SUPPORTING INFORMATION

Quantitation in the regioselectivity of acylation of glycosyl diglycerides: Total synthesis of a *Streptococcus pneumoniae* α-glucosyl diglyceride

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Derivation of eqn (1)

In a situation of perfect regioselectivity, the relative integrations of the resonances at δ *x* ppm (labelled position) and δ *y* ppm (unlabelled position) are equal to the ratio of ^{13}C abundances (*L* and *N*) at the two carbonyls:

$$\frac{\int x}{\int y} = \frac{L}{N} \tag{S1}$$

where *L* is the ^{13}C atom% of the labelled carbonyl and *N* is the natural abundance of ^{13}C (taken as 1.109%) at the unlabelled carbonyl.

Eqn (S1) can be modified to describe scenarios where a proportion of the molecules have undergone acyl migration. In this case, let *z* be the proportion of the mixture of regioisomers with the desired regiochemistry:

$$\frac{\int x}{\int y} = \frac{zL + N(1-z)}{zN + L(1-z)} \tag{S2}$$

Rearranging eqn (S2) to isolate *z* gives eqn (S3), which can be used to calculate the proportion of molecules in the sample that have the desired regiochemistry:

$$z = \frac{N - L \cdot \left(\frac{\int x}{\int y}\right)}{N \cdot \left(\frac{\int x}{\int y}\right) - L \cdot \left(\frac{\int x}{\int y}\right) + N - L} \tag{S3}$$

In equation S3, *z* has a value between 0 and 1. The percentage ratio of regioisomers in the mixture, *R*, is obtained directly from *z* (eqns S4 and 1):

$$R = 100z \tag{S4}$$

$$R = \frac{N - L \cdot \left(\frac{\int x}{\int y}\right)}{N \cdot \left(\frac{\int x}{\int y}\right) - L \cdot \left(\frac{\int x}{\int y}\right) + N - L} \times 100 \tag{1}$$

Simplified Version

If the atom% ^{13}C at the labelled carbon is sufficiently high (>99%), eqn 1 can be simplified by assuming the signals at δ *x* ppm and δ *y* ppm are both due entirely to the presence of the ^{13}C-labelled carbon. In this situation, *N* can be treated as having a value of 0, and *L* can be treated as having a value of 1. Substituting these values into eqn 1 gives eqn S5:

$$R = \frac{\frac{\int x}{\int y}}{\frac{\int x}{\int y} + 1} \times 100 \qquad \text{(S5)}$$

This simplified version gives values of *R* that closely agree with that of Eqn 1 for modest levels of regioselectivity where the integrals of the resonances at δ *x* ppm (labelled position) and δ *y* ppm (unlabelled position) are similar, but for high levels of regioselectivity where the integrals are substantially different, eqn 1 diverges from eqn S5 and the former should be used.

Protocol for quantitative NMR analysis

NMR samples were prepared such that the concentration of analyte and $Cr(acac)_3$ were 80–90 mM and 50 mM, respectively. Inverse-gated proton-decoupled ^{13}C spectra were collected over a spectral width of 31250 Hz, with an acquisition time of 0.7 s, and 21875 complex points. A flip angle of 45° and a relaxation delay of 10 s were used. Samples were heated to 25 °C and spun at 20 Hz. Signal averaging over a minimum of 2000 scans was sufficient for quantitation. The resultant quantitative ^{13}C spectra were deconvolved using peak-fitting in MestreNova 10; peaks were fitted to a generalized Lorentzian equation. Peak positions, peak widths at half-height, and optimal combination of Lorentzian and Gaussian (L/G) shapes for each individual peak were allowed to vary to optimize fit. Peak areas for the fitted curves were normalized against the smaller peak.

Measurement of isotopic labelling of 1-^{13}C-palmitic acid

1-^{13}C-labelled palmitic acid (99 atom%) was purchased from Sigma-Aldrich. This material was assumed to comprise a mixture of two compounds: 1) 1-^{13}C-palmitic acid, with natural isotopic abundances at the remaining 15 carbons, and 2) unlabelled palmitic acid, with natural isotopic abundances at all 16 carbons. The predicted ESI-MS $[M–H]^-$ profiles for each of these two compounds are listed in Table S1.

Table S1: Theoretical isotope profiles for natural and ^{13}C-labelled palmitic acids.[1]

m/z	*relative abundance*		*normalized abundance (ρ)*	
	Natural	*1-^{13}C-labelled*	*Natural*	*1-^{13}C-labelled*
255	100	0.00	**0.8348**	0.0000
256	17.8814	100	0.1493	0.8437
257	1.906	16.7998	0.0159	0.1417
258	*negligible*	1.7243	0.0000	**0.0145**

The signals at *m*/*z* 255 and 258 are specific for the natural and ^{13}C-labelled palmitic acids, respectively. The ratio of the signal intensities (*I*) at *m*/*z* 255 and 258 can be expressed as function of the ratio of the natural and labelled palmitic acids in the sample:

$$\frac{I_{255}}{I_{258}} = \frac{(1-L)\cdot\rho_{255}}{\rho_{258}\cdot L} \qquad \text{(S6)}$$

where I_{255} and I_{258}, and ρ_{255} and ρ_{258} are the respective signal intensities and normalized abundances for the signals at *m*/*z* 255 and 258 in the natural and labelled palmitic acids, respectively; and *L* is the total proportion of labelled palmitic acid in the sample. Rearranging the equation to isolate *L* gives:

$$L = \frac{\rho_{255}}{\rho_{258}\cdot\frac{I_{255}}{I_{258}} + \rho_{255}} \qquad \text{(S7)}$$

A 1.0 mg/mL sample of commercial 1-^{13}C-palmitic acid was prepared in MeOH, then diluted 100-fold into isopropanol. 50 µL of the resulting sample solution was then combined with 10 µL 3% aq. NH_4OH and the mixture analyzed directly on a Finnigan hybrid LTQ-FTMS. The ratios of signal intensities at *m*/*z* 255 and 258 were calculated separately from peak heights and peak areas (Fig S1). Substitution of the measured I_{255}/I_{258} values, and the ρ_{255}/ρ_{258} values from Table 1, into eqn (S7) yielded *L* as 0.9924 based on peak height, and 0.9956 based on peak area. The mean of these values (99.4 atom% ^{13}C) was used in regioselective fidelity calculations.

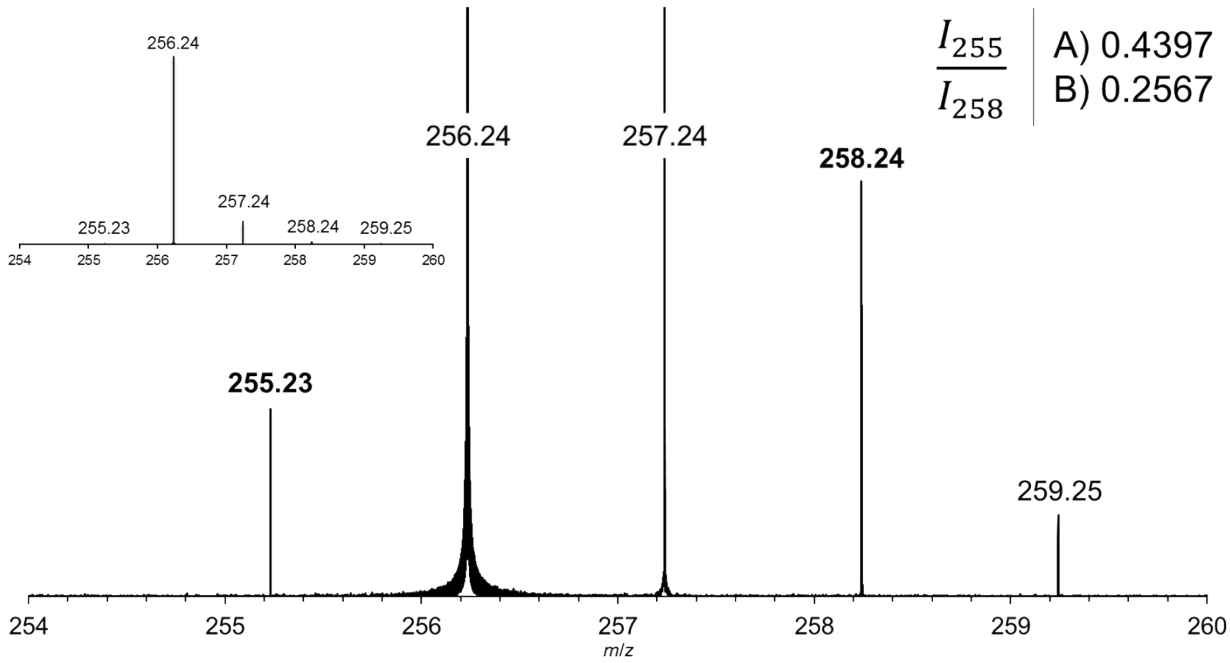


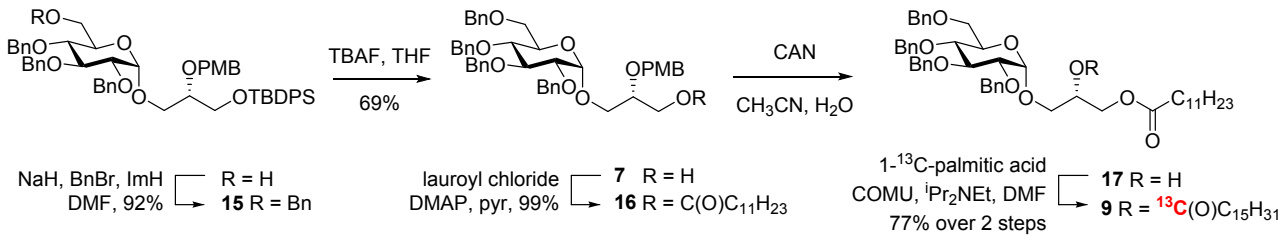
Figure S1: Negative ion FTMS-ESI SIM spectrum of commercial 1-^{13}C-palmitic acid. Signal averaging was performed over 50 scans. I255/I258 values were calculated based on A) peak heights and B) peak areas.

Experimental

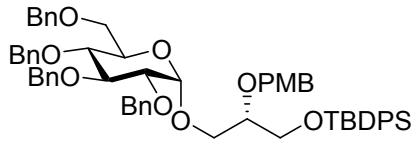
General

Proton nuclear magnetic resonance spectra (^{1}H NMR, 400, 500 MHz) and proton decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR, 100, 125 MHz) were obtained in deuterated chloroform with residual protonated solvent, or the central ^{13}C triplet signal as internal standard. Abbreviations for multiplicity are s, singlet; d, doublet; t, triplet; q, quartet; p, pentet. Fourier-transform infrared spectra were obtained as neat samples on an attenuated total reflectance instrument using a diamond-coated zinc selenide sample accessory. Optical rotations were obtained using a JASCO DIP-1000 polarimeter. $[\alpha]_D$ values are given in 10^{-1} cm^2 g^{-1}. 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 GF_{254} and chromatograms were visualized with ceric ammonium molybdate (Hanessian's stain). High resolution mass spectra (HRMS) were obtained by ionizing samples using electro-spray ionization (ESI) and a time-of-flight mass analyzer. Dry DMF was obtained by drying over 4 Å molecular sieves. Hexanes refers to petroleum ether, boiling range 40-60 °C. Dichloromethane and THF were dried over alumina according to the method of Pangborn *et al*.[3]

For mass spectrometry of SPN Glc-DAG-s2, samples (10 µM in isopropanol:methanol:chloroform (4:2:1, v:v:v) containing 2.5 mM lithium acetate) were introduced into an Orbitrap Fusion Lumos mass spectrometer (Thermo Scientific, San Jose, California) via electrospray ionization (ESI). ESI-MS and MS^n spectra were acquired at a mass resolving power of 500,000. For MS^n experiments, $[M+Li]^+$ precursor ions were monoisotopically mass selected using the isolation quadrupole (± 0.5 m/z), then subjected to higher energy collisional dissociation (HCD) (for MS^2). Collision energies were individually optimized for each precursor or product ion of interest. Spectra shown are the average of 100 scans.

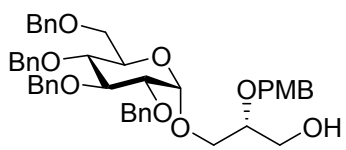


1-O-*tert*-Butyldiphenylsilyl-2-*O*-(4-methoxybenzyl)-sn-glyceryl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (15)



A solution of 1-O-*tert*-butyldiphenylsilyl-2-*O*-(4-methoxybenzyl)-3-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-sn-glyceride[4] (550 mg, 623 μmol) in DMF (11.0 mL) was added to a stirred suspension of 60% NaH in mineral oil (99.6 mg, 2.49 mmol) and imidazole (17.0 mg, 249 μmol) in DMF (15.5 mL) at 0 °C, then the reaction mixture was warmed to rt and stirred under nitrogen for 30 min. The mixture was cooled to 0 °C, and a solution of BnBr (296 μL, 2.49 mmol) in DMF (1.00 mL) was added and the reaction mixture was warmed to rt and stirred under nitrogen for 24 h. The reaction mixture was diluted by slow addition of MeOH (15 mL) at 0 °C, then the solvent was evaporated in vacuo. The residue was dissolved in EtOAc (100 mL) and washed sequentially with water (3 × 50 mL), then brine (3 × 50 mL), then dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The residue was purified by flash chromatography (45:4:1 ✂ 11:4:1 hexanes/CH_2Cl_2/acetone), affording the compound **15** as a colourless oil (529 mg, 92%); $[\alpha]^{25}_D$ +24.4 (*c* 1.00 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.08 (9 H, s, $C(CH_3)_3$), 3.54–3.65 (3 H, m, H2,2',4'), 3.66–3.82 (6 H, m, H1,1',3,5',6',6'), 3.76 (3 H, s, OCH_3), 3.90–3.97 (1 H, m, H3'), 4.02 (1 H, dd, *J* 9.2, 9.2 Hz, H3'), 4.45 (1 H, d, *J* 12.0 Hz, CH_2Ph), 4.51 (1 H, d, *J* 10.9 Hz, CH_2Ph), 4.59–4.64 (3 H, m, CH_2Ph), 4.68 (1 H, d, *J* 12.0 Hz, CH_2Ph), 4.74 (1 H, d, *J* 12.0 Hz, CH_2Ph), 4.83–4.89 (2 H, m, CH_2Ph), 4.93 (1 H, d, *J* 3.4 Hz, H1'), 5.01 (1 H, d, *J* 10.9 Hz, CH_2Ph), 6.79–6.83 (2 H, m, *meta*-*Ar*OMe), 7.21–7.46 (28 H, Ar), 7.65–7.74 (4 H, m, *ortho*-Si*Ph*$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.3 (*C*$(CH_3)_3$), 27.0 (3C, C(*C*$H_3)_3$), 55.3 (OCH_3), 63.6 (C1), 68.4 (C6'), 68.5 (C3), 70.3 (C5'), 72.0, 72.8, 73.6, 75.1, 75.7 (5 C, *C*H_2Ph), 77.7 (C4'), 78.3 (C2), 80.1 (C2'), 82.1 (C3'), 97.5 (C1'), 113.7 (2 C, *meta*-*Ar*OCH_3), 127.6, 127.67, 127.72, 127.74, 127.79, 127.82, 127.87, 127.92, 128.0, 128.38, 128.42, 128.44, 129.3, 129.73, 129.74, 130.9 (30 C, Ar, Ph), 133.4, 133.5 (2 C, *ipso*-Si*Ph*$_2$), 135.66, 135.72 (4 C, *ortho*-Si*Ph*$_2$), 138.0, 138.46, 138.51, 139.0 (4 C, *ipso*-CH_2*Ph*), 159.1 (*para*-*Ar*OCH_3); IR ν 666.9, 696.1, 736.2, 822.7, 909.7, 935.8, 1027.9, 1038.8, 1070.1, 1085.2, 1156.5, 1210.1, 1247.2, 1302.4, 1360.4, 1391.1, 1428.0, 1454.0, 1496.8, 1513.1, 1587.3, 1612.2, 1709.3, 2858.1, 2929.8, 3031.5, 3064.5 cm^{-1}; HRMS (ESI$^+$) calcd for $C_{61}H_{72}NO_9Si$ $[M+NH_4]^+$ *m*/*z* 990.4971, found 990.5028.

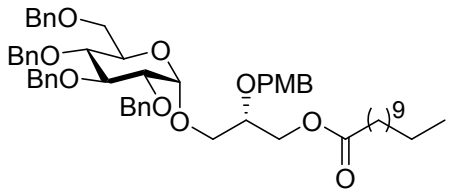
2-*O*-(4-Methoxybenzyl)-sn-glyceryl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (7)



$TBAF{\cdot}H_2O$ (456 mg, 1.63 mmol) was added to a stirred solution of compound **15** (529 mg, 544 mmol) in THF (5.44 mL). The mixture was stirred for 16 h under an atmosphere of nitrogen, then the solvent was

evaporated in vacuo. The residue was dissolved into EtOAc (50 mL) and washed with water (2 × 25 mL) and brine (2 × 25 mL), then dried ($MgSO_4$), filtered and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (117:2:1 ✂ 17:2:1 PhMe/CH_2Cl_2/MeOH) to give compound **7** as a colourless oil (275 mg, 69%): $[\alpha]^{27}_D$ +50.9 (*c* 1.00 in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.41 (1 H, br s, OH), 3.54–3.87(10 H, m, H1,1',2,2',3,3',4',5',6',6'), 3.79 (3 H, s, OCH_3), 4.01 (1 H, dd, *J* 9.3, 9.3 Hz, H3'), 4.49 (1 H, d, *J* 12.1 Hz, CH_2Ph), 4.51 (1 H, d, *J* 11.2 Hz, CH_2Ph), 4.59 (1 H, d, *J* 11.5 Hz, CH_2Ph), 4.62 (1 H, d, *J* 12.1 Hz, CH_2Ph), 4.66–4.69 (2 H, m, CH_2Ph), 4.80 (1 H, d, *J* 11.2 Hz, CH_2Ph), 4.81 (1 H, d, *J* 3.4 Hz, H1'), 4.859 (1 H, d, *J* 10.8 Hz, CH_2Ph), 4.864 (1 H, d, *J* 10.8 Hz, CH_2Ph), 4.99 (1 H, d, *J* 11.0 Hz, CH_2Ph), 6.84–6.90 (2 H, m, *meta*-*Ar*OMe), 7.14–7.19 (2 H, m, *ortho*-*Ar*OMe), 7.23–7.41 (20 H, Ph); ^{13}C NMR (150 MHz, $CDCl_3$) δ 55.3 (OCH_3), 62.6 (C1), 68.1 (C6'), 68.5 (C3), 70.5 (C5'), 71.7 (*C*H_2ArOMe), 73.4, 73.5, 75.1, 75.7 (4 C, *C*H_2Ph), 77.0 (C2), 77.7 (C4'), 80.0 (C2'), 82.1 (C3'), 97.6 (C1'), 113.9 (2 C, *meta*-*Ar*OCH_3), 127.65, 127.74, 127.8, 127.9, 128.00, 128.03, 128.4, 128.5, 129.5, 130.4 (23 C, Ar,Ph), 137.9, 138.25, 138.30, 138.8 (4 C, *ipso*-CH_2*Ph*), 159.3 (*para*-*Ar*OCH_3); IR ν 696.0, 735.0, 821.0, 910.7, 1027.3, 1067.3, 1156.5, 1208.8, 1247.0, 1302.4, 1359.1, 1396.8, 1453.8, 1496.8, 1513.1, 1586.0, 1612.0, 1721.2, 2869.0, 2911.2, 3007.3, 3031.0, 3062.9, 3467.6 cm^{-1}; HRMS (ESI^+) calcd for $C_{45}H_{54}NO_9$ $[M+NH_4]^+$ *m*/*z* 752.3793, found 752.3795.

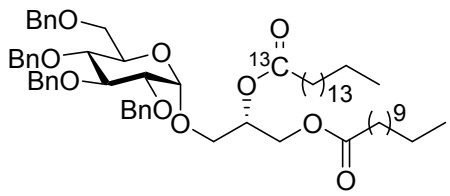
1-*O*-Dodecanoyl-2-*O*-(4-methoxybenzyl)-sn-glyceryl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (16)



Lauroyl chloride (62.9 μL, 272 μmol) was added to a solution of compound **7** (50.0 mg, 68.0 μmol) and DMAP (8.31 mg, 68.0 μmol), in pyridine (6.80 mL), and the mixture was stirred at rt under an atmosphere of nitrogen for 24 h. The solvent was evaporated in vacuo, and the residue was dissolved into Et_2O (100 mL), and then washed sequentially with 0.1 M aq. HCl (5 × 50 mL), water (3 × 20 mL), sat. aq. $NaHCO_3$ (3 × 20 mL), and brine (2 × 20 mL), then dried ($MgSO_4$), filtered, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (45:4:1 ✂ 11:4:1 hexanes/CH_2Cl_2/acetone) to give the monoglyceride **16** as a colourless oil (62.0 mg, 99%): $[\alpha]^{27}_D$ +24.0 (*c* 1.00 in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.88 (3 H, t, *J* 7.0 Hz, CH_2CH_3), 1.17–1.35 (16 H, m, acyl), 1.55–1.64 (2 H, m, acyl-*β*), 2.29 (2 H, t, *J* 7.6 Hz, acyl-*α*), 3.52 (1 H, dd, *J* 10.4, 6.0 Hz, H3), 3.57 (1 H, dd, *J* 9.6, 3.6 Hz, H2), 3.59 (1 H, dd, *J* 10.6, 1.9 Hz, H6'), 3.65 (1 H, dd, *J* 9.8, 9.3 Hz, H4'), 3.69 (1 H, dd, *J* 10.6, 3.6 Hz, H6'), 3.73–3.78 (2 H, m, H3', H5'), 3.76 (3 H, s, OCH_3), 3.79–3.84 (1 H, m, H2), 3.97 (1 H, dd, *J* 9.6, 9.3 Hz, H3'), 4.13 (1 H, dd, *J* 11.7, 5.4 Hz, H1), 4.30 (1 H, dd, *J* 11.7, 4.1 Hz, H1'), 4.45 (1 H, d, *J* 12.1 Hz,

CH_2Ph), 4.46 (1 H, d, *J* 10.7 Hz, CH_2Ph), 4.58–4.62 (3 H, m, CH_2Ph), 4.64 (1 H, d, *J* 12.1 Hz, CH_2Ph), 4.74 (1 H, d, *J* 11.9 Hz, CH_2Ph), 4.82 (1 H, d, *J* 11.2 Hz, CH_2Ph), 4.827 (1 H, d, *J* 10.8 Hz, CH_2Ph), 4.828 (1 H. d, *J* 3.6 Hz, H1'), 4.97 (1 H, d, *J* 10.9 Hz, CH_2Ph), 6.80–6.85 (2 H, m, *meta*-*Ar*OMe), 7.11–7.41 (22 H, Ph); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.3 (CH_2CH_3), 22.8 (CH_2CH_3), 25.0 (acyl-*β*), 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 32.0 ($CH_2CH_2CH_3$), 34.3 (acyl-*α*), 55.4 (OCH_3), 62.6 (C1), 67.9 (C6'), 68.5 (C3), 70.5 (C5'), 71.9 (CH_2ArOMe), 73.1, 73.6 (2 C, CH_2Ph), 75.1 (C2), 75.2, 75.8 (2 C, CH_2Ph), 77.7 (C4'), 80.1 (C2'), 82.0 (C3'), 97.6 (C1'), 113.9 (2 C, *meta*-*Ar*OCH_3), 127.68, 127.76, 127.8, 127.9, 128.00, 128.02, 128.1, 128.46, 128.48, 128.49, 128.52, 129.5, 130.4 (23 C, Ar,Ph), 138.0, 138.38, 138.42, 138.91 (4 C, *ipso*-CH_2*Ph*), 159.3 (*para*-*Ar*OCH_3), 173.7 (sn-1-CO_2); IR ν 697.1, 735.3, 807.0, 911.2, 1028.5, 1041.5, 1070.3, 1085.8, 1157.2, 1208.6, 1247.9, 1302.7, 1359.4, 1454.3, 1497.0, 1513.7, 1586.3, 1612.5, 1736.7, 2853.7, 2923.9, 3031.5, 3063.9 cm^{-1}; HRMS (ESI$^+$) calcd for $C_{57}H_{76}NO_{10}$ $[M+NH_4]^+$ *m*/*z* 934.5464, found 934.5412.

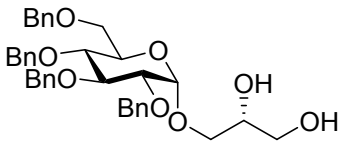
1-*O*-Dodecanoyl-2-*O*-(1-^{13}C-hexadecanoyl)-sn-glyceryl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (9)



CAN (119 mg, 216 μmol) was added to a stirred solution of compound **16** (62.0 mg. 67.6 μmol) in a mixture of CH_3CN (5.48 mL) and water (608 μL) at rt. The mixture was stirred at rt for 2 h, then diluted with EtOAc (20 mL) and water (10 mL) and stirred for 10 min. The organic layer was separated and the aqueous layer extracted with EtOAc (2 × 10 mL). The combined organic phases were washed with water (2 × 10 mL) and brine (2 × 10 mL), then dried ($MgSO_4$), filtered, and the solvent was evaporated in vacuo. ^{i}Pr$_2$NEt (47.1 μL, 270 μmol) was added to a solution of the crude residue (53.9 mg, 67.6 μmol), DMAP (1.24 mg, 10.1 μmol), COMU (116 mg, 270 μmol), and 1-^{13}C-palmitic acid (69.6 mg, 270 μmol) in DMF (4.06 mL) at rt. The mixture was stirred at rt for 24 h, then was diluted with EtOAc (50 mL) and washed with water (3 × 20 mL), brine (2 × 20 mL), then dried ($MgSO_4$), filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography (1% ⩤ 50% EtOAc/toluene) to give the diglyceride **9** as a colourless oil (53.9 mg, 77%): $[\alpha]^{26}_D$ +41.6 (*c* 1.00 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (6 H, t, *J* 6.8 Hz, CH_3), 1.21–1.38 (38 H, m, acyl), 1.55–1.69 (4 H, m, acyl-*β*), 2.27–2.33 (4 H, m, acyl-*α*), 3.57 (1 H, dd, *J* 9.7, 3.5 Hz, H2'), 3.58 (1 H, dd, *J* 10.9, 5.6 Hz, H3), 3.63 (1 H, dd, *J* 10.3, 2.0 Hz, H6'), 3.66 (1 H, dd, *J* 9.2, 9.0 Hz, H4'), 3.73 (1 H, dd, *J* 10.3, 3.4 Hz, H6'), 3.76 (1 H, ddd, *J* 9.2, 3.4, 2.0 Hz, H5'), 3.77 (1 H, dd, *J* 10.9, 5.4 Hz, H3'), 3.96 (1 H, dd, *J* 9.7, 9.0 Hz, H3'), 4.21 (1 H, dd, *J* 12.0, 6.1 Hz, H1), 4.43 (1 H, dd, *J* 12.0, 3.7 Hz, H1'), 4.48 (1 H, d, *J* 12.0 Hz, CH_2Ph), 4.50 (1 H, d, *J* 11.0 Hz, CH_2Ph), 4.61 (1 H, d, *J* 12.0 Hz, CH_2Ph), 4.61 (1 H, d, *J* 12.0

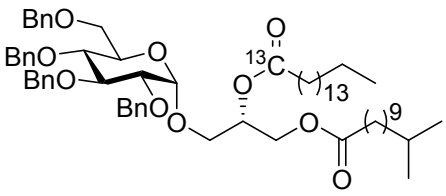
Hz, CH_2Ph), 4.64 (1 H, d, *J* 12.0 Hz, CH_2Ph), 4.77 (1 H, d, *J* 12.0 Hz, CH_2Ph), 4.78 (1 H, d, *J* 3.5 Hz, H1'), 4.82 (1 H, d, *J* 11.0 Hz, CH_2Ph), 4.85 (1 H, d, *J* 10.8 Hz, CH_2Ph), 4.97 (1 H, d, *J* 10.8 Hz, CH_2Ph), 5.27 (1 H, dddd, *J* 6.1, 5.6, 5.4, 3.7 Hz, H2), 7.13–7.39 (22 H, Ph); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.3 (2 C, CH_2CH_3), 22.8 (2 C, CH_2CH_3), 25.0 (2 C, acyl-*β*), 29.25, 29.29, 29.44, 29.48, 29.5, 29.63, 29.65, 29.76, 29.79, 29.82, 29.84 (16 C), 32.0, 32.1 (2 C, $CH_2CH_2CH_3$), 34.2, 34.6 (2 C, acyl-*α*), 62.6 (C1), 66.5 (C3), 68.5 (C6'), 70.0 (C2), 70.7 (C5'), 73.3, 73.6, 75.2, 75.8 (4 C, CH_2Ph), 77.6 (C4'), 80.2 (C2'), 81.9 (C3'), 97.9 (C1'), 127.69, 127.78, 127.83, 127.95, 127.98, 128.0, 128.1, 128.47, 128.50, 128.59 (20 C, Ph), 138.0, 138.40, 138.43, 138.93 (4 C, *ipso*-CH_2*Ph*), 173.1 (sn-2-CO_2), 173.5 (sn-1-CO_2); IR ν 696.7, 733.9, 800.5, 1027.9, 1071.6, 1153.7, 1260.5, 1361.0, 1454.6, 1497.0, 1698.7, 1741.9, 2853.4, 2923.1 cm^{-1}; HRMS (ESI$^+$) calcd for $C_{64}{}^{13}CH_{98}NO_{10}$ $[M+NH_4]^+$ *m*/*z* 1053.7219, found 1053.7223.

sn-Glycer-3-yl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (2)



The diol was prepared by addition of CAN (554 mg, 1.01 mmol) to a solution of compound **7** (232 mg, 316 μmol) in CH_3CN (25.6 mL) and water (2.84 mL), and following the same procedure employed above. The residue was purified by flash chromatography (17:2:1 ✂ 7:2:1 PhMe/CH_2Cl_2/MeOH) to give compound **2** as a colourless oil (166 mg, 86%):$[\alpha]^{26}_D$ +50.9 (*c* 1.00 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.18, 2.73 (2 H, br s, 2 × OH), 3.45 (1 H, dd, *J* 10.4, 6.9 Hz, H3), 3.56–3.72 (5 H, m, H1,1',4',6',6'), 3.59 (1 H, dd, *J* 3.6, 9.8 Hz, H2'), 3.79 (1 H, dd, *J* 10.4, 3.5 Hz, H3'), 3.81–3.90 (2 H, m, H2,5'), 3.98 (1 H, dd, *J* 9.3, 9.3 Hz, H3'), 4.48 (1 H, d, *J* 12.0 Hz, CH_2Ph), 4.50 (1 H, d, *J* 10.7 Hz, CH_2Ph), 4.60 (1 H, d, *J* 12.0 Hz, CH_2Ph), 4.65 (1 H, d, *J* 11.9 Hz, CH_2Ph), 4.75 (1 H, d, *J* 3.6 Hz, H1'), 4.80 (1 H, d, *J* 11.9 Hz, CH_2Ph), 4.84 (1 H, d, *J* 10.7 Hz, CH_2Ph), 4.85 (1 H, d, *J* 10.7 Hz, CH_2Ph), 4.96 (1 H, d, *J* 10.7 Hz, CH_2Ph), 7.14–7.39 (20 H, m, Ph); ^{13}C NMR (150 MHz, $CDCl_3$) δ 63.7 (C6'), 68.5 (C1), 70.4 (C2), 70.6 (C5'), 71.4 (C3), 73.6, 73.7, 75.1, 75.8 (4 C, CH_2Ph), 77.7 (C4'), 79.9 (C2'), 82.1 (C3'), 98.4 (C1'), 127.7, 127.8, 127.86, 127.94, 127.97, 128.06, 128.2, 128.3, 128.46, 128.47, 128.50, 128.64 (20 C, Ph), 137.8, 137.9, 138.1, 138.7 (4 C, *ipso*-CH_2*Ph*); IR ν 696.5, 735.5, 802.8, 914.3, 1027.3, 1066.9, 1156.2, 1208.6, 1261.1, 1360.3, 1453.8, 1496.8, 1722.4, 2918.9, 3030.7, 3442.2 cm^{-1}; HRMS (ESI$^+$) calcd for $C_{37}H_{42}NaO_8$ $[M+Na]^+$ *m*/*z* 637.2772, found 637.2781.

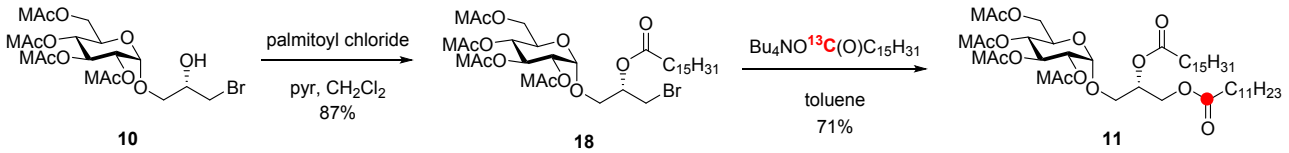
1-*O*-(10-Methyldodecanoyl)-2-*O*-(1-^{13}C-hexadecanoyl)-sn-glyceryl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (5)



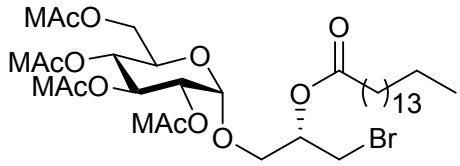
DCC (14.6 mg, 70.9 μmol) was added to a solution of compound **2** (41.5 mg, 67.5 μmol), DMAP (1.00 mg, 8.17 μmol) and 10-methyldodecanoic acid[5] (15.2 mg, 70.9 μmol) in CH_2Cl_2 (645 μL) at 0 °C. The mixture was stirred under nitrogen at 0 °C for 12 h, then the solvent was removed under a nitrogen needle and the residue was purified by flash chromatography (10% ⩤ 40% EtOAc/toluene). The chromatography was performed with chilled solvents and silica gel, with an elution time of ~8 min. The total time required to purify the intermediate monoglyceride was <15 min, and at no time did the temperature ever exceed 10 °C. Solvent evaporation at rt afforded the intermediate monoglyceride as a colourless oil, which was used immediately without further analysis. DCC (16.7 mg, 81.0 μmol) was added to a solution of the intermediate monoglyceride (53.8 mg, 67.5 μmol), DMAP (1.00 mg, 8.17 μmol) and 1-^{13}C-palmitic acid (20.8 mg, 81.0 μmol) in CH_2Cl_2 (675 μL) at 0 °C. The mixture was stirred under nitrogen at rt for 24 h, then the solvent was removed in vacuo. The residue was diluted with EtOAc (20 mL) and washed with water (3 × 10 mL), brine (2 × 10 mL), then dried ($MgSO_4$), filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography (1% ⩤ 50% EtOAc/toluene) to give the diglyceride **5** as a white waxy residue* (55.3 mg, 79%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83–0.93 (9 H, m, 3 × CH_3), 1.09–1.19 (2 H, m, C*H*$_2$CH(CH$_3$)$_2$), 1.20–1.35 (36 H, m, acyl), 1.47–1.65 (5 H, m, C*H*(CH$_3$)$_2$,acyl-*β*), 2.18–2.23 (2 H, m, *sn*-2 acyl-*α*), 3.55 (1 H, dd, *J* 9.7, 3.5 Hz, H2'), 3.56 (1 H, dd, *J* 10.9, 5.6 Hz, H3), 3.61 (1 H, dd, *J* 10.3, 2.0 Hz, H6'), 3.64 (1 H, dd, *J* 9.2, 9.0 Hz, H4'), 3.71 (1 H, dd, *J* 10.3, 3.4 Hz, H6'), 3.74 (1 H, ddd, *J* 9.2, 3.4, 2.0 Hz, H5'), 3.75 (1 H, dd, *J* 10.9, 5.4 Hz, H3'), 3.94 (1 H, dd, *J* 9.7, 9.0 Hz, H3'), 4.20 (1 H, dd, *J* 12.0, 6.1 Hz, H1), 4.42 (1 H, dd, *J* 12.0, 3.7 Hz, H1'), 4.47 (1 H, d, *J* 12.0 Hz, C*H*$_2$Ph), 4.50 (1 H, d, *J* 11.0 Hz, C*H*$_2$Ph), 4.61 (1 H, d, *J* 12.0 Hz, C*H*$_2$Ph), 4.64 (1 H, d, *J* 12.0 Hz, C*H*$_2$Ph), 4.77 (1 H, d, *J* 12.0 Hz, C*H*$_2$Ph), 4.78 (1 H, d, *J* 3.5 Hz, H1'), 4.82 (1 H, d, *J* 11.0 Hz, C*H*$_2$Ph), 4.85 (1 H, d, *J* 10.8 Hz, C*H*$_2$Ph), 4.97 (1 H, d, *J* 10.8 Hz, C*H*$_2$Ph), 5.27 (1 H, dddd, *J* 6.1, 5.6, 5.4, 3.7 Hz, H2), 7.13–7.39 (22 H, Ph); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.3 (CH_3), 22.80 (2 C, CH(*C*H$_3$)$_2$), 22.82 (*C*H$_2$CH$_3$), 25.0 (2 C, acyl-*β*), 27.6 (*C*H$_2$CH$_2$CH(CH$_3$)$_2$), 28.1 (2 C, *C*H(CH$_3$)$_2$), 29.26, 29.29, 29.4, 29.50, 29.54, 29.61, 29.65, 29.75, 29.79, 29.82, 29.85 (12 C), 30.1 (2 C,*C*H$_2$CH$_2$CH$_3$, *C*H$_2$CH$_2$CH$_2$CH(CH$_3$)$_2$), 34.3 (2 C, acyl-*α*), 39.2 (2 C, *C*H$_2$CH(CH$_3$)$_2$), 62.6 (C1), 66.5 (C3), 68.5 (C6'), 70.0 (C2), 70.7 (C5'), 73.2, 73.6, 75.2, 75.8 (4 C, *C*H$_2$Ph), 77.6 (C4'), 80.2 (C2'), 82.0 (C3'), 97.9 (C1'), 127.69, 127.78, 127.83, 127.91, 127.95, 127.98, 128.0, 128.1, 128.47, 128.50, 128.59 (20 C, Ph), 138.0, 138.40, 138.44, 138.94 (4 C, *ipso*-

* The product was contaminated with *N*,*N*-dicyclohexylurea, however this did not interfere with the analysis.

CH_2Ph), 173.1 (sn-2-CO_2), 173.5 (sn-1-CO_2); HRMS (ESI^+) calcd for $C_{65}{}^{13}CH_{100}NO_{10}$ $[M+NH_4]^+$ *m*/*z* 1067.7375, found 1067.7433.

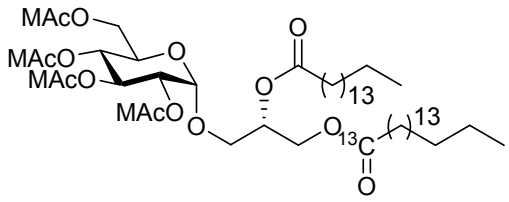


(2'*R*)-3'-Bromo-2'-palmitoyloxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (18)



Palmitoyl chloride (0.103 ml, 0.337 mmol) was added to a stirred solution of (2'*R*)-3'-bromo-2'-hydroxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside **10**[6] (0.102 g, 0.168 mmol) in dry CH_2Cl_2 (4 mL) and pyridine (0.270 mL, 3.37 mmol) at 0 °C. The reaction mixture was allowed to warm to rt and stirring was continued overnight. The reaction mixture was diluted with CH_2Cl_2, washed sequentially with water, sat. aq. $CuSO_4$ and sat. aq. $NaHCO_3$. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash chromatography of the residue (EtOAc/pet. spirits 1:1) afforded compound **18** as a colourless oil (0.123 g, 87%), $[\alpha]_D^{25}$ = +66.6 (c 0.68, $CHCl_3$): ^1H NMR (400 MHz, $CDCl_3$) δ 0.87 (3 H, t, *J* 7 Hz, CH_2CH_3), 1.24–1.31 (24 H, m, alkyl), 1.59–1.65 (2 H, m, α-CH_2) 2.33–2.37 (2 H, m, β-CH_2), 3.39, 3.40, 3.42, 3.45 (4 × 3 H, 4s, CH_3OCH_2), 3.52 (1 H, dd, $J_{1',2'}$ 4.6, $J_{1',1'}$ 10.7 Hz, H1'), 3.60 (1 H, dd, $J_{1',2'}$ 6.3, $J_{1',1'}$ 10.6 Hz, H1'), 3.72 (1 H, dd, $J_{2',3'}$ 4.9, $J_{3',3'}$ 10.8 Hz, H3'), 3.89 (1 H, dd, $J_{2',3'}$ 4.7, $J_{3',3'}$ 10.8 Hz, H3'), 3.95, 4.00, 4.08 (3 × 2 H, 3s, CH_3OCH_2), 4.02 (2 H, m, CH_3OCH_2), 4.07–4.11 (1 H, m, H5), 4.19 (1 H, dd, $J_{5,6}$ 2.2, $J_{6,6}$ 12.4 Hz, H6), 4.38 (1 H, dd, $J_{5,6}$ 4.2, $J_{6,6}$ 12.4 Hz, H6), 4.99 (1 H, dd, $J_{1,2}$ 3.8, $J_{2,3}$ 10.1 Hz, H2), 5.09–5.17 (3 H, m, H1,2',4), 5.53 (1 H, t, $J_{2,3}$ 9.7, $J_{3,4}$ 9.7 Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.0, 29.3, 29.4, 29.4, 29.5, 29.6, 29.75, 29.79, 29.8, 30.0, 32.1, 34.3 (fatty acyl), 59.5, 59.57, 59.59, 59.60, 61.8, 67.41, 67.45, 68.5, 69.42, 69.44, 69.5, 69.6, 70.5, 70.6, 70.7, 96.3 (C1), 169.3, 169.6, 169.7, 170.1 (4 C, $MeOCH_2C$=O), 172.9 (sn-2-CO_2); HRMS (ESI^+) calcd for $C_{37}H_{63}BrO_{16}NH_4$ $[M+NH_4]^+$ *m*/*z* 860.3638, found 860.3646.

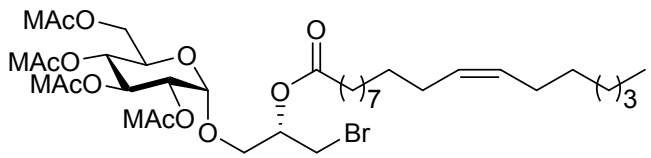
1′-*O*-(1''-^{13}C-Palmitoyl)-2′-*O*-palmitoyl-*sn*-glyceryl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (12)



Tetrabutylammonium hydroxide solution in H_2O (1.5 M, 0.089 mL, 0.137 mmol) was added to a suspension of 1-^{13}C-palmitic acid (0.039 g, 0.152 mmol) in H_2O. The resulting mixture was vigorously stirred at rt overnight. The solvent was evaporated and the crude residue was co-evaporated with toluene several times to give the tetrabutylammonium salt of palmitic acid acid. A mixture of tetrabutylammonium palmitate (0.069 g, 0.137 mmol) and compound **18** (0.058 g, 0.069 mmol) in toluene (2 ml) was heated to 85 °C and stirred vigorously for 25 min. The solvents were evaporated under high vacuum. Flash chromatography of the residue (EtOAc/pet spirits 2:3) afforded compound **12** as a colourless oil (0.050 g, 71%), $[\alpha]_D^{25}$ = +57.8 (c 0.65 in $CHCl_3$): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81–0.94 (6 H, m, CH_2CH_3), 1.25–1.42 (48 H, m, alkyl), 1.48–1.66 (4 H, m, α-CH_2), 2.23–2.35 (4 H, m, β-CH_2), 3.38, 3.39, 3.41, 3.43 (4 × 3 H, 4s, CH_3O), 3.62 (1 H, dd, $J_{2',3'}$ 5.5, $J_{3',3'}$ 11.2 Hz, H3'), 3.79 (1 H, dd, $J_{2',3'}$ 4.6, $J_{3',3'}$ 11.2 Hz, H3'), 3.94, 3.98, 4.07 (3 × 2 H, 3s, CH_3OCH_2), 4.04 (2 H, m, CH_3OCH_2), 4.06–4.08 (1 H, m, H5), 4.12 (1 H, ddd, $J_{1',1'}$ 11.9, $J_{1',2'}$ 6.0, $J_{1',3'}$ 3.2 Hz, H1'), 4.17 (1 H, dd, $J_{5,6}$ 2.3, $J_{6,6}$ 12.4 Hz, H6), 4.32 (1 H, ddd, $J_{1',1'}$ 11.8, $J_{1',2'}$ 4.2, $J_{1',3'}$ 2.8 Hz, H1'), 4.37 (1 H, dd, $J_{5,6}$ 4.1, $J_{6,6}$ 12.4 Hz, H6), 4.96 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 12.4 Hz, H2), 5.08–5.23 (3 H, m, H1,2',4), 5.5 (1 H, t, $J_{2,3}$ 9.7, $J_{3,4}$ 9.7 Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.0, 29.2, 29.40, 29.42, 29.5, 29.61, 29.63, 29.75, 29.77, 29.78, 29.79, 29.81, 32.0, 32.0, 34.3, 34.4, 59.4, 59.51, 59.53, 59.54, 61.7, 62.1, 67.0, 67.4, 68.5, 69.32, 69.39, 69.40, 69.6, 70.5, 70.7, 96.2 (C1), 169.3, 169.6, 169.7, 170.1 (4 C, $MeOCH_2C$=O), 172.9 (sn-2-CO_2), 173.2 (sn-1-CO_2); HRMS (ESI$^+$) calcd for $C_{52}{}^{13}CH_{94}O_{18}NH_4$ $[M+NH_4]^+$ 1037.6812, found 1037.6778.

Total synthesis of *Streptococcus pneumoniae* Glc-DAG-s2

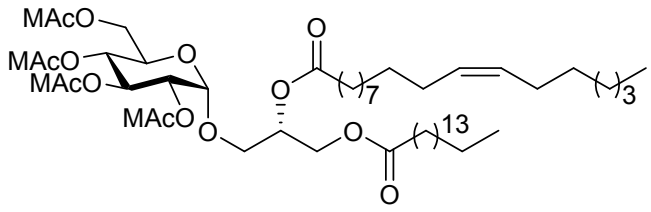
(2'*R*)-3'-Bromo-2'-vaccenoyloxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (13)



Vaccenoyl chloride (0.054 ml, 0.162 mmol) was added to a stirred solution of (2'*R*)-3'-bromo-2'-hydroxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside **10**[6] (0.049 g, 0.081 mmol) in dry CH_2Cl_2 (2 mL) and pyridine (0.130 mL, 1.62 mmol) at 0 °C. The reaction mixture was allowed to warm to rt and stirring was continued overnight. The reaction mixture was diluted with CH_2Cl_2, washed sequentially with water, sat. aq. $CuSO_4$ and sat. aq. $NaHCO_3$. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash chromatography of the residue (EtOAc/pet. spirits 1:1) afforded **13** as

colourless oil (0.061 g, 87 %), $[\alpha]_D^{25}$ = +62.3 (c 1.29 in $CHCl_3$): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (3 H, t, *J* 8 Hz, CH_2CH_3), 1.24–1.31 (20 H, m, alkyl), 1.59–1.65 (2 H, m, α-CH_2) 1.98–2.04 (4 H, m, $H_2CCH{=}CHCH_2$), 2.33–2.37 (2 H, m, β-CH_2), 3.39, 3.41, 3.43, 3.45 (4 × 3 H, 4s, CH_3OCH_2), 3.52 (1 H, dd, $J_{1',2'}$ 4.6, $J_{1',1'}$ 10.7 Hz, H1'), 3.60 (1 H, dd, $J_{1',2'}$ 6.2, $J_{1',1'}$ 10.6 Hz, H1'), 3.72 (1 H, dd, $J_{2',3'}$ 4.9, $J_{3',3'}$ 10.9 Hz, H3'), 3.89 (1 H, dd, $J_{2',3'}$ 4.7, $J_{3',3'}$ 10.8 Hz, H3'), 3.95, 4.00, 4.08 (3 × 2 H, 3s, CH_3OCH_2), 4.02 (2 H, m, CH_3OCH_2), 4.07–4.11 (1 H, m, H5), 4.19 (1 H, dd, $J_{5,6}$ 2.3, $J_{6,6}$ 12.3 Hz, H6), 4.38 (1 H, dd, $J_{5,6}$ 4.3, $J_{6,6}$ 12.4 Hz, H6), 4.99 (1 H, dd, $J_{1,2}$ 3.8, $J_{2,3}$ 10.1 Hz, H2), 5.09–5.17 (3 H, m, H1,2',4), 5.30–5.38 (2 H, m, HC=CH), 5.53 (1 H, t, $J_{2,3}$ 9.6, $J_{3,4}$ 9.8 Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.0, 27.3, 27.4, 29.1, 29.2, 29.3, 29.4, 29.58, 29.64, 29.8, 29.9, 30.0, 31.9, 34.3 (fatty acyl), 59.48, 59.56, 59.59, 59.60, 61.8, 67.41, 67.45, 68.5, 69.42, 69.43, 69.47, 69.6, 70.4, 70.5, 70.7, 96.3 (C1), 129.9, 130.1 (2 C, HC=CH), 169.3, 169.6, 169.7, 170.1 (4 C, $MeOCH_2\mathbf{C}{=}O$), 172.9 (sn-2-CO_2); HRMS (ESI^+) calcd for $C_{39}H_{65}BrO_{16}NH_4$ $[M+NH_4]^+$ *m*/*z* 886.3794, found 886.3830.

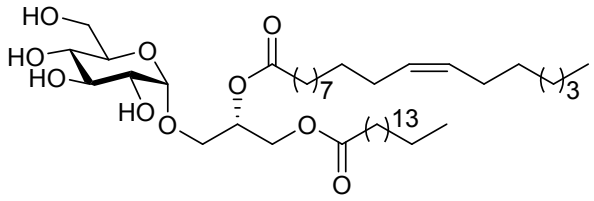
1'-*O*-Palmitoyl-2'-*O*-vaccenoyl-*sn*-glyceryl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (14)



Tetrabutylammonium hydroxide solution in H_2O (1.5 M, 0.219 mL, 0.147 mmol) was added to a suspension of palmitic acid (0.042 g, 0.163 mmol) in H_2O (1.5 mL). The resulting mixture was vigorously stirred at rt overnight. The solvent was evaporated and the crude residue was co-evaporated with toluene several times to give the tetrabutylammonium salt of palmitic acid. A mixture of tetrabutylammonium palmitate (0.073 g, 0.147 mmol) and compound **13** (0.064 g, 0.074 mmol) in toluene (2 mL) was heated to 85 °C and stirred vigorously for 25 min. The solvents were evaporated under high vacuum. Flash chromatography of the residue (EtOAc/pet spirits 2:3) afforded compound **14** as a colourless oil (0.040 g, 52%), $[\alpha]_D^{25}$ = +46.8 (c 1.82 in $CHCl_3$): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81–0.94 (6 H, m, CH_2CH_3), 1.25–1.42 (46 H, m, alkyl), 1.54–1.67 (4 H, m, α-CH_2), 1.97–2.03 (4 H, m, $H_2CCH{=}CHCH_2$), 2.27–2.35 (4 H, m, β-CH_2), 3.39, 3.40, 3.42, 3.44 (4 × 3 H, 4s, CH_3O), 3.62 (1 H, dd, $J_{2',3'}$ 5.5, $J_{3',3'}$ 11.2 Hz, H3'), 3.80 (1 H, dd, $J_{2',3'}$ 4.6, $J_{3',3'}$ 11.2 Hz, H3'), 3.94, 3.98, 4.07 (3 × 2 H, 3s, CH_3OCH_2), 4.00–4.04 (2 H, m, CH_3OCH_2), 4.04–4.08 (1 H, m, H5), 4.13 (1 H, dd, $J_{1',2'}$ 6.0, $J_{1',1'}$ 11.9 Hz, H1'), 4.18 (1 H, dd, $J_{6,5}$ 2.3, $J_{6,6}$ 12.4 Hz, H6), 4.33 (1 H, dd, $J_{1',2'}$ 4.3, $J_{1',1'}$ 11.9 Hz, H1'), 4.38 (1 H, dd, $J_{5,6}$ 4.1, $J_{6,6}$ 12.4 Hz, H6), 4.97 (1 H, dd, $J_{1,2}$ 3.8, $J_{2,3}$ 10.1 Hz, H2), 5.09–5.23 (3 H, m, H1,2',4), 5.52 (1 H, t, $J_{2,3}$ 9.7, $J_{3,4}$ 9.7 Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 14.3, 22.80, 22.83, 25.01, 25.04, 27.4, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 29.80, 29.81, 29.84, 29.87, 29.92, 31.9, 32.1, 34.2, 34.4 (fatty acyl), 59.48, 59.55, 59.56, 59.58, 61.7, 62.1, 67.1, 67.4, 68.5, 69.4, 69.5, 69.6,

69.8, 70.6, 70.7, 96.3 (C1), 129.9, 130.1 (2C, HC=CH), 169.3, 169.6, 169.7, 170.1 (4 C, $MeOCH_2C$=O), 173.1 (sn-2-CO_2), 173.4 (sn-1-CO_2); HRMS (ESI^+) calcd for $C_{55}H_{96}O_{18}NH_4$ $[M+NH_4]^+$ *m/z* 1062.6935, found 1062.6955.

1'-*O*-Palmitoyl-2'-*O*-vaccenoyl-*sn*-glyceryl α-D-glucopyranoside (1; Glc-DAG-s2)



A solution of *t*-butylamine (0.180 ml, 1.7 mmol) and compound **14** (0.037 g, 0.035 mmol) in $CHCl_3$ (0.2 mL) and MeOH (0.8 mL) was stirred at 0 °C for 10 min and then at 10 °C for 1 h. The solvents were evaporated under high vacuum without heating. Flash chromatography of the residue (MeOH/$CHCl_3$ 5:95) afforded compound **1** (Glc-DAG-s2) as a white semisolid (0.015 g, 57%), $[\alpha]_D^{25}$ = +50.0 (c 0.75 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (6 H, m, CH_2CH_3), 1.10–1.42 (46 H, m, alkyl), 1.52–1.66 (4 H, m, α-CH_2) 1.98–2.06 (4 H, m, H_2CCH=$CHCH_2$), 2.13 (1H, br. s, OH), 2.26–2.36 (4 H, m, β-CH_2), 2.98 (1H, br. s, OH), 3.48–3.65 (4 H, m, H2,4,5,3'), 3.70–3.91 (4 H, m, H3,6,6,3'), 4.15 (1 H, dd, $J_{1',2'}$ 6.1, $J_{1',1'}$ 12 Hz, H1'), 4.22 (1 H, br. s, OH), 4.39 (1 H, dd, $J_{1',2'}$ 3.3, $J_{1',1'}$ 12 Hz, H1'), 4.47 (1H, br. s, OH), 4.85 (1 H, s, H1), 5.20–5.29 (1 H, m, H2'), 5.30–5.41 (2 H, m, HC=CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.64, 22.67, 24.9, 27.2, 29.0, 29.2, 29.3, 29.50, 29.54, 29.6, 29.71, 29.78, 31.8, 31.9, 34.1 (fatty acyl), 34.26, 61.6, 62.5, 66.2, 69.8, 71.8, 72.0, 74.2, 99.1 (C1), 129.8, 129.9 (2C, HC=CH), 173.3 (sn-2-CO_2), 173.8 (sn-1-CO_2); HRMS (ESI^+) calcd for $C_{43}H_{80}O_{10}NH_4$ $[M+NH_4]^+$ *m/z* 774.6089, found 774.6094.

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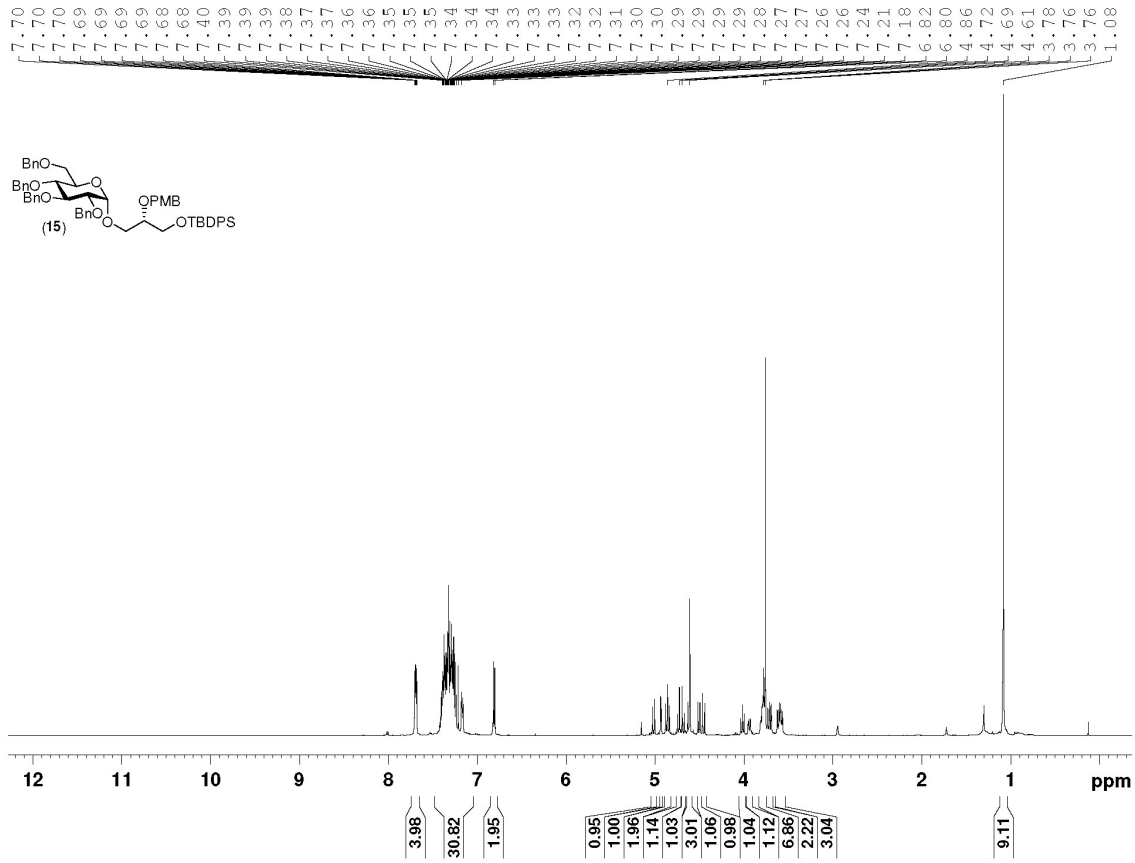
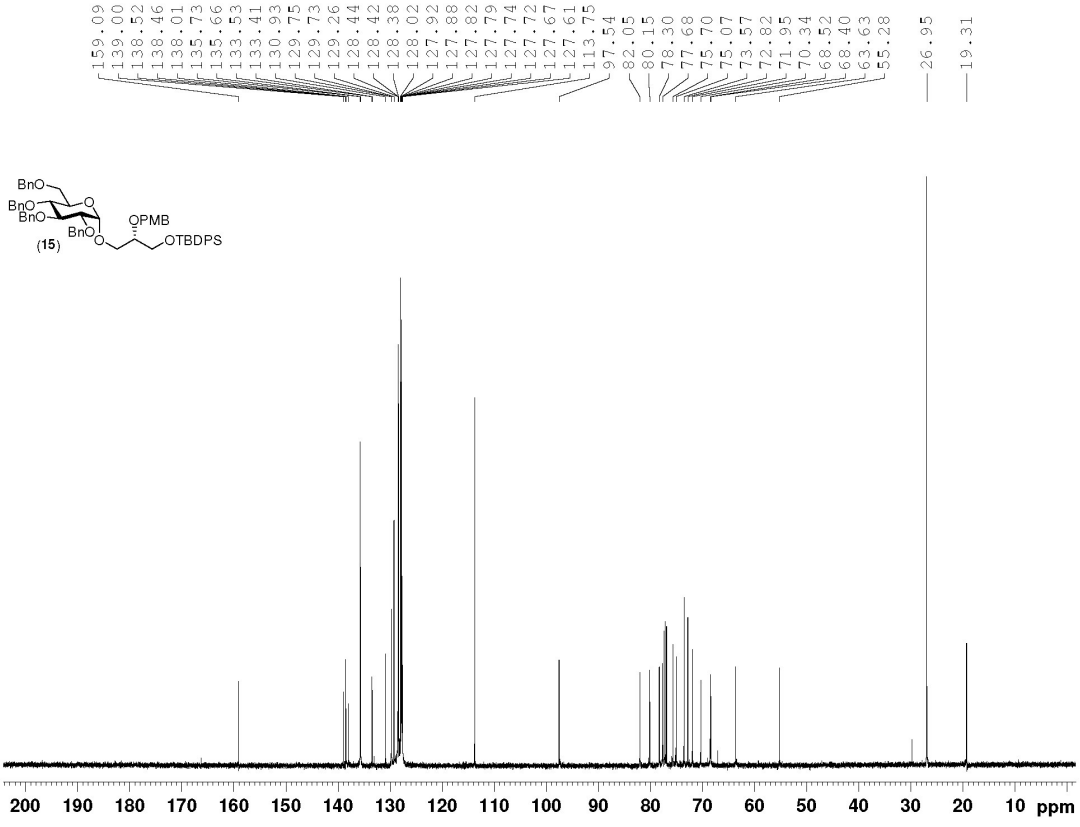
NMR data

1-O-*tert*-Butyldiphenylsilyl-2-*O*-(4-methoxybenzyl)-sn-glyceryl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (15)

^{1}H NMR

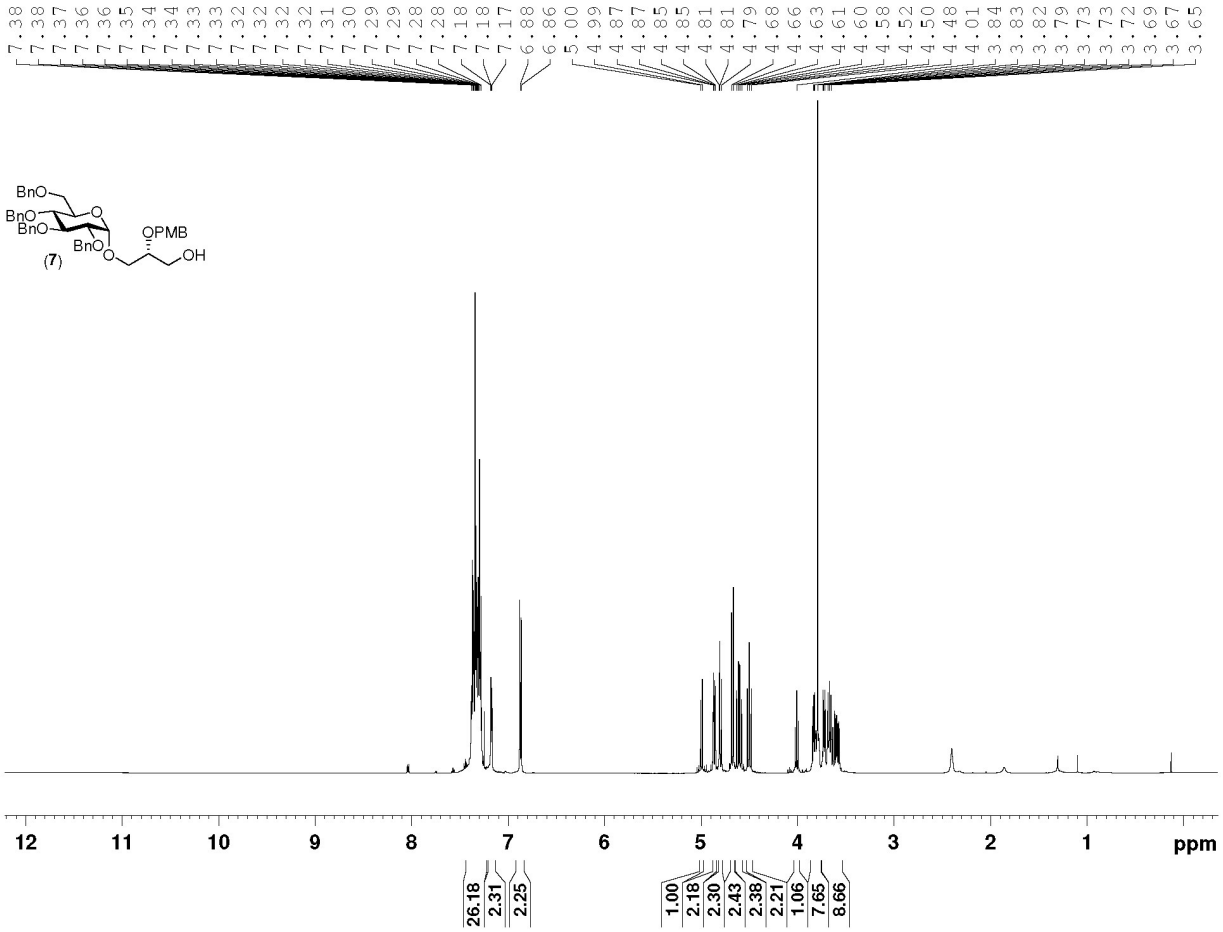


^{13}C NMR

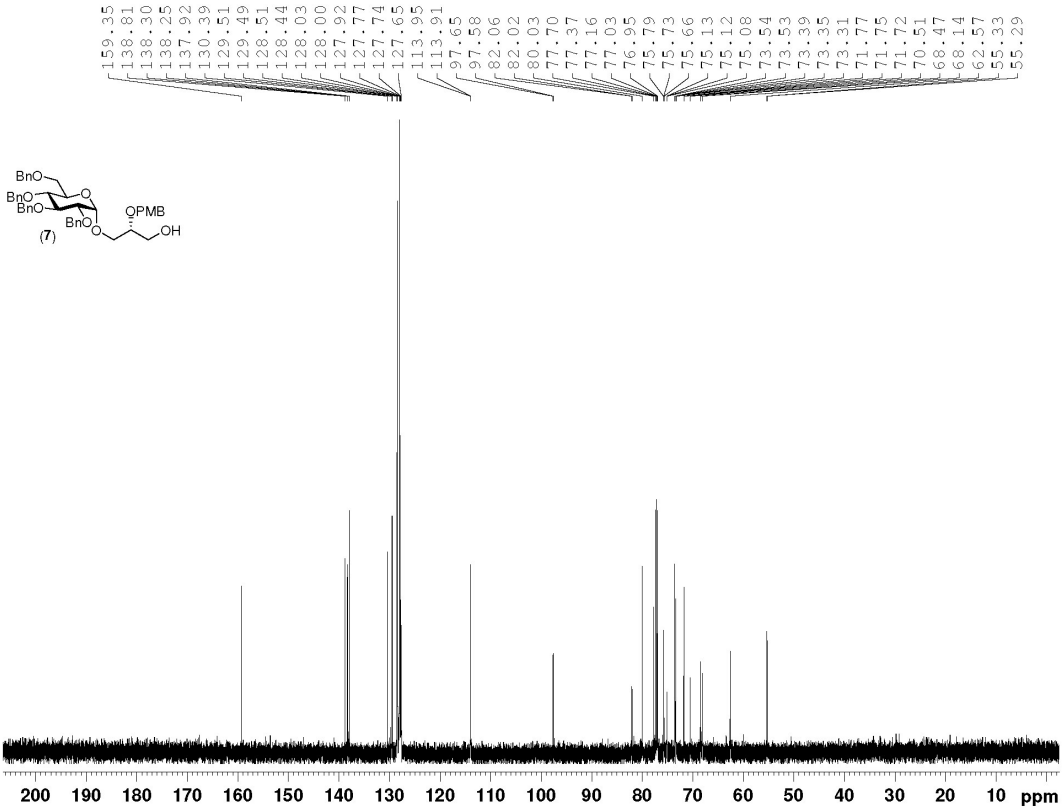


2-*O*-(4-Methoxybenzyl)-sn-glyceryl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (7)

^{1}H NMR

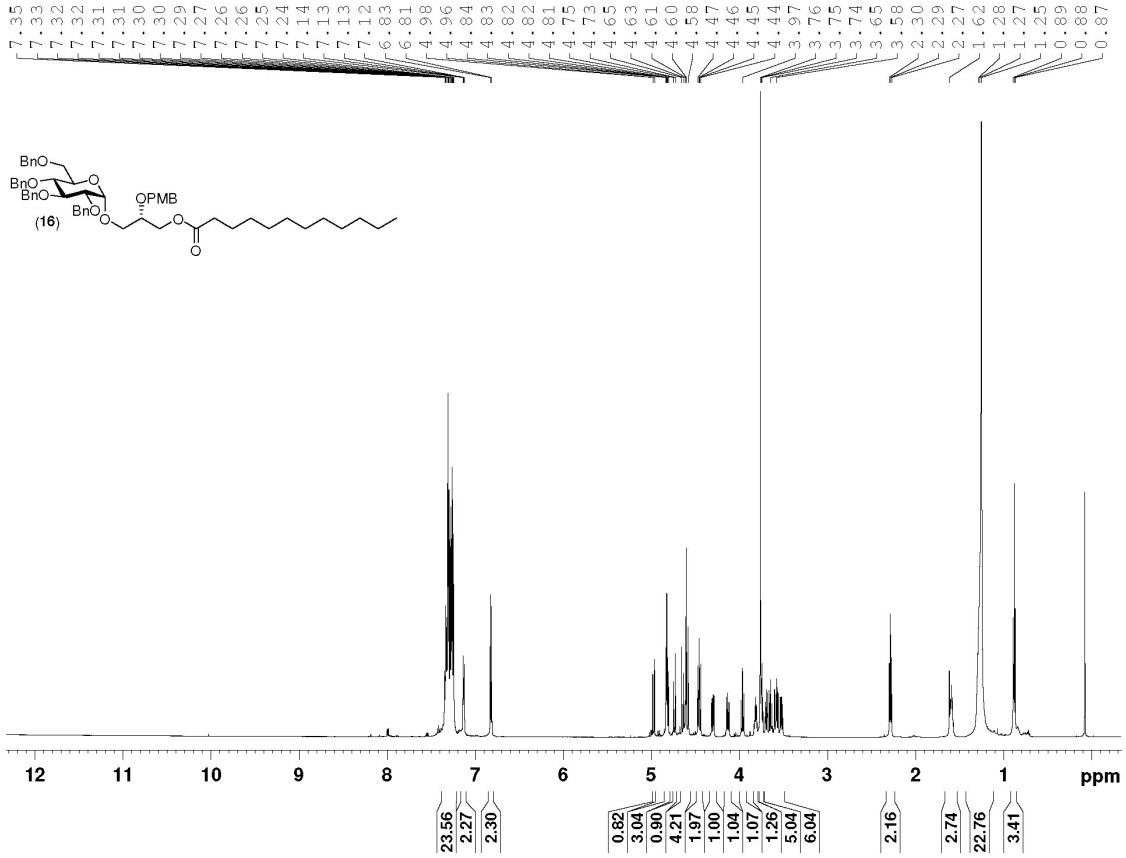


^{13}C NMR

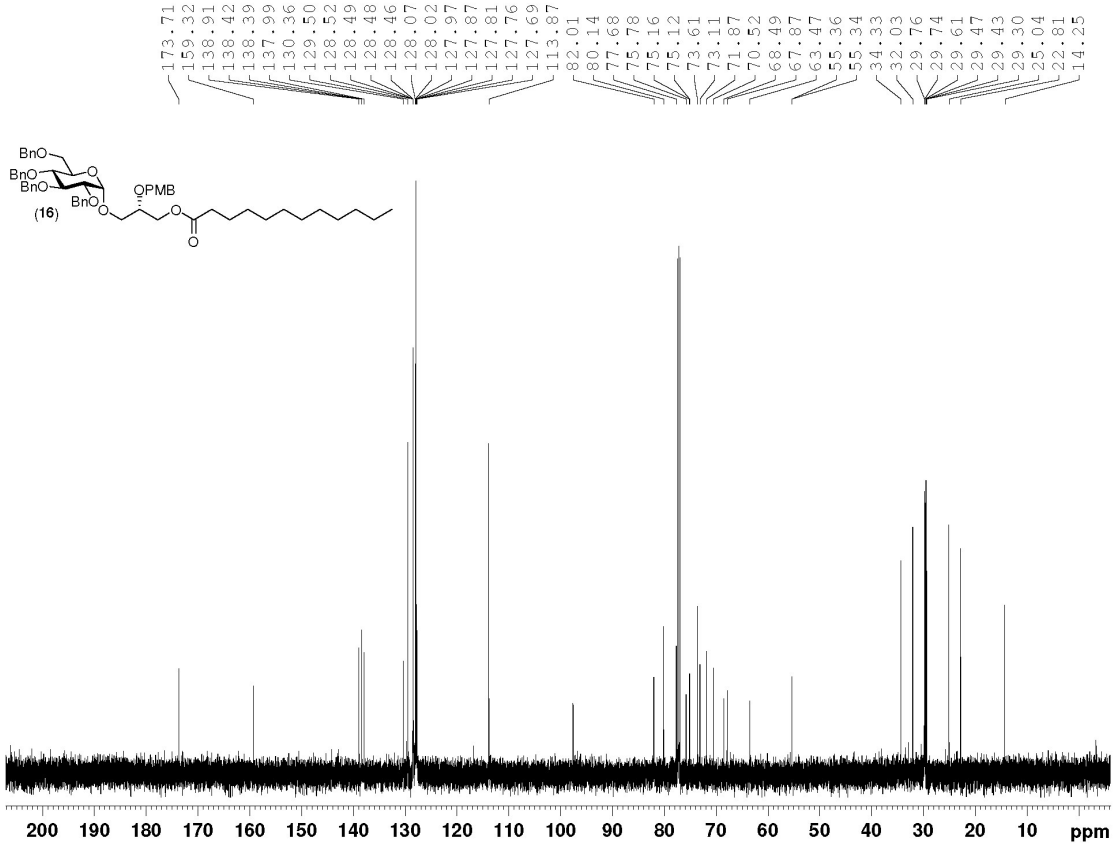


1-*O*-Dodecanoyl-2-*O*-(4-methoxybenzyl)-sn-glyceryl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (16)

^{1}H NMR

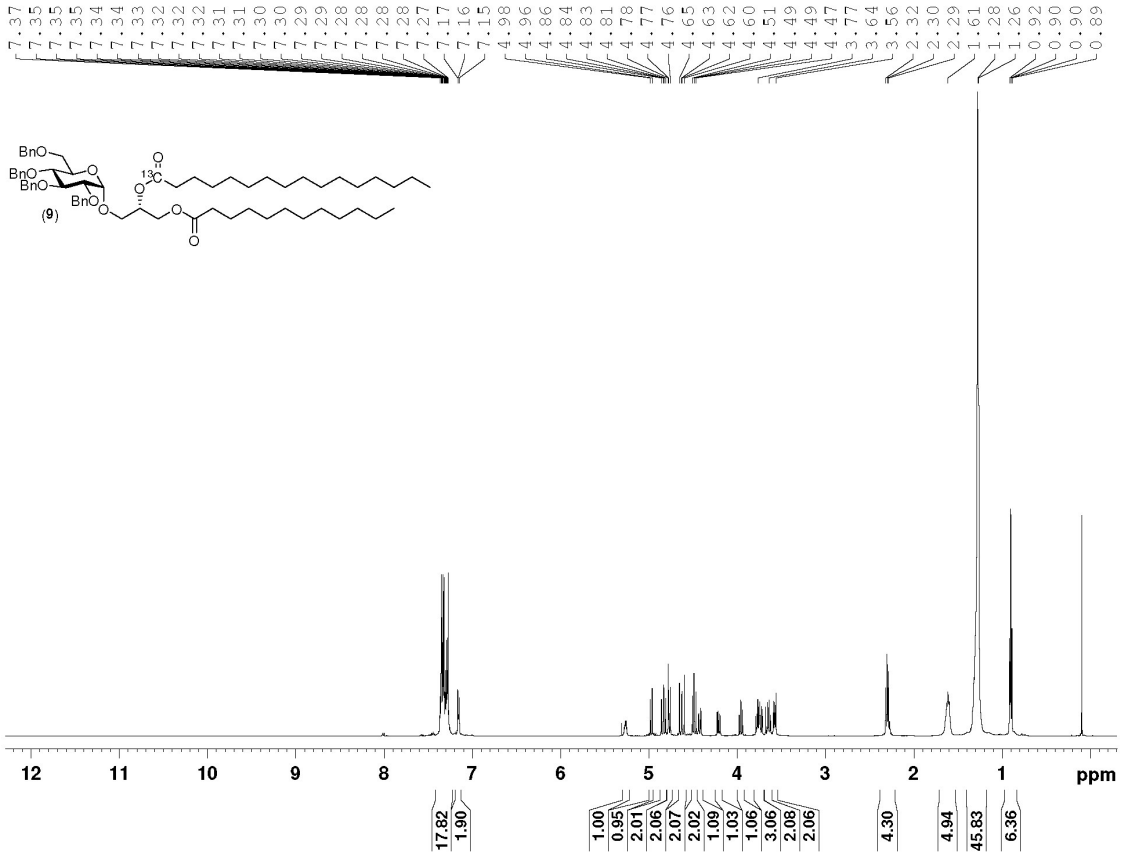


^{13}C NMR

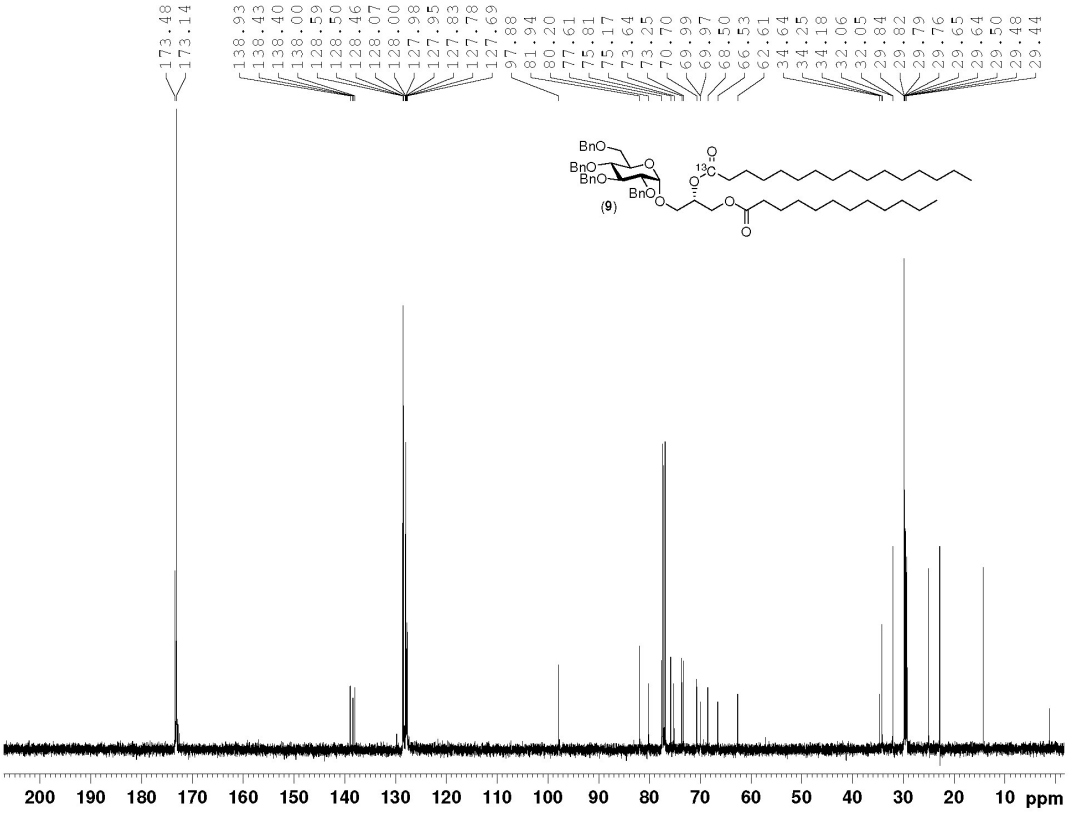


1-*O*-Dodecanoyl-2-*O*-(1-^{13}C-hexadecanoyl)-sn-glyceryl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (9)

^{1}H NMR

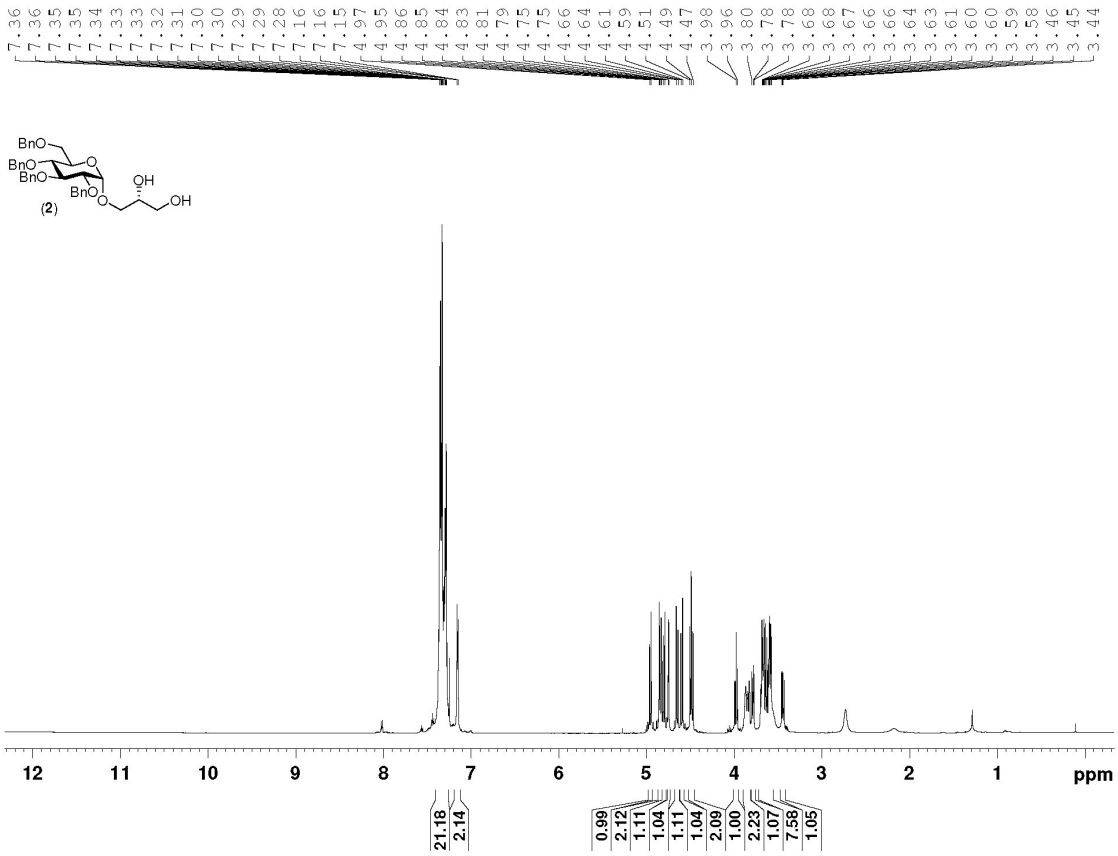


^{13}C NMR

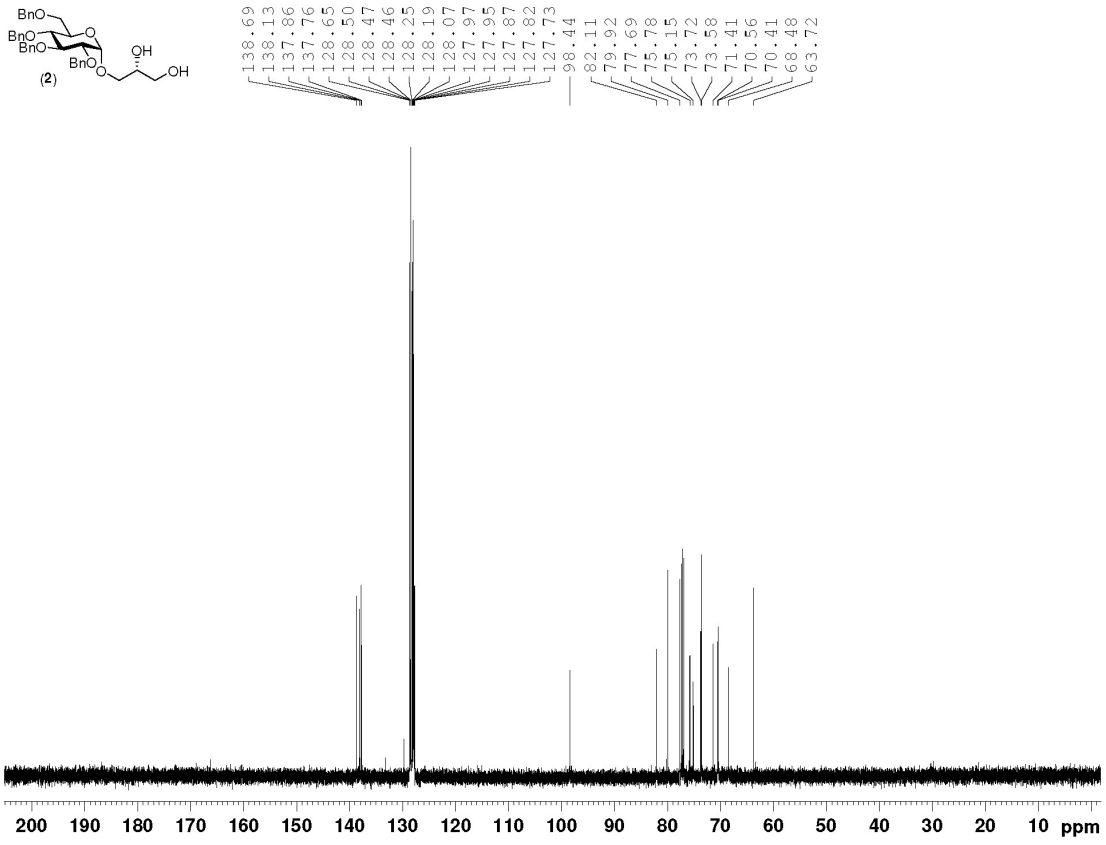


sn-Glycer-3-yl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (2)

^{1}H NMR

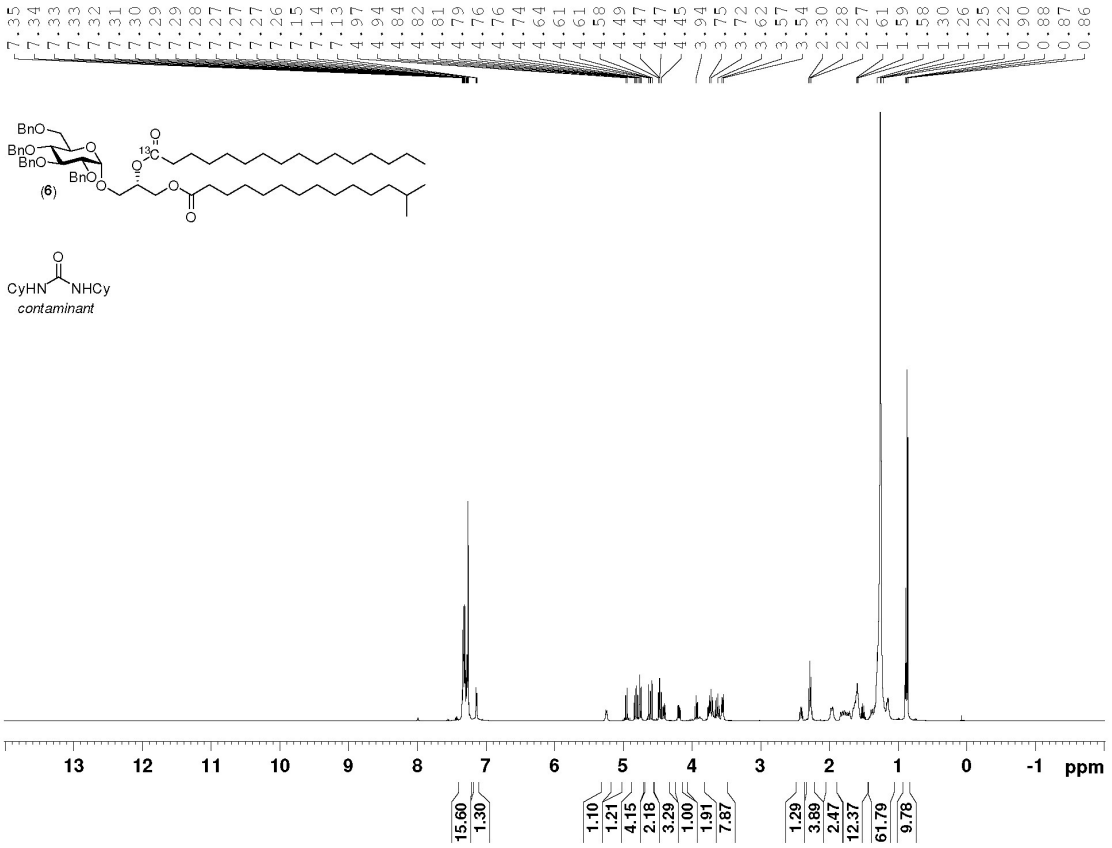


^{13}C NMR

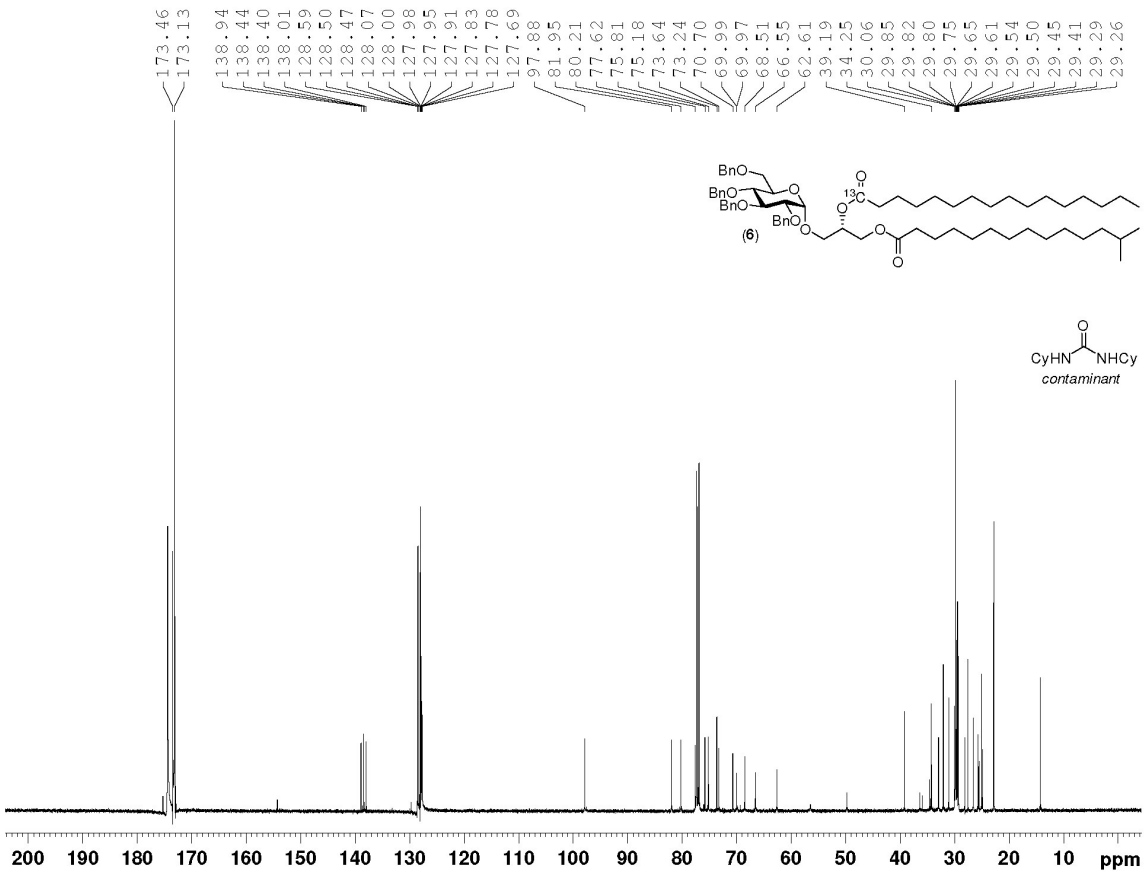


1-*O*-(10-Methyldodecanoyl)-2-*O*-(1-^{13}C-hexadecanoyl)-sn-glyceryl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (5)

^{1}H NMR

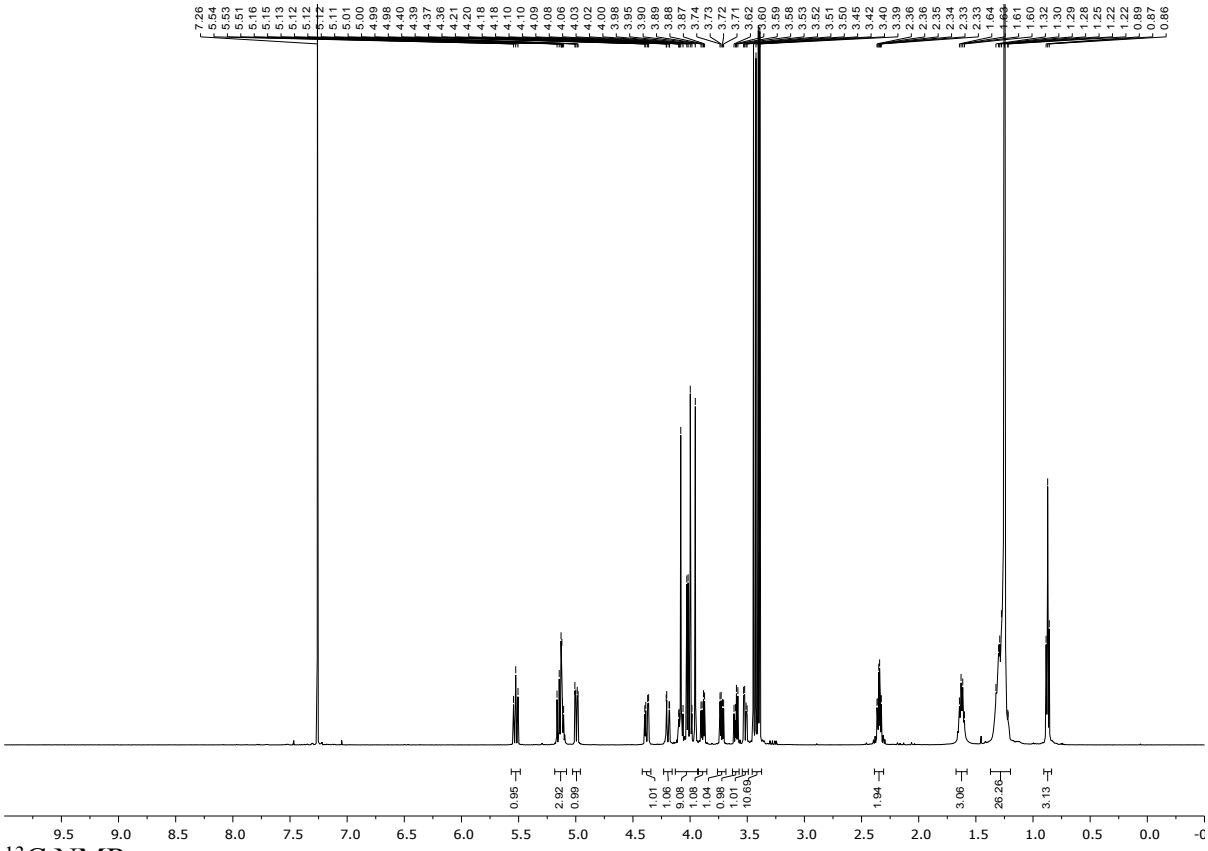


^{13}C NMR

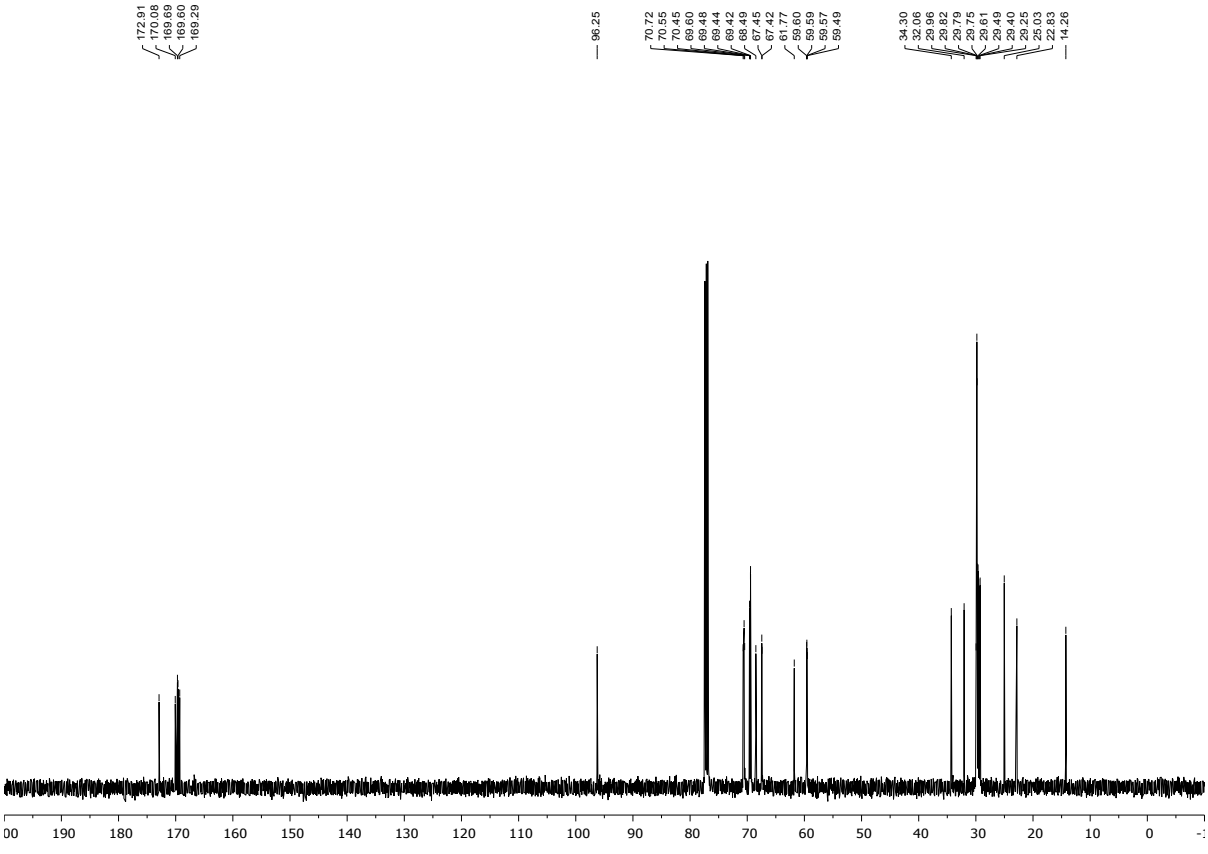


(2'*R*)-3'-Bromo-2'-palmitoyloxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (18)

^{1}H NMR

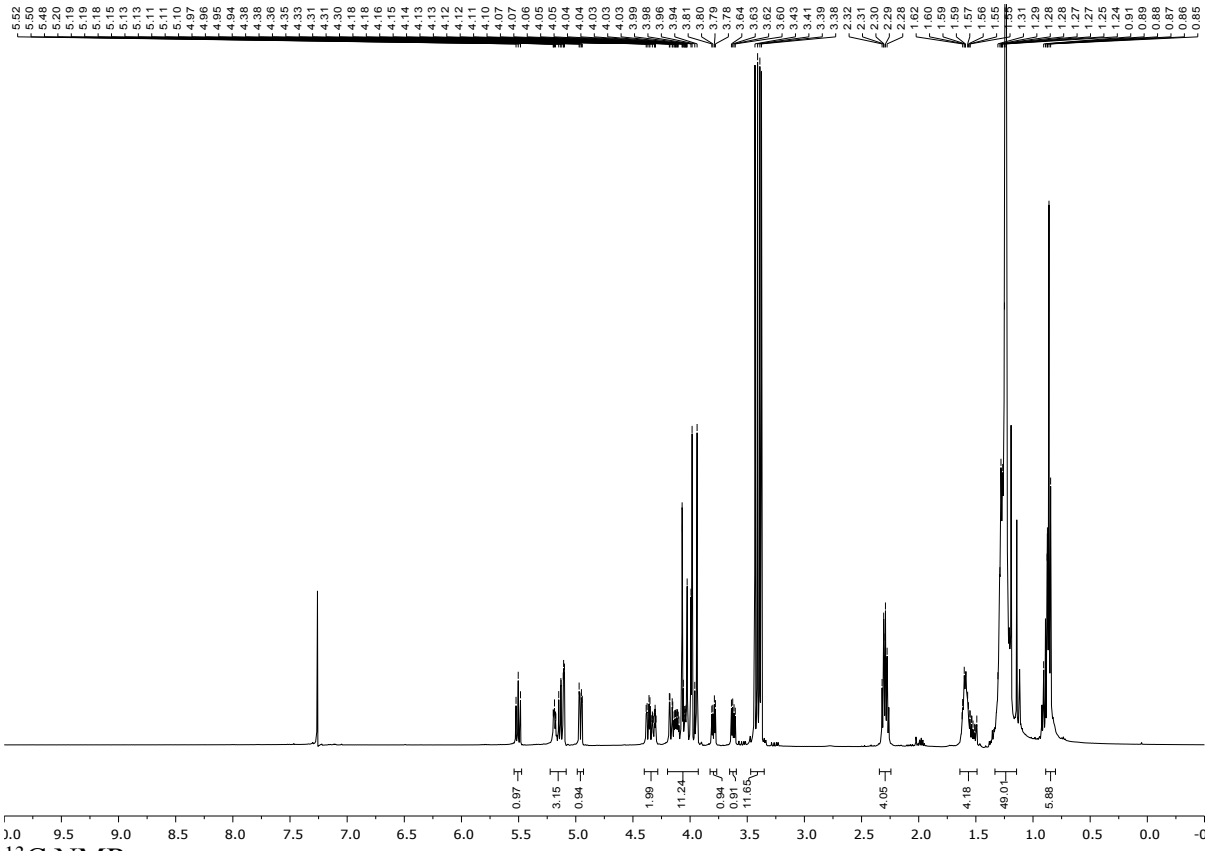


^{13}C NMR

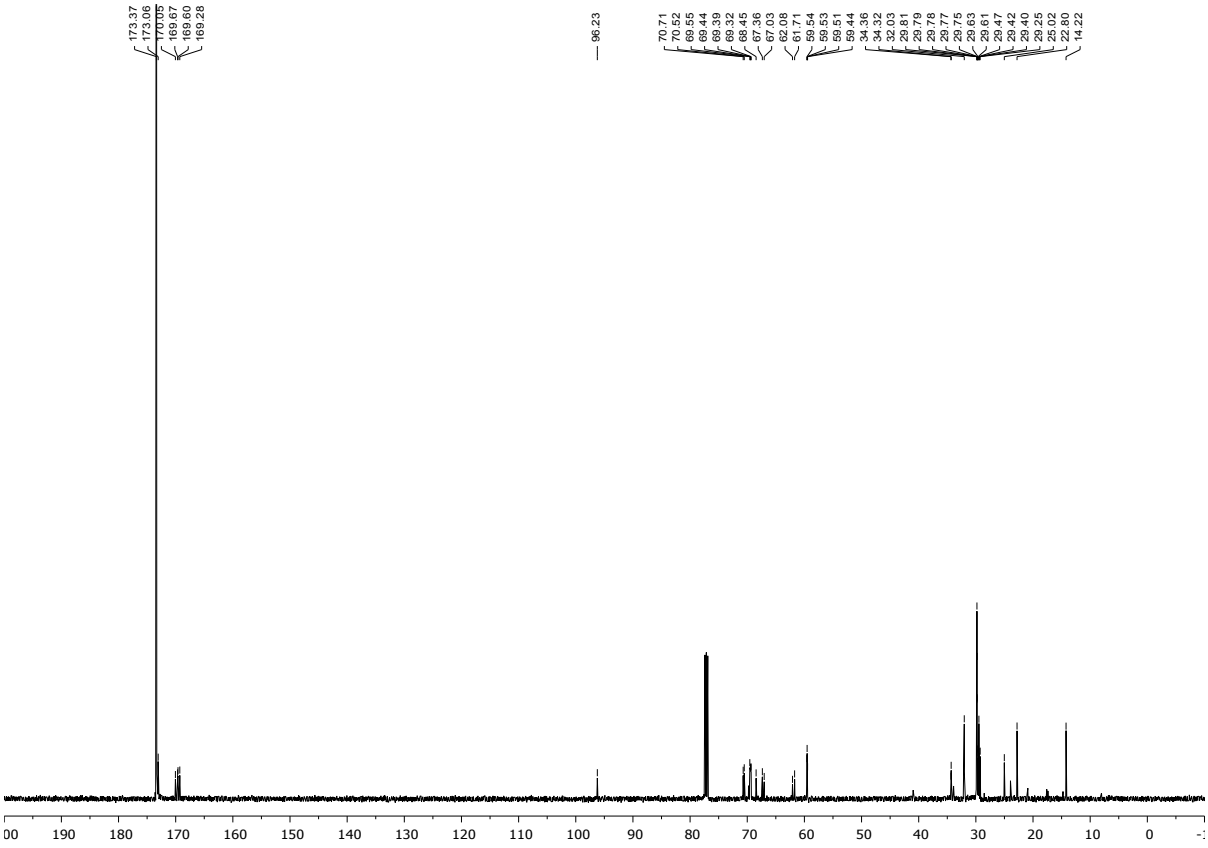


1′-*O*-(1″-^{13}C-Palmitoyl)-2′-*O*-palmitoyl-*sn*-glyceryl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (12)

^{1}H NMR

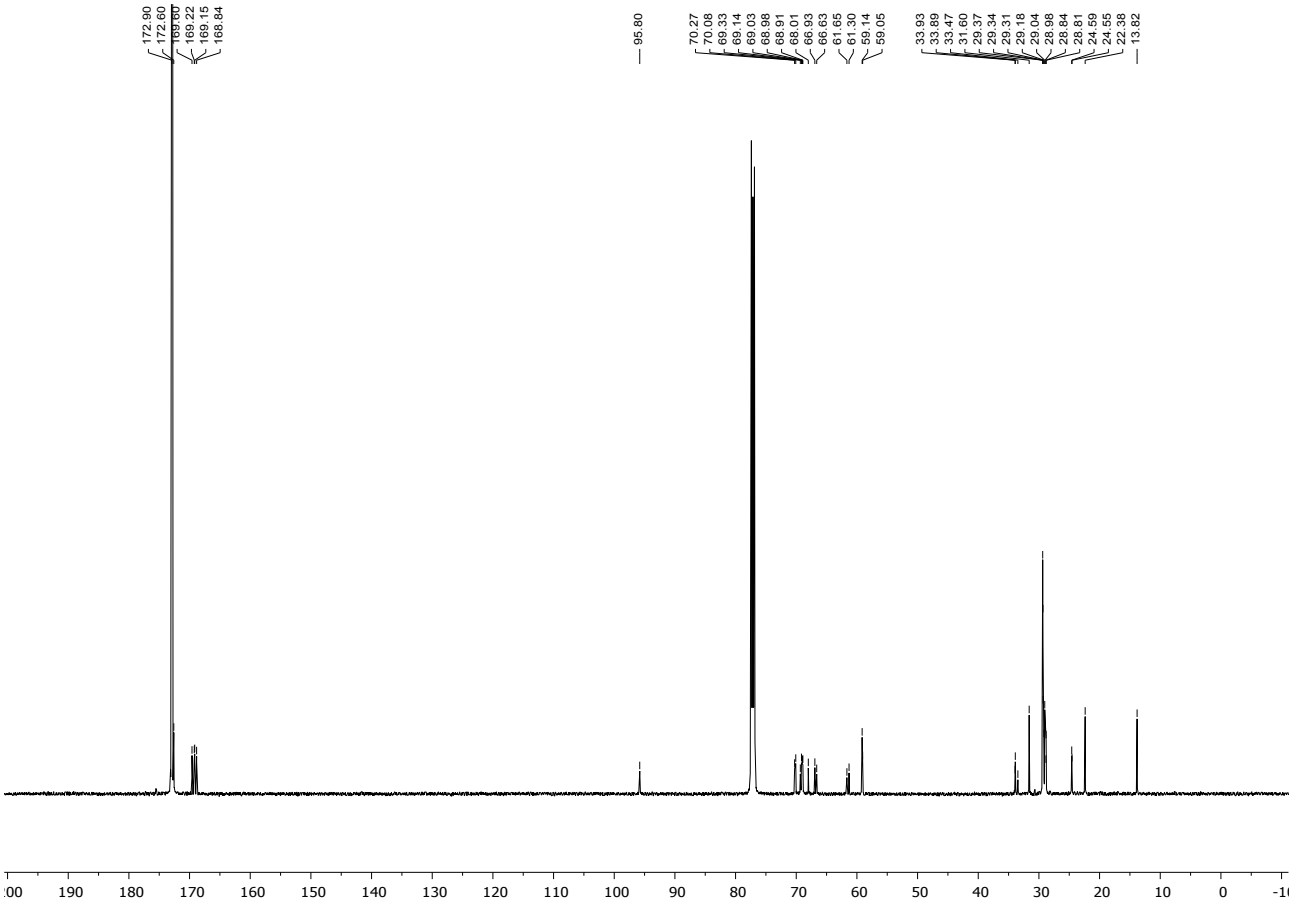


^{13}C NMR



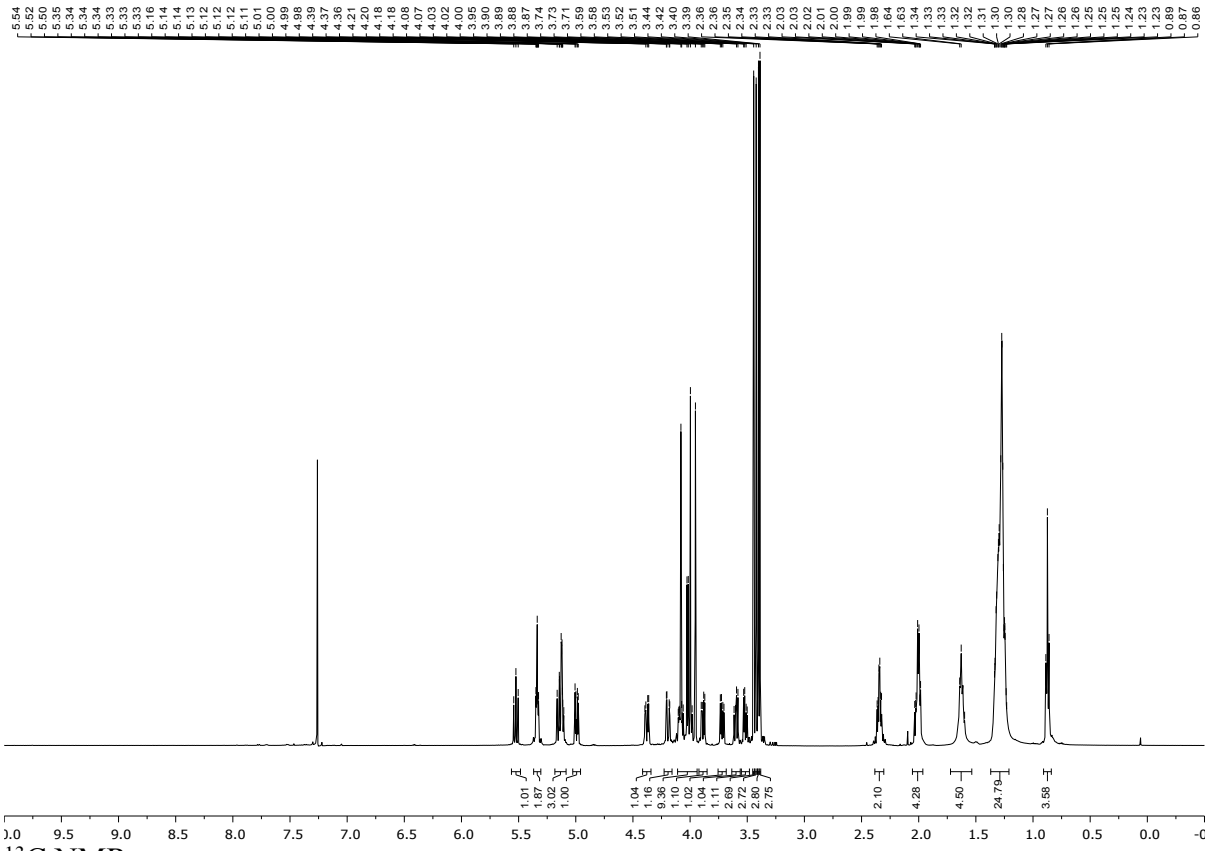
1′-*O*-(1″-^{13}C-Palmitoyl)-2′-*O*-palmitoyl-*sn*-glyceryl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (12) + Cr(Acac)$_2$

^{13}C NMR

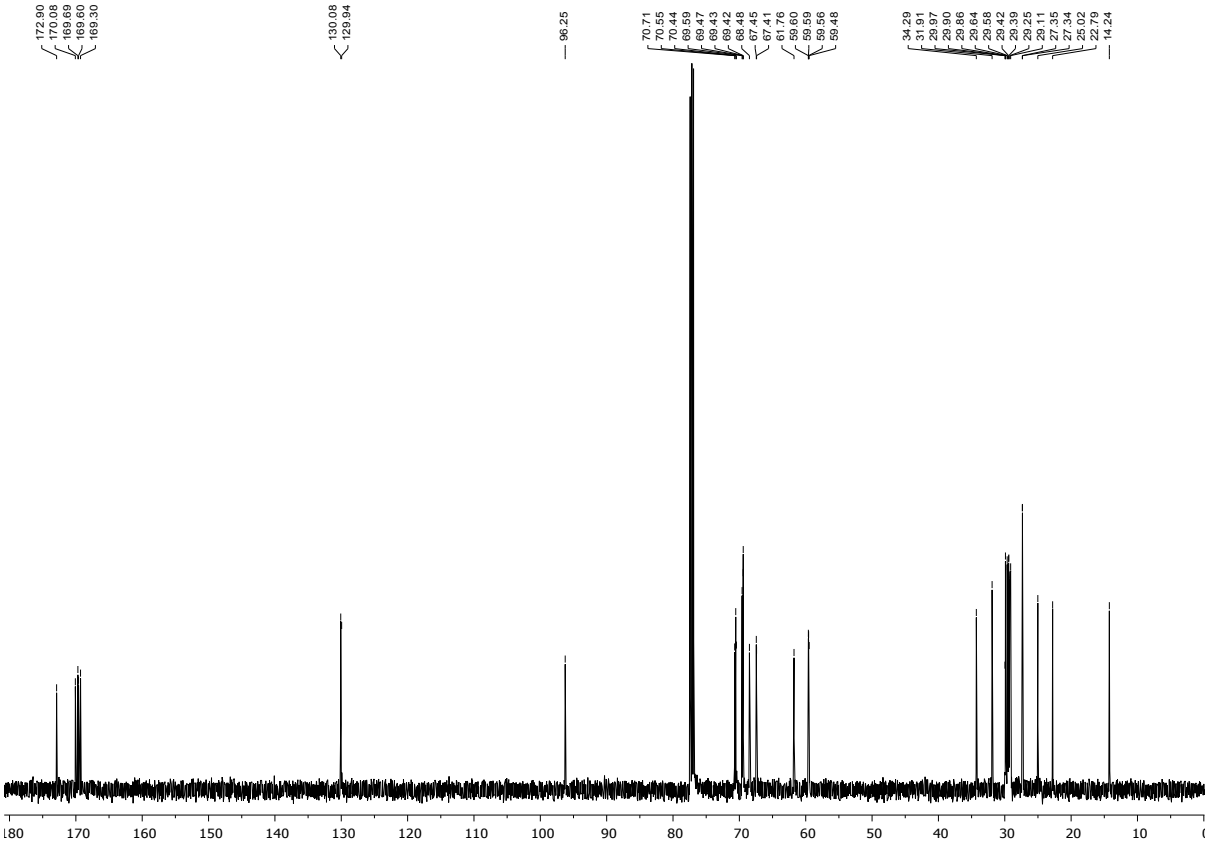


(2'*R*)-3'-Bromo-2'-vaccenoyloxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (13)

^{1}H NMR

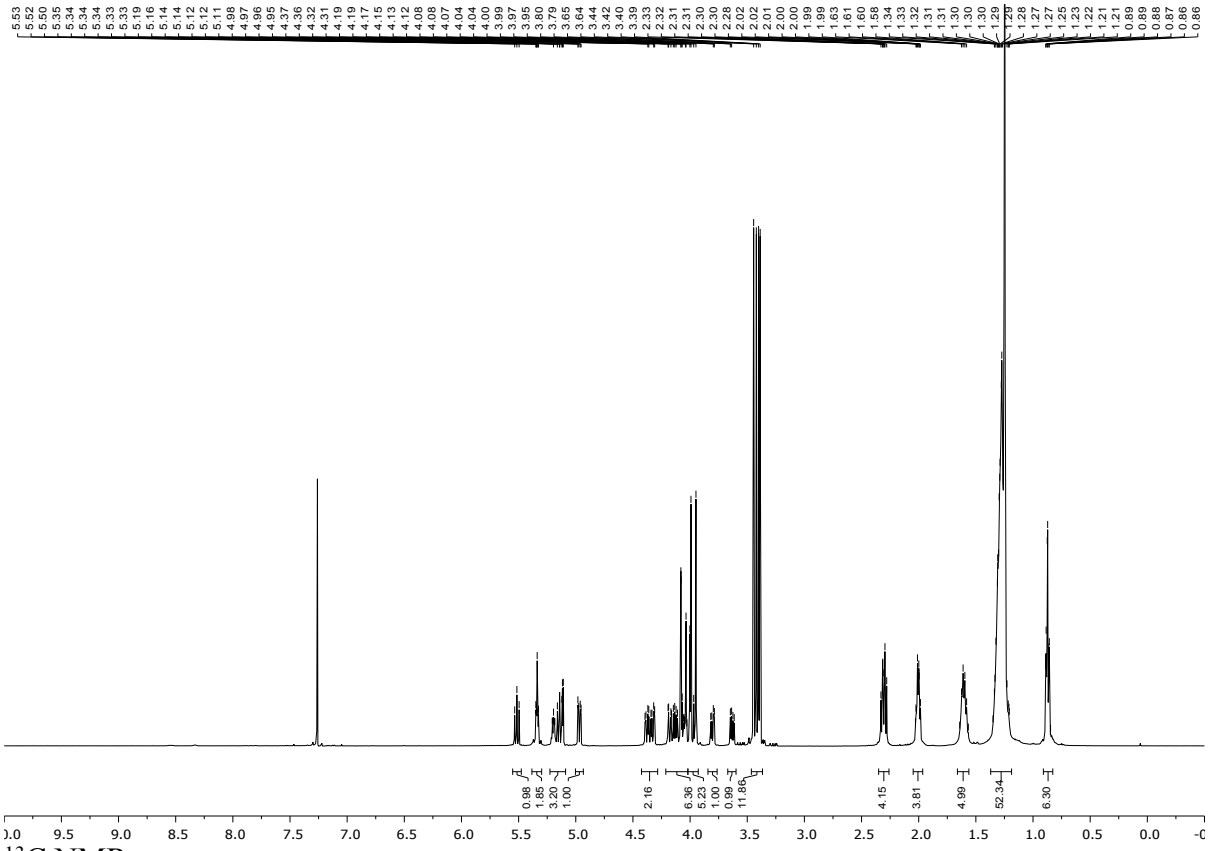


^{13}C NMR

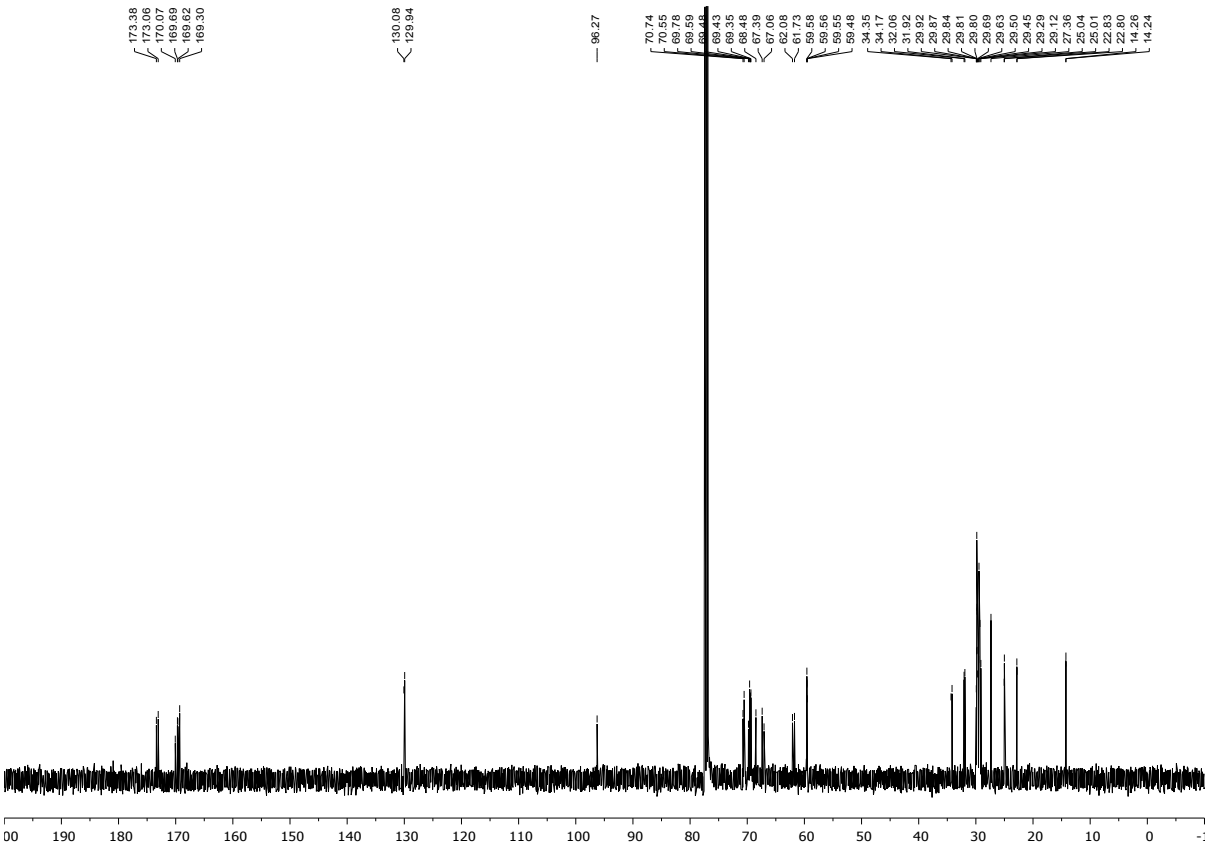


1'-*O*-Palmitoyl-2′-*O*-vaccenoyl-*sn*-glyceryl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (14)

^{1}H NMR

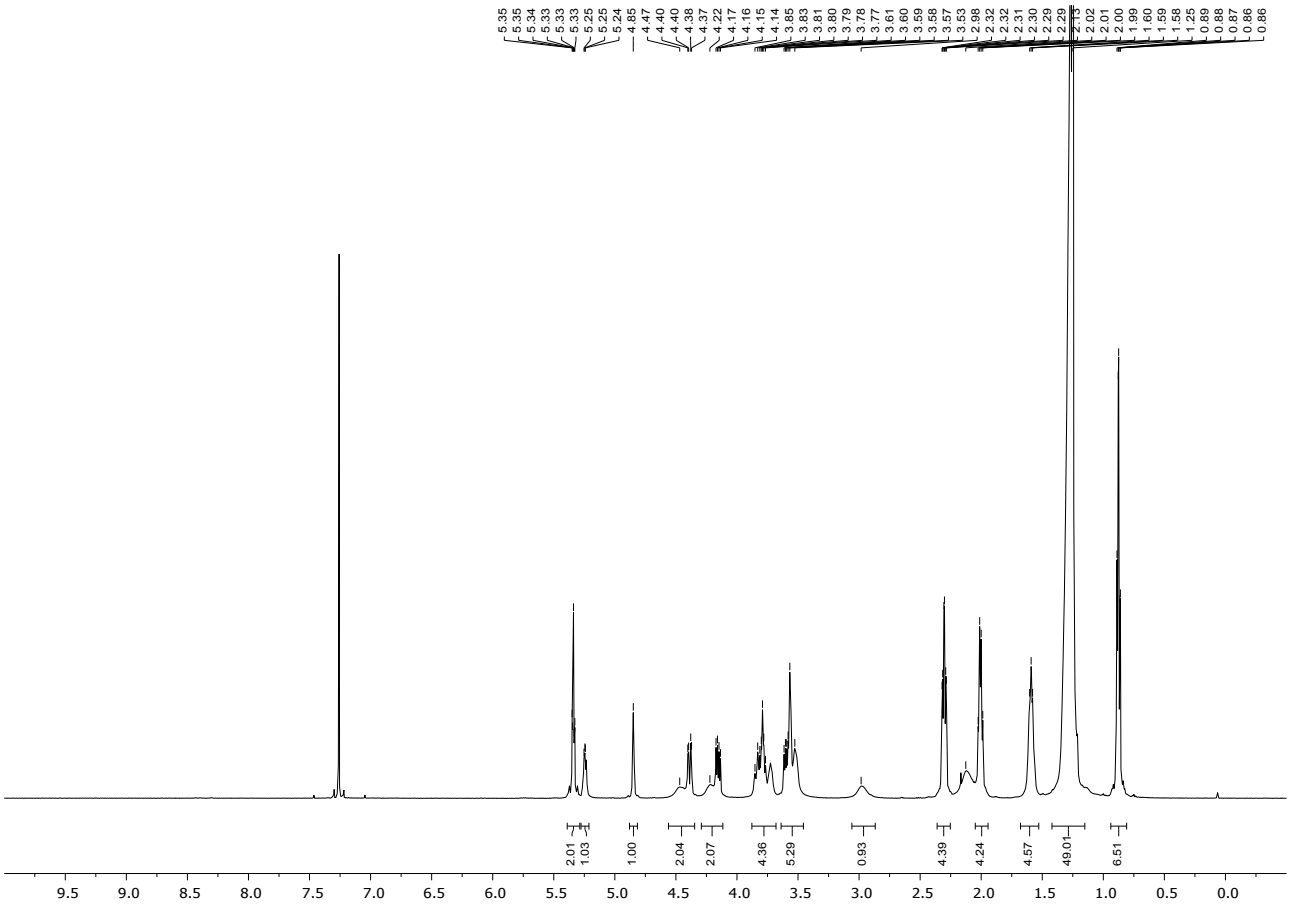


^{13}C NMR

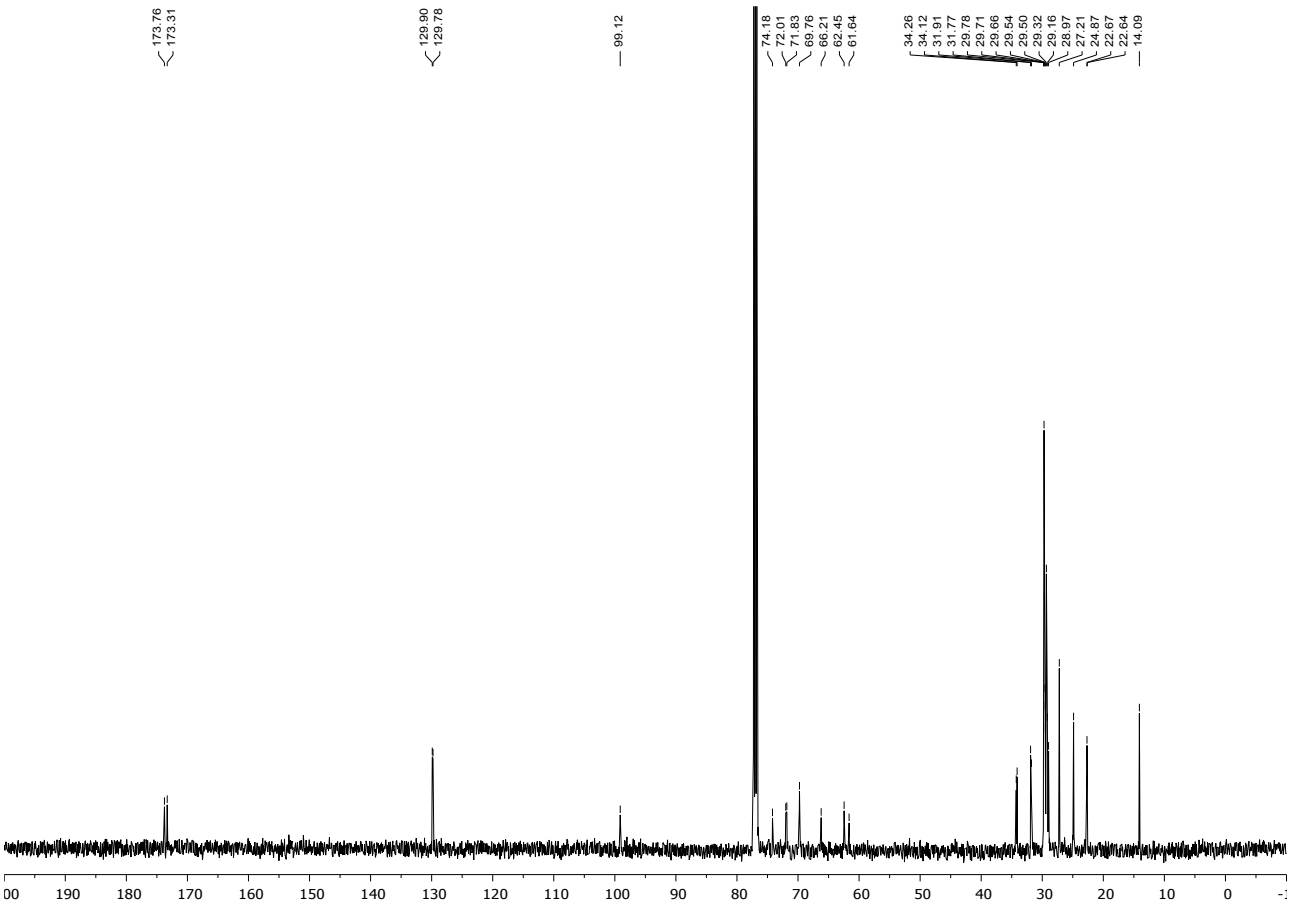


1'-*O*-Palmitoyl-2'-*O*-vaccenoyl-sn-glyceryl α-D-glucopyranoside (1, Glc-DAG-s2)

^{1}H NMR



^{13}C NMR



ESI MS^2 data (positive ion mode)