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Synthesis of Tritiated Leukotriene Antagonist SK&F 104353

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Summary

Tritium labeled isotopomers of (2S,3R)-3-[(2-carboxyethyl)thio]-3-[2-(8-phenyloctyl)phenyl]-2-hydroxypropionic acid (SK&F 104353) with high specific activity were prepared by catalytic tritiation of the corresponding halogenated and unsaturated precursors. These precursors were synthesized from their respective benzaldehydes using a core sequence (Darzens Condensation, epoxide opening and base-induced retroaldol reaction) to generate the required alpha-hydroxy propionic acid moiety. Upon tritiation, the halogenated substrate provided the dimethyl ester derivative having a specific activity of 55 Ci/mmol, while the unsaturated precursor led directly to [^{3}H]SK&F 104353 with a specific activity of 138 Ci/mmole.

Key Words: [^{3}H]Leukotriene antagonist, Tritium-halogen exchange, Catalytic tritiation.

Introduction

A central part of the strategy used in the synthesis of unlabeled SK&F 104353[1] and [^{14}C]SK&F 104353[2] was employed to prepare advanced intermediates suitable for conversion into [^{3}H]SK&F 104353 labeled in two different regions. That strategy (see preceeding paper) involved conversion of a benzaldehyde derviative to an exclusively *trans* glycidic ester by a Darzens Condensation, followed by regiorandom but stereospecific ring

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opening with methyl 3-mercaptopropionate. The undesired regioisomer of the epoxide cleavage product was destroyed by base-induced retroaldol reaction, and the desired isomer was isolated by simple flash column chromatographic separation. In the works cited, the latter was converted to ^{14}C-labeled or unlabeled SK&F 104353 by resolution and hydrolysis.[2]

We decided to utilize this core sequence to prepare analogs of the desired regioisomer obtained from epoxide opening. In one case, halogen was introduced in the terminal phenyl group and in the other, unsaturation in the octyl chain. An appropriate sequence of tritiation, resolution and hydrolysis in either case would yield a tritiated analog of SK&F 104353. Since we desired products having high specific activities, substrates with multiple unsaturation and multiple halogens were chosen as synthetic targets.

Results

Synthesis of **10**. Our first approach entailed preparation of a triply halogenated analog as precursor to [^{3}H]SK&F 104353. The synthesis of the halogenated substrate **10** began with nitration of carboxylic acid **2**. As anticipated, mononitration occurred exclusively in the terminal phenyl ring under a variety of reaction conditions.[3] However, little control of ortho/para selectivity could be achieved, with the observed o/p ratios ranging from 44/56 to 53/47 as measured by HPLC (220 nm detection). Therefore, compound **2** was nitrated with KNO_3/trifluoroacetic acid by the method of Spitzer and Stewart,[4] to give a quantitative yield of 2-[8-(2- and 4-nitrophenyl)octyl]benzoic acid (**3**) with an o/p ratio of 50/50. The nitro acid **3** was reduced quantitatively by catalytic hydrogenation to amino acid **4**; a portion of this isomeric mixture was separated by flash chromatography and the two isomers were characterized individually in order to confirm their expected structures. The bulk of the amino acid mixture **4** was converted to the tribromo compound **5** by electrophilic bromination-substitutive deamination according to the procedure of

Doyle,[5] to give a 51% yield of product. The ^{1}H NMR spectrum of the material was consistent with the expected mixture of 2,3,5- and 3,4,5-tribromo isomers.

Tribromo acid **5** was converted to aldehyde **7** in two steps by standard procedures in 93% overall yield. Small samples of the two isomers of **7** were separated chromatographically and characterized spectroscopically, confirming the gross structure and the bromine substitution patterns. Darzens reaction on the bulk of the isomer mixture **7** gave glycidic ester **8** (98%). Reaction with methyl 3-mercaptopropionate in the presence of triethylamine gave a mixture of **9** and **10**. Both normal and reverse-phase HPLC analysis revealed four peaks, due to the presence of the two tribromo isomers for each regioisomer. The four peaks were cleanly and rapidly reduced to two upon retroaldol destruction of **9**, and the desired product **10** was isolated in 31% yield, following chromatographic purification. HPLC analysis of **10** showed two components in the ratio of 49.6/50.4. Separation of these two isomers and spectroscopic characterization established their structures as the 2,3,5- and 3,4,5- isomers of **10**.

Tritiation of **10**. A 41 mg portion of **10** in ethyl acetate/DMF/triethylamine was reacted with excess carrier-free tritium gas in the presence of preactivated 10% Pd/C, to provide, after workup and removal of labile tritium, 1.5 Ci of **11** with a radiochemical purity of 88% (HPLC). HPLC purification of a portion of this gave **11** of >97% radiochemical purity, specific activity of 55 Ci/mmole, and a UV spectrum identical to that of an unlabeled standard. ^{3}H NMR confirmed that the tritium label was located in the terminal phenyl ring. Studies with unlabeled **11** demonstrated that the enantiomers could be separated easily on a micromole scale by chiral-HPLC using a Supelcosil LC-(R)-UREA column.

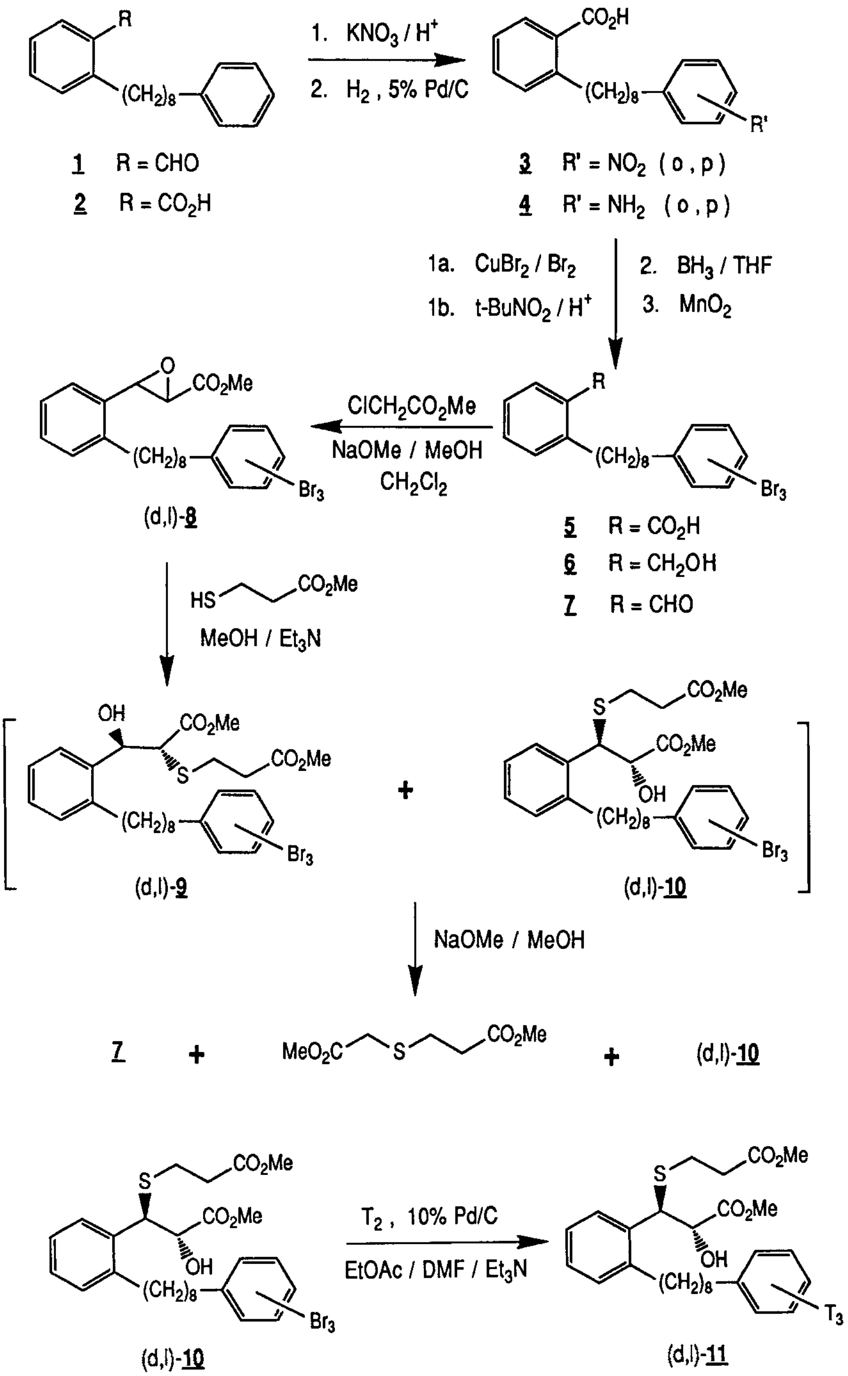
Synthesis of **20**. Our second approach featured the synthesis of optically pure diacetylenic analog **20**, leading directly to the desired enantiomer of SK&F 104353 upon tritiation. This scheme took advantage of the readily available starting diacetylenic benzaldehyde **14**, prepared directly in 86% yield from palladium catalyzed coupling between

2-bromobenzaldehyde (**12**) and 8-phenyl-1,7-octadiyne (**13**).[6] Following our central strategy outlined previously, benzaldehyde **14** was converted to *trans* epoxy ester **15** by the Darzens Condensation in 84% yield. Epoxide opening with methyl 3-mercaptopropionate led to a mixture of regioisomers **16** and **17**, which were subjected to base-induced retroaldol cleavage. After flash chromatography, the desired regioisomer **17** was isolated in 41% yield. Optical resolution was accomplished by derivatization of the regioisomer **17** with N-(2,2,2-trichloroethoxycarbonyl)-(S)-prolyl acid chloride,[7] followed by repeated normal-phase preparative HPLC,[8] furnishing the desired 2S,3R diastereomer **18** (18% from **17**). Upon treatment with aqueous lithium hydroxide in dimethoxyethane, diacetylenic analog **20** was obtained in 64% yield after HPLC purification. A sample of **20** was hydrogenated in the presence of 10% Pd/C in 1:1 (v/v) ethyl acetate/DMF.[9] The reduced product was treated with diazomethane, and the resulting dimethyl ester was demonstrated by chiral-HPLC to possess the required 2S,3R absolute stereochemistry.[10]

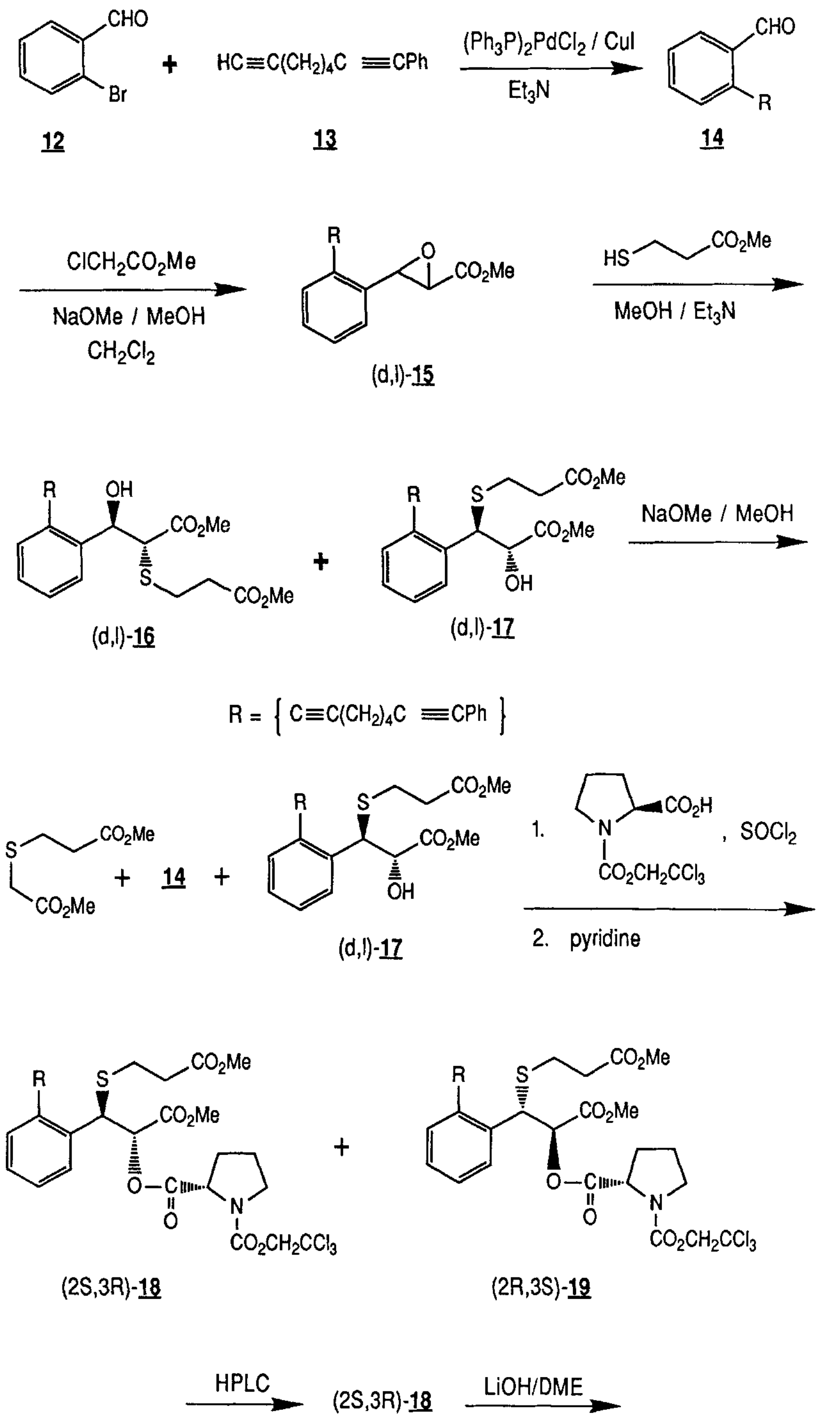
Tritiation of **20**. A 20 mg sample of **20** was reduced with an excess of carrier-free tritium in the presence of preactivated 10% Pd/C in 1:1 (v/v) ethyl acetate/DMF, resulting in 4.5 Ci of crude [^{3}H]SK&F 104353 with a radiochemical purity of 58%. Small portions were purified by reverse-phase HPLC to afford pure materials with radiochemical purities in excess of 97% and a specific activity of 138 Ci/mmole.[11] ^{3}H NMR revealed that the tritium label resided exclusively in the alkyl side chain.

Radiochemical Stabilities and Storage. [^{3}H]SK&F 104353, when standing in solution (either ethanol or toluene) at room temperature and exposed to air, completely oxidized to its sulfoxide derivative in a period of 48 hours.[12] This is in stark contrast to unlabeled SK&F 104353, which is completely stable under these conditions. The best way to store **21** was by dissolving it, immediately after lyophilization of HPLC solvents, in thoroughly degassed ethanol under argon and keeping the solution at -80 °C. Under these conditions, a decomposition rate of 0.6 % per week was observed over a period of 35 weeks.

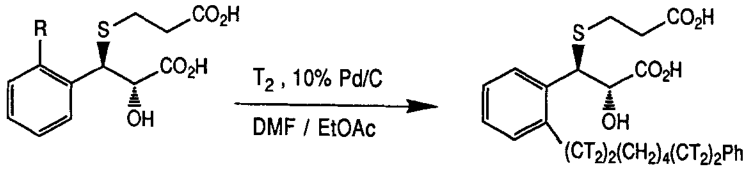
Scheme I


R
(CH2)8
1. KNO3 / H+
2. H2, 5% Pd/C
CO2H
(CH2)8
R'
1 R = CHO
2 R = CO2H
3 R' = NO2 (o, p)
4 R' = NH2 (o, p)
1a. CuBr2 / Br2
1b. t-BuNO2 / H+
2. BH3 / THF
3. MnO2
O
CO2Me
(CH2)8
Br3
(d,l)-8
ClCH2CO2Me
NaOMe / MeOH
CH2Cl2
R
(CH2)8
Br3
5 R = CO2H
6 R = CH2OH
7 R = CHO
HS CO2Me
MeOH / Et3N
OH
CO2Me
S
CO2Me
(CH2)8
Br3
(d,l)-9
+
S
CO2Me
CO2Me
OH
(CH2)8
Br3
(d,l)-10
NaOMe / MeOH
7
+
MeO2C S CO2Me
+
(d,l)-10
S
CO2Me
CO2Me
OH
(CH2)8
Br3
(d,l)-10
T2, 10% Pd/C
EtOAc / DMF / Et3N
S
CO2Me
CO2Me
OH
(CH2)8
T3
(d,l)-11

Scheme II


12
13
14
HC≡C(CH2)4C≡CPh
(Ph3P)2PdCl2 / CuI
Et3N
ClCH2CO2Me
NaOMe / MeOH
CH2Cl2
(d,l)-15
HS CO2Me
MeOH / Et3N
(d,l)-16
(d,l)-17
NaOMe / MeOH
R = { C≡C(CH2)4C≡CPh }
14
1. , SOCl2
2. pyridine
(2S,3R)-18
(2R,3S)-19
HPLC
(2S,3R)-18
LiOH/DME

Scheme II (continued)



(2S,3R)-**20** (2S,3R)-**21**

R = $\{C{\equiv}C(CH_2)_4C{\equiv}CPh\}$

Experimental

General Procedures

All solvents were distilled under argon prior to use. Pd/C catalyst was purchased from Engelhard. Mass spectral data were collected using a Finnegan 1020 mass spectrometer in electron impact mode. ^1H NMR were recorded by either a JEOL 270-MHz or a Bruker AM400 spectrometer in $CDCl_3$ with TMS as reference. ^3H NMR were recorded on an IBM AF-300 spectrometer (^3H at 320 MHz) in ^1H-decoupled mode in C_6D_6. Referencing of chemical shifts was achieved by the ghost referencing method reported in the literature.[13] Radioactive concentrations were determined by liquid scintillation counting using [^3H]hexadecane as internal standard in Bioflour scintillation fluid. Radio-HPLC profiles were recorded on a Ramona-D radioactivity detector with TruCount scintillation fluid at 5 mL/min in a 0.75 mL flow cell. Tritiations were performed at the National Tritium Labeling Facility at Berkeley, CA.

2-(8-Phenyloctyl)benzoic acid (2)

A solution of 2-(8-phenyloctyl)benzaldehyde (23.56 g, 80 mmol) in acetone (250 mL) was treated at room temperature by dropwise addition of an excess of Jones reagent. The reaction was stirred for 2 h after addition, then isopropanol (3 mL) was added and stirring was continued for another 30 min. The reaction was filtered through a pad of silica gel, the filtrate evaporated *in vacuo*, and the residue recrystallized from hexane to provide 17.75 g (71%) of **2** as off-white crystals.

2-[8-(2/4-Nitrophenyl)octyl]benzoic acid (3)

A 36.42 g (117.5 mmol) portion of **2** and potassium nitrate (12.0 g, 118 mmol) were dissolved in trifluoroacetic acid (400 mL), and the solution was stirred at room temperature for 16 h. Most of the solvent was removed on a rotary evaporator, and the residue was dissolved in ethyl ether. This solution was washed four times with water, twice with brine, then dried over magnesium sulfate. The solution was filtered and evaporated *in vacuo* to give 42.40 g (101%) of a mixture of 2-[8-(2-nitrophenyl)octyl]benzoic acid and 2-[8-(4-nitrophenyl)octyl]benzoic acid as a viscous oil. TLC (SiO_2, 80:20:1 hexane/ethyl acetate/acetic acid): 2 spots of similar intensity R_f 0.30 and 0.32; ^{1}H NMR (270 MHz): 1.24 (8H, m, CH_2), 1.62 (4H, m, CH_2), 2.69 (t, J = 7.5 Hz, 4-nitro isomer octyl C8**H**), 2.86 (0.8 H, t, J = 7.8 Hz, 2-nitro isomer octyl C8**H**), 3.01 (2H, t, J = 7.5 Hz, octyl C1**H**), 7.24-7.33 (4.4H, m, Ar**H**), 7.45 (1.4H, m, Ar**H**), 7.85 (0.4H, d, J = 7.8 Hz, 2-nitro isomer carboxy ring C6**H**), 8.02 (0.7 H, d, J = 7.8 Hz, 4-nitro isomer carboxy ring C6**H**), 8.11 (1H, d, J = 8.8 Hz, 1/2 of AB quartet, 4-nitrophenyl C3**H**, C5**H**); MS, m/z (%): 355 (M^+), 337 (7), 320 (15), 170 (29), 158 (48), 148 (66), 144 (68), 130 (78), 120 (100).

2-[8-(2/4-Aminophenyl)octyl]benzoic acid (4)

A mixture of nitrophenyl isomers **3** (40.0 g, 113 mmol) was dissolved in absolute ethanol (200 mL) and hydrogenated under 40 psi of hydrogen gas with 5% Pd/C (1.04 g). After 95 minutes, the mixture was filtered through Celite and the solvent removed first on a rotary evporator then at <0.1 Torr at 50 °C for 3 h. Resulting was 38.16 g (104%) of a mixture of 2-[8-(2-aminophenyl)octyl]benzoic acid and 2-[8-(4-aminophenyl)octyl]benzoic acid as a tan oil. TLC (SiO_2, 49:49:2 hexane/ethyl acetate/acetic acid): 2 spots of equal intensity R_f 0.42 and 0.68. A 300 mg sample of this was subjected to flash chromatography (70:30:1 hexane/ethyl acetate/acetic acid) to give 2-[8-(2-aminophenyl)octyl]benzoic acid (faster running)--^{1}H NMR (270 MHz): 1.34 (8H, m, CH_2), 1.57 (4H, m, CH_2), 2.43 (2H, t, J = 7.7 Hz, benzylic **H**), 3.01 (2H, t, J = 7.8 Hz, benzylic **H**), 6.67-6.76 (2H, m, Ar**H**), 6.99-7.05 (2H, m, Ar**H**), 7.10-7.26 (2H, m, Ar**H**), 7.40 (1H, t, J = 7.3 Hz, carboxy ring C4**H**), 7.95 (1H, d, J = 7.8 Hz, carboxy ring C6**H**); MS, m/z (%): 326 (23), 325 (M^+, 100), 190 (12); and 2-[8-(4-aminophenyl)octyl]benzoic acid (slower running)--^{1}H NMR (270 MHz): 1.29 (8H, m, CH_2), 1.55 (4H, m, CH_2), 2.48 (2H, t, J = 7.6 Hz, benzylic **H**), 2.98 (2H, t, J = 7.7 Hz, benzylic **H**), 6.64 (2H, d, J = 8.3 Hz, 1/2 of AB quartet, aniline ring Ar**H**), 6.76 (2H, br s, N**H**), 6.96 (2H, d, J = 8.3 Hz, 1/2 of AB quartet, aniline ring Ar**H**), 7.22-7.27 (2H, m, carboxy ring C3**H**, C5**H**), 7.47 (1H, t, J = 6.9 Hz, carboxy ring C4**H**), 7.99 (1H, d, J = 7.8 Hz, carboxy ring C6**H**); MS, m/z (%): 326 (23), 325 (M^+, 100), 253 (3), 184 (14), 171 (18).

2-[8-(2,3,5/3,4,5-Tribromophenyl)octyl]benzoic acid (5)

Cupric bromide (2.61 g, 11.7 mmol) was added to acetonitrile (200 mL), and the suspension was heated to 50 °C with stirring. A solution of bromine (10.58 mL, 205 mmol) in acetonitrile (50 mL) was added, followed by dropwise addition of a solution of 2-[8-(2/4-aminophenyl)octyl]benzoic acid (19.03 g, 58.6 mmol) in acetonitrile (30 mL). Thirty minutes later, a solution of t-butylnitrite (8.32 mL, 70.3 mmol) in acetonitrile (25 mL) was added in small portions over 30 minutes. The reaction mixture was maintained at 50-55 °C for an additional 1.5 h, then cooled to room temperature, and poured into 700 mL of 3 N aqueous HCl. The mixture was extracted three times with 1:1 (v/v) ether/ethyl acetate, and the combined extracts were washed with 3 N aqueous HCl, dilute aqueous sodium sulfite, water, and brine. The solution was dried over magnesium sulfate, and the solvent was removed *in vacuo* to give 27.74 g of a dark brown gum. Flash chromatography on 500 g of silica gel eluted with 90:10:1 hexane/ethyl acetate/acetic acid yielded 16.26 g (51%) of a mixture of 2-[8-(2,3,5-tribromophenyl)octyl]benzoic acid and 2-[8-(3,4,5-tribromophenyl)octyl]benzoic acid as a pale yellow oil. TLC (SiO_2, 80/20/1 hexane/ethyl acetate/acetic acid): elonged spot R_f 0.41; ^1H NMR (270 MHz): 1.40 (8H, m, CH_2), 1.60 (4H, m, CH_2), 2.47 (0.9H, t, J = 7.7 Hz, minor isomer benzylic **H**), 2.72 (1.1H, t, J = 7.8 Hz, major isomer benzylic **H**), 3.02 (2H, t, J = 7.8 Hz, benzylic **H**), 7.22-7.29 (2.4H, m, Ar**H**), 7.36 (1.1**H**, s, 3,4,5-isomer bromo ring C2**H** and C6**H**), 7.46 (1H, t, J = 7.4 Hz, carboxy ring C4**H**), 7.59 (0.4H, d, J = 1.9 Hz, 2,3,5-isomer bromo ring C2**H** and C6**H**), 8.20 (1H, d, J = 7.8 Hz, carboxy ring C6**H**); MS, m/z (%): 548 (M^+, 1), 546 (M^+, 2), 469 (50), 468 (22), 467 (100), 466 (16), 465 (55), 451 (23), 449 (49), 447 (25), 331 (30), 329 (92), 327 (98), 325 (35), 251 (38), 250 (24), 249 (80), 248 (36), 247 (46).

2-[8-(2,3,5/3,4,5-Tribromophenyl)octyl]benzyl alcohol (**6**)

A solution of 2-[8-(2,3,5/3,4,5-tribromophenyl)octyl]benzoic acid (15.1 g, 27.6 mmol) in dry THF (43 mL) was added slowly to 1M borane in THF (35 mL), and the reaction was stirred at room temperature for 15 h. The reaction was quenched by addition of methanol (4 mL), and the solvent was removed by rotary evaporation. The residue was partitioned between ether and water, the ether phase was washed with 1N aqueous sodium hydroxide, brine, and dried over magnesium sulfate. Removal of solvent yielded 15.16 g (103%) of 2-[8-(2,3,5-tribromophenyl)octyl]benzyl alcohol and 2-[8-(3,4,5-tribromophenyl)octyl]benzyl alcohol as a clear oil. TLC (SiO_2, 80:20:1 hexane/ethyl acetate/acetic acid) R_f 0.20 and 0.23; ^1H NMR (270 MHz): 1.30-1.40 (8H m, CH_2), 1.60 (4H, m, CH_2), 2.50 (1H, t, J = 7.8 Hz, major isomer octyl C8**H**), 2.67 (2H, t, J = 7.8 Hz, octyl C1**H**), 2.75 (0.6H, t, J = 7.8 Hz, minor isomer octyl C8**H**), 4.72 (2H, s, CH_2OH), 7.18-7.29 (3.8H, m, Ar**H**), 7.35-7.39 (0.7H, m, Ar**H**), 7.39 (1.1H, s, 3,4,5-isomer bromo ring C2**H** and C6**H**), 7.62 (0.4H, d, J = 2.2 Hz, 2,3,5-isomer bromo ring Ar**H**); MS, m/z

(%): (no M^+), 518 (16), 517 (11), 516 (47), 515 (13), 514 (49), 455 (10), 453 (21), 451 (11), 437 (37), 433 (19), 331 (29), 329 (88), 327 (100), 325 (48), 251 (23), 250 (21), 249 (49), 248 (36), 247 (30), 246 (19).

2-[8-(2,3,5/3,4,5-Tribromophenyl)octyl]benzaldehyde (7)

2-[8-(2,3,5/3,4,5-Tribromophenyl)octyl]benzyl alcohol (14.16 g, 26.6 mmol) was dissolved in ethyl acetate (300 mL). Activated manganese dioxide (40 g) was added, and the mixture was swirled and stirred for 1.25 h. The mixture was filtered through Celite, then the solvent was removed *in vacuo* to give 12.7 g (90%) of a mixture of 2-[8-(2,3,5-tribromophenyl)-octyl]benzaldehyde and 2-[8-(3,4,5-tribromophenyl)octyl]benzaldehyde as a pale yellow oil. TLC (SiO_2, 96:4 hexane/acetone): R_f 0.44 and 0.50. A partial separation was achieved by flash chromatography (SiO_2, 0% to 3% ethyl acetate in hexane), which gave initial and final fractions highly enriched (90% by HPLC: LiChrosorb Si60, 0.25% isopropanol in hexane, 1.0 mL/min; UV at 220 nm; R_t's 5.98 and 6.94 min) in the individual components and a large center fraction consisting of a mixture of the two (42% at R_t 5.98 min and 55% at R_t 6.94 min). The faster running component was 2-[8-(2,3,5-tribromophenyl)octyl]benzaldehyde: ^1H NMR (270 MHz): 1.30-1.34 (8H, m, CH_2), 1.59 (4H, m, CH_2), 2.74 (2H, t, J = 7.8 Hz, octyl C8**H**), 3.02 (2H, t, J = 7.8 Hz, octyl C1**H**), 7.24-7.28 (2H, m, Ar**H**), 7.35 (1H, t, J = 7.3 Hz, aldehyde ring C5**H**), 7.50 (1H, dt, J = 1.5 Hz, J = 7.3 Hz, aldehyde ring C4**H**), 7.61 (1H, d, J = 2.4 Hz, bromo ring Ar**H**), 7.83 (1H, dd, J = 1.5 Hz, H = 7.8 Hz, aldehyde ring C6**H**), 10.29 (1H, s, CHO); MS, m/z (%); 534 (M^+, 11), 533 (13), 532 (M^+, 34), 531 (21), 530 (M^+, 36), 529 (16), 528 (M^+, 12), 453 (49), 452 (27), 451 (100), 449 (58), 331 (28), 329 (85), 327 (95), 325 (33), 251 (24), 250 (19), 249 (51), 248 (32), 247 (30), 246 (16); the slower running component was 2-[8-(3,4,5-tribromophenyl)octyl]benzaldehyde:^1H NMR (270 MHz): 1.29-1.34 (8H, m, CH_2), 1.59 (4H, m, CH_2), 2.49 (2H, t, J = 7.8 Hz, octyl C8**H**), 3.01 (2H, t, J = 7.8 Hz, octyl C1**H**), 7.25-7.28 (1H, m, Ar**H**), 7.35 (1H, t, J = 7.3 Hz, aldehyde ring C5**H**), 7.38 (2H, s, bromo ring C2**H** and C6**H**), 7.52 (1H, dt, J = 1.5 Hz, J = 7.3 Hz, aldehyde ring C4**H**), 7.83 (1H, dd, J = 1.5 Hz, J = 7.8 Hz, aldehyde ring C6**H**), 10.28 (1H, s, CHO); MS, m/z (%): 534 (M^+, 9), 533 (10), 532 (M^+, 29), 531 (16), 530 (M^+, 29), 529 (11), 528 (M^+, 11), 453 (36), 452 (19), 451 (75), 449 (46), 331 (27), 329 (86), 327 (100), 325 (36), 251 (35), 250 (25), 249 (74), 248 (37), 247 (43), 246 (18).

Methyl *trans*-2,3-Epoxy-3-[2-[8-(2,3,5/3,4,5-tribromophenyl)octyl]-phenyl]propionate (**8**)

2-[8-(2,3,5/3,4,5-Tribromophenyl)octyl]benzaldehyde (4.81 g, 9.1 mmol, 42:55 ratio of 2,3,5 to 3,4,5 by HPLC) was dissolved in dichloromethane (100 mL), and 25% sodium methoxide/methanol (4.77 mL, 20.8 mmol) was added. The mixture was cooled with a dry ice/acetone bath, and methyl chloroacetate (1.98 mL, 22.6 mmol) was added dropwise over a 20 minute period. The cooling bath was removed, and the reaction was stirred for 90 minutes. The reaction was diluted with more dichloromethane. The solution was washed twice with water, twice with brine, dried over magnesium sulfate, and evaporated *in vacuo* to give 5.34 g (98%) of a mixture of methyl 2,3-epoxy-3-[2-[8-(2,3,5-tribromophenyl)octyl]phenyl]propionate and methyl 2,3-epoxy-3-[2-[8-(3,4,5-tribromophenyl)octyl]phenyl]propionate as a pale oil. TLC (SiO_2, 92:8 cyclohexane/ethyl acetate, R_f 0.26 and 0.32); HPLC (LiChrosorb Si60, 0.2% isopropanol in hexane, 1.0 mL/min, UV at 220 nm): R_t 6.92 min (45.1%) and 7.70 min (47.4%); ^1H NMR (270 MHz): 1.32 (8H, m, CH_2), 1.58 (4H, m, CH_2), 2.47 (1H, t, J = 7.5 Hz, benzylic **H**), 2.60-2.78 (3H, m, benzylic **H**), 3.41 (1H, d, J = 2.0 Hz, C2**H**), 3.84 (3H, s, CO_2CH_3), 4.26 (1H, m, C3**H**), 7.16-7.29 (4.8H, m, Ar**H**), 7.39 (0.95H, s, 3,4,5-isomer bromo ring C2**H** and C6**H**), 7.61 (0.46H, d, J = 2.0 Hz, 2,3,5-isomer bromo ring Ar**H**); MS, m/z (%): 606 (M^+, 4), 604 (M^+, 14), 602 (M^+, 14), 600 (M^+, 4), 549 (21), 547 (70), 545 (69), 543 (30), 533 (20), 531 (70), 529 (65), 527 (26), 331 (31), 329 (89), 327 (100), 325 (37), 251 (18), 250 (15), 249 (37), 248 (79), 247 (24), 246 (17).

Methyl *erythro*-3-[(2-Carbomethoxyethyl)thio]-3-[2-[8-(2,3,5/3,4,5-tribromophenyl)octyl]phenyl]-2-hydroxypropionate (10)

To a solution of **8** (5.34 g, 8.9 mmol) in methanol (100 mL) and triethylamine (10 mL) was added methyl 3-mercaptopropionate (5.88 mL, 53.1 mmol). The solution was stirred at room temperature under argon for 16.5 h. The volatile components of the mixture was removed *in vacuo*, and a 150 mg portion of the oily residue was removed. The remainder was dissolved in dry methanol (100 mL), and to the solution was added 25% sodium methoxide/methanol (5 mL). The mixture was stirred at room temperature for 1 h, then was acidified by addition of dilute aqueous HCl. The resulting suspension was diluted with water, then extracted twice with ethyl acetate (150 mL). The combined extracts were washed twice with water, twice with brine, then dried over magnesium sulfate and evaporated *in vacuo* to yield 7.22 g of a light yellow liquid. The product was purified by flash chromatography (ethyl acetate/hexane mixtures: 1 L of 20:80, then 1.5 L of 25:75, then 1 L of 28:72). Resulting was 1.98 g (31%) of **10**. HPLC analysis (LiChrosorb Si60, 2.0 mL/min, 2.5:97.5 isopropanol/hexane, UV at 220 nm) showed only two components, R_t 8.85 and 10.12 min in the ratio of 49.6/50.4. A small portion was subjected to semi-preparative HPLC (LiChrosorb RP-18, 5.0 mL/min, 80:20 acetonitrile/water) to separate the two isomers: 2,3,5-tribromo isomer, R_t = 48.8 min, ^{1}H NMR (400 MHz): 1.33 (8H, m, CH_2), 1.58 (4H, m, CH_2), 2.51 (2H, t, J = 7.8 Hz, CH_2), 2.54 (2H, t, J = 7.3 Hz, CH_2), 2.60 (1H, m, 1/2 CH_2), 2.70 (1H, m, 1/2 CH_2), 2.78 (2H, m, CH_2), 3.65 (3H, s, CO_2CH_3), 3.67 (3H, s, CO_2CH_3), 4.55 (1H, d, J = 4.9 Hz, C2**H**), 4.60 (1H, br s, C3**H**), 7.12-7.26 (3H, m, dialkylphenyl C3**H**, C4**H** and C5**H**), 7.40 (2H, s, tribromophenyl C2**H** and C6**H**), 7.61 (1H, dd, J = 6.5 Hz, J = 2.3 Hz, dialkylphenyl C6**H**); 3,4,5-tribromo isomer, R_t = 33.5 min; ^{1}H NMR (400 MHz): 1.37 (8H, m, CH_2), 1.59 (4H, m, CH_2), 2.55 (2H, t, J = 7.3 Hz, CH_2), 2.59 (1H, m, 1/2 CH_2), 2.69 (1H, m, 1/2 CH_2), 2.74-2.81 (4H, m, CH_2), 3.56 (3H, s, CO_2CH_3), 3.65 (3H, s,

CO_2CH_3), 4.55 (1H, d, J = 4.8 Hz, C2H or C3H), 4.61 (1H, d, J = 4.8 Hz, C3H or C2H), 7.13-7.26 (3H, m, dialkylphenyl C3H, C4H and C5H), 7.28 (1H, d, J = 2.2 Hz, tribromophenyl CH), 7.61 (1H, dd, J = 6.5 Hz, J = 2.2 Hz, dialkylphenyl C6H), 7.63 (1H, d, J = 2.2 Hz, tribromophenyl CH).

Methyl *erythro*-3-[(2-Carbomethoxyethyl)thio]-3-[2-(8-[^{3}H]phenyloctyl)-phenyl]-2-hydroxypropionate (11)

A 41 mg (57 mmol) portion of **10** was stirred in 5 mL of 2:2:1 (v/v/v) ethyl acetate/DMF/triethylamine with 80 mg of preactivated 10% Pd/C under an initial 720 mm pressure of tritium gas. After 4.5 h, excess tritium gas was removed, and the labile tritium was exchanged away by repeated addition and evaporation of methanol. The solution remaining was filtered, evaporated *in vacuo*, and the residue was extracted with benzene to separate the product from triethylamine hydrobromide. Obtained in benzene solution was 1490 mCi of **11** having a radiochemical purity of 88% by HPLC (Waters µBondapak C_{18} column, 70:30 acetonitrile/water). A portion of this material was purified by semipreparative reverse-phase HPLC (same mobile phase), giving 46.5 mCi of **11** of radiochemical purity >97%, specific activity of 55 Ci/mmol, and having a UV spectrum superimposable on that of an unlabeled standard. ^{3}H NMR (320 MHz): 7.16-7.32 (m, ArT).

2-(8-Phenyl-1,7-octadiynyl)benzaldehyde (14)

A mixture of 8-phenyl-1,7-octadiyne (**13**, 7.0 g, 38.5 mmol), 2-bromobenzaldehyde (**12**, 6.9 g, 37.3 mmol), bis(triphenylphosphine)palladium (II) chloride (576 mg, 0.8 mmol), CuI (140 mg, 0.7 mmol) and triethylamine (70 mL) were stirred under argon, and heated to 95 °C over a period of 20 min. After an additional 15 min, precipitate appeared. Triethylamine (20 mL) was added to facilitate stirring. After an additional 35 min, the reaction was cooled to 0°C, filtered, and washed with ethyl acetate (50 mL). The filtrate was diluted with 1N aqueous HCl solution (50 mL), and extracted with ethyl acetate (200 mL). The organic phase was washed with saturated sodium bicarbonate (30 mL), water (20 mL), brine (20 mL), and dried over sodium sulfate. Filtration, concentration *in vacuo*, and purification on a silica gel column using gradient elution of dichloromethane in hexane gave **14** as an oil (9.2 g, 86%). HPLC (two Altex Ultrasphere ODS C_{18} columns, 9:1 acetonitrile/water, 1.0 mL/min, UV at 230 nm): R_t 11.8 min; ^1H NMR (270 MHz): 1.76-1.87 (4H, m, homopropargylic **H**), 2.50 (2H, t, J = 6.5 Hz, propargylic **H**), 2.57 (2H, t, J = 6.5 Hz, propargylic **H**), 7.25-7.52 (9H, m, Ar**H**), 10.55 (1H, s, CHO); MS, m/z (%): 285 (M^+-1, 4), 257 (24), 243 (6), 229 (26), 215 (30), 165 (10), 141 (12), 128 (24), 115 (100).

Methyl *trans*-2,3-Epoxy-3-[2-(8-phenyl-1,7-octadiynyl)phenyl]propionate (15)

The Darzens reaction was carried out on 5.1 g (17.8 mmol) of **14** in the same way as on **7** to give **15** as an oil (5.4 g, 84%). HPLC (two Altex Ultrasphere ODS C_{18} columns, 9:1 acetonitrile/water, 1.0 mL/min, UV at 230 nm): R_t 10.9 min; ^1H NMR (270 MHz): 1.75-1.78 (4H, m, homopropargylic **H**), 2.45-2.51 (4H, m, propargylic **H**), 3.43 (1H, d, J = 1.95 Hz, C2**H**), 3.82 (3H, s, CO_2CH_3), 4.54 (1H, d, J = 1.71 Hz, C3**H**), 7.15-7.41 (9H, m, Ar**H**); MS, m/z (%): 357 (M^+-1, 1), 325 (2), 311 (2), 299 (87), 281 (20), 271 (60), 215 (29), 202 (11), 178 (13), 165 (18), 152 (17), 141 (26), 128 (35), 115 (76), 105 (100).

Methyl *erythro*-3-[(2-Carbomethoxyethyl)thio]-3-[2-(8-phenyl-1,7-octyldiynyl)phenyl]-2-hydroxypropionate (17)

The mercaptopropionate addition/retroaldol reaction sequence was carried out on 5.0 g (14.0 mmol) of **15** in the same way as on **8** to provide **17** as an oil (2.8 g, 41%). HPLC (two Altex Ultrasphere ODS C_{18} columns, 4:1 acetonitrile/water, 1.0 mL/min, UV at 230 nm): R_t 13.2 min, R_t 12.5 min for regiosiomer **16**; ^1H NMR (270 MHz): 1.81-1.84 (4H, s, homopropargylic **H**), 3.65 (3H, s, CO_2CH_3), 3.66 (s, 3H, CO_2CH_3), 4.63 (1H, dd, J = 7.3 Hz, J =

4.4 Hz, C2H), 4.94 (1H, d, J = 4.4 Hz, C3H), 7.16-7.38 (9H, m, ArH); MS, m/z (%): 477 (M^+-1, 35), 405 (2), 391 (34), 373 (15), 331 (10), 313 (9), 301 (100), 281 (9), 269 (44), 253 (35), 241 (46), 228 (28), 215 (17), 205 (12), 165 (14), 159 (17), 141 (21), 128 (32), 115 (60).

(2S,3R)-Methyl 3-[(2-Carboxyethyl)thio]-3-[2-(8-phenyl-1,7-octyldiynyl)-phenyl]-2-[N-(2,2,2-trichloroethoxycarbonyl)-(S)-prolyloxy]propionate (**18**)

The N-(2,2,2-trichloroethoxycarbonyl)-(S)-prolyl acid chloride was prepared by stirring thionyl chloride (8 mL) and the corresponding acid (9.0 g, 31.0 mmol) at 0 °C under argon for 3 h. Concentration *in vacuo* gave a thick oil which was dissolved in pyridine (5 mL). To a stirred solution of **17** (1.9 g, 4.0 mmol) in pyridine (15 mL) at 0 °C under argon was added the acid chloride solution in one portion. The reaction was then stirred at room temperature overnight. Concentration *in vacuo* and purification on a silica gel column using gradient elution of ethyl acetate in hexane afforded the diastereomeric mixture as an oil (2.2 g, 75%). The diastereomers were separated by preparative HPLC (Dynamax silica gel column (8 µm, 2.14 mm I.D. x 25 cm), 98:2 (v/v) dichloroethane/ethyl acetate, 20.0 mL/min, UV at 260 nm, R_t 29-37 min for **18**, R_t 33-42 min for the undesired **19**). A second HPLC purification was necessary in order to obtain pure **18** (550 mg, 18%). Analytical HPLC (two IBM silica gel columns (5 µm, 4.6 mm I.D. x 25 cm), 97:3 (v/v) dichloroethane/ethyl acetate, 1.0 mL/min, UV at 260 nm) R_t 16.0 min.

(2S,3R)-3-[(2-Carboxyethyl)thio]-3-[2-(8-phenyl-1,7-octyldiynyl)phenyl]-2-hydroxypropionic acid (**20**)

To a stirred solution of **19** (550 mg, 0.73 mmol) in dimethoxyethane at 5 °C under argon was added dropwise 2 mL of an aqueous lithium hydroxide solution (1.3 g, of lithium hydroxide in 20 mL of water). The reaction mixture was stirred at 5 °C for 5 h, then acidified with 3.3N aqueous HCl solution, diluted with water (5 mL), and extracted with ethyl acetate (2 x 50 mL). The combined organic phase was washed with water (10 mL), brine (10 mL), and dried over sodium sulfate. Concentration *in vacuo*, and purification on a silica gel column using gradient elution of ethyl acetate in 100:1 (v/v) hexane/formic acid gave crude **20** (292 mg), which was further purified by preparative HPLC (Dynamax C$_{18}$ column (8 µm, 2.14 cm I.D. x 25 cm), 30:70:1 (v/v/v) water/methanol/acetic acid, 15.0 mL/min, UV at 260 nm, R$_t$ 18 min). Collected fractions were concentrated *in vacuo* below 25 °C to remove as much methanol as possible. The resulting solution was then extracted with ethyl acetate. The organic phase was washed with water, brine, dried over sodium sulfate. Filtration, concentration *in vacuo*, and drying under vacuum led to **20** as an oil (212 mg, 64%). HPLC (Waters µBondapak C$_{18}$ column, 75:25:1 (v/v/v) methanol/water/acetic acid, 1.0 mL/min, UV at 260 nm) R$_t$ 7.6 min; ^{1}H NMR (270 MHz): 1.78-1.82 (4H, m, homopropargylic **H**), 4.58 (1H, d, J = 5.9 Hz, C2**H** or C3**H**), 4.92 (1H, d, J = 5.9 Hz, C3**H** or C2**H**), 7.23-7.41 (9H, m, Ar**H**); MS, m/z (%): 449 (M$^+$-1, 10), 377 (14), 359 (2), 316 (37), 303 (100), 283 (26), 273 (52), 253 (27), 241 (30), 228 (24), 215 (21), 191 (11), 185 (15), 173 (24), 165 (18), 160 (15), 147 (19), 141 (21), 128 (37), 115 (73).

(2S,3R)-3-[(2-Carboxyethyl)thio]-3-[2-(8-phenyl[^{3}H]octyl)phenyl]-2-hydroxypropionic acid (**21**)

A solution of **20** (20 mg) in 4 mL of 1:1 (v/v) ethyl acetate/DMF was degassed and then exposed to tritium gas under atmospheric pressure. To this solution was added in one portion 10% Pd/C (40 mg) which had been dried at room temperature under vacuum overnight and presaturated with tritium gas. The reaction was stirred at room temperature for 23 h. After filtration, the material was diluted with methanol and then lyophilized. This procedure was repeated two more times to remove tritium labiles. The resulting material was dissolved in ethanol (4.7 mL) with a total radioactivity of 4.5 Ci and a radiochemical purity of 58% by HPLC (Waters µBondapak C_{18} column, 25:75:1 (v/v/v) water/methanol/acetic acid, 1.0 mL/min, Ramona-D radioactivity detection, R_t at 15.7 min). Small portions were purified by preparative HPLC (Dynamax C_{18} column (8 µm, 2.14 cm I.D. x 25 cm), 25:75:1 (v/v/v) water/methanol/acetic acid, 15.0 mL/min, UV at 260 min, R_t at 47.5 min). ^3H NMR (320 MHz): 1.60-1.80 (br s, homobenzylic **T**), 2.38 (br s, benzylic **T**), 2.80 (br s, benzylic **T**). Freshly collected HPLC eluates with radiochemical purity >97% were immediately lyophilized and then dissolved in ethanol. To minimize decomposition, **21** had to be dissolved in a thoroughly degassed solvent (prepared by freeze-pump-thaw procedure) under an argon atmosphere and stored at a low temperature (-80°C).

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8. Repeated HPLC was necessary due to the difficulty in separating these two diastereomers. In the preparative HPLC employed, these two components did not have a baseline resolution.

9. Various conditions using other cataysts and solvents were screened. This system was found to be the fastest in terms of reduction duration.

10. A 20 mg sample of **20** was hydrogenated in the presence of 10% Pd/C (20 mg) in 4 mL of 1:1 DMF/ethyl acetate at room temperature under atmospheric pressure for 5 h. After filtration and concentration *in vacuo*, the reduction product was treated with diazomethane to generate its dimethyl ester derivative, which was analyzed by a chiral HPLC method (two Supelcosil LC-(R)-UREA columns (5 μm, 4.6 mm I.D. x 25 cm), 100:1 (v/v) hexane/isopropanol, 1.0 mL/min, UV at 230 nm). The desired 2S,3R enantiomer had the longer retention time.

11. The specific activity of **21** was computed from its mass concentration and radioactive concentration. The former was determined by an UV-weight basis HPLC method (Whatman Partisil ODS-3 column (5 μm, 4.6 mm I.D. x 25 cm), mobile phase: 25:75 (v/v)

water/0.02M nBu_4ClO_4 in methanol, 1.0 mL/min, UV at 230nm) against a reference standard of SK&F 104353.

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