Asymmetric Synthesis of a Mechanism-Based Inhibitor of Oxidosqualene Cyclase

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Oxidosqualene cyclase (E.C. 5.4.99.7) catalyzes the conversion of (3*S*)-2,3-oxidosqualene to lanosterol in vertebrates.[1a] A variety of inhibitors have been developed to study the active site of this remarkable enzyme,[1b] including cyclizable substrate analogs such as dioxidosqualenes,[2] 10,15-didesmethyl-2,3-oxidosqualene,[3] and 29-methylidene-2,3-oxidosqualene (29-MOS).[4] Each of the epoxide-containing inhibitors tested to date has been synthesized as a racemic mixture, even though only the (3*S*) enantiomer is recognized and processed by the cyclase. In order to facilitate structural characterization of the inhibitor–enzyme adduct, we have developed an asymmetric synthesis of the mechanism-based oxidosqualene cyclase inhibitor (3*S*)-29-MOS (**13**). This synthesis, which employs the Sharpless asymmetric dihydroxylation[5] as the key step, provides access to a high specific activity, enantiomerically-enriched substrate analog for biochemical studies.

Epoxidation of squalene with magnesium monoperoxyphthalic acid in the presence of Adogen 646 (methyltrialkyl(C_8–C_{10})ammonium chloride) in THF: water (3:4 v/v) provided a mixture of internal epoxides **1a** and **1b** in 25% yield (Scheme 1).[4,6] Oxidative cleavage with periodic acid gave the corresponding C-14 and C-18 aldehydes **2a** and **2b** in 86% yield after silica chromatography. Reduction of this mixture of aldehydes with sodium borohydride afforded a mixture of alcohols (71% yield), which were then separated with reverse phase column chromatography on C_{18}/Porasil B (CH_3CN/H_2O). The C-18 alcohol **3**, obtained in 43% yield, was then acetylated (Ac_2O, Et_3N, and DMAP) to give acetate **4** in 85% yield.

Asymmetric dihydroxylation (AD) of **4** was accomplished using AD-mix-β.[5] This polyene was not an ideal substrate for the dihydroxylation reaction. Considerable effort was required to optimize the reaction. Thus, selectivity for **6a** was attained by varying the reaction time and the amount of osmium tetraoxide while keeping the ratio of OsO_4 and ligand fixed at 1:5. The results are summarized in Table 1. The most practical concentration was determined to be 1.0% OsO_4 with a reaction time of 7 h. Under these conditions, the desired terminal (3*R*)-diol **6a** was obtained in 26% yield, the internal diols (**5a, 5b,** and **6b**) in 4.2%, 4.3%, and 13% yields, respectively, and the polyols **7** in 29% yield. The products were isolated by column chromatography; the 6,7- and 10,11-diols **5a** and **5b** eluted together and the desired 2,3-diol **6a** eluted with the 14,15-diol **6b**. The diol **6a** was produced in 94.6% ee as shown by GC of the corresponding (−)-α-MTPA ester.

Table 1. Results of Varying OsO_4 Concentration

% OsO_4	reactn time (h)	% A[a,d]	% B[b,d]	% C[c,d]
0.2	16	12	18	28
0.5	7	10	18	16
0.5	12	11	20	37
1.0	7	9	39	29

[a] Fraction A is a mixture of **5a** and **5b**. [b] Fraction B is a mixture of **6a** and **6b**. [c] Fraction C is a mixture of **7a** and **7b**. [d] Isolated yield.

Isomers **6a** and **6b** could not be separated by silica gel column chromatography and were converted to a mixture of secondary mesylates with MsCl in the presence of Et_3N and DMAP.[7] The desired 2-hydroxy-3-mesyl acetate **8** was obtained in 86% yield. Cyclization with inversion of the 3*R* center to the (3*S*) epoxide and concomitant deprotection of the acetate of **8** in the presence of K_2CO_3 in methanol yielded the corresponding (3*S*)-2,3-epoxy alcohol. Oxidation of the alcohol with PDC in the presence of sodium acetate[8] yielded the 2,3-epoxy aldehyde **9** in 82% yield.

The bis(trifluoroethyl)methoxycarbonylphosphonate ylide was allowed to react with aldehyde **9** in the presence of 18-crown-6 ether in THF[9] to give the epoxy methyl ester **10** in 92% isolated yield. The *Z* isomer was then separated by silica gel chromatography and reduced with lithium aluminum hydride[10] to provide the epoxy allylic alcohol **11** in 78% yield. Oxidation of **11** under optimum conditions[11] (MnO_2, Na_2CO_3, and hexane) provided the corresponding enal **12** in 69% yield. Reduction of **12** with sodium borotritide (specific activity = 15 Ci/mmol) yielded **[^{3}H]11** (5.31 mCi, 2.35 Ci/mmol) in 80% yield. Oxidation of **[^{3}H]19** (MnO_2, Na_2CO_3, hexane) provided **[^{3}H]12** in 69% yield (specific activity = 1.8 Ci/mmol). Olefination of **[^{3}H]12** afforded **[^{3}H]13** in quantitative yield (specific activity = 1.8 Ci/mmol).

The increased potency of **[^{3}H]13** as an affinity label for both rat and pig liver oxidosqualene cyclase was tested. The labeling experiment[12] was performed by incubating the purified cyclase[12a] with **[^{3}H]13** (4.6 μM) at pH 7.4 for 15 min at 37 °C. The protein solution was then analyzed by SDS-polyacrylamide gel electrophoresis, and the extent of covalent labeling by the inhibitor was determined in the dried, enhanced gel by fluorescence autoradiography. Using 10 000 dpm of **[^{3}H]13** gave the same intensity of covalent modification of enzyme achieved with 20 000 dpm of racemic [^{3}H]29-MOS.

(1) (a) For a current review of the enzymology and chemistry of squalene and oxidosqualene cyclases, see: Abe, I.; Rohmer, M.; Prestwich, G. D. *Chem. Rev.* **1993**, *93*, 2189–2206. (b) For a review of the medicinal chemistry of inhibitors of squalene synthesis and metabolism, see: Abe, I.; Tomesch, J. C.; Wattanasin, S.; Prestwich, G. D. *Natural Prod. Rept.*, **1994**, 279–302.

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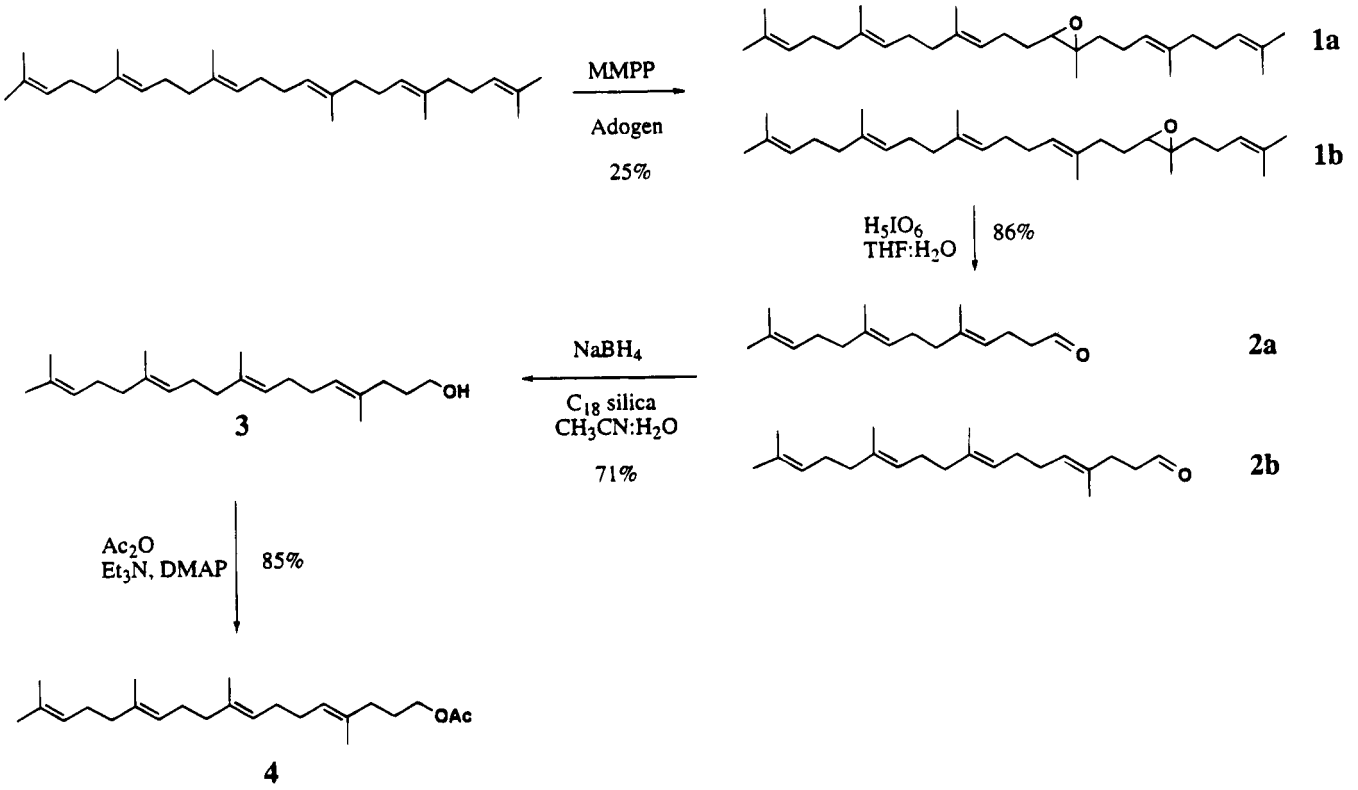
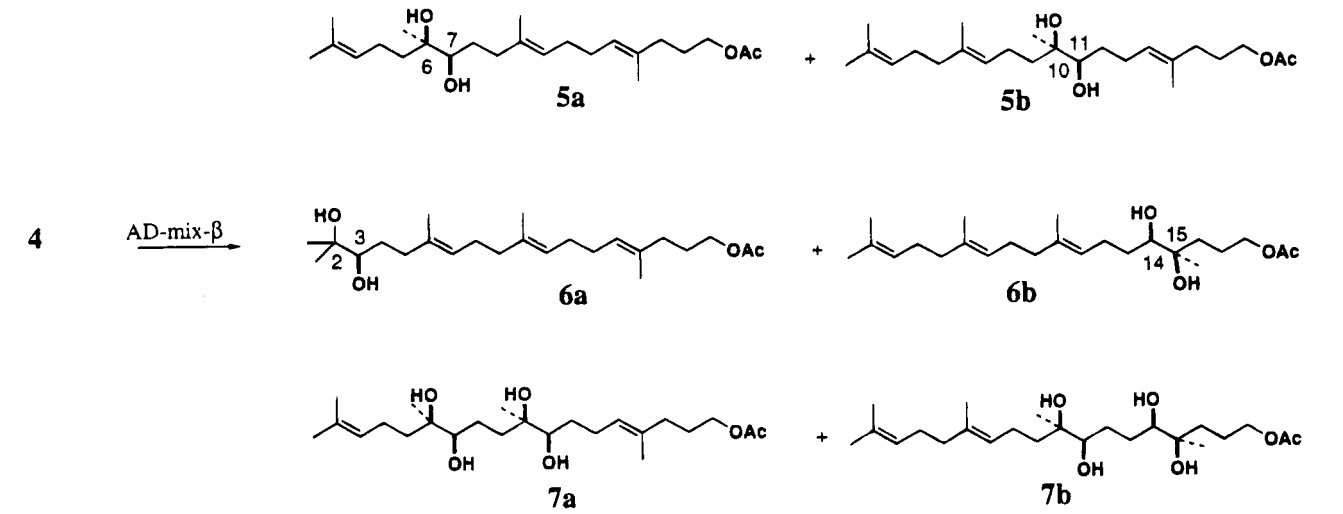
0022-3263/94/1959-5488$04.50/0

Scheme 1



Scheme 2



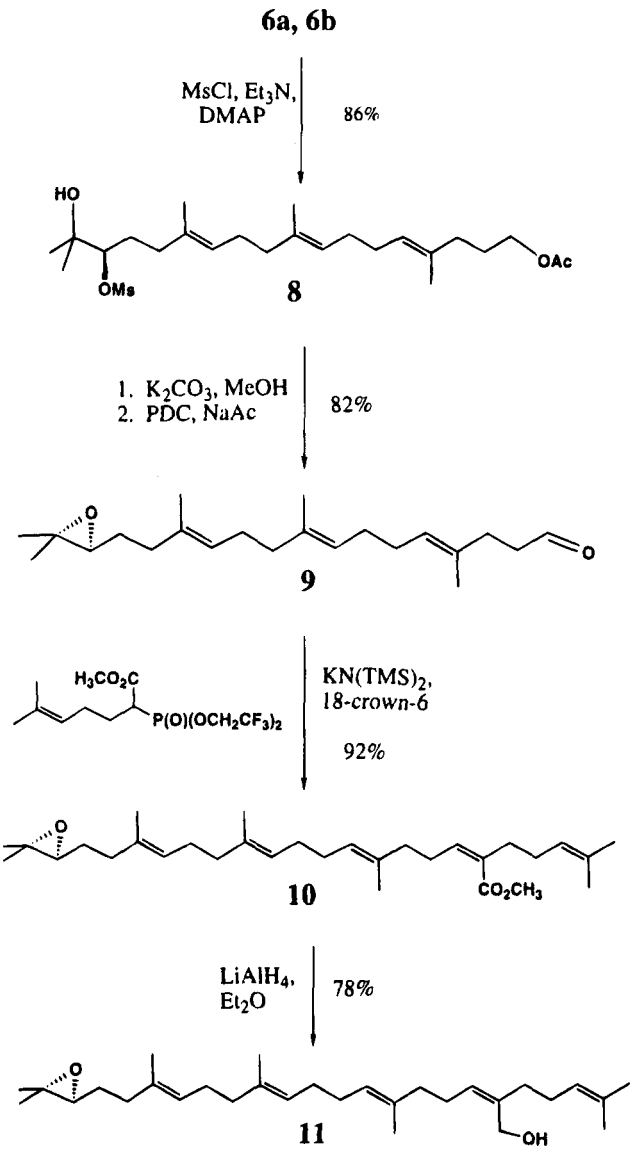
The availability of the (3*S*) enantiomer **13** of 29-MOS via direct asymmetric synthesis allows continuation of several studies. First, the nature of the cyclized moiety attached to the two Asp residues in the active-site DDTAEA motif[12b] can be determined more effectively with nonracemic material. Second, the chiral suicide substrate can be employed with other nonvertebrate cyclases to probe active-site geometry with greater sensitivity. Finally, to our knowledge, this is the first tritium-labeled enzyme substrate prepared using the AD reaction.

Experimental Section

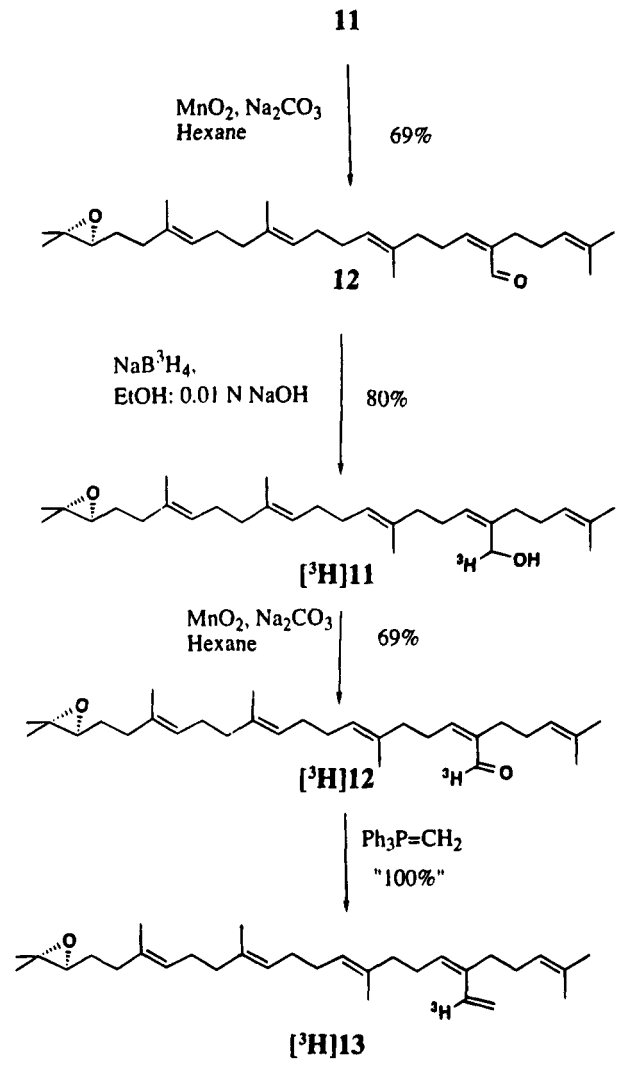
All chemicals and reagents were purchased from Aldrich Chemical Co. with the exception of [^{3}H]$NaBH_4$, which was obtained from New England Nuclear Co. All air-sensitive reactions were performed in an atmosphere of nitrogen or argon. Tetrahydrofuran was freshly distilled from sodium benzophenone ketyl. CH_2Cl_2, Et_3N, and MsCl were freshly distilled from CaH_2. After the first listing of NMR data below, only diagnostic resonances are reported.

(2*E*,6*E*,14*E*,18*E*)-10,11-Epoxy-2,6,10,15,19,23-hexamethyl-2,6,14,18,22-tetracosapentaene and (2*E*,10*E*,14*E*,18*E*)-6,7-Epoxy-2,6,10,15,19,23-hexamethyl-2,10,14,18,22-tetracosapentaene (1a and 1b). In a 1000-mL flask, 20 g of squalene (48.7 mmol), 1.3 g of Adogen (3.2 mmol, 0.07 equiv), and 28.4 g of MMPP (58.4 mmol, 1.2 equiv) in 180 mL of THF and 240 mL of water were stirred vigorously for 2 days between 0 °C and room temperature. The reaction was quenched with 300 mL of 10% $NaHCO_3$. The reaction mixture was then extracted with ether (3 × 300 mL), dried with $MgSO_4$, and concentrated to give 21.4 g of crude product. The epoxides were chromatographed on silica gel by elution with ethyl acetate–hexane (EA/H) mixtures yielding 3.5 g (25%) of the internal epoxides **1a** and **1b** as a colorless oil:[6] FT-IR 2979 cm^{-1}, 1669, 1448, 1352, 1108; ^{1}H NMR δ 1.23, 1.24 (s,s, 3 H together), 1.56–1.68 (br s, 21 H), 1.93–2.15 (m 20 H), 2.69–2.71 (t,t 1:1, 1 H together, J = 6.4 and 6.5 Hz), 5.02–5.20 (m, 5 H); ^{13}C NMR δ 15.98, 16.50, 16.59, 17.65, 23.75, 23.87, 24.87, 25.66, 26.57, 26.65, 26.75, 27.30, 28.17,

Scheme 3



Scheme 4



28.26, 28.99, 36.30, 38.88, 39.70, 60.71, 63.29, 123.31, 123.59, 124.11, 124.24, 124.38, 124.88, 131.13, 131.41, 134.25, 135.341, 134.95, 134.74.

(6*E*,8*E*)-5,9,13-Trimethyl-4,8,12-tetradecatrien-1-al and (4*E*,8*E*,12*E*)-4,9,13,17-Tetramethyl-4,8,12,16-octadecatetraen-1-al (2a and 2b). The internal epoxides **1a** and **1b** were oxidatively cleaved to the corresponding aldehydes. The epoxides (5.2 g) in a solution of 50 mL of THF and 16 mL of water were cooled to 0 °C. Periodic acid (2.08 g, 1.5 equiv) was then added and the mixture was stirred for 30 min at 0 °C and at room temperature for 9.5 h. The reaction was quenched with 10% $NaHCO_3$, 400 mL of water was added, the solution was extracted with ether (3 × 200 mL), the organics were dried ($MgSO_4$) and concentrated *in vacuo*, and the residue was chromatographed on silica yielding 4.03 g (86%) of the two aldehydes as colorless oil **2a** and **2b**: FT-IR 1718 cm^{-1}; ^1H NMR δ 1.57–1.72 (br s), 1.76–2.14 (m), 2.27–2.37 (m), 2.43–2.55 (m), 9.76–9.77 (t,t 1:1, 1 H, J = 1.81 Hz and 1.10 Hz).

(4*E*,8*E*,12*E*)-4,9,13,17-Tetramethyl-4,8,12,16-octadecatetraen-1-ol (3). The mixture of aldehydes **2a** and **2b** (1.9 g, 6.7 mmol) was dissolved in 35 mL of ethanol. Sodium borohydride (253 mg, 1.1 equiv) was added and the mixture was stirred at room temperature. After 1 h, the reaction was quenched with saturated NH_4Cl, diluted with water, and extracted with diethyl ether (3 × 100 mL). The combined organic extracts were then washed with brine, dried ($MgSO_4$), and concentrated. The crude mixture was then purified by flash chromatography yielding 1.4 g of alcohols. The alcohols were then separated by preparative reverse phase chromatography with C_{18}/Porasil B silica using 75–100% acetonitrile/water gradient. The more hydrophobic fractions yielded 0.6 g of the desired alcohol **3** as a colorless oil: FT-IR 3354 cm^{-1}, 1665 cm^{-1}; ^1H NMR δ 1.63 (s, 12 H), 1.68 (s, 3 H), 2.00 (br m, 14 H), 2.24 (t, J = 6 Hz, 2 H), 3.63 (t, J = 6 Hz and 2 Hz, 2 H), 5.14 (br m, 5 H); ^{13}C NMR δ 39.71, 62.74. Anal. Calcd for $C_{22}H_{38}O$: C, 82.95; H, 12.02. Found: C, 79.61; H, 11.23.

(4*E*,8*E*,12*E*)-4,9,13,17-Tetramethyl-4,8,12,16-octadecatetraenyl Acetate (4). The alcohol **3** (159 mg, 0.5 mmol) was dissolved in 5 mL of anhydrous methylene chloride. Acetic anhydride (47 mL, 0.5 mmol, 1 equiv), pyridine (80.8 mL, 1.0 mmol, 2 equiv) and DMAP (3 mg, 0.05 equiv) were added sequentially. The mixture was stirred at room temperature. After 20 min the reaction was diluted with ethyl ether, and the organics were washed (0.1 M HCl, water, $NaHCO_3$, and brine), dried ($MgSO_4$), concentrated, and chromatographed on silica gel to give 153 mg (85%) of protected alcohol **4**: FT-IR 1713 cm^{-1}; ^1H NMR δ 1.99–2.08 (m, 19 H), 4.02 (t, 2 H, J = 6.6 Hz); ^{13}C NMR δ 64.11, 170.92. Anal. Calcd for $C_{24}H_{40}O_2$: C, 79.94; H, 11.18. Found: C, 80.00; H, 11.14.

(4*E*,8*E*,12*E*)-16,17-Dihydroxy-4,9,13,17-tetramethyl-4,8,-12-octadecatrienyl Acetate (6a) and (4*E*,8*E*,12*E*)-4,5-Dihydroxy-9,13,17-tetramethyl-8,12,16-octadecatrienyl Acetate (6b). In a 25-mL flask, 5 mL of *tert*-butyl alcohol, 5 mL of water, 2.92 g of AD-mix-β, 1.0% OsO_4, 5.0% ligand, methanesulfonamide (190 mg, 1 equiv) and 390 mg (2.08 mmol) of acetate **4** were stirred at 0 °C for 7 h. Then 2.9 g of sodium metabisulfite was added to the mixture, which was stirred for 1 h, while warming to room temperature. Methylene chloride (10 mL) was added and the aqueous layer was then extracted (3 × 10 mL of ethyl acetate). The combined organic layers were washed (2 N NaOH), dried ($MgSO_4$), concentrated, and chromatographed on silica yielding 76 mg of the 2,3- and 14,15-diols **6a** and **6b** as a yellow oil. The reaction yield was 39% based on 28% recovery

of starting material. **6a + 6b**: FT-IR 3428 cm^{-1} (C–OH), 2954, 1745, 1717, 1239, 1037; ^1H NMR δ 1.10 (s), 1.15 (s), 1.20 (s), 3.3–3.4 (m, 1 H), 4.04 (m, 2 H), 4.12 (m, 3 H); ^{13}C NMR δ 39.64, 39.71, 64.25, 64.93, 73.97, 74.23, 76.84, 78.24, 170.91. Anal. Calcd for $C_{24}H_{42}O_4$: C, 73.05; H, 10.73. Found: C, 73.25; H, 10.68.

The (–)-α-MTPA ester was prepared from diol **6a** with (–)-α-MTPA chloride in CH_2Cl_2 and purified (SiO_2). ^1H NMR of (–)-α-MTPA ester of **6a**: δ 1.09 (s, 3 H), 1.13 (s, 3 H), 1.60–1.65 (m, 9 H), 1.81–2.08 (m, 17 H), 3.12 (m, 1 H), 4.04 (m, 2 H), 4.12 (t, 3 H, J = 6.6 Hz), 5.13–5.21 (br m, 3 H). The ee was determined by GC analysis of the MTPA ester: column HP-1, 20 m × 32 mm, 1.05 μm film thickness, injector 150 °C, detector 300 °C. Oven program: 275 °C (1 min), 5 °C/min to 290 °C (10 min). Retention times: 6.029 and 6.364 min.

(4*E*,8*E*,12*E*)-17-Hydroxyl-16-(mesyloxy)-4,9,13,17-tetramethyl-4,8,12-octadecatrienyl Acetate (8). The diol mixture of **6a** and **6b** (130 mg, 0.37 mmol) was dissolved in 20 mL of methylene chloride and cooled to –20 °C. DMAP (4.5 mg, 0.1 equiv), methanesulfonyl chloride (34 μl, 1.2 equiv), and triethylamine (77 μl, 1.5 equiv) were added, and the reaction was stirred at –20 °C for 1 h and at 0 °C for 4 h. The reaction was quenched with 10% aqueous $NaHCO_3$ and the reaction mixture extracted with ethyl ether. The combined extracts were dried ($MgSO_4$), concentrated, and chromatographed on silica with 5% ethyl acetate/methylene chloride to give 105 mg (86%) of the desired mesylate **8** as a colorless oil: FT-IR 3333 cm^{-1}, 2923, 1446, 1379, 1057, 837; ^1H NMR δ 1.23 (s, 3 H), 1.26 (s, 3 H), 2.12–2.22 (m, 2 H), 3.14 (s, 3 H), 4.03 (t, 2 H, J = 6.8 Hz), 4.55–4.59 (m, 1 H); ^{13}C NMR δ 62.81, 74.09, 78.24, 170.88. Anal. Calcd for $C_{25}H_{44}O_6S$: C, 63.52; H, 9.38. Found: C, 63.77; H, 9.40.

(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. The mesylate **8** (26 mg, 0.060 mmol) was then dissolved in methanol and cooled to –10 °C. Potassium carbonate (33 mg, 0.24 mmol, 4 equiv) was then added, and the mixture was stirred at 0 °C for 6 h and at room temperature for 10 h. The reaction was diluted with water and extracted with 1:1 EA/H. The extracts were combined, dried ($MgSO_4$), concentrated, and chromatographed on silica. The reaction yielded 15.7 mg (89%) of the deprotected epoxy alcohol as a colorless oil: FT-IR 3383 cm^{-1}, 2958, 1249, 1117; ^1H NMR δ 1.25 (s 3 H), 1.30 (s, 3 H), 2.70 (t, 1 H, J = 6.2 Hz), 3.62 (t, 2 H, J = 6.3 Hz); ^{13}C NMR δ 58.31, 62.81, 64.20. Anal. Calcd for $C_{22}H_{38}O_2$: C, 78.98; H, 11.45. Found: C, 78.69; H, 11.45.

(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 (9). The epoxy alcohol (15 mg, 0.045 mmol) was dissolved in 0.5 mL of methylene chloride with PDC (33.84 mg, 2 equiv) and sodium acetate (6.7 mg, 0.081 mmol) and stirred at room temperature for 4 h. The solution was concentrated, diluted with hexane, and loaded directly on to a silica column and eluted with 5% EA/H to yield 13 mg (95%) of the epoxy aldehyde **9**: FT-IR 1728 cm^{-1}, 1666, 1123[1]; ^1H NMR δ 2.31 (t, 2 H, J = 7.4 Hz), 2.49 (dt, 2 H, J = 1.7 and 7.2 Hz), 2.70 (t, 1 H, J = 6.2 Hz), 9.75 (t, 1 H, J = 1.7 Hz); ^{13}C NMR δ 42.08, 58.20, 64.09, 202.52. Anal. Calcd for $C_{22}H_{37}O_2$: C, 79.22; H, 11.18. Found: C, 79.40; H, 11.16.

(3*S*)-(6*E*,10*E*,14*E*,18*Z*,22*E*)-2,3-Epoxy-2,6,10,15,23-pentamethyl-19-(methoxycarbonyl)-6,10,14,18,22-docosapentaene (10). The bis(trifluoroethyl) phosphonate (13.3 mg, 0.033 mmol) and 18-crown-6 (8.7 mg, 0.033 mmol) were added to 5 mL of anhydrous THF cooled to –78 °C. Potassium bis(trimethylsilyl)amide (66 mL, 0.033 mmol, 1.0 equiv) was then added slowly. The reaction was stirred at –78 °C for 30 min. The aldehyde **9** (10 mg, 0.03 mmol, 0.9 equiv) was then added dropwise and the mixture was stirred for an additional 2 h. The reaction was then quenched with saturated NH_4Cl and the reaction mixture extracted with ethyl ether. The organic extracts were then combined, dried ($MgSO_4$), concentrated under vacuum, and chromatographed on silica yielding 12.5 mg (77%) of the desired ester **10**: FAB-MS M – H = 469, 1.9% intensity; FT-IR 1713 cm^{-1}; ^1H NMR δ 1.68 (s, 3 H), 2.22–2.27 (m, 2 H), 2.48–2.55 (m, 2 H), 3.73 (s, 3 H), 5.82–5.90 (m, 1 H); ^{13}C NMR δ 46.82, 51.02, 58.21, 64.18, 141.92, 168.50. Anal. Calcd for $C_{32}H_{50}O_3$: C, 79.10; H, 10.70. Found: C, 77.39; H, 10.72.

(3*S*)-(6*E*,10*E*,14*E*,18*Z*)-2,3-Epoxy-2,6,10,15,23-pentamethyl-19-(hydroxymethyl)-6,10,14,18,22-tetracosapentaene (11). The epoxy methyl ester **10** (6 mg, 0.012 mmol) was dissolved in anhydrous diethyl ether and lithium aluminum hydride (1.4 mg, 0.038 mmol, 3 equiv) and stirred at room temperature for 1 h. The reaction mixture was then cooled to 0 °C and the reaction carefully quenched with saturated NH_4Cl. The solution was then filtered, and the organic layer was dried ($MgSO_4$), concentrated, and chromatographed on silica yielding 4.1 mg (78%) of 26-hydroxy-2,3-epoxysqualene **11**: FT-IR 3333 cm^{-1}, 2923, 1446, 1379, 1057, 837; ^1H NMR δ 2.71 (t, 1 H J = 6.2 Hz), 4.11 (s, 2 H), 5.31 (t, J = 7.3 Hz,1 H); ^{13}C NMR δ 58.34, 60.31, 64.21, 138.32.

(3*S*)-(6*E*,10*E*,14*E*,18*Z*)-2,3-Epoxy-2,6,10,15,23-pentamethyl-19-formyl-6,10,14,18,22-tetracosapentaene (12). The 2,3-epoxy allylic alcohol **11** (10.8 mg, 0.024 mmol) was dissolved in 4 mL of hexane and cooled to 0 °C. Sodium carbonate (106 mg, 1.2 mmol, 50 equiv) and MnO_2 (41 mg, 0.48 mmol, 20 equiv) were then added sequentially. The reaction was stirred at 0 °C for 88 h and at room temperature for 6 h. The mixture was filtered and then chromatographed on deactivated silica (8% EA/H containing 1% triethylamine). The reaction yielded 7.5 mg of the desired aldehyde **12** (69% yield): FT-IR 3333 cm^{-1}, 2923, 1446, 1379, 1057, 837; ^1H NMR δ 2.70 (t, J = 6.2 Hz, 1 H), 4.11 (br d, J = 5.1 Hz, 2 H), 6.44 (br t, J = 7.3 Hz, 1 H) 10.08 (s, 1 H); ^{13}C NMR δ 58.29, 64.17, 139.90, 148.99, 149.14, 190.92.

[^{3}H]-(3*S*)-(6*E*,10*E*,14*E*,18*Z*)-2,3-Epoxy-2,6,10,15,23-pentamethyl-19-hydroxymethyl)-6,10,14,18,22-tetracosapentaene ([^{3}H]11). The 2,3-epoxy enal **12** (2.0 mg, 4.5 μmol) was dissolved in 1 mL of 9:1 ethanol:0.01 N NaOH and cooled to 0 °C. Approximately 15 mCi of [^{3}H]$NaBH_4$ (specific activity = 1.8 Ci/mmol) was then added, and the reaction was stirred with cooling for 40 min and at room temperature for 40 min. The reaction was then quenched with saturated NH_4Cl, and the reaction mixture was extracted with 1:1 EA/H, dried ($MgSO_4$), concentrated, and chromatographed. The reaction yielded 5.31 mCi (1 mg, specific activity = 2.35 Ci/mmol) of allylic alcohol. This product had an R_f value identical to that of the unlabeled allylic alcohol **11**. The product was identified by coelution on TLC with the unlabeled material.

[^{3}H]-(3*S*)-(6*E*,10*E*,14*E*,18*Z*)-2,3-Epoxy-2,6,10,15,23-pentamethyl-19-formyl-6,10,14,18,22-tetracosapentaene ([^{3}H]12). The tritiated allylic alcohol **11** (1 mg, 2.3 μmol) was then dissolved in 1 mL of hexane and cooled to 0 °C. Sodium carbonate (12 mg, 50 equiv) and MnO_2 (4 mg, 20 equiv) were then added, and the mixture was stirred at 0 °C for 88 h and at room temperature for 6 h. The mixture was then filtered, concentrated, and chromatographed to yield 2.29 mCi of the enal **[^{3}H]12** in 69% yield (0.6 mg). The product coeluted with the unlabeled material on TLC.

[^{3}H]-(3*S*)-(6*E*,10*E*,14*E*,18*Z*)-2,3-Epoxy-2,6,10,15,23-pentamethyl-19-vinyl-6,10,14,18,22-tetracosapentaene ([^{3}H]-13). The reaction was first performed with nonradiolabeled starting material. Olefination of tritiated **20** was accomplished following the same conditions. The enal **[^{3}H]12** (0.6 mg, 1.4 μmol) was dissolved in 1 mL of THF and cooled to –78 °C. The methyltriphenylphosphine ylide (105 μl, 0.17 M, 5 equiv) was then added. The reaction was stirred at –78 °C for 1 h and at room temperature for 1 h. The reaction was then quenched with 10% $NaHCO_3$, and the reaction mixture was extracted with 1:1 EA/H, dried ($MgSO_4$), concentrated, and chromatographed on silica to yield [^{3}H]-(3*S*)-29-methylidene-2,3-oxidosqualene **21** in quantitative yield (1.8 Ci/mmol). The product was identified by coelution on TLC with the unlabeled material. The spectral data are presented for the nonradiolabeled product from the trial run: FT-IR 1665 cm^{-1}, 1595, 1121; ^1H NMR δ 1.25 (s, 3 H), 1.30 (s, 3 H), 1.60 (br s, 12 H), 1.68 (br s, 3 H), 1.95–2.22 (m, 20 H), 2.70 (t, J = 6.3 Hz, 1 H), 5.05 (dd, J = 1.0 and 17.4 Hz, 1 H), 5.08–5.18 (m, 4 H), 5.37 (br t, J = 7.5 Hz, 1 H), 6.67 (dd, J = 10.4, 17.4 Hz, 1 H); ^{13}C NMR δ 58.29, 64.17, 139.90, 148.99, 149.14, 190.92.

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