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Studies on Peptides. LXIII.[1,2] Synthesis of the Octacosapeptide Corresponding to Positions 1 through 28 of Porcine Gastric Inhibiting Polypeptide (GIP)

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The octacosapeptide amide corresponding to positions 1 through 28 of porcine gastric inhibitory polypeptide (GIP) was synthesized in a conventional manner. This peptide fragment of GIP exhibited no suppression of gastric acid secretion stimulated by tetragastrin in Heihenhein pouch dogs.

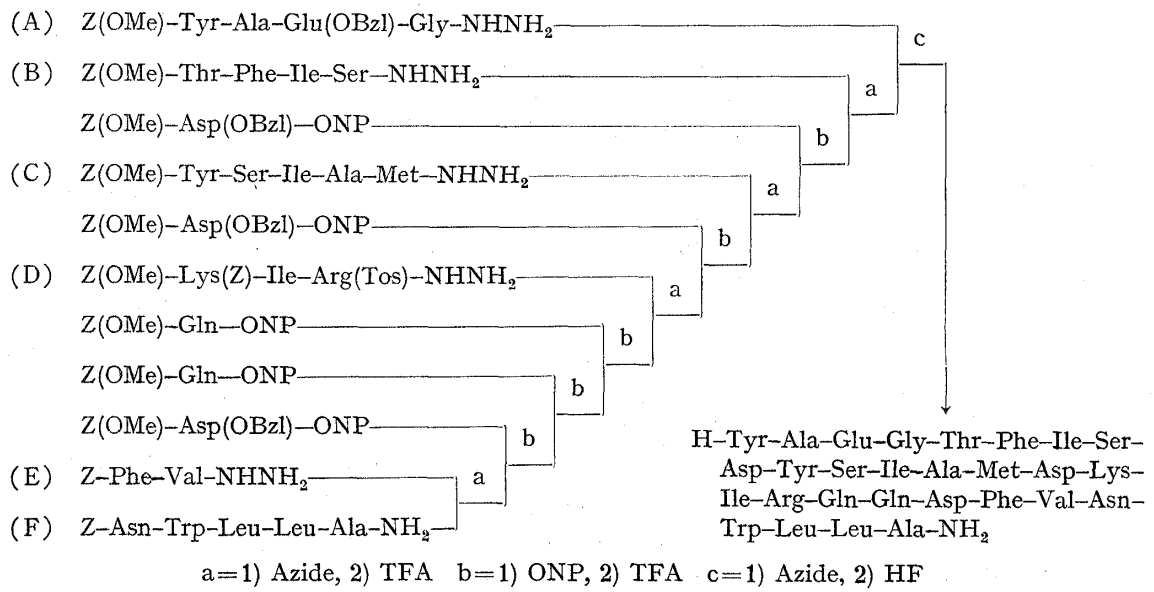
When Brown and Dryburgh[4] elucidated the complete amino acid sequence of porcine gastric inhibitory polypeptide (GIP) in 1971, it became evident that the structural similarities exist in the N-terminal portion of GIP with porcine glucagon[5] and secretin.[6] In the first 26 amino acid residues of GIP, 15 and 9 residues occur in the same position compared to those of glucagon and secretin respectively. Because of structural similarities of these three polypeptides, it seems worthwhile to investigate the structure-activity relationship of GIP, especially the contribution, if any, of the N-terminal portion of this molecule to its characteristic physiological properties. Prior to the chain elongation of the protected octadecapeptide (positions 26—43), the synthesis of which was described in the preceding paper,[1] we have synthesized the octacosapeptide amide corresponding to positions 1 through 28 of GIP, the chain length of which is comparable to glucagon (29 amino acid sequence) and secretin (27 amino acid sequence). It should be mentioned also that vasoactive intestinal polypeptide (VIP)[7] is known to have the similar chain length (27 amino acid sequence) with more structural similarity to secretin.

Synthetic strategy employed here is essentially the same as those described in the preceding paper.[1] Amino acid derivatives bearing protecting groups removable by hydrogen fluoride[8] were employed, *i.e.*, Asp(OBzl), Glu(OBzl), Lys(Z) and Arg(Tos). In the present synthesis, as a temporary protection of the α-amino function of necessary intermediates, the Z group removable by catalytic hydrogenation was used until the Phe residue (position 22) was introduced and in the latter stage of the synthesis, the Z(OMe) group[9] removable by TFA played

1) Part LXII: H. Ogawa, M. Kubota, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **24**, 2428 (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 Biochemistry Nomenclature; *Biochemistry*, **5**, 2485 (1966), *ibid.*, **6**, 362 (1967), *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Troc=trichloroethyloxycarbonyl, Tos=*p*-toluenesulfonyl, OBzl=benzyl ester, ONP=*p*-nitrophenyl ester, OPCP=pentachlorophenyl ester, OSU=N-hydroxysuccinimide ester, OQCl=5-chloro-8-quinolyl ester, DCC=dicyclohexylcarbodiimide, HOBT=N-hydroxybenzotriazole, TFA=trifluoroacetic acid, DMF=dimethylformamide, DMSO=dimethylsulfoxide, OBu^t=*tert*-butyl ester.
3) Location: *Sakyo-ku, Kyoto, 606, Japan.*
4) J.C. Brown and J.R. Dryburgh, *Can. J. Biochem.*, **49**, 867 (1971).
5) W.W. Bromer, G.L. Sinn, and O.K. Behrens, *J. Am. Chem. Soc.*, **79**, 2807 (1957).
6) V. Mutt, E.J. Jorpes, and S. Magnusson, *Eur. J. Biochem.*, **5**, 513 (1970).
7) V. Mutt and S.I. Said, *Eur. J. Biochem.*, **42**, 581 (1974).
8) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
9) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).

a role for this purpose. As also mentioned previously,[1] anisole containing 2% ethanedithiol was employed to minimize destruction of the Trp residue during the TFA treatment.

As the building blocks for construction of the entire amino acid sequence of the octacosapeptide amide, six relatively small fragments were selected as shown in Fig. 1, *i.e.*, A (position 1—4), B (position 5—8), C (10—14), D (16—18), E (22—23) and F (24—28). Of these, Z–Phe–Val–$NHNH_2$ (E) is the known compound.[10]



a=1) Azide, 2) TFA b=1) ONP, 2) TFA c=1) Azide, 2) HF

Fig. 1. Synthetic Route to the Octacosapeptide Amide (GIP sequence 1—28)

The N-terminal tetrapeptide unit, Tyr–Ala–Glu–Gly, was prepared in three different protected forms. First, Z(OMe)–Tyr–OH was condensed with H–Ala–OMe by DCC and the resulting protected dipeptide ester, Z(OMe)–Tyr–Ala–OMe, was treated with hydrazine hydrate as usual to afford the corresponding hydrazide, Z(OMe)–Tyr–Ala–$NHNH_2$. Next, according to Honzl and Rudinger,[11] this hydrazide was condensed with two known dipeptide derivatives, H–Glu(OBzl)–Gly–OH[12] and H–Glu(OBu^t)–Gly–OH[13] to give Z(OMe)–Tyr–Ala–Glu(OBzl)–Gly–OH and Z(OMe)–Tyr–Ala–Glu(OBu^t)–Gly–OH respectively. In addition to these syntheses, we wish to accumulate data in demonstrating the usefulness of Troc-hydrazine[14] for the preparation of peptide hydrazides containing the Glu(OBzl) residue. This substituted hydrazine was introduced in 1971 and applied at the first time to the synthesis of dogfish MSH,[15] in which Z(OMe)–Ser–Met–Glu(OBzl)–$NHNH_2$ was prepared starting with Z(OMe)–Glu(OBzl)–NHNH–Troc. In the present synthesis, as shown in Fig. 2, Z(OMe)–Glu(OBzl)–Gly–OH was condensed with Troc–$NHNH_2$ by DCC in the presence of HOBT[16] to give Z(OMe)–Glu(OBzl)–Gly–NHNH–Troc in 86% yield. This, after removal of the Z(OMe) group with TFA, was condensed with the azide derived from Z(OMe)–Tyr–Ala–$NHNH_2$ and the resulting protected hydrazide, Z(OMe)–Tyr–Ala–Glu(OBzl)–Gly–NHNH–Troc, was treated with zinc in acetic acid for quantitative removal of the Troc group. Contaminating zinc in

10) E. Schnabel, H. Herzog, P. Hoffmann, E. Klauke, and I. Ugi, *Angew. Chem.*, **7**, 380 (1968).
11) J. Honzl and J. Rudinger, *Collection. Czech. Chem. Commun.*, **26**, 2333 (1961).
12) H. Yajima and H. Kawatani, *Chem. Pharm. Bull.* (Tokyo), **19**, 1905 (1971).
13) H. Yajima, Y. Okada, Y. Kinomura, N. Mizokami, and H. Kawatani, *Chem. Pharm. Bull.* (Tokyo), **17**, 1237 (1969).
14) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **19**, 420 (1971).
15) H. Watanabe, M. Kubota, H. Yajima, A. Tanaka, M. Nakamura, and T. Kawabata, *Chem. Pharm. Bull.* (Tokyo), **22**, 1889 (1974).
16) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

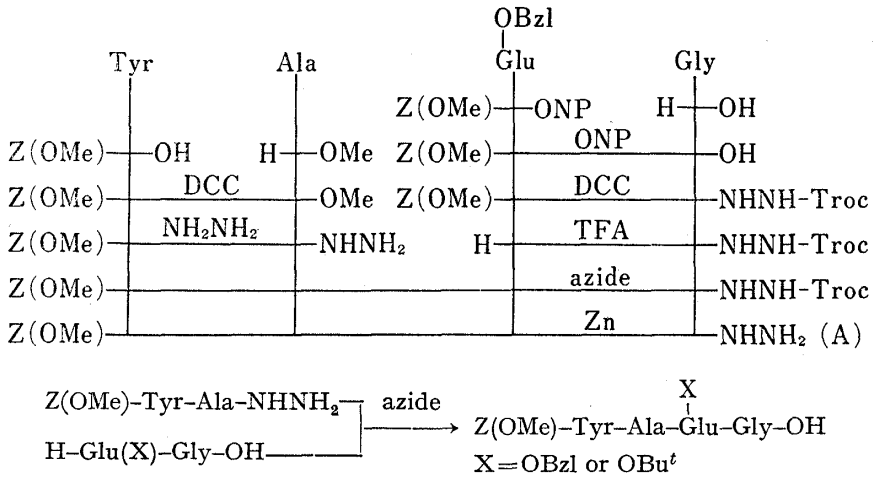


Fig. 2. Synthetic Scheme of the Protected Tetrapeptide Unit Z(OMe)-(GIP 1—4)-$NHNH_2$ and Two Related Peptides

the product was then removed by treatment with a chelating reagent, ethylenediamine tetraacetate (EDTA). Homogeneity of the desired hydrazide, Z(OMe)-Tyr-Ala-Glu(OBzl)-Gly-$NHNH_2$ (A), was assessed by three criteria; thin-layer chromatography, elemental analysis and acid hydrolysis. The positive hydrazine test[17] supported further the assigned formula of this compound. We decided to use this hydrazide for the present synthesis and the Glu(OBu^t) derivative for the total synthesis of GIP as we will mention in the next paper.

Next, Z(OMe)-Thr-Phe-Ile-Ser-OMe was prepared by two alternative ways; the one by the 2+2 condensation method and the other by the stepwise elongation method. In the first route, as shown in Fig. 3, Z(OMe)-Thr-Phe-OMe and Z(OMe)-Ile-Ser-OMe were prepared by the DCC condensation of respective amino acid derivatives. The former was converted to the corresponding hydrazide, Z(OMe)-Thr-Phe-$NHNH_2$, which was then condensed by the modified azide procedure with the TFA treated sample of the latter. In the 2nd route, the above TFA treated sample of Z(OMe)-Ile-Ser-OMe was allowed to condense with Z(OMe)-Phe-OH by the *p*-nitrophenyl ester procedure[18] to give Z(OMe)-Phe-Ile-Ser-OMe, which

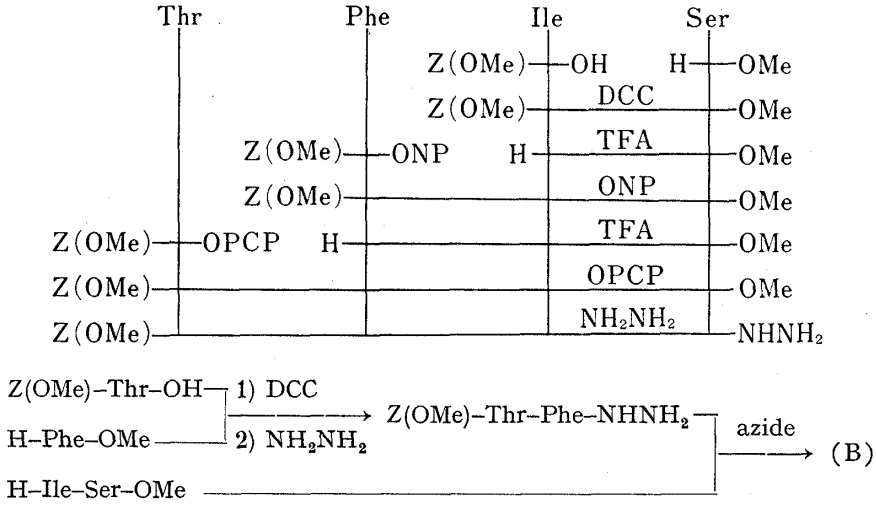


Fig. 3. Synthetic Scheme of the Protected Tetrapeptide Hydrazide Z(OMe)-(GIP 5—8)-$NHNH_2$ (B)

17) H. Ertel and L. Horner, *J. Chromatog.*, **7**, 268 (1962); K. Hofmann, R. Schmiechen, R.D. Wells, Y. Wolman, and N. Yanaihara, *J. Am. Chem. Soc.*, **87**, 611 (1965).
18) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1956).

after the similar TFA treatment, was condensed with Z(OMe)–Thr–OH by the pentachlorophenyl ester procedure.[19] Identity of two samples of Z(OMe)–Thr–Phe–Ile–Ser–OMe was confirmed by comparison of their *Rf*, mp and rotation figures. Conversion of this protected tetrapeptide ester to the corresponding hydrazide, Z(OMe)–Thr–Phe–Ile–Ser–$NHNH_2$ (B) was conducted in the usual manner.

In order to prepare the protected pentapeptide hydrazide, Z(OMe)–Tyr–Ser–Ile–Ala–Met–$NHNH_2$ (C), the corresponding pentapeptide ester, Z(OMe)–Tyr–Ser–Ile–Ala–Met–OMe was first prepared by condensation of Z(OMe)–Tyr–Ser–$NHNH_2$ and H–Ile–Ala–Met–OMe as shown in Fig. 4. The former hydrazide was derived in the usual manner from Z(OMe)–Tyr–Ser–OMe, which was prepared by the DCC condensation of Z(OMe)–Tyr–OH and H–Ser–OMe. For the synthesis of the above tripeptide ester, Z(OMe)–Ile–OH was allowed to condense by the N-hydroxysuccinimide ester procedure[20] with the TFA treated sample of Z(OMe)–Ala–Met–OMe. This dipeptide ester was prepared by the DCC condensation of respective amino acid derivatives under the nitrogen atmosphere. Such care was taken throughout syntheses of Met-containing peptides to minimize its oxidation.

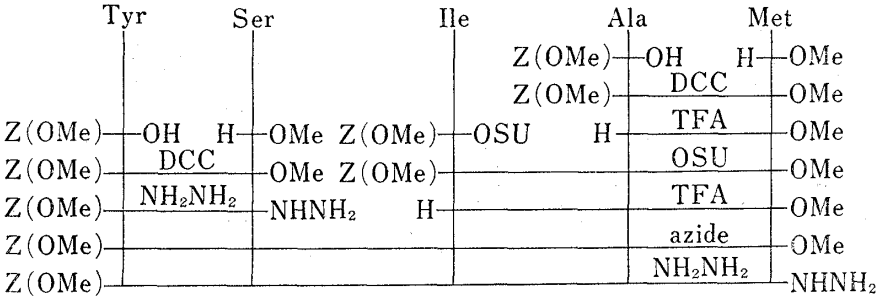


Fig. 4. Synthetic Scheme of the Protected Pentapeptide Hydrazide Z(OMe)–(GIP 10—14)–$NHNH_2$ (C)

The tripeptide hydrazide, Z(OMe)–Lys(Z)–Ile–Arg(Tos)–$NHNH_2$ (D), was synthesized in a stepwise manner starting with H–Arg(Tos)–OMe as shown in Fig. 5. The DCC condensation of H–Arg(Tos)–OMe and Z–Ile–OH gave Z–Ile–Arg(Tos)–OMe. Previously, this compound was isolated as an amorphous powder.[21] At this convenience, we characterized well its physical constants and this, after hydrogenation, was condensed with Z(OMe)–Lys(Z)–OH by the 5-chloro-8-quinolyl ester procedure.[22] The resulting protected tripeptide ester, Z(OMe)–Lys(Z)–Ile–Arg(Tos)–OMe was smoothly converted in the usual manner to (D). Besides of this synthesis, we attempted to prepare the Arg(Z_2) analogue of this tripeptide unit. Z–Arg(Z_2)–OH was prepared according to Weygand and Nintz.[23] However, the yield was only 27% in our experiments and 37% in the literature. Because of this difficulty, this approach was abandoned.

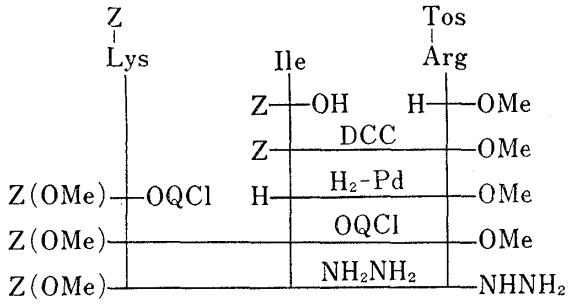


Fig. 5. Synthetic Scheme of the Protected Tripeptide Hydrazide, Z(OMe)–(GIP 16—18)–$NHNH_2$ (D)

19) J. Kovacs and A. Kapoor, *J. Am. Chem. Soc.*, **87**, 118 (1965).
20) G.W. Anderson, J.E. Zimmermann, and F.M. Callahan, *J. Am. Chem. Soc.*, **86**, 1839 (1964).
21) H. Yajima, Y. Okada, H. Watanabe, and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **22**, 1067 (1974).
22) H.D. Jakubke and A. Voigt, *Chem. Ber.*, **97**, 2944 (1966).
23) F.R. Weygand and E. Nintz, *Z. Naturforsch, B.*, **20**, 430 (1965).

The C-terminal pentapeptide amide, Z–Asn–Trp–Leu–Leu–Ala–NH_2 (F) was synthesized in the stepwise manner starting with H–Ala–NH_2 as illustrated in Fig. 6. This amide was condensed with Z–Leu–OH by DCC as usual to give Z–Leu–Ala–NH_2. In the literature,[24] this compound was derived from Z–Leu–Ala–OMe by ammonolysis. Physical constants of our compound were agreed well with those reported. Combination of hydrogenation for the removal of the Z group and the active ester procedure was employed to elongate the peptide chain to (F), *i.e.*, the pentachlorophenyl, trichlorophenyl and *p*-nitrophenyl ester procedures for introduction of Z–Leu–OH, Z–Trp–OH and Z–Asn–OH respectively. The protected pentapeptide amide (F), though it is a relatively small peptide, is less soluble in ethyl acetate and also in methanol. Therefore, purification was carried out by batchwise washing with 5% citric acid and 5% sodium bicarbonate followed by precipitation from DMF with ethyl acetate.

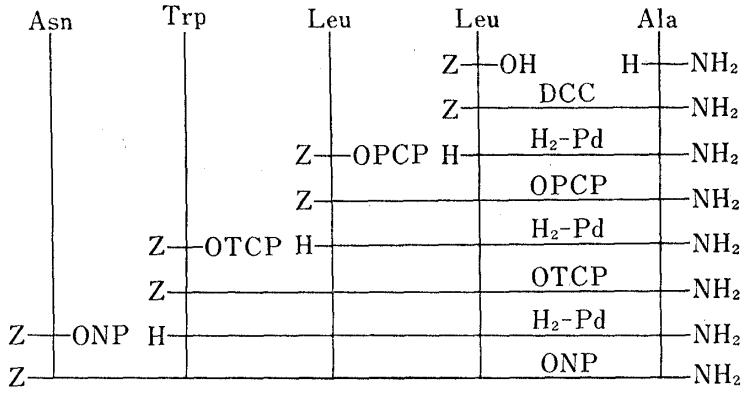


Fig. 6. Synthetic Scheme of the Protected Pentapeptide Amide, Z–(GIP 24—28)–NH_2 (F)

Relatively small fragments prepared as outlined above were then assembled by the azide procedure to minimize the risk of racemization. Two residues of Gln and Asp(OBzl) were introduced by the *p*-nitrophenyl ester procedure as shown in Fig. 1. In order to secure each coupling step, a slight excess carboxyl component (1.2 to 1.5 equivalent) was employed. Most of intermediates are less soluble in ethyl acetate, even in DMF and therefore purification was carried out by batchwise washing with acid and base as stated above followed by repeated precipitation from DMF or DMSO with ethyl acetate or methanol. Since relatively small carboxyl components were employed, such washing and precipitation procedures were sufficient enough to purify the desired compounds. All of intermediates, as well as the protected octacosapeptide amide, Z(OMe)–Tyr–Ala–Glu(OBzl)–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–NH_2, abbreviated as Z(OMe)–(GIP 1—28)–NH_2, were characterized by three criteria; thin-layer chromatography, elemental analysis and hydrolysis with 6N hydrochloric acid or 3N *p*-toluenesulfonic acid.[25] As pointed out by Iselin,[26] the recovery of Tyr in acid hydrolysates of protected peptides was generally low. However nearly quantitative recovery could be seen in hydrolysates with 3N *p*-toluenesulfonic acid.

All of protecting groups from the protected octacosapeptide amide was removed by hydrogen fluoride.[8] As a scavenger, anisole containing ethanedithiol was employed. The resulting product was immediately converted to the corresponding acetate by Amberlite CG-4B (acetate form) and then applied to a column of Sephadex G-25, which was eluted with 10% acetic acid. Eluates were examined by UV absorbancy at 280 mμ. Fractions

24) W.J. Polglose and E.L. Smith, *J. Am. Chem. Soc.*, **71**, 3081 (1974).
25) T.Y. Liu and Y.H. Chang, *J. Biol. Chem.*, **246**, 2842 (1971).
26) B. Iselin, *Helv. Chim. Acta*, **45**, 1510 (1962).

corresponding to the front main peak were collected and the solvent was removed by lyophilization. In this step, a last trace of the scavenger was removed. Since the product was found less soluble in cold water, a small amount of cold water was added and the insoluble product was collected by filtration. Fortunately this procedure removed a minor impurity with low *Rf* value and thus we were able to isolate a thin-layer chromatographically pure looking product in this step. To pursue further purification, partition chromatography on Sephadex G-25 was employed using the solvent system of *n*-butanol–acetic acid–water (4: 5: 1) according to Yamashiro.[27] When examined eluates by UV absorbancy at 280 mμ, a single peak was detected. The product thus isolated exhibited a sharp single spot on thin layer chromatography in three different solvent systems. A hydrolysate with 3N *p*-toluenesulfonic acid contained the constituent amino acids in ratios predicted by theory. Recovery of Trp was nearly quantitative. Complete digestion of our synthetic octacosapeptide amide by aminopeptidase (AP-M)[28] was possible and thus the optical purity of our synthetic peptide could be established.

Biological assay was conducted in the *in vivo* systems. Despite of our synthetic efforts devoted here, the synthetic octacosapeptide amide was devoid of any characteristic GIP activities, such as suppression of gastric acid secretion stimulated by tetragastrin in Heidenhein pouch dogs and insulin release in rats. Because of the structural similarities of this fragment with those of glucagon and secretin, activities responsible to two latter polypeptides was thought to be examined in future. Under these circumstances, synthetic fragments described herein was then used for the chain elongation of the C-terminal octadecapeptide toward the total synthesis of GIP.

Experimental

General experimental methods employed are essentially the same as those described in the part LXII. Thin layer chromatography was performed on silicagel (Kiesel gel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8: 3: 1), Rf_2 *n*-BuOH–H_2O–AcOH (4: 5: 1), Rf_3 *n*-BuOH–AcOH–pyridine–H_2O (4: 1: 1: 2), Rf_4 *n*-BuOH–AcOH–H_2O–AcOEt (1: 1: 1: 1).

Z(OMe)-Tyr-Ala-OMe——DCC (10.32 g) was added to a mixture of Z(OMe)-Tyr-OH (20.99 g) and H-Ala-OMe (prepared from 6.98 g of the hydrochloride with 7 ml of Et_3N) in DMF (40 ml) and the mixture was stirred at room temperature for 24 hr. The solution was filtered, the filtrate was condensed *in vacuo* 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 then recrystallized from AcOEt and ether; yield 11.98 g (56%), mp 134—137°, $[\alpha]_D^{27}$ −20.4°, (c=1.7, MeOH), Rf_1 0.72. *Anal.* Calcd. for $C_{22}H_{26}O_7N_2$: C, 61.38; H, 6.08; N, 6.50. Found: C, 61.36; H, 6.35; N, 6.52.

Z(OMe)-Tyr-Ala-NHNH$_2$——To a solution of Z(OMe)-Tyr-Ala-OMe (3.87 g) in MeOH (100 ml), 80% hydrazine hydrate (4.5 ml) was added. The crystalline mass formed on standing at room temperature overnight was collected by filtration, washed with MeOH and then recrystallized from DMF and MeOH; yield 3.04 g (79%), mp 249—252°, $[\alpha]_D^{22}$ −8.5° (c=1.0, DMF), Rf_1 0.58. *Anal.* Calcd. for $C_{21}H_{26}O_6N_4$: C, 58.59; H, 6.08; N, 13.01. Found: C, 58.49; H, 6.12; N, 12.99.

Z(OMe)-Tyr-Ala-Glu(OBu^t)-Gly-OH——To an ice-cold solution of Z(OMe)-Tyr-Ala-NHNH$_2$ (1.72 g) in DMF (20 ml), 3.78N HCl–DMF (2.1 ml) and isoamyl nitrite (0.67 ml) were added. After stirring for 5 min, when the hydrazine test became negative the solution was neutralized with Et_3N (1.7 ml) and then combined with a solution of H-Glu(OBu^t)-Gly-OH (0.78 g) and Et_3N (0.42 ml) in H_2O (10 ml). The mixture was stirred at 4° for 48 hr, the solvent was evaporated and the residue was treated with ether and 5% citric acid. The resulting powder was washed with 5% citric acid and H_2O and precipitated from DMF with ether; yield 1.02 g (52%), mp 117—120°, $[\alpha]_D^{22}$ −10.1°, (c=0.9, DMF), Rf_1 0.25. Amino acid ratios in 3N Tos-OH hydrolysate: Tyr 0.99, Ala 1.00, Glu 0.99, Gly 0.93 (average recovery 94%). *Anal.* Calcd. for $C_{32}H_{42}O_{11}N_4 \cdot 1.5H_2O$: C, 56.04; H, 6.61; N, 8.17. Found: C, 56.37; H, 6.39; N, 8.22.

Z(OMe)-Tyr-Ala-Glu(OBzl)-Gly-OH——In the usual manner, Z(OMe)-Glu(OBzl)-Gly-OH (2.25 g) was treated with TFA (4 ml) in the presence of anisole (2 ml) in an ice-bath for 40 min and the excess TFA was removed by evaporation. A gummy precipitate formed by addition of dry ether, was dried over KOH pellets

27) D. Yamashiro, *Nature*, **201**, 76 (1964).
28) G. Pfleiderer and P.G. Celliers, *Biochem. Z.*, **339**, 186 (1963); H. Watanabe, H. Ogawa, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **23**, 375 (1975).

in vacuo for 3 hr and then dissolved in DMF (10 ml) containing Et_3N (0.7 ml). To this solution, the azide (prepared from 2.35 g of Z(OMe)-Tyr-Ala-$NHNH_2$ with 3.13 N HCl–DMF, 0.8 ml of isoamyl nitrite and 2.5 ml of Et_3N) in DMF (20 ml) was added 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 ether and 5% citric acid gave a gelatinous mass, which was washed batchwisely with 5% citric acid and H_2O and then precipitated from DMF with ether; yield 3.06 g (89%), mp 157—160°, $[\alpha]_D^{22}$ −15.5° (c=1.2, DMF), Rf_1 0.25. Amino acid ratios in 3 N Tos-OH hydrolysate: Tyr 0.91, Ala 1.00, Glu 0.99, Gly 1.00 (average recovery 95%). *Anal.* Calcd. for $C_{35}H_{40}O_{11}N_4$: C, 60.86; H, 5.82; N, 8.08. Found: C, 60.60; H, 5.96; N, 8.35.

Z(OMe)-Glu(OBzl)-Gly-NHNH-Troc——DCC (1.13 g) was added to a mixture of Z(OMe)-Glu(OBzl)-Gly-OH (2.29 g) and Troc-$NHNH_2$ (1.13 g) in tetrahydrofuran (25 ml) and the solution, after stirring at room temperature for 48 hr, was 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. Treatment of the residue with n-hexane gave a fine powder; yield 2.78 g (86%), mp 72—76°, $[\alpha]_D^{22}$ −6.0° (c=0.9, MeOH), Rf_1 0.86. *Anal.* Calcd. for $C_{26}H_{29}O_9N_4Cl_3$: C, 48.19; H, 4.51; N, 8.64. Found: C, 48.16; H, 4.77, N, 8.94.

Z(OMe)-Tyr-Ala-Glu(OBzl)-Gly-NHNH-Troc——In the usual manner, Z(OMe)-Glu(OBzl)-Gly-NHNH-Troc (2.43 g) was treated with TFA (3 ml) in the presence of anisole (1.5 ml) in an ice-bath for 60 min and the excess TFA was removed by evaporation. The residue was washed with n-hexane, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (15 ml). Et_3N (0.5 ml) and the azide (prepared from 1.86 g of Z(OMe)-Tyr-Ala-$NHNH_2$ with 0.65 ml of isoamyl nitrite, 2.8 ml of 3.13 N HCl–DMF and 1.2 ml of Et_3N) in DMF (20 ml) were added and the mixture was stirred at 4° for 48 hr. A few drops of AcOH was added and the solvent was evaporated. Treatment of the residue with ether and 5% citric acid afforded a powder, which was washed batchwisely with 5% citric acid and H_2O and precipitated from DMF with ether; yield 2.78 g (88 %), mp 136—139°, $[\alpha]_D^{24}$ −11.4° (c=1.3, DMF), Rf_1 0.70. *Anal.* Calcd. for $C_{38}H_{43}O_{12}N_6Cl_3$: C, 51.73; H, 4.91; N, 9.52. Found: C, 51.96; H, 4.99; N, 9.63.

Z(OMe)-Tyr-Ala-Glu(OBzl)-Gly-$NHNH_2$——To a solution of Z(OMe)-Tyr-Ala-Glu(OBzl)-Gly-NHNH-Troc (2.52 g) in AcOH (30 ml), zinc powder (4.0 g) was added and the solution was stirred at room temperature for 90 min and further at 45° for 30 min. After addition of DMF (20 ml), the solution was filtered, the filtrate was condensed *in vacuo* and the residue was dissolved in H_2O (50 ml) containing EDTA (9.6 g). The solution was stirred for 30 min and then neutralized with sodium bicarbonate. The resulting gelatinous mass was collected by filtration and precipitated from DMF with AcOEt; yield 1.66 g (82%), mp 215—219°, $[\alpha]_D^{25}$ −15.7° (c=1.1, DMF), Rf_1 0.51. Amino acid ratios in 3 N Tos-OH hydrolysate: Tyr 1.00, Ala 0.99, Glu 1.12, Gly 1.00 (average recovery 96%). *Anal.* Calcd. for $C_{35}H_{42}O_{10}N_6$: C, 59.47; H, 5.99; N, 11.89. Found: C, 59.38; H, 6.21; N, 11.75.

Z(OMe)-Ile-Ser-OMe——DCC (4.54 g) was added to a mixture of Z(OMe)-Ile-OH (5.91 g) and H-Ser-OMe (prepared from 3.73 g of the hydrochloride with 3.4 ml of Et_3N) in DMF (30 ml). The solution, after stirring at room temperature for 48 hr, was 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 resulting solid was recrystallized from AcOEt and ether; yield 5.46 g (69%), mp 155—158°, $[\alpha]_D^{25}$ −12.5° (c=0.9, MeOH), Rf_1 0.89. *Anal.* Calcd. for $C_{19}H_{28}O_7N_2$: C, 57.56; H, 7.11; N, 7.06. Found: C, 57.73; H, 7.31; N, 7.19.

Z(OMe)-Phe-Ile-Ser-OMe——In the usual manner, Z(OMe)-Ile-Ser-OMe (7.81 g) was treated with TFA (12 ml) in the presence of anisole (4 ml) in an ice-bath for 50 min and the excess TFA was evaporated. The residue was washed with n-hexane, dried over KOH pellets *in vacuo* and then dissolved in DMF (30 ml). After addition of Et_3N (5.6 ml), Z(OMe)-Phe-ONP[29] (10.81 g) and HOBT (2.70 g), the mixture was stirred at room temperature for 24 hr and the solvent was evaporated. 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 gelatinous residue was purified by precipitation from DMF with ether; yield 7.38 g (68%), mp 214—218°, $[\alpha]_D^{25}$ −27.6° (c=0.9, MeOH), Rf_1 0.86. *Anal.* Calcd. for $C_{28}H_{37}O_8N_3 \cdot 1/2H_2O$: C, 60.85; H, 6.93; N, 7.60. Found: C, 61.01; H, 6.72; N, 7.62.

Z(OMe)-Thr-Phe-OMe——DCC (8.25 g) was added to a mixture of Z(OMe)-Thr-OH (12.0 g) and H-Phe-OMe (prepared from 8.63 g of the hydrochloride with 5.6 ml of Et_3N) in DMF (120 ml) and the solution, after stirring overnight, was 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 11.64 g (66%), mp 64—68°, $[\alpha]_D^{25}$ 9.2° (c=0.7, MeOH), Rf_1 0.91. *Anal.* Calcd. for $C_{23}H_{28}O_7N_2 \cdot 1/2H_2O$: C, 60.91; H, 6.44; N, 6.17. Found: C, 61.24; H, 6.55; N, 6.35.

Z(OMe)-Thr-Phe-$NHNH_2$——To a solution of Z(OMe)-Thr-Phe-OMe (5.33 g) in MeOH (100 ml), 80% hydrazine hydrate (7.5 ml) was added. The solid mass formed on standing at room temperature overnight, was collected by filtration, washed with MeOH and then precipitated from DMF with ethanol; yield 4.78 g

29) E. Klieger, *Ann. Chem.*, **724**, 204 (1969).

(90%), mp 211—215°, $[\alpha]_D^{25}$ −2.1° (c=1.3, DMF). Rf_1 0.52. *Anal.* Calcd. for $C_{22}H_{28}O_6N_4$: C, 59.44; H, 6.34; N, 12.60. Found: C, 59.34; H, 6.28; N, 12.38.

Z(OMe)-Thr-Phe-Ile-Ser-OMe——a) In the usual manner, Z(OMe)-Phe-Ile-Ser-OMe (7.07 g) was treated with TFA (10 ml) in the presence of anisole (3 ml) in an ice-bath for 50 min and the excess TFA was removed by evaporation. A fine powder formed by addition of dry ether was collected by filtration, dried over KOH pellets *in vacuo* and then dissolved in DMF (30 ml). Et_3N (3.6 ml) and Z(OMe)-Thr-OPCP[30] (8.51 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 washed batchwisely with 5% citric acid, 5% sodium bicarbonate and H_2O and the precipitated from DMF with ether; yield 6.27 g (75%), mp 222—226°, $[\alpha]_D^{25}$ −7.3° (c=1.2, DMF). Rf_1 0.60. *Anal.* Calcd. for $C_{32}H_{44}O_{10}N_4$: C, 59.61; H, 6.87; N, 8.69. Found: C, 59.77; H, 6.57; N, 8.43.

b) In the usual manner, Z(OMe)-Ile-Ser-OMe (1.47 g) was treated with TFA (3 ml) in the presence of anisole (1 ml) in an ice-bath for 40 min and the excess TFA was evaporated. The residue, after washing with *n*-hexane, was dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (10 ml). Et_3N (0.52 ml) and the azide (derived from 2.49 g of Z(OMe)-Thr-Phe-$NHNH_2$ with 3.6 ml of 3.13 N HCl–DMF, 0.81 ml of isoamyl nitrite and 1.57 ml of Et_3N) in DMF (20 ml) were combined and the mixture was stirred at 4° for 48 hr. The solvent was evaporated and the residue was treated with ether. The resulting powder was washed batchwisely as stated above and precipitated from DMF with ether; yield 2.0 g (84%), mp 228—232°, $[\alpha]_D^{25}$ −7.2° (c=0.7, DMF), Rf_1 0.60. *Anal.* Found: C, 59.57; H, 6.64; N, 8.44.

Z(OMe)-Thr-Phe-Ile-Ser-$NHNH_2$ (II)——To a solution of Z(OMe)-Thr-Phe-Ile-Ser-OMe (7.09 g) in DMF (100 ml), 80% hydrazine hydrate (10 ml) was added and the solution was kept on standing overnight. The gelatinous mass formed was collected by filtration, washed with MeOH and then precipitated from DMSO with MeOH; yield 5.43 g (77%), mp 265—270°, $[\alpha]_D^{25}$ −8.0° (c=0.9, DMSO), Rf_1 0.36. Amino acid ratios in an acid hydrolysate: Thr 1.00, Phe 1.04, Ile 1.02, Ser 0.92 (average recovery 92%). *Anal.* Calcd. for $C_{31}H_{44}O_9N_6$: C, 57.74; H, 6.87; N, 13.03. Found: C, 57.45; H, 6.96; N, 12.97.

Z(OMe)-Ala-Met-OMe——DCC (15.89 g) was added to a mixture of Z(OMe)-Ala-OH (17.73 g) and H-Met-OMe (prepared from 16.78 g of the hydrochloride with 12 ml of Et_3N) in methylene chloride (200 ml) and the solution was stirred at room temperature for 24 hr. DC-urea formed during the reaction was removed by filtration. 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 *n*-hexane and recrystallized from methylene chloride and *n*-hexane; yield 20.08 g (72%), mp 108—110°, $[\alpha]_D^{25}$ −27.8° (c=1.2, MeOH), Rf_1 0.82. *Anal.* Calcd. for $C_{18}H_{26}O_6N_2S$: C, 54.25; H, 6.57; N, 7.03. Found: C, 53.99; H, 6.55; N, 6.85.

Z(OMe)-Ile-Ala-Met-OMe——Z(OMe)-Ala-Met-OMe (6.12 g) was treated with TFA (10 ml) in the presence of anisole (3 ml) in an ice-bath for 60 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 (20 ml). To this solution, Et_3N (4.2 ml) and Z(OMe)-Ile-OSU[29] (9.01 g) were combined and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was dissolved in methylene chloride, which was washed with 5% citric acid, 5% sodium bicarbonate and H_2O–NaCl, dried over sodium sulfate and then evaporated. The resulting solid was recrystallized from methylene chloride and *n*-hexane; yield 6.52 g (83%), mp 183—186°, $[\alpha]_D^{24}$ −13.0° (c=0.9, DMF), Rf_1 0.90. *Anal.* Calcd. for $C_{24}H_{37}O_7N_3S$: C, 56.33; H, 7.28; N, 8.21. Found: C, 56.52; H, 7.39; N, 8.06.

Z(OMe)-Tyr-Ser-OMe——DCC (4.13 g) was added to a mixture of Z(OMe)-Tyr-OH dicyclohexylamine salt[31] (10.53 g) and H-Ser-OMe hydrochloride (3.12 g) in DMF (40 ml) and the solution, after stirring at room temperature for 48 hr, was filtered. The filtrate was condensed *in vacuo* and the residue was dissolved in AcOEt, which was washed with 5% sodium carbonate, 5% citric acid and H_2O–NaCl, dried over sodium sulfate and then evaporated. The residue was triturated with ether and recrystallized from AcOEt and ether; yield 6.10 g (68%), mp 135—137°, $[\alpha]_D^{25}$ −4.0° (c=1.4, MeOH), Rf_1 0.49. *Anal.* Calcd. for $C_{22}H_{26}O_8N_2$: C, 59.18; H, 5.87; N, 6.27. Found: C, 59.10; H, 5.79; N, 6.32.

Z(OMe)-Tyr-Ser-$NHNH_2$——In the usual manner, 80% hydrazine hydrate (32.2 ml) was added to a solution of Z(OMe)-Tyr-Ser-OMe (14.38 g) in MeOH (250 ml). The gelatinous mass formed on standing overnight was collected by filtration and precipitated from DMF with MeOH; yield 10.26 g (71%), mp 225—230°, $[\alpha]_D^{27}$ −13.0° (c=0.6, DMF), Rf_1 0.49. *Anal.* Calcd. for $C_{21}H_{26}O_7N_4$: C, 56.49; H, 5.87; N, 12.55. Found: C, 56.74; H, 5.80; N, 12.52.

Z(OMe)-Tyr-Ser-Ile-Ala-Met-OMe——Z(OMe)-Ile-Ala-Met-OMe (10.23 g) was treated with TFA (20 ml) in the presence of anisole (4.4 ml) in an ice-bath for 60 min. The excess TFA was removed by evaporation and the residue, after washing with *n*-hexane, was dried over KOH pellets *in vacuo* and then dissolved in DMF (20 ml). To this solution, Et_3N (2.8 ml) and the azide (prepared from 10.72 g of Z(OMe)-Tyr-Ser-$NHNH_2$ with 13 ml of 3.78 N HCl–DMF, 4.2 ml of isoamyl nitrite and 6.7 ml of Et_3N) in DMF (60 ml) were combined. The mixture, after stirring at 4° for 48 hr, was condensed *in vacuo*. Treatment of the residue with AcOEt

30) H. Yajima, Y. Kiso, and K. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **22**, 1079 (1974).
31) H. Yajima, F. Tamura, Y. Kiso, and M. Kurobe, *Chem. Pharm. Bull.* (Tokyo), **21**, 1380 (1973).

afforded a fine powder, which was washed with 5% citric acid, 5% sodium bicarbonate and H_2O and then precipitated from DMF with AcOEt; yield 14.05 g (77%), mp 228—232°, $[\alpha]_D^{25}$ −15.4° (c=0.8, DMF), Rf_1 0.77. *Anal.* Calcd. for $C_{36}H_{51}O_{11}N_5S$: C, 56.74; H, 6.74; N, 9.19. Found: C, 57.01; H, 6.77; N, 9.19.

Z(OMe)-Tyr-Ser-Ile-Ala-Met-NHNH$_2$ (III)——In the usual manner, 80% hydrazine hydrate (4 ml) was added to a solution of Z(OMe)-Tyr-Ser-Ile-Ala-Met-OMe (3.05 g) in DMF (20 ml). The gelatinous mass formed on standing overnight was collected by filtration and precipitated from DMSO with MeOH; yield 2.60 g (85%), mp 247—251°, $[\alpha]_D^{25}$ −2.0° (c=1.0, DMSO), Rf_1 0.45. Amino acid ratios in 3 N Tos-OH hydrolysate: Tyr 1.13, Ser 0.92, Ile 1.18, Ala 1.00, Met 1.02 (average recovery 91%). *Anal.* Calcd. for $C_{35}H_{51}O_{10}N_7S$: C, 55.17; H, 6.74; N, 12.87. Found: C, 54.89; H, 6.73; N, 12.93.

Z-Ile-Arg(Tos)-OMe——DCC (7.22 g) was added to a mixture of Z-Ile-OH (11.04 g) and H-Arg(Tos)-OMe (prepared from 12.12 g of the hydrochloride with 4.5 ml of Et_3N) in DMF (40 ml) and tetrahydrofuran (100 ml). The mixture was stirred at room temperature for 24 hr and the solution was filtered. The filtrate was condensed and the residue was extracted with AcOEt, which after washing with 5% sodium carbonate, 1 N HCl and H_2O, was dried over sodium sulfate and then evaporated. Treatment of the residue with *n*-hexane gave a solid, which was recrystallized from AcOEt and *n*-hexane; yield 11.50 g (61%), mp 66—69°, $[\alpha]_D^{30}$ −10.3° (c=1.2, MeOH), Rf_1 0.92. (lit.[21] $[\alpha]_D$ −2.9° in DMF). *Anal.* Calcd. for $C_{28}H_{39}O_7N_5S$: C, 57.02; H, 6.66; N, 11.87. Found: C, 56.84; H, 6.75; N, 11.62.

Z(OMe)-Lys(Z)-Ile-Arg(Tos)-OMe——Z-Ile-Arg(Tos)-OMe (4.53 g) in DMF (20 ml) containing conc. HCl (1 ml) was hydrogenated over a Pd catalyst for 10 hr and the solution was filtered. To this filtrate, Et_3N (2.8 ml) and Z(OMe)-Lys(Z)-OQCl[32] (6.91 g) were combined and the mixture was stirred at room temperature for 24 hr. The solvent was evaporated and the residue was dissolved in AcOEt, which was washed with 0.5 N HCl, 5% sodium carbonate and H_2O–NaCl, dried over sodium sulfate and then evaporated. The residue was triturated with ether and recrystallized from DMF and ether; yield 6.51 g (74%), mp 106—109°, $[\alpha]_D^{25}$ −3.6° (c=0.8, DMF) Rf_1 0.90. *Anal.* Calcd. for $C_{43}H_{59}O_{11}N_7S$: C, 58.55; H, 6.74; N, 11.11. Found: C, 58.42; H, 6.69; N, 11.19.

Z(OMe)-Lys(Z)-Ile-Arg(Tos)-NHNH$_2$ (IV)——To a solution of Z(OMe)-Lys(Z)-Ile-Arg(Tos)-OMe (6.41 g) in DMF (30 ml), 80% hydrazine hydrate (7.2 ml) was added. The solution, after standing at room temperature for 48 hr, was condensed *in vacuo*. Treatment of the residue with ethanol afforded the solid, which was precipitated from DMF with AcOEt; yield 4.89 g (77%), mp 177—181°, $[\alpha]_D^{25}$ −7.4° (c=1.5, DMF), Rf_1 0.67. Amino acid ratios in an acid hydrolysate: Lys 1.00, Ile 0.99, Arg 0.94 (average recovery 96%). *Anal.* Calcd. for $C_{42}H_{59}O_{10}N_9S$: C, 57.18; H, 6.74; N, 14.29. Found: C, 57.17; H, 6.76; N, 14.24.

Z-Leu-Ala-NH$_2$——Z-Ala-NH$_2$[33] (13.74 g) in tetrahydrofuran (50 ml) was hydrogenated over a Pd catalyst in the presence of 3.78 N HCl–dioxane (19.6 ml) for 5 hr. The catalyst was removed by filtration and the filtrate was condensed. Treatment of the residue with ether afforded a fine powder; yield 7.74 g. This was then dissolved in DMF (50 ml). To this solution, Et_3N (8.7 ml) and a solution of Z-Leu-OH (20.08 g) in tetrahydrofuran (200 ml) were combined. DCC (12.81 g) was then added to this solution and the mixture was stirred at room temperature for 24 hr. The solution was filtered and the filtrate was condensed *in vacuo*. The resulting solid residue was washed batchwisely with 1 N HCl, 5% sodium carbonate and H_2O and then recrystallized from tetrahydrofuran and AcOEt; yield 16.99 g (82%), mp 185—190°, $[\alpha]_D$ −26.1° (c=0.9, EtOH), (lit.[24] prepared by ammonolysis of Z-Leu-Ala-OMe, mp 189°, $[\alpha]_D^{29}$ −26° in ethanol). Rf_1 0.57. *Anal.* Calcd. for $C_{17}H_{25}O_4N_3$: C, 60.87; H, 7.51; N, 12.52. Found: C, 61.09; H, 7.74; N, 12.48.

Z-Leu-Leu-Ala-NH$_2$——Z-Leu-Ala-NH$_2$ (15.66 g) dissolved in DMF(50 ml) containing a few drops of AcOH was hydrogenated over a Pd catalyst for 6 hr. To this filtered solution, Et_3N (6.6 ml) and Z-Leu-OPCP[34] (28.72 g) were combined. After the solution was stirred at room temperature for 24 hr, the solvent was evaporated. The solid residue was washed with ether, 1 N HCl, 5% sodium carbonate and H_2O and then recrystallized from ethanol and ether; yield 14.52 g (69%), mp 203—208°, $[\alpha]_D^{22}$ −21.5° (c=0.9, DMF), Rf_1 0.56. *Anal.* Calcd. for $C_{23}H_{36}O_5N_4$: C, 61.58; H, 8.09; N, 12.49. Found: C, 61.31; H, 8.18; N, 12.25.

Z-Trp-Leu-Leu-Ala-NH$_2$——Z-Leu-Leu-Ala-NH$_2$ (14.35 g) in DMF (100 ml) containing a few drops of AcOH was hydrogenated over a Pd catalyst for 10 hr. The solution was filtered. Et_3N (4.5 ml) and Z-Trp-OTCP[35] (19.16 g) were added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the solid residue was treated with AcOEt. The resulting gelatinous mass was washed batchwisely with 5% citric acid, 5% sodium bicarbonate and H_2O and then precipitated from DMF with AcOEt; yield 18.95 g (93%), mp 219—225°, $[\alpha]_D^{22}$ −31.9° (c=1.0, DMF), Rf_1 0.61. *Anal.* Calcd. for $C_{34}H_{46}O_6N_6 \cdot 1/2H_2O$: C, 63.43; H, 7.35; N, 13.05. Found: C, 63.61; H, 7.51; N, 13.28.

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32) H. Yajima, H. Ogawa, H. Watanabe, N. Fujii, M. Kurobe, and S. Miyamoto, *Chem. Pharm. Bull.* (Tokyo), **23**, 371 (1975).
33) H. Matsuo, Y. Kawazoe, M. Sato, M. Ohnishi, and T. Tatsuno, *Chem. Pharm. Bull.* (Tokyo), **18**, 1788 (1970).
34) K. Kovacs, M.Q. Ceprini, C.A. Duppaz, and G.N. Schmit, *J. Org. Chem.*, **32**, 3696 (1967).
35) J. Pless and R.A. Boissonnas, *Helv. Chim. Acta*, **46**, 1609 (1963).

Z-Asn-Trp-Leu-Leu-Ala-NH_2 (VI)——Z-Trp-Leu-Leu-Ala-NH_2 (4.50 g) in DMF (30 ml) containing a few drops of AcOH was hydrogenated over a Pd catalyst as stated above. To this filtered solution, Et_3N (1.1 ml), Z-Asn-ONP (3.87 g) and HOBT (1.35 g) were combined and the mixture was stirred at room temperature for 24 hr. The solvent was evaporated and the residue was washed with AcOEt. The resulting gelatinous mass was washed batchwisely as stated above and precipitated from AcOEt; yield 4.81 g (80%), mp 294—297°, $[\alpha]_D^{28}$ −24.7° (c=0.9, DMF), Rf_1 0.44. Amino acid ratios in 3 N Tos-OH hydrolysate: Asp 1.00, Trp 0.92, Leu 1.92, Ala 1.17 (average recovery 88%). *Anal.* Calcd. for $C_{38}H_{52}O_8N_8 \cdot 1/2H_2O$: C, 60.21; H, 7.04; N, 14.78. Found: C, 60.09; H, 6.90; N, 14.86.

Z-Phe-Val-Asn-Trp-Leu-Leu-Ala-NH_2——Z-Asn-Trp-Leu-Leu-Ala-NH_2 (5.14 g) in DMF (30 ml) containing a few drops of AcOH was hydrogenated over a Pd catalyst for 8 hr. The catalyst was removed by filtration and the filtrate was neutralized with Et_3N. This solution was kept in an ice-bath until the following azide was prepared. To a solution of Z-Phe-Val-$NHNH_2$ (4.12 g) in DMF (30 ml), 3.13 N HCl–DMF (6.4 ml) and isoamyl nitrite (1.6 ml) were added under cooling with ice-NaCl. The solution was stirred for 5 min until the hydrazine test became negative. This solution, after neutralization with Et_3N (4.2 ml) was stirred at 4° for 48 hr. The solvent was evaporated and the residue was treated with AcOEt and H_2O and the resulting solid was washed batchwisely with 5% citric acid, 5% sodium bicarbonate and H_2O and precipitated from DMF with AcOEt; yield 5.86 g (87%), mp 282—287°, $[\alpha]_D^{25}$ −25.9° (c=1.2, DMF), Rf_1 0.53. Amino acid ratios in 3 N Tos-OH hydrolysate: Phe 1.00, Val 0.91, Asp 0.91, Trp 0.91, Leu 2.33, Ala 1.17 (average recovery 93%). *Anal.* Calcd. for $C_{92}H_{70}O_{10}N_{10} \cdot 1/2H_2O$: C, 62.19; H, 7.12; N, 13.94. Found: C, 62.09; H, 7.15; N, 14.25.

Z(OMe)-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-NH_2——The above protected hexapeptide amide (4.30 g) dissolved in DMF (50 ml) containing a few drops of AcOH was hydrogenated over a Pd catalyst as stated above. To this filtered solution, Et_3N (1.2 ml), Z(OMe)-Asp(OBzl)-ONP[36] (3.25 g) and HOBT (0.58 g) were combined and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, the residue was treated with AcOEt to form a gelatinous mass, which was washed batchwisely as stated above and precipitated from DMF with AcOEt; yield 4.55 g (86%), mp 236—240°, $[\alpha]_D^{25}$ −31.2° (c=1.0, DMF), Rf_1 0.60. Amino acid ratios in 3 N Tos-OH hydrolysate: Asp 1.94, Phe 1.00, Val 0.85, Trp 0.72, Leu 2.08, Ala 1.36 (average recovery 82%). *Anal.* Calcd. for $C_{64}H_{83}O_{14}N_{11} \cdot H_2O$: C, 61.56; H, 6.86; N, 12.34. Found: C, 61.46; H, 7.14; N, 12.76.

Z(OMe)-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-NH_2——The above protected heptapeptide amide (2.46 g) was treated with TFA (3 ml) in the presence of anisole (4.3 ml) containing 2% ethanedithiol in an ice-bath for 50 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 DMF (30 ml). Et_3N (0.28 ml), Z(OMe)-Gln-ONP[37] (1.29 g) and HOBT (0.27 g) were 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 batchwisely as stated above and then precipitated from DMF with AcOEt; yield 1.87 g (69%), mp 238—245°, $[\alpha]_D^{25}$ −35.7° (c=0.9, DMF), Rf_1 0.40. Amino acid ratios in 3 N Tos-OH hydrolysate: Glu 1.13, Asp 1.95, Phe 1.24, Val 0.95, Trp 0.74, Leu 2.00, Ala 1.25 (average recovery 88%). *Anal.* Calcd. for $C_{69}H_{91}O_{16}N_{13}$: C, 60.99; H, 6.75; N, 13.40. Found: C, 60.72; H, 6.88; N, 13.70.

Z(OMe)-Gln-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-NH_2——The above protected octapeptide amide (1.38 g) was treated with TFA (2 ml) in the presence of anisole (2.5 ml) containing 2% ethanedithiol as stated above and dry ether was added. The resulting powder was collected by filtration, washed with ether, dried over KOH pellets *in vacuo* and then dissolved in DMF (20 ml). Et_3N (0.3 ml), Z(OMe)-Gln-ONP (0.88 g) and HOBT (0.14 g) were added and the mixture was stirred at room temperature for 48 hr. AcOEt was added and the resulting gelatinous mass was washed batchwisely as stated above and then precipitated from DMSO with AcOEt; yield 1.06 g (70%), mp 240—246°, $[\alpha]_D^{25}$ −30.3° (c=0.5, DMSO), Rf_1 0.34. Amino acid ratios in 3 N Tos-OH hydrolysate: Glu 1.82, Asp 1.72, Phe 1.17, Val 0.86, Trp 0.79, Leu 1.85, Ala 1.00 (average recovery 98%). *Anal.* Calcd. for $C_{74}H_{99}O_{18}N_{15}$: C, 59.78; H, 6.71; N, 14.13. Found: C, 59.58; H, 6.70; N, 14.31.

Z(OMe)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-NH_2——The above protected decapeptide amide (1.05 g) was treated with TFA (2 ml) in the presence of anisole containing 2% ethanadithiol as stated above. The resulting TFA salt, obtained as a fine powder by addition of dry ether, was dried over KOH pellets *in vacuo* for 3 hr and then dissolved in a mixture of DMSO (3 ml) and DMF (7 ml). Et_3N (0.1 ml) and the azide (prepared according to Honzl and Rudinger from 1.76 g of Z(OMe)-Lys(Z)-Ile-Arg(Tos)-$NHNH_2$ with 3.13 N HCl-DMF, 0.3 ml of isoamyl nitrite and 0.84 ml of Et_3N) in DMF (20 ml) were added and the mixture was stirred at 4° for 48 hr. After evaporation of the solvent, the residue was treated with H_2O and the resulting powder was purified by batchwise washing followed by precipitation twice from DMF with AcOEt; yield 1.02 g (66%), mp 249—255°, $[\alpha]_D^{25}$ −20.5° (c=0.9, DMF). Rf_1 0.37. Amino acid ratios in 3 N Tos-OH hydrolysate: Lys 0.93, Ile 0.75, Arg+Arg(Tos) 0.90, Glu 2.20, Asp 2.05, Phe 0.94, Val

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

1.00, Trp 0.90, Leu 1.76, Ala 1.00 (average recovery 92%). *Anal.* Calcd. for $C_{107}H_{146}O_{25}N_{22}S \cdot 2H_2O$: C, 58.18; H, 6.84; N, 13.95. Found: C, 58.68; H, 6.80; N, 13.40.

Z(OMe)-Asp(OBzl)-Lys(Z)-Ile-Arg(Tos)-Gln-Gln-Asp(OBzl)-Phe-Val-Asn-Trp-Leu-Leu-Ala-NH_2——The above protected tridecapeptide amide (1.01 g) was treated with TFA (2 ml) in the presence of anisole (2.5 ml) containing 2% ethanedithiol in an ice-bath for 50 min. The resulting TFA salt was isolated as stated above and then dissolved in DMF (20 ml). Et_3N (0.13 ml) and Z(OMe)-Asp(OBzl)-ONP (0.42 g) and HOBT (0.06 g) were combined and the mixture was stirred at room temperature for 48 hr. Isolation of the product was carried out in essentially the same manner as described above; yield 0.82 g (75%), mp 268—272°, $[\alpha]_D^{25}$ −9.7° (c=0.3, DMF), Rf_1 0.49. Amino acid ratios in 3 N Tos-OH hydrolysate: Asp 2.78, Lys 0.96, Ile 0.91, Arg+Arg(Tos) 0.92, Glu 2.22, Phe 1.30, Val 1.00, Trp 0.67, Leu 2.30, Ala 0.98 (average recovery 86%). *Anal.* Calcd. for $C_{118}H_{157}O_{28}N_{23}S$: C. 59.60; H, 6.65; N, 13.55. Found: C, 59.47; H, 6.51; N, 13.26.

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-NH_2——The above tetradecapeptide amide (0.80 g) was treated with TFA (1 ml) in the presence of anisole (1.5 ml) containing 2% ethanedithiol in an ice-bath for 50 min. The resulting TFA salt was isolated as stated above and then dissolved in a mixture of DMF (6 ml) and DMSO (4 ml). Et_3N (0.05 ml) and the azide (prepared from 0.76 g of Z(OMe)-Tyr-Ser-Ile-Ala-Met-$NHNH_2$, 0.7 ml of 3.13 N HCl–DMF, 0.16 ml of isoamyl nitrite and 0.42 ml of Et_3N) in DMF (20 ml) were combined and the mixture was stirred at 4° for 48 hr. Isolation of the product was carried out by batchwise washing as stated above followed by precipitation from DMF with MeOH, yield 0.93 g (93%), mp 261—267°, $[\alpha]_D^{26}$ −7.6° (c=0.3, DMF), Rf_1 0.44. Amino acid ratios in 3 N Tos-OH hydrolysate: Tyr 1.19, Ser 1.20, Ile 2.17, Ala 2.33, Met 0.81, Asp 3.05, Lys 0.90, Arg+Arg(Tos) 0.83, Glu 2.15, Phe 0.86, Val 1.00, Trp 0.92, Leu 1.81, Ala 2.33 (average recovery 86%). *Anal.* Calcd. for $C_{144}H_{196}O_{35}N_{28}S_2 \cdot 5H_2O$: C, 57.01; H, 6.84; N, 12.93. Found: C, 57.44; H, 6.71; N, 12.56.

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-NH_2——The above protected nonadecapeptide amide (0.92 g) was treated with TFA (1 ml) in the presence of anisole (1.5 ml) containing 2% ethanedithiol in an ice-bath for 50 min. The TFA salt isolated as stated above was dissolved in DMF (20 ml). Et_3N (0.1 ml) and Z(OMe)-Asp(OBzl)-ONP (0.25 g) and HOBT (0.04 g) were added and the mixture was stirred at room temperature for 48 hr. Isolation of the product was carried out in essentially the same manner as described above and the desired product was precipitated from DMF with AcOEt; yield 0.68 g (70%), mp 263—270°, $[\alpha]_D^{25}$ −10.6° (c=0.5, DMF), Rf_1 0.42. Amino acid ratios in 3 N Tos-OH hydrolysate: Asp 4.28, Tyr 1.20, Ser 0.81, Ile 1.67, Ala 2.09, Met 1.00, Lys 1.13, Arg+Arg(Tos) 0.90, Glu 2.20, Phe 1.17, Val 1.00, Trp 0.87, Leu 2.21 (average recovery 93%). *Anal.* Calcd. for $C_{155}H_{207}O_{38}N_{29}S_2 \cdot 5H_2O$: C, 57.47; H, 6.75; N, 12.54. Found: C, 57.64; H, 6.53; N, 12.06.

Z(OMe)-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-NH_2——The above protected eicosapeptide amide (0.66 g) was treated with TFA (1 ml) in the presence of anisole (1.5 ml) containing 2% ethanedithiol in an ice-bath for 50 min. The TFA salt isolated as stated above was dissolved in DMF (10 ml) containing Et_3N (0.03 ml). To this ice-cold solution, the azide (prepared from 0.41 g of Z(OMe)-Thr-Phe-Ile-Ser-$NHNH_2$ with 0.4 ml of 3.13 N HCl–DMF, 0.1 ml of isoamylnitrite and 0.3 ml of Et_3N) in DMF (20 ml) and the mixture was stirred at 4° for 48 hr. Isolation of the product was carried out as stated above; yield 0.71 g (93%), mp 252—259°, $[\alpha]_D^{25}$ −10.5° (c=1.2, DMF), Rf_1 0.47. Amino acid ratios in 3 N Tos-OH hydrolysate: Thr 1.07, Phe 2.28, Ile 3.08, Ser 2.30, Asp 3.80, Tyr 1.03, Ala 2.19, Met 0.67, Lys 0.94, Arg+Arg(Tos) 1.02, Glu 2.33, Val 1.00, Trp 0.64, Leu 1.95 (average recovery 87%). *Anal.* Calcd. for $C_{177}H_{239}O_{41}N_{33}S_2 \cdot 5H_2O$: C, 57.65; H, 6.80; N, 12.53. Found: C, 57.75; H, 6.56; N, 12.16.

Z(OMe)-Tyr-Ala-Glu(OBzl)-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-NH_2——The above protected tetracosapeptide amide (0.49 g) was treated with TFA (1 ml) in the presence of anisole (2 ml) containing 2% ethanedithiol in an ice-bath for 50 min. The TFA salt isolated as stated above was dissolved in DMF (10 ml) containing Et_3N (0.02 ml). To this solution, the azide (prepared from 0.29 g of Z(OMe)-Tyr-Ala-Glu(OBzl)-Gly-$NHNH_2$, 0.26 ml of 3.13 N HCl–DMF, 0.06 ml of isoamyl nitrite and 0.17 ml of Et_3N) in DMF (10 ml) and the mixture was stirred at 4° for 48 hr. The product was purified by batchwise washing as stated above followed by precipitation from DMF with MeOH; yield 0.53 g (94%), mp 264—271°, $[\alpha]_D^{25}$ −20.1° (c=1,1, DMF), Rf_1 0.50. Amino acid ratios in an acid hydrolysate: Tyr 1.63, Ala 3.16, Glu 2.88, Gly 1.30, Thr 1.03, Phe 2.11, Ile 3.26, Ser 1.78, Asp 4.44, Met 0.74, Lys 1.10, Arg 1.10, Val 1.00, Leu 1.90 (average recovery 83%). *Anal.* Calcd. for $C_{203}H_{269}O_{51}N_{37}S_2 \cdot 5H_2O$: C, 58.08; H, 6.69; N, 12.34. Found: C, 58.43; H, 6.39; N, 11.43.

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-NH_2. H-(GIP 1—28)-NH_2——The above protected octacosapeptide amide (136 mg) was treated with hydrogen fluoride (approximately 3 ml) in the presence of anisole (1 ml) and ethanedithiol (0.2 ml) in an ice-bath for 60 min. The excess hydrogen fluoride was removed by evaporation at 0° *in vacuo* and the residue was treated with ether. The resulting powder was collected by filtration and then dissolved in 10% AcOH (5 ml), which was treated with Amberlite CG-4B (acetate form, Type 2, approximately 2 g) for 30 min. The resin was removed by filtration, the filtrate, after addition of ethanedithiol (0.05 ml), was incubated at 40° for 5 hr and then lyophilized. An ice-cold water (5 ml) was added and the insoluble material collected by filtration, was dissolved in 10% AcOH (5 ml). This solution was applied to a column

of Sephadex G-25 (1.7×135 cm), which was eluted with 10% AcOH. Individual fractions (5 ml each) were collected and the UV absorbancy at 280 mμ was determined. Fractions corresponding to the front main peak (tube No. 23—51) were collected and the solvent was removed by lyophilization to give a fluffy powder; yield 70 mg (yield in the deblocking step 48%). This powder (60 mg) was dissolved in the upper phase (4 ml) of the solvent system consisting of *n*-butanol–AcOH–H_2O (4: 1: 5) and the solution was applied to a column of Sephadex G-25 (1.7×63 cm) equilibrated previously with the lower phase of the above solvent system. The column was then developed with the upper phase of this solvent. Individual fractions (5 ml each) were collected and absorbancy at 280 mμ was determined. The desired fractions (tube No. 9—20) were collected and the solvent was removed by lyophilization to give a white fluffy powder; yield 43 mg (yield in the purification step 34%), $[\alpha]_D^{26}$ −29.5° (c=0.2, 10% AcOH), Rf_2 0.35, Rf_3 0.80, Rf_4 0.52. Amino acid ratios in 3 N Tos-OH hydrolysate: Tyr 2.11, Ala 3.03, Glu 2.69, Gly 1.20, Thr 1.27, Phe 1.81, Ile 2.84, Ser 2.00, Asp 4.02 Met 0.85, Lys 1.02, Arg 0.95, Val 1.00, Trp 0.71, Leu 1.98 (average recovery 89%). Amino acid ratios in AP-M digestt: Tyr 1.87, Ala 2.22, Glu 1.00, Gln+Ser 3.77 (calcd. as Ser), Gly 0.93, Phe 1.83, Ile 2.70, Asp 2.34, Asn+Thr 1.88 (Calcd. as Thr), Met 1.00, Lys 0.90, Arg 1.01, Val 1.00, Trp 0.78, Leu 1.70 (average recovery 81%). *Anal.* Calcd. for $C_{151}H_{225}O_{44}N_{37}S \cdot 3CH_3COOH \cdot 24H_2O$: C, 48.26; H, 7.35; N, 13.27. Found: C, 48.28; H, 6.91; N, 13.54.

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