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Research Article

Synthesis of [^{35}S]aryl sulfonyl chlorides from [^{35}S]elemental sulfur

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Summary

A new synthetic methodology that captures electrophilic $^{35}S_8$ with aryl Grignard reagents or lithiates provides entry to a wide variety of [^{35}S]aryl sulfonyl chlorides upon oxidation and chlorination. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: [^{35}S]aryl sulfonyl chlorides; [^{35}S]alkyl sulfonyl chlorides

Introduction

High specific activity [^{35}S]methanesulfonamide[1] and [^{35}S]benzenesulfonamide[2] radioligands are finding increased use as tools to study receptors in biological systems. The coupling of amines with [^{35}S]sulfonyl chlorides is generally a fast and high-yielding procedure that can be adapted to a variety of substrates and can provide radioligands that offer superior sensitivity and better resolution for autoradiography when compared to tritiated and iodinated radioligands, respectively.

While initial attempts to produce high specific activity [^{35}S]benzenesulfonic acid by [^{35}S]sulfonation of benzene suffered from dilution of specific activity, presumably due to ubiquitous sulfates in the reagents or vessels, both [^{35}S]methanesulfonate and [^{35}S]benzenesulfonate are now commercially available at high specific activities (800–1200 Ci/mmol).[†] Following a simple one-step procedure using oxalyl chloride, the corresponding [^{35}S]sulfonyl chlorides are typically formed in over 90% radiochemical yields.[1,2]

In order to support a discovery program at Merck, we needed to prepare high specific activity [^{35}S]aryl sulfonamide radioligands. Initially, we

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[†][^{35}S]Benzenesulfonate and [^{35}S]methanesulfonate are commercially available by custom synthesis from Perkin-Elmer Life Sciences.

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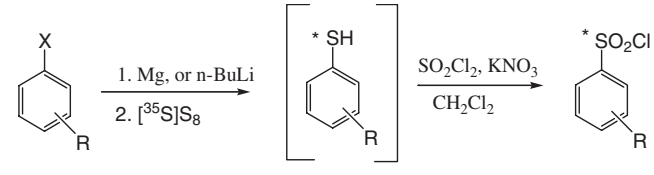
considered [^{35}S]sulfonation as an approach to making the desired [^{35}S]aryl sulfonates. However, the potential for deactivation of the aromatic ring by substituents and poor regiocontrol, as well as the potential for dilution of specific activity, made [^{35}S]sulfonation a less attractive option. A new synthetic methodology using [^{35}S]elemental sulfur (S_8) as a sulfur source was developed to overcome these limitations and was further developed as a general method to synthesize multisubstituted [^{35}S]aryl sulfonic acids and their corresponding [^{35}S]sulfonyl chlorides.

Results and discussion

While high specific activity [^{35}S]elemental sulfur has demonstrated limited stability in storage, herein we report the trapping of aryl Grignard reagents or lithiates[3] with commercially available, electrophilic high specific activity [^{35}S]S_8 to generate the corresponding [^{35}S]thiol or [^{35}S]polythiane species. Subsequent oxidation with KNO_3 and chlorination with SO_2Cl_2[4] generated the [^{35}S]aryl sulfonyl chlorides directly as shown in Scheme 1 below.

Using this procedure, a variety of multisubstituted aryl [^{35}S]sulfonyl chlorides were produced as highlighted in Table 1 below. While [^{35}S]sulfona-



X = Br, I
R = H, Cl, di-Cl, CF_3, CN

Scheme 1.

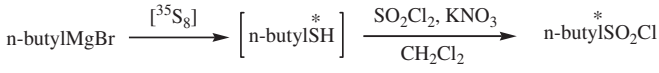
Table 1.

Starting halide	[^{35}S]Sulfonyl chloride	Radiochemical yield (%)
1-Bromo-3,4-dichlorobenzene	3,4-Dichlorobenzenesulfonyl chloride	15[b]
1-Bromo-2,4-dichlorobenzene	2,4-Dichlorobenzenesulfonyl chloride	25[a]
1-Iodo-3,5-dichlorobenzene	3,5-Dichlorobenzenesulfonyl chloride	30[a]
2-Bromonaphthylene	2-Naphthylenesulfonyl chloride	16[b]
Bromobenzene	Benzenesulfonyl chloride	30[b]
1-Iodo-3-trifluoromethylbenzene	3-Trifluoromethylbenzene sulfonyl chloride	14[a]
3-Iodobenzonitrile	3-Cyanobenzenesulfonyl chloride	27[a]
n-Butyl bromide	*n*-Butanesulfonyl chloride	25[a]

[a] Crude [^{35}S]sulfonyl chloride was hydrolyzed, purified, and reconstituted.
[b] Crude [^{35}S]sulfonyl chloride used 'as is'.

tion of naphthylene gives a mixture of the 1 and 2 isomers (ratio 85/15),[‡] the 2-isomer can be exclusively prepared by this new method from 2-bromonaphthylene. Deactivation of the aromatic ring by halogens, coupled with poor regioselectivity reduces the utility of [^{35}S]sulfonation of halobenzenes. Using standard lithium-halogen exchange or Grignard formation procedures,[5] followed by quenching with [$^{35}S_8$] and treatment with KNO_3/SO_2Cl_2, several [^{35}S]dichlorobenzenesulfonyl chlorides were produced as single regioisomers. In the synthesis of [^{35}S]2,4-dichlorobenzenesulfonyl chloride, lithium-halogen exchange on 1-bromo-2,4 dichlorobenzene was performed at low temperature to avoid potential benzyne formation. In general, the resulting [^{35}S]sulfonyl chloride was used 'as is' or hydrolyzed to the [^{35}S]sulfonic acid, purified by reverse phase chromatography, and reconstituted to the [^{35}S]sulfonyl chloride under standard conditions.[1]

We have also demonstrated the synthesis of an alkyl [^{35}S]sulfonyl chloride using this procedure, which provides an alternative to the Strecker synthesis of [^{35}S]alkylsulfonic acids. More specifically, [^{35}S]*n*-butanesulfonyl chloride was produced from *n*-butyl magnesium bromide (Scheme 2).



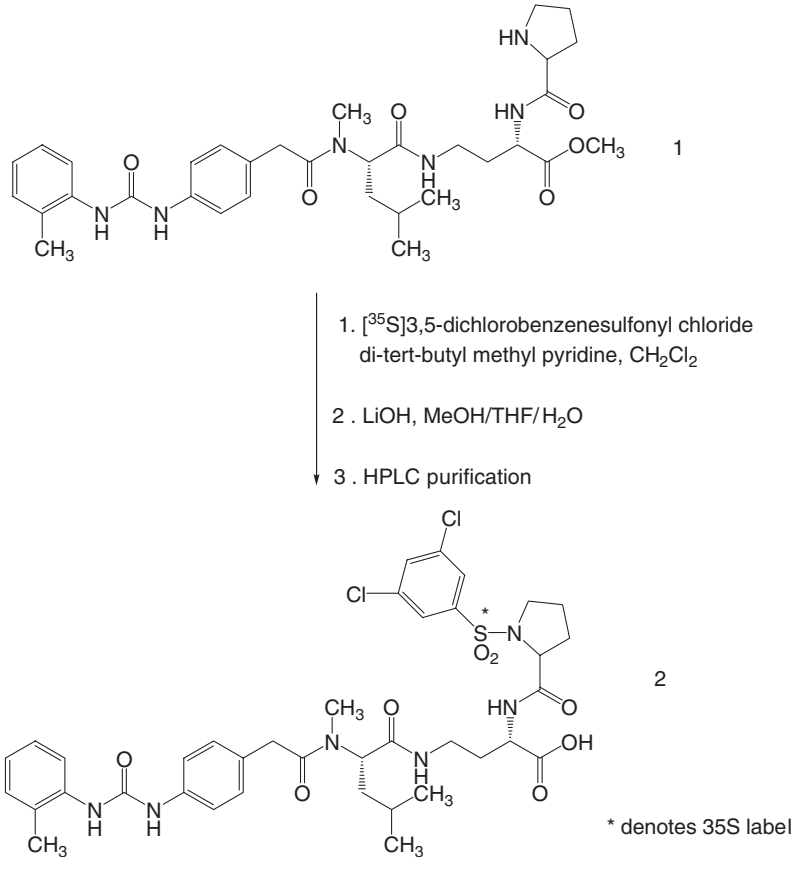
Scheme 2.

While the overall yields to several of the [^{35}S]aryl sulfonyl chlorides are moderate, this methodology remains a useful one in that many of the desired [^{35}S]aryl sulfonyl chlorides cannot be obtained any other way. Derivatization of the resulting [^{35}S]sulfonyl chlorides with amines provided several [^{35}S]sulfonamide radioligands with specific activities in the range of 700–1100 Ci/mmol indicating that no dilution had taken place (Scheme 3).

Experimental

Radioactivity measurements were carried out using Lumi-Scint Luminometer LSC liquid scintillation spectrometer using Packard Ultima Gold as scintillation medium. Analytical HPLC analyses were performed using a Dupont Zorbax RX C8 column (4.6 mm × 25 cm or Dupont Zorbax RX C18 column (4.6 mm × 25 cm), 1 ml/min, Rainin UV-1 detector at 240 nm, Packard Flo-1 Beta radioactivity monitor, Spectra-Physics SP8810 LC pump and controller, and software run on an IBM computer. [^{35}S]Elemental sulfur was obtained from Perkin-Elmer. Mass spectra were performed on a Hewlett Packard Series 1100 MSD mass spectrometer using an XDBC8 column with

[‡] Personal communication from Perkin-Elmer regarding the initial [^{35}S]isomeric ratio.



Scheme 3.

pH 3.5 formate buffer/acetonitrile gradient. The identities of the appropriate labeled sulfonyl chlorides or sulfonic acids were established by HPLC co-elution with authentic material or known derivatives.

[^{35}S]3,4-Dichlorobenzenesulfonyl chloride

To a flame dried flask containing Mg (23 mg, 0.95 mmol) and a crystal of iodine was added 1-iodo-3,4-dichlorobenzene (175 mg, 0.65 mmol) in 500 µl ether. The Grignard reaction was initiated with heating to 35°C for 30 min. After cooling to room temperature, [^{35}S]S_8 (25 mCi, stock solution in benzene) was added and the mixture aged for 2 h. HPLC analysis (Zorbax RX C8, 50/50 A/B, A = CH_3CN, B = 0.1% TFA, r_t = 16.3 min) showed the mixture contained many radiochemical entities (presumably due to the polymeric nature of the S_8).[§] The

[§] HPLC analysis does not always demonstrate the presence of free thiol due to the polymeric nature of elemental sulfur. Reaction with KNO_3/SO_2Cl_2 apparently breaks the polymer and leads to formation of the free sulfonyl chloride.

reaction was quenched with 1 ml of 1.0 N HCl, diluted with methylene chloride (10 ml). The phases were separated and the organic phase dried over $MgSO_4$ and filtered. A count of the solution indicated 7 mCi. The dichloromethane solution was treated directly with KNO_3 (10 mg) and SO_2Cl_2 (35 µl) and aged 16 h at room temperature. HPLC analysis (Zorbax RX C8, 50/50 A/B, A = CH_3CN, B = 0.1% TFA, r_t = 16.3 min) showed formation of the desired [^{35}S]3,4-dichlorobenzenesulfonyl chloride in 40% radiochemical yield. The organic phase was stirred vigorously with aqueous sodium bicarbonate solution to hydrolyze the sulfonyl chloride and the phases separated. The resulting aqueous phase was loaded on an Oasis HLB 1 g cartridge. Following elution with 3 volumes of water, followed by 20/80 CH_3CN/water afforded 4 mCi of 98% radiochemically pure [^{35}S]3,4-dichlorobenzenesulfonic acid HPLC analysis (Zorbax RX C8, 20/80 A/B, A = CH_3CN, B = 0.1% TFA, r_t = 11.6 min). The aqueous phase was concentrated by a stream of N_2 and the resulting solid dried *in vacuo* for 1 h. The solids were slurried into methylene chloride (5 ml) and 1 µl of DMF and 50 µl of oxalyl chloride were added. The reaction mixture was aged at room temperature overnight, then quenched with water (2 × 5 ml), washed with 10% aqueous sodium bicarbonate solution (5 ml) and dried twice over $MgSO_4$ to give 3.8 mCi of [^{35}S]3,4-dichlorobenzenesulfonyl chloride.

[^{35}S]2,4-Dichlorobenzenesulfonyl chloride

1-Bromo-2,4-dichlorobenzene (375 mg, 1.67 mmol) was dissolved in THF (1 ml) and ether (2 ml). The mixture was cooled to −90°C and *n*-BuLi (1.04 ml, 1.6 M) was added over 10 min. A toluene solution of [^{35}S]S_8 (14 mCi) was added over 10 min and the mixture aged for 15 min. The reaction was warmed to −50°C over 15 min then quenched with 1.0 N HCl (3 ml). The aqueous phase was extracted with methylene chloride (2 × 4 ml) and the combined organic phase dried over $MgSO_4$. To the crude reaction mixture was added KNO_3 (40 mg) and SO_2Cl_2 (100 µl) and the solution aged for 4 h at room temperature. To the mixture was added 100 µl of water and 50 µl of pyridine to hydrolyze the sulfonyl chloride and the stirred mixture aged overnight. The organic phase was extracted with water (1 × 8 ml) and the resulting aqueous phase loaded on an Oasis HLB 1 g cartridge. After washing with 3 volumes of water the [^{35}S]acid was eluted with 20/80 CH_3CN/water to afford 3.6 mCi of 98% radiochemically pure [^{35}S]2,4 dichlorobenzene sulfonic acid HPLC analysis (Zorbax RX C8, 20/80 A/B, A = CH_3CN, B = 0.1% TFA, r_t = 11.5 min). The aqueous phase was concentrated and the resulting solid dried *in vacuo* for 1 h. The solids were slurried into methylene chloride (5 ml) and 1 µl of DMF and 50 µl of oxalyl chloride were added. The reaction mixture was aged at room temperature overnight, then quenched with water (2 × 5 ml), washed with 10% aqueous sodium bicarbonate solution

(5 ml) and dried twice over $MgSO_4$ to give 3.5 mCi of [^{35}S]2,4-dichlorobenzene sulfonyl chloride.

[^{35}S]3,5-Dichlorobenzenesulfonyl chloride

To a flame dried flask containing Mg (23 mg, 0.95 mmol) and a crystal of iodine was added 1-iodo-3,5-dichlorobenzene (185 mg, 0.7 mmol) in 500 µl ether. The Grignard reaction was initiated with heating to 35°C for 30 min. After cooling to room temperature, [^{35}S]S_8 (12 mCi, stock solution in benzene) was added and the mixture aged for 2 h. The reaction was quenched with 1 ml of 1.0 N HCl, diluted with methylene chloride (10 ml). The phases were separated and the organic phase dried over $MgSO_4$ and filtered. A count of the solution indicated 7 mCi. The dichloromethane solution was treated directly with KNO_3 (10 mg) and SO_2Cl_2 (35 µl) and aged 16 h at room temperature. HPLC analysis (Zorbax RX C8, 50/50 A/B, A = CH_3CN, B = 0.1% TFA, r_t = 15.9 min) showed formation of the desired [^{35}S]3,5-dichlorobenzenesulfonyl chloride formation in 47% radiochemical yield. The organic phase was stirred vigorously with aqueous sodium bicarbonate solution to hydrolyze the sulfonyl chloride and the phases separated. The resulting aqueous phase was loaded on an Oasis HLB 1 g cartridge. Elution with 3 volumes of water, followed by 20/80 CH_3CN/water afforded 4 mCi of 98% radiochemically pure [^{35}S]3,5-dichlorobenzenesulfonic acid. HPLC analysis (Zorbax RX C8, 20/80 A/B, A = CH_3CN, B = 0.1% TFA, r_t = 11 min). The aqueous phase was concentrated by a stream of N_2 and the resulting solid dried *in vacuo* for 1 h. The solids were slurried into methylene chloride (5 ml) and 1 µl of DMF and 50 µl of oxalyl chloride were added. The reaction mixture was aged at room temperature overnight, then quenched with water (2 × 5 ml), washed with 10% aqueous sodium bicarbonate solution (5 ml) and dried twice over $MgSO_4$ to give 3.8 mCi of [^{35}S]3,5-dichlorobenzenesulfonyl chloride.

[^{35}S]2-Napthylenesulfonyl chloride

To a flame dried flask containing Mg (51 mg, 2.12 mmol) and a crystal of iodine was added 2-bromonapthylene (250 mg, 1.2 mmol) in 2 ml of ether. The Grignard reaction was initiated with heating to 35°C for 30 min. After cooling to room temperature, [^{35}S]S_8 (150 mCi, stock solution in benzene) was added and the mixture aged for 2 h. The reaction was quenched with 2 ml of aqueous ammonium chloride and diluted with methylene chloride (10 ml). The phases were separated and the organic phase dried over $MgSO_4$ and filtered. A count of the solution indicated 75 mCi. The dichloromethane solution was treated directly with KNO_3 (10 mg) and SO_2Cl_2 (75 µl) and aged 16 h at room temperature. HPLC analysis (Zorbax RX C18, 30/70 A/B

to 100 A over 20 min, A = CH_3CN, B = 0.1% TFA, r_t = 18 min) showed formation of the desired [^{35}S]2-naphthylenesulfonyl chloride in 47 (25 mCi) radiochemical yield.

[^{35}S]Benzenesulfonyl chloride

To a 0°C solution of phenyl magnesium bromide in THF (1 ml, 1.0 M, 1 mmol) under N_2 was added [^{35}S]S_8 (stock solution in benzene, 50 µl, 2.2 mCi). The mixture was aged 1 h at room temperature. HPLC analysis (Zorbax RX C18, 30/70 A/B to 100 A over 20 min, A = CH_3CN, B = 0.1% TFA, r_t = 14 min) showed [^{35}S]thiophenol formation in 50% radiochemical yield.[4] The reaction was quenched with 1 ml of 1.0 N HCl and diluted with methylene chloride (5 ml). The phases were separated and the organic phase dried over $MgSO_4$ and filtered. A count of the solution indicated 1.75 mCi. The dichloromethane solution was treated directly with KNO_3 (5 mg) and SO_2Cl_2 (25 µl) and aged 16 h at room temperature. HPLC analysis (Zorbax RX C18, 30/70 A/B to 100 A over 20 min, A = CH_3CN, B = 0.1% TFA, r_t = 13.2 min) indicated formation of [^{35}S]benzenesulfonyl chloride in 40% yield.

[^{35}S]3-Trifluoromethylbenzenesulfonyl chloride

To a solution of 1-iodo-3-trifluoromethylbenzene (189 mg, 0.695 mmol) in anhydrous THF (1.0 ml) was added *n*-BuLi (435 µl, 0.696 mmol) at −78°C under N_2. After stirring at −78°C for 30 min, 100 µl of the aryl lithiate solution was added dropwise to a solution of [^{35}S]S_8 (100 mCi, stock solution in toluene). The reaction mixture was allowed to warm to room temperature and aged for 1 h, then cooled to 0°C, and quenched with 1 ml of 1.0 N HCl. The mixture was extracted with methylene chloride (4 × 2 ml) and the combined organic phase dried over $MgSO_4$ and filtered. The dichloromethane solution was treated directly with KNO_3 (25 mg) and SO_2Cl_2 (50 µl) and aged 16 h at room temperature. The organic phase was stirred vigorously with water and the phases separated the resulting aqueous phase was loaded on an Oasis HLB 1 g cartridge. After washing with 3 volumes of water the [^{35}S]acid was eluted with 20/80 CH_3CN/water to afford 14 mCi of 97% radiochemically pure [^{35}S]3-trifluoromethylbenzene sulfonic acid. HPLC analysis (Zorbax RX C8, 20/80 A/B, A = CH_3CN, B = 0.1% TFA, r_t = 8 min). The aqueous phase was concentrated and the resulting solid dried *in vacuo* for 1 h. The solids were slurried into methylene chloride (5 ml) and 1 µl of DMF and 50 µl of oxalyl chloride were added. The reaction mixture was aged at room temperature overnight, then quenched with water (2 × 5 ml), washed with 10% aqueous sodium bicarbonate solution (5 ml) and dried twice over $MgSO_4$ to give 13.8 mCi of [^{35}S]3-trifluromethylbenzenesulfonyl chloride.

[^{35}S]3-Cyanobenzenesulfonyl chloride

To a solution of 1-iodo-3-cyanobenzene (51 mg, 0.267 mmol) in anhydrous THF (1.0 ml) was added *n*-BuLi (165 µl, 0.267 mmol) at −78°C under N_2. After stirring at −78°C for 30 min, 100 µl of the [^{35}S]S_8 (18 mCi) in toluene (100 µl) was added dropwise to the anion solution. The reaction mixture was quenched with 1 ml of saturated ammonium chloride at −78°C. The mixture was extracted with methylene chloride (4 × 2 ml) and the combined organic phase dried over $MgSO_4$ and filtered. The dichloromethane solution was treated directly with KNO_3 (25 mg) and SO_2Cl_2 (50 µl) and aged 16 h at room temperature. To the organic phase was added 50 µl of pyridine and 100 µl of water and stirred vigorously to hydrolyze the [^{35}S]sulfonyl chloride. The phases were separated and the resulting aqueous phase was loaded on an Oasis HLB 1 g cartridge. After washing with 3 volumes of water the [^{35}S]acid was eluted with 20/80 CH_3CN/water to afford 5 mCi of 97% radiochemically pure [^{35}S]3-cyanobenzenesulfonic acid. HPLC analysis (Zorbax RX C8, 20/80 A/B, A = CH_3CN, B = 0.1% TFA, r_t = 12 min) The aqueous phase was concentrated and the resulting solid dried *in vacuo* for 1 h. The solids were slurried into methylene chloride (5 ml) and 1 µl of DMF and 50 µl of oxalyl chloride were added. The reaction mixture was aged at room temperature overnight, then quenched with water (2 × 5 ml), washed with 10% aqueous sodium bicarbonate solution (5 ml) and dried twice over $MgSO_4$ to give 4.8 mCi of [^{35}S]3-cyanobenzenesulfonyl chloride.

[^{35}S]n-Butanesulfonyl chloride

To a solution of butyl magnesium bromide (1 ml, 2.0 M, 2 mmol) under N_2 was added [^{35}S]S_8 (stock solution in benzene, 75 µl, 3 mCi). The mixture was aged 1 h at room temperature. The reaction was quenched with 1 ml of 1.0 N HCl, diluted with methylene chloride (5 ml). The phases were separated and the organic phase dried over $MgSO_4$ and filtered. A count of the solution indicated 1.4 mCi. The dichloromethane solution was treated directly with KNO_3 (5 mg) and SO_2Cl_2 (25 µl) and aged 16 h at room temperature. HPLC analysis (Zorbax RX C18, 30/70 A/B to 100 A over 20 min, A = CH_3CN, B = 0.1% TFA, r_t = 9 min) demonstrated [^{35}S]butanesulfonyl chloride formation in 60% radiochemical yield.

[^{35}S]Sulfonamide **2**

3.9 mg of amine **1** and 4 mg of di-*tert*-butylmethyl pyridine was dissolved in 20 µl of methylene chloride. [^{35}S]3,5-Dichlorobenzenesulfonyl chloride in methylene chloride (5.5 mCi) was concentrated by atmospheric distillation to a volume of approximately 75 µl and added to the amine solution. The mixture was aged at room temperature for 2 h, then quenched directly into methanol

(1 ml), THF (1 ml) and water (1 ml). 5 mg of lithium hydroxide was added and the mixture aged for 1 h at room temperature. HPLC analysis (Zorbax RX C18, 50/50 A/B, A = CH_3CN, B = 0.1% TFA, r_t = 15 min) indicated the desired [^{35}S]sulfonamide **2** in 80% overall radiochemical yield. The crude tracer was purified by preparative HPLC (Zorbax RX C18, 50/50 A/B, A = CH_3CN, B = 0.1% TFA) to afford 3.45 mCi of pure [^{35}S]sulfonamide **2**. Mass spectral analysis (MS *m/e* 817 $[M+H]^+$ unlabeled, *m/e* 820 $[M+H]^+$ labeled) demonstrated the specific activity of [^{35}S]sulfonamide **2** was 755 Ci/mmol.

Conclusion

A new synthetic methodology has been developed that captures electrophilic $^{35}S_8$ with aryl or alkyl Grignard reagents or lithiates. The methodology provides access to a wide variety of substituted [^{35}S]aryl sulfonyl chlorides upon oxidation and chlorination.[¶]

Acknowledgements

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[¶] It has been discovered that the resulting aqueous waste from the oxidation/chlorination reaction gives off volatile radioactivity and must be stored in a capped container to prevent contamination.

