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Short Research Article

Synthesis of $^{13}C_6$, ^{3}H, and ^{14}C labeled Sch 414319 and ^{35}S labeling of an analog, Sch 225336†

CAROLEE FLADER LAVEY*, DAVID HESK, SHARON HENDERSHOT, DAVID KOHARSKI, SURINDERJIT SALUJA and PAUL MC NAMARA

Schering-Plough Research Institute, 2015 Galloping Hill Road, K15-4-4545, Kenilworth, NJ 07033, USA

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Introduction

Sch 414319 (**1**, Figure 1), a cannabinoid inverse agonist, can potentially be used for the treatment of psoriasis, multiple sclerosis, and rheumatoid arthritis. Three labeled forms of Sch 414319 were synthesized within our group. Carbon-13 labeled Sch 414319 was prepared for use as a standard in a mass spectrometry based bioanalytical assay. Tritiated material and a carbon-14 labeled compound were synthesized to assess metabolism. In addition, a sulfur-35 labeled form of the mesylated analog, Sch 225336 (**2**, Figure 1), was prepared to study the binding kinetics of the CB2 receptor. The synthesis of each labeled compound is described in this paper.

Results and discussion

The synthesis of [^{3}H]Sch 414319 is summarized in Scheme 1, where Sch 414319 was deprotonated using *n*-butyllithium and the resulting anion was quenched with low specific activity tritiated water (50 Ci/ml). An analysis of a portion of the compound by ^{3}H-NMR showed that the tritium was distributed throughout the three aromatic rings.

The synthesis of [$^{13}C_6$]Sch 414319 was based on the route developed and optimized by Chemical Research and Chemical Development (unpublished results) at Schering-Plough Research Institute[1] and is outlined in Scheme 2. The label was introduced using [$^{13}C_6$]fluorobenzene, which was deprotonated using *sec*-butyllithium and the resulting anion quenched with carbon dioxide[2] to give benzoic acid **4**. Aniline **5** was generated by the treatment of **4** with sodium azide and was subsequently converted to thiophenol **6** via diazonium salt formation followed by reaction with potassium ethyl xanthate[3]. Disulfide **7** was formed by the oxidation of thiophenol **6** using sodium perborate[4]. An impurity was present that co-eluted with the disulfide during column chromatography—the impure disulfide was used in the subsequent reaction and the impurity was removed at a later stage by column chromatography. Sulfide **9** was generated by deprotonating chlorosulfone **8** with *n*-butyllithium and quenching the anion with disulfide **7**. The 2-fluoro[$^{13}C_6$]thiophenol (**6**) that was generated in this reaction could be recovered and potentially re-used in future syntheses. Crude sulfide **9** was oxidized to sulfone **10** using a urea/hydrogen peroxide complex and trifluoroacetic anhydride. Free amine **11** was formed by reaction of the trifluoroacetanilide in **10** with lithium hydroxide and was subsequently converted to [$^{13}C_6$]Sch 414319 (**12**) using triflic anhydride. Compound **12** was purified by column chromatography and recrystallized twice from toluene. A 1.1% impurity persisted and was removed ultimately by converting [$^{13}C_6$]Sch 414319 to its piperazine salt (**13**).

The first batch of [^{14}C]Sch 414319 (**19**) was prepared using a route analogous to that for [$^{13}C_6$]Sch 414319 (**12**). The radiochemical yield was 1.7% for this eight-step synthesis, starting from fluoro[U-^{14}C]benzene. Subsequently, an improved route to the carbon-14 labeled compound was devised (Scheme 3) that

*Correspondence to: Carolee Flader Lavey, Schering-Plough Research Institute, 2015 Galloping Hill Road, K15-4-4545, Kenilworth, NJ 07033, USA. E-mail: carolee.lavey@spcorp.com

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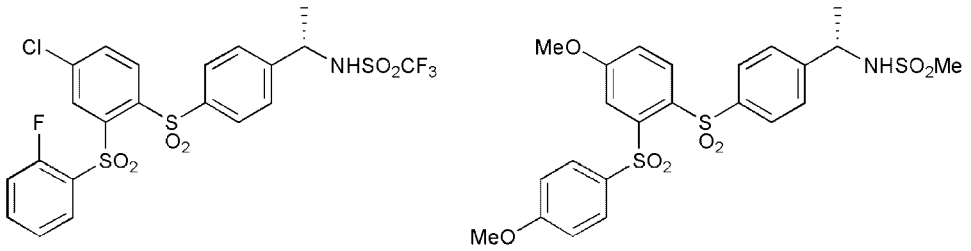
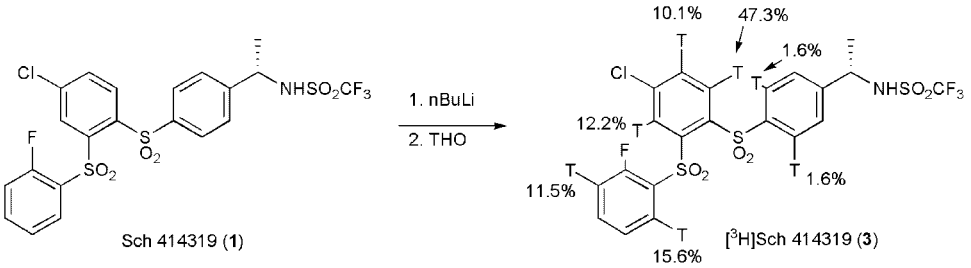


Figure 1 Sch 414319 (**1**); Sch 225336 (**2**)



Scheme 1

resulted in an 8.1% radiochemical yield of **19** after six steps. For this synthesis, fluoro[U-^{14}C]benzene was deprotonated using *sec*-butyllithium and the resulting anion was quenched with sulfur dioxide to give sulfinic acid **14**. Sulfinate ester **15** was generated by conversion of the sulfinic acid to the sulfonyl chloride using thionyl chloride, followed by reaction with ethanol and pyridine[5]. Compound **15** was reacted directly with the anion generated from chlorosulfone **8** to give sulfoxide **16**, which was oxidized to sulfone **17** using a urea/hydrogen peroxide complex and trifluoroacetic anhydride. The last two steps in the synthesis of [^{14}C]Sch 414319 (**19**) parallel those of the carbon-13 synthesis, with the conversion of the trifluoroacetanilide in compound **17** to the free amine (compound **18**) using lithium hydroxide, followed by reaction with triflic anhydride to give the final product (**19**).

Sch 225336, an analog of Sch 414319, was labeled with sulfur-35 as shown in Scheme 4 in order to aid in the study of the binding kinetics of the CB2 receptor. For this synthesis, methane[^{35}S]sulfonic acid was reacted with oxalyl chloride to give [^{35}S]mesyl chloride. Amine **20** was mesylated using this crude reagent[6] to give [^{35}S]Sch 225336 (**21**). The radiochemical yield was 28.7% from methane[^{35}S]sulfonic acid.

Experimental

Materials: [$^{13}C_6$]Fluorobenzene was purchased from Cambridge Isotope Labs, fluoro[U-^{14}C]benzene was purchased from either GE Healthcare or Perkin Elmer, tritiated water was purchased from GE Healthcare, and methane [^{35}S]sulfonic acid was purchased from Perkin Elmer. All anhydrous solvents and reagents not listed above (including gases) were purchased from commercial suppliers (Acros Organics, Fisher, or Aldrich Chemical Company) and used as received. Compounds **1**, **8**, and **20** were obtained from either Chemical Research or Chemical Development at Schering-Plough Research Institute.

Liquid scintillation counting: Quantitation of radioactivity was performed using a Packard 2200CA liquid scintillation analyzer and Scintiverse BD cocktail.

Thin layer chromatography (TLC): Thin layer chromatography was performed using Whatman LK6DF (silica gel 60) 5×20 cm, 0.25 mm plates. The plates were scanned on a Bioscan 1000 linear analyzer using the solvent systems indicated.

High performance liquid chromatography: [^{3}H] and [^{14}C]Sch 414319 and [^{35}S]Sch 225336 were analyzed by HPLC for radiochemical and chemical purity. [$^{13}C_6$]Sch 414319 was analyzed for chemical purity by HPLC. In addition, reaction progress was frequently monitored by HPLC. Chemical purity was determined using a Waters 2487 Dual programmable wavelength detector and radiochemical purity using a Radiomatic C150TR radioflow detector with Radiomatic Flo-Scint III liquid scintillation cocktail. The following systems were used:

1. Zorbax SB-C18 column, 4.6 mm ID $\times$ 150 mm, 254 nm, (65:35:0.1) acetonitrile:water: trifluoroacetic

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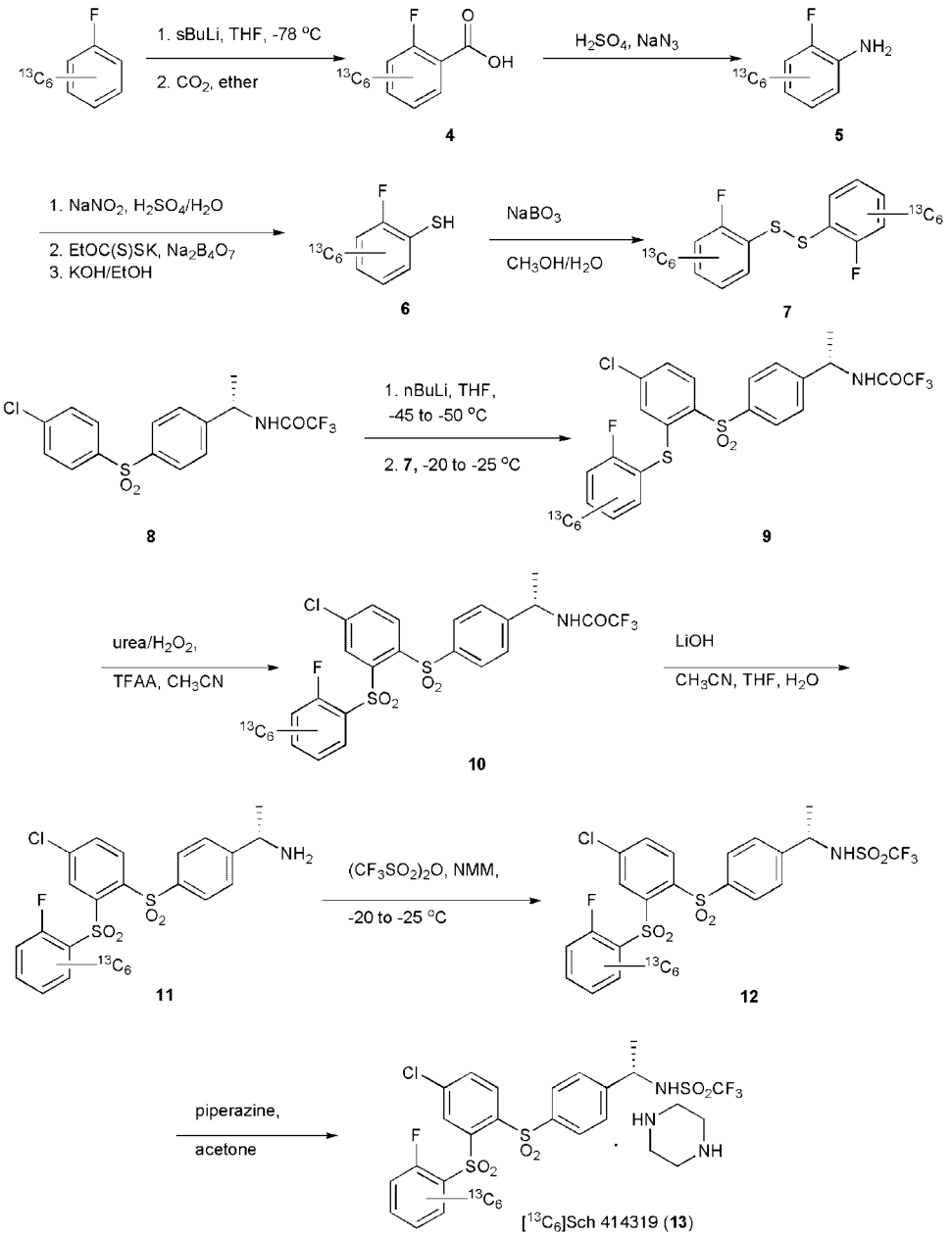

Scheme 2

acid at 1 ml/min for 15 min followed by a gradient to acetonitrile.

2. Zorbax SB-C18 column, 4.6 mm ID × 150 mm, 254 nm, (60:40:0.1) acetonitrile:water: trifluoroacetic acid at 1 ml/min for 15 min followed by a gradient to acetonitrile.
3. Zorbax Extend C18 column, 3 mm ID × 150 mm, 254 nm, 40:60 acetonitrile: 0.05 M aqueous triethylammonium acetate pH 9 at 0.5 ml/min for 15 min followed by a gradient to acetonitrile.
4. Zorbax Extend C18 column, 4.6 mm ID × 150 mm, 254 nm, 50:50 acetonitrile: 0.05 M aqueous triethylammonium acetate pH 8 at 1 ml/min for 15 min followed by a gradient to acetonitrile.

Synthesis of (^{3}H)Sch 414319 (3)

A solution of tetrahydrofuran (1 ml) and Sch 414319 (60 mg, 0.10 mmol) was cooled to −78°C under N_2 in a flame-dried round-bottomed flask. *n*-Butyllithium (0.25 ml, 1.6 M, 0.4 mmol) was added dropwise and the resulting orange solution was stirred at −78°C for 15 min before THO (561.6 mCi, 50 Ci/ml) was added.

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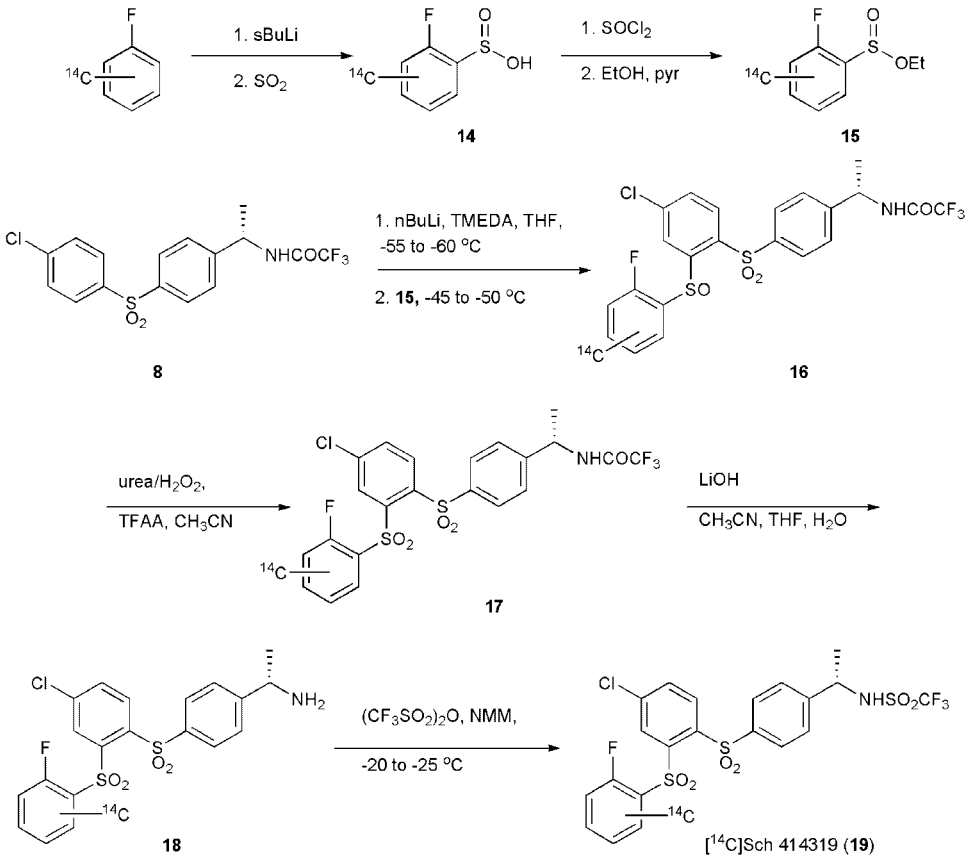

Scheme 3

The reaction was stirred at −78°C for 1 h, then quenched by the addition of water (2 ml) and methylene chloride (5 ml) and warmed to RT. The aqueous layer was acidified and extracted with methylene chloride (3 × 5 ml). The combined organic layers were washed with water (2 ml), dried (sodium sulfate) and evaporated, and the resulting crude [^{3}H]Sch 414319 (76.6 mCi, RCP 29.6%) was dissolved in EtOH (10 ml) for storage. Compound **3** was purified by RP-HPLC (Zorbax Extend C18, 10 mm ID × 250 mm, 254 nm, 50:50 acetonitrile: 0.05 M aqueous triethylammonium acetate pH 8 at 5 ml/min); 17 mCi of [^{3}H]Sch 414319 at a specific activity of 1.05 Ci/mmol was obtained. The radiochemical purity as determined by radio-HPLC (system 4) and radio-TLC plate scanning (5:95 2 M methanolic ammonia:methylene chloride) was 98.7 and 98.6%, respectively. ^{3}H-NMR of [^{3}H]Sch 414319 ($CDCl_3$): δ 8.59 (t, H6′), 8.38 (d, H3′), 8.26 (t, H6), 7.98 (d, H2″), 7.84 (d, H4′), 7.13 (t, H3).

Synthesis of ($^{13}C_6$)Sch 414319 piperazine salt (13)

2-Fluoro($^{13}C_6$)benzoic acid (4). Into a dry, 100 ml, round-bottomed flask were added tetrahydrofuran (25 ml) and [$^{13}C_6$]fluorobenzene (1.0 g, 9.8 mmol). The clear, colorless solution was cooled to −78°C and *sec*-butyllithium (8.6 ml, 1.14 M, 9.8 mmol) was added dropwise over 30 min. The cloudy yellow solution was stirred at −78°C for 2.5 h, then transferred via a glass syringe to an open 250 ml flask (previously dried) containing dry ice and ether (100 ml). The cloudy, white mixture was warmed to 0°C and quenched with aqueous hydrochloric acid (30 ml, 1 N). The layers were separated and the ether layer was washed with water (30 ml), then extracted with sodium hydroxide (3 × 30 ml, 0.2 M). The combined aqueous extracts were washed with ether (20 ml), then acidified with aqueous hydrochloric acid (1 M) and extracted with ether (5 × 40 ml). The combined organic extracts were washed with water (2 × 30 ml) and brine (30 ml), dried (sodium sulfate) and evaporated to give **4** as an off-white solid (1.27 g, 89%). ^{1}H NMR (d_6-DMSO): δ 13.21 (s, 1 H), 8.07 (m, 0.5 H), 7.83 (m, 0.5 H), 7.66 (m, 0.5 H), 7.51 (m, 0.5 H), 7.43 (m, 0.5 H), 7.09 (m, 0.5 H).

2-Fluoro($^{13}C_6$)aniline (5). A solution of 2-fluoro[$^{13}C_6$]-benzoic acid (**4**, 1.27 g, 8.7 mmol) in sulfuric acid (4.4 ml, concentrated) was heated to 50°C in a 25 ml

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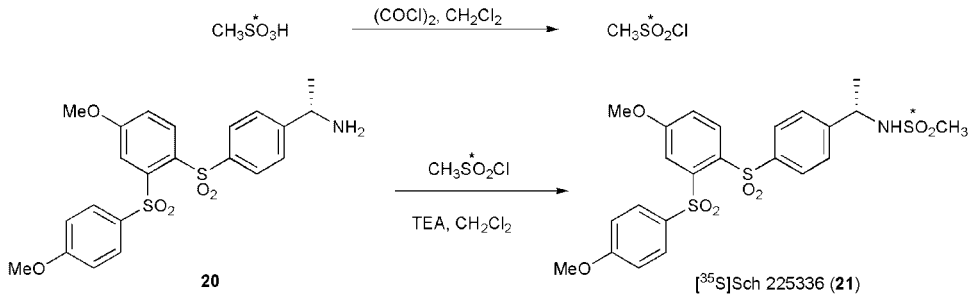

Scheme 4

round-bottomed flask equipped with a reflux condenser. Sodium azide (0.62 g, 9.6 mmol) was added in seven portions over 1 h – bubbling ensued and the reaction turned yellow. The solution was heated at 50°C for 6 h, then cooled to RT, diluted with water (5 ml), cooled to 0°C, and made basic using aqueous sodium hydroxide (12 M). The aqueous solution was extracted with methylene chloride (3 × 30 ml). The combined organic layers were washed with brine (20 ml), dried (sodium sulfate) and evaporated at room temperature to give the crude product as a brown liquid. The residue was purified by column chromatography (methylene chloride) to give **5** as a brown/orange oil (0.82 g, 81%). ^{1}H NMR ($CDCl_3$): δ 7.18 (m, 0.5 H), 7.14 (m, 0.5 H), 6.97 (m, 0.5 H), 6.89 (m, 0.5 H), 6.78 (m, 0.5 H), 6.74 (m, 0.5 H), 6.58 (m, 0.5 H), 6.49 (m, 0.5 H), 3.71 (bs, 2 H).

2-Fluoro($^{13}C_6$)thiophenol (6). A cloudy orange mixture of 2-fluoro[$^{13}C_6$]aniline (**5**, 0.82 g, 7 mmol) in sulfuric acid:water (1:6, 7.4 ml) was cooled to 0°C. Sodium nitrite (0.58 g, 8.4 mmol) in water (3.4 ml) was added dropwise and the diazonium salt, a clearer orange solution, was stirred at 0°C for 2 h. In a separate flask, a mixture of potassium ethyl xanthate[7] (2.02 g, 12.6 mmol), sodium tetraborate decahydrate (7.74 g, 20.3 mmol), and water (31 ml) was heated to 72°C. After the yellow mixture became clear, the diazonium salt was added dropwise. The reaction immediately turned cloudy yellow and an orange oil formed. The mixture was heated at 72°C for 1 h, stirred at RT for 1 h, then allowed to stand overnight. Water (30 ml) was added and the aqueous solution was extracted with methylene chloride (3 × 50 ml). The combined organic layers were dried (sodium sulfate) and evaporated to give the crude intermediate xanthate (1.75 g) as an orange oil. This compound was hydrolyzed by refluxing in a solution of potassium hydroxide (0.90 g, 16.1 mmol) in ethanol (7.8 ml) for 5 h. After the reaction was cooled to RT, it was evaporated to remove the ethanol. The dark orange solid that resulted was partitioned between water (30 ml) and methylene chloride (50 ml). The layers were separated and the aqueous layer was extracted with methylene chloride (2 × 50 ml). The combined organic layers were dried (sodium sulfate) and evaporated to give a mixture of **6** and disulfide **7** (0.92 g) as an orange oil. The material was directly oxidized to the disulfide.

Di-2-fluoro($^{13}C_6$)benzenedisulfide (7). A mixture of 2-fluoro[$^{13}C_6$]thiophenol (**6**) and di-2-fluoro[$^{13}C_6$]benzenedisulfide (**7**) (0.92 g total) was dissolved in methanol (17 ml) and water (6.9 ml). Sodium perborate (1.37 g, 13.72 mmol) was added and the yellow solution became thick yellow. After 20 min, the reaction progress was checked by HPLC (system 1). When all starting thiophenol was gone, water (5 ml) was added and the aqueous solution was extracted with methylene chloride (3 × 30 ml). The combined organic layers were dried (sodium sulfate) and evaporated to give the crude product as a brown oil (0.7 g). The material was purified by column chromatography (hexanes) to give purified **7** (0.158 g) and a mixture of **7** and a byproduct (0.192 g).‡

***N*-(1-{4-(4-Chloro-2-(2-fluoro($^{13}C_6$)phenylsulfanyl)benzenesulfonyl)phenyl}ethyl)-2,2,2-trifluoroacetamide (9)**. A solution of *N*-{1-[4-(4-chlorobenzenesulfonyl)-phenyl] ethyl}-2,2,2-trifluoroacetamide (**8**, 0.21 g, 0.54 mmol) and 1,10-phenanthroline (trace amount) in tetrahydrofuran (2.3 ml) was cooled to −45 to −50°C. n-Butyllithium (0.99 ml, 1.08 M, 1.07 mmol) was added dropwise until the solution turned dark red. The reaction was stirred at −45 to −50°C for 45 min, then added dropwise via Teflon cannula to a solution of the

‡The byproduct is a less polar impurity that forms during the oxidation reaction. Both the purified di-2-fluoro[$^{13}C_6$]benzenedisulfide (**7**) and the mixture of di-2-fluoro[$^{13}C_6$]benzenedisulfide and the byproduct are used independently in the following two reactions, then the purified products from those reactions are combined and subjected together to the last reactions in the series.

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purified disulfide **7** (0.158 g, 0.59 mmol) in tetrahydrofuran (1.6 ml) at −20 to −25°C. The orange solution was stirred for 15 min, then checked by HPLC (system 1). When the reaction was complete, it was warmed to 0°C and acetic acid (0.0936 ml, 1.62 mmol) was added. After 10 min, water (20 ml) and ethyl acetate (30 ml) were added and the pH was raised to 10 using aqueous sodium hydroxide (25%). The layers were separated, water (20 ml) was added and the pH was again raised to 10. The layers were separated and the organic layer was washed with aqueous sodium chloride (20%, 10 ml), dried (sodium sulfate) and evaporated to give crude **9** (0.461 g, chemical purity 64%) as an orange oil.[§] The material was used without purification in the following reaction. [The same reaction was carried out using the mixture of di-2-fluoro[$^{13}C_6$]benzenedisulfide and a byproduct to give the crude product (0.592 g) as a yellow oil.]

(S)-*N*-(1-(4-((4-Chloro-2-((2-fluoro($^{13}C_6$)phenyl)sulfoxyl)-phenyl)sulfonyl)phenyl)-ethyl)-2,2,2-trifluoroacetamide (10). To a solution of crude **9** (0.461 g, chemical purity 64%, 0.56 mmol) in ethyl acetate (1.8 ml) was added urea/hydrogen peroxide (0.238 g, 2.52 mmol). The reaction, under argon, was placed in a RT water bath and acetonitrile (0.22 ml) was added. Trifluoroacetic anhydride (0.32 ml, 2.24 mmol) was added dropwise to the yellow mixture over 1 h—the reaction became clear yellow. The reaction was stirred for 2.5 h at RT then checked by HPLC (system 1). When all of **9** was gone, the reaction was diluted with ethyl acetate (5 ml) and cooled to 10–15°C. Sodium sulfite (0.213 g, 1.68 mmol) in water (0.9 ml) was added dropwise—the reaction turned cloudy, then clear yellow. The pH was raised to 8 with aqueous sodium hydroxide (25%, 0.6 ml) and the aqueous solution was extracted with ethyl acetate (4 × 15 ml). The combined organic layers were washed with brine (15 ml), dried (sodium sulfate) and evaporated to give the crude product as an orange oil, which was purified by column chromatography (2:8 ethyl acetate:hexanes) to give the product (**10**) as a white solid. This material was combined with the purified product generated from the disulfide+byproduct starting material to give **10** (0.29 g) as a white solid.

(S)-4-((4-Chloro-2-((2-fluoro($^{13}C_6$)phenyl)sulfonyl)phenyl)sulfonyl)-alpha-methylbenzenemethanamine (11). A mixture of lithium hydroxide (0.0375 g, 1.56 mmol) in water (1.4 ml) was stirred for 5 min until it turned clear. A solution of **10** (0.29 g, 0.52 mmol) in acetonitrile (0.7 ml) and tetrahydrofuran (1 ml) was added—the reaction turned cloudy, then clear light yellow. The reaction was stirred for 19 h, then checked by HPLC (system 2). When all starting material was gone, aqueous sodium chloride (10%, 5 ml) and methyl *tert*-butyl ether (10 ml) were added. The layers were separated and the organic layer was washed again with aqueous sodium chloride (10%, 5 ml), then dried (sodium sulfate) and evaporated. The residue was coevaporated with acetonitrile (3 × 10 ml) to give **11** (0.206 g, 86%) as a light yellow oil that was dried under vacuum overnight.

(S)-*N*-(1-(4-((4-Chloro-2-((2-fluoro($^{13}C_6$)phenyl)-sulfonyl)-phenyl)sulfonyl)phenyl)-ethyl)trifluoromethanesulfonamide (12). A mixture of **11** (0.206 g, 0.448 mmol) in acetonitrile (5.1 ml) was heated in a 50°C oil bath to put the compound into solution, then cooled to −20 to −25°C. *N*-methylmorpholine (0.054 ml, 0.493 mmol) was added followed by the dropwise addition of triflic anhydride (0.0753 ml, 0.448 mmol) over 30 min. After 10 min, the reaction progress was checked by HPLC (system 2). If starting material remained, an appropriate amount of *N*-methylmorpholine and triflic anhydride was added and the reaction was again checked after 10 min. When the reaction was complete, it was diluted with water (10 ml) and ethyl acetate (10 ml) and the pH was lowered to 4.5 with aqueous hydrochloric acid (1 N). Sodium chloride (0.03 g) was added and the aqueous layer was extracted with ethyl acetate (2 × 30 ml). The combined organic layers were dried (sodium sulfate) and evaporated to give crude [$^{13}C_6$]Sch 414319 (0.6 g) as a yellow oil, which was purified by column chromatography (2:8 ethyl acetate:hexanes) to give **12** (0.191 g, 72%) as a white foam. The compound was recrystallized twice from toluene, then converted to the piperazine salt (**13**) to remove an impurity that was present in 1.1%.

Piperazine salt of ($^{13}C_6$)Sch 414319 (13). A mixture of piperazine (0.215 g, 0.25 mmol) in acetone (0.2 ml) was heated in a 50°C oil bath until it was clear and colorless. Compound **12** (0.148 g, 0.25 mmol) in acetone (1 ml) was added and the mixture was heated for 15 min at 50°C, then slowly cooled to −10°C over 1.5 h. The acetone was removed by passing a stream of nitrogen over the flask until 0.3 ml remained. Toluene (3 ml) was added and the reaction became cloudy. The flask was stored at −20°C overnight to induce crystallization, then the solvent was filtered away to give **13** (0.161 g, 95%) as a white solid with a chemical purity of 97.5%. FAB Mass Spectrometry of unlabeled Sch 414319 **1**: *m/z* 586.266 (M), 490, 452, 437, 426, 341, 333, 317, 277, and 269. FAB mass spectrometry of **13**: *m/z* 592.229 (M), 490, 458, 443, 426, 341, 339, 323, 277, and 275. ^{1}H NMR of **13** (d_6-DMSO): 8.40 (d, 1 H),

[§]The basic aqueous layers can be acidified and extracted with methylene chloride to recover 2-fluoro[$^{13}C_6$]thiophenol (**6**).

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8.35 (m, 1 H), 8.14 (dd, 1 H), 8.10 (m, 1 H), 7.79 (m, 1 H), 7.74 (d, 1 H), 7.53 (d, 1 H), 7.51 (m, 1 H), 7.36 (m, 1 H), 4.42 (q, 1 H), 2.82 (s, 8 H), 1.20 (d, 3 H).

Synthesis of (^{14}C)Sch 414319 (19)

(^{14}C)-*o*-Fluorobenzene sulfinic acid (14). Into a dry, 25 ml, round-bottomed flask was added tetrahydrofuran (3 ml) under argon. Three freeze/thaw cycles were performed, and then [^{14}C]-fluorobenzene (90 mCi, 64.75 mCi/mmol, 1.39 mmol) was vacuum transferred from a break-seal ampoule into the flask over 1 hr. After the flask was removed from the vacuum transfer system, it was cooled to −78°C under argon. *sec*-Butyllithium (0.72 M, 1.93 ml) was added dropwise over 15 min and the resulting clear, light yellow solution was stirred at −78°C for 2.5 h. Into a dry, 100 ml, round-bottomed flask was added tetrahydrofuran (30 ml) under argon. The solvent was cooled to −50 to −60°C and then sulfur dioxide was bubbled into the solvent for 10 min while the flask was minimally open to the air. The anion was added quickly via a teflon cannula to the sulfur dioxide/tetrahydrofuran solution. The flask originally containing the anion was rinsed with tetrahydrofuran (2 × 1 ml) and this was added to the reaction flask via a teflon cannula. The reaction was warmed slowly to 0°C under argon over 1.3 h (if the reaction warmed to −40°C too quickly, then the deprotonated fluorobenzene decomposed to a benzyne intermediate). The resulting cloudy white mixture was warmed to room temperature while open to the air. The stir bar was removed and the reaction was evaporated to give a white solid. Hydrochloric acid (5 ml, 1 N) was added and the suspension was evaporated and then coevaporated with acetonitrile (4 × 5 ml) to remove residual water. The resulting white solid was extracted with methylene chloride (2 × 5 ml) and ethyl acetate (2 × 5 ml). The combined organic extracts were dried (sodium sulfate) and evaporated in a 50 ml round-bottomed flask to give a light yellow oil that was dried in a vacuum desiccator overnight. Crude **14** (0.271 g, assume quantitative yield) was used without purification in the following reaction.

Ethyl (^{14}C)-*o*-fluorobenzene sulfinate ester (15). Into a 50 ml, round-bottomed flask containing crude [^{14}C]-*o*-fluorobenzene sulfinic acid (**14**, assume 1.39 mmol) was added toluene (4.5 ml). The mixture was sonicated and then stirred under argon while thionyl chloride (0.51 ml, 6.95 mmol) was added dropwise. The reaction was stirred at room temperature for 1.5 h, then evaporated. The residue was coevaporated with toluene (3 × 2 ml) to remove residual thionyl chloride. To the cloudy yellow oil with white solid were added toluene (0.6 ml) and ether (2.4 ml). The mixture was sonicated, then cooled to 0°C under argon. A solution of ethanol (0.088 ml, 1.50 mmol) in pyridine (0.24 ml) was added dropwise. The cloudy, light yellow reaction was warmed to room temperature and stirred for 2 h. Distilled water (2 ml) and ether (7 ml) were added. The layers were separated and the organic layer was washed with hydrochloric acid (2 ml, 1 N) and brine (2 ml). The aqueous layers were washed with methylene chloride (2 ml) and this was added to the ether solution. The combined organic layers were dried (sodium sulfate) and evaporated to afford a yellow oil (75.28 mCi) that had a radiochemical purity (RCP) of 59% by radio-TLC (1:9 ethyl acetate:hexanes). The crude material was purified using a Waters silica gel 'sep-pak' (5 g, 1:99 ethyl acetate:hexanes) to give [^{14}C]-sulfinate ester **15** as a yellow oil (39.34 mCi, RCP 99%, 44% yield from [^{14}C]-fluorobenzene).

(S)-*N*-(1-(4-((4-Chloro-2-((^{14}C)-2-fluorophenyl)sulfoxyl)-phenyl)sulfonyl)phenyl)-ethyl)-2,2,2-trifluoroacetamide (16). To a dry 25 ml, round-bottomed flask were added (S)-*N*-[1-[4-[[4-chlorophenyl]sulfonyl] phenyl]-ethyl]-2,2,2-trifluoroacetamide (**9**, 0.262 g, 0.669 mmol), 1,10-phenanthroline (1 mg), tetrahydrofuran (2.7 ml), and *N,N,N',N'*-tetramethylethylenediamine (0.101 ml, 0.669 mmol). The solution was cooled to −55 to −60°C under argon. *n*-Butyllithium (1.28 ml, 1 M, 1.40 mmol) was added dropwise (the indicator was used to determine when 1 eq. of base had been added, even though the base had been previously titrated). The resulting dark brown solution was stirred under argon for 45 min at −55 to −60°C and then added via a teflon cannula to a solution of ethyl [^{14}C]-*o*-fluorobenzene sulfinate ester (**15**, 39.34 mCi, 64.75 mCi/mmol, 0.608 mmol) in tetrahydrofuran (1.9 ml) at −45 to −50°C. The flask originally containing the anion was rinsed with tetrahydrofuran (2 × 1 ml) and this was added to the reaction flask via a teflon cannula. After 15 min, the reaction conversion was 55% by HPLC (system 1). The reaction was warmed to 0°C and acetic acid (0.104 ml, conc.) was added. The cloudy yellow mixture was warmed to room temperature and stirred for 10 min. Water (4 ml) and ethyl acetate (4 ml) were added and the pH was raised to 11 with sodium hydroxide (0.04 ml, 25%). The layers were separated and the organic layer was extracted with pH 11 water (3 ml). The layers were separated and the combined aqueous layers were extracted with ethyl acetate (3 ml). The combined organic layers were washed with water (2 ml), brine (2 ml), dried (sodium sulfate) and evaporated to give [^{14}C]-sulfoxide **16** as a yellow/orange oil (37.34 mCi, RCP 60%). The material was used without purification in the following reaction.

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(S)-*N*-(1-(4-((4-Chloro-2-(((14C)-2-fluorophenyl)sulfonyl)-phenyl)sulfonyl)phenyl)-ethyl)-2,2,2-trifluoroacetamide (17). Into a 25 ml, round-bottomed flask were added [^{14}C]-sulfoxide **16** (37.34 mCi, 64.75 mCi/mmol, 0.577 mmol), ethyl acetate (2 ml), urea/hydrogen peroxide (0.244 g, 2.60 mmol), and acetonitrile (0.23 ml) under argon. The cloudy yellow mixture was placed in a room temperature water bath. Trifluoroacetic anhydride (0.326 ml, 2.31 mmol) was added dropwise over 30 min; the reaction turned clear yellow. After 2.5 h, the reaction was complete by HPLC (system 1). Ethyl acetate (4 ml) was added and the reaction was cooled to 10–15°C. Sodium sulfite (0.218 g, 1.73 mmol) in water (1.2 ml) was added dropwise; the reaction turned cloudy, then clear yellow. Sodium hydroxide (0.4 ml, 25%) was added to raise the pH to 5–9. The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 3 ml). The combined organic layers were washed with brine (2 ml), dried (sodium sulfate) and evaporated to give crude [^{14}C]-sulfone **17** as a yellow oil (33.93 mCi, RCP 59%). The crude product was purified by silica gel chromatography (2:8 → 4:6 ethyl acetate:hexanes) to give [^{14}C]-sulfone **17** as a white solid (20.5 mCi, RCP 96.9%, 55% yield from [^{14}C]-sulfinate ester **15**, HPLC system 2).

S-4-((4-Chloro-2-(((14C)-2-fluorophenyl)sulfonyl)phenyl)-sulfonyl)-alpha-methylbenzenemethanamine (18). Into a 15 ml, round-bottomed flask was added compound **17** (20.5 mCi, 64.75 mCi/mmol, 0.317 mmol), tetrahydrofuran (2 ml), and acetonitrile (0.7 ml). Lithium hydroxide (22.8 mg, 0.951 mmol) in water (0.9 ml) was added dropwise; the reaction became clear and then cloudy yellow. The reaction was stirred at room temperature overnight, then diluted with *t*-butylmethyl ether (4 ml) and washed with sodium chloride (2 × 2 ml, 10%). The combined aqueous extracts were washed with *t*-butylmethyl ether (2 ml). The combined organic extracts were dried (sodium sulfate) and filtered into a 25 ml round-bottomed flask. It was estimated that there was 20.2 mCi of crude [^{14}C]-amine **18**. (The radioactivity of the product was determined by counting the radioactivity in the aqueous washes and subtracting this number from that of the starting material.) The solvent was removed by passing nitrogen over the solution, and the [^{14}C]-amine was used immediately in the following reaction. For the purposes of calculating the amounts of the reactants needed for the next reaction, it was assumed that the product was 90% pure.

(S)-*N*-(1-(4-((4-Chloro-2-(((14C)-2-fluorophenyl)-sulfonyl)-phenyl)sulfonyl)phenyl)-ethyl)trifluoromethanesulfonamide (19). Into a 25 ml round-bottomed flask containing crude [^{14}C]-amine **18** (18.2 mCi, 64.75 mCi/mmol, 0.281 mmol) was added acetonitrile (3.2 ml). The solution was cooled to −20 to −25°C under argon. *N*-methylmorpholine (0.034 ml, 0.309 mmol) was added followed by the dropwise addition of triflic anhydride (0.0473 ml, 0.281 mmol). After 10 min, the reaction progress was checked by HPLC (system 2). If the reaction was incomplete, the appropriate equivalents of *N*-methylmorpholine and triflic anhydride (corresponding to the amount of starting material still present) were added to the reaction and the reaction progress was again checked by HPLC. This addition was repeated one more time. Water (3 ml) and ethyl acetate (5 ml) were added. Hydrochloric acid (0.03 ml, 1 N) was added to lower the pH to 4–4.5. The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 3 ml). The combined organic layers were washed with brine (2 ml), dried (sodium sulfate) and evaporated to give crude trifluoromethanesulfonamide **19** as a yellow oil (16.9 mCi). The crude material was first purified using a Waters silica gel 'sep-pak' (5 g, 1:2 ethyl acetate:hexanes) to give compound **19** as a yellow oil (9.81 mCi, RCP 88.9%), which was further purified using NP-HPLC (Phenomenex Prodigy 5 μ silica (30), 10 mm ID x 250 mm, 8 ml/min, 254 nm, 18:82 ethyl acetate:hexanes, column cleaned with ethyl acetate between injections) to give [^{14}C]Sch 414319 (**19**) as a white solid (7.3 mCi, RCP 99.2%).

Synthesis of (^{35}S)Sch 225336 (21)

(^{35}S)Mesyl chloride. To a 10 ml Reactiware Vial was added a solution of methane [^{35}S]sulfonic acid (10.0 mCi, 1438 Ci/mmol) in water (97 μl). Ethanol (800 μl) and potassium hydroxide (5 μl, 1 N) were added and the solvent was removed by passing nitrogen over the solution. Ethanol (800 μl) was added again and then removed by passing nitrogen over the solution. Anhydrous methylene chloride (1500 μl) and oxalyl chloride (200 μl, 2.1 mmol) were added and the clear, colorless solution was stirred under argon (gas evolves). After 1 h, 10% dimethylformamide in methylene chloride (30 μl) was added and more gas evolved. The reaction was stirred at room temperature under argon for 18 h. Methylene chloride (1.5 ml) was added and the reaction was cooled to 0°C. The organic solution was washed with ice cold aqueous sodium bicarbonate (2 × 2 ml, 1%), room temperature sodium bicarbonate (2 ml, 1%), and room temperature sodium hydrogensulfite (1 ml, 2%). The organic solution was dried (sodium sulfate) for 1 h, then filtered through a plug of sodium sulfate into a flame-dried 15 ml pear-shaped flask. The methylene chloride solution was distilled under atmospheric pressure at 60°C until the volume was reduced to approximately 100 μl. This

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[^{35}S]mesyl chloride solution was used immediately in the following reaction.

([^{35}S)Sch 225336 (21). To a dry 0.3 ml Reactiware vial were added compound **20** (10 mg, 21.7 μmol), methylene chloride (20 μl), and triethylamine (5 μl, 35.9 μmol). The solution of [^{35}S]mesyl chloride in methylene chloride (~100 μl) was added. The flask originally containing the [^{35}S]mesyl chloride solution was rinsed with methylene chloride (2 × 50 μl) and this was added to the reaction flask. The solution was vigorously stirred for 15 min, then the solvent was removed by passing nitrogen over it. The resulting residue was taken up in acetonitrile (180 μl) and 0.05 M aqueous triethylammonium acetate pH 9 (120 μl) and analyzed by HPLC (system 3). The radiochemical purity of [^{35}S]Sch 225336 was 60.7%. The material was purified by RP-HPLC (Zorbax Extend C18, 9.4 mm ID x 250 mm, 254 nm, 40:60 acetonitrile: 0.05 M aqueous triethylammonium acetate pH 9 at 4 ml/min) to give [^{35}S]Sch 225336 (**21**, 2.87 mCi, RCP 99.2%), which was stored as a solution in ethanol (6 ml).

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