Synthesis of Carbon-14 Labeled Leukotriene Antagonist SK&F 104353

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Summary

Enantiomerically pure (2S,3R)-3-[(2-carboxyethyl)thio]-3-[2-(8-phenyloctyl)phenyl]-2-hydroxy[2-^{14}C]propionic acid ([^{14}C]SK&F 104353) was synthesized from methyl chloro[2-^{14}C]acetate *via* a five step sequence. Darzens Condensation with 2-(8-phenyloctyl)benzaldehyde gave a *trans* epoxy ester which underwent epoxide opening with methyl 3-mercaptopropionate to furnish a pair of regioisomers. The desired isomer was isolated by subjecting the mixture to retroaldol cleavage conditions to remove the unwanted component. Subsequent optical resolution as a derivative of (S)-proline and hydrolysis of the 2S,3R diastereomer afforded the title compound with a 5% overall radiochemical yield.

Key Words: [^{14}C]Leukotriene antagonist, Darzens Condensation, Methyl chloro[2-^{14}C]acetate, Epoxide opening, Retroaldol cleavage, Optical resolution.

Introduction

(2S,3R)-3-[(2-Carboxyethyl)thio]-3-[2-(8-phenyloctyl)phenyl]-2-hydroxypropionic acid (**1**, SK&F 104353) is a novel high-affinity leukotriene receptor antagonist[1] which exhibits potent and selective inhibition of leukotriene-induced responses both *in vitro* and *in vivo*.[2] It is a useful experimental tool for study of the role leukotrienes play in various allergic disorders and has potential therapeutic utility in disease states such as

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bronchial asthma.[3] Both high and low specific activity radiolabeled isotopomers of **1** were required for various studies. Two synthetic approaches to high specific activity compounds are described in the accompanying paper; in this article we describe the preparation of enantiomerically pure [^{14}C]**1** for use in metabolism and pharmacokinetic studies.

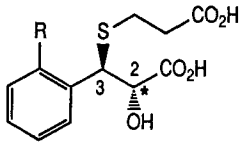
The synthetic route involved epoxide ester **3**[1] labeled at C2 as the key intermediate. This intermediate was chosen because it could be elaborated easily to the desired product by epoxide opening with a requisite thiol nucleophile,[4] and it could be generated from the known[5] 2-(8-phenyloctyl)benzaldehyde **2** *via* the Darzens Condensation with an alpha-haloacetate, readily available in ^{14}C-labeled form.

Results

Commercially available methyl chloro[2-^{14}C]acetate was the starting material. Darzens Condensation with benzaldehyde **2** gave *trans* epoxy ester **3** in 70% isolated yield as the sole isomer when carried out in dichloromethane. The stereochemistry was confirmed to be as previously reported,[1] based on the observation of the coupling constants (J = 1.5-1.9 Hz) between the vicinal protons in the epoxide ring as measured by ^{1}H NMR.[6]

Epoxide opening of **3**, conducted in 10:1 (v/v) methanol-triethylamine with 6-fold molar excess of methyl 3-mercaptopropionate at room temperature, led to a nearly quantitative yield of two *erythro* regioisomers **4** and **5**, resulting from stereospecific (inversion) but regiorandom cleavage of the oxirane. The ratio of the desired isomer **4** to the unwanted isomer **5** was determined to be 55:43 by reverse-phase radio-HPLC. Thus, formation of the *trans* epoxide **3** as the sole product of the Darzens Condensation is critical. Any of the *cis* epoxy isomer **10** present would have given rise to two additional undesired byproducts--*threo* stereoisomers **11** and **12** (Scheme II).

The two regioisomers **4** and **5** could not be separated by silica gel column chromatography. Treatment of the regioisomeric mixture with 3

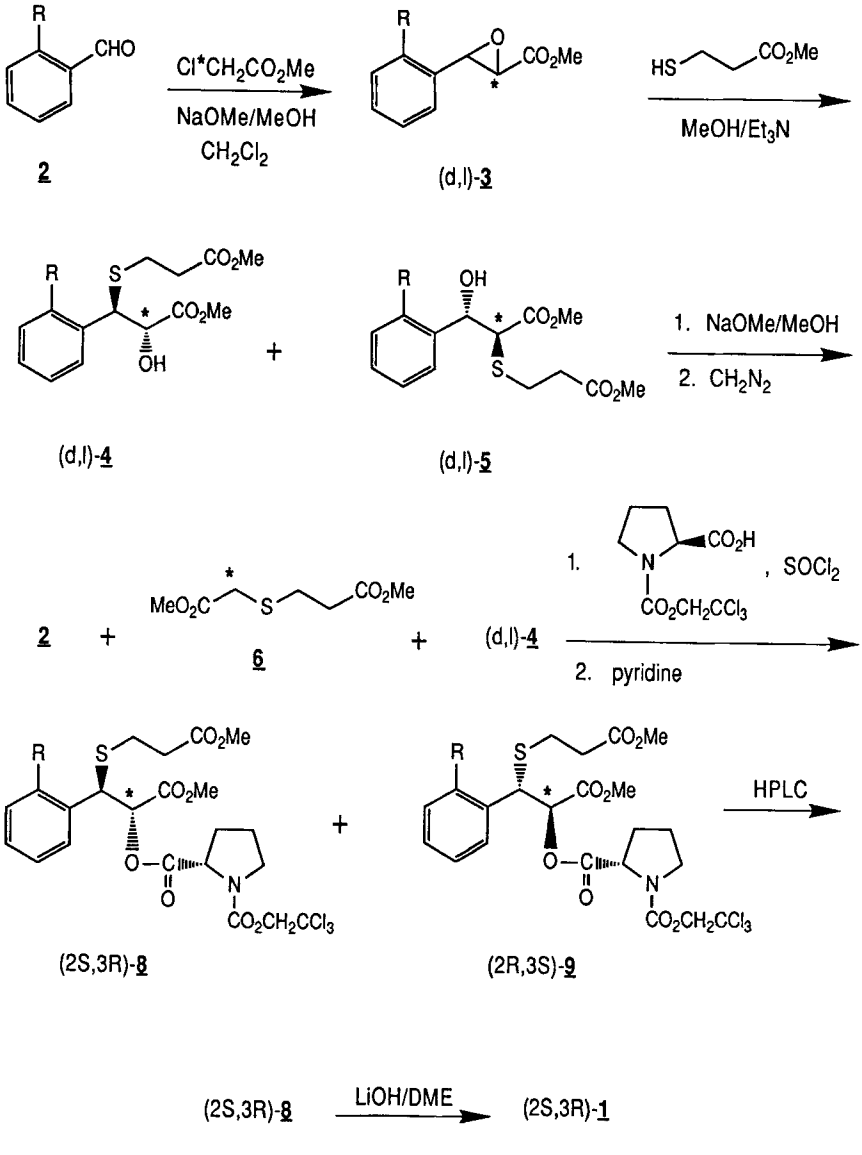


(2S,3R)-**1**, ([^{14}C]SK&F 104353)

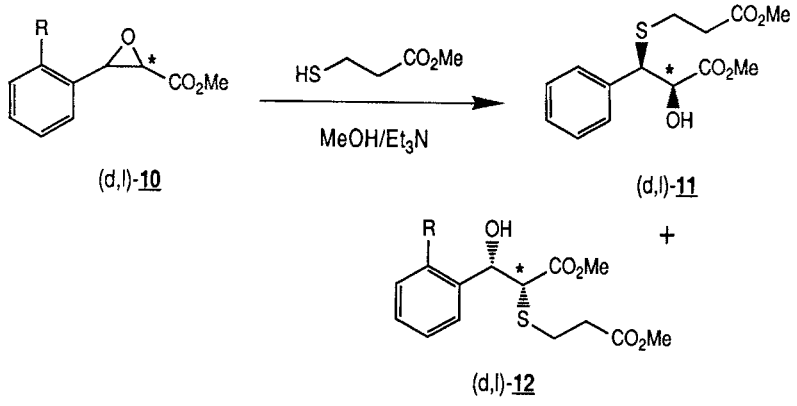
R = $(CH_2)_8Ph$

• = ^{14}C

Scheme I



Scheme II



(d,l)-10 (d,l)-11 (d,l)-12

equivalents of sodium methoxide in methanol caused **5** to undergo retroaldol cleavage to form benzaldehyde **2** and dimethyl 3-thia[2-^{14}C]hexa-1,6-dioate (**6**), thus allowing easy isolation of **4**. Under these conditions, however, the methyl ester **4** was partially hydrolyzed. Therefore, the crude retroaldol product mixture was treated with diazomethane to regenerate the methyl ester.[7] Subsequent silica gel column chromatography furnished **4** in 46% yield from **3**.

Optical resolution was achieved by derivatizing racemic **4** with N-(2,2,2-trichloroethoxycarbonyl)-(S)-proline (**7**)[8] followed by HPLC separation of the resulting diastereomers to provide each of the enantiomers **8** and **9** in 47% yield. The 2S,3R diastereomer **8** was hydrolyzed with aqueous lithium hydroxide in dimethoxyethane at 5 °C. After repeated HPLC purification, [^{14}C]SK&F 104353 was obtained in 36% yield from **8** with a radiochemical purity in excess of 98%. The 2S,3R absolute stereochemistry of **1** was proven by a chiral HPLC method[15] which also established the stereochemistry of **8**. A total of 5.4 mCi of **1** with a specific activity of 16.1 mCi/mmole was obtained, representing a 5% overall radiochemical yield from methyl chloro[2-^{14}C]acetate.

Experimental

General Procedures

Dichloromethane and pyridine were distilled under argon from calcium

hydride prior to use. All other solvents used were HPLC grade. Methyl 3-mercaptopropionate, thionyl chloride, sodium methoxide in methanol and (S)-proline were purchased from Aldrich. Methyl chloro[2-^{14}C]acetate was purchased from New England Nuclear. 2-(8-Phenyloctyl)benzaldehyde was synthesized *via* the oxazoline route described in reference 5. Mass spectral data were collected on unlabeled analogs using a Finnigan 1020 mass spectrometer in the electron impact mode. ^1H NMR spectra were obtained with either a JEOL 270-MHz or a Bruker AM400 spectrometer in deuterated chloroform with tetramethylsilane as reference. Radioactive concentrations were determined by liquid scintillation counting using [^{14}C]hexadecane as internal standard in Bioflour scintillation cocktail. Radio-HPLC employed a Ramona-D radioactivity detector with TruCount scintillation fluid at 5 mL/min in a 0.75 mL flow cell. Ramona computer software programs were utilized to perform radioactive peak area integrations.

Methyl 3-[2-(8-Phenyloctyl)phenyl]-*trans*-2,3-epoxy[2-^{14}C]propionate (3)

To methyl chloro[2-^{14}C]acetate (239 mg, 2.2 mmol, 47.8 mCi/mmol, 105.4 mCi) under a helium atmosphere in a flask cooled by a dry-ice/acetone bath was added sequentially CH_2Cl_2 (10 mL), 2-(8-phenyloctyl)benzaldehyde (**2**, 650 mg, 2.2 mmol) in CH_2Cl_2 (1 mL), and sodium methoxide in methanol (0.6 mL, 2.6 mmol, 25% by weight). The resulting mixture was stirred magnetically while warming to room temperature over a period of 1 h, and maintained at ambient temperature overnight. The mixture was filtered through silica gel and Supersil in a sintered glass funnel, and the adsorbent was washed with diethyl ether (100 mL). The filtrate was concentrated *in vacuo*. The resulting residue was purified by preparative HPLC (Dynamax silica gel column (8 μm, 2.54 cm I.D. x 25 cm) eluted with 97:3 (v/v) hexane/ethyl acetate at 14.0 mL/min) to give 570 mg of epoxy ester **3** as an oil (70% yield).[9] The radiochemical purity was 96% by HPLC (two Altex Ultrasphere ODS C_{18} columns (5 μm, 4.6 mm I.D. x 25 cm), 9:1 (v/v) acetonitrile/water at 1.5 mL/min, retention time at 12 min). ^1H NMR (270

MHz): 1.31 (8H, br s) 1.57 (4H, br s), 2.56-2.70 (4H, m, benzylic **H**), 3.40 (1H, d, J = 1.9 Hz, C2-**H**), 3.82 (3H, s, CO_2CH_3), 4.25 (1H, d, J = 1.5 Hz, C3-**H**), 7.16-7.27 (9H, m, Ph-**H**). EIMS, m/z (relative intensity): 366 (M^+, 9), 344 (14), 309 (100), 293 (82), 276 (11), 260 (21), 228 (13), 192 (12), 188 (32), 177 (40), 159 (42), 158 (43), 145 (88), 143 (91).

Methyl *erythro*-3-[(2-Carbomethoxyethyl)thio]-3-[2-(8-phenyloctyl)-phenyl]-2-hydroxy-[2-^{14}C]propionate (**4**)

Epoxy ester **3** (570 mg, 1.6 mmol, 47.5 mCi/mmol) was diluted with 1.3 g (3.6 mmol) of cold carrier to give a calculated specific activity of 14.7 mCi/mmol. To a stirred solution of a 969 mg (2.6 mmol) portion of the diluted material in 16 mL of 9:1 (v/v) methanol/triethylamine at room temperature under argon was added dropwise over a period of 10 min a solution of methyl 3-mercaptopropionate (1.8 mL, 15.7 mmol) in 4 mL of the same solvent mixture. The reaction was stirred at room temperature overnight. Concentration *in vacuo* and flash chromatographic purification on a silica gel column using a step gradient of ethyl acetate in hexane (desired product eluting with 30% ethyl acetate) furnished a mixture of regioisomers **4** and **5** (1.32 g, 103% yield). Analysis of this product by radio-HPLC indicated a 43:55[10] mixture of **5**:**4** (two Altex Ultrasphere ODS C_{18} columns (5 μm, 4.6 mm I.D. x 25 cm), 4:1 (v/v) acetonitrile/water at 2 mL/min, retention time of **5**: 10.7 min, retention time of **4**: 11.9 min). To a solution of this mixture (2.7 mmol) in methanol (20 mL) at room temperature under argon was added dropwise over a period of 10 min sodium methoxide in methanol (1.6 mL, 8.4 mmol, 25% by weight). The mixture was stirred at room temperature for 1 h, cooled to 5 °C, and acidified with 3.3 N aqueous HCl solution. After addition of water (15 mL), the mixture was extracted with ethyl acetate (2 x 25 mL). Concentration of the extracts *in vacuo* gave the crude product. The material was dissolved in diethyl ether at 5 °C and methylated with freshly prepared ethereal diazomethane.[7] Concentration *in vacuo* and purification on a silica gel column using gradient elution of ethyl acetate in hexane (desired product

eluting with 30% ethyl acetate) gave the desired regioisomer **4** as an oil (610 mg, 46% yield).[11] The radiochemical purity by HPLC (same as above) was 91%. No trace of **5** was detected. ^{1}H NMR (measured on unlabeled analog, 400 MHz): 1.34 (8H, br s), 1.58 (4H, br s), 2.52-2.81 (8H, m), 3.09 (1H, d, J = 6.2 Hz, O-**H**), 3.64 (3H, s, CO_2CH_3), 3.66 (3H, s, CO_2CH_3), 4.55 (1H, d, J = 4.8 Hz, C3-**H**), 4.60 (1H, dd, J = 6.2 Hz, J = 4.9 Hz, C2-**H**), 7.13-7.65 (9H, m, Ph-**H**).[12] EIMS, m/z (relative intensity): 397 (M^+-89, 75), 366 (1), 307 (1), 289 (3), 219 (2), 192 (4), 188 (7), 159 (12), 131 (54), 117 (72), 105 (52), 91 (100).

The regioisomeric mixture could be separated by neutral alumina column chromatography using gradient elution of ethyl acetate in hexane. An unlabeled sample of regioisomer **5**, eluting at 30% ethyl acetate, was obtained by this method. ^{1}H NMR (400 MHz): 1.33 (8H, br s), 1.57 (4H, br s), 2.42-2.67 (8H, m), 2.87 (1H, d, J = 5.0 Hz, O-**H**), 3.63 (3H, s, CO_2CH_3), 3.64 (1H, d, J = 8.7 Hz, C3-**H**), 3.80 (3H, s, CO_2CH_3), 5.27 (1H, dd, J = 8.8 Hz, J = 5.2 Hz, C2-**H**), 7.17-7.43 (9H, m, Ph-**H**).[13] The desired isomer **4** was eluted with 40% ethyl acetate in hexane.

Methyl (2S,3R)-3-[(2-Carbomethoxyethyl)thio]-3-[2-(8-phenyloctyl)-phenyl]-2-[N-(2,2,2-trichloroethoxycarbonyl)-(S)-prolyloxy]-[2-^{14}C]-propionate (8)

Thionyl chloride (5 mL) and N-(2,2,2-trichloroethoxycarbonyl)-(S)-proline (2.1 g, 7.2 mmol, prepared from (S)-proline according to a literature procedure)[8] were stirred together under argon at 0 °C for 4 h. Concentration *in vacuo* gave the acid chloride, which was dissolved in 3 mL of pyridine. This solution was added to a stirred solution of racemic **4** (610 mg, 1.3 mmol) in 12 mL of pyridine under argon at 0 °C. The reaction was stirred at room temperature overnight. Concentration *in vacuo* and purification on a silica gel column using gradient elution of ethyl acetate in hexane furnished a 1:1 diastereomeric mixture (950 mg, 99% yield) as determined by radio-HPLC (two IBM silica gel columns (5 μm, 4.6 mm I.D. x 25 cm), 97:3

(v/v) dichloroethane/ethyl acetate at 1.5 mL/min, retention time of **8**: 9.8 min, retention time of **9**: 11.6 min).

Separation of the diastereomers was accomplished by preparative normal-phase HPLC (two Dynamax silica gel columns (8 μm, 2.14 cm I.D. x 25 cm), 98:2 (v/v) dichloroethane/ethyl acetate at 35.0 mL/min, UV detection at 260 nm, 90 mg per injection). The desired 2S,3R **8** had a retention time of 42.5 min. The retention time of unwanted 2R,3S **9** was 52.5 min. Fractions collected from HPLC elution were combined and concentrated *in vacuo*, giving diastereomer **8** as an oil (450 mg, 47% yield). The radiochemical purity by HPLC (same as above) was 94%. No trace of **9** was detected.

(2S,3R)-3-[(2-Carboxyethyl)thio]-3-[2-(8-phenyloctyl)phenyl]-2-hydroxy-[2-^{14}C]propionic acid (1)

To a stirred solution of **8** (450 mg, 0.6 mmol) in 8 mL of dimethoxyethane under argon at 5 °C was added dropwise 2 mL of aqueous lithium hydroxide solution (1.3 g of lithium hydroxide in 20 mL of water) over a period of 5 min. The reaction was continued at 5 °C for 4 h, and then acidified with 3.3 N aqueous HCl solution. Extraction with ethyl acetate (2 x 35 mL) and concentration *in vacuo* gave the crude hydrolyzed product. At this stage, this crude material was combined with another batch of crude diacid prepared analogously from 262 mg (0.35 mmol) of **8**. Purification on a silica gel column using gradient elution of ethyl acetate in 100:2 (v/v) hexane/formic acid gave 620 mg of diacid **1**. This material was subjected to repeated HPLC purification (Dynamax C_{18} column (8 μm, 2.54 cm I.D. x 25 cm), 20:80:1 (v/v/v) water/methanol/acetic acid at 20.0 mL/min, UV detection at 260 nm, retention time at 18 min). Fractions collected were concentrated *in vacuo* below 25 °C to evaporate a major portion of methanol present. The resulting milky solution was extracted with ethyl acetate (3 x 60 mL). The combined organic phases were washed with water (2 x 20 mL), brine (25 mL), and dried over sodium sulfate. Concentration *in vacuo* followed by drying under high vacuum gave 5.4 mCi of **1** as an oil. The

chemical purity[14] was 96.2%. The enantiomeric purity[15] was 100%; and the radiochemical purity[16] was 98.6%. The specific activity was 16.1 mCi/mmol. ^1H NMR (270 MHz): 1.33 (8H, br s), 1.61 (4H, br s), 2.57-2.88 (8H, m), 4.58 (1H, d, J = 5.1 Hz), 4.64 (1H, d, J = 5.5 Hz), 7.13-7.64 (9H, m, Ph-**H**). EIMS, m/z (relative intensity): 424 (M^+-24, 2), 396 (28), 383 (100), 352 (2), 336 (7), 308 (26), 290 (13), 276 (3), 252 (3), 235 (3), 219 (4), 203 (7), 185 (19), 174 (73), 159 (87).

Storage and Stability

[^{14}C]SK&F 104353 was stored as a solution in ethanol under argon at -80 °C with a radioactive concentration of 0.54 mCi/mL and specific activity of 16.1 mCi/mmol. After a 19 month period, the radiochemical purity remained unchanged.

Acknowlegements

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9. The same procedure was repeated starting with 112 mCi of methyl chloro[2-^{14}C]acetate on a similar scale, resulting in a 69% isolated yield of epoxy ester **3**.

10. The ratio of **5**:**4** changed to 30:68 by UV detection at 230 nm. This was attributed to the higher molar absorption coefficient exhibited by **4**. Thus radioactivity detection was more reliable in this instance.

11. Prolonged reaction in the retroaldol cleavage resulted in decreased yield of **4**. A 3 h cleavage period afforded a 35% isolated yield.

12. Assignment of the hydroxy, C-2 and C-3 protons was carried by deuterium oxide exchange measurement. Under such conditions, the doublet at 3.09 ppm (O-**H**) disappeared while the quartet at 4.60 ppm (C2-**H**) collapsed to a doublet.

13. The hydroxy, C-2 and C-3 protons were assigned by deuterium oxide exchange experiment similar to that performed in reference 12.

14. Chemical purity of the final diacid **1** was determined by comparison of the HPLV-UV absorbance to an unlabeled reference standard of known chemical purity. Conditions: Waters μBondapak C_{18} column, 35:65 (v/v) 0.02 M potassium dihydrogen phosphate (pH = 2.5)/ acetonitrile at 1.0 mL/min, UV at 210 nm, retention time at 7.7 min.

15. Enantiomeric purity of **1** as its dimethyl ester (diazomethane) was determined by chiral HPLC (Supelcosil LC-(R)-UREA column (5 μm, 4.6 mm I.D. x 25 cm), 100:1 (v/v) hexane/isopropanol at 0.5 mL/min, UV at 230 nm. Retention times: (2R,3S) 25.3 min; (2S,3R) 26.5 min).

16. The radiochemical purity of **1** was determined by HPLC (Whatman Partisil 10 SAX column (4.6 mm I.D. x 25 cm), 0.025 M ammonium bicarbonate in 1:1 (v/v) methanol/water at 1.0 mL/min. Retention time at 5.9 min).