Amino Acids and Peptides. XXIX. Synthesis of Peptide Fragments Related to Active Center of Eglin c and Studies on the Relationship between Their Structure and Their Inhibitory Activity against Cathepsin G and α-Chymotrypsin[1,2]

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H-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg-Tyr-OMe, corresponding to the sequence 41—49 of eglin c, inhibited human leukocyte cathepsin G and α-chymotrypsin. In order to gain further insight into the relationship between the structure and the inhibitory activity against cathepsin G and α-chymotrypsin, peptide fragments related to the above nonapeptide were synthesized by a conventional solution method and their inhibitory activities were examined. The smallest peptide which exhibited inhibitory effects on the above enzymes was H-Pro-Val-Thr-Leu-OMe, corresponding to the sequence 42—45 of eglin c.

Keywords eglin c active center; related peptide; chemical synthesis; inhibitor; leukocyte cathepsin G; α-chymotrypsin; structure–activity relationship

Eglin c, isolated from the leech *Hirudo medicinalis*,[3] consists of 70 amino acid residues[4] (Fig. 1) and effectively inhibits chymotrypsin and subtilisin as well as leukocyte elastase and cathepsin G. The latter two enzymes have become the focus of increasing attention 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 therapeutic agent of emphysema and inflammation. Rink *et al.*[7] prepared N^{α}-acetyleglin c by means of genetic techniques,[7] although its molecular weight is too large for practical therapeutic use.

Previously, we reported that a small peptide, H-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg-Tyr-OMe, which corresponds to the sequence 41—49 of eglin c, and is a binding loop of eglin c involved in direct contact with subtilisin,[8] exhibited significant inhibitory activity against cathepsin G and α-chymotrypsin but not against leukocyte elastase,

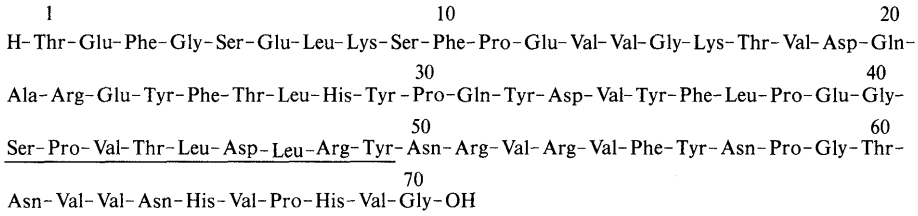

Fig. 1. Primary Structure of Eglin c

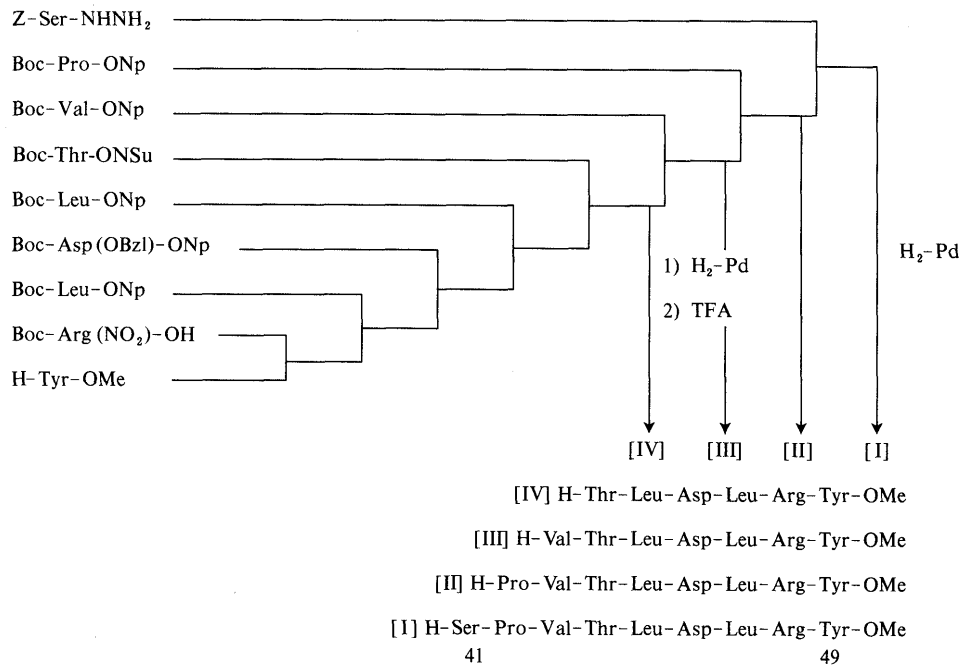

Fig. 2. Synthetic Scheme for N-Terminally Shortened Peptides of Eglin c (41—49)

TABLE I. Yield, $[\alpha]_D$ Value, *Rf* Values and Amino Acid Ratios of Peptides [I]—[VIII] and Related Peptides

Compound	Yield (%)	$[\alpha]_D$ (°) (Solvent)	TLC		Amino acid analysis (average recovery, %)							
			Rf^2	Rf^4	Ser	Pro	Val	Thr	Leu	Asp	Arg	Tyr
Boc-(42—49)-OMe	92.8	−72.0 (MeOH)	0.50			1.1	1.0	1.0	2.2	1.1	1.0	0.9 (81)
Boc-(43—49)-OMe	86.4	−86.0 (MeOH)	0.39				1.0	0.9	1.9	1.0	1.0	0.8 (80)
Boc-(44—49)-OMe	76.5	−3.9 (MeOH)	0.38					1.0	2.1	1.0	1.0	1.0 (79)
H-(41—49)-OMe [I]	61.5	−114.0 (3% AcOH)		0.64	1.0	1.1	1.1	1.0	2.2	1.1	1.1	1.0 (74)
H-(42—49)-OMe [II]	54.0	−88.0 (3% AcOH)		0.63		1.0	1.0	1.0	2.1	1.1	1.0	0.9 (70)
H-(43—49)-OMe [III]	95.1	−30.0 (3% AcOH)		0.68			1.1	1.0	2.2	1.1	1.0	0.9 (74)
H-(44—49)-OMe [IV]	62.9	−29.5 (3% AcOH)		0.70				1.0	2.1	1.1	1.0	0.9 (80)
H-(42—48)-OMe [V]	95.9	−116.0 (3% AcOH)		0.45		0.9	1.1	1.0	2.0	1.0	0.9 (77)	
H-(42—47)-OMe [VI]	92.5	−100.0 (3% AcOH)		0.54		0.9	1.0	1.0	2.0	1.0 (79)		
H-(42—46)-OMe [VII]	92.2	−96.0 (3% AcOH)		0.64		0.9	1.1	1.0	1.0	1.0 (78)		
H-(42—45)-OMe [VIII]	86.2	−89.0 (3% AcOH)		0.68		0.9	1.0	0.9	1.0 (78)			

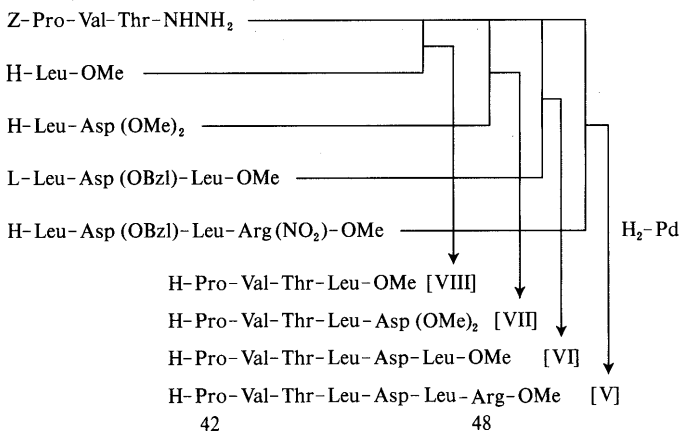

Fig. 3. Synthetic Scheme for C-Terminally Shortened Peptides of Eglin c (42—49)

TABLE II. K_i Values of Peptides [I]—[VIII] and Related Peptides

Compound	K_i (mM)	
	Cathepsin G[a]	α-Chymotrypsin[a]
Boc-(42—49)-OMe	0.040	0.010
Boc-(43—49)-OMe	0.050	0.030
Boc-(44—49)-OMe	0.23	0.12
H-(41—49)-OMe [I]	0.022	0.0070
H-(42—49)-OMe [II]	0.040	0.012
H-(43—49)-OMe [III]	0.23	0.040
H-(44—49)-OMe [IV]	1.7	0.090
H-(45—49)-OMe[b]	>2.0	>2.0
H-(42—48)-OMe [V]	0.16	0.025
H-(42—47)-OMe [VI]	0.63	0.27
H-(42—46)-OMe [VII]	0.39	0.19
H-(42—45)-OMe [VIII]	0.10	0.30

a) The reaction mixture contained 0.5 mM inhibitor. *b*) See ref. 1. The reaction was carried out in Tris–HCl buffer (0.1 M, pH 8.0) containing 0.2 M NaCl.

although eglin c itself potently inhibited all three enzymes.[1)]

This paper deals with systematic synthesis of peptides related to the above nonapeptide and examination of their inhibitory effects on cathepsin G and α-chymotrypsin.

For the peptide synthesis, the guanidino group of Arg was protected with an NO_2 group, which can be removed by catalytic hydrogenation, in combination with the (TFA)-labile Boc-group as the N^{α}-protecting group.

Since H–Ser–Pro–Val–Thr–OMe (eglin c 41—44) and H–Leu–Asp–Leu–Arg–Tyr–OMe (eglin c 45—49) did not exhibit any detectable inhibitory activity,[1)] four kinds of N-terminally shortened peptides of eglin c (41—49) were synthesized as illustrated in Fig. 2. Starting with H–Tyr–OMe, stepwise coupling of Boc-amino acid was carried out to give the desired protected peptides. The protected peptide was hydrogenated, followed by treatment with TFA. The homogeneity of peptides obtained was ascertained by thin-layer chromatography (TLC) and amino acid analysis of an acid hydrolysate, and these results are summarized in Table I.

Next, C-terminally shortened peptides of eglin c (42—49) were synthesized as shown in Fig. 3. The homogeneity of peptides obtained was ascertained by TLC and amino acid analysis of acid hydrolysate, and the results are summarized in Table I.

The inhibitory effects of the above peptides on cathepsin G and α-chymotrypsin were examined and the results are summarized in Table II. As can be seen, inhibitory activity against cathepsin G and α-chymotrypsin decreased in parallel with the decrease of chain length from the N-terminus. With regard to inhibitory activity against cathepsin G, eglin c (42—49) exhibited similar activity to eglin c (41—49) ($K_i = 4.0 \times 10^{-5}$ and 2.0×10^{-5} M, respectively), while the K_i value of eglin c (43—49) was 2.3×10^{-4} M. Therefore, the Pro residue at position 42 is required for manifestation of potent inhibitory activity. Moreover, amino blocking group increased inhibitory ac-

tivity against cathepsin G.

From the results of the examination of the inhibitory activity of the C-terminally shortened peptides, it was found that the smallest active center peptide of eglin c, which can exhibit inhibitory activity against cathepsin G and α-chymotrypsin, was H–Pro–Val–Thr–Leu–OMe, (eglin c 42—45), because eglin c (41—44) did not inhibit either of the enzymes.[1] The fact that elongation of the Leu residue at position 45 of eglin c manifested inhibitory activity is compatible with the report that the Leu^{45}–Asp^{46} sequence is the reactive site of eglin c.[8]

In conclusion, these results suggest to us that modification at the N- and/or C-terminus of the Pro–Val–Thr–Leu sequence might afford a potent cathepsin G inhibitor of small molecular size which would be suitable for practical therapeutic use for emphysema.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co.). Amino acid compositions of an acid hydrolysate (6 N HCl, 110 °C, 20 h) were determined with an amino acid analyzer, K-101 AS (Kyowa Seimitsu Co.). On TLC (Kieselgel G, Merck), Rf^1, Rf^2, Rf^3 and Rf^4 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), *n*-BuOH, AcOH and H_2O (4:1:5, upper phase) and *n*-BuOH, pyridine, AcOH and H_2O (4:1:1:2).

Boc-Arg(NO_2)-Tyr-OMe A mixed anhydride [prepared from Boc–Arg(NO_2)–OH (10 g, 0.031 mol) and ethyl chloroformate (3.0 ml, 0.031 mol) as usual] in DMF (100 ml) was combined with H–Tyr–OMe·HCl (7.3 g, 0.031 mol) in DMF (100 ml) containing Et_3N (4.4 ml, 0.031 mol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. The oily residue was purified by silica gel column (3.7 × 62 cm) chromatography, equilibrated and eluted with 3% MeOH in $CHCl_3$, yield 9.2 g (59.0%), amorphous powder, $[\alpha]_D^{25}$ −0.8° (c=0.5, DMF), Rf^1 0.30, Rf^2 0.70. *Anal.* Calcd for $C_{21}H_{31}N_6O_8 \cdot 1/4H_2O$: C, 50.3; H, 6.64; N, 16.8. Found: C, 50.4; H, 6.72; N, 16.5.

Boc-Leu-Arg(NO_2)-Tyr-OMe Boc–Leu–ONp (4.0 g, 0.011 mol) and H–Arg(NO_2)–Tyr–OMe·HCl [prepared from Boc–Arg(NO_2)–Tyr–OMe (5.5 g, 0.011 mol) and 6.0 N HCl/dioxane (17.3 ml, 0.11 mol) as usual] were dissolved in DMF (150 ml) containing Et_3N (1.6 ml, 0.011 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 evaporated down. The residue was purified by silica gel column (3.3 × 41 cm) chromatography, equilibrated and eluted with 3% MeOH in $CHCl_3$, and the product was crystallized from AcOEt and petroleum ether, yield 6.0 g (89.0%), mp 72—75 °C (dec.), $[\alpha]_D^{25}$ −22.0° (c=0.5, DMF), Rf^1 0.20, Rf^2 0.54. *Anal.* Calcd for $C_{27}H_{43}N_7O_9$: C, 53.2; H, 7.11; N, 16.1. Found: C, 53.4; H, 7.32; N, 15.9.

Boc-Asp(OBzl)-Leu-Arg(NO_2)-Tyr-OMe Boc–Asp(OBzl)–ONp (4.0 g, 9.0 mmol) and H–Leu–Arg(NO_2)–Tyr–OMe·TFA [prepared from Boc–Leu–Arg(NO_2)–Tyr–OMe (5.9 g, 9.7 mmol), TFA (7.2 ml, 97 mmol) and anisole (2.1 ml, 19 mmol) as usual] were dissolved in DMF (100 ml) containing Et_3N (1.6 ml, 11 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 evaporated down. The residue was purified by silica gel column (3.7 × 40 cm) chromatography, equilibrated and eluted with 2% MeOH in $CHCl_3$. The product was recrystallized from AcOEt and petroleum ether, yield 5.3 g (67.7%), mp 82—85 °C (dec.), $[\alpha]_D^{25}$ −16.8° (c=1.0, DMF), Rf^1 0.35, Rf^2 0.70. *Anal.* Calcd for $C_{38}H_{54}N_8O_{12}$: C, 56.0; H, 6.68; N, 13.8. Found: C, 56.0, H, 6.97; N, 13.4.

Boc-Leu-Asp(OBzl)-Leu-Arg(NO_2)-Tyr-OMe Boc–Leu–ONp (4.0 g, 11 mol) and H–Asp(OBzl)–Leu–Arg(NO_2)–Tyr–OMe·TFA [prepared from Boc–Asp(OBzl)–Leu–Arg(NO_2)–Tyr–OMe (5.2 g, 6.3 mmol), TFA (4.8 ml, 64 mmol) and anisole (1.4 ml, 13 mmol) as usual] were dissolved in DMF (100 ml) containing Et_3N (1.1 ml, 7.8 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 afford a gelatinous solid, which was collected by filtration and recrystallized from MeOH, yield 3.9 g (66.7%), mp 186—191 °C, $[\alpha]_D^{25}$ −24.2° (c=1.0, DMF), Rf^1 0.38, Rf^2 0.81. *Anal.* Calcd for $C_{44}H_{65}N_9O_{13} \cdot 1/4H_2O$: C, 56.7; H, 7.08; N, 13.5. Found: C, 56.4; H, 7.03; N, 13.5.

Boc-Thr-Leu-Asp(OBzl)-Leu-Arg(NO_2)-Tyr-OMe Boc–Thr–ONSu (1.6 g, 51 mmol) and H–Leu–Asp(OBzl)–Leu–Arg(NO_2)–Tyr–OMe·TFA [prepared from Boc–Leu–Asp(OBzl)–Leu–Arg(NO_2)–Tyr–OMe (3.8 g, 4.1 mmol), TFA (3.1 ml, 41 mmol) and anisole (0.9 ml, 8.2 mmol) as usual] were dissolved in DMF (100 ml) containing Et_3N (0.7 ml, 5.0 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 a gelatinous solid, which was collected by filtration and recrystallized from MeOH, yield 3.2 g (74.1%), mp 151—155 °C, $[\alpha]_D^{25}$ −25.4° (c=1.0, DMF), Rf^1 0.36, Rf^2 0.66. *Anal.* Calcd for $C_{48}H_{72}N_{10}O_{15} \cdot 1/2H_2O$: C, 55.5; H, 7.09; N, 13.5. Found: C, 55.6; H, 7.17; N, 13.4.

Boc-Val-Thr-Leu-Asp(OBzl)-Leu-Arg(NO_2)-Tyr-OMe Boc–Val–ONp (1.1 g, 3.3 mmol) and H–Thr–Leu–Asp–(OBzl)–Leu–Arg(NO_2)–Tyr–OMe·TFA [prepared from Boc–Thr–Leu–Asp(OBzl)–Leu–Arg(NO_2)–Tyr–OMe (2.8 g, 2.7 mmol), TFA (4.1 ml, 55 mmol) and anisole (0.6 ml, 5.5 mmol) as usual] were dissolved in DMF (70 ml) containing Et_3N (0.5 ml, 3.5 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 afford a gelatinous solid, which was collected by filtration and recrystallized from MeOH, yield 1.9 g (64.2%), mp 215—218 °C, $[\alpha]_D^{25}$ −20.2° (c=1.0, DMF), Rf^1 0.32, Rf^2 0.75. *Anal.* Calcd for $C_{53}H_{81}N_{11}O_{16} \cdot H_2O$: C, 55.5; H, 7.30; N, 13.4; Found: C, 55.3; H, 7.20; N, 13.3.

Boc-Pro-Val-Thr-Leu-Asp(OBzl)-Leu-Arg(NO_2)-Tye-OMe Boc–Pro–ONp (0.6 g, 1.8 mmol) and H–Val–Thr–Leu–Asp(OBzl)–Leu–Arg–(NO_2)–Tyr–OMe·TFA [prepared from Boc–Val–Thr–Leu–Asp(OBzl)–Leu–Arg(NO_2)–Tyr–OMe (1.6 g, 1.4 mmol), TFA (2.2 ml, 30 mmol) and anisole (0.32 ml, 3.0 mmol) as usual] were dissolved in DMF (60 ml) containing Et_3N (0.24 ml, 1.7 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 afford a gelatinous solid, which was collected by filtration and recrystallized from MeOH, yield 1.7 g (95.4%), mp 193–198 °C, $[\alpha]_D^{25}$ −46.0° (c=0.1, DMF), Rf^1 0.27, Rf^2 0.72. *Anal.* Calcd for $C_{58}H_{88}N_{12}O_{17} \cdot 3/2H_2O$: C, 55.6; H, 7.32; N, 13.4. Found: C, 55.4; H, 7.22; N, 13.3.

Z-Ser-Pro-Val-Thr-Leu-Asp(OBzl)-Leu-Arg(NO_2)-Tyr-OMe Z–Ser–N_3 [prepared from Z–Ser–$NHNH_2$ (0.32 g, 1.3 mmol) and isopentyl nitrite (0.18 ml) as usual] in DMF (20 ml) was added to a solution of H–Pro–Val–Thr–Leu–Asp(OBzl)–Leu–Arg(NO_2)–Tyr–OMe·TFA [prepared from Boc–Pro–Val–Thr–Leu–Asp(OBzl)–Leu–Arg(NO_2)–Tyr–OMe (1.3 g, 1.1 mmol), TFA (1.6 ml, 22 mmol) and anisole (0.23 ml, 2.2 mmol) as usual] in DMF (30 ml) containing Et_3N (0.18 ml, 1.3 mmol). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, AcOEt and H_2O were added to the residue to afford a gelatinous solid, which was collected by filtration and recrystallized from MeOH, yield 0.9 g (64.0%), mp 127—131 °C, $[\alpha]_D^{25}$ −32.0° (c=0.1, DMF), Rf^1 0.15, Rf^2 0.60. *Anal.* Calcd for $C_{64}H_{91}N_{13}O_{19} \cdot 3H_2O$: C, 54.9; H, 6.98; N, 13.0. Found: C, 55.0; H, 7.23; N, 13.0.

Boc-Leu-Arg(NO_2)-OMe The title compound was prepared from Boc–Leu–ONp (5.0 g, 0.014 mol) and H–Arg(NO_2)–OMe·HCl (3.2 g, 0.012 mol) and purified by silica gel column chromatography, yield 5.0 g (78.9%), mp 72—74 °C, $[\alpha]_D^{25}$ −24.4° (c=0.5, MeOH), Rf^1 0.66, Rf^2 0.70. *Anal.* Calcd for $C_{18}H_{34}N_6O_7$: C, 48.7; H, 7.68; N, 18.8. Found: C, 48.1; H, 7.78; N, 18.5.

Boc-Asp(OBzl)-Leu-Arg(NO_2)-OMe The title compound was prepared from Boc–Asp(OBzl)–ONp (4.4 g, 9.0 mmol) and H–Leu–Arg(NO_2)–OMe [prepared from Boc–Leu–Arg(NO_2)–OMe (4.5 g, 10 mmol), TFA (7.6 ml, 102 mmol) and anisole (2.1 ml, 20 mmol) as usual] and purified by silica gel column chromatography, yield 5.4 g (82.4%), mp 67—72 °C, $[\alpha]_D^{25}$ −29.7° (c=0.3, DMF), Rf^1 0.54. *Anal.* Calcd for $C_{29}H_{45}N_7O_{10}$: C, 53.5; H, 6.96; N, 15.1. Found: C, 53.7; H, 7.21; N, 14.8.

Boc-Leu-Asp(OBzl)-Leu-Arg(NO_2)-OMe The title compound was prepared from Boc–Leu–ONp (1.3 g, 3.7 mmol) and H–Asp(OBzl)–Leu–Arg(NO_2)–OMe [prepared from Boc–Asp(OBzl)–Leu–Arg(NO_2)–OMe (2.0 g, 3.1 mmol), TFA (2.4 ml, 32 mmol) and anisole (0.7 ml, 6.4 mmol) as usual] and recrystallized from AcOEt, yield 1.6 g (67.3%), mp

158—162 °C, $[\alpha]_D^{25}$ −33.3° (c=0.3, DMF), Rf^1 0.56. *Anal.* Calcd for $C_{35}H_{55}N_8O_{11}$: C, 55.0; H, 7.38; N, 14.7. Found: C, 54.9; H, 7.52; N, 14.5.

Boc-Asp(OBzl)-Leu-OMe The title compound was prepared from Boc-Asp(OBzl)-ONp (4.0 g, 9.0 mmol) and H-Leu-OMe·HCl (1.8 g, 9.9 mmol) and purified by silica gel column chromatography, yield 2.5 g (63.1%), mp 43—45 °C, $[\alpha]_D^{25}$ −31.0° (c=0.4, MeOH), Rf^1 0.78, Rf^2 0.85. *Anal.* Calcd for $C_{23}H_{34}N_2O_7$: C, 61.3; H, 7.61; N, 6.22. Found: C, 61.2; H, 7.64; N, 6.23.

Boc-Leu-Asp(OBzl)-Leu-OMe The title compound was prepared from Boc-Leu-ONp (1.9 g, 5.4 mmol) and H-Asp(OBzl)-Leu-OMe·TFA [prepared from Boc-Asp(OBzl)-Leu-OMe (2.4 g, 5.3 mmol), TFA (3.0 ml, 41 mmol) and anisole (1.2 ml, 11 mmol) as usual] and recrystallized from ether, yield 1.4 g (47.4%), mp 113—116 °C, $[\alpha]_D^{25}$ −17.2° (c=0.5, MeOH), Rf^1 0.63. *Anal.* Calcd for $C_{29}H_{45}N_3O_8$: C, 61.8; H, 8.05; N, 7.45. Found: C, 61.8; H, 8.04; N, 7.61.

Boc-Leu-Asp(OMe)$_2$ The title compound was prepared from Boc-Leu-ONp (3.1 g, 8.8 mmol) and H-Asp(OMe)$_2$·HCl (2.0 g, 10 mmol) and recrystallized from ether, yield 1.9 g (57.2%), mp 90—94 °C, $[\alpha]_D^{25}$ −88.0° (c=0.1, MeOH), Rf^1 0.65, Rf^2 0.66. *Anal.* Calcd for $C_{17}H_{30}N_2O_7$: C, 54.5; H, 8.08; N, 7.48. Found: C, 54.5; H, 8.07; N, 7.55.

Z-Pro-Val-Thr-OMe The title compound was prepared from Z-Pro-ONp (7.7 g, 0.021 mol) and H-Val-Thr-OMe·TFA [prepared from Boc-Val-Thr-OMe[9] (6.9 g, 0.021 mol), TFA (16.1 ml, 0.22 mol) and anisole (4.5 ml, 0.041 mol) as usual] and recrystallized from AcOEt, yield 7.2 g (74.5%), mp 176—180 °C, $[\alpha]_D^{25}$ −37.0° (c=0.2, DMF), Rf^1 0.58, Rf^2 0.69. *Anal.* Calcd for $C_{23}H_{33}N_3O_7$: C, 59.6; H, 7.18; N, 9.07. Found: C, 59.8; H, 7.17; N, 9.11.

Z-Pro-Val-Thr-NHNH$_2$ Hydrazine hydrate (90%, 3.2 ml, 0.057 mol) was added to a solution of Z-Pro-Val-Thr-OMe (6.0 g, 0.013 mol) in MeOH (150 ml). The reaction mixture was stored at room temperature overnight to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 5.8 g (96.1%), mp 247—259 °C, $[\alpha]_D^{25}$ −32.0° (c=0.1, DMSO), Rf^1 0.21, Rf^2 0.57. *Anal.* Calcd for $C_{22}H_{33}N_5O_6$: C, 57.0; H, 7.18; N, 15.1. Found: C, 57.2; H, 7.17; N, 15.0.

Z-Pro-Val-Thr-Leu-OMe The title compound was prepared from Z-Pro-Val-Thr-N_3 [prepared from Z-Pro-Val-Thr-NHNH$_2$ (0.4 g, 0.86 mmol) and isopentyl nitrite as usual] and H-Leu-OMe·HCl (0.19 g, 1.05 mmol) and recrystallized from MeOH, yield 0.25 g (50.9%), mp 141—146 °C, $[\alpha]_D^{25}$ −42.0° (c=0.1, DMF), Rf^1 0.58, Rf^2 0.75. *Anal.* Calcd for $C_{29}H_{44}N_4O_8$: C, 60.4; H, 7.69; N, 9.72. Found: C, 60.1; H, 7.56; N, 9.78.

Z-Pro-Val-Thr-Leu-Asp(OMe)$_2$ The title compound was prepared from Z-Pro-Val-Thr-N_3 [prepared from Z-Pro-Val-Thr-NHNH$_2$ (0.93 g, 2.0 mmol) and isopentyl nitrite] and H-Leu-Asp(OMe)$_2$ [prepared from Boc-Leu-Asp(OMe)$_2$ (0.9 g, 2.4 mmol), TFA (1.5 ml, 20 mmol) and anisole (0.5 ml, 4.6 mmol)] and recrystallized from MeOH, yield 0.53 g (33.9%), mp 185—189 °C, $[\alpha]_D^{25}$ −45.3° (c=0.1, DMF), Rf^1 0.58, Rf^2 0.67. *Anal.* Calcd for $C_{34}H_{51}N_5O_{11}\cdot H_2O$: C, 56.4; H, 7.38; N, 9.68. Found: C, 56.7; H, 7.18; N, 9.78.

Z-Pro-Val-Thr-Leu-Asp(OBzl)-Leu-OMe The title compound was prepared from Z-Pro-Val-Thr-N_3 [prepared from Z-Pro-Val-Thr-NHNH$_2$ (0.79 g, 1.7 mmol) and isopentyl nitrite (0.24 ml, 1.7 mmol)] and H-Leu-Asp(OBzl)-Leu-OMe·TFA [prepared from Boc-Leu-Asp(OBzl)-Leu-OMe (0.8 g, 1.4 mmol), TFA (2.2 ml, 29 mmol) and anisole (0.3 ml, 2.8 mmol)] and recrystallized from MeOH, yield 0.53 g, (59.3%), mp 220—223 °C, $[\alpha]_D^{25}$ −64.0° (c=0.1, DMF), Rf^1 0.55, Rf^2 0.85. *Anal.* Calcd for $C_{46}H_{66}N_6O_{12}\cdot 2H_2O$: C, 59.3; H, 7.58; N, 9.03. Found: C, 59.2; H, 7.33; N, 9.02.

Z-Pro-Val-Thr-Leu-Asp(OBzl)-Leu-Arg(NO$_2$)-OMe The title compound was prepared from Z-Pro-Val-Thr-N_3 [prepared from Z-Pro-Val-Thr-NHNH$_2$ (0.79 g, 1.7 mmol) and isopentyl nitrite (0.24 ml, 1.7 mmol)] and H-Leu-Asp(OBzl)-Leu-Arg(NO$_2$)-OMe·TFA [prepared from Boc-Leu-Asp(OBzl)-Leu-Arg(NO$_2$)-OMe (0.77 g, 1.0 mmol), TFA (1.5 ml, 20 mmol) and anisole (0.22 ml, 2.0 mmol)] and recrystallized from MeOH, yield 0.26 g (23.9%), mp 214—216 °C, $[\alpha]_D^{25}$ −29.0° (c=0.2, DMF), Rf^1 0.27, Rf^2 0.67. *Anal.* Calcd for $C_{52}H_{77}N_{11}O_{15}\cdot 7H_2O$: C, 51.1; H, 7.50; N, 12.6. Found: C, 51.5; H, 7.30; N, 12.4.

General Procedure for Deprotection of Protected Peptides A protected peptide (100 mg) in 50% AcOH (10 ml) was hydrogenated over a Pd catalyst. After removal of Pd and the solvent, the residue was dissolved in H_2O and lyophilized to give an amorphous powder. The Boc group, if necessary, was removed by using TFA-anisole. Yield, $[\alpha]_D$ and Rf values and the result of amino acid analysis of an acid hydrolysate are summarized in Table I.

Assay Procedure of Inhibitory Activity Toward Cathepsin G and α-Chymotrypsin Cathepsin G[10] was purified by affinity chromatography using a Suc-L-Tyr-D-Leu-D-Val-*p*NA Sepharose column from crude extract of human leukocytes. α-Chymotrypsin was purchased from Sigma Chemical Co., St. Louis. Enzymatic activities were determined by the method described previously[11] using Suc-Ile-Pro-Phe-*p*NA.[12] The effects of synthetic peptides on the enzymes were determined as follows. Synthetic peptide was dissolved in MeOH (final concentration of MeOH was 4—7%) and the enzymatic activity was assayed in the presence and absence of the peptide examined. K_i values were calculated according to the method described previously.[13]

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References and Notes

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2) Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. 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: Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; OBzl, benzyl ester; ONp, *p*-nitrophenyl ester; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; ACOEt, ethyl acetate; TFA, trifluoroacetic acid; AcOH, acetic acid; *n*-BuOH, *n*-butanol; Suc, succinyl; *p*NA, *p*-nitroanilide.
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