Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Corynomycolic acid-containing glycolipids signal through the pattern recognition receptor Mincle

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Supplementary Figures

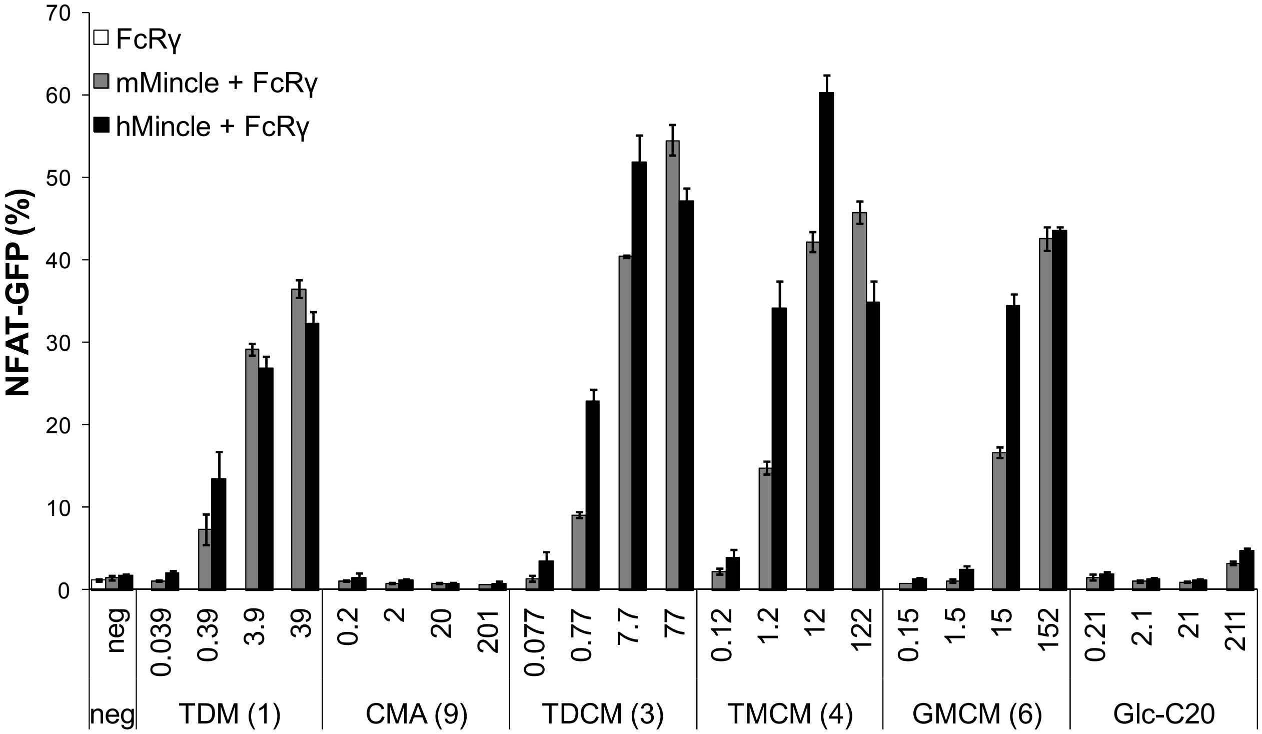


Figure S1.

Data from Figure 3A, replotted according to molecular weight. Amounts correspond to nnmol of (glyco)lipid per well. Molar quantity of TDM was calculated using an estimated molecular mass of 2582 g mol^{-1}.

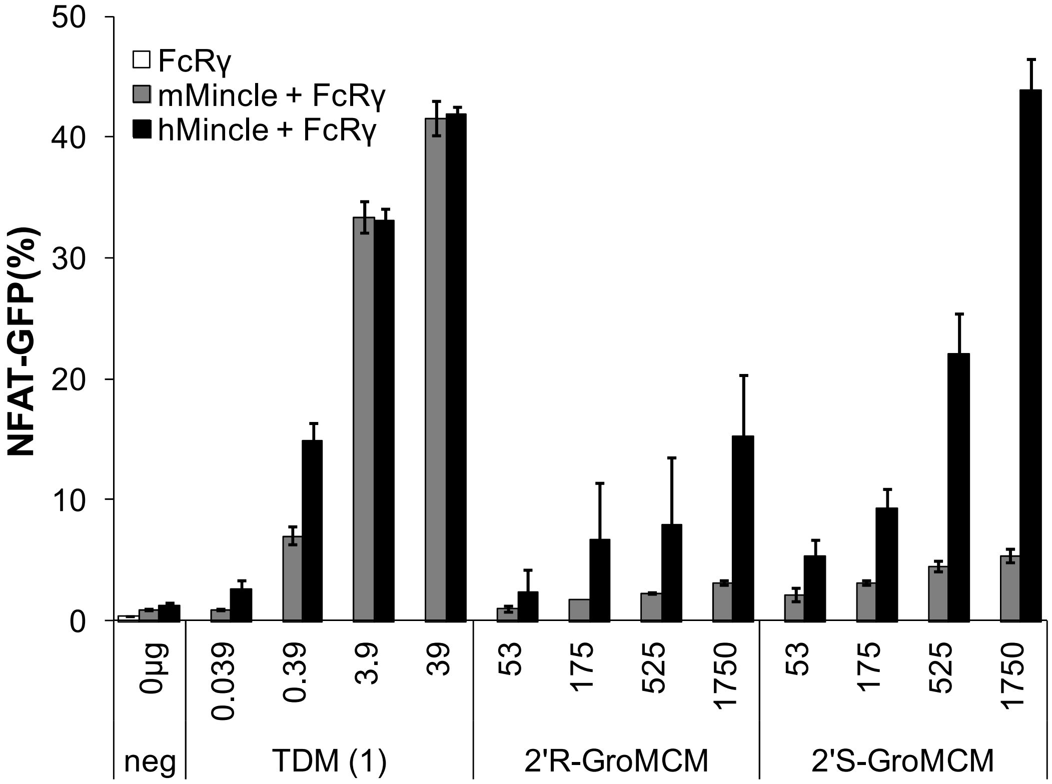


Figure S2.

Data from Figure 3B, replotted according to molecular weight. Amounts correspond to nnmol of (glyco)lipid per well. Molar quantity of TDM was calculated using an estimated molecular mass of 2582 g mol^{-1}.

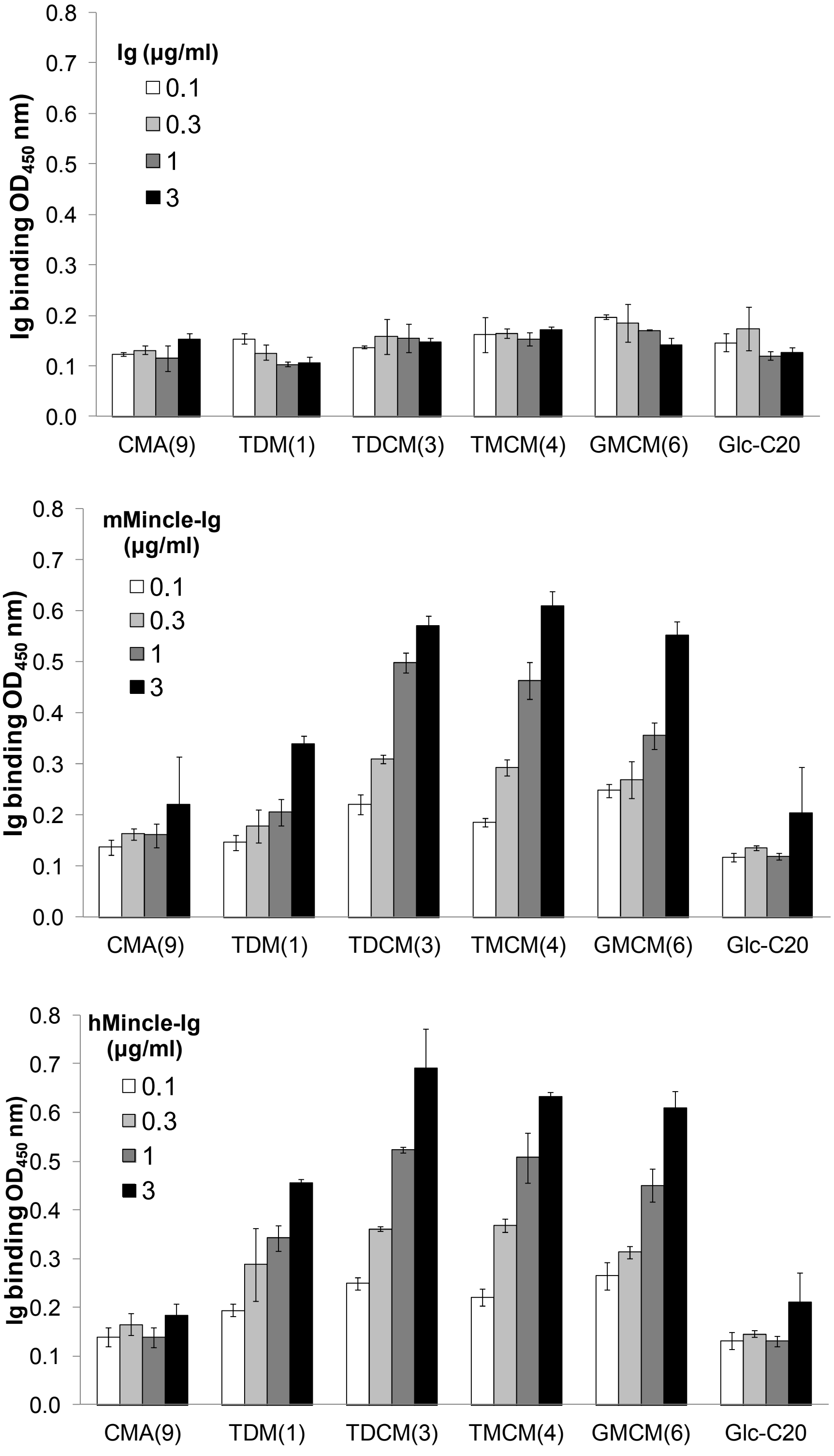


Figure S3.

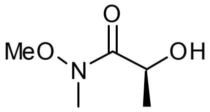
ELISA-based detection of mycolates by mouse (mMincle) and human (hMincle) Ig fusions. (Top) hIgG1-Fc (hIg), (middle) hMincle-Ig, and (bottom) mMincle-Ig were incubated with the indicated amount of plate-coated (glyco)lipid. Bound protein was detected with anti–hIgG-HRP followed by the addition of colorimetric substrate. For further details see Ref.[1]

Synthetic Chemistry

General methods

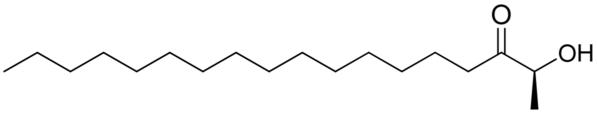
Proton nuclear magnetic resonance spectra (^{1}H NMR, 400 or 500 MHz) and proton decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR, 100 or 125 MHz) were obtained in deuterochloroform, methanol-d_4 (CD_3OD) OR DMSO-d_6 with residual protonated solvent or solvent carbon signals as internal standards. Abbreviations for multiplicity are s, singlet; d, doublet; t, triplet; q, quartet; p, pentet. Flash chromatography was carried out on silica gel 60 according to the procedure of Still *et al.*[2] Analytical thin layer chromatography (t.l.c.) was conducted on aluminium-backed 2 mm thick silica gel 60 F_{254} and chromatograms were visualized with ceric ammonium molybdate (Hanessian's stain), potassium permanganate or 5% H_2SO_4/MeOH, with charring as necessary. Melting points were obtained using a hot-stage or capillary apparatus and are corrected. High resolution mass spectra (HRMS) were obtained using an ESI-TOF-MS; all samples were run using 0.1% formic acid. Dry CH_2Cl_2, THF, and Et_2O were obtained from a dry solvent apparatus (Glass Contour of SG Water, Nashua, U.S.A.) as per the procedure of Pangborn *et al.*[3] Dry DMF was dried over 4 Å molecular sieves. Pet. spirits refers to petroleum ether, boiling range 40-60 °C. IR spectra were obtained using a FT-IR spectrometer with a zinc selenide/diamond attenuated total reflectance sample accessory as a thin film.

*(S)-2-Hydroxy-*N*-methoxy-*N*-methylpropionamide (10)*



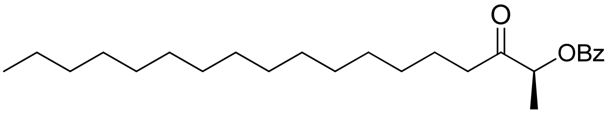
Isopropylmagnesium chloride in THF (21.2 mL, 2 M, 42.4 mmol) was added dropwise over 30 min to a stirred mixture of (*S*)-ethyl lactate (960 µL, 8.57 mmol) and *N*,*O*-dimethylhydroxylamine (2.06 g, 21.2 mmol) in dry THF (25 mL) at -20 °C under N_2. The mixture was stirred at 0 °C for 30 min then neutralized with sat. aq. NH_4Cl (75 mL). The aqueous phase was extracted with CH_2Cl_2 (5 × 20 mL) and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give compound **10** as an oil (1.03 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (3 H, d, *J* 6.3 Hz, $CHC\mathbf{H}_3$), 3.24 (3 H, s, NCH_3), 3.35 (1 H, d, *J* 7.8 Hz, OH), 3.71 (3 H, s, OCH_3), 4.48 (1 H, dt, *J* 6.4, 7.8 Hz, C**H**OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.72, 32.22, 61.15, 64.72, 175.54. Spectral data was consistent with that reported.[4]

(S)-2-Hydroxyoctadecan-3-one (11)



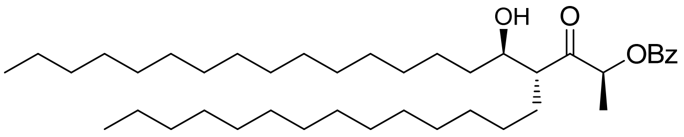
Pentadecylmagnesium bromide (13.5 mL of 1.0 M in THF, 13.5 mmol) was slowly added by cannula to a solution of Weinreb amide **10** (600 mg, 4.50 mmol) in THF (20 mL) at −20 °C. The mixture was stirred at −20 °C for 2 h then was warmed to 0 °C, and stirred for 1 h, and then was stirred at r.t. for 16 h. Sat. aq. NH_4Cl (15 mL) and CH_2Cl_2 (15 mL) were added and the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with sat. aq. NaCl, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (5→10% EtOAc/petrol) to afford compound **11** as a colourless amorphous solid (719 mg, 56%), $[\alpha]_D$ +37.4 (c. 1.05 in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.87 (3 H, t, *J* = 7.0 Hz, $CH_2C\mathbf{H_3}$), 1.20-1.32 (24 H, m, $(CH_2)_{12}$), 1.38 (3 H, d, *J* = 7.2 Hz, $CHC\mathbf{H_3}$), 1.60-1.63 (2 H, m, 2H, $C\mathbf{H_2}CH_2CO$), 2.38-2.53 (2 H, m, $C\mathbf{H_2}CO$), 3.55 (1 H, q, *J* = 7.0 Hz, $C\mathbf{H}OH$), 4.21-4.25 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3 (CH_3), 20.0, 22.8, 23.7, 29.3, 29.4, 29.50, 29.58, 29.72, 29.77, 29.8, 29.81, 29.82, 29.83, 32.0, 37.7, 72.7 (CHOH), 212.8 (C=O); IR (film) ν_{max} 3745.0, 2919.6, 2843.9, 2307.7, 1980.2, 1967.1, 1949.6, 1710.7, 1465.2, 1031.2 cm^{-1}; HRMS (ESI$^+$): *m/z* 285.2806 $[M+H]^+$ (calcd. $[C_{18}H_{36}O_2+H]^+$ 285.2788).

(S)-3-Oxooctadecan-2-yl benzoate (12)



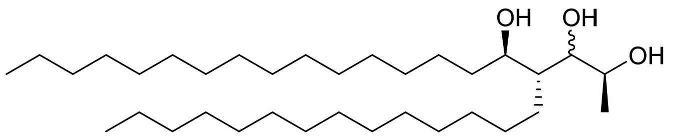
Diisopropylethylamine (0.53 mL, 3.09 mmol) was added to a stirred mixture of ketone **11** (440 mg, 1.54 mmol), benzoic anyhydride (525 mg, 2.32 mmol) and 4-dimethylaminopyridine (18.8 mg, 0.15 mmol) in CH_2Cl_2 (5 mL) under N_2 at r.t. The mixture was stirred for 16 h then H_2O (16.7 μL) was added and the mixture vigorously stirred for 45 min. The mixture was washed with H_2O (20 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (2→5% EtOAc/petrol) to afford compound **12** as a colourless solid (461 mg, 77%); $[\alpha]_D$ +18.3 (c. 1.10 in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.87 (3 H, t, *J* = 7.0 Hz, $CH_2C\mathbf{H_3}$), 1.23-1.31 (24 H, m, $(CH_2)_{12}$), 1.52 (3 H, d, *J* = 6.6 Hz, $CHC\mathbf{H_3}$), 1.59-1.62 (2 H, m, 2H, $C\mathbf{H_2}CH_2CO$), 2.45-2.61 (2 H, m, $C\mathbf{H_2}CO$), 5.32 (1 H, q, *J* = 6.6 Hz, $C\mathbf{H}OH$), 7.46 (2 H, t, *J* = 7.8 Hz, Ar), 7.59 (1 H, t, *J* = 7.2 Hz, Ar), 8.08 (2 H, d, *J* = 7.2 Hz, Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 16.5, 22.8, 23.3, 29.3, 29.4, 29.50, 29.54, 29.58, 29.73, 29.78, 29.79, 29.81, 29.82, 29.83, 32.0, 38. 4, 75.3, 128.6, 129.6, 129.9, 133.4, 166.0, 208.1; IR (film) ν_{max} 2919.4, 2850.3, 2307.7, 1714.2, 1465.2, 1449.7, 1406.0, 1313.9, 1273.9, 1119.1, 1066.3, 1026.8, 714.8 cm^{-1}; HRMS (ESI$^+$): *m/z* 389.3146 [M+H]$^+$ (calcd. $[C_{25}H_{40}O_3+H]^+$ 389.3050).

(2S,4R,5R)-5-Hydroxy-3-oxo-4-tetradecylicosan-2-yl benzoate (13)



A solution of the ketone **12** (686 mg, 1.76 mmol) in ether (15 mL) was added by cannula to a stirred mixture of dicyclohexylchloroborane[5] (675 mg, 3.17 mmol) and dimethylethylamine (194 mg, 2.64 mmol) in ether (10 mL) at −78 °C under N_2. After 15 min, the mixture warmed to 0 °C and stirred for one hour then cooled to -78 °C before the addition of hexadecanal[6] (1.29 g, 5.36 mmol) in ether (15 mL). The mixture was stirred at -78 °C for one hour then kept at –20 °C for 17h. The mixture warmed to 0 °C, methanol (30 mL) and aq. NaH_2PO_4/Na_2HPO_4 (1.0 M pH 7, 30 mL) were added, followed by 30% aq. H_2O_2 (30 mL). The mixture was stirred at 0 °C for 1 h then was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (2-5% EtOAc/petrol) affording the *anti* adduct (847 mg, 76%) as an amorphous solid. $[\alpha]_D$ +27.9 (c. 1.15 in $CHCl_3$); IR (film) ν_{max} 3422.7, 2917.6, 2849.9, 2354.2, 2342.7, 1731.7, 1720.8, 1467.2, 1262.7, 1121.0, 1072.0, 1031.2, 709.4 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.0 Hz, 2 × CH_3), 1.20-1.47 (54 H, m, 27 × CH_2), 1.54 (3 H, d, *J* = 7.0 Hz, CHC$\mathbf{H_3}$), 1.63-1.78 (2 H, m, C$\mathbf{H_2}$CH), 2.56 (1 H, d, *J* = 5.5 Hz, OH), 2.82 (1 H, q, *J* = 6.5, 13.0 Hz, CHCO), 3.73 (1 H, m, C**H**OH), 5.46 (q, *J* = 7.0, 14.0 Hz, 1H), 7.44-7.47 (m, 2H), 7.56-7.59 (m, 1H), 8.07-8.09 (m, 2H);^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 16.1, 22.8, 25.9, 27.6, 29.4, 29.5, 29.5, 29.6, 29.7, 29.81, 29.83, 29.84, 29.9, 32.1, 35.7, 52.8, 72.4, 75,4, 128.8, 129.6, 129.9, 133.5, 165.8, 212.7; HRMS (ESI$^+$): *m/z* 629.5535 [M+H]$^+$ (calcd. $[C_{41}H_{72}O_4+H]^+$ 629.5503).

(2S,4R,5R)-4-Tetradecylicosane-2,3,5-triol (14)



$NaBH_4$ (120 mg, 3.18 mmol) was added to a stirred mixture of the ketone **13** (1.00 g, 1.59 mmol), MeOH (3 mL) and Et_2O (6 mL) at r.t. under N_2. After 2 h the mixture was poured into sat. aq. $NaHCO_3$ and extracted with Et_2O (3 × 20 mL). The combined organic extracts were washed with

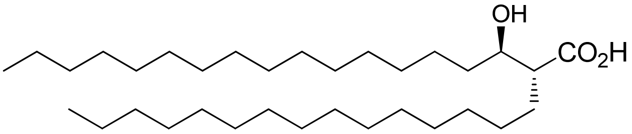
H_2O (20 mL) then sat. aq. NaCl (20 mL), dried ($MgSO_4$), filtered and conc. *in vacuo* to give a crude diol (949 mg, 95%).

A solution of NaOMe in MeOH (1 M, 2 mL) was added to a solution of the crude diol (949 mg, 1.50 mmol) in THF (6 mL) under N_2. The mixture was stored at –20 °C for 17 h, then was poured into sat. aq. NH_4Cl (25 mL) and extracted with Et_2O (3 × 15 mL). The combined organic extracts were washed with H_2O (20 mL) then brine (20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the triol **14** as a mixture of diastereoisomers (796 mg, quant). The mixture was separated by flash chromatography (10→20% acetone/CH_2Cl_2) for characterization purposes:

Diastereoisomer A (first to elute): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* 6.9 Hz, 2 × $CH_2C\mathbf{H_3}$), 1.14 (3 H, d, *J* 6.2 Hz, 3 H), 1.16-1.70 (55 H, 27 × CH_2,$C\mathbf{H}C_{14}H_{29}$), 2.40 (1 H, br s, OH), 2.47 (1 H, br s, OH), 3.34 (1 H, br s, OH), 3.70 (1 H, m, CHOH), 3.77-3.88 (2 H, m, 2 × CHOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.28 (2 × CH_3), 19.32 ($CH\mathbf{C}H_3$), 22.85, 25.27, 26.44, 28.00, 29.52, 29.76, 29.79, 29.81, 29.82, 29.84, 29.86, 30.07, 32.09, 35.93 (27 × CH_2), 43.04 ($\mathbf{C}HC_{14}H_{29}$), 68.78, 73.69, 76.41 (3 × CHOH).

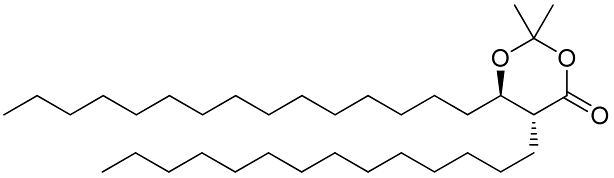
Diastereoisomer B (second to elute): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* 6.9 Hz, 2 × $CH_2C\mathbf{H_3}$), 1.20 (3 H, d, *J* 6.4 Hz, 3 H), 1.22-1.65 (55 H, 27 × CH_2,$C\mathbf{H}C_{14}H_{29}$), 2.98 (3 H, br s, 3 × OH), 3.72 (1 H, dd, *J* 4.3, 5.7, CHOH), 3.75 (1 H, td, *J* 3.10, 7.8 Hz, CHOH), 3.91 (1 H, qt, *J* 4.3, 6.3 Hz, CHOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.28 (2 × CH_3), 18.18 ($CH\mathbf{C}H_3$), 22.85, 25.63, 26.63, 28.97, 29.52, 29.67, 29.81, 29.82, 29.84, 29.86, 29.87, 30.33, 32.09, 35.71 (27 × CH_2), 44.47 ($\mathbf{C}HC_{14}H_{29}$), 69.17, 73.49, 77.20 (3 × CHOH). HRMS (ESI$^+$): *m/z* 549.5217 $[M+Na]^+$ (calcd. $[C_{34}H_{70}O_3+Na]^+$ 549.5217).

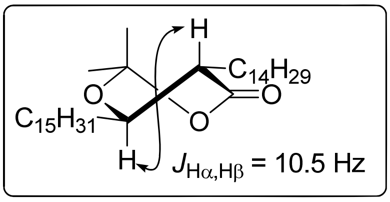
*(2**R**,3**R**)-3-Hydroxy-2-tetradecyloctadecanoic acid (9; (+)-2**R**,3**R**-corynomycolic acid)*



14.5% w/w $NaIO_4/SiO_2$^{6} (134 mg, 0.63 mmol) was added to a stirred mixture of the triols **15** (165 mg, 0.31 mmol) and CH_2Cl_2 (5 mL) under N_2. The mixture was stirred at r.t. for 1 h then filtered through a sintered frit and evaporated to dryness under reduced pressure. The crude aldehyde was dissolved in *tert*-BuOH (5 mL) then water (1 mL), *cis*-2-butene (247 µL, 2.82 mmol), NaH_2PO_4 (64.8 mg, 0.47 mmol) and $NaClO_2$ (85 mg, 0.94 mmol)) were added. The resulting mixture was stirred for 1.5 h, and then was concentrated under reduced pressure and the residue partitioned between CH_2Cl_2 (20 mL) and H_2O (25 mL). MeOH (2 mL) was added to break the emulsion formed, the organic layer was separated, and the aqueous layer extracted with CH_2Cl_2 (5 × 10 mL). The solvent was evaporated from the organic phase and the residue was purified by flash chromatography (83:15:2 pet. spirits/EtOAc/AcOH) to give **2** as a white solid (140 mg, 90%), m.p. 78-79 °C (lit.[7] m.p. 77-78 °C); $[\alpha]_D^{24}$ 8.5 ° (c 1.02, $CHCl_3$), lit.[7] $[\alpha]_D$ 7.8 ° (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, 3:1 $CDCl_3/CD_3OD$) δ 0.83 (6 H, t, *J* 6.8 Hz, 2 × C**H**$_3$), 1.15-1.65 (54 H, m, 27 × CH_2), 2.70 (1 H, dt, *J* 5.0, 9.7 Hz, C**H**CO_2H), 3.59-3.63 (1 H, dt, *J* 3.9, 7.5 Hz, CHOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.27 (2 × CH_3), 22.85, 25.86, 27.49, 29.52, 29.58, 29.67, 29.74, 29.76, 29.80, 29.82, 29.86, 32.08, 35.65 (27C, CH_2), 51.00 (**C**HCO_2H), 72.31 (**C**HOH), 179.20 (CO_2H); HRMS (ESI$^+$): *m/z* 495.4790 $[M-H]^-$ (calcd. for $[C_{32}H_{64}O_3-H]^-$ 495.4790).

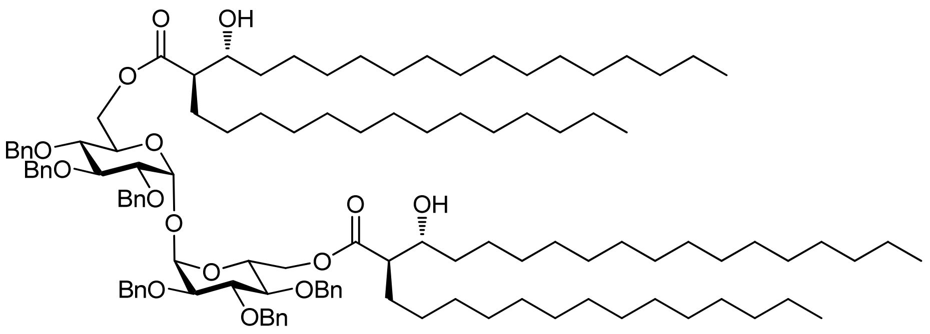
*(5*R*,6*R*)-2,2-Dimethyl-6-pentadecyl-5-tetradecyl-1,3-dioxan-4-one*





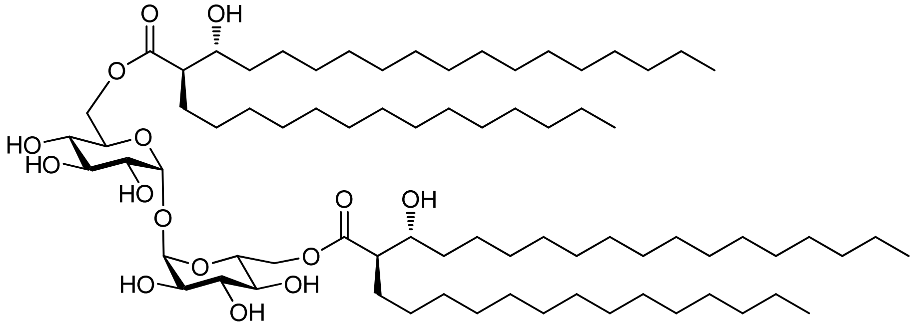
Pyridinium *p*-toluenesulfonate (1.0 mg, 0.0039 mmol) was added to a solution of corynomycolic acid (39.0 mg, 0.0785 mmol), 2-methoxypropene (30 μL, 0.314 mmol) and dry CH_2Cl_2 (2 mL) under N_2. The solution was stirred at r.t. for 30 min, $NaHCO_3$ (6.3 mg, 0.075 mmol) was added and stirring continued for 5 min. Celite (0.5 g) was added and the mixture evaporated under reduced pressure. The dried residue was loaded onto silica and purified by flash chromatography (0-10% Et_2O/pet. spirits with 1% Et_3N) to give the acetonide (39.0 mg, 93%). $[\alpha]_D^{24}$ 0.9 ° (c 1.05, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* 7.0 Hz, 2 × CH_3), 1.13-1.70 (53 H, m, CH_2), 1.55 (3 H, s, $C(CH_3)_2$), 1.56 (3 H, s, $C(CH_3)_2$), 1.75-1.86 (1 H, m, CHC**H**$_2$), 2.32 (1 H, ddd, *J* 4.4, 5.3, 10.5 Hz, H5), 3.90 (1 H, ddd, *J* 2.3, 8.8, 10.5 Hz, H6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.26 (2 × CH_3), 22.84, 25.30, 25.38, 26.77, 27.51, 29.48, 29.52, 29.54, 29.69, 29.74, 29.78, 29.81, 29.83, 29.85, 30.07, 32.03, 33.94 (27 × CH_2, 2 × CH_3), 45.60 (**C**C=O), 70.95 (CHOR), 105.40 (**C**$(CH_3)_2$), 171.19 (C=O); HRMS (ESI+): *m/z* 537.5250 $[M+H]^+$ (calcd. for $[C_{35}H_{68}O_3+H]^+$ 537.5241).

*6,6'-Di-*O*-((2''*R*,3''*R*)-3''-hydroxy-2''-tetradecyloctadecanoyl)-2,2',3,3',4,4'-hexa-*O*-benzyltrehalose (16)*



$CsHCO_3$ (48.5 mg, 0.250 mmol) was added to a mixture of (+)-2*R*,3*R*-corynomycolic acid **9** (54.6 mg, 0.110 mmol), the tosylate **15** (59.7 mg, 0.0500 mmol), dry DMF (1 mL) and dry THF (5 mL) under N_2. The mixture was stirred and heated at 70 °C for 18 h, poured into H_2O (30 mL) and extracted with Et_2O (1 × 20 mL, 2 × 10 mL). The combined organic extracts were washed with H_2O (20 mL), then brine (20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (20% EtOAc/pet. spirits) affording compound **16** (12.1 mg, 13%) as well as the monosubstituted product (15.3 mg, 20%). The monosubstituted material was again subjected to the above conditions to afford a further 8.6 mg (9%; total yield 21%) of compound **16**. $[\alpha]_D^{25}$ +57.5 ° (c 1.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (12 H, t, *J* 7.0 Hz, 4 × CH_3), 1.15-1.70 (108 H, m, 54 × CH_2), 2.44 (4 H, m, $CHCO_2$,OH), 3.54 (4 H, m, H2,2',4,4'), 3.63 (2 H, m, C**H**OH), 4.04 (2 H, t, *J* = 9.3 Hz, H3,3'), 4.10 (2 H, dd, *J* = 3.5, 12.3 Hz, H6a,6'a),4.21 (4 H, m, H5,5',H6b,H6'b), 4.55 (2 H, d, *J* = 10.7 Hz, CH_2Ar); 4.69 (4 H, 2 × d, *J* = 11.8 Hz, CH_2Ar); 4.87 (4 H, m, CH_2Ar); 4.99 (2 H, d, *J* = 10.8 Hz, CH_2Ar), 5.17 (2 H, d, *J* = 4.4 Hz, H1,1'); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.28 (4 × CH_3), 22.85, 25.88, 27.66, 29.52, 29.63, 29.76, 29.79, 29.83, 29.88, 30.47, 32.09, 35.59 (54 × CH_2), 51.47 (**C**HCO_2H), 62.43 (2 × CH_2), 69.23 (2 × CH), 72.29 (2 × CH), 75.82 (6 × CH_2), 77.93, 79.76, 81.68, 94.04 (8 × CH), 127.52, 127.78, 127.91, 128.05, 128.07, 128.56, 128.61 (30 × ArCH), 137.96, 138.10, 138.72 (6 × ArC), 175.35 (C=O); HRMS (ESI$^+$): *m/z* 1862.3319 $[M+Na]^+$ (calcd. $[C_{118}H_{182}O_{15}+Na]^+$ 1862.3371).

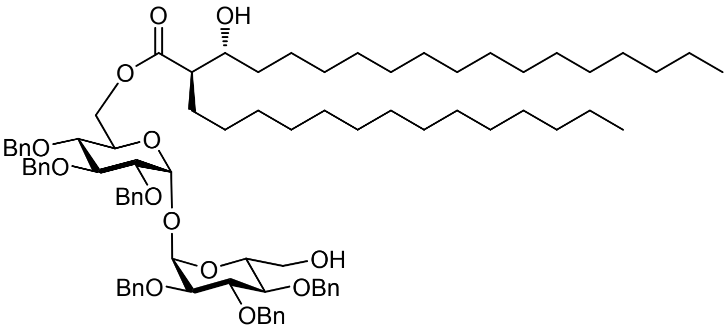
6,6'-Di-O-((2''R,3''R)-3''-hydroxy-2''-tetradecyloctadecanoyl)-trehalose (3; TDCM)



H_2 was bubbled through a stirred mixture of the protected mycolate **16** (5.0 mg, 2.72 μmol), 20% $Pd(OH)_2/C$ (6.0 mg), THF (2.5 mL), and MeOH (2.5 mL) for 30 min then the mixture was stirred under H_2 atmosphere for 17 h. The mixture was filtered through Celite, which was then rinsed with

MeOH (50 mL). The filtrate was evaporated to dryness and the residue purified by flash chromatography ($CHCl_3$→20:10:1 $CHCl_3$/MeOH/H_2O) to give TDCM **(3)** an amorphous solid (2.5 mg, 71%). $[\alpha]_D^{24}$ +74.9 ° (c 0.125, $CHCl_3$), lit.[8] $[\alpha]_D$ +55.1 ° (c 0.13, $CHCl_3$), lit.[9] $[\alpha]_D$ 69.1 ° (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, 1:1 $CD_3OD/CDCl_3$) δ 0.86 (12 H, t, *J* 6.8 Hz, 4 × CH_3), 1.10-1.70 (108 H, m, 54 × CH_2), 2.41 (2 H, ddd, *J* = 4.7, 7.6, 10.3 Hz, $CHCO_2$), 3.32 (2 H, obscured dd, H4), 3.47 (2 H, dd, *J* = 3.7, 9.8 Hz, H2), 3.66 (2 H, m, C**H**OH), 3.75(2 H, t, *J* = 9.3 Hz, H3), 4.04 (2 H, ddd, *J* = 1.9, 5.8, 9.6 Hz, H5), 4.17 (2 H, dd, *J* = 7.0, 11.9 Hz, H6a), 4.45 (2 H, dd, *J* = 1.9, 11.9 Hz, H6b), 5.05 (2 H, d, *J* = 3.7 Hz, H1); ^{13}C NMR (100 MHz, 1:1 $CD_3OD/CDCl_3$) δ 14.36 (4 × CH_3), 23.29, 26.06, 28.03, 29.68, 30.00, 30.031, 30.04, 30.15, 30.25, 30.29, 30.34, 32.58, 35.35 (54 × CH_2), 53.54 (**C**HCO_2), 70.8, 71.5, 72.5, 73.1, 73.8, 78.5 (2 × CHOH,C2,3,4,5,6), 94.8 (2 × C1), 176.0 (C=O); HRMS (ESI$^+$): *m/z* 1322.0535 $[M+Na]^+$ (calcd. $[C_{76}H_{146}O_{15}+ Na]^+$ 1322.0554).

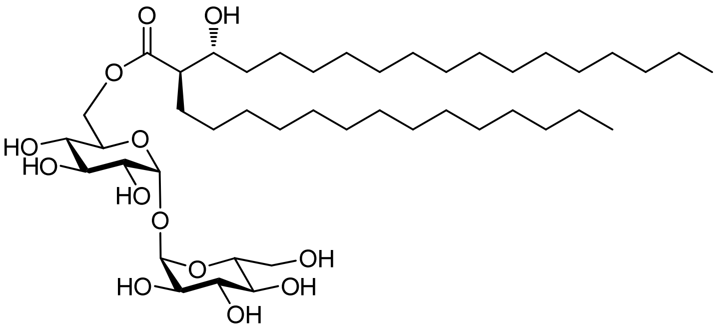
2,2',3,3',4,4'-Hexa-*O*-benzyl-6-O-(2''*R*,3''*R*)-3''-hydroxy-2''-tetradecyloctadecanoyl-trehalose (18)



$CsHCO_3$ (34 mg, 0.176 mmol) was dried under vacuum at 175 °C for 45 min then added to a mixture of (2*R*,3*R*)-3-hydroxy-2-tetradecyloctadecanoic acid (**9**) (17.5 mg, 0.035 mmol), the tosylate (**17**)[10] (55 mg, 0.053 mmol), dry DMF (0.5 mL) and dry THF (2.5 mL) under N_2. The mixture was heated at 70 °C for 42 h by which time the THF had evaporated. The mixture was diluted with H_2O (20 mL) and extracted with Et_2O (2 × 10 mL). The combined organic extracts were washed with H_2O (20 mL), then brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (8:2:0.02→6:4:0.04 pet. spirits/EtOAc/AcOH) to give compound **18** as a colourless gum (24.6 mg, 51%). $[\alpha]_D^{23}$ 75.5 ° (c 0.89, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* 6.9 Hz, 2 × CH_3), 1.15-1.70 (54 H, m, 27 × CH_2), 2.30-2.45 (2 H, m, OH,C**H**CO_2), 3.50-3.65 (7 H, m, H2,2',4,4',6'a,6'b,C**H**OH), 4.00-4.27 (6 H, m, H3,3',5,5',6a,6b), 4.53-4.76 (6 H, m, 6 × C**H**$_2$Ph), 4.84-4.91 (4 H, m, 4 × C**H**$_2$Ph), 5.00 (2 H, 2 × d, 2 × C**H**$_2$Ph), 5.15 (1 H, d, $J_{1,2}$ 3.5 Hz, H1), 5.17 (1 H, d, $J_{1',2'}$ 3.5 Hz, H1'), 7.23-7.38 (30 H, m, 30 × ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.27 (2 × CH_3), 22.84, 25.87, 27.66, 29.51, 29.63, 29.76, 29.78, 29.79, 29.81, 29.86, 32.07, 35.59 (27 × CH_2), 51.51 (**C**HCO_2H), 61.70, 62.50

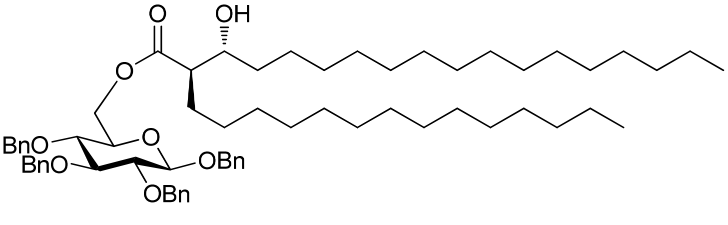
(C6,6'), 69.21 (**C**H(OH)$CHCO_2$), 71.43, 72.33 (2 × CH), 73.15, 73.28, 75.20, 75.36, 75.72, 75.83 (6 × CH_2), 77.53, 78.01, 79.68, 79.76, 81.65, 81.71 (6 × CH), 93.89, 94.12 (C1,1'), 127.56, 127.61, 127.70, 127.78, 127.81, 127.88, 128.00, 128.03, 128.06, 128.22, 128.52, 128.54, 128.56, 128.60 (30 × ArCH), 138.04, 138.07, 138.11, 138.34, 138.74, 138.87, 175.32 (7 × ArC); HRMS (ESI^+): *m/z* 1383.8626 $[M+Na]^+$ (calcd. $[C_{86}H_{120}O_{13}+Na]^+$ 1383.8621).

*6-O-(2''*R*,3''*R*)-3''-Hydroxy-2''-tetradecyloctadecanoyl-trehalose (4; TMCM)*



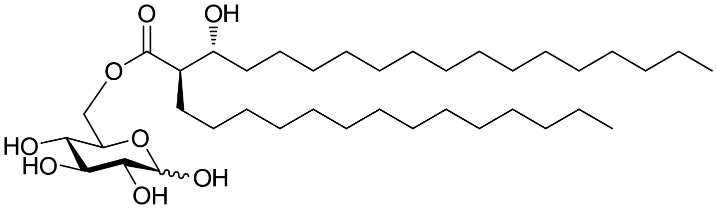
H_2 was bubbled through a stirring mixture of compound **18** (18.0 mg, 0.013 mmol), 20% $Pd(OH)_2/C$ (4.0 mg), AcOH (50 μL), THF (1.0 mL), and MeOH (1.5 mL) for 30 min then the mixture was stirred under an H_2 atmosphere for 17 h. The mixture was filtered through Celite which was rinsed with MeOH (50 mL). The filtrate was evaporated to dryness and the residue purified by flash chromatography (90:9:1 →20:9:1 $CHCl_3/MeOH/H_2O$) to give TMCM (**4**) as a colourless gum (9.0 mg, 83%). $[\alpha]_D^{23}$ 81.0 ° (c 0.23, $CHCl_3$), lit.[11] $[\alpha]_D$ +68 ° (c 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* 6.9 Hz, 2 × CH_3), 1.15-1.70 (54 H, m, 27 × CH_2), 2.44 (1 H, ddd, *J* 4.2, 7.2, 10.4 Hz, $CHCO_2$), 3.34 (2 H, 2 × d, *J* 9.1 Hz, H4,4'), 3.46 (2 H, apparent ddd, *J* 3.7, 9.8, H2,2'), 3.64-3.72 (2 H, m, H6'a,C**H**(OH)$CHCO_2$), 3.75-3.86 (4 H, m, H3,3',H5,H6'b), 4.07 (1 H, ddd, *J* 1.9, 5.2, 10.0 Hz, H5'), 4.18 (1 H, dd, *J* 5.3, 11.9 Hz, H6a), 4.46 (1 H, dd, *J* 2.1, 11.9 Hz, H6b), 5.09 (2 H, 2 × overlapping d, H1,1'); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.45 (2 × CH_3), 23.75, 26.57, 28.53, 29.77, 30.49, 30.58, 30.65, 30.72, 30.78, 30.79, 30.81, 33.09, 35.55 (27 × CH_2), 54.15 (**C**HCO_2H), 62.66, 64.38 (C6,6'), 71.43, 71.94, 72.03, 73.19, 73.22, 73.56, 73.86, 74.43, 74.52, 95.24, 95.33 (10 × CH), 176.18 (C=O); HRMS (ESI^+): *m/z* 838.6242 $[M+NH_4]^+$ (calcd. $[C_{44}H_{84}O_{13}+ NH_4]^+$ 838.6250).

*Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-((2'*R*,3'*R*)-3'-hydroxy-2'-tetradecyloctadecanoyl)-β-D-glucopyranoside (20)*



$CsHCO_3$ (58.5 mg, 0.302 mmol) was added to a mixture of the acid **9** (30.0 mg, 0.0604 mmol), the tosylate **19**[12] (50.1 mg, 0.0721 mmol), dry DMF (0.5 mL) and dry THF (2.5 mL) under N_2. The mixture was heated at 70 °C for 48 h by which time the THF had evaporated. The mixture was poured into H_2O (20 mL) and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with H_2O (20 mL), then brine (20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (10→20% EtOAc/pet. spirits) giving **20** as a colourless gum (49.1 mg, 80%). $[\alpha]_D^{24}$ 1.0 ° (c 0.685, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (6 H, t, *J* 6.9 Hz, 2 × CH_3), 1.10-1.80 (54 H, m, 27 × CH_2), 2.43-2.50 (2 H, m, OH,$CHCO_2$), 3.48-3.56, 3.64-3.73 (5 H, 2 × m, H2,3,4,5,C**H**OH), 4.20-4.24 (1 H, m, H6a), 4.51-4.57 (2 H, m, H1,6b), 4.61 (1 H, d, *J* 11.1 Hz, C**H**$_2$Ph), 4.63 (1 H, d, *J* 12.0 Hz, C**H**$_2$Ph), 4.71 (1 H, d, *J* 10.9 Hz, C**H**$_2$Ph), 4.79 (1 H, d, *J* 10.9 Hz, C**H**$_2$Ph), 4.87-4.97 (4 H, m, 2 × CH_2Ph), 7.23-7.38 (20 H, m, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.28 (2C, CH_3), 22.84, 26.03, 27.68, 29.52, 29.62, 29.74, 29.75, 29.79, 29.80, 29.81, 29.85, 29.86, 32.08, 35.81 (27C, CH_2), 51.45 (**C**HCO_2), 63.03, 71.27 (2C, OCH_2), 72.46, 73.01 (2C, OCH), 75.09, 75.28, 75.90 (3C, OCH_2), 77.96, 82.45, 84.69 (3C, OCH), 102.47 (C1), 127.84, 128.00, 128.02, 128.12, 128.17, 128.28, 128.50, 128.55, 128.56, 128.65 (20C, Ph-**C**H), 137.30, 137.85, 138.42, 138.53 (4C, Ph-**C**$_q$), 175.36 (C=O); HRMS (ESI$^+$): *m/z* 1036.7596 $[M+NH_4]^+$ (calcd. for $[C_{66}H_{98}O_8+NH_4]^+$ 1036.7600).

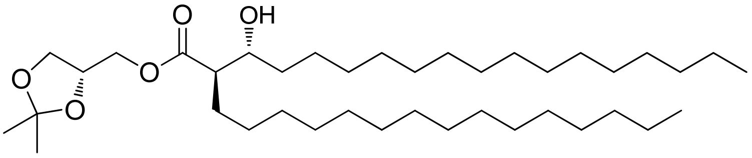
6-O-(2'R,3'R)-3'-Hydroxy-2'-tetradecyloctadecanoyl-D-glucose (6; GMCM)



$Pd(OH)_2/C$ (10%, 10 mg) was added to a mixture of **20** (12.3 mg, 0.012 mmol), MeOH (3 mL), THF (2 mL) and AcOH (0.1 mL). The mixture was placed on a Parr apparatus and shaken under H_2 (5 psi) for 2.5 h, then at 10 psi for a further 1 h. Additional $Pd(OH)_2/C$ (11 mg) was added and the mixture was shaken under H_2 (5 psi) for 16 h. The mixture was filtered through Celite and the filter plug rinsed sequentially with MeOH/THF (3:2, 6 mL), MeOH (6 mL) and THF (6 mL). The combined filtrates were concentrated *in vacuo* and the residue purified by flash chromatography (90:9:1 $CHCl_3/MeOH/H_2O$) to give GMCM (**6**) as a colourless gum (7.7 mg, 93%). $[\alpha]_D^{22}$ 34.2 ° (c 0.320, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD) δ 0.90 (6 H, t, *J* 6.9 Hz, 2 × CH_3), 1.10-1.70 (54 H, m, 27 × CH_2), 2.41-2.46 (2 H, m, OH,$CHCO_2$), 3.14 (0.45 H, t, *J* 8.4 Hz, H2β), 3.27-3.37 (2.55 H, 2 × m, H2α,4α,4β), 3.48 (0.45 H, ddd, *J* 2.4, 6.4, 9.2 Hz, H5β), 3.63-3.74 (1.55 H, m, C**H**OH,H3α,3β), 3.96 (0.55 H, ddd, *J* 2.2, 4.9, 9.6 Hz, H5α), 4.16, 4.19 (1 H, 2 × overlapping dd, *J*

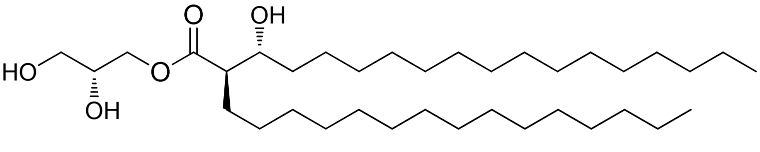
5.5, 11.8 Hz, 2 × H6a), 4.42-4.49 (1.45 H, 2 × H6b,H1β), 5.08 (0.55 H, *J* 3.7 Hz, H1α); ^{13}C NMR (100 MHz, CD_3OD) δ 14.46 (2C, CH_3), 23.76, 26.50, 26.58, 28.54, 28.55, 29.69, 29.74, 30.50, 30.62, 30.66, 30.72, 30.80, 33.09, 35.56, 35.60 (27C, CH_2), 54.16, 54.20 (**C**HCO_2αβ), 64.56, 64.84 (C6α,β), 70.69 (C5α), 71.85, 71.94 (CH), 73.51 (C3), 73.85 (CH), 74.73 (CHOH), 75.42 (C5β), 76.22 (C2β), 77.92 (CH), 94.01 (C1α), 98.25 (C1β), 176.16, 176.24 (C=Oαβ); HRMS (ESI$^+$): *m/z* 676.5717 $[M+NH_4]^+$ (calcd. for $[C_{38}H_{74}O_8+NH_4]^+$ 676.5722).

(2*R*,3*R*)-((*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 3-hydroxy-2-tetradecyloctadecanoate (22)



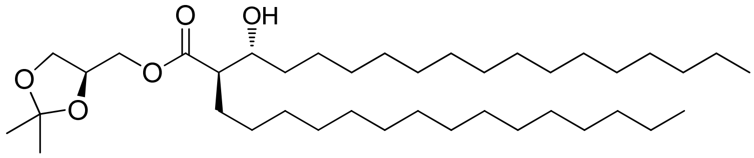
$CsHCO_3$ (63.4 mg, 0.327 mmol) was added to a stirred mixture of the acid **9** (32.5 mg, 0.065 mmol), the mesylate **21**[13] (16.5 mg, 0.079 mmol), dry DMF (0.3 mL), and dry THF (1.5 mL) under N_2. The mixture was stirred and heated at 70 °C for 17 h then an additional portion of the mesylate **21** (9.5 mg, 0.045 mmol) was added and stirred at 70 °C continued for 6 h before another portion of mesylate **21** (26 mg, 0.123 mmol) was added. After a further 16 h the mixture was poured into H_2O (20 mL), and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with H_2O (20 mL) then sat. aq. NaCl (20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (12→15% EtOAc/pet. spirits) to give **22** as a colourless gum (25.9 mg, 65%). $[\alpha]_D^{23}$ 4.2 ° (c 1.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* 6.9 Hz, 2 × C**H**$_3$CH$_2$), 1.20-1.71 (60 H, m, 27 × C**H**$_2$,C(C**H**$_3$)$_2$), 2.45 (2 H, m, C**H**CHO**H**), 3.63-3.67 (1 H, m, C**H**OH), 3.76 (1 H, dd, *J* 5.9, 8.5 Hz, C**H**$_2$OC), 4.07 (1 H, dd, *J* 6.4, 8.5 Hz, C**H**$_2$OC), 4.16 (1 H, dd, *J* 4.3, 9.9 Hz, C**H**$_2$OC(O)), 4.24 (1 H, dd, *J* 3.3, 9.9 Hz, C**H**$_2$OC(O)), 4.33 (1 H, m, C**H**OC); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.27 (2C, CH_3), 22.84 (CH_2), 25.46 (1C, CH_3), 25.85 (CH_2), 26.87 (1C, CH_3), 27.57, 29.51, 29.59, 29.68, 29.70, 29.72, 29.73, 29.74, 29.76, 29.79, 29.81, 29.85, 32.08, 35.71 (25C, CH_2), 51.46 (CH), 64.60, 66.44 (2C, CH_2), 72.48, 73.54 (2C, CH), 109.97 (**C**(CH_3)$_2$), 175.45 (C=O); HRMS (ESI$^+$): *m/z* 611.5609 $[M+H]^+$ (calcd. for $[C_{38}H_{74}O_5+H]^+$ 611.5609).

(2*R*,3*R*)-((*R*)-2,3-Dihydroxypropyl) 3-hydroxy-2-tetradecyloctadecanoate (2'*R*-8; 2'*R*-GroMCM)



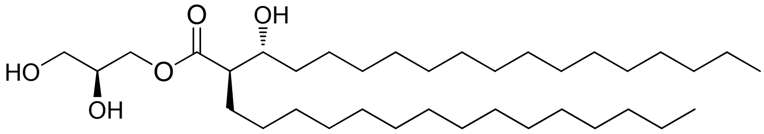
The acetal **22** (25.9 mg, 0.0424 mmol) was dissolved in a mixture of THF/TFA/H_2O (17:8:3, 2.8 mL). The mixture was placed on a rotary evaporator at 40 °C and the pressure reduced from 200 mbar to 35 mbar over 10 min. After a further 20 min at 35 mbar the residue was dissolved in CH_2Cl_2 and evaporated onto silica. Flash chromatography (35→45% EtOAc/pet. spirits) gave **2'*R*-8** as an amorphous solid (23.8 mg, 98%), m.p. 54-55 °C. $[\alpha]_D^{23}$ 8.8 ° (c 0.87, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* 6.9 Hz, 2 × C**H**$_3$CH$_2$), 1.15-1.75 (54 H, m, 27 × C**H**$_2$), 2.37-2.49 (2 H, m, C**H**CHOH,OH), 2.62 (1 H, br s, OH), 3.15 (1 H, br s, OH), 3.59-3.69 (3 H, m, 2 × C**H**$_2$OH,C**H**(OH)C$_{15}$H$_{31}$), 3.94 (1 H, m, C**H**(OH)CH$_2$OH), 4.23-4.26 (2 H, m, C**H**$_2$OC(O)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.28 (2C, CH_3), 22.85, 25.62, 27.62, 29.52, 29.58, 29.67, 29.69, 29.74, 29.75, 29.77, 29.79, 29.82, 29.85, 29.86, 29.93, 29.99, 32.08, 35.51 (27C, CH_2), 52.34 (CO$_2$**C**H), 63.45, 65.37 (2C, OCH_2), 70.12, 73.10 (2C, OCH), 175.60 (C=O); HRMS (ESI$^+$): *m/z* 593.5113 $[M+Na]^+$ (calcd. for $[C_{35}H_{70}O_5+Na]^+$ 593.5115).

*(2*R*,3*R*)-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 3-hydroxy-2-tetradecyloctadecanoate (24)*



$CsHCO_3$ (15.4 mg, 0.0794 mmol) was added to a stirred mixture of the acid **9** (7.9 mg, 0.016 mmol), the mesylate **23**[14] (12.1 mg, 0.0576 mmol), dry DMF (0.5 mL), and dry THF (2.5 mL) under N_2. The mixture was heated at 70 °C for 48 h and then poured into H_2O (20 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with H_2O (20 mL) then sat. aq. NaCl (20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (12→15% EtOAc/pet. spirits) to give **24** as a colourless gum (6.9 mg, 71%). $[\alpha]_D^{22}$ 8.0 ° (c 0.39, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* 6.9 Hz, 2 × C**H**$_3$CH$_2$), 1.20-1.75 (60 H, m, 27 × C**H**$_2$,C(C**H**$_3$)$_2$), 2.47 (1 H, dt, *J* 5.6, 9.2 Hz, C**H**CHOH), 2.49 (1 H, d, *J* 8.4 Hz, OH), 3.62-3.70 (1 H, m, C**H**OH), 3.76 (1 H, dd, *J* 5.6, 8.4 Hz, C**H**$_2$OC), 4.08 (1 H, dd, *J* 6.4, 8.4 Hz, C**H**$_2$OC), 4.12 (1 H, dd, *J* 4.8, 11.6 Hz, C**H**$_2$OC(O)), 4.24 (1 H, dd, *J* 6.0, 11.6 Hz, C**H**$_2$OC(O)), 4.34 (1 H, m, C**H**OC); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.28 (2C, CH_3), 22.85 (CH_2), 25.41 (CH_3), 25.88 (CH_2), 26.86 (CH_3), 27.55, 29.52, 29.59, 29.68, 29.72, 29.73, 29.75, 29.79, 29.82, 29.86, 32.08, 35.75 (25C, CH_2), 51.65 (**C**HCO$_2$), 64.75, 66.40 (2C, OCH_2), 72.41, 73.65 (2C, OCH), 110.02 (**C**(CH$_3$)$_2$), 175.30 (C=O); HRMS (ESI$^+$): *m/z* 633.5429 $[M+Na]^+$ (calcd. for $[C_{38}H_{74}O_5+Na]^+$ 633.5428).

(2R,3R)-((S)-2,3-Dihydroxypropyl) 3-hydroxy-2-tetradecyloctadecanoate (2'S-8; 2'S-GroMCM)



The acetonide **21** (21.6 mg, 0.0354 mmol) was dissolved in THF/TFA/H_2O (8:3:1, 1 mL) and the resulting mixture stirred at r.t. for 1 h. The mixture was concentrated *in vacuo* at 25 °C and the residue purified by flash chromatography (30→40% EtOAc/pet. spirits) to give **2'*S*-8** as a colourless amorphous solid (19 mg, 94%), m.p. 53-54 °C. $[\alpha]_D^{20}$ 13.7 ° (c 0.38, $CHCl_3$); ^{1}H NMR (400 MHz, 3:1 $CDCl_3$/MeOH) δ 0.84 (3 H, t, *J* 6.9 Hz, 2 × C**H**$_3$CH$_2$), 1.15-1.70 (54 H, m, 27 × CH$_2$), 2.40 (1 H, ddd, *J* 4.5, 7.2, 10.2 Hz, C**H**CHOH), 3.50-3.64 (3 H, m, C**H**$_2$OH,C**H**(OH)$C_{15}H_{31}$), 3.80-3.86 (1 H, m, C**H(**OH)CH$_2$OH), 4.08 (1 H, dd, *J* 6.4, 11.4 Hz, C**H**$_2$OC(O)), 4.27 (1 H, dd, *J* 4.4, 11.4 Hz, C**H**$_2$OC(O)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.27 (2C, CH_3), 22.85, 25.64, 27.62, 29.52, 29.58, 29.67, 29.69, 29.74, 29.77, 29.79, 29.82, 29.85, 29.86, 32.08, 35.55 (27C, CH_2), 52.21 (**C**HCO$_2$), 63.41, 65.38 (2C, CH_2), 70.12, 73.08 (2C, CH), 175.66 (C=O); HRMS (ESI$^+$): *m/z* 593.5115 $[M+Na]^+$ (calcd. for $[C_{35}H_{70}O_5+Na]^+$ 593.5115).

Mincle reporter assay

2B4-NFAT-GFP reporter cells expressing mouse Mincle/FcRγ or human Mincle/FcRγ were prepared as previously described.[15] In order to stimulate the cells, TDM, TDCM, TMCM, GMCM, 2'*R*- and 2'*S*-GroMCM, and CMA were dissolved in chloroform:methanol (2:1) at 1 mg/ml were diluted in isopropanol and added to 96-well plates at 20 μl/well, followed by evaporation of the solvent as previously described.[1] Activation of NFAT-GFP was monitored by flow cytometry.

References

(1) Ishikawa, E.; Ishikawa, T.; Morita, Y. S.; Toyonaga, K.; Yamada, H.; Takeuchi, O.; Kinoshita, T.; Akira, S.; Yoshikai, Y.; Yamasaki, S. *J. Exp. Med.* **2009**, *206*, 2879-2888.

(2) Still, W. C.; Kahn, M.; Mitra, A. M. *J. Org. Chem.* **1978**, *43*, 2923-2925.

(3) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.

(4) Paterson, I. *Synthesis* **1998**, *1998*, 639-652.

(5) Paterson, I.; Temal-Laïb, T. *Org. Lett.* **2002**, *4*, 2473-2476.

(6) Zhong, Y.-L.; Shing, T. K. M. *J. Org. Chem.* **1997**, *62*, 2622-2624.

(7) Ratovelomanana-Vidal, V.; Girard, C.; Touati, R.; Tranchier, J. P.; Hassine, B. B.; Genêt, J. P. *Adv. Synth. Catal.* **2003**, *345*, 261-274.

(8) Nishizawa, M.; Minagawa, R.; Garcia, D. M.; Hatakeyama, S.; Yamada, H. *Tetrahedron Letters* **1994**, *35*, 5891-5894.

(9) Nishizawa, M.; Yamamoto, H.; Imagawa, H.; Barbier-Chassefiere, V.; Petit, E.; Azuma, I.; Papy-Garcia, D. *J Org Chem* **2007**, *72*, 1627-1633.

(10) Yamaryo-Botte, Y.; Rainczuk, A. K.; Lea-Smith, D. J.; Brammananth, R.; van der Peet, P. L.; Meikle, P.; Ralton, J. E.; Rupasinghe, T. W. T.; Williams, S. J.; Coppel, R. L.; Crellin, P. K.; McConville, M. J. *ACS Chemical Biology* **2015**, doi: 10.1021/cb5007689.

(11) Datta, A. K.; Takayama, K.; Nashed, M. A.; Anderson, L. *Carbohydr. Res.* **1991**, *218*, 95-109.

(12) Prandi, J. *Carbohydr. Res.* **2011**, *347*, 151-154.

(13) Lemaire, M.; Posada, F.; Gourcy, J. G.; Jeminet, G. *Synthesis* **1995**, 627-629.

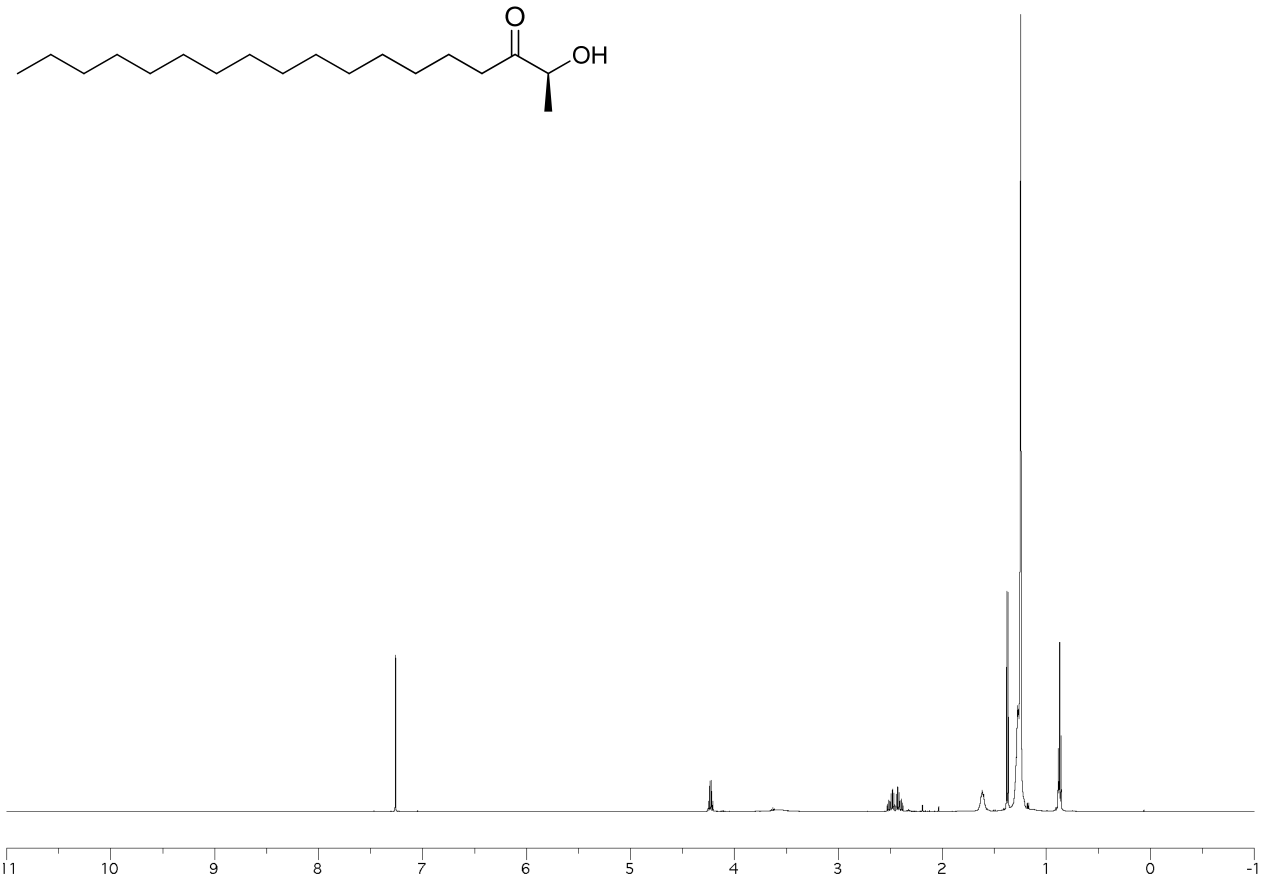
(14) Clinch, K.; Evans, G. B.; Fröhlich, R. F. G.; Furneaux, R. H.; Kelly, P. M.; Legentil, L.; Murkin, A. S.; Li, L.; Schramm, V. L.; Tyler, P. C.; Woolhouse, A. D. *J. Med. Chem.* **2009**, *52*, 1126-1143.

(15) Yamasaki, S.; Ishikawa, E.; Sakuma, M.; Hara, H.; Ogata, K.; Saito, T. *Nat. Immunol.* **2008**, *9*, 1179-1188; Hattori, Y.; Morita, D.; Fujiwara, N.; Mori, D.; Nakamura, T.; Harashima, H.; Yamasaki, S.; Sugita, M. *J. Biol. Chem.* **2014**, *289*, 15405-15412.

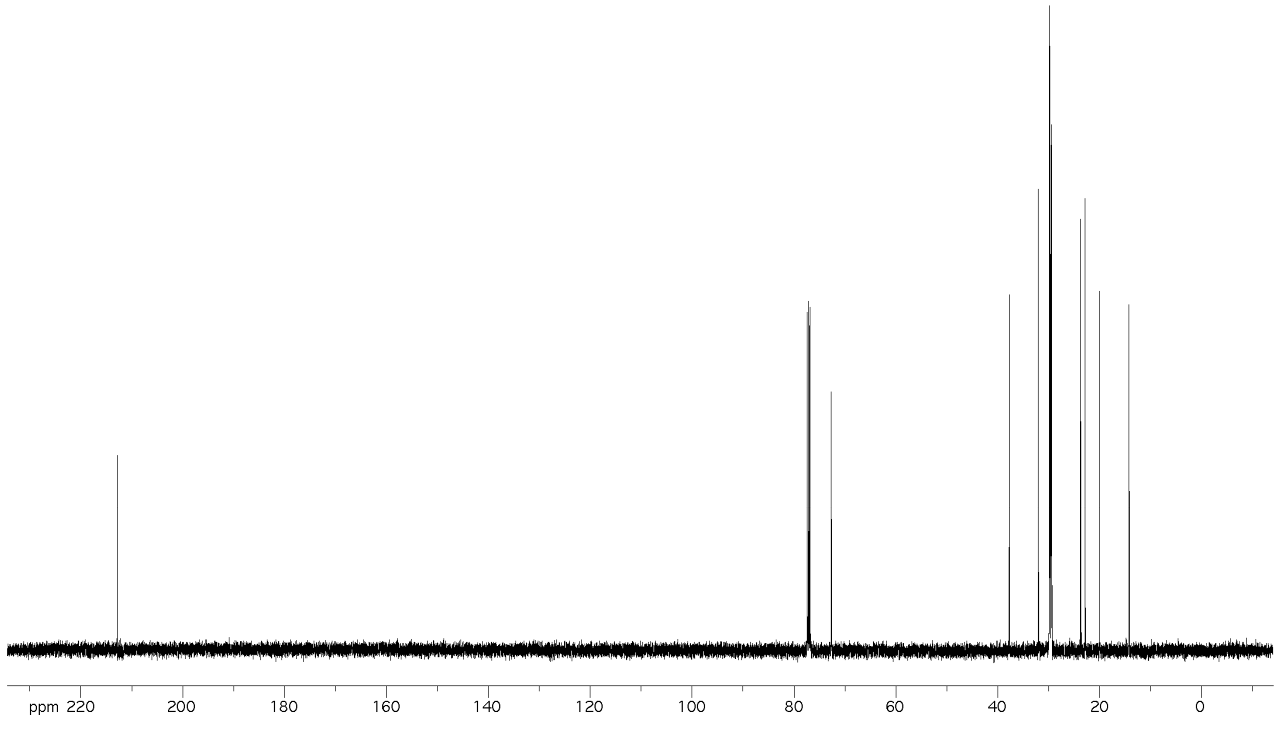
NMR spectra

(S)-2-Hydroxyoctadecan-3-one (11)

^{1}H NMR spectrum

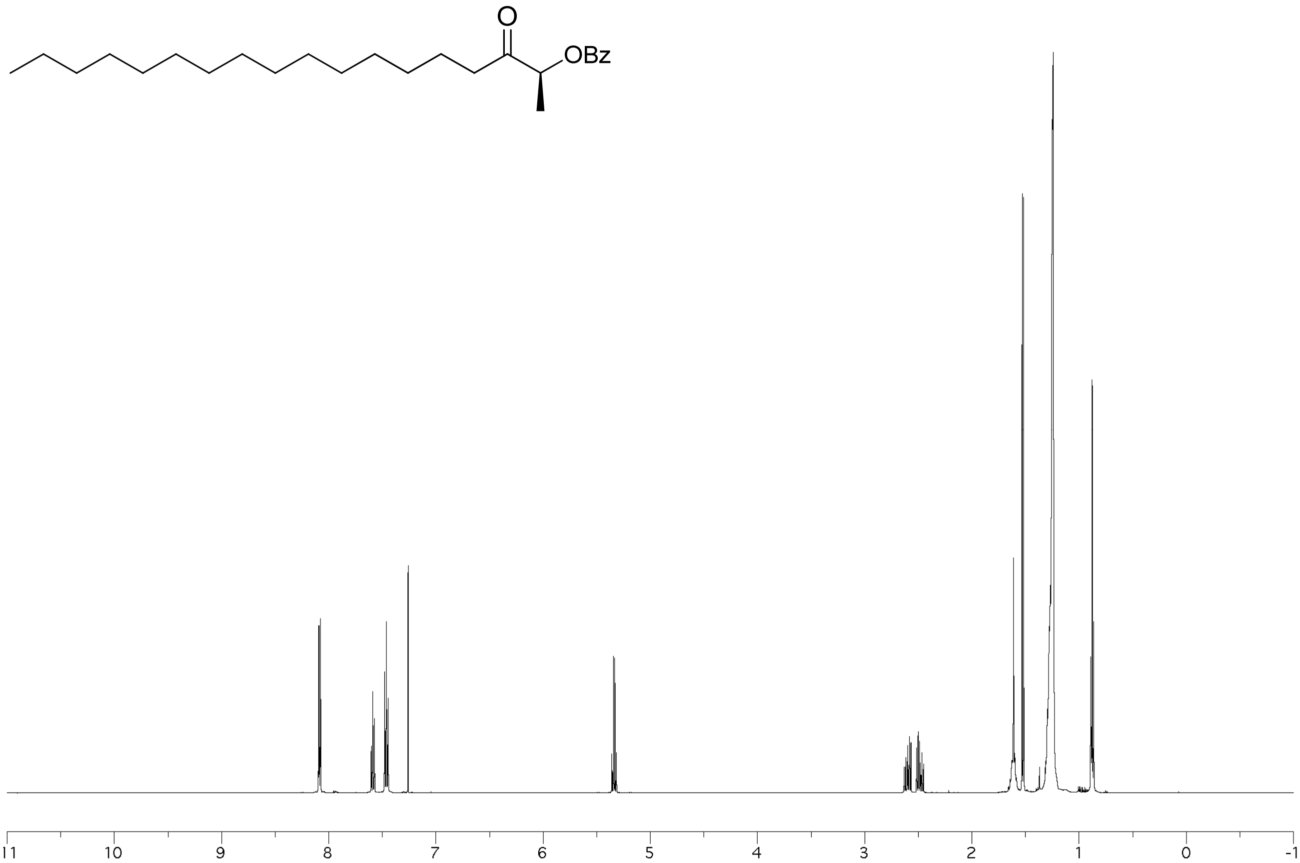


^{13}C NMR spectrum



(S)-3-Oxooctadecan-2-yl benzoate (12)

^{1}H NMR spectrum



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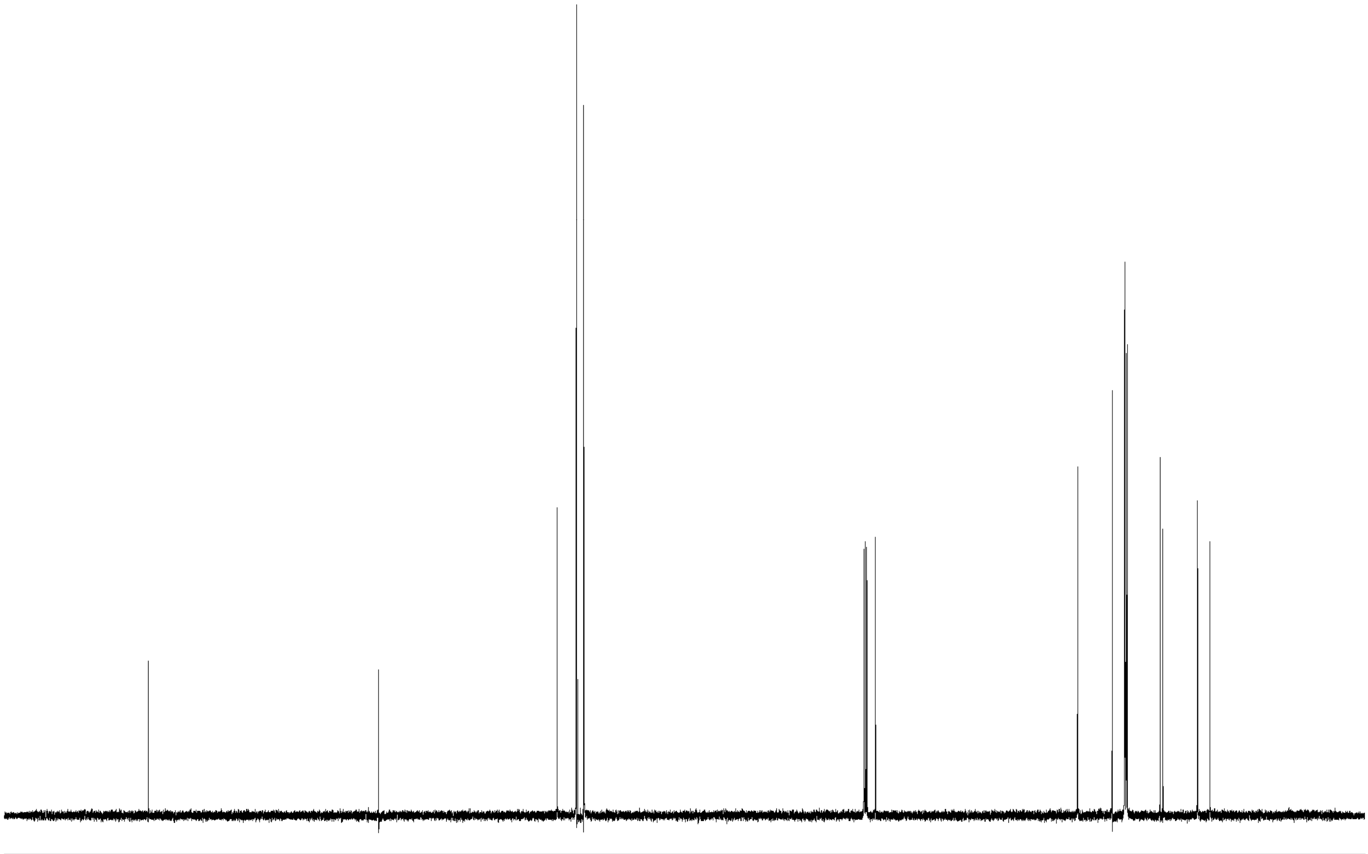
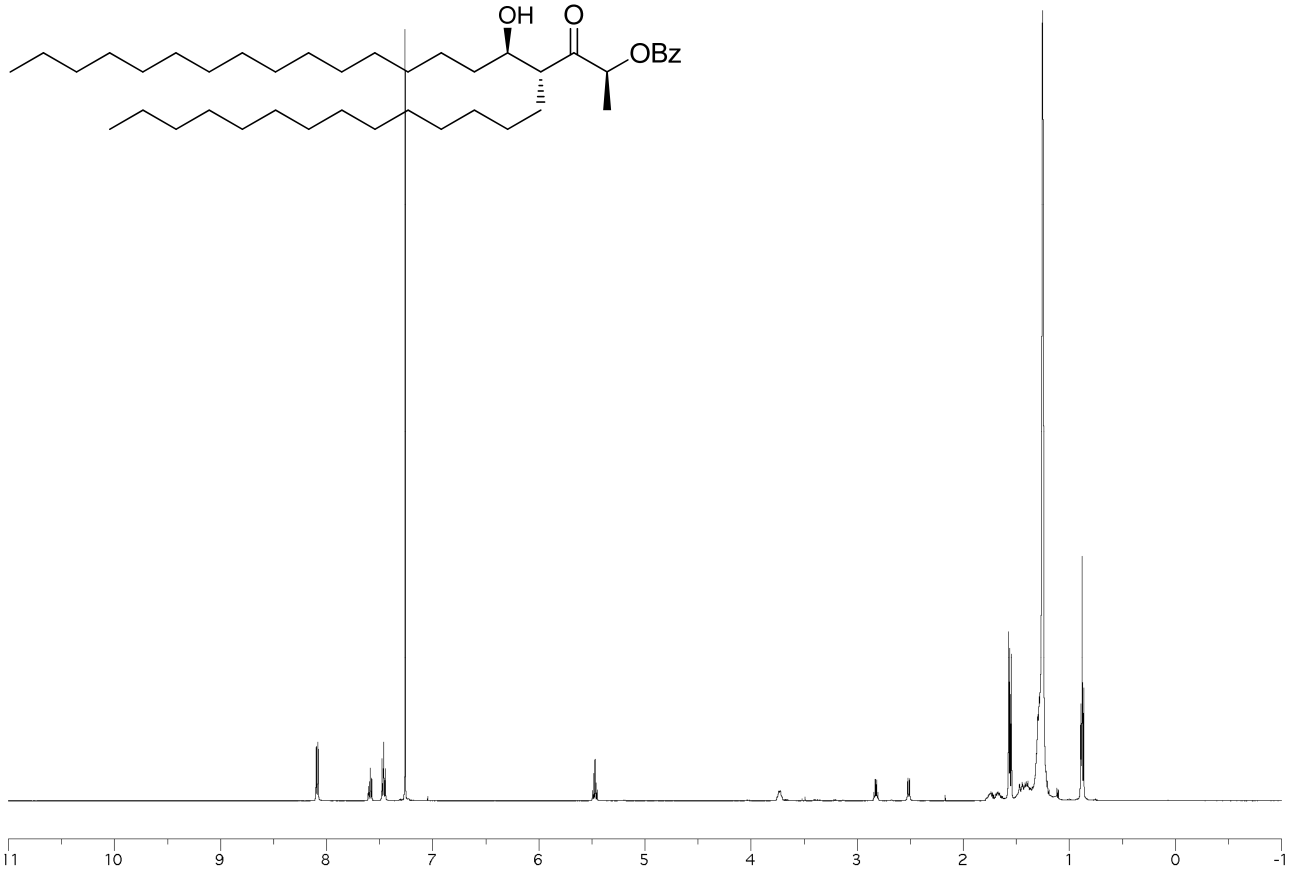
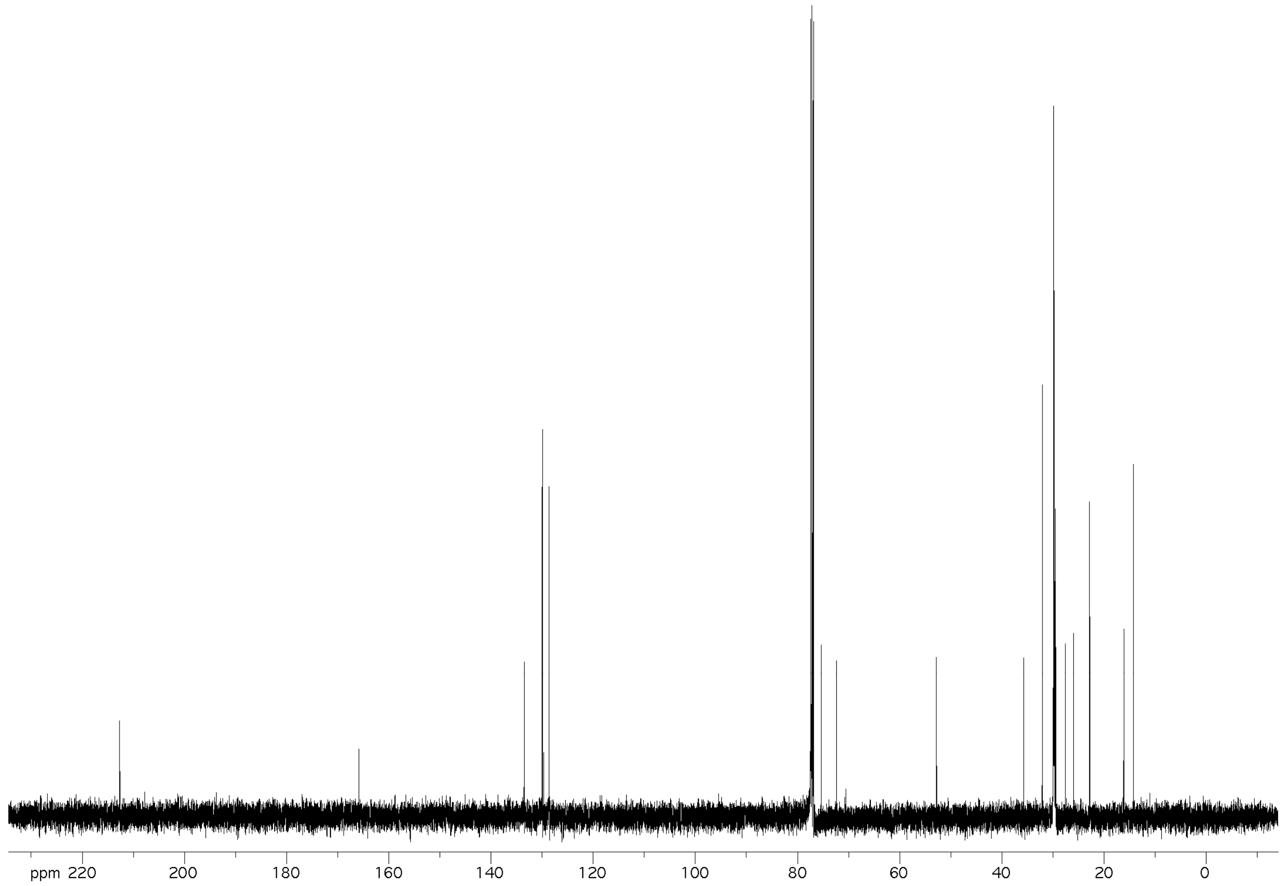


(2S,4R,5R)-5-Hydroxy-3-oxo-4-tetradecylicosan-2-yl benzoate (13)

^{1}H NMR spectrum

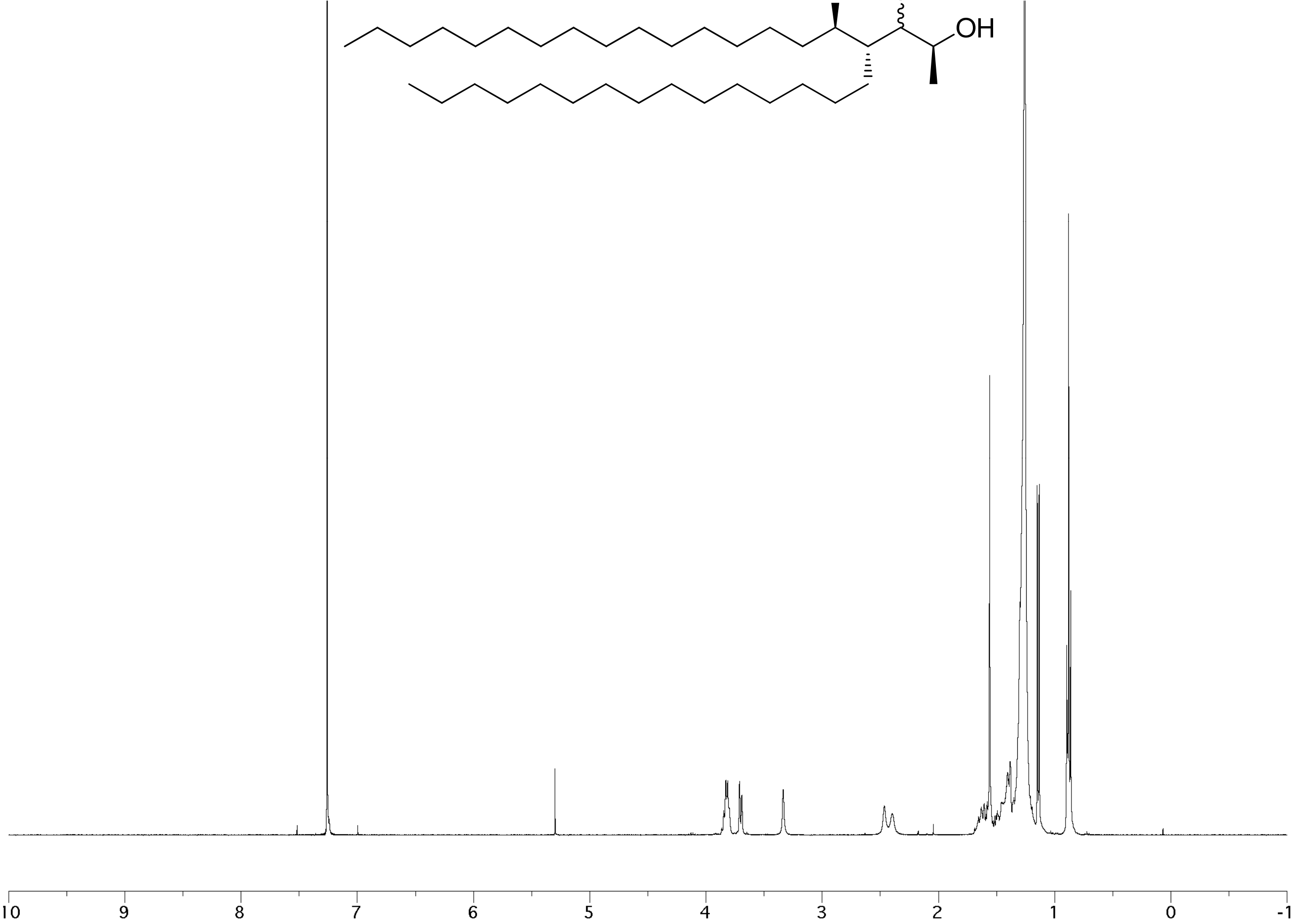


^{13}C NMR spectrum

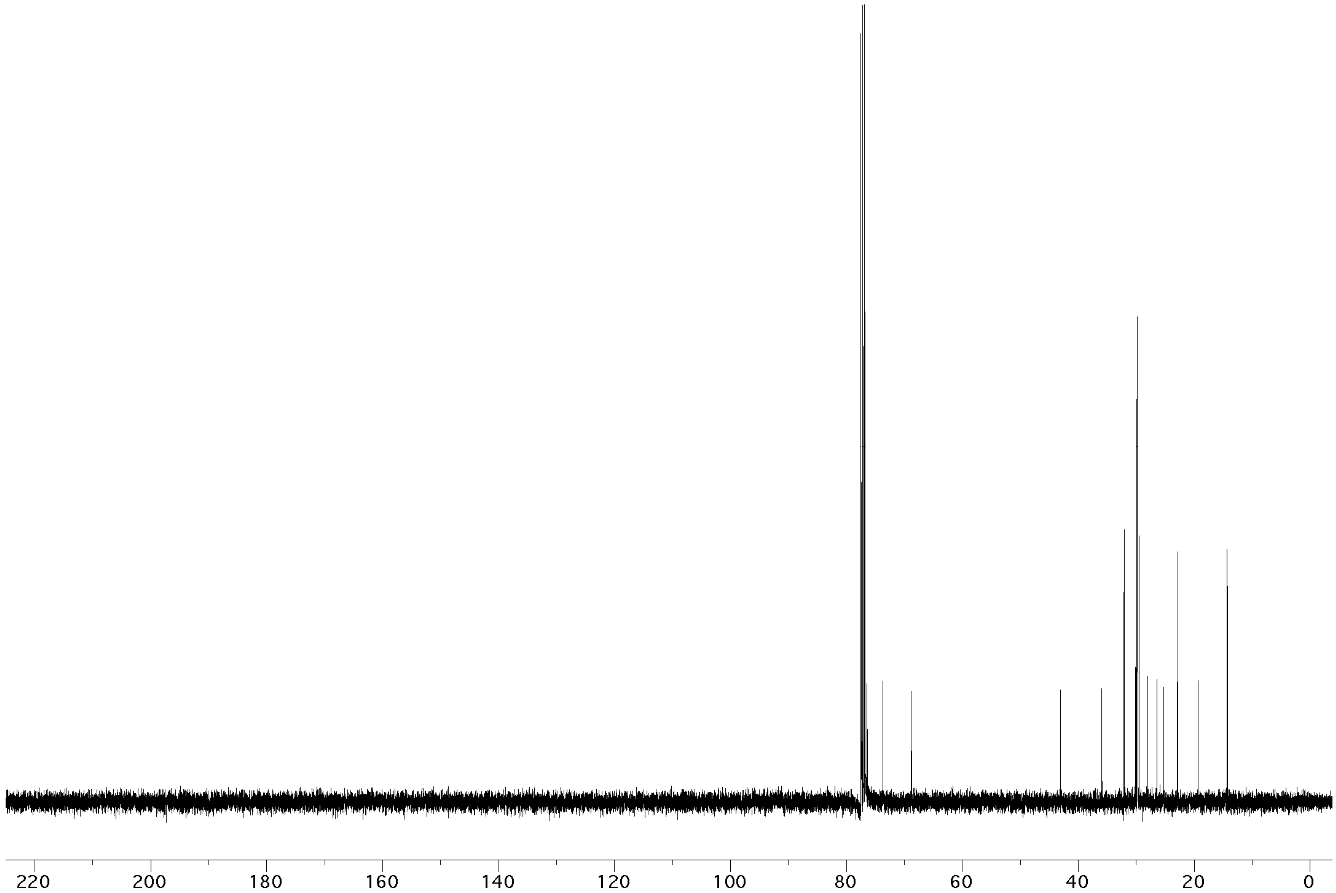


(2S,4R,5R)-4-Tetradecylicosane-2,3,5-triol (14) – First eluted diastereoisomer

^{1}H NMR spectrum

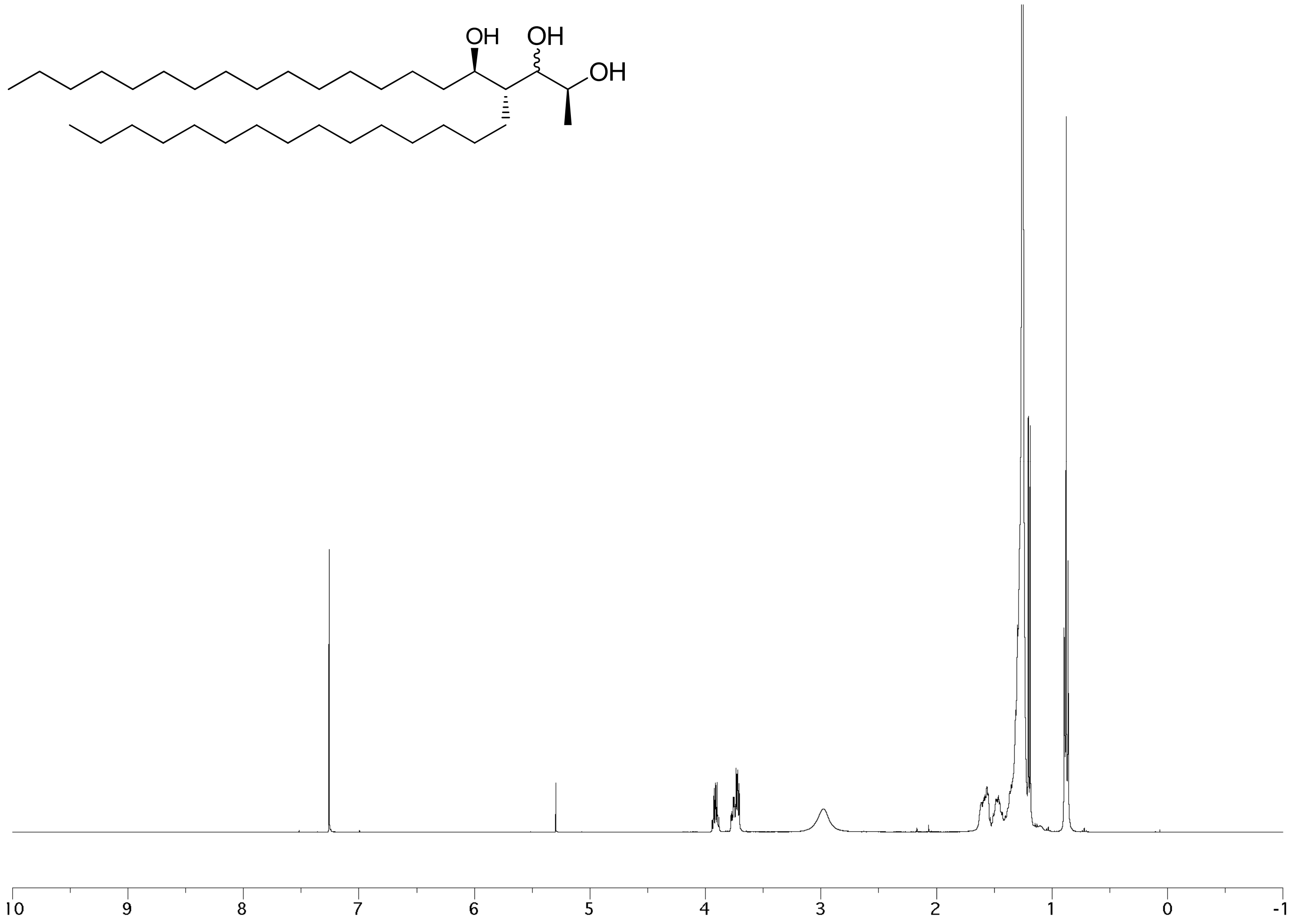


^{13}C NMR spectrum

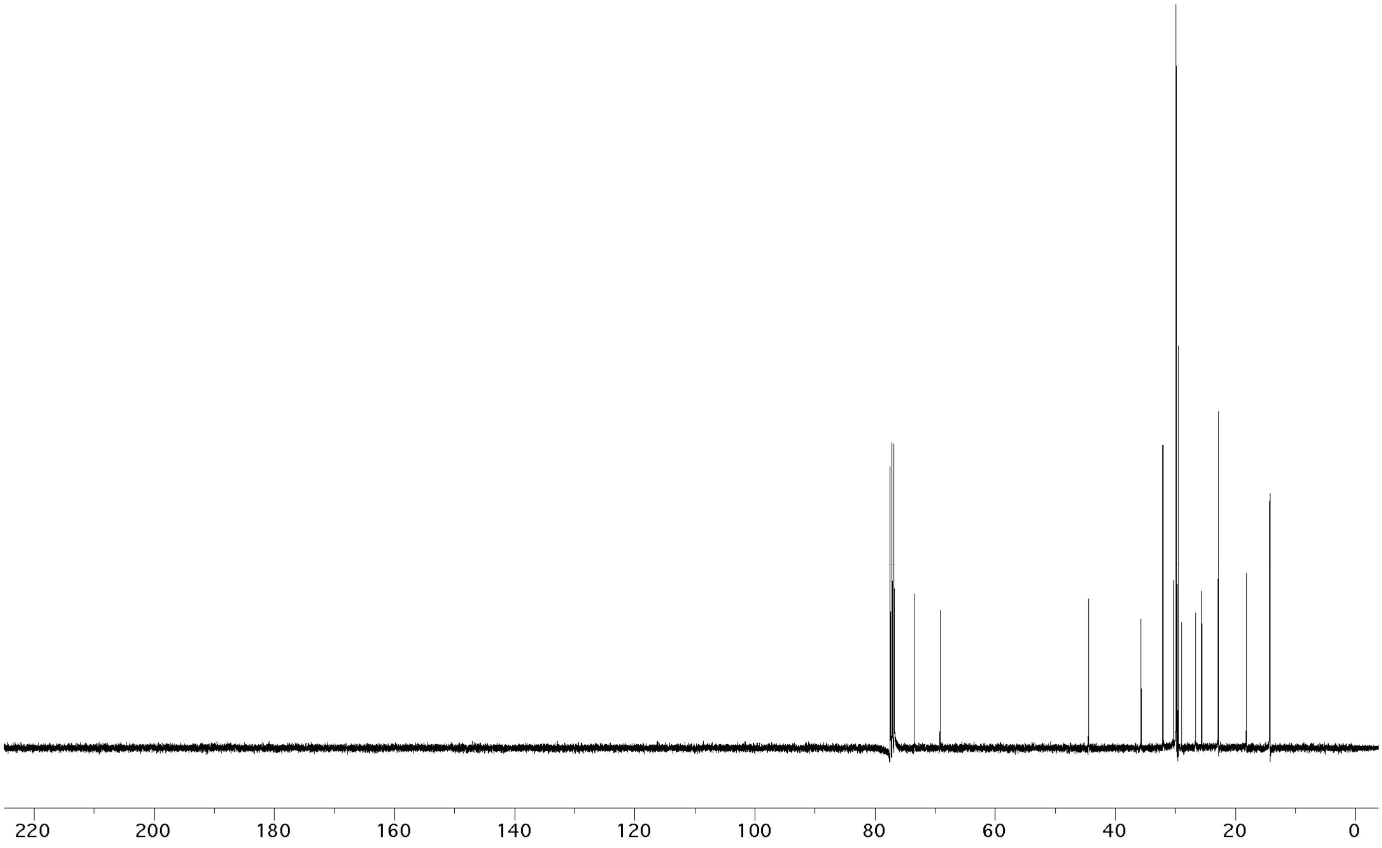


(2S,4R,5R)-4-Tetradecylicosane-2,3,5-triol (14) – Second eluted diastereoisomer

^{1}H NMR spectrum

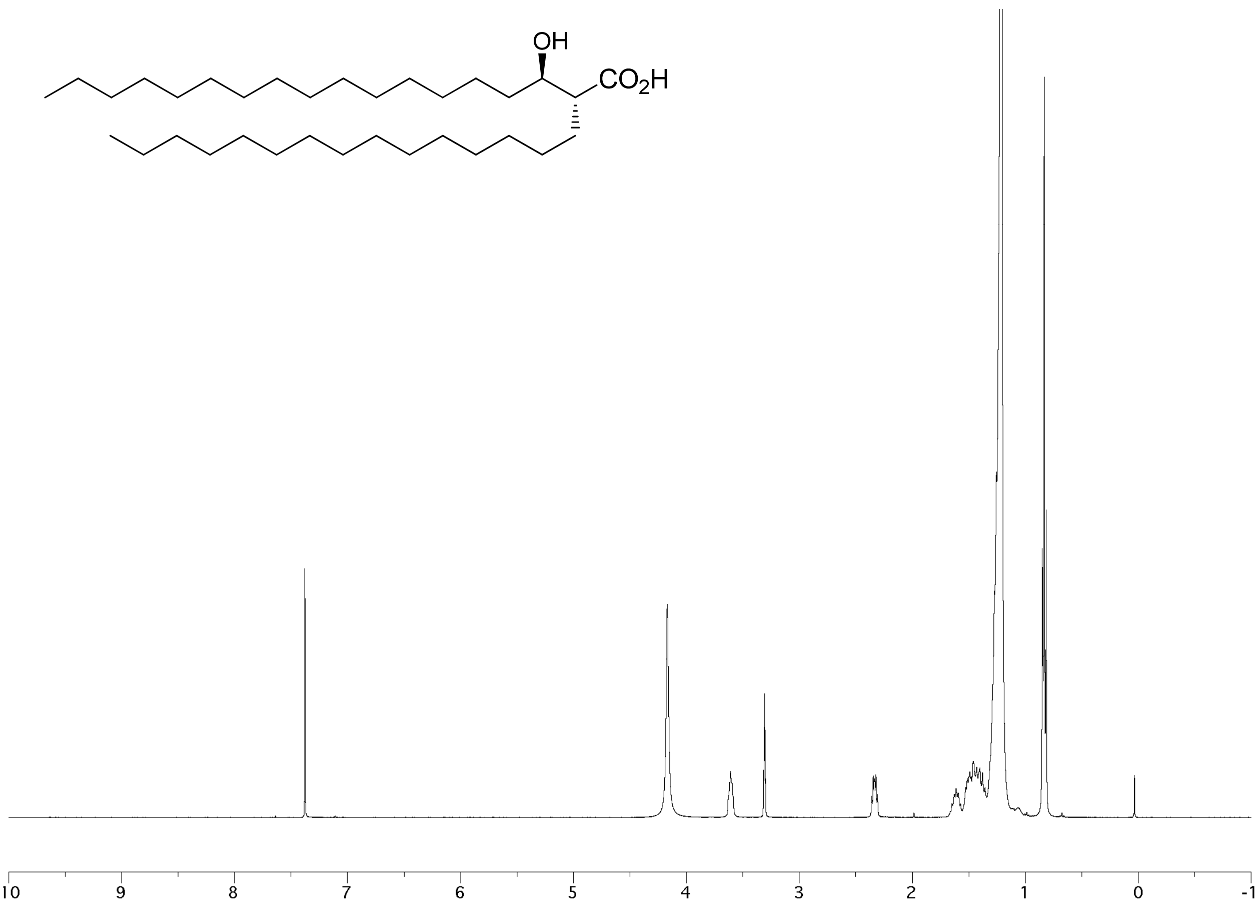


^{13}C NMR spectrum

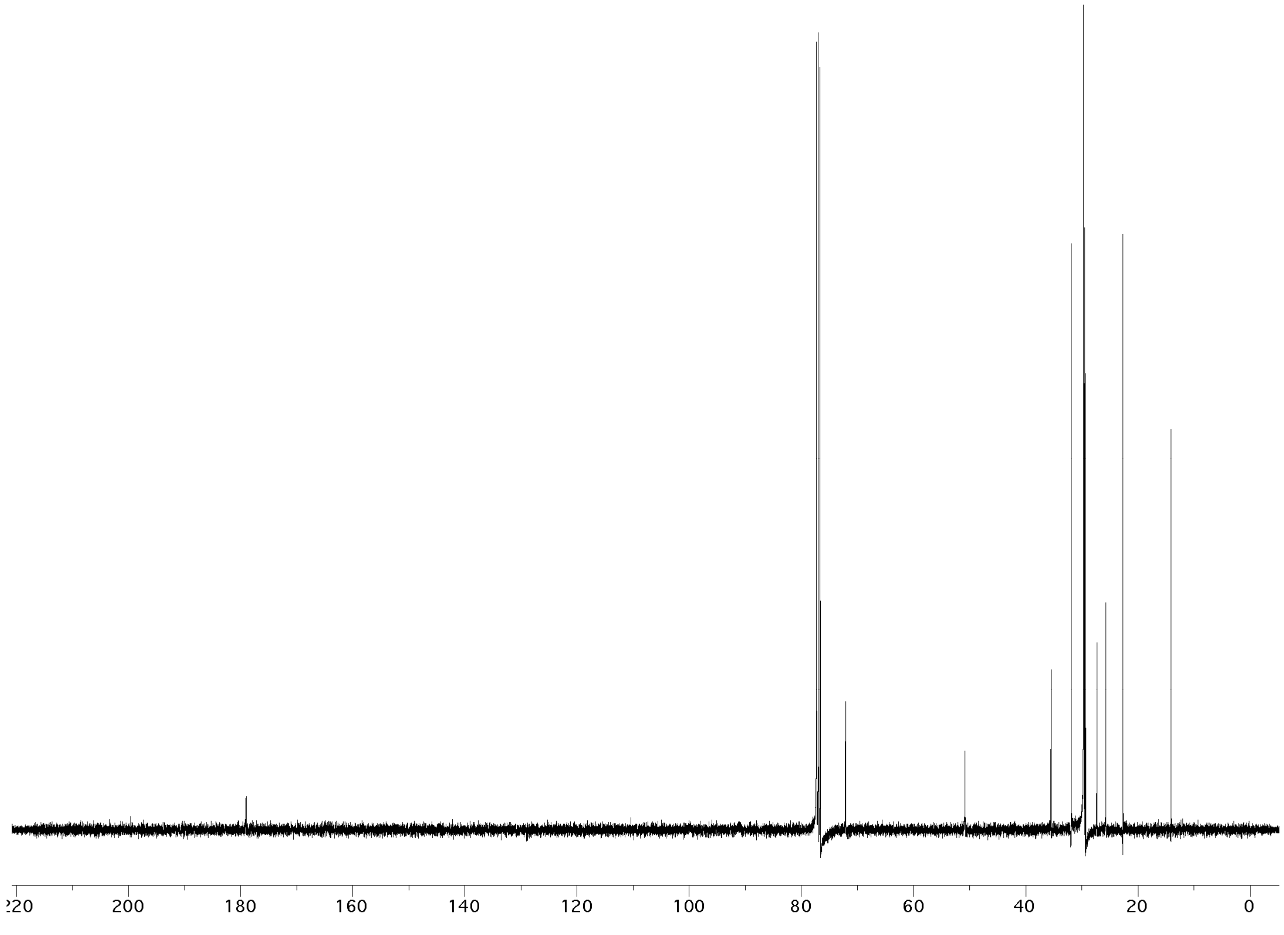


*(2*R*,3*R*)-3-Hydroxy-2-tetradecyloctadecanoic acid (9; (+)-2*R*,3*R*-corynomycolic acid)*

^{1}H NMR spectrum

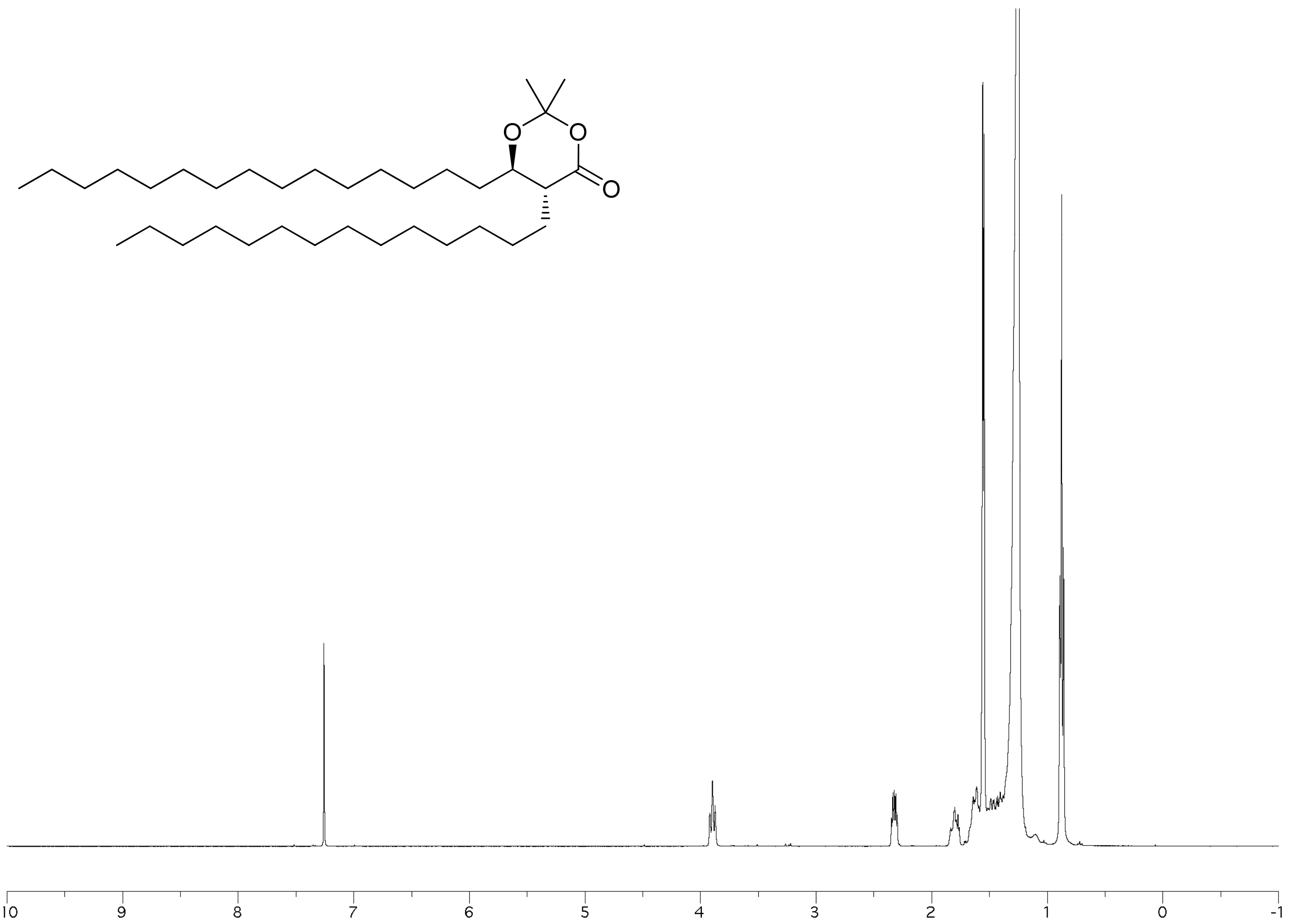


^{13}C NMR spectrum

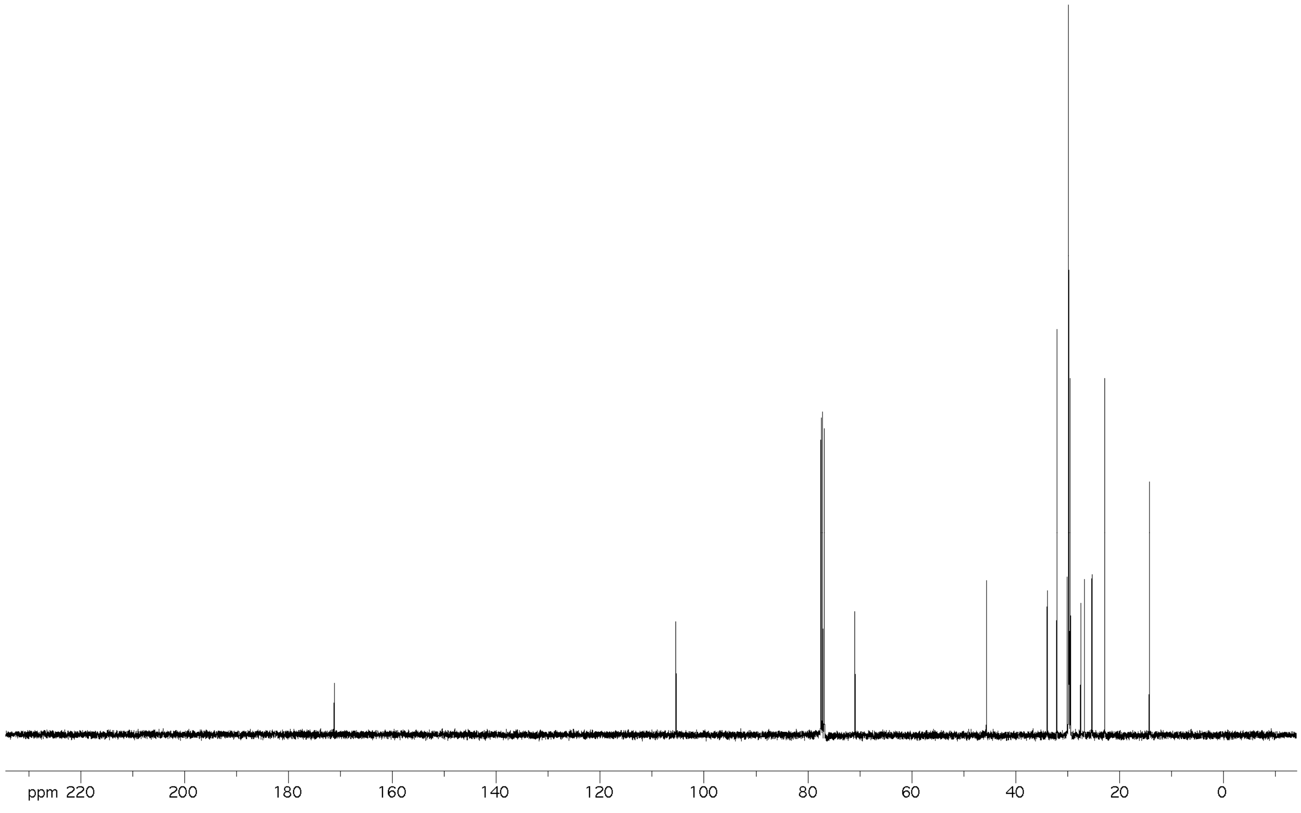


(5R,6R)-2,2-Dimethyl-6-pentadecyl-5-tetradecyl-1,3-dioxan-4-one

^{1}H NMR

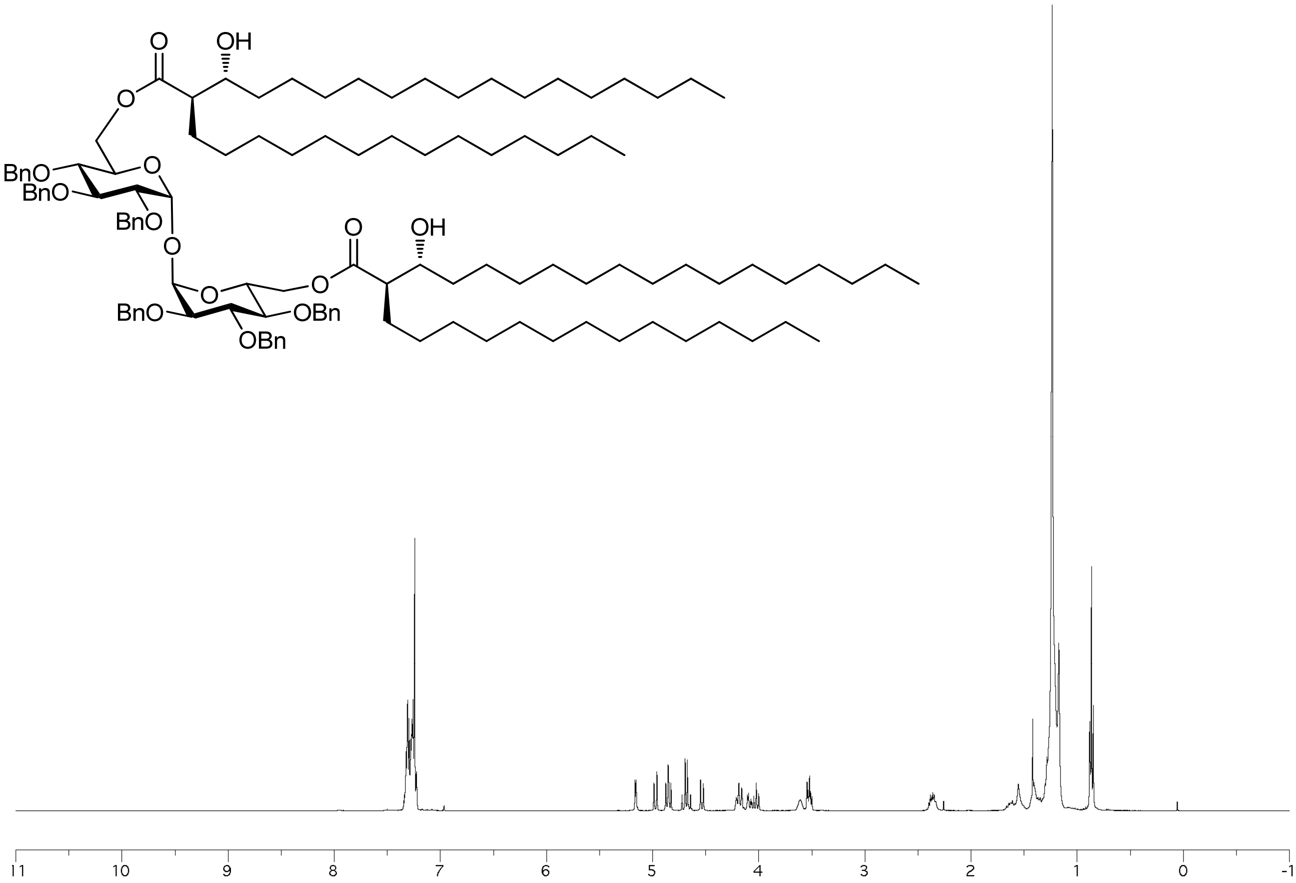


^{13}C NMR

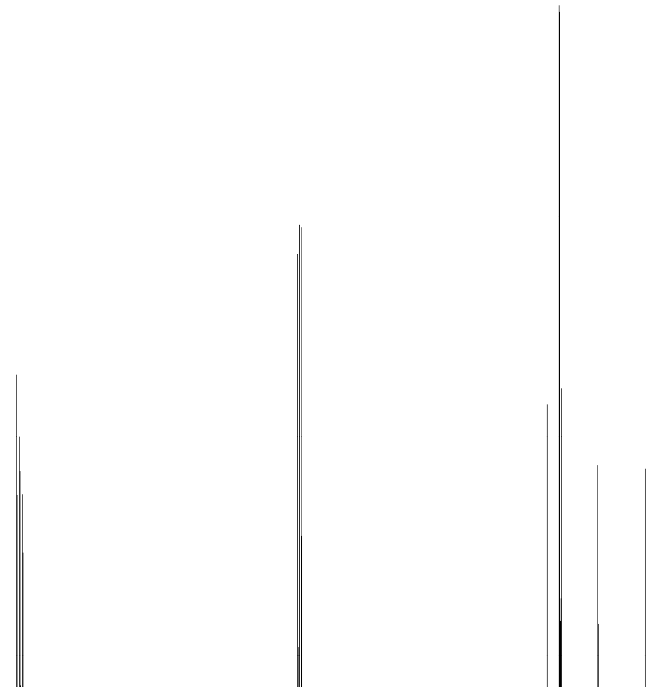


6,6'-Di-O-((2''R,3''R)-3''-hydroxy-2''-tetradecyloctadecanoyl)-2,2',3,3',4,4'-hexa-O-benzyltrehalose (16)

^{1}H NMR

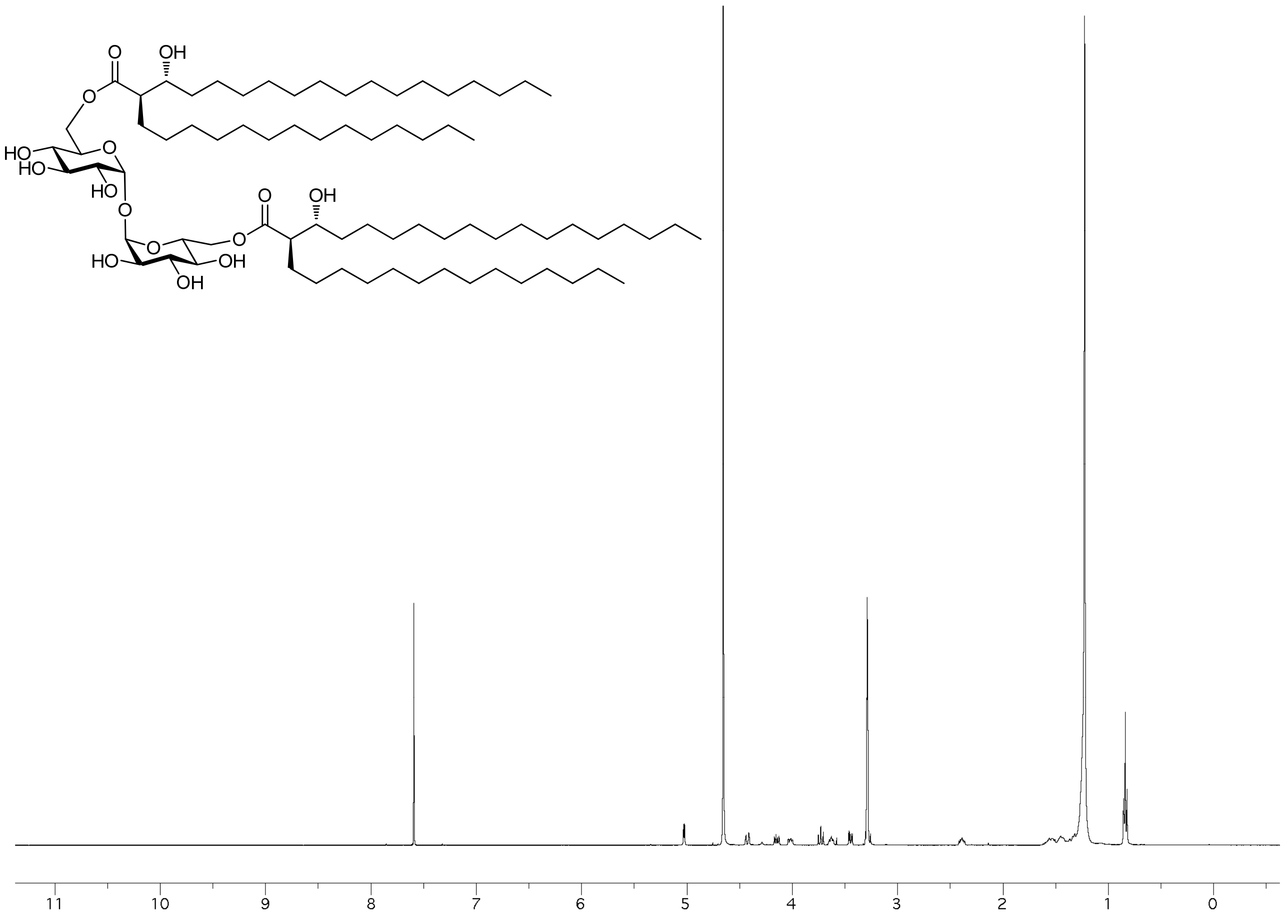


^{13}C NMR

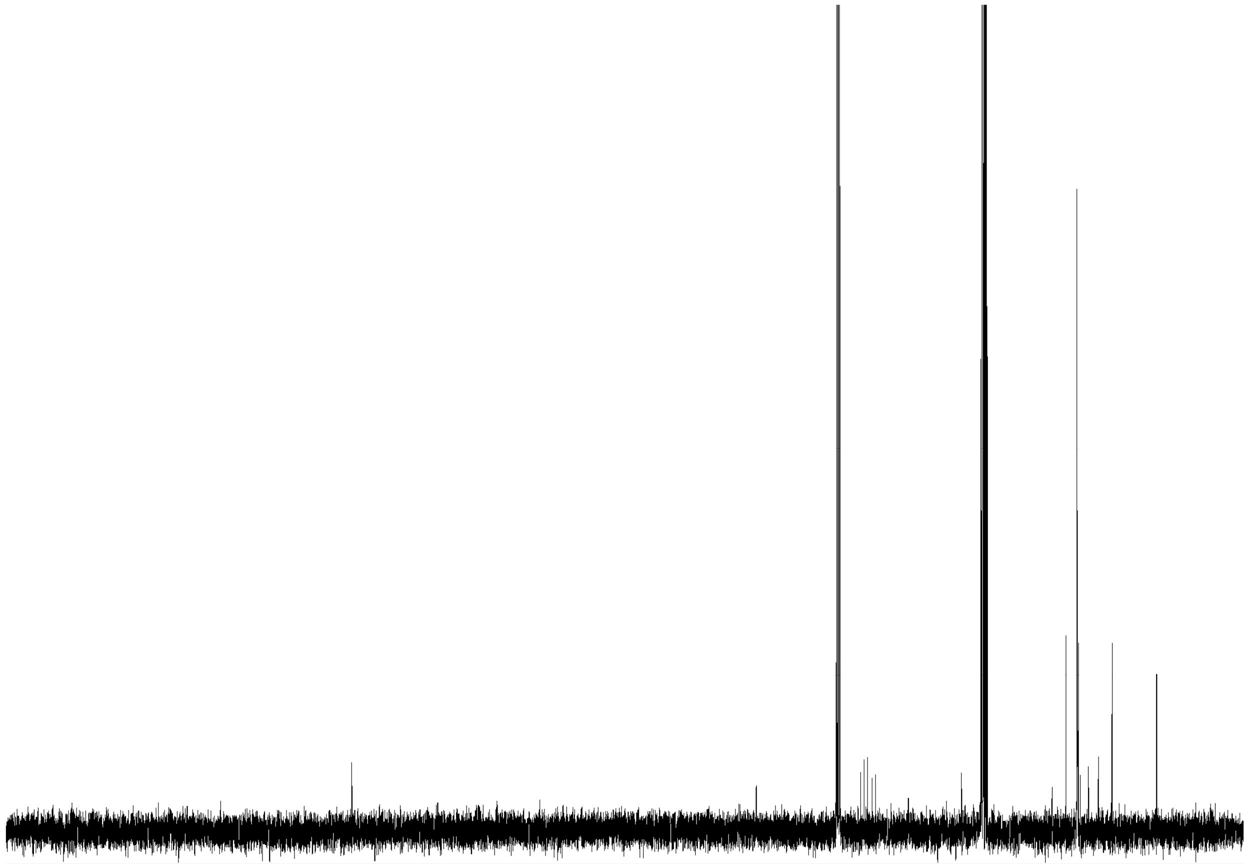


6,6'-Di-O-((2''R,3''R)-3''-hydroxy-2''-tetradecyloctadecanoyl)-trehalose (3; TDCM)

^{1}H NMR

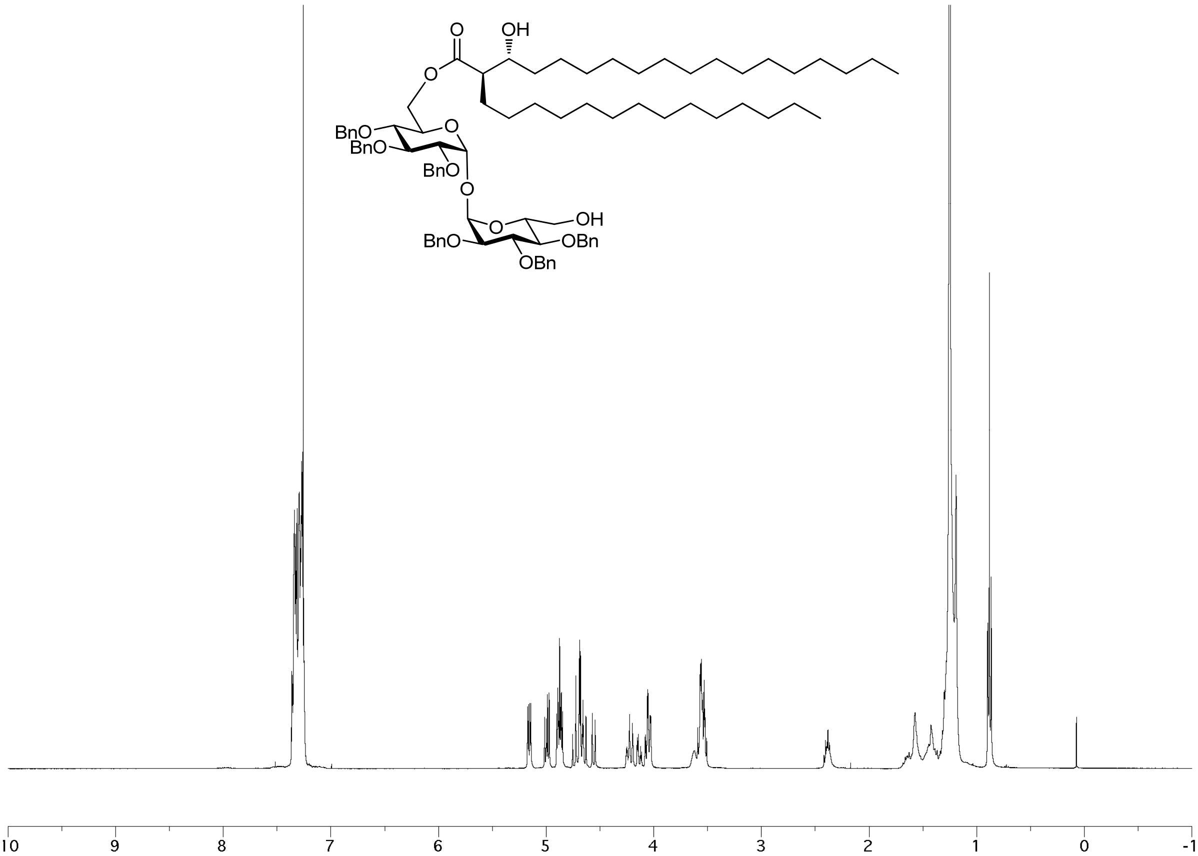


^{13}C NMR



2,2',3,3',4,4'-Hexa-O-benzyl-6-O-(2''R,3''R)-3''-hydroxy-2''-tetradecyloctadecanoyl-trehalose (18)

^{1}H NMR



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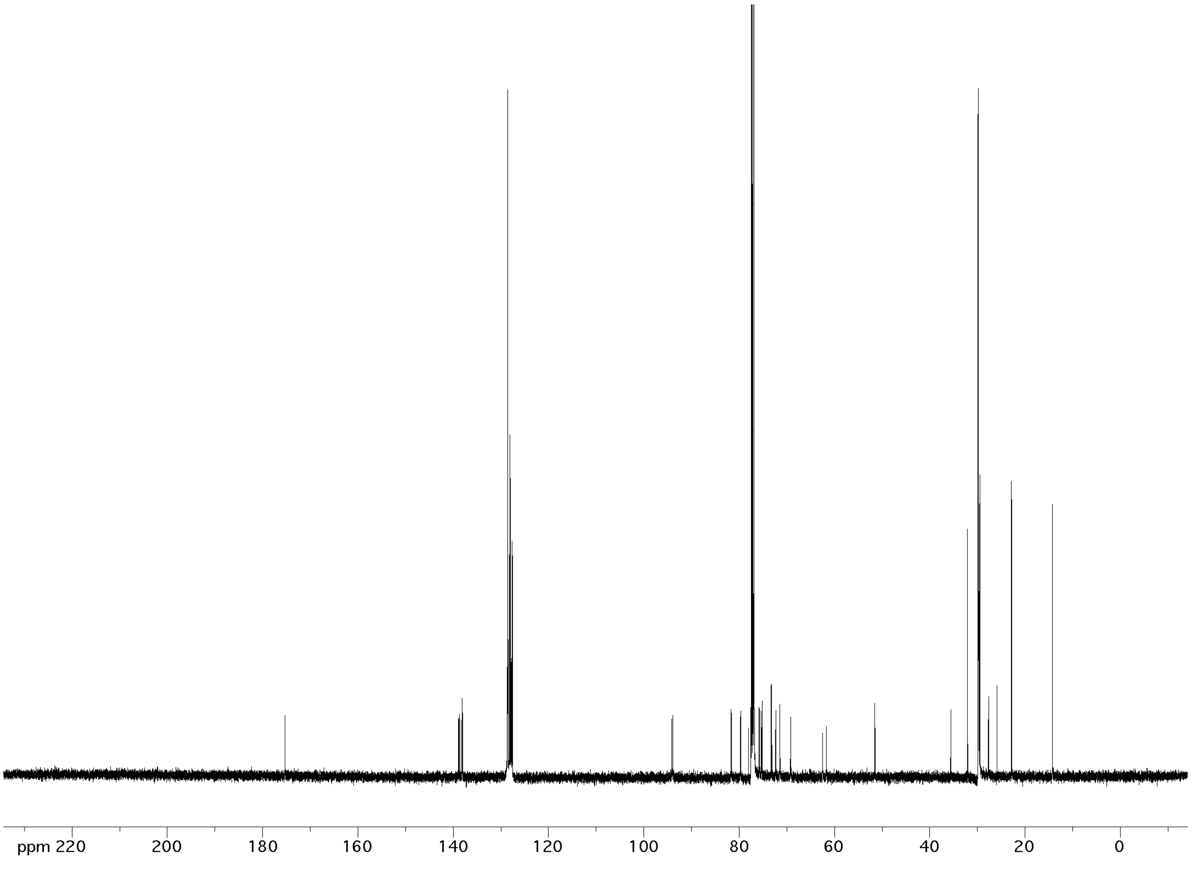
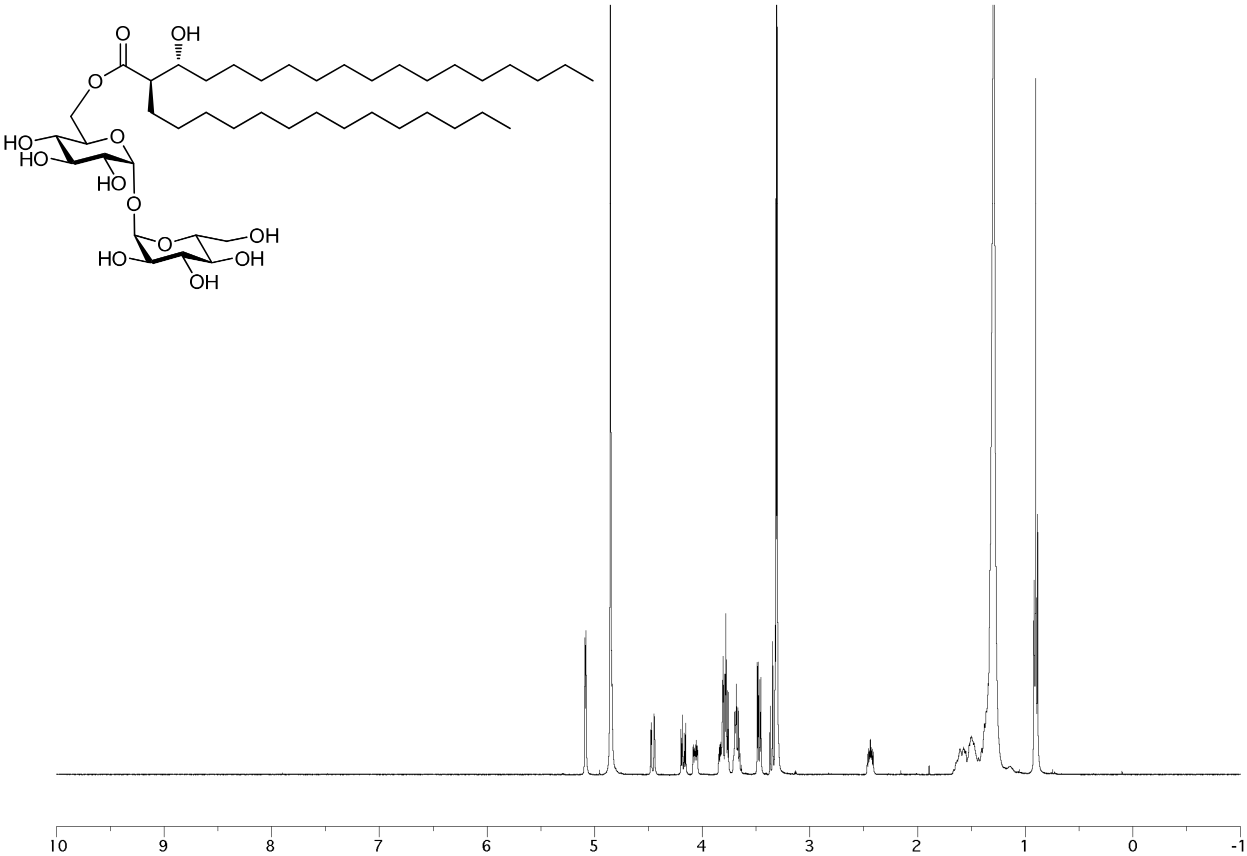
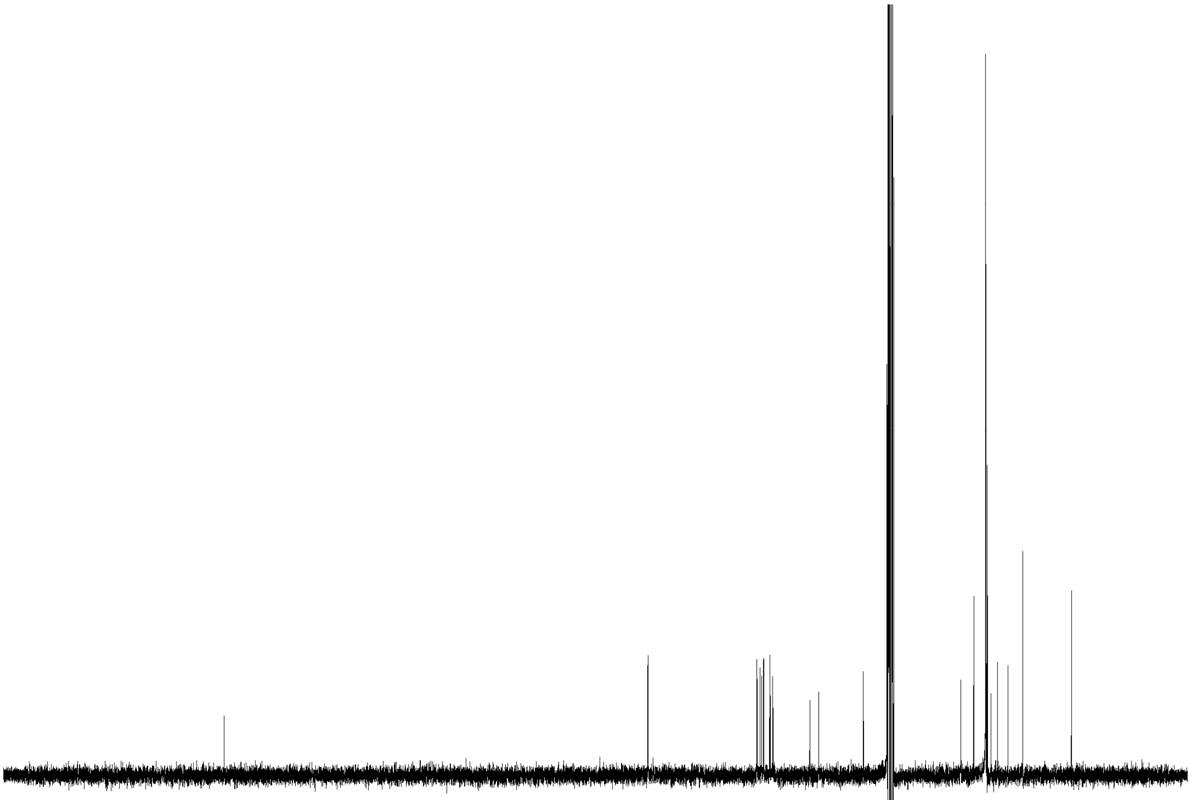


6-O-(2''R,3''R)-3''-Hydroxy-2''-tetradecyloctadecanoyl-trehalose (4; TMCM)

^{1}H NMR

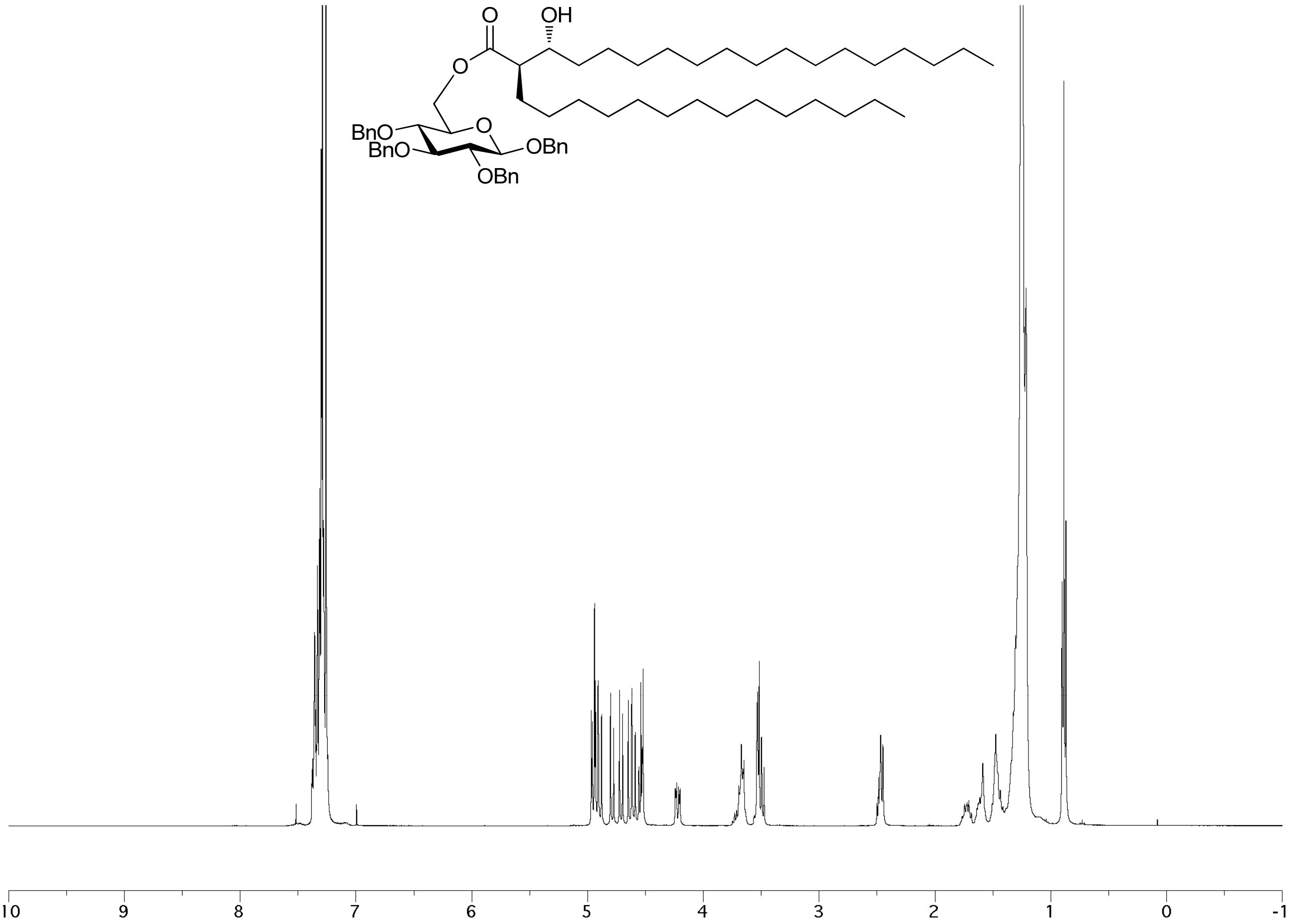


^{13}C NMR

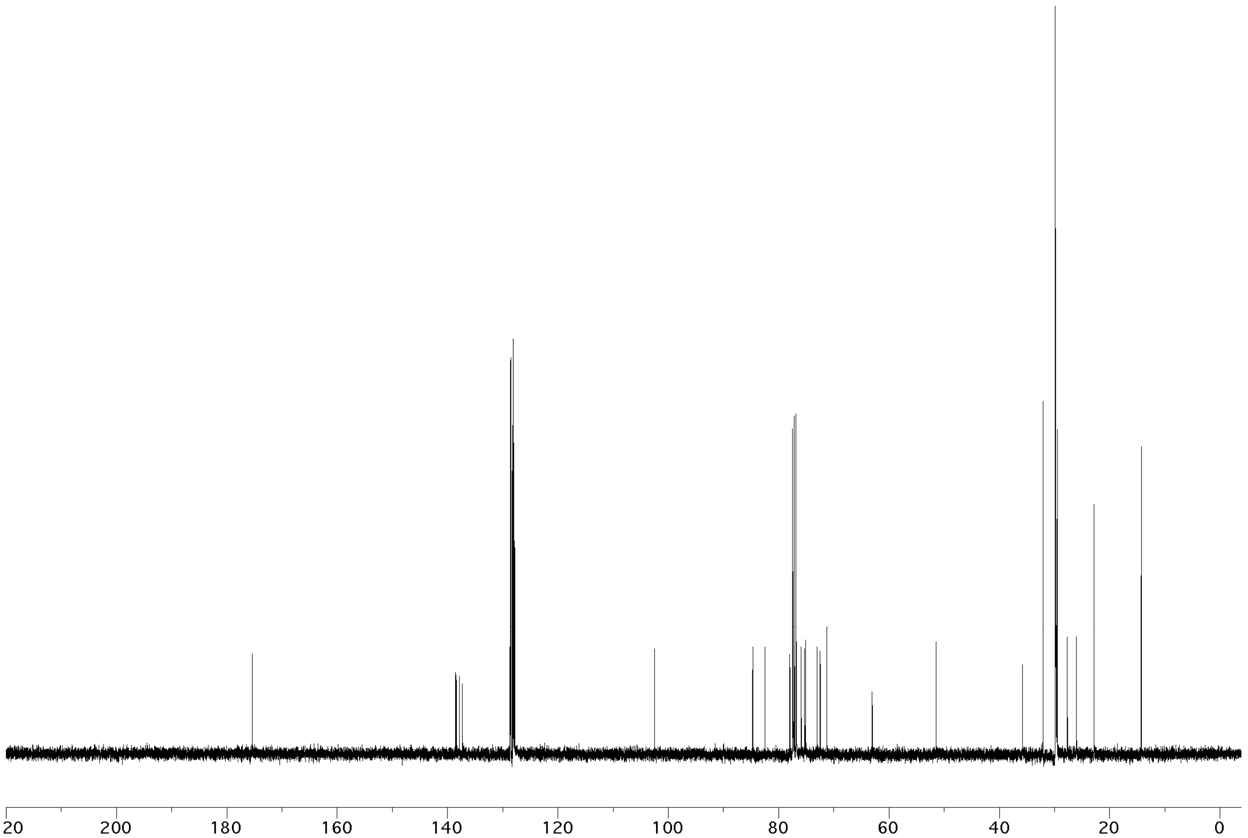


Benzyl 2,3,4-tri-O-benzyl-6-O-((2'R,3'R)-3'-hydroxy-2'-tetradecyloctadecanoyl)-β-D-glucopyranoside (20)

^{1}H NMR spectrum

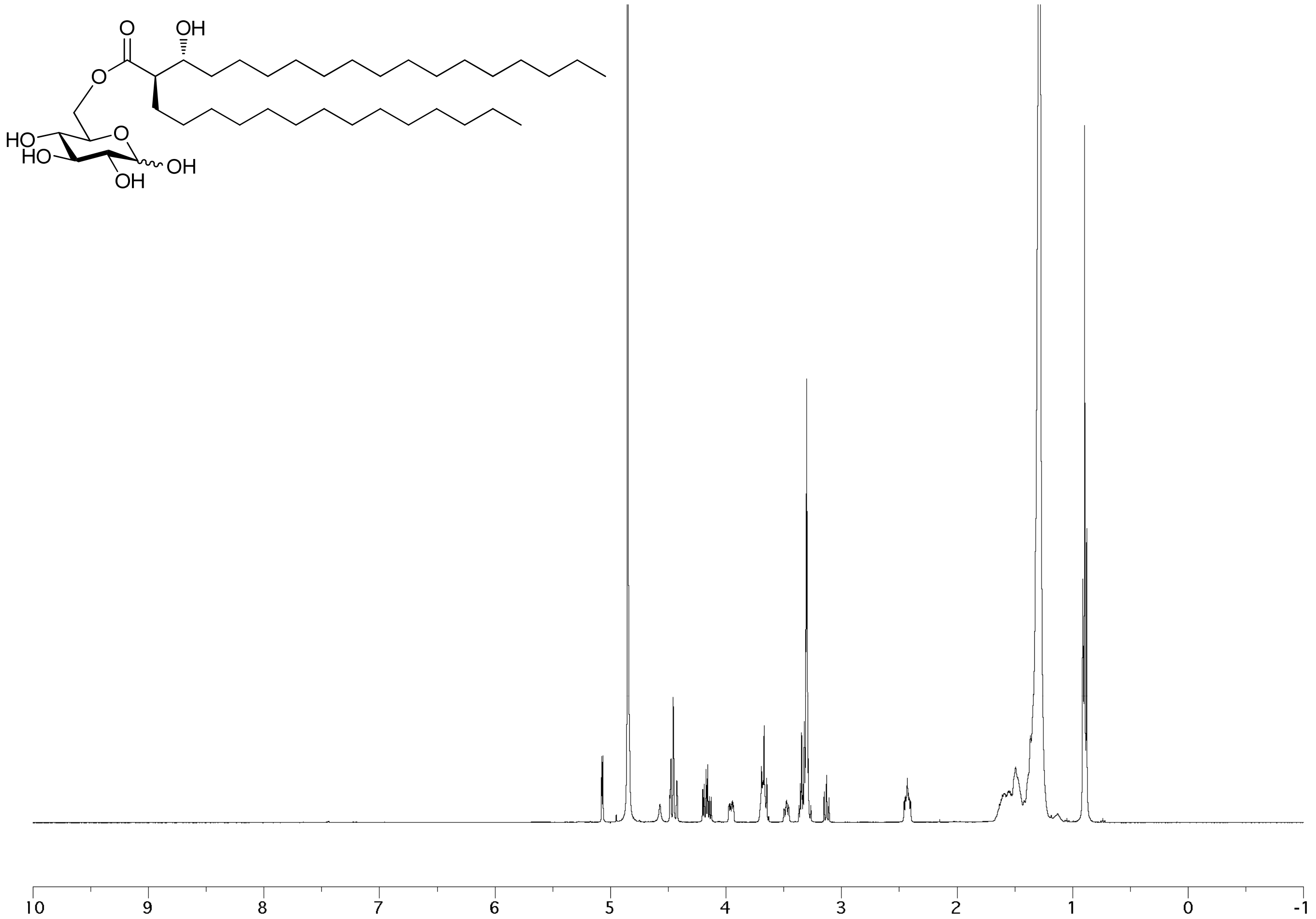


^{13}C NMR spectrum

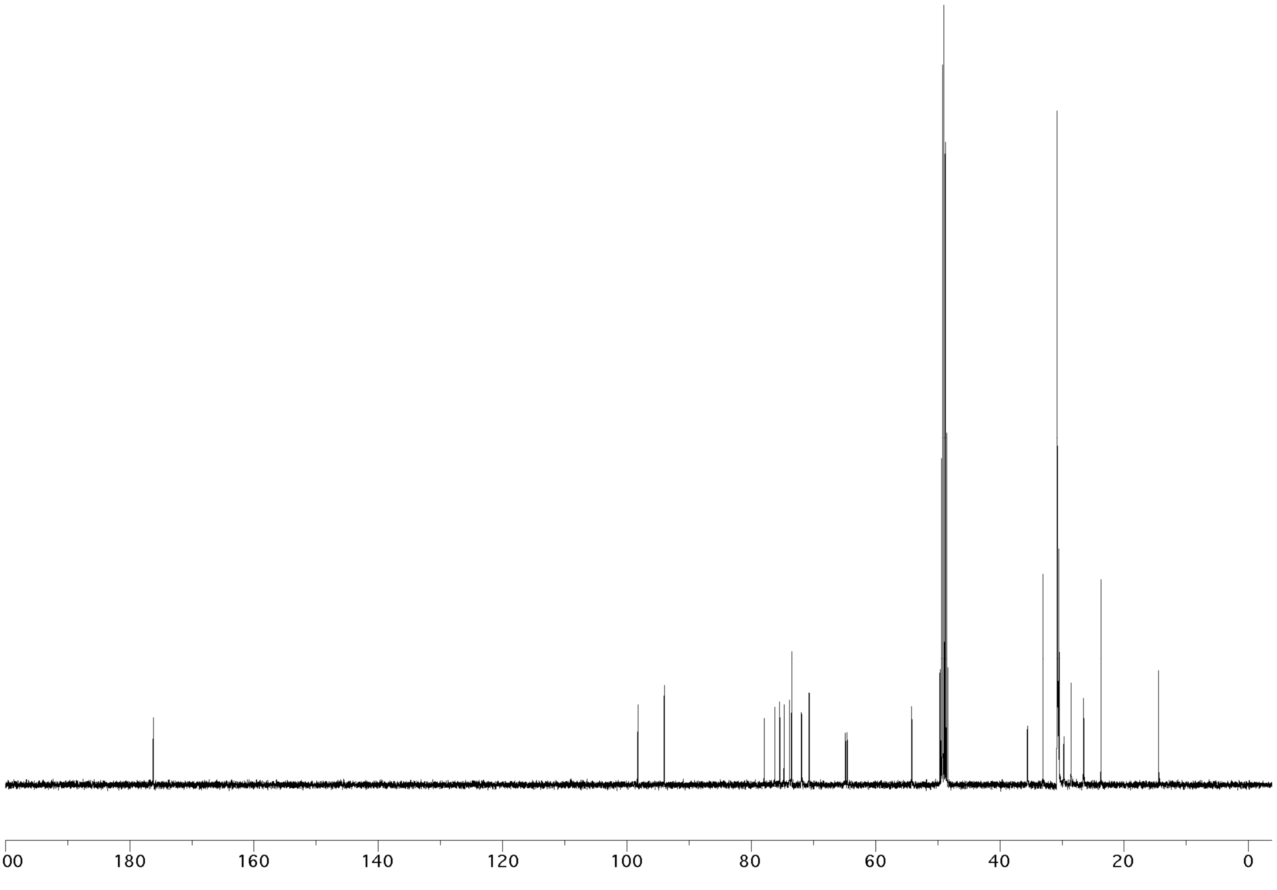


6-O-(2′R,3′R)-3′-Hydroxy-2′-tetradecyloctadecanoyl-D-glucose (6; GMCM)

^{1}H NMR spectrum

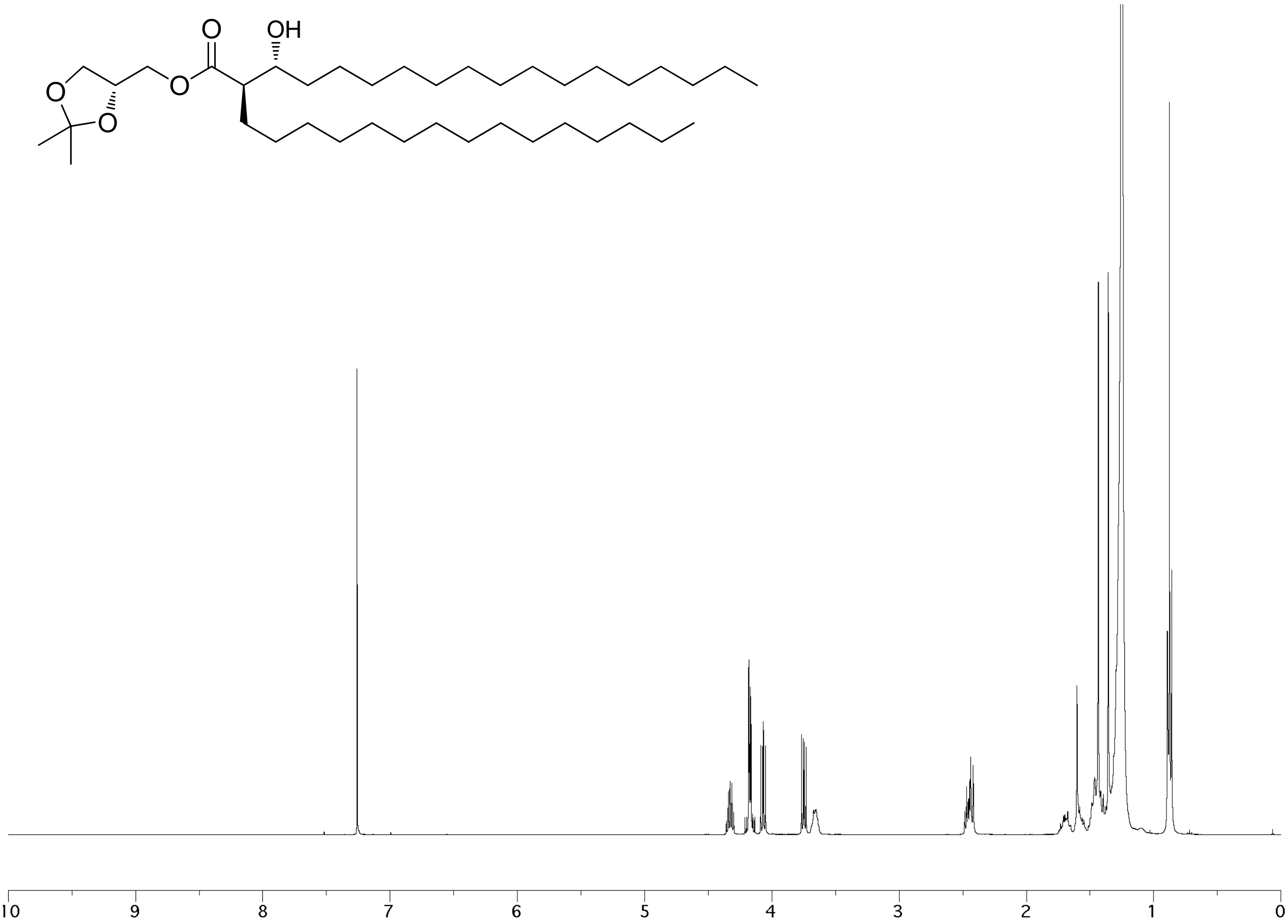


^{13}C NMR spectrum

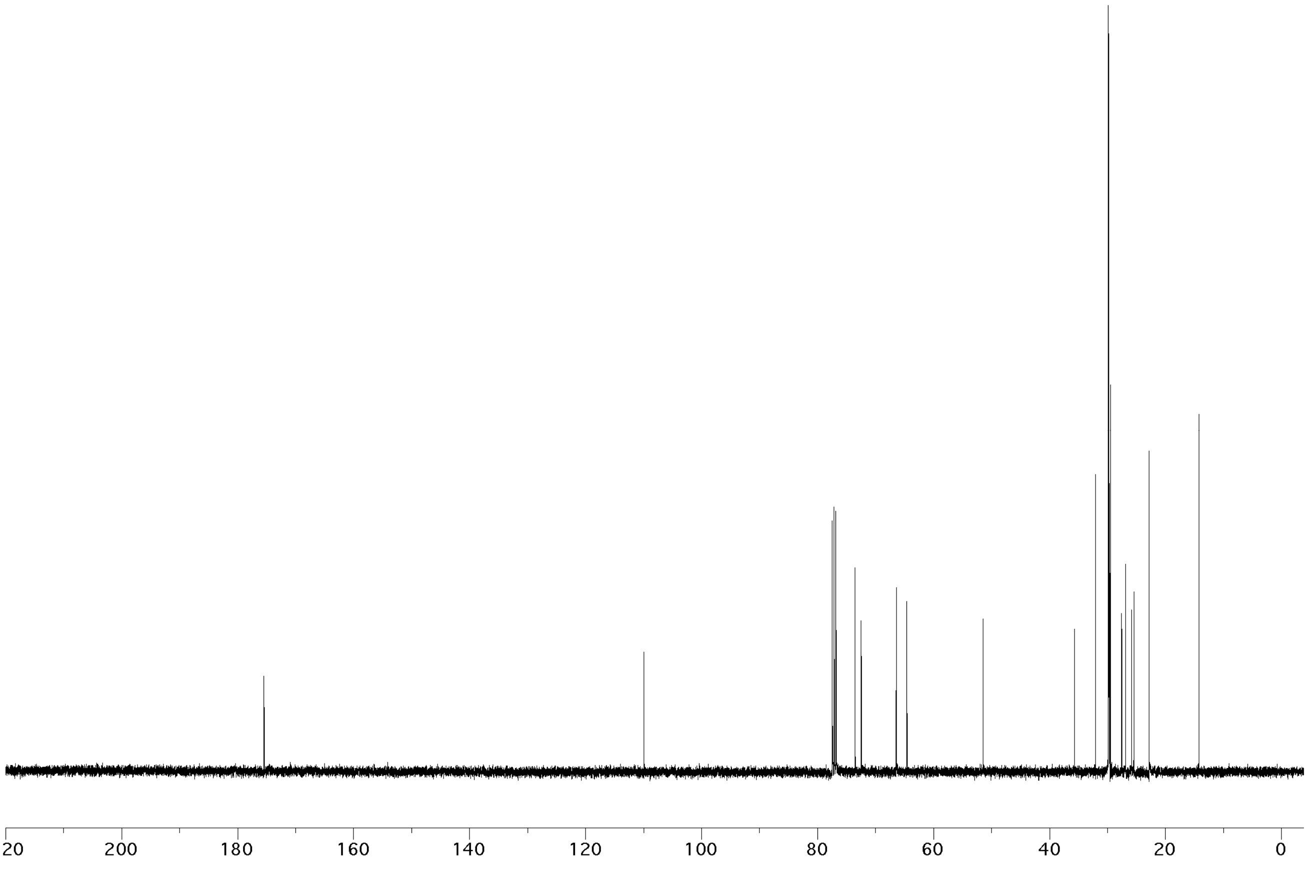


(2R,3R)-((R)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 3-hydroxy-2-tetradecyloctadecanoate (22)

^{1}H NMR spectrum

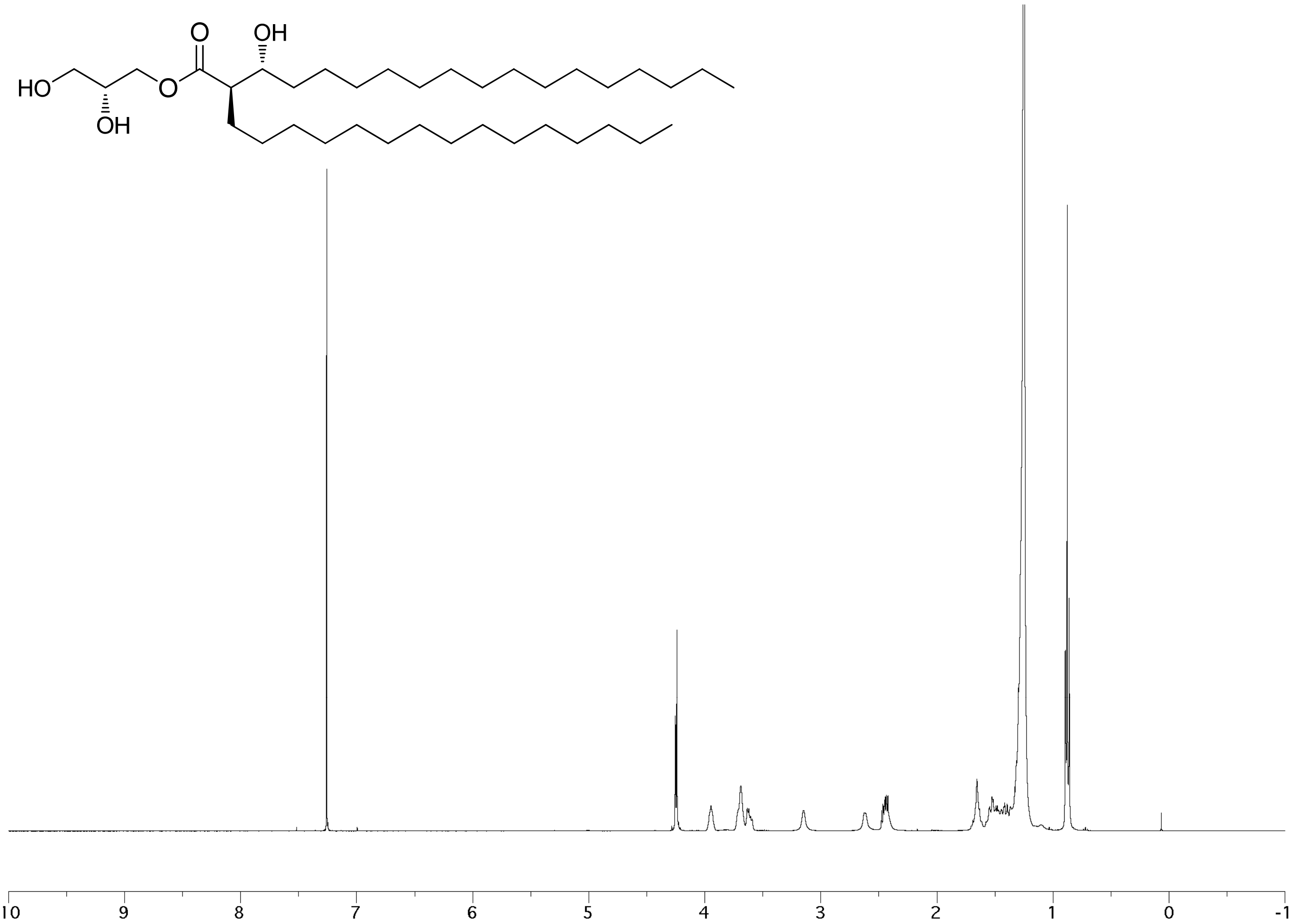


^{13}C NMR spectrum

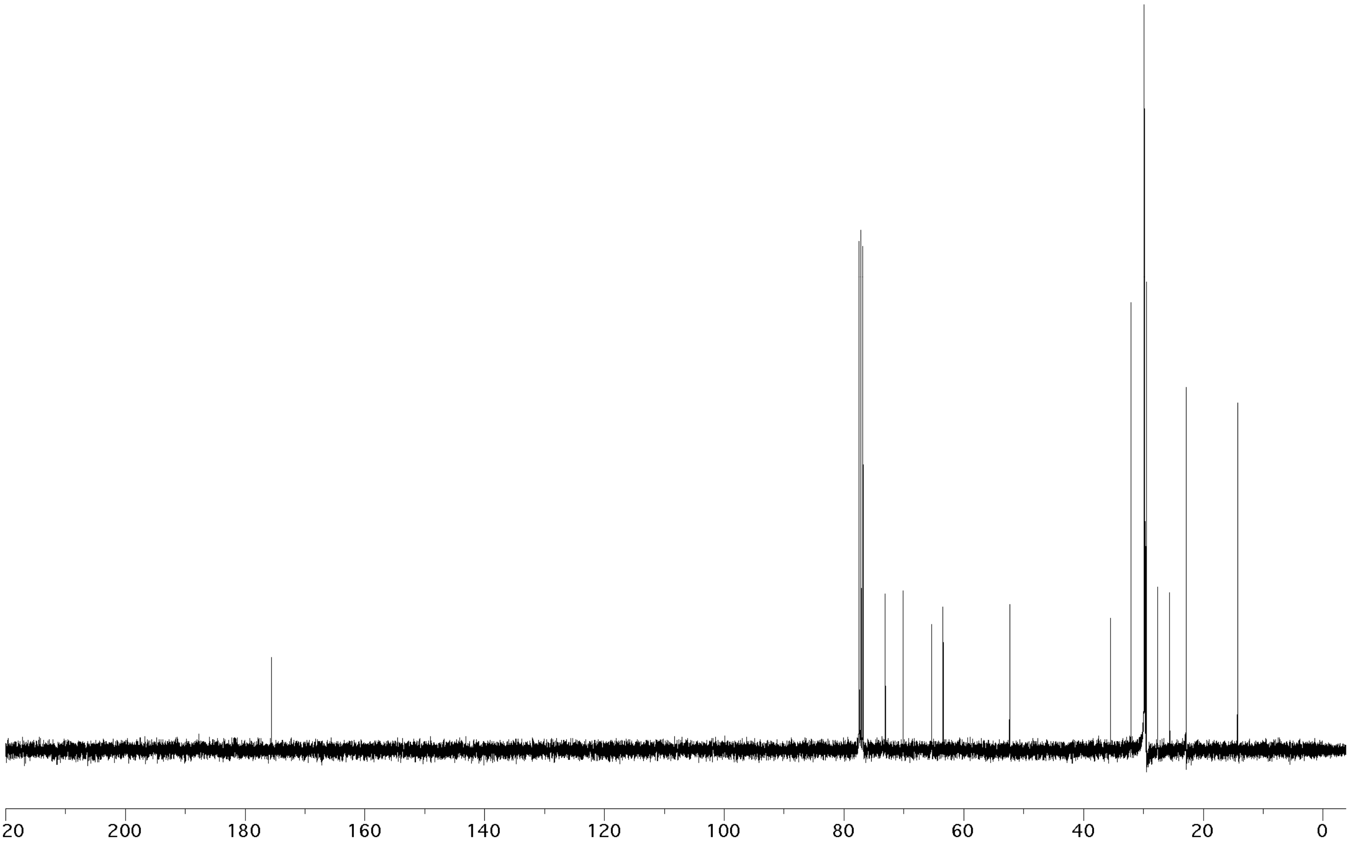


(2R,3R)-((R)-2,3-Dihydroxypropyl) 3-hydroxy-2-tetradecyloctadecanoate (2'R-8; 2'R-GroMCM)

^{1}H NMR spectrum

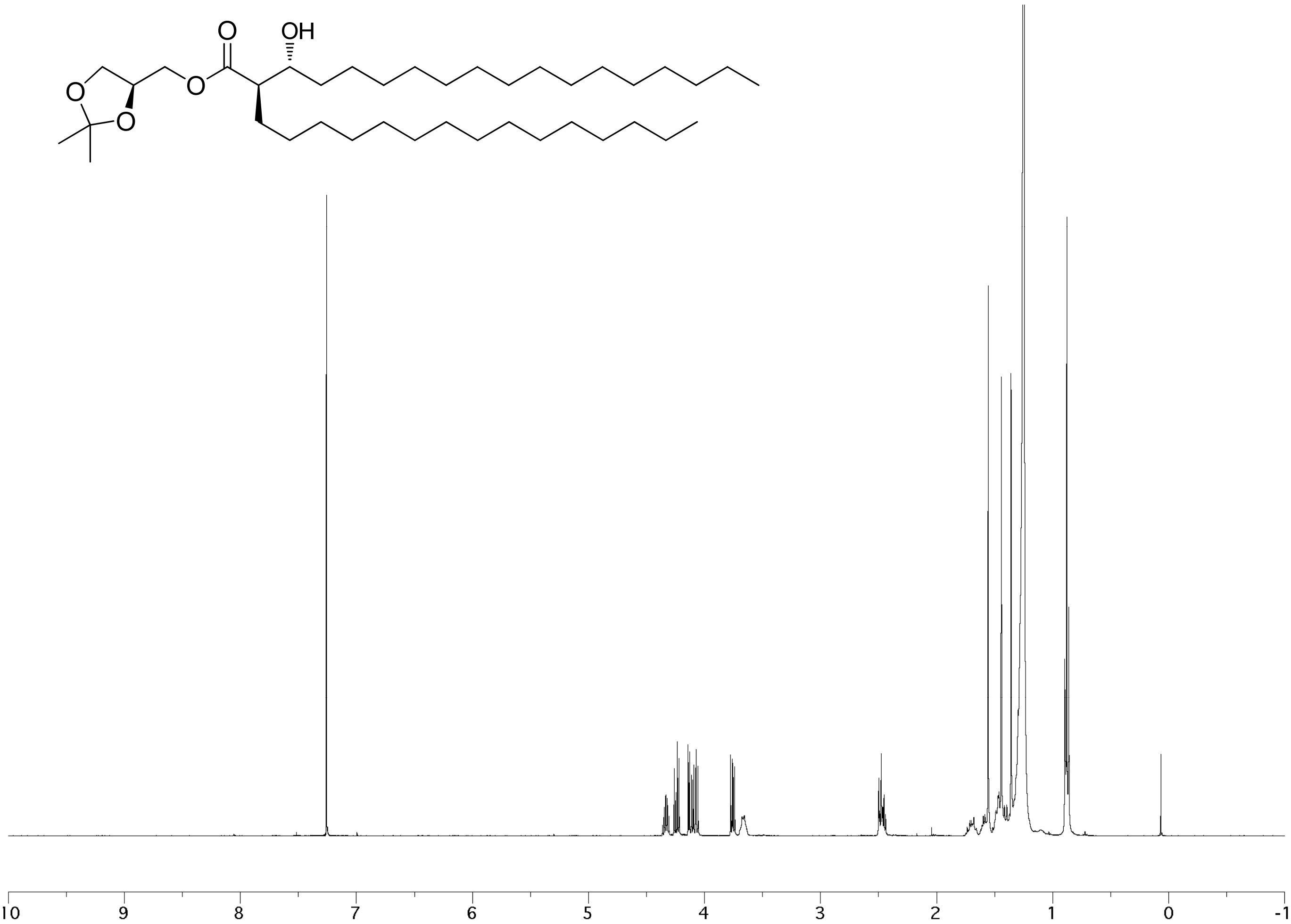


^{13}C NMR spectrum

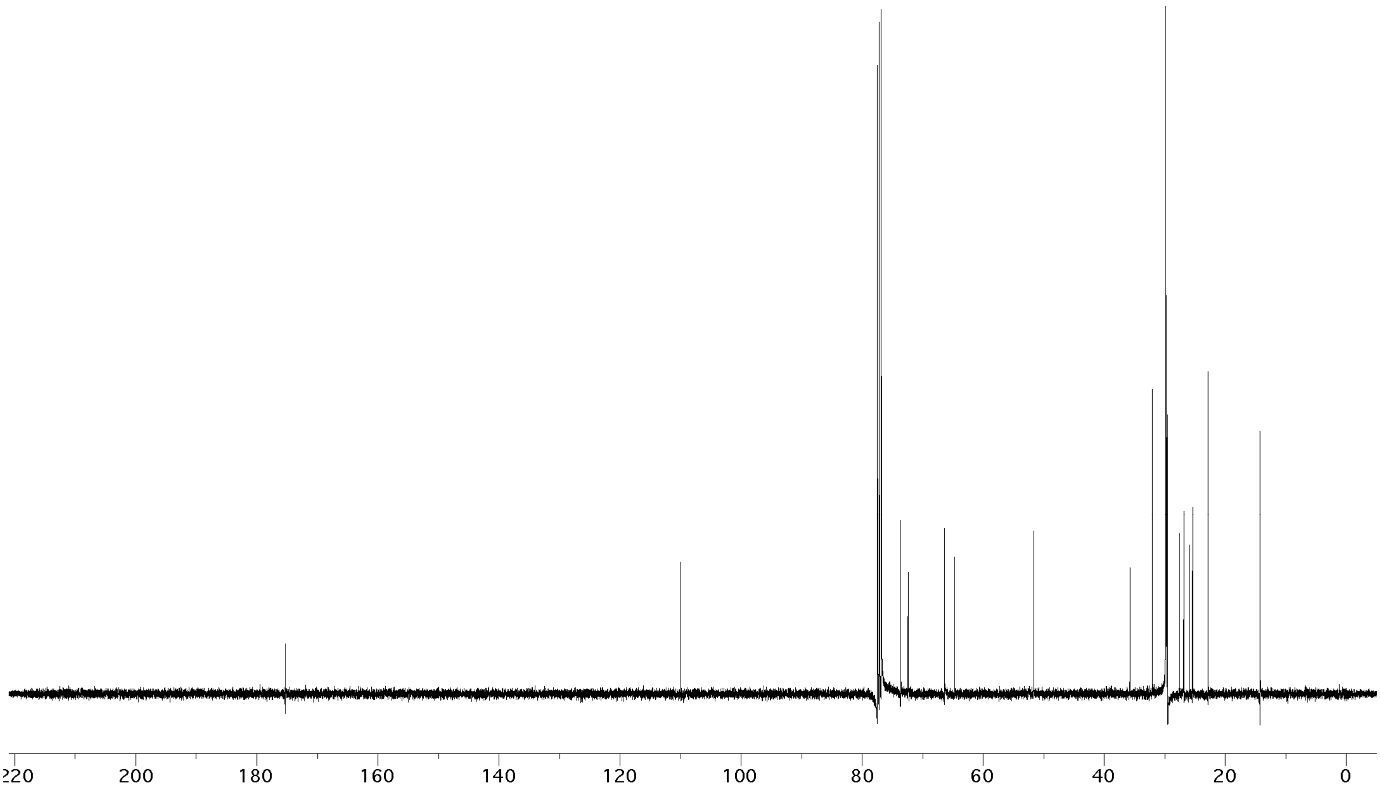


(2R,3R)-((S)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 3-hydroxy-2-tetradecyloctadecanoate (23)

^{1}H NMR spectrum

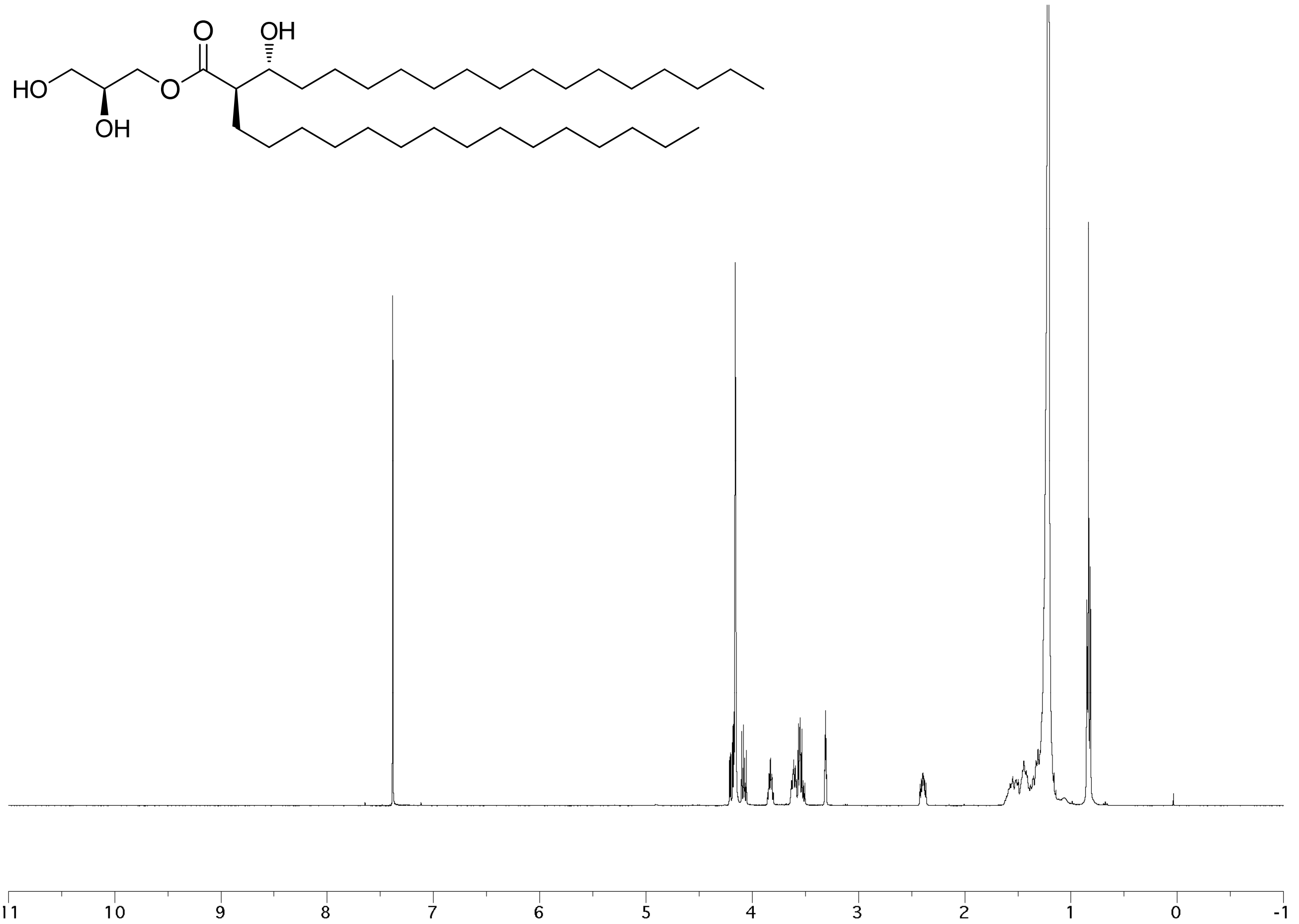


^{13}C NMR spectrum



(2R,3R)-((S)-2,3-Dihydroxypropyl) 3-hydroxy-2-tetradecyloctadecanoate (2'S-8; 2'S-GroMCM)

^{1}H NMR spectrum



^{13}C NMR spectrum