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143. The Preparation of Merrifield-Resins Through Total Esterification With Cesium Salts

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(26. II. 73)

Summary. The reaction of chloromethylated polystyrene-*co*-1%-divinylbenzene resin with the cesium salts of N-protected amino acids proceeds fast and without side reactions to give N-protected amino acyl resin esters free of quaternary ammonium sites or reactive chloride.

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In solid-phase peptide synthesis [1] the most widely used starting material is an N-protected amino acid bound *via* a benzyl ester linkage to an insoluble copolymer of styrene and divinylbenzene (*Merrifield*-resin)[2]. This derivative is accessible either in a one-step reaction or through intermediates, both approaches starting with chloromethyl resin. In the former scheme the chloromethyl resin is reacted with the triethylammonium salt of a Boc-amino acid[3] in ethyl acetate [1], ethanol [3], *t*-butyl alcohol [4] or DMF [5]. Other organic bases have also proven useful in this reaction, namely, tetramethyl ammonium hydroxide [6], diisopropyl ethylamine [7], and dicyclohexyl amine [8]. Indirect schemes of synthesis involve the preparation of acetoxymethyl resin [9] followed by saponification or aminolysis [10] and Boc-amino acylation of the resulting hydroxymethyl resin with the aid of a coupling agent, such as N,N'-carbonyldiimidazole [11], triphenylphosphine/2,2'-dipyridyl disulfide [12], DCCI [11], or N,N-dimethylformamide dineopentyl acetal [13]. Boc-amino acid resin esters have also been obtained from chloromethyl resin in a two-step procedure *via* a dimethyl methylene sulfonium resin salt as an intermediate [14][4].

Although all of these methods have been demonstrated to yield useful products, each one of them also suffers one or more of the following drawbacks:

1. The use of triethylammonium salts gives rise to a sizeable amount of quaternary ammonium sites on the resin [17]. The ion-exchange property of such groups can interfere [18] with those monitoring methods that depend on elution of anions from amine-containing resins, and they could conceivably retain TFA from the deprotection step and release it in a subsequent coupling step, resulting in the termination of peptide chains.

2. If there are unsubstituted chloromethyl groups left, they can react with triethyl amine to form additional quaternary ammonium groups in each neutralization step [19] giving rise to the problems mentioned above. In addition, they might alkylate other available nucleophiles, *e.g.*, the N-terminal amino groups of a peptide chain or alkylatable functional groups (His, Cys, Met) [20]. These are irreversible and yield-diminishing side-reactions.

3. Because of side-reactions or incomplete reaction it is difficult with presently available procedures to accurately determine the degree of substitution in advance.

For these reasons a search for a one-step procedure for obtaining Boc-amino acid resin esters devoid of quaternary ammonium sites or unreacted chloromethyl groups was undertaken, the result of which is presented here.

Although the lithium salt of a Boc-amino acid has been reported not to react with chloromethyl resin [21] the potassium salts of acetic acid [9] and of sebacid acid monomethyl ester [22] react at elevated temperatures. However, the conditions used in the latter procedures (125°, 24 h and 150°, 10 h respectively) appear too drastic for

[2] For a recent review on solid-phase peptide synthesis see ref. [2].

[3] The abbreviations recommended by the IUPAC-IUB Commission of Biomedical Nomenclature (J. biol. Chemistry *241*, 2491 (1966); *242*, 555 (1967)) have been used throughout. In addition, Boc = *t*-butyloxycarbonyl, TFA = trifluoroacetic acid, DMF = N,N-dimethylformamide, DCCI = N,N'-dicyclohexylcarbodiimide.

[4] Not discussed here are special-purpose resins, such as 'activateable' resins [15] and a resin with a 'spacer' between polymer and anchoring point of the first amino acid [16].

Boc-amino acids. The sodium salt of a Boc-amino acid has been used to attach an amino acid to a soluble chloromethyl polymer [23]. In this instance, when a two-fold excess of salt and three days reaction time was used the product still contained over 10% of unreacted chloromethyl groups, thus indicating a rather sluggish reaction rate.

A cesium salt, containing a cation larger than lithium, sodium or potassium might be expected to be more lipophilic and therefore more compatible with the resin. In addition, in a polar solvent, such as DMF, the salt of a carboxylic acid should be dissociated to a greater extent if the cation is large than if it is small. Since the carboxylate rather than the ion pair is the nucleophile that displaces the chloride, a high degree of dissociation is very desirable: It will result in a high concentration of the reacting species and consequently increase the reaction rate. In agreement with this reasoning, such a dependence of the esterification rate on the size of the cation was found to exist when various salts of a Boc-amino acid were allowed to react with chloromethyl resin (Fig. 1). Under otherwise identical conditions (DMF,

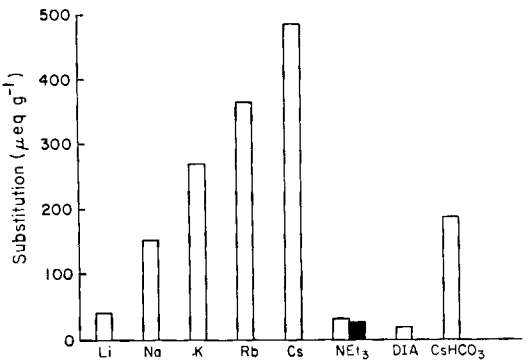

Fig. 1. *Incorporation of Boc-valine into chloromethyl resin* (substitution, 1950 μeq/g) *using various salts under otherwise identical conditions* (DMF, room temp., 5 h). The black bar represents quaternary ammonium groups obtained with the triethylammonium salt. When the cesium salt was prepared *in situ* from Boc-valine and cesium hydrogencarbonate, thus introducing one equivalent of water, the substitution was only half that found when dry Boc-Val-OCs was used (DIA = diisopropyl ethylamine).

room temperature, 5 h, 20% molar excess of salt over cholоromethyl groups on the resin) the cesium salt yielded the highest incorporation of Boc-valine into the resin. It was more than one order of magnitude greater than for the lithium salt and nearly double that of the potassium salt. In order to demonstrate the absence of amino or ammonium groups the resins were treated with pyridinium picrate and washed thoroughly [24]. There was no (< 1 μeq/g) picrate retained in the resins that had been prepared with alkali salts or with diisopropyl ethylamine, while in the case of the triethyl ammonium salt 25 μeq of picrate per gram of resin were found to be ionically bound to quaternary ammonium sites.

Because of their superior reaction rate cesium salts were used through the remainder of this study. When the temperature was raised to 50°, esterification with

Table 1. *Reaction of cesium salts with chloromethyl resins in DMF at 50°*

Salt		Chloromethyl Resin		Total Volume per g of Resin (ml)	Reaction Time (h)	Substitution[a] (μeq/g)	Yield[b]
Name	Equivalents	Equivalents	Substitution (μeq/g)				
Boc-Phe-OCs	1.0	5.0	1950	6	16	Phe, 400	100%
Boc-Val-OCs	1.2	1.0	1950	20	1.5	Val, 1230	63%
Boc-Val-OCs	1.2	1.0	1950	20	6	Val, 1670	85%
Boc-Val-OCs	1.2	1.0	1950	20	20	Val, 1830	94%
Bpoc-Val-OCs[c]	1.0	4.5	530	8	15	Val, 120[d]	100%
Boc-D-Val-L-Lac-OCs [25][c]	1.0	3.5	1950	7	16	Val, 540	96%
Boc-D-Val-L-Lac-OCs [25][c]	1.0	1.3	820	6	24	Val, 590	94%
Boc-D-Val-L-Lac-OCs [25][c]	1.0	1.0	550	7	16	Val, 490	89%
Boc-Gly-OCs	1.0	1.0	820	7	16	Gly, 590	72%
Boc-Ala-OCs	1.0	1.0	820	7	16	Ala, 630	77%
Boc-Pro-OCs	1.0	1.0	820	7	16	Pro, 670	82%
Boc-Leu-OCs	1.0	1.0	820	7	16	Leu, 710	87%
Boc-Phe-OCs	1.0	1.0	820	7	16	Phe, 700	85%
Boc-Val-OCs	1.0	1.0	820	7	16	Val, 780	95%

[a] By amino acid analysis after hydrolysis.
[b] Based on limiting reactant and substitution, thus, discounting mechanical losses.
[c] Bpoc = 2-(*p*-biphenyl)isopropyloxycarbonyl; Lac = lactyl.
[d] Dr. *R. S. Feinberg*, The Rockefeller University, personal communication.

Boc-Val-OCs was complete in less than twelve hours (Fig. 2). The chloride content of the resin decreased concomitantly with the incorporation of valine. Furthermore, there was no deprotection of the amino group ($< 0.05\%$ in 22 h) under the reaction conditions. It is therefore concluded that essentially no side reactions occur.

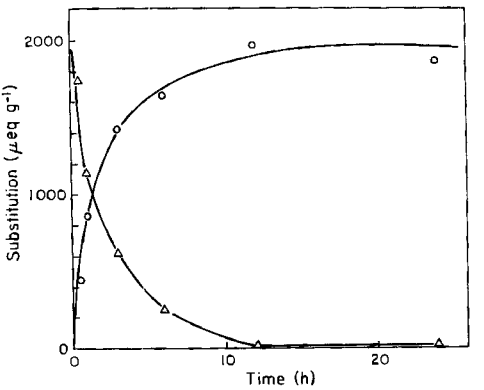

Fig. 2. *The esterification of chloromethyl resin with Boc-valine cesium salt as monitored by the decrease of the chloride content of the resin* (△) *and by the increase in valine substitution* (○). Conditions: DMF, 50°, 20% molar excess of salt over chloromethyl resin.

Since the reaction is quantitative the degree of substitution is conveniently predetermined by choosing either the cesium salt or the chloromethyl resin as the limiting reactant. Thus, when one equivalent of Boc-L-phenylalanine cesium salt was reacted at 50° with five equivalents of chloromethyl resin a quantitative yield of Boc-Phe-resin was obtained (Table 1). Conversely, with an excess (1.2 to 3-fold) of cesium salt over chloromethyl groups almost all of the chloride was displaced (Table 2).

Table 2. *Reaction of chloromethyl resins with varying excesses of Boc-Val-OCs in DMF* (6–8 ml per g of resin). Reaction time, 16 h, temperature, 50°.

Substitution of Chloromethyl-Resin (μeq/g)	Molar Excess of Boc-Val-OCs (Resin = 1.0)	Residual Chloride[a] (μeq/g)
550	3.0	0
150	3.0	2
50	3.0	0
550	1.5	8
150	1.5	3
50	1.5	2
1950	1.2	6
50	1.2	8

[a]) By potentiometric titration with $AgNO_3$ after quantitative displacement with hot pyridine. These values are corrected for the blank value (4 μeq/g) obtained with not chloromethylated polystyrene-*co*-1%-divinylbenzene resin.

The residual chloride represents a small fraction of chloromethyl groups that react only under far more drastic conditions (pyridine, 110°). Having such limited reactivity, however, these chloromethyl groups are of little concern, for they will most likely also be inert to substitution by other nucleophiles, such as tertiary amines or functional groups of the peptide chains.

For the preparation of resins to be used for peptide syntheses the scheme which achieves total esterification and leaves only little chloride unreacted is recommended. In a typical run 1.0 g of chloromethyl resin (substitution, 550 μeq/g), 232 mg of Boc-Val-OCs (660 μmol, 20% excess) and 9 ml of DMF were combined and stirred in a sealed vessel at 50° overnight. The resin was washed thoroughly with DMF, DMF/water 9:1 (*v/v*), DMF and ethanol, then dried and a sample was hydrolyzed. Amino acid analysis of the hydrolyzate indicated a substitution of 550 μequivalents of Boc-valine per gram of resin which corresponds to a yield of 98% based on the initial amount of chloromethyl groups on the resin.

The present method represents a smooth way to prepare *Merrifield*-resins in good yield and of a specific substitution. The high purity of these resins may help to reduce the number of unexplained side-products encountered in solid-phase peptide synthesis.

The author wishes to express his gratitude to Prof. *R. B. Merrifield* who suggested the need for a total esterification procedure for his helpful advice and to Mr. *Arunkumar Dhundale* and Mrs. *Maureen Sanz* for their technical assistance. This investigation was supported by NIH Grant HE 12 157, *U. S. Public Health Service* Grant AM 1260 and by the *Hoffmann-La Roche Foundation*.

Experimental Part

In order to correct for the weight change during reactions substitutions of resins are expressed in μ-equivalents per gram of benzyl polymer (·$CH_2C_6H_4$-resin) according to the formula [26] $s = a/(1 - a \cdot e)$ where s = substitution in μ-equivalents per gram; a = analytical concentration of substituent on the resin in μ-equivalents per gram, and e = equivalent weight of the substituent bound to the benzyl polymer in grams per μ-equivalent.

All chemicals and solvents used were reagent grade. DMF ('Spectroquality', *Matheson, Coleman & Bell*, East Rutherford, N.J.) was stored over Molecular Sieve Type 4A (same supplier). Alkali hydroxides and hydrogencarbonates were purchased from *ROC/RIC* Corp., Sun Valley, Calif., Boc-amino acids from *Fox Chemical Co.*, Los Angeles, Calif., *Beckman* Instruments, Inc., Palo Alto, Calif. or Protein Research Foundation, Japan; Polystyrene-*co*-1%-divinylbenzene resin (*Bio-Beads* S-X1, 200–400 Mesh) from *Bio-Rad Laboratories*, Richmond, Calif. Amino acid analyses (*Beckman* Spinco amino acid analyzer Model 21) were by Miss *L. Apacible* of Rockefeller University.

Chloromethyl Resins. Polystyrene-*co*-1%-divinylbenzene resin was chloromethylated with chloromethylmethylether and stannic chloride [27] [1] as described previously [10]. The degree of chloromethylation was varied by using different amounts of catalyst. Thus, when 10 g samples of resin in 60 ml of chloromethylmethylether were combined with 0.3, 0.12 or 0.06 ml of $SnCl_4$ in 10 ml hexane and allowed to react for 1 h at 0° substitutions of 550, 150 and 50 μ-equivalents of chloride per g of resin respectively were obtained. The substitution of chloride was determined by quantitative displacement with pyridine at 100–110° followed by potentiometric titration of the released chloride with $AgNO_3$ [28].

Cesium Salts of Boc-amino Acids. 2 g of a Boc-amino acid was dissolved in 10–15 ml of ethanol and diluted with 3–5 ml water. The pH of this solution was adjusted to 7.0 (pH-meter) by adding aqueous cesium hydrogencarbonate. The neutral solution was then flash evaporated. After repeated evaporation to dryness with bezene the Boc-amino acid cesium salts were obtained as white powders or solids. They were dried over P_2O_5 for 5 h and used without further purification.

Other Salts. The lithium, sodium, potassium and rubidium salts (Fig. 1) were prepared similarly to the cesium salt using the corresponding hydroxides or hydrogen carbonates. The triethylammonium and diisopropyl ethylammonium salts were prepared *in situ* by combining equivalent amounts of Boc-valine and base.

Boc-Amino Acyl Resin Esters. One millimol of dry Boc-amino acid cesium salt, one milliequivalent of chloromethyl resin and 6–8 ml of DMF per g of resin were placed in a screw-capped vial provided with a magnetic stirring bar. The suspension was stirred overnight while kept at 50° by means of a thermostated water bath. The resin was filtered, washed thoroughly with DMF, DMF/H_2O 9:1 (*v/v*), DMF and ethanol, and dried. The substitution of the resin was determined by amino acid analysis after hydrolysis with conc. HCl/propionic acid 1:1 (*v/v*) at 140° for 3 h [29]. The results of this and other experiments are summarized in Table 1 and 2.

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