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Simple preparation and radiochemical estimation of phenolic polystyrenes suitable for solid (gel) phase peptide synthesis

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INTRODUCTION

The classical supports for the Merrifield solid (gel) phase peptide synthesis are the halomethyl- and hydroxymethyl- derivatives of bead-form crosslinked polystyrene[1]. The substituent groups are used to attach the carboxyl group of an *N*-protected amino acid which is to form the *C*-terminal of the peptide to be synthesized. Thus, the peptide chains undergoing synthesis are anchored, throughout each polystyrene bead, by benzyl ester linkages.

Currently, there is much interest in schemes of solid (gel) phase peptide synthesis in which the growing peptide chains are anchored, by phenyl ester linkages, to bead-form phenolic polystyrenes[2-6]. Initially, interest in this type of support was generated by the known base lability of the phenyl ester linkage. This lability has been exploited to detach peptides from the resin, in fully protected form, either by peroxide catalysed cleavage[7] or by autocatalysed transesterification with 2-dimethylaminoethanol[8]. Interest has been stimulated further by the discovery that premature acidolytic cleavage during peptide synthesis is minimal[6].

Bead-form phenolic polystyrene for solid (gel) phase peptide synthesis has, to date, been prepared in acetate form, by the suspension copolymerization of styrene, *p*-acetoxystyrene and divinylbenzene[4]. Phenol groups are generated subsequently by hydrazinolysis of the acetoxy groups. *p*-acetoxystyrene is expensive and tedious to prepare and the necessary suspension polymerization can be difficult to effect successfully to obtain discrete copolymer beads of appropriate size.

Here we report a simple approach to the preparation of suitable phenolic polystyrenes by direct functionalization of commercial polystyrene beads of similar quality to those used to prepare the conventional Merrifield supports. A radiochemical technique for the accurate estimation of the phenol content of the derivatized beads is described.

EXPERIMENTAL

Bromination of polystyrene beads

Styrene–1% divinylbenzene copolymer beads (40–75 μm) (Koch–Light Ltd, Colnbrook) were prewashed according to the method of Relles and Schluenz[9]. This involved treatment, at 60–80°C, with aqueous M NaOH, aqueuos M HCl, aqueous 2M NaOH/dioxan (1/2), aqueous 2m HCl/dioxan (1/2), water and dimethyl formamide followed by treatment at ambient temperature, with methanolic 2M HCl, water, methanol, methanol/dichloromethane (1/3) and methanol/dichloromethane (1/10). The washing procedure led to a loss of dry bead weight of 10%.

Controlled bromination was effected by an adaption of the method of Merrifield[10]. The washed resin (30 g, 0.276 mol) was suspended in carbon tetrachloride (250 cm^3) containing iodine (0.375 g, 0.003 mol) and a solution of bromine (20.1 cm^3, 62.3 g, 0.780 mol) added, with stirring. Polymer samples (~2 g) were removed from the reactor at intervals and were each washed immediately with carbon tetrachloride, dioxan, water and methanol before being vacuum dried at 50°C and the bromine content determined (*Table 1*).

Lithiation and oxidation of brominated polystyrenes

n-Butyllithium (3.2 g, 0.05 mol) was obtained by evaporating an aliquot of commercial 1.6 M n-butyllithium in hexane (Aldrich Chemical Co., Gillingham) by argon bubbling at 70°C in a Corley–Sachs–Anfinsen reactor[11]. The reactor temperature was adjusted to 60°C and dry toluene (30 cm^3) added, followed by the sample (2 g) of brominated polystyrene. Reaction was allowed to proceed, with gentle argon bubbling, over 2 h after which the lithiated polymer was washed successively with three aliquots of dry toluene (30 cm^3). Excess toluene was drawn off and t-butylhydroperoxide (15 cm^3, 0.10 mol) was added to the swollen beads. Reaction was allowed to proceed with argon bubbling for 2 h at 25°C after which the reaction solvent was drawn off and the beads washed successively with tetrahydrofuran, tetrahydrofuran/water (3/1), tetrahydrofuran/water (1/1), water and tetrahydrofuran. The beads were suspended in trifluoroacetic acid/dichloromethane (1/1) for 20 min and washed with 10% triethylamine in dichloromethane, tetrahydrofuran, tetrahydrofuran/water (3/1), tetrahydrofuran/water (1/1), water, ethanol and diethyl ether. The resulting polymer beads were vacuum dried at 50°C.

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Radiochemical estimation of polystyrene phenol content

[^{14}C]-Acetic anhydride (2Ci, vacuum ampoule, Radiochemical Centre, Amersham), was added to redistilled acetic anhydride (500 cm^3) via a vacuum line. The labelled anhydride was stored over phosphorus (V) oxide. Immediately before use an aliquot (50 cm^3) of labelled anhydride was heated under reflux with dicyclohexylcarbodiimide (2 cm^3) for 0.5 h and fractionally distilled collecting the fraction boiling at 138°C. Duplicate samples (0.5 g) of phenolic polymer were treated with labelled acetic anhydride (1 cm^3, 10 mmol, 1 μCi) and triethylamine (1.4 cm^3, 10 mmol) in dichloromethane (10 cm^3). After 3 h the [^{14}C]-labelled acetoxy polymer was washed successively with dichloromethane and ethanol. It was then treated with a 10% hydrazine solution in dimethylformamide/dioxan (10 cm^3). After 1 h the deacetylation mixture was drawn off and the resin washed with dimethylformamide and with ethanol.

The combined deacetylation mixture and wash solution were diluted to 50 cm^3 with ethanol. Standard solutions were prepared by dissolving [^{14}C]-labelled acetic anhydride (1 cm^3, 1 μCi), and the 10% solution of hydrazine (10 cm^3) in dimethylformamide (10 cm^3) and diluting to 50 cm^3 with ethanol. The activity of the samples and standards were determined with a Phillips Liquid Scintillation Analyser (PW4510/01). Aliquots (1 cm^3) of the standard and sample were added to a 2/1 (v/v) toluene/triton × 100 scintillation cocktail[12], containing 2,5-diphenyloxazole (0.4% w/v) and 1,4-di-2-(5-phenyloxazolyl)-benzene (0.02% w/v), in premeasured vials and the activity was redetermined. The phenolic content was calculated from the equation:

$$\text{Phenolic content (mol g}^{-1}) = \frac{A_x \times 0.0106}{A_0 \times g}$$

where A_x = combined activity of two duplicate measurements (this compensates for the loss of half of the label in the acetylation process); A_0 = activity of standard containing 0.0106 moles of [^{14}C]-acetic anhydride; g = weight of polymer sample in grams.

RESULTS AND DISCUSSION

Controlled bromination of bead-form 1% crosslinked polystyrene was effected to give derivatives of differing functionality (*Table 1*).

Samples of bromine content 0.99, 1.77 and 3.65 g^{-1} were selected for hydroxylation. The method, which involved conversion of the polymers to the respective lithium derivatives by treatment with n-butyllithium followed by reaction with t-butylhydroperoxide, was adapted from a procedure described by Chang and Edward[13] for the conversion of aryllithium compounds to phenol. The phenolic contents of the derivatized polymer were 0.12, 0.25 and 0.50 mmol g^{-1}, respectively, with residual bromine contents of 0.07, 0.11 and 0.15 mmol g^{-1}.

Table 1 Bromine contents of 1% crosslinked polystyrene beads

Bromination time/h	% Bromine	Extent of substitution/ mmol g^{-1}	Bromine atoms per aromatic ring
0.75	6.2	0.77	0.14
1.5	7.9	0.99	0.18
3	11.2	1.40	0.26
6	14.2	1.77	0.33
24	22.4	2.80	0.51
48	26.0	3.25	0.60
72	29.2	3.65	0.67

The phenol content was estimated by fully acetylating the polymer samples with [^{14}C]-labelled acetic anhydride in the presence of triethylamine. The [^{14}C]-labelled *O*-acetyl groups were removed from the resin by treatment with hydrazine solution and finally, measuring the amount of [^{14}C] radioactivity resolubilized. The efficacy of this procedure was confirmed by estimating a phenolic poly(acryloyl morpholine) derivative[14], known phenol content 1.0 mmol g^{-1}. This gave a value for the phenolic content of 0.95 mmol g^{-1} by the radiochemical method.

Reaction of the bead form 0.5 mmol g^{-1} phenolic polystyrene derivative in dichloromethane with t-butoxycarbonyl glycine and diisopropylcarbodiimide in the presence of 4-dimethylaminopyridine overnight led to a t-butoxycarbonyl glycine content of 0.3 mmol g^{-1}. This is accepted widely as an ideal loading for laboratory scale solid (gel) phase peptide synthesis[1].

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