Amino Acids and Peptides. XVI.¹⁾ Synthesis of *N*-Terminal Tetrapeptide Analogs of Fibrin α -Chain and Their Inhibitory Effects on Fibrinogen/Thrombin Clotting

Koichi KAWASAKI,*^a Katsuhiko HIRASE,^a Masanori MIYANO,^a Toshiki TSUJI,^a and Masanori IWAMOTO^b

Faculty of Pharmaceutical Sciences, Kobe-Gakuin University,^a Ikawadani-cho, Nishi-ku, Kobe 651-21, Japan and Research Institute, Daiichi Seiyaku Co., Ltd.,^b 1-chome, Kitakasai, Edogawa-ku, Tokyo 134, Japan. Received June 26, 1992

***N*-Terminal tetrapeptide analogs of fibrin α -chain were synthesized by the solution method using a new active ester, the ester of the oxime of *p*-nitroacetophenone, and by the solid-phase method. Their inhibitory effects on fibrinogen/thrombin clotting were examined. Of the synthetic peptides, amide analogs of Gly-Pro-Arg-Pro exhibited a more potent inhibitory effect.**

Keywords peptide synthesis; active ester; fibrin α -chain; anticoagulant; fibrinogen/thrombin clotting

N-Terminal tetrapeptide analogs, H-Gly-Pro-Arg-Pro-OH (I) and H-Gly-Pro-Arg-Sar-OH (II), were synthesized by the solid-phase method and reported to be potent inhibitors of fibrin polymerization by Laudano and Doolittle.²⁾ Their inhibitory effect was due to their binding to fibrinogen, not to thrombin. On the basis of the inhibition mechanism, the development of a new type of anticoagulant is considered. In the preceding paper,³⁾ we reported the synthetic studies on the *N*-terminal tripeptide analogs of fibrin α -chain. We describe here the synthesis of *N*-terminal tetrapeptide analogs of fibrin α -chain and their inhibitory effects on fibrinogen/thrombin clotting (FTC). A total of 29 tetrapeptides were synthesized by the solution method and the solid-phase method.

A standard sample, H-Gly-Pro-Arg-Pro-OH (I), was prepared as shown in Fig. 1. Z-Gly-OH and H-Pro-OBzl were coupled by the dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt) method,⁴⁾ followed by hydrazine treatment to give Z-Gly-Pro-NHNH₂. Z(OMe)-Arg(NO₂)-OH and H-Pro-OBzl were coupled by the DCC/HOBt method, followed by TFA treatment to give H-Arg(NO₂)-Pro-OBzl. It was reacted with Z-Gly-Pro-NHNH₂ via azide formation to give protected tetrapeptide. The protected tetrapeptide obtained was then hydrogenated to I.

For the synthesis of some analogs of I, Z-Gly-Pro-OH was used as an essential component, which was prepared by a new active ester method as shown in Fig. 2. *p*-Nitroacetophenone was converted to its oxime (HONA) and was reacted with Z-Gly-OH by the DCC method to

give an active ester, Z-Gly-ONA. It was then reacted with H-Pro-OH to give Z-Gly-Pro-OH in a yield of 83%. The active ester crystallized and reacted easily. To compare the coupling yield, Z-Gly-Pro-OH was prepared by the *p*-nitrophenyl ester method⁵⁾ as well (yield, 80%). The results showed that the HONA ester method might be as useful as the *p*-nitrophenyl ester method for peptide synthesis.

As examples for the preparation of tetrapeptide analogs, synthetic schemes for H-Sar-Pro-Arg-Sar-OH, H-Gly-Sar-Arg-Pro-OH, H-Gly-Pro-Arg-Pro-hexamethyleneimine and H-Gly-Pro-Arg-Pro-*p*-nitroaniline are shown in Fig. 3. At the final deprotection step, hydrogenation was employed for *N*^G-nitroarginine⁶⁾-containing peptides and trifluoromethanesulfonic acid (TFMSA) treatment⁷⁾ for *N*^G-mesitylenesulfonylarginine [Arg(Mts)]⁸⁾-containing peptides and *N*^G-triisopropylbenzenesulfonylarginine [Arg(Tis)]⁹⁾-containing peptides.

Table I summarizes synthetic Gly-Pro-Arg-Pro analogs—in which one or two amino acids are replaced with other amino acids—and their inhibitory effects on FTC.³⁾ Since IC₅₀ of synthetic peptides varied widely with used lots of fibrinogen and thrombin, IC₅₀ of I was always measured for comparison when the inhibitory effect of the synthetic peptides was measured. Relative activity was calculated by dividing IC₅₀ of I by that of each synthetic peptide. I and II showed almost the same potency as reported.²⁾ Replacement of Gly with Pro (III) or Sar (IV), both imino acids, resulted in a sharp cut of the inhibitory effect. Similar results were obtained when Gly¹ was

Fig. 1. Synthetic Scheme for H-Gly-Pro-Arg-Pro-OH (I)

Fig. 2. Synthetic Scheme for Z-Gly-ONA and Z-Gly-Pro-OH

Fig. 3. Synthetic Schemes for Fibrin Related Tetrapeptides

TABLE I. Inhibitory Effects of *N*-Terminal Tetrapeptide Analogs of Fibrin α -Chain on FTC

Peptides	Relative activities
H-Gly-Pro-Arg-Pro-OH (I)	1.00
H-Gly-Pro-Arg-Sar-OH (II)	0.97
H-Pro-Pro-Arg-Pro-OH (III)	0.03
H-Sar-Pro-Arg-Pro-OH (IV)	0.04
H-Sar-Pro-Arg-Sar-OH (V)	0.03
H-D-Ala-Pro-Arg-Pro-OH (VI)	0.04
H-D-Ala-Pro-Arg-Val-OH (VII)	0.04
H- β -Ala-Pro-Arg-Pro-NH ₂ (VIII)	0.02
H-Gly-Sar-Arg-Pro-OH (IX)	0.04
H-Gly-Phe-Arg-Pro-OH (X)	0.04
H-Gly-D-Pro-Arg-Pro-NH ₂ (XI)	0.04
H-Gly-D-Pro-Arg-D-Pro-NH ₂ (XII)	0.04
H-Gly-Pro-D-Arg-Pro-NH ₂ (XIII)	0.07
H-Gly-Pro-Arg(NO ₂)-Pro-NH ₂ (XIV)	0.07
H-Gly-Pro-Lys-Pro-NH ₂ (XV)	0.02
H-Gly-Pro-Orn-Pro-NH ₂ (XVI)	0.02
H-Gly-Pro-Arg-D-Pro-OH (XVII)	0.03
H-Gly-Pro-Arg-(Me) β -Ala-OH (XVIII)	0.03

replaced with D-Ala and with β -Ala. The *N*-terminal Gly and its primary amine are very important for the effect. 1,4-Disubstituted analogs [Sar^{1,4} analog (V) and D-Ala¹, Val⁴ analog (VII)] also resulted in the loss of the effect. Replacement of Pro⁴ with Sar did not affect the inhibitory effect as reported,²⁾ but replacement of Pro² with Sar resulted in a remarkable decrease in the inhibitory effect. Similar results were obtained when Pro² was replaced with Phe or D-Pro². L-Pro² is essential but Pro⁴ is not essential and potentiates the effect. Arg³ was replaced with D-Arg, Arg(NO₂), Lys or Orn and the replaced analogs showed

TABLE II. Inhibitory Effects of Tetrapeptide Amide Analogs on FTC

Peptides	Relative activities
H-Gly-Pro-Arg-Pro-OH (I)	1.00
H-Gly-Pro-Arg-Pro-NH ₂ (XIX)	3.52
H-Gly-Pro-Arg-Sar-NH ₂ (XX)	1.11
H-Gly-Pro-Arg-Pro-N(C ₂ H ₅) ₂ (XXI)	1.03
H-Gly-Pro-Arg-Pro-morpholine (XXII)	1.14
H-Gly-Pro-Arg-Pro-hexamethyleneimine (XXIII)	3.25
H-Gly-Pro-Arg-Pro-cyclohexylamine (XXIV)	2.37
H-Gly-Pro-Arg-Pro-cycloheptylamine (XXV)	2.86
H-Gly-Pro-Arg-Pro-cyclooctylamine (XXVI)	2.37
H-Gly-Pro-Arg-Pro-aniline (XXVII)	1.31
H-Gly-Pro-Arg-Pro- <i>p</i> -nitroaniline (XXVIII)	2.21
H-Gly-Pro-Arg-Pro-NHNH ₂ (XXIX)	2.70

remarkable decreases in the effect. L-Arg³ is essential for the activity. When Pro⁴ was replaced with D-Pro or *N*-methyl- β -alanine [(Me) β -Ala], the analogs exhibited a very low inhibitory effect. Such remarkable loss of the inhibitory effect was not expected because the relative activity of H-Gly-Pro-Arg-OH was 0.33³⁾ and Pro⁴ and Sar⁴ analogs (I and II) have an almost equal inhibitory effect. Considering the results listed in Table I, the three amino acids (Gly, Pro, Arg) at the *N*-terminal portion of the fibrin α -chain are essential for the inhibitory effect on FTC. Gly-Pro-Arg was reported as the minimum chain length for the inhibitory effect on FTC.²⁾ Our present results indicate that each of the three amino acids plays a very important role.

As shown in Table I, no potent inhibitor was obtained by the substitution of an amino acid in the tetrapeptide with another amino acid. Since we had previously found that some tripeptide amide analogs exhibited a potent inhibitory effect,³⁾ we prepared tetrapeptide amide analogs listed in Table II and examined their inhibitory effects on FTC.

The amide of I (XIX) exhibited a potent inhibitory effect and its effect was 3.52 times as high as that of I. The amide of II (XX) also exhibited a little higher inhibitory effect. Various amide analogs of I were then prepared and their inhibitory effects on FTC were examined. The inhibitory effect of the diethylamide compound (XXI) was almost equal to that of I. The morpholide compound (XXII) showed a slightly higher inhibitory effect. The hexamethyleneimine compound (XXIII) was a potent inhibitor whose inhibitory effect was 3.25 times as high as that of I. Cyclohexylamide, cycloheptylamine and cyclooctylamine compounds (XXIV, XXV, XXVI) also exhibited potent inhibitory effects (their relative activities were 2.37, 2.86 and 2.37 respectively). The anilide compound (XXVII) showed a slightly higher inhibitory effect than I, and the *p*-nitroanilide derivative (XXVIII) was more effective than the anilide. Analog XXIX in which the acidic carboxyl group was replaced by a basic hydrazide also exhibited a potent inhibitory effect. Compared with I, amidation of the *C*-terminal proline carboxyl group tended to increase the inhibitory effect on FTC. Such a tendency was also observed when the *C*-terminal arginine carboxyl group of H-Gly-Pro-Arg-OH was converted to the amides.³⁾ Why amidation of the *C*-terminal carboxyl group increases the inhibitory effect is not clear, but some

TABLE III. Inhibitory Effects of Synthetic Peptides on Thrombin-Induced Hydrolysis of S-2238

Peptides	Concentration (mM)	% inhibition
H-Gly-Pro-Arg-Pro-NH ₂ (XIX)	1.0	-3.8
H-Gly-Pro-Arg-Pro-hexamethyleneimine (XXIII)	1.0	15.5
H-Gly-Pro-Arg-Pro-cyclohexylamine (XXIV)	1.0	18.5
H-Gly-Pro-Arg-Pro-cycloheptylamine (XXV)	1.0	16.4
H-Gly-Pro-Arg-Pro-cyclooctylamine (XXVI)	1.0	16.4
H-Gly-Pro-Arg-Pro-NHNH ₂ (XXIX)	1.0	10.1

ionic factors may affect the binding of synthetic peptides to fibrin.

Of the synthetic peptides, XIX, XXIII, XXIV, XXV, XXVI and XXIX were examined for their anti-thrombin activity on thrombin/S-2238 (D-phenylalanylpipeconylarginine *p*-nitroanilide)³⁾ and results are shown in Table III. XIX did not inhibit thrombin activity but other peptides inhibited the thrombin activity by 10–18%. The inhibitory effect of I was reported as the result of inhibition of fibrin polymerization, not of thrombin catalytic activity.³⁾ From the results shown in Table III, it is concluded that the inhibitory effect of XIX may be produced by the inhibition of fibrin polymerization, and that the inhibitory effect of the other peptide may be produced partly by the inhibition on fibrin polymerization, partly by the inhibition of thrombin catalytic activity.

H-Gly-Pro-Arg-Pro-OH (I) was reported as a potent anticoagulant²⁾ and here we found more potent anticoagulants resulting from modification of Pro⁴⁾.

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-H₂O (4:1:5, upper phase), Rf^2 = BuOH-AcOH-pyridine-H₂O (4:1:1:2), Rf^3 = CHCl₃-MeOH-H₂O (8:3:1, lower phase), Rf^4 = AcOEt-benzene (1:1), Rf^5 = CHCl₃-MeOH-AcOH (90:8:2). Rotations were measured with a JASCO DIP-360 polarimeter. The synthetic peptides were hydrolyzed in 6N HCl at 110°C for 24–36 h. During the hydrolysis, nitroarginine was mainly converted to Arg, but partially converted to Orn. Thus, the Arg content of Arg(NO₂)-containing peptide in an acid hydrolysate was calculated as Arg+Orn contents. Amino acid compositions of acid hydrolysates were determined with a Hitachi 835 amino acid analyzer. The inhibitory effect of the synthetic peptides on FTC and anti-thrombin activities of the peptides on thrombin/S-2238 system were examined as reported.³⁾ For solid-phase peptide synthesis, *p*-methylbenzhydrylamine resin and chloromethyl resin were purchased from Peptide Institute, Inc. Introduction of an amino acid to the chloromethyl resin was performed by the cesium salt method.¹⁰⁾ Solid-phase peptide synthesis was performed by a 430A Peptide synthesizer (Applied Biosystem) or by the manual method. *N*²-Amino groups of amino acids were protected with a Boc group, and a guanidino group of Arg was protected with a mesitylenesulfonyl group. *N*^ω-Amino groups of Lys and Orn were protected with a Z group. Synthetic protocol for the manual method is shown in Table IV. Final deprotection in the solid-phase method was performed by HF or TFMSA treatment. Reverse phase high performance liquid chromatography (RP-HPLC) was conducted with Waters 600 on Asahipak ODP-90 or YMC Pack AQ-ODS-5 using a mixture of 0.1% TFA-containing CH₃CN/H₂O as an eluent. CM-cellulose column chromatography was conducted using ammonium acetate buffer in stepwise elution from 0.01 to 0.05 M concentration. Conversion of AcOH salt (or TFA salt) of a synthetic peptide to its hydrochloride was achieved by lyophilization from H₂O containing an equivalent amount of HCl. Fast atom bombardment mass spectra (FAB-MS) were measured on a VG Analytical ZAV-SE spectrometer.

General Procedure for the Mixed Anhydride Method Equimolar reagents were used. Isobutyl chloroformate¹¹⁾ and Et₃N (or NMM) were added to a *N*²-protected amino acid (or peptide) solution in DMF at

TABLE IV. Synthetic Protocol for Solid-Phase Peptide Synthesis

Step	reagents	Reaction time (min)
1	NMM/DCM	10 × 2
2	DCM	3 × 3
3	Boc- or Z(OMe)-amino acid (2 eq) in DMF (or DCM), 1 mol DCC/DCM (2 eq) ^{a)}	120
4	50% MeOH/DCM	5 × 3
5	DCM	2 1
6	50% TFA/DCM, anisole	2 1
		45 1
7	DCM	3 × 3

a) 1 M HOBt/DMF (2 eq) was added when an Arg derivative was reacted.

–10°C and stirred for 15 min. The mixture was combined with an amino-component and stirred overnight. The solvent was removed and the product was extracted with AcOEt, followed by washing with 5% Na₂CO₃, 5% citric acid and H₂O. The AcOEt layer was dried with Na₂SO₄ and evaporated off. The product was purified by a suitable method.

General Procedure for the DCC and DCC/HOBt Methods Equimolar reagents were used. DCC was added to a solution of *N*²-protected amino acid (or peptide, and HOBt) at –10°C and the mixture was stirred overnight. The solvent was removed *in vacuo* and the product was extracted with AcOEt, followed by washing with 5% Na₂CO₃, 5% citric acid and H₂O. The AcOEt layer was dried over Na₂SO₄ and evaporated off. The residue was purified by a suitable method.

General Procedure for TFA Treatment *N*²-Boc or *N*²-Z(OMe) amino acids (or peptides) was dissolved in TFA containing 5% anisole (a peptide/TFA: 100 mg/1 ml) and the solution was stirred for 15 min at 0°C and 30 min at room temperature. Chilled ether was added and the resulting precipitate was collected by filtration (or centrifugation). The precipitate was used without further purification as follows; the precipitate was dissolved in DMF and neutralized with Et₃N (or NMM). The mixture was used for a coupling reaction.

General Procedure for TFMSA Treatment on a Protected Peptide A protected peptide was dissolved in a mixture of TFA and thioanisole, and TFMSA were added to the solution. 10 molar excess of thioanisole and 20 molar excess of TFMSA to the peptide were used. The mixture was stirred at 0°C for 1 h and at room temperature for 1 h. Chilled ether was added and the resulting precipitate was collected by filtration or centrifugation. The precipitate was washed with ether repeatedly and dissolved in 5% AcOH, followed by washing with ether again. The aqueous layer was treated with Amber lite IRA-400 (acetate) and lyophilized.

***p*-Nitroacetophenone Oxime** *p*-Nitroacetophenone (2 g, 12 mmol) dissolved in dioxane (25 ml) was added to a solution of hydroxylamine hydrochloride (1 g, 14.4 mmol) and sodium acetate (1.18 g, 14.4 mmol) in H₂O (25 ml), and the mixture was stirred at 50°C for 3 h. The solvent was removed *in vacuo* and the residue was washed with H₂O. Recrystallized from MeOH. Yield 1.8 g (83%), mp 173–174°C. *Anal.* Calcd for C₈H₈N₂O₃: C, 53.33; H, 4.47; N, 15.54. Found: C, 53.15; H, 4.40; N, 15.33.

Z-Gly-ONA A mixture of Z-Gly-OH (2.09 g, 10 mmol), *p*-nitroacetophenone oxime (1.8 g, 10 mmol) and DCC (2.06 g, 10 mmol) in DMF (25 ml) was stirred overnight and filtered. The solvent was removed *in vacuo* and the residue was recrystallized from MeOH. Yield 3.01 g (81%), mp 112–114°C, Rf^3 0.71. *Anal.* Calcd for C₁₈H₁₇N₃O₆: C, 58.22; H, 5.40; N, 11.32. Found: C, 58.49; H, 4.67; N, 11.28.

Z-Gly-Pro-OH Z-Gly-ONA (2 g, 5.38 mmol) dissolved in dioxane (15 ml) was added to a mixture of H-Pro-OH (620 mg, 5.38 mmol), NMM (0.60 ml, 5.5 mmol) and H₂O (5 ml), and the whole was stirred overnight. The solvent was removed *in vacuo* and the residue was dissolved in a mixture of AcOEt and H₂O. The H₂O layer was washed with AcOEt and acidified with 6N HCl. The resulting precipitate was extracted with AcOEt. The AcOEt was removed *in vacuo* and the residue was recrystallized from AcOEt. Yield 1.37 g (83%), mp 153–155°C, Rf^3 0.46, $[\alpha]_D^{20}$ –69.9° (c = 1.0, MeOH). Identified with the authentic sample³⁾ by the mixed mp method and infrared (IR) spectrum method. Amino acids ratios in an acid hydrolysate: Gly 1.00, Pro 0.97 (average recovery 88%).

Z-Gly-Pro-OH was also prepared by the reaction of Z-Gly-ONp¹²⁾ and H-Pro-OH in the same manner as described above. Yield 80%, mp 152–155°C, R_f^3 0.46, $[\alpha]_D^{20}$ –70.2° ($c=1.0$, MeOH). The product was identified with an authentic sample by the mixed mp method and IR spectrum.

Z-Gly-Pro-OBzl Z-Gly-OH (5.0 g, 24 mmol) and H-Pro-OBzl·HCl (5.8 g, 24 mmol) were coupled in DMF by the DCC/HOBt method. After removal of the solvent, the residue was dissolved in AcOEt and washed with 10% citric acid, 10% Na₂CO₃ and H₂O. After drying over Na₂SO₄, the solvent was evaporated off and the residue was purified by silica gel column chromatography using CHCl₃-MeOH as an eluent. Oily material. Yield 8.0 g (84%), R_f^4 0.62, $[\alpha]_D^{24}$ –77.2° ($c=1.0$, MeOH). *Anal.* Calcd for C₂₂H₂₄N₂O₅: C, 66.65; H, 6.10; N, 7.07. Found: C, 66.34; H, 6.21; N, 6.79. Amino acids ratios in an acid hydrolysate: Gly 1.00, Pro 1.02 (average recovery 86%).

Z-Gly-Pro-NHNH₂ Z-Gly-Pro-OBzl (8.70 g, 21.9 mmol) and hydrazine hydrate (4.4 ml, 87.8 mmol) were dissolved in EtOH (30 ml) and the solution was stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was dissolved in CHCl₃, followed by washing with H₂O. The solvent was removed *in vacuo* and the residue was precipitated from CHCl₃/petroleum ether and dried. Yield 5.6 g (80.4%), amorphous powder, R_f^3 0.57, $[\alpha]_D^{24}$ –70.9° ($c=1.0$, MeOH). *Anal.* Calcd for C₁₅H₂₀N₄O₄·1/4H₂O: C, 55.46; H, 6.36; N, 17.24. Found: C, 55.39; H, 6.36; N, 17.12. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.09 (average recovery 80%).

Z(OMe)-Arg(NO₂)-Pro-OBzl Z(OMe)-Arg(NO₂)-OH (10 g, 26 mmol) and H-Pro-OBzl [prepared from its hydrochloride (6.3 g, 26 mmol) and Et₃N (3.4 ml, 26 mmol)] were coupled by the DCC/HOBt method in DMF in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃ as an eluent. Yield 10.48 g (71%), amorphous powder, R_f^4 0.71, $[\alpha]_D^{24}$ –56.7° ($c=1.0$, MeOH). *Anal.* Calcd for C₂₇H₃₄N₆O₈: C, 56.83; H, 6.01; N, 14.73. Found: C, 56.55; H, 6.00; N, 14.73.

Z-Gly-Pro-Arg(NO₂)-Pro-OBzl To a DMF solution (20 ml) of Z-Gly-Pro-NHNH₂ (1.67 g, 5.26 mmol) and 7.4 N HCl/dioxane (2.31 ml), isoamyl nitrite (0.71 ml, 5.26 mmol) was added at –10°C and the whole was stirred for 10 min. The mixture was neutralized with Et₃N and combined with a DMF solution of H-Arg(NO₂)-Pro-OBzl [prepared from 2 g (3.51 mmol) of Z(OMe) derivative by TFA treatment, followed by Et₃N treatment]. After stirring overnight, the solvent was removed *in vacuo* and the residue was dissolved in AcOEt, followed by washing with 10% Na₂CO₃, 5% citric acid and H₂O. After removal of the solvent, the residue was purified by silica gel column chromatography using MeOH-containing CHCl₃ as an eluent. Yield 1.80 g (74%), mp 114–120°C, R_f^5 0.69, $[\alpha]_D^{25}$ –85.8° ($c=1.0$, MeOH). *Anal.* Calcd for C₃₃H₄₂N₉O₈: C, 57.04; H, 6.11; N, 16.13. Found: C, 56.91; H, 6.20; N, 15.88. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.91, Arg + Orn 0.92 (average recovery 86%).

H-Gly-Pro-Arg-Pro-OH (I) Z-Gly-Pro-Arg(NO₂)-Pro-OBzl (0.50 g, 0.72 mmol) was hydrogenated over Pd catalyst in AcOH-containing MeOH for 12 h. The product was purified by RP-HPLC and converted to its hydrochloride by lyophilization from HCl-containing H₂O. Yield 255 mg (71%), hygroscopic powder, R_f^1 0.10, R_f^2 0.22, $[\alpha]_D^{25}$ –69.4° ($c=1.0$, MeOH). *Anal.* Calcd for C₁₈H₃₁N₇O₅·2HCl·1/2H₂O: C, 42.61; H, 6.75; N, 19.32. Found: C, 42.45; H, 6.97; N, 19.32. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.89, Arg 0.84 (average recovery 79%).

H-Gly-Pro-Arg-Sar-OH (II) Prepared by the manual solid-phase method. Final deprotection was done by HF treatment. The product was purified by HPLC (YMC Pack AQ-ODS-5). Yield 63 mg (17%), highly hygroscopic powder, R_f^2 0.34, $[\alpha]_D^{25}$ –50.8° ($c=1.0$, H₂O), FAB-MS m/z 400 [M⁺ + 1]. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.05, Arg 1.09, Sar 1.09 (average recovery 83%).

Z-Pro-Pro-NHNH₂ Z-Pro-OH (7.73 g, 31 mmol) and H-Pro-OMe (prepared from 5.13 g of its hydrochloride, 31 mmol) were coupled by the DCC/HOBt method in DMF in the usual manner. The product was treated with NH₂NH₂·H₂O (4.77 ml, 93 mmol) in MeOH. The solvent was evaporated off and the residue was dissolved in CHCl₃, followed by washing with H₂O. The CHCl₃ layer was condensed and petroleum ether was added to give a solid. Yield 9.31 g (83%), mp 68–71°C, R_f^3 0.19, $[\alpha]_D^{18}$ –112.0° ($c=1.0$, MeOH). *Anal.* Calcd for C₁₈H₂₄N₄O₄: C, 59.98; H, 6.73; N, 15.54. Found: C, 59.73; H, 6.83; N, 15.33.

Z-Pro-Pro-Arg(NO₂)-Pro-OBzl Isoamyl nitrite (0.64 ml, 4.77 mmol) was added to a solution of Z-Pro-Pro-NHNH₂ (1.72 g, 4.77 mmol) and 6 N HCl/dioxane (2.34 ml, 14.04 mmol) in DMF (15 ml) at –30°C and the mixture was stirred for 15 min, followed by neutralization with

Et₃N. The mixture was combined with a solution of H-Arg(NO₂)-Pro-OBzl [prepared from 1.64 g (2.87 mmol) of its Z(OMe) derivative by TFA treatment, followed by Et₃N treatment] in DMF (15 ml) and the whole was stirred overnight in a cold room. The solvent was removed *in vacuo* and the residue was dissolved in AcOEt. The organic layer was washed with 10% Na₂CO₃, 0.5 N HCl and H₂O, and dried. The solvent was removed *in vacuo* and the product was purified by silica gel column chromatography using MeOH-containing CHCl₃. Yield 1.41 g (67%), amorphous powder, decomposed at 91–94°C, R_f^5 0.48, $[\alpha]_D^{23}$ –119.9° ($c=1.0$, MeOH). *Anal.* Calcd for C₃₆H₄₆N₈O₉: C, 58.84; H, 6.32; N, 15.25. Found: C, 58.58; H, 6.23; N, 15.05.

H-Pro-Pro-Arg-Pro-OH (III) Above protected tetrapeptide (536 mg, 0.73 mmol) was hydrogenated over Pd catalyst in 10% AcOH/MeOH for 10 h. The product was purified by CM-cellulose column chromatography using ammonium acetate buffer as an eluent. Yield 269 mg (70%), hygroscopic powder, R_f^2 0.07, $[\alpha]_D^{20}$ –156.0° ($c=0.5$, H₂O). *Anal.* Calcd for C₂₁H₃₅N₇O₅·AcOH·1/2H₂O: C, 51.67; H, 7.56; N, 18.34. Found: C, 51.51; H, 7.65; N, 18.43. Amino acid ratios in an acid hydrolysate: Arg 1.00, Pro 3.29 (average recovery 74%).

Z-Sar-Pro-OBzl Prepared from Z-Sar-OH (11 g, 49 mmol) and H-Pro-OBzl (prepared from 10.2 g of its hydrochloride by treatment with Et₃N, 42 mmol) in DMF (140 ml) by the DCC/HOBt method in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃ as an eluent. Yield 10.8 g (54%), amorphous material, R_f^4 0.55, $[\alpha]_D^{28}$ –71.4° ($c=1.0$, MeOH). *Anal.* Calcd for C₂₃H₂₆N₂O₅: C, 67.29; H, 6.40; N, 6.83. Found: C, 67.43; H, 6.53; N, 6.62.

Z-Sar-Pro-Arg(NO₂)-Pro-OBzl Z-Sar-Pro-OBzl (4 g, 9.7 mmol) was converted to the corresponding hydrazide by treating with hydrazine hydrate (4 ml, 80 mmol) in MeOH (10 ml). The solvent was removed and the residue was dissolved in CHCl₃, followed by washing with H₂O. The solvent was removed to give an oily material (3 g, R_f^3 0.70, hydrazine test; positive) which was used for the next reaction. To a solution of the hydrazide (1.39 g, 4.16 mmol) in DMF (20 ml), 6 N HCl/dioxane (1.69 ml) and isoamyl nitrite (0.56 ml, 4.17 mmol) were added at –10°C and the mixture was stirred for 15 min, followed by neutralization with Et₃N. The mixture was combined with a solution of H-Arg(NO₂)-Pro-OBzl [prepared from 1.02 g (1.79 mmol) of its Z(OMe)-derivative by TFA treatment, followed by Et₃N treatment] in DMF (10 ml) and the whole was stirred overnight in a cold room. The solvent was removed *in vacuo* and the residue was extracted with AcOEt, followed by washing with 10% Na₂CO₃, 0.5 N HCl and H₂O. After removal of the solvent, the residue was purified by silica gel column chromatography using MeOH-containing CHCl₃. Recrystallized from EtOH-ether. Yield 0.72 g (57%), mp 88–91°C, R_f^5 0.41, $[\alpha]_D^{20}$ –71.4° ($c=1.0$, MeOH). *Anal.* Calcd for C₃₄H₄₄N₈O₆·1/2H₂O: C, 56.89; H, 6.31; N, 15.61. Found: C, 56.66; H, 6.56; N, 15.28.

H-Sar-Pro-Arg-Pro-OH (IV) The above protected tetrapeptide (307 mg, 0.43 mmol) was hydrogenated over Pd catalyst in MeOH overnight. The product was purified by RP-HPLC using YMC Pack AQ-ODS-5 column and converted to its hydrochloride. Yield 147 mg (61%), hygroscopic powder, R_f^6 0.41, $[\alpha]_D^{24}$ –100.5° ($c=0.5$, H₂O). *Anal.* Calcd for C₁₉H₃₃N₇O₅·2HCl·2.5H₂O: C, 40.93; H, 7.23; N, 17.58. Found: C, 40.59; H, 7.29; N, 17.21. Amino acid ratios in an acid hydrolysate: Arg 1.00, Pro 2.34 (average recovery 81%).

Z(OMe)-Arg(NO₂)-Sar-OBzl To a solution of Z(OMe)-Arg(NO₂)-OH (10 g, 26 mmol) and H-Sar-OBzl [prepared from its tosylate (11.25 g, 32 mmol) by DIEA treatment] in DMF (150 ml), DCC (5.40 g, 26 mmol) and HOBt (3.5 g, 26 mmol) were added at –10°C and the mixture was stirred overnight. After removal of the solvent, the product was extracted with AcOEt, followed by washing with 10% Na₂CO₃, 5% citric acid and H₂O. The solvent was removed and the residue was purified by silica gel column chromatography using MeOH-containing CHCl₃ as an eluent. Yield 11.8 g (83%), oily material, R_f^5 0.55, $[\alpha]_D^{22}$ –18.9° ($c=1.0$, MeOH). *Anal.* Calcd for C₂₅H₃₂N₆O₈: C, 55.14; H, 5.92; N, 15.43. Found: C, 54.79; N, 5.79; N, 15.04.

Z-Sar-Pro-Arg(NO₂)-Sar-OBzl Isoamyl nitrite (0.8 ml, 5.95 mmol) was added to a solution of Z-Sar-Pro-NHNH₂ (2 g, 5.95 mmol) and 6 N HCl/dioxane (3 ml) in DMF (10 ml) at –10°C and the mixture was stirred for 15 min followed by neutralization with DIEA. The mixture was combined with a solution of H-Arg(NO₂)-Sar-OBzl [prepared from its Z(OMe) derivative (2.5 g, 4.59 mmol) by TFA treatment, followed by DIEA treatment] in DMF (10 ml) and the whole was stirred overnight in a cold room. The solvent was removed *in vacuo* and the residue was extracted with AcOEt, followed by washing with 10% Na₂CO₃, 0.5 N

HCl and H₂O. After removal of the solvent, the product was purified by silica gel column chromatography using MeOH-containing CHCl₃. Yield 1.17 g (37%), amorphous powder, R_f^5 0.38, $[\alpha]_D^{21}$ -70.3° ($c=1.0$, MeOH). *Anal.* Calcd for C₃₂H₄₂N₈O₉: C, 56.29; H, 6.20; N, 16.41. Found: C, 56.36; H, 6.24; N, 16.17.

H-Sar-Pro-Arg-Sar-OH (V) The above protected tetrapeptide (280 mg, 0.41 mmol) was hydrogenated over Pd catalyst in 5% AcOH/MeOH (25 ml) at 40 °C for 15 h. The product was purified by Dowex 50 ($\times 2$) column chromatography using the pH gradient method in 0.05 M pyridine acetate buffer (pH 3.5–5.8). Yield 110 mg (56%), amorphous powder, R_f^6 0.65, $[\alpha]_D^{22}$ -42.6° ($c=1.0$, MeOH). *Anal.* Calcd for C₁₇H₃₁N₇O₅·AcOH·2H₂O: C, 47.78; H, 8.23; N, 20.53. Found: C, 47.33; H, 8.46; N, 20.24. Amino acid ratios in an acid hydrolysate: Sar 2.00, Pro 1.08, Arg 0.92 (average recovery 78%).

Z-D-Ala-Pro-Arg(NO₂)-Pro-OBzl Isobutyl chloroformate (0.43 ml, 3.3 mmol) was added to a solution of Z-D-Ala-OH (0.74 g, 3.3 mmol) and Et₃N (0.46 ml, 3.3 mmol) at -15°C and the mixture was stirred for 15 min. After neutralization with Et₃N, the mixture was combined with a solution of H-Pro-Arg(NO₂)-Pro-OBzl [prepared from its Boc-derivative (2 g, 3.3 mmol) by TFA treatment, followed by neutralization with Et₃N] and the whole was stirred overnight in a cold room. The solvent was removed *in vacuo* and the residue was extracted with AcOEt, followed by washing with 5% Na₂CO₃, 0.5 N HCl and H₂O. The solvent was removed and the residue was purified by silica gel column chromatography using MeOH-containing CHCl₃. Yield 1.65 g (70%), mp 115–120 °C, R_f^7 0.55, $[\alpha]_D^{24}$ -69.0° ($c=1.0$, MeOH). *Anal.* Calcd for C₃₄H₄₄N₈O₉: C, 57.61; H, 6.25; N, 15.80. Found: C, 57.30; H, 6.49; N, 15.96.

H-D-Ala-Pro-Arg-Pro-OH (VI) Above protected tetrapeptide (269 mg, 0.38 mmol) was hydrogenated over Pd catalyst in a mixture of MeOH (15 ml) and 1 N HCl (0.76 ml) for 20 h. The product passed through Sephadex G-25 column (3 $\times$ 150 cm) in H₂O. Yield 122 mg (63%), amorphous powder, R_f^2 0.22, $[\alpha]_D^{24}$ -124.3° ($c=0.5$, H₂O). *Anal.* Calcd for C₁₉H₃₅O₅N₇·2HCl·H₂O: C, 43.20; H, 7.08; N, 18.48. Found: C, 43.04; H, 7.11; N, 18.47.

Z-D-Ala-Pro-Arg(NO₂)-Val-OBzl Z-D-Ala-OH (0.74 g, 3.3 mmol) and H-Pro-Arg(NO₂)-Pro-OBzl [prepared from 2 g (3.3 mmol) of its Boc derivative¹³] by TFA treatment in the usual manner] were reacted by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃. Yield 1.25 g (53%), mp 95–100 °C, R_f^3 0.88, $[\alpha]_D^{22}$ -36.0° ($c=0.9$, MeOH). *Anal.* Calcd for C₃₄H₄₆N₈O₉: C, 57.45; H, 6.52; N, 15.76. Found: C, 57.59; H, 6.38; N, 15.84. Amino acid ratios in an acid hydrolysate: Ala 1.00, Pro 1.06, Arg+Orn 0.89, Val 0.91 (average recovery 86%).

H-D-Ala-Pro-Arg-Val-OH (VII) The above protected tetrapeptide (262 mg, 0.37 mmol) was hydrogenated with Pd catalyst in MeOH in the usual manner. The product was purified by Sephadex G-25 column chromatography using H₂O as an eluent. Yield 119 mg (71%), hygroscopic powder, R_f^2 0.48, $[\alpha]_D^{21}$ -82.7° ($c=0.5$, H₂O). *Anal.* Calcd for C₁₈H₃₁N₇O₅·3/2H₂O: C, 48.69; H, 8.19; N, 20.93. Found: C, 48.25; H, 8.02; N, 21.31. Amino acid ratios in an acid hydrolysate: Ala 1.06, Pro 1.09, Arg 0.88, Val 1.00 (average recovery 86%).

β -Ala-Pro-Arg-Pro-NH₂ (VIII) Prepared by the solid-phase method. The final deprotection was done by HF treatment. The product (315 mg) was purified by CM-cellulose column chromatography using 0.02–0.05 M ammonium acetate buffer. Lyophilized from HCl-containing H₂O. Yield 129 mg (41%), hygroscopic powder, R_f^2 0.38, $[\alpha]_D^{27}$ -70.2° ($c=1.0$, H₂O). *Anal.* Calcd for C₁₉H₃₄N₈O₄·2HCl·1.5H₂O: C, 42.38; H, 7.29; N, 20.80. Found: C, 42.08; H, 7.54; N, 21.01. Amino acid ratios in an acid hydrolysate: β -Ala 1.00, Pro 1.98, Arg 1.10 (average recovery 90%).

Z-Gly-Sar-OBzl A mixed anhydride [prepared from Z-Gly-OH (1.67 g, 8 mmol), Et₃N (1.1 ml, 8 mmol) and isobutyl chloroformate (1.0 ml, 8 mmol) at -10°C in DMF (15 ml)] was added to a solution of H-Sar-OBzl [prepared from its tosylate (1.76 g, 5 mmol) with Et₃N (0.7 ml, 5 mmol)] in DMF (15 ml) and the mixture was stirred overnight. The solvent was removed *in vacuo* and the residue was extracted with AcOEt followed by washing with 10% Na₂CO₃, 10% citric acid and H₂O. The solvent was removed and the residue was recrystallized from EtOH. Yield 1.25 g (68%), mp 88–90 °C, R_f^4 0.67. *Anal.* Calcd for C₂₀H₂₂N₂O₅: C, 64.85; H, 5.99; N, 7.56. Found: C, 64.80; H, 5.96; N, 7.71.

Z-Gly-Sar-NHNH₂ Z-Gly-Sar-OBzl (1 g, 2.7 mmol) was converted to the hydrazide by treatment with NH₂NH₂·H₂O (0.41 ml, 8.1 mmol) in MeOH (10 ml). The product was recrystallized from EtOH. Yield 0.45 g

(57%), mp 139–140 °C, R_f^4 0.24. *Anal.* Calcd for C₁₃H₁₈N₄O₄: C, 53.05; H, 6.16; N, 19.04. Found: C, 52.88; H, 6.44; N, 19.36.

Z-Gly-Sar-Arg(NO₂)-Pro-OBzl Isoamyl nitrite (0.78 ml, 5.8 mmol) and 7.4 N HCl/dioxane (2.34 ml, 17.3 mmol) were added to a solution of Z-Gly-Sar-NHNH₂ (1.70 g, 5.8 mmol) in DMF (10 ml) at -10°C and the mixture was stirred for 15 min followed by neutralization with Et₃N. The mixture was combined with a DMF (10 ml) solution of TFA·H-Arg(NO₂)-Pro-OBzl [prepared from its Z(OMe) derivative (2.70 g, 4.8 mmol) by TFA treatment] and Et₃N (0.68 ml, 4.9 mmol) and the whole was stirred overnight in a cold room. The solvent was removed *in vacuo* and the residue was extracted with AcOEt, followed by washing with 5% Na₂CO₃, 5% citric acid and H₂O. The solvent was removed and the residue was purified by silica gel column chromatography using MeOH-containing CHCl₃ as an eluent. Yield 1.83 g (57%), mp 88–90 °C, R_f^5 0.61, $[\alpha]_D^{22}$ -34.1° ($c=1.0$, DMF). *Anal.* Calcd for C₃₁H₄₀N₈O₉: C, 55.68; H, 6.03; N, 16.76. Found: C, 55.51; H, 6.03; N, 16.69. Amino acid ratios in an acid hydrolysate: Gly 1.00, Sar 1.10, Arg+Orn 0.47, Pro 1.02 (average recovery 70%).

H-Gly-Sar-Arg-Pro-OH (IX) Above protected tetrapeptide (500 mg, 0.78 mmol) was hydrogenated over Pd catalyst in 3% AcOH/MeOH (20 ml). The product was purified by CM-cellulose column chromatography using 0.01–0.05 M ammonium acetate buffer (pH 6.8). The material was lyophilized from 2 eqmol HCl-containing H₂O. Yield 191 mg (52%), hygroscopic powder, R_f^2 0.14, $[\alpha]_D^{23}$ -26.9° ($c=1.0$, H₂O). FAB-MS m/z 400 ($M^+ + 1$). Amino acid ratios in an acid hydrolysate: Gly 1.00, Sar 0.94, Arg 0.89, Pro 1.09 (average recovery 88%).

Z-Gly-Phe-Arg(NO₂)-Pro-OBzl Isoamyl nitrite (0.80 ml, 2.08 mmol) and 7.4 N HCl/dioxane (0.89 ml, 6.24 mmol) were added to a solution of Z-Gly-Phe-NHNH₂ (0.70 g, 2.08 mmol) in DMF (7 ml) at -10°C and the solution was stirred for 15 min. After neutralization with Et₃N, the solution was added to a solution of H-Arg(NO₂)-Pro-OBzl [prepared from its Z(OMe) derivative (1.19 g, 2.29 mmol) by TFA treatment, followed by Et₃N treatment] in DMF (12 ml) and the whole was stirred overnight in a cold room. The solvent was removed *in vacuo* and the residue was extracted with AcOEt, followed by washing with 5% citric acid, 5% Na₂CO₃ and H₂O. The AcOEt was removed and the residue was purified by silica gel column chromatography using MeOH-containing CHCl₃. Yield 0.63 g (45%), amorphous powder, R_f^3 0.92, $[\alpha]_D^{26}$ -49.5° ($c=1.0$, MeOH). *Anal.* Calcd for C₃₂H₂₇N₇O₉: C, 60.28; H, 4.26; N, 15.37. Found: C, 59.97; H, 4.22; N, 15.03. Amino acid ratios in an acid hydrolysate: Gly 1.00, Phe 0.93, Arg+Orn 0.84, Pro 1.00 (average recovery 80%).

H-Gly-Phe-Arg-Pro-OH (X) The above protected tetrapeptide (0.50 g, 0.67 mmol) was hydrogenated over Pd catalyst in MeOH for 12 h. The product was purified by HPLC (YMC Pack AQ 5) using 0.1% TFA-containing CH₃CN/H₂O and converted to its hydrochloride by lyophilization from HCl-containing H₂O. Yield 213 mg (58%), hygroscopic powder, R_f^2 0.49, $[\alpha]_D^{25}$ -28.8° ($c=1.0$, H₂O). *Anal.* Calcd for C₂₂H₃₃N₇O₅·2HCl·1.5H₂O: C, 45.91; H, 6.65; N, 17.03. Found: C, 45.66; H, 6.49; N, 16.67.

H-Gly-D-Pro-Arg-Pro-NH₂ (XI) Prepared by the solid-phase method. The final deprotection was performed by HF treatment. The product (317 mg) was purified by CM-cellulose column chromatography using 0.01–0.05 M ammonium acetate buffer. Lyophilized from 1% AcOH. Yield 184 mg (58%), hygroscopic powder, R_f^2 0.38, $[\alpha]_D^{27}$ -10.8° ($c=1.0$, H₂O). *Anal.* Calcd for C₁₈H₃₂N₈O₄·2AcOH·2H₂O: C, 45.50; H, 7.63; N, 19.29. Found: C, 45.88; H, 7.79; N, 19.09. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.11, Arg 1.04 (average recovery 93%).

H-Gly-D-Pro-Arg-D-Pro-NH₂ (XII) Prepared by the solid-phase method. The final deprotection was performed by HF treatment and the product (316 mg) was purified by CM-cellulose column chromatography using 0.01–0.05 M ammonium acetate buffer. Lyophilized from 1% AcOH. Yield 202 mg (64%), hygroscopic powder, R_f^2 0.38, $[\alpha]_D^{27}$ $+40.6^\circ$ ($c=1.0$, H₂O), FAB-MS m/z : 425 ($M^+ + 1$). Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.27, Arg 1.06 (average recovery 90%).

H-Gly-Pro-D-Arg-Pro-NH₂ (XIII) Prepared by the solid-phase method and the final deprotection was performed by HF treatment. The product (288 mg) was purified by CM-cellulose column chromatography using 0.01–0.05 M ammonium acetate buffer. Lyophilized from HCl-containing H₂O. Yield 184 mg (64%), hygroscopic powder, R_f^2 0.37, $[\alpha]_D^{27}$ -55.8° ($c=1.0$, H₂O). *Anal.* Calcd for C₁₈H₃₂N₈O₄·2HCl·5/3H₂O: C, 40.99; H, 7.13; N, 21.24. Found: C, 41.29; H, 7.07; N, 20.96. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.05, Arg 1.07 (average

recovery 90%).

Boc-Arg(NO₂)-Pro-NH₂ Z-Pro-NH₂ (1.56 g, 6.3 mmol) was hydrogenated over Pd catalyst in MeOH for 3 h and the solvent was removed. The residue was dissolved in DMF (7 ml) and combined with a solution of Boc-Arg(NO₂)-OH (2 g, 6.3 mmol) and Et₃N (1.3 ml, 9.5 mmol) in DMF (10 ml). Diphenylphosphoryl azide (DPPA, 2.05 ml, 9.5 mmol) was added to the mixture at -10 °C and the whole was stirred overnight in a cold room, followed by evaporation of the solvent *in vacuo*. The product was extracted with CHCl₃ and purified by silica gel column chromatography using MeOH-containing CHCl₃. Yield 1.50 g (58%), mp 72–75 °C, *R*_f³ 0.58, [α]_D²⁸ -25.4° (*c*=1.0, MeOH). *Anal.* Calcd for C₁₉H₂₇N₇O₆: C, 50.77; H, 6.05; N, 21.81. Found: C, 50.49; H, 6.25; N, 21.55.

Z-Gly-Pro-Arg(NO₂)-Pro-NH₂ A mixed anhydride was prepared from Z-Gly-Pro-OH (580 mg, 1.90 mmol), Et₃N (0.26 ml, 1.90 mmol) and isobutylchloroformate (0.25 ml, 1.90 mmol) at -10 °C in DMF (4 ml) and was stirred for 15 min. The anhydride solution was combined with a DMF solution (6 ml) of H-Arg(NO₂)-Pro-NH₂ [prepared from its Boc-derivative (650 mg, 1.58 mmol) by TFA treatment, followed by Et₃N treatment] and the mixture was stirred overnight in a cold room. The solvent was removed and the product was extracted with CHCl₃, followed by washing with 5% NaHCO₃, 5% citric acid and H₂O. The CHCl₃ was removed and the residue was recrystallized from CHCl₃. Yield 650 mg (70%), mp 109–114 °C, *R*_f³ 0.48, [α]_D²⁶ -71.9° (*c*=1.0, MeOH). *Anal.* Calcd for C₂₆H₃₇N₉O₇·2H₂O: C, 50.07; H, 6.62; N, 19.29. Found: C, 49.85; H, 6.29; N, 19.11. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.98, Arg+Orn 0.92 (average recovery 82%).

H-Gly-Pro-Arg(NO₂)-Pro-NH₂ (XIV) 25% HBr/AcOH (4.36 ml) was added to a solution of the above protected tetrapeptide (500 mg, 0.85 mmol) in AcOH (4 ml) and the mixture was stirred at 0 °C for 30 min and at 20 °C for 30 min. Dry ether was added to give a precipitate which was collected by centrifugation. The product was purified by CM-cellulose column chromatography using 5–20 mM ammonium acetate buffer. The product was converted to its hydrochloride by lyophilization from HCl-containing H₂O. Yield 243 mg (57%), amorphous powder, *R*_f² 0.38, [α]_D²⁶ -107.2° (*c*=1.0, H₂O). *Anal.* Calcd for C₁₈H₃₁N₉O₆·HCl·1.5H₂O: C, 40.56; H, 6.61; N, 23.65. Found: C, 40.33; H, 6.53; N, 23.88. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.91, Arg+Orn 0.91 (average recovery 71%).

H-Gly-Pro-Lys-Pro-NH₂ (XV) Prepared by the solid-phase method. N^ε-Amino group of Lys was protected with a Z group. Final deprotection was done by treatment with TFMSA in the usual manner. The crude product (248 mg) was purified by CM-cellulose column chromatography using 0.01–0.05 M ammonium acetate buffer. Lyophilized from HCl-containing H₂O. Yield 114 mg (46%), highly hygroscopic powder, *R*_f² 0.39, [α]_D²⁵ -110.9° (*c*=1.0, H₂O), FAB-MS *m/z*: 398 (M⁺+1). Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.17, Lys 1.03 (average recovery 88%).

H-Gly-Pro-Orn-Pro-NH₂ (XVI) Prepared by the solid-phase method. The final deprotection was done by TFMSA treatment. The crude product (280 mg) was purified by CM-cellulose column chromatography using 0.01–0.05 M ammonium acetate buffer. Lyophilized from HCl-containing H₂O. Yield 126 mg (45%), hygroscopic powder, *R*_f² 0.36, [α]_D²⁷ -116.3° (*c*=0.4, H₂O), FAB-MS *m/z*: 383 (M⁺+1). Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.09, Orn 1.23 (average recovery 83%).

H-Gly-Pro-Arg-D-Pro-NH₂ (XVII) Prepared by the solid-phase method. The final deprotection was done by TFMSA treatment. The crude product (318 mg) was purified by CM-cellulose column chromatography using 0.01–0.05 M ammonium acetate buffer. Yield 178 mg (56%), hygroscopic powder, *R*_f² 0.37, [α]_D²⁷ -50.8° (*c*=1.0, H₂O). *Anal.* Calcd for C₁₈H₃₂N₈O₄·2AcOH·H₂O: C, 46.97; H, 7.52; N, 19.91. Found: C, 47.21; H, 7.41; N, 19.80. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.85, Arg 0.98 (average recovery 86%).

Z(OMe)-Arg(NO₂)-[(Me)β-Ala]-OBzl Prepared from Z(OMe)-Arg(NO₂)-OH (2.97 g, 7.8 mmol) and H-(Me)β-Ala-OBzl [prepared from its tosylate (2.88 g, 7.8 mmol) by treatment with Et₃N (1.09 ml, 7.9 mmol)] in DMF (20 ml) by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃. Yield 2.32 g (53%), amorphous powder, *R*_f⁵ 0.61, [α]_D²² -18.2° (*c*=1.0, MeOH). *Anal.* Calcd for C₂₆H₃₄N₆O₈·1/2H₂O: C, 55.00; H, 6.23; N, 14.81. Found: C, 55.02; H, 6.00; N, 14.86.

Z-Gly-Pro-Arg(NO₂)-[(Me)β-Ala]-OBzl Prepared from Z-Gly-Pro-OH (980 mg, 3.23 mmol) and H-Arg(NO₂)-[(Me)β-Ala]-OBzl [prepared

from its Z(OMe) derivative (1.45 g, 3.23 mmol) by TFA treatment, followed by Et₃N treatment] in DMF (15 ml) by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃. Yield 1.41 g (80%), mp 70–74 °C, *R*_f¹ 0.70, [α]_D¹⁸ -68.4° (*c*=1.0, MeOH). *Anal.* Calcd for C₃₂H₄₂N₈O₉: C, 56.28; H, 6.21; N, 16.41. Found: C, 56.03; H, 6.12; N, 16.43.

H-Gly-Pro-Arg-[(Me)β-Ala]-OH (XVIII) The above protected tetrapeptide (300 mg, 0.44 mmol) was hydrogenated over Pd catalyst in a mixture of MeOH (20 ml) and AcOH (4 ml) at 40 °C for 16 h. The solvent was evaporated off and the product was purified by CM-cellulose column chromatography using 0.01–0.05 M ammonium acetate buffer. Yield 113 mg (54%), hygroscopic powder, *R*_f² 0.36, [α]_D²³ -89.9° (*c*=0.5, H₂O), FAB-MS *m/z*: 415 (M⁺+1). Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 0.96, Arg 0.89, (Me)β-Ala 0.84 (average recovery 80%).

H-Gly-Pro-Arg-Pro-NH₂ (XIX) Prepared by the solid-phase method and the final deprotection was done by TFMSA treatment. The crude product (289 mg) was purified by CM-cellulose column chromatography using 0.01–0.05 M ammonium acetate buffer. Lyophilized from HCl-containing H₂O. Yield 78 mg (27%), hygroscopic powder, *R*_f² 0.37, [α]_D²⁷ -111.4° (*c*=1.0, H₂O). *Anal.* Calcd for C₁₈H₃₂N₈O₄·2HCl·1.5H₂O: C, 41.22; H, 7.11; N, 21.36. Found: C, 40.97; H, 7.01; N, 21.19. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.30, Arg 1.10 (average recovery 90%).

H-Gly-Pro-Arg-Sar-NH₂ (XX) Prepared by the solid-phase method and the final deprotection was done by TFMSA treatment. The crude product (302 mg) was purified by HPLC (YMC Pack AQ-5) using 0.1% TFA-containing H₂O/CH₃CN as an eluent. The product was converted to its hydrochloride by lyophilization from HCl-containing H₂O. Yield 142 mg (47%), hygroscopic powder, *R*_f² 0.38, [α]_D²⁵ -41.2° (*c*=1.0, H₂O). *Anal.* Calcd for C₁₆H₃₀N₈O₄·2HCl·H₂O: C, 39.26; H, 7.00; N, 22.89. Found: C, 39.89; H, 7.26; N, 22.49. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.09, Arg 0.95, Sar 0.92 (average recovery 74%).

H-Pro-N(C₂H₅)₂ Isobutyl chloroformate (2.6 ml, 20 mmol) was added to a solution of Z-Pro-OH (5 g, 20 mmol) and Et₃N (2.8 ml, 20 mmol) in DMF (30 ml) at -10 °C and the mixture was stirred for 15 min. HN(C₂H₅)₂ (4.1 ml, 40 mmol) was added and the whole was stirred overnight. The solvent was removed and the product was extracted with AcOEt followed by washing with 5% Na₂CO₃, 5% citric acid and H₂O. The AcOEt was removed and the residue was hydrogenated over Pd catalyst in a mixture of AcOH (1 ml) and MeOH (20 ml) for 1 h. The product was precipitated from MeOH/ether. Yield 1.43 g (42%), syrupy material, *R*_f³ 0.58, [α]_D²⁷ -57.3° (*c*=1.3, MeOH). For elemental analysis, the product was purified by CM-cellulose column chromatography using 0.01–0.5% NH₄OH as an eluent. *Anal.* Calcd for C₉H₁₈N₂O·1/2H₂O: C, 60.30; H, 10.68; N, 16.46. Found: C, 60.11; H, 10.93; N, 16.36.

Z(OMe)-Arg(NO₂)-Pro-N(C₂H₅)₂ Prepared from Z(OMe)-Arg(NO₂)-OH (2.82 g, 7.4 mmol) and H-Pro-N(C₂H₅)₂·AcOH (0.83 g, 4.9 mmol) by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃ as an eluent. Yield 2.20 g (84%), amorphous powder, *R*_f³ 0.73, [α]_D²³ -46.6° (*c*=1.0, MeOH). *Anal.* Calcd for C₂₄H₃₇N₇O₅: C, 53.82; H, 6.96; N, 18.31. Found: C, 53.98; H, 7.11; N, 17.97. Amino acid ratio in an acid hydrolysate: Arg+Orn 1.00, Pro 1.02 (average recovery 89%).

Boc-Gly-Pro-Arg(NO₂)-Pro-N(C₂H₅)₂ Prepared from Boc-Gly-Pro-OH (510 mg, 1.87 mmol) and H-Arg(NO₂)-Pro-N(C₂H₅)₂ [prepared from its Z(OMe) derivative (1 g, 1.87 mmol) by TFA treatment, followed by Et₃N treatment] in DMF (10 ml) by the mixed anhydride method in the usual manner. The solvent was removed *in vacuo* and the product was extracted with *n*-BuOH, followed by washing with 5% citric acid, 5% Na₂CO₃ and H₂O. The *n*-BuOH layer was condensed *in vacuo* and the product was precipitated by the addition of ether. Yield 960 mg (82%), mp 118–122 °C, *R*_f² 0.77, [α]_D²³ -89.7° (*c*=1.0, MeOH). *Anal.* Calcd for C₂₇H₄₇N₉O₈·1/2H₂O: C, 51.09; H, 7.62; N, 19.86. Found: C, 51.04; H, 7.60; N, 19.72. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.38, Arg+Orn 1.16 (average recovery 84%).

H-Gly-Pro-Arg-Pro-N(C₂H₅)₂ (XXI) The above protected tetrapeptide (500 mg, 0.80 mmol) was hydrogenated over Pd catalyst in MeOH (10 ml) for 18 h. The solvent was removed and the residue was dissolved in 6 N HCl/dioxane (2 ml) and stirred for 1 h at room temperature. Chilled ether was added to give a precipitate which was repeatedly washed with ether. The precipitate was lyophilized from H₂O. Yield 240 mg (54%), hygroscopic powder, *R*_f² 0.39, [α]_D²⁷ -119.3° (*c*=1.0,

H₂O). *Anal.* Calcd for C₂₂H₄₀N₈O₄·2HCl·2H₂O: C, 44.82; H, 7.86; N, 19.01. Found: C, 44.92; H, 7.98; N, 18.53. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.05, Arg 0.98 (average recovery 92%).

Z-Pro-Morpholine Prepared from Z-Pro-OH (5.0 g, 20 mmol) and morpholine (2.61 ml, 30 mmol) by the mixed anhydride method in the usual manner. The product was recrystallized from EtOH. Yield 5.45 g (85%), mp 147–149 °C, *R*_f³ 0.79, $[\alpha]_D^{26}$ –22.1° (*c*=1.0, MeOH). *Anal.* Calcd for C₁₇H₂₂N₂O₄: C, 64.13; H, 6.97; N, 8.80. Found: C, 64.07; H, 7.02; N, 8.81.

Boc-Arg(NO₂)-Pro-Morpholine DPPA (6.14 ml, 28 mmol) was added to a solution of Boc-Arg(NO₂)-OH (7.33 g, 23 mmol), Et₃N (3.17 ml, 23 mmol) and H-Pro-morpholine [prepared from its Z derivative (6.1 g, 19 mmol) by catalytic hydrogenation] in DMF (30 ml) at –10 °C and the mixture was stirred overnight in a cold room. The solvent was removed *in vacuo* and the product was extracted with CHCl₃, followed by washing with NaCl-saturated 5% Na₂CO₃, 5% citric acid and H₂O. The CHCl₃ was removed and the residue was purified by silica gel column chromatography using MeOH-containing CHCl₃ as an eluent. Yield 5.68 g (69%), mp 79–82 °C, *R*_f³ 0.65, $[\alpha]_D^{26}$ –22.9° (*c*=1.0, MeOH). *Anal.* Calcd for C₂₀H₃₃N₇O₉: C, 49.47; H, 7.26; N, 20.19. Found: C, 49.35; H, 7.34; N, 19.89. Amino acid ratio in an acid hydrolysate: Arg + Orn 0.92, Pro 1.00 (average recovery 86%).

Z-Gly-Pro-Arg(NO₂)-Pro-Morpholine The above protected dipeptide (1 g, 2.06 mmol) was treated with TFA to remove the Z(OMe) group and reacted with Z-Gly-Pro-OH (630 mg, 2.06 mmol) by the mixed anhydride method in the usual manner. The product was recrystallized from AcOEt. Yield 930 mg (67%), mp 95–98 °C, *R*_f³ 0.70, $[\alpha]_D^{26}$ –85.8° (*c*=1.0, MeOH). *Anal.* Calcd for C₃₀H₄₃N₉O₉·1/2H₂O: C, 52.78; H, 6.50; N, 18.46. Found: C, 52.86; H, 6.52; N, 18.11. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.13, Arg + Orn 0.92 (average recovery 78%).

H-Gly-Pro-Arg-Pro-Morpholine (XXII) The above protected tetrapeptide (450 mg, 67 mmol) was hydrogenated over Pd catalyst in MeOH in the usual manner. The product was purified by CM-cellulose column chromatography using 0.01–0.05 M ammonium acetate buffer. The product was converted to its HCl salt by lyophilization from HCl-containing H₂O. Yield 231 mg (70%), hygroscopic powder, *R*_f² 0.30, $[\alpha]_D^{26}$ –114.2° (*c*=1.0, H₂O). *Anal.* Calcd for C₂₂H₃₈N₈O₄·2HCl·4H₂O: C, 42.38; H, 7.76; N, 18.00. Found: C, 42.71; H, 7.64; N, 18.38. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.10, Arg 0.92 (average recovery 74%).

Boc-Pro-Hexamethyleneimine Prepared from Boc-Pro-OH (5 g, 23 mmol) and hexamethyleneimine (2.60 ml, 23 mmol) by the DCC/HOBt method in DMF in the usual manner. The product was recrystallized from AcOEt. Yield 4.70 g (69%), mp 55–58 °C, *R*_f⁵ 0.68, $[\alpha]_D^{23}$ –33.0° (*c*=1.0, MeOH). *Anal.* Calcd for C₁₆H₂₈N₂O₃: C, 64.83; H, 9.52; N, 9.45. Found: C, 64.55; H, 9.59; N, 9.17.

Boc-Arg(Mts)-Pro-Hexamethyleneimine The above protected Pro derivative (1.30 g, 4.4 mmol) was treated with TFA to remove Boc-group and reacted with Boc-Arg(Mts)-OH (2 g, 4.4 mmol) by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃ as an eluent. Yield 1.30 g (47%), mp 91–95 °C, *R*_f⁵ 0.69, $[\alpha]_D^{24}$ –22.4° (*c*=1.0, MeOH). *Anal.* Calcd for C₃₃H₅₀N₆O₆S: C, 58.65; H, 7.94; N, 13.24. Found: C, 58.38; H, 7.83; N, 12.97.

Z-Gly-Pro-Arg(Mts)-Pro-Hexamethyleneimine Z-Gly-Pro-OH (1.50 g, 4.8 mmol) and H-Arg(Mts)-Pro-hexamethyleneimine [prepared from its Boc derivative (1.0 g, 1.60 mmol) by TFA treatment] was reacted by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃. Yield 990 mg (75%), mp 108–111 °C, *R*_f⁵ 0.58, $[\alpha]_D^{24}$ –72.1° (*c*=1.0, MeOH). *Anal.* Calcd for C₄₁H₅₉N₈O₈S·1/2H₂O: C, 59.11; H, 7.26; N, 13.45. Found: C, 59.07; H, 7.04; N, 13.24.

H-Gly-Pro-Arg-Pro-Hexamethyleneimine (XXIII) The above protected tetrapeptide (600 mg, 0.78 mmol) was treated with TFMSA in the usual manner. The product was purified by HPLC (YMC Pack AQ-5) using 0.1% TFA-containing CH₃CN/H₂O as an eluent. The product was converted to its hydrochloride by lyophilization from HCl-containing H₂O. Yield 206 mg (49%), hygroscopic powder, *R*_f² 0.41, $[\alpha]_D^{27}$ –104.3° (*c*=1.0, H₂O). *Anal.* Calcd for C₂₄H₄₂N₈O₄·2HCl·1.5H₂O: C, 47.52; H, 7.80; N, 18.47. Found: C, 47.86; H, 7.70; N, 18.19. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.91, Arg 1.07 (average recovery 80%).

Boc-Pro-Cyclohexylamine Prepared from Boc-Pro-OH (5 g, 23 mmol) and cyclohexylamine (2.6 ml, 46 mmol) by the DCC/HOBt method in the

usual manner. Recrystallized from AcOEt. Yield 5.42 g (80%), mp 142–144 °C, *R*_f⁵ 0.72, $[\alpha]_D^{24}$ –48.1° (*c*=1.0, MeOH). *Anal.* Calcd for C₁₆H₂₈N₂O₃: C, 64.83; H, 9.52; N, 9.45. Found: C, 65.02; H, 9.80; N, 9.45.

Boc-Arg(Mts)-Pro-Cyclohexylamine Boc-Arg(Mts)-OH (2 g, 4.4 mmol) and H-Pro-cyclohexylamine [prepared from its Boc derivative (950 mg, 4.4 mmol) by TFA treatment in the usual manner] were reacted by the DCC/HOBt method in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃. Yield 1.88 g (67%), mp 115–118 °C, *R*_f⁵ 0.51, $[\alpha]_D^{24}$ –30.8° (*c*=1.0, MeOH). *Anal.* Calcd for C₃₁H₅₀N₆O₆S: C, 58.65; H, 7.94; N, 13.24. Found: C, 58.93; H, 8.18; N, 12.91.

Z-Gly-Pro-Arg(Mts)-Pro-Cyclohexylamine Z-Gly-Pro-OH (1.50 g, 4.8 mmol) and H-Arg(Mts)-Pro-cyclohexylamine [prepared from its Boc derivative (1 g, 1.58 mmol) by TFA treatment in the usual manner] were reacted by the DCC/HOBt method in the usual manner. The product was purified by silica gel column chromatography. Yield 950 mg (72%), mp 122–125 °C, *R*_f⁵ 0.66, $[\alpha]_D^{24}$ –76.0° (*c*=1.0, MeOH). *Anal.* Calcd for C₄₁H₅₈N₈O₈S: C, 59.83; H, 7.10; N, 13.61. Found: C, 59.79; H, 7.15; N, 13.47. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.04, Arg 1.04 (average recovery 98%).

H-Gly-Pro-Arg-Pro-Cyclohexylamine (XXIV) Z-Gly-Pro-Arg(Mts)-Pro-cyclohexylamine (600 mg, 0.73 mmol) was treated with TFMSA in the usual manner. The product was purified by HPLC (YMC Pack AQ-ODS-5) using 0.1% TFA-containing CH₃CN/H₂O as an eluent. The product was converted to HCl salt by lyophilization from HCl-containing H₂O. Yield 237 mg (56%), hygroscopic powder, *R*_f² 0.38, $[\alpha]_D^{27}$ –105.2° (*c*=1.0, H₂O). *Anal.* Calcd for C₂₄H₄₂N₈O₄·2HCl·2H₂O: C, 46.82; H, 7.86; N, 18.20. Found: C, 46.64; H, 7.56; N, 17.91. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.11, Arg 1.03 (average recovery 88%).

Boc-Pro-Cycloheptylamine Prepared from Boc-Pro-OH (5.00 g, 23 mmol) and cycloheptylamine (3.6 ml, 28 mmol) by the DCC/HOBt method in the usual manner. Recrystallized from AcOEt. Yield 5.80 g (81%), *R*_f⁵ 0.75, $[\alpha]_D^{24}$ –45.3° (*c*=1.0, MeOH). *Anal.* Calcd for C₁₇H₃₀N₂O₃: C, 65.77; H, 9.74; N, 9.02. Found: C, 65.71; H, 9.97; N, 9.10.

Boc-Arg(Mts)-Pro-Cycloheptylamine Boc-Arg(Mts)-OH (3.5 g, 7.7 mmol) and H-Pro-cycloheptylamine [prepared from its Boc derivative (2 g, 6.4 mmol) by TFA treatment in the usual manner] was reacted by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography. Yield 4.07 g (79%), mp 112–116 °C, *R*_f⁵ 0.78, $[\alpha]_D^{24}$ –30.6° (*c*=1.0, MeOH). *Anal.* Calcd for C₃₂H₅₃N₆O₆S: C, 59.14; H, 8.22; N, 12.93. Found: C, 59.27; H, 8.37; N, 12.63.

Z-Gly-Pro-Arg(Mts)-Pro-Cycloheptylamine Z-Gly-Pro-OH (1.5 g, 4.8 mmol) and H-Arg(Mts)-Pro-cycloheptylamine [prepared from its Boc derivative (1.1 g, 1.6 mmol) by TFA treatment in the usual manner] were reacted by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃ as an eluent. Yield 1.1 g (82%), mp 118–121 °C, *R*_f⁵ 0.50, $[\alpha]_D^{24}$ –71.1° (*c*=1.0, MeOH). *Anal.* Calcd for C₄₂H₆₀N₈O₈S·H₂O: C, 59.00; H, 7.31; N, 13.10. Found: C, 59.26; H, 7.27; N, 13.09. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.99, Arg 1.03 (average recovery 97%).

H-Gly-Pro-Arg-Pro-Cycloheptylamine (XXV) Z-Gly-Pro-Arg(Mts)-Pro-cycloheptylamine (600 mg, 0.72 mmol) was treated with TFMSA in the usual manner. The product was purified by HPLC on YMC Pack AQ-ODS-5 using 0.1% TFA-containing CH₃CN/H₂O. The product was converted to HCl salt by lyophilization from HCl-containing H₂O. Yield 290 mg (58%), hygroscopic powder, *R*_f² 0.40, $[\alpha]_D^{27}$ –117.3° (*c*=1.0, H₂O). *Anal.* Calcd for C₂₅H₄₄N₈O₄·2HCl·5/4H₂O: C, 48.74; H, 7.93; N, 18.18. Found: C, 49.10; H, 7.94; N, 17.82.

Boc-Pro-Cyclooctylamine Boc-Pro-OH (3 g, 14 mmol) and cyclooctylamine (2 g, 14 mmol) were reacted by the DCC/HOBt method in the usual manner. Recrystallized from AcOEt. Yield 3.73 g (82%), mp 135–138 °C, *R*_f⁵ 0.83, $[\alpha]_D^{23}$ –50.1° (*c*=1.0, MeOH). *Anal.* Calcd for C₁₈H₃₂N₂O₃: C, 66.63; H, 9.94; N, 8.63. Found: C, 66.50; H, 10.17; N, 8.72.

Boc-Arg(Mts)-Pro-Cyclooctylamine Boc-Arg(Mts)-OH (1 g, 2.2 mmol) and H-Pro-cyclooctylamine [prepared from its Boc derivative (0.71 g, 2.2 mmol) by TFA treatment in the usual manner] were reacted by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using MeOH-containing CHCl₃ as an eluent. Yield 1.1 g (75%), mp 101–103 °C, *R*_f⁵ 0.80, $[\alpha]_D^{23}$ –30.7° (*c*=1.0, MeOH). *Anal.* Calcd for C₃₃H₅₄N₆O₆S: C, 59.74; H, 8.21; N, 12.68. Found: C, 59.76; H, 8.51; N, 12.38.

Z-Gly-Pro-Arg(Mts)-Pro-Cyclooctylamine Z-Gly-Pro-OH (610 mg, 2 mmol) and H-Arg(Mts)-Pro-cyclooctylamine [prepared from its Boc derivative (840 mg, 1.3 mmol) by TFA treatment in the usual manner] were reacted by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography. Yield 700 mg (63%), mp 113–116 °C, R_f^3 0.67, $[\alpha]_D^{25}$ –73.0° ($c=1.0$, MeOH). *Anal.* Calcd for $C_{43}H_{62}N_8O_8S$: C, 60.68; H, 7.34; N, 13.17. Found: C, 60.41; H, 7.26; N, 12.90. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.04, Arg 1.04 (average recovery 98%).

H-Gly-Pro-Arg-Pro-Cyclooctylamine (XXVI) Z-Gly-Pro-Arg(Mts)-Pro-cyclooctylamine (500 mg, 0.59 mmol) was treated with TFMSA in the usual manner. The product was purified by HPLC on YMC Pack AQ-ODS-5 using 0.1% TFA-containing CH_3CN/H_2O and converted to HCl salt by lyophilization from HCl-containing H_2O . Yield 280 mg (79%), hygroscopic powder, R_f^2 0.40, $[\alpha]_D^{27}$ –116.1° ($c=1.0$, H_2O). *Anal.* Calcd for $C_{26}H_{46}N_8O_4 \cdot 2HCl \cdot 7/4H_2O$: C, 48.86; H, 8.12; N, 17.53. Found: C, 48.86; H, 7.99; N, 17.53. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.13, Arg 1.06 (average recovery 83%).

Z-Pro-Aniline Z-Pro-OH (5 g, 20 mmol) and aniline (1.8 ml, 20 mmol) were reacted by the DCC/HOBt method in the usual manner. The product was recrystallized from EtOH. Yield 4.8 g (74%), mp 111–114 °C, R_f^5 0.89, $[\alpha]_D^{26}$ –64.5° ($c=1.0$, MeOH). *Anal.* Calcd for $C_{19}H_{20}N_2O_2 \cdot 1/2EtOH$: C, 70.99; H, 6.55; N, 8.28. Found: C, 70.30; H, 6.46; N, 8.89.

Boc-Arg(NO₂)-Pro-Aniline DPPA (1.2 ml, 5.54 mmol) was added to a solution of Boc-Arg(NO₂)-OH (1.77 g, 5.54 mmol), Et_3N (0.76 ml, 5.54 mmol) and H-Pro-aniline [prepared from its Z derivative (0.95 g, 3.1 mmol) by hydrogenation in MeOH] in DMF (10 ml) and the mixture was stirred overnight in a cold room. The solvent was removed *in vacuo* and the product was extracted with AcOEt followed by washing with 5% Na_2CO_3 , 5% citric acid and H_2O . The solvent was evaporated off and the residue was recrystallized from MeOH. Yield 1.22 g (81%), mp 142–146 °C, R_f^5 0.38, $[\alpha]_D^{26}$ –67.8° ($c=1.0$, MeOH). *Anal.* Calcd for $C_{22}H_{33}N_7O_6$: C, 53.76; H, 6.77; N, 19.95. Found: C, 53.71; H, 6.82; N, 19.52. Amino acid ratio in an acid hydrolysate: Arg+Orn 0.93, Pro 1.00 (average recovery 83%).

Z-Gly-Pro-Arg(NO₂)-Pro-Aniline Z-Gly-Pro-OH (370 mg, 1.22 mmol) and H-Arg(NO₂)-Pro-aniline [prepared from its Boc derivative (500 mg, 1.22 mmol) by TFA treatment in the usual manner] were reacted by the mixed anhydride method in the usual manner. The product was extracted with $CHCl_3$ followed by washing with 5% Na_2CO_3 , 5% citric acid and H_2O . The $CHCl_3$ was evaporated off and the residue was precipitated from $CHCl_3$ /petroleum ether. Yield 450 mg (65%), mp 137–141 °C, R_f^3 0.67, $[\alpha]_D^{26}$ –107.0° ($c=1.0$, MeOH). *Anal.* Calcd for $C_{32}H_{41}N_9O_8 \cdot H_2O$: C, 55.08; H, 6.21; N, 18.07. Found: C, 55.06; H, 6.02; N, 18.32. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.99, Arg+Orn 0.87 (average recovery 84%).

H-Gly-Pro-Arg-Pro-Aniline (XXVII) Z-Gly-Pro-Arg(NO₂)-Pro-aniline (400 mg, 0.58 mmol) was hydrogenated over Pd catalyst in MeOH for 10 h. The product was purified by CM-cellulose column chromatography, followed by HPLC on YMC Pack ODS-5. The purified product was converted to HCl salt by lyophilization from HCl-containing H_2O . Yield 162 mg (49%), hygroscopic powder, R_f^2 0.57, $[\alpha]_D^{26}$ –135.6° ($c=1.0$, H_2O), FAB-MS m/z : 501 ($M^+ + 1$). Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.11, Arg 1.03 (average recovery 87%).

Z-Pro-p-Nitroaniline PCl_3 (0.35 ml, 4 mmol) was added to a solution of *p*-nitroaniline (1.1 g, 8 mmol) in dry pyridine (6 ml) at –10 °C and the mixture was stirred for 15 min. Z-Pro-OH (2 g, 8 mmol) was added and the mixture was stirred for 3 h at 60 °C and overnight at room temperature. The solvent was removed and the product was extracted with AcOEt, followed by washing with 5% Na_2CO_3 , 5% citric acid and H_2O . The solvent was removed and the product was recrystallized from AcOEt. Yield 1.40 g (77%), mp 134–137 °C, R_f^3 0.67, $[\alpha]_D^{24}$ –72.0° ($c=1.0$, MeOH). *Anal.* Calcd for $C_{19}H_{19}N_3O_5$: C, 61.78; H, 5.18; N, 11.37. Found: C, 61.55; H, 5.09; N, 11.26.

Boc-Arg(Mts)-Pro-p-Nitroaniline Z-Pro-*p*-nitroaniline (1 g, 2.7 mmol) was dissolved in 30% HBr/AcOH (3 ml) and the solution was stirred for 1 h at room temperature. Chilled ether was added to give a precipitate which was collected by centrifugation and washed with ether. The material was dissolved in a mixture of DMF (8 ml) and Et_3N (0.39 ml), and reacted with Boc-Arg(Mts)-OH (1.5 g, 3.2 mmol) by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography. Yield 1.4 g (77%), mp 134–137 °C, R_f^3 0.71, $[\alpha]_D^{24}$ –72.0° ($c=1.0$, MeOH). *Anal.* Calcd for $C_{31}H_{43}N_7O_8S$: C, 55.26; H, 6.43; N, 14.55. Found: C, 54.99; H, 6.73; N, 14.19.

Z-Gly-Pro-Arg(Mts)-Pro-p-Nitroaniline Z-Gly-Pro-OH (550 mg, 1.8 mmol) and H-Arg(Mts)-Pro-*p*-nitroaniline [prepared from its Boc derivative (1 g, 1.5 mmol) by TFA treatment in the usual manner] were reacted by the mixed anhydride method in the usual manner. The product was recrystallized from AcOEt/ether. Yield 1.05 g (81%), mp 131–134 °C, R_f^3 0.67, $[\alpha]_D^{24}$ –106.3° ($c=1.0$, MeOH). *Anal.* Calcd for $C_{41}H_{51}N_9O_{10}S \cdot 1/2H_2O$: C, 56.54; H, 6.02; N, 14.47. Found: C, 56.36; H, 6.06; N, 14.22. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.86, Arg 1.00 (average recovery 84%).

H-Gly-Pro-Arg-Pro-p-Nitroaniline (XXVIII) Z-Gly-Pro-Arg(Mts)-Pro-*p*-nitroaniline (500 mg, 0.58 mmol) was deblocked by TFMSA treatment in the usual manner. The product was purified by HPLC on a YMC Pack ODS-5 column and the material was converted to HCl salt by lyophilization from H_2O containing equivalent HCl. Yield 180 mg (51%), hygroscopic powder, R_f^2 0.57, $[\alpha]_D^{27}$ –152.1° ($c=1.0$, H_2O), FAB-MS m/z : 545 ($M^+ + 1$). Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 1.89, Arg 0.92 (average recovery 89%).

Z-Arg(Tis)-Pro-NHNHBoc Z-Arg(Tis)-OH (1.6 g, 2.8 mmol) and H-Pro-NHNHBoc (642 mg, 2.8 mmol) were reacted by the DCC/HOBt method in the usual manner. The product was recrystallized from ether. Yield 1.96 g (89%), mp 97–102 °C, R_f^5 0.75, $[\alpha]_D^{26}$ –51.8° ($c=1.0$, MeOH). *Anal.* Calcd for $C_{39}H_{60}N_7O_8S$: C, 59.52; H, 7.68; N, 12.46. Found: C, 59.25; H, 7.57; N, 12.17. Amino acid ratio in an acid hydrolysate: Arg 1.00, Pro 1.05 (average recovery 80%).

Z-Gly-Pro-Arg(Tis)-Pro-NHNHBoc Z-Gly-Pro-OH (919 mg, 3 mmol) and H-Arg(Tis)-Pro-NHNHBoc [prepared from its Z derivative (1.65 g, 2.1 mmol) by hydrogenation in MeOH in the usual manner] were reacted by the DCC/HOBt method in the usual manner. The product was purified by silica gel column chromatography. Yield 1.6 g (67%), mp 127–131 °C, R_f^5 0.74, $[\alpha]_D^{26}$ –84.2° ($c=1.0$, MeOH). *Anal.* Calcd for $C_{46}H_{70}N_9O_{10}S$: C, 58.77; H, 7.40; N, 13.41. Found: C, 58.50; H, 7.51; N, 13.19. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.11, Arg 1.06 (average recovery 87%).

H-Gly-Pro-Arg-Pro-NHNH₂ (XXIX) Z-Gly-Pro-Arg(Tis)-Pro-NHNHBoc (500 mg, 0.53 mmol) was deblocked by TFMSA treatment in the usual manner. The product was purified by CM-cellulose column chromatography and was obtained in 0.05 M $AcONH_4$ eluate. Lyophilized from 1% AcOH. Yield 242 mg (73%), hygroscopic powder, R_f^2 0.36, $[\alpha]_D^{26}$ –112.9° ($c=1.0$, H_2O). *Anal.* Calcd for $C_{18}H_{33}N_9O_4 \cdot 3AcOH \cdot 3/2H_2O$: C, 44.62; H, 7.48; N, 19.48. Found: C, 44.73; H, 7.35; N, 19.13. Amino acid ratios in an acid hydrolysate: Gly 1.00, Pro 2.09, Arg 1.04 (average recovery 83%).

Acknowledgement This work was supported in part by the Science Research Promotion Fund of the Japan Private School Promotion Foundation.

References and Notes

- Standard abbreviations for amino acids, protecting groups, and peptides are used [*Eur. J. Biochem.*, **138**, 9 (1984)]. Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Boc = *tert*-butoxycarbonyl. Other abbreviations include: DMF = dimethylformamide, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid, DIEA = diisopropylethylamine, Mts = mesitylenesulfonyl, Tis = 2,4,6-triisopropylbenzenesulfonyl, DPPA = diphenylphosphoryl azide, HOBt = 1-hydroxybenzotriazole, FTC = fibrinogen/thrombin clotting, NMM = *N*-methylmorpholine.
- A. P. Laudano and R. F. Doolittle, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 3085 (1978).
- K. Kawasaki, T. Tsuji, K. Hirase, M. Miyano, Y. Imoto, and M. Iwamoto, *Chem. Pharm. Bull.*, **39**, 584 (1991).
- W. König and R. Geiger, *Chem. Ber.*, **103**, 788, 2024 (1970).
- M. Bodanszky, *Nature* (London), **175**, 685 (1955).
- K. Hofmann and M. Bergmann, *J. Biol. Chem.*, **138**, 243 (1941).
- H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. Chem. Soc., Chem. Commun.*, **1974**, 107.
- H. Yajima, M. Takeyama, J. Hanai, and K. Mitani, *J. Chem. Soc., Chem. Commun.*, **1978**, 482.
- H. Echner and W. Voelter, *Z. Naturforsch.*, **42b**, 1591 (1987).
- B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).
- J. R. Vaughan, Jr. and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).
- T. Wieland and B. Heinke, *Ann. Chem.*, **615**, 184 (1958).
- K. Kawasaki, T. Tsuji, K. Hirase, M. Miyano, S. Inouye, and M. Iwamoto, *Chem. Pharm. Bull.*, accepted.