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Amino Acids and Peptides. XXV.[1a] Phosphorus in Organic Synthesis. XIV.[1b] Application of Diphenyl Phosphorazidate (DPPA) to the Synthesis of the N-Terminal Hexapeptide of the Vasoactive Intestinal Peptide[2]

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The synthesis of the N-terminal hexapeptide of the vasoactive intestinal peptide (VIP), His-Ser-Asp-Ala-Val-Phe(II) was carried out by the both fragment condensation and stepwise elongation approaches using mainly diphenyl phosphorazidate(DPPA) as a coupling reagent.

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

As a series of experiments on the application of diphenyl phosphorazidate ($N_3PO(OPh)_2$, DPPA) to the peptide synthesis,[4,5] we reported[1] the synthesis of the protected N-terminal hexapeptide (I) of secretin by the fragment condensation approach and revealed that the DPPA method may be as good as the classical azide method. We describe here the application of the DPPA method to the synthesis of the N-terminal hexapeptide[6] (II) of the vasoactive intestinal peptide (VIP),[7] a gastrointestinal hormone, by the both fragment condensation and stepwise elongation approaches.

Z–Val[8] was coupled with Phe–OMe using DPPA in the presence of triethylamine (TEA) in dimethylformamide (DMF),[1,4,5] giving Z–Val–Phe–OMe in 94% yield. Removal of the Z group was done by the catalytic process over palladium-carbon to furnish Val–Phe–OMe. Similar attachment of Z–Ala followed by deblocking efficiently afforded Ala–Val–Phe–OMe (III). Fragment condensation of III with Z–Ser–Asp (OBu^t)[1] by the DPPA method gave Z–Ser–Asp(OBu^t)–Ala–Val–Phe–OMe (IV) in 77% yield. Stepwise elongation approach was also applied to III. Thus the tripeptide (III) was coupled with Z–Asp(OBu^t) by the DPPA method to yield Z–Asp(OBu^t)–Ala–Val–Phe–OMe in 94% yield. The Z group was removed catalytically from the tetrapeptide derivative. Z–Ser was introduced to the resulting Asp(OBu^t)–Ala–Val–Phe–OMe to give IV in 81% yield by the diethyl phosphorocyanidate ($NC\cdot PO(OEt)_2$, DEPC) method, which was also a new coupling procedure developed by our

1) *a*) Part XXIV: K. Ozawa, T. Shioiri, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **25**, 122 (1977); *b*) Part XIII: *Idem, ibid.*, **25**, 122 (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) The synthesis of II using the dicyclohexylcarbodiimide and *p*-nitrophenyl ester methods was reported: Y.S. Klausner, V. Mutt, and M. Bodanszky, *Bioorg. Chem.*, **2**, 78 (1972).
7) On the total synthesis of VIP, see M. Bodanszky, Y.S. Klausner, C.Y. Lin, V. Mutt, and S.I. Said, *J. Am. Chem. Soc.*, **96**, 4973 (1974).
8) All optically active amino acids are of L-configuration. Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commision on Biochemical Nomenclature, *Pure Appl. Chem.*, **40**, 315 (1974).

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

II: His–Ser–Asp–Ala–Val–Phe

Chart 1

laboratory.[5,9] The samples of the pentapeptide (IV) by the both approaches showed identical physical properties, suggesting that the fragment condensation by the DPPA method may be free of racemization. Deblocking of IV followed by the attachment of Z–His afforded the protected N-terminal hexapeptide (V) of VIP. Removal of OBu^t and Z functions from V was carried out by the treatment with trifluoroacetic acid followed by hydrogenolysis. The resulting ester was hydrolyzed with chymotrypsin[6] to furnish the free hexapeptide (II).

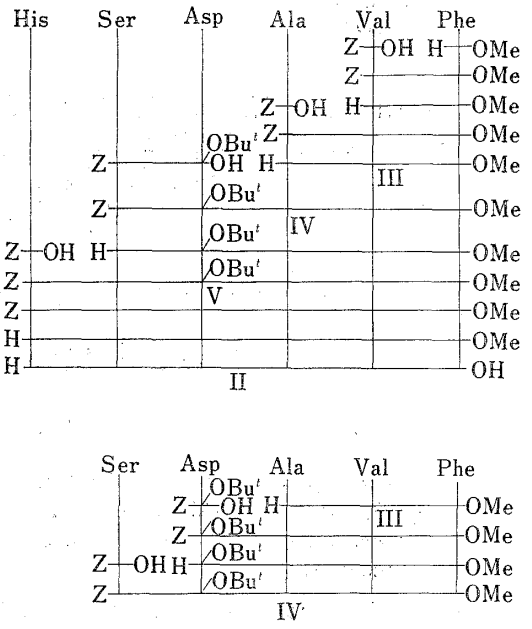

Chart 2 Comparison of the Fragment Condensation Approach with Stepwise Elongation Approach

Experimental

Unless otherwise stated, melting points were measured on a hot stage apparatus and uncorrected. After the coupling reaction in 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.

Z-Val-Phe-OMe——To a stirred mixture of Z-Val (2.52 g, 10 mM) and Phe-OMe·HCl (2.16 g, 10 mM) in DMF (30 ml) was added DPPA (3.30 g, 12 mM) in DMF (10 ml) at −5—2°, followed by the addition of TEA (2.23 g, 22 mM) in DMF (10 ml). The mixture was stirred under ice-cooling for 2 hr, and then at room temperature for 40 hr. After dilution with ethyl acetate (300 ml), the mixture was washed as usual. Drying the organic extracts followed by evaporation gave an oily solid, which was recrystallized from benzene–hexane to give Z-Val-Phe-OMe (3.87 g, 94%), mp 130—132° (lit.[10] 132—134° (benzene–pet.ether)). Recrystallization from methanol afforded a colorless crystalline powder, mp 141—142° (lit.[11] 139—140° (methanol)), $[\alpha]_D^{20}$ +16.4° (c=1.1, dioxane) (lit.[10] 14.7°±2° (c=1.1, dioxane)). *Anal.* Calcd. for $C_{23}H_{28}O_5N_2$: C, 66.97; H, 6.84; N, 6.79. Found: C, 66.81; H, 6.81; N, 6.88.

Val-Phe-OMe——A stirred solution of Z-Val-Phe-OMe (3.50 g, 8.5 mM) in methanol (100 ml) was hydrogenated for 5 hr in the presence of 5% palladium–carbon (1 g) and 18% methanolic hydrogen chloride (2 g, 10 mM). The catalyst was removed by filtration and the filtrate was evaporated. The crystalline residue was dried over potassium hydroxide *in vacuo* to give the hydrochloride[11] of Val-Phe-OMe (2.68 g, quantitative), which was used directly for the next step.

Z-Ala-Val-Phe-OMe——The hydrochloride of Val-Phe-OMe (1.57 g, 5 mM) and Z-Ala (1.23 g, 5.5 mM) were dissolved in DMF (50 ml) and cooled to −5—0° with stirring. DPPA (1.65 g, 6 mM) in DMF (10 ml) was added, followed by the addition of TEA (1.12 g, 11 mM) in DMF (10 ml). The mixture was stirred with ice-cooling for 4 hr, and then at room temperature for 5 hr. Ethyl acetate (700 ml) and benzene (200 ml) were added, and the mixture was washed as usual. Drying followed by evaporation gave a colorless semisolid, which was recrystallized from chloroform–hexane to give Z-Ala-Val-Phe-OMe (2.06 g, 85%) as a colorless crystalline powder, mp 208—210° (lit.[6] 204—205°), $[\alpha]_D^{20}$ −49.2° (c=1, methanol). *Anal.* Calcd. for $C_{26}H_{33}O_6N_3$: C, 64.58; H, 6.88; N, 8.69. Found: C, 64.63; H, 6.80; N, 8.58.

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Ala-Val-Phe-OMe (III)——A stirred solution of Z-Ala-Val-Phe-OMe (560 mg, 1.16 mM) in methanol (50 ml) was hydrogenated for 7 hr in the presence of 5% palladium–carbon (150 mg). Filtration followed by evaporation gave III (405 mg, quantitative) as a colorless oil, which was used directly for the next step.

Z-Asp(OBu^t)-Ala-Val-Phe-OMe——To a stirred mixture of III (944 mg, 2.7 mM) and Z-Asp(OBu^t)[12] (1.05 g, 3.23 mM) in DMF (32 ml) was added DPPA (889 mg, 3.23 mM) in DMF (4 ml) with ice-cooling, followed by the addition of TEA (326 mg, 3.23 mM) in DMF (4 ml). The mixture was stirred with ice-cooling for 6 hr, and then at room temperature for 20 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 crystalline powder, which was washed with diethyl ether (30 ml) to give the tetrapeptide derivative (1.66 g, 94%). Recrystallization from methanol and diethyl ether afforded a colorless crystalline powder, mp 204—206°, $[\alpha]_D^{20}$ −12.0° (c=1, DMF). *Anal.* Calcd. for $C_{34}H_{46}O_9N_4$: C, 62.37; H, 7.08; N, 8.56. Found: C, 62.23; H, 6.99; N, 8.41.

Asp(OBu^t)-Ala-Val-Phe-OMe——A stirred solution of Z-Asp(OBu^t)-Ala-Val-Phe-OMe (1.15 g, 1.76 mM) in methanol (100 ml) was hydrogenated over 5% palladium–carbon (200 mg) for 7 hr. Filtration followed by evaporation afforded the deblocked tetrapeptide (917 mg, quantitative), which was used directly for the next step.

Z-Ser-Asp(OBu^t)-Ala-Val-Phe-OMe (IV)——i) To a stirred mixture of Z-Ser-Asp(OBu^t)[1] (548 mg, 1.28 mM) and III (405 mg, 1.16 mM) in DMF (24 ml) was added DPPA (383 mg, 1.39 mM) in DMF (3 ml) with ice-cooling, followed by the addition of TEA (156 mg, 1.54 mM) in DMF (3 ml). The mixture was stirred with ice-cooling for 6 hr, and then at room temperature for 20 hr. Ethyl acetate–benzene (4: 1, 1 liter) was added, and the mixture was washed as usual. Drying followed by evaporation afforded a colorless solid, which was washed with diethyl ether (50 ml) and recrystallized from methanol to give IV (655 mg, 77%) as a colorless crystalline solid, mp 211—213°, $[\alpha]_D^{20}$ −16.4° (c=1, DMF). *Anal.* Calcd. for $C_{37}H_{51}O_{11}N_5 \cdot 1/2H_2O$: C, 59.18; H, 6.98; N, 9.33. Found: C, 59.40; H, 7.03; N, 9.48.

ii) To a stirred mixture of Z-Ser (411 mg, 1.72 mM) and Asp(OBu^t)-Ala-Val-Phe-OMe (745 mg, 1.43 mM) in DMF (10 ml) was added DEPC[9] (281 mg, 1.72 mM) in DMF (3 ml) with ice-cooling, followed by the addition of TEA (174 mg, 1.72 mM) in DMF (3 ml). The mixture was stirred with ice-cooling for 6 hr, and at room temperature for 20 hr. Ethyl acetate-benzene (4: 1, 500 ml) were added, and the mixture was washed as usual. The organic extracts were dried and evaporated to give a crystalline solid, which was washed with diethyl ether (30 ml) to give IV (856 mg, 81%). Recrystallization from methanol afforded a colorless crystalline solid, mp 211—213°, $[\alpha]_D^{20}$ −16.2° (c=1, DMF), which was completely identical with the sample obtained in i).

Ser-Asp(OBu^t)-Ala-Val-Phe-OMe——A stirred solution of IV (1.11 g, 1.5 mM) in methanol (100 ml) was hydrogenated over 5% palladium–carbon (200 mg) for 15 hr. Filtration followed by evaporation gave the deblocked pentapeptide (910 mg, quantitative), which was used directly for the next step.

Z-His-Ser-Asp(OBu^t)-Ala-Val-Phe-OMe (V)——To a stirred mixture of Z-His (500 mg, 1.73 mM) and Ser-Asp (OBu^t)-Ala-Val-Phe-OMe (910 mg, 1.5 mM) in DMF (10 ml) was added DPPA (577 mg, 2.08 mM) in DMF (2 ml) with ice-cooling, followed by the addition of TEA (175 mg, 1.73 mM) in DMF (3 ml). The mixture was stirred with ice-cooling for 4 hr, and then at room temperature for 15 hr. Ethyl acetate (500 ml) was added to the mixture, which was washed as usual. Drying the organic extracts followed by concentration to 50 ml gave the protected hexapeptide (761 mg, 58%) as colorless crystals. Recrystallization from methanol afforded a colorless crystalline powder, mp 199—201°, $[\alpha]_D^{20}$ −15.2° (c=1, DMF). *Anal.* Calcd. for $C_{43}H_{58}O_{12}N_8 \cdot H_2O$: C, 57.57; H, 6.74: N, 12.49. Found: C, 57.69; H, 6.62; N, 12.43.

His-Ser-Asp-Ala-Val-Phe (II)——The protected hexapeptide (V, 202 mg, 0.23 mM) was dissolved in trifluoroacetic acid (5 ml). After 1 hr stirring at room temperature, the trifluoroacetic acid was removed *in vacuo* and diethyl ether was added to the residue. The precipitate was washed on a filter with diethyl ether and dried over potassium hydroxide *in vacuo* to give the trifluoroacetate of Z-His-Ser-Asp-Ala-Val-Phe-OMe (190 mg, quantitative). A solution of this material (190 mg) in methanol (100 ml) was stirred and hydrogenated in the presence of 5% palladium–carbon (50 mg) for 24 hr. Filtration and evaporation gave His-Ser-Asp-Ala-Val-Phe-OMe (150 mg). This hexapeptide ester (150 mg) was dissolved in 1% aq. ammonium carbonate (15 ml), and 0.2% α-chymotrypsin in 1 mM acetic acid (0.3 ml) was added. After 4 hr at room temperature, 0.2% α-chymotrypsin in 1 mM acetic acid (0.3 ml) was added and the mixture was allowed to stand at 20° overnight. Lyophilization gave a colorless powder, which was dissolved in water (10 ml) and the solution kept on a boiling water bath for 5 min and then cooled with an ice-bath. The resulting precipitates were filtered, and the filtrate was lyophilized. The residue was purified by chromatography on a column of Sephadex G-10 (2.5×70 cm) with water to give His-Ser-Asp-Ala-Val-Phe (II) (55 mg, 36%) as a colorless powder, mp 175—180°. Paper chromatography (Toyo filter paper No. 51A): single spot ninhydrin and Pauly positive; *Rf* 0.17 (*n*-butanol–acetic acid–water, 7: 1: 2) and 0.37 (*n*-butanol–acetic acid–pyridine–water, 36: 6: 24: 20). Amino acid ratios in an acid hydrolysate and AP-M digest (number in bracket): His 0.88 (0.95), Ser 0.95 (0.92), Asp 1.19 (1.10), Ala 1.14 (1.05), Val 0.99 (1.09), Phe 0.92 (1.01); average recovery 80% (80%).

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