Chem. Pharm. Bull. 25(1) 122—125 (1977)

UDC 547.466.1.04 : 547.558.1' 437.04

Amino Acids and Peptides. XXIV.[1] Phosphorus in Organic Synthesis. XIII.[2] Application of Diphenyl Phosphorazidate (DPPA) to the Synthesis of the Protected N-Terminal Hexapeptide of Secretin[3]

Kenji Ozawa, Takayuki Shioiri, and Shun-Ichi Yamada

Faculty of Pharmaceutical Sciences, University of Tokyo[4]

(Received May 18, 1976)

The diphenyl phosphorazidate (DPPA) method, a new coupling procedure in the peptide synthesis, was successfully applied to the synthesis of Z-His(Z)-Ser-Asp(OBu^t)-Gly-Thr-Phe-OBu^t(I) corresponding to sequence 1—6 of secretin. Comparison of the DPPA method with the classical azide method was made by the fragment coupling of Ser-Asp with Gly-Thr-Phe, revealing that the former may be as good as the latter.

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

Recent reports[5] from our laboratories have revealed that diphenyl phosphorazidate[6] ($N_3PO(OPh)_2$, DPPA), in combination with triethylamine, may be an efficient reagent for the synthesis of peptides without racemization. We describe here the application of the DPPA method to the synthesis of the protected hexapeptide (I) corresponding to sequence 1—6 of secretin,[7] a gastrointestinal hormone.

Z-His(Z)-Ser-Asp(OBu^t)-Gly-Thr-Phe-OBu^t[8] **(I)**

The synthesis of I started from Phe–OBu^t, which was coupled with Z–Thr by DPPA in the presence of triethylamine (TEA) in dimethylformamide (DMF).[5] The resulting Z–Thr–Phe–OBu^t, obtained in 85% yield, was hydrogenated over 5% palladium-carbon to give Thr–Phe–OBu^t. Attachment of Z–Gly to Thr–Phe–OBu^t was attained in 65% yield by the DPPA method in company with the formation of the carbamoyl azide (II) which was produced by the Curtius rearrangement[9] of Z–Gly. The Z-group of the tripeptide derivative, Z–Gly–Thr–Phe–OBu^t, was removed by catalytic hydrogenolysis to give Gly–Thr–Phe–OBu^t (III).

On the other hand, Z–Ser–Asp(OBu^t)–OEt, obtained by the coupling of Z–Ser with Asp(OBu^t)–OEt in 90% yield, was hydrolyzed to yield Z–Ser–Asp(OBu^t) (IV). Fragment condensation of Z–Ser–Asp(OBu^t) (IV) with Gly–Thr–Phe–OBu^t (III) by the DPPA method

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2) Part XII: K. Ninomiya, T. Shioiri, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **24**, 2711 (1976).
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afforded Z–Ser–Asp(OBu^t)–Gly–Thr–Phe–OBu^t (VI) in 46% yield. To compare the DPPA method with the classical azide method, Z–Ser–Asp(OBu^t)–OEt was converted to the corresponding azide (V) *via* the hydrazide.[10] The azide (V) was coupled with the tripeptide (III) to give the pentapeptide derivative (VI) in 51% yield. The physical properties of VI obtained by the both methods were virtually identical. Thus the DPPA method may be as good as the classical azide method and have a few advantages over the azide method as it is more clean and less troublesome.

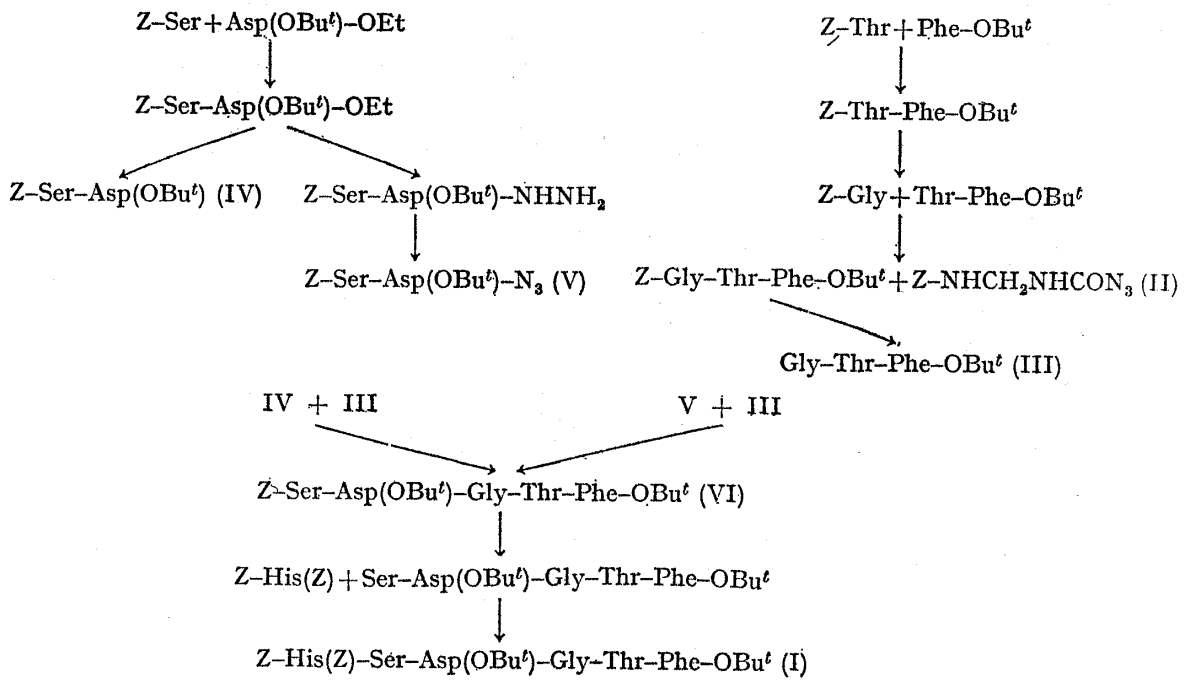

Chart 1

Catalytic deblocking of VI afforded Ser–Asp(OBu^t)–Gly–Thr–Phe–OBu^t, which was condensed with Z–His(Z) by the DPPA method to give the protected N-terminal hexapeptide (I) of secretin in 50% yield.[11]

Application of the DPPA method to the synthesis of other physiologically active peptides is now under way.

Experimental

Unless otherwise stated, melting points were measured on a hot stage apparatus and uncorrected. Silica gel (Wakogel C-200) was used for column chromatography. The organic solutions were dried over anhydrous sodium sulfate before vacuum evaporation.

Z-Thr-Phe-OBu^t——To a stirred mixture of Z-Thr (5.88 g, 23 mM) and Phe-OBu^t[12] (5.138 g, 23 mM) in DMF (80 ml) was added DPPA (6.78 g, 24.7 mM) in DMF (40 ml) at −3∼0°, followed by TEA (2.59 g, 24.7 mM) in DMF (30 ml). After the addition, the mixture was stirred at 0° for 2 hr and at room temperature for 40 hr. The reaction mixture was diluted with ethyl acetate (500 ml)–benzene (100 ml), and successively washed with 10% aq. citric acid (50×2 ml), water (50 ml), sat. aq. sodium chloride (50×2 ml), sat. aq. sodium bicarbonate (50×2 ml), water (50 ml), and sat. aq. sodium chloride (50×2 ml). Drying followed by evapora-

10) *Cf.*) F. Chillemi, *Gazz. Chim. Ital.*, **96**, 359 (1966) [*C. A.*, **65**, 3961*c* (1966)].
11) As a preliminary experiment, deblocking of the protected hexapeptide (I) was carried out by the treatment of trifluoroacetic acid and then catalytic hydrogenolysis to give His-Ser-Asp-Gly-Thr-Phe, which was identified with the sample kindly sent by Dr. M.A. Ondetti of the Squibb Institute for Medical Research, U.S.A.; M.A. Ondetti, A. Deer, J.T. Sheehan, J. Pluščec, and O. Kocy, *Biochemistry*, **7**, 4069 (1968). The free hexapeptide had no secretin activity which was kindly assayed by Dr. S. Tachibana of Eisai Co., Ltd.
12) G.W. Anderson and F.M. Callahan, *J. Am. Chem. Soc.*, **82**, 3359 (1960).

tion gave a yellow oil, which was purified by a silica gel column chromatography with benzene–ethyl acetate (4: 1) to give Z-Thr-Phe-OBu^t (9.03 g, 85%) as colorless crystals (ethyl acetate–pet. ether), mp 101—102.5°, $[\alpha]_D^{20}$ +14.4° (c=1.5, acetone). *Anal.* Calcd. for $C_{25}H_{32}O_6N_2$: C, 65.77; H, 7.07; N, 6.14. Found: C, 66.11; H, 7.25; N, 6.01.

Thr-Phe-OBu^t——Z-Thr-Phe-OBu^t (1.01 g, 2.2 mM) in ethanol (60 ml) was hydrogenated over 5% palladium–carbon (0.8 g) for 5 hr. Filtration followed by evaporation gave a colorless oil (0.72 g, quantitative), which was solidified on standing in a freezer for several days. Recrystallization from diethyl ether–hexane afforded colorless needles, mp 75—77°. *Anal.* Calcd. for $C_{17}H_{26}O_4N_2$: C, 63.33; H, 8.13; N, 8.69. Found: C, 63.26; H, 8.04; N, 8.65.

Z-Gly-Thr-Phe-OBu^t——To a stirred mixture of Thr-Phe-OBu^t (7.855 g, 24.4 mM) and Z-Gly (6.17 g, 29.5 mM) in DMF (90 ml) was added simultaneously DPPA (7.94 g, 28.9 mM) in DMF (40 ml) and TEA (2.58 g, 25.5 mM) in DMF (30 ml) at −3∼0°. After the addition, the mixture was stirred at 0∼3° for 3.5 hr and then at room temperature for 25 hr. The mixture was diluted with ethyl acetate (700 ml), and successively washed with 10% aq. citric acid (60×2 ml), water (60 ml), sat. aq. sodium chloride (60×2 ml), sat. aq. sodium bicarbonate (60×2 ml), water (60 ml), and sat. aq. sodium chloride (60×2 ml). Drying followed by evaporation afforded a yellow–brown oil, which was fractionated by a silica gel column chromatography with benzene–ethyl acetate (1: 1).

The first fraction to be eluted was the carbamoyl azide (II) (2.71 g, 37% from Z-Gly) as colorless crystals (ethyl acetate–pet. ether), mp 133—134°; IR ν_{max}^{Nujol} cm^{-1}: 3360. 2160, 1690, 760, 710; NMR in $CDCl_3$ δ (ppm from tetramethylsilane): 4.5 (2H, triplet, J=7 Hz, NHC$\underline{H}_2$NH), 5.08 (2H, singlet, OCH_2), 7.28 (5H, singlet, C_6H_5); Mass Spectrum m/e: 249 (M^+), 206, 193, 183, 182, 158, 91. *Anal.* Calcd. for $C_{10}H_{11}O_3N_5$: C, 48.19; H, 4.41; N, 28.11. Found: C, 48.09; H, 4.43; N, 27.84.

The second fraction to be eluted was Z-Gly-Thr-Phe-OBu^t (8.187 g, 65%) as colorless crystals (ethyl acetate–pet. ether), mp 63—65°, $[\alpha]_D^{20}$ −8.2° (c=1.1, methanol); Mass Spectrum m/e: 513 (M^+). *Anal.* Calcd. for. $C_{27}H_{35}O_7N_3\cdot H_2O$: C, 61.00; H, 7.02; N, 7.94. Found: C, 61.27; H, 6.85; N, 7.83.

Gly-Thr-Phe-OBu^t(III)——Z-Gly-Thr-Phe-OBu^t (4 g, 7.8 mM) in methanol (200 ml) was hydrogenated over 5% palladium–carbon (1.3 g) for 6 hr. Filtration followed by evaporation gave a colorless oil (2.95 g, quantitative), which was used directly for the next step.

Z-Ser-Asp(OBu^t)-OEt——To a stirred mixture of Z-Ser (5.70 g, 24 mM) and Asp (OBu^t)-OEt[13] (4.612 g, 21 mM) in DMF (100 ml) was added DPPA (7.88 g, 29 mM) in DMF (45 ml) at 0°, followed by TEA (2.88 g, 28.5 mM) in DMF (50 ml). After the addition, the mixture was stirred at 2—4° for 2 hr and then at room temperature for 20 hr. The mixture was diluted with ethyl acetate (2 liters), and successively washed with 5% aq. citric acid (50×2 ml), water (50 ml), sat. aq. sodium chloride (50×2 ml), sat. aq. sodium bicarbonate (50×2 ml), water (50 ml), and sat. aq. sodium chloride (50×2 ml). Drying followed by evaporation gave a yellow oil, which was purified by a silica gel column chromatography with benzene–ethyl acetate (1: 1) to give Z-Ser-Asp (OBu^t)-OEt (8.44 g, 90%) as colorless solid (ethyl acetate–pet. ether), mp 75—76°, $[\alpha]_D^{20}$ −8.6° (c=1, methanol). *Anal.* Calcd. for $C_{21}H_{30}O_8N_2$: C, 57.52; H, 6.90; N, 6.39. Found: C, 57.35; H, 6.84 N, 6.64.

Z-Ser-Asp(OBu^t) (IV)——A mixture of Z-Ser-Asp(OBu^t)-OEt (2.725 g, 6.2 mM) and 1 N aq. sodium hydroxide (7.17 ml) in dioxane (20 ml) was stirred at room temperature for 2 hr 15 min. After the addition of 1 N aq. sodium hydroxide (1 ml), the mixture was stirred for 10 min and neutralized with 10% aq. citric acid with cooling. Most of dioxane was evaporated at 25°, and the residue was acidified with citric acid and extracted with ethyl acetate (200×2 ml). The extracts were washed with water (30×2 ml) and sat. aq. sodium chloride (30×2 ml). Drying followed by evaporation afforded a colorless oil (2.453 g, 92%), which was solidified by tritulation with benzene. Recrystallization from ethyl acetate–pet. ether afforded colorless needles, mp 69—72°, $[\alpha]_D^{20}$ +11.04° (c=1, methanol). *Anal.* Calcd. for $C_{19}H_{26}O_8N_2\cdot H_2O$: C, 53.26; H, 6.59; N, 6.54. Found: C, 53.86; H, 6.27; N, 6.40.

Z-Ser-Asp(OBu^t)-NHNH$_2$——A solution of Z-Ser-Asp(OBu^t)-OEt (2.112 g, 4.8 mM) and hydrazine hydrate (1 ml) in ethanol (15 ml) was stirred at room temperature overnight to give white precipitates of the hydrazide, which were filtered. Evaporation of the filtrate afforded the solid residue, which was dried over conc. sulfuric acid and suspended with a small amount of ethyl acetate. Filtration gave colorless crystals of the hydrazide. Total yield was 1.842 g (90%). Recrystallization from ethyl acetate–ethanol gave colorless crystals, mp 189.5—190°. *Anal.* Calcd. for $C_{19}H_{28}O_7N_4$: C, 53.77; H, 6.65; N, 13.18. Found: C, 53.58; H, 6.65; N, 13.24.

Z-Ser-Asp(OBu^t)-Gly-Thr-Phe-OBu^t (VI)——i) By the DPPA Method: To a stirred mixture of Z-Ser-Asp (OBu^t) (IV) (318 mg, 0.78 mM) and Gly-Thr-Phe-OBu^t (III) (295 mg, 0.78 mM) in DMF (14 ml) was added DPPA (240 mg, 0.87 mM) in DMF (5 ml) and TEA (82.2 mg, 0.81 mM) in DMF (5 ml) at −5∼0°. The mixture was stirred at −5∼−2° for 3.5 hr, and then at room temperature overnight. Ethyl acetate (500 ml) was added to the mixture, which was successively washed with 10% aq. citric acid (40×2 ml), water (40 ml), and

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sat. aq. sodium chloride (40×5 ml). Drying followed by evaporation afforded a yellow oil, which was purified by a silica gel column chromatography with ethyl acetate–benzene (4: 1) to give VI as a colorless oil (274.6 mg, 46%), which was solidified with acetone. Recrystallization from ethyl acetate–pet. ether gave colorless crystals, mp 159—160.5°, $[\alpha]_D^{20}$ −11.9° (c=1, ethanol). *Anal.* Calcd. for $C_{38}H_{54}O_{12}N_5$: C, 59.13; H, 6.92; N, 9.07. Found: C, 58.83; H, 6.90; N, 8.98.

ii) By the Azide Method: To a stirred DMF (6 ml) solution of Z-Ser-Asp(OBu^t)-NHNH$_2$ (281 mg, 0.66 mM) was added conc. hydrochloric acid (0.66 ml) and water (1 ml) at −30°, followed by the addition of sodium nitrite (68 mg, 0.66×1.5 mM) in water (2 ml) at −20°. After stirred at −20° for 7 min, the mixture was diluted with ethyl acetate (25 ml) and washed with cold sat. aq. sodium bicarbonate (8×2 ml), water (10×2 ml), and sat. aq. sodium chloride (8 ml). The resulting solution of Z-Ser-Asp(OBu^t)-N$_3$ (V) was dried for 10 min with cooling, and decanted. Gly-Thr-Phe-OBu^t (252 mg, 0.61 mM) in ethyl acetate (5 ml) was added to the decanted solution, and the mixture was stirred at 0° for 10 hr, kept in a freezer (−20°) overnight, and then stirred at room temperature for 10 hr. The solution was washed with sat. aq. sodium bicarbonate (5×2 ml), water (5 ml), sat. aq. sodium chloride (5×2 ml), 5% aq. citric acid (5×2 ml), water (5 ml), and sat. aq. sodium chloride (5×2 ml). Drying followed by evaporation gave an oil, which was treated with ethyl acetate to give VI as colorless crystals (242 mg, 51%). Recrystallization from ethyl acetate–pet. ether gave colorless crystals, mp 160—161°, $[\alpha]_D^{20}$ −12.2° (c=1, ethanol). *Anal.* Calcd. for $C_{38}H_{52}O_{12}N_5$: C, 59.13; H, 6.92; N, 9.07. Found: C, 58.81; H, 6.91; N, 8.88.

Ser-Asp(OBu^t)-Gly-Thr-Phe-OBu^t——The pentapeptide (VI) (100 mg) in methanol (30 ml) was hydrogenated over 5% palladium–carbon (30 mg) for 20 hr with stirring. Filtration and evaporation at 20—30° gave a colorless oil (82 mg, 99%), which was used directly for the next step.

Z-His(Z)-Ser-Asp(OBu^t)-Gly-Thr-Phe-OBu^t (I)——To a stirred mixture of Z-His(Z)·methanol[14] (606 mg, 1.37 mM) and Ser-Asp(OBu^t)-Gly-Thr-Phe-OBu^t (616 mg, 0.97 mM) in DMF (17 ml) was added DPPA (370 mg, 1.35 mM) in DMF (7 ml) at −3°, followed by the addition of TEA (136 mg, 1.35 mM) in DMF (7 ml). The mixture was stirred at 0° overnight, and at 10° for 2 days. Ethyl acetate (500 ml) was added to the mixture, which was successively washed with 2% aq. citric acid (50 ml), water (50 ml), sat. aq. sodium chloride (50×2 ml), 5% aq. potassium bicarbonate (50×2 ml), water (50 ml), and sat. aq. sodium chloride (50×2 ml). Drying followed by evaporation at 25—30° gave a mixture of oil and crystals, which was treated with diethyl ether (50 ml) to give I (500 mg, 50%) as a colorless powder. Recrystallization from diethyl ether–hexane–ethyl acetate gave colorless crystals, mp 123—125°, $[\alpha]_D^{18}$ −14.6° (c=1, ethanol); IR ν_{max}^{KBr} cm^{-1}: 1725, 1650; NMR in $CDCl_3$ δ (ppm from tetramethylsilane): 1.15 (3H, doublet, J=7 Hz, CH_3 of Thr), 1.35 (9H, singlet, $(CH_3)_3C$), 5.05 (2H, singlet, CH_2 of Z), 5.35 (2H, singlet, CH_2 of Z), 7—7.6 (20H, multiplet, aromatic protons). *Anal.* Calcd. for $C_{44}H_{59}O_{13}N_8 \cdot 4H_2O$: C, 53.92; H, 6.89; N, 11.44. Found: C, 54.25; H, 6.26; N, 11.46.

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