Supporting Information

for

Highly efficient chemoenzymatic synthesis and facile purification of α-Gal pentasaccharyl ceramide Galα3nLc$_4$βCer

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General methods for compound purification and characterization

Chemicals were purchased and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on 800 MHz Bruker Avance III spectrometers. High resolution electrospray ionization (ESI) mass spectra were obtained using Thermo Electron LTQ-Orbitrap Hybrid MS at the Mass Spectrometry Facility in the University of California, Davis. Silica gel 60 Å (230–400 mesh, Sorbent Technologies) was used for flash column chromatography. Redi*Sep*®*R*$_f$ C18 Aq Gold cartridges were bought from Teledyne Isco Inc .Thin-layer chromatography (TLC, Sorbent Technologies) was performed on silica gel plates using anisaldehyde sugar staining or 5% sulfuric acid in ethanol staining for detection. Phytoshingosine was purchased from TCI America. D-Galactose (Gal) and *N*-acetyl-D-glucosamine (GlcNAc) were purchased from Fisher Scientific (Pittsburgh, Pennsylvania, USA. Guanidine 5'-triphosphate (GTP) was bought from Hangzhou Meiya Pharmacy (Hangzhou, China). Adenosine 5'-triphosphate (ATP) was bought from Beta Pharma Scientific, Inc (Branford, CT). Uridine-5'-triphosphate (UTP) was bought from Chemfun Medical Technology Co. Recombinant enzymes *Streptococcus pneumoniae TIGR4* galactokinase (SpGalK),[1] *Pasteurella multocida N*-acetylglucosamine uridyltransferase (PmGlmU),[2] *Bifidobacterium longum* strain ATCC55813 *N*–acetylhexosamine-1-kinase (BiNahK),[3] *Pasteurella multocida* inorganic pyrophosphatase (PmPpA),[4] *Neisseria meningitides* β1–3-*N*–acetylglucosaminyltransferase (NmLgtA),[5] *Bifidobacterium longum* UDP-sugar pyrophosphorylase (BLUSP),[6] bovine α1–3-galactosyltransferase (Bα1–3GalT),[7] *Neisseria meningitidis* β1–4-galactosyltransferase (NmLgtB)[4] were expressed and purified as described previously. One unit (U) is defined as the amount of the enzyme that catalyzes the conversion of 1 μmol of substrate per minute at 30 °C for NmLgtA or 37 °C for all other enzymes.

(2R,3R,4E)-2-Azido-3-O-benzoyloxy-1-O-tertbutyldiphenylsilyloxy-octadec-4-ene (**11**)[8]

Compound **10** was synthesized from commercially available phytosphigosine (**6**) as reported previously.[9] For the synthesis of compound **11**, to a solution of compound **10** (3.9 g, 6.9 mmol) in dry CH_2Cl_2 (25 mL), Et_3N (5.8 mL, 41.4 mmol) and 4-dimethyl amino pyridine (DMAP) (0.84 mg, 0.69 mmol) were added and the mixture was stirring at 0 °C. Benzoyl chloride (1.6 mL, 13.8 mmol) was then added to the stirring reaction mixture drop-wisely. The reaction was allowed to warm up to r.t. and stirred for overnight, until TLC analysis (hexane:ethyl acetate = 9:1 by volume and detected with *p*-anisaldehyde sugar stain) showed total consumption of the starting material. The reaction was then diluted with CH_2Cl_2, washed with HCl (1 N), saturated $NaHCO_3$ solution, and brine (10% NaCl solution), and was dried with Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the product was purified by silica gel chromatography using hexane:EtOAc = 20:1 (by volume) as an eluent to produce compound **11** (4.3 g, 95%) as a colorless oil. ^{1}H NMR (800 MHz, $CDCl_3$) δ 8.02–8.01 (m, 2H), 7.69–7.65 (m, 4H), 7.59–7.56 (m, 1H), 7.45–7.39 (m, 6H), 7.3 (t, *J* = 7.5 Hz, 2H), 5.90 (dt, *J* = 15.2, 6.4 Hz, 1H), 5.69–5.67 (m, 1H), 5.53–5.49 (m, 1H), 3.85–3.82 (m, 1H), 3.76–3.74 (m, 2H), 2.05–2.00 (m, 2H), 1.35–1.24 (m, 22H), 1.08(br s, 9H), 0.89 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (200 MHz, $CDCl_3$) δ 165.35, 138.68, 135.74, 135.73, 133.26, 133.00, 132.87, 130.24, 130.04, 130.00, 129.91, 129.85, 128.58, 127.99, 127.98, 127.94, 123.38, 77.36, 77.20, 77.04, 74.49, 65.94, 63.53, 32.53, 32.12, 29.88, 29.86, 29.85, 29.76, 29.61, 29.55, 29.31, 28.89, 26.87, 22.89, 19.30, 14.32. ESI HRMS (m/z) calculated for $C_{41}H_{57}N_3O_3Si$ (M + H) 668.4241, found 668.4278.

(2S,3R,4E)-2-Azido-3-O-benzoyloxy-octadec-4-ene-1-ol (**12**)[8]

To a solution of compound **11** (3.75 g, 5.61 mmol) in dry THF (40 mL) in a plastic flask, 65–70% HF·pyridine solution (1.8 mL) was added drop-wisely to the stirred mixture at 0 °C. The reaction mixture was stirred at r.t. for about 4 h until complete consumption of compound **11** as judged by TLC analysis (hexane:ethyl acetate = 8:1 by volume and detected with *p*-anisaldehyde sugar stain). The reaction mixture was then quenched using solid $NaHCO_3$. EtOAc (50 mL) and H_2O (50 mL) were then added. The aqueous phase was extracted twice with EtOAc. The combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$ and dried over Na_2SO_4. After filtratrion, the solvent was removed under reduced pressure and the product was purified by silica gel chromatography using hexane:EtOAc = 6:1 (by volume) as an eluent to produce compound **12** (2.3g, 97%) as a off-white residue. ^{1}H NMR (800 MHz, $CDCl_3$) δ 8.07–8.02 (m, 2H), 7.60 –7.56 (m, 1H), 7.45 (t, *J* = 7.2 Hz, 2H), 5.96 (dt, *J* = 15.2, 6.4 Hz, 1H), 5.65–5.58 (m, 2H), 3.83–3.79 (m, 1H), 3.77–3.73 (m, 1H), 3.65–3.61 (m, 1H), 2.18–2.16 (m, 1H), 2.11–2.03 (m, 2H), 1.43–1.34 (m, 2H), 1.31–1.20 (m, 22H), 0.88 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (200 MHz, $CDCl_3$) δ 165.68, 138.99, 133.50, 129.96, 129.89, 128.65, 123.40, 74.81, 66.37, 62.14, 32.54, 32.09, 29.85, 29.84, 29.82, 29.81, 29.74, 29.58, 29.53, 29.30, 28.85, 22.86, 14.30. ESI HRMS (m/z) calculated for $C_{25}H_{39}N_3O_3$ (M + Na) 452.2883, found 452.2835.

*O-(2,3,4,6-Tetra-O-benzoyl-*β*-D-galactopyranosyl)-(1→4)-*(*2,3,6-tri-O-benzoyl-*β*-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-azido-3-O-benzoyloxy-octadec-4-ene* (**14**)[10]

To a solution of perbenzoylated lactosyl trichloroacetimidate **13** (1.1 g, 0.90 mmol)[10] and acceptor **12** (300 mg, 0.69 mmol) in 25 mL of dry CH_2Cl_2, powdered molecular sieves (4Å, 1.0 g) were added. The mixture was stirred under argon at r.t. for 30 min. The reaction mixture was cooled down to -18 °C and $BF_3{\cdot}OEt_2$ (377 µL, 1.0 mmol) was added. The reaction mixture was then stirred at -20 °C until TLC analysis (hexane:ethyl acetate = 3:1 by volume and detected with *p*-anisaldehyde sugar stain) showed fully conversion of the acceptor (30–45 min). The reaction was quenched with Et_3N, and the solid was filtered off. The filtrate was concentrated under vacuum, and the residue was purified by silica gel chromatography using hexane:EtOAc = 3:1 (by volume) as an eluent to produce compound **14** (930 mg, 90%) as a colorless oil. ^{1}H NMR (800 MHz, $CHCl_3$): δ 8.01–7.15 (40 H, Ar-H), 5.82 (t, *J* = 9.6 Hz, 1H), 5.73–5.70 (m, 2H), 5.67 (dt, *J* = 16.0, 6.4 Hz, 1H), 5.51–5.48 (m, 2H), 5.43–5.37 (m, 2H), 4.87 (d, *J* = 8.0 Hz, 1H), 4.73 (d, *J* = 8.0 Hz, 1H), 4.57 (dd, *J* = 12.0, 1.6 Hz, 1H), 4.48 (dd, *J* = 12.0, 4.8 Hz, 1H), 4.28 (t, *J* = 9.6 Hz, 1H), 3.91–3.88 (m, 2H), 3.86–3.84 (m, 2H), 3.74–3.69 (m, 2H), 3.55–3.53 (m, 1H), 1.88 (q, *J* = 7.4 Hz, 2H), 1.30–1.2 (m, 2H), 1.25–1.20 (m, 20H), 0.88 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (800 MHz, $CHCl_3$): d 165.82, 165.57, 165.42, 165.24, 165.03, 164.94, 164.83, 138.98, 133.56, 133.44 (2C), 133.40, 133.36, 133.28, 133.25, 133.22, 133.07 , 130.01, 129.92–122.39 (Ar-C), 101.03, 100.83, 75.87, 74.79, 73.11, 72.86, 71.77, 71.62, 71.41, 69.88, 68.29, 67.52, 63.41, 62.26, 61.06, 32.28, 31.95, 29.72, 29.69, 29.67, 29.60, 29.39, 29.38, 29.15, 28.60, 22.72, 14.16. ESI HRMS (m/z) calculated for $C_{86}H_{87}N_3O_{20}$ (M + H) 1482.5955, found 1482.5921.

*Gram-scale synthesis of Lac*β*Sph (***5***): O-(*β*-D-galactopyranosyl)-(1→4)-*(β*-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-aminooctadec-4-ene-1,3-diol* (**5**)[11]

To a solution of **14** (2.9 g, 1.95 mmol) in dry MeOH (50 mL), NaOMe (250 mg) was added. After being stirred at r.t. for 6 h, the reaction mixture was neutralized with Dowex 50W (H^+), filtered and concentrated under reduced pressure. This intermediate was used in the next step without further purification. To the dry intermediate (1.25 g, 1.92 mmol) in pyridine-water (1:1 v/v, 30 mL), 1,3-propanedithiol[12] (1.9 mL, 19 mmol) and Et_3N (2 mL) were added and the mixture was stirred at 50 °C for 48 h. The reaction mixture was concentrated and purified by silica gel chromatography using chloroform:methanol:water (5:4:1 v/v/v) as an eluent to produce compound **5** (1.10 g, 94%) as a white amorphous powder. ^{1}H NMR (800 MHz, MeOD) δ 5.81–5.74 (m, 1H), 5.49 (dd, *J* = 12.4, 7.2 Hz, 1H), 4.36 (d, *J* = 8.0 Hz, 1H), 4.31 (d, *J* = 8.0 Hz, 1H), 4.06 (t, *J* = 6.4 Hz, 1H), 3.91 (dd, *J* = 12.0, 2.4 Hz, 1H), 3.89–3.82 (m, 2H), 3.82 (dd, *J* = 3.2, .8 Hz, 1H), 3.80–3.76 (m, 2H), 3.70 (dd, *J* = 12.0, 4.8 Hz, 1H), 3.61–3.51 (m, 4H), 3.48 (dd, *J* = 9.6, 3.2 Hz, 1H), 3.44–3.40 (m, 1H), 3.28 (dd, *J* = 8.8, 7.2 Hz, 1H), 3.02 – 2.97 (m, 1H), 2.11–2.07 (m, 2H), 1.45–1.39 (m, 2H), 1.36–1.25 (m, 20H), 0.90 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (200 MHz, MeOD) δ 136.02, 130.54, 105.31, 104.37, 80.70, 77.30, 76.73, 76.47, 75.02, 74.88, 74.04, 72.74, 70.92, 70.50, 62.70, 61.99, 56.50, 33.63, 33.28, 31.00, 30.96, 30.84, 30.68, 30.58, 30.54, 23.94, 14.64. ESI HRMS (m/z) calculated for $C_{30}H_{57}NO_{12}$ (M + H) 624.3954, found: 624.3945.

*One-pot four-enzyme synthesis of Lc$_3$βSph (**4**): O-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-aminooctadec-4-ene-1,3-diol* **(4)**

To prepare the trisaccharide **4**, NmLgtA (0.5 mg, 0.29 U/mg when LacβMU was used as the acceptor substrate)[5] was added to a centrifuge tube (10 mL) containing 25 mg of LacβSph (**5**), GlcNAc (1.2 eq., 10 mg), ATP (1.5 eq., 33 mg), UTP (1.5 eq, 30 mg.), $MgCl_2$ (20 mM), Tris-HCl buffer (100 mM, pH = 8.0), BiNahK (0.7 mg, 1.4 U/mg when GlcNAc was used as the substrate),[3] PmGlmU (1.25 mg), PmPpA (1 mg), and water. The total volume was brought up to 9.5 mL. The reaction mixture was incubated at 37 °C for 52 h. The reaction progress was monitored using mass spectrometry and TLC ($CHCl_3$:MeOH:H_2O = 5:4:1, by volume). After the reaction reached to an optimum yield, reaction mixture was diluted with the same volume of ethanol and the solution was incubated at 4 °C for 30 min. The precipitates were removed by centrifugation and the supernatant was concentrated. The residue was dissolved in 2–3 mL of water at 40–45 °C and the solution was directly loaded to a preconditioned Redi*Sep*®*R$_f$* C18 cartridge (5.5 g media) through a 10 mL plastic syringe. After loading, the cartridge was washed with acidic deionized water (10 mL with 0.01% TFA v/v) to wash out non-lipid components. The product was eluted from the C18 cartridge with 37% acetonitrile and 0.01% TFA in water (v/v). The elute solvent was collected in 1–1.5 mL fractions. Unreacted LacβSph was eluted with 50% acetonitrile in 0.01% TFA/H_2O. The whole process took 20–30 minutes. The target glycolipid **4** (27 mg, 83%) was isolated after lyophilization. ^{1}H NMR (800 MHz, MeOD) δ 6.03–5.75 (m, 1H), 5.48 (dd, *J* = 15.2, 7.2 Hz, 1H), 4.64 (d, *J* = 8.8 Hz, 1H), 4.36 (d, *J* = 8.8 Hz, 1H), 4.35 (d, *J* = 8.8 Hz, 1H), 4.31 (t, *J* = 5.6 Hz, 1H), 4.05 (d, *J* = 3.2 Hz, 1H), 3.98 (dd, *J* = 12.0, 8.8 Hz, 1H), 3.95–3.89 (m, 2H), 3.88–3.82 (m, 2H), 3.78 (dd, *J* = 12.0, 7.2 Hz, 1H), 3.72–3.66 (m, 3H), 3.64–3.52 (m, 6H), 3.48–3.43 (m, 2H), 3.38 (m, 1H), 3.33 (t, *J* = 9.6 Hz, 1H), 3.29–3.26 (m, 1H), 2.16–2.10 (m, 2H), 2.00 (s, 3H), 1.45–1.41 (m, 2H), 1.35–1.23 (m, 20H), 0.90 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (200 MHz, MeOD) δ 174.77, 137.02, 128.48, 105.16, 104.42, 103.93, 83.57, 80.32, 78.15, 76.92, 76.84, 76.51, 75.98, 74.71, 72.06, 71.78, 71.07, 70.14, 67.27, 62.71, 61.85, 57.85, 56.86, 33.57, 33.27, 30.99, 30.96, 30.95,

30.84, 30.68, 30.60, 30.37, 23.94, 23.27, 14.64. ESI HRMS (m/z) calculated for $C_{38}H_{70}N_2O_{17}$ (M + H) 827.4747, found 827.4798.

*One-pot four-enzyme synthesis of nLc$_4$βSph (**3**): O-(*β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-*β-*D*-glucopyranosyl)-(1→3)-(*β-*D*-galactopyranosyl)-(1→4)-(*β-*D*-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-aminooctadec-4-ene-1, 3-diol* (**3**)[13]

To prepare the tetrasaccharide **3**, NmLgtB (0.3 mg, 0.07 U/mg when GlcNAcβMU was used as the acceptor substrate)[4] was added to a centrifuge tube (10 mL) containing 20 mg of the trisaccharide acceptor **4**, Gal (1.2 eq., 5 mg), ATP (1.5 eq., 20 mg), UTP (1.5 eq., 18 mg), $MgCl_2$ (20 mM), Tris-HCl buffer (100 mM, pH = 8.0), SpGalK (0.40 mg), BLUSP (0.5 mg, 56 U/mg when glucose-1-phosphate was used as the substrate),[1] PmPpA (0.8 mg) and water. The total volume was bought up to 6 mL. The reaction mixture was incubated at 37 °C for 30 h. The reaction progress was monitored using mass spectrometry and TLC ($CHCl_3$:MeOH:H_2O = 5:4.5:1.5). After the reaction was reached to an optimum yield, reaction mixture was diluted with the same volume of ethanol and incubated at 4 °C for 30 min. The precipitates were removed by centrifugation and the supernatant was concentrated. The residue was dissolved in 2–3 mL of water at 40–45 °C. Then the solution was directly loaded to a preconditioned Redi*Sep*®*R$_f$* C18 cartridge (5.5 g media) through a 10 mL plastic syringe and washed with acidic deionized water (10 mL with 0.01% TFA v/v) to wash out non-lipid components. The elute solvent was collected in 1–1.5 mL fractions. The product was eluted from the C18 cartridge with 35% acetonitrile and 0.01% TFA in water (v/v).The whole process takes about 20–30 minutes. The target glycolipid **3** (22 mg, 92%) was obtained as white powder after lyophilization. ^{1}H NMR (800 MHz, MeOD) δ 5.86–5.82 (m, 1H), 5.48 (dd, *J* = 15.2, 7.2 Hz, 1H), 4.66 (d, *J* = 8.8 Hz, 1H), 4.36 (d, *J* = 8.8 Hz, 1H), 4.35 (d, *J* = 4.8 Hz, 1H), 4.34 (d, *J* = 4.8 Hz, 1H), 4.24 (t, *J* = 5.6 Hz, 1H), 4.04 (d, *J* = 3.2 Hz, 1H), 3.96–3.90 (m, 3H), 3.90–3.85 (m, 3H), 3.83 (dd, *J* = 11.9, 4.8 Hz, 1H), 3.81 (d, *J* = 3.2 Hz, 1H), 3.79–3.74 (m, 3H), 3.72–3.67 (m, 2H), 3.66–3.60 (m, 3H), 3.60–3.51 (m, 6H), 3.48 (dd, *J* = 9.6, 3.2 Hz, 1H), 3.46–3.43 (m, 1H), 3.42–3.40 (m, 1H), 3.28 (m, 1H), 2.14–2.07 (m, 2H), 1.99 (s, 3H), 1.45–1.39 (m, 2H), 1.36–1.24 (m, 20H), 0.90 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (200 MHz, MeOD) δ 174.56, 136.81, 128.94, 105.26, 105.17, 104.42, 104.02, 83.64, 80.71, 80.34, 77.38, 76.89, 76.83, 76.72, 76.51, 75.04, 74.74, 74.10, 72.80, 71.76, 70.53, 70.11, 62.76, 62.67, 61.91, 61.87, 57.12, 56.78, 33.59, 33.28, 31.00, 30.97, 30.96, 30.84, 30.68, 30.60, 30.42, 23.94, 23.24, 14.64. ESI HRMS (m/z) calculated for $C_{44}H_{80}N_2O_{22}$ (M + H) 989.5275, found: 989.5287.

*One-pot four-enzyme synthesis of Galα3nLc$_4$βSph (**2**): O-(α-D-galactopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-aminooctadec-4-ene-1,3-diol* (**2**)[14]

To prepare the pentasaccharide **2**, Bα1–3GalT (0.3 mg, 10.6 U/mg when lactose was used as the acceptor substrate)[7] was added to a centrifuge tube (10 mL) containing 12 mg of the tetrasaccharide acceptor **3**, Gal (1.2 eq., 3 mg), ATP (2 eq., 14 mg), UTP (2 eq., 12 mg), $MgCl_2$ (20 mM), $MnCl_2$ (20 mM), Tris-HCl buffer (100 mM, pH = 7.5), SpGalK (0.6 mg), BLUSP (0.9 mg, 56 U/mg when glucose-1-phosphate was used as the substrate),[1] PmPpA (0.5 mg) and water. The total volume

was bought up to 3 mL). The reaction mixture was incubated at 37 °C for 48 h. The reaction progress was monitored using mass spectrometry and TLC ($CHCl_3$:MeOH:H_2O = 5:4.5:1.5, by volume). After the reaction was reached to an optimum yield, reaction mixture was diluted with the same volume of ethanol and incubated at 4 °C for 30 min. The precipitates were removed by centrifugation and the supernatant was concentrated. The residue was dissolved in 2–3 mL of water at 40–45 °C. Then the solution was directly loaded to a preconditioned Redi*Sep*®*R*$_f$ C18 cartridge (5.5 g media) through a 10 mL plastic syringe and washed with acidic deionized water (10 mL with 0.1% TFA v/v) to wash out non-lipid components. The elute solvent was collected in 1–1.5 mL fractions. The product was eluted from the C18 cartridge with 32% acetonitrile in 0.1% TFA/water (v/v) and the unreacted acceptor was eluted using 35% or a higher concentration (50%) of acetonitrile in 0.1% TFA/H_2O. The whole process took about 20–30 minutes. The target glycolipid **2** (12.8 mg, 88%) was obtained after lyophilization. ^{1}H NMR (800 MHz, MeOD) δ 5.89–5.81 (m, 1H), 5.48 (dd, *J* = 15.2, 7.2 Hz, 1H), 5.03 (d, *J* = 2.4 Hz, 1H), 4.66 (d, *J* = 8.0 Hz, 1H), 4.43 (d, *J* = 8.0 Hz, 1H), 4.36 (t, *J* = 8.8 Hz, 2H), 4.31 (t, *J* = 5.6 Hz, 1H), 4.22 (t, *J* = 5.6 Hz, 1H), 4.04 (dd, *J* = 7.2, 3.2 Hz, 2H), 3.99–3.96 (m, 1H), 3.95–3.89 (m, 4H), 3.87 (dd, *J* = 12.0, 4.0 Hz, 1H), 3.85–3.82 (m, 3H), 3.80–3.74 (m, 3H), 3.73–3.68 (m, 4.5 H), 3.67 (d, *J* = 3.2 Hz, .5H), 3.66–3.60 (m, 4H), 3.60–3.52 (m, 5H), 3.47–3.43 (m, 1H), 3.42–3.38 (m, 2H), 3.29 (m, 1H), 2.13–2.09 (m, 2H), 1.99 (s, 3H), 1.44–1.41 (m, 2H), 1.36–1.25 (m, 20H), 0.90 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (200 MHz, MeOD) δ 174.50, 136.96, 128.41, 105.20, 105.10, 104.39, 103.84, 97.90, 83.54, 80.91, 80.16, 80.10, 76.86, 76.83, 76.78, 76.66, 76.45, 74.63, 74.03, 72.41, 71.69, 71.50, 71.27, 71.20, 70.99, 70.30, 70.06, 67.17, 66.81, 62.88, 62.64, 61.86, 61.74, 56.99, 56.77, 33.54, 33.24, 30.97, 30.93, 30.92, 30.81, 30.65, 30.57, 30.33, 23.91, 23.18, 14.61. ESI HRMS (m/z) calculated for $C_{50}H_{91}N_2O_{27}$ (M + H) 1151.5804, found: 1151.5882.

*Synthesis of Galα3nLc$_4$βCer (**1**): O-(α-D-galactopyranosyl)-(1→3)-(*β*-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→3)-(*β*-D-galactopyranosyl)-(1→4)-(*β*-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-(hexadecaneacetamido)-octadec-4-ene-1, 3-diol* (**1**)

To a solution of sphingolipid **2** (8 mg, 0.007 mmol) in dry DMF (1 mL), HOBt (1.5 mg, 0.009 mmol), EDC·HCl (1.6 mg, 0.009 mmol), palmitic acid (2.2 mg, 0.009 mmol), and Et_3N (105 mL, 0.01 mmol) were added to the solution. The reaction mixture was stirred under N_2 atmosphere at room temperature for 24 h. The reaction progress was monitored using mass spectrometry. After completion, the solution was concentrated under reduced pressure and passed through Sephadex™ LH 20 using CH_3OH) as eluant to produce pure compound **1** (8 mg, 85%). ^{1}H NMR (800 MHz, MeOD) δ 5.70–5.66 (m, 1H), 5.45 (dd, *J* = 15.2, 7.2 Hz, 1H), 5.03 (d, *J* = 2.4 Hz, 1H), 4.66 (d, *J* = 8.8 Hz, 1H), 4.44 (d, *J* = 8.0 Hz, 1H), 4.36 (d, *J* = 7.2 Hz, 1H), 4.30 (d, *J* = 8.0 Hz, 1H), 4.22 (t, *J* = 6.4 Hz, 1H), 4.19 (dd, *J* = 9.6, 4.0 Hz, 1H), 4.07 (t, *J* = 8.0 Hz, 1H), 4.05–4.02 (m, 2H), 3.98–3.96 (m, 1H), 3.92–3.86 (m, 5H), 3.84–3.82 (m, 3H), 3.80–3.74 (m, 3H), 3.74–3.66 (m, 6H), 3.66–3.60 (m, 10H), 3.53 (t, *J* = 8.8 Hz, 1H), 3.43–3.40 (m, 3H), 3.30–3.27 (m, 1H), 2.17 (t, *J* = 7.2 Hz, 2H), 2.05–2.00 (m, 2H), 1.99 (s, 3H), 1.62–1.53 (m, 2H), 1.42–1.36 (m, 2H), 1.29 (s, 42H), 0.90 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (201 MHz, MeOD) δ 176.13, 174.55, 135.32, 131.57, 105.27, 105.22, 104.68, 104.44, 97.97, 83.56, 81.01, 80.51, 80.19, 76.91, 76.86, 76.72, 76.70, 76.46, 75.02, 74.15, 73.18, 72.47, 71.84, 71.58, 71.35, 71.26, 70.38, 70.15, 70.09, 66.89, 62.95, 62.69, 62.64, 61.96, 57.05, 54.88, 37.58, 33.67, 33.31, 33.30, 31.09, 31.06, 31.04, 31.03, 31.01, 31.00, 30.98, 30.92, 30.84, 30.72, 30.71, 30.68, 30.66, 30.63, 27.38, 23.96, 23.23, 14.66. ESI HRMS (m/z) calculated for $C_{66}H_{120}N_2O_{28}$ (M + H) 1389.8100, found 1389.8165.

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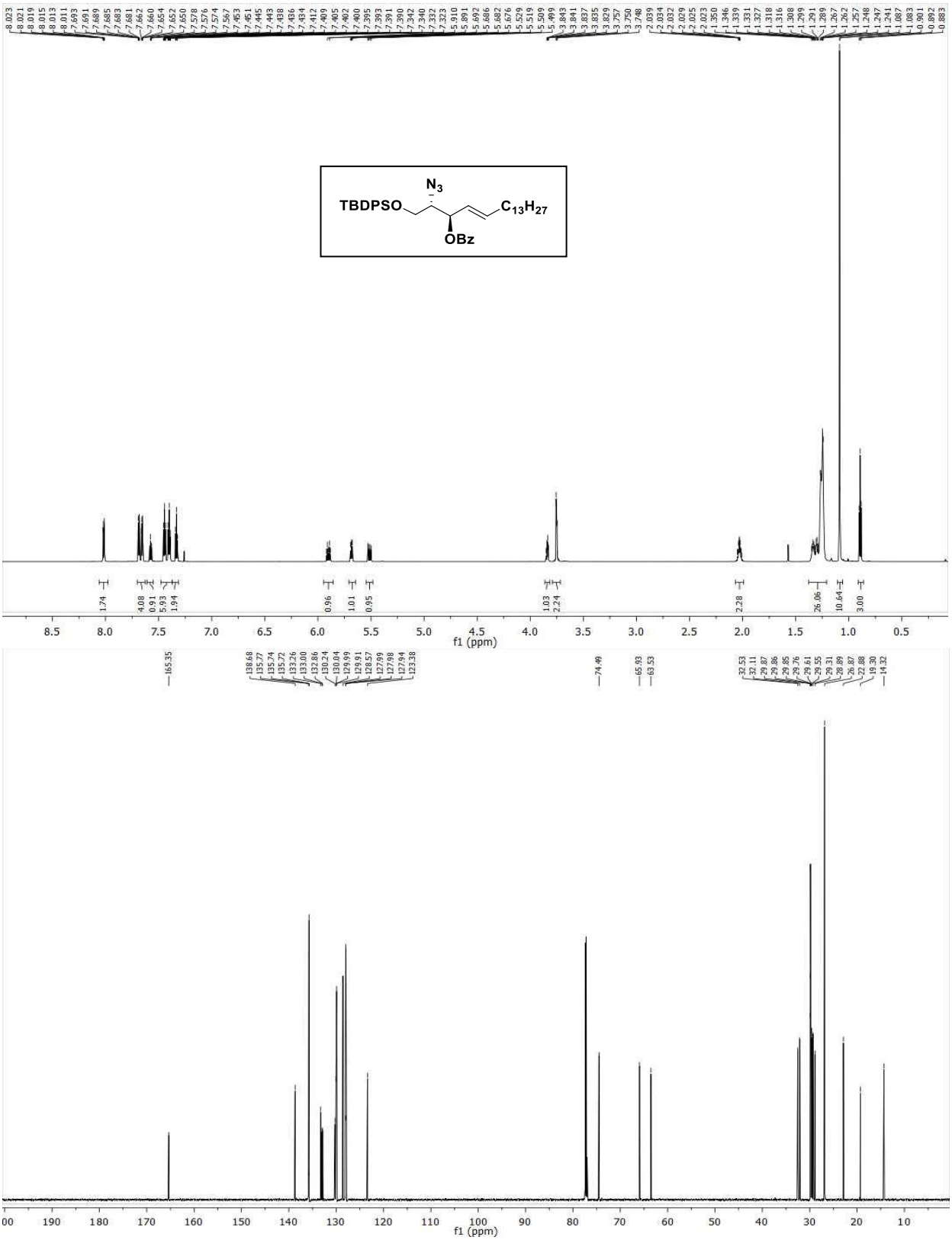
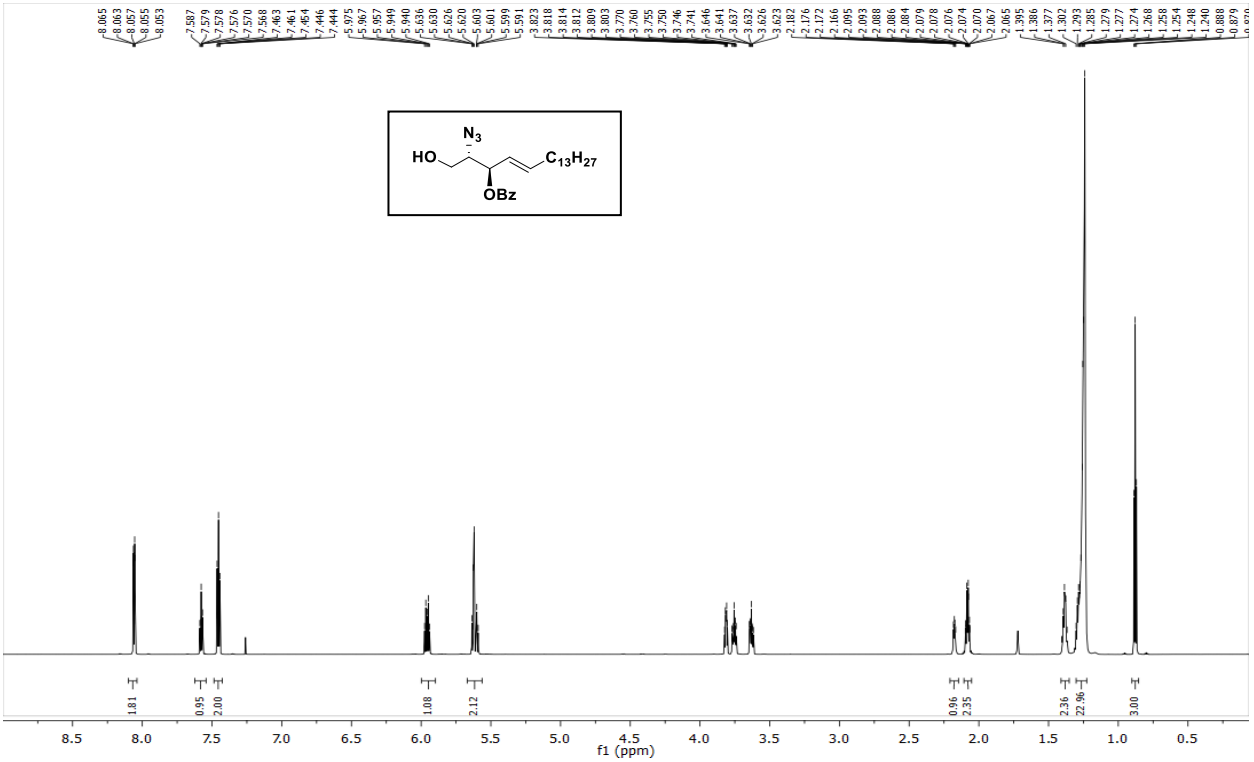
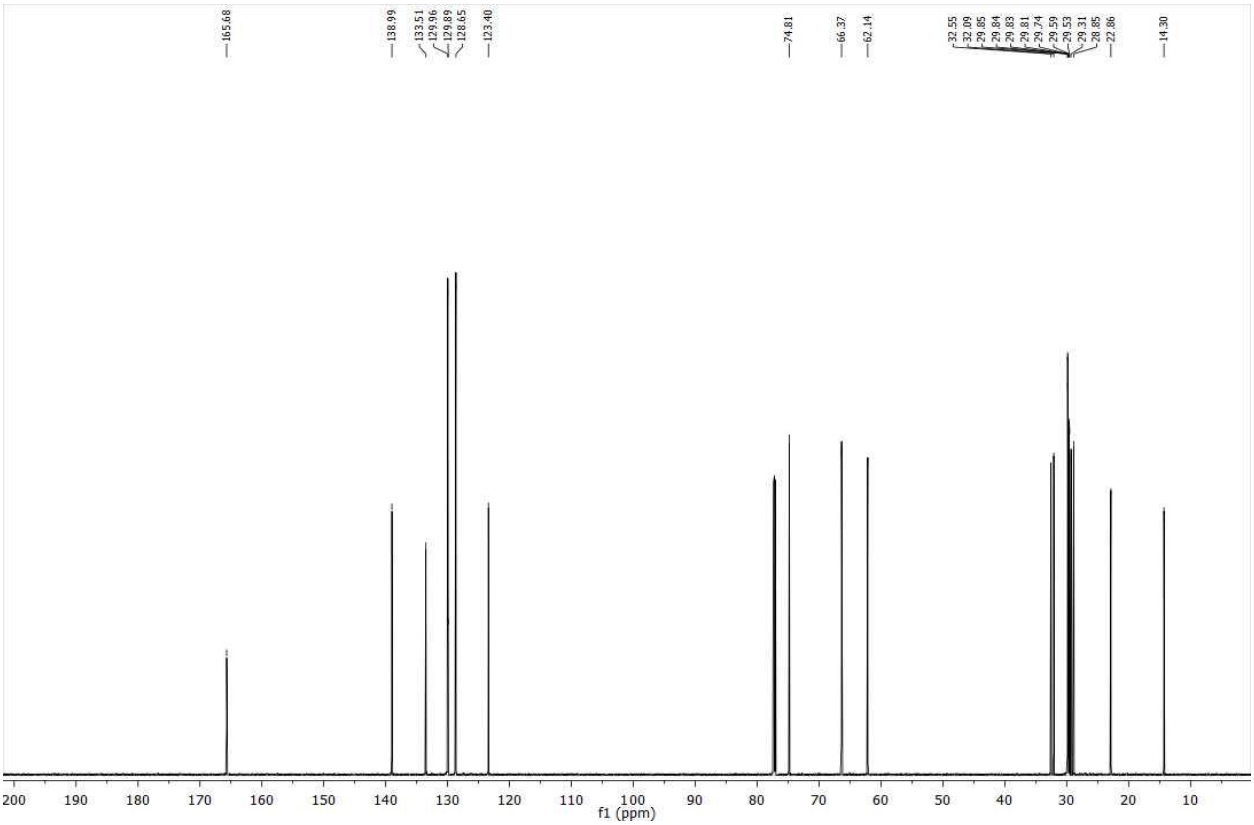
^{1}H NMR (800 MHz, $CDCl_3$) and ^{13}C NMR spectra of (2R,3R,4E)-2-azido-3-*O*-benzoyloxy-1-*O*-tertbutyldiphenylsilyloxy-octadec-4-ene (**11**)

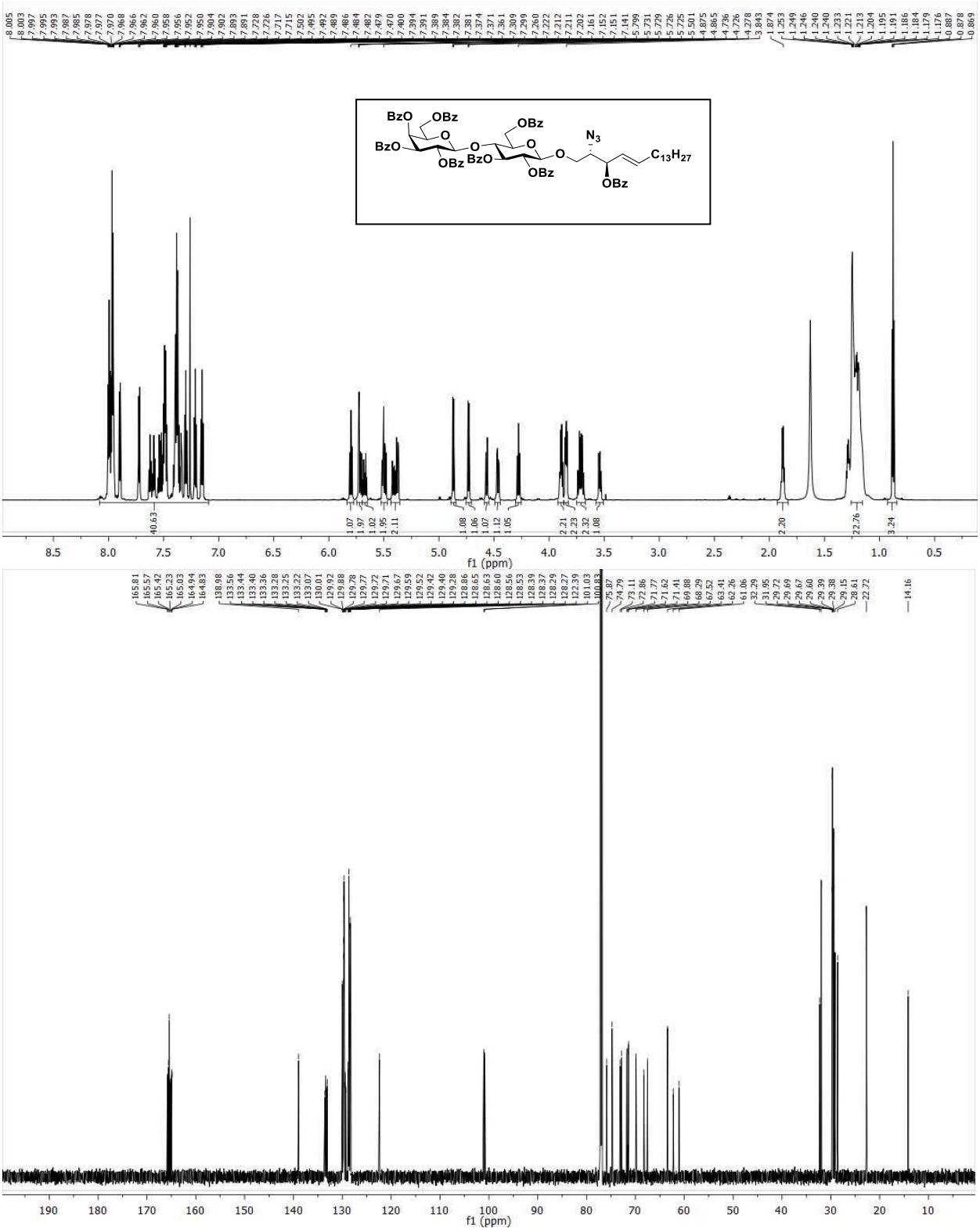


^{1}H NMR (800 MHz, $CDCl_3$) and ^{13}C NMR spectra of (2S,3R,4E)-2-azido-3-*O*-benzoyloxy-octadec-4-ene-1-ol (**12**)

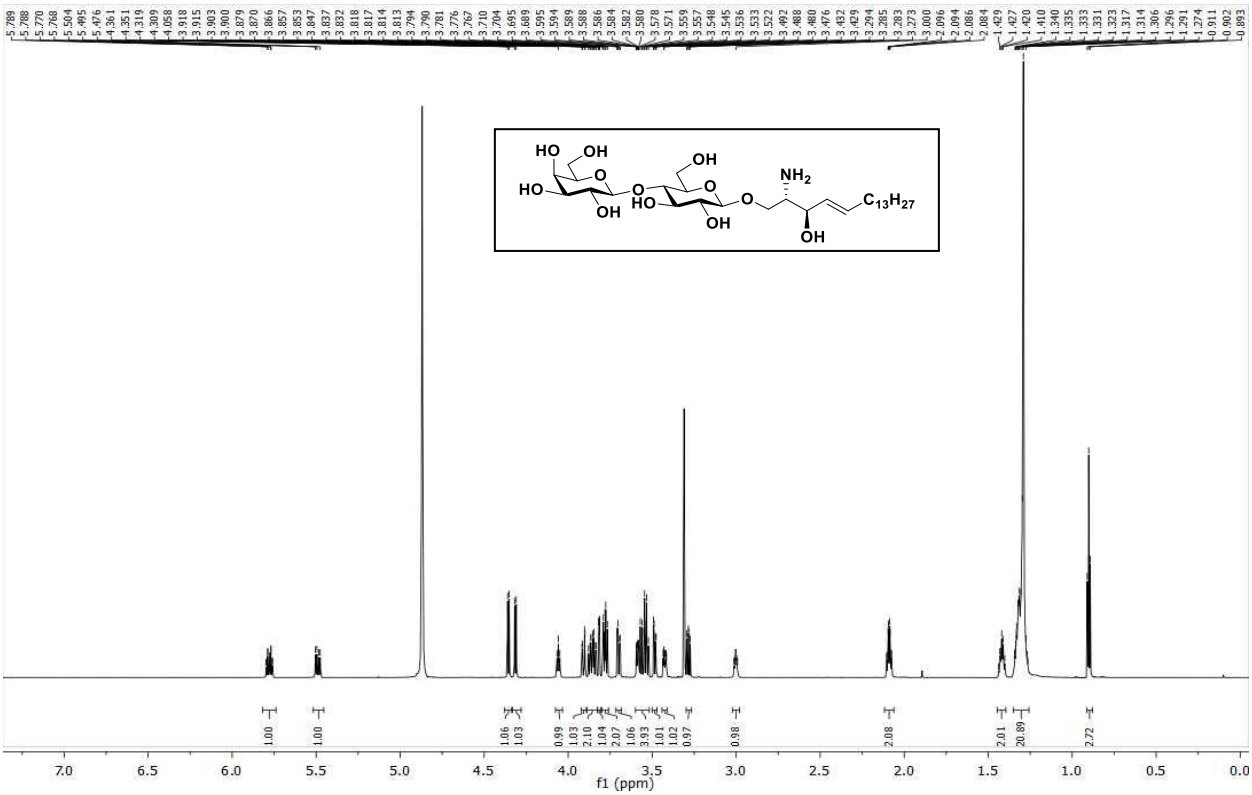


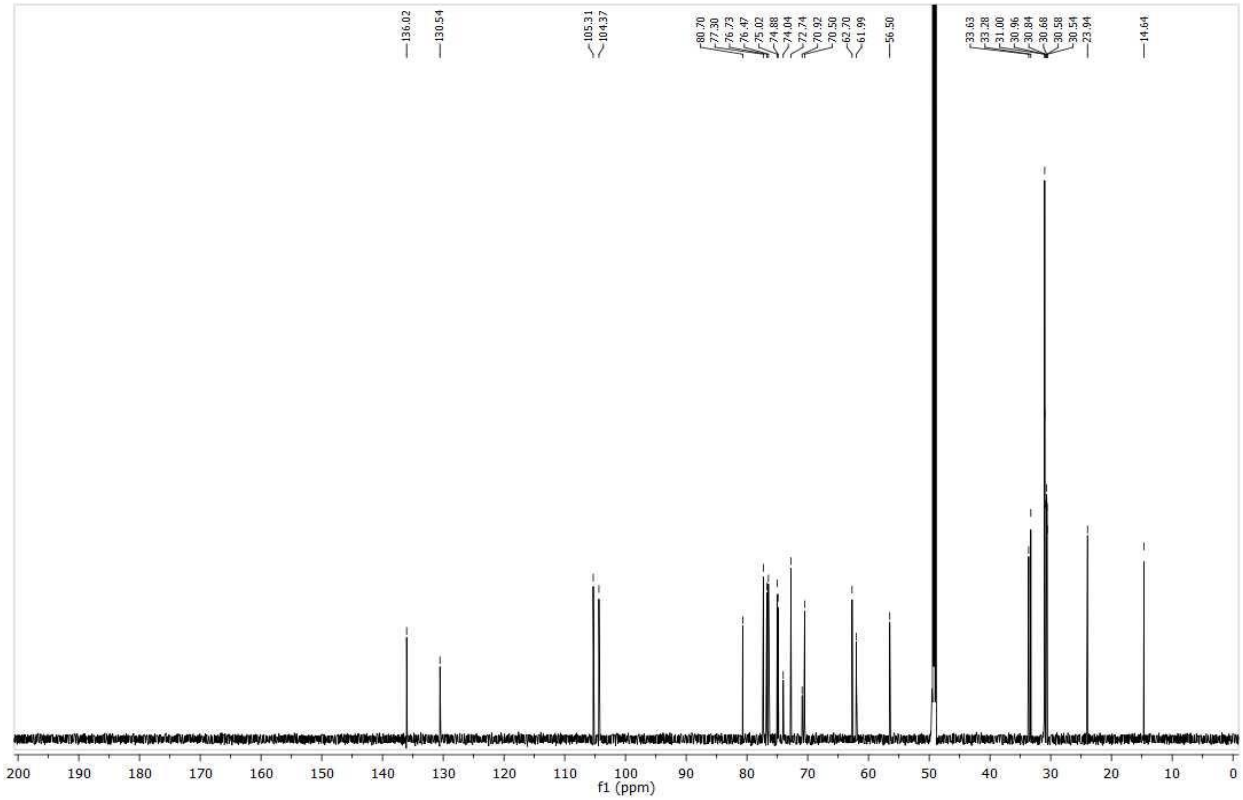


^{1}H NMR (800 MHz, $CDCl_3$) and ^{13}C NMR spectra of *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-azido-3-*O*-benzoyloxy-octadec-4-ene (**14)**

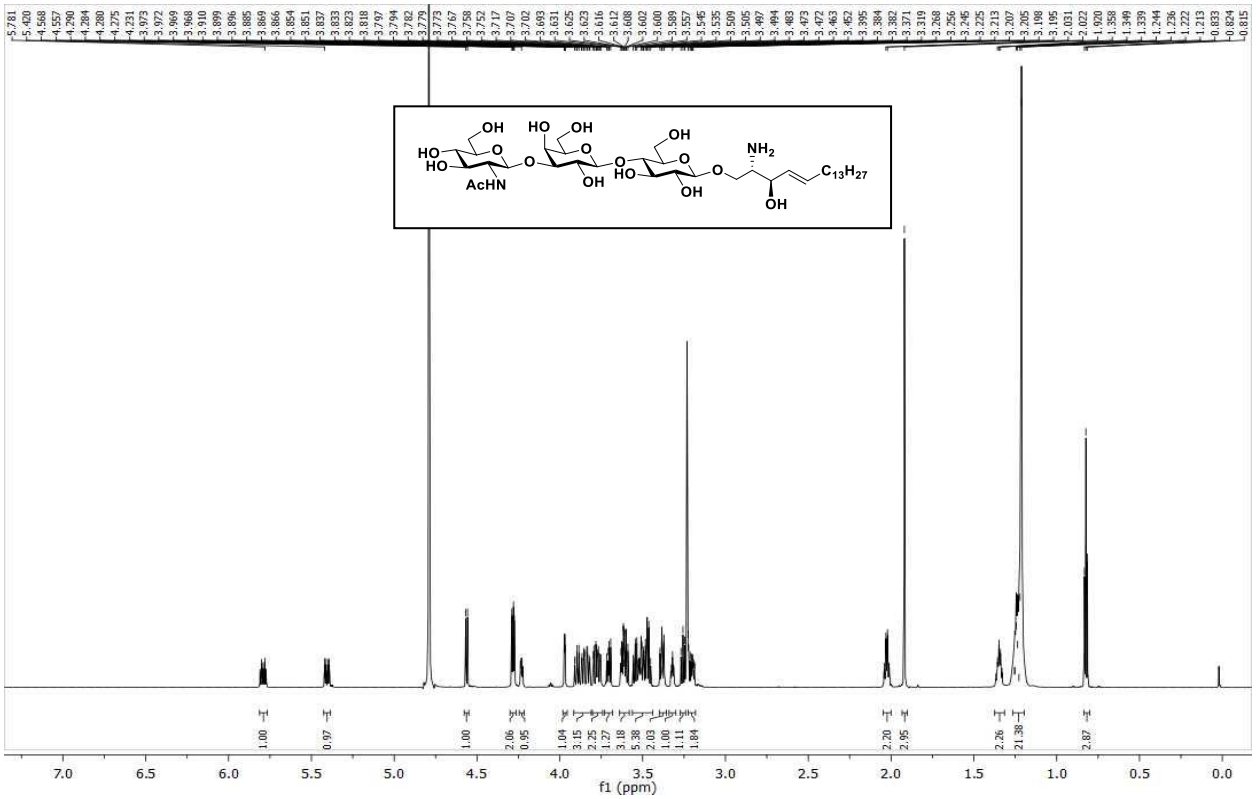


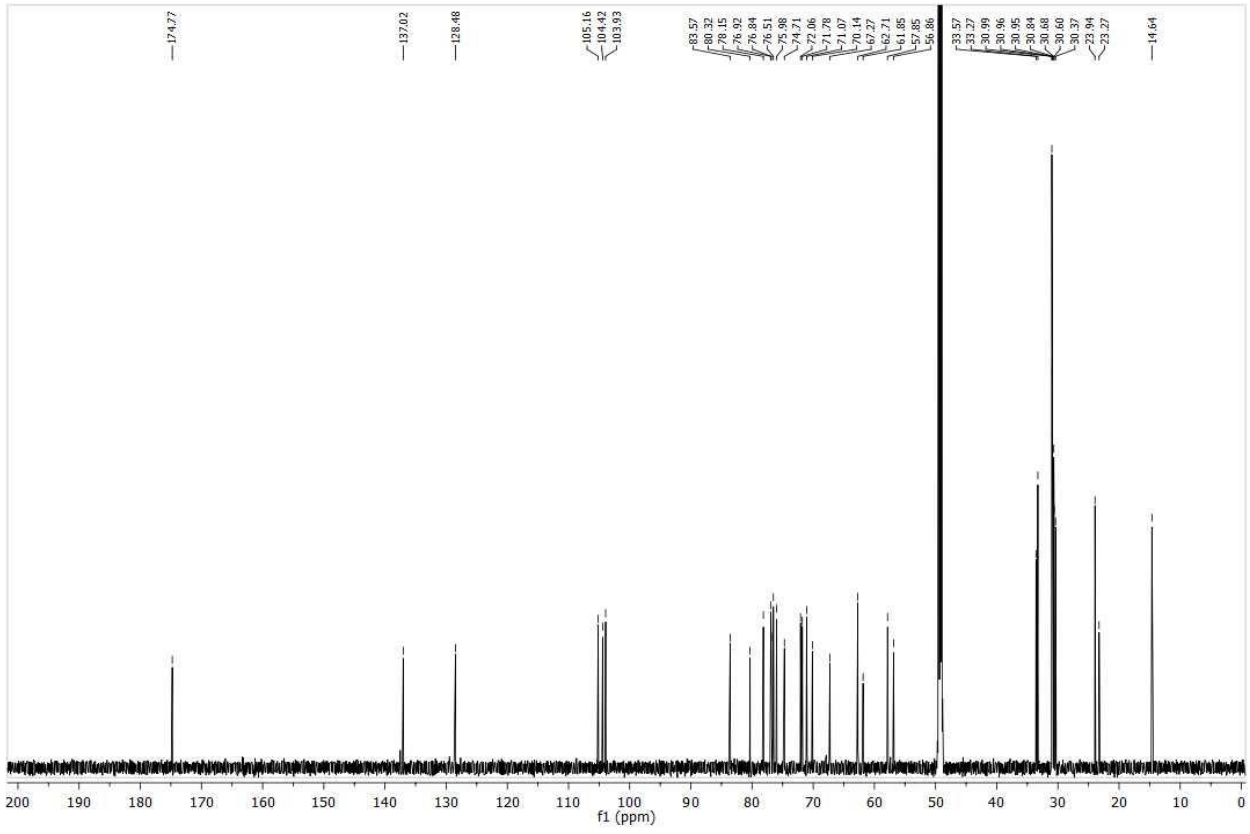
^{1}H NMR (800 MHz, CD_3OD) and ^{13}C NMR spectra of LacβSph (**5**)



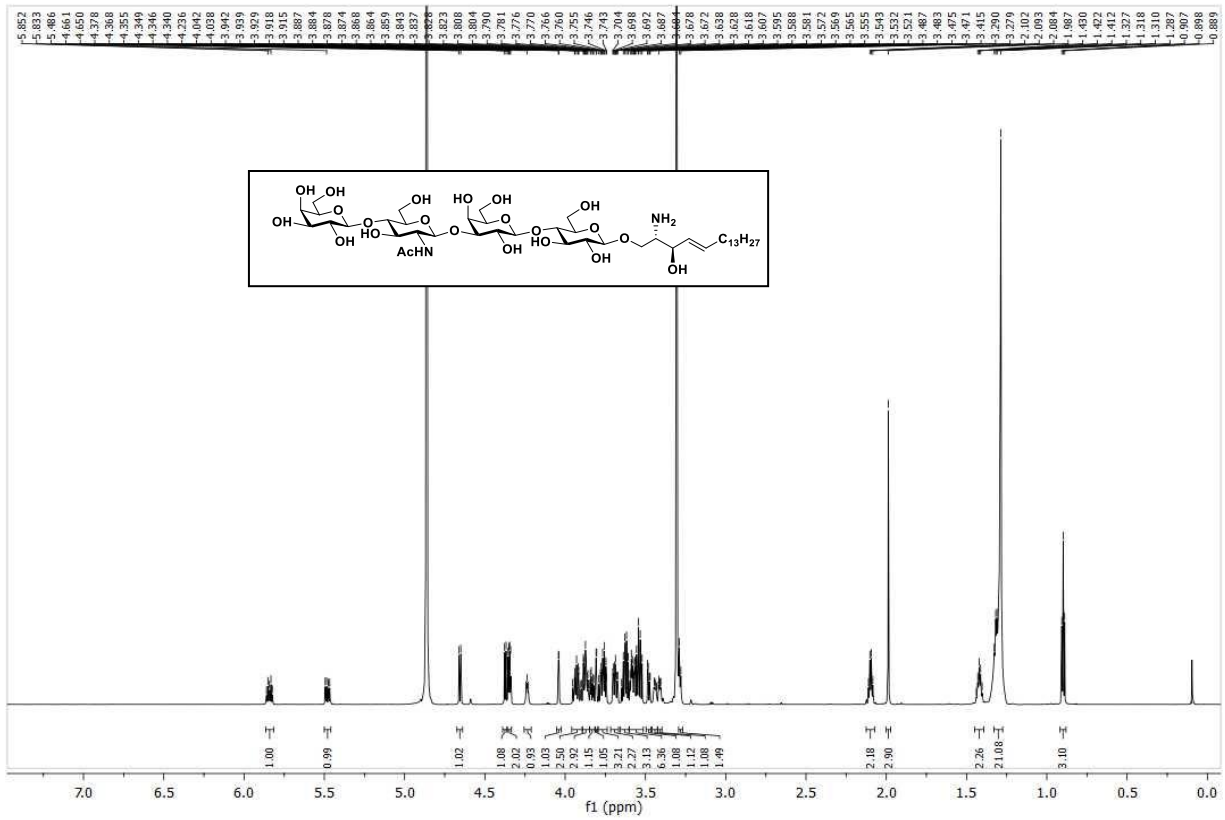


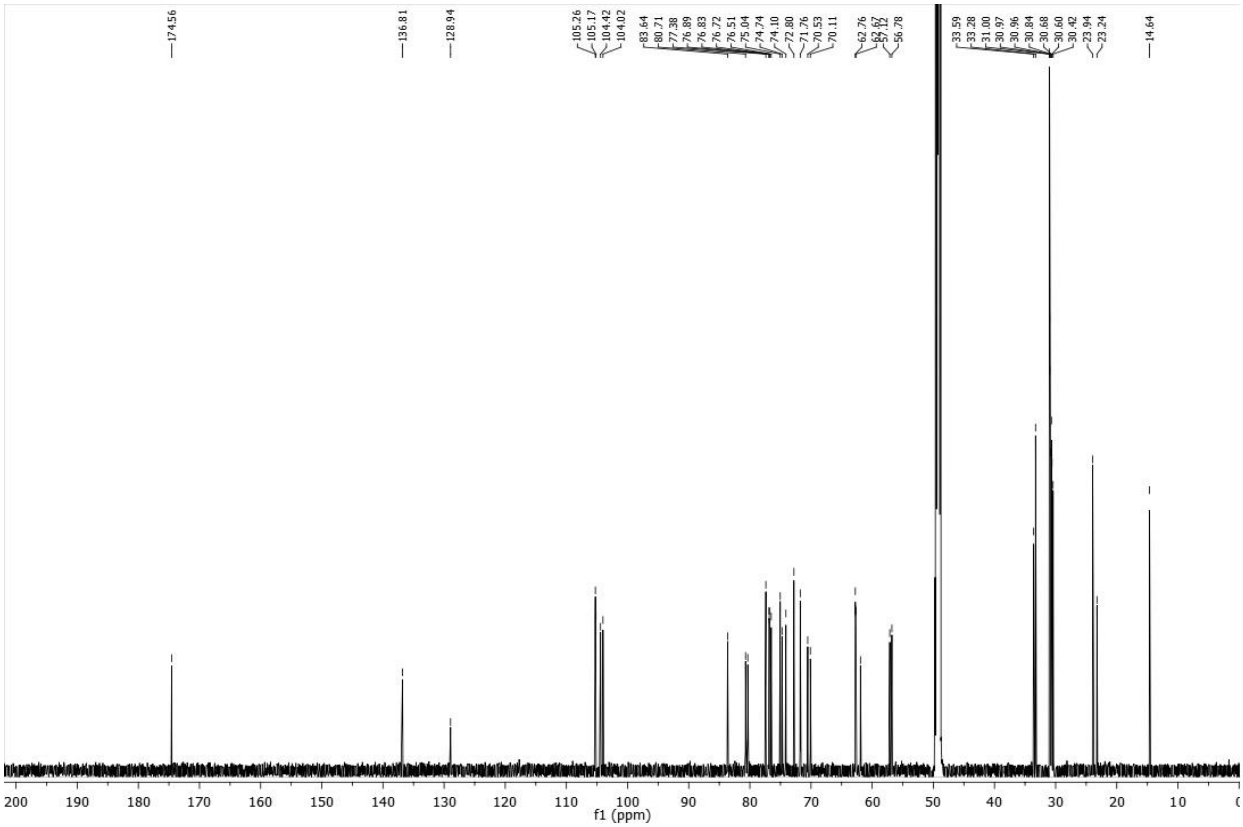
^{1}H NMR (800 MHz, CD_3OD) and ^{13}C NMR spectra of Lc$_3$βSph (**4**)



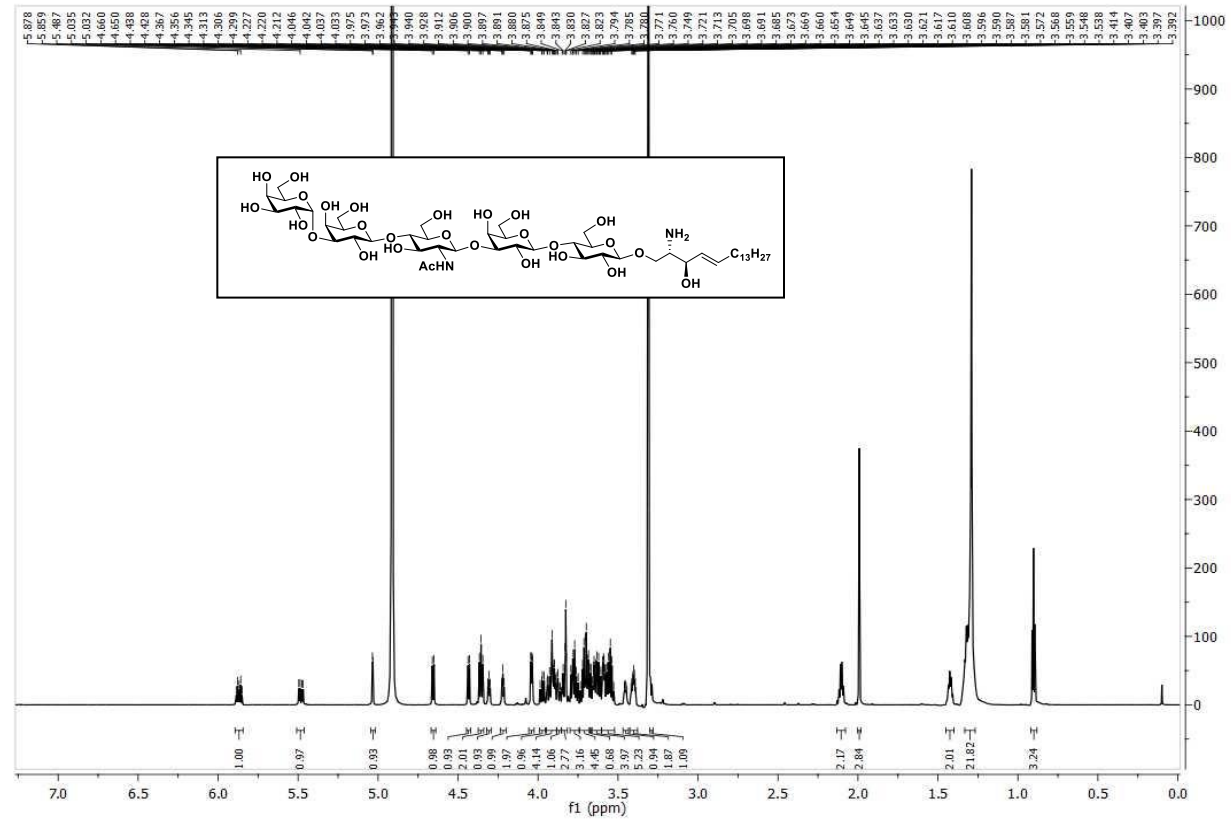


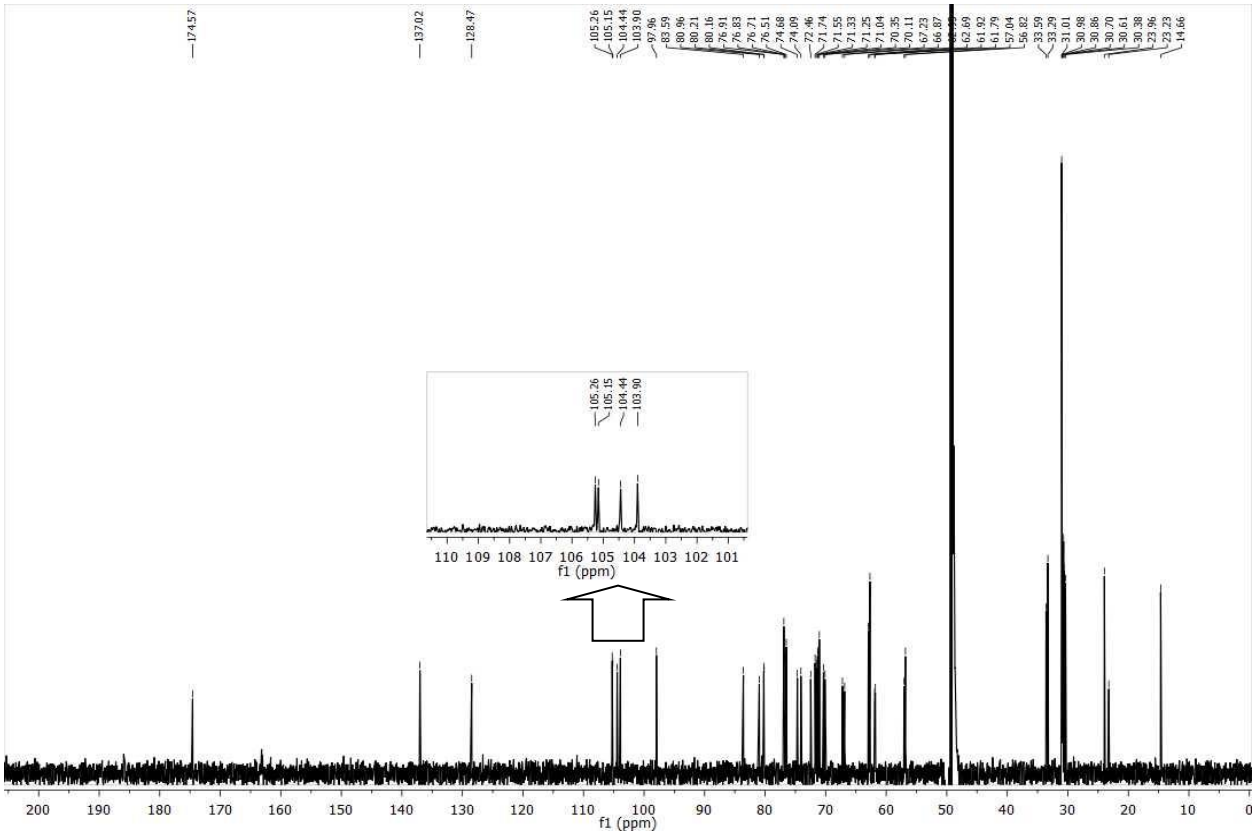
^{1}H NMR (800 MHz, CD_3OD) and ^{13}C NMR spectra of nLc$_4$βSph (**3**)





^{1}H NMR (800 MHz, CD_3OD) and ^{13}C NMR spectra of Galα3nLc$_4$βSph (**2**)





^{1}H NMR (800 MHz, CD_3OD) and ^{13}C NMR spectra of Galα3nLc$_4$βCer (**1**)