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AN EFFICIENT METHOD FOR THE SYNTHESIS OF PEPTIDES BY THE USE OF PHENYLPHOSPHONIC ESTERS

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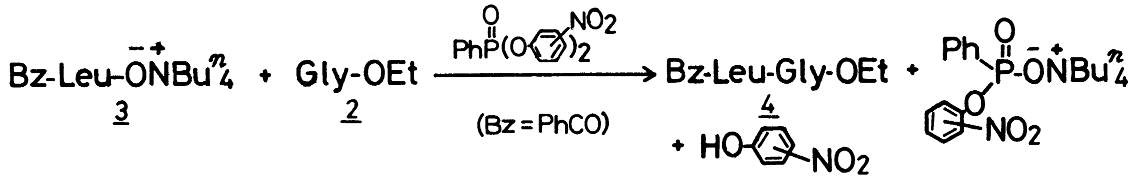
Treatment of the tetrabutylammonium salt of N-benzoyl-L-leucine and glycine ethyl ester with bis(o- or p-nitrophenyl) phenylphosphonate in DMF resulted in the formation of the corresponding dipeptide in excellent yield with high optical purity. Similarly, racemization-free Z-L-Phe-Gly-OEt was prepared in high yield.

Among a wide variety of methods for the formation of peptides, a mixed anhydride method which involves mixed anhydride type intermediates derived from organophosporus compounds and amino acids have so far become of much interest based on the fact that acyl phosphates play an important role in biosynthesis of peptides. However, little have been known about the practical methods except by the employment of diphenyl phosphoroazidate$^{1)}$ and diethyl phosphorocyanidate$^{2)}$ as phosphorus components reported by Yamada et al. Although these coupling agents have demonstrated successful formation of racemization-free peptides, they are sometimes highly toxic and relatively expensive.$^{3)}$ More recently, Gorecka and co-workers have employed diethyl phosphorobromidate as a coupling reagent.$^{3)}$ Their method tends to form racemates by the effect of tertiary amines such as triethylamine and N-methylmorpholine as indicated in the Anderson test, different from other known peptide-forming methods. Accordingly, careful control of the reaction conditions is necessary to avoid racemization. The high reactivity of the bromidate may also raise some problems such as stability during storage and side-reactions in the synthesis of peptides having various functional groups in side chains.

During our investigation on new method of phosphorylation, we have found that tetrabutylammonium acetate is useful for the selective removal of a p-nitrophenyl group in phosphoric triesters, under neutral conditions at room temperature.$^{4)}$ The reaction may proceed by way of a highly reactive intermediate, acetyl phosphate derivative, which is in turn attacked by the nucleophiles existing in the reaction medium. This fact prompted us to develop an efficient method for the synthesis of peptides via mixed anhydrides with phosphorus compounds.

In order to investigate optimum reaction conditions we have chosen the reaction of N-benzoyl-L-leucine($\underline{1}$) with glycine ethyl ester($\underline{2}$) employed in the Young test,$^{5)}$ known as the most severe racemization test. Phosphorus compounds used here can be readily prepared from phenols and phosphorus halides such as ethyl phosphorodichloridate and phenylphosphonic dichloride in the presence of triethylamine and all the aryl esters are stable crystals. The carboxyl component 1 was used as the tetrabutylammonium salt 3 which was readily prepared either by the action of alkaline tetrabutylammonium hydrogen sulfate on 1 or by the application of tetrabutylammonium hydroxide in the presence of sodium hydroxide. The crystalline salt 3 was stocked over P_2O_5 and was applied to the peptide synthesis. More conveniently the residue 3, after extraction with chloroform and subsequent evaporation of the solvent, was directly used without purification. Thus, equimolar amounts of N-benzoyl-L-leucine tetrabutylammonium (3) and glycine ethyl ester (2) was treated with phosphorus esters such as bis(p-nitrophenyl) phenylphosphonate and ethyl bis(p-nitrophenyl) phosphate, respectively, and N-benzoyl-L-leucylglycine ethyl ester (4) was obtained in good to excellent yields. Each optical purity of the dipeptide was estimated by comparison with specific rotation value (-34°) of the pure L-isomer.



The following is procedures for the preparation of tetrabutylammonium N-benzoyl-L-leucinate (3).

Method A: Slightly excess tetrabutylammonium hydroxide (10% methanol solution) and N-benzoyl-L-leucine (1) in chloroform was treated with dilute sodium hydroxide solution and dried over anhydrous magnesium sulfate. After evaporation of the solvent the tetrabutylammonium salt 3 was obtained and dried on phosphorus pentoxide in vacuo.

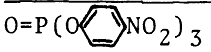
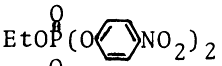
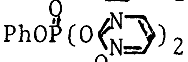
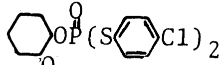
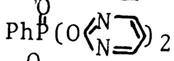
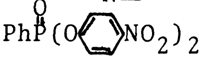
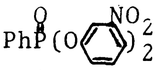
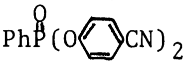
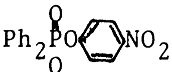
Method B: A cold aqueous solution of tetrabutylammonium hydrogen sulfate (1.2 equiv) and sodium hydroxide (2.4 equiv) was treated with a chloroform solution of 1 (1.0 equiv), and the aqueous mixture was extracted with chloroform three times. The combined organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The residual ammonium salt 3 was directly used without further purification.

The following is general procedure for the Young test by the present method.

To a stirred mixture of equimolar amounts of the ammonium salt 3 and ethyl glycinate (2) in a solvent was added a phosphorus compound (1.2 equiv). The mixture was stirred under the reaction conditions shown in Table 1 and 2, and diluted with ethyl acetate. The organic solution was successively washed with water (once), 2N hydrochloric acid (1), water (2), saturated sodium hydrogen carbonate (1), water (1), and saturated brine (1), and then dried ($MgSO_4$). After evaporation of ethyl acetate crude N-benzoyl-L-leucylglycine ethyl ester (4) was purified by silica gel column or preparative thin layer chromatography.

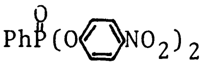
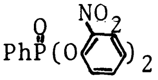
The results are summerized in Table 1 and 2. It is noted that phenylphosphonic esters gave better results than phosphoric and diphenylphosphinic esters. Among aryloxy components in the phosphorus esters examined here, o-and p-nitrophenoxy groups are especially effective. Thus, the reaction by employing bis(o- or p-nitrophenyl) phenylphosphonate proceeded smoothly even at or below 0°C and the

Table 1. The reaction of equimolar amounts of 2 and 3 with various phosphorus compounds.

Phosphorus compound	Reaction conditions$^{1)}$	Yield, %	L-Isomer, %	$[\alpha]_D$(T, °C)
	r.t., 2h	90	28	-9.6°(26)
	r.t., 5days	43	82	-27.8 (28)
	r.t., 14h	71	69	-23.5 (30)
	r.t., 15h	27	91	-30.9 (30)
$(PhO)_2\overset{O}{\overset{\Vert}{P}}OC_6Cl_5$	r.t., 22h	60	52	-17.6 (28)
	r.t., 15h	71	54	-18.3 (28)
	r.t., 24h	69	89	-30.2 (29)
	r.t., 1h	81	65	-22.4 (30)
	0°C, 5h then r.t., overnight	75	85	-28.8 (27)
	0°C, 22h then r.t., 5h	84	86	-29.2 (25)
	0°C, 2h then r.t., 4.5h	74	98	-33.5 (30)
$Ph_2\overset{O}{\overset{\Vert}{P}}OC_6Cl_5$	r.t., 6h	81	86	-29.4 (26)

1) N, N-Dimethylformamide was used as a solvent.

Table 2. Results of the Young test using bis(nitrophenyl) phenylphosphonates under various conditions.

Phosphorus compound	Solv.	Reaction conditions	Yield, %	L-Isomer, %	$[\alpha]_D$(T, °C)
	DMF	0°C, 3.5h	81	80	-27.2°(30)
	DMF	-10°C, 8h	88	96	-32.5 (24)
	DMF	-10°C, 5h then r.t., overnight$^{1)}$	84	96	-32.5 (30)
	DMF	-10°C, 7h, then r.t., overnight$^{2)}$	93	95	-32.3 (30)
	DMF	-10°C, 8h$^{3)}$	82	97	-33.0 (24)
	DMF	0°C, 6.5h	88	91	-31.1 (25)
	DMF	0°C, 5h then r.t., 1.5h$^{4)}$	88	90	-30.7 (26)
	DMF	-10°C, 8h	87	96	-32.7 (25)
	DMF	-10°C, 8h$^{5)}$	80	97	-33.0 (23)
	THF	-10°C, 10h then 0°C, 10h	90	94	-32.0 (23)
	CH_2Cl_2	-10°C, 10h then 0°C, 10h	78	79	-27.0 (23)
	CH_3CN	-10°C, 8h	82	93	-31.5 (22)

1) 2,6-Lutidine (1 equiv) was added. 2) 1.2 Molar amounts of the ammonium salt 3 were used. 3) Glycine ethyl ester hydrochloride (1 equiv) with triethylamine (1.2 equiv) was used. 4) Calculated amount of N-benzoyl-L-leucine (1 equiv) was converted to the ammonium salt 3 according to Method B and 3 was directly employed after evaporation of chloroform. 5) N-Benzoyl-L-leucine (1.0 equiv) in place of the ammonium salt 3 was used with triethylamine (1.2 equiv).

almost pure L-isomer was obtained in excellent yield. A favorable solvent in the present reaction is N,N-dimethylformamide (DMF) in which even large peptides are soluble. The use of DMF as a solvent widens the scope of the present method. Employment of triethylamine and free N-benzoyl-L-leucine (1) in place of the use of the tetrabutylammonium salt gave the dipeptide 4 also with high optical purity, however, the chemical yield was lower. The result indicates that the ammonium salt 3 is highly nucleophilic to accelerate the formation of the mixed anhydride intermediate. Furthermore, liberation of a phenol during the reaction eliminates the use of an acid captor such as tertiary amine. These facts make it possible to simplify the procedure and to eliminate a main racemization factor. On the other hand, the chloride ion effect6) was not noticed when the amine component 2 was used as its hydrochloride with slightly excess of triethylamine (1.2 equiv). Moreover, addition of 2,6-lutidine (1.0 equiv) did not affect the reaction of 2 and 3 with bis(p-nitrophenyl) phenylphosphonate.

In a similar fashion, a racemization-free dipeptide was prepared by the use of bis(o-nitrophenyl) phenylphosphonate: Z-L-Phe-Gly-OEt [91% yield, $[\alpha]_D^{26}$ -16.7° (c 2, EtOH)].7)

When the reaction of the ammonium salt 3 of N-Bz-L-Leu with bis(p-nitrophenyl) phenylphosphonate was carried out in the absence of the amine component 2, p-nitrophenyl leucinate was not formed at all as monitored by tlc and almost completely racemized N-benzoylleucine was recovered. This result suggests that an initially formed mixed anhydride is directly converted to the corresponding dipeptide by the attack of an amine component on the carbonyl carbon atom.

The present reaction provides an efficient method for racemization-free peptide synthesis particularly because of the following facts: (1) simplicity of the procedure, (2) excellent synthetic yield and optical purity, (3) possible use of an amine component as hydrochloride, and of DMF as a solvent, (4) the bis(nitrophenyl) phenylphosphonates being inexpensive, stable, and available in large scale. Further work on the scope and utility of the method is now in progress.

References and Note

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7) Literature values: $[\alpha]_D^{20}$-16.8°(c 2, EtOH)8); $[\alpha]_D^{25}$-16.0°(c 2, EtOH).9)
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