Total Synthesis of Urogastrone (Human Epidermal Growth Factor, h-EGF).[1] Part 1. Synthesis of the Fully Protected Urogastrone

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The first total synthesis of urogastrone (h-EGF) has been achieved by the segment condensation method in solution applying the maximum protection strategy with a two-step deprotection procedure. The polypeptide chain was constructed from 10 small segments which were synthesized in a conventional fashion using Boc for the amino protecting group, Acm for the thiol protecting group, and Pac for the carboxy protecting group except for segments (48—53) and (1—5), which were protected with Bzl and Troc hydrazide, respectively. The larger fragment condensation reactions were successfully carried out by the WSCD-HOBt method and the azide method to obtain the fully protected tripentacosapeptide corresponding to the sequence of urogastrone.

Since the discovery of epidermal growth factors (EGFs) some 30 years ago,[2] there has been a continuing interest in determining their physiological role in the control of growth and function of cells. EGFs were isolated from the submaxillary gland of male mouse and from human urine and proved to stimulate the proliferation and differentiation of cells of ectodermal and mesodermal origin.[3,4] Although mouse EGF (m-EGF) was readily accessible in adequate amounts, the isolation of human EGF (h-EGF) was a formidable task because of its low abundance in human urine. On the other hand, urogastrone was isolated from human urine as a potent inhibitor of gastric acid secretion[5,6] and subsequently shown to be a polypeptide of 53 amino acid residues with three disulphide bonds[7] (Figure 1). This polypeptide is now regarded as being identical with h-EGF, since it was found to be biologically and structurally related to m-EGF.[8,9] However, the biological evaluation of this peptide has been hampered by the difficulty in isolating it from human urine.

We therefore undertook the chemical synthesis of urogastrone in order to obtain sufficient for detailed biological and biochemical studies. In an earlier preliminary communication,[1] we reported the first total synthesis of urogastrone: this and following papers are devoted to a full account of this work. In this first paper, we describe the synthesis of the protected polypeptide corresponding to the entire amino acid sequence of urogastrone.

Success in the synthesis of complex and large peptides such as urogastrone which contain disulphide bonds entirely depends upon a successful selection of the cysteine thiol protecting groups and the subsequent deprotection of these. In our synthesis of urogastrone, we chose, for this protection, the Acm group[10] which could be removed by treatment with $Hg(OAc)_2$ without affecting the other groups.† We thus adopted a two-step deprotection strategy applying a maximum protection procedure, in which the other side-chain protecting groups were chosen among those removable by treatment with HF. These groups were as follows: Glu(OBzl), Asp(Chx),[11] Ser(Bzl), Arg(Tos), Tyr(Cl_2Bzl),[12] Lys(ClZ),[13] and Trp(For).[14,15] The Chx group for Asp was chosen in order to avoid succinimide formation and the Cl_2Bzl group for Tyr was selected in order to avoid *ortho*-alkylation during HF treatment. The For group for Trp was used in order to suppress the t-butylation and oxidation of the indole nucleus during the Boc-deprotection process. The Lys ε-amino group was protected with the ClZ group, to prevent the side-chain amino branching, because it is much more stable than the Z group itself to TFA treatment. For protection of the two His residues incorporated in the urogastrone sequence, the Tos group was employed: this could be cleaved with pyridinium chloride[16] after incorporation of His(Tos) into the peptides.

The synthesis of the protected urogastrone was carried out using the solution method and the peptide chain was elongated from 10 segments as indicated in Scheme 1. Those segments were synthesized using Boc for the temporary amino protecting group. The carboxy groups of the segments were protected by the Pac group except for those of segments (1—5) (**10**) and (48—53) (**1**). The C-terminal of segment (1—5) (**10**) was protected as the Troc hydrazide[17] with a view to adopting the azide method for further elongation and that of segment (48—53) (**1**) was protected as the Bzl ester.

Segment (48—53) (**1**) was prepared in a stepwise manner starting from Boc-Arg(Tos)-OBzl by the Su active ester method for ^{49}Trp and ^{48}Lys, and the WSCD–HOBt procedure for ^{52}Leu, ^{51}Glu, and ^{50}Trp. In each condensation reaction, the Boc group of the peptides was removed by treatment with TFA in the presence of anisole. The yield of segment (48—53) (**1**) was 43% (Scheme 2). Segments (40—47) (**2**) and (37—39) (**3**) were prepared starting from Boc-Leu-OPac and Boc-Gly-OPac, respectively, by the mixed anhydride method (Bu^iOCOCl–NMM) for ^{46}Asp, ^{45}Arg, and ^{38}Ile, the WSCD–HOBt method for ^{44}Tyr, ^{43}Gln, ^{41}Arg, ^{40}Glu, and ^{37}Tyr, and the Su active ester method for ^{42}Cys. These segments were obtained in 43 and

† All amino acid residues mentioned have the L-configuration. Abbreviations used here for amino acids, protecting groups, and condensation and deprotection reagents are those recommended by the I.U.P.A.C.-I.U.B. Commission on Biochemical Nomenclature (Pure Appl. Chem., 1984, **56**, 595): Boc = t-butoxycarbonyl, Pac = phenacyl, Acm = acetamidomethyl, Bzl = benzyl, Chx = cyclohexl, Tos = toluene-*p*-sulphonyl, Cl_2Bzl = 2,6-dichlorobenzyl, ClZ = 2-chlorobenzyloxycarbonyl, For = formyl, Troc = 2,2,2-trichloroethoxycarbonyl, WSCD = 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide, HOBt = *N*-hydroxybenzotriazole, Su = *N*-hydroxysuccinimide, Np = *p*-nitrophenol, Bu^iOCOCl = isobutyl chlorocarbonate, EDT = ethane-1,2-dithiol, Me_2S = dimethyl sulphide, TFA = trifluoroacetic acid, AcOH = acetic acid, NMM = *N*-methylmorpholine, DMF = *N*,*N*-dimethylformamide, DMSO = dimethyl sulphoxide, NMP = *N*-methylpyrrolidone, THF = tetrahydrofuran, IPE = di-isopropyl ether.

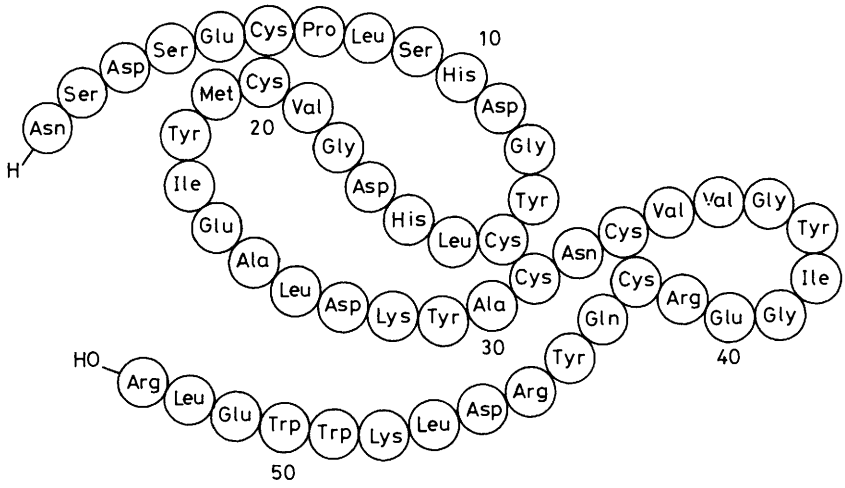
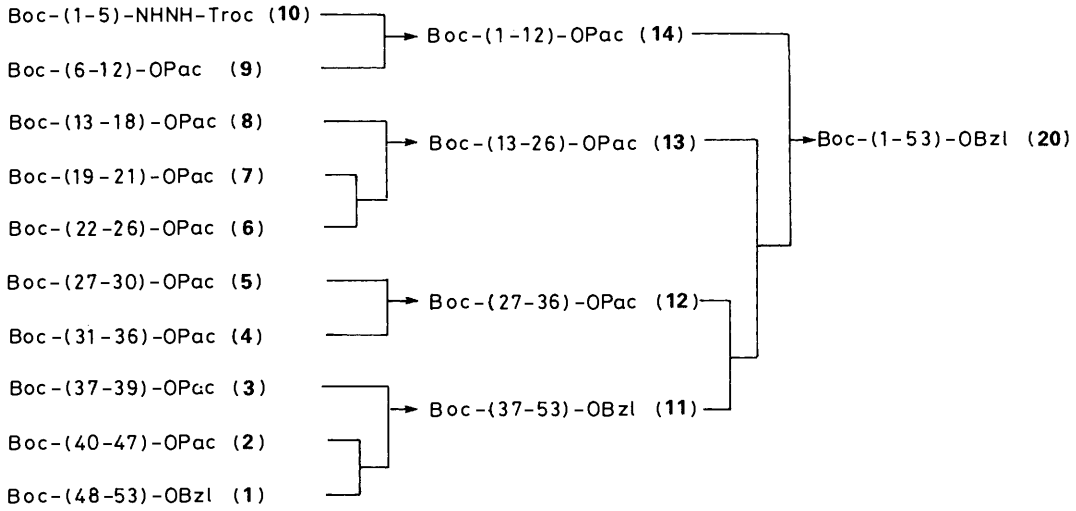
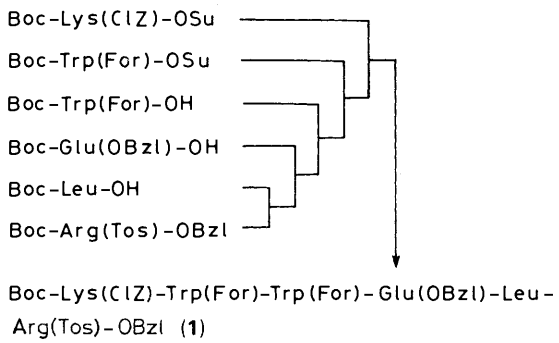


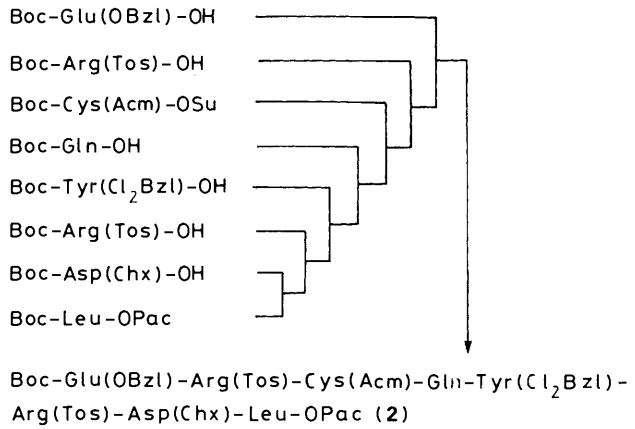
Figure 1. Structure of urogastrone (h-EGF)



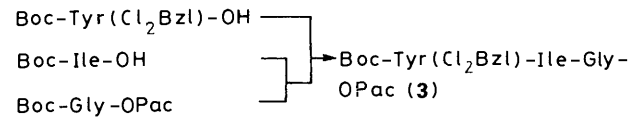
Scheme 1. Synthetic scheme for the protected urogastrone, Boc-(1—53)-OBzl (20)



Scheme 2. Synthetic scheme for the protected hexapeptide ester, Boc-(48—53)-OBzl (**1**)

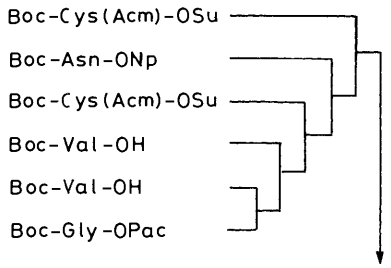


Scheme 3. Synthetic scheme for the protected octapeptide ester, Boc-(40—47)-OPac (**2**)



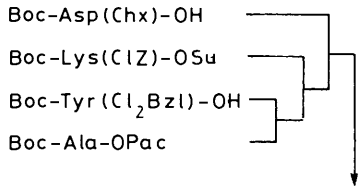
Scheme 4. Synthetic scheme for the protected tripeptide ester, Boc-(37—39)-OPac (**3**)

85% yields, respectively (Schemes 3 and 4). Segments (31—36) (**4**) and (27—30) (**5**) were synthesized from Boc-Gly-OPac and Boc-Ala-OPac, respectively. In these syntheses, the ^{35}Val and ^{34}Val were introduced by the mixed anhydride method, the ^{33}Cys, ^{31}Cus, and ^{28}Lys residues were introduced by the Su active ester method, the ^{32}Asn was introduced by the Np active ester method, and the ^{29}Tyr and ^{27}Asp residues were attached by the WSCD–HOBt method. Segment (31—36) (**4**) was obtained in 49% yield and segment (27—30) (**5**) in 76% yield (Schemes 5 and 6).



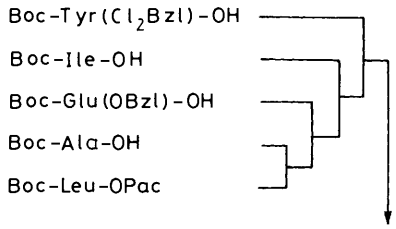
Boc-Cys(Acm)-Asn-Cys(Acm)-Val-Val-Gly-OPac (**4**)

Scheme 5. Synthetic scheme for the protected hexapeptide ester, Boc-(31—36)-OPac (**4**)



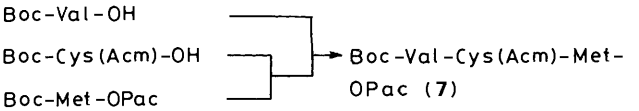
Boc-Asp(Chx)-Lys(ClZ)-Tyr(Cl_2Bzl)-Ala-OPac (**5**)

Scheme 6. Synthetic scheme for the protected tetrapeptide ester, Boc-(27—30)-OPac (**5**)

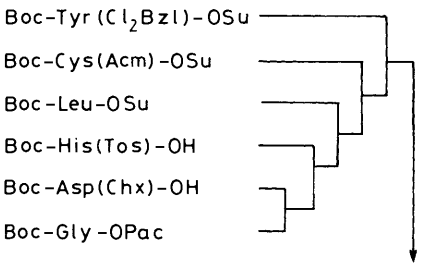


Boc-Tyr(Cl_2Bzl)-Ile-GLu(OBzl)-Ala-Leu-OPac (**6**)

Scheme 7. Synthetic scheme for the protected pentapeptide ester, Boc-(22—26)-OPac (**6**)



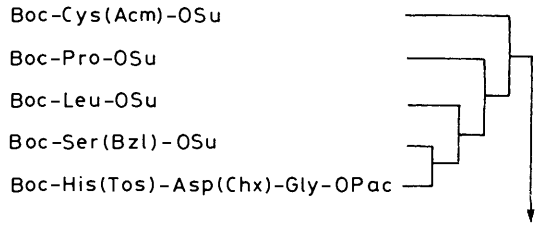
Scheme 8. Synthetic scheme for the protected tripeptide ester, Boc-(19—21)-OPac (**7**)



Boc-Tyr(Cl_2Bzl)-Cys(Acm)-Leu-His-Asp(Chx)-Gly-OPac (**8**)

Scheme 9. Synthetic scheme for the protected hexapeptide ester, Boc-(13—18)-OPac (**8**)

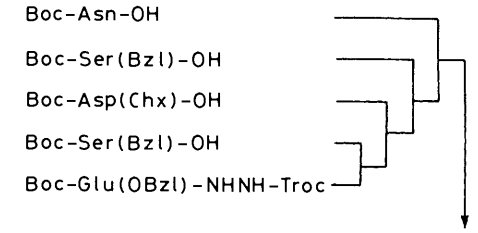
Segments (22—26) (**6**) and (19—21) (**7**) were obtained from Boc-Leu-OPac and Boc-Met-OPac, respectively. All the amino acid residues in these segments were introduced by the WSCD–HOBt method. The yields of these segments were 65 and 43% (Schemes 7 and 8). Segment (13—18) (**8**) was prepared from Boc-Gly-OPac in 59% yield by using the mixed anhydride method for the ^{17}Asp and ^{16}His residues and the Su active ester method for the ^{15}Leu, ^{14}Cys, and ^{13}Tyr residues (Scheme 9). Segment (6—12) (**9**) was prepared starting from Boc-His(Tos)-Asp(Chx)-Gly-OPac used for the preparation of segment (13—18) (**8**). In this synthesis, all the amino acid residues were introduced by the Su active ester method (Scheme 10). The yield



Boc-Cys(Acm)-Pro-Leu-Ser(Bzl)-His-Asp(Chx)-Gly-OPac (**9**)

Scheme 10. Synthetic scheme for the protected heptapeptide ester, Boc-(6—12)-OPac (**9**)

was 33%. Segment (1—5) (**10**) was prepared from Boc-Glu-(OBzl)-NHNH-Troc in 75% yield by using the WSCD–HOBt method for the ^{3}Asp, ^{2}Ser, and ^{1}Asn residues and the mixed anhydride method for the ^{4}Ser residue (Scheme 11).



Boc-Asn-Ser(Bzl)-Asp(Chx)-Ser(Bzl)-Glu(OBzl)-NHNH-Troc (**10**)

Scheme 11. Synthetic scheme for the protected pentapeptide ester, Boc-(1—5)-NHNH-Troc (**10**)

These 10 segments were further coupled to make four larger fragments as indicated in Scheme 1. After removal of the Boc group with TFA–anisole and the Pac and Troc groups with Zn–AcOH, the condensation reactions were performed in DMF using the WSCD–HOBt method for fragments (**11**), (**12**), and (**13**) and the azide method for fragment (**14**).

For the preparation of fragment (**11**), segment (40—47) was first coupled, after removal of the Pac group, to the Boc-deprotected segment (48—53) by the WSCD–HOBt method. In order to check racemization, which might occur during the fragment condensation between ^{47}Leu and ^{48}Lys, the diastereoisomer [D-^{47}Leu]-Boc-(40—53)-OBzl was prepared by Sakakibara's method,[18] deprotected with HF, and subjected to h.p.l.c. analysis in comparison with the above condensation product. This analysis showed that the racemized product was not contained in the condensation product as can be seen in Figure 2. Therefore, the product (40—53) was subjected to a further coupling reaction to segment (37—39) (**3**). After removal of the Boc group in segment (40—53) and the Pac group in segment (37—39) (**3**), both were coupled by the WSCD–HOBt method to give, in 81% yield, fragment (**11**), whose purity was secured by amino acid analysis (see Table).

Fragment (**12**) was synthesized from segments (31—36) (**4**) and (27—30) (**5**) by a similar fragment condensation (Scheme 1). After removal of the Boc group in segment (31—36) (**4**) and the Pac group in segment (27—30) (**5**), both were condensed by the WSCD–HOBt method. The product (27—36) (**12**) was also subjected to analysis for racemization and found not to contain the diastereoisomer [D-^{30}Ala]-(27—36) as shown in Figure 2. The yield of fragment (**12**) was 88% and the amino acid analysis was shown in the Table.

Table. Amino acid analysis of protected urogastrone and intermediates. The number in parentheses gives the theoretical amino acid ratio in each protected peptide fragment

Position / Residue	1—12 (**14**)	13—26 (**13**)	27—36 (**12**)	37—53 (**11**)	27—53 (**18**)	13—53 (**19**)	1—53 (**20**)
Asp	3.28 (3)	0.99 (1)	1.93 (2)	1.12 (1)	3.28 (3)	4.14 (4)	7.54 (7)
Ser	2.89 (3)						2.56 (3)
Glu	1.16 (1)	0.99 (1)		3.16 (3)	3.08 (3)	4.05 (4)	5.09 (5)
Pro	1.02 (1)						1.26 (1)
Gly	1.01 (1)	0.99 (1)	1.00 (1)	0.98 (1)	2.07 (2)	3.04 (3)	4.13 (4)
Ala		1.34 (1)	1.01 (1)		1.58 (1)	3.76 (2)	4.12 (4)
Val		0.98 (1)	1.65 (2)		2.08 (2)	2.93 (3)	2.93 (3)
Met		0.85 (1)				0.82 (1)	0.80 (1)
Ile		0.97 (1)		0.90 (1)	0.88 (1)	1.89 (2)	1.85 (2)
Leu	1.00 (1)	2.00 (2)		2.00 (2)	2.00 (2)	4.00 (4)	5.00 (5)
Tyr		1.93 (2)	0.95 (1)	2.00 (2)	3.05 (3)	4.90 (5)	4.80 (5)
Lys			0.94 (1)	0.99 (1)	2.07 (2)	1.94 (2)	1.90 (2)
His	1.10 (1)	1.12 (1)				1.08 (1)	2.14 (2)
Arg				3.17 (3)	3.03 (3)	2.89 (3)	2.85 (3)
Trp				0.96 (2)	0.88 (2)	0.79 (2)	0.82 (2)
Cys	0.08 (1)	0.18 (2)	0.88 (2)	0.42 (1)	1.01 (3)	0.30 (5)	0.45 (6)

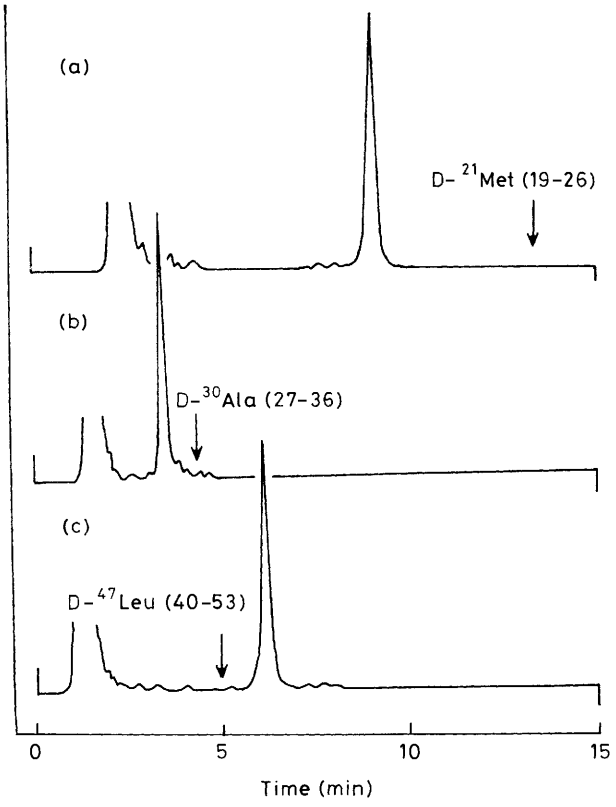


Figure 2. H.p.l.c. profile of deprotected fragments (19—26) (a), (27—36) (b), and, (40—53) (c). Column: Nucleosil $5C_{18}$ (4 × 150 mm); eluant: (a) 20% MeCN in 0.1M phosphate buffer (pH 4.8), (b) 14% MeCN in 0.1M phosphate buffer (pH 4.8), (c) 23% MeCN in 0.1M phosphate buffer (pH 4.8); flow rate: 1.0 ml/min; detection: absorbance at 210 nm

Fragment (**13**) was synthesized in a similar manner from segments (22—26) (**6**), (19—21) (**7**), and (13—18) (**8**) (Scheme 1). Removal of the Boc group in segment (22—26) (**6**) and the Pac group in segment (19—21) (**7**), followed by condensation of these deprotected products by the WSCD–HOBt method gave the larger segment (19—26) in 91% yield. Since there was a fear of racemization between ^{21}Met and ^{22}Tyr during the condensation reaction, h.p.l.c. analysis was carried out on the product in comparison with the diastereoisomer [D-^{21}Met]-(19—26) and the result showed that the product was pure as shown in Figure 2. Then, segment (13—18) (**8**) was coupled, after removal of the Pac group, to the Boc-removed segment (19—26) by the WSCD–HOBt method to give fragment (**13**) in 87% yield. The amino acid analysis is shown in the Table.

The synthesis of fragment (**14**) was accomplished by condensation of segments (6—12) (**9**) and (1—5) (**10**) using the azide method (Scheme 1). Removal of the Troc group in segment (1—5) (**10**), followed by condensation of the resulting product to the Boc-deprotected segment (6—12) by the Rudinger method [19] yielded in 90% yield fragment (**14**), whose purity was assessed by amino acid analysis as shown in the Table.

Having succeeded in the preparation of fragments (**11**), (**12**), (**13**), and (**14**), the stage was set to commence to make the full sequence of urogastrone. For this purpose, we first attempted to remove the Pac ester group in fragments (**12**), (**13**). and (**14**). Although the ester group in fragment (**14**) was readily deprotected by reduction with Zn–AcOH in DMF to give its free acid (**17**) in 88% yield, the application of this method to fragments (**12**) and (**13**) were unsuccessful owing to their poor solubility in DMF. We therefore examined another Zn reduction method and found that anthranilic acid–pyridine in a mixture of NMP and DMSO was effective for the removal of the Pac groups in fragments (**12**) and (**13**). Thus, the Pac ester in fragment (**12**) was cleaved in a 2:1:1 ratio of NMP–DMSO–pyridine at 50 °C for 2 h to give its free acid (**15**) in 86% yield (Scheme 12). The ester group in fragment (**13**) was removed in a 20:5:1 ratio of the same solvents at 35 °C for 6 h to give its free acid (**16**) in 92% yield (Scheme 13). The side reaction of the aminosuccinimide formation at the ^{17}Asp–^{18}Gly sequence in fragment (**13**) was suppressed by limiting the ratio of pyridine and controlling the reaction temperature.

Finally, the resulting free acids (**15**), (**16**), and (**17**) were in turn coupled to fragment (**11**), *via* (**18**) and (**19**) by the WSCD–HOBt method after removal of the Boc group at each successive stage (Scheme 14). Every coupling reaction was carried out by using a 0.1-fold excess of the acid component in NMP at 0—5 °C and the amino component was extinguished on t.l.c. The protected urogastrone (**20**) was thus obtained in 90% overall yield. The data of the amino acid analysis of the protected urogastrone (**20**) and the intermediates are listed in the Table.

We have been able to synthesize the fully protected urogastrone as described above. Removal of the protective

Boc-Asp(Chx)-Lys(ClZ)-Tyr(Cl_2Bzl)-Ala-Cys(Acm)-Asn-Cys(Acm)-Val-Val-Gly-OPac (**12**)

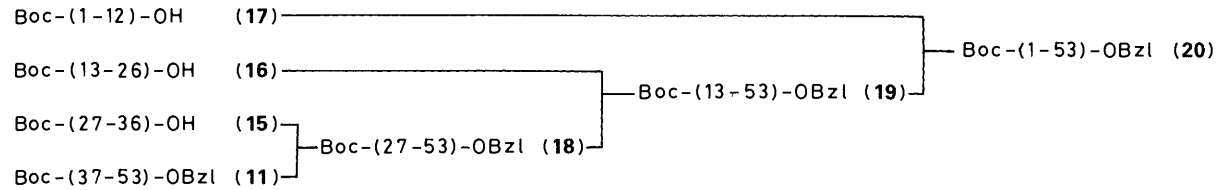
Zn-anthranilic acid (10 eqiv.) / NMP-DMSO-pyridine (2:1:1), 50 °C, 2 h → Boc-(27-36)-OH (**15**)

Scheme 12. Synthetic scheme for the protected decapeptide, Boc-(27— 36)-OH (**15**)

Boc-Tyr(Cl_2Bzl)-Cys(Acm)-Leu-His-Asp(Chx)-Gly-Val-Cys(Acm)-Met-Tyr(Cl_2Bzl)-Ile-Glu(OBzl)-Ala-Leu-OPac (**13**)

Zn-anthranilic acid (10 equiv. / NMP-DMSO-pyridine (20:5:1), 35 °C, 6 h → Boc-(13-26)-OH (**16**)

Scheme 13. Synthetic scheme for the protected tetradecapeptide, Boc- (13—26)-OH (**16**)



Scheme 14. Synthetic scheme for the protected tripentacontapeptide ester, Boc-(1—53)-OBzl (**20**)

groups and formation of the disulphide bonds are the subject of the following paper.

Experimental

M.p.s were measured on a Thomas-Hoover apparatus and are uncorrected. Optical rotations were determined on a JASCO DIP-140 polarimeter. Thin-layer chromatography (t.l.c.) was carried out on silica gel (Kieselgel $60F_{254}$, E. Merck): R_F values refer to the following solvent systems (v/v): R_{F1}, $CHCl_3$–MeOH–AcOH (8:1:1); R_{F2}, $CHCl_3$–MeOH–AcOH (8:2:1); R_{F3}, $CHCl_3$–MeOH–AcOH (95:5:3); R_{F4}, $CHCl_3$–MeOH–AcOEt (8:1:1); R_{F5}, $CHCl_3$–MeOH–ACOEt (8:2:1); R_{F6}, $CHCl_3$–MeOH–AcOEt (4:1:1); R_{F7}, benzene–acetone (4:1); R_{F8}, AcOEt–hexane (1:1); R_{F9}, AcOEt–hexane (1:2); R_{F10}, $CHCl_3$–MeOH (10:1); R_{F11}, $CHCl_3$–MeOH (9:1); R_{F12}, $CHCl_3$–AcOEt (1:1); R_{F13}, $CHCl_3$–AcOEt (2:1). Acid hydrolyses were performed in 6M HCl containing 5% phenol for 24 h. The amino acid compositions of the hydrolysates were determined with a Hitachi model 835 amino acid analyser and are not corrected for amino acid destruction. High-performance liquid chromatography (h.p.l.c.) was carried out on a Hitachi model 655 liquid chromatography system by using a column of Nucleosil $5C_{18}$ (4.0 × 150 mm) with u.v. detection at 210 nm. Solvent systems were as follows: eluant A, 20% MeCN in 0.1M phosphate buffer (pH 4.8); eluant B, 14% MeCN in 0.1M phosphate buffer (pH 4.8); eluant C, 23% MeCN in 0.1M phosphate buffer (pH 4.8).

General Procedures for Deprotection of the Boc Group.—*Deprotection procedure A.* Protected peptides were treated with TFA–anisole (9:1, v/v) with ice-bath cooling for 60 min. After evaporation of TFA, 4M HCl in dioxane (2 mol equiv.) was added to the residues and the mixtures were stirred for 10 min and concentrated under reduced pressure. The residues were triturated with dry ether and the resulting solids were filtered off, washed with dry ether, and dried *in vacuo.*

Deprotection procedure B. Protected peptides were treated with TFA–anisole (9:1, v/v) with ice-bath cooling for 60 min. After evaporation of TFA, the residues were dried over KOH pellets under reduced pressure.

General Procedure for Purification of Fully Protected Products.—*Procedure A.* When products were soluble in AcOEt, the products were extracted with AcOEt and the extracts were washed successively with 5% HCl, 1M aqueous $NaHCO_3$, and saturated brine, dried ($MgSO_4$), and concentrated under reduced pressure.

Procedure B. When products were insoluble in AcOEt, the crude products were precipitated by adding appropriate solvents. The resulting solids were filtered off, washed successively with 5% HCl, 1M aqueous $NaHCO_3$, water, and ether, and dried *in vacuo.*

Boc-Arg(Tos)-OBzl.—To a solution of Boc-Arg(Tos)-OH (43.3 g, 0.101 mol) and di-isopropylethylamine (22.9 ml, 0.131 mol) in DMF (400 ml) was added a solution of benzyl bromide (24.9 g, 0.145 mol) in DMF (50 ml) with ice-bath cooling. The reaction mixture was stirred at the same temperature for 1 h and at room temperature overnight. After evaporation of the solvent, the product was subjected to purification procedure A to afford the ester as an amorphous solid (57.3 g, 97.3%), $[\alpha]_D^{23}$ −10.0° (*c* 1.0 in DMF), R_{F6} 0.85 (Found: C, 57.15; H, 6.35; N, 6.05. $C_{25}H_{34}N_4O_6S$ requires C, 57.90; H, 6.61; N, 6.18%).

Boc-Leu-Arg(Tos)-OBzl.—HCl·H-Arg(Tos)-OBzl [prepared from Boc-Arg(Tos)-OBzl (24.7 g, 47.6 mmol) according to deprotection procedure A], Boc-Leu-OH (12.1 g, 52.4 mmol), and HOBt (7.08 g, 52.4 mmol) were dissolved in CH_2Cl_2 (200 ml), and WSCD (8.12 g, 52.4 mmol) was added with ice-bath cooling. Stirring was continued for 3 h at the same temperature and an additional 1 h at room temperature, after which the solution was concentrated under reduced pressure. The product was subjected to purification procedure A to afford the protected dipeptide ester as an amorphous solid (29.8 g, 99.1%), m.p. 68—70 °C, $[\alpha]_D^{23}$ −15.8° (*c*, 1.0 in DMF), R_{F11} 0.52 (Found: C, 59.0; H, 7.15; N, 10.3. $C_{31}H_{45}N_5O_7S$ requires C, 58.93; H, 7.18; N, 11.09%).

Boc-Glu(OBzl)-Leu-Arg(Tos)-OBzl.—TFA·H-Leu-Arg-(Tos)-OBzl [prepared from Boc-Leu-Arg(Tos)-OBzl (12.61 g, 19.96 mmol) according to deprotection procedure B], Boc-Glu(OBzl)-OH (7.35 g, 22.4 mmol), and HOBt (2.76 g, 20.4 mmol) were dissolved in DMF (120 ml), and WSCD (3.17 g, 20.4 mmol) was added with ice-bath cooling and the mixture was stirred at 5 °C overnight. The mixture was then evaporated, and the residue was subjected to purification procedure A and

crystallized from IPE to give the protected tripeptide ester (14.3 g, 83.9%), m.p. 70—73 °C, $[\alpha]_D^{23}$ −11.4° (*c*, 1.0 in DMF), R_{F6} 0.68 (Found: C, 59.0; H, 7.15; N, 10.3. $C_{43}H_{58}N_6O_{10}S$ requires C, 60.69; H, 6.87; N, 9.88%).

Boc-Trp(For)-Glu(OBzl)-Leu-Arg(Tos)-OBzl.—HCl·H-Glu(OBzl)-Leu-Arg(Tos)-OBzl [prepared from Boc-Glu(OBzl)-Leu-Arg(Tos)-OBzl (10.0 g, 11.75 mmol) according to deprotection procedure A], Boc-Trp(For)-OH (4.30 g, 13.0 mmol), and HOBt (1.90 g, 14.1 mmol) were dissolved in DMF (40 ml), and WSCD (2.03 g, 13.0 mmol) was added at −10 °C. The mixture was then stirred at the same temperature for 1.5 h and at 5 °C for additional 6 h. The mixture was evaporated and the product was subjected to purification procedure A, followed by silica gel column chromatography (50 g) eluting with $CHCl_3$–AcOEt (1:1). The product was crystallized from ether to give the protected tetrapeptide ester (8.50 g, 68.2%), m.p. 96—100 °C, $[\alpha]_D^{23}$ −11.1° (*c*, 1.0 in DMF), R_{F6} 0.65 (Found: C, 61.1; H, 6.35; N, 10.2. $C_{55}H_{68}N_8O_{12}S$ requires C, 62.01; H, 6.43; N, 10.52%).

Boc-Trp(For)-Trp(For)-Glu(OBzl)-Leu-Arg(Tos)-OBzl.—HCl·H-Trp(For)-Glu(OBzl)-Leu-Arg(Tos)-OBzl [prepared from Boc-Trp(For)-Glu(OBzl)-Leu-Arg(Tos)-OBzl (5.97 g, 5.60 mmol) according to deprotection procedure A] was dissolved in DMF (80 ml) containing NMM (0.56 g, 5.60 mmol). Boc-Trp(For)-OSu (2.40 g, 5.60 mmol) was added to the above solution and the mixture was stirred at 5 °C overnight. The solution was then concentrated under reduced pressure and the residue was subjected to purification procedure A. Crystallization from ether afforded the protected pentapeptide ester (6.50 g, 90.7%), m.p. 157—160 °C, $[\alpha]_D^{23}$ −13.0° (*c*, 1.0 in DMF), R_{F6} 0.55 (Found: C, 62.65; H, 5.95, N, 10.8. $C_{67}H_{78}N_{10}O_{14}S$ requires C, 62.90; H, 6.14; N, 10.95%).

Boc-Lys(ClZ)-Trp(For)-Trp(For)-Glu(OBzl)-Leu-Arg(Tos)-OBzl (**1**).—TFA·H-Trp(For)-Trp(For)-Glu(OBzl)-Leu-Arg(Tos)-OBzl [prepared from Boc-Trp(For)-Trp(For)-Glu(OBzl)-Leu-Arg(Tos)-OBzl (6.00 g, 4.69 mmol) according to deprotection procedure B] was dissolved in DMF (100 ml) containing TEA (475 mg, 4.69 mmol). Boc-Lys(ClZ)-OSu (2.44 g, 4.77 mmol) was added to the above solution with ice-bath cooling and the mixture was stirred at 5 °C overnight. The mixture was evaporated under reduced pressure and the residue was subjected to purification procedure B to yield the protected hexapeptide ester (6.20 g, 83.9%), m.p. 183—192 °C, $[\alpha]_D^{23}$ −15.3° (*c*, 1.0 in DMF), R_{F6} 0.47 (Found: C, 61.05; H, 6.05; N, 10.45. $C_{81}H_{95}Cl_1N_{12}O_{17}S$ requires C, 61.72; H, 6.07; N, 10.66%).

Boc-Leu-OPac.—To a solution of Boc-Leu-OH·H_2O (100 g, 0.401 mol) and Et_3N (40.5 g, 0.401 mol) in DMF (1 400 ml) was added phenacyl bromide (79.8 g, 0.401 mol) with ice-bath cooling. The reaction mixture was stirred at the same temperature for 2 h and at room temperature overnight. The solution was then filtered and concentrated under reduced pressure and, the product was subjected to purification procedure A. The residue was crystallized from hexane to give the ester (124.8 g, 89.1%), m.p. 71—72 °C, $[\alpha]_D^{23}$ −36.6° (*c*, 1.0 in DMF), R_{F9} 0.49 (Found: C, 65.4; H, 7.7; N, 4.1. $C_{19}H_{27}NO_5$ requires C, 65.31; H, 7.79; N, 4.01%).

Boc-Asp(Chx)-Leu-OPac.—HCl·H-Leu-OPac [prepared from Boc-Leu-OPac (3.44 g, 9.85 mmol) according to deprotection procedure A] was neutralized with NMM (0.996 g, 9.85 mmol) in CH_2Cl_2 (30 ml) at −50 °C and added to a solution of the mixed anhydride of Boc-Asp(Chx)-OH (3.11 g, 9.85 mmol) [prepared by treating the latter with isobutyl chlorocarbonate (1.35 g, 9.85 mmol) in the presence of NMM (0.996 g, 9.85 mmol) in CH_2Cl_2 (30 ml)]. After being stirred for 3 h at the same temperature, the reaction mixture was evaporated under reduced pressure and the residue was subjected to purification procedure A. The product was crystallized from IPE to give the protected dipeptide ester (4.80 g, 89.1%), m.p. 72—74 °C, $[\alpha]_D^{23}$ −29.5° (*c*, 1.0 in DMF), R_{F8} 0.63 (Found: C, 63.75; H, 7.45; N, 5.2. $C_{29}H_{42}N_2O_8$ requires C, 63.72; H, 7.74; N, 5.12%).

Boc-Arg(Tos)-Asp(Chx)-Leu-OPac.—TFA·H-Asp(Chx)-Leu-OPac [prepared from Boc-Asp(Chx)-Leu-OPac (2.50 g, 4.57 mmol) according to deprotection procedure B] was neutralized with NMM (0.460 g, 4.57 mmol) in CH_2Cl_2 (30 ml) at −50 °C and added to a solution of the mixed anhydride of Boc-Arg(Tos)-OH (1.78 g, 4.16 mmol) [prepared by treating the latter with isobutyl chlorocarbonate (0.570 g, 4.16 mmol) in the presence of NMM (0.420 g, 4.16 mmol) in THF (30 ml)]. After being stirred for 3 h at the same temperature, the reaction mixture was evaporated under reduced pressure and the product was purified by purification procedure A. The product was crystallized from IPE to give the protected tripeptide ester (3.05 g, 77.9%), m.p. 73—78 °C, $[\alpha]_D^{23}$ −19.8° (*c*, 1.0 in DMF), R_{F4} 0.65 (Found: C, 58.8; H, 7.1; N, 10.7. $C_{42}H_{60}N_6O_{11}S$ requires C, 58.86; H, 7.06; N, 9.81%).

Boc-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac.—HCl·H-Arg(Tos)-Asp(Chx)-Leu-OPac [prepared from Boc-Arg(Tos)-Asp-(Chx)-Leu-OPac (9.08 g, 10.6 mmol) according to deprotection procedure A], Boc-Tyr(Cl_2Bzl)-OH (5.11 g, 11.6 mmol), and HOBt (1.80 g, 11.6 mmol) were dissolved in DMF (150 ml), and WSCD (1.71 g, 12.7 mmol) was added with ice-bath cooling and the reaction mixture was stirred at the same temperature overnight. After evaporation of the mixture, the residue was subjected to purification procedure A and the product was crystallized from AcOEt–ether to afford the protected tetrapeptide ester (10.9 g, 86.5%), m.p. 103—106 °C, $[\alpha]_D^{23}$ −16.1° (*c*, 1.0 in DMF), R_{F4} 0.57 (Found: C, 58.5; H, 6.1; N, 8.2. $C_{58}H_{73}Cl_2N_7O_{13}S$ requires C, 59.08; H, 6.24; N, 8.31%).

Boc-Gln-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac.—HCl·H-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac [prepared from Boc-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac (14.1 g, 12.0 mmol) according to deprotection procedure A], Boc-Gln-OH (3.54 g, 14.3 mmol), and HOBt (2.11 g, 15.6 mmol) were dissolved in DMF (150 ml), and WSCD (2.23 g, 14.3 mmol) was added with ice-bath cooling. After being stirred at the same temperature overnight, the reaction mixture was concentrated under reduced pressure. The product was subjected to purification procedure A, followed by crystallization from AcOEt–ether to give the protected pentapeptide ester (15.2 g, 96.8%), m.p. 110—115 °C, $(\alpha]_D^{23}$ −16.7° (*c*, 1.0 in DMF), R_{F5} 0.56 (Found: C, 56.85; H, 6.15; N, 9.4. $C_{63}H_{81}Cl_2N_9O_{15}S$ requires C, 57.9; H, 6.25; N, 9.64%).

Boc-Cys(Acm)-Gln-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac.—TFA·H-Gln-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac [prepared from Boc-Gln-Tyr(Cl_2Bzl)-Arg(Tos)-Asp-(Chx)-Leu-OPac (13.7 g, 10.5 mmol) according to deprotection procedure B], was dissolved in DMF (150 ml) containing Et_3N (1.06 g, 10.5 mmol). Boc-Cys(Acm)-OSu (4.48 g, 11.5 mmol) was added to the above solution with ice-bath cooling and the reaction mixture was stirred at 5 °C overnight. After evaporation of the solvent, the residue was subjected to purification procedure A, followed by crystallization from ether to give the protected hexapeptide ester (14.4 g, 92.8%), m.p. 127—129 °C, $[\alpha]_D^{23}$ −16.6° (*c*, 1.0 in DMF), R_{F6} 0.48 (Found: C, 55.85; H, 6.05; N, 10.2. $C_{69}H_{91}Cl_2N_{11}O_{17}S$ requires C, 55.94; H, 6.19; N, 10.40%).

Boc-Arg(Tos)-Cys(Acm)-Gln-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac.—TFA·H-Cys(Acm)-Gln-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac [prepared from Boc-Cys(Acm)-Gln-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac (5.90 g, 3.98 mmol) according to deprotection procedure B], Boc-Arg(Tos)-OH (1.88 g, 4.38 mmol), and HOBt (0.650 g, 4.78 mmol) were dissolved in DMF (60 ml), and WSCD (0.680 g, 4.38 mmol) was added with ice-bath cooling. After being stirred at 5 °C overnight, the reaction mixture was concentrated under reduced pressure and the product was subjected to purification procedure A, followed by crystallization from THF–ether to give the protected heptapeptide ester (6.05 g, 84.8%), m.p. 137—140 °C, $[\alpha]_D^{23}$ −13.5° (*c*, 1.0 in DMF), R_{F6} 0.56 (Found: C, 54.5; H, 5.6; N, 9.1. $C_{82}H_{109}Cl_2N_{15}O_{20}S_3$ requires C, 54.96; H, 6.13; N, 11.72%).

Boc-Glu(OBzl)-Arg(Tos)-Cys(Acm)-Gln-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac (**2**).—TFA·H-Arg(Tos)-Cys(Acm)-Gln-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac [prepared from Boc-Arg(Tos)-Cys(Acm)-Gln-Tyr(Cl_2Bzl)-Arg(Tos)-Asp(Chx)-Leu-OPac (3.83 g, 2.14 mmol) according to deprotection procedure B], Boc-Glu(OBzl)-OH (0.866 g, 2.57 mmol), and HOBt (0.376 g, 2.78 mmol) were dissolved in DMF (40 ml), and WSCD (0.399 g, 2.57 mmol) was added with ice-bath cooling. After being stirred at the same temperature overnight, the reaction mixture was evaporated under reduced pressure and the product was subjected to purification procedure B to yield the protected octapeptide ester (4.03 g, 93.6%), m.p. 178–180 °C, $[\alpha]_D^{23}$ −30.5° (*c*, 1.0 in DMF), R_{F6} 0.52 (Found: C, 55.15; H, 6.05; N, 11.0. $C_{94}H_{122}Cl_2N_{16}O_{23}S_3$ requires C, 56.14; H, 6.11, N, 11.14%).

Boc-Gly-OPac.—To a solution of Boc-Gly-OH (17.5 g, 0.100 mmol) and Et_3N (10.1 g, 0.100 mmol) in AcOEt (200 ml) was added phenacyl bromide (19.9 g, 0.100 mmol) with ice-bath cooling and the reaction mixture was stirred at the same temperature overnight. The solution was then filtered and concentrated under reduced pressure, the product was subjected to purification procedure A and crystallized from hexane to give the ester (26.4 g, 90.4%), m.p. 58—59 °C, R_{F9} 0.40 (Found: C, 61.4; H, 6.5; N, 4.75. $C_{15}H_{19}NO_5$ requires C, 61.42; H, 6.53; N, 4.78%).

Boc-Ile-Gly-OPac.—HCl·H-Gly-OPac [prepared from Boc-Gly-OPac (2.55 g, 8.71 mmol) according to deprotection procedure A] was neutralized with NMM (0.881 g, 8.71 mmol) in CH_2Cl_2 (20 ml) at −50 °C and allowed to react with the mixed anhydride of Boc-Ile-OH (2.01 g, 8.71 mmol) [prepared by treating the latter with isobutyl chlorocarbonate (1.91 g, 8.71 mmol) in the presence of NMM (0.881 g, 8.71 mmol) in CH_2Cl_2 (20 ml)]. After being stirred at the same temperature for 2 h, the reaction mixture was concentrated under reduced pressure and the product was subjected to purification procedure A and crystallized from AcOEt–hexane to give the protected dipeptide ester (3.25 g, 91.8%), m.p. 135—137 °C, $[\alpha]_D^{23}$ −14.1° (*c*, 1.0 in DMF), R_{F13} 0.46 (Found: C, 62.15; H, 7.4; N, 6.9. $C_{21}H_{30}N_2O_6$ requires C, 62.05; H, 7.44, N, 6.89%).

Boc-Tyr(Cl_2Bzl)-Ile-Gly-OPac (**3**).—HCl·H-Ile-Gly-OPac [prepared from Boc-Ile-Gly-OPac (2.08 g, 5.11 mmol) according to deprotection procedure A], Boc-Tyr(Cl_2Bzl)-OH (2.25 g, 5.11 mmol), and HOBt (0.759 g, 5.62 mmol) were dissolved in DMF (60 ml), and WSCD (0.793 g, 5.11 mmol) was added with ice-bath cooling. After being stirred at the same temperature overnight, the reaction mixture was concentrated under reduced pressure and the product was subjected to purification procedure A, followed by crystallization from AcOEt–hexane to give the protected tripeptide ester (3.45 g, 92.7%), m.p. 187—188 °C, $[\alpha]_D^{23}$ −18.9° (*c*, 1.0 in DMF), R_{F11} 0.54 (Found: C, 60.75; H, 6.2; N, 5.7. $C_{37}H_{43}Cl_2N_3O_8$ requires C, 60.99; H, 5.95; N, 5.77%).

Boc-Val-Gly-OPac.—HCl·H-Gly-OPac [prepared from Boc-Gly-OPac (4.39 g, 15.0 mmol) according to deprotection procedure A] was neutralized with NMM (1.52 g, 15.0 mmol) in CH_2Cl_2 (30 ml) at −50 °C and allowed to react with the mixed anhydride of Boc-Val-OH (3.26 g, 15.0 mmol) [prepared by treating the latter with isobutyl chlorocarbonate (2.05 g, 15.0 mmol) in the presence of NMM (1.52 g, 15.0 mmol) in CH_2Cl_2 (30 ml)]. After being stirred for 3 h at the same temperature, the reaction mixture was concentrated under reduced pressure and the product was subjected to purification procedure A and crystallized from AcOEt–hexane to give the protected dipeptide ester (5.30 g, 90.0%), m.p. 134—136 °C, $[\alpha]_D^{23}$ −28.7° (*c*, 1.0 in DMF), R_{F8} 0.33 (Found: C, 61.2; H, 7.0, N, 7.15. $C_{20}H_{28}N_2O_6$ requires C, 61.21; H, 7.19; N, 7.14%).

Boc-Val-Val-Gly-OPac.—HCl·H-Val-Gly-OPac [prepared from Boc-Val-Gly-OPac (3.53 g, 9.00 mmol) according to deprotection procedure A] was neutralized with NMM (0.910 g, 9.00 mmol) in CH_2Cl_2 (60 ml) at −20 °C and allowed to react with the mixed anhydride of Boc-Val-OH (1.96 g, 9.00 mmol) [prepared by treating the latter with isobutyl chlorocarbonate (1.23 g, 9.00 mmol) in the presence of NMM (0.910 g, 9.00 mmol) in CH_2Cl_2 (20 ml)]. After being stirred for 3 h at the same temperature, the reaction mixture was concentrated under reduced pressure, and the residue was treated with ether. The resulting powder was subjected to purification procedure B, followed by recrystallization from $CHCl_3$–ether to give the protected tripeptide ester (3.59 g, 81.1%), m.p. 193—194 °C, $[\alpha]_D^{23}$ −18.0° (*c*, 1.0 in DMF), R_{F12} 0.45 (Found: C, 61.0; H, 7.45; N, 8.55. $C_{25}H_{37}N_3O_7$ requires C, 61.08; H, 7.59; N, 8.55%).

Boc-Cys(Acm)-Val-Val-Gly-OPac.—HCl·H-Val-Val-Gly-OPac [prepared from Boc-Val-Val-Gly-OPac (2.49 g, 5.07 mmol) according to deprotection procedure A] was dissolved in DMF (40 ml) containing Et_3N (0.513 g, 5.07 mmol) and Boc-Cys(Acm)-OSu (2.37 g, 6.08 mmol) was added with ice-bath cooling. After being stirred at 5 °C overnight, the reaction mixture was concentrated under reduced pressure, and the product was subjected to purification procedure A. Recrystallization from DMF–ether gave the protected tetrapeptide ester (3.06 g, 90.7%), m.p. 187—190 °C, $[\alpha]_D^{23}$ −53.1° (*c*, 1.0 in DMF), R_{F2} 0.82 (Found: C, 55.8; H, 7.1; N, 10.15. $C_{31}H_{47}N_5O_9S$ requires C, 55.93; H, 7.12; N, 10.52%).

Boc-Asn-Cys(Acm)-Val-Val-Gly-OPac.—TFA·H-Cys(Acm)-Val-Val-Gly-OPac [prepared from Boc-Cys(Acm)-Val-Val-Gly-OPac (5.02 g, 7.54 mmol) according to deprotection procedure B] was dissolved in DMF (70 ml) containing NMM (0.762 g, 7.53 mmol), and Boc-Asn-ONp (3.99 g, 11.3 mmol) was added at 5 °C. After being stirred at the same temperature for 72 h, the reaction mixture was concentrated under reduced pressure, and the residue was treated with AcOEt. The resulting powder was washed with MeOH to give the protected peptapeptide ester (4.65 g, 79.1%), m.p. 211—212 °C, $[\alpha]_D^{23}$ −24.7° (*c*, 1.0 in DMF), R_{F2} 0.57 (Found: C, 53.55; H, 6.75; N, 12.45. $C_{35}H_{53}N_7O_{11}S$ requires C, 53.90; H, 6.85; N, 12.57%).

Boc-Cys(Acm)-Asn-Cys(Acm)-Val-Val-Gly-OPac (**4**).—TFA·H-Asn-Cys(Acm)-Val-Val-Gly-OPac [prepared from Boc-Asn-Cys(Acm)-Val-Val-Gly-OPac (4.52 g, 5.80 mmol) according to deprotection procedure B] was dissolved in DMF (100 ml) containing NMM (0.606 g, 5.99 mmol) and Boc-Cys(Acm)-OSu (3.11 g, 7.99 mmol) was added at room temperature. After

being stirred at the same temperature overnight, the reaction mixture was concentrated under reduced pressure, and the residue was treated with AcOEt. The resulting precipitate was washed with MeOH to give the protected hexapepetide ester (5.19 g, 93.9%), m.p. 216—223 °C, $[\alpha]_D^{23}$ −21.4° (*c*, 1.0 in DMF), R_{F2} 0.46 (Found: C, 51.1; H, 6.55; N, 13.0. $C_{41}H_{63}N_9O_{13}S_2$ requires C, 51.61; H, 6.66; N, 13.21%).

Boc-Ala-OPac.—To a solution of Boc-Ala-OH (18.9 g, 0.100 mol) and Et_3N (10.1 g, 0.100 mol) in AcOEt (200 ml) was added phenacyl bromide (19.9 g, 0.100 mol) with ice-bath cooling and the reaction mixture was stirred at the same temperature overnight. The solution was then filtered and concentrated under reduced pressure, and the product was subjected to purification procedure A, and crystallized from hexane to give the ester (28.7 g, 93.5%), m.p. 124—125 °C, $[\alpha]_D^{23}$ −44.8° (*c*, 1.0 in DMF), R_{F9} 0.53 (Found: C, 62.5; H, 6.85; N, 4.5. $C_{16}H_{21}NO_3$ requires C, 62.53; H, 6.89; N, 4.56%).

Boc-Tyr(Cl$_2$Bzl)-Ala-OPac.—HCl·H-Ala-OPac [prepared from Boc-Ala-OPac (4.61 g, 15.0 mmol) according to deprotection procedure A], Boc-Tyr(Cl_2Bzl)-OH (6.60 g, 15.0 mmol), and HOBt (2.03 g, 15.0 mmol) were dissolved in DMF (60 ml), and WSCD (2.33 g, 15.0 mmol) was added at −10 °C. After being stirred at the same temperature for 5 h, the reaction mixture was concentrated under reduced pressure and the product was subjected to purification procedure A, followed by crystallization from ether to give the protected dipeptide ester (8.26 g, 87.5%), m.p. 146—148 °C, $[\alpha]_D^{23}$ −11.9° (*c*, 1.0 in DMF), R_{F7} 0.40 (Found: C, 57.85; H, 5.25; N, 4.25. $C_{32}H_{34}Cl_2N_2O_7 \cdot 2H_2O$ requires C, 57.75; H, 5.45; N, 4.21%).

Boc-Lys(ClZ)-Tyr(Cl$_2$Bzl)-Ala-OPac.—TFA·H-Tyr(Cl_2-Bzl)-Ala-OPac (prepared from Boc-Tyr(Cl_2Bzl)-Ala-OPac (3.15 g, 5.00 mmol) according to deprotection procedure B] was dissolved in DMF (60 ml) containing NMM (0.465 g, 4.60 mmol) and Boc-Lys(ClZ)-OSu (2.35 g, 4.60 mmol) was added with ice-bath cooling. After being stirred at 5 °C overnight, the reaction mixture was concentrated under reduced pressure and the residue was treated with ether to give the protected tripeptide ester (4.05 g, 97.4%), m.p. 190—191 °C, $[\alpha]_D^{23}$ −19.3° (*c*, 1.0 in DMF), R_{F3} 0.45 (Found: C, 59.65; H, 5.55; N, 6.05. $C_{46}H_{51}Cl_3N_4O_{10}$ requires C, 59.65; H, 5.55; N, 6.05%).

Boc-Asp(Chx)-Lys(ClZ)-Tyr(Cl$_2$Bzl)-Ala-OPac (**5**).—HCl·H-Lys(ClZ)-Tyr(Cl_2Bzl)-Ala-OPac [prepared from Boc-Lys-(ClZ)-Tyr(Cl_2Bzl)-Ala-OPac (0.463 g, 0.500 mmol) according to deprotection procedure A], Boc-Asp(Chx)-OH (0.189 g, 0.600 mmol), and HOBt (81.0 mg, 0.600 mmol) were dissolved in DMF (10 ml), and WSCD (93 mg, 0.600 mmol) was added with ice-bath cooling. After being stirred at 5 °C overnight, the reaction mixture was concentrated under reduced pressure, and the product was subjected to purification procedure A, followed by crystallization from ether to give the protected tetrapeptide ester (500 mg, 89.0%), m.p. 163—166 °C, $[\alpha]_D^{23}$ −22.7° (*c*, 1.0 in DMF), R_{F3} 0.50 (Found: C, 59.45; H, 5.8; N, 6.2. $C_{56}H_{66}Cl_3N_5O_{13}$ requires C, 59.87; H, 5.92; N, 6.23%).

Boc-Ala-Leu-OPac.—HCl·H-Leu-OPac [prepared from Boc-Leu-OPac (9.50 g, 27.2 mmol) according to deprotection procedure A], Boc-Ala-OH (5.67 g, 30.0 mmol), and HOBt (4.41 g, 32.6 mmol) were dissolved in DMF (150 ml), and WSCD (4.64 g, 30.0 mmol) was added at −20 °C. After being stirred at the same temperature for 2 h, the reaction mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by silica gel column chromatography (220 g) eluting with $CHCl_3$ to give the protected dipeptide ester as an oil (9.37 g, 81.8%), $[\alpha]_D^{23}$ −51.46° (*c*, 1.0 in DMF), R_{F4} 0.76 (Found: C, 62.15; H, 7.75; N, 6.55. $C_{22}H_{32}N_2O_6$ requires C, 62.84; H, 7.67; N, 6.66%).

Boc-Glu(OBzl)-Ala-Leu-OPac.—HCl·H-Ala-Leu-OPac [prepared from Boc-Ala-Leu-OPac (9.18 g, 21.8 mmol) according to deprotection procedure A], Boc-Glu(OBzl)-OH (8.09 g, 24.0 mmol), and HOBt (3.53 g, 26.1 mmol) were dissolved in DMF (150 ml), and WSCD (3.72 g, 24.0 mmol) was added with ice-bath cooling. After being stirred at the same temperature for 2 h, the reaction mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by crystallization from IPE to give the protected tripeptide ester (12.4 g, 88.9%), m.p. 110—112 °C, $[\alpha]_D^{23}$ −51.6° (*c*, 1.0 in DMF), R_{F6} 0.83 (Found: C, 63.9; H, 7.0; N, 6.65. $C_{34}H_{45}N_3O_9$ requires C, 63.83; H, 7.09; N, 6.57%).

Boc-Ile-Glu(OBzl)-Ala-Leu-OPac.—HCl·H-Glu(OBzl)-Ala-Leu-OPac [prepared from Boc-Glu(OBzl)-Ala-Leu-OPac (1.67 g, 2.60 mmol) according to deprotection procedure A], Boc-Ile-OH (0.690 g, 2.93 mmol), and HOBt (0.420 g, 3.11 mmol) were dissolved in DMF (30 ml), and WSCD (0.440 g, 2.83 mmol) was added with ice-bath cooling. After being stirred at the same temperature for 2 h, the reaction mixture was concentrated under reduced pressure and the residue was subjected to purification procedure B to give the protected tetrapeptide ester (1.91 g, 97.4%), m.p. 167—168 °C, $[\alpha]_D^{23}$ −21.8° (*c*, 1.0 in DMF), R_{F6} 0.73 (Found: C, 64.25; H, 7.6; N, 7.5. $C_{40}H_{56}N_4O_{10}$ requires C, 63.81; H, 7.44; N, 7.50%).

Boc-Tyr(Cl$_2$Bzl)-Ile-Glu(OBzl)-Ala-Leu-OPac (**6**).—HCl·H-Ile-Glu(OBzl)-Ala-Leu-OPac [prepared from Boc-Ile-Glu-(OBzl)-Ala-Leu-OPac (7.91 g, 10.5 mmol) according to deprotection procedure A], Boc-Tyr(Cl_2Bzl)-OH (4.62 g, 10.5 mmol), and HOBt (1.56 g, 11.6 mmol) were dissolved in DMF (70 ml), and WSCD (1.80 g, 11.6 mmol) was added with ice-bath cooling. After 3 h of being stirred at the same temperature, the mixture was concentrated under reduced pressure and the residue was subjected to purification procedure B to give the protected pentapeptide ester (10.3 g, 91.7%), m.p. 195–196 °C, $[\alpha]_D^{23}$ −18.6° (*c*, 1.0 in DMF), R_{F6} 0.75 (Found: C, 62.35; H, 6.45; N, 6.75. $C_{56}H_{69}Cl_2N_5O_{12}$ requires C, 62.56; H, 6.47; N, 6.51%).

Boc-Met-OPac.—To a solution of Boc-Met-OH (10.0 g, 40.1 mmol) and Et_3N (4.06 g, 40.1 mmol) in AcOEt (200 ml) was added phenacyl bromide (8.62 g, 40.1 mmol) with ice-bath cooling and the mixture was stirred at room temperature for 6 h. The mixture was filtered and the filtrate was subjected to purification procedure A, followed by crystallization from IPE to give the ester (12.5 g, 85.1%), m.p. 103—105 °C. $[\alpha]_D^{23}$ −33.6° (*c*, 1.0 in DMF), R_{F9} 0.55 (Found: C, 58.85; H, 6.5; N; 3.75. $C_{18}H_{25}NO_5S$ requires C, 58.84; H, 6.86; N, 3.81%).

Boc-Cys(Acm)-Met-OPac.—HCl·H-Met-OPac [prepared from Boc-Met-OPac (0.447 g, 1.22 mmol) according to deprotection procedure A], Boc-Cys(Acm)-OH (0.390 g, 1.33 mmol), and HOBt (0.180 g, 1.33 mmol) were dissolved in DMF (12 ml), and WSCD (0.210 g, 1.35 mmol) was added with ice-bath cooling. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by silica gel column chromatography (13 g) eluting with $CHCl_3$. The product was crystallized from ether to give the protected dipeptide ester (0.410 g, 62.6%), m.p. 58—62 °C, $[\alpha]_D^{23}$ −28.6° (*c*, 1.0 in DMF), R_{F10} 0.73 (Found: C, 53.65; H, 6.5; N, 7.7. $C_{24}H_{35}N_3O_7S_2$ requires C, 53.23; H, 6.51; N, 7.76%).

Boc-Val-Cys(Acm)-Met-OPac (**7**).—HCl·H-Cys(Acm)-Met-

OPac [prepared from Boc-Cys(Acm)-Met-OPac (11.1 g, 20.5 mmol) according to deprotection procedure A], Boc-Val-OH (4.90 g, 22.6 mmol), and HOBt (3.05 g, 22.6 mmol) were dissolved in DMF (200 ml), and WSCD (3.49 g, 22.6 mmol) was added with ice-bath cooling. After being stirred at the same temperature for 2 h, the mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by crystallization from ether to give the protected tripeptide ester (9.10 g, 69.2%), m.p. 142—145 °C, $[\alpha]_D^{23}$ −51.4° (*c*, 1.0 in DMF), R_{F3} 0.27 (Found: C, 53.95; H, 6.6; N, 8.8. $C_{29}H_{44}N_4O_8S_2$ requires C, 54.36; H, 6.92; N, 8.74%).

Boc-Asp(Chx)-Gly-OPac.—HCl·H-Gly-OPac [prepared from Boc-Gly-OPac (4.90 g, 16.7 mmol) according to deprotection procedure A] was neutralized with NMM (1.70 g, 16.7 mmol) in CH_2Cl_2 (40 ml) at −50 °C and allowed to react with the mixed anhydride of Boc-Asp(Chx)-OH (5.27 g, 16.7 mmol) [prepared by treating the latter with isobutyl chlorocarbonate (2.28 g, 16.7 mmol) in the presence of NMM (1.70 g, 16.7 mmol) in CH_2Cl_2 (60 ml)]. After being stirred at the same temperature for 3 h, the mixture was concentrated under reduced pressure and the product was subjected to purification procedure A to give the protected dipeptide ester as an oil (7.81 g, 98.6%), $[\alpha]_D^{23}$ −14.1° (*c*, 1.0 in DMF), R_{F8} 0.50 (Found: C, 60.6; H, 6.9; N, 5.65. $C_{25}H_{34}N_2O_8$ requires C, 61.21; H, 6.99; N, 5.71%).

Boc-His(Tos)-Asp(Chx)-Gly-OPac.—TFA·H-Asp(Chx)-Gly-OPac [prepared from Boc-Asp(Chx)-Gly-OPac (7.45 g, 15.7 mmol) according to deprotection procedure B] was neutralized with NMM (1.60 g, 15.7 mmol) in CH_2Cl_2 (90 ml) at −50 °C and allowed to react with the mixed anhydride of Boc-His(Tos)-OH (6.41 g, 15.7 mmol) [prepared by treating the latter with isobutyl chlorocarbonate (2.14 g, 15.7 mmol) in the presence of NMM (1.60 g, 15.7 mmol) in CH_2Cl_2 (60 ml)]. After being stirred for 3 h at the same temperature, the mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by recrystallization from AcOEt–ether to give the protected tripeptide ester (10.6 g, 88.0%), m.p. 134—136 °C, $[\alpha]_D^{23}$ −23.0° (*c*, 1.0 in DMF), R_{F9} 0.54 (Found: C, 59.25; H, 5.95; N, 8.55. $C_{38}H_{47}N_5O_{11}S$ requires C, 58.38; H, 6.06; N, 8.96%).

Boc-Leu-His(Tos)-Asp(Chx)-Gly-OPac.—Boc-His(Tos)-Asp(Chx)-Gly-OPac (8.96 g, 11.7 mmol) was treated with TFA (120 ml) at −15 °C for 2 h. Excess of TFA was then evaporated and the residue was triturated with dry ether: the resulting solid was filtered off and dissolved in DMF (120 ml) containing Et_3N (1.18 g, 11.7 mmol). Boc-Leu-OSu (3.84 g, 11.7 mmol) was added to the above solution with ice-bath cooling and the reaction mixture was stirred at 5 °C overnight. It was then concentrated under reduced pressure and the resulting residue was treated with ether to afford a powder, which was subjected to purification procedure B and recrystallized from DMF–ether to give the protected tetrapeptide ester (8.64 g, 84.0%), m.p. 165—167 °C, $[\alpha]_D^{23}$ −27.8° (*c*, 1.0 in DMF), R_{F4} 0.82 (Found: C, 58.6; H, 6.2; N, 8.6. $C_{44}H_{58}N_6O_{12}S$ requires C, 59.05; H, 6.53; N, 9.39%).

Boc-Leu-His-Asp(Chx)-Gly-OPac.—To a solution of Boc-Leu-His(Tos)-Asp(Chx)-Gly-OPac (7.07 g, 8.04 mmol) in DMF (200 ml) was added pyridinium chloride (9.30 g, 80.4 mmol) at the room temperature and the mixture was stirred for 2 h. The reaction mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by crystallization from AcOEt–ether to give the protected tetrapeptide ester (5.73 g, 96.2%), m.p. 133–135 °C, $[\alpha]_D^{23}$ −34.5° (*c*, 1.0 in DMF), R_{F4} 0.50 (Found: C, 59.4; H, 6.9; N, 11.45. $C_{37}H_{52}N_6O_{10}$ requires C, 59.99; H, 7.07; N, 11.34%).

Boc-Cys(Acm)-Leu-His-Asp(Chx)-Gly-OPac.—2TFA·H-Leu-His-Asp(Chx)-Gly-OPac [prepared from Boc-Leu-His-Asp(Chx)-Gly-OPac (6.05 g, 8.17 mmol) according to deprotection procedure B] was dissolved in DMF (100 ml) containing Et_3N (1.65 g, 16.3 mmol) and Boc-Cys(Acm)-OSu (3.18 g, 8.17 mmol) was added with ice-bath cooling. After being stirred at 5 °C overnight, the mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by recrystallization from $CHCl_3$–ether to give the protected pentapeptide ester (6.90 g, 92.3%), m.p. 172—177 °C, $[\alpha]_D^{23}$ −31.2° (*c*, 1.0 in DMF), R_{F6} 0.37 (Found: C, 55.1; H, 6.65; N, 12.25. $C_{43}H_{62}N_8O_{12}S$ requires C, 56.44; H, 6.83; N, 12.25%).

Boc-Tyr(Cl_2Bzl)-Cys(Acm)-Leu-His-Asp(Chx)-Gly-OPac (**8**).—2TFA·H-Cys(Acm)-Leu-His-Asp(Chx)-Gly-OPac [prepared from Boc-Cys(Acm)-Leu-His-Asp(Chx)-Gly-OPac (6.67 g, 7.29 mmol) according to deprotection procedure B] was dissolved in DMF (120 ml) containing Et_3N (1.48 g, 14.6 mmol) and Boc-Tyr(Cl_2Bzl)-Osu (3.92 g, 7.29 mmol) was added with ice-bath cooling. After being stirred at 5 °C overnight, the reaction mixture was concentrated under reduced pressure and the residue was treated with ether to afford a power, which was subjected to purification procedure B and recrystallized from DMF–ether to give the protected hexapeptide ester (8.25 g, 91.5%), m.p. 178—181 °C, $[\alpha]_D^{23}$ −33.6° (*c*, 1.0 in DMF), R_{F6} 0.36 (Found: C, 56.6; H, 5.9; N, 9.75. $C_{59}H_{75}Cl_2N_9O_{14}S$ requires C, 57.28; H, 6.11; N, 10.19%.

Boc-Ser(Bzl)-His(Tos)-Asp(Chx)-Gly-OPac.—TFA·H-His-(Tos)-Asp(Chx)-Gly-OPac (2.55 g, 3.21 mmol) was dissolved in DMF (40 ml) containing Et_3N (0.325 g, 3.21 mmol) and Boc-Ser(Bzl)-OSu (1.26 g, 3.21 mmol) was added with ice-bath cooling. After being stirred at 5 °C overnight, the mixture was concentrated under reduced pressure and the residue was treated with ether. The resulting powder was subjected to purification procedure B to give the protected tetrapeptide ester (2.51 g, 81.5%), m.p. 151–153 °C, $[\alpha]_D^{23}$ −33.1° (*c*, 1.0 in DMF), R_{F4} 0.53 (Found: C, 59.55; H, 6.0; N, 8.6. $C_{48}H_{58}N_6O_{13}S$ requires C, 60.11; H, 6.10; N, 8.76%).

Boc-Ser(Bzl)-His-Asp(Chx)-Gly-OPac.—To a solution of Boc-Ser(Bzl)-His(Tos)-Asp(Chx)-Gly-OPac (2.36 g, 2.46 mmol) in DMF (40 ml) was added pyridinium chloride (2.24 g, 19.4 mmol) at room temperature. After being stirred for 2 h, the mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by crystallization from AcOEt–ether to give the protected tetrapeptide ester (1.78 g, 89.9%), m.p. 113—116 °C, $[\alpha]_D^{23}$ −27.0° (*c*, 1.0 in DMF), R_{F4} 0.64 (Found: C, 60.75; H, 6.4; N, 10.27. $C_{41}H_{52}N_6O_{11}$ requires C, 61.18; H, 6.51; N, 10.44%).

Boc-Leu-Ser(Bzl)-His-Asp(Chx)-Gly-OPac.—2TFA·H-Ser-(Bzl)-His-Asp(Chx)-Gly-OPac [prepared from Boc-Ser(Bzl)-His-Asp(Chx)-Gly-OPac (1.59 g, 1.98 mmol) according to deprotection procedure B] was dissolved in DMF (25 ml) containing Et_3N (0.400 g, 3.96 mmol) and Boc-Leu-OSu (0.650 g, 1.98 mmol) was added with ice-bath cooling. After being stirred at 5 °C overnight, the mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by recrystallization from AcOEt–ether to give the protected pentapeptide ester (1.57 g, 86.4%), m.p. 118—123 °C, $[\alpha]_D^{23}$ −68.4° (*c*, 1.0 in DMF), R_{F6} 0.56 (Found: C, 60.55, H, 6.8, N, 10.4. $C_{47}H_{63}N_7O_{12}$ requires C, 61.49; H, 6.92; N, 10.68%).

Boc-Pro-Leu-Ser(Bzl)-His-Asp(Chx)-Gly-OPac.—2TFA·H-Leu-Ser(Bzl)-His-Asp(Chx)-Gly-OPac [prepared from Boc-Leu- Ser(Bzl)-His-Asp(Chx)-Gly-OPac (8.78 g, 9.56 mmol)

according to deprotection procedure B] was dissolved in DMF (130 ml) containing Et_3N (1.93 g, 19.1 mmol) and Boc-Pro-OSu (2.99 g, 9.56 mmol) was added with ice-bath cooling. After being stirred at 5 °C overnight, the mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by recrystallization from $CHCl_3$–ether to give the protected hexapeptide ester (8.82 g, 90.9%), m.p. 128—134 °C, $[\alpha]_D^{23}$ −49.9° (*c*, 1.0 in DMF), R_{F6} 0.52 (Found: C, 60.85; H, 6.75; N, 10.85. $C_{52}H_{70}N_8O_{13}$ requires C, 61.52; H, 6.95; N, 11.04%).

Boc-Cys(Acm)-Pro-Leu-Ser(Bzl)-His-Asp(Chx)-Gly-OPac (**9**).—2TFA·H-Pro-Leu-Ser(Bzl)-His-Asp(Chx)-Gly-OPac [prepared from Boc-Pro-Leu-Ser(Bzl)-His-Asp(Chx)-Gly-OPac (8.68 g, 8.55 mmol) according to deprotection procedure B] was dissolved in DMF (150 ml) containing Et_3N (1.73 g, 17.1 mmol) and Boc-Cys(Acm)-OSu (3.33 g, 8.55 mmol) was added with ice-bath cooling. After being stirred at 5 °C overnight, the mixture was concentrated under reduced pressure and the residue was subjected to purification procedure A, followed by silica gel column chromatography (50 g) with $CHCl_3$–MeOH (50:1). The product was crystallized from AcOEt–ether to give the protected heptapeptide ester (5.75 g, 56.5%), m.p. 103—106 °C, $[\alpha]_D^{23}$ −55.1° (*c*, 1.0 in DMF), R_{F6} 0.38 (Found: C, 57.6; H, 6.8; N, 11.45. $C_{58}H_{80}N_{10}O_{15}S$ requires C, 58.57; H, 6.78; N, 11.78%).

Boc-Glu(OBzl)-NHNH-Troc.—A solution of Boc-Glu(OBzl)-OH (33.7 g, 0.100 mol) and NMM (10.1 g, 0.100 mol) in CH_2Cl_2 (400 ml) was cooled to −15 °C and treated with isobutyl chlorocarbonate (13.7 g, 0.100 mol). After the mixture had been stirred for 40 min, 2,2,2-trichloroethyl carbazate (20.7 g, 0.100 mol) was added at −40 °C and the stirring was continued at − 15 °C for 1 h. The solvent was removed under reduced pressure and the residue was subjected to purification procedure A to give the hydrazide as an oil (51.7 g, 98.1%), $[\alpha]_D^{23}$ −14.3° (*c*, 1.0 in MeOH), R_{F6} 0.74 (Found: C, 43.65; H, 4.7; N, 7.45. $C_{20}H_{26}Cl_3N_3O_7$ requires C, 45.60; H, 4.97; N, 7.98%).

Boc-Ser(Bzl)-Glu(OBzl)-NHNH-Troc.—HCl·H-Glu(OBzl)-NHNH-Troc [prepared from Boc-Glu(OBzl)-NHNH-Troc (20.0 g, 38.0 mmol) according to deprotection procedure A] was neutralized with NMM (3.85 g, 38.0 mmol) in CH_2Cl_2 (150 ml) at −40 °C and allowed to react with the mixed anhydride of Boc-Ser(Bzl)-OH (11.2 g, 38.0 mmol) [prepared by treating the latter with isolbutyl chlorocarbonate (5.19 g, 38.0 mmol) in the presence of NMM (3.85 g, 38.0 mmol) in CH_2Cl_2 (150 ml)]. After being stirred at −15 °C for 1 h, the mixture was concentrated under reduced pressure and the product was subjected to purification procedure A to give the protected dipeptide hydrazide as an oil (26.3 g, 98.2%), $[\alpha]_D^{23}$ −14.6° (*c*, 1.0 in MeOH), R_{F6} 0.58 (Found: C, 50.85; H, 5.4; N, 7.7. $C_{30}H_{37}Cl_3N_4O_9$ requires C, 51.18; H, 5.30; N, 7.96%).

Boc-Asp(Chx)-Ser(Bzl)-Glu(OBzl)-NHNH-Troc.—HCl·H-Ser(Bzl)-Glu(OBzl)-NHNH-Troc [prepared from Boc-Ser(Bzl)-Glu(OBzl)-NHNH-Troc (25.3 g, 35.9 mmol) according to deprotection procedure A], Boc-Asp(Chx)-OH (12.5 g, 39.6 mmol), and HOBt (5.35 g, 39.6 mmol) were dissolved in DMF (450 ml), and WSCD (6.13 g, 39.5 mmol) was added with ice-bath cooling. After being stirred at the same temperature for 2 h, the mixture was concentrated under reduced pressure and the product was subjected to purification procedure A, followed by crystallization from IPE to give the protected tripeptide hydrazide (30.2 g, 93.3%), m.p. 93—97 °C, $[\alpha]_D^{23}$ −15.7° (*c*, 1.0 in DMF), R_{F6} 0.67 (Found: C, 53.2; H, 5.75; N, 7.85. $C_{40}H_{52}Cl_3N_5O_{12}$ requires C, 53.31; H, 5.82; N, 7.77%).

Boc-Ser(Bzl)-Asp(Chx)-Ser(Bzl)-Glu(OBzl)-NHNH-Troc.—HCl·H-Asp(Chx)-Ser(Bzl)-Glu(OBzl)-NHNH-Troc [prepared from Boc-Asp(Chx)-Ser(Bzl)-Glu(OBzl)-NHNH-Troc (16.6 g, 18.5 mmol) according to deprotection procedure A], Boc-Ser(Bzl)-OH (6.00 g, 20.3 mmol), and HOBt (2.99 g, 22.1 mmol) were dissolved in DMF (160 ml), and WSCD (3.15 g, 20.3 mmol) was added with ice-bath cooling. After being stirred at the same temperature for 2 h, the mixture was concentrated under reduced pressure and the product was subjected to purification procedure A, followed by crystallization from IPE to give the protected tetrapeptide hydrazide (8.40 g, 92.3%), m.p. 132—136°, $[\alpha]_D^{23}$ −13.1° (*c*, 1.0 in DMF), R_{F6} 0.80 (Found: C, 55.45; H, 6.0; N, 7.55. $C_{50}H_{63}Cl_3N_6O_{14}$ requires C, 55.69; H, 5.89; N, 7.79%).

Boc-Asn-Ser(Bzl)-Asp(Chx)-Ser(Bzl)-Glu(OBzl)-NHNH-Troc (**10**).—HCl·H-Ser(Bzl)-Asp(Chx)-Ser(Bzl)-Glu(OBzl)-NHNH-Troc [prepared from Boc-Ser(Bzl)-Asp(Chx)-Ser(Bzl)-Glu(OBzl)-NHNH-Troc (0.194 g, 0.180 mmol) according to deprotection procedure A], Boc-Asn-OH (0.046 g, 0.198 mmol), and HOBt (0.026 g, 0.198 mmol) were dissolved in DMF (14 ml), and WSCD (30.7 mg, 0.198 mmol) was added with ice-bath cooling. After being stirred at the same temperature for 2 h, the mixture was subjected to purification procedure A, followed by crystallization from ether to give the protected pentapeptide hydrazide (0.190 g, 88.4%), m.p. 161—164 °C, $[\alpha]_D^{23}$ −20.7° (*c*, 1.0 in DMF), R_{F6} 0.65 (Found: C, 54.2; H, 5.85; N, 9.35. $C_{54}H_{69}Cl_3N_8O_{16}$ requires C, 54.39; H, 5.83; N, 9.40%).

Boc-Glu(OBzl)-Arg(Tos)-Cys(Acm)-Gln-Tyr(Cl$_2$Bzl)-Arg(Tos)-Asp(Chx)-Leu-OH [*Boc*-(40—47)-*OH*].—Boc-(40—47)-OPac (**2**) (3.18 g, 1.58 mmol) was dissolved in a mixture of DMF (60 ml) and AcOH (60 ml), and Zn powder (2.5 g) was added under N_2 at room temperature. After being stirred at the same temperature for 1 h, the mixture was filtered and concentrated under reduced pressure. The residue was treated with ether and the resulting precipitate was washed 5% aqueous HCl and water to give the protected octapeptide (2.90 g, 97.0%), m.p. 180—182 °C, $[\alpha]_D^{23}$ −15.1° (*c* 1.0 in DMF), R_{F1} 0.15 (Found: C, 52.2; H, 6.0; N, 11.55. $C_{86}H_{116}Cl_2N_{16}O_{22}S_3$ requires C, 54.57; H, 6.18; N, 11.84%).

Boc-Tyr(Cl$_2$Bzl)-Ile-Gly-OH [*Boc*-(37—39)-*OH*].—Boc-(37—39)-OPac (**2**) (0.729 g, 1.00 mmol) was dissolved in a mixture of DMF (10 ml) and AcOH (10 ml), and Zn powder (0.70 g) was added under N_2 at room temperature. After being stirred at the same temperature for 2 h, the mixture was filtered and concentrated under reduced pressure. The residue was treated with ether and the resulting precipitate was washed with 5% aqueous HCl, water, and ether to give the protected tripeptide (0.502 g, 82.3%), m.p. 190—199 °C, $[\alpha]_D^{23}$ −32.7° (*c*, 1.0 in DMF), R_{F1} 0.60 (Found: C, 56.85; H, 5.95; N, 6.5. $C_{29}H_{37}Cl_2N_3O_7$ requires C, 57.05; H, 6.11; N, 6.88%).

Boc-(40—53)-*OBzl*.—HCl·H-(48—53)-OBzl [prepared from Boc-(48—53)-OBzl (**1**) (3.01 g, 1.91 mmol) according to deprotection procedure A], Boc-(40—47)-OH (3.97 g, 2.10 mmol), and HOBt (0.334 g, 2.48 mmol) were dissolved in DMF (80 ml), and WSCD (0.326 g, 2.10 mmol) was added at −10 °C. After being stirred at 5 °C overnight, the mixture was concentrated under reduced pressure and the product was subjected to purification procedure A, followed by precipitation from AcOEt–ether to give the protected tetradecapeptide ester as a powder (6.13 g, 95.8%), m.p. 214—217 °C, $[\alpha]_D^{23}$ −7.6° (*c*, 1.0 in DMF), R_{F1} 0.49 (Found: C, 57.45; H, 6.05; N, 11.6. $C_{162}H_{201}Cl_3N_{28}O_{36}S_4$ requires C, 58.06; H, 6.05; N, 11.70%). Amino-acid analysis: Glu, 3.04; Arg, 2.92; Cys, 0.27; Tyr, 1.00; Asp, 1.03; Leu, 1.96; Lys, 0.96; Trp 0.85.

Boc-(37—53)-*OBzl* (**11**).—HCl·H-(40—53)-OBzl [pre-

pared from Boc-(40—53)-OBzl (4.02 g, 1.20 mmol) according to deprotection procedure A], Boc-(37—39)-OH (0.793 g, 1.32 mmol), and HOBt (0.210 g, 1.56 mmol) were dissolved in DMF (60 ml), and WSCD (0.205 g, 1.32 mmol) was added at −10 °C. After being stirred at 5 °C for 48 h, the mixture was concentrated under reduced pressure and the residue was subjected to purification procedure B, followed by precipitation from DMF–MeOH–ether to give the heptadecapeptide ester as a powder (3.89 g, 84.4%), m.p. 230—232 °C, $[\alpha]_D^{23}$ −6.1° (*c*, 1.0 in DMF), R_{F1} 0.41. Amino-acid analysis: Asp, 1.12; Glu, 3.16; Gly, 0.98; Cys, 0.42; Ile, 0.90; Leu, 2.00; Tyr, 2.00; Lys, 0.99; Arg, 3.17; Trp, 0.96.

Boc-Asp(Chx)-Lys(ClZ)-Tyr(Cl_2Bzl)-Ala-OH [*Boc*-(27—30)-*OH*].—Boc-(27—30)-OPac (**5**) (11.8 g, 10.5 mmol) was dissolved in a mixture of DMF (200 ml) and AcOH (60 ml), and Zn powder (7.0 g) was added under N_2 at room temperature. After being stirred at the same temperature for 3 h, the mixture was filtered and concentrated under reduced pressure. The residue was dissolved in AcOEt (300 ml) and the solution was washed with 5% aqueous HCl and water, dried ($MgSO_4$), and evaporated under reduced pressure. The resulting residue was washed with ether–hexane to give the protected tetrapeptide (10.2 g, 96.9%), m.p. 183—184 °C, $[\alpha]_D^{23}$ −20.1° (*c*, 1.0 in DMF), R_{F3} 0.24 (Found: C, 57.1; H, 6.0; N, 7.0. $C_{48}H_{60}Cl_3N_5O_{12}$ requires C, 57.34; H, 6.01; N, 6.97%).

Boc-(27—36)-*OPac* (**12**).—HCl·H-(31—36)-OPac [prepared from Boc-(31—36)-OPac (**4**) (3.05 g, 3.20 mmol) according to deprotection procedure A], Boc-(27—30)-OH (3.54 g, 3.52 mmol), and HOBt (0.553 g, 4.10 mmol) were dissolved in DMF (130 ml), and WSCD (0.546 g, 3.52 mmol) was added at −10 °C. After being stirred at the same temperature overnight, the mixture was concentrated under reduced pressure and the product was subjected to purification procedure B, followed by reprecipitation from DMF–MeOH to give the protected decapeptide ester (5.20 g, 88.2%), m.p. 259—263 °C, $[\alpha]_D^{23}$ −21.8° (*c*, 1.0 in DMF), R_{F2} 0.59 (Found: C, 54.05; H, 6.25; N, 10.7. $C_{84}H_{113}Cl_3N_{14}O_{22}S_2$ requires C, 54.79; H, 6.19, N, 10.65%). Amino-acid analysis: Asp, 1.93; Gly, 1.00; Ala, 1.01; Cys, 0.88; Val, 1.65; Tyr, 0.95; Lys, 0.94.

Boc-Tyr(Cl_2Bzl)-Cys(Acm)-Leu-His-Asp(Chx)-Gly-OH·HCl [*Boc*-(13—18)-*OH·HCl*].—Boc-(13—18)-OPac (**8**) (3.00 g, 2.43 mmol) was dissolved in a mixture of DMF (30 ml) and AcOH (30 ml), and Zn powder (3.00 g) was added under N_2 at room temperature. The reaction mixture was stirred at the same temperature for 1 h, after which it was filtered and concentrated under reduced pressure. The residue was treated with ether and the resulting precipitate was washed with 5% aqueous HCl, water, and ether to give the protected hexapeptide (1.73 g, 61.6%), m.p. 182—185 °C, $[\alpha]_D^{23}$ −27.7°, R_{F2} 0.26 (Found: C, 52.45; H, 6.05; N, 10.4. $C_{51}H_{69}Cl_2N_9O_{13}S$·HCl requires C, 53.01; H, 6.02; N, 10.91%).

Boc-Val-Cys(Acm)-Met-OH [*Boc*-(19—21)-*OH*].—Boc-Val-Cys(Acm)-Met-OPac (**7**) (3.20 g, 5.00 mmol) was dissolved in a mixture of DMF (30 ml) and AcOH (30 ml), and Zn powder (3.27 g) was added under N_2 at room temperature. The reaction mixture was stirred at the same temperature for 1 h, after which it was filtered and concentrated under reduced pressure. The residue was treated with 5% aqueous HCl, and the resulting precipitate was washed with 5% aqueous HCl, water, and ether to give the protected tripeptide (2.41 g, 92.2%), m.p. 198—200 °C, $[\alpha]_D^{23}$ −22.7° (*c*, 1.0 in DMF), R_{F2} 0.71 (Found: C, 47.95; H, 7.05; N, 10.45. $C_{21}H_{38}N_4O_7S_2$ requires C, 48.26; H, 7.33; N, 10.72%).

Boc-(19—26)-*OPac*.—HCl·H-Tyr(Cl_2Bzl)-Ile-Glu(OBzl)-Ala-Leu-OPac [HCl·H-(22—26)-OPac] [prepared from Boc-(22—26)-OPac (**6**) (5.06 g, 5.00 mmol) according to deprotection procedure A], Boc-Val-Cys(Acm)-Met-OH [Boc-(19—21)-OH] (2.61 g, 5.00 mmol), and HOBt (0.743 g, 5.50 mmol) were dissolved in DMF (100 ml), and WSCD (0.776 g, 5.00 mmol) was added at −10 °C. The reaction mixture was stirred at −5 °C overnight after which it was evaporated under reduced pressure. The product was subjected to purification procedure B followed by reprecipitation from DMF–MeOH–ether to give the protected octapeptide ester (6.75 g, 91.2%), m.p. 262—264 °C, $[\alpha]_D^{23}$ −17.4° (*c*, 1.0 in DMF), R_{F10} 0.34 (Found: C, 57.85; H, 6.6; N, 8.3. $C_{72}H_{97}Cl_2N_9O_{16}S_2$ requires C, 58.45; H, 6.61; N, 8.52%).

Boc-(13—26)-*OPac* (**13**).—HCl·H-(19—26)-OPac [prepared from Boc-(19—26)-OPac (2.11 g, 1.43 mmol) according to deprotection procedure A], Boc-(13—18)-OH·HCl (1.73 g, 1.50 mmol), and HOBt (0.232 g, 1.72 mmol) were dissolved in DMF (40 ml), and WSCD (0.244 g, 1.57 mmol) was added at −10 °C. The reaction mixture was stirred at 5 °C overnight after which the solvent was removed under reduced pressure and the residue was treated with AcOEt and ether. The resulting powder was subjected to purification procedure B and reprecipitation from DMF–MeOH gave the protected tetradecapeptide ester (3.07 g, 86.7%), m.p. 264—267 °C, $[\alpha]_D^{23}$ −13.8° (*c*, 1.0 in DMF), R_{F2} 0.66 (Found: C, 47.95; H, 6.25; N, 9.95. $C_{118}H_{156}Cl_4N_{18}O_{26}S_3$ requires C, 47.14; H, 6.34; N, 10.16%). Amino-acid analysis: Asp, 0.99; Glu, 0.99; Gly, 0.99; Ala, 1.34; Cys, 0.18; Val, 0.98; Met, 0.85; Ile, 0.97; Leu, 2.00; Tyr, 1.93; His, 1.12.

Boc-Asn-Ser(Bzl)-Asp(Chx)-Ser(Bzl)-Glu(OBzl)-NHNH$_2$.—Boc-Asn-Ser(Bzl)-Asp(Chx)-Ser(Bzl)-Glu(OBzl)-NHNH-Troc (**10**) (6.89 g, 6.00 mmol) was dissolved in a mixture of DMF (100 ml) and AcOH (50 ml), and Zn powder (5.0 g) was added under N_2 at room temperature. The reaction mixture was stirred for 1 h after which Zn powder was filtered off and the filtrate was concentrated under reduced pressure. The residue was treated with 1M aqueous $NaHCO_3$ and the resulting precipitate was washed with water and ether to give the protected pentapeptide hydrazide (5.05 g, 82.8%), m.p. 232—234 °C, $[\alpha]_D^{23}$ −55.6° (*c*, 1.0 in DMF), R_{F6} 0.55 (Found: C, 60.0; H, 6.65; N, 10.65. $C_{51}H_{68}N_8O_{14}$ requires C, 60.22; H, 6.74; N, 11.02%).

Boc-(1—12)-*OPac* (**14**).—Boc-Asn-Ser(Bzl)-Asp(Chx)-Ser-(Bzl)-Glu(OBzl)-NHNH$_2$ [Boc-(1—5)-NHNH$_2$] (4.97 g, 4.89 mmol) was dissolved in DMF (110 ml) and 4.68M HCl in dioxane (2.09 ml, 4.89 mmol) was added. The mixture was cooled to −10 °C and t-butyl nitrite (0.580 ml, 4.89 mmol) was added. After 15 min, Et_3N (0.991 g, 9.79 mmol) and a solution of 2TFA·H-Cys(Acm)-Pro-Leu-Ser(Bzl)-His-Asp(Chx)-Gly-OPac [2TFA·H-(6—12)-OPac] [prepared from Boc-(6—12)-OPac (**9**) (5.35 g, 4.50 mmol) according to deprotection procedure B] in DMF (60 ml) containing Et_3N (1.40 g, 13.8 mmol) were added and the reaction mixture was stirred at 5 °C overnight. It was then evaporated under reduced pressure and the product was subjected to purification procedure B and reprecipitation from DMF–ether to give the protected dodecapeptide ester (8.37 g, 89.7%), m.p. 168—170 °C, $[\alpha]_D^{23}$ −31.3° (*c*, 1.0 in DMF), R_{F2} 0.61 (Found: C, 59.25; H, 6.4; N. 10.4. $C_{104}H_{136}N_{16}O_{27}S$ requires C, 60.22; H, 6.61; N, 10.80%). Amino-acid analysis: Asp, 3.28; Ser, 2.89; Glu, 1.16; Pro, 1.02; Gly, 1.01; Cys, 0.08; Leu, 1.00; His, 1.10.

Boc-(27—36)-*OH* (**15**).—To a solution of Boc-(27—36)-OPac (**12**) (2.00 g, 1.09 mmol) in a mixture of NMP (30 ml), DMSO (15 ml), and pyridine (15 ml) were added anthranilic acid (1.41 g, 10.9 mmol) and Zn powder (3.00 g) under N_2 at

50 °C. The reaction mixture was then stirred at the same temperature for 2 h after which it was filtered and concentrated under reduced pressure. The residue was treated with 5% aqueous HCl and the resulting precipitate was washed with 5% HCl, water, and ether to give the protected decapeptide (1.62 g, 86.2%), m.p. 252—255 °C, $[\alpha]_D^{23}$ −13.6° (*c*, 1.0 in DMSO), R_{F2} 0.38 (Found: C, 52.25; H, 6.2; N, 11.2. $C_{76}H_{107}Cl_3N_{14}O_{21}S_2$ requires C, 52.97; H, 6.26; N, 11.38%).

Boc-(13—36)-*OH·HCl* (**16**).—To a solution of Boc-(13—26)-OPac (**13**) (2.00 g, 0.807 mmol) in a mixture of NMP (60 ml), DMSO (15 ml), and pyridine (3 ml) were added anthranilic acid (1.11 g, 8.07 mmol) and Zn powder (3.0 g) under N_2 at 35 °C. After the reaction mixture had been stirred at the same temperature for 6 h, the mixture was filtered and concentrated under reduced pressure. The residue was treated with 5% aqueous HCl and the resulting precipitate was washed with 5% aqueous HCl, water, and ether to give the protected tetradecaptide (1.77 g, 92.1%), m.p. 254—256 °C, $[\alpha]_D^{23}$ −14.0° (*c*, 1.0 in DMSO), R_{F2} 0.18 (Found: C, 54.9; H, 6.25; N, 10.25. $C_{110}H_{151}Cl_5N_{18}O_{25}S_3$) requires C, 55.08; H, 6.34; N, 10.51%).

Boc-(1—12)-*OH·HCl* (**17**).—Boc-(1—12)-OPac (**14**) (2.00 g, 0.964 mmol) was dissolved in a mixture of DMF (20 ml) and AcOH (20 ml), and Zn powder (3.00 g) was added under N_2 at room temperature. The reaction mixture was stirred at the same temperature for 1 h, after which the mixture was filtered and concentrated under reduced pressure. The residue was treated with 5% aqueous HCl and the resulting precipitate was washed with 5% aqueous HCl, water, and ether to give the protected dodecapeptide (1.69 g, 88.0%), m.p. 171—174 °C, $[\alpha]_D^{23}$ −33.0° (*c*, 1.0 in DMF), R_{F2} 0.25 (Found: C, 56.75; H, 6.6; N, 10.85. $C_{96}H_{131}Cl_1N_{16}O_{26}$ requires C, 57.86; H, 6.63; N, 11.25%).

Boc-(27—53)-*OBzl* (**18**).—HCl·H-(37—53)-OBzl [prepared from Boc-(37—53)-OBzl (**11**) (4.54 g, 1.18 mmol) according to deprotection procedure A], Boc-(27—36)-OH (**15**) (2.24 g, 1.30 mmol), and HOBt (0.207 g, 1.53 mmol) were dissolved in NMP (70 ml), and WSCD (0.219 g, 1.32 mmol) was added at −10 °C. After being stirred at 5 °C for 48 h, the mixture was concentrated under reduced pressure and the product was subjected to purification procedure B, and reprecipitation from DMF–MeOH–AcOEt to give the protected heptacosapeptide ester (6.16 g, 95.8%), m.p. 235 °C (decomp.), $[\alpha]_D^{23}$ −10.4° (*c*, 1.0 in DMSO), R_{F6} 0.60. Amino-acid analysis: Asp, 3.28; Glu, 3.08; Gly, 2.07; Ala, 1.58; Cys, 1.01; Val, 2.08; Ile, 0.88; Leu, 2.00; Tyr, 3.05; Lys, 2.07; Arg, 3.03; Trp, 0.88.

Boc-(13—53)-*OBzl* (**19**).—HCl·H-(27—53)-OBzl [prepared from Boc-(27—53)-OBzl (**18**) (7.95 g, 1.46 mmol) according to deprotection procedure A], Boc-(13—26)-OH·HCl (**16**) (3.84 g, 1.61 mmol), and HOBt (0.257 g, 1.90 mmol) were dissolved in NMP (150 ml), and WSCD (0.272 g, 1.75 mmol) was added at −10 °C. After being stirred at 5 °C for 48 h, the mixture was concentrated under reduced pressure and the product was subjected to purification procedure B, and reprecipitation from NMP–MeOH–AcOEt to give the protected hentetracontapeptide ester (11.1 g, 98.6%), m.p. 231 °C (decomp.), $[\alpha]_D^{23}$ −15.1° (*c*, 1.0 in DMSO), R_{F6} 0.50. Amino-acid analysis: Asp, 4.14; Glu, 4.05; Gly, 3.04; Ala, 3.76; Cys, 0.30; Val, 2.93; Met, 0.82; Ile, 1.89; Leu, 4.00; Tyr, 4.90; Lys, 1.94; His, 1.08; Arg, 2.89; Trp, 0.79.

Boc-(1—53)-*OBzl* (**20**).—2HCl·H-(13—53)-OBzl [prepared from Boc-(13—53)-OBzl (**19**) (10.6 g, 1.38 mmol) according to deprotection procedure A], Boc-(1—12)-OH·HCl (**17**) (3.03 g, 1.52 mmol), and HOBt (0.243 g, 1.80 mmol) were dissolved in NMP (200 ml), and WSCD (0.258 g, 1.66 mmol) was added at −10 °C. After being stirred at 5 °C for 96 h, the mixture was concentrated under reduced pressure and the product was subjected to purification procedure B to give the protected tripentacontapeptide ester (12.6 g, 95.7%), m.p. 251 °C (decomp.), $[\alpha]_D^{23}$ −18.6° (*c*, 1.0 in DMSO), R_{F6} 0.51. Amino-acid analysis: Asp, 7.54; Ser, 2.56; Glu, 5.09; Pro, 1.26; Gly, 4.13; Ala, 4.12; Cys, 0.45; Val, 2.93; Met, 0.80; Ile, 1.85; Leu, 5.00; Tyr, 4.80; Lys, 1.90; His, 2.14; Arg, 2.85; Trp, 0.82.

Racemization during Condensaation Reaction for Fragments (19—26), (27—36), *and* (40—53).—The racemic fragment Boc-(19—21)-OH was prepared according to Sakakibara's method and coupled to HCl·H-(22—26)-OPac in a manner similar to that for Boc-(19—26)-OPac. The condensation product was treated with Zn powder in DMF–AcOH as described for Boc-(19—26)-OH and further deprotected as follows. Fragment Boc-(19—26)-OH (100 mg) was treated with HF–anisole (9:1, v/v, 1 h, 0 °C) and, after removal of HF, the residue was triturated with ether to give a precipitate. This product was dissolved in 50% aqueous AcOH and analysed by h.p.lc. The result is shown in Figure 2. The racemized fragments Boc-(27—30)-OH and Boc-(40—47)-OH were similarly prepared and coupled to HCl·H-(31—36)-OPac and HCl·H-(48—53)-OBzl, respectively. The protecting groups in the condensation product Boc-(27—36)-OPac were removed as above. For removal of the protecting groups in the fragment Boc-(40—53)-OPac, see the subsequent paper. The retention times: fragment (19—26), L 9.0 min, D 13.2 min (solvent system A); fragment (27—36), L 3.4 min, D 4.3 min (solvent system B); fragment (40—53), L 6.1 min, D 5.0 min (solvent system C).

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