Synthesis of Protected Peptide Fragments Related to Porcine Motilin[1)]

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Syntheses are described of three protected peptide fragments which correspond to positions 1—6, 7—11, and 12—22, respectively, of the entire amino acid sequence of porcine motilin, a gastric motoractivity-promoting polypeptide. Stable tosyl group was used for the protection of ε-amino group of lysine and guanidino group of arginine. These protected peptide fragments were mainly constructed in stepwise manner using benzyloxycarbonyl amino acid by mixed anhydride method in the presence of *N*-hydroxysuccinimide. For preparation of the C-terminal undecapeptide, fragment condensation method was employed using two protected tripeptide azides. The protected peptides and intermediates could be readily purified by simple procedure.

Brown *et al.*[2)] reported the determination of primary structure of porcine motilin, a gastric motoractivity-promoting polypeptide. Synthesis by Wünsch *et al.*[3)] of a norleucine analog based on that structure revealed an incorrect assingment of the amino acid at position 14, and the structure was revised at this position from Glu to Gln.[4)] Yajima *et al.*[5)] have synthesized the docosapeptide corresponding to the revised sequence of porcine motilin.

Limited availability of natural material of this hormone prompted us to prepare it in sufficient amount for further biological and immunological studies. The present paper describes the synthesis of three protected peptide fragments related to porcine motilin which may be employed for the construction of the entire molecule. These protected peptide fragments differ from those used by other investigators for synthesis, of motilin, which correspond to the sequences 1—6 7—11, and 12—22, respectively, as shown in Chart 1.

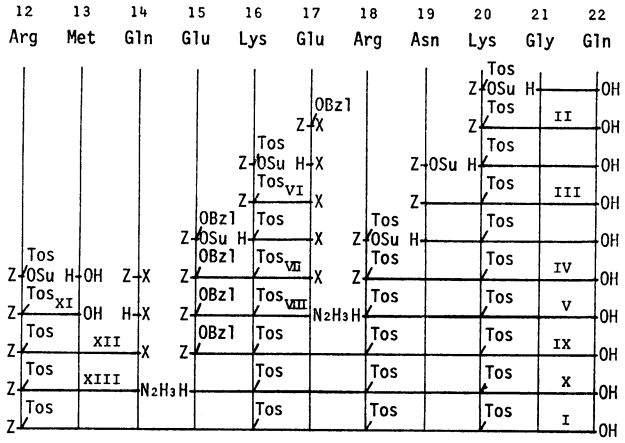
$$\overset{1}{\text{Phe}}\text{-}\overset{2}{\text{Val}}\text{-}\overset{3}{\text{Pro}}\text{-}\overset{4}{\text{Ile}}\text{-}\overset{5}{\text{Phe}}\text{-}\overset{6}{\text{Thr}}\text{-}\overset{7}{\text{Tyr}}\text{-}\overset{8}{\text{Gly}}\text{-}\overset{9}{\text{Glu}}\text{-}\overset{10}{\text{Leu}}\text{-}\overset{11}{\text{Gln}}\text{-}\overset{12}{\text{Arg}}\text{-}\overset{13}{\text{Met}}\text{-}\overset{14}{\text{Gln}}\text{-}\overset{15}{\text{Glu}}\text{-}$$
$$\overset{16}{\text{Lys}}\text{-}\overset{17}{\text{Glu}}\text{-}\overset{18}{\text{Arg}}\text{-}\overset{19}{\text{Asn}}\text{-}\overset{20}{\text{Lys}}\text{-}\overset{21}{\text{Gly}}\text{-}\overset{22}{\text{Gln}}$$

Chart 1. Primary structure of porcine motilin.

Stable tosyl group was selected for the protection of ε-amino group of lysine and guanidino group of arginine and Z-group was used for protection of α-amino group, since both of the protecting groups can be readily removed by sodium–liquid ammonia or anhydrous HF[6)] treatment at the final step in the synthesis of porcine motilin.

The route for the preparation of C-terminal protected undecapeptide (Fragment 1) (I) is shown in Scheme 1. Protected pentapeptide (IV) was prepared in stepwise manner using Z amino acids by mixed anhydride method in the presence of *N*-hydroxysuccinimide[7)] and then the chain was elongated by acylation with the azides of *N*-benzyloxycarbonyl tripeptides to give protected undecapeptide (I).

Coupling of Z–Lys(Tos)–OSu with triethylammonium salt of H–Gly–Gln–OH[8,9)] produced Z–Lys(Tos)–Gly–Gln–OH (II), which was partially deblocked by hydrogenolysis. Interaction of the resulting product with Z–Asn–OSu[10)] gave Z–Asn–Lys(Tos)–Gly–Gln–OH (III), which was hydrogenated. The partially deblocked tetrapeptide was coupled with Z–Arg(Tos)–OSu and the resulting protected pentapeptide

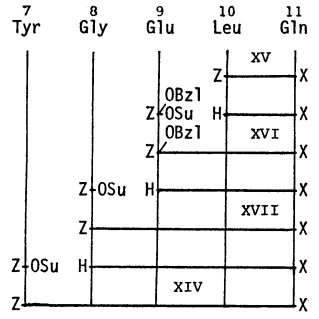


Scheme 1. Synthesis of fragment 1.
X=*t*-butoxycarbonyl hydrazide.

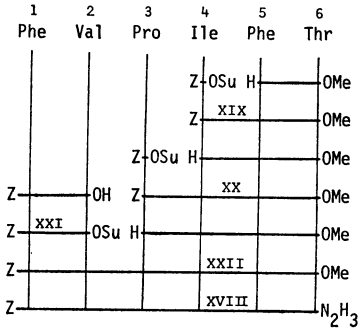
(IV) was subjected to hydrogenolysis to yield H–Arg(Tos)–Asn–Lys(Tos)–Gly–Gln–OH (V). Acylation of V with azide derived from Z–Glu(OBzl)–Lys(Tos)–Glu–NHNH–Boc (VII) gave Z–Glu(OBzl)–Lys(Tos)–Glu–Arg(Tos)–Asn–Lys(Tos)–Gly–Gln–OH (IX). The protected tripeptide hydrazide, VIII, was obtained by the stepwise chain elongation starting from H–Glu–NHNH–Boc derived from Z–Glu(OBzl)–NHNH–Boc.

Hydrogenolysis of IX yielded partially protected octapeptide, H–Glu–Lys(Tos)–Glu–Arg(Tos)–Asn–Lys(Tos)–Gly–Gln–OH (X). This was acylated with azide derived from Z–Arg(Tos)–Met–Gln–NHNH–Boc (XII) to give protected undecapeptide, Z–Arg(Tos)–Met–Gln–Glu–Lys(Tos)–Glu–Arg(Tos)–Asn–Lys(Tos)–Gly–Gln–OH (I). The product was purified by reprecipitation from MeOH with ethyl acetate. Preparation of the protected tripeptide hydrazide XII was accomplished by the interaction of H–Gln–NHNH–Boc[11)] with a mixed anhydride of Z–Arg(Tos)–Met–OH.[12)]

The protected pentapeptide hydrazide (Fragment 2) (XIV) was prepared by the route shown in Scheme 2. Z–Leu–Gln–NHNH–Boc (XV) was obtained by coupling of a mixed anhydride of Z–Leu–OH with H–Gln–NHNH–Boc. This compound was employed as intermediate for preparation of an LH–RH analog.[13)] Hydrogenation of XV gave H–Leu–Gln–NHNH–Boc, which was coupled with Z–Glu(OBzl)–OSu to give protected tripeptide hydrazide (XVI). Subsequent introduction of Gly and Tyr residues was also per-



Scheme 2. Synthesis of fragment 2.



Scheme 3. Synthesis of fragment 3.

formed by active ester procedure.[7] The final product was purified by reprecipitation from MeOH with ethyl acetate to afford the protected pentapeptide hydrazide, Z–Tyr–Gly–Glu–Leu–Gln–NHNH–Boc (XIV).

The route for the preparation of the protected hexapeptide hydrazide (Fragment 3) (XVIII) is shown in Scheme 3.

Z–Phe–Thr–OMe[14] was used as the starting material and hydrogenated to give H–Phe–Thr–OMe, which was coupled with Z–Ile–OSu.[15] The resulting protected tripeptide methyl ester (XIX) was hydrogenated and then the product was acylated with Z–Pro–OSu[7] to give Z–Pro–Ile–Phe–Thr–OMe (XX). Hydrogenation of XX and then acylation with Z–Phe–Val–OSu (XXI) gave Z–Phe–Val–Pro–Ile–Phe–Thr–OMe (XXII), which was converted to the corresponding hydrazide, Z–Phe–Val–Pro–Ile–Phe–Thr–$NHNH_2$ (Fragment 3), by the action of hydrazine.

High purity of the protected peptide fragments, 1, 2, and 3, prepared in this study was proved by elemental analysis, amino acid analysis of their acid hydrolysates and thin-layer chromatography in two solvent systems. The syntheses of these protected peptide fragments proceeded smoothly and homogeneous materials were obtained by simple purification steps. The protected peptide hydrazides, which were prepared mainly by active ester method *via* mixed anhydride or azide procedure, in this study may be suitable intermediates for construction of a large quantity of porcine motilin *via* fragment condensation.[16]

Experimental

The melting points were uncorrected. Optical rotations were measured on a JASCO DIP4 Automatic Polarimeter. Analytical samples were dried *in vacuo* over P_2O_5 at 60—70 °C for 20 h. Amino acid analysis was performed with a HITACHI Model KLA-3B amino acid analyzer. Acid hydrolysis of a sample for amino acid analysis was conducted with 6M HCl at 110 °C for 24 h in a sealed tube. Designations of solvent systems for TLC on silica gel G (Merck) are : R_f^{I} *n*-BuOH–AcOH–H_2O (4 : 1 : 5) upper layer; R_f^{II} *n*-BuOH–pyridine–AcOH–H_2O (30 : 20 : 6 : 24). All solvents were of reagent grade and were distilled before use. Evaporations were carried out *in vacuo* at 40—45 °C in rotary evaporators.

Z-Lys(Tos)-Gly-Gln-OH (II). Z–Glu–Gln–OH[9] (3.00 g) was hydrogenated over Pd in MeOH (80 ml) containing 10% AcOH (10 ml) in the usual manner. The catalyst was removed by filtration, the filtrate was evaporated and the residue was dried *in vacuo* over P_2O_5 to give H–Gly–Gln–OH[8,9] R_f^{I} 0.11. To a solution of Z–Lys(Tos)–OH (3.86 g) and *N*-methylmorpholine (0.91 ml) in THF (50 ml) cooled to −15 °C, isobutyl chloroformate (1.11 ml) was added and, after 1 min, an ice-cold solution of *N*-hydroxysuccinimide (1.12 g) in THF (5 ml) was added. The mixture was kept at 0 °C for 5 min and at 20 °C for additional 15 min. The resulting active ester was combined with an ice-cold solution of the above deblocked dipeptide and TEA (1.24 ml) in H_2O (50 ml). The mixture was kept at 20 °C for 20 h, and a bulk of THF was evaporated. The residual aqueous solution was adjusted to pH 8 with TEA and washed with ethyl acetate three times. The solution was acidified with 1 N citric acid and extracted with ethyl acetate. The extracts were dried over Na_2SO_4 and evaporated to give a solid, which was collected by filtration and dried. Reprecipitation from MeOH with ethyl acetate gave II; yield, 3.90 g (70.8%); mp 147—148 °C; $[\alpha]_D^{14}$ −9.6° (*c* 0.52 MeOH); R_f^{I} 0.64, R_f^{II} 0.72. Found: C, 54.75; H, 6.12; N, 11.09%. Calcd for $C_{28}H_{37}O_9N_5S$: C, 54.27; H, 6.02; N, 11.30%.

Z-Asn-Lys(Tos)-Gly-Gln-OH (III). Hydrogenation of II (1.50 g) was carried out over Pd in MeOH (50 ml) and H_2O (50 ml) in the usual manner. Isolation of the product was performed in the manner as described for the hydrogenation of Z-Gly-Gln-OH, R_f^{I} 0.33. The hydrogenated material was acylated with the *N*-hydroxysuccinimide ester of Z-Asn-OH (0.96 g) and the product was isolated in the same manner as described for preparation of II. Reprecipitation from MeOH with ethyl acetate gave III; yield, 1.28 g (72.3%); mp 148—150 °C; $[\alpha]_D^{18}$ −27.3° (*c* 0.55 MeOH); R_f^{I} 0.45, R_f^{II} 0.67. Found: C, 51.76; H, 5.89; N, 13.30%. Calcd for $C_{32}H_{47}O_{11}N_7S \cdot 1/2H_2O$: C, 51.74; H, 5.97; N, 13.19%.

Z-Arg(Tos)-Asn-Lys(Tos)-Gly-Gln-OH (IV). Compound III (1.18 g) was hydrogenated over Pd in MeOH (50 ml) containing 10% AcOH (10 ml) in the usual manner, R_f^{I} 0.18. The hydrogenated material was acylated with the *N*-hydroxysuccinimide ester of Z–Arg(Tos)–OH (1.11 g) and the product was isolated in the same manner as described for preparation of II. Reprecipitation from MeOH with ethyl acetate gave IV; yield, 1.53 g (91.6%); mp 125—129 °C; $[\alpha]_D^{18}$ −13.0° (*c* 0.54 DMF); R_f^{I} 0.49, R_f^{II} 0.69. Found: C, 51.17; H, 5.93; N, 14.68%. Calcd for $C_{45}H_{61}O_{14}N_{11}S_2 \cdot H_2O$: C, 50.89; H, 5.98 N, 14.51%.

Z-Lys(Tos)-Glu-NHNH-Boc (VI). Z–Glu(OBzl)–NHNH–Boc (2.60 g) was hydrogenated over Pd in MeOH (50 ml) containing 1 M HCl (5.35 ml) in the usual manner, R_f^{I} 0.50. The hydrogenated material was coupled with the *N*-hydroxysuccinimide ester of Z–Lys(Tos)–OH (2.55 g) in the same manner as described for preparation of II. The solvents were evaporated. The residue was extracted with ethyl acetate, which was washed with 1 N citric acid and

saturated NaCl and dried over Na_2SO_4. The solvent was evaporated and the residue was solidified by addition of ether. The product was reprecipitated from ethyl acetate with ether; yield, 3.30 g (91.0%); mp 79 °C (dec.); $[\alpha]_D^{25}$ −28.2° (*c* 0.48 MeOH); R_f^I 0.89, R_f^{II} 0.85. Found: C, 55.25; H, 6.77; N, 9.76%. Calcd for $C_{31}H_{43}O_{10}N_5S$: C, 54.93; H, 6.39; N, 10.33%.

Z-Glu(OBzl)-Lys(Tos)-Glu-NHNH-Boc (VII). Compound VI (1.09 g) was hydrogenated over Pd in MeOH (50 ml) in the usual manner, R_f^I 0.60. The hydrogenated material was acylated with the *N*-hydroxysuccinimide ester of Z-Glu(OBzl)-OH (0.71 g) and the product was isolated in the same manner as described for preparation of VI. Reprecipitation from MeOH with ethyl acetate gave VII; yield, 1.03 g (72.0%); mp 81 °C (dec.); $[\alpha]_D^{18}$ −26.8° (*c* 0.50 DMF); R_f^I 0.90, R_f^{II} 0.88. Found: C, 55.56; H, 6.15; N, 8.78%. Calcd for $C_{43}H_{56}O_{13}N_6S\cdot 2H_2O$: C, 55.36; H, 6.48; N, 9.01%.

Z-Arg(Tos)-Met-OH[12] *(XI).* A solution of Z-Arg(Tos)-OH (2.54 g) and *N*-methylmorpholine (0.56 ml) in THF (30 ml) was cooled to −15 °C and isobutyl chloroformate (0.66 ml) was added. After 30 s, *N*-hydroxysuccinimide (0.86 g) dissolved in THF (10 ml) was added. The mixture was combined with an ice-cold solution of H-Met-OH (0.75 g) and TEA (0.70 ml) in H_2O (20 ml). The mixture was kept at 20 °C for 20 h and THF was evaporated. Isolation of the product was carried out in the manner as described for the preparation of II. The residue which was solidified on addition of ether was collected, dried, and reprecipitated from ethyl acetate with ether to give product XI; yield, 2.02 g (66.7%); mp 101—105 °C; $[\alpha]_D^{18}$ −7.4° (*c* 1.01 MeOH); R_f^I 0.83, R_f^{II} 0.80. Found: C, 52.03; H, 5.98; N, 11.54%. Calcd for $C_{26}H_{35}O_7N_5S_2\cdot 1/2H_2O$: C, 51.81; H, 6.02; N, 11.62%.

Z-Arg(Tos)-Met-Gln-NHNH-Boc (XII). Z-Gln-NHNH-Boc[11] (1.00 g) was hydrogenated over Pd in MeOH (50 ml) in the usual manner, R_f^I 0.36. A solution of XI (1.66 g) and *N*-methylmorpholine (0.28 ml) in THF (30 ml) was cooled to −15 °C and isobutyl chloroformate (0.33 ml) was added. After 1 min, the resulting mixed anhydride was combined with an ice-cold solution of the above hydrogenated material in THF (10 ml). The mixture was kept at 0 °C for 5 min and at 20 °C for additional 15 min. The solution was evaporated. The residue was dissolved in ethyl acetate, and the solution was washed successively with 1 N citric acid, saturated NaCl, saturated Na_2HCO_3 and saturated NaCl. The organic layer was dried over Na_2SO_4 and evaporated. The residue which was solidified on addition of ether was collected, dried, and recrystallized from MeOH with ether to give XII; yield, 1.29 g (60.8%); mp 182—183 °C; $[\alpha]_D^{18}$ −16.6° (*c* 0.51 DMF); R_f^I 0.80, R_f^{II} 0.81. Found: C, 51.09; H, 6.38; N, 14.95%. Calcd for $C_{36}H_{53}O_{10}N_9S_2\cdot 1/2H_2O$: C, 51.17; H, 6.44; N, 14.92%.

Z-Glu(OBzl)-Lys(Tos)-Glu-Arg(Tos)-Asn-Lys(Tos)-Gly-Gln-OH (IX). Compound IV (1.00 g) was hydrogenated over Pd in MeOH (50 ml) containing 10% AcOH (10 ml) in the usual manner to give V, R_f^I 0.27. A solution of VII (1.64 g) in TFA (5 ml) was kept at 20 °C for 20 min and addition of ether gave precipitate, which was collected by filtration and dried over KOH. This material (VIII) (1.53 g) was dissolved in DMF (20 ml), and the solution cooled at −15 °C, and 6 M HCl in dioxane (0.96 ml) and isopentyl nitrite (0.28 ml) were added. The mixture was left at −10 °C for 5 min and neutralized with TEA (0.81 ml). To the solution was added an ice-cold solution of the above hydrogenated material V and TEA (0.13 ml) in DMF (30 ml). The mixture was stirred at 4 °C for 20 h and the solvent was evaporated. The residue was extracted with five portions of *n*-BuOH which were washed with six portions of 10% AcOH. Evaporation of *n*-BuOH layers gave a viscous oil, which was solidified from MeOH with ethyl acetate, collected and reprecipitated from MeOH with ethyl acetate to give IX; yield, 1.56 g (97.0%); mp 165—166 °C; $[\alpha]_D^{18}$ −13.4° (*c* 0.52 DMF); R_f^I 0.60, R_f^{II} 0.72. Found: C, 53.36; H, 6.00; N, 12.10%. Calcd for $C_{75}H_{99}O_{23}N_{15}S_3$: C, 53.78; H, 5.96; N, 12.54%.

Z-Arg(Tos)-Met-Gln-Glu-Lys(Tos)-Glu-Arg(Tos)-Asn-Lys(Tos)-Gly-Gln-OH (I) (Fragment 1). Compound IX (0.80 g) was hydrogenated over Pd in MeOH (50 ml) and 50% AcOH (50 ml) for 40 h in the usual manner to give X, R_f^I 0.38. A solution of XII (0.80 g) in TFA (3.5 ml) was kept at 20 °C for 30 min and addition of peroxide free ether gave precipitate, which was collected by filtration and dried over KOH. This material (XIII) was dissolved in DMF (20 ml) and the solution cooled at −15 °C and 6 M HCl in dioxane (0.48 ml) and isopentyl nitrite (0.14 ml) were added. The mixture was left at −10 °C for 5 min and neutralized with TEA (0.53 ml). To the solution was added an ice-cold solution of the above hydrogenated material X and TEA (0.06 ml) in DMF (15 ml). The mixture was stirred at 4 °C for 20 h and the solvent was evaporated. The residue was extracted with five portions of *n*-BuOH which were washed with six portions of 15% AcOH. Evaporation of the *n*-BuOH layers gave an oil, which was solidified from MeOH with ethyl acetate, collected and reprecipitated from MeOH with ethyl acetate to give I; yield, 0.61 g (59.3%); mp 164—165 °C; $[\alpha]_D^{18}$ −17.0° (*c* 0.53 DMF); R_f^I 0.38, R_f^{II} 0.61; amino acid ratios in acid hydrolysate: Met, 0.84; Glu, 4.15; Asp, 0.96; Gly, 0.90; Arg(Tos) and Lys(Tos) were not determined. Found: C, 50.12; H, 6.04; N, 13.66%. Calcd for $C_{91}H_{128}O_{29}N_{22}S_5\cdot 2H_2O$: C, 49.90; H, 6.07; N, 14.07%.

Z-Leu-Gln-NHNH-Boc (XV). A solution of Z-Leu-OH (3.82 g) and *N*-methylmorpholine (1.46 ml) in THF (50 ml) was cooled to −15 °C and isobutyl chloroformate (1.74 ml) was added. After 30 s, the resulting mixed anhydride was combined with an ice-cold solution of H-Gln-NHNH-Boc, derived from Z-Gln-NHNH-Boc[11] (4.37 g), and TEA (1.68 ml) in THF (40 ml). The mixture was kept at 4 °C for 5 min and at 20 °C for further 15 min. The solvent was evaporated. Isolation of the product XV was carried out in the manner as described for the preparation of II; yield, 4.02 g (66.0%); mp 139—140 °C; $[\alpha]_D^{20}$ −44.2° (*c* 0.50 MeOH); R_f^I 0.79, R_f^{II} 0.87. Found: C, 56.47; H, 7.43; N, 13.89%. Calcd for $C_{28}H_{37}O_7N_5$: C, 56.79; H, 7.35; N, 13.80%.

Z-Glu(OBzl)-Leu-Gln-NHNH-Boc (XVI). Compound XV (2.00 g) was hydrogenated over Pd in MeOH (50 ml) containing 1 M HCl (3.9 ml) in the usual manner, R_f^I 0.48. The hydrogenated material was coupled with the *N*-hydroxysuccinimide ester of Z-Glu(OBzl)-OH (1.74 g) in the same manner as described for preparation of II. The solvent was evaporated. The residue was solidified by addition of H_2O, which was collected by filtration and dried. Reprecipitation from MeOH with ethyl acetate gave XVI; yield, 2.30 g (81.1%); mp 175—180 °C; $[\alpha]_D^{18}$ −26.6° (*c* 0.53 DMF); R_f^I 0.89, R_f^{II} 0.92. Found: C, 59.73; H, 6.93; N, 11.44%. Calcd for $C_{36}H_{50}O_{10}N_6$: C, 59.49; H, 6.93; N, 11.56%.

Z-Gly-Glu-Leu-Gln-NHNH-Boc (XVII). Compound XVI (2.20 g) was hydrogenated over Pd in MeOH (80 ml) containing 10% AcOH (10 ml) in the usual manner, R_f^I 0.38. The hydrogenated material was coupled with the *N*-hydroxysuccinimide ester of Z-Gly-OH (0.76 g) in

the same manner as described for preparation of II. The solvents were evaporated. The residue was dissolved in ethyl acetate, and the solution was washed with 1 N citric acid and H_2O and dried over Na_2SO_4. The solvent was evaporated to give solid which was collected by filtration and dried. Reprecipitation from MeOH with ethyl acetate gave XVII; yield, 1.44 g (68.6%); mp 163—166 °C; $[\alpha]_D^{20}$ −22.9° (*c* 0.50 DMF); R_f^I 0.75, R_f^{II} 0.76. Found: C, 53.38; H, 6.93; N, 14.46%. Calcd for $C_{31}H_{47}O_{11}N_7$: C, 53.67; H, 6.83; H, 14.13%.

Z–Tyr–Gly–Glu–Leu–Gln–NHNH–Boc (XIV) (Fragment 2). Compound XVII (1.41 g) was hydrogenated over Pd in MeOH (50 ml) containing 10% AcOH (5 ml) in the usual manner, R_f^I 0.35. DCC (0.50 g) was added to a solution of Z–Tyr–OH (0.77 g) and *N*-hydroxysuccinimide (0.28 g) in THF (40 ml) at 4 °C. The mixture was stirred at 4 °C for 20 h. The dicyclohexylurea was removed by filtration, and the solvent was evaporated. The residue, which was solidified on addition of ether–petroleum ether (1 : 2), was collected and dried. The resulting active ester was added to an ice-cold solution of the above hydrogenated material and TEA (0.28 ml) in DMF (20 ml). The mixture was kept at 20 °C for 20 h and DMF was evaporated. The residue was dissolved in ethyl acetate, which was washed with 1 N citric acid and H_2O and dried. The solvent was evaporated and the residue was solidified by addition of MeOH–ethyl acetate, collected, and reprecipitated from MeOH with ethyl acetate to give XIV; yield, 1.46 g (84.0%); mp 164—165 °C; $[\alpha]_D^{16}$ −46.4° (*c* 0.54 DMF); R_f^I 0.79, R_f^{II} 0.77; amino acid ratios in acid hydrolysate: Tyr, 0.89; Gly, 0.99; Glu, 2.10; Leu, 1.03. Found: C, 54.87; H, 6.62; H, 12.86%. Calcd for $C_{40}H_{56}O_{13}N_8 \cdot H_2O$: C, 54.91; H, 6.68; N, 12.81%.

Z–Ile–Phe–Thr–OMe (XIX). *Z–Phe–Thr–OMe*[14] (2.90 g) was hydrogenated over Pd in MeOH (50 ml) containing 1 M HCl (7 ml) in the usual manner, R_f^I 0.55. Z–Ile–OSu[15] (3.80 g) was added to an ice-cold solution of the above hydrogenated material and TEA (0.98 ml) in DMF (50 ml). The mixture was kept at 20 °C for 40 h and the solvent was evaporated. To the residue was added 1 N citric acid. The precipitate thus formed was collected by filtration and then washed with hot MeOH to yield XIX; yield, 3.15 g (85.4%); mp 210—211 °C; $[\alpha]_D^{20}$ −13.1° (*c* 0.50 DMSO); R_f^I 0.87, R_f^{II} 0.85. Found: C, 63.66; H, 7.11; N, 8.19%. Calcd for $C_{28}H_{37}O_7N_3$: C, 63.74; H, 7.07; N, 7.96%.

Z–Pro–Ile–Phe–Thr–OMe (XX). Compound XIX (3.00 g) was hydrogenated over Pd in MeOH (80 ml) containing 10% AcOH (10 ml) in the usual manner, R_f^I 0.52. Z–Pro–OSu[7] (2.95 g) was added to an ice-cold solution of the above hydrogenated material and TEA (0.79 ml) in DMF (50 ml). The mixture was kept at 20 °C for 20 h and the solvent was evaporated. The residue was solidified by addition of 5% AcOH, which was collected by filtration, washed with H_2O and dried. The solid was then washed with hot MeOH to give XX; yield, 3.35 g (94.4%); mp 235—236 °C; $[\alpha]_D^{20}$ −38.2° (*c* 0.50 DMSO); R_f^I 0.86, R_f^{II} 0.85. Found: C, 63.00; H, 7.02; N, 9.27%. Calcd for $C_{33}H_{44}O_8N_4$: C, 63.44; H, 7.10; N, 8.97%.

Z–Phe–Val–OSu (XXI). To an ice-cold solution of Z–Phe–Val–OH[17] (3.05 g) and *N*-hydroxysuccinimide (0.88 g) in THF (40 ml) was added DCC (1.58 g), and the mixture was stirred at 4 °C for 18 h. The suspension was filtered, and the filtrate was evaporated. The solid residue, which was obtained on addition of ether, was collected, dried, and recrystallized from EtOH; 3.25 g (85.2%); mp 156—157 °C; $[\alpha]_D^{20}$ −11.6° (*c* 2.30 dioxane); Found: C, 63.23; H, 6.14; N, 8.46%. Calcd for $C_{26}H_{29}O_7N_3$: C, 63.02; H, 5.90; N, 8.48%.

Z–Phe–Val–Pro–Ile–Phe–Thr–OMe (XXII). Compound XX (1.73 g) was hydrogenated over Pd in a mixture of MeOH (80 ml), 10% AcOH (30 ml) and *n*-BuOH (20 ml) in the usual manner, R_f^I 0.51. Z–Phe–Val–OSu (1.64 g) was added to an ice-cold solution of the above hydrogenated material and TEA (0.38 ml) in DMF (20 ml). The mixture was kept at 20 °C for 20 h and the solvent was evaporated. Isolation of the product was carried out in the manner as described for the preparation of I; yield, 0.99 g (40.3%); mp 172—174 °C; $[\alpha]_D^{16}$ −58.5° (*c* 0.51 DMF); R_f^I 0.86, R_f^{II} 0.90. Found: C, 63.31; H, 7.25; N, 9.89%. Calcd for $C_{47}H_{62}O_9N_6 \cdot H_2O$: C, 63.50; H, 7.26; H, 9.45%.

Z–Phe–Val–Pro–Ile–Phe–Thr–NHNH$_2$ (XVIII) (Fragment 3). Hydrazine hydrate (90%) (0.92 ml) was added to an ice-cold solution of XXII (0.95 g) in DMF (3 ml) and the solution was kept at 20 °C for 20 h. The solvent was evaporated and the residue was extracted with five portions of *n*-BuOH which were washed with six portions of 10% AcOH. Evaporations of the *n*-BuOH layers gave an oil which was solidified by addition of ether. The solid was collected and dried; yield, 0.58 g (60.7%); mp 209—211 °C; $[\alpha]_D^{20}$ −45.9° (*c* 0.51 DMSO); R_f^I 0.75, R_f^{II} 0.80; amino acid ratios in acid hydrolysate: Phe, 2.04; Val, 1.12; Pro, 0.93; Ile, 1.00; Thr, 0.92. Found: C, 62.64; H, 7.06; N, 12.55%. Calcd for $C_{46}H_{62}O_9N_8 \cdot H_2O$: C, 62.14; H, 7.26; N, 12.60%.

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1) The amino acids except glycine are of the L-configuration. Abbreviations used are: Z, benzyloxycarbonyl; OBzl, benzyl ester; OSu, *N*-hydroxysuccinimide ester; Tos, *p*-toluenesulfonyl; Boc, *t*-butoxycarbonyl; *n*-BuOH, 1-butanol; TEA, triethylamine; DMF, *N,N*-dimethylformamide; TFA, trifluoroacetic acid; HCl, hydrochloric acid or hydrogen chloride; DCC, dicyclohexylcarbodiimide; DMSO, dimethyl sulfoxide.

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