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Studies on Peptides. LXIV.[1,2] Synthesis of the Tritetracontapeptide corresponding to the Entire Amino Acid Sequence of Porcine Gastric Inhibitory Polypeptide (GIP)

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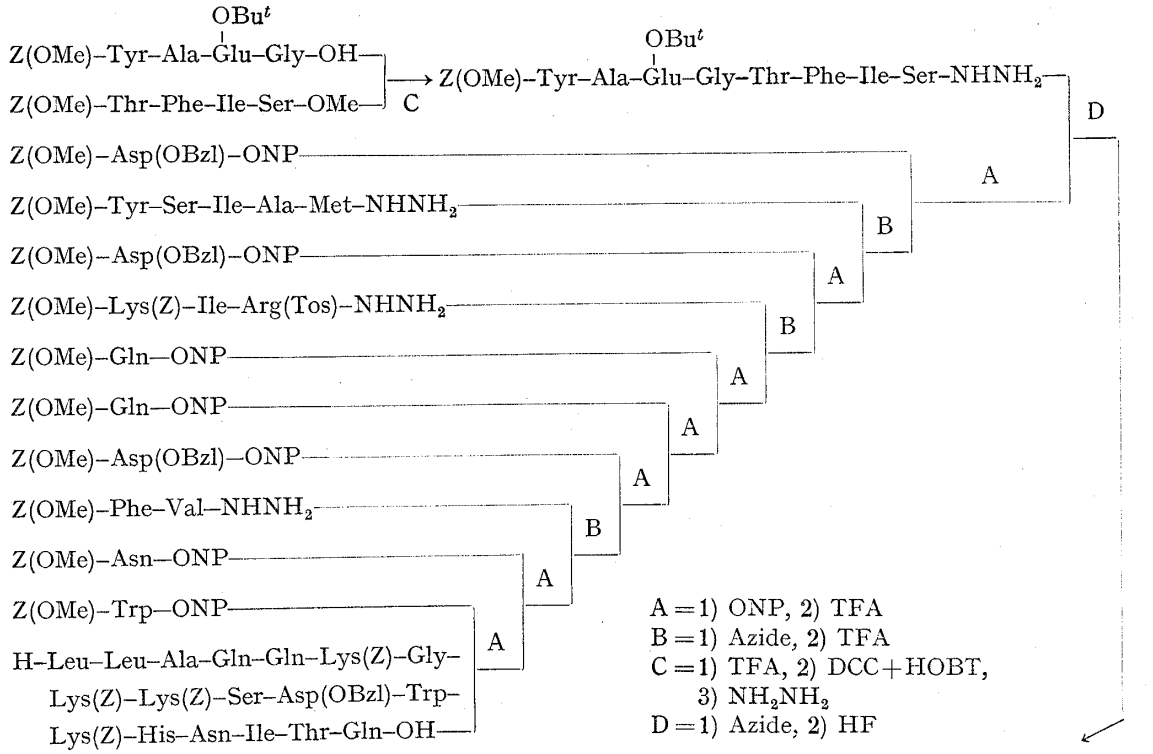
The tritetracontapeptide corresponding to the entire amino acid sequence of porcine gastric inhibitory polypeptide (GIP) was synthesized starting with the C-terminal octadecapeptide, the synthesis of which was previously reported. The hydrogen fluoride procedure was employed to remove all protecting groups employed at the final stage of the synthesis. Trichloroethyloxycarbonylhydrazine was used to prepare alternatively the nonacosapeptide, a cyanogen bromide fragment of GIP, H-(GIP 15—43)-OH. Synthetic GIP suppressed gastric acid secretion stimulated by histamine as well as tetragastrin in Heidenhein pouch dogs. H-(GIP 15—43)-OH exhibited approximately one quarter of the activity of the whole molecule. Association of the insulin release activity in GIP was synthetically confirmed.

In the preceding two papers, we have described the syntheses of two segments of porcine gastric inhibitory polypeptide (GIP); the one was the C-terminal octadecapeptide (position 26—43)[4] and the other the N-terminal octacosapeptide amide (1—28).[1] Through these synthetic studies, it was found that further chain elongation is necessary for synthetic peptides to exsert its inherent physiological responses.

During the course of structural elucidation of GIP, Brown and Pederson[5] reported that of two fragments formed by cyanogen bromide cleavage of the Met residue at position 14, a fragment corresponding to the C-terminal portion of the molecule retained still the GIP activity in approximately 70% of the natural source. Informations from the above synthetic studies together with the natural source suggest that the observable GIP activity can only be expected in peptides with relatively long chain length, possibly longer than the C-terminal octadecapeptide.[4]

In this paper, we wish to describe the synthesis of the tritetracontapeptide which covers the entire amino acid sequence of GIP.[6] Improved synthesis of intermediates is also enclosed.

1) Part LXIII: M. Kubota, H. Ogawa, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **24**, 2435 (1976).
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: *Biochemistry*, **5**, 2485 (1966), *ibid.*, **6**, 362 (1967), *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Troc=trichloroethyloxycarbonyl, Tos=*p*-toluenesulfonyl, OBu^t=*tert*-butyl ester, OBzl=benzyl ester, ONP=*p*-nitrophenyl ester, DCC=dicyclohexylcarbodiimide, HOBT=N-hydroxybenzotriazole, TFA=trifluoroacetic acid, DMSO=dimethylsulfoxide, THF=tetrahydrofuran.
3) Location: *a) Sakyo-ku, Kyoto; b) Kumano, Sakyo-ku, Kyoto; c) Ikutaku, Kobe.*
4) H. Ogawa, M. Kubota, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **24**, 2428 (1976).
5) J.C. Brown and R.A. Pederson, *J. Physiol.*, **210**, 52p (1970).
6) J.C. Brown, *Can. J. Biochem.*, **49**, 255 (1971); J.C. Brown and J.R. Dryburgh, *Can. J. Biochem.*, **49**, 867 (1971).



H–Tyr–Ala–Glu–Gly–Thr–Phe–Ile–Ser–Asp–Tyr–Ser–Ile–Ala–Met–Asp–Lys–Ile–Arg–Gln–Gln–Asp–Phe–Val–Asn–Trp–Leu–Leu–Ala–Gln–Gln–Lys–Gly–Lys–Lys–Ser–Asp–Trp–Lys–His–Asn–Ile–Thr–Gln–OH (porcine gastric inhibitory polypeptide)

Fig. 1. Synthetic Route to Gastric Inhibitory Polypeptide (GIP)

Biological activity of synthetic GIP and the nonacosapeptide, *i.e.*, the cyanogen bromide fragment of GIP, was examined.

As illustrated in Fig. 1, synthesis of GIP was carried out starting with the protected octadecapeptide,[4] which was synthesized by selecting 5 peptide fragments as building blocks: I (position 41—43), II (38—39), III (33—35), IV (29—32) and V (26—28). Additional 5 peptide fragments used in the present synthesis are those selected for the previous synthesis of the N-terminal octacosapeptide amide,[1] except the N-terminal tetrapeptide unit. Z(OMe)–Tyr–Ala–Glu(OBu^t)–Gly–OH (IX-a), instead of the corresponding Glu(OBzl) derivative, was adopted. This was condensed with H–Thr–Phe–Ile–Ser–OMe (deprotected IX-b) by DCC plus HOBT[7] and the resulting octapeptide ester was converted to the corresponding hydrazide, Z(OMe)–Tyr–Ala–Glu(OBu^t)–Gly–Thr–Phe–Ile–Ser–NHNH$_2$ (IX), which was used as one building block at the final condensation step of the synthesis. Thus, actually 4 peptide fragments; *i.e.*, Z(OMe)–Phe–Val–NHNH$_2$ (VI), Z(OMe)–Lys(Z)–Ile–Arg(Tos)–NHNH$_2$ (VII), Z(OMe)–Tyr–Ser–Ile–Ala–Met–NHNH$_2$ (VIII) and IX, were condensed by the modified azide procedure[8] to minimize racemization. Because of difficulty in the direct preparation of peptide hydrazides containing Asp(OBzl) or Asp(OBu^t), three residues of Z(OMe)–Asp(OBzl)–OH (position 9, 15 and 21) were introduced stepwisely by the *p*-nitrophenyl ester procedure.[9] This procedure is known to be preferable to introduce Asn and Gln residues too. In addition, the azide procedure of Trp containing peptides should be avoided. This residue (position 25) was, therefore, introduced also stepwisely by the same active ester procedure. Thus synthetic strategy adopted for the previous synthesis of the N-terminal octacosapeptide amide was

7) J.C. Sheehan and G.P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955), W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
8) J. Honzl and J. Rudinger, *Collection Czech. Chem. Commun.*, **26**, 2333 (1961).
9) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1956).

similarly extended to the present synthesis. After each coupling step, remaining amino components were washed out batchwisely with 3% acetic acid and relatively small acylating components used in excess could readily removed by repeated precipitation from hot DMF with either methanol or ethyl acetate and in some instance with THF.

Because of poor solubility of protected intermediates, acylations were performed in a mixture of DMSO and DMF. After reactions, somewhat elevated temperature (bath temperature 50—55°) was necessary for evaporation of the solvents *in vacuo*. A few drops of mercaptoethanol was added before evaporation to prevent air oxidation of the Met residue. It should be mentioned that the same TFA deblocking procedure of the Z(OMe) group[10] from Trp-containing peptides employed previously[1,4] could also be extended to the present chain elongation reactions. We could carry out the synthesis without producing any brown color, when anisole containing 2% ethanedithiol[11] was employed as a scavenger.

In the first synthesis of GIP, the final protected tritetracontapeptide Z(OMe)-Tyr-Ala-Glu(OBu^t)-Gly-Thr-Phe-Ile-Ser-Asp(OBzl)-Tyr-Ser-Ile-Ala-Met-Asp(OBzl)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys-(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH abbreviated as Z(OMe)-(GIP 1—43)-OH, was deprotected without further purification. Purification of this compound was now achieved by applying the column chromatography on Sephadex LH-20. When the crude material formed after the azide condensation of IX was passed through the column using DMSO as an eluent, the desired compound and the rearrangement product resulted from the above azide were well separated. Thus all intermediates including the final protected tritetracontapeptide were characterized by three criteria; thin-layer chromatography, elemental analysis and amino acid analysis with 3N Tos-OH hydrolysates.[12]

Despite of many advantageous features in the present synthetic scheme illustrated in Fig. 1, a large number of coupling reactions required forced to decrease the amount of the products in each step considerably. As a tool for reducing these coupling steps in some

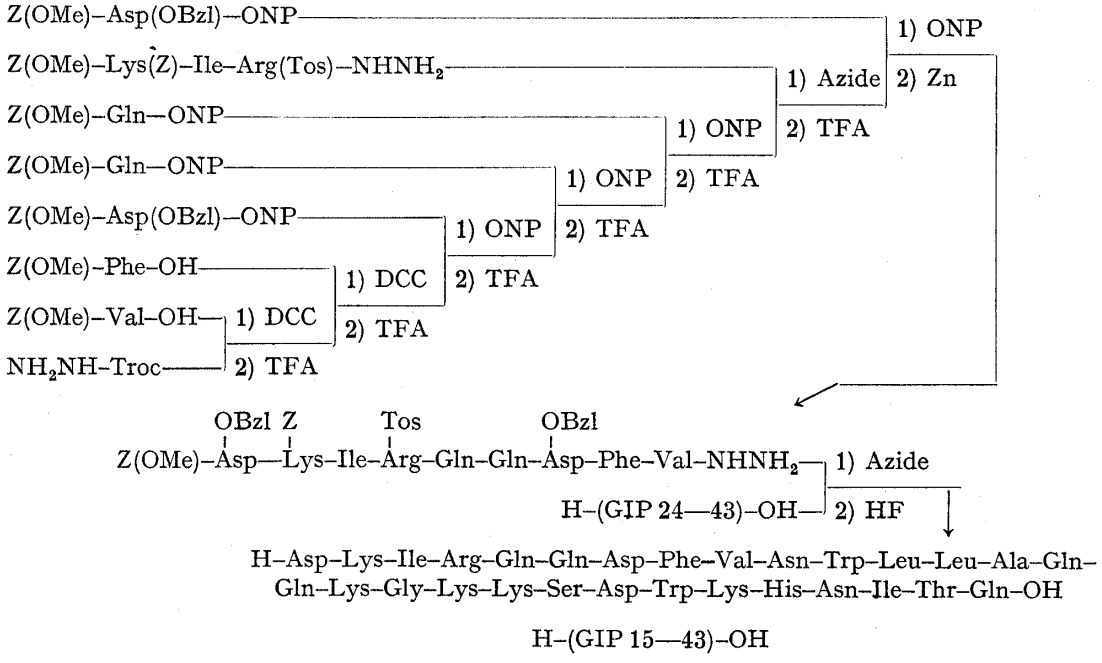


Fig. 2. Synthetic Scheme of the Protected Octapeptide Hydrazide, Z(OMe)-(GIP 15—23)-$NHNH_2$ and the Nonacosapeptide, H-(GIP 15—43)-OH

10) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).
11) J.J. Shar, A.B. Robinson, and M.D. Kamen, *J. Am. Chem. Soc.*, **95**, 6097 (1973).
12) T.Y. Liu and Y.H. Chang, *J. Biol. Chem.*, **246**, 2842 (1971).

extent, we decided to use Troc–$NHNH_2$[13] for the synthesis of peptide hydrazides containing Asp(OBzl). Successful application of this substituted hydrazine for the analogous synthesis of Glu(OBzl)-containing hydrazides[1,14] encouraged us to perform this new approach.

The protected nonapeptide hydrazide, Z(OMe)–Asp(OBzl)–Lys(Z)–Ile–Arg(Tos)–Gln–Gln–Asp(OBzl)–Phe–Val–$NHNH_2$ (position 15—23), was prepared starting with Z(OMe)–Val–NHNH–Troc as illustrated in Fig. 2. Z(OMe)–Val–OH was first condensed with Troc–$NHNH_2$ by DCC and the resulting Z(OMe)–Val–NHNH–Troc, after the TFA treatment, was condensed with Z(OMe)–Phe–OH again by DCC. Chain elongation of Z(OMe)–Phe–Val–NHNH–Troc obtained here was carried out in essentially the same manner as stated above using available amino acid derivatives, Z(OMe)–Asp(OBzl)–ONP and Z(OMe)–Gln–ONP and the tripeptide hydrazide, Z(OMe)–Lys(Z)–Ile–Arg(Tos)–$NHNH_2$. The Troc group adopted here is stable during the TFA treatment, but could be removed from the protected nonapeptide Troc-hydrazide, Z(OMe)–Asp(OBzl)–Lys(Z)–Ile–Arg(Tos)–Gln–Gln–Asp(OBzl)–Phe–Val–NHNH–Troc, by treatment with zinc in a mixture of acetic acid and DMF. Purity of the resulting nonapeptide hydrazide was assessed by elemental and amino acid analyses, as well as the positive hydrazine test[15] on thin-layer chromatography. Fortunately, this protected hydrazide is freely soluble in ice-cold DMF–DMSO and therefore was easy handled for the azide condensation with the deprotected eicosapeptide, H–Asn–Trp–Leu–Leu–Ala–Gln–Gln–Lys(Z)–Gly–Lys(Z)–Lys(Z)–Ser–Asp(OBzl)–Trp–Lys(Z)–His–Asn–Ile–Thr–Gln–OH. In order to purify the desired product, column chromatography on Sephadex LH-20 was employed using DMSO–DMF as an eluent. This procedure was also effective to remove the contaminant resulted from the relatively large nonapeptide azide component as described earlier, if the amount of the sample applied was suitably adjusted depending on the column size. Thus, the protected nonacosapeptide, Z(OMe)–Asp(OBzl)–Lys(Z)–Ile–Arg(Tos)–Gln–Gln–Asp(OBzl)–Phe–Val–Asn–Trp–Leu–Leu–Ala–Gln–Gln–Lys(Z)–Gly–Lys(Z)–Lys(Z)–Ser–Asp(OBzl)–Trp–Lys(Z)–His–Asn–Ile–Thr–Gln–OH, was synthesized by two alternative ways; the one was obtained after six consecutive chain elongation steps starting with the eicosapeptide and the other after one step condensation. However, in the latter step, column chromatographic purification was required. It should be mentioned that this sequence corresponds to the cyanogen bromide fragment reported by Brown and Pederson.[5] A part of this sample was treated with hydrogen fluoride[16] for biological assays as we will describe latter.

In order to remove all protecting groups employed, Z(OMe)–(GIP 1—43)–OH was exposed to hydrogen fluoride in an ice bath for 60 minutes. Anisole containing 2% ethanedithiol and skatole served as scavengers to avoid alkylation.[17] The resulting deblocked peptide was immediately converted to the corresponding acetate with Amberlite CG-400 (acetate form) and purified by column chromatography on Sephadex G-25 to remove scavengers. To elute the desired compound, 0.2 M acetic acid was used in this step and absorbancy at 280 mμ due to Trp served to monitor the chromatographic purification. Fractions corresponding to the front main peak were collected and the product was submitted to the 2nd chromatographic purification on CM-cellulose. Conditions reported in the isolation of GIP from the natural source[18] was adopted. Indeed elution with 0.01 M ammonium bicarbonate (pH 7.8) was found effective to isolate the desired compound rather than ammonium acetate buffers.

13) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **19**, 420 (1971).
14) H. Watanabe, M. Kubota, H. Yajima, A. Tanaka, M. Nakamura, and T. Kawabata, *Chem. Pharm. Bull.* (Tokyo), **22**, 1889 (1974).
15) H. Ertel and L. Horner, *J. Chromatog.*, **7**, 268 (1962), K. Hofmann, R. Schmiechen, D.R. Wells, Y. Wolman, and N. Yanaihara, *J. Am. Chem. Soc.*, **87**, 611 (1965).
16) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
17) S. Sano and S. Kawanishi, *J. Am. Chem. Soc.*, **97**, 3480 (1975); R.S. Feinberg and R.B. Merrifield, *ibid.*, **97**, 3485 (1975).
18) J.C. Brown, V. Mutt, and R.A. Pederson, *J. Physiol.*, **209**, 57 (1970).

The tritetracontapeptide thus purified exhibited a sharp single spot on thin-layer chromatography in two different solvent systems and migrated as a single component in the field of disc electrophoresis at pH 2.3. Its purity was further assessed by amino acid analyses of 3 N Tos-OH hydrolysates and aminopeptidase (AP-M) digests.[19] In the latter case, Gln and Asn peaks overlapped with those of Thr and Ser respectively. However, the presence of these amino acids could be figured out from the difference in recoveries between acid and enzymatic hydrolysates. Nearly the theoretical amount of Trp was detected. Experimental evidences cited above may justify the conclusion that our synthetic GIP possesses a high degree of homogeneity and the L-configuration of constituent amino acids.

When administered by continuous infusion to Heidenhein pouch dogs, the synthetic GIP (1 μg/kg/hr) suppressed the gastric acid secretion stimulated by histamine hydrochloride (10 μg/kg/hr) as well as tetragastrin (4 μg/kg/hr) in the range of 60%. The same level of inhibition was achieved, when H-(GIP 15—43)-OH (4 μg/kg/hr) was employed. Thus, it became evident, through these synthetic studies, that the tritetracontapeptide corresponding to the entire amino acid sequence of GIP proposed by Brown and Dryburgh[6] has definitely an enterogastron-like activity and within this sequence, the C-terminal portion, longer than the C terminal octadecapeptide, endows this characteristic physiological response. The activity of the nonacosapeptide, H-(GIP 15—43)-OH, can be figured out as about 1/4 of that of the full molecule. These results were fully reproducible. As far as our results were compared with those described by Pederson and Brown,[20] it could be presumed that our synthetic tritetracontapeptide is about four times active than that reported in GIP from the natural source.

Ability of synthetic GIP in insulin release[21] was next examined. Intravenous administration of synthetic GIP (1 μg/kg/hr) to rats elicited significant release of insulin. Association of the incretin-like activity in the GIP molecule has thus synthetically been confirmed too. Structure-activity relationship of this incretin-like activity will be reported in a separated paper.

Experimental

General experimental methods employed here are essentially the same as those described in the Part LXII[4] of this series. 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, Rf_3, Rf_4, Rf_5 *n*-BuOH–pyridine–AcOH–H_2O (1: 1: 1: 1), (4: 1: 1: 2), (30: 6: 20: 24), (30: 20: 6: 24) respectively. Solvents of DMSO–DMF was evaporated *in vacuo*, after addition of a few drops of mercaptoethanol.

Z(OMe)-Tyr-Ala-Glu(OBu^t)-Gly-Thr-Phe-Ile-Ser-OMe——Z(OMe)-Thr-Phe-Ile-Ser-OMe[1] (0.92 g) was treated with TFA (1.5 ml) in the presence of anisole (0.5 ml) in an ice-bath for 60 min and dry ether was added. The resulting powder collected by filtration, was dissolved in 1 N HCl–dioxane (1.4 ml) and the solvent was evaporated *in vacuo*. Addition of dry ether afforded a fine powder, which was collected by filtration and then dissolved in DMF (10 ml). Et_3N (0.2 ml), Z(OMe)-Tyr-Ala-Glu(Bu^t)-Gly-OH[1] (0.73 g), HOBT (0.18 g) and DCC (0.27 g) were successively added and the mixture was stirred at room temperature for 48 hr. The solution was filtered, the filtrate was condensed *in vacuo* and the residue was treated with AcOEt to form a fine powder, which was washed batchwisely with 5% citric acid and H_2O and then precipitated from DMF with AcOEt; yield 1.03 g (70%), mp 240—245°, $[\alpha]_D^{20}$ −12.5° (c=1.0, DMF), Rf_1 0.71. *Anal.* Calcd. for $C_{55}H_{76}O_{17}N_8$: C, 58.91; H, 6.83; N, 9.99. Found: C, 58.66; H, 6.71; N, 9.70.

Z(OMe)-Tyr-Ala-Glu(OBu^t)-Gly-Thr-Phe-Ile-Ser-NHNH$_2$ (IX)——To a solution of the above protected octapeptide ester (0.99 g) in DMF (30 ml), 80% hydrazine hydrate (0.6 ml) was added and the solution, after standing at room temperature overnight, was condensed *in vacuo*. Treatment of the residue with EtOH afforded a fine powder which was precipitated from DMF with MeOH; yield 0.75 g (76%), mp 249—255°, $[\alpha]_D^{25}$ −6.7° (c=0.4, DMSO), Rf_2 0.70. Amino acid ratios in an acid hydrolysate: Tyr 0.74, Ala 1.10, Glu 0.94,

19) G. Pfleiderer and P.G. Celliers, *Biochem. Z.*, **339**, 186 (1963).
20) R.A. Pederson and J.C. Brown, *Gastroenterology*, **62**, 393 (1972).
21) R.A. Pederson, H.E. Schubert, and J.C. Brown, *Can. J. Physiol. Pharmacol.*, **53**, 217 (1975); T.W. Jun, *Gastroenterology*, **68**, 621 (1975).

Gly 1.06, Thr 1.00, Phe 1.25, Ile 1.18, Ser 0.93 (average recovery 84%). *Anal.* Calcd. for $C_{54}H_{76}O_{16}N_{10}$: C, 57.84; H, 6.83; N, 12.49. Found: C, 57.62; H, 7.08; N, 12.48.

Z(OMe)-Val-NHNH-Troc——DCC (13.80 g) was added to a mixture of Z(OMe)-Val-OH (17.16 g) and Troc-$NHNH_2$ (15.20 g) in AcOEt (100 ml) and the solution, after stirring at room temperature for 24 hr, was filtered. The filtrate was washed with 5% citric acid, 5% sodium bicarbonate and H_2O–NaCl, dried over sodium sulfate and then evaporated. The residue was triturated with ether and then recrystallized from AcOEt and ether; yield 25.33 g (88%), mp 137—138°, $[\alpha]_D^{20}$ −11.8° (c=0.9, DMF), Rf_1 0.83. *Anal.* Calcd. for $C_{17}H_{22}O_6N_3Cl_3$: C, 43.37; H, 4.71; N, 8.92. Found: C, 43.34; H, 4.80; N, 9.19.

Z(OMe)-Phe-Val-NHNH-Troc——Z(OMe)-Val-NHNH-Troc (10.21 g) was treated with TFA (15 ml) in the presence of anisole (5 ml) in an ice-bath for 40 min. The excess TFA was evaporated and the residue, after washing with *n*-hexane, was dissolved in AcOEt and basified with 5% sodium bicarbonate. The organic phase was separated, washed with H_2O–NaCl, dried over sodium sulfate and then filtered. This filtrate was combined with a solution of Z(OMe)-Phe-OH (7.24 g) in THF (30 ml). After addition of DCC (4.54 g), the mixture was stirred at room temperature for 24 hr and then filtered. The filtrate was condensed and the residue was dissolved in AcOEt, which was washed with 5% citric acid, 5% sodium bicarbonate and H_2O–NaCl, dried over sodium sulfate and then evaporated. The residue was triturated with ether and recrystallized from AcOEt and ether; yield 8.53 g (63%), mp 181—186°, $[\alpha]_D^{20}$ −16.5° (c=1.0, DMF), Rf_1 0.82. *Anal.* Calcd. for $C_{26}H_{31}O_7N_4Cl_3$: C, 50.53; H, 5.05; N, 9.06. Found: C, 50.61; H, 5.21; N, 9.10.

Z(OMe)-Asp(OBzl)-Phe-Val-NHNH-Troc——In the usual manner, Z(OMe)-Phe-Val-NHNH-Troc (7.53 g) was treated with TFA (10 ml) in the presence of anisole (3 ml) in an ice-bath for 40 min. The excess TFA was removed by evaporation and the residue, after washing with *n*-hexane, was dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (30 ml). Et_3N (1.6 ml), HOBT (1.65 g), Z(OMe)-Asp(OBzl)-ONP[22] (8.08 g) were added and the mixture was stirred at room temperature for 24 hr. The solvent was evaporated and the residue was triturated with ether. The resulting powder was washed batchwisely with 5% citric acid, 5% sodium bicarbonate and H_2O–NaCl and precipitated from DMF with ether; yield 8.06 g (82%), mp 192—195°, $[\alpha]_D^{20}$ −25.1° (c=0.9, DMF), Rf_1 0.91. *Anal.* Calcd. for $C_{37}H_{42}O_{10}N_5Cl_3$: C, 53.98; H, 5.14; N, 8.50. Found: C, 54.17; H, 5.09; N, 8.60.

Z(OMe)-Gln-Asp(OBzl)-Phe-Val-NHNH-Troc——In the usual manner, Z(OMe)-Asp(OBzl)-Phe-Val-NHNH-Troc (5.90 g) was treated with TFA (6 ml) in an ice-bath for 40 min. Addition of dry ether afforded a fine powder, which was collected by filtration and then dissolved in DMF (50 ml) containing Et_3N (2 ml). Z(OMe)-Gln-ONP[23] (4.74 g) and HOBT (0.97 g) were added and the mixture was stirred at room temperature for 24 hr. The solvent was evaporated *in vacuo* and the residue was treated with AcOEt. The resulting powder was washed batchwisely with 5% citric acid, 5% sodium bicarbonate and H_2O and then precipitated from DMF with AcOEt; yield 6.12 g (90%), mp 213—217°, $[\alpha]_D^{20}$ −24.3° (c=0.9, DMF), Rf_1 0.60. *Anal.* Calcd. for $C_{42}H_{50}O_{12}N_7Cl_3$: C, 53.02; H, 5.29; N, 10.30. Found: C, 52.93; H, 5.57; N, 10.39.

Z(OMe)-Gln-Gln-Asp(OBzl)-Phe-Val-NHNH-Troc——Z(OMe)-Gln-Asp(OBzl)-Phe-Val-NHNH-Troc (11.84 g) was treated with TFA (15 ml) in the presence of anisole (8 ml) as stated above and the resulting TFA salt isolated as a fine powder was dissolved in DMF (60 ml), to which Et_3N (3.5 ml), Z(OMe)-Gln-ONP (6.39 g) and HOBT (1.68 g) were added. The mixture was stirred at room temperature for 48 hr and the solvent was evaporated. Treatment of the residue with AcOEt afforded the solid, which after washing with 10% citric acid and H_2O, was precipitated from DMF with THF; yield 10.99 g (82%), mp 236—240°, $[\alpha]_D^{20}$ −22.7° (c=1.0, DMF), Rf_1 0.49. *Anal.* Calcd. for $C_{47}H_{58}O_{14}N_9Cl_3$: C, 52.30; H, 5.42; N, 11.68. Found: C, 52.56; H, 5.45; N, 11.87.

Z(OMe)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-NHNH-Troc——The above protected pentapeptide Troc-hydrazide (2.91 g) was treated with TFA (5.0 ml) in the presence of anisole (2.9 ml) as stated above. The resulting TFA salt isolated after addition of dry ether, was dissolved in ice-cold DMF (15 ml) containing Et_3N (0.4 ml). This solution was kept in an ice-bath until the following azide was ready. Z(OMe)-Lys(Z)-Ile-Arg(Tos)-$NHNH_2$[1] (2.86 g) was dissolved in DMF (25 ml) with an aid of 2.03 N HCl–DMF (3.45 ml). Isoamyl nitrite (0.49 ml) was added under cooling with ice-NaCl and the mixture was stirred for 5 min until the hydrazine test became negative. The solution was then neutralized with Et_3N (1.53 ml) and combined with the above solution containing H-Gln-Gln-Asp(OBzl)-Phe-Val-NHNH-Troc. The mixture, after stirring at 4° for 48 hr, was condensed *in vacuo* and the residue was treated with AcOEt. The resulting powder was washed batchwisely with 10% citric acid and H_2O and precipitated twice from DMF with MeOH; yield 3.91 g (82%), mp 245—250°, $[\alpha]_D^{20}$ −18.9° (c=1.0, DMSO), Rf_3 0.78. *Anal.* Calcd. for $C_{80}H_{105}O_{21}N_{16}SCl_3$: C, 54.43; H, 6.00; N, 12.70. Found: C, 54.71; H, 6.12; N, 12.64.

Z(OMe)-Asp(OBzl)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-NHNH-Troc——The above protected octapeptide Troc-hydrazide (1.77 g) was treated with TFA (3.0 ml) in the presence of anisole (2.2 ml) as stated above. After 60 min, dry ether was added and the resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in a mixture of DMF (20 ml) and DMSO (5 ml), to which

22) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **22**, 1061 (1974).
23) E. Schröder and E. Klieger, *Ann. Chem.*, **673**, 196 (1964).

Et_3N (0.28 ml), HOBT (0.14 g) and Z(OMe)-Asp(OBzl)-ONP (0.76 g) were added. The mixture was stirred at room temperature for 48 hr and then condensed *in vacuo*. Treatment of the residue with AcOEt afforded a fine powder, which was washed batchwisely with 10% citric acid and H_2O and then precipitated from DMF with AcOEt; yield 1.65 g (84%), mp 225—232°, $[\alpha]_D^{20}$ −19.8° (c=1.0, DMF), Rf_1 0.49. *Anal.* Calcd. for $C_{91}H_{116}O_{24}N_{17}SCl_3$: C, 55.47; H, 5.93; N, 12.09. Found: C, 55.50; H, 5.82; N, 12.04.

Z(OMe)-Asp(OBzl)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-NHNH$_2$——The above protected nonapeptide Troc-hydrazide (1.48 g) was dissolved in a mixture of DMF (20 ml), DMSO (20 ml) and AcOH (20 ml) and zinc dust (3.0 g) was added. The mixture was stirred at room temperature for 3 hr, meanwhile the progress of the reaction was pursued by thin-layer chromatography. The solution was filtered with an aid of filter-cell and the filtrate was condensed *in vacuo* to approximately 5 ml, to which 10% EDTA solution in 10% AcOH (100 ml) was added. The solution, after stirring for 20 min, was basified with sodium bicarbonate. The gelatinous mass formed was stored in a refrigerator overnight, collected by filtration, washed with H_2O and then precipitated from DMF with MeOH; yield 0.87 g (64%), mp 250—255°, $[\alpha]_D^{20}$ −24.8° (c=0.9, DMF), Rf_1 0.29, Rf_2 0.57. Amino acid ratios in an acid hydrolysate: Asp 2.14, Lys 0.85, Ile 1.00, Arg 0.86, Glu 1.96, Phe 1.06, Val 0.92 (average recovery 99%). *Anal.* Calcd. for $C_{88}H_{115}O_{22}N_{17}S$: C, 58.88; H, 6.46; N, 13.27. Found: C, 59.03; H, 6.69; N, 13.06.

Z(OMe)-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 25—43)-OH——The protected octadecapeptide, Z(OMe)-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH (2.91 g) was immersed well with anisole containing 2% ethanedithiol (5.5 ml) and TFA (5.5 ml) was added. The solution was stirred under N_2 gas in an ice-bath for 60 min, when dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF–DMSO (30 ml—10 ml), to which Et_3N (0.56 ml), HOBT (0.14 g) and Z(OMe)-Trp-ONP[24] (0.74 g) were combined. The mixture was stirred at room temperature overnight and then condensed *in vacuo*. The residue was treated with AcOEt and 3% AcOH and the resulting powder was washed batchwisely with 3% AcOH and H_2O and then precipitated twice from DMF with AcOEt; yield 2.35 g (76%), mp 155—160°, $[\alpha]_D^{20}$ −16.7° (c=1.0, DMSO), Rf_2 0.79. Amino acid ratios in 3 N Tos-OH hydrolysate: Trp 1.44, Leu 2.29, Ala 1.16, Glu 3.38, Lys 4.21, Gly 1.16, Ser 0.92, Asp 1.96, His 0.98, Ile 1.00, Thr 0.94 (average recovery 85%). *Anal.* Calcd. for, $C_{153}H_{203}O_{39}N_{31}$: C, 59.26; H, 6.60; N, 14.01. Found: C, 59.24; H, 6.69; N, 13.73.

Z(OMe)-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 24—43)-OH——Z(OMe)-(GIP 25—43)-OH (1.55 g) was treated with TFA (2.8 ml) in the presence of anisole (2.8 ml) containing 2% ethanedithiol as stated above and dry ether was added. The resulting powder was collected, washed with ether, dried over KOH pellets *in vacuo* and then dissolved in DMF–DMSO (20 ml—10 ml). Et_3N (0.28 ml), HOBT (68 mg) and Z(OMe)-Asn-ONP[25] (0.42 g) were combined and the mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was treated with AcOEt and 3% AcOH. The resulting powder was washed batchwisely as stated above and then precipitated from DMF with MeOH; yield 0.81 g (51%), mp 260—266°, $[\alpha]_D^{20}$ −23.8° (c=1.0, DMSO), Rf_1 0.07, Rf_2 0.81. Amino acid ratios in 3 N Tos-OH hydrolysate: Asp 3.10, Trp 1.24, Leu 2.24, Ala 1.18, Glu 3.26, Lys 4.47, Gly 1.20, Ser 0.93, His 0.88, Ile 1.00, Thr 0.93 (average recovery 83%). *Anal.* Calcd. for $C_{157}H_{209}O_{41}N_{33}\cdot 3H_2O$: C, 57.69; H, 6.63; N, 14.14. Found: C, 57.48; H, 6.56; N, 13.84.

Z(OMe)-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 22—43)-OH——Z(OMe)-(GIP 24—43)-OH (0.81 g) was treated with TFA (1.4 ml) in the presence of anisole (1.4 ml) containing 2% ethanedithiol as stated above and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMSO–DMF (7 ml—8 ml) containing Et_3N (0.11 ml). Z(OMe)-Phe-Val-NHNH$_2$ (0.22 g) was dissolved in DMF (5 ml) and under cooling with ice–NaCl, 3.13 N HCl–DMF (0.35 ml) and isoamyl nitrite (0.07 ml) were added consecutively. The solution was stirred for 5 min, until the hydrazine test became negative. The solution was then neutralized with Et_3N (0.23 ml) and then combined with the above solution containing eicosapeptide. The mixture was stirred at 4° for 48 hr and then condensed *in vacuo*. Treatment of the residue with AcOEt and 3% AcOH afforded a fine powder, which was purified as mentioned above; yield 0.72 g (83%), mp 250—254°, $[\alpha]_D^{20}$ −14.4° (c=0.9, DMSO), Rf_1 0.07, Rf_2 0.81. Amino acid ratios in 3 N Tos-OH hydrolysate: Phe 1.39, Val 1.00, Asp 3.00, Trp 1.47, Leu 2.18, Ala 1.23, Glu 3.37, Lys 4.20, Gly 1.23, Ser 0.90, His 0.75, Ile 0.89, Thr 0.88 (average recovery 80%). *Anal.* Calcd. for $C_{171}H_{227}O_{43}N_{35}\cdot 6H_2O$: C, 57.54; H, 6.75; N, 13.74. Found: C, 57.70; H, 6.67; N, 13.31.

Z(OMe)-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 21—43)-OH——Z(OMe)-(GIP 22—43)-OH (0.96 g) was treated with TFA (1.5 ml) in the presence of anisole (1.5 ml) containing 2% ethanedithiol as stated above. The resulting TFA salt precipitated by dry ether as a fine powder, was dried over KOH pellets *in vacuo* and then dissolved in DMF–DMSO (5 ml—5 ml), to which Et_3N (0.15 ml), HOBT (40 mg) and Z(OMe)-Asp(OBzl)-

24) N. Fujii and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **23**, 1596 (1975).
25) E. Schröder and E. Klieger, *Ann. Chem.*, **673**, 208 (1964).

ONP (0.25 g) were combined. The mixture, after stirring at room temperature for 24 hr, was condensed *in vacuo* and the residue was treated with AcOEt and 3% AcOH. The resulting powder was washed batchwisely as stated above and then precipitated twice from DMF with AcOEt; yield 0.75 g (74%), mp 243—245°, $[\alpha]_D^{20}$ −17.9° (c=1.0, DMSO), Rf_1 0.17. Amino acid ratios in 3 N Tos-OH hydrolysate: Asp 4.35, Phe 1.44, Val 1.00, Trp 1.51, Leu 2.39, Ala 1.31, Glu 3.46, Lys 4.06, Gly 1.28, Ser 0.81, His 0.78, Ile 0.84, Thr 0.80 (average recovery 85%). *Anal.* Calcd. for $C_{182}H_{238}O_{46}N_{36}\cdot 3H_2O$: C, 58.75; H, 6.61; N, 13.55. Found: C, 59.10; H, 6.54; N, 13.20.

Z(OMe)-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 20—43)-OH——Z(OMe)-(GIP 21—43)-OH (0.75 g) was treated with TFA (1.1 ml) in the presence of anisole (1.1 ml) containing 2% ethanedithiol as stated above. The TFA salt precipitated by dry ether as a fine powder, was dried over KOH pellets *in vacuo* and then dissolved in DMF–DMSO (5 ml—3 ml), to which Et_3N (0.12 ml), HOBT (30 ml) and Z(OMe)-Gln-ONP (0.16 g) were combined. The mixture, after stirring at room temperature for 24 hr, was condensed *in vacuo* and the residue was treated with ether. The resulting powder was washed batchwisely as stated above and then precipitated twice from DMF with a mixture of MeOH and AcOEt (1: 1); yield 0.56 g (74%), mp 231—234°, $[\alpha]_D^{20}$ −13.7° (c=1.0, DMSO), Rf_2 0.70. Amino acid ratios in 3 N Tos-OH hydrolysate: Glu 4.47, Asp 3.75, Phe 1.27, Val 1.00, Trp 1.39, Leu 2.27, Ala 1.24, Lys 3.35, Gly 1.16, Ser 0.79, His 0.81, Ile 0.92, Thr 0.96 (average recovery 79%). *Anal.* Calcd. for $C_{187}H_{246}O_{48}N_{38}\cdot 3H_2O$: C, 58.36; H, 6.60; N, 13.83. Found: C, 58.43; H, 6.57; N, 13.39.

Z(OMe)-Gln-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 19—43)-OH——Z(OMe)-(GIP 20—43)-OH (0.52 g) was treated with TFA (0.75 ml) in the presence of anisole (0.75 ml) containing 2% ethanedithiol as stated above. The resulting TFA salt, after drying over KOH pellets *in vacuo* for 3 hr, was dissolved in DMF–DMSO (3 ml—2 ml), to which Et_3N (0.12 ml), HOBT (20 mg) and Z(OMe)-Gln-ONP (0.11 g) were combined. The solution was stirred at room temperature for 48 hr and then condensed *in vacuo*. The residue was treated with AcOEt. The resulting powder was washed batchwisely as stated above and then precipitated twice from DMF with THF; yield 0.41 g (76%), mp 230—233°, $[\alpha]_D^{20}$ −26.3° (c=0.9, DMSO), Rf_1 0.25. Amino acid ratios in 3 N Tos-OH hydrolysate: Glu 5.29, Asp 4.17, Phe 1.27, Val 1.00, Trp 1.24, Leu 1.00, Ala 1.29, Lys 4.41, Gly 1.37, Ser 0.96, His 0.84, Ile 1.00, Thr 0.91 (average recovery 89%). *Anal.* Calcd. for $C_{192}H_{254}O_{50}N_{40}\cdot 2H_2O$: C, 58.26; H, 6.57; N, 14.16. Found: C, 58.12; H, 6.53; N, 13.94.

Z(OMe)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 16—43)-OH——Z(OMe)-(GIP 19—43)-OH (0.41 g) was treated with TFA (0.6 ml) in the presence of anisole (0.6 ml) containing 2% ethanedithiol as stated above. The resulting TFA salt, after drying over KOH pellets *in vacuo* for 3 hr, was dissolved in DMF–DMSO (2 ml—1 ml). Et_3N (0.04 ml) and the azide (derived from 0.18 g of Z(OMe)-Lys(Z)-Ile-Arg(Tos)-$NHNH_2$[1]) with 0.14 ml of 3.13 N HCl–DMF, 0.03 ml of isoamyl nitrite and 0.09 ml of Et_3N) in DMF (3 ml) were combined and the solution, after stirring at 4° for 48 hr, was condensed *in vacuo*. The product was isolated as stated above by batchwise washing followed by precipitation from DMF with AcOEt; yield 0.34 (71%), mp 240—243°, $[\alpha]_D^{25}$ −16.6° (c=0.3, DMSO), Rf_1 0.07, Rf_2 0.77. Amino acid ratios in 3 N Tos-OH hydrolysate: Lys 4.52, Ile 1.98, Arg 0.73, Glu 5.23, Asp 3.83, Phe 0.82, Val 1.28, Trp 1.43, Leu 2.05, Ala 1.00, Gly 1.11, Ser 1.00, His 0.85, Thr 1.08 (average recovery 77%). *Anal.* Calcd. for $C_{225}H_{301}O_{57}N_{47}S\cdot 6H_2O$: C, 57.29; H, 6.68; N, 13.95. Found: C, 57.06; H, 6.77; N, 14.26.

Z(OMe)-Asp(OBzl)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 15—43)-OH——(a) Z(OMe)-(GIP 16—43)-OH (0.33 g) was treated with TFA (0.5 ml) in the presence of anisole (0.5 ml) containing 2% ethanedithiol as stated above. The resulting TFA salt, after drying over KOH pellets *in vacuo* for 3 hr, was dissolved in DMF–DMSO (1 ml—1 ml), to which Et_3N (0.04 ml), HOBT (20 mg) and Z(OMe)-Asp(OBzl)-ONP (0.11 g) were added. The mixture was stirred at room temperature for 72 hr and then condensed *in vacuo*. The product was isolated as stated above by batchwise washing followed by precipitation from DMF with AcOEt; yield 0.24 g (69%), mp 213—217°, $[\alpha]_D^{25}$ −22.7° (c=1.0, DMSO), Rf_1 0.60, Rf_2 0.73. Amino acid ratios in 3 N Tos-OH hydrolysate: Asp 4.53, Lys 4.63, Ile 1.75, Arg not det. Glu 5.45, Phe 1.10, Val 0.78, Trp 1.23, Leu 1.82, Ala 1.00, Gly 1.11, Ser 0.88, His 0.70, Thr 0.87 (average recovery 88%). *Anal.* Calcd. for $C_{236}H_{312}O_{60}N_{48}S\cdot 12H_2O$: C, 56.35; H, 6.73; N, 13.37. Found: C, 56.37; H, 6.42; N, 13.04.

(b) Z(OMe)-(GIP 24—43)-OH (1.61 g) was treated with TFA (3.0 ml) in the presence of anisole (3.0 ml) containing 2% ethanedithiol as stated above. The resulting TFA salt was dissolved in DMF–DMSO (10 ml—5 ml) containing Et_3N (0.21 ml). To this ice-cold solution, the azide (prepared from 1.35 g of Z(OMe)-Asp(OBzl)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-$NHNH_2$ with 0.80 ml of 2.03 N HCl–DMF, 1.12 ml of 10% isoamyl nitrite solution in DMF and 0.34 ml of Et_3N) in DMF–DMSO (8 ml—8 ml) was combined. The solution was stirred at 4° for 48 hr and then an additional azide (0.5 equivalent) was added and stirring was continued for an additional 24 hr. The solution, after neutralization with AcOH, was condensed *in vacuo* and the residue was treated with AcOEt and 3% AcOH. The resulting powder was washed batchwisely with 3% AcOH and H_2O and then dissolved in a small amount of a mixture of DMF–DMSO (1: 1, v/v) and the solution was applied to a column of Sephadex LH-20 (3.0×140 cm) which was eluted with the same solvent sys-

tem. Individual fractions (5 ml each) were collected and absorbancy at 280 mμ was determined. The fractions corresponding to the front main peak were collected (tube No. 56—69) and the solvent was evaporated *in vacuo*. Treatment of the residue with AcOEt afforded a fine powder, which was washed with ether; yield 1.33 g (55%), $[\alpha]_D^{20}$ −22.7° (c=1.0, DMSO), Rf_2 0.75. Amino acid ratios in 3 N Tos-OH hydrolysate: Asp 4.82, Lys 4.91, Arg not det., Glu 5.36, Phe 1.25, Val 0.71, Trp 1.44, Leu 2.18, Ala 1.19, Gly 1.26, Ser 1.00, His 0.71, Thr 0.96 (average recovery 85%).

Z(OMe)-Tyr-Ser-Ile-Ala-Met-Asp(OBzl)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 10—43)-OH——Z(OMe)-(GIP 15—43)-OH (1.08 g) was treated with TFA (2.5 ml) in the presence of anisole (2.4 ml) containing 2% ethanedithiol as stated above and the resulting TFA salt, after drying over KOH pellets *in vacuo* for 3 hr, was dissolved in DMF–DMSO (3 ml—2 ml). Et_3N (0.1 ml) and the azide (prepared from 0.26 g of Z(OMe)-Tyr-Ser-Ile-Ala-Met-$NHNH_2$[1] with 0.36 ml of 2.03 N HCl–DMF, 0.05 ml of isoamyl nitrite and 0.16 ml of Et_3N) in DMF (3 ml) were added. The mixture, after stirring at 4° for 48 hr, was condensed *in vacuo*. The product was isolated by batchwise washing with 3% AcOH and H_2O followed by precipitation from DMF with MeOH; yield 0.71 g (60%), mp 246—250°, $[\alpha]_D^{25}$ −8.4° (c=0.3, DMF), Rf_1 0.61. Amino acid ratios in 3 N Tos-OH hydrolysate: Tyr 1.11, Ser 1.94, Ile 3.29, Ala 2.35, Met 1.09, Asp 5.04, Lys 4.90, Arg not det., Glu 5.27, Phe 1.00, Val 0.73, Trp 1.39, Leu 2.38, Gly 1.14, His 0.82, Thr 0.78 (average recovery 95%). *Anal.* Calcd. for $C_{262}H_{351}O_{67}N_{53}S_2 \cdot 4H_2O$: C, 57.72; H, 6.64; N, 13.62. Found: C, 57.45; H, 6.76; N, 13.88.

Z(OMe)-Asp(OBzl)-Tyr-Ser-Ile-Ala-Met-Asp(OBzl)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 9—43)-OH——Z(OMe)-(GIP 10—43)-OH (0.71 g) was treated with TFA (1.6 ml) in the presence of anisole (1.5 ml) containing 2% ethanedithiol as stated above. The resulting TFA salt, after drying over KOH pellets *in vacuo* for 3 hr, was dissolved in DMF-DMSO (3 ml—2 ml), to which Et_3N (0.07 ml), HOBT (36 mg) and Z(OMe)-Asp(OBzl)-ONP (0.20 g) were combined. The mixture was stirred at room temperature for 48 hr and the solvent was evaporated *in vacuo*. The product was isolated as mentioned above by batchwise washing followed by precipitation from DMF with ethanol; yield 0.60 g (81%), mp 230—233°, $[\alpha]_D^{20}$ −18.8° (c=0.9, DMSO), Rf_1 0.66. Amino acid ratios in 3 N Tos-OH hydrolysate: Asp 6.14, Tyr 0.75, Ser 1.78, Ile 2.72, Ala 2.35, Met 0.68, Lys 5.05, Arg not det., Glu 5.39, Phe 1.05, Val 1.00, Trp 1.33, Leu 2.51, Gly 1.38, His 0.77, Thr 0.90 (average recovery 86%). *Anal.* Calcd. for $C_{273}H_{362}O_{70}N_{54}S_2 \cdot 9H_2O$: C, 57.06; H, 6.67; N, 13.16. Found: C, 57.33; H, 6.48; N, 12.86.

Z(OMe)-Tyr-Ala-Glu(OBu^t)-Gly-Thr-Phe-Ile-Ser-Asp(OBzl)-Tyr-Ser-Ile-Ala-Met-Asp(OBzl)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 1—43)-OH——Z(OMe)-(GIP 9—43)-OH (0.60 g) was treated with TFA (1.5 ml) in the presence of anisole (1.2 ml) containing 2% ethanedithiol as stated above and the resulting TFA salt, after drying over KOH pellets *in vacuo* for 3 hr, was dissolved in DMF–DMSO (2 ml—1 ml). Et_3N (0.05 ml) and the azide (prepared from 0.18 g of Z(OMe)-Tyr-Ala-Glu(OBu^t)-Gly-Thr-Phe-Ile-Ser-$NHNH_2$, 0.1 ml of 2.98 N HCl–DMF, 0.02 ml of isoamyl nitrite and 0.07 ml of Et_3N) in DMF–DMSO (1 ml—1 ml) were added. The mixture was stirred at 4° for 48 hr and the solvent was evaporated. Treatment of the residue with 3% AcOH and AcOEt afforded a fine powder, which after washing with 3% AcOH and H_2O, was dissolved in a small amount of DMSO. The solution was applied to a column of Sephadex LH-20 (3.0×140 cm), which was eluted with DMSO. Individual fractions (5 ml each) were collected and absorbancy at 280 mμ was determined. Fractions corresponding to the front main peak (tube No. 65—70) were combined and the solvent, after addition of a few drops of ethanedithiol, was evaporated *in vacuo*. Treatment of the residue with AcOEt afforded a fine powder; yield 0.32 g (46%), mp 230—243°, $[\alpha]_D^{20}$ −15.6° (c=0.4, DMSO), Rf_2 0.82. Amino acid ratios in 3 N Tos-OH hydrolysate: Tyr 1.48, Ala 2.85, Glu 6.20, Gly 2.35, Thr 2.00, Phe 1.67, Ile 3.68, Ser 2.33, Asp 6.40, Met 0.57, Lys 5.25, Arg not det., Val 1.00, Trp 1.25, Leu 2.41, His 0.68 (average recovery 92%). *Anal.* Calcd. for $C_{318}H_{426}O_{83}N_{62}S_2 \cdot 8H_2O$: C, 57.40; H, 6.70; N, 13.05. Found: C, 57.30; H, 6.71; N, 13.08.

H-Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-Arg-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys-Gly-Lys-Lys-Ser-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln-OH, H-(GIP 1—43)-OH——Z(OMe)-(GIP 1—43)-OH (100 mg) was treated with HF (approximately 5 ml) in the presence of anisole (2 ml) containing 2% ethanedithiol and skatole (80 mg) in an ice-bath for 60 min. The excess HF was evaporated under reduced pressure at 0° and dry ether was added. The resulting powder was collected by filtration and dissolved in a small amount of H_2O, which was treated with Amberlite CG-400 (acetate form approximately 3 g) for 30 min. The resin was removed by filtration and the filtrate was lyophilized to give a fluffy powder; yield 76 mg. This powder was then dissolved in a small amount of 0.2 M AcOH and the solution was applied to a column of Sephadex G-25 (1.8×140 cm), which was eluted with the same solvent. Individual fractions (4 ml each) were collected and absorbancy at 280 mμ was detected. Fractions corresponding to the front main peak (tube No. 35—46, Fig.3-a) were collected and the solvent was removed by lyophilization to give a fluffy white powder; yield 30 mg (deblocking step 40%). For further purification, the product (20 mg) was dissolved in H_2O (2 ml) and the solution was applied to a column of CM-cellulose (2.4×1.5 cm), which was eluted with 0.01 M NH_4HCO_3 (pH 7.8). Individual fractions (5 ml each) were collected and absorbancy at 280 mμ

was determined. Fractions corresponding to the main peak (tube No. 15—24, Fig. 3-b) were collected and the solvent was removed by lyophilization. For desalting, the residue was dissolved in a small amount of H_2O and the solution was applied to a column of Sephadex G-25 (1.8 × 140 cm), which was eluted with 0.2 M AcOH. The desired fractions were collected as described above and the product was finally lyophilized as a fluffy white powder; yield 12 mg (CMC purification step 60%). Rf_4 0.54, Rf_5 0.77, $[\alpha]_D^{25}$ −23.7° (c=0.3, H_2O). Disc electrophoresis mobility on 15% polyacrylamide gel (0.5 × 5.5 cm, 5 mA/tube) at pH 2.3 (0.37 M glycine buffer) was 0.6 cm toward the cathode after 120 min (stained by coomassie brilliant blue). Amino acid ratios in 3 N Tos-OH hydrolysate: Tyr 1.65, Ala 3.16, Glu 6.53, Gly 2.14, Thr 1.97, Phe 2.13, Ile 3.71, Ser 2.48, Asp 6.46, Met 0.63, Lys 5.60, Arg 0.85, Val 1.00, Trp 1.20, Leu 2.37, His 0.79 (average recovery 93%). Amino acid ratios in AP-M digest (numbers in parentheses indicate the theory): Tyr 1.70 (2), Ala 2.97 (3), Glu 0.99 (1), Gly 2.24 (2), Phe 1.66 (2), Ile 4.02 (4), Asp 3.60 (4), Met 0.64 (1), Lys 5.43 (5), Arg 0.93 (1), Val 1.00 (1), Trp 1.63 (2), Leu 2.15 (2), His 0.90 (1), Gln+Thr 6.58 (5+2, calcd. as Thr), Asn+Ser 4.45 (2+3 calcd. as Ser) (average recovery 82%). *Anal.* Calcd. for $C_{230}H_{350}O_{68}N_{62}S \cdot 9AcOH \cdot 35H_2O$: C, 47.47; H, 7.32; N, 13.84. Found: C, 47.17; H, 6.81; N, 13.56.

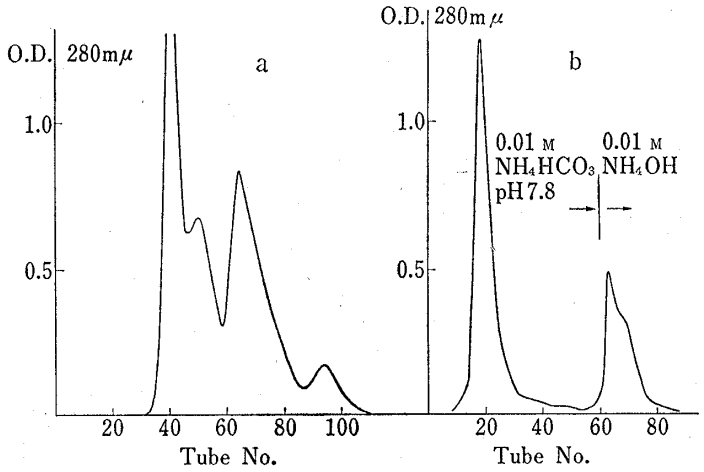


Fig. 3-a. Purification of Synthetic GIP on Sephadex G-25

Column: 1.8×140 cm
Fraction: 4.0 ml each
Eluate 0.2 M AcOH

Fig. 3-b. Purification of Synthetic GIP on CM-cellulose

Column: 1.8×4.5 cm
Fraction: 5.0 ml each
flow rate: 80 ml/hr

When the above CM-cellulose column was further eluted with 0.01 M NH_4OH, some impure material was obtained (6 mg), which was not further examined.

H-Asp-Lys-Ile-Arg-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys-Gly-Lys-Lys-Ser-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln-OH, H-(GIP 15—43)-OH——The protected nonacosapeptide, Z(OMe)-(GIP 15—43)-OH(96 mg) was treated with HF (approximately 5 ml) in the presence of anisole containing 2% ethanedithiol and skatole (50 mg) in an ice-bath for 60 min to remove all protecting groups. The excess HF was removed by evaporation at 0°. Dry ether was added and the resulting powder was collected by filtration, dissolved in H_2O and the solution was treated with Amberlite CG-4B (acetate form, approximately 1 g) for 30 min. The resin was removed by filtration and the filtrate was lyophilized. The resulting powder was dissolved in a small amount of 0.2 M AcOH (2 ml) and the solution was applied to a column of Sephadex G-25 (3.0 × 140 cm), which was eluted with the same solvent. Individual fractions (5 ml each) were collected and absorbancy at 280 mμ was determined. Fractions corresponding to the front main peak (tube No. 67—107) were collected and the solvent was removed by lyophilization to give a fluffy white powder; yield 23 mg (32%). Thin-layer chromatographically pure looking sample was obtained. Rf_3 0.50, Rf_5 0.48. Amino acid ratios in 3 N Tos-OH hydrolysate: Asp 5.04, Lys 4.81, Ile 1.80, Arg 0.92, Glu 5.42, Phe 1.00, Val 0.84, Trp 1.40, Leu 2.10, Ala 1.13, Gly 0.97, Ser 0.85, His 0.65, Thr 0.78 (average recovery 86%).

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