Supporting Information

Effect of lipid length and branching of monoacylglycerides on Mincle agonist activity.

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Chemistry Experimental

General Chemicals:
Prior to use, toluene (ROMIL) was dried and stored over Na wire, and the following solvents were distilled: acetone (Fisher Scientific), ethyl acetate (Fisher Scientific) and petroleum ether (Merck), DMSO (Merck). Behenic acid (BDH Biochem), MgSO₄ (Pure Science), NaCl (Chem Solute), Et₂O (LabServ), DMAP (Lab Supply), EDCI (Chem Impex), TFA (Pancreac), THF (ROMIL), NaHCO₃ (Pure Science), HCl (Chem Solute), isopropanol (Fischer Scientific), KMnO₄ (AnalR), 11-methyldodecanoic acid (Larodan AB), 13-methyltetradecanoic acid (Larodan AB), 15-methylhexadecanoic acid (Larodan AB), 17-methyloctadecanoic acid (Larodan AB), 18-methylnonadecanoic acid (Larodan AB), 21-methyldocosanoic acid (Larodan AB), Palmitic Acid (Fulka), Oleic acid (Sigma Aldich), Stearic acid (Fisher Scientific), Hexacosanoic acid (Merck), CDCl₃ (Aldrich), Diisopropylamine (Sigma Aldich), Sodium Hydride (Sigma Aldrich), Triphenylphosphene (Acros Organics), Methyl iodide (Sigma Aldrich), Boron trifluoride diethyl etherate (Janssen Chimica), Triethysilane (Sigma Aldrich), Pyridinium Chlorochromate (Sigma Aldrich), and 10-bromodecanoic acid (Sigma Aldrich) were used as received. Reactions were monitored by TLC analysis by dipping in 10% H₂SO₄ in EtOH followed by charring or dipping in a solution of KMnO₄ (0.05 M), K₂CO₃ (0.4 M), and NaOH (0.06%) in water. Column chromatography was performed using Pure Science silica gel (40-63 µm). All solvents were removed by evaporation under reduced pressure. High resolution mass spectra were recorded on an Agilent 6530 Q-TOF mass spectrometer utilising a JetStream™ electro-spray ionisation (ESI) source in positive or negative mode. Optical rotations were recorded on a Autopol II (Rudolph Research Analytical) at 589 nm (sodium D-line). Infrared (IR) spectra were recorded as thin films using either a Bruker Platinum-ATR spectrometer and are reported in wave numbers (cm⁻¹).
Nuclear magnetic resonance spectra were obtained at 20 °C in CDCl$_3$ or C$_5$D$_5$N using a Varian INOVA operating at 500 MHz. Chemical shifts are given in ppm (δ) relative to the solvent residual peak. NMR peak assignments were made using COSY, HSQC, and HMBC 2D experiments.

25-Methylhexacos-10-enolic acid (13). 10-bromodecanoic acid (225 mg, 1 mmol) and triphenylphosphine (262 mg, 1 mmol) were combined and heated at 80 °C for 30 mins under Ar atmosphere. The resulting red oil was suspended in 8 mL of dry DMSO and subjected to further heating for 20 mins. The mixture was then cooled to 0 °C, and butyl lithium (2.0 M in cyclohexane, 1.25 mL, 2.5 mmol) was added drop wise. The reaction was stirred for one hour before aldehyde 12 (500 mg, 2 mmol) was added. The reaction mixture was warmed to r.t. and further stirred for 3 hr. The suspension was then diluted with EtOAc, washed with 1M HCl and brine, dried with anhydrous magnesium sulfate, filtered, and concentrated in vacuo to give a red oil. The crude product was purified by silica gel flash chromatography (PE) to yield 13 as a white solid (172 mg, 0.84 mmol, 21%). R$_f$ = 0.8 (PE); IR (film) = 2998, 2923, 2853, 1708, 1462, 1411, 932, 739; $^1$H NMR (500 MHz, CDCl$_3$) δ 5.47–5.41 (m, 2H, H-10, H-11), 2.37–2.29 (m, 2H, H-2), 1.6–1.58 (m, 4H, H-9, H-1), 1.55–1.46 (m, 1H, H-25), 1.32–1.21 (m, 32H, H-4–8, H-13–23), 1.17–1.10 (m, 2H, H-24), 0.86 (d, $J_{25,26a+b}$ = 7.1 Hz, 6H, H-26); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 129.97 (C-10), 129.78 (C-11), 39.05 (C-24), 35.58 (C-2), 33.98 (C-12), 32.13 (C-10), 29.94, 29.76, 29.73, 29.69, 29.68, 29.66, 29.65, 29.63, 29.60, 29.58, 29.56, 29.42, 29.32, 29.28, 29.23, 29.22, 29.21, 29.17, 29.05, 29.04, 28.99 (C-4–8, C-13–22), 27.96 (C-25), 27.41 (C-23), 24.67 (C-3), 22.65 (C-26); HRMS (ESI) m/z calculated for [C$_{27}$H$_{53}$O$_2$]$^+$: 409.4040, found 409.4029.

**General procedure for esterification:**

MAGs of different chain lengths were synthesised according to a procedure by Khan et al.$^1$ (S)-(R)-1,2-O-Isopropylidenglycerol (1 mmol, 1 equiv.) and the carboxylic acid (2 mmol, 2 equiv.) were co-evaporated together with dry toluene (5 mL), then suspended in dry toluene. To the reaction mixture, EDCI (3.3 mmol, 3.3 equiv.) and DMAP (1 mmol, 1 equiv.) were added and the resulting suspension was heated to 70 °C for 48 h. The reaction mixture was then cooled to r.t. and diluted with of EtOAc (5 mL). The organic layer was washed with water (5 mL), sat. aq. NaHCO$_3$
(5 mL), and brine (5 mL). The combined aqueous phases were re-extracted with of EtOAc (5 mL) and the combined organic phases were dried with anhydrous MgSO₄, filtered, and concentrated in vacuo. The product was purified using gradient silica-gel column chromatography (PE to PE/EtOAc, 4:1, v/v).

1,2-O-Isopropylidene-1-O-(11-methyldodecanoyl)-sn-glycerol (16a). By subjecting diol 14 (30 mg, 0.22 mmol), 11-methyldodecanoic acid 15a (97 mg, 0.45 mmol), EDCI (139 mg, 0.726 mmol) and DMAP (27 mg, 0.34 mmol) to the general procedure for esterification (8 h), the title compound 16a was obtained as a colourless oil (46.2 mg, 0.140 mmol, 64%). R₆ = 0.4 (PE/EtOAc, 9:1, v/v); [α]₂³¹D = +4.8 (c = 1 CHCl₃); IR (film) = 2924, 2854, 1740, 1459, 1380, 1251, 1157, 1083, 1056, 1042, 964, 861, 841, 737, 681 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 4.31 (p, J₂,₃ = J₂,₁ = 6.2 Hz, 1H, H₂), 4.16 (dd, J₃a,₃b = 11.5 Hz, J₃a,₂ = 4.7 Hz, 1H, H₃a), 3.74–3.71 (m, 1H, H₁b), 2.34 (t, J₅,₆ = 7.5 Hz, 1H, H₅), 1.66–1.58 (m, 12H, H₇–₁₂), 1.18–1.11 (m, 2H, H₁₃), 0.85 (d, J₁₄,₁₅a+b = 6.6 Hz, 6H, H₁₅); ¹³C NMR (125 MHz, CDCl₃) δ 173.55 (C₄), 109.70 (C₂'), 73.54 (C-2), 66.23 (C-1), 64.40 (C-3), 38.92 (C-13), 34.00 (C-5), 29.77, 29.58, 29.50, 29.34, 29.13, 29.00 (C-7–₁₁), 27.84 (C-14), 27.27 (C-12), 26.56 (C-1'), 25.28 (C-1'), 24.78 (C-6), 22.54 (C-15); HRMS (ESI) m/z calculated for [C₁₉H₃₆NaO₄]⁺: 351.2506, found 351.2523.

1,2-O-Isopropylidene-1-O-(13-methyltetradecanoyl)-sn-glycerol (16b). By subjecting diol 14 (22 mg, 0.16 mmol), 13-methyltetradecanoic acid 15b (82 mg, 0.33 mmol), EDCI (105 mg, 0.549 mmol) and DMAP (20 mg, 0.1664 mmol) to the general procedure for esterification (8 h), the title compound 16b was obtained as a colourless oil (45 mg, 0.125 mmol, 78%). R₆ = 0.3 (PE/EtOAc, 9:1, v/v); [α]₂³¹D = +7.6 (c = 1, CHCl₃); IR (film) = 2923, 2851, 1741, 1465, 1370, 1250, 1159, 1095, 1009, 871, 841, 743, 691 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 4.31 (p, J₂,₃ = 6.2 Hz, J₂,₁ = 4.69 Hz, 1H, H₂), 4.16 (dd, J₃a,₃b = 11.5 Hz, J₃a,₂ = 4.7 Hz, 1H, H₃a), 4.09 (t, J₃b,₂ = 5.8 Hz, 1H, H₃b), 4.07–4.05 (m, 1H, H₁a), 3.74 (dd, J₁b,₁a = 8.5 Hz, J₁b,₂ = 6.2 Hz, 1H, H₁b), 2.34 (t, J₅,₆ = 7.6 Hz, 2H, H₅), 1.66–1.58 (m, 2H, H₆–₇), 1.54–1.46 (m, 1H, H₁₆), 1.43 (s, 3H, H-
1', 1.37 (s, 3H, H-1'), 1.32–1.21 (m, 16H, H-7–14), 1.18–1.11 (m, 2H, H-15), 0.86 (d, J_{16,17\alpha+\beta} = 6.6 Hz, 6H, H-17); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.64 (C-4), 109.79 (C-2'), 73.66 (C-2), 66.33 (C-1), 64.49 (C-3), 39.03 (C-15), 34.25 (C-5), 29.92, 29.68, 29.63, 29.58, 29.43, 29.23, 29.10 (C-7–13), 27.95 (C-16), 27.40 (C-14), 26.65 (C-1'), 25.35 (C-1'), 24.87 (C-6), 22.70 (C-17). HRMS (ESI) $m/z$ calculated for [C$_{21}$H$_{40}$NaO$_4$]$^+$: 379.2819, found 379.2835.

1,2-O-Isopropylidene-3-O-(13-methyltetradecanoyl)-sn-glycerol (16c). By subjecting diol 14 (25 mg, 0.18 mmol), 13-methyltetradecanoic acid 15b (87 mg, 0.36 mmol), EDCI (113 mg, 0.594 mmol) and DMAP (22 mg, 0.18 mmol) to the general procedure for esterification (8 h), the title compound 16c was obtained as a colourless oil (50 mg, 0.14 mmol, 77%). $R_f = 0.3$ (PE/EtOAc, 9:1, v/v); $[\alpha]_D^{21.1} = -4.2$ (c = 2, CHCl$_3$); IR (film) = 2921, 2851, 1743, 1464, 1370, 1250, 1173, 1056, 1089, 871, 841, 756, 653 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 4.31 (p, $J_{2,3} = J_{2,1} = 6.2$ Hz, 1H, H-2), 4.16 (dd, $J_{3a,3b} = 11.5$ Hz, $J_{3a,2} = 4.7$ Hz, 1H, H-3a), 4.09 (t, $J_{3b,2} = 5.8$ Hz, 1H, H-3b), 4.07–4.05 (m, 1H, H-1a), 3.74 (dd, $J_{1b,1a} = 8.5$ Hz, $J_{1b,2} = 6.2$ Hz, 1H, H-1b), 2.34 (t, $J_{5,6} = 7.6$ Hz, 2H, H-5), 1.66–1.58 (m, 2H, H-6), 1.54–1.46 (m, 1H, H-16), 1.43 (s, 3H, H-1'), 1.37 (s, 3H, H-1'), 1.32–1.21 (m, 16H, H-7–14), 1.18–1.11 (m, 2H, H-15), 0.86 (d, $J_{16,17\alpha+\beta} = 6.6$ Hz, 6H, H-17); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.51 (C-4), 109.67 (C-2'), 73.52 (C-2), 66.18 (C-1), 64.37 (C-3), 38.92 (C-15), 33.98 (C-5), 29.80, 29.57, 29.51, 29.47, 29.32, 29.12, 29.99 (C-7–13), 27.84 (C-16), 27.29 (C-14), 26.54 (C-1'), 25.27 (C-1'), 24.76 (C-6), 22.48 (C-17); HRMS (ESI) $m/z$ calculated for [C$_{21}$H$_{41}$O$_4$]$^+$: 357.2999, found 357.2991.

1,2-O-Isopropylidene-1-O-(17-methyloctadecanoyl)-sn-glycerol (16d). By subjecting diol 14 (33.5 mg, 0.25 mmol), 17-methyloctadecanoic acid 15c (150 mg, 0.50 mmol), EDCI (160 mg, 0.83 mmol) and DMAP (31 mg, 0.25 mmol) to the general procedure for esterification (8 h), the title compound 16d was obtained as a white solid (75 mg, 0.18 mmol, 73%). $R_f = 0.3$ (PE/EtOAc, 9:1, v/v); $[\alpha]_D^{22.1} = +5.1$ (c = 2, CHCl$_3$); IR (film) = 2916, 2848, 1733, 1469, 1212, 1153, 1049, 1048, 871, 846, 747, 683 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 4.31 (p, $J_{2,3} = J_{2,1} = 5.1$ Hz, 1H, H-2), 4.16 (dd, $J_{3a,3b} = 11.6$ Hz, $J_{3a,2} = 4.8$ Hz, 1H, H-3a), 4.12–4.03 (m, H, H-3b, H-1a), 3.73 (dd, $J_{1b,1a} = 8.9$ Hz, $J_{1b,2} =$
5.8 Hz, 1H, H-1b), 2.33 (t, J_{5,6} = 7.3 Hz, 2H, H-5), 1.61 (p, J_{6,5} = J_{6,7} = 7.3 Hz, 2H, H-6), 1.50 (m, 1H, H-20), 1.43 (s, 3H, H-1’), 1.36 (s, 3H, H-1’), 1.33–1.21 (m, 24H, H-7–18), 1.16–1.10 (m, 2H, H-19), 0.85 (d, J_{20,21a+b} = 6.6 Hz, 6H, H-21); ^{13}C NMR (125 MHz, CDCl_{3}) δ 173.63 (C-4), 109.80 (C-2’), 73.65 (C-2), 66.33 (C-1), 64.49 (C-3), 39.05 (C-19), 34.10 (C-5), 29.94, 29.72, 29.68, 29.67, 29.66, 29.63, 29.58, 29.44, 29.24, 29.11 (C-7–17), 27.95 (C-20), 27.41 (C-18), 26.67 (C-1’), 25.38 (C-1’), 24.88 (C-6), 22.65 (C-21); HRMS (ESI) m/z calculated for [C_{25}H_{48}NaO_{4}]^{+}: 435.3445, found 435.3449.

1,2-O-Isopropylidene-1-O-(18-methylnonadecanoyl)-sn-glycerol (16e). By subjecting diol 14 (32 mg, 0.24 mmol), 18-methylnonadecanoic acid 15d (150 mg, 0.48 mmol), EDCI (153 mg, 0.79 mmol) and DMAP (29 mg, 0.24 mmol) to the general procedure for esterification (48 h), the title compound 16e was obtained as a white solid (77 mg, 0.18 mmol, 75%). R_f = 0.4 (PE/EtOAc, 9:1, v/v); [α]_{D}^{19.5} = +3.7 (c = 2, CHCl_{3}); IR (film) = 2916, 2849, 1731, 1453, 1253, 1158, 1100, 1055, 931, 874, 791, 582 cm^{-1}; ^{1}H NMR (500 MHz, CDCl_{3}) δ 4.30 (p, J_{2,3} = J_{2,1} = 6.2 Hz, 1H, H-2), 4.15 (dd, J_{3a,3b} = 11.6 Hz, J_{3a,2} = 4.9 Hz, 1H, H-3a), 4.11–4.02 (m, 2H, H-5), 1.64–1.57 (m, 2H, H-6), 1.53–1.46 (m, 1H, H-21), 1.42 (s, 3H, H-1’), 1.35 (s, 3H, H-1’), 1.32–1.19 (m, 26H, H-7–19), 1.17–1.10 (m, 2H, H-20), 0.85 (d, J_{21,22a+b} = 6.6 Hz, 6H, H-22); ^{13}C NMR (125 MHz, CDCl_{3}) δ 173.64 (C-4), 109.81 (C-2’), 73.66 (C-2), 66.35 (C-1), 64.50 (C-3), 39.05 (C-20), 34.11 (C-5), 29.94, 29.71, 29.68, 29.67, 29.63, 29.58, 29.44, 29.23, 29.11 (C-7–18), 27.95 (C-21), 27.41 (C-19), 26.67 (C-1’), 25.39 (C-1’), 24.88 (C-6), 22.65 (C-22); HRMS (ESI) m/z calculated for [C_{26}H_{50}NaO_{4}]^{+}: 449.3601, found 449.3616.

1,2-O-Isopropylidene-1-O-(21-methyldecanooyl)-sn-glycerol (16f). By subjecting diol 14 (20 mg, 0.15 mmol), 21-methyldecanoic acid 15e (107 mg, 0.30 mmol), EDCI (88 mg, 0.46 mmol) and DMAP (18 mg, 0.15 mmol) to the general procedure for esterification (8 h), the title compound 16f was obtained as a white solid (51.5 mg, 0.11 mmol, 73%). R_f = 0.4 (PE/EtOAc, 9:1, v/v); [α]_{D}^{19.0} = +4.9 (c = 2, CHCl_{3}); IR (film) = 2987, 2917, 2849, 1733, 1471, 1372, 1239, 1158, 1114, 1051,
96, 890, 771, 583, 463 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.33 (p, \(J_{2,3} = J_{2,1} = 5.9\) Hz, 1H, H-2), 4.18 (dd, \(J_{3a,3b} = 11.5\) Hz, \(J_{3a,2} = 4.7\) Hz, 1H, H-3a), 4.14–4.05 (m, 2H, H-3b, H-1a), 3.76 (dd, \(J_{1b,1a} = 8.5\) Hz, \(J_{1b,2} = 6.2\) Hz, 1H, H-1b), 2.36 (t, \(J_{5,6} = 7.6\) Hz, 2H, H-5), 1.66–1.60 (m, 2H, H-6), 1.56–1.48 (s, 1H, H-24), 1.45 (s, 3H, H-1’), 1.38 (s, 3H, H-1’), 1.34–1.21 (m, 38 H, H-7–22), 1.18–1.11 (m, 2H, H-23), 0.85 (d, \(J_{24,25a+b} = 6.6\) Hz, 6H, H-25); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 173.67 (C-4), 109.83 (C-2’), 73.67 (C-2), 66.36 (C-1), 64.53 (C-3), 39.07 (C-23), 34.13 (C-5), 29.97, 29.75, 29.72, 29.69, 29.67, 29.62, 29.47, 29.27, 29.14 (C-7–21), 27.98 (C-24), 27.44 (C-22), 26.70 (C-1’), 25.41 (C-1’), 24.91 (C-6), 22.68 (C-25); HRMS (ESI) \(m/z\) calculated for \([C_{29}H_{56}NaO_4]^+\): 491.4071, found 491.4074.

1,2-O-Isopropylidene-3-O-(21-methyldoconoyl)-sn-glycerol (16g). By subjecting diol 14 (20 mg, 0.15 mmol), 21-methyldoconoic acid 15e (107 mg, 0.30 mmol), EDCI (88 mg, 0.46 mmol) and DMAP (18 mg, 0.15 mmol) to the general procedure for esterification (8 h), the title compound 16g was obtained as a white solid (52 mg, 0.11 mmol, 74%). \(R_f = 0.4\) (PE/EtOAc, 9:1, v/v); \([\alpha]_D^{17.5} = -1.6\) (c = 2, CHCl\(_3\)); IR (film) = 2986, 2927, 2846, 1732, 1414, 1301, 1222, 1188, 1028, 983, 846, 718, 550, 449 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.30 (p, \(J_{2,3} = J_{2,1} = 5.8\) Hz, 1H, H-2), 4.18 (dd, \(J_{3a,3b} = 11.5\) Hz, \(J_{3a,2} = 4.7\) Hz, 1H, H-3a), 4.14–4.06 (m, 2H, H-3b, H-1a), 3.76 (dd, \(J_{1b,1a} = 8.5\) Hz, \(J_{1b,2} = 6.2\) Hz, 1H, H-1b), 2.36 (t, \(J_{5,6} = 7.6\) Hz, 2H, H-5), 1.67–1.59 (m, 2H, H-6), 1.55–1.48 (m, 1H, H-24), 1.45 (s, 3H, H-1’), 1.39 (s, 3H, H-1’), 1.34–1.22 (m, 32H, H-7–22), 1.18–1.13 (m, 2H, H-23), 0.86 (d, \(J_{24,25a+b} = 6.6\) Hz, 6H, H-25); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 173.69 (C-4), 109.83 (C-2’), 73.66 (C-2), 66.35 (C-1), 64.52 (C-3), 39.07 (C-23), 34.16 (C-5), 234.13, 29.96, 29.75, 29.72, 29.69, 29.66, 29.61, 29.47, 29.14 (C-7–21), 27.98 (C-24), 27.44 (C-22), 26.70 (C-1’), 25.41 (C-1’), 24.90 (C-6), 22.68 (C-25); HRMS (ESI) \(m/z\) calculated for \([C_{29}H_{56}NaO_4]^+\): 491.4071, found 491.4074.
1,2-\textit{O}-Isopropylidene-1-\textit{O}-(25-methylhexacos-10-enoyl)-\textit{sn}-glycerol (16h). By subjecting diol 14 (7.5 mg, 0.056 mmol), \textit{(E/Z)}-25-methylhexacos-10-enoi acid 13 (45 mg, 0.112 mmol), EDCI (36 mg, 1.84 mmol) and DMAP (7 mg, 0.056 mmol) to the general procedure for esterification (8 h), the title compound 16h was obtained as a white solid (21 mg, 0.039 mmol, 71\%). Rf = 0.5 (PE/EtOAc, 9:1, v/v); [\alpha]_D^{21} +8.6 (c = 2, CHCl_3); IR (film) = 2922, 2852, 1741, 1644, 1465, 1380, 1251, 1158, 1085, 1056, 975, 841, 871, 720, 582, 489 cm\(^{-1}\); \textit{1}H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 5.37–5.30 (m, 2H, H-13, H-14), 4.29 (p, \(J_{2,3} = J_{2,1} = 5.8\) Hz, 1H, H-2), 4.16 (dd, \(J_{3a,3b} = 11.5\) Hz, \(J_{3a,2} = 4.7\) Hz, 1H, H-3a), 4.10–4.15 (m, 2H, H-3b, H-1a), 3.73 (dd, \(J_{1b,1a} = 8.0\) Hz, \(J_{1b,2} = 6.7\) Hz, 1H, H-1b), 2.33 (t, \(J_{5,6} = 7.4\) Hz, 2H, H-5), 2.02–1.95 (m, 2H, H12), 1.64–1.60 (m, 2H, H-6), 1.54–1.47 (m, 1H, H-28), 1.42 (s, 3H, H-1'), 1.36 (s, 3H-H-1'), 1.33–1.20 (m, 32H, H-7–11, H-15–25), 1.17–1.07 (m, 4H, H-26–27), 0.85 (d, \(J_{28,29a+b} = 6.6\) Hz, 6H, H-29); \textit{13}C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 173.50 (C-4), 129.83 (C13), 129.65 (C-14), 109.68 (C-2'), 73.54 (C-2), 66.22 (C-1), 64.38 (C-3), 38.94 (C-27), 33.98 (C-5), 29.82, 29.65, 29.60, 29.58, 29.56, 29.52, 29.47, 29.45, 29.33, 29.23, 29.12, 29.10, 28.99 (C-7–12, C15–25), 27.84 (C-28), 27.09 (C-26), 26.55 (C-1'), 25.27 (C-1'), 24.77 (C-6), 22.53 (C-29); HRMS (ESI) \textit{m}/\textit{z} calculated for [C\(_{33}\)H\(_{62}\)NaO\(_4\)]\(^{+}\): 545.4540, found 545.4541.

1,2-\textit{O}-Isopropylidene-3-\textit{O}-(25-methylhexacos-10-enoyl)-\textit{sn}-glycerol (16i). By subjecting diol 14 (12.5 mg, 0.095 mmol), \textit{(E/Z)}-25-methylhexacos-10-enoi acid 13 (78 mg, 0.191 mmol), EDCI (60 mg, 0.313 mmol) and DMAP (12 mg, 0.095 mmol) to the general procedure for esterification (8 h), the title compound 16i was obtained as a white solid (37 mg, 0.071 mmol, 75\%). Rf = 0.5 (PE/EtOAc, 9:1, v/v); [\alpha]_D^{21} -7.4 (c = 1 CHCl\(_3\)); IR (film) = 2921, 2852, 1741, 1645, 1465, 1369, 1213, 1158, 1063, 1056, 917, 843, 720, 657, 515, 463 cm\(^{-1}\); \textit{1}H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 5.35–5.37 (m, 2H, H-13, H-14), 4.29 (p, \(J_{2,3} = J_{2,1} = 5.8\) Hz, 1H, H-2), 4.16 (dd, \(J_{3a,3b} = 11.5\) Hz, \(J_{3a,2} = 4.7\) Hz, 1H, H-3a), 4.10–4.15 (m, 2H, H-3b, H-1a), 3.73 (dd, \(J_{1b,1a} = 8.0\) Hz, \(J_{1b,2} = 6.7\) Hz, 1H, H-1b), 2.33 (t, \(J_{5,6} = 7.4\) Hz, 2H, H-5), 2.02–1.95 (m, 2H, H12), 1.64–1.60 (m, 2H, H-6), 1.54–1.49 (m, 1H, H-28), 1.42 (s, 3H, H-1'), 1.36 (s, 3H, H-1'), 1.33–1.19 (m, 34H, H-7–11, H-15–26), 1.17–1.07 (m, 2H, H-27), 0.85 (d, \(J_{28,29a+b} = 6.6\) Hz, 6H, H-29); \textit{13}C NMR (125 MHz,
CDCl$_3$ δ 173.60 (C-4), 129.94 (C13), 129.76 (C-14), 109.79 (C-2’), 73.64 (C-2), 66.33 (C-1), 64.49 (C-3), 39.04 (C-27), 34.09 (C-5), 29.93, 29.72, 29.70, 29.68, 29.67, 29.65, 29.64, 29.62, 29.58, 29.55, 29.43, 29.31, 29.23, 29.21, 29.10 (C-7–12, C15–25), 27.95 (C-28), 27.41 (C-26), 26.66 (C-1’), 25.37 (C-1’), 24.87 (C-6), 22.64 (C-29); HRMS (ESI) $m/z$ calculated for [C$_{33}$H$_{63}$O$_4$]$^+$: 523.4721, found 523.7442.

1,2-O-Isopropylidene-1-O-(hexacosanoyl)-sn-glycerol (19f). By subjecting diol 14 (30 mg, 0.23 mmol), hexacosanoic acid 17e (182.5 mg, 0.46 mmol), EDCI (145.5 mg, 0.759 mmol) and DMAP (28 mg, 0.23 mmol) to the general procedure for esterification (8 h), the title compound 19f was obtained as a white solid (86 mg, 0.17 mmol, 73%). R$_f$ = 0.5 (PE/EtOAc, 9:1, v/v); [$\alpha$]$^D_{22}$ = +9.4 (c = 4, CHCl$_3$); IR (film) = 2945, 2914, 2837, 1731, 1463, 1267, 1192, 1054, 1041, 919, 896, 753, 681 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 4.33 (p, $J_{2,3} = J_{2,1} = 5.4$ Hz, 1H, H-2), 4.16 (dd, $J_{3a,3b} = 11.5$ Hz, $J_{3a,2} = 4.7$ Hz, 1H, H-3a), 4.11–4.03 (m, 2H, H-3b, H-1a), 3.75 (dd, $J_{1b,1a} = 8.5$ Hz, $J_{1b,2} = 6.2$ Hz, 1H, H-1b), 2.34 (t, $J_{5,6} = 7.4$ Hz, 2H, H-5), 1.63 (p, $J_{6,7} = J_{6,5} = 7.5$ Hz, 2H, H6), 1.43 (s, 3H, H-1’), 1.36 (s, 3H, H-1’), 1.33–1.20 (m, 44H, H-7–28), 0.87 (t, $J_{29,28} = 6.9$Hz, 3H, H-29); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.67 (C-4), 109.83 (C-2’), 73.67 (C-2), 66.35 (C-1), 64.52 (C-3), 34.12 (C-5), 31.93, 29.72, 29.69, 29.67, 29.61, 29.47, 29.38, 29.27, 29.13 (C-7–27), 26.69 (C-1’), 25.41 (C-1’), 24.90 (C-6), 22.71 (C-28), 14.14 (C-29); HRMS (ESI) $m/z$ calculated for [C$_{32}$H$_{62}$NaO$_4$]$^+$: 533.4540, found 533.4551.

1,2-O-Isopropylidene-3-O-(hexacosanoyl)-sn-glycerol (19g). By subjecting diol 14 (30 mg, 0.23 mmol), hexacosanoic acid 17e (182.5 mg, 0.46 mmol), EDCI (145.5 mg, 0.759 mmol) and DMAP (28 mg, 0.23 mmol) to the general procedure for esterification (8 h), the title compound 19g was obtained as a white solid (83.5 mg, 0.16 mmol, 71%). R$_f$ = 0.5 (PE/EtOAc, 9:1, v/v); [$\alpha$]$^D_{22}$ = -8.9 (c = 4 CHCl$_3$); IR (film) = 2931, 2852, 1714, 1439, 1251, 1146, 1109, 1084, 1047, 951, 863, 843, 746, 681, 547 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 4.33 (p, $J_{2,3} = J_{2,1} = 5.3$ Hz, 1H, H-2), 4.16 (dd, $J_{3a,3b} = 11.5$ Hz, $J_{3a,2} = 4.7$ Hz, 1H, H-3a), 4.11–4.03 (m, 2H, H-3b, H-1a), 3.73 (t, $J_{1b,2} = 7.3$, 1H, H-1b), 2.34 (t, $J_{5,6} = 7.6$ Hz, 2H, H-5), 1.63 (p, $J_{6,7} = J_{6,5} = 7.5$ Hz, 2H, H6), 1.43 (s, 3H, H-1’), 1.36 (s, 3H, H-1’), 1.33–
1.20 (m, 44H, H-7–28), 0.87 (t, J_{29,28} = 6.8 Hz, 3H, H-29); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.60 (C-4), 109.79 (C-2'), 73.66 (C-2), 66.34 (C-1), 64.50 (C-3), 34.10 (C-5), 31.94, 29.72, 29.67, 29.66, 29.61, 29.47, 29.38, 29.26, 29.13 (C-7–27), 26.67 (C-1'), 25.38 (C-1'), 24.89 (C-6), 22.69 (C-28), 14.12 (C-29); HRMS (ESI) m/z calculated for [C$_{32}$H$_{62}$NaO$_4$]$^+$: 533.4540, found 533.4553.

1,2-O-Isopropylidene-1-O-(2-tetradecyloctadecanoyl)-sn-glycerol (20a). By subjecting diol 14 (25 mg, 0.19 mmol), 2-tetradecyloctadecanoic acid 18 (183 mg, 0.38 mmol), EDCI (120 mg, 0.62 mmol) and DMAP (23 mg, 0.19 mmol) to the general procedure for esterification (8 h), the title compound 20a was obtained as a white solid (89 mg, 0.15 mmol, 77%). R$_f$ = 0.6 (PE/EtOAc, 9:1, v/v); $[\alpha]_D^{21.1}$ = +9.4 (c = 4 CHCl$_3$); IR (film) = 2943, 2914, 2865, 1754, 1417, 1345, 1234, 1160, 1114, 1097, 1009, 952, 826, 715, 621, 519 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 4.30 (p, J$_{2,1}$ = 5.8 Hz, 1H, H-2), 4.16 (d, J$_{3a,2}$ = 5.4 Hz, 1H, H-3a), 4.07 (dd, J$_{1a,1b}$ = 8.4 Hz, J$_{1a,2}$ = 6.4 Hz, 2H, H-3b, H-1a), 3.74 (dd, J$_{1b,1a}$ = 8.4, J$_{1b,2}$ = 6.1 Hz, 1H, H-1b), 2.39–2.33 (m, 1H, H-5), 1.61–1.55 (m, 4H, H-6, H-22), 1.43 (s, 3H, H-1’), 1.36 (s, 3H, H-1’), 1.31–1.16 (m, 52H, H-7–20, H-23–34), 0.87 (t, J$_{21,20}$ = J$_{35,34}$ = 6.9Hz, 6H, H-21, H-35); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 176.40 (C-4), 109.71 (C-2'), 73.62 (C-2), 66.46 (C-1), 64.17 (C-3), 46.66 (C-5), 32.44, 32.39, 31.92, 29.67, 29.36, 27.44, (C-6–19, C-22–33), 26.72 (C-1’), 25.42 (C-1’), 22.69 (C-20, C-34), 14.10 (C-21, C-35); HRMS (ESI) m/z calculated for [C$_{38}$H$_{74}$NaO$_4$]$^+$: 617.5479, found 617.5483.

1,2-O-Isopropylidene-3-O-(2-tetradecyloctadecanoyl)-sn-glycerol (20b). By subjecting diol 14 (25 mg, 0.19 mmol), 2-tetradecyloctadecanoic acid 18 (183 mg, 0.38 mmol), EDCI (120 mg, 0.62 mmol) and DMAP (23 mg, 0.19 mmol) to the general procedure for esterification (8 h), the title compound 20b was obtained as a white solid (80 mg, 0.13 mmol, 78%). R$_f$ = 0.6 (PE/EtOAc, 9:1, v/v); $[\alpha]_D^{18.6}$ = -8.2 (c = 3, CHCl$_3$); IR (film) = 2924, 2914, 2818, 1732, 1409, 1312, 1214, 1187, 1110, 1031, 953, 825, 742, 534 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 4.30 (p, J$_{2,1}$ = 5.9 Hz, 1H, H-2), 4.16 (d, J$_{3a,2}$ = 5.4 Hz, 1H, H-3a), 4.07 (dd, J$_{1a,1b}$ = 8.4 Hz, J$_{1a,2}$ = 6.4 Hz, 2H, H-3b, H-1a), 3.74 (dd, J$_{1b,1a}$ = 8.4, J$_{1b,2}$ = 6.3 Hz, 1H, H-1b), 2.39–2.31 (m, 1H, H-5), 1.60–1.54 (m, 4H, H-6, H-22), 1.43 (s, 3H, H-1’), 1.36 (s, 3H, H-1’), 1.31–1.16 (m, 52H, H-7–20, H-23–34), 0.88 (t, J$_{21,20}$ = J$_{35,34}$ = 6.9Hz,
6H, H-21, H-35); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 176.48 (C-4), 109.80 (C-2'), 73.72 (C-2), 66.57 (C-1), 64.27 (C-3), 46.71 (C-5), 32.53, 32.48, 32.01, 29.78, 29.74, 29.69, 29.64, 29.58, 29.45, 27.53 (C-6–19, C-22–33), 26.81 (C-1'), 25.50 (C-1'), 22.78 (C-20, C-34), 14.21 (C-21, C-35); HRMS (ESI) m/z calculated for [C$_{38}$H$_{74}$NaO$_4$]$^+$: 617.5479, found 617.5485.

**General procedure for isopropylidene deprotection:**

A solution of isopropylidene protected iso-branched, linear, or α-branched MAGs in TFA:THF:H$_2$O mixture (5 mL, 3:8:1, v/v/v) was added and the reaction was stirred at room temperature on rotary evaporator.$^2$ After 20-30 min, the reaction mixture was concentrated in vacuo and the resulting residue was purified using silica-gel column chromatography (PE/EtOAc, 10:1-2:1, v/v).

**1-O-(11-methyldodecanoyl)-sn-glycerol (6a).** By subjecting 16a (46 mg, 0.140 mmol), to the general procedure for isopropylidene deprotection (20 mins), the title compound 6a was obtained as a colourless oil (34 mg, 0.11 mmol, 85%). $R_f = 0.3$ (PE/EtOAc, 2:1, v/v); $[\alpha]_{D}^{13}$ = +5.4 (c = 1, CHCl$_3$); IR (film) = 3435, 3004, 2923, 2851, 1711, 1424, 1372, 1221, 1091, 532 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 4.21 (dd, $J_{3a,3b} = 11.7$ Hz, $J_{3a,2} = 4.6$ Hz, 1H, H-3a), 4.15 (dd, $J_{3b,3a} = 11.7$ Hz, $J_{3b,2} = 6.2$ Hz, 1H, H-3b), 3.93 (p, $J_{2,3} = J_{2,1} = 5.5$ Hz, 1H, H-2), 3.70 (dd, $J_{1a,1b} = 11.4$ Hz, $J_{1a,2} = 3.8$ Hz, 1H, H-1a), 3.60 (dd, $J_{1b,1a} = 11.4$ Hz, $J_{1b,2} = 5.8$ Hz, 1H, H-1b), 2.54 (br s, 1H, OH), 2.35 (t, $J_{5,6} = 7.6$ Hz, 2H, H-5), 2.11 (br s, 1H, OH), 1.66–1.59 (m, 2H, H-6), 1.54–1.46 (m, 1H, H-14), 1.33–1.23 (m, 12H, H-7–12), 1.18–1.11 (m, 2H, H-13), 0.86 (d, $J_{14,15a+b} = 6.6$ Hz, 6H, H-15); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 174.37 (C-4), 70.26 (C-2), 65.16 (C-3), 63.31 (C-1), 39.03 (13), 34.15 (C-5), 29.88, 29.61, 29.44, 29.24, 29.11 (C-7–11), 27.95 (C-14), 27.38 (C-12), 24.90 (C-6), 22.65 (C-15); HRMS (ESI) m/z calculated for [C$_{16}$H$_{32}$NaO$_4$]$^+$: 311.2193, found 311.2191.

**1-O-(13-methyltetradecanoyl)-sn-glycerol (6b).** By subjecting 16b (45 mg, 0.125 mmol), to the general procedure for isopropylidene deprotection (25 mins), the title compound 6b was obtained as a white solid (36 mg, 0.11 mmol, 91%). $R_f = 0.3$ (PE/EtOAc, 2:1, v/v); $[\alpha]_{D}^{22}$ = +6.5 (c = 1, CHCl$_3$); IR (film) = 3434, 3004, 2917, 2849, 1732,
1467, 1372, 1171, 1045, 720 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.21 (dd, \(J_{3a,3b} = 11.6\) Hz, \(J_{3a,2} = 4.7\) Hz, 1H, H-3a), 4.14 (dd, \(J_{3b,3a} = 11.6\) Hz, \(J_{3b,2} = 6.1\) Hz, 1H, H-3b), 3.93 (p, \(J_{2,3} = J_{2,1} = 5.1\) 1H, H-2), 3.71 (dd, \(J_{1a,1b} = 11.3\) Hz, \(J_{1a,2} = 4.1\) Hz, 1H, H-1a), 3.60 (dd, \(J_{1a,1b} = 11.3\) Hz, \(J_{1b,2} = 5.6\) Hz, 1H, H-1b), 2.36 (t, \(J_{5,6} = 7.6\) Hz, 2H, H-5), 1.67–1.59 (m, 2H, H-6), 1.55–1.48 (m, 1H, H-16), 1.35–1.22 (m, 16H, H-7–16), 1.18–1.12 (m, 2H, H-15), 0.86 (d, \(J_{16,17a+b} = 7.2\) Hz, 6H, H-17); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 174.40 (C-4), 70.27 (C-2), 65.16 (C-3), 63.33 (C-1), 39.05 (15), 34.16 (C-5), 29.94, 29.71, 29.65, 29.60, 29.45, 29.25, 29.13 (C-7–13), 27.42 (C-16), 27.42 (C-14), 24.92 (C-6), 22.67 (C-17); HRMS (ESI) \(m/z\) calculated for \([C_{18}H_{36}NaO_4]^+\): 339.2506, found 339.2514.

3-\(O\)-(13-methyltetradecanoyl)-\(sn\)-glycerol (6c). By subjecting 16c (50 mg, 0.14 mmol), to the general procedure for isopropylidene deprotection (30 mins), the title compound 6c was obtained as a white solid (41 mg, 0.13 mmol, 92%). \(R_f\) = 0.3 (PE/EtOAc: 2:1, v/v); \([\alpha]_D^{27.1} = -5.3\) (c = 1, CHCl\(_3\)); IR (film) = 3435, 3014, 2927, 2843, 1722, 1462, 1376, 1045, 987, 856, 719 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.19 (dd, \(J_{3a,3b} = 11.6\) Hz, \(J_{3a,2} = 4.7\) Hz, 1H, H-3a), 4.14 (dd, \(J_{3b,3a} = 11.6\) Hz, \(J_{3b,2} = 6.1\) Hz, 1H, H-3b), 3.96–3.92 (m, 1H, H-2), 3.68 (d, \(J_{1b,2} = 3.9\) Hz, 1H, H-1a), 3.62–3.56 (m, 1H, H-1b), 2.77 (br s, 1H, OH), 2.34 (t, \(J_{5,6} = 7.6\) Hz, 2H, H-5), 1.62 (p, \(J_{6,7} = J_{6,5} = 7.6\) Hz, 2H, H-6), 1.55–1.45 (m, 1H, H-16), 1.34–1.99 (m, 16H, H-7–14), 1.17–1.11 (m, 2H, H-15), 0.86 (d, \(J_{16,17a+b} = 7.2\) Hz, 6H, H-17); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 174.41 (C-4), 70.25 (C-2), 65.13 (C-3), 63.33 (C-1), 39.04 (15), 34.15 (C-5), 29.93, 29.70, 29.68, 29.67, 29.64, 29.60, 29.44, 29.24, 29.12 (C-7–13), 27.96 (C-16), 27.41 (C-14), 24.90 (C-6), 22.66 (C-17); HRMS (ESI) \(m/z\) calculated for \([C_{18}H_{36}NaO_4]^+\): 339.2506, found 339.2511.

1-\(O\)-(17-methyloctadecanoyl)-\(sn\)-glycerol (6d). By subjecting 16d (75 mg, 0.18 mmol), to the general procedure for isopropylidene deprotection (20 mins), the title compound 6d was obtained as a white solid (59.6 mg, 0.16 mmol, 89%). \(R_f\) = 0.3 (PE/EtOAc: 2:1, v/v); \([\alpha]_D^{7.0} = +2.8\) (c = 1, CHCl\(_3\)); IR (film) = 3425, 3114, 2926, 2849, 1734, 1467, 1363, 1171, 1152, 1008, 907, 887, 732 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.19 (dd, \(J_{3a,3b} = 11.6\) Hz, \(J_{3a,2} = 4.7\) Hz, 1H, H-3a), 4.14 (dd, \(J_{3b,3a} = 11.6\) Hz, \(J_{3b,2} = 6.1\) Hz, 1H, H-3b), 3.95–3.90 (m, 1H, H-2), 3.68 (d, \(J_{1a,2} = 3.9\) Hz, 1H, H-1a), 3.62–3.56 (m, 1H, H-1b), 2.61 (br s, 1H, OH), 2.34
(t, $J_{5,6} = 7.6$ Hz, 2H, H-5), 2.17 (br s, 1H, OH), 1.62 (p, $J_{6,5} = J_{6,7} = 7.6$ Hz, 2H, H-6), 1.55–1.47 (m, 1H, H-20), 1.34–1.22 (m, 24H, H-7–18), 1.17–1.11 (m, 2H, H-19), 0.86 (d, $J_{20,21a+b} = 7.2$ Hz, 6H, H-21); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 174.39 (C-4), 70.26 (C-2), 65.15 (C-3), 63.31 (C-1), 39.05 (19), 34.15 (C-5), 29.94, 29.72, 29.69, 29.68, 29.67, 29.64, 29.59, 29.45, 29.24, 29.12, 29.10 (C-7–17), 27.96 (C-20), 27.42 (C-18), 24.90 (C-6), 22.66 (C-21); HRMS (ESI) $m/z$ calculated for [C$_{22}$H$_{45}$O$_4$]$^+$: 373.3312, found 373.3318.

1-O-(18-methylnonadecanoyl)-sn-glycerol (6e). By subjecting 16e (77 mg, 0.18 mmol), to the general procedure for isopropylidene deprotection (30 mins), the title compound 6e was obtained as a white solid (65 mg, 0.167 mmol, 93%). $R_f = 0.2$ (PE/EtOAc, 2:1, v/v); $[\alpha]_D^{19.4} = +2.2$ (c = 1.2, CHCl$_3$); IR (film) = 3411, 3304, 2916, 2849, 1724, 1463, 1351, 1172, 1018, 906, 867, 729 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.20 (dd, $J_{3a,3b} = 11.7$ Hz, $J_{3a,2} = 4.7$ Hz, 1H, H-3a), 4.15 (dd, $J_{3b,2} = 11.7$ Hz, $J_{3b,3} = 4.7$ Hz, 1H, H-3b), 3.93 (p, $J_{2,3} = J_{2,1} = 5.2$ Hz, 1H, H-2), 3.69 (dd, $J_{1a,lb} = 11.5$ Hz, $J_{1a,2} = 3.9$ Hz, 1H, H-1a), 3.59 (dd, $J_{1b,1a} = 11.5$ Hz, $J_{1b,2} = 5.9$ Hz, H-1b), 2.35 (t, $J_{5,6} = 7.5$ Hz, 2H, H-5), 1.62 (p, $J_{6,7} = J_{6,5} = 7.4$ Hz, 2H, H-6), 1.55–1.48 (m, 1H, H-21), 1.34–1.19 (m, 26H, H-7–19), 1.18–1.12 (m, 2H, H-20), 0.86 (d, $J_{21,22a+b} = 7.1$ Hz, 6H, H-22); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 174.38 (C-4), 70.26 (C-2), 65.14 (C-3), 63.32 (C-1), 39.05 (20), 34.15 (C-5), 29.94, 29.72, 29.69, 29.67, 29.64, 29.60, 29.45, 29.24, 29.12 (C-7–18), 27.96 (C-21), 27.42 (C-19), 24.90 (C-6), 22.66 (C-22); HRMS (ESI) $m/z$ calculated for [C$_{23}$H$_{47}$O$_4$]$^+$: 387.3469, found 387.3474.

1-O-(21-methyloctadecanoyl)-sn-glycerol (6f). By subjecting 16f (51.5 mg, 0.11 mmol), to the general procedure for isopropylidene deprotection (30 mins), the title compound 6f was obtained as a white solid (43 mg, 0.10 mmol, 91%). $R_f = 0.3$ (PE/EtOAc, 2:1, v/v); $[\alpha]_D^{10.8} = +2.4$ (c = 1, CHCl$_3$); IR (film) = 3384, 2922, 2852, 1737, 1457, 1341, 1172, 1012, 916, 854, 769, 543 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.20 (dd, $J_{3a,3b} = 11.7$ Hz, $J_{3a,2} = 4.6$ Hz, 1H, H-3a), 4.14 (dd, $J_{3b,3a} = 11.7$ Hz, $J_{3b,2} = 6.2$ Hz, 1H, H-3b), 3.94 (p, $J_{2,3} = J_{2,1} = 5.3$ Hz, 1H, H-2), 3.68 (d, $J_{1a,2} = 3.9$ Hz, 1H, H-1a), 3.62–3.56 (d, $J_{1b,2} = 3.8$ Hz, 1H, H-1b), 2.61 (br s, 1H, OH), 2.35 (t, $J_{5,6} = 7.6$ Hz, 2H, H-5), 2.18 (br s, 1H, OH), 1.62 (p, $J_{6,7} = J_{6,5} = 7.6$ Hz, 2H, H-6), 1.54–1.45 (m, 1H, H-24), 1.31–1.25 (m, 30H, H-7–22), 1.17–1.14 (m, 2H, H-
23), 0.86 (d, $J_{24,25a+b} = 7.2$ Hz, 6H, H-25); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 174.38 (C-4), 70.27 (C-2), 65.16 (C-3), 63.32 (C-1), 39.05 (23), 34.15 (C-5), 29.93, 29.70, 29.64, 29.59, 29.44, 29.24, 29.12, 29.10 (C-7–21), 27.96 (C-24), 27.41 (C-22), 24.91 (C-6), 22.66 (C-25); HRMS (ESI) $m/z$ calculated for [C$_{26}$H$_{52}$NaO$_4$]$^+$: 451.3758, found 451.3762.

3-O-(21-methyldeconoyl)-sn-glycerol (6g). By subjecting diol 16g (52 mg, 0.11 mmol), to the general procedure for isopropylidene deprotection (30 mins), the title compound 6g was obtained as a white solid (43.5 mg, 0.101 mmol, 92%). $R_t = 0.3$ (PE/EtOAc, 2:1, v/v); $[\alpha]_D^{21.8} = -1.8$ (c = 2, CHCl$_3$); IR (film) = 3384, 2922, 2852, 1737, 1457, 1341, 1172, 1012, 916, 854, 769, 543 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) 4.21 (dd, $J_{3a,3b} = 11.6$ Hz, $J_{3a,2} = 4.7$ Hz, 1H, H-3a), 4.16 (dd, $J_{3b,3b} = 11.6$ Hz, $J_{3b,2} = 4.7$ Hz, 1H, H-3b), 5.96 (p, $J_{23} = J_{2,1} = 5.1$ Hz, 1H, H-2), 3.70 (dd, $J_{1a,1b} = 11.6$ Hz, $J_{1a,2} = 3.9$ Hz, 1H, H-1a), 3.64 (dd, $J_{1b,1a} = 11.6$ Hz, $J_{1b,2} = 5.9$ Hz, H-1b), 2.64 (br s, 1H, OH), 2.39 (t, $J_{5,6} = 7.4$ Hz, 2H, H-5), 1.66–1.61 (m, 2H, H-6), 1.55–1.47 (m, 1H, H-24), 1.30–1.18 (m, 32H, H-7–22), 1.15–1.12 (m, 2H, H-23), 0.86 (d, $J_{24,25a+b} = 7.1$ Hz, 6H, H-25); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 174.28 (C-4), 70.15 (C-2), 65.03 (C-3), 63.22 (C-1), 38.95 (23), 34.04 (C-5), 28.84, 29.62, 29.64, 29.59, 29.58, 29.56, 29.49, 29.34, 29.14, 29.12, 29.02, 29.00 (C-7–21), 27.85 (C-24), 27.31 (C-22), 24.80 (C-6), 22.55 (C-25); HRMS (ESI) $m/z$ calculated for [C$_{26}$H$_{53}$O$_4$]$^+$: 429.3938, found 429.3924.

1-O-(hexacosanoyl)-sn-glycerol (7f). By subjecting 19f (86 mg, 0.17 mmol), to the general procedure for isopropylidene deprotection (30 mins), the title compound 7f was obtained as a white solid (63 mg, 0.16 mmol, 93%). $R_t = 0.3$ (PE/EtOAc, 2:1, v/v); $[\alpha]_D^{20.5} = +8.4$ (c = 2, CHCl$_3$); IR (film) = 3361, 2924, 2852, 1731, 1452, 1332, 1187, 1007, 957, 823, 791, 673 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) 4.20 (dd, $J_{3a,3b} = 11.7$ Hz, $J_{3a,2} = 4.6$ Hz, 1H, H-3a), 4.15 (dd, $J_{3b,3b} = 11.6$ Hz, $J_{3b,2} = 6.2$ Hz, 1H, H-3b), 3.93 (p, $J_{2,3} = J_{2,1} = 5.2$ Hz, 1H, H-2), 3.69 (dd, $J_{1a,1b} = 11.4$ Hz, $J_{1a,2} = 4$ Hz, 1H, H-1a), 3.59 (dd, $J_{1b,1a} = 11.4$ Hz, $J_{1b,2} = 5.8$ Hz, H-1b), 2.36 (t, $J_{5,6} = 8.0$ Hz, 2H, H-5), 1.67–1.61 (m, 2H, H-6), 1.31–1.27 (m, 44H, H-7–28), 0.88 (t, $J_{28,29} = 7.0$ Hz, 3H, H-29); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.62 (C-4), 73.66 (C-2), 66.34 (C-3), 64.51 (C-1), 34.11 (C-5), 31.94, 29.71, 29.69, 29.67, 29.47, 29.38, 29.26, 29.13 (C-7–27), 24.90 (C-6), 22.71 (C-28), 14.13 (C-29); HRMS (ESI) $m/z$ calculated for [C$_{29}$H$_{59}$O$_4$]$^+$: 471.4408, found 471.4422.
3-O-(hexacosanoyl)-sn-glycerol (7g). By subjecting 19g (83 mg, 0.16 mmol), to the general procedure for isopropylidene deprotection (25 mins), the title compound 7g was obtained as a white solid (58 mg, 0.145 mmol, 91%). R_f = 0.3 (PE/EtOAc, 2:1, v/v); [α]_D^{27} = -8.8 (c = 2, CHCl_3); IR (film) = 3341, 2921, 2851, 1724, 1487, 1331, 1165, 1009, 967, 867, 761, 656 cm⁻¹; ¹H NMR (500 MHz, CDCl_3) δ 4.23 (dd, J_{3a,3b} = 11.7 Hz, J_{3a,2} = 4.5 Hz, 1H, H-3a), 4.15 (dd, J_{3b,3b} = 11.7 Hz, J_{3b,2} = 6.2 Hz, 1H, H-3b), 3.95 (p, J_{2,3} = J_{2,1} = 5.2 Hz, 1H, H-2), 3.69 (dd, J_{1a,1b} = 11.5 Hz, J_{1a,2} = 4.0 Hz, 1H, H-1a), 3.59 (dd, J_{1b,1a} = 11.5 Hz, J_{1b,2} = 5.8 Hz, H-1b), 2.36 (t, J_{5,6} = 7.4 Hz, 2H, H-5), 1.67–1.61 (m, 2H, H-6), 1.31–1.27 (m, 44H, H-7–28), 0.89 (t, J_{28,29} = 7.0 Hz, 3H, H-29); ¹³C NMR (125 MHz, CDCl_3) δ 173.66 (C₁), 73.66 (C₂), 66.34 (C₃), 64.52 (C-1), 34.12 (C-5), 31.94, 29.71, 29.69, 29.67, 29.66, 29.61, 29.47, 29.38, 29.26, 29.13 (C-7–27), 24.90 (C-6), 22.70 (C-28), 14.14 (C-29); HRMS (ESI) m/z calculated for [C_{29}H_{58}O₄]^+: 471.4408, found 471.4413.

1-O-tetradecyloctadecanoyl-sn-glycerol (8a). By subjecting 20a (89 mg, 0.15 mmol), to the general procedure for isopropylidene deprotection (20 mins), the title compound 8a was obtained as a white solid (66 mg, 0.137 mmol, 91%). R_f = 0.4 (PE/EtOAc, 2:1, v/v); [α]_D^{19} = +7.6 (c = 2, CHCl_3); IR (film) = 3381, 2924, 2862, 1727, 1476, 1345, 1174, 1008, 926, 853, 787, 5490 cm⁻¹; ¹H NMR (500 MHz, CDCl_3) δ 4.21 (dd, J_{3a,3b} = 11.7 Hz, J_{3a,2} = 4.7 Hz, 1H, H-3a), 4.15 (dd, J_{3b,3b} = 11.7 Hz, J_{3b,2} = 6.1 Hz, 1H, H-3b), 3.92 (p, J_{2,3} = J_{2,1} = 5.2 Hz, 1H, H-2), 3.69 (dd, J_{1a,1b} = 11.5 Hz, J_{1a,2} = 3.9 Hz, 1H, H-1a), 3.59 (dd, J_{1b,1a} = 11.5 Hz, J_{1b,2} = 5.7 Hz, H-1b), 2.39–2.34 (m, 1H, H-5), 2.28 (br s, 1H, OH), 1.62–1.57 (m, 2H, H-6), 1.49–1.39 (m, 2H, H-22), 1.32–1.05 (m, 54H, H-7–20, H-23–35), 0.86 (t, J_{21,20} = J_{35,34} = 7.0 Hz, 6H, H-21, H-35); ¹³C NMR (125 MHz, CDCl_3) δ 177.27 (C-4), 70.3 (C-2), 65.00 (C-3), 63.34 (C-1), 46.70 (C-5), 32.48, 32.40, 31.92, 29.69, 29.67, 29.60, 29.54, 29.47, 29.36, 27.46, 27.43, 22.69 (C-6–20, C22–33), 14.12 (C-21, C-35); HRMS (ESI) m/z calculated for [C_{33}H_{71}O_{4}]^+: 555.5347, found 555.5349.
3-O-2-tetradecyloctadecanoyl-sn-glycerol (8b). By subjecting diol 20b (80 mg, 0.13 mmol), to the general procedure for isopropylidene deprotection (30 mins), the title compound 8b was obtained as a white solid (53 mg, 0.11 mmol, 89%). \( \Delta f = 0.4 \) (PE/EtOAc, 2:1, v/v); \([\alpha]_D^{20.4} = -7.8 \) (c = 2, CHCl₃); IR (film) = 3381, 2924, 2835, 1714, 1443, 1328, 1179, 1013, 906, 854, 761, 541 cm⁻¹; \(^1\)H NMR (500 MHz, CDCl₃) 4.21 (dd, \( J_{3a,3b} = 11.7 \) Hz, \( J_{3a,2} = 4.8 \) Hz, 1H, H-3a), 4.15 (dd, \( J_{3b,3b} = 11.7 \) Hz, \( J_{3b,2} = 6.0 \) Hz, 1H, H-3b), 3.92 (p, \( J_{2,3} = J_{2,1} = 5.2 \) Hz, 1H, H-2), 3.69 (dd, \( J_{1a,1b} = 11.5 \) Hz, \( J_{1a,2} = 3.9 \) Hz, 1H, H-1a), 3.59 (dd, \( J_{1b,1a} = 11.5 \) Hz, \( J_{1b,2} = 5.8 \) Hz, H-1b), 2.39–2.33 (m, 1H, H-5), 2.28 (br s, 1H, OH), 1.61–1.57 (m, 2H, H-6), 1.48–1.41 (m, 2H, H-22), 1.31–1.24 (m, 54H, H-7–20, H-23–35), 0.86 (t, \( J_{21,20} = J_{35,34} = 7.0 \) Hz, 6H, H-21, H-35); \(^{13}\)C NMR (125 MHz, CDCl₃) δ 177.26 (C-4), 70.36 (C-2), 64.99 (C-3), 63.35 (C-1), 46.70 (C-5), 32.40, 31.92, 29.70, 29.64, 29.60, 29.54, 29.47, 29.36, 27.43, 22.69 (C-6–20, C22-34), 14.12 (C-21, C-34); HRMS (ESI) \( m/z \) calculated for [C₉₃H₁₇₁NaO₄]⁺: 577.5166, found 577.5172.

**General procedure for hydrogenation:**

A solution of isopropylidene protected MAGs dissolved in DCM (5 mL) was added Pd(OH)₂/C. H₂-gas was allowed to bubble through the reaction mixture for 12 hours. The suspension was then diluted with CH₂Cl₂, filtered through celite and concentrated in vacuo. The resulting residue was purified using silica-gel column chromatography (PE/EtOAc, 10:1-2:1, v/v).

1-O-(25-methylhexacosanoyl)-sn-glycerol (6h). By subjecting 16h (21 mg, 0.039 mmol) and Pd(OH)₂/C (40 mg) to the general procedure for hydrogenation, the title compound 6h was obtained as a white solid (17 mg, 0.034 mmol, 89%). \( \Delta f = 0.4 \) (PE/EtOAc, 2:1, v/v); \([\alpha]_D^{21.5} = +6.4 \) (c = 1, CHCl₃); IR (film) = 3374, 2932, 2851, 1734, 1453, 1346, 1189, 1012, 918, 857, 799, 673, 576 cm⁻¹; \(^1\)H NMR (500 MHz, CDCl₃) 4.20 (dd, \( J_{3a,3b} = 11.7 \) Hz, \( J_{3a,2} = 4.7 \) Hz, 1H, H-3a), 4.15 (dd, \( J_{3b,3a} = 11.7 \) Hz, \( J_{3b,2} = 4.7 \) Hz, 1H, H-3b), 3.93 (p, \( J_{2,3} = J_{2,1} = 5.2 \) Hz, 1H, H-2), 3.69 (dd, \( J_{1a,1b} = 11.7 \) Hz, \( J_{1a,2} = 3.9 \) Hz, 1H, H-1a), 3.60 (dd, \( J_{1b,1a} = 11.7 \) Hz, \( J_{1b,2} = 5.9 \) Hz, H-1b), 2.39–2.33 (m, 2H, H-5), 1.65–1.61 (m, 2H, H-6), 1.55–1.48 (m, 1H, H-28), 1.32–1.21 (m, 40H, H-7–26), 1.1–1.12 (m, 2H, H-27), 0.86 (d, \( J_{28,29a+b} = 7.1 \) Hz, 6H, H-29); \(^{13}\)C NMR (125 MHz, CDCl₃) δ 174.28 (C-4), 70.16 (C-2), 65.06 (C-3), 63.21 (C-
3-O-(25-methylhexacosanoyl)-sn-glycerol (6i). By subjecting 16i (37 mg, 0.071 mmol) and Pd(OH)$_2$/C (53 mg) to the general procedure for hydrogenation, the title compound 6i was obtained as a white solid (30 mg, 0.062 mmol, 87%). R$_f$ = 0.4 (PE/EtOAc, 2:1, v/v); $[\alpha]_D^{21.6}$ = -4.8 (c = 1, CHCl$_3$); IR (film) = 3314, 2912, 2841, 1715, 1452, 1347, 1189, 1009, 927, 853, 712, 576 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) 4.20 (dd, $J_{3a,3b}$ = 11.7 Hz, $J_{3a,2}$ = 4.7 Hz, 1H, H-3a), 4.15 (dd, $J_{3b,3b}$ = 11.6 Hz, $J_{3b,2}$ = 4.7 Hz, 1H, H-3b), 3.93 (p, $J_{2,3} = J_{2,1}$ = 5.2 Hz, 1H, H-2), 3.69 (dd, $J_{1a,1b}$ = 11.6 Hz, $J_{1a,2}$ = 3.9 Hz, 1H, H-1a), 3.59 (dd, $J_{1b,1a}$ = 11.6 Hz, $J_{1b,2}$ = 5.9 Hz, H-1b), 2.39–2.33 (m, 2H, H-5), 1.6–1.61 (m, 2H, H-6), 1.55–1.48 (m, 1H, H-28), 1.32–1.21 (m, 40H, H-7–26), 1.15–1.12 (m, 2H, H-27), 0.86 (d, $J_{28,29a+1b}$ = 7.1 Hz, 6H, H-29); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 174.38 (C-4), 70.26 (C-2), 65.16 (C-3), 63.31 (C-1), 39.05 (27), 34.15 (C-5), 29.94, 29.72, 29.69, 29.68, 29.67, 29.64, 29.59, 29.44, 29.24, 29.12 (C-7–25), 27.96 (C-28), 27.41 (C-26), 24.90 (C-6), 22.66 (C-29); HRMS (ESI) m/z calculated for [C$_{30}$H$_{60}$NaO$_4$]$^+$: 507.4401, found 507.4402.
Biological Methods:

2B4-NFAT-GFP reporter cells assay:

Purified lipids (1 mg/mL) in chloroform/methanol (2:1, v/v) were serially diluted with isopropanol and added to the wells of 96-well plates, followed by evaporation of the solvent. The concentration of 2B4-NFAT-GFP reporter cells expressing hMincle + FcRγ, mMincle + FcRγ, or FcRγ was adjusted to $4 \times 10^5$ cells/mL and 100 uL/well were added to MAG-coated plates (0.1, or 1 nmol/well) for 18 h. The reporter cells were harvested, stained with DAPI, and analysed for NFAT-GFP expression using flow cytometry.

![NFAT-GFP expression](image)

**Figure 1:** NFAT-GFP 2B4 reporter cells expressing mMincle + FcRγ, or FcRγ-only were stimulated using MAG-coated plates (0.1 or 1 nmol/well) for 18 h. The cells were then harvested and examined for NFAT-GFP expression. Data reported is a representative of two independent experiments performed in duplicate (mean ± SEM).

Human Monocyte Assay:

The use of human leukocyte from 20 healthy donors with written informed consent was approved by New Zealand Northern A Health and Disability Ethics Committee (approval number 15/NTA/178). Human monocytes were purified from whole blood by negative selection using RosettaSep Human Monocyte Enrichment Cocktail (StemCell) according to the manufacturer’s instructions. The density centrifugation was carried out using Ficoll-Paque (1.078 g/L, GE...
Healthcare Life Sciences) and stained with CellTrace™ CFSE Cell Proliferation Kit (Thermo Fisher). The cell concentration was adjusted to 1 x 10^6 cell/mL in complete RMPI (10% FCS, 1% PenStrep) and 100 μL were added to individual well, with plate-coated MAGs or TDB (0.1, or 1 nmol/well). Supernatant was collected after 24 h incubation at 37 °C (5% CO₂).

**Cytokine Analysis:** hIL-8 (BD Biosciences) levels were determined via sandwich ELISA according to the manufacturer’s instructions.

**MMT Assay:**

A standard MTT assay was performed using HL-60 cell line. Cells suspended in cRPMI media (1×10^6 cells/mL) were added to a 96 well plate (100 μL/well) coated with 6b, 7d, 7f, 8a or cyclohexamide (positive control) at concentrations of 0.001, 0.01, 0.1, 1, 10, or 100 nmol/well, and incubated at 37 °C (5% CO₂) for 22 h. Untreated cells served as a negative control. The supernatant was then removed, and the cells were treated with 100 μL of 1mg/mL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution and incubated for a further 3 h. After this time, 150 μL of MTT solvent (4 mM HCl, 0.1% NP40 in isopropanol) was added into each well. The solution was pipetted to fully dissolve the MTT formazan and optical density was measured at 590 nm.

**Endotoxin Testing:**

All synthesised MAGs were confirmed to be endotoxin free at a sensitivity of ≤0.1 EU/mL by using the Pierce Limulus amebocyte lysate (LAL) chromogenic endotoxin quantitation kit (Thermo Scientific).

**References:**

NMR Spectra

$^1$H NMR, CDCl$_3$, 500 MHz
$^{13}$C NMR, CDCl$_3$, 125 MHz

16a
$^1$H NMR, CDCl$_3$, 500 MHz

16b
$^{13}$C NMR, CDCl$_3$, 125 MHz

16b
$^{13}$C NMR, CDCl$_3$, 125 MHz

![Chemical Structure Image]
$^1$H NMR, CDCl$_3$, 500 MHz

16e
$^1$H NMR, CDCl3, 125 MHz

31
$^1$H NMR, CDCl$_3$, 500 MHz

16g
$^{13}$C NMR, CDCl$_3$, 125 MHz

16g
$^{13}$C NMR, CDCl$_3$, 125 MHz
$^1$H NMR, CDCl$_3$, 500 MHz

16h
$^{13}$C NMR, CDCl$_3$, 125 MHz
$^1$H NMR, CDCl$_3$, 500 MHz

16i
$^{13}$C NMR, CDCl$_3$, 125 MHz

![Chemical Structure](image)
$^1$H NMR, CDCl$_3$, 500 MHz

19f
$^{13}$C NMR, CDCl$_3$, 125 MHz

19f
$^1$H NMR, CDCl$_3$, 500 MHz

19g
$^1$H NMR, CDCl$_3$, 500 MHz

![NMR spectrum](image)

20a
$^1$H NMR, CDCl$_3$, 500 MHz

6a
$^{13}$C NMR, CDCl$_3$, 125 MHz
$^{1}H$ NMR, CDCl$_3$, 500 MHz

[Chemical structure image]
$^{13}$C NMR, CDCl$_3$, 125 MHz
$^1$H NMR, CDCl$_3$, 500 MHz
$^{13}$C NMR, CDCl$_3$, 125 MHz

![Chemical Structure](image)

$6c$
$^1$H NMR, CDCl$_3$, 500 MHz

6d
$^{13}$C NMR, CDCl$_3$, 125 MHz
$^1$H NMR, CDCl$_3$, 500 MHz

6e
$^1$H NMR, CDCl$_3$, 500 MHz
$^1$H NMR, CDCl$_3$, 500 MHz
$^{13}$C NMR, CDCl$_3$, 125 MHz

$6g$
$^{1}H$ NMR, CDCl$_3$, 500 MHz
$^{13}$C NMR, CDCl$_3$, 125 MHz

6h
$^{13}$C NMR, CDCl$_3$, 125 MHz

**Chemical Structure: 6i**

![Chemical Structure](image)
$^1$H NMR, CDCl$_3$, 500 MHz

![NMR spectrum image](image-url)
$^{13}$C NMR, CDCl$_3$, 125 MHz

![Chemical Structure](attachment:image.png)
$^{13}$C NMR, CDCl$_3$, 125 MHz

![Chemical Structure](image_url)

$7g$
$^1$H NMR, CDCl$_3$, 500 MHz
$^{13}$C NMR, CDCl$_3$, 125 MHz

![Chemical Structure](image)
$^1$H NMR, CDCl$_3$, 500 MHz

Diagram showing a chemical structure labeled 8b with ppm values indicated on the spectrum.
$^{13}$C NMR, CDCl$_3$, 125 MHz