Synthesis of 10(*S*)-Hydroxyeicosatetraenoic Acid: A Novel Cytochrome P-450 Metabolite of Arachidonic Acid

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10(*S*)-(−)-hydroxyeicosa-5(*Z*),8(*Z*),11(*Z*),14(*Z*)-tetraenoic acid methyl ester (**2**) was synthesized in eight steps starting from enantiomerically pure (*R*)-glyceraldehyde acetonide. The 10(*R*)-(+)-enantiomer was prepared using an identical method except that the starting material was (*S*)-glyceraldehyde acetonide. By use of these authentic standards, it was possible to confirm that arachidonic acid was converted to a mixture of 10(*S*)-(−)-hydroxyeicosa-5(*Z*),8(*Z*),11(*Z*),14(*Z*)-tetraenoic acid (**1**) and the 10(*R*)-(+)-enantiomer by phenobarbital-induced rat liver microsomes.

Introduction

Hydroxyeicosatetraenoic acids[2] (HETEs) are formed in numerous cell types by enzymatically-mediated hydroxylation of arachidonic acid. There are three distinct enzymes involved in HETE biosynthesis:lipoxygenase (LOX), prostaglandin H (PGH) synthase, and cytochrome P-450. LOX- and PGH-synthase-mediated arachidonic acid metabolism is normally highly stereoselective. For example, platelet 12-LOX produces 12(*S*)-HETE[3,4] and PGH synthase catalyzes the formation of 11(*R*)-HETE.[5,6] However, cytochrome P-450-mediated metabolism of arachidonic acid does not generally occur with such high stereoselectivity or regioselectivity. Thus, incubation of arachidonic acid with rat liver microsomes results in the formation of at least eight different HETEs.[7,8] Pretreatment of the rats with β-naphthoflavone results in the conversion of arachidonic acid to an additional three metabolites: 16-, 17-, and 18-HETEs.[9] During a mechanistic study of the P-450-dependent pathway of arachidonic acid metabolism using phenobarbital (PB)-induced rat liver microsomes, we observed three new HETEs as major products in the microsomal incubation mixture.[10] From gas chromatography/mass spectrometry (GC/MS) analysis, it appeared that the three HETEs had hydroxyl groups in each of the different bis-allylic positions (C-7, C-10, and C-13). There have been two previous reports on the formation of bis-allylic HETEs. Oliw *et al.*[11] identified 13-HETE as an arachidonic acid metabolite in rat liver microsomes, and Guerriero *et al.* identified 13-HETE (as its ethyl ester derivative) as a metabolite of red algae.[12] We report the synthesis of 10(*S*)-(−)-(5*Z*,8*Z*,11*Z*,14*Z*)-HETE (**1**) and its 10(*R*)-(+)-enantiomer (Scheme 1). The availability of these compounds has provided confirmation of the structural assignment of 10-HETEs from the microsomal incubation and allowed assignment of their absolute configuration.

Results and Discussion

Chiral HPLC analysis of 10-HETE isolated from an incubation of arachidonic acid with PB-induced rat liver microsomes suggested that it was almost a 50:50 mixture of two enantiomers.[10] In order to confirm this observation, it was decided to first synthesize 10(*S*)-HETE and then to use a similar method for preparation of the 10-(*R*)-enantiomer. The starting compound for the synthesis of 10(*S*)-HETE (**1**, Scheme 1) was (*R*)-glyceraldehyde acetonide (**4**), prepared by the lead tetraacetate cleavage[13] of (D)-mannitol acetonide (**3**) in 80% yield. A Wittig condensation of **4** with phosphonium salt **5**[14] using 1 equiv of lithium hexamethyldisilazide and tetrahydrofuran:toluene (1:1)[15] at −78 °C provided the acetonide diene **6** in 47% yield. Acid hydrolysis of **6** afforded the diol **7** in 83% yield. The primary hydroxyl group of **7** was selectively protected using *tert*-butyldimethylsilyl (TBDMS) chloride in the presence of 4-(dimethylamino)-pyridine to give **8**.[16] Protection of the secondary hydroxyl group was performed using *tert*-butyldiphenylsilyl (TBDPS) chloride to give **9** (in 81% yield). Selective deprotection of the bis-silyl ether **9** was performed using pyridinium *p*-toluenesulfonate (PPTS). This removed the TBDMS group and provided the TBDPS-protected secondary alcohol **10** in 76% yield.[16] Oxidation of **10** using pyridinium chlorochromate gave the aldehyde **11** (73%). The phosphonium salt[17] **12** for the Wittig reaction was

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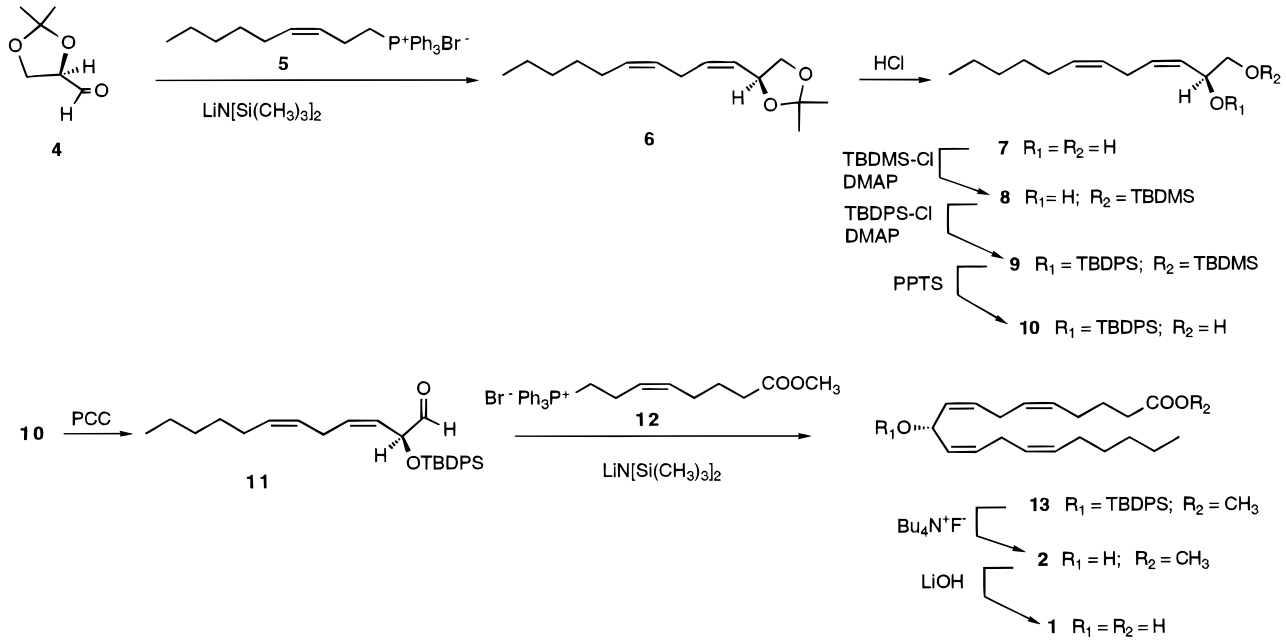
Scheme 1



prepared from methyl 8-hydroxy-5-octenoate.[18] The Wittig condensation of **11** with **12** gave the TBDPS-protected 10(*S*)-HETE methyl ester **13** in 49% yield. The TBDPS group was removed with aqueous tetrabutylammonium fluoride to give 10(*S*)-(−)-HETE as its methyl ester derivative **2** ($[\alpha]^{20}_D$ −11.6; *c* 0.1, EtOH). The hydrolysis of methyl ester **2** using lithium hydroxide gave 10(*S*)-(−)-HETE (**1**, 43%) as an unstable oil that had to be used immediately.

The synthesis of 10(*R*)-(+)-HETE was completed using the same reaction sequence (Scheme 1) except that (*S*)-glyceraldehyde acetonide was used as the starting material. Analyses of 10(*S*)-HETE and 10(*R*)-HETE methyl esters using chiral phase HPLC revealed a single enantiomer (>98% enantiomeric excess) in each case.[19] By comparison of chiral HPLC and GC/MS data, material isolated from the microsomal incubations was shown to be a racemic mixture of 10(*S*)-(−)-HETE and 10(*R*)-(+)-HETE.[10] It was found that the 10-HETEs were extremely unstable under acidic conditions, and this may explain why they have not been observed previously. The 10(*S*)- and 10(*R*)-HETEs prepared in the present study have been used to examine the retention of stereochemistry during acid-catalyzed rearrangement and to examine the enantioselective metabolism of 10-HETE by 15-LOX.[10]

HETEs have been shown to exhibit a range of potent biological activities. 12(R)-HETE is a potent vasoconstrictor in the cornea[20] and 12(*S*)-HETE induces microvascular endothelial cell retraction,[21] whereas 20-HETE inhibits ion transport in the kidney[22] and is a vasoconstrictor.[23] The availability of authentic 10-HETE enantiomers from the present study will also allow their biological activity to be assessed. Future studies will be performed to determine whether 10-HETE is formed *in vivo* and whether it is glucuronidated.[24] Finally, the availability of 10-HETE enantiomers will make it possible to assess their ability to form cyclooxygenase metabolites by reaction with PGH synthase.[25] Such prostaglandin-like metabolites could conceivably possess potent biological activities.

Experimental Section

General Methods. Unless otherwise stated, solvents and reagents were purchased and used without further purification. Tetrahydrofuran was distilled from sodium benzophenone prior to use. Nuclear magnetic resonance spectra were recorded using a 200, 300, or 400 MHz spectrometer. Thin layer chromatography (TLC) was performed using 2.5 × 10 cm, 250 μm analytical plates coated with silica gel G. Column chromatography was performed under air pressure on TLC grade 60 Å silica gel. The solvent mixtures indicated for TLC are volume/volume mixtures.

(*Z,Z*)-4-Deca-1,4-dien-1-yl-2,2-dimethyl-1,3-dioxolane (6). A portion of 14 mM solution of lithium bis(trimethylsilyl)amide in THF was added dropwise over a period of 30 min to a magnetically stirred suspension of phosphonium salt **5** (6.25 g, 13.4 mmol) in THF (10 mL) and toluene (10 mL) at 0 °C. The reaction mixture was stirred for an additional 30 min at 0 °C. The mixture was cooled to −78 °C, and **4** (2.10 g, 16.2 mmol) in THF (5 mL) was added dropwise. Stirring was continued at 0 °C for 2 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl (10 mL) and extracted with ethyl acetate (100 mL). The organic layer was washed with water (2 × 25 mL), dried ($MgSO_4$), and concentrated to give a crude oil. This was purified by flash chromatography using 5% ethyl acetate in hexane to yield **6** as a colorless oil

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(1.8 g, 47%): TLC R_f (5% ethyl acetate/hexane) = 0.69; ^{1}H NMR ($CDCl_3$) δ 5.25−5.60 (m, 4 H), 4.84 (q, J = 8.1 Hz, 1 H), 4.04 (dd, J = 6.0 and 7.9 Hz, 1 H), 3.50 (t, J = 8.1 Hz, 1 H), 2.83 (t, J = 7.2 Hz, 2 H), 2.01 (q, J = 6.9 Hz, 2 H), 1.37 (s, 3 H), 1.33 (s, 3 H) 1.20−1.30 (m, 6 H), 0.86 (t, J = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 133.2, 131.1, 127.3, 126.7, 71.9, 69.4, 31.4, 29.2, 27.2, 26.7, 26.1, 25.9, 22.5, 14.0; MS (*m/z*, electrospray) 239 (19, M + 1), 221 (54), 199 (51), 181 (57), 163 (100), 120 (19), 110 (21).

(2*S*)-(*Z*,*Z*)-3,6-Dodecadiene-1,2-diol (7). Hydrochloric acid (concd, 1.7 mL) was added dropwise to the solution of diene **6** (1.3 g, 5.4 mmol) in methanol (30 mL) at 0 °C. Stirring was continued at 0 °C for 2 h. The reaction mixture was neutralized to pH = 7 with concentrated aqueous NH_4OH. Methanol was removed under vacuum. Water (10 mL) was added to the residue, which was then extracted with ethyl acetate (2 × 25 mL), dried ($MgSO_4$), and evaporated. The residue was purified by silica gel flash chromatography using hexane:acetone (9:1) to give **7** as a colorless oil (0.9 g, 83%): TLC R_f (30% ethyl acetate/hexane) = 0.42; ^{1}H NMR ($CDCl_3$) δ 5.20−5.70 (m, 4 H), 4.50−4.65 (m, 1 H), 3.45−3.60 (m, 2 H), 2.70−2.90 (m, 2 H), 1.90−2.10 (m, 2 H), 1.20−1.45 (m, 6 H), 0.85−0.95 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 132.7, 131.2, 128.1, 126.2, 68.6, 66.3, 31.5, 29.2, 27.2, 26.2, 22.5, 14.0.

(2*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]-(Z,Z)-3,6-dodecadien-2-ol (8). 4-(Dimethylamino)pyridine (347 mg, 2.83 mmol) and *tert*-butyldimethylsilyl chloride (426 mg, 2.83 mmol) were added to the solution of diol **7** (560 mg, 2.83 mmol) in methylene chloride (15 mL). The reaction mixture was stirred at room temperature for 5 h. Methylene chloride (50 mL) was added and the mixture washed with saturated aqueous NaCl. The organic extract was dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography with 10% ethyl acetate in hexane as eluent to give **8** as an oil (660 mg, 75%): TLC R_f (30% ethyl acetate/hexane) = 0.65; ^{1}H NMR ($CDCl_3$) δ 5.25−5.60 (m, 4 H), 4.45−4.55 (m, 1 H), 3.50−3.65 (m, 1 H), 3.40−3.50 (m, 1 H), 2.70−2.90 (m, 2 H), 2.20 (bs, 1H), 1.90−2.20 (m, 2 H), 1.22−1.45 (m, 6 H), 0.85-0.95 (m, 12 H), 0.10 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 132.4, 130.9, 129.1, 127.0, 68.4, 66.6, 32.5, 31.5, 29.3, 27.2, 26.3, 25.9, 22.6, 18.3, 14.0; HRMS calcd for $C_{14}H_{27}O_2Si$ (M − 57) 255.1780, found 255.1773.

(2*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]-2-[(*tert*-butyldiphenylsilyl)oxy]-(*Z*,*Z*)-3,6-dodecadiene (9). A solution of diene **8** (295 mg, 0.945 mmol) and 4-(dimethylamino)pyridine (231 mg, 1.89 mmol) in methylene chloride (5 mL) was stirred with *tert*-butyldiphenylsilyl chloride (520 mg, 1.89 mmol) at room temperature for 15 h. The reaction mixture was partitioned between methylene chloride (50 mL) and saturated aqueous NaCl. The combined organic exracts were dried ($MgSO_4$) and concentrated. The residual oil was purified by flash chromatography using 2% ethyl acetate in hexane to yield **9** as a oil (423 mg, 81%). Further purification was carried out by HPLC (silica column, 10 mm × 25 cm, 5 μm) using hexane/2-propanol/acetic acid (99.05:0.9:0.05 v/v/v) as the mobile phase at a wavelength 235 nm with a flow rate 3 mL/min. The retention time for the compound **9** was 13.86 min: TLC R_f (10% ethyl acetate/hexane) = 0.74; ^{1}H NMR ($CDCl_3$) δ 7.62−7.83 (m, 4 H), 7.25−7.48 (m, 6 H), 4.95−5.48 (m, 4 H), 4.36−4.64 (m, 1 H), 3.55−3.72 (m, 1 H), 3.38−3.54 (m, 1 H), 2.30−2.52 (m, 2H), 1.75−2.00 (m, 2 H), 1.15−1.38 (m, 6 H), 1.04 (s, 9 H), 0.80−0.95 (m, 12 H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 136.0, 135.9, 134.3, 130.8, 130.3, 130.1, 129.8, 129.6, 129.4, 127.8, 127.6, 127.4, 127.3, 70.5, 67.7, 31.5, 29.3, 29.2, 29.1, 27.1, 27.0, 26.9, 26.8, 26.1, 25.9, 22.5, 19.3, 18.4, 14.1; HRMS calcd for $C_{30}H_{45}O_2Si_2$ (M − 57) 493.2958, found 493.2944.

(2*S*)-2-[(*tert*-Butyldiphenylsilyl)oxy]-(*Z*,*Z*)-3,6-dodecadien-1-ol (10). Pyridinium *p*-toluenesulfonate (97 mg, 0.38 mmol) was added to the solution of **9** (423 mg, 0.77 mmol) in ethanol (10 mL). The mixture was stirred at 60 °C for 15 h. The reaction mixture was cooled to room temperature and then partitioned between methylene chloride (50 mL) and saturated aqueous NaCl. The combined organic extract was dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography using 5% ethyl acetate in hexane to give **10** as an oil (254 mg, 76%). Further purification was carried out by HPLC (Silica column, 10 mm × 25 cm, 5 μm) using hexane/2-propanol/acetic acid (99.05:0.9:0.05 v/v/v) as the mobile phase at a wavelength 235 nm with a flow rate 3 mL/min. The retention time for the compound **10** was 17.04 min: TLC R_f (10% ethyl acetate/hexane) = 0.36; ^{1}H NMR ($CDCl_3$) δ 7.62−7.78 (m, 4 H), 7.29−7.55 (m, 6 H), 4.91−5.55 (m, 4 H), 4.45−4.65 (m, 1 H), 3.32−3.62 (m, 2 H), 2.15−2.55 (m, 2 H), 1.71−2.09 (m, 2 H), 1.15−1.40 (m, 6 H), 1.05 (s, 9 H), 0.86 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 135.9, 135.8, 133.8, 133.6, 131.1, 130.8, 130.7, 129.8, 129.7, 129.4, 127.7, 127.5, 126.8, 70.7, 67.0, 31.4, 29.2, 27.1, 27.0, 25.9, 22.5, 19.3, 14.0; HRMS calcd for $C_{24}H_{31}O_2Si$ (M − 57) 379.2093, found 379.2089.

(2*S*)-2-[(*tert*-Butyldiphenylsilyl)oxy]-(*Z*,*Z*)-3,6-dodecadien-1-al (11). Pyridinium chlorochromate (154 mg, 0.71 mmol), anhydrous sodium acetate (154 mg), and 4 Å molecular sieves (154 mg) were ground together and then suspended with stirring in methylene chloride (10 mL) at room temperature. Alcohol **10** (100 mg) in methylene chloride (1 mL) was added dropwise to the mixture, which was then stirred for 30 min. The reaction mixture was diluted with ether (10 mL), and the mixture was filtered through a Celite pad. The solvent was evaporated under vacuum, and the residual oil was purified by flash chromatography using 2% ethyl acetate in hexane to give **11** as an oil (75 mg, 73%): TLC R_f (10% ethyl acetate/hexane) = 0.47; ^{1}H NMR ($CDCl_3$) δ 9.50 (s, 1 H), 7.55−7.75 (m, 4 H), 7.32−7.50 (m, 6 H), 5.05−5.70 (m, 4 H), 4.75 (d, J = 7.0 Hz, 1 H), 2.40−2.55 (m, 2 H), 1.75−1.95 (m, 2 H), 1.15−1.40 (m, 6 H), 1.10 (s, 9 H), 0.85 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 199.2, 135.8, 134.3, 131.2, 130.0, 129.9, 127.8, 127.7, 126.1, 124.9, 76.1, 31.4, 29.2, 27.1, 26.9, 26.5, 22.5, 19.3, 14.1.

(10*S*)-Methyl 10-[(*tert*-butyldiphenylsilyl)oxy]eicosa-5(*Z*),8(*Z*),11(*Z*),14(*Z*)-tetraenoate (13). Lithium bis(trimethylsilyl)amide (580 μL, 0.58 mmol) was added dropwise to a suspension of phosphonium salt **12** (308 mg, 0.62 mmol) in dry THF (5 mL) at 0 °C over a period of 20 min. After the addition was complete, the reaction was stirred for 15 min at 0 °C and for 30 min at room temperature. The reaction mixture was cooled to −78 °C, aldehyde **11** (180 mg, 0.415 mmol) in THF (3 mL) was added dropwise, and stirring was continued at 0 °C for 3 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl (10 mL) and extracted with ethyl acetate (100 mL). The combined organic extract was washed with water (2 × 25 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography using 3% ethyl acetate in hexane to yield **13** as a colorless oil (116 mg, 49%). Further purification was carried out by HPLC (silica column, 10 mm × 25 cm, 5 μm) using hexane/2-propanol/acetic acid (99.05:0.9:0.05 v/v/v) as the mobile phase at a wavelength of 235 nm with a flow rate 3 mL/min. The retention time for the compound **13** was 15.14 min: TLC R_f (5% ethyl acetate/hexane) = 0.50; ^{1}H NMR ($CDCl_3$) δ 7.64−7.68 (m, 4 H), 7.30−7.42 (m, 6 H), 4.95−5.65 (m, 9 H), 3.62 (s, 3 H), 2.24−2.36 (m, 4 H), 2.20 (t, J = 7.4 Hz, 2H), 1.81−1.91 (m, 4 H), 1.57−1.65 (m, 2 H), 1.21−1.30 (m, 6 H), 1.01 (s, 9 H), 0.85 (t, J = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 174.3, 135.9, 134.1, 132.1, 131.8, 131.3, 130.6, 129.4, 129.0, 128.9, 128.4, 127.9, 127.4, 127.1, 65.9, 51.3, 33.4, 32.4, 31.4, 29.2, 29.1, 27.1, 26.9, 26.8, 26.4, 25.9, 25.8, 24.7, 22.5, 19.2, 14.0; HRMS calcd for $C_{33}H_{43}O_3Si$ (M − 57) 515.2981, found 515.2994.

10(*S*)-(−)-Hydroxyeicosa-5(*Z*),8(*Z*),11(*Z*),14(*Z*)-tetraenoic Acid Methyl Ester (2). Tetrabutylammonium fluoride (300 mL, 0.3 mmol) was added dropwise to a solution of **13** (116 mg, 0.2 mmol) in THF (5 mL) under nitrogen, and stirring was continued at room temperature for 2 h. The reaction mixture was partitioned between ethyl acetate and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography using 3% acetone in hexane to give **2** as a colorless oil (41 mg, 61%). Further purification was carried out by HPLC (silica column, 10 mm × 25 cm, 5 μm) using hexane/2-propanol (100:0.5 v/v) as the mobile phase at a wavelength 210 nm with a flow rate 3 mL/min. The retention time for compound **2** was 38.26 min: $[\alpha]^{20}_D$ −11.6 (c 0.1, EtOH); TLC R_f (30% ethyl acetate/hexane) = 0.57; ^{1}H NMR (CD_3CN) δ 5.18−5.55 (m, 9 H), 3.62 (s, 3 H), 2.68−3.05 (m, 4 H), 2.30 (t, J = 7.4 Hz, 2 H), 1.95−2.25 (m, 4 H), 1.55−1.75 (m, 2 H), 1.15−1.50 (m, 6 H), 0.89 (t, J = 6.5 Hz, 3

H); ^{13}C NMR (CD_3CN) δ 174.6, 133.1, 130.5, 129.7, 129.5, 129.4, 128.4, 64.0, 51.9, 34.0, 32.3, 30.0, 27.8, 27.3, 26.8, 25.6, 23.3, 14.3; HRMS calcd for $C_{21}H_{33}O_2$ ($MH^+ - H_2O$) 317.2480, found 317.2480.

10(*S*)-(−)-Hydroxyeicosa-5(*Z*),8(*Z*),11(*Z*),14(*Z*)-tetraenoic Acid (1). LiOH (3 N, 600 μL) was added to a solution of 10(*S*)-(−)-hydroxyeicosatetraenoic acid methyl ester (2.0 mg, 0.006 mmol) in dimethoxyethane (400 μL), and the mixture was stirred at 60 °C for 2 h. The reaction mixture was acidified carefully to pH 6 using dilute HCl (1 N) and extracted using CH_2Cl_2 (3 × 5 mL). The organic extracts were combined, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give **1** as a colorless oil (0.82 mg, 43%): TLC R_f (30% ethyl acetate/hexane) = 0.46; ^{1}H NMR (CD_3CN) δ 5.30−5.47 (m, 8 H), 5.17−5.25 (m, 1 H), 2.74−2.95 (m, 4 H), 2.27 (t, J = 7.4 Hz, 2 H), 2.02−2.18 (m, 4 H), 1.58−1.68 (m, 2 H), 1.25−1.40 (m, 6 H), 0.89 (t, J = 6.7 Hz, 3 H); ^{13}C NMR (CD_3CN) δ 175.0 133.1, 133.0, 131.6, 130.4, 129.7, 129.5, 129.3, 128.3, 64.0, 33.6, 32.3, 30.0, 27.9, 27.2, 26.8, 25.5, 23.3, 14.3; HRMS calcd for $C_{20}H_{31}O_2$ ($MH^+ - H_2O$) 303.2324, found 303.2323. This compound was unstable on a silica column and so could not be further purified by HPLC. However, it could be readily prepared in >98% purity by saponification of methyl ester **2**, as described above, when required.

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Supporting Information Available: NMR spectra for **1a,b**, **6-11**, and **13** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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