Solid-phase synthesis of biaryl cyclic peptides containing a histidine-tyrosine linkage

Iteng Ng-Choi[a], Àngel Oliveras[a], Marta Planas[a,*] and Lidia Feliu[a,*]

[a]*Laboratori d'Innovació en Processos i Productes de Síntesi Orgànica (LIPPSO), Departament de Química, Universitat de Girona, Maria Aurèlia Capmany 69, 17003 Girona, Spain*

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ABSTRACT

A solid-phase strategy for the synthesis of biaryl cyclic peptides containing a side-chain to side-chain His-Tyr linkage was developed. The key step was the macrocyclization of a linear peptidyl resin incorporating a 5-bromohistidine and a 3-boronotyrosine via the formation of the biaryl bond by means of a microwave-assisted Suzuki-Miyaura reaction. This method allowed direct access to biaryl cyclic peptides containing a 3- or 5-amino acid ring and bearing the histidine residue at the N- or the C-terminus, being especially conducive for analogues in which this amino acid is located at the C-terminus. This study also served to establish a strategy for the synthesis of biaryl cyclic peptides derived from the two hemispheres of the natural biaryl bicyclic peptides aciculitins.

1. Introduction

Over the last decades, much attention has been focused on biaryl cyclic peptides containing an aryl-aryl bond between the side chains of two aromatic amino acids. The interest in this type of peptides has risen because they exhibit interesting biological activities, in which the biaryl motif plays a crucial role [1]. Among these biaryl moieties, aryltyrosines are found in simple peptides as well as in complex macrocycles, such as the antimicrobial peptides arylomycins [2,3], the proteasome inhibitor TMC-95 [4-7], the neurotensin antagonist RP-66453 [8,9], or the antibiotic vancomycin [10,11]. Similarly, arylhistidines are present in the active site of heme-copper oxidases, and in cytotoxic and antifungal marine peptides, such as aciculitins [12-14].

Biaryl cyclic peptides are also significant from a structural point of view, constituting an important synthetic challenge [1]. The formation of the biaryl bond has been achieved via a Suzuki-Miyaura cross-coupling reaction, a Pd-catalyzed C-H activation or a Cu-mediated oxidative coupling. The former reaction has allowed the synthesis of peptides containing a Trp-Tyr [4,15], a Phe-Phe [15-18], a Phe-Tyr [15,17,19] or a Tyr-Tyr [15,19-21] linkage. The C-H activation reaction has been used to form the biaryl bonds Trp-Phe and Trp-Tyr [22,23], while the Cu-mediated oxidative phenol coupling has provided access to peptides bearing a Tyr-hydroxyphenylglycine motif [24]. Despite the benefits of the solid supported chemistry, most of these reactions have been performed in solution. In fact, the solid-phase synthesis of biaryl peptides has been scarcely reported. This methodology has only been applied by four research groups to obtain cyclic peptides featuring a covalent bond Phe-Phe [16-18], Phe-Tyr [17,19], Tyr-Tyr [19], Trp-Phe or Trp-Tyr [22].

In this context, the synthesis of biaryl cyclic peptides containing a 5-arylhistidine is especially noteworthy because the arylation of the 4(5)-position of the imidazole ring has proven to be difficult. So far, several methods based on transition metal-catalyzed reactions have been developed for the synthesis in solution of 4(5)-arylimidazoles, but most of them provide moderate yields and/or require drastic conditions [25-36]. Regarding 5-arylhistidine derivatives, to the best of our knowledge, their synthesis in solution has only been described in two reports through microwave-assisted Pd-catalyzed direct C-H activation [37] or a Suzuki-Miyaura cross-coupling [38]. Taking advantage of the solid supported chemistry, we extended the latter methodology to the solid-phase preparation of 5-arylhistidine-containing peptides via arylation of a resin-bound 5-bromohistidine residue with commercially available arylboronic acids [39,40]. It was observed that microwaves were crucial to enhance the arylation of histidine, shortening the reaction time and providing higher overall purities [38]. This strategy enabled the first solid-phase synthesis of biaryl linear tri- and tetrapeptides containing a 5-arylhistidine [40]. Later, we applied this approach to the modification of lead antimicrobial peptides with activity against plant pathogens of economic importance [39]. The resulting 5-arylhistidine-containing peptides displayed antibacterial and antifungal activity, and low hemolysis. This low cytotoxicity was attributed to the presence of the imidazole ring of histidine.

Within our current interest on biaryl cyclic peptides, we envisioned that the above strategy devised for the preparation of linear peptides containing a 5-arylhistidine could be applied to the solid-phase synthesis of biaryl macrocyclic sequences incorporating a biaryl bond between the side-chain of a histidine and of a tyrosine. To the best of our knowledge, the synthesis of 5-arylhistidine-containing cyclic peptides has not yet been reported. Thus, the main purpose of this work was the solid-phase preparation of biaryl cyclic peptides **I** and **II**, which contain a biaryl linkage between the 5-position of the imidazole of the histidine and the 3-position of the phenyl group of the tyrosine (Fig. 1). The histidine is located at the N-terminus in biaryl cyclic peptides with general structure **I**, whereas compounds **II** bear this residue at the C-terminus. The size of the ring is changed by incorporating different combinations of lysines and leucines, as representative cationic and hydrophobic amino acids. The synthesis of these compounds could be accomplished through the cyclization of a linear peptidyl resin via the formation of the biaryl bond under the conditions of the Suzuki-Miyaura reaction. Herein, the feasibility of this strategy to prepare biaryl cyclic peptides **I** and **II** of different ring sizes was studied.

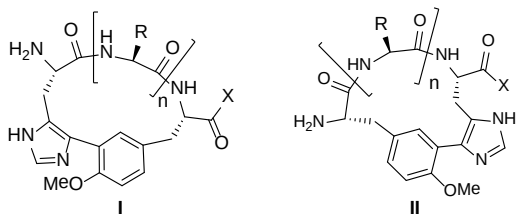


Fig. 1. General structure of biaryl cyclic peptides **I** and **II**.

2. Results and discussion

2.1. Biaryl cyclic peptides containing a histidine residue at the N-terminus

We first investigated the solid-phase preparation of biaryl cyclic peptides **BPC782**, **BPC784**, and **BPC786** containing a side-chain to side-chain His-Tyr linkage with the histidine residue located at the N-terminus (Fig. 2).

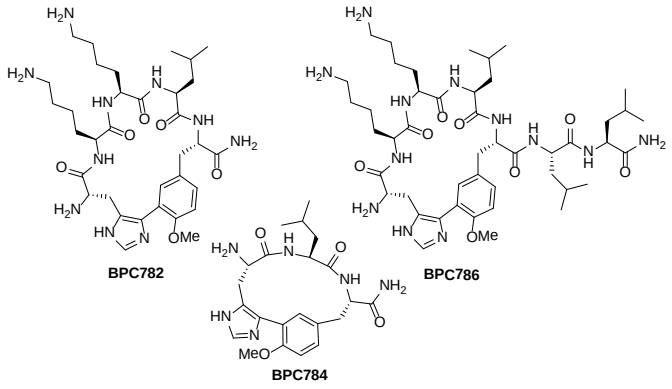


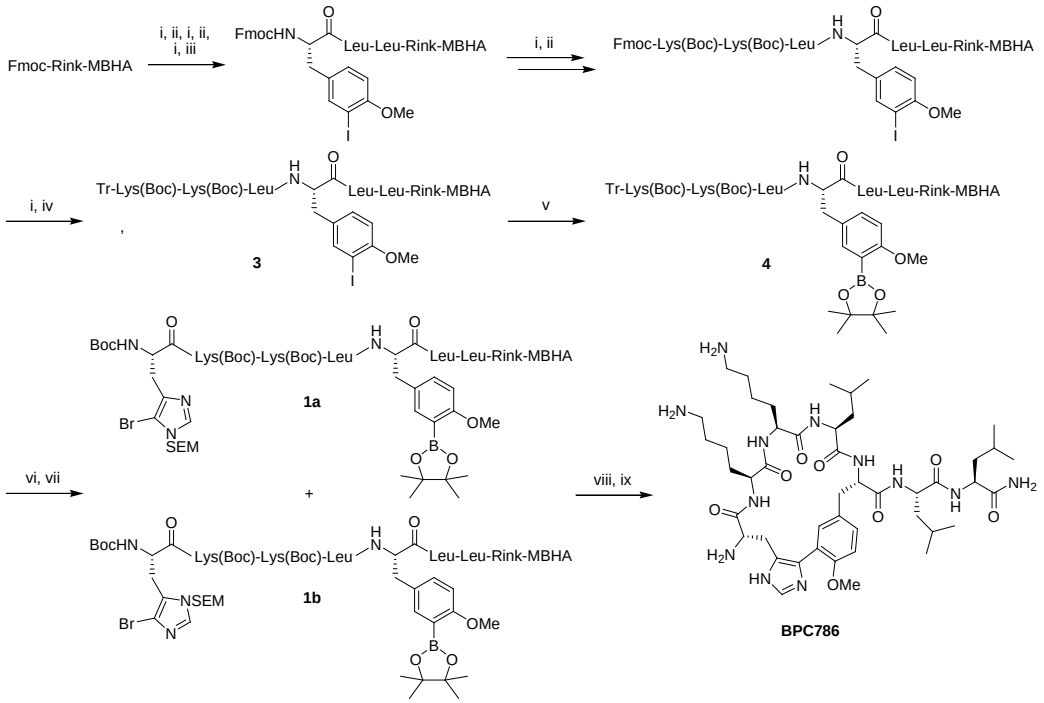
Fig. 2. Structure of biaryl cyclic peptides containing a His at the N-terminus.

In particular, we first assayed the synthesis of **BPC786** which comprises a 5-residue ring and a Leu-Leu spacer at the C-terminus. The synthetic strategy involved the preparation of the linear peptidyl resins **1**, incorporating a 5-bromohistidine at the N-terminus and a 3-boronotyrosine at the C-terminus, followed by their cyclization through a microwave-assisted Suzuki-Miyaura cross-coupling (Scheme 1). Synthesis of the regioisomeric peptidyl resins Boc-His(5-Br,1-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Leu-Leu-Rink-MBHA (**1a**) and Boc-His(5-Br,3-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Leu-Leu-Rink-MBHA (**1b**) started from a Fmoc-Rink-MBHA resin. First, the iodopeptidyl resin Fmoc-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-I,Me)-Leu-Leu-Rink-MBHA was obtained following a standard 9-fluorenylmethoxycarbonyl (Fmoc)/*tert*-butyl (*t*Bu) strategy through sequential Fmoc removal and coupling steps. The Fmoc group was removed using piperidine/DMF (3:7) (2 + 10 min). Couplings of Fmoc-Leu-OH and Fmoc-Lys(Boc)-OH were mediated by *N*,*N*-diisopropylcarbodiimide (DIPCDI) and ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) in DMF for 1 h. Fmoc-Tyr(3-I,Me)-OH (**2**) was anchored using 1-[(1-(cyano-2-ethoxy-2-oxoethylylidineaminooxy)dimethylaminomorpholino)] uronium hexafluorophosphate (COMU), Oxyma and *N*,*N*-diisopropylethylamine (DIEA) in DMF overnight. This tyrosine derivative was synthesized in solution from Boc-Tyr(3-I,Me)-OMe [40] through subsequent removal of the *tert*-butoxycarbonyl (Boc) group, methyl ester hydrolysis, and Fmoc protection of the N^{α}-amino group.

Once the iodopeptidyl resin Fmoc-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-I,Me)-Leu-Leu-Rink-MBHA was obtained, the Fmoc group was replaced by a trityl (Tr) group due to the instability of the former to the basic Miyaura borylation conditions (Scheme 1). After Fmoc removal, the N^{α}-amino group of the resin was protected by treatment with trityl chloride (TrCl) in presence of DIEA for 4 h. An aliquot of the resulting resin Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-I,Me)-Leu-Leu-Rink-MBHA (**3**) was exposed to trifluoroacetic acid (TFA)/H_2O/triisopropylsilane (TIS) (95:2.5:2.5) for 2 h, affording H-Lys-Lys-Leu-Tyr(3-I,Me)-Leu-Leu-NH_2 in 84% HPLC purity, which was characterized by mass spectrometry.

Next, borylation of the iodopeptidyl resin **3** led to Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Leu-Leu-Rink-MBHA (**4**). This reaction was performed under the conditions previously described in our group [19], which involved the use of bis(pinacolato)diboron (B_2Pin_2) (4 equiv.), $PdCl_2$(dppf) (0.18 equiv.), 1,1'-bis(diphenylphosphanyl)ferrocene (dppf) (0.09 equiv.), and KOAc (6 equiv.) in anhydrous DMSO at 80 ºC for 8 h (Scheme 1). It was observed that longer reaction times promoted the protodeborylation of the tyrosine residue of the boronopeptidyl resin **4**. Acidolytic cleavage of an aliquot of **4** gave the boronopeptide H-Lys-Lys-Leu-Tyr(3-B(OH)$_2$,Me)-Leu-Leu-NH_2 in 81% HPLC purity. This boronic acid resulted from the hydrolysis of the pinacol boronic ester group during HPLC analysis, which was confirmed by mass spectrometry.

The trityl group of the resin-bound tyrosine boronic ester **4** was then selectively removed with TFA/H_2O/CH_2Cl_2 (0.2:1:98.8) (2×1 + 2×20 min). Finally, the regioisomeric mixture of Boc-His(5-Br,1-SEM)-OH (**5a**) and Boc-His(5-Br,3-SEM)-OH (**5b**), prepared as previously reported [40], was coupled using DIPCDI and Oxyma in DMF for 3 h to yield the expected regioisomeric peptidyl resins **1**. An aliquot of **1** was treated with TFA/H_2O/TIS (95:2.5:2.5) under stirring for 3 h. H-His(5-Br)-Lys-Lys-Leu-Tyr(3-B(OH)$_2$,Me)-Leu-Leu-NH_2 (**6**) was obtained in 75% HPLC purity, and was characterized by mass spectrometry.

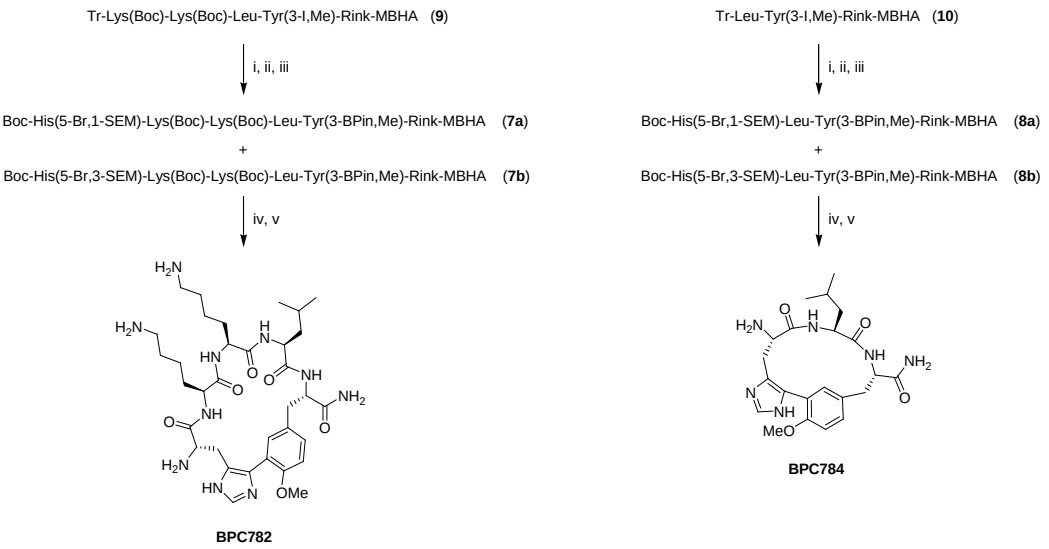


Scheme 1. Solid-phase synthesis of the biaryl cyclic peptide **BPC786**. Reagents and conditions: (i) Piperidine/DMF (3:7) (2 + 10 min). (ii) Fmoc-Leu-OH or Fmoc-Lys(Boc)-OH, DIPCDI, Oxyma, DMF, 1 h. (iii) Fmoc-Tyr(3-I,Me)-OH (**2**), COMU, Oxyma, DIEA, DMF, overnight. (iv) TrCl, DIEA, DMF, 4 h. (v) B_2Pin_2, $PdCl_2$(dppf), dppf, KOAc, DMSO, 80 ºC, 8 h. (vi) TFA/H_2O/CH_2Cl_2. (0.2:1:98.8) (2×1 + 2×20 min). (vii) Boc-His(5-Br,1-SEM)-OH (**5a**) and Boc-His(5-Br,3-SEM)-OH (**5b**), DIPCDI, Oxyma, DMF, 3 h. (viii) $Pd_2(dba)_3$, KF, SPhos or P(*o*-tolyl)$_3$, DME/EtOH/H_2O, MW, 140 ºC, 30 min. (ix) TFA/H_2O/TIS (95:2.5:2.5), 3 h, stirring.

With the linear peptidyl resins 1 in hand, their macrocyclization was initially attempted under the conditions of the Suzuki-Miyaura cross-coupling (Scheme 1). Thus, resins 1 were first exposed to $Pd_2(dba)_3$ (0.2 equiv.), P(*o*-tolyl)$_3$ (0.4 equiv.), and KF (4 equiv.) in degassed 1,2-dimethoxyethane (DME)/EtOH/H_2O (9:9:2) under microwave irradiation at 140 ºC for 30 min. The resulting resin was cleaved, and HPLC and ESI-MS analysis of the crude reaction mixture revealed the formation of the expected biaryl cyclic peptide **BPC786** (30% purity) together with the protodeborylated and debrominated derivative H-His-Lys-Lys-Leu-Tyr(Me)-Leu-Leu-NH_2 (29% purity), a common byproduct of this reaction [41]. The cyclization was also assayed using SPhos [42-45] instead of P(*o*-tolyl)$_3$. Giralt and co-workers reported that the presence of Buchwald's SPhos ligand avoids racemization of α-amino acids such as tyrosine derivatives in Suzuki-Miyaura reactions [42]. Under these conditions a similar result was obtained, **BPC786** was formed in 31% purity. H-His-Lys-Lys-Leu-Tyr(Me)-Leu-Leu-NH_2 and the oxidized and debrominated byproduct H-His-Lys-Lys-Leu-Tyr(3-OH,Me)-Leu-Leu-NH_2 were also detected (20 and 11% purity, respectively). **BPC786** was then isolated by reverse-phase column chromatography, analyzed and characterized by HPLC and mass spectrometry, being obtained in 79% purity.

In view of these results, we then studied the application of this methodology to the synthesis of biaryl cyclic peptides **BPC782** and **BPC784** (Scheme 2). The former is a **BPC786** analogue that does not contain the Leu-Leu spacer at the C-terminus while **BPC784** consists of a 3-residue ring. The synthesis of **BPC782** required the preparation of the linear peptidyl resins Boc-His(5-Br,1-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**7a**) and Boc-His(5-Br,3-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**7b**) whereas the linear precursors of **BPC784** were resins Boc-His(5-Br,1-SEM)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**8a**) and Boc-His(5-Br,3-SEM)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**8b**). These linear peptidyl resins **7** and **8** were synthesized following the protocol described for peptidyl resins **1**. Accordingly, iodopeptidyl resins Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-I,Me)-Rink-MBHA (**9**) and Tr-Leu-Tyr(3-I,Me)-Rink-MBHA (**10**) were prepared. Acidolytic cleavage of an aliquot of **9** and **10** provided the corresponding iodopeptides in 87 and 72% HPLC purity, respectively. Borylation of **9** and **10**, followed by trityl group removal and subsequent coupling of bromohistidines **5** yielded the expected peptidyl resins **7** and **8**, respectively. Treatment of an aliquot of these resins with TFA/H_2O/TIS (95:2.5:2.5) led to H-His(5-Br)-Lys-Lys-Leu-Tyr(3-B(OH)$_2$,Me)-NH_2 (**11**) and H-His(5-Br)-Leu-Tyr(3-B(OH)$_2$,Me)-NH_2 (**12**) in 60% HPLC purity which were characterized by mass spectrometry.

Cyclization of **7** and **8** was performed through a microwave-assisted intramolecular Suzuki-Miyaura reaction under the conditions described above for **1** using SPhos as ligand. After cleavage of the resin resulting from the cyclization of **7**, HPLC and mass spectrometry analysis of the crude reaction mixture revealed the formation of the biaryl cyclic peptide **BPC782** as major product in 35% purity, together with a mixture of the common Suzuki-Miyaura arylation byproducts. After column chromatography purification, **BPC782** was obtained in 68% HPLC purity and was characterized by mass spectrometry. Similarly, cyclization of **8** led to the formation of **BPC784** in 30% purity, which was purified and obtained in 90% purity. Its structure was confirmed by mass spectrometry. Thus, the synthesis of **BPC782**, **BPC784** and **BPC786** revealed that the cyclization is not influenced by the presence of a Leu-Leu spacer and that similar results are obtained for the formation of a 3- or 5-residue ring.



Scheme 2. Solid-phase synthesis of biaryl cyclic peptides **BPC782** and **BPC784**. Reagents and conditions: (i) B_2Pin_2, $PdCl_2$(dppf), dppf, KOAc, DMSO, 80 ºC, 8 h. (ii) TFA/H_2O/CH_2Cl_2 (0.2:1:98.8) (2×1 + 2×20 min). (iii) Boc-His(5-Br,1-SEM)-OH (**5a**) and Boc-His(5-Br,3-SEM)-OH (**5b**), DIPCDI, Oxyma, DMF, 3 h. (iv) $Pd_2(dba)_3$, KF, SPhos, DME/EtOH/H_2O, MW, 140 ºC, 30 min. (v) TFA/H_2O/TIS (95:2.5:2.5), 3 h, stirring.

2.2. Biaryl cyclic peptides containing a histidine residue at the C-terminus

We next turned our attention to the solid-phase synthesis of biaryl cyclic peptides **BPC788**, **BPC790** and **BPC792** in which the histidine residue was located at the C-terminus (Fig. 3).

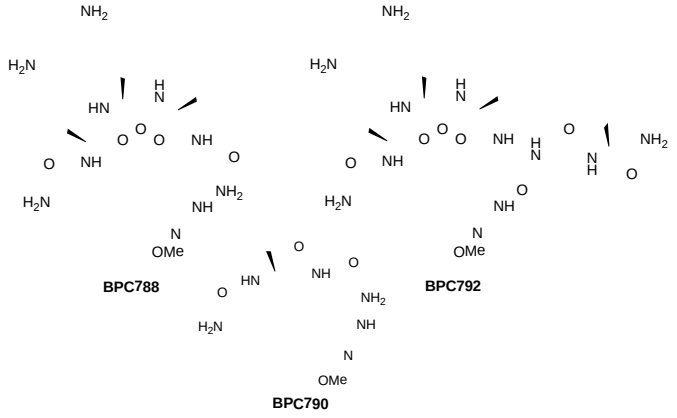
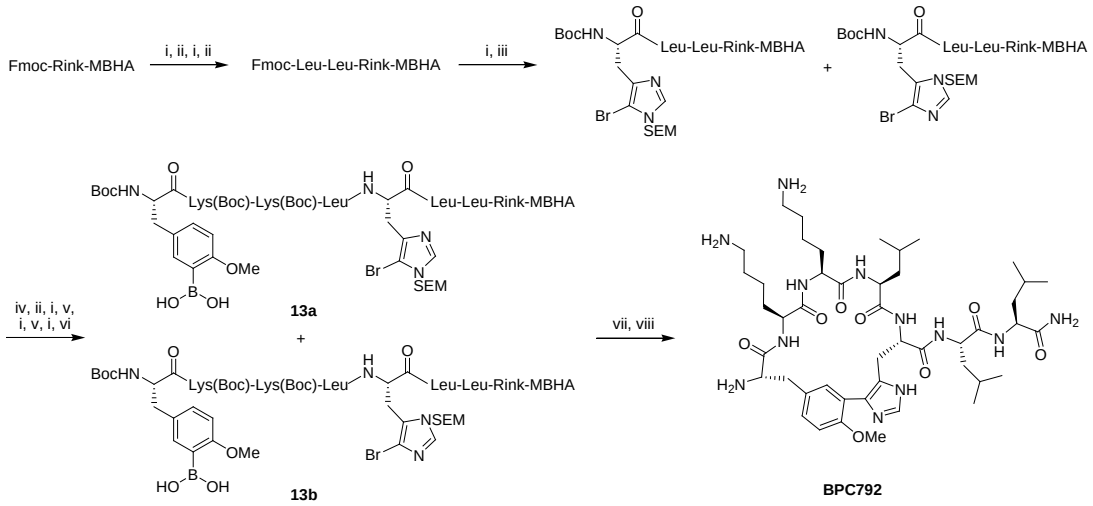


Fig.3. Structure of biaryl cyclic peptides containing a His at the C-terminus.

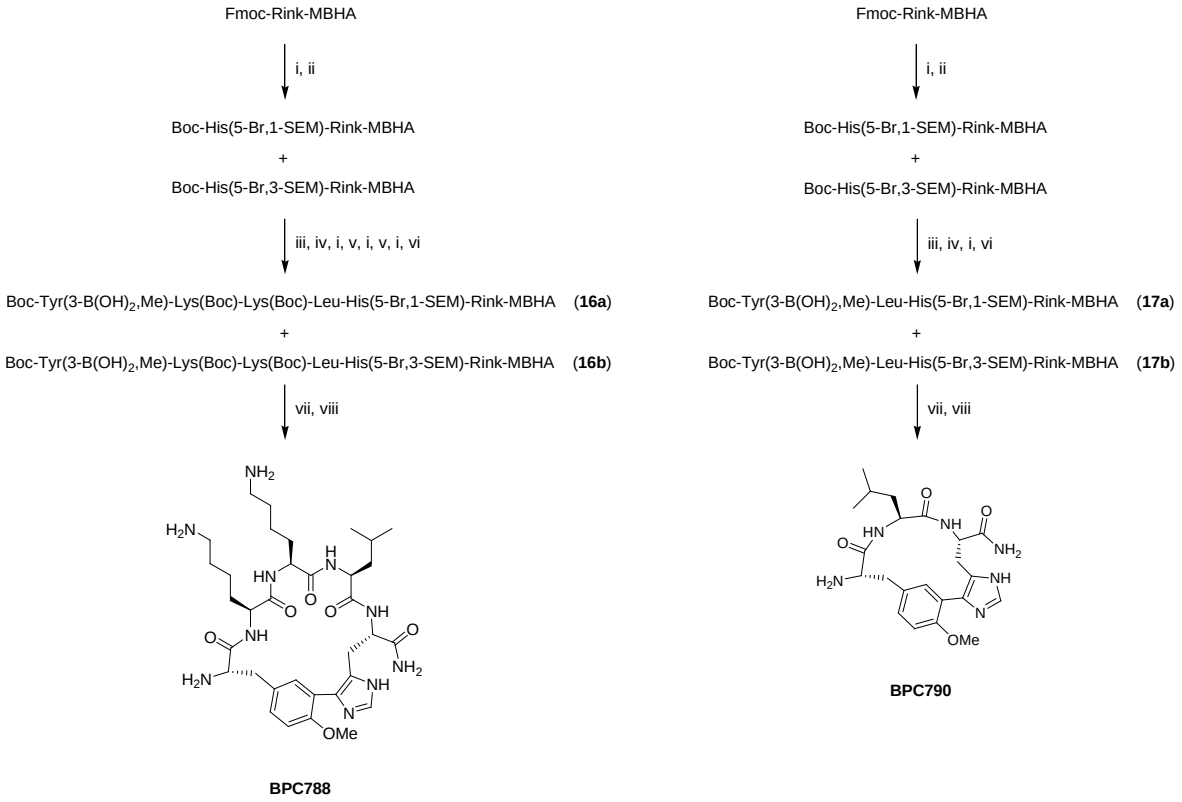
The preparation of **BPC792**, incorporating a 5-residue ring and a Leu-Leu spacer at the C-terminus, was first investigated. A similar strategy to that of biaryl cyclic peptides that contain the histidine residue at the N-terminus was followed (Scheme 3). In this case, the linear peptidyl resins Boc-Tyr(3-B(OH)$_2$,Me)-Lys(Boc)-Lys(Boc)-Leu-His(5-Br,1-SEM)-Leu-Leu-Rink-MBHA (**13a**) and Boc-Tyr(3-B(OH)$_2$,Me)-Lys(Boc)-Lys(Boc)-Leu-His(5-Br,3-SEM)-Leu-Leu-Rink-MBHA (**13b**), incorporating a 3-boronotyrosine at the N-terminus and a 5-bromohistidine at the C-terminus, were required. First, Fmoc-Leu-Leu-Rink-MBHA was synthesized following an Fmoc/*t*Bu strategy as previously described. After Fmoc removal, Boc-His(5-Br,1-SEM)-OH (**5a**) and Boc-His(5-Br,3-SEM)-OH (**5b**) were coupled using COMU, Oxyma and DIEA in DMF overnight. Then, the Boc group was selectively removed under mild conditions by treatment with trimethylsilyltriflate (TMSOTf) in presence of 2,6-lutidine (10×30 min) [46]. Peptide elongation was carried out by sequential coupling and deprotection steps using Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH and Boc-Tyr(3-B(OH)$_2$,Me)-OH (**14**) as amino acid derivatives. Boronotyrosine **14** was prepared in solution through Miyaura borylation of Boc-Tyr(3-I,Me)-OMe [40], followed by hydrolysis of the pinacolate and saponification of the methyl ester. It should be noticed that coupling of **14** to the solid support was mediated by DIPCDI and Oxyma in DMF and should not exceed a 3 h reaction time in order to avoid side products. An aliquot of the resulting resins **13** was treated with TFA/H_2O/TIS (95:2.5:2.5) under stirring for 3 h to provide H-Tyr(3-B(OH)$_2$,Me)-Lys-Lys-Leu-His(5-Br)-Leu-Leu-NH_2 (**15**) in 65% HPLC purity, which was characterized by mass spectrometry.

The cyclization of the regioisomeric peptidyl resins **13** was carried out through a Suzuki-Miyaura arylation under the conditions used for the synthesis of biaryl cyclic peptides bearing a histidine at the N-terminus which involved treatment with $Pd_2(dba)_3$, SPhos, and KF under microwave irradiation at 140 ºC for 30 min (Scheme 3). HPLC and mass spectrometry analysis of the crude reaction mixture obtained from the acidolytic cleavage of the resulting resin indicated the presence of the desired biaryl cyclic peptide **BPC792** as major product (55% purity) together with the debrominated and protodeborylated byproduct H-Tyr(Me)-Lys-Lys-Leu-His-Leu-Leu-NH_2 (22% purity). **BPC792** was finally purified by reverse-phase column chromatography being obtained in 92% HPLC purity, and characterized by mass spectrometry.

Synthesis of **BPC788**, a **BPC792** analog lacking the Leu-Leu spacer, and of **BPC790**, consisting of a 3-residue ring, was performed following the same synthetic approach used for **BPC792** (Scheme 4). The corresponding linear peptidyl resins **16** and **17** were prepared as described for **13**. After acidolytic cleavage of an aliquot of each resin, the corresponding expected linear peptides **18** and **19** were detected in 59% and 52% HPLC purity, respectively.



Scheme 3. Solid-phase synthesis of biaryl cyclic peptide **BPC792**. Reagents and conditions: (i) Piperidine/DMF (3:7) (2 + 10 min). (ii) Fmoc-Leu-OH, DIPCDI, Oxyma, DMF, 1 h. (iii) Boc-His(5-Br,1-SEM)-OH (**5a**) and Boc-His(5-Br,3-SEM)-OH (**5b**), COMU, Oxyma, DIEA, DMF, overnight. (iv) TMSOTf, 2,6-lutidine, CH_2Cl_2 (10×30 min). (v) Fmoc-Lys(Boc)-OH, DIPCDI, Oxyma, DMF, 1 h. (vi) Boc-Tyr(3-B(OH)$_2$,Me)-OH (**14**), DIPCDI, Oxyma, DMF, 3 h. (vii) $Pd_2(dba)_3$, KF, SPhos, DME/EtOH/H_2O, MW, 140 ºC, 30 min. (viii) TFA/H_2O/TIS (95:2.5:2.5), 3 h, stirring.



Scheme 4. Solid-phase synthesis of biaryl cyclic peptides **BPC788** and **BPC790**. Reagents and conditions: (i) Piperidine/DMF (3:7) (2 + 10 min). (ii) Boc-His(5-Br,1-SEM)-OH (**5a**) and Boc-His(5-Br,3-SEM)-OH (**5b**), COMU, Oxyma, DIEA, DMF, overnight. (iii) TMSOTf, 2,6-lutidine, CH_2Cl_2 (10×30 min). (iv) Fmoc-Leu-OH, DIPCDI, Oxyma, DMF, 1 h. (v) Fmoc-Lys(Boc)-OH, DIPCDI, Oxyma, DMF, 1 h. (vi) Boc-Tyr(3-B(OH)$_2$,Me)-OH (**14**), DIPCDI, Oxyma, DMF, 3 h. (vii) $Pd_2(dba)_3$, KF, SPhos, DME/EtOH/H_2O, MW, 140 ºC, 30 min. (viii) TFA/H_2O/TIS (95:2.5:2.5), 3 h, stirring.

The intramolecular Suzuki-Miyaura cross-coupling of **16** and **17** afforded the expected biaryl cyclic peptides **BPC788** and **BPC790**, respectively. **BPC788** was detected in 32% HPLC purity together with a mixture of the byproducts H-Tyr(Me)-Lys-Lys-Leu-His-NH_2 (15% purity) and H-Tyr(3-OH,Me)-Lys-Lys-Leu-His-NH_2 (5% purity). Interestingly, **BPC790** was successfully obtained (49% HPLC purity) and only traces of the common byproducts were detected. Column chromatography purification provided these biaryl cyclic peptides in 87 and 96% HPLC purity, respectively, and they were characterized by mass spectrometry. These results pointed out that the macrocyclization through formation of a His-Tyr linkage is favored when the histidine residue is located at the C-terminus.

2.3. Biaryl cyclic peptides derived from the northern and southern hemispheres of aciculitins

Taking into account the biological properties of aciculitins as well as their synthetic interest [12-14], we decided to extend the above methodology to the solid-phase synthesis of analogues of the northern and southern hemisphere of these bicyclic peptides (Fig. 4). In particular, we focused our attention on the preparation of the northern hemisphere analog **20** and of the southern hemisphere derivative **21**.

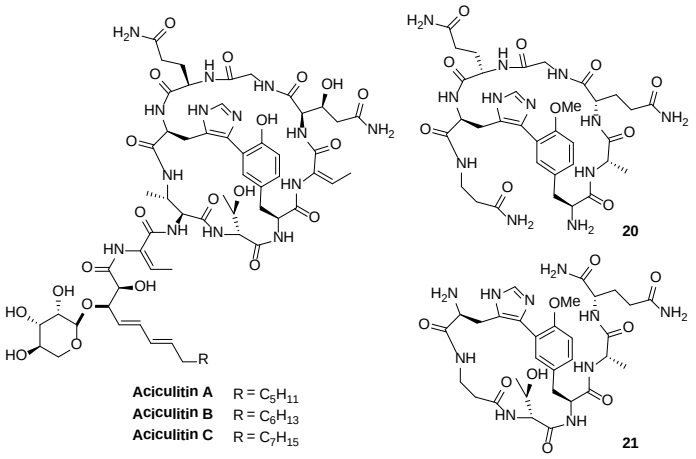


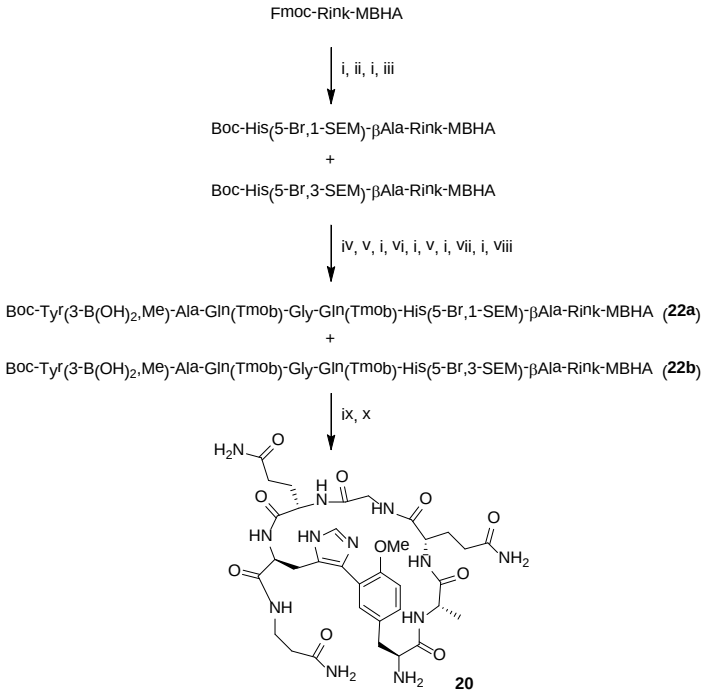
Fig. 4. Structure of aciculitins A-C and of biaryl cyclic peptides **20** and **21**.

Biaryl cyclic peptides **20** and **21** were designed based on commercially available L-amino acids. In particular, the 3-hydroxyglutamine, the 2,3-diaminobutyric acid and the 2-amino-2-butenoic acid residues were replaced by a glutamine, a β-alanine and an alanine, respectively.

We initially tested the synthesis of the biaryl cyclic peptide **20** which contains a histidine residue at the C-terminus. The β-alanine was chosen as anchoring point to the solid support. This peptide was synthesized according to the strategy described above for peptides **BPC788**, **BPC790** and **BPC792**. Thus, the regioisomeric heptapeptidyl resins Boc-Tyr(3-B(OH)$_2$,Me)-Ala-Gln(Tmob)-Gly-Gln(Tmob)-His(5-Br,1-SEM)-βAla-Rink-MBHA (**22a**) and Boc-Tyr(3-B(OH)$_2$,Me)-Ala-Gln(Tmob)-Gly-Gln(Tmob)-His(5-Br,3-SEM)-βAla-Rink-MBHA (**22b**) were prepared (Scheme 5). An aliquot of these peptidyl resins was acidolytically cleaved, yielding H-Tyr(3-B(OH)$_2$,Me)-Ala-Gln-Gly-Gln-His(5-Br)-βAla-NH$_2$ (**23**) in >99% purity, which was characterized by mass spectrometry.

These resins were then subjected to the Suzuki-Miyaura macrocyclization reaction as described above. HPLC and mass spectrometry analysis of the crude reaction mixture showed the presence of the desired biaryl cyclic peptide **20** in 64% purity. Two peaks appeared in the HPLC chromatogram, both corresponding to **20** as demonstrated by HPLC-MS analysis of the crude reaction mixture carried out at different temperatures (20 to 60 ºC). These two peaks coalesced at 40 and 60 ºC which pointed out that they could be attributed to two different conformers. This peptide was purified by column chromatography, being obtained in 87% purity and characterized by mass spectrometry.

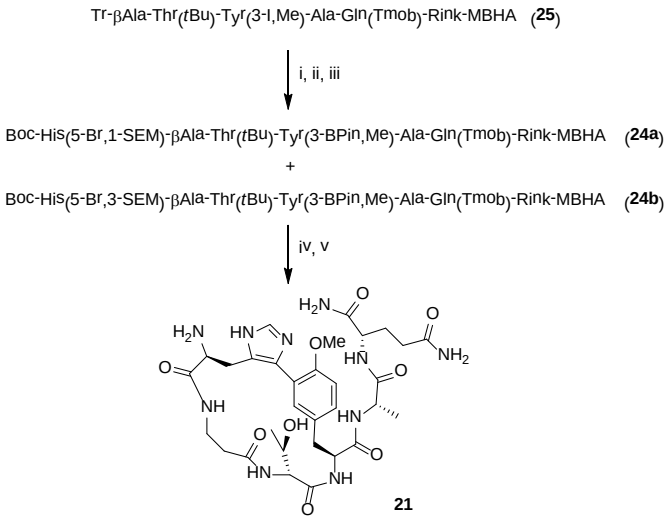
In the case of the biaryl cyclic peptide **21**, bearing a histidine at the N-terminus, the glutamine residue was selected as the anchoring point. Thus, the synthesis involved the preparation of



Scheme 5. Solid-phase synthesis of the biaryl cyclic peptide **20**. Reagents and conditions: (i) Piperidine/DMF (3:7) (2 + 10 min). (ii) Fmoc-βAla-OH, DIPCDI, Oxyma, DMF, 1 h. (iii) Boc-His(5-Br,1-SEM)-OH (**5a**) and Boc-His(5-Br,3-SEM)-OH (**5b**), COMU, Oxyma, DIEA, DMF, overnight. (iv) TMSOTf, 2,6-lutidine, CH_2Cl_2 (10×30 min). (v) Fmoc-Gln(Tmob)-OH, DIPCDI, Oxyma, DMF, 1 h. (vi) Fmoc-Gly-OH, DIPCDI, Oxyma, DMF, 1 h. (vii) Fmoc-Ala-OH, DIPCDI, Oxyma, DMF, 1 h. (viii) Boc-Tyr(3-B(OH)$_2$,Me)-OH, DIPCDI, Oxyma, DMF, 3 h. (ix) $Pd_2(dba)_3$, KF, SPhos, DME/EtOH/H_2O, MW, 140 ºC, 30 min. (x) TFA/H_2O/TIS (95:2.5:2.5), 3 h, stirring.

the peptidyl resins Boc-His(5-Br,1-SEM)-βAla-Thr(^{t}Bu)-Tyr(3-BPin,Me)-Ala-Gln(Tmob)-Rink-MBHA (**24a**) and Boc-His(5-Br,3-SEM)-βAla-Thr(^{t}Bu)-Tyr(3-BPin,Me)-Ala-Gln(Tmob)-Rink-MBHA (**24b**), containing a 5-bromohistidine at the N-terminus and a 3-boronotyrosine at the C-terminus (Scheme 6). To achieve this objective, we first synthesized the peptidyl resin Tr-βAla-Thr(tBu)-Tyr(3-I,Me)-Ala-Gln(Tmob)-Rink-MBHA (**25**) following the strategy used for resins **3**, **9** and **10**. An aliquot of the resulting resin **25** was treated with a mixture of TFA/H_2O/TIS (95:2.5:2.5) for 2 h, affording the expected iodopeptide H-βAla-Thr-Tyr(3-I,Me)-Ala-Gln-NH$_2$ in 87% purity.

Then, resin **25** was exposed to bis(pinacolato)diboron (B_2Pin_2) (4 equiv.), $PdCl_2$(dppf) (0.18 equiv.), 1,1'-bis(diphenylphosphanyl)ferrocene (dppf) (0.09 equiv.), and KOAc (6 equiv.) in anhydrous DMSO at 80 ºC for 8 h (Scheme 4). Acidolytic cleavage of an aliquot of the resulting resin **26** gave H-βAla-Thr-Tyr(3-B(OH)$_2$,Me)-Ala-Gln-NH$_2$ in 62% purity. Selective removal of the trityl group, followed by coupling of the regioisomeric histidine derivatives **5** afforded the expected peptidyl resins **24** which were subjected to Suzuki-Miyaura macrocyclization. Acidolytic cleavage of these resins rendered the expected biaryl cyclic peptide **21** in 12% purity, as confirmed by mass spectrometry. This peptide was purified by reverse-phase column chromatography, being obtained in 84% HPLC purity, and characterized by mass spectrometry.



Scheme 6. Solid-phase synthesis of the biaryl cyclic peptide **21**. Reagents and conditions: (i) B_2Pin_2, $PdCl_2$(dppf), dppf, KOAc, DMSO, 80 ºC., 8 h. (ii) TFA/H_2O/CH_2Cl_2 (0.2:1:98.8), (2×1 + 2×20 min). (iii) Boc-His(5-Br,1-SEM)-OH (**5a**) and Boc-His(5-Br,3-SEM)-OH (**5b**), DIPCDI, Oxyma, DMF, 3 h. (iv) $Pd_2(dba)_3$, SPhos, KF, DME/EtOH/H_2O, MW, 140 ºC, 30 min. (v) TFA/H_2O/TIS (95:2.5:2.5), 3 h, stirring.

The synthesis of **20** and **21** confirmed that the cyclization through the formation of a biaryl bond His-Tyr via a Suzuki-Miyaura cross-coupling gives better results when the histidine residue is located at the C-terminus. This study constitutes the first approach towards the synthesis of the naturally occurring biaryl bicyclic peptides aciculitins.

3. Conclusions

In summary, we describe the first solid-phase synthesis of biaryl cyclic peptides containing a His-Tyr linkage. The key feature of our synthetic methodology is the cyclization via the formation of a biaryl bond between a 5-bromohistidine and a 3-boronotyrosine. This strategy allowed the preparation of biaryl cyclic peptides incorporating a 3- or 5-residue ring and with the histidine residue at the N- or the C-terminus. Best results were obtained when the histidine was at the C-terminus. In addition, this study was extended to the synthesis of analogues of the northern and southern hemispheres of aciculitins. We envisaged that this methodology could be considered a useful starting point for the total synthesis of aciculitins or of other biaryl bicyclic peptides.

4. Experimental section

4.1. General information

Manual peptide synthesis was performed in polypropylene syringes fitted with a polyethylene porous disk. Solvents and soluble reagents were removed by suction. Most chemicals were purchased from commercial suppliers Sigma–Aldrich (Madrid, Spain), Iris Biotech GmbH (Marktredwitz, Germany), Scharlab (Sentmenat, Spain) or Panreac (Castellar del Vallès, Spain) and used without further purification.

Peptides were analyzed under standard analytical HPLC conditions with a Dionex liquid chromatography instrument composed of an UV/Vis Dionex UVD170U detector, a P680 Dionex bomb, an ASI-100 Dionex automatic injector, and CHROMELEON 6.60 software. Detection was performed at 220 nm. Solvent A was 0.1% aq. TFA and solvent B was 0.1% TFA in CH_3CN. Conditions A: Analysis was carried out with a Kromasil 100 C_{18} (4.6 mm × 40 mm, 3.5 μm) column with 2–100% B over 7 min at a flow rate of 1 mL/min. Conditions B: Analysis was carried out with a Kromasil 100 C_{18} (4.6 mm × 250 mm, 5 μm) column with 2–100% B over 28 min at a flow rate of 1 mL/min. Conditions C: Analysis was carried out with a Kromasil 100 C_{18} (4.6 mm × 250 mm, 5 μm) column with 2–25% B over 3 min followed by 25-35% B over 30 min and 35-100% B over 1 min at a flow rate of 1 mL/min. Peptides were also analyzed with a 1260 Infinity II liquid chromatography instrument (Agilent Technologies) composed of a Diode Array Detector HS, a Quaternary Pump VL, a 1260 Vial sampler and OpenLab CDS ChemStation software. Conditions D: Analysis was carried out with a linear gradient of 2–100% B over 12 min at a flow rate of 1 mL/min. Conditions E: Analysis was carried out with a linear gradient of 2–100% B over 12 min at a flow rate of 1 mL/min at 40 ºC.

Peptide purifications were performed on a Combi*Flash* Rf200 automated flash chromatography system using Redi*Sep* Rf Gold reversed-phase C_{18} column packed with high performance C_{18} derivatized silica.

ESI-MS analyses were performed at the Serveis Tècnics de Recerca of the University of Girona with an Esquire 6000 ESI ion Trap LC/MS (Bruker Daltonics) instrument equipped with an electrospray ion source. The instrument was operated in the positive ESI(+) ion mode. Samples (5 μL) were introduced into the mass spectrometer ion source directly through an HPLC autosampler. The mobile phase (80:20 CH_3CN/H_2O at a flow rate of 100 μL/min) was delivered by a 1100 Series HPLC pump (Agilent). Nitrogen was employed as both the drying and nebulising gas. Nitrogen was employed as both the drying and nebulising gas.

HRMS were recorded on a Bruker MicroTof-QIITM instrument using an electrospray ionization source at the Serveis Tècnics de Recerca of the University of Girona. Samples were introduced into the mass spectrometer ion source by direct infusion using a syringe pump and were externally calibrated using sodium formate. The instrument was operated in the positive ion mode.

^{1}H and ^{13}C NMR spectra were measured with a Bruker 300 or 400 MHz NMR spectrometer at the Serveis Tècnics de Recerca of the University of Girona. Chemical shifts were reported as δ values (ppm) directly referenced to the solvent signal.

Microwave-assisted reactions were performed with a single mode Discover S-Class labstation microwave (CEM) (0-300 W). The time, temperature, and power were controlled with the Synergy software. The temperature was monitored through an infrared sensor in the floor of the cavity.

4.2. Synthesis of methyl 3-iodo-4-methoxy-L-tyrosinate

Boc-Tyr(3-I,Me)-OMe [40] (4.50 g, 10.34 mmol, 1 equiv.) was dissolved in TFA/CH_2Cl_2 (1:1, 30 mL) and stirred at room temperature for 2 h. After this time, the solvent mixture was removed under vacuum. Diethyl ether was then added and evaporated, this process was repeated three times to completely remove the TFA. The resulting product was dried in vacuo overnight to afford H-Tyr(3-I,Me)-OMe as a white solid (3.25 g, 94% yield). t_R = 6.65 min (93% purity) (Conditions A). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59 [d, *J* = 2.2 Hz, 1 H, CH-2_{arom}], 7.18 [dd, *J* = 2.2 and 8.4 Hz, 1 H, CH-6_{arom}], 6.76 [d, *J* = 8.4 Hz, 1 H, CH-5_{arom}], 4.18 [t, *J* = 6.6 Hz, 1 H, CH-α], 3.84 [s, 3 H, CO_2CH_3], 3.74 [s, 3 H, OCH_3], 3.16 [d, *J* = 6.6 Hz, 2 H, CH_2-β] ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 169.47 [CO_2CH_3], 157.14 [C-4_{arom}], 139.79 [CH-2_{arom}], 130.89 [C-1_{arom}], 128.60 [CH-6_{arom}], 111.58 [CH-5_{arom}], 86.37 [C-3_{arom}], 56.44 [OCH_3], 53.20 [CH-α], 52.77 [CO_2CH_3], 34.47 [CH_2-β] ppm.

4.3. Synthesis of 3-iodo-4-methoxy-L-tyrosine

A solution of LiOH (1.24 g, 28.91 mmol, 3 equiv.) in water (17 mL) was added to a solution of H-Tyr(3-I,Me)-OMe (3.23 g, 9.64 mmol, 1 equiv.) in MeOH/THF (1:1, 34 mL). The reaction mixture was stirred at room temperature for 2 h. After this time, the organic solvents were evaporated under reduced pressure and water (60 mL) was added to the resulting residue. The solution was adjusted to pH 5 by addition of glacial AcOH and the resulting precipitate was filtered, washed with cold diethyl ether, and dried in vacuo overnight, yielding H-Tyr(3-I,Me)-OH as a white solid (3 g, 96% yield). t_R = 6.21 min (>99% purity) (Conditions A). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.64 [d, *J* = 2.1 Hz, 1 H, CH-2$_{arom}$], 7.24 [dd, *J* = 2.1 and 8.4 Hz, 1 H, CH-6$_{arom}$], 6.91 [d, *J* = 8.4 Hz, 1 H, CH-5$_{arom}$], 3.78 [s, 3 H, OCH$_3$], 3.31 [dd, *J* = 4.5 and 7.7 Hz, 1 H, CH-α], 3.00 [dd, *J* = 4.5 and 14.1 Hz, 1 H, CH$_2$-β], 2.75 [dd, *J* = 7.7 and 14.1 Hz, 1 H, CH$_2$-β] ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 156.37 [C-4$_{arom}$], 139.60 [CH-2$_{arom}$], 132.22 [C-1$_{arom}$], 130.76 [CH-6$_{arom}$], 111.36 [CH-5$_{arom}$], 85.98 [C-3$_{arom}$], 56.33 [OCH$_3$], 55.74 [CH-α], 35.87 [CH$_2$-β] ppm.

*4.4. Synthesis of N(α)-(9-fluorenylmethoxycarbonyl)-3-iodo-4-methoxy-L-tyrosine (**2**)*

A solution of H-Tyr(3-I,Me)-OH (3 g, 9.34 mmol, 1 equiv.) in dioxane (32 mL) was adjusted to pH 7-8 by addition of aqueous 10% Na_2CO_3. The reaction mixture was stirred at room temperature for 30 min and Fmoc-OSu (3.31 g, 9.81 mmol, 1.05 equiv.) was then added. The mixture was stirred for 24 h at room temperature and then concentrated in vacuo. EtOAc (40 mL) was added and the organic solution was washed with 1 N HCl (30 mL) and H_2O (3×30 mL). The aqueous layers were combined, adjusted to pH 1 and extracted with EtOAc (3×40 mL). All the organic layers were combined, washed with brine (30 mL) and dried over anhydrous magnesium sulfate. Removal of the solvent followed by digestion of the resulting precipitate in pentane/diethyl ether (1:1, 50 mL) for 2 h afforded a white solid, which was purified by column chromatography. Elution with CH_2Cl_2/MeOH (95:5) gave Fmoc-Tyr(3-I,Me)-OH (**2**) as a white solid (2.85 g, 57% yield). t_R = 9.00 min (>99% purity) (Conditions A). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.78 [bb, 1 H, CO$_2$H], 7.88 [d, *J* = 7.6 Hz, 2 H, 2 CH$_{arom}$-Fmoc], 7.71 [d, *J* = 2.0 Hz, 1 H, CH-2$_{arom}$], 7.66-7.62 [m, 2 H, 2 CH$_{arom}$-Fmoc], 7.43-7.39 [m, 2 H, 2 CH$_{arom}$-Fmoc], 7.34-7.25 [m, 3 H, 2 CH$_{arom}$-Fmoc, CH-6$_{arom}$], 6.90 [d, *J* = 8.4 Hz, 1 H, CH-5$_{arom}$], 4.22-4.10 [m, 4 H, CH-α, CH$_2$-Fmoc, CH-Fmoc], 3.77 [s, 3 H, OCH$_3$], 3.01 [dd, *J* = 4.4 and 13.8 Hz, 1 H, CH$_2$-β], 2.77 [dd, *J* = 10.4 and 13.8 Hz, 1 H, CH$_2$-β] ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.20 [CO$_2$H], 156.37 [C-4$_{arom}$], 155.97 [CONH], 143.74, 140.67 [4 C$_{arom}$-Fmoc], 139.46 [CH-2$_{arom}$], 132.20 [C-1$_{arom}$], 130.40 [CH-6$_{arom}$], 127.63, 127.10, 125.29, 120.11 [8 CH$_{arom}$-Fmoc], 111.28 [CH-5$_{arom}$], 85.73 [C-3$_{arom}$], 65.69 [CH$_2$-Fmoc], 56.27 [OCH$_3$], 55.61 [CH-α], 46.57 [CH-Fmoc], 34.95 [CH$_2$-β] ppm.

4.5. Synthesis of methyl 3-borono-N(α)-tert-butoxycarbonyl-4-methoxy-L-tyrosinate

A solution of Boc-Tyr(3-I,Me)-OMe [40] (920 mg, 2.11 mmol, 1 equiv.) in degassed anhydrous DMSO (9 mL) was added to a solution of bis(pinacolato)diboron (B_2Pin_2) (1.08 g, 4.23 mmol, 2 equiv.), $PdCl_2$(dppf) (100 mg, 0.12 mmol, 0.06 equiv.), and KOAc (840 mg, 8.45 mmol, 4 equiv.) in degassed anhydrous DMSO (4.5 mL). The mixture was stirred under nitrogen at 80 ºC for 7 h. After this time, brine (50 mL) was added and the resulting solution was extracted with EtOAc (3×50 mL). The combined organic extracts were washed with brine (3×50 mL), and dried over anhydrous magnesium sulphate. Removal of the solvent gave a dark brown oil, which was purified by column chromatography. Elution with hexane/EtOAc (4:1) afforded Boc-Tyr(3-B(OH)$_2$,Me)-OMe together with Boc-Tyr(3-BPin,Me)-OMe. A solution of CH_3CN/H_2O (1:1) was then added and stirred at 75 ºC for 4 h. The resulting solution was lyophilized to afford a white solid, which was purified by column chromatography. Elution with hexane/EtOAc (1:1) yielded Boc-Tyr(3-B(OH)$_2$,Me)-OMe as a white solid (450 mg, 59% yield). t_R = 7.51 min (>99% purity) (Conditions A). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.57 [d, *J* = 2.8 Hz, 1 H, CH-2$_{arom}$], 7.20 [dd, *J* = 2.8 and 8.4 Hz, 1 H, CH-6$_{arom}$], 6.84 [d, *J* = 8.4 Hz, 1 H, CH-5$_{arom}$], 5.79 [bs, 2 H, B(OH)$_2$], 4.98 [bs, 1 H, CONH], 4.56-4.54 [m, 1 H, CH-α], 3.89 [s, 3 H, OCH$_3$], 3.73 [s, 3 H, CO$_2$CH$_3$], 3.09 [dd, *J* = 5.6 and 13.8 Hz, 1 H, CH$_2$-β], 3.01 [dd, *J* = 6.0 and 13.8 Hz, 1 H, CH$_2$-β], 1.41 [s, 9 H, C(CH$_3$)$_3$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.53 [*C*O$_2$CH$_3$] 163.85 [C-4$_{arom}$], 155.37 [CONH], 137.87 [CH-2$_{arom}$], 133.75, 128.64 [CH-6$_{arom}$, C-1$_{arom}$], 110.84, 110.34 [CH-5$_{arom}$, C-3$_{arom}$], 80.30 [*C*(CH$_3$)$_3$], 55.95 [OCH$_3$], 54.73 [CH-α], 52.41 [CO$_2$*C*H$_3$], 37.60 [CH$_2$-β], 28.41 [(*C*H$_3$)$_3$C] ppm.

*4.6. Synthesis of 3-borono-N(α)-tert-butoxycarbonyl-4-methoxy-L-tyrosine (**14**)*

An aqueous solution of LiOH (3 mL, 4.17 mmol, 3 equiv) was added to a solution of Boc-Tyr(3-B(OH)$_2$,Me)-OMe (450 mg, 1.27 mmol, 1 equiv.) in MeOH/THF (1:1, 6 mL). The reaction mixture was stirred at room temperature for 1.5 h. After this time, the organic solvents were evaporated under reduced pressure and water (25 mL) was added to the resulting residue. The solution was adjusted to pH 5-6 by addition of 1 N HCl followed by extraction with EtOAc (3×25 mL). The organic layers were combined, washed with brine (25 mL), and dried over anhydrous magnesium sulfate. Removal of the solvent afforded Boc-Tyr(3-B(OH)$_2$,Me)-OH (**14**) as a white solid (410 mg, 95% yield). t_R = 6.84 min (>99% purity) (Conditions A). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.60 [s, 1 H, CH-2$_{arom}$], 7.25-7.22 [m, 1 H, CH-6$_{arom}$], 6.82 [d, *J* = 8.8 Hz, 1 H, CH-5$_{arom}$], 5.06-5.04 [m, 1 H, CONH], 4.51-4.49 [m, 1 H, CH-α], 3.87 [s, 3 H, OCH$_3$], 3.13 [dd, *J* = 4.8 and 13.6 Hz, 1 H, CH$_2$-β], 3.03 [dd, *J* = 5.2 and 13.6 Hz, 1 H, CH$_2$-β], 1.40 [s, 9 H, C(CH$_3$)$_3$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.45 [*C*O$_2$H] 163.74 [C-4$_{arom}$], 155.49 [CONH], 137.79 [CH-2$_{arom}$], 134.02, 128.57 [CH-6$_{arom}$, C-1$_{arom}$], 110.82, 110.30 [CH-5$_{arom}$, C-3$_{arom}$], 80.33 [*C*(CH$_3$)$_3$], 55.70 [OCH$_3$], 54.70 [CH-α], 37.28 [CH$_2$-β], 28.43 [(*C*H$_3$)$_3$C] ppm.

*4.7. General method for the synthesis of the 3-iodotyrosylpeptidyl resins **3**, **9**, **10** and **25***

Peptidyl resins were synthesized manually by the solid-phase method with standard Fmoc chemistry. MBHA resin (0.4 mmol/g) was used as solid support and it was swollen with CH_2Cl_2 (1×20 min) and DMF (1×20 min), and washed with piperidine/DMF (3:7, 1×5 min) and DMF (6×1 min). Then, the resin was treated with Fmoc-Rink linker (4 equiv.), DIPCDI (4 equiv.) and Oxyma (4 equiv.) in DMF at room temperature overnight. After this time, the resin was washed with DMF (6×1 min). Elongation of the peptide chain was performed through sequential Fmoc removal and coupling of the corresponding amino acids. Fmoc group removal was achieved with a mixture of piperidine/DMF (3:7, 2 + 10 min). Coupling of the corresponding commercially available amino acids Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Tmob)-OH, Fmoc-Ala-OH, Fmoc-Thr(*t*Bu)-OH, and Fmoc-βAla-OH (4 equiv.) was performed by using DIPCDI (4 equiv.) and Oxyma (4 equiv.) in DMF at room temperature for 1 h, whereas coupling of Fmoc-Tyr(3-I,Me)-OH (**2**) (2 equiv.) was carried out using COMU (2 equiv.), Oxyma (2 equiv.) and DIEA (4 equiv.) in DMF at room

temperature overnight. The completion of the reactions was monitored by the Kaiser test [47]. After each coupling and deprotection step, the resulting resin was washed with DMF (6×1 min).

Upon completion of the peptide sequence, the N-terminal Fmoc group was removed and a trityl group was introduced using TrCl (10 equiv.) and DIEA (10 equiv.) in DMF at room temperature for 4 h. Then, the resulting resin was washed with DMF (6×1 min) and CH_2Cl_2 (3×1 min), and air-dried. The completion of this reaction was monitored by the Kaiser test [47]. An aliquot of the resulting peptidyl resin was cleaved with TFA/H_2O/TIS (95:2.5:2.5) whilst stirring for 2 h at room temperature. Following TFA evaporation and diethyl ether extraction, the crude peptide was dissolved in H_2O/CH_3CN (1:1), lyophilized, analysed by HPLC and characterized by mass spectrometry.

*4.7.1. Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-I,Me)-Leu-Leu-Rink-MBHA (**3**)*

The iodohexapeptidyl resin **3** was prepared following the general method described above. Acidolytic cleavage of an aliquot of this peptidyl resin afforded H-Lys-Lys-Leu-Tyr(3-I,Me)-Leu-Leu-NH_2 in 84% purity. t_R = 18.99 min (Conditions B). MS (ESI): *m/z* = 458.7 $[M + 2H]^{2+}$, 916.5 $[M + H]^+$, 938.5 $[M + Na]^+$.

*4.7.2. Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-I,Me)-Rink-MBHA (**9**)*

The iodotetrapeptidyl resin **9** was prepared following the general method described above. Acidolytic cleavage of an aliquot of this peptidyl resin afforded H-Lys-Lys-Leu-Tyr(3-I,Me)-NH_2 in 87% purity. t_R = 4.80 min (Conditions D). MS (ESI): *m/z* = 345.6 $[M + 2H]^{2+}$, 690.3 $[M + H]^+$.

*4.7.3. Tr-Leu-Tyr(3-I,Me)-Rink-MBHA (**10**)*

The iododipeptidyl resin **10** was prepared following the general method described above. Acidolytic cleavage of an aliquot of this peptidyl resin afforded H-Leu-Tyr(3-I,Me)-NH_2 in 72% purity. t_R = 18.13 min (Conditions B). MS (ESI): *m/z* = 434.0 $[M + H]^+$.

*4.7.4. Tr-βAla-Thr(^{t}Bu)-Tyr(3-I,Me)-Ala-Gln(Tmob)-Rink-MBHA (**25**)*

The iododipeptidyl resin **25** was prepared following the general method described above. Acidolytic cleavage of an aliquot of this peptidyl resin afforded H-βAla-Thr-Tyr(3-I,Me)-Ala-Gln-NH_2 in 87% purity. t_R = 5.87 min (Conditions A).

4.8. General method for the solid-phase Miyaura borylation

A 2-10 mL round-bottomed flask was charged with the corresponding 3-iodotyrosylpeptidyl resin, B_2Pin_2 (4 equiv.), $PdCl_2$(dppf) (0.18 equiv.), and dppf (0.09 equiv.). A thoroughly sonicated solution of KOAc (6 equiv.) in degassed anhydrous DMSO (20 μL/mg of resin) was then added, and the mixture was heated at 80 ºC for 8 h. Upon completion of the reaction, the resin was washed with DMSO (6×1 min), MeOH (6×1 min), CH_2Cl_2 (6×1 min), and diethyl ether (3×1 min). An aliquot of the resulting boronopeptidyl resin was cleaved with TFA/H_2O/TIS (95:2.5:2.5) whilst being stirred for 2 h at room temperature. Following TFA evaporation and diethyl ether extraction, the crude peptide was dissolved in H_2O/CH_3CN (1:1), lyophilized, analysed by HPLC, and characterized by mass spectrometry.

*4.8.1. Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Leu-Leu-Rink-MBHA (**4**)*

The boronohexapeptidyl resin **4** was prepared starting from Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-I,Me)-Leu-Leu-Rink-MBHA (**3**) (170 mg) following the general method for solid-phase Miyaura borylation. Acidolytic cleavage of an aliquot of this peptidyl resin gave H-Lys-Lys-Leu-Tyr(3-B$(OH)_2$,Me)-Leu-Leu-NH_2 (81% purity), resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 15.87 min (Conditions C). MS (ESI): *m/z* = 417.7 $[M + 2H]^{2+}$, 834.6 $[M + H]^+$.

4.8.2. Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Rink-MBHA

This boronotetrapeptidyl resin was prepared starting from Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-I,Me)-Rink-MBHA (**9**) (57 mg) following the general method for solid-phase Miyaura borylation. Acidolytic cleavage of an aliquot of this peptidyl resin gave H-Lys-Lys-Leu-Tyr(3-B$(OH)_2$,Me)-NH_2 (69% purity), resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 16.85 min (Conditions B). MS (ESI): *m/z* = 304.7 $[M + 2H]^{2+}$, 608.4 $[M + H]^+$.

4.8.3. Tr-Leu-Tyr(3-BPin,Me)-Rink-MBHA

This boronodipeptidyl resin was prepared starting from Tr-Leu-Tyr(3-I,Me)-Rink-MBHA (**10**) (90 mg) following the general method for solid-phase Miyaura borylation. Acidolytic cleavage of an aliquot of this peptidyl resin gave H-Leu-Tyr(3-B$(OH)_2$,Me)-NH_2 (50% purity), resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 14.84 min (Conditions B). MS (ESI): *m/z* = 352.1 $[M + H]^+$.

*4.8.4. Tr-βAla-Thr(^{t}Bu)-Tyr(3-BPin,Me)-Ala-Gln(Tmob)-Rink-MBHA (**26**)*

This boronodipeptidyl resin was prepared starting from Tr-βAla-Thr(^{t}Bu)-Tyr(3-I,Me)-Ala-Gln(Tmob)-Rink-MBHA (**25**) (175 mg) following the general method for solid-phase Miyaura borylation. Acidolytic cleavage of an aliquot of this peptidyl resin gave H-βAla-Thr-Tyr(3-B$(OH)_2$,Me)-Ala-Gln-NH_2 (62% purity), resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 5.20 min (Conditions A). MS (ESI): *m/z* = 610.3 $[M + H]^+$, 632.3 $[M + Na]^+$.

*4.9. General method for the solid-phase synthesis of the linear peptidyl resins **1**, **7** and **8***

The corresponding boronopeptidyl resins were treated with TFA/H_2O/CH_2Cl_2 (0.2:1:98.8, 2×1 + 2×20 min), and washed with DMF (3×1 min), DIEA/CH_2Cl_2 (1:19, 3×1 min), CH_2Cl_2 (3×1 min), and DMF (3×1 min). Then, coupling of the regioisomeric mixture of Boc-His(5-Br,1-SEM)-OH (**5a**) and Boc-His(5-Br,3-SEM)-OH (**5b**) (3 equiv.) was carried out using DIPCDI (3 equiv.) and Oxyma (3 equiv.) in DMF at room temperature for 3 h. The resins were then washed with DMF (6×1 min) and CH_2Cl_2 (3×1 min), and air-dried. The completion of the reactions was monitored by the Kaiser test [47]. An aliquot of the resulting peptidyl resins was cleaved with TFA/H_2O/TIS (95:2.5:2.5) whilst being stirred for 3 h at room temperature. Following TFA evaporation and diethyl ether extraction, the corresponding crude peptide was dissolved in H_2O/CH_3CN (1:1), lyophilized, analysed by HPLC, and characterized by mass spectrometry.

*4.9.1. Boc-His(5-Br,1-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Leu-Leu-Rink-MBHA (**1a**) and Boc-His(5-Br,3-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Leu-Leu-Rink-MBHA (**1b**)*

Resins **1** were synthesized starting from Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Leu-Leu-Rink-MBHA (**4**) (100 mg) following the general method described above. Acidolytic cleavage of an aliquot of these peptidyl resins afforded H-His(5-Br)-Lys-Lys-Leu-Tyr(3-B$(OH)_2$,Me)-Leu-Leu-NH_2 (**6**) (75% purity), resulting from the hydrolysis of the pinacol boronate

during HPLC analysis. t_R = 16.25 min (Conditions C). MS (ESI): *m/z* = 1049.5, 1051.5 $[M + H]^+$.

*4.9.2. Boc-His(5-Br,1-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**7a**) and Boc-His(5-Br,3-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**7b**)*

Resins **7** were synthesized starting from Tr-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (50 mg) following the general method described above. Acidolytic cleavage of an aliquot of these peptidyl resins afforded H-His(5-Br)-Lys-Lys-Leu-Tyr(3-$B(OH)_2$,Me)-NH_2 (**11**) (60% purity), resulting from the hydrolysis of the pinacol boronate during HPLC analysis. t_R = 14.98 min (Conditions B). MS (ESI): *m/z* = 823.3, 825.3 $[M + H]^+$.

*4.9.3. Boc-His(5-Br,1-SEM)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**8a**) and Boc-His(5-Br,3-SEM)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**8b**)*

Resins **8** were synthesized starting from Tr-Leu-Tyr(3-BPin,Me)-Rink-MBHA (85 mg) following the general method described above. Acidolytic cleavage of an aliquot of these peptidyl resins afforded H-His(5-Br)-Leu-Tyr(3-$B(OH)_2$,Me)-NH_2 (**12**) (60% purity), resulting from the hydrolysis of the pinacol boronate during HPLC analysis. t_R = 15.38 min (Conditions B). MS (ESI): *m/z* = 567.1, 569.1 $[M + H]^+$.

*4.10. General method for the solid-phase synthesis of the linear peptidyl resins **13**, **16**, **17** and **22***

These peptidyl resins were synthesized manually by the solid-phase method with standard Fmoc chemistry. MBHA resin (0.4 mmol/g) was used as solid support and it was swollen with CH_2Cl_2 (1×20 min) and DMF (1×20 min), and washed with piperidine/DMF (3:7, 1×5 min) and DMF (6×1 min). Then, resins were treated with Fmoc-Rink linker (4 equiv.), DIPCDI (4 equiv.) and Oxyma (4 equiv.) in DMF at room temperature overnight. After this time, resins were washed with DMF (6×1 min). Elongation of the peptide chain was performed through sequential Fmoc removal and coupling of the corresponding amino acids. Fmoc group removal was achieved with a mixture of piperidine/DMF (3:7, 2 + 10 min), and then resins were washed with DMF (6×1 min). Couplings of the corresponding Fmoc-amino acids (4 equiv.) were performed using DIPCDI (4 equiv.) and Oxyma (4 equiv.) in DMF at room temperature for 1 h. Coupling of Boc-His(5-Br,1-SEM)-OH (**5a**) and Boc-His(5-Br,3-SEM)-OH (**5b**) (2 equiv.) was mediated by COMU (2 equiv.), Oxyma (2 equiv.) and DIEA (4 equiv.) in DMF at room temperature overnight. Coupling of Boc-Tyr(3-$B(OH)_2$,Me)-OH (**14**) (3 equiv.) was carried out using DIPCDI (3 equiv.) and Oxyma (3 equiv.) in DMF at room temperature for 3 h. After each coupling step, the resulting resins were washed with DMF (6×1 min). The completion of the reactions was monitored by the Kaiser test [47]. The Boc group of the 5-bromohistidine residue was removed by treatment with a mixture of TMSOTf and 2,6-lutidine in CH_2Cl_2 (final concentrations: 2.5 M TMSOTf and 3.75 M 2,6-lutidine) at room temperature (10×30 min). The resulting resins were washed with CH_2Cl_2 (5×1 min), MeOH (3×5 min) and DMF (5×1 min) [46].

Upon completion of the peptide sequence, an aliquot of the resulting peptidyl resins was cleaved with TFA/H_2O/TIS (95:2.5:2.5) whilst being stirred for 3 h at room temperature. Following TFA evaporation and diethyl ether extraction, the corresponding crude peptide was dissolved in H_2O/CH_3CN (1:1), lyophilized, analysed by HPLC, and characterized by mass spectrometry.

*4.10.1. Boc-Tyr(3-$B(OH)_2$,Me)-Lys(Boc)-Lys(Boc)-Leu-His(5-Br,1-SEM)-Leu-Leu-Rink-MBHA (**13a**) and Boc-Tyr(3-$B(OH)_2$,Me)-Lys(Boc)-Lys(Boc)-Leu-His(5-Br,3-SEM)-Leu-Leu-Rink-MBHA (**13b**)*

Peptidyl resins **13** were prepared following the general method described above. Acidolytic cleavage of an aliquot of these peptidyl resins afforded H-Tyr(3-$B(OH)_2$,Me)-Lys-Lys-Leu-His(5-Br)-Leu-Leu-NH_2 (**15**) in 65% purity. t_R = 14.71 min (Conditions C). MS (ESI): *m/z* = 1049.5, 1051.5 $[M + H]^+$.

*4.10.2. Boc-Tyr(3-$B(OH)_2$,Me)-Lys(Boc)-Lys(Boc)-Leu-His(5-Br,1-SEM)-Rink-MBHA (**16a**) and Boc-Tyr(3-$B(OH)_2$,Me)-Lys(Boc)-Lys(Boc)-Leu-His(5-Br,3-SEM)-Rink-MBHA (**16b**)*

Peptidyl resins **16** were prepared following the general method described above. Acidolytic cleavage of an aliquot of these peptidyl resins afforded H-Tyr(3-$B(OH)_2$,Me)-Lys-Lys-Leu-His(5-Br)-NH_2 (**18**) in 59% purity. t_R = 13.53 min (Conditions B). MS (ESI): *m/z* = 412.1, 413.1 $[M + 2H]^{2+}$, 823.4, 825.4 $[M + H]^+$.

*4.10.3. Boc-Tyr(3-$B(OH)_2$,Me)-Leu-His(5-Br,1-SEM)-Rink-MBHA (**17a**) and Boc-Tyr(3-$B(OH)_2$,Me)-Leu-His(5-Br,3-SEM)-Rink-MBHA (**17b**)*

Peptidyl resins **17** were prepared following the general method described above. Acidolytic cleavage of an aliquot of these peptidyl resins afforded H-Tyr(3-$B(OH)_2$,Me)-Leu-His(5-Br)-NH_2 (**19**) in 52% purity. t_R = 14.30 min (Conditions B). MS (ESI): *m/z* = 567.1, 569.1$[M + H]^+$.

*4.10.4. Boc-Tyr(3-$B(OH)_2$,Me)-Ala-Gln(Tmob)-Gly-Gln(Tmob)-His(5-Br,1-SEM)-βAla-Rink-MBHA (**22a**) and Boc-Tyr(3-$B(OH)_2$,Me)-Ala-Gln(Tmob)-Gly-Gln(Tmob)-His(5-Br,3-SEM)-βAla-Rink-MBHA (**22b**)*

Peptidyl resins **22** were prepared following the same procedure described above. Acidolytic cleavage of an aliquot of these peptidyl resins afforded H-Tyr(3-$B(OH)_2$,Me)-Ala-Gln-Gly-Gln-His(5-Br)-βAla-NH_2 (**23**) in >99% purity. t_R = 5.15 min (Conditions A). MS (ESI): *m/z* = 909.1, 911.1 $[M + H]^+$, 931.0, 933.0 $[M + Na]^+$.

4.11. General method for the solid-phase intramolecular Suzuki-Miyaura arylation

A 15 mL reaction vessel containing a magnetic stir bar was charged with the corresponding linear peptidyl resins, $Pd_2(dba)_3$ (0.2 equiv.), SPhos (0.4 equiv.), and KF (4 equiv.). Thoroughly degassed DME/EtOH/H_2O (9:9:2, 0.25-0.51 mL) was then added under nitrogen. The reaction mixture was heated at 140 ºC under microwave irradiation for 30 min. After the reaction time, upon cooling, the solvent was removed and the resin was washed with DMF (6×1 min), EtOH (6×1 min), CH_2Cl_2 (6×1 min), and diethyl ether (3×1 min). The resulting biaryl cyclic peptidyl resins were cleaved with TFA/H_2O/TIS (95:2.5:2.5) whilst being stirred for 3 h at room temperature. Following TFA evaporation and diethyl ether extraction, the corresponding crude peptide was dissolved in H_2O/CH_3CN (1:1), lyophilized, analysed by HPLC and mass spectrometry. Biaryl cyclic peptides were purified by reverse-phase column chromatography, analysed by HPLC, and characterized by HRMS.

*4.11.1. Biaryl cyclic peptide **BPC782***

Starting from resins Boc-His(5-Br,1-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**7a**) and Boc-His(5-Br,3-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**7b**) (50 mg), Suzuki-Miyaura cyclization followed

by acidolytic cleavage gave biaryl cyclic peptide **BPC782** (t_R = 13.78 min, 35% purity) together with H-His-Lys-Lys-Leu-Tyr(3-OH,Me)-NH_2 (t_R = 14.35 min, 11% purity) and H-His-Lys-Lys-Leu-Tyr(Me)-NH_2 (t_R = 15.45 min, 12% purity) (Conditions B). Elution with H_2O/MeOH/TFA (90:10:0.2) gave **BPC782** in 68% purity (t_R = 5.26 min) (Conditions A) and in 6% yield. MS (ESI): *m/z* = 350.2 $[M + 2H]^{2+}$, 699.5 $[M + H]^+$. HRMS (ESI): calcd. for $C_{34}H_{56}N_{10}O_6$ $[M + 2H]^{2+}$ 350.2187, found 350.2192; calcd. for $C_{34}H_{55}N_{10}O_6$ $[M + H]^+$ 699.4301, found 699.4323.

*4.11.2. Biaryl cyclic peptide **BPC784***

Starting from resins Boc-His(5-Br,1-SEM)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**8a**) and Boc-His(5-Br,3-SEM)-Leu-Tyr(3-BPin,Me)-Rink-MBHA (**8b**) (73 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC784** (t_R = 12.65 min, 30% purity) together with H-His-Leu-Tyr(3-OH,Me)-NH_2 (t_R = 14.47 min, 11% purity), H-His(5-Br)-Leu-Tyr(3-OH,Me)-NH_2 (t_R = 14.89 min, 12% purity) and H-His-Leu-Tyr(Me)-NH_2 (t_R = 15.98 min, 7% purity) (Conditions B). Elution with H_2O/TFA (100:0.2) gave **BPC784** in 90% purity (t_R = 5.10 min) (Conditions A) and in 7% yield. MS (ESI): *m/z* = 443.2 $[M + H]^+$. HRMS (ESI): calcd. for $C_{22}H_{31}N_6O_4$ $[M + H]^+$ 443.2401, found 443.2394.

*4.11.3. Biaryl cyclic peptide **BPC786***

Starting from resins Boc-His(5-Br,1-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Leu-Leu-Rink-MBHA (**1a**) and Boc-His(5-Br,3-SEM)-Lys(Boc)-Lys(Boc)-Leu-Tyr(3-BPin,Me)-Leu-Leu-Rink-MBHA (**1b**) (50 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC786** (t_R = 15.79 min, 31% purity) together with H-His-Lys-Lys-Leu-Tyr(3-OH,Me)-Leu-Leu-NH_2 (t_R = 15.50 min, 11% purity) and H-His-Lys-Lys-Leu-Tyr(Me)-Leu-Leu-NH_2 (t_R = 18.35 min, 20% purity) (Conditions C). Elution with H_2O/MeOH/TFA (85:15:0.2) gave **BPC786** in 79% purity (t_R = 6.10 min) (Conditions A) and in 5% yield. MS (ESI): *m/z* = 925.6 $[M + H]^+$, 947.6 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{46}H_{77}N_{12}O_8$ $[M + H]^+$ 925.5982, found 925.5940; $C_{46}H_{76}N_{12}O_8Na$ $[M + Na]^+$ 947.5801, found 947.5763.

*4.11.4. Biaryl cyclic peptide **BPC788***

Starting from resins Boc-Tyr(3-B$(OH)_2$,Me)-Lys(Boc)-Lys(Boc)-Leu-His(5-Br,1-SEM)-Rink-MBHA (**16a**) and Boc-Tyr(3-B$(OH)_2$,Me)-Lys(Boc)-Lys(Boc)-Leu-His(5-Br,3-SEM)-Rink-MBHA (**16b**) (68 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC788** (t_R = 15.46 min, 32% purity) together with H-Tyr(3-OH,Me)-Lys-Lys-Leu-His-NH_2 (t_R = 15.26 min, 5% purity) and H-Tyr(Me)-Lys-Lys-Leu-His-NH_2 (t_R = 16.16 min, 15% purity) (Conditions B). Elution with H_2O/TFA (100:0.2) gave **BPC788** in 87% purity (t_R = 5.19 min) (Conditions A) and in 8% yield. MS (ESI): *m/z* = 350.2 $[M + 2H]^{2+}$, 699.5 $[M + H]^+$, 721.4 $[M + Na]^+$, 737.4 $[M + K]^+$. HRMS (ESI): calcd. for $C_{34}H_{55}N_{10}O_6$ $[M + H]^+$ 699.4301, found 699.4305; calcd. for $C_{34}H_{54}N_{10}O_6Na$ $[M + Na]^+$ 721.4120, found 721.4128.

*4.11.5. Biaryl cyclic peptide **BPC790***

Starting from resins Boc-Tyr(3-B$(OH)_2$,Me)-Leu-His(5-Br,1-SEM)-Rink-MBHA (**17a**) and Boc-Tyr(3-B$(OH)_2$,Me)-Leu-His(5-Br,3-SEM)-Rink-MBHA (**17b**) (50 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC790** (t_R = 12.87 min, 49% purity) (Conditions B). Elution with H_2O/CH_3CN (88:12) gave **BPC790** in 96% purity (t_R = 5.10 min) (Conditions A) and in 8% yield. MS (ESI): *m/z* = 443.1 $[M + H]^+$. HRMS (ESI): calcd. for $C_{22}H_{31}N_6O_4$ $[M + H]^+$ 443.2401, found 443.2382.

*4.11.6. Biaryl cyclic peptide **BPC792***

Starting from resins Boc-Tyr(3-B$(OH)_2$,Me)-Lys(Boc)-Lys(Boc)-Leu-His(5-Br,1-SEM)-Leu-Leu-Rink-MBHA (**13a**) and Boc-Tyr(3-B$(OH)_2$,Me)-Lys(Boc)-Lys(Boc)-Leu-His(5-Br,3-SEM)-Leu-Leu-Rink-MBHA (**13b**) (50 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC792** (t_R = 14.22 min, 55% purity) together with H-Tyr(Me)-Lys-Lys-Leu-His-Leu-Leu-NH_2 (t_R = 15.59 min, 22% purity) (Conditions C). Elution with H_2O/MeOH/TFA (90:10:0.2) gave **BPC792** in 92% purity (t_R = 5.96 min) (Conditions A) and in 9% yield. MS (ESI): *m/z* = 463.2 $[M + 2H]^{2+}$, 925.6 $[M + H]^+$, 947.6 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{46}H_{79}N_{12}O_8$ $[M + 3H]^{3+}$ 309.2042, found 309.2056; calcd. for $C_{46}H_{78}N_{12}O_8$ $[M + 2H]^{2+}$ 463.3027, found 463.3045; calcd. for $C_{46}H_{77}N_{12}O_8$ $[M + H]^+$ 925.5982, found 925.6021.

*4.11.7. Biaryl cyclic peptide **20***

Starting from resins Boc-Tyr(3-B$(OH)_2$,Me)-Ala-Gln(Tmob)-Gly-Gln(Tmob)-His(5-Br,1-SEM)-βAla-Rink-MBHA (**22a**) and Boc-Tyr(3-B$(OH)_2$,Me)-Ala-Gln(Tmob)-Gly-Gln(Tmob)-His(5-Br,3-SEM)-βAla-Rink-MBHA (**22b**) (50 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave the biaryl cyclic peptide **20** (t_R = 11.49 and 11.78 min, 64% purity) (Conditions B). Elution with H_2O/TFA (100:0.2) gave **20** in 87% purity (t_R = 2.43 min) (Conditions E) and in 6% yield. MS (ESI): *m/z* = 785.5 $[M + H]^+$. HRMS (ESI): calcd. for $C_{34}H_{49}N_{12}O_{10}$ $[M + H]^+$ 785.3689, found 785.3715; calcd. for $C_{34}H_{48}N_{12}O_{10}Na$ $[M + Na]^+$ 807.3509, found 807.3533.

*4.11.8. Biaryl cyclic peptide **21***

Starting from resins Boc-His(5-Br,1-SEM)-βAla-Thr(^{t}Bu)-Tyr(3-BPin,Me)-Ala-Gln(Tmob)-Rink-MBHA (**24a**) and Boc-His(5-Br,3-SEM)-βAla-Thr(^{t}Bu)-Tyr(3-BPin,Me)-Ala-Gln(Tmob)-Rink-MBHA (**24b**) (50 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave the biaryl cyclic peptide **21** (t_R = 4.84 min, 12% purity) (Conditions A). Elution with H_2O/TFA (100:0.2) gave **21** in 84% purity (t_R = 5.01 min) (Conditions A) and in 7% yield. MS (ESI): *m/z* = 701.3 $[M + H]^+$, 723.3 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{31}H_{45}N_{10}O_9$ $[M + H]^+$ 701.3365, found 701.3369.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/xxxx.

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