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

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ABSTRACT

The feasibility of the solid-phase intramolecular 4(5)-arylation of a histidine residue to obtain biaryl cyclic peptides bearing a His-Phe linkage was established. The synthetic strategy involved the preparation of a linear peptidyl resin incorporating a 5-bromohistidine and a 4-boronophenylalanine, and its cyclization through the formation of the biaryl bond between the imidazole of histidine and the phenyl group of phenylalanine via a microwave-assisted Suzuki-Miyaura cross-coupling. This methodology was applied to the preparation of biaryl cyclic peptides consisting of a 3- or 5-residue ring, incorporating the His residue at the N- or the C-terminus and bearing a Leu-Leu spacer or a $-NH_2$ group at the C-terminus. In the case of the 3-residue ring peptides, the position of the His did not influence the macrocyclization. In contrast, to obtain the 5-member ring biaryl cyclic peptides, the His residue should be located at the N-terminus. It was also observed that the Leu-Leu spacer is crucial for the intramolecular arylation. These results suggest that this approach could be useful for the preparation of a diversity of synthetic and natural biaryl cyclic peptides bearing a His-Phe linkage.

KEYWORDS

Cross-coupling, cyclization, macrocycles, microwave chemistry, Suzuki-Miyaura

INTRODUCTION

Unsymmetrical biaryl systems are present in many naturally occurring cyclic peptides that show a variety of important biological activities including antimicrobial or cytotoxic (Feliu and Planas 2005). In recent years, much attention has been turned to the incorporation of biaryl amino acids into biologically active peptides (Haug et al. 2007; Le Quement et al. 2011; Ng-Choi et al. 2014; Mendive-Tapia et al. 2015; García-Pindado et al. 2017). It has been reported that the presence of the biaryl motif restricts the conformational flexibility of peptides, enhances their proteolytic stability, increases their selectivity, and improves their bioavailability (Willemse et al. 2017). In particular, 5-arylhistidines are present in cytotoxic and antifungal marine peptides and it has been observed that the imidazole ring is crucial for their activity (Bewley et al. 1996). Moreover, we have shown that the incorporation of a 5-arylhistidine in a linear antimicrobial peptide renders sequences with antibacterial and antifungal activity, and low hemolysis. This low cytotoxicity has been attributed to the presence of the imidazole ring of histidine (Ng-Choi et al. 2012).

Despite the significance of biaryl motifs in drug discovery, the preparation of biaryl cyclic peptides still remains an important synthetic challenge. In fact, a scarce number of research groups has addressed the solid-phase synthesis of cyclic peptides incorporating a Phe-Phe, Phe-Tyr, Tyr-Tyr, Trp-Phe or a Trp-Tyr linkage (Afonso et al. 2011, 2012; Meyer et al. 2012; Mendive-Tapia et al. 2015; García-Pindado et al. 2017; Ng-Choi et al. 2019a). In this context, we have recently developed a solid-phase strategy for the synthesis of biaryl cyclic peptides containing a His-Tyr bond (Ng-Choi et al. 2019b). A linear peptidyl resin incorporating a 5-bromohistidine and a 3-boronotyrosine was used as precursor and the cyclization was performed through a microwave-assisted Suzuki-Miyaura cross-coupling. This approach provided access to 3- or 5-member biaryl cyclic peptides with a His at the N- or at the C-terminus, and was extended to the preparation of analogues of the northern and southern hemispheres of aciculitins. To the best of our knowledge, this is the first report on the solid-phase cyclization of peptides

by arylation of a His residue. In fact, it has been described that the derivatization of the position 4(5) of the imidazole ring of a His is troublesome.

Herein, we were interested in studying the feasibility of the above strategy to the solid-phase synthesis of biaryl cyclic peptides incorporating a His-Phe linkage, which to the best of our knowledge has not yet been reported. In particular, we planned to obtain such biaryl cyclic peptides by formation of a biaryl bond between the position 4(5) of the imidazole ring of His and the position 4 of the benzene ring of Phe. Accordingly, the synthesis of the biaryl cyclic peptides depicted in Figures 1 and 2, consisting of a 3- or 5-residue ring and bearing the His at the N- or C-terminus was investigated.

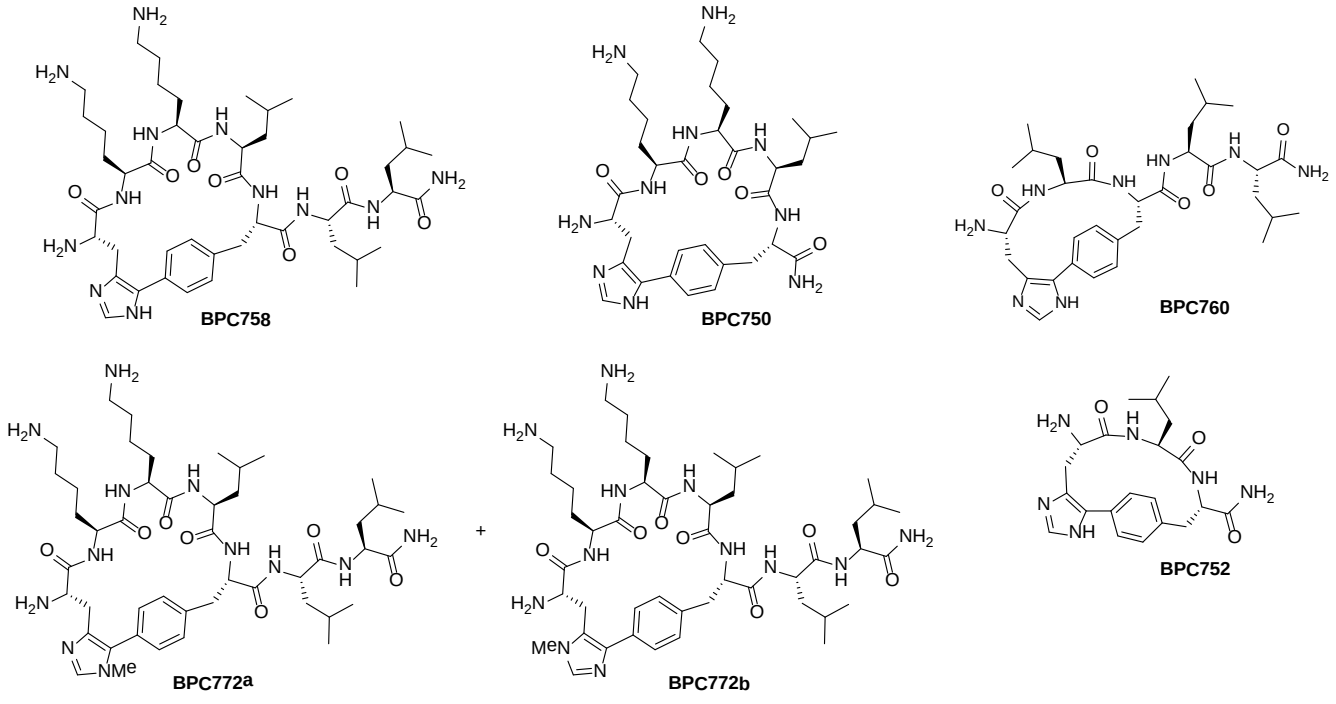


Figure 1. Structure of the biaryl cyclic peptides incorporating a His at the N-terminus

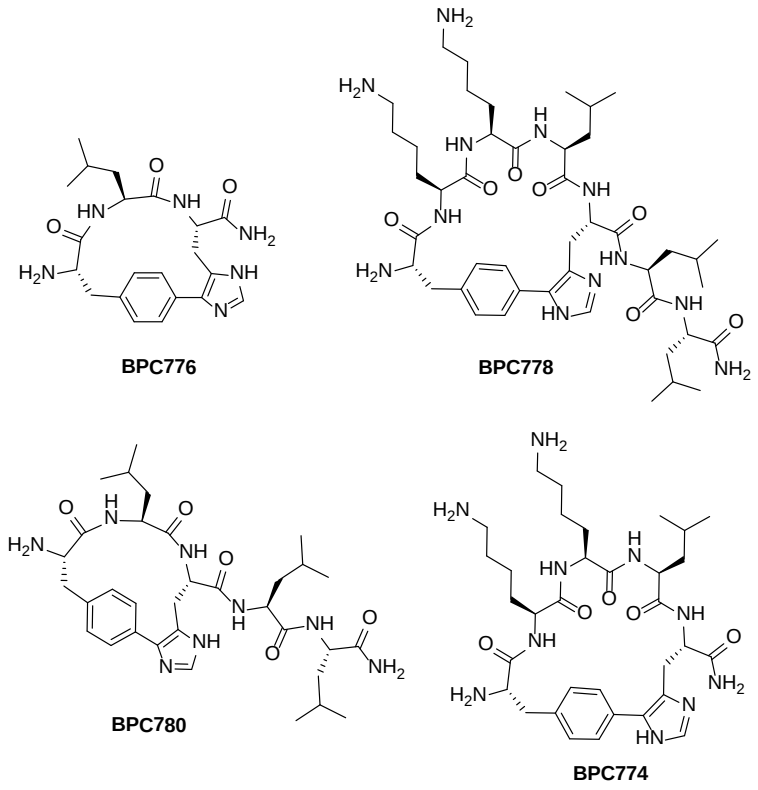


Figure 2. Structure of the biaryl cyclic peptides incorporating a His at the C-terminus

MATERIALS AND METHODS

General Methods: Manual peptide synthesis was performed in polypropylene syringes (2 or 5 mL) 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 a 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 a 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 a 2–25% B over 3 min followed by a 25-35% B over 30 min and a 35-100% B over 1 min at a flow rate of 1 mL/min. Peptides were also analyzed with an Agilent Technologies LC 1200 series liquid chromatography instrument at 220 nm. Solvent A was 0.1% aq TFA and solvent B was 0.1% TFA in CH_3CN. Conditions D: Analysis was carried out with a Kromasil 100 C_{18} (4.6 mm × 250 mm, 5 μm) column with a 2–15% B over 1 min followed by a 15% B over 1 min, 15-60% B over 26 min, 60% over 1 min, and a 60-100% B over 1 min at a flow rate of 1 mL/min

ESI-MS analyses were performed 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 μLmin^{-1}) was delivered by a 1200 Series HPLC pump (Agilent). Nitrogen was employed as both the drying and nebulising gas. HRMS were recorded on a Bruker MicroTof-QIITM instrument using ESI ionization source at Serveis Tècnics 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.

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.

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.

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

Synthesis of amino acid derivatives

Methyl 5-bromo-N(α)-tert-butoxycarbonyl-N(τ)-methyl-L-histidinate and methyl 5-bromo-N(α)-tert-butoxycarbonyl-N(π)-methyl-L-histidinate

DBU (70 μL, 0.43 mmol, 1.5 equiv.) was added to a solution of Boc-His(5-Br)-OMe (100 mg, 0.29 mmol, 1 equiv.) (Cerezo et al. 2008) in anhydrous DMF (730 μL) at 0 ºC. The reaction mixture was stirred at this temperature under nitrogen for 1.5 h. After this time, iodomethane was added (27 μL, 0.43 mmol, 1.5 equiv.), the mixture was stirred for 4 h and allowed to warm to room temperature. Then, the reaction mixture was poured into cold water (15 mL) and the product was extracted with EtOAc/toluene

(1:1, 3×5 mL). The organic layers were combined, washed with brine (10 mL), and dried over anhydrous magnesium sulfate. Removal of the solvent, followed by vacuum drying yielded Boc-His(5-Br,1-Me)-OMe and Boc-His(5-Br,3-Me)-OMe as a yellow oil (3.21 g, 61% yield). t_R = 6.39 min (>99% purity) (Conditions A). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 [s, 0.75 H, CH-2$_{imid}$], 7.33 [s, 0.25 H, CH-2$_{imid}$], 5.90-5.88 [br. s, 0.75 H, CONH], 5.20-5.18 [br. s, 0.25 H, CONH], 4.58-4.54 [m, 0.75 H, CH-α], 4.49-4.43 [m, 0.25 H, CH-α], 3.76 [s, 0.75 H, NCH_3], 3.72 [s, 2.25 H, NCH_3], 3.66 [s, 0.75 H, OCH_3], 3.56 [s, 2.25 H, OCH_3], 3.11-2.95 [m, 2 H, CH_2-β], 1.43 [s, 6.75 H, $(CH_3)_3C$], 1.39 [s, 2.25 H, $(CH_3)_3C$] ppm. MS (ESI): *m/z* = 362.1, 364.1[M + H]$^+$.

5-Bromo-N(α)-tert-butoxycarbonyl-N(τ)-methyl-L-histidine and 5-bromo-N(α)-tert-butoxycarbonyl-N(π)-methyl-L-histidine

An aqueous solution of LiOH (14 mg, 0.33 mmol, 3 equiv.) in H_2O (210 μL) was added to a solution of Boc-His(5-Br,1-Me)-OMe and Boc-His(5-Br,3-Me)-OMe (40 mg, 0.11 mmol, 1 equiv.) in MeOH/THF (1:1, 420 μL). The reaction mixture was stirred at room temperature for 2 h. After this time, the organic solvents were evaporated under reduced pressure and water (500 μL) was added to the resulting residue. The resulting solution was adjusted to pH 5 by addition of 1 N HCl followed by extraction with EtOAc (3×5 mL). The organic layers were combined, washed with brine (5 mL), and dried over anhydrous magnesium sulfate. Removal of the solvent afforded Boc-His(5-Br,1-Me)-OH and Boc-His(5-Br,3-Me)-OH as a white solid (37 mg, 95% yield). t_R = 5.92 min (24% purity) and 6.03 min (76% purity) (Conditions A). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 7.77 [s, 0.75 H, CH-2$_{imid}$], 7.52 [s, 0.25 H, CH-2$_{imid}$], 7.03-7.02 [bs, 0.25 H, CONH], 6.84-6.82 [br. s, 0.75 H, CONH], 4.23-4.18 [m, 0.75 H, CH-α], 4.10-4.04 [m, 0.25 H, CH-α], 3.58 [s, 0.75 H, NCH_3], 3.54 [s, 2.25 H, NCH_3], 2.82-2.80 [m, 2 H, CH_2-β], 1.36 [s, 6.75 H, $(CH_3)_3C$], 1.32 [s, 2.25 H, $(CH_3)_3C$] ppm. ^{13}C NMR (300 MHz, [D_6]DMSO): δ =

173.15, 172.71 [CO_2H], 155.07 [CONH], 138.31, 138.24 [CH-2_{imid}], 135.81, 125.57 [C-4_{imid}], 114.27, 101.76 [C-5_{imid}], 78.14, 78.10 [$C(CH_3)_3$], 52.89, 52.60 [CH-α], 32.57, 32.12 [NCH_3], 28.68, 28.14 [$(CH_3)_3C$, CH_2-β] ppm. MS (ESI): *m/z* = 248.0, 250.0 $[M - Boc + H]^+$, 348.0, 350.0 $[M + H]^+$.

Solid-Phase Synthesis of the Linear Peptidyl Resins 1 and 6-9 Containing a 5-Bromohistidine Residue at the N-terminus

General Method for the Synthesis of 4-Iodophenylalanylpeptidyl Resins

These 4-iodophenylalanylpeptidyl 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). Couplings of the corresponding amino acids Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH or Fmoc-Phe-OH were performed by using DIPCDI (4 equiv.) and Oxyma (4 equiv.) in DMF at room temperature for 1 h, except for coupling of Fmoc-Phe(4-I)-OH (2 equiv.) (Ng-Choi et al. 2019a) which was carried out by 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 (1970). Fmoc group removal was achieved with a mixture of piperidine/DMF (3:7, 2 + 10 min). 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 the 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 (1970). An aliquot of the resulting peptidyl resin was cleaved with TFA/H_2O/TIS (95:2.5:2.5) for 2 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.

Tr-Lys(Boc)-Lys(Boc)-Leu-Phe(4-I)-Leu-Leu-Rink-MBHA (2)

The iodohexapeptidyl resin **2** was prepared following the general method for the synthesis of 4-iodophenylalanylpeptidyl resins. Acidolytic cleavage of an aliquot of this peptidyl resin afforded H-Lys-Lys-Leu-Phe(4-I)-Leu-Leu-NH_2 in 94% purity. t_R = 18.81 min (Conditions B). MS (ESI): *m/z* = 443.6 $[M + 2H]^{2+}$, 886.4 $[M + H]^+$, 908.4 $[M + Na]^+$.

Tr-Lys(Boc)-Lys(Boc)-Leu-Phe(4-I)-Rink-MBHA

This iodotetrapeptidyl resin was prepared following the general method for the synthesis of 4-iodophenylalanylpeptidyl resins. Acidolytic cleavage of an aliquot of this peptidyl resin afforded H-Lys-Lys-Leu-Phe(4-I)-NH_2 in 70% purity. t_R = 16.89 min (Conditions B). MS (ESI): *m/z* = 330.6 $[M + 2H]^{2+}$, 660.2 $[M + H]^+$, 682.2 $[M + Na]^+$.

Tr-Leu-Phe(4-I)-Leu-Leu-Rink-MBHA

This iodotetrapeptidyl resin was prepared following the general method for the synthesis of 4-iodophenylalanylpeptidyl resins. Acidolytic cleavage of an aliquot of this peptidyl resin afforded H-Leu-Phe(4-I)-Leu-Leu-NH_2 in 92% purity. t_R = 21.28 min (Conditions B). MS (ESI): *m/z* = 630.2 $[M + H]^+$.

Tr-Leu-Phe(4-I)-Rink-MBHA

This iododipeptidyl resin was prepared following the general method for the synthesis of 4-iodophenylalanylpeptidyl resins. Acidolytic cleavage of an aliquot of this peptidyl resin afforded H-Leu-Phe(4-I)-NH_2 in 57% purity. t_R = 18.34 min (Conditions B). MS (ESI): *m/z* = 404.0 $[M + H]^+$.

General Method for Solid-Phase Miyaura Borylation

A 2-10 mL round-bottomed flask was charged with the corresponding iodophenylalanylpeptidyl resin, bis(pinacolato)diboron (B_2Pin_2) (4 equiv.), $PdCl_2$(dppf) (0.18 equiv.), and 1,1'-bis(diphenylphosphanyl)ferrocene (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 24 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) for 2 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.

Tr-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (3)

The boronohexapeptidyl resin **3** was prepared starting from Tr-Lys(Boc)-Lys(Boc)-Leu-Phe(4-I)-Leu-Leu-Rink-MBHA (**2**) (220 mg) following the general method for solid-phase Miyaura borylation. Acidolytic cleavage of an aliquot of this peptidyl resin gave H-Lys-Lys-Leu-Phe(4-B$(OH)_2$)-Leu-Leu-NH_2 (82% purity), resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 14.20 min (Conditions C). MS (ESI): *m/z* = 402.8 $[M + 2H]^{2+}$, 804.6 $[M + H]^+$.

Tr-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Rink-MBHA

This boronotetrapeptidyl resin was prepared starting from Tr-Lys(Boc)-Lys(Boc)-Leu-Phe(4-I)-Rink-MBHA (157 mg) following the general method for solid-phase Miyaura borylation. Acidolytic cleavage of an aliquot of this peptidyl resin gave H-Lys-Lys-Leu-Phe(4-B$(OH)_2$)-NH_2 (77% purity), resulting

from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 13.04 min (Conditions B). MS (ESI): *m/z* = 289.7 $[M + 2H]^{2+}$, 578.3 $[M + H]^+$.

Tr-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA

This boronotetrapeptidyl resin was prepared starting from Tr-Leu-Phe(4-I)-Leu-Leu-Rink-MBHA (280 mg) following the general method for solid-phase Miyaura borylation. Acidolytic cleavage of an aliquot of this peptidyl resin gave H-Leu-Phe(4-$B(OH)_2$)-Leu-Leu-NH_2 (81% purity), resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 17.90 min (Conditions B). MS (ESI): *m/z* = 548.3 $[M + H]^+$.

Tr-Leu-Phe(4-BPin)-Rink-MBHA

This boronodipeptidyl resin was prepared starting from Tr-Leu-Phe(4-I)-Rink-MBHA (102 mg) following the general method for solid-phase Miyaura borylation. Acidolytic cleavage of an aliquot of this peptidyl resin gave H-Leu-Phe(4-$B(OH)_2$)-NH_2 (35% purity), resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 13.12 min (Conditions B). MS (ESI): *m/z* = 322.1 $[M + H]^+$.

General Method for the Solid-Phase Synthesis of the Linear Peptidyl Resins 1 and 6-9

The corresponding boronopeptidyl resin was 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 and Boc-His(5-Br,3-SEM)-OH (3 equiv.) (Cerezo et al. 2008) or Boc-His(5-Br,1-Me)-OH and Boc-His(5-Br,3-Me)-OH (3 equiv.) was carried out by using DIPCDI (3 equiv.) and Oxyma (3 equiv.) in DMF at room temperature for 3 h. The corresponding resins were 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 (1970). 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 or for 2 h at room temperature without stirring. In both cases, following TFA evaporation and diethyl ether extraction, the corresponding crude peptide was dissolved in H_2O/CH_3CN, lyophilized, analysed by HPLC, and characterized by mass spectrometry.

Boc-His(5-Br,1-SEM)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (1a) and Boc-His(5-Br,3-SEM)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (1b)

Resins **1** were synthesized starting from Tr-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (**3**) (250 mg) following the general method described above using Boc-His(5-Br,1-SEM)-OH and Boc-His(5-Br,3-SEM)-OH (Cerezo et al. 2008). Acidolytic cleavage of an aliquot of these peptidyl resins was performed by treatment with TFA/H_2O/TIS (95:2.5:2.5) under stirring for 3 h at room temperature. H-His(5-Br)-Lys-Lys-Leu-Phe(4-$B(OH)_2$)-Leu-Leu-NH_2 was obtained in 73% purity, resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 14.47 min (Conditions C). MS (ESI): *m/z* = 1019.5, 1021.5 $[M + H]^+$.

Boc-His(5-Br,1-Me)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (6a) and Boc-His(5-Br,3-Me)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (6b)

Resins **6** were synthesized starting from Tr-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (50 mg) following the general method described above using Boc-His(5-Br,1-Me)-OH and Boc-His(5-Br,3-Me)-OH. Acidolytic cleavage of an aliquot of these peptidyl resins was performed by treatment with TFA/H_2O/TIS (95:2.5:2.5) for 2 h at room temperature without stirring. The regioisomeric peptides H-His(5-Br,1-Me)-Lys-Lys-Leu-Phe(4-$B(OH)_2$)-Leu-Leu-NH_2 and H-His(5-Br,3-Me)-Lys-Lys-Leu-Phe(4-$B(OH)_2$)-Leu-Leu-NH_2 were obtained in 88% purity, resulting from the hydrolysis of the

pinacol boronic ester during HPLC analysis. t_R = 19.78 and 20.02 min (Conditions B). MS (ESI): *m/z* = 517.2, 518.2 $[M + 2H]^{2+}$, 1033.5, 1035.5 $[M + H]^+$.

Boc-His(5-Br,1-SEM)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Rink-MBHA (7a) and Boc-His(5-Br,3-SEM)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Rink-MBHA (7b)

Resins **7** were synthesized starting from Tr-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Rink-MBHA (160 mg) following the general method described above using Boc-His(5-Br,1-SEM)-OH and Boc-His(5-Br,3-SEM)-OH (Cerezo et al. 2008). Acidolytic cleavage of an aliquot of the resulting peptidyl resins was performed by treatment with TFA/H_2O/TIS (95:2.5:2.5) under stirring for 3 h at room temperature. H-His(5-Br)-Lys-Lys-Leu-Phe(4-B$(OH)_2$)-NH_2 was obtained in 60% purity, resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 13.50 min (Conditions B). MS (ESI): *m/z* = 793.3, 795.3 $[M + H]^+$.

Boc-His(5-Br,1-SEM)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (8a) and Boc-His(5-Br,3-SEM)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (8b)

Resins **8** were synthesized starting from Tr-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (70 mg) following the general method described above using Boc-His(5-Br,1-SEM)-OH and Boc-His(5-Br,3-SEM)-OH (Cerezo et al. 2008). Acidolytic cleavage of an aliquot of these peptidyl resins was performed by treatment with TFA/H_2O/TIS (95:2.5:2.5) under stirring for 3 h at room temperature. H-His(5-Br)-Leu-Phe(4-B$(OH)_2$)-Leu-Leu-NH_2 was obtained in 79% purity, resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 17.84 min (Conditions B). MS (ESI): *m/z* =763.2, 765.2 $[M + H]^+$.

Boc-His(5-Br,1-SEM)-Leu-Phe(4-BPin)-Rink-MBHA (9a) and Boc-His(5-Br,3-SEM)-Leu-Phe(4-BPin)-Rink-MBHA (9b)

Resins **9** were synthesized starting from Tr-Leu-Phe(4-BPin)-Rink-MBHA (60 mg) following the general method described above using Boc-His(5-Br,1-SEM)-OH and Boc-His(5-Br,3-SEM)-OH (Cerezo et al. 2008). Acidolytic cleavage of an aliquot of these peptidyl resins was performed by treatment with TFA/H_2O/TIS (95:2.5:2.5) under stirring for 3 h at room temperature. H-His(5-Br)-Leu-Phe(4-$B(OH)_2$)-NH_2 was obtained in 64% purity, resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 13.76 min (Conditions B). MS (ESI): *m/z* = 537.1, 539.1 $[M + H]^+$.

General Method for the Solid-Phase Synthesis of the Linear Peptidyl Resins Containing a 5-Bromohistidine Residue at the C-Terminus

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). Couplings of the corresponding Fmoc-amino acids were performed by using DIPCDI (4 equiv.) and Oxyma (4 equiv.) in DMF at room temperature for 1 h, while couplings of the previously synthesized Boc-amino acids, Boc-His(5-Br,1-SEM)-OH and Boc-His(5-Br,3-SEM)-OH (2 equiv.) (Cerezo et al. 2008) or Boc-Phe(4-BPin)-OH (2 equiv) (Ng-Choi et al. 2019a), were carried out by using COMU (2 equiv.), Oxyma (2 equiv.) and DIEA (4 equiv.) in DMF at room temperature overnight. 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 (1970). 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). 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), and the resulting resins were washed with CH_2Cl_2 (5×1 min), MeOH (3×5 min) and DMF (5×1 min) (Zhang et al. 1998). 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, lyophilized, analysed by HPLC, and characterized by mass spectrometry.

Boc-Phe(4-BPin)-Leu-His(5-Br,1-SEM)-Leu-Leu-Rink-MBHA (10a) and Boc-Phe(4-BPin)-Leu-His(5-Br,3-SEM)-Leu-Leu-Rink-MBHA (10b)

Resins **10** were prepared starting from MBHA resin (0.4 mmol/g) following the general method described above. Acidolytic cleavage of an aliquot of these peptidyl resins afforded H- Phe(4-$B(OH)_2$)-Leu-His(5-Br)-Leu-Leu-NH_2 (69% purity), resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 17.68 min (Conditions B). MS (ESI): *m/z* = 763.3, 765.3 $[M + H]^+$, 785.3, 787.3 $[M + Na]^+$.

Boc-Phe(4-BPin)-Leu-His(5-Br,1-SEM)-Rink-MBHA and Boc-Phe(4-BPin)-Leu-His(5-Br,3-SEM)-Rink-MBHA

These resins were prepared starting from MBHA resin (0.4 mmol/g) following the general method described above. Acidolytic cleavage of an aliquot of these peptidyl resins afforded H-Phe(4-$B(OH)_2$)-Leu-His(5-Br)-NH_2 (60% purity), resulting from the hydrolysis of the pinacol boronic ester during HPLC analysis. t_R = 13.30 min (Conditions B). MS (ESI): *m/z* = 537.1, 539.1 $[M + H]^+$.

Solid-phase synthesis of the biaryl cyclic peptides

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 incorporating the borono and bromo functionalities, $Pd_2(dba)_3$ (0.2 equiv.), P(*o*-tolyl)$_3$ (0.4 equiv.), and KF (4 equiv.). Thoroughly degassed DME/EtOH/H_2O (9:9:2, 0.16-0.40 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 resins were 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.

Biaryl Cyclic Peptide BPC750

Starting from resins Boc-His(5-Br,1-SEM)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Rink-MBHA (**7a**) and Boc-His(5-Br,3-SEM)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Rink-MBHA (**7b**) (55 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC750** (t_R = 7.41 min, 14% purity) together with H-His-Lys-Lys-Leu-Phe(4-B(OH)$_2$)-NH_2 (t_R = 8.45 min, 25% purity), H-His-Lys-Lys-Leu-Tyr-NH_2 (t_R = 8.91 min, 37% purity) and H-His-Lys-Lys-Leu-Phe-NH_2 (t_R = 11.69 min, 7% purity) (Conditions D). MS (ESI): *m/z* = 669.4 $[M + H]^+$.

Biaryl Cyclic Peptide BPC752

Starting from resins Boc-His(5-Br,1-SEM)-Leu-Phe(4-BPin)-Rink-MBHA (**9a**) and Boc-His(5-Br,3-SEM)-Leu-Phe(4-BPin)-Rink-MBHA (**9b**) (92 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC752** (t_R = 13.81 min, 13% purity) (Conditions B). Elution with H_2O/TFA (100:0.2) yielded **BPC752** in 91% purity (t_R = 4.50 min) (Conditions A). MS (ESI): *m/z* = 413.1 $[M + H]^+$. HRMS (ESI): calcd. for $C_{21}H_{29}N_6O_3$ $[M + H]^+$ 413.2296, found 413.2291.

Biaryl Cyclic Peptide BPC758

Starting from resins Boc-His(5-Br,1-SEM)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (**1a**) and Boc-His(5-Br,3-SEM)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (**1b**) (50 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC758** (t_R = 14.53 min, 44% purity) together with H-His-Lys-Lys-Leu-Tyr-Leu-Leu-NH_2 (**5**) (t_R = 15.24 min, 9% purity) and H-His-Lys-Lys-Leu-Phe-Leu-Leu-NH_2 (**4**) (t_R = 18.61 min, 17% purity) (Conditions C). Elution with H_2O/MeOH/TFA (90:10:0.2) yielded **BPC758** in 93% purity (t_R = 6.02 min) (Conditions A). MS (ESI): *m/z* = 895.6 $[M + H]^+$. HRMS (ESI): calcd. for $C_{45}H_{76}N_{12}O_7$ $[M + 2H]^{2+}$ 448.2974, found 448.2957; calcd. for $C_{45}H_{75}N_{12}O_7$ $[M + H]^+$ 895.5876, found 895.5850.

Biaryl Cyclic Peptide BPC760

Starting from resins Boc-His(5-Br,1-SEM)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (**8a**) and Boc-His(5-Br,3-SEM)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (**8b**) (60 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC760** (t_R = 17.45 min, 33% purity) together with H-His-Leu-Tyr-Leu-Leu-NH_2 (t_R = 17.81 min, 14% purity) and H-His-Leu-Phe-Leu-Leu-NH_2 (t_R = 18.92 min, 18% purity) (Conditions B). Elution with H_2O/MeOH/TFA (90:10:0.2) yielded **BPC760** in

>99% purity (t_R = 6.20 min) (Conditions A). MS (ESI): *m/z* = 639.4 $[M + H]^+$. HRMS (ESI): calcd. for $[M + H]^+$ $C_{33}H_{51}N_8O_5$ 639.3977, found 639.3956.

Biaryl Cyclic Peptide BPC772

Starting from resins Boc-His(5-Br,1-Me)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (**6a**) and Boc-His(5-Br,3-Me)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (**6b**) (50 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptides **BPC772a** and **BPC772b** (t_R = 19.61 and 19.68 min, 32% purity) together with H-His(Me)-Lys-Lys-Leu-Tyr-Leu-Leu-NH_2 (t_R = 19.93 min, 10% purity) and H-His(Me)-Lys-Lys-Leu-Phe-Leu-Leu-NH_2 (t_R = 20.54 min, 13% purity) (Conditions B). Elution with H_2O/MeOH/TFA (95:5:0.2) yielded **BPC772a** and **BPC772b** in 91% purity (t_R = 6.01 min) (Conditions A). MS (ESI): *m/z* = 909.6 $[M + H]^+$, 931.6 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{46}H_{78}N_{12}O_7$ $[M + 2H]^{2+}$ 455.3053, found 455.3046; calcd. for $C_{46}H_{80}N_{12}O_8$ $[M + H_2O + 2H]^{2+}$ 464.3106, found 464.3101; calcd. for $C_{47}H_{80}N_{12}O_9$ $[M + HCO_2H + 2H]^{2+}$ 478.3080, found 478.3162.

Biaryl Cyclic Peptide BPC776

Starting from resins Boc-Phe(4-BPin)-Leu-His(5-Br,1-SEM)-Rink-MBHA and Boc-Phe(4-BPin)-Leu-His(5-Br,3-SEM)-Rink-MBHA (50 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC776** (t_R = 7.42 min, 10% purity) together with H-Phe(4-$B(OH)_2$)-Leu-His-NH_2 (t_R = 7.95 min, 9% purity), H-Tyr-Leu-His-NH_2 (t_R = 8.23 min, 4% purity) and H-Phe-Leu-His-NH_2 (t_R = 9.98 min, 4% purity) (Conditions D). MS (ESI): *m/z* = 413.2 $[M + H]^+$.

Biaryl Cyclic Peptide BPC780

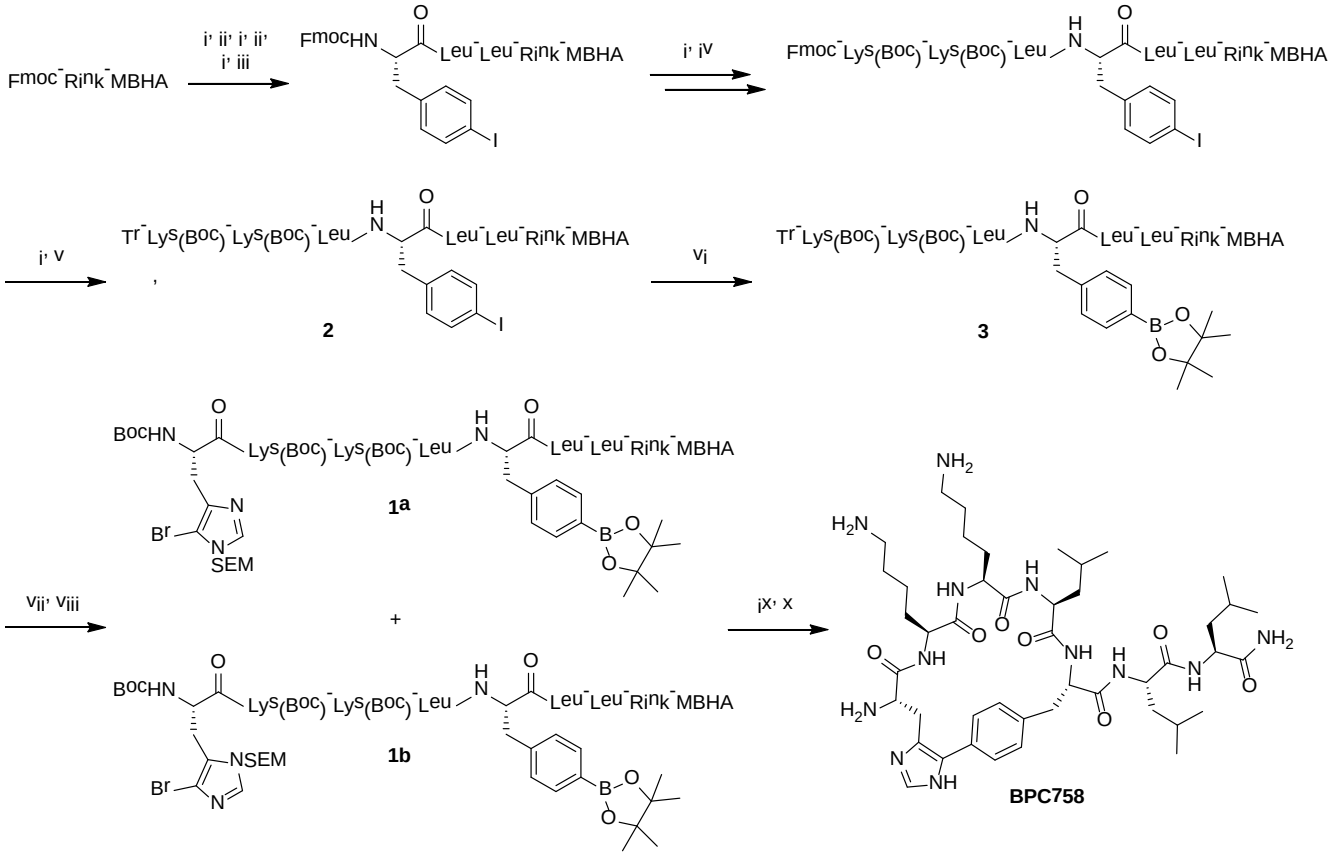
Starting from resins Boc-Phe(4-BPin)-Leu-His(5-Br,1-SEM)-Leu-Leu-Rink-MBHA (**10a**) and Boc-Phe(4-BPin)-Leu-His(5-Br,3-SEM)-Leu-Leu-Rink-MBHA (**10b**) (70 mg), Suzuki-Miyaura cyclization followed by acidolytic cleavage gave biaryl cyclic peptide **BPC780** (t_R = 17.62 min, 30% purity) (Conditions B). Elution with H_2O/CH_3CN/TFA (70:30:0.1) yielded pure **BPC780** in >99% purity (t_R = 6.43 min) (Conditions A). MS (ESI): *m/z* = 639.4 $[M + H]^+$, 661.4 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{33}H_{52}N_8O_5$ $[M + 2H]^{2+}$ 320.2025, found 320.2028; calcd. for $C_{33}H_{51}N_8O_5$ $[M + H]^+$ 639.3977, found 639.3998.

RESULTS AND DISCUSSION

Solid-Phase Synthesis of Cyclic Biaryl Peptides Containing a Histidine Residue at the N-terminus

We planned to synthesize the biaryl cyclic peptides containing a His at the N-terminus depicted in Figure 1. They consist of a 3- or 5- amino acid ring and incorporate an amide group or a Leu-Leu spacer at the C-terminus. The use of 2-(trimethylsilyl)ethoxymethyl (SEM) or methyl as imidazole protecting group was evaluated.

The strategy for the solid-phase synthesis of these biaryl cyclic peptides involved as key steps: (i) the preparation of a linear peptidyl resin incorporating a 4-boronophenylalanine at the C-terminus and a 5-bromohistidine at the N-terminus, and (ii) the cyclization of this linear peptidyl resin via a microwave-assisted Suzuki-Miyaura cross-coupling (Scheme 1). The synthesis of the linear precursor was envisaged through preparation of the corresponding N-terminal trityl protected 4-iodophenylalanine peptidyl resin, solid-phase Miyaura borylation, trityl group removal and coupling of a conveniently protected 5-bromohistidine.



Scheme 1. Solid-phase synthesis of the biaryl cyclic peptide **BPC758**. 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-Phe(4-I)-OH, COMU, Oxyma, DIEA, DMF, overnight. (iv) Fmoc-Leu-OH or Fmoc-Lys(Boc)-OH, DIPCDI, Oxyma, DMF, 1 h. (v) TrCl, DIEA, DMF, 4 h. (vi) B_2Pin_2, $PdCl_2(dppf)$, dppf, KOAc, DMSO, 80 ºC, 24 h. (vii) TFA/H_2O/CH_2Cl_2 (0.2:1:98.8) (2×1 + 2×20 min). (viii) Boc-His(5-Br,1-SEM)-OH and Boc-His(5-Br,3-SEM)-OH, DIPCDI, Oxyma, DMF, 3 h. (ix) See Table 1. (x) TFA/H_2O/TIS (95:2.5:2.5), 3 h, stirring.

First, the synthesis of the biaryl cyclic peptide **BPC758** was investigated (Scheme 1). According to the above strategy, the peptidyl resins **1** were required as linear precursors. Thus, we synthesized the N-terminal trityl protected 4-iodophenylalanine peptidyl resin **2** starting from a Fmoc-Rink-MBHA resin

following a standard 9-fluorenylmethoxycarbonyl (Fmoc)/*tert*-butyl (*t*Bu) strategy. Elongation of the peptide sequence was performed through sequential Fmoc group removal and coupling steps. The Fmoc group was removed using piperidine/DMF (3:7). Coupling of amino acids was mediated by *N*,*N*-diisopropylcarbodiimide (DIPCDI) and ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) in DMF, except for the anchoring of Fmoc-Phe(4-I)-OH (Ng-Choi et al. 2019a) to the Rink linker which was achieved using 1-[(1-(cyano-2-ethoxy-2-oxoethylidineaminooxy)dimethylaminomorpholino)] uronium hexafluorophosphate (COMU), Oxyma and *N*,*N*-diisopropylethylamine (DIEA) in DMF. After assembling the Fmoc-protected peptide sequence, the Fmoc group was then replaced by a trityl group to overcome the instability of Fmoc under the basic Miyaura borylation conditions. Thus, after treatment with piperidine/DMF (3:7) the resin was subjected to trityl chloride (TrCl) and DIEA. An aliquot of the resulting resin **2** was treated with a mixture of trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/H_2O (95:2.5:2.5) for 2 h, providing the corresponding iodopeptide in 94% purity, which was also characterized by mass spectrometry.

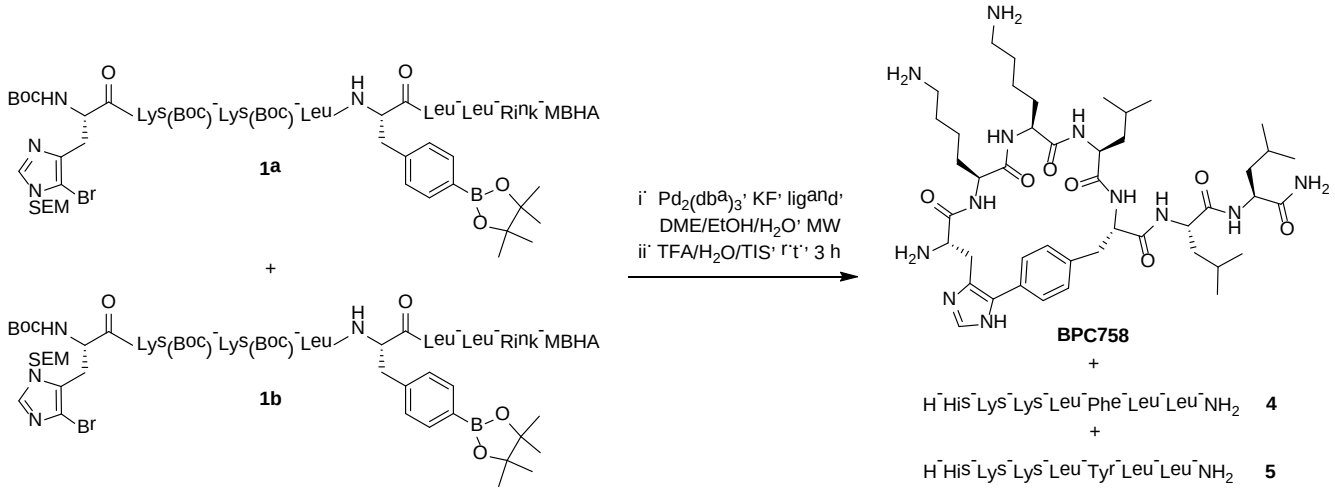
Next, the solid-phase Miyaura borylation of the 4-iodophenylalanine of peptidyl resin **2** was performed according to the conditions previously reported in our group (Afonso et al. 2010). Resin **2** was treated with bis(pinacolato)diboron (B_2Pin_2) (4 equiv.), $PdCl_2$(dppf) (0.18 equiv.), 1,1'-bis(diphenylphosphanyl)ferrocene (dppf) (0.09 equiv.), and KOAc (6 equiv.) in anhydrous DMSO at 80 ºC for 24 h. Acidolytic cleavage of an aliquot of the resulting resin **3** afforded the corresponding 4-boronophenylalanine peptide H-Lys-Lys-Leu-Phe(4-$B(OH)_2$)-Leu-Leu-NH_2 in 82% purity. This boronic acid was formed by hydrolysis of the pinacol boronic ester during HPLC analysis, as confirmed by mass spectrometry.

Linear peptidyl resins **1** were obtained from resin-bound peptide boronic esters **3** through trityl group removal with TFA/H_2O/CH_2Cl_2 (0.2:1:98.8) and coupling of the conveniently protected regioisomeric 5-bromohistidines Boc-His(5-Br,1-SEM)-OH and Boc-His(5-Br,3-SEM)-OH (Cerezo et

al. 2008) using DIPCDI and Oxyma in DMF for 3 h. Thus, peptidyl resins **1** were obtained as a mixture of two regioisomers (**1a** and **1b**). An aliquot of these peptidyl resins was acidolytically cleaved with TFA/TIS/H_2O (95:2.5:2.5) under stirring for 3 h. The corresponding linear boronic acid peptide H-His(5-Br)-Lys-Lys-Leu-Phe(4-$B(OH)_2$)-Leu-Leu-NH_2 was obtained in 73% HPLC purity and was characterized by mass spectrometry.

With the peptidyl resins **1** in hand, we proceeded to study their macrocyclization through a Suzuki-Miyaura reaction. After each assay, an aliquot of the resulting resin was cleaved and the crude reaction mixture was analyzed by HPLC and mass spectrometry (Table 1). Initially, the reaction was attempted under the conditions described for the synthesis of biaryl cyclic peptides containing a Phe-Phe linkage (Afonso et al. 2011): $Pd_2(dba)_3$ (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 120 ºC for 30 min. However, the desired biaryl cyclic peptide **BPC758** was detected in 29% purity together with a high amount of common side products, such as the protodeborylated and debrominated derivative H-His-Lys-Lys-Leu-Phe-Leu-Leu-NH_2 (**4**) (16% purity), and the oxidized and debrominated analogue H-His-Lys-Lys-Leu-Tyr-Leu-Leu-NH_2 (**5**) (19% purity) (entry 1, Table 1). An increase of the temperature up to 140 ºC improved the purity of **BPC758** to 44% and decreased the amount of the byproduct **5** to 9% (entry 2, Table 1). A similar result was achieved when the reaction was performed at 140 ºC using a larger excess of reagents (entry 3, Table 1). When the reaction was attempted with SPhos as ligand, the formation of **BPC758** slightly decreased (32 and 37% purity) and the percentage of **5** increased (33 and 29% purity, respectively) (entries 4 and 5, Table 1). The resulting biaryl cyclic peptide **BPC758** was purified by column chromatography, being obtained in 93% purity, and it was analyzed by mass spectrometry.

Table 1. Suzuki-Miyaura macrocyclization of regioisomeric peptidyl resins **1**.



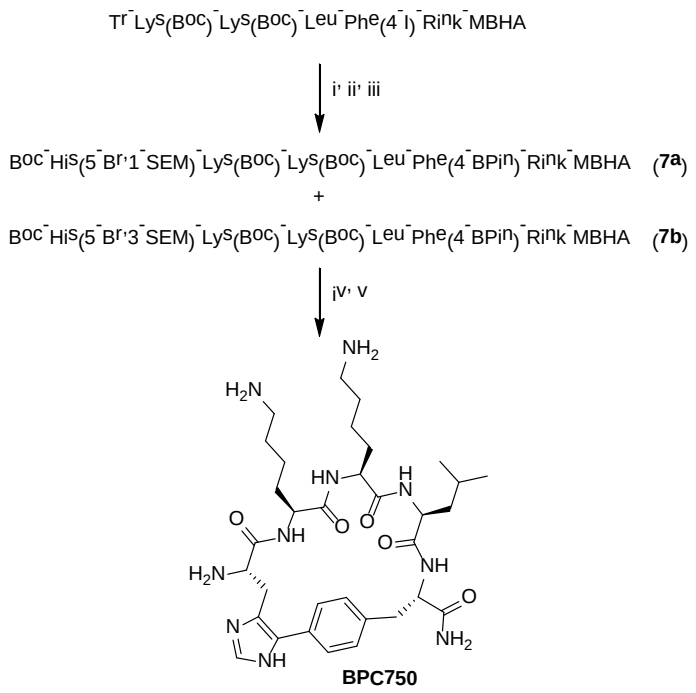
Entry	Catalyst (equiv.)	Ligand (equiv.)	Base (equiv.)	T (°C)	t (min)	**BPC758** (%)[a]	**4** (%)[a]	**5** (%)[a]
1	$Pd_2(dba)_3$ (0.2)	P(*o*-tolyl)$_3$ (0.4)	KF (4)	120	30	29	16	19
2	$Pd_2(dba)_3$ (0.2)	P(*o*-tolyl)$_3$ (0.4)	KF (4)	140	30	44	17	9
3	$Pd_2(dba)_3$ (0.3)	P(*o*-tolyl)$_3$ (0.6)	KF (6)	140	30	41	-	-
4	$Pd_2(dba)_3$ (0.2)	SPhos (0.4)	KF (4)	120	30	32	10	33
5	$Pd_2(dba)_3$ (0.2)	SPhos (0.4)	KF (4)	140	30	37	18	29

[a] Percentage determined by HPLC at 220 nm from the crude reaction mixture.

The influence of the protecting group of the histidine residue on the intramolecular Suzuki-Miyaura cross-coupling was tested by replacing the SEM group with a methyl. A similar strategy to the one represented in Scheme 1 was followed. The required Boc-His(5-Br,1-Me)-OH and Boc-His(5-Br,3-Me)-OH were prepared by methylation of the imidazole ring of Boc-His(5-Br)-OMe (Cerezo et al. 2008) with iodomethane followed by final methyl ester hydrolysis (58% overall yield). The trityl group of resin Tr-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (**3**) was removed and treated with the aforementioned histidine derivatives. The resulting resins incorporating both the 5-bromohistidine and the 4-boronophenyalanine residues, Boc-His(5-Br,1-Me)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-MBHA (**6a**) and Boc-His(5-Br,3-Me)-Lys(Boc)-Lys(Boc)-Leu-Phe(4-BPin)-Leu-Leu-Rink-

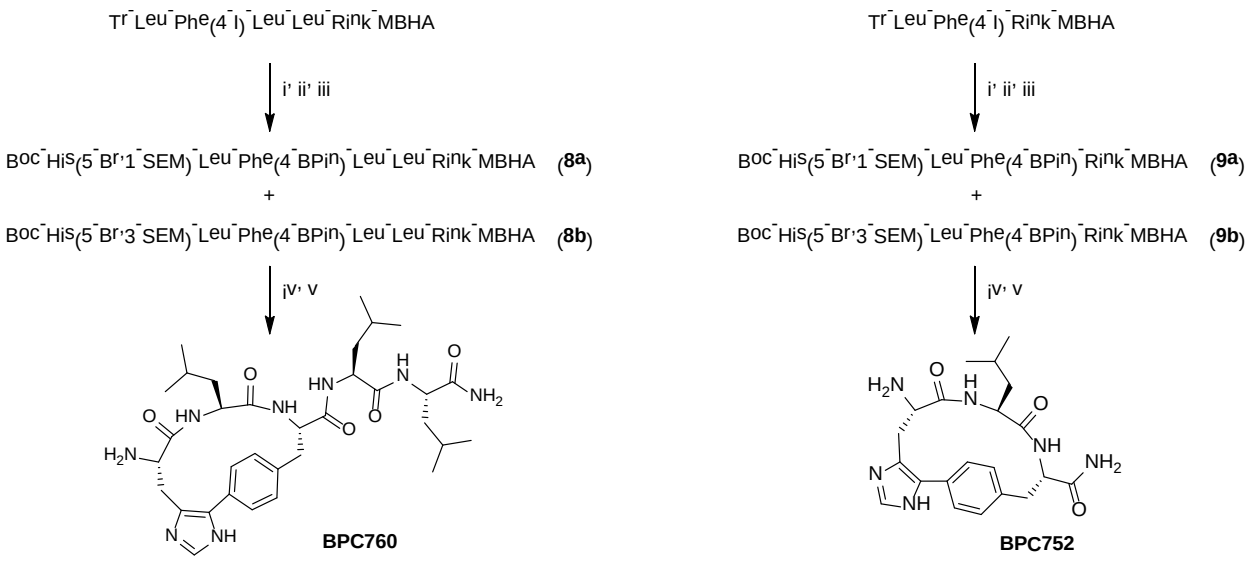
MBHA (**6b**)**,** were then subjected to the best Suzuki-Miyaura cyclization conditions previously established: $Pd_2(dba)_3$ (0.2 equiv.), P(*o*-tolyl)$_3$ (0.4 equiv.), and KF (4 equiv.) in degassed 1,2-dimethoxyethane (DME)/EtOH/H_2O (9:9:2) under microwave irradiation at 140 ºC for 30 min. After cleavage, the expected regioisomeric biaryl cyclic peptides **BPC772a** and **BPC772b** were obtained in 32% purity. These results were similar to those of **BPC758**, which denote that the presence of the bulkier SEM group did not hinder the intramolecular arylation. **BPC772** was purified by column chromatography (91% purity) and was characterized by mass spectrometry.

Next, we attempted the synthesis of the biaryl cyclic peptide **BPC750**, analog to **BPC758** but without a Leu-Leu spacer at the C-terminus (Scheme 2). For this purpose, peptidyl resins **7** bearing the 4-boronophenylalanine residue bound to the Rink linker were prepared. The intramolecular Suzuki-Miyaura cross-coupling using the above conditions yielded the expected biaryl cyclic peptide **BPC750** in 14% HPLC purity together with the linear peptides H-His-Lys-Lys-Leu-Phe-NH_2, H-His-Lys-Lys-Leu-Tyr-NH_2, H-His-Lys-Lys-Leu-Phe(4-B$(OH)_2$)-NH_2 resulting from protodeborylation, debromination and/or oxidation of the linear precursor **7**. The lower purity observed for these biaryl cyclic peptide in the crude reaction mixture compared to that of their analogue containing a Leu-Leu spacer could be attributed to the steric hindrance posed by the resin.



Scheme 2. Synthesis of the biaryl cyclic peptide **BPC750**. Reagents and conditions: (i) B_2Pin_2, $PdCl_2$(dppf), dppf, KOAc, DMSO, 80 ºC, 24 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 and Boc-His(5-Br,3-SEM)-OH, DIPCDI, Oxyma, DMF, 3 h. (iv) $Pd_2(dba)_3$, KF, P(*o*-tolyl)$_3$, DME/EtOH/H_2O, MW, 140 ºC, 30 min. (v) TFA/H_2O/TIS (95:2.5:2.5), 3 h, stirring.

To investigate if this approach could also be applied to the formation of 3-member ring biaryl cyclic peptides, resins **8** and **9** were synthesized, which differ on the presence of a Leu-Leu spacer at the C-terminus (Scheme 3). Cyclization of resins **8** and **9** afforded the biaryl cyclic peptides **BPC760** and **BPC752** in 33% and 13% HPLC purity, respectively, together with linear peptides resulting from protodeborylation, debromination and/or oxidation of the corresponding precursors **8** and **9**. **BPC760** and **BPC752** were purified by column chromatography (>99% and 91% purity, respectively) and were characterized by mass spectrometry. Therefore, similar results were obtained for the preparation of biaryl cyclic peptides incorporating 3 or 5 amino acids in their ring and it was observed that the presence of a Leu-Leu spacer at the C-terminus favoured the cyclization.

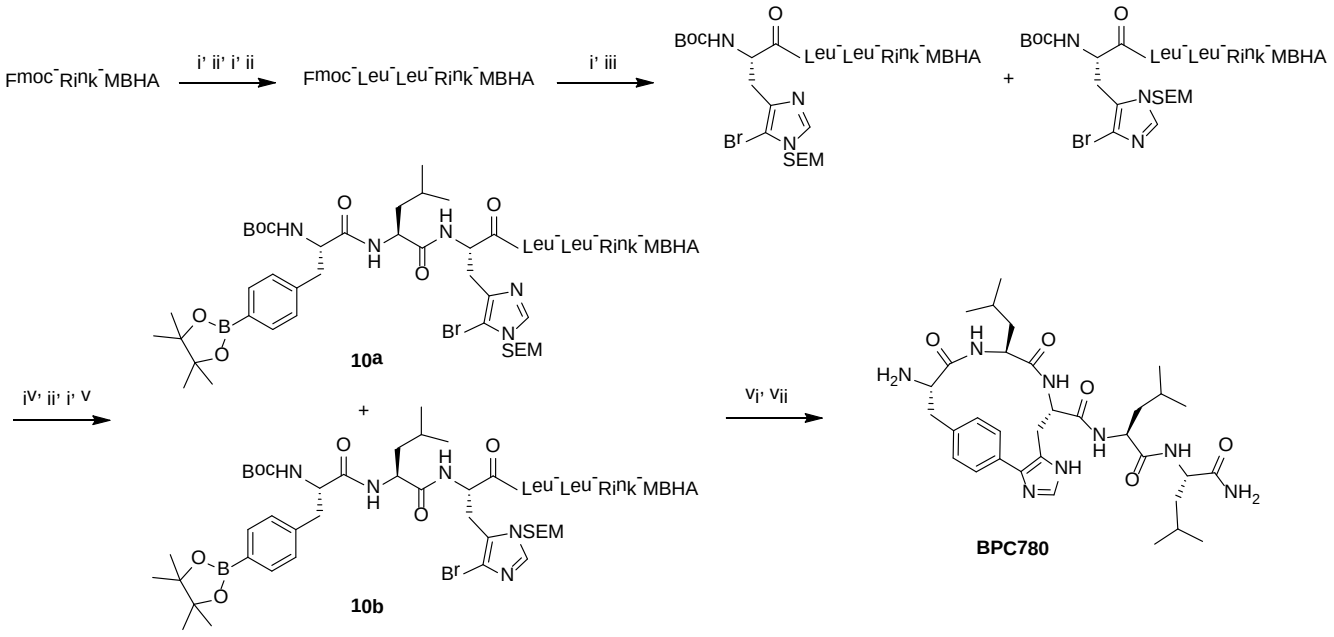


Scheme 3. Synthesis of the biaryl cyclic peptides **BPC760** and **BPC752**. Reagents and conditions: (i) B_2Pin_2, $PdCl_2$(dppf), dppf, KOAc, DMSO, 80 ºC, 24 h. (ii) TFA/H_2O/CH_2Cl_2 (0.2:1:98.8) (2×1 + 2×20 min). (iii) Boc-His(5-Br,1-SEM)-OH and Boc-His(5-Br,3-SEM)-OH, DIPCDI, Oxyma, DMF, 3 h. (iv) $Pd_2(dba)_3$, KF, P(*o*-tolyl)$_3$, DME/EtOH/H_2O, MW, 140 ºC, 30 min. (v) TFA/H_2O/TIS (95:2.5:2.5), 3 h, stirring.

Solid-Phase Synthesis of Cyclic Biaryl Peptides Containing a Histidine Residue at the C-terminus

The feasibility of synthesizing 5-arylhistidine-containing cyclic peptides in which the His residue is located at the C-terminus was investigated (Figure 2). In particular, taking into account the previous results, we studied the synthesis of biaryl cyclic peptides containing a 3- or 5-residue ring, and incorporating an amide group or a Leu-Leu spacer at the C-terminus.

Towards this aim, a similar strategy to that described for biaryl cyclic peptides bearing a His at the N-terminus was planned. In this case, it would involve the preparation of a linear peptidyl resin incorporating a 4-boronophenylalanine at the N-terminus and a 5-bromohistidine at the C-terminus, which it would be then cyclized by means of a microwave-assisted intramolecular Suzuki-Miyaura cross-coupling (Scheme 4).



Scheme 4. Synthesis of biaryl cyclic peptide **BPC780**. 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 and Boc-His(5-Br,3-SEM)-OH, COMU, Oxyma, DIEA, DMF, overnight. (iv) TMSOTf, 2,6-lutidine, CH_2Cl_2 (10×30 min). (v) Boc-Phe(4-BPin)-OH, COMU, Oxyma, DIEA, DMF, overnight. (vi) $Pd_2(dba)_3$, KF, P(*o*-tolyl)$_3$, DME/EtOH/H_2O, MW, 140 ºC, 30 min. (vii) TFA/H_2O/TIS (95:2.5:2.5), 3 h, stirring.

This approach was first applied to the synthesis of the 3-member ring biaryl cyclic peptide **BPC780** which incorporates a Leu-Leu spacer at the C-terminus. The synthesis of the required linear peptidyl resins **10** started from a Fmoc-Rink-MBHA resin and followed a Fmoc/*t*Bu strategy. The two Fmoc-Leu-OH residues were incorporated by sequential Fmoc removal and coupling steps. The Fmoc group was cleaved with piperidine/DMF (3:7) and couplings were performed with DIPCDI and Oxyma in DMF. Next, after Fmoc removal, the regioisomeric mixture of Boc-His(5-Br,1-SEM)-OH and Boc-His(5-Br,3-SEM)-OH (Cerezo et al. 2008) was coupled using COMU, Oxyma and DIEA in DMF. The Boc group of the resulting 5-bromohistidine peptidyl resins was selectively removed with trimethylsilyl

trifluoromethanesulfonate (TMSOTf) in the presence of 2,6-lutidine (Zhang et al. 1998). Peptide elongation was performed by coupling of the corresponding Fmoc-Leu-OH and of Boc-Phe(4-BPin)-OH (Ng-Choi et al. 2019a) as the N-terminal residue. An aliquot of the resulting resins **10** was cleaved with TFA/TIS/H_2O (95:2.5:2.5) for 3 h yielding the corresponding 4-boronophenylalanyl peptide H-Phe(4-$B(OH)_2$)-Leu-His(5-Br)-Leu-Leu-NH_2 in 69% purity. Formation of the boronic acids took place during HPLC analysis which was confirmed by mass spectrometry.

With the boronopeptidyl resins **10** in hand, the intramolecular Suzuki-Miyaura arylation was carried out using $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, and KF under microwave irradiation at 140 ºC for 30 min, which were the best conditions for the formation of the biaryl bond in cyclic peptides with a His at the N-terminus (Scheme 4). After acidolytic cleavage, the crude reaction mixture was analyzed by HPLC and mass spectrometry. Results showed that the intramolecular arylation of resins **10** did provide the biaryl cyclic peptide **BPC780** in 30% HPLC purity. Purification through column chromatography rendered **BPC780** in >99% purity and was characterized by mass spectrometry.

This methodology was applied to the synthesis of **BPC774**, **BPC776** and **BPC778** (Figure 2). The 3-member ring biaryl cyclic peptide **BPC776** without the Leu-Leu spacer was obtained in lower purity (10%) than its analog **BPC780**. This result is in accordance to that obtained for biaryl cyclic peptides with a His at the N-terminus. Unexpectedly, in the case of the 5-member ring cyclic peptides **BPC778** and **BPC774**, differing on the presence of a Leu-Leu spacer, the cyclization of the corresponding linear peptidyl resins was not successful. Mass spectrometry analysis of the crude reaction mixtures showed only traces of the expected biaryl cyclic peptide **BPC778**, whereas **BPC774** was not observed. Taking all these results together, it can be concluded that the synthesis of biaryl cyclic peptides bearing a His at the C-terminus is more difficult than that of analogues with a His at the N-terminus, specially in the case of the 5-member ring peptides.

CONCLUSIONS

This paper reports the development of a strategy for the solid-phase synthesis of biaryl cyclic peptides containing a His-Phe linkage. It has been evaluated the position of the His in the sequence and the presence of a spacer at the C-terminus. It was observed that the intramolecular Suzuki-Miyaura cross-coupling was favoured when a Leu-Leu spacer is located at the C-terminus. Moreover, in the case of the biaryl cyclic peptides consisting of a 3-residue ring, similar results were obtained irrespective of the His position. In contrast, the formation of the 5-member biaryl cyclic peptides was only achieved when the His was at the N-terminus.

This methodology takes full advantage of the solid-phase synthesis because, apart from macrocyclization, borylation is also performed on the solid support. This protocol avoids the synthesis and purification of the amino acid boronic ester in solution and at the same time it facilitates the removal of the palladium catalyst used in the Suzuki-Miyaura reaction. Therefore, this approach could be useful for the preparation of cyclic peptides containing a biaryl linkage between a His and a Phe in a flexible manner under mild conditions.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The Authors declare that they have no conflict of interest.

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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