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SYNTHESIS OF THE MERRIFIELD RESIN ESTERS OF N-PROTECTED AMINO ACIDS WITH THE AID OF HYDROGEN BONDING[1]

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A synthesis of the Merrifield resin esters of N-protected amino acids was attempted with the aid of hydrogen-bonding property of fluoride ion with carboxylic acids to obtain the esters in satisfactory yields. This will provide an alternatively efficient and economical method for the preparation of the key intermediate of the solid phase peptide synthesis.

A number of methods have been developed to synthesize the so-called Merrifield resin esters of Boc-amino acids (amino acid esters with the chloromethylated co-polystyrene-2% divinylbenzene resin), which is considered to be the key intermediate for solid-phase peptide synthesis. Esterification procedures using chloromethyl resin can be summarized as follows: 1) that using triethylammonium salts of Boc-amino acids in refluxing ethanol,[2] 2) the reaction of the tetramethylammonium salts of amino acids in dimethylformamide (DMF) at room temperature,[3] 3) the recent method of Gisin[4] utilizing the cesium salts of Boc-amino acids, which are the most effective among several alkali metal salts, in DMF at 50°C, where carboxylate anions associated with large (soft) counterions are considered to be more reactive than those associated with small (hard) counterions, 4) the procedure using 18-crown-6 as a catalyst to enhance the nucleophilicity of carboxylate anions of the potassium salts of Boc-amino acids in DMF,[5] and 5) that using 1,5-diaza-bicyclo[4,3,0]nonene-5 (DBN) or 1,8-diazabicyclo[5,4,0]undecene-7 (DBU) salts of N-protected amino acids in DMF at 50°C.[6]

Recently, Miller and his associates[7] successfully carried out alkylations of phenolic hydroxyl groups and carboxylic acids with alkyl halides with the aid of the hydrogen-bonding ability of fluoride anion. Furthermore, Emsley and Hoyte[8] recently showed that the strongest hydrogen bonding exists between fluoride anion and formic acid by using Ab initio-LCAO-MO-SCF calculations. Therefore, taking these results into consideration, we tried using the hydrogen-bonding property of fluoride with carboxylic acids for the Merrifield resin ester synthesis, which led us to the successful synthesis of the above-mentioned resin esters. The reaction scheme was:

Table. Esterification of N-Protected Amino Acids to Resin Support

Boc-L-AA-OH	Yield,[a] %		
	at 80° (24 hr)	at 50° (40 hr)	at 50° (24 hr)
Gly	97	92	89
Val	100	98	92
	2[b]	--	--
	--	--	45[c]
	--	--	98[d]
Ala	92	86	101
Ile·1/2 H_2O	81	93	--
Pro	92	93	--
Phe	--	97[e]	85[e]
N^G-Tos-Arg	47	--	--
N^{im}-Tos-His	59	--	--

[a] Unless otherwise noted, yields are determined with amino acid analysis (see text). [b] Carried out without using potassium fluoride. [c] Carried out without stirring for 28 hr and the yield was estimated from weight increase. [d] Synthesized by Gisin's method[4] utilizing cesium salt. [e] Ten-ml of DMF was used.

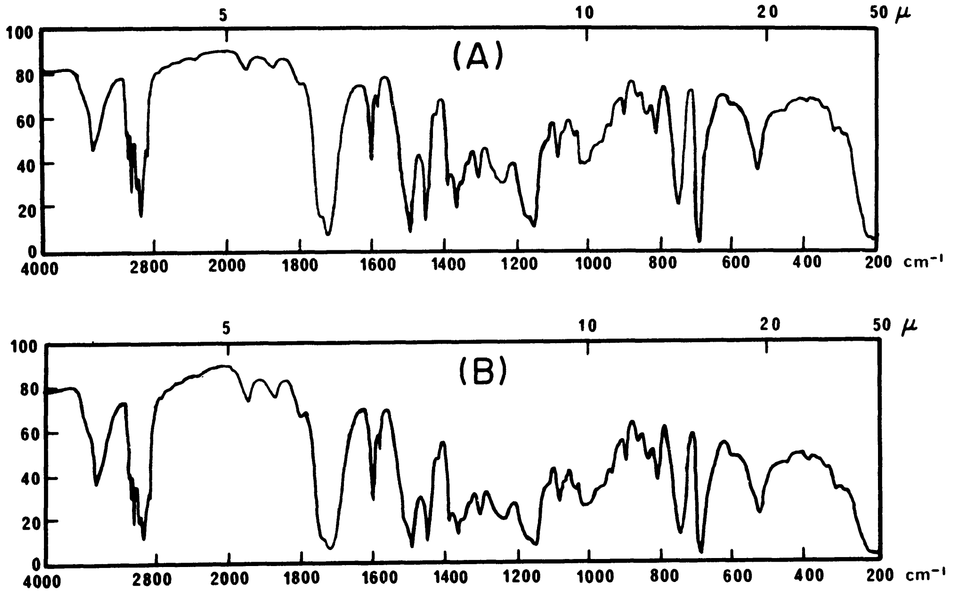
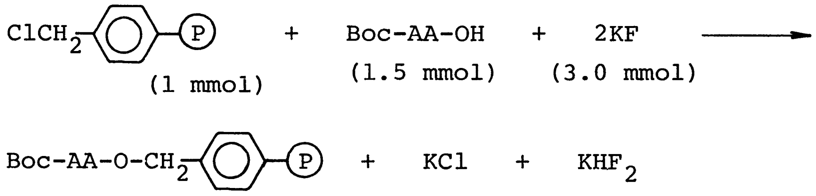


Figure. Infrared spectra of Boc-Valine resin ester (KBr).
A: Obtained by Gisin's method.[4]
B: Obtained by the present method.



Abbreviations used: Ⓟ = polystyrene backbone,
Boc-AA-OH = t-Butoxycarbonylamino acid

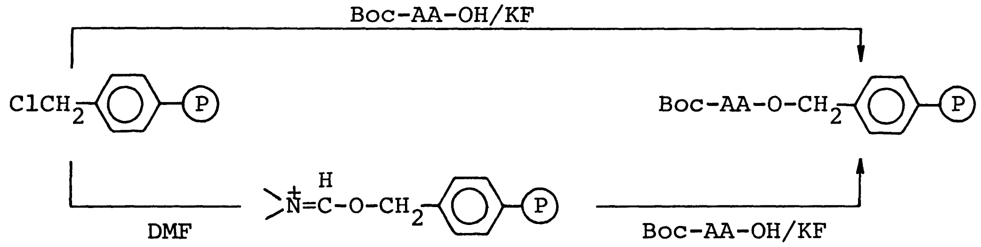
As preliminary experiments, reactions of Boc-amino acids (1.5 mmol) and chloromethyl resin[9] (1.0 mmol) in 4 ml of DMF in the presence of potassium fluoride (1.5 x 2 mmol) at 80°C for 24 hr afforded the desired esters in almost quantitative yields. The results are shown in the Table.

Subsequently, the reaction was performed with the temperature lowered to 50°C and using reaction times of 40 and 24 hr; the esters were obtained in excellent yields. The results, also included in the Table, show that the 24-hr period was enough for the reaction to be completed even at 50°C.

In the typical experimental procedure, chloromethylated polymer[9] (1 mmol) and anhydrous potassium fluoride (1.5 x 2 mmol) were added to the solution of Boc-valine (1.5 mmol) in 4 ml of DMF in a 15-mm O.D. test tube equipped with a small magnetic stirring bar; the reaction vessel was stoppered tightly with silicone rubber. The reaction mixture was stirred at 50°C in an oil bath for 24 hr and filtered. The resin thus obtained was washed thoroughly with DMF, 50% aqueous DMF, 50% aqueous ethanol, and finally ethanol, and then dried *in vacuo* at 80°C to constant weight. Incorporation of Boc-valine into the resin was qualitatively estimated to be 106 and 96% from the weight increase and elemental analysis of nitrogen (1.43%), respectively. Hydrolysis[10] of the resin with conc. HCl in propionic acid (1:1; v/v) at 130°C for 3 hr followed by amino acid analysis exhibited 92% introduction of Boc-valine, after correction for the weight increase. The infrared spectra of Boc-valine resin esters obtained with our method and Gisin's method[4] were identical, as shown in the Figure. Efficient stirring was necessary to complete the reaction, otherwise only partial esterification was observed as shown in the Table.

The fact that the terminal amino group in the products was not detected with the qualitative color test reported by Kaiser et al.[11] shows that the protecting Boc group is not harmed in the present ester synthesis.

In the case of esterification of Boc-isoleucine, which contains a half mole of water of crystallization, the reaction was not affected by a small amount of moisture. This is very convenient for practical purposes as water of crystallization in the starting amino acids need not be removed. Although coupling of basic amino acids, such as Boc-N^G-tosyl-L-arginine and Boc-N^{im}-tosyl-L-histidine, to the resin resulted in lower yields, our results were still better than those[12] prepared by the method of Merrifield[2] or Dorman and Love.[13] Boc-isoleucine resin ester prepared by the present method contained no alloisoleucine above the blank, as examined by amino acid analysis, indicating that little or no racemization had occurred during the esterification. Two competitive pathways are considered to exist for the nucleophilic substitution of Boc-amino acids onto polymeric benzyl chloride in the synthesis to afford the same ester as shown below:[14]



The present results thus provide an efficient and convenient method for the preparation of the Merrifield resin ester, which requires neither the potassium salt of Boc-amino acid to be prepared in advance nor the use of an expensive crown ether.[5]

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