DOI: 10.1002/ejoc.200600833

Synthesis of a 24-Membered Cyclic Peptide-Biphenyl Hybrid

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Keywords: Biaryls / Calpain inhibitors / Macrocycles / Peptide-biphenyl hybrids / Solid-phase synthesis

The synthesis of a 24-membered macrocyclic peptide-biphenyl hybrid with four amino acid residues and two biphenyl fragments was performed by a combination of solid-phase and solution methodologies. The acyclic precursor was prepared by a solid phase methodology whereas the final macrocyclization was carried out by diisopropylcarbodiimide (DIPCDI) and *N*-hydroxybenzotriazole (HOBt) in solution. The target molecule was a moderate inhibitor of μ-calpain.

Introduction

Peptides and related compounds are the focus of intense research activity. These compounds have been used as tools to study biochemical and biophysical processes,[1] as chiral catalysts,[2] as building blocks for the preparation of technological materials,[3] and, mainly, as pharmaceuticals.[4] Although many peptides show biological activity, they are frequently questioned as drugs because of poor absorption, lack of transportation across cell membranes, in vivo instability, and incapacity to achieve a bioactive conformation. As a result of these drawbacks, many peptide analogues have been prepared. Two general strategies for the design of peptide analogues are the insertion of non-peptidic fragments into a peptide chain (either main or side)[5] and the generation of cyclic structures.[6]

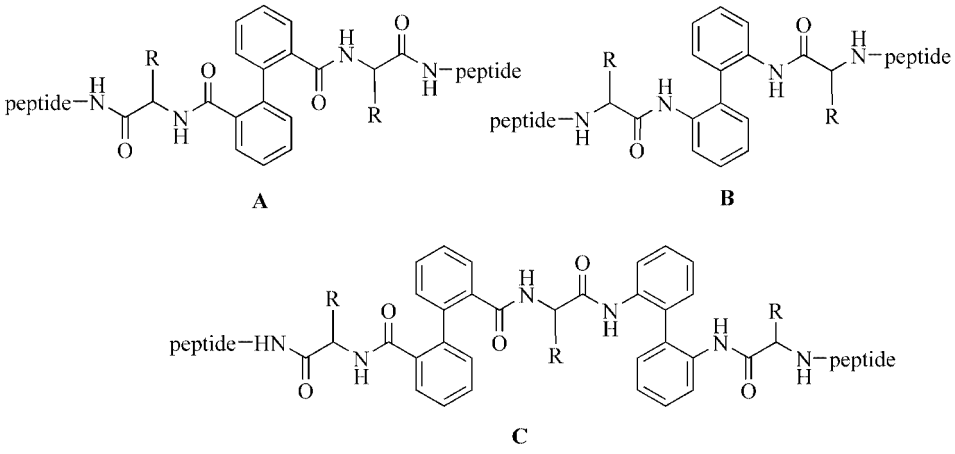

Figure 1. Generic structures of peptide-biphenyl hybrids of types **A**–**C**.

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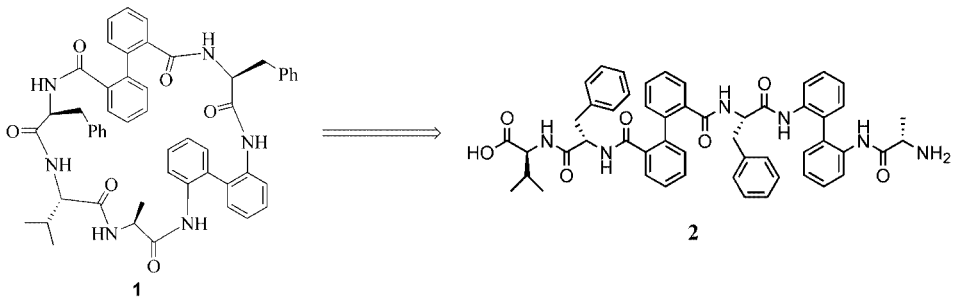
Recently, we have focused on the synthesis, structure, and properties of peptide-biphenyl hybrids (**A**–**C**, Figure 1), which are derivatives of 1,1′-biphenyl with amino acid or peptide chains at the positions C(2) and C(2′).[7–10] Since biphenyl derivatives[11–13] and peptides possess useful characteristics, the combination of the two functionalities has resulted in the generation of compounds with structural[7] and biological properties of great interest. Of these, the most interesting is their capacity to inhibit calpain.[8,9] This enzyme is a cysteine protease with an active physiological role,[14] which, when over-activated, causes a variety of pathological conditions.[15–16]



Scheme 1. Retrosynthetic analysis of macrocycle **1**.

It is well established that the intramolecular tethering of the *N*- and *C*-terminals of peptide chains, generating a macromolecular structure, provides compounds with lowered conformational flexibility as well as increased stability vs. peptidases. As a step forward in our research on peptide-biaryl hybrids, here we have prepared a cyclic analogue of a peptide-biphenyl hybrid of type **C**, with the objective to assess the effect of this structural restriction on its properties as well as to prove the feasibility of the synthetic strategy. The target model molecule was **1**, which was intended to be prepared by cyclization, in solution, of the lineal precursor **2** (Scheme 1), which in turn would be synthesized in the solid-phase mode following an adapted methodology from that recently developed by our group.[9]

Results and Discussion

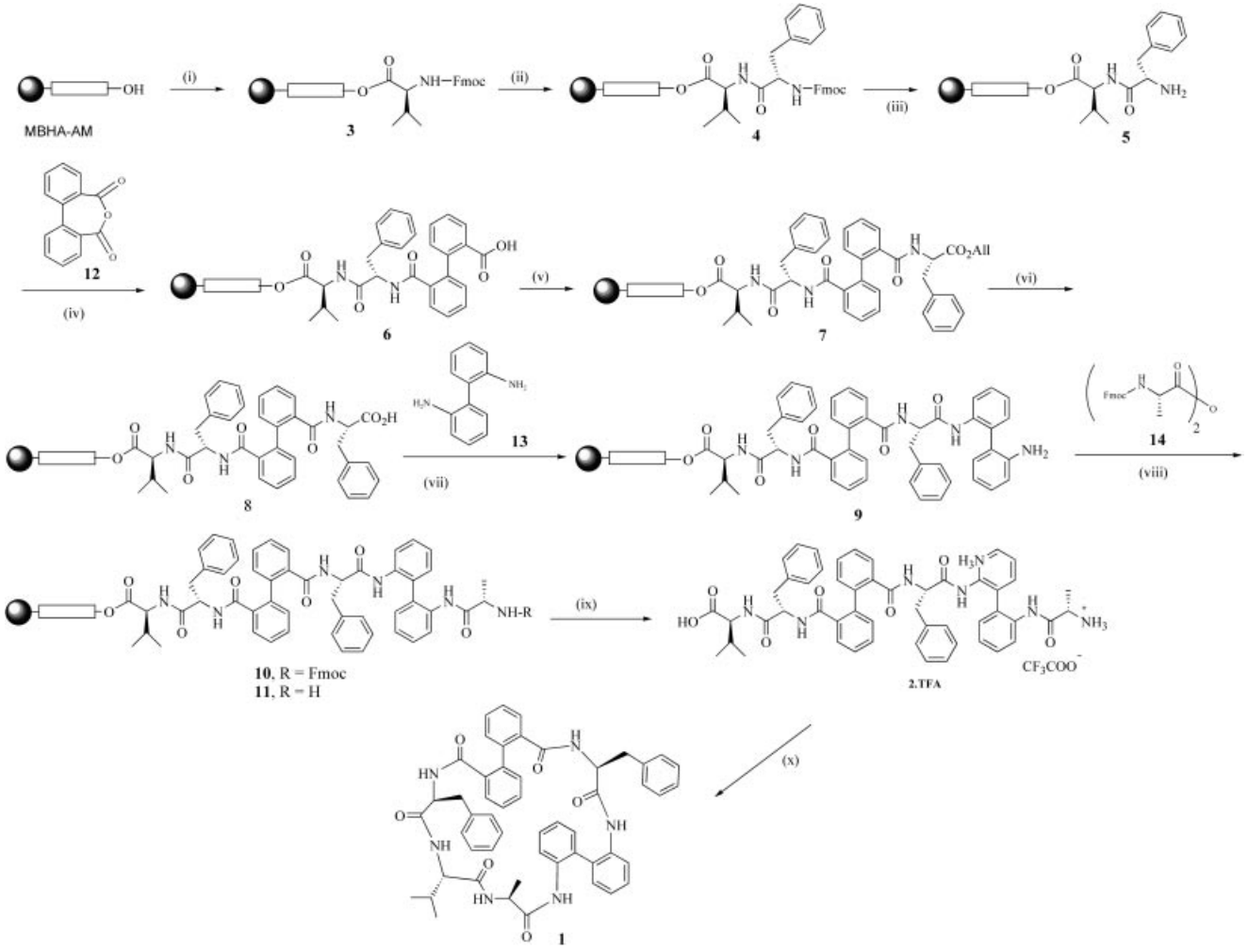
Our synthetic plan for the solid-phase synthesis of the target peptide-biphenyl hybrid is based on a standard strategy for the solid-phase synthesis of peptides,[17] with the initial amino acid linked to the resin by the *C*-terminal, although taking into account that when the biphenyl unit, which contains two carboxylic moieties, is incorporated, the chain changes sense.[5c] In our previous synthesis of peptide-biphenyl hybrids, we used methyl ester as temporary protection of carboxylic acid and a Rink amide linker bonded to a MBHA-PS-resin, which resulted in primary amides after resin cleavage. Although the solid-phase synthesis of the target acyclic peptide-biphenyl **2** could be performed in a similar way, two significant modifications were made. On the one hand, the Wang acid linker (AB) was used, thereby allowing us to obtain a carboxylic acid after removal from the resin; and, on the other hand, an orthogonal protection for the carboxylic group was required.

The synthesis is shown in Scheme 2.[18] The MBHA-PS resin was modified with the AB linker (DIPCDI/HOBt) and reacted with the first protected amino acid, Fmoc-Val-OH (DIPCDI/DMAP), to give **3**, which was sequentially treated with piperidine and coupled with Fmoc-Phe-OH (DIPCDI/HOBt) to afford the dipeptide **4**. This dipeptide, in turn, was *N*-deprotected (piperidine) to **5** and treated with diphenic anhydride (**12**) (Et_3N) to give the peptide-biphenyl hybrid **6**. The next amino acid had to be coupled by the amino group with a suitable protection at the carboxyl group. Previously, we found that protection as methyl ester was efficient, releasing the free carboxylic acid by basic hydrolysis. However, this strategy was not viable in this case since the link to the resin would also be hydrolyzed. Therefore, we used the allyl ester as carboxylic protection.

Thus, the acid **6** was coupled to H-Phe-OAll (PyBOP/HOAt/DIEA) to provide **7**, which, by treatment with a catalytic amount of $Pd(PPh_3)_4$ in the presence of $PhSiH_3$,[19] gave the hybrid **8**. This acid was activated with CDI and treated with excess of 2,2′-biphenyl-1,1′-diamine (**13**) to furnish the peptide-biphenyl hybrid **9** with two biphenyl fragments. The amine **9** was reacted with the anhydride **14** (Et_3N) to give an *N*-protected peptide-biphenyl hybrid **10**, which was deprotected (piperidine) to the amine **11**. The structure and purities of all the reaction products were assessed by a combination of HPLC and MS of the corresponding acid obtained after treatment with TFA used to remove the solid support. HPLCs of all intermediates **4**–**11** showed high purities (> 90%). Once the synthetic sequence in the solid phase was completed, the peptide was removed from the resin (TFA) to give, after solvent treatment (CH_3CN-H_2O) and lyophilization, the target acyclic compound **2** as its trifluoroacetate salt (**2·TFA**).

With the acyclic precursor **2** in hand, we performed the key macrocyclization step in solution.[20] Preliminary experiments were performed to determine the most suitable conditions.[21] We found that the treatment of a 2 *mM* solution of **2** in DCM with DIPCDI/HOBt/DIEA gave the macrocycle **1** in 70% yield, after purification by preparative HPLC. Overall, the complete synthetic sequence (12 steps) was done starting from 500 mg of MBHA-PS-resin, with an initial loading of 0.7 mmol/g, affording 81.8 mg of the macrocycle **1** in 27% overall yield. The macrocyclic peptide-biphenyl hybrid **1** was tested as an inhibitor of μ-calpain. Our results show that this hybrid showed modest inhibitory capacity, with an IC_{50} value of 29 μM.[22]

The macrocyclic peptide-biphenyl hybrid compound **1** is a mixture of atropisomers. Thus, it was observed that, at room temperature, the HPLC of **1** presents two close broad peaks center at 10.44 and 10.61 min with identical UV spectra. These two peaks tend to coalesce (10.35 min) on increasing the temperature to 60 °C (Figure 2), which indicates that these peaks correspond to equilibrating conformers. The presence of several conformers was confirmed by ^1H NMR (Figure 3b); thus, **1** is a mixture of 4 conformers in DMSO at 60 °C, probably because of the restricted rota-



Scheme 2. Synthesis of macrocyclic peptide-biphenyl hybrid **1**. (i) (a) DIPCDI (2.0 equiv.), HOBt (2.0 equiv.), DMF, (b) Fmoc-Val-OH (10.0 equiv.), DIPCDI (10.0equiv.), DMAP (0.1 equiv.), DCM/DMF (9:1); (ii) (a) piperidine/DMF (1:4), (b) Fmoc-Phe-OH (3.0 equiv.), DIPCDI (3.0 equiv.), HOBt (3.0 equiv.), DMF; (iii) piperidine/DMF (1:4); (iv) **12** (5.0 equiv.), Et_3N (5.0 equiv.), DMF; (v) HCl·H-Phe-OAll (3.0 equiv.), PyBOP (3.0 equiv.), HOAt (3.0 equiv.), DIEA (6.0 equiv.), DMF; (vi) $Pd(PPh_3)_4$ (0.1 equiv.), $PhSiH_3$ (10.0 equiv.), DCM; (vii) (a) CDI (10.0 equiv.), DMF, (b) **13** (5.0 equiv.), DIPCDI (5.0 equiv.), HOBt (5.0 equiv.), DMF; (viii) (a) **14** (3.0 equiv.), Et_3N (3.0 equiv.), DCM, (b) piperidine/DMF (1:4); (ix) TFA/DCM (95:5); (x) DIPCIDI (3.0 equiv.), HOBt (3.0 equiv.), DIEA, (3.0 equiv.), DCM (2 mM).

tion around the aryl-aryl bond of the biphenyl moieties, as found in other acyclic peptide-biphenyl hybrids.[7a] However, the macrocycle **1** showed lower conformational mobility than its acyclic analogue, as inferred from the comparison of their ^{1}H-NMR spectra (Figure 3).

Conclusions

The macrocyclic peptide-biphenyl hybrid **1**, which contains two fragments of biphenyl and four amino acid residues, was synthesized in high overall yield by a combination of solid-phase and solution methodologies, including an efficient macrocyclization to a 24-membered ring. The present strategy provides access to macrocyclic compounds with amino acid and biaryl fragments which, like the acyclic counterpart, may show structural, biological, and technological properties of interest. We are currently working on the preparation of other macrocyclic peptide-biphenyl hybrids as well as the determination of their conformational preferences and properties.

Experimental Section

General Methods: 4-Methylbenzhydrylamine resin VHL (0.7 mmol/g, 100–200 mesh), AB handle and Fmoc-amino acids were purchased from NovaBiochem (Läufelfingen, Switzerland). Dimethylformamide (DMF), dichloromethane (DCM) and tetrahydrofuran (THF) were purchased from SDS (Peypin, France) and used as received. Analytical HPLC was carried out on a Waters® 2695 Separations Module instrument, including a Waters® 996 Photodiode Array Detector with UV detection from 210 to 500 nm. Linear gradients of CH_3CN and H_2O were run at 1 mL/min flow rate, from 100:0 (0.1% of TFA in H_2O: 0.1% of TFA in CH_3CN) to 0:100 (0.1% of TFA in H_2O: 0.1% of TFA in CH_3CN) over 10 or 15 min,

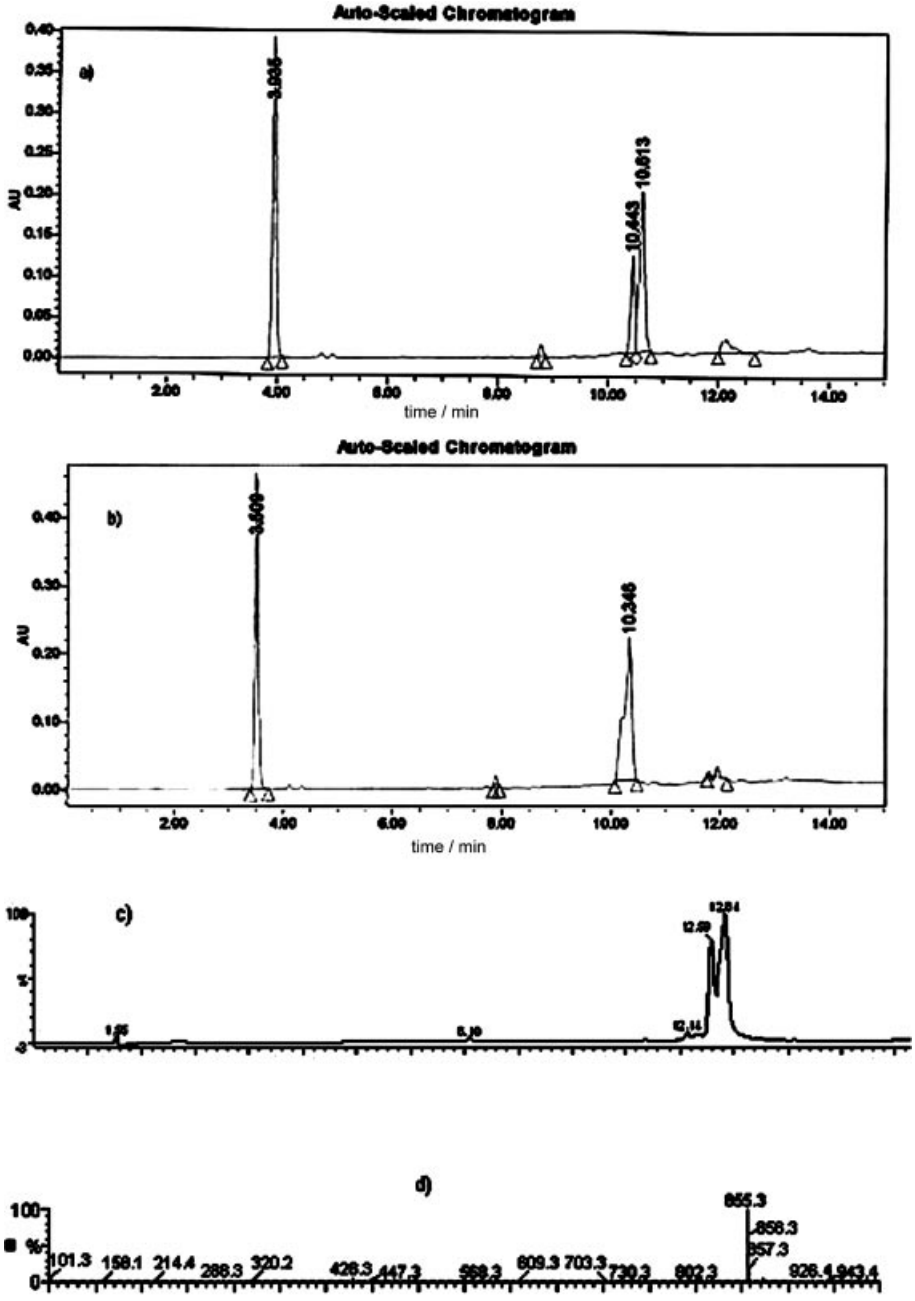


Figure 2. Crude product, HPLC analysis (a) at room temperature and (b) at 60 °C; purified macrocycle **1**, (c) HPLC analysis at room temperature; (d) mass spectrum.

with a Symmetry® C-18 column (5.0 μm, 4.6 mm×150 mm). All HPLC-MS spectra were obtained on a Micromass ZQ mass spectrometer in electrospray positive ionization (ES^+) mode in-line with a Waters® 2795 HPLC system (Separations module, Alliance™) and Waters® 2487 Dual absorbance Detector (PDA). Linear gradients of CH_3CN and H_2O were run at a flow rate of 0.5 mL/min, from 100:0 (0.1% of HCOOH in H_2O:CH_3CN) to 0:100 (0.1% of HCOOH in H_2O:CH_3CN) over 10 or 15 min, with a X-Terra® C-18 column (5.0 μm, 4.0 × 50 mm). All reactions were performed in polypropylene syringes (10 mL), each fitted with a polyethylene porous disc. Ninhydrine tests were performed in the reactions involving resin-bonded amines[23] and the reactions involving resin-bonded carboxylic acids were monitored by the malachite green test,[24] and when necessary, the coupling reaction was repeated until a negative test was obtained. IR spectra were measured in a Perkin–Elmer Spectrum One FT-IR spectrometer; the frequencies in the IR spectra are shown in cm^{-1}. ^1H NMR spectra were measured in Bruker DRX-600, Varian UNITY 500 and Varian INOVA 300 spectrometers. Chemical shifts (δ) are reported in parts per million and the coupling constants are indicated in Hz. ^1H-NMR spectra were referenced to the chemical shift of either TMS (δ = 0.00 ppm) or the residual proton in the deuterated solvent. MS analyses were recorded in Hewlett–Packard 1100 MSD (ESI) spectrometers. Combustion analyses were performed in a Carlo–Erba EA 1180-Elemental Analyzer by E. Barbero (CSIC). Optical rotations were determined in a Perkin–Elmer 241 MC polarimeter at room temperature (ca. 295 K). Melting points were measured on a Kofler hot-stage apparatus.

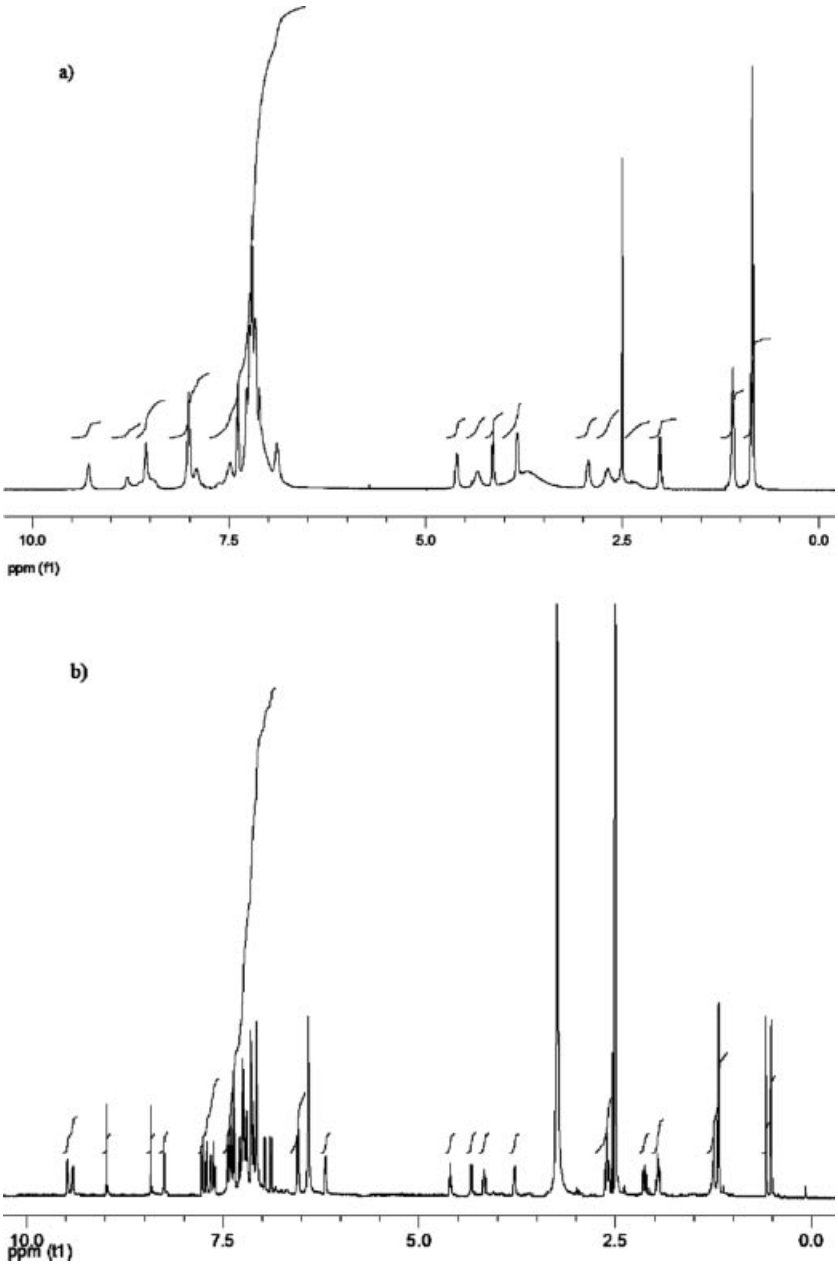


Figure 3. ^{1}H-NMR spectra (600 MHz, [D_6]DMSO) of acyclic peptide-biphenyl hybrid **2·TFA** at 60 °C (a) and of macrocycle **1** at 50 °C (b).

Incorporation of the AB Acid Linker into the PS-MBHA Resin (preparation of MBHA-AM): After washing the PS-MBHA resin (500 mg, 0.35 mmol) with DCM (5× 1 min), TFA 40% / DCM (1× 1 min, 1× 30 min), DCM (5× 1 min), 10% DIEA / DCM (3× 3 min) and DCM (5× 1 min), a solution of the AB handle (137.2 mg, 0.7 mmol) and HOBt (94.6 mg, 0.7 mmol) in DMF was added, followed by DIPCDI (108.4 μL). The mixture was stirred overnight.

Sequential Coupling of MBHA-AM with Fmoc-Amino Acids and Removal of Fmoc Group (preparation of 5): The resin was washed with DCM (5× 1 min) and DMF (5× 1 min) and sequentially treated with solutions of DIPCDI (2.0 equiv.) and HOBt (2.0 equiv.), and with a solution of Fmoc-Val-OH (10.0 equiv.), and left to react for 2.5 hours. The Fmoc group was then removed from the resin by treatment with piperidine/DMF (1:4) (2× 15 min). The next Fmoc-aa-OH (Fmoc-Phe-OH) was introduced by reaction with a solution of Fmoc-Phe-OH (3.0 equiv.), DIPCDI (3.0 equiv.) and HOBt (3.0 equiv.) for 2.5 h. The Fmoc-protecting group was removed with piperidine/DMF (1:4) (2× 15 min) to give amine **5**, which was used in the next step.

Incorporation of the First Biphenyl Fragment (preparation of 6): The peptidyl resin **5** was swollen in DMF. Diphenic anhydride (**12**, 5.0 equiv.) dissolved in the minimum amount of DMF was then added followed by Et_3N (5.0 equiv.). The mixture was shaken at room temperature for over 2.5 h and the solvent was removed by filtration. The resin was washed with DMF (5× 1 min), DCM (5× 1 min) and dried in vacuo.

Coupling with HCl·H-Phe-OAll (preparation of 7): The resin **6** with the free carboxylic functionality was swollen in DMF and then

DIEA (3.0 equiv.), PyBOP (3.0 equiv.) and HOAt (3.0 equiv.), dissolved in the minimum amount of DMF, were added. The mixture was shaken for 5 min and then a solution of HCl·H-Phe-OAll (3.0 equiv.) and DIEA (3.0 equiv.) in DMF was added. The reaction mixture was stirred at room temperature for 2.5 h and the resin was then filtered and washed with DMF (5× 1 min).

Removal of the Allyl Group (preparation of 8): The peptide resin **7** was washed with DCM (5× 1 min), Ar was passed through the resin, a solution of $PhSiH_3$ (10.0 equiv.) in DCM was added and the resin was manually stirred. Then, a solution of $Pd(PPh_3)_4$ (0.10 equiv.) in DCM was added and the reaction was shaken for 10 min. The peptide resin was washed with DMC (8× 30 s) and the process was repeated once to assure completeness of the reaction to obtain the acid **8**.

Incorporation of the Second Biphenyl Fragment (preparation of 9). Activation of the Carboxylic Acid: The resin-linked acid **8** was swollen in DMF and a solution of CDI (10.0 equiv.) in DMF was added. The mixture was shaken at room temperature over 2.5 h to assure complete activation of the acid. The resin was then filtered, washed with DMF (5× 1 min) and DCM (5× 1 min) and dried in vacuo.

Coupling with Biphenyl-2,2′-diamine (13): The activated resin was swollen in DMF and then a solution of **13** (5.0 equiv.) in DMF was added followed by DIPCDI (5.0 equiv.) and HOBt (5.0 equiv.), dissolved in the minimum amount of DMF. The mixture was shaken for over 2 h at room temperature. The resin was finally filtered, washed with DMF (5× 1 min), DCM (5× 1 min) and dried in vacuo to give **9**, which was used in the next step.

Synthesis of (*S*,*S*,*S*,*S*)-2-{2-[(2′-{1-[2′-(2-Aminopropionylamino)biphenyl-2-ylcarbamoyl]-2-phenylethylcarbamoyl}biphenyl-2-carbonyl)-amino]-3-phenylpropionylamino}-3-methylbutyric Acid (2). Preparation of the Symmetric Anhydride 14: DCC (3.0 equiv.) was added to a solution of Fmoc-Ala-OH (6.0 equiv.) in DCM and the reaction mixture was stirred at room temperature for over 30 min. The DCU formed was filtered off and the resulting solution was used in the next step.

Coupling with the Symmetric Anhydride, Removal of the Fmoc Group and Cleavage from the Resin: The resin **9** with the free amino functionality was swollen in DCM and the solution of the symmetric anhydride, previously prepared, was added, followed by Et_3N (3.0 equiv.). The mixture was shaken for 4.5 h. The resin was finally filtered, washed with DCM (5× 1 min) and dried in vacuo to furnish **10**. The resin was then washed with DMF (5× 1 min) and treated with piperidine/DMF (1:4) (2× 15 min) to give the amine **11**. Cleavage was accomplished by treatment of the resin **11** with TFA/DCM (95:5) for over 2 h. The filtrate was collected, concentrated to dryness and the resulting residue was dissolved in the minimum amount of CH_3CN. Water was then added and after lyophilization compound **2** was obtained as the trifluoroacetate salt. ^{1}H NMR (600 MHz, [D$_6$]DMSO, 60 °C): δ = 9.31 (broad s, 1 H, NH-biph.), 8.85 (s, 1 H, NH-biph.), 8.59 (broad s, 1 H, NH-Phe$_1$; 1 H, NH-Phe$_2$), 8.02 (broad s, 1 H, NH$_2$-Ala), 7.96 (broad s, 1 H, NH-Val), 7.47 (m, 1 H, H$_{arom.}$), 7.39 (m, 2 H, H$_{arom.}$), 7.27–7.10 (m, 21 H, H$_{arom.}$), 6.89 (m, 2 H, H$_{arom.}$), 4.60 (m, 1 H, C*Hα*-Phe), 4.33 (m, 1 H, C*Hα*-Phe), 4.14 (m, 1 H, C*Hα*-Val), 3.83 (m, 1 H, C*Hα*-Ala), 2.92 [m, 1 H, C(*Hα*Hβ)-Phe$_1$; 1 H, C(*Hα*Hβ)-Phe$_2$], 2.69 [m, 1 H, C(Hα*Hβ*)-Phe$_1$; 1 H, C(Hα*Hβ*)-Phe$_2$], 2.01 [m, 1 H, C*H*(CH$_3$)$_2$-Val], 1.09 (m, 3 H, C*H*$_3$-Ala), 0.85 [d, *J* = 6.9, 3 H, C(C*H*$_3$)$_2$-Val], 0.83 [d, *J* = 6.9, 3 H, C(C*H*$_3$)$_2$-Val]. ESI-MS: *m*/*z* = 872.93 ([MH]$^+$, 100%). $C_{52}H_{52}N_6O_7$ (873.01) calcd. C 71.54, H 6.00; N 9.63; found: C 71.36, H 5.88, N 9.44.

Synthesis of Macrocycle 1: A solution of **2·TFA** (134.9 mg, 0.14 mmol) in dry DCM (70 mL, 2 mM) was sequentially treated with DIPCDI (64 μL, 3.0 equiv.), HOBT (56 mg, 3.0 equiv.), and DIEA (70 μL, 3.0 equiv.). The mixture was stirred at room temperature overnight. The solvent was then removed to give a crude product, which was purified by HPLC to give 81.8 mg (70% yield, 27% overall yield for the whole 12-steps sequence) of macrocycle **1**. White solid; m.p. 165–168 °C. $[\alpha]_D$ = +40 (*c* = 0.02, MeOH). IR (KBr): $\tilde{\nu}$ = 3416, 3057, 3028, 2963, 2927, 1646, 1518, 1439, 1201, 1174, 753, 699. ^{1}H NMR (500 MHz, 27 °C, $CDCl_3$, mixture of atropisomers): δ = 9.24 (br. d, *J* = 8.4), 8.80 (br. s), 8.69 (br. s), 8.10–7.85 (m), 7.65–6.90 (m), 6.85 (m), 6.75 (m), 6.58 (br. d, *J* = 6.9), 6.40 (br. s), 6.16 (br. s), 6.09 (br. s), 6.02 (br. d, *J* = 6.8), 5.96 (br. d, *J* = 7.8), 5.79 (br. d, *J* = 6.3), 5.55 (m), 4.94 (m), 4.79 (m), 4.65 (m), 4.52 (m), 4.45–4.25 (m), 4.09 (m), 3.90 (m), 3.82 (br. t, *J* = 6.3), 3.70 (br. t, *J* = 5.2), 3.63 (br. d, *J* = 8.3), 3.50 (br. d, *J* = 6.8), 3.47 (br. d, *J* = 5.4), 3.40 (br. t, *J* = 5.1), 3.16 (m), 3.01 (m), 2.99–2.83 (m), 2.77–2.51 (m), 2.29 (m), 1.93 (m), 1.32 (d, *J* = 7.2), 1.27 (d, *J* = 6.9), 1.22 (d, *J* = 5.4), 1.17 (d, *J* = 7.2), 0.97 (d, *J* = 7.3), 0.92–0.77 (m), 0.73 (d, *J* = 6.5), 0.71 (d, *J* = 5.7), 0.66 (d, *J* = 6.9), 0.60 (d, *J* = 7.5), 0.49 (dd, *J* = 6.8, 2.3). ^{1}H NMR (300 MHz, 40 °C, CD_3CN, mixture of atropisomers): δ = 8.77 (s), 8.68 (br. d, *J* = 9.3), 8.27 (m), 8.16 (m), 8.05 (m), 7.92 (d, *J* = 8.1), 7.84 (dd, *J* = 7.8, 1.2), 7.70 (m), 7.62 (td, *J* = 7.7, 1.4), 7.55–6.93 (m), 6.89 (m), 6.87–6.76 (m), 6.68 (m), 6.60 (br. d, *J* = 7.8), 6.53 (m), 6.39 (m), 4.67 (td, *J* = 9.3, 4.4), 4.58–4.42 (m), 4.34 (m), 4.11 (m), 3.78 (m), 3.67 (t, *J* = 5.4), 3.35 (m), 3.12 (t, *J* = 6.1), 2.76 (d, *J* = 3.8), 2.71 (d, *J* = 4.4), 2.57 (dd, *J* = 14.2, 9.7), 2.30 (d, *J* = 9.3), 2.25 (dd, *J* = 9.3, 3.3), 1.32 (m), 1.23 (d, *J* = 7.3), 1.13 (d, *J* = 4.9), 1.10 (d, *J* = 8.8), 0.95–0.85 (m), 0.83–0.74 (m), 0.71 (d, *J* = 5.8), 0.64 (d, *J* = 6.9). ^{1}H NMR (600 MHz, [D$_6$]DMSO, 50 °C, mixture of atropisomers): δ = 9.48 (d, *J* = 7.9, 1 H, NH-Phe$_2$), 9.42 (d, *J* = 8.7, 1 H, NH-Phe$_1$), 8.98 (s, 1 H, NH-biph.), 8.42 (s, 1 H, NH-biph.), 8.25 (broad d, 1 H, NH-Ala), 7.76 (d, *J* = 7.8, 1 H, H$_{arom}$), 7.71 (d, *J* = 7.8, 1 H, H$_{arom}$), 7.61 (t, *J* = 7.5, 1 H, Ha$_{rom}$), 7.65 (d, *J* = 8.3, 1 H, NH-Val), 7.45–7.35 (m, 5 H, H$_{arom.}$), 7.29–7.18 (m, 6 H, H$_{arom.}$), 7.15–7.06 (m, 7 H, H$_{arom.}$), 6.96 (d, *J* = 7.4, 1 H, H$_{arom}$), 6.89 (*d*, *J* = 7.4, 1 H, H$_{arom}$), 6.54 (m, 2 H, H$_{arom}$), 6.19 (d, *J* = 7.5, 1 H, H$_{arom}$), 4.61 (m, 1 H, C*Hα*-Phe$_1$), 4.33 (m, 1 H, C*Hα*-Val), 4.17 (m, 1 H, C*Hα*-Phe$_2$), 3.78 (m, 1 H, C*Hα*-Ala), 2.60 [m, 1 H, C(*Hα*CHβ)-Phe$_1$; 1 H, C(*Hα*CHβ)-Phe$_2$], 2.13 [m, 1 H, C(HαC*Hβ*)-Phe$_1$], 1.95 [m, 1 H, C(HαC*Hβ*)-Phe$_2$; 1 H, C*H*(CH$_3$)$_2$-Val], 1.19 (d, *J* = 7.0, 3 H, C*H*$_3$-Ala), 0.58 [d, *J* = 6.8, 3 H, C(C*H*$_3$)$_2$-Val], 0.52 [d, *J* = 6.8, 3 H, C(C*H*$_3$)$_2$-Val]. ESI-MS: *m*/*z* = 855.3 ([MH]$^+$, 100%). $C_{52}H_{50}N_6O_6$ (854.99) calcd. C 73.05, H 5.89, N 9.83; found: C 73.28, H 5.91, N 9.63.

Acknowledgments

This work was partially supported by Ministerio de Ciencia y Tecnología (MCyT) (BQU2003-00089 and 2002-02047), Ministerio de Educacíon y Ciencia (MEyC) (CTQ2004-01978), and the Fundació Parc Científic de Barcelona (Barcelona Science Park Foundation). We thank Mercedes Alonso (CSIC) for determining the biological activity of **1** and Mónica Luengo (Barcelona Science Park, University of Barcelona) for assistance in the HPLC purification.

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[18] a) All the amino acids are of the L-series, and the abbreviations are based on the standard three-letter code; b) The reagents and solvents are abbreviated as follows: AB = Wang acid linker, All = allyl, CDI = 1,1′-carbonyldiimidazole, DIEA = *N*,*N*′-diisopropylethylamine, DIPCDI = *N*,*N*′-diisopropylcarbodiimide, DMAP = 4-(dimethylamino)pyridine, DCM = dichloromethane, DMF = *N*,*N*-dimethylformamide, DPPA = diphenyl phosphorazidate, EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, Fmoc = 9-fluorenylmethoxycarbonyl, HOAt = 1-hydroxy-7-azabenzotriazole, HOBt = 1-hydroxybenzotriazole, MBHA = 4-methylbenzhydrylamine, PS = polystyrene, PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, TFA = trifluoroacetic acid.

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(the distance between the two ends varies from 3.28 to 5.47 Å) were quite abundant.
[21] EDC/HOBt/DIEA and DPPA/$NaHCO_3$ also gave the target compound, although the DIPCDI/HOBt method gave the macrocycle **1** with higher purity.
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Received: September 23, 2006
Published Online: January 17, 2007

