

The Synthesis of Very Long Chain Polyunsaturated Fatty Acids (VLC-PUFAs)

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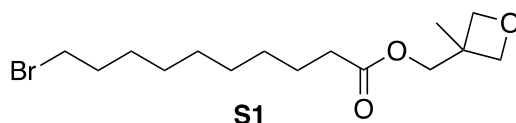
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Experimentals

General. Glassware was either dried in an oven at 130 °C or flame dried under a N₂ atmosphere prior to use. Unless described otherwise reactions were performed using common dry, inert atmosphere techniques. Solvents were purified according to the guidelines in Purification of Common Laboratory Chemicals (Perrin, Armarego, and Perrin: Oxford, 1966). Dichloromethane, triethylamine, and pyridine were distilled from CaH₂. Reactions were monitored by TLC and visualized by a dual short wave/long wave UV lamp and stained with an ethanolic solution of potassium permanganate or *p*-anisaldehyde. Flash column chromatography was performed using F60 grade silica gel, 230- 400 mesh. NMR spectra were recorded on Varian Unity-300, Varian VXR-500, Varian Inova-500, or Varian Inova-400 spectrometers. Chemical shifts for ¹H NMR were reported as δ, parts per million (ppm), relative to the signal of tetramethylsilane at 0 ppm or the CHCl₃ signal at 7.26 ppm. Chemical shifts for ¹³C NMR were reported as δ, parts per million, relative to the center line signal of the CDCl₃ triplet at 77.2 ppm or CD₃OD septet at 49.3 ppm or DMSO-*d*⁶ septet at 39.5 ppm. The abbreviations s, d, t, q, dd, p, and m stand for the resonance multiplicity singlet, doublet, triplet, quartet, doublet of doublet, pentet, and multiplet, respectively. IR spectra were recorded on a Nicolet iS10 FTIR Spectrometer. Mass spectra were recorded at the Mass Spectrometry Facility in the Department of Chemistry at the University of Utah using a Finnigan MAT 95 double focusing high

resolution mass spectrometer. Concentration refers to removal of solvent under reduced pressure (house vacuum at ca. 20 mmHg).

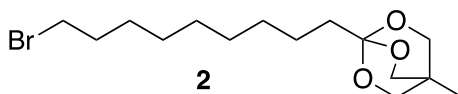
Animals. Mice were housed in the vivarium at the Moran Eye Center and maintained in a standard 12 h light and 12 h dark cycle environment, with water and food ad libitum (Teklad Global Soy-Free Rodent Diet, Envigo). All animal handling procedures used in this study were approved by appropriate institutional animal care and use committees and were carried out according to NIH guidelines.



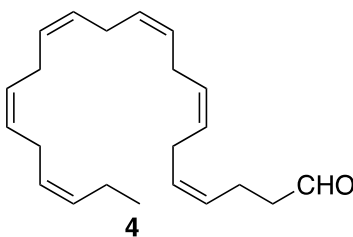
(3-methyloxetan-3-yl)methyl 10-bromodecanoate (S1). To a solution of 10-bromo-1-decanoic acid (2.60 g, 10.3 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added 1 drop of DMF and oxalyl chloride (2.0 mL, 23 mmol). The resulting reaction mixture was allowed to warm to rt and stirred for 2 h. The resulting acid chloride was immediately used in the subsequent step.

To a solution of the acid chloride from above in CH_2Cl_2 (100 mL) at 0 °C was sequentially added a solution of 3-methyl-3-oxetanemethanol (1.0 g, 9.8 mmol) in CH_2Cl_2 (25 mL) followed by trimethylamine (3.00 mL, 21.6 mmol). The resulting reaction mixture was allowed to warm to rt and stirred at that temperature for 3 h. The reaction was quenched with water (100 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were washed with sat. NaHCO_3 (aq., 100 mL) and brine (100 mL), dried (Na_2SO_4) and concentrated. Flash chromatography (19:1 hexanes:ethyl acetate) gave 3.0 g (91%) of **S1** as colorless oil. ^1H NMR (500 MHz, CDCl_3) δ 4.50 (d, J = 5.8 Hz, 2H), 4.36 (d, J = 5.8 Hz, 2H), 4.14 (s, 2H), 3.39 (t, J = 6.8 Hz, 2H), 2.34 (t, J = 7.3

Hz, 2H), 1.83 (p, $J = 6.8$ Hz, 2H), 1.63-1.61 (m, 2H), 1.40-1.39 (m, 2H), 1.32-1.29 (m, 11H); ^{13}C NMR (125 MHz, CDCl_3) δ 174.0, 79.7, 68.6, 39.2, 34.3, 34.1, 32.9, 29.4, 29.3, 29.2, 28.8, 28.2, 25.1, 21.3; EI-LRMS calcd for $\text{C}_{15}\text{H}_{28}\text{BrO}_3$ $[\text{M}+\text{H}]^+$ (m/z) 335.1 found 335.1.

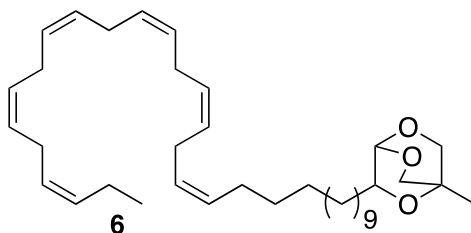


1-(9-bromononyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (2). To a solution of **2** (3.0 g, 9.0 mmol) in CH_2Cl_2 (100 mL) at 0°C was slowly added $\text{BF}_3\cdot\text{OEt}_2$ (0.30 mL, 2.4 mmol). The resulting reaction mixture was allowed to warm to rt and then stirred at that temperature overnight. The reaction mixture was cooled to 0°C and the reaction was quenched by the slow addition of NEt_3 (1 mL). The resulting mixture was warmed to rt and to this was added H_2O (50 mL). Following separation, the aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL), the organic layers combined and washed with sat. NaHCO_3 (aq., 100 mL) and brine (100 mL) and then dried (Na_2SO_4). Concentration and flash chromatography (19:1 ethyl acetate/hexanes) gave 2.4 g (80%) of **2** as a thick colorless oil. IR (neat) 2925, 2853, 1733, 1458, 1397, 1352, 1261, 1055, 990, 886 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 3.88 (s, 6H), 3.38 (t, $J = 6.8$ Hz, 2H), 1.83 (p, $J = 6.8$ Hz, 2H), 1.66-1.62 (m, 2H), 1.43-1.38 (m, 4H), 1.27-1.26 (m, 8H), 0.79 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3) δ 109.2, 72.7, 36.9, 34.2, 33.0, 30.4, 29.6, 29.5, 29.4, 28.9, 28.3, 23.3, 14.7; EI-LRMS calcd for $\text{C}_{15}\text{H}_{28}\text{BrO}_3$ $[\text{M}+\text{H}]^+$ (m/z) 335.1 found 335.1.



(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenal (4). To a solution of docohexaenoic acid (1.00 g, 2.80 mmol) in THF (15 mL) at 0 °C was added LiAlH₄ (0.500 g, 13.1 mmol). The resulting reaction mixture was stirred for 2h at 0 °C before the reaction was quenched by the slow addition of sat. Na₂SO₄ (aq., 20 mL). The mixture was allowed to warm to rt and stirred at that temperature for 1 h. Filtration and concentration gave DHA alcohol as a thick colorless oil which was used in the next step without further purification.

To a solution of oxalyl chloride (1.00 mL, 11.7 mmol) in CH₂Cl₂ (20 mL) at -78 °C was added DMSO (2.00 mL, 28.2 mmol) slowly. The DHA alcohol from above in CH₂Cl₂ (10 mL) was added to the resulting reaction mixture after it had stirred for 15 minutes. After addition of the alcohol the reaction mixture was stirred for an additional 2h at -78 °C and triethylamine (2.00 mL, 14.3 mmol) was added. The reaction mixture was allowed to warm to rt after which it was quenched with water. The phases were separated and the aqueous phase was extracted with CH₂Cl₂ (3 x 25 mL). The organic extracts were combined, washed with brine (100 mL), and dried (Na₂SO₄). Concentration and flash chromatography (1:20 ethyl acetate:hexane) gave 425 mg (86%) of DHA aldehyde **4** as a pale yellow oil. IR (neat) 3011, 2962, 2921, 2714, 1726, 1658, 1434, 1390, 1352, 1265, 1055, 917, 702 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 9.74 (s, 1H), 5.40-5.29 (m, 12H), 2.83-2.79 (m, 10H), 2.43-2.38 (m, 2H), 2.09-2.02 (m, 4H), 1.62-1.58 (m, 2H), 2.34-1.25 (m, 20H), 0.96 (t, J= 7.4Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 202.7, 132.1, 130.5, 128.7, 128.6, 128.4, 42.83, 128.2, 128.0, 127.9, 127.6, 127.1, 44.0, 29.8, 129.7, 29.6, 29.5, 29.4, 29.3, 27.4, 25.8, 25.7, 25.6, 22.2, 20.7, 14.4

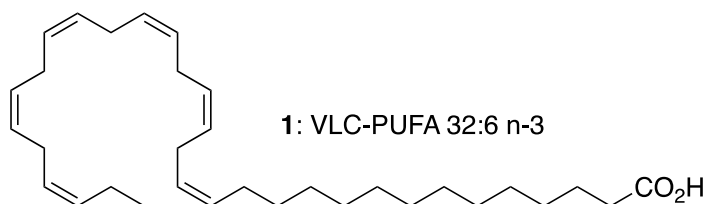


1-((13Z,16Z,19Z,22Z,25Z,28Z)-hentriaconta-13,16,19,22,25,28-hexaen-1-yl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (6): To fresh magnesium powder (100.0 mg, 4.10 mmol) at rt was added 1 drop of dibromoethane and a solution of orthoester **2** (800.0 mg, 2.4 mmol) in THF (15 mL). The reaction mixture was stirred for 2 h and then cooled to 0 °C. To this was added a solution of DHA aldehyde **4** (80.0 mg, 0.250 mmol) in THF (5 mL). The resulting mixture was immediately warmed to rt and then stirred at this temperature for an additional 2h after which the reaction was quenched with sat. NH₄Cl (aq., 20 mL). The aqueous phase was extracted with EtOAc (3 x 20 mL), the extracts were combined, and dried (Na₂SO₄). Concentration and flash chromatography (a gradient of 1:9 acetate:hexanes to 1:4 ethyl acetate:hexanes) gave 345 mg of a mixture of alcohol **5** and dimeric material as a white solid.

To the solution of alcohol **5** in CH₂Cl₂ (10 mL) at 0 °C was added trimethylamine (0.300 mL, 2.15 mmol) and MsCl (0.050 mL, 0.65 mmol). The reaction mixture was allowed to warm to rt and stirred at that temperature for 2 h after which the reaction was quenched with water (10 mL). The aqueous phase was extracted with CH₂Cl₂ (3 x 10 mL), the organic extracts were combined, dried (Na₂SO₄), and concentrated. Flash chromatography (1:4 ethyl acetate:hexanes) gave 300 mg mixture of mesylated product and dimer from **2** as a light yellow oil.

To a mixture of the mesylated product and dimer from above (300 mg) in THF (15 mL) at 0 °C was added LiAlH₄ (150 mg, 3.95 mmol) slowly. After the addition was complete

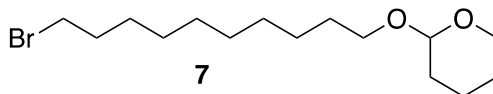
the reaction mixture was warmed to rt and stirred at that temperature for 8h after which the reaction was quenched with sat. Na₂SO₄ (aq., 2 ml). After stirring for 1h the resulting mixture was filtered. Concentration and flash chromatography (1:9 ethyl acetate:hexane) gave 20 mg (14% from **4**) of **6** as a colorless oil. IR (neat) 3416, 2923, 2852, 1711, 1463, 1173, 1074 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.42-5.30 (m, 12H), 3.89 (s, 6H), 2.84-2.80 (m, 10H), 2.09-2.02 (m, 4H), 1.67-1.63 (m, 2H), 1.43-1.24 (m, 18H), 0.97 (t, *J* = 7.3 Hz, 3H), 0.79 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 132.2, 130.7, 128.8, 128.7, 128.5, 128.4, 128.3, 128.0, 127.7, 127.2, 109.3, 72.7, 36.9, 30.4, 29.9, 29.8, 29.7, 29.5, 27.4, 25.8, 25.7, 23.3, 20.7, 14.7, 14.4.



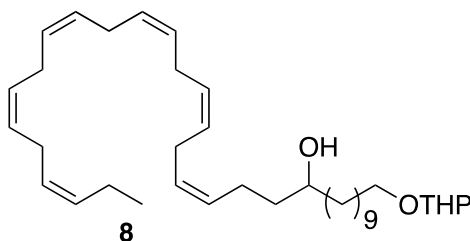
(14Z,17Z,20Z,23Z,26Z,29Z)-dotriaconta-14,17,20,23,26,29-hexaenoic acid (1). To a solution of **6** (20.0 mg, 0.0360 mmol) in mixture of THF (5 mL) and H₂O (0.5 mL) at rt was added TsOH•H₂O (0.6 mg, 0.003 mmol). The resulting reaction mixture was stirred overnight, concentrated, and used in next step without additional purification.

To a solution of the ester from above in THF (5 mL) was added 15% NaOH (0.2 mL). The resulting reaction mixture was stirred for 1h at 50 °C after which the reaction mixture was cooled to rt and the reaction was quenched with 1M HCl (aq, 5 mL). The aqueous phase was extracted with EtOAc (3 × 5 mL), the organic extracts were combined and dried (Na₂SO₄). Concentration and flash chromatography (1:4 EtOAc:hexane) gave 14 mg (83%) of **1** as a colorless thick oil. IR (neat) 3416, 2923, 2852, 1711, 1463, 1173, 1074 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.42-5.30 (m, 12H), 2.85-2.80 (m, 10H), 2.34 (t,

$J = 7.3$ Hz, 2H), 2.09-2.03 (m, 4H), 1.63 (p, $J = 7.3$ Hz, 2H), 1.34-1.22 (m, 18H), 0.97 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (125 MHz, CDCl_3) δ 179.8, 132.2, 130.7, 128.8, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.7, 127.2, 34.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 27.4, 25.8, 25.7, 24.9, 20.7, 14.5; HRMS (ESI) calcd for $\text{C}_{32}\text{H}_{52}\text{ONa}$ $[\text{M}+\text{Na}]^+$ (m/z) 475.3916 found 475.3910.

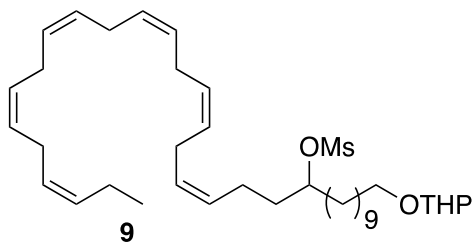


2-((10-bromodecyl)oxy)tetrahydro-2H-pyran (7). To a solution of 10-bromo-1-decanol (4.00 g, 15.9 mmol) in THF (16 mL) at rt was added 3,4-dihydro-2H-pyran (16.0 mL, 175 mmol) and PPTS (402 mg, 1.6 mmol). The reaction mixture was stirred at rt for 16h and then concentrated. The resulting residue was purified by flash chromatography (1:19 ethyl acetate:hexanes) giving 4.50 g (88%) of **7** as a colorless liquid. IR (neat) 2924, 2852, 1453, 1439, 1351, 1259, 1134, 1119, 1030, 987, 868 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 4.57-4.55 (dd $J = 3.1$ Hz, 1H), 3.72-3.83 (ddd, $J = 9.6, 6.9, 6.9$, 1H), 3.73-3.69 (m, 1H), 3.51-3.46 (m, 1H), 3.40 (t, $J = 6.9$ Hz, 1H), 3.34 (dd, $J = 6.7, 3.9$, 2H), 1.86-1.80 (m, 3H), 1.70-1.67 (dddd, $J = 13.1, 9.1, 3.1, 3.0$, 1H), 1.63-1.59 (m, 4H), 1.44-1.39 (m, 3H), 1.36-1.24 (m, 9H); ^{13}C NMR (125 MHz, CDCl_3) δ 98.9, 67.8, 67.4, 62.4, 62.4, 34.1, 32.9, 32.0, 30.9, 29.8, 29.6, 29.5, 29.5, 28.8, 28.3, 26.3, 25.6, 19.8, 19.8, 19.5, 14.1; EI-LRMS calcd for $\text{C}_{15}\text{H}_{30}\text{BrO}_2$ $[\text{M}+\text{H}]^+$ (m/z) 321.3 found 321.2.

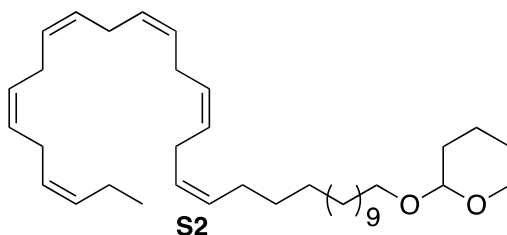


(14Z,17Z,20Z,23Z,26Z,29Z)-1-((tetrahydro-2H-pyran-2-yl)oxy)dotriaconta-

14,17,20,23,26,29-hexaen-11-ol (8): To freshly crushed magnesium turnings (300.0 mg, 12.3 mmol) at rt was added 3 drops of dibromoethane followed by a solution of **7** (2.00 g, 6.23 mmol) in THF (12 mL). After stirring for 2 h, the reaction mixture was cooled to 0 °C and to this was added a solution of DHA aldehyde **4** (425 mg, 1.36 mmol) in THF (2 mL). The resulting mixture was warmed to rt and stirred at that temperature for 2 h before the reaction was quenched with sat. NH₄Cl (aq., 20 mL). The aqueous phase was extracted with EtOAc (3 x 20 mL) and the combined organic extracts were dried (Na₂SO₄). Concentration and flash chromatography (a gradient of 1:19 ethyl acetate:hexanes to 3:17 ethyl acetate:hexanes) gave 550 mg (73%) of **8** as a colorless oil. IR (neat) 3415, 3012, 2926, 2853, 1453, 1261, 1136, 1121, 1077, 1032, 906, 705 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.43-5.29 (m, 12H), 4.55-4.52 (m, 1H), 3.88-3.81 (m, 1H), 3.70-3.71 (ddd, *J* = 9.5 Hz, 6.8 Hz, 6.8 Hz, 1H), 3.65-3.59 (m, 1H), 3.50-3.47 (ddd, 5.4 Hz, 5.4 Hz, 4.4 Hz, 1H), 3.36-3.65 (ddd, *J* = 9.4 Hz, 6.6 Hz, 6.6 Hz, 1H), 2.88-2.74 (m, 10H), 2.22-2.13 (m, 2H), 2.07 (p, *J* = 7.3 Hz, 2H), 1.84-1.80 (m, 1H), 1.73-1.66 (m, 1H), 1.60-1.35 (m, 8H), 1.34-1.22 (m, 16H), 0.97 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 132.2, 130.0, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.2, 99.0, 71.7, 67.9, 62.5, 37.7, 37.3, 30.9, 29.9, 29.8, 29.7, 29.6, 26.4, 25.8, 25.7, 23.8, 20.7, 19.9, 14.5; HRMS (ESI) calcd for C₃₇H₆₂O₃Na [M+Na]⁺ (m/z) 577.4597 found 577.4597.

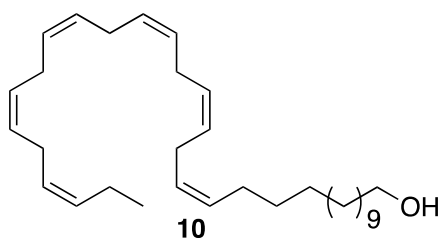


(14Z,17Z,20Z,23Z,26Z,29Z)-1-((tetrahydro-2H-pyran-2-yl)oxy)dotriaconta-14,17,20,23,26,29-hexaen-11-yl methanesulfonate (9). To a solution of alcohol **8** (390 mg, 0.71 mmol) in CH₂Cl₂ (20 mL) at 0 °C was added Et₃N (0.40 mL, 5.4 mmol) and methanesulfonyl chloride (0.16 mL, 2.1 mmol). The reaction mixture was warmed to rt and stirred at that temperature for 2h before the reaction was quenched with water (10 mL). The aqueous phase was extracted with CH₂Cl₂ (3 x 20 mL). The organic phases were combined, washed with brine (20 mL), and dried (Na₂SO₄). Concentration and flash chromatography (1:4 ethyl acetate: hexane) gave 410 mg of **9** (92%) as a light yellow oil. IR (neat) 3439, 3012, 2923, 2852, 1722, 1455, 1349, 1171, 1118, 1075, 1022, 970, 901, 721 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.42-5.29 (m, 12H), 4.72 (p, *J* = 6.3, 1H), 4.56-4.53 (dd, *J* = 4.2 Hz, 3.2Hz, 1H), 3.86-3.81 (ddd, *J* = 7.8 Hz, 2.9 Hz, 1H), 3.72-3.67 (dt, *J* = 9.6 Hz, 4.8 Hz, 4.8 Hz, 1H), 3.50-3.48 (m, 1H), 3.38-3.32 (ddd, *J* = 9.6 Hz, 6.7 Hz, 6.7 Hz, 1H), 2.99 (s, 3H), 2.86-2.80 (m, 10H), 2.22-2.12 (m, 2H), 2.07 (p, *J* = 7.4 Hz, 2H), 1.85-1.80 (m, 1H) 1.62-1.54 (m, 3H), 1.55-1.49 (m, 6H), 1.43-1.25 (m, 16H), 0.97 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 132.2, 129.2, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.2, 99.0, 83.7, 67.8, 62.5, 38.9, 34.6, 34.5, 31.0, 29.9, 29.8, 29.7, 29.6, 29.5, 26.4, 25.8, 25.7, 25.1, 23.0, 20.7, 19.9, 14.4; HRMS (ESI) calcd for C₃₈H₆₄O₅SNa [M+Na]⁺ (*m/z*) 655.4372 found 655.4367.



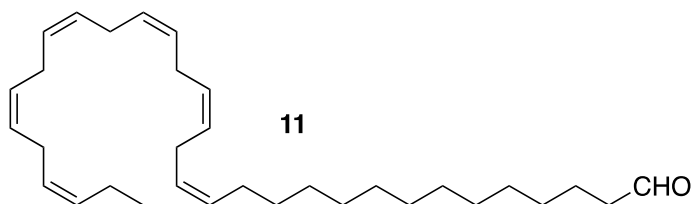
2-(((14Z,17Z,20Z,23Z,26Z,29Z)-dotriaconta-14,17,20,23,26,29-hexaen-1-yl)oxy)tetrahydro-2H-pyran (S2): To a solution of **9** (410 mg, 0.65 mmol) in THF (30 mL)

at 0 °C was added LiAlH₄ (300 mg, 8 mmol). The resulting reaction mixture was allowed to warm to rt and then stirred at that temperature for 8h. The reaction mixture was cooled to 0 °C and the reaction was carefully quenched with sat. Na₂SO₄ (aq., 3 mL) at 0 °C. The resulting mixture was warmed to rt and stirred at that temperature for 1h. Filtration and concentration gave **S2** as a thick colorless oil which was used in the next step without additional purification. ¹H NMR (500 MHz, CDCl₃) δ 5.41-5.28 (m, 12H), 4.57 (t, *J* = 3.4 Hz, 1H), 3.87-3.84 (ddd, *J* = 11.0 Hz, 7.5 Hz, 3.29 Hz, 2.9 Hz, 1H), 3.87-3.78 (ddd, *J* = 9.6 Hz, 6.8 Hz, 6.8 Hz, 1H), 3.50-3.48 (m, 1H), 3.39-3.36 (m, 1H), 2.87-2.79 (m, 10H), 2.10-2.03 (m, 4H), 1.87-1.78 (m, 1H), 1.74-1.67 (m, 1H), 1.62-1.48 (m, 6H), 1.34-1.23 (m, 22H), 0.97 (t, *J* = 7.6 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 132.1, 130.5, 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.9, 127.6, 127.1, 98.9, 67.7, 62.3, 30.9, 29.9, 29.8, 29.7, 29.6, 29.4, 27.4, 26.4, 25.7, 25.6, 20.7, 19.7, 14.4; EI-HRMS calcd for C₃₇H₆₂O₂Na [M+Na]⁺ (m/z) 561.4648 found 61.4645.

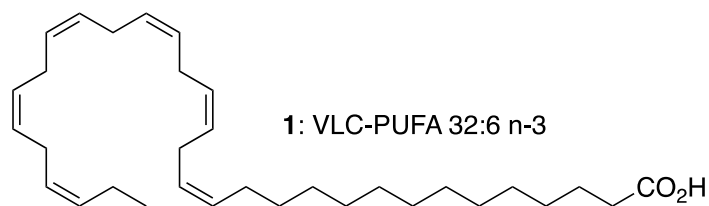


(14Z,17Z,20Z,23Z,26Z,29Z)-dotriaconta-14,17,20,23,26,29-hexaen-1-ol (10). To a solution of THP acetal **S2** (400.0 mg, 0.743 mmol) in a mixture of THF (10 mL) and MeOH (10 mL) at rt was added TsOH•H₂O (12 mg, 0.065 mmol). After the reaction mixture had stirred overnight it was concentrated. The resulting residue was purified by flash chromatography (1:4 ethyl acetate:hexanes) to give 280 mg of **10** (95%) as a colorless oil. IR (neat) 3382, 3012, 2923, 2852, 1723, 1462, 1368, 1067, 721 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.43-5.29 (m, 12H), 3.64 (t, *J* = 6.7, 2H), 2.88-2.80 (m, 10H), 2.10-2.02 (m, 4H),

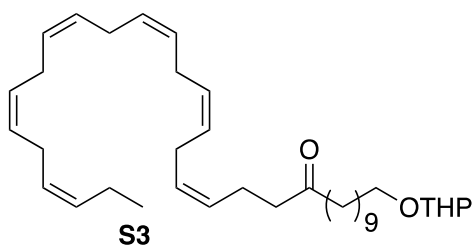
1.59-1.54 (m, 4H), 1.34-0.98 (m, 20H), 0.97 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (125 MHz, CDCl_3) δ 132.1, 130.6, 128.7, 128.4, 128.3, 128.2, 128.0, 127.7, 127.1, 63.1, 32.9, 29.9, 29.8, 29.7, 29.6, 29.5, 27.4, 25.9, 25.8, 25.7, 20.7, 14.4; HRMS calcd for $\text{C}_{32}\text{H}_{55}\text{O}$ $[\text{M}+\text{H}]^+$ (m/z) 455.4253 found 455.4250.



(14Z,17Z,20Z,23Z,26Z,29Z)-dotriaconta-14,17,20,23,26,29-hexaenal (11). To a solution DMSO (1.0 mL, 14 mmol) in CH_2Cl_2 (10 mL) at -78 °C was added oxalyl chloride (0.50 mL, 5.8 mmol). After the reaction mixture had stirred for 0.5h, alcohol **10** (280 mg, 0.62 mmol) in CH_2Cl_2 (8 mL) was then added to it and the resulting mixture was stirred for an additional 2h at -78 °C. To this was added Et_3N (1.0 mL, 7.2 mmol), the resulting reaction mixture was allowed to warm to rt, and the reaction was quenched with water (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL), the organic extracts were combined and filtered through a pad of Na_2SO_4 (Na_2SO_4 pad was rinsed with CH_2Cl_2). Concentration and flash chromatography (1:9 ethyl acetate:hexanes) gave 250 mg of **11** (90%) as a light yellow oil. IR (neat) 3012, 2960, 2921, 2714, 1722, 1658, 1434, 1390, 1352, 1265, 1065, 917, 702 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 9.74 (t, $J = 1.7$ Hz, 1H), 5.42-5.27 (m, 12H), 2.86-2.79 (m, 10H), 2.42-2.38 (m, 2H), 2.10-2.02 (m, 2H), 1.64-1.58 (m, 2H), 1.36-1.25 (m, 20H), 0.96 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (125 MHz, CDCl_3) δ 202.7, 132.1, 130.5, 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.9, 127.6, 127.1, 44.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.4, 25.8, 25.7, 25.6, 22.2, 20.7, 14.4. HRMS (ESI) calcd for $\text{C}_{32}\text{H}_{52}\text{ONa}$ $[\text{M}+\text{Na}]^+$ (m/z) 475.3916 found 475.3910.

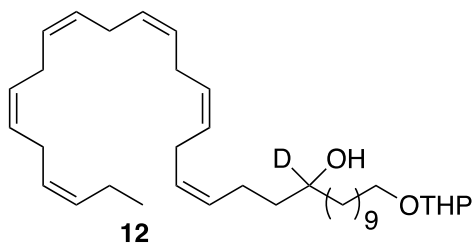


(14Z,17Z,20Z,23Z,26Z,29Z)-dotriaconta-14,17,20,23,26,29-hexaenoic acid (1). To a solution of aldehyde **11** (1.20 g, 2.56 mmol) in DMF (26 mL) at was added Oxone[®] (1.73 g, 2.82 mmol). After the reaction mixture had stirred for 1 h it was filtered through celite (the celite pad was rinsed with CH₂Cl₂). DMF was removed via repeated coevaporation with hexane. Concentration and flash chromatography (1:10 ethyl acetate:hexanes with 4% acetic acid) gave 611 mg of **1** (51%) as a colorless thick oil. IR (neat) 3416, 2923, 2852, 1711, 1463, 1173, 1074 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.42-5.30 (m, 12H), 2.85-2.80 (m, 10H), 2.35 (t, *J* = 7.5 Hz, 2H), 2.11-2.03 (m, 4H), 1.68-1.60 (m, 2H), 1.36-1.25 (m, 18H), 0.97 (t, *J* = 7.6 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 179.8, 132.2, 130.7, 128.8, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.7, 127.2, 34.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 27.4, 25.8, 25.7, 24.9, 20.7, 14.5; EI-LRMS calcd for C₃₂H₅₂O₂Na[M-H]⁺ (m/z) 491.3865 found 491.3857.



(14Z,17Z,20Z,23Z,26Z,29Z)-1-((tetrahydro-2H-pyran-2-yl)oxy)dotriaconta-14,17,20,23,26,29-hexaen-11-one (S3): To a solution of oxalyl chloride (0.10 mL, 1.4 mmol) in CH₂Cl₂ (5 mL) at -78 °C was added DMSO (0.2 mL, 3 mmol). After the resulting mixture had stirred for 0.5 h, a solution of alcohol **8** (100. mg, 0.180 mmol) in CH₂Cl₂ (4

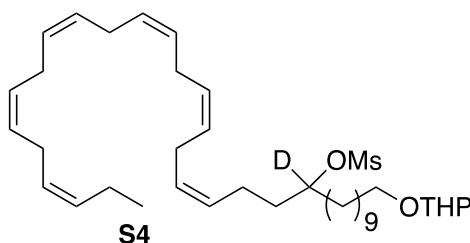
mL) was added slowly to the reaction mixture. After stirring for an additional 2h at -78 °C, Et₃N (0.40 mL, 2.9 mmol) added to reaction mixture. The reaction mixture was warmed to rt and the reaction was quenched with water (10 mL). The aqueous phase was extracted with CH₂Cl₂ (3 × 10 mL), the organic extracts were combined, dried (Na₂SO₄), and concentrated. Flash chromatography (1:19 EtOAc:hexanes) gave 49 mg (50%) of **S3** as a colorless oil. IR (neat) 3398, 2925, 2853, 1712, 1454, 1353, 1261, 1200, 1119, 1076, 1023, 985, 905, 868, 811, 721 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.41-5.28 (m, 12H), 4.56 (s, 1H), 3.87-3.84 (m, 1H), 3.72-3.69 (m, 1H), 3.50-3.47 (m, 1H), 3.39-3.34 (m, 1H), 2.83-2.79 (m, 10H), 2.44 (t, *J* = 7.3 Hz, 2H), 2.38-2.30 (m, 4H), 2.07 (p, *J* = 7.3 Hz, 2H), 1.85-1.79 (m, 1H), 1.72-1.68 (m, 1H), 1.57-1.49 (m, 6H), 1.33-1.26 (m, 14H), 0.96 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 210.7, 132.2, 129.0, 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.2, 99.0, 67.8, 62.5, 43.1, 42.5, 38.8, 38.4, 30.9, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 26.6, 26.4, 25.8, 25.7, 25.6, 24.5, 24.0, 21.8, 19.8, 14.5; EI-LRMS calcd for C₃₇H₆₀O₃ [M+Na]⁺ (m/z) 575.4 found 575.6.



(14Z,17Z,20Z,23Z,26Z,29Z)-1-((tetrahydro-2H-pyran-2-yl)oxy)dotriaconta-

14,17,20,23,26,29-hexaen-11-d-11-ol (12): To a solution of ketone **S3** (70.0 mg, 0.14 mmol) in THF (2 mL) at 0 °C was added LiAlD₄ (30 mg, 0.7 mmol). The resulting reaction mixture was stirred for 2h after which the reaction was quenched with sat. Na₂SO₄ (aq. 1 mL) at 0 °C. The resulting mixture was stirred for 0.5 h, filtered, and concentrated. The resulting residue was purified by flash chromatography (1:5 EtOAc:hexanes) to give 63

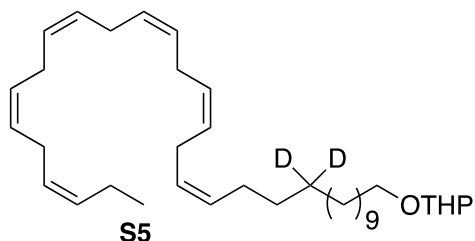
mg (90%) of **12** as a colorless oil. IR (neat) 3332, 2973, 2879, 1658, 1374, 1087, 1045, 879, 621 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 5.42-5.28 (m, 12H), 4.56 (bs, 1H), 3.87-3.83 (m, 1H), 3.73-3.70 (m, 1H), 3.49-3.47 (m, 1H), 3.37-3.34 (m, 1H), 2.83-2.80 (m, 10H), 2.19-2.12 (m, 2H) 2.09 (p, $J = 7.3$ Hz, 2H), 1.84-1.79 (m, 1H), 1.69-1.65 (m, 1H) 1.58-1.22 (m, 25H), 0.96 (t, $J = 7.3$ Hz, 3H) ^{13}C NMR (125 MHz, CDCl_3) δ 132.2, 130.6, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.2, 99.0, 67.9, 62.5, 37.6, 37.2, 31.0, 29.9, 29.8, 29.7, 26.4, 25.8, 25.7, 23.7, 20.7, 19.9, 14.5; EI-LRMS calcd for $\text{C}_{37}\text{H}_{61}\text{DO}_3$ $[\text{M}+\text{Na}]^+$ (m/z) 578.4 found 578.7.



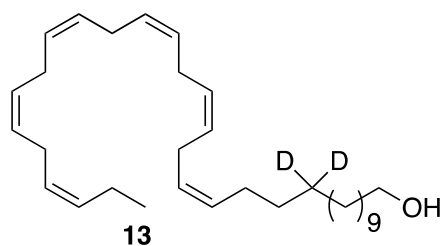
(14Z,17Z,20Z,23Z,26Z,29Z)-1-((tetrahydro-2H-pyran-2-yl)oxy)dotriaconta-

14,17,20,23,26,29-hexaen-11-yl-11-d methanesulfonate (S4). To a solution of **12** (63 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added MsCl (44 mL, 0.56 mmol) and Et_3N (160 μL , 1.1 mmol). The reaction mixture was warmed to rt and stirred at that temperature for 2h. The reaction was quenched with H_2O (5 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 x 5 mL). The organic extracts were combined and concentrated. The resulting residue was purified by flash chromatography (1:4 EtOAc:hexanes) to give 44 mg (60%) of **S4** as a colorless oil. IR (neat) 3012, 2924, 2853, 1974, 1454, 1352, 1261, 1120, 1077, 1033, 975, 906, 719 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 5.42-5.28 (m, 12H), 4.57 (bs, 1H), 3.88-3.84 (m, 1H), 3.73-3.71 (m, 1H), 3.50-3.48 (m, 1H), 3.38-3.36 (m, 1H), 2.99 (s, 3H), 2.81-2.77 (m, 10H), 2.19-2.12 (m, 2H) 2.10 (p, $J = 7.3$ Hz, 2H), 1.85-1.60 (m, 5H), 1.58-1.50 (m, 5H) 1.41-1.22 (m, 16H), 0.97 (t, $J = 7.3$ Hz, 3H) ^{13}C NMR (125 MHz,

CDCl₃) δ 132.2, 129.2, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 127.2, 99.0, 67.9, 62.6, 34.5, 34.4, 30.9, 29.9, 29.8, 29.7, 29.6, 26.4, 25.8, 25.7, 25.1, 22.9, 20.7, 19.9, 14.5; EI-LRMS calcd for C₃₈H₆₃DO₅SNa [M+Na]⁺ (m/z) 656.4 found 656.7.

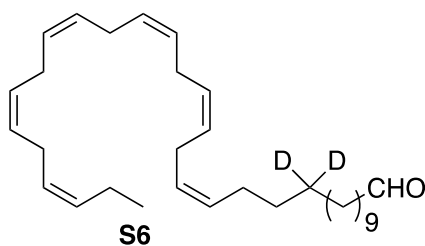


2-(((14Z,17Z,20Z,23Z,26Z,29Z)-dotriaconta-14,17,20,23,26,29-hexaen-1-yl-11,11-d₂oxy)tetra-hydro-2H-pyran (S5): To a solution of **S4** (44 mg, 0.070 mmol) in THF (2 mL) at 0 °C was added LiAlD₄ (30 mg, 0.7 mmol). The resulting reaction mixture was warmed to rt and stirred at that temperature for 8h. The reaction mixture was cooled to 0 °C and the reaction was quenched with sat. Na₂SO₄ (aq. 1 mL), warmed to rt and stirred at that temperature for 0.5h. The mixture was filtered and the filtrate was concentrated to give **S5** as a colorless oil which was used in the next step without additional purification. IR (neat) 3012, 2922, 2852, 1454, 1121, 1078, 1033, 711 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.38-5.28 (m, 12H), 4.57 (bs, 1H), 3.88-3.85 (m, 1H), 3.73-3.71 (m, 1H), 3.50-3.48 (m, 1H) 3.38-3.36 (m, 1H), 2.84-2.77 (m, 10H), 2.22-2.14 (m, 3H) 2.05 (p, *J* = 7.3 Hz, 2H), 1.83-1.76 (m, 1H), 1.73-1.68 (m, 1H), 1.58-1.42 (m, 7H) 1.34-1.24 (m, 16H), 0.96 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 132.2, 130.7, 128.8, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.7, 127.2, 99.0, 67.9, 62.5, 31.0, 29.9, 29.8, 29.7, 29.6, 27.4, 26.4, 25.8, 25.7, 20.7, 19.9, 14.5; EI-LRMS calcd for C₃₇H₆₀D₂O₂Na [M+Na]⁺ (m/z) 563.4 found 563.7.



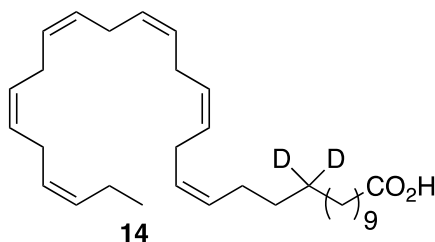
(14Z,17Z,20Z,23Z,26Z,29Z)-dotriaconta-14,17,20,23,26,29-hexaen-11,11-d₂-1-ol (13):

To a solution of **S5** (40.0 mg, 87.8 mmol) in a mixture of THF (1 mL) and MeOH (1 mL) was added TsOH.H₂O (1.5 mg, 7.0 mmol). After the resulting reaction mixture had stirred for 16h it was concentrated. The resulting residue was purified by flash chromatography (1:4 EtOAc:hexane) to give 17.3 mg (55%) of **13** as a colorless oil. IR (neat) 3311, 3013, 2962, 2852, 2166, 1652, 1462, 1392, 1266, 1056, 928, 711 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.42-5.28 (m, 12H), 3.63 (t, *J* = 6.8, 2H), 2.84-2.77 (m, 10H), 2.10-2.02 (m, 4H), 1.59-1.53 (m, 2H), 1.34-0.98 (m, 18H), 0.97 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 132.1, 130.7, 128.8, 128.7, 128.4, 128.3, 128.1, 128.0, 127.7, 127.2, 63.3, 33.0, 29.9, 29.8, 29.7, 29.6, 29.5, 27.4, 25.9, 25.8, 25.7, 20.7, 14.4; EI-LRMS calcd for C₃₂H₅₂D₂ONa [M+Na]⁺ (*m/z*) 479.4 found 479.6.



(14Z,17Z,20Z,23Z,26Z,29Z)-dotriaconta-14,17,20,23,26,29-hexaenal-11,11-d₂ (S6). To a solution of DMSO (44 mL, 0.60 mmol) in CH₂Cl₂ (1 mL) at -78 °C was added oxalyl chloride (26 μL, 0.3 mmol). After 0.5h, alcohol **13** (17.3 mg, 0.0380 mmol) in CH₂Cl₂ (2 mL) was added to the reaction mixture. After an additional 2h at -78 °C Et₃N (130 μL, 0.90 mmol) was added to the reaction mixture. The mixture was warmed to rt and the reaction

was quenched with water (5 mL). The aqueous phase was extracted with CH₂Cl₂ (3 x 5 mL), the organic extracts were combined and filtered through a pad of Na₂SO₄ (the Na₂SO₄ pad was rinsed with CH₂Cl₂). Concentration and flash chromatography (1:19 ethyl acetate:hexanes) gave 16.2 mg (95%) of **S6** as a colorless oil. IR (neat) 3012, 2923, 2853, 2715, 2198, 1728, 1658, 1462, 1391, 1263, 1069, 929, 710 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 9.74 (s, 1H), 5.42-5.30 (m, 12H), 2.84-2.80 (m, 10H), 2.41 (td, *J* = 7.6, 1.4 Hz, 2H), 2.09-2.02 (m, 2H), 1.62 (q, *J* = 7.4 Hz, 2H), 1.34-1.25 (m, 18H), 0.97 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 202.0, 132.2, 130.7, 128.8, 128.7, 128.4, 128.3, 128.1, 128.0, 127.7, 127.2, 44.1, 29.9, 29.8, 29.6, 29.5, 29.4, 27.4, 25.8, 25.7, 22.3, 20.7, 14.4; EI-LRMS calcd for C₃₂H₅₀D₂ONa [M+Na]⁺ (*m/z*) 477.4 found 477.5.



(14Z,17Z,20Z,23Z,26Z,29Z)-dotriaconta-14,17,20,23,26,29-hexaenoic-11,11-d₂ acid (14): To a solution of **S6** (8.0 mg, 0.17 mmol) in DMF (2 mL) at rt was added oxone (218 mg, 0.36 mmol). The resulting reaction mixture was stirred at rt for 4 h after which it was filtered through a pad of celite (the celite pad was rinsed with CH₂Cl₂) and concentrated. The resulting residue was purified by flash chromatography (1:4 EtOAc:hexanes) giving 3.5 mg (42%) of **14** as a colorless oil. IR (neat) 2921, 2851, 1707, 1457, 1409, 1173, 1055, 801, 721 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.42-5.30 (m, 12H), 2.85-2.80 (m, 10H), 2.34 (t, *J* = 7.3 Hz, 2H), 2.09-2.02 (m, 4H), 1.63 (p, *J* = 7.3 Hz, 2H), 1.34-1.21 (m, 18H), 0.96 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 178.2, 132.2, 130.7, 128.8, 128.5, 128.4, 128.3, 128.1, 128.0, 127.7, 127.2, 110.2, 33.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3,

27.4, 25.8, 25.7, 24.9, 22.9, 20.7, 14.5; EI-LRMS calcd for $C_{32}H_{50}D_2O_2$ $[M-H]^-$ (m/z) 469.4
found 469.5.