Amino Acids and Peptides. XXXVII. Synthesis of Stereoisomeric Nonapeptides Corresponding to Sequence 41—49 of Eglin c and Examination of Their Inhibitory Activity against Human Leukocyte Cathepsin G and α-Chymotrypsin[1,2]

Ayumi FUJII,[a] Satoshi TSUBOI,[a] Keiichi ASADA,[a] Yoko NAGAMATSU,[b] Junichiro YAMAMOTO,[b] and Yoshio OKADA*,[a]

Faculty of Pharmaceutical Sciences[a] and Faculty of Nutrition,[b] Kobe-Gakuin University, Nishi-ku, Kobe 651-21, Japan. Received January 17, 1994; accepted March 11, 1994

A nonapeptide, H-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg-Tyr-OH, corresponding to sequence 41—49 of eglin c inhibited leukocyte cathepsin G and α-chymotrypsin with K_i values of 2.2×10^{-5} and 7.2×10^{-6} M, respectively, although eglin c itself inhibited leukocyte elastase, cathepsin G and α-chymotrypsin with K_i values of 6.0×10^{-9}, 5.5×10^{-9} and 2.5×10^{-9} M, respectively. The inhibitory activity of the nonapeptide decreased following incubation with cathepsin G due to the cleavage of the Leu[45]–Asp[46] peptide bond. Therefore, Leu[45] and/or Asp[46] were replaced with D-amino acids and the inhibitory activities of the resultant nonapeptides were examined. Their inhibitory activities against cathepsin G and α-chymotrypsin were much weaker than those of the all-L-type nonapeptide, suggesting that the amino acids at the active site, Leu[45] and Asp[46] are required to be in the L-configuration for potent activity.

Keywords eglin c (41-49); analog; chemical synthesis; leukocyte 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 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 candidate therapeutic agent for the treatment of emphysema and inflammation. Rink *et al.* prepared N^{α}-acetyleglin c by use of gene technology,[7] but its molecular weight is too large for practical therapeutic use. Our attention was therefore directed to the systematic synthesis of eglin c and related peptides with the objectives of elucidating the structure–inhibitory activity relationship and of obtaining peptide inhibitors of small molecular size for both leukocyte elastase and cathepsin G.

Previously, it was reported that eglin c (41—49), corresponding to the active center of eglin c, inhibited cathepsin G and α-chymotrypsin with K_i values of 2.2×10^{-5} and 7.2×10^{-6} M, respectively, but did not leukocyte elastase even though eglin c itself inhibited leukocyte elastase, cathepsin G and α-chymotrypsin with K_i values of 6.0×10^{-9}, 5.5×10^{-9} and 2.5×10^{-9} M, respectively.[8-10] Although eglin c (41—49) exhibited specific inhibitory activity against cathepsin G or α-chymotrypsin, this inhibitory activity decreased as a function of incubation time with cathepsin G or α-chymotrypsin, presumably due to the cleavage of the Leu[45]–Asp[46] bond, which is the active site of eglin c, by the enzyme as shown in Fig. 1.

In order to obtain more potent inhibitors, analogs (II—VII) of H-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg-Tyr-OH, eglin c (41—49) (I)[10] were designed by replacing Leu[45] and/or Asp[46] with D-amino acids to prevent the cleavage of the peptide bond, and synthesized by the solution method as shown in Fig. 2.

As illustrated in Fig. 2, each protected nonapeptide (**1**—**7**) was constructed from the N-terminal tetrapeptide and a C-terminal pentapeptide, which contains the modified residue(s). For the construction of **1**, Boc-Ser-Pro-Val-Thr-$NHNH_2$[9] was employed as the N-terminal peptide block. However, Z-Ser-Pro-Val-Thr-$NHNH_2$ was used for the other peptides (**2**—**7**), because the yield was better. Boc or Z-Ser-Pro-Val-Thr-$NHNH_2$ was coupled with various kinds of C-terminal pentapeptide esters to give the protected nonapeptides. The protected nonapeptides (**1**—**7**) were treated with HF in the presence of thioanisole and *m*-cresol at 0 °C for 90 min to afford the desired peptides (I—VII) after purification by Sephadex G-25 chromatography and preparative HPLC. The homogeneity of each peptide was ascertained by TLC, analytical HPLC and amino acid analysis.

Next, their inhibitory activities against leukocyte elas-

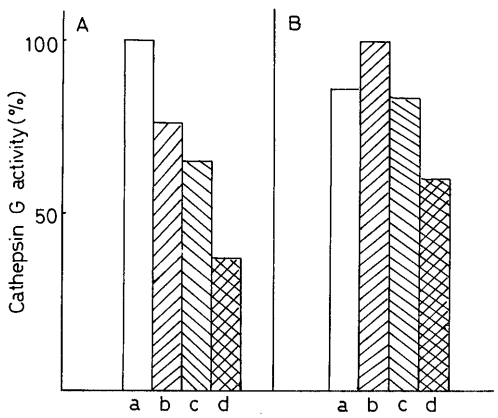

Fig. 1. Effects of Preincubation on Inhibitory Activity of Eglin c (41—49)

Preincubation time: A, 0 min; B, 90 min. Final concentration of eglin c (41—49): a, control; b, 10 μM; c, 20 μM; d, 40 μM.

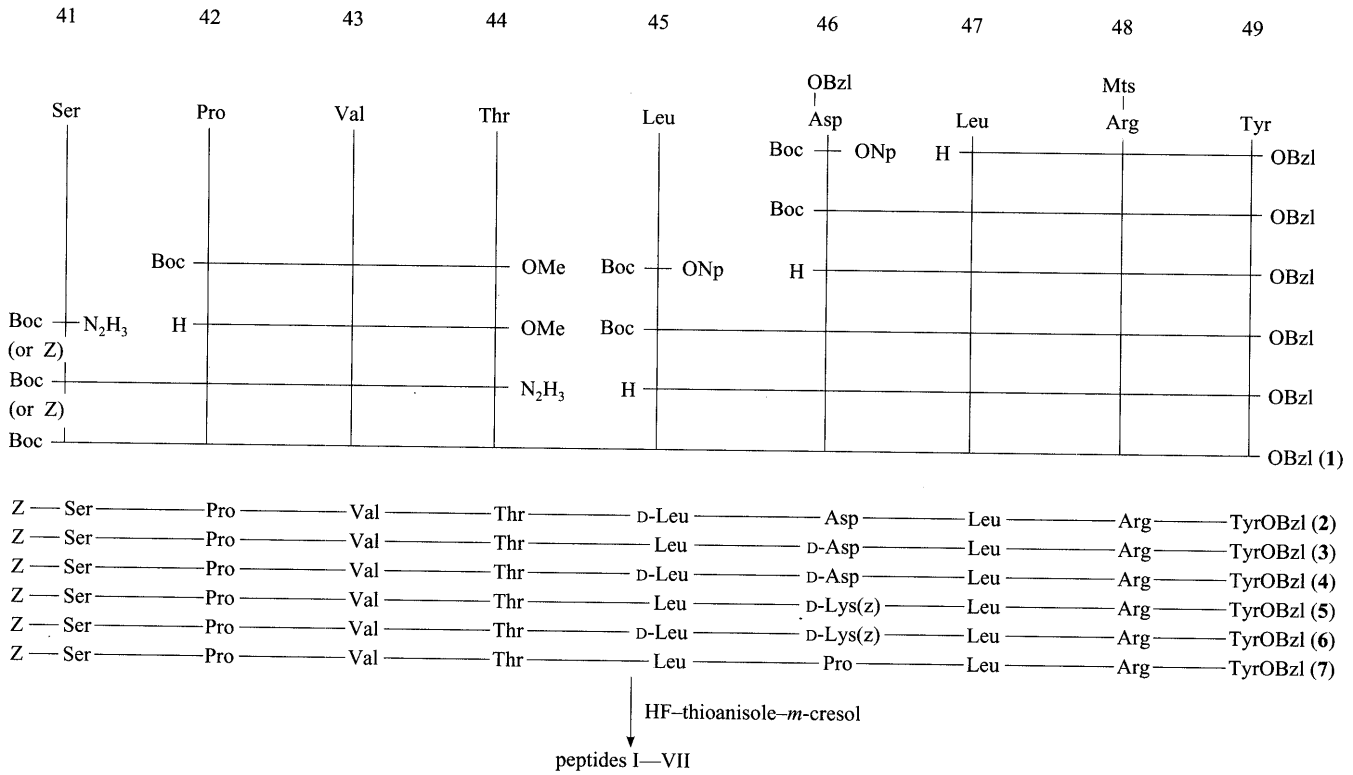

Fig. 2. Synthetic Scheme for Eglin c (41—49) and Its Analogs

TABLE I. Remaining Activities of Various Enzymes and K_i Values of H–Ser–Pro–Val–Thr–X–Y–Leu–Arg–Tyr–OH, Eglin c (41—49) and Its Analogs

Compound X	Compound Y		Remaining activity (%)[a] (peptide: 0.2 mM)				K_i (mM)			
			Cathepsin G	α-Chym.	LE	PE	Cathepsin G	α-Chym.	LE	PE
Leu	Asp	(I)	14	53	100	43	0.022	0.007	>1.5	0.133
D-Leu	Asp	(II)	50	ND	ND	ND	0.10	ND	ND	ND
Leu	D-Asp	(III)	91	ND	100	ND	1.5	ND	>1.5	ND
D-Leu	D-Asp	(IV)	90	ND	ND	ND	1.2	ND	ND	ND
Leu	D-Lys	(V)	100	100	100	100	>1.5	>1.5	>1.5	>1.5
D-Leu	D-Lys	(VI)	100	88	100	95	>1.5	0.20	>1.5	>1.5
Leu	Pro	(VII)	80	88	100	27	0.27	0.20	>1.5	0.08

a) The remaining activity was estimated by comparison with the amidolytic activity of enzyme without inhibitor (100%). α-Chym., α-chymotrypsin; LE, leukocyte elastase; PE, porcine pancreatic elastase. ND: not tested.

tase and porcine pancreatic elastase, as well as cathepsin G and α-chymotrypsin, were examined and the results are summarized in Table I. The Leu^{45}–Asp^{46} moiety was converted to D-Leu–Asp (II), Leu–D-Asp (III), or D-Leu–D-Asp (IV), which are not cleaved by cathepsin G. However, these analogs exhibited weaker competitive inhibitory activity against cathepsin G than the parent peptide (I). Substitution of the Asp^{46} residue with D-Asp caused a greater decrease of the inhibitory activity of the peptide than the substitution of the Leu^{45} residue with D-Leu. It was deduced that the β-carboxyl group of D-Asp does not play a role in the interaction between the peptide and the enzyme, resulting in weaker inhibitory activity than that of the parent peptide. Next, the Asp residue was substituted with D-Lys to give peptides V and VI. Peptide V did not exhibit any detectable inhibitory activity against the enzymes tested. Peptide VI exhibited weak inhibitory activity against only α-chymotrypsin, indicating that there is some difference in active center structure between cathepsin G and α-chymotrypsin. Next, the Asp^{46} residue was replaced with Pro to afford peptide VII, which is not cleaved by cathepsin G. This peptide, however, exhibited weaker inhibitory activities against cathepsin G and α-chymotrypsin than the parent peptide (I). Although peptide VII did not show any inhibitory activity against leukocyte elastase, as expected, it inhibited porcine pancreatic elastase more potently than the parent peptide (I), indicating that there is also a difference in active site structure between leukocyte elastase and porcine pancreatic elastase.

In conclusion, amino acid substitution at the active site of I failed to increase the inhibitory activity, presumably because the conformation of the parent peptide was changed inappropriately as a result of the substitution.

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 and Rf^2 values refer to the systems of $CHCl_3$, MeOH and H_2O (90 : 8 : 2) and $CHCl_3$, MeOH and H_2O (8 : 3 : 1, lower phase), respectively.

Boc-D-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl Boc-D-Asp(OBzl)-ON*p* (0.59 g, 1.32 mmol) and H-Leu-Arg(Mts)-Tyr-OBzl [prepared from Boc-Leu-Arg(Mts)-Tyr-OBzl[10] (0.9 g, 1.1 mmol) and 5.1 N HCl/dioxane (1.1 ml, 5.5 mmol) as usual] were dissolved in DMF (20 ml) containing Et_3N (0.15 ml, 1.1 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. Petroleum ether was added to the residue to afford the crude product. The crude product in $CHCl_3$ (2 ml) was applied to a silica gel column (1.2 × 25 cm), which was equilibrated and eluted with $CHCl_3$. The solvent of the eluate (350—600 ml) was removed by evaporation and petroleum ether was added to the residue to afford crystals. Yield 0.47 g (41.5%), mp 122—123 °C, $[\alpha]_D^{25}$ −16.0° (*c* = 1.0, MeOH), Rf^1 0.56. *Anal.* Calcd for $C_{53}H_{69}N_7O_{12}S \cdot 1/2H_2O$: C, 61.4; H, 6.80; N, 9.45. Found: C, 61.3; H, 6.84; N, 9.70.

Boc-Leu-D-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl Boc-Leu-ON*p* (0.15 g, 0.41 mmol) and H-D-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl [prepared from Boc-D-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl (0.35 g, 0.34 mmol) and 5.1 N HCl/dioxane (0.33 ml, 1.7 mmol) as usual], were dissolved in DMF (20 ml) containing Et_3N (0.05 ml, 0.34 ml) and 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. Petroleum ether was added to the residue to afford crystals, which were collected and recrystallized from AcOEt. Yield 112 mg (28.4%), mp 123—124 °C, $[\alpha]_D^{25}$ −9.3° (*c* = 1.0, MeOH), Rf^1 0.50. *Anal.* Calcd for $C_{59}H_{80}N_8O_{13}S \cdot 1/2H_2O$: C, 61.6; H, 7.18; N, 9.74. Found: C, 61.5; H, 7.12; N, 10.0.

Boc-D-Leu-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl The title compound was prepared from Boc-D-Leu-OPyCl[11] (0.7 g, 2.1 mmol) and H-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl [prepared from Boc-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl[10] (2.0 g, 1.9 mmol) and 5.5 N HCl/dioxane (3.5 ml, 19 mmol) as usual], yield 1.5 g (68.2%), mp 171—175 °C, $[\alpha]_D^{25}$ −16.8° (*c* = 1.0, MeOH), Rf^1 0.61. *Anal.* Calcd for $C_{59}H_{80}N_8O_{13}S \cdot H_2O$: C, 61.1; H, 7.13; N, 9.66. Found: C, 61.2; H, 7.08; N, 9.87.

Boc-D-Leu-D-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl The title compound was prepared from Boc-D-Leu-OPyCl[11] (0.6 g, 1.8 mmol) and H-D-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl [prepared from Boc-D-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl (1.5 g, 1.5 mmol) and 5.5 N HCl/dioxane (2.7 ml, 15 mmol) as usual], yield 1.5 g (88.2%), mp 90—96 °C, $[\alpha]_D^{25}$ +3.4° (*c* = 1.0, MeOH), Rf^1 0.61. *Anal.* Calcd for $C_{59}H_{80}N_8O_{13}S \cdot H_2O$: C, 61.1; H, 7.25; N, 9.39. Found: C, 61.4; H, 7.25; N, 9.39. Found: C, 61.4; H, 7.13; N, 9.66.

Boc-D-Lys(Z)-Leu-Arg(Mts)-Tyr-OBzl The title compound was prepared from Boc-D-Lys(Z)-ON*p* (2.8 g, 5.5 mmol) and H-Leu-Arg(Mts)-Tyr-OBzl [prepared from Boc-Leu-Arg(Mts)-Tyr-OBzl (4.1 g, 5.0 mmol) and 5.5 N HCl/dioxane as usual], yield 4.3 g (79.6%), mp 87—91 °C, $[\alpha]_D^{25}$ −12.3° (*c* = 1.0, MeOH), Rf^1 0.60. *Anal.* Calcd for $C_{56}H_{76}N_8O_{12}S \cdot 1/2H_2O$: C, 61.5; H, 7.09; N, 10.1. Found: C, 61.3; H, 7.02; N, 10.2.

Boc-Leu-D-Lys(Z)-Leu-Arg(Mts)-Tyr-OBzl The title compound was prepared from Boc-Leu-OPyCl[11] (0.75 g, 2.2 mmol) and H-D-Lys(Z)-Leu-Arg(Mts)-Tyr-OBzl [prepared from Boc-D-Lys(Z)-Leu-Arg(Mts)-Tyr-OBzl (2.0 g, 1.8 mmol) and 5.5 N HCl/dioxane (10 mmol) as usual], yield 1.7 g (78.7%), mp 86—88 °C, $[\alpha]_D^{25}$ −15.3° (*c* = 0.9, MeOH), Rf^1 0.63. *Anal.* Calcd for $C_{62}H_{87}N_9O_{13}S \cdot 1/2H_2O$: C, 61.7; H, 7.35; N, 10.4. Found: C, 62.0; H, 7.32; N, 10.3.

Boc-D-Leu-D-Lys(Z)-Leu-Arg(Mts)-Tyr-OBzl The title compound was prepared from Boc-D-Leu-ON*p* (0.75 g, 2.2 mmol) and H-D-Lys(Z)-Leu-Arg(Mts)-Tyr-OBzl [prepared from Boc-D-Lys(Z)-Leu-Arg(Mts)-Tyr-OBzl (2.0 g, 1.8 mmol) and 5.5 N HCl/dioxane (3.3 ml, 18 mmol) as usual], yield 1.7 g (78.7%), mp 92—98 °C, $[\alpha]_D^{25}$ −6.2° (*c* = 1.0, MeOH), Rf^1 0.53. *Anal.* Calcd for $C_{62}H_{87}N_9O_{13}S \cdot 1/2H_2O$: C, 61.7; H, 7.35; N, 10.4. Foud: C, 61.7; H, 7.47; N, 10.4.

Boc-Pro-Leu-Arg(Mts)-Tyr-OBzl The title compound was prepared from Boc-Pro-ON*p* (0.6 g, 1.7 mmol) and H-Leu-Arg(Mts)-Tyr-OBzl·HCl [prepared from Boc-Leu-Arg(Mts)-Tyr-OBzl[10] (1.3 g, 1.6 mmol) and 5.5 N HCl/dioxane (2.9 ml, 16 mmol) as usual], yield 1.2 g (82.5%), mp 103—106 °C, $[\alpha]_D^{25}$ −44.3° (*c* = 1.0, MeOH), Rf^1 0.48. *Anal.* Calcd for $C_{47}H_{65}N_7O_{10}S \cdot 2/3H_2O$: C, 60.6; H, 7.17; N, 10.5. Found: C, 60.6; H, 7.33; N, 10.2.

Boc-Leu-Pro-Leu-Arg(Mts)-Tyr-OBzl The title compound was prepared from Boc-Leu-ON*p* (0.5 g, 1.32 mmol) and H-Pro-Leu-Arg(Mts)-Tyr-OBzl·HCl [prepared from Boc-Pro-Leu-Arg(Mts)-Tyr-OBzl (1.01 g, 1.1 mmol) and 5.5 N HCl/dioxane (2.0 ml, 11 mmol) as usual], yield 780 mg (68.6%), mp 111—115 °C, $[\alpha]_D^{25}$ −61.2° (*c* = 1.0, MeOH), Rf^1 0.50. *Anal.* Calcd for $C_{53}H_{76}N_8O_{11}S \cdot H_2O$: C, 60.6; H, 7.48; N, 10.7. Found: C, 60.5; H, 7.53; N, 10.4.

Z-Ser-Pro-Val-Thr-OMe The title compound was prepared from Z-Ser-N_3 [prepared from Z-Ser-$NHNH_2$ (2.1 g, 8.4 mmol), 7.2 N HCl (2.3 ml, 17 mmol) and isopentyl nitrite (1.2 ml, 8.4 mmol) as usual] and H-Pro-Val-Thr-OMe·TFA [prepared from Boc-Pro-Val-Thr-OMe[9] (3.0 g, 7.0 mmol), TFA (5.2 ml, 70 mmol) and anisole (2.3 ml, 21 mmol) as usual], yield 2.1 g (54.5%), mp 141—145 °C, $[\alpha]_D^{25}$ −86.2° (*c* = 1.0, MeOH), Rf^1 0.62. *Anal.* Calcd for $C_{26}H_{38}N_4O_9 \cdot 1/2H_2O$: C, 55.8; H, 7.02; N, 10.0. Found: C, 55.8; H, 6.88; N, 10.1.

Z-Ser-Pro-Val-Thr-$NHNH_2$ Hydrazine hydrate (98%, 1.1 ml, 20 mmol) was added to a solution of Z-Ser-Pro-Val-Thr-OMe (2.0 g, 3.6 mmol) in MeOH (30 ml). The solution was stored at room temperature overnight. Crystals that formed were collected by filtration and washed with MeOH, yield 1.9 g (96%), mp 223—230 °C, $[\alpha]_D^{25}$ −41.2° (*c* = 1.0, DMF), Rf^1 0.08, Rf^2 0.69. *Anal.* Calcd for $C_{25}H_{38}N_6O_8$: C, 54.5; H, 6.96; N, 15.3. Found: C, 54.3; H, 6.90; N, 15.4.

Boc-Ser-Pro-Val-Thr-Leu-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl (1) Boc-Ser-Pro-Val-Thr-N_3 [prepared from Boc-Thr-Pro-Val-Thr-$NHNH_2$[9] (68 mg, 0.13 mmol) and isopentyl nitrite (0.02 ml, 0.13 mmol) as usual] was added to a cold solution of H-Leu-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl [prepared from Boc-Leu-Asp(OBzl)-Leu-Arg(Mts)-Tyr-OBzl[10] (100 mg, 0.088 mmol) and 5.5 N HCl/dioxane (0.2 ml, 0.88 mmol) as usual] in DMF (20 ml) containing Et_3N (0.01 ml, 0.088 mmol). The reaction mixture was stirred at 4 °C for 2 d. 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. Petroleum ether was added to the residue to give crystals, which were collected and recrystallized from AcOEt. Yield, melting point, $[\alpha]_D$ value, *Rf* value and elemental analysis are summarized in Table II.

General Procedure for Synthesis of Z-Ser-Pro-Val-Thr-X-Y-Leu-Arg(Mts)-Tyr-OBzl (2—7) [X: Leu, D-Leu; Y: Asp(OBzl), D-Asp(OBzl), D-Lys(Z), Pro] Z-Ser-Pro-Val-Thr-N_3 [prepared from Z-Ser-Pro-Val-Thr-$NHNH_2$ (1.0 g, 1.8 mmol), 5.5 N HCl/dioxane (0.7 ml, 3.6 mmol) and isopentyl nitrite (0.25 ml, 1.8 mmol) as usual] in DMF (70 ml) was added to a solution of H-X-Y-Leu-Arg(Mts)-Tyr-OBzl·HCl [X: Leu, D-Leu; Y: Asp(OBzl), D-Asp(OBzl), D-Lys(Z), Pro], [prepared from Boc-X-Y-Leu-Arg(Mts)-Tyr-OBzl (0.9 mmol) and 5.5 N HCl (1.6 ml, 9.0 mmol) as usual] in DMF (50 ml) containing Et_3N (0.14 ml) under cooling with ice-salt. The reaction mixture was stirred at 4 °C for 2 d. 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. Petroleum ether was added to the residue to give crystals, which were collected and recrystallized from MeOH and AcOEt. Yield, melting point, $[\alpha]_D$ value, *Rf* value and the result of the elemental analysis are summarized in Table II.

General Procedure for Deprotection of Protected Nonapeptides with HF The protected nonapeptide (50 mg) was treated with HF (10 ml) in the presence of thioanisole (0.30 ml) and *m*-cresol (0.1 ml) in an ice-bath for 90 min. After removal of HF, dry ether was added to the residue to afford a precipitate, which was collected by filtration and dissolved in H_2O (10 ml). The pH of the solution was adjusted to 8 with 1 M NH_4OH and the solution was kept at room temperature for 30 min and applied to a column of Sephadex G-25 (2.2 × 58 cm), which was equilibrated and eluted with 3% AcOH. The desired fractions (3 g each) were combined and the solvent was removed by lyophilization to give a fluffy powder. This product was further purified by reversed phase HPLC. Yield, $[\alpha]_D$ value and the result of amino acid analysis of the acid hydrolysate are summarized in Table III.

Assay Procedure Human leukocyte elastase[12] and cathepsin G[13] were prepared in our laboratory by newly developed affinity chro-

TABLE II. Yield, Melting Point, $[\alpha]_D$ Value, *Rf* Value and Analytical Data of Boc or Z–Ser–Pro–Val–Thr–X–Y–Leu–Arg–Tyr–OBzl

Compound X	Y		Yield (%)	mp (°C)	$[\alpha]_D$ (°) (Solvent)	Formula	Elemental analysis Calcd (Found) C	H	N	TLC *Rf*[1]
Leu	Asp(OBzl)	(1)	82.1	133—137	−25.3 (DMF)	$C_{76}H_{110}N_{12}O_{19}S \cdot H_2O$	59.0 (58.8	7.32 7.27	10.9 10.8)	0.46
D-Leu	Asp(OBzl)	(2)	85.7	112—116	−41.6 (MeOH)	$C_{79}H_{106}N_{12}O_{19}S \cdot H_2O$	60.1 (59.9	6.90 6.72	10.8 10.7)	0.63
Leu	D-Asp(OBzl)	(3)	60.7	125—126	−21.3 (MeOH)	$C_{79}H_{106}N_{12}O_{19}S \cdot 2H_2O$	59.5 (59.4	6.94 6.70	10.2 10.5)	0.32
D-Leu	D-Asp(OBzl)	(4)	85.7	105—112	−24.5 (MeOH)	$C_{79}H_{106}N_{12}O_{19}S \cdot H_2O$	60.1 (59.9	6.90 7.06	10.8 10.5)	0.67
Leu	D-Lys(Z)	(5)	59.7	99—104	−38.9 (MeOH)	$C_{82}H_{113}N_{13}O_{19}S \cdot 3H_2O$	58.9 (58.9	7.18 7.17	10.9 11.2)	0.51
D-Leu	D-Lys(Z)	(6)	90.8	112—118	−28.3 (MeOH)	$C_{82}H_{113}N_{13}O_{19}S \cdot 2H_2O$	59.6 (59.6	7.13 7.21	11.0 11.2)	0.56
Leu	Pro	(7)	66.7	125—130	−76.7 (MeOH)	$C_{73}H_{102}N_{12}O_{17}S \cdot 2H_2O$	58.9 (58.7	7.18 7.17	11.3 11.2)	0.33

TABLE III. Yield, $[\alpha]_D$ Value and Amino Acid Analysis of H–Ser–Pro–Val–Thr–X–Y–Leu–Arg–Tyr–OH, Eglin c (41—49) and Its Analogs

Compound X	Y		Yield (%)	$[\alpha]_D$ (°) (3% AcOH)	Amino acid analysis (average recovery) Ser	Pro	Val	The	Leu	Asp	Arg	Tyr	(%)
Leu	Asp	(I)	27.3	−86.2	0.90	1.18	1.00	0.86	2.01	0.86	1.13	0.86	(76.6)
D-Leu	Asp	(II)	9.8	−45.5	0.94	1.05	0.96	0.82	2.03	1.00	0.96	0.70	(69.3)
Leu	D-Asp	(III)	63.6	−44.9	0.96	1.08	1.01	0.86	1.97	1.00	0.98	0.80	(73.3)
D-Leu	D-Asp	(IV)	14.7	−37.6	0.91	1.01	1.00	0.92	2.08	1.03	0.88	0.73	(66.5)
Leu	D-Lys	(V)	9.3	−49.6	0.93	1.10	1.00	0.85	1.88	1.09 (Lys)	1.22	0.83	(71.8)
D-Leu	D-Lys	(VI)	19.7	−26.5	0.86	1.01	1.04	0.85	2.01	1.00 (Lys)	0.95	0.98	(74.3)
Leu	Pro	(VII)	26.1	−105.7	0.92	1.96	1.00	0.90	2.16	—	1.26	0.86	(88.7)

matography procedures. α-Chymotrypsin and bovine pancreatic elastase were purchased from Miles Co. Ltd., Elkhardt, and Sigma Co., St. Louis, respectively.

Assay of leukocyte elastase, cathepsin G, α-chymotrypsin and porcine pancreatic elastase: Enzymatic activities were determined by the methods described prevously[12] using Suc–Ala–Tyr–Leu–Val–*p*NA[14] for leukocyte elastase, Suc–Ile–Pro–Phe–*p*NA[15] for cathepsin G and α-chymotrypsin and Suc–$(Ala)_3$–*p*NA for porcine pancreatic elastase. The enzyme reaction was carried out in tris-HCl buffer (0.1 M, pH 8.0 for leukocyte elastase and porcine pancreactic elastase and 0.1 M, pH 7.5 for cathepsin G and α-chymotrypsin). The amidolytic activities of the above enzymes were assayed by measuring the *p*-nitroaniline (410 nm) released from each specific substrate.

Effects of synthetic peptides on enzymes: Enzymatic activity was assayed in the presence or absence of the peptide examined. The K_i values for the peptides were calculated according to the method previously described.[16]

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References and Notes

1) Part XXXVI: H. Taguchi, Y. Nishiyama, A. C. M. Camargo, Y. Okada, *Chem. Pharm. Bull.*, **41**, 2038 (1993).
2) The customary L indication of amino acid residues is omitted; only D isomers are indicated. Standard abbreviations for amino acids, peptides and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 3485 (1966); **6**, 362 (1967); **11**, 1726 (1972). Other abbreviations used are: Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Suc, succinyl; *p*NA, *p*-nitroanilide; OBzl, benzyl ester; ONp, *p*-nitrophenyl ester; ONSu, *N*-hydroxysuccinimide ester; OPyCl, 6-chloro-2-pyridyl ester; DCC, *N,N'*-dicyclohexylcarbodiimide; DPPA, diphenylphosphorylazide; TFA, trifluoroacetic acid; AcOH, acetic acid; AcOEt, ethyl acetate; DMF, dimethylformamide.
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