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Research Article

Use of fluorous and solid-phase electrophiles as scavengers for excess amine in the preparation of sulfur-35 labelled radioligands

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Summary

A method for scavenging excess amines in sulfur-35-labeled radioligand preparations using fluorous scavengers has been developed in an effort to simplify the purification. This fluorous scavenging has been shown to be effective at removing excess amines from several [^{35}S]sulfonylation mixtures. In many cases this results in one less semi-preparative HPLC purification, and thus in higher radiochemical yield and time savings. Fluorous scavenging was compared to the use of a solid-phase resin (PS-isocyanate) and determined to be approximately equal with respect to recovery of radioactive product. However, the fluorous scavengers were shown to be more effective than solid-phase resin for removing basic amine components. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: radioligands; fluorous scavenging; solid-phase resin scavenging

Introduction

The synthesis of high specific activity [^{35}S]sulfonamides has been reported[1] as an often effective alternative to tritiated and radioiodinated radioligands[2] and photoaffinity probes.[3–5] Typically, 10–50 mCi of the appropriate carrier-free [^{35}S]sulfonyl chloride (1–10 μg, ~1000 Ci/mmol) is reacted with a large excess of precursor amine (2–5 mg). Purification is then conducted using semi-preparative HPLC. Due to the large amount of precursor amine mass (relative to [^{35}S]sulfonamide product), the first semi-preparative HPLC purification is usually inadequate for removing all mass and radiochemical impurities which

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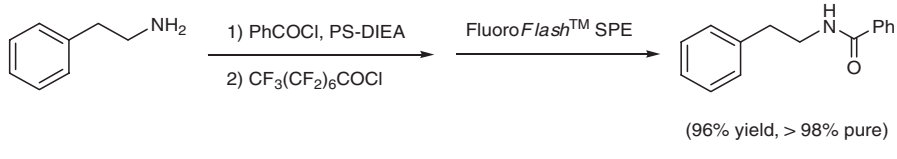
leads to multiple semi-preparative HPLC runs and lowered radiochemical recovery. Although *pKa* differences between sulfonamide product and amine precursor often exist, acid extraction is usually an ineffective separation approach due to the high lipophilicity of the starting material.

Solid-phase scavenging has been shown to be useful for rapid purification for a wide range of reaction mixtures.[6] A recent variation of this purification approach was reported by Lindsley and coworkers[7] which makes use of fluorous-phase separation principles[8] by tethering a fluorous chain to a scavenging electrophilic or nucleophilic group. The primary advantage of fluorous scavengers over solid-phase resins is that the reactions can be performed in a homogeneous manner which improves scavenging efficiency and reproducibility. The fluorous-tethered byproduct or starting material can then be removed by Fluoro*Flash*™ solid-phase extraction (SPE) to give the ''non-tagged'' desired product (Scheme 1).

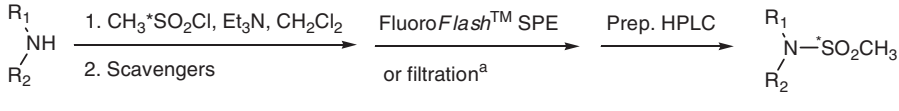
In order to reduce the need for repetitive preparative HPLC purifications, we were interested in the application of scavenging to high specific activity radioligand synthesis. However, we were concerned that non-specific absorption of the very low mass radiolabelled product to solid-phase resins would significantly decrease radiochemical recovery. For this reason we felt application of fluorous methods may hold advantages over solid-phase counterparts. Herein, we compare the scavenging efficiency and radioactivity recovery for fluorous and solid-phase resin scavenging (Scheme 2).

Results and discussion

A previously reported [^{35}S]sulfonylation reaction[2] was selected to assess scavenging efficiency with two fluorous scavengers, pentadecafluorooctanoyl chloride and 2-(perfluorooctyl) ethyl isocyanate. Both scavengers gave similar results for rate of amine scavenging and radioactivity recovery with a 1 h



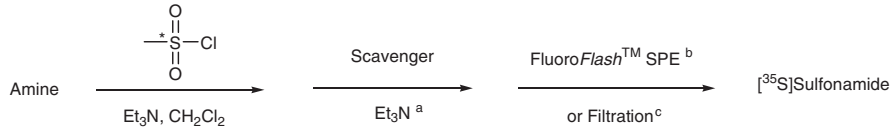
Scheme 1. An example of using fluorous scavengers in organic synthesis[7]

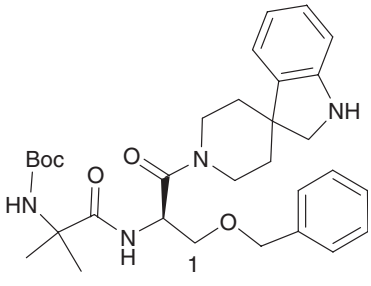
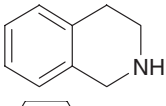
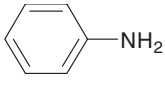
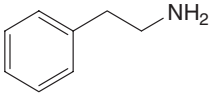


Scheme 2. Depiction of scavenging excess amine in [^{35}S]sulfonamide synthesis. *Denotes sulfur-35. [a]Workup for solid-phase resin scavenging reaction

reaction time, providing nearly complete removal of amine **1** following filtration through a Fluoro*Flash*™ SPE sep-pak (Table 1, entry 1). Due to better accessibility and lower cost, pentadecafluorooctanoyl chloride was used

Table 1. The scavenging of amines with different scavengers



Entry	Amine	Scavenger	Amine remaining (%)[d] 1 h	Amine remaining (%)[d] 24 h	Product recovery (%)[e]
1		$CF_3(CF_2)_6COCl$	1.1[f]	N/D	76
		$CF_3(CF_2)_7(CH_2)_2$ NCO	1.6[f]	N/D	92
		PS-isocyanate solid-phase resin	80.5[f]	11.6[f]	91
			38.2[g]	3.6[g]	90
2		$CF_3(CF_2)_6COCl$	0.6[f]	N/D	94
3		$CF_3(CF_2)_6COCl$	0[f]	N/D	99
4		$CF_3(CF_2)_6COCl$	0.8[f]	N/D	99
		PS-isocyanate solid-phase resin	17.0[f]	12.5[f]	93
			15.5[g]	6.5[g]	92

[a] Et_3N is necessary only when $CF_3(CF_2)_6COCl$ is used.
[b] Workup for fluorous scavenging.
[c] Workup for resin scavenging.
[d] Amine remaining (%) was calculated using the corresponding [^{35}S]sulfonamide as internal standard after correction for product recovery.
[e] Product recovery is defined as the ratio of the amount of [^{35}S]sulfonamide recovered *vs* the amount of [^{35}S]sulfonamide used in the scavenging reaction.
[f] 3 equivalents of scavenger was used.
[g] 10 equivalents of scavenger was used.
* Denotes sulfur-35.

to scavenge three other [^{35}S]sulfonylation mixtures with 1° or 2° amines with varying basicity. The scavenging efficiencies and product recoveries were nearly identical for all reactions (Table 1, entries 2–4), which indicates this method should be general for a wide range of amines. Solid-phase scavenging with PS-isocyanate resin was evaluated using two amine [^{35}S]sulfonylation reaction mixtures (Table 1, entries 1 and 4). In both cases good radioactivity recoveries were obtained, however the rates for scavenging reaction were slower and the amount of amine starting material remaining after 24 h was significantly higher compared to fluorous scavenging.

In conclusion, we have shown that solid-phase and fluorous scavenging can be effectively applied to high specific activity [^{35}S]sulfonamide radioligand synthesis in order to simplify the purification process. Due to the homogenous nature of the reaction using fluorous scavengers, this method provided more complete and faster removal of undesired reaction components compared to the use of PS-isocyanate resin.

Experimental

General

[^{35}S]sulfonylation reaction mixtures in methylene chloride were obtained using a standard procedure from methane[^{35}S]sulfonyl chloride.[2] Pentadecafluorooctanoyl chloride, 1,2,3,4-tetrahydroisoquinoline, aniline, phenethylamine and solvents were obtained from Aldrich. 2-(Perfluorooctyl) ethyl isocyanate and Fluoro*Flash*™ SPE sep-paks were obtained from Fluorous Technologies, Inc. (FTI). PS-isocyanate (1.5 mmol/g) was obtained from Argonaut Technologies, Inc. Anhydrous solvents were dried over 4 Å molecular sieves for at least 24 h prior to use. Analytical HPLC assays were performed using an Agilent 1100 series HPLC system with a G1315B diode-array UV detector, a Packard Radiomatic™ 500TR flow monitor and Phenomenex Luna C18(2) column (5 μm, 4.6 × 250 mm) with one of the following two methods: (a) 1.0 ml/min, 25°C, isocratic 35% acetonitrile −0.1% aqueous TFA for 20 min and 100% acetonitrile for 10 min, (b) 1.0 ml/min, 25° C, gradient 20% acetonitrile −0.1% aqueous TFA to 80% acetonitrile −0.1% aqueous TFA over 20 min and 100% acetonitrile for 10 min. The radioactive product was identified by HPLC co-elution with authentic unlabeled standard. The amount of radioactivity was determined by scintillation counting using a BIOSCAN liquid scintillation counter.

Fluorous scavenging

To a portion of a [^{35}S]sulfonylation mixture of amine (2–5 mg) and the corresponding [^{35}S]sulfonamide (0.5–11 mCi) in methylene chloride (∼200 μl) was added acetonitrile (1 ml). The mixture was then exposed to a stream of

nitrogen to evaporate off methylene chloride and some of acetonitrile to a final volume of ~200 µl. At this point, an HPLC assay of the solution was performed using method (a) or (b) to determine the pre-scavenging amount of amine present relative to [^{35}S]sulfonamide. To this solution was then added 3 equivalents of a fluorous scavenger (along with 3 equivalents of triethylamine with pentadecafluorooctanoyl chloride). After 1 h, the reaction mixture was diluted with 10 ml of 25% acetonitrile −0.1% aqueous TFA to give a turbid suspension, which was loaded onto a pre-conditioned 2 g Fluoro*Flash*™ SPE sep-pak.[‡] The non-fluorous tethered [^{35}S]sulfonamide was eluted with 20 ml of 60% methanol-water and the [^{35}S]sulfonamide was isolated in acetonitrile or methanol using a Phenomenex strata™-X sep-pak.[§] Finally, the product solution was counted to determine radioactivity recovery and assayed on HPLC to determine amine remaining (%).

Resin scavenging

A portion of a [^{35}S]sulfonylation mixture containing amine (2–5 mg) and the corresponding [^{35}S]sulfonamide (0.5–1 mCi) in 200–400 µl of methylene chloride was assayed by HPLC using method (a) or (b) to determine the pre-scavenging amount of amine present relative to [^{35}S]sulfonamide. To this solution was added PS-isocyanate resin (3 or 10 equivalents), and the resulting slurry was then stirred slowly at room temperature for 1 h. The reaction was assayed by HPLC using method (a) or (b) to determine amine remaining (%). The reaction mixture was stirred for another 23 h at which point 5 ml of methylene chloride was added. The resulting slurry was stirred for 5 min before the resin was removed by filtration to give the [^{35}S]sulfonamide in methylene chloride. The solution was counted to determine radioactivity recovery and assayed by HPLC to determine amine remaining (%).

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[‡] Pre-conditioning of a 2 g Fluoro*Flash*™ SPE sep-pak consisted of washing with 20 ml of methanol or acetonitrile followed by 20 ml of water.

[§] A Phenomenex strata™-X sep-pak workup procedure was performed as follows: a sep-pak was pre-conditioned with 2 × volume of methanol or acetonitrile followed by 2 × volume of water. The product solution which was diluted with an appropriate amount of water was loaded onto the sep-pak, and washed with 2 × volume of water. The product was then eluted with 5–10 ml of methanol or acetonitrile.

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