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Solid-Supported Synthesis of Cryptand-like Macrobicyclic Peptides

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A straightforward method for the solid-supported synthesis of cryptand-like bicyclic peptides (**1**−**5**) on a backbone amide linker has been described. For the branching, two novel easily available building blocks, viz. *N*-(4-methoxytrityl)-*N*-(2-nitrobenzenesulfonyl)-protected *N*,*N*-bis(2-aminoethyl)-*β*-alanine (**6**) and *N*-(9-fluorenylmethoxycarbonyl) protected iminodiacetic acid monoallyl ester (**7**), have been employed. The key steps of the synthesis are as follows: (i) stepwise coupling of one amino acid and **6** to the secondary amino group of the linker; (ii) removal of the 2-nitrobenzenesulfonyl group and SPPS by the Fmoc chemistry, using **7** as the penultimate and *tert*-butoxycarbonyl (Boc) protected glycine as the last amino acid; (iii) removal of the 4-methoxytrityl protection and subsequent SPPS by the Fmoc chemistry; (iv) removal of the allyl and Fmoc groups, followed by cyclization; and (v) removal of the Boc and *tert*-butyl groups, followed by cyclization. Final cleavage from the support and removal of benzyl-derived protecting groups gives the desired bicyclic products.

Introduction

Conformationally constrained cyclic peptides have gained interest as structural probes with which the binding characteristics of receptors may be elucidated.[1] Additional cyclization may increase their rigidity further, and hence bicyclic peptides and their analogues constitute an even more useful set of tools for such studies.[2] The spatially defined locations of the side chain functional groups may be systematically varied by changing the amino acid content, which can be utilized as a basis for the interpretation of the structure−affinity studies. Bearing this in mind, it is quite surprising that only a few studies on the solid-supported synthesis of bicyclic peptides are available. Side-chain lactam-bridged bicyclic peptides have been obtained by incorporating two appropriately protected lysine,[3] N^{ϵ}-(1,3-diaminopropionyl)-lysine,[4] or 2-amino-3-[4-(aminomethyl)phenylacetyl]aminopropionic acid[5] building blocks in the peptide chain and coupling the side chain amino groups to the *β*-carboxy group of two aspartate residues. Another approach exploits conjugate group mediated cyclization: a support-bound peptide bearing one 3,5-dihydroxybenzoyl and two 2-fluoro-5-nitrobenzoyl residues is subjected to a SnAr displacement of the fluoro substituents by the benzoyl hydroxy groups.[6] We have recently reported on a solid-supported synthesis of spirobicyclic peptides[7] and bicyclic peptides containing three parallel peptide chains.[8] These syntheses have been based on bis(aminomethyl)malonic acid[9] and bis(aminomethyl)-*β*-alanine[10] as branching units. While the former compound allowed rather convenient solid-supported cyclization to spiro bicyclic peptides, bicyclic peptides containing three parallel chains were obtained in an overall yield less than 5% on using the latter building block for branching. We now report on a more efficient solid-supported synthesis that allows preparation of cryptand-like bicyclic peptides having two parallel and one opposite peptide chains. The method is based on exploitation of a nitrogen atom as the branching

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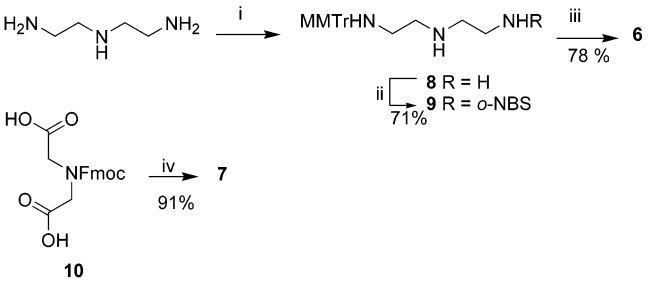
SCHEME 1[a]



[a] Reagents and conditions: (i) MMTrCl, Py. (ii) 2-Nitrobenzenesulfonyl chloride, Et_3N, dioxane. (iii) (1) Ethyl acrylate, DBU, DCM, EtOH; (2) KOH, H_2O, dioxane; (iv) (1) DIC, DMF, DCM; (2) AllOH.

point. Accordingly, peptides **1**−**5**, containing leucine, aspartate, phenylalanine, serine, histidine, and β-alanine residues, were prepared on a backbone amide linker,[11] using branching units **6** and **7** to construct the cryptand-like structure. The otherwise difficult second cyclization is in all likelihood facilitated by rapid pyramidal inversion around the central nitrogen atom in **6** and **7**, which allows the peptide chains to adopt a mutual orientation required for the lactamization without any retardation by incompatible chirality of the branching units.

Results and Discussion

Synthesis of the Branchig Units (6, 7). The branching units (**6**, **7**) used to construct the bicyclic peptides (**1**−**5**) were easily obtained from commercially available starting materials (Scheme 1). *N*,*N*-Bis(2-aminoethyl)-3-aminopropionic acid bearing the 4-methoxytrityl (MMTr) and 2-nitrobenzenesulfonyl (*o*-NBS) protections at the primary amino groups (**6**) was prepared from diethylenetriamine. The 4-methoxytrityl group was first introduced by treating the starting material with 4-methoxytrityl chloride in pyridine (**8**),[12] and the remaining primary amino group was reacted with 2-nitrobenzenesulfonyl chloride in dioxane, resulting in precipitation of 3-aza-N^1-(4-methotrityl)-N^5-(2-nitrobenzenesulfonyl)pentane-1,5-diamine (**9**) as a hydrochloride. Michael-type addition of **9** to the β-carbon atom of ethyl acrylate, followed by alkaline hydrolysis of the ester linkage, then gave *N*-(4-methoxytrityl)-*N*-(2-nitrobenzenesulfonyl)-3-aminopropionic acid (**6**) in an overall 55% yield (calculated from **8**). The monoallyl ester of *N*-(9-fluorenylmethoxycarbonyl) (Fmoc) protected iminodiacetic acid (**7**) was, in turn, obtained in a 90% yield by diisopropylcarbodiimide (DIC) promoted conversion of the commercially available *N*-protected iminodiacetic acid (**10**) to its anhydride that was reacted with allyl alcohol.

Synthesis of the Bicyclic Peptides (1−5). The synthesis of bicyclic peptides (**1**−**5**) is outlined in Scheme 2. A 4-alkoxybenzaldehyde derived backbone amide linker (**11**),[11b] prepared as described previously,[7] was first attached by 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-*b*]pyridinium hexafluorophosphate 3-oxide (HATU)-promoted coupling[13] to aminomethyl polystyrene derivatized with a Leu-Leu-Gly spacer. A loading of 180 μmol·g^{-1} was obtained, according to the amount of benzofulvene released from the Fmoc-protected support-bound linker (**12**) upon deblocking. The exposed secondary amino group of the pre-linked β-alanine residue was acylated with either *N*-Fmoc-Phe-OH (**13**) or *N*-Fmoc-Asp(OBn)-OH (**14**), using a standard anhydride method. It is worth noting that removal of the Fmoc group by a piperidine treatment did not result in aspartimide formation in this position, which often is encountered with peptides having a benzyl-protected aspartate residue.[14] Branching unit **6** was then coupled by HATU activation to the terminal α-amino group to obtain **15** and **16**. The *o*-NBS protection was removed with mild basic thiophenol treatment[15] (PhSH−DBU−DMF 1:1:18), and the first peptide arm was assembled by the HATU/Fmoc chemistry, using branching unit **7** as the penultimate amino acid (**17**, **18**). The *N*-MMTr protection that well tolerated the coupling and deprotection conditions employed was then removed with dichloroacetic acid in dichloromethane (3:97), and the second peptide arm was constructed (**19**−**23**). The allyl group of branching unit **7** and the Fmoc group of the second peptide arm were removed in this order, and then cyclization efficiencies were studied by using bicyclic peptide (**1**) as a model. Three different coupling reagents, viz. HATU, benzotriazol-1-yl-*N*-oxy-tris-(pyrrolidino)phosphonium hexafluorophosphate (PyBOP),[16] and 7-azabenzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP),[17] were employed without additives. A mixture of DMSO and NMP (1:4, v/v) was used as a solvent. This mixture has been reported to minimize on-resin oligomerization,[4] since a combination of high solvent polarity and good peptide-resin swelling impede hydrogen bond formation between the support-bound chains.[18] The first cyclization was rather facile. Each of the coupling reagents at a 2-fold excess in the presence of *N*,*N*-diisopropylethylamine (DIEA, 4 equiv) gave almost complete cyclization in 5 h, as shown by HPLC analysis of small aliquots released from **24**. No sign of imide formation at the iminodiacetyl residue (**7**) was observed during the peptide chain assembly or cyclization. After completion of the first cyclization, the Boc and *tert*-butyl protections were removed in a single step with TFA in dichloromethane (DCM), and then the efficiency of the second cyclization was studied. The coupling procedures indicated above now resulted in incomplete cyclization: 87%, 75%, and 69% with PyBOP, PyAOP, and HATU, respectively [yields calculated from peak areas of **1** (b) and the corresponding starting material (a), Figure 2]. These coupling efficiencies are inconsistent with the earlier results, according

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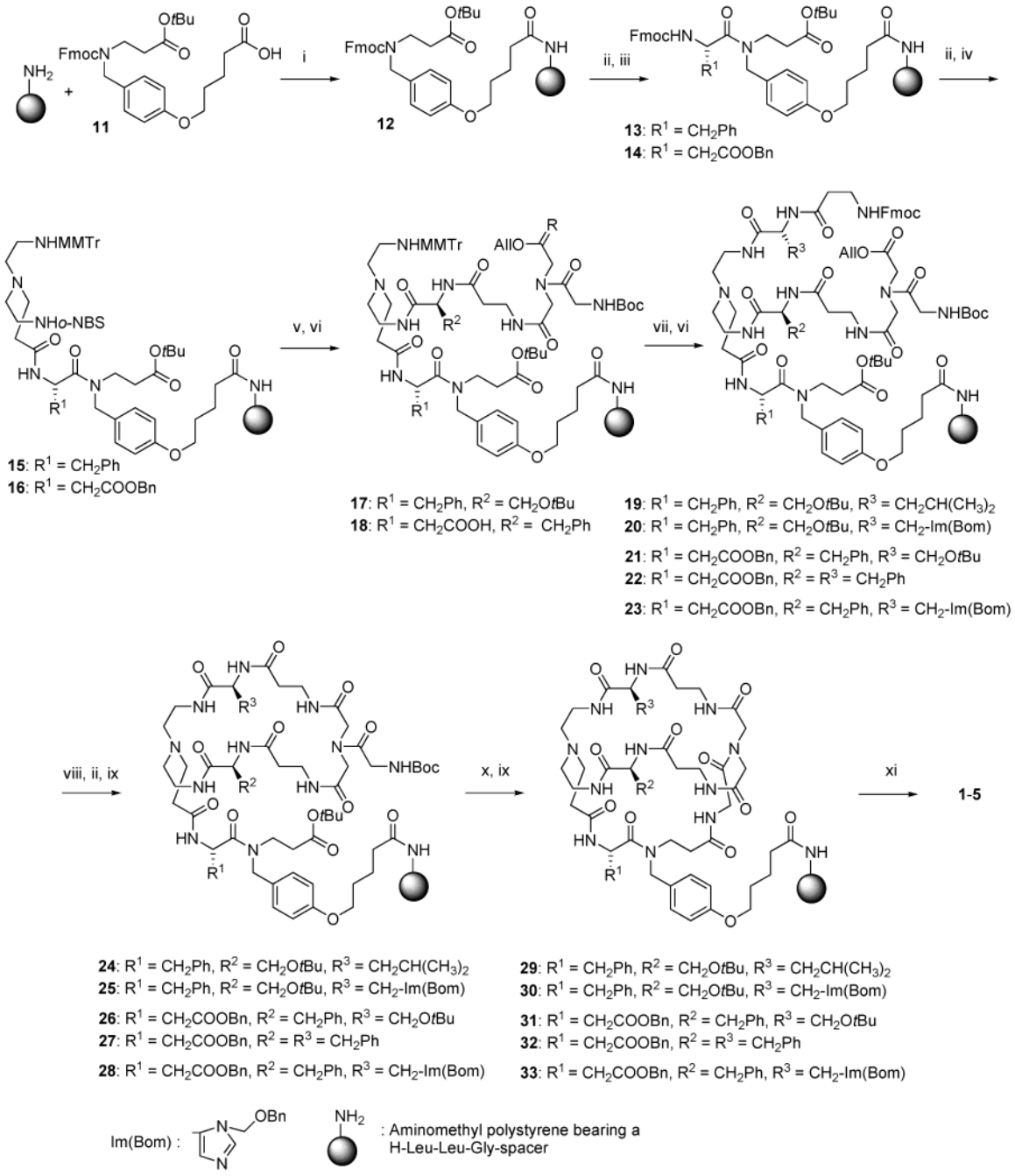
SCHEME 2[a]



[a] Reagents and conditions: (a) (i) HATU, DIEA, DMF. (ii) Piperidine, DMF. (iii) $(FmocPhe)_2O$ or $(FmocAsp)_2O$, DMF, DCM. (iv) ***6***, HATU, DIEA, DMF. (v) PhSH, DBU, DMF. (vi) Fmoc SPPS. (vii) DCA, DCM. (viii) $Pd(AcO)_2$, Ph_3P, Bu_3SnH, AcOH, DCM. (ix) PyAOP, DIEA, DMSO, NMP. (x) TFA, DCM. (xi) HBr, AcOH, anisole, TFA.

to which the cyclization with HOBt-based reagents is usually slower than that with HOAt-based reagents, and with uronium salts slower than with phosphonium salts.[1c,19] The excess of the coupling reagent and DIEA was then increased to 5- and 10-fold, respectively. Under such conditions, the yield of cyclization exceeded 90% with all the coupling agents employed. No marked differences in the overall isolated yields of **1** (15−17%) were observed. Even though PyBOP seemed to be the most efficient cyclization reagent, the isolated yield was highest with PyAOP.[20] Accordingly, PyAOP/DIEA (5/10 equiv, 5 h) was then used for the cyclization of monocyclic peptides **24**−**28** to their bicyclic counterparts. The same treatment was used also for the first cyclization of deprotected **19**−**23**, even though each of the coupling reagents gave already almost complete cyclization at a 2-fold excess indicated above for **24**. Acidolytic cleavage and deprotection, followed by RP HPLC purification (Figures 3 and 4), gave the bicyclic peptides (**1**−**5**) in a

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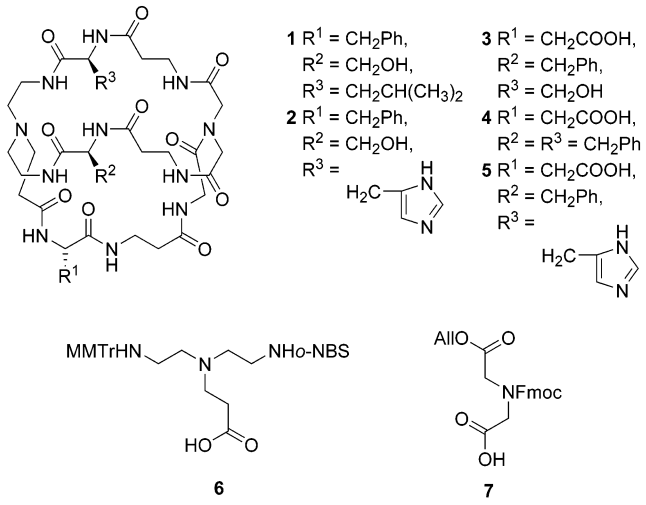

FIGURE 1. Strucures **1**−**7**.

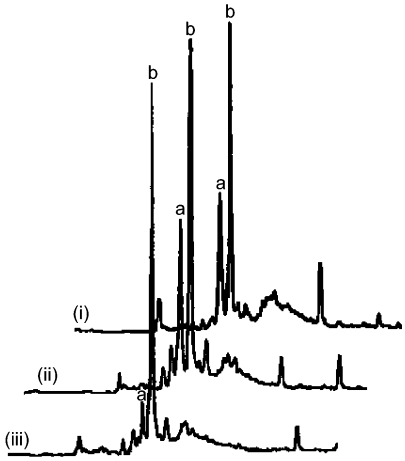

FIGURE 2. Analytical HPLC chromatograms of the crude product mixtures of **1** obtained by using 2 equiv of different coupling reagents in the second cyclization: (i) HATU, (ii) PyAOP, and (iii) PyBOP. The reaction time was 5 h (at room temperature), 4 equiv of DIEA was used as a base, and DMSO−NMP (1:4) was used as a solvent. Notation: (a) starting material, t_r = 13.56 min; (b) bicyclic product (**1**), t_r = 13.94 min. For the chromatographic conditions, see protocol A in the Experimental Section.

5−17% yield (Table 1). The cyclization efficiency itself was not the reason for the lower yields of **2**−**5** compared to **1**, but the presence of trifunctional amino acid residues (Asp, His) and sequence-dependent competition between on-support polymerization and the desired cyclization increased the amount of impurities, as indicated by the presence of a broad signal in the HPLC chromatogram of the crude product (Figure 3). The final products (**1**−**5**) were characterized, in addition to RP HPLC (Figure 4), by HRMS (Table 1) and ^{1}H NMR spectroscopy (Supporting Information).

In summary, easily available branching units **6** and **7** allow solid-supported synthesis of cryptand-like bicyclic peptides in a reasonable yield. The cyclizations occur more readily than with building blocks containing a spiro carbon, such as tris(aminomethyl)acetate or bis(aminomethyl)malonate. It is also worth noting that branching unit **7** is not susceptible to imide formation encountered with structurally related aspartate and glutamate.[21] The amino acids used to construct the peptide bridges may well contain side chain functionalities, allowing randomization of the amino acid composition and hence a library synthesis.

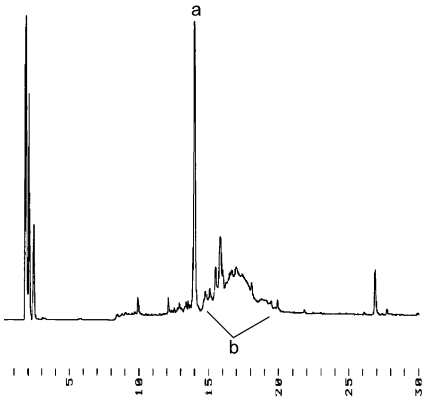

FIGURE 3. An example of analytical RP HPLC chromatograms of a crude product mixture of bicyclic peptide **3**. Notation: (a) bicyclic product (**3**), t_r = 13.98 min, (b) mainly polymerized material. For the chromatographic conditions, see protocol B in the Experimental Section.

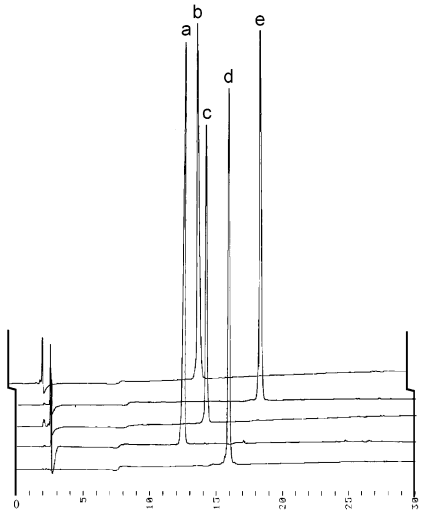

FIGURE 4. Analytical RP HPLC chromatograms of purified bicyclic peptides **1**−**5**. Notation: (a) **2**, t_r = 12.46 min, (b) **5**, t_r = 14.18 min, (c) **3**, t_r = 14.22 min, (d) **1**, t_r = 15.90 min, (e) **4**, t_r = 18.19 min. For the chromatographic conditions, see protocol B in the Experimental Section.

TABLE 1. Required {$[(M + 2H^+)/2]_{req}$} and Found {$[(M + 2H^+)/2]_{found}$} LC/ES-HRMS Molecular Masses and Isolated Yields of the Bicyclic Peptides (1−5) Prepared

bicyclic peptide	yield/%	$[(M + 2H^+)/2]_{found}$	$[(M + 2H^+)/2]_{req}$
1	17	436.7350	436.7349
2	8	448.7240	448.7223
3	5	437.7075	437.7063
4	8	467.7262	467.7245
5	7	462.7199	462.7198

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Experimental Section

3-Aza-N^1-(4-methoxytrityl)-N^5-(2-nitrobenzenesulfonyl)-pentane-1,5-diamine (9). 2-Nitrobenzenesulfonyl chloride (0.92 g, 4.1 mmol) in dioxane (30 mL) was slowly added to a mixture of 3-aza-N^1-(4-methoxytrityl)pentane-1,5-diamine[12] (**8**, 1.6 g, 4.1 mmol) and dioxane (7 mL). Triethylamine (0.58 mL, 4.1 mmol) was added, and the reaction mixture was stirred at room temperature overnight. The product precipitated as a hydrochloride was filtered, washed several times with toluene, and dried under reduced pressure. The salt was partitioned between DCM (100 mL) and 0.1 mol L^{-1} aq sodium hydroxide (100 mL), and the mixture was shaken vigorously. The organic phase was washed with brine, dried over Na_2SO_4, and evaporated to dryness, giving **9** (1.7 g, 71%) as a yellowish foam. The product was used without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11 (m, 1H), 7.78−7.66 (m, 3H), 7.44−7.15 (m, 12H), 6.80 (m, 2H), 3.78 (s, 3H), 3.12 (m, 2H), 2.71 (m, 2H), 2.59 (m, 2H), 2.21 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.8, 148.1, 146.2, 138.1, 133.5, 133.4, 132.6, 131.0, 129.7, 128.5, 127.8, 126.2, 125.3, 113.1, 70.2, 55.2, 49.5, 47.9, 43.1; HRMS (ESI) $[M + H]^+$ required 561.2166, found 561.2168.

***N*-(4-Methoxytrityl)-*N*-(2-nitrobenzenesulfonyl)-3-aminopropionic Acid (6).** Compound **9** (1.7 g, 3.0 mmol) was dissolved in a mixture of DCM (2.0 mL) and EtOH (2.0 mL), and ethyl acrylate (0.48 mL, 4.4 mmol) and DBU (0.44 mL, 3.0 mmol) were added. The mixture was stirred at room temperature for 3 days and then evaporated to dryness. The residue was dissolved in dioxane (60 mL) containing water (4 mL) and KOH (0.8 g, 15 mmol), and the mixture was stirred at room temperature overnight to hydrolyze the ester linkage. The volatile substances were removed, and the residue was dissolved in pyridine (50 mL). The mixture was shaken with a Dowex-50 resin (pyridinium ion form) for 1 h. The resin was filtered off, and the filtrate was evaporated to dryness. The crude product was purified by Silica gel chromatography (0.2% pyridine and 10% MeOH in DCM) to yield **6** (1.5 g, 78%) as a white foam. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04 (m, 1H), 7.68−7.57 (m, 3H), 7.45−7.12 (m, 12H), 6.77 (m 2H), 3.74 (s, 3H), 3.19 (m, 2H), 2.81−2.72 (m, 6H), 2.42−2.34 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz,) δ 175.7, 157.9, 148.1, 145.8, 137.6, 133.4, 133.4, 132.6, 130.6, 129.8, 128.5, 127.9, 126.4, 124.8, 113.2, 70.6, 55.2, 53.9, 53.0, 50.2, 40.3, 39.6, 31.2; HRMS (FAB) $[M + H]^+$ requires 633.238, found 633.237.

***N*-(9-Fluorenylmethoxycarbonyl)iminodiacetic Acid Monoallyl Ester (7).** DIC (0.44 mL, 2.8 mmol) was slowly added to a solution of *N*-Fmoc-iminodiacetic acid (**10**, 1.0 g, 2.8 mmol) in a mixture of DMF (1.0 mL) and DCM (4.0 mL). After 1 h of stirring, allyl alcohol (2.0 mL, 28 mmol) was added, and the agitation of the reaction mixture was continued overnight at room temperature. All volatile materials were removed, and the residue was dissolved in a small amount of ethyl acetate. Partially precipitated diisopropylurea was filtered off, and the filtrate was evaporated to dryness. The crude reaction product was purified by Silica gel chromatography (5% MeOH in DCM) to yield **7** (1.0 g, 91%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76−7.73 (m, 2H), 7.54−7.51 (m, 2H), 7.41−7.36 (m, 2H), 7.31−7.27 (m, 2H), 5.90 (m, 1H), 5.37−5.26 (m, 2H), 4.64 (m, 2H), 4.46 (m, 2H), 4.23 (m, 1H), 4.20 (s, 1H), 4.19 (s, 1H), 4.10 (s, 1H), 4.08 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8, 173.4, 170.1, 169.3, 155.9, 155.8, 143.5, 143.5, 141.3, 131.2, 131.2, 127.8, 127.1, 124.9, 120.0, 119.3, 68.5, 68.4, 66.4, 66.3, 49.8, 49.7, 49.6, 49.3, 47.0, 46.9; HRMS (EI) M^+ requires 395.1369, found 395.1367.

Synthesis of Bicyclic Peptides (1−5). A 300-mg sample of resin **12**[7] having a loading of 180 μmol g^{-1}, determined by the release of benzofulvene from the linker, was used for the synthesis of each bicyclic peptide (**1**−**5**). The Fmoc group was removed with piperidine in DMF (1:4, v/v), and the exposed secondary amino group was acylated by an anhydride method, i.e. by treating the support with 15 equiv of N^{α}-Fmoc protected anhydride of phenylalanine or monobenzylated aspartate in DCM (25 °C, 6 h) to obtain **13** or **14**, respectively. The Fmoc group was removed, and branching unit **6** was coupled (5 equiv of **6**, 5 equiv of HATU, and 10 equiv of DIEA in DMF, 25 °C, 1 h), giving **15** and **16**. The *o*-NBS group was removed by treatment with a mixture of PhSH, DBU, and DMF (5:5:90, 25 °C, 2 h), and the first peptide arm was assembled by using the HATU/Fmoc chemistry described above. Elongation of **15** in a stepwise manner with Fmoc-Ser(*t*Bu)-OH, Fmoc-β-Ala-OH, branching unit **7**, and Boc-Gly-OH gave **17**, while **18** was obtained by using Fmoc-Phe-OH instead of Fmoc-Ser(*t*Bu)-OH for the otherwise similar derivatization of **16**. Prolonged reaction time and higher excess of reagents had to be used for the coupling of Boc-Gly-OH to the secondary amino function of the branching unit **7** (10 equiv of Boc-Gly-OH, 10 equiv of HATU, 20 equiv of DIEA in DMF−DCM, 1:9, 25 °C, 4 h). The 4-methoxytrityl group was then removed from **17** and **18** with DCA in DCM (3:97, v/v), and the second peptide arm was constructed by again using the HATU/Fmoc chemistry. Coupling of either Fmoc-Leu-OH or Fmoc-His(Bom)-OH, followed by Fmoc-β-Ala, to **17** gave **19** and **20**, and coupling of Fmoc-Ser(^{t}Bu)-OH, Fmoc-Phe-OH, or Fmoc-His(Bom)-OH, again followed by Fmoc-β-Ala, to **18** gave peptides **21**−**23**. The allyl ester protection was removed by adding $Pd(OAc)_2$ (2 equiv), $P(Ph)_3$ (12 equiv), AcOH (12 equiv), and Bu_3SnH (24 equiv) to the resins (**19**−**23**) swelled in DCM.[22] The suspensions were allowed to shake for 1 h under argon, and the resins were then filtered, washed with DCM, MeOH, and Et_3N−DMF (1:3, v/v), and dried. The Fmoc group was then removed from the second peptide arm with the conventional piperidine treatment, and the resins were washed with DMF, DCM, Et_3N−DCM (1:3, v/v), and MeOH and dried. For the first cyclization, PyAOP (5 equiv) and DIEA (10 equiv) were added onto the resins swelled in DMSO−NMP (1:4, v/v), and the reaction was allowed to proceed for 5 h at 25 °C. The resins (**24**−**28**) were filtered, washed several times with DMF, DCM, and MeOH, and dried. The unreacted amino functions were then capped with Ac_2O in THF containing *N*-methylimidazole and lutidine. Before the second cyclization, the Boc and *t*-Bu groups were removed in a single step by treatment with TFA−DCM (1:3, v/v, 25 °C, 1.5h), and the resins were washed/neutralized with DCM, pyridine−DCM, DIEA−DCM, and MeOH and dried. The cyclization was then carried out as described above for the first lactamization. The resins were washed several times with DMF, DCM, TFA−DCM (1:9), and MeOH and dried. The bicyclic peptides (**1**−**5**) were cleaved from supports **29**−**33** with a mixture of 33% HBr in AcOH−anisole−TFA (5:5:90 v/v/v, 25 °C, 2 h), evaporated to dryness, and purified by RP HPLC. Each compound was obtained as a white powder, the isolated yields ranging from 5% to 17% (Table 1). The purity (>90%) and retention times of the compounds synthesized are illustrated in Figure 4. The authenticity of the products was verified by HRMS (ESI) (Table 1) and ^{1}H NMR spectroscopy (Supporting Information).

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Supporting Information Available: General procedures and spectral data for compounds 1−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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