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Rapid Anchoring of Cesium Salts of Boc-Protected Amino Acids On Chloromethyl Polystyrene Resin Using Dibenzo-18-crown-6 as a catalyst

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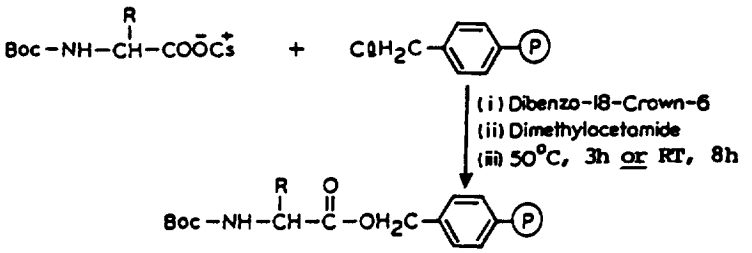
Abstract : Cesium salts of Boc-protected amino acids react rapidly with chloromethyl polystyrene (Merrifield resin) in presence of catalytic amount of dibenzo-18-crown-6 to give anchored benzyl ester resin in almost quantitative yields. © 1997 Elsevier Science Ltd.

Chloromethylated polystyrene cross linked with divinylbenzene (DVB) has been widely used in solid phase peptide synthesis[1,2]. The first N-protected amino acid is attached via a benzyl ester linkage to the DVB-linked polystyrene matrix. Quantitative esterification is usually required in order to avoid side reactions of reagents or side chains of amino acids. Several methods have been reported for anchoring the salts of N-protected amino acids on to Merrifield resin viz. triethylammonium[3], diisopropyl ethylammonium[4] and tetramethylammonium salt[5] etc. However all these methods have one or the other disadvantages, viz. prolonged reaction times and drastic reaction conditions which may lead to racemization and poor yields. The most popular method of Gisin[6] utilising the cesium salts requires 16-24 h at 50°C in N,N-dimethylformamide (DMF).

For the present studies cesium salts of Boc-amino acids were chosen because they are more reactive with chloromethyl resin than other alkali metals[6]etc,moreover dibenzo-18-crown-6 is operative more efficiently in Cs^+ metal cation binding[7,8] than in other metals viz. Na^+,$K^+$and Rb^+. Binding ability of Cs^+, which forms 2:1 sandwich complex with dibenzo-18-crown-6 is well documented[9,10]. Its ability to bind strongly the Cs^+allows the corresponding anionic component to function as a better nucleophile.

We wish to report the quick and quantitative displacement of chloride from chloromethyl resin by cesium salts of Boc-amino acids in N,N-dimethylacetamide (DMAc) at 50 °C and at room temperature using dibenzo-18-crown-6 as a catalyst.



R = Side chains

Several solvents were used for esterification. Reaction of 1 equiv of chloromethyl resin and 2 equiv of each Boc amino acid cesium salt and dibenzo-18-crown-6 at 50°C for 5 h in dichloromethane(CH_2Cl_2), DMF and DMAc resulted almost in complete esterification. Yield of Boc-amino acid benzyl ester resin were 90% in CH_2Cl_2 and more than 96% in DMF and DMAc. Both DMF and DMAc were found to be the best solvents for the reaction. Earlier we have observed that dicyclohexylammonium salts of N-protected amino acids in presence of catalytic amount of KI in DMAc react faster than in DMF to give N-protected amino acyl resin ester in good yields[11]. In the present studies also the rate of esterification was faster 3 h. when DMAc is used as a solvent, however it took 4 to 5 h for completion in DMF (**Figure 1**).

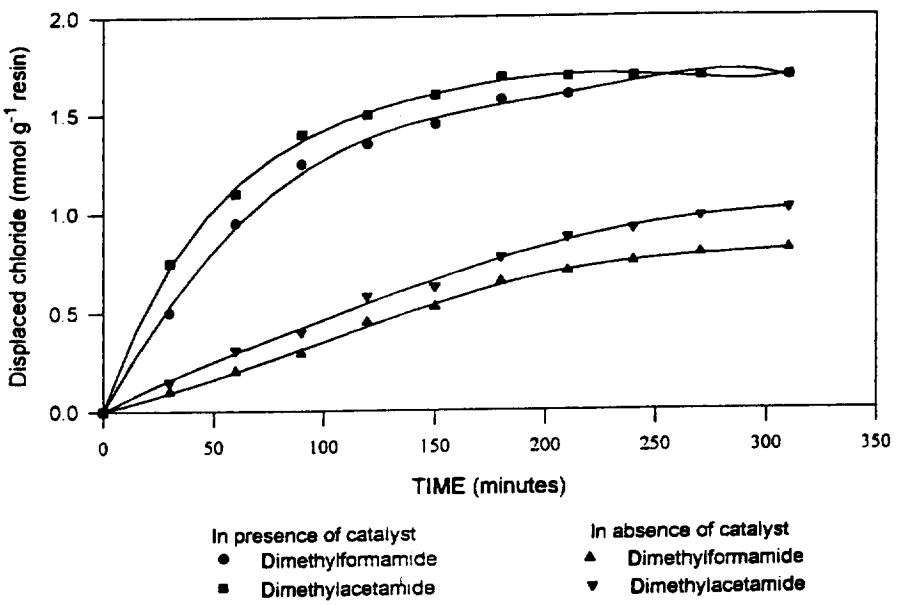


Figure 1. The time course of esterification of Boc-Alanine-OCs in different solvents with and without catalyst as monitored by the increase in displaced chloride ions by Boc-Alanine.

For anchoring the amino acids on to the Merrifield resin preparation of cesium salts of N-protected amino acid is required. The cesium salts are prepared as per published procedure [12]

Studies varying the temperature, time and molar ratios of reactants were undertaken in DMAc.

It was found that at room temperature (8 h), Boc Ala-OCs could displace all the chlorides if resin(1 equiv) dibenzo-18-crown-6 (2 equiv) and cesium salt (1.2 equiv) were used. However the reaction took 3 h for completion when the temperature was raised to 50°C. Time course of the reaction at 50°c.is given by the example of Boc-alanine cesium salt(table-1).

Time	Residual chloride (m. mol/g)
1 h	0.5
2 h	0.2
3 h	0.05

Table-1.Time course of esterification of Merrifield Resin with Boc-Ala-OCs in presence of dibenzo-18-crown-6 in DMAc at 50°C

In conclusion the present method is a distinct instance of improvement over the other existing methods. All the N- Boc amino acids were quickly and quantitatively attatched to the solid support using this method (see Table-II).

Name	Yield(%)	Name	Yield(%)
N-Boc-Gly-OCs	91	N-Boc-Ala-OCs	94
N-Boc-Phe-OCs	95	N-Boc-Val-OCs	98.8
N-Boc-Leu-OCs	97	N-Boc-Ile-OCs	95
N-Boc-Pro-OCs	94	N-Boc-Asp(OBzl)-OCs	96
N-Boc-Arg(Mtr)-OCs	94	N-Boc-Trp-OCs	93

Table-II. Reaction of cesium salts of some N-Boc amino acids with chloromethyl resin# at 50°C.

Experimental details and analysis are given below

Preparation of t-Butyloxycarbonyl alanine methyl polystyrene ester.

In all the experiments chloromethylated polystyrene (Cl contents 1.7 m.mol g^{-1} resin was used). Boc-Ala-OCs (0.4 mmol) and dibenzo-18-crown-6 (0.34 mmol) were added to chloromethylated polystyrene (Cl contents 0.34 mmol.) 200 mg. in 5 ml DMAc.The mixture was left at 50° C for 3 h with swirling from time to time. The resulting product was filtered and washed twice with DMF (10ml each) followed by water(10ml) and methanol(10ml) and finally dried using diethyl ether. The amount of incorporated amino acid was estimated by amino acid analysis and picric acid titration method[13,14].

Esterification of Fmoc-amino acids to 4-alkoxybenzyl alcohol (Wang's resin[15,16]) resin and 2-methoxy 4-alkoxybenzyl alcohol (SASRIN)[17] resin by existing methods is time consuming and results in partial racemization[18]. In the present method all the N-Boc amino acids coupled to Merrifield resin in 3 h at 50°C with 0.2 to 0.5% racemization while at room temprature 8 h, the racemization was minimal ie from 0.1 to 0.2 %. Therefore this method can also be used for the attachment of Fmoc-amino acid cesium salts to the Wang resin chloride and SASRIN chloride[19] at room temperature. The results will be published elsewhere.

ACKNOWLEDGEMENT

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ABBREVIATIONS : Cs : cesium, Mtr : 4-methoxy-2,3,6-trimethylbenzenesulphonyl, Bzl: benzyl.

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