Pergamon

Tetrahedron 54 (1998) 7179–7202

TETRAHEDRON

Synthesis of the First Tricyclic Homodetic Peptide. Use of Coordinated Orthogonal Deprotection to Achieve Directed Ring Closure≠

Ralph Hirschmann,*[a] Wenqing Yao,[a] Byron Arison,*[b] Laurie Maechler,*[c] Avery Rosegay,[b] Paul A. Sprengeler,[a] and Amos B. Smith, III*[a]

[a]*Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104*

[b]*Merck Research Laboratories, Rahway, New Jersey 07065 and*

[c]*Panlabs Inc., 11804 North Creek Parkway South, Bothell, Washington 98011*

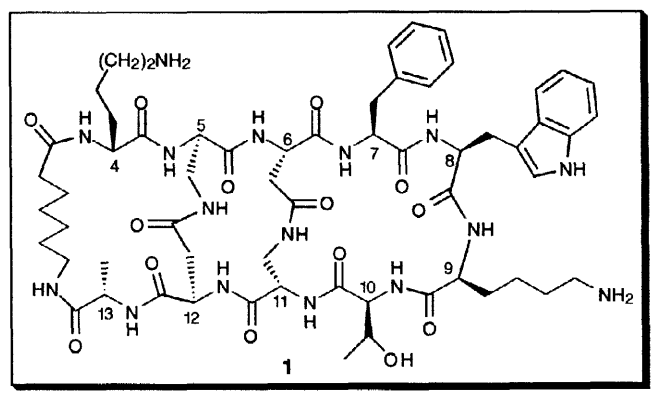
Received 2 February 1998; revised 5 March 1998; accepted 9 March 1998

Abstract: The discovery of somatostatin antagonists at one or more receptor subtypes remains an important goal. We therefore undertook the synthesis of the homodetic tricyclic peptide (**1**) hoping to cause conformational distortion and thereby achieve this biological goal. The synthetic strategy called for five dimensional orthogonal amino protection. The carboxyl and amino protecting groups were selected to assure the desired selective ring closures. The amino protecting groups were also chosen to permit differentiation between the two lysine ε-amino groups. An improved general cyclization procedure was achieved, which provided the complex *c*-octapeptide **13** in 93% yield. Biological assay results for **1** are also presented.

Keywords: *Peptide analogues/mimetics; protecting groups; cyclization; solid phase synthesis*

Introduction

The peptide hormone somatostatin (SRIF) **2**, a cyclic tetradecapeptide was first isolated, characterized and synthesized by Brazeau et al[1] in the early 1970's. Its ability to inhibit the release of several hormones and neurotransmitters including growth hormone, insulin, and glucagon, makes it of importance

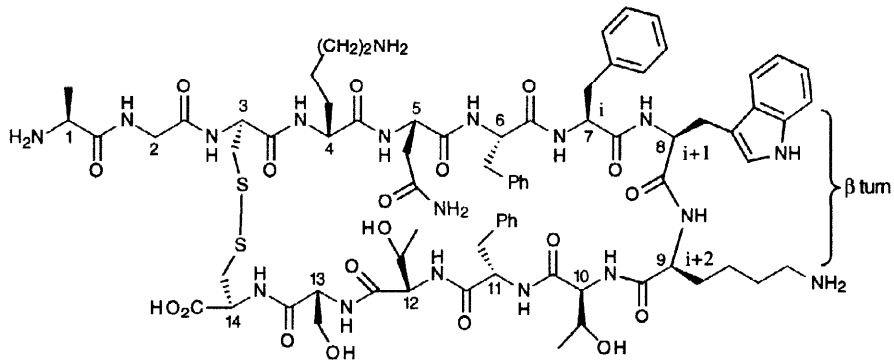


≠Dedicated to our colleague and friend, Professor Madeleine M. Joullié, in celebration of forty years of distinguished teaching and research at the University of Pennsylvania.

0040-4020/98/$19.00
PII: S0040-4020(98)00354-8

in physiology and pathophysiology. The far more biostable agonist octreotide (a.k.a. Sandostatin®) has already found use in medicine.[2] Conversely, a selective somatostatin antagonist would be of pharmacologic interest and could serve to clarify the function of the relevant receptor subtypes.[3]

In 1976 Holladay and Puett[4] insightfully proposed a solution conformation for SRIF incorporating a γ-turn based on the CD spectrum. Earlier, Rivier and coworkers[5] had reported that replacement of Trp^8 by D-Trp enhanced potency by ten fold. Ramachandran[6] had proposed that replacement of an L-amino acid by a D-amino acid in the *i+1* position of a β-turn would enhance the stability of the turn. Veber *et al.* speculated therefore that SRIF contains a β-turn rather than a γ-turn and that Trp is in the *i+1* position of the β-turn, thus explaining the activity enhancing effect of the D-Trp residue. The Merck group[7,8] set out to test this hypothesis and presented compelling experimental evidence for a β-turn involving the Phe^7-Trp^8-Lys^9-Thr^{10} tetrapeptide sequence, generating an antiparallel β-pleated sheet.



2

Figure 1. Bioactive conformation of somatostatin (**2**).

The emerging model[9] suggested that the β-carbon atoms of Asn^5 and Thr^{12} are within bonding distance and that the β-carbons of Phe^6 and Phe^{11} also appeared to be in close proximity permitting hydrophobic stacking. Subsequent molecular modeling suggested that the replacement of Asn^5/Thr^{12} by a cystine bridge might generate a bicyclic analog of SRIF without causing conformational perturbation of either the amide backbone or the sidechains at the β-turn. It had been shown by Rivier *et al.*[10] via an Ala scan that the Asn^5 and the Thr^{12} side chains are not required for either binding or agonism. The Merck group therefore undertook the synthesis of bicyclic **3** which was indeed found to be a highly potent SRIF agonist, supporting the proposed conformation (c.f., Figure 1).

The side chains of Phe^6 and Phe^{11} also appeared to be within bonding distance. However, it had been shown at the Salk Institute[11] that replacement of these amino acids by Ala reduced the potency by 99 and 97%, respectively. This did not bode well for the likelihood that **5**, wherein both of these Phe residues were replaced, would retain significant biological activity. Nevertheless **5** was synthesized and was found to have high potency. This

paradox was resolved by assigning primarily a conformational rather than a receptor-binding role to Phe^6 and Phe^{11}. The former involves hydrophobic stacking of the two aromatic rings, a function which cannot be satisfied when two Ala residues replace Phe^6 and Phe^{11}. On the other hand, introduction of two dicarba bridges (**4**) led to an inactive cyclic dodecapeptide for reasons which are not fully understood. Brady, *et al.*[12] have suggested that the difference in biological activity between **3** and **4** may be largely due to the difference in the preferred geometry between the disulfide and the biscarba analog. A third bicyclic peptide (**6**) was also found to be highly active.[13]

To generate significant conformational distortion of the backbone of SRIF, and thereby generate an SRIF antagonist, we undertook the design and synthesis of the homodetic tricyclic peptide **1** (Scheme 1).[14,15] To our knowledge no tricyclic homodetic peptide had heretofore been prepared. The synthesis was viewed as a significant chemical challenge,[16] both in terms of the required protecting group strategy and because it was anticipated that generation of the third ring would induce significant strain.

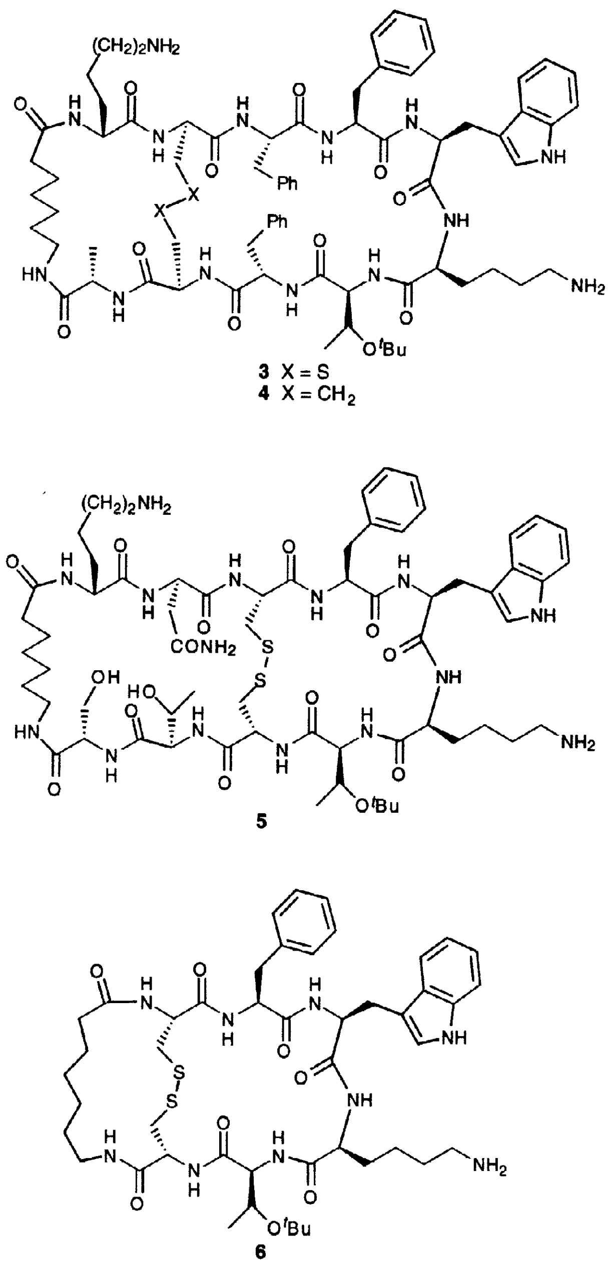


Figure 2. The structures of compounds **3-6**.

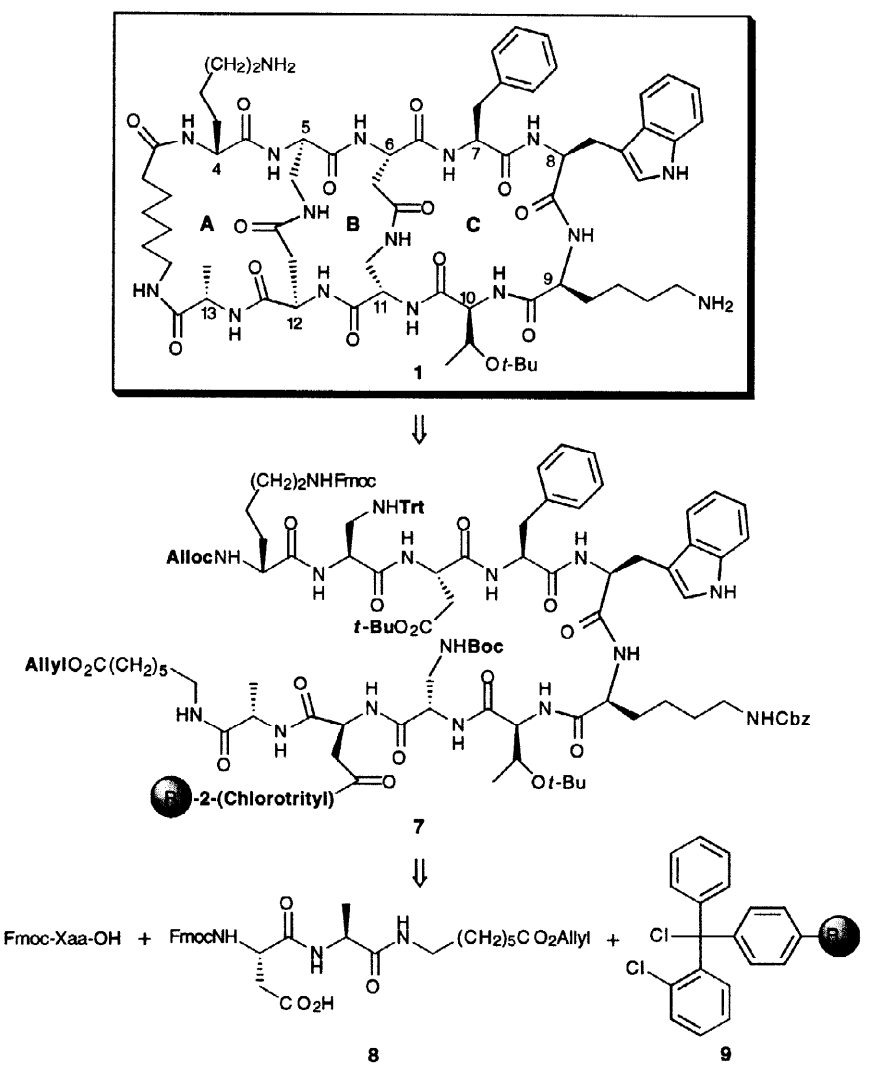
Many biologically active peptides are cyclic molecules,[17] including for example, oxytocin, vasopressin, atrial natriuretic factor, as well as diverse antibotics such as gramicidin, gentamycin, tyrocidine, valinomycin, and the phalloidin toxins. It is also now well appreciated that cyclization induces a constraint, thereby providing important information about side chain and backbone conformations.[18] For example, cyclization can be an invaluable tool in the elucidation of the bioactive conformation of a linear molecule. This information also contributes to the successful conversion of peptides to nonpeptide peptidomimetics.[19] Hence, the development of methods for the synthesis of cyclic, homodetic peptides, both in solution and on solid support, has been the target of extensive research.[20-22] This task is made even more complex in the case of **1**, because we wished to be able to deprotect the two lysine ε-amino groups selectively.[23]

Synthetic Strategy

In addition to the inherent strain, construction of the tricyclic homodetic peptide **1** appeared formidable because multiple orthogonal protection[22] and selective and coordinated deprotection strategies would be required to avoid unwanted ring closures. The choice of the amino protecting groups was made even more complex, because we wished to be able to remove selectively the protecting groups from the ε-amino groups of lysine. Thus in order to execute consecutive ring closures in a controlled manner, we required paired carboxyl and amine protecting groups that could be simultaneously *and selectively* removed. Towards this end, we chose *t*-Boc/*t*-Butyl, Alloc/Allyl, and finally the trityl/2-chlorotrityl solid support as the three pairs of amine/carboxyl protecting group partners. To incorporate the capability to remove the protecting groups of the ε-amino groups of the two lysines selectively, five dimensional orthogonal amino protection with coordinated protecting groups for the carboxyl groups would also be required.

To achieve these goals we planned to anchor the partially protected tripeptide **8** to solid support (**9**) via a β-aspartyl ester linkage and then assemble the linear sequence in a stepwise manner using Fmoc chemistry.[24] We would employ 0.75% TFA to remove the peptide from solid support. This operation would also serve to remove the trityl group from the Dpr^5 β-amino group while leaving the other protecting groups intact. Cyclization of the Asp^{12} and Dpr^5 side chains would then be carried out at high dilution using DPPA, generating the first ring which embodies the B and C rings of **1**. Catalytic $Pd(PPh_3)_2Cl_2$ and Bu_3SnH would next selectively remove the Alloc and Allyl

Scheme 1

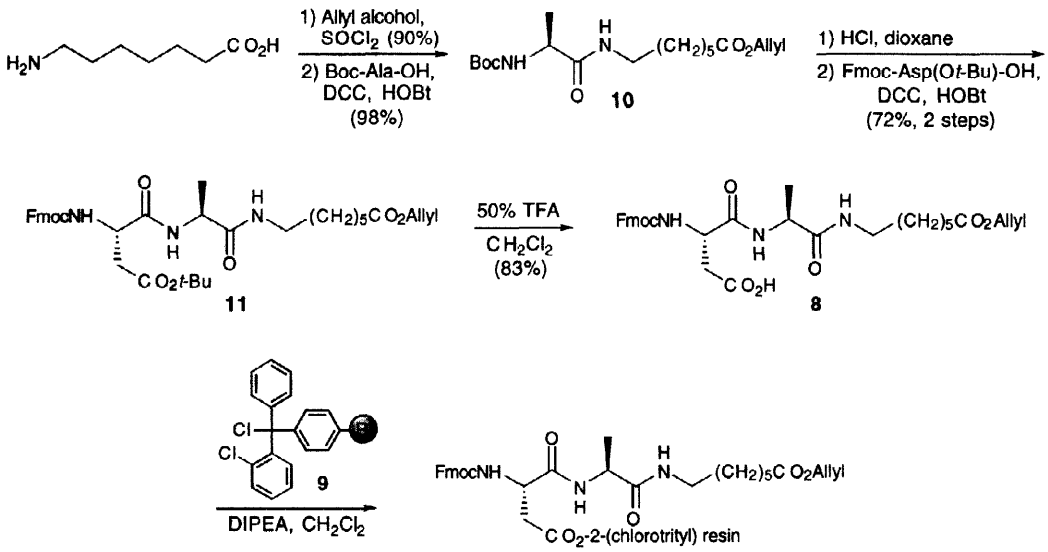


protecting groups permitting generation of ring A. Formation of rings B and C would be accomplished via TFA mediated cleavage of the *t*-Boc/*t*-Butyl ester protecting group of the Dpr[11] and Asp[6], respectively, followed by ring closure. Finally, stepwise removal of the ε-amino protecting groups of Lys[4] (Fmoc) and Lys[9] (ε-Cbz) using respectively piperidine and Pd black catalyzed transfer hydrogenation would afford **1**.

Synthesis of the First Tricyclic Homodetic Peptide

Construction and Initial Cyclization of the Linear Precursor. The synthesis of tripeptide **12** is outlined in Scheme 2. Allyl protection of the carboxyl group of 7-aminoheptanoic acid followed by the standard DCC-HOBt coupling with Boc-Ala-OH gave dipeptide allyl ester **10**. Removal of the Boc group with HCl in dioxane, followed by acylation with Fmoc-Asp(*t*-Bu)-OH gave **11**. Tripeptide **8** was then prepared by removal of the *t*-Bu group of **11** with 50% TFA in CH_2Cl_2. Coupling of **8** to a solid support was achieved by esterification with 2-chlorotrityl chloride resin **9**[25] to afford **12.**

Scheme 2

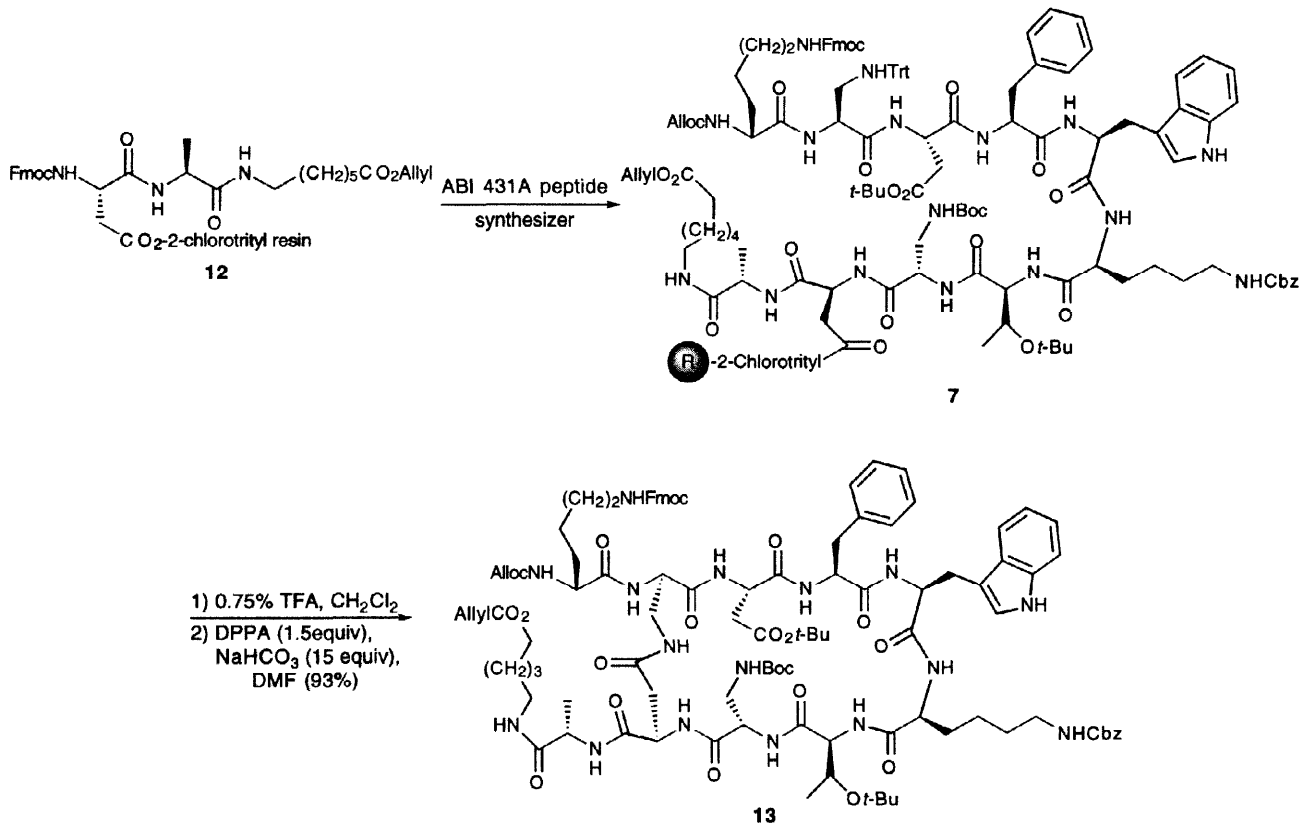


Several linkers have been described[26] which permit the release of partially protected peptide fragments from solid supports under relatively mild conditions. In our hands, the 2-chlorotrityl polystyrene support proved to be the most reliable. It permits one to avoid both side reactions, such as racemisation during attachement of the first amino acid,[27] and formation of piperazines at the dipeptide step.[28] These side reactions are encountered when benzyl alcohol-based solid supports are employed. Moreover, removal of the partially protected peptide fragment from the solid support can be effected under very mild acidic conditions. An additional important advantage of the trityl linker is the fact that the resultant

stable, sterically hindered trityl cation does not attack the side chains of Trp and Tyr,[29] one of the principal shortcoming of linkers that generate benzyl cations during the acid catalyzed cleavage of peptides from the solid support.[30]

Peptide **7** was assembled as outlined in Scheme 3 using Fmoc chemistry and the HBTU/DIPEA coupling reagents. Treatment of **7** with a solution of 0.25% TFA in methylene chloride for 30 min effected cleavage from the solid support. The resulting product, however, was found to be partially tritylated at position 4 (SRIF numbering). Further treatment with 0.75% TFA in CH_2Cl_2 provided a product shown to be homogeneous by reverse phase analytical HPLC and 500-MHz ^1H NMR analysis (cf. >95%). This material was used directly for the subsequent cyclization.

Scheme 3



With the partially protected linear peptide secured, we turned our attention to the requisite cyclizations. For the construction of cyclopeptides, cyclization is often the step which limits the overall yield due to competition between dimerization and cyclization even under highly dilute conditions. This may be attributed to the rigidity of amide bonds of the linear peptide due to the strong π character and the preference for a transoid conformation which can make intramolecular amide bond formation unfavorable.

We followed the procedure developed by Brady and coworkers employing DPPA.[13] However to achieve optimal yields, we found it critical to ensure exclusion of moisture.[15] The

formation of the first ring (embodying the B and C rings, Scheme 3) was mediated by diphenylphosphoryl azide (DPPA, 1.5 equiv) and solid sodium bicarbonate (15 equiv) in anhydrous DMF at 4 °C (final concentration ca. 0.008 M). The cyclization was shown by analytical RP-HPLC to be complete in less than 24 hours. The monocyclic **13** was then isolated in 93% yield after purification by flash chromatography.

Further Cyclization Studies. The high yield for the cyclization step is noteworthy. The preference for a linear peptide favoring β-turn formation would facilitate the cyclization process. Indeed it has been reported that cyclization yields depend primarily on the linear sequence.[31,32] Molecular modeling calculations performed by Cavelier-Frontin on 4-Ala-chlamydocin, a HC-toxin analog, suggested that factors affecting the energy of the transition-state, such as steric hindrance to ring formation and competition with dimerization, also determine the yield of cyclization. In an interesting study, Brady and coworkers[33] found that treating the linear peptide H-D-Phe-Pro-Phe-Pro-Phe-Pro-OH (**14**) with DPPA and triethylamine as base, provided cyclo-(D-Phe-Pro-Phe-Pro-Phe-Pro) (**15**) in 57% yield, while under the identical conditions, H-Phe-Pro-D-Phe-Pro-Phe-Pro-OH (**16**) gave only 2% of the same cyclization product **15**.

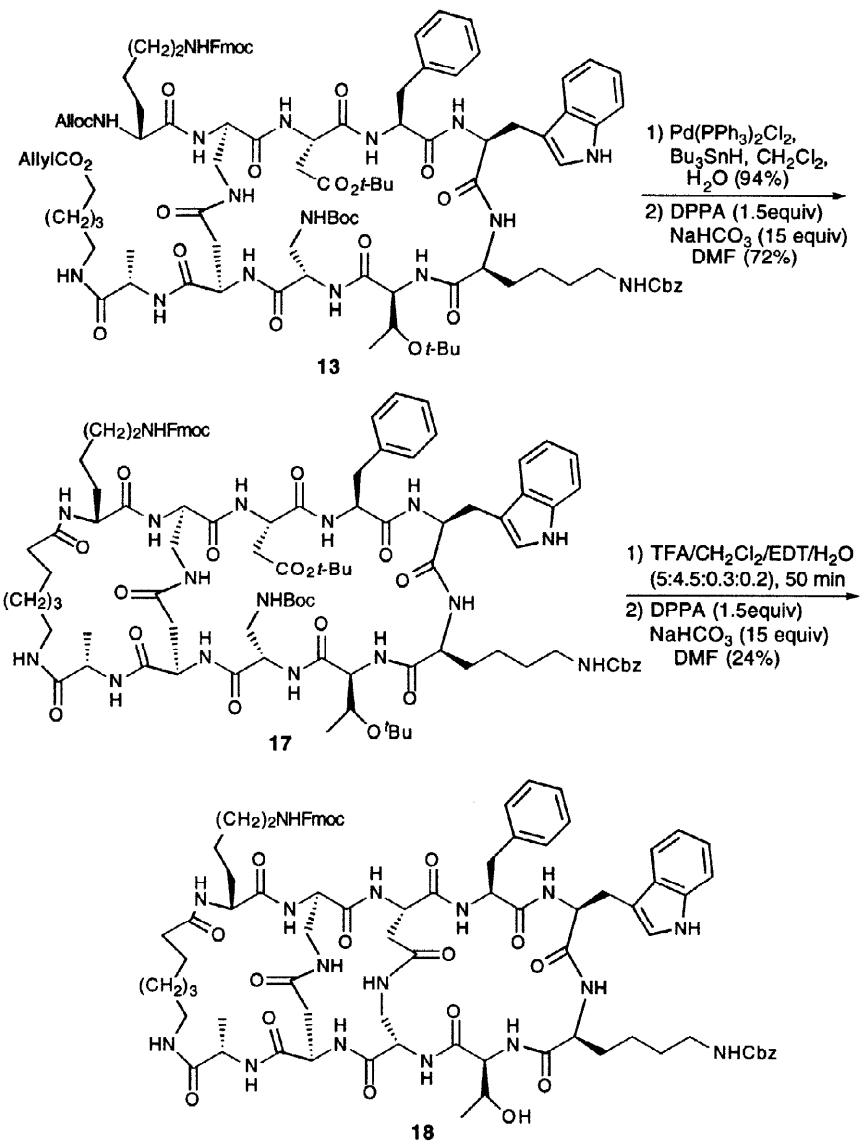
To ascertain whether our markedly higher yield in the synthesis of **13** was due to the linear sequence or whether other factors might also be involved, the linear peptide precursors **14** and **15** were prepared following a similar procedure to that used for the synthesis of **1**. Interestingly, the cyclization of **14** under our improved conditions (1.5 equiv DPPA, 15 equiv $NaHCO_3$ in dry DMF, 4-8 mM peptide concentration) provided **15** in 85% yield, while **16** provided monocyclic peptide **15** in 46% yield and the dimer in 42%. This clearly confirms that the linear sequence does have an effect on the yield, but in addition it demonstrates that the reaction conditions can be tuned to optimize the cyclization yield.

In an unrelated peptidomimetic project directed at the synthesis of RGD containing peptides, we prepared a diverse set of monocyclic hexapeptides using our cyclization conditions.[34,35] In every instance, the cyclization yield was excellent ranging from 85 to 97% (structures not shown). In particular, we found that both the rigorous exclusion of residual water from the cyclization medium and the use of a large excess of solid sodium bicarbonate (15 equiv) were essential for high cyclization yields,[36] especially with sequences which were difficult to cyclize or which contained sequences prone to form imides such as Asp-Xaa (Xaa = Gly or Ser). Sodium bicarbonate, present to buffer the medium, is essentially insoluble in DMF, but any residual water present in the cyclization medium would partially dissolve the base and thereby promote side reactions. This rationale is further supported in the later stages of the synthesis of **1** (vide infra).

With **13** in hand, deprotection of the Alloc and Allyl groups was accomplished simultaneously in 94% yield (Scheme 4) using $Pd(PPh_3)_2Cl_2$ as catalyst and Bu_3SnH as the hydride source.[37] Subsequent cyclization under our improved DPPA conditions provided **17** in 72% yield after silica gel chromatography. The simultanous removal of Boc and *t*-Bu groups from **17** was then readily achieved by treatment with 50% TFA in CH_2Cl_2, with 3%

1,2-dithioethane and 2% water as ion scavengers. The free carboxyl was next activated for cyclization by DPPA under dilute conditions. However, in this case the desired cyclization product **18** was formed in only 24% yield, with high molecular weight compounds representing the major byproducts. The presence of two highly constrained bridges might make **18** hard to generate and, once formed, susceptible to decomposition via imide formation and subsequent bridge cleavage. This may explain, at least in part, the low yield in this cyclization step. Support for the latter interpretation was provided by the observation that treatment of **18** under mild conditions (20% piperidine in DMF) gave a complex mixture

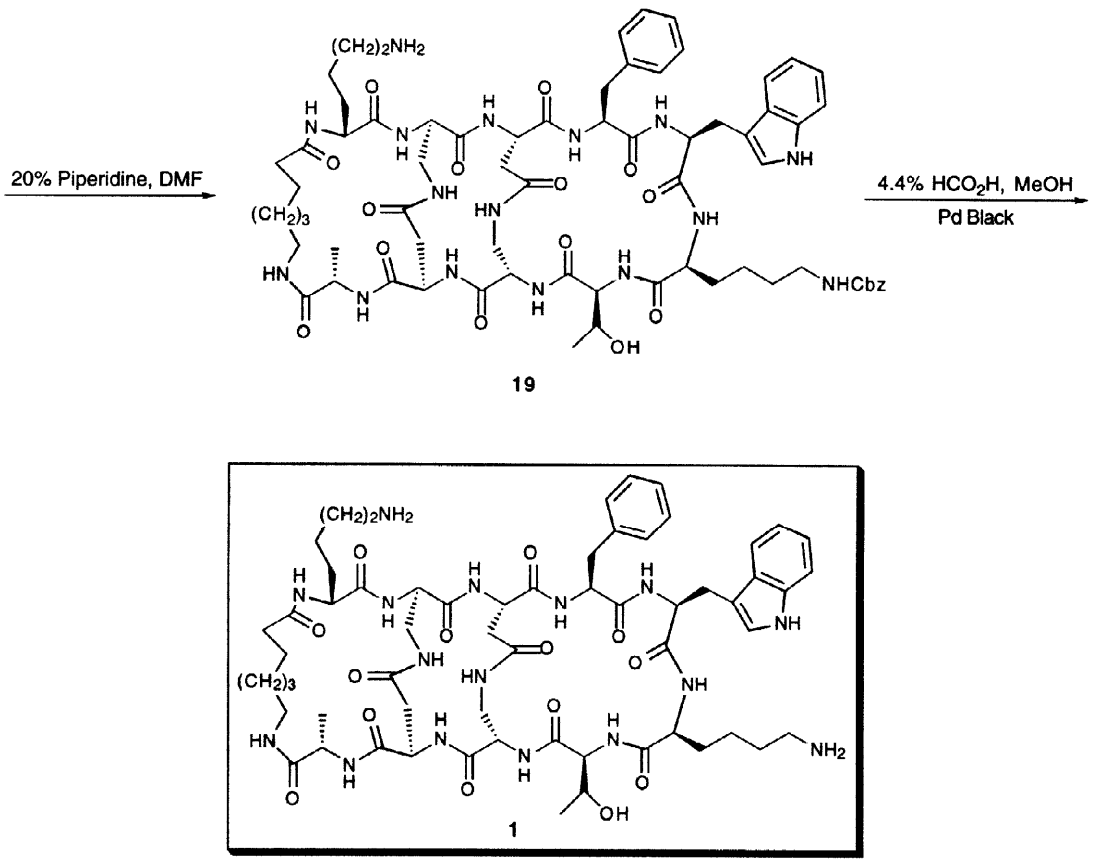
Scheme 4



of products containing **19** plus a wide range of high molecular weight side-products as demonstrated by HPLC and mass spectrometry. Nevertheless, **19** could be purified by

RP-HPLC and subjected to the final Cbz removal via hydrogenation to afford a mixture containing the desired product **1** (Scheme 5).

Scheme 5



Purification of the Target Molecule. The crude material was purified by preparative RP-HPLC (C18 column). Analytical RP-HPLC using a C18 column in conjunction with two different linear gradient and one isocratic solvent systems resulted *in all three cases* in a single symmetric peak, suggesting homogeneity (Figure 3a). However, ^{1}H NMR analysis indicated the presence of an impurity (Figure 4a) which had initially been thought to represent a minor conformational isomer of **1** (vide infra). Assignment of the ^{1}H spectum of **1**, however, indicated that the resonances of the minor component were due to an impurity that was structurally different from **1**. Using a C4 column (Vydac) analytical RP-HPLC enabled us to remove a 10-14% impurity (Figure 3b). A second purification employing a semi-preparative C4 column afforded a product which was shown by 500-MHz ^{1}H NMR to be pure **1** (Figure 4b). These results serve as an important reminder that, the widely accepted notion in peptide chemistry notwithstanding, single symmetric peaks by analytical HPLC in more than two different solvent gradient systems does not *assure* purity. It is also worth noting that although C18 columns are the most widely used stationary

phase in reversed phase HPLC, we have found C4 and C8 columns to be superior for the purification of not only cyclic but linear peptides as well. This was observed not only in the experiments described above where a C4 column was required, but also in a related project,[13,35] in which two c-hexapeptides could not be resolved by a C18 column using a variety of solvent systems, but were readily separated on a C8 column.

Biological Activity and Discussion

The tricyclic peptide **1** failed to displace completely ^{125}I-Tyr^{11} somatostatin from SRIF receptors on the membranes of mouse anterior pituitary AtT-20 cells. Maximal displacement of 50% (three experiments) was observed at 1 μM, with no additional dose-related displacement up to 10 μM, the highest concentration tested (Figure 5).[38]

The much reduced binding affinity of the tricyclic peptide may be a consequence of at least two factors. As mentioned above,[39] replacement of either Phe^6 or Phe^{11} of SRIF by alanine resulted in the striking loss of biological activity; the observation that the bicyclic compound **5**, in which both Phe^6 and Phe^{11} were replaced by a cystine bridge, retained high biological activity, suggested that the phenylalanines do not directly interact with the receptor, and the Phe^6 and Phe^{11} of the SRIF play an important structural role in stabilizing the bioactive conformation around the β–turn through hydrophobic bonding.[40] This interpretation was recently re-examined. Brady *et al.*[41] suggested that in cyclo(Phe-D-Trp-Lys-Thr-Phe-Pro) the bridging region Phe^{11}-Pro^6 (the residues that may mimic

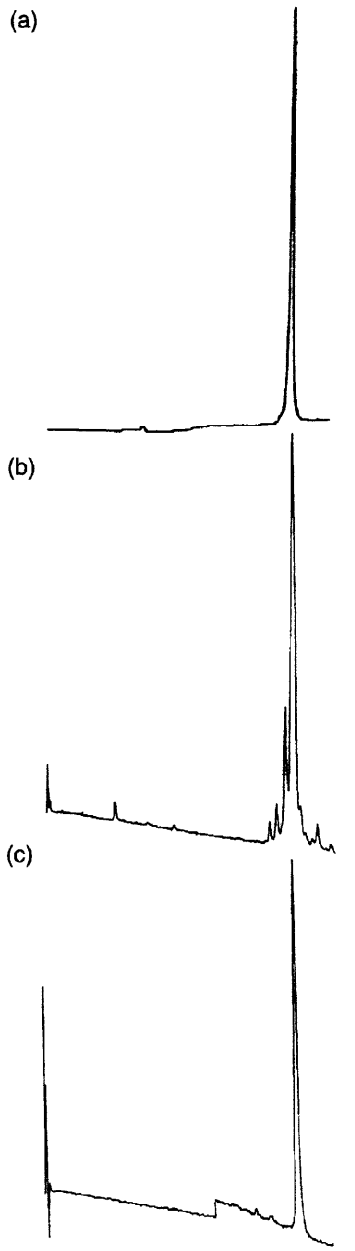


Figure 3. HPLC traces of **1**: (a) symmetrical peak after initial purification analyzed with a C18 Dynamax column (buffer B); (b) initially purified material analyzed with a C4 Vydac column (buffer B) showing one major and several minor impurities; (c) single symmetrical peak after final purification analyzed with a C4 Vydac column (buffer B).

Phe^{6} and Phe^{11} in SRIF) not only plays a structural role, but may also be recognized as a component of ligand-receptor interaction *via* the phenyl ring. Thus in the potent analog **5**, the disulfide bridge may act as a surrogate for the aromatic moiety of Phe^{11}. Such π-sulfur interactions are thought to contribute to the structural stability in certain globular proteins.[42] The non-bonded interaction between benzene and dimethylsulfide contributes 0.8 kcal/mole.[43] More recently, Kaupmann and co-workers[44] used site directed mutagenesis to suggest that the transmembrane domains V, VI, and VII of the SSTR2 receptor determine its selectivity. More importantly, Kaupmann's results suggested that phenyl nuclei of Phe^{11}-Phe^{6} of the SRIF, as well as the S-S bridge of -Cys^{11}-Cys^{6}-Phe^{11}- in octreotide are directly involved in the receptor binding.[44] The amide bond that **1** contains in this region of the molecule may be too polar a functionality, thereby reducing binding.

A second possible factor contributing to the low affinity of **1** for the SRIF receptor is that a conformational change may have occurred upon changing from the monocyclic SRIF (**2**) and bicyclic structures such as **3** and **5** containing disulfide bridges to tricyclic **1** containing only amide bridges. It is known that amide bonds derived from primary amino groups prefer to adopt a trans geometry whereas disulfide bonds prefer a 90° dihedral angle. In addition, computer modeling of **1** suggests that four distinct conformations of similar energy can arise from the two central bridges as shown in Figure 6. If this model is correct, only one of the four conformers of **1** (e.g., Figure 6a) has a conformation similar to the published model[45] leading to loss of potency.

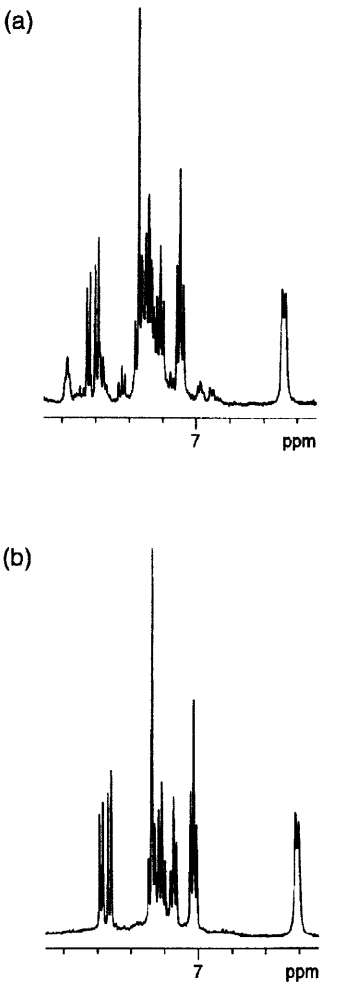


Figure 4. 400-MHz ^{1}H NMR spectrum of **1** (a) after RP-HPLC purification using C18 Dynamax column only; (b) after RP-HPLC purification with both a C18 Dynamax and a C4 Vydac column.

NMR studies on **1** indicate that, unlike cyclic hexapeptide L-363,301, there are no uniquely slow exchangeable NHs suggesting that a well defined β-turn is not present. Another significant conformational feature in the NMR of **1** is the approximate 0.7 ppm upfield displacement of the Phe ortho protons (6.47 ppm) and one of the Phe beta protons (2.23 ppm), indicated that these protons are close to and above the plane of an aromatic residue. ROESY and COSY spectra provide further evidence that the Phe is positioned above the indole ring of Trp. Thus, instead of the Trp^8 and Lys^9 being close to each other in space as observed in the SRIF supragonists, the Phe^7 and Trp^8 are close together in **1**. Therefore in solution the orientation of the Trp and Lys side chains in **1** do not favor receptor binding.

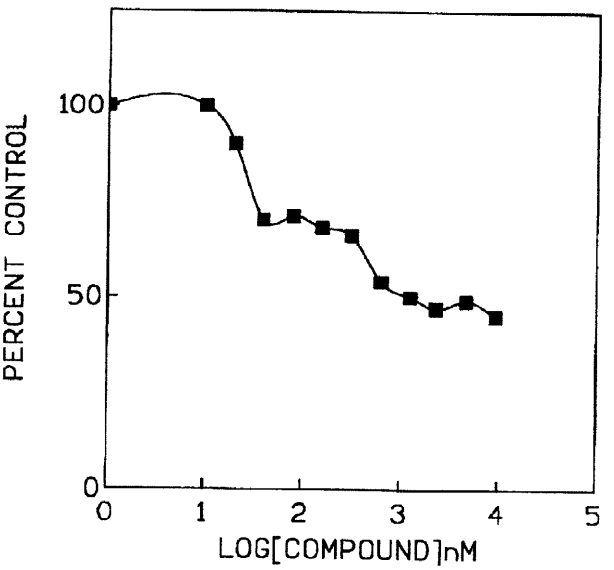


Figure 5. Dose response curve for the displacement of $^{125}I\text{-}Tyr^{11}$ somatostatin by **1** on AtT-20 cells.

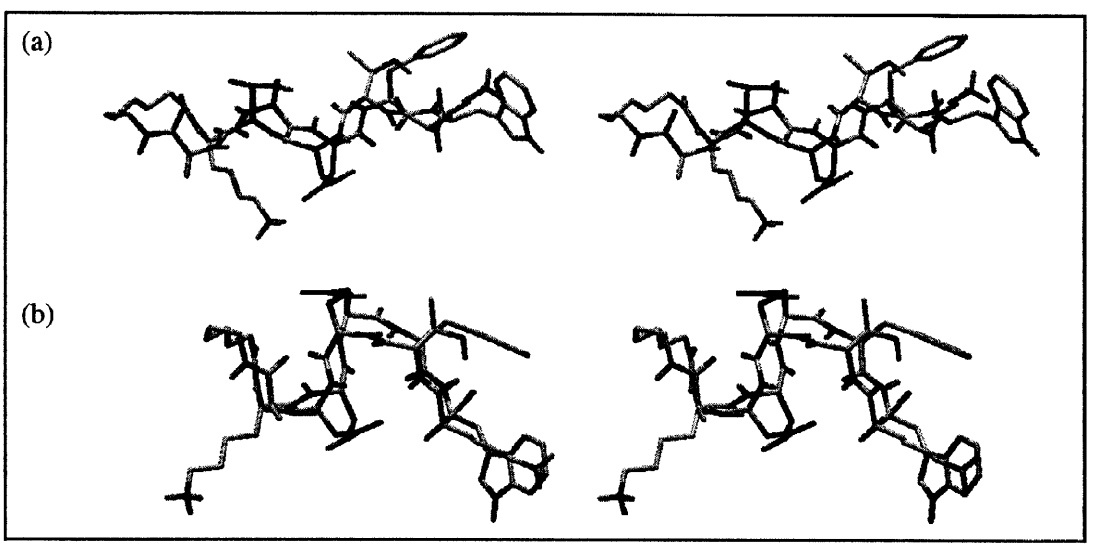


Figure 6. Molecular modeling (MM2 minimized, stereo view) of **1** showing the possible orientations of the bridgehead atoms (black) relative to the plane defined by the rings: (a) above and below and (b) below and above.

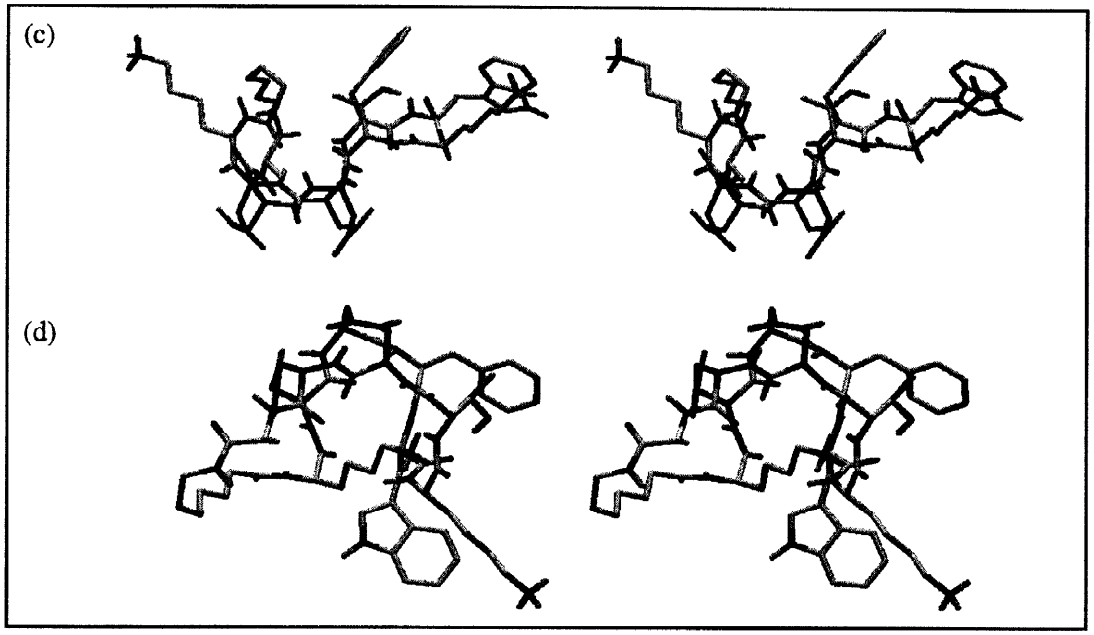


Figure 6 (continued). Molecular modeling (MM2 minimized, stereo view) of **1** showing the possible orientations of the bridgehead atoms (black) relative to the plane defined by the rings: (c) both below and (d) both above.

EXPERIMENTAL SECTION

Unless otherwise noted, all solvents and reagents were obtained from commercial souces and used without further purification. Analytical reverse-phase HPLC was carried out employing a LKB system (2152 LC controller, 2150 HPLC pump, 2141 variable wavelength monitor on a C18 Dynamax 300Å (0.46-25 cm) column; semi or preparative reverse-phase HPLC separations were achieved using a Ranin solvent delivery system equipped with a dynamax detector (model UV-D) utilizing either C18 Dynamax 300Å (21.4 x 250 mm) column or C8 Vydac column (10 x 250 mm). The mobile phase consisted of 0.1% TFA in water (buffer A) and 0.1% TFA in acetonitrile (buffer B). The FAB-mass spectra were obtained on a ZAB-E VG analytical spectrometer. ^{1}H and ^{13}C NMR spectra were obtained with a Brucker AM500 spectrometer. Chemical shifts are reported in δ values relative to tetramethylsilane for proton and solvent for carbon spectra. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter.

Fmoc-DPR(Boc)-OH. To a solution of 12.9 g of PIFA in 30 mL CH_3CN was added 9.9 g of Fmoc-Asn-OH followed by 60 mL DMF. The resultant mixture was vigorously stirred until a clear solution was generated. To this clear solution was added 30 mL H_2O. After stirring at room temp for 15 min, 2.4 mL of pyridine was added dropwise. The solution was then stirred at room temp for 23 h after which time the solvent was removed,

and the residue was crystallized from water and dried to give 9.7 g pure product. To a 0 °C solution of 4.89 g of the above product (Fmoc-DPR-OH) in 80 mL THF and 15 mL DMF was added 3 mL DIPEA, followed by 4.55 g of Boc anhydride. The mixture was stirred at room temperature for 6.0 h and concentrated in vacuo. The resultant residue was then dissolved in ethyl acetate, washed with water and brine, dried over Na_2SO_4, filtered and concentrated. Flash chromatography on silica gel with hexane/EtOAc (1:2) afforded 4.6 g (72% yield) of pure product: $[\alpha]_D^{25}$ +1.59° (c 1.13, EtOAc); mp 94-96 °C; Rf 0.20 ($CHCl_3$/MeOH/AcOH = 9:1:0.05). ^1H NMR (500 MHz, CD_3OD) δ 1.42 (s, 9 H), 3.39-3.42 (m, 1 H), 3.52-3.54 (m, 1 H), 4.2 (t, *J* = 6.96 Hz, 1 H), 4.3-4.8 (m, 3 H), 7.29 (t, *J* = 7.41 Hz, 2 H), 7.37 (t, *J* = 7.41 Hz, 2 H), 7.65 (t, *J* = 6.48 Hz, 2 H), 7.77 (d, *J* = 7.52 Hz, 2 H). ^{13}C NMR (125 MHz, CD_3OD) δ 28.71, 42.62, 48.34, 55.91, 68.16, 80.48, 120.9, 126.26, 128.15, 128.76, 142.54, 145.26, 158.49, 158.59, 173.72; high resolution mass spectrum (CI) *m/z* 444.2161 [$(M+NH_4)^+$; calcd for $C_{23}H_{26}N_2O_6$: 444.2135].

Anal. Calcd for $C_{23}H_{26}N_2O_6$: C, 64.45; H, 6.36; N, 6.27. Found: C, 64.78; H, 6.25; N, 6.57.

Allyl 7-aminoheptanoic ester. To 10 ml allyl alcohol precooled to -12 °C was added 3.0 mL $SOCl_2$ dropwise, followed by addition of 580 mg (4.0 mmol) of solid 7-aminoheptanoic acid. The resulting solution was warmed to room temperature and stirred for 24 h. The solvent was then evaporated and the residue was precipitated with dry diethyl ether to afford 0.89 g (90% yield) of the desired product as a very hygroscopic solid: mp106-108 °C; ^1H NMR (500 MHz, CD_3OD) δ 1.40-1.41 (m, 4 H), 1.64-1.66 (m, 4 H), 2.37 (t, *J* = 7.30 Hz, 2 H), 2.92 (t, *J* = 7.59 Hz, 2 H), 4.57 (d, *J* = 5.48 Hz, 2 H), 5.20 (d, *J* = 10.4 Hz, 1 H), 5.29 (d, *J* = 17.2 Hz, 1 H), 5.97-5.89 (m, 1 H); ^{13}C NMR (125 MHz, CD_3OD) δ 25.7, 27.05, 28.33, 29.52, 34.73, 40.68, 65.99, 118.2, 133.8, 174.9; high resolution mass spectrum (CI) *m/z* 186.1483 [M^+; calcd for $C_{10}H_{19}NO_2$•HCl: 186.1494].

BOC-Ala-Hep-OAllyl (10). To a solution of 0.953 g (5.04 mmol) Boc-Ala-OH and 0.93 g (4.2 mmol) allyl 7-aminoheptanoic ester in 10 mL DMF was added 0.877 mL (5.04 mmol) diisopropylethylamine at 0 °C, followed by 0.963 g EDIC. After the reaction mixture was stirred at 0 °C for 0.5 h, the solution was warmed to room temperature and stirred for an additional 4 h. The reaction mixture was then diluted with methylene chloride, and the organic layer was separated and sequentially washed with 10% citric acid, 10% $NaHCO_3$, and brine, then dried over anhydrous Na_2SO_4, filtered, and concentrated. Flash chromatography of the crude material on silica with CH_2Cl_2/CH_3OH (90:3) provided 1.46g of **10** (98% yield) as an oil: $[\alpha]_D^{25}$ -22.43° (c 0.78, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.34-1.29 (m, 7 H), 1.35 (s, 9 H), 1.37-1.53 (m, 2 H), 1.60-1.66 (m, 2 H), 2.32 (t, *J* = 7.44 Hz, 2 H), 3.21-3.25 (m, 2 H), 4.14 (s, 1 H), 4.56-4.58 (m, 2 H), 5.21 (m, 1 H), 5.32 (dd, *J* = 1.5, 3.1 Hz, 1 H), 5.29 (dd, *J* = 1.5, 3.0 Hz, 1 H), 5.87-5.96 (m, 1 H), 6.4 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.41. 24.68, 26.36, 28.26, 28.63, 29.25, 34.03, 39.26, 50.02, 64.88, 79.93,

118.04, 132.24, 155.51, 172.55, 173.22; high resolution mass spectrum (FAB) *m/z* 379.2213 [(M+Na)$^+$; calcd for $C_{18}H_{32}N_2O_5$: 379.2209].

Anal. Calcd for $C_{18}H_{32}N_2O_5$: C, 60.63; H, 9.05; N, 7.86. Found: C, 60.41; H, 8.78; N, 7.70.

Fmoc-Asp(^{t}Butyl)-Ala-Hep-Allyl (11). To a solution of 1.42 g (3.98 mmol) BOC-Ala-Hep-OAllyl (**10**) in 10 mL CH_2Cl_2 and 0.5 mL H_2O was added 10.0 mL trifluoroacetic acid dropwise at room temperature. The resulting solution was stirred at room temperature for 1.5 h. The solution was then concentrated to half of its orginal volume and concentrated from dry benzene (three times) to give the crude material, which was dissolved in 10 mL DMF and cooled to 0 °C. To this solution was added 1.64 g Fmoc-Asp(^{t}Bu)-OH and 0.9 g (6.0 mmol) HOBt, followed by 1.04 mL (6.0 mmol) diisopropylethylamine and 1.2 g (6.0 mmol) EDIC. The solution was stirred at 0 °C for 0.5 h and then at room temperature for 2 h. The reaction mixture was diluted with 40 mL EtOAC and 10 mL 5% citric acid, and the organic layer was separated and sequentially washed with 5% $NaHCO_3$ (3x15 mL) and brine, then dried over Na_2SO_4, filtered, and concentrated. The resulting residue was recrystallized from EtOAc/Hexane to afford 1.78g of **11** (72% yield) as a white crystalline solid: $[\alpha]_D^{25}$ +19.12° (c 0.57, DMSO); mp 110-112 °C, ^{1}H NMR (500 MHz, d_6-DMSO) δ 1.20 (d, *J* = 6.94 Hz, 3 H), 1.24 (bs, 4 H), 1.38 (bs, 12 H), 1.46-1.53 (m, 2 H), 2.31 (t, *J* = 7.26 Hz, 2 H), 2.46 (dd, *J* = 9, 16.1 Hz, 1 H), 2.7 (dd, *J* = 4.89, 16.1 Hz, 1 H), 3.03-2.99 (m, 2 H), 4.19-4.39 (m, 5 H), 4.53-4.54 (m, 2 H), 5.23 (dd, *J* = 1.32, 2.3 Hz, 1 H), 5.32 (dd, *J* = 1.3, 2.3 Hz, 1 H), 5.87-5.94 (m, 1 H), 7.32 (t, *J* = 7.41 Hz, 2 H), 7.42 (t, *J* = 7.39 Hz, 2 H), 7.65-7.75 (m, 4 H), 7.89 (d, *J* = 7.5 Hz, 2 H), 7.93 (d, *J* = 7.28 Hz, 1 H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 18.3, 24.29, 25.84, 27.62, 28.04, 28.73, 33.26, 37.42, 38.36, 46.55, 48.31, 51.33, 64.1, 65.71, 80.1, 117.53, 120.03, 125.17, 126.99, 127.57, 132.74, 140.65, 143.67, 155.72, 169.41, 170.05, 171.59, 172.42; high resolution mass spectrum (FAB) *m/z* 672.3261 [(M+Na)$^+$; calcd for $C_{36}H_{47}N_3O_8$: 672.3255].

Anal. Calcd for $C_{36}H_{47}N_3O_8$: C, 66.53; H, 7.29; N, 6.47. Found: C, 66.34; H, 7.13; N, 6.46.

Fmoc-Asp-Ala-Hep-Allyl (8). To a solution of 1.73 g (2.78 mmol) Fmoc-Asp(^{t}Butyl)-Ala-Hep-Allyl (**11**) in 9.0 mL CH_2Cl_2 and 1.0 mL H_2O was added 10 mL trifloroacetic acid. After stirring at room temp for 1.0 h, the solvent was evaporated and the residue was crystallized from EtOAc/hexane to afford 1.37 g of **8** (83% yield) as a white solid: $[\alpha]_D^{25}$ + 10.82° (c 0.425, DMSO); mp: 172-174 °C; ^{1}H NMR (500 MHz, d_6-DMSO) δ 1.20 (d, *J* = 7.03 Hz, 3 H), 1.22-1.25 (m, 3 H), 1.37 (t, *J* = 6.3 Hz, 2 H), 1.51 (t, *J* = 6.69 Hz, 2 H), 2.3 (t, *J* = 7.36 Hz, 2 H), 2.53 (dd, *J* = 8.7, 16.6 Hz, 1 H), 2.73 (dd, *J* = 5.1, 16.6 Hz, 1 H), 3.01 (m, 2 H), 4.16-4.31 (m, 6 H), 4.34-4.38 (m, 1 H), 4.53 (dd, *J* = 5.4 Hz, 2 H), 5.2 (d, *J* = 10.4 Hz, 1 H), 5.28 (dd, *J* = 1.3, 17.2 Hz, 1 H), 5.86-5.93 (m, 1 H), 7.33 (t, *J* = 7.3 Hz, 2 H), 7.42 (t, *J* = 7.43 Hz, 2 H), 7.7 (t, *J* = 7.65 Hz, 4 H), 7.95 (d, *J* = 7.34 Hz, 1 H), 7.89 (d, *J* =

7.53 Hz, 2 H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 18.27, 24.32, 25.86, 28.05, 28.74, 33.29, 36.22, 39.01, 46.59, 48.36, 51.30, 64.14, 65.77, 117.58, 120.06, 125.24, 127.05, 127.6, 132.77, 140.67, 143.75, 155.82, 170.35, 171.63, 171.84, 172.48; high resolution mass spectrum (FAB) *m/z* 616.2635 [$(M+Na)^+$; calcd for $C_{32}H_{39}N_3O_8$: 616.2657].

Anal. Calcd for $C_{32}H_{39}N_3O_8$-H_2O: C, 62.82; H, 6.76; N, 6.87. Found: C, 63.17; H, 6.48; N, 6.79.

Attachment of Fmoc-Asp-Ala-Hep-Allyl to 2-chlorotrityl chloride resin. A sample of 2-chlorotrityl chloride resin (1.0 g) was suspended in 10 mL CH_2Cl_2 at room temperature for five minutes. To the above suspension was added 0.6 g (1.0 mmol) Fmoc-Asp-Ala-Hep-Allyl (**8**), followed by 3.0 mL diethylpropylamine. The resulting mixture was stirred at room temperature for 4.0 h, then quenched by addition 10 mL of 20% diisopropylethylamine in methanol. The suspension was filtered and washed sequentially with DMF (3 x 10 mL), methanol (1 x 10mL), DMF (3 x 10mL), *i*-prOH (3 x 10mL), and diethyl ether. The resin was then dried under vacuum to give 1.25 g peptide resin **12**. Substitution was determined to be 0.35 mmol/g.

Alloc-Lys(Fmoc)-Dpr(trt)-Asp(tBu)-Phe-D-Trp-Lys(Cbz)-Thr(tBu)-Dpr(Boc)-Asp(O-2-chlorotrityl Resin-Ala-Hep-Allyl (**7**). Assembly of protected peptides on solid support was carried out using either a Beckman Instruments Model 990B peptide synthesizer or an Applied Biosystems, Inc. Model 431A automated peptide synthesizer. N-α-Fmoc amino acids, purchased from Bachem, Inc. or prepared according to the literature procedures, with appropriately protected side chains were employed throughout. Starting from Fmoc-Asp-Ala-Hep-Allyl crosslinked 2-chlorotrityl polystyrene resin (**12**) (1.25 g, 0.35 mmol/g), **7** was assembled according to standard procedure[46] with some modification. Fmoc removal (20% piperidine in DMF), 2.0 mmol individual amino acid along with HBTU were used in the coupling step. The coupling reaction was carried out at room temperature for 2.0 h. Thus, incorporated in order, were: Fmoc-Dpr(Boc)-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Lys(Cbz)-OH, Fmoc-Trp-OH, Fmoc-Phe-OH, Fmoc-Asp(*t*Bu)-OH, Fmoc-Dpr(Trt)-OH, Alloc-Lys(Fmoc)-OH. An aliquat of the resin was taken after each coupling step and cleaved with 0.5% TFA in CH_2Cl_2 or a Kaiser test was performed to monitor the coupling reaction. After completing the synthesis on solid support, the resin was dried under vacuum to afford 2.3 g peptide resin.

Alloc-Lys(Fmoc)-Dpr-Asp(tBu)-Phe-D-Trp-Lys(Cbz)-Thr(tBu)-Dpr(Boc)-Asp-Ala-Hep-Allyl. Peptide resin **7** (1.39 g) was treated with 22 mL 0.75% trifluoroacetic acid in methylene chloride at room temperature. After 0.5 h, the suspension was filtered and washed with 0.5% trifluoroacetic acid in methylene chloride. The filtrate was evaporated to half of its volume and concentrated from benzene, and the residue was precipitated with dry diethyl ether to afford 0.75 g of the crude linear peptide of more than 95% purity (Condition: Analytic column: C18 Dynamax 300Å (0.46-25 cm) , Buffer A, 0.1% TFA in H_2O; buffer B:

0.1% TFA in Acetonitrile, 70-25'-95% buffer B, flow rate at 0.56 ml/min, monitored at 254 nm). This material was used in the next cyclization step without further purification. $[\alpha]_D^{25}$ -3.62° (c 0.442, DMSO); ^{1}H NMR (500 MHz, d_6-DMSO) δ 0.95 (d, J = 6.13 Hz, 3 H), 1.14 (s, 9 H), 1.18 (d, J = 6.99 Hz, 3 H), 1.23 (m, 5 H), 1.29 (s, 9 H), 1.35 (s, 9 H), 1.35-1.41 (m, 5 H), 1.50-1.51 (m, 6 H), 1.60-1.69 (m, 1 H), 1.70-1.74 (m, 1 H), 2.30 (t, J = 7.34 Hz, 2 H), 2.35-2.41 (m, 2 H), 2.43-2.48 (m, 1 H), 2.60-2.78 (m, 4 H), 2.89-3.0 (m, 9 H), 3.0-3.2 (m, 2 H), 3.2-3.3 (m, 4 H), 3.85-3.96 (m, 3 H), 4.16 (t, J = 7.23 Hz, 1 H), 4.20 (t, J = 6.82 Hz, 1 H), 4.29 (d, J = 6.74 Hz, 3 H), 4.31-4.40 (m, 3 H), 4.41-4.50 (m, 3 H), 4.50-4.60 (m, 6 H), 4.98 (s, 2 H), 5.17-5.21 (m, 2 H), 5.26-5.30 (m, 2 H), 5.87-5.93 (m, 2 H), 6.6 (m, 1 H), 6.96 (t, J = 7.5 Hz, 1 H), 7.05 (t, J = 7.26 Hz, 1 H), 7.15 (d, J = 7.22 Hz, 4 H), 7.19 (d, J = 6.72 Hz, 3 H), 7.24 (m, 1 H), 7.31-7.36 (m, 8 H), 7.41 (t, J = 7.24 Hz, 3 H), 7.60-7.70 (m, 6 H), 7.78 (bs, 2 H), 7.88 (d, J = 7.47 Hz, 2 H), 7.92 (d, J = 7.26 Hz, 1 H), 8.12-8.20 (m, 2 H), 8.21-8.28 (m, 2 H); high resolution mass spectrum (FAB) m/z 2024.0283 [(M+Na)$^+$; calcd for $C_{103}H_{140}N_{16}O_{25}$: 2024.0073].

Alloc-Lys(Fmoc)-Dpr-Asp(O^{t}Bu)-Phe-D-Trp-Lys(Cbz)-Thr(^{t}Bu)-Dpr(Boc)-Asp-Ala-Hep-Allyl

(**13**). To a suspension of 326.6 mg (0.1632 mmol) Alloc-Lys(Fmoc)-Dpr(NH_3^+)-Asp(tButyl)-Phe-Trp-Lys(Cbz)-Thr(tButyl)-Dpr(Boc)-Asp(OH)-Ala-Hep-Allyl and 205.6 mg (2.448 mmol) solid $NaHCO_3$ in 28.1 mL dry DMF at 0 °C was added 68.8mg (0.245mmol) DPPA dropwise. The resulting reaction mixture was stirred at 4 °C for 24 h, after which time analytical RP-HPLC indicated that the cyclization was complete. The reaction mixture was filtered and washed with dry DMF, and the filtrate was concentrated. Flash chromatography of the crude material with CH_2Cl_2/CH_3OH (90:6) afforded 300.9 mg (93% yield) of monocyclic peptide **13** as a white amorphous solid: $[\alpha]_D^{25}$ -20.88° (c 0.455, DMF); decomposed at 117-120 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 1.19 (s, 9 H), 1.25 (d, J = 6.33 Hz, 3 H), 1.28-1.34 (m, 4 H), 1.37 (s, 9 H), 1.38-1.39 (m, 7 H), 1.41 (s, 9 H), 1.42-1.53 (m, 7 H), 1.56-1.61 (m, 2 H), 1.71-1.74 (m, 2 H), 1.80-1.85 (m, 1 H), 1.85-1.92 (m, 1 H), 2.31 (t, J = 7.42 Hz, 4 H), 2.67 (dd, J = 9.20, 15.12 Hz, 1 H), 2.79-2.81 (dd, J = 4.28, 15.24 Hz, 1 H), 2.82-2.95 (m, 2 H), 3.09-3.12 (m, 4 H), 3.17 (t, J = 6.98 Hz, 2 H), 3.20-3.25 (m, 1 H), 3.27-3.30 (m, 1 H), 3.33-3.41 (m, 1 H), 3.48-3.56 (m, 3 H), 4.05-4.09 (m, 1 H), 4.10-4.18 (m, 2 H), 4.22-4.24 (m, 1 H), 4.30-4.38 (m, 6 H), 4.42-4.46 (m, 1 H), 4.48-4.54 (m, 1 H), 4.51-4.57 (m, 4 H), 4.61-4.67 (m, 1 H), 4.72-4.77 (m, 1 H), 5.04 (s, 2 H), 5.16-5.20 (m, 2 H), 5.26-5.31 (m, 2 H), 5.87-5.95 (m, 1 H), 7.01-7.05 (m, 3 H), 7.07-7.11 (m, 2 H), 7.11-7.15 (m, 3 H), 7.24-7.31 (m, 7 H), 7.32-7.38 (m, 3 H), 7.45-7.51 (m, 1 H), 7.60-7.61 (m, 2 H), 7.75-7.78 (m, 2 H); high resolution mass spectrum (FAB) m/z 2005.9979 [(M+Na)$^+$; calcd for $C_{103}H_{138}N_{16}O_{24}$: 2005.9968].

H-Lys(Fmoc)-Dpr-Asp(^{t}Bu)-Phe-D-Trp-Lys(Cbz)-Thr(^{t}Bu)-Dpr(Boc)-Asp-Ala-Hep-Allyl-OH.

To a solution of 480 mg **13** and 6.8 mg $PdCl_2(PPh_3)_2$ in 14 mL CH_2Cl_2 and 0.7 mL H_2O was added 156 μL (0.58 mmol) Bu_3SnH dropwise. After 5 min, the color of the reaction mixture turned from yellow to brownish; at this point, TLC indicated that the reaction was complete. The reaction mixture was concentrated, and the resulting residue was precipitated with dry ether and washed with additional ether (3 x 20 mL). The solid was further purified by a semi-preparative RP-HPLC (Condition: 21.4 mm C18 Dynamax column, 45-25'-95 % buffer B, flow rate: 12 mL/min; monitored at 254 nm; the major fractions (RT: 23 min) were pooled and lyophilized to provide 423.3 mg (94% yield) of the peptide: $[\alpha]_D^{25}$ -13.5° (c 0.2, CH_3OH); mp: 153-156 °C; ^1H NMR (500 MHz, CD_3OD) δ 0.85-1.02 (m, 1 H), 1.1-1.20 (m, 2 H), 1.16 (s, 9 H), 1.22-1.33 (m, 6 H), 1.37-1.48 (m, 26 H), 1.50-1.53 (m, 4 H), 1.54-1.65 (m, 4 H), 1.70-1.86 (m, 3 H), 1.92-2.08 (m, 2 H), 2.26 (t, J = 7.45 Hz, 2 H), 2.60-2.65 (m, 1 H), 2.79-2.81 (m, 1 H), 2.90-2.96 (m, 2 H), 3.0-3.11 (m, 6 H), 3.18-3.20 (m, 2 H), 3.21-3.35 (m, 2 H), 3.87 (t, J = 6.37 Hz, 2 H), 4.0 (bs, 1 H), 4.10-4.19 (m, 3 H), 4.20-4.36 (m, 4 H), 4.40-4.45 (m, 1 H), 4.46-4.52 (m, 1 H), 4.50-4.56 (m, 1 H), 4.64 (s, 1H), 5.01-5.08 (m, 2 H), 6.99 (t, J = 7.36 Hz, 1 H), 7.08 (t, J = 7.26 Hz, 1 H), 7.11-7.20 (m, 3 H), 7.21-7.31 (m, 10 H), 7.36 (dd, J = 7.50, 15.08 Hz, 4 H), 7.60 (d, J = 7.28 Hz, 2 H), 7.78 (d, J = 7.57 Hz, 2 H); high resolution mass spectrum (FAB) *m/z* 1882.9619 [$(M+Na)^+$; calcd for $C_{96}H_{130}N_{16}O_{22}$+H: 1882.9522].

Cyclo-[Lys(Fmoc)-Dpr-Asp(^{t}Bu)-Phe-D-Trp-Lys(Cbz)-Thr(^{t}Bu)-Dpr(Boc)-Asp-Ala-Hep]

(17). To a solution of 96.9 mg (0.052 mmol) of the above peptide and 65.5 mg solid $NaHCO_3$ in 8.9 mL dry DMF, precooled to 0 °C, was added 17.2 μL (0.078 mmol) of DPPA. The solution was stirred at 4 °C for 24 h, after which time analytical RP-HPLC indicated that the cyclization was complete. The mixture was filtered and washed with DMF, and the filtrate was evaporated to give a residue which was purified by flash chromatography with CH_2Cl_2/CH_3OH (90:9) to afford 68.8 mg of **17** (72% yield): $[\alpha]_D^{25}$ -29.14° (c 0.35, CH_3OH); decomposed at: 186-189 °C; ^1H NMR (500 MHz, CD_3OD) δ 1.18-1.22 (m, 16 H), 1.27-1.30 (m, 7 H), 1.36 (d, J = 7.27 Hz, 3 H), 1.41 (s, 9 H), 1.49-1.45 (m, 11 H), 1.45-1.53 (m, 6 H), 1.53-1.56 (m, 1 H), 1.66-1.71 (m, 1 H), 1.75-1.78 (m, 2 H), 1.85-1.92 (m, 1 H), 2.19-2.30 (m, 2 H), 2.30-2.40 (m, 2 H), 2.51-2.60 (m, 1 H), 2.87-2.99 (m, 3 H), 3.00-3.16 (m, 4 H), 3.19-3.22 (m, 2 H), 3.31-3.45 (m, 3 H), 3.55-3.57 (m, 1 H), 4.14-4.16 (m, 2 H), 4.24-4.31 (m, 6 H), 4.34-4.38 (m, 1 H), 4.47-4.56 (m, 2 H), 4.56-4.61 (m, 1 H), 4.71-4.73 (m, 1 H), 5.03 (s, 2 H), 6.99-7.05 (m, 3 H), 7.07-7.11 (m, 3 H), 7.11-7.15 (m, 3 H), 7.26-7.39 (m, 9 H), 7.43-7.49 (m, 1 H), 7.58-7.62 (m, 2 H), 7.77 (t, J = 7.47 Hz, 2 H); high resolution mass spectrum (FAB) *m/z* 1864.9373 [$(M+Na)^+$; calcd for $C_{96}H_{128}N_{16}O_{21}$: 1864.9338].

Cyclo-[Lys(Fmoc)-Dpr-Asp(OH)-Phe-D-Trp-Lys(Cbz)-Thr-Dpr(NH_2)-Asp-Ala-Hep]. To a solution of 45.2 mg **17** in 2.25 mL CH_2Cl_2, 0.15 mL EDT, and 0.1 mL H_2O was added 2.5 mL TFA dropwise at room temperature. After the resultant solution was stirred at room temp for 70 min, the reaction mixture was concentrated to half of its original volume and then concentrated from dry benzene (2 x 5 mL). The resultant material was triturated with ice-cooled dry ether, and the precipitate was collected by filtration and washed with ether (4 x 10 mL). The crude solid was purified by a semi-preparative RP-HPLC (Condition: 21.4 mm C18 dynamax column, 50-25'-95 % buffer B, flow rate =12 mL/min. monitored at 254 nm. The major fractions (RT: 12.5 min) were pooled and lyophilized to provide 29.4 mg of the bicyclic peptide (74% yield) as a white amorphous solid: $[\alpha]_D^{25}$ -28.67° (c 0.15, CH_3OH); decomposed at 168-171 °C; ^1H NMR (500 MHz, CD_3OD) δ 1.22 (d, J = 6.34 Hz, 3 H), 1.25-1.38 (m, 7 H), 1.35 (d, J = 7.18 Hz, 3 H), 1.42-1.60 (m, 8 H), 1.61-1.68 (m, 2 H), 1.72-1.81 (m, 2 H), 1.82-1.90 (m, 1 H), 2.18-2.23 (m, 1 H), 2.23-2.31 (m, 1 H), 2.62-2.73 (m, 3 H), 2.75-2.83 (m, 2 H), 2.91-2.98 (m, 1 H), 3.07-3.12 (m, 5 H), 3.23-3.21 (m, 4 H), 3.33-3.49 (m, 3 H), 3.50-3.56 (m, 1 H), 4.10-4.25 (m, 4 H), 4.28-4.38 (m, 6 H), 4.50-4.60 (m, 3 H), 5.06 (s, 2 H), 6.95-7.0 (m, 2 H), 7.01-7.07 (m, 1 H), 7.08-7.16 (m, 5 H), 7.24-7.35 (m, 6 H), 7.32-7.40 (m, 3 H), 7.55-7.62 (m, 3 H), 7.61-7.70 (m, 1 H), 7.78 (d, J = 7.55 Hz, 2 H); high resolution mass spectrum (FAB) *m/z* 1630.7874 [$(M+H)^+$; calcd for $C_{83}H_{104}N_{16}O_{19}$+H: 1630.7819].

Cyclo-[Lys(Fmoc)-Dpr-Asp-Phe-D-Trp-Lys(Cbz)-Thr-Dpr-Asp-Ala-Hep] **(18)**. To a suspension of 28.4 mg (0.0174 mmol) of the above bicyclic peptide and 22 mg (0.26 mmol) solid $NaHCO_3$ in 3 mL dry DMF precooled to 0 °C was added 7.2 mg (0.021 mmol) DPPA. The solution was kept at 4 °C for 24 h. The reaction mixture was then filtered, washed with DMF, and the filtrate was evaporated to afford a crude residue which was purified by a semi-preparative RP-HPLC (Condition: 21.4 mm C18 dynamax column, 50-25'-95 % buffer B, flow rate: 12 ml/min; monitored at 254 nm). The major fractions with RT of 14.2 min were pooled and lyophilized to provide 6.8 mg of **18** (24% yield) as a white amorphous solid: $[\alpha]_D^{25}$ -12.57° (c 0.175, CH_3OH); decomposed at: 176-179 °C; ^1H NMR (500 MHz, CD_3OD) δ 1.16-1.23 (m, 2 H), 1.22 (d, J = 6.39 Hz, 3 H), 1.23-1.35 (m, 5 H), 1.37 (d, J = 7.16 Hz, 3 H), 1.39-1.61 (m, 13 H), 1.68-1.92 (m, 2 H), 2.24 (t, J = 6.14 Hz, 2 H), 2.46-2.55 (m, 2 H), 2.69-2.81 (m, 2 H), 2.82-2.92 (m, 3 H), 2.94-3.03 (m, 1 H), 3.07-3.18 (m, 5 H), 3.20-3.26 (m, 2 H), 3.52-3.68 (m, 2 H), 3.70-3.82 (m, 1 H), 3.90-3.98 (m, 1 H), 4.07-4.12 (m, 2 H), 4.15-4.18 (m, 1 H), 4.24-4.33 (m, 3 H), 4.46-4.50 (m, 2 H), 4.50-4.70 (m, 3 H), 4.98 (bs, 2 H), 6.60-6.65 (m, 2 H), 6.94-7.02 (m, 2 H), 7.04-7.07 (m, 3 H), 7.11-7.14 (m, 1 H), 7.22-7.31 (m, 7 H), 7.35-7.39 (m, 3 H), 7.55-7.57 (m, 1 H), 7.63 (d, J = 7.37 Hz, 2 H), 7.78 (d, J = 7.45 Hz, 2

H); high resolution mass spectrum (FAB) *m/z* 1633.7479 [(M+Na)$^+$; calcd for $C_{83}H_{102}N_{16}O_{18}$: 1633.7455].

Cyclo-(Lys-Dpr-Asp-Phe-D-Trp-Lys-Thr-Dpr-Asp-Ala-Hep) (**1**). Solid **18** (55.8 mg) was treated with 3.0 mL of 20% piperidine in DMF for 20 min at room temperature. The reaction mixture was then concentrated in vacuo. The resulting residue (**19**) along with 45 mg 10% Pd/C was mixed with 4.0 mL CH_3OH and hydrogenated under 39 psi H_2 for 2.5 h. The reaction mixture was then filtered through celite and washed with CH_3OH. The filtrate was concentrated in vacuo and the resultant material was purified by reverse-phase HPLC (Condition: 21.4 mm C18 dynamax column, 10-25'-95 % buffer B, flow rate:12 mL/min; monitored at 254 nm) to afford 5.3 mg of **1** (12% yield) (RT: 14.8 min) along with a wide range of higher molecular weight byproducts. The desired material was further purified using a semi-preparative Vydac-C4 column to afford **1** as a white amorphous solid: $[\alpha]_D^{25}$ -22.91 (c 0.092, H_2O); ^1H NMR (400 MHz, D_2O) δ 1.10-1.31 (m, 6 H), 1.23 (d, J = 6.3 Hz, 3 H), 1.32-1.48 (m, 4 H), 1.35 (d, J = 7.4 Hz, 3 H), 1.49-1.61 (m, 5 H), 1.63-1.82 (m, 5 H), 1.82-1.92 (m, 1 H), 2.14-2.34 (m, 4 H), 2.79-3.06 (m, 9 H), 3.09-3.19 (m, 2 H), 3.27 (dd, J = 10.8, 14.8 Hz, 1 H), 3.53 (dd, J = 4.4, 15.2 Hz, 1 H), 3.59-3.72 (m, 3 H), 3.76-3.91 (m, 2 H), 4.01-4.09 (m, 1 H), 4.23-4.27 (m, 1 H), 4.43-4.48 (m, 3 H), 4.49-4.51 (m, 1 H), 4.53-4.61 (m, 1 H), 4.61-4.65 (m, 1 H), 6.41 (d, J = 7.2 Hz, 2 H), 7.02 (t, J = 7.5 Hz, 2 H), 7.15 (t, J = 7.6 Hz, 1 H), 7.22 (t, J = 7.2 Hz, 1 H), 7.27 (s, 1 H), 7.27 (t, J = 8.4 Hz, 1 H), 7.53 (d, J = 8.0 Hz, 1 H), 7.58 (d, J = 7.6 Hz, 1 H); high resolution mass spectrum (FAB) *m/z* 1277.6438 [(M+Na)$^+$; calcd for $C_{60}H_{86}N_{16}O_{14}$: 1277.6407].

Cyclization studies on substrates H-D-Phe-Pro-Phe-Pro-Phe-Pro-OH (14) and H-Phe-Pro-D-Phe-Pro-Phe-Pro-OH (16). Linear peptides **14** and **16** were prepared starting from Fmoc-Pro-O-2-chlorotrityl resin using a similar method to that used for the synthesis of alloc-Lys(Fmoc)-Dpr-Asp(tBu)-Phe-D-Trp-Lys(Cbz)-Thr(tBu)-Dpr(Boc)-Asp-Ala-Hep-allyl.

A suspension of 33.6 mg TFA·H-D-Phe-Pro-Phe-Pro-Phe-Pro-OH (**14**) and 49 mg $NaHCO_3$ in 9.7 mL DMF was treated with 12.56 µL (0.05833 mmol, 1.5 eq.) DPPA at 0 °C for 48 h. The solution was then filtered and washed with DMF, and the filtrate was concentracted in vacuo and purified by RP-HPLC (Column: 0.46x250 mm, C18 dynamax, gradient: 10-10'-45%, then 45-15'-95% buffer B, monitored at 214 nm) to afford 24.2 mg (85% yield) cyclo-(D-Phe-Pro-Phe-Pro-Phe-Pro) (**15**) as a white amorphous solid: high resolution mass spectrum (FAB) *m/z* 754.3533 [(M+Na)$^+$; calcd for $C_{42}H_{48}N_6O_6$: 754.3525].

Under the identical conditions, a suspension of 11.0 mg (0.01273 mmol) TFA·H-Phe-Pro-D-Phe-Pro-Phe-Pro-OH (**16**) and 16 mg $NaHCO_3$ in 3.2 mL DMF was treated with 4.11 µL DPPA to afford 4.3 mg (46% yield) cyclo-(D-Phe-Pro-Phe-Pro-Phe-Pro) (**15**) and 3.9 mg

(42% yield) dimer product: cyclo-(D-Phe-Pro-Phe-Pro-Phe-Pro)2: high resolution mass spectrum (FAB) *m/z* 1487.7113 [(M+Na)$^+$; calcd for $C_{84}H_{96}N_{12}O_{12}$: 1487.7168].

Acknowledgments

Financial support was provided by the National Institutes of Health (Institute of General Medical Sciences) through grant GM-41821 and by Bachem, Inc. (CA), Merck Research Laboratories, Zeneca, and Smith Kline Beecham. We also thank Dr. George Furst and Mr. John Dykins of the University of Pennsylvania Spectroscopic Service Centers for assistance in securing certain high-field NMR and mass spectra, respectively.

References

1. Brazeau, P.; Vale, W.; Burgus, R.; Ling, N.; Butcher, M.; Rivier, J.; Guillemin, R. (1973) *Science* **179**, 78.
2. (a) Bauer, W.; Briner, U.; Doeper, W.; Haller, R.; Hugenin, R.; Marbach, P.; Petcher, T. J.; Pless, J. (1982) *Life Sciences*, **31**, 1133. (b) Plewe, G.; Beyer, J.; Krause, U.; Neufeld, M.; Del Pozo, E. (1984) *Lancet* 782. (c) Hoeldtke, R. D.; O'Dorisio, T. M.; Boden, G. (1986) *Lancet* 602.
3. Brown, P. J.; Schonbrunn, A. (1993) *J. Biol. Chem.* **268**, 6668. Rasolonjanahary, R.; Sévenet, T.; Voegelein, F. G.; Kordon, C. (1995) *Eur. J. Pharmacol.* **285**, 19.
4. (a) Holladay, L. A.; Puett, D. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1199. (b) Holladay, L.A., Rivier, J.; Puett, D. (1977) *Biochemistry* **16**, 4895.
5. Rivier, J.; Brown, M.; Vale, W. (1975) *Biochem. Biophys. Res. Comm.* **65**, 746.
6. Chamdrasekaren, R.; Lakshminarayanau, A. V.; Pandya, U. V.; Ramachandran, G. N. (1973) *Biochem. Biophys. Acta.* **303**, 14.
7. Veber, D. F.; Holly, F. W.; Paleveda, W. J.; Nutt, R. F.; Bergstrand, S. J.; Torchiana, M.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2636.
8. Veber, D. F.; Strachan, R. G.; Bergstrand, S. J.; Holly, F. W.; Homnick, C. F.; Hirschmann, R.; Torchiana, M.; Saperstein, R. (1976) *J. Am. Chem. Soc.* **98**, 2367.
9. Veber, D.; Holly, F.; Paleveda, W.; Nutt, R.; Bergstrand, S. J.; Torchina, M.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2636.
10. Rivier, J.; Brown, M.; Rivier, C.; Ling, N.; Vale, W. (1976) in *Proc. 14th Euro. Pept. Symp.* (Loffet, A., ed.), p. 427, Wepion, Belgium.
11. Vale, W.; Rivier, J.; Ling, N.; Brown, M. (1978) *Metabolism* **27**, 139.
12. (a) Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. (1979) *J. Org. Chem.* **44**, 3101. (b) Brady, S. F.; Freidinger, R. M.; Paleveda, W. J.; Colton, C. D.; Homnick, C. F.; Whitter, W. L.; Curley, P.; Nutt, R. F.; Veber, D. F. (1987) *J. Org. Chem.* **52**, 764.

13. Veber, D. F.; Holly, F. W.; Nutt, R. F.; Bergstrand, S. J.; Brady, S. F.; Hirschmann, R.; Glitzer, M. S.; Saperstein, R. (1979) *Nature* **280**, 512.
14. Homomeric cyclic peptides (in which the ring is built of amino acids) are said to be homodetic when the amino acid constituents of the ring are joined together only through amide bonds, see: Bodanszky, M.; Klausner, Y. S.; Ondetti, M. A. (1966) in *Peptide Synthesis* 2nd ed., p 190, John Wiley & Sons, New York.
15. A preliminary announcement has been published: Hirschmann, R.; Yao, W.; Arison, B.; Maechler, L.; Rosegay, A.; Sprengeler, P. A.; Smith, A. B. III (1996) *Tetrahedron Lett.* **37**, 5637.
16. Ösapay, G.; Taylor, J. W. (1992) *J. Am. Chem. Soc.* **114**, 6966.
17. (a) Hruby, V. J. (1993) *Biopolymer* **33**, 1073. (b) Hruby, V. J. (1982) *Life Sci.* **31**, 189.
18. Thorsett, E. D.; Harris, E. E.; Aster, S. D.; Peterson, E. R.; Snyder, J. P.; Springer, J. P.; Hirshfield, J.; Tristam, E. W.; Patchett, A. A.; Ulm, E. H.; Vassil, T. C. (1986) *J. Med. Chem.* **29**, 251.
19. (a) Veber, D. F.; Freidinger, R. M.; Perlow, D. S.; Paleveda, W. J. Jr.; Holly, F. W.; Strachan, R. G.; Nutt, R. F.; Arison, B. H.; Homnick, C.; Randall, W. C.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. (1981) *Nature* **292**, 55. (b) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Smith, A. B. III; Strader, C. D.; Cascieri, M. A.; Candelore, M. R.; Donaldson, C.; Vale, W.; Maechler, L. (1992) *J. Am. Chem. Soc.* **114**, 9217. (c) McDowell, R. S.; Gadek, T. R.; Barker, P. L.; Burdick, D. J.; Chan, K. S.; Quan, C. L.; Skelton, N.; Struble, M.; Thorsett, E. D.; Tischler, M.; Tom, J. Y. K.; Webb, T. R.; Burnier, J. P. (1994) *J. Am. Chem. Soc.* **116**,5069. (d) McDowell, R. S.; Blackburn, B. K.; Gadek, T. R.; McGee, L. R.; Rawson, T.; Reynolds, M. E.; Robarge, K. D.; Somers, T. C.; Thorsett, E. D.; Tischler, M.; Webb, R. R. II;Venuti, M. C. (1994) *J. Am. Chem. Soc.* **116**, 5077. (e) Dutta, A. S.; Gormley, J. J.; Woodburn, J. R.; Paul, P. K. C.; Osguthorpe, D. J.; Campbell, M. M. (1993) *Bioorg. Med. Chem. Lett.* **3**, 943.
20. Review : Kates, S. A.; Sole, N. A.; Albericio, F.; Barany, G. (1994) in *Peptides: Design, Synthesis, and Biological Activity* (Basava, C. & Anantharamaiah, G. M., eds.), pp.39-58, Birkhäuser, Boston.
21. (a) Rovero, P.; Quartara, L.; Fabbri, G. (1991) *Tetrahedron Lett.* **32**, 2639. (b) Tromelin, A.; Fulachier, M. H.; Mourier, G.; Ménez, A. (1992) *Tetrahedron Lett.* **33**, 5197. (d) McMurray, J. S. (1991) *Tetrahedron Lett.* **32**, 7679. (e) Trzeciak, A.; Bannwarth, W. (1992) *Tetrahedron Lett.* **33**, 4557. (f) Bloomberg, G. B.; Askin, D.; Gargaro, A. R.; Tanner, M. J. A. (1993) *Tetrahedron Lett.* **34**, 4709. (h) Kapurniotu, A.; Taylor, J. W. (1993) *Tetrahedron Lett.* **34**, 7031. For a review see: reference 15.
22. Barany, G.; Merrifield, R. B. (1977) *J. Am. Chem. Soc.* **99**, 7363.
23. Spanevello, R. A.; Hirschmann, R.; Raynor, K.; Reisine, T.; Nutt, R. F. (1991) *Tetrahedron Lett.* **32**, 4675.

24. (a) Carpino, L. A.; Han, G. Y., (1970) *J. Am. Chem. Soc.* **92**, 5748; (b) Chang, C. D.; Meienhofer, J. (1978) *Int. J. Peptide Protein Res.* **11**, 246. (c) Atherton, E.; Fox, H.; Harkiss, D.; Logan, C. J.; Sheppard, R. C.; Williams, B. J. (1978) *J. Chem. Soc., Chem. Commun.* 537. (d) For a review, see: Fields, G. B.; Noble, R. L. (1990) *Int. J. Peptide Protein Res.* **35**, 161.
25. Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiu, G.; Sotiriu, P.; Yao, W.; Schäfer, W. (1989) *Tetrahedron Lett.* **30**, 3943. Barlos, K.; Gatos, D.; Kapolos, S.; Papaphotiu, G.; Schäfer, W.; Yao, W. (1989) *Tetrahedron Lett.* **30**, 3947.
26. For reviews see: Fields, G. B.; Noble, R. L. (1990) *Int. J. Peptide Protein Res.* **35**, 161. Benz, H. (1994) *Synthesis* 337. Typical examples are cleavage from the resin by: nucleophiles, Findeis, M. A.; Kaiser, E. T. (1989) *J. Org. Chem.* **54**, 3478; mild acid, Rink, H. (1987) *Tetrahedron Lett.* **28**, 3787; Mergler, M.; Tanner, R.; Gosteli, J.; Grogg, P. (1988) *Tetrahedron Lett.* **29**, 4005; Albericio, F.; Barany, G. (1991) *Tetrahedron Lett.* **32**, 1015; base, Liu, Y. Z.; Ding, S. H.; Chu, J. Y.; Felix, A. M. (1990) *Int. J. Peptide Protein Res.* **35**, 95; fluoride, Mullen, D. G.; Barany, G. (1987) *Tetrahedron Lett.* **28**, 491; Ramage, R.; Barron, C. A.; Bielecki, S.; Holden, R.; Thomas, D. W. (1992) *Tetrahedron* **48**, 499; and Pd(0) catalysis, Kunz, H.; Dombo, B. (1988) *Angew. Chem. Int. Ed. Engl.* **27**, 711.
27. Atherton, E.; Benoiton, N. L.; Brown, E.; Sheppard, R. C.; Williams, B. J. (1986) *J. Chem. Soc., Chem. Commun.* 336. Grandas, A.; Jorba, X.; Giralt, E.; Fedroso, E. (1989) *Int. J. Peptide Protein Res.* **33**, 386.
28. Barlos, K.; Gatos, D.; Kutsogianni, S.; Papahotiou, G.; Poulos, C.; Tsegenidis, T. (1991) *Int. J. Peptide Protein Res.* **38**, 562. Barlos, K.; Gatos, D.; Papaphotiou, G.; Schafer, W. (1993) *Liebigs Ann. Chem.* 215.
29. Barlos, K.; Gatos, D.; Kutsogianni, S.; Papahotiou, G.; Poulos, C.; Tsegenidis, T. (1993) *Ann. Chem.* 215. Hirschmann, R.; Yao, W.; Cascieri, M. A.; Strader, C. D.; Maechler, L.; Cichy-Knight, M. A.; Hynes, J.; Van Rijn, R.; Sprengeler, P.; Smith, A. B., III. (1996) *J. Med. Chem.* **39**, 2441.
30. Tam, J. P. (1988) In *Macromolecular Sequencing and Synthesis: Selected Methods and Applications*, David H. Schlesinger, Ed., Alan R. Liss, Inc., New York, 153-184.
31. Hoffmann, E.; Beck-Sickinger, A. G.; Jung, G. (1991) *Liebigs Ann. Chem.* 585-590. Schmidt, U.; Lieberknecht, A.; Griesser, H.; Utz, R.; Beuttler, T.; Bartkowiak, F. (1986) *Synthesis* 361. Ueda, K.; Waki, M.; Izumiya, N. (1987) *Int. J. Peptide Protein Res.* **30**, 33. Schmidt, R.; Neubert, K. (1991) *Int. J. Peptide Protein Res.* **37**, 502.
32. (a) Cavelier-Frontin, F.; Pèpe, G.; Verducci, J.; Siri, D.; Jacquier, R. (1992) *J. Am. Chem. Soc.* **114**, 8885. (b) Kessler, H.; Haase, B. (1992) *Int. J. Peptide Protein Res.* **39**, 36.
33. Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. (1979) *J. Org. Chem.* **44**, 3101.
34. Yao, W., unpublished results.

35. Hirschmann, R.; Yao, W.; Cascieri, M. A.; Strader, C. D.; Maechler, L.; Cichy-Knight, M. A.; Hynes, J., Jr.; Van Rijn, R. D.; Sprengeler, P. A.; Smith, A. B., III (1996) *J. Med. Chem.* **39**, 2441.
36. Lender, A.; Yao, W. Q.; Sprengeler, P. A.; Spanevello, R. A.; Furst, G. T.; Hirschmann, R.; Smith, A. B., III (1993) *Int. J. Peptide Protein Res.* **42**, 509.
37. Dangles, O.; Guibé, F.; Balavoine, G.; Lavielle, S.; Marquet, A. (1987) *J. Org. Chem.* **52**, 4984.
38. The binding affinity of **1** was determined as previously described; see reference 35.
39. Vale, W.; Rivier, J.; Ling, N.; Brown, M. (1978) *Metabolism* **27**, 139.
40. Veber, D. F.; Holly, F. W.; Paleveda, W. J.; Nutt, R. F.; Bergstrand, S. J.; Torchiana, M.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2636-2640. Hirschmann, R. (1982) In *Chemistry of Natural Products. Proceedings of the Sino-American Symposium of Natural Products*, Wang Yu, Ed., Science Press, Beijing, China, 44-54.
41. Brady, S. F.; Paleveda, J. W.; Arison, B. H.; Saperstein, R.; Brady, E. J.; Raynor, K.; Reisine, T.; Veber, D. F.; Freidinger, R. M. (1993) *Tetrahedron* **49**, 3449.
42. Morgan, R. S.; McAdon, J. M. (1980) *Int. J. Peptide Protein Res.* **15**, 177.
43. Némethy, G.; Scheraga, H. A. (1981) *Biochem. Biophys. Res. Commun.* **98**, 482.
44. Kaupmann, K.; Bruns, C.; Raulf, F.; Weber, H. P.; Mattes, H.; Lübbert, H. (1995) *EMBO J.* **14**, 727.
45. Arison, B. H.; Hirschman, R.; Palveda, W. J.; Brady, S. F.; Veber, D. F. (1981) *Biochem. Biophys. Res. Comm.* **100**, 1148.
46. Applied Biosystem Inc. Publication, No 35, Fast Moc 0.25 and 0.10 mmol on the Model 431A.