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LARGE-SCALE SYNTHESIS OF ANTICOAGULANT DECAPEPTIDE MDL 28050

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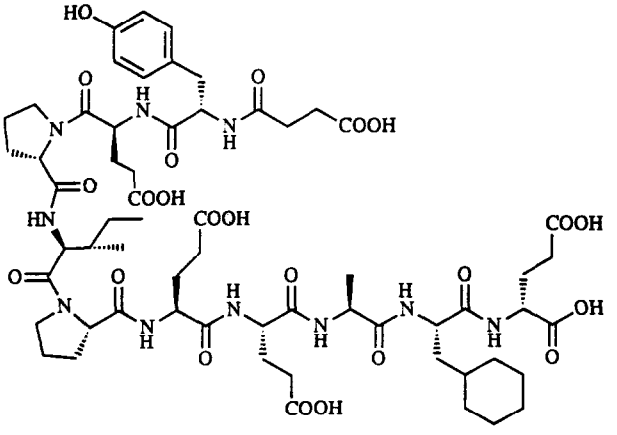
ABSTRACT: A solution phase synthesis of the anticoagulant decapeptide Suc-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-*D*-Glu-OH (**1**, MDL 28050) on a large scale is described. Our strategy employed in the 24-step total synthesis relies on a convergent approach. The basic feature is the preparation and the coupling of two protected pentapeptides, **2** and **3**. Several key intermediates were purified by crystallizations including the protected decapeptide **21**. Only a single purification required preparative HPLC. Using this synthetic route, we prepared 98% pure final product **1** on a 40-g scale. The overall yield of this process is about 20%.

Anticoagulants based on residues 45-65 of the leech protein hirudin exhibit highly specific and potent interactions with thrombin.[1] The hirudin-based anticoagulant decapeptide, Suc-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-*D*-Glu-OH (**1**, MDL 28050), which features a succinyl-capped N-terminus, the unnatural amino acid residue H-Cha-OH at position nine, and H-*D*-Glu-OH at the C-terminus represents a new type of thrombin inhibitor which prevents thrombin-induced fibrin clot formation in human plasma without blocking the enzyme's active site.[2]



Suc-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-*D*-Glu-OH (**1**, MDL 28050)

Abbreviations: BrZ, o-bromobenzyloxycarbonyl; Bzl, benzyl; Cha, cyclohexylalanyl; DCC, 1,3-dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DME, 1,2-dimethoxyethane; HOSu, N-hydroxysuccinimide; IPCF, isopropyl chloroformate; NMM, N-methylmorpholine; Pac, phenacyl; PTSA, p-toluenesulphonic acid; Suc, succinyl; Amino acid symbols denote the *L* configuration except where otherwise noted.

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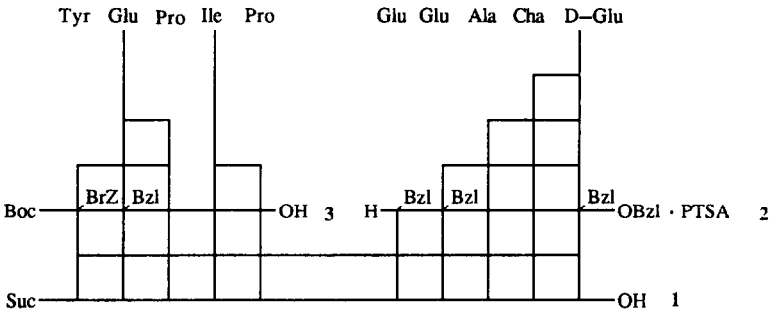
Preparation of small amounts of decapeptide **1** for initial biological screening was performed on an Applied Biosystems peptide synthesizer with a Boc-*D*-Glu-Merrifield solid support.[2] As larger quantities of peptide **1** were requested for clinical studies, the development of a practical and cost-effective synthesis became critical. Herein we report the details of our solution phase synthesis developed for the large-scale preparation of decapeptide **1**. Besides the usual advantages of a solution phase peptide synthesis[3], our reaction scheme has the specific advantage of permitting the purification of several key intermediates by crystallizations which allowed us to delay any elaborate chromatography until the final product **1** had been obtained.

A convergent [5+5] coupling strategy was deemed most practical for the solution phase synthesis[4] of the decapeptide **1**. The basic feature of this strategy is the coupling of two protected pentapeptides, **2** and **3**, to construct the decapeptide framework (Scheme I). The two pentapeptides were synthesized by different strategies. The protected Glu-Glu-Ala-Cha-*D*-Glu unit **2** was prepared starting from the C-terminal residue H-*D*-Glu(Bzl)-OBzl · PTSA (**6**) by sequential coupling with the required amino acid derivatives (Scheme I). The alternative, namely the coupling of Boc-Glu(Bzl)-Glu(Bzl)-Ala-OH with H-Cha-*D*-Glu(Bzl)-OBzl · PTSA in a [3+2] strategy, interestingly, afforded only starting materials. The left-hand Tyr-Glu-Pro-Ile-Pro portion **3** of our target molecule **1** was prepared by a convergent [3+2] coupling of a protected Tyr-Glu-Pro unit with the Ile-Pro unit (Scheme I). This specific coupling sequence, which dealt most efficiently with the "moderate" yield of the proline coupling steps, was considered to be the most economic approach to prepare peptide **3**.

Scheme I. [5+5] Synthetic Strategy for Decapeptide 1

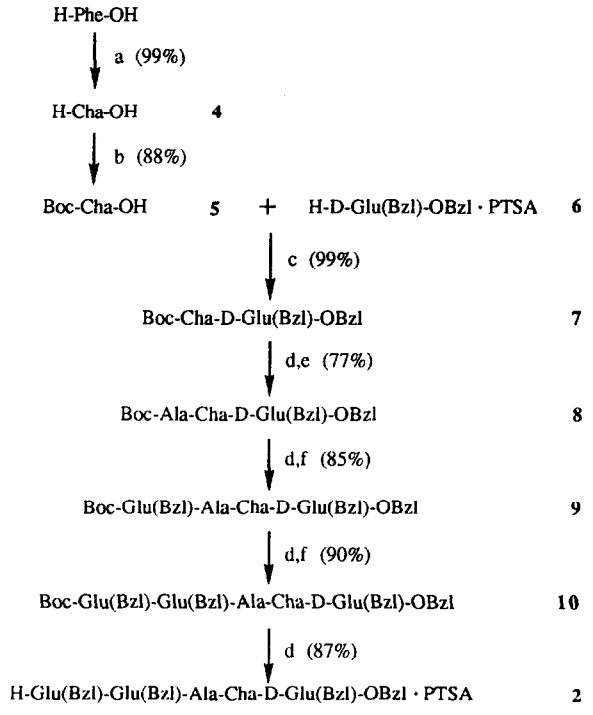


The selection of protecting groups for this large-scale synthesis, namely Boc, Bzl, Pac, and BrZ, was guided[5] mainly by the cost of the corresponding amino acid precursors, their compatibility in our reaction scheme, the physical properties of the corresponding intermediates and their ease of removal. In general, the mixed anhydride (IPCF/NMM)[6] coupling method was employed. Seven of the nine peptide bonds were constructed in high yield by this method with a simple aqueous workup procedure for the isolation of the coupling product. Although the Glu-Pro and Ile-Pro linkage could be formed by this technique as well (64% and 40% yield respectively), the N-hydroxysuccinimide ester method was employed instead affording these dipeptides in better yield.

RESULTS AND DISCUSSION

Linear Synthesis of Pentapeptide 2. Starting from the C-terminal amino acid residue of decapeptide **1**, the commercially available H-*D*-Glu(Bzl)-OBzl · PTSA (**6**) was coupled with Boc-Cha-OH (**5**) by the mixed anhydride method to afford dipeptide **7** (Scheme II). The unnatural amino acid **5** which is rather expensive commercially was prepared in large quantity from H-Phe-OH by catalytic hydrogenation[7] and subsequent protection with $(Boc)_2O$. Removal of the Boc group from dipeptide **7** was performed with excess TFA. After the addition of PTSA and trituration, analytically pure H-Cha-*D*-Glu(Bzl)-OBzl · PTSA was isolated as a crystalline solid. This compound was coupled with Boc-Ala-OH to give tripeptide **8** in excellent yield. By repeating the above described deprotection/coupling sequence, tripeptide **8** was converted to tetrapeptide **9**, and then further via Boc-protected pentapeptide **10** to the target molecule **2**. The ability to isolate each peptide intermediate as a pure solid PTSA salt from the corresponding deprotection step provided easy purification and greatly facilitated execution of the sequence on a large scale. Marfey analysis[8] of pentapeptide **2** showed less than 0.5% racemization. The overall yield of pentapeptide **2** from H-*D*-Glu(Bzl)-OBzl · PTSA was 51%.

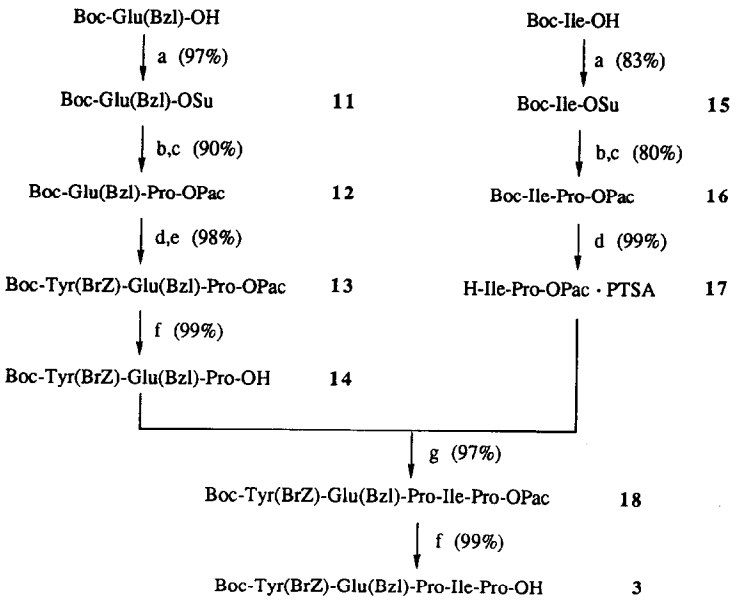
Scheme II. Linear Synthesis of Pentapeptide 2



(a) H_2, PtO_2; (b) $(Boc)_2O$, NaOH; (c) IPCF, NMM, THF; (d) TFA, PTSA · H_2O; (e) Boc-Ala- OH, IPCF, NMM, THF; (f) Boc-Glu(Bzl)-OH, IPCF, NMM, THF.

[3+2] Synthesis of Pentapeptide 3. The N-hydroxysuccinimide ester method was used for the preparation of dipeptides containing a proline residue. Thus, Boc-Glu(Bzl)-OH was treated with HOSu in the presence of DCC to afford the activated ester **11**. Unprotected H-Pro-OH was used to couple with ester **11** in DMF[9], and the resulting dipeptide was protected at the C-terminus as Pac-ester **12** (Scheme III). The alternative, i.e. coupling of ester **11** with H-Pro-OPac, was also possible, but gave dipeptide **12** in inferior yield. Boc-deprotection of dipeptide **12** followed by mixed anhydride coupling of the resulting product with commercially available Boc-Tyr(BrZ)-OH[10] provided tripeptide **13**. Cleavage of the Pac group[11] by zinc reduction gave Boc-Tyr(BrZ)-Glu(Bzl)-Pro-OH (**14**) as a thick oil. Meanwhile, the dipeptide building block H-Ile-Pro-OPac·PTSA (**17**) was prepared from Boc-Ile-OH and H-Pro-OH by the N-hydroxysuccinimide ester method as depicted in Scheme III. The [3+2] coupling of tripeptide **14** and dipeptide **17** by the mixed anhydride method then afforded pentapeptide **18**. Again, crude **18** was reduced with zinc to give the target molecule **3** (<1% racemization by Marfey analysis[8]). Both zinc reduction products, **14** and **3**, were purified by simple silica gel plug-filtration. The overall yield of pentapeptide **3** was 81% from Boc-Glu(Bzl)-OH and 63% from Boc-Ile-OH.

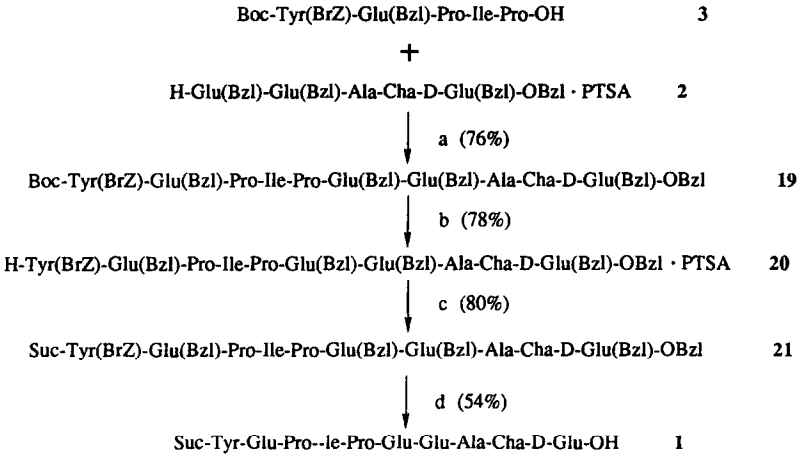
Scheme III. Convergent [3+2] Synthesis of Pentapeptide 3



(a) DCC, HOSu, DME; (b) H-Pro-OH, Et_3N, DMF; (c) $PhCOCH_2Br$, Et_3N, EtOAc; (d) TFA, PTSA·H_2O; (e) Boc-Tyr(BrZ)-OH, IPCF, NMM, THF; (f) Zn, 90% AcOH; (g) IPCF, NMM, THF.

[5+5] Synthesis of Decapeptide 1. Pentapeptides 2 and 3 were coupled by the mixed anhydride method to provide decapeptide 19 in 76% yield (Scheme IV). Removal of the Boc group followed by PTSA salt formation gave decapeptide PTSA salt 20. Compound 20 was acylated with succinic anhydride in the presence of NMM to afford the succinyl-capped decapeptide 21. Both decapeptides 20 and 21 were isolated as noncrystalline solids which allowed us to obtain them with sufficient purity and permitted us to avoid any tedious column chromatography. Final deprotection of decapeptide 21 by palladium-catalyzed hydrogenolysis afforded crude decapeptide 1. This material was purified by reverse phase preparative HPLC to provide a fluffy, white solid after lyophilization (98% pure by HPLC, <1% racemization by Marfey analysis[8], mp 158-160 °C). The overall yield of decapeptide 1 from pentapeptide 3 is 26%.

Scheme IV. Convergent [5+5] Synthesis of Decapeptide 1



(a) IPCF, NMM, THF; (b) TFA, PTSA·H_2O; (c) Succinic anhydride, NMM, CH_2Cl_2; (d) H_2, Pd/C, aq. AcOH.

CONCLUSIONS

This process illustrates the efficacy of a convergent solution phase approach for the synthesis of decapeptide 1. Particularly, the high coupling yield for the peptide intermediates, the minimal racemization and simple purifications provided us with an efficient way to prepare the target molecule 1 with satisfactory purity. The overall yield of decapeptide 1, including the final HPLC purification, is 14% from H-*D*-Glu(Bzl)-OBzl · PTSA (6, 12 steps), or 17% from Boc-Ile-OH (12 steps), or 23% from Boc-Glu(Bzl)-OH (10 steps).

EXPERIMENTAL SECTION

General. Protected amino acids were purchased from Bachem Inc., except Boc-Tyr(BrZ)-OH, which was purchased from Calbiochem Inc. Anhydrous THF (99+%), TFA (99+%), *L*-proline (99+%), and *L*-phenylalanine (99+%) were purchased from Aldrich Chemical Co., Inc. Other solvents and reagents were analytical grade. Rotary evaporations were performed at 30-35 °C and 20 torr. Melting points were obtained on a Thomas Hoover melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian XL 300 and/or Varian GEMINI-300 spectrometers at 300 MHz for ^1H and 75 MHz for ^{13}C. All chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane. Mass spectra were obtained on a Finnigan MAT4600 spectrometer at 70 eV. Methane was the reagent gas used for chemical ionization spectra. The high resolution (HR) and fast atom bombardment (FAB) mass spectra were obtained on a ZAB2-SE reverse geometry double focusing mass spectrometer (VG Analytical, Ltd.). Relative intensities are shown in parentheses. Optical rotations were measured with a Perkin-Elmer 241 polarimeter in a 1-dm cell. Elemental and thermogravimetric (Tg) analyses were performed by the Analytical Department, Marion Merrell Dow Research Institute, Cincinnati Center. Thin layer chromatography (TLC) was performed on silica gel 60 precoated plates (0.25 mm, Merck). Product purities were analyzed on a Waters 600 Millipore HPLC system (4.6 mm x 25 cm Vydac 218TP54 5μ stationary phase, gradient elution of 15% to 40% CH_3CN in H_2O (0.05% TFA) in 25 min at 2.0 mL/min, 215 nm detection). For amino acid analysis of **1**, the peptide (50 nmol) was hydrolyzed with HCl (6 N, 0.1% phenol) for 48 h at 105 °C. The analysis was performed on a Beckman System 6300 amino acid analyzer.

H-Cha-OH (4). A solution of H-Phe-OH (50 g, 0.30 mol) in AcOH (200 mL) and H_2O (140 mL) was hydrogenated in a Parr apparatus with PtO_2 (2.5 g) at 50 psi and 50 °C for 18 h. The mixture was cooled to room temperature, diluted with MeOH (250 mL) and H_2O (250 mL), and filtered to remove the catalyst. The filtrate was diluted with Et_2O (1.0 L), stirred for 18 h, and filtered to give a white powdery **4** (38 g, 74%). The filtrate was concentrated to a slurry (200 mL) which was filtered to give a second crop of **4** (13 g, 25%): mp 297-300 °C (lit.[7] 295-297 °C); ^1H NMR (TFA/$CDCl_3$) δ 7.31 (s, 3H) 4.3 (m, 1H, NCH) 2.0 (m, 1H) 1.8 (m, 5H) 1.5 (m, 1H) 1.2 (m, 4H) 1.0 (m, 2H); ^{13}C NMR (TFA/$CDCl_3$) δ 174.8, 52.2, 38.0, 33.7, 33.0, 32.1, 25.9, 25.7, 25.5; MS m/z 172 (MH^+, 100), 126(75). $[\alpha]_D^{20}$ +10.4° (c 0.99, 0.01 N HCl). HRMS: calc'd. for $C_9H_{18}NO_2$ (MH^+): 172.1337. Found: 172.1338.

Boc-Cha-OH · dicyclohexylamine (5). H-Cha-OH (**4**, 75 g, 0.44 mol) was dissolved in DME (1.4 L) containing H_2O (1.1 L) and NaOH (16.5 g, 0.41 mol) at 4 °C. To this solution was added di-*t*-butyldicarbonate (105 g, 0.48 mol), and the resulting mixture was warmed to room temperature and stirred for 18 h. The clear solution was acidified (pH 2) with $NaHSO_4$ (375 mL, 1 M), the DME was removed by rotary evaporation, and the resulting aqueous mixture was extracted with CH_2Cl_2 (3 x 500 mL). The combined organic layers were dried with $MgSO_4$ and filtered. The filtrate was treated with dicyclohexylamine (87 mL, 0.44 mol). The solution was concentrated to give a solid residue. This material was recrystallized from *t*-BuOMe (1.5 L) to yield the title compound **5** (174 g, 88%): mp 168-170 °C; ^1H NMR ($CDCl_3$) δ 8.8 (br s, 2H) 5.3 (m, 1H) 4.0 (m, 1H, NCH) 3.0 (m, 2H) 2.0 (m, 3H) 1.42 (s, 9H) 1.9-1.0 (m, 28H) 0.9 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 177.5, 155.4, 78.3, 53.9, 52.5, 41.8, 34.4, 33.9, 33.3, 29.2, 28.4, 26.6, 26.4, 26.3, 25.2, 24.8; MS m/z 272 (MH^+, 1), 216(1.5), 172(100). $[\alpha]_D^{20}$ +1.58° (c 1.01, $CHCl_3$). Anal. calc'd. for $C_{26}H_{48}NO_2$ (452.4): C, 68.98; H, 10.68; N, 6.18. Found: C, 69.14; H, 11.00; N, 6.28.

Boc-Cha-*D*-Glu(Bzl)-OBzl (7). A solution of Boc-Cha-OH · DCHA (**5**, 50 g, 0.11 mol) in EtOAc (800 mL) was mixed with aqueous $NaHSO_4$ (600 mL, 2 M) and stirred at room temperature for 20 min. The layers were separated, and the aqueous layer was extracted with EtOAc (2 x 600 mL). The combined organic layers were dried with $MgSO_4$ and concentrated by rotary evaporation to a colorless oil (41 g). The oil was dissolved in anhydrous THF (800 mL), cooled to -5 °C, and treated with NMM (11 mL, 0.11 mol) followed by IPCF (110 mL, 1 M in toluene). The mixture was stirred for 15 min, treated with NMM (11 mL, 0.11 mol) followed by H-*D*-Glu(Bzl)OBzl · PTSA (**6**, 55 g, 0.11 mol), and stirred for 1.5 h at -5 °C. The mixture was treated with sat'd NH_4Cl (80 mL) and filtered. The filtrate was concentrated, and the residue was dissolved in CH_2Cl_2 (1 L) and washed with aqueous HCl (240 mL, 1 M). The organic layer was dried with $MgSO_4$ and filtered. The filtrate was concentrated to give dipeptide **7** (65 g, 99%) as a glass: ^1H NMR ($CDCl_3$) δ 7.3 (m, 10H) 6.82 (d, 1H, J = 7.5 Hz) 5.15 (s, 2H) 5.10 (s, 2H) 4.7 (m, 1H, NCH) 4.6 (m, 1H) 4.1 (m, 1H, NCH) 2.4 (m, 2H) 2.2 (m, 1H) 2.0 (m, 1H) 1.7 (m, 9H) 1.35 (s, 9H) 1.1 (m, 4H); MS m/z 581 (MH^+, 50), 525 (100), 481 (50). $[\alpha]_D^{20}$ -16.8° (c 2.35, $CHCl_3$). HRMS: Calc'd. for $C_{33}H_{45}N_2O_7$ (MH^+): 581.3226. Found: 581.3204.

Boc-Ala-Cha-*D*-Glu(Bzl)-OBzl (8). Dipeptide **7** (65 g, 0.11 mol) was cooled in an ice bath and treated with TFA (250 mL). The mixture was stirred for 15 min, treated with PTSA · H_2O (21 g, 0.11 mol), and stirred for 30 min. The resulting solution was concentrated by rotary evaporation, and the residual syrup was dissolved in CH_2Cl_2 (200 mL). The solution was concentrated again to give a glass. The glass was triturated with *t*-BuOMe (300 mL) and stirred for 1 h at room temperature to give white crystals. The crystals were filtered and washed with Et_2O (300 mL) to give H-Cha-*D*-Glu(Bzl)-OBzl · PTSA (53 g, 81%): mp 119-121 °C; ^1H NMR ($CDCl_3$) δ 8.43 (d, 1H, J = 7.5 Hz) 7.8 (br s, 2H) 7.69 (d, 2H, J = 7.5 Hz) 7.2 (m, 11H) 7.01 (d, 2H, J = 7.5 Hz) 5.0 (m, 4H) 4.5 (m, 1H, NCH) 4.4 (m, 1H, NCH) 2.4 (m, 2H) 2.25 (s, 3H)

2.2 (m, 1H) 2.0 (m, 1H) 1.6 (m, 3H) 1.4 (m, 4H) 1.2 (m, 1H) 0.9 (m, 3H) 0.7 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 172.3, 171.5, 169.6, 141.4, 140.2, 135.8, 135.3, 128.8, 128.5, 128.4, 128.2, 128.1, 128.0, 126.1, 67.2, 66.3, 52.1, 51.8, 38.9, 33.4, 33.0, 32.4, 30.2, 26.3, 26.1, 25.7, 21.3; MS m/z 481 (MH^+, 63), 173 (43), 126 (18), 91 (100). $[\alpha]_D^{20}$ +27.4° (c 1.03, $CHCl_3$). Anal. calc'd. for $C_{35}H_{44}N_2O_8S$ (652.8): C, 64.39; H, 6.79; N, 4.29; S, 4.91. Found: C, 64.33; H, 7.05; N, 4.22; S, 5.10.

A solution of Boc-Ala-OH (15.4 g, 0.081 mol) in anhydrous THF (530 mL) was cooled to -5 °C, and treated with NMM (9 mL, 0.081 mol) followed by IPCF (81 mL, 1 M in toluene). The mixture was stirred for 20 min, treated with NMM (9 mL, 0.081 mol) followed by H-Cha-*D*-Glu(Bzl)-OBzl · PTSA (53 g, 0.081 mol), and stirred for 2 h at -5 °C. The mixture was treated with sat'd NH_4Cl (80 mL) and filtered. The filtrate was concentrated by rotary evaporation, and the residue was dissolved in CH_2Cl_2 (1 L). The solution was washed with aqueous HCl (260 mL, 1 M) and sat'd $NaHCO_3$ (300 mL), dried with $MgSO_4$, and concentrated to give a colorless oil. The oil was triturated with hexane (200 mL), and this mixture was stirred for 18 h at room temperature and filtered to give white, powdery tripeptide 8 (51 g, 95%) after drying: mp 103-105 °C; ^{1}H NMR ($CDCl_3$) δ 7.3 (m, 10H) 7.05 (d, 1H, *J* = 7.0 Hz) 6.50 (d, 1H, *J* = 7.0 Hz) 5.1 (m, 4H) 5.0 (m, 1H) 4.6 (m, 1H, NCH) 4.5 (m, 1H, NCH) 4.1 (m, 1H, NCH) 2.4 (m, 2H) 2.2 (m, 1H) 2.0 (m, 1H) 1.7 (m, 6H) 0.9-1.5 (m, 7H) 1.43 (s, 9H) 1.32 (d, 3H, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 172.7, 172.5, 172.0, 155.9, 135.7, 135.2, 128.5, 128.4, 128.3, 128.2, 128.1, 80.6, 67.1, 66.4, 51.7, 50.9, 50.6, 39.0, 34.1, 33.6, 32.2, 30.2, 28.2, 26.7, 26.3, 26.1, 25.9, 17.8; MS m/z 652 (MH^+, 31), 596 (26), 328 (15), 92 (100). $[\alpha]_D^{20}$ -34.4° (c 1.05, $CHCl_3$). Anal. calc'd. for $C_{36}H_{49}N_3O_8$ (651.8): C, 66.32; H, 7.58; N, 6.45. Found: C, 66.24; H, 7.74; N, 6.36.

Boc-Glu(Bzl)-Ala-Cha-*D*-Glu(Bzl)-OBzl (9). Tripeptide 8 (51 g, 0.078 mol) in CH_2Cl_2 (50 mL), was treated with TFA (250 mL) at 4 °C. The solution was stirred for 15 min, treated with PTSA · H_2O (15 g, 0.078 mol), and stirred for 15 min. The resulting solution was concentrated by rotary evaporation, and the residual syrup was dissolved in CH_2Cl_2 (200 mL). The solution was concentrated again to give a glass. The glass was triturated with Et_2O (300 mL) and stirred for 30 min at room temperature. The solid was collected by filtration to give H-Ala-Cha-*D*-Glu(Bzl)-OBzl · PTSA (53 g, 94%) after drying: mp 174-176 °C; ^{1}H NMR (DMSO-d_6) δ 8.60 (d, 1H, *J* = 8.0 Hz) 8.49 (d, 1H, *J* = 8.0 Hz) 8.0 (br s, 3H) 7.48 (d, 2H, *J* = 8.0 Hz) 7.3 (m, 10H) 7.10 (d, 2H, *J* = 8.0 Hz) 5.10 (s, 2H) 5.06 (s, 2H) 4.4 (m, 1H, NCH) 4.3 (m, 1H, NCH) 3.8 (m, 1H, NCH) 2.4 (m, 2H) 2.28 (s, 3H) 2.1 (m, 1H) 1.9 (m, 1H) 1.28 (d, 3H, *J* = 7.0 Hz) 0.8-1.7 (m, 13H); ^{13}C NMR (DMSO-d_6) δ 172.0, 171.9, 171.4, 169.2, 145.6, 137.7, 136.1, 135.9, 128.6, 128.5, 128.1, 127.9, 125.5, 66.1, 65.6, 51.1, 50.5, 48.0, 33.3, 32.9, 32.1, 29.7, 26.0, 25.9, 25.7, 25.6, 20.8, 17.2; MS m/z 552 (MH^+, 100) 173 (30), 115 (18), 102 (25). $[\alpha]_D^{20}$ +4.47° (c 1.03, MeOH). Anal. calc'd. for $C_{38}H_{49}N_3O_9S$ (723.9): C, 63.05; H, 6.82; N, 5.81; S, 4.43. Found: C, 63.32; H, 6.90; N, 5.79; S, 4.45.

A solution of Boc-Glu(Bzl)-OH (53 g, 0.16 mol) in anhydrous THF (1 L) was cooled to -5 °C, and treated with NMM (17 mL, 0.16 mol) followed by IPCF (156 mL, 1 M in toluene). The mixture was stirred for 15 min, treated with NMM (17 mL, 0.16 mol) followed by H-Ala-Cha-*D*-Glu(Bzl)-OBzl · PTSA (113 g, 0.16 mol), and stirred for 2 h at 0 °C. The mixture was treated with sat'd NH_4Cl (200 mL), stirred for 30 min, and filtered. The filtrate was concentrated by rotary evaporation, and the residue was dissolved in CH_2Cl_2 (1.5 L). The solution was washed with aqueous HCl (600 mL, 1 M) and sat'd $NaHCO_3$ (600 mL), dried with $MgSO_4$, and concentrated. The oily residue was triturated with Et_2O (2 L), and this mixture was stirred for 1 h at room temperature and filtered to give white, powdery tetrapeptide 9 (122 g, 90%): mp 137-138 °C; ^{1}H NMR ($CDCl_3$) δ 7.2-7.4 (m, 15H) 7.16 (d, 1H, *J* = 7.5 Hz) 7.00 (d, 1H, *J* = 8.5 Hz) 6.70 (d, 1H, *J* = 6.5 Hz) 5.70 (d, 1H, *J* = 6.5 Hz) 5.1 (m, 4H) 5.0 (m, 2H) 4.6 (m, 1H, NCH) 4.5 (m, 1H, NCH) 4.3 (m, 1H, NCH) 4.0 (m, 1H, NCH) 2.5 (m, 4H) 1.42 (s, 9H) 1.36 (d, 3H, *J* = 7.0 Hz) 0.8-2.3 (m, 17H); ^{13}C NMR ($CDCl_3$) δ 178.9, 173.6, 172.6, 172.4, 172.2, 172.0, 171.5, 156.3, 135.9, 135.4, 128.6, 128.5, 128.44, 128.40, 128.3, 128.2, 128.1, 128.0, 80.8, 66.9, 66.8, 66.2, 55.1, 51.7, 51.0, 50.3, 38.7, 34.2, 33.6, 31.8, 30.7, 30.3, 28.2, 26.7, 26.4, 26.1, 26.0, 17.6; FAB-MS m/z 871 (MH^+, 32), 781 (14), 722 (10), 552 (14), 481 (7), 328 (7), 185 (100), 126 (14). $[\alpha]_D^{20}$ -9.8° (c 1.10, MeOH). Anal. calc'd. for $C_{48}H_{62}N_4O_{11}$ (871.0): C, 66.19; H, 7.18; N, 6.43. Found: C, 66.25; H, 7.27; N, 6.31.

Boc-Glu(Bzl)-Glu(Bzl)-Ala-Cha-*D*-Glu(Bzl)-OBzl (10). Tetrapeptide 9 (121 g, 0.14 mol) was cooled in an ice bath and treated with TFA (275 mL). The mixture was stirred for 20 min, and then treated with PTSA · H_2O (26.5 g, 0.14 mol). The mixture was warmed to room temperature and then stirred for 1 h. The resulting solution was concentrated by rotary evaporation, and the residual syrup was triturated with Et_2O/MeOH (2.5 L, 4/1) to give, after filtration, H-Glu(Bzl)-Ala-Cha-*D*-Glu(Bzl)-OBzl · PTSA (119 g, 91%) as a white powder: mp 191-193 °C; ^{1}H NMR (DMSO-d_6) δ 8.62 (d, 1H, *J* = 8.0 Hz) 8.48 (d, 1H, *J* = 8.0 Hz) 8.1 (m, 3H) 7.47 (d, 2H, *J* = 8.0 Hz) 7.3 (m, 16H) 7.10 (d, 2H, *J* = 8.0 Hz) 5.1 (m, 6H) 4.4 (m, 3H) 3.8 (m, 1H, NCH) 2.5 (m, 2H) 2.4 (m, 2H) 2.27 (s, 3H) 1.18 (d, 3H, *J* = 7.0 Hz) 0.6-2.1 (m, 17H); ^{13}C NMR (DMSO-d_6) δ 172.2, 172.0, 171.7, 171.4, 171.3, 167.5, 145.6, 137.7, 136.1, 136.0, 135.9, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 125.5, 66.0, 65.7, 65.6, 51.3, 51.1, 50.2, 48.2, 33.4, 32.9, 32.0, 29.7, 28.8, 26.4, 26.0, 25.9, 25.7, 25.5, 20.8, 18.1; FAB-MS m/z 771 (MH^+, 100), 681 (28), 591 (2), 552 (5), 481 (15), 391 (7), 328 (24), 291 (20), 263 (14), 192 (31), 126 (35). $[\alpha]_D^{20}$ -4.2° (c 1.10, MeOH). Anal. calc'd. for $C_{50}H_{62}N_4O_{12}S$ (943.1): C, 63.67; H, 6.63; N, 5.94; S, 3.40. Found: C, 63.37; H, 6.85; N, 5.91; S, 3.45.

A solution of Boc-Glu(Bzl)-OH (43 g, 0.13 mol) in anhydrous THF (1 L) was cooled to -5 °C, and treated with NMM (14 mL, 0.13 mol) followed by IPCF (126 mL, 1 M in toluene). The mixture was stirred for 20 min at 0 °C, treated with NMM (14 mL, 0.13 mol) followed by H-Glu(Bzl)-Ala-Cha-*D*-Glu(Bzl)-OBzl · PTSA (119 g, 0.13 mol), and stirred for 2 h at 0 °C. The mixture was treated with sat'd NH_4Cl (100 mL), stirred for 30 min, and filtered. The filtrate was concentrated by rotary evaporation, and the residue was dissolved in CH_2Cl_2 (1.5 L). The solution was washed with aqueous HCl (620 mL, 1 M) and sat'd $NaHCO_3$ (700 mL), dried with $MgSO_4$, and concentrated to give pentapeptide **10** (137 g, 100%) as a white foam: ^1H NMR ($CDCl_3$) δ 7.95 (d, 1H, *J* = 7.0 Hz) 7.54 (d, 1H, *J* = 8.0 Hz) 7.3 (m, 20H) 7.1 (m, 2H) 5.6 (d, 1H, *J* = 7.0 Hz) 5.1 (m, 8H) 4.6 (m, 1H, NCH) 4.4 (m, 1H, NCH) 4.3 (m, 1H, NCH) 4.1 (m, 1H, NCH) 3.8 (m, 1H, NCH) 2.5 (m, 7H) 1.43 (s, 9H) 1.40 (d, 3H, *J* = 7.0 Hz) 0.8-2.3 (m, 18H). $[\alpha]_D^{20}$ -7.8° (c 1.53, $CHCl_3$). Anal. calc'd. for $C_{60}H_{75}O_{14}$ (1090.3): C, 66.08; H, 6.94; N, 6.43. Found: C, 66.28; H, 6.93; N, 6.48.

H-Glu(Bzl)-Glu(Bzl)-Ala-Cha-*D*-Glu(Bzl)-OBzl · PTSA (2). Pentapeptide **10** (137 g, 0.12 mol) was cooled in an ice bath and treated with TFA (300 mL). The mixture was stirred for 20 min, treated with PTSA · H_2O (24.4 g, 0.13 mol), and stirred for 1 h. The resulting solution was concentrated by rotary evaporation, and the residual syrup was dissolved in CH_2Cl_2 (400 mL). This solution was concentrated again to give a glass. The glass was dissolved in MeOH (500 mL), and this solution was triturated with Et_2O/*t*-BuOMe (2 L, 1/1) to give, after filtration, peptapeptide **2** (130 g, 87%) as a white powder: mp 182-184 °C; ^1H NMR (DMSO-d_6) δ 8.60 (d, 1H, *J* = 8.0 Hz) 8.39 (d, 1H, *J* = 8.0 Hz) 8.21 (d, 1H, *J* = 7.0 Hz) 8.1 (br s, 2H) 7.91 (d, 1H, *J* = 8.0 Hz) 7.47 (d, 2H, *J* = 8.0 Hz) 7.3 (m, 21H) 7.09 (d, 2H, *J* = 8.0 Hz) 5.1 (m, 8H) 4.3 (m, 4H, NCH) 3.8 (m, 1H, NCH) 2.5 (m, 4H) 2.4 (m, 2H) 2.27 (s, 3H) 1.13 (d, 3H, *J* = 7.0 Hz) 0.7-2.1 (m, 19H); ^{13}C NMR (DMSO-d_6) δ 173.7, 172.2, 172.1, 171.9, 171.6, 171.3, 169.9, 167.8, 145.7, 137.6, 136.1, 136.0, 135.8, 128.4, 128.3, 128.0, 127.9, 127.80, 127.75, 127.70, 125.5, 66.0, 65.7, 65.5, 51.8, 51.3, 51.0, 50.2, 48.0, 33.3, 32.9, 32.0, 30.0, 29.7, 28.9, 27.5, 26.2, 26.0, 25.9, 25.7, 25.5, 20.8, 17.8; FAB-MS m/z 990 (MH^+, 8), 900 (12), 810 (10), 720 (5), 645 (3), 553 (9), 461 (18), 369 (41), 277 (88), 185 (100). $[\alpha]_D^{20}$ -11.2° (c 1.00, MeOH). Anal. calc'd. for $C_{62}H_{75}N_5O_{15}S \cdot 1.2\ H_2O$ (1184.0)[12]: C, 62.90; H, 6.59; N, 5.91. Found: C, 62.89; H, 6.52; N, 6.00.

Boc-Glu(Bzl)-OSu (11). A mixture of Boc-Glu(Bzl)-OH (70 g, 0.21 mmol) and anhydrous DME (415 mL) was cooled to 5 °C, treated with HOSu (24 g, 0.21 mol), and stirred for 30 min. This mixture was treated with a solution of DCC (47 g, 0.23 mol) dissolved in DME (50 mL), and stirred for 18 h at 5 °C. The resulting cold mixture was filtered, and the filtrate was concentrated by rotary evaporation. The residue was treated with hexane (400 mL), and this mixture was stored for 18 h at -20 °C. The mixture was filtered to give ester **11** (88 g, 97%) as white needles: mp 100-102 °C; ^1H NMR ($CDCl_3$) δ 7.4 (m, 5H) 5.2 (m, 1H) 5.13 (s, 2H) 4.8 (m, 1H, NCH) 2.83 (s, 4H) 2.60 (t, 2H, *J* = 7.2 Hz) 2.3 (m, 1H) 2.2 (m, 1H) 2.2 (m, 1H) 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.3, 168.5, 168.0, 154.0, 145.0, 135.5, 128.6, 128.2, 77.4, 66.6, 51.4, 29.8, 28.2, 28.1, 25.6; MS m/z 435 (MH^+, 3), 379 (14), 335 (100). $[\alpha]_D^{20}$ -22.7° (c 1.01, dioxane). Anal. calc'd. for $C_{21}H_{26}N_2O_8$ (434.5): C, 58.06; H, 6.03; N, 6.45. Found: C, 58.18; H, 6.22; N, 6.50.

Boc-Glu(Bzl)-Pro-OPac (12). A mixture of H-Pro-OH (25 g, 0.22 mol), DMF (400 mL), and Et_3N (30 mL, 0.22 mol) at room temperature was treated with a solution of ester **11** (85 g, 0.2 mol) in DMF (280 mL), and stirred for 18 h. The resulting mixture was concentrated by rotary evaporation at 60 °C. The residue was dissolved in EtOAc (1 L), and this solution was washed with aqueous HCl (2 x 200 mL, 1 M) and sat'd brine (2 x 200 mL). The organic layer was dried with $MgSO_4$ and concentrated to give a white foam (ca. 96 g). This foam was dissolved in EtOAc (600 mL), and this solution was treated with Et_3N (28 mL, 0.2 mol) and 2-bromoacetophenone (39 g, 0.2 mol) at room temperature. The resulting mixture was stirred for 18 h at room temperature and filtered. The filtrate was washed with sat'd $NaHCO_3$ (2 x 300 mL) and brine (2 x 300 mL), dried with $MgSO_4$, and concentrated to give a white cake. The cake was treated with hexane (600 mL), and the resulting slurry was stirred for 2 h and filtered to give dipeptide **12** (97 g, 90%) as a white powder: mp 94-95 °C; ^1H NMR ($CDCl_3$) δ 7.89 (d, 2H, *J* = 7.5 Hz) 7.62 (t, 1H, *J* = 7.5 Hz) 7.49 (t, 2H, *J* = 7.5 Hz) 7.3 (m, 5H) 5.50 (d, 1H, *J* = 17.0 Hz) 5.32 (d, 1H, *J* = 8.0 Hz) 5.23 (d, 1H, *J* = 17.0 Hz) 5.12 (s, 2H) 4.7 (m, 1H, NCH) 4.6 (m, 1H, NCH) 3.7 (m, 2H, NCH_2) 2.5 (m, 2H) 2.3 (m, 2H) 2.2 (m, 2H) 2.0 (m, 1H) 1.8 (m, 1H) 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 191.8, 172.8, 171.3, 170.8, 155.6, 135.8, 134.0, 133.9, 128.9, 128.5, 128.2, 128.1, 127.7, 79.7, 66.4, 66.3, 58.7, 50.8, 47.0, 29.4, 29.1, 28.3, 27.9, 24.9; MS m/z 553 (MH^+, 63), 497 (81), 453 (82), 121 (100). $[\alpha]_D^{20}$ -69.9° (c 1.07, $CHCl_3$). Anal. calc'd. for $C_{30}H_{36}N_2O_8$ (552.6): C, 65.20; H, 6.57; N, 5.07. Found: C, 65.32; H, 6.79; N, 5.03.

Boc-Tyr(BrZ)-Glu(Bzl)-Pro-OPac (13). Dipeptide **12** (95 g, 0.17 mol) was cooled to 4 °C, and was dissolved in TFA (150 mL) over a 15 min period. This solution was treated with PTSA · H_2O (33 g, 0.17 mol), and the resulting mixture was stirred for 30 min at room temperature. The resulting solution was concentrated by rotary evaporation, and the residual syrup was treated with CH_2Cl_2 (170 mL) and Et_2O (180 mL). The resulting mixture was stirred for 2 h and filtered to give H-Glu(Bzl)-Pro-OPac · PTSA (107 g, 99%) as a white powder: mp 188-190 °C; ^1H NMR (DMSO-d_6/D_2O) δ 7.96 (d, 2H, *J* = 7.5 Hz) 7.72 (t, 1H, *J* = 7.5 Hz) 7.60 (t, 2H, *J* = 7.5 Hz) 7.54 (d, 2H, *J* = 7.5 Hz) 7.30 (s, 5H) 7.29 (d, 2H, *J* = 7.5 Hz) 5.56 (d, 1H, *J* = 17.0 Hz) 5.47 (d, 1H, *J* = 17.0 Hz) 5.08 (s, 2H) 4.6 (m, 1H, NCH) 4.2 (m, 1H, NCH) 3.7 (m, 1H, NCH_2) 3.5 (m, 1H, NCH_2) 2.6 (m, 2H) 2.3 (m, 1H) 2.29 (s, 3H) 2.2 (m, 1H) 2.0 (m, 4H); ^{13}C NMR (DMSO-d_6/D_2O) δ 193.0, 172.3, 171.4, 167.6, 146.0, 138.1, 136.3, 134.5, 134.0, 129.3, 128.8, 128.7, 128.4, 128.3, 128.2, 125.8, 67.0, 65.9, 58.6, 50.0, 46.8, 28.7, 28.2, 25.3, 24.5, 20.8; MS m/z 453 (MH^+, 3), 407 (16), 345 (11), 317 (100), 227 (12), 209 (51), 173 (19), 137 (82), 119

(74), 91 (92). Anal. calc'd. For $C_{32}H_{36}N_2O_9S$ (624.7): C, 61.53; H, 5.80; N, 4.48. Found: C, 61.92; H, 5.85; N, 4.46.

A solution of Boc-Tyr(BrZ)-OH (74 g, 0.15 mol, Calbiochem) and anhydrous THF (1 L) was cooled to -5 °C, and treated with NMM (16 mL, 0.15 mol) followed by IPCF (149 mL, 1 M in toluene). The mixture was stirred for 20 min at -5 °C, treated with NMM (16 mL, 0.15 mol) followed by H-Glu(Bzl)-Pro-OPac · PTSA (93 g, 0.15 mol) in THF (700 mL), and stirred for 3 h at -5 °C. The mixture was treated with sat'd NH_4Cl (140 mL) and filtered. The filtrate was concentrated by rotary evaporation, and the residue was dissolved in CH_2Cl_2 (1.5 L). The solution was washed with aqueous HCl (400 mL, 1 M) and sat'd $NaHCO_3$ (400 mL), dried with $MgSO_4$, and concentrated to give dipeptide **13** (137 g, 99%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.86 (d, 2H, *J* = 7.5 Hz) 7.6 (m, 2H) 7.47 (m, 3H) 7.2 (m, 9H) 7.09 (d, 2H, *J* = 8.0 Hz) 6.76 (d, 1H, *J* = 7.5 Hz) 5.47 (d, 1H, *J* = 17.0 Hz) 5.35 (s, 2H) 5.20 (d, 1H, *J* = 17.0 Hz) 5.1 (m, 2H) 4.90 (d, 1H, *J* = 8.0 Hz) 4.8 (m, 1H, NCH) 4.6 (m, 1H, NCH) 4.3 (m, 1H, NCH) 3.6 (m, 2H, NCH_2) 3.0 (m, 2H) 2.2 (m, 7H) 1.8 (m, 1H) 1.39 (s, 9H); MS m/z 930/928 (MH^+, 5), 830/828 (25), 616 (45), 508 (28), 169 (25), 123 (55), 112 (100), 91 (55). $[\alpha]_D^{20}$ -43.7° (c 1.10, $CHCl_3$). Anal. calc'd. for $C_{47}H_{50}BrN_3O_{12}$ (929.9): C, 60.78; H, 5.43; N, 4.52. Found: C, 60.97; H, 5.58; N, 4.37.

Boc-Tyr(BrZ)-Glu(Bzl)-Pro-OH (14). A solution of tripeptide **13** (135 g, 0.15 mol) in AcOH (900 mL) and H_2O (90 mL) was treated with Zn dust (95 g, 1.5 mol), and the resulting mixture was stirred for 18 h at room temperature. The inorganic solid was filtered and washed with AcOH (80 mL), and the combined filtrates were concentrated by rotary evaporation. To the residue was added toluene (200 mL), and the mixture was concentrated again. This residue was purified by flash chromatography (eluting with 1/1, hexane/CH_2Cl_2, to separate acetophenone from **14**, and then eluting with 40% EtOH/CH_2Cl_2). The product-containing fractions were combined and concentrated to give tripeptide **14** (118 g, 100%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.80 (d, 1H, *J* = 8.0 Hz) 7.60 (d, 1H, *J* = 7.5 Hz) 7.47 (d, 1H, *J* = 7.5 Hz) 7.3 (m, 8H) 7.12 (d, 2H, *J* = 7.5 Hz) 7.00 (d, 2H, *J* = 7.5 Hz) 5.50 (d, 1H, *J* = 7.5 Hz) 5.31 (s, 2H) 5.11 (d, 1H, *J* = 14.0 Hz) 5.03 (d, 1H, *J* = 14.0 Hz) 4.8 (m, 1H, NCH) 4.4 (M, 2H, NCH) 4.6 (m, 2H, NCH_2) 4.5 (m, 2H, NCH_2) 3.1 (m, 2H) 2.4 (m, 2H) 1.6-2.3 (m, 6H) 1.38 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.7, 171.0, 170.9, 155.9, 153.4, 150.0, 135.9, 134.6, 134.4, 133.0, 130.7, 130.6, 130.3, 130.2, 128.6, 128.5, 128.4, 128.3, 127.8, 123.5, 121.1, 120.6, 90.5, 69.7, 66.6, 59.5, 54.7, 49.8, 47.6, 38.3, 30.4, 29.5, 28.8, 28.4, 28.2, 27.8, 25.1; FAB-MS m/z 812/810 (MH^+, 17) 754/752 (9), 641/639 (6), 595/593 (7), 569/567 (9), 171/169 (100). $[\alpha]_D^{20}$ -15.4° (c 1.02, $CHCl_3$). Anal. calc'd. for $C_{39}H_{44}BrN_3O_{11}$ (810.7): C, 57.78; H, 5.47; N, 5.18. Found: C, 57.65; H, 5.51; N, 5.01.

Boc-Ile-OSu (15). A mixture of Boc-Ile · 1/2 H_2O (50 g, 0.21 mol) and anhydrous DME (415 mL) was cooled to 5 °C, treated with HOSu (24 g, 0.21 mol), and stirred for 1 h to give a cloudy solution. This solution was treated with a solution of DCC (47 g, 0.23 mol) in DME (50 mL), stirred for 24 h at 5 °C, and filtered with a cold DME (300 mL) wash. The combined filtrates were concentrated by rotary evaporation, and the residue dissolved in DME (150 mL). To this solution was added hexane (1 L), and the resulting slurry was stirred for 1 h at room temperature and then stored for 18 h at -20 °C. The slurry was filtered to give ester **15** (57 g, 83%) as white needles: mp 78-80 °C; ^{1}H NMR (DMSO-d_6) δ 7.60 (d, 1H, *J* = 7.0 Hz) 4.2 (m, 1H, NCH) 2.8 (br s, 4H) 1.9 (m, 1H) 1.5 (m, 1H) 1.40 (s, 9H) 1.3 (m, 1H) 0.96 (d, 3H, *J* = 7.0 Hz) 0.85 (*t*, 3H, *J* = 7.0 Hz); ^{13}C NMR (DMSO-d_6) δ 170.0, 167.9, 155.4, 67.6, 56.7, 36.1, 28.1, 25.5, 24.6, 14.9, 11.0; MS m/z 329 (MH^+, 2), 273 (45), 229 (100). $[\alpha]_D^{20}$ -26.8° (c 1.0, dioxane). Anal. calc'd. for $C_{15}H_{24}N_2O_6$ (328.4): C, 54.86; H, 7.37; N, 8.53. Found: C, 54.84; H, 7.55; N, 8.68.

Boc-Ile-Pro-OPac (16). A mixture of H-Pro-OH (20 g, 0.17 mol), DMF (540 mL),and Et_3N (24 mL, 0.17 mol) at room temperature was treated with ester **15** (51 g, 0.15 mol) and stirred for 18 h. The resulting mixture was filtered, and the filtrate was concentrated by rotary evaporation at 56 °C. The residue was dissolved in EtOAc (850 mL), and this solution was washed with aqueous HCl (2 x 400 mL, 1 M) and sat'd brine (2 x 400 mL). The organic layer was dried with $MgSO_4$ and concentrated to give a white foam (ca. 61 g). This foam was dissolved in EtOAc (600 mL), and this solution was treated with Et_3N (22 mL, 0.16 mol) and 2-bromoacetophenone (31 g, 0.15 mol) at room temperature. The resulting mixture was stirred for 18 h and filtered. The filtrate was washed with sat'd $NaHCO_3$ (2 x 200 mL) and brine (2 x 200 mL), dried with $MgSO_4$, and concentrated. The residue was treated with Et_2O (30 mL) and hexane (220 mL), and the resulting slurry was stirred for 1 h at room temperature and then stored for 18 h at -20 °C. The slurry was filtered to give dipeptide **16** (55 g, 80%) as a white powder: mp 84-85 °C; ^{1}H NMR ($CDCl_3$) δ 7.89 (d, 2H, *J* = 7.5 Hz) 7.61 (*t*, 1H, *J* = 7.5 Hz) 7.49 (*t*, 2H, *J* = 7.5 Hz) 5.54 (d, 1H, *J* = 17.0 Hz) 5.23 (d, 1H, *J* = 17.0 Hz) 5.16 (d, 1H, *J* = 8.0 Hz) 4.7 (m, 1H, NCH) 4.3 (m, 1H, NCH) 3.8 (m, 1H, NCH) 3.7 (m, 1H, NCH) 2.4 (m, 2H) 2.2 (m, 1H) 2.1 (m, 1H) 1.8 (m, 1H) 1.6 (m, 1H) 1.44 (s, 9H) 1.1 (m, 1H) 1.00 (d, 3H, *J* = 7.5 Hz) 0.89 (*t*, 3H, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 191.9, 171.5, 171.4, 155.8, 134.0, 128.9, 127.7, 79.5, 66.2, 58.7, 56.2, 47.3, 37.9, 29.2, 28.4, 24.9, 24.2, 15.3, 11.2; MS m/z 447 (MH^+, 100), 391 (59), 347 (37). $[\alpha]_D^{20}$ -95.4° (c 0.97, $CHCl_3$). Anal. calc'd. for $C_{24}H_{34}N_2O_6$ (446.6): C, 64.55; H, 7.68; N, 6.27. Found: C, 64.46; H, 7.74; N, 6.35.

H-Ile-Pro-OPac·PTSA (17). A solution of dipeptide **16** (31 g, 0.07 mol) in CH_2Cl_2 (25 mL) was cooled in an ice bath and treated with TFA (100 mL). The mixture was stirred for 15 min, treated with PTSA · H_2O (13 g, 0.07 mol), and

stirred for 20 min in an ice bath. The resulting solution was concentrated by rotary evaporation, and the residual syrup was dissolved in CH_2Cl_2 (150 mL). This solution was concentrated again to give a glass. The glass was triturated with a solution of CH_2Cl_2 (20 mL) and Et_2O (200 mL), and the resulting mixture was stirred for 1 h at room temperature and filtered to give dipeptide **17** (36 g, 99%) as a white powder: mp 155-156 °C; ^1H NMR ($CDCl_3$) δ 7.9 (br s, 3H) 7.86 (d, 2H, *J* = 7.5 Hz) 7.77 (d, 1H, *J* = 7.5 Hz) 7.58 (*t*, 1H, *J* = 7.5 Hz) 7.45 (*t*, 2H, *J* = 7.5 Hz) 7.13 (d, 2H, *J* = 7.5 Hz) 5.44 (d, 1H, *J* = 17.0 Hz) 5.23 (d, 1H, *J* = 17.0 Hz) 4.6 (m, 1H, NCH) 4.2 (m, 1H, NCH) 3.8 (m, 1H, NCH) 3.5 (m, 1H, NCH) 2.32 (s, 3H) 2.2 (m, 2H) 2.0 (m, 1H) 1.9 (m, 2H) 1.5 (m, 1H) 1.2 (m, 1H) 0.99 (d, 3H, *J* = 7.0 Hz) 0.76 (*t*, 3H, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 192.5, 171.7, 168.0, 142.1, 140.4, 134.2, 129.1, 129.0, 127.9, 126.3, 66.1, 59.1, 55.9, 47.4, 36.2, 28.7, 24.6, 23.6, 21.0, 14.9, 13.9, 10.9; MS m/z 347 (MH^+, 11), 329 (2), 239 (9), 211 (100). $[\alpha]_D^{20}$ -63.9° (c 1.02, $CHCl_3$). HRMS: calc'd. for $C_{19}H_{27}N_2O_4$ (MH^+): 347.1970. Found: 347.1963.

Boc-Tyr(BrZ)-Glu(Bzl)-Pro-Ile-Pro-OPac (18). A solution of tripeptide **14** (118 g, 0.15 mol) in anhydrous THF (1.5 L) was cooled to -5 °C, and treated with NMM (16 mL, 0.15 mol) followed by IPCF (145 mL, 1 M in toluene). The mixture was stirred for 15 min, treated with NMM (16 mL) followed by dipeptide **17** (73 g, 0.15 mol) in THF (200 mL), and stirred for 3 h at -5 °C. The mixture was treated with sat'd NH_4Cl (170 mL) and filtered. The filtrate was concentrated by rotary evaporation, and the residue was dissolved in CH_2Cl_2 (2.5 L). The solution was washed with aqueous HCl (450 mL, 1 M) and sat'd $NaHCO_3$ (500 mL), dried with $MgSO_4$, and concentrated to give pentapeptide **18** (159 g, 97%) as a glass: ^1H NMR (DMSO-d_6) δ 8.14 (d, 1H, *J* = 8.0 Hz) 8.05 (d, 1H, *J* = 8.0 Hz) 7.98 (d, 2H, *J* = 7.5 Hz) 7.7 (m, 2H) 7.6 (m, 3H) 7.47 (t, 1H, *J* = 7.5 Hz) 7.3 (m, 8H) 7.16 (d, 2H, *J* = 7.5 Hz) 6.96 (d, 1H, *J* = 8.0 Hz) 5.60 (d, 1H, *J* = 17.0 Hz) 5.45 (d, 1H, *J* = 17.0 Hz) 5.32 (s, 2H) 5.15 (d, 1H, *J* = 14.0 Hz) 5.06 (d, 1H, *J* = 14.0 Hz) 4.6 (m, 1H, NCH) 4.5 (m, 1H, NCH) 4.4 (m, 2H, NCH) 4.2 (m, 1H, NCH) 3.8 (m, 1H, NCH_2) 3.6 (m, 3H, NCH_2) 2.9 (m, 1H) 2.7 (m, 1H) 2.5 (m, 2H) 1.6-2.3 (m, 11H) 1.5 (m, 1H) 1.27 (s, 3H) 1.1 (m, 1H) 0.88 (d, 3H, *J* = 7.5 Hz) 0.79 (t, 3H, *J* = 7.5 Hz); ^{13}C NMR (DMSO-d_6) δ 192.7, 172.5, 171.4, 171.3, 171.2, 170.1, 169.3, 155.2, 152.8, 149.2, 136.1, 134.1, 134.0, 133.7, 132.7, 130.8, 130.7, 130.3, 128.9, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6, 123.0, 120.6, 78.1, 69.2, 66.5, 65.4, 58.9, 58.2, 55.4, 54.3, 49.3, 46.9, 36.5, 36.3, 29.1, 29.0, 28.9, 28.8, 28.1, 27.8, 27.7, 26.8, 24.5, 24.4, 23.9, 14.8, 10.9, 10.8; FAB-MS m/z 1140/1138 (MH^+, 83) 1040/1038 (6), 641/639 (6), 597/595 (6), 234 (100), 171/169 (83). $[\alpha]_D^{20}$ -73.7° (c 0.98, $CHCl_3$). Anal. calc'd. for $C_{58}H_{68}BrN_5O_{14} \cdot 0.5\ H_2O$ (1146.4)[12]: C, 60.76; H, 6.07; N, 6.11. Found: C, 60.66; H, 5.99; N, 6.28.

Boc-Tyr(BrZ)-Glu(Bzl)-Pro-Ile-Pro-OH (3). A solution of pentapeptide **18** (159 g, 0.14 mol) in AcOH (900 mL) and H_2O (90 mL) was treated with Zn dust (92 g, 1.4 mol), and the resulting mixture was stirred for 18 h at room temperature. The inorganic solid was filtered, and the filtrate was concentrated by rotary evaporation. The residue was mixed with toluene (200 mL) and concentrated again. This residue was purified by flash chromatography (eluting with 1/1, hexane/CH_2Cl_2, to separate acetophenone from **3**, and then eluting with 40% EtOH/CH_2Cl_2). The product-containing fractions were combined and concentrated to give pentapeptide **3** (143 g, 100%) as a white foam: ^1H NMR (DMSO-d_6) δ 8.15 (d, 1H, *J* = 8.0 Hz) 7.94 (d, 1H, *J* = 8.0 Hz) 7.71 (d, 1H, *J* = 7.5 Hz) 7.58 (d, 1H, *J* = 7.5 Hz) 7.46 (t, 1H, *J* = 7.5 Hz) 7.3 (m, 9H) 7.14 (d, 2H, *J* = 7.5 Hz) 6.96 (d, 1H, *J* = 7.5 Hz) 5.31 (s, 2H) 5.14 (d, 1H, *J* = 14.0 Hz) 5.07 (d, 1H, *J* = 14.0 Hz) 4.6 (m, 1H, NCH) 4.4 (m, 2H, NCH) 4.2 (m, 2H, NCH) 3.6 (, 1H, NCH_2) 3.5 (m, 3H, NCH_2) 2.9 (m, 1H) 2.7 (m, 1H) 2.5 (m, 2H) 1.6-2.1 (m, 11H) 1.5 (m, 1H) 1.27 (s, 9H) 1.1 (m, 1H) 0.94 (d, 3H, *J* = 7.5 Hz) 0.80 (t, 3H, *J* = 7.5 Hz); FAB-MS m/z 1022/1020 (MH^+, 12), 960/958 (11), 326 (5), 171/169 (100). $[\alpha]_D^{20}$ -62.6° (c 0.99, $CHCl_3$). Anal. calc'd. for $C_{50}H_{62}BrN_5O_{13} \cdot H_20$ (1039.0)[12]: C, 57.80; H, 6.21; N, 6.74. Found: C, 57.77; H, 6.18; H, 6.66.

Boc-Tyr(BrZ)-Glu(Bzl)-Pro-Ile-Pro-Glu(Bzl)-Glu(Bzl)-Ala-Cha-*D*-Glu(Bzl)-OBzl (19). A solution of pentapeptide **3** (138 g, 0.12 mol) in anhydrous THF (1.5 L) was cooled to -5 °C, and treated with NMM (13 mL, 0.12 mol) followed by IPCF (117 mL, 1 M in toluene). The mixture was stirred for 25 min, treated with NMM (13 mL) followed by pentapeptide **2** (136 g, 0.12 mol) and THF (300 mL), and stirred for 2 h at -5 °C. The mixture was treated with sat'd NH_4Cl (100 mL) and filtered. The filtrate was concentrated by rotary evaporation, and the residue was dissolved in CH_2Cl_2 (1.5 L). The solution was washed with aqueous HCl [(600 mL, 1 M) and sat'd $NaHCO_3$ (600 mL), dried with $MgSO_4$, and concentrated to give a white foam. The foam was purified by flash chromatography (1/40, MeOH/CH_2Cl_2), and the product-containing fractions were combined and concentrated to give **19** (177 g, 76%) as a white foam: ^1H NMR (DMSO-d_6) δ 8.35 (d, 1H, *J* = 8.0 Hz) 8.1 (m, 1H) 8.0 (m, 2H) 7.96 (d, 1H, *J* = 8.0 Hz) 7.88 (d, 1H, *J* = 8.0 Hz) 7.72 (d, 1H, *J* = 7.5 Hz) 7.59 (d, 1H, *J* = 7.5 Hz) 7.47 (t, 1H, *J* = 7.5 Hz) 7.3 (m, 29H) 7.16 (d, 2H, *J* = 7.5 Hz) 6.98 (d, 1H, *J* = 7.5 Hz) 5.32 (s, 2H) 5.1 (m, 10H) 4.6 (m, 1H, NCH) 4.3 (m, 9H, NCH) 3.7 (m, 1H, NCH_2) 3.5 (m, 3H, NCH_2) 2.9 (m, 1H) 2.7 (m, 1H) 2.4 (m, 8H) 1.0-2.1 (m, 32H) 1.30 (s, 9H) 1.16 (d, 3H, *J* = 7.5 Hz) 0.84 (d, 3H, *J* = 7.5 Hz) 0.78 (t, 3H, *J* = 7.5 Hz); FAB-MS m/z 1994/1992 (MH^+, 19), 1894 (100), 1786 (4), 1566 (6), 1413/1411 (9), 1297 (9), 1200 (2), 1087 (30), 997 (2), 979 (2), 641/639 (5), 597/595 (5), 536 (5), 171/169 (86). $[\alpha]_D^{20}$ -23.3° (c 1.02, $CHCl_3$). Anal. calc'd. for $C_{105}H_{127}BrN_{10}O_{24}$ (1993.3): C, 63.29; H, 6.43; N, 7.03. Found: C, 63.22; H, 6.48; N, 6.76.

H-Tyr(BrZ)-Glu(Bzl)-Pro-Ile-Pro-Glu(Bzl)-Glu(Bzl)-Ala-Cha-*D*- Glu(Bzl)-OBzl · PTSA (20). Decapeptide **19** (176 g, 0.088 mol) was cooled to 4 °C, and was dissolved in TFA (600 mL) over a 30 min period. This solution was treated with PTSA · H_2O (16.8 g, 0.088 mol), and the resulting mixture was stirred for 10 min at room temperature. The

resulting solution was concentrated by rotary evaporation, and the residual syrup was dissolved in CH_2Cl_2 (1.2 L). This solution was diluted with Et_2O (2 L) with stirring for 30 min, and the resulting slurry was stored for 18 h at -20 °C. Filtration of this slurry gave decapeptide **20** (142 g, 78%) as a white powder: mp 93-97 °C; ^1H NMR (DMSO-d_6) δ 8.86 (d, 1H, J = 8.0 Hz) 8.34 (d, 1H, J = 8.0 Hz) 8.1 (m, 4H) 7.96 (d, 1H, J = 8.0 Hz) 7.88 (d, 1H, J = 8.0 Hz) 7.72 (d, 1H, J = 7.5 Hz) 7.58 (d, 1H, J = 7.5 Hz) 7.50 (d, 2H, J = 7.5 Hz) 7.48 (t, 1H, J = 7.5 Hz) 7.3 (m, 32H) 7.12 (d, 2H, J = 7.5 Hz) 5.32 (s, 2H) 5.1 (m, 10H) 4.6 (m, 1H, NCH) 4.3 (m, 8H, NCH) 4.1 (m, 1H, NCH) 3.6 (m, 1H, NCH_2) 3.5 (m, 3H, NCH_2) 3.0 (m, 2H) 2.4 (m, 8H) 2.29 (s, 3H) 0.9-2.1 (m, 32H) 1.16 (d, 3H, J = 7.5 Hz) 0.85 (d, 3H, J = 7.5 Hz) 0.77 (t, 3H, J = 7.5 Hz); FAB-MS m/z 1894/1892 (MH^+, 100), 1804/1802 (9), 1032 (9), 597/595 (3), 569/567 (3), 481 (2), 350/348 (8), 171/169 (22). $[\alpha]_D^{20}$ -29.8° (c 0.98, $CHCl_3$). Anal. calc'd. for $C_{107}H_{127}BrN_{10}O_{25}S \cdot 1.5\ H_2O$ (2092.2)[12]: C, 61.42; H, 6.26; N, 6.70. Found: C, 61.47; H, 6.28; N, 6.48.

Suc-Tyr(BrZ)-Glu(Bzl)-Pro-Ile-Pro-Glu(Bzl)-Glu(Bzl)-Ala-Cha-*D*-Glu(Bzl)-OBzl (21). A solution of decapeptide **20** (136 g, 0.065 mol) and succinic anhydride (20 g, 0.2 mol) in CH_2Cl_2 (2 L) was cooled to 4 °C, treated with NMM (22 mL, 0.2 mol), and stirred for 18 h at 4 °C. The resulting mixture was washed with aqueous HCl (2 x 600 mL, 1 M), and the organic solution was dried with $MgSO_4$ and concentrated by rotary evaporation. The residue was dissolved in CH_2Cl_2 (1.4 L), and this solution was diluted with *t*-BuOMe (1.2 L) and Et_2O (300 mL). The resulting mixture was stirred for 18 h at room temperature and filtered to give decapeptide **21** (104 g, 80%) as a white powder: mp 120-130 °C; ^1H NMR (DMSO-d_6) δ 8.40 (d, 2H, J = 8.0 Hz) 8.2 (m, 2H) 8.0 (m, 4H) 7.70 (1H, J = 7.5 Hz) 7.57 (d, 1H, J = 7.5 Hz) 7.46 (t, 1H, J = 7.5 Hz) 7.3 (m, 29) 7.12 (d, 2H, J = 8.0 Hz) 5.31 (s, 2H) 5.1 (m, 10H) 4.5 (m, 2H, NCH) 4.3 (m, 8H, NCH) 3.6 (m, 1H, NCH_2) 3.5 (m, 3H, NCH_2) 3.0 (m, 1H) 2.8 (m, 1H) 2.4 (m, 8H) 2.2 (m, 4H) 1.0-2.1 (m, 32H) 1.18 (d, 3H, J = 7.5 Hz) 0.84 (d, 3H, J = 7.5 Hz) 0.76 (t, 3H, J = 7.5 Hz); ^{13}C NMR (DMSO-d_6) δ 152.9, 149.2, 136.2, 136.1, 135.9, 134.1, 132.8, 130.8, 130.7, 130.4, 128.4, 128.1, 128.0, 127.9, 127.89, 127.82, 127.7, 123.1, 120.7, 69.3, 66.0, 65.5, 65.4, 59.3, 59.1, 54.5, 53.7, 53.6, 52.1, 52.0, 51.7, 51.1, 50.4, 49.6, 48.3, 47.2, 46.8, 46.7, 36.6, 36.2, 36.1, 33.4, 33.0, 31.9, 31.7, 31.6, 31.5, 30.0, 29.9, 29.8, 29.7, 29.4, 29.3, 29.0, 27.2, 27.1, 27.0, 26.6, 26.2, 26.1, 26.0, 25.9, 25.7, 25.5, 24.5, 24.47, 24.45, 24.1, 22.8, 17.7, 15.0, 10.8; FAB-MS m/z 1995/1993 (MH^+, 30), 1517/1515 (8), 1298 (15), 1087 (38), 997 (20), 979 (4), 905 (3), 794 (4), 771 (3), 695 (6), 536 (4), 481 (6), 350/348 (18), 328 (11), 171/169 (100). $[\alpha]_D^{20}$ -39.2° (c 1.00, $CHCl_3$). Anal. calc'd. for $C_{104}H_{123}BrN_{10}O_{25} \cdot 5\ H_2O$ (2083.1)[12]: C, 59.96; H, 6.43; N, 6.72. Found: C, 59.97; H, 6.20; N, 6.66.

Suc-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-*D*-Glu-OH (1, MDL 28050). A solution of decapeptide **21** (103 g, 0.049 mol) in AcOH (2 L) was hydrogenated in a Parr apparatus with 10% Pd/C (40 g) at 50 psi at room temperature for 1 h. The resulting mixture was filtered to remove the catalyst, and the filtrate was concentrated by rotary evaporation to give a yellow solution (800 mL). This solution was diluted with Et_2O (3 L), and the resulting slurry was stirred for 1 h at room temperature and filtered to give a sticky solid (85 g). The solid was dissolved in 20% CH_3CN/H_2O (230 mL), and then diluted with a urea solution of 10% $AcOH/H_2O$ (230 mL, 6 M). This product solution was purified in 10 runs by preparative reverse-phase HPLC (Prochrom column: 23 x 5 cm I.D.; stationary phase: DuPont Zorbax T/M Pro-10/200 C_8 GR-2; gradient elution method: 10 to 23% CH_3CN/H_2O containing 0.1% AcOH in 10 min, increasing to 30% CH_3CN in 30 min, and increasing to 70% CH_3CN in 1 min; flow rate: 120 mL/min; detection: 285 nm). Fractions with >98% purity of **1** were combined, filtered to remove inorganic contaminants, and lyophilized to give decapeptide **1** (37 g, 54%) as a white powder: mp 158-161 °C; ^1H NMR (DMSO-d_6) δ 12.6 (br s, 6H) 9.2 (br s, 1H) 7.8-8.2 (m, 8H) 7.00 (d, 2H, J = 7.5 Hz) 6.33 (d, 2H, J = 7.5 Hz) 4.13-4.6 (m, 10H, NCH) 3.7 (m, 1H, NCH_2) 3.4(m, 3H, NCH_2) 2.8 (m, 1H) 2.6 (m, 1H) 2.2 (m, 8H) 0.9-2.1 (m, 36H) 1.17 (d, 3H, J = 7.5 Hz) 0.84 (d, 3H, J = 7.0 Hz) 0.80 (t, 3H, J = 7.0 Hz); ^{13}C NMR (DMSO-d_6) δ 174.3, 174.2, 174.1, 173.9, 173.8, 173.3, 172.1, 171.8, 171.4, 171.3, 171.2, 170.9, 170.7, 170.2, 169.6, 155.8, 130.2, 127.9, 114.8, 59.4, 59.0, 54.7, 54.0, 52.2, 51.8, 51.0, 50.3, 49.6, 48.2, 47.3, 46.9, 36.8, 36.3, 33.4, 33.1, 32.1, 30.2, 30.1, 29.9, 29.5, 29.2, 29.1, 27.5, 27.2, 26.8, 26.4, 26.2, 25.8, 25.6, 24.6, 24.5, 24.2, 17.9, 15.1, 11.0; FAB-MS m/z 1329 (MH^+, 100), 1066 (10), 937 (16), 840 (3), 727 (25), 490 (10), 393 (27). $[\alpha]_D^{20}$ -77.0° (c 1.00, 4/1, CH_3CN/H_2O). HPLC purity, 98%. Amino acid analysis: Tyr, 0.99; Glu, 4.09; Pro, 1.96; Ile, 0.94; Ala, 1.02. Anal. calc'd. for $C_{61}H_{88}N_{10}O_{23} \cdot 3.5\ H_2O$ (1392.5)[12]: C, 52.62; H, 6.88; N, 10.06. Found: C, 52.62; H, 6.81; N, 9.94.

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