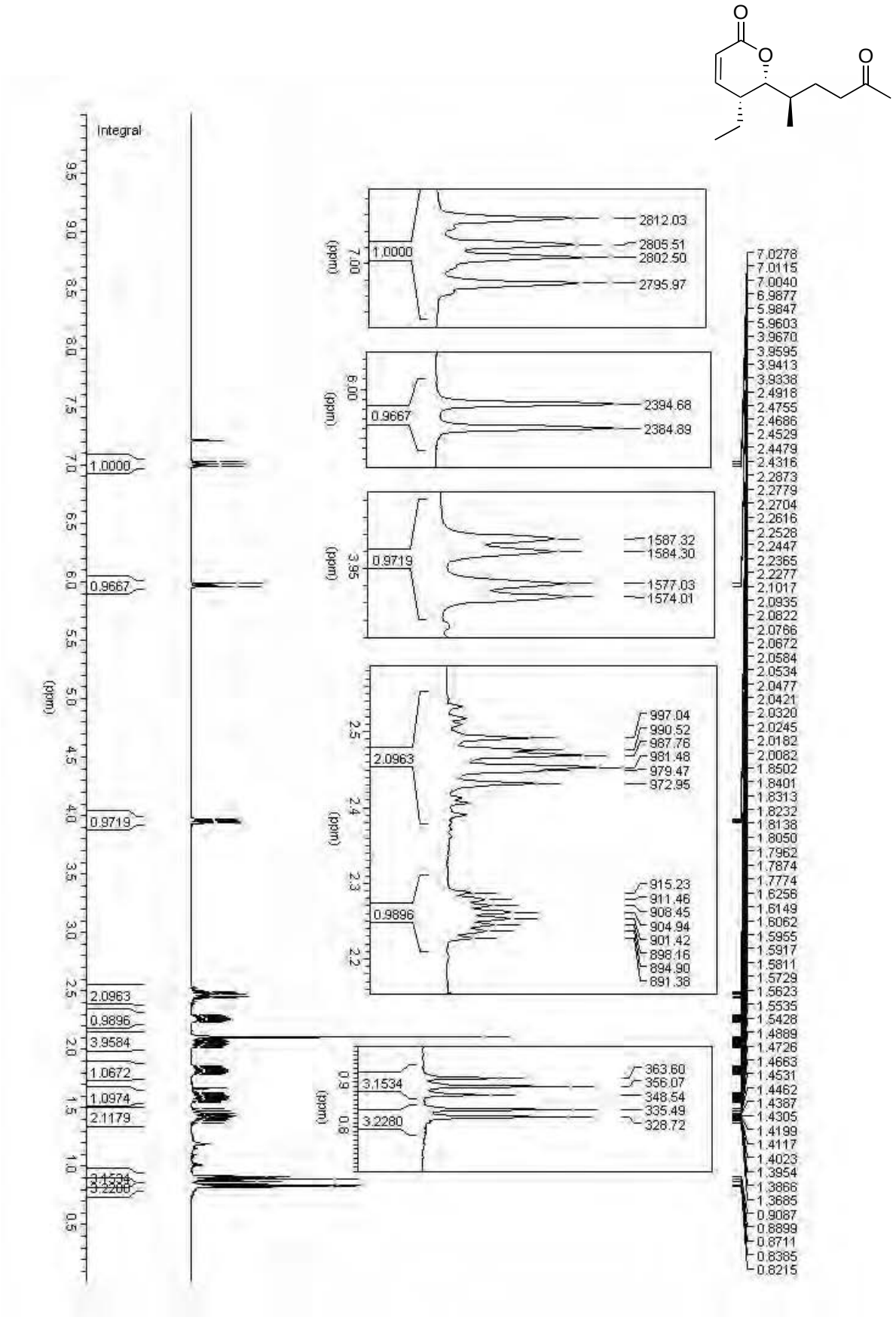
(5*R*,6*R*)-5-ethyl-6-((R)-4-(2-methyl-1,3-dioxolan-2-yl)butan-2-yl)-5,6-dihydropyran-2-one (III.9)



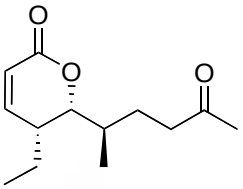
(5*R*,6*R*)-5-Ethyl-6-((R)-4-(2-methyl-1,3-dioxolan-2-yl)butan-2-yl)-5,6-dihydropyran-2-one (III.9)

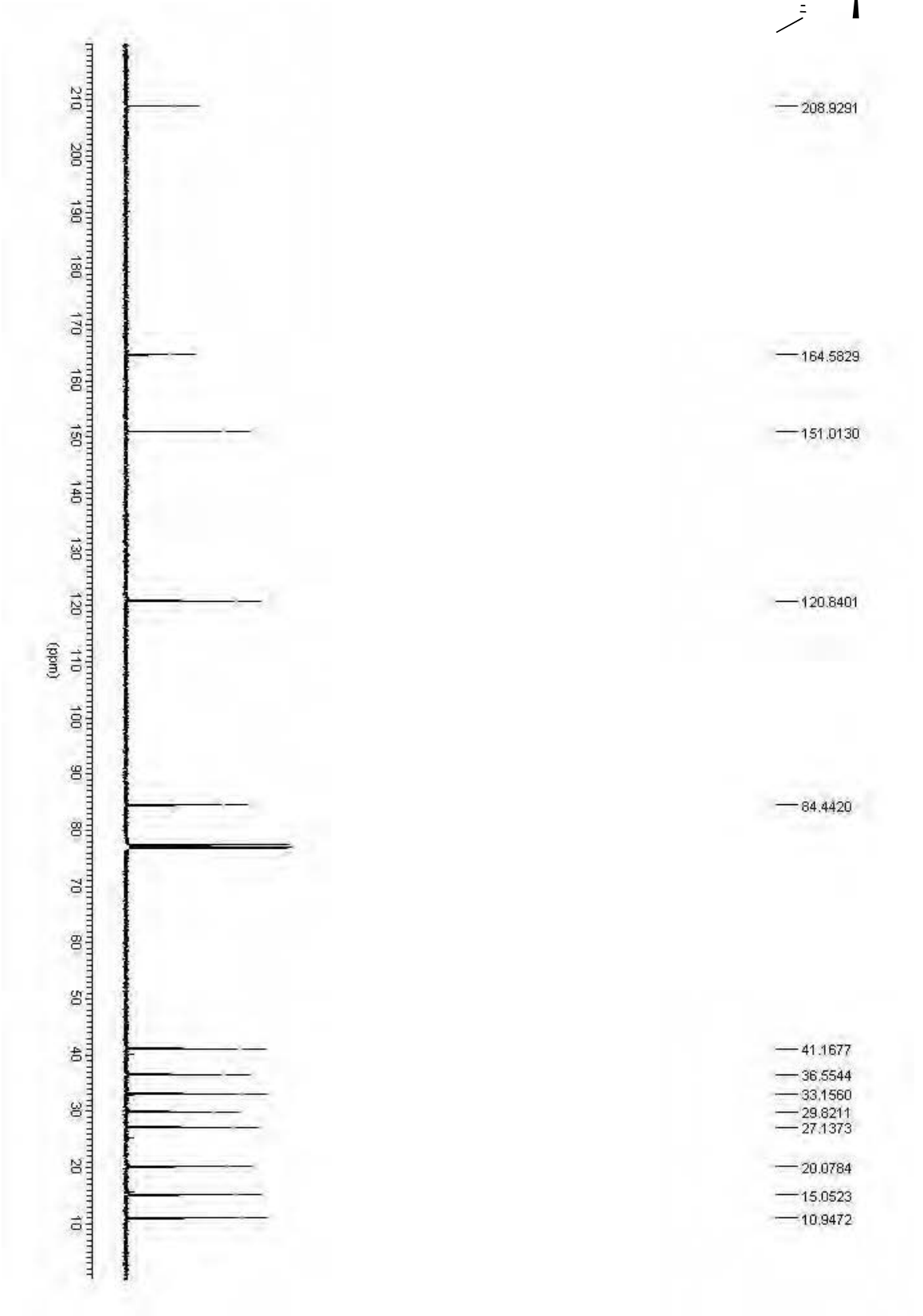


(5*R*,6*R*)-5-Ethyl-6-((R)-5-oxohexan-2-yl)-5,6-dihydropyran-2-one (III.10)

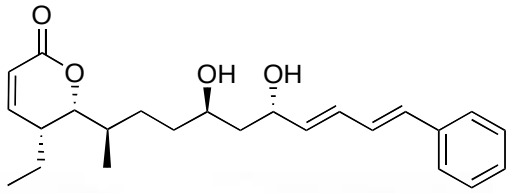


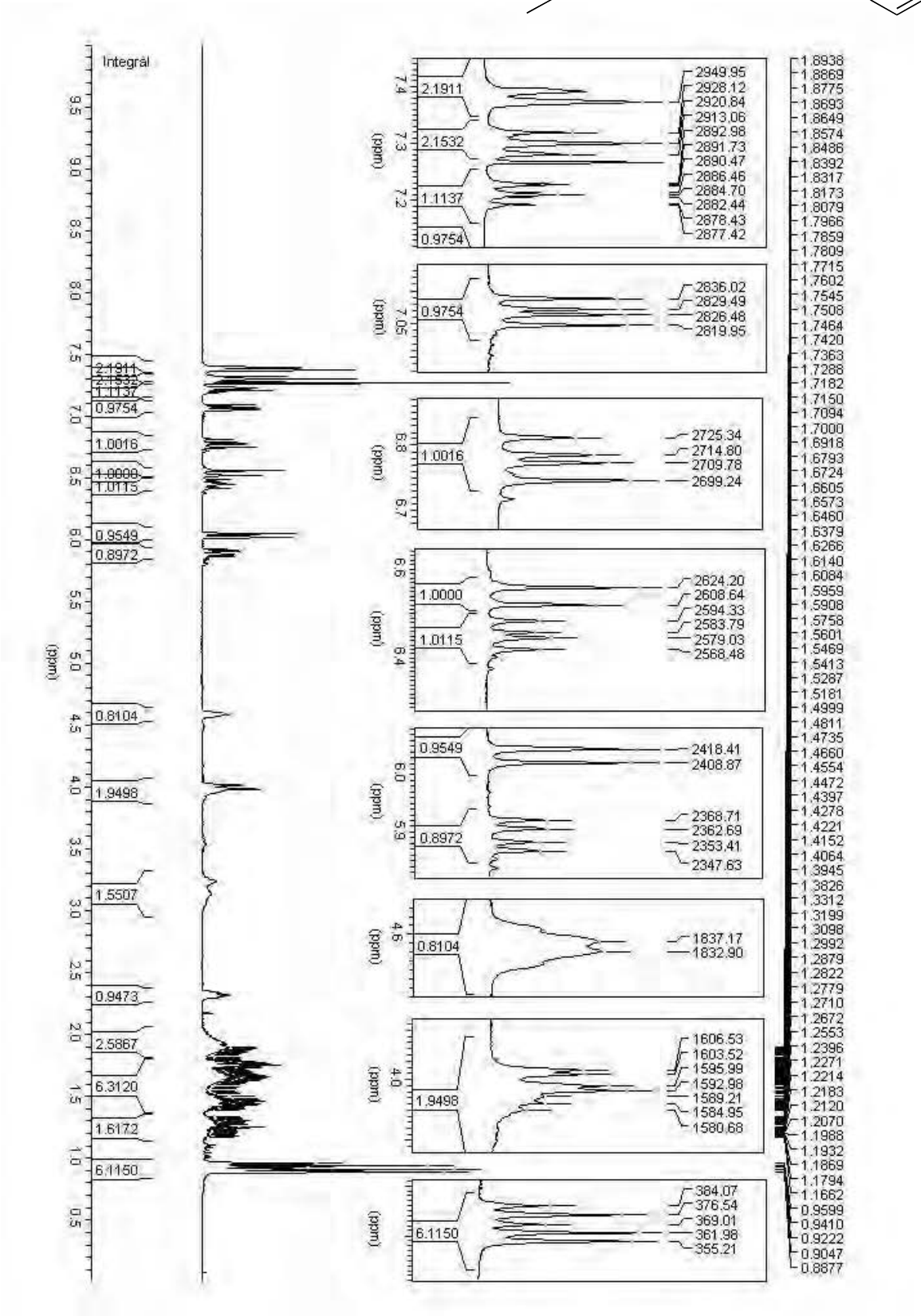
(5*R*,6*R*)-5-ethyl-6-((R)-5-oxohexan-2-yl)-5,6-dihydropyran-2-one (III.10)



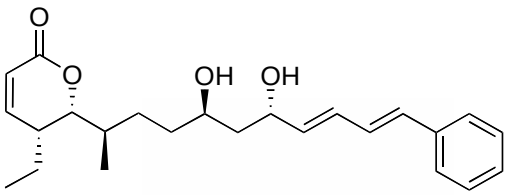


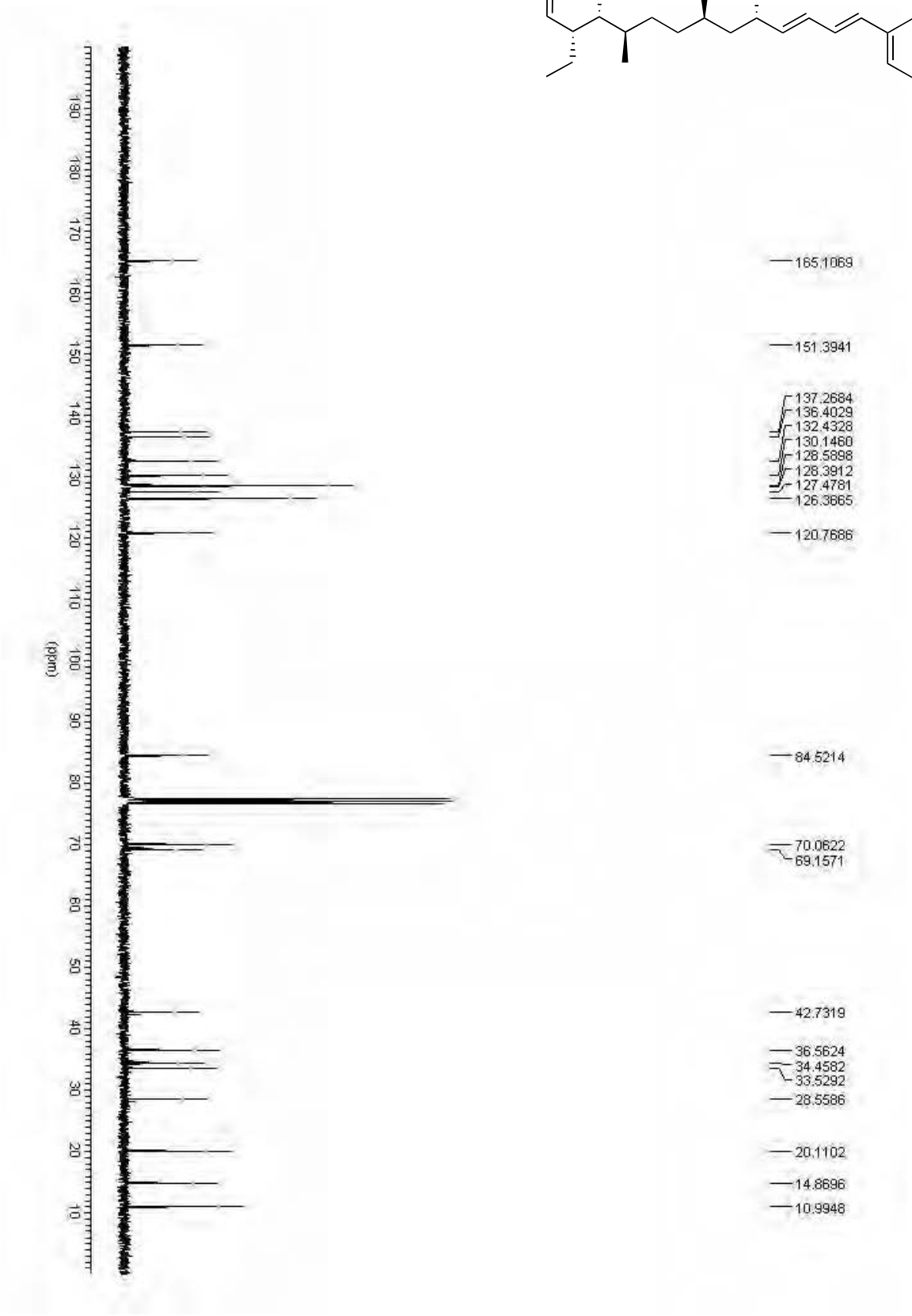
(–)-Bitungolide F



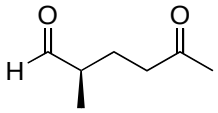


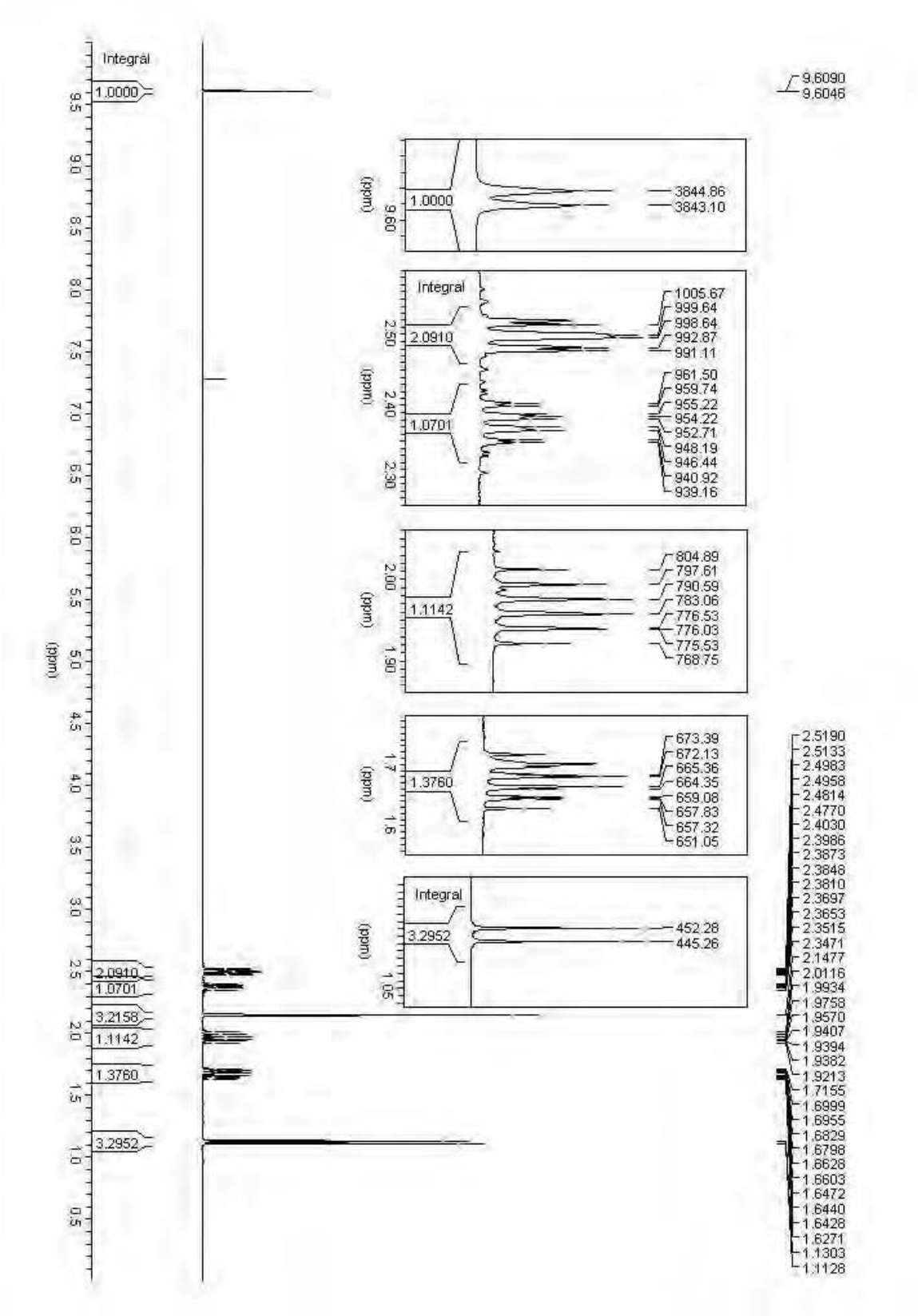
(–)-Bitungolide F



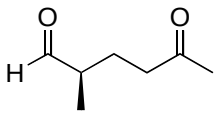


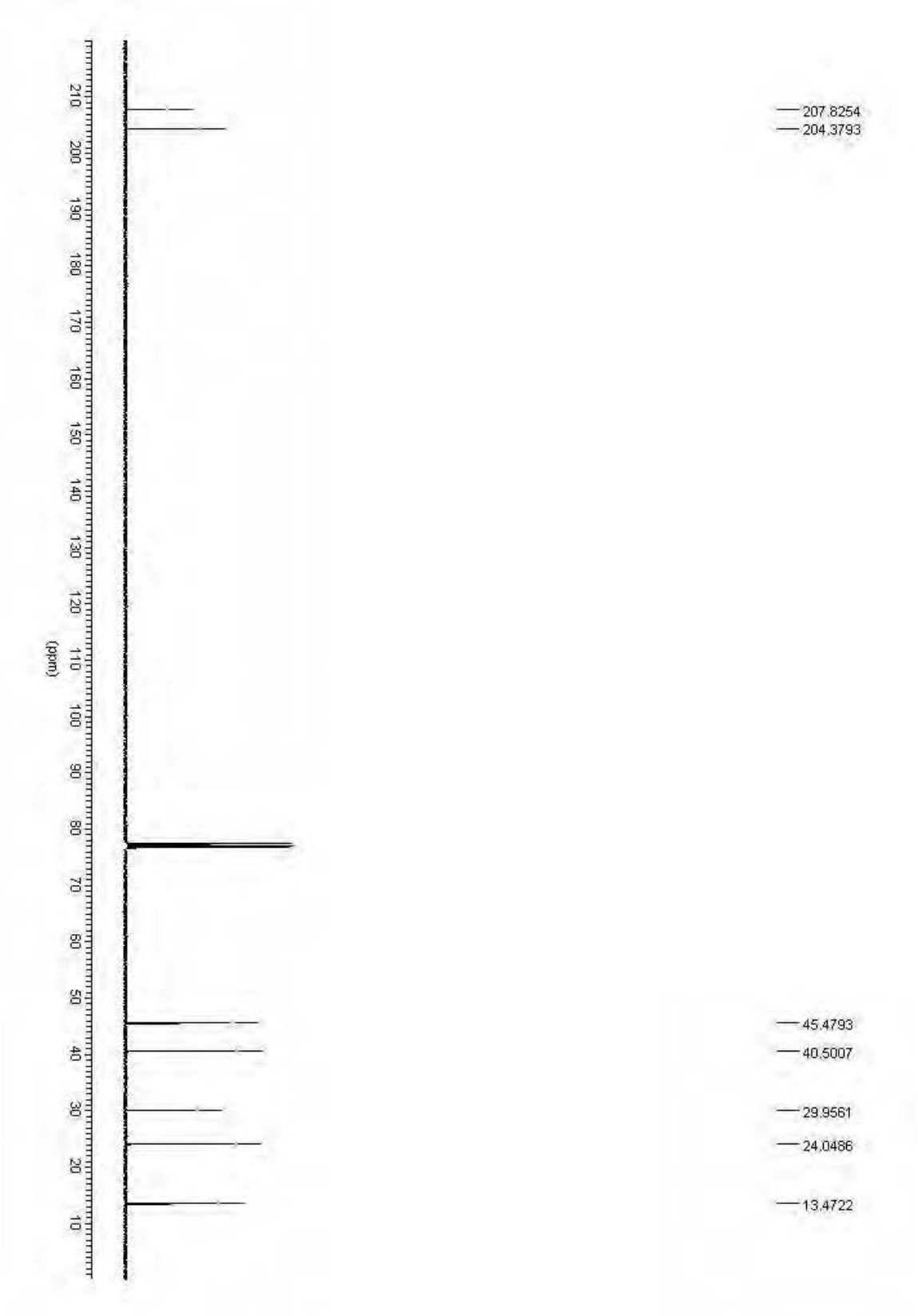
(*R*)-2-Methyl-5-oxo-hexanal (III.27)



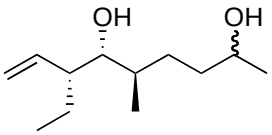


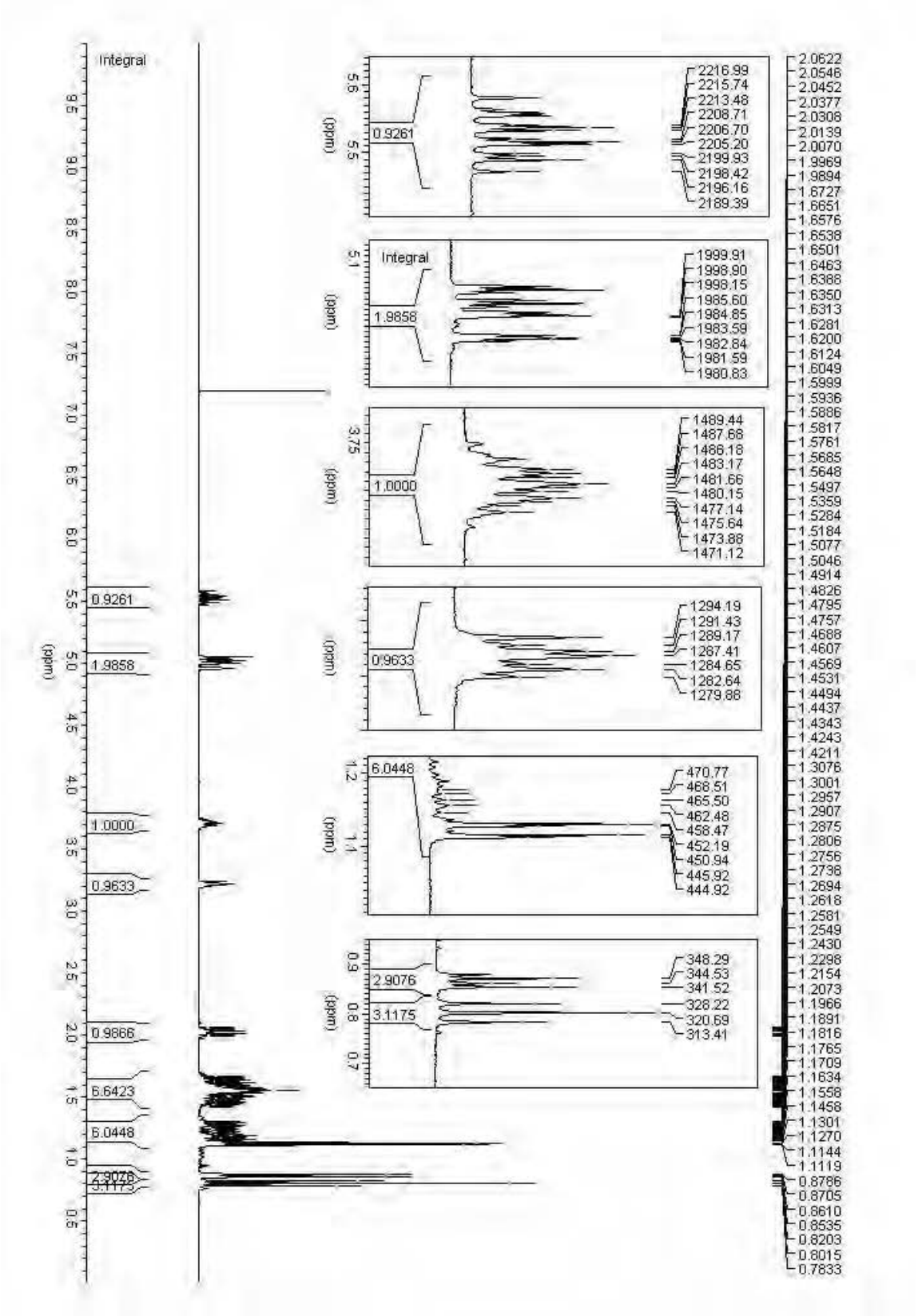
(*R*)-2-Methyl-5-oxo-hexanal (III.27)



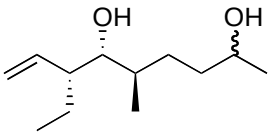


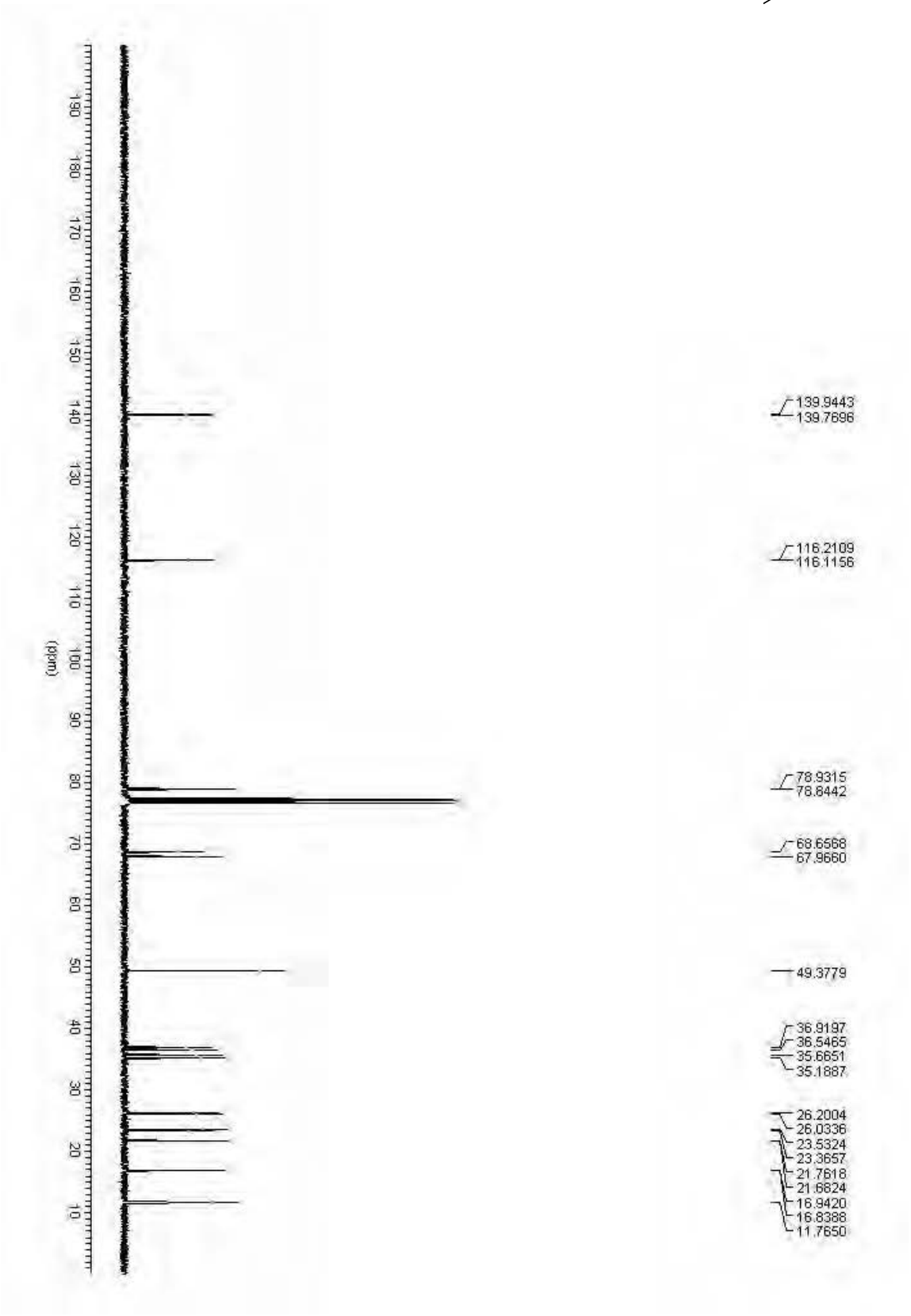
(5*R*,6*R*,7*R*)-7-Ethyl-5-methyl-non-8-ene-2,6-diol (III.30)



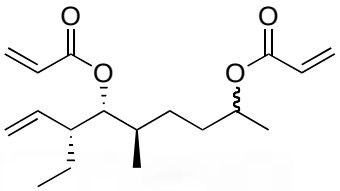


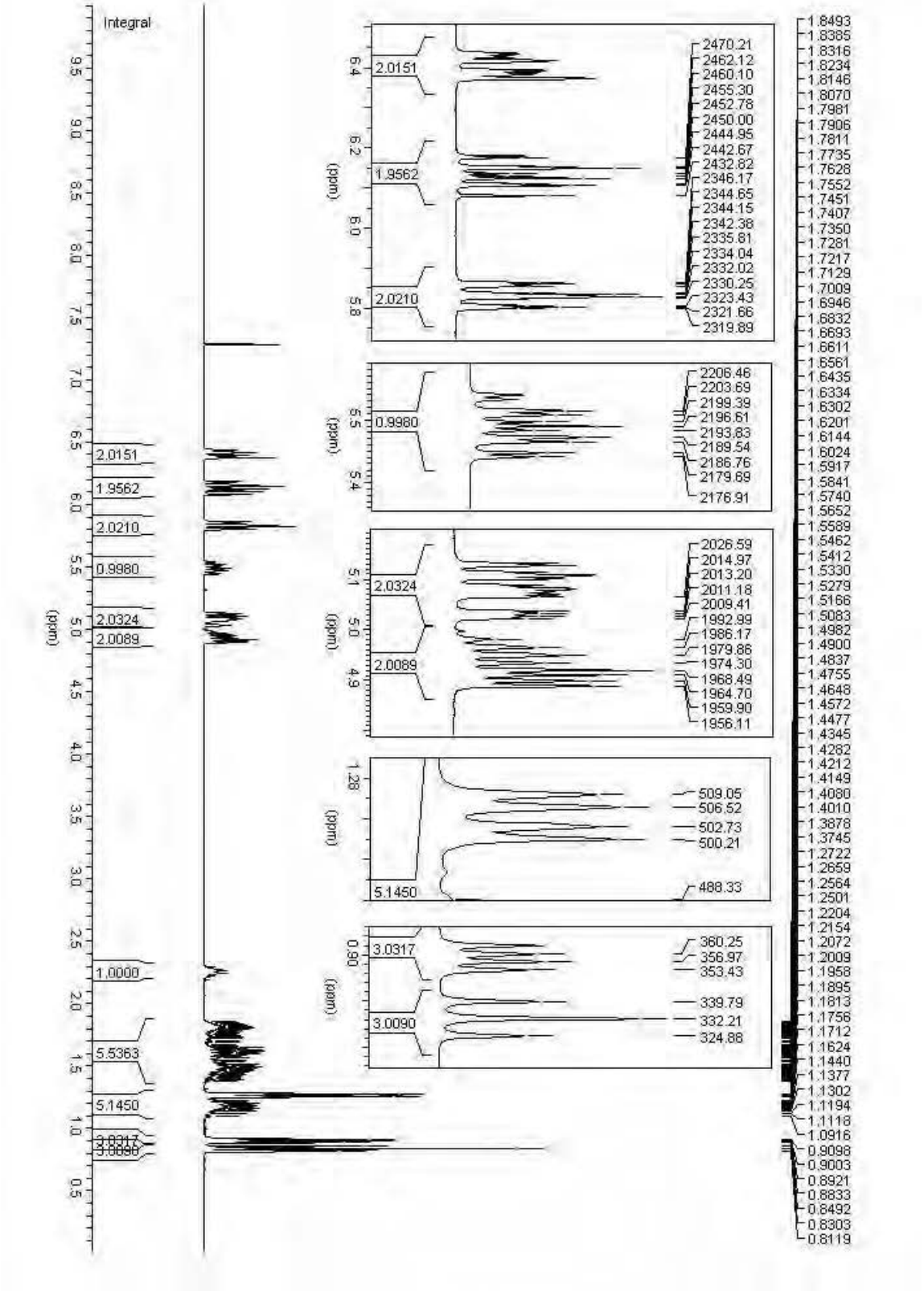
(5*R*,6*R*,7*R*)-7-Ethyl-5-methyl-non-8-ene-2,6-diol (III.30)



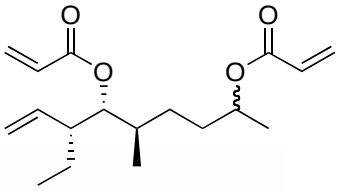


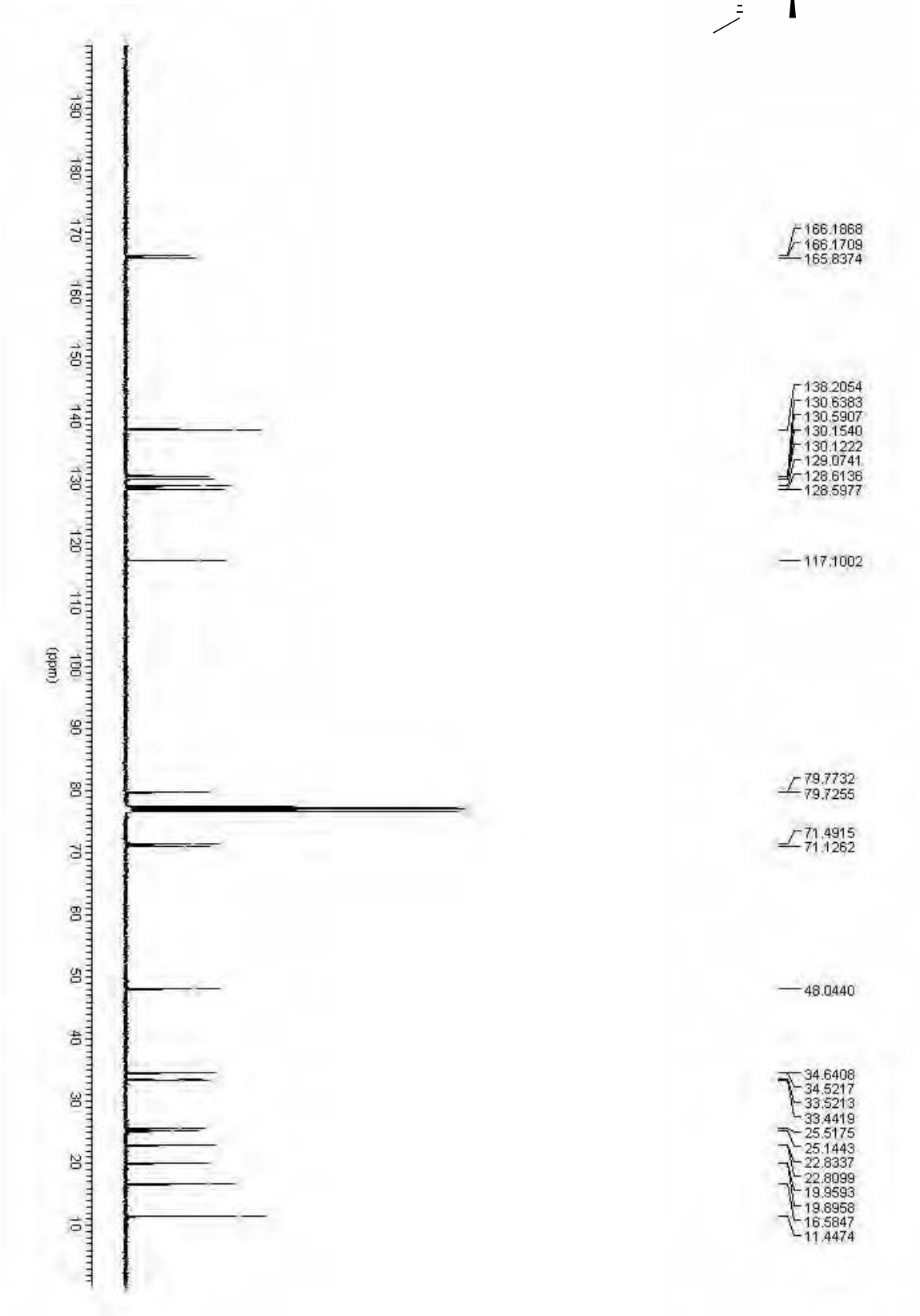
Acrylic acid (1*R*,2*R*)-5-acryloyloxy-1-((*R*)-1-ethyl-allyl)-2-methyl-hexyl ester (III.31)



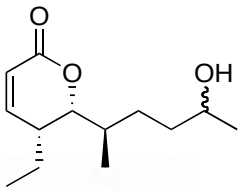


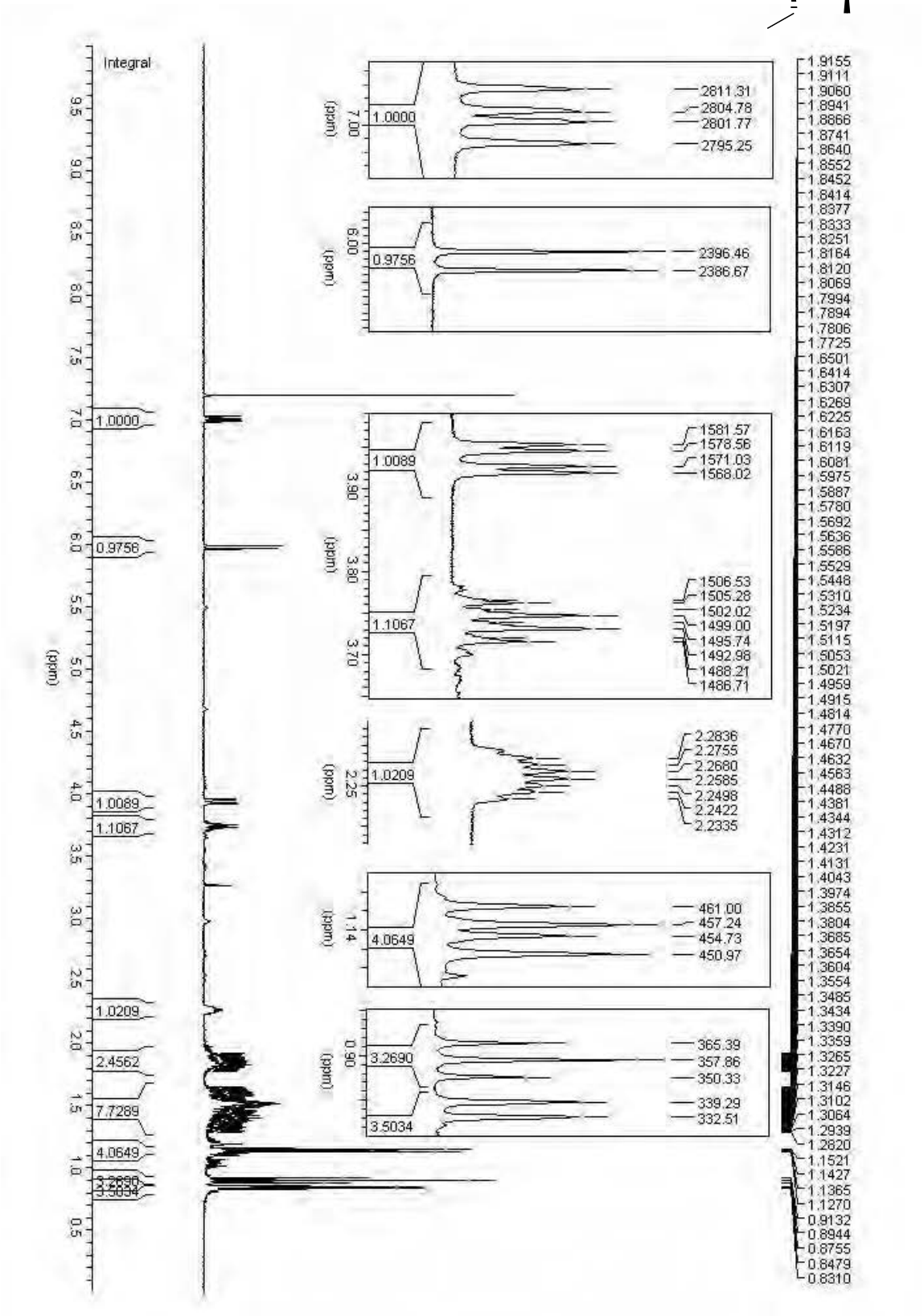
Acrylic acid (1*R*,2*R*)-5-acryloyloxy-1-((*R*)-1-ethyl-allyl)-2-methyl-hexyl ester (III.31)





(5*R*,6*R*)-5-Ethyl-6-((*R*)-4-hydroxy-1-methyl-pentyl)-5,6-dihydro-pyran-2-one (III.33)





5*R*,6*R*)-5-Ethyl-6-((*R*)-4-hydroxy-1-methyl-pentyl)-5,6-dihydro-pyran-2-one (III.33)