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Synthesis of Porcine Motilin and Its D-Phe[1]-Analog by the Use of Methanesulfonic Acid[1)]

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Motilin and [D-Phe[1]]-motilin were synthesized *via* the corresponding sulfoxide (Met[13]). The protected Met(O)[13]-motilin and [D-Phe[1], Met(O)[13]]-motilin, in which Met residue was substituted by Met(O) residue, were constructed from three suitable subunits, respectively. The protected peptides were deblocked by methanesulfonic acid in the presence of anisole to obtain Met(O)[13]-motilin and its D-Phe[1]-derivative, and the resulting Met(O)-peptides were finally reduced by thiol to give the desired docosapeptides, motilin and D-Phe[1]-motilin.

Keywords——motilin; D-Phe[1]-motilin; gastric motor activity stimulating polypeptide; methanesulfonic acid; HONB–DCC method; docosapeptide

Since the complete amino acid sequence of porcine motilin was defined to be the docosapeptide, H-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-OH,[3)] this gastric motor activity stimulating polypeptide has been synthesized using hydrogen fluoride[4,5)] or sodium in liquid ammonia[6)] as a deprotecting tool at the final step of the synthesis, respectively. Besides, TFA was used for the synthesis of two analogs, [Leu[13], Glu[14]]- and [norleucine[13], Glu[14]]-motilin, by Wünsch, *et al.*[7)]

We wish to report an alternate synthesis of motilin, in which a newly introduced deprotecting reagent, methanesulfonic acid (MSA),[8)] was employed. With the advent of this strategy, a hitherto unknown analog, [D-Phe[1]]-motilin, was also prepared. The reason we selected these Met-containing peptides as a synthetic target is to see whether the characteristic methyl-transfer reaction[9)] from anisole in the MSA procedure can be prevented by the use of methionine-sulfoxide (Met(O)).

Our synthetic scheme for motilin is outlined in Fig. 1. The α-amino group of the intermediates was protected by the BOC-group, while amino acid derivatives bearing protecting groups removable by MSA were Lys(Z), Glu(OBzl) and Arg(MBS). Among them, Arg(MBS)

1) Amino acids, peptides and their derivatives in this communication are of L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, BOC=*tert*-butoxycarbonyl, MBS=*p*-methoxybenzenesulfonyl, OBu^t=*tert*-butyl ester, OBzl=benzyl ester, HONB=N-hydroxy-5-norbornene-2,3-dicarboximide, DCC=N,N′-dicyclohexylcarbodiimide, DCU=N,N′-dicyclohexylurea, TFA=trifluoroacetic acid, TEA=triethylamine, DMF=N,N -dimethylformamide, THF=tetrahydrofuran, DCHA=dicyclohexylamine.

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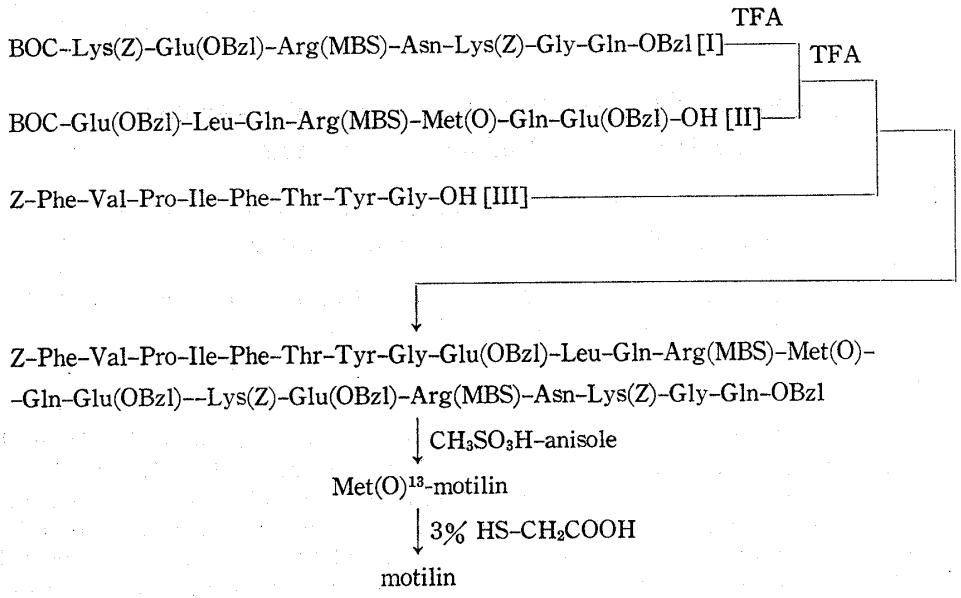

Fig. 1. Synthetic Route to Porcine Motilin

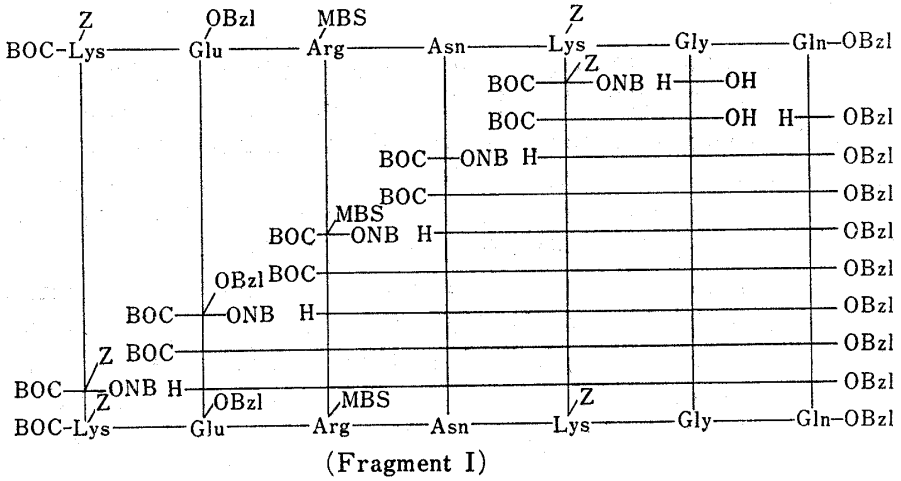

Fig. 2. Preparation of Protected Heptapeptide (**16**—**22**)

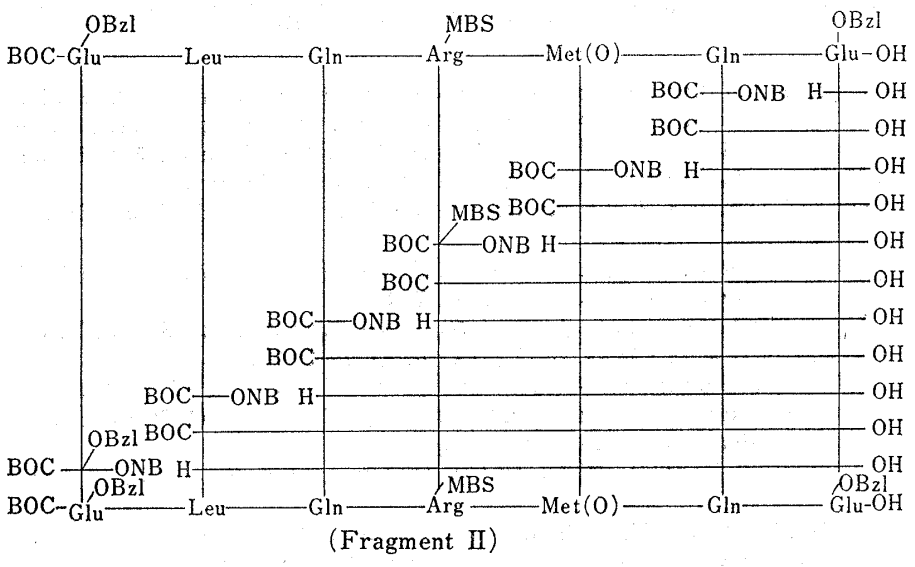

Fig. 3. Preparation of Protected Heptapeptide (**9**—**15**)

is a derivative recently found in our laboratory.[10]

As shown in Fig. 1, three peptide fragments, BOC-Lys(Z)-Glu(OBzl)-Arg(MBS)-Asn-Lys-(Z)-Gly-Gln-OBzl (I), BOC-Glu(OBzl)-Leu-Gln-Arg(MBS)-Met(O)-Gln-Glu(OBzl)-OH (II) and Z-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-OH (III), served as the building blocks for the construction of the protected docosapeptide. The three fragments were prepared mainly in the stepwise chain elongation manner using HONB activated esters of acylamino acids.[11] The synthetic routes to these fragments were shown in Fig. 2, 3 and 4, respectively.

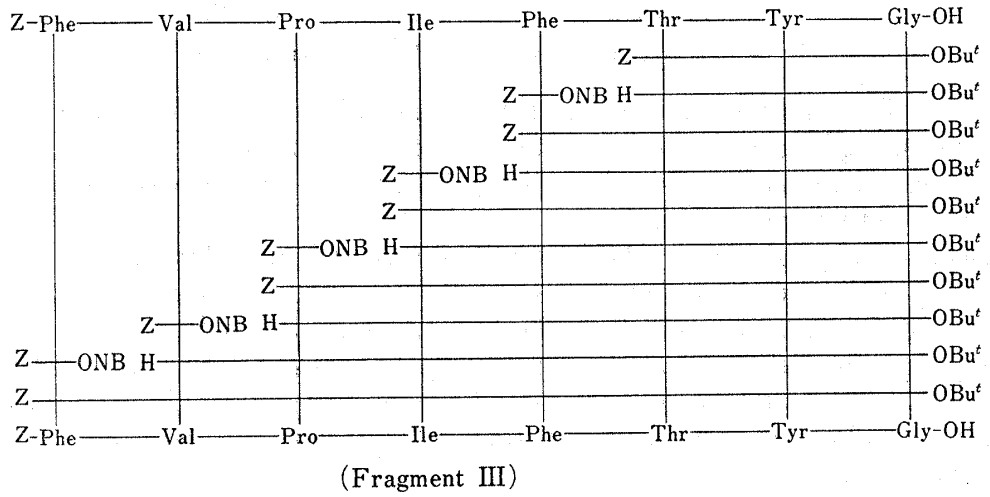

Fig. 4. Preparation of Protected Octapeptide (**1**—**8**)

For construction of the entire amino acid sequence of the docosapeptide, the fragment I was treated with TFA to remove N^{α}-BOC group and the resulting free base of I was condensed with the fragment II by the HONB–DCC method,[11] minimizing racemization during the coupling reaction,[12] to afford BOC-Glu(OBzl)-Leu-Gln-Arg(MBS)-Met(O)-Gln-Glu(OBzl)-Lys(Z)-Glu(OBzl)-Arg(MBS)-Asn-Lys(Z)-Gly-Gln-OBzl (IV). The BOC-group of the tetradecapeptide IV was removed by the treatment with TFA, and the resulting free base was coupled with the fragment III by the HONB–DCC method. The crude protected docosapeptide thus obtained was exposed to MSA in the presence of anisole as a scavenger at room temperature for 60 min to remove all the protecting groups. The resulting deblocked peptide was immediately converted to the corresponding acetate with Amberlite IRA-410 (acetate form) and purified by column chromatography on Biogel P-2 and carboxymethylcellulose (ammonium acetate buffer, pH 6.8) to give chromatographically pure [Met(O)[13]]-motilin. Motilin-sulfoxide thus obtained was dissolved in 3% aqueous thioglycolic acid and the solution was kept to stand at 50° for 20 hr to reduce the sulfoxide. The reduced peptide was purified by column chromatography on carboxymethylcellulose to remove the thioglycolic acid. The final product was homogeneous judged by thin-layer chromatography and paper electrophoresis (checked by ninhydrin, Sakaguchi and Pauly reagents). The amino acid analyses of 6 N HCl hydrolysates and of aminopeptidase M hydrolysates were in good accord with the theoretical values.

For synthesis of [D-Phe[1]]-motilin, Z-D-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-OH (V) was used as a subunit instead of the fragment III. The synthesis of the fragment V was outlined in Fig. 5.

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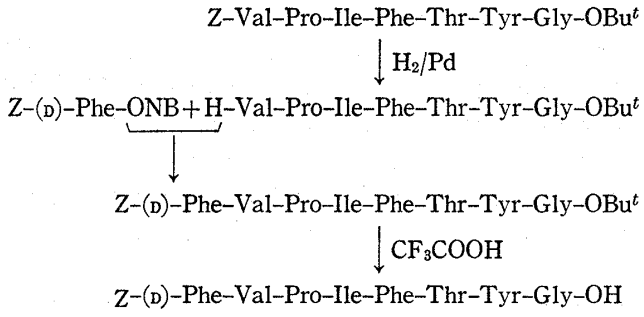

Fig. 5. Preparation of Protected D-Phe-octapeptide (**1**—**8**)

[D-Phe^1]-motilin was obtained in the same manner as described for the synthesis of motilin. The analog thus obtained was found to be homogeneous by thin layer chromatography and paper electrophoresis, and the amino acid analysis of the acid hydrolysate was also in accord with the theoretical values.

Biological activity of the synthetic peptides was examined by the duodenal muscle contraction (rabbit)[13] and it was found that the activity of the synthetic motilin was identical with that of an authentic sample.[4] The activities of [Met(O)^{13}]-motilin and [D-Phe^1]-motilin were approximately 4/5 and 1/5, respectively, of that of the synthetic motilin. These results may indicate that the N-terminal part of the molecule of motilin plays an important role in the biological action and that the methionine residue in this molecule is not essential for the biological activity.

Experimental

All melting points were taken in open capillaries and are uncorrected. Rotations were determined with a Perkin-Elmer Model 141 polarimeter. Amino acid analyses were performed on a Hitachi KLA-3B amino acid analyzer. Acid hydrolyses were carried out according to the method of Matsubara and Sasaki.[14] Evaporations were carried out in a rotary evaporator under reduced pressure at a temperature of 40—45°. Catalytic hydrogenations were performed at room temperature with palladium black as catalyst. The purity of the products was tested by thin-layer chromatography (TLC) using Merck precoated silica gel plate $60F_{254}$. Solvent systems used were: $CHCl_3$–MeOH–AcOH (9:1:0.5, Rf^1), AcOEt–pyridine–AcOH–H_2O (30:10:3:5, Rf^2), *n*-BuOH–pyridine–AcOH–H_2O (30:20:6:24, Rf^3), *n*-BuOH–AcOEt–AcOH–H_2O (1:1:1:1, (Rf^4).

BOC-Lys(Z)-Gly-OH·DCHA (1)——To a solution of glycine (2.7 g, 36 mmol) and TEA (4.6 ml) in a mixture of DMF (100 ml) and H_2O (10 ml) was added a solution of BOC–Lys(Z)–ONB (19.4 g, 36 mmol) in THF (50 ml). The solution was stirred for 12 hr at 20° and then evaporated. The residue was dissolved in AcOEt (200 ml) and the solution was washed with 0.1 N HCl (300 ml) and H_2O, and dried over anhydr. Na_2SO_4. The AcOEt solution was evaporated to a small volume, and to this was added DCHA (4.5 g). After removal of the solvent the residue was crystallized from AcOEt–pet. ether: 12.5 g (67.5%), mp 140—141°, $[\alpha]_D^{27}$ −7.4° (c=0.5 in DMF), Rf^1 0.48. *Anal.* Calcd. for $C_{33}H_{54}N_4O_7$: C, 64.05; H, 8.80; N, 9.06. Found: C, 63.70; H, 9.19; N, 8.94.

BOC-Lys(Z)-Gly-Gln-OBzl (2)——BOC–Gln–OBzl (3.7 g, 11 mmol) was treated with TFA (50 ml) containing 6 N HCl (2 ml) for 20 min at 20°. After evaporation the residue was triturated with dry ether. The precipitate was collected and dried over NaOH pellets *in vacuo*. The powder was dissolved in THF (50 ml) and to this was added TEA (1.54 ml).

Compound **1** (6.0 g, 10 mmol) was suspended in AcOEt (100 ml), and the suspension was washed with 0.1 N H_2SO_4 (100 ml) and dried over anhydr. Na_2SO_4. The AcOEt layer was evaporated and the residue was dissolved in THF (50 ml). To this were added HONB (1.97 g) and DCC (2.26 g) at 0°, and the mixture was stirred for 3 hr. The mixture was filtered to remove the formed DCU and to the filtrate was added the above mentioned THF solution containing the amine component. After 12 hr at room temperature, the solvent was removed by evaporation. The residue was dissolved in AcOEt (100 ml) and then washed

13) T. Segawa, M. Nakano, Y. Kai, H. Kawatani, and H. Yajima, *J. Pharm. Pharmac.*, **28**, 650 (1976); We wish to express our deep appreciation to Prof. Tomio Segawa, Hiroshima University School of Medicine, for the assay.

14) H. Matsubara and K. Sasaki, *Biochem. Biophys. Res. Commun.*, **35**, 176 (1969).

with 0.1 N HCl and 5% aqueous $NaHCO_3$, and evaporated. The residue was crystallized from AcOEt: 5.2 g (79.5%), mp 148—149°, $[\alpha]_D^{27}$ −12.2° (c=0.5 in DMF), Rf^1 0.40. *Anal.* Calcd. for $C_{33}H_{45}N_5O_9$: C, 60.44; H, 6.92; N, 10.68. Found: C, 60.45; H, 6.86; N, 10.84.

BOC-Asn-Lys(Z)-Gly-Gln-OBzl (3)——Compound **2** (7.1 g, 10 mmol) was treated with TFA (80 ml) containing 6 N HCl (1.9 ml) for 20 min at 20°. The usual work-up provided a precipitate which was dissolved in DMF (100 ml) and to this was added TEA (1.55 ml) at 0°.

BOC–Asn–OH (2.32 g, 10 mmol) was coupled with HONB (1.97 g) in the presence of DCC (2.26 g), and the resulting ester was combined with the above mentioned amine component. After 12 hr at room temperature, the solvent was removed and the residue was purified by reprecipitation from MeOH–ether: 6.1 g (79.2%), mp 181—183°, $[\alpha]_D^{27}$ −13.4° (c=0.5 in DMF), Rf^1 0.25. *Anal.* Calcd. for $C_{37}H_{51}N_7O_{11}$: C, 57.73; H, 6.68; N, 12.74. Found: C, 57.30; H, 6.61; N, 12.74.

BOC-Arg(MBS)-Asn-Lys(Z)-Gly-Gln-OBzl (4)——Compound **3** (6.0 g, 7.8 mmol) was treated with TFA (70 ml) for 20 min at 20°. The usual work-up provided a precipitate which was dried over NaOH pellets *in vacuo*, then dissolved in DMF (60 ml) together with TEA (1.1 ml).

BOC–Arg(MBS)–OH (3.5 g, 7.8 mmol) was coupled with HONB (1.58 g) by the DCC method in THF (50 ml). After filtration the filtrate was added to the above mentioned amine component. The material isolated by the usual manner was further purified by reprecipitation from EtOH–AcOEt: 5.5 g (62%), mp 141—144°, $[\alpha]_D^{27}$ −5.8° (c=0.5 in DMF), Rf^2 0.68. *Anal.* Calcd. for $C_{50}H_{69}N_{11}O_{15}S\cdot H_2O$: C, 53.89; H, 6.42; N, 13.82; S, 2.88. Found: C, 53.87; H, 6.27; N, 14.22; S, 3.00.

BOC-Glu(OBzl)-Arg(MBS)-Asn-Lys(Z)-Gly-Gln-OBzl (5)——Compound **4** (4.93 g, 4.5 mmol) was treated with TFA (50 ml) for 20 min at room temperature. The usual work-up provided a powder which was used for the coupling with BOC–Glu(OBzl)–OH (1.51 g, 4.5 mmol) *via* the corresponding HONB-ester in a similar manner described above. After 12 hr, the solution was evaporated and the residue was washed with H_2O and then triturated with ether. The resulting powder was purified by reprecipitation from MeOH–ether: 5.7 g (95.0%), mp 169—170°, $[\alpha]_D^{27}$ −7.0° (c=0.5 in DMF), Rf^2 0.80. *Anal.* Calcd. for $C_{62}H_{82}N_{12}O_{18}S\cdot 0.5H_2O$: C, 56.22; H, 6.31; N, 12.69; S, 2.42. Found: C, 56.27; H, 6.40; N, 12.66; S, 2.46.

BOC-Lys(Z)-Glu(OBzl)-Arg(MBS)-Asn-Lys(Z)-Gly-Gln-OBzl (I)——Compound **5** (5.70 g, 4.2 mmol) was treated with TFA and the TFA salt isolated as a powder was dissolved in DMF (30 ml) containing TEA (0.63 ml). To this was added BOC–Lys(Z)–ONB (2.32 g, 4.3 mmol), and the solution was stirred for 12 hr at room temperature. After evaporation, the residue was triturated with H_2O and AcOEt and then purified by reprecipitation from MeOH–acetonitrile: 5.7 g (86.5%), mp 172—173°, $[\alpha]_D^{27}$ −8.6° (c=0.5 in DMF), Rf^2 0.88. *Anal.* Calcd. for $C_{76}H_{100}N_{14}O_{21}S\cdot H_2O$: C, 57.20; H, 6.44; N, 12.29; S, 2.01. Found: C, 57.15; H, 6.40; N, 12.54; S, 2.05. Amino acid ratios in acid hydrolysate: Lys 2.04(2), Arg 1.00(1), Asp 0.96(1), Glu 1.96(2), Gly 1.18(1).

BOC-Gln-Glu(OBzl)-OH (6)——To a solution of BOC–Gln–OH (11.1 g, 45 mmol) and HONB (9.66 g) in THF (100 ml) was added DCC (10.2 g) at 0° and the mixture was stirred for 3 hr at 0°. After filtration and evaporation, the residue was dissolved in DMF (100 ml). To this was added a suspension of H-Glu(OBzl)–OH in H_2O (20 ml) together with TEA (6.3 ml). The mixture was stirred vigorously at room temperature for 12 hr and then evaporated. The usual work-up gave a precipitate which was crystallized from AcOEt: 13.5 g (64.5%), mp 151°, $[\alpha]_D^{24}$ −13.8° (c=0.5 in MeOH), Rf^1 0.19. *Anal.* Calcd. for $C_{22}H_{31}N_3O_8$: C, 56.76; H, 6.71; N, 9.03. Found: C, 56.78; H, 6.79; N, 9.02.

BOC-Met(O)-Gln-Glu(OBzl)-OH (7)——Compound **6** (11.2 g, 24 mmol) was treated with TFA and the TFA salt isolated was dissolved in DMF (100 ml) together with TEA (6.4 ml), and to this was added BOC–Met(O)–ONB, prepared from 6.10 g (23 mmol) of BOC–Met(O)–OH by the usual manner. After 13 hr, the solvent was evaporated and the residue was dissolved in *n*-BuOH (150 ml). The *n*-BuOH solution was washed with 0.1 N HCl and H_2O, and evaporated. The residue was treated with ether to give a powder, which was further purified by reprecipitation from MeOH–ether: 10.5 g (74.5%), mp 152—155°, $[\alpha]_D^{27}$ −4.2° (c=1.0 in DMF), Rf^2 0.23. *Anal.* Calcd. for $C_{27}H_{40}N_4O_{10}S\cdot H_2O$: C, 51.43; H, 6.71; N, 8.89; S, 5.04. Found: C, 51.40; H, 6.20; N, 9.08; S, 5.09.

BOC-Arg(MBS)-Met(O)-Gln-Glu(OBzl)-OH (8)——Compound **7** (9.17 g, 15 mmol) was treated with TFA, and the TFA salt isolated was coupled with BOC–Arg(MBS)–OH (6.66 g, 15 mmol) *via* the corresponding HONB ester in DMF (100 ml). After the usual work-up and extraction with *n*-BuOH (150 ml), the solvent was removed by evaporation and the residue was chromatographed on silica gel (150 g) and eluted with the solvent Rf^2. The fractions (170—350 ml) containing the desired product were combined and evaporated. The residue was treated with ether to give a fine powder: 7.0 g (50.0%), mp 115—119° (dec.), $[\alpha]_D^{27}$ −12.2° (c=1.0 in MeOH), Rf^2 0.22. *Anal.* Calcd. for $C_{40}H_{58}N_8O_{14}S_2\cdot H_2O$: C, 50.20; H, 6.32; N, 11.71; S, 6.70. Found: C, 50.39; H, 6.18; N, 11.59; S, 6.26.

BOC-Gln-Arg(MBS)-Met(O)-Gln-Glu(OBzl)-OH (9)——Compound **8** (6.5 g, 6.9 mmol) was treated with TFA and the resulting TFA salt was acylated with BOC–Gln–OH (1.57 g, 6.9 mmol) *via* the corresponding HONB ester in DMF (50 ml) for 12 hr. After addition of AcOH (1 ml) to the reaction mixture, the solvent was removed and the residue was triturated with ether and then purified by reprecipitation from MeOH–ether: 7.0 g (95.2%), mp 165—169°, $[\alpha]_D^{27}$ −10.8° (c=0.5 in DMF), Rf^2 0.13. *Anal.* Calcd. for $C_{45}H_{66}N_{10}O_{16}S_2\cdot H_2O$: C, 49.81; H, 6.32; N, 12.91; S, 5.91. Found: C, 49.69; H, 6.15; N, 12.59; S, 5.62.

BOC-Leu-Gln-Arg(MBS)-Met(O)-Gln-Glu(OBzl)-OH (10)——Compound **9** (7.0 g, 6.5 mmol) was treated with TFA, and the TFA salt isolated was coupled with BOC–Leu–ONB (2.7 g, 6.5 mmol) in DMF (50 ml). The reaction mixture was evaporated and the residue was chromatographed on silica gel (100 g) with the Rf^2 solvent. The desired fractions (110—290 ml) were collected and evaporated. The residue was then triturated with ether to give a fine powder: 5.1 g (66.5%), mp 185—189°, $[\alpha]_D^{27}$ −15.0° (c=0.5 in DMF), Rf^2 0.18. *Anal.* Calcd. for $C_{51}H_{77}N_{11}O_{17}S_2 \cdot H_2O$: C, 50.35; H, 6.71; N, 12.66; S, 5.27. Found: C, 50.17; H, 6.24; N, 12.49; S, 4.97.

BOC-Glu(OBzl)-Leu-Gln-Arg(MBS)-Met(O)-Gln-Glu(OBzl)-OH (II)——Compound **10** (5.0 g, 4.24 mmol) was treated with TFA, and the product was coupled with BOC–Glu(OBzl)–ONB (2.2 g, 4.2 mmol) in DMF (40 ml). After the usual work-up the substance was purified by reprecipitation from DMF–AcOEt: 5.1 g (86.8%), mp 175—177°, $[\alpha]_D^{27}$ −10.2° (c=0.5 in DMF), Rf^2 0.37. *Anal.* Calcd. for $C_{63}H_{90}N_{12}O_{20}S_2 \cdot H_2O$: C, 53.37; H, 6.54; N, 11.85; S, 4.52. Found: C, 53.49; H, 6.49; N, 11.81; S, 4.52. Amino acid ratios in acid hydrolysate: Arg 1.02(1), Glu 4.39(4), Met 0.98(1), Leu 1.00(1).

Z-Phe-Thr-Tyr-Gly-OBu^t (11)——The Z-group of Z–Thr–Tyr–Gly–OBu^t[15] (9.7 g, 18 mmol) was quantitatively removed by hydrogenolysis and the free base was coupled with Z–Phe–ONB (8.3 g, 18 mmol). After the usual work-up, the substance was crystallized from AcOEt–pet. ether: 12.0 g (86.5%), mp 161—162°, $[\alpha]_D^{24}$ −5.3° (c=1.0 in DMF), Rf^1 0.42. *Anal.* Calcd. for $C_{36}H_{44}N_4O_9$: C, 63.89; H, 6.55; N, 8.28. Found: C, 63.68; H, 6.72; N, 8.32.

Z-Ile-Phe-Thr-Tyr-Gly-OBu^t (12)——Compound **11** (9.47 g, 14 mmol) was hydrogenated and the free base was coupled with Z–Ile–ONB (5.95 g, 14 mmol) in THF (150 ml) for 12 hr. After evaporation, the residue was triturated with AcOEt and then purified by reprecipitation from EtOH–ether to give a fine powder: 6.7 g (61.0%), mp 195—197°, $[\alpha]_D^{24}$ −15.0° (c=0.5 in DMF), Rf^1 0.53. *Anal.* Calcd. for $C_{42}H_{55}N_5O_{10} \cdot 0.5H_2O$: C, 63.14; H, 7.07; N, 8.77. Found: C, 62.93; H, 7.05; N, 8.91.

Z-Pro-Ile-Phe-Thr-Tyr-Gly-OBu^t (13)——Compound **12** (6.6 g, 8.3 mmol) was hydrogenated and the free base was coupled with Z–Pro–ONB (3.4 g, 8.3 mmol) in DMF (50 ml) for 12 hr. After the usual work-up, the substance was purified by reprecipitation from MeOH–ether: 6.9 g (94.0%), mp 204—206°, $[\alpha]_D^{24}$ −28.8° (c=0.5 in DMF), Rf^1 0.48. *Anal.* Calcd. for $C_{47}H_{62}N_6O_{11} \cdot H_2O$: C, 62.38; H, 7.13; N, 9.29. Found: C, 62.56; H, 6.93; N, 9.53.

Z-Val-Pro-Ile-Phe-Thr-Tyr-Gly-OBu^t (14)——Compound **13** (5.9 g, 6.6 mmol) was hydrogenated and the free base was coupled with Z–Val–ONB (2.7 g, 6.6 mmol) for 12 hr. After the usual work-up, the residue was triturated with ether and then reprecipitated from MeOH–ether: 4.7 g (71.8%), mp 144—146°, $[\alpha]_D^{24}$ −69.1° (c=1.0 in MeOH), Rf^1 0.71. *Anal.* Calcd. for $C_{52}H_{71}N_7O_{12} \cdot H_2O$: C, 62.20; H, 7.33; N, 9.77. Found: C, 62.17; H, 7.05; N, 9.85.

Z-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-OBu^t (15)——Compound **14** (3.6 g, 3.6 mmol) was hydrogenated and the free base was coupled with Z–Phe–ONB (1.68 g, 3.6 mmol) in DMF (50 ml) for 13 hr. The reaction mixture was evaporated and the residue was triturated with AcOEt to give a powder which was purified by reprecipitation from DMF–AcOEt: 3.2 g (79.1%), mp 196—198°, $[\alpha]_D^{27}$ −38.4° (c=0.5 in DMF), Rf^1 0.62. *Anal.* Calcd. for $C_{61}H_{80}N_8O_{13} \cdot H_2O$: C, 63.64; H, 7.18; N, 9.74. Found: C, 63.41; H, 7.18; N, 9.64. Amino acid ratios in acid hydrolysate: Thr 0.96(1), Pro 1.06(1), Gly 1.02(1), Val 1.00(1), Ile 1.00(1), Tyr 0.56(1), Phe 1.96(2).

Z-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-OH (III)——Compound **15** (1.13 g, 1 mmol) was dissolved in TFA (10 ml) and allowed to stand at room temperature for 25 min. The TFA was removed by evaporation and the residue was triturated under ether to give a powder, which was collected by filtration and purified by reprecipitation from n-BuOH: 910 mg (83.5%), mp 184—186°, $[\alpha]_D^{27}$ −41.4° (c=0.5 in DMF), Rf^2 0.49. *Anal.* Calcd. for $C_{57}H_{72}N_8O_{13} \cdot H_2O$: C, 62.50; H, 6.81; N, 10.23. Found: C, 62.61; H, 6.85; N, 10.58.

Z-(D)-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-OBu^t (16)——Compound **14** (500 mg, 0.5 mmol)was hydrogenated and the free base was coupled with Z–(D)–Phe–ONB (294 mg). After the usual work-up, the substance was purified by reprecipitation from DMF–ether: 400 mg (71%), mp 197—199°, $[\alpha]_D^{22}$ −28.2° (c=0.5 in DMF), Rf^1 0.60. *Anal.* Calcd. for $C_{61}H_{80}N_8O_{13} \cdot H_2O$: C, 63.64; H, 7.17; N, 9.74. Found: C, 63.51; H, 7.17; N, 9.94.

Z-(D)-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-OH (V)——Compound **16** (367 mg, 0.32 mmol) was treated with TFA (4 ml) and worked up in the same manner as described on compound III to give a fine powder: 340 mg (93%), mp 142—145°, $[\alpha]_D^{21}$ −29.0° (c=0.5 in DMF), Rf^2 0.50. *Anal.* Calcd. for $C_{57}H_{72}N_8O_{13} \cdot 2H_2O$: C, 61.49; H, 6.87; N, 10.06. Found: C, 61.22; H, 6.75; N, 10.27.

BOC-Glu(OBzl)-Leu-Gln-Arg(MBS)-Met(O)-Gln-Glu(OBzl)-Lys(Z)-Glu(OBzl)-Arg(MBS)-Asn-Lys(Z)-Gly-Gln-OBzl (IV)——Compound I (1.58 g, 1 mmol) was treated with TFA (15 ml) for 15 min at 20°. To this was added 6 N HCl (0.2 ml) at 0°, and the usual work-up provided a precipitation which was dissolved in DMF (20 ml) together with compound II (1.40 g, 1 mmol) and N-ethylmorphorine (0.14 ml). The solution was cooled to −10° and to this were added HONB (250 mg) and DCC (288 mg) with stirring. After stirring at −10° for 3 hr and at 20° for 12 hr, the reaction mixture was filtered and the filtrate was evaporated. The substance obtained was chromatographed on silica gel (40 g) using the solvent Rf^2, and the fractions

15) S. Kobayashi, S. Shinagawa, M. Obayashi, T. Fukuda, and M. Fujino, *Chem. Pharm. Bull.* (Tokyo), **23**, 2033 (1975).

(70—190 ml) were combined and then evaporated. The residue was triturated with AcOEt and ether: 2.40 g (84.0%), mp 191—192°, $[\alpha]_D^{27}$ −4.0° (c=0.25 in DMF), Rf^2 0.52. *Anal.* Calcd. for $C_{134}H_{180}N_{26}O_{38}S_3 \cdot 2H_2O$: C, 55.58; H, 6.40; N, 12.58; S, 3.32. Found: C, 55.45; H, 6.21; N, 12.51; S, 3.32. Amino acid ratios in acid hydrolysate: Lys 1.96(2), Arg 1.92(2), Asp 1.04(1), Glu 6.05(6), Gly 1.00(1), Met 0.84(1), Leu 0.88(1).

[Met(O)^{13}]-motilin——Compound IV (2.0 g, 0.7 mmol) was treated with TFA (20 ml) for 15 min at 20°. After addition of 6 N HCl (0.2 ml), the TFA was removed by evaporation. The usual work-up provided a fine powder quantitatively. A part of the powder (527 mg, 0.2 mmol) was dissolved in DMF (5 ml), and to this was added N-ethylmorphorine (0.03 ml).

Compound III (215 mg, 0.2 mmol) and HONB (71 mg) were dissolved in DMF (5 ml), and to this was added DCC (62 mg) at 0°. After stirring at 0° for 2 hr and at 20° for 5 hr, the mixture was filtered and the filtrate was added to the above mentioned DMF solution. After 12 hr, the solvent was removed by evaporation and the residue was treated with AcOEt to give a powder: 720 mg (92%). The crude protected docosapeptide thus obtained was treated with MSA (7 ml) in the presence of anisole (0.7 ml) at room temperature for 60 min. To the reaction mixture was added dry ether at 0° and the precipitate formed was washed well with dry ether. The precipitate was dissolved in H_2O (50 ml) and the solution was passed through a column (1×10 cm) of Amberlite IRA-410 (AcO^-). The eluate and washings were combined and lyophilized to give a powder: 450 mg (84%). The powder was dissolved in a small amount of 5% AcOH (1 ml) and the solution was applied to a column (1×97 cm) of Biogel P-2, which was eluted with the same solvent. The fraction (36—60 ml) were collected and lyophilized. The powder was dissolved in H_2O (50 ml) and the solution was applied to a column (1.8×43 cm) of CM-cellulose, which was eluted with pH 6.8 ammonium acetate buffer (gradient elution: 0.005 M/0.15 M=800 ml/800 ml). The fractions (480—540 ml) containing the pure product were combined and lyophilized: 180 mg (total yield, 36%), $[\alpha]_D^{24}$ −52.4° (c=0.5 in 5% AcOH), Rf^3 (cellulose) 0.48, Rf^4 (cellulose) 0.59. Amino acid ratios in acid hydrolysate: Lys 1.77(2), Arg 2.16(2), Asp 1.00(1), Thr 1.00(1), Glu 6.06(6), Pro 1.08(1), Gly 2.16(2), Val 1.00(1), Met 0.92(1), Ile 0.92(1), Leu 0.92(1), Tyr 1.13(1), Phe 2.03(2) (average recovery, 76.9%).

Motilin——Met(O)^{13}-Motilin (100 mg) was dissolved in 3% aqueous thioglycolic acid (10 ml) and kept to stand at 50° for 20 hr and to this solution was then added H_2O (90 ml). The solution was applied to a column of CM-cellulose (1.2×30 cm) which was eluted with pH 6.7 ammonium acetate buffer (gradient: 0.005 M/0.15 M=250 ml/250 ml). The fractions (160—195 ml) were combined and lyophilized to a constant weight to give a white fluffy powder: 70 mg (70%), $[\alpha]_D^{26}$ −50.0° (c=0.5 in 5% AcOH), Rf^3 (cellulose) 0.52, Rf^4 (cellulose) 0.60. Amino acid ratios in acid hydrolysate: Lys 1.94(2), Arg 1.89(2), Asp 1.00(1), Thr 0.94(1), Glu 6.35(6), Pro 0.94(1), Gly 1.94(2), Val 0.94(1), Met 1.00(1), Ile 0.94(1), Leu 1.00(1), Tyr 1.00(1), Phe 1.89(2) (average recovery, 86%). Amino acid ratios in aminopeptidase M hydrolysate: Lys 1.80(2), Arg 2.00(2), Glu 3.16(3), Pro 1.04(1), Gly 1.96(2), Val 1.04(1), Met 1.00(1), Ile 0.94(1), Leu 1.00(1), Tyr 1.00(1), Phe 2.00(2), Thr+Gln 4.05(4), Asn 1.15(1) (average recovery, 84%).

[D-Phe^1, Met(O)^{13}]-motilin——The deblocked tetradecapeptide (657 mg) derived from compound IV was coupled with compound V (269 mg) *via* the corresponding HONB-ester as described for Met(O)^{13}-motilin to give the crude protected peptide: 800 mg. The crude peptide (520 mg) was treated with MSA (8 ml) in the presence of anisole (0.8 ml) at room temperature for 60 min. After purification of the product through a Biogel P-2 column and a CM-cellulose column the desired product was obtained: 141 mg (total yield, 35%), $[\alpha]_D^{22}$ −67.8° (c=0.5 in 5% AcOH), Rf^3 (cellulose) 0.48, Rf^4 (cellulose) 0.59. Amino acid ratios in acid hydrolysate: Lys 1.86(2), Arg 2.11(2), Asp 1.10(1), Thr 1.00(1), Glu 5.85(6), Pro 0.97(1), Gly 2.00(2), Val 1.00(1), Met 1.07(1), Ile 1.00(1), Leu 1.01(1), Tyr 1.03(1), Phe 2.00(2) (average recovery, 83%).

[D-Phe^1]-motilin——[D-Phe^1, Met(O)^{13}]-Motilin (80 mg) was treated with thioglycolic acid and the product was purified through a CM-cellulose column as described for motilin to give the pure product: 50 mg (62%), $[\alpha]_D^{22}$ −64.6° (c=0.5 in 5% AcOH), Rf^3 (cellulose) 0.52, Rf^4 (cellulose) 0.62. Amino acid ratios in acid hydrolysate: Lys 1.92(2), Arg 2.00(2), Asp 1.00(1), Thr 0.87(1), Glu 5.67(6), Pro 1.04(1), Gly 1.87(2), Val 0.96(1), Met 1.04(1), Ile 0.96(1), Leu 1.00(1), Tyr 0.96(1), Phe 1.83(2) (average recovery, 88%).

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