Design, Synthesis and Evaluation of Cytotoxic Properties of Bisamino Glucosylated Antitumor Ether Lipids against Cancer Cells and Cancer Stem Cells

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Supplementary information

Synthetic procedures for the synthesis of intermediates and final compounds

1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-*α/β*-D-glucopyranoside (12)

D-glucosamine hydrochloride **11** (3.0 g, 13.92 mmol) was dissolved in water (15.0 ml). To the solution was added Et_3N (2.8 g, 27.80 mmol) and $CuSO_4$ x $5H_2O$ (0.036 g, 0.015 mmol). Triflic azide (16.00 mmol), prepared as previously reported,[23] was then added to the reaction mixture. The resulting blue mixture was stirred vigorously for 18 h and then concentrated under vacuum at room temperature. The residue was dissolved in pyridine (30.0 ml) and DMAP (0.15 g, 1.20 mmol) was added followed by addition of acetic anhydride (9.0 ml, 96.00 mmol). After stirring for 18 h at room temperature, the reaction was stopped with methanol (10.0 ml), and concentrated to dryness. The resulting residue was dissolved in ethyl acetate (120.0 ml) and washed with saturated sodium bicarbonate (×3) and distilled water (×2). The organic layer was dried over anhydrous sodium sulphate, concentrated to dryness,. and purified by flash chromatography (Hexanes:EtOAc, 3:2) to yield **12** as an off white solid (4.5 g, 85 %). The NMR data was in agreement with previously reported data.[23] ES-MS: m/z $[M + Na]^+$ calc'd for $C_{14}H_{19}N_3O_9Na^+$: 396.1, found: 396.2.

Phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-*α/β*-D-glucopyranoside (13)

To a solution of **12** (4.5 g, 12.10 mmol) in DCM (60.0 ml) at room temperature was added thiophenol (2.4 ml, 24.00 mmol) and $BF_3.Et_2O$ (3.0 ml, 24.00 mmol). After stirring overnight at room temperature, the reaction was stopped with saturated sodium bicarbonate solution and the organic layer washed with same solution (×3) and distilled water (×2). This was then dried over anhydrous sodium sulphate and concentrated to dryness The residue was purified by flash chromatography (Hexanes:EtOAc, 3:2) to afford **13** as a α:β (4:1) mixture in the form of a brownish white solid (3.9 g, 76 %). The NMR data was in agreement with previously reported data.[23] ES-MS: m/z $[M + Na]^+$ calc'd for $C_{18}H_{21}N_3O_7SNa^+$: 446.1, found: 446.3

Phenyl -2-azido-2-deoxy-1-thio-*α/β*-D-glucopyranoside (14)

To a dispersion of **13** (3.9 g, 9.21 mmol) in methanol was added NaOMe (1.0 g). The mixture was stirred for 1 h and stopped with 1.0 g of ion exchange resin (H^+). When the reaction mixture became clear, the resin was filtered and the mixture was concentrated under vacuum. The residue was purified by flash chromatography (100 % ethyl acetate) to give **14** as an off-white solid. α/β (3/1) mixture (2.0 g, 73 %). Characteristic ^1H NMR (300 MHz, $CDCl_3$) δ 5.53 (d, *J* = 4.4 Hz, 0.75H, *α* H-1), 4.54 (d, *J* = 10.2 Hz, 0.25H, *β* H-1). ES-MS: m/z $[M + Na]^+$ calc'd for $C_{12}H_{15}N_3O_4SNa^+$: 320.1, found: 320.3.

Phenyl-2-azido-2-deoxy-1-thio-*6-O*-(-2,4,6-triisopropylbenzylsulphonyl-*α/β*-D-glucopyranoside (15)

Compound **14** (3.2 g, 10.76 mmol), catalytic DMAP, and triisopropylbenzylsulphonylchloride (TIBS) were added together in a flask and cooled to 0 °C under vacuum for 20-30 mins, after which it was connected to a nitrogen atmosphere. 50.0 ml of dry pyridine was added and the reaction was stirred for 24 h at room temperature, after which it was stopped by addition of methanol (10.0 ml). The methanol and pyridine were removed under high vacuum. The crude mixture was dissolved in EtOAc and washed with 5 % HCl (×2), saturated sodium bicarbonate solution (×2), and water (×1), to give a dark brown organic layer. The organic solvent was removed under vacuum and crude residue was purified by flash chromatography (Hexanes/EtOAc, 3:2) to give **15** as a brown solid (2.5 g, 4.43 mmol). Yield 41 % (4.8:5.2, α:β). Characteristic ^1H NMR (300 MHz, $CDCl_3$) δ 7.60 – 7.37 (m, 2H, TIBS aromatic proton), 7.32 – 7.16 (m, 5H, thiophenyl proton), 5.53 (d, *J* = 4.3 Hz, 0.48H, *α* H-1), 4.44 (d, *J* = 9.1 Hz, 0.52H, *β* H-1), 1.37 – 1.14 (m, 18H, TIBS isopropyl $-CH_3$). ES-MS: m/z $[M + Na]^+$ calc'd for $C_{27}H_{37}N_3O_6S_2Na^+$: 586.2, found: 586.4.

Phenyl -2, 6-diazido-2, 6-dideoxy-1-thio-α/β-D-glucopyranoside (16)

Compound **15** (2.5 g, 4.43 mmol) was dissolved in anhydrous DMF (25.0 ml) under dry conditions, followed by the addition of NaN_3 (2.3 g, 35.44 mmol). The reaction stirred overnight at 70 °C. The DMF was removed under high vacuum and the residue was suspended in ethyl acetate and filtered to remove excess sodium azide. The organic layer was then concentrated under vacuum and

purified by flash chromatography (100 % EtOAc) to give **16** as a brownish gel (1.36 g, 4.40 mmol). Yield 99 % (5.5:4.5, α:β). Characteristic ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 – 7.19 (m, 5H, thiophenyl aromatic protons), 5.64 (d, *J* = 4.8 Hz, 0.55H, α H-1), 4.51 (d, *J* = 9.9 Hz, 0.45H, β H-1). ES-MS: m/z $[M + Na]^+$ calc'd for $C_{12}H_{14}N_6O_3SNa^+$: 345.1, found: 345.5.

Phenyl – 3,4-diacetyl-2, 6-diazido-2, 6-dideoxy-1-thio-*α/β*- D-glucopyranoside (17)

To a solution of compound **16** (1.4 g, 4.40 mmol) in pyridine (25.0 ml) was added DMAP (0.1 g) and acetic anhydride (5.0 ml). The solution was stirred overnight at room temperature and upon completion, excess acetic anhydride was quenched with methanol (5.0 ml). The solvents were removed under high vacuum to give a brownish residue,. and dissolved in DCM, washed with 5 % HCl solution (×2), saturated sodium bicarbonate (×3) and distilled water (×2). The organic layer was dried over anhydrous sodium sulphate and concentrated under vacuum to a give a brown gel residue. The residue was then purified by flash chromatography (Hexanee:EtOAc, 2:3) to give compound **17** as a brown gel (1.5 g, 4.40 mmol), yield 98% (4.6:5.4, α:β). Characteristic proton NMR data: ^{1}H NMR (300 MHz, Chloroform-d) δ 7.65 – 7.24 (m, 15H, thiophenyl aromatic protons), 5.66 (d, J = 5.6 Hz, 0.46H, αH-1), 4.51 (d, J = 10.2 Hz, 0.54H), 2.13 – 1.99 (m, 6H, acetate -CH_3). ES-MS: calcd $C_{16}H_{18}N_6O_5SNa^+$m/z: 429.10 found $[M+Na]^+$ m/z: 429.1

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(3',4'-O-diacetyl-2', 2',6'-diazido-, 6',2'-dideoxy–α-D-glucopyranosyl)-*sn*-glycerol (19)

Compound **17** (0.16 g, 0.40 mmol) and compound **18** (0.17 g, 0.48 mmol) were dissolved in anhydrous DCM (10.0 ml) under argon atmosphere. NIS (0.18 g, 0.80 mmol) and silver triflate (0.02 g, 0.08 mmol) were added. The reaction mixture was stirred for 3 h. Upon completion, the reaction mixture was diluted with DCM (20.0 ml) and filtered over celite. The resulting organic layer was washed with saturated sodium thiosulphate solution (×2), saturated sodium bicarbonate (×3), and water (×2). The organic layer was then dried over anhydrous sodium sulphate and then concentrated under vacuum to give a brownish gel residue. The residue was purified by flash chromatography (Hexanes/EtOAc, 2:3) to isolate compound **19** as a brown gel from a mixture of **19** and **20.** Yield 33 % (0.082 g, 0.13 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.52 – 5.43 (m, 1H, H-3), 5.06 (d, *J* = 3.5 Hz, 1H, α H-1), 5.00 (dd, *J* = 10.2, 9.1 Hz, 1H, H-4), 4.07 (dt, *J* = 10.2, 4.4 Hz, 1H), 3.91 (dd, *J* = 9.7, 2.5 Hz, 1H), 3.69 – 3.51 (m, 5H), 3.48 (s, 3H, -*OCH*$_3$), 3.47 – 3.35 (m, 1H), 3.29 (m, 3H, H-2, H-6), 2.09 (s, 3H, acetate *CH*$_3$), 2.05 (s, 3H, acetate *CH*$_3$), 1.58 (m, 2H), 1.26 (s, 26H, lipid tail), 0.89 (t, *J* = 6.6 Hz, 3H, lipid terminal –*CH*$_3$).^{13}C NMR (75 MHz, $CDCl_3$) δ 170.13, 170. 01, 98.04, 79.11, 71.86, 70.10, 69.72, 69.46, 68.90, 67.91, 60.90, 57.96, 50.96, 31.93, 29.70, 29.36, 26.11, 22.69, 20.62, 14.11. ES-MS: m/z $[M + Na]^+$ calc'd for $C_{30}H_{54}N_6O_8Na^+$m/z: 649.4, found: 649.4

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diazido-2',6'-dideoxy–α-D-glucopyranosyl)-*sn*-glycerol (21)

Compound **19** was treated according to the general method of acetate deprotection to give **21** as a brown gel. Yield 90 % (0.063 g, 0.12 mmol) ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.98 (d, *J* = 3.5 Hz, 1H, H-1), 4.00 (dd, *J* = 10.4, 8.6 Hz, 1H, H-3), 3.94 – 3.78 (m, 3H), 3.64 – 3.53 (m, 5H), 3.52 – 3.41 (m, 8H), 3.18 (dd, *J* = 10.3, 3.5 Hz, 1H, H-2), 1.58 (m, 2H), 1.27 (s, 26H, lipid tail), 0.89 (t, *J* = 6.6 Hz, 3H, lipid terminal –*CH*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 98.21, 79.24, 71.90, 71.72, 71.39, 70.90, 69.67, 67.18, 62.82, 57.91, 51.36, 31.94, 29.72, 29.67, 29.64, 29.62, 29.52, 29.37, 26.10, 22.70, 14.12. ES-MS: m/z $[M + Na]^+$ calc'd for $C_{26}H_{50}N_6O_6Na^+$: 565.4, found: 565.3.

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-*sn*-glycerol (1)

Compound **21** was deprotected as described under general method for azide reduction to give **1** as yellowish white solid. Yield 65 % (0.04 g, 0.08 mmol). ^{1}H NMR (300 MHz, MeOD) δ 4.8 (d, *J* = 3.6 Hz, 1H, H-1), 3.85 (m, 1H), 3.66 – 3.40 (m, 11H), 3.18 (dd, *J* = 9.3 Hz, 1H, H-4), 3.07 – 2.95 (m, 1H, H-6a), 2.77 (dd, *J* = 13.4, 6.9 Hz, 1H, H-6b) 2.60 (dd, *J* = 10.0, 3.6 Hz, 1H, H-2), 1.62 – 1.56 (m, 2H), 1.32 (s, 26H), 0.93 (t, *J* = 6.5 Hz, 3H**).**^{13}C NMR (75 MHz, MeOD) δ 100.82, 80.67, 76.13, 73.90, 73.50, 72.73, 71.24, 68.25, 58.21, 57.36, 43.78, 33.12, 30.83, 30.80, 30.64, 30.52, 27.30, 23.78, 14.50. HRMS: m/z $[M + Na]^+$ calc'd for $C_{26}H_{54}N_2O_6Na^+$: 513.3880, found: 513.3956. Elemental Analysis: calc'd: C, 63.64; H, 11.09; N, 5.71, found: C, 63.41; H, 10.83; N 5.38.

1, 3, 4, 6-tetra-*O*-acetyl-2-deoxy-2-*N*-phthalimido-D-glucopyranoside (22)

Glucosamine hydrochloride **11** (3.0 g, 14.00 mmol) and NaOH (1.1 g, 28.00 mmol) were dissolved in 50.0 ml of water. The resulting mixture was stirred at room temperature for 30 mins. Phthalic anhydride (2.4 g, 157.0 mmol) was added to the solution. The mixture was stirred at room temperature for 18 h and concentrated. The residue was dissolved in pyridine (30.0 mL), and 19.8 ml acetic added. The resulting solution was stirred overnight at room temperature, quenched with 6.0ml methanol, and concentrated under high vacuum. The remaining solid was dissolved in CH_2Cl_2 (40.0 mL), and washed each with 40.0 ml 10 % HCl (×1), saturated $NaHCO_3$ solution (×3), H_2O (×1), and brine (×1) successively. The organic layer was dried over anhydrous $MgSO_4$., concentrated under reduced pressure, and purified to give **22** (3.3 g, 49 %). NMR data were consistent with literature.[33]

Phenyl 3,4,6-tri-*O*-acetyl-2-*N*-phthalimido-2-deoxy-1-thio-β-D-glucopyranose (23)

To a solution of **22** (1.5 g, 3.16 mmol) in DCM (20.0 ml) was added thiophenol (1.2 ml, 9.48 mmol) and $BF_3.Et_2O$ (1.0 ml, 9.48 mmol). After stirring overnight at room temperature, the reaction was stopped and washed with saturated sodium bicarbonate solution (×3), water (×2), and was dried over anhydrous sodium sulphate. The organic layer was concentrated and purified by flash chromatography

(Hexanes:EtOAc, 3:2) to afford **23** as a brownish white solid (1.3 g yield 77 %). The NMR data was in agreement with previously reported data.[23]

Phenyl-2-phthalimido-2-deoxy-1-thio-β-D-glucopyranose (24)

To a dispersion of **23** (1.3 g, 2.85 mmol) in methanol was added NaOMe (0.15 g). The mixture was stirred until complete dissolution of **23** (15 mins) and 1.0 g of ion exchange resin (H+) was added. When the reaction mixture was clear, the resin was filtered, concentrated, and purified by flash chromatography (100 % ethyl acetate) to give **24** as a white solid. Yield 80 %. NMR data for compound **24**: ^{1}H NMR (300 MHz, MeOD) δ = 8.04 – 7.74 (m, 4H, phthalimido aromatic protons)), 7.49 – 7.17 (m, 5H, thiophenyl aromatic protons), 5.61 (d, *J* =10.4, 1H, H-1), 4.28 (dd, *J* =10.2, 7.8, 1H, H-3), 4.08 (dd, *J* =10.4, 1H), 3.97 (dd, *J* =12.0, 2.0, 1H), 3.78 (dd, *J* =12.1, 5.1, 1H), 3.59 – 3.41 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ = 135.67, 134.47, 132.84, 130.00, 128.71, 124.49, 124.20, 85.49, 82.69, 73.87, 72.28, 62.86, 57.82. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{20}H_{19}NO_6SNa^+$: 424.1, found: 424.1.

Phenyl-2-N-phthalimido-2-deoxy-6-(*O*-toluenesulphonyl)-1-thio-β-D-glucopyranose (25)

To a solution of compound **24** (0.7 g, 1.74 mmol) in anhydrous pyridine (15.0 ml) at 0 °C was added *p*-toluenesulphonyl chloride (0.4 g, 2.09 mmol) and DMAP (0.05 g) under nitrogen atmosphere. The reaction was warmed up to room temperature and stirred overnight, after which it was stopped with 5.0 ml methanol. The mixture was concentrated under high vacuum and purified by flash chromatography (Hexanes/EtOAc, 9:1) to give **25** as a white foam (0.7 g, 1.3 mmol). Yield 74 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.93 – 7.68 (m, 6H, aromatic protons), 7.44 – 7.13 (m, 7H, aromatic protons), 5.53 (d, *J* = 10.3, 1H, H-1), 4.45 – 4.25 (m, 3H, H-3), 4.19 – 4.02 (m, 2H, H-2), 3.78 – 3.46 (m, 2H), 3.31 (br s, 1H, OH), 3.05 (br s, 1H, OH), 2.45 (s, 3H, toluene *CH_3*).^{13}C NMR (75 MHz, $CDCl_3$) δ = 134.32, 132.61, 129.96, 128.87, 128.07, 83.51, 77.24, 72.62, 70.95, 68.58, 55.23, 21.69. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{27}H_{25}NO_8S_2Na^+$: 578.1, found: 578.2.

Phenyl -2-N-phthalimido-2-deoxy-6-azido-6-deoxy-1-thio-β-D-glucopyranose (26)

Compound **25** (2.8 g, 5.19 mmol) was dissolved in anhydrous DMF (25.0 ml) under nitrogen gas atmosphere, then NaN_3 (2.7 g, 41.53 mmol) was added and stirred at 70 °C overnight. The DMF was removed under high vacuum and the residue suspended in ethyl acetate, filtered to remove excess sodium azide, re-concentrated, and partially purified by flash chromatography using Ethylacetate to give **26** as a brownish gel (1.99 g, 4.67 mmol) Yield 90 %. Compound **26** was not characterized by NMR before it was used for next reaction. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{20}H_{18}N_4O_5SNa^+$: 449.1, found: 449.1.

Phenyl 3,4-diacetyl-2-N-phthalimido-2-deoxy-6-azido-6-deoxy-1-thio-β-D-glucopyranose (27)

To a solution of compound **26** (1.5 g, 3.66 mmol) in pyridine (25.0 ml) was added a catalytic amount of DMAP, and acetic anhydride (3.0 ml). The solution was stirred overnight at room temperature. Upon completion of reaction, excess acetic anhydride was quenched with methanol (5.0 ml), and solvents removed under high vacuum to give a brownish residue. The residue was dissolved in DCM, washed with 5 % HCl solution (×2), saturated sodium bicarbonate (×3) and distilled water (×2). The organic layer was dried over anhydrous Na_2SO_4, concentrated, and purified by flash chromatography (Hexanes/EtOAc, 2:3) to give **27** as a light yellow solid. Yield 87 % (1.6 g, 3.20 mmol). Characteristic ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.80 (m, 4H, phthalimido aromatic protons), 7.49 – 7.18 (m, 5H, thiophenyl aromatic protons), 5.57 (d, *J* = 10.2, 1H, H-1), 4.29 - 4.25 (m, 1H), 4.14 (dd, *J* = 10.2,1H, H-2), 3.66 – 3.55 (m, 2H, H-6a), 3.55 – 3.42 (m, 2H, H-6b), 3.37 (d, *J* =4.0, 1H), 3.30 (d, *J* = 5.9, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = δ 170.13, 170.01, 134.41, 133.28, 128.95, 128.31, 123.54, 83.67, 78.59, 73.04, 72.23, 55.57, 51.59, 21.46, 20.87. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{24}H_{22}N_4O_7SNa^+$: 533.1, found: 533.1.

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(3',4'-O-diacetyl-2'-N-phthalimido-6'-azido-2',6'-dideoxy–β-D-glucopyranosyl)-*sn*-glycerol (28)

Compound **27** (0.2 g, 0.39 mmol) was glycosylated with lipid alcohol **21** (0.13 g, 0.43 mmol) according to the general method of glycosylation, to give glycolipid **28** as a yellowish white solid. Yield 47 % (0.13 g, 0.18 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.81 (m, 4H, phthalimido aromatic protons), 5.86 (dd, *J* = 10.8, 9.0, 1H, H-3), 5.40 (d, *J* = 8.5 Hz, 1H, H-1), 5.06 (dd, *J* = 10., 9.0, 1H,H-4), 4.33 (dd, *J* = 10.8, 8.4, 1H, H-2), 3.98 – 3.83 (m, 2H), 3.63 (dd, *J* = 10.7, 4.9, 1H), 3.52 – 3.41 (m, 2H), 3.38 – 3.08 (m, 8H), 2.06 (s, 3H, acetate *CH_3*), 1.88 (s, 3H, acetate *CH_3*), 1.66 – 1.58 (m, 2H), 1.27 (s, 26H, lipid tail), 0.89 (t, *J* = 6.6 Hz, 3H, lipid terminal –*CH_3*). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.11, 169.63, 134.25, 123.54, 98.47, 78.60, 73.61, 71.66, 70.41, 70.38, 69.82, 68.71, 57.59, 54.64, 51.23, 31.94, 29.71, 29.67, 29.61, 29.48, 29.37, 26.01, 22.70, 20.66, 20.48, 14.12. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{38}H_{58}N_4O_{10}Na^+$: 753.4, found: 753.5.

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(-2'-amino-6'-azido-2',6'-dideoxy–β-D-glucopyranosyl)-*sn*-glycerol (29)

Compound **31** (0.1 g, 0.14 mmol) was deprotected using the general method of simultaneous deprotection of acetate and phthalimido group to give **32** as a yellowish white solid. Yield 55 % (0.04 g, 0.08 mmol). ^{1}H NMR (300 MHz, MeOD) δ = 4.29 (d, *J* = 8.1, 1H, H-1), 3.95 (dd, *J* = 10.5, 4.3, 1H), 3.71 (dd, *J* = 10.5, 4.2, 1H), 3.64 – 3.51 (m, 4H), 3.51 – 3.38 (m, 7H), 3.29 – 3.20 (m, 2H, H-3), 2.70 – 2.55 (dd, *J* = 8.1,1H, H-2), 1.59-1.52 (m 2H), 1.32 (s, 26H, lipid tail), 0.88 (t, *J* = 6.6 Hz, 3H, lipid terminal –*CH_3*).^{13}C NMR (75 MHz, MeOD) δ = 104.73, 80.48, 79.10, 77.41, 77.31, 72.65, 71.46, 69.44, 58.33, 58.04, 52.79, 33.10, 30.80, 30.61, 30.50, 27.25, 23.76,

14.47. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{26}H_{52}N_4O_6Na$: 539.4, found: 539.4.

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(-2',6'-diamino-2',6'-dideoxy-β-D-glucopyranosyl)-*sn*-glycerol (2)

Compound **29** (0.04 g, 0.08 mmol) was treated according to the general method for azide reduction to give compound **2** as an off white solid. Yield 78 % (0.03 g, 0.06 mmol). ^{1}H NMR (300 MHz, MeOD) δ = 4.26 (d, *J* = 8.0, 1H, H-1), 3.95 (dd, *J* = 10.8, 4.6, 1H), 3.70 (dd, *J* = 10.6, 4.2, 1H), 3.66 – 3.45 (m, 8H), 3.33 – 3.14 (m, 3H), 3.06 (dd, *J* = 13.4, 2.7, 1H), 2.61 (dd, *J* = 9.6, 8.0, 1H,H-2), 1..66 -1.54 (m, 2H), 1.32 (s, 26H, lipid tail), 0.87 (t, *J* = 6.6 Hz, lipid terminal –*CH$_3$*).^{13}C NMR (75 MHz, MeOD) δ = 104.98, 80.58, 78.08, 77.51, 73.51, 72.68, 71.41, 69.74, 58.45, 58.17, 43.99, 33.12, 30.83, 30.79, 30.64, 30.52, 27.28, 23.78. HRMS: m/z [M + Na]$^+$ calc'd for $C_{26}H_{54}N_2O_6Na^+$m/z: 513. 3612, found: 513.3612. Elemental Analysis: calc'd: C, 63.64; H, 11.09; N, 5.71, found: C, 63.39; H, 10.88; N, 5.98.

1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(-2'-N-phthalimido-6'-azido-2',6'-dideoxy–β-D-glucopyranosyl)-*sn*-glycerol (30)

Compound **28** (0.05 g, 0.07 mmol) was dissolved in methanol followed by the addition of catalytic NaOMe (0.1 equiv) and stirred for 20 mins. The reaction was stopped by with ion exchange resin (H$^+$), filtered, concentrated, and purified by flash chromatography using 100 % ethyl acetate to give **30** as a yellowish white solid. Yield 62 % (0.025 g, 0.04 mmol) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 – 7.77 (m, 4H, phthalimido aromatic protons), 5.20 (d, *J* = 8.3 Hz, 1H, H-1), 4.34 (dd, *J* = 10.9, 8.5 Hz, 1H, H-3), 4.13 (dd, *J* = 10.9, 8.3 Hz, 1H, H-2), 3.87 (dd, *J* = 10.7, 4.6 Hz, 1H, H-6a), 3.76 - 3.66 (m, 1H), 3.62 – 3.49 (m, 4H, H-4, H-5, H-6b), 3.49 – 3.39 (m, 2H), 3.35 -3.28 (m, 2H), 3.26 (d, *J* = 4.0 Hz, 1H), 3.23 – 3.07 (m, 5H), 1.45 – 1.36 (m, 2H), 1.26 (s, 26H, lipid tail), 0.90 (t, *J* = 6.6 Hz, 3H lipid terminal –*CH$_3$*).^{13}C NMR (75 MHz, $CDCl_3$) δ 168.40, 134.18, 131.70, 123.42, 98.68, 78.67, 77.46, 75.23, 72.78, 71.69, 69.95, 68.32, 57.56, 56.56, 51.48, 31.94, 29.72, 29.67, 29.62, 29.47, 29.38, 26.00, 22.70, 14.13. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{34}H_{54}N_4O_8Na^+$: 669.4, found: 669.4

1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(-2'-N-phthalimido-6'-amino-2',6'-dideoxy–β-D-glucopyranosyl)-*sn*-glycerol (3)

Compound **30** (0.025 g, 0.04 mmol) was subjected to the general method for azide reduction to give compound **3** (0.017 g, 0.03 mmol) as light yellow solid. Yield 67 %. ^{1}H NMR (300 MHz, MeOD) δ 8.03 – 7.61 (m, 4H), 5.20 (d, *J* = 8.5 Hz, 1H, H-1), 4.33 (dd, *J* = 10.7, 8.6 Hz, 1H, H-3), 4.00 (dd, *J* = 10.7, 8.5 Hz, 1H, H-2), 3.87 (dd, *J* = 11.0, 4.2 Hz, 1H), 3.72 – 3.55 (m, 2H), 3.43 – 3.26 (m, 17H), 3.26 – 3.07 (m, 6H, H-6a), 2.88 (dd, *J* = 13.5, 7.5 Hz, 1H, H-6b),), 1.58 – 1.46 (m, 2H), 1.32 (s, 26H), 0.83 (t, *J* = 6.8 Hz, 3H).^{13}C NMR (75 MHz, MeOD) δ 168.40, 134.18, 131.70, 123.42, 98.68, 78.67, 77.46, 75.23, 72.78, 71.69, 69.95, 57.56, 56.56, 52.45, 51.48, 31.94, 29.72, 29.67, 29.62, 29.47, 29.38, 26.00, 22.70, 14.13. HRMS: m/z [M + Na]$^+$ calc'd for $C_{34}H_{56}N_2O_8Na^+$: 643.3934, found: 643.3857. Elemental Analysis: calc'd: C, 65.78; H, 9.09; N, 4.51, found: C, 65.69; H, 8.99; N, 4.61.

1-*O*-Hexadecyl-2-deoxy-3-O-(3',4'-O-diacetyl-2'-N-phthalimido-6'-azido-2',6'-dideoxy–β-D-glucopyranosyl)-*sn*-glycerol (32)

Compound **27** (0.2 g, 0.39 mmol) and the previously reported lipid compound **31** (0.17 g, 0.48 mmol) were dissolved in anhydrous DCM (10.0 ml) under argon atmosphere. NIS (0.18 g, 0.80 mmol) and silver triflate (0.02 g, 0.08 mmol) were added and stirring at room temperature for 3 h. Upon completion, the reaction mixture was diluted with DCM (20.0 ml), filtered over celite, washed with saturated sodium thiosulphate solution (×2), saturated sodium bicarbonate (×3), and water (×2) successively. The organic layer was then dried over anhydrous sodium sulphate, concentrated, and purified by flash chromatography (Hexanes/EtOAc, 2:3) to give **32** (0.14 g, 0.2 mmol) as a white solid. Yield 51 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.85 (dd, *J* = 5.5, 3.1, 2H, phthalimido aromatic protons), 7.73 (dd, *J* = 5.5, 3.1, 2H, phthalimido aromatic protons), 5.79 (dd, *J* = 10.8, 9.0, 1H, H-3), 5.38 (d, *J* = 8.5, 1H, H-1), 5.05 (dd, *J* = 10.1, 9.0, 1H, H-4), 4.30 (dd, *J* = 10.8, 8.5, 1H, H-2), 3.96 – 3.81 (m, 2H), 3.63 – 3.52 (m, 1H), 3.43 (d, *J* = 13.6, 6.9, 1H), 3.28 – 3.16 (m, 3H), 3.15 – 2.99 (m, 2H), 2.03 (s, 3H, acetate *CH$_3$*), 1.85 (s, 3H, acetate *CH$_3$*), 1.76 – 1.58 (m, 2H), 1.26 (s, 26H, lipid tail), 0.89 (t, *J* = 6.6 Hz, 3H, lipid terminal –*CH$_3$*).^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.12, 169.62, 134.27, 123.57, 97.99, 73.60, 71.82, 71.00, 70.53, 70.37, 67.04, 66.96, 54.68, 51.24, 31.91, 29.73, 29.68, 29.59, 29.48, 29.34, 26.07, 22.67, 14.10. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{37}H_{56}N_4O_9Na^+$: 723.4, found: 723.5.

1-*O*-Hexadecyl-2-deoxy-3-O-(2'-amino-6'-azido-2',6'-dideoxy–β-D-glucopyranosyl)-*sn*-glycerol (33)

Compound **32**(0.14 g, 0.20 mmol) was subjected to general method for simultaneous deprotection of acetate and phthalimido group to give **33** (0.074 g, 0.12 mmol). Yield 60 %. ^{1}H NMR (300 MHz, MeOD) δ = 4.28 (d, *J* = 7.9, 1H, H-1), 3.98 (dd, *J* = 9.6, 6.3, 1H), 3.69 – 3.61 (m, 1H), 3.57 - 3.53 (m, 2H), 3.51 – 3.41 (m, 5H, H-6), 3.30 – 3.21 (m, 2H, H-3), 2.63 (dd, *J* = 9.8,7.9, 1H), 1.96 – 1.82 (m, 2H, -OCH$_2$-**CH$_2$**.CH$_2$O-), 1.66 - 1.57 (m, 2H), 1.32 (s, 26H, lipid tail), 0.86 (t, *J* = 6.7 Hz, 3H, lipid terminal –*CH$_3$*).^{13}C NMR (75 MHz, MeOD) δ = 104.54, 77.34, 72.72, 72.10, 68.77, 67.91, 58.37, 52.81, 33.12, 31.16, 30.82, 30.65, 30.52, 27.31, 23.78, 14.50. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{33}H_{52}N_4O_7Na^+$: 639.4, found: 639.4.

1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-*sn*-glycerol (4)

Compound **33** (0.074 g, 0.12 mmol) was subjected to the general method for azide reduction to give compound **4** (0.039 g, 0.08 mmol) as a white solid. Yield 70 %. ^{1}H NMR (300 MHz, MeOD) δ = 4.25 (d, *J* = 8.0, 1H, H-1), 3.99 – 3.61 (m, 2H), 3.50-3.60 (m, *J* = 6.3, 3.8, 3H), 3.45 (t, *J* = 6.5, 3H), 3.31 – 3.14 (m, 2H, H-3), 3.06 (dd, *J* = 13.4, 2.8, 1H, H-6a), 2.76 (dd, *J* = 13.4, 7.0, 1H, H-6b), 2.59 (dd, *J* = 9.5, 8.0, 1H, H-

2), 1.96 – 1.82 (m, 2H, -OCH_2-***CH_2***.CH_2O-), 1.64 - 1.55 (m, 2H), 1.32 (s, 26H, lipid tail), 0.85 (t, *J* = 6.7 Hz, 3H, lipid terminal –*CH_3*). ^{13}C NMR (75 MHz, MeOD) δ = 104.78, 78.00, 77.55, 73.57, 72.10, 68.70, 67.89, 58.50, 44.00, 33.11, 31.21, 30.82, 30.66, 30.51, 27.32, 23.77, 14.50. HRMS: m/z [M + Na]$^+$ calc'd for $C_{25}H_{52}N_2O_5Na^+$: 483.3774, found: 483.3781. Elemental Analysis: calc'd: C, 65.18; H, 11.38; N, 6.08, found: C, 65.43; H, 11.21; N, 5.79.

1-*O*-Hexadecyloxy-2S/R, 3-di (-3,4-diacetyl-6'azido-2-*N*-phthalimido-2',6'-dideoxy- β-D- glucopyranosyl)-glycerol (35)

Compound **27**(0.300 g, 0.59 mmol) and lipid diol **37** (0.063 g, 0.20 mmol) were subjected to the general procedure for the glycosylation reaction to give the diglycosylated glycolipid **35** (0.15 g, 0.13 mmol) as a brownish white solid. Yield 65 % (S:R, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 – 7.65 (m, 8H, phthalimido aromatic protons), 5.71 (td, *J* = 10.4, 9.1 Hz, 2H, H-3a, H-3b), 5.53 (d, *J* = 8.5 Hz, 1H, H-1a), 5.20 (d, *J* = 8.4 Hz, 1H, H-1b), 5.02 (dd, *J* = 10.1, 8.9 Hz, 1H), 4.94 – 4.81 (m, 1H), 4.28-4.15 (m, 2H, H-2a), 3.97 – 3.67 (m, 4H), 3.67 – 3.28 (m, 6H, H-2b), 3.26 – 2.98 (m, 3H), 2.06 (s, 6H, acetate *CH_3*), 1.86 (s, 3H, acetate *CH_3*), 1.64 (s, 3H, acetate CH_3),1.66– 1.57 (m, 2H), 1.28 (s, 26H, lipid tail), 0.89 (t, *J* = 6.7 Hz, 3H, lipid terminal –*CH_3*.) ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.30, 134.19, 123.81, 123.52, 96.74, 96.69, 76.57, 73.25, 72.90, , 71.53,70.40, 70.39, 70.36, 70.15, 70.14, 70.12, 69.77, 69.76,54.68, 54.41, 51.22, 51.23, 51.09, 31.89, 31.85, 29.65, 25.97, 25.87, 21.71, 22.65, 21.47, 20.68, 20.43, 14.13.ES-MS: m/z [M + Na]$^+$ calc'd for $C_{55}H_{72}N_8O_{17}Na^+$: 1139.5, found: 1139.4.

1-*O*-Hexadecyloxy-2S/R,3-di(-2'-amino-6'azido-2',6'-dideoxy-β-D-glucopyranosyl)-glycerol (36)

Compound **35** (0.15 g, 0.13 mmol) was subjected to the general method for simultaneous deprotection of acetate and phthalimido protective group to give **36** (0.048 g, 0.07 mmol) as a white solid. Yield 53 % (S:R, 1:1). ^{1}H NMR (300 MHz, MeOD) δ 4.49 (d, *J* = 8.0Hz, 1H, H-1a), 4.33 (dd, *J* = 8.4, 1H, H-1b), 4.16 – 3.97 (m, 2H), 3.78 (dd, *J* = 10.7, 5.5 Hz, 1H), 3.67 (dd, *J* = 5.1, 2.7 Hz, 2H), 3.59 – 3.39 (m, 2H), 3.36 – 3.22 (m, 7H, H-3a, H3b), 2.75 – 2.54 (m, 2H, H-2a, H-2b), 1.60 m, 2H), 1.31 (s, 26H, lipid tail), 0.93 (t, *J* = 4.5Hz, 3H, lipid terminal –*CH_3*).^{13}C NMR (75 MHz, MeOD) δ 104.87, 104.28, 78.63, 77.23, 77.05, 72.64, 72.60, 72.52, 71.57, 70.72, 58.48, 58.35, 52.79, 33.10, 30.81, 30.65, 30.49, 27.31, 23.76, 14.47. ES-MS m/z [M + Na]$^+$ calc'd for $C_{31}H_{60}N_8O_9Na^+$: 711.4, found: 711.4

1-*O*-Hexadecyloxy-2S/R, 3-di(-2',6'-diamino-2',6'-dideoxy-β-D-glucopyranosyl)-glycerol (5)

Compound **36** (0.048 g, 0.07 mmol) was subjected to general method for azide reduction to give compound **5** (0.032 g, 0.05 mmol) as a white solid. Yield 69 % (S:R, 1:1). ^{1}H NMR (300 MHz, MeOD) δ 4.44 (d, *J* = 8.3 Hz, 1H, H-1a), 4.29 (d, *J* = 8.4 Hz, 1H, H-1b), 4.03 (d, *J* = 14.5 Hz, 2H), 3.8 - 3.59 (m, 3H), 3.56 – 3.35 (m, 5H), 3.28 – 3.17 (m, 4H, H-3a, H-3b), 3.15 – 3.01 (m, 2H, H-6a'', H-6b''), 2.81 – 2.69 (m, 2H, H-6a', H-6b'), 2.64 – 2.58 (m, 2H, H-2a, H-2b), 1.60 (s, 2H), 1.32 (s, 26H, lipid tail), 0.92 (t, *J* = 6.8 Hz, 3H, lipid terminal –*CH_3*). ^{13}C NMR (75 MHz, MeOD) δ 103.58, 103.27, 77.43, 76.41, 76.38, 72.11, 72.03, 70.19, 70.13, 69.47, 69.45, 57.17, 57.14, 48.04, 42.55, 42.47, 42.44, 31.61, 29.42, 29.31, 29.19, 25.77, 19.91, 23.67, 21.21, 11.80. HRMS: m/z [M + Na]$^+$ calc'd for $C_{31}H_{64}N_4O_9Na^+$: 659.4571, found: 659.2064. Elemental Analysis: calc'd: C, 58.46; H, 10.13; N, 8.80, found: C, 58.74; H, 10.22; N, 9.03.

3-Hexadecyloxy-2R-hydroxylpropyl-1-para-toluenesulphonate (38)

The lipid diol **37** (2.0 g, 6.32 mmol) was dissolved in 20.0 ml DCM, cooled to 0 °C, then Et_3N (1.8 ml, 1.28 g), toluenesulphonychloride (6.95 mmol, 1.33 g) and DMAP (0.04 g, 0.32 mmol) were added sucessively. The temperature was allowed to warm up to room temperature and the mixture stirred for 4 h. At the end of the reaction, mixture was diluted with ethylacetate (60.0 ml), washed with saturated aqueous ammonium chloride (×3), and brine (×3). The organic layer was dried over anhydrous sodium sulphate, concentrated, and purified using flash chromatography (hexanes/ethyl acetate, 4:1) to give **38** (1.8 g, 3.80 mmol) as a white flaky solid. Yield 60 %.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.2 Hz, 2H, aromatic protons), 7.33 (d, *J* = 8.1 Hz, 2H, aromatic protons), 4.11 – 4.00 (m, 2H, TsO-CH_2), 3.99 – 3.89 (m, 1H, HO-CH), 3.46 – 3.31 (m, 4H), 2.80 (d, *J* = 5.4 Hz, 1H, OH), 2.42 (s, 3H, Toluene –*CH_3*), 1.55 – 1.41 (m, 2H), 1.25 (s, 26H, lipid tail), 0.87 (t, *J* = 6.4 Hz, 3H, lipid terminal –*CH_3*).^{13}C NMR (75 MHz, $CDCl_3$) δ 144.90, 132.77, 129.88, 127.99, 71.73, 70.77, 70.56, 68.25, 31.93, 29.71, 29.68, 29.64, 29.61, 29.48, 29.37, 26.01, 22.68, 21.58, 14.11. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{26}H_{46}NO_5Na^+$: 493.2964, found: 493.6788

3-Hexadecyloxy-2R-hdroxyl propyl-1-azide (39)

Compound **38** (1.3 g, 2.76 mmol) and sodium azide (10 equiv) were suspended in anhydrous DMF and was stirred at 90 °C for 18 h. Upon completion, the mixture was concentrated, diluted with ethyl acetate, and filtered to remove excess sodium azide. The filterate was re-concentrated and purified with flash chromatography (hexanes/ethyl acetate, 9:1) to give **39** (0.85 g, 2.50 mmol) as a white wax-like solid. Yield 91 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.91 – 3.86 (m, 1H, HO-CH), 3.48 – 3.34 (m, 4H), 3.31 (dd, *J* = 5.5, 2.9 Hz, 2H, ***-CH_2***N_3), 3.17 (s, 1H, OH), 1.55 – 1.41 (m, 2H), 1.25 (s, 26H, lipid tail), 0.85 (t, *J* = 6.6 Hz, 3H, terminal lipid -*CH_3*). ^{13}C NMR (75 MHz, $CDCl_3$) δ 71.92, 71.71, 69.59, 53.54, 31.93, 29.71, 29.67, 29.61, 29.52, 29.47, 29.37, 26.05, 22.67, 14.03. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{19}H_{39}N_3O_2Na^+$: 364.3, found: 364.5

1-*O*-Hexadecyloxy-2R-(-3',4',6'-triacetyl-2-*N*-phthalimido-2'-deoxy-β-D-glucopyranosyl)-3-azido-*sn*-glycerol (40)

Compounds **23**(0.2 g, 0.38 mmol) and **39** (0.1 g, 0.31 mmol) were treated as describe in the general method for glycosylation reaction, to give **40** (0.12 g, 0.16 mmol) as a

brownish white solid. Yield 53 %.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 – 7.63 (m, 4H, phthalimido aromatic protons), 5.80 (dd, *J* = 10.7, 9.1 Hz, 1H, H-3), 5.53 (d, *J* = 8.5 Hz, 1H, H-1), 5.16 (dd, *J* = 10.7 Hz, 1H, H-4), 4.39 – 4.28 (m, 2H, H-2), 4.19 (dd, *J* = 12.2, 2.5 Hz, 1H), 3.96 – 3.73 (m, 2H, H-5), 3.60 (d, *J* = 10.0, 4.8 Hz, 1H), 3.48 – 3.30 (m, 4H), 3.27 – 3.16 (m, 1H), 2.12 (s, 3H, acetate –*CH*$_3$), 2.04 (s, 3H, acetate –*CH*$_3$), 1.88 (s, 3H, acetate –*CH*$_3$), 1.48 (m, 2H), 1.09 (s, 26H, lipid tail), 0.88 (t, *J* = 6.6 Hz, 3H, Lipid terminal –*CH*$_3$).^{13}C NMR (75 MHz, $CDCl_3$) δ 170.58, 170.11, 169.47, 134.21, 131.56, 123.50, 98.61, 78.66, 71.96, 71.79, 70.74, 70.24, 69.03, 62.16, 54.64, 52.46, 31.93, 29.69, 29.66, 29.62, 29.58, 29.45, 29.36, 26.06, 22.69, 20.75, 20.63, 20.44, 14.11. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{39}H_{58}N_4O_{11}Na^+$: 781.4, found: 781.4

1-*O*-Hexadecyloxy-2R-(2'-amino-2'-deoxy-β-D-glucopyranosyl)-3-azido-*sn*-glycerol (6)

Compound **40** (0.12 g, 0.16 mmol) was treated according to the general method for simultaneous removal of acetate and phthalimido to give **6** (0.04 g, 0.08 mmol) as an off white solid. Yield 49 %. ^{1}H NMR (300 MHz, MeOD) δ 4.45 (d, *J* = 8.0 Hz, 1H, H-1), 4.12 – 3.95 (m, 1H), 3.74 -3.65 (m, 3H), 3.63 – 3.54 (m, 2H), 3.51- 3.38 (m, 4H), 3.32 -3.27 (m, *J* = 8.4 Hz, 2H, H-3), 2.66 (dd, *J* = 8.0, 6.8, 1H, H-2), 1.64 – 1.49 (m, 2H), 1.32 (s, 26H), 0.93 (t, *J* = 7.1, Hz, 3H lipid terminal –*CH*$_3$). ^{13}C NMR (75 MHz, MeOD) δ 104.02, 78.30, 78.13, 77.23, 72.70, 71.78, 71.70, 62.78, 61.56, 58.30, 53.17, 33.15, 30.87, 30.83, 30.76, 30.70, 30.66, 30.55, 27.30, 23.81, 20.97, 14.57. HRMS: m/z [M + Na]$^+$ calc'd for $C_{25}H_{50}N_4O_6Na^+$: 525.3628, found: 525.3064. Elemental Analysis: calc'd: C, 59.73; H, 10.03; N, 11.15, found: C, 59.65; H, 10.13; N, 11.24

1-*O*-Hexadecyloxy-2R-(2'-amino-2'-deoxy-β-D-glucopyranosyl)-3-amino-*sn*- glycerol (7)

Compound **6** (0.03 g, 0.06 mmol) was treated according to the general method for azide reduction to give compound **7** (0.02 g, 0.04 mmol) as a white solid. Yield 65 %. ^{1}H NMR (300 MHz, MeOD) δ 4.40 (d, *J* = 8.1 Hz, 1H, H-1), 3.93 – 3.82 (m, 1H, -O-*CH*), 3.75 – 3. 67 (m, 3H), 3.60 – 3.41 (m, 4H), 3.36 – 3.22 (m, 2H, H-3), 2.97 – 2.71 (m, 2H, -*CH*$_2$NH$_2$), 2.63 (t, *J* = 8.4 Hz, 1H, H-2), 1.58 (m, 2H), 1.32 (s, 26H), 0.87 (t, *J* = 6.9 Hz, 3H).^{13}C NMR (75 MHz, MeOD) δ 104.46, 80.02, 78.22, 77.78, 72.70, 72.67, 71.78, 62.81, 58.45, 43.77, 33.11, 30.80, 30.66, 30.51, 27.31, 23.77, 14.49. HRMS: m/z [M + Na]$^+$ calc'd for $C_{25}H_{52}N_2O_6Na^+$: 499.3723, found: 499.2997. Elemental Analysis: calc'd: C, 62.99; H, 11.00; N, 5.88, found: C, 63.05; H, 11.15; N, 6.01

2(R/S)-Azido-3-hexadecyloxy-1-propanol (44)

Compound **41** was synthesized as previously reported without any modification.[28] Diisopropylazodicarboxylate (DIAD; 3.2 ml, 15.00 mmol) was added to a solution of racemic mixture of 3-*O*- hexadecyl-*sn*-glycerol **34** (3.4 g, 13.00 mmol) in 180.0 ml of DCM at 0 °C. After the mixture was stirred for 3h under Nitrogen gas, Me_2SiN_3 was added. The mixture was stirred at the same temperature for additional 3 h, then at room temperature until glycerol **34** had completely reacted. The mixture was concentrated to give a yellow residue which was dissolved in a minimal amount of DCM and passed through a pad of silica gel in a sintered glass funnel. The pad was rinsed with hexane/EtOAc (50:1) until the excess yellow DIAD began to elute. After concentration of the eluted silyloxyazide, the residue was dissolved in 30.0 ml THF and treated with a solution of $(nBu)_4NF$ (1.0 M, 25.0 ml) in THF. The mixture was stirred for 3 h at room temperature and diluted with 250.0 ml Et_2O, washed with water (×2), and brine (×2). The organic layer was separated, dried over anhydrous sodium sulphate and concentrated under vacuum. The crude product was purified by flash column chromatography (hexane/EtOAc, 4:1) to give compound **41** as a colorless gel. The NMR corresponds to previously reported data.[28] Yield 50 %.

1-*O*-Hexadecyloxy-2S/R-azido,3-(-3',4',6'-triacetyl-2-*N*-phthalimido-2'-deoxy-β-D-glucopyranosyl)-glycerol (42)

The azido lipid **41** (0.28 g, 0.82 mmol) and the glycoside donor **23** (0.52 g, 0.99 mmol) were treated according to general method for glycosylation reaction to give the protected glycolipid **42** as an isomeric mixture (S:R, 1:1) (0.36 g, 0.48 mmol) of a white solid. Yield 58 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 – 7.69 (m, 4H, phthalimido aromatic protons), 5.79 (ddd, *J* = 10.7, 9.1, 4.7 Hz, 1H, H-3), 5.39 (dd, *J* = 10.9, 8.5 Hz, 1H, H-1), 5.16 (dd, *J* = 10.2, 9.1 Hz, 1H, H-4), 4.39 – 4.24 (m, 2H, H-2), 4.20 – 4.05 (m, 2H), 4.01 – 3.81 (m, 2H, H-5), 3.65 – 3.55 (m, 1H), 3.55 – 3.45 (m, 1H), 3.44 – 3.32 (m, 1H), 3.31 – 3.10 (m, 2H), 2.09 (s, 3H), 2.01 (s, 3H), 1.84 (s, 3H), 1.46 - 138 (m, 2H), 1.30 (s, 26H, lipid tail), 0.85 (t, *J* = 6.6 Hz, 3H, terminal lipid *CH*$_3$).^{13}C NMR (75 MHz, $CDCl_3$) δ 170.60, 170.06, 169.42, 134.27, 134.23, 131.47, 123.54, 98.56, 98.46, 71.96, 71.68, 71.61, 70.65, 70.59, 70.25, 69.94, 69.05, 68.91, 68.90, 61.93, 61.90, 60.54, 60.32, 59.90, 54.49, 31.90, 29.67, 29.63, 29.59, 29.55, 29.46, 29.39, 29.33, 25.90, 22.66, 20.98, 20.71, 20.58, 20.41, 14.18, 14.09. ES-MS: m/z [M + Na]$^+$ calc'd for $C_{39}H_{58}N_4O_{11}Na^+$: 781.4, found: 781.4

1-*O*-Hexadecyloxy-2S/R-amino-3-(-3',4',6'-triacetyl-2-*N*-phthalimido-2'-deoxy-β-D-glucopyranosyl)-glycerol (43)

Compound **42** (0.36 g, 0.48 mmol) was treated according to the general procedure for azide reduction to give **43** (0.25 g, 0.34 mmol) as a white solid. Yield 71 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 – 7.63 (m, 4H, phthalimido aromatic protons), 5.79 (t, *J* = 9.9 Hz, 1H,H-3), 5.35 (d, *J* = 8.4 Hz, 1H, H-1), 5.16 (t, *J* = 9.6 Hz, 1H, H-4), 4.37 – 4.29 (m, 1H, H-2), 4.22 – 4.08 (m, 1H), 3.92 – 3.77 (m, 2H, H-5), 3.75 – 3.59 (m, 1H), 3.48 (dd, *J* = 9.6, 5.3 Hz, 1H), 3.40 (dd, *J* = 9.6, 6.8 Hz, 1H), 3.26 – 3.06 (m, 3H), 3.06 - 2.94 (m, 1H, -*CH*NH$_2$), 2.25 (br. s, 2H, amino protons), 2.10 (s, 3H), 2.02 (s, 3H), 1.85 (s, 3H), 1.49 – 1.34 (m, 2H), 1.23 (s, 26H, lipid tail), 0.86 (t, *J* = 6.4 Hz, 3H, lipid terminal -*CH*$_3$).^{13}C NMR (75 MHz, $CDCl_3$) δ 170.67, 170.12, 169.47, 134.34, 131.37, 123.62, 98.58, 98.37, 72.64, 72.48, 72.32, 71.88, 71.42, 70.71, 69.01, 62.02, 58.10, 54.65, 50.66, 50.61, 31.90, 29.68, 29.64, 29.58, 29.52, 29.45, 29.34,

26.05, 22.67, 20.74, 20.61, 20.43, 18.40, 14.10. ES-MS: m/z $[M + Na]^+$ calc'd for $C_{39}H_{60}N_2O_{11}Na^+$: 755.4, found: 755.4

1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-amino-2'-deoxy-β-D- glucopyranosyl)-glycerol (8)

Compound **43** (0.03 g, 0.04 mmol) was treated according to the general procedure for simultaneous removal acetate and phthalimido protecting group to give **8** (0.01 g, 0.023 mmol) as a white solid. Yield 57 % (S:R, 1:1). ^{1}H NMR (300 MHz, MeOD) δ 4.70 (dd, *J* = 8.3, 3.1 Hz, 1H, H-1), 4.19 – 4.02 (m, 1H), 4.01 – 3.87 (m, 2H), 3.79 – 3.73 (m, 1H), 3.72 – 3.61 (m, 3H), 3.65 – 3.57 (m, 1H, H-3), 3.57 – 3.48 (m, 2H), 3.49 - 3.32 (m, 2H, -*CH*NH_2), 2.96 (dd, *J* = 10.3, 8.4 Hz, 1H, H-2), 1.71 – 1.55 (m, 2H), 1.33 (s, 26H, lipid tail), 0.92 (t, *J* = 6.4 Hz, 3H, lipid terminal –*CH_3*).^{13}C NMR (75 MHz, MeOD) δ 100.58, 100.18, 78.63, 73.85, 72.93, 71.74, 71.65, 68.58, 68.43, 62.15, 62.10, 52.72, 52.69, 33.09, 30.80, 30.77, 30.64, 30.53, 30.48, 27.13, 23.75. HRMS: m/z $[M + Na]^+$ calc'd for $C_{25}H_{52}N_2O_6Na^+$: 499.3723, found: 499.3450. Elemental Analysis: calc'd: C, 62.73; H, 11.00; N, 5.88, found: C, 62.99; H, 10.78; N, 5.55

1-*O*-Hexadecyloxy-2S/R-*N*-hexadecylacyl-3-(-3',4',6'-triacetyl-2-*N*-phthalimido-2'-deoxy-β-D-glucopyranosyl)-glycerol (45)

Compound **43** (0.13 g, 18.00 mmol) was dissolved in 10.0 ml of anhydrous DMF. Palmitic acid **44** (0.054 g, 0.21 mmol) and the coupling agent TBTU (0.08 g, 0.25 mmol) were subsequently added under argon atmosphere, and stirred for 5 h at room temperature. After complete disappearance of **43**, the reaction mixture was concentrated and purified by flash chromatography (hexanes/EtOAc, 4:1) to give **45** (0.1 g, 0.11 mmol) as a white compound which was characterized as deblocked analogue **9** Yield 60 %. ES-MS: m/z $[M + Na]^+$ calc'd for $C_{55}H_{90}N_2O_{12}Na^+$: 993.6, found: 993.4

1-*O*-Hexadecyloxy-2S/R-*N*-hexadecylacyl-3-(-2'-amino-2'-deoxy-β-D-glucopyranosyl)-glycerol (9)

Compound **45** (0.1 g, 0.11 mmol) was subjected to the treatment of the general method for simultaneous deprotection of acetate and phthalimido protecting group to give compound **9** (0.05 g, 0.06 mmol) as a white solid (S:R, 1:1). Yield was 58 %.^{1}H NMR (300 MHz, MeOD) δ 4.59 (dd, *J* = 8.3, 6.3 Hz, 1H, H-1 R/S), 4.42 – 4.20 (m, 1H, H-4 R/S), 3.92 (dd, *J* = 12.1, 4.3 Hz, 2H, H-6a R/S), 3.83 – 3.63 (m, 3H, H-6b R/S), 3.66 – 3.44 (m, 6H, H-3 R/S), 2.89 – 2. 71 (m, 1H, H-2 R/S), 2.29 – 2.17 (m, 2H, -NHCO-*CH_2*), 1.71 - 1.51 (m, 4H), 1.33 (s, 50H, two lipid tails), 0.93 (t, *J* = 6.2 Hz, 6H, terminal –*CH_3* of the two lipid tails). ^{13}C NMR (75 MHz, MeOD) δ 176.79, 100.75, 100.45, 78.56, 74.02, 72.49, 72.45, 71.81, 71.02, 70.71, 70.53, 62.32, 57.54, 55.16, 50.52, 50.39, 49.88, 37.29, 37.19, 33.11, 30.85, 30.81, 30.74, 30.59, 30.52, 30.30, 27.33, 27.13, 23.77, 14.48. HRMS: m/z $[M + Na]^+$ calc'd for $C_{41}H_{82}N_2O_7Na^+$: 737.6020, found: 737.3607. Elemental Analysis: calc'd: C, 68.86; H, 11.56; N, 3.92, found: C, 68.66; H, 11.73; N, 3.89

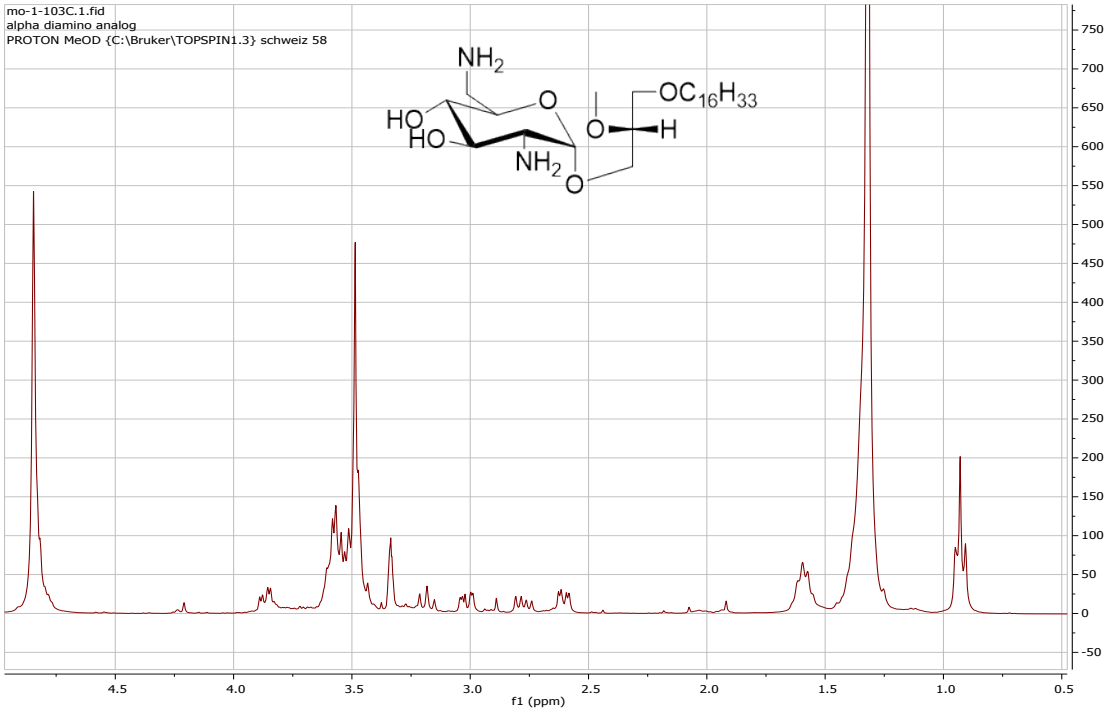
1-*O*-Hexadecyloxy-2S/R-*N*-methylcarbamoyl-3-(-3',4',6'-triacetyl-2-*N*-phthalimido-2'-deoxy-β-D-glucopyranosyl)-glycerol (47)

To a solution of compound **43** (0.12 g, 0.16 mmol) in DCM was added methylchloroformate **46** (0.03 g, 0.31 mmol) and Et_3N (0.04 g, 0.35 mmol) at 0 °C. The mixture was stirred overnight and concentrated under vacuum to give residue, which was purified by flash chromatography (hexanes/EtOAc, 3:2) to give the carbamate glycolipid **47** (0.1 g, 0.13 mmol) as a white solid (S:R, 1:1).[15] Yield 80 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 – 7.68 (m, 4H, phthalimido aromatic protons), 5.79 (dd, *J* = 10.7, 9.1 Hz, 1H, H-3), 5.35 (d, *J* = 8.4 Hz, 1H, H-1), 4.83 (d, *J* = 7.4 Hz, 1H, carbamate -NH), 4.42 – 4.23 (m, 2H), 4.19 – 4.15 (m, 1H), 3.88 – 3.71 (m, 4H), 3.53 (s, 3H, carbamate -*CH_3*), 3.35 - 28 (m, 2H), 3.15 – 3.08 (m, 2H), 2.11 (s, 3H), 2.02 (s, 3H), 1.85 (s, 3H), 1.45 – 1.34 (m, 2H), 1.27 (s, 26H, lipid tail), 0.88 (t, *J* = 6.4 Hz, 3H, lipid terminal -*CH_3*).^{13}C NMR (75 MHz, $CDCl_3$) δ 170.69, 170.11, 169.47, 134.32, 134.28, 131.42, 123.62, 98.49, 98.39, 71.89, 71.39, 71.32, 70.67, 70.63, 68.95, 68.91, 68.76, 68.66, 61.98, 54.59, 31.91, 29.69, 29.64, 29.58, 29.45, 29.34, 26.00, 25.97, 22.67, 20.73, 20.61, 20.43, 14.11. ES-MS: m/z $[M + Na]^+$ calc'd for $C_{41}H_{62}N_2O_{13}Na^+$: 813.4, found: 813.3

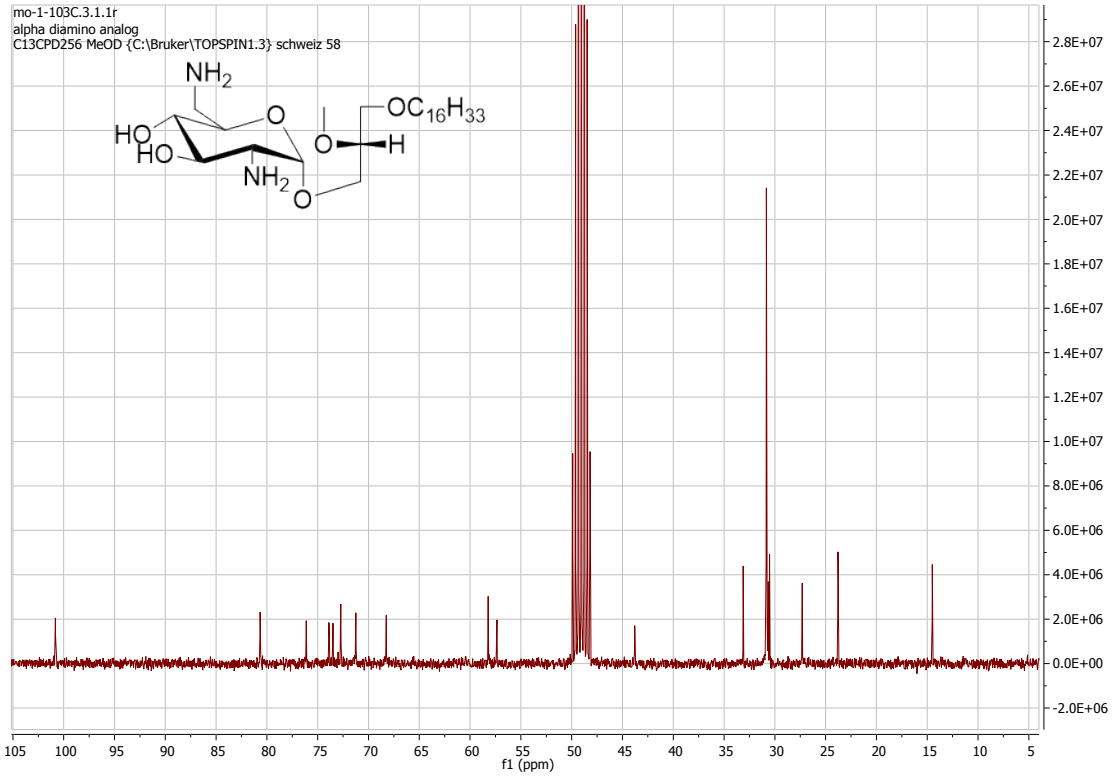
1-*O*-Hexadecyloxy-2S/R-*N*-methylcarbamoyl-(-2'-amino-2'-deoxy-β-D-glucopyranosyl)-glycerol (10)

The protected carbamate glycolipid **47** (0.1 g, 0.13 mmol) was deprotected using the general method for simultaneous removal of acetate and phthalimido protecting group to give compound **10** (0.033 g, 0.06 mmol) as a white solid (S:R, 1:1). Yield 47 %. ^{1}H NMR (300 MHz, MeOD) δ 4.26 (dd, *J* = 8.1, 2.4 Hz, 1H, H-1), 4.00 – 3.89 (m, 2H), 3.87 (d, *J* = 1.5 Hz, 1H), 3.76 – 3.68 (m, 2H), 3.66 (s, 3H, carbamate –*CH_3*), 5.55 - 3.44 (m 4H, H-3), 3.38 – 3.22 (m, 3H), 265 – 2.58 (m, 1H, H-2) 1.60 – 1.55 (m, 2H), 1.31 (s, 26H, lipid tail), 0.92 (t, *J* = 6.8 Hz, 3H, lipid terminal –CH_3). ^{13}C NMR (75 MHz, MeOD) δ 105.05, 104.71, 78.23, 77.57, 77.51, 72.44, 71.84, 71.74, 70.97, 70.60, 70.28, 62.80, 62.69, 58.36, 58.34, 52.56, 52.23, 33.11, 30.82, 30.79, 30.65, 30.59, 30.50, 27.27, 23.77, 14.49. HRMS: m/z $[M + Na]^+$ calc'd for $C_{27}H_{54}N_2O_8Na^+$: 557.3778, found: 557.3364. Elemental Analysis: calc'd: C, 60.65; H, 10.18; N, 5.24 found: C, 60.82; H, 10.31; N, 5.19.

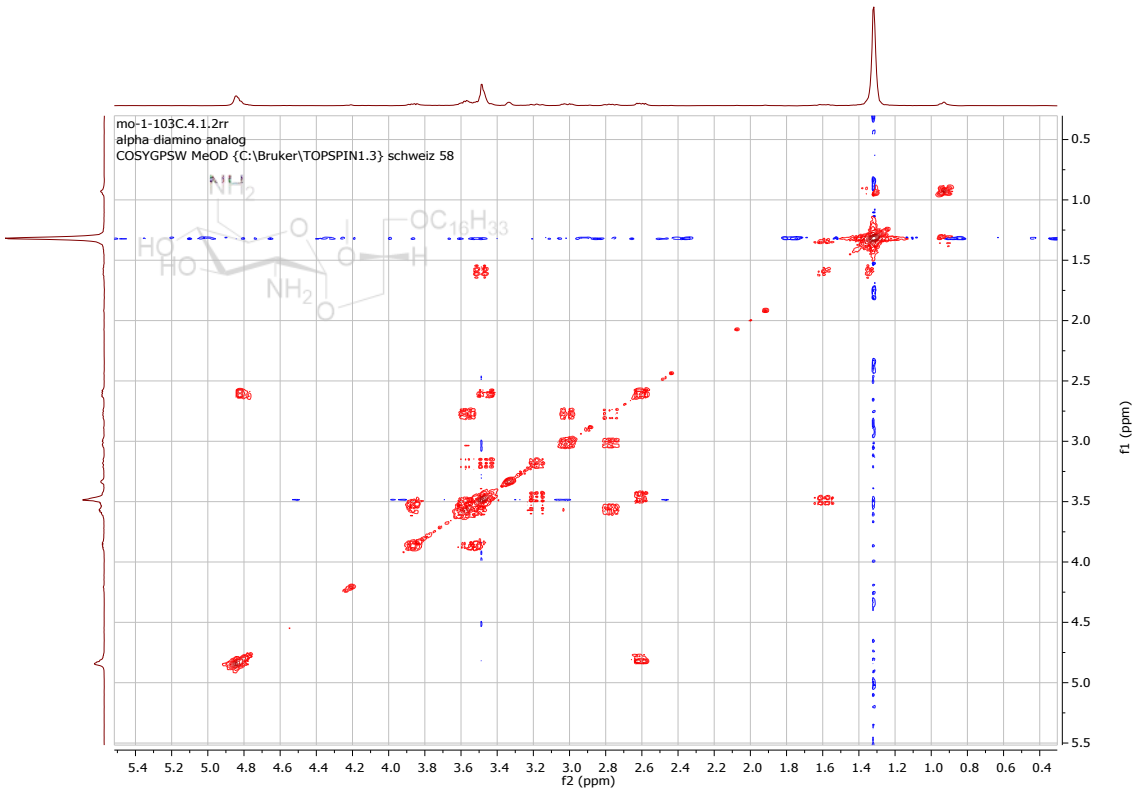
^{1}H NMR Spectra of compound **1**



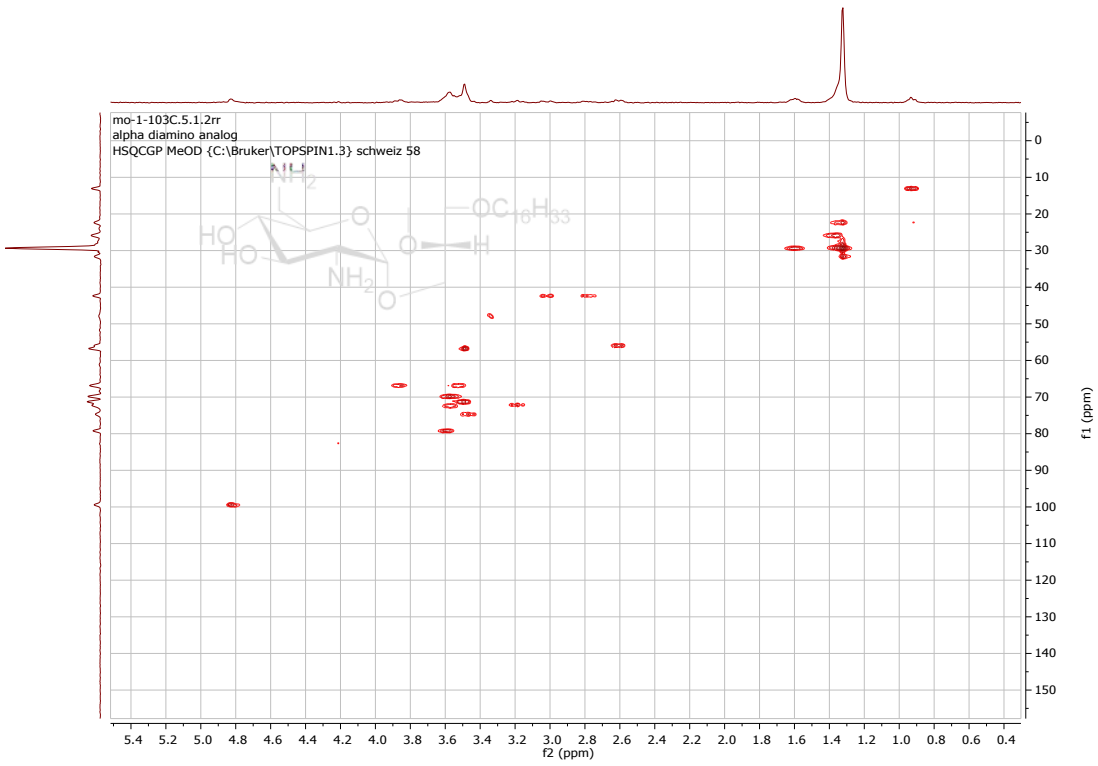
^{13}C NMR Spectra of Compound **1**



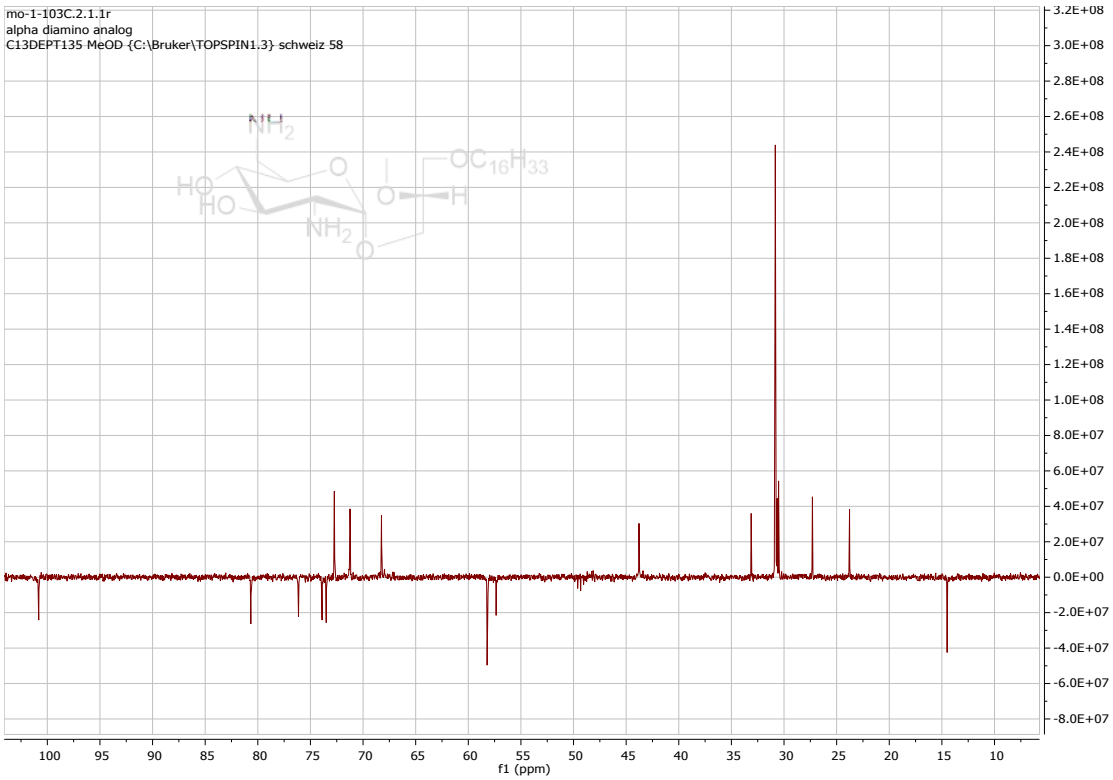
COSY NMR Spectra of Compound **1**



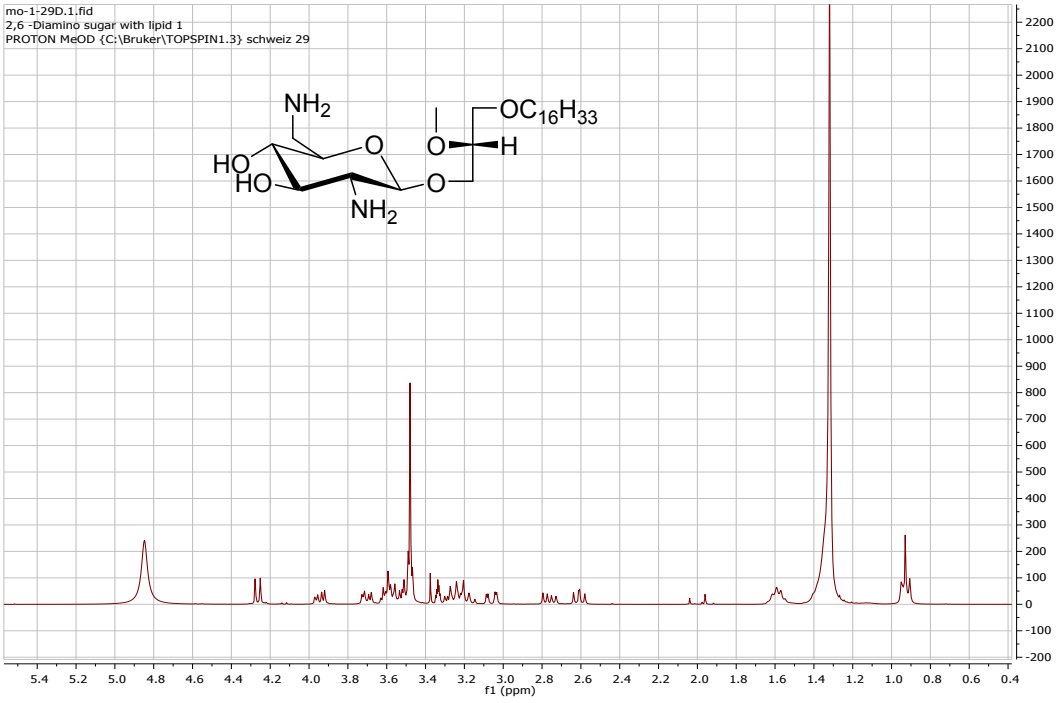
HSQC Spectra of Compound **1**



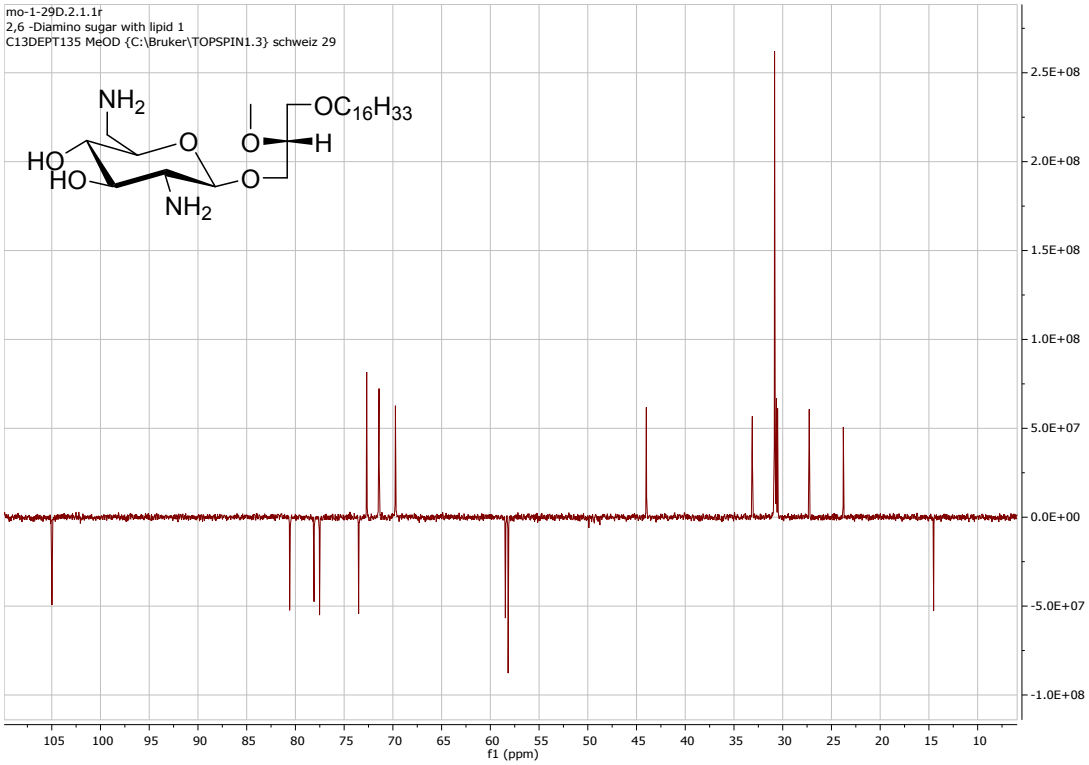
DEPT135 NMR Spectra of Compound **1**



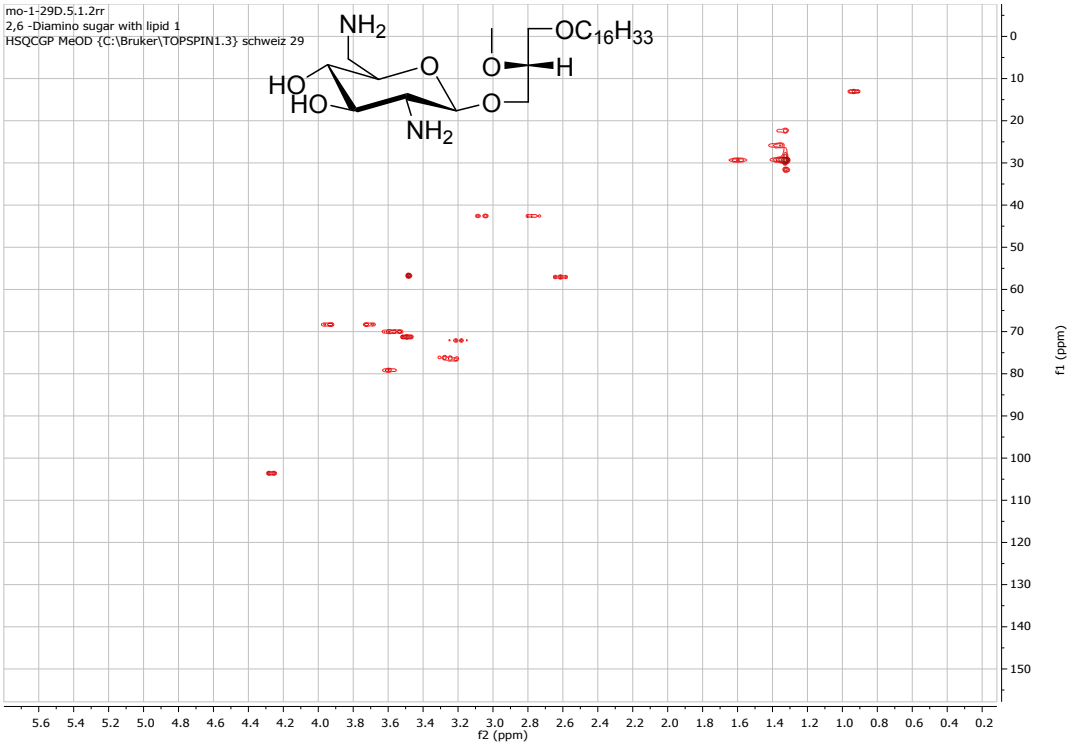
^{1}H NMR Spectra of Compound **2**



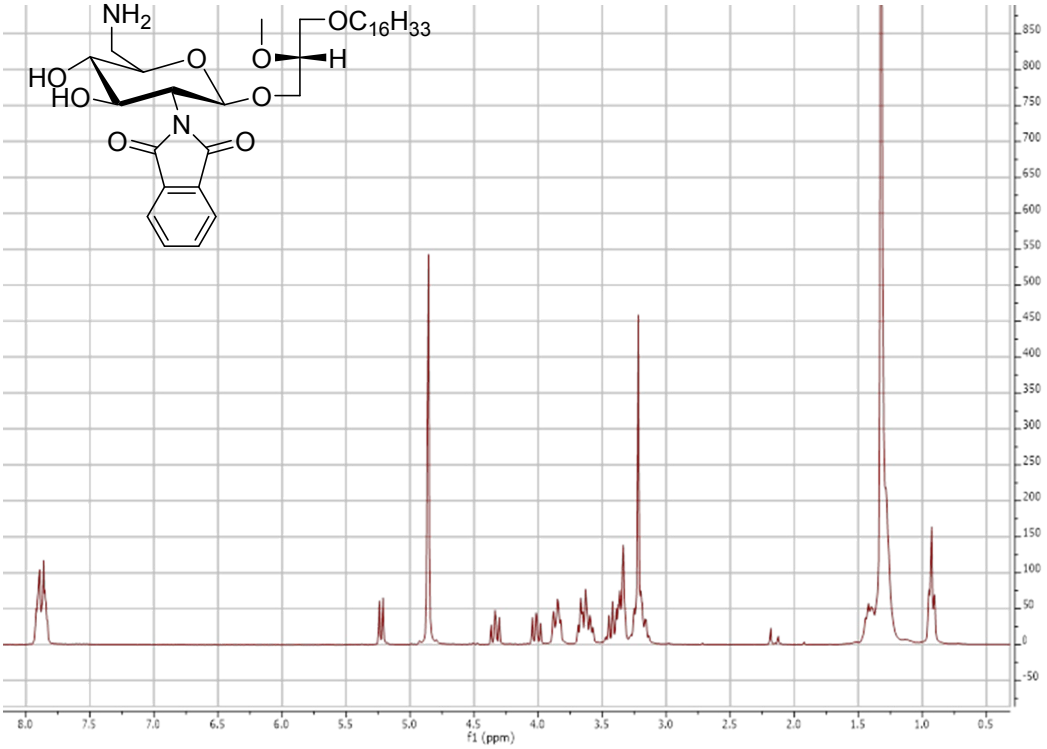
DEPT135 NMR Spectra of compound **2**



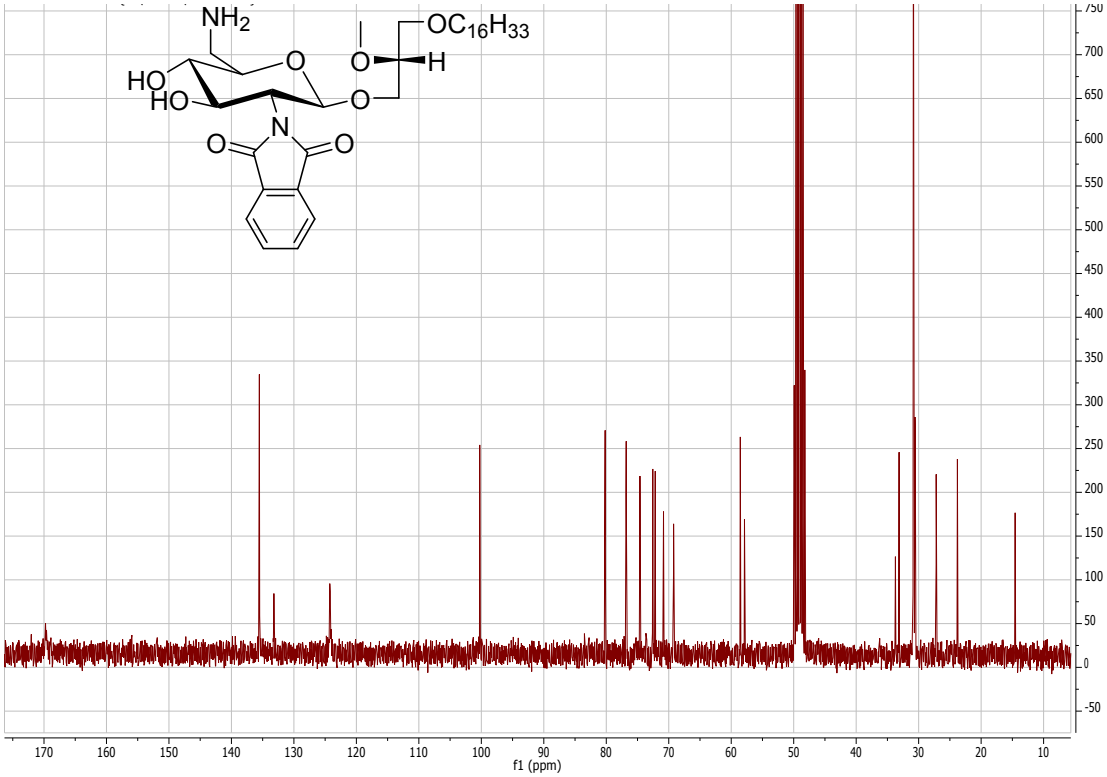
HSQC NMR of Spectra of compound **2**



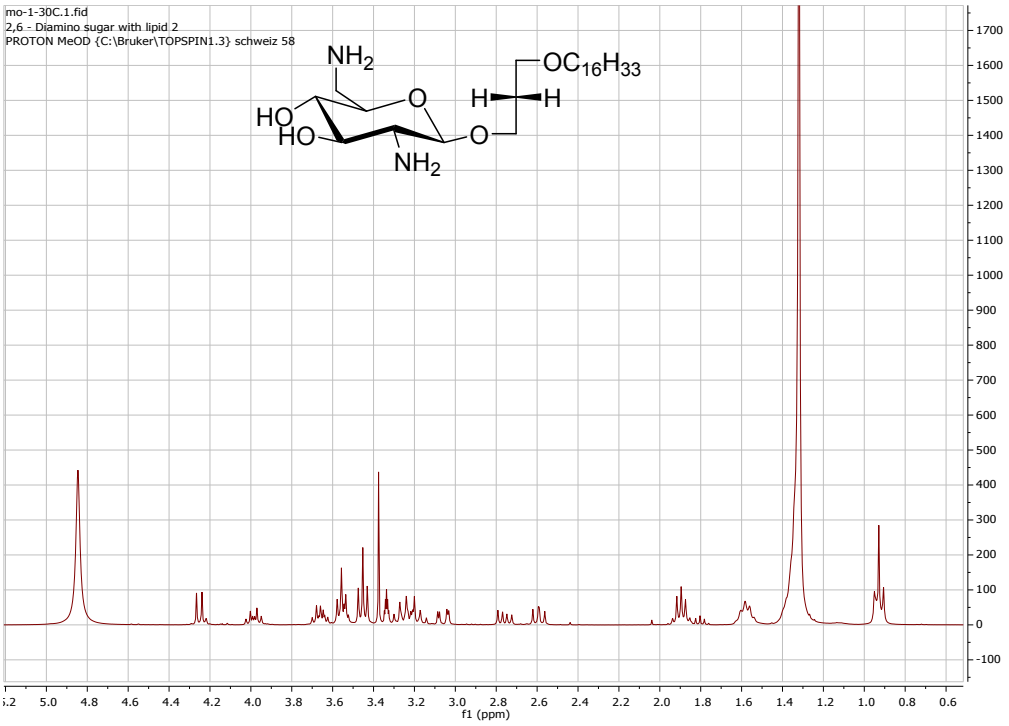
^{1}H NMR Spectra of compound **3**



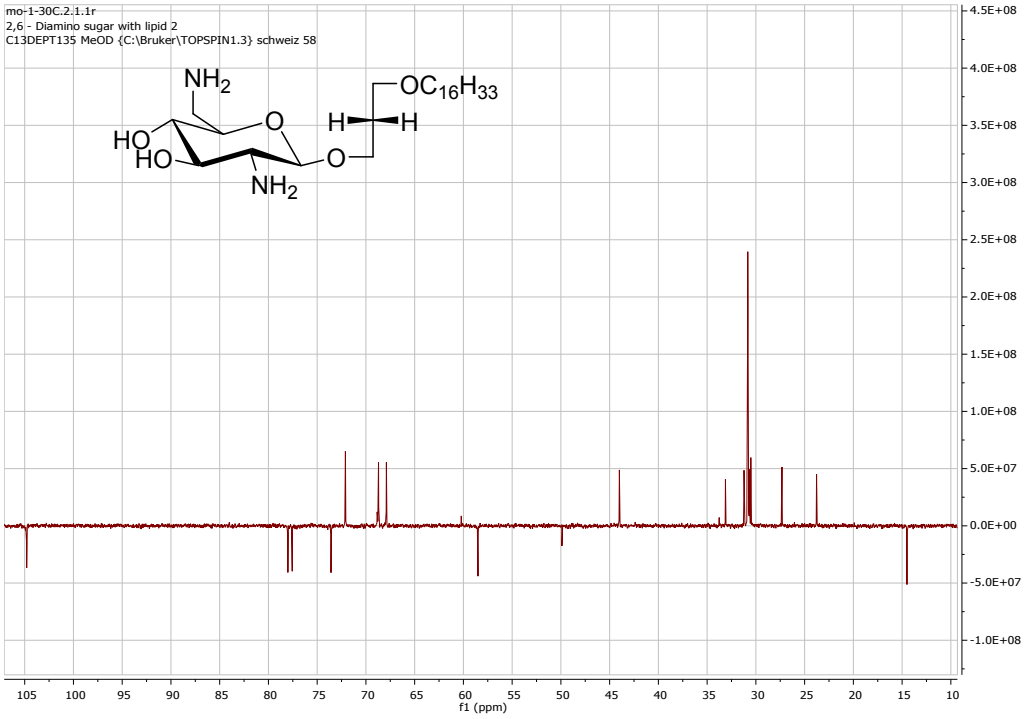
^{13}C NMR spectra of compound **3**



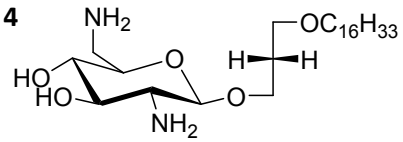
1H NMR Spectra of compound **4**

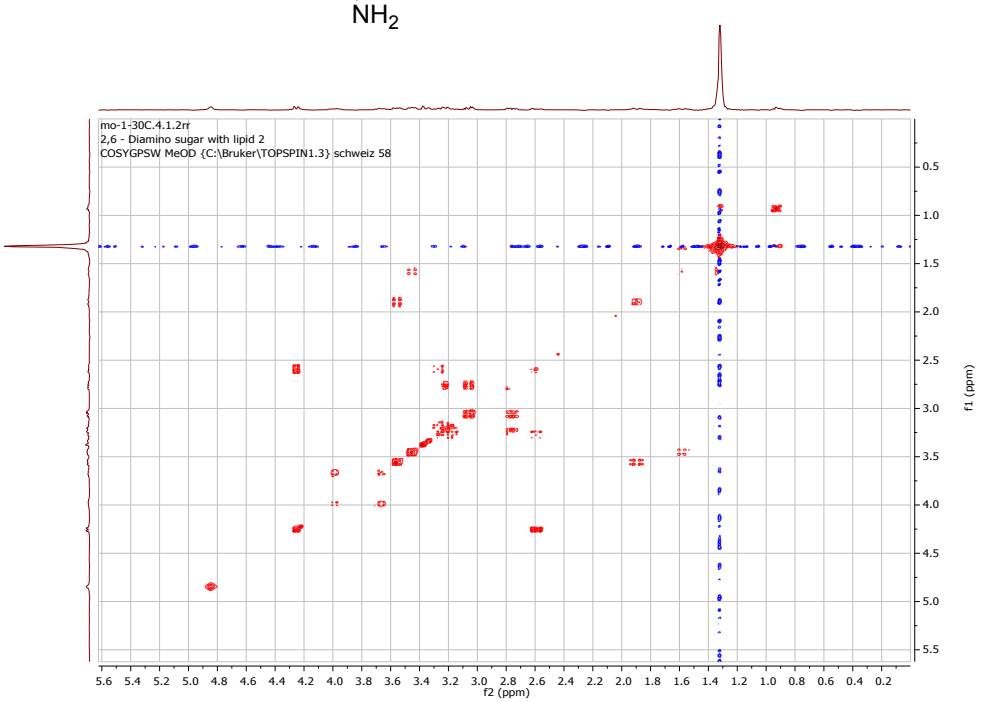


DEPT135 NMR Spectra of compound **4**

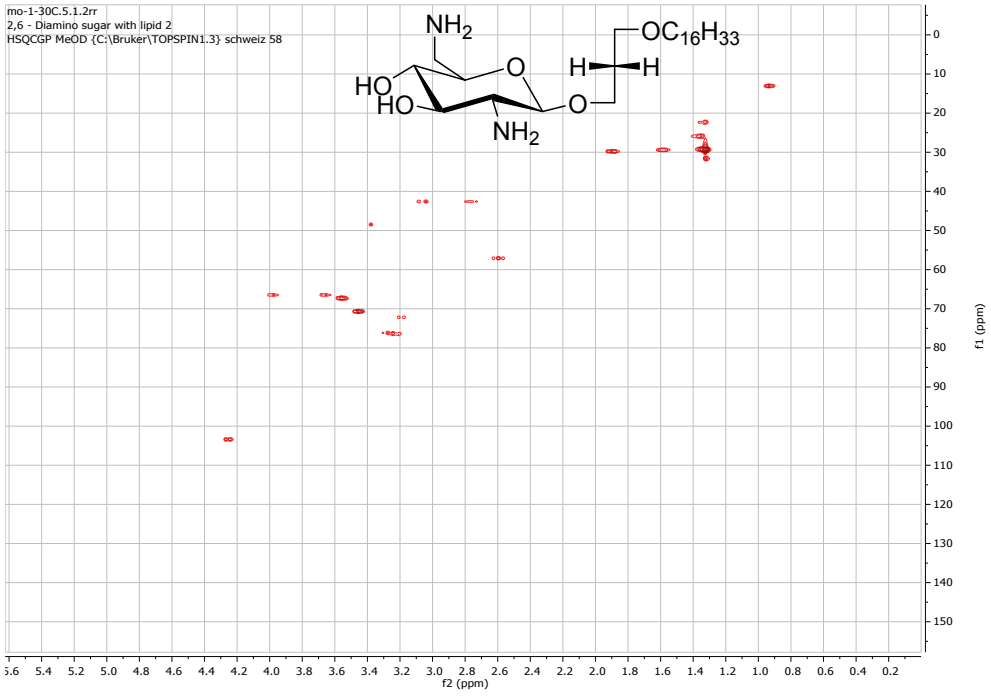


COSY NMR Spectra of compound **4**

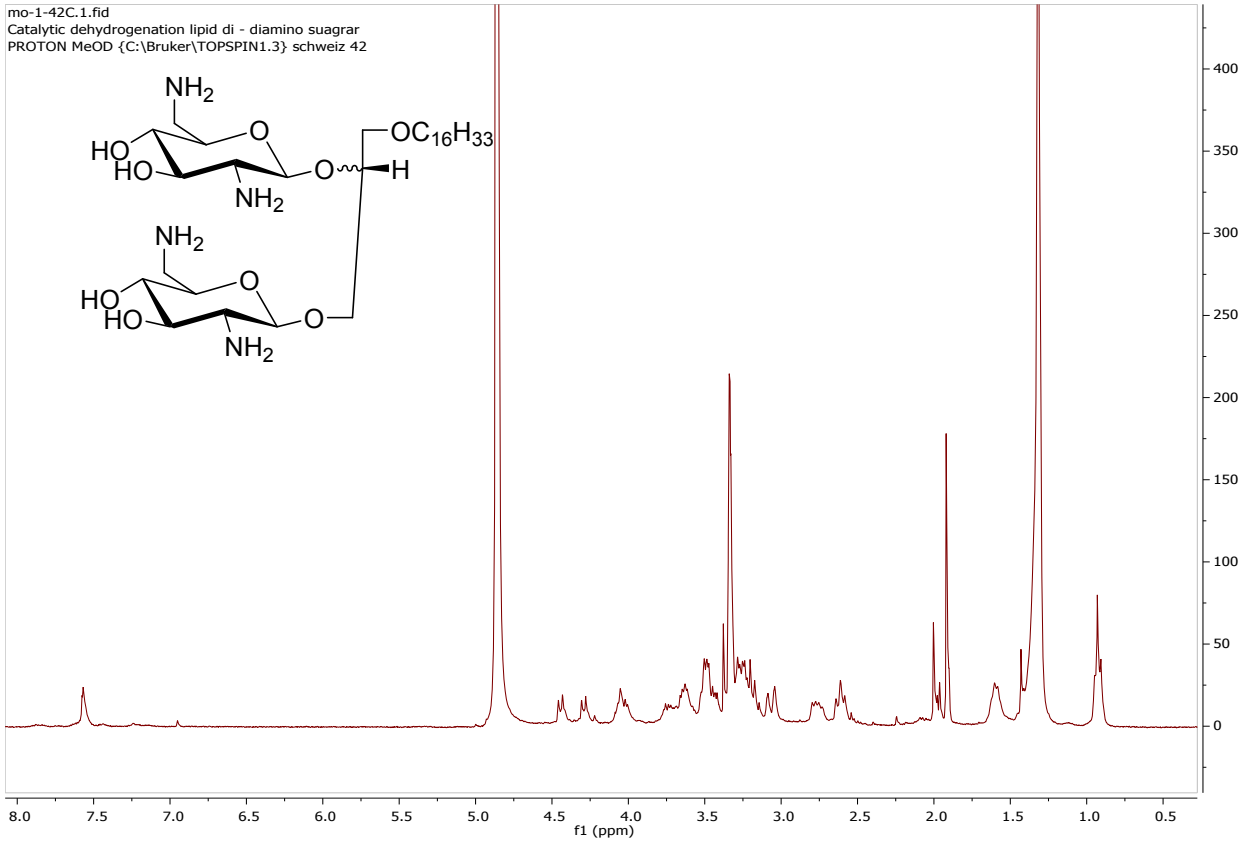




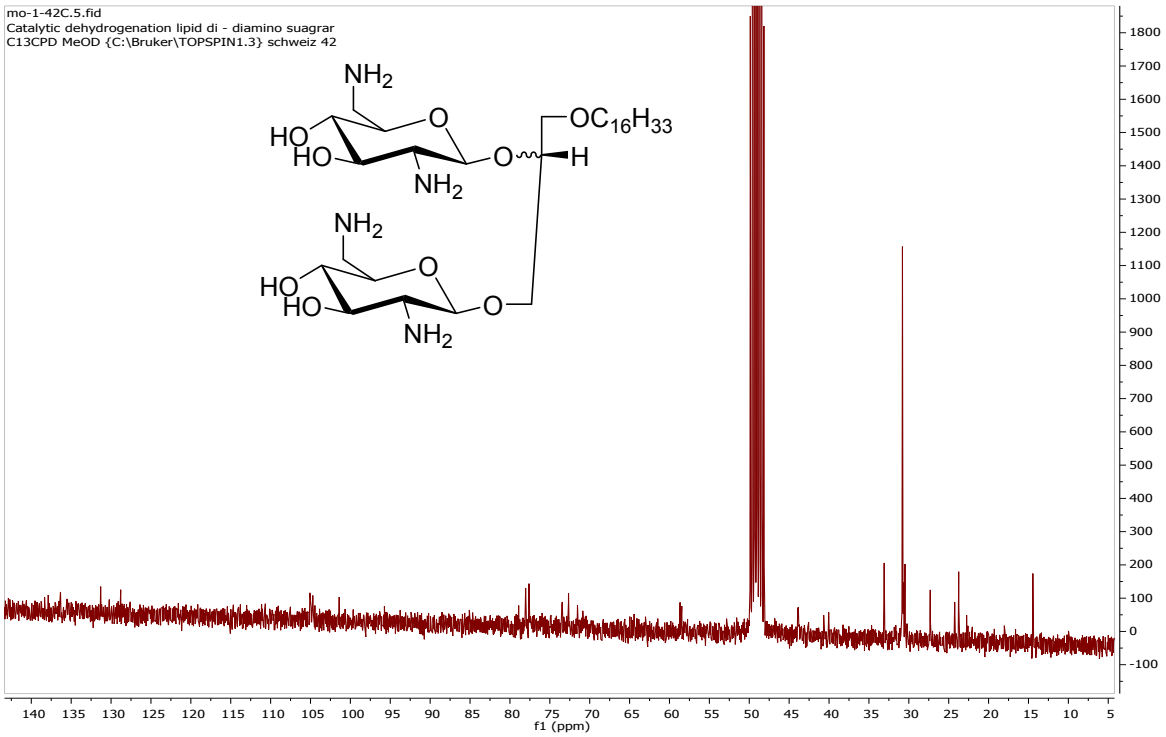
HSQC Spectra of compound **4**



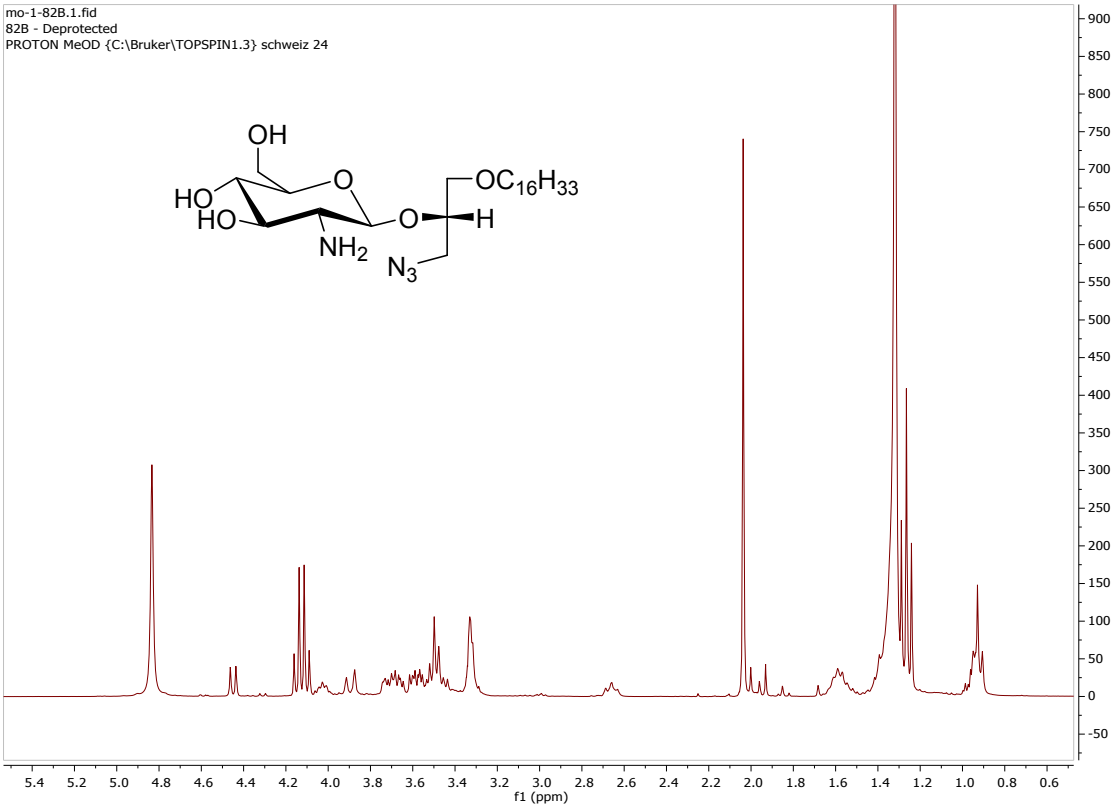
^{1}H NMR spectra of compound **5**



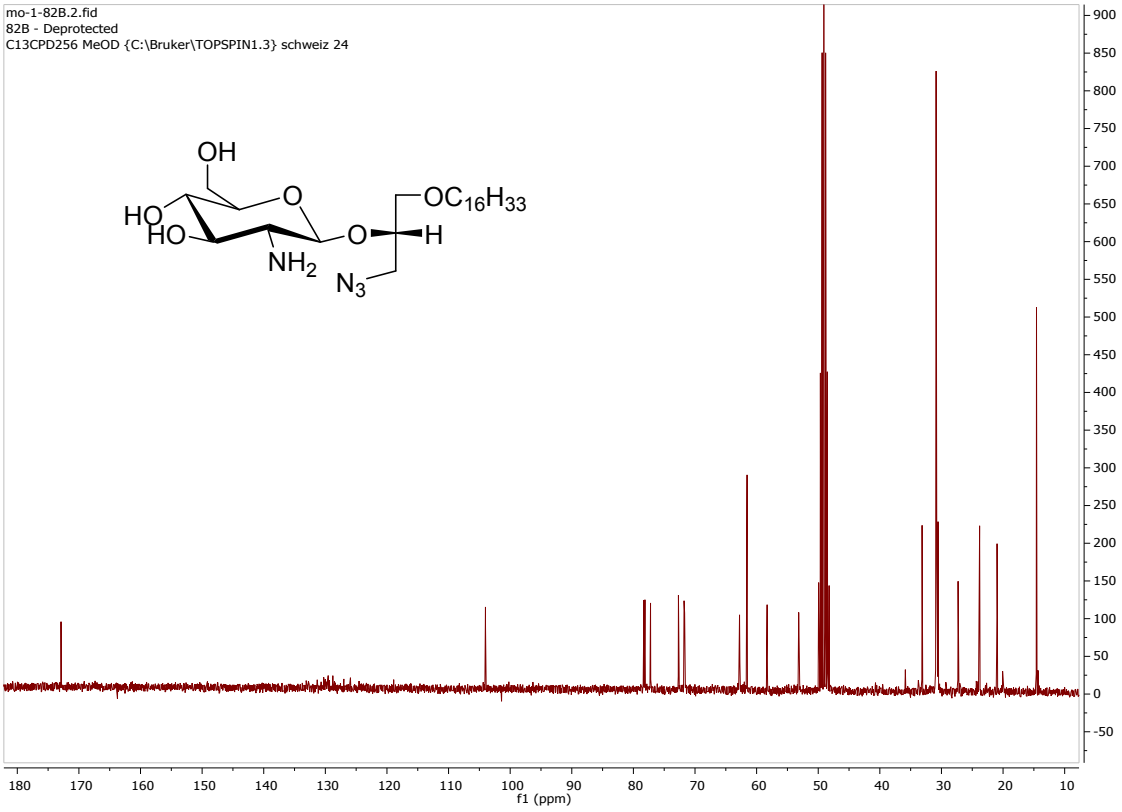
^{13}C NMR Spectra of compound **5**



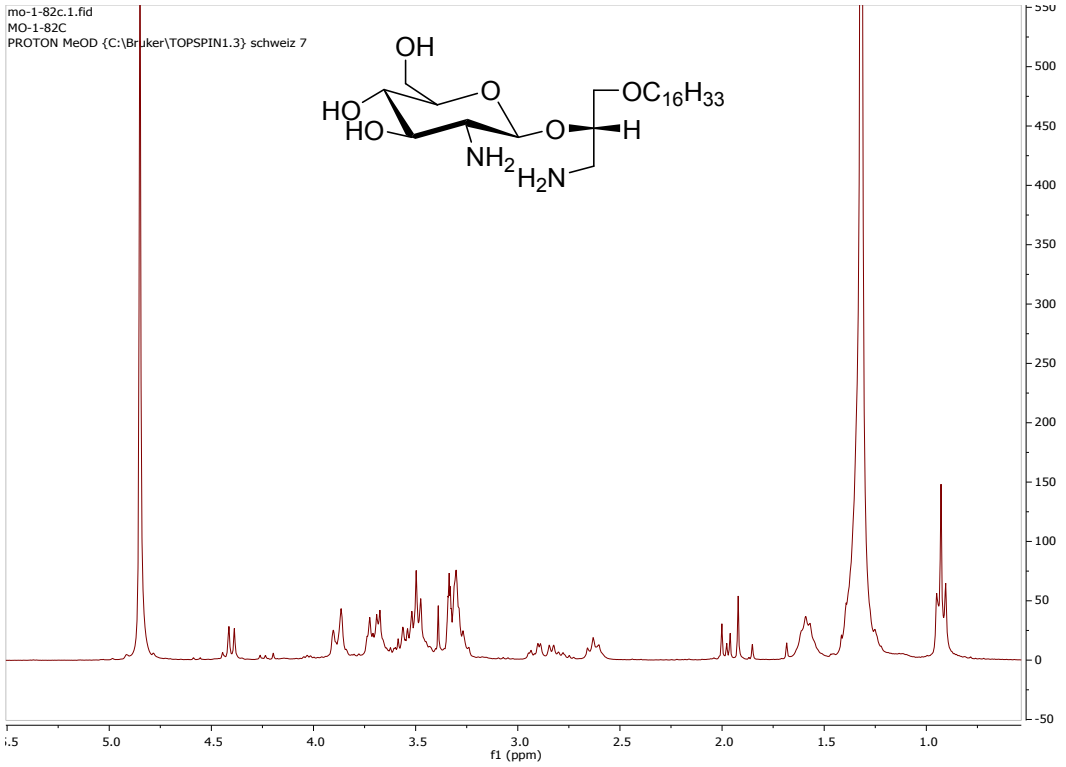
^{1}H NMR Spectra compound **6**



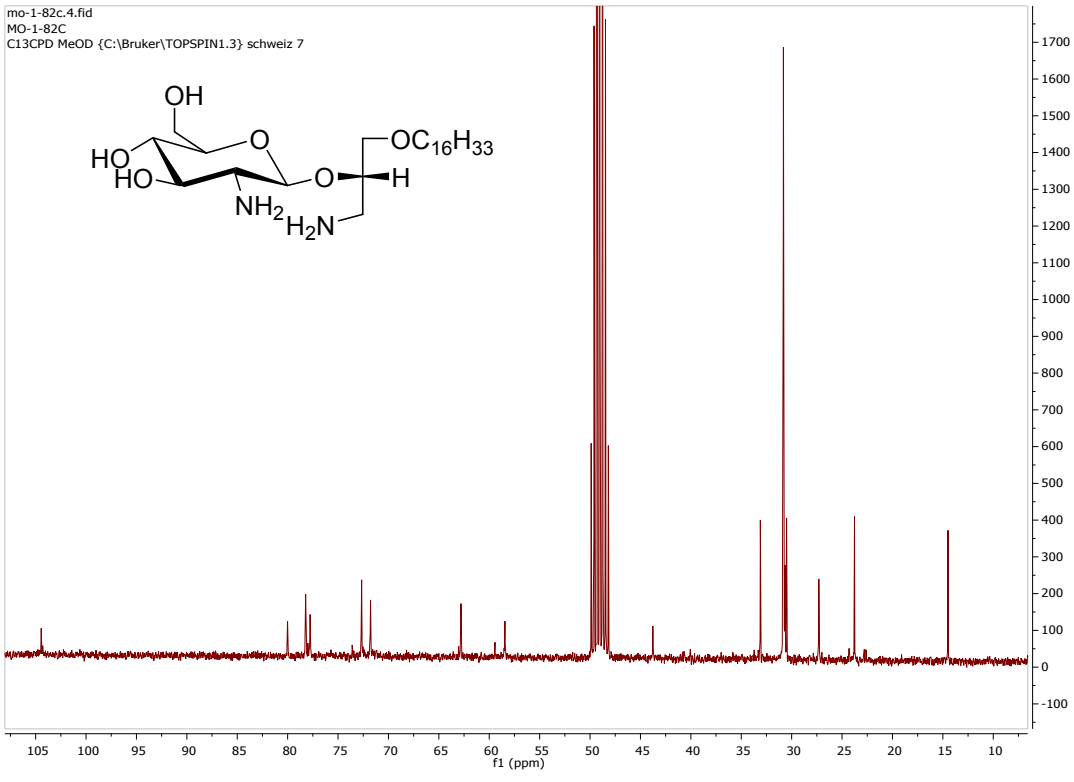
^{13}C NMR spectra of Compound **6**



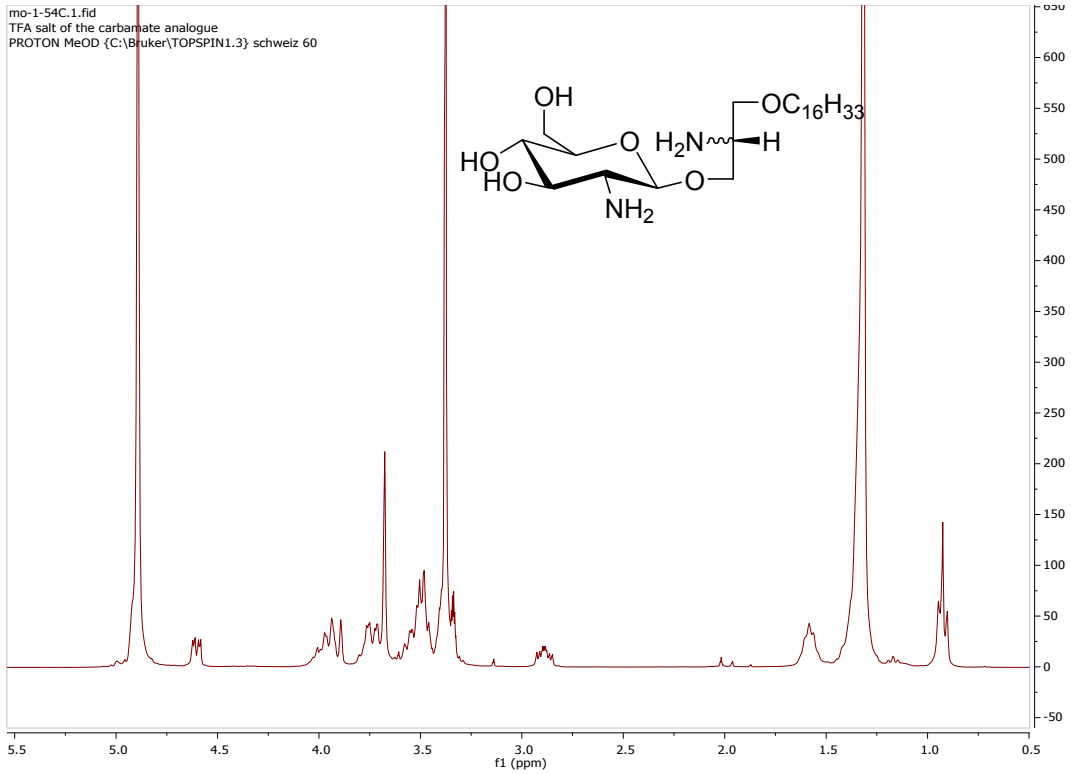
^{1}H NMR Spectra Compound **7**



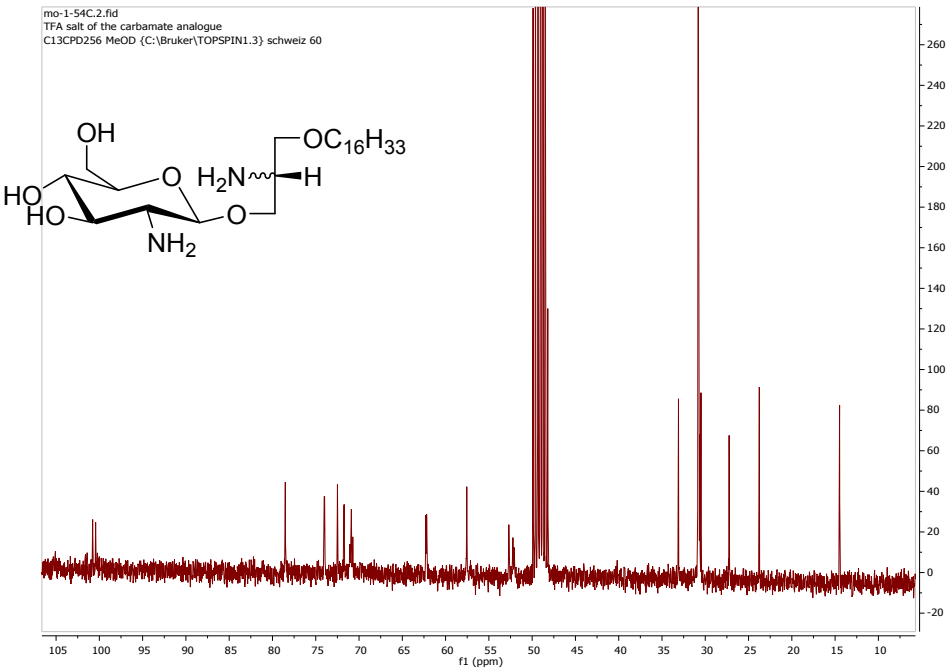
^{13}C NMR Spectra Compound **7**



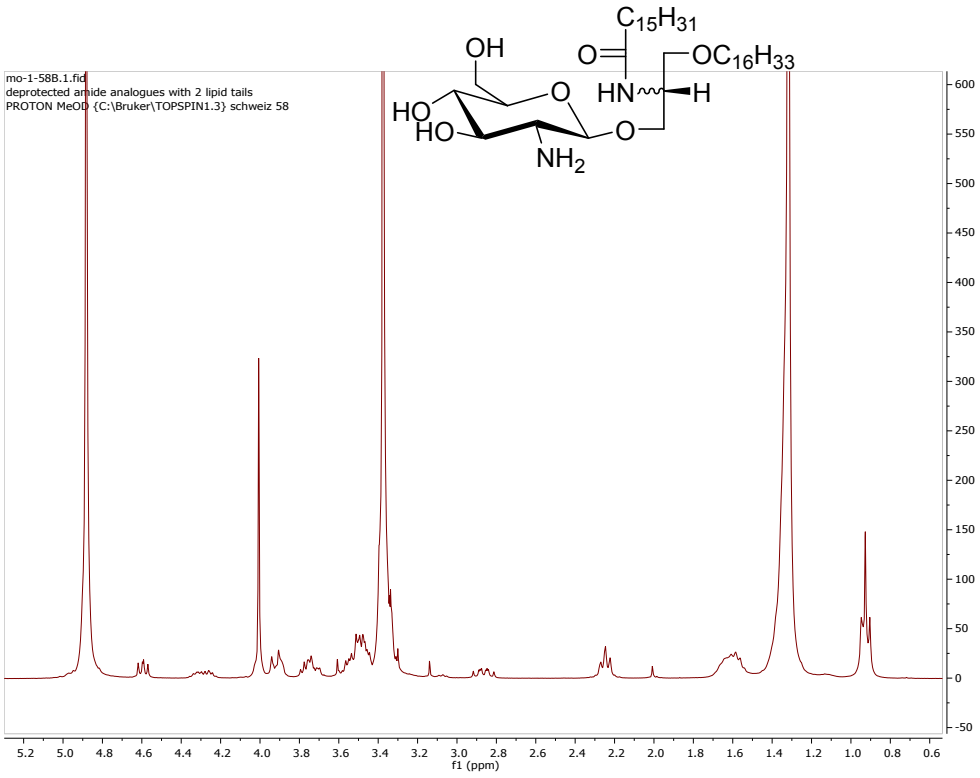
^{1}H NMR Spectra Compound **8**



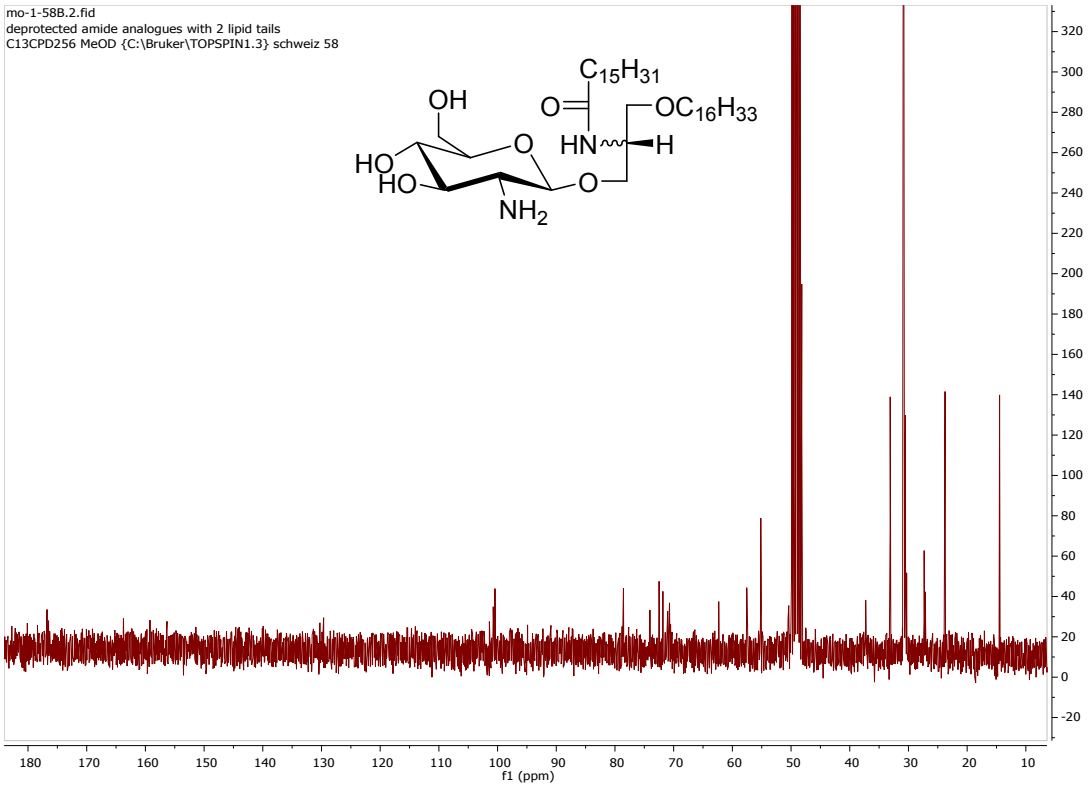
^{13}C NMR Spectra Compound **8**



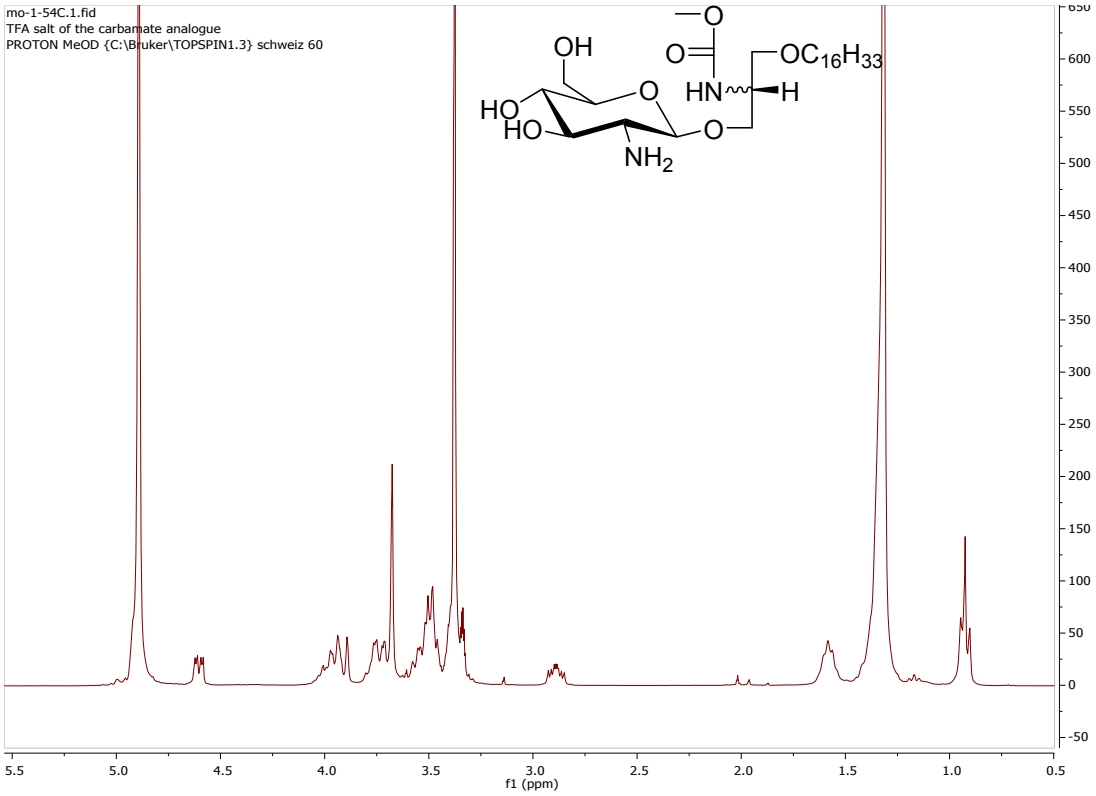
^{1}H NMR Spectra Compound **9**



^{13}C NMR of compound **9**



^{1}H NMR Spectra Compound **10**



^{13}C NMR of compound **10**