Amino Acids and Peptides. Part 38.[1] Development of a New Amino-protecting Group, 2-Adamantyloxycarbonyl, and its Application to Peptide Synthesis†,[2]

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A new ε-amino protecting group, 2-adamantyloxycarbonyl (2-Adoc), was developed, and its application to the solid-phase synthesis of protected peptides was demonstrated in combination with N^{α}-fluoren-9-ylmethoxycarbonyl (Fmoc) protection and trifluoroacetic acid (TFA)-cleavable resin support. The 2-Adoc group was applied successfully also to the solution-phase peptide synthesis depending on *tert*-butoxycarbonyl (Boc)-chemistry.

For the chemical synthesis of polypeptides consisting of >40–50 amino acid residues, the conventional solid-phase peptide synthesis is circumscribed mainly by unavoidable accumulation of the deleted peptides which bear considerable resemblance to the target molecule and are difficult to detect and remove from the desired product.[3] An attractive method to overcome the above difficulties is the fragment condensation approach, which has been successfully employed in solution-phase peptide synthesis.[4] Convergent solid-phase peptide synthesis, which involves the preparation of protected peptide fragments by stepwise solid-phase peptide synthesis, followed by purification and their assembly in solution[5] or on a solid support,[6] should be a promising approach in terms of the ease of purification of the final product. In this strategy, the fragments must be soluble in the appropriate solvent suitable for the fragment condensation reaction and the subsequent removal from the desired product by reprecipitation, chromatography, or washing (in the case of solid-phase fragment assembly); therefore an improvement in the solubility of the protected peptide fragments can provide a successful, convergent, solid-phase peptide synthesis. Our studies have therefore been directed to the development of new side-chain-protecting groups with the objective of increasing both the solubility of the peptide fragment in organic solvents and the stability to the conditions during the synthesis of peptide fragments for use in the convergent solid-phase peptide synthesis as well as in solution-phase peptide synthesis.

Previously, we have reported that the 2-adamantyl (2-Ada) ester employed for protection of the β-carboxy function of Asp was suitably soluble in organic solvents and stable to acid,[7,8] while the 1-adamantyl (1-Ada) ester was susceptible to acid.[7] We therefore expected that the 2-adamantyloxycarbonyl [2-Adoc, Fig. 1(*a*)] group would be stable to trifluoroacetic acid (TFA) and would increase the solubility of protected peptides, while 1-adamantyloxycarbonyl [1-Adoc, Fig. 1(*b*)] was susceptible to acid.[9]

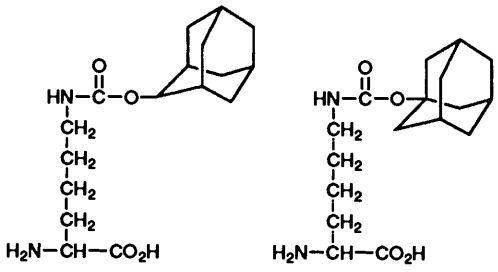


Fig. 1 Structures of (*a*) Lys(2-Adoc) and (*b*) Lys(1-Adoc)

This report deals with the development of a new amino-protecting group, 2-adamantyloxycarbonyl (2-Adoc), which is suitable for ε-amino protection of Lys in convergent solid-phase peptide synthesis in combination with N^{α}-Fmoc protection and TFA-labile solid support, and solution-phase peptide synthesis depending on Boc-chemistry.

Results and Discussion

H-Lys(2-Adoc)-OH [Fig. 1(*a*)] was prepared from a Lys_2–Cu complex and 2-adamantyl chloroformate (2-Adoc-Cl)‡ in the usual manner,[10,11] and its stability and susceptibility to various acids and bases were examined by measurements of regenerated Lys concentration with an amino acid analyser after each treatment. As summarized in Table 1, the 2-Adoc group was stable to 7.6 mol dm^{-3} HCl in 1,4-dioxane, TFA, 25% HBr in AcOH, and 1 mol dm^{-3} trimethylsilyl bromide (TMSBr)thioanisole/TFA for up to 24 h. The 2-Adoc group could be removed by trifluoromethanesulfonic acid (TFMSA) or anhydrous HF in a few minutes at 0 °C, but it was cleaved very slowly by methanesulfonic acid (MSA), and MSA treatment was not practical for the deprotection of 2-Adoc. 2-Adoc was stable to 20 v/v-% piperidine in DMF, Fmoc-deprotecting reagent, and 10% triethylamine in DMF, 10% aq. $NaHCO_3$ and 2 mol dm^{-3} aq. NaOH, for up to 24 h. We therefore concluded that the 2-Adoc group was suitable for ε-amino protection of Lys in combination with both Fmoc and Boc N^{α}-protection.

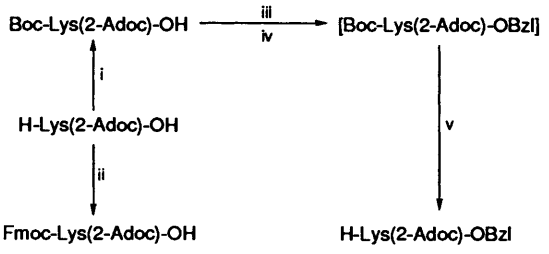
Next, various Lys(2-Adoc) derivatives were synthesized as shown in Scheme 1. H-Lys(2-Adoc)-OH was treated with

† Abbreviations used in this report for amino acids, peptides and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, 1966, **5**, 2485; 1967, **6**, 362; 1972, **11**, 1726. The following additional abbreviations are used: Acm, acetamidomethyl; AcOEt, ethyl acetate; AcOH, acetic acid; 1-Ada, 1-adamantyl; 2-Ada, 2-adamantyl; 1-Adoc, 1-adamantyloxycarbonyl; 2-Adoc, 2-adamantyloxycarbonyl; Boc, *tert*-butoxycarbonyl; Bop, benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate; Bzl, benzyl; Chp, cycloheptyl; Chx, cyclohexyl; DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DCM, dichloromethane; DIEA, diisopropylethylamine; DMF, dimethylformamide; DTT, dithiothreitol; EDTA, disodium dihydrogen ethylenediaminetetraacetate dihydrate; Fmoc, fluoren-9-ylmethoxycarbonyl; Fmoc-OSu, fluoren-9-ylmethyl *N*-succinimidyl carbonate; HOBt, 1-hydroxybenzotriazole; LSIMS, liquid secondary-ion mass spectrometry; MeBzl, 4-methylbenzyl; MSA, methanesulfonic acid; TFA, trifluoroacetic acid; TFMSA, trifluoromethanesulfonic acid; TMSBr, trimethylsilyl bromide; TosOH, toluene-*p*-sulfonic acid; Z, benzyloxycarbonyl.

‡ 2-Adoc-Cl was prepared from adamantan-2-ol and phosgene according to the method for the preparation of 1-Adoc-Cl.[9] 2-Adoc-Cl is commercially available from Watanabe Chemical Industries (Hiroshima, Japan).

Table 1 Stability of N^{ε}-2-Adoc group towards various acids and bases

Conditions	% Lys regenerated					
	5 min	20 min	40 min	60 min	120 min	24 h
7.6 mol dm^{-3} HCl, 1,4-dioxane	0	0	0	0	0	0
TFA	0	0	0	0	0	0
25% HBr, AcOH	0	0	0	0	0	1
1 mol dm^{-3} TMSBr, thioanisole, TFA	0	0	0	0	0	1
MSA	7	14	23	25	35	100
TFMSA	99	100	100	100	100	98
HF		100		100		
10% Et_3N, DMF	0	0	0	0	0	0
10% $NaHCO_3$	0	0	0	0	0	0
2 mol dm^{-3} NaOH	0	0	0	0	0	0
20% Piperidine, DMF	0	0	0	0	0	0



Scheme 1 *Reagents:* (i) Boc_2O; (ii) Fmoc-OSu; (iii) Cs_2CO_3; (iv) benzyl bromide; (v) 5.2 mol dm^{-3} HCl–1,4-dioxane

di-*tert*-butyl dicarbonate (Boc_2O)[12] or fluoren-9-ylmethyl *N*-succinimidyl carbonate (Fmoc-OSu)[13] in the usual manner to give Boc-Lys(2-Adoc)-OH or Fmoc-Lys(2-Adoc)-OH, respectively. The crystalline HCl·H-Lys(2-Adoc)-OBzl was prepared by HCl treatment of Boc-Lys(2-Adoc)-OBzl, which was synthesized from Boc-Lys(2-Adoc)-OH, Cs_2CO_3 and benzyl bromide,[14] while TosOH·H-Lys(2-Adoc)-OBzl prepared by the reflux of H-Lys(2-Adoc)-OH, TosOH and benzyl alcohol in benzene,[15] could not be crystallized.

First, in order to evaluate the 2-Adoc group for the solid-phase synthesis of protected peptides in combination with N^{α}-Fmoc protection and TFA-cleavable resin support, we synthesized three protected peptides corresponding to the sequences 25–35 (**1**), 36–45 (**2**) and 46–53 (**3**) of metallothionein-like growth inhibitory factor (GIF).[16] Their amino acid sequences and side-chain protections are shown in Fig. 2. The desired sequences were constructed on Wang resin[17] by Bop[18]-

Fmoc-Cys(Acm)-Lys(2-Adoc)-Cys(Acm)-Thr(Bzl)-Ser(Bzl)-Cys(Acm)-Lys(2-Adoc)-Lys(2-Adoc)-Ser(Bzl)-Cys(Acm)-Cys(Acm)-OH
Fmoc-(GIF 25–35)-OH **1**

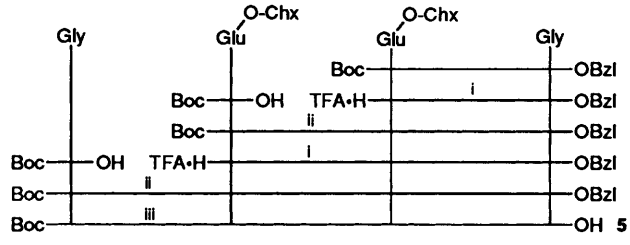
Fmoc-Ser(Bzl)-Cys(Acm)-Cys(Acm)-Pro-Ala-Glu(*O*-Chp)-Cys(Acm)-Glu(*O*-Chp)-Lys(2-Adoc)-Cys(Acm)-OH Fmoc-(GIF 36–45)-OH **2**

Fmoc-Ala-Lys(2-Adoc)-Asp(*O*-2-Ada)-Cys(Acm)-Val-Cys(Acm)-Lys(2-Adoc)-Gly-OH Fmoc-(GIF 46–53)-OH **3**

Fig. 2 Amino acid sequences and side-chain protections of Fmoc-(GIF 25–35)-OH **1**, Fmoc-(GIF 36–45)-OH **2**, and Fmoc-(GIF 46–53)-OH **3**

mediated coupling, and then were cleaved from the resin by TFA–phenol (95:5) to retain N^{α}-Fmoc and side-chain protecting groups. The cleavage products were reprecipitated from appropriate solvents. Their analytical HPLC profiles are shown in Fig. 3. Relatively high homogeneity of the cleavage products on amino acid analysis and HPLC indicated that the 2-Adoc group was perfectly stable during the successive piperidine treatments and the TFA cleavage. Those fragments containing the 2-Adoc group were easily soluble in DMF in practically sufficient concentration for their use in fragment condensation. For instance, compound **1** was soluble in DMF in higher concentration than was the N^{ε}-benzyloxycarbonyl (Z) homologue of compound **1**, > 50 mg cm^{-3} and < 30 mg cm^{-3}, respectively. These protected peptide fragments **1**–**3** will be employed for the convergent solid-phase synthesis of GIF.

Next, to evaluate the 2-Adoc group for the solution-phase peptide synthesis depending on Boc-chemistry, a dodecapeptide **4**, corresponding to the sequence 53–64 of mouse GIF (mGIF, Fig. 4)[19] was synthesized using 2-Adoc group protection. To construct the above peptide, Boc-(mGIF 53–56)-OH **5** and Boc-(mGIF 57–64)-OBzl **6** were prepared by stepwise elongation according to Schemes 2 and 3, respectively. After removal of the



Scheme 2 *Reagents:* (i) TFA; (ii) Bop, Et_3N; (iii) H_2/Pd

Boc group of compound **6**, the resultant amine was condensed with tetrapeptide **5** by the Bop-HOBt method.[20] The 2-Adoc group was perfectly stable during the synthesis, including the deprotection of the Boc group. Finally, the protected dodecapeptide was treated by the low and high HF methods,[21] successively, to give the desired dodecapeptide **4**. Amino acid compositions of the 6 mol dm^{-3} HCl hydrolysate (110 °C; 18 h) and LSIMS gave satisfactory results, and analytical HPLC of compound **4** showed > 90% homogeneity (Fig. 5).

Conclusions.—The results obtained here show that newly developed 2-Adoc group is suitable for ε-amino protection of Lys in convergent solid-phase peptide synthesis in combination with N^{α}-Fmoc protection and TFA-labile resin. In addition, the 2-Adoc group is also suitable for the solution-phase peptide synthesis depending on Boc chemistry. Higher solubility of the protected peptide fragments is required for both convergent solid-phase peptide synthesis and solution-phase peptide synthesis; therefore, numerous applications of N^{ε}-2-Adoc protection for the synthesis of large peptides are expected from this group's good chemoselectivity and high solubility.

Experimental

M.p.s were determined with a Yanagimoto micro apparatus and are uncorrected. On TLC (Kieselgel G, Merck), R_{f1}- and

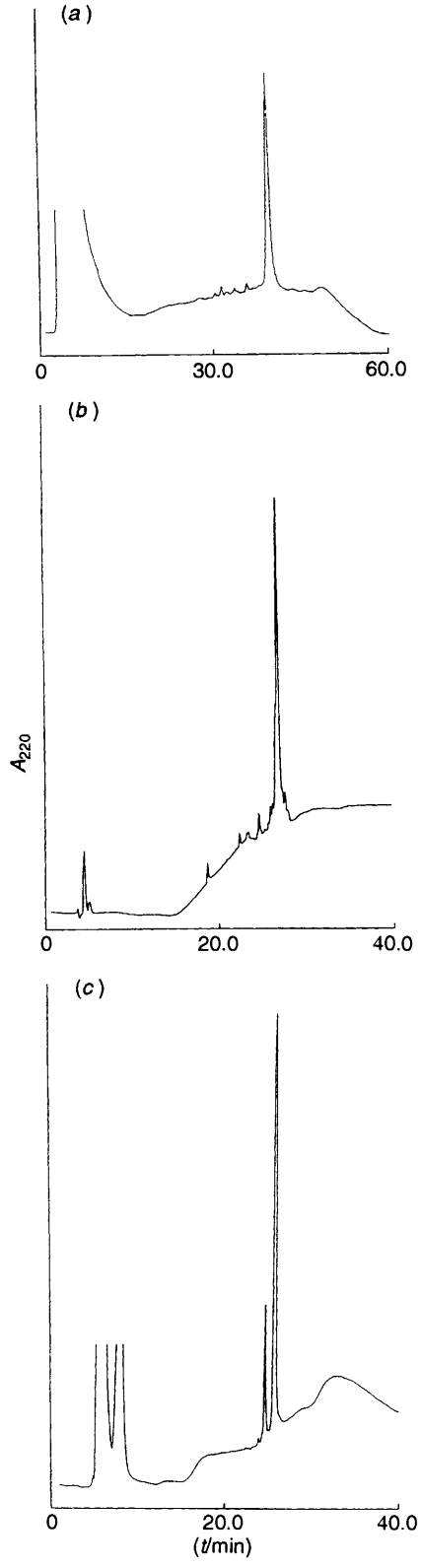


Fig. 3 Analytical HPLC of the crude products; (*a*) Fmoc-(GIF 25–35)-OH **1**, (*b*) Fmoc-(GIF 36–45)-OH **2**, and (*c*) Fmoc-(GIF 46–53)-OH **3** (column and mobile-phase system are given in the Experimental section)

R_{f2}-values refer to (1) $CHCl_3$–MeOH–AcOH (90:8:2), and (2) $CHCl_3$–MeOH–water (8:3:1, lower phase), respectively. Optical rotations were measured with an automatic DIP-360 polarimeter (Japan Spectroscopic Co. Ltd., Japan), and $[\alpha]_D$-values are in units of 10^{-1} deg cm^2 g^{-1}. Liquid secondary-ion mass spectroscopy (LSIMS) was performed on a Hitachi

H-Gly-Glu-Glu-Gly-Ala-Lys-Ala-Glu-Ala-Glu-Lys-Cys-OH **4**

Fig. 4 Amino acid sequence of mGIF 53–64 **4**

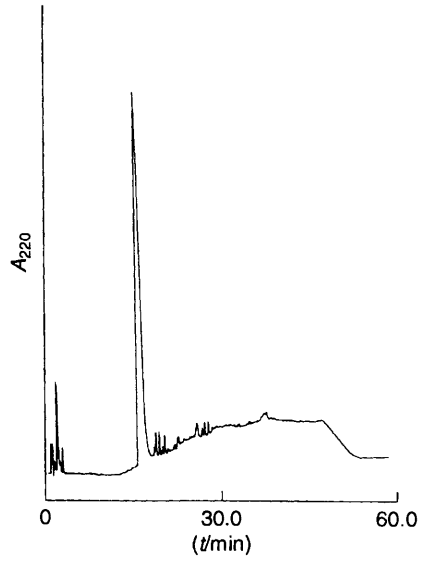


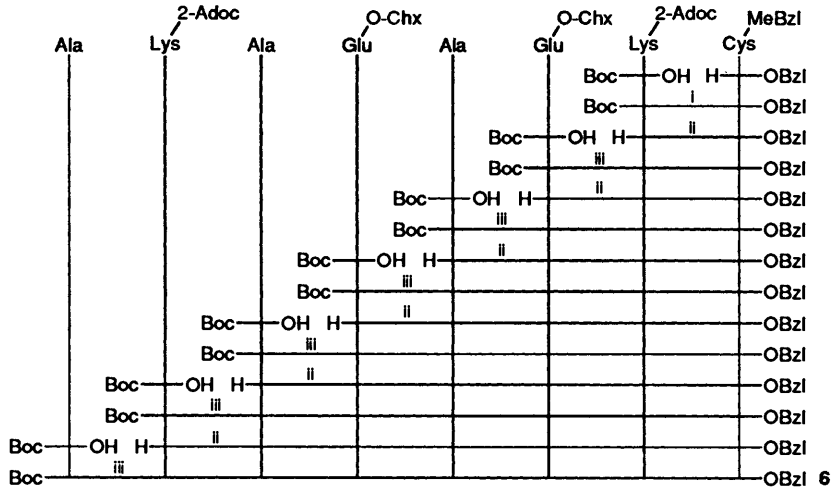
Fig. 5 Analytical HPLC of dodecapeptide **4** (column and mobile-phase system are given in the Experimental section)

M-2000 using a mixture of glycerol and magic bullet [dithiothreitol–dithioerythritol (3:1)] as matrix. Peptide-resins were hydrolysed in 12 mol dm^{-3} HCl–propionic acid (1:1, v/v) at 110 °C for 24 h, and peptides were hydrolysed in 6 mol dm^{-3} HCl at 110 °C for 18 h. Amino acid compositions of acid hydrolysates except for Cys were determined with an automated amino acid analyser (K-101AS or K-202SN, Kyowa Seimitsu Co. Ltd.). On analytical HPLC, columns 1, 2 and 3 refer to YMC-Pack A-302 ODS (4.6 × 150 mm), YMC-Pack Protein RP (4.6 × 250 mm) and Waters Nava-Pak C18 (3.9 × 150 mm) columns, respectively. In the mobile-phase system, A and B refer to water and MeCN, respectively, both containing 0.05 v/v-% TFA.

2-Adoc-Cl was a generous gift from Watanabe Chemical Industries (Hiroshima, Japan). Fmoc-amino acids except for Fmoc-Glu(O-Chp)-OH and Fmoc-Lys(2-Adoc)-OH were also purchased from Watanabe Chemical Industries. Fmoc-Gly- and Fmoc-Cys(Acm)-O-Wang resin (0.53 mmol g^{-1} and 0.61 mmol g^{-1}, respectively) were purchased from Kokusan Chemical Works (Japan). DCM and DMF for solid-phase synthesis were also purchased from Kokusan Chemical Works, and used without purification. Hexane refers to n-hexane. pH was measured with indicator paper (Merck).

H-Lys(2-Adoc)-OH.—The *title compound* was prepared from Lys·HCl (10.0 g, 54.8 mmol), $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$ (24.0 g, 100 mmol) and 2-Adoc-Cl (14.1 g, 65.7 mmol) according to the synthetic procedure for H-Lys(Z)-OH.[10,11] The crude product was recrystallized from boiling water containing a small amount of EDTA; yield 8.7 g (48.9%), m.p. 241–245 °C; $[\alpha]_D^{25}$ +10.5 (*c* 1.0, 50% AcOH); R_{f2} 0.25 (Found: C, 61.5; H, 8.85; N, 8.5. $C_{17}H_{28}N_2O_4 \cdot 0.5H_2O$ requires C, 61.2; H, 8.77; N, 8.40%).

Examination of Stability and Susceptibility of N^{α}*-2-Adoc Group to Various Acids and Bases.*—H-Lys(2-Adoc)-OH (20 μmol) was dissolved in the test solution (1 cm^3), and aliquots of the solution were collected at 5, 20, 40, 60, 120 min and 24 h. 0.5 mol dm^{-3} aq. Na_2CO_3 or 1 mol dm^{-3} HCl was added to the ice-cooled solution to adjust the pH to 2. The resultant mixtures were stored at −5 °C until amino acid analysis. The results are summarized in Table 1.



Scheme 3 *Reagents:* (i) DCC–HOBt; (ii) TFA; (iii) Bop, Et_3N

Fmoc-Lys(2-*Adoc*)-*OH*.—The *title compound* was prepared from H-Lys(2-Adoc)-OH (6.5 g, 20 mmol) and Fmoc-OSu (7.1 g, 22 mmol) according to the published procedure.[13] The oily material obtained was dissolved in AcOEt (50 cm^3), and a solution of DCHA (3.6 g, 20 mmol) in diethyl ether (100 cm^3) was added to the solution under ice-cooling. The resultant crystalline product was collected by filtration, washed with diethyl ether, and recrystallized from $CHCl_3$–hexane. Removal of DCHA with saturated aq. citric acid gave an amorphous powder (7.4 g, 67.7%), $[\alpha]_D^{25}$ −14.2 (*c* 1.0, DMF), R_{f1} 0.50 (Found: C, 67.7; H, 6.9; N, 4.9. $C_{32}H_{38}N_2O_6 \cdot 1.2H_2O$ requires C, 67.6; H, 7.17; N, 4.93%).

Boc-Lys(2-*Adoc*)-*OH*·*DCHA*.—The *title compound* was prepared from H-Lys(2-Adoc)-OH (16.3 g, 50 mmol), Boc_2O (11.9 g, 55 mmol) and DCHA (9.1 g, 50 mmol) in the usual manner;[12] yield 20.5 g (67.7%), m.p. 132–134 °C; $[\alpha]_D^{23}$ +3.54 (*c* 1.0, MeOH); R_{f2} 0.54 (Found: C, 67.25; H, 9.9; N, 6.85. $C_{34}H_{59}N_3O_6$ requires C, 67.4; H, 9.82; N, 6.94%).

H-Lys(2-*Adoc*)-*OBzl*·*HCl*.—To a solution of Boc-Lys(2-Adoc)-OH [prepared from Boc-Lys(2-Adoc)-OH·DCHA (30.3 g, 50 mmol) in the usual manner] in MeOH (200 cm^3)–water (20 cm^3) was added 20% aq. Cs_2CO_3 to adjust the pH to 7. The resultant solution was evaporated to dryness. The residue was redissolved in DMF (150 cm^3), and the solution was evaporated down. This process was repeated once more. To a solution of the residual solid in DMF (150 cm^3) was added benzyl bromide (9.4 g, 55 mmol). The reaction mixture was stirred at room temperature for 4 h, and then was evaporated down. The residual oil was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4, and evaporated down. 5.2 Mol dm^{-3} HCl in 1,4-dioxane (96.2 cm^3, 0.5 mol) was added to the ice-cooled residue. The above solution was stirred for 1 h, and was then evaporated down. Diethyl ether was added to the residue to afford *crystals*, which were collected by filtration, and washed with diethyl ether (yield 14.7 g, 65.2%), m.p. 97–99 °C; $[\alpha]_D^{24}$ −1.78 (*c* 1.0, MeOH); R_{f2} 0.66 (Found: C, 63.9; H, 8.05; N, 6.1. $C_{24}H_{35}ClN_2O_4$ requires C, 63.9; H, 7.82; N, 6.21%).

Fmoc-Glu(O-*Chp*)-*OH*.—The *title compound* was prepared from H-Glu(*O*-Chp)-OH[22] (750 mg, 3.1 mmol) and Fmoc-OSu (1.1 g, 3.4 mmol) according to the published procedure.[13] The oily material obtained was dissolved in diethyl ether (30 cm^3), and a solution of DCHA (561 mg, 3.1 mmol) in diethyl ether (30 cm^3) was added to the first, ice-cooled solution. The resultant crystalline product was collected by filtration, washed with diethyl ether, and recrystallized from $CHCl_3$–hexane. Removal of DCHA with saturated aq. citric acid gave an amorphous *powder* (940 mg, 67.1%), $[\alpha]_D^{24}$ −15.0 (*c* 1.0, DMF); R_{f2} 0.55 (Found: C, 67.1; H, 6.6; N, 2.9. $C_{26}H_{31}NO_6 \cdot H_2O$ requires C, 67.1; H, 6.88; N, 2.90%).

General Procedure for Solid-phase Synthesis.—Solid-phase peptide synthesis was carried out manually according to the following program: (1) DMF, 5 × 6 cm^3, 1 min; (2) 20 v/v-% piperidine–DMF, 2 × 6 cm^3, 3 min + 1 × 6 cm^3, 20 min; (3) DMF, 10 × 6 cm^3, 1 min; (4) Fmoc-amino acid (0.5 mmol) in DMF (2.5 cm^3), Bop (0.5 mmol) in DMF (2.5 cm^3), 1 mol dm^{-3} DIEA–DMF (1 cm^3), 30 min; (5) DMF, 5 × 6 cm^3, 1 min. A Ninhydrin test[23] was performed after step 4, and double coupling was performed if required. After completion of all couplings, the peptide resin was washed successively with DMF (5 × 6 cm^3, 1 min), DCM (5 × 6 cm^3, 1 min), MeOH (5 × 6 cm^3, 1 min) and hexane (5 × 6 cm^3, 1 min), and then was dried *in vacuo* over KOH pellets.

Fmoc-(*GIF* 25–35)-*OH* **1**.—Fmoc-amino acids corresponding to the desired sequence were incorporated onto Fmoc-Cys(Acm)-O-Wang resin (0.2 mmol) according to the general procedure (yield 653 mg, 78.9%). An aliquot of the peptide resin (300 mg) was treated with TFA–phenol (95:5; 5 cm^3) at room temperature for 60 min. After removal of the resin by filtration and the TFA by evaporation, dry diethyl ether was added to the residue to afford a precipitate, which was collected by filtration, washed with diethyl ether, and reprecipitated from DMF–diethyl ether (yield 106 mg, 49.1%), R_{f2} 0.43, t_R 39.41 min [88.9% (column 1, A:B 80:20 for 5 min, 80:20 to 20:80 in 25 min, 20:80 for 10 min, and 20:80 to 80:20 in 10 min)]. Amino acid compositions of acid hydrolysate were Thr:Ser:Lys 0.96(1):1.70(2):3.00(3) (recovery of Lys 86.8%).

N^{ε}-*Z-Homologue of Compound* **1**.—The title compound was synthesized using Fmoc-Lys(Z)-OH instead of Fmoc-Lys(2-Adoc)-OH in the same manner as described above (0.1 mmol scale) (yield 119 mg, 48.8%). Amino acid compositions of acid hydrolysate were Thr:Ser:Lys 0.86(1):1.70(2):3.00(3) (recovery of Lys 80.4%).

Fmoc-(GIF 36–45)-OH **2**.—Fmoc-amino acids corresponding to the desired sequence were incorporated onto Fmoc-Cys(Acm)-O-Wang resin (0.2 mmol) according to the general procedure (yield 546 mg, 78.0%). An aliquot of the peptide resin (300 mg) was treated with TFA–phenol (95:5; 5 cm^3) at room temperature for 60 min. After removal of the resin by filtration and the TFA by evaporation, dry diethyl ether was added to the residue to afford a precipitate, which was collected by filtration, washed with diethyl ether, and reprecipitated from DMF–water and TFA–diethyl ether, successively (yield 102 mg, 58.3%); R_{f2} 0.33, t_R 27.20 min [76.2% (column 2, A:B 80:20 for 5 min, 80:20 to 20:80 in 15 min, 20:80 for 20 min, and 20:80 to 80:20 in 10 min)]. Amino acid compositions of the acid hydrolysate were Ser:Glu:Ala:Pro:Lys 0.82(1):2.26(2):1.00(1):1.15(1):1.14(1) (recovery of Ala 90.3%).

Fmoc-(GIF 46–53)-OH **3**.—Fmoc-amino acids corresponding to the desired sequence were incorporated onto Fmoc-Gly-O-Wang resin (0.2 mmol) according to the general procedure (yield 761 mg, 103%). An aliquot of the peptide resin (300 mg) was treated with TFA–phenol (95:5; 5 cm^3) at room temperature for 60 min. After removal of the resin by filtration and the TFA by evaporation, dry diethyl ether was added to the residue to afford a precipitate, which was collected by filtration, washed with diethyl ether, and reprecipitated from DMF–MeOH (yield 116 mg, 77.9%), R_{f2} 0.37, t_R 25.03 min (column 2, A:B 80:20 for 5 min, 80:20 to 10:90 in 15 min, 10:90 for 10 min, and then 10:90 to 80:20 in 10 min). Amino acid compositions of the acid hydrolysate were Asp:Gly:Ala:Val:Lys 1.17(1):1.00(1):0.98(1):1.08(1):2.21(2) (recovery of Gly 96.6%).

Boc-Lys(2-Adoc)-Cys(MeBzl)-OBzl.—To an ice–salt-cooled solution of Boc-Lys(2-Adoc)-OH [prepared from Boc-Lys(2-Adoc)-OH·DCHA (4.0 g, 6.6 mmol) in the usual manner], H-Cys(MeBzl)-OBzl [prepared from TosOH·H-Cys(MeBzl)-OBzl (3.5 g, 7.26 mmol) in the usual manner] and HOBt (1.0 g, 6.6 mmol) in DCM (100 cm^3) was added DCC (1.4 g, 6.6 mmol). The reaction mixture was stirred at 4 °C overnight. After removal of dicyclohexylurea, the solution was diluted with $CHCl_3$ (100 cm^3), washed successively with 10% aq. $NaHCO_3$ and water, dried over Na_2SO_4, and then evaporated down. Hexane was added to the residue to afford *crystals*, which were collected by filtration, and reprecipitated from diethyl ether–hexane (yield 3.5 g, 73.5%), m.p. 80–82 °C; $[\alpha]_D^{24}$ −33.7 (*c* 1.0, DMF); R_{f1} 0.67 (Found: C, 66.6; H, 7.7; N, 5.85. $C_{40}H_{55}N_3O_7S$ requires C, 66.6; H, 7.68; N, 5.82%).

Boc-Glu(O-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl.—To an ice-cooled solution of H-Lys(2-Adoc)-Cys(MeBzl)-OBzl·TFA [prepared from Boc-Lys(2-Adoc)-Cys(MeBzl)-OBzl (3.3 g, 4.57 mmol), anisole (1.5 cm^3, 13.7 mmol) and TFA (3.4 cm^3) in the usual manner] and Boc-Glu(*O*-Chx)-OH (1.5 g, 4.57 mmol) in DCM (100 cm^3) containing Et_3N (0.7 cm^3, 4.57 mmol) was added Bop reagent (2.0 g, 4.57 mmol) and Et_3N (0.7 cm^3, 4.57 mmol). The reaction mixture was stirred at room temperature for 2 h, washed successively with 5% aq. $NaHCO_3$ and water, dried over Na_2SO_4, and then evaporated down. The residual oil, dissolved in $CHCl_3$ (5 cm^3), was applied to a silica gel column (2.8 × 51 cm), which was equilibrated with $CHCl_3$, and eluted successively with $CHCl_3$ (1100 cm^3), 0.5% MeOH in $CHCl_3$ (850 cm^3) and 1.0% MeOH in $CHCl_3$ (500 cm^3). The solvent of the effluent (1450–2450 cm^3) was removed by evaporation. Light petroleum was added to the residue to afford *crystals*, which were collected by filtration (3.4 g, 79.8%), m.p. 69–70 °C; $[\alpha]_D^{24}$ −32.1 (*c* 1.0, DMF); R_{f1} 0.76 (Found: C, 63.9; H, 7.5; N, 6.0. $C_{51}H_{72}N_4O_{10}S·1.5H_2O$ requires C, 63.8; H, 7.56; N, 5.83%). Amino acid compositions of the acid hydrolysate were Glu:Lys 1.04(1):1.00(1) (recovery of Lys 98.3%).

Boc-Ala-Glu(O-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl.—To an ice-cooled solution of H-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl·TFA [prepared from Boc-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl (1.0 g, 1.07 mmol), anisole (0.3 cm^3, 3.0 mmol) and TFA (1.6 cm^3, 21 mmol) in the usual manner] and Boc-Ala-OH (223 mg, 1.18 mmol) in DCM (20 cm^3) containing Et_3N (150 mm^3, 1.07 mmol) were added Bop reagent (522 mg, 1.18 mmol) and Et_3N (165 mm^3, 1.18 mmol). The reaction mixture was stirred at room temperature for 3 h, diluted with $CHCl_3$ (50 cm^3), washed successively with 5% aq. $NaHCO_3$ and water, dried over Na_2SO_4, and then evaporated down. Diethyl ether was added to the residue to afford *crystals*, which were collected by filtration, and recrystallized from AcOEt–hexane (yield 1.0 g, 93.1%), m.p. 116–119 °C; $[\alpha]_D^{24}$ −32.4 (*c* 1.0, DMF); R_{f1} 0.60 (Found: C, 63.0; H, 7.7; N, 7.0. $C_{54}H_{77}N_5O_{11}S·1.2H_2O$ requires C, 63.2; H, 7.57; N, 6.83%). Amino acid compositions of the acid hydrolysate were Glu:Ala:Lys 1.11(1):1.21(1):1.00(1) (recovery of Lys 85.0%).

Boc-Glu(O-Chx)-Ala-Glu(O-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl.—To an ice-cooled solution of H-Ala-Glu-(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl·TFA [prepared from Boc-Ala-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl (2.0 g, 1.99 mmol), anisole (0.6 cm^3, 5.97 mmol) and TFA (3.0 cm^3, 39.8 mmol) in the usual manner] and Boc-Glu(*O*-Chx)-OH (721 mg, 2.19 mmol) in DCM (50 cm^3) containing Et_3N (280 mm^3, 1.99 mmol) were added Bop reagent (970 mg, 2.19 mmol) and Et_3N (310 mm^3, 2.19 mmol). The reaction mixture was stirred at room temperature for 2 h, diluted with $CHCl_3$ (50 cm^3), washed successively with 5% aq. $NaHCO_3$ and water, dried over Na_2SO_4, and then evaporated down. Diethyl ether was added to the residue to afford *crystals*, which were collected by filtration, and recrystallized from AcOEt–hexane (yield 2.3 g, 95.1%), m.p. 147–149 °C; $[\alpha]_D^{24}$ −25.2 (*c* 1.0, DMF); R_{f2} 0.80 (Found: C, 60.7; H, 7.3; N, 6.55. $C_{65}H_{94}N_6O_{14}S·4H_2O$ requires C, 60.6; H, 7.36; N, 6.53%). Amino acid compositions of the acid hydrolysate were Glu:Ala:Lys 2.22(2):1.23(1):1.00(1) (recovery of Lys 91.2%).

Boc-Ala-Glu(O-Chx)-Ala-Glu(O-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl.—To an ice-cooled solution of H-Glu(*O*-Chx)-Ala-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl·TFA [prepared from Boc-Glu(*O*-Chx)-Ala-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl (1.6 g, 1.32 mmol), anisole (427 mm^3, 3.95 mmol) and TFA (2.0 cm^3, 26.4 mmol) in the usual manner] and Boc-Ala-OH (274 mg, 1.45 mmol) in DCM (50 cm^3) were added Bop reagent (641 mg, 1.45 mmol) and Et_3N (390 mm^3, 2.77 mmol). The reaction mixture was stirred at room temperature for 3 h, diluted with $CHCl_3$ (50 cm^3), washed successively with 5% aq. $NaHCO_3$ and water, dried over Na_2SO_4, and then evaporated down. Diethyl ether–hexane was added to the residue to afford *crystals*, which were collected by filtration, and recrystallized from EtOH–hexane (yield 1.23 g, 72.4%), m.p. 175–180 °C; $[\alpha]_D^{24}$ −28.5 (*c* 1.0, DMF); R_{f1} 0.54, R_{f2} 0.79 (Found: C, 60.7; H, 7.3; N, 7.4. $C_{68}H_{99}N_7O_{15}S·3H_2O$ requires C, 60.9; H, 7.44; N, 7.31%). Amino acid compositions of the acid hydrolysate were Glu:Ala:Lys 2.09(2):2.26(2):1.00(1) (recovery of Lys 100.7%).

Boc-Lys(2-Adoc)-Ala-Glu(O-Chx)-Ala-Glu(O-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl.—To an ice-cooled solution of H-Ala-Glu(*O*-Chx)-Ala-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl·TFA [prepared from Boc-Ala-Glu(*O*-Chx)-Ala-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl (950 mg, 738 μmol), anisole (239 mm^3, 2.22 mmol) and TFA (1.1 cm^3, 14.8 mmol) in the usual manner] and Boc-Lys(2-Adoc)-OH [prepared from Boc-Lys(2-Adoc)-OH·DCHA (492 mg, 812 μmol) in the usual manner] in DCM (50 cm^3) were added Bop reagent (359 mg,

812 μmol) and Et_3N (217 mm^3, 1.55 mmol). The reaction mixture was stirred at room temperature for 2 h, diluted with $CHCl_3$ (50 cm^3), washed successively with 5% aq. $NaHCO_3$ and water, dried over Na_2SO_4, and then evaporated down. Diethyl ether was added to the residue to afford *crystals*, which were collected by filtration, and recrystallized from AcOEt–hexane (yield 1.12 g, 95.2%), m.p. 225–229 °C; $[\alpha]_D^{24}$ −24.8 (*c* 1.0, DMF); R_{f1} 0.33 (Found: C, 61.7; H, 7.6; N, 7.4. $C_{85}H_{125}N_9O_{18}S\cdot 3H_2O$ requires C, 62.0; H, 7.65; N, 7.65%). Amino acid compositions of the acid hydrolysate were Glu:Ala:Lys 2.24(2):2.16(2):2.00(2) (recovery of Lys 92.7%).

Boc-Ala-Lys(2-*Adoc*)-*Ala-Glu*(O-*Chx*)-*Ala-Glu*(O-*Chx*)-Lys(2-*Adoc*)-*Cys*(*MeBzl*)-*OBzl* **6**.—To an ice-cooled solution of H-Lys(2-Adoc)-Ala-Glu(*O*-Chx)-Ala-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl·TFA [prepared from Boc-Lys(2-Adoc)-Ala-Glu(*O*-Chx)-Ala-Glu(*O*-Chx)-Lys(2-Adoc)-Cys-(MeBzl)-OBzl (918 mg, 576 μmol), anisole (189 mm^3, 1.73 mmol) and TFA (860 mm^3, 11.5 mmol) in the usual manner] and Boc-Ala-OH (131 mg, 691 μmol) in DMF (50 cm^3) were added Bop reagent (306 mg, 691 μmol) and Et_3N (177 mm^3, 1.27 mmol). The reaction mixture was stirred at room temperature for 2 h, and then was evaporated down. Aq. acetone was added to the residue to afford a *precipitate*, which was collected by filtration, and washed with acetone (yield 810 mg, 84.5%), m.p. 245–248 °C; $[\alpha]_D^{24}$ −27.9 (*c* 1.0, DMF); R_{f1} 0.21, R_{f2} 0.56 (Found: C, 61.4; H, 7.5; N, 8.3. $C_{88}H_{130}N_{10}O_{19}S\cdot 3H_2O$ requires C, 61.5; H, 7.63; N, 8.15%). Amino acid compositions of the acid hydrolysate were Glu:Ala:Lys 2.07(2):2.84(3):2.00(2) (recovery of Lys 94.2%).

Boc-Glu(O-*Chx*)-*Gly-OBzl*.—To an ice–salt-cooled solution of H-Gly-OBzl [prepared from H-Gly-OBzl·TosOH (4.0 g, 11.5 mmol) in the usual manner] in DMF (240 cm^3) containing Et_3N (1.8 cm^3, 12.7 mmol) were added Boc-Glu(*O*-Chx)-OH (3.8 g, 11.5 mmol), HOBt (1.6 g, 11.5 mmol) and DCC (2.9 g, 13.8 mmol). The reaction mixture was stirred at 4 °C overnight. After removal of dicyclohexylurea and the solvent, the residual oil was extracted with AcOEt. The extract was washed successively with 5% aq. Na_2CO_3, 10% aq. citric acid and water, dried over Na_2SO_4, and then was evaporated down. Light petroleum was added to the residue to afford a precipitate. The crude material in $CHCl_3$ was applied to a silica gel column (1.2 × 25 cm), which was equilibrated and eluted with $CHCl_3$ (700 cm^3). After removal of the solvent from the effluent (400–700 cm^3), light petroleum was added to the residue to afford *crystals*, which were collected by filtration (yield 3.8 g, 70.0%), m.p. 56–58 °C; $[\alpha]_D^{28}$ −6.7 (*c* 1.0, DMF), R_{f1} 0.60 (Found: C, 62.9; H, 7.7; N, 5.9. $C_{25}H_{36}N_2O_7$ requires C, 63.0; H, 7.61; N, 5.88%). Amino acid compositions of the acid hydrolysate were Glu:Gly 1.03:1.00 (recovery of Gly 90.1%).

Boc-Glu(O-*Chx*)-*Glu*(O-*Chx*)-*Gly-OBzl*.—To a solution of H-Glu(*O*-Chx)-Gly-OBzl·TFA [prepared from Boc-Glu(*O*-Chx)-Gly-OBzl (3.0 g, 6.29 mmol), anisole (2.0 cm^3, 18.9 mmol) and TFA (4.7 cm^3, 62.9 mmol)] and Boc-Glu(*O*-Chx)-OH (2.28 g, 6.92 mmol) in DCM (80 cm^3) were added successively Bop reagent (3.06 g, 6.92 mmol) and Et_3N (1.85 cm^3, 13.2 mmol). The reaction mixture was stirred at room temperature for 3 h, diluted with $CHCl_3$ (100 cm^3), washed successively with 5% aq. $NaHCO_3$ and water, dried over Na_2SO_4, and then evaporated down. Diethyl ether–light petroleum was added to the residue to afford a *precipitate*, which was collected by filtration, and washed with hexane (yield 3.06 g, 72.9%), m.p. 81–83 °C; $[\alpha]_D^{24}$ −13.3 (*c* 1.0, DMF); R_{f1} 0.58, R_{f2} 0.71 (Found: C, 63.0; H, 7.9; N, 6.0. $C_{36}H_{53}N_3O_{10}$ requires C, 62.9; H, 7.77; N, 6.11%).

Boc-Gly-Glu(O-*Chx*)-*Glu*(O-*Chx*)-*Gly-OBzl*.—To a solution of H-Glu(*O*-Chx)-Glu(*O*-Chx)-Gly-OBzl·TFA [prepared from Boc-Glu(*O*-Chx)-Glu(*O*-Chx)-Gly-OBzl (2.0 g, 3.0 mmol), anisole (1.0 cm^3, 9.0 mmol) and TFA (2.2 cm^3, 30 mmol)] and Boc-Gly-OH (631 mg, 3.6 mmol) in DCM (50 cm^3) were added successively Bop reagent (1.46 g, 3.3 mmol) and Et_3N (0.9 cm^3, 6.6 mmol). The reaction mixture was stirred at room temperature for 2 h, diluted with $CHCl_3$ (50 cm^3), washed successively with 5% aq. $NaHCO_3$ and water, dried over Na_2SO_4, and then evaporated down. Diethyl ether was added to the residue to afford *crystals*, which were collected by filtration, and recrystallized from EtOH (yield 1.87 g, 86.0%), m.p. 121–122 °C; $[\alpha]_D^{24}$ −7.0 (*c* 1.0, DMF); R_{f1} 0.75 (Found: C, 61.1; H, 7.7; N, 7.5. $C_{38}H_{56}N_4O_{11}$ requires C, 61.3; H, 7.58; N, 7.52%). Amino acid compositions of the acid hydrolysate were Glu:Gly 2.11(2):2.00(2) (recovery of Gly 82.3%).

Boc-Gly-Glu(O-*Chx*)-*Glu*(O-*Chx*)-*Gly-OH* **5**.—Boc-Gly-Glu(*O*-Chx)-Glu(*O*-Chx)-Gly-OBzl (800 mg, 1.1 mmol) was dissolved in aq. MeOH, and catalytically hydrogenated over Pd for 6 h. After removal of the solvent, diethyl ether was added to the residue to afford a *precipitate*, which was collected by filtration, and washed with diethyl ether (yield 720 mg, 100.1%), m.p. 105–107 °C; $[\alpha]_D^{24}$ −6.5 (*c* 1.0, DMF); R_{f1} 0.45 (Found: C, 56.9; H, 7.9; N, 8.3. $C_{31}H_{50}N_4O_{11}$ requires C, 56.9; H, 7.70; N, 8.56%).

Boc-Gly-Glu(O-*Chx*)-*Glu*(O-*Chx*)-*Gly-Ala-Lys*(2-*Adoc*)-*Ala-Glu*(O-*Chx*)-*Ala-Glu*(O-*Chx*)-*Lys*(2-*Adoc*)-*Cys*(*MeBzl*)-*OBzl*.—To a solution of H-Ala-Lys(2-Adoc)-Ala-Glu(*O*-Chx)-Ala-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl·TFA [prepared from Boc-Ala-Lys(2-Adoc)-Ala-Glu(*O*-Chx)-Ala-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl (300 mg, 180 μmol), anisole (58 mm^3, 540 μmol) and TFA (270 mm^3, 3.6 mmol)] and Boc-Gly-Glu(*O*-Chx)-Glu(*O*-Chx)-Gly-OH (137 mg, 216 μmol) in DMF (15 cm^3) were added successively HOBt (29 mg, 216 μmol), Bop reagent (96 mg, 216 μmol) and Et_3N (55 mm^3, 396 μmol). The reaction mixture was stirred at room temperature overnight. MeOH was added to the solution to afford a *precipitate*, which was collected by filtration, and washed with aq. MeOH (yield 257 mg, 65.5%), m.p. 280 °C (decomp.); $[\alpha]_D^{24}$ −25.5 (*c* 0.2, DMF) (Found: C, 60.7; H, 7.8; N, 9.0. $C_{114}H_{170}N_{14}O_{27}S\cdot 3H_2O$ requires C, 60.7; H, 7.60; N, 8.70%). Amino acid compositions of the acid hydrolysate were Glu:Gly:Ala:Lys 4.06(4):2.00(2):2.84(3):1.90(2) (recovery of Gly 89.2%).

H-Gly-Glu-Glu-Gly-Ala-Lys-Ala-Glu-Ala-Glu-Lys-Cys-OH **4**.—Boc-Gly-Glu(*O*-Chx)-Glu(*O*-Chx)-Gly-Ala-Lys(2-Adoc)-Ala-Glu(*O*-Chx)-Ala-Glu(*O*-Chx)-Lys(2-Adoc)-Cys(MeBzl)-OBzl (75 mg, 34 μmol) in the low-HF cocktail (*m*-cresol–thioanisole–dimethyl sulfide–HF 0.5:0.5:6.5:2.5 cm^3) was stirred at −5 °C for 45 min. After removal of HF and dimethyl sulfide, the residual mixture was dissolved in HF (9 cm^3) and the solution was stirred at −5 °C for 45 min. After removal of HF, dry diethyl ether was added to the residue to afford a precipitate, which was collected by filtration, washed with diethyl ether, and dissolved in 3% aq. AcOH (5 cm^3). The resultant solution was treated with Amberlite IRA-45 (acetate form), and lyophilized to give a fluffy powder. To a solution of the crude product in 3% aq. AcOH (2 cm^3) was added DTT (100 mg), and the resultant mixture was stirred at room temperature overnight. The solution was then applied to a Sephadex G-15 (2.6 × 61.5 cm) column, which was equilibrated and eluted with 3% aq. AcOH. Individual fractions (3.5 cm^3) were collected, and the desired fractions (35–46) were combined and lyophilized to give a fluffy powder (40.4 mg, 98.8%), $[\alpha]_D^{24}$ −49.0 (*c* 0.2, water), t_R 16.09 min [91.4% (column 3, A:B 97:3 for 5 min, 97:3 to 80:20 in 15 min,

80:20 to 40:60 in 10 min, 40:60 for 10 min)]. LSIMS: 1221.7 (MH^+). Amino acid compositions of the acid hydrolysate were Glu:Gly:Ala:Lys 4.16(4):2.00(2):3.02(3):1.99(2) (recovery of Gly 79.6%).

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