# 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–$NHNH_2$. Z(OMe)–Arg($NO_2$)–OH and H–Pro–OBzl were coupled by the DCC/HOBt method, followed by TFA treatment to give H–Arg($NO_2$)–Pro–OBzl. It was reacted with Z–Gly–Pro–$NHNH_2$ *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 method[5] 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 $N^G$-nitroarginine[6]-containing peptides and trifluoromethanesulfonic acid (TFMSA) treatment[7] 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 $IC_{50}$ of synthetic peptides varied widely with used lots of fibrinogen and thrombin, $IC_{50}$ of I was always measured for comparison when the inhibitory effect of the synthetic peptides was measured. Relative activity was calculated by dividing $IC_{50}$ 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 $Gly^1$ was

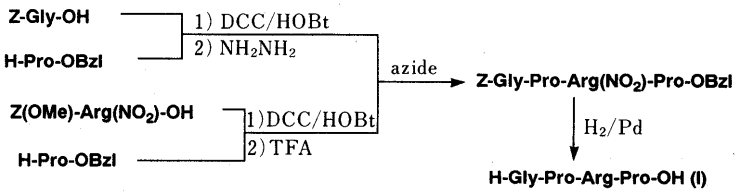


Fig. 1. Synthetic Scheme for H–Gly–Pro–Arg–OH (I)

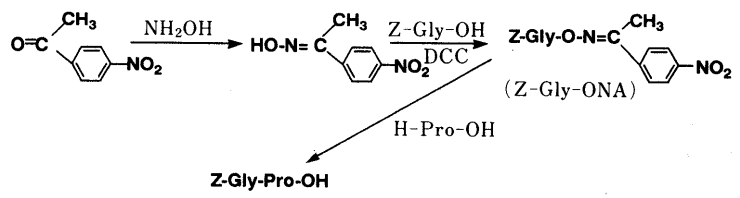


Fig. 2. Synthetic Scheme for Z–Gly–ONA and Z–Gly–Pro–OH

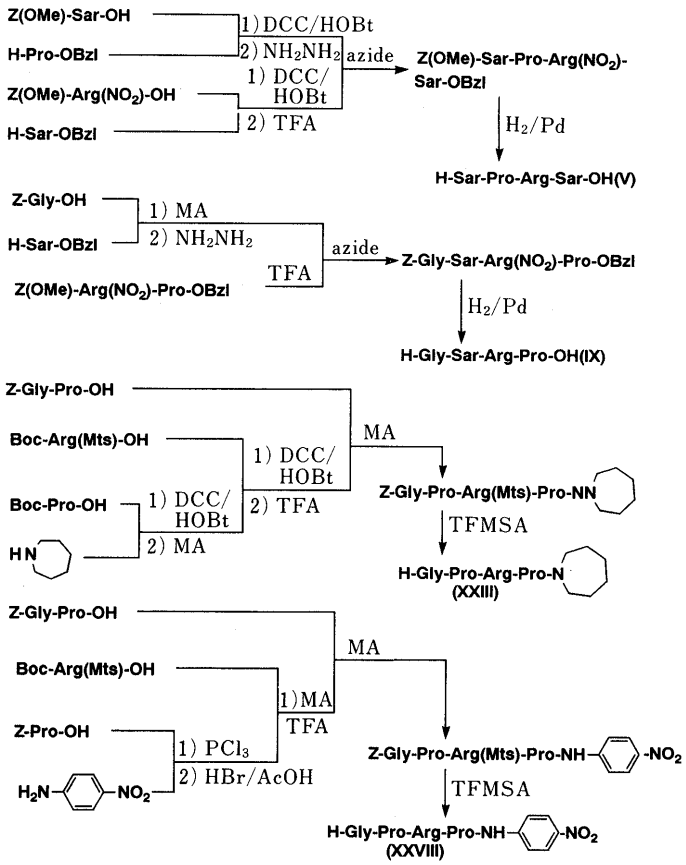


Fig. 3. Synthetic Schemes for Fibrin Related Tetrapeptides

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 $D\text{-}Ala^1$, $Val^4$ analog (VII)] also resulted in the loss of the effect. Replacement of $Pro^4$ with Sar did not affect the inhibitory effect as reported,[2] but replacement of $Pro^2$ with Sar resulted in a remarkable decrease in the inhibitory effect. Similar results were obtained when $Pro^2$ was replaced with Phe or $D\text{-}Pro^2$. $L\text{-}Pro^2$ is essential but $Pro^4$ is not essential and potentiates the effect. $Arg^3$ was replaced with D-Arg, Arg($NO_2$), 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–$N(C_2H_5)_2$ (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–*p*-nitroaniline (XXVIII) | 2.21 |
| H–Gly–Pro–Arg–Pro–$NHNH_2$ (XXIX) | 2.70 |

remarkable decreases in the effect. $L\text{-}Arg^3$ is essential for the activity. When $Pro^4$ 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 0.33[3] and $Pro^4$ and $Sar^4$ 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 hexamethyleneimide compound (XXIII) was a potent inhibitor whose inhibitory effect was 3.25 times as high as that of I. Cyclohexylamide, cycloheptylamide and cyclooctylamide 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 anticoagulant[2] and here we found more potent anticoagulants resulting from modification of $Pro^4$.

## 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–$H_2O$ (4 : 1 : 5, upper phase), $Rf^2$ = BuOH–AcOH–pyridine–$H_2O$ (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). Rotations were measured with a JASCO DIP-360 polarimeter. The synthetic peptides were hydrolyzed in 6 N 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($NO_2$)-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. $N^{\alpha}$-Amino groups of amino acids were protected with a Boc group, and a guanidino group of Arg was protected with a mesitylenesulfonyl group. $N^{\omega}$-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 $CH_3CN/H_2O$ 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 $H_2O$ containing an equivalent amount of HCl. Fast atom bombardment mass spectra (FAB-MS) were measured on a VG Analitical ZAV-SE spectrometer.

**General Procedure for the Mixed Anhydride Method** Equimolar reagents were used. Isobutyl chloroformate[11] and $Et_3N$ (or NMM) were added to a $N^{\alpha}$-protected amino acid (or peptide) solution in DMF at −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% $Na_2CO_3$, 5% citric acid and $H_2O$. The AcOEt layer was dried with $Na_2SO_4$ and evaporated off. The product was purified by a suitable method.

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.

**General Procedure for the DCC and DCC/HOBt Methods** Equimolar reagents were used. DCC was added to a solution of $N^{\alpha}$-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% $Na_2CO_3$, 5% citric acid and $H_2O$. The AcOEt layer was dried over $Na_2SO_4$ and evaporated off. The residue was purified by a suitable method.

**General Procedure for TFA Treatment** $N^{\alpha}$-Boc or $N^{\alpha}$-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 $Et_3N$ (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 molars excess of thioanisole and 20 molars 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 $H_2O$ (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 $H_2O$. Recrystallized from MeOH. Yield 1.8 g (83%), mp 173—174 °C. *Anal.* Calcd for $C_8H_8N_2O_3$: 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 $C_{18}H_{17}N_3O_6$: 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 $H_2O$ (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 $H_2O$. The $H_2O$ layer was washed with AcOEt and acidified with 6 N 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 sample[3] 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–ONp[12] and H–Pro–OH in the same manner as described above. Yield 80%, mp 152—155 °C, $Rf^3$ 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% $Na_2CO_3$ and $H_2O$. After drying over $Na_2SO_4$, the solvent was evaporated off and the residue was purified by silica gel column chromatography using $CHCl_3$–MeOH as an eluent. Oily material. Yield 8.0 g (84%), $Rf^4$ 0.62, $[\alpha]_D^{24}$ −77.2° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{22}H_{24}N_2O_5$: 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–NHNH$_2$** 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 $CHCl_3$, followed by washing with $H_2O$. The solvent was removed *in vacuo* and the residue was precipitated from $CHCl_3$/petroleum ether and dried. Yield 5.6 g (80.4%), amorphous powder, $Rf^3$ 0.57, $[\alpha]_D^{24}$ −70.9° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{15}H_{20}N_4O_4 \cdot 1/4H_2O$: 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(NO$_2$)-Pro–OBzl** Z(OMe)–Arg(NO$_2$)–OH (10 g, 26 mmol) and H–Pro–OBzl [prepared from its hydrochloride (6.3 g, 26 mmol) and $Et_3N$ (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 $CHCl_3$ as an eluent. Yield 10.48 g (71%), amorphous powder, $Rf^4$ 0.71, $[\alpha]_D^{24}$ −56.7° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{27}H_{34}N_6O_8$: C, 56.83; H, 6.01; N, 14.73. Found: C, 56.55; H, 6.00; N, 14.73.

**Z–Gly–Pro–Arg(NO$_2$)–Pro–OBzl** To a DMF solution (20 ml) of Z–Gly–Pro–NHNH$_2$ (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 $Et_3N$ and combined with a DMF solution of H–Arg(NO$_2$)–Pro–OBzl [prepared from 2 g (3.51 mmol) of Z(OMe) derivative by TFA treatment, followed by $Et_3N$ treatment]. After stirring overnight, the solvent was removed *in vacuo* and the residue was dissolved in AcOEt, followed by washing with 10% $Na_2CO_3$, 5% citric acid and $H_2O$. After removal of the solvent, the residue was purified by silica gel column chromatography using MeOH-containing $CHCl_3$ as an eluent. Yield 1.80 g (74%), mp 114—120 °C, $Rf^5$ 0.69, $[\alpha]_D^{25}$ −85.8° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{33}H_{42}N_9O_8$: 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(NO$_2$)–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 $H_2O$. Yield 255 mg (71%), hygroscopic powder, $Rf^1$ 0.10, $Rf^2$ 0.22, $[\alpha]_D^{25}$ −69.4° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{18}H_{31}N_7O_5 \cdot 2HCl \cdot 1/2H_2O$: 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 mannual 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, $Rf^2$ 0.34, $[\alpha]_D^{25}$ −50.8° ($c$=1.0, $H_2O$), 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–NHNH$_2$** 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 $NH_2NH_2 \cdot H_2O$ (4.77 ml, 93 mmol) in MeOH. The solvent was evaporated off and the residue was dissolved in $CHCl_3$, followed by washing with $H_2O$. The $CHCl_3$ layer was condensed and petroleum ether was added to give a solid. Yield 9.31 g (83%), mp 68—71 °C, $Rf^3$ 0.19, $[\alpha]_D^{18}$ −112.0° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{18}H_{24}N_4O_4$: C, 59.98; H, 6.73; N, 15.54. Found: C, 59.73; H, 6.83; N, 15.33.

**Z–Pro–Pro–Arg(NO$_2$)–Pro–OBzl** Isoamyl nitrite (0.64 ml, 4.77 mmol) was added to a solution of Z–Pro–Pro–NHNH$_2$ (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 $Et_3N$. The mixture was combined with a solution of H–Arg(NO$_2$)–Pro–OBzl [prepared from 1.64 g (2.87 mmol) of its Z(OMe) derivative by TFA treatment, followed by $Et_3N$ 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% $Na_2CO_3$, 0.5 N HCl and $H_2O$, and dried. The solvent was removed *in vacuo* and the product was purified by silica gel column chromatography using MeOH-containing $CHCl_3$. Yield 1.41 g (67%), amorphous powder, decomposed at 91—94 °C, $Rf^5$ 0.48, $[\alpha]_D^{23}$ −119.9° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{36}H_{46}N_8O_9$: 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, $Rf^2$ 0.07, $[\alpha]_D^{20}$ −156.0° ($c$=0.5, $H_2O$). *Anal.* Calcd for $C_{21}H_{35}N_7O_5 \cdot AcOH \cdot 1/2H_2O$: 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 $Et_3N$, 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 $CHCl_3$ as an eluent. Yield 10.8 g (54%), amorphous material, $Rf^4$ 0.55, $[\alpha]_D^{28}$ −71.4° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{23}H_{26}N_2O_5$; C, 67.29; H, 6.40; N, 6.83. Found: C, 67.43; H, 6.53; N, 6.62.

**Z–Sar–Pro–Arg(NO$_2$)–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 $CHCl_3$, followed by washing with $H_2O$. The solvent was removed to give an oily material (3 g, $Rf_3$ 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 $Et_3N$. The mixture was combined with a solution of H–Arg(NO$_2$)–Pro–OBzl [prepared from 1.02 g (1.79 mmol) of its Z(OMe)-derivative by TFA treatment, followed by $Et_3N$ 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% $Na_2CO_3$, 0.5 N HCl and $H_2O$. After removal of the solvent, the residue was purified by silica gel column chromatography using MeOH-containing $CHCl_3$. Recrystallized from EtOH–ether. Yield 0.72 g (57%), mp 88—91 °C, $Rf^5$ 0.41, $[\alpha]_D^{20}$ −71.4° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{34}H_{44}N_8O_9 \cdot 1/2H_2O$: 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, $Rf^6$ 0.41, $[\alpha]_D^{24}$ −100.5° ($c$=0.5, $H_2O$). *Anal.* Calcd for $C_{19}H_{33}N_7O_5 \cdot 2HCl \cdot 2.5H_2O$: 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(NO$_2$)-Sar–OBzl** To a solution of Z(OMe)–Arg(NO$_2$)–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% $Na_2CO_3$, 5% citric acid and $H_2O$. The solvent was removed and the residue was purified by silica gel column chromatography using MeOH-containing $CHCl_3$ as an eluent. Yield 11.8 g (83%), oily material, $Rf^5$ 0.55, $[\alpha]_D^{22}$ −18.9° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{25}H_{32}N_6O_8$: C, 55.14; H, 5.92; N, 15.43. Found: C, 54.79; N, 5.79; N, 15.04.

**Z–Sar–Pro–Arg(NO$_2$)–Sar–OBzl** Isoamyl nitrite (0.8 ml, 5.95 mmol) was added to a solution of Z–Sar–Pro–NHNH$_2$ (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(NO$_2$)–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% $Na_2CO_3$, 0.5 N

HCl and $H_2O$. After removal of the solvent, the product was purified by silica gel column chromatography using MeOH-containing $CHCl_3$. Yield 1.17 g (37%), amorphous powder, $Rf^5$ 0.38, $[\alpha]_D^{21}$ −70.3° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{32}H_{42}N_8O_9$: 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 (×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, $Rf^6$ 0.65, $[\alpha]_D^{22}$ −42.6° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{17}H_{31}N_7O_5 \cdot AcOH \cdot 2H_2O$: 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($NO_2$)-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 $Et_3N$ (0.46 ml, 3.3 mmol) at −15 °C and the mixture was stirred for 15 min. After neutralization with $Et_3N$, the mixture was combined with a solution of H-Pro-Arg($NO_2$)-Pro-OBzl [prepared from its Boc-derivative (2 g, 3.3 mmol) by TFA treatment, followed by neutralization with $Et_3N$] 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% $Na_2CO_3$, 0.5 N HCl and $H_2O$. The solvent was removed and the residue was purified by silica gel column chromatography using MeOH-containing $CHCl_3$. Yield 1.65 g (70%), mp 115—120 °C, $Rf^7$ 0.55, $[\alpha]_D^{24}$ −69.0° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{34}H_{44}N_8O_9$: 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 (3×150 cm) in $H_2O$. Yield 122 mg (63%), amorphous powder, $Rf^2$ 0.22, $[\alpha]_D^{24}$ −124.3° ($c$=0.5, $H_2O$). *Anal.* Calcd for $C_{19}H_{35}O_5N_7 \cdot 2HCl \cdot H_2O$: C, 43.20; H, 7.08; N, 18.48. Found: C, 43.04; H, 7.11; N, 18.47.

**Z-D-Ala-Pro-Arg($NO_2$)-Val-OBzl** Z-D-Ala-OH (0.74 g, 3.3 mmol) and H-Pro-Arg($NO_2$)-Pro-OBzl [prepared from 2 g (3.3 mmol) of its Boc derivative[13]) 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 $CHCl_3$. Yield 1.25 g (53%), mp 95—100 °C, $Rf^3$ 0.88, $[\alpha]_D^{22}$ −36.0° ($c$=0.9, MeOH). *Anal.* Calcd for $C_{34}H_{46}N_8O_9$: 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 $H_2O$ as an eluent. Yield 119 mg (71%), hygroscopic powder, $Rf^2$ 0.48, $[\alpha]_D^{21}$ −82.7° ($c$=0.5, $H_2O$). *Anal.* Calcd for $C_{18}H_{31}N_7O_5 \cdot 3/2H_2O$: 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-$NH_2$ (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 $H_2O$. Yield 129 mg (41%), hygroscopic powder, $Rf^2$ 0.38, $[\alpha]_D^{27}$ −70.2° ($c$=1.0, $H_2O$). *Anal.* Calcd for $C_{19}H_{34}N_8O_4 \cdot 2HCl \cdot 1.5H_2O$: 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), $Et_3N$ (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 $Et_3N$ (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% $Na_2CO_3$, 10% citric acid and $H_2O$. The solvent was removed and the residue was recrystallized from EtOH. Yield 1.25 g (68%), mp 88—90 °C, $Rf^4$ 0.67. *Anal.* Calcd for $C_{20}H_{22}N_2O_5$: C, 64.85; H, 5.99; N, 7.56. Found: C, 64.80; H, 5.96; N, 7.71.

**Z-Gly-Sar-$NHNH_2$** Z-Gly-Sar-OBzl (1 g, 2.7 mmol) was converted to the hydrazide by treatment with $NH_2NH_2 \cdot H_2O$ (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, $Rf^4$ 0.24. *Anal.* Calcd for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.16; N, 19.04. Found: C, 52.88; H, 6.44; N, 19.36.

**Z-Gly-Sar-Arg($NO_2$)-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-$NHNH_2$ (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 $Et_3N$. The mixture was combined with a DMF (10 ml) solution of TFA·H-Arg($NO_2$)-Pro-OBzl [prepared from its Z(OMe) derivative (2.70 g, 4.8 mmol) by TFA treatment] and $Et_3N$ (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% $Na_2CO_3$, 5% citric acid and $H_2O$. The solvent was removed and the residue was purified by silica gel column chromatography using MeOH-containing $CHCl_3$ as an eluent. Yield 1.83 g (57%), mp 88—90 °C, $Rf^5$ 0.61, $[\alpha]_D^{22}$ −34.1° ($c$=1.0, DMF). *Anal.* Calcd for $C_{31}H_{40}N_8O_9$: 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 eq mol HCl-containing $H_2O$. Yield 191 mg (52%), hygroscopic powder, $Rf^2$ 0.14, $[\alpha]_D^{23}$ −26.9° ($c$=1.0, $H_2O$), 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($NO_2$)-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-$NHNH_2$ (0.70 g, 2.08 mmol) in DMF (7 ml) at −10 °C and the solution was stirred for 15 min. After neutralization with $Et_3N$, the solution was added to a solution of H-Arg($NO_2$)-Pro-OBzl [prepared from its Z(OMe) derivative (1.19 g, 2.29 mmol) by TFA treatment, followed by $Et_3N$ 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% $Na_2CO_3$ and $H_2O$. The AcOEt was removed and the residue was purified by silica gel column chromatography using MeOH-containing $CHCl_3$. Yield 0.63 g (45%), amorphous powder, $Rf^3$ 0.92, $[\alpha]_D^{26}$ −49.5° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{32}H_{27}N_7O_8$: 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 $CH_3CN/H_2O$ and converted to its hydrochloride by lyophilization from HCl-containing $H_2O$. Yield 213 mg (58%), hygroscopic powder, $Rf^2$ 0.49, $[\alpha]_D^{25}$ −28.8° ($c$=1.0, $H_2O$). *Anal.* Calcd for $C_{22}H_{33}N_7O_5 \cdot 2HCl \cdot 1.5H_2O$: 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-$NH_2$ (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, $Rf^2$ 0.38, $[\alpha]_D^{27}$ −10.8° ($c$=1.0, $H_2O$). *Anal.* Calcd for $C_{18}H_{32}N_8O_4 \cdot 2AcOH \cdot 2H_2O$: 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-$NH_2$ (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, $Rf^2$ 0.38, $[\alpha]_D^{27}$ +40.6° ($c$=1.0, $H_2O$), 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-$NH_2$ (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 $H_2O$. Yield 184 mg (64%), hygroscopic powder, $Rf^2$ 0.37, $[\alpha]_D^{27}$ −55.8° ($c$=1.0, $H_2O$). *Anal.* Calcd for $C_{18}H_{32}N_8O_4 \cdot 2HCl \cdot 5/3H_2O$: 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($NO_2$)-Pro-$NH_2$** Z-Pro-$NH_2$ (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($NO_2$)-OH (2 g, 6.3 mmol) and $Et_3N$ (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 $CHCl_3$ and purified by silica gel column chromatography using MeOH-containing $CHCl_3$. Yield 1.50 g (58%), mp 72—75 °C, $Rf^3$ 0.58, $[\alpha]_D^{28}$ $-25.4°$ ($c=1.0$, MeOH). *Anal.* Calcd for $C_{19}H_{27}N_7O_6$: C, 50.77; H, 6.05; N, 21.81. Found: C, 50.49; H, 6.25; N, 21.55.

**Z-Gly-Pro-Arg($NO_2$)-Pro-$NH_2$** A mixed anhydride was prepared from Z-Gly-Pro-OH (580 mg, 1.90 mmol), $Et_3N$ (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($NO_2$)-Pro-$NH_2$ [prepared from its Boc-derivative (650 mg, 1.58 mmol) by TFA treatment, followed by $Et_3N$ treatment] and the mixture was stirred overnight in a cold room. The solvent was removed and the product was extracted with $CHCl_3$, followed by washing with 5% $NaHCO_3$, 5% citric acid and $H_2O$. The $CHCl_3$ was removed and the residue was recrystallized from $CHCl_3$. Yield 650 mg (70%), mp 109—114 °C, $Rf^3$ 0.48, $[\alpha]_D^{26}$ $-71.9°$ ($c=1.0$, MeOH). *Anal.* Calcd for $C_{26}H_{37}N_9O_7\cdot 2H_2O$: 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($NO_2$)-Pro-$NH_2$ (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 $H_2O$. Yield 243 mg (57%), amorphous powder, $Rf^2$ 0.38, $[\alpha]_D^{26}$ $-107.2°$ ($c=1.0$, $H_2O$). *Anal.* Calcd for $C_{18}H_{31}N_9O_6\cdot HCl\cdot 1.5H_2O$: 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-$NH_2$ (XV)** Prepared by the solid-phase method. $N^{\varepsilon}$-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 $H_2O$. Yield 114 mg (46%), highly hygroscopic powder, $Rf^2$ 0.39, $[\alpha]_D^{25}$ $-110.9°$ ($c=1.0$, $H_2O$), 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-$NH_2$ (XVI)** Prepared by the solid-phase method. The final deprotection was done by TFAMSA 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 $H_2O$. Yield 126 mg (45%), hygroscopic powder, $Rf^2$ 0.36, $[\alpha]_D^{27}$ $-116.3°$ ($c=0.4$, $H_2O$), 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-$NH_2$ (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, $Rf^2$ 0.37, $[\alpha]_D^{27}$ $-50.8°$ ($c=1.0$, $H_2O$). *Anal.* Calcd for $C_{18}H_{32}N_8O_4\cdot 2AcOH\cdot H_2O$: 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 rescovery 86%).

**Z(OMe)-Arg($NO_2$)-[(Me)β-Ala]-OBzl** Prepared from Z(OMe)-Arg($NO_2$)-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 $Et_3N$ (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 $CHCl_3$. Yield 2.32 g (53%), amorphous powder, $Rf^5$ 0.61, $[\alpha]_D^{22}$ $-18.2°$ ($c=1.0$, MeOH). *Anal.* Calcd for $C_{26}H_{34}N_6O_8\cdot 1/2H_2O$: C, 55.00; H, 6.23; N, 14.81. Found: C, 55.02; H, 6.00; N, 14.86.

**Z-Gly-Pro-Arg($NO_2$)-[(Me)β-Ala]-OBzl** Prepared from Z-Gly-Pro-OH (980 mg, 3.23 mmol) and H-Arg($NO_2$)-[(Me)β-Ala]-OBzl [prepared from its Z(OMe) derivative (1.45 g, 3.23 mmol) by TFA treatment, followed by $Et_3N$ 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 $CHCl_3$. Yield 1.41 g (80%), mp 70—74 °C, $Rf^1$ 0.70, $[\alpha]_D^{18}$ $-68.4°$ ($c=1.0$, MeOH). *Anal.* Calcd for $C_{32}H_{42}N_8O_9$: 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, $Rf^2$ 0.36, $[\alpha]_D^{23}$ $-89.9°$ ($c=0.5$, $H_2O$), 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-$NH_2$ (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 $H_2O$. Yield 78 mg (27%), hygroscopic powder, $Rf^2$ 0.37, $[\alpha]_D^{27}$ $-111.4°$ ($c=1.0$, $H_2O$). *Anal.* Calcd for $C_{18}H_{32}N_8O_4\cdot 2HCl\cdot 1.5H_2O$: 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-$NH_2$ (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 $H_2O/CH_3CN$ as an eluent. The product was converted to its hydrochloride by lyophilization from HCl-containing $H_2O$. Yield 142 mg (47%), hygroscopic powder, $Rf^2$ 0.38, $[\alpha]_D^{25}$ $-41.2°$ ($c=1.0$, $H_2O$). *Anal.* Calcd for $C_{16}H_{30}N_8O_4\cdot 2HCl\cdot H_2O$: 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($C_2H_5$)$_2$** Isobutyl chloroformate (2.6 ml, 20 mmol) was added to a solution of Z-Pro-OH (5 g, 20 mmol) and $Et_3N$ (2.8 ml, 20 mmol) in DMF (30 ml) at $-10$ °C and the mixture was stirred for 15 min. $HN(C_2H_5)_2$ (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% $Na_2CO_3$, 5% citric acid and $H_2O$. 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, $Rf^3$ 0.58, $[\alpha]_D^{27}$ $-57.3°$ ($c=1.3$, MeOH). For elemental analysis, the product was purified by CM-cellulose column chromatography using 0.01—0.5% $NH_4OH$ as an eluent. *Anal.* Calcd for $C_9H_{18}N_2O\cdot 1/2H_2O$: C, 60.30; H, 10.68; N, 16.46. Found: C, 60.11; H, 10.93; N, 16.36.

**Z(OMe)-Arg($NO_2$)-Pro-N($C_2H_5$)$_2$** Prepared from Z(OMe)-Arg($NO_2$)-OH (2.82 g, 7.4 mmol) and H-Pro-N($C_2H_5$)$_2\cdot$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 $CHCl_3$ as an eluent. Yield 2.20 g (84%), amorphous powder, $Rf^3$ 0.73, $[\alpha]_D^{23}$ $-46.6°$ ($c=1.0$, MeOH). *Anal.* Calcd for $C_{24}H_{37}N_7O_5$: 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($NO_2$)-Pro-N($C_2H_5$)$_2$** Prepared from Boc-Gly-Pro-OH (510 mg, 1.87 mmol) and H-Arg($NO_2$)-Pro-N($C_2H_5$)$_2$ [prepared from its Z(OMe) derivative (1 g, 1.87 mmol) by TFA treatment, followed by $Et_3N$ 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% $Na_2CO_3$ and $H_2O$. 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, $Rf^2$ 0.77, $[\alpha]_D^{23}$ $-89.7°$ ($c=1.0$, MeOH). *Anal.* Calcd for $C_{27}H_{47}N_9O_8\cdot 1/2H_2O$: 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($C_2H_5$)$_2$ (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 $H_2O$. Yield 240 mg (54%), hygroscopic powder, $Rf^2$ 0.39, $[\alpha]_D^{27}$ $-119.3°$ ($c=1.0$,

$H_2O$). *Anal.* Calcd for $C_{22}H_{40}N_8O_4 \cdot 2HCl \cdot 2H_2O$: 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, $Rf^3$ 0.79, $[\alpha]_D^{26}$ −22.1° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{17}H_{22}N_2O_4$: C, 64.13; H, 6.97; N, 8.80. Found: C, 64.07; H, 7.02; N, 8.81.

**Boc–Arg($NO_2$)–Pro–Morpholine** DPPA (6.14 ml, 28 mmol) was added to a solution of Boc–Arg($NO_2$)–OH (7.33 g, 23 mmol), $Et_3N$ (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 $CHCl_3$, followed by washing with NaCl-saturated 5% $Na_2CO_3$, 5% citric acid and $H_2O$. The $CHCl_3$ was removed and the residue was purified by silica gel column chromatography using MeOH-containing $CHCl_3$ as an eluent. Yield 5.68 g (69%), mp 79—82 °C, $Rf^3$ 0.65, $[\alpha]_D^{26}$ −22.9° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{20}H_{35}N_7O_7$: 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($NO_2$)–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, $Rf^3$ 0.70, $[\alpha]_D^{26}$ −85.8° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{30}H_{43}N_9O_9 \cdot 1/2H_2O$: 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 $H_2O$. Yield 231 mg (70%), hygroscopic powder, $Rf^2$ 0.30, $[\alpha]_D^{26}$ −114.2° ($c$=1.0, $H_2O$). *Anal.* Calcd for $C_{22}H_{38}N_8O_4 \cdot 2HCl \cdot 4H_2O$: 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, $Rf^5$ 0.68, $[\alpha]_D^{23}$ −33.0° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{16}H_{28}N_2O_3$: 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 $CHCl_3$ as an eluent. Yield 1.30 g (47%), mp 91—95 °C, $Rf^5$ 0.69, $[\alpha]_D^{24}$ −22.4° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{33}H_{50}N_6O_6S$: 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 $CHCl_3$. Yield 990 mg (75%), mp 108—111 °C, $Rf^5$ 0.58, $[\alpha]_D^{24}$ −72.1° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{41}H_{59}N_8O_8S \cdot 1/2H_2O$: 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 $CH_3CN/H_2O$ as an eluent. The product was converted to its hydrochloride by lyophilization from HCl-containing $H_2O$. Yield 206 mg (49%), hygroscopic powder, $Rf^2$ 0.41, $[\alpha]_D^{27}$ −104.3° ($c$=1.0, $H_2O$). *Anal.* Calcd for $C_{24}H_{42}N_8O_4 \cdot 2HCl \cdot 1.5H_2O$: 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, $Rf^5$ 0.72, $[\alpha]_D^{24}$ −48.1° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{16}H_{28}N_2O_3$: 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 $CHCl_3$. Yield 1.88 g (67%), mp 115—118 °C, $Rf^5$ 0.51, $[\alpha]_D^{24}$ −30.8° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{31}H_{50}N_6O_6S$: 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, $Rf^5$ 0.66, $[\alpha]_D^{24}$ −76.0° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{41}H_{58}N_8O_8S$: 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 $CH_3CN/H_2O$ as an eluent. The product was converted to HCl salt by lyophilization from HCl-containing $H_2O$. Yield 237 mg (56%), hygroscopic powder, $Rf^2$ 0.38, $[\alpha]_D^{27}$ −105.2° ($c$=1.0, $H_2O$). *Anal.* Calcd for $C_{24}H_{42}N_8O_4 \cdot 2HCl \cdot 2H_2O$: 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%), $Rf^5$ 0.75, $[\alpha]_D^{24}$ −45.3° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{17}H_{30}N_2O_3$: 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, $Rf^5$ 0.78, $[\alpha]_D^{24}$ −30.6° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{32}H_{53}N_6O_6S$: 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 $CHCl_3$ as an eluent. Yield 1.1 g (82%), mp 118—121 °C, $Rf^5$ 0.50, $[\alpha]_D^{24}$ −71.1° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{42}H_{60}N_8O_8S \cdot H_2O$: 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 $CH_3CN/H_2O$. The product was converted to HCl salt by lyophilization from HCl-containing $H_2O$. Yield 290 mg (58%), hygroscopic powder, $Rf^2$ 0.40, $[\alpha]_D^{27}$ −117.3° ($c$=1.0, $H_2O$). *Anal.* Calcd for $C_{25}H_{44}N_8O_4 \cdot 2HCl \cdot 5/4H_2O$: 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, $Rf^5$ 0.83, $[\alpha]_D^{23}$ −50.1° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{18}H_{32}N_2O_3$: 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 $CHCl_3$ as an eluent. Yield 1.1 g (75%), mp 101—103 °C, $Rf^5$ 0.80, $[\alpha]_D^{23}$ −30.7° ($c$=1.0, MeOH). *Anal.* Calcd for $C_{33}H_{54}N_6O_6S$: 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, $Rf^3$ 0.67, $[\alpha]_D^{23}$ −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, $Rf^2$ 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, $Rf^5$ 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($NO_2$)-Pro-Aniline** DPPA (1.2 ml, 5.54 mmol) was added to a solution of Boc-Arg($NO_2$)-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, $Rf^5$ 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($NO_2$)-Pro-Aniline** Z-Gly-Pro-OH (370 mg, 1.22 mmol) and H-Arg($NO_2$)-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, $Rf^3$ 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($NO_2$)-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, $Rf^2$ 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, $Rf^3$ 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, $Rf^3$ 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, $Rf^3$ 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, $Rf^2$ 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, $Rf^5$ 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, $Rf^5$ 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-NHNH$_2$ (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, $Rf^2$ 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**

1) 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*-butyloxycarbonyl. 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.
2) A. P. Laudano and R. F. Doolittle, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 3085 (1978).
3) K. Kawasaki, T. Tsuji, K. Hirase, M. Miyano, Y. Imoto, and M. Iwamoto, *Chem. Pharm. Bull.*, **39**, 584 (1991).
4) W. König and R. Geiger, *Chem. Ber.*, **103**, 788, 2024 (1970).
5) M. Bodanszky, *Nature* (London), **175**, 685 (1955).
6) K. Hofmann and M. Bergmann, *J. Biol. Chem.*, **138**, 243 (1941).
7) H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. Chem. Soc., Chem. Commun.*, **1974**, 107.
8) H. Yajima, M. Takeyama, J. Hanai, and K. Mitani, *J. Chem. Soc., Chem. Commun.*, **1978**, 482.
9) H. Echner and W. Voelter, *Z. Naturforsch.*, **42b**, 1591 (1987).
10) B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).
11) J. R. Vaughan, Jr. and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).
12) T. Wieland and B. Heinke, *Ann. Chem.*, **615**, 184 (1958).
13) K. Kawasaki, T. Tsuji, K. Hirase, M. Miyano, S. Inouye, and M. Iwamoto, *Chem. Pharm. Bull.*, accepted.