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Research Article

Synthesis of carbon-14 labeled 4-[4-[2-[2-[bis(4-chlorophenyl)methoxy]ethyl sulfonyl][1-^{14}C]ethoxy]phenyl]-1,1,1-trifluoro-2-butanone

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Summary

Carbon-14 labeled 4-[4-[2-[2-[bis(4-chlorophenyl)methoxyethylsulfonyl] [1-^{14}C]-ethoxy]phenyl]-1,1,1-trifluoro-2-butanone was prepared in a six step radioactive synthesis from 2-bromo[1-^{14}C]acetic acid. The overall radiochemical yield was 2.2%. The specific activity of the final product was found to be 42 μCi/mg with a radiochemical purity of >98%. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: carbon-14; cPLA2; anti-inflammatory

Introduction

Leukotrienes and prostaglandins are derived from arachidonic acid and are potent lipid mediators of inflammation and pain. Cytosolic phospholipase A2 (cPLA2) catalyzes the selective release of arachidonic acid from the *sn*-2 position of phospholipids and is believed to play a key cellular role in the generation of arachidonic acid.[1] 4-[4-[2-[2-[bis (4-chlorophenyl)methoxy]ethylsulfonyl]ethoxy]phenyl]-1,1,1-trifluoro-

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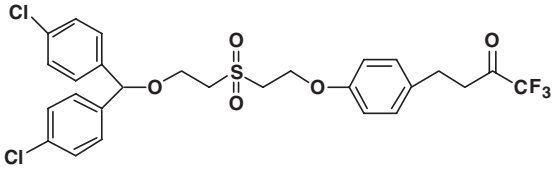


1a

Figure 1. 4-[4-[2-[2-[bis(4-chlorophenyl)methoxy]ethylsulfonyl]ethoxy]phenyl]-1,1,1-trifluoro-2-butanone

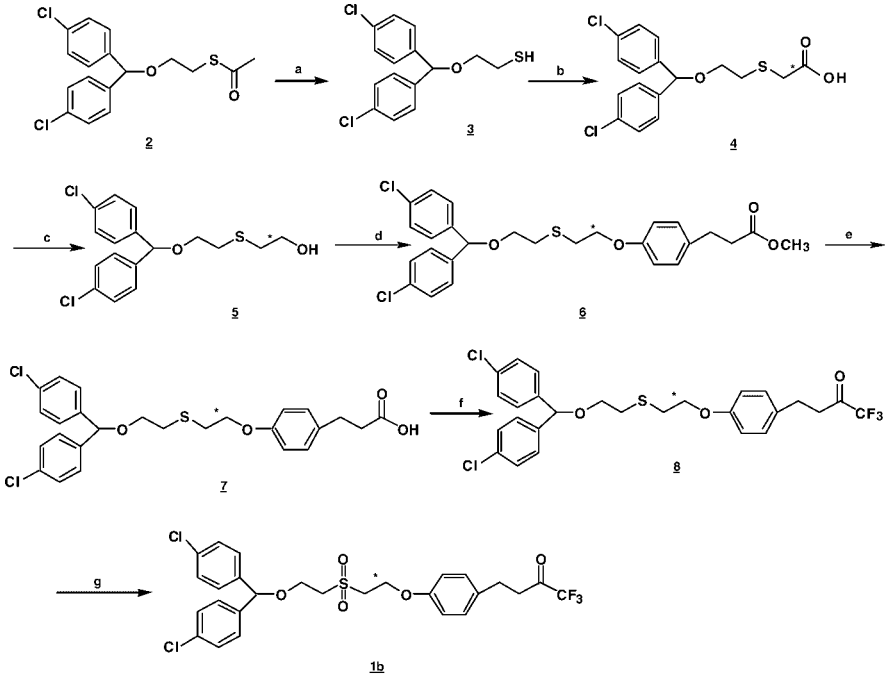
2-butanone **1a**, was found to be a selective inhibitor of cPLA2 (IC50 = 2.8 μM) in that it did not inhibit secreted phospholipase A2 *in vitro*, nor phospholipase C and phospholipase D in cells (Figure 1).[1] This compound was also active in inhibiting arachidonate and eicosanoid production in U937 cells, neutrophils, platelets, monocytes, and mast cells.[1]

Compound **1a** represents a novel inhibitor of cPLA2, which partitions into the phospholipid bilayer and competes with phospholipid substrate for the active site.[1] This potent inhibition of the enzyme translated into anti-inflammatory activity when applied topically (5%, w/v) to a phorbol ester- induced chronic inflammation model in mouse ears, inhibiting edema and neutrophil infiltration, as well as, prostaglandin and leukotriene levels in the skin.[1]

This paper reports the synthesis of 4-[4-[[2-bis(4-chlorophenyl)-methoxy]ethyl]sulfonyl][1-^{14}C]ethoxyphenyl]-1,1,1-trifluoro-2-butanone, **1b**, for use in subsequent metabolism and pharmacokinetic studies, as well as, experimental skin flux studies (Scheme 1).

Results and discussion

The synthesis of carbon-14 labeled 4-[4-[2-[2-[bis(4-chlorophenyl) methoxy]ethylsulfonyl][1-^{14}C]ethoxy]phenyl]-1,1,1-trifluoro-2-butanone, **1b**, was achieved starting from 2-bromo[1-^{14}C]acetic acid in six steps. In this procedure, 2-[bis(4-chlorophenyl)methoxy]ethane thioacetate **2** was deprotected to yield 2-[bis(4-chlorophenyl)methoxy]ethanethiol **3** which was then reacted immediately with 2-bromo[1-^{14}C]acetic acid to yield 2-[bis(4-chlorophenyl)methoxy]ethanethio[1-^{14}C]acetic acid **4**. Reduction of the radiolabeled acid **4** to the corresponding alcohol **5** with $LiAlH_4$ yielded 2-[2-[bis(4-chlorophenyl)methoxy]ethylthio][1-^{14}C] ethanol **5**. Coupling of the alcohol with methyl 3-(4-hydroxyphenyl)propionate



Reagents: a, NaOH, THF; b, $BrCH_2CO_2H$, NaOH, H_2O; c, $LiAlH_4$, THF; d, methyl 3-(4-hydroxyphenyl) propionate, triphenylphosphine, diethyl azodicarboxylate, toluene; e, KOH, EtOH, H_2O; f, ClCOCOCl, $(CF3CO)_2O$, pyridine,CH_2Cl_2, toluene; g, m-chloroperbenzoic acid, CH_2Cl_2, HPLC.

Scheme 1. Synthesis of Carbon-14 labeled 4-[4-[2-[2-bis(4-chlorophenyl)methoxy] ethylsulfonyl][1-^{14}C]ethoxy]phenyl]-1,1,1-trifluoro-2-butanone

yielded methyl 3-[4-[2-[2-[bis(4-chlorophenyl)methoxy]ethylthio][1-^{14}C]ethoxy]phenyl]propionate (**6**). Saponification of **6** with potassium hydroxide yielded 3-[4-[2-[2-[bis(4-chlorophenyl)methoxy]ethylthio][1-^{14}C] ethoxy]phenyl]propionic acid **7**. Conversion of carboxylic acid **7** to the trifluoromethyl ketone **8** via trifluoroacetic anhydride and pyridine yielded 4-[4-[2-[2-[bis(4-chlorophenyl) methoxy]ethylthio][1-^{14}C]ethoxy]- phenyl]-1,1,1-trifluoro-2-butanone **8**.[2] Subsequent oxidation of the sulfide **8** via 3-chloroperoxybenzoic acid, yielded the crude final product **1b** which was purified via HPLC.

The radiochemical yield of this synthesis was 2.2% which resulted from numerous reactions proceeding in only modest yield and difficulties encountered during purification procedures. Flash chromatography was used to purify several of the radiolabeled intermediates, however subsequent radio-HPLC analysis indicated that some closely eluting radioactive impurities could not be removed via flash

chromatography and were thus carried on to the next reaction. LC-MS and NMR studies have shown that in the presence of water the trifluoromethyl ketone of compounds **8** and **1b** exists in equilibrium with the corresponding gem-diol moiety.[3] This equilibrium causes serious tailing of the peak for both **8** and **1b** during reverse phase HPLC using conventional aqueous buffer/acetonitrile conditions.[3] Purification of the final product, **1b**, from close eluting radiolabeled impurities of unknown structure was only possible using reverse-phase HPLC, which resulted in significant tailing of the desired compound and incomplete recovery of product.

Materials and methods

Previously prepared non-radioactive proprietary precursors and final product were synthesized according to published procedures and used as standards throughout this study.[4] 2-Bromo[1-^{14}C]acetic acid (52 mCi/mmol) was purchased from ViTrax Co. All other reagents were obtained from Aldrich and were either ACS grade or the highest quality material commercially available. HPLC purification and analysis was performed on a Rainin Dynamax HPLC system consisting of two SD-200 pumps, a Rainin UV-I detector and an *INUS* β-RAM radioactive flowthrough detector. Zorbax R_x C-18 columns were obtained from VWR Scientific Products. YMC basic 5 μm ODS (2) columns were obtained from Waters.

High-performance liquid chromatography

Method A. In this method samples are loaded onto a Zorbax R_xC-18 column ($4.6 \times 250\,mm^2$) with a mobile phase of 60% CH_3CN and 40% H_2O containing 0.1% TFA at a flow rate of 1.0 ml/min. The UV-1 detector was set at 265 nm.

Method B. In this method samples are loaded onto a Zorbax R_xC-18 column ($4.6 \times 250\,mm^2$) with a mobile phase of 80% CH_3CN and 20% H_2O containing 0.1% TFA at a flow rate of 1.0 ml/min. The UV-1 detector was set at 265 nm.

Method C. In this method samples are loaded onto a Zorbax R_xC-18 column ($4.6 \times 250\,mm^2$) with a mobile phase of 70% CH_3CN and 30% H_2O containing 0.1% TFA at a flow rate of 1.0 ml/min. The UV-1 detector was set at 240 nm.

Method D. In this method samples are loaded onto a YMC basic 5 μm ODS (2) column (100 × 250 mm^2) with a mobile phase of 60% CH_3CN and 40% H_2O at a flow rate of 4.0 ml/min. The UV-1 detector was set at 230 nm.

Experimental

2-[Bis(4-chlorophenyl)methoxy]ethanethiol **3**

Into a 100 ml RB flask containing 2-[bis(4-chlorophenyl)methoxy] ethane thioacetate **2** (0.679 g, 1.91 mmol) was added anhydrous THF (9 ml), absolute EtOH (9 ml) and the solution allowed to stir at 22°C under argon. To this solution was added a freshly prepared solution of NaOH (4.38 ml, 1 M, 4.38 mmol) in deoxygenated water and stirred for 1 h after which time the reaction mixture was then acidified with HCl (5.25 ml, 1 N), and extracted with toluene (90 ml). The organic layer was rinsed with H_2O (10 ml, 3x), brine (10 ml), dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield **3** (0.6 g, 1.91 mmol) as an oil which was used immediately in the next step.

2-[Bis(4-chlorophenyl)methoxy]ethanethio[1-^{14}C]acetic acid **4**

Into a 100 ml RB flask containing **3** (0.6 g, 1.91 mmol) was added anhydrous THF (6.7 ml), absolute EtOH (6.7 ml) and the solution allowed to stir at 22°C under argon. To this solution was added a freshly prepared solution of NaOH (4.5 ml, 1 M, 4.5 mmol) in deoxygenated water and a mixture of 2-bromo[1-^{14}C]acetic acid (50 mCi, 52 mCi/mmol, 0.133 g, 0.96 mmol) and non-radioactive 2-bromoacetic acid (0.133 g, 0.96 mmol) in one portion. The reaction mixture was stirred at 22°C for 1 h and then diluted with H_2O (45 ml), acidified with HCl (5.35 ml, 1 N) and extracted with EtOAc (90 ml). The organic phase was rinsed with H_2O (25 ml, 2 ×), brine (25 ml), dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield **4** (38 mCi, 0.568 g, 1.53 mmol, 76%). HPLC analysis of **4** (Method A) showed a radiochemical purity of 97%. In this system **4** has a retention time of approximately 10.5 min.

*2-[2-[Bis(4-chlorophenyl)methoxy]ethylthio][1-^{14}C]ethanol (***5***).*

Into a 250 ml RB flask containing **4** (38 mCi, 0.568 g, 1.53 mmol) dissolved in anhydrous THF (60 ml) was added $LiAlH_4$ (0.20 g,

5.3 mmol) in small portions. After the evolution of hydrogen had ceased, the reaction mixture was heated to reflux for 1 h. The reaction mixture was then cooled to 0°C and treated successively with H_2O (0.2 ml added slowly), NaOH (0.2 ml, 15% solution) and H_2O (0.6 ml). After 15 min, the resulting solid was filtered and the filtrate diluted with EtOAc (200 ml), and the organic layer washed with brine (25 ml), dried ($MgSO_4$) and filtered. The filtrate was concentrated *in vacuo* and purified via flash chromatography (SiO_2, 20% EtOAc/hexane to 30% EtOAc/hexane) to yield **5** (20.2 mCi, 0.293 g, 0.816 mmol, 53%). HPLC analysis of **5** (Method A) showed a radiochemical purity of 90%. In this system **5** has a retention time of approximately 11.7 min.

Methyl 3-[4-[2-[2-[bis(4-chlorophenyl)methoxy]ethylthio] [1-^{14}C]ethoxy]phenyl] propionate, **6**

Into a 25 ml RB flask containing **5** (20.2 mCi, 0.293 g, 0.816 mmol) dissolved in toluene (4.5 ml) was added methyl 3-(4-hydroxyphenyl)-propionate (0.200 g, 1.110 mmol), and triphenylphosphine (0.285 g, 1.110 mmol) and stirred at room temperature. To this solution was added diethyl azodicarboxylate (175 µl, 1.110 mmol) over 5 min and the reaction stirred at room temperature for 18 h after which time, the reaction mixture was diluted with EtOAc (40 ml), washed with saturated $NaHCO_3$ (20 ml), dried ($MgSO_4$), and filtered. The filtrate was concentrated *in vacuo* and tritiated with hexane:toluene (6:4, 10 ml) and the resulting triphenylphosphine oxide filtered and the filtrate concentrated *in vacuo*. The residue was dissolved in a minimum amount of 10% EtOAc/hexane and sonicated to assist in solublization. The crude product was then purified via flash chromatography (SiO_2, 5–10% EtOAc/hexane) to yield **6** (12.4 mCi, 0.260 g, 0.500 mmol, 61%). The balance of activity was isolated as **5**. HPLC analysis of **6** (Method B) showed a radiochemical purity of 91%. In this system **6** has a retention time of approximately 12.3 min.

3-[4-[2-[2-[Bis(4-chlorophenyl)methoxy]ethylthio][1-^{14}C]ethoxy]phenyl]propionic acid, **7**

Into a 25 ml RB flask containing **6** (12.4 mCi, 0.260 g, 0.500 mmol) and aqueous EtOH (80%, 11 ml) was added KOH (150 mg, 2.673 mmol) dissolved in H_2O (1 ml) and the resulting mixture heated at 60°C for 1 h. The solvent was then removed *in vacuo* and the residue diluted with H_2O

(10 ml), acidified to pH 2 with HCl (2 N) and extracted with CH_2Cl_2 (20 ml, 2 ×). The organic layers were combined, washed with brine and dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo* to yield **7** (10.7 mCi, 218 mg, 0.432 mmol, 86%) as a white solid. HPLC analysis of **7** (Method B) showed a radiochemical purity of 78%. In this system **7** has a retention time of approximately 6.3 min. The balance of activity was found as a single peak eluting before the desired product. The identify of this impurity was not determined.

4-[4-[2-[2-[Bis(4-chlorophenyl)methoxy]ethylthio][1-^{14}C]ethoxy] phenyl]-1,1,1-trifluoro-2-butanone, **8**

Into a 25 ml RB flask containing crude **7** (10.7 mCi, 218 mg, 0.432 mmol) dissolved in anhydrous CH_2Cl_2 (5.5 ml) was added oxalyl chloride (0.34 g 2.67 mmol), and DMF (30 µl) and the reaction mixture stirred at room temperature for 1 h. The solvent was then removed *in vacuo* and the residue dissolved in toluene (17 ml) and cooled (0–5°C, ice-bath). To this solution was added trifluoroacetic anhydride (0.70 g, 4.77 mmol), and pyridine (0.22 g, 2.78 mmol). The reaction mixture was then allowed to warm to room temperature and stirred for 2 h. The reaction mixture was diluted with H_2O (1 ml), stirred for 15 min, and then extracted with EtOAc (60 ml). The organic layer was washed with H_2O (15 ml), saturated $NaHCO_3$ (15 ml), brine (15 ml), dried ($MgSO_4$), and filtered. The filtrate was concentrated *in vacuo* and purified via flash chromatography (SiO_2, 15–35% EtOAc/hexane) to yield **8** (3.1 mCi). HPLC analysis of **8** (Method C) showed a radiochemical purity of 91%. In this system **8** has a retention time of approximately 16.5 min, while **7** has a retention time of approximately 11.5 min. In this chromatographic system there is significant tailing of the peak for **8**. Radiochemical purity was determined by including all of the area under the peak until the peak returned to baseline (~3 min).

4-[4-[2-[2-[Bis(4-chlorophenyl)methoxy]ethylsulfonyl][1-^{14}C] ethoxy]phenyl]-1,1,1-trifluoro-2-butanone, **1b**

Into a 50 ml RB flask containing **8** dissolved in CH_2Cl_2 (17 ml) was added 3-chloroperoxybenzoic acid (0.32 g, 0.57 mmol) and the mixture stirred at room temperature for 2 h, diluted with EtOAc (80 ml), the organic layer washed with saturated $NaHCO_3$ (15 ml), brine (15 ml), dried ($MgSO_4$) and filtered. The filtrate was concentrated *in vacuo* and

the crude product **1b** was purified via HPLC (Method D) to yield **1b** (1.1 mCi, 26.2 mg, specific activity 42 μCi/mg). During this purification, there is significant tailing of the peak as it elutes from the HPLC column and the product was collected from 21 to 29 min post-injection. HPLC analysis (Method C) of **1b** indicated a radiochemical purity of >98%). In this system **1b** has a retention time of approximately 5.8 min while **8** has a retention time of approximately 16.5 min.

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