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Amino Acids and Peptides. XI.[1] Phosphorus in Organic Synthesis. VI.[2] Application of Diphenyl Phosphorazidate to the Synthesis of Peptides containing Various Functions[3]

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Application of diphenyl phosphorazidate (DPPA) to the synthesis of peptides revealed that the DPPA method is quite general because it can be applied to the formation of peptides containing various functional groups. No difficulties were encountered when the side chains of serine, threonine, valine, glutamine, asparagine, methionine, histidine, tryptophan, and pyroglutamic acid were present in the carboxyl component. Arginine and cysteine showed no trouble when their side chain functions were protected with the nitro and benzyl groups, respectively. Serine, tyrosine, and histidine could be used as the amino component without any protection of the side chains. Fragment coupling of N-benzyloxycarbonylleucylleucine with valylphenylalanine methyl ester hydrochloride by DPPA showed that no racemization occurred.

In previous papers of this series, we reported diphenyl phosphorazidate (DPPA) may be used for the racemization-free peptide synthesis[5] and the mechanism of the DPPA method have been discussed.[1] We report here application of DPPA to the synthesis of peptides containing various functions in side chains.[6] The results are summarized in Tables I—III.[7]

Protection of both aliphatic hydroxyl groups as in serine and threonine and phenolic group as in tyrosine is in some cases required during the peptide bond formation; otherwise ester formation, β-elimination or other undesirable side reactions might occur. However the DPPA method proceeded smoothly as shown in examples 1—4. It is well-known that the ω-amido group of both asparagine and glutamine are dehydrated to give nitriles by N,N'-dicyclohexylcarbodiimide, but no dehydration was observed by DPPA (examples 4—6). N-Benzyloxycarbonyl derivatives of sulfur containing amino acids as methionine and cysteine whose mercapto function was protected with benzyl group smoothly coupled with amino acid esters by DPPA. Although many peptide syntheses are carried out without protection of the basic imidazole ring in the side chain of histidine, unprotection poses a number of problems for the synthesis of histidine containing peptides. DPPA allowed to couple smoothly N-benzyloxycarbonylhistidine with leucine methyl ester. Arginine showed no trouble when its basic guanidino group was protected with the ordinary nitro group. As in the case of N-benzyloxycarbonyl amino acids, N-*tert*-butyloxycarbonyl derivatives smoothly combined with amino acid esters by DPPA (see examples 11 and 12).

The sequence of pyroglutamyl-histidine is known as N-terminal sequence of hypothalamo-hypophysiotropic hormones[8] such as thyrotropin-releasing hormone and luteinizing hormone-

1) Part X: T. Shioiri and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), 22, 855 (1974).
2) Part V: Ref. 1).
3) Preliminary communication: T. Shioiri, K. Ninomiya, and S. Yamada, *J. Am. Chem. Soc.*, 94, 6203 (1972). Presented in part at the 10th Symposium on Peptide Chemistry, Sapporo, September 26, 1972, Abstracts p. 15.
4) Location: *Hongo, Bunkyo-ku, Tokyo, 113, Japan.*
5) T. Shioiri and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), 22, 849 (1974).
6) *cf.* E. Schröder and K. Lübke, "The Peptides," Vol. I, Academic Press, New York and London, 1965.
7) All optically active amino acids are of L-configuration.
8) A. Arimura, H. Matsuo, and Y. Baba, *Tanpakushitsu Kakusan Koso*, 17, 479 (1972); K. Folkers, N.-G. Johansson, F. Hooper, B. Currie, H. Sievertsson, J.-K. Chang, and C.Y. Bowers, *Angew. Chem. Intern. Ed. Engl.*, 12, 255 (1973).

releasing hormone. Although pyroglutamylhistidine methyl ester was already synthesized[9] from pyroglutamic acid and histidine methyl ester by N,N'-dicyclohexylcarbodiimide, the yield was 55% and it seems rather troublesome to separate the dipeptide ester and N,N'-dicyclohexylurea. Thus the coupling of pyroglutamic acid with histidine methyl ester was carried out in acetonitrile by DPPA. As the dipeptide ester precipitated out during the reaction, the reaction mixture was simply filtered and pyroglutamylhistidine methyl ester was obtained in 72% yield.

Leucylleucylvalylphenylalanine methyl ester was reported as a renin inhibiting peptide.[10] As a model for the fragment coupling, N-benzyloxycarbonylleucylleucine was coupled with

Table I. Preparation of Peptide Derivatives by the DPPA Method

Example	Peptide derivative[a,b]	Reaction time		Yield (%)
		hr at 0°	hr at room temp.	
1	Z-Ser-Tyr-OMe	0.5	4	63
2	Z-Thr-Phe-OMe	2	21	72.5
3	Z-Val-Tyr-OMe	2	18	69
4	Z-Gln-Ser-OMe	2	21	80
5	Z-Asn-Gly-OEt[c]	1	19	70
6	Z-Asn-Phe-OMe[d,e]	1	20	80
7	Z-Met-Gly-OEt[d]	4	14	90
8	Z-Cys(Bzl)-Val-OMe	2	22	76
9	Z-His-Leu-OMe	4	21	95
10	Z-Arg(NO_2)-Leu-OMe	1	19	83
11	Boc-Trp-Gly-OEt[d]	4	14	89.5
12	Boc-Trp-Val-OMe	2	22	85
13	Glu-His-OMe[d,f]	3	14	72
14	Z-Leu-Leu⋮Val-Phe-OMe[g]	4	21	87
15	Z-Leu-Leu⋮Val-Phe-OMe[g,h]	3	16	63

a) Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1971).
b) Hydrochlorides of the amino components were used and the reaction solvent was dimethylformamide unless otherwise stated.
c) The reaction solvent was a mixture of dimethylformamide and ethyl acetate (1: 1).
d) Free esters were used as amino components.
e) The reaction solvent was a mixture of dimethylformamide and ethyl acetate (1: 2).
f) The reaction solvent was acetonitrile.
g) The dotted line indicates the point of coupling.
h) After Z-Leu-Leu-OMe was saponified, the reaction mixture was directly subjected to coupling.

Table II. Physical Data of Peptide Derivatives

Exa.	Peptide derivative	mp (°C)		$[\alpha]_D$ Obs.[c] (temp., *c*, solv.)	Lit.[b]
		obs.[a]	lit.[b]		
1	Z-Ser-Tyr-OMe	114—116	115—116[d]	+11.7 (21, 2.1, DMF)	+13.5
2	Z-Thr-Phe-OMe	106—108	105—107[e]	+ 5.6 (21, 0.9, DMF)	+ 4.0
3	Z-Val-Tyr-OMe	157—158	155.5—156[f]	+13.6 (25, 4.8, pyridine)	+10.2
4	Z-Gln-Ser-OMe	166—167 178—182	167[g] 182—185	− 9.6 (20, 1.0, MeOH)	—
5	Z-Asn-Gly-OEt	188—190	188—189[h]	− 6.0 (25, 1.1, DMF)	− 4.4

9) J. Bøler, J.-K. Chang, F. Enzmann, and K. Folkers, *J. Med. Chem.*, **14**, 475 (1971).
10) A. Ide, K. Shigezane, S. Shigezane, T. Mizoguchi, and S. Saito, *Yakugaku Zasshi*, **90**, 850 (1970).

Exa.	Peptide derivative	mp (°C) obs.[a]	mp (°C) lit.[b]	$[\alpha]_D$ Obs.[c] (temp., c, solv.)	Lit.[d]
6	Z-Asn-Phe-OMe	198—200	193—194[i]	+15.0 (23, 1.0, AcOH)	+15.3
7	Z-Met-Gly-OEt	98— 99	96— 97[h]	−17.9 (27, 4.7, EtOH)	−18.6
8	Z-Cys(Bzl)-Val-OMe	79— 81	new	−29.8 (20, 1.0, MeOH)	new
9	Z-His-Leu-OMe	126.5—127	124—126[j]	−25.0 (23, 0.3, EtOH)	−25.2
10	Z-Arg(NO_2)-Leu-OMe	163—164	170—171[k]	−20.7 (30, 0.9, MeOH)	−23.4
11	Boc-Trp-Gly-OEt	118—119	117.5—118[h]	−12.8 (20, 1.1, EtOH)	−12.9
12	Boc-Trp-Val-OMe	oil	new[l]	—	new
13	□—Glu-His-OMe	208—209	210—212[m]	− 4.2 (22, 1.1, MeOH)	− 1.5
14	Z-Leu-Leu-Val-Phe-OMe	209—210	209.5—210[j]	−25.2 (20, 0.9, DMF)	−25.4
15	Z-Leu-Leu-Val-Phe-OMe	207—209	209.5—210[j]	−25.2 (20, 1.1, DMF)	−25.4

a) Measured on a hot stage apparatus, and uncorrected.
b) Literature values of melting points and optical rotations were cited from the same literatures.
c) Observed rotations were measured at the same temperature using the same solvent and approximately the same concentration as those of literature values.
d) S. Guttmann and R.A. Boissonnas, *Helv. Chim. Acta*, **41**, 1852 (1958).
e) H.C. Beyermann and J.S. Bontekoe, *Rec. trav. chim.*, **83**, 255 (1964).
f) H. Schwarz, F.M. Bumpus, and I.H. Page, *J. Am. Chem. Soc.*, **79**, 5697 (1957).
g) E. Sondheimer and R.W. Holley, *J. Am. Chem. Soc.*, **76**, 2816 (1954) reported that Z-Gln-Ser-OMe melted at 182—185° (transition at 167°). Our sample melted at 166—167°, and after resolidification it melted at 178—182°.
h) Y. Takeuchi and S. Yamada, *Chem. Pharm. Bull.* (Tokyo) **22**, 841 (1974).
i) E. Schröder, *Ann.*, **688**, 250 (1965).
j) Reference 10 in the text. Decomposition at the mp.
k) K. Hofmann, W.D. Peckham, and A. Rheiner, *J. Am. Chem. Soc.*, **78**, 238 (1956).
l) Characterized as the hydrazide, mp 216—217° (decomp.), $[\alpha]_D^{22}$ −31.6° (*c*=0.6, MeOH).
m) Reference 9 in the text. D. Gillesen, A.M. Felix, W. Lergier, and R.O. Studer, *Helv. Chim. Acta*, **53**, 63 (1970) reported mp 199—201° and $[\alpha]_D^{25}$ −4.3° (*c*=1, MeOH).

TABLE III. Analysis of Peptide Derivatives

Exa.	Peptide derivative	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
1	Z-Ser-Tyr-OMe	$C_{21}H_{24}O_7N_2$	60.56	5.81	6.73	60.31	5.78	6.66
2	Z-Thr-Phe-OMe	$C_{22}H_{26}O_6N_2$	63.75	6.32	6.76	63.45	6.29	7.04
3	Z-Val-Tyr-OMe	$C_{23}H_{28}O_6N_2$	64.47	6.59	6.54	64.21	6.55	6.43
4	Z-Gln-Ser-OMe	$C_{17}H_{23}O_7N_3$	53.53	6.08	11.02	53.12	5.99	10.83
5	Z-Asn-Gly-OEt	$C_{16}H_{21}O_6N_3$	54.69	6.02	11.96	54.47	5.85	11.92
6	Z-Asn-Phe-OMe	$C_{22}H_{25}O_6N_3$	61.81	5.90	9.83	61.71	5.87	9.92
7	Z-Met-Gly-OEt	$C_{17}H_{24}O_5N_2S$	55.43	6.56	7.61	55.54	6.59	7.50
8	Z-Cys(Bzl)-Val-OMe	$C_{24}H_{30}O_5N_2S$	62.87	6.60	6.11	62.86	6.57	6.01
9	Z-His-Leu-OMe	$C_{21}H_{28}O_5N_4$	60.56	6.78	13.45	60.31	6.80	13.31
10	Z-Arg(NO_2)-Leu-OMe	$C_{21}H_{32}O_7N_6$	52.49	6.71	17.49	52.17	6.68	17.57
11	Boc-Trp-Gly-OEt	$C_{20}H_{27}O_5N_3$	61.68	6.99	10.79	61.78	6.93	10.73
12	Boc-Trp-Val-$NHNH_2$	$C_{21}H_{31}O_4N_5$	60.41	7.48	16.78	60.30	7.44	16.71
13	□—Glu-His-OMe	$C_{12}H_{16}O_4N_4 \cdot 1/2H_2O$	49.82	5.92	19.37	50.12	5.90	19.11
14	Z-Leu-Leu-Val-Phe-OMe	$C_{35}H_{50}O_7N_4$	65.80	7.89	8.77	65.78	7.76	8.90
15	Z-Leu-Leu-Val-Phe-OMe	$C_{35}H_{50}O_7N_4$	65.80	7.89	8.77	65.63	7.90	8.84

valylphenylalanine methyl ester hydrochloride using DPPA to give N-benzyloxycarbonyl-leucylleucylvalylphenylalanine methyl ester whose melting point and optical rotation agreed with those of the literature,[10] in which the tetrapeptide derivative was prepared from the same starting materials by the azide method. On the other hand N-benzyloxycarbonyl-leucylleucine methyl ester was saponified with aqueous sodium hydroxide in dimethylformamide. The reaction mixture was, *in situ*, subjected to coupling with valylphenylalanine methyl ester hydrochloride by DPPA giving the tetrapeptide derivative in 63% yield. In many cases of the fragment coupling, the carboxyl component was isolated in a free state and the coupling with the amino component was carried out after activation of the free carboxyl group. However, the DPPA method has the advantage that a saponified reaction mixture can be directly used for the fragment coupling practically free of racemization.

We are now applying the DPPA method to the synthesis of some larger peptides which are possibly biologically active.

Experimental

The silica gel column chromatography was performed with Wakogel C-200.

Preparation of Peptide Derivatives by the DPPA Method; General Procedure——To a stirred mixture of the carboxyl component (1.5 mmoles) and the hydrochloride of the amino component (1.65—1.8 mmoles) in dimethylformamide (5 ml) was added DPPA (1.65—1.8 mmoles) in dimethylformamide (5 ml) at 0°, followed by the addition of triethylamine (3.15—3.3 mmoles) in dimethylformamide (5 ml) at 0° during 5—10 min. The mixture was stirred at 0°, and then at room temperature (20—25°). The reaction time was described in Table I.

The reaction mixture was diluted with a mixture of benzene and ethyl acetate (2: 1 or 1: 2, 150 ml), successively washed with 5% hydrochloric acid (2×10 ml), water (2×10 ml), saturated aqueous sodium chloride (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 *in vacuo* afforded the crude peptide derivative.

Variations from the general description and purifications of the crude peptide derivatives were shown in each case below. Physical and analytical data were shown in Tables II and III.

1) **Z-Ser-Tyr-OMe**——Purification was made by column chromatography with a mixture of ethyl acetate and chloroform (1: 1).

2) **Z-Thr-Phe-OMe**——Purification was made by column chromatography with a mixture of ethyl acetate and chloroform (1: 3), followed by recrystallization from a mixture of benzene and *n*-hexane.

3) **Z-Val-Tyr-OMe**——Purified by column chromatography with a mixture of ethyl acetate and chloroform (1: 10).

4) **Z-Gln-Ser-OMe**——The reaction mixture was diluted with a mixture of ethyl acetate (100 ml) and benzene (50 ml), and treated successively with 5% aqueous hydrochloric acid (10 ml), water (10 ml), and saturated aqueous sodium chloride (2×10 ml). The aqueous layer was adjusted to pH ~7 with saturated aqueous sodium bicarbonate. After salting-out with crystalline sodium chloride, the mixture was extracted with ethyl acetate (200 ml). The extracts were dried over sodium sulfate and evaporated to give a white solid, which was recrystallized from a mixture of methanol and diethyl ether affording colorless prisms of Z–Gln–Ser–OMe.

5) **Z-Asn-Gly-OEt**——The reaction mixture was diluted with a mixture of ethyl acetate (200 ml) and chloroform (50 ml) to give a small amount of precipitated crystals of Z–Asn–Gly–OEt. The filtrate was treated as in the general description giving the crude peptide, which was washed with a mixture of diethyl ether and *n*-hexane to afford pure Z–Asn–Gly–OEt.

6) **Z-Asn-Phe-OMe**——The colorless crystals of Z–Asn–Phe–OMe were precipitated during the reaction. After dilution with *n*-hexane, the crystals were collected. A small crop of Z–Asn–Phe–OMe was obtained from the usual neutral extracts.

7) **Z-Met-Gly-OEt**——Purified by column chromatography with a mixture of ethyl acetate and *n*-hexane (1: 2).

8) **Z-Cys(Bzl)-Val-OMe**——Purified by column chromatography with a mixture of ethyl acetate and *n*-hexane (1: 3), followed by recrystallization from a mixture of ethyl acetate and petroleum ether.

9) **Z-His-Leu-OMe**——Washing of the diluted reaction mixture was carried out as usual except using 0.5 mole aqueous boric acid in place of 5% aqueous hydrochloric acid. The crude crystals of Z–His–Leu–OMe were washed with a mixture of diethyl ether and *n*-hexane (1: 1), followed by recrystallization from aqueous acetone to give colorless crystals.

10) **Z-Arg(NO_2)-Leu-OMe**——Purified by washing the crude peptide with diethyl ether, followed by recrystallization from a mixture of methanol and diethyl ether to give colorless small prisms.

11) **Boc-Trp-Gly-OEt**——Washing of the diluted reaction mixture was carried out as usual except using 5% aqueous citric acid instead of 5% hydrochloric acid. The crude peptide was purified by column chromatography with ethyl acetate and chloroform (1: 3), followed by recrystallization from a mixture of ethyl acetate and petroleum ether to give colorless needles.

12) **Boc-Trp-Val-OMe and Boc-Trp-Val-$NHNH_2$**——As in 11), 5% aqueous citric acid was used instead of 5% hydrochloric acid. Purification of the crude peptide was made by column chromatography with a mixture of ethyl acetate and *n*-hexane (1: 2) to give a colorless oil of Boc-Trp-Val-OMe.

An oily Boc-Trp-Val-OMe (0.21 g) in methanol (2 ml) was treated with 100% hydrazine hydrate (0.2 ml) at room temperature for 4 days. Dilution of the reaction mixture with diethyl ether gave colorless crystals (0.125 g) of Boc-Trp-Val-$NHNH_2$. Recrystallization from a mixture of methanol and diethyl ether gave colorless small needles.

13) **└Glu—His-OMe**——The white precipitates which appeared during the reaction were filtered, and washed with acetonitrile. Recrystallization from a mixture of methanol and diethyl ether gave colorless leaflets of └Glu—His-OMe.

14) **Z-Leu-Leu-Val-Phe-OMe from Z-Leu-Leu-OH and H-Val-Phe-OMe·HCl**——The crude product was washed with a mixture of diethyl ether and *n*-hexane (1: 1). Recrystallization from ethyl acetate gave colorless small needles.

15) **Z-Leu-Leu-Val-Phe-OMe from Z-Leu-Leu-OMe and H-Val-Phe-OMe·HCl**——To a stirred solution of Z-Leu-Leu-OMe (0.39 g, 1 mmole) in dimethylformamide (10 ml) was added 2N aqueous sodium hydroxide (0.5 ml, 1 mmole) with ice-cooling. The mixture was stirred at 0° for 2 hr. To this mixture was successively added DPPA (0.30 g, 1.1 mmoles), triethylamine (0.105 g, 1 mmole), and H-Val-Phe-OMe·HCl (0.32 g, 1 mmole) in dimethylformamide (5 ml). The mixture was stirred at 0° for 3 hr, and then at room temperature for 16 hr. The reaction mixture was treated as in 14) to give Z-Leu-Leu-Val-Phe-OMe.

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