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Amino Acids and Peptides. XXVI.[1a] Phosphorus in Organic Synthesis. XV.[1b] Application of Diphenyl Phosphorazidate (DPPA) and Diethyl Phosphorocyanidate (DEPC) to the Synthesis of the N-Terminal Decapeptide of Gastric Inhibitory Polypeptide[2]

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The synthesis of the N-terminal decapeptide of gastric inhibitory polypeptide (GIP), Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr(IV), was carried out in two ways ($F_1+F_{2-4}+F_{5-10}$ and $F_{1-4}+F_{5-10}$) using diphenyl phosphorazidate (DPPA) and diethyl phosphorocyanidate (DEPC) as coupling reagents.

Keywords——peptide; gastrointestinal hormone; organophosphorus compound; condensation; amino acid

Previous communications from our laboratories have described the application of two O,O′-disubstituted phosphoropseudohalidates, diphenyl phosphorazidate ($N_3PO(OPh)_2$, DPPA)[4,5] and diethyl phosphorocyanidate ($NCPO(OEt)_2$, DEPC)[5,6] to the peptide synthesis.

Using DPPA mainly as a coupling reagent, N-terminal fragments (I and II) of two gastrointestinal hormones, secretin[7] and the vasoactive intestinal peptide,[1] were prepared by the solution method. The synthesis of motilin (III), another gastrointestinal hormone, by the combination of the solution and the solid phase methods using DPPA and DEPC was also communicated.[5] We describe here the application of these two new coupling reagents to the synthesis of the N-terminal decapeptide (IV) of gastric inhibitory polypeptide (GIP)[8] which also belongs to gastrointestinal hormones.

Z OBu^t
Z–His–Ser–Asp–Gly–Thr–Phe–OBu^t (I)

His–Ser–Asp–Ala–Val–Phe (II)

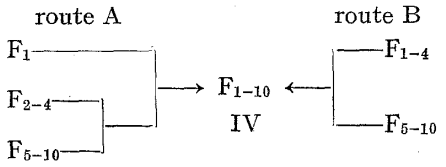
Phe–Val–Pro–Ile–Phe–Thr–Tyr–Gly–Glu–Leu–Gln–Arg–Met–Gln–Glu–Lys–Glu–Arg–Asn–Lys–Gly–Gln (III)

Tyr–Ala–Glu–Gly–Thr–Phe–Ile–Ser–Asp–Tyr (IV)

Chart 1

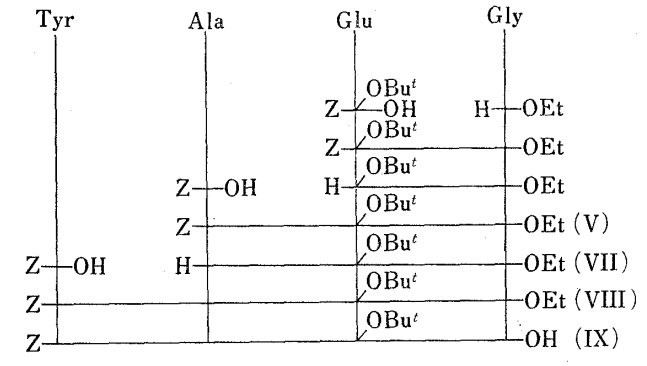
1) *a*) Part XXV: Y. Hamada, T. Shioiri, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **25**, 221 (1977); *b*) Part XIV: *Idem, ibid.*, **25**, 221 (1977).
2) Presented in part at the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, April 1975, Abstracts, II, p. 6.
3) Location: *7-3-1, Hongo, Bunkyo-ku, Tokyo, 113, Japan.*
4) T. Shioiri, K. Ninomiya, and S. Yamada, *J. Am. Chem. Soc.*, **94**, 6203 (1972); T. Shioiri and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **22**, 849, 855, and 859 (1974).
5) S. Yamada, N. Ikota, T. Shioiri, and S. Tachibana, *J. Am. Chem. Soc.*, **97**, 7174 (1975).
6) S. Yamada, Y. Kasai, and T. Shioiri, *Tetrahedron Letters*, **1973**, 1595; T. Shioiri, Y. Yokoyama, Y. Kasai, and S. Yamada, *Tetrahedron*, **32**, 2211 (1976).
7) K. Ozawa, T. Shioiri, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **25**, 122 (1977).
8) *a*) After our work had been finished, the total synthesis of GIP was communicated: H. Yajima, H. Ogawa, M. Kubota, T. Tobe, M. Fujimura, K. Henmi, K. Torizuka, H. Adachi, H. Imura, and T. Taminato, *J. Am. Chem. Soc.*, **97**, 5593 (1975); *b*) The synthesis of the protected tridecapeptide derivative having the sequence of N-terminal residues 1—13 of GIP was also reported: R. Macrae and G.T. Young, *J. Chem. Soc.*, Perkin I, **1975**, 1185.

Our approach for the synthesis of IV involved two routes, which are schematically shown in Chart 2. As α-amino protecting groups we invariably used the Z-group,[9] which was split off by catalytic hydrogenolysis. The side chain functions of Glu, Thr, Ser, and Asp were protected with the Bu^t group.



F_{m-n}: peptide fragment corresponding to sequence m-n

Chart 2



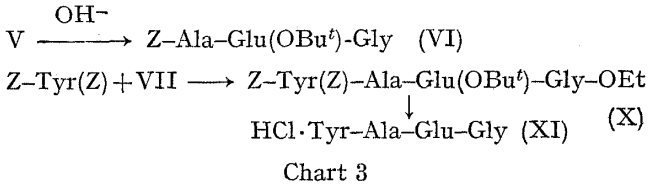


Chart 3

Preparation of the required fragments F_{2-4} and F_{1-4} is described in Chart 3. Stepwise attachment of Z-Glu(OBu^t) and Z-Ala to Gly-OEt was conveniently carried out by both DPPA and DEPC methods. Alkaline hydrolysis of the protected tripeptide (V) gave the fragment F_{2-4} (VI). Catalytic hydrogenolysis of V afforded the N-terminal free tripeptide (VII), which was condensed with Z-Tyr by the DEPC method to give the protected tetrapeptide (VIII). The fragment F_{1-4} (IX) was obtained from VIII by alkaline hydrolysis. The tripeptide (VII) was also condensed with Z-Tyr(Z) by the DPPA method to give the tetrapeptide derivative (X), which was successively subjected to alkaline hydrolysis, catalytic hydrogenolysis, and acid treatment, giving the hydrochloride of free tetrapeptide (XI).

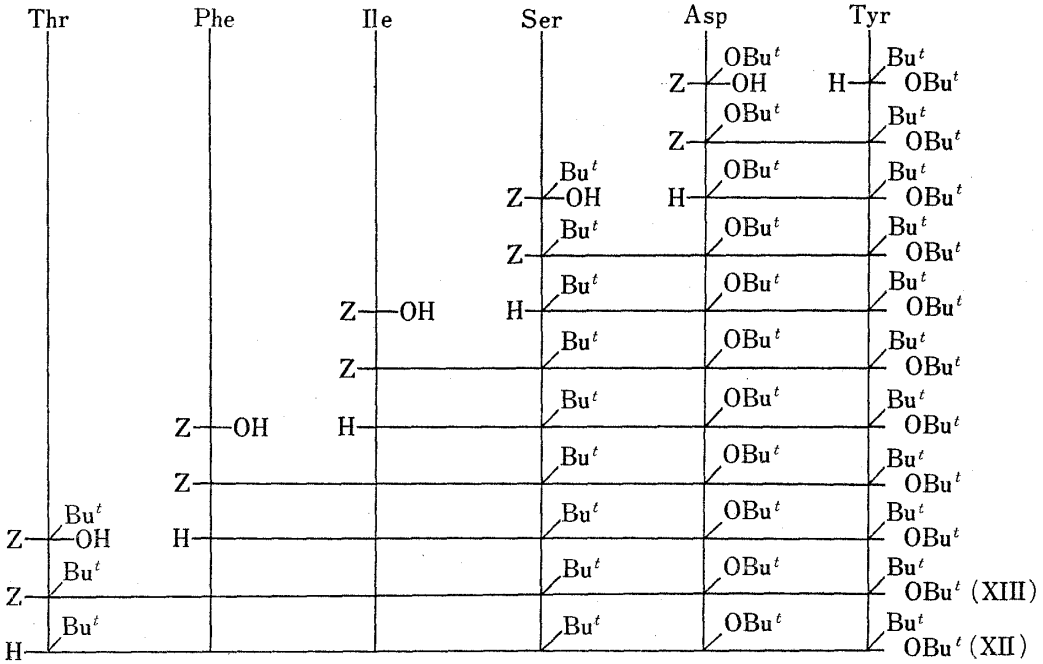


Chart 4

9) All optically active amino acids are of L-configuration. Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, *Pure Appl. Chem.*, **40**, 315 (1974).

The synthesis of the fragment F_{5-10} (XII) started from the hydrochloride of Tyr(Bu^t)-OBu^t, with which were continuously linked Z-Asp(OBu^t), Z-Ser(Bu^t), Z-Ile, Z-Phe, and Z-Thr(Bu^t) by both DPPA and DEPC methods. The resulting hexapeptide derivative (XIII) was catalytically deblocked to give the required fragment F_{5-10} (XII), shown in Chart 4.

Final assembling of each fragment was achieved in two ways (routes A and B in Chart 2). In the route A, the fragment F_{2-4} (VI) was coupled with F_{5-10} (XII) using both DPPA and DEPC methods to give the protected nonapeptide, to which, after deblocking, was attached Z-Tyr to produce the protected decapeptide F_{1-10} (XIV). In the route B, coupling of the fragment F_{1-4} (IX) with F_{5-10} (XII) was carried out by the DEPC method to give XIV as shown in Chart 5. Removal of protecting groups from XIV was accomplished by the treatment with trifluoroacetic acid, followed by catalytic hydrogenation, giving the decapeptide F_{1-10} (IV) corresponding to sequence 1—10 of GIP.[10]

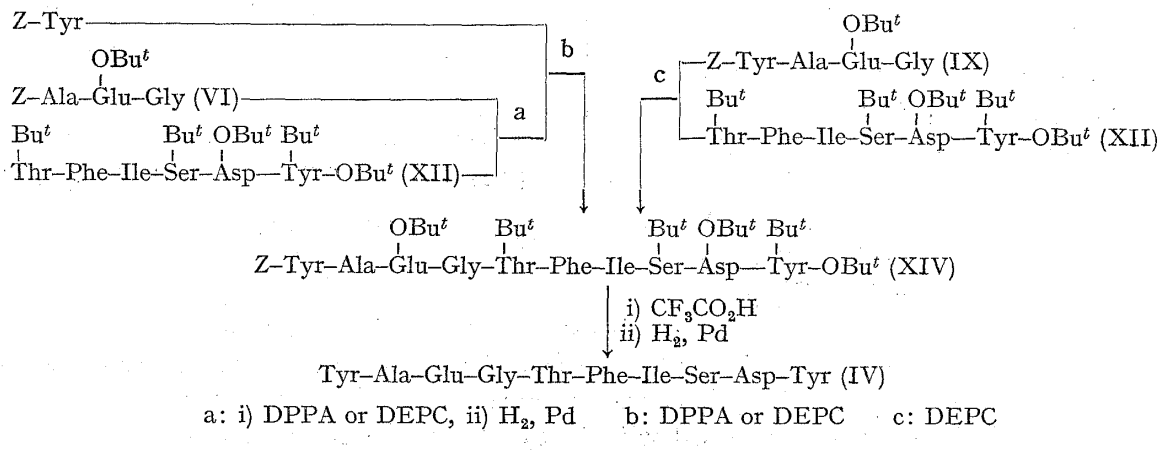


Chart 5

The results described above have well proved that DPPA and DEPC may give very satisfactory results with a wide range of non-functional and suitably protected functional amino acids and may be generally useful as coupling reagents for both fragment condensation and stepwise elongation approaches in the peptide synthesis.

Experimental

Unless otherwise stated, melting points were measured on a hot stage apparatus and uncorrected. After the coupling reaction in dimethylformamide (DMF) (A ml) was over, the reaction mixture was diluted with ethyl acetate and benzene and washed successively with 10% aq. citric acid (A×2 ml), water (A×1 ml), sat. aq. sodium chloride (A×1 ml), sat. aq. sodium bicarbonate (A×2 ml), water (A×2 ml), and sat. aq. sodium chloride (A×2 ml), the processes being designated as "washed as usual." The organic extracts were dried over anhydrous magnesium sulfate or sodium sulfate. DMF and TEA refer to dimethylformamide and triethylamine, respectively.

Z-Glu(OBu^t)-Gly-OEt——i) To a stirred mixture of Z-Glu(OBu^t)[11] (8.44 g, 25 mM) and Gly-OEt·HCl (3.48 g, 25 mM) in DMF (80 ml) was added DEPC (4.08 g, 25 mM) in DMF (10 ml) with ice-cooling, followed by the addition of TEA (5.06 g, 50 mM) in DMF (10 ml). The mixture was stirred with ice-cooling for 4 hr, and then at room temperature for 15 hr. Ethyl acetate–benzene (4: 1, 1 liter) were added, and the mixture was washed as usual and dried. Evaporation followed by recrystallization from diethyl ether–hexane gave colorless crystals (9.25 g, 87%), mp 67—68°, $[\alpha]_D^{20}$ −18.5° (c=1.04, methanol). *Anal.* Calcd. for $C_{21}H_{30}O_7N_2$: C, 59.72; H, 7.11; N, 6.64. Found: C, 59.65; H, 6.91; N, 6.61.

ii) To a stirred mixture of Z-Glu(OBu^t) (1.6 g, 4.7 mM) and Gly-OEt·HCl (0.81 g, 5.8 mM) in DMF (35 ml) were added DPPA (1.55 g, 5.6 mM) and TEA (1.04 g, 10 mM) at 0——5°. The reaction mixture was stirred for 1 hr at this temperature and then at room temperature for 24 hr. The mixture was poured into ethyl

10) The synthetic fragments F_{1-4}(XI) and F_{1-10} (IV) were devoid of any GIP activities. We are grateful to Dr. S. Tachibana, Eisai Co., Ltd., for the biological assay.

11) E. Schnabel, *Ann.*, **696**, 220 (1966); E. Schröder and E. Klieger, *Ann.*, **673**, 196 (1964).

acetate (150 ml)–benzene (15 ml), and washed as usual. Drying followed by evaporation gave crystals, which were recrystallized from ethyl acetate–petroleum ether to give colorless crystals (1.8 g, 90%), identical with the sample obtained in i).

Z-Ala-Glu(OBu^t)-Gly-OEt (V)——i) Z-Glu(OBu^t)-Gly-OEt (1.27 g, 3 mM) in ethanol (50 ml) was hydrogenated over 5% palladium–carbon (0.2 g) at room temperature for 15 hr in the presence of pyridine hydrochloride (347 mg, 3 mM). The reaction mixture was filtered to remove the catalyst, solvent removed *in vacuo* and the residue was dried over phosphorus pentoxide *in vacuo*. The hydrochloride thus obtained and Z-Ala (803 mg, 3.6 mM) were dissolved in DMF (10 ml). DEPC (978 mg, 6 mM) in DMF (3 ml) was added with stirring and ice-cooling, followed by the addition of TEA (668 mg, 6.6 mM) in DMF (3 ml). The reaction mixture was stirred with ice-cooling for 4 hr, and then at room temperature for 12 hr. Ethyl acetate–benzene (1: 1, 150 ml) were added, and the mixture was washed as usual. Drying the organic extracts followed by evaporation gave a yellow semisolid, which was recrystallized from ethyl acetate–hexane to give a colorless powder (1.80 g, 91%), mp 126—128°, $[\alpha]_D^{20}$ −31.7° (c=1.01, methanol). *Anal.* Calcd for $C_{24}H_{35}O_8N_3$: C, 58.42; H, 7.09; N, 8.52. Found: C, 58.39; H, 7.19; N, 8.64.

ii) Z-Glu(OBu^t)-Gly-OEt (1.52 g, 3.1 mM) in methanol (50 ml) containing glacial acetic acid (0.25 ml, 3.1 mM) was hydrogenated over palladium black (0.5 g) at room temperature for 4 hr. The reaction mixture was filtered, the solvent removed *in vacuo*, and the residue was dried. The acetate thus obtained was coupled with Z-Ala (0.68 g, 3.05 mM) in DMF (30 ml) using DPPA (0.99 g, 3.6 mM) and TEA (0.64 g, 6.3 mM) as described above. The crude tripeptide derivative was recrystallized from ethyl acetate–petroleum ether to give a colorless powder (1.1 g, 63%) identical with the sample obtained in i).

Z-Ala-Glu(OBu^t)-Gly (VI)——A mixture of Z-Ala-Glu(OBu^t)-Gly-OEt (V) (2.47 g, 5 mM) and 1 N aq. sodium hydroxide (6 ml, 6 mM) in acetone (24 ml) was stirred with ice-cooling for 1 hr. The mixture was concentrated *in vacuo* below 20° (bath temperature), and the residue was dissolved in water (30 ml). After being washed with diethyl ether (25×2 ml), the aqueous layer was acidified with citric acid, and extracted with ethyl acetate (50×2 ml). The organic extracts were washed with water (10×2 ml) and sat. aq. sodium chloride (10×2 ml), and dried. Evaporation followed by recrystallization from diethyl ether–hexane gave colorless crystals (2.0 g, 86%), mp 130—132°, $[\alpha]_D^{20}$ −32.0° (c=1, methanol). *Anal.* Calcd. for $C_{22}H_{31}O_8N_3$: C, 56.77; H, 6.67; N, 9.03. Found: C, 56.72; H, 6.82; N, 8.92.

Z-Tyr-Ala-Glu(OBu^t)-Gly-OEt(VIII)——Z-Ala-Glu(OBu^t)-Gly-OEt (V) (987 mg, 2 mM) was dissolved in ethanol (50 ml), and the solution was hydrogenated over 5% palladium–carbon (200 mg) for 15 hr. The catalyst was filtered, and the filtrate was concentrated to give Ala-Glu(OBu^t)-Gly-OEt (VII) (720 mg, quantitative). DEPC (391 mg, 2.4 mM) in DMF (1 ml) was added to a stirred mixture of the tripeptide (720 mg, 2 mM) and Z-Tyr (757 mg, 2.4 mM) in DMF (12 ml) with stirring and ice-cooling, followed by the addition of TEA (243 mg, 2.4 mM) in DMF (2 ml). The mixture was stirred with ice-cooling for 4 hr, and then at room temperature for 12 hr. The mixture was diluted with ethyl acetate–benzene (4: 1, 300 ml), and washed as usual. Drying the organic extracts followed by evaporation afforded crystals, which were washed with diethyl ether (20 ml) to give VIII (1.20 g, 92%). Recrystallization from ethanol–diethyl ether furnished colorless crystals, mp 161—163°, $[\alpha]_D^{20}$ −11.2° (c=1, DMF). *Anal.* Calcd. for $C_{33}H_{44}O_{10}N_4$: C, 60.34; H, 6.77; N, 8.55. Found: C, 59.93; H, 6.83; N, 8.38.

Z-Tyr(Z)-Ala-Glu(OBu^t)-Gly-OEt (X)——Z-Ala-Glu(OBu^t)-Gly-OEt (V) (1.0 g, 1.26 mM) in methanol (25 ml) was hydrogenated over palladium black (0.5 g) for 4 hr at room temperature in the presence of glacial acetic acid (0.13 ml). The usual work-up afforded the acetate of Ala-Glu (OBu^t)-Gly-OEt (VII), which was coupled with Z-Tyr(Z)[12] (1.08 g, 2.4 mM) using DPPA (0.67 g, 2.4 mM) and TEA (0.52 g, 5.1 mM) in DMF (30 ml) as usual. The crude tetrapeptide was recrystallized from ethyl acetate to give colorless crystals (1.2 g, 75%), mp 130—131°, $[\alpha]_D^{20}$ −35.9° (c=0.49, DMF). *Anal.* Calcd. for $C_{41}H_{50}O_{12}N_4$: C, 62.27; H, 6.37; N, 7.08. Found: C, 61.45; H, 6.12; N, 7.41.

Z-Tyr-Ala-Glu(OBu^t)-Gly (IX)——Z-Tyr-Ala-Glu(OBu^t)-Gly-OEt (VIII) (657 mg, 1 mM) was dissolved in ethanol (7.2 ml) with the aid of heating, and cooled with ice. After the addition of 1 N aq. sodium hydroxide (2.4 ml), the mixture was stirred with ice-cooling for 10 min and then at room temperature for 1 hr. The ethanol was removed *in vacuo* below 30° (bath temperature). The residue was dissolved in water (8 ml), and the aqueous layer was extracted with ethyl acetate (5 ml). Water (20 ml) was further added to the aqueous layer, which was acidified with citric acid and extracted with ethyl acetate (150 ml). The organic extracts were washed with water (10×2 ml) and sat. aq. sodium chloride (10×2 ml). Drying followed by evaporation gave colorless crystals, which were washed with diethyl ether (10 ml) to give IX (620 mg, 99%). Recrystallization from ethanol–ethyl acetate gave colorless crystals, mp 101—103°, $[\alpha]_D^{20}$ −9.2° (c=1, DMF). *Anal.* Calcd. for $C_{31}H_{40}O_{10}N_4 \cdot 1.5H_2O$: C, 56.77; H, 6.62; N, 8.54. Found: C, 56.74; H, 6.33; N, 8.91.

Z-Tyr(Z)-Ala-Glu(OBu^t)-Gly——Z-Tyr(Z)-Ala-Glu(OBu^t)-Gly-OEt (X) (0.4 g, 0.52 mM) was hydrolyzed with 1 N aq. sodium hydroxide (1.02 ml, 1.02 mM) in acetone (20 ml) for 1 hr at room temperature. The reaction mixture was diluted with water (30 ml), and the acetone was removed *in vacuo*. The aqueous solution was washed with ethyl acetate, acidified with citric acid, and extracted with ethyl acetate. The organic ex-

12) E. Katchalski and M. Sela, *J. Am. Chem. Soc.*, **75**, 5284 (1953).

tracts were washed with water, dried, and concentrated to give crystalline precipitates (0.155 g, 41%). Recrystallization from chloroform gave colorless crystals, mp 151—153°, $[\alpha]_D^{20}$ +29.8° (c=0.49, DMF). *Anal.* Calcd. for $C_{39}H_{46}O_{12}N_4$: C, 61.41; H, 6.08; N, 7.34. Found: C, 61.20; H, 6.14; N, 7.42.

HCl·Tyr-Ala-Glu-Gly (XI)——Z-Tyr(Z)-Ala-Glu(OBu^t)-Gly (0.55 g, 0.72 mM) in methanol (50 ml) containing conc. hydrochloric acid (0.45 ml) was hydrogenated over palladium black (0.2 g) for 4 hr at room temperature. Filtration of the catalyst followed by evaporation afforded Tyr-Ala-Glu(OBu^t)-Gly (0.45 g), which was dried over sodium hydroxide. Tyr-Ala-Glu(OBu^t)-Gly(0.33 g) was treated with 1.5 N methanolic hydrogen chloride (6.8 ml) for 1.5 hr. The solvent was removed *in vacuo* and the residue was dried over sodium hydroxide. The crude compound was recrystallized from methanol–diethyl ether to give HCl·Tyr-Ala-Glu-Gly (XI) (0.26 g, 89%), mp 115—118°. Amino acid analysis of an acid hydrolyzate: Tyr 1.1, Ala 0.8, Glu 1.2, Gly 0.8.

Z-Asp(OBu^t)-Tyr(Bu^t)-OBu^t——i) To a stirred mixture of Z-Asp (OBu^t)[13] (2.94 g, 9.1 mM) and Tyr-(Bu^t)-OBu^t·HCl[14] (2.50 g, 7.6 mM) in DMF (20 ml) was added DEPC (1.78 g, 10.9 mM) in DMF (2 ml) with ice-cooling followed by the addition of TEA (1.69 g, 16.7 mM) in DMF (3 ml). The reaction mixture was stirred with ice-cooling for 4 hr, and then at room temperature for 18 hr. Ethyl acetate–benzene (4: 1, 400 ml) were added, and the mixture was washed as usual. Drying the organic extracts followed by evaporation gave a pale yellow oil (5.05 g), which was purified by column chromatography on silica gel (Wakogel C-200, 100 g) with benzene to give colorless solid (3.71 g, 82%). Recrystallization from pentane afforded colorless prisms, mp 71—73°, $[\alpha]_D^{20}$ −9.9° (c=1, methanol). *Anal.* Calcd. for $C_{33}H_{46}O_8N_2$: C, 66.20; H, 7.74; N, 4.68. Found: C, 66.43; H, 7.86; N, 4.90.

ii) Z-Asp (OBu^t) (0.97 g, 3.0 mM) was coupled with Tyr(Bu^t)-OBu^t·HCl (1.19 g, 3.6 mM) using DPPA (0.99 g, 3.6 mM) and TEA (0.66 g, 6.5 mM) in DMF (30 ml) in the same manner as above, giving Z-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (1.7 g, 95%).

Z-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t——i) Z-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (5.8 g, 8.3 mM) was dissolved in methanol (80 ml), and the solution was stirred with 5% palladium–carbon (1 g) under a stream of hydrogen. After 10 hr, the catalyst was removed by filtration and the filtrate was evaporated to give Asp(OBu^t)-Tyr-(Bu^t)-OBu^t (3.86 g, quantitative). This dipeptide and Z-Ser(Bu^t)[13,15] (2.70 g, 9.14 mM) were dissolved in DMF (40 ml). DEPC (1.49 g, 9.14 mM) in DMF (5 ml) was added with stirring and ice-cooling, followed by the addition of TEA (925 mg, 9.14 mM) in DMF (5 ml). The reaction mixture was stirred with ice-cooling for 4 hr and then at room temperature for 12 hr. Benzene (1 liter) was added, and the mixture was washed as usual. Drying followed by evaporation gave a yellow oil (6.2 g), which was purified by column chromatography over silica gel (Wakogel C-200, 120 g) with ethyl acetate–benzene (1: 6) to give a colorless oil (5.20 g, 84%). The oil was solidified on standing in a freezer, mp 46—48°, $[\alpha]_D^{20}$ −11.9° (c=1, methanol). *Anal.* Calcd. for $C_{40}H_{59}O_{10}N_3$: C, 64.75; H, 8.02; N, 5.66. Found: C, 64.82; H, 8.15; N, 5.90.

ii) Z-Ser(Bu^t) (0.62 g, 2.1 mM) was coupled with Asp (OBu^t)-Tyr(Bu^t)-OBu^t (0.95 g, 2.1 mM) using DPPA (0.57 g, 2.1 mM) and TEA (0.21 g 2.1 mM) in DMF (15 ml) as described above. The yield of the tripeptide was 1.18 g (78%).

Z-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t——i) A solution of Z-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (1.23 g, 1.66 mM) in methanol (50 ml) was stirred with 5% palladium–carbon (0.3 g) under a stream of hydrogen for 15 hr. Filtration followed by evaporation gave an amorphous powder (940 mg, quantitative). This tripeptide and Z-Ile (663 mg, 2.5 mM) were dissolved in DMF (10 ml). DEPC (538 mg, 3.3 mM) in DMF (2 ml) was added with stirring and ice-cooling, followed by the addition of TEA (253 mg, 2.5 mM) in DMF (3 ml). The reaction mixture was stirred with ice-cooling for 6 hr, and then at room temperature for 12 hr. Ethyl acetate–benzene (4: 1, 200 ml) were added, and the mixture was washed as usual. Drying followed by evaporation gave a yellow oil (1.3 g), which was purified by column chromatography on silica gel (Wakogel C-200, 70 g) with ethyl acetate-benzene (1: 4) to give an amorphous powder (960 mg, 68%). Recrystallization from aq. methanol gave a colorless crystalline powder, mp 122—124°, $[\alpha]_D^{20}$ −14.1° (c=1, methanol). *Anal.* Calcd. for $C_{46}H_{70}O_{11}N_4$: C, 64.61; H, 8.25; N, 6.55. Found: C, 64.40; H, 8.38; N, 6.35.

ii) Z-Ile (1.86 g, 7.0 mM) and Ser (Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (1.93 g, 3.5 mM) were dissolved in tetrahydrofuran (30 ml). DPPA (1.93 g, 7 mM) in tetrahydrofuran (5 ml) was added with stirring and ice-cooling, followed by the addition of TEA (708 mg, 7.0 mM) in tetrahydrofuran (5 ml). The reaction mixture was stirred with ice-cooling for 6 hr, and then at room temperature for 18 hr. Evaporated residue was chromatographed over silica gel (Wakogel C-200, 300 g) using ethyl acetate–benzene (1: 6) to give the tetrapeptide derivative (1.55 g, 52%).

Z-Phe-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t——i) A solution of Z-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (1.14 g, 1.33 mM) in methanol (50 ml) was stirred at room temperature with 5% palladium–carbon (0.2 g) under a stream of hydrogen for 15 hr. Filtration of the catalyst followed by evaporation gave Ile-Ser-(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (960 mg, quantitative). The tetrapeptide and Z-Phe (599 mg, 2 mM) were

13) E. Wünsch and A. Zwick, *Chem. Ber.*, **99**, 105 (1966).
14) H.C. Beyerman and J.S. Bontekoe, *Rec. trav. Chim.*, **81**, 691 (1962).
15) E. Wünsch and J. Jentsch, *Chem. Ber.*, **97**, 2490 (1964); E. Schröder, *Ann.*, **670**, 127 (1963).

dissolved in tetrahydrofuran (8 ml). DEPC (440 mg, 2.66 mM) fn tetrahydrofuran (1 ml) was added with stirring and ice-cooling, followed by the addition of TEA (202 mg, 2 mM) in tetrahydrofuran (1 ml). The reaction mixture was stirred with ice-cooling for 6 hr, and then at room temperature for 14 hr. After the evaporation, the residue was purified over silica gel (Wakogel C-200, 100 g) using chloroform–methanol (96:4). Recrystallization from 90% aq. methanol gave colorless crystals (855 mg, 64%), mp 220—223°, $[\alpha]_D^{20}$ +16.6° (c=1, chloroform). *Anal.* Calcd. for $C_{55}H_{79}O_{12}N_5$: C, 65.91; H, 7.95; N, 6.99. Found: C, 65.99; H, 8.07; N, 7.07.

ii) To a stirred mixture of Z-Phe (685 mg, 2.29 mM) and Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (1.38 g, 1.91 mM) in DMF (9 ml) was added DPPA (630 mg, 2.29 mM) in DMF (2 ml) with ice-cooling, followed by the addition of TEA (232 mg, 2.29 mM) in DMF (4 ml). The reaction mixture was stirred with ice-cooling for 6 hr, and then at room temperature for 18 hr. Ethyl acetate–benzene (4: 1, 200 ml) were added and the mixture was washed as usual. Drying of the organic extracts followed by evaporation gave colorless crystals, which were washed with diethyl ether (20 ml). The yield of the pentapeptide was 1.23 g (64%).

Z-Thr(Bu^t)-Phe-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (XIII)——i) Z-Phe-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (1.50 g, 1.5 mM) in methanol (200 ml) was hydrogenated over 5% palladium–carbon (0.2 g) for 20 hr at room temperature. The catalyst was filtered, and the filtrate was evaporated to give Phe-Ile-Ser-(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (1.30 g, quantitative). The pentapeptide and Z-Thr(Bu^t)[15] (558 mg, 1.8 mM) were dissolved in DMF (12 ml). DEPC (294 mg, 1.8 mM) in DMF (1 ml) was added with stirring and ice-cooling, followed by the addition of TEA (182 mg, 1.8 mM) in DMF (2 ml). The reaction mixture was stirred with ice-cooling for 4 hr, and then at room temperature for 12 hr. Ethyl acetate–benzene (4: 1, 360 ml) were added, and the mixture was washed as usual. Drying the organic extracts followed by evaporation afforded a pale yellow solid (1.76 g). Recrystallization from 90% aq. methanol afforded colorless granules (1.43 g, 82%), mp 201—203°, $[\alpha]_D^{20}$ +18.1° (c=1, chloroform). Recrystallization from ethanol raised the mp to 206—208°. *Anal.* Calcd. for $C_{63}H_{94}O_{14}N_6 \cdot 0.5H_2O$: C, 64.74; H, 8.21; N, 7.19. Found: C, 64.77; H, 8.23; N, 7.23.

ii) To a stirred mixture of Z-Thr(Bu^t) (186 mg, 0.6 mM) and Phe-Ile-Ser (Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (440 mg, 0.5 mM) in DMF (3 ml) was added DPPA (165 mg, 0.6 mM) in DMF (1 ml) with ice-cooling, followed by the addition of TEA (61 mg, 0.6 mM) in DMF (1 ml). The reaction mixture was stirred with ice-cooling for 4 hr, and then at room temperature. Ethyl acetate–benzene (4: 1, 150 ml) were added, and the mixture was washed as usual. Drying the organic extracts followed by evaporation afforded a yellow solid (690 mg), which was recrystallized from 90% aq. methanol to give colorless granules (330 mg, 57%).

Z-Ala-Glu(OBu^t)-Gly-Thr(Bu^t)-Phe-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t——i) Z-Thr(Bu^t)-Phe-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (XIII) (464 mg, 0.4 mM) was dissolved in methanol (100 ml), and the solution was stirred with 5% palladium–carbon (100 mg) for 24 hr under a stream of hydrogen. The catalyst was filtered, and the solvent was removed *in vacuo* to give Thr(Bu^t)-Phe-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (XII) (410 mg, quantitative). This hexapeptide (XII) and Z-Ala-Glu(OBu^t)-Gly(VI) (279 mg, 0.6 mM) were dissolved in DMF (5 ml). DEPC (98 mg, 0.6 mM) in DMF (1 ml) was added with stirring and ice-cooling, followed by the addition of TEA (61 mg, 0.6 mM) in DMF (1 ml). The reaction mixture was stirred with ice-cooling for 4 hr, and then at room temperature for 20 hr. Chloroform (400 ml) was added, and the mixture was successively washed with 5% aq. citric acid (70×2 ml), water (70 ml), sat. aq. sodium chloride (70 ml), sat. aq. sodium bicarbonate (70×2 ml), water (70×2 ml), and sat. aq. sodium chloride (70×2 ml), and dried. The organic layer was concentrated to 20 ml, to which was added hot 90% aq. methanol. The protected nonapeptide was precipitated as a colorless powder (525 mg, 89%), mp 239—241°, $[\alpha]_D^{20}$ −2.9° (c=1, acetic acid). *Anal.* Calcd. for $C_{77}H_{117}O_{19}N_9$: C, 62.79; H, 8.01; N, 8.56. Found: C, 62.45; H, 8.10; N, 8.48.

ii) To a stirred mixture of Z-Ala-Glu(OBu^t)-Gly(VI) (84 mg, 0.18 mM) and Thr(Bu^t)-Phe-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t(XII) (155 mg, 0.15 mM) in chloroform (1 ml) was added DPPA (50 mg, 0.18 mM) in chloroform (0.5 ml) with ice-cooling, followed by the addition of TEA (18 mg, 0.18 mM) in chloroform (0.5 ml). The reaction mixture was stirred with ice-cooling for 4 hr, and then at room temperature for 18 hr. Chloroform (100 ml) was added, and the mixture was successively washed with 5% aq. citric acid (20×2 ml), water (20 ml), sat. aq. sodium chloride (20 ml), sat. aq. sodium bicarbonate (20×2 ml), water (20×2 ml), and sat. aq. sodium chloride (20×2 ml). Drying followed by evaporation afforded a yellow solid, which was washed with hot 90% aq. methanol (5 ml) to give the protected nonapeptide (100 mg, 45%).

Z-Tyr-Ala-Glu(OBu^t)-Gly-Thr(Bu^t)-Phe-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (XIV)——i) A solution of Z-Ala-Glu (OBu^t)-Gly-Tyr(Bu^t)-Phe-Ile-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t (525 mg, 0.36 mM)in acetic acid–methanol–water (16: 4: 1, 105 ml) was stirred with 5% palladium–carbon (200 mg) for 3 days under a stream of hydrogen. The catalyst was filtered, and the methanol was removed at 30° (bath temperature) *in vacuo*. The acetic acid and water were removed by lyophilization. The colorless powder thus obtained was dissolved in acetic acid (2 ml). After the addition of chloroform (500 ml), the solution was successively washed with 5% aq. sodium bicarbonate (100×3 ml), water (100×2 ml), and sat. aq. sodium chloride (100 ml). Drying of the organic layer followed by evaporation gave a colorless semisolid (401 mg, 86%). This material (280 mg, 0.21 mM) and Z-Tyr (132 mg, 0.42 mM) were dissolved in DMF (10 ml). DEPC (75 mg, 0.46 mM) in DMF (2 ml) was added with stirring and ice-cooling, followed by the addition of TEA (42 mg, 0.42 mM) in DMF (3 ml). The reaction mixture was stirred with ice-cooling for 2 hr, and then at room temperature for 17 hr. Chloroform (450 ml) was added, and the mixture was successively washed with 5% aq. citric acid (50×2 ml), water

(50 ml), sat. aq. sodium chloride (50 ml), sat. aq. sodium bicarbonate (50×2 ml), water (50×2 ml), and sat. aq. sodium chloride (50×2 ml). The chloroform extracts were dried and concentrated to 25 ml, to which was added hot 90% aq. methanol. The decapeptide derivative (XIV) was obtained as a colorless powder (275 mg, 80%), mp 244—246°, $[\alpha]_D^{20}$ −3.2° (c=1, DMF). *Anal.* Calcd. for $C_{86}H_{126}O_{21}N_{10}\cdot H_2O$: C, 62.44; H, 7.82; N, 8.50. Found: C, 62.38; H, 7.82; N, 8.58. Amino acid analysis of an acid hydrolyzate: Tyr 1.69; Ala 1.06, Glu 1.12; Gly 1.09; Thr 0.90; Phe 1.10, Ile 1.05, Ser 0.74, Asp 1.12.

ii) To a stirred mixture of Z-Tyr-Ala-Glu(OBu^t)-Gly(IX) (189 mg, 0.3 mM) and Thr(Bu^t)-Phe-Ile-Ser-(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-OBu^t(XII) (145 mg, 0.14 mM) in DMF (10 ml) was added DEPC (54 mg, 0.33 mM) in DMF (2 ml) with ice-cooling, followed by the addition of TEA (30 mg, 0.3 mM) in DMF (3 ml). The reaction mixture was stirred with ice-cooling for 2 hr, and then at room temperature for 18 hr. Chloroform (300 ml) was added, and the mixture was successively washed with 5% aq. citric acid (50×2 ml), water (50 ml), sat. aq. sodium chloride (50 ml), sat. aq. sodium bicarbonate (50×2 ml), water (50×2 ml), and sat. aq. sodium chloride (50×2 ml). The chloroform layer was dried, and concentrated to 15 ml. Addition of hot 90% aq. methanol (30 ml) to the concentrated solution gave a colorless powder (125 mg, 55%).

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr (IV)——The protected decapeptide (XIV) (135 mg, 0.083 mM) was dissolved in trifluoroacetic acid (8 ml), and the solution was stirred at room temperature for 1 hr. The trifluoroacetic acid was removed *in vacuo*. Chloroform (20 ml) was added to the residue, and the mixture was evaporated. This work-up was repeated three times. The residue was dried over potassium hydroxide to give Z-Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr (107 mg, quantitative) as a colorless powder, mp 201—203°. This material was suspended in 90% aq. acetic acid (60 ml), and the mixture was hydrogenated over palladium black (50 mg) for 24 hr. Another portion of the fresh palladium black (50 mg) was added, and the hydrogenation was continued for 24 hr more. The catalyst was filtered, and the filtrate was lyophilized to give the decapeptide as a colorless powder (101 mg, quantitative), mp 199—206°. A portion of this product (55 mg) was dissolved in aq. ammonia, and the solution was applied to a column of Sephadex G-10 (2.5×70 cm) with 1% aq. ammonium carbonate. Lyophilization afforded IV as a colorless powder, mp 205—220° (decomp), $[\alpha]_D^{20}$ −24.3° (c=0.16, water). Paper chromatography on Toyo filter paper No. 51A: single spot ninhydrin and Pauly positive; Rf 0.2 (n-butanol–acetic acid–water, 7: 1: 2); Rf 0.58 (n-butanol–acetic acid–pyridine–water, 30: 6: 24: 20). *Anal.* Calcd. for $C_{54}H_{72}O_{19}N_{10}\cdot 6.5H_2O$: C, 50.57; H, 6.69; N, 10.92. Found: C, 50.84; H, 6.45; N, 10.41. Amino acid analysis of an acid hydrolyzate: Tyr 2.05, Ala 1.0, Glu 1.05, Gly 1.05, Thr 1.0, Phe 0.97, Ile 0.91, Ser 0.97, Asp 1.05. Average recovery 98.5%.

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