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Amino Acids and Peptides. IX.[1] Phosphorus in Organic Synthesis. IV.[2] Diphenyl Phosphorazidate. A New Convenient Reagent for the Peptide Synthesis[3]

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Preliminary investigations on the interaction between diethyl phosphorazidate and benzoic acid revealed that phosphoryl azides may be a convenient reagent for a modified Curtius reaction and the amide bond formation. Among phosphoryl azides, diphenyl phosphorazidate (DPPA) which was easily prepared by the action of sodium azide on diphenyl phosphorochloridate was chosen for the investigation on the racemization-free peptide synthesis. The Young test under various conditions proved that DPPA may be a new convenient reagent for the racemization-free peptide synthesis. The results are summarized in Tables.

It is well known that mixed anhydrides of carboxylic acids with phosphoric acids (RCO_2-$PO(OR')_2$) play an important role in the biosynthesis of proteins.[5] We have had a considerable interest on the chemical properties of the mixed anhydrides of this type in hoping to realize the shift of the *in vivo* reactions to the *in vitro* ones in the biomimetic sence.[6] After several trials, we found a new practical method of the peptide synthesis based on the reaction between phosphoryl azides and carboxylic acids.

As a preliminary experiment, benzoic acid was allowed to react with diethyl phosphorochloridate in the presence of triethylamine to give benzoic diethylphosphoric anhydride,[7] which quantitatively afforded benzazide on treatment with sodium azide.

$$PhCO_2H + ClPO(OEt)_2 \xrightarrow{Et_3N} PhCO_2PO(OEt)_2 \xrightarrow{NaN_3} PhCON_3 \quad (Ph=\text{phenyl})$$

Two steps are required to convert carboxylic acids to carboxylic acid azides in this case. However, we considered that the reaction would proceed in a single operation if phosphoryl azides were used instead of phosphoryl chlorides, as followed.

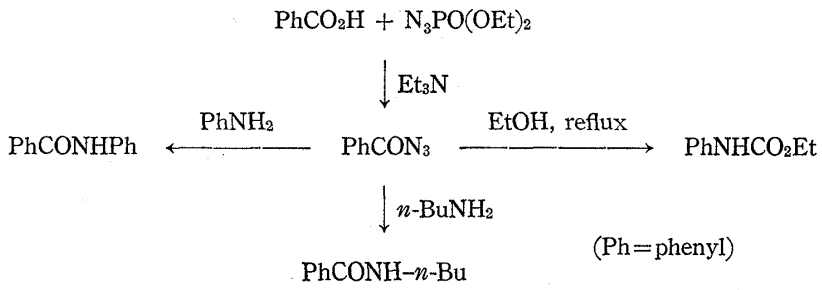
$$RC\bar{O}_2\overset{+}{B}H + N_3PO(OR')_2 \longrightarrow [RCO_2PO(OR')_2 + \bar{N}_3\overset{+}{B}H] \longrightarrow RCON_3 + \overset{+}{B}H\bar{O}PO(OR')_2$$

B: base

Indeed, when diethyl phosphorazidate[8] was allowed to react with benzoic acid in methylene chloride in the presence of triethylamine, a thin-layer chromatography of the reaction mixture revealed the formation of benzazide. Aniline was added to the reaction mixture

1) Part VIII: Y. Takeuchi and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **22**, 841 (1974).
2) Part III: Ref. 1).
3) Preliminary communication: T. Shioiri, K. Ninomiya, and S. Yamada, *J. Am. Chem. Soc.*, **94**, 6203 (1972). Presented in part at the 9th Symposium on Peptide Chemistry, Shizuoka, November 24, 1971, Abstracts, p. 5; and at the Symposium on Organophosphorus Compounds, Tokyo, January 20, 1972, Abstracts, p. 17.
4) Location: *7-3-1, Hongo, Bunkyo-ku, Tokyo, 113, Japan.*
5) A.L. Lehninger, "Biochemistry. The Molecular Basis of Cell Structure and Function," Worth Publishers, New York, 1970, pp. 689—711.
6) R. Breslow, *Chem. Soc. Rev.*, **1**, 553 (1972).
7) F. Cramer and M. Winter, *Chem. Ber.*, **94**, 989 (1961).
8) F.L. Scott, R. Riordan, and P.D. Morton, *J. Org. Chem.*, **27**, 4255 (1962).

to give benzanilide in 71% yield. N-*n*-Butylbenzamide was also obtained in 50% yield by the action of *n*-butylamine. On the other hand, ethanol was added to the above reaction mixture, and refluxing the mixture caused the Curtius rearrangement to furnish ethyl carbanilate in 58% yield.



The Curtius reaction proceeds in two-step operation, but refluxing a mixture of benzoic acid, triethylamine, and diethyl phosphorazidate in ethanol directly gave ethyl carbanilate in 62% yield.

Since these reactions would be initiated by the attack of the carboxylate anion to the phosphorus atom of the phosphoryl azide, these reactions were considered to proceed much more smoothly when a phosphoryl azide containing a more electron withdrawing group at the phosphorus atom is used. The group we chose was phenyl.

Thus, diphenyl phosphorazidate (DPPA)[9] was prepared by the action of sodium azide on diphenyl phosphorochloridate in acetone. DPPA is a stable, non-explosive liquid, bp 157° (0.17 mmHg), and shows a characteristic strong absorption of the azido group at 2200 cm^{-1} in the infrared spectrum (neat).[8]

Using this phosphoryl azide, we have investigated the peptide bond formation and the modified Curtius reaction, the former of which will be the subject of this paper.[10]

Although there are so many methods for the peptide formation reaction, considerable efforts to find out the racemization-free methods of peptide synthesis are still actively continuing. Recently many interesting methods using ingeniously designed coupling reagents have been discovered.[1,11] However, the well-known azide method[12] is still the only reliable method extensively employed for the racemization-free fragment condensation in the peptide synthesis, though this has serious disadvantages, mainly troublesome of the operation, low yields and contamination of the product with by-products. The coupling of a carboxyl component with an amino component by DPPA was presumed to proceed *via* a carboxylic acid azide[13] and to be substituted for the azide method.

To investigate the racemization during the peptide bond formation, we adopted the supersensitive Young test[14] involving the synthesis of benzoylleucylglycine ethyl ester from benzoyl-L-leucine and glycine ethyl ester.

Anderson, *et al.*[15] extensively investigated the factors which affect yield and racemization in peptide synthesis by the mixed carbonic anhydride method, and reported that ethyl

9) In our preliminary communication,[3] the name "diphenylphosphoryl azide" was chosen to stress the transfer of the azido function from phosphoryl azides to carboxylic acids. However, we will adopt the name "diphenyl phosphorazidate" according to the nomenclature by Chemical Abstracts from this paper.

10) The details of the modified Curtius reaction using DPPA will be reported elsewhere.

11) For a summary of reagents for peptide formation, see Y.S. Klausner and M. Bodanszky, *Synthesis*, **1972**, 453.

12) T. Curtius, *Ber.*, **35**, 3226 (1902); E. Schröder and K. Lübke, "The Peptides," Vol. I, Academic Press, New York and London, 1965, p. 79.

13) Some comments on the mechanism of the DPPA method will be presented in the following paper of this series.

14) M.W. Williams and G.T. Young, *J. Chem. Soc.*, **1963**, 881.

15) G.W. Anderson, J.E. Zimmermann, and F.M. Callahan, *J. Am. Chem. Soc.*, **89**, 5012 (1967).

acetate gave the best yields and no racemate in the Anderson test[16] under the specified conditions. Although the DPPA method will proceed through a kind of carboxylic phosphoric anhydride[13] but not a mixed carbonic anhydride, we first examined the Young test in ethyl acetate.

The use of equimolecular benzoyl-L-leucine and free glycine ethyl ester resulted in a rather low yield of the product though its optical purity was high. This will be due to the poor formation of the carboxylate anion from the carboxyl component. The increase of the amount of the amino component gave rise to the increase of the yield accompanying the high optical purity of the product. The use of 1 mole of benzoyl-L-leucine and 2 moles of DPPA and glycine ethyl ester, respectively, gave the excellent result as shown in Table I.

Table I. The Young Test in Ethyl Acetate[a]

Bz-L-Leu-OH (equiv.)	DPPA (equiv.)	H-Gly-OEt (equiv.)	Bz-Leu-Gly-OEt			
			Yield (%)	mp (°C)	$[\alpha]_D$	L-Isomer (%)
1	1.1	1	37.5	153—156	−30.3	89
1	1.1	2	68	158—159	−32.8	96.5
1	1.1	3	77	155—157	−31.9	94
1	2	2	89	157—159	−32.5	96

a) The reactions were carried out at 0° for 24 hr.

Next we investigated the coupling in dimethylformamide which is very common to the peptide synthesis. Again the use of 2 equivalents of glycine ethyl ester gave the product with high optical purity in good yield. In contrast with the coupling in ethyl acetate, 1.2 equivalents of DPPA is sufficient for the coupling in dimethylformamide. As the use of 2 equivalents of the amino component is, however, not advantageous, investigations were made to use tertiary amines to generate the carboxylate anion. As is shown in Table II, triethylamine can be used favorably as well as N-methylmorpholine which is demonstrated by Anderson, *et al.*[15] as an amine suitable for both the Anderson and Young tests. Furthermore, no apparent increase of racemization and decrease of yield were observed when glycine ethyl ester hydrochloride and an equivalent of triethylamine were used in place of free glycine ester, showing that no or little "chloride effect"[17] was present in this new coupling procedure.

Table II. Effect of Bases on the Young Test[a]

Bz-L-Leu-OH (equiv.)	Base (equiv.)	DPPA (equiv.)	H-Gly-OEt (equiv.)	Bz-Leu-Gly-OEt			
				Yield (%)	mp (°C)	$[\alpha]_D$	L-Isomer (%)
1	—	1.2	2	89	157—158	−31.5	93
1	1[b]	1.1	1.1	80	153—156	−30.3	89
1	1[c]	1.1	1.1	80	154—157	−31.5	93
1	2[c]	1.1	1.1[d]	81	154—156	−31.1	92

a) The reactions were carried out in dimethylformamide at 0° for 24 hr.
b) N-methylmorpholine
c) triethylamine
d) H-Gly-OEt·HCl was used.

16) G.W. Anderson and R.W. Young, *J. Am. Chem. Soc.*, **74**, 5307 (1952).
17) a) M.W. Williams and G.T. Young, *J. Chem. Soc.*, **1964**, 3701; b) A.W. Williams and G.T. Young, *J. Chem. Soc. Perkin I*, **1972**, 1194.

Accordingly, glycine ethyl ester hydrochloride which is much easier to handle was used together with triethylamine in subsequent experiments.

In a general procedure, DPPA (1.1 equivalents) in an appropriate solvent was added to a stirred mixture of benzoyl-L-leucine (1 equivalent) and glycine ethyl ester hydrochloride (1.1 equivalents) in the same solvent at or below 0°, followed by the addition of triethylamine (2.1 equivalents), and the mixture was stirred at or below room temperature for several hours.

Table III shows the effect of reaction temperatures in dimethylformamide. As expected, the content of L-isomer increased with decreasing the coupling temperature in company with decrease of the yield.

Table III. The Young Test in Dimethylformamide[a]

Bz-L-Leu-OH (equiv.)	DPPA (equiv.)	H-Gly-OEt·HCl (equiv.)	Reac. temp (°C)	Reac. time (hr)	Bz-Leu-Gly-OEt Yield (%)	mp (°C)	$[\alpha]_D$	L-Isomer (%)
1	1.1	1.1	21	6	87	156—158	−30.9	91
1	1.1	1.1	0	0.5	69.5	156—158	−31.2	92
			20	0.5				
1	1.3	1.3[b]	0	0.5	88	157—158	−31.0	91
			20	3				
1	1.1	1.1	−15	0.5				
			0	0.5	76	158—160	−31.1	91.5
			20	1				
1	2	1.1	−20	1				
			0	1	84.5	157—158	−31.5	93
			20	2				
1	1.1	1.1	−20	7				
			0	15	83	158—159	−32.1	94.5
			20	2				
1	1.1	1.1	−20	6	59	158—160	−32.9	97
1	1.1	1.1	−20	24	71	157—159	−32.9	97

a) 2.1 Equiv. of triethylamine were used unless otherwise stated.
b) 2.3 Equiv. of triethylamine were used.

Table IV. Solvent Effect on the Young Test[a]

Solvent	Bz-Leu-Gly-OEt Yield (%)	mp (°C)	$[\alpha]_D$	L-Isomer (%)
CH_2Cl_2	73	147—153	−27	79.5
CH_2Cl_2[b]	76	155—157	−29.1	86
$((CH_3)_2N)_3PO$[c]	69	154—155	−27.7	81.5
CH_3CN	83	154—156	−28.9	85
$CH_3CON(CH_3)_2$	81	155—158	−30.5	90
Dioxane–$CH_3CO_2CH_2CH_3$ (2:1)	84.5	156—158	−31.0	91
$CH_3CO_2CH_2CH_3$	84.5	155—157	−31.0	91
Tetrahydrofuran	85	156—158	−31.2	92

a) Unless otherwise stated, the reactions were carried out at 0° for 0.5 hr and then at 20° for 4 hr using 1 equiv. of Bz-L-Leu-OH, 1.1 equiv. of DPPA, 1.1 equiv. of H-Gly-OEt·HCl, and 2.1 equiv. of triethylamine.
b) The reaction was carried out at 0° for 24 hr.
c) The reaction was carried out at 7—11° for 10 min, and at 20° for 6 hr.

Solvents also exerted considerable influence in the Young test not only on racemization but on yield. Dimethylformamide, tetrahydrofuran, dioxane, and ethyl acetate were more advantageous than methylene chloride, hexamethylphosphoramide, and acetonitrile as shown

in Table IV. Usability of a highly polar solvent dimethylformamide in which even large peptides are often soluble widens the scope of this new method.

The principal advantages of DPPA over the other coupling reagents are that DPPA may make possible to couple peptides with peptide esters without racemization, the coupling proceeds very mildly, and DPPA is readily available.[18]

The mechanism of the DPPA method and application to the synthesis of peptides containing various functions will be reported shortly.

Experimental

Unless otherwise stated, melting points were determined on a hot stage apparatus and uncorrected. Infrared spectra were determined in nujol mulls.

Benzoic Diethylphosphoric Anhydride——To benzoic acid (12.2 g, 0.1 mole) in dry diethyl ether (150 ml) was added triethylamine (10.1 g, 0.1 mole) at 0°, followed by the addition of diethyl phosphorochloridate (17.2 g, 0.1 mole) in dry diethyl ether (20 ml) during 5 min. The mixture was stirred at room temperature overnight, and filtered. The filtrate was evaporated *in vacuo* to give a slightly yellow oil. Distillation at 154—164° (0.16 mmHg) gave a colorless oil (21.6 g, 84%), whose infrared spectrum (neat) was identical with that of the literature.[7]

Benzazide——To sodium azide (0.39 g, 6 mmoles) in water (1.5 ml) was added with stirring benzoic diethylphosphoric anhydride (1.29 g, 5 mmoles) in diethyl ether (5 ml) at room temperature. The mixture was stirred at room temperature for 1.5 hr. The aqueous layer was extracted with diethyl ether. The combined ethereal extracts were washed with water, saturated aqueous sodium chloride, and dried over sodium sulfate. Evaporation gave 0.74 g (quantitative) of a colorless oil, which solidified with ice-cooling. The infrared spectrum was identical with that of the authentic benzazide prepared by the action of sodium azide on benzoyl chloride.[19]

Reaction of Benzoic Acid with Diethyl Phosphorazidate in the Presence of Triethylamine——To a mixture of benzoic acid (0.366 g, 3 mmoles) and triethylamine (0.31 g, 3 mmoles) in methylene chloride (8 ml) was added diethyl phosphorazidate (0.54 g, 3 mmoles) in methylene chloride (4 ml). The mixture was stirred at room temperature for 43 hr, diluted to 18 ml with methylene chloride, and was divided into three parts (6 ml, 6 ml, and 5.6 ml).

i) Benzanilide: To the above reaction mixture (6 ml) was added aniline (0.3 ml), and the mixture was allowed to stand at room temperature for 4 days. The mixture was evaporated to give a residue, which was dissolved in ethyl acetate. The ethyl acetate solution was successively washed with 10% aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. Drying over sodium sulfate followed by evaporation gave 143 mg (71%) of colorless crystals, mp 160—161°. The infrared spectrum was identical with that of the authentic benzanilide.[20]

ii) N-*n*-Butylbenzamide: To the above methylene chloride solution (6 ml) was added *n*-butylamine (0.3 ml), and the mixture was allowed to stand at room temperature for 2 days. Evaporation followed by a preparative layer chromatography (silica G.F. plates of 1.5 mm thickness, benzene–diethyl ether (5: 1)) gave N-*n*-butylbenzamide (90 mg, 50%), whose infrared spectrum was virtually identical with that of the authentic sample prepared from benzoyl chloride and *n*-butylamine.[21]

iii) Ethyl Carbanilate: To the above methylene chloride solution (5.6 ml) was added ethanol (10 ml), and the mixture was distilled until the boiling point reached 78°. The mixture was refluxed for 2 hr. Evaporation followed by a preparative layer chromatography (silica G.F. plates of 1.5 mm thickness, benzene) gave ethyl carbanilate (92 mg, 58%), whose infrared spectrum was virtually identical with that of the authentic sample prepared from aniline and ethyl chloroformate.[22]

Ethyl Carbanilate (a Modified Curtius Procedure)——A mixture of benzoic acid (0.62 g, 5 mmoles), triethylamine (0.505 g, 5 mmoles), and diethyl phosphorazidate (0.895 g, 5 mmoles) in ethanol (15 ml) was stirred at reflux for 19 hr. Evaporation gave a colorless viscous oil, which was treated with ethyl acetate and 2% aqueous hydrochloric acid. The ethyl acetate layer was washed with water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. Drying over sodium sulfate followed by evaporation gave a colorless oil, which was purified by a preparative layer chromatography (silica G.F. plates of 1.5 mm thickness, benzene) to give 0.51 g (62%) of ethyl carbanilate.

18) DPPA is now commercially available from Go-ei Shoji, Co. & Ltd., Osaka, Japan, Willow Brook Laboratories, Inc., and Aldrich Chemical Co. Inc., Wisconsin, U.S.A.
19) E.W. Barrett and C.W. Porter, *J. Am. Chem. Soc.*, **63**, 3434 (1941).
20) "IRDC Data Card," Nankodo Co., Tokyo, 1960, No. 33.
21) N.J. Leonard and E.W. Nommensen, *J. Am. Chem. Soc.*, **71**, 2808 (1949).
22) *cf.* Th. Wilm and G. Wischin, *Ann.*, **147**, 157 (1868).

Diphenyl Phosphorazidate (DPPA)——A mixture of diphenyl phosphorochloridate (56.8 g, 0.21 moles) and sodium azide (16.3 g, 0.25 moles) in dry acetone was stirred at room temperature for 21 hr. The filtered solution was concentrated, and the residue was distilled at 152—155° (0.15 mmHg) to give 53.3 g (92%) of a colorless oil. For analysis a sample was redistilled at 157° (0.17 mmHg). *Anal.* Calcd. for $C_{12}H_{10}O_3N_3P$: C, 52.37; H, 3.66; N, 15.27; mol. wt. 275.20. Found: C, 52.12; H, 3.70; N, 15.31. Mass Spectrum *m/e*: 275 (M^+).

Benzoyl-L-leucine——Prepared according to the literature;[14] mp 106—107°, $[\alpha]_D^{24}$ −7.1° (c=2.54, ethanol) (lit.[14] mp 106°, $[\alpha]_D^{23}$ −6.9° (c=2.6, ethanol)). Its optical purity was confirmed by its conversion to the cyclohexylammonium salt, mp 144—145°, $[\alpha]_D^{20}$ +14.8° (c=4.26, ethanol) (lit.[14] mp 145—146°, $[\alpha]_D^{19}$ +14.4° (c=4.25, ethanol)).

The Young Test; General Procedure——To a stirred mixture of benzoyl-L-leucine (0.47 g, 2 mmoles) and glycine ethyl ester hydrochloride (0.31 g, 2.2 mmoles) in dimethylformamide (5 ml) was added DPPA (0.605 g, 2.2 mmoles) in dimethylformamide (5 ml) at or below 0°, followed by the addition of triethylamine (0.42 g, 4.2 mmoles) in dimethylformamide (5 ml) during 5—10 min. The mixture was stirred at or below 0° and then at room temperature (*ca.* 20°). More precise reaction conditions in each case were described in each Table.

The reaction mixture was diluted with a mixture of benzene (50 ml) and ethyl acetate (100 ml), and successively washed with 5% aqueous hydrochloric acid (2×10 ml), water (10 ml), saturated aqueous sodium chloride (2×10 ml), saturated aqueous sodium bicarbonate (2×10 ml), water (10 ml), and saturated aqueous sodium chloride (2×10 ml). Drying over sodium sulfate followed by evaporation gave colorless crystals, which were subjected to a silica gel column chromatography (Wakogel C-200, 70—140 g). Elution with a mixture of chloroform and ethyl acetate (10: 1) gave colorless crystals of benzoylleucylglycine ethyl ester whose structure was confirmed by thin layer chromatographic and infrared spectral comparisons with the authentic ones.[14] These crystals were weighed, and the mp (lit. values[14]: L-isomer, 156.5—157°; racemate, 146°) was determined. Optical purity of each product was expressed as "L-isomer, %" which was calculated by the equation: observed $[\alpha]_D \times 100/-34°$ (optical rotation of pure benzoyl-L-leucylglycine ethyl ester).[14,17b]