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SOLID PHASE PEPTIDE SYNTHESIS BY OXIDATION-REDUCTION CONDENSATION SPEED-UP BY USE OF TRI-*n*-BUTYLPHOSPHINE AS REDUCING REAGENT

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Use of tri-*n*-butylphosphine as a reducing reagent in solid phase peptide synthesis by oxidation-reduction condensation is studied. Attachment of the first amino acid to hydroxymethyl resin reached the amount suitable for the succeeding peptide bond elongation within 1 hr. Key of this reaction is to keep the concentration as high as possible.

Oxidation-reduction condensation method for peptide synthesis has been shown to be convenient and useful by some examples[1]. In these syntheses triphenylphosphine has been used exclusively as a reducing reagent. But, it was found that use of much less reactive tris(4-bromophenyl) phosphite completely prevented the racemization in the reaction in N,N-dimethylformamide solution as communicated in the preceding paper[2]. Namely, acylating ability in the oxidation-reduction condensation is much affected by the structure of the phosphorous reagent. Then, use of much more reactive tri-*n*-butylphosphine could be expected to speed-up the reaction. Recently Matsueda, *et al.* reported the attachment of the first amino acid to hydroxymethyl resin by the oxidation-reduction condensation method by use of triphenylphosphine and 2,2'-dithiodipyridine(DTP)[3]. They recommended the reaction time of 24 hr and use of 3 equivalent amounts of the reagents. Esterification to chloromethyl resin also requires the reaction time of 24 hr or more[4]. In this communication rapid esterification of the first amino acid to the hydroxymethyl resin by use of tri-*n*-butylphosphine and DTP is reported.

In a typical experiment, 10 mg hydroxymethylated polystyrene-2%-divinylbenzene beads are suspended in 0.1 ml methylene chloride containing 0.02 mmol each of Boc-Phe-OH, $(n\text{-Bu})_3P$ and DTP. The mixture is shaken for 1 hr at 25°. Filtration and washing are done by the usual manner. Boc group is removed by trifluoroacetic acid-methylene chloride(1:1). The whole resin is hydrolyzed in 6*N*-HCl/propionic acid and phenylalanine liberated is quantitated by ninhydrin method. In a similar manner several experiments are carried out by changing the amounts of the reagents and/or the reaction time. Amount of phenylalanine incorporated is plotted against the reaction time in Figure 1.

As expected this phosphine showed excellent reactivity. Incorporation of amino acid almost finished after 1 hr. Amount of the amino acid esterified depends

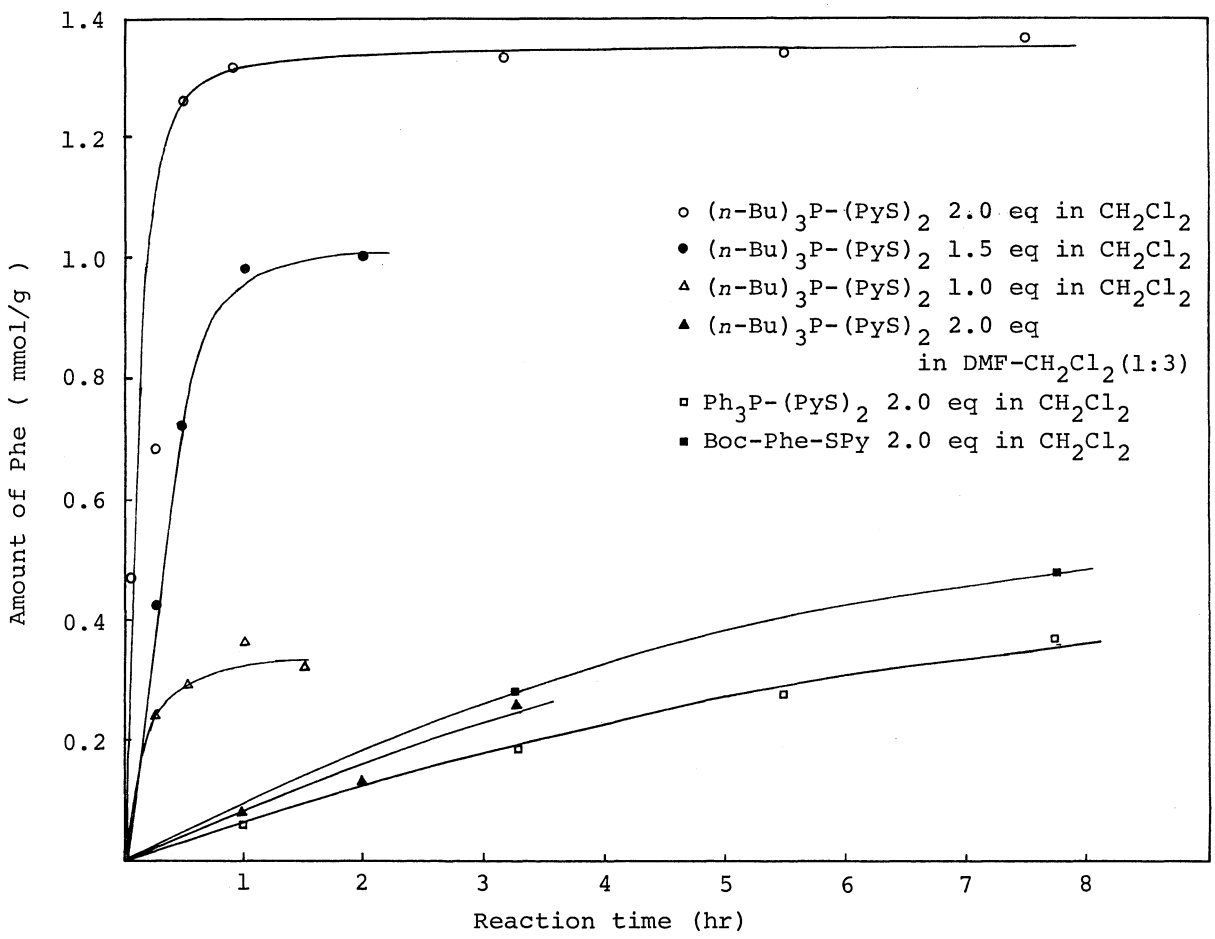

Figure 1. Attachment of Phe to the Hydroxymethyl Resin

only on the amounts of the reagents. So its control is easier than the usual control by reaction time.

Bodanszky *et al.* revealed the *N*-carboxyanhydride formation from Boc-amino acid as the result of over-activation by *N,N'*-dicyclohexylcarbodiimide[5]. Fortunately this side reaction could not be detected in the reaction using $(n\text{-Bu})_3P$ and DTP. When Boc-Phe-OH was treated with $(n\text{-Bu})_3P$ and DTP in methylene chloride for 12 hr at room temperature, Boc-Phe-SPy was isolated in 55 % yield. 2-Pyridylthio ester is an active ester reported by Young[6]. As is shown in Figure 1, esterification of Boc-amino acid through this active ester is much slower than the above mentioned reaction. Namely, 2-pyridylthio ester is not an active acylating intermediate in the oxidation-reduction condensation at least in the early step of the reaction.

$$\text{Boc-Phe-OH} + (n\text{-Bu})_3P + (\text{PyS})_2 \xrightarrow{\text{HOR}} \begin{cases} \text{Boc-Phe-OR} + (n\text{-Bu})_3P{=}O + 2\text{PySH} \\ \text{Boc-Phe-SPy} + (n\text{-Bu})_3P{=}O + \text{PySH} \end{cases}$$

Solvent dependence is remarkable in this reaction. When *N,N*-dimethylformamide was used as solvent Boc-phenylalanine was not esterified after shaking for 7 hr. Rate and total amount of the amino acid esterified are decreased in *N,N*-dimethyl-

formamide-methylene chloride mixed solution. Such decrease in rate of condensation is reported in the aminolyses of *o*-hydroxyphenyl, 2-pyridyl and 2-pyridylthio esters[7].

Swelling is said to be a very important factor in the solid phase peptide synthesis on the polystyrene-divinylbenzene copolymer support. But, superiority of 1%-cross-linked resin was not observed in our experiments as shown in Table 1. It is more important in this reaction to keep the volume of the solvent as small as possible. 2%-cross-linked resin is favored for this porpose.

Table 1. Effects of Concentration and Cross-linking*

Cross-linking	Solvent	Concentration	Phe esterified
2 %	CH_2Cl_2 10ml/g	0.20 *M*	0.27 mmol/g
1 %	CH_2Cl_2 15ml/g	0.13 *M*	0.10 mmol/g
1 %	CH_2Cl_2 15ml/g	0.20 *M*	0.24 mmol/g

* OH=2.0 mmol/g.

On these findings attachment of various amino acids was tried by using the 2%-cross-linked resin and methylene chloride as solvent. Results are listed in Table 2. Boc-glutamine was not esterified in these conditions.

Table 2. Attachment of Various Amino Acids to the Hydroxymethyl Resin by Use of Tri-*n*-butylphosphine and 2,2'-dithiodipyridine*

Boc-Amino Acid	Reagents(eq/OH)	Amount esterified(mmol/g)
Gly	1.0	0.33
Ala	1.0	0.29
Phe	1.2	0.51
Val	1.0	0.35
Pro	2.1	0.37
Met	2.1	0.36
Ser(Bzl)	2.0	0.47
Tyr(Bzl)	2.0	0.14
His(Tos)	2.0	1.04
Asn	3.0	0.14

* In methylene chloride at 25° for 1 hr. OH=2.0 mmol/g.

As the result of high activation, racemization was increased as compared with the reaction by use of triphenylphosphine; L-isomer content of Bz-L-Leu-Gly-OEt was 62 % by Young test[8] when the reaction was carried out in methylene chloride at room temperature. Accordingly, this new procedure can not be used for the introduction and the succeeding peptide bond formation by fragment method. But, it is expected to be useful for the rapid stepwise peptide bond elongation as reported by Corley, *et al.*[9]. Study for such use is now in progress.

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