Supplemental information for:

Mass spectrometry-directed structure elucidation and total synthesis of ultra-long chain (*O*-acyl)-*ω*-hydroxy fatty acids

Sarah E. Hancock[1,2†], Ramesh Ailuri[2,3], David L. Marshall[4], Simon H. J. Brown[2,5], Jennifer T. Saville[3‡], Venkateswara R. Narreddula[4], Nathan R. Boase[6], Berwyck L. J. Poad[4], Adam J. Trevitt[3], Mark D. P. Willcox[7], Michael J. Kelso[2,3], Todd W. Mitchell[1,2*] and Stephen J. Blanksby[4,6*]

[1]School of Medicine, University of Wollongong, Wollongong NSW, AUSTRALIA

[2]Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong NSW, AUSTRALIA

[3]School of Chemistry, University of Wollongong, Wollongong NSW, AUSTRALIA

[4]Central Analytical Research Facility, Institute for Future Environments, Queensland University of Technology, Brisbane QLD AUSTRALIA

[5]School of Biological Sciences, University of Wollongong, Wollongong NSW, AUSTRALIA

[6]School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology, Brisbane QLD, AUSTRALIA

[7]School of Optometry and Vision Science, University of New South Wales, Sydney, NSW, AUSTRALIA

***Please address correspondence to:**

toddm@uow.edu.au

stephen.blanksby@qut.edu.au

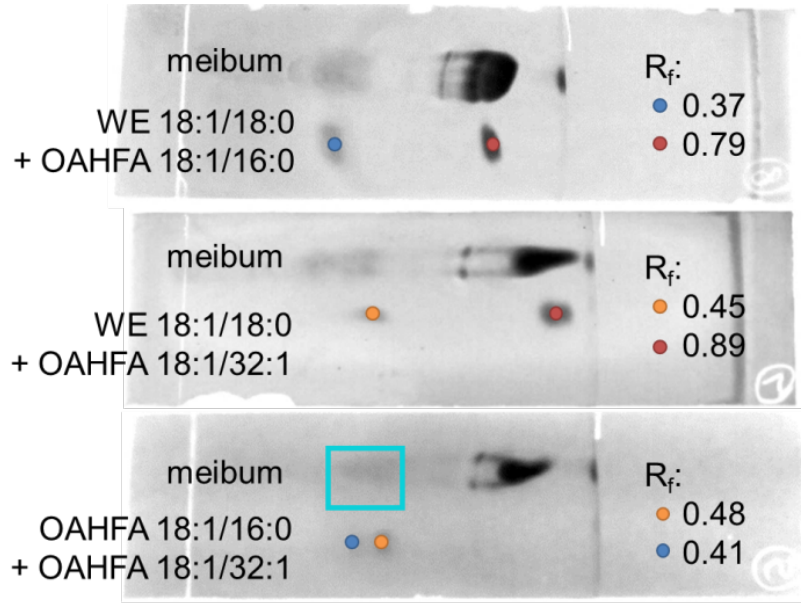
†Present address: School of Medical Sciences, University of New South Wales, Sydney AUSTRALIA

‡Present address: Genetics and Molecular Pathology, SA Pathology at Women's and Children's Hospital, Adelaide, SA, AUSTRALIA

Contents

1 Experimental section

1.1 Thin-layer chromatography



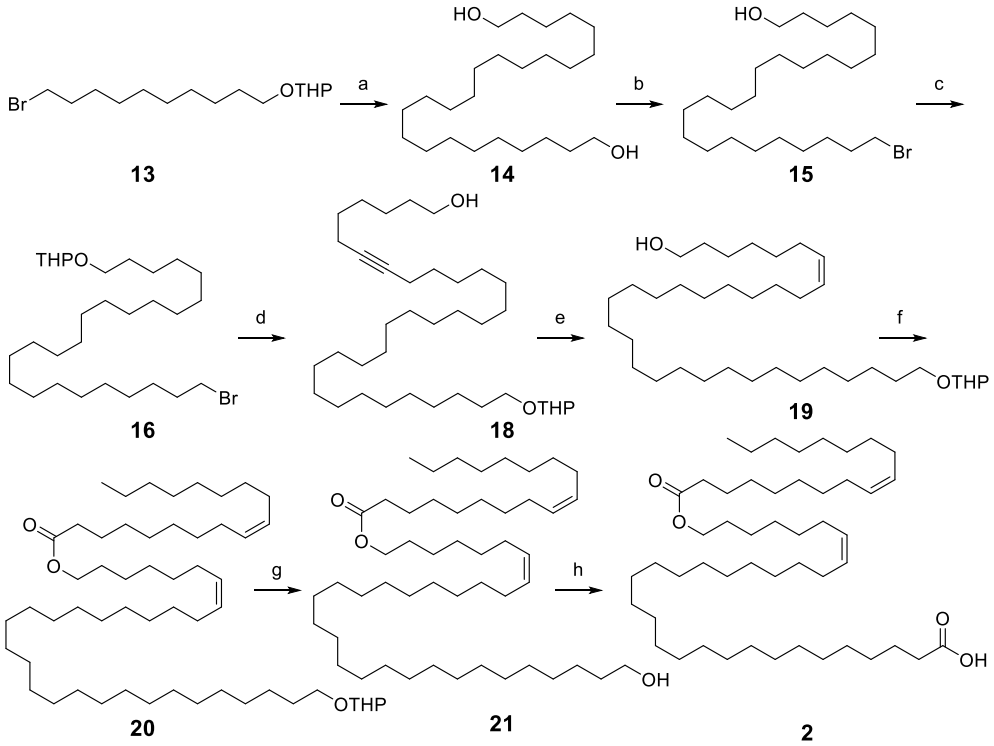
Supplemental Figure S1: Enrichment of OAHFA from meibum by TLC. Synthetic WE 18:1/18:0 (red), OAHFA 18:1(*n*-9, *cis*)/*ω*-*O*-16:0 (blue) and OAHFA 18:1(*n*-9, *cis*)/*ω*-O-32:1(*n*-7, *cis*) **2** (orange) were run alongside whole meibum to identify the retention factor (R_f) of meibum OAHFA.

1.2 OAHFA synthesis

1.2.1 General

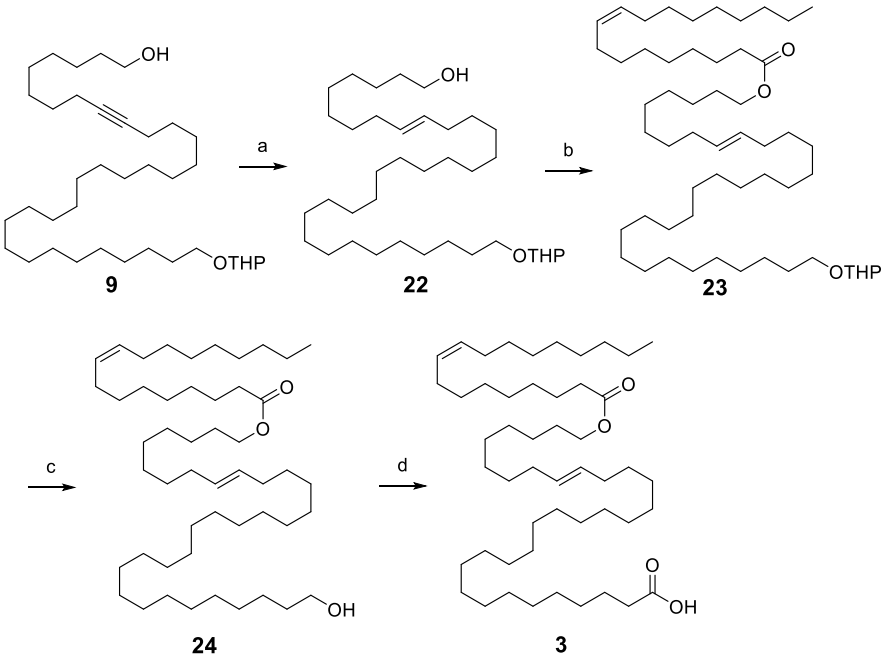
Column chromatography was performed using Merck silica gel 60 (230-400 mesh). Reaction monitoring by TLC was carried out using Silicycle Silica Gel 60 F_{254} (0.2 mm) plates. Compounds were visualized by examination under UV light and/or by staining with anisaldehyde. Infrared (IR) spectra were recorded on neat samples using a Nicolet Avatar 360 FT-IR spectrometer. Characteristic absorption bands are quoted in wavenumbers [cm^{-1}]. ^{1}H and ^{13}C NMR spectra were recorded on a Varian-Inova-500 MHz spectrometer. Chemical shifts (δ) are relative to tetramethylsilane (TMS) at 0 ppm. High resolution electrospray ionisation mass spectra (HRMS) were recorded using a factory modified Waters QToF Ultima Mass Spectrometer (Wilmslow, UK). Melting points were determined using a Griffin melting point apparatus and are uncorrected.

1.2.2 Synthesis of OAHFA 18:1(*n*-9, *cis*)/*ω*-*O*-32:1(*n*-7, *cis*) (2)



Supplemental Scheme S1: Synthesis of OAHFA 18:1(*n*-9, *cis*)/*ω*-*O*-32:1(*n*-7, *cis*) **2**. Reagents and conditions: (a) (i) Mg, THF, 4 h, reflux then 1,4-dibromobutane in THF, Li_2CuCl_4, -10 ºC to rt, 12 h; (ii) TsOH, methanol, reflux, 16 h, 57% (over two steps); (b) HBr, cyclohexane, reflux, 6 h, 64%; (c) Dihydropyran, TsOH, THF, 0 ºC-rt, 16 h, 82%; (d) (i) tert-butyldimethyl(oct-7-yn-1-yloxy)silane **17**, *n*-BuLi, THF-HMPA, -78 ºC to -40 ºC, 1 h; **16** in THF, TBAI, -78 ºC to 60 ºC, 24 h (ii) TBAF, THF, 0 ºC-rt, 12 h, 56% (over two steps); (e) Lindlar catalyst, quinoline, benzene, rt, 12 h, 92%; (f) oleic acid, DCC, DMAP, CH_2Cl_2, 0 ºC-rt, 24 h, 72%; (g) CSA, methanol-THF, 0 ºC-rt, 12 h, 82%; (h) Jones´ reagent, acetone, rt, 1 h, 87%.

1.2.3 Synthesis of OAHFA 18:1(n-9, *cis*)/*ω*-O-32:1(n-9, *trans*) (3)



Supplemental Scheme S2: Synthesis of OAHFA 18:1(*n*-9, *cis*)/*ω*-*O*-32:1(*n*-9, *trans*) **3**. Reagents and conditions: (a) $LiAlH_4$, diglyme, 130 ºC, 24 h, 62%; (b) oleic acid, DCC, DMAP, CH_2Cl_2, 0 ºC-rt, 24 h, 74%; (c) CSA, methanol-THF, 0 ºC-rt, 12 h, 92%; (d) Jones´ reagent, acetone, rt, 1 h, 86%.

1.2.4 Synthesis of OAHFA 18:1(*n*-9, *cis*)/*ω*-*O*-16:0

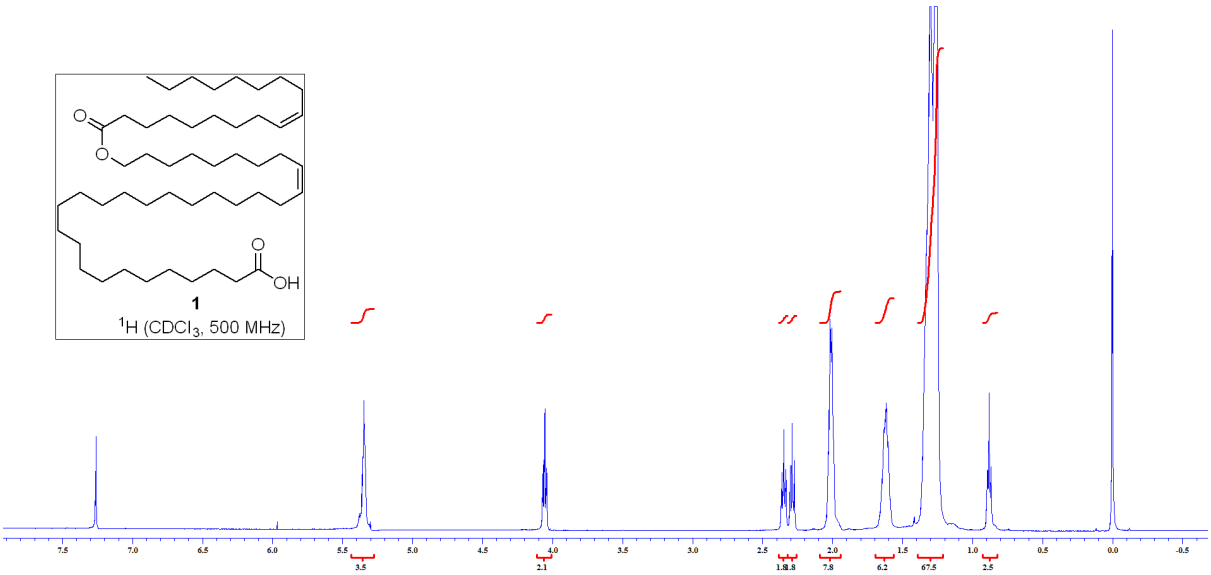
This OAHFA was synthesized as described previously.(1)

1.2.5 Experimental procedures and compound characterisation

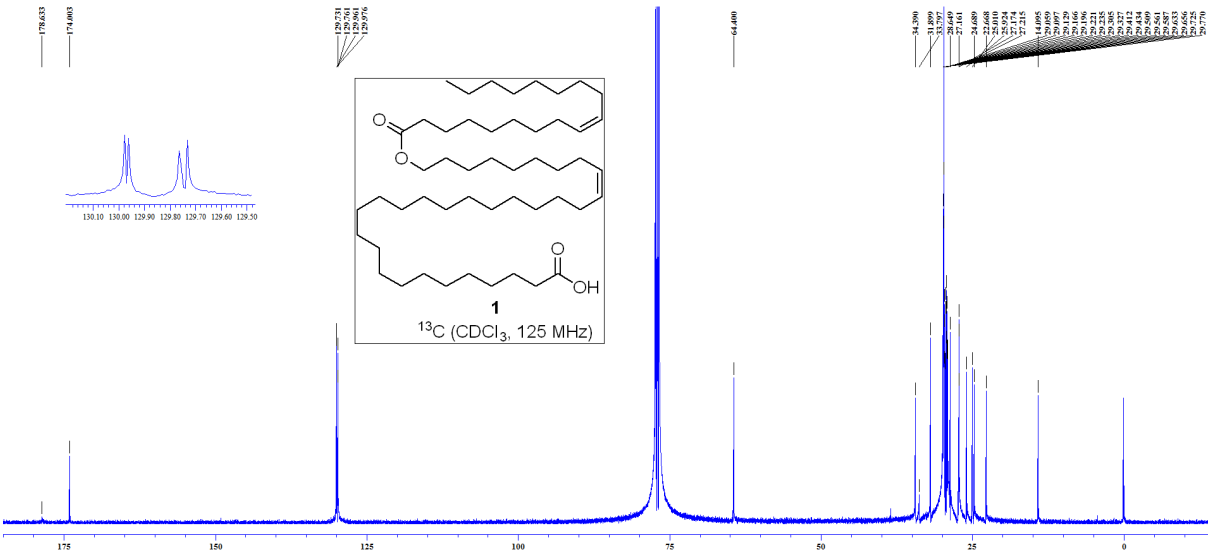
(Z)-32-(Oleoyloxy)dotriacont-23-enoic acid (1)

Jones' reagent (0.056 mL, 2.5 M, 0.14 mmol) was added dropwise to a solution of alcohol **12** (70 mg, 0.09 mmol) in acetone (2 mL) at rt and the mixture was stirred for 1 hour. Excess Jones' reagent was neutralized with 0.1 mL of isopropanol and the mixture was filtered over a pad of celite (washed with 5 mL of ethyl acetate, EtOAc). The organic layer was washed with saturated sodium chloride (5 mL), dried over anhydrous sodium sulfate and concentrated *in*

vacuo. The crude product was purified by flash chromatography on silica gel (EtOAc:Pet. sp. 1:9) to give **1** (62 mg, 87%) as a white solid. M.P. 58-60 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, 3H, *J*=6.3 Hz), 1.22-1.40 (m, 66H), 1.56-1.68 (m, 6H), 1.92-2.06 (m, 8H), 2.29 (t, 2H, *J*= 7.3 Hz), 2.35 (t, 2H, *J*=7.3 Hz), 4.06 (t, 2H, *J*=6.8 Hz), 5.30-5.40 (m, 4H) (Supplemental Figure S2). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 22.7, 24.7, 25.0, 25.9, 27.16, 27.17, 27.2, 28.6, 29.06, 29.1, 29.13, 29.17, 29.2, 29.22, 29.24, 29.3, 29.33, 29.4, 29.43, 29.5, 29.56, 29.59, 29.6, 29.66, 29.7, 29.77, 31.9, 33.8, 34.4, 64.4, 129.7, 129.8, 129.96, 129.98, 174.0, 178.6 (Supplemental Figure S3). IR(neat) ν : 3736, 2916, 2849, 2156, 2020, 1979, 1725, 1693, 1471, 1426, 1378, 1337, 1298, 1254, 1208, 1178, 1070, 949, 823, 718, 682, 656, 623 cm^{-1}. HRMS calculated for $C_{50}H_{95}O_4$ $[M+H]^+$ 759.7230, found 759.7246.HI Da



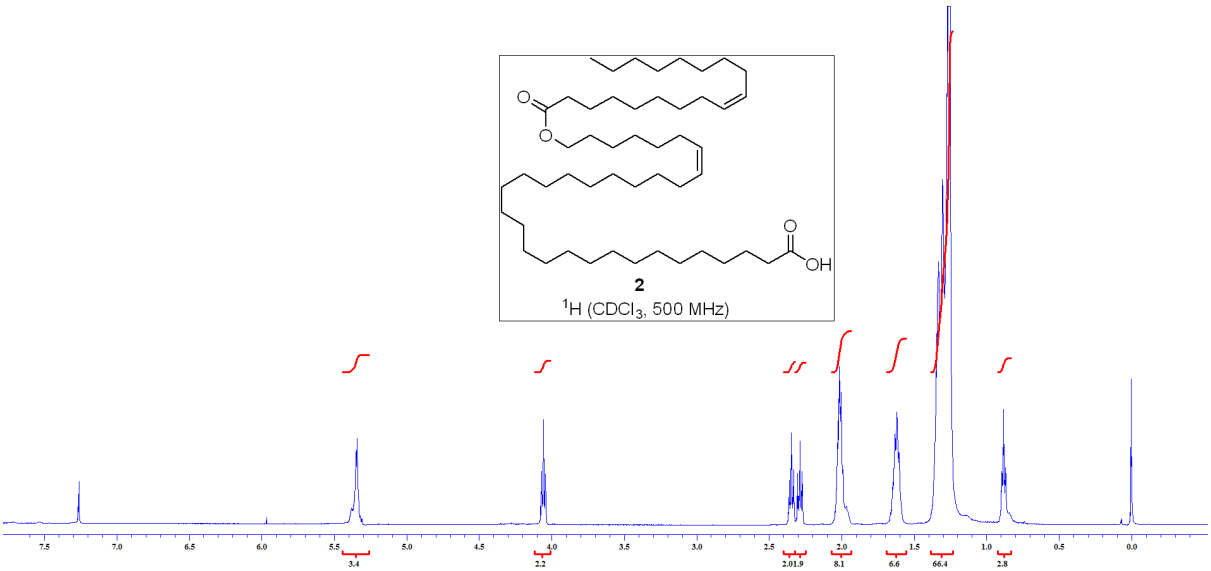
Supplemental Figure S2: ^{1}H NMR of synthetic OAHFA 18:1(n-9, *cis*)/*ω*-O-32:1(n-9, *cis*) **1**



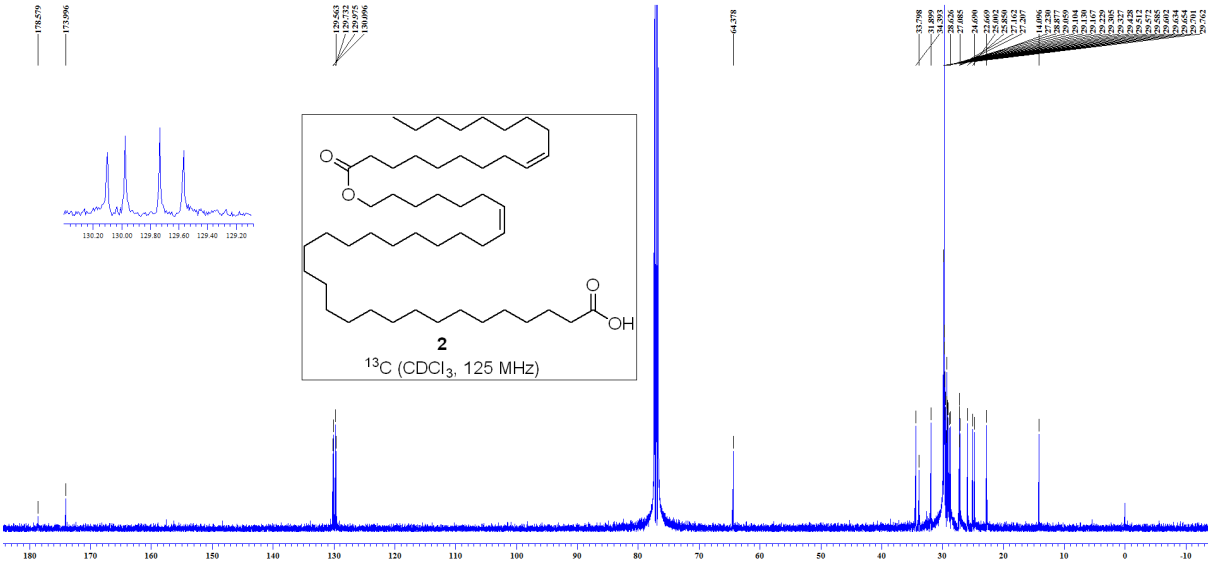
Supplemental Figure S3: ^{13}C NMR of synthetic OAHFA 18:1(n-9, *cis*)/*ω*-O-32:1(n-9, *cis*) **1**

(Z)-32-(Oleoyloxy)dotriacont-25-enoic acid (2)

Jones´ oxidation of alcohol **21** (80 mg, 0.107 mmol) using the method described for **1** afforded **2** (70 mg, 87% yield) as a white solid. M.P. 52-54 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 0.88 (t, 3H, *J*=6.3 Hz), 1.20-1.40 (m, 66H), 1.56-1.68 (m, 6H), 1.92-2.06 (m, 8H), 2.29 (t, 2H, *J*= 7.3 Hz), 2.35 (t, 2H, *J*=7.3 Hz), 4.06 (t, 2H, *J*=6.8 Hz), 5.30-5.40 (m, 4H) (Supplemental Figure S4). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 22.7, 24.7, 25.0, 25.9, 27.09, 27.16, 27.2, 27.23, 28.6, 28.9, 29.06, 29.1, 29.13, 29.17, 29.2, 29.3, 29.33, 29.4, 29.5, 29.57, 29.59, 29.6, 29.63, 29.65, 29.7, 29.8, 31.9, 33.8, 34.4, 64.4, 129.6, 129.7, 130.0, 130.1, 174.0, 178.6 (Supplemental Figure S5). IR(neat) ν : 3355, 3017, 2923, 2852, 1711, 1465, 1215, 1180, 751, 667 cm^{-1}. HRMS calculated for $C_{50}H_{93}O_4$ $[M-H]^-$ 757.7074, found 757.7108.



Supplemental Figure S4: ^{1}H NMR of synthetic OAHFA 18:1(n-9, *cis*)/*ω*-O-32:1(n-7, *cis*) **2**

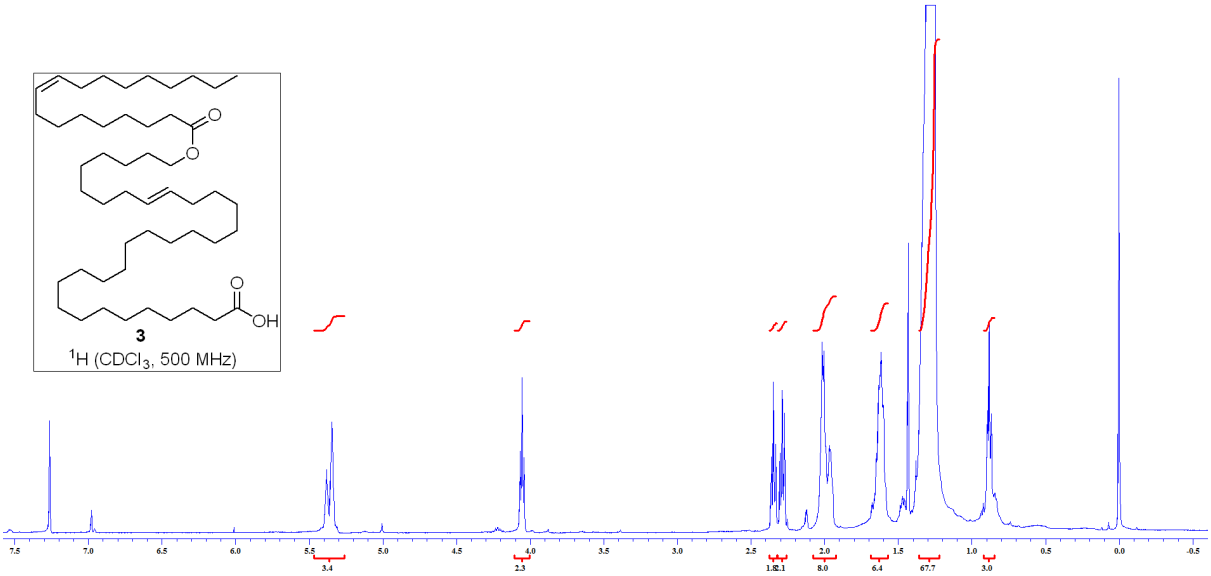


Supplemental Figure S5: ^{13}C NMR of synthetic OAHFA 18:1(n-9, *cis*)/*ω*-O-32:1(n-9, *cis*) **2**

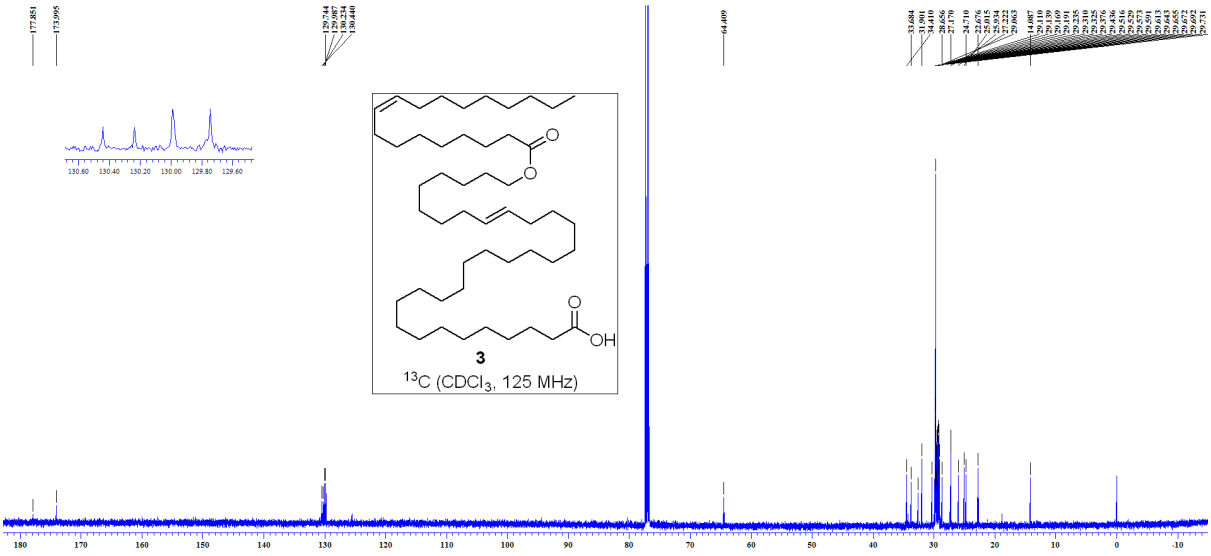
(E)-32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-9-en-1-yl oleate (3)

Jones´ oxidation of alcohol **24** (40 mg, 0.05 mmol) using the method described for **1** afforded **3** (35 mg, 86%) as a white solid. M.P. 62-65 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, 3H, *J*=6.3 Hz), 1.16-1.40 (m, 66H), 1.56-1.70 (m, 6H), 1.92-2.06 (m, 8H), 2.29 (t, 2H, *J*=7.3 Hz),

2.35 (t, 2H, *J*=7.3 Hz), 4.05 (t, 2H, *J*=6.6 Hz), 5.32-5.40 (m, 4H) (Supplemental Figure S6). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 22.7, 24.7, 25.0, 25.9, 27.2, 27.22, 28.7, 29.06, 29.1, 29.14, 29.17, 29.19, 29.2, 29.3, 29.36, 29.38, 29.4, 29.5, 29.53, 29.57, 29.59, 29.6, 29.64, 29.66, 24.69, 29.73, 30.3, 31.9, 32.6, 33.7, 34.4, 64.4, 129.7, 130.0, 130.2, 130.4, 174.0, 177.9 (Supplemental Figure S7). IR(neat) ν : 3741, 2916, 2849, 2160, 2029, 1979, 1726, 1692, 1538, 1472, 1428, 1378, 1297, 1253, 1208, 1180, 1071, 965, 824, 717, 680 cm^{-1}. HRMS calculated for $C_{50}H_{93}O_4$ $[M-H]^-$ 757.7074, found 757.7070.



Supplemental Figure S6: ^{1}H NMR of synthetic OAHFA 18:1(n-9, *cis*)/*ω*-O-32:1(n-9, *trans*) **3**



Supplemental Figure S7: ^{13}C NMR of synthetic OAHFA 18:1(n-9, *cis*)/*ω*-O-32:1(n-9, *trans*) **3**

2-((11-Bromoundecyl)oxy)tetrahydro-2H-pyran (4)

p-Toluenesulfonic acid (TsOH, 7 mg, 0.039 mmol) was added to a stirring solution containing 11-bromoundecan-1-ol (2.0 g, 7.96 mmol) and dihydropyran (1.08 mL, 11.94 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred overnight. The reaction mixture was diluted with water (10 mL) and the mixture extracted with CH_2Cl_2 (2 × 10 mL), washed with saturated sodium chloride (10 mL) and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the crude residue was purified by flash chromatography on silica gel (2% EtOAc in Pet. sp) to give **4** (2.53 g, 95%) as a colourless liquid. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.25-1.38 (m, 12H), 1.38-1.46 (m, 2H), 1.48-1.62 (m, 6H), 1.68-1.75 (m, 1H), 1.80-1.88 (m, 3H), 3.36-3.42 (m, 3H), 3.46-3.52 (m, 1H), 3.70-3.76 (m, 1H), 3.84-3.90 (m, 1H), 4.56-4.59 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 19.7, 25.5, 26.2, 28.2, 28.7, 29.4, 29.4, 29.5, 29.7, 30.8, 32.8, 34.0, 62.3, 67.7, 98.8. IR(neat) ν : 2927, 2856, 2307, 1215, 1117, 1076, 1024, 905, 868, 748, 668 cm^{-1}. HRMS calculated for $C_{16}H_{31}O_2BrNa$ $[M+Na]^+$ 357.1405, found 357.1401.

Docosane-1,22-diol (5)

To a suspension of Mg turnings (79 mg, 3.28 mmol) in dry tetrahydrofuran (THF, 5 mL) under argon was added **4** (1.0 g, 2.982 mmol). A drop of 1, 2 dibromoethane was added and the reaction mixture was heated at reflux for 4 h. The mixture was cooled to –10 °C and freshly prepared Li_2CuCl_4 (0.29 mL, 0.1 M in THF) and a solution of **4** (1.09 g, 3.280 mmol) in THF (5 mL) were added. The temperature was slowly increased to rt and after 12 h the mixture was poured into a saturated solution of ammonium chloride (10 mL). The aqueous phase was extracted with EtOAc (2 × 10 mL) and the combined organic phases were washed with saturated sodium chloride (10 mL), dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude mixture was dissolved in anhydrous methanol (20 mL) and heated at reflux overnight in the presence of TsOH (5 mg). After removing the solvent under vacuum the residue was recrystallised from heptane to yield **5** (632 mg, 62%) as a white solid. M.P. 98-100 ºC; Lit.(2) 96-98 ºC; ^{1}H NMR (500 MHz, CD_3OD) δ: 1.20-1.36 (m, 36H), 1.48-1.58 (m, 4H), 3.55 (t, 4H, *J*=6.6 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ: 25.7, 29.4, 29.7, 32.7, 63.2. IR(neat) ν : 3650, 3283, 3121, 2917, 2849, 2350, 2321, 1967, 1956, 1420, 1363, 1060, 1006, 838, 719, 604 cm^{-1}. HRMS calculated for $C_{22}H_{47}O_2$ $[M+H]^+$ 343.3576, found 343.3572.

22-Bromodocosan-1-ol (6)

To a stirred solution of diol **5** (500 mg, 1.46 mmol) in cyclohexane (5 mL) was added 48% HBr (0.29 mL, 1.75 mmol). The mixture was heated at reflux for 6 h before the reaction mixture was concentrated *in vacuo*, diluted with water (20 mL) and extracted with chloroform (2 × 10 mL). The organic layers were combined, dried over anhydrous sodium sulfate and concentrated. The crude residue was purified by flash chromatography on silica gel (1:4 EtOAc:Pet. sp.) to give **6** (408 mg, 69%) as a pale yellow solid. M.P. 67-69 ºC; Lit.(3) 66-68 ºC; ^{1}H NMR (500

MHz, $CDCl_3$) δ: 1.24-1.36 (m, 34H), 1.38-1.46 (m, 2H), 1.54-1.62 (m, 2H), 1.82-1.89 (m, 2H), 3.41 (t, 2H, *J* = 6.8 Hz), 3.64 (t, 2H, *J* = 6.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 25.7, 28.2, 28.8, 29.4, 29.5, 29.6, 29.7, 32.8, 32.8, 34.0, 63.1. IR(neat) ν : 3728, 3118, 2916, 2849, 2358, 2312, 2028, 2023, 1993, 1950, 1911, 1424, 1420, 1006, 838, 719 602 cm^{-1}. HRMS calculated for $C_{22}H_{46}OBr$ $[M+H]^+$ 405.2732, found 405.2746.

2-((22-Bromodocosyl)oxy)tetrahydro-2H-pyran (7)

TsOH (2 mg, 0.009 mmol) was added to a solution containing 22-bromodocosan-1-ol **6** (400 mg, 0.986 mmol) and dihydropyran (DHP, 0.1 mL, 1.18 mmol) in anhydrous THF (5 mL) at 0 ^oC. The solution was allowed to warm to room temperature and stirred overnight. The mixture was diluted with water (5 mL), extracted with EtOAc (2 × 5 mL), washed with saturated sodium chloride soultion (5 mL) and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and the crude residue purified by flash chromatography on silica gel (2% EtOAc in Pet. sp.) to afford **7** (444 mg, 92%) as a white solid. M.P. 42-45 ºC; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.18-1.37 (m, 35H), 1.39-1.46 (m, 1H), 1.48-1.62 (m, 6H), 1.66-1.76 (m, 1H), 1.78-1.90 (m, 3H), 3.35-3.44 (m, 3H), 3.46-3.53 (m, 1H), 3.69-3.76 (m, 1H), 3.84-3.92 (m, 1H), 4.56-4.61 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 19.7, 25.5, 26.2, 28.8, 29.43, 29.5, 29.5, 29.6, 29.6, 29.7, 29.8, 29.8, 30.8, 32.8, 34.0, 62.3, 67.7, 98.8. IR(neat) ν : 3123, 2916, 2849, 2355, 2314, 2028, 2017, 1993, 1979, 1943, 1923, 1472, 1424, 1355, 1062, 1033, 1006, 839, 718, 644, 603 cm^{-1}. HRMS calculated for $C_{27}H_{53}O_2BrNa$ $[M+Na]^+$ 511.3127, found 511.3134.

32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-9-yn-1-ol (9)

To a solution of *tert*-butyl(dec-9-yn-1-yloxy)dimethylsilane(4) **8** (154 mg, 0.57 mmol) in THF (5 mL) and hexamethylphosphoramide (HMPA) (1 mL) at -78 °C under argon was slowly added *n*-butyllithium (*n*-BuLi,1.6 M in hexane) (0.43 mL, 0.69 mmol). The temperature was

raised to -40 ºC and maintained for 60 minutes. The reaction mixture was again cooled to -78 °C and 2-((22-bromodocosyl)oxy)tetrahydro-2H-pyran **7** (421 mg, 0.86 mmol) in THF (2 mL) was added dropwise. After complete addition of **7** the temperature was allowed to warm slowly to rt. At this stage tetrabutylammonium iodide (TBAI, 2.0 mg, 0.005 mmol) was added and the mixture stirred at 50 °C for 24 h. The reaction was quenched by addition of saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with saturated sodium chloride (10 mL), dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was redissolved in anhydrous THF (5 mL) and a solution containing tetrabutylammonium fluoride (TBAF, 1.0 M in THF, 1.15 mL, 1.15 mmol) was added at 0 °C. After 5 min the ice-bath was removed and the reaction stirred at rt for 12 h. Water (5 mL) was added and the mixture was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel (EtOAc:Pet. sp. 1:9) to give the alkynol **9** (206 mg, 64%) as a white solid. M.P. 57-59 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.18-1.40 (m, 44H), 1.42-1.62 (m, 12H), 1.66-1.76 (m, 1H), 1.78-1.87 (m, 1H), 2.13 (t, 4H, *J*=6.8 Hz), 3.35-3.42 (m, 1H), 3.47-3.53 (m, 1H), 3.64 (t, 2H, *J*= 6.3 Hz), 3.70-3.76 (m, 1H), 3.84-3.91 (m, 1H), 4.56-4.60 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 18.7, 18.8, 19.7, 25.5, 25.7, 26.2, 28.8, 28.9, 29.1, 29.1, 29.2, 29.3, 29.5, 29.6, 29.6, 29.61, 29.6, 29.7, 29.7, 29.7, 29.8, 30.8, 32.8, 62.3, 63.1, 67.7, 80.1, 80.3, 98.8. IR(neat) ν : 3727, 3574, 3018, 2927, 2855, 2312, 1512, 1215, 1121, 1075, 1022, 868, 747, 668 cm^{-1}. HRMS calculated for $C_{37}H_{70}O_3Na$ $[M+Na]^+$ 585.5223, found 585.5197.

(Z)-32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-9-en-1-ol **(10)**

Quinoline (2 mg) was added to a solution of alkynol **9** (106 mg, 0.19 mmol) in benzene (3 mL) at room temperature. After stirring for 5 min, Lindlar catalyst (5 mg) was added and the reaction

was placed under H_2 (1 atm) and stirred for 12 h. The reaction mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel (10% EtOAc in Pet. sp.) to give the (*Z*)-alkenol **10** (104 mg, 98%) as a white solid. M.P. 46-48 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.22-1.38 (m, 48H), 1.48-1.62 (m, 8H), 1.68-1.74 (m, 1H), 1.78-1.88 (m, 1H),1.94-2.08 (m, 4H), 3.34-3.41 (m, 1H), 3.47-3.53 (m, 1H), 3.61-3.67 (m, 2H), 3.70-3.76 (m, 1H), 3.84-3.90 (m, 1H), 4.56-4.60 (m, 1H), 5.30-5.38 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 19.7, 25.5, 25.7, 26.3, 27.1, 27.2, 29.1, 29.4, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7, 29.7, 29.8, 30.8, 32.8, 62.3, 63.1, 67.2, 98.9, 129.7, 130.1. IR(neat) ν : 3580, 3018, 2926, 2854, 2377, 2320, 1723, 1675, 1499, 1215, 1076, 1027, 864, 749, 668 cm^{-1}. HRMS calculated for $C_{37}H_{72}O_3Na$ $[M+Na]^+$ 587.5379, found 587.5399.

(Z)-32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-9-en-1-yl oleate (11)

To a stirred solution of (*Z*)-alkenol **10** (92 mg, 0.16 mmol) in anhydrous CH_2Cl_2 (3 mL) under argon was added 4-dimethylaminopyridine (DMAP, 9 mg, 0.08 mmol) and dicyclohexylcarbodiimide (DCC, 83 mg, 0.41 mmol). After cooling to 0 °C, oleic acid (0.06 mL, 0.19 mmol) was added and the ice bath was removed. The reaction was allowed to warm to room temperature and stirred for 24 h. Water (4 mL) was then added along with CH_2Cl_2 (10 mL). The organic layer was washed with more water (2 x 5 mL) and the combined aqueous phases were extracted with CH_2Cl_2 (2 x 5 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel (EtOAc:Pet. sp, 0:100 to 2:98) to give **11** (105 mg, 78%) as a white waxy solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, 3H, *J*=6.3 Hz), 1.20-1.38 (m, 70H), 1.48-1.64 (m, 8H), 1.68-1.74 (m, 1H), 1.78-1.88 (m, 1H), 1.92-2.06 (m, 8H), 2.29 (t, 2H, *J*=7.3 Hz), 3.34-3.40 (m, 1H), 3.45-3.53 (m, 1H), 3.70-3.77 (m, 1H), 3.84-3.90 (m, 1H), 4.05 (t, 2H, *J*=6.8 Hz), 4.56-4.60 (m, 1H), 5.30-5.40 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 19.7,

22.7, 25.0, 25.5, 25.9, 26.2, 27.2, 27.2, 27.2, 28.6, 29.1, 29.1, 29.2, 29.2, 29.2, 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7, 29.7, 29.7, 29.8, 29.8, 30.8, 31.9, 34.4, 38.5, 62.3, 64.4, 67.7, 98.8, 129.71, 129.74, 129.94, 129.95, 173.9. IR(neat) ν : 3112, 3080, 2916, 2849, 2366, 2028, 1993, 1988, 1967, 1950, 1923, 1911, 1700, 1696, 1472, 1424, 1178, 1063, 1006, 945, 838, 718, 607 cm^{-1}. HRMS calculated for $C_{55}H_{104}O_4Na$ $[M+Na]^+$ 851.7832, found 851.7874.

(Z)-32-Hydroxydotriacont-9-en-1-yl oleate (12)

Tetrahydropyran (THP)-protected alcohol **11** (90 mg, 0.11 mmol) was dissolved in THF:methanol (1:4, 3 mL) at 0 ºC and camphorsulfonic acid (CSA, 3 mg) was added. The reaction was stirred at rt for 12 h before the solvents were removed *in vacuo*. The residue was diluted with water and extracted with chloroform (2 × 10 mL). The combined organic layers were washed with saturated sodium chloride (5 mL), dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel (12% EtOAc in Pet. sp.) to give the alcohol **12** (76 mg, 95 %) as a white solid. M.P. 55-57 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.89 (t, 3H, *J*=6.8 Hz), 1.20-1.38 (m, 66H), 1.52-1.66 (m, 8H), 1.94-2.06 (m, 8H), 2.28 (t, 2H, *J*=7.3 Hz), 3.61-3.68 (m, 2H), 4.05 (t, 2H, *J*=6.8 Hz), 5.30-5.40 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 22.7, 25.0, 25.7, 25.9, 27.2, 27.2, 28.7, 29.1, 29.1, 29.2, 29.2, 29.2, 29.3, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7, 29.7, 29.7, 29.8, 31.9, 32.8, 34.4, 38.5, 63.1, 64.4, 129.7, 129.8, 129.9, 130.0, 174.0. IR(neat) ν : 3590, 3291, 3120, 2916, 2849, 2311, 1967, 1962, 1957, 1724, 1473, 1424, 1363, 1178, 1059, 1006, 839, 718, 601 cm^{-1}. HRMS calculated for $C_{50}H_{96}O_3Na$ $[M+Na]^+$ 767.7257, found 767.7261.

Tetracosane-1,24-diol (14)

To a suspension of Mg turnings (136 mg, 5.60 mmol) in dry THF (10 mL) under argon atmosphere was added 2-((10-bromodecyl)oxy)tetrahydro-2H-pyran **13**(5) (1.2 g, 3.73 mmol).

A drop of 1,2-dibromoethane was added and the reaction mixture was heated at reflux for 4 h. The mixture was cooled to –10 °C and freshly prepared Li_2CuCl_4 (0.5 mL, 0.1 M in THF) and a solution of 1,4-dibromobutane (403 mg, 1.867 mmol) in THF (3 mL) were added. The temperature was slowly increased to rt. After 12 h, the resulting mixture was poured into a saturated solution of ammonium chloride (10 mL) and extracted with EtOAc (2 × 10 mL). The combined organic extracts were washed with brine solution (10 mL), dried over sodium sulphate and concentrated under reduced pressure. The crude mixture was dissolved in dry methanol (5 mL) and heated at reflux for 3 h in the presence of TsOH (5 mg). The hot suspension was filtered and the solids recrystallised from heptane to give tetracosane-1,24-diol **14** (399 mg, 57% over two steps) as a white solid. M.P. 106-108 ºC; Lit.(6) 106-107.5 ºC. ^{1}H NMR (500 MHz, CD_3OD) δ : 1.20-1.39 (m, 40H), 1.49-1.58 (m, 4H), 3.57 (t, 4H, *J*=6.3 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ: 26.2, 29.1, 30.03, 30.08, 32.9, 62.6. IR(neat) ν : 3650, 3270, 2916, 2848, 1457, 1214, 1060, 746 cm^{-1}. HRMS calculated for $C_{24}H_{50}O_2Na$ $[M+Na]^+$ 393.3709, found 393.3717.

24-Bromotetracosan-1-ol (15)

Bromination of tetracosane-1,24-diol **14** (350 mg, 0.944 mmol) using the method described for compound **6** afforded **15** (262 mg, 64%) as a white solid. M.P. 68-70 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 1.20-1.38 (m, 38H), 1.39-1.47 (m, 2H), 1.52-1.62 (m, 2H), 1.81-1.99 (m, 2H), 3.40 (t, 2H, *J*= 6.8 Hz), 3.64 (t, 2H, *J*=6.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 25.7, 28.2, 28.8, 29.4, 29.5, 29.59, 29.60, 29.64, 29.67, 29.69, 32.8, 32.9, 34.0, 63.1. IR(neat) ν : 3294, 2916, 2849, 1462, 1215, 1059, 751, 731, 668 cm^{-1}. HRMS calculated for $C_{24}H_{49}BrONa$ $[M+Na]^+$ 455.2864, found 455.2772.

2-((24-Bromotetracosyl)oxy)tetrahydro-2H-pyran (16)

THP-protection of **15** (250 mg, 0.576 mmol) using the method described for compound **7** afforded **16** (244 mg, 82%) as a white solid. M.P. 44-46 ºC; Lit.(7) 45.5-46.5 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.10-1.46 (m, 40H), 1.46-1.62 (m, 6H), 1.64-1.75 (m, 1H), 1.76-1.89 (m, 3H), 3.34-3.46 (m, 3H), 3.46-3.52 (m, 1H), 3.68-3.76 (m, 1H), 3.82-3.90 (m, 1H), 4.56-4.60 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 19.6, 25.5, 26.2, 28.2, 28.7, 29.4, 29.46, 29.50, 29.57, 29.58, 29.62, 29.66, 29.72, 30.8, 32.8, 33.8, 62.2, 67.6, 98.7. IR(neat) ν : 3013, 2925, 2853, 2332, 1457, 1363, 1215, 1133, 1076, 1027, 907, 859, 732, 718, 668 cm^{-1}. HRMS calculated for $C_{29}H_{57}BrO_2Na$ $[M+Na]^+$ 539.3440, found 539.3428.

32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-7-yn-1-ol (18)

To a solution of tert-butyldimethyl(oct-7-yn-1-yloxy)silane **17** (122 mg, 0.509 mmol) in THF (3 mL) and HMPA (1 mL) at -78 °C was slowly added *n*-BuLi (1.6 M in hexane) (0.31 mL). The temperature was raised to -40 ºC and maintained for 60 minutes before being cooled again to -78 °C. Bromide **16** (240 mg, 0.463 mmol) in THF (2 ml) was then added dropwise and the temperature allowed to rise slowly to room temperature. TBAI (2 mg, 0.004 mmol) was added and the reaction was heated at 50°C for 24 hours. The reaction was quenched by addition of saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was re-dissolved in dry THF (5 mL) and TBAF (1.0 M in THF, 1.63 mL, 1.63 mmol) was added at 0 °C. After 5 min the ice-bath was removed and the reaction stirred at rt for 12h. After quenching by addition of water (3 mL) the mixture was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Purification of the crude residue by silica gel flash column

chromatography (EtOAc:Pet.sp: 1:9) afforded **18** (145 mg, 56% over 2 steps) as a white solid. M.P. 53-55 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 1.12-1.40 (m, 44H), 1.42-1.64 (m, 12H), 1.66-1.76 (m, 1H), 1.78-1.87 (m, 1H), 2.10-2.18 (m, 4H), 3.34-3.40 (m, 1H), 3.45-3.54 (m, 1H), 3.64 (t, 2H, *J*= 6.3 Hz), 3.70-3.74 (m, 1H), 3.82-3.90 (m, 1H), 4.54-4.60 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 18.7, 18.8, 19.7, 25.2, 25.5, 26.2, 28.6, 28.9, 29.1, 29.2, 29.5, 29.56, 29.60, 29.61, 29.63, 29.66, 29.67, 29.69, 29.7, 30.8, 32.7, 62.3, 63.0, 67.7, 80.0, 80.4, 98.8. IR(neat) ν : 3650, 3019, 2927, 2855, 2370, 1457, 1214, 1118, 1072, 1036, 746, 668, 618 cm^{-1}. HRMS calculated for $C_{37}H_{70}O_3Na$ $[M+Na]^+$ 585.5223, found 585.5248.

(Z)-32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-7-en-1-ol (19)

Lindlar reduction of **18** (140 mg, 0.248 mmol) using the method described for compound **10** gave **19** (129 mg, 92%) as a white solid. M.P. 49-51 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 1.08-1.42 (m, 48H), 1.48-1.64 (m, 8H), 1.66-1.74 (m, 1H), 1.78-1.88 (m, 1H),1.92-2.08 (m, 4H), 3.34-3.40 (m, 1H), 3.46-3.54 (m, 1H), 3.64 (t, 2H, *J*= 6.3 Hz), 3.70-3.75 (m, 1H), 3.82-3.90 (m, 1H), 4.54-4.60 (m, 1H), 5.30-5.40 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 19.7, 25.5, 25.6, 26.2, 27.1, 27.2, 29.1, 29.3, 29.5, 29.52, 29.57, 29.60, 29.61, 29.65, 29.67, 29.7, 29.76, 30.8, 32.8, 62.3, 63.0, 67.7, 98.8, 129.7, 130.0. IR(neat) ν : 3590, 3019, 2927, 2856, 2328, 1680, 1465, 1214, 1074, 1032, 748, 668 cm^{-1}. HRMS calculated for $C_{37}H_{72}O_3Na$ $[M+Na]^+$ 587.5379, found 587.5386.

(Z)-32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-7-en-1-yl oleate (20)

Acylation of **19** (120 mg, 0.212 mmol) with oleic acid using the method described for compound **11** afforded **20** (126 mg, 72% yield) as a white waxy solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 0.88 (t, 3H, *J*= 6.3 Hz), 1.10-1.45 (m, 70H), 1.46-1.64(m, 8H), 1.66-1.76 (m, 1H), 1.78-1.88 (m, 1H), 1.92-2.08 (m, 8H), 2.29 (t, 2H, *J*= 7.3 Hz), 3.34-3.40 (m, 1H), 3.45-3.52

(m, 1H), 3.70-3.76 (m, 1H), 3.82-3.90 (m, 1H), 4.05 (t, 2H, *J*= 6.8 Hz), 4.56-4.59 (m, 1H), 5.30-5.40 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 19.7, 22.7, 25.0, 25.5, 25.9, 26.3, 27.1, 27.2, 27.22, 27.23, 28.6, 28.9, 29.1, 29.14, 29.2, 29.5, 29.52, 29.53, 29.6, 29.63, 29.66, 29.7, 29.76, 30.8, 31.9, 34.4, 62.3, 64.4, 67.7, 98.8, 129.6, 129.7, 130.0, 130.1, 174.0. IR(neat) ν : 3013, 2926, 2855, 2339, 1680, 1465, 1215, 1119, 1076, 1027, 748, 668 cm^{-1}. HRMS calculated for $C_{55}H_{104}O_4Na$ $[M+Na]^+$ 851.7832, found 851.7873.

(Z)-32-Hydroxydotriacont-7-en-1-yl oleate (21)

THP deprotection of **20 (**120 mg, 0.144 mmol) using the method described for compound **12** afforded **21** (87 mg, 82 %) as a white solid. M.P. 46-48 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 0.90 (t, 3H, *J*= 6.8 Hz), 1.10-1.45 (m, 66H), 1.51-1.66 (m, 8H), 1.92-2.04 (m, 8H), 2.29 (t, 2H, *J*= 7.3 Hz), 3.64 (t, 2H, *J*= 6.8 Hz), 4.05 (t, 2H, *J*= 6.8 Hz), 5.30-5.40 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 22.7, 25.0, 25.7, 25.9, 27.1, 27.2, 27.21, 28.6, 28.9, 29.1, 29.1, 29.2, 29.3, 29.3, 29.4, 29.5, 29.5, 29.57, 29.60, 29.65, 29.7, 29.8, 31.9, 32.8, 34.4, 63.1, 64.4, 129.6, 129.7, 130.0, 130.1, 174.0. IR(neat) ν : 3589, 3017, 2927, 2855, 2328, 1680, 1455, 1216, 748, 668 cm^{-1}. HRMS calculated for $C_{50}H_{96}O_3Na$ $[M+Na]^+$ 767.7257, found 767.7292.

(E)-32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-9-en-1-ol (22)

Compound **9** (90 mg, 0.16 mmol) was dissolved in diglyme (4 mL) under argon at room temperature and $LiAlH_4$ (60 mg, 1.60 mmol) in diglyme (1 mL) was added. The resulting mixture was heated to 130 ºC and stirred for 24 h. The reaction mixture was quenched with aqueous NaOH (0.1 M, 2 mL) and EtOAc (10 mL) was added. The organic layer was separated and dried over anhydrous sodium sulfate before being concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel (10% EtOAc in Pet. sp.) to give **22** (56 mg, 62%) as a white solid. M.P. 49-51 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.08-2.18 (m, 62H),

3.34-3.42 (m, 1H), 3.46-3.54 (m, 1H), 3.64 (t, 2H, *J*=6.8 Hz), 3.70-3.76 (m, 1H), 3.84-3.92 (m, 1H), 4.56-4.60 (m, 1H), 5.32-5.42 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 19.7, 25.5, 25.7, 26.2, 27.2, 27.3, 29.06, 29.10, 29.13, 29.17, 29.22, 29.27, 29.31, 29.45, 29.49, 29.52, 29.55, 29.59, 29.61, 29.66, 29.69, 29.8, 30.8, 32.6, 32.80, 62.30, 63.0, 67.7, 98.8, 130.3, 130.4. IR(neat) ν : 3590, 3019, 2927, 2856, 2328, 1680, 1465, 1214, 1074, 1032, 748, 668 cm^{-1}. HRMS calculated for $C_{37}H_{72}O_3Na$ $[M+Na]^+$ 587.5379, found 587.5386.

(E)-32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-9-en-1-yl oleate (23)

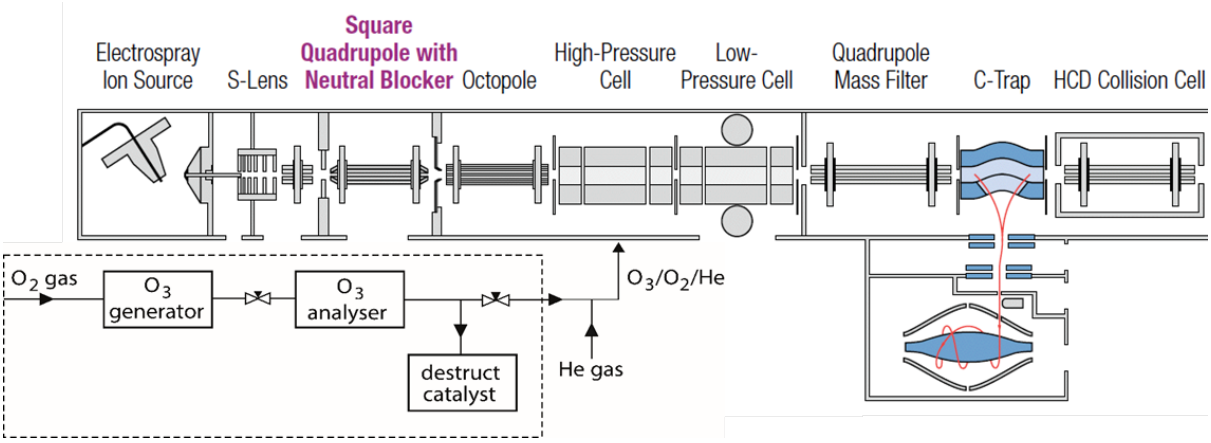
Acylation of *trans* alkenol **22** (52 mg, 0.09 mmol) with oleic acid using the method described for compound **11** afforded ester **23** (56 mg, 74%) as a white solid. M.P. 42-45 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, 3H, *J*=6.3 Hz), 1.20-1.39 (m, 70H), 1.50-1.64 (m, 8H), 1.66-1.76 (m, 1H), 1.78-1.88 (m, 1H), 1.92-2.05 (m, 8H), 2.29 (t, 2H, *J*=7.3 Hz), 3.35-3.42 (m, 1H), 3.46-3.54 (m, 1H), 3.70-3.76 (m, 1H), 3.84-3.92 (m, 1H), 4.05 (t, 2H, *J*=6.8 Hz), 4.56-4.60 (m, 1H), 5.32-5.40 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 19.7, 22.7, 25.0, 26.2, 27.1, 27.2, 28.7, 29.0, 29.10, 29.13, 29.16, 29.18, 29.21, 29.30, 29.32, 29.37, 29.41, 29.49, 29.51, 29.52, 29.59, 29.62, 29.65, 29.68, 29.70, 29.76, 29.78, 30.8, 31.9, 32.6, 34.4, 38.5, 62.3, 64.38, 67.7, 98.8, 129.7, 129.9, 130.2, 130.5, 174.0. IR(neat) ν : 3013, 2926, 2855, 2339, 1680, 1465, 1215, 1119, 1076, 1027, 748, 668 cm^{-1}. HRMS calculated for $C_{55}H_{104}O_4Na$ $[M+Na]^+$ 851.7832, found 851.7873.

(E)-32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-9-en-1-yl oleate (24)

THP deprotection of **23** (50 mg, 0.06 mmol) using the method described for compound **12** afforded **24** (41 mg, 92%) as a white solid. M.P. 56-59 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, 3H, *J*=6.8 Hz), 1.18-1.38 (m, 66H), 1.54-1.66 (m, 8H), 1.94-2.04 (m, 8H), 2.29 (t, 2H, *J*=7.3 Hz), 3.61-3.68 (m, 2H), 4.05 (t, 2H, *J*=6.8 Hz), 5.31-5.41 (m, 4H). ^{13}C NMR (125 MHz,

$CDCl_3$) δ: 14.1, 22.7, 25.0, 25.7, 25.9, 27.16, 27.21, 28.7, 29.05, 29.10, 29.13, 29.17, 29.21, 29.31, 29.37, 29.43, 29.51, 29.57, 29.60, 29.65, 29.7, 29.8, 31.90, 32.6, 32.8, 34.4, 63.1, 64.4, 129.7, 1230.0, 130.2, 130.4, 174.0. IR(neat) ν : 3589, 3017, 2927, 2855, 2328, 1680, 1455, 1216, 748, 668 cm^{-1}. HRMS calculated for $C_{50}H_{96}O_3Na$ $[M+Na]^+$ 767.7257, found 767.7292.

1.3 Mass Spectrometry



Supplemental Figure S8: Schematic of the modified Orbitrap Elite mass spectrometer used in this study. Ozone produced by an external generator is infused into the helium bath gas and delivered to the segmented linear ion trap.

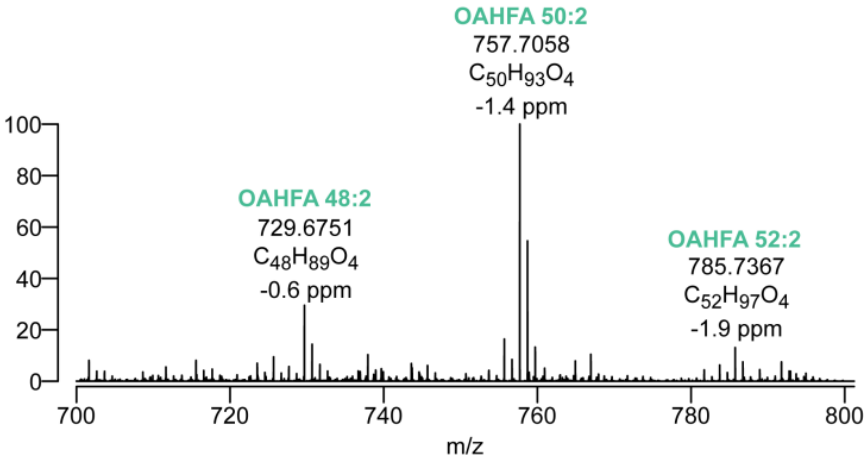
Supplemental Table S1: Predicted neutral losses (or gains) for OzID product ions from monounsaturated lipids showing the dependence on position of a carbon-carbon double bond with respect to the methyl terminus of the hydrocarbon chain (n-x). Columns are shown for a fatty acid (FA) and ω-hydroxy fatty acid (ω-HFA).

DB-position	**Neutral loss (FA)**		**Neutral loss (ω-HFA)**	
n-	Aldehyde	Criegee	Aldehyde	Criegee
1	-2	-18	14	-2
2	12	-4	28	12
3	26	10	42	26
4	40	24	56	40
5	54	38	70	54
6	68	52	84	68
7	82	66	98	82
8	96	80	112	96
9	110	94	126	110
10	124	108	130	124
11	138	122	144	138
12	152	136	168	152

2 Results

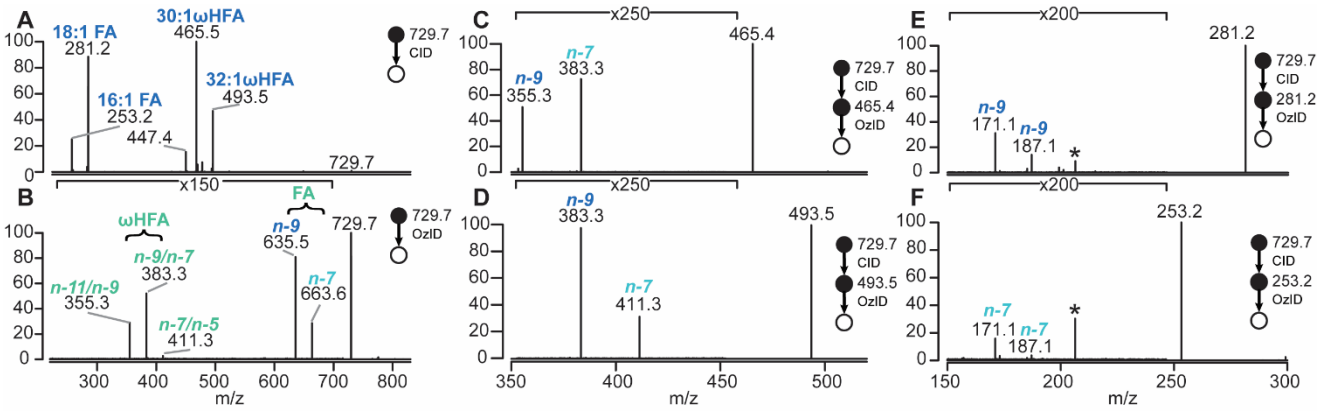
2.1 Structural elucidation of meibum OAHFA 50:2

2.1.1 Negative high-resolution MS of meibum

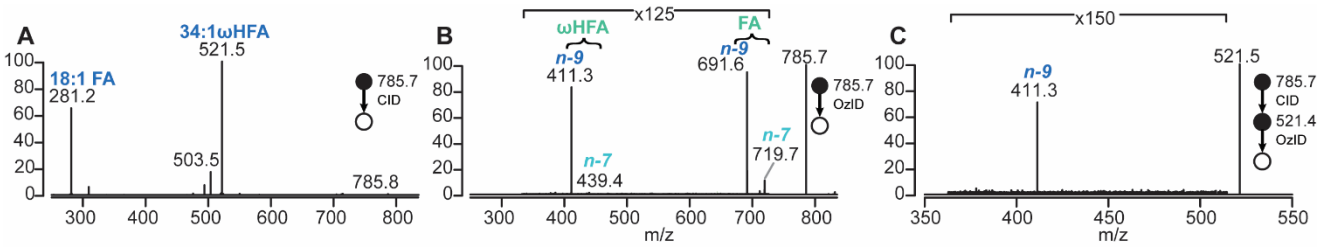


Supplemental Figure S9: Negative ion mass spectrum of pooled human meibum samples showing the three most abundant OAHFA (as $[M-H]^-$): 50:2, *m/z* 757.7; 48:2, *m/z* 729.7; and 52:2, *m/z* 785.7

2.1.2 OzID of meibum OAHFA 48:2 and 52:2

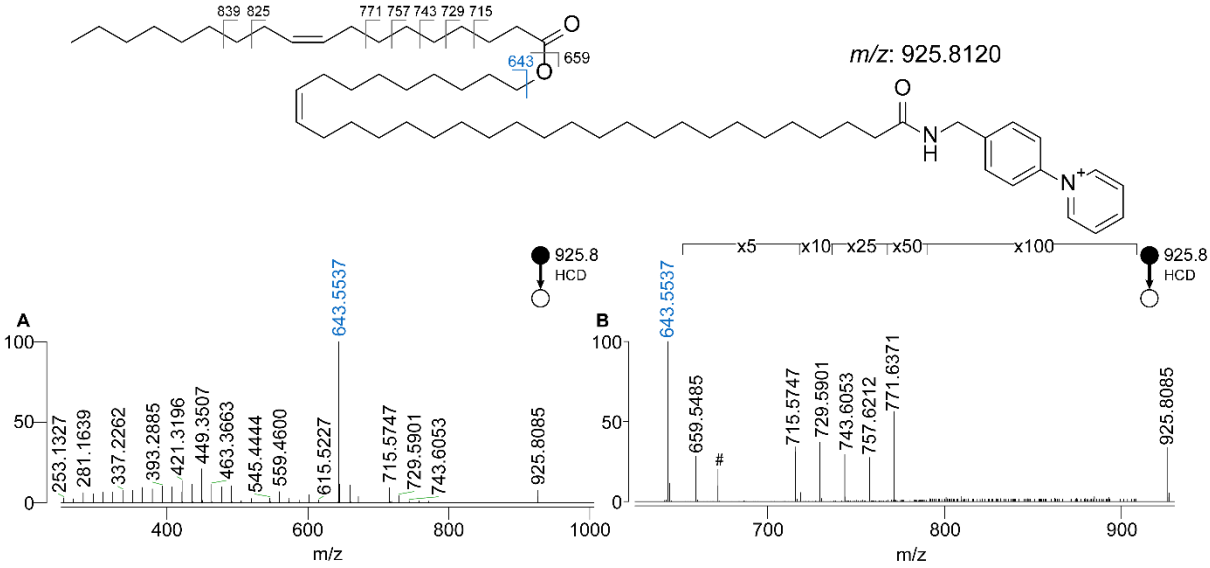


Supplemental Figure S10: Negative ion (A) CID and (B) OzID mass spectra obtained from OAHFA 48:2 present in meibum. (C) CID/OzID mass spectrum localising double bond position in the *ω*-HFA 30:1 or (D) *ω*-HFA 32:1; as well as, (E) FA 18:1 or (F) FA 16:1. * indicates signal artefacts unrelated to OzID.



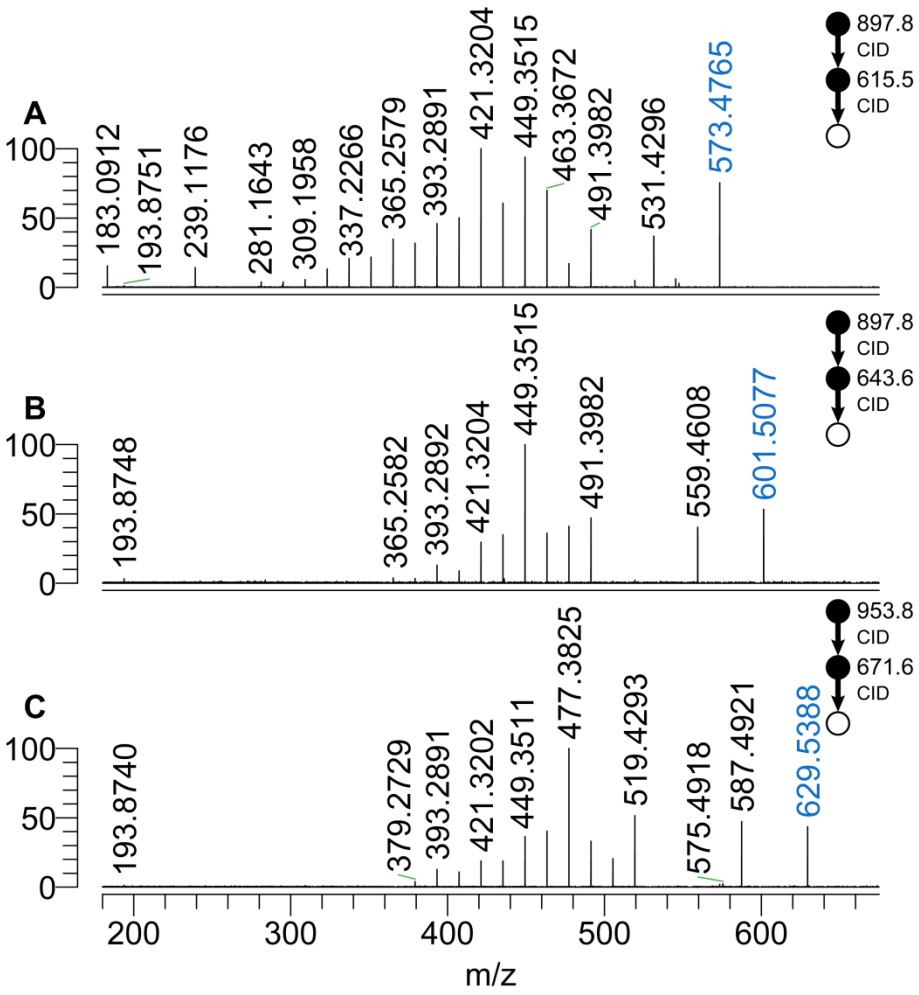
Supplemental Figure S11: Negative ion (A) CID and (B) OzID mass spectra from meibum-derived OAHFA 52:2. (C) CID/OzID mass spectrum localising double bond position in the *ω*-HFA 34:1 reveals double bond positional isomers. CID/OzID of FA 18:1 are not shown as it was below the limit of detection.

2.1.3 CID of [M+AMPP]$^+$ OAHFA 50:2



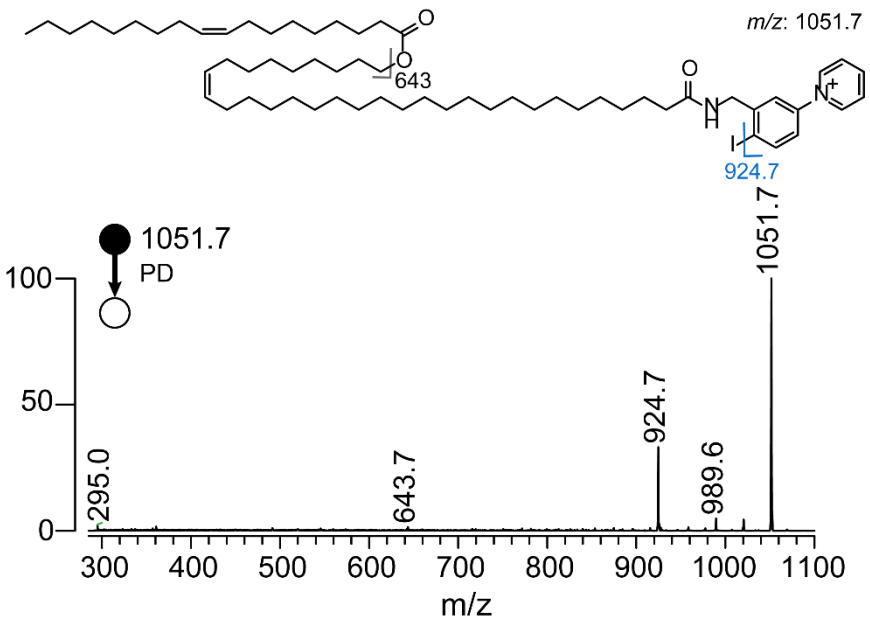
Supplemental Figure S12: (A) Positive ion CID spectrum (aquired using HCD mode) of [M+AMPP]$^+$ meibum OAHFA 50:2. The major product ion is *m/z* 643.6, resulting from the neutral loss of the 18:1 fatty acid. (B) Magnification of the region of HCD spectrum related to the FA 18:1(*n*-9) shows little charge-remote fragmentation near the methyl end. # indicates neutral loss of a 16:1 fatty acid from the meibum OAHFA 16:1/34:1 [M+AMPP]$^+$ isomer also found at *m/z* 925.8.

2.1.4 MS³ of meibum OAHFA 48:2 and 52:2 as [M+AMPP]⁺



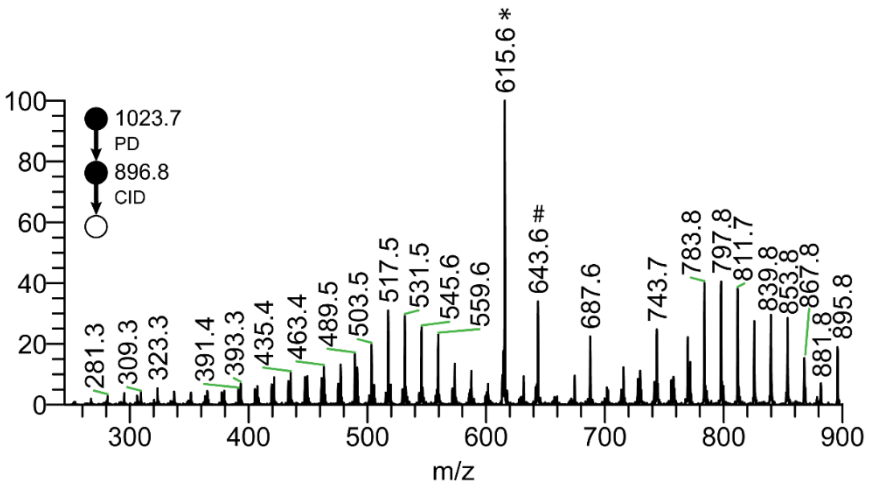
Supplemental Figure S13: MS³ of dehydrated HFA formed from CID of meibum-derived (A) OAHFA 48:2 (18:1/30:1), (B) OAHFA 48:2 (16:1/32:1) and (C) OAHFA 52:2 (18:1/34:1). MS³ spectra for all three OAHFA exhibit the 42 Da loss diagnostic for an ω-HFA (*i.e.*, neutral loss of 42 Da, C_3H_6)

2.1.5 PD of meibum OAHFA 50:2 as $[M+4\text{-I-AMPP}]^+$



Supplemental Figure S14: Photodissociation at 266 nm of the mass-selected $[OAHFA\ 50:2+4\text{-I-AMPP}]^+$ ion arising from posititve ion ESI of an OAHFA-enriched fraction derivatizated with the 4-I-AMPP reagent.

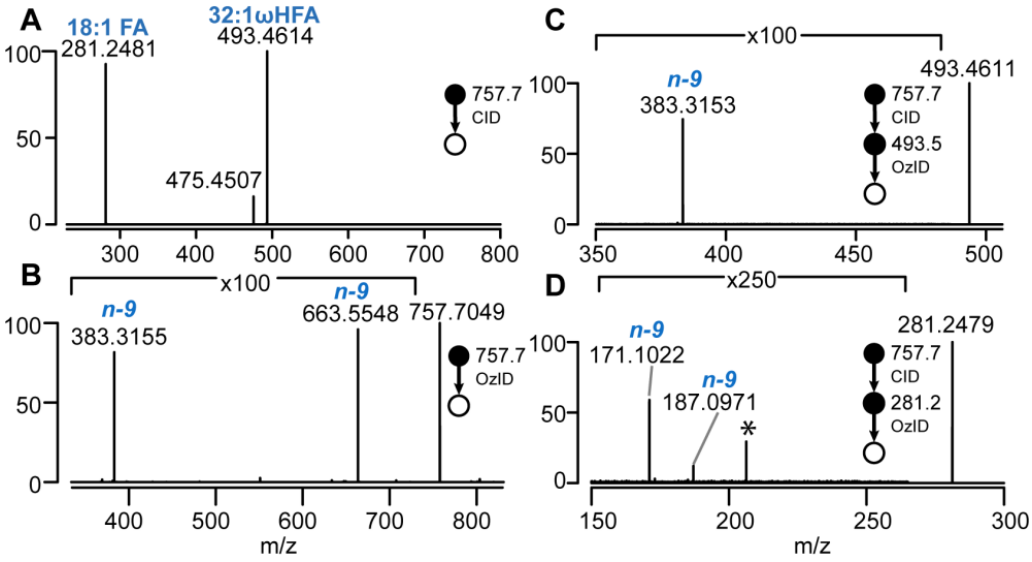
2.1.6 RDD of meibum OAHFA 48:2 as $[M+4\text{-I-AMPP}]^{\bullet+}$



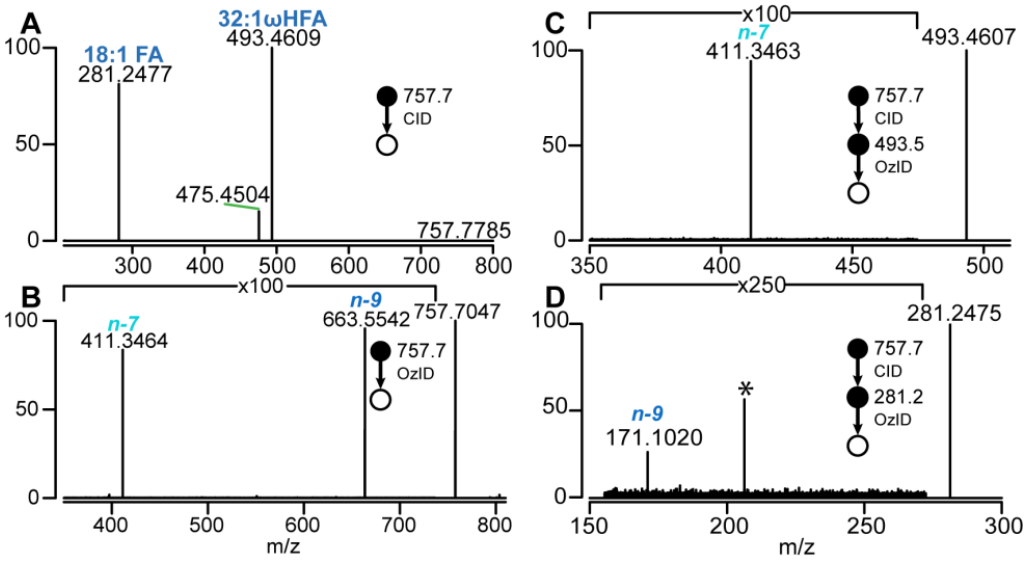
Supplemental Figure S15: Radical-directed dissociation of OAHFA 48:2 $[M+4\text{-I-AMPP}]^{\bullet+}$. * indicates ω-HFA 30:1 from OAHFA 18:1/30:1, # indicates ω-HFA 32:1 from OAHFA 16:1/32:1.

2.2 Synthetic OAHFA analysis by mass spectrometry

2.2.1 OzID of synthetic OAHFA

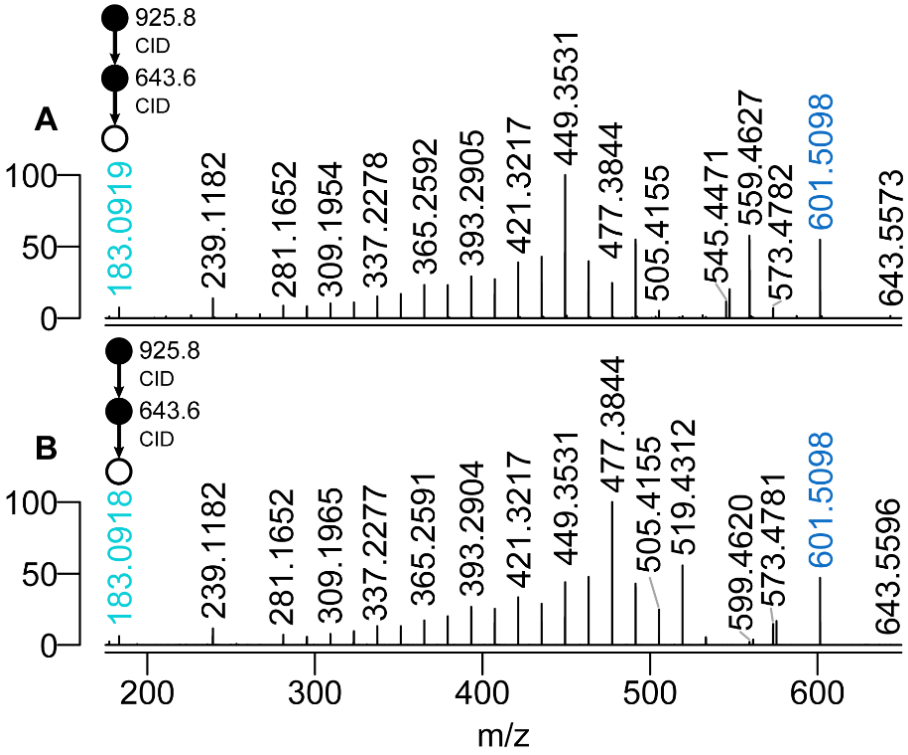


Supplemental Figure S16: Negative ion (A) CID and (B) OzID mass spectra of synthetic OAHFA 18:1(*n*-9, *cis*)/*ω*-*O*-32:1(*n*-9, *cis*) **1**. Also shown are CID/OzID mass spectra of the product ions corresponding to (C) *ω*-HFA 32:1 and (D) FA 18:1. * indicates an artefact signal unrelated to OzID.



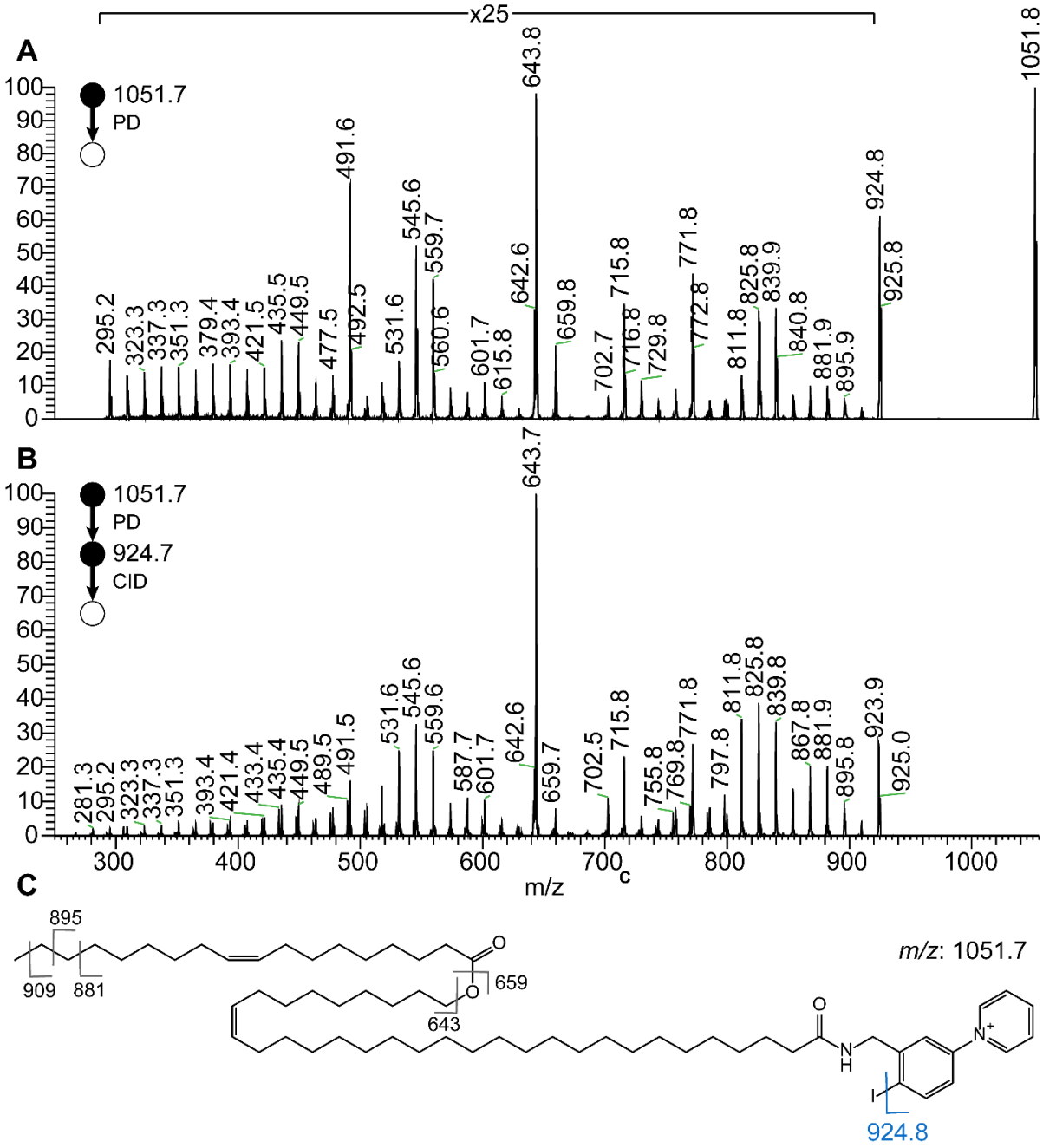
Supplemental Figure S17: Negative ion (A) CID and (B) OzID mass spectra of synthetic OAHFA 18:1(*n*-9, *cis*)/(*ω*-*O*-32:1(*n*-7, *cis*) **2**. Also shown are CID/OzID mass spectra of the product ions corresponding to (C) *ω*-HFA 32:1 (C) and (D) FA 18:1. * indicates an artefact signal unrelated to OzID.

2.2.2 MS³ of synthetic OAHFA as [M+AMPP]⁺



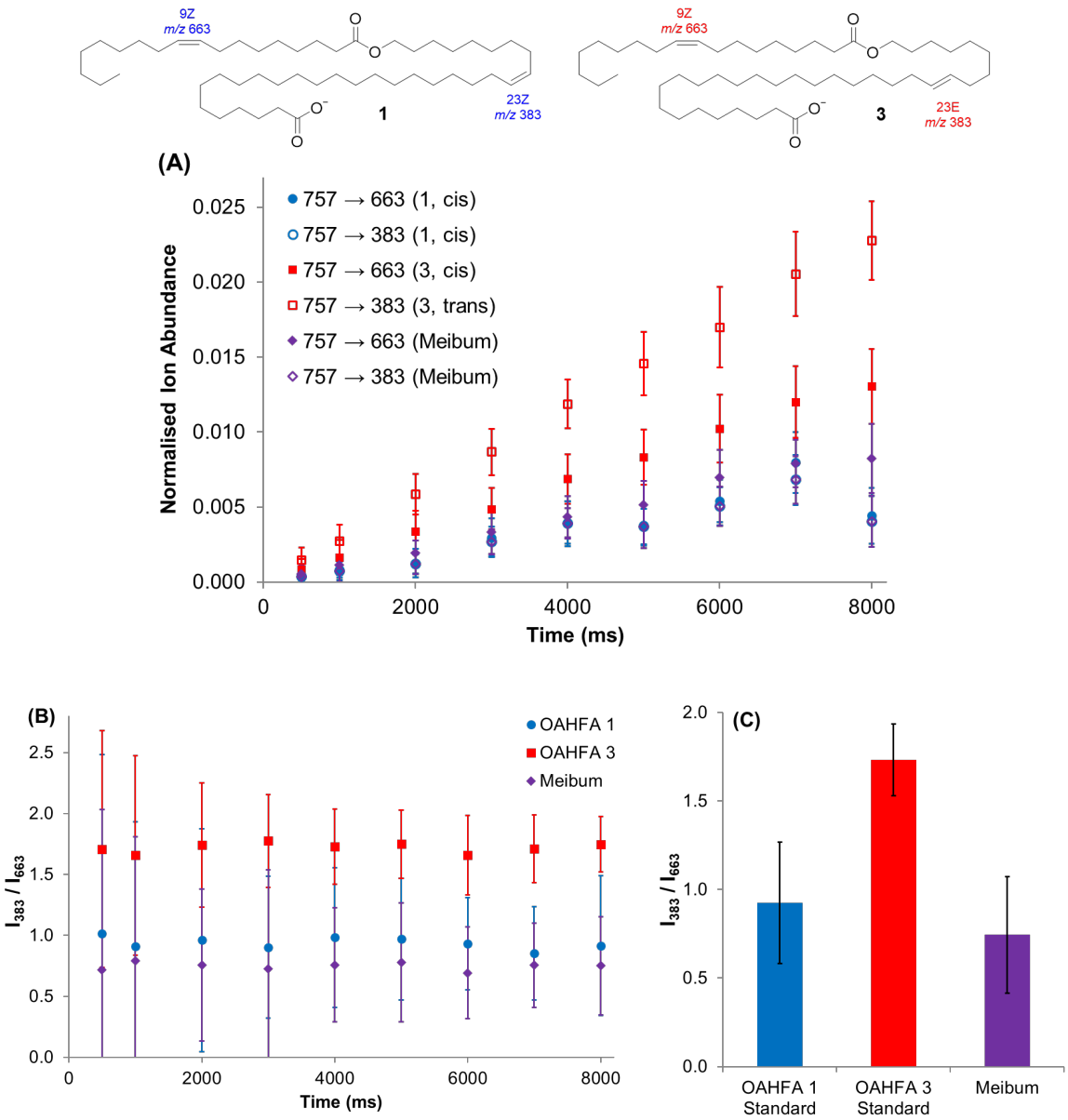
Supplemental Figure S18: Positive ion MS^3 spectra of the terminal alkene $[32:1HFA\text{-}H_2O+AMPP]^+$ (*m/z* 643) arising from CID of synthetic (A) OAHFA 18:1(*n*-9, *cis*)/*ω*-32:1(*n*-9, *cis*) **1** (B) and 18:1(*n*-9, *cis*)/*ω*-32:1(*n*-7, *cis*) **2** derivatized with AMPP. The ion at *m/z* 601 is characteristic for the terminal alkene formed from CID of an *ω*-HFA.

2.2.3 PD/RDD of synthetic OAHFA as [M+4-I-AMPP]+



Supplemental Figure S19: Positive ion (A) photodissociation and (B) radical-directed dissociation of synthetic OAHFA 18:1(*n*-9, *cis*)/*ω*-*O*-32:1(*n*-9, *cis*) derivatised with 4-I-AMPP (C) Structure of derivatized synthetic OAHFA.

2.3 Comparison of *cis*/*trans* isomers by OzID



Supplemental Figure S20: (A) OzID of the [M-H]- anions from synthetic OAHFA 18:1(n-9, cis)/ω-32:1(n-9, cis) [1, blue], OAHFA 18:1(n-9, cis)/ω-32:1(n-9, trans) [3, red] and OAHFA 50:2 from meibum (purple). Normalised product ion abundances corresponding to oxidative cleavage of the FA (m/z 663) and HFA (m/z 383) portions of the molecule are shown as filled and open circles, respectively. Ion abundances represent an average of at least 20 scans. (B) The ratio of OzID product ion abundances (taken from A) for each ionized lipid at each reaction time. (C) Fitting each dataset in B with the form y = k yields an average ozonolysis peak abundance ratio for each ionized lipid. In each plot, the error bars represent twice the standard deviation on the mean (i.e., ±2σ).

3 References

1. Brown, S. H. J., C. M. E. Kunnen, E. Duchoslav, N. K. Dolla, M. J. Kelso, E. B. Papas, P. Lazon de la Jara, M. D. P. Willcox, S. J. Blanksby, and T. W. Mitchell. 2013. A Comparison of Patient Matched Meibum and Tear Lipidomes. *Invest. Ophthalmol. Vis. Sci.* **54**: 7417–7424.

2. Drescher, S., A. Meister, A. Blume, G. Karlsson, M. Almgren, and B. Dobner. 2007. General synthesis and aggregation behaviour of a series of single-chain 1,omega-bis(phosphocholines). *Chem. Weinh. Bergstr. Ger.* **13**: 5300–5307.

3. Greaves, J., K. R. Munro, S. C. Davidson, M. Riviere, J. Wojno, T. K. Smith, N. C. O. Tomkinson, and L. H. Chamberlain. 2017. Molecular basis of fatty acid selectivity in the zDHHC family of S-acyltransferases revealed by click chemistry. *Proc. Natl. Acad. Sci. U. S. A.* **114**: E1365–E1374.

4. Sandtorv, A. H., and H.-R. Bjørsvik. 2015. Scope and Mechanistic Limitations of a Sonogashira Coupling Reaction on an -Imidazole Backbone. *Eur. J. Org. Chem.* **2015**: 4658–4666.

5. Chatterjee, T., M. Sarma, and S. K. Das. 2010. Donor–acceptor amphiphilic 2,2′-bipyridine chromophores: synthesis, linear optical, and thermal properties. *Tetrahedron Lett.* **51**: 6906–6910.

6. Slezak, F. B., J. P. Stallings, D. H. Wagner, and J. H. Wotiz. 1961. Novel Polyacetylenic Diols1 and Polyacetylenic Polyurethanes. *J. Org. Chem.* **26**: 3137–3140.

7. Schill, G., and C. Merkel. 1978. Synthesis of Long Chain Terminally Bifunctionalised Alkane and Alkyne Derivatives. *Chem. Ber.* **111**: 1446–1452.