Supplemental information for:

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

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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 (Rf) 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 F₂₅₄ (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]. ¹H and ¹³C 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:CuCl₂, -10 °C 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 °C-rt, 16 h, 82%; (d) (i) tert-butyldimethyl(oct-7-yn-1-yl)oxy)silane 17, n-BuLi, THF-HMPA, -78 °C to -40 °C, 1 h; 16 in THF, TBAI, -78 °C to 60 °C, 24 h (ii) TBAF, THF, 0 °C-rt, 12 h, 56% (over two steps); (e) Lindlar catalyst, quinoline, benzene, rt, 12 h, 92%; (f) oleic acid, DCC, DMAP, CH₃Cl₂, 0 °C-rt, 24 h, 72%; (g) CSA, methanol-THF, 0 °C-rt, 12 h, 82%; (h) Jones’ reagent, acetone, rt, 1 h, 87%.
1.2.3 Synthesis of OAHFA 18:1(n-9, cis)/ω-0-32:1(n-9, trans) (3)

Supplemental Scheme S2: Synthesis of OAHFA 18:1(n-9, cis)/ω-0-32:1(n-9, trans) 3. Reagents and conditions: (a) LiAlH₄, diglyme, 130 °C, 24 h, 62%; (b) oleic acid, DCC, DMAP, CH₂Cl₂, 0 °C-rt, 24 h, 74%; (c) CSA, methanol-THF, 0 °C-rt, 12 h, 92%; (d) Jones’ reagent, acetone, rt, 1 h, 86%.

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

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
The crude product was purified by flash chromatography on silica gel (EtOAc:Pet. sp. 1:9) to give I (62 mg, 87%) as a white solid. M.P. 58-60 °C. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 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$_3$) $\delta$: 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. 

Supplemental Figure S2: $^1$H NMR of synthetic OAHFA 18:1(n-9, cis)/$\omega$-O-32:1(n-9, cis) I
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 °C. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 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$_3$) $\delta$: 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] $^{\text{[757.7074]}}$, 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 °C. $^1$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$_3$) $\delta$: 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, 29.45, 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, 129.3, 130.0, 130.2, 130.4, 174.0, 177.9 (Supplemental Figure S7). IR(neat) $\nu$: 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)/$\omega$-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 dihyropyran (1.08 mL, 11.94 mmol) in dry CH$_2$Cl$_2$ (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$_2$Cl$_2$ (2 × 10 mL), washed with saturated sodium chloride (10 mL) and dried over anhydrous Na$_2$SO$_4$. 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. $^1$H 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$_2$BrNa [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 Li₂CuCl₄ (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 °C; Lit.(2) 96-98 °C; ¹H NMR (500 MHz, CD₃OD) δ: 1.20-1.36 (m, 36H), 1.48-1.58 (m, 4H), 3.55 (t, 4H, J=6.6 Hz). ¹³C NMR (125 MHz, CD₃OD) 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 C₂₂H₄₇O₂ [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 °C; Lit.(3) 66-68 °C; ¹H NMR (500 MHz, CD₃OD) δ: 1.20-1.36 (m, 36H), 1.48-1.58 (m, 4H), 3.55 (t, 4H, J=6.6 Hz). ¹³C NMR (125 MHz, CD₃OD) 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 C₂₂H₄₇O₂ [M+H]^+ 343.3576, found 343.3572.
MHz, CDCl₃) 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). ¹³C NMR (125 MHz, CDCl₃) 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 C₂₂H₄₆OBr [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
°C. 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 solution (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, CDCl₃)
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). ¹³C NMR (125 MHz, CDCl₃) 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 C₂₇H₅₃O₂BrNa [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 ºC 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 °C 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 NH₄Cl (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; ¹H NMR (500 MHz, CDCl₃) 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). ¹³C NMR (125 MHz, CDCl₃) 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, 29.6, 29.6, 29.6, 29.6, 29.7, 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) v : 3727, 3574, 3018, 2927, 2855, 2312, 1512, 1215, 1022, 868, 747, 668 cm⁻¹. HRMS calculated for C₃₇H₇₀O₃Na [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\textsubscript{2} (1 atm) and stirred for 12 h. The reaction mixture was filtered through a pad of celite and the filtrate was concentrated \textit{in vacuo}. The crude residue was purified by flash chromatography on silica gel (10% EtOAc in Pet. sp.) to give the (Z)-alkenol \textit{10} (104 mg, 98\%) as a white solid. M.P. 46-48 °C. \textit{\textsuperscript{1}H} NMR (500 MHz, CDCl\textsubscript{3}) \textit{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). \textit{\textsuperscript{13}C} NMR (125 MHz, CDCl\textsubscript{3}) \textit{δ}: 19.7, 25.5, 25.7, 26.3, 27.1, 27.2, 29.1, 29.4, 29.5, 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) \nu : 3580, 3018, 2926, 2854, 2377, 2320, 1723, 1675, 1499, 1215, 1076, 1027, 864, 749, 668 cm\textsuperscript{-1}. HRMS calculated for C\textsubscript{37}H\textsubscript{72}O\textsubscript{3}Na [M+Na\textsuperscript{+}] 587.5379, found 587.5399.

\textit{(Z)-32-((Tetrahydro-2H-pyran-2-yl)oxy)dotriacont-9-en-1-yl olate (11)}

To a stirred solution of (Z)-alkenol \textit{10} (92 mg, 0.16 mmol) in anhydrous CH\textsubscript{2}Cl\textsubscript{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\textsubscript{2}Cl\textsubscript{2} (10 mL). The organic layer was washed with more water (2 x 5 mL) and the combined aqueous phases were extracted with CH\textsubscript{2}Cl\textsubscript{2} (2 x 5 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated \textit{in vacuo}. The crude residue was purified by flash chromatography on silica gel (EtOAc:Pet. sp, 0:100 to 2:98) to give \textit{11} (105 mg, 78\%) as a white waxy solid. \textit{\textsuperscript{1}H} NMR (500 MHz, CDCl\textsubscript{3}) \textit{δ}: 0.88 (t, 3H, \textit{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, \textit{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, \textit{J}=6.8 Hz), 4.56-4.60 (m, 1H), 5.30-5.40 (m, 4H). \textit{\textsuperscript{13}C} NMR (125 MHz, CDCl\textsubscript{3}) \textit{δ}: 14.1, 19.7,
22.7, 25.0, 25.5, 26.2, 27.2, 27.2, 27.2, 28.6, 29.1, 29.1, 29.2, 29.2, 29.3, 29.4, 29.5,
29.5, 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, 2366, 2028,
1993, 1988, 1967, 1950, 1923, 1911, 1700, 1696, 1472, 1424, 1178, 1063, 1006, 945, 838, 718,
607 cm⁻¹. HRMS calculated for C₅₅H₁₀₄O₄Na [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 °C 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 °C.

¹H NMR (500 MHz, CDCl₃) 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). ¹³C NMR (125 MHz, CDCl₃) 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 C₅₀H₉₆O₃Na [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 Li₂CuCl₄ (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 °C; Lit.(6) 106-107.5 °C. ¹H NMR (500 MHz, CD₃OD) δ: 1.20-1.39 (m, 40H), 1.49-1.58 (m, 4H), 3.57 (t, 4H, J=6.3 Hz). ¹³C NMR (125 MHz, CD₃OD) 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 C₂₄H₄₀O₂Na [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 °C. ¹H NMR (500 MHz, CDCl₃) 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). ¹³C NMR (125 MHz, CDCl₃) 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 C₂₄H₄₀BrONa [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 °C; Lit.(7) 45.5-46.5 °C. $^1$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, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5. 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$_2$Na [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 °C 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$_4$Cl (5 mL) and extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine, dried over Na$_2$SO$_4$ 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 °C. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta : 1.12-1.40\) (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\(_3\)) \(\delta : 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) \(\nu : 3650, 3019, 2855, 1457, 1214, 1118, 1072, 1036, 746, 668, 618\) cm\(^{-1}\). HRMS calculated for C\(_{37}\)H\(_{70}\)O\(_3\)Na [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 °C. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta : 1.08-1.42\) (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\(_3\)) \(\delta : 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) \(\nu : 3590, 3019, 2855, 1457, 1214, 1074, 1032, 748, 668\) cm\(^{-1}\). HRMS calculated for C\(_{37}\)H\(_{72}\)O\(_3\)Na [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\(_3\)) \(\delta : 0.88\) (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$_4$Na [M+Na]$^+$ 851.7832, found 851.7873.

(Z)-32-Hydroxydotriacont-7-en-1-yl olate (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, 29.4, 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$_3$Na [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). 13C NMR (125 MHz, CDCl3) 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 C37H72O3Na [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 °C. 1H NMR (500 MHz, CDCl3) 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). 13C NMR (125 MHz, CDCl3) 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 C55H104O4Na [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 °C. 1H NMR (500 MHz, CDCl3) 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). 13C NMR (125 MHz,
CDCl₃ 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, 130.0, 130.2, 130.4, 174.0. IR(neat) ν: 3589, 3017, 2927, 2855, 2328, 1680, 1455, 1216, 748, 668 cm⁻¹. HRMS calculated for C₅₀H₉₆O₃Na [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) |
|-------------|------------------|---------------------|
|             | 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 (acquired 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. 6 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 MS$^3$ of meibum OAHFA 48:2 and 52:2 as [M+AMPP]$^+$

Supplemental Figure S13: MS$^3$ 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). MS$^3$ spectra for all three OAHFA exhibit the 42 Da loss diagnostic for an ω-HFA (i.e., neutral loss of 42 Da, C$_3$H$_6$)
2.1.5 PD of meibum OAHFA 50:2 as [M+4-I-AMPP]⁺

Supplemental Figure S14: Photodissociation at 266 nm of the mass-selected [OAHFA 50:2+4-I-AMPP]⁺ ion arising from positive ion ESI of an OAHFA-enriched fraction derivatized with the 4-I-AMPP reagent.

2.1.6 RDD of meibum OAHFA 48:2 as [M+4-I-AMPP]²⁺

Supplemental Figure S15: Radical-directed dissociation of OAHFA 48:2 [M+4-I-AMPP]²⁺. * 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 MS³ spectra of the terminal alkene [32:1HFA-H₂O+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(ω-9, cis)/ω-O-32:1(ω-9, cis) derivatised with 4-I-AMPP (C) Structure of derivatized synthetic OAHFA.
2.3 Comparison of \textit{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).
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