REGIO- AND STEREO-CONTROLLED SYNTHESIS OF [24,30-^{14}C]-LABELED-2,3-EPOXYSQUALENE

Xiao-yi Xiao and Glenn D. Prestwich*

Department of Chemistry
State University of New York
Stony Brook, New York, New York 11794-3400

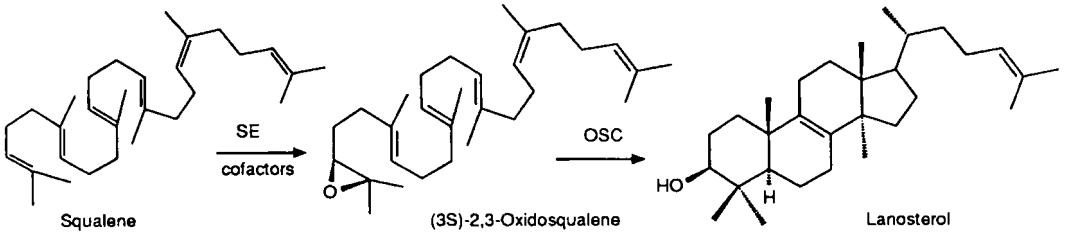
SUMMARY

[24,30-^{14}C]-(3*S*)-2,3-Epoxysqualene and its racemate were synthesized by two convenient routes. The products have the label in a metabolically-nonlabile position relative to the demethylation of lanosterol to cholesterol.

Key words: oxidosqualene cyclase, cholesterol biogenesis, enzyme assay, carbon-14 labeling, asymmetric synthesis

INTRODUCTION

(3*S*)-2,3-Epoxysqualene is a key intermediate in the biochemical synthesis of triterpenes and sterols in vertebrates, plants, and fungi.[1,2] In the study of the mechanism and inhibition of vertebrate oxidosqualene cyclase (OSC, EC 5.4.99.7), which catalyzes the conversion of (3*S*)-2,3-epoxysqualene to lanosterol, we required the natural (3*S*) enantiomer of the labeled 2,3-epoxysqualene. Moreover, it was desirable to have the ^{14}C-label in such a position that the radiolabel would be retained during subsequent oxidative demethylations at C-4 which transform lanosterol to cholesterol.



Racemic ^{14}C-labeled epoxysqualene was first synthesized from biosynthetically-produced [^{14}C]squalene.[3] Surprisingly, even though the (3*R*) enantiomer could disrupt careful kinetic analysis of inhibitor action with purified OSC, the chemical synthesis of

*Address correspondence to this author.

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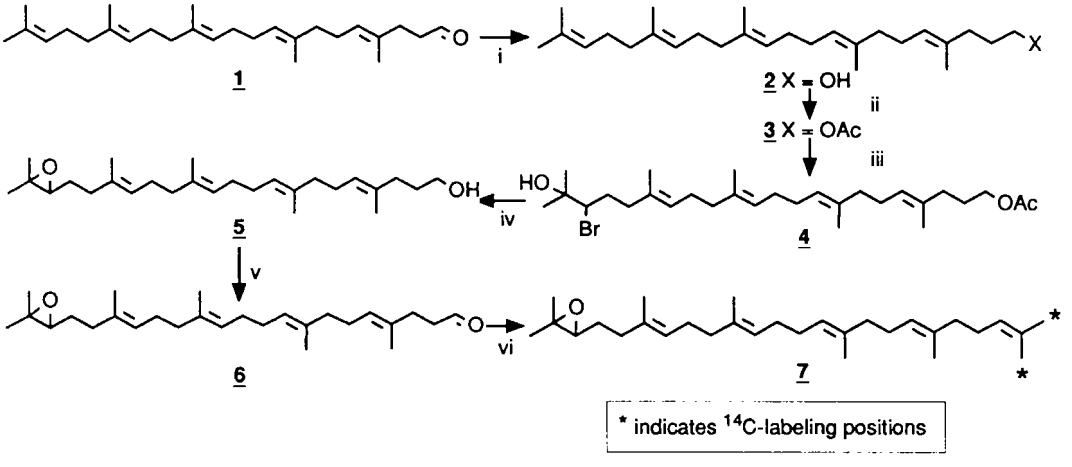
optically-active ^{14}C-labeled 2,3-epoxysqualene is unreported. We describe herein two routes for the synthesis of [24,30-^{14}C]-2,3-epoxysqualene in racemic and in the optically-active (3*S*) form.

Results and Discussion

The epoxytrisnorsqualene aldehyde **6** needed for the condensation with a ^{14}C-labeled Wittig reagent was synthesized starting from trisnorsqualene (TNS) aldehyde (**1**) as shown in Scheme I.[4] After reduction of aldehyde **1** to alcohol **2** and acetylation, acetate **3** was treated with *N*-bromosuccinimide (NBS) in wet THF between 0 - 20 °C. Initial attempts to make the terminal bromohydrin directly from TNS aldehyde (**1**) or TNS alcohol (**2**) failed. Thus, treatment of **1** with NBS in wet THF at 0 °C caused complete decomposition of the aldehyde within 1 hr, while the same condition cleanly converted TNS alcohol (**2**) to a cyclic ether at 84% yield by attack of Br^+ to C-5 (numbering from hydroxy group on **2**) and the subsequent cyclization of OH to C-4.

The resulting bromohydrin **4** was isolated and simultaneously cyclized and deprotected with K_2CO_3 in methanol to afford the racemic epoxytrisnorsqualenol **5** in quantitative yield. Oxidation with pyridinium dichromate (PDC) provided aldehyde **6** needed to prepare the racemic [24,30-^{14}C]-2,3-epoxysqualene (**7**).

Scheme I.

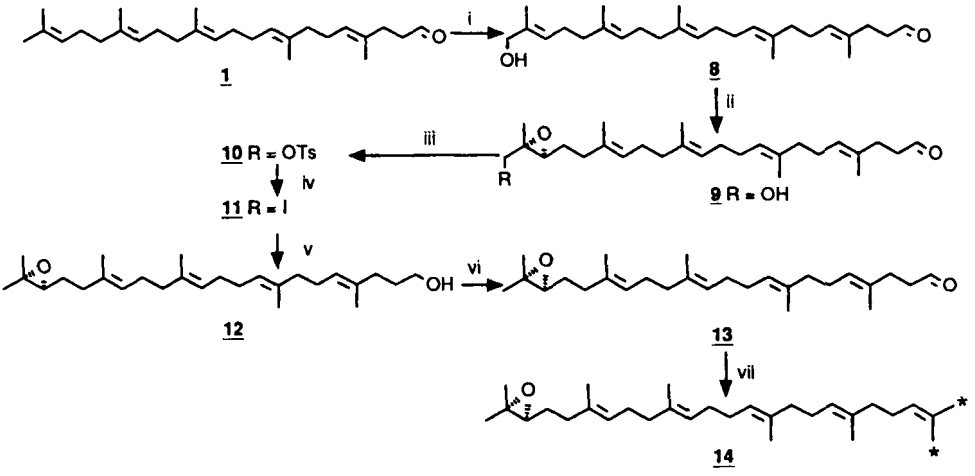


(i) $NaBH_4$, CH_3OH; (ii) Ac_2O, pyridine; (iii) NBS, wet THF; (iv) K_2CO_3, MeOH; (v) PDC, NaOAc, CH_2Cl_2; (vi) $(^{14}CH_3)_2C{=}PPh_3$, THF.

The synthesis of the optically-active precursor (3*S*)-epoxytrisnorsqualene aldehyde **13** began with TNS aldehyde (**1**) (Scheme II). Allylic oxidation with SeO_2 / *t*-BuOOH in CH_2Cl_2 at 0 °C afforded 20% of the desired *(E)*-allylic alcohol **8** after column chromatography separation of other isomers. Sharpless epoxidation[5] in the presence of L-(+)-diethyltartrate produced the (*S*)-epoxy alcohol **9** at 74% yield with more than 95% e.e. as shown by the ^1H NMR of the respective (+)-α-MTPA ester derivative.[6] Deoxygenation of the alcohol **9** to the corresponding epoxy alcohol **12** was achieved by tosylation, iodination, and reduction with

$NaBH_3CN$ in HMPA at room temperature.[7] The resulting (*S*)-epoxytrisnorsqualene alcohol **12** was then oxidized (PDC, CH_2Cl_2) to the desired (*S*)-epoxytrisnorsqualene aldehyde **13**.

Scheme II.



(i) SeO_2, *t*-BuOOH, CH_2Cl_2; (ii) *t*-BuOOH, L-(+)-diethyltartrate, $Ti(O\text{-}iPr)_4$, 3 Å molecular sieves, CH_2Cl_2; (iii) TsCl, pyridine, $CHCl_3$; (iv) NaI, acetone; (v) $NaBH_3CN$, HMPA; (vi) PDC, NaOAc, CH_2Cl_2; (vii) $(^{14}CH_3)_2C{=}PPh_3$, THF.

The radiolabeled isopropylidene group was introduced by Wittig olefination of either the racemic **6** or the (3*S*)-epoxy aldehyde **13** with the ^{14}C-labeled isopropyl phosphorylid made from ethyltriphenylphosphonium bromide and [^{14}C]iodomethane.[4,8] Cyclization of the ^{14}C-labeled (3*S*)-2,3-epoxysqualene (**14**) by the action of OSC from either hog liver microsomes or baker's yeast showed greater than 85% conversion to ^{14}C-labeled lanosterol as determined by radio-TLC analysis.[4]

The optically-active (3*S*)-^{14}C-labeled 2,3-epoxysqualene with the radiolabel located distal from the proto-sterol nucleus provides four advantages over conventional racemic compounds with uniform labeling[3] or the optically-active compound generated *in situ* from [24,30-^{14}C]-squalene by the action of squalene epoxidase (SE) also present with OSC in hog liver microsomes.[4,9] First, the ^{14}C-label is not lost during the subsequent oxidative demethylations at C-4 which convert lanosterol to cholesterol. Second, potential interference of the (3*R*) enantiomer with kinetic analysis of purified enzymes is eliminated. Third, the ^{14}C-label is more efficiently utilized. Fourth, the use of microsomal SE, OSC inhibitors and a variety of cofactors, as well as a relatively tedious chemical purification from the crude enzyme reaction, can be completely avoided.

MATERIALS AND METHODS

General Methods. All glassware, syringes, and needles were oven-dried at 110 °C, assembled while hot, and cooled under dry nitrogen. All reactions were conducted under a slight positive pressure of dry nitrogen. Dry methylene chloride was obtained by distillation from calcium hydride. Anhydrous tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Hexamethylphosphoramide (HMPA) and pyridine were dried over calcium hydride overnight and distilled under reduced pressure. Acetone was dried over anhydrous potassium carbonate overnight and distilled prior to use. Methanol and chloroform were used as supplied. NMR spectra were obtained on a GE QE-300 spectrometer, with chloroform-*d* as the solvent. IR data were obtained on a Perkin Elmer 1600 series FT-IR spectrometer. Radioactive samples were counted in an LKB 1218 RackBeta liquid scintillation counter using ScintiVerse II (Fisher Scientific) scintillation cocktail.

(4*E*,8*E*,12*E*,16*E*)-4,8,13,17,21-pentamethyl-4,8,12,16,20-docosapentaen-1-yl acetate (3). Trisnorsqualene alcohol **2** (0.9491 g, 2.45 mmol)[4] was treated with acetyl anhydride (1.2 ml, 12.7 mmol, 5 equiv.) in 5 ml dry pyridine for 16 hr at room temperature. The reaction was then added to 100 ml diethyl ether followed by careful addition of sodium bicarbonate aqueous solution (10%, 50 ml). The organic layer was collected and washed with water (50 ml x 3), dried over $MgSO_4$, concentrated under vacuum, and flash chromatographed on silica gel with 2% ethyl acetate / hexane, producing 0.9893 g (94%) of pure acetate **3**. ^{1}H NMR ($CDCl_3$): δ 1.58 (15 H, br s), 1.65 (3 H, s), 2.01 (3 H, s), 1.90-2.12 (20 H, m), 4.01 (2 H, t, J = 6.7 Hz), 5.00-5.20 (5 H, m); ^{13}C NMR ($CDCl_3$): δ 13.99, 15.86, 17.52, 20.79, 22.56, 25.52, 25.55, 26.52, 26.69, 28.15, 31.50, 35.69, 39.64, 64.03, 124.17, 124.20, 124.32, 124.99, 130.95, 133.44, 134.68, 134.86, 170.81; FT-IR (neat): 2921, 1744, 1666, 1445, 1383, 1241 cm^{-1}.

(4*E*,8*E*,12*E*,16*E*)-20-Bromo-21-hydroxy-4,8,13,17,21-pentamethyl-4,8,12,16-docosatetraen-1-yl-acetate (4). Acetate **3** (0.9859 g, 2.30 mmol) was dissolved in 10 ml THF / H_2O (10/1, v/v) and treated with NBS (0.4298 g, 2.41 mmol, 1.05 equiv.) for 2 hr between 0 °C to room temperature. Diethyl ether (100 ml) and ammonium chloride aqueous solution (10%, 10 ml) were then added. The organic layer was washed with water (20 ml x 3), dried over $MgSO_4$, concentrated under vacuum, and column chromatographed on silica gel with ethyl acetate / hexane (gradient) to afford 0.4061 g (34%) desired bromohydrin **4** as a colorless oil (about 15% starting material was recovered). ^{1}H NMR ($CDCl_3$): δ 1.29 (3 H, s), 1.30 (3 H, s), 1.56 (12 H, br s), 1.60-1.80 (2 H, m), 2.00 (3 H, s), 1.90-2.15 (18 H, m), 3.93, (1 H, dd, J = 1.7, 11.3 Hz), 3.99 (2 H, t, J = 6.7 Hz), 5.02-5.15 (3 H, m), 5.17 (1 H, br t, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$): δ 13.99, 15.73, 15.90, 20.83, 22.54, 25.89, 26.39, 26.50, 26.71, 28.13, 31.46, 31.99, 35.65, 38.05, 39.53, 64.08, 70.44, 72.28, 124.26, 124.34, 124.96, 125.85, 132.85, 133.47, 134.69, 134.74, 170.97; FT-IR (neat): 3486, 2924, 1740, 1667, 1448, 1368, 1243, 1042 cm^{-1}.

(4*E*,8*E*,12*E*,16*E*)-20,21-Epoxy-4,8,13,17,21-pentamethyl-4,8,12,16-docosatetraen-1-ol (5). Bromohydrin **4** (0.3861 g, 0.73 mmol) was dissolved in methanol and added to anhydrous potassium carbonate (0.50 g, 3.6 mmol, 5 equiv.) and stirred 15 min at room temperature. Water (0.5 ml) was then added and stirring was continued for 1 hr. Addition of more water (50 ml), evaporation of methanol under vacuum, followed by extraction with diethyl ether (50 ml x 3) and concentration afforded the crude product. Flash column chromatography on silica gel with 20% ethyl acetate / hexane produced the desired epoxy alcohol **5** quantitatively (0.2886 g). ^{1}H NMR ($CDCl_3$): δ 1.26 (3 H, s), 1.30 (3 H, s), 1.58 (15 H, br s), 1.60 (3 H, s), 1.95-2.15 (20 H, m), 2.70 (1 H, t, J = 6.1 Hz), 3.63 (2 H, m), 5.07-5.30 (4 H, m); ^{13}C NMR ($CDCl_3$): δ 13.44, 15.84, 15.99, 16.12, 18.72, 24.87, 25.90, 26.55, 26.63, 27.47, 28.23, 30.73, 35.97, 36.29, 38.73, 39.65, 58.31, 62.77, 64.20, 119.20, 124.38, 124.78, 124.91, 133.94, 134.57, 134.93; FT-IR (neat): 3410, 2925, 1667, 1446, 1380, 1058 cm^{-1}.

(4*E*,8*E*,12*E*,16*E*)-20,21-Epoxy-4,8,13,17,21-pentamethyl-4,8,12,16-docosatetraen-1-al (6). A methylene chloride solution of trisnorepoxysqualene alcohol **5** (0.2855 g, 0.71 mmol, in 20 ml CH_2Cl_2) was added to sodium acetate (112 mg, 1.4 mmol, 2 equiv.) and PDC (0.5335 g, 1.4 mmol, 2 equiv.) at room temperature. The mixture was stirred 17 hr before addition of sodium bicarbonate aqueous solution (10%, 100 ml). Extraction with diethyl ether (100 ml x 3), drying over $MgSO_4$, concentration under vacuum, and flash column chromatography on silica gel with 8% ethyl acetate / hexane afforded 0.2015 g (71%) of the aldehyde **6**. ^{1}H NMR ($CDCl_3$): δ 1.24 (3 H, s), 1.28 (3 H, s), 1.55-1.65 (12 H, m), 1.9-2.1 (16 H, m), 2.23 (2 H, t, J = 7.4 Hz), 2.49 (2 H, dt, J = 1.7, 7.2 Hz), 2.68 (1 H, t, J = 6.2 Hz), 5.05-5.25 (4 H, m), 9.72, (1 H, t, J = 1.7 Hz); ^{13}C NMR ($CDCl_3$): δ 15.93, 18.67, 24.83, 26.47, 26.58, 27.40, 28.17, 31.78, 36.24, 39.45, 39.60, 42.08, 58.20, 64.09, 124.26, 124.43, 124.82, 125.33, 132.76, 133.91, 134.69, 134.88, 202.52; IR (neat): 2918, 2714, 1728, 1667, 1446, 1384, 1123, 872 cm^{-1}.

[24,30-^{14}C]-Labeled-(3*S*,3*R*)-2,3-epoxysqualene (7). Following several identical reactions with unlabeled reagents, *n*-BuLi (1.6 M in hexane, 125 µl, 200 µmol, 1.0 equiv.) was added to a THF (10 ml) solution of ethyltriphenylphosphonium bromide (74.2 mg, 200 µmol) under an atmosphere of argon. After stirring 1 hr at room temperature, 0.5 ml (10 µmol, 1.1 equiv. to iodomethane) of the deep orange ylid solution was added dropwise to 0.5 ml THF solution (pre-cooled to -78 °C) of [^{14}C]iodomethane (54 mCi/mmol, 500 µCi, 9.3 mmol) under Ar and stirred 1.5 hr at -78 °C. Next, *n*-BuLi (1.6 M in hexane, 12.5 µl, 20 µmol, 2.0 equiv. to the isopropyltriphenylphosphonium salt formed) was added dropwise to the resulting milk-white mixture at -78 °C to form the deep red isopropylidenephosphorane solution. After stirring 1 hr at -78 °C, (3*S*,3*R*)-2,3-epoxytrisnorsqualene aldehyde (**6**) (3.9 mg, 10 µmol, 1.1 equiv. to isopropyl ylid formed) in 0.5 ml THF was added dropwise at -78 °C and stirred 1 hr at -78 °C and 20 hr at -5 °C. The reaction mixture was then diluted with 5 ml of hexane / ethyl acetate (1/1, v/v) and purified on silica gel to give 2.5 mg (60%, 301 µCi, 51 mCi/mmol) of the desired [24,30-^{14}C]-(3*S*,3*R*)-2,3-epoxysqualene (**7**).

Spectroscopic data of (3*S*,3*R*)-2,3-epoxysqualene from reaction with radio-inert reagents: ^{1}H NMR ($CDCl_3$): δ 1.25 (3 H, s), 1.29 (3 H, s), 1.59 (12 H, br s), 1.61 (3 H, s), 1.67 (3 H, s), 1.9-2.2 (20 H, m), 2.70 (1 H, t, *J* = 6.2 Hz), 5.0-5.2 (5 H, m); ^{13}C NMR ($CDCl_3$): δ 15.96, 17.63, 18.70, 24.85, 25.66, 26.60, 26.71, 27.41, 28.22, 36.27, 39.70, 58.24, 64.13, 124.15, 124.21, 124.35, 124.88, 131.16, 133.07, 134.81, 134.86, 135.03; IR (neat): 2961, 2922, 2854, 1667, 1447, 1378, 1122, 843, 682 cm^{-1}. These data are identical with those of 2,3-epoxysqualene from direct epoxidation of squalene with *m*CPBA.

(4*E*,8*E*,12*E*,16*E*)-22-Hydroxy-4,8,13,17,21-pentamethyl-4,8,12,16,20-docosapentaen-1-al (**8**). A suspension of SeO_2 (0.4102 g, 3.70 mmol, 0.5 equiv.) and methylene chloride cooled to 0 °C was treated with *t*-BuOOH (90%, 1.48 g, 14.8 mmol, 2 equiv.) and stirred 30 min in the dark. Trisnorsqualene aldehyde **1** (2.7762 g, 7.22 mmol, dissolved in 10 ml CH_2Cl_2)[4] was added over 5 min and stirring was continued for 2 hr at 0 °C in the dark. The reaction mixture was then diluted with 200 ml diethyl ether and washed with aq. $NaHCO_3$ (10%, 100 ml x 3). The organic solution was dried over $MgSO_4$, concentrated under vacuum, and flash chromatographed on silica gel with ethyl acetate / hexane (gradient) to afford 0.5669 g (20%) of the desired terminal *E*-allylic alcohol **8**, besides other regio-isomers and unreacted starting material. ^{1}H NMR ($CDCl_3$): δ 1.60 (12 H, br s), 1.67 (3 H, s), 1.92-2.17 (16 H, m), 2.26-2.36 (2 H, m), 2.47-2.56 (2 H, m), 3.94-4.06 (2 H, m), 5.05-5.25 (4 H, m), 5.35-5.48 (1 H, m), 9.7 (1 H, t, *J* = 1.4 Hz); ^{13}C NMR ($CDCl_3$): δ 15.89, 17.55, 25.55, 26.13, 26.20, 26.43, 26.66, 27.80, 28.13, 31.75, 39.22, 39.40, 39.61, 41.96, 42.03, 68.76, 121.39, 123.86, 124.09, 124.19, 124.30, 124.43, 125.32, 125.86, 134.62, 136.73, 202.45; FT-IR (neat): 3408, 2925, 1726, 1675, 1625, 1446, 1382 cm^{-1}.

(20*S*,21*S*)-(4*E*,8*E*,12*E*,16*E*)-22-Hydroxy-20,21-epoxy-4,8,13,17,21-pentamethyl-4,8,12,16-docosatetraen-1-al (**9**). To a suspension of 3 Å molecular sieves (330 mg) and methylene chloride (5 ml) pre-cooled to -10 °C, L-(+)-diethyltartrate (0.34 ml, 2.0 mol, 2.4 equiv.), titanium (IV) isopropoxide (0.60 ml, 2.0 mmol, 2.4 equiv.) and *t*-BuOOH (1 M in CH_2Cl_2, dried over $MgSO_4$, 1.25 ml, 1.25 mmol, 1.5 equiv.) were added subsequently. The mixture was stirred 10 min at -10 °C and then cooled down to -25 °C. *(E)*-Allylic alcohol **9** (0.3327 g, 0.83 mmol, dissolved in 5 ml CH_2Cl_2) was added dropwise over 10 min, and stirring was continued for 16 hr at this temperature. Citric acid (10%, 10 ml) was added while warming up to room temperature. After stirring 30 min at room temperature, the mixture was extracted with diethyl ether (50 ml x 3), dried over $MgSO_4$, and concentrated under vacuum. Flash column chromatography on silica gel with 20% ethyl acetate / hexane produced 0.2568 g (74%) of the desired epoxy alcohol **9**. NMR analysis of the respective MTPA ester indicated that the epoxy alcohol was >95% e.e. ^{1}H NMR ($CDCl_3$): δ 1.23 (3 H, s), 1.49 (6 H, br s), 1.51 (6 H, br s), 1.84-2.50 (16 H, m), 2.20 (2 H, br t, *J* = 7.5 Hz), 2.40 (2 H, br t, *J* = 7.5 Hz), 2.91 (1 H, t, *J* = 6.2 Hz), 3.42 (1 H, dd, *J* = 6.9, 12.2 Hz), 3.56 (1 H, dd, *J* = 4.2, 12.2 Hz), 4.95-5.00 (4 H, m), 9.6 (1 H, t, *J* = 1.6 Hz); ^{13}C NMR ($CDCl_3$): δ 15.69, 26.31, 26.44, 26.52, 26.66, 27. 96, 31.60, 35.94, 39.24, 39.36, 39.42, 41.86, 59.99, 60.96, 65.60, 124.14, 124.28, 124.76, 125.16, 132.65, 133.59, 134.43, 134.63, 202.13;

FT-IR (neat): 3441, 2926, 1744, 1553, 1450, 1387 cm^{-1}. ^1H NMR of MTPA ester: ($CDCl_3$) δ 1.26 (3 H, s), 1.60 (9 H, br s), 1.67 (3 H, s), 1.93-2.20 (16 H, m), 2.31 (2 H, br t, J = 7.5 Hz), 2.51 (2 H, br t, J = 7.5 Hz), 2.85 (1 H, t, J = 6.1 Hz), 3.56 (3 H, s), 4.13 (1 H, d, J = 11.6 Hz), 4.40 (1 H, d, J = 11.6 Hz), 5.05-5.20 (4 H, m), 9.75 (1 H, t, J = 1.8 Hz).

(20*S*,21*S*)-(4*E*,8*E*,12*E*,16*E*)-22-Iodo-20,21-epoxy-4,8,13,17,21-pentamethyl-4,8,12,16-docosatetraen-1-al (**11**). Epoxy alcohol **9** (41.1 mg, 0.099 mmol) was dissolved in chloroform (5 ml) and cooled to 0 °C. Pyridine (17 μl, 0.2 mmol, 2 equiv.) and *p*-toluenesulfonyl chloride (28 mg, 0.15 mmol, 1.5 equiv.) were then added sequentially. The reaction was stirred 5 hr at 0 °C before dilution with diethyl ether (50 ml). The solution was washed with water (20 ml x 3), dried over $MgSO_4$, concentrated under vacuum, and flash chromatographed on silica gel to yield 41.0 mg (73%) of epoxy tosylate **10** as a colorless oil. Part of the tosylate **10** (33.4 mg, 0.058 mmol) was dissolved in 2 ml anhydrous acetone and treated with dry sodium iodide (290 mg, 1.9 mmol, 30 equiv.) for 20 hr at room temperature. Diethyl ether (20 ml) was added and the solution was washed with water (5 ml x 3), dried over $MgSO_4$, concentrated under vacuum, and chromatographed on silica gel with 8% ethyl acetate / hexane, affording 30 mg (97%) of the desired epoxy iodide **11**. ^1H NMR ($CDCl_3$): δ 1.25 (3 H, s), 1.59 (6 H, br s), 1.61 (6 H, br s), 1.93-2.21 (16 H, m), 2.31 (2 H, br t, J = 7.2 Hz), 2.51 (2 H, dt, J = 1.7, 7.2 Hz), 2.86 (1 H, t, J = 6.2 Hz), 3.08 (1 H, d, J = 9.8 Hz), 3.22 (1 H, d, J = 9.8 Hz), 5.05-5.30 (4 H, m), 9.75 (1 H, t, J = 1.7 Hz); ^{13}C NMR ($CDCl_3$): δ 13.97, 14.03, 15.95, 16.01, 26.52, 26.61, 27.57, 28.22, 28.94, 29.66, 31.83, 36.06, 39.50, 39.60, 39.65, 42.13, 59.98, 66.18, 124.35, 124.47, 125.27, 125.39, 132.83, 133.50, 134.78, 134.92, 202.54; FT-IR (neat): 2920, 2851, 2715, 1726, 1667, 1446, 1385 cm^{-1}.

(20*S*)-(4*E*,8*E*,12*E*,16*E*)-20,21-Epoxy-4,8,13,17,21-pentamethyl-4,8,12,16-docosatetraen-1-ol (**12**). Epoxy iodide **11** (28.8 mg, 0.055 mmol) was dissolved in 1.5 ml dry HMPA and added with sodium cyanoborohydride ($NaBH_3CN$, 34.4 mg, 0.55 mmol, 10 equiv.) at room temperature. The reaction was stirred 40 hr and quenched with aq. NaH_2PO_4 (0.1 M, 10 ml). The mixture was then extracted with 50% ethyl acetate / hexane (20 ml x 3) and the combined organic solution was dried over $MgSO_4$, concentrated under vacuum, and flash chromatographed on silica gel with 20% ethyl acetate / hexane, producing 14.6 mg (66%) alcohol **12**, which was identical both chromatographically and spectroscopically to the racemic compound **5**.

(20*S*)-(4*E*,8*E*,12*E*,16*E*)-20,21-Epoxy-4,8,13,17,21-pentamethyl-4,8,12,16-docosatetraen-1-al (**13**). Epoxytrisnorsqualene alcohol **12** (5.1 mg, 0.013 mmol) was treated with sodium acetate (2 mg, 0.024 mmol, 1.8 equiv.) and PDC (10 mg, 0.027 mmol, 2 equiv.) in methylene chloride (1 ml) at room temperature. After stirring 4.5 hr, sodium bicarbonate aqueous solution (10%, 2 ml) was added and the mixture was extracted with diethyl ether (10 ml x 3). The combined organic solution was dried over $MgSO_4$, concentrated under vacuum, and chromatographed on silica gel with 8% ethyl acetate /

hexane to yield 4.1 mg (81%) of the epoxy aldehyde **13**. Chromatographic and spectroscopic data of **13** were identical to those of the racemic compound **6**.

[24,30-^{14}C]-Labeled-(3*S*)-2,3-epoxysqualene (14). Following exactly the same procedure as for the synthesis of the racemic squalene epoxide **7**, (3*S*)-2,3-epoxytris-norsqualene aldehyde (**13**) (3.9 mg, 0.01 mmol) was converted to 2.6 mg (61%, 314 μCi, 52 mCi/mmol) [24,30-^{14}C]-labeled-(3*S*)-2,3-epoxysqualene (**14**), which was chromatographically identical to the racemic compound **7** by radio-TLC analysis.

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