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Studies on Peptides. CXXVI.[1,2] Synthesis of the Protected Tetracosapeptide Corresponding to Positions 30—53 of Mouse Epidermal Growth Factor (EGF)

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As a starting material for the synthesis of mouse epidermal growth factor, a half of the molecule, the protected tetracosapeptide ester (positions 30—53), was synthesized by successive condensations of eight peptide fragments: (positions 51—53), (49—50), (46—48), (42—45), (39—41), (37—38), (34—36), (30—33).

Keywords——synthesis of epidermal growth factor (EGF); 2,2,2-trichloroethyloxycarbonylhydrazine; mesitylenesulfonylarginine; side reaction of tryptophan; trifluoromethanesulfonic acid deprotection

Epidermal growth factor (EGF) is a polypeptide hormone isolated from the submaxillary gland of male mouse[3] and its structure, consisting of fifty-three amino acid residues with three disulfide bridges, was elucidated by Savage *et al.*[4] in 1973 (Fig. 1). Later, the structural homology (70%) between EGF and urogastrone,[5] isolated from human urine, was pointed out. Thus, this submaxillary principle was found to possess, together with EGF activity, a powerful inhibitory action against gastric acid secretion.[6] In 1983, Gray *et al.*[7] and Scott *et al.*[8] elucidated the nucleotide sequence of an EGF complementary deoxyribonucleic acid (cDNA) clone and suggested that EGF is synthesized as a large protein precursor of 1168 or 1217 amino acids. The chemically elucidated EGF structure was thus firmly supported by studies on its precursor. At present, there is known to be structural similarity between EGF and not only urogastrone, but also several gut-pancreatic polypeptides, such as human pancreatic secretory trypsin inhibitor[9] and gastric inhibitory polypeptide (GIP),[10] and a common evolutionary origin, together with a possible functional relationship, for these peptides has been suggested.[11] More recently, expression of the urogastrone gene in yeast was reported.[12]

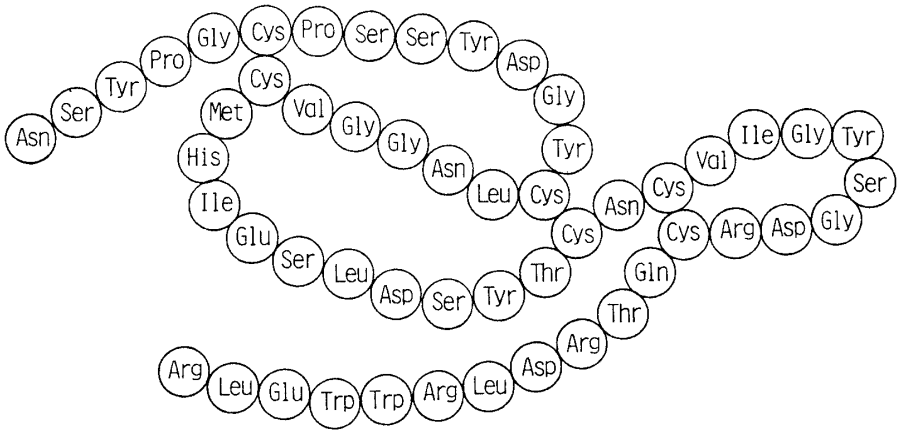

Fig. 1. Structure of Epidermal Growth Factor

Based on the early observation by Taylor *et al.*,[13] that upon air-oxidation, reduced EGF regenerated the EGF activity with the reconstitution of three disulfide bridges, we undertook the solution synthesis of a linear tripentacontapeptide corresponding to the entire amino acid sequence of EGF by condensation of fifteen peptide fragments and one amino acid, followed by deprotection with TFMSA/TFA,[14] and were able to isolate, after air-oxidation, a peptide with powerful anti-gastric activity according to the scheme illustrated in Fig. 2. During the course of this synthesis, we had to take special care in constructing the peptide backbone and encountered considerable difficulty in carrying out the air-oxidation step reproducibly to obtain the active product. We wish to present a detailed account of these investigations in two consecutive papers. In the first paper, synthesis of the protected tetracosapeptide corresponding to positions 30—53 of EGF is described (Fig. 3).

In the present synthesis, we used the thioanisole–mediated deprotection procedure[15] in the final step of the synthesis. The advantages of this new deprotecting procedure have been demonstrated by syntheses of several peptide hormones[16] and have recently been reviewed.[17] Thus, in the presence of the TFA–labile Boc or Z(OMe) group for N^{α}-protection, amino acid derivatives bearing protecting groups removable by the combination of TFMSA and thioanisole were employed, *i.e.*, Arg(Mts),[18] Cys(MBzl), Glu(OBzl), Asp(OBzl), and Ser(Bzl). The Bzl protecting group of Asp residue at particular positions was removed by hydrogenolysis during the fragment synthesis in order to avoid the base-catalyzed succinimide formation, since this side reaction is known to be sequence-dependent,[19] as will be discussed

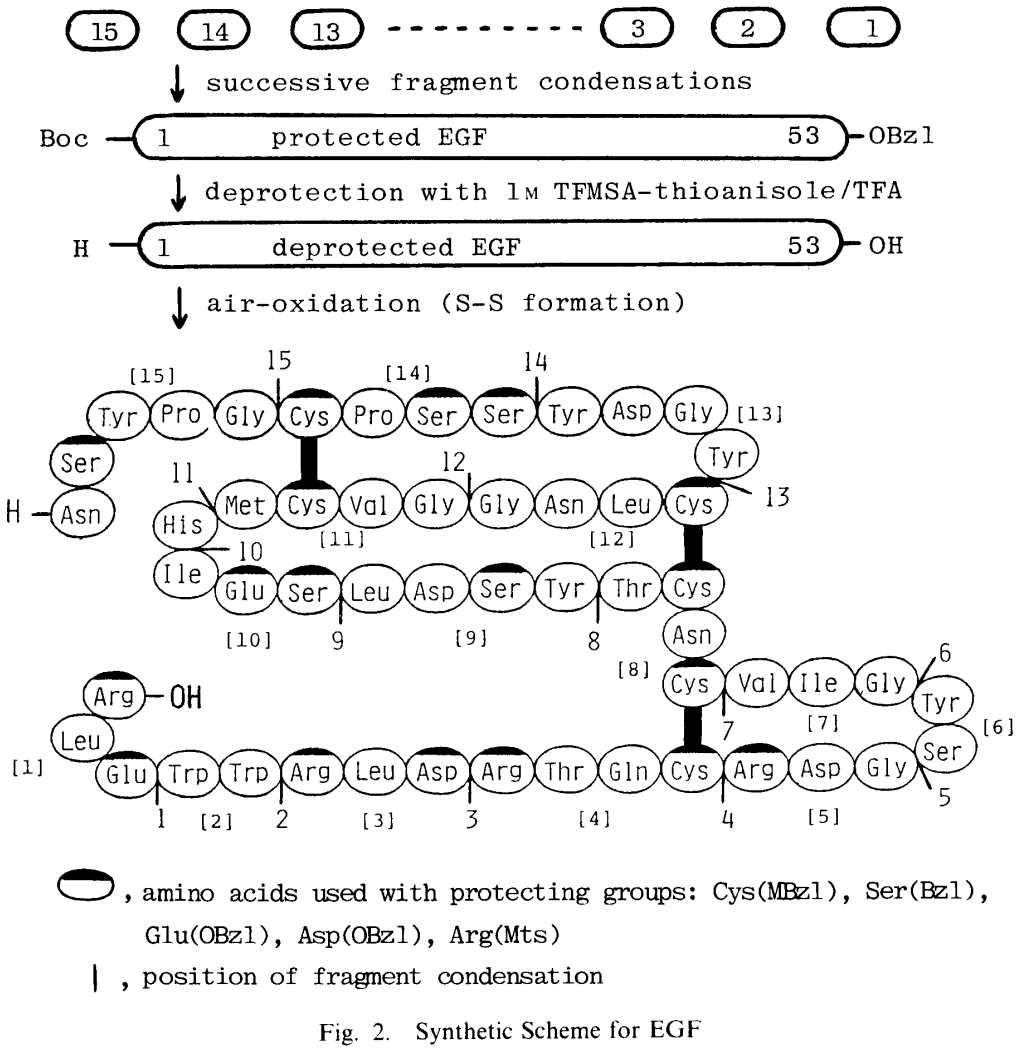

Fig. 2. Synthetic Scheme for EGF

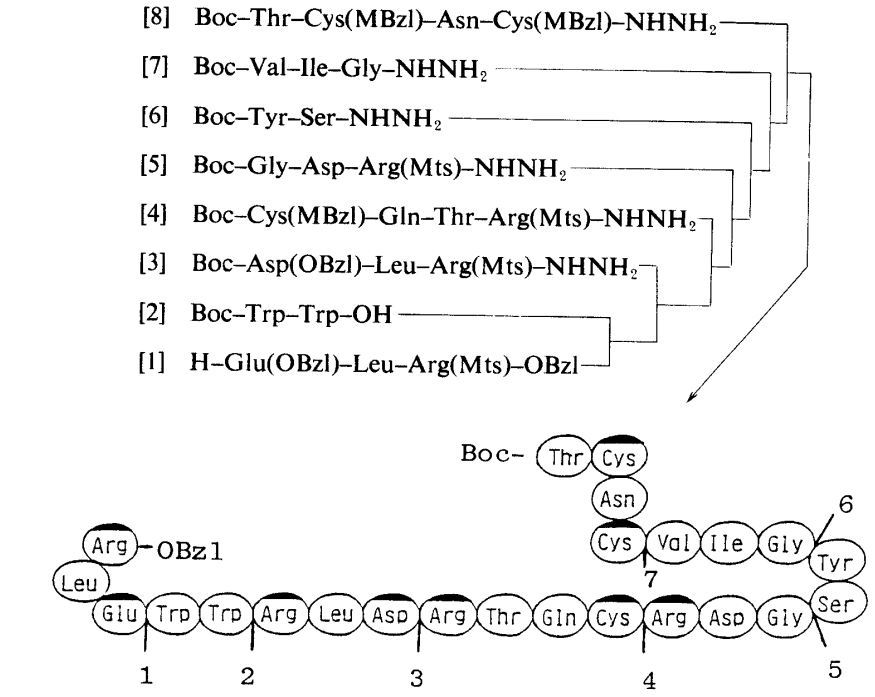

Fig. 3. Synthetic Route to the Protected Tetracosapeptide, EGF (30—53)

in a later section of this paper.

It seems worthwhile to note that this hormone does not contain any Ala, Phe or even Lys residues within a compact molecule folded by three disulfide bridges. However, the Trp–Trp sequence is located near the C-terminal end of the molecule, which from a synthetic viewpoint presents a certain difficulty as regards the suppression of side reactions involving its functional group.[20] In order to suppress alkylation at the indole moiety of Trp residue during the N^{α}-deprotection with TFA, anisole containing EDT[21] was employed as a cation scavenger.

In order to construct the EGF peptide backbone, we selected fifteen peptide fragments and decided to apply the azide procedure[22] as a main tool to assemble these fragments successively, since much less risk of racemization is involved in this procedure. Thus, these fragments were prepared in the form of the hydrazides, except for the fragment [2] consisting of two Trp residues, since the azide reaction is not ideal for Trp-containing peptides. Thus, this fragment was introduced by the use of DCC in the presence of HOBt[23] in order to suppress possible racemization. Hydrazides containing the Asp(OBzl) or Glu(OBzl) residue were synthesized with the aid of substituted hydrazine, Troc–$NHNH_2$,[24] the protecting group of which can be removed by Zn[25] or Cd[26] in acetic acid without affecting other functional groups.

Based on the strategy described above, we undertook the synthesis of EGF. The synthesis of a half of the molecule, the protected tetracosapeptide derived from condensation of eight fragments, from [1] to [8], is reported herein.

First, the necessary eight fragments were prepared. Fragment [1], Z(OMe)–Glu(OBzl)–Leu–Arg(Mts)–OBzl, was prepared in a stepwise manner starting with H–Arg(Mts)–OBzl, by successive condensations of Z(OMe)–Leu–OH and Z(OMe)–Glu(OBzl)–OH *via* the mixed anhydride[27] and the Np active ester,[28] respectively, as shown in Fig. 4.

Fragment [2], Boc–Trp–Trp–OH, was prepared by the Np method and characterized as its DCHA salt. The Boc group was adopted as the N^{α}-protecting group for the rest of the

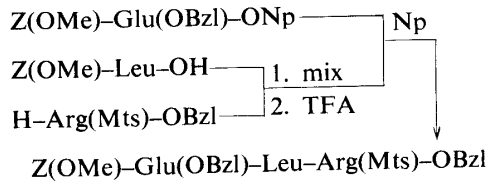

Fig. 4. Synthetic Scheme for the Protected Tripeptide Ester, Z(OMe)-(EGF 51—53)-OBzl [1]

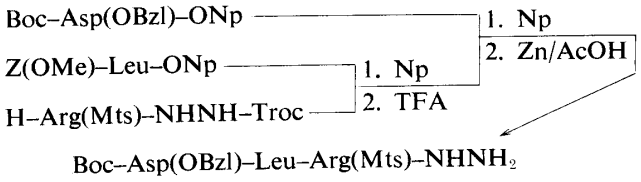

Fig. 5. Synthetic Scheme for the Protected Tripeptide Hydrazide, Boc-(EGF 46—48)-$NHNH_2$ [3]

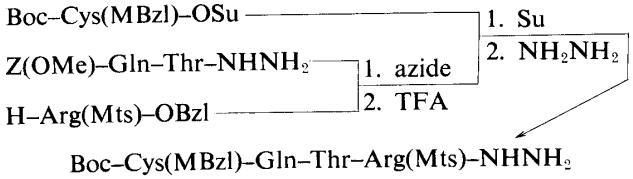

Fig. 6. Synthetic Scheme for the Protected Tetrapeptide Hydrazide, Boc-(EGF 42—45)-$NHNH_2$ [4]

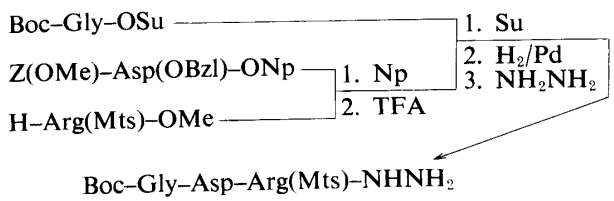

Fig. 7. Synthetic Scheme for the Protected Tripeptide Hydrazide, Boc-(EGF 39—41)-$NHNH_2$ [5]

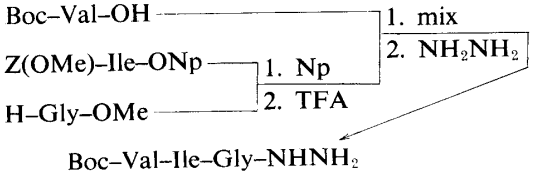

Fig. 8. Synthetic Scheme for the Protected Tripeptide Hydrazide, Boc-(EGF 34—36)-$NHNH_2$ [7]

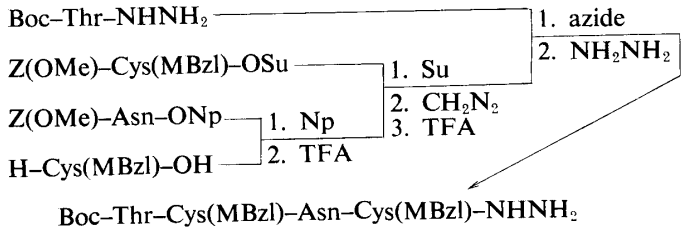

Fig. 9. Synthetic Scheme for the Protected Tetrapeptide Hydrazide, Boc-(EGF 30—33)-$NHNH_2$ [8]

fragments, since less side reaction at the Trp residue was involved during the N^{α}-deprotection with TFA compared with the TFA deprotection of the Z(OMe) group,[29] when anisole containing EDT was employed as a cation scavenger.

Fragment [3], Boc-Asp(OBzl)-Leu-Arg(Mts)-$NHNH_2$, was prepared starting with Z(OMe)-Arg(Mts)-NHNH-Troc as shown in Fig. 5. This, after TFA treatment, was condensed in a stepwise manner with Z(OMe)-Leu-OH and Boc-Asp(OBzl)-OH by the Np procedure. From the resulting protected tripeptide, the Troc group was removed by treatment with Zn powder in a mixture of acetic acid and methanol.

An available hydrazide, Z(OMe)-Gln-Thr-$NHNH_2$,[30] was used to prepare the next fragment [4], Boc-Cys(MBzl)-Gln-Thr-Arg(Mts)-$NHNH_2$. This was condensed with H-Arg(Mts)-OBzl, *via* the azide, to give Z(OMe)-Gln-Thr-Arg(Mts)-OBzl, then the next residue, Boc-Cys(MBzl)-OH was introduced by the Su method[31] to give Boc-Cys(MBzl)-

Gln–Thr–Arg(Mts)–OBzl, which was smoothly converted to [4] by the usual hydrazine treatment as shown in Fig. 6.

The next fragment, Boc–Gly–Asp–Arg(Mts)–$NHNH_2$ [5], was prepared according to the scheme illustrated in Fig. 7. In a model experiment, we observed that the Asp(OBzl) residue linked to Arg(Mts) suffered base-catalyzed side reaction, *i.e.*, succinimide formation, to some extent. Thus we decided to prepare this segment without protecting the β-carboxyl function of the Asp residue. Starting with H–Arg(Mts)–OMe, instead of H–Arg(Mts)–NHNH–Troc, Boc–Gly–Asp–Arg(Mts)–OMe was prepared in a stepwise manner by successive active ester condensations of Z(OMe)–Asp(OBzl)–OH and Boc–Gly–OH, followed by hydrogenolysis. Although column chromatography was required to purify the intermediate, Boc–Gly–Asp(OBzl)–Arg(Mts)–OMe, the hydrogenated ester was smoothly converted to [5] by the usual hydrazine treatment.

Fragment [6], Boc–Tyr–Ser–$NHNH_2$, was prepared by the usual azide condensation of Boc–Tyr–$NHNH_2$ with H–Ser–OMe, followed by hydrazine treatment without particular difficulty.

Fragment [7], Boc–Val–Ile–Gly–$NHNH_2$, was also prepared in a stepwise manner as shown in Fig. 8. The Np and the mixed anhydride procedures were employed to introduce Z(OMe)–Ile–OH and Boc–Val–OH, respectively. In coupling sterically hindered amino acids, Val and Ile, the latter method was effective. The homogeneous protected tripeptide ester, Boc–Val–Ile–Gly–OMe, was readily converted to [7] as usual.

Next fragment [8], Boc–Thr–Cys(MBzl)–Asn–Cys(MBzl)–$NHNH_2$, was prepared in a stepwise manner also as shown in Fig. 9. The Asn and Cys(MBzl) residues were introduced by the active ester procedure and the N-terminal residue, Thr, by the azide method. The resulting protected tetrapeptide ester, Boc–Thr–Cys(MBzl)–Asn–Cys(MBzl)–OMe, was converted to [8] without any particular solubility problem. This hydrazide could be purified by precipitation from DMF with methanol.

According to the route illustrated in Fig. 3, the eight fragments were successively condensed by Honzl and Rudinger's azide procedure, except for fragment [2], as described above. This di-Trp unit was condensed by means of the DCC + HOBt procedure to suppress possible racemization. For this purpose, the TFA-treated sample of [1] was further treated

TABLE I. Amino Acid Analysis of the Protected Tetracosapeptide and Its Intermediates

A.A.	49—53 (5)	46—53 (8)	42—53 (12)	39—53 (15)	37—53 (17)	34—53 (20)	30—53 (24)
Asp		0.98 (1)	1.00 (1)	1.97 (2)	2.02 (2)	2.07 (2)	3.03 (3)
Thr			1.10 (1)	0.91 (1)	0.96 (1)	0.96 (1)	1.64 (2)
Ser					0.90 (1)	0.77 (1)	0.76 (1)
Glu	1.03 (1)	1.01 (1)	2.00 (2)	2.07 (2)	2.00 (2)	2.10 (2)	2.12 (2)
Gly				0.95 (1)	1.00 (1)	1.96 (2)	1.98 (2)
Cys			N.D.	N.D.	N.D.	N.D.	N.D.
Val						0.88 (1)	0.74 (1)
Ile						0.85 (1)	0.71 (1)
Leu	1.00 (1)	2.00 (2)	2.00 (2)	2.00 (2)	2.00 (2)	2.00 (2)	2.00 (2)
Tyr					0.95 (1)	1.01 (1)	0.92 (1)
Trp[a)]	1.87 (2)	1.95 (2)	1.31 (2)	1.72 (2)	1.65 (2)	N.D.	N.D.
Arg	0.98 (1)	1.99 (2)	3.08 (3)	3.92 (4)	4.02 (4)	4.18 (4)	4.04 (4)
Recov. (%)	90	91	91	88	84	88	96

Numbers in parentheses are theoretical values.

a) Trp was determined by 4 M CH_3SO_3H hydrolysis.

with 5% $NaHCO_3$ and the resulting free amino component was subjected to condensation with [2] by using DCC+HOBt at 4 °C. This Et_3N-free procedure seems to be ideal for suppression of possible racemization at the C-terminal Trp residue as well as the acylurea formation of [2], a side reaction of DCC.

Every fragment condensation was performed in DMF without any particular solubility problem. Most products were purified by precipitation from DMF with an appropriate solvent, such as ethyl acetate, or isopropyl ether or methanol and the protected tetracosapeptide ester, Boc-(EGF 30—53)-OBzl, was similarly purified from a mixture of DMSO-DMF with methanol. Throughout this synthesis, Leu was taken as a diagnostic amino acid in acid hydrolysis. By comparison of the recovery of Leu with those of newly incorporated amino acids, satisfactory condensation of each fragment, was confirmed (Table I). By the combination of TLC and elemental analysis, the purity of each product was further confirmed. Thus, we were able to synthesize a half of the EGF molecule with a high degree of homogeneity.

Further chain elongation of this tetracosapeptide to the tripentacontapeptide corresponding to the entire amino acid sequence of EGF will be described in the following paper, together with unusual phenomena in the azide reaction associated with the His-containing fragment.

Experimental

General experimental procedures employed in this paper are essentially the same as described in Part LXXXVIII[32] of this series.

Unless otherwise mentioned, products were purified by one of the following procedures: Procedure A: For purification of protected peptide esters 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 protected peptides 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.

Procedure C: For purification of protected peptides with free carboxyl groups, the crude product was dissolved in 3% ammonia and the aqueous phase, after being washed with AcOEt, was acidified with citric acid. The resulting powder (if an oil was obtained, it was extracted with AcOEt) was washed with 5% citric acid and H_2O and recrystallized or precipitated from appropriate solvents.

Thin layer chromatography (TLC) was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems (v/v): Rf_1 $CHCl_3$–MeOH (10:0.5); Rf_2 $CHCl_3$–MeOH–H_2O (8:3:1); Rf_3 $CHCl_3$–MeOH–AcOH (9:1:0.5).

Z(OMe)-Arg(Mts)-OBzl——Z(OMe)-Arg(Mts)-OH[18] [prepared from 20.29 g (32.7 mmol) of the CHA salt] was esterified with DCHA (9.1 ml, 45.7 mmol) and benzyl bromide (5.4 ml, 45.7 mmol), and after being stirred for 24 h at room temperature, the mixture was concentrated. The product was purified by procedure A and recrystallized from AcOEt and isopropyl ether; yield 19.12 g (96%), mp 50—52 °C, $[\alpha]_D^{20}$ +1.4° (c=0.7, MeOH), Rf_1 0.38. *Anal.* Calcd for $C_{31}H_{38}N_4O_7S$: C, 60.96; H, 6.27; N, 9.17. Found: C, 61.06; H, 6.32; N, 8.88.

Z(OMe)-Leu-Arg(Mts)-OBzl——Z(OMe)-Arg(Mts)-OBzl (18.55 g, 30.4 mmol) was treated with TFA–anisole (39.5 ml–9.9 ml) in an ice-bath for 60 min, then excess TFA was removed by evaporation *in vacuo* and the residue was treated with *n*-hexane. The resulting oily precipitate was dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMF (50 ml) together with Et_3N (4.3 ml, 30.4 mmol). A mixed anhydride [prepared from 17.37 g (36.4 mmol) of Z(OMe)-Leu-OH·DCHA salt] was added to the above ice-chilled solution and the mixture, after being stirred in an ice-bath for 4 h, was concentrated. The product was purified by procedure A and recrystallized from AcOEt and isopropyl ether; yield 20.53 g (93%), mp 60—62 °C, $[\alpha]_D^{20}$ −9.0° (c=0.6, MeOH), Rf_1 0.52. *Anal.* Calcd for $C_{37}H_{49}O_5N_8S$: C, 61.39; H, 6.82; N, 9.68. Found: C, 61.51; H, 7.12; N, 9.48.

Z(OMe)-Glu(OBzl)-Leu-Arg(Mts)-OBzl [1]——Z(OMe)-Leu-Arg(Mts)-OBzl (19.34 g, 26.7 mmol) was treated with TFA–anisole (46 ml–11.6 ml) as stated above, then excess TFA was removed by evaporation, and the residue was treated with *n*-hexane. The resulting powder was dried over KOH pellets *in vacuo* for 4 h, then dissolved in DMF (100 ml) together with Et_3N (7.4 ml, 53.4 mmol) and Z(OMe)-Glu(OBzl)-ONp (15.35 g, 29.4 mmol). The mixture was stirred at room temperature for 24 h and the solvent was removed by evaporation. The product was purified by

procedure A and recrystallized from AcOEt and isopropyl ether; yield 22.20 g (88%), mp 71—73 °C, $[\alpha]_D^{26}$ −6.5° (c = 0.5, MeOH), Rf_1 0.40. Amino acid ratios in 6 N HCl hydrolysate: Glu 1.05, Leu 1.00, Arg 1.01 (recovery of Leu, 97%). *Anal.* Calcd for $C_{49}H_{62}N_6O_{11}S$: C, 62.40; H, 6.63; N, 8.91. Found: C, 62.56; H, 6.69; N, 8.67.

Boc–Trp–Trp–OH·DCHA [2]——Boc–Trp–ONp (15.41 g, 36.2 mmol) in THF (50 ml) was allowed to react with H–Trp–OH (18.49 g, 90.5 mmol) in H_2O (100 ml) in the presence of Et_3N (17.7 ml, 126.4 mmol) at room temperature for 12 h. After removal of the solvent by evaporation, the product was purified by procedure C then converted to the corresponding DCHA salt. The salt was recrystallized from AcOEt and isopropyl ether; yield 18.98 g (78%), mp 183—185 °C, $[\alpha]_D^{26}$ −3.6° (c = 0.6, MeOH), Rf_3 0.59. *Anal.* Calcd for $C_{39}H_{53}N_5O_5$: C, 69.72; H, 7.95; N, 10.42. Found: C, 70.00; H, 7.97; N, 10.39.

Z(OMe)–Leu–Arg(Mts)–NHNH–Troc——Z(OMe)–Arg(Mts)–NHNH–Troc[33] (13.84 g, 19.5 mmol) was treated with TFA–anisole (31.6 ml–6.3 ml) as usual, then excess TFA was removed by evaporation. Treatment of the residue with *n*-hexane afforded a gummy product, which was dried over KOH pellets *in vacuo* for 3 h, then dissolved in DMF (70 ml) together with Et_3N (5.4 ml, 39 mmol) and Z(OMe)–Leu–ONp (8.12 g, 19.5 mmol). The mixture was stirred at room temperature for 24 h, and then concentrated *in vacuo*. The product was purified by procedure A and triturated with ether. The resulting powder was recrystallized from AcOEt and ether; yield 11.23 g (70%), mp 104—107 °C, $[\alpha]_D^{20}$ −24.6° (c = 0.6, MeOH), Rf_1 0.39. *Anal.* Calcd for $C_{33}H_{46}Cl_3N_7O_9S$: C, 48.15; H, 5.63; N, 11.91. Found: C, 48.24; H, 5.39; N, 11.75.

Boc–Asp(OBzl)–Leu–Arg(Mts)–NHNH–Troc——Z(OMe)–Leu–Arg(Mts)–NHNH–Troc (7.85 g, 9.5 mmol) was treated with TFA–anisole (21 ml–4.2 ml) and the N^{α}-deprotected peptide, isolated as stated above, was dissolved in DMF (40 ml), together with Et_3N (2.7 ml, 19.1 mmol) and Boc–Asp(OBzl)–ONp (4.24 g, 9.5 mmol). The mixture was stirred at room temperature for 24 h. The solvent was removed by evaporation and the product was purified by procedure A followed by recrystallization from AcOEt and isopropyl ether; yield 7.38 g (80%), mp 102—105 °C, $[\alpha]_D^{20}$ −30.8° (c = 0.6, MeOH). Rf_1 0.41. *Anal.* Calcd for $C_{40}H_{57}Cl_3N_8O_{11}S$: C, 49.82; H, 5.96; N, 11.62. Found: C, 49.73; H, 6.19; N, 11.33.

Boc–Asp(OBzl)–Leu–Arg(Mts)–NHNH$_2$ [3]——Boc–Asp(OBzl)–Leu–Arg(Mts)–NHNH–Troc (7.68 g, 8.0 mmol) in a mixture of AcOH–MeOH (1 : 1, 40 ml) was treated with Zn powder (10.4 g, 160 mmol) at room temperature for 12 h. Fresh Zn powder (5.2 g, 80 mmol) was added and the solution, after being stirred for an additional 5 h, was filtered. The residue was dissolved in AcOEt. The organic phase was successively washed with 3% EDTA, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated down. Treatment of the residue with isopropyl ether afforded a powder; yield 5.96 g (94%), mp 98—100 °C, $[\alpha]_D^{26}$ −14.1° (c = 0.6, MeOH), Rf_2 0.69. Amino acid ratios in 6 N HCl hydrolysate: Asp 1.00, Leu 1.06, Arg 1.00 (recovery of Asp, 88%). *Anal.* Calcd for $C_{37}H_{56}O_8N_9S$: C, 56.32; H, 7.15; N, 14.22. Found: C, 55.92; H, 7.32; N, 14.52.

Z(OMe)–Gln–Thr–Arg(Mts)–OBzl——Z(OMe)–Arg(Mts)–OBzl (11.38 g, 18.6 mmol) was treated with TFA–anisole (24.2 ml–6.0 ml) in an ice-bath for 80 min, then excess TFA was removed by evaporation. The residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (60 ml) containing Et_3N (2.6 ml, 18.6 mmol). The azide [prepared from 9.51 g (22.4 mmol) of Z(OMe)–Gln–Thr–NHNH$_2$][30] in DMF (50 ml) and Et_3N (3.4 ml, 24.6 mmol) were added to the above ice-chilled solution of H–Arg(Mts)–OBzl and the mixture was stirred at 4 °C for 12 h. Additional azide [prepared from 1.58 g (3.7 mmol) of the hydrazide] in DMF (10 ml) and Et_3N (0.5 ml, 3.7 mmol) were added and the stirring was continued for an additional 12 h. The solvent was removed by evaporation and the product was purified by procedure A followed by recrystallization from MeOH and AcOEt; yield 11.58 g (74%), mp 110—113 °C, $[\alpha]_D^{22}$ −3.0° (c = 0.7, DMF), Rf_2 0.55. *Anal.* Calcd for $C_{40}H_{53}N_7O_{11}S$: C, 57.19; H, 6.36; N, 11.67. Found: C, 57.26; H, 6.46; N, 11.69.

Boc–Cys(MBzl)–Gln–Thr–Arg(Mts)–OBzl——Z(OMe)–Gln–Thr–Arg(Mts)–OBzl (8.60 g, 10 mmol) was treated with TFA–anisole (16.6 ml–3.3 ml) as usual, then excess TFA was removed by evaporation and the residue was treated with ether. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (50 ml), together with Et_3N (1.4 ml, 10 mmol), Boc–Cys(MBzl)–OSu (4.94 g, 11 mmol), and NMM (1.1 ml, 11 mmol). The mixture was stirred at room temperature for 12 h and the solvent was removed by evaporation. The product was purified by procedure A followed by recrystallization from MeOH and ether; yield 9.58 g (94%), mp 94—97 °C, $[\alpha]_D^{20}$ −15.0° (c = 0.4, DMF). Rf_2 0.58. *Anal.* Calcd for $C_{47}H_{66}O_8N_{12}S_2$: C, 56.49; H, 6.66; N, 11.22. Found: C, 56.23; H, 6.71; N, 10.98.

Boc–Cys(MBzl)–Gln–Thr–Arg(Mts)–NHNH$_2$ [4]——Boc–Cys(MBzl)–Gln–Thr–Arg(Mts)–OBzl (9.58 g, 9.6 mmol) in DMF (50 ml) was treated with 80% hydrazine hydrate (4.8 ml, 96 mmol) at room temperature for 12 h. The solvent was evaporated off and the residue was treated with ether. The resulting powder was precipitated from DMF with ether; yield 8.81 g (99%), mp 179—181 °C, $[\alpha]_D^{26}$ −7.0° (c = 0.6, DMSO), Rf_2 0.32. Amino acid ratios in 6 N HCl hydrolysate: Glu 1.12, Thr 1.03, Arg 1.00 (recovery of Arg, 98%) *Anal.* Calcd for $C_{40}H_{62}N_{10}O_{11}S_2$: C, 52.04; H, 6.77; N, 15.17. Found: C, 52.05; H, 6.81; N, 15.32.

Z(OMe)–Asp(OBzl)–Arg(Mts)–OMe——Z(OMe)–Arg(Mts)–OMe (8.89 g, 17.0 mmol) was treated with TFA–anisole (18 ml–3.6 ml) as usual, then excess TFA was removed by evaporation and the residue was washed with *n*-hexane. The resulting oily precipitate was dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMF (40 ml) together with Et_3N (4.6 ml, 34.0 mmol). Z(OMe)–Asp(OBzl)–ONp (7.61 g, 15.0 mmol) was added and the mix-

ture was stirred at 4 °C for 12 h. After addition of a few drops of AcOH, the solvent was evaporated off. The product was purified by procedure A followed by recrystallization from AcOEt and isopropyl ether; yield 9.41 g (85%), mp 49—52 °C, $[\alpha]_D^{25}$ −4.3° (*c*=0.9, MeOH). Rf_2 0.88. *Anal.* Calcd for $C_{36}H_{45}N_5O_{10}S$: C, 58.44; H, 6.13; N, 9.47. Found: C, 58.54; H, 6.41; N, 10.03.

Boc–Gly–Asp(OBzl)–Arg(Mts)–OMe——Z(OMe)–Asp(OBzl)–Arg(Mts)–OMe (5.09 g, 6.9 mmol) was treated with TFA–anisole (11.2 ml–2.2 ml) and the N^{α}-deprotected product isolated as stated above was dissolved in DMF (30 ml) containing Et_3N (0.96 ml, 6.9 mmol). Boc–Gly–OSu (1.87 g, 6.9 mmol) and NMM (0.70 ml, 6.9 mmol) were added and the mixture was stirred at room temperature for 24 h. The solvent was removed by evaporation and the product was purified by procedure A followed by silica gel chromatography (7.2 × 10 cm) with $CHCl_3$–MeOH (10 : 0.5). The product was recrystallized from AcOEt and isopropyl ether; yield 3.30 g (65%), mp 59—62 °C, $[\alpha]_D^{25}$ −24.3° (*c*=0.5, MeOH), Rf_1 0.21. *Anal.* Calcd for $C_{34}H_{48}N_6O_{10}S$: C, 55.72; H, 6.60; N, 11.47. Found: C, 55.68; H, 6.90; N, 11.77.

Boc–Gly–Asp–Arg(Mts)–NHNH$_2$ [5]——Boc–Gly–Asp(OBzl)–Arg(Mts)–OMe (2.63 g, 3.6 mmol) dissolved in a mixture of MeOH and H_2O (9 : 1, 30 ml) was hydrogenated over a Pd catalyst (in the presence of a few drops of AcOH) for 1 h. The solution was filtered and the filtrate was concentrated. The residue was dissolved in MeOH (20 ml) and treated with 80% hydrazine hydrate (2.2 ml, 36 mmol) at room temperature overnight. The solvent was evaporated off and the residue was extracted with *n*-BuOH. The organic phase was washed with H_2O and then evaporated down. The residue was treated with ether and the resulting powder was recrystallized from MeOH and ether; yield 1.87 g (81%), mp 130—133 °C, $[\alpha]_D^{26}$ −26.3° (*c*=0.8, MeOH), Rf_3 0.22. Amino acid ratios in 6 N HCl hydrolysate: Gly 1.00, Asp 1.02, Arg 0.90 (recovery of Gly, 62%) *Anal.* Calcd for $C_{26}H_{42}N_8O_9S \cdot 4.5H_2O$: C, 43.14; H, 7.10; N, 15.48. Found: C, 43.36; H, 7.19; N, 14.97.

Boc–Tyr–Ser–OMe——The azide [prepared from 6.0 g (20.3 mmol) of Boc–Tyr–$NHNH_2$] and Et_3N (3.1 ml, 22.4 mmol) were added to an ice-chilled solution of H–Ser–OMe [prepared from 4.74 g (30.5 mmol) of the hydrochloride] and the mixture, after being stirred at 4 °C for 12 h, was concentrated. The product was purified by procedure A followed by recrystallization from AcOEt and *n*-hexane; yield 5.10 g (66%), mp 64—66 °C, $[\alpha]_D^{15}$ +1.5° (*c*=0.7, MeOH) Rf_2 0.68. *Anal.* Calcd for $C_{18}H_{26}N_2O_7$: C, 56.53; H, 6.85; N, 7.33. Found: C, 56.37; H, 6.98; N, 7.36.

Boc–Tyr–Ser–NHNH$_2$ [6]——Boc–Tyr–Ser–OMe (4.13 g, 11.3 mmol) in MeOH (20 ml) was treated with 80% hydrazine hydrate (7.1 ml, 110 mmol) at room temperature overnight. The solvent was removed by evaporation and the residue was extracted with *n*-BuOH. The organic phase was washed with H_2O and then concentrated. The residue was triturated with ether and the resulting powder was recrystallized from MeOH and ether; yield 2.53 g (59%), mp 169—172 °C, $[\alpha]_D^{26}$ +5.1° (*c*=0.6, DMF) Rf_2 0.36. Amino acid ratios in 6 N HCl hydrolysate; Tyr 0.89, Ser 1.00 (recovery of Ser 90%) *Anal.* Calcd for $C_{17}H_{26}N_4O_6 \cdot 0.5H_2O$: C, 52.16; H, 6.95; N, 14.32. Found: C, 52.50; H, 6.81; N, 14.62.

Z(OMe)–Ile–Gly–OMe——The title compound was prepared by the standard Np procedure using Z(OMe)–Ile–ONp (9.67 g, 23.0 mmol) and H–Gly–OMe [prepared from 4.66 g (37.1 mmol) of the HCl salt]. The product was purified by procedure A followed by recrystallization from AcOEt and ether; yield 6.89 g (81%), mp 137—139 °C, $[\alpha]_D^{15}$ −25.0° (*c*=0.5, MeOH), Rf_1 0.68. *Anal.* Calcd for $C_{18}H_{26}N_2O_6$: C, 59.00; H, 7.15; N, 7.65. Found: C, 59.01; H, 7.22; N, 7.61.

Boc–Val–Ile–Gly–OMe——Z(OMe)–Ile–Gly–OMe (6.76 g, 18.5 mmol) was treated with TFA–anisole (16 ml–4 ml) as usual, then excess TFA was removed by evaporation and the residue was treated with *n*-hexane. The resulting oily precipitate was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (30 ml) containing Et_3N (2.6 ml, 18.5 mmol). A mixed anhydride [prepared from 8.83 g (22.2 mmol) of Boc–Val–OH·DCHA salt] was added to the above ice-chilled solution and the mixture was stirred in an ice-bath for 12 h. The solvent was evaporated off and the residue was purified by procedure B. Recrystallization from MeOH and ether afforded a powder; yield 5.08 g (69%), mp 174—176 °C, $[\alpha]_D^{15}$ −13.8° (*c*=0.5, DMF), Rf_2 0.65. *Anal.* Calcd for $C_{19}H_{35}N_3O_6$: C, 56.84; H, 8.79; N, 10.47. Found: C, 56.65; H, 8.71; N, 10.40.

Boc–Val–Ile–Gly–NHNH$_2$ [7]——Boc–Val–Ile–Gly–OMe (2.85 g, 7.1 mmol) in MeOH (30 ml) was treated with 80% hydrazine hydrate (4.4 ml, 71 mmol) at room temperature overnight. The solution was concentrated and the residue was triturated with H_2O. The resulting powder was washed with H_2O, and recrystallized from MeOH and ether; yield 1.54 g (54%), mp 184—186 °C, $[\alpha]_D^{26}$ −5.5° (*c*=0.5, DMSO), Rf_2 0.50. Amino acid ratios in 6 N HCl hydrolysate: Val 0.91, Ile 0.85, Gly 1.00 (recovery of Gly 98%) *Anal.* Calcd for $C_{18}H_{35}N_5O_5$: C, 53.84; H, 8.79; N, 17.44. Found: C, 53.76; H, 8.92; N, 17.63.

Z(OMe)–Asn–Cys(MBzl)–OH——Z(OMe)–Asn–ONp (17.30 g, 42 mmol), and Et_3N (5.8 ml, 42 mmol) were added to a solution of H–Cys(MBzl)–OH (5.0 g, 21 mmol) and Et_3N (2.9 ml, 21 mmol) in DMF–H_2O (1 : 2, 120 ml) and mixture was stirred at room temperature for 24 h. The solvent was removed by evaporation and the residue was treated with 5% citric acid. The resulting powder was purified by washing with 0.1 N HCl and H_2O followed by precipitation from DMF with AcOEt; yield 6.17 g (57%), mp 185—187 °C, $[\alpha]_D^{25}$ +6.4° (*c*=0.5, DMSO), Rf_3 0.27. *Anal.* Calcd for $C_{24}H_{29}N_3O_8S \cdot 0.5H_2O$: C, 54.53; H, 5.72; N, 7.95. Found: C, 54.70; H, 5.95; N, 7.81.

Z(OMe)–Cys(MBzl)–Asn–Cys(MBzl)–OH——Z(OMe)–Asn–Cys(MBzl)–OH (3.86 g, 7.4 mmol) was treated with TFA–anisole (8.0 ml–1.6 ml), then excess TFA was removed by evaporation and the residue was treated with *n*-

hexane. The resulting powder was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (20 ml) containing Et_3N (1.0 ml, 7.4 mmol). Z(OMe)–Cys(MBzl)–OSu (4.48 g, 8.9 mmol) and Et_3N (1.2 ml, 8.6 mmol) were added and the mixture was stirred at room temperature for 12 h. The solvent was removed by evaporation and the residue was treated with 5% citric acid. The resulting powder was purified by washing with 5% citric acid and H_2O followed by precipitation from DMF with AcOEt; yield 4.54 g (82%), mp 191—193 °C, $[\alpha]_D^{25}$ −33.6° (c=0.7, DMSO), Rf_2 0.26. *Anal.* Calcd for $C_{35}H_{42}N_4O_{10}S_2$: C, 56.59; H, 5.70; N, 7.54. Found: C, 56.83; H, 5.93; N, 7.34.

Z(OMe)–Cys(MBzl)–Asn–Cys(MBzl)–OMe——Z(OMe)–Cys(MBzl)–Asn–Cys(MBzl)–OH (7.68 g, 10.3 mmol) in DMF (70 ml) was methylated with ethereal diazomethane as usual. After evaporation of the DMF, the residue was treated with AcOEt. The resulting powder was washed with 5% $NaHCO_3$, H_2O and precipitated from DMF with MeOH; yield 6.91 g (88%), mp 179—181 °C, $[\alpha]_D^{25}$ −22.6° (c=0.8, DMSO), Rf_2 0.74. *Anal.* Calcd for $C_{36}H_{44}N_4O_{10}S_2$: C, 57.12; H, 5.86; N, 7.40. Found: C, 56.90; H, 5.93; N, 7.56.

Boc–Thr–Cys(MBzl)–Asn–Cys(MBzl)–OMe——Z(OMe)–Cys(MBzl)–Asn–Cys(MBzl)–OMe (3.90 g, 5.2 mmol) was treated with TFA–anisole (9 ml–2.2 ml), then dry ether was added. The resulting powder was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (20 ml) containing Et_3N (0.7 ml, 5.2 mmol). The azide [prepared from 2.40 g (10.3 mmol) of Boc–Thr–$NHNH_2$] in DMF (15 ml) and Et_3N (1.6 ml, 11.4 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4 °C for 48 h. Additional azide [prepared from 0.60 g (2.6 mmol) of the hydrazide] and Et_3N (0.36 ml, 2.6 mmol) were added and the mixture, after being stirred for a further 12 h, was concentrated. The product was purified by procedure B followed by precipitation from DMF with MeOH; yield 2.65 g (65%), mp 136—139 °C, $[\alpha]_D^{20}$ −16.6° (c=0.7, DMSO), Rf_2 0.66. *Anal.* Calcd for $C_{36}H_{51}N_5O_{11}S_2$: C, 54.46; H, 6.48; N, 8.82. Found: C, 54.16; H, 6.62; N, 8.74.

Boc–Thr–Cys(MBzl)–Asn–Cys(MBzl)–$NHNH_2$ [8]——The above protected tetrapeptide ester (3.00 g, 3.8 mmol) in DMF–MeOH (1 : 1, 30 ml) was treated with 80% hydrazine hydrate (2.4 ml, 38 mmol) at room temperature overnight. The resulting solid was precipitated from DMF with MeOH; yield 2.40 g (80%), mp 201—204 °C, $[\alpha]_D^{26}$ −39.5° (c=0.5, DMSO), Rf_2 0.57. Amino acid ratios in 6 N HCl hydrolysate: Asp 1.00, Thr 0.80 (recovery of Asp, 90%). *Anal.* Calcd for $C_{35}H_{51}N_7O_{10}S_2 \cdot H_2O$: C, 52.35; H, 6.53; N, 12.21. Found: C, 52.28; H, 6.51; N, 12.34.

Boc–Trp–Trp–Glu(OBzl)–Leu–Arg(Mts)–OBzl——Z(OMe)–Glu(OBzl)–Leu–Arg(Mts)–OBzl (12.04 g, 12.8 mmol) was treated with TFA–anisole (20.7 ml–4.1 ml) in an ice-bath for 80 min, then *n*-hexane was added. The resulting oily precipitate was washed with *n*-hexane and treated with 5% $NaHCO_3$. The resulting powder was washed with H_2O, dried over KOH pellets *in vacuo* for 3 h, then dissolved in DMF (50 ml) together with Boc–Trp–Trp–OH [prepared from 8.57 g (12.8 mmol) of the DCHA salt] and HOBT (1.95 g, 12.8 mmol). DCC (3.95 g, 19.1 mmol) was added and the mixture was stirred at 4 °C for 12 h. DCC (1.32 g, 6.4 mmol) was further added and stirring was continued for an additional 12 h. The solution was filtered and the filtrate was concentrated. The product was purified by procedure A and recrystallized from AcOEt and isopropyl ether; yield 14.09 g (88%), mp 99—102 °C, $[\alpha]_D^{26}$ −32.3° (c=0.7, MeOH), Rf_1 0.48. *Anal.* Calcd for $C_{67}H_{82}N_{10}O_{12}S \cdot 0.5H_2O$: C, 63.84; H, 6.64; N, 11.11. Found: C, 63.83; H, 6.77; N, 11.41.

Boc–Asp(OBzl)–Leu–Arg(Mts)–Trp–Trp–Glu(OBzl)–Leu–Arg(Mts)–OBzl [Boc–(EGF 46—53)–OBzl]——The above protected pentapeptide ester (11.0 g, 8.8 mmol) was treated with TFA–anisole–EDT (22.5 ml–3.8 ml–0.7 ml) in an ice-bath for 80 min, then ether was added. The resulting powder was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (50 ml) containing Et_3N (1.2 ml, 8.8 mmol). The azide [prepared from 6.94 g (8.8 mmol) of Boc–Asp(OBzl)–Leu–Arg(Mts)–$NHNH_2$] and Et_3N (1.4 ml, 10 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4 °C for 12 h.

Fresh azide [prepared from 1.39 g (1.8 mmol) of the hydrazide] and Et_3N (0.25 ml, 1.8 mmol) were added and stirring was continued for an additional 12 h. After addition of a few drops of AcOH, the solvent was removed by evaporation and the product was purified by procedure B followed by column chromatography on silica gel (7.2 × 17 cm) with $CHCl_3$–MeOH (40 : 1). The fractions containing a substance of Rf_1 0.32 were combined, the solvent was removed by evaporation, and the residue was triturated with AcOEt and ether to afford a powder; yield 8.89 g (53%), mp 156—159 °C, $[\alpha]_D^{28}$ −26.7° (c=0.4, DMSO), Rf_1 0.32. *Anal.*Calcd for $C_{99}H_{126}N_{16}O_{19}S_2$: C, 62.31; H, 6.66; N, 11.75. Found: C, 62.09; H, 6.69; N, 11.56.

Boc–Cys(MBzl)–Gln–Thr–Arg(Mts)–Asp(OBzl)–Leu–Arg(Mts)–Trp–Trp–Glu(OBzl)–Leu–Arg(Mts)–OBzl [Boc–(EGF 42—53)–OBzl]——The above protected octapeptide ester (7.66 g, 4.0 mmol) was treated with TFA–anisole–EDT (20.7 ml–3.5 ml–0.7 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF (40 ml) containing Et_3N (0.56 ml, 4.0 mmol). The azide [prepared from 5.56 g (6.0 mmol) of Boc–Cys(MBzl)–Gln–Thr–Arg(Mts)–$NHNH_2$] and Et_3N (0.93 ml, 6.6 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4 °C for 12 h. Fresh azide [prepared from 1.11 g (1.2 mmol) of the hydrazide] and Et_3N (0.17 ml, 1.2 mmol) were added. Stirring was continued for an additional 12 h. The solvent was removed by evaporation and the product was purified by procedure B followed by precipitation twice from DMF with MeOH; yield 7.16 g (66%), mp 211—214 °C, $[\alpha]_D^{28}$ −13.5° (c=0.5, DMSO), Rf_2 0.69. *Anal.* Calcd for $C_{134}H_{176}N_{24}O_{28}S_4$: C, 59.62; H, 6.57; N, 12.46. Found: C, 59.74; H, 6.57; N, 12.17.

Boc–Gly–Asp–Arg(Mts)–Cys(MBzl)–Gln–Thr–Arg(Mts)–Asp(OBzl)–Leu–Arg(Mts)–Trp–Trp–Glu(OBzl)–Leu–

Arg(Mts)-OBzl [Boc-(EGF 39—53)-OBzl]——The above protected dodecapeptide ester (7.5 g, 2.8 mmol) was treated with TFA–anisole–EDT (17 ml–3.6 ml–0.7 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF (70 ml) containing Et_3N (0.39 ml, 2.8 mmol). The azide [prepared from 1.96 g (3.1 mmol) of Boc–Gly–Asp–Arg(Mts)–$NHNH_2$] and Et_3N (0.47 ml, 3.4 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4 °C for 12 h. After addition of fresh azide [prepared from 0.54 g (0.8 mmol) of the hydrazide] and Et_3N (0.12 ml, 0.8 mmol), stirring was continued for an additional 12 h. After addition of a few drops of AcOH, the solvent was removed by evaporation and the product was purified by procedure B followed by precipitation three times from DMF with MeOH; yield 7.03 g (79%), mp 211—224 °C, $[\alpha]_D^{28}$ −14.4° (c=0.7, DMSO), Rf_2 0.54. *Anal.* Calcd for $C_{155}H_{206}N_{30}O_{35}S_5 \cdot H_2O$: C, 57.67; H, 6.50; N, 13.02. Found: C, 57.56; H, 6.44; N, 13.00.

Boc-Tyr-Ser-Gly-Asp-Arg(Mts)-Cys(MBzl)-Gln-Thr-Arg(Mts)-Asp(OBzl)-Leu-Arg(Mts)-Trp-Trp-Glu-(OBzl)-Leu-Arg(Mts)-OBzl [Boc-(EGF 37—53)-OBzl]——The above protected pentadecapeptide ester (6.87 g, 2.1 mmol) was treated with TFA–anisole–EDT (16.7 ml–2.8 ml–0.54 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF (60 ml) containing Et_3N (0.30 ml, 2.1 mmol). The azide [prepared from 0.98 g (2.6 mmol) of Boc–Tyr–Ser–$NHNH_2$] and Et_3N (0.40 ml, 2.9 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4 °C for 12 h. Fresh azide [prepared from 0.25 g (0.6 mmol) of the hydrazide] and Et_3N (0.09 ml, 0.6 mmol) were further added and stirring was continued for an additional 12 h. The solvent was removed by evaporation and the product was purified by procedure B followed by precipitation twice from DMF with MeOH; yield 7.08 g (96%), mp 225—228 °C, $[\alpha]_D^{28}$ −10.4° (c=0.4, DMSO), Rf_2 0.47. *Anal.* Calcd for $C_{167}H_{220}N_{32}O_{39}S_5 \cdot 2H_2O$: C, 57.37; H, 6.46; N, 12.82. Found: C, 57.45; H, 6.37; N, 12.70.

Boc-Val-Ile-Gly-Tyr-Ser-Gly-Asp-Arg(Mts)-Cys(MBzl)-Gln-Thr-Arg(Mts)-Asp(OBzl)-Leu-Arg(Mts)-Trp-Trp-Glu(OBzl)-Leu-Arg(Mts)-OBzl [Boc-(EGF 34—53)-OBzl]——The above protected heptadecapeptide ester (7.08 g, 2.1 mmol) was treated with TFA–anisole–EDT (17.0 ml–3.5 ml–0.69 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF (70 ml) containing Et_3N (0.29 ml, 2.1 mmol). The azide [prepared from 0.99 g (2.5 mmol) of Boc–Val–Ile–Gly–$NHNH_2$] and Et_3N (0.38 ml, 2.7 mmol) were added. The whole was stirred for 12 h, additional azide [prepared from 0.33 g (0.8 mmol) of the hydrazide] and Et_3N (0.11 ml, 0.8 mmol) were added, and stirring was continued for a further 12 h. The solvent was removed by evaporation and the product was purified by procedure B followed by precipitation twice from DMF with MeOH; yield 7.13 g (93%), mp 257—260 °C, $[\alpha]_D^{28}$ −19.4° (c=0.4, DMSO), Rf_2 0.43. *Anal.* Calcd for $C_{180}H_{243}N_{35}O_{42}S_5 \cdot 2H_2O$: C, 57.41; H, 6.61; N, 13.02. Found: C, 57.37; H, 6.91; N, 12.96.

Boc-Thr-Cys(MBzl)-Asn-Cys(MBzl)-Val-Ile-Gly-Tyr-Ser-Gly-Asp-Arg(Mts)-Cys(MBzl)-Gln-Thr-Arg-(Mts)-Asp(OBzl)-Leu-Arg(Mts)-Trp-Trp-Glu(OBzl)-Leu-Arg(Mts)-OBzl [Boc-(EGF 30—53)-OBzl]——The above protected eicosapeptide ester (6.74 g, 1.8 mmol) was treated with TFA–anisole–EDT (18.0 ml–3.1 ml–0.61 ml) and the deprotected peptide isolated as stated above was dissolved in DMF–DMSO (1 : 1, 70 ml) containing Et_3N (0.25 ml, 1.8 mmol). The azide [prepared from 1.87 g (2.4 mmol) of Boc–Thr–Cys(MBzl)–Asn–Cys(MBzl)–$NHNH_2$] and Et_3N (0.36 ml, 2.6 mmol) were added. The whole was stirred for 12 h, and additional azide [prepared from 1.15 g (1.5 mmol) of the hydrazide] and Et_3N (0.20 ml, 1.5 mmol) were added. Stirring was continued for a further 12 h and the solvent was removed by evaporation. The product was purified by procedure B followed by precipitation twice from DMSO–DMF (1 : 1) with MeOH; yield 7.10 g (90%), mp 263 °C (dec.), $[\alpha]_D^{28}$ −17.2° (c=0.4, DMSO), Rf_2 origin. *Anal.* Calcd for $C_{210}H_{282}N_{40}O_{50}S_7 \cdot 3H_2O$: C, 56.74; H, 6.53; N, 12.61. Found: C, 56.71; H, 6.62; N, 12.67.

References and Notes

1) Part CXXV: N. Fujii, K. Akaji, Y. Hayashi, and H. Yajima, *Chem. Pharm. Bull.*, **33**, 362 (1985).
2) Amino acids and peptide derivatives mentioned in this investigation were of the L-configuration. The following abbreviations are used: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, Mts = mesitylene-2-sulfonyl, Bzl = benzyl, DCC = dicyclohexylcarbodiimide, Np = *p*-nitrophenyl, Su = *N*-hydroxysuccinimidyl, Troc = 2,2,2-trichloroethoxycarbonyl, CHA = cyclohexylamine, DCHA = dicyclohexylamine, NMM = *N*-methylmorpholine, EDTA = ethylenediaminetetraacetic acid disodium salt, DMF = dimethylformamide, THF = tetrahydrofuran, TFMSA = trifluoromethanesulfonic acid, TFA = trifluoroacetic acid, DMSO = dimethylsulfoxide, EDT = ethanedithiol, HOBT = *N*-hydroxybenztriazole.
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