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Synthesis and evaluation of a scintillant-containing solid support for use in combinatorial chemistry

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Abstract

A scintillant monomer, 2,5-diphenyl-4-vinyloxazole, has been synthesised and co-polymerised with 4-ethylstyrene, divinylbenzene and 4-vinylbenzyl chloride in the presence of a toluene porogen, to form a scintillant-containing macroporous resin. Despite prolonged exposure to organic solvent, this resin retains the ability to scintillate efficiently in the presence of ionising radiation. © 2000 Elsevier Science Ltd. All rights reserved.

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The scintillation proximity assay (SPA) developed and marketed by Amersham Pharmacia Biotech, is a dynamic procedure for detecting and quantifying intermolecular interactions.[1] In this elegant approach, a receptor molecule is labelled with SPA beads. SPA beads are solid polymeric microspheres that contain 2,5-diphenyloxazole, a well-known scintillant molecule that is a major component of most commercially available scintillation cocktails.[2] In a typical SPA procedure, a tritiated ligand is incubated with a SPA-labelled receptor. If the receptor binds the ligand (Fig. 1a), the tritium is brought into close proximity to the encapsulated scintillant, resulting in the emission of light. This response may be monitored and quantified in a scintillation counter. The short pathlength of tritium β-particles (average pathlength=1.5 μm in water[3]) ensures that a signal is detected only when the receptor actually binds the ligand. Unbound material is too remote to elicit a significant signal (Fig. 1b).

SPA beads are prepared by incubating a DMSO solution of 2,5-diphenyloxazole with, for example, Sepharose 4B beads. Addition of water to this system results in the DMSO being washed away from the beads whilst the scintillant molecules, being insoluble in aqueous media, are precipitated within the pores of the beads. Unfortunately, this manufacturing process ensures that SPA beads are completely incompatible with the use of organic solvents. Exposure to organic solvent results in dissolution of

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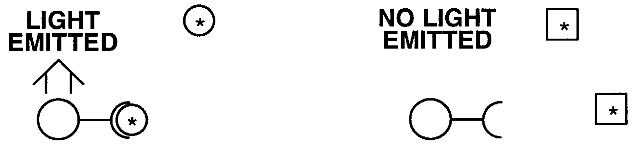


a) Tritiated ligand held in close proximity to the SPA bead causing light to be emitted

b) Tritiated molecules too remote from the SPA bead to elicit a response

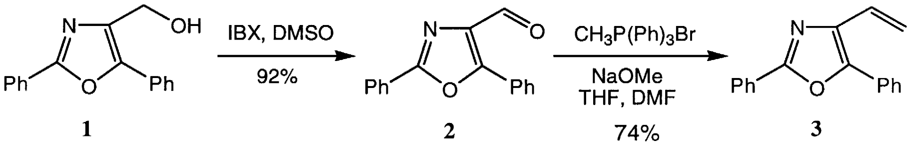
Fig. 1. Schematic representation of the scintillation proximity assay (SPA)

the precipitated scintillant molecules rendering the beads useless for subsequent assay. Indeed, even in aqueous solvents leaching of this type has been reported as problematic.[4]

The fact that exposure to organic solvents renders conventional SPA beads inactive precludes their use as supports for solid phase combinatorial chemistry and this has limited the application of SPA in the field of combinatorial chemistry. Our interest in developing new assay techniques for application in solid phase combinatorial chemistry prompted us to investigate whether chemically-functionalised polystyrene based beads, that incorporate 2,5-diphenyloxazole molecules covalently, could be used for solid phase synthesis and still retain the ability to scintillate in the presence of ionising radiation. We wish to report here the construction and evaluation of a chemically-functionalised scintillant-containing support[5] and in the following communication[6] we describe the use of this resin in solid phase synthetic chemistry and a subsequent scintillation-based assay.

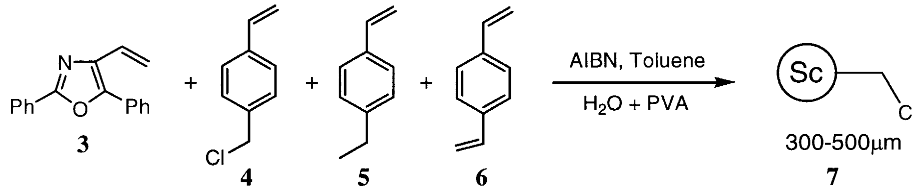
To construct a scintillant-containing macroporous resin we first required the ready availability of an appropriate monomer that contained a 2,5-diphenyoxazole scintillant moiety. Recently,[7] we reported the synthesis of aldehyde **2** formed by oxidising alcohol **1** with pyridinium chlorochromate (PCC). Consequently, 2,5-diphenyl-4-vinyloxazole **3** was selected as the monomer of choice since it was anticipated that this monomer could be synthesised readily from aldehyde **2** via a standard Wittig olefination reaction.[8]

Whilst the small-scale PCC oxidation of alcohol **1** proceeds in good yield (67%),[7] on a multi-gram scale the reaction was capricious and yields lower. Fortunately, when 2-iodoxybenzoic acid (IBX)[9] was used in place of PCC for this oxidation, multi-gram amounts of aldehyde **2** could be synthesised from alcohol **1** reproducibly and in excellent yield. As we had hoped, the subsequent Wittig reaction of aldehyde **2** proceeded smoothly, again on a multi-gram scale, giving the desired scintillant monomer **3** in good yield[10] (Scheme 1).



Scheme 1. Synthesis of 2,5-diphenyl-4-vinyloxazole **3**

Having established an efficient synthetic route to monomer **3**, we proceeded with the construction of a chemically-functionalised scintillant-containing polystyrene-based resin. In general, the resins used in solid phase synthetic chemistry and combinatorial chemistry are constructed by a suspension polymerisation procedure. Accordingly, monomer **3** was co-polymerised with 4-vinylbenzyl chloride **4**, 4-ethylstyrene **5** and a cross-linker, divinylbenzene **6**, using standard suspension polymerisation techniques.[11] This procedure was carried out in the presence of toluene, which served as a porogen and water containing poly(vinyl alcohol) (90% hydrolysed, average M_w, 90 000–146 000) which acted as a droplet stabiliser (Scheme 2).



Scheme 2. Synthesis of scintillant-containing macroporous resin **7**

The 20% cross-linked, chemically-functionalised scintillant-containing macroporous polymeric beads **7** that resulted from the suspension polymerisation were collected by filtration, washed exhaustively, dried and then sieved to provide resin beads with sizes ranging between 300 and 500 μm in diameter. The beads were then subjected to Soxhlet extraction using tetrahydrofuran. At various time intervals, a sample of resin was removed from the Soxhlet thimble and these samples, together with a blank control resin (prepared in an identical fashion to the scintillant containing resin but with monomer **3** being replaced by styrene), were subjected to a scintillation counting assay. Three portions of each resin sample, of known mass, were placed in scintillation vials. To each vial, an equal aliquot of a ^{14}C hexadecane/toluene stock solution was added and the vials monitored in a scintillation counter. The averaged counts per minute (cpm) obtained per mg of resin are shown in Fig. 2. As the figure shows clearly, even after prolonged Soxhlet extraction times, the scintillant-containing macroporous resin **7** still retains the ability to scintillate efficiently in the presence of ionising radiation (column c). In addition, the counts obtained for the scintillant-containing resin **7** (columns a, b and c) are far in excess of the background counts obtained for the corresponding non-scintillant-containing control resin (column d).

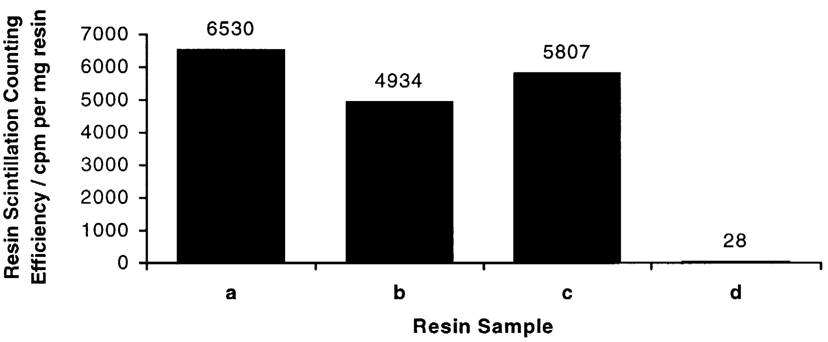


Fig. 2. Scintillation counting efficiencies of scintillant-containing resin **7** (after varying periods of Soxhlet extraction) and a non-scintillant-containing control resin. a=Resin **7**, Soxhlet time=0 h; b=resin **7**, Soxhlet time=24 h; c=resin **7**, Soxhlet time=48 h; d=non-scintillant-containing control resin. The activity of the ^{14}C hexadecane/toluene stock solution was 121 000 cpm per aliquot (assessed in triplicate by counting in commercial scintillation cocktails). In all cases, the total mass of resin per vial was measured to the nearest 0.1 mg with masses varying between 5.1 and 8.4 mg

In conclusion, we have synthesised 2,5-diphenyl-4-vinyloxazole **3** and used this monomer to construct chemically-functionalised macroporous polystyrene beads that scintillate efficiently in the presence of ionising radiation. In addition, we have demonstrated that this scintillant-containing resin retains the ability to scintillate even after prolonged exposure to organic solvent. In the following communication, we describe how this chemically-functionalised scintillant-containing resin may be used successfully for both solid phase synthetic organic chemistry and subsequent scintillation-based assay.[6]

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