Synthesis of Spirobicyclic Peptides on a Solid Support

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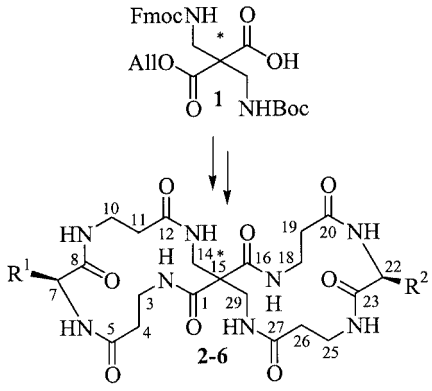
The spirobicyclic peptides **2**–**6** were synthesized as stereoisomeric pairs using an orthogonally protected bis(aminomethyl)malonic acid building block **1** as the branching unit. Peptide **2** was synthesized by two methods. Either the chain assembly and first cyclization were carried out on a Wang resin, and the second cyclization in solution (Scheme 1), or the whole synthesis was performed on a solid-supported backbone amide linker derived from 4-alkoxybenzaldehyde (Scheme 3). The applicability of the latter method was further evaluated by synthesis of four additional spirobicyclic peptides **3**–**6**.

Introduction

Conformationally constrained cyclic analogs of biologically active peptides are useful tools for defining the structural requirements for binding to receptors.[1] Additional cyclization still increases the rigidity, and hence the binding properties of bicyclic peptides and their analogs are of special interest.[2] In spite of this, relatively few synthesis of bicyclic peptides have been described either in solution[3] or on a solid support.[4]

Homodetic spiropeptides, i.e. spirobicyclic peptides obtained by backbone cyclization, constitute an interesting subclass of bicyclic peptides that may exhibit binding properties characteristic for spiroheteromacrocyclic compounds.[5] We now report on the synthesis of such peptides by two alternative manners. Previously, the orthogonally protected bis(aminomethyl)malonic acid building block **1** has been prepared by us and exploited in the construction of backbone cyclized/branched peptide conjugates.[6] The same building block has now been used to obtain homodetic spirobicyclic peptides (Figure 1). In fact, it appears to be the smallest building block that may be designed for such a purpose. The peptide chain containing **1** may be assembled and the first cyclization is carried out on a Wang resin, while the second cyclization is done in solution after release of the lariat-like peptide from the support. Alternatively, the whole synthesis may be performed on a solid support bearing a 4-alkoxybenzaldehyde-derived backbone amide handle. Although the former method appears to give somewhat higher yields, as indicated by the preparation of heptapeptide **2** by both procedures, the latter approach, as a pure solid support synthesis, undoubtedly is the method of choice for parallel synthesis of peptide libraries. Hence, the applicability of this method has been further tested by preparation of the four additional spirobicyclic peptides **3**−**6**. To the best of our knowledge, this is the first description for the synthesis of spiropeptides.



2 $R^1 = CH_2Ph$, $R^2 = H$
3 $R^1 = R^2 = CH_2Ph$
4 $R^1 = CH_2CH(CH_3)_2$, $R^2 = H$
5 $R^1 = R^2 = CH_2CH(CH_3)_2$
6 $R^1 = CH_2Ph$, $R^2 = CH_2CH(CH_3)_2$

Figure 1. Orthogonally protected bis(aminomethyl)malonic acid **1** as a constituent of the spirobicyclic peptides **2**−**6**

Results and Discussion

Synthesis of Spirobicyclic Peptides

Two alternative methods were applied to obtain the spirobicyclic heptapeptide **2**. The first of them (Method 1) is outlined in Scheme 1. Accordingly, a Wang resin was first loaded with *N*-Fmoc-β-alanine using a standard symmetrical anhydride method (**7**, loading 90 μmol·g^{-1}). The linear peptide chain containing **1** was then assembled by the Fmoc

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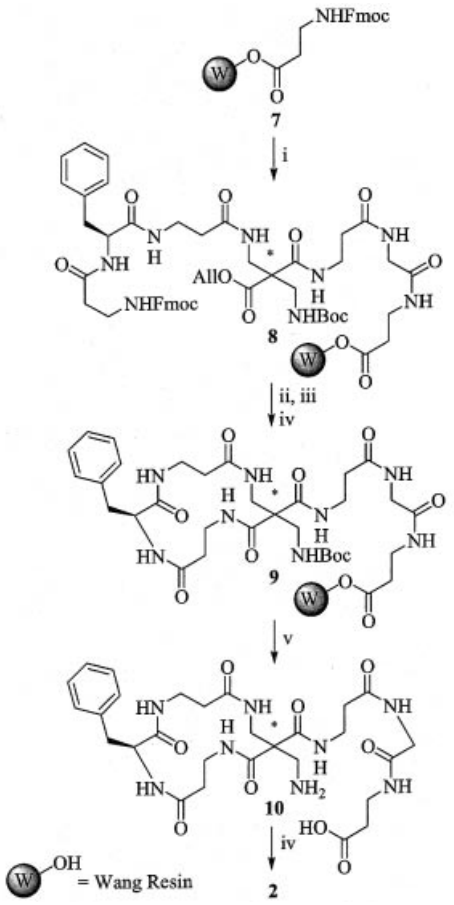


Scheme 1. i) SPPS; ii) $(Ph_3P)_4Pd^0$, 0.5 M HCl, morpholine, DMSO, THF; iii) piperidine, DMF; iv) HATU, DIPEA, DMF; v) TFA, CH_2Cl_2

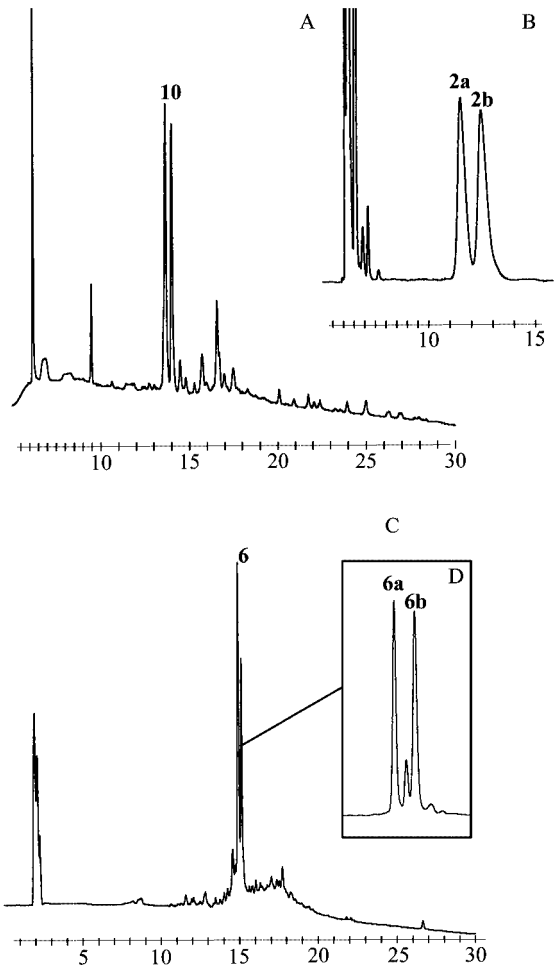
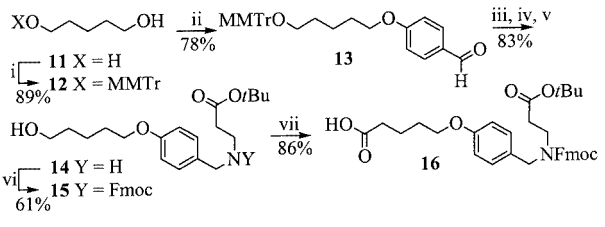


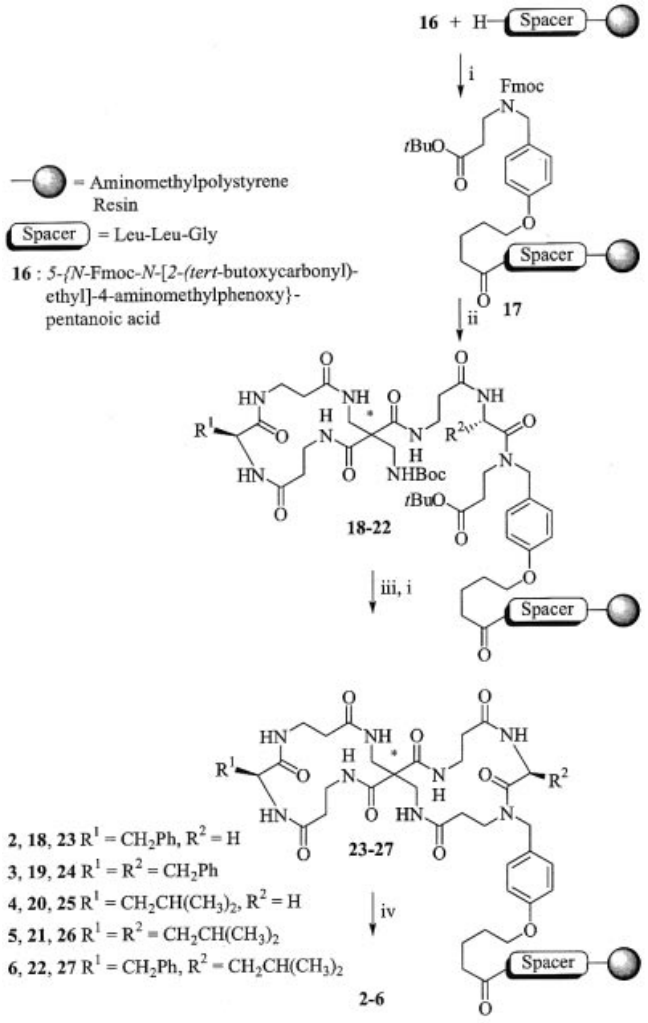
Figure 2. HPLC chromatograms of the synthesis products as diastereomeric pairs; A: Lariat-like peptide (**10**, t_r = 13.64 and 14.00 min); B: spirobicyclic peptide (**2a**, t_r = 11.5 min and **2b**, t_r = 12.4 min) from the cyclization in solution (Method 1); C: an example of a spirobicyclic peptide (**6**, t_r = 14.9 and 15.2 min) synthesized by Method 2; D: purified **6** in isocratic system (**6a**: t_r = 9.0 min; **6b**: t_r = 10.4 min); gradient from aqueous TFA to acetonitrile in 30 min (A and C), 5% acetonitrile in 0.1% aqueous TFA (**B**), 20% acetonitrile in 0.1% aqueous TFA (D), Hypersil Hypurity C18 (150 × 4.6 mm, 5 μm), flow 1.0 mL·min^{-1}, detection at 215 nm

chemistry exploiting established activation methods (HATU activation[7] for **1** and DCC/HOBt activation[8] for the other amino acids). After completion of the chain assembly, the *N*-terminal Fmoc group and the allyl protection[9] of the branching unit were removed, and the first cyclization was carried out (5 equiv. HATU, 10 equiv. DIPEA in DMF, 4 h at room temperature). Insertion of **1** in the peptide chain creates a stereogenic center at the branching carbon atom, and therefore the monocyclic lariat-like peptide **10** when cleaved from the support was obtained as a pair of diastereomers. The side chain (R^1) of the Phe residue and carbonyl carbon atom 16 (for numbering, see Figure 1) can either be on the same or opposite site of the ring. The overall yield of the diastereomers purified by HPLC was 50%. Before the second cyclization in solution, the solution of **10** was neutralized and desalted. The cyclization was performed in diluted solution (1 mM **10** in DMF, 5 equiv. HATU, 10 equiv. DIPEA, 4 h at room temperature) to avoid dimerization. The spirobicyclic diastereomers were easily separated by isocratic HPLC (**2a** and **2b**, see B in Figure 2). Both isomers were obtained in a 13% overall isolated yield.

The same spiroheptapeptide **2** was also prepared entirely on a solid support (Method 2). The linker employed was the 4-alkoxybenzaldehyde backbone amide linker, introduced originally by Bourne et al.[10] for the Boc chemistry. The first building block, β-Ala-O*t*Bu, was, however, coupled to the linker precursor, viz. 4-[5-(4-methoxytrityl)oxypentyloxy]benzaldehyde (**13**), by reductive amination in solution (Scheme 2), and the secondary amine was then protected with an Fmoc group. Detritylation and Jones oxidation then gave the linker **16**, which was attached to an



Scheme 2. Synthesis of the linker **16**: i) MMTrCl, Py; ii) 4-hydroxybenzaldehyde, DEAD, Ph_3P, THF; iii) H-β-Ala-O*t*Bu·HCl, DIPEA, Na_2SO_4, CH_2Cl_2; iv) $NaBH_3CN$, AcOH, MeOH; v) AcOH; vi) FmocCl, DIPEA, dioxane; vii) CrO_3, H_2O, H_2SO_4, acetone



Scheme 3. i) HATU, DIPEA, DMF; ii) SPPS; iii) TFA, CH_2Cl_2; iv) HBr, AcOH, TFA

aminomethyl-PS support bearing a short peptide spacer (Leu-Leu-Gly) (**17**, loading 90 μmol·g^{-1}, Scheme 3). To the subsequent chain assembly, the Fmoc chemistry was applied. The use of the *t*Bu ester of β-Ala as the first residue is essential, since it eliminates the diketopiperazine formation that has frequently been encountered with backbone amide linkers during the Fmoc deprotection of the penultimate amino acid.[11] Diketopiperazine is a six-membered ring, and therefore it is not formed when using a β-amino acid. After the Fmoc deprotection, the exposed secondary amino group of **17** was acylated with $(FmocGly)_2O$ [15 equiv. in DCM/DMF (9:1), 5 h at room temperature]. An acid anhydride was used as the acylating agent, since symmetrical anhydrides have been reported to give higher yields in this kind of difficult acylations than the corresponding acids activated with normal coupling reagents.[11a,12] The monocyclic lariat-like peptide **18** was then obtained as described above for the preparation of **9** by Method 1. The *N*-Boc protection of the branching unit and the *Ot*Bu protection of the linker-bound β-alanine were removed in a single step (25% TFA in DCM, 1 h at room temperature), and the second on-resin cyclization was performed (5 equiv. HATU, 10 equiv. DIPEA in DMF, 4 h at room temperature). Cleavage by HBr/AcOH/TFA (1:3:40, v/v/v, 3 h at 4 °C, repeated three times) released the bicyclic peptide as a stereoisomer pair (**2**). The overall isolated yield after HPLC purification was 6% for both isomers.

As indicated above, **2** was obtained in a higher yield by performing only the first cyclization on a solid support. The drawback of this method, however, is that a laborious HPLC purification is needed before the second cyclization. For this reason, and since a pure solid support undoubtedly is the method of choice for the parallel synthesis of peptide libraries, the rest of the spirobicyclic peptides **3**−**6** were synthesized by Method 2. It is also worth noting that performance of two subsequent homodetic cyclizations on a single support still is a challenge, and hence additional examples of such procedures are desirable. The yields of the isolated diastereomeric pairs ranged from 10 to 15%. In each case diastereomeric mixtures were first purified and the isomers were then separated by isocratic elution. The attempts to obtain the enantiomerically pure bis(aminomethyl)malonic acid building block **1** failed, and hence separation of the final products **2**−**6** by simple isocratic elution (4−20% MeCN in 0.1% aqueous TFA) to pure diastereomers was applied. The authenticity of all products was verified by HPLC/ESI-MS (Table 1). In addition, **2a** and **2b** prepared in a larger quantity were chacterized by HRMS, ^{1}H NMR and ^{13}C NMR (including PDQF, HMQC and HMBC spectra).

Table 1. Required (M_{req}) and found ($[M + H]^+_{found}$, LC/ESI-MS/MS) molecular masses for the spirobicyclic peptides **2**−**6**

Diastereomer pair	$[M + H]^+_{found}$	M_{req}
2	615.7	614.3
3	705.8	704.3
4	581.7	580.3
5	637.8	636.4
6	671.8	670.3

All the spirobicyclic peptides prepared contain four β-alanine residues, having only two positions to be randomized with natural α-amino acids. While this to some extent reduces the diversity of the library obtained, it ensures efficient backbone cyclization.[6] Further studies are undoubtedly needed to find out whether the diversity may be still increased by using only natural amino acids.

Experimental Section

General Remarks: The NMR spectra were recorded with Bruker 200 NMR, JEOL JNM-GX 400 and JEOL JNM-A 500 spectrometers. The chemical shifts are given in ppm from internal TMS. The mass spectra were recorded with 7070E VG or PE SCIEX API 365 LC/ESI-MS/MS mass spectrometers. Hypersil C18 columns (150 × 4.6 mm, 5 μm, or 250 × 10 mm, 5 μm) were used in the RP HPLC analyses and purifications.

Synthesis of the Spirobicyclic Peptides 2−6

Synthesis of the Diastereomer Pair 2a,b. Method 1: *N*-Fmoc-protected β-alanine (0.24 mmol) and diisopropylcarbodiimide (DIC, 0.12 mmol) were dissolved in CH_2Cl_2 (1.0 mL) and allowed to react for 20 min at 25 °C. Volatile materials were removed, and the residue was suspended in DMF (1.0 mL). The dissolved material was added onto the Wang resin (265 mg, initial loading of the hydroxy groups: 1.03 mmol·g^{-1}) pre-swelled in DMF. The suspension was allowed to react for 1 h at 25 °C, giving a loading of 90 μmol·g^{-1} (determined on the basis of Fmoc). The unchanged hydroxy groups on the support were capped by acetic anhydride before the synthesis. The linear peptide chain **8** was first assembled by Fmoc chemistry using well-established methods of activation, viz. HATU activation for building block **1** and DCC/HOBt activation for the other amino acids. After chain assembly, the allyl protecting group of the carboxy function of the branching residue derived from **1** was removed by treatment with $(Ph_3P)_4Pd^0$ (55 mg, 2 equiv.) in a mixture of DMSO, THF, 0.5 mol·L^{-1} aq. HCl, and morpholine (2:2:1:0.1, v/v/v/v; 5.0 mL) for 45 min at 25 °C under argon. The resin was then washed with DMSO, THF, DMF, Et_3N/DMF (1:1, v/v), CH_2Cl_2, and MeOH in this order, and the Fmoc group was removed with a mixture of piperidine and DMF (1:5, v/v, 15 min), followed by washings with DMF, Et_3N/DMF (1:1, v/v), CH_2Cl_2, and MeOH in this order. After allyl and Fmoc deprotections, the resin was suspended in DMF (4.0 mL), and HATU (46 mg, 5 equiv.) and *N*,*N*-diisopropylethylamine (DIPEA; 42 μL, 10 equiv.) in DMF (1.0 mL) were added to the mixture. After 4 h of shaking at 25 °C, the resin was filtered, washed with DMF, CH_2Cl_2, and MeOH, and dried. The product was cleaved from the resin with a mixture of TFA and CH_2Cl_2 (1:1, v/v, 25 °C, 30 min), and the crude monocyclic peptide was purified by HPLC (Figure 2, A), yielding 9.2 mg (50%) of the TFA salt of the two stereoisomers of **10** (ESI-MS: *m*/*z* = 633.7 $[M + H]^+$). The monocyclic peptide **10** was released from the TFA salt by adding sodium hydroxide (5 mM, 2 equiv.) and desalting the mixture by HPLC (elution with water for 10 min, then a linear gradient from water to MeCN in 10 min, flow rate 1 mL·min^{-1}). The eluent was removed under reduced pressure, and the residue was dissolved in DMF (12 mL). To achieve cyclization through the terminal amino and carboxy functions, HATU (23 mg, 5 equiv.) and DIPEA (21 μL, 10 equiv.) were added, and the mixture was stirred for 4 h at 25 °C. The solvents were evaporated to dryness, the residue was dissolved in 0.1% aq. TFA, and purified by HPLC (Figure 2, B). The spirobicyclic products **2a** and **2b** were separated. The isolated yield for both stereoisomers was 2.0 mg (26% from **10**, overall 13%). **Method 2:** A short peptide spacer (Leu-Leu-Gly) was first attached to an aminomethylpolystyrene resin. Initial loading (0.6 mmol·g^{-1}) was reduced (90 μmol·g^{-1}) and the unchanged amino groups were carefully capped with acetic anhydride. The solid support **17** (130 mg, loading 90 μmol·g^{-1}) employed in the peptide synthesis was then obtained by acylating the tripeptide-derivatized aminomethylpolystyrene resin with **16** (HATU/DIPEA activation in DMF). Treatment with piperidine/DMF (1:5, 15 min) exposed the secondary amino group, which was subsequently acylated with $(Fmoc\text{-}Gly)_2O$ (15 equiv.) at 25 °C for 6 h. The resin-bound lariat-like monocyclic peptide **18**, bearing an *N*-terminal Boc and *O*-terminal *t*Bu protection, was then assembled using the same activation and deprotection methods as described above for the preparation of **9** by Method 1. The Boc and *t*Bu protections were removed in a single step by TFA/CH_2Cl_2 (1:4, v/v, 25 °C, 1 h), followed by washings with CH_2Cl_2, Py/CH_2Cl_2 (1:19, v/v), and MeOH in this order. The resin was dried under reduced pressure, and suspended in DMF (1.5 mL). HATU (22 mg, 5 equiv.) in DMF (0.4 mL) and DIPEA (20 μL, 10 equiv.) were added, and the mixture was shaken for 4 h at 25 °C. The resin was filtered, washed with DMF, CH_2Cl_2, and MeOH, and dried. Cleavage by HBr/AcOH/TFA (1:3:40, v/v/v, 4 °C, 3 h, three times) released the stereoisomer pair **2**. The overall isolated yield after HPLC purification was 6% for each stereisomer.

Diastereomer 2a: ^{1}H NMR (500 MHz, [D$_6$]acetone + D$_2$O, ppm): δ = 7.30−7.26 (m, 4 H, Ph), 7.22−7.18 (m, 1 H, Ph), 4.51 (dd, *J* = 9.7 Hz, 1 H and 5.4 Hz, HC-7), 3.88 (d, *J* = 16.4 Hz, 1 H, *H*HC-22), 3.79 and 3.70 (d, 1 H, *J* = 14.4 Hz and d, 1 H, *J* = 14.5 Hz, *H*HC-14 and *H*HC-29), 3.71 (d, *J* = 16.4 Hz, 1 H, H*H*C-22), 3.67−3.63 (m, 1 H, *H*HC-3), 3.57 and 3.56 (d, 1 H, *J* = 14.5 Hz and d, 1 H, *J* = 14.4 Hz, H*H*C-14 and H*H*C-29), 3.60−3.35 (m, 6 H, H_2C-16, H_2C-18 and H_2C-25), 3.34−3.27 (m, 1 H, H*H*C-3), 3.19 (dd, *J* = 13.9 Hz, 1 H and 5.4 Hz, C*H*HPh), 2.89 (dd, *J* = 13.9 Hz, 1 H and 9.7 Hz, CH*H*Ph), 2.67−2.55 and 2.48−2.36 (m, 3 H and m, 2 H, *H*HC-4, H_2C-11 and H_2C-19), 2.43−2.39 (m, 2 H, H_2C-26), 2.30−2.24 (m, 1 H, H*H*C-4) (Figure 3). ^{13}C NMR (125 MHz, [D$_6$]acetone + D$_2$O, ppm): δ = 177.03 and 176.99 (C-12 and C-27), 176.4 (C-20), 175.5 (C-5), 174.9 (C-8), 173.4 (C-23), 173.1 and 172.9 (C-1 and C-16), 140.5, 131.9, 131.1, and 129.3 (Ph), 62.1 (C-15), 58.4 (C-7), 46.4 (C-22), 44.5 and 44.1 (C-14 and C-29), 39.4 (*C*H_2Ph), 39.1 and 37.9 (C-10 and C-18), 38.9 (C-3), 38.4 (C-25), 38.3 and 36.8 (C-11 and C-19), 38.2 (C-26), 37.3 (C-4). PDQF, HMQC and HMBC spectra were also utilized. HRMS (EI): calcd. for $C_{28}H_{38}N_8O_8$ $[M^+]$ 614.281; found 614.283.

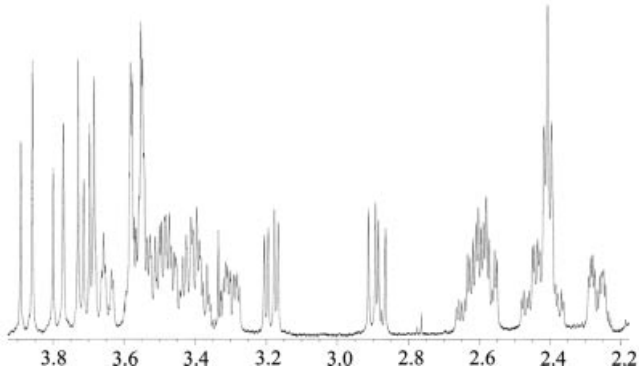


Figure 3. ^{1}H NMR (500 MHz, [D$_6$] + D$_2$O) spectrum of **2a**

Diastereomer 2b: ^{1}H NMR (500 MHz, [D$_6$]acetone + D$_2$O, ppm): δ = 7.32−7.26 (m, 4 H, Ph), 7.21−7.18 (m, 1 H, Ph), 4.51 (dd, *J* = 9.5 Hz, 1 H and 5.5 Hz, HC-7), 3.87 (d, *J* = 14.4 Hz, 1 H, *H*HC-29), 3.81 (s, 2 H, H_2C-22), 3.74 (d, *J* = 14.7 Hz, 1 H, *H*HC-14), 3.75−3.70 (m, 1 H, *H*HC-18), 3.69−3.64 (m, 1 H, *H*HC-25), 3.57 (d, *J* = 14.7 Hz, 1 H, H*H*C-14), 3.58−3.52 (m, 1 H, *H*HC-10), 3.51−3.46 (m, 1 H, *H*HC-3), 3.48 (d, *J* = 14.4 Hz, 1 H, H*H*C-29), 3.43−3.38 (m, 1 H, H*H*C-3), 3.37−3.24 (m, 3 H, H*H*C-18, H*H*C-25 and H*H*C-10), 3.17 (dd, *J* = 14.0 Hz, 1 H and 5.4 Hz, C*H*HPh), 2.87 (dd, *J* = 14.0 Hz and 9.4 Hz, CH*H*Ph), 2.60−2.48 (m, 4 H, *H*HC-4, H_2C-19 and *H*HC-11), 2.45−2.37 (m, 3 H, H*H*C-4 and H_2C-26), 2.26 (ddd, 1 H, *J* = 15.2, 5.2 Hz and 2.7 Hz, H*H*C-4) (Figure 4). ^{13}C NMR (125 MHz, [D$_6$]acetone + D$_2$O, ppm): δ = 177.1 (C-12), 176.7 (C-27), 176.3 (C-20), 175.5 (C-5), 174.7 (C-8), 173.3 (C-23), 173.03 and 172.98 (C-1 and C-16), 140.5, 131.9, 131.1, and 129.2 (Ph), 62.2 (C-15), 58.2 (C-7), 46.3 (C-22), 44.2 (C-14), 44.0 (C-29), 39.04 and 39.03 (C-3 and *C*H_2Ph), 38.84 and 38.77 (C-18 and C-26), 38.11 and 38.07 (C-11 and C-25), 38.0 (C-10), 37.5 (C-19), 36.9 (C-4). PDQF, HMQC and HMBC spectra were also utilized. HRMS (EI): calcd. for $C_{28}H_{38}N_8O_8$ $[M^+]$ 614.281; found 614.284.

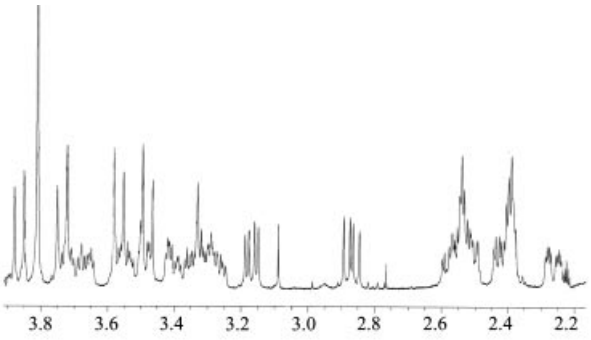


Figure 4. ^{1}H NMR (500 MHz, [D_6] + D_2O) spectrum of **2b**

Syntheses of the Diastereomer Pairs 3−6: The spirobicyclic peptides **3−6** were synthesized analogously to **2** by Method 2. The overall isolated yields as stereoisomeric pairs were 10−15%. All stereoisomers were first purified (Figure 2, C), and the stereoisomers were then separated by an isocratic elution (4−20% MeCN in 0.1% aqueus TFA, Figure 2, D). The authenticity of the products was verified by HPLC/ESI-MS. Calulated and found molecular masses M_{req} and $[M + H]^+$ for all the spirobicyclic peptides **2−6** are shown in Table 1.

Synthesis of the Linker 16

4-[5-(4-Methoxytrityloxy)pentyloxy]benzaldehyde (13): 4-Methoxytrityl chloride (5.0 g, 16 mmol) was added to a stirred mixture of 1,5-pentanediol (**11**, 7.4 mL, 70 mmol) and pyridine (50 mL). The mixture was agitated overnight at room temperature, and then all volatile substances were removed. The residue was dissolved in CH_2Cl_2, washed with water and brine, dried with Na_2SO_4, and the solvents were evaporated to dryness. The residue was purified by silica gel chromatography (30% EtOAc in petroleum ether) to yield 5.4 g (89%) of **12** as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ = 7.37−7.06 (m, 12 H, MMTr), 6.72 (m, 2 H, MMTr), 3.68 (s, 3 H, CH_3O), 3.51 (t, *J* = 6.2 Hz, 2 H, C*H*$_2$OH), 2.96 (t, *J* = 6.4 Hz, 2 H, MMTrOC*H*$_2$), 1.62−1.28 (m, 6 H, CH_2(C*H*$_2$)$_3$CH_2). The tritylated pentanol **12** (1.0 g, 2.7 mmol), 4-hydroxybenzaldehyde (0.36 g, 2.9 mmol) and Ph_3P (0.77 g, 2.9 mmol) were dissolved in THF (20 mL). Diethyl azodicarboxylate (DEAD, 0.60 g, 3.4 mmol) was added dropwise to the mixture, which was stirred overnight at room temperature. The volatile substances were removed under reduced pressure, and the crude reaction product was purified by silica gel chromatography (CH_2Cl_2) to yield 0.99 g (78%) of **13** as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 9.87 (s, 1 H, CHO), 7.80 (d, *J* = 8.6 Hz, 2 H, Ph), 7.45−7.19 (m, 12 H, MMTr), 6.96 (d, *J* = 8.6 Hz, 2 H, Ph), 6.81 (d, *J* = 8.7 Hz, 2 H, MMTr), 4.01 (t, *J* = 6.4 Hz, 2 H, C*H*$_2$OPh), 3.78 (s, 3 H, CH_3O), 3.09 (t, *J* = 6.3 Hz, 2 H, MMTrOC*H*$_2$), 1.79 (m, 2H,C*H*$_2$CH$_2$OPh), 1.69 (m, 2 H, MMTrOCH$_2$C*H*$_2$), 1.57 (m, 2 H, C*H*$_2$(CH$_2$)$_2$OPh). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 190.8 (CHO), 164.2, 158.4, 144.9, 136.1, 131.9, 130.3, 129.7, 128.4, 127.7, 126.7, 114.7, 112.9 (MMTr and Ph), 86.0 (C_q, MMTr), 68.2 (*C*H$_2$OPh), 63.1 (MMTrO*C*H$_2$), 55.2 (CH_3O), 29.7 (*C*H$_2$CH$_2$OPh), 28.8 (MMTrOCH$_2$*C*H$_2$), 22.7 [*C*H$_2$(CH$_2$)$_2$OPh]. MS (EI): *m*/*z* (%) = 273 (100) [$MMTr^+$], 480 (14) [M^+]. HRMS (EI): calcd. for $C_{32}H_{32}O_4$ [M^+] 480.2301; found 480.2307.

***tert*-Butyl 3-{[4-(5-Hydroxypentyloxy)benzyl]amino}propanoate (14):** The aldehyde **13** (0.90 g, 1.9 mmol), the *tert*-butyl ester of β-alanine hydrochloride (0.34 g, 1.9 mmol), *N*,*N*-diisopropylethylamine (DIPEA, 0.33 mL, 1.9 mmol), and excess of Na_2SO_4 were stirred in CH_2Cl_2 (20 mL) for 2 d at room temperature. Solids were filtered off, and the filtrate was concentrated to yield a yellow oil. The oil was dissolved in a cold (+4 °C) mixture of AcOH and MeOH (AcOH/MeOH, 3:200, v/v, 40 mL), and $NaBH_3CN$ (0.24 g, 3.7 mmol) was added to the mixture, which was allowed to warm to room temperature, stirred for additional 5 h, and the solvents were evaporated to dryness. The resulting oil was diluted with AcOH (30 mL), stirred overnight at room temperature, and then the volatiles were removed. The residue was diluted with CH_2Cl_2, washed with water and brine, dried with Na_2SO_4, and the solvents were evaporated to dryness. The crude product was purified by silica gel chromatography (10% MeOH in CH_2Cl_2) to yield 0.52 g (83%) of **14** as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 7.37 (d, *J* = 8.5 Hz, 2 H, Ph), 6.90 (d, *J* = 8.5 Hz, 2 H, Ph), 4.11 (s, 2 H, PhC*H*$_2$NH), 3.94 (t, *J* = 6.4 Hz, 2 H, C*H*$_2$OPh), 3.66 (t, *J* = 6.4 Hz, 2 H, HOC*H*$_2$), 3.12 (t, *J* = 6.7 Hz, 2 H, NHC*H*$_2$CH$_2$), 2.75 (t, *J* = 6.7 Hz, 2 H, CH_2COO), 1.80 (m, 2 H, C*H*$_2$CH$_2$OPh), 1.62 (m, 2 H, HOCH$_2$C*H*$_2$), 1.53 [m, 2 H, C*H*$_2$(CH$_2$)$_2$OPh], 1.43 [s, 9 H, C(CH$_3$)$_3$]. ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 170.7 (*C*O*t*Bu), 159.9, 131.3, 122.8, 115.1 (Ph), 82.5 (C_q in *t*Bu), 67.9 (*C*H$_2$OPh), 62.6 (HOCH$_2$), 51.5 (PhCH$_2$NH), 42.6 (NH*C*H$_2$CH$_2$), 32.3 (*C*H$_2$COO), 31.6 (*C*H$_2$CH$_2$OPh), 28.9 (HOCH$_2$*C*H$_2$), 28.0 [C(*C*H$_3$)$_3$], 22.3 [*C*H$_2$(CH$_2$)$_2$OPh]. MS (EI): *m*/*z* (%) = 208 (100) [M − (CH$_2$)$_2$COO*t*Bu]$^+$, 280 (28) [M − *t*Bu]$^+$, 337 (3) [M^+]. HRMS (EI): calcd. for $C_{19}H_{31}NO_4$ [M^+] 337.2253; found 337.2251.

***tert*-Butyl 3-{(9-Fluorenylmethoxycarbonyl)[4-(5-hydroxypentyloxy)benzyl]amino}propanoate (15):** 9-Fluorenylmethoxycarbonyl chloride (FmocCl; 1.8 g, 7.1 mmol) was added portionwise to a stirred solution of **14** (2.2 g, 6.4 mmol) and DIPEA (1.1 mL, 6.4 mmol) in dioxane (30 mL). The reaction mixture was stirred overnight at room temperature, and the solvents were evaporated to dryness. The residue was purified by silica gel chromatography (0 to 4% MeOH in CH_2Cl_2) to yield 2.2 g (61%) of **15** as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 7.74 (m, 2 H, Fmoc), 7.59 (d, *J* = 7.1 Hz, 1 H, Ph), 7.48 (d, *J* = 7.1 Hz, 1 H, Ph), 7.38 (m, 2 H, Fmoc), 7.33−7.24 (m, 2 H, Fmoc), 7.11 (d, *J* = 8.0 Hz, 1 H, Ph), 6.94 (d, *J* = 7.9 Hz, 1 H, Ph), 6.79 (m, 2 H, Fmoc), 4.53 (m, 2 H, CH_2O in Fmoc), 4.39, 4.30 (s and s, 2 H, PhC*H*$_2$N), 4.24 (m, 1 H, H9 Fmoc), 3.94 (t, *J* = 6.4 Hz, 2 H, C*H*$_2$OPh), 3.67 (m, 2 H, HOC*H*$_2$), 3.44, 3.27 (m and m, 2 H, NC*H*$_2$CH$_2$), 2.46, 2.17 (m and m, 2 H, CH$_2$C*H*$_2$COO), 1.80 (m, 2 H, C*H*$_2$CH$_2$OPh), 1.70−1.50 (m, 4 H, HOCH$_2$C*H*$_2$C*H*$_2$), 1.43 [s, 9 H, C(CH$_3$)$_3$]. ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 171.1, 170.8 (*C*O*t*Bu), 158.3 (Ph), 156.4, 155.9 (C=O in Fmoc), 143.9, 141.3, 129.1, 128.5, 127.6, 127.0, 124.8, 119.9, 114.5 (Fmoc and Ph), 80.7 (C_q in *t*Bu), 67.7 (*C*H$_2$OPh), 67.1, 67.0 (*C*H$_2$O in Fmoc), 62.7 (*C*H$_2$OH, 50.3, 50.1 (PhC*H*$_2$N), 47.4, 47.3 (C9, Fmoc), 43.2, 42.1 (NC*H*$_2$CH$_2$), 34.4, 34.1 (*C*H$_2$COO*t*Bu), 32.4 (*C*H$_2$CH$_2$OPh), 29.0 (HOCH$_2$*C*H$_2$), 28.0 [C(*C*H$_3$)$_3$], 22.3 [*C*H$_2$(CH$_2$)$_2$OPh]. MS (EI): *m*/*z* (%) = 280 (100) [M − Fmoc − *t*Bu]$^+$, 502 (5) [M − *t*Bu]$^+$, 559 (1.5) [M^+]. HRMS (EI): calcd. for $C_{34}H_{41}NO_6$ [M^+] 559.2934; found 559.2933.

5-(4-{[(2-*tert*-Butoxycarbonylethyl)(9-fluorenylmethoxycarbonyl)amino]methyl}phenoxy)pentanoic Acid (16): A solution of CrO_3 (0.42 g, 4.2 mmol), H_2SO_4 (0.42 mL) and water (1.3 mL) was added dropwise to the stirred solution of **15** (2.1 g, 3.8 mmol) in acetone (75 mL). The mixture was agitated for 2 h at room temperature. $CHCl_3$ (75 mL) and pyridine (0.6 mL) were added to the mixture, and then the resulting organic phase was filtered, washed with brine, dried with Na_2SO_4, and the solvents were evaporated to dry-

ness. The residue was purified by silica gel chromatography (10% MeOH in CH_2Cl_2) to yield 1.9 g (86%) of **16** as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 7.74 (m, 2 H, Fmoc), 7.59 (d, *J* = 7.1 Hz, 1 H, Ph), 7.48 (d, *J* = 7.1 Hz, 1 H, Ph), 7.40−7.20 (m, 4 H, Fmoc), 7.11 (d, *J* = 8.1 Hz, 1 H, Ph), 6.93 (d, *J* = 7.9 Hz, 1 H, Ph), 6.79 (m, 2 H, Fmoc), 4.54 (m, 2 H, C*H*$_2$O in Fmoc), 4.39, 4.30 (s and s, 2 H, PhC*H*$_2$N), 4.24 (m, 1 H, 9-H Fmoc), 3.95 (m, 2 H, C*H*$_2$OPh), 3.46, 3.27 (m and m, 2 H, NC*H*$_2$CH$_2$), 2.44 (m, 3 H, HOOCC*H*$_2$ and NCH$_2$C*H*$_2$COO*t*Bu), 2.18 (m, 1 H, NCH$_2$C*H*$_2$COO*t*Bu), 1.84 (m, 4 H, C*H*$_2$C*H*$_2$CH$_2$OPh), 1.43 [s, 9 H, C(CH$_3$)$_3$]. ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 178.9 (COOH), 171.2, 170.9 (*C*OO*t*Bu), 158.3 (Ph), 156.4,156.0 (C=O in Fmoc), 144.0, 141.4, 129.2, 128.6, 127.7, 127.1, 124.9, 120.0, 114.5 (Fmoc and Ph), 80.7 (C$_q$ in *t*Bu), 67.3 (*C*H$_2$OPh), 67.2 (*C*H$_2$O in Fmoc), 50.4, 50.1 (Ph*C*H$_2$N), 47.5, 47.3 (C-9 Fmoc), 43.2, 42.2 (N*C*H$_2$CH$_2$), 34.4, 34.2 (CH$_2$*C*H$_2$COO*t*Bu), 33.6 (*C*H$_2$CH$_2$OPh), 28.6 (HOOC*C*H$_2$), 28.1 [C(*C*H$_3$)$_3$], 21.4 [*C*H$_2$(CH$_2$)$_2$OPh]. MS (EI): *m*/*z* (%) = 178 (100) [benzofulvene$^+$], 294 (17) [M$^+$ − Fmoc − *t*Bu], 516 (1) [M$^+$ − *t*Bu], 573 (0.5) [M$^+$]. HRMS (EI): calcd. for $C_{34}H_{39}NO_7$ [M$^+$] 573.273; found 573.271.

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