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PEPTIDE SYNTHESIS BY OXIDATION-REDUCTION CONDENSATION BY USE OF TRIARYL PHOSPHITE AS REDUCING REAGENT

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Use of triaryl phosphite as reducing reagent in peptide synthesis by oxidation-reduction condensation was studied. Aryl esters having electron-withdrawing substituents gave optically pure peptides in good yields by reacting in N,N-dimethylformamide solution.

In the preceding report it has been shown that oxidation-reduction condensation method for peptide synthesis by the use of triphenylphosphine and 2,2'-dithiodipyridine(DTP) has the versatile utility.[1] Since side reaction is not observed in the synthesis of arginine, asparagine and glutamine containing peptides, all coupling steps can be performed by the same procedure with the same reagents. Biologically active oxytocin[2], Phe^2,Lys-vasopressin[3] and LH-RH[4] have been synthesized by this method.

Only one restriction of this method is the racemization observed when the reaction is carried out in the polar solvents such as acetonitrile and N,N-dimethylformamide(DMF) at higher temperature than -30°. Use of such solvents is indispensable for the synthesis of long chain peptides especially containing arginine, asparagine or glutamine. To overcome this problem use of other trivalent phosphorus compounds was investigated.

At the first stage, comparison of some substituted phenylphosphines was made as for reaction rate and racemization degree. While no remarkable change was seen in these experiments, it was found that less reactive chlorophenylphosphine gave the product of higher optical purity. On these findings reaction of triaryl phosphite possessing much less nucleophillic reactivity was tried.

2 mmol each of benzoyl-L-leucine, ethyl glycinate, triphenyl phosphite (10% excess) and DTP are mixed in 10 ml of anhydrous DMF and stirred for 15 hr at 30°. After evaporation off of the solvent in vacuo, residual materials are chromatographed on silica gel column to give ethyl benzoyl-L-leucylglycinate in 89% yield. From its $[\alpha]_D$ value L-isomer content was calculated to be 94%. On this encouraging result this reaction was further investigated in detail.

Methyl benzyloxycarbonyl-L-nitroarginyl-L-leucinate(Z-L-Arg(NO_2)-L-Leu-OMe) was selected as a model compound and its yields of formation were compared by using various phosphite esters under various conditions. Results are summerized in Table 1.

Table 1. Preparation of Z-L-Arg(NO_2)-L-Leu-OMe by Oxidation-Reduction Condensation by Use of Triaryl Phosphite as Reducing Reagent.

R in $(R\text{-}C_6H_4O)_3P$	Disulfide	Solvent (ml/mM)	Conditions	Yield, %
p-CH_3O	2-Pyridyl	DMF (1)	40° 3 hr	27
H		(1)	40° 3 hr	59
p-Br		(1)	25° 5 hr	81
p-Br		(2.5)	25° 5 hr	78
p-Br		(5)	25° 5 hr	72
p-Br		(1)	40° 3 hr	83
p-O_2N*		(1)	40° 3 hr	87

* $(p\text{-}O_2NC_6H_4O)_2POC_6H_5$

From these results it is clear that electron-withdrawing substituents increase the yield of the peptide. The other important factor is the concentration; namely, higher concentration gives the better result. Usually 1 M solution is used. Under such conditions reaction completes almost within 5 hr at 25° or 3 hr at 40°.

Recently Mitin reported the method for peptide synthesis with use of triphenyl phosphite in the presence of imidazole as catalyst.[5] Pyridine catalyzed reaction is also reported.[6]

Pyrid-2-thione, produced in the above mentioned oxidation-reduction condensation reaction, would catalyze such ester exchange reaction. To make clear the relation between the two type reactions these catalytic reactions were compared through the synthesis of the same model peptide. As is shown in Table 2, reaction is accelerated by electron-withdrawing substituent. Among the catalysts examined imidazole gave a little better result. But, in all cases yields do not exceed that in the oxidation-reduction condensation reaction. This is probably due to the formation of strongly acidic monoaryl phosphite, which blocks the amino component.

Table 2. Preparation of Z-L-Arg(NO_2)-L-Leu-OMe by Ester Exchange Reaction by Use of Triaryl Phosphite.

R in $(R\text{-}C_6H_4O)_3P$	Catalyst (equiv)	Solvent (ml/mM)	Conditions	Yield, %
p-CH_3O	Pyrid-2-thione (1)	DMF (1)	40° 3 hr	32
H	Pyrid-2-thione (1)			56
p-Br	Pyrid-2-thione (1)			70
p-Br	Pyrid-2-thione (0.1)			66
p-O_2N*	Pyrid-2-thione (1)			76
p-Br	Pyrid-2-one (0.1)			63
p-Br	Imidazole (0.1)			71

* $(p\text{-}O_2NC_6H_4O)_2POC_6H_5$

Racemization degree was estimated by the Young test[7] and results are listed in Table 3. Optically pure ethyl benzoyl-L-leucylglycinate(Bz-L-Leu-Gly-OEt) was obtained by oxidation-reduction condensation method with use of tris(*p*-bromophenyl) phosphite. But, racemization was slightly increased in the case of nitrophenyl ester. This is probably caused by competitive ester exchange reaction. In fact, partial racemization was occurred in the ester exchange type reaction especially when active phenyl ester was used.

Table 3. Young Racemization Test for Peptide Synthesis by Use of Triaryl Phosphite in DMF*1

R in $(R\text{-}C_6H_4O)_3P$	Co-reagent	Conditions	Yield, %	$[\alpha]_D^{25}$ *2	L-isomer, %
p-Br	DTP	25° 5 hr	80	-33.5°	99
p-Br	DTP	40° 1 hr	83	-34.3°	100
p-O_2N*3	DTP	40° 1 hr	73	-29.8°	88
H	Imidazole	40° 1 hr	53	-30.4°	89
p-O_2N*3	Imidazole	40° 1 hr	77	-23.7°	70

*1) 1ml/mM

*2) c3.1, EtOH

*3) $(p\text{-}O_2NC_6H_4O)_2POC_6H_5$

As for side reactions, Z-L-Asn-L-Cys(Bzl)-OMe and Z-L-Gln-L-Cys(Bzl)-OMe were obtained both in 75% yield without accompanying the nitrile formation. Lactam formation was not also observed during the above mentioned experiments synthesizing Z-L-Arg(NO_2)-L-Leu-OMe. From these facts this modified oxidation-reduction condensation method can be said to be very useful for coupling of long chain peptide fragments soluble in DMF.

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