[Chem. Pharm. Bull. 24(10)2428—2434(1976)]

UDC 547.466.1.057.09 : 615.31.076.9

Studies on Peptides. LXII.[1-3] Synthesis of the Protected Octadecapeptide corresponding to Positions 26 through 43 of Porcine Gastric Inhibitory Polypeptide (GIP)

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(Received January 26, 1976)

The protected octadecapeptide corresponding to positions 26 through 43 of porcine gastric inhibitory polypeptide was synthesized by a conventional method. In this deprotected peptide, any significant inhibitory effect against the gastric acid secretion stimulated by histamine hydrochloride was not noted in heidenhein pouch dogs.

Brown, *et al.*[5] isolated, from porcine duodenal mucosa, a peptide with the inhibitory property against the gastric acid secretion and succeeded in elucidating its complete amino acid sequence in 1971.[6] This peptide, termed as gastric inhibitory polypeptide (GIP), consists of 43 amino acid residues in a straight chain. Structural similarity was soon realized between the N-terminal portion of GIP and other candidate hormones of the gut.[7] Positions of 15 amino acid residues in GIP were identical with those of glucagon[8] and 9 with those of secretin.[9] The latter is known to possess the similar chain length with more structural similarity to vasoactive intestinal polypeptide (VIP).[10] In addition to the gastric inhibitory activity mentioned above, two other characteristic physiological effects are supposed to be endowed within this tritetracontapeptide sequence; *i.e.*, inhibitory effect on motor activity in pouch of the body and antrum of the stomach (enterogastron effect) and release of insulin (incretin effect). As far as synthetic studies of GIP are concerned, only partial syntheses have been reported to date by Cambel[11] and Wünsch, *et al.*[12]

We have synthesized the tritetracontapeptide corresponding to the entire amino acid sequence of GIP and examined preliminary physiological properties of this synthetic peptide and some intermediates. In the following three consecutive papers, we wish to describe the

1) Preliminary communication of this paper: H. Yajima, H. Ogawa, M. Kubota, T. Tobe, M. Fujimura, K. Henmi, K. Torizuka, H. Adachi, I. Imura, and T. Taminato, *J. Am. Chem. Soc.*, **97**, 5593 (1975).
2) Part LXI: K. Koyama, H. Watanabe, H. Kawatani, Z. Iwai, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **24**, 2558 (1976).
3) Amino acids, peptides and their derivatives mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by IUPAC-I UB Commission of Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966), *ibid.*, **6**, 362 (1967), *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Tos=*p*-toluenesulfonyl, OBzl=benzyl ester, ONP=*p*-nitrophenyl ester, OPCP=pentachlorophenyl ester, OQCl=5-chloro-8-quinolyl ester, DCC=dicyclohexylcarbodiimide, TFA=trifluoroacetic acid, DMF=dimethylformamide, DMSO=dimethylsulfoxide.
4) Location: *Sakyo-ku, Kyoto, 606, Japan.*
5) J.C. Brown, V. Mutt, and R.A. Pederson, *J. Physiol.*, **209**, 57 (1970).
6) J.C. Brown, *Can. J. Biochem.*, **49**, 255 (1971); J.C. Brown, and J.R. Dryburgh, *Can. J. Biochem.*, **49**, 867 (1971).
7) M.I. Grossman, *Gastroenterology*, **67**, 730 (1974).
8) W.W. Bromer, G.L. Sinn, and O.K. Behrens, *J. Am. Chem. Soc.*, **79**, 2807 (1957).
9) V. Mutt, E.J. Jorpes, and S. Magnusson, *Eur. J. Biochem.*, **5**, 513 (1970).
10) V. Mutt and S.I. Said, *Eur. J. Biochem.*, **42**, 581 (1974).
11) R. Camble, "Chemistry and Biology of Peptides," ed. by J. Meienhofer, Ann. Arbor Science Pub., Michigan, U.S., 1972, p. 382.
12) K. Kovacs, J.K. Petres, G. Wendelberger, and E. Wünsch, *Z. Physiol. Chem.*, **354**, 890 (1973); J.K. Petres, K. Kovacs, K.H. Deimer, and E. Wünsch, *ibid.*, **354**, 895 (1973).

detailed account of our synthetic studies related to GIP, which may cast some light on the structure-activity relationship of this important upper intestinal polypeptide. In the first paper, synthesis of the C-terminal octadecapeptide (position 26—43) was described.

Synthetic strategy adopted for the total synthesis of GIP is essentially the same as that employed for our previous synthesis of another intestinal polypeptide, motilin,[13] since both compounds possess the Lys and Met residues. However, two residues of Trp, sensitive to acids, are present in GIP, but not in the latter. Special care was, therefore, undertaken to prevent destruction of the Trp residue throughout the present synthesis.

The α-amino function of intermediates was protected by the TFA labile Z(OMe) group.[14] Amino acid derivatives bearing protecting groups removable by hydrogen fluoride[15] were employed, *i.e.*, Lys(Z) and Asp(OBzl) in the synthesis of the C-terminal octadecapeptide and in addition, Arg(Tos) in the latter stage. These protecting groups survive mostly intact under the limited TFA treatment.

In order to prevent destruction of the Trp residue during the various TFA deblocking steps, anisole containing 2% ethanedithiol was employed. Previously, Marshall[16] mentioned that destruction of Trp during the deblocking of the Boc group by TFA or hydrochloric acid could be reduced by the use of anisole containing mercaptoethanol and we also confirmed his observation.[17] Later, Kamen, *et al.*[18] recommended, in the solid phase synthesis of lysozyme, the use of a mixture of anisole and ethanedithiol rather than mercaptoethanol. Indeed, when Z(OMe)–Trp–OH immersed well with a stocked solution of anisole containing 2% ethanedithiol was exposed to the action of TFA under nitrogen gas in an ice-bath for

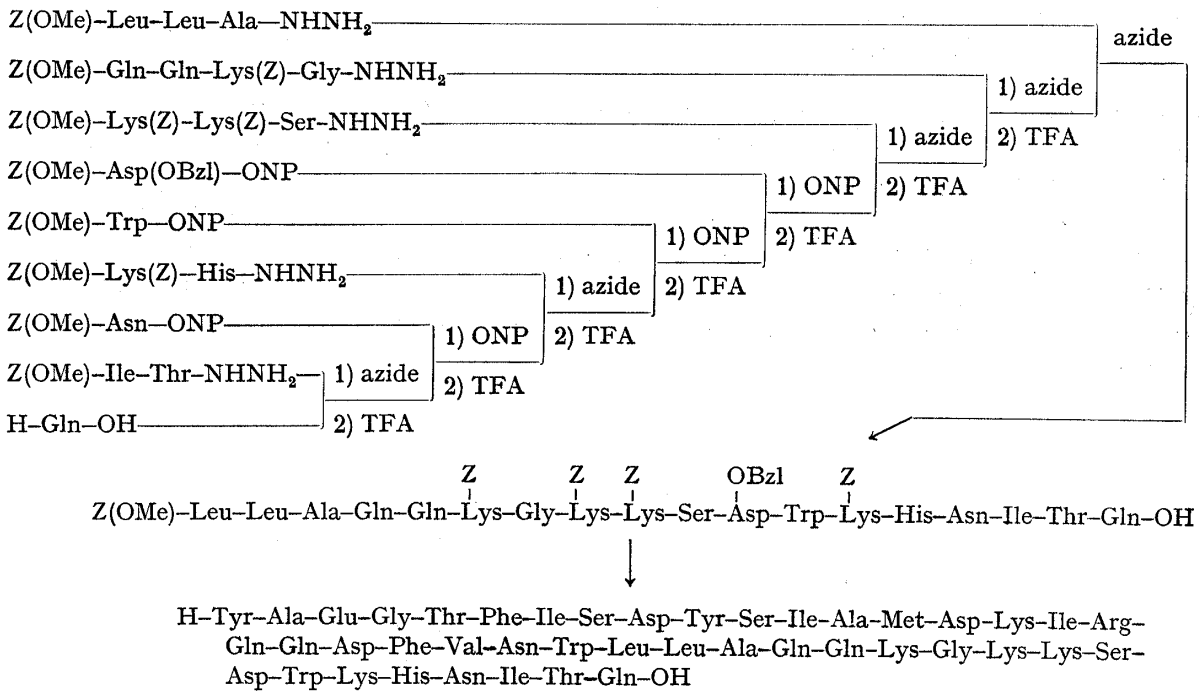

Fig. 1. Synthetic Route to the Protected Octadecapeptide, Z(OMe)–(GIP 26—43)–OH

13) H. Yajima, Y. Kai, and H. Kawatani, *J. C. S. Chem. Commun.*, **1975**, 159.
14) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962); H. Yajima, F. Tamura, and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **18**, 2574 (1970), H. Yajima, F. Tamura, Y. Kiso, and M. Kurobe, *ibid.*, **21**, 1380 (1973).
15) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
16) G.R. Marshall, *Advan. Exp. Med.*, **2**, 48 (1969).
17) H. Yajima, H. Kawatani, and H. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **18**, 1333 (1970).
18) J.J. Shar, A.B. Robinson, and M.D. Kamen, *J. Am. Chem. Soc.*, **95**, 6097 (1973).

120 minutes or even more longer period, no brown color was produced. Thus, the TFA treatment for 45 to 60 minutes under these conditions was selected for the removal of the Z(OMe) group from Trp-containing intermediates throughout this synthesis and the Trp content of synthetic peptides was estimated in 3N Tos–OH hydrolysates.

Along the line of strategy mentioned above, synthesis of the protected octadecapeptide corresponding to positions 26 through 43 of GIP was carried out as illustrated in Fig. 1. Five peptide fragments served as the building blocks for construction of the C-terminal portion of GIP, *i.e.*, I (41—42), II (38—39), III (33—35), IV (29—32) and V (26—28).

Z(OMe)–Ile–Thr–$NHNH_2$ (I) and Z(OMe)–Lys(Z)–His–$NHNH_2$ (II) were prepared by the DCC condensation[19] of the respective amino acid derivatives followed by exposure the resulting esters to hydrazine hydrate. In the latter case, the crude protected dipeptide ester, Z(OMe)–Lys(Z)–His–OMe, was exposed to methanol containing acetic acid to remove the contaminating dicyclohexylamidino derivative as mentioned by Rink and Riniker.[20] In order to prepare the protected tripeptide hydrazide, Z(OMe)–Lys(Z)–Lys(Z)–Ser–$NHNH_2$ (III), Z(OMe)–Lys(Z)–OH was first condensed as illustrated in Fig. 2, with H–Ser–OMe by the DCC procedure to give Z(OMe)–Lys(Z)–Ser–OMe, which after treatment with TFA, was next condensed with Z(OMe)–Lys(Z)–OH by the 5-chloro-8-quinolyl ester procedure according to Jakubuke,[21] since this active ester is a nice crystalline compound and 5-chloro-8-hydroxyquinoline, liberated after the condensation reaction, could be easily removed by washing with dilute hydrochloric acid.

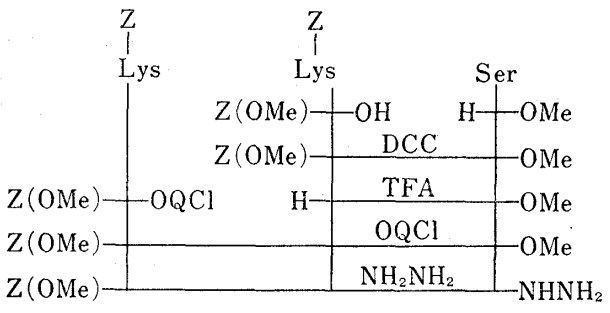

Fig. 2. Synthetic Scheme of the Protected Tripeptide Hydrazide, Z(OMe)-(GIP 33—35)-$NHNH_2$

For the synthesis of the protected tetrapeptide hydrazide, Z(OMe)–Gln–Gln–Lys(Z)–Gly–$NHNH_2$ (IV), Z(OMe)–Lys(Z)–OH was introduced by means of DCC and two residues of Z(OMe)–Gln–OH in a stepwise manner by the *p*-nitrophenyl ester procedure.[22] The resulting protected tetrapeptide ester was converted to the corresponding hydrazide (IV) by the usual hydrazine treatment as shown in Fig. 3.

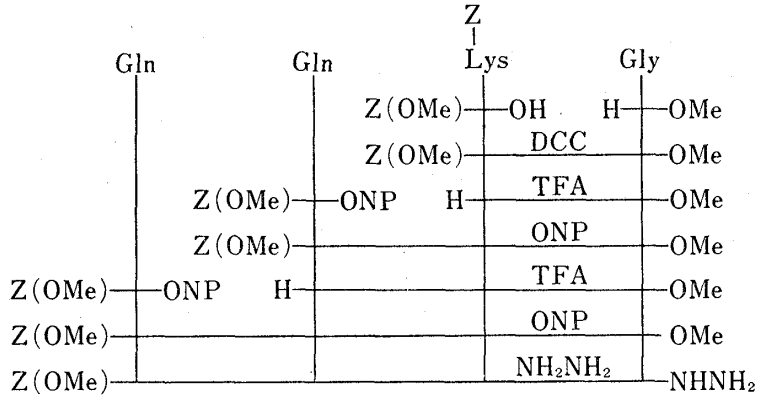

Fig. 3. Synthetic Scheme of the Protected Tetrapeptide Hydrazide, Z(OMe)-(GIP 29—32)-$NHNH_2$

19) J.C. Sheehan and G.P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
20) H. Rink and B. Riniker, *Helv. Chim. Acta*, **57**, 831 (1974).
21) H.D. Jakubuke and A. Voigt, *Chem. Ber.*, **99**, 2944 (1966).
22) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

The tripeptide fragment, Z(OMe)–Leu–Leu–Ala–$NHNH_2$ (V) was also prepared by the stepwise chain elongation method starting with H–Ala–OMe. The first residue of Z(OMe)–Leu–OH was introduced by the DCC procedure and the 2nd residue by the pentachlorophenyl ester procedure.[23] The resulting tripeptide ester was treated with hydrazine hydrate as stated above. This scheme is illustrated in Fig. 4.

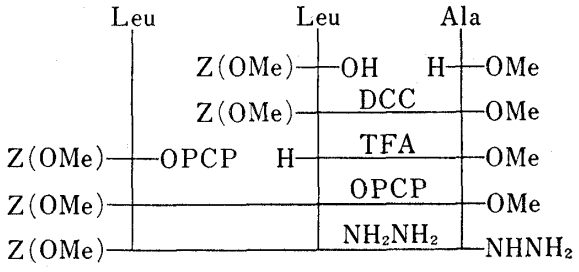

Fig. 4. Synthetic Scheme of the Protected Tripeptide Hydrazide, Z(OMe)–(GIP 26—28)–$NHNH_2$

For construction of the entire amino acid sequence of the octadecapeptide, five fragments thus synthesized were assembled by the azide procedure[24] to minimize racemization and three amino acid residues, Z(OMe)–Asn–OH, Z(OMe)–Trp–OH, and Z-(OMe)–Asp(OBzl)–OH, were introduced by the stepwise *p*-nitrophenyl ester procedure. Poor solubility in DMF prompted the use of a mixture of DMF and DMSO for acylation beyond the octapeptide stage. After introduction of the Trp residue (position 37), the Z(OMe) group was selectively removed by TFA in the presence of anisole containing 2% ethanedithiol as stated above. No brown color was produced during the necessary TFA treatment as observed in a model experiment. Since we selected relatively small acylating compounds, they could be readily removed by washing or precipitation following each coupling step. Batchwise washing with dilute acetic acid and water followed by repeated precipitation from DMF or a mixture of DMF and DMSO with methanol were efficient enough to purify every intermediate, as well as the final 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. Purity of synthetic peptides was assessed by thin-layer chromatography, elemental analysis and amino acid analysis of 3N Tos–OH hydrolysates. The well characterized protected octadecapeptide thus synthesized served, after deprotection of the Z(OMe) group, as an amino component for the total synthesis of GIP, which we will describe in the latter paper.

In order to obtain some information about the chain length-activity relationship of GIP, a part of the protected octadecapeptide was treated with trifluoromethanesulphonic acid to remove all protecting groups.[25] Though the inhibitory action of this partially purified peptide (4 μg/kg/hr) was tested against gastric acid secretion stimulated by histamine hydrochloride (10 μg/kg/hr) in heidenhein pouch dogs,[26] any reproducible and significant results were not obtained. The result indicated that further chain elongation is necessary to produce the expected higher level of GIP activity.

Experimental

Melting points are uncorrected. Rotations were determined with a Union digital polarimeter PM-101. Analytical samples were dried *in vacuo* over P_2O_5 at 50—60°. The amino acid compositions of acid hydrolysates were determined with Hitachi amino acid analyser Model KLA-5 and values are uncorrected for amino acid destruction. Solvents were freshly distilled and evaporations were carried out *in vacuo* at a temperature of 40—50° in a rotary evaporator. Thin layer chromatography was performed on silicagel (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), Rf_3 $CHCl_3$–MeOH–AcOH (9: 1: 0.5).

Z(OMe)-Ile-Thr-OMe——DCC (45.40 g) was added to a mixture of Z(OMe)-Ile-OH (60.0 g) and H-Thr-OMe (prepared from 41.50 g of the hydrochloride with 34 ml of Et_3N) in DMF–AcOEt (200—250 ml). The

23) K. Kovacs and A Kapoor, *J. Am. Chem. Soc.*, **87**, 118 (1965).
24) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Commun.*, **26**, 2333 (1961).
25) H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. C. S., Chem. Commun.*, **1974**, 107.
26) R.A. Pederson and J.C. Brown, *Gastroenterol.*, **62**, 393 (1972).

solution was stirred at room temperature for 48 hr, filtered and then condensed *in vacuo*. Addition of AcOEt to the residue afforded a gelatinous mass, which was washed with 10% citric acid and H_2O and then recrystallized from MeOH and ether; yield 67.09 g (82%), mp 140—142°, $[\alpha]_D^{20}$ +6.9° (c=1.2, DMF), Rf_1 0.83. *Anal.* Calcd. for $C_{20}H_{30}O_7N_2$: C, 58.52; H, 7.37; N, 6.83. Found: C, 58.77; H, 7.41; N, 7.04.

Z(OMe)-Ile-Thr-NHNH$_2$——To a solution of Z(OMe)-Ile-Thr-OMe (41.05 g) in MeOH (300 ml), 80% hydrazine hydrate (25 ml) was added. A gelatinous mass formed on standing overnight, was collected by filtration, washed with ethanol and then precipitated from DMF with ethanol; yield 35.10 g (86%), mp 233—238°, $[\alpha]_D^{20}$ +14.5° (c=1.0, DMF), Rf_1 0.39. *Anal.* Calcd. for $C_{19}H_{30}O_6N_4$: C, 55.59; H, 7.37; N, 13.65. Found: C, 55.71; H, 7.43; N, 13.65.

Z(OMe)-Ile-Thr-Gln-OH——To a solution of Z(OMe)-Ile-Thr-NHNH$_2$ (18.26 g) in DMF (100 ml), 3.13 N HCl–DMF (32.0 ml) and isoamylnitrite (6.7 ml) were added under cooling with ice-NaCl. The solution was stirred for 5 min, when the hydrazine test[27] became negative. The solution, after neutralization with Et_3N (14 ml), was combined with a solution of H-Gln-OH (10.96 g) in H_2O (100 ml) containing Et_3N (16.7 ml) and the mixture was stirred at 4° for 48 hr. After addition of a few drops of AcOH, the solvent was evaporated and the residue was treated with AcOEt. The resulting solid was washed batchwisely with 5% citric acid and H_2O and then precipitated from DMF with MeOH; yield 17.81 g (75%), mp 181—184°, $[\alpha]_D^{25}$ +7.7° (c=0.6, DMF), Rf_1 0.15, Rf_2 0.65. *Anal.* Calcd. for $C_{24}H_{36}O_9N_4$: C, 54.95; H, 6.92; N, 10.68. Found: C, 54.79; H, 7.22; N, 10.59.

Z(OMe)-Asn-Ile-Thr-Gln-OH——Z(OMe)-Ile-Thr-Gln-OH (5.25 g) was treated with TFA (7 ml) in the presence of anisole (7.6 ml) in an ice-bath for 60 min, when dry ether was added. The resulting powder was dried over KOH pellets *in vacuo* and then dissolved in DMF (100 ml). To this solution, Et_3N (2.8 ml), HOBT (1.35 g) and Z(OMe)-Asn-ONP[28] (6.26 g) were successively added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was treated with ether to form a gelatinous mass, which was washed batchwisely with 10% citric acid and H_2O and then precipitated from DMF with ethanol; yield 4.60 g (72%); mp 216—219°, $[\alpha]_D^{20}$ −9.0° (c=1.0, DMF), Rf_1 0.09, Rf_2 0.61. *Anal.* Calcd. for $C_{28}H_{42}O_{11}N_6 \cdot 1/2H_2O$: C, 51.92; H, 6.69; N, 12.97. Found: C, 51.90; H, 6.92; N, 13.03.

Z(OMe)-Lys(Z)-His-NHNH$_2$——DCC (4.95 g) was added to a solution of Z(OMe)-Lys(Z)-OH (8.89 g) and H-His-OMe (prepared from 4.84 g of the dihydrochloride with 5.6 ml of Et_3N) in DMF (120 ml) and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was treated with AcOEt. The gelatinous mass formed was collected by filtration and recrystallized from MeOH and ether. The product was then dissolved in MeOH (100 ml)–2 N AcOH (25 ml) and the solution, after heating at 60° for 5 hr, was filtered and condensed *in vacuo*. Treatment of the residue with ether afforded a fine powder, which was dissolved in MeOH (100 ml) and 80% hydrazine hydrate (8.0 ml) was added. After standing overnight, the solution was condensed and the residue was treated with ethanol. The gelatinous mass formed on standing in a refrigerator for 3 hr, was collected by filtration and precipitated from DMF with ethanol; yield 8.85 g (74%), mp 180—182°, $[\alpha]_D^{25}$ −8.3° (c=0.5, DMF), Rf_1 0.55. *Anal.* Calcd. for $C_{29}H_{37}O_7N_7$: C, 58.47; H, 6.26; N, 16.46. Found: C, 58.41; H, 6.15; N, 16.62.

Z(OMe)-Lys(Z)-His-Asn-Ile-Thr-Gln-OH——Z(OMe)-Asn-Ile-Thr-Gln-OH (5.38 g) was treated with TFA (10 ml) in the presence of anisole (7.3 ml) in an ice-bath for 60 min. Dry ether was added to form a fine powder, which was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in a mixture of DMF (50 ml) and H_2O (5 ml). To this ice-cold solution, Et_3N (2.4 ml) and the azide (derived from 5.96 g of Z(OMe)-Lys(Z)-His-NHNH$_2$ with 8.2 ml of 3.78 N HCl–DMF, 1.48 ml of isoamylnitrite and 5.7 ml of Et_3N) in DMF (70 ml) were added and the mixture was stirred at 4° for 48 hr. After addition of a few drops of AcOH, the solvent was evaporated and the residue was treated with AcOEt. The resulting solid was washed batchwisely with H_2O and then precipitated from DMF with MeOH; yield 5.33 g (61%), mp 219—221°, $[\alpha]_D^{20}$ −11.6° (c=1.0, DMF), Rf_1 0.09, Rf_2 0.60. *Anal.* Calcd. for $C_{48}H_{67}O_{15}N_{11}$: C, 55.53; H, 6.51; N, 14.84. Found: C, 55.27; H, 6.58; N, 14.70.

Z(OMe)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH——In the usual manner, Z(OMe)-Lys(Z)-His-Asn-Ile-Thr-Gln-OH (3.11 g) was treated with TFA (3.5 ml) in the presence of anisole (4.9 ml) in an ice-bath for 45 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 (50 ml) containing Et_3N (1.3 ml). Z(OMe)-Trp-ONP (2.20 g) and HOBT (0.4 g) were combined and the mixture was stirred at room temperature for 48 hr. After addition of a few drops of AcOH, the solvent was evaporated and the residue was treated with AcOEt. The resulting powder was washed batchwisely with 5% AcOH and H_2O and then precipitated from DMF with MeOH; yield 1.92 g (52%), mp 217—220°, $[\alpha]_D^{20}$ −15.9° (c=1.0, DMF), Rf_1 0.15, Rf_2 0.58. *Anal.* Calcd. for $C_{59}H_{77}O_{16}N_{13} \cdot 3H_2O$: C, 55.42; H, 6.54; N, 14.24. Found: C, 55.29; H, 6.44; N, 14.14.

Z(OMe)-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 36—43)-OH——The above protected heptapeptide, Z(OMe)-(GIP 37—43)-OH, (2.45 g) was treated with TFA (7.6 ml) in the presence of anisole (10 ml) containing 2% ethanedithiol in an ice-bath for 60 min. Dry ether was added and the resulting

27) H. Ertel and L. Horner, *J. Chromatog.*, **7**, 268 (1962).
28) E. Schröder and E. Klieger, *Ann. Chem.*, **673**, 208 (1964).

fine powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (20 ml) and DMSO (5 ml) containing Et_3N (0.8 ml). Z(OMe)-Asp(OBzl)-ONP (1.53 g) and HOBT (0.27 g) were added and the mixture was stirred at room temperature for 24 hr. A few drops of AcOH was added and the solvent was evaporated *in vacuo*. The residue was treated with AcOEt and the resulting powder, after washing batchwisely with 5% AcOH and H_2O, was precipitated from DMF with MeOH; yield 1.42 g (50%), mp 211—215°, $[\alpha]_D^{25}$ −16.7° (c=0.4, DMF), Rf_1 0.10, Rf_2 0.80. *Anal.* Calcd. for $C_{70}H_{88}O_{19}N_{14}$: C, 58.81; H, 6.21; N, 13.72. Found: C, 58.51; H, 6.33; N, 13.79.

Z(OMe)-Lys(Z)-Ser-OMe——DCC (4.95 g) was added to a mixture of Z(OMe)-Lys(Z)-OH (8.89 g) and H-Ser-OMe (prepared from 3.42 g of the hydrochloride with 3.1 ml of Et_3N) in DMF (50 ml) and the solution was stirred at room temperature for 48 hr. The solution was filtered, the filtrate was condensed and the residue was dissolved in AcOEt, which was washed with 5% sodium carbonate, 10% citric acid and H_2O–NaCl, dried over sodium sulfate and then evaporated. Treatment of the residue with ether afforded the solid, which was recrystallized from AcOEt and ether; yield 7.34 g (67%), mp 108—110°, $[\alpha]_D^{20}$ +1.7° (c=1.2, DMF), Rf_1 0.93, Rf_3 0.50. *Anal.* Calcd. for $C_{27}H_{35}O_9N_3$: C, 59.44; H, 6.47; N, 7.70. Found: C, 59.39; H, 6.62; N, 7.83.

Z(OMe)-Lys(Z)-Lys(Z)-Ser-OMe——In the usual manner, Z(OMe)-Lys(Z)-Ser-OMe (5.46 g) was treated with TFA (4 ml) in the presence of anisole (5.4 ml) in an ice-bath for 50 min. The excess TFA was removed by evaporation and the residue was washed with petroleum ether, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in tetrahydrofuran (120 ml). To this solution, Et_3N (1.4 ml) and Z(OMe)-Lys(Z)-OQCl[29] (6.06 g) were added and the mixture was stirred at room temperature for 24 hr. The solvent was evaporated and the residue was treated with ether. The resulting powder was recrystallized from tetrahydrofuran and ether; yield 5.89 g, 73% mp 151—153°, $[\alpha]_D^{20}$ −3.7° (c=1.1, DMF), Rf_1 0.57. *Anal.* Calcd. for $C_{41}H_{53}O_{12}N_5 \cdot H_2O$: C, 59.62; H, 6.59; N, 8.48. Found: C, 59.86; H, 6.62; N, 8.58.

Z(OMe)-Lys(Z)-Lys(Z)-Ser-NHNH$_2$——To a solution of Z(OMe)-Lys(Z)-Lys(Z)-Ser-OMe (5.77 g) in MeOH (200 ml), 80% hydrazine hydrate (2.8 ml) was added. The gelatinous mass formed on standing overnight, was collected by filtration and then precipitated from DMF with tetrahydrofuran; yield 4.65 g (81%), mp 198—202°, $[\alpha]_D^{25}$ −8.2° (c=1.0, DMF), Rf_1 0.58. *Anal.* Calcd. for $C_{40}H_{53}O_{11}N_7$: C, 59.46; H, 6.61; N, 12.14. Found: C, 59.16; H, 6.69; N, 12.14.

Z(OMe)-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 33—43)-OH——The protected octapeptide, Z(OMe)-(GIP 36—43)-OH (2.04 g) was treated with TFA (5.5 ml) in the presence of anisole (7.7 ml) containing 2% ethanedithiol in an ice-bath for 50 min. The fine powder formed by addition of dry ether was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMSO (8 ml) and DMF (30 ml) containing Et_3N (0.6 ml). With this ice-cold solution, the azide (prepared from 1.72 g of Z(OMe)-Lys(Z)-Lys(Z)-Ser-NHNH$_2$ with 1.4 ml of 3.13 N HCl–DMF, 0.32 ml of isoamylnitrite and 0.95 ml of Et_3N) in DMF (40 ml) was combined and the mixture was stirred at 4° for 48 hr. After addition of a few drops of AcOH, the solvent was evaporated. Treatment of the residue with AcOEt afforded the solid, which was washed batchwisely with 5% AcOH and H_2O and then precipitated from DMF with MeOH; yield 1.98 g (68%), mp 213—217°, $[\alpha]_D^{25}$ −14.3° (c=1.0, DMF), Rf_1 0.15, Rf_2 0.74. Amino acid ratios in 3 N Tos-OH hydrolysate: Lys 2.92, Ser 0.97, Asp 1.89, His 1.02, Ile 0.99, Thr 1.00, Glu 1.19 (average recovery 93%). *Anal.* Calcd. for $C_{101}H_{129}O_{27}N_{19} \cdot 2H_2O$: C, 58.39; H, 6.45; N, 12.81. Found: C, 58.46; H, 6.46; N, 12.58.

Z(OMe)-Gln-Lys(Z)-Gly-OMe——In the usual manner, Z(OMe)-Lys(Z)-Gly-OMe[30] (3.09 g) was treated with TFA (6 ml) in the presence of anisole (1.5 ml) at 0° for 60 min, when dry petroleum ether was added to form an oily precipitate. The supernatant was removed by decantation and the residue, after drying over KOH pellets *in vacuo* for 3 hr, was dissolved in DMF (20 ml). To this solution, Et_3N (1.7 ml) and Z(OMe)-Gln-ONP[31] (3.88 g) were added. The mixture was stirred at room temperature for 48 hr and then the solvent was evaporated. The gelatinous mass formed by addition of AcOEt was washed batchwisely with 5% citric acid, 5% sodium bicarbonate and H_2O–NaCl and then precipitated from DMF with AcOEt; 3.06 g (79%), mp 183—186°, $[\alpha]_D^{20}$ −11.2° (c=0.9, DMF), Rf_1 0.58. *Anal.* Calcd. for $C_{31}H_{41}O_{10}N_5$: C, 57.84; H, 6.42; N, 10.88. Found: C, 57.96; H, 6.30; N, 10.69.

Z(OMe)-Gln-Gln-Lys(Z)-Gly-OMe——As stated above, Z(OMe)-Gln-Lys(Z)-Gly-OMe (1.93 g) was treated with TFA (4 ml) in the presence of anisole (0.8 ml) in an ice-bath for 60 min, when dry ether was added to form a fine powder, which was collected by filtration, dried over KOH pellets *in vacuo* for 2 hr and then dissolved in DMF (30 ml). Et_3N (0.84 ml) and Z(OMe)-Gln-ONP (1.94 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 to form the gelatinous mass, which was washed with 5% citric acid, 5% sodium bicarbonate and H_2O and then precipitated from DMF with AcOEt; yield 2.18 g (94%), mp 238—242°, $[\alpha]_D^{20}$ −7.9° (c=1.0, DMF), Rf_1 0.55. *Anal.* Calcd. for $C_{36}H_{49}O_{12}N_7$: C, 56.01; H, 6.39; N, 12.70. Found: C, 55.76; H, 6.33; N, 12.67.

Z(OMe)-Gln-Gln-Lys(Z)-Gly-NHNH$_2$——In the usual manner, 80% hydrazine hydrate (2 ml) was added

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to a solution of Z(OMe)-Gln-Gln-Lys(Z)-Gly-OMe (1.54 g) in DMF (30 ml) and the solution, after standing at room temperature for 3 days, was condensed *in vacuo*. The residue was washed with ethanol and then precipitated from DMSO with ethanol; yield 1.16 g (75%), mp 225—229°, $[\alpha]_D^{25}$ −43.0° (c=0.1, DMSO), Rf_1 0.41. *Anal.* Calcd. for $C_{35}H_{49}O_{11}N_9$: C, 54.46; H, 6.39; N, 16.33. Found: C, 54.25; H, 6.31; N, 16.16.

Z(OMe)-Gln-Gln-Lys(Z)-Gly-Lys(Z)-Lys(Z)-Ser-Asp(OBzl)-Trp-Lys(Z)-His-Asn-Ile-Thr-Gln-OH, Z(OMe)-(GIP 29—43)-OH——The above protected undecapeptide, Z(OMe)-(GIP 33—43)-OH (1.98 g) was treated with TFA (4 ml) in the presence of anisole (5 ml) containing 2% ethanedithiol in an ice-bath for 50 min. The fine powder formed by addition of dry ether was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMSO (8 ml) and DMF (30 ml) containing Et_3N (0.4 ml). To this ice-cold solution, the azide (prepared from 1.12 g of Z(OMe)-Gln-Gln-Lys(Z)-Gly-$NHNH_2$ with 1.0 ml of 3.13 N HCl–DMF, 0.22 ml of isoamylnitrite and 0.6 ml of Et_3N) in DMF (5 ml) was added and the mixture was stirred at 4° for 72 hr. After addition of a few drops of AcOH, the solvent was evaporated and the residue was treated with AcOEt. The resulting fine powder was washed batchwisely with 5% AcOH and H_2O and precipitated twice from DMF with hot MeOH; yield 1.55 g (61%), mp 232—238°, $[\alpha]_D^{25}$ −14.7° (c=0.3, DMSO), Rf_1 0.08, Rf_2 0.85. *Anal.* Calcd. for $C_{127}H_{166}O_{35}N_{26}\cdot 3H_2O$: C, 57.11; H, 6.49; N, 13.64. Found: C, 56.95; H, 6.52; N, 13.88.

Z(OMe)-Leu-Ala-OMe——DCC (4.13 g) was added to a solution of Z(OMe)-Leu-OH (7.0 g) in tetrahydrofuran (70 ml) and this solution was combined with a solution of H-Ala-OMe (prepared from 2.78 g of the hydrochloride with 2.8 ml of Et_3N) in DMF (20 ml). The mixture was stirred at room temperature for 48 hr. DCC urea formed during the reaction was removed by filtration and the filtrate was condensed *in vacuo*. The residue was extracted with AcOEt, which was washed with 5% citric acid, 5% sodium bicarbonate and H_2O–NaCl, dried over sodium sulfate and then evaporated. Treatment of the residue with petroleum ether afforded a fine powder, which was recrystallized from AcOEt and petroleum ether; yield 3.95 g (52%), mp 112—115°, $[\alpha]_D^{20}$ −11.4° (c=0.9, AcOEt), Rf_1 0.75. *Anal.* Calcd. for $C_{19}H_{28}O_6N_2$: C, 59.98; H, 7.41; N, 7.36. Found: C, 59.71; H, 7.40; N, 7.22.

Z(OMe)-Leu-Leu-Ala-OMe——Z(OMe)-Leu-Ala-OMe (3.66 g) was treated with TFA (4 ml) in the presence of anisole (1 ml) in an ice-bath for 60 min. Petroleum ether was added to form an oily precipitate. The supernatant was removed by decantation, the residue was dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (20 ml). Et_3N (2.8 ml) and Z(OMe)-Leu-OPCP (6.79 g) were added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was extracted with AcOEt, which was washed with 5% citric acid, 5% sodium bicarbonate and H_2O, dried over sodium sulfate and then evaporated. Treatment of the residue with petroleum ether afforded the solid, which was recrystallized from AcOEt and petroleum ether; yield 4.15 g (88%), mp 168—170°, $[\alpha]_D^{20}$ +9.3° (c=1.1, DMF), Rf_1 0.89. *Anal.* Calcd. for $C_{25}H_{39}O_7N_3$: C, 60.83; H, 7.96; N, 8.51. Found: C, 60.55; H, 8.04; N, 8.51.

Z(OMe)-Leu-Leu-Ala-$NHNH_2$——To a solution of Z(OMe)-Leu-Leu-Ala-OMe (4.94 g) in MeOH (60 ml), 80% hydrazine hydrate (5 ml) was added. Condensation of the solvent afforded the solid, which was recrystallized from MeOH; yield 4.60 g (93%), mp 170—173°, $[\alpha]_D^{20}$ −32.2° (c=1.2, DMF), Rf_1 0.68. *Anal.* Calcd. for $C_{24}H_{39}O_6N_5$: C, 58.39; H, 7.96; N, 14.18. Found: C, 58.09; H, 7.90; N, 14.21.

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, Z(OMe)-(GIP 26—43)-OH——The protected pentadecapeptide, Z(OMe)-(GIP 29—43)-OH (7.06 g) was treated with TFA (14.6 ml) in the presence of anisole (15 ml) containing 2% ethanedithiol in an ice-bath for 60 min. The resulting TFA salt was isolated as stated above and then dissolved in a mixture of DMF (80 ml) and DMSO (20 ml). Et_3N (1.13 ml) and the azide (prepared from 2.0 g of Z(OMe)-Leu-Leu-Ala-$NHNH_2$, 2.72 ml of 3.13 N HCl–DMF, 0.6 ml of isoamylnitrite and 1.76 ml of Et_3N) in DMF (100 ml) were added and the mixture was stirred at 4° for 48 hr. After addition of a few drops of AcOH, the solvent was evaporated and the residue was treated with AcOEt and 3% AcOH. The resulting powder was washed with 3% AcOH and hot MeOH and then precipitated twice from DMF with MeOH; yield 7.51 g (95%), mp 236—239°, $[\alpha]_D^{25}$ −19.1° (c=0.4, DMSO), Rf_1 0.10, Rf_2 0.81. Amino acid ratios in 3 N Tos-OH hydrolysate: Leu 1.89, Ala 1.00, Glu 3.34, Lys 4.33, Gly 1.10, Ser 0.82, Asp 2.09, Trp 0.92, His 0.95, Ile 1.17, Thr 0.92 (average recovery 85%). *Anal.* Calcd. for $C_{142}H_{193}O_{38}N_{29}\cdot H_2O$: C, 58.16; H, 6.70; N, 13.85. Found: C, 58.01; H, 6.81; N, 13.61.

In order to prepare the sample for bioassay, the above protected octadecapeptide (58 mg) was treated with trifluoromethanesulphonic acid[25] (1 ml) in the presence of anisole (0.5 ml) in an ice-bath for 30 min and at room temperature for 30 min and dry ether was added. The resulting powder was collected by filtration and dissolved in H_2O (5 ml). This solution, after treatment with Amberlite CG-400 (acetate form, approximately 2 g), was filtered and then applied to a column of Sephadex G-25 (1.0×140 cm), which was eluted with 0.2 M AcOH. Individual fractions (5 ml each) were collected and absorbancy at 280 mμ was determined. Fractions corresponding to the front main peak (tube No. 26—40) were combined, the solvent was evaporated and the residue was lyophilized to give a fluffy white powder; yield 37 mg, 87% Rf_2 0.11. Amino acid 0.88, His 3 N Tos-OH hydrolysate: Leu 1.81, Ala 0.92, Glu 3.34, Lys 3.50, Gly 1.00, Ser 0.73, Asp 1.83, Trp ratios in 1.14, Ile 0.64, Thr 0.76 (average recovery 86%).

Acknowledgement This investigation was supported by the grant of Ministry of Education, Science and Cultare (grant No. 947072). The authors express their sincere appreciations to the unanimous support of The Mitsubishi Foundation for our studies on gastrointestinal peptide hormones.