[Chem. Pharm. Bull. 34(2) 603—612 (1986)]

Studies on Peptides. CXXXV.[1,2] Preparation of Seven Peptide Fragments for the Synthesis of Human Calcitonin Gene-Related Peptide (hCGRP)

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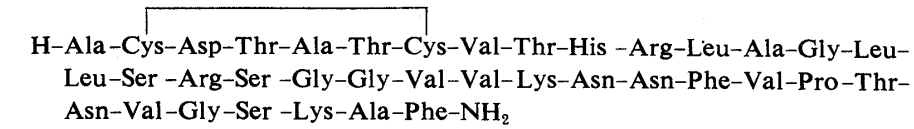
(Received July 15, 1985)

Seven peptide fragments were synthesized by known amide-forming reactions as building blocks for the solution-phase synthesis of the heptatriacontapeptide amide corresponding to the entire amino acid sequence of human calcitonin gene-related peptide (hCGRP). *S*-1-Adamantylcysteine [O. Nishimura, C. Kitada, and M. Fujino, *Chem. Pharm. Bull.*, **26**, 1576 (1978)] was employed, together with amino acid derivatives bearing protecting groups removable by 1 M trifluoromethanesulfonic acid–thioanisole in trifluoroacetic acid.

Keywords——human calcitonin gene-related peptide synthesis; *S*-1-adamantylcysteine; *S*-*p*-methoxybenzylcysteine; Asp side reaction; N^G-mesitylenesulfonylarginine

Structural analysis of the messenger ribonucleic acid (RNA) encoding a precursor of rat calcitonin revealed that this gene encodes, besides calcitonin, a novel neuropeptide named calcitonin gene-related peptide (rCGRP).[3] Immunochemically, this rat 37-residue peptide amide is supposed to be produced in nervous tissues *via* tissue-specific RNA processing. Shortly after this finding, Morris *et al.*[4] succeeded in the isolation of CGRP from patients with medullary thyroid carcinoma using antibody raised against rCGRP (positions 28—37) and determined its sequence using a fast atom bombardment (FAB) mass spectrometric mapping approach. This human peptide (hCGRP) is a 37-residue peptide amide with one disulfide bridge; this structure differs from the predicted rCGRP by substitution of four amino acids as shown in Fig. 1. This chemically deduced structure was confirmed by nucleotide sequence analysis of its precursor.[5—7]

We have synthesized the heptatriacontapeptide amide corresponding to the entire amino acid sequence of hCGRP by the conventional solution-phase method according to the route



	1	3	25	35
human	Ala	Asp	Asn	Lys
rat	Ser	Asn	Asp	Glu

Fig. 1. Structure of Human Calcitonin Gene-Related Peptide (hCGRP)

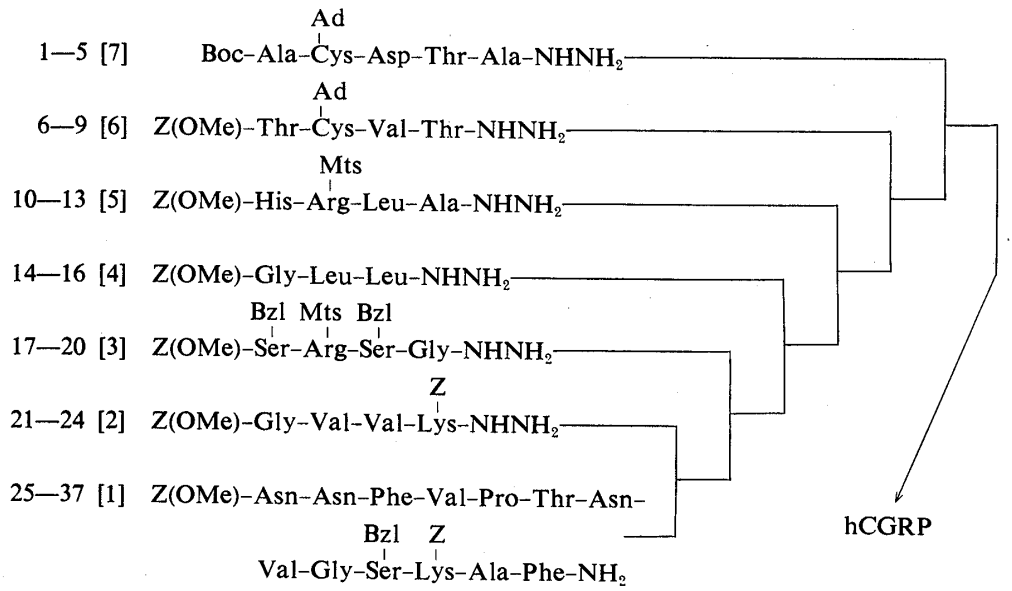


Fig. 2. Synthetic Route to Human Calcitonin Gene-Related Peptide

shown in Fig. 2, which shows the seven peptide fragments selected as building blocks to construct the entire peptide backbone of this newly found neuropeptide. In this paper, we wish to describe the preparation of these fragments.

In the present synthesis, Cys(Ad) was employed for the first time. This Ad group was originally introduced by Nishimura *et al.*[8] in 1978 as an S-protecting group of cysteine removable by $(CH_3COO)_2Hg$ in TFA, but has never been applied to practical peptide synthesis. As reported in the preceding paper,[1] this protecting group was found to be more stable to TFA and more resistant to air-oxidation as compared with the S-MBzl group[9] which we currently employed for the synthesis of Cys-containing peptides. This protecting group was found to be cleaved by 1 M TFMSA–thioanisole in TFA,[10] together with other protecting groups, such as Z, Bzl and Mts,[11] or selectively by $(CF_3COO)_3Tl$.[1] These attractive features of the Ad group prompted us to examine its usefulness in practical peptide synthesis.

Except for Cys(Ad), the method we employed in this synthesis is essentially the same as that employed for our recent synthesis of EGF (epidermal growth factor).[12] In combination with the TFA-labile Z(OMe) group,[13] amino acid derivatives bearing protecting groups removable by 1 M TFMSA–thioanisole in TFA were employed, *i.e.*, Lys(Z), Ser(Bzl), Asp(OBzl), Arg(Mts)[11] and Cys(Ad), mentioned above. Of these, the Bzl ester of the Asp residue (position 3) was removed by hydrogenolysis at the stage of fragment synthesis, that is, before introduction of Cys(Ad), in order to avoid base-catalyzed ring-closure of Asp(OBzl), since this unfavorable side reaction is known to be sequence-dependent.[14]

The C-terminal tridecapeptide amide, Z(OMe)–Asn–Asn–Phe–Val–Pro–Thr–Asn–Val–Gly–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2 [1], was synthesized according to the scheme illustrated in Fig. 3 by azide condensation[15] of Z(OMe)–Phe–Val–Pro–Thr–$NHNH_2$ and a TFA-treated sample of Z(OMe)–Asn–Val–Gly–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2, followed by stepwise additions of two Asn residues *via* the corresponding Np ester.[16] This approach was much easier for the preparation of [1] than the condensation of relatively large fragments, such as 6+7 condensation. The C-terminal heptapeptide amide, Z(OMe)–Asn–Val–Gly–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2 [1-a], was prepared using two available fragments, Z(OMe)–Lys(Z)–Ala–$NHNH_2$[17] and Z(OMe)–Val–Gly–$NHNH_2$,[18] as shown in Fig. 4. First, the former was condensed with H–Phe–NH_2 *via* the azide, then the result-

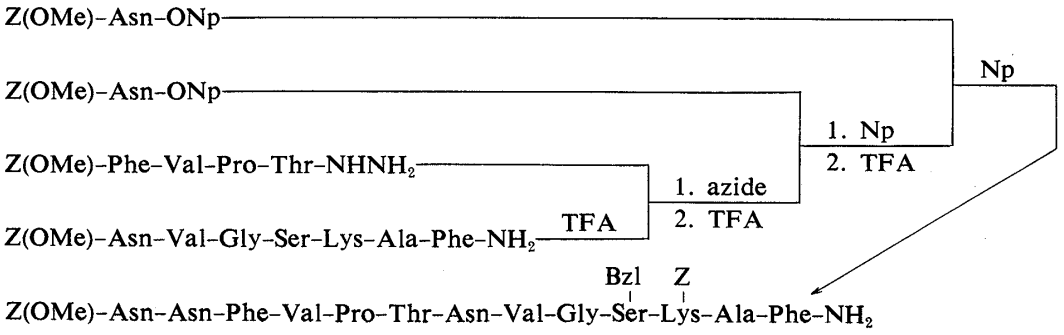


Fig. 3. Synthetic Scheme for the Protected Tridecapeptide Amide, Z(OMe)–(hCGRP 25—37)–NH_2 [1]

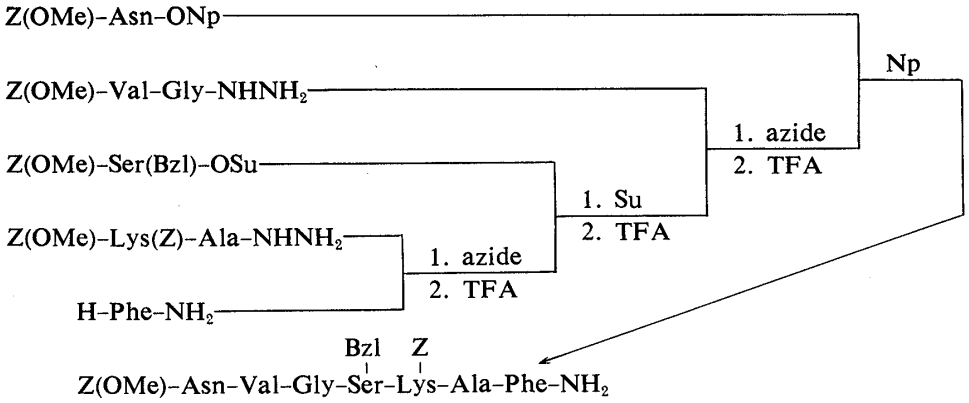


Fig. 4. Synthetic Scheme for the Protected Heptapeptide Amide, Z(OMe)–(hCGRP 31—37)–NH_2 [1-a]

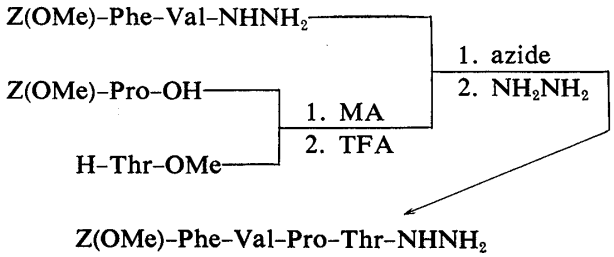


Fig. 5. Synthetic Scheme for the Protected Tetrapeptide Hydrazide, Z(OMe)–(hCGRP 27—30)–$NHNH_2$ [1-b]

ing tripeptide amide, after TFA treatment, was coupled with Z(OMe)–Ser(Bzl)–OH *via* the Su ester.[19] The peptide chain of Z(OMe)–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2 thus obtained was next elongated to [1-a] by condensation of Z(OMe)–Val–Gly–$NHNH_2$ and Z(OMe)–Asn–OH *via* the azide and the Np ester,[16] respectively. The next fragment, Z(OMe)–Phe–Val–Pro–Thr–$NHNH_2$ [1-b], was prepared as shown in Fig. 5. Previously, bond-formation of Val–Pro by DCC[20] was reported to give predominantly the acyl-urea,[21] a by-product of DCC.[22] Thus, H–Pro–Thr–OMe was first prepared by the mixed anhydride (MA) procedure[23] followed by TFA treatment. This was coupled with Z(OMe)–Phe–Val–$NHNH_2$[24] *via* the azide as was done in our porcine motilin synthesis.[18] The resulting tetrapeptide ester was next smoothly converted to the corresponding hydrazide. Assembly of the two fragments, [1-a] and [1-b], thus obtained and subsequent condensations of two Asn residues as shown in Fig. 3 proceeded smoothly without particular difficulty. The purity of the prod-

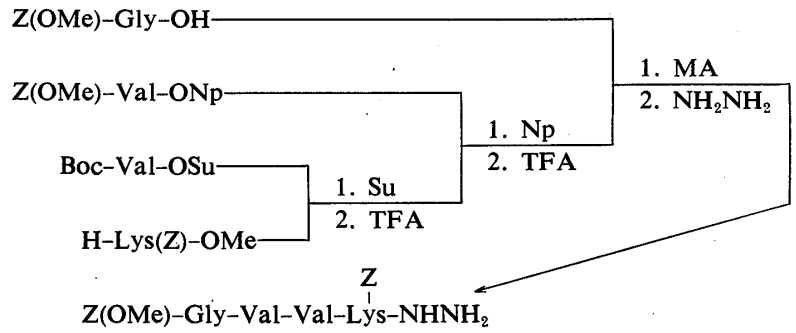


Fig. 6. Synthetic Scheme for the Protected Tetrapeptide Hydrazide, Z(OMe)–(hCGRP 21—24)–$NHNH_2$ [2]

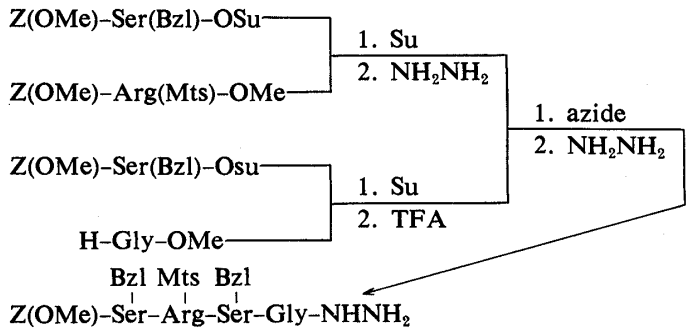


Fig. 7. Synthetic Scheme for the Protected Tetrapeptide Hydrazide, Z(OMe)–(hCGRP 17—20)–$NHNH_2$ [3]

uct [1] was confirmed by thin layer chromatography (TLC), amino acid analysis after 6 N HCl hydrolysis, and elemental analysis, as was also done with other fragments.

The next fragment, Z(OMe)–Gly–Val–Val–Lys(Z)–$NHNH_2$ [2], was synthesized in a stepwise manner as shown in Fig. 6. The Su, Np and MA procedures were employed to introduce the respective amino acid residues. The resulting protected tetrapeptide ester was smoothly converted to [2] by usual hydrazine treatment. Thus, the fragment possessing the Gly residue at the N-terminal, instead of the sterically bulky Val residue (positions 22 and 23), was prepared.

Fragment [3], Z(OMe)–Ser(Bzl)–Arg(Mts)–Ser(Bzl)–Gly–$NHNH_2$, was prepared as shown in Fig. 7 by condensation of two dipeptide units followed by usual hydrazine treatment. Z(OMe)–Ser–(Bzl)–Gly–OMe was prepared easily by the Su procedure, but Z(OMe)–Ser(Bzl)–Arg(Mts)–OMe prepared by the same Su ester procedure required column chromatographic purification. The latter purified sample, after conversion to the corresponding hydrazide, was condensed with a TFA-treated sample of the former dipeptide ester *via* the azide.

Fragment [4], Z(OMe)–Gly–Leu–Leu–$NHNH_2$, was synthesized in a stepwise manner starting with the available dipeptide ester, Z(OMe)–Leu–Leu–OMe.[25] This, after TFA treatment, was allowed to react with Z(OMe)–Gly–ONp. The resulting ether-soluble tripeptide ester was converted to [4] by the usual hydrazine treatment without characterization.

Fragment [5], Z(OMe)–His–Arg(Mts)–Leu–Ala–$NHNH_2$, was synthesized in a stepwise manner according to the scheme illustrated in Fig. 8. The Su, MA and azide procedures were employed to introduce the respective amino acid residues, Leu, Arg(Mts) and His. The MA procedure was particularly effective to obtain a fairly pure Arg(Mts)-containing peptide, Z(OMe)–Arg(Mts)–Leu–Ala–OMe. The protected tetrapeptide ester thus obtained was converted to [5] as usual.

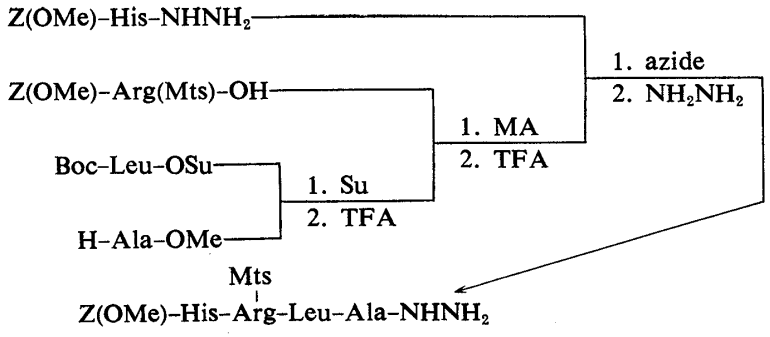


Fig. 8. Synthetic Scheme for the Protected Tetrapeptide Hydrazide, Z(OMe)–(hCGRP 10—13)–$NHNH_2$ [5]

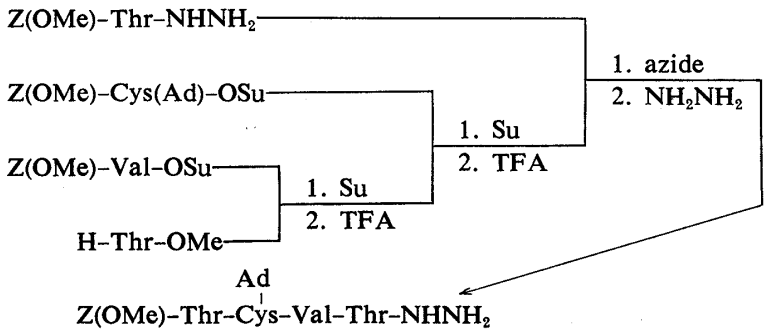


Fig. 9. Synthetic Scheme for the Protected Tetrapeptide Hydrazide, Z(OMe)–(hCGRP 6—9)–$NHNH_2$ [6]

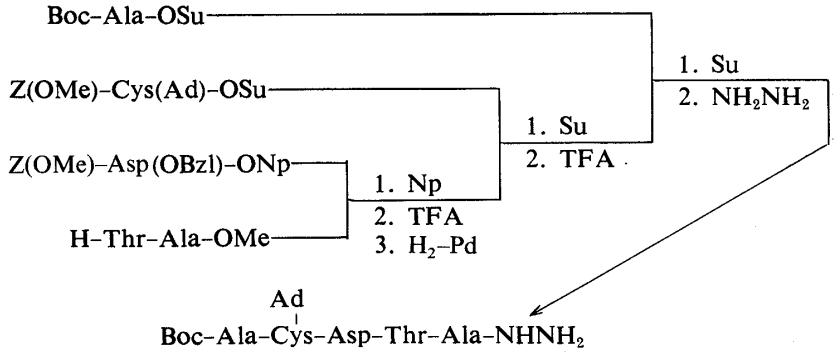


Fig. 10. Synthetic Scheme for the Protected Pentapeptide Hydrazide, Boc–(hCGRP 1—5)–$NHNH_2$ [7]

The next fragment [6], Z(OMe)–Thr–Cys(Ad)–Val–Thr–$NHNH_2$, was synthesized according to the scheme illustrated in Fig. 9. Z(OMe)–Val–Thr–OMe was prepared by the Su procedure, then Z(OMe)–Cys(Ad)–OH was introduced by the same Su procedure in a satisfactory yield. The last Thr residue was introduced by the azide procedure and the resulting tetrapeptide ester was converted to [6] as usual. Thus, a Cys(Ad)-containing peptide was synthesized for the first time without producing any extra spot on TLC due to degradation of the S-Ad group during the TFA and hydrazine treatments.

The N-terminal fragment, Boc–Ala–Cys(Ad)–Asp–Thr–Ala–$NHNH_2$ [7], was synthesized in a stepwise manner also starting with the known dipeptide ester, Z(OMe)–Thr–Ala–OMe,[26] as shown in Fig. 10. This dipeptide ester, after TFA treatment, was allowed to react with Z(OMe)–Asp(OBzl)–ONp to yield Z(OMe)–Asp(OBzl)–Thr–Ala–OMe. Next, from the resulting tripeptide ester, the Z(OMe) group was removed by TFA treatment as usual

and then the Bzl group was removed by hydrogenolysis in order to avoid base-catalyzed succinimide formation. The Asp–Thr sequence is one of the base-sensitive sequences.[11] The peptide chain or H–Asp–Thr–Ala–OMe thus obtained was elongated by the successive Su condensations of Z(OMe)–Cys(Ad)–OH and Boc–Ala–OH and the resulting protected pentapeptide ester was converted to [7] as usual.

The seven peptide fragments thus prepared were used to construct the entire peptide backbone of hCGRP as will be reported in the following paper.

Experimental

General experimental methods employed here are essentially the same as described in Part CXXXIII[25] of the present series.

N^{α}-Deprotection——The N^{α}-protecting group, Z(OMe) or Boc, was cleaved by TFA (*ca.* 10 ml per 1 g of a peptide) in the presence of anisole (2 eq or more) at ice-bath temperature for 60 min. After evaporation of TFA *in vacuo* at 15—20 °C, the residue was treated with dry ether. If a powder was obtained, it was collected by filtration, dried over KOH pellets *in vacuo* for 3 h and then used for the condensation reaction. If an oily precipitate was obtained, it was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 h and then used for the condensation reaction.

Condensation Reactions——The DCC and the active ester condensation reactions were performed at room temperature. Each hydrazide was converted to the corresponding azide by treatment with isoamyl nitrite and the azide reaction was performed at 4 °C. A mixed anhydride was prepared using isobutyl chloroformate and allowed to react with an amino component in an ice-bath for 5 h.

Purification——Unless otherwise mentioned, products were purified by one of the following procedures.

Procedure A: For purification of a protected peptide soluble in AcOEt, the extract was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and concentrated. The residue was crystallized or precipitated from appropriate solvents.

Procedure B: For purification of a peptide less soluble in AcOEt, the crude product was triturated with ether and 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and crystallized or precipitated from appropriate solvents. For purification of peptides containing the Asp residue, 5% citric acid and H_2O were used for washing. For purification of peptides containing the His residue, 5% $NaHCO_3$ and H_2O were used for washing.

Procedure C: For purification of a peptide partially soluble in H_2O, the crude product was extracted with *n*-BuOH and the extract was washed with H_2O saturated with *n*-BuOH, then concentrated. The residue was crystallized or precipitated from appropriate solvents.

TLC was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems (v/v): Rf_1 $CHCl_3$–MeOH–H_2O (8 : 3 : 1), Rf_2 $CHCl_3$–MeOH–AcOH (9 : 1 : 0.5).

Z(OMe)–Lys(Z)–Ala–Phe–NH_2——The azide [prepared from 10.59 g (20.0 mmol) of Z(OMe)–Lys(Z)–Ala–$NHNH_2$[17]] in DMF (50 ml) and Et_3N (2.78 ml, 20.0 mmol) were added to an ice-chilled solution of H–Phe–NH_2 [obtained from 6.56 g(20.0 mmol) of the Z(OMe)-derivative] in DMF (30 ml). The mixture, after being stirred for 14 h, was concentrated and the product was purified by procedure B, followed by precipitation from DMF with MeOH; yield 9.17 g (69%), mp 239—242 °C, $[\alpha]_D^{18}$ −16.5° (c=0.9, DMF), Rf_1 0.72. *Anal.* Calcd for $C_{35}H_{43}N_5O_8$: C, 63.52; H, 6.55; N, 10.58. Found: C, 63.45; H, 6.58; N, 10.47.

Z(OMe)–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2——A TFA-treated sample of Z(OMe)–Lys(Z)–Ala–Phe–NH_2 (8.99 g, 13.60 mmol) was dissolved in DMF–DMSO (1 : 1, 100 ml), together with Et_3N (3.78 ml, 27.2 mmol) and Z(OMe)–Ser(Bzl)–OSu (8.06 g, 17.5 mmol). The solution, after being stirred for 14 h, was concentrated and the product was purified by procedure B, followed by precipitation from DMF with MeOH; yield 9.72 g (85%), mp 209—211 °C, $[\alpha]_D^{18}$ −8.2° (c=0.9, DMF), Rf_1 0.69. *Anal.* Calcd for $C_{45}H_{54}N_6O_{10}$: C, 64.42; H, 6.49; N, 10.02. Found: C, 64.35; H, 6.49; N, 10.03.

Z(OMe)–Val–Gly–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2——The azide [prepared from 1.61 g (4.57 mmol) of Z(OMe)–Val–Gly–$NHNH_2$[18]] in DMF (8 ml) and Et_3N (0.64 ml, 4.57 mmol) were added to an ice-chilled solution of H–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2 [obtained from 3.19 g (3.81 mmol) of the Z(OMe)-derivative] and the mixture, after being stirred for 12 h, was concentrated. The product was purified by procedure B followed by precipitation from DMSO with MeOH; yield 3.35 g (88%), mp 234—235 °C, $[\alpha]_D^{18}$ −4.2° (c=0.7, DMSO), Rf_1 0.74. *Anal.* Calcd for $C_{52}H_{66}N_8O_{12}$: C, 62.76; H, 6.69; N, 11.26. Found: C, 62.70; H, 6.59; N, 11.26.

Z(OMe)–Asn–Val–Gly–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2 [1-a]——A TFA-treated sample of the above protected hexapeptide amide (3.37 g, 3.39 mmol) was dissolved in DMF–DMSO (1 : 1, 20 ml), together with Et_2N (0.94 ml, 6.78 mmol), Z(OMe)–Asn–ONp (1.84 g, 4.41 mmol) and HOBt[26] (0.2 g, 1.48 mmol). The mixture, after being stirred for 3 h, was concentrated and the product was purified by procedure B, followed by precipitation from DMF with

MeOH; yield 3.61 g (96%), mp 209—211 °C, $[\alpha]_D^{18}$ −9.6° (c=0.5, DMSO), Rf_1 0.68. Amino acid ratios in 6 N HCl hydrolysate: Asp 0.99, Thr 0.93, Gly 0.98, Ala 1.07, Val 0.97, Phe 1.00, Lys 1.07 (recovery of Phe 94%). *Anal.* Calcd for $C_{56}H_{72}N_{10}O_{14}$: C, 60.63; H, 6.54; N, 12.63. Found: C, 60.34; H, 6.54; N, 12.52.

Z(OMe)–Pro–Thr–OMe——This dipeptide ester was prepared by the mixed anhydride (MA) procedure and purified by recrystallization from AcOEt and ether; yield 69%, mp 122—123 °C, $[\alpha]_D^{18}$ −54.2° (c=0.9, MeOH), Rf_1 0.92. *Anal.* Calcd for $C_{19}H_{26}N_2O_7$: C, 57.90; H, 6.64; N, 7.10. Found: C, 57.86; H, 6.77; N, 6.98.

Z(OMe)–Phe–Val–Pro–Thr–OMe——The azide [prepared from 7.01 g (15.84 mmol) of Z(OMe)–Phe–Val–$NHNH_2$[24)]] in DMF (50 ml) and Et_3N (2.20 ml, 15.84 mmol) were added to an ice-chilled solution of H–Pro–Thr–OMe [obtained from 6.25 g (15.84 mmol) of the Z(OMe)-derivative] in DMF (100 ml) and the mixture, after being stirred at −15 °C for 48 h, was concentrated. The product was purified by procedure A, followed by precipitation from AcOEt with ether; yield 7.12 g (70%), mp 79—81 °C, $[\alpha]_D^{18}$ −42.1° (c=0.6, MeOH), Rf_1 0.92. *Anal.* Calcd for $C_{33}H_{44}N_4O_9$: C, 61.86; H, 6.92; N, 8.75. Found: C, 61.97; H, 7.13; N, 8.58.

Z(OMe)–Phe–Val–Pro–Thr–$NHNH_2$ [1-b]——Z(OMe)–Phe–Val–Pro–Thr–OMe (7.12 g, 11.11 mmol) in MeOH (100 ml) was treated with 80% hydrazine hydrate (3.48 ml, 5 eq) at room temperature for 24 h. The solvent was removed by evaporation and the residue was treated with H_2O to form a powder, which was recrystallized from MeOH and EtOH; yield 6.20 g (87%), mp 138—139 °C, $[\alpha]_D^{18}$ −44.0° (c=0.8, DMF), Rf_1 0.61. Amino acid ratios in 6 N HCl hydrolysate: Thr 1.01, Pro 1.28, Val 1.00, Phe 1.11 (recovery of Val 85%). *Anal.* Calcd for $C_{32}H_{44}N_6O_8 \cdot 1/2H_2O$: C, 59.15; H, 6.98; N, 12.94. Found: C, 59.34; H, 6.84; N, 12.93.

Z(OMe)–Phe–Val–Pro–Thr–Asn–Val–Gly–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2——The azide [prepared from 1.05 g (1.63 mmol) of Z(OMe)–Phe–Val–Pro–Thr–$NHNH_2$] in DMF (5.0 ml) and Et_3N (0.23 ml, 1.63 mmol) were added to an ice-chilled solution of H–Asn–Val–Gly–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2 [obtained from 1.21 g (1.09 mmol) of the Z(OMe)-derivative] in DMF–DMSO (1 : 1, 10 ml) and the mixture, after being stirred for 14 h, was concentrated. The product was purified by procedure B, followed by precipitation from DMSO with MeOH; yield 1.53 g (90%), mp 276—278 °C, $[\alpha]_D^{18}$ −21.8° (c=0.6, DMSO), Rf_1 0.56. Amino acid ratios in 6 N HCl hydrolysate: Asp 1.05, Thr 0.93, Ser 0.97, Pro 1.12, Gly 1.07, Ala 1.13, Val 1.84, Phe 2.00, Lys 1.06 (recovery of Phe 79%). *Anal.* Calcd for $C_{79}H_{104}N_{14}O_{19} \cdot 1/2H_2O$: C, 60.71; H, 6.77; N, 12.55. Found: C, 60.58; H, 6.59; N, 12.88.

Z(OMe)–Asn–Phe–Val–Pro–Thr–Asn–Val–Gly–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2——A mixture of a TFA-treated sample of the above undecapeptide amide (1.53 g, 0.98 mmol), Et_3N (0.27 ml, 2.0 mmol), Z(OMe)–Asn–ONp (0.62 g, 1.47 mmol) and HOBt (66 mg, 0.49 mmol) was stirred for 3 h and the solvent was removed by evaporation. The product was purified by procedure B, followed by precipitation from DMSO with MeOH; yield 1.60 g (97%), mp 273—275 °C, $[\alpha]_D^{18}$ −22.7° (c=0.5, DMSO), Rf_1 0.49. *Anal.* Calcd for $C_{83}H_{110}N_{16}O_{21} \cdot H_2O$: C, 59.13; H, 6.70; N, 13.29. Found: C, 59.10; H, 6.49; N, 13.31.

Z(OMe)–Asn–Asn–Phe–Val–Pro–Thr–Asn–Val–Gly–Ser(Bzl)–Lys(Z)–Ala–Phe–NH_2 [1]——A mixture of a TFA-treated sample of the above dodecapeptide amide (1.60 g, 0.97 mmol), Et_3N (0.27 ml, 1.95 mmol), Z(OMe)–Asn–ONp (0.61 g, 1.46 mmol) and HOBt (66 mg, 0.49 mmol) in DMF–DMSO (1 : 1, 10 ml) was stirred for 5 h. The solvent was removed by evaporation and the product was purified by procedure B, followed by precipitation from DMSO with MeOH; yield 1.67 g (96%), mp 276—277 °C, $[\alpha]_D^{18}$ −22.4° (c=0.6, DMSO), Rf_1 0.41. Amino acid ratios in 6 N HCl hydrolysate: Asp 3.15, Thr 0.97, Ser 1.04, Pro 1.10, Gly 1.05, Ala 1.08, Val 1.87, Phe 2.00, Lys 1.13 (recovery of Phe 92%). *Anal.* Calcd for $C_{87}H_{116}N_{18}O_{23} \cdot H_2O$: C, 58.04; H, 6.61; N, 14.01. Found: C, 57.88; H, 6.54; N, 14.18.

Boc–Val–Lys(Z)–OMe——This dipeptide ester was prepared by the Su procedure and purified by recrystallization from AcOEt and ether; yield 91%, mp 95.5—97 °C, $[\alpha]_D^{25}$ −21.0° (c=1.0, MeOH), Rf_1 0.60. *Anal.* Calcd for $C_{25}H_{39}N_3O_7$: C, 60.83; H, 7.97; N, 8.51. Found: C, 60.52; H, 8.05; N, 8.90.

Z(OMe)–Val–Val–Lys(Z)–OMe——A mixture of a TFA-treated sample of Boc–Val–Lys(Z)–OMe (7.90 g, 16 mmol), Et_3N (4.60 ml, 32 mmol) and Z(OMe)–Val–ONp (7.70 g, 19.2 mmol) in DMF (100 ml) was stirred for 24 h and concentrated. The product was purified by procedure B, followed by precipitation from DMF with ether; yield 9.50 g (90%), mp 210—213 °C, $[\alpha]_D^{25}$ −1.0° (c=1.0, DMF), Rf_1 0.90. *Anal.* Calcd for $C_{34}H_{48}N_4O_9$: C, 62.18; H, 7.37; N, 8.53. Found: C, 61.92; H, 7.53; N, 8.49.

Z(OMe)–Gly–Val–Val–Lys(Z)–OMe——A mixed anhydride [prepared from 3.20 g (13.2 mmol) of Z(OMe)–Gly–OH] in THF (30 ml) was added to an ice-chilled solution of H–Val–Val–Lys(Z)–OMe [obtained from 7.50 g (11 mmol) of the Z(OMe)-derivative] in DMF (100 ml) and the mixture, after being stirred for 3 h, was concentrated. The product was purified by procedure B, followed by precipitation from DMF with ether; yield 6.00 g (77%), mp 191—194 °C, $[\alpha]_D^{25}$ −4.0° (c=1.0, DMF), Rf_1 0.83. *Anal.* Calcd for $C_{36}H_{51}N_5O_{10} \cdot H_2O$: C, 59.08; H, 7.39; N, 9.57. Found: C, 59.48; H, 7.35; N, 9.56.

Z(OMe)–Gly–Val–Val–Lys(Z)–$NHNH_2$ [2]——The above methyl ester (5.50 g, 7.7 mmol) in DMF (100 ml) was treated with 80% hydrazine hydrate (4.60 ml, 77 mmol) for 24 h. The product was precipitated from DMF with H_2O; yield 4.70 g (85%), mp 232—236 °C, $[\alpha]_D^{25}$ +7.5° (c=1.1, DMF), Rf_1 0.59. Amino acid ratios in 6 N HCl hydrolysate: Gly 1.00, Val 1.59, Lys 1.02 (recovery of Gly 71%). *Anal.* Calcd for $C_{35}H_{51}N_7O_9$: C, 58.89; H, 7.20; N, 13.74. Found: C, 58.67; H, 7.50; N, 13.71.

Z(OMe)–Ser(Bzl)–Gly–OMe——This dipeptide ester was prepared by the Su procedure and purified by

procedure A, followed by recrystallization from AcOEt and ether; yield 81%, mp 84—86 °C, $[\alpha]_D^{18}$ +1.4° (c=0.7, MeOH), Rf_1 0.81. *Anal.* Calcd for $C_{22}H_{26}N_2O_7$: C, 61.38; H, 6.09; N, 6.51. Found: C, 61.19; H, 6.13; N, 6.68.

Z(OMe)–Ser(Bzl)–Arg(Mts)–OMe——A mixture of a TFA-treated sample of Z(OMe)–Arg(Mts)–OMe (12.77 g, 23.9 mmol), Et_3N (5.0 ml, 35.8 mmol) and Z(OMe)–Ser(Bzl)–OSu (10.91 g, 23.9 mmol) in DMF (60 ml) was stirred for 18 h and concentrated. The product was purified by procedure A, followed by column chromatography on silica gel (2.8 × 30 cm) using $CHCl_3$–MeOH (20 : 1). It was precipitated with isopropyl ether; yield 10.20 g (61%), mp 58—61 °C, $[\alpha]_D^{18}$ −3.3° (c=0.9, MeOH), Rf_1 0.71. *Anal.* Calcd for $C_{35}H_{45}N_5O_9S$: C, 59.05; H, 6.37; N, 9.84. Found: C, 59.30; H, 6.58; N, 9.90.

Z(OMe)–Ser(Bzl)–Arg(Mts)–NHNH$_2$——Z(OMe)–Ser(Bzl)–Arg(Mts)–OMe (6.0 g, 8.43 mmol) in MeOH (50 ml) was treated with 80% hydrazine hydrate (2.63 ml, 5 eq) for 24 h and the product was recrystallized from AcOEt and ether; yield 5.20 g (87%), mp 166—167 °C, $[\alpha]_D^{18}$ −34.0° (c=1.0, MeOH), Rf_1 0.66. *Anal.* Calcd for $C_{34}H_{45}N_7O_9S$: C, 57.37; H, 6.37; N, 13.78. Found: C, 57.72; H, 6.37; N, 13.70.

Z(OMe)–Ser(Bzl)–Arg(Mts)–Ser(Bzl)–Gly–OMe——The azide [prepared from 4.96 g (6.97 mmol) of Z(OMe)–Ser(Bzl)–Arg(Mts)–NHNH$_2$] in DMF (10 ml) and Et_3N (0.97 ml, 6.97 mmol) were added to an ice-chilled solution of H–Ser(Bzl)–Gly–OMe [prepared from 2.50 g (5.81 mmol) of the Z(OMe)-derivative] in DMF (30 ml) and the mixture, after being stirred for 18 h, was concentrated. The product was purified by procedure A, followed by recrystallization from MeOH and ether; yield 4.01 g (73%), mp 112—113 °C, $[\alpha]_D^{18}$ −3.5° (c=0.9, MeOH), Rf_1 0.81. *Anal.* Calcd for $C_{47}H_{59}N_7O_{12}S$: C, 59.67; H, 6.29; N, 10.36. Found: C, 59.48; H, 6.23; N, 10.53.

Z(OMe)–Ser(Bzl)–Arg(Mts)–Ser(Bzl)–Gly–NHNH$_2$ [3]——The above tetrapeptide ester (4.01 g, 4.24 mmol) in MeOH (20 ml) was treated with 80% hydrazine hydrate (1.06 ml, 5 eq) overnight. The product was purified by recrystallization from MeOH and EtOH; yield 3.29 g (82%), mp 134—136 °C, $[\alpha]_D^{18}$ +1.5° (c=0.7, DMF), Rf_1 0.76. Amino acid ratios in 6 N HCl hydrolysate: Ser 1.89, Gly 1.00, Arg 1.03 (recovery of Gly 81%). *Anal.* Calcd for $C_{46}H_{59}N_9O_{11}S$: C, 58.40; H, 6.29; N, 13.33. Found: C, 58.12; H, 6.20; N, 13.27.

Z(OMe)–Gly–Leu–Leu–NHNH$_2$ [4]——A mixture of a TFA-treated sample of Z(OMe)–Leu–Leu–OMe[25] (12.0 g, 30.0 mmol), Et_3N (9.17 ml, 66.0 mmol) and Z(OMe)–Gly–ONp (13.0 g, 36.0 mmol) in DMF (100 ml) was stirred for 18 h and the solvent was removed by evaporation. The oily product isolated by procedure A was dissolved in MeOH (100 ml) and treated with 80% hydrazine hydrate (12.0 ml, 200 mmol) for 6 h at room temperature. The solvent was removed by evaporation and the residue was treated with H_2O to form a powder, which was washed with H_2O and recrystallized from MeOH and isopropyl ether; yield 9.70 g (82%), mp 131—132 °C, $[\alpha]_D^{20}$ −23.0° (c=0.5, DMF), Rf_1 0.71. Amino acid ratios in 6 N HCl hydrolysate: Gly 1.00, Leu 2.01 (recovery of Gly 100%). *Anal.* Calcd for $C_{23}H_{37}N_5O_6$: C, 57.60; H, 7.78; N, 14.60. Found: C, 57.74; H, 7.73; N, 14.41.

Boc–Leu–Ala–OMe——This dipeptide ester was prepared by the Su procedure and recrystallized from AcOEt and n-hexane; yield 85%, mp 114—116 °C, $[\alpha]_D^{25}$ −35.0° (c=1.0, MeOH), Rf_1 0.76. *Anal.* Calcd for $C_{15}H_{28}N_2O_5$: C, 56.94; H, 8.92; N, 8.85. Found: C, 57.30; H, 9.16; N, 9.06.

Z(OMe)–Arg(Mts)–Leu–Ala–OMe——A mixed anhydride [prepared from 8.70 g (16.7 mmol) of Z(OMe)–Arg(Mts)–OH] in THF (80 ml) was added to an ice-chilled solution of H–Leu–Ala–OMe [obtained from 4.70 g (15 mmol) of the Boc-derivative] in DMF (50 ml) and the mixture, after being stirred for 5 h, was concentrated. The product was purified by procedure A, followed by column chromatography on silica gel using $CHCl_3$–MeOH (30 : 1). The product was finally recrystallized from AcOEt and n-hexane; yield 4.50 g (60%), mp 83—84 °C, $[\alpha]_D^{25}$ −21.0° (c=1.0, MeOH), Rf_1 0.59. *Anal.* Calcd for $C_{34}H_{50}N_6O_9S$: C, 56.80; H, 7.01; N, 11.69. Found: C, 56.68; H, 7.05; N, 11.36.

Z(OMe)–His–Arg(Mts)–Leu–Ala–OMe——The azide [prepared from 3.58 g (10.73 mmol) of Z(OMe)–His–NHNH$_2$] in DMF (20 ml) and Et_3N (5.09 ml, 36.6 mmol) were added to an ice-chilled solution of H–Arg(Mts)–Leu–Ala–OMe [obtained from 5.14 g (7.15 mmol) of the Z(OMe)-derivative] in DMF (20 ml) and the mixture, after being stirred for 48 h, was concentrated. The residue was dissolved in AcOEt and the extract was washed with H_2O, dried over Na_2SO_4 and concentrated. Trituration of the residue with isopropyl ether gave a powder, which was recrystallized from AcOEt and isopropyl ether; yield 4.77 g (80%), mp 111—115 °C, $[\alpha]_D^{18}$ −18.0° (c=1.0, MeOH), Rf_1 0.58. *Anal.* Calcd for $C_{40}H_{57}N_9O_{10}S \cdot 1.5H_2O$: C, 54.40; H, 6.85; N, 14.28. Found: C, 54.51; H, 6.62; N, 14.36.

Z(OMe)–His–Arg(Mts)–Leu–Ala–NHNH$_2$ [5]——The above tetrapeptide ester (4.77 g, 5.57 mmol) in MeOH (40 ml) was treated with 80% hydrazine hydrate (1.74 ml, 5 eq) for 24 h. The solvent was removed by evaporation and the product was precipitated from AcOEt with ether; yield 4.36 g (91%), mp 138—140 °C, $[\alpha]_D^{18}$ −3.8° (c=0.8, DMF), Rf_1 0.47. Amino acid ratios in 6 N HCl hydrolysate: Ala 1.00, Leu 0.99, His 0.95, Arg 0.99 (recovery of Ala 90%). *Anal.* Calcd for $C_{39}H_{57}N_{11}O_9S$: C, 53.59; H, 6.80; N, 17.63. Found: C, 53.61; H, 6.77; N, 17.59.

Z(OMe)–Val–Thr–OMe——This dipeptide ester was prepared by the Su procedure and recrystallized from MeOH and ether; yield 85%, mp 134—136 °C, $[\alpha]_D^{18}$ −20.0° (c=0.7, MeOH), Rf_1 0.85. *Anal.* Calcd for $C_{19}H_{28}N_2O_7$: C, 57.56; H, 7.12; N, 7.07. Found: C, 57.65; H, 7.26; N, 7.06.

Z(OMe)–Cys(Ad)–Val–Thr–OMe——A mixture of a TFA-treated sample of Z(OMe)–Val–Thr–OMe (1.92 g, 4.84 mmol), Et_3N (1.48 ml, 10.65 mmol) and Z(OMe)–Cys(Ad)–OSu (3.0 g, 5.81 mmol) in DMF (20 ml) was stirred for 24 h and concentrated. The product was purified by procedure A, followed by recrystallization from MeOH and ether; yield 2.57 g (84%), mp 140—142 °C, $[\alpha]_D^{18}$ −33.1° (c=0.7, MeOH), Rf_1 0.86. *Anal.* Calcd for $C_{32}H_{47}N_3O_8S$: C, 60.64; H, 7.74; N, 6.63. Found: C, 60.80; H, 7.48; N, 6.70.

Z(OMe)–Thr–Cys(Ad)–Val–Thr–OMe——The azide [prepared from 1.40 g (4.71 mmol) of Z(OMe)–Thr–$NHNH_2$] in DMF (10 ml) and Et_3N (0.65 ml, 4.71 mmol) were added to an ice-chilled solution of H–Cys(Ad)–Val–Thr–OMe [obtained from 2.0 g, (3.14 mmol) of the Z(OMe)-derivative] in DMF (20 ml) and the mixture, after being stirred for 24 h, was concentrated. The product was purified by procedure B, followed by precipitation from DMF with ether; yield 1.85 g (80%), mp 219—221 °C, $[\alpha]_D^{18}$ −25.7° (c=1.0, DMF), Rf_1 0.91. *Anal.* Calcd for $C_{36}H_{54}N_4O_{10}S$: C, 58.83; H, 7.41; N, 7.62. Found: C, 58.85; H, 7.27; N, 7.62.

Z(OMe)–Thr–Cys(Ad)–Val–Thr–$NHNH_2$ [6]——The above tetrapeptide ester (1.0 g, 1.36 mmol) in DMF (20 ml) was treated with 80% hydrazine hydrate (0.85 ml, 10 eq) overnight and the solvent was removed by evaporation. Trituration of the residue with EtOH afforded a powder, which was precipitated from DMF with EtOH; yield 0.80 g (80%), mp 239—240 °C, $[\alpha]_D^{18}$ −7.0° (c=1.0, DMF), Rf_2 0.33. Amino acid ratios in 6 N HCl hydrolysate: Thr 1.74, Val 1.00, Cys N.D. (recovery of Val 78%). *Anal.* Calcd for $C_{35}H_{54}N_6O_9S \cdot H_2O$: C, 55.83; H, 7.23; N, 11.46. Found: C, 55.86; H, 7.34; N, 11.40.

Z(OMe)–Asp(OBzl)–Thr–Ala–OMe——A mixture of a TFA-treated sample of Z(OMe)–Thr–Ala–OMe[27] (1.65 g, 4.48 mmol), Et_3N (0.62 ml, 4.48 mmol), Z(OMe)–Asp(OBzl)–ONp (2.74 g, 5.38 mmol) and HOBt (73 mg, 0.54 mmol) in DMF (20 ml) was stirred for 5 h and the solvent was removed by evaporation. The residue was purified by procedure B, followed by recrystallization from THF and ether; yield 2.13 g (83%), mp 153—155 °C, $[\alpha]_D^{18}$ −35.5° (c=0.5, MeOH), Rf_1 0.89. *Anal.* Calcd for $C_{27}H_{32}N_3O_9 \cdot 1/2H_2O$: C, 57.72; H, 6.23; N, 7.21. Found: C, 57.83; H, 6.20; H, 7.28.

Z(OMe)–Cys(Ad)–Asp–Thr–Ala–OMe——A TFA-treated sample of Z(OMe)–Asp(OBzl)–Thr–Ala–OMe (1.50 g, 2.62 mmol) in DMF (50 ml) was hydrogenated over a Pd catalyst for 3 h and the catalyst was removed by filtration. Et_3N (1.09 ml, 7.86 mmol) and Z(OMe)–Cys(Ad)–OSu (1.63 g, 3.14 mmol) were added to the filtrate and the mixture was stirred for 14 h. The solvent was removed by evaporation and the residue was dissolved in AcOEt. The organic phase was washed with 5% citric acid and H_2O, dried over Na_2SO_4 and concentrated. Trituration of the residue with ether afforded a powder which was recrystallized from AcOEt and ether; yield 1.33 g (70%), mp 219—221 °C, $[\alpha]_D^{18}$ −4.0° (c=0.5, DMF), Rf_1 0.54. *Anal.* Calcd for $C_{34}H_{48}N_4O_{11}S$: C, 56.65; H, 6.71; N, 7.77. Found: C, 55.77; H, 6.90; N, 7.78.

Boc–Ala–Cys(Ad)–Asp–Thr–Ala–OMe——A mixture of a TFA-treated sample of Z(OMe)–Cys(Ad)–Asp–Thr–Ala–OMe (1.33 g, 1.84 mmol), Et_3N (0.51 ml, 3.67 mmol) and Boc–Ala–OSu (0.68 g, 2.39 mmol) in DMF (10 ml) was stirred for 14 h and concentrated. The product was purified as described above and finally recrystallized from AcOEt and ether; yield 0.96 g (72%), mp 206—207 °C, $[\alpha]_D^{18}$ −32.6° (c=0.7, MeOH), Rf_1 0.47. *Anal.* Calcd for $C_{33}H_{53}N_5O_{11}S \cdot 1/2H_2O$: C, 53.79; H, 7.38; N, 9.51. Found: C, 53.81; H, 7.29; N, 9.44.

Boc–Ala–Cys(Ad)–Asp–Thr–Ala–$NHNH_2$ [7]——The above pentapeptide ester (0.96 g, 1.32 mmol) in MeOH (10 ml) was treated with 80% hydrazine hydrate (0.41 ml, 5 eq) for 48 h. The solvent was removed by evaporation, the residue was dissolved in H_2O, and the solution was acidified with AcOH. The resulting powder was recrystallized from MeOH and EtOH; yield 0.77 g (80%), mp 189—191 °C, $[\alpha]_D^{18}$ −41.0° (c=0.6, MeOH), Rf_1 0.39. Amino acid ratios in 6 N HCl hydrolysate: Asp 1.06, Thr 1.00, Ala 1.95, Cys N.D. (recovery of Thr 80%). *Anal.* Calcd for $C_{32}H_{53}N_7O_{10}S$: C, 52.80; H, 7.34; N, 13.47. Found: C, 52.66; H, 7.40; N, 13.27.

Acknowledgement This investigation was supported in part by a Grant-in-Aid (No. 60771852) from the Ministry of Education, Science and Culture, Japan.

References and Notes

1) Part CXXXIV: N. Fujii, A. Otaka, S. Funakoshi, H. Yajima, O. Nishimura, and M. Fujino, *Chem. Pharm. Bull.*, **34**, 869 (1986).
2) Amino acids and peptide derivatives mentioned in this investigation are of the L-configuration. The following abbreviations are used: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Su = *N*-hydroxysuccinimidyl, Np = *p*-nitrophenyl, DCC = dicyclohexylcarbodiimide, Boc = *tert*-butoxycarbonyl, Mts = mesitylenesulfonyl, Ad = 1-adamantyl, Bzl = benzyl, DMF = dimethylformamide, DMSO = dimethylsulfoxide, HMPA = hexamethylphosphoramide, TFMSA = trifluoromethanesulfonic acid, TFA = trifluoroacetic acid, HOBt = *N*-hydroxybenzotriazole.
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