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Use of a scintillant-containing macroporous resin in both solid phase synthesis and subsequent on-bead scintillation-based analysis

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Abstract

A chemically-functionalised scintillant-containing macroporous resin has been used successfully in a two stage solid phase organic synthesis. The second step in this synthesis involved the covalent attachment of a tritiated acetate group to the resin. The resultant radiolabelled scintillant-containing resin beads scintillate spontaneously and with high efficiency due to the close proximity of the tritium atoms to the scintillant molecules within the beads.

Keywords: oxazoles; polymer support; solid-phase synthesis; labelling.

In the preceding communication[1] we described the construction and evaluation of a chemically-functionalised scintillant-containing macroporous resin. We demonstrated successfully that this resin, unlike conventional scintillation proximity assay (SPA) beads,[2] could be exposed to exhaustive Soxhlet extraction without losing the ability to scintillate efficiently in the presence of ionising radiation. Our long term objective in this area is to develop a range of scintillant-containing resins and to exploit these novel materials by developing a simultaneous assay/deconvolution strategy for generic application in solid phase combinatorial chemistry. To achieve this goal, we need to be able to use scintillant-containing resins for both solid phase synthesis and subsequent on-bead SPA style assay of the compounds they bear. In this communication, we wish to report our preliminary results that demonstrate that a scintillant-containing resin can indeed be used successfully as a support for solid phase synthesis whilst retaining the ability to scintillate in a subsequent scintillation-based assay.

The scintillant-containing macroporous resin **1**, the construction of which is described in the preceding communication,[1] was formed by a suspension co-polymerisation reaction of 2,5-diphenyl-4-

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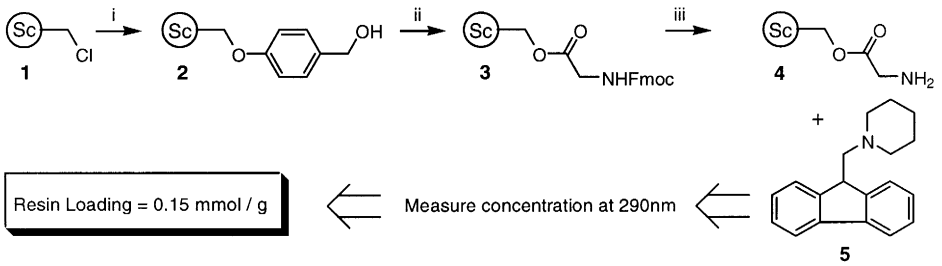
vinyloxazole, 4-vinylbenzyl chloride, 4-ethylstyrene and divinylbenzene. The product resin contained chemically reactive benzyl chloride groups, and thus may be best viewed as a scintillant-containing macroporous analogue of Merrifield's resin.[3] From the monomer composition used to construct the resin, the theoretical loading of benzyl chloride groups was calculated to be 1.2 mmol/g. Prior to using this resin for synthesis, we first needed to quantify the resin loading accurately. One method used routinely for evaluating resin loading employs an Fmoc release assay.[4] Here, an Fmoc-protected amino acid is attached to the resin and then the Fmoc group is removed subsequently by treatment with piperidine. The amount of liberated 1-fluoren-9-ylmethylpiperidine **5** relates directly to the resin loading and is quantified readily by UV–vis spectroscopy.[5]

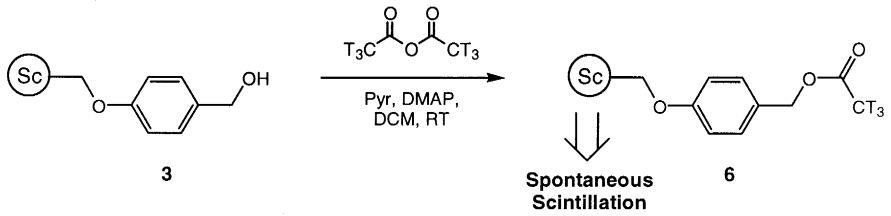
Prior to attachment of an Fmoc-protected amino acid, scintillant-containing resin **1** was first converted into a scintillant-containing macroporous analogue of Wang resin[6] **2** by reaction with 4-hydroxybenzyl alcohol in the presence of sodium methoxide.[7] Unsymmetrical active ester methodology[8] was then used to couple Fmoc–glycine to the scintillant-containing Wang resin **2**. The unsymmetrical active ester of Fmoc–glycine was prepared in situ by the addition of 2,6-dichlorobenzoyl chloride to a solution of pyridine and *N*,*N*-dimethylformamide that contained Fmoc–glycine and a suspension of scintillant resin **2**. The resin loading was then established using the Fmoc release assay. Aliquots, of known mass, of the Fmoc–glycine-substituted resin **3** were incubated with piperidine and the amount of 1-fluoren-9-ylmethylpiperidine **5** that was released was quantified by monitoring the absorbance of the reaction mixture at 290 nm (Scheme 1).



Scheme 1. Solid phase synthesis utilising scintillant-containing resin **1**. *Reagents and conditions:* (i) NaOMe, 4-hydroxybenzylalcohol, dimethylacetamide, 50°C; (ii) Fmoc–Gly–OH, 2,6-dichlorobenzoyl chloride, pyridine, DMF; (iii) 1–10 mg aliquots of resin **3**, 20% piperidine in DMF

The value for the resin loading that we obtained from this study was 0.15 mmol/g. This relatively low loading was not unexpected, since the resin was of a highly cross-linked macroporous type that had been formed in the presence of a toluene porogen. Consequently, many of the chemically reactive sites will reside in regions of the polymer matrix that are inaccessible to both solvents and reagents. Indeed recently, Sherrington[9] has reported the observation that a similar type of macroporous resin (derived from 25% chloromethylstyrene, 65% divinylbenzene and 10% styrene with toluene as the porogen) gave only 40% substitution when reacted with an alcohol in dimethylacetamide.

Our long term goal is to use scintillant-containing resins for both the synthesis of library molecules and, subsequently, to identify library molecules that bind through non-covalent interactions to a target radiolabelled receptor molecule, for example, an enzyme. To demonstrate this principle, we chose to mimic an infinitely tight binding interaction between library molecule and receptor by the covalent attachment of a radiolabel to a scintillant-containing resin. Accordingly, scintillant-containing Wang resin **2** was acetylated with tritiated acetic anhydride using standard conditions for acetylation[10] (Scheme 2). After a reaction time of 16 h, the resin was washed exhaustively with dichloromethane to remove all of the unreacted tritiated acetic anhydride and the tritiated acetic acid, formed as a by-product in the reaction.



Scheme 2. Acetylation of scintillant-containing resin **3** with tritiated acetic anhydride

The radiolabelled beads **6** that resulted from this transformation were found to scintillate spontaneously due to the close proximity of the tritium atoms to the scintillant molecules incorporated covalently within the resin. To quantify this phenomenon, aliquots of the beads were placed in the bottom of glass scintillation vials and the vials monitored in an appropriate scintillation counter. To establish the scintillating counting efficiency of the 'dry' beads, after exposure to exhaustive Soxhlet extraction and two steps of solid phase synthetic chemistry, 10 cm^3 of Ultima Gold, a commercial scintillation cocktail, was added to each vial. Recounting the vials gave the maximum possible counts that could be obtained from each sample.

As Table 1 shows, the average cpm obtained per mg of resin when counted 'dry' is 555 cpm/mg and the average counted in Ultima Gold scintillation cocktail is 970 cpm/mg. In previous work,[11] we have demonstrated that systems containing just 2,5-diphenyloxazole as the scintillant give approximately 70% scintillation counting efficiency when compared with Ultima Gold scintillation cocktail. With this factor taken into consideration, the radiolabelled scintillant-containing beads **6** are actually scintillating with approximately 80% efficiency.

Table 1
Scintillation counting results obtained for radiolabelled scintillant-containing resin **6**

Mass of resin / mg	Dry resin cpm	Dry resin cpm / mg resin	Resin in Ultima Gold cpm	Resin in Ultima Gold cpm / mg resin
6.39	3396	531	6847	1072
8.17	4699	575	8874	1086
7.31	4287	586	7207	986
7.78	4300	553	6544	841
7.33	3901	532	6352	866
Average cpm / mg		**555**	Average cpm / mg	**970**

Since the activity of the tritiated acetic anhydride was known, the figures obtained from the counting experiment in Ultima Gold also provided a method for establishing resin loading. When the resin loading was calculated in this manner, a value of 0.14 mmol/g was obtained. This value is in excellent agreement with the value of 0.15 mmol/g obtained using the more conventional Fmoc-release assay.

In conclusion, we have employed a chemically-functionalised scintillant-containing macroporous resin in two separate solid phase syntheses. In the second synthesis, by attaching a radiolabel to the beads covalently, we were able to form beads that scintillated spontaneously and with high efficiency. We have thus demonstrated successfully that scintillant-containing resins may be employed in solid phase combinatorial chemistry and subsequent SPA style assay. We now intend to exploit these novel materials by utilising them in the synthesis and subsequent on-bead assay of solid phase combinatorial libraries. Work to this effect is currently underway in our laboratory and we will report our findings in due course.

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