Amino Acids and Peptides. XIII.[1] Synthetic Studies on N-Terminal Tripeptide Amide Analogs of Fibrin α-Chain

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N-Terminal tripeptide analogs of fibrin α-chain were synthesized and their inhibitory effect on fibrinogen/thrombin clotting was examined. A new water-soluble active ester, 3-pyridinium ester, was used for the synthesis. Among the synthetic peptides, H-Gly-Pro-Arg-hexamethyleneimine exhibited the highest inhibitory effect on fibrinogen-thrombin clotting.

Keywords clotting; water-soluble active ester; peptide synthesis; fibrinogen–thrombin clotting; anticoagulant

The N-terminal tripeptide of fibrin α-chain (H–Gly–Pro–Arg–OH) and its analogs (H–Gly–Pro–Arg–Pro–OH and H–Gly–Pro–Arg–Sar–OH) were reported to be potent inhibitors of fibrin polymerization by Laudano and Doolittle[2]; the inhibitory effect of the latter two analogs was more potent than that of the former. The inhibitory effect was due to the binding of the peptides to fibrinogen, not to thrombin. Based on the mechanism of the inhibition, the development of a new type of anticoagulants should be possible. In the present study, various tripeptide analogs of the *N*-terminal portion of fibrin α-chain were synthesized to examine their inhibitory effect on fibrinogen–thrombin clotting (FTC). A new water-soluble active ester, methyl-pyridinium iodide ester was developed and used for synthesis of the peptides. Benzyloxycarbonyl (Z)[3] and *tert*-butoxycarbonyl (Boc)[4] groups were used as α-amino protecting groups and mesitylenesulfonyl (Mts)[5] and nitro groups[6] were used as guanidino protecting groups.

Since the fourth amino acids of the above inhibitors (H–Gly–Pro–Arg–Pro–OH and H–Gly–Pro–Arg–Sar–OH) have a secondary amine, various Gly–Pro–Arg amide analogs with such an amide were prepared. As the standard sample for comparison of the inhibitory effects of synthetic peptides on FTC, H–Gly–Pro–Arg–Pro–OH, the preparation of which by the solid-phase method was reported by Laudano and Doolittle,[2] was synthesized as shown in Fig. 1. The peptide was prepared by stepwise elongation from C-terminal H–Pro–OBzl.[7] The coupling reactions were done by the mixed anhydride method[8] and removals of Boc groups were done with trifluoroacetic acid (TFA). The final deblocking of the protected tetrapeptide was done by hydrogenation.

Tripeptide amide derivatives were prepared from Z–Gly–Pro–OH[9] and Arg amide derivatives by the mixed anhydride method followed by hydrogenation or trifluoromethanesulfonic acid (TFMSA)[10] treatment. For preparation of Z–Gly–Pro–OH, a new water-soluble active ester, pyridinium ester, was used as shown in Fig. 2. For preparation of pyridinium ester, 2-hydroxypyridine, 3-hydroxypyridine and 4-hydroxypyridine were treated with methyl iodide in methanol. Among them, 3-hydroxypyridine was converted to the corresponding pyridinium salt quantitatively, while 2- and 4-hydroxypyridines were not converted quantitatively even when a large excess of methyl

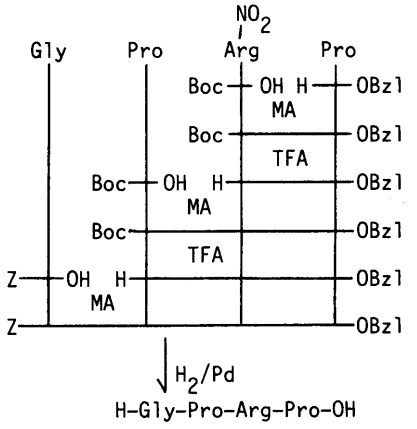

Fig. 1. Synthetic Scheme for the Tetrapeptide
MA: mixed anhydride method.

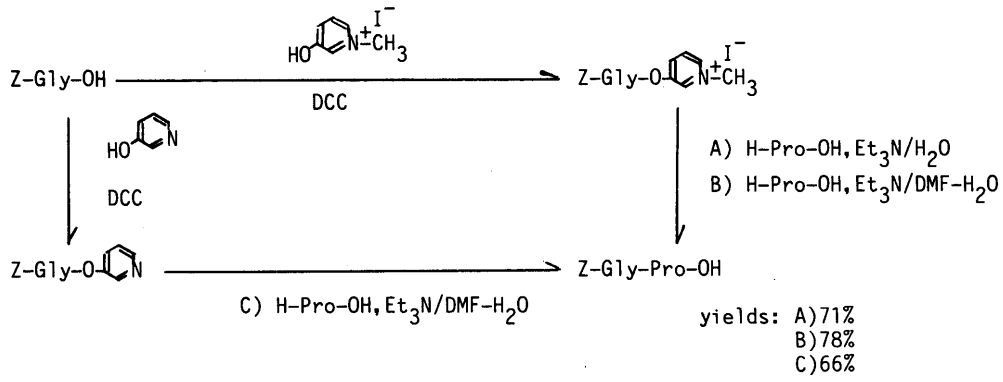

Fig. 2. Synthetic Scheme for Z–Gly–Pro–OH

iodide was used. 3-Hydroxypyridinium salt was thus used for preparation of a pyridinium ester. Z–Gly–OH was converted to the pyridinium ester by the dicyclohexylcarbodiimide (DCC) method[11] and the ester was reacted with H–Pro–OH in a mixture of DMF and water to give Z–Gly–Pro–OH in 78% yield. The same reaction proceeded even in water and the yield was 71%. The results suggested that the pyridinium ester method might be useful not only for peptide synthesis but also for acylation of compounds (such as proteins or sugars) in aqueous media. The 3-pyridine ester of Z–Gly–OH was also prepared by the DCC method to compare with the pyridinium ester. The pyridyl ester was reacted with H–Pro–OH to give Z–Gly–Pro–OH in 66% yield. The pyridinium ester was soluble in water and insoluble in organic solvents such as ethyl acetate and ether, but the pyridyl ester was soluble in the organic solvents and hardly soluble in water. Purification of the product (Z–Gly–Pro–OH) by the pyridinium ester method was achieved by washing the ethyl acetate extract with water. The occurrence of racemization during the coupling reaction was checked by nuclear magnetic resonance (NMR) spectroscopy according to the method reported by Weinstein *et al.*[12] For that purpose, Z–Ala–Phe–OMe was prepared by the pyridinium ester method and its NMR spectrum was measured. The methyl signal of Ala in the synthetic Z–Ala–Phe–OMe was compared with those of standard Z–L-Ala–L-Phe–OMe and Z–D-Ala–L-Phe–OMe.[12] As shown in Fig. 3, methyl signals of standard samples corresponding to D-Ala and L-Ala appeared at 1.28 and 1.33 ppm, respectively. The methyl signal of Ala in synthetic Z–Ala–Phe–OMe appeared at 1.33 ppm and no signal was observed at 1.28 ppm. These results indicate that the pyridinium ester method can be used for peptide synthesis.

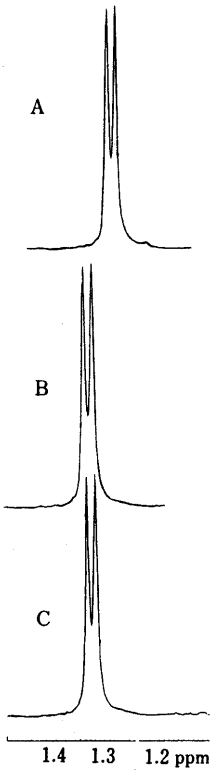

Fig. 3. NMR Spectrum Showing the Methyl Signals of Ala in Z–Ala–Phe–OMe

Solvent: $CDCl_3$. A, standard sample (D–L); B, standard sample (L–L); C, sample prepared by the pyridinium ester method.

Synthetic schemes for Gly–Pro–Arg–pyrrolidine and Gly–Pro–Arg–hexamethyleneimine are shown in Fig. 4 as examples of the synthetic methods used for various tripeptide amides. Boc–Arg(Mts)–OH and hexamethyleneimine were coupled by the mixed anhydride method, followed by TFA treatment to give H–Arg(Mts)–hexamethyleneimine. This was coupled with Z–Gly–Pro–OH by the mixed anhydride method followed by treatment with TFMSA to give H–Gly–Pro–Arg–hexamethyleneimine. Other tripeptide amides were prepared in a similar way, and hydrogenation was carried out for the final deprotection when N^G-nitroarginine[H–Arg(NO_2)–OH] was used instead of N^G-mesitylenesulfonylarginine.

The *p*-nitroanilide of the tripeptide was also prepared and the amide formation of Arg was achieved by the phosphazo

Table I. Relative Inhibitory Effects of Synthetic Peptides on FTC

Synthetic peptides	Relative activities
H–Gly–Pro–Arg–Pro–OH	1.00[a]
H–Gly–Pro–Arg–OH	0.33
H–Gly–Sar–OH	0.08
H–Gly–Pro–Arg–NH_2	0.14
H–Gly–Pro–Arg–$NHCH_3$	0.14
H–Gly–Pro–Arg–$N(CH_3)$	0.60
H–Gly–Pro–Arg–$N(C_2H_5)_2$	0.62
H–Gly–Pro–Arg–$N(C_3H_7)_2$	0.53
H–Gly–Pro–Arg–pyrrolidine	0.56
H–Gly–Pro–Arg–piperidine	0.93
H–Gly–Pro–Arg–4-hydroxypiperidine	1.20
H–Gly–Pro–Arg–3-methylpiperidine	1.29
H–Gly–Pro–Arg–hexamethyleneimine	1.85
H–Gly–Pro–Arg–cyclohexylamine	0.57
H–Gly–Pro–Arg–cyclooctylamine	0.48
H–Gly–Pro–Arg–aniline	0.20
H–Gly–Pro–Arg–*p*-nitroaniline	0.18

a) IC_{50} of H–Gly–Pro–Arg–Pro–OH: 28—65 μM.

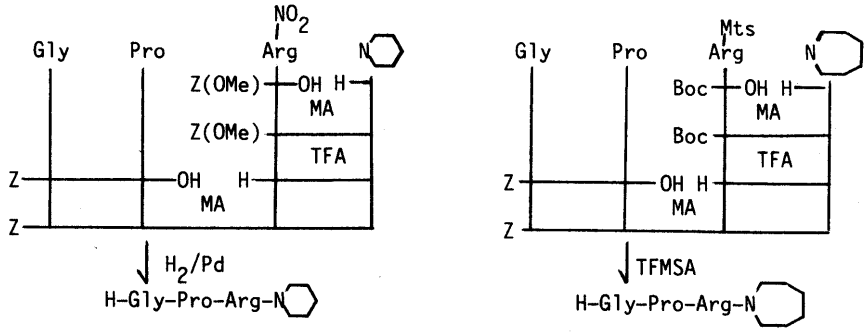

Fig. 4. Synthetic Schemes for the Tripeptides

Table II. Inhibitory Effects of Synthetic Peptides on Thrombin-Induced Hydrolysis of S-2238

Peptides	Concentration (mM)	% inhibition
H–Gly–Pro–Arg–piperidine	1.0	4.3
H–Gly–Pro–Arg–4-hydroxypiperidine	1.0	−2.5
H–Gly–Pro–Arg–3-methylpiperidine	1.0	1.8

method[13] instead of the mixed anhydride method.

Inhibitory effects of the synthetic peptides on fibrinogen–thrombin clotting[1a] were examined and the results are summarized in Table I. Since IC_{50} of each synthetic peptide varied when different lot of fibrinogen and thrombin were used, IC_{50} of H–Gly–Pro–Arg–Pro–OH was always measured for comparison when the inhibitory effect of the synthetic peptides was examined. Relative activities were calculated by dividing the IC_{50} of H–Gly–Pro–Arg–Pro–OH by that of each peptide.

As expected, the tripeptide amides with secondary amines were found to have a more potent inhibitory effect on FTC, compared with the carboxyl-free tripeptide or the tripeptide amide of a primary amine; the inhibitory effect of H–Gly–Pro–Arg–$N(CH_3)_2$ was more potent than those of H–Gly–Pro–Arg–OH and H–Gly–Pro–Arg–$NHCH_3$. The amide compounds of cyclic imines (such as piperidine, 4-hydroxypiperidine, 3-methylpiperidine and hexamethyleneimine) exhibited potent inhibitory effects and among them, the amide analog of hexamethyleneimine exhibited the highest inhibitory effect. Its inhibitory effect was more potent than that of H–Gly–Pro–Arg–Pro–OH, which was reported as a potent inhibitor by Laudano and Doolittle.[2] The inhibitory effect of the amide compound of piperidine was nearly equal to that of H–Gly–Pro–Arg–Pro–OH. The amide compounds of 4-hydroxypiperidine and 3-methylpiperidine were more effective than H–Gly–Pro–Arg–Pro–OH. Substitution of Pro with sarcosine (Sar) resulted in a decrease of the inhibition. The effect of the anilide was weaker than that of the cyclohexylamide and the introduction of a nitro group on the anilide did not cause any change.

Among the synthetic peptides, H–Gly–Pro–Arg–piperidine, H–Gly–Pro–Arg–4-hydroxypiperidine and H–Gly–Pro–Arg–3-methylpiperidine were examined for their antithrombin activities on thrombin/S-2238 (D-phenylalanylpipeconylarginine *p*-nitroanilide)[14] as shown in Table II. Since the peptides had almost no effect on the hydrolysis by thrombin, the inhibitory effects may result from their action on fibrin polymerization, but not on thrombin catalytic activity.

Experimental

Melting points are uncorrected. Solvent systems for ascending thin-layer chromatography on Silica gel G (type 60, E. Merck) are indicated as follows: Rf^1 = BuOH–AcOH–MeOH–H_2O (4:1:5, upper phase), Rf^2 = BuOH–pyridine–AcOH–H_2O (4:1:1:2), Rf^3 = $CHCl_3$–MeOH–H_2O (8:3:1, lower phase), Rf^4 = AcOEt–benzene (1:1), Rf^5 = $CHCl_3$–MeOH–AcOH (90:8:2). Inhibitory effect of the peptides on FTC and antithrombin activities of the peptides on thrombin/S-2238 system were examined as reported.[1a] NMR spectra were taken in $CDCl_3$ on a Brucker AM-400 (400 MHz). Chemical shifts are given in δ values (ppm) with tetramethylsilane as an internal standard. Amino acid compositions of acid hydrolysates were determined with a Hitachi 835 amino acid analyzer. Rotations were measured with a JASCO DIP-360 polarimeter.

Z–Gly–Pro–Arg(NO_2)–OBzl Prepared from Z–Gly–Pro–OH (1.5 g, 5 mmol) and H–Arg(NO_2)–OBzl·2TosOH[18] (3.2 g, 4.9 mmol) by the mixed anhydride method in the usual manner.[8] The product was purified by silica gel column (2.5 × 25 cm) chromatography using 1% MeOH–$CHCl_3$ as an eluent. Yield 1.52 g (52%), mp 68—71 °C, Rf^3 0.62, $[\alpha]_D^{22}$ −55.8° (c = 1.0, MeOH). *Anal.* Calcd for $C_{28}H_{35}N_7O_8$: C, 56.3; H, 5.9; N, 16.4. Found: C, 56.3; H, 6.0; N, 16.2.

H–Gly–Pro–Arg–OH Z–Gly–Pro–Arg(NO_2)–OBzl (500 mg, 0.8 mmol) was hydrogenated over a Pd catalyst in a mixture of AcOH (0.5 ml) and MeOH (20 ml) in the usual manner. The product was lyophilized from water to give a hygroscopic powder. Yield 263 mg (81%), Rf^2 0.10, $[\alpha]_D^{18}$ −55.2° (c = 1.0, H_2O). *Anal.* Calcd for $C_{13}H_{24}N_6O_4 \cdot AcOH \cdot 1/3H_2O$: C, 45.7; H, 7.3; N, 21.3. Found: C, 46.0; H, 7.4; N, 21.5. Amino acid ratios in an acid hydrolysate: Gly 1.00; Arg 0.90; Pro 1.14 (average recovery 87%).

Boc–Arg(NO_2)–Pro–OBzl Prepared from Boc–Arg(NO_2)–OH (3.5 g, 11 mmol) and HCl·H–Pro–OBzl (2.65 g, 11 mmol) in DMF by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 1% MeOH–$CHCl_3$ as an eluent. Yield 3.6 g (59%), syrupy material, Rf^3 0.85, $[\alpha]_D^{21}$ −53.1° (c = 0.9, MeOH). *Anal.* Calcd for $C_{23}H_{34}N_6O_7$: C, 54.5; H, 6.8; N, 16.6. Found: C, 54.8; H, 6.6; N, 16.4.

Boc–Pro–Arg(NO_2)–Pro–OBzl Prepared from Boc–Pro–OH (2.67 g, 12.4 mmol) and H–Arg(NO_2)–Pro–OBzl [prepared from 6.3 g (12.4 mmol) of the corresponding Boc derivative by TFA treatment followed by Et_3N treatment] by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 2% MeOH–$CHCl_3$ as an eluent. Yield 5.53 g (83%), mp 100—105 °C, Rf^5 0.58, $[\alpha]_D^{21}$ −96.2° (c = 0.9, MeOH). *Anal.* Calcd for $C_{28}H_{41}N_7O_8$: C, 55.7; H, 6.9; N, 16.2. Found: C, 55.7; H, 7.0; N, 16.1.

Z–Gly–Pro–Arg(NO_2)–Pro–OBzl Prepared from Z–Gly–OH (0.9 g, 4.3 mmol) and H–Pro–Arg(NO_2)–Pro–OBzl [prepared from 2 g (3.3 mmol) of the corresponding Boc derivative by TFA treatment followed by Et_3N treatment] by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 1.83 g (77%), mp 103—107 °C, Rf^5 0.56, $[\alpha]_D^{21}$ −89.2° (c = 1.2, MeOH). *Anal.* Calcd for $C_{33}H_{42}N_8O_9$: C, 57.0; H, 6.1; N, 16.1. Found: C, 56.9; H, 6.2; N, 15.9.

H–Gly–Pro–Arg–Pro–OH The above protected tetrapeptide (500 mg, 0.7 mmol) was hydrogenated over a Pd catalyst in a mixture of MeOH and 1 N HCl in the usual manner. The product was lyophilized to give a hygroscopic powder. Yield 258 mg (78%), Rf^2 0.22, $[\alpha]_D^{21}$ −89.2° (c = 1.2, MeOH). *Anal.* Calcd for $C_{18}H_{31}N_7O_5 \cdot HCl \cdot 1/2H_2O$: C, 42.6; H, 6.8; N, 19.3. Found: C, 42.5; H, 7.0; N, 19.3. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.24, Arg 0.93 (average recovery 77%).

3-Hydroxymethylpyridinium Iodide A solution of 3-hydroxypyridine (1 g, 11 mmol) and methyl iodide (19.5 ml, 220 mmol) in MeOH (4 ml) was refluxed at 45 °C for 6 h. After removal of the solvent *in vacuo*, the aqueous solution of the product was washed repeatedly with AcOEt and lyophilized. Yield 2.15 g (86%), mp 95—97 °C, Rf^2 0.70. *Anal.* Calcd for C_6H_8INO: C, 30.4; H, 3.4; N, 5.9. Found: C, 30.1; H, 3.4; N, 5.9.

3-(Z–Gly)-methylpyridinium Iodide Ester (Z–Gly–OMPI) DCC (2.27 g, 11 mmol) was added to a solution of Z–Gly–OH in DMF (10 ml) at 0 °C and the mixture was stirred for 20 min. A chilled solution of 1-methyl-3-hydroxypyridinium iodide (2.17 g, 9.2 mmol) in DMF (10 ml) was added to the above mixture and the whole was stirred overnight. The solvent was evaporated off, and the aqueous solution of the residue was washed with ethyl and lyophilzed. The residue was triturated with *n*-propanol. Yield 2.1 g (54%), amorphous powder, Rf^2 0.78. *Anal.* Calcd for $C_{16}H_{17}IN_2O_4 \cdot 5/3H_2O$: C, 41.9; H, 4.5; N, 6.1. Found: C, 41.9; H, 4.4; N, 6.0.

3-(Z–Gly)pyridyl Ester (Z–Gly–OPy) Prepared from Z–Gly–OH (5 g, 24 mmol) and 3-hydroxypyridine (2.27 g, 24 mmol) in DMF by the DCC method in the usual manner. The product was purified by silica gel column chromatography using 2% MeOH–$CHCl_3$ as an eluent. Yield 4.52 g (66%), syrupy material, Rf^4 0.42. *Anal.* Calcd for $C_{15}H_{14}N_2O_4 \cdot H_2O$: C, 59.2; H, 5.3; N, 9.2. Found: C, 58.9; H, 5.1; N, 8.9.

Z–Gly–Pro–OH a) Pyridinium Ester Method: A solution of Z–Gly–OMPI (0.3 g, 1 mmol), H–Pro–OH (0.12 g, 1 mmol) and *N*-methylmorpholine (NMM, 0.11 ml, 1 mmol) in H_2O (3 ml) was stirred overnight and acidified with citric acid. The AcOEt extract of the resulting precipitate was washed with H_2O and dried with Na_2SO_4. The solvent was evaporated off and the residue was recrystallized from AcOEt. Yield 0.22 g (71%). A 78% yield was obtained when the same reaction was done in a mixture

of DMF–H_2O, mp 153—156 °C, Rf^3 0.49, $[\alpha]_D^{22}$ −71.3° (c = 1.0, MeOH). *Anal.* Calcd for $C_{15}H_{18}N_2O_5 \cdot 1/4H_2O$: C, 58.0; H, 6.0; N, 9.0. Found: C, 57.8; H, 5.8; N, 9.2. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.09 (average recovery 86%).

b) Pyridyl Ester Method: Z–Gly–OPy (1.2 g, 4.24 mmol) and H–Pro–OH (0.49 g, 4.24 mmol) were reacted in a mixture of DMF (10 ml), H_2O (3 ml) and Et_3N (0.59 ml, 4.3 mmol). After removal of the solvent, the product was purified as described above. Yield 0.85 g (66%), mp 150—152 °C, Rf^3 0.49, $[\alpha]_D^{28}$ −65.8° (c = 1.0, MeOH). Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.08 (average recovery 73%).

3-(Z-Ala)-methylpyridinium Iodide Ester (Z-Ala-OMPI) A solution of Z–Ala–OH (2 g, 9 mmol) and DCC (2.2 g, 10.8 mmol) in CH_2Cl_2 was stirred for 20 min at −5 °C and the resulting precipitate was removed by filtration. The filtrate was combined with a DMF solution of 1-methyl-3-hydroxypyridinium iodide (2.12 g, 9 mmol) and the mixture was stirred overnight. The solvent was evaporated off and the aqueous solution of the residue was washed with ether followed by lyophilization. Yield 3.51 g (88%), amorphous powder, Rf^2 0.73, $[\alpha]_D^{24}$ −13.1° (c = 1.0, MeOH). *Anal.* Calcd for $C_{17}H_{19}IN_2O_4 \cdot 2H_2O$: C, 42.7; H, 4.9; N, 5.9. Found: C, 43.1; H, 5.0; N, 6.1.

Z-Ala-Phe-OMe A mixture of Z–Ala–OMPI (1.98 g, 4.5 mmol), NMM (0.5 ml, 4.5 mmol) and H–Phe–OMe·HCl (0.97 g, 4.5 mmol) in DMF was stirred overnight and the solvent was evaporated off. The AcOEt extract of the residue was washed successively with 5% Na_2CO_3, 5% citric acid and H_2O, and concentrated. The product was recrystallized from AcOEt–petroleum ether. Yield 1.24 g (63%), mp 96—100 °C, Rf^4 0.76, $[\alpha]_D^{26}$ −10.4° (c = 1.0, EtOH) [lit.[19] mp 99—100 °C, $[\alpha]_D^{22}$ −9.3° (c = 1.0, EtOH)]. Amino acid ratios in an acid hydrolysate: Ala 1.00, Phe 1.03 (average recovery 95%). ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, CH_3).

Z(OMe)-Arg(NO_2)-NH_2 Prepared from Z(OMe)–Arg(NO_2)–OH (3 g, 7.8 mmol) and 28% ammonia water (9.67 ml) in THF (60 ml) by the mixed anhydride method in the usual manner. The resulting precipitate was collected by filtration and washed with MeOH. Yield 2.08 g (70%), mp 220—223 °C, Rf^3 0.59, $[\alpha]_D^{26}$ −5.0° (c = 1.0, DMF). *Anal.* Calcd for $C_{15}H_{22}N_6O_6$: C, 47.1; H, 5.8; N, 22.0. Found: C, 47.4; H, 5.8; N, 21.7.

Z-Gly-Pro-Arg(NO_2)-NH_2 Prepared from Z–Gly–Pro–OH (0.56 g, 1.8 mmol) and H–Arg(NO_2)–NH_2 [prepared from the corresponding Z(OMe) derivative (0.53 g, 1.4 mmol) by TFA treatment followed by TEA treatment] were coupled by the diphenylphosphoryl azide (DPPA)[15] method in DMF. The DMF was removed *in vacuo* and the residue was extracted with BuOH followed by washing the extract with 10% Na_2CO_3, 0.5 N HCl and H_2O. The BuOH layer was concentrated and the product was precipitated by addition of ether. Yield 0.20 g (71%), amorphous powder, Rf^3 0.54, $[\alpha]_D^{26}$ −37.3° (c = 1.0, MeOH). *Anal.* Calcd for $C_{21}H_{30}N_8O_7$: C, 49.8; H, 6.0; N, 22.1. Found: C, 49.9; H, 5.9; N, 21.9.

Z(OMe)-Arg(NO_2)-$NHCH_3$ Prepared from Z–Arg(NO_2)–OH (4.03 g, 10 mmol) and 40% NH_2CH_3 (1.7 ml, 20 mmol) by the mixed anhydride method in DMF in the usual manner. The product was purified by silica gel column (2.7 × 15 cm) chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 1.68 g (41%), amorphous powder, Rf^3 0.70, $[\alpha]_D^{19}$ −4.4° (c = 1.0, MeOH). *Anal.* Calcd for $C_{16}H_{24}N_6O_6$: C, 48.5; H, 6.1; N, 21.2 Found: C, 48.4; H, 6.2; N, 21.0.

Z-Gly-Pro-Arg(NO_2)-$NHCH_3$ Prepared from Z–Gly–Pro–OH (0.57 g, 1.86 mmol) and H–Arg(NO_2)–$NHCH_3$ (prepared from the corresponding Z(OMe) derivative by TFA treatment followed by TEA treatment) by the mixed anhydride method. The product was purified in the same manner as described for Z–Gly–Pro–Arg(NO_2)–NH_2. Recrystallized from AcOEt. Yield 0.66 g (69%), mp 98—101 °C, Rf^3 0.65, $[\alpha]_D^{19}$ −46.1° (c = 1.0, MeOH). *Anal.* Calcd for $C_{22}H_{32}N_8O_7$: C, 50.8; H, 6.2; N, 21.5. Found: C, 50.6; H, 6.4; N, 21.5.

H-Gly-Pro-Arg-$NHCH_3$ Prepared from Z–Gly–Pro–Arg(NO_2)–$NHCH_3$ (202 mg, 0.4 mmol) in a mixture of AcOH and MeOH by catalytic hydrogenation. The product was lyophilized from water to give a hygroscopic powder. Yield 147 mg (82%), Rf^2 0.28, $[\alpha]_D^{21}$ −70.0° (c = 1.0, H_2O). *Anal.* Calcd for $C_{14}H_{27}N_7O_3 \cdot 2AcOH \cdot 3/4H_2O$: C, 45.5; H, 7.8; N, 20.6. Found: C, 45.4; H, 7.5; N, 20.8. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.17, Arg 0.97 (average recovery 77%).

Z(OMe)-Arg(NO_2)-$N(CH_3)_2$ Prepared from Z(OMe)–Arg(NO_2)–OH (3.04 g, 7.9 mmol) and 44% dimethylamine (0.81 ml, 7.9 mmol) in DMF by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 1.03 g (32%), mp 68—70 °C, Rf^5 0.40, $[\alpha]_D^{20}$ −11.3° (c = 1.0, MeOH). *Anal.* Calcd for $C_{17}H_{26}N_6O_6 \cdot 1/3H_2O$: C, 49.0; H, 6.5; N, 20.2. Found: C, 48.9; H, 6.3; N, 20.4.

Z-Gly-Pro-Arg(NO_2)-$NH(CH_3)_2$ Prepared from Z–Gly–Pro–OH (0.29 g, 0.95 mmol) and H–Arg(NO_2)–$N(CH_3)_2$ [prepared from the corresponding Z(OMe) derivative (0.39 g, 0.95 mmol) by TFA treatment followed by TEA treatment] in DMF by the mixed anhydride method in the usual manner. Yield 0.34 g (67%), amorphous powder, Rf^3 0.70, $[\alpha]_D^{23}$ −62.9° (c = 1.0, MeOH). *Anal.* Calcd for $C_{23}H_{34}N_8O_7$: C, 51.7; H, 6.4; N, 21.0. Found: C, 51.5; H, 6.5; N, 20.8.

H-Gly-Pro-Arg-$N(CH_3)_2$ Prepared from Z–Gly–Pro–Arg(NO_2)–$N(CH_3)_2$ (204 mg, 0.38 mmol) in MeOH by catalytic hydrogenation in the usual manner. Yield 146 mg (80%), amorphous powder, Rf^2 0.14, $[\alpha]_D^{20}$ −82.0° (c = 0.5, H_2O). *Anal.* Calcd for $C_{15}H_{29}N_7O_3 \cdot 2AcOH \cdot H_2O$: C, 46.2; H, 8.0; N, 19.9. Found: C, 46.5; H, 8.1; N, 20.2.

Z(OMe)-Arg(NO_2)-$N(C_2H_5)_2$ Prepared from Z(OMe)–Arg(NO_2)–OH (3 g, 7.8 mmol) and $(C_2H_5)_2NH$ (1.08 ml, 10 mmol) by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 1.62 g (47%), mp 56—59 °C, Rf^5 0.56, $[\alpha]_D^{17}$ −24.4° (c = 1.0, MeOH). *Anal.* Calcd for $C_{19}H_{30}N_6O_6$: C, 52.0; H, 6.9; N, 19.2. Found: C, 51.9; H, 6.8; N, 18.9.

Z-Gly-Pro-Arg(NO_2)-$N(C_2H_5)_2$ Prepared from Z–Gly–Pro–OH (0.55 g, 1.8 mmol) and H–Arg(NO_2)–$N(C_2H_5)_2$ [prepared from the corresponding Z(OMe) derivative (0.72 g, 1.6 mmol) by TFA treatment followed by TEA treatment] in DMF by the mixed anhydride method in the usual manner. Yield 0.47 g (51%), mp 77—79 °C, Rf^3 0.69, $[\alpha]_D^{19}$ −61.4° (c = 1.0, $CHCl_3$). *Anal.* Calcd for $C_{25}H_{38}N_8O_7$: C, 53.4; H, 6.8; N, 19.9. Found: C, 53.4; H, 6.8; N, 19.7.

H-Gly-Pro-Arg-$N(C_2H_5)_2$ Prepared from Z–Gly–Pro–Arg(NO_2)–$N(C_2H_5)_2$ (219 mg, 0.39 mmol) in a mixture of AcOH and MeOH by catalytic hydrogenation in the usual manner. Yield 154 mg (79%), hygroscopic powder, Rf^2 0.24, $[\alpha]_D^{18}$ −99.8° (c = 0.5, H_2O). *Anal.* Calcd for $C_{17}H_{33}N_7O_3 \cdot 2AcOH \cdot 1/2H_2O$: C, 49.2; H, 8.3; N, 19.1. Found: C, 48.9; H, 8.5; N, 19.4. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro 1.08; Arg 0.94 (average recovery 88%).

Z-(OMe)-Arg(NO_2)-$N(C_3H_7)_2$ Prepared from Z(OMe)–Arg(NO_2)–OH (2 g, 5.2 mmol) and $NH(C_3H_7)_2$ (1.42 ml, 10.4 mmol) in DMF by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 2.96 g (61%), amorphous powder, Rf^3 0.67, $[\alpha]_D^{21}$ −22.4° (c = 1.0, MeOH). *Anal.* Calcd for $C_{21}H_{34}N_6O_6$: C, 54.1; H, 7.4; N, 18.0. Found: C, 53.9; H, 7.4; N, 18.0.

Z-Gly-Pro-Arg(NO_2)-$N(C_3H_7)_2$ Prepared from Z–Gly–Pro–OH (0.34 g, 0.88 mmol) and H–Arg(NO_2)–$N(C_3H_7)_2$ [prepared from its Z(OMe) derivative (0.43 g, 0.92 mmol) by TFA treatment followed by TEA treatment] by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 0.41 g (76%), mp 79—82 °C, Rf^3 0.68, $[\alpha]_D^{20}$ −73.6° (c = 1.0, MeOH). *Anal.* Calcd for $C_{27}H_{42}N_8O_7$: C, 54.9; H, 7.2; N, 19.0. Found: C, 54.6; H, 7.4; N, 18.8.

H-Gly-Pro-Arg-$N(C_3H_7)_2$ Prepared from Z–Gly–Pro–Arg(NO_2)–$N(C_3H_7)_2$ (255 mg, 0.43 mmol) by catalytic hydrogenation in a mixture of MeOH and AcOH. Yield 172 mg (75%), hygroscopic powder, Rf^2 0.17, $[\alpha]_D^{20}$ −78.2° (c = 0.5, H_2O). *Anal.* Calcd for $C_{19}H_{37}N_7O_3 \cdot 2AcOH \cdot 2H_2O$: C, 48.7; H, 8.7; N, 17.3. Found: C, 48.4; H, 8.5; N, 17.3. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro 1.11; Arg 0.90 (average recovery 78.6%).

Z(OMe)-Arg(NO_2)-Pyrrolidine Prepared from Z(OMe)–Arg(NO_2)–OH (2.99 g, 7.8 mmol) and pyrrolidine (1.25 ml, 15 mmol) in DMF by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 1.72 g (50%), mp 73—75 °C, Rf^5 0.40, $[\alpha]_D^{22}$ −10.4° (c = 1.0, MeOH). *Anal.* Calcd for $C_{19}H_{28}N_6O_6$: C, 52.3; H, 6.5; N, 19.3. Found: C, 52.1; H, 6.5; N, 19.2.

Z-Gly-Pro-Arg(NO_2)-Pyrrolidine Prepared from Z–Gly–Pro–OH (0.37 g, 1.2 mmol) and H–Arg(NO_2)–pyrrolidine [prepared from its Z(OMe) derivative (0.52 g, 1.2 mmol) by TFA treatment followed by TEA treatment] by the mixed anhydride method in the usual manner. The product was extracted with BuOH and the extract was washed successively with 10% Na_2CO_3, 0.5 N HCl, 5% $NaHCO_3$ and H_2O. The solvent was evaporated off and the residue was precipitated from EtOH–ether. Yield 0.31 g (46%), mp 105—108 °C, Rf^3 0.73, $[\alpha]_D^{17}$ −77.5° (c = 1.0, MeOH). *Anal.* Calcd for $C_{25}H_{36}N_8O_7 \cdot 1/4H_2O$: C, 53.1; H, 6.5; N, 19.8. Found: C, 53.1; H, 6.5; N, 19.5.

H-Gly-Pro-Arg-Pyrrolidine Prepared from Z–Gly–Pro–Arg(NO_2)–pyrrolidine (224 mg, 0.4 mmol) in a mixture of AcOH and MeOH by hydrogenation in the usual manner. Yield 178 mg (89%), hygroscopic powder, Rf^2 0.32, $[\alpha]_D^{21}$ −74.4° (c = 0.5, H_2O). *Anal.* Calcd for

$C_{17}H_{31}N_7O_3 \cdot 2AcOH \cdot 3/4H_2O$: C, 49.0; H, 7.9; N, 19.0. Found: C, 48.6; H, 7.9; N, 19.2. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro 1.12; Arg 0.91 (average recovery 78%).

Z(OMe)-Arg(NO_2)-Piperidine Prepared from Z(OMe)-Arg(NO_2)-OH (3 g, 7.8 mmol) and piperidine (1.38ml, 14 mmol) by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 1.81 g (51%), amorphous powder, Rf^5 0.60, $[\alpha]_D^{23}$ −13.2° (c=1.0, MeOH). *Anal.* Calcd for $C_{20}H_{30}N_6O_6 \cdot H_2O$: C, 51.3; H, 6.9; N, 17.9. Found: C, 51.5; H, 6.6; N, 18.1.

Z-Gly-Pro-Arg(NO_2)-Piperidine Prepared from Z-Gly-Pro-OH (810 mg, 2.64 mmol) and H-Arg(NO_2)-piperidine [prepared from its Z(OMe) derivative (990 mg, 2.3 mmol) by TFA treatment followed by TEA treatment] by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 0.66 g (52%), mp 107—110 °C, Rf^5 0.60, $[\alpha]_D^{19}$ −61.1° (c=1.0, MeOH). *Anal.* Calcd for $C_{26}H_{38}N_8O_7$: C, 54.3; H, 6.7; N, 19.5. Found: C, 54.2; H, 6.7; N, 19.2.

H-Gly-Pro-Arg(NO_2)-Piperidine Prepared from Z-Gly-Pro-Arg(NO_2)-piperidine (133 mg, 0.23 mmol) in a mixture of AcOH and MeOH by hydrogenation. Yield 94 mg (79%), hygroscopic powder, Rf^2 0.17, $[\alpha]_D^{20}$ −95.1° (c=0.5, H_2O). *Anal.* Calcd for $C_{18}H_{33}N_7O_3 \cdot 2AcOH \cdot 3/2H_2O$: C, 48.7; H, 8.2; N, 18.1. Found: C, 48.4; H, 8.4; N, 17.9. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro 1.10; Arg 0.98 (average recovery 91%).

Z(OMe)-Arg(NO_2)-4-Hydroxypiperidine Prepared from Z(OMe)-Arg(NO_2)-OH (5 g, 13 mmol) and 4-hydroxypiperidine (2.5 g, 25 mmol) in DMF by the DCC method. The DMF was evaporated off and the residue was dissolved in BuOH. This solution was washed successively with 10% Na_2CO_3, 5% citric acid and water. The BuOH was evaporated off and the residue was purified by silica gel column chromatography using 2% MeOH–$CHCl_3$ as an eluent. Yield 2.92 g (48%), amorphous powder, Rf^3 0.86, $[\alpha]_D^{26}$ −10.5° (c=1.0, MeOH). *Anal.* Calcd for $C_{20}H_{30}N_6O_7$: C, 51.5; H, 6.5; N, 18.0. Found: C, 51.2; H, 6.5; N, 17.7.

Boc-Gly-Pro-Arg(NO_2)-4-Hydroxypiperidine Prepared from Boc-Gly-Pro-OH[16] (0.68 g, 2.5 mmol) and H-Arg(NO_2)-4-hydroxypiperidine [prepared from its Z(OMe) derivative (1.29 g, 2.8 mmol) by TFA treatment followed by TEA treatmen] by the mixed anhydride method in the usual manner. The product was dissolved in BuOH and the solution was washed successively with 10% Na_2CO_3, 5% citric acid and water. The BuOH was evaporated off and the residue was precipitated from MeOH–ether and lyophilized from water. Yield 1.23 g (88%), amorphous powder, Rf^3 0.64, $[\alpha]_D^{29}$ −41.8° (c=1.0, DMF). *Anal.* Calcd for $C_{23}H_{40}N_8O_8$: C, 49.6; H, 7.2; N, 20.1. Found: C, 49.5; H, 7.3; N, 19.9.

H-Gly-Pro-Arg-4-Hydroxypiperidine Boc-Gly-Pro-Arg(NO_2)-4-hydroxypiperidine (600 mg, 1.1 mmol) was treated with HF at 0 °C for 1 h in the presence of anisole. The product was purified by CM-cellulose column chromatography using 0.01 M $AcONH_4$ containing an AcOH gradient (0—2%) as an eluent. Yield 0.39 g (75%), hygroscopic powder, Rf^1 0.22, $[\alpha]_D^{22}$ −30.5° (c=1.0, MeOH). *Anal.* Calcd for $C_{18}H_{33}N_7O_4 \cdot AcOH \cdot 1/2H_2O$: C, 50.0; H, 8.0; N, 20.4. Found: C, 49.8; H, 8.1; N, 20.0. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro 1.14; Arg 1.09 (average recovery 89%).

Z(OMe)-Arg(NO_2)-3-Methylpiperidine Prepared from Z(OMe)-Arg(NO_2)-OH (2 g, 5.2 mmol) and 3-methylpiperidine (0.62 g, 6.3 mmol) in DMF by the DCC-HOBt method[17] in the usual manner. The product was purified by silica gel column chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 2.1 g (86%), mp 74—75 °C, Rf^5 0.76, $[\alpha]_D^{22}$ −11.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{21}H_{32}N_6O_6$: C, 54.3; H, 6.9; N, 18.1. Found: C, 54.1; H, 6.9; N, 17.9.

Z-Gly-Pro-Arg(NO_2)-3-Methylpiperidine Prepared from Z-Gly-Pro-OH (790 mg, 2.6 mmol) and H-Arg(NO_2)-3-methylpiperidine [derived from its Z(OMe) derivative (1 g, 2.2 mmol) by TFA treatment] in DMF by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 1.0 g (68%), mp 105—107 °C, Rf^3 0.67, $[\alpha]_D^{22}$ −70.2° (c=1.0, MeOH). *Anal.* Calcd for $C_{27}H_{40}N_8O_7$: C, 55.1; H, 6.8; N, 19.0. Found: C, 55.4; H, 6.7; N, 18.8.

H-Gly-Pro-Arg-3-Methylpiperidine Prepared from Z-Gly-Pro-Arg(NO_2)-3-methylpiperidine (800 mg, 1.36 mmol) by hydrogenation with a Pd catalyst in the usual manner. Yield 511 mg (71%), hygroscopic powder, Rf^2 0.23, $[\alpha]_D^{23}$ −62.0° (c=1.0, H_2O). *Anal.* Calcd for $C_{19}H_{35}N_7O_3 \cdot 2AcOH \cdot 3/2H_2O$: C, 49.6; H, 8.3; N, 17.6. Found: C, 49.4; H, 8.6; N, 18.0.

Boc-Arg(Mts)-Hexamethyleneimine Prepared from Boc-Arg(Mts)-OH (3 g, 6.6 mmol) and hexamethyleneimine (0.9 ml, 7.9 mmol) in DMF by the DCC–HOBt method in the usual manner. The product was purified by silica gel column chromatography using 2% MeOH–$CHCl_3$ as an eluent. Yield 2.33 g (66%), Rf^5 0.49, $[\alpha]_D^{24}$ −9.3° (c=1.0, MeOH). *Anal.* Calcd for $C_{26}H_{43}N_5O_5S \cdot 1/4H_2O$: C, 57.6; H, 8.1; N, 12.5. Found: C, 57.8; H, 8.1; N, 12.5.

Z-Gly-Pro-Arg(Mts)-Hexamethyleneimine Prepared from Z-Gly-Pro-OH (0.71 g, 2.3 mmol) and H-Arg(Mts)-hexamethyleneimine [prepared from its Boc derivative (1 g, 1.9 mmol) by TFA treatment followed by TEA treatment] by the mixed anhydride method in the usual manner. After purification by an extraction procedure with AcOEt, the product was precipitated from AcOEt–ether. Yield 1.08 g (78%), mp 98—101 °C, Rf^3 0.69, $[\alpha]_D^{24}$ −60.3° (c=1.0, MeOH). *Anal.* Calcd for $C_{36}H_{51}N_7O_7S$: C, 59.6; H, 7.1; N, 13.5. Found: C, 59.3; H, 7.2; N, 13.2.

H-Gly-Pro-Arg-Hexamethyleneimine Prepared from Z-Gly-Pro-Arg(Mts)-hexamethyleneimine (500 mg, 0.69 mmol) by 1 M TFMSA–TFA treatment[12] in the presence of thioanisole for 1 h at 0 °C and 1 h at room temperature. Cold ether was added to the reaction mixture to give a precipitate, which was treated with Amberlite IRA 400 resin (acetate form) in 5% AcOH. After lyophilization, the product was purified by HPLC (YMC R-ODS-5 column, 20 × 250 mm; gradient system of 0.1% TFA–H_2O: 0.1% TFA–CH_3CN, 80 : 20—50 : 50). The product was converted to its hydrochloride by lyophilization from a mixture of H_2O and 1 N HCl. Yield 230 mg (70%), hygroscopic powder, Rf^2 0.23, $[\alpha]_D^{27}$ −95.6° (c=1.0, H_2O). *Anal.* Calcd for $C_{19}H_{35}N_7O_3 \cdot 2HCl \cdot 7/4H_2O$: C, 44.4; H, 7.9; N, 19.1. Found: C, 44.4; H, 7.8; N, 19.0. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro 1.00; Arg 0.88 (average recovery 79%).

Boc-Arg(Mts)-Cyclohexylamine Prepared from Boc-Arg(Mts)-OH (1 g, 2.19 mmol) and cyclohexylamine (0.3 ml, 2.62 mmol) in DMF by the DCC–HOBt method[17] in the usual manner. Yield 1.11 g (94%), mp 99—105 °C, Rf^5 0.25, $[\alpha]_D^{24}$ +3.1° (c=1.0, MeOH). *Anal.* Calcd for $C_{26}H_{43}N_5O_5S$: C, 58.1; H, 8.1; N, 13.0. Found: C, 57.9; H, 8.1; N, 12.8.

Z-Gly-Pro-Arg(Mts)-Cyclohexylamine Prepared from Z-Gly-Pro-OH (0.76 g, 2.47 mmol) and H-Arg(Mts)-cyclohexylamine [prepared its Boc derivative (1.11 g, 2.06 mmol) by 4 N HCl–dioxane treatment followed by TEA treatment] by the DCC–HOBt method in the usual manner. Yield 1.26 g (84%), mp 115—120 °C, Rf^2 0.76, $[\alpha]_D^{24}$ −36.1° (c=1.0, MeOH). *Anal.* Calcd for $C_{36}H_{51}N_7O_7S$: C, 59.6; H, 7.1; N, 13.5. Found: C, 59.3; H, 7.3; N, 13.4.

H-Gly-Pro-Arg-Cyclohexylamine Z-Gly-Pro-Arg(Mts)-cyclohexylamine (0.6 g, 0.83 mmol) was treated with 1 M TFMSA–TFA in the presence of thioanisole at 0 °C for 1 h and at room temperature for 1 h. Ether was added to give a precipitate, which was treated with Amberlite IRA 400 (acetate form) in 5% AcOH. After lyophilization, the product was purified by HPLC (YMC R-ODS-5, 20 × 250 mm; gradient system of 0.1% TFA–H_2O: 0.1% TFA-CH_3CN, 80 : 20—50 : 50). The product was converted to its hydrochloride by lyophilization from a mixture of 1 N HCl and H_2O. Yield 0.19 g (49%), hygroscopic powder, Rf^2 0.21, $[\alpha]_D^{25}$ −80.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{19}H_{35}N_7O_3 \cdot 2HCl \cdot 5/4H_2O$: C, 45.2; H, 7.9; N, 19.4. Found: C, 45.2; H, 7.9; N, 19.4. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro 1.05; Arg 1.08 (average recovery 92%).

Boc-Arg(Mts)-Cyclooctylamine Prepared from Boc-Arg(Mts)-OH (3 g, 6.75 mmol) and cyclooctylamine (1.09 ml, 7.92 mmol) by the DCC–HOBt method in the usual manner. Recrystallized from AcOEt–ether. Yield 3.37 g (90%), mp 96—102 °C, Rf^5 0.60, $[\alpha]_D^{26}$ −3.4° (c=1.0, MeOH). *Anal.* Calcd for $C_{28}H_{45}N_5O_5S$: C, 59.4; H, 8.5; N, 12.4. Found: C, 59.7; H, 8.7; N, 12.0.

Z-Gly-Pro-Arg(Mts)-Cyclooctylamine Prepared from Z-Gly-Pro-OH (0.65 g, 2.12 mmol) and H-Arg(Mts)-cyclooctylamine [prepared from the corresponding Boc derivative (1 g, 1.77 mmol) by HCl–dioxane treatment followed by TEA treatment] by the DCC-HOBt method in the usual manner. Recrystallized from AcOEt–ether. Yield 1.25 g (93%), mp 115—120 °C, Rf^1 0.77, $[\alpha]_D^{24}$ −34.6° (c=1.0, MeOH). *Anal.* Calcd for $C_{38}H_{55}N_7O_7S$: C, 60.5; H, 7.4; N, 13.0. Found: C, 60.8; H, 7.7; N, 12.8. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro 1.07; Arg 1.01 (average recovery 87%).

H-Gly-Pro-Arg-Cyclooctylamine Prepared from the corresponding protected peptide by treatment with 1 M TFMSA–TFA (13.3 ml)–thioanisole (0.8 ml) for 1 h at 0 °C and 1 h at room temperature. The product was precipitated by addition of ether, washed with ether and treated with Amberlite IRA-400 (acetate form) in 5% AcOH. After lyophilization, the product was purified by HPLC [YMC R-ODS-5; gradient system of (0.1% TFA–H_2O)/(0.1% TFA–CH_3CN), 80 : 20—50 : 50]. Yield 0.14 g (41%), hygroscopic powder, Rf^2 0.23, $[\alpha]_D^{26}$ −84.4° (c=1.0, H_2O). *Anal.* Calcd for $C_{21}H_{39}N_7O_3 \cdot 2HCl \cdot 3/2H_2O$: C, 46.9; H, 8.3; N, 18.2. Found: C, 47.1; H, 8.0; N, 18.2. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro

1.11; Arg 1.06 (average recovery 81%).

Boc-Arg(NO_2)-Aniline Prepared from Boc-Arg(NO_2)-OH (1.6 g, 5 mmol) and aniline (0.46 ml, 5 mmol) in DMF by the mixed anhydride method in the usual manner. Recrystallized from AcOEt–petroleum ether. Yield 1.77 g (90%), mp 77—80 °C, Rf^3 0.46, $[\alpha]_D^{28}$ −2.6° (c = 1.9, MeOH). *Anal.* Calcd for $C_{17}H_{26}N_6O_5$: C, 51.8; H, 6.6; N, 21.3. Found: C, 51.9; H, 6.8; N, 21.0.

Z-Gly-Pro-Arg(NO_2)-Aniline Prepared from Z-Gly-Pro-OH (0.77 g, 2.53 mmol) and H-Arg(NO_2)-NHC_6H_5 [prepared from its Boc derivative (1 g, 2.53 mmol) by TFA treatment followed by TEA treatment] by the mixed anhydride method in the usual manner. Recrystallized from AcOEt–petroleum ether. Yield 1.18 g (80%), mp 113—115 °C, Rf^3 0.67, $[\alpha]_D^{28}$ −49.6° (c = 1.0, MeOH). *Anal.* Calcd for $C_{27}H_{34}N_8O_7 \cdot 1/2H_2O$: C, 54.8; H, 5.8; N, 18.9. Found: C, 55.0; H, 5.9; N, 18.6.

H-Gly-Pro-Arg-Aniline Prepared from the protected peptide by hydrogenation in a mixture of MeOH and AcOH. The product was purified by CM-cellulose column (2.7 × 19.5 cm) chromatography using $AcONH_4$ buffer (pH 6.8). After repeated lyophilization, the product was converted to its HCl salt by lyophilization from a mixture of 1 N HCl and H_2O. Yield 0.25 g (51%), hygroscopic powder, Rf^2 0.35, $[\alpha]_D^{26}$ −85.0° (c = 1.0, H_2O). *Anal.* Calcd for $C_{19}H_{29}N_7O_3 \cdot 2HCl \cdot 7/4H_2O$: C, 44.9; H, 6.8; N, 19.3. Found: C, 44.6; H, 6.8; N, 18.9. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro 1.14; Arg 0.94 (average recover 78%).

Z-Arg(Mts)-*p*-Nitroaniline Prepared from Z-Arg(Mts)-OH (3 g, 5 mmol) and *p*-nitroaniline (0.69 g, 5 mmol) by the phosphazo method.[13] The product was purified by silica gel column (2.9 × 25 cm) chromatography using 2% MeOH–$CHCl_3$ as an eluent. Yield 1.65 g (53%), mp 95—97 °C, Rf^5 0.76, $[\alpha]_D^{28}$ +2.0° (c = 1.0, MeOH). *Anal.* Calcd for $C_{29}H_{34}N_6O_7S \cdot 1/2H_2O$: C, 56.2; H, 5.7; N, 13.6. Found: C, 56.7; H, 5.8; N, 13.2.

Z-Gly-Pro-Arg(Mts)-*p*-Nitroaniline Prepared from Z-Gly-Pro-OH (0.67 g, 2.17 mmol) and H-Arg(Mts)-*p*-nitroaniline [prepared from its Z derivative (1.33 g, 2.17 mmol) by HBr–AcOH treatment followed by TEA treatment] by the mixed anhydride method in the usual manner. The product was purified by silica gel column (1.9 × 14.5 cm) chromatography using 1% MeOH–$CHCl_3$ as an eluent. Yield 0.77 g (47%), mp 96—100 °C, Rf^5 0.80, $[\alpha]_D^{28}$ −30.5° (c = 1.0, MeOH). *Anal.* Calcd for $C_{36}H_{44}N_8O_8S \cdot H_2O$: C, 55.2; H, 5.9; N, 14.3. Found: C, 55.8; H, 5.8; N, 13.9.

H-Gly-Pro-Arg-*p*-Nitroaniline Prepared from the protected peptide (0.5 g, 0.64 mmol) by 1 M TFMSA–TFA (13 ml)–thioanisole (0.75 ml) treatment in the usual manner. The product was purified by CM-cellulose column (1.9 × 12 cm) chromatography using $AcONH_4$ buffer (pH 6.8) as an eluent. After repeated lyophilization, the material was converted to its HCl salt by lyophilization from a mixture of H_2O and 1 N HCl. Yield 0.21 g (64%), hygroscopic powder, Rf^2 0.45, $[\alpha]_D^{26}$ −33.4° (c = 1.0, H_2O). *Anal.* Calcd for $C_{19}H_{28}O_5N_8 \cdot 2HCl \cdot 3/2H_2O$: C, 41.6; H, 6.1; N, 20.4. Found: C, 41.3; H, 6.3; N, 20.2. Amino acid ratios in an acid hydrolysate: Gly 1.00; Pro 1.04; Arg 1.03 (average recovery 72%).

Z-Gly-Sar-OH Prepared from Z-Gly-ONp (2.16 g, 6.54 mmol) and H-Sar-OH (0.58 g, 6.54 mmol). The product was purified by silica gel column (1.8 × 21.3 cm) chromatography using 1% MeOH–$CHCl_3$ as an eluent. Yield 1.14 g (62%), amorphous powder, Rf^5 0.60. *Anal.* Calcd for $C_{13}H_{16}N_2O_5$: C, 55.7; H, 5.8; N, 10.0. Found: C, 55.4; H, 5.9; N, 9.7.

Z-Gly-Sar-Arg(NO_2)-OBzl Prepared from Z-Gly-Sar-OH (0.96 g, 3.42 mmol) and H-Arg(NO_2)-OBzl·TosOH (1.65 g, 3.42 mmol) in DMF by the mixed anhydride method in the usual manner. The product was purified by silica gel column (1.8 × 21 cm) chromatography using 3% MeOH–$CHCl_3$ as an eluent. Yield 1.04 g (53%), mp 72—75 °C, Rf^3 0.66, $[\alpha]_D^{25}$ −14.8° (c = 1.0, MeOH). *Anal.* Calcd for $C_{26}H_{34}N_7O_8$: C, 54.5; H, 6.0; N, 17.1. Found: C, 54.4; H, 6.1; N, 16.8.

H-Gly-Sar-Arg-OH Prepared from the protected peptide (0.5 g, 0.87 mmol) in MeOH by hydrogenation. The product was purified by CM-cellulose column (1.7 × 12 cm) chromatography using $AcONH_4$ buffer (pH 6.8) and converted to its HCl salt by lyophilization from a mixture of 1 N HCl and H_2O. Yield 0.17 g (54%), hygroscopic powder, Rf^2 0.33, $[\alpha]_D^{26}$ −9.6° (c = 1.0, H_2O). *Anal.* Calcd for $C_{11}H_{22}N_6O_4 \cdot 2HCl \cdot 3/2H_2O$: C, 32.8; H, 6.8; N, 20.9. Found: C, 32.7; H, 6.6; N, 21.0. Amino acid ratios in an acid hydrolysate: Gly 1.00; Sar 0.89; Arg 0.92 (average recovery 89%).

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References and Notes

1) *a*) Part XII: K. Kawasaki, K. Hirase, T. Tsuji, M. Miyano and M. Iwamoto, *Thrombosis Res.*, **56**, 757 (1989); *b*) Standard abbreviations for amino acids, protecting groups and peptides are used [*Eur. J. Biochem.*, **138**, 9 (1984)]; other abbreviations include DMF = dimethylformamide, Mts = mesitylenesulfonyl, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid, TEA = triethylamine, DCC = dicyclohexylcarbodiimide, HOBt = 1-hydroxybenzotriazole, ONp = *p*-nitrophenyl, FTC = fibrinogen–thrombin clotting, N^G = the nitrogen of a guanidino group, NMM = *N*-methylmorpholine.
2) A. P. Laudano and R. F. Doolittle, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 3085 (1978).
3) M. Bergmann and L. Zervas, *Chem. Ber.*, **65**, 1192 (1932).
4) D. S. Tarbell, T. Yamamoto and B. M. Pope, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 730 (1972).
5) H. Yajima, M. Takeyama, J. Kanaki and K. Mitani, *J. Chem. Soc., Chem. Commun.*, **1978**, 482.
6) M. Bergmann, L. Zervas and H. Rinke, *Z. Physiol. Chem.*, **224**, 40 (1934).
7) J. Ramachandran and C. H. Li, *J. Org. Chem.*, **28**, 173 (1963).
8) J. R. Vaughan, Jr. and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).
9) G. W. Anderson, J. E. Zimmerman and F. M. Callahan, *J. Am. Chem. Soc.*, **86**, 1839 (1964).
10) H. Yajima, N. Fujii, H. Ogawa and H. Kawatani, *J. Chem. Soc., Chem. Commun.*, **1974**, 107.
11) *a*) H. G. Khorana, *J. Chem. Soc.*, **1952**, 2081; *b*) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
12) *a*) B. Halpern, L. F. Chew and B. Weinstein, *J. Am. Chem. Soc.*, **89**, 5051 (1967); *b*) B. Weinstein and A. E. Pritchard, *J. Chem. Soc., Perkin Trans. 1*, **1972**, 1015.
13) S. Goldschmidt and G. Rosculet, *Chem. Ber.*, **93**, 2387 (1960).
14) M. Iwamoto and B. Blombach, *Thrombo. Res.*, **33**, 427 (1984).
15) T. Shioiri, K. Ninomiya and S. Yamada, *J. Am. Chem. Soc.*, **94**, 6203 (1972).
16) *a*) T. Yamada, K. Suegane, S. Kuwata and H. Watanabe, *Bull. Chem. Soc. Jpn.*, **50**, 1088 (1977); *b*) K. Khodadadeh, K. Heppenheimer and E. R. Heidemann, *Makromol. Chem.*, **178**, 1897 (1977).
17) W. Konig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
18) H. Otsuka, K. Inouye, M. Nakayama and F. Shinozaki, *Bull. Chem. Soc. Jpn.*, **39**, 882 (1966).
19) L. Zervas, D. Borovas and E. Gazis, *J. Am. Chem. Soc.*, **85**, 3660 (1963).