α-Glucuronosyl and α-glucosyl diacylglycerides, natural killer T cell-activating lipids from bacteria and fungi

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Table S1. Sequences of TCR lines used in this work.

Cell line	TCR-α	CDR3α	TCR-β	CDR3β	Isolated using CD1d tetramers loaded with	Ref.
A10B8.1	Vα10-Jα50	CAIASSSFSKLVF	Vβ8.1-Jβ2.7	CASRLGGYEQYF	α-GalCer	[1]
A10B8.3	Vα10-Jα50	CAIASSSFSKLVF	Vβ8.3-Jβ2.1	CASRTGLAAEQFF	α-GlcADAG	[2]
VB8 STD	Vα14-Jα18	CVVGDRGSALGRLHF	Vβ8.2-Jβ2.1	CASGDAGGNYAEQFF	α-GalCer	[3]

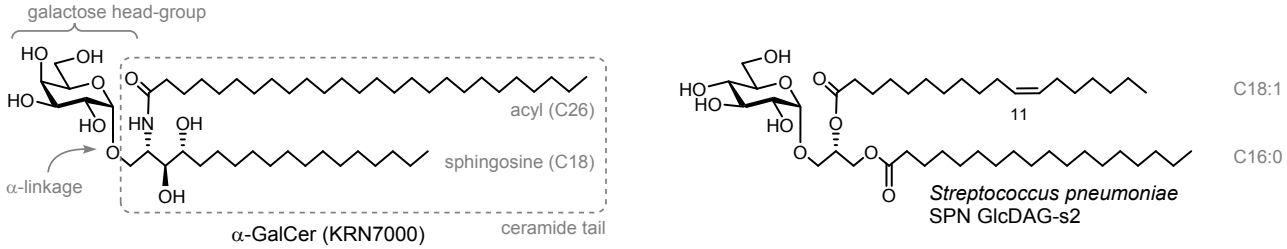


Figure S1. Structures of α-GalCer and SPN-GlcDAG-s2

Immunology Methods

Flow Cytometry

All antibodies were acquired from BD Biosciences unless otherwise stated. Fluorochrome-conjugated mouse specific antibodies include anti-αβTCR APC (clone H57-597) and anti-CD69 PE (clone H1.2F3). 7-Amino-actinomycin D (7-AAD) viability dye (Sigma) was included in all flow cytometry-staining experiments for dead cell exclusion in cytometric analysis. Streptavidin (SAV)-PE was purchased from BD Biosciences.

Mouse CD1d (and respective alanine CD1d mutants) for plate bound and tetramer assays was produced in-house in High Five insect cells as previously described.[4-6] Cell suspensions were first incubated for 10 minutes on ice with Fc-receptor block (anti-CD16/CD32, clone 2.4G2, produced in house), prior to antibody staining. Cells were analysed on a BD LSRFortessa™ III, or alternatively, cells were sorted on a BD FACSAria III™ (Becton Dickinson). Samples were analysed using FlowJo software (Tree Star Inc).

Glycolipids

α-GalCer (C26) was purchased from Alexis Biochemicals. GD3 (C26:0) was purchased from Matreya LLC. α-GalCer (C24:1 'PBS-44') was provided by Prof. Paul Savage (Brigham Young University, USA). SPN-Glc was synthesized as reported.[7] Glycolipids were prepared in either tyloxapol-based detergent (0.5% or 0.05% v/v tyloxapol) in tris-buffered saline (TBS) pH 8, or Tween 20-based detergent (0.5% Tween-20), 56 mg/mL sucrose, 7.5 mg/mL L-histidine in PBS. All glycolipids were stored at -20ºC. Solutions of glycolipids were sonicated for ~30 min prior to each use.

Generation of TCR-transduced cell lines

T cell lines expressing αβ TCR sequences were generated by cloning full-length genes encoding α- and β-chains into a pMIGII vector containing a *cis*-acting hydrolase element P2A linked gene system (see Table S1). The generation of TCR transduced mouse NKT cell lines was performed similarly as described by Holst *et al*.[8] Briefly, HEK293T cells were transfected using FuGENE-6 transfection reagent (Promega) with pMIGII expression vector containing both verified TCRα and TCRβ sequences, pMIGII expression vector containing the CD3 subunits encoding sequences,[9] packaging vector pEQ-Pam-3-E and packaging vector pVSV-G.[8] Retrovirus-containing supernatant was collected every 12 h, filtered in a 0.45 μm filter (Sartorius) and used to transduce mouse BW5147.TCR $\alpha^-\beta^-$ thymoma cells (termed BW58).[10] The pMIGII, expression and packaging vectors were provided by Dr. Dario Vignali (St. Jude's Research Hospital, USA), and the CD3 expression vector was provided by Prof. Stephen Turner (Monash University, Australia). BW58 cell lines were cultured in complete Dulbecco's Modified Eagle Medium (cDMEM, Gibco) supplemented with 10% FBS (v/v), 15 mM HEPES (Gibco), 1 mM sodium pyruvate (Gibco), 0.1 mM non-essential amino acids (Gibco), 50 μM β-mercaptoethanol

(Sigma), 100 U/mL penicillin (Invitrogen), 100 μg/mL streptomycin (Invitrogen) and 2 mM L-glutamine (Invitrogen), at 37 ºC, 5% CO_2.

For plate bound activation assays, lipid-loaded CD1d was coated in 96-well flat-bottom plates (10 μg/ml) for 3 hours at 37 ºC. BW58 cell lines were co-cultured with plate bound complex for 16 h in cDMEM. Cells were labelled with CD69 mAb to asses CD69 upregulation (staining panel also included anti-TCRβ and 7AAD), and/or culture supernatants were collected for IL-2 cytokine analysis using cytometric bead array (CBA) flex set for mice (BD Biosciences), according to the manufacturer's instructions.

Chemistry methods

General

Pyridine was distilled over KOH before use. Dichloromethane and THF were dried over alumina according to the method of Pangborn *et al*.[11] Reactions were monitored using TLC, performed with silica gel 60 F_{254}. Detection was effected by charring in a mixture of 5% sulfuric acid in methanol, 10% phosphomolybdic acid in EtOH, and/or visualizing with UV light. Flash chromatography was performed according to the method of Still et al.[12] using silica gel 60. $[\alpha]_D$ values are given in deg 10^{-1} cm^2 g^{-1}. NMR experiments were conducted on 400, 500 or 600 MHz instruments, with chemical shifts referenced relative to residual protiated solvent and are in ppm. $^1H-^1H$ COSY spectra were used to confirm proton assignments and HMQC and HMBC spectra used for carbon assignments. Mass spectra were acquired in the ESI-QTOF mode.

Ethyl (*S*)-10-methyl octadecenoate (10)

Hoveyda Grubbs 2nd generation catalyst (HG-II) (2.79 mg, 4.46 µg) was added to a solution of (*S*)-2,6-dimethyltetradecene **9**[13] (0.025 g, 0.0114 mmol) and ethyl heptenoate (0.052 g, 0.33 mmol) in dry dichloromethane (1.14 ml) and stirred for 24 h under reflux. Upon consumption of starting material, the reaction mixture was concentrated under reduced pressure. EtOAc (5.0 ml) was added to the residue and the solution was subjected to hydrogenation under high pressure (37.5 bar) in a Buchi® hydrogenator overnight at rt after which the solvent was removed under reduced pressure. Flash chromatography (2 % diethyl ether/ petroleum spirits) of the crude mixture afforded the title compound as a colourless oil (0.031g, 85%); $[\alpha]^{25}_D$ 0.87 (*c* 0.5 in $CHCl_3$) (lit.[14] $[\alpha]^{25}_D$ 0.14 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (3 H, d, *J* 6.5 Hz, $CHCH_3$), 0.87 (3 H, t, *J* 6.8 Hz, CH_3), 1.10–1.03 (2 H, m, CH_2), 1.31–1.20 (28 H, m, 12 × CH_2, 1 × CH, $COOCH_2CH_3$), 1.61 (2 H, m, $COCH_2CH_2$), 2.27 (2 H, t, *J* 7.6 Hz, $COCH_2$), 4.11 (2 H, d, *J* 7.1 Hz, $C{=}OOCH_2$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.2, 14.4, 19.8, 22.8, 25.1, 27.1, 27.2, 29.3, 29.4, 29.5, 29.6, 29.8, 30.0, 30.1, 32.0, 32.8, 32.8, 34.5, 37.2, 60.2, 174.0 (C=O); HRMS calcd for $C_{21}H_{42}O_2$ $[M+H]^+$ 327.3218, found 327.3216.

(*S*)-10-Methyl octadecanoic acid (11, *S*-TBSA)

Aqueous KOH solution (2 M, 0.73 mL, 1.47 mmol) was added to a solution of the ethyl ester **10** (0.032 g, 0.097 mmol) in MeOH/THF (1:1, 2 ml) and stirred under reflux overnight. The solution was acidified with citric acid (1 M), extracted with EtOAc, washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure to afford (*S*)-TBSA as colourless oil (0.028 g, 98%); $[\alpha]^{25}_D$ 0.43 (*c* 0.5 in $CHCl_3$) (lit.[15] $[\alpha]^{25}_D$ 0.21 in $CHCl_3$) ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (3 H, d, *J* 6.5 Hz, $CHCH_3$), 0.88 (3 H, t, *J* 6.8 Hz, CH_2CH_3), 1.12–1.04 (2 H, m, CH_2), 1.36–1.21 (25 H, m, 12 × CH_2, 1 × CH), 1.67–1.59 (2 H, m, $COCH_2CH_2$), 2.35 (2 H, t, *J* 7.5 Hz, $OCOCH_2$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.2, 19.8, 22.8, 24.8,

27.3, 29.2, 29.4, 29.5, 29.6, 29.8, 30.0, 30.1, 32.0, 32.8, 34.0, 37.2, 179.4 (C=O); $C_{18}H_{36}O_2$ $[M+H]^+$ 285.2749, found 285.2746.

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

Palmitoyl chloride (0.1 ml, 0.331 mmol) was added to a stirred solution of (2'*S*)-3'-bromo-2'-hydroxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside **12**[16] (0.102 g, 0.165 mmol) in dry CH_2Cl_2 (3 ml) and pyridine (0.266 ml, 3.30 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 with water, sat aq. $CuSO_4$, followed by sat. aq. $NaHCO_3$. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash chromatography of the residue (50% EtOAc /petroleum ether) afforded **13** as a colorless oil (0.132 g, 95%). $[\alpha]_D^{26}$ +55.9 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3 H, t, *J* 6.7 Hz, CH_2CH_3), 1.24–1.31 (24 H, m, 12 × CH_2), 1.56–1.63 (2 H, m, β-CH_2), 2.32 (2 H, td, *J* 2.4, 7.4 Hz, α-CH_2), 3.37, 3.38, 3.40, 3.42 (12 H, 4 s, 4 × OCH_3), 3.52 (1 H, dd, *J* 4.6, 10.8 Hz, H1'), 3.60 (1 H, dd, *J* 6.3, 10.8 Hz, H1'), 3.72 (1 H, dd, *J* 6.2, 10.6 Hz, H3'), 3.89 (1 H, dd, *J* 4.7, 10.8 Hz, H3'), 3.96, 4.00, 4.10 (6 H, 3 s, 3 × CH_3OCH_2), 4.02 (2 H, m, CH_3OCH_2), 4.06–4.13 (1 H, m, H5), 4.17 (1 H, dd, *J* 2.1, 12.4 Hz, H6a), 4.36 (1 H, dd, *J* 4.2, 12.4 Hz, H6b), 4.97 (1 H, dd, *J* 3.7, 10.2 Hz, H2), 5.10–5.14 (3 H, m, H1,2',4), 5.50 (1 H, t, *J* 9.7 Hz, H3); ^{13}C NMR ($CDCl_3$, 100 MHz) 14.2, 22.8, 25.0, 29.3, 29.41, 29.44, 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 ($MeOCH_2C$=O), 172.9 (sn-2-C=O); HRMS (ESI^+) calcd for $C_{37}H_{63}BrO_{16}$ $(M+H)^+$ 842.3299. Found 842.3296; calcd for $C_{37}H_{63}O_{16}Br$ $(M + NH_4)^+$ 860.3638. Found 860.3646.

(2'*S*)-3'-Bromo-2'-oleoyloxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (14)

Oleoyl chloride (0.27 ml, 0.816 mmol) was added to a stirred solution of (2'*S*)-3'-bromo-2'-hydroxypropyl-2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside **12**[16] (247 mg, 0.408 mmol) in dry CH_2Cl_2 (9 ml) and pyridine (0.657 ml, 8.16 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 with water, sat. aq. $CuSO_4$ and sat. aq. $NaHCO_3$. The organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash chromatography of the residue (EtOAc/pet. spirits) afforded **14** as a colourless oil (337 mg, 95%). $[\alpha]_D^{24}$ +52.3 (*c* 1, $CHCl_3$) (lit.[16] $[\alpha]_D^{25}$ +55.8 (*c* 1.15, $CHCl_3$)); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (3 H, t, *J* 6.9 Hz, CH_2CH_3), 1.18–1.39 (20 H, m, alkyl), 1.60–1.66 (2 H, m, β-CH_2), 1.96–2.05 (4 H, m, CH_2CH–$CHCH_2$), 2.29–2.38 (2 H, m, α-CH_2), 3.39, 3.40, 3.42, 3.44 (12H, 4 s, 4 × CH_3OCH_2), 3.51 (1 H, dd, *J* 4.6, 10.6 Hz, H1'), 3.59 (1 H, dd, *J* 6.2, 10.7 Hz, H1'), 3.72 (1 H, dd, *J* 4.9, 10.9 Hz, H3'), 3.88 (1 H, dd, *J* 4.8, 10.8 Hz, H3'), 3.95, 3.99, 4.08 (6 H, 3 s, 3 × CH_3OCH_2), 4.01–4.11 (3 H, m, CH_3OCH_2, H5), 4.19 (1 H, dd, *J* 2.3, 12.4 Hz, H6a), 4.38 (1 H, dd, *J* 4.2, 12.4 Hz, H6b), 4.99 (1 H, dd, *J* 3.8, 10.1 Hz, H2), 5.09–5.17 (3 H, m, H1,4,2'), 5.29–5.37 (2 H, m,

HC=CH), 5.52 (1 H, t, *J* 9.7 Hz, H3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 22.8, 25.0, 27.3, 27.4, 29.20, 29.24, 29.3, 29.43, 29.44, 29.6, 29.8, 29.9, 30.0, 32.0, 34.3 (fatty acyl), 59.5, 59.56, 59.57, 59.59, 61.8, 67.40, 67.44, 68.5, 69.41, 69.42, 69.5, 69.6, 70.4, 70.5, 70.7, 96.2 (C1), 129.8, 130.2 (HC=CH), 169.3, 169.6, 169.7, 170.1 ($MeOCH_2C$=O), 172.9 (sn-2-C=O); HRMS (ESI$^+$) calcd for $C_{35}H_{65}BrO_{16}$ $(M + Na)^+$ 891.3348. Found 891.3343.

1'-*O*-((*R*)-10-Tuberculostearyl)-2'-*O*-palmitoyl-sn-glyceryl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (15)

Tetrabutylammonium hydroxide (60 % w/w in H_2O) (0.058 ml, 0.092 mmol) was added to a suspension of (*R*)-tuberculostearic acid[7] (0.030 g, 0.103 mmol) in H_2O (1.5 ml). The resulting mixture was vigorously stirred at rt overnight. The solvent was evaporated under reduced pressure and the crude residue was co-evaporated with toluene several times to afford the tetrabutylammonium salt of (*R*)-tuberculostearic acid. Tetrabutylammonium (*R*)-tuberculostearate (0.054 g, 0.101 mmol) was added to a solution of **13** (0.043 g, 0.050 mmol) in toluene (0.5 ml) and the resulting mixture was heated to 85 °C and stirred vigorously for 25 min. The solvents were evaporated under reduced pressure. Flash chromatography of the residue (40% EtOAc/petroleum ether) afforded **15** as a colourless oil (0.038 g, 70%). $[\alpha]_D^{25}$ +29.6 (*c* 0.25, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) 0.83 (3 H, d, *J* 6.4 Hz, CHC*H*$_3$), 0.87 (6 H, t, *J* 6.8 Hz, 2 × CH_3), 1.10–1.14 (2 H, m, tuberculostearyl CH_2), 1.25–1.42 (49 H, m, 24 × CH_2, 1 × CH), 1.61 (4 H, dd, *J* 6.8, 13.7 Hz, 2 × β-CH_2), 2.31 (4 H, q, *J* 7.7 Hz, 2 × α-CH_2), 3.39, 3.41, 3.43, 3.45 (12 H, 4 s, 4 × OCH_3), 3.63 (1 H, dd, *J* 11.2, 5.5 Hz, H3'), 3.81 (1 H, dd, *J* 11.2, 4.5 Hz, H3'), 3.95, 4.00, 4.02, 4.08 (8 H, 4 s, 4 × OCOC*H*$_2$), 4.06–4.08 (1 H, m, H5), 4.11–4.20 (2 H, m, H1a',6a), 4.31–4.41 (2 H, m, H1b',6b), 4.97 (1 H, dd, *J* 3.7, 10.1 Hz, H2), 5.12–5.23 (3 H, m, H1,4,2'), 5.52 (1 H, t, *J* 9.7 Hz, H3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.2 (2 × CH_2CH_3), 19.7 ($CHCH_3$ (tuberculostearyl)), 22.7, 24.89, 24.95, 27.1, 29.2, 29.2, 29.3, 29.4, 29.6, 29.6, 29.7, 29.7, 30.0, 30.1, 32.0, 32.8, 34.1, 34.2 (fatty acyl), 59.4, 59.48, 59.55, 59.57, 67.3, 68.4, 69.2, 69.3, 69.4, 69.5, 69.7, 70.4, 70.6, 76.7, 77.1, 77.4, 96.2 (C1), 170.0 ($MeOCH_2C$=O), 173.0 (sn-2-C=O), 173.3 (sn-1-C=O); HRMS (ESI$^+$) calcd for $C_{56}H_{100}O_{18}Na$ $(M+Na)^+$ 1083.6802. Found 1083.6801.

1'-*O*-((*S*)-10-Tuberculostearyl)-2'-*O*-palmitoyl-sn-glyceryl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (16)

Tetrabutylammonium hydroxide (60 % w/w in H_2O) (0.041 ml, 0.064 mmol) was added to a suspension of (*S*)-tuberculostearic acid **11** (0.021 g, 0.071 mmol) in H_2O (1 ml). The resulting mixture was vigorously stirred at rt overnight. The solvent was evaporated under reduced pressure and the crude residue was co-evaporated with toluene several times to afford tetrabutylammonium salt of (*S*)-tuberculostearic acid. Tetrabutylammonium (*S*)-tuberculostearate (0.038 g, 0.712 mmol) was added to a solution of **13** (0.030 g, 0.036 mmol) in toluene (0.5 ml) and the resulting mixture was heated to 85

°C and stirred vigorously for 25 min. The solvents were evaporated under reduced pressure. Flash chromatography of the residue (40% EtOAc/petroleum ether) afforded **16** as a colourless oil (0.019 g, 50%). $[\alpha]_D^{25}$ +29.5 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) 0.83 (3 H, d, *J* 6.4 Hz, $CHCH_3$), 0.87 (6 H, t, *J* 6.8 Hz, 2 × CH_3), 1.10–1.14 (2 H, m, tuberculostearyl CH_2), 1.25–1.42 (49 H, m, 24 × CH_2, 1 × CH), 1.61 (4 H, dd, *J* 6.8, 13.7 Hz, 2 × β-CH_2), 2.31 (4 H, q, *J* 7.7 Hz, 2 × α-CH_2), 3.39, 3.41, 3.43, 3.45 (12 H, 4 s, 4 × OCH_3), 3.63 (1 H, dd, *J* 11.2, 5.5 Hz, H3'), 3.81 (1 H, dd, *J* 11.2, 4.5 Hz, H3'), 3.95, 4.00, 4.02, 4.08 (8 H, 4 s, 4 × OCO*C*H_2), 4.06–4.08 (1 H, m, H5), 4.11–4.20 (2 H, m, H1',6), 4.31–4.41 (2 H, m, H1',6), 4.97 (1 H, dd, *J* 3.7, 10.1 Hz, H2), 5.12–5.23 (3 H, m, H1,4,2'), 5.52 (1 H, t, *J* 9.7 Hz, H3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.2 (2 × CH_2CH_3), 19.7 (CH*C*H_3 (tuberculostearyl)), 22.7, 24.9, 24.96, 27.1, 29.2, 29.2, 29.3, 29.4, 29.6, 29.6, 29.7, 29.7, 30.0, 30.1, 32.0, 32.8, 34.1, 34.2, (fatty acyl), 59.4, 59.48, 59.55, 59.57, 67.3, 68.4, 69.2, 69.3, 69.4, 69.5, 69.7, 70.4, 70.6, 76.7, 77.1, 77.4, 96.2 (C1), 170.0 (MeOCH$_2$*C*=O), 173.0 (sn-2-C=O), 173.3 (sn-1-C=O); HRMS (ESI$^+$) calcd for $C_{58}H_{100}O_{18}Na$ $(M+Na)^+$ 1083.6802. Found 1083.6803.

1'-*O*-Oleoyl-2'-*O*-palmitoyl-sn-glyceryl 2,3,4,6-tetra-*O*-methoxy-acetyl-α-D-glucopyranoside (17)

Tetrabutylammonium hydroxide solution in H_2O (1.5 M, 0.056 ml, 0.085 mmol) was added to a suspension of oleic acid (30 µl, 0.095 mmol) in H_2O (1 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 oleic acid. A mixture of tetrabutylammonium oleate (45 mg, 0.085 mmol) and **13** (40 mg, 0.047 mmol) in toluene (1 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 **17** as a colourless oil (31 mg, 63%). $[\alpha]_D^{24}$ +50.8 (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* 6.9 Hz, CH_2CH_3), 1.18–1.36 (44 H, m, alkyl), 1.55–1.66 (4 H, m, β-CH_2), 1.96–2.06 (4 H, m, CH_2CH=$CHCH_2$), 2.27–2.35 (4 H, m, α-CH_2), 3.39, 3.40, 3.42, 3.45 (12 H, 4 s, 4 × CH_3OCH_2), 3.63 (1 H, dd, *J* 5.5, 11.2 Hz, H3'), 3.81 (1 H, dd, *J* 4.5, 11.2 Hz, H3'), 3.95–4.12 (8 H, m, 4 × CH_3OCH_2), 4.04–4.07 (1 H, m, H5), 4.13 (1 H, dd, *J* 5.6, 12.3 Hz, H1'), 4.18 (1 H, dd, *J* 2.3, 12.4 Hz, H6), 4.33 (1 H, dd, *J* 4.3, 11.8 Hz, H1'), 4.38 (1 H, dd, *J* 4.1, 12.4 Hz, H6), 4.97 (1 H, dd, *J* 3.8, 10.1 Hz, H2), 5.09–5.23 (3 H, m, H1,4,2'), 5.30–5.39 (2 H, m, HC=CH), 5.49–5.55 (1 H, m, H3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 22.83, 22.84, 25.0, 25.1, 27.3, 27.4, 29.27, 29.29, 29.4, 29.46, 29.47, 29.51, 29.7, 29.81, 29.83, 29.85, 29.91, 32.0, 32.1, 34.2, 34.4 (fatty acyl), 59.49, 59.56, 59.58, 59.59, 61.7, 62.1, 67.1, 67.4, 68.5, 69.36, 69.44, 69.5, 69.6, 69.8, 70.6, 70.8, 96.3 (C1), 129.8, 130.2 (HC=CH), 169.3, 169.6, 169.7, 170.1 (MeOCH$_2$*C*=O), 173.1 (sn-2-C=O), 173.4 (sn-1-C=O); HRMS (ESI$^+$) calcd for $C_{55}H_{96}O_{18}$ $(M + NH_4)^+$ 1062.6935. Found 1062.6918.

1'-*O*-Palmitoyl-2'-*O*-oleoyl-sn-glyceryl 2,3,4,6-tetra-*O*-methoxy-acetyl-α-D-glucopyranoside (18)

Tetrabutylammonium hydroxide solution in H_2O (1.5 M, 0.478 ml, 0.738 mmol) was added to a suspension of palmitic acid (210 mg, 0.820 mmol) in H_2O (8 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 (0.354 g, 0.738 mmol) and **14** (343 mg, 0.382 mmol) in toluene (2.5 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 **18** as a colourless oil (0:220 mg, 55%). $[\alpha]_D^{24}$ +47.7 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (6 H, t, *J* 6.9 Hz, CH_2CH_3), 1.20–1.38 (44 H, m, alkyl), 1.55–1.64 (4 H, m, β-CH_2), 1.97–2.03 (4 H, m, CH_2CH=$CHCH_2$), 2.26–2.34 (4 H, m, α-CH_2), 3.38, 3.39, 3.41, 3.44 (12 H, 4 s, 4 × CH_3OCH_2), 3.62 (1 H, dd, *J* 5.5, 11.2 Hz, H3'), 3.80 (1 H, dd, *J* 4.6, 11.2 Hz, H3'), 3.93–4.08 (8 H, m, 4 × CH_3OCH_2), 4.03–4.07 (1 H, m, H5), 4.12 (1 H, dd, *J* 6.0, 11.9 Hz, H1'), 4.17 (1 H, dd, *J* 2.3, 12.4 Hz, H6), 4.32 (1 H, dd, *J* 4.3, 11.8 Hz, H1'), 4.37 (1 H, dd, *J* 4.1, 12.4 Hz, H6), 4.96 (1 H, dd, *J* 3.8, 10.1 Hz, H2), 5.08–5.23 (3 H, m, H1,2',4), 5.28–5.38 (2 H, m, HC=CH), 5.51 (1 H, t, *J* 9.7 Hz, H3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.2, 22.8, 22.8, 24.99, 25.00, 27.30, 27.34, 29.22, 29.27, 29.34, 29.41, 29.43, 29.44, 29.5, 29.61, 29.64, 29.75, 29.77, 29.78, 29.82, 29.85, 29.88, 32.02, 32.04, 34.1, 34.3 (fatty acyl), 59.45, 59.49, 59.53, 59.54, 61.7, 62.1, 67.0, 67.4, 68.5, 69.3, 69.4, 69.45, 69.6, 69.8, 70.5, 70.7, 96.2 (C1), 129.8, 130.1 (HC=CH), 169.3, 169.6, 169.7, 170.1 ($MeOCH_2C$=O), 173.0 (sn-2-C=O), 173.4 (sn-1-C=O); HRMS (ESI$^+$) calcd for $C_{55}H_{96}O_{18}$ $(M + NH_4)^+$ 1062.6935. Found 1062.6921.

1'-*O*-((*R*)-10-Tuberculostearyl)-2'-*O*-palmitoyl-sn-glyceryl α-D-glucopyranoside (5)

A solution of *tert*-butylamine (0.109 ml, 1.05 mmol) and **15** (0.023 g, 0.021 mmol) in $CHCl_3$ (0.165 ml) and MeOH (0.385 ml) was stirred at 0 °C for 10 min and then at 10 °C for an hour. The solvents were evaporated under reduced pressure at 10-15 °C. Flash chromatography of the residue (5% MeOH/$CHCl_3$) afforded **5** as a white semisolid (0.013 g, 82%). $[\alpha]_D^{26}$ +45.1 (*c* 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, d_6-DMSO) δ 0.80 (3 H, d, *J* 6.4 Hz, $CHCH_3$), 0.84 (6 H, t, *J* 6.7 Hz, 2 × CH_2CH_3), 1.06 (2 H, m, CH_2-tuberculostearyl), 1.23–1.42 (49 H, m, 24 × CH_2, 1 × CH), 1.38–1.48 (4 H, m, 2 × β-CH_2), 2.25 (4 H, q, *J* 7.3 Hz, 2 × α-CH_2), 3.07 (1 H, td, *J* 5.4, 9.3 Hz, H3), 3.17 (1 H, ddd, *J* 3.7, 6.5, 9.9 Hz, H2), 3.43–3.51 (4 H, m, H4,6,6,3a'), 3.57 (1 H, ddd, *J* 1.8, 5.6, 11.4 Hz, H5), 3.69 (1 H, dd, *J* 5.7, 10.7 Hz, H3b'), 4.14 (1 H, dd, *J* 7, 12 Hz, H1a'), 4.31 (1 H, dd, *J* 2.7, 12 Hz, H1b'), 4.40 (1 H, t, *J* 5.8 Hz, OH), 4.64 (1 H, d, *J* 3.6 Hz, H1), 4.66 (1 H, d, *J* 6.5 Hz, OH), 4.77 (1 H, d, *J* 4.8 Hz, OH), 4.86 (1 H, d, *J* 5.3 Hz, OH), 5.12 (1 H, m, H2'); ^{13}C NMR (150 MHz, d_6-DMSO) δ 13.8, 19.5, 22.1, 23.5, 24.4, 26.5, 28.7, 29.1, 31.3, 32.1, 33.3, 33.5, 36.4 (fatty acyl), 60.7 (C6), 62.3, 64.9, 65.0, 68.5, 69.6, 69.9, 71.7,

72.9, 73.0, 98.9 (C1), 172.3 (sn-2-C=O), 172.5 (sn-1-C=O); HRMS (ESI^+) calcd for $C_{44}H_{88}NO_{10}$ $(M+NH_4)^+$ 790.6403. Found 790.6403

1'-*O*-((*S*)-10-Tuberculostearyl)-2'-*O*-palmitoyl-sn-glyceryl α-D-glucopyranoside (6)

A solution of *tert*-butylamine (0.085 ml, 0.815 mmol) and **16** (0.017 g, 0.016 mmol) in $CHCl_3$ (0.165 ml) and MeOH (0.385 ml) was stirred at 0 °C for 10 min and then at 10 °C for an hour. The solvents were evaporated under reduced pressure at 10-15 °C. Flash chromatography of the residue (5% MeOH/$CHCl_3$) afforded **6** as a white semisolid (0.011 g, 89%). $[\alpha]_D^{25}$ +44.4 (*c* 0.5, $CHCl_3$). ^1H NMR (400 MHz, d_6-DMSO) δ 0.81 (3 H, d, *J* 6.4 Hz, CHC*H*$_3$), 0.85 (6 H, t, *J* 6.7 Hz, 2 × CH_2CH_3), 1.06 (2 H, m, CH_2-tuberculostearyl), 1.23–1.42 (49 H, m, 24 × CH_2, 1 × CH), 1.38–1.48 (4 H, m, 2 × β-CH_2), 2.26 (4 H, q, *J* 7.3 Hz, 2 × α-CH_2), 3.07 (1 H, td, *J* 5.4, 9.3 Hz, H3), 3.17 (1 H, ddd, *J* 3.7, 6.5, 9.9 Hz, H2), 3.36–3.56 (4 H, m, H3a',4,6,6), 3.57 (1 H, ddd, *J* 1.8, 5.6, 11.4 Hz, H5), 3.70 (1 H, dd, *J* 5.7, 10.7 Hz, H3b'), 4.15 (1 H, dd, *J* 7, 12 Hz, H1a'), 4.32 (1 H, dd, *J* 2.7, 12 Hz, H1b'), 4.41 (1 H, t, *J* 5.8 Hz, OH), 4.64 (1 H, d, *J* 3.6 Hz, H1), 4.68 (1 H, d, *J* 6.5 Hz, OH), 4.76 (1 H, d, *J* 4.8 Hz, OH), 4.87 (1 H, d, *J* 5.3 Hz, OH), 5.12 (1 H, m, H2'); ^{13}C NMR (150 MHz, d_6-DMSO) δ 13.9, 19.5, 22.0, 23.5, 24.4, 26.3, 28.4, 28.7, 29.0, 29.3, 31.3, 32.1, 33.3, 33.5, 36.4 (fatty acyl), 60.7, 62.3, 64.9, 65.0, 68.5, 69.6, 69.9, 71.7, 72.9, 73.0, 98.9 (C1), 172.3 (sn-2-C=O), 172.5 (sn-1-C=O); HRMS (ESI^+) calcd for $C_{44}H_{88}NO_{10}$ $(M+NH_4)^+$ 790.6403. Found 790.6402.

1'-*O*-Oleoyl-2'-*O*-palmitoyl-sn-glyceryl α-D-glucopyranoside (7)

A solution of *tert*-butylamine (516 µl, 4.92 mmol) and **17** (257 mg, 0.246 mmol) in CH_2Cl_2 (1:6 ml) and MeOH (6:5 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 **7** as a white semi-solid (110 mg, 59%). $[\alpha]_D^{24}$ +48.1 (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$:CD_3OD) δ 0.66 (6 H, t, *J* 6.8 Hz, CH_2CH_3), 0.98–1.16 (44 H, m, alkyl), 1.35–1.43 (4 H, m, β-CH_2), 1.76–1.83 (4 H, m, CH_2CH=CHCH_2), 2.07–2.13 (4 H, m, α-CH_2), 3.17 (1 H, t, *J* 9.4 Hz, H4), 3.21 (1 H, dd, *J* 3.8, 9.7 Hz, H2), 3.33 (1 H, ddd, *J* 3.0, 4.3, 9.9 Hz, H5), 3.38–3.45 (2 H, m, H3,3a'), 3.50–3.59 (2 H, m, H6,6), 3.61 (1 H, dd, *J* 5.6, 10.8 Hz, H3b'), 3.95 (1 H, dd, *J* 6.4, 12.0 Hz, H1a'), 4.19–4.23 (1 H, m, H1b'), 4.60 (1 H, d, *J* 3.8 Hz, H1), 5.00–5.06 (1 H, m, H2'), 5.07–5.16 (2 H, m, HC=CH); ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD) δ 13.6, 22.4, 24.60, 24.62, 26.88, 26.91, 28.83, 28.84, 28.85, 28.93, 29.02, 29.04, 29.1, 29.2, 29.37, 29.39, 29.42, 29.44, 29.47, 31.6, 31.7, 33.8, 34.0 (fatty acyl), 61.2 (C6), 62.4 (C1'), 65.8 (C3'), 69.8, 69.9 (C4,2'), 71.8 (C2), 72.0 (C5), 73.5 (C3), 99.1 (C1), 129.4, 129.7 (HC=CH), 173.4 (sn-2-C=O), 173.8 (sn-1-C=O); HRMS (ESI^+) calcd for $C_{43}H_{80}O_{10}$ $(M + NH_4)^+$ 774.6090. Found 774.6069.

1'-*O*-Palmitoyl-2'-*O*-oleoyl-sn-glyceryl α-D-glucopyranoside (8)

A solution of *tert*-butylamine (438 µl, 4.17 mmol) and **18** (218 mg, 0.209 mmol) in CH_2Cl_2 (1.3 ml) and MeOH (5.5 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 **8** as a white semi-solid (128 mg, 81%). $[\alpha]_D^{24}$ +49.3(*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD) δ 0.72 (6 H, t, *J* 7.0 Hz, CH_2CH_3), 1.03–1.22 (44H, m, alkyl), 1.39–1.50 (4 H, m, β-CH_2), 1.81–1.89 (4 H, m, CH_2CH=$CHCH_2$), 2.12–2.20 (4 H, m, α-CH_2), 3.24 (1 H, t, *J* 9.5 Hz, H4), 3.27 (1 H, dd, *J* 3.7, 9.7 Hz, H2), 3.39 (1 H, ddd, *J* 3.7, 10.0 Hz, H5), 3.44–3.50 (2 H, m, H3,3'), 3.58–3.64 (2 H, m, H6a,6b), 3.67 (1 H, dd, *J* 5.5, 10.8 Hz, H3'), 3.99–4.03 (1 H, m, H1a'), 4.26 (1 H, dd, *J* 3.4, 12.0 Hz, H1b'), 4.66 (1 H, d, *J* 3.7 Hz, H1), 5.06–5.13 (1 H, m, H2'), 5.14–5.24 (2 H, m, HC=CH); ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD) δ 13.8, 22.5, 24.7, 27.01, 27.04, 28.9, 28.97, 28.99, 29.1, 29.15, 29.20, 29.35, 29.36, 29.48, 29.50, 29.51, 29.54, 29.57, 29.60, 31.7, 31.8, 34.0, 34.1 (fatty acyl), 61.3 (C6), 62.4 (C1'), 66.0 (C3'), 69.90, 69.95 (C4, C2'), 71.9 (C2), 72.0 (C5), 73.6 (C3), 99.1 (C1), 129.5, 129.8 (HC=CH), 173.5 (sn-2-C=O), 173.9 (sn-1-C=O); HRMS (ESI$^+$) calcd for $C_{43}H_{80}O_{10}$ $(M + NH_4)^+$ 774.6090. Found 774.6089.

1'-*O*-((*R*)-10-Tuberculostearyl)-2-*O*-palmitoyl-sn-glyceryl α-D-glucopyranosiduronic acid (1)

(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO) (0.53 mg, 3.42 µmol), BAIB (12.2 mg, 0.037 mmol) and $NaHCO_3$ (7.2 mg, 0.0855 mmol) were added to a solution of **5** (13.2 mg, 0.017 mmol) in acetonitrile/water (1:1, 1.0 ml) at 0 °C and stirred vigorously for 24 h. Additional TEMPO (0.53 mg, 3.42 µmol) and BAIB (12.2 mg, 0.037 mmol) were added after 24 h and stirring was continued overnight. The reaction mixture was quenched with 2M sodium thiosulfate, acidified with HCl and extracted with diethyl ether. The organic portion was dried over (Na_2SO_4), filtered and concentrated under reduced pressure. Flash chromatography of the residue (70:20:1 MeOH/$CHCl_3$/H_2O and 1% acetic acid) afforded **1** as a colorless semisolid (13.2 mg, 85%). $[\alpha]_D^{26}$ +35.8 (*c* 0.25, DMSO). ^{1}H NMR (500 MHz, d_6-DMSO) δ 0.79 (3 H, d, *J* 6.4 Hz, $CHCH_3$), 0.83 (6 H, t, *J* 6.7 Hz, 2 × CH_2CH_3), 1.04 (2 H, m, CH_2-tuberculostearyl), 1.21–1.35 (49 H, m, 24 × CH_2, 1 × CH), 1.38–1.48 (4 H, m, 2 × β-CH_2), 2.21–2.27 (4 H, m, 2 × α-CH_2), 3.22–3.24 (1 H, m, H2), 3.54 (1 H, m, H3a'), 3.69 (1 H, m, H3b'), 3.76 (1 H, d, *J* 10.1 Hz, H5), 4.15 (1 H, dd, *J* 7.2, 12 Hz, H1a'), 4.32 (1 H, dd, *J* 2.7, 12 Hz, H1b'), 4.68 (1 H, s, H1), 4.76 (1 H, d, *J* 4.8 Hz, OH), 4.87 (1 H, d, *J* 5.3 Hz, OH), 5.11–5.21 (1 H, m, H2'); ^{13}C NMR (150 MHz, d_6-DMSO) δ 13.8, 19.6 ($CHCH_3$ (tuberculostearyl), 22.1, 24.33, 24.35, 26.30, 26.33, 28.33, 28.35, 28.58, 28.60, 28.64, 28.8, 28.9, 29.2, 31.1, 31.9, 33.3, 33.4, 36.33, 36.35, 36.37, 62.1, 65.4, 69.5 (C1',2',3'), 71.3, 71.7, 71.8, 72.6, 99.3 (C1), 170.9 (C6), 172.2, 172.4 (C=O); HRMS (ESI$^+$) calcd for $C_{44}H_{82}O_{11}Na$ $[M+Na]^+$ 809.5749. Found 809.5745.

1'-*O*-((*S*)-10-Tuberculostearyl)-2-*O*-palmitoyl-sn-glyceryl α-D-glucopyranosiduronic acid (2)

TEMPO (0.34 mg, 1.99 µmol), bis(acetoxy)iodobenzene (BAIB) (7.97 mg, 0.0219 mmol) and $NaHCO_3$ (4.72 mg, 0.0498 mmol) were added to a solution of **6** (7.7 mg, 0.001 mmol) in acetonitrile/water (1:1, 1.0 ml) at 0 °C and stirred vigorously for 24 h. Additional TEMPO (0.34 mg, 1.99 µmol), BAIB (7.97 mg, 0.0219 mmol) were added after 24 h and stirred overnight. The reaction was quenched with 2M sodium thiosulfate, acidified with HCl and extracted with diethyl ether. The organic layer was dried over (Na_2SO_4), filtered and concentrated under reduced pressure. Flash chromatography of the residue (70:20:1 MeOH/$CHCl_3$/H_2O and 1% acetic acid) afforded **2** as a colorless semisolid (5.4 mg, 70%). $[\alpha]_D^{26}$ +45.4 (*c* 0.6, $CHCl_3$). ^{1}H NMR (500 MHz, d_6-DMSO) δ 0.79 (3 H, d, *J* 6.4 Hz, CHC*H*$_3$), 0.83 (6 H, t, *J* 6.7 Hz, 2 × CH_2C*H*$_3$), 1.04 (2 H, m, CH_2-tuberculostearyl)), 1.21–1.35 (49 H, m, 24 × CH_2, 1 × CH), 1.38–1.48 (4 H, m, 2 × β-CH_2), 2.21–2.27 (4 H, m, 2 × α-CH_2), 3.22–3.24 (1 H, m, H2), 3.54 (1 H, m, H3a'), 3.69 (1 H, m, H3b'), 3.76 (1 H, d, *J* 10.1 Hz, H5), 4.15 (1 H, dd, *J* 7.2, 12 Hz, H1a'), 4.32 (1 H, dd, *J* 2.7, 12 Hz, H1b'), 4.68 (1 H, s, H1), 4.76 (1 H, d, *J* 4.8 Hz, OH), 4.87 (1 H, d, *J* 5.3 Hz, OH), 5.11–5.21 (1 H, m, H2'); ^{13}C NMR (150 MHz, d_6-DMSO) δ 13.8, 19.6 (CH*C*H$_3$ (tuberculostearyl)), 22.1, 24.33, 24.35, 26.3, 26.33, 28.33, 28.35, 28.58, 28.60, 28.64, 28.8, 28.9, 29.2, 31.1, 31.9, 33.3, 33.4, 36.33, 36.35, 36.37, 62.1, 65.4, 69.5 (C1',2',3'), 71.3, 71.7, 71.8, 72.6, 99.3 (C1), 170.9 (C6), 172.2, 172.4 (C=O); HRMS (ESI$^+$) calcd for $C_{44}H_{82}O_{11}Na$ $[M+Na]^+$ 809.5749. Found 809.5746.

1'-*O*-Oleoyl-2'-*O*-palmitoyl-sn-glyceryl α-D-glucopyranosiduronic acid (3)

Bobbitt's salt [4-(acetylamino)-2,2,6,6-tetramethyl-1-oxo-piperidinium tetrafluoroborate] (30 mg, 0.099 mmol) was added to a vigorously stirred solution of **7** (25 mg, 0.033 mmol) in MeCN (266 µl) and aq. $KHCO_3$ (1.5 M, 133 µl). The mixture was stirred for 2 d and then concentrated. Sequential flash chromatography of the residue ($CHCl_3$/MeOH/H_2O/TFA(0.1%) and then toluene:EtOAc:MeOH:H_2O:TFA(0.1%)) afforded **3** as a colourless glass (13 mg, 50%). $[\alpha]_D^{24}$ +28.7 (*c* 0.45, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD:TFA 95:5:0.1) δ 0.81 (6 H, t, *J* 6.9 Hz, CH_2C*H*$_3$), 1.12–1.34 (44 H, m, alkyl), 1.48–1.60 (4 H, m, β-CH_2), 1.91–1.99 (4 H, m, C*H*$_2$CH=CHC*H*$_2$), 2.22–2.30 (4 H, m, α-C*H*$_2$), 3.47–3.67 (4 H, m, H2,3,4,3'), 3.80 (1 H, dd, *J* 5.1, 10.8 Hz, H3'), 4.03 (1 H, d, *J* 9.4 Hz, H5), 4.10 (1 H, dd, *J* 6.2, 12.0 Hz, H1'), 4.34 (1 H, dd, *J* 3.6, 12.0 Hz, H1'), 4.85 (1 H, s, H1), 5.15–5.21 (1 H, m, H2'), 5.23–5.35 (2 H, m, HC=CH); ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD:TFA 95:5:0.1) δ 14.1, 22.7, 24.85, 24.88, 27.19, 27.22, 29.12, 29.16, 29.22, 29.33, 29.37, 29.53, 29.67, 29.72, 29.74, 29.77, 31.9, 34.1, 34.2 (fatty acyl), 62.4, 66.8, 69.9, 71.4, 71.6, 73.1, 99.3 (C1), 129.7, 130.0 (HC=CH), 172.1 (C6), 173.6 (sn-2-C=O), 174.0 (sn-1-C=O); HRMS (ESI$^+$) calcd for $C_{43}H_{78}O_{11}$ $(M - H)^-$ 769.5471. Found 769.5478.

1'-*O*-Palmitoyl-2'-*O*-oleoyl-sn-glyceryl α-D-glucopyranosiduronic acid (4)

Bobbitt's salt (54 mg, 0.180 mmol) was added to a vigorously stirred solution of **8** (34 mg, 0.045 mmol) in MeCN (420 µl) and aq. $KHCO_3$ (1.5 M, 180 µl). The mixture was stirred for 2 d and then concentrated. Sequential flash chromatography of the residue ($CHCl_3$/MeOH/H_2O/TFA(0.1%) and toluene:EtOAc:MeOH:H_2O:TFA(0.1%)) afforded **4** as a colourless glass (16 mg, 45%). $[\alpha]_D^{24}$ +33.0 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD:TFA 95:5:0.1) 0.82 (6 H, t, *J* 6.9Hz, CH_2CH_3),1.11–1.35 (44 H, m, alkyl), 1.47–1.61 (4 H, m, β-CH_2), 1.89–2.01 (4 H, m, CH_2CH=$CHCH_2$), 2.19–2.33 (4 H, m, α-CH_2), 3.43–3.70 (4 H, m, H2,3,4,3'), 3.82 (1 H, s, H3'), 4.04 (1 H, s, H5), 4.11 (1 H, dd, *J* 5.9, 12.1 Hz, H1'), 4.35 (1 H, dd, *J* 3.3, 12.1 Hz, H1'), 4.89 (1 H, s, H1), 5.19 (1 H, s, H2'), 5.24–5.35 (2 H, m, HC=CH); ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD:TFA 95:5:0.1) δ 14.0, 22.57, 22.58, 24.75, 24.77, 27.09, 27.12, 28.98, 29.03, 29.06, 29.13, 29.20, 29.22, 29.26, 29.41, 29.43, 29.55, 29.56, 29.58, 29.60, 29.64, 29.67, 31.81, 31.83, 34.0, 34.1 (fatty acyl), 62.3, 66.7, 69.7, 71.3, 71.5, 73.1, 99.3 (C1), 129.6, 129.9 (HC=CH), 171.7 (C6), 173.5 (sn-2-C=O), 173.9 (sn-1-C=O); HRMS (ESI$^+$) calcd for $C_{43}H_{78}O_{11}$ $(M - H)^-$ 769.5471. Found 769.5455.

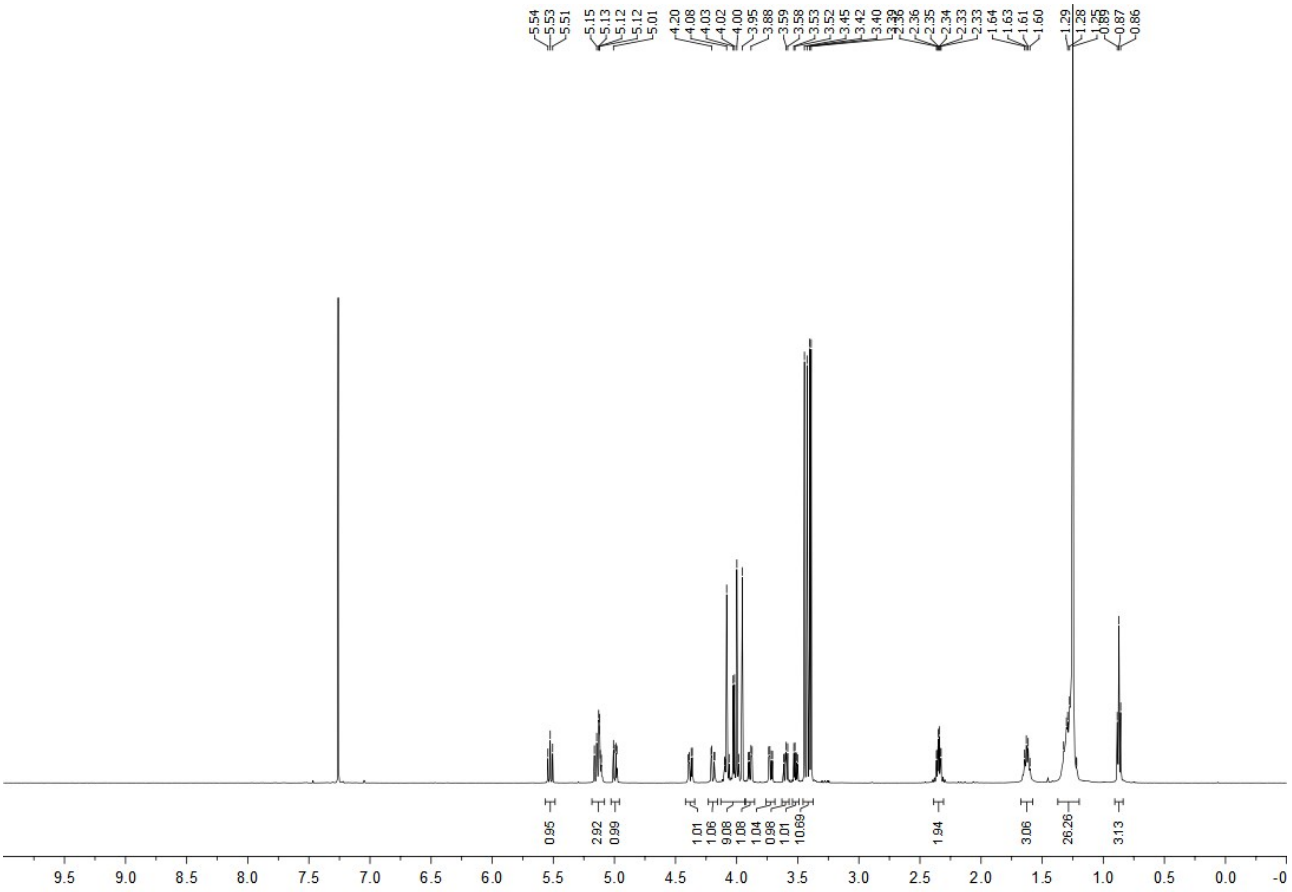
References

1. A. P. Uldrich, O. Patel, G. Cameron, D. G. Pellicci, E. B. Day, L. C. Sullivan, K. Kyparissoudis, L. Kjer-Nielsen, J. P. Vivian, B. Cao, A. G. Brooks, S. J. Williams, P. Illarionov, G. S. Besra, S. J. Turner, S. A. Porcelli, J. McCluskey, M. J. Smyth, J. Rossjohn and D. I. Godfrey, *Nat. Immunol.*, 2011, **12**, 616.
2. C. Almeida, S. Sundararaj, J. Le Nours, T. Praveena, B. Cao, S. Burugupalli, D. G. M. Smith, O. Patel, M. Brigl, D. G. Pellicci, S. J. Williams, A. P. Uldrich, D. I. Godfrey and J. Rossjohn, *Nat. Commun.*, 2019, in press.
3. D. G. Pellicci, O. Patel, L. Kjer-Nielsen, S. S. Pang, L. C. Sullivan, K. Kyparissoudis, A. G. Brooks, H. H. Reid, S. Gras, I. S. Lucet, R. Koh, M. J. Smyth, T. Mallevaey, J. L. Matsuda, L. Gapin, J. McCluskey, D. I. Godfrey and J. Rossjohn, *Immunity*, 2009, **31**, 47.
4. N. A. Borg, K. S. Wun, L. Kjer-Nielsen, M. C. Wilce, D. G. Pellicci, R. Koh, G. S. Besra, M. Bharadwaj, D. I. Godfrey, J. McCluskey and J. Rossjohn, *Nature*, 2007, **448**, 44.
5. J. L. Matsuda, O. V. Naidenko, L. Gapin, T. Nakayama, M. Taniguchi, C. R. Wang, Y. Koezuka and M. Kronenberg, *J. Exp. Med.*, 2000, **192**, 741.
6. T. Mallevaey, A. J. Clarke, J. P. Scott-Browne, M. H. Young, L. C. Roisman, D. G. Pellicci, O. Patel, J. P. Vivian, J. L. Matsuda, J. McCluskey, D. I. Godfrey, P. Marrack, J. Rossjohn and L. Gapin, *Immunity*, 2011, **34**, 315.
7. S. Burugupalli, M. B. Richardson and S. J. Williams, *Org. Biomol. Chem.*, 2017, **15**, 7422.
8. J. Holst, A. L. Szymczak-Workman, K. M. Vignali, A. R. Burton, C. J. Workman and D. A. Vignali, *Nat. Protoc.*, 2006, **1**, 406.
9. A. L. Szymczak, C. J. Workman, Y. Wang, K. M. Vignali, S. Dilioglou, E. F. Vanin and D. A. Vignali, *Nat. Biotechnol.*, 2004, **22**, 589.
10. F. Letourneur and B. Malissen, *Eur. J. Immunol.*, 1989, **19**, 2269.
11. A. B. Pangborn, M. A. Giardello, R. H. Grubbs, R. K. Rosen and F. J. Timmers, *Organometallics*, 1996, **15**, 1518.
12. W. C. Still, M. Kahn and A. M. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.
13. I. O. Roberts and M. S. Baird, *Chem. Phys. Lipids*, 2006, **142**, 111.
14. W. Rosenbrook, D. A. Riley and P. A. Lartey, *Tetrahedron Lett.*, 1985, **26**, 3.
15. I. O. Roberts and M. S. Baird, *Chem Phys Lipids*, 2006, **142**, 111.
16. S. Shah, M. Nagata, S. Yamasaki and S. J. Williams, *Chem. Commun.*, 2016, **52**, 10902.

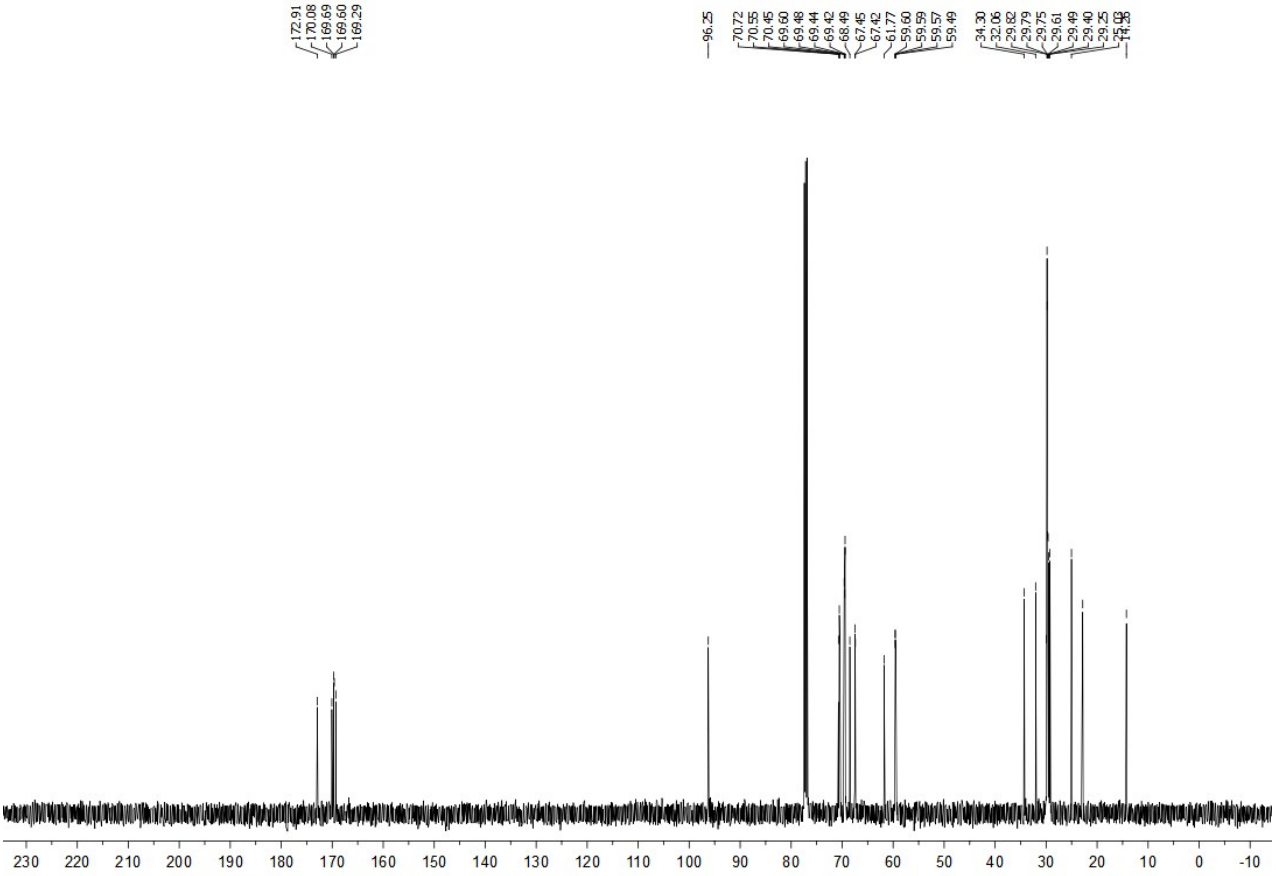
NMR Spectra

(2'*S*)-3'-Bromo-2'-palmitoyloxypropyl 2,3,4,6-tetra-*O*-methoxy-acetyl-α-D-glucopyranoside (13)

^{1}H NMR

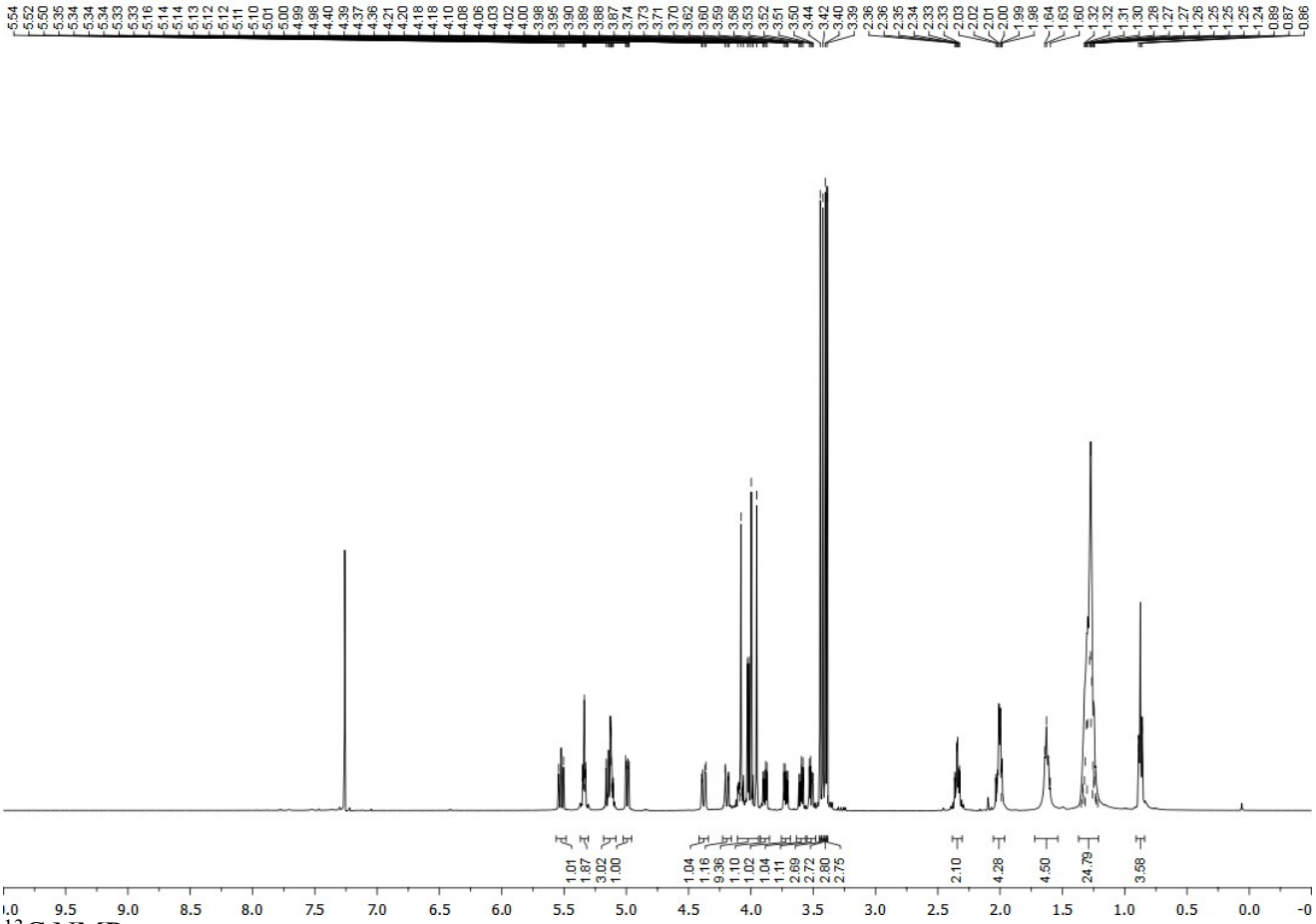


^{13}C NMR



(2'*S*)-3'-Bromo-2'-oleoyloxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (14)

^{1}H NMR



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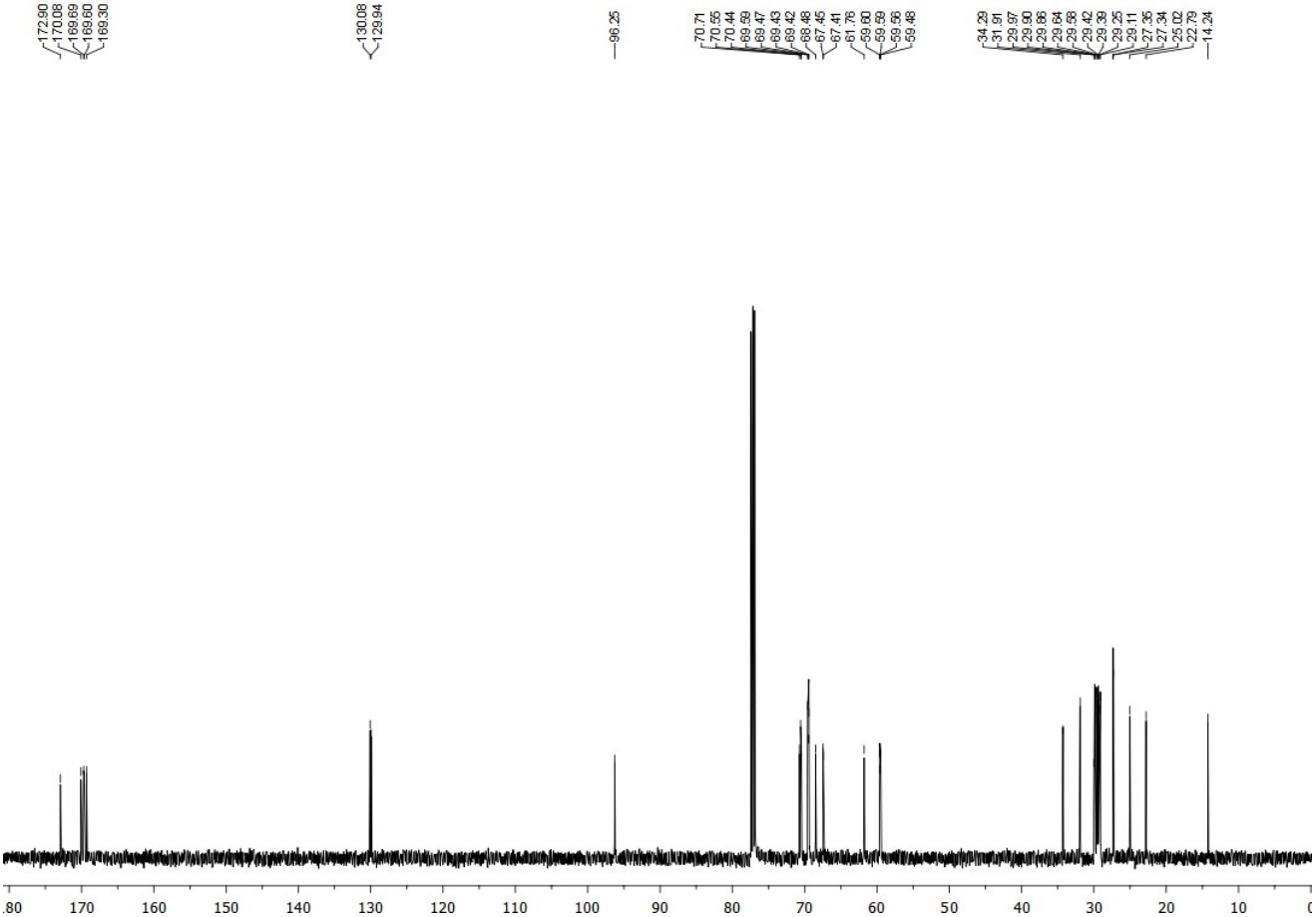

1'-*O*-((*R*)-10-Tuberculostearyl)-2'-*O*-palmitoyl-sn-glyceryl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (15)

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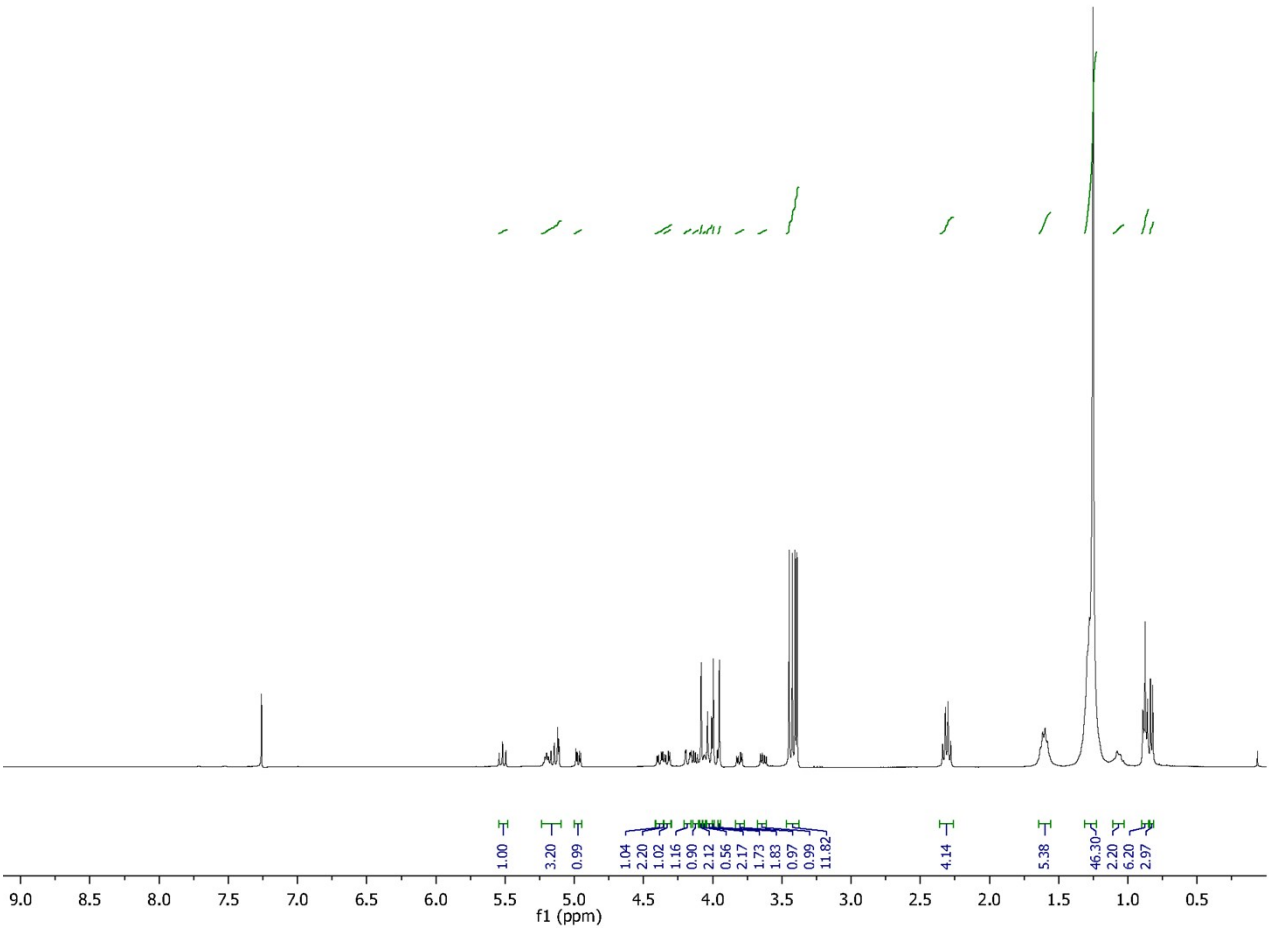

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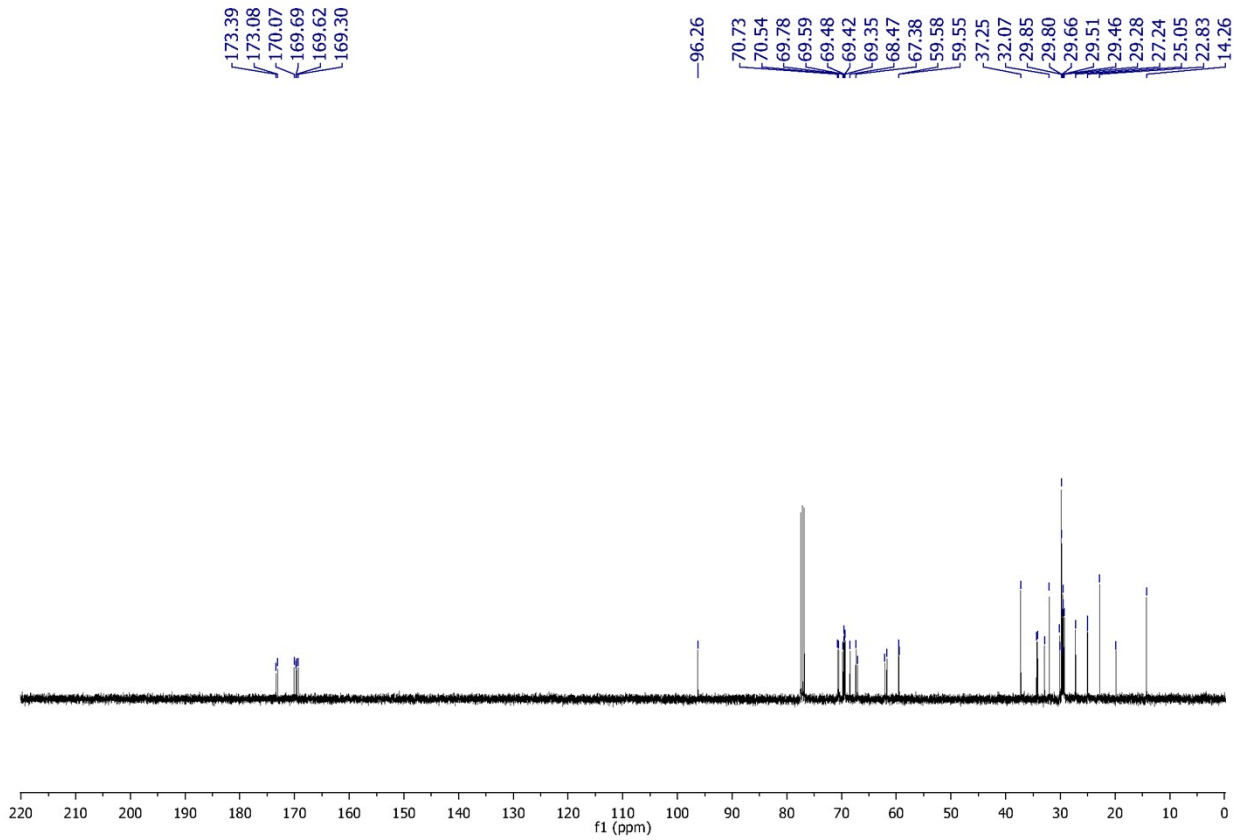
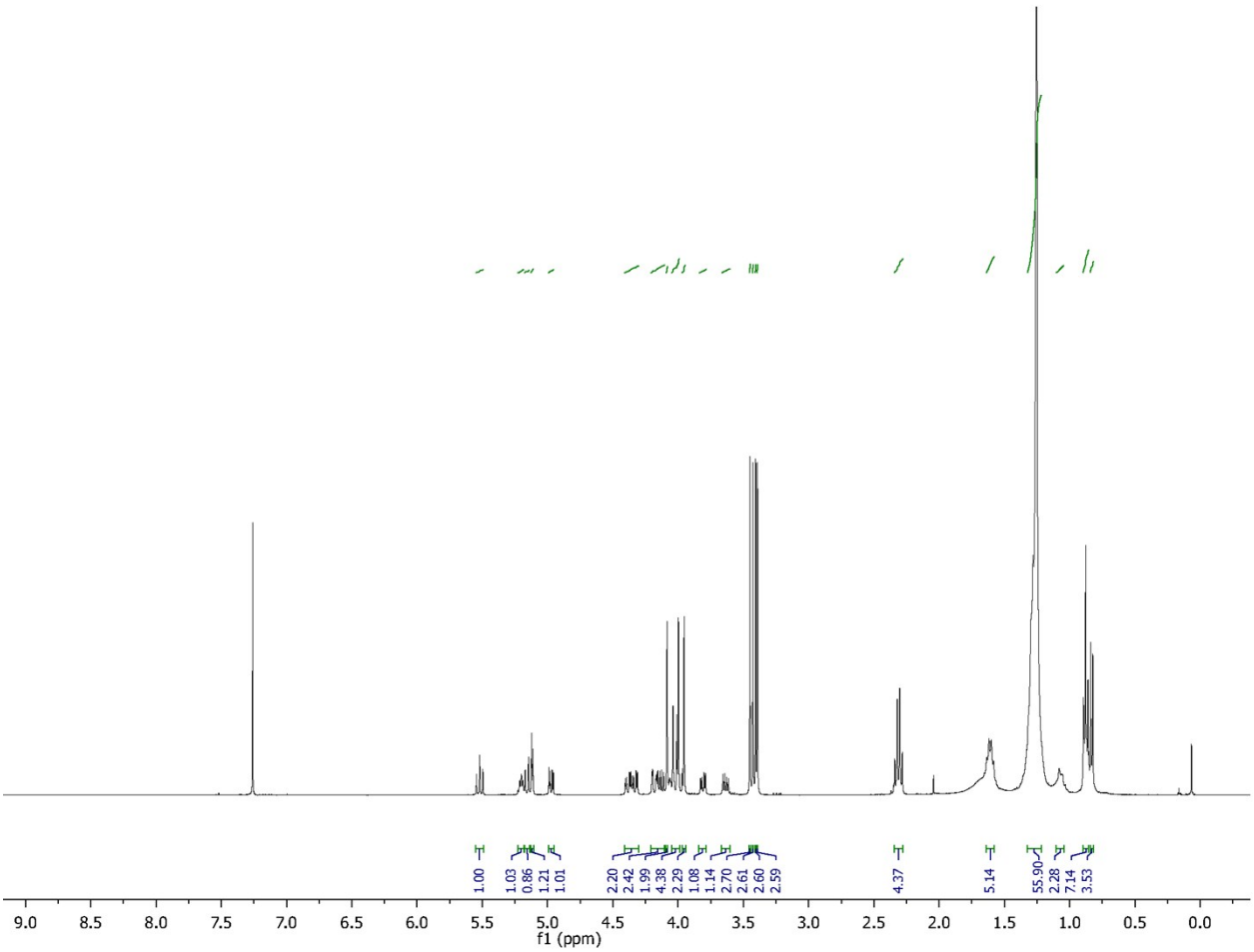


1'-*O*-((*S*)-10-Tuberculostearyl)-2'-*O*-palmitoyl-sn-glyceryl 2,3,4,6-tetra-*O*-methoxyacetyl-α-D-glucopyranoside (16)

^{1}H NMR



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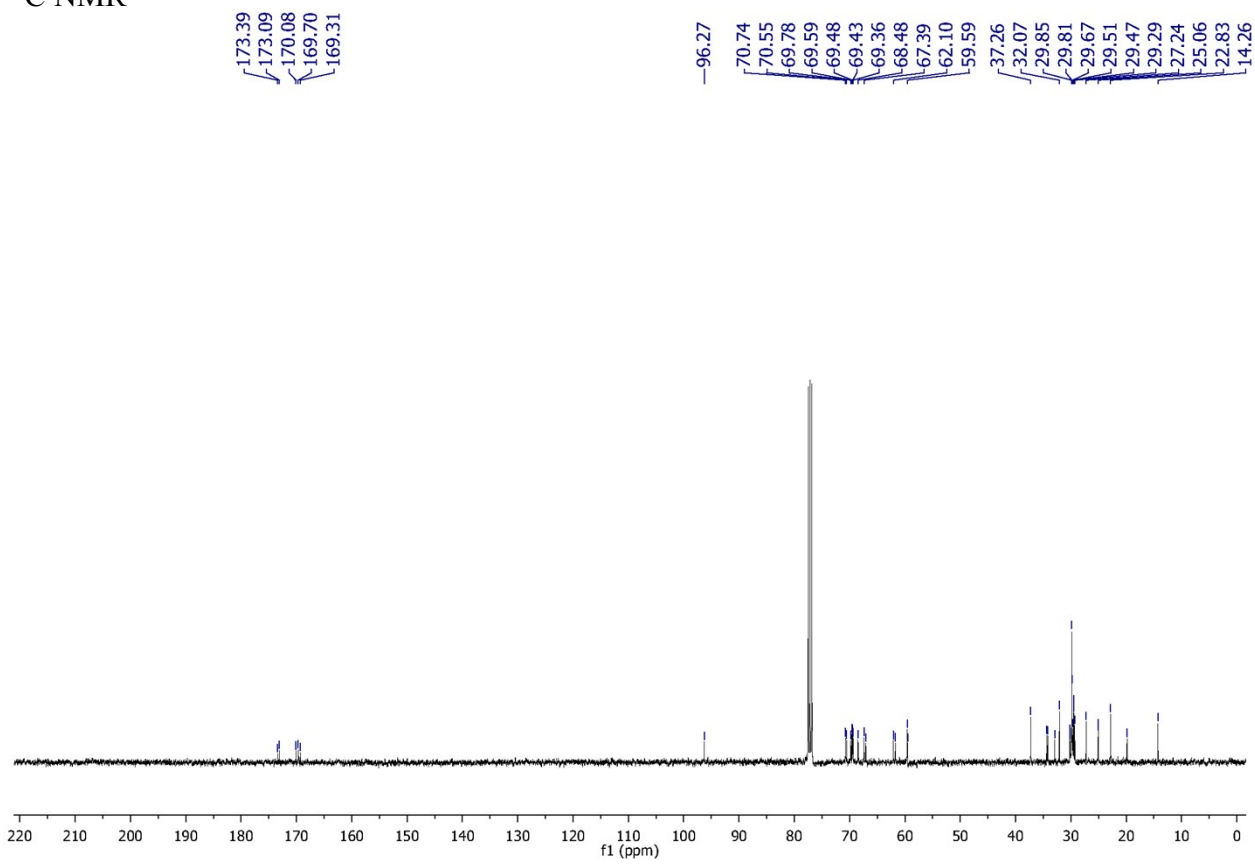
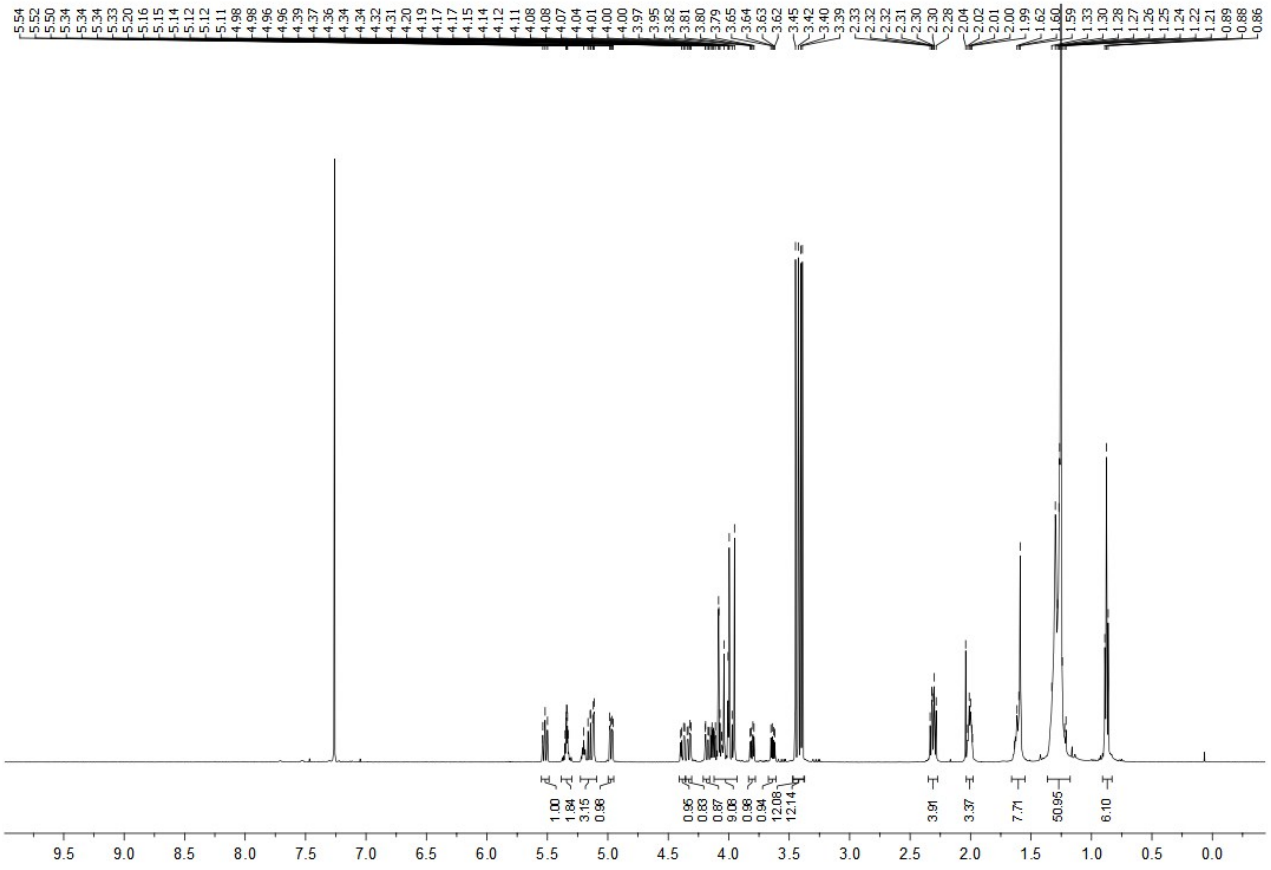


1'-*O*-Oleoyl-2'-*O*-palmitoyl-sn-glyceryl 2,3,4,6-tetra-*O*-methoxy-acetyl-α-D-glucopyranoside (17)

^{1}H NMR



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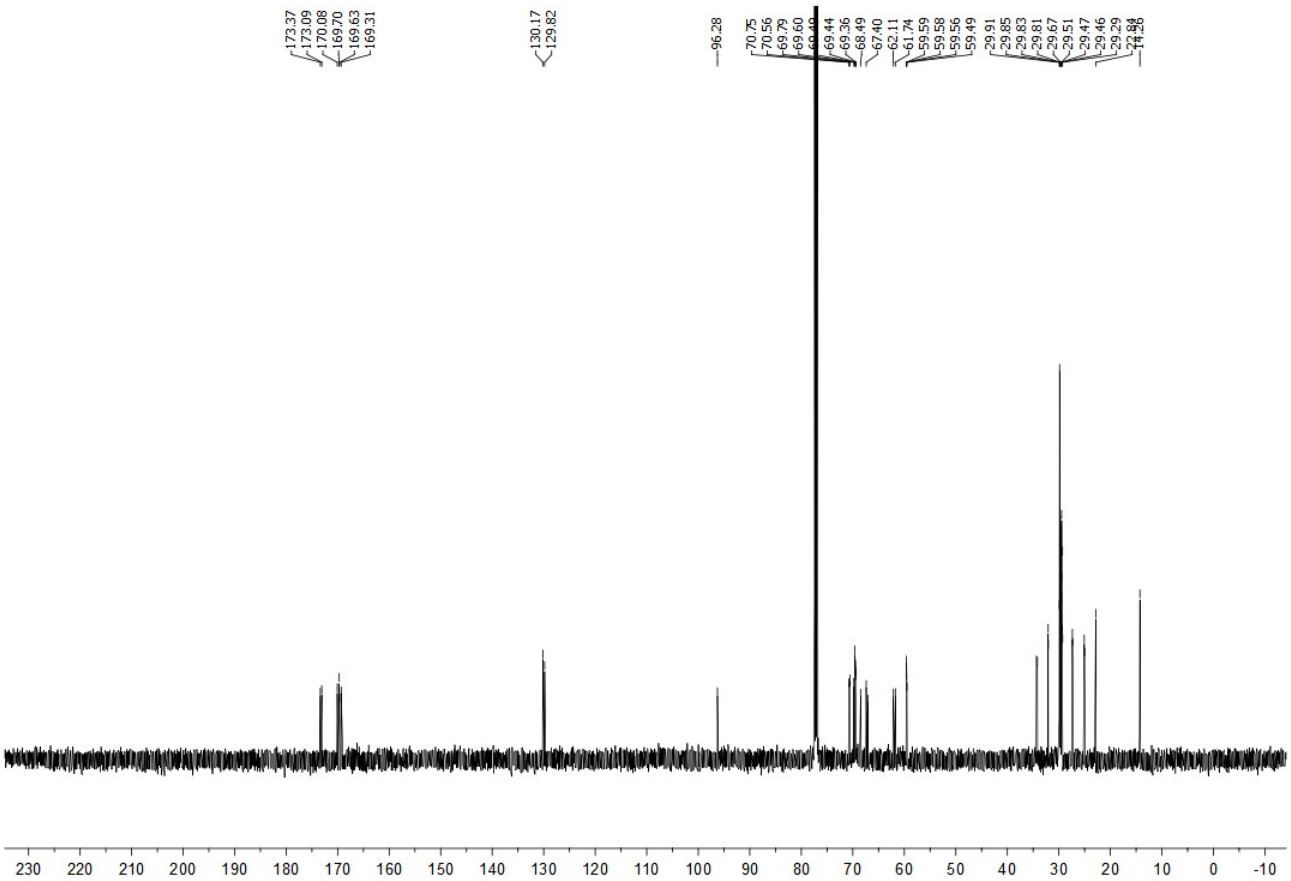
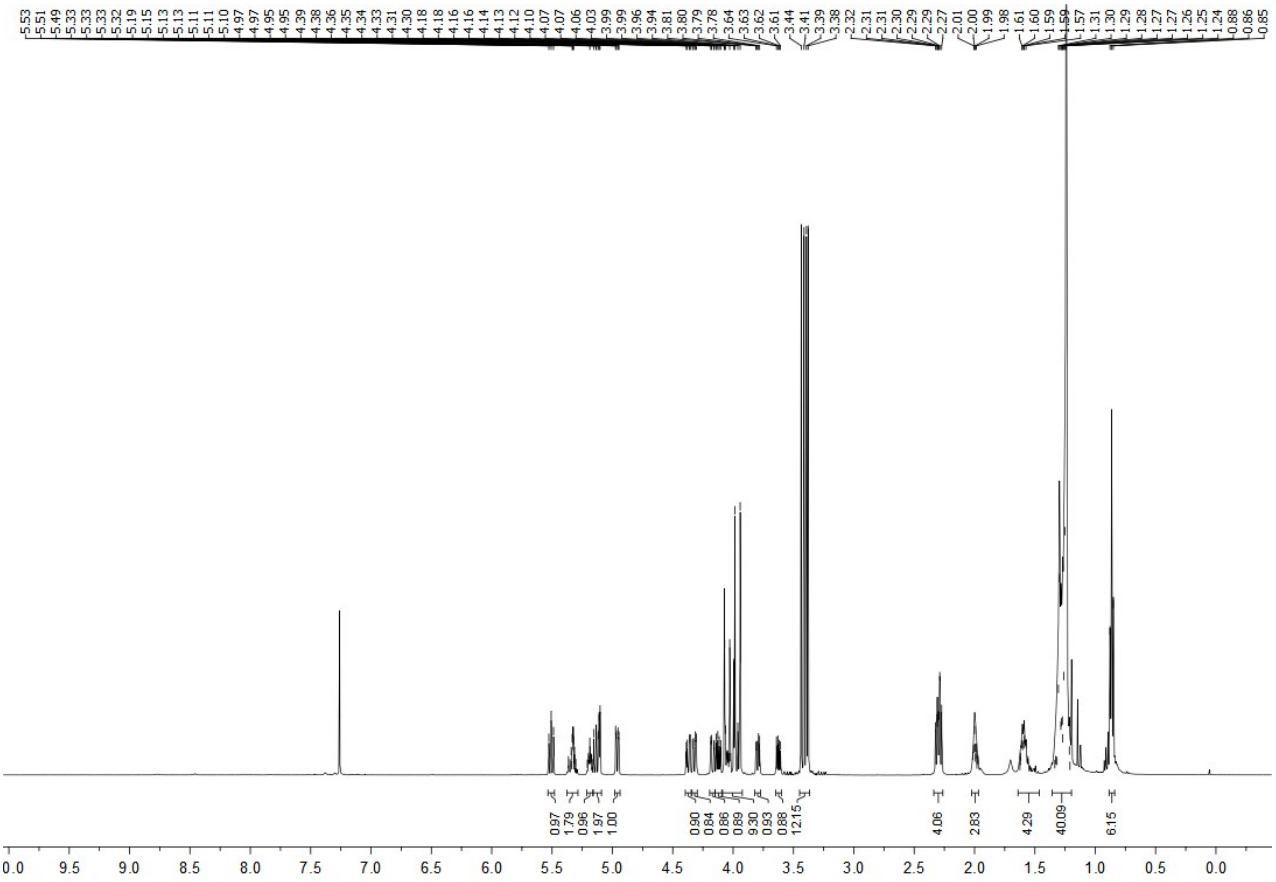


1'-*O*-Palmitoyl-2'-*O*-oleoyl-sn-glyceryl 2,3,4,6-tetra-*O*-methoxy-acetyl-α-D-glucopyranoside (18)

^{1}H NMR



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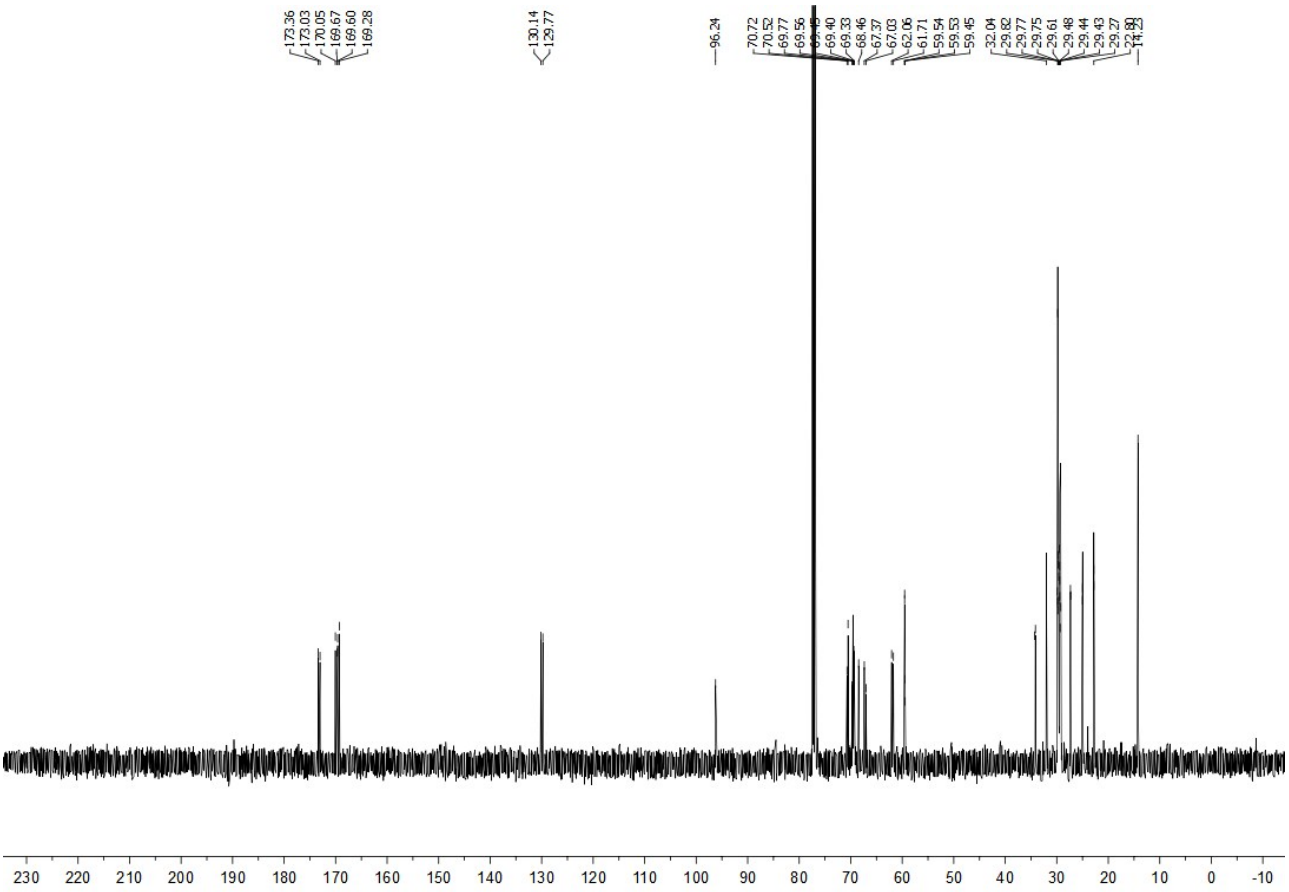
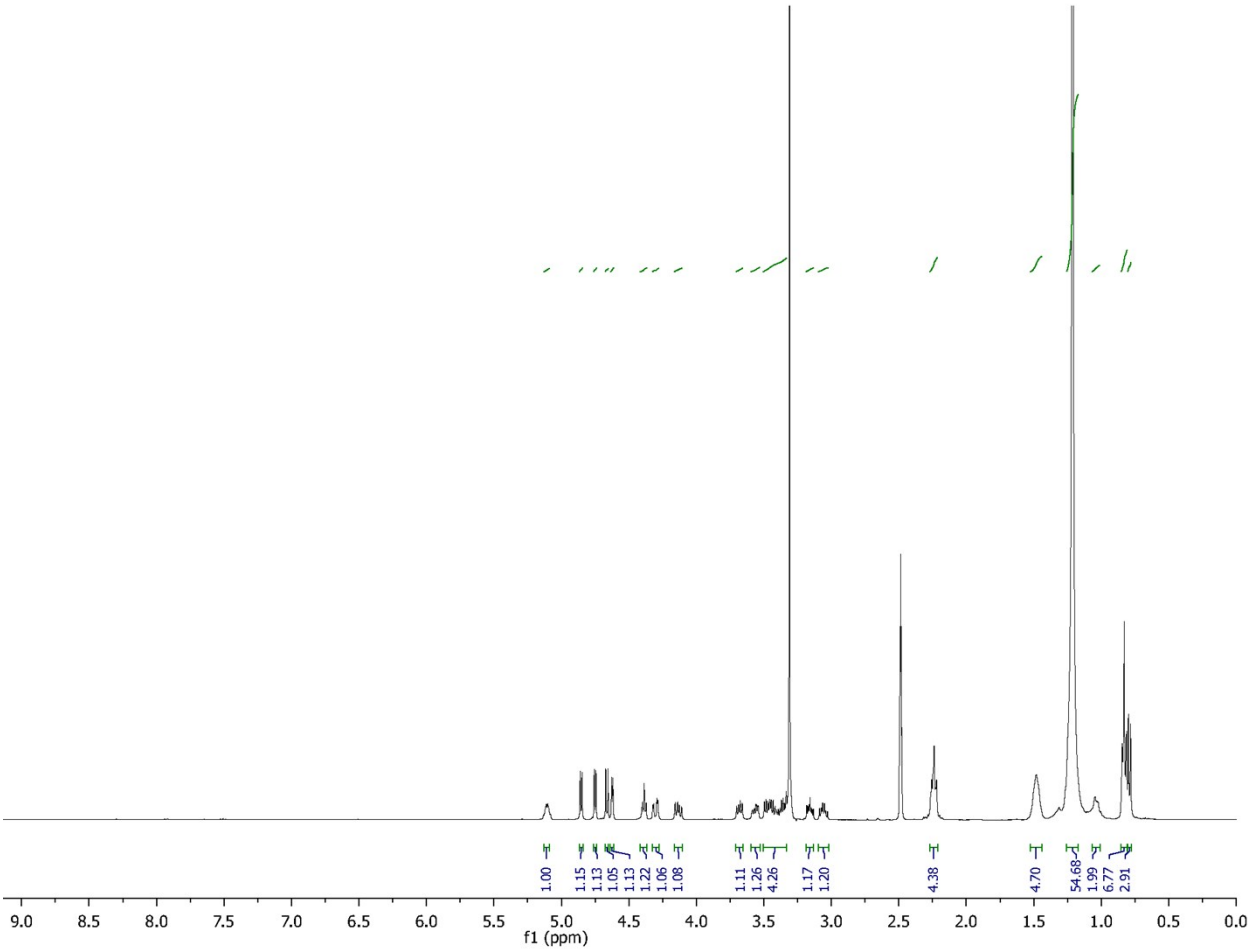
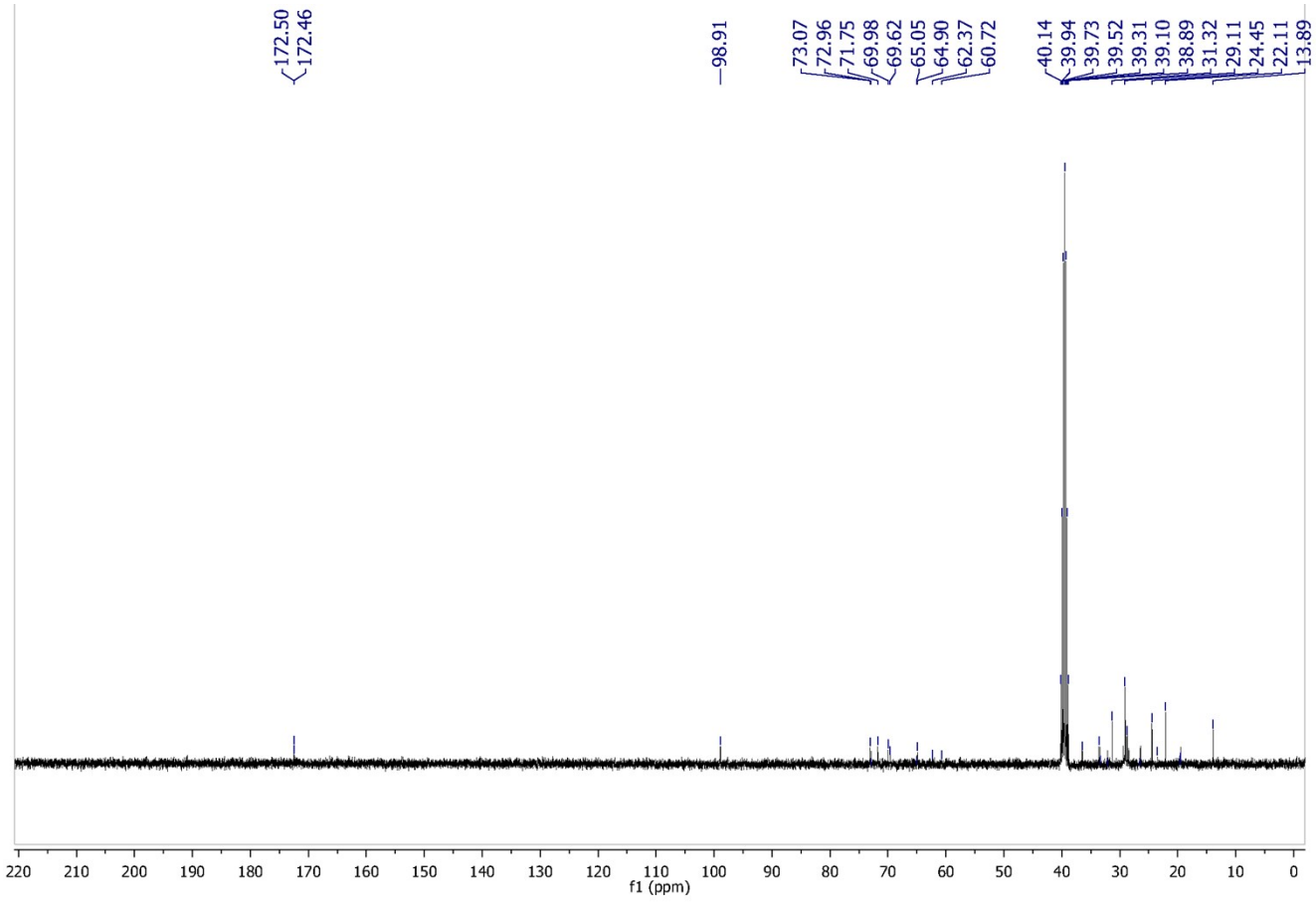


1'-*O*-((*R*)-10-Tuberculostearyl)-2'-*O*-palmitoyl-sn-glyceryl α-D-glucopyranoside (5)

^{1}H NMR

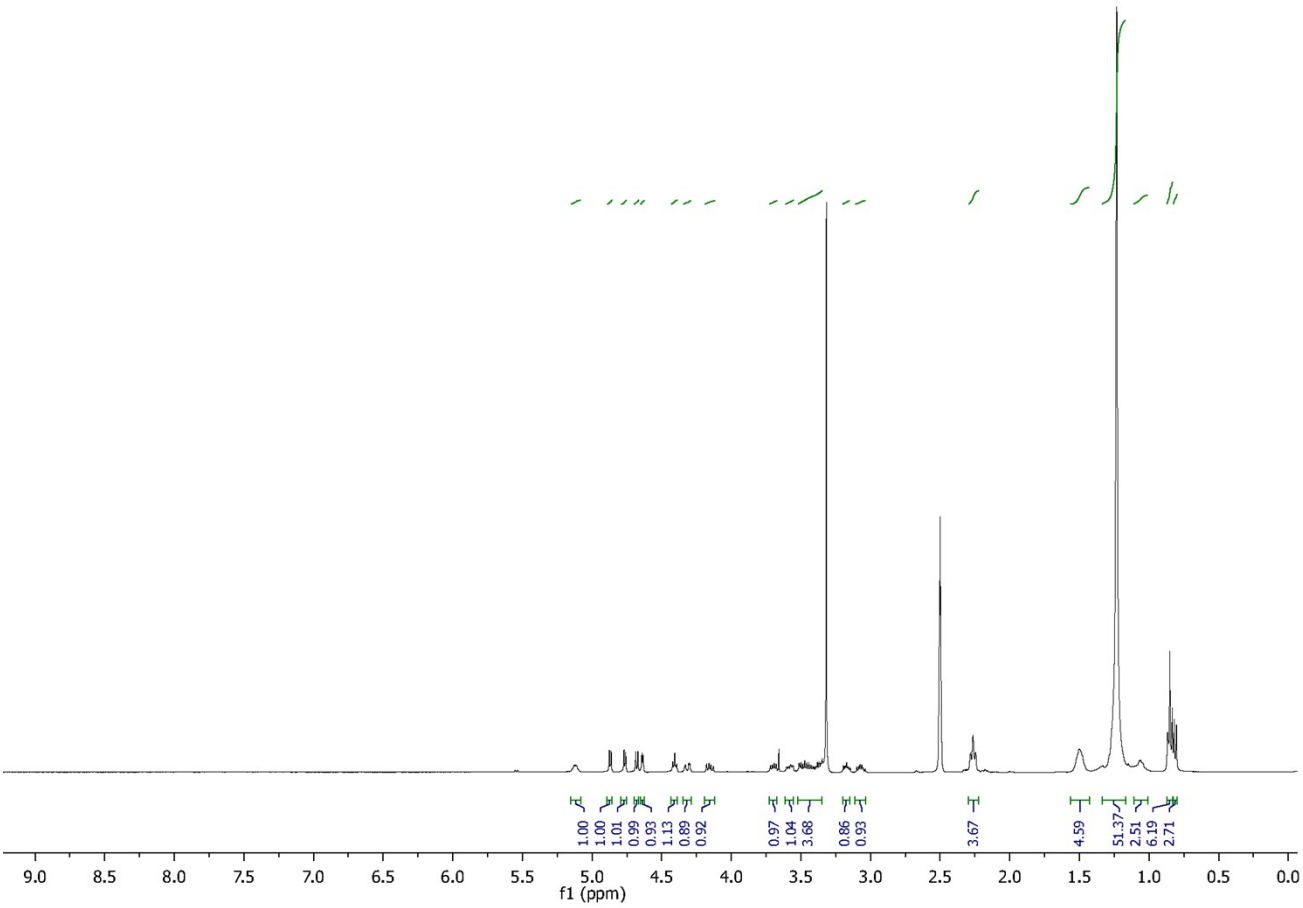


^{13}C NMR



1'-*O*-((*S*)-10-Tuberculostearyl)-2'-*O*-palmitoyl-sn-glyceryl α-D-glucopyranoside (6)

^{1}H NMR



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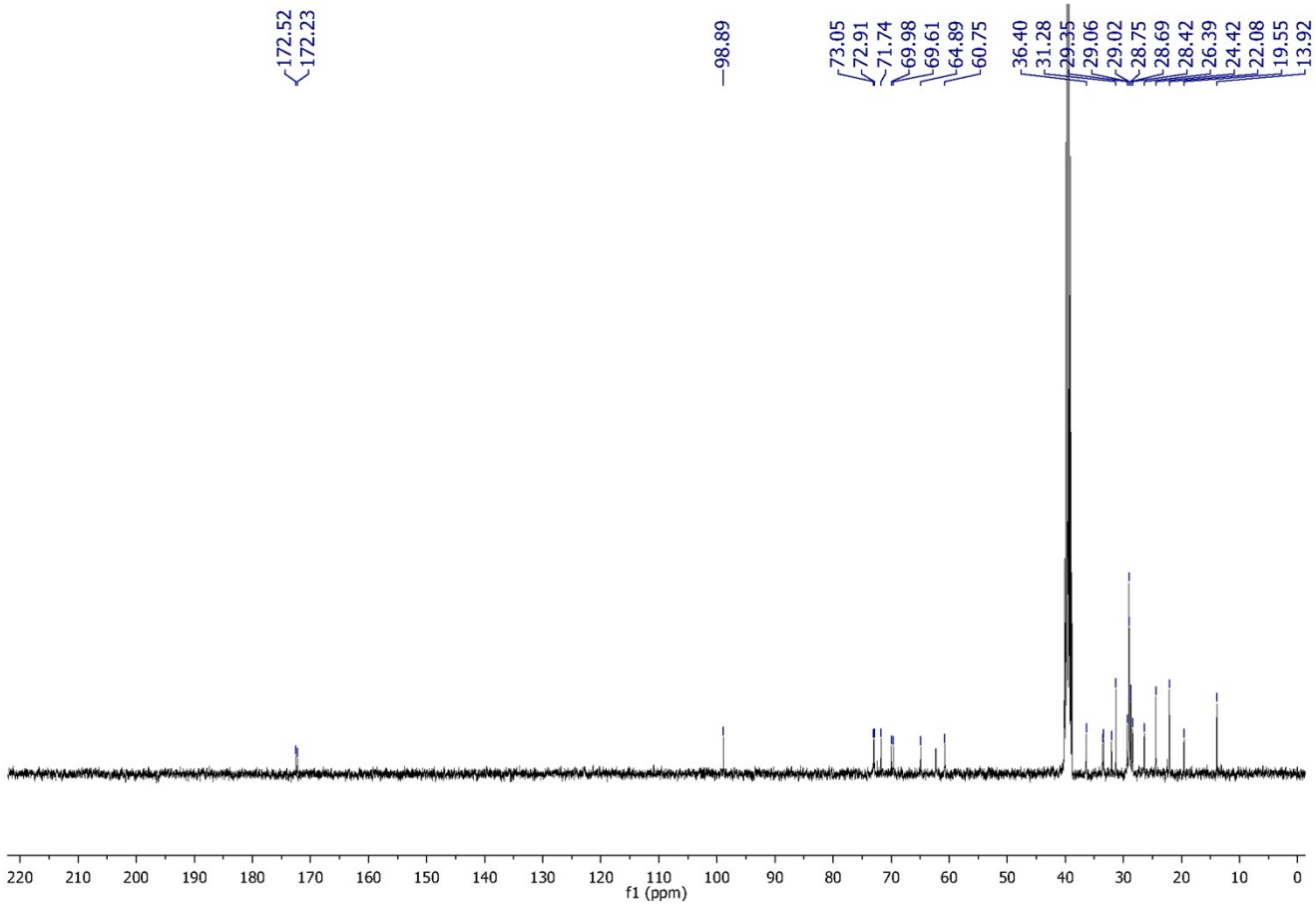
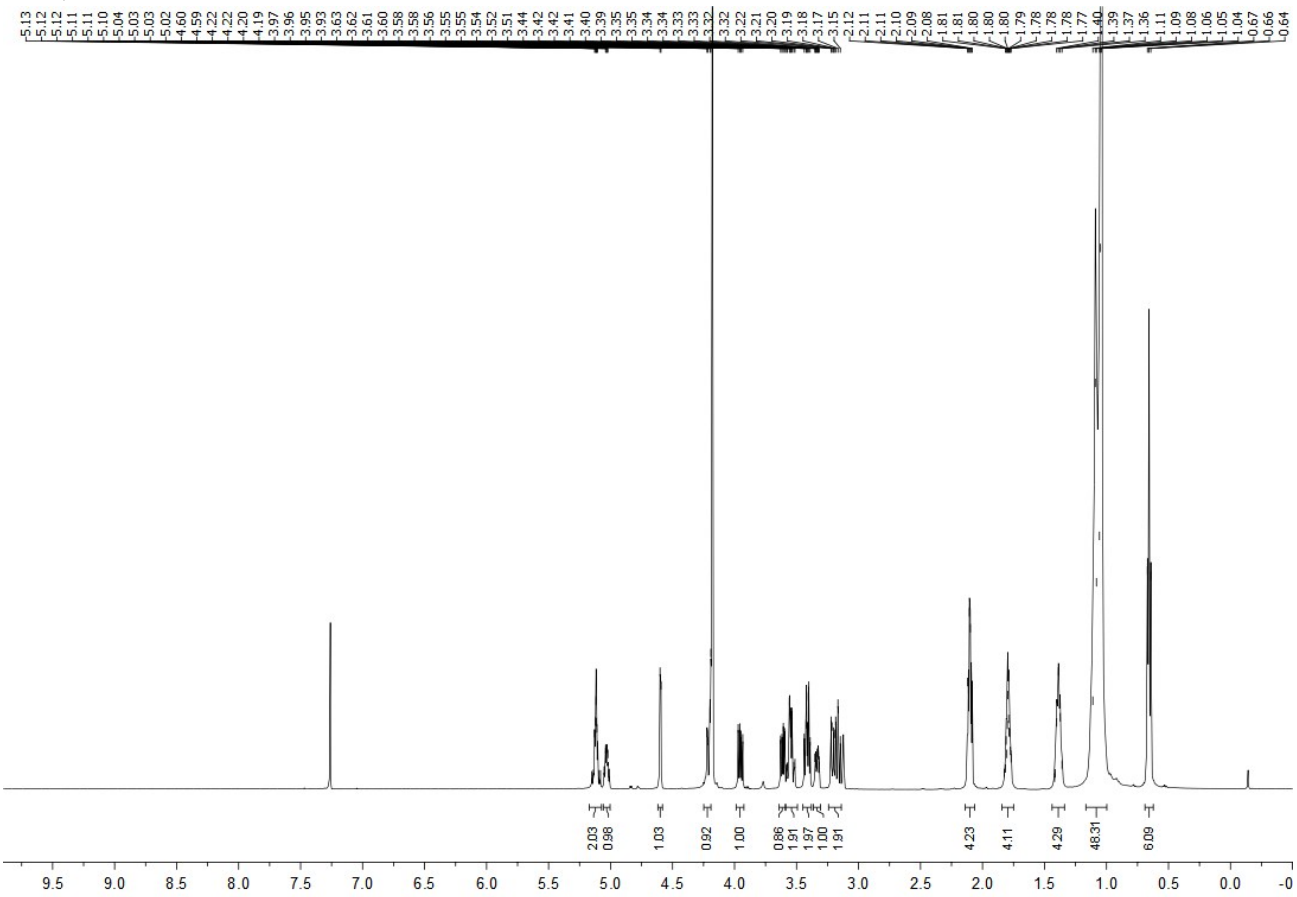


1'-*O*-Oleoyl-2'-*O*-palmitoyl-sn-glyceryl α-D-glucopyranoside (7)

^{1}H NMR



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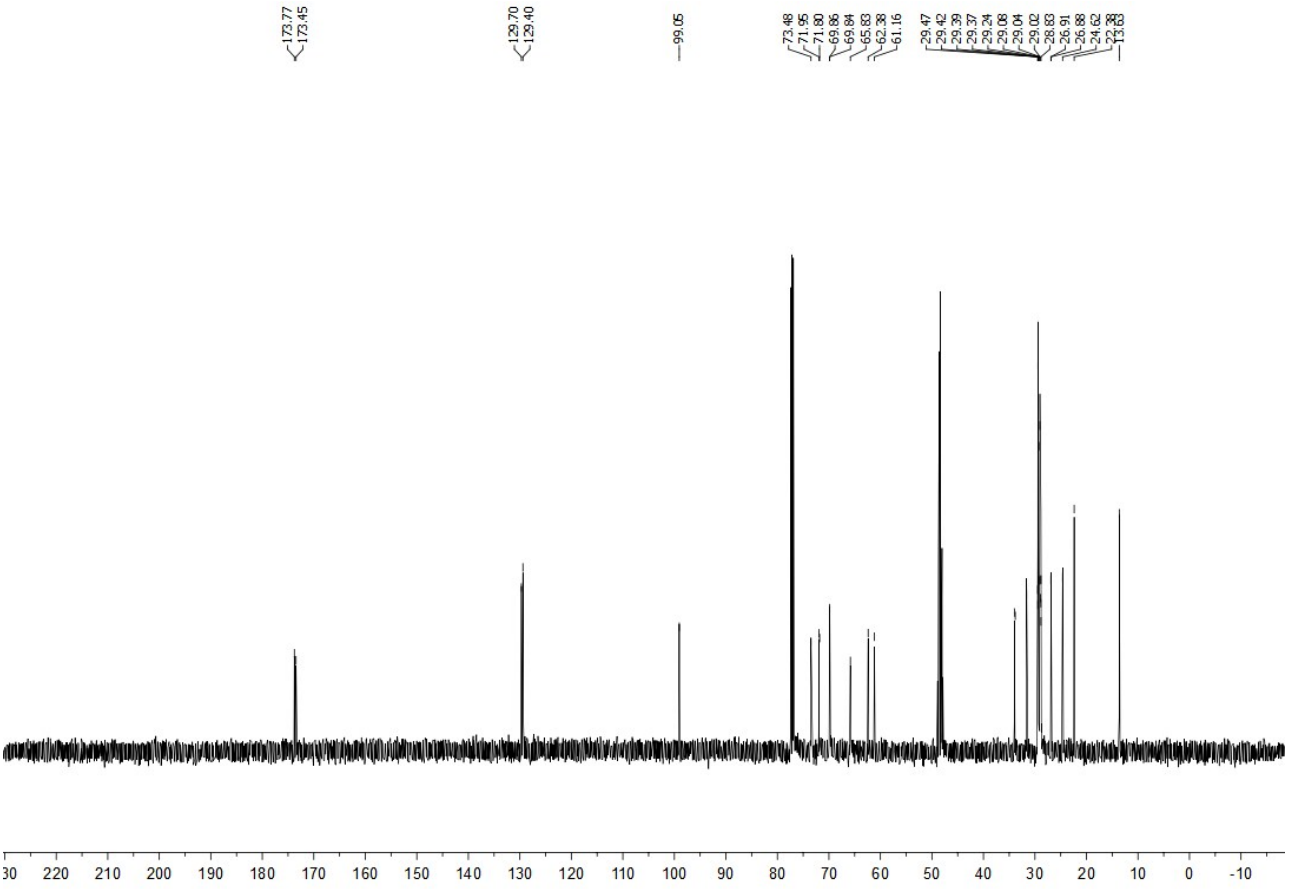
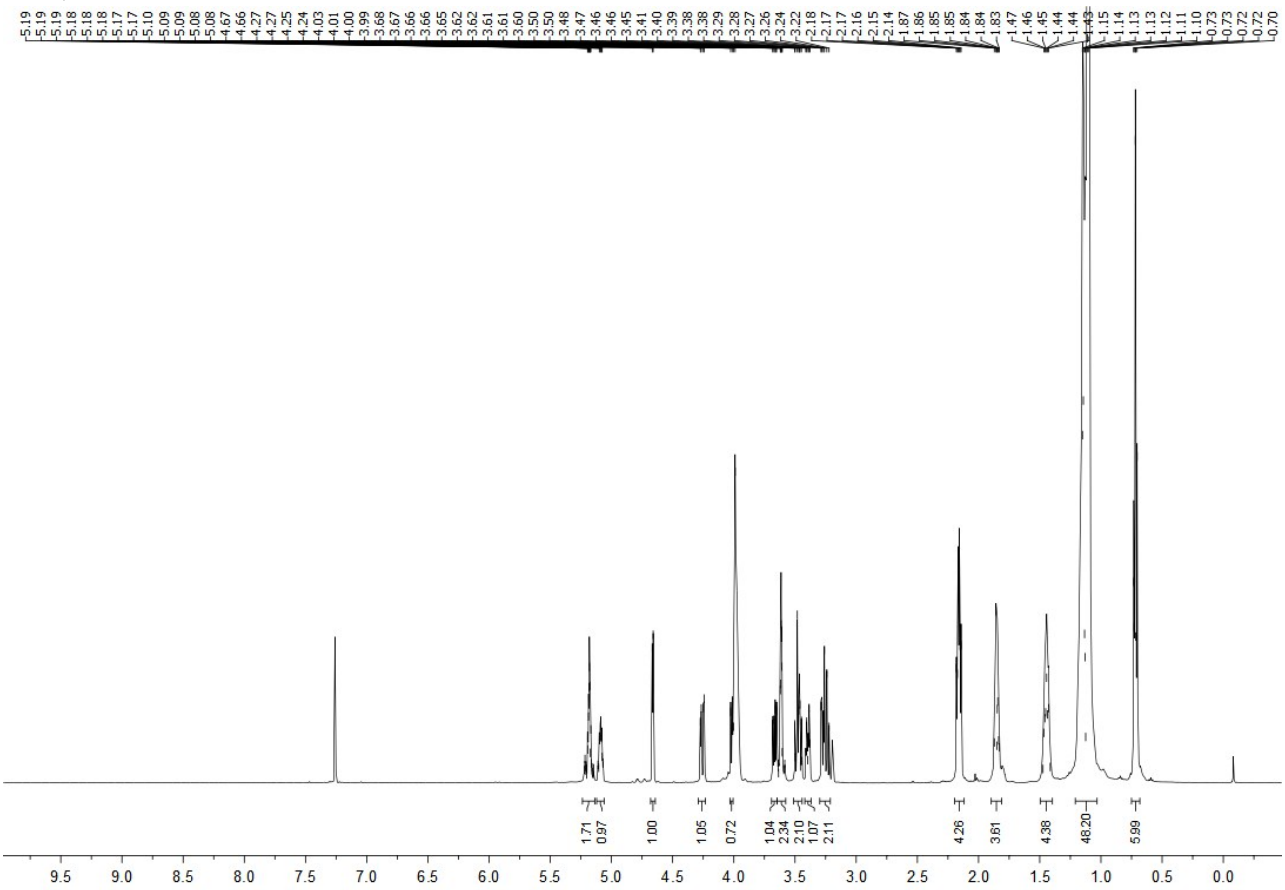


1'-*O*-Palmitoyl-2'-*O*-oleoyl-sn-glyceryl α-D-glucopyranoside (8)

^{1}H NMR



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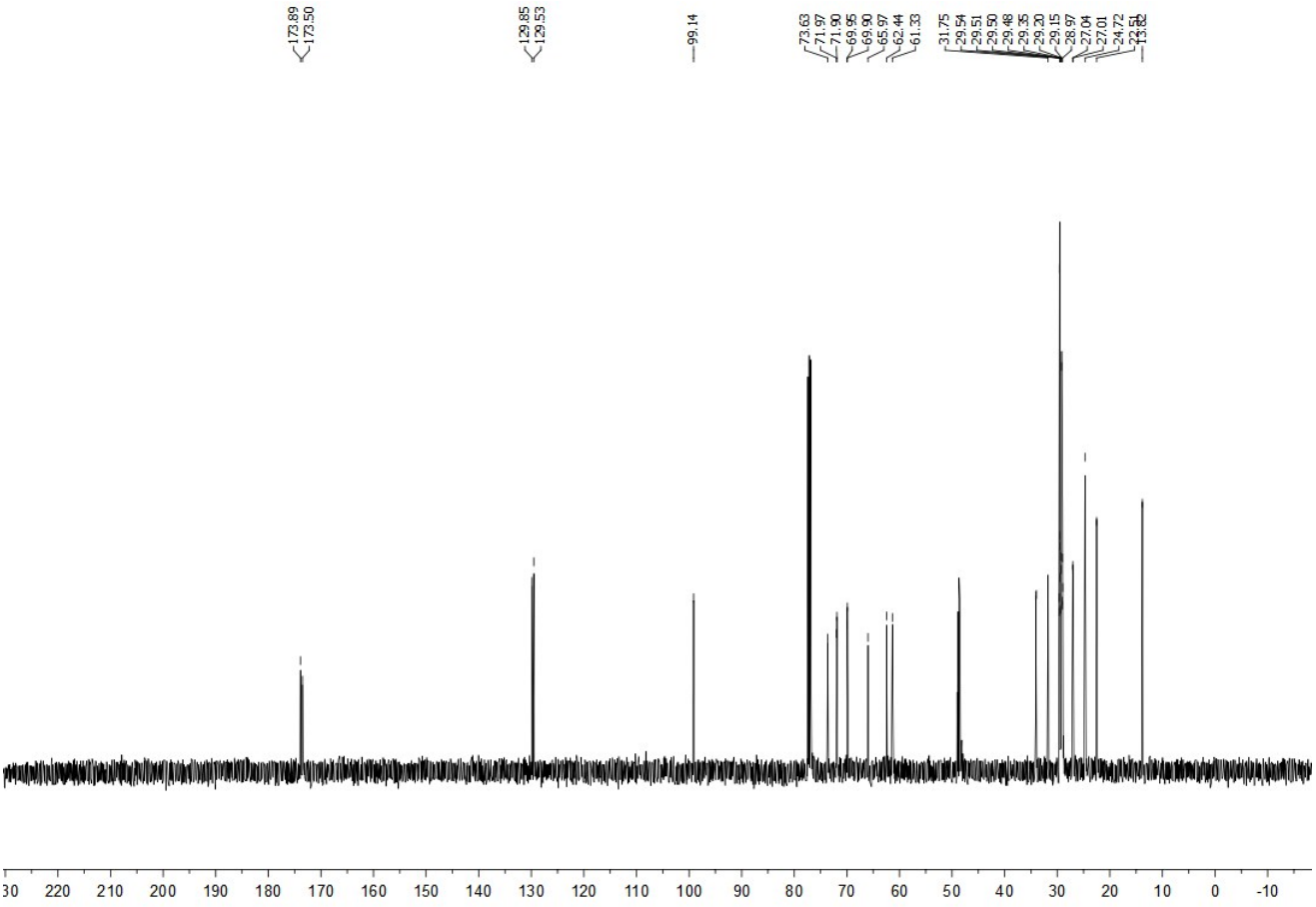
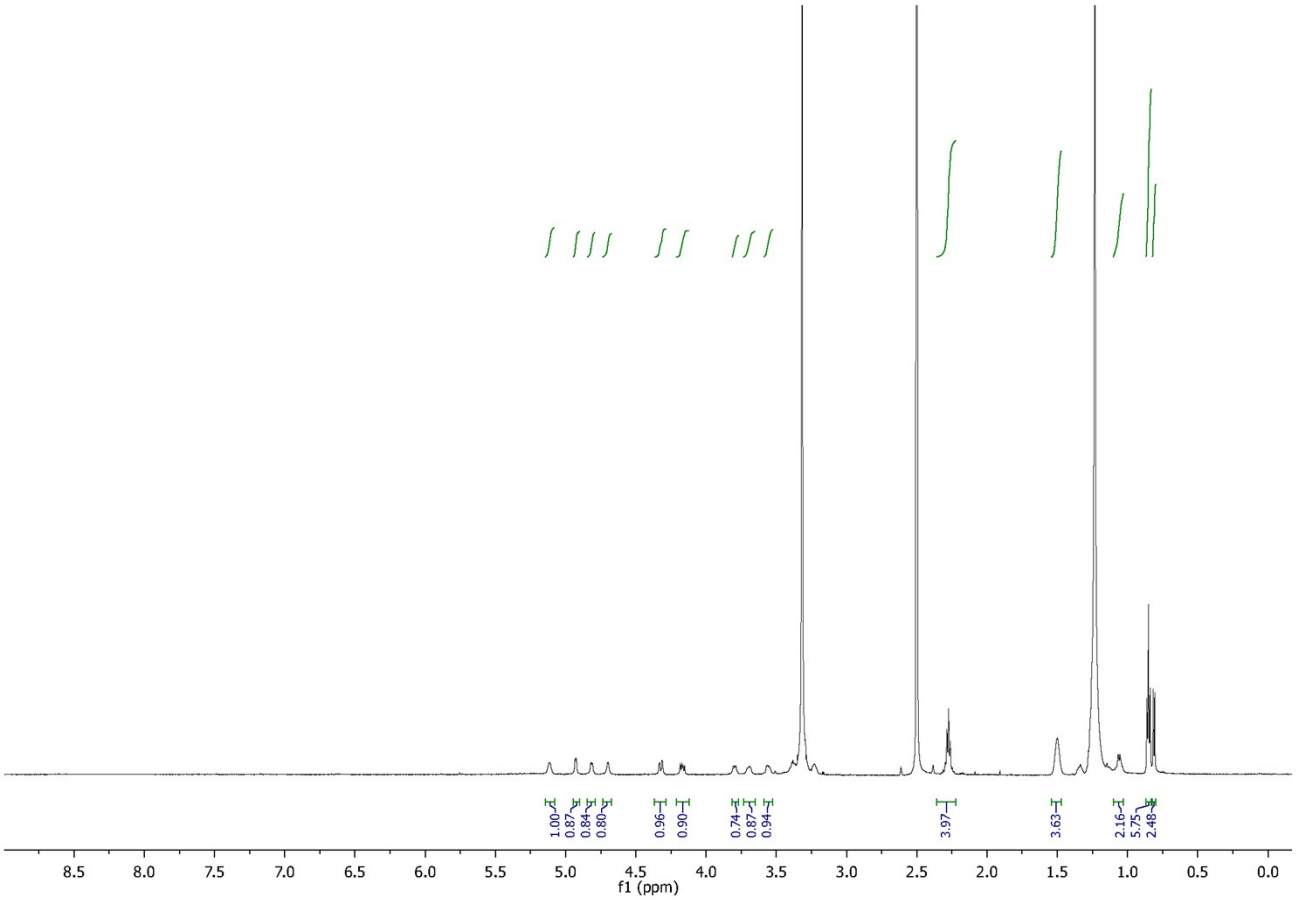
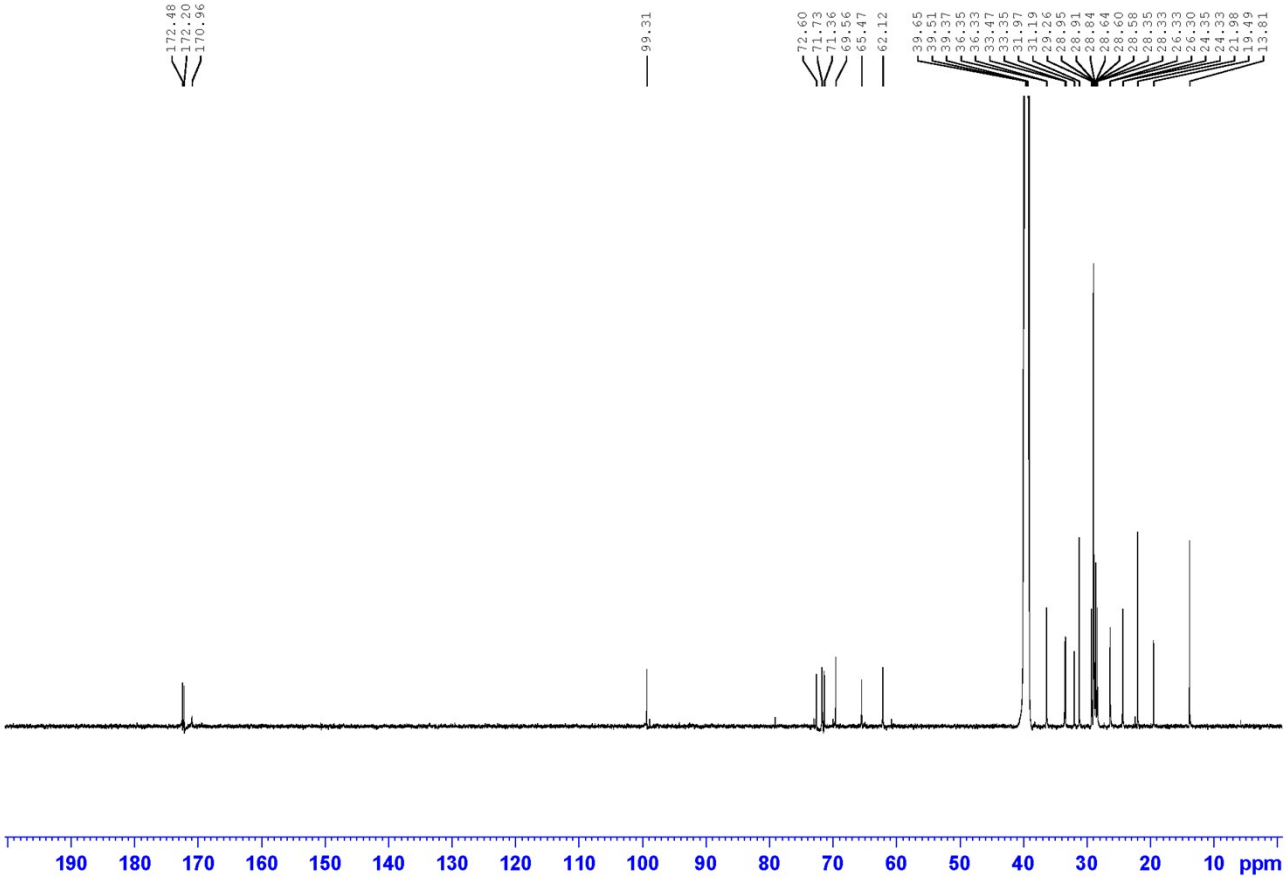


1'-*O*-((*R*)-10-Tuberculostearyl)-2-*O*-palmitoyl-sn-glyceryl α-D-glucopyranosiduronic acid (1)

^{1}H NMR

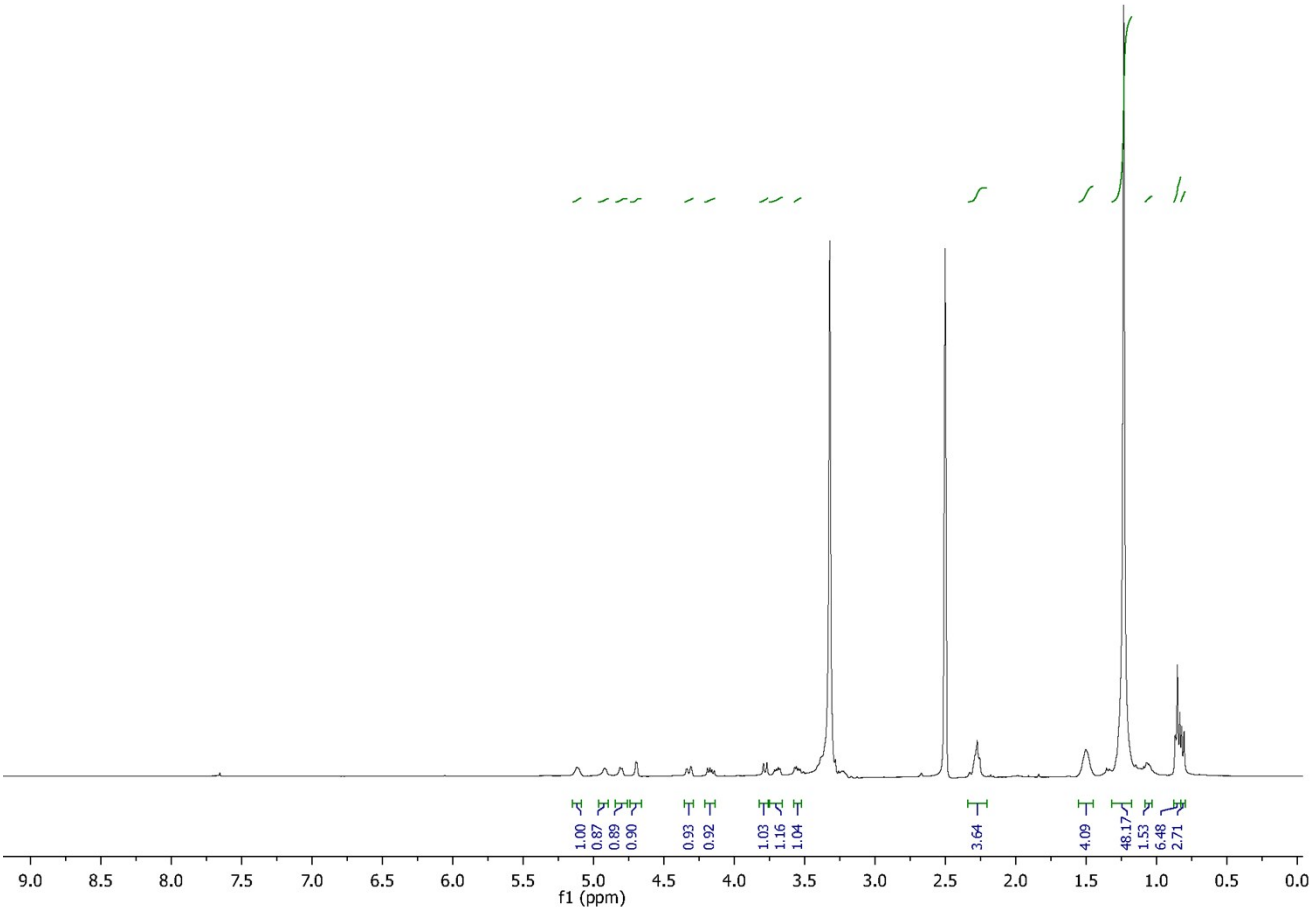


^{13}C NMR



1'-*O*-((*S*)-10-Tuberculostearyl)-2-*O*-palmitoyl-sn-glyceryl α-D-glucopyranosiduronic acid (2)

^{1}H NMR



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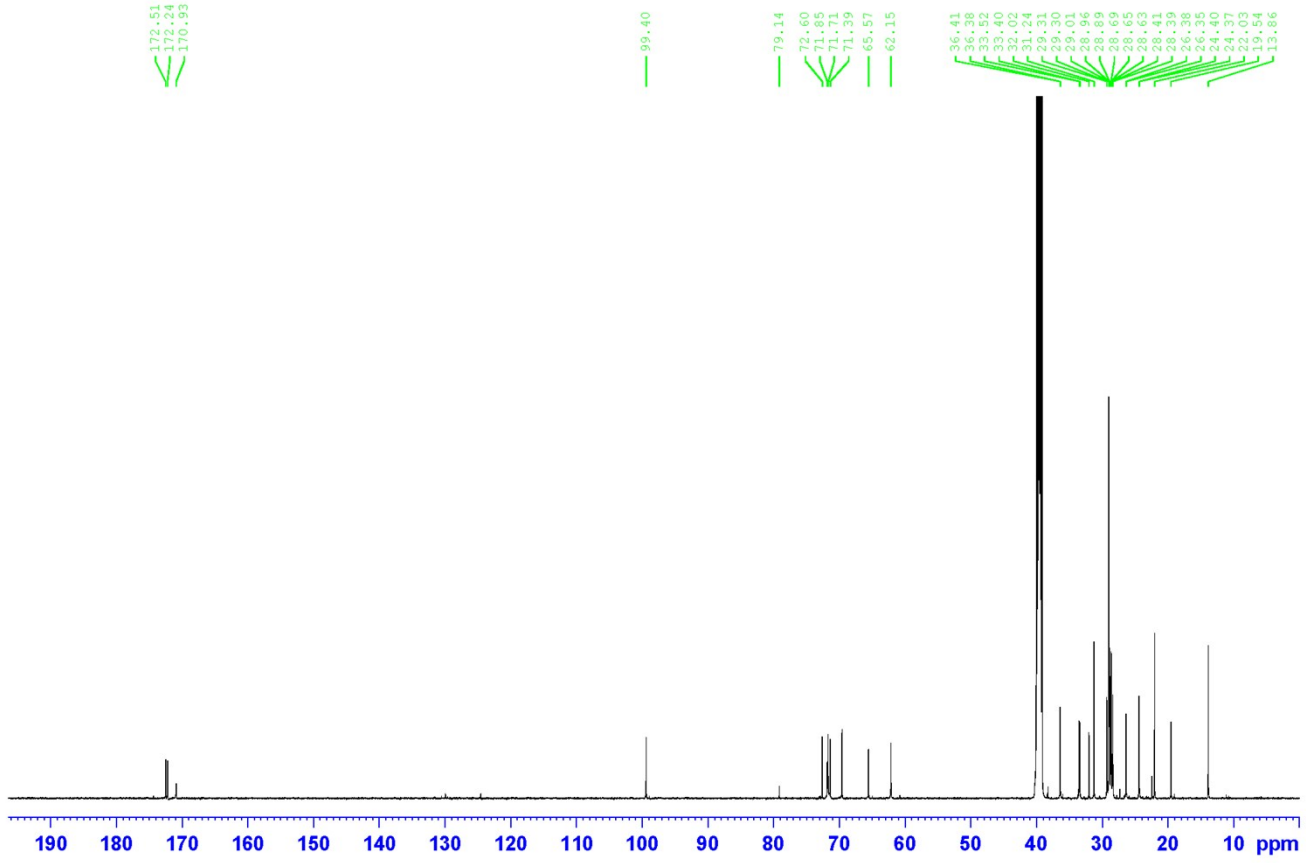
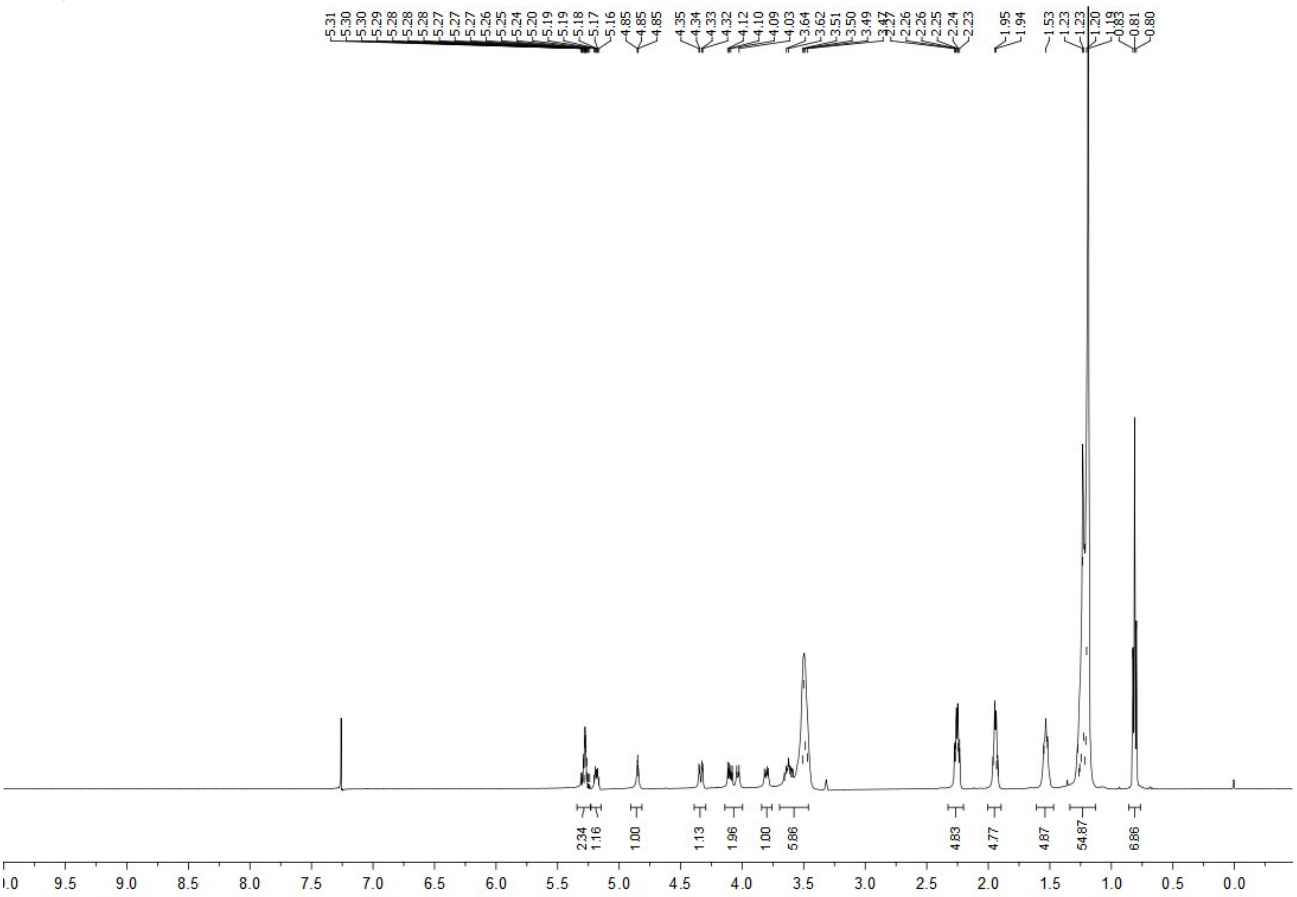


1'-*O*-Oleoyl-2'-*O*-palmitoyl-sn-glyceryl α-D-glucopyranosiduronic acid (3)

^{1}H NMR



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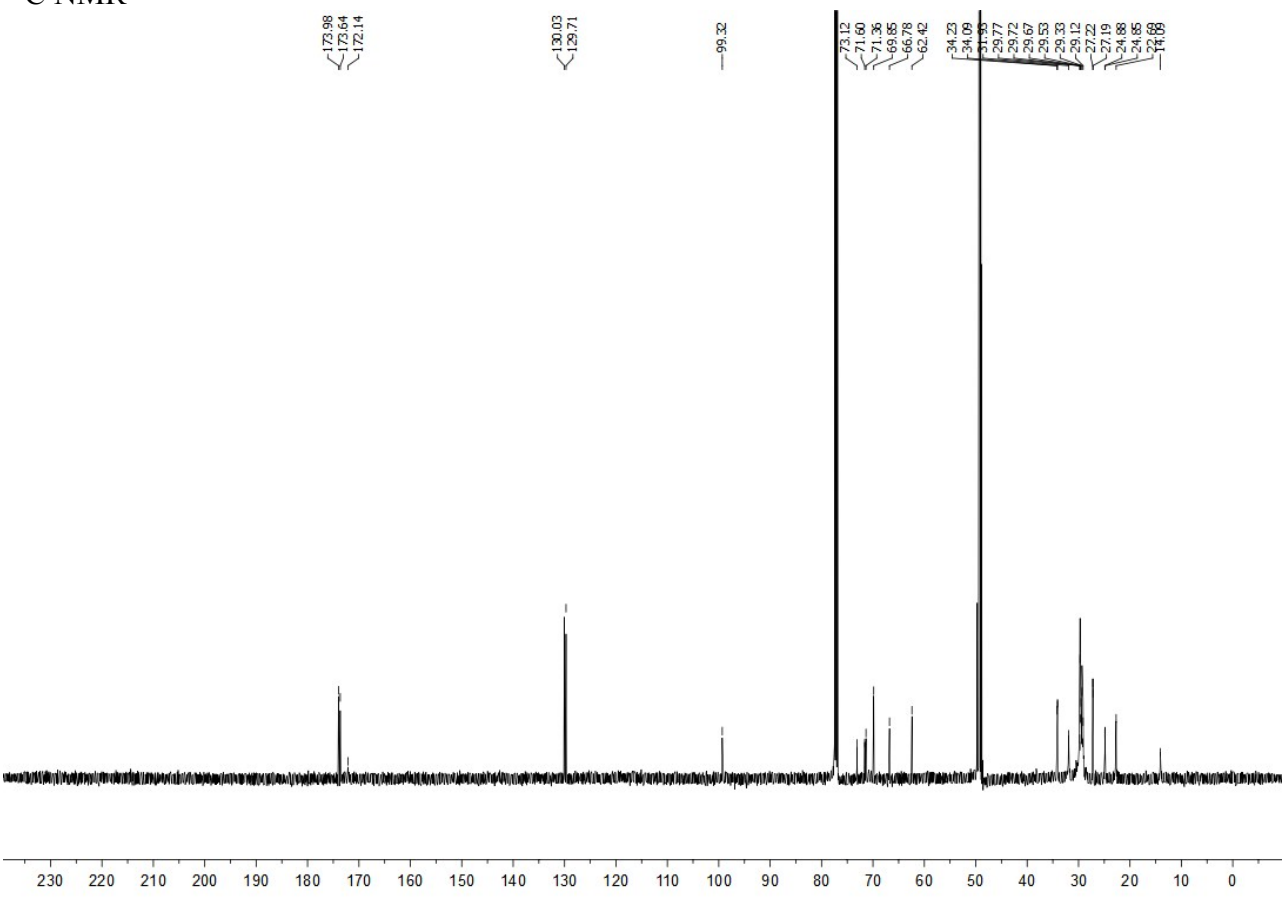
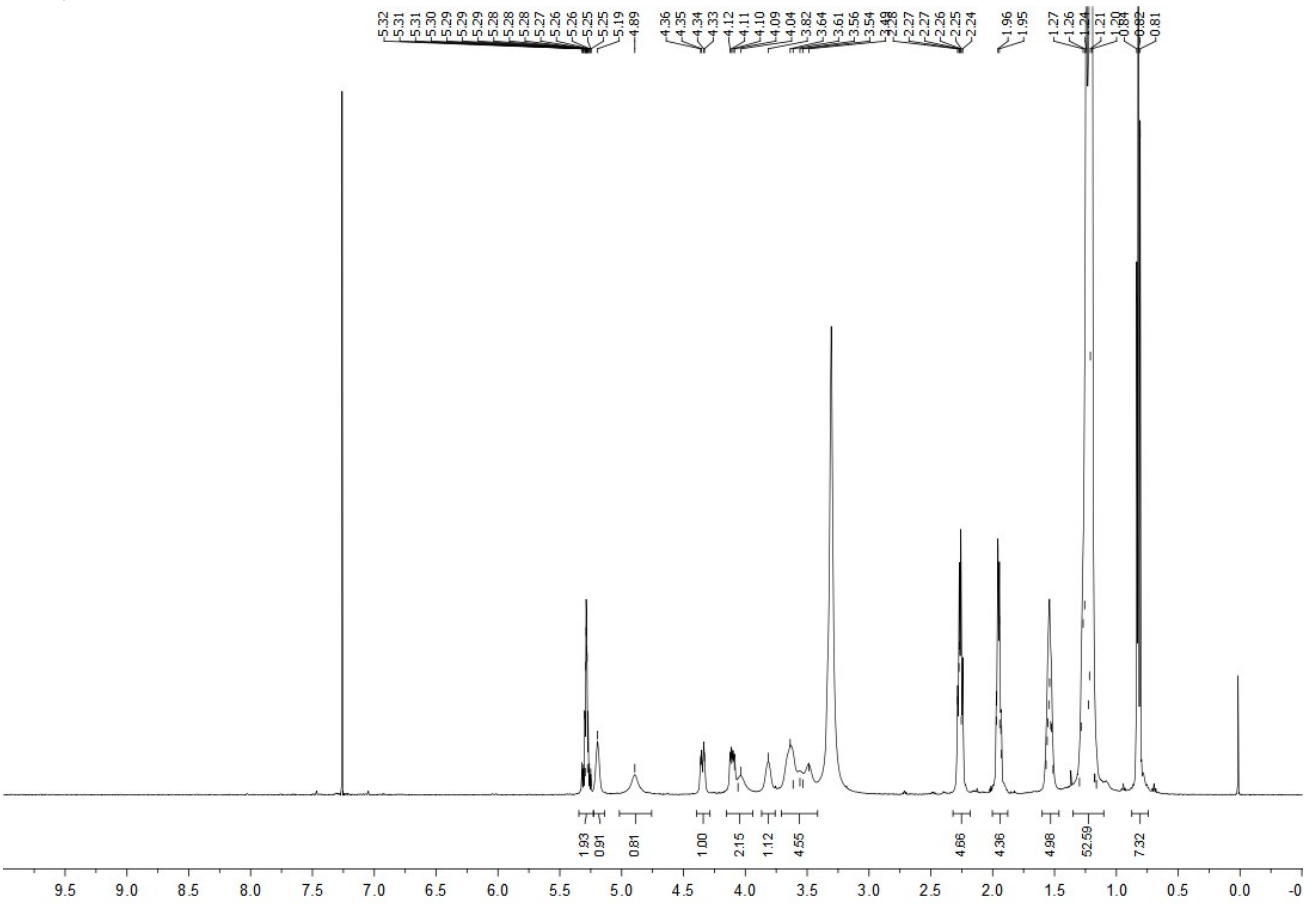


1'-*O*-Palmitoyl-2'-*O*-oleoyl-sn-glyceryl α-D-glucopyranosiduronic acid (4)

^{1}H NMR



^{13}C NMR