Amino Acids and Peptides. XXVIII. Synthesis of Peptide Fragments Related to Eglin c and Studies on the Relationship between Their Structure and Effects on Human Leukocyte Elastase, Cathepsin G and α-Chymotrypsin[1,2]

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Various peptide fragments related to eglin c, which consists of 70 amino acid residues, were synthesized by a conventional solution method and their inhibitory effects on leukocyte elastase, cathepsin G and α-chymotrypsin were examined. Among them, H-Arg-Glu-Tyr-Phe-OMe (eglin c 22—25) and H-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg-Tyr-OMe (eglin c 41—49) inhibited cathepsin G and α-chymotrypsin but not leukocyte elastase, while H-Thr-Asn-Val-Val-OMe (eglin c 60—63) inhibited leukocyte elastase but not cathepsin G or α-chymotrypsin, although eglin c potently inhibited leukocyte elastase, cathepsin G and α-chymotrypsin. These results indicated that the interaction sites of eglin c with leukocyte elastase, cathepsin G and α-chymotrypsin might be different.

Keywords eglin c-related peptide; chemical synthesis; inhibitory effect; leukocyte elastase; cathepsin G; α-chymotrypsin; structure–activity relationship

Eglin c, isolated from the leech *Hirudo medicinalis*,[3] consists of 70 amino acid residues[4] and effectively inhibits chymotrypsin and subtilisin as well as leukocyte elastase and cathepsin G. The latter two enzymes have attracted our interest in recent years due to their possible involvement in connective tissue turnover and diseases such as emphysema, rheumatoid arthritis and inflammation.[5,6] Therefore, eglin c is a potential candidate as a therapeutic agent for emphysema and inflammation. Rink *et al.* prepared N^{α}-acetyleglin c by a gene engineering technique,[7] although its molecular weight is too large for practical therapeutic use.

Under these circumstances, our studies were directed to the synthesis of small-molecular inhibitors for leukocyte elastase and cathepsin G. This paper deals with the synthesis of eglin c-related peptides and studies on the relationship between their structure and their inhibitory effects on leukocyte elastase, cathepsin G and α-chymotrypsin with the objective of finding relatively small molecules which can inhibit leukocyte elastase and cathepsin G.

The primary structures of eglin c and the peptide fragments synthesized are shown in Fig. 1. For the peptide synthesis, amino acid derivatives bearing protecting groups removable by methanesulfonic acid,[8] *i.e.* Lys(Z) and Arg(Mts), by TMSBr,[9] *i.e.* His(Bom), and by catalytic hydrogenation, *i.e.* Asp(OBzl) and Glu(OBzl), were employed in combination with the TFA-labile Boc group as the N^{α}-protecting group. The Bzl group of β- or γ-carboxyl functional group of Asp or Glu was removed by catalytic hydrogenation prior to use for fragment condensation.

The N-terminal undecapeptide, H–(1—11)–OMe (I) was synthesized according to the route shown in Fig. 2.

The synthetic scheme for H–(12—21)–OMe (II) is illustrated in Fig. 3.

H–(22—25)–OMe (III) was prepared as follows. Starting with H–Phe–OMe, Z–Tyr–$NHNH_2$, Boc–Glu(OBzl)–ONp and Boc–Arg(Mts)–OH were coupled successively by the azide method, the active ester method and the DPPA method, respectively, followed by catalytic hydrogenation and then treatment with MSA to give peptide III. H–(26—30)–OMe (IV) was prepared as follows. Starting with H–Tyr–Pro–OMe,[10] Z–His–$NHNH_2$, Z–Leu–ONp and Boc–Thr–ONSu[11] were coupled successively by the azide method and the active ester method, respectively, followed by treatment with HCl/dioxane.

H–(31—40)–OMe (V) was synthesized as illustrated in Fig. 4.

H–(41—49)–OMe (VI) was prepared according to the route shown in Fig. 5.

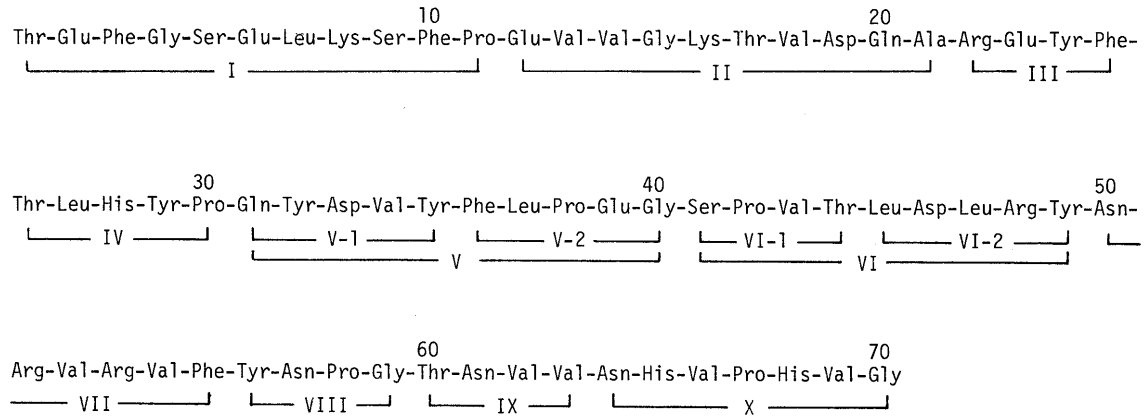

Fig. 1. Primary Structure of Eglin c and Peptide Fragments (I—X)

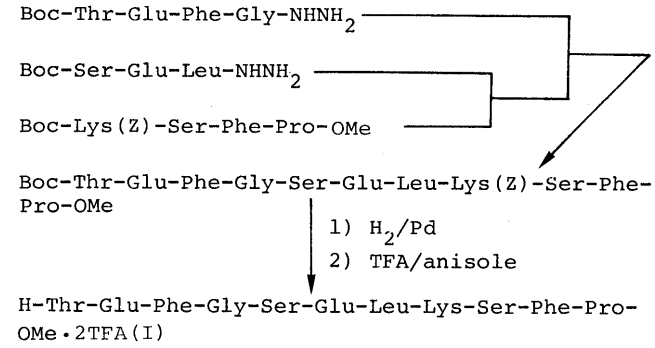

Fig. 2. Synthetic Scheme for H–(1—11)–OMe (I)

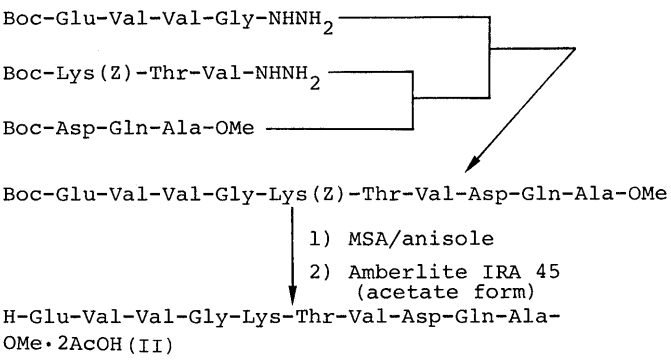

Fig. 3. Synthetic Scheme for H–(12—21)–OMe (II)

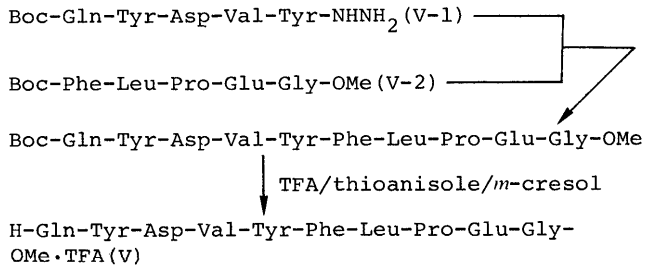

Fig. 4. Synthetic Scheme for H–(31—40)–OMe (V)

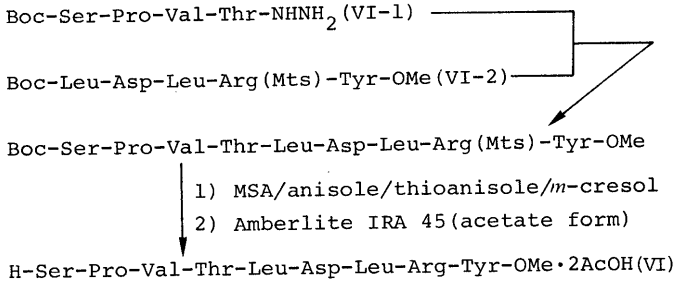

Fig. 5. Synthetic Scheme for H–(41—49)–OMe (VI)

H–(50—55)–OMe (VII) was prepared as follows. Starting with H–Val–Phe–OMe,[12] Z–Arg(Mts)–OH, Z–Val–OPyCl,[13] Z–Arg(Mts)–OH and Boc–Asn–ONp were coupled successively by the DPPA method, a newly developed active ester method, the DPPA method and the active ester method, respectively, followed by treatment with MSA.

H–(56—59)–OMe (VIII) was prepared as follows. Starting with H–Pro–Gly–OMe,[14] Z–Asn–ONp and Boc–Tyr–$NHNH_2$ were coupled successively by the active ester method and the azide method, respectively, followed by treatment with HCl/dioxane.

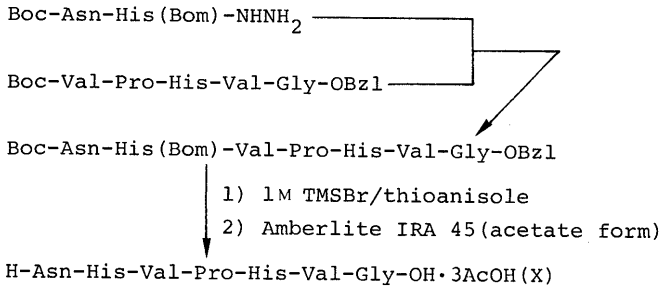

Fig. 6. Synthetic Scheme for H–(64—70)–OH (X)

H–(60—63)–OMe (IX) was synthesized as follows. Starting with H–Val–Val–OMe,[15] Z–Asn–ONp and Boc–Thr–OH were coupled successively by the active ester method and the DPPA method, respectively, followed by treatment with HCl/dioxane.

Finally, the C-terminal heptapeptide, H–(64—70)–OH (X) was synthesized according to the route shown in Fig. 6. The imidazole nitrogen of the His residue at position 65 was protected with the Bom group, because Boc–Asn–His–OMe and Boc–Asn–His–$NHNH_2$ were very soluble in water and the yields were very poor; furthermore, Boc–Ala(CN)–His–OMe was obtained instead of the desired peptide.

The homogeneity of the peptides obtained (I—X) was ascertained by thin-layer chromatography (TLC), amino acid analysis of an acid hydrolysate and elemental analysis, and the results are summarized in Table I.

Next, the inhibitory effects of peptides I—X on leukocyte elastase, cathepsin G and α-chymotrypsin were examined; the results are summarized in Table II. As can be seen in this table, H–(41—49)–OMe (VI) significantly inhibited cathepsin G and α-chymotrypsin, whose enzymatic similarity was reported previously,[16] with K_i values of 4.0×10^{-5} and 2.0×10^{-5} M, respectively, but it had no effect on leukocyte elastase.

Previously, Bode *et al.*,[17] reported that the nine residues of the binding loop (40—48) are involved in direct contact with subtilisin as a result of the determination of the crystal structure of the complex formed between eglin c and subtilisin Carlsberg by X-ray analysis. However, from our results, it can be deduced that this binding loop can not react with the active site of leukocyte elastase. In the eglin c molecule, Thr^{44}, Asp^{46} and Arg^{48} form electrostatic and hydrogen bonds with Arg^{53}, Arg^{51} and Gly^{70}, respectively.[17] These interactions are as effective in stabilizing the reactive site loop as are the disulfide bridges present in the Kunitz and Kazarl type inhibitors. Peptide VI does not have such bonds. This might be a possible reason why the K_i values of peptide VI towards cathepsin G and α-chymotrypsin are 10^5 times larger than those of eglin c. Peptide III inhibited cathepsin G and α-chymotrypsin with K_i values of 8.0×10^{-4} and 7.0×10^{-5} M, respectively, and it slightly increased leukocyte elastase activity. H–(60—63)–OMe (IX) inhibited leukocyte elastase with K_i value of 1.6×10^{-4} M, but had no effect on cathepsin G and α-chymotrypsin. It was also reported that eglin c in the crystalline complex is shortened by seven residues at its amino terminus.[17] Dodt *et al.*,[18] incubated eglin c with an exopeptidase, cathepsin C, and isolated N-terminally

TABLE I. Yield, Melting Point, $[\alpha]_D$, *Rf* Values and Amino Acid Analysis Data

Compound	Yield (%)	mp (°C)	$[\alpha]_D$ (°) (*c*, solvent)	*Rf* values	Amino acid analysis (average recovery)	Final deprotection method[a]
H–(1—11)–OMe[b] ·2TFA(I)	40.6	154—157	−44.8 (0.5, H_2O)	0.20[e] 0.88[f]	Thr 0.97; Glu 2.19; Phe 2.11; Gly 0.96; Ser 2.01; Leu 0.98; Lys 0.95; Pro 1.00 (79.3%)	A
H–(12—21)–OMe ·2AcOH(II)	96.2	Amorphous	−38.2 (1.0, 10% AcOH)	0.77[e]	Glx 2.01; Val 2.78; Gly 1.01; Lys 1.02; Thr 0.92; Asp 1.18; Ala 1.00 (80.1%)	B
H–(22—25)–OMe ·2AcOH(III)	41.9	Amorphous	−2.03 (0.5, H_2O)	0.27[e] 0.83[f]	Arg 1.12; Glu 1.06; Tyr 0.81; Phe 1.00 (72.4%)	B
H–(26—30)–OMe ·2HCl(IV)	96.1	174—178	−52.8 (1.0, 10% AcOH)	0.52[e]	Thr 1.00; Leu 1.00; His 1.01; Tyr 0.92; Pro 1.00 (79.9%)	C
H–(31—35)–OMe ·TFA(V-1)	92.0	142—148	−15.0 (1.0, MeOH)	0.32[d]	Asp 0.95; Glu 1.04; Val 0.34; Tyr 1.51(82.3%)	A
H–(36—40)–OMe ·HCl(V-2)	87.1	131—136.5	−73.8 (0.8, MeOH)	0.48[e] 0.18[c]	Glu 1.06; Gly 1.00; Leu 0.63; Phe 0.71; Pro 1.02 (80.0%)	C
H–(31—40)–OMe ·TFA(V)	59.7	163—172	−61.5 (0.6, MeOH)	0.26[d]	Asp 0.93; Glu 2.34; Gly 0.82; Val 0.50; Leu 0.81; Tyr 1.33; Phe 1.00; Pro 1.09 (76.3%)	A
H–(41—44)–OMe ·HCl(VI-1)	66.0	205—209.5 (dec.)	−83.4 (0.9, MeOH)	0.38[e] 0.27[c]	Ser 1.00; Thr 1.00; Val 0.85; Pro 1.07 (84.1%)	C
H–(45—49)–OMe ·2AcOH(VI-2)	49.0	91—97	−14.5 (0.9, MeOH)	0.34[d]	Asp 1.06; Leu 2.12; Tyr 0.90; Arg 0.92 (60.0%)	B
H–(41—49)–OMe ·2AcOH(VI)	58.0	Amorphous	−79.0 (0.6, MeOH)	0.11[d] 0.50[e]	Asp 1.04; Thr 0.82; Ser 0.91; Val 1.02; Leu 1.80; Tyr; 0.90; Arg 0.69; Pro 1.06 (80.9%)	B
H–(50—55)–OMe ·3AcOH(VII)	87.8	Amorphous	−36.3 (0.6, 10% AcOH)	0.78[f]	Asn 0.95; Arg 1.93; Val 1.98; Phe 1.00 (82.1%)	B
H–(56—59)–OMe ·HCl(VIII)	99.0	163—166	−45.0 (0.9, 10% AcOH)	0.21[d]	Tyr 0.96; Asn 1.06; Pro 1.02; Gly 1.00 (82.1%)	C
H–(60—63)–OMe ·HCl(IX)	81.9	215—220	−80.0 (0.5, 10% AcOH)	0.65[e]	Thr 1.02; Asn 1.00; Val 1.74 (64.8%)	C
H–(64—70)–OH[b] ·3AcOH(X)	60.7	Amorphous	−78.9 (0.5, 10% AcOH)	0.64[f]	Asn 1.30; His 1.98; Val 1.85; Pro 0.99; Gly 1.00 (75.4%)	D

a) See Experimental section. *b*) This salt was deduced from the result of elemental analysis. *c*) Rf^2, *d*) Rf^4, *e*) Rf^5, *f*) Rf^6.

TABLE II. Inhibitory Effects of Eglin c Fragments

Fragment	Remaining activity (%)		
	LE	Cathepsin G	α-Chymotrypsin
H–(1—11)–OMe(I)	81	82	92
H–(12—21)–OMe(II)	107	108	106
H–(22—25)–OMe(III)	130	75	60
H–(26—30)–OMe(IV)	105	103	94
H–(31—35)–OMe(V-1)	90	80	100
H–(36—40)–OMe(V-2)	102	85	92
H–(31—40)–OMe(V)	108	64	95
H–(41—44)–OMe(VI-1)	100	102	105
H–(45—49)–OMe(VI-2)	106	95	89
H–(41—49)–OMe(VI)	94	12	21
H–(50—55)–OMe(VII)	106	95	70
H–(56—59)–OMe(VIII)	108	106	98
H–(60—63)–OMe(IX)	66	96	97
H–(64—70)–OH(X)	100	104	103

The final concentrations of inhibitors and substrates were 0.5 mM. The reaction was carried out in Tris–HCl buffer (0.1 M, pH 8.0) containing 0.2 M NaCl for LE (leukocyte elastase) and in Tris–HCl buffer (0.1 M, pH 7.5) containing 0.2 M NaCl for cathepsin G and α-chymotrypsin. The substrate for LE was Suc–Ala–Tyr–Leu–Val–*p*NA and that for cathepsin G and α-chymotrypsin was Suc–Ile–Pro–Phe–*p*NA.

shortened eglin c, eglin c (5—70) and eglin c (7—70). These eglin c derivatives exhibited similar inhibitory activity to that of native eglin c against chymotrypsin, indicating that the N-terminal part of eglin c did not have a significant role in the manifestation of inhibitory activity. In fact, H–(1—11)–OMe (I) did not exhibit significant inhibitory activity against the above three enzymes. H–(31—40)–OMe (V) inhibited only cathepsin G, indicating a difference in enzymatic character between cathepsin G and α-chymotrypsin.

In conclusion, it can be emphasized that the interaction sites of eglin c with leukocyte elastase and with cathepsin G and α-chymotrypsin might be different.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co., Ltd.). Amino acid compositions of acid hydrolysates (6 N HCl, 110 °C, 18 h; for peptides containing a Val–Val bond, 6 N HCl, 110 °C, 72 h) were determined with an amino acid analyzer, K-101 AS (Kyowa Seimitsu Co., Ltd.). On TLC (Kieselgel G, Merck), Rf^1, Rf^2, Rf^3, Rf^4, Rf^5, Rf^6, Rf^7 and Rf^8 values refer to the systems of $CHCl_3$, MeOH and AcOH (90 : 8 : 2), $CHCl_3$, MeOH and H_2O (8 : 3 : 1, lower phase), $CHCl_3$, MeOH and H_2O (89 : 10 : 1), *n*-BuOH, AcOH and H_2O (4 : 1 : 5, upper phase), *n*-BuOH, pyridine, AcOH and H_2O (4 : 1 : 1 : 2), *n*-BuOH, pyridine, AcOH and H_2O (1 : 1 : 1 : 1), $CHCl_3$, ether (4 : 1), and *n*-BuOH, H_2O, AcOEt and NH_4OH (5 : 1 : 2 : 1), respectively.

Boc–Glu(OBzl)–Phe–Gly–OMe Boc–Glu(OBzl)–ONp (5.5 g, 11.9 mmol) and H–Phe–Gly–OMe·HCl [prepared from Z–Phe–Gly–OMe[19] (3.7 g, 9.9 mmol) by catalytic hydrogenation as usual] were dissolved in DMF (150 ml) containing Et_3N (2.1 ml, 15.0 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated to a small volume to give crystals, which were collected by filtration and recrystallized from AcOEt, yield 4.2 g (75.1%), mp 151—154 °C, $[\alpha]_D^{27}$ −6.9° (*c* = 1.0, MeOH), Rf^1 0.89, Rf^2 0.98. *Anal.* Calcd for $C_{29}H_{37}N_3O_9$: C, 62.7; H, 6.71; N, 7.56. Found: C, 62.7; H, 6.75; N, 7.62.

Boc–Thr–Glu(OBzl)–Phe–Gly–OMe Boc–Thr–ONSu[11] (4.8 g, 0.015 mol) and H–Glu(OBzl)–Phe–Gly–OMe·HCl [prepared from Boc–Glu(OBzl)–Phe–Gly–OMe (6.5 g, 0.012 mol) and 6.5 N HCl/dioxane (23.4 ml, 0.15 mol)] were dissolved in DMF (100 ml) containing Et_3N (2.0 ml, 0.014 mol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with

AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated. The oily residue in $CHCl_3$ (6 ml) was applied to a silica gel column (3.3 × 48.5 cm). The column was eluted with $CHCl_3$. Individual fractions (200 ml each) were collected. The solvent of the eluate (tube Nos. 16—25) was removed by evaporation and petroleum ether was added to the residue to afford a powder, yield 6.0 g (78.2%), mp 55—60 °C, $[\alpha]_D^{27}$ −10.2° (c=1.0, MeOH), Rf^1 0.64, Rf^2 0.76. *Anal.* Calcd for $C_{33}H_{44}N_4O_{10}\cdot 1/2H_2O$: C, 59.7; H, 6.83; N, 8.65. Found: C, 59.5; H, 6.81; N, 8.42.

Boc-Thr-Glu-Phe-Gly-OMe Boc–Thr–Glu(OBzl)–Phe–Gly–OMe (1.0 g, 1.52 mmol) in MeOH (50 ml) was hydrogenated over Pd catalyst, yield 0.83 g (96.0%), mp 78—82 °C, $[\alpha]_D^{27}$ −3.1° (c=1.0, MeOH), Rf^1 0.23, Rf^2 0.60. *Anal.* Calcd for $C_{25}H_{36}N_4O_{10}\cdot 1/2H_2O$: C, 54.7; H, 6.79; N, 9.81. Found: C, 54.8; H, 6.56; N, 9.64.

Boc-Thr-Glu-Phe-Gly-NHNH$_2$ [Boc-(1—4)-NHNH$_2$] Hydrazine hydrate (90%, 0.86 ml, 17 mmol) was added to a solution of Boc–Thr–Glu–Phe–Gly–OMe (2.0 g, 3.53 mmol) in EtOH (30 ml). The mixture was stored at room temperature overnight. After removal of the solvent, the residue was neutralized with AcOH. The resulting oily material in 3% AcOH (2 ml) was applied to a column of Sephadex G-25 (1.7 × 108 cm), equilibrated and eluted with 3% AcOH. Individual fractions (5 g each) were collected and the solvent of the effluent (tube Nos. 32—35) was removed by lyophilization to give an amorphous powder, yield 1.4 g (70.6%), $[\alpha]_D^{27}$ −14.0° (c=1.0, MeOH), Rf^2 0.15, Rf^5 0.78, Rf^6 0.89. *Anal.* Calcd for $C_{27}H_{38}N_6O_9\cdot H_2O$: C, 51.4; H, 6.90; N, 14.4. Found: C, 51.5; H, 6.69; N, 14.4.

Boc-Glu(OBzl)-Leu-OMe H–Leu–OMe [prepared from H–Leu–OMe·HCl (3.5 g, 0.019 mol) and Et_3N (2.8 ml, 0.020 mol)] and Boc–Glu(OBzl)–ONp (7.3 g, 0.016 mol) were dissolved in DMF (100 ml). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and H_2O, dried over Na_2SO_4 and concentrated. The oily residue in $CHCl_3$ (6 ml) was applied to a silica gel column (3.0 × 48 cm), which was eluted with $CHCl_3$. Individual fractions (100 ml each) were collected. The solvent of the effluent (tube Nos. 8—18) was removed by evaporation. Petroleum ether was added to the residue to give crystals, which were collected by filtration, yield 5.5 g (73.8%), mp 60—62 °C, $[\alpha]_D^{27}$ −23.4° (c=1.0, MeOH), Rf^1 0.85. *Anal.* Calcd for $C_{24}H_{36}N_2O_7$: C, 62.1; H, 7.81; N, 6.03. Found: C, 61.8; H, 7.72; N, 5.90.

Boc-Ser-Glu(OBzl)-Leu-OMe Boc–Ser–N_3 [prepared from Boc–Ser–NHNH$_2$ (1.5 g, 6.8 mmol) and isopentyl nitrile (1.0 ml, 7.1 mmol) as usual] was added to a solution of H–Glu(OBzl)–Leu–OMe·HCl [prepared from Boc–Glu(OBzl)–Leu–OMe (2.2 g, 4.7 mmol) and 6.4 N HCl/dioxane (7.3 ml, 47.0 mmol)] in DMF (70 ml) containing Et_3N (0.66 ml, 4.7 mmol). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and H_2O, dried over Na_2SO_4 and concentrated. The oily residue in $CHCl_3$ (2 ml) was applied to a silica gel column (1.0 × 32 cm), equilibrated with $CHCl_3$ and eluted with 1% MeOH in $CHCl_3$. Individual fractions (50 ml each) were collected. The eluate (tube Nos. 4—6) was concentrated, yield 2.0 g (77.2%), $[\alpha]_D^{27}$ −45.7° (c=0.2, MeOH), Rf^1 0.52, Rf^2 0.58. *Anal.* Calcd for $C_{27}H_{41}N_3O_9$: C, 58.8; H, 7.49; N, 7.62. Found: C, 58.8; H, 7.72; N, 7.51.

Boc-Ser-Glu-Leu-OMe Boc–Ser–Glu(OBzl)–Leu–OMe (0.5 g, 0.91 mmol) in MeOH (20 ml) was hydrogenated over a Pd catalyst, yield 0.3 g (71.2%), mp 65—68 °C, $[\alpha]_D^{27}$ −44.4° (c=1.0, MeOH), Rf^1 0.32, Rf^2 0.30. *Anal.* Calcd for $C_{20}H_{35}N_3O_9\cdot 1/4H_2O$: C, 51.6; H, 7.69; N, 9.02. Found: C, 51.4; H, 7.90; N, 8.87.

Boc-Ser-Glu-Leu-NHNH$_2$ [Boc-(5—7)-NHNH$_2$] Hydrazine hydrate (90%, 0.4 ml, 8.0 mmol) was added to a solution of Boc–Ser–Glu–Leu–OMe (1.1 g, 2.3 mmol) in EtOH (8 ml). The reaction mixture was stored at room temperature overnight. After removal of the solvent, the residue in MeOH (4 ml) was applied to a column of Sephadex LH-20 (2.7 × 128 cm), equilibrated and eluted with MeOH. Individual fractions (5 g each) were collected and the solvent of the effluent (tube Nos. 68—74) was removed by evaporation. Ether was added to the residue to give crystals, yield 0.72 g (67.9%), mp 135—144 °C, $[\alpha]_D^{27}$ −8.7° (c=1.0, DMF), Rf^2 0.22, Rf^4 0.52. *Anal.* Calcd for $C_{15}H_{35}N_5O_8\cdot 1/4H_2O$: C, 49.0; H, 7.68; N, 15.0. Found: C, 49.2; H, 7.82; N, 14.8.

Z-Ser-Phe-Pro-OMe Z–Ser–N_3 [prepared from Z–Ser–NHNH$_2$ (2.7 g, 11.0 mmol) and isopentyl nitrite (1.5 ml, 11.0 mmol) as usual] in DMF (50 ml) was added to a solution of H–Phe–Pro–OMe·HCl [prepared from Z–Phe–Pro–OMe[20] (3.4 g, 8.3 mmol) by catalytic hydrogenation] in DMF (80 ml) containing Et_3N (1.2 ml, 8.3 mmol). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and H_2O, dried over Na_2SO_4 and concentrated. The oily residue in $CHCl_3$ (6 ml) was applied to a silica gel column (2.1 × 56 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (100 ml each) were collected. The solvent of the effluent (tube Nos. 7—11) was removed by evaporation to give an amorphous powder, yield 4.4 g (100%), $[\alpha]_D^{27}$ −3.6° (c=0.1, MeOH), Rf^1 0.63. *Anal.* Calcd for $C_{26}H_{31}N_3O_7\cdot 1/4H_2O$: C, 62.1; H, 6.23; N, 8.37. Found: 62.2; H, 6.32; N, 8.37.

Boc-Lys(Z)-Ser-Phe-Pro-OMe [Boc-(8—11)-OMe] Boc–Lys(Z)–ONp (7.8 g, 0.015 mol) and H–Ser–Phe–Pro–OMe [prepared from Z–Ser–Phe–Pro–OMe (6.4 g, 0.013 mol) by catalytic hydrogenation] were dissolved in DMF (150 ml) containing Et_3N (2.0 ml, 0.013 mol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and H_2O, dried over Na_2SO_4 and concentrated. The oily residue in $CHCl_3$ (5 ml) was applied to a silica gel column (3.0 × 57 cm), equilibrated and eluted with $CHCl_3$, followed by 1% MeOH in $CHCl_3$. Individual fractions (200 ml each) were collected. The solvent of the effluent (tube Nos. 33—77) was removed by evaporation to give an amorphous powder, yield 8.9 g (94.6%), $[\alpha]_D^{27}$ −51.3° (c=1.0, MeOH), Rf^1 0.48, Rf^2 0.77. *Anal.* Calcd for $C_{37}H_{51}N_5O_{10}$: C, 60.9; H, 7.30; N, 9.38. Found: C, 60.9; H, 7.11; N, 9.59.

Boc-Ser-Glu-Leu-Lys(Z)-Ser-Phe-Pro-OMe Boc–Ser–Glu–Leu–N_3 [prepared from Boc–Ser–Glu–Leu–NHNH$_2$ (0.35 g, 0.76 mmol) and isopentyl nitrite (0.11 ml, 0.76 mmol) as usual] in DMF (5 ml) was added to a solution of H–Lys(Z)–Ser–Phe–Pro–OMe·TFA [prepared from Boc–Lys(Z)–Ser–Phe–Pro–OMe (0.36 g, 0.50 mmol) and TFA (0.37 ml, 5.0 mmol)] in DMF (10 ml) containing Et_3N (0.07 ml, 0.50 mmol) under cooling with ice-salt. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 3% AcOH, dried over Na_2SO_4 and concentrated. The residue was purified by gel-filtration on Sephadex LH-20, yield 0.2 g (36.5%), mp 112—118 °C, $[\alpha]_D^{27}$ −46.7° (c=0.3, MeOH), Rf^2 0.50. *Anal.* Calcd for $C_{51}H_{74}N_8O_{10}\cdot 3/2H_2O$: C, 56.6; H, 7.17; N, 10.4. Found: C, 56.5; H, 7.17; N, 10.4.

Boc-Thr-Glu-Phe-Gly-Ser-Glu-Leu-Lys(Z)-Ser-Phe-Pro-OMe [Boc-(1—11)-OMe] Boc–Thr–Glu–Phe–Gly–N_3 [prepared from Boc–Thr–Glu–Phe–Gly–NHNH$_2$ (0.65 g, 1.14 mmol) and isopentyl nitrite (0.16 ml, 1.14 mmol) as usual] in DMF (5 ml) was added to a solution of H–Ser–Glu–Leu–Lys(Z)–Ser–Phe–Pro–OMe·TFA [prepared from Boc–Ser–Glu–Leu–Lys(Z)–Ser–Phe–Pro–OMe (0.60 g, 0.57 mmol) and TFA (0.42 ml, 5.70 mmol) as usual] in DMF (10 ml) containing Et_3N (0.3 ml, 2.28 mmol) under cooling with ice-salt. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, MeOH was added to the residue to give a gelatinous powder, yield 0.13 g (15.2%), mp 199—203 °C, $[\alpha]_D^{27}$ −42.5° (c=0.1, DMF), Rf^2 0.24, Rf^4 0.73. *Anal.* Calcd for $C_{71}H_{100}N_{12}O_{23}\cdot H_2O$: C, 56.6; H, 6.82; N, 11.1. Found: C, 56.3; H, 6.96; N, 11.3.

Z-Val-Val-Gly-OMe Z–Val–OPyCl[13] (3.2 g, 9.8 mmol) was added to a solution of H–Val–Gly–OMe·HCl [prepared from Z–Val–Gly–OMe[21] (2.9 g, 8.9 mmol) by catalytic hydrogenation] in DMF (40 ml) containing Et_3N (1.3 ml, 8.9 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, AcOEt and H_2O were added to the residue to give crystals, which were collected by filtration and washed with MeOH, yield 3.6 g (96.0%), mp 178—180 °C, $[\alpha]_D^{27}$ −1.5° (c=0.9, DMF), Rf^1 0.71. *Anal.* Calcd for $C_{21}H_{31}N_3O_6$: C, 59.8; H, 7.43; N, 9.96. Found: C, 59.8; H, 7.47; N, 9.91.

Boc-Glu(OBzl)-Val-Val-Gly-OMe The title compound was prepared from Boc–Glu(OBzl)–ONp (8.21 g, 0.018 mol) and H–Val–Val–Gly–OMe·HCl [prepared from Z–Val–Val–Gly–OMe (6.3 g, 0.015 mol) by catalytic hydrogenation], yield 5.5 g (61.3%), mp 180—183 °C, $[\alpha]_D^{27}$ −15.0° (c=0.9, DMF), Rf^1 0.61. *Anal.* Calcd for $C_{30}H_{46}N_4O_9$: C, 59.4; H, 7.64; N, 9.24. Found: C, 59.2; H, 7.74; N, 9.16.

Boc-Glu-Val-Val-Gly-OMe Boc–Glu(OBzl)–Val–Val–Gly–OMe (2.0 g, 3.3 mmol) in DMF (30 ml) was hydrogenated over a Pd catalyst. After removal of the Pd and the solvent, ether was added to the residue to give crystals, which were collected by filtration, yield, 1.7 g (98.0%), mp 205—207 °C, $[\alpha]_D^{27}$ −15.9° (c=1.0, DMF), Rf^1 0.47. *Anal.* Calcd for $C_{23}H_{40}N_4O_9\cdot 1/4H_2O$: C, 53.0; H, 7.84; N, 10.7. Found: C, 53.0; H, 7.67; N, 11.0.

Boc-Glu-Val-Val-Gly-NHNH$_2$ [Boc-(12—15)-NHNH$_2$] Hydrazine hydrate (90%, 0.63 ml, 12.6 mmol) was added to a solution of Boc–Glu–Val–Val–Gly–OMe (1.3 g, 2.52 mmol) in DMF (20 ml). The mixture was stored at room temperature overnight. After removal of the

solvent, EtOH was added to the residue to give crystals, which were collected by filtration, yield 0.8 g (62.0%), mp 169—171 °C, $[\alpha]_D^{27}$ −10.9° (c=0.9, DMF), Rf^1 0.20. *Anal.* Calcd for $C_{22}H_{40}N_6O_8 \cdot 3/2H_2O$: C, 48.6; H, 7.99; N, 15.5. Found: C, 48.4; H, 8.00; N, 15.7.

Z–Thr–Val–OMe The title compound was prepared from Z–Thr–OH (10.1 g, 0.04 mol) and H–Val–OMe·HCl (6.7 g, 0.04 mol) by the DCC (9.9 g, 0.048 mol) method, yield 12.7 g (86.6%), mp 63—65 °C, $[\alpha]_D^{27}$ −24.7° (c=1.0, MeOH), Rf^1 0.69. *Anal.* Calcd for $C_{18}H_{26}N_2O_6$: C, 59.0; H, 7.15; N, 7.65. Found: C, 59.1; H, 7.08; N, 7.78.

Boc–Lys(Z)–Thr–Val–OMe The title compound was prepared from Boc–Lys(Z)–ONp (4.1 g, 8.2 mmol) and H–Thr–Val–OMe·HCl [prepared from Z–Thr–Val–OMe (3.0 g, 8.2 mmol) by catalytic hydrogenation], yield 4.7 g (95.9%), amorphous, $[\alpha]_D^{27}$ −33.0° (c=1.0, MeOH), Rf^1 0.54. *Anal.* Calcd for $C_{29}H_{46}N_4O_9$: C, 58.6; H, 7.81; N, 9.42. Found: C, 58.3; H, 7.65; N, 9.46.

Boc–Lys(Z)–Thr–Val–NHNH$_2$ [Boc–(16—18)–NHNH$_2$] Hydrazine hydrate (90%, 1.9 ml, 38.0 mmol) was added to a solution of Boc–Lys(Z)–Thr–Val–OMe (2.0 g, 3.8 mmol) in EtOH (30 ml). The reaction mixture was stored at room temperature overnight to give crystals, which were collected by filtration and recrystallized from MeOH, yield 1.1 g (56.0%), mp 199—201 °C, $[\alpha]_D^{27}$ −39.2° (c=1.0, MeOH), Rf^1 0.26, Rf^2, 0.78. *Anal.* Calcd for $C_{28}H_{46}N_6O_8$: C, 56.5; H, 7.81; N, 14.1. Found: C, 56.4; H, 7.80; N, 14.2.

Boc–Asp(OBzl)–Gln–Ala–OMe The title compound was prepared from Boc–Asp(OBzl)–ONp (1.0 g, 2.3 mmol) and H-Gln–Ala–OMe·HCl [prepared from Z–Gln–Ala–OMe[22] (0.68 g, 2.3 mmol) by catalytic hydrogenation] in DMF (10 ml) containing Et_3N (0.26 ml, 1.9 mmol). The crude product in $CHCl_3$ (2 ml) was applied to silica gel column (2.0×27 cm), equilibrated with $CHCl_3$ and eluted with the following solvents, $CHCl_3$ (350 ml), 1% MeOH in $CHCl_3$ (200 ml) and 2% MeOH in $CHCl_3$ (150 ml). The solvent of the effluent obtained with 2% MeOH in $CHCl_3$ was removed by evaporation. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 0.64 g (63.5%), mp 159—161 °C, $[\alpha]_D^{27}$ −126.3° (c=0.9, DMF), Rf^1 0.62, Rf^2 0.76. *Anal.* Calcd for $C_{25}H_{36}N_4O_9$: C, 56.0; H, 6.78; N, 10.4. Found: C, 55.8; H, 6.74; N, 10.6.

Boc–Asp–Gln–Ala–OMe [Boc–(19—21)–OMe] Boc–Asp(OBzl)–Gln–Ala–OMe (500 mg, 0.932 mmol) in MeOH (10 ml) and DMF (4 ml) was hydrogenated over Pd catalyst, yield 403 mg (96.9%), mp 164—168 °C, $[\alpha]_D^{27}$ −43.1° (c=1.0, MeOH), Rf^1 0.18, Rf^2 0.37. *Anal.* Calcd for $C_{18}H_{30}N_4O_9$: C, 48.4; H, 6.79; N, 12.5. Found: C, 48.2; H, 6.89; N, 12.6.

Boc–Lys(Z)–Thr–Val–Asp–Gln–Ala–OMe Boc–Lys(Z)–Thr–Val–N$_3$ [prepared from Boc–Lys(Z)–Thr–Val–NHNH$_2$ (1.57 g, 2.6 mmol) and isopentyl nitrite (0.37 ml, 2.6 mmol) as usual] was added to a solution of H–Asp–Gln–Ala–OMe·TFA [prepared from Boc–Asp–Gln–Ala–OMe (1.0 g, 2.2 mmol) and TFA (1.6 ml, 22 mmol) containing anisole (0.48 ml, 4.4 mmol)] in DMF (60 ml) containing Et_3N (0.74 ml, 5.2 mmol). The reaction mixture was stirred at 4 °C. After removal of the solvent, AcOEt and H_2O were added to the residue to give crystals, which were collected by filtration and recrystallized from DMF and MeOH, yield 1.86 g (93.5%), mp 218—219 °C, $[\alpha]_D^{27}$ −21.6° (c=0.7, DMSO), Rf^2 0.24. *Anal.* Calcd for $C_{41}H_{64}N_8O_{15} \cdot H_2O$: C, 53.1; H, 7.19; N, 12.1. Found: C, 53.2; H, 7.18; N, 12.3.

Boc–Glu–Val–Val–Gly–Lys(Z)–Thr–Val–Asp–Gln–Ala–OMe [Boc–(12—21)–OMe] The title compound was prepared from Boc–Glu–Val–Val–Gly–N$_3$ [prepared from Boc–Glu–Val–Val–Gly–NHNH$_2$ (110 mg, 0.21 mmol) and isopentyl nitrite (0.03 ml, 0.21 mmol) as usual] and H–Lys(Z)–Thr–Val–Asp–Gln–Ala–OMe·TFA (125 mg, 0.14 mmol) in the usual manner, yield 104 mg (56.7%), mp 230—231 °C, $[\alpha]_D^{27}$ −35.1° (c= 0.3, DMSO), Rf^3 0.65, Rf^4 0.78. *Anal.* Calcd for $C_{58}H_{92}N_{12}O_{21} \cdot 3/2H_2O$: C, 52.8; H, 7.27; N, 12.7. Found: C, 52.5; H. 7.09; N, 13.0.

Boc–Glu(OBzl)–Tyr–Phe–OMe The title compound was prepared from Boc–Glu(OBzl)–ONp (3.9 g, 8.4 mmol) and H–Tyr–Phe–OMe·HCl [prepared from Z–Tyr–Phe–OMe[23] (4.5 g, 9.4 mmol) by catalytic hydrogenation] in the usual manner, yield 4.3 g (76.8%), mp 86—89 °C, $[\alpha]_D^{27}$ −5.6° (c=1.0, DMF), Rf^1 0.42, Rf^2 0.77. *Anal.* Calcd for $C_{33}H_{39}N_3O_9$: C, 65.3; H, 6.55; N, 6.35. Found: C, 65.6; H, 6.68; N, 6.40.

Boc–Arg(Mts)–Glu(OBzl)–Tyr–Phe–OMe Boc–Arg(Mts)–OH (2.3 g, 5.59 mmol) and H–Glu(OBzl)–Tyr–Phe–OMe·HCl [prepared from Boc–Glu(OBzl)–Tyr–Phe–OMe (4.2 g, 6.35 mmol) and 7.5 N HCl/dioxane (8.5 ml, 63.6 mmol)] were dissolved in DMF (30 ml) and the mixture was cooled with ice. DPPA (1.75 ml, 6.35 mmol) and Et_3N (1.8 ml, 12.8 mmol) were added to the above cold solution. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated. The oily residue in MeOH (5 ml) was applied to a column of Sephadex LH-20 (2.7×129 cm), equilibrated and eluted with MeOH. Individual fractions (5 g each) were collected and the solvent of the effluent (tube Nos. 60—74) was removed by evaporation. Petroleum ether was added to the residue to give crystals, which were collected by filtration, yield 2.5 g (46.8%), mp 85—87 °C, $[\alpha]_D^{27}$ +8.2° (c=0.5, DMF), Rf^1 0.64, Rf^2 0.71. *Anal.* Calcd for $C_{51}H_{65}N_7O_{12}S$: C, 59.9; H, 6.65; N, 9.57. Found: C, 60.1; H, 6.51; N, 9.27.

Boc–Arg(Mts)–Glu–Tyr–Phe–OMe [Boc–(22—25)–OMe] Boc–Arg(Mts)–Glu(OBzl)–Tyr–Phe–OMe (2.5 g, 2.50 mmol) in MeOH (50 ml) was hydrogenated over a Pd catalyst, yield 2.2 g (96.5%), mp 94—100 °C (dec.), $[\alpha]_D^{27}$ −1.4° (c=0.5, DMF), Rf^1 0.14, Rf^2 0.30. *Anal.* Calcd for $C_{44}H_{59}N_7O_{12}S \cdot 1/4H_2O$: C, 57.8; H, 6.58; N, 10.7. Found: C, 57.7; H, 6.87; N, 10.5.

Z–His–Tyr–Pro–OMe The title compound was prepared from Z–His–N$_3$ [prepared from Z–His–NHNH$_2$ (1.2 g, 4.2 mmol) and isopentyl nitrite (0.58 ml, 4.2 mmol)] and H–Tyr–Pro–OMe·HCl[10] [prepared from Z–Tyr–Pro–OMe (1.5 g, 3.5 mmol) by catalytic hydrogenation], yield 1.9 g (95.9%), mp 105—110 °C, $[\alpha]_D^{27}$ −21.6° (c=0.7, MeOH), Rf^2 0.68. *Anal.* Calcd for $C_{29}H_{33}N_5O_7 \cdot 1/2H_2O$: C, 60.8; H, 6.09; N, 12.2. Found: C, 60.8; H, 5.99; N, 12.4.

Boc–Leu–His–Tyr–Pro–OMe The title compound was prepared from Boc–Leu–ONp (1.22 g, 3.3 mmol) and H–His–Tyr–Pro–OMe·HCl [prepared from Z–His–Tyr–Pro–OMe (1.5 g, 2.7 mmol) by catalytic hydrogenation]. The crude product in $CHCl_3$ (2 ml) was applied to a silica gel column (2.0×43.0 cm), which was eluted with the following solvents: $CHCl_3$ (260 ml), 1% MeOH in $CHCl_3$ (150 ml), 2% MeOH in $CHCl_3$ (200 ml) and 3% MeOH in $CHCl_3$ (360 ml). The solvent of the effluent (3% MeOH in $CHCl_3$) was removed by evaporation. Ether was added to the residue to afford crystals, yield 1.0 g (59.4%), mp 125—128 °C, $[\alpha]_D^{27}$ −51.9° (c=1.0, MeOH), Rf^2 0.69. *Anal.* Calcd for $C_{32}H_{46}N_6O_8 \cdot 1/2H_2O$: C, 59.0; H, 7.28; N, 12.9. Found: C, 58.9; H, 7.28; N, 12.7.

Boc–Thr–Leu–His–Tyr–Pro–OMe [Boc–(26—30)–OMe] The title compound was prepared from Boc–Thr–ONSu[11] (591 mg, 1.87 mmol) and H–Leu–His–Tyr–Pro–OMe·TFA [prepared from Boc–Leu–His–Tyr–Pro–OMe (800 mg, 1.24 mmol) and TFA (0.92 ml, 12.4 mmol) containing anisole (0.27 ml, 2.49 mmol)]. The crude product in $CHCl_3$ (5 ml) was applied to a silica gel column (1.9×25.2 cm), which was eluted with the following solvents: $CHCl_3$ (150 ml), 1% MeOH in $CHCl_3$ (200 ml), 2% MeOH in $CHCl_3$ (900 ml) and 3% MeOH in $CHCl_3$ (1650 ml). The solvent of the appropriate effluent (3% MeOH in $CHCl_3$) was removed by evaporation. Ether was added to the residue to afford crystals, yield 447 mg (50.4%), mp 138—140 °C, $[\alpha]_D^{27}$ −62.9° (c=1.0, MeOH), Rf^2 0.51, Rf^3 0.30. *Anal.* Calcd for $C_{36}H_{53}N_7O_{10} \cdot 9/4H_2O$: C, 55.1; H, 7.11; N, 12.5. Found: C, 55.0; H, 6.93; N, 12.3.

Boc–Val–Tyr–OMe The title compound was prepared from Boc–Val–OH (6.5 g, 0.030 mol) and H–Tyr–OMe·HCl (6.9 g, 0.030 mol) by the DCC (7.4 g, 0.036 mol), HOBt (4.1 g, 0.030 mol) method,[24] yield 10.6 g (90%), mp 148—153 °C, $[\alpha]_D^{27}$ −17.7° (c=0.9, MeOH), Rf^1 0.49. *Anal.* Calcd for $C_{20}H_{30}N_2O_6$: C, 60.9; H, 7.67; N, 7.10. Found: C, 60.7; H, 7.82; N, 7.23.

Boc–Asp(OBzl)–Val–Tyr–OMe The title compound was prepared from Boc–Asp(OBzl)–ONp (7.7 g, 0.018 mol) and H–Val–Tyr–OMe·HCl [prepared from Boc–Val–Tyr–OMe (9.0 g, 0.023 mol) and 4 N HCl/dioxane (25 ml, 0.10 mol)] as usual and recrystallized from AcOEt, yield 6.8 g (63%), mp 148—153 °C, $[\alpha]_D^{27}$ −27.0° (c=0.9, MeOH), Rf^1 0.52, Rf^3 0.71. *Anal.* Calcd for $C_{31}H_{41}N_3O_6$: C, 62.1; H, 6.89; N, 7.01. Found: C, 62.0; H, 6.94; N, 7.10.

Boc–Gln–Tyr–NHNH$_2$ Hydrazine hydrate (90%, 0.83 ml, 17 mmol) was added to a solution of Boc–Gln–Tyr–OMe (2.3 g, 5.4 mmol) [prepared from Boc–Gln–ONp (15.0 g, 14 mmol) and H–Tyr–OMe·HCl (3.9 g, 17 mmol) in the usual manner] in MeOH (30 ml). The reaction mixture was allowed to stand at room temperature overnight. The precipitate was collected by filtration and recrystallized from MeOH, yield 1.9 g (83%), mp 231.5—236.5 °C, $[\alpha]_D^{27}$ +21.7° (c=0.8, HMPA), Rf^2 0.38. *Anal.* Calcd for $C_{19}H_{29}N_5O_6$: C, 53.9; H, 6.90; N, 16.5. Found: C, 53.9; H, 6.95; N, 16.3.

Boc–Gln–Tyr–Asp(OBzl)–Val–Tyr–OMe Boc–Gln–Tyr–N$_3$ [prepared from Boc–Gln–Tyr–NHNH$_2$ (1.5 g, 3.5 mmol) and isopentyl nitrite (0.58 ml, 4.2 mmol) as usual] in DMF (20 ml) and DMSO (20 ml) was added to a solution of H–Asp(OBzl)–Val–Tyr–OMe·HCl [prepared from Boc–Asp(OBzl)–Val–Tyr–OMe (2.5 g, 4.2 mmol) and 5.0 N HCl/dioxane (2.5 ml, 12.5 mmol) as usual] in DMF (20 ml) containing Et_3N (0.58 ml, 4.2 mmol). The reaction mixture was stirred at 4 °C for 48 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid and H_2O, dried over Na_2SO_4 and evaporated

down. The residue in $CHCl_3$: MeOH : H_2O (16 : 3 : 1, lower phase, 3 ml) was purified by silica gel column (2 × 36 cm) chromatography, yield 1.1 g (35.7%), mp 173—175.5 °C, $[\alpha]_D^{27}$ −30.7° (c=0.8, MeOH), Rf^1 0.40, Rf^2 0.64. *Anal.* Calcd for $C_{45}H_{58}N_6O_{13}\cdot H_2O$: C, 59.5; H, 6.65; N, 9.24. Found: C, 59.6; H, 6.56; N, 9.34.

Boc-Gln-Tyr-Asp-Val-Tyr-OMe [Boc-(31—35)-OMe] The title compound was prepared from Boc-Gln-Tyr-Asp(OBzl)-Val-Tyr-OMe (0.50 g, 0.57 mmol) by catalytic hydrogenation over a Pd catalyst, yield 0.42 g (94%), mp 171—190 °C, $[\alpha]_D^{27}$ −18.9° (c=0.8, DMF), Rf^1 0.04, Rf^2 0.06. *Anal.* Calcd for $C_{38}H_{52}N_6O_{13}\cdot H_2O$: C, 55.7; H, 6.64; N, 10.3. Found: C, 55.7; H, 6.63; N, 10.3.

Boc-Gln-Tyr-Asp-Val-Tyr-$NHNH_2$ [Boc-(31—35)-$NHNH_2$] Hydrazine hydrate (90%, 0.44 ml, 8.8 mmol) was added to a solution of Boc-Gln-Tyr-Asp-Val-Tyr-OMe (1.4 g, 1.8 mmol) in MeOH (15 ml). The reaction mixture was stored at room temperature overnight. A precipitate was collected by filtration and washed with 10% AcOH, H_2O and EtOH, yield 0.85 g (61%), mp 207—213 °C (dec.). $[\alpha]_D^{27}$ −32.8° (c=0.6, DMF), Rf^4 0.74, Rf^5 0.75. *Anal.* Calcd for $C_{37}H_{52}N_8O_{12}\cdot 1/2H_2O$: C, 54.9; H, 6.59; N, 13.8. Found: C, 54.5; H, 6.81; N, 14.2.

Boc-Glu(OBzl)-Gly-OMe The title compound was prepared from Boc-Glu(OBzl)-ONp (9.5 g, 0.021 mol) and H-Gly-OMe·HCl (3.19 g, 0.025 mol) and recrystallized from AcOEt and ether, yield 7.6 g (89%), mp 64—68 °C, $[\alpha]_D^{27}$ −10.5° (c=0.9, MeOH), Rf^1 0.52, Rf^3 0.73. *Anal.* Calcd for $C_{20}H_{28}N_2O_7$: C, 58.8; H, 6.91; N, 6.86. Found: C, 58.7; H, 6.91; N, 6.87.

Boc-Pro-Glu(OBzl)-Gly-OMe The title compound was prepared from Boc-Pro-ONp (4.7 g, 0.014 mol) and H-Glu(OBzl)-Gly-OMe·HCl [prepared from Boc-Glu(OBzl)-Gly-OMe (6.3 g, 0.015 mol) and 7.5 N HCl/dioxane (6.0 ml, 0.045 mol)], yield 5.4 g (72%), amorphous powder, $[\alpha]_D^{27}$ −53.7° (c=1.1, MeOH), Rf^1 0.76, Rf^3 0.66. *Anal.* Calcd for $C_{25}H_{35}N_3O_8$: C, 59.4; H, 6.98; N, 8.31. Found: C, 59.4; H, 7.12; N, 8.36.

Boc-Leu-Pro-Glu(OBzl)-Gly-OMe The title compound was prepared from Boc-Leu-ONp (2.0 g, 5.9 mmol) and H-Pro-Glu(OBzl)-Gly-OMe·HCl [prepared from Boc-Pro-Glu(OBzl)-Gly-OMe (3.0 g, 5.9 mmol) and 3.8 N HCl/dioxane (8.0 ml, 30.0 mmol)] and purified by silica gel column (2.5 × 39 cm) chromatography, yield 2.3 g (63%), amorphous solid, $[\alpha]_D^{27}$ −70.6° (c=0.9, MeOH), Rf^1 0.49, Rf^3 0.49. *Anal.* Calcd for $C_{31}H_{46}N_4O_9\cdot 1/2H_2O$: C, 59.3; H, 7.54; N, 8.92. Found: C, 59.6; H, 7.40; N, 8.97.

Boc-Phe-Leu-Pro-Glu(OBzl)-Gly-OMe The title compound was prepared from Boc-Phe-ONp (1.2 g, 3.2 mmol) and H-Leu-Pro-Glu(OBzl)-Gly-OMe·HCl [prepared from Boc-Leu-Pro-Glu(OBzl)-Gly-OMe (2.0 g, 3.2 mmol) and 3.0 N HCl/dioxane (5.3 ml, 16.0 mmol)]. The crude product was purified by silica gel column (2.5 × 33 cm) chromatography, yield 1.5 g (60%), mp 65—67 °C, $[\alpha]_D^{27}$ −65.8° (c=1.0, MeOH), Rf^1 0.46. *Anal.* Calcd for $C_{40}H_{55}N_5O_{10}\cdot 1/2H_2O$: C, 62.0; H, 7.28; N, 9.03. Found: C, 62.2; H, 7.29; N, 9.07.

Boc-Phe-Leu-Pro-Glu-Gly-OMe [Boc-(36—40)-OMe] The title compound was prepared from Boc-Phe-Leu-Pro-Glu(OBzl)-Gly-OMe (3.79 g, 4.8 mmol) by catalytic hydrogenation over a Pd catalyst, yield 3.2 g (98%), mp 115—117 °C, $[\alpha]_D^{27}$ −74.0° (c=0.9, MeOH), Rf^1 0.45, Rf^2 0.36. *Anal.* Calcd for $C_{33}H_{49}N_5O_{10}$: C, 58.7; H, 7.31; N, 10.4. Found: C, 59.0; H, 7.70; N, 10.2.

Boc-Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly-OMe [Boc-(31—40)-OMe] Boc-Gln-Tyr-Asp-Val-Tyr-N_3 [prepared from Boc-Gln-Tyr-Asp-Val-Tyr-$NHNH_2$ (0.32 g, 0.40 mmol) and isopentyl nitrite (0.067 ml, 0.48 mmol) as usual] in DMF (10 ml) was added to a solution of H-Phe-Leu-Pro-Glu-Gly-OMe·HCl [prepared from Boc-Phe-Leu-Pro-Glu-Gly-OMe (0.14 g, 0.20 mmol) and 3.3 N HCl/dioxane (1.0 ml, 3.80 mmol)] in DMF (10 ml) containing Et_3N (0.03 ml, 0.20 mmol). The reaction mixture was stirred at 4 °C for 72 h. After removal of the solvent, AcOEt and H_2O were added to the residue to give a precipitate. The crude product was purified by gel-filtration on Sephadex LH-20, yield 0.41 g, (76%), mp 182—186 °C, $[\alpha]_D^{27}$ −41.2° (c=0.9, DMF), Rf^2 0.20, Rf^4 0.67, Rf^5 0.67. *Anal.* Calcd for $C_{65}H_{85}N_{11}O_{20}\cdot 4H_2O$: C, 55.1; H, 6.90; N, 10.9. Found: C, 55.4; H, 6.63; N, 10.6.

Boc-Pro-Val-Thr-OMe The title compound was prepared from Boc-Pro-ONp (6.82 g, 0.02 mol) and H-Val-Thr-OMe·HCl [prepared from Boc-Val-Thr-OMe[13] (7.6 g, 0.023 mol) and 4.0 N HCl/dioxane (17 ml, 0.68 mol)]. The crude product was recrystallized from AcOEt and petroleum ether, yield 7.7 g (88.1%), mp 182—184 °C, $[\alpha]_D^{27}$ −81.7° (c=1.0, MeOH), Rf^1 0.85, Rf^7 0.14. *Anal.* Calcd for $C_{20}H_{25}N_3O_7\cdot 1/4H_2O$: C, 55.3; H, 8.24; N, 9.68. Found: C, 55.0; H, 8.37; N, 9.50.

Boc-Ser-Pro-Val-Thr-OMe [Boc-(41—44)-OMe] Boc-Ser-N_3 [prepared from Boc-Ser-$NHNH_2$ (1.3 g, 6.0 mmol) and isopentyl nitrite (1.0 ml, 7.2 mmol)] in DMF (10 ml) was added to a solution of H-Pro-Val-Thr-OMe [prepared from Boc-Pro-Val-Thr-OMe (2.8 g, 6.5 mmol) and 3.2 N HCl/dioxane (10.2 ml, 32.5 mmol)] in DMF (10 ml) containing Et_3N (0.84 ml, 6.0 mmol). The reaction mixture was stirred at 4 °C for 48 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid and H_2O, dried over Na_2SO_4 and concentrated. Petroleum ether was added to the residue to give crystals, which were collected by filtration and recrystallized from AcOEt and petroleum ether, yield, 1.4 g (45.2%), mp 68—77 °C, $[\alpha]_D^{27}$ −41.2° (c=1.0, MeOH), Rf^1 0.71, Rf^3 0.58. *Anal.* Calcd for $C_{23}H_{40}N_4O_9\cdot 1/4H_2O$: C, 53.0; H, 7.83; N, 10.7. Found: C, 53.3; H, 8.07; N, 10.3.

Boc-Ser-Pro-Val-Thr-$NHNH_2$ [Boc-(41—44)-$NHNH_2$] Hydrazine hydrate (98%, 0.4 ml, 8.0 mmol) was added to a solution of Boc-Ser-Pro-Val-Thr-OMe (0.8 g, 1.6 mmol) in MeOH (10 ml). The reaction mixture was stored at room temperature overnight. A precipitate was collected by filtration and washed with MeOH, yield 0.60 g (75%), mp 225—227 °C, $[\alpha]_D^{27}$ −43.0° (c=1.0, HMPA), Rf^1 0.10, Rf^2 0.42. *Anal.* Calcd for $C_{22}H_{44}N_6O_8$: C, 51.1; H, 7.80; N, 12.3. Found: C, 50.9; H, 7.84; N, 16.3.

Z-Arg(Mts)-Tyr-OMe DPPA (1.45 ml, 5.3 mmol) was added to a solution of Z-Arg(Mts)-OH (2.2 g, 4.4 mmol) and H-Tyr-OMe·HCl (1.2 g, 5.3 mmol) in DMF (15 ml), followed by addition of Et_3N (1.35 ml, 9.7 mmol) under cooling with ice-salt. The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and H_2O, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to give crystals. The crude product was purified by silica gel column (2.5 × 39 cm) chromatography, yield 1.6 g (47.3%), mp 82—90 °C, $[\alpha]_D^{27}$ −0.5° (c=1.0, MeOH), Rf^1 0.33, Rf^3 0.18. *Anal.* Calcd for $C_{33}H_{41}N_5O_8S\cdot 1/2H_2O$: C, 58.6; H, 6.25; N, 10.3. Found: C, 58.5; H, 6.45; N, 10.0.

Boc-Leu-Arg(Mts)-Tyr-OMe The title compound was prepared from Boc-Leu-ONp (0.88 g, 2.5 mmol) and H-Arg(Mts)-Tyr-OMe [prepared from Z-Arg(Mts)-Tyr-OMe (1.48 g, 2.2 mmol) by catalytic hydrogenation]. The crude material was purified by silica gel column (2.8 × 31 cm) chromatography, yield 1.9 g (77%), mp 110—118 °C, $[\alpha]_D^{27}$ −10.9° (c=0.8, MeOH), Rf^1 0.31, Rf^3 0.15. *Anal.* Calcd for $C_{36}H_{54}N_6O_9S\cdot 1/2H_2O$: C, 57.2; H, 7.33; N, 11.1. Found: C, 57.2; H, 7.43; N, 10.8.

Boc-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OMe The title compound was prepared from Boc-Asp(OBzl)-ONp (0.85 g, 2.0 mmol) and H-Leu-Arg-(Mts)-Tyr-OMe [prepared from Boc-Leu-Arg(Mts)-Tyr-OMe (1.5 g, 2.0 mmol) and 3.8 N HCl/dioxane (5.0 ml, 19.0 mmol)]. The crude material was recrystallized from AcOEt and petroleum ether, yield 0.5 g (79%), mp 98—109 °C, $[\alpha]_D^{27}$ −20.4° (c=1.0, MeOH), Rf^1 0.40, Rf^3 0.50. *Anal* Calcd for $C_{47}H_{65}N_7O_{12}S\cdot 3/2H_2O$: C, 57.7; H, 7.00; N, 10.0. Found: C, 57.7; H, 6.85; N, 9.85.

Boc-Leu-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OMe The title compound was prepared from Boc-Leu-ONp (0.74 g, 2.1 mmol) and H-Asp-(OBzl)-Leu-Arg(Mts)-Tyr-OMe [prepared from Boc-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OMe (2.0 g, 2.1 mmol and 3.8 N HCl/dioxane (11.2 ml, 42.0 mmol)]. The crude product was purified by silica gel column (2.5 × 38 cm) chromatography, yield 1.6 g (69%), mp 108—114.5 °C, $[\alpha]_D^{27}$ −25.3° (c=0.9, MeOH), Rf^1 0.49, Rf^3 0.50. *Anal.* Calcd for $C_{53}H_{76}N_8O_{13}S$: C, 59.8; H, 7.19; N, 10.5. Found: C, 59.9; H, 7.49; N, 10.3.

Boc-Leu-Asp-Leu-Arg(Mts)-Tyr-OMe [Boc-(45—49)-OMe] The title compound was prepared from Boc-Leu-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OMe (1.38 g, 1.3 mmol) by catalytic hydrogenation, yield 1.24 g (98%), mp 127—132 °C, $[\alpha]_D^{27}$ −33.6° (c=1.0, MeOH), Rf^1 0.34, Rf^2 0.44. *Anal.* Calcd for $C_{46}H_{70}N_8O_{13}S$: C, 56.6; H, 7.23; N, 11.5. Found: C, 56.4; H, 7.29; N, 11.2.

Boc-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-OMe [Boc-(41—49)-OMe] Boc-Ser-Pro-Val-Thr-N_3 [prepared from Boc-Ser-Pro-Val-Thr-$NHNH_2$ (0.68 g, 1.34 mmol) and isopentyl nitrite (0.21 ml, 1.50 mmol)] in DMF (20 ml) and DMSO (2 ml) was added to a solution of H-Leu-Asp-Leu-Arg(Mts)-Tyr-OMe [prepared from Boc-(45—49)-OMe (0.72 g, 0.74 mmol) and 3.0 N HCl/dioxane (2.5 ml, 7.40 mmol)] in DMF (15 ml) containing Et_3N (0.10 ml, 0.74 mmol). The reaction mixture was stirred at 4 °C for 72 h. After removal of the solvent, AcOEt and H_2O were added to the residue to afford crystals. The crude material was purified by gel-filtration on Sephadex LH-20, yield 0.74 g (73.6%), mp 140—156 °C, $[\alpha]_D^{27}$ −30.3° (c=0.9, DMF), Rf^1 0.16, Rf^2 0.28. *Anal.* Calcd for $C_{63}H_{98}N_{12}O_{19}S$: C, 55.7; H, 7.26; N, 12.4. Found: C, 55.4; H, 7.49; N, 12.1.

Z-Arg(Mts)-Val-Phe-OMe DPPA (3.1 ml, 11 mmol) and Et_3N (5.9 ml, 42 mmol) were added to a solution of Z-Arg(Mts)-OH [prepared from

Z–Arg(Mts)–OH·CHA (5.0 g, 9.2 mmol) and 1 N HCl (9.2 ml)] and H–Val–Phe–OMe [prepared from Z–Val–Phe–OMe[12] (4.5 g, 11 mmol) by catalytic hydrogenation] in DMF (60 ml) under cooling with ice. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and H_2O, dried over Na_2SO_4 and concentrated. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH and ether, yield 4.9 g (71.3%), mp 137—139 °C, $[\alpha]_D^{27}$ −20.6° (c=0.95, MeOH), Rf^1 0.60, Rf^2 0.79. *Anal.* Calcd for $C_{38}H_{50}N_6O_8S$: C, 60.8; H, 6.72; N, 11.2. Found: C, 60.6; H, 6.78; N, 10.9.

Z–Val–Arg(Mts)–Val–Phe–OMe Z–Val–OPyCl (2.6 g, 8.2 mmol) and H–Arg(Mts)–Val–Phe–OMe·HCl [prepared from Z–Arg(Mts)–Val–Phe–OMe (5.1 g, 6.8 mmol) by catalytic hydrogenation] were dissolved in DMF (100 ml) containing Et_3N (0.95 ml, 6.8 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, AcOEt and H_2O were added to the residue to give crystals, yield 5.2 g (89.6%), mp 212—215 °C, $[\alpha]_D^{27}$ −9.3° (c=0.5, DMF), Rf^1 0.54, Rf^2 0.89. *Anal.* Calcd for $C_{43}H_{59}N_7O_9S$: C, 60.8; H, 7.01; N, 11.5. Found: C, 60.6; H, 7.11; N, 11.4.

Z–Arg(Mts)–Val–Arg(Mts)–Val–Phe–OMe The title compound was prepared from Z–Arg(Mts)–OH [prepared from Z–Arg(Mts)–OH·CHA (3.9 g, 7.2 mmol) and 1 N HCl (7.2 ml)] and H–Val–Arg(Mts)–Val–Phe–OMe [prepared from Z–Val–Arg(Mts)–Val–Phe–OMe (4.1 g, 4.8 mmol) by catalytic hydrogenation] by the DPPA method as described in the synthesis of Z–Arg(Mts)–Val–Phe–OMe, yield 4.6 g (80.7%), mp 165—168 °C, $[\alpha]_D^{27}$ −14.6° (c=0.5, DMF), Rf^1 0.43, Rf^2 0.82. *Anal.* Calcd for $C_{58}H_{81}N_{11}O_{12}S_2 \cdot 1/2H_2O$: C, 58.2; H, 6.91; N, 12.9. Found: C, 58.0; H, 7.04; N, 12.8.

Boc–Asn–Arg(Mts)–Val–Arg(Mts)–Val–Phe–OMe [Boc–(50—55)–OMe] The title compound was prepared from Boc–Asn–ONp (296 mg, 0.84 mmol) and H–Arg(Mts)–Val–Arg(Mts)–Val–Phe–OMe [prepared from Z–Arg(Mts)–Val–Arg(Mts)–Val–Phe–OMe (500 mg, 0.42 mmol) by catalytic hydrogenation], yield 382 mg (71.8%), mp 215—218 °C, $[\alpha]_D^{27}$ −15.5° (c=1.1, DMF), Rf^1 0.37, Rf^2 0.74. *Anal.* Calcd for $C_{58}H_{89}N_{13}O_{13}S_2 \cdot H_2O$: C, 55.5; H, 7.11; N, 14.5. Found: C, 55.3; H, 7.03; N, 14.3.

Z–Asn–Pro–Gly–OMe The title compound was prepared from Z–Asn–ONp (4.5 g, 0.012 mol) and H–Pro–Gly–OMe·HCl [prepared from Z–Pro–Gly–OMe[14] (4.0 g, 0.011 mol) by catalytic hydrogenation]. The crude product was purified by silica gel column (2.5 × 55.2 cm) chromatography, yield 2.3 g (47.5%), mp 63—65 °C, $[\alpha]_D^{27}$ −64.5° (c=1.1, DMF), Rf^1 0.48, Rf^2 0.78. *Anal.* Calcd for $C_{20}H_{26}N_4O_7$: C, 55.3; H, 6.03; N, 12.9. Found: C, 55.1; H, 6.13; N, 12.7.

Boc–Tyr–Asn–Pro–Gly–OMe [Boc–(56—59)–OMe] The title compound was prepared from Boc–Tyr–N_3 [prepared from Boc–Tyr–$NHNH_2$ (4.2 g, 0.012 mol) and isopentyl nitrite (2.45 ml, 0.012 mol) as usual] and H–Asn–Pro–Gly–OMe·HCl [prepared from Z–Asn–Pro–Gly–OMe (5.1 g, 0.012 mol) by catalytic hydrogenation], yield 5.3 g (80.3%), mp 207.5—209.5 °C, $[\alpha]_D^{27}$ −48.6° (c=0.9, DMF), Rf^1 0.28, Rf^2 0.55. *Anal.* Calcd for $C_{26}H_{37}N_5O_9$: C, 55.4; H, 6.62; N, 12.4. Found: C, 55.1; H, 6.60; N, 12.3.

Boc–Asn–Val–Val–OMe The title compound was prepared from Boc–Asn–ONp (3.5 g, 10 mmol) and H–Val–Val–OMe·HCl [prepared from Z–Val–Val–OMe[15] (3.0 g, 8 mmol) by catalytic hydrogenation], yield 2.6 g (73.3%), mp 204—207 °C, $[\alpha]_D^{27}$ −23.8° (c=1.0, DMF), Rf^1 0.40, Rf^2 0.82. *Anal.* Calcd for $C_{20}H_{36}N_4O_7$: C, 54.0; H, 8.16; N, 12.6. Found: C, 54.0; H, 8.31; N, 12.8.

Boc–Thr–Asn–Val–Val–OMe [Boc–(60—63)–OMe] DPPA (3.55 g, 12.9 mmol) and Et_3N (3.31 ml, 23.6 mmol) were added to a solution of Boc–Thr–OH (2.35 g, 11.0 mmol) and H–Asn–Val–Val–OMe·HCl [prepared from Boc–Asn–Val–Val–OMe (4.0 g, 8.9 mmol) and 7.5 N HCl/dioxane (6.0 ml, 44.7 mmol)] in DMF (50 ml) under cooling with ice. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and H_2O, dried over Na_2SO_4 and concentrated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from AcOEt, yield 4.28 g (89.2%), mp 195—198 °C, $[\alpha]_D^{27}$ −22.0° (c=0.7, DMSO), Rf^1 0.24, Rf^2 0.84. *Anal.* Calcd for $C_{24}H_{43}N_5O_9$: C, 52.8; H, 7.96; N, 12.8. Found: C, 52.9; H, 7.89; N, 12.6.

Boc–Asn–His(Bom)–OMe Boc–Asn–ONp (4.0 g, 0.011 mol) was added to a solution of H–His(Bom)–OMe·TFA [prepared from Boc–His(Bom)–OMe·HCl[25] (4.8 g, 0.011 mol) and TFA (8.3 ml, 0.11 mol) containing anisole (2.4 ml, 0.022 mol)] in DMF (60 ml) containing Et_3N (3.2 ml, 0.022 mol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with 10% citric acid. The extract was washed with AcOEt. The pH of the water layer was adjusted to 8 with Na_2CO_3 to give a precipitate, which was extracted with AcOEt. The extract was washed with H_2O, dried over Na_2SO_4 and concentrated. Petroleum ether was added to the residue to afford crystals, yield, 2.92 g (51.3%), mp 141—142 °C, $[\alpha]_D^{27}$ −6.3° (c=1.0, MeOH), Rf^2 0.75, Rf^3 0.53. *Anal.* Calcd for $C_{24}H_{35}N_5O_7 \cdot 5/2H_2O$: C, 52.4; H, 7.33; N, 12.7. Found: C, 52.6; H, 7.03; N, 12.5.

Boc–Asn–His(Bom)–$NHNH_2$ Hydrazine hydrate (90%, 1.0 ml, 20 mmol) was added to a solution of Boc–Asn–His(Bom)–OMe (2.0 g, 4.0 mmol) in MeOH (40 ml). The reaction mixture was stored at room temperature overnight. After removal of the solvent, $CHCl_3$ and H_2O were added to the residue to give crystals, yield 1.96 g (97.0%), mp 125—127 °C, $[\alpha]_D^{27}$ −34.3° (c=1.0, MeOH), Rf^2 0.46, Rf^3 0.18. *Anal.* Calcd for $C_{23}H_{35}N_7O_6 \cdot H_2O$: C, 52.8; H, 7.14; N, 18.7. Found: C, 52.7; H, 6.84; N, 18.8.

Boc–Asn–His–OMe Boc–Asn–OH (9.3 g, 0.04 mol), triphenyl phosphite (19.8 g, 0.06 mol) and imidazole (4.1 g, 0.06 mol) were added to a solution of H–His–OMe [prepared from H–His–OMe·2HCl (9.7 g, 0.04 mol) and Et_3N (11.2 ml, 0.08 mol) under ice-cooling] in DMF (60 ml) under cooling with ice. The reaction mixture was stirred at 40 °C overnight. After removal of the solvent, AcOEt (100 ml) and Na_2CO_3 (60 ml) were added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 3.3 g (28%), mp 183—185 °C, $[\alpha]_D^{27}$ −3.64° (c=0.1, DMF), Rf^2 0.41. *Anal.* Calcd for $C_{16}H_{25}N_5O_9$: C, 50.1; H, 6.59; N, 18.3. Found: C, 50.0; H, 6.69; N, 18.2.

Boc–Ala(CN)–His–OMe Boc–Asn–ONp (0.49 g, 1.39 mmol) was added to a solution of H–His–OMe [prepared from H–His–OMe·2HCl (0.49 g, 2.02 mmol) and Et_3N (0.57 ml, 4.04 mmol)] in DMF (8 ml) under cooling with ice. The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3 (1000 ml) and Na-saturated water, dried over Na_2SO_4 and concentrated, yield 0.14 g (28.1%), $[\alpha]_D^{27}$ −0.0° (c=0.1, DMF), Rf^2 0.65. *Anal.* Calcd for $C_{16}H_{23}N_5O_5 \cdot 1/2H_2O$: C, 51.3; H, 6.47; N, 18.7. Found: C, 51.4; H, 6.58; N, 18.4.

Boc–Asn–His–$NHNH_2$ Hydrazine hydrate (90%, 0.73 ml, 13.0 mmol) was added to a solution of Boc–Asn–His–OMe (1.0 g, 2.6 mmol) in DMF (20 ml). The reaction mixture was stored at room temperature for 2 d. After removal of the solvent, EtOH and ether were added to the residue to give crystals, which were collected by filtration and recrystallized from MeOH and ether, yield 96 mg (9.7%), mp 135—137 °C, $[\alpha]_D^{27}$ −24.8° (c=0.97, MeOH), Rf^2 0.19, Rf^4 0.26. *Anal.* Calcd for $C_{15}H_{25}N_7O_5 \cdot 1/4H_2O \cdot MeOH$: C, 45.7; H, 6.97; N, 23.4. Found: C, 45.9; H, 6.88; N, 23.7.

Boc–Ala(CN)–His–$NHNH_2$ Hydrazine hydrate (90%, 0.04 ml, 0.80 mmol) was added to a solution of Boc–Ala(CN)–His–OMe (0.1 g, 0.26 mmol) in DMF (2 ml). The reaction mixture was stored at room temperature. The crystals that formed were collected by filtration and recrystallized from EtOH, yield 54.7 mg (54.9%), mp 197—198 °C, $[\alpha]_D^{27}$ −25.0° (c=1.0, MeOH), Rf^2 0.19, Rf^4 0.33. *Anal.* Calcd for $C_{15}H_{23}N_7O_4$: C, 49.3; H, 6.36; N, 26.8. Found: C, 49.4; H, 6.44; N, 26.5.

Boc–Val–Gly–OBzl A mixed anhydride [prepared from Boc–Val–OH (3.56 g, 0.015 mol) and ethyl chloroformate (1.5 ml, 0.015 mol) as usual] in THF (100 ml) was added to a solution of H–Gly–OBzl·Tos–OH (5.00 g, 0.015 mol) in DMF (50 ml) containing Et_3N (2.1 ml, 0.015 mol) under cooling with ice. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated. Petroleum ether was added to the residue to afford crystals. The crude product was purified by silica gel column (3.0 × 42.0 cm) chromatography, yield 4.17 g (76.0%), mp 67—69 °C, $[\alpha]_D^{27}$ −25.8° (c=1.0, MeOH), Rf^3 0.58, Rf^7 0.49. *Anal.* Calcd for $C_{19}H_{28}N_2O_5$: C, 62.6; H, 7.74; N, 7.69. Found: C, 62.7; H, 7.81; N, 7.60.

Boc–Val–Pro–$NHNH_2$ Hydrazine hydrate (90%, 2.0 ml, 0.036 mol) was added to a solution of Boc–Val–Pro–OMe[26] (4.35 g, 0.012 mol) in EtOH (15 ml). The reaction mixture was stored at room temperature overnight. After removal of the solvent, the residue was extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4 and concentrated, yield 3.5 g (81.4%), amorphous, $[\alpha]_D^{27}$ −75.6° (c=1.0, MeOH), Rf^1 0.45, Rf^2 0.80. *Anal.* Calcd for $C_{19}H_{26}N_2O_5 \cdot 1/2H_2O$: C, 53.8; H, 8.68; N, 16.6. Found: C, 53.7; H, 8.68; N, 16.3.

Boc–Val–Pro–His–OMe Boc–Val–Pro–N_3 [prepared from Boc–Val–Pro–$NHNH_2$ (13.8 g, 0.042 mol) and isopentyl nitrite (5.9 ml, 0.042 mol) as usual] in DMF (100 ml) was added to a solution of H–His–OMe [prepared from H–His–OMe·2HCl (10.2 g, 0.042 mol) and Et_3N (11.7 ml,

0.084 mol)] in DMF (50 ml) under cooling with ice. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and H_2O, dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel column (3.8 × 62.0 cm) chromatography to give an oily material, yield 13.0 g (66.7%), $[\alpha]_D^{27}$ −55.3° (c = 1.0, MeOH), Rf^1 0.33, Rf^2 0.77. Amino acid ratios in an acid hydrolysate: Val 1.00; Pro 1.00; His 0.95 (average recovery 78.9%).

Boc–Val–Pro–His–$NHNH_2$ Hydrazine hydrate (90%, 4.7 ml, 0.084 mol) was added to a solution of Boc–Val–Pro–His–OMe (13.0 g, 0.028 mol) in EtOH (60 ml). The mixture was stored at room temperature overnight. After removal of the solvent, the residue was extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford a precipitate, which was collected by filtration, yield 8.65 g (66.5%), $[\alpha]_D^{27}$ −69.2° (c = 1.0, MeOH), Rf^2 0.53, Rf^4 0.38. *Anal.* Calcd for $C_{21}H_{35}N_7O_5 \cdot H_2O$: C, 53.1; H, 7.87; N, 20.6. Found: C, 53.2; H, 7.57; N, 20.4.

Boc–Val–Pro–His–Val–Gly–OBzl Boc–Val–Pro–His–N_3 [prepared from Boc–Val–Pro–His–$NHNH_2$ (500 mg, 1.07 mmol) and isopentyl nitrite (0.2 ml, 1.07 mmol) as usual] in DMF (20 ml) was added to a solution of H–Val–Gly–OBzl·TFA [prepared from Boc–Val–Gly–OBzl (390 mg, 1.07 mmol) and TFA (0.8 ml, 10.7 mmol) containing anisole (0.6 ml, 5.35 mmol)] in DMF (10 ml) containing Et_3N (0.2 ml, 1.07 mmol) under cooling with ice. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and H_2O, dried over Na_2SO_4 and concentrated. Ether was added to the residue to afford crystals, yield 539 mg (72.3%), mp 110—115 °C, $[\alpha]_D^{27}$ −72.7° (c = 1.0, MeOH), Rf^2 0.74, Rf^3 0.26. *Anal.* Calcd for $C_{35}H_{51}N_7O_8 \cdot H_2O$: C, 58.7; H, 7.48; N, 13.7. Found: C, 58.6; H, 7.45; N, 13.5.

Boc–Asn–His(Bom)–Val–Pro–His–Val–Gly–OBzl [Boc–(64—70)–OBzl] Boc–Asn–His(Bom)–N_3 [prepared from Boc–Asn–His(Bom)–$NHNH_2$ (500 mg, 0.99 mmol) and isopentyl nitrite (0.14 ml, 0.99 mmol) as usual] was added to a solution of H–Val–Pro–His–Val–Gly–OBzl·TFA [prepared from Boc–Val–Pro–His–Val–Gly–OBzl (460 mg, 0.66 mmol) and TFA (0.49 ml, 6.6 mmol) containing anisole (0.15 ml, 1.32 mmol)] in DMF (5 ml) containing Et_3N (0.19 ml) under cooling with ice. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, AcOEt and 5% Na_2CO_3 were added to the residue to give crystals, yield 509 mg (72.2%), mp 202.5—203 °C, $[\alpha]_D^{27}$ −41.9° (c = 1.3, DMF), Rf^2 0.50, Rf^4 0.17. *Anal.* Calcd for $C_{53}H_{74}N_{12}O_{12} \cdot 1/2H_2O$: C, 58.9; H, 7.01; N, 15.5. Found: C, 58.7; H, 6.86; N, 15.6.

General Procedure of Final Deprotection A: A protected peptide was treated with TFA in the presence of anisole and *m*-cresol in the case of a Tyr-containing peptide, in an ice-bath for 15 min and at room temperature for 60 min, and dry ether was added to the solution. The resulting powder was collected by filtration.

B: A protected peptide was treated with MSA in the presence of anisole or thioanisole and *m*-cresol in the case of a Tyr-containing peptide, in an ice-bath for 30 min and at room temperature for 90 min, and dry ether was added to the solution. The resulting powder was collected by centrifugation, and dissolved in H_2O. The solution was treated with Amberlite IRA 45 (acetate form) for 30 min and filtered. The pH of the filtrate was adjusted to 8 with 1 N NH_4OH. After 30 min, the pH of the solution was readjusted to 6.5 with 1 N AcOH and the mixture was lyophilized to give a hygroscopic powder.

C: A protected peptide was treated with HCl/dioxane in an ice-bath for 10 min and at room temperature for 60 min and ether was added to the solution. The resulting powder was collected by filtration.

D: A protected peptide was treated with 1 M TMSBr–thioanisole/TFA in the presence of *m*-cresol in an ice-bath for 3 h, and dry ether was added to the solution. The resulting powder was collected by centrifugation and dissolved in H_2O; the resulting solution was treated with Amberlite IRA 45 (acetate form) for 30 min. The pH of the filtrate was adjusted to 8 with 1 N NH_4OH and after 30 min, readjusted to 6.5 with 1 N AcOH. The solvent was removed by lyophilization to give a hygroscopic powder.

Assay Procedure of Leukocyte Elastase, Cathepsin G and α-Chymotrypsin Leukocyte elastase[27,28] and cathepsin G[29] were purified by affinity chromatography using a Suc–L-Tyr–D-Leu–D-Val–pNA Sepharose column from crude extracts of human leukocytes. α-Chymotrypsin was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Enzymatic activities were determined by the method described previously[28] using Suc–Ala–Tyr–Leu–Val–pNA[30] for leukocyte elastase and Suc–Ile–Pro–Phe–pNA[31] for cathepsin G and α-chymotrypsin. The effects of synthetic peptides on the enzymes were determined as follows. Synthetic peptide was dissolved in MeOH (the final concentration of MeOH was 4—7%). Enzymatic activity was assayed in the presence and absence of the peptide to be examined.

References and Notes

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2) Amino acids, peptide and their derivatives mentioned in this paper are of the L-configuration except in the case of glycine. Standard abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 3458 (1966); *idem*, *ibid.*, **6**, 362 (1967); *idem*, *ibid.*, **11**, 1726 (1972). Other abbreviations used are: Ala(CN), β-cyanoalanine; Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; OBzl, benzyl ester; Bom, benzyloxymethyl; ONp, *p*-nitrophenyl ester; OPyCl, 6-chloro-2-pyridyl ester; DCC, *N,N'*-dicyclohexylcarbodiimide; DPPA, diphenylphosphorylazide; HOBt, *N*-hydroxybenzotriazole; DMF, dimethylformamide; HMPA, hexamethylphosphoramide; CHA, cyclohexylamine; Tos–OH, *p*-toluenesulfonic acid; AcOEt, ethyl acetate; TFA, trifluoroacetic acid; MSA, methanesulfonic acid; TMSBr, trimethylsilyl bromide; AcOH, acetic acid; *n*-BuOH, *n*-butanol; DMSO, dimethyl sulfoxide; Suc, succinyl; pNA, *p*-nitroanilide.
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