Tetrahedron Letters No. 42, pp 3795 - 3798, 1976. Pergamon Press. Printed in Great Britain.

PREPARATION OF AMINOMETHYL-POLYSTYRENE RESIN BY DIRECT AMIDOMETHYLATION

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(Received in UK 13 August 1976; received in UK for publication 7 September 1976)

Aminomethyl derivatives of crosslinked polystyrene resins have become important for the preparation of supports for solid phase peptide synthesis. We have used an aminomethyl-resin in the preparation of a more acid-resistant support to minimize the loss of peptide intermediates during synthesis[1,2]. In addition, aminomethyl-resins have been used for the preparation of supports bearing spacer[3] or photolabile groups[4], supports for peptide sequencing[5] and polymeric carbodiimides[6].

Aminomethyl-resins 9 have been synthesized by reaction of chloromethyl-resins 5 with excess ammonia[4] or amines[5]. The potential for undesired crosslinking by alkylation of these aminomethyl sites with unreacted chloromethyl groups could be avoided by reaction of chloromethyl-resin 5 with potassium phthalimide[1,2,3,6] to give phthalimidomethyl-resin 7, followed by hydrazinolysis to the amine 9 (Route A). This procedure required the chloromethylation[7] of polystyrene resin 1 with chloromethyl methyl ether 2. However, the recent designation[8] of this reagent and its normal contaminant, bis(chloromethyl)ether, as potent carcinogens has prompted us to devise a new synthesis of aminomethyl-copoly(styrene-divinylbenzene)resin.

We find direct amidomethylation by the Tscherniac-Einhorn reaction[9] to be a superior route to the phthalimidomethyl-resin 7, which is then readily converted in refluxing ethanolic hydrazine to the aminomethyl-resin 9 (Route B). This synthesis uses the readily available N-(hydroxymethyl)phthalimide 3a or N-(chloromethyl)phthalimide 3b together with copoly(styrene-divinylbenzene)resin 1 and an acid catalyst, such as HF, CF_3SO_3H, or $SnCl_4$. An alternative route (C) utilizing N-(hydroxymethyl)trifluoroacetamide 4 is also attractive because of the mild conditions required to liberate the amine (10% KOH in ethanol at 25°).

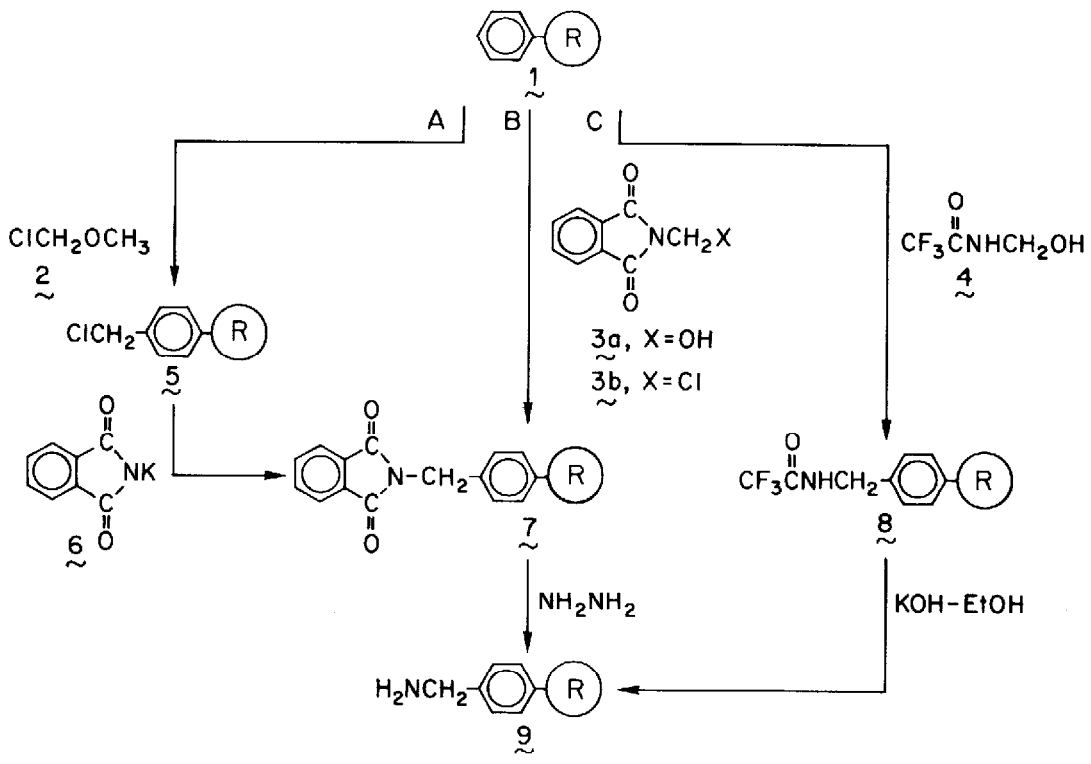


Figure 1. Synthetic Routes to Aminomethyl-copoly(styrene-divinylbenzene)resin

Typical experiment: Copoly(styrene-1%-divinylbenzene)resin 1 (Bio-Rad Laboratories, S-X1, 200-400 mesh beads) was suspended in 1:1 (v/v) trifluoroacetic acid-dichloromethane (10 ml/g resin) containing either 3a or 3b (Eastman Organic Chemicals) (5 mmole/g resin). Trifluoromethanesulfonic acid catalyst (1 mmole/g resin) was added and the mixture was shaken at room temperature for 4 hr. The resin was filtered and washed successively with 1:1 trifluoroacetic acid-dichloromethane, dichloromethane, ethanol and methanol, and dried under vacuum at 25°. Elemental analysis of the product, phthalimidomethyl-resin 7, indicated a substitution of 0.20 mmole N/g starting with 3a and 1.06 mmole/g starting with 3b. Characteristic i.r. bands were observed at 1720 and 1775 cm^{-1}. Treatment of 7 by refluxing for 10 hr. in ethanol containing 5% hydrazine (Eastman 95%+) gave aminomethyl-resin 9. The amine content of 9 was determined by picrate titration[10], which agreed with the nitrogen content.

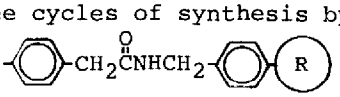
We have also reacted the polystyrene beads 1 with 3b (30 mmole/g resin) in anhydrous HF (5 ml/g resin) at 25° for periods up to 6 hr to give phthalimido-

methyl-resins having substitutions of 0.1 to 1.0 mmole N/g. Reaction of 1 and 3b in 0.1 M $SnCl_4$ in methylene chloride (10 ml/g resin) was also satisfactory.

Table I. Amidoalkylation of copoly(styrene-1%-divinylbenzene)resin[a]

Run	Reagent[b]	Catalyst[c]	Solvent[d]	Time (hr)	Substitution[e] (mmol N/g)
1	(4)	CF_3SO_3H	CH_2Cl_2-CF_3COOH (1:1)	1	0.10
2	"	"	"	2	0.28
3	"	"	"	3	0.37
4	"	"	"	4	0.51
5	(3a)	"	"	4	0.20
6	(3b)	None	"	22	<0.03
7	"	CF_3SO_3H	"	4	1.06
8	"	"	CH_2Cl_2	4	0.32
9	"	$SnCl_4$	CH_2Cl_2	4	1.35
10	"	HF	HF	4	0.52
11	"	HF	HF	6	1.06

[a] Bio-Beads, S-X1, 200-400 mesh. The incorporation of trifluoroacetamidomethyl groups onto the resin was indicated by strong infrared absorptions (KBr) at 1720, 1200 and 1170 cm^{-1}. The phthalimidomethyl-resin gave characteristic carbonyl absorptions at 1775 and 1720 cm^{-1}.
[b] 5 mmol/g-resin for reactions 1-9; 30 mmol/g-resin for reactions 10 and 11.
[c] 1 mmol/g-resin for reactions 1-5 and 7-9.
[d] 10 ml/g-resin. All reactions were run at room temperature.
[e] Determined by elemental nitrogen analysis.

The suitability of the new aminomethyl-resins for solid phase peptide synthesis was demonstrated by the preparation of the simple model tetrapeptide, Leu-Ala-Gly-Val[7]. Thus, aminomethyl-resin (0.27 mmole/g) prepared by route B was coupled with Boc-valyl-4-(oxymethyl)phenylacetic acid[1,2] using activation by dicyclohexylcarbodiimide. Residual amino groups were acetylated, and the resulting Boc-valyl-4-(oxymethyl)phenylacetamidomethyl-resin (0.19 mmole Val/g) was carried through three cycles of synthesis by standard procedures to give Boc-Leu-Ala-Gly-Val-OCH_2-  -$CH_2\overset{O}{\overset{\|}{C}}NHCH_2$-. Following cleavage from the resin with HF, the unpurified product contained 99.1% Leu-Ala-Gly-Val, as shown by ion exchange chromatography. These results are comparable to those obtained

earlier[1,2] on aminomethyl-resins prepared by route A and on chloromethyl-resin[11].

The new syntheses of aminomethyl-resin provide several advantages: (1) they avoid the use of the carcinogenic reagent chloromethyl methyl ether in the laboratory; (2) they avoid the need for commercial chloromethyl-resin and allow the use of unsubstituted styrene-divinylbenzene resins which can be subjected to much more vigorous washing procedures; (3) they avoid the problems related to excess chloromethyl or hydroxymethyl sites; (4) the use of HF as both catalyst and solvent may avoid the clustering of sites thought possible by the use of $SnCl_4$ or $ZnCl_2$ as catalyst; (5) the syntheses require one less reaction, the reactions are easy to perform, and they allow ready control of the degree of substitution.

This work was supported in part by Grant AM 01260 from the U.S. Public Health Service and by a grant from the Hoffmann-La Roche Foundation.

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