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REACTION OF POTASSIUM SALTS OF BOC AMINO ACIDS WITH CHLOROMETHYL POLYSTYRENE CATALYZED BY 18-CROWN-6

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In the synthesis of peptides by the solid phase procedure as described by Merrifield,[1] the first step involves attachment of an N-protected amino acid to a chloromethyl polystyrene-co divinylbenzene resin to form a benzyl ester. Several common methods of esterification involve 1) the use of triethylammonium salts of amino acid derivatives in refluxing ethanol,[2] 2) the modified Loffett procedure involving reaction of the tetramethylammonium salt in dimethylformamide at room temperature,[3] and 3) the method of Gisin utilizing the cesium salts of Boc amino acids in DMF at 50°C.[4] Complete reaction of chloromethyl groups (quantitative esterification) is usually desirable in order to avoid side reactions of reagents[4] or amino acid side chains[5] with any remaining chloromethyl groups; however only the cesium salt method results in a nearly quantitative esterification.

The ability of compounds known as crown ethers to bind cations is well documented by several recent reviews.[6] This ability to bind cations will often allow the corresponding anionic component to function as an improved nucleophile. Notable in regard to esterification is the report by Liotta et al.[7] that the complex of 18-crown-6 and potassium acetate will react quantitatively with benzyl bromide in acetonitrile at 25°C to form benzyl acetate, whereas little or no reaction occurred in the absence of crown ether.

Although Gisin found that cesium salts of Boc amino acids are more reactive with a chloromethyl resin than are the other alkali metal salts,[8] we used the potassium salts because they have higher association constants with the crown ethers that are readily available.[9]

We now report the use of 18-crown-6 to catalyze the quantitative displacement of chloride from a chloromethyl resin by the potassium salts of Boc amino acids in DMF at 50°C and at room temperature.

Preliminary results using 1.0 equivalents each of 18-crown-6, the potassium salt of Boc Leu,[10] and the chloromethyl resin at 100°C for 18 hr[11] resulted in virtually complete esterification in several solvent systems; acetonitrile (96%), ethyl acetate (91%), and dimethylformamide (100%).[12] Lowering the temperature to 50°C gave the following results: ethanol (5%), dimethylsulfoxide (7%), chloroform (11%), dioxane (20%), benzene (23%), acetonitrile (27%), ethyl acetate (32%), tetrahydrofuran (35%), acetone (56%), methylene chloride (92%), and dimethylformamide (96%). Although initially promising, the high incorporation values determined for CH_2Cl_2 were later found to be an artifact caused by reaction of Boc Leu O^- with

CH_2Cl_2 in the presence of 18-crown-6. (See Table I.) The product of the reaction is presumably the chloromethyl ester of Boc Leu, but we made no attempt to establish this.

Table I. Chloride liberation during reaction in CH_2Cl_2 at 50° for 18 hours.

Reagents (mmoles)			Cl^- liberated
Boc Leu OK	18-Crown-6	Resin CH_2Cl	(mmoles)[a]
0.192	0.195	0.161	0.165
--	0.197	0.168	0.008
0.203	--	0.203	0.012
0.202	0.199	--	0.160
--	--	--	0.008[b]

[a] The amount of Cl^- liberated at 35° and at room temperature was 50% and 28%, respectively, of that liberated at 50°.

[b] From titration of CH_2Cl_2 and washings.

Since DMF was the best solvent for the reaction, studies varying the temperature, time and molar ratios of reactants were undertaken in DMF with the potassium salt of Boc Leu in order to determine the minimum requirements for quantitative esterification. It was found that at room temperature (18 hr) Boc Leu OK could displace all of the chloride if 2.0 equivalents of both the salt and the crown ether (relative to the chloromethyl resin) were used. (See Table II.) When 11 other Boc amino acid potassium salts were subjected to the same reaction conditions, four (Pro, Phe, Val, and Sar) gave quantitative esterification. However, all of the Boc amino acids tested could completely displace the chloride from the chloromethyl resin in DMF at 50°C by using 1.2 or, in some cases, 1.5 equivalents of both the potassium salt and the crown ether. (See Table III.)

Table II. Reaction of Boc Leu potassium salt with chloromethyl polystyrene in DMF at room temperature.

equivalents of		% reaction	equivalents of		% reaction
crown	salt		crown	salt	
0	2.0	33	1.0	1.5	85
0	3.0	37	1.0	2.0	91
0.1	1.0	65	1.2	1.0	79
0.25	1.0	72	1.2	1.2	90
0.5	1.0	80	1.5	1.5	92
0.5	2.0	81	1.75	1.75	97
1.0	1.0	83	2.0	1.0	77
1.0	1.2	88	2.0	2.0	101
			3.0	3.0	99

Table III. Percent incorporation of Boc amino acids into a chloromethyl resin in DMF with varying equivalents of the K salt and crown ether.

Boc AA OK	Room temperature, 18 hr, 2.0 eq.	50°, 16 hr,[13] 1.0 eq.	50°, 18 hr, 1.2 eq.	50°, 18 hr, 1.5 eq.
Boc-Leu	101	96	101	-
Boc-Pro	99	96	96	-
Boc-Phe	97	96	99	-
Boc-Val	96	93	96	-
Boc-Sar	96	89	97	-
Boc-Ile	85	93	93	97
Boc-ε-Z-Lys	85	95	99	-
Boc-D-Ala	84	88	92	97
Boc-N^{im}-Tos-His	79	84	100	-
Boc-Gly	76	91	96	-
Boc-Ser (OBzl)	71	89	92	95
Boc-Tyr (OBzl)	67	85	89	99

Boc = t-butyloxycarbonyl; Tos - *p*-toluenesulfonyl; Z = benzyloxycarbonyl; Bzl = benzyl

In solid phase synthesis, sometimes it is desirable to ensure complete reaction of a Boc amino acid (*e.g.* if it is isotopically labeled) rather than complete reaction of all chloromethyl groups. In the present work, this was accomplished at room temperature by using one equivalent of the potassium salt of Boc Gly or Boc Leu, one equivalent of 18-crown-6 and a 5-fold excess of chloromethyl resin in DMF for 18 hours.

Finally, a comparison of the relative reactivities of the Cs, Rb, and K salts of Boc Leu with an equivalent of 18-crown-6 gave the following results at room temperature, DMF, 18 hr: Cs-75%, Rb-75%, and K-83%; and at 50°C, DMF, 18 hr: Cs-87%, Rb-89%, and K-96%, thus confirming the rationale for used K rather than Cs or Rb salts.

In conclusion it has been demonstrated that 18-crown-6 will function as a catalyst in the quantitative esterification of a chloromethyl resin by the potassium salt of a Boc amino acid under mild reaction conditions. The catalysis, though useful, is less dramatic than we anticipated, probably because the reaction takes place too close to the backbone of the polystyrene to allow unhindered approach of the crown ether.

References and Notes

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(8) Under conditions of DMF at room temperature for 5 hr, Gisin[4] found that the Cs salt of Boc Val gave better incorporation onto a chloromethyl resin by approximate factors of 1.3, 1.8, 3.2 and 11 with respect to the Rb, K, Na, and Li salts of Boc Val.

(9) The log of the stability constants of the 1:1 complex of alkali earth cations and 18-crown-6; A) in MeOH: Na = 4.32, K = 6.10, Cs = 4.62 and B) in H_2O: Na = < 0.3, K = 2.06, Cs = 0.8. Y.A. Ovchinnikov, V.T. Ivanov, and A.M. Shkrob, "Membrane-Active Complexones", Elsevier Scientific Publishing Co., New York, 1974, p. 62.

(10) In a typical preparation of Boc amino acid potassium salts, 1.0 g of a Boc amino acid was dissolved in a mixture of EtOH (6 ml), H_2O (4 ml) and 1.0 equivalent of a 1.0 N KOH solution. The solvent was removed by azeotropic distillation in the presence of toluene and dried in vacuo over P_2O_5. The resultant hygroscopic white salt was used without further treatment.

(11) In a typical reaction 200 mg of a 1% cross-linked chloromethyl resin (Lab Systems, 0.75 mmol Cl^-/g-resin), Boc Leu OK (0.150 mmol), and 18-crown-6 (0.150 mmol) were combined in a 13 mm O.D. screw cap test tube equipped with a small magnetic stirring bar. 3.0 ml of solvent was added and the mixture was stirred in an oil bath regulated at 100° ± 3°C.

(12) The extent of reaction of a Boc amino acid with the resin was determined by filtering the reaction mixture, washing the resin with the reaction solvent (3 x 2 ml), with DMF (3 x 2 ml), with 75% DMF-H_2O (3 x 2 ml), and with EtOH (3 x 2 ml). The filtrate was titrated for chloride (ref. 5, pp. 55-56). The results were consistent with a determination of active chloride remaining on the resin[8] (ref. 5, p. 27), and with amino acid analysis. Hydrolyses were carried out in propionic acid at 130° for 3 hrs. (F. Westall and H. Hesser, Anal. Biochem., 61, 610 (1974)).

(13) Under similar conditions Gisin found the following percent incorporations with cesium salts: Boc Leu-87%, Boc Pro-82%, Boc Phe-85%, Boc Val-95%, Boc-L-Ala-77%, and Boc Gly-72%.

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