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Studies on Peptides. LIV.[1,2] Synthesis of the Protected Tetradecapeptide corresponding to Positions 9 through 22 of Porcine Motilin, a Gastric Motor Activity Stimulating Polypeptide

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The protected tetradecapeptide corresponding to positions 9 through 22 of porcine motilin, Z(OMe)-Glu(OBzl)-Leu-Gln-Arg(Tos)-Met-Gln-Glu(OBzl)-Lys(Z)-Glu(OBzl)-Arg-(Tos)-Asn-Lys(Z)-Gly-Gln-OH, was synthesized to serve as a starting material for the total synthesis of this gastric motor activity stimulating polypeptide.

In 1973, Brown, *et al.*[4] disclosed at the first time the primary structure of the porcine intestinal peptide having the characteristic gastric motor activity stimulating property. This peptide, named motilin, consists of 22 amino acids in a streight chain which has one Met residue at position 13. Shortly after this publication, the biologically active Met-substituted peptide, [13-Nle]-motilin, was synthesized by Wünsch, *et al.*,[5] who utilized protecting groups removable by trifluoroacetic acid (TFA). In 1974, Schubert and Brown[6] made a minor correction of their 1973 formula. The Gln residue, instead of Glu, was placed at position 14 in their new structure.

In two consecutive papers, we wish to describe the first synthesis of the docosapeptide corresponding to this newly revised sequence of porcine motilin (I). Four peptide fragments: V (sequence 1—5), IV (sequence 6—8), III (sequence 10—13) and II (sequence 14—22), served as stems to construct the entire amino acid sequence of motilin.

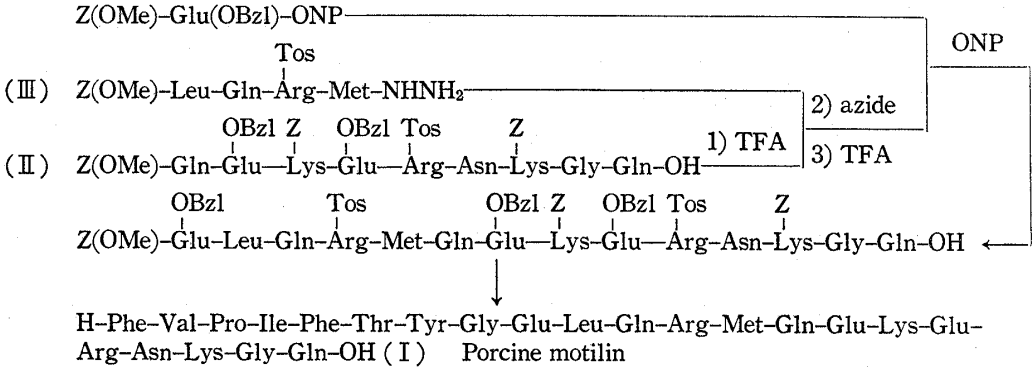

Fig. 1. Synthetic Route to the Protected Tetradecapeptide, Z(OMe)-(motilin 9–22)-OH

1) Part LIII: K. Koyama, Y. Mori, Y. Kiso, S. Hirabayashi, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **23**, 2301 (1975).
2) Amino acids, peptides and their derivatives mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature: *Biochem.*, **5**, 2485 (1966): *ibid.*, **6**, 362 (1967): *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Tos=*p*-toluenesulfonyl, OBzl=benzyl ester, OSU=N-hydroxy succinimide ester, OQCl=5-chloro-8-quinolyl ester, ONP=*p*-nitrophenyl ester, ODNP=2,4-dinitrophenyl ester.
3) Location: *Sakyo-ku, Kyoto.*
4) J.C. Brown, M.A. Cook, and J.R. Dryburgh, *Can. J. Biochem.*, **51**, 533 (1973).
5) E. Wünsch, J.C. Brown, K.H. Deimer, F. Dres, E. Jaeger, J. Mousiol, R. Scharf, H. Stocker, P. Thamm, and G. Wendlberger, *Z. Naturforsch*, **28c**, 235 (1973).
6) H. Schubert and J.C. Brown, *Can. J. Biochem.*, **52**, 7 (1974).

In this paper, synthesis of the protected tetradecapeptide corresponding to the C-terminal portion (sequence 9—22) of porcine motilin was described. For this synthesis, two peptide fragments, (III) and (II) were condensed and the Glu residue at position 9 was introduced by the active ester procedure as illustrated in Fig. 1.

The Z(OMe) group[7] removable by TFA played a role as temporary protection for the α-amino function of necessary intermediates containing Glu(OBzl), Arg(Tos) and Lys(Z). These side chain protecting groups adopted survive mostly intact under the limited TFA treatment and are known to be cleaved by hydrogen fluoride[8] at the final step of the synthesis.

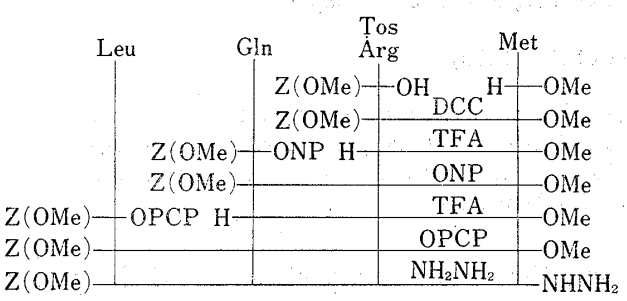

Fig. 2. Synthetic Scheme of the Protected Tetrapeptide Hydrazide, Z(OMe)-(motilin 10—13)-$NHNH_2$

For the synthesis of the protected tetrapeptide hydrazide, Z(OMe)–Leu–Gln–Arg(Tos)–Met–$NHNH_2$ (III) abbreviated as Z(OMe)-(motilin 10—13)-$NHNH_2$, the stepwise elongation method was employed as shown in Fig. 2. First, Z(OMe)–Arg(Tos)–OH was condensed with H–Met–OMe by dicyclohexylcarbodiimide (DCC) to give Z(OMe)–Arg(Tos)–Met–OMe as amorphous powder. To carry out the synthesis of Met-containing peptides, every reaction was performed under the nitrogen atmosphere to prevent its possible oxidation to Met sulfoxide. The Z(OMe) group from the above protected dipeptide ester was removed by TFA in the presence of anisole and the resulting H–Arg(Tos)–Met–OMe, after extraction from the basified solution with ice-cold ethyl acetate, was submitted to the next condensation with Z(OMe)–Gln–ONP to give Z(OMe)–Gln–Arg(Tos)–Met–OMe in satisfactory yield. Next, the pentachlorophenyl ester procedure[9] was employed to introduce Z(OMe)–Leu–OH to the TFA treated sample of the above protected tripeptide ester. The resulting protected tetrapeptide ester, Z(OMe)–Leu–Gln–Arg(Tos)–Met–OMe, was converted to III by hydrazine hydrate in the usual manner. Homogeneity of III was assessed by aicd hydrolysis and elemental analysis.

For the synthesis of the protected nonapeptide, Z(OMe)–Gln–Glu(OBzl)–Lys(Z)–Glu(OBzl)–Arg(Tos)–Asn–Lys(Z)–Gly–Gln–OH (II) abbreviated as Z(OMe)–(motilin 14—22)–OH, the C-terminal pentapeptide unit, Arg–Asn–Lys–Gly–Gln, was synthesized by two alternate routes; the one by the stepwise elongation method starting with H–Gln–OH and the other by the azide condensation of the 4+1 units as shown in Fig. 3.

In the former route, various active ester procedures were employed. The triethylammonium salt of H–Gln–OH was condensed with Z–Gly–OH by the N-hydroxysuccinimide ester procedure.[10] After the reaction, the solvent was evaporated and the residue was dissolved in water. The aqueous phase was washed with ethyl acetate to remove the unreacted active ester and then acidified with citric acid. However, no precipitation of the product, Z–Gly–Gln–OH, occurred immediately. Extraction of the product with ethyl acetate was unsatisfactory. When the solution was kept on standing at room temperature for a week or so, Z–Gly–Gln–OH began to crystallize. Thus the first crop of the desired product was obtained after such time consuming purification step. The Z group from this dipeptide was removed by catalytic hydrogenation to give H–Gly–Gln–OH. In the literatures, this dipeptide was

7) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962): H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **17**, 1962 (1969): H. Yajima, F. Tamura, and Y. Kiso, *ibid.*, **18**, 2574 (1970): H. Yajima, F. Tamura, Y. Kiso, and M. Kurobe, *ibid.*, **21**, 1380 (1973).
8) S. Sakakibara, Y. Shimonishi, Y. Kishida, H. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
9) J. Kovacs, M.Q. Ceprini, C.A. Dupraz, and G.N. Schmidt, *J. Org. Chem.*, **32**, 3696 (1957).
10) G.W. Anderson, J.E. Zimmerman, and F. Callahan, *J. Am. Chem. Soc.*, **85**, 3039 (1963).

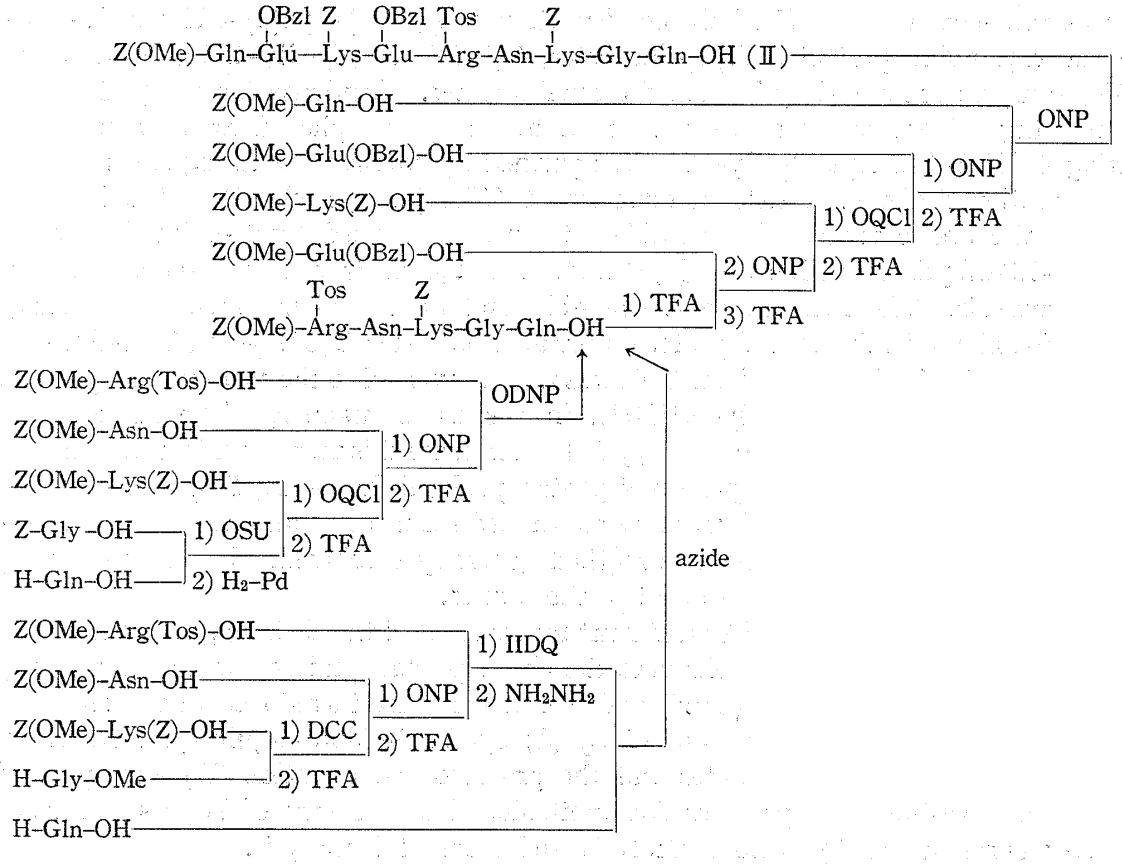

Fig. 3. Synthetic Scheme of the Protected Nonapeptide, Z(OMe)-(motilin 14–22)-OH

derived from Z-Gly-Gln(N^{γ}-xanthyl)-OH by the hydrogen bromide treatment[11] and the former protected dipeptide was prepared by using 7-hydroxy-2-ethylbenzoisoxazolium fluoroborate.[12]

Next, to this free dipeptide, Z(OMe)-Lys(Z)-OH was introduced by the 5-chloro-8-quinolyl ester procedure according to Jakubke.[13] In this coupling step, the N-hydroxysuccinimide ester method was unfavourable, since Z(OMe)-Lys(Z)-OSU was saponified mostly during the coupling reaction. The resulting Z(OMe)-Lys(Z)-Gly-Gln-OH, after treatment with TFA, was condensed with Z(OMe)-Asn-OH by the *p*-nitrophenyl ester procedure[14] to give Z(OMe)-Asn-Lys(Z)-Gly-Gln-OH. Removal of the Z(OMe) group from this tetrapeptide was performed as usual by the TFA treatment. To the resulting tetrapeptide, Z(OMe)-Arg(Tos)-OH was introduced by the 2,4-dinitrophenyl ester procedure[15] without isolating the corresponding active ester. The progress of the active ester forming step, as well as the coupling reaction, was pursued by thin-layer chromatography. The desired protected pentapeptide, Z(OMe)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH, was no longer extractable with ethyl acetate. Therefore, purification was carried out by batchwise washing with citric acid and water followed by recrystallization from methanol and ether or precipitation from dimethylformamide (DMF) with ethyl acetate.

According to the scheme illustrated in Fig. 3, we have prepared also this protected pentapeptide by an alternate route, since the isolation of the first crop of the sample, H-Gly-Gln-

11) Y. Shimonishi, S. Sakakibara, and S. Akabori, *Bull. Chem. Soc. Japan*, **35**, 1966 (1962).
12) D.S. Kemp, S.J. Wrobal, Jr., S.W. Wang, Z. Bernstein, and J. Rebek Jr., *Tetrahedron*, **30**, 3969 (1974).
13) H.D. Jakubke, *Chem. Ber.*, **99**, 2944 (1966); *idem*, *Z. Chem.*, **6**, 52 (1966).
14) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
15) J.A. Farrington, P.J. Hextall, G.W. Kenner, and J.M. Turner, *J. Chem. Soc.*, **1957**, 1407; M. Bodanszky and M.A. Ondetti, *Chem. and Ind.*, **1966**, 26.

OH, was time-consuming. The DCC condensation of Z(OMe)–Lys(Z)–OH and H–Gly–OMe gave the crystalline protected dipeptide ester, Z(OMe)–Lys(Z)–Gly–OMe, from which the Z(OMe) group was removed by TFA in the usual manner. Condensation of Z(OMe)–Asn–OH with resulting dipeptide ester was performed by the *p*-nitrophenyl ester procedure. The resulting Z(OMe)–Asn–Lys(Z)–Gly–OMe was purified by batchwise washing and recrystallization as mentioned above. Next, introduction of Z(OMe)–Arg(Tos)–OH to the TFA treated sample of the above protected tripeptide ester was performed by N-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (IIDQ),[16] instead of 2,4-dinitrophenyl ester adopted in the former synthesis. The reaction went smoothly and after similar batchwise washing and precipitation methods, the desired protected tetrapeptide ester, Z(OMe)–Arg(Tos)–Asn–Lys(Z)–Gly–OMe, was obtained in 70% yield. This was then converted to the corresponding hydrazide, Z(OMe)–Arg(Tos)–Asn–Lys(Z)–Gly–$NHNH_2$, in the usual manner, The azide procedure modified by Honzl and Rudinger[17] was applied to condense this tetrapeptide unit with H–Gln–OH to give the above mentioned protected pentapeptide, Z(OMe)–Arg(Tos)–Asn–Lys(Z)–Gly–Gln–OH, in 72% yield. The 2nd route we mentioned seems to be an unusual peptide elongation method compared to the stepwise method generally employed. However overall yield in the 2nd route was not inferior to that of the former.

Next, combination of the TFA treatment for removal of the Z(OMe) group and the active ester procedure was employed to elongate the above pentapeptide chain to the nonapeptide stage (II). Two residues of Glu(OBzl) and one of Gln were introduced in the order by the *p*-nitrophenyl ester procedure and one of Lys(Z) by the 5-chloro-8-quinolyl ester procedure mentioned above. All intermediates and the protected nonapeptide (II) were purified by the batchwise washing and precipitation methods. Homogeneity of II was confirmed by thin-layer chromatography, acid hydrolysis and elemental analysis.

Condensation of two fragments, II and III thus synthesized, was achieved by the modified azide procedure.[17] III was converted to the corresponding azide, which was allowed to react with the TFA treated sample of II. In order to purify the product, column chromatography on silica was employed. The solvent system of chloroform, methanol and water (8: 3: 1 v/v) was found effective to separate the unreacted amino component as well as the rearrangement product of the azide from the desired protected tridecapeptide, Z(OMe)–Leu–Gln–Arg(Tos)–Met–Gln–Glu(OBzl)–Lys(Z)–Glu(OBzl)–Arg(Tos)–Asn–Lys(Z)–Gly–Gln–OH. The acid hydrolysate of the main component contained the constituent amino acids in ratios predicted by theory, indicating the homogeneity of the product resulted from this coupling reaction.

Next, the Glu residue located at position 9 was introduced to the TFA treated sample of the above protected tridecapeptide by the *p*-nitrophenyl ester procedure. This route is more easier to operate in this case than the use of the azide procedure of peptides containing the Glu(OBzl) residue, since in the latter route, the corresponding hydrazide has to be prepared starting with a substituted hydrazine, such as trichloroethyloxycarbonylhydrazine.[18] Thus addition of one Glu residue we adopted seems to be a method of choice to introduce one amino acid residue bearing the Bzl group without risk of racemization.

The protected tetradecapeptide, Z(OMe)–Glu(OBzl)–Leu–Gln–Arg(Tos)–Met–Gln–Glu(OBzl)–Lys(Z)–Glu(OBzl)–Arg(Tos)–Asn–Lys(Z)–Gly–Gln–OH, synthesized as stated above was purified by column chromatography on silica. The solvent system of chloroform, methanol and water (8: 3: 1) containing a half volume of DMF was necessary to elute the desired compound. Homogeneity of the protected tetradecapeptide, a starting material for the total synthesis of motilin, was confirmed by thin-layer chromatography, acid hydrolysis and elemental analysis.

16) Y. Kiso and H. Yajima, *Chem. Commun.*, **1972**, 942; Y. Kiso, Y. Kai, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **21**, 2507 (1973).
17) J. Honzl and J. Rudinger, *Collection Czech. Chem. Commun.*, **26**, 2333 (1961).
18) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **19**, 420 (1971).

Experimental

Thin-layer chromatography was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8:3:1), Rf_2 *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2).

Z-Gly-Gln-OH——Z-Gly-OSU (29.03 g) in THF (200 ml) was added to a solution of H-Gln-OH (16.08 g) in H_2O (100 ml) containing Et_3N (30.8 ml) and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was dissolved in H_2O (200 ml), which after washing with AcOEt, was acidified with citric acid. This aqueous solution was saturated with NaCl and kept on standing at room temperature for 4 days. The crystalline mass formed was collected by filtration, washed with a small amount of H_2O and then recrystallized twice from MeOH; yield 21.10 g (62%), mp 149—151°, $[\alpha]_D^{28}$ +5.4° (c=0.5, MeOH). Rf_1 0.15, Rf_2 0.54. (lit.[12] mp 158.5—159°, $[\alpha]_D$ +2.4° in EtOH). Amino acid ratios in an acid hydrolysate: Gly 1.00, Glu 1.10 (recovery 99%). *Anal.* Calcd. for $C_{13}H_{19}O_6N_3 \cdot 1/2H_2O$: C, 52.01; H, 5.82; N, 12.14. Found: C, 51.86; H, 5.60; N, 11.87.

H-Gly-Gln-OH——Z-Gly-Gln-OH (10.12 g) in MeOH (100 ml) containing AcOH (1 ml) was hydrogenated in the usual manner over a Pd catalyst for 12 hr. The solution was filtered, the filtrate was condensed *in vacuo* and the residue was treated with AcOEt. The resulting solid was recrystallized from H_2O and EtOH; yield 5.79 g (95%), mp 198—200°, $[\alpha]_D^{26}$ −1.6° (c=1.0, H_2O), Rf_2 0.08. (lit.[11] mp 206° decomp., $[\alpha]_D$ −1.8° in H_2O). *Anal.* Calcd. for $C_7H_{13}O_4N_3 \cdot 1/2H_2O$: C, 39.62; H, 6.65; N, 19.81. Found: C, 39.94; H, 6.74; N, 19.93.

Z(OMe)-Lys(Z)-Gly-Gln-OH——Z(OMe)-Lys(Z)-OQCl (21.82 g) in DMF (100 ml) was combined with a solution of H-Gly-Gln-OH (6.10 g) in H_2O (100 ml) containing Et_3N (8.4 ml) and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in 5% NH_4OH (100 ml), which after washing with AcOEt, was acidified with 5% citric acid. The resulting precipitate was extracted with AcOEt. The organic phase was washed with H_2O–NaCl, dried over Na_2SO_4 and then evaporated. Treatment of the residue with ether afforded the solid, which was recrystallized with EtOH; yield 13.75 g (73%), mp 163—166°, $[\alpha]_D^{27}$ −6.6° (c=1.0, MeOH). Rf_1 0.19, Rf_2 0.79. *Anal.* Calcd. for $C_{30}H_{39}O_{10}N_5$: C, 57.22; H, 6.24; N, 11.12. Found: C, 56.94; H, 6.25; N, 11.01.

Z(OMe)-Asn-Lys(Z)-Gly-Gln-OH——Z(OMe)-Lys(Z)-Gly-Gln-OH (12.60 g) was treated with TFA (20 ml) in the presence of anisole (10 ml) in an ice-bath for 30 min, when dry ether was added. The resulting powder was collected by filtration, washed with dry ether, dried over KOH pellets *in vacuo* for 5 hr and then dissolved in a mixture of DMF (250 ml) and H_2O (150 ml). To this solution, Et_3N (8.4 ml), HOBT (3.5 g) and Z(OMe)-Asn-ONP (8.35 g) were added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated *in vacuo* and the residue was treated with AcOEt. The resulting solid was washed batchwisely with 5% citric acid and H_2O and then recrystallized from DMF and AcOEt; yield 11.92 g (80%), mp 227—229°, $[\alpha]_{27}^{D}$ −1.8° (c=0.8, DMF). Rf_1 0.34, Rf_2 0.63. *Anal.* Calcd. for $C_{34}H_{45}O_{12}N_7 \cdot H_2O$: C, 53.60; H, 6.22; N, 12.87. Found: C, 53.55; H, 6.27; N, 13.10.

Z(OMe)-Lys(Z)-Gly-OMe——DCC (31.55 g) was added to a solution of Z(OMe)-Lys(Z)-OH (66.67 g) and H-Gly-OMe (prepared from 18.90 g of the hydrochloride with 20.7 ml of Et_3N) in DMF (450 ml) and the mixture was stirred at room temperature for 72 hr. The solvent was filtered, the filtrate was condensed *in vacuo* and the residue was treated with AcOEt. The resulting powder was washed batchwisely with 5% citric acid, 5% Na_2CO_3 and H_2O and then recrystallized from MeOH and ether; yield 59.50 g (77%), mp 107—109°, $[\alpha]_{26}^{D}$ −10.9° (c=0.6, MeOH). Rf_1 0.83. *Anal.* Calcd. for $C_{26}H_{33}O_8N_3$: C, 60.57; H, 6.45; N, 8.15. Found: C, 60.81; H, 6.49; N, 8.21.

Z(OMe)-Asn-Lys(Z)-Gly-OMe——Z(OMe)-Lys(Z)-Gly-OMe (15.46 g) was treated with TFA (31 ml) in the presence of anisole (7.8 ml) at 0° for 60 min, when dry petroleum ether was added. The resulting oily precipitate was washed with petroleum ether, dried over KOH pellets *in vacuo* and then dissolved in DMF (200 ml). To this solution, Et_3N (4.2 ml) and Z(OMe)-Asn-ONP (15.0 g) were added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was treated with AcOEt. The resulting powder was washed with 5% citric acid, 5% $NaCO_3$ and H_2O and then precipitated from DMF with AcOEt; yield 9.52 g (50%), mp 184—186°, $[\alpha]_D^{26}$ −5.9° (c=0.9, DMF). Rf_1 0.56. *Anal.* Calcd. for $C_{30}H_{39}O_{10}N_5$: C, 57.22; H, 6.24; N, 11.12. Found: C, 57.35; H, 6.13; N, 11.08.

Z(OMe)-Arg(Tos)-Asn-Lys(Z)-Gly-OMe——As stated above, Z(OMe)-Asn-Lys(Z)-Gly-OMe (6.30 g) was treated with TFA (12.6 ml) in the presence of anisole (3.2 ml) in an ice-bath for 60 min. The excess TFA was evaporated *in vacuo* and 3.15 N HCl–dioxane (6.3 ml) was added. The excess solvent was again evaporated *in vacuo*. The fine powder formed by addition of dry ether was collected by filtration and then dissolved in DMF (40 ml). To this solution, Et_3N (1.4 ml) and Z(OMe)-Arg(Tos)-OH (7.43 g) in THF (50 ml) were added. IIDQ (6.0 ml) was further combined and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was treated with AcOEt. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and then recrystallized from MeOH and ether; yield 6.62 g (70%), mp 134—140°, $[\alpha]_D^{26}$ −4.9° (c=0.7, DMF). Rf_1 0.65. *Anal.* Calcd. for $C_{43}H_{57}O_{13}N_9S$: C, 54.94; H, 6.11; N, 13.41. Found: C, 54.93; H, 6.09; N, 13.36.

19) H.E. Ertel and L. Horner, *J. Chromatog.*, 7, 268 (1962).

Z(OMe)-Arg(Tos)-Asn-Lys(Z)-Gly-NHNH$_2$——To a solution of Z(OMe)-Arg(Tos)-Asn-Lys(Z)-Gly-OMe (9.40 g) in MeOH (60 ml) and DMF (20 ml), 80% hydrazine hydrate (2.8 ml) was added and the mixture was kept on standing at room temperature for 48 hr. The solvent was evaporated *in vacuo* and the residue was treated with EtOH. The resulting powder was collected by filtration, washed with EtOH and dried over H_2SO_4 *in vacuo*; yield 7.20 g (77%), mp 165—168°, $[\alpha]_D^{27}$ −3.0° (c=0.9, DMF). Rf_1 0.43. *Anal.* Calcd. for $C_{42}H_{57}O_{12}N_{11}S \cdot 1/2H_2O$: C, 53.15; H, 6.05; N, 16.24. Found: C, 53.09; H, 6.10; N, 16.39.

Z(OMe)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH——(a) DCC (0.12 g) and DNP-OH (0.12 g) were added to a solution of Z(OMe)-Arg(Tos)-OH (0.30 g) in DMF (10 ml) and the solution was stirred at room temperature for 3 hr. Thin-layer chromatographic examination revealed the presence of a new spot, Rf_1 0.82 and the spot corresponding to Z(OMe)-Arg(Tos)-OH disappeared. This solution was added to a solution of H-Asn-Lys(Z)-Gly-Gln-OH (prepared 0.33 g of the corresponding Z(OMe)-derivative by treatment with 0.7 ml of TFA in the presence of 0.3 ml of anisole as stated above followed by neutralization with 0.2 ml of Et_3N) in 50% aqueous DMF (15 ml). The mixture, after stirring at room temperature for 48 hr, was condensed *in vacuo* and the residue was treated with AcOEt. The resulting solid was washed batchwisely with 5% citric acid and H_2O and then recrystallized from DMF and AcOEt; yield 0.32 g (60%), mp 167—171°, $[\alpha]_D^{28}$ −0.9° (c=1.0, DMF). Rf_1 0.10, Rf_2 0.54. *Anal.* Calcd. for $C_{47}H_{63}O_{15}N_{11}S$: C, 53.55; H, 6.02; N, 14.62. Found: C, 53.40; H, 5.94; N, 14.56.

(b) To a solution of Z(OMe)-Arg(Tos)-Asn-Lys(Z)-Gly-NHNH$_2$ (4.70 g) in DMF (50 ml), 3.58 N HCl-DMF (2.8 ml) and isoamylnitrite (0.74 ml) were added at −5°. The solution was stirred at this temperature for 5 min, when the hydrazine test[19] became negative. This solution, after neutralization with Et_3N (1.38 ml), was combined with a solution of H-Gln-OH (1.10 g) in H_2O (10 ml) containing Et_3N (1.73 ml) and the mixture was stirred at 4° for 48 hr. The solvent was evaporated *in vacuo* and the residue was treated with 5% citric acid and AcOEt. The resulting powder was washed batchwisely with 5% citric acid and H_2O and then precipitated from THF with AcOEt; yield 3.80 g (72%), mp 168—171°, $[\alpha]_D^{27}$ −1.8° (c=1.3, DMF). Rf_1 0.10, Rf_2 0.61. *Anal.* Found: C, 53.80; H, 6.18; N, 14.47.

Z(OMe)-Glu(OBzl)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH——Z(OMe)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH (10.54 g) was treated with TFA (21 ml) in the presence of anisole (5.3 ml) in an ice-bath for 60 min. The excess TFA was evaporated *in vacuo* and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 5 hr and then dissolved in DMF (100 ml) containing Et_3N (2.8 ml). To this solution, Z(OMe)-Glu(OBzl)-ONP (7.80 g) was added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was treated with 5% citric acid and AcOEt. The resulting powder was washed batchwisely with 5% citric acid and H_2O and then precipitated from DMF with AcOEt; yield 7.71 g (61%), mp 195—197°, $[\alpha]_D^{28}$ −2.9° (c=1.0, DMF). Rf_1 0.13, Rf_2 0.80. *Anal.* Calcd. for $C_{59}H_{76}O_{18}N_{12}S$: C, 55.65; H, 6.02; N, 13.20. Found: C, 55.42; H, 6.03; N, 13.17.

Z(OMe)-Lys(Z)-Glu(OBzl)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH——The above protected hexapeptide (7.41 g) was treated with TFA (20 ml) in the presence of anisole (3.7 ml) as stated above. The TFA salt formed by addition of dry ether was dried over KOH pellets *in vacuo* and then dissolved in DMF (100 ml) containing Et_3N (1.6 ml). To this solution, Z(OMe)-Lys(Z)-OQCl (5.30 g) was added and the mixture, after stirring at room temperature for 48 hr, was condensed *in vacuo*. The residual solid formed by treatment with 5% citric acid and AcOEt was washed batchwisely with 5% citric acid and H_2O and precipitated twice from DMF and AcOEt; yield 6.90 g (78%), mp 170—175°, $[\alpha]_D^{27}$ −7.6° (c=1.0, DMF). Rf_1 0.27, Rf_2 0.82. *Anal.* Calcd. for $C_{73}H_{94}O_{21}N_{14}S$: C, 57.09; H, 6.17; N, 12.77. Found: C, 56.91; H, 6.37; N, 12.52.

Z(OMe)-Glu(OBzl)-Lys(Z)-Glu(OBzl)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH——The above protected heptapeptide (5.33 g) was treated with TFA (13 ml) in the presence of anisole (2.5 ml) as stated above. The TFA salt formed by addition of dry ether was dried over KOH pellets and then dissolved in DMF (50 ml) containing Et_3N (1.0 ml). To this solution, Z(OMe)-Glu(OBzl)-ONP (2.70 g) was added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was purified as stated above; yield 5.69 g (96%), mp 191—193°, $[\alpha]_D^{27}$ −6.4° (c=1.0, DMF). Rf_1 0.17, Rf_2 0.81. *Anal.* Calcd. for $C_{85}H_{107}O_{24}N_{15}S$: C, 58.17; H, 6.15; N, 11.97. Found: C, 58.13; H, 6.19; N, 12.06.

Z(OMe)-Gln-Glu(OBzl)-Lys(Z)-Glu(OBzl)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH (II)——The above protected octapeptide (3.0 g) was treated with TFA (8 ml) in the presence of anisole (1.5 ml) as stated above. The TFA salt similarly collected was dissolved in DMF (30 ml) containing Et_3N (0.48 ml) and the solution, after addition of Z(OMe)-Gln-ONP (1.0 g) was stirred at room temperature for 48 hr. The solvent was evaporated and the residual solid formed by addition of 5% citric acid and AcOEt, was washed batchwisely with 5% citric acid and H_2O and then dissolved in the solvent of $CHCl_3$–MeOH–H_2O (8: 3: 1). The solution was filtered and then condensed. The residue was treated with AcOEt to form the fine powder; yield 2.62 g (81 %), mp 207—211°, $[\alpha]_D^{27}$ −6.2° (c=1.0, DMF). Rf_1 0.16, Rf_2 0.76. Amino acid ratios in an acid hydrolysate: Glu 4.41, Lys 1.87, Arg 0.95, Asp 1.00, Gly 1.00 (average recovery 91%). *Anal.* Calcd. for $C_{90}H_{115}O_{26}N_{17}S$–$H_2O$: C, 56.86; H, 6.20; N, 12.53. Found: C, 56.86; H, 6.20; N, 12.65.

Z(OMe)-Arg(Tos)-Met-OMe——H-Met-OMe hydrochloride (5.32 g) was dissolved in a small amount of H_2O and Et_3N (3.4 ml) was added under cooling with ice. The resulting precipitate was extracted with AcOEt (50 ml), which after drying over Na_2SO_4, was combined with a solution of Z(OMe)-Arg(Tos)-OH (9.85 g) in DMF (80 ml). To this solution, DCC (4.94 g) was added and the mixture was stirred at room tempera-

ture for 48 hr under the nitrogen gas. The solution was filtered and the filtrate was condensed *in vacuo*. The residue was dissolved in AcOEt, which was washed with 5% citric acid, 5% NH_4OH and H_2O–NaCl, dried over Na_2SO_4 and then evaporated to give an oily residue. Treatment of the residue with ether gave the powder, which was recrystallized from AcOEt and ether; yield 12.34 g (97%), mp 59—61°, $[\alpha]_D^{27}$ −8.8° (c=0.6, MeOH), Rf_1 0.70; Rf_2 0.89. *Anal.* Calcd. for $C_{28}H_{39}O_8N_5S_2$: C, 52.71; H, 6.16; N, 10.98. Found: C, 52.99; H, 6.21; N, 10.69.

Z(OMe)-Gln-Arg(Tos)-Met-OMe——Z(OMe)-Arg(Tos)-Met-OMe (3.19 g) was treated with TFA (7 ml) in the presence of anisole (3 ml) in an ice-bath for 40 min and the solvent was evaporated *in vacuo*. The residue was dissolved in a small amount of H_2O, which after washing with AcOEt, was basified with K_2CO_3 and the resulting precipitate was extracted with AcOEt. This organic phase, after washing with 5% K_2CO_3, was dried over Na_2SO_4 and then combined with a solution of Z(OMe)-Gln-ONP (2.37 g) in DMF (50 ml) containing Et_3N (0.8 ml). After the mixture was stirred at room temperature for 48 hr, the solvent was evaporated. Treatment of the residue with ether afforded the solid, which was washed batchwisely with 5% citric acid, H_2O and ether and then recrystallized from DMF and AcOEt; yield 3.70 g (95%), mp 133—135°, $[\alpha]_D^{28}$ −7.4° (c=0.9, DMF). Rf_1 0.49, Rf_2 0.86. *Anal.* Calcd. for $C_{33}H_{47}O_{10}N_7S_2$: C, 51.75; H, 6.19; N, 12.80. Found: C, 52.02; H, 6.05; N, 12.78.

Z(OMe)-Leu-Gln-Arg(Tos)-Met-OMe——As stated above, Z(OMe)-Gln-Arg(Tos)-Met-OMe (3.83 g) was treated with TFA (7 ml) in the presence of anisole (1.9 ml) in an ice-bath for 40 min. Dry ether was added and the resulting precipitate was collected by filtration, washed with dry ether and then dissolved in DMF (50 ml). To this solution, Et_3N (1.4 ml) and Z(OMe)-Leu-OPCP (3.92 g) were combined and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was treated with ether. The resulting powder was washed batchwisely with 5% citric acid, 5% NH_4OH and H_2O and then recrystallized from AcOEt and ether; yield 4.01 g (91%). mp 145—148°, $[\alpha]_D^{24}$ −23.2° (c=0.8, MeOH). Rf_1 0.69, Rf_2 0.85. *Anal.* Calcd. for $C_{39}H_{58}O_{11}N_8S_2 \cdot 1.5H_2O$: C, 51.69; H, 6.79; N, 12.37. Found: C, 51.78; H, 6.76; N, 12.14.

Z(OMe)-Leu-Gln-Arg(Tos)-Met-$NHNH_2$ (III), Z(OMe)-(motilin 10—13)-$NHNH_2$——To a solution of Z(OMe)-Leu-Glu-Arg(Tos)-Met-OMe (3.52 g) in MeOH (50 ml), 80% hydrazine hydrate (2.5 ml) was added and the solution was kept on standing overnight. The gelatinous mass formed was collected by filtration and recrystallized from MeOH; yield 3.12 g (88%), mp 178—182°, $[\alpha]_D^{24}$ −8.9° (c=1.1, DMF). Rf_1 0.22, Rf_2 0.84. Amino acid ratios in an acid hydrolysate: Leu 1.00, Glu 1.20, Arg 0.79, Met 0.89 (average recovery 88%). *Anal.* Calcd. for $C_{38}H_{58}O_{10}N_{10}S_2$: C, 51.91; H, 6.65; N, 15.94. Found: C, 52.03; H, 6.71; N, 15.72.

Z(OMe)-Leu-Gln-Arg(Tos)-Met-Gln-Glu(OBzl)-Lys(Z)-Glu(OBzl)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH——Z(OMe)-Gln-Gln(OBzl)-Lys(Z)-Glu(OBzl)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH (2.61 g) was treated with TFA (6 ml) in the presence of anisole (1.3 ml) and the TFA salt isolated as stated above was dissolved in DMF (20 ml) containing Et_3N (0.58 ml). This solution was combined with a solution of Z(OMe)-Leu-Gln-Arg-(Tos)-Met-azide (prepared in the usual manner from 1.72 g of the corresponding hydrazide with 1.04 ml of 3.78 N HCl–DMF, 0.31 ml of isoamylnitrite and 0.54 ml of Et_3N) in DMF (15 ml). The mixture was stirred at 4° for 48 hr and the solvent was evaporated *in vacuo*. Treatment of the residue with H_2O and AcOEt afforded the fine powder, which was dissolved in a small amount of the solvent of $CHCl_3$–MeOH–H_2O (8: 3: 1) and the solution was applied to a column of silica (5.9×8.5 cm), which was eluted with the same solvent system. The eluates which contained the substance of Rf_1 0.37 were combined and the solvent was evaporated. The residue was treated with H_2O and the resulting powder was again dissolved in the same solvent (10 ml). The solution was filtered, the filtrate was condensed and the residue was treated with AcOEt to afford the fine powder; yield 2.52 g (70%), mp 241—243°, $[\alpha]_D^{27}$ −5.2° (c=1.0, DMF). Rf_1 0.37, Rf_2 0.83. Amino acid ratios in an acid hydrolysate: Leu 0.97, Glu 5.18, Arg 1.93, Met 0.76, Lys 1.71, Asp 1.07, Gly 1.00 (average recovery 95%). *Anal.* Calcd. for $C_{119}H_{161}O_{33}H_{25}S_3 \cdot 3H_2O$: C, 54.55; H, 6.43; N, 13.37. Found: C, 54.46; H, 6.48; N, 13.61.

Z(OMe)-Glu(OBzl)-Leu-Gln-Arg(Tos)-Met-Glu(OBzl)-Lys(Z)-Glu(OBzl)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH——The above protected tridecapeptide (2.0 g) was treated with TFA (5 ml) in the presence of anisole (1.0 ml) and the TFA salt isolated as stated above was dissolved in DMF (50 ml) containing Et_3N (0.22 ml). To this solution, Z(OMe)-Glu(OBzl)-ONP (0.82 g) was combined and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated *in vacuo* and the residue was treated with H_2O and AcOEt. The resulting powder was purified by column chromatography on silica (5.9×8.0 cm). The desired compound was eluted with a mixture of $CHCl_3$–MeOH–H_2O (8: 3: 1) and DMF (1: 1 v/v). The eluates which contained the substance of Rf_1 0.23 were combined and the solvent was evaporated *in vacuo*. The residue was treated with H_2O. The resulting powder was dissolved in a small amount of the above mixed solvent and the solution, after filtration, was condensed. The residue was treated with ether to afford the fine powder; yield 1.58 g (73%), mp 215—217°, $[\alpha]_D^{27}$ −2.2° (c=1.0, DMF). Rf_1 0.23, Rf_2 0.83. Amino acid ratios in an acid hydrolysate: Glu 6.19, Leu 0.87, Arg 1.92, Met 0.78, Lys 1.82, Asp 1.12, Gly 1.00 (average recovery 93%). *Anal.* Calcd. for $C_{131}H_{174}O_{36}N_{26}S_3 \cdot 3H_2O$: C, 55.41; H, 6.39, N, 12.83. Found: C, 55.12; H, 6.32; N, 12.96.