Synthesis and Microarray-Assisted Binding Studies of Core Xylose and Fucose Containing N-Glycans

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1. General Methods

Materials. Chemicals were purchased from Sigma-Aldrich or Acros Organics and were used without further purification. All reaction solvents were dried over activated 4Å or 3Å molecular sieves. Thin layer chromatography was carried out using Merck aluminium sheets silica gel 60 F_{254} and visualized by UV irradiation (254 nm) or by staining with vanillin solution
The recombinant enzymes α-1,3-fucosyltransferases (AtFucTA) from *Arabidopsis Thaliana,* α-1,6-fucosyltransferase, (AgFUT6) from *Anopheles gambiae,* core α-1,3-fucosyltransferase CeFUT6 and β-1,4-N-acetylgalactosaminyltransferase from *C. elegans,* bovine milk β-1,4-galactosyltransferase were expressed as described previously.[1,2,3,4,5] β-N-acetylglucosaminidase from *Canavalia ensiformis* (Jack bean) was purchased from Sigma Aldrich. Uridine 5′-diphosphogalactose disodium salt (UDP-Gal), uridine 5′-diphospho-N-acetylgalactosamine disodium salt (UDP-GalNAc) and guanosine 5′-diphospho-β-L-fucose sodium salt (GDP-Fuc) were purchased from Carbosynth.
All aqueous solutions were prepared from nanopure water produced with a Diamond UV water purification system (Branstead International).
Canavalia ensiformis lectin (ConA), Wheat germ agglutinin (WGA), *Ricinus communis* agglutinin -120 (RCA-120), *Aleuria aurantia* lectin (AAL), *Pisum sativum* agglutinin (PSA), *Galantus nivalis* agglutinin (GNA), *Griffonia* (*Bandeiraea) simplicifolia* lectin *II* (BSL-II) and *Wisteria floribunda* lectin (WFL) were purchased from VectorLabs. Lectins were labeled with Hilyte Plus™ 647 and Hilyte Plus™ 555 protein labeling kits from AnaSpec. Recombinant human DC-SIGN/Fc Chimera was purchased from Sino Biological Inc.. Anti-human IgG (Fc specific)-Cy3, developed in goat, was purchased from Sigma-Aldrich and Anti-human IgM-Alexa fluor 647 (μ specific) was purchased from Invitrogen. Anti-human antibodies were used in dilution 1:1000. Schistosomiasis infected sera samples were obtained from 41 individuals living in Piida community, Butiaba, Uganda. Glycan arrays (36x18) were prepared as indicated in the section 6 and compartmentalized with 8 Well ProPlate Module 7 x 16 mm Well from Electron Microscopy Sciences. NHS activated glass slides, Nexterion® H were purchased from Schott AG.

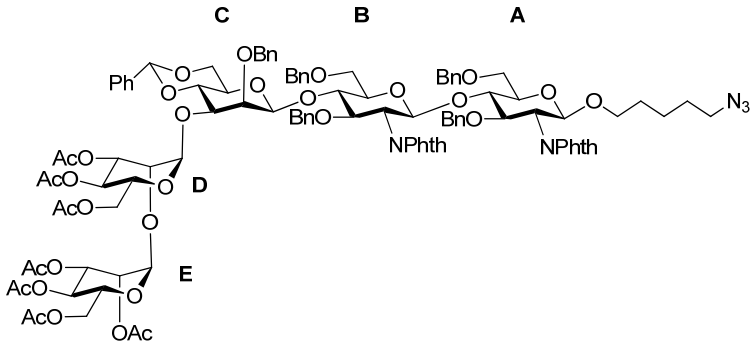
Instrumentation. Microwave irradiation was performed on Biotage Initiator monomode oven, (Biotage AB). Hydrogenation reactions were performed in continuous-flow hydrogenation reactor H-Cube® from ThalesNano Nanotechnology Inc. Purifications of compounds were performed on: size-exclusion chromatography using LP2 gel from Biorad, SampliQ high performance graphitized carbon cartridges (1 mL) from Agilent Technologies, C18 Sep-Pak Cartridges (1 mL) from Waters (Milford), flash chromatography using Merck 62Å 230-400 mesh silica gel or on a Biotage SP4 automated flash chromatography system, (Biotage AB) employing

[1] Serna, S.; Yan, S.; Martin-Lomas, M.; Wilson, I. B. H.; Reichardt, N. C. *J. Am. Chem. Soc.* **2011**, *133*, 16495–16502.
[2] Yan, S.; Serna, S.; Reichardt, N. C.; Paschinger, K.; Wilson, I. B. H. *J. Biol. Chem.* **2013***, 288,* 21015-21028
[3] Serna, S.; Hokke, C. H.; Weissenborn, M.; Flitsch, S.; Martin-Lomas,M.; Reichardt, N. C. *ChemBioChem* **2013**, *14*, 862 – 869.
[4] Boeggeman, E. E.; Ramakrishnan, B.; Qasba, P. K. Protein Expres. Purif. , **2003**, *30*, 219–229.
[5] Kawar, Z. S.; Van Die, I.; Cummings, R. D. *J. Biol. Chem.* **2002**, *277*, 34924–34932.

prepacked silica cartridges. All aqueous solutions were prepared from nanopure water produced with a Diamond UV water purification system (Branstead International).
Pooled glycan containing fractions were lyophilized on an ALPHA-2-4 LSC freeze-dryer from Christ. All organic solvents were concentrated using rotary evaporation.
^{1}H and ^{13}C spectra were acquired on Bruker 500 MHz spectrometer or on Bruker BioSpin GmbH 800 MHz. ^{13}C-HSQC spectra recorded with Bruker BioSpin GmbH 800 MHz use a folded window in the F1(^{13}C) dimension (with a sweep width value of 40 ppm). Chemical shifts were reported in ppm (δ) and referenced to the residual signal of the solvent used (MeOD 4.87 ppm; $CDCl_3$ 7.26ppm; D_2O 4.79 ppm). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; m, multiplet. Coupling constants (*J*) are reported in Hz. High resolution mass spectra were acquired on a Waters LCT Premier XE instrument, (Waters) equipped with a standard ESI source by direct injection. The instrument was operated with a capillary voltage of 1.0 kV and a cone voltage of 200 V. Cone and desolvation gas flow were set to 50 and 600 L/h, respectively; source and desolvation temperatures were 100 °C. MALDI-TOF mass analyses were performed on an Ultraflextreme III time-of-flight mass spectrometer equipped with a pulsed N_2 laser (337 nm) and controlled by FlexControl 3.3 software (Bruker Daltonics). The acquisitions were carried out in positive ion reflectron mode at a laser frequency of 500 Hz. Microarrays were printed employing a robotic non contact piezoelectric SciFLEXARRAYER spotter S11 (Scienion). Fluorescence measurements were performed in an Agilent G2565BA microarray scanner system (Agilent Technologies) with 10 µm resolution, using 2 lasers (532 nm, 633 nm). Quantification of fluorescence was achieved by ProScanArray® Express software from Perkin Elmer.

2. Chemical synthesis of compounds G43 and G54

S1

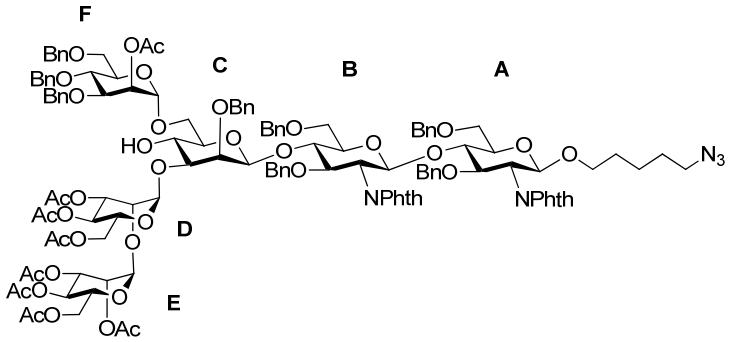


5-azidopentyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S1): The acceptor[6] (58 mg , 0.41 mmol) and the disaccharide donor[7] (40 mg, 0.50 mmol) were stirred in dry CH_2Cl_2 (1 mL) in the presence of 3Å molecular sieves for 1h at room temperature, then TMSOTf added (1 µL, 0.05 mmol, 0.13 eq) and resulting reaction mixture stirred for 2h, quenched with triethylamine, filtrated through a plug of Celite® and concentrated. The residue was purified by flash column chromatography (hexanes: EtOAc, 1:1) to yield the title compound as a white solid (66 mg, 80%); **^{1}H NMR (500 MHz, $CDCl_3$) δ** 7.97-6.71 (m, 40H, Ar), 5.43 (s, 1H, H-1D), 5.40-5.29 (m, 5H, CHPh, H-3D, H-1B, H-3E, H-4D), 5.24 (t, *J* = 9.9 Hz, 1H, H-4E), 5.20 (dd, *J* = 3.4, 1.9 Hz, 1H, H-2E), 4.96-4.81 (m, 5H, C*H*$_2$Ph, H-1A), 4.71 (d, *J* = 1.8 Hz, 2H, H-1C, H-1E), 4.60 (t, *J* = 13.3 Hz, 1H, C*H*$_2$Ph), 4.57-4.49 (m, 4H, C*H*$_2$Ph), 4.41 (d, *J* = 12.2 Hz, 1H, C*H*$_2$Ph), 4.32 (dd, *J* = 10.7, 8.4 Hz, 1H, H-3B), 4.27-4.17 (m, 3H, H-4A, H-2B, H-6aD), 4.17-4.08 (m, 5H, H-4A, H-3A, H-2A, H-2D, H-6aC), 4.07-3.95 (m, 4H, H-6bD, H-5E, H-4C, H-6aE), 3.85-3.74 (m, 3H, H-2C, H-3C, H-5D), 3.74-3.64 (m, 3H, H-6bE, H-6aB, OC*H*$_2$), 3.59-3.51 (m, 2H, H-6bB, H-6aA), 3.48-3.40 (m, 2H, H-6bA, H-6bC), 3.36-3.22 (m, 3H H-5A, H-5B, OC*H*$_2$), 3.11 (td, *J* = 9.6, 4.8 Hz, 1H, H-5C), 2.93-2.81 (m, 2H, C*H*$_2$N$_3$), 2.11 (s, 3H, C*H*$_3$CO), 2.10 (s, 3H, C*H*$_3$CO), 2.07 (s, 3H, C*H*$_3$CO), 2.06 (s, 3H, C*H*$_3$CO), 2.01 (s, 3H, C*H*$_3$CO), 1.98 (s, 3H, C*H*$_3$CO), 1.94 (s, 3H, C*H*$_3$CO), 1.43-1.26 (m, 4H, CH_2, linker), 1.07 (m, 2H, CH_2 linker); **^{13}C NMR from HSQC experiment (126 MHz, $CDCl_3$) δ:** 134.1, 133.7, 128.4, 127.9, 127.8, 127.6, 127.3, 126.1, 123.6, 123.2, 123.1, 101.4, 99.3, 98.8, 98.1, 97.1, 78.5, 78.4, 78.2, 77.0, 76.9,75.9, 75.4, 74.9, 74.7,74.6, 74.4, 74.3, 73.4, 72.8, 69.8, 69.6, 69.1, 69.0, 68.9, 68.8, 68.6, 68.4, 68.3, 68.2, 67.6, 67.6, 67.0, 66.4, 66.0, 62.4, 62.0, 61.9, 56.6, 55.7, 51.1, 28.7, 28.2, 23,1, 20.8, 20.8, 20.7.

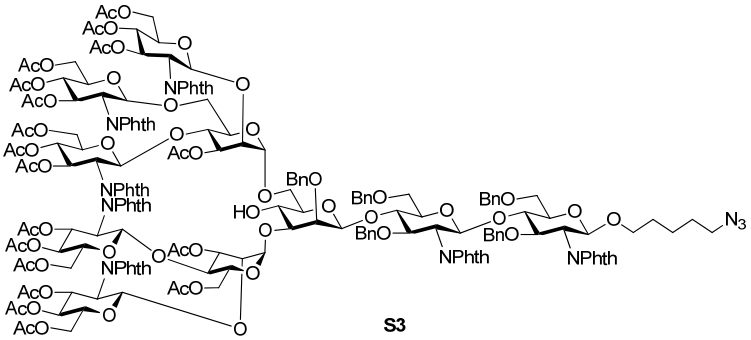
[6] Serna, S.; Etxebarria, J.; Ruiz, N.; Martin-Lomas, M.; Reichardt, N-C. *Chem. Eur. J*, **2010**, *16*, 13163–13175.

[7] Upreti, M.; Ruhel, D.; Vishwakarma, R. A.; *Tetrahedron*, **2000**, *56*, 6577-6584.

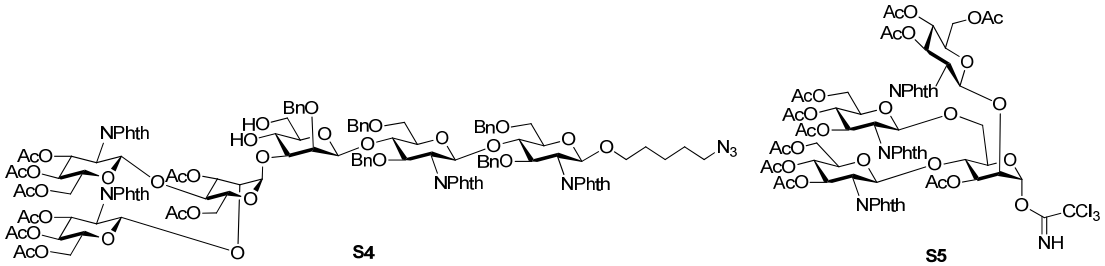
S2



5-azidopentyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1→3)]-2-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S2): Following the general procedure for benzylidene acetal deprotection (see experimental part), compound **S1** (66 mg, 32.5 µmol) was treated with $BF_3{\cdot}OEt_2$. After purification, the diol acceptor was obtained (45 mg, 70%). Intermediate diol (65 mg. 33.4 µmol) and donor **5** (23 mg, 36.1 µmol) in dry CH_2Cl_2 (1 mL) were stirred for 1h with 3Å molecular sieves, the mixture was cooled to -78°C and TMSOTf (2 µL, 11.1 µmol, 0.33 eq) added. After 1h, triethylamine was added to quench the acid and the crude was filtrated through a plug of Celite® and concentrated. The residue was purified by flash column chromatography (toluene:EtOAc, 3:1) to yield the title compound as a white solid (26 mg ,32%); **^{1}H NMR (500 MHz, $CDCl_3$) δ** 7.84-6.67 (m, 48H, Ar), 5.38 (dd, *J* = 10.0, 3.4 Hz, 1H, H-3E), 5.32-5.26 (m, 4H, H-4D, H-3D, H-1D, H-1B), 5.26-5.23 (m, 2H, H-4E, H-2F), 5.22 (dd, *J* = 3.3, 1.9 Hz, 1H, H-2E), 5.00 (d, *J* = 12.1 Hz, 1H, OCH_2Ph), 4.96-4.88 (m, 2H, OCH_2Ph, H-1A), 4.82 (dd, *J* = 12.5, 10.5 Hz, 2H, OCH_2Ph), 4.76 (s, 2H, H-1E, H-1F), 4.72 (d, *J* = 10.9 Hz, 1H, OCH_2Ph), 4.67 (s, 1H, H-1C), 4.60 (dd, *J* = 12.4, 9.4 Hz, 2H, OCH_2Ph), 4.56-4.44 (m, 6H, OCH_2Ph), 4.36 (d, *J* = 10.8 Hz, 1H, OCH_2Ph), 4.28-4.16 (m, 5H, OCH_2Ph, H-3B, H-4A, H-2B, H-6aD), 4.16-4.05 (m, 8H, H-6bD, H-6aE, H-2A, H-4C, H-5E, H-4B, H-3A, H-2D), 4.02 (m, 2H, H-6bE, OCH_2Ph), 3.92 (dd, *J* = 10.7, 3.3 Hz, 1H, H-6aC), 3.84-3.74 (m, 4H, H-5D, H-5F, H-2C, H-3F), 3.74-3.58 (m, 6H, H-4F, H-6aA, H-6bA, H-6aF, H-6bF, CH_2O), 3.57-3.44 (m, 3H, H-6bA, H-6aB, H-3C), 3.40 (dd, *J* = 10.9, 3.9 Hz, 1H, H-6bB), 3.33-3.27 (m, 2H, H-5A, H-5B), 3.27-3.20 (m, 1H CH_2O,), 3.13 (t, *J* = 9.5, 3.2 Hz, 1H, H-2C), 2.92-2.80 (m, 2H, ,-C*H*$_2$N$_3$), 2.10 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 1.37-1.28 (m, 4H, 2x CH_2), 1.11-1.00 (m, 2H, CH_2); **^{13}C NMR from HSQC experiment (126 MHz, $CDCl_3$) δ:** 133.8, 128.4, 128.1, 128.0, 127.8, 127.7, 127.1, 123.4, 101.4, 100.2, 98.5, 98.4, 98.2, 97.2, 81.2, 78.8, 78.4, 77.9, 77.1, 76.9, 76.5, 76.0, 75.2, 75.0, 74.7,74.5, 74.5, 74.2, 73.4, 72.8, 72.0, 71.6, 70.2, 69.8, 69.1, 69.0, 68.9, 68.6, 68.5, 68.3, 68.0, 67.8, 67.4, 66.6, 62.5, 56.7, 55.8, 51.2, 28.5, 23.2, 20.9, 20.8, 19.7.



S3



S4 S5

5-azidopentyl {3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-O-[(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)]-O-3,6-di-O-acetyl-α-D-mannopyranosyl-(1→3)}-O-{3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-O-[3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-O-[3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-O-(3-O-acetyl-α-D-mannopyranosyl)-(1→6)}-O-2-O-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose (S3): Acceptor **S4**[1] (61 mg, 25.3 µmol), donor **S5**[8] (65 mg, 40.2 µmol) and 3Å molecular sieves were stirred in dry CH_2Cl_2 (2 mL) for 45 min. The reaction was cooled to 0°C and 10% of TMSOTf in CH_2Cl_2 (5 µL, 2.7 µmol) was added. After 2h the reaction was quenched with triethylamine, concentrated and purified by flash column chromatography (hexane:acetone, 3:2) to obtain the title compound as a white foam (70 mg, 72%); **^{1}H NMR (500 MHz, CDCl3) δ** 7.98-7.46 (m, 28H, Ar), 7.39-7.18 (m, 15H, Ar), 7.17-7.11 (m, 1H, Ar), 7.02-6.64 (m, 9H, Ar), 5.85-5.73 (m, 2H,), 5.60-5.44 (m, 4H), 5.40 (d, *J* = 8.4 Hz, 1H), 5.21 (dd, *J* = 5.5, 2.8 Hz, 1H), 5.19-5.11 (m, 4H), 5.08 (t, *J* = 9.6 Hz, 1H), 5.03-4.86 (m, 6H), 4.83 (d, *J* = 12.8 Hz, 1H), 4.78 (s, 1H), 4.73 (d, *J* = 12.0 Hz, 1H), 4.62-4.42 (m, 7H), 4.42-4.22 (m, 9H), 4.21-3.85 (m, 20H), 3.76 (d, *J* = 9.1 Hz, 1H), 3.71-3.61 (m, 2H), 3.56 (d, *J* = 10.3 Hz, 3H), 3.53-3.44 (m, 4H), 3.44-3.30 (m, 6H), 3.31-3.21 (m, 2H), 3.16 (d, *J* = 9.9 Hz, 1H), 3.05-2.80 (m, 5H), 2.66-2.54 (m, 2H), 2.11 (s, 3H, CH_3CO), 2.10-2.06 (m, 9H, 3x CH_3CO), 2.05 (s, 6H, 2x CH_3CO), 2.04-2.01 (m, 9H, 3x CH_3CO), 2.00 (d, *J* = 6.4 Hz, 6H, 2x CH_3CO), 1.95 (s, 3H, CH_3CO), 1.91 (s, 3H, CH_3CO), 1.87-1.83 (m, 9H, 3x CH_3CO), 1.80 (2xs, 6H, 2x CH_3CO), 1.42-1.29 (m, 4H, 2xCH_2), 1.12-1.01 (m, 2H, CH_2); **^{13}C NMR (126 MHz, $CDCl_3$)δ:** 171.0, 171.0, 170.7, 170.6, 170.1, 170.0, 169.9, 169.9, 169.8, 169.5, 169.4, 169.4, 168.2, 167.5, 167.2, 138.9, 138.7, 138.6, 138.4, 137.8, 134.4, 133.9, 133.6, 131.8, 131.7, 131.6, 131.5, 131.3, 128.6, 128.5, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.3, 127.3, 127.1, 126.9, 123.8, 123.8, 123.6, 123.5, 123.1, 123.1, 101.0, 99.0, 98.9, 98.1, 97.8, 97.6, 97.3, 97.0, 96.3, 78.9, 77.7, 77.2, 77.1, 76.9, 76.9, 75.8, 75.3, 74.8, 74.5, 74.5, 74.4, 74.3, 73.2, 72.8, 72.6, 71.6, 71.5, 71.5, 71.4, 71.0, 70.7, 70.5, 70.4, 70.3, 70.2, 69.4, 69.3, 69.0, 68.9, 68.8,

[8] Unverzagt, C.; Gundel, G.; Eller, S.; Schuberth, R.; Seifert, J.; Weiss, H.; Niemietz, M.; Pischl, M.; Raps, C. *Chem. Eur. J*, **2009**, *15*, 12292-12302.

68.6, 68.5, 68.3, 68.2, 67.7, 67.4, 62.3, 62.1, 61.7, 61.3, 56.6, 55.7, 55.0, 54.7, 54.6, 54.4, 54.3, 51.1, 29.7, 28.7, 28.3, 23.0, 20.9, 20.8, 20.7, 20.7, 20.6, 20.6, 20.5, 20.4, 20.4, 20.2.

3. General procedures

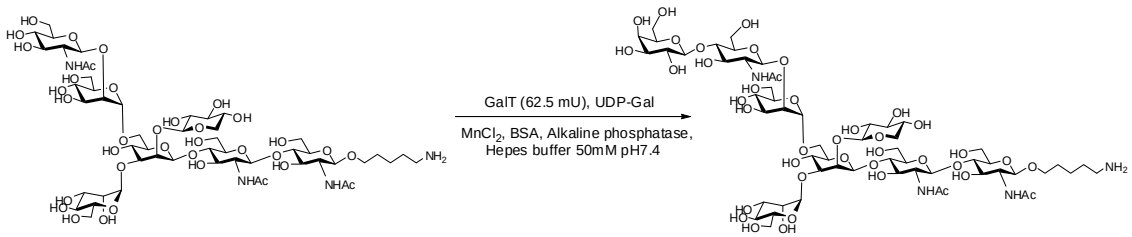
3.1 **General procedure A:** Deprotection of compounds G1, G3, G5, G6

A mixture of the glycan (1 eq.) and ethylenediamine in *n*BuOH (1:4; 42 ml/mmol) was heated to 120 ºC under microwave irradiation for 3 cycles of 30 minutes each. The reaction mixture was concentrated and coevaporated with toluene (3 times) and EtOH (3 times). The crude product was dissolved in anhydrous pyridine (0.06 mL/µmol), cooled to 0 ºC and Ac_2O (0.028 mL/µmol) was added dropwise. The reaction was warmed to room temperature and stirred overnight. The mixture was concentrated and co-evaporated with toluene (3 times) to remove traces of pyridine. The crude product was purified by size exclusion chromatography (CH_2Cl_2 :MeOH, 1:2). The compound was dissolved in MeOH and 0.5M NaOMe in MeOH was added (0.2 eq. per each acetate group). After 2 h of stirring at room temperature, the reaction mixture was quenched with Amberlite® IR120 (H), filtered and concentrated. The crude product was dissolved in MeOH containing 0.1% of trifluoroacetic acid (TFA) and hydrogenated with H-Cube® reactor on 10% Pd/C cartridge (full H_2 mode; 0.5 ml/min; 50 ºC). The solution of the final compound was quenched with $NaHCO_3$ and purified on graphitized carbon cartridge.

3.2 **General procedure B:** Deprotection of compounds G2, G4, G43 and G54

A mixture of the **glycans** (1 eq.) and ethylenediamine in *n*BuOH (1:4; 42 ml/mmol) was heated to 120 ºC under microwave irradiation for 3 cycles of 30 minutes each. The reaction mixture was concentrated and coevaporated with toluene (3 times) and EtOH (3 times). The crude product was dissolved in anhydrous pyridine (0.06 mL/µmol), cooled to 0 ºC and Ac_2O (0.028 mL/µmol) was added drop wise. The reaction was warmed to room temperature and stirred overnight. The mixture was concentrated and coevaporated with toluene (3 times). The crude product was purified by flash column chromatography (SiO_2, EtOAc:MeOH, 99:1). Sodium (25 mg) was added to liquid ammonia (15 mL) at -78 ºC and the resulting blue mixture was stirred for 5 minutes. A solution of the protected product in anhydrous THF (0.11 mL/µmol) was added drop wise and the resulting mixture was stirred at -78 ºC until the blue color disappeared (1 h). The reaction was quenched by adding solid NH_4Cl until neutral pH and evaporated to dryness. The crude product was purified by graphitized carbon cartridge with the elution conditions (H_2O:MeOH, 1:1).

3.3 **General procedure C:** Reaction with bovine milk β-1,4-galactosyltransferase

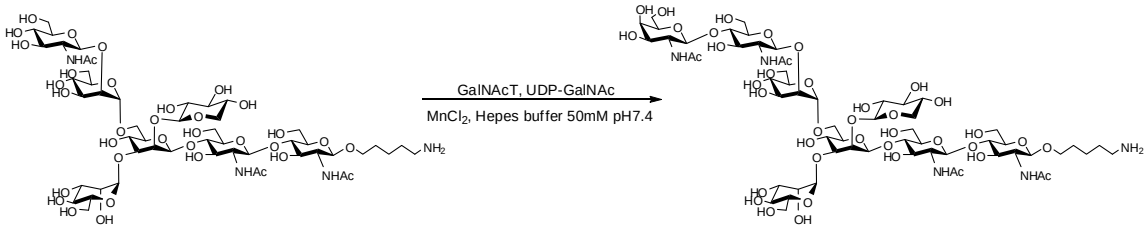


A solution (500 µL/mg) of glycan (1eq.), UDP-Gal (Uridine 5′-diphosphogalactose disodium salt) (1.2 eq.), bovine serum albumin BSA (0.5 mg), bovine milk β-1,4-galactosyltransferase, alkaline phosphatase and $MnCl_2$ (10 mM) in HEPES buffer (50 mM, pH=7.4) was incubated at 37 ºC overnight. The resulting mixture was heated at 95ºC for 5 minutes to precipitate the enzyme. After centrifugation the supernatant was purified on the C18 cartridge (ACN/water + 0.1% TFA). Fractions were analyzed by MALDI-TOF and the compound containing fractions were pooled and freeze dried to obtain the title compound

3.4 **General procedure D:** Reaction with fucosyltransferases

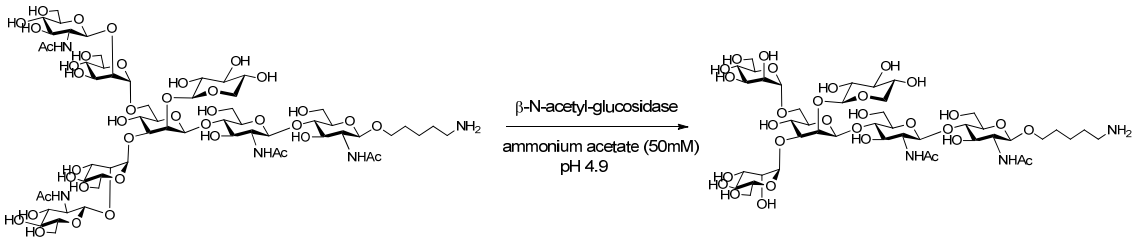
A solution (500 µL/mg) of glycan (1 eq.), GDP-Fuc (guanosine 5′-diphospho-β-L-fucose sodium salt) (1.2eq./fucose residue), CeFUT6, AtFucTA, CeFUT1 or AgFUT6 and 20 mM $MnCl_2$ in MES buffer (40 mM, pH=6.5) was incubated at room temperature overnight. The resulting mixture was heated at 95ºC for 5 minutes to precipitate the enzyme. After centrifugation the supernatant was purified on the C18 cartridge with the elution conditions (ACN/water, 1/1 + 0.1% TFA). Fractions were analyzed by MALDI-TOF and the compound containing fractions were pooled and freeze dried.

3.5 **General procedure E:** Reaction with β-1,4-N-acetylgalactosaminyltransferase



A solution (500 µL/mg) of glycan (1 eq.), UDP-GalNAc (Uridine 5′-diphospho-N-acetylgalactosamine disodium salt) (1.2 eq.), concentrated *P. pastoris* extract containing GalNAcT and $MnCl_2$ (10 mM) in HEPES buffer (40 mM, pH=7.4) was incubated at room temperature for 72h. The resulting mixture was heated at 95ºC for 5 minutes to precipitate the enzyme. After centrifugation the supernatant was purified on the C18 cartridge with the elution conditions (ACN/water, 1/1 + 0.1% TFA). Fractions were analyzed by MALDI-TOF and the compound containing fractions were pooled and freeze dried.

3.6 **General procedure F:** Reaction with Jack bean β-N-acetylglucosaminidase



A solution (500 µL/mg) of compound glycan (1 eq.), Jack bean β-N-acetylglucosaminidase in ammonium acetate buffer (50mM, pH 4.9) was incubated at room temperature for 96h. The resulting mixture was heated at 95ºC for 5 minutes to precipitate the enzyme. After centrifugation the supernatant was purified on the C18 cartridge with the elution conditions (ACN/water, 1/1 + 0.1% TFA). Fractions were analyzed by MALDI-TOF and the compound containing fractions were pooled and freeze dried.

4. List of final compounds

5-Aminopentyl [α-D-mannopyranosyl]-(1→6),[β-D-xylopyranosyl]-(1→2)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (G1): Following the general procedure A, compound **17** (24.5 mg, 11.17 µmol) was deprotected to give **G1** as a white solid (5.32 mg, 49 % over 4 steps); **^{1}H NMR (500 MHz, D_2O) δ** 4.93 (s, 1H), 4.87 (s, 1H), 4.62 (d, *J* = 7.5 Hz, 1H), 4.50 (d, *J* = 7.5 Hz, 1H), 4.46 (d, *J* = 7.5 Hz, 1H), 4.28 (m, 1H), 3.99– 3.59 (m, 33H), 3.54-3.37 (m, 4H), 3.27 (t, *J* = 11.1Hz, 1H), 2.94 (t, *J* = 7.54 Hz, 2H, C*H*$_2$NH$_2$), 2.09, 2.04 (2xs, 6H, -NH*Ac*), 1.66-1.58 (m, 4H, CH_2 linker), 1.43-1.37 (m, 2H, CH_2 linker); **^{13}C NMR (126 MHz, D_2O) δ** 174.7, 174.4, 104.2, 101.3, 101.0, 100.7, 99.7, 79.7, 79.3, 78.0, 75.3, 74.5, 74.4, 73.2, 72.7, 72.4, 72.1, 70.4, 70.1, 69.8, 69.2, 67.0, 66.7, 65.8, 65.0, 60.9, 60.1, 55.0, 39.5, 28.1, 27.0, 22.2, 22.1; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{38}H_{67}N_3O_{25}H$ 966.4142, found 966.4161.

5-Aminopentyl [α-D-mannopyranosyl]-(1→3),[β-D-xylopyranosyl]-(1→2)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (G2): Following the general procedure B, compound **12** (31 mg, 15.08 µmol) was deprotected to give **G2** as a white solid (7.43 mg, 50 % over 3 steps); **^{1}H NMR (500 MHz, D_2O) δ** 5.14 (s, 1H), 4.86 (s, 1H), 4.60 (d, *J* = 7.6 Hz, 1H), 4.50 (d, *J* = 7.8 Hz, 1H), 4.47(d, *J* = 7.6 Hz, 1H), 4.26 (d, *J* = 3.0 Hz, 1H), 4.06– 4.04 (m, 1H), 4.03-3.99 (m, 2H), 3.93-3.57 (m, 27H), 3.52-3.43 (m, 3H), 3.38 (t, *J* = 8.6 Hz, 1H), 3.25 (t, *J* = 11.3 Hz, 1H), 2.93 (t, *J* = 7.2 Hz, 2H), 2.08, 2.04 (2xs, 6H, -NH*Ac*), 1.67-1.56 (m, 4H, CH_2 linker), 1.42-1.36 (m, 2H CH_2 linker); **^{13}C NMR (126 MHz, D_2O, peaks assigned from HSQC) δ** 104.8, 102.2, 101.3, 101.0, 100.1, 79.3, 79.0, 78.7, 77.3, 76.2, 74.5, 74.5, 73.5, 73.2, 72.4, 72.0, 70.3, 70.3, 70.1, 70.0, 69.2, 66.8, 66.3, 64.9, 64.9, 60.3, 60.1, 60.1, 55.1, 55.0, 39.5, 28.2, 27.2, 22.3, 22.2, 22.2; **HRMS (ESI) *m/z*** $[M+Na]^+$ calculated for $C_{38}H_{67}N_3O_{25}Na$ 988.3956, found 988.3972.

5-Aminopentyl [2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]-(1→3),[β-D-xylopyranosyl]-(1→2)- β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (G3): Following the general procedure A, compound **10** (36 mg, 15.74 µmol) was deprotected to give **G3** as a white solid (12.5 mg, 65% over 4 steps); **^{1}H NMR (500 MHz, D_2O) δ** 5.15 (s, 1H, H1M), 4.86 (s, 1H, H1M'), 4.60 (d, *J* = 7.7 Hz, 1H), 4.53-4.49 (m, 2H), 4.45 (d, *J* = 7.6 Hz, 1H, H1X), 4.25 (d, *J* = 2.8 Hz, 1H, H2M), 4.16 (m, 1H, H2M'), 4.04-4.00 (m, 1H), 3.93-3.85 (m, 10H), 3.80– 3.42(m, 28H), 3.42-3.39 (m, 1H), 3.27-3.23 (m, 1H), 2.92 (t, *J* = 7.6 Hz, 2H, - C*H*$_2$NH$_2$), 2.07, 2.06, 2.04 (3xs, 9H, -NH*Ac*), 1.66-1.57 (m, 4H, CH_2 linker), 1.42-1.37 (m, 2H, CH_2 linker); **^{13}C NMR (126 MHz, D_2O) δ** 174.7, 174.6, 174.4, 104.9, 101.3, 101.0, 100.1, 99.7, 99.2, 79.3, 79.1, 78.7, 77.1, 76.6, 76.3, 75.8, 75.3, 74.5, 73.6, 73.3, 73.2, 72.4, 71.9, 70.1, 69.9, 69.4, 69.3, 67.2, 66.5, 64.9, 61.7, 60.6, 60.2, 60.1, 55.3, 55.1, 55.0, 39.5, 28.1, 27.3, 22.3, 22.1, 22.1, 22.1; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{46}H_{80}N_4O_{30}H$ 1169.493, found 1169.4907.

5-Aminopentyl [α-D-mannopyranosyl]-(1→3), [2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]- (1→6), [β-D-xylopyranosyl]-(1→2)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (G4): Following the general procedure B compound **16** (40 mg, 14.96 µmol) was deprotected to give **G4** as a white solid (11.85 mg, 56 %); **^{1}H NMR (500 MHz, D_2O) δ** 5.13 (s, 1H), 4.91 (s, 1H), 4.88 (s, 1H), 4.60 (d, *J* = 7.8 Hz, 1H), 4.56 (d, *J* = 8.4 Hz, 1H), 4.50 (m, *J*=7.8 Hz, 1H), 4.45 (d, *J* = 7.5 Hz, 1H), 4.27 (d, *J* = 2.8 Hz, 1H), 4.12-4.11 (m, 1H), 4.06-4.04 (m, 1H), 4.02-3.98 (m, 2H), 3.94-3.36 (m, 43H), 3.25 (t, 11.2 Hz, 1H), 2.92 (t, *J*=7.5 Hz, 2H, C*H*$_2$NH$_2$), 2.09, 2.06, 2.04 (3xs, 9H, -NH*Ac*), 1.65-1.58 (m, 4H, CH_2 linker), 1.40-1.37 (m, 2H, CH_2 linker); **^{13}C NMR (126 MHz, D_2O) δ** 174.8, 174.6, 174.4, 105.1, 102.2, 101.3, 101.0, 100.6, 99.6, 96.9, 79.5, 79.3, 77.3, 76.2, 75.8, 75.3, 74.5, 74.4, 74.3, 73.5, 73.3, 72.8, 72.4, 72.0, 70.3, 70.1, 69.9, 69.9, 69.7, 69.5, 69.3, 67.3, 66.7, 66.4, 65.4, 64.9, 61.6, 61.2, 60.6, 60.1, 60.0, 55.3, 55.0, 39.5, 28.2, 28.1, 27.4, 27.2, 22.3, 22.2, 22.1; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{52}H_{90}N_4O_{35}H$ 1331.5458, found 1331.5494.

5-Aminopentyl [2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]-(1→3),[α-D-mannopyranosyl]-(1→6),[β-D-xylopyranosyl]-(1→2)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (G5): Following the general procedure A, compound **15** (24.8 mg, 8.93 µmol) was deprotected to give **G5** as a white solid (58% over 4 steps); **^{1}H NMR (500 MHz, D_2O) δ** 5.15 (s, 1H), 4.93 (d, 1H, *J*=1.7 Hz), 4.88 (s, 1H), 4.61 (d, *J* = 8.0 Hz, 1H), 4.53 (d, 1H, *J*=8.3 Hz), 4.50 (d, *J* = 8.2Hz, 1H), 4.45 (d, *J* = 7.6 Hz, 1H), 4.27 (d, *J*=3.2 Hz, 1H), 4.20-4.15 (dd, *J*=3.2, 1.4 Hz, 1H), 4.05-3.37 (m, 45H), 3.26 (t, *J* = 11.1 Hz, 1H), 2.99 (t, *J*=7.8 Hz, 1H, linker), 2.09, 2.06, 2.04 (3xs, 9H, -NH*Ac*), 1.71-1.65 (m, 2H, linker), 1.64-1.58 (m, 2H, linker), 1.43-1.39 (m, 2H, linker); **^{13}C NMR (126 MHz, D_2O) δ** 105.1, 101.3, 101.0, 100.4, 99.7, 99.5, 99.1, 79.5, 79.3, 79.2, 77.0, 76.5, 75.6, 74.5, 74.2, 73.7, 73.3, 73.2, 72.7, 72.4, 71.8, 70.3, 70.2, 70.1, 69.8, 69.7, 69.7, 69.4, 69.2, 67.2, 66.6, 66.5, 65.2, 65.2, 64.9, 64.8, 61.7 , 60.6, 60.6, 60.1, 54.9, 55.1, 39.4, 28.1, 26.5, 22.3, 22.1; **HRMS (ESI)** *m/z* $[M+H]^+$ calculated for $C_{52}H_{90}N_4O_{35}H$ 1331.5458, found 1331.5502.

5-Aminopentyl di[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]-(1→3),(1→6)-[β-D-xylopyranosyl]-(1→2)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (G6): Following the general procedure A, compound **14** (95 mg, 54.2 μmol) was deprotected to give **G6** as a white solid (50.2 mg, 60 % over 4 steps);**^{1}H NMR (500 MHz, D_2O) δ** 5.13 (s, 1H), 4.90 (s, 1H), 4.86 (s, 1H), 4.59 (d, *J* = 7.7 Hz, 1H), 4.57 (d, *J* = 8.4 Hz, 1H), 4.52 (d, *J*=8.3 Hz, 1H), 4.51 (d, *J*=7.6 Hz, 1H), 4.45(d, *J* = 7.7 Hz, 1H), 4.26 (d, *J* = 2.6 Hz 1H), 4.15-4.12 (m, 1H), 4.12-4.07 (m, 1H), 4.05 –3.35 (m, 51H), 3.24 (t, *J* = 11.2 Hz, 1H), 2.91 (t, *J*=7.8 Hz, 2H), 2.07, 2.05, 2.04, 2.02 (4xs, 12H, -NH*Ac*), 1.65-1.55 (m, 4H, linker), 1.40-1.34 (m, 2H, linker); **^{13}C NMR (126 MHz, D_2O) δ** 174.8, 174.7, 174.5, 105.1, 101.4, 101.0, 100.5, 99.7, 99.6, 99.2, 96.9, 79.6, 79.4, 76.6, 76.3, 75.8, 75.4, 74.5, 74.4, 73.7, 73.4, 73.3, 72.9, 72.5, 72.1, 70.2, 69.9, 69.6, 69.5, 69.3, 67.3, 67.2, 66.5, 65.0, 61.8, 61.6, 60.7, 60.1, 55.4, 55.1, 39.6, 28.1, 27.3, 22.4, 22.2, 22.2; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{60}H_{103}N_5O_{40}$ H 1534.6252, found 1534.6322.

5-aminopentyl α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (G43): Following the general procedure B, compound **S1** (40 mg, 16.54 μmol) was deprotected to give **G43** (8.76 mg, 46% over 3 steps); **^{1}H NMR (500 MHz, D2O) δ** 5.35 (d, *J* = 1.9 Hz, 1H, H-$1_{\alpha Man}$), 5.05 (d, *J* = 1.9 Hz, 1H, H-$1_{\alpha Man}$), 4.91 (d, *J* = 1.8 Hz, 1H, H-$1_{\alpha Man}$), 4.77 (s, 1H), 4.60 (d, *J* = 7.7 Hz, 1H), 4.49 (d, J = 8.0 Hz, 1H), 4.24 (d, *J* = 1.7 Hz, 1H), 4.10 (dd, *J* = 3.3, 1.7 Hz, 1H), 4.07 (dd, *J* = 3.3, 1.8 Hz, 1H), 4.01-3.96 (m, 2H), 3.95-3.54 (m, 32H), 3.53-3.45 (m, 1H), 2.94-2.87 (m, 2H, linker), 2.08 (s, 3H, -NH*Ac*), 2.03 (s, 3H, -NH*Ac*), 1.66-1.51 (m, 4H, 2x CH_2 linker), 1.42-1.33 (m, 2H, CH_2 linker); **^{13}C NMR (126 MHz, D_2O, peaks assigned from HSQC) δ:**174.7, 174.4, 102.2, 101.4, 101.0, 100.8, 100.3, 99.6, 80.6, 79.6, 79.3, 78.4, 74.5, 74.3, 74.2, 73.4, 73.2, 72.7, 72.4, 71.9, 70.4, 70.3, 70.1, 70.1, 70.0, 69.9, 69.8, 67.0, 66.8, 66.7, 65.8, 61.7, 61.1, 60.9, 60.1, 60.0, 55.0, 54.9, 39.5, 28.1, 27.3, 22.1; **HRMS (ESI) *m/z*** $[M+Na]^+$ calculated for $C_{45}H_{79}N_3O_{31}Na$ 1180.4595, found 1180.4685.

5-aminopentyl {2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-O-[2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)]-O-α-D-mannopyranosyl-(1→3)}-O-{2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-O-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)]-O-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-O-α-D-mannopyranosyl)-(1→6)}-O-β-D-mannopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranose (G54): Following the general procedure B, compound **S2 (**30 mg, 7.76 μmol) was deprotected to give **G54** (3.26 mg, 21% over 3 steps); **^{1}H NMR (500 MHz, D2O) δ** 5.13 (s, 1H, H-$1_{\alpha Man}$), 4.84 (s, 1H, H-$1_{\alpha Man}$), 4.61 (d, *J* = 7.5 Hz, 1H), 4.55-4.47 (m, 6H), 4.24 (d, *J* = 2.8 Hz, 1H), 4.20 (d, *J* = 3.1 Hz, 1H), 4.16-4.02 (m, 3H), 4.01-3.36 (m, 57H), 2.93 (dd, *J* = 8.5, 6.7 Hz, 2H, linker), 2.15-1.99 (m, 21H, 7x -NH*Ac*), 1.69-1.53 (m, 4H, 2xCH_2, linker), 1.39 (m, *J* = 6.3 Hz, 2H, CH_2)**; ^{13}C NMR (126 MHz, D_2O, peaks assigned from HSQC) δ** 101.6, 101.6, 101.4, 101.4, 101.0, 100.4, 99.9, 99.8, 99.1, 97.0, 80.1, 79.3, 79.3, 79.2, 78.3, 78.1, 76.2, 76.0, 75.9, 75.8, 74.52, 74.51, 74.4, 73.5, 73.4, 73.3, 72.4, 71.9, 70.17, 70.15, 70.12, 70.11, 69.8, 69.48, 69.46, 68.3, 68.1, 65.5, 65.2, 61.3, 60.7, 60.6, 60.09, 60.06, 55.7, 55.6, 55.2, 54.8, 39.4, 28.1, 26.8, 22.5, 22.4, 22.4, 22.2, 22.14, 22.12; **HRMS (ESI) *m/z*** $[M+Na]^+$ calculated for $C_{79}H_{134}N_8O_{51}Na$ 2033.8036, found 2033.8073.

5. List of compounds enzymatically diversified in solution

Heptasaccharide G7: Following the general procedure D, compound **G7** (1.35 mg, 98.5%) was prepared from **G3** (1.22 mg, 1.04 µmol) using core α-1,6-fucosyltransferase AgFUT6 (30 µL, 1.05 mg/mL) and GDP-Fuc (792 µg, 1.25 µmol). ; **^{1}H NMR (500 MHz, D_2O) δ** 5.15 (s, 1H, H-$1_{\alpha Man}$), 4.91 (d, *J* = 4.0 Hz, 1H, H-1_{Fuc}), 4.86 (s, 1H, H-$1_{\beta Man}$), 4.68 (d, *J* = 7.6 Hz, 1H), 4.51 (t, *J* = 8.4 Hz, 2H), 4.46 (d, *J* = 7.6 Hz, 1H), 4.26-4.25 (m, 1H, H-2_{Man}), 4.17-4.16 (m, 1H, H-2_{Man}), 4.16-4.12 (m, 1H, H-5_{Fuc}), 4.02 (dd, *J* = 11.5, 5.4 Hz, 1H), 3.96-3.44 (m, 38H), 3.40-3.35 (m, 1H), 3.26 (t, *J* = 11.1 Hz, 1H), 2.99 (t, *J* = 7.6 Hz, 2H, linker), 2.08, 2.06, 2.04 (3xs, 9H, NH*Ac*), 1.69-1.65 (m, 2H, linker), 1.63-1.58 (m, 2H, linker), 1.44-1.38 (m, 2H, linker), 1.24 (d, *J* = 6.7 Hz, 3H, CH_{3Fuc}); **^{13}C NMR (126 MHz, D_2O) δ**,105.0, 101.2, 101.0, 100.1, 99.7, 99.4, 99.2, 79.2, 78.7, 78.6, 77.17, 76.6, 76.3, 75.7, 73.6, 73.4, 73.3, 73.1, 72.3, 72.0, 71.8, 70.0, 69.8, 69.6, 69.5, 69.5, 69.4, 69.3, 68.1, 68.0, 67.2, 66.8, 66.5, 66.5, 64.9, 64.9, 61.7, 60.3, 60.3, 55.2, 55.1, 39.3, 28.1, 26.4, 22.3(x3), 22.1, 15.4; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{52}H_{90}N_4O_{34}H$ 1315.5509, found 1315.5499.

Octasaccharide G8: Following the general procedure D, compound **G8** (1.24 mg, 99%) was prepared from **G5** (1.03 mg, 0.755 µmol) using core α-1,6-fucosyltransferase AgFUT6 (30 µL, 1.05 mg/ml), and GDP-Fuc (589 µg, 0.93 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.15 (s, 1H, H-$1_{\alpha Man}$), 4.93 (s, 1H, H-$1_{\alpha Man}$), 4.92 (d, *J* = 4.0 Hz, 1H, H-1_{Fuc}), 4.88 (s, 1H), 4.68 (d, *J* = 8.1 Hz, 1H), 4.53 (d, *J* = 8.1 Hz, 1H), 4.51 (d, *J* = 8.1 Hz, 1H), 4.45 (d, *J* = 7.6 Hz, 1H), 4.27-4.26 (m, 1H, H-2_{Man}), 4.18-4.16 (m, 1H, H-2_{Man}), 4.15-4.12 (m, 1H, H-5_{Fuc}), 4.04-3.51 (m, 45H), 3.40-3.36 (m, 1H), 3.26 (t, *J* = 11.3 Hz, 1H), 2.99 (t, *J* = 7.5 Hz, 2H, linker), 2.10, 2.06, 2.04 (3xs, 9H, NH*Ac*), 1.71-1.65 (m, 2H, linker), 1.63-1.58 (m, 2H, linker), 1.43-1.38 (m, 2H, linker), 1.24 (d, *J* = 6.7 Hz, 3H, CH_{3Fuc}); **^{13}C NMR (126 MHz, D_2O) δ** 105.2, 101.1, 101.0, 100.6, 99.7, 99.5, 99.1, 79.6, 79.6, 79.4, 78.6, 76.9, 76.5, 75.6, 74.4, 74.3,73.6, 73.3, 73.3, 72.8, 72.6, 72.1, 71.8, 70.2, 70.0, 69.9, 69.8, 69.6, 69.5, 69.3, 68.1, 68.0, 67.2, 66.8, 66.7, 66.6, 66.5, 65.4, 65.3, 64.9, 64.9, 61.8, 60.7, 60.6, 55.20, 54.3, 54.8, 39.3, 28.1, 26.4, 22.3, 22.1, 22.1 (x2), 15.3; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{58}H_{100}N_4O_{39}H$ 1477.6037, found 1477.6073.

Nonasaccharide G9: Following the general procedure D, compound **G9** (0.82 mg, 75%) was prepared from **G6** (1 mg, 0.651 µmol) using core α-1,6-fucosyltransferase AgFUT6 (30 µL, 1.05 mg/ml) and GDP-Fuc (589 µg, 0.93 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.16 (s, 1H, H-$1_{\alpha Man}$), 4.92 (s, 2H, H-$1_{\alpha Man,}$ H-$1_{\alpha Fuc}$), 4.89 (s, 1H, H-$1_{\beta Man}$), 4.68 (d, *J* = 7.9 Hz, 1H), 4.57 (d, *J* = 8.3 Hz, 1H), 4.53 (d, *J* = 8.3 Hz, 1H), 4.51 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 7.6 Hz, 1H), 4.27-4.25 (m, 1H, H-2_{Man}), 4.18-4.16 (m, 1H, H-2_{Man}), 4.14-4.12 (m, 2H), 4.04-4.00 (m, 2H), 3.94-3.37 (m, 49H), 3.26 (t, *J* = 11.1 Hz, 1H), 3.00 (t, *J* = 7.7 Hz, 2H, linker), 2.10, 2.07, 2.06, 2.04 (4xs, 12H, NH*Ac*), 1.69-1.66 (m, 2H, linker), 1.62-1.59 (m, 2H, linker), 1.42-1.38 (m, 2H, linker), 1.24 (d, *J* = 6.6 Hz, 3H, CH_{3Fuc}); **^{13}C NMR (126 MHz, D_2O) δ**105.2, 101.2, 101.1, 100.6, 99.7, 99.3, 99.2, 99.2, 99.1, 81.8, 79.6, 79.6, 79.4, 79.2, 77.1, 76.6, 76.3, 76.2, 75.7, 74.3, 73.9, 73.3, 73.3, 73.1, 73.0, 72.2, 71.8, 71.70, 71.1, 70.2, 69.8,69.5, 69.4, 68.2, 68.0, 67.1, 66.8, 66.5, 66.3, 66.3, 65.5, 65.1, 64.9, 64.2,

61.7, 61.7, 60.1, 60.6, 60.3, 55.2, 55.1, 54.9, 53.5, 39.3, 28.1, 26.5, 22.2, 22.2, 22.1, 15.4; **HRMS (ESI) *m/z*** $[M+Na]^+$ calculated for $C_{66}H_{113}N_5O_{44}Na$ 1702.6651, found 1702.6577.

Hexasaccharide G10: Following the general procedure D, compound **G10** (0.72 mg, 99%) was prepared from **G2** (548 µg, 0.57 µmol) using core α-1,3-fucosyltransferase CeFUT1 (26 µL, 0.9 mg/ml), and GDP-Fuc (430 µg, 0.68 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.15 (s, 1H, H-1_{Fuc}), 5.14 (s, 1H, H-$1_{\alpha Man}$), 4.84 (s, 1H, H-$1_{\beta Man}$), 4.74-4.70 (m, 1H, H-5_{Fuc}), 4.55 (d, *J* = 7.9 Hz, 1H), 4.49 (t, *J* = 7.6 Hz, 2H), 4.26 (d, *J* = 3.2 Hz, 1H, H-2_{Man}), 4.06-3.39 (m, 29H), 3.26 (t, *J* = 11.2 Hz, 1H), 2.95 (t, *J*=7.7 Hz, 2H, linker), 2.06, 2.03 (2xs, 9H, -NH*Ac*), 1.68-1.57 (m, 4H, linker), 1.41-1.37 (m, 2H, linker), 1.28 (d, *J* = 6.7 Hz, 3H, CH_{3Fuc}); **^{13}C NMR (126 MHz, D_2O)** δ 104.9, 102.2, 100.9, 100.4, 100.1, 98.4, 79.9, 78.9, 77.4, 76.2, 75.3, 75.3, 74.7, 73.9, 73.5, 73.3, 72.2, 72.0, 70.3, 70.3, 70.2, 69.9, 69.4, 69.1, 67.7, 66.8, 66.7, 66.3, 64.9, 64.7, 61.2, 61.0, 60.1, 55.7, 55.2, 39.4, 28.1, 26.9(2x), 22.3, 22.2, 15.5; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{44}H_{77}N_3O_{29}$ H 1112.4716 found 1112.4758.

Heptasaccharide G11: Following the general procedure D, compound G11 (??? mg, 85 %) was prepared from **G3** (1.27 mg, 1.08 µmol) using AtFucTA (50 µL, 0.55 mg/ml) and GDP-Fuc (guanosine 5'-diphospho-β-L-fucose sodium salt) (0.8 mg, 1.27 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.16 (s, 1H, H-1_{Fuc}), 5.14 (d, *J* = 4.2 Hz, 1H, H-$1_{\alpha Man}$), 4.72 (m, 1H, H-5_{Fuc}), 4.85 (s, 1H, H-$1_{\beta Man}$), 4.55 (d, *J* = 7.91 Hz, 1H), 4.53 (d, *J* = 8.4 Hz, 1H), 4.50 (d, *J* = 7.91 Hz, 1H), 4.48 (d, *J* = 7.7 Hz, 1H), 4.25 (d, *J* = 2.27 Hz, 1H, H-2_{Man}), 4.19-4.17 (m, 1H, H-2_{Man}), 4.03 (dd, *J* = 11.7, 5.5 Hz, 1H), 3.99 (dd, *J* = 10.5, 3.26 Hz, 1H), 3.94-3.43 (m, 34H), 3.38 (dd, *J* = 9.8, 7.9 Hz, 1H), 3.27 (t, *J* = 11.1 Hz, 1H), 2.91 (t, *J*=7.6 Hz, 2H, linker), 2.06, 2.05, 2.03 (3xs, 9H, -NH*Ac)*, 1.66-1.57 (m, 4H, linker), 1.42-1.37 (m, 2H, linker), 1.28 (d, *J* = 6.9 Hz, 3H, CH_{3Fuc}); **^{13}C NMR (126 MHz, D_2O) δ** 104.9, 101.0,100.4, 100.1 , 99.7, 99.1, 98.3, 79.9, 79.7, 79.1, 77.0, 76.6, 76.1, 75.9 75.4, 74.7, 73.3, 73.7, 73.2, 72.2, 71.9, 70.2, 70.2, 69.8, 69.4, 69.3, 69.2, 67.8, 67.2, 66.8, 66.1, 64.9, 64.9, 61.8, 60.9, 60.5, 60.3, 60.2, 55.6, 55.3, 55.3, 39.7, 28.1, 27.3, 22.2 (3x), 22.1, 15.5; **HRMS (MALDI-tof) *m/z*** $[M+Na]^+$ calculated for $C_{52}H_{90}N_4O_{34}Na$ 1337.5329, found 1337.5347.

Octasaccharide G12: Following the general procedure D, compound **G12** (0.47 mg, 84%) was prepared from **G5** (0.56 mg, 0.38 µmol) using core α-1,3-fucosyltransferase AtFucTA (25µL, 0.25 mg/mL), and GDP-Fuc (291 µg, 0.46 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.15 (s, 1H, H-$1_{\alpha Man}$), 5.13 (d, *J* = 3.9 Hz, 1H, H-1_{Fuc}), 4.91 (d, *J* = 1.3 Hz, 1H, H1- $H_{\alpha Man}$), 4.85 (s, 1H, H1 $_{\beta Man}$), 4.71-4.69 (m, 1H, H-5_{Fuc}), 4.54 (d, *J* = 8.3 Hz, 1H, H1- H_{GlcNAc}), 4.52 (d, *J* = 8.7 Hz, 1H, H1- H_{GlcNAc}), 4.49 (d, *J* = 8.3 Hz, 1H, H1- H_{GlcNAc}), 4.45 (d, *J* = 7.4 Hz, 1H, H1- H_{xyl}), 4.25 (d, *J* = 3.4 Hz, 1H, H2- H_{Mann}), 4.16-4.15 (m, 1H, H2- H_{Man}), 4.02 (dd, *J* = 11.6, 5.3 Hz, 1H), 3.98-3.95 (m, 2H), 3.93-3.83 (m, 12H), 3.81-3.80 (m,1H), 3.80-3.72 (m, 9H), 3.68-3.43 (m, 17H), 3.37(dd, *J* = 9.4, 7.8 Hz, 1H), 3.26 (t, *J*=11 Hz, 1H), 2.98 (t, *J*=7.8 Hz, 2H, linker), 2.05, 2.05, 2.02 (3xs, 9H, NHAc), 1.68-1.64 (m, 2H, linker), 1.60-1.57 (m, 2H, linker), 1.41-1.37 (m, 2H, linker), 1.27 (d, *J* = 6.6 Hz, 3H, CH_3 Fuc); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ** 107.8, 103.6, 103.2, 103.0, 102.3, 102.3, 101.7, 100.9, 83.3, 81.8, 79.6, 79.2, 78.4, 78.0, 77.1, 77.1, 76.9, 76.5, 76.4, 75.9, 75.8, 75.4, 74.9, 74.7, 74.4, 73.1, 72.5, 72.4, 72.0, 71.9, 71.8, 69.8, 69.4, 69.3, 69.1, 68.0, 67.8, 67.8, 64.4, 64.4, 63.5, 63.5, 63.0, 62.6, 62.4, 58.2, 58.1, 57.7, 41.9, 30.7, 29.0, 24.8, 24.8, 24.8, 18.1; **HRMS (ESI) *m/z*** $[M+Na+H]^{2+}$ calculated for $C_{58}H_{101}N_4O_{39}Na$ 851.8362, found 851.8347.

Nonasaccharide G13: Following the general procedure D, compound **G13** (0.62 mg, 99%) was prepared from **G6** (0.57 mg, 0.37 µmol) using core α-1,3-fucosyltransferase AtFucTA (25µL, 0.25 mg/mL), and GDP-Fuc (267 µg, 0.42 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.16 (s, 1H, H-$1_{\alpha Man}$), 5.15 (d, *J*=3.7 Hz, 1H, H-1_{Fuc}), 4.91 (s, 1H, H-$1_{\alpha Man}$), 4.87 (s, 1H, H-$1_{\beta Man}$),4.75 (m, 1H, H-5_{Fuc}), 4.55 (m, 3H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.46 (d, *J* = 7.6 Hz, 1H), 4.27-4.26 (m, 1H, H-$2_{Man\beta}$), 4.17-4.16(m, 1H, H-$2_{Man\alpha}$), 4.12-4.11(m, 1H, H-$2_{Man\alpha}$), 4.05-3.43 (m, 47H), 3.40 (t, *J*=8.8 Hz 1H), 3.27 (t, *J*=11.5 Hz, 1H), 3.00 (t, *J*=7.8 Hz, 2H, linker), 2.07, 2.06, 2.03 (3xs, NH*Ac*, 12H), 1.71-1.58 (m, 4H, linker), 1.43-1.37 (m, 2H, linker), 1.28 (d, *J* = 6.5 Hz, 3H, CH_{3Fuc}); **^{13}C NMR(201 MHz, D_2O) δ:** 104.7, 100.5, 100.1, 99.9, 99.3, 99.1, 98.6, 97.8, 96.5, 80.2, 78.8, 76.6,76.0, 75.7, 75.3, 74.9, 74.8, 74.1, 74.1, 73.8, 73.3, 72.8, 72.8, 72.4, 71.8, 71.5, 71.2, 69.7, 69.7, 69.4, 69.0, 68.9, 68.8, 68.7, 68.0, 67.3, 66.7, 66.2, 65.9, 64.9, 64.5, 64.4, 61.2, 61.2, 61.1, 60.5, 60.5, 60.1, 60.1, 60.1, 59.5, 59.4, 55.1, 54.8, 54.5, 38.9, 27.6, 26.0, 21.8, 21.7, 21.6, 15.0; **HRMS (MALDI-tof) *m/z*** $[M+Na]^+$ calculated for $C_{66}H_{113}N_5O_{44}Na$ 1702.6651, found 1702.6577.

Heptasaccharide G14: Following the general procedure C, compound **G14** (0.63 mg, 99%) was prepared from **G3** (0.5 mg, 0.427 µmol) using 5mU of bovine milk β-1,4-galactosyltransferase and, UDP-Gal (0.39 mg, 0.64 µmol); **^{1}H NMR (500 MHz, D_2O)** δ 5.16 (s, 1H, H-$1_{\alpha Man}$), 4.86 (s, 1H, H-$1_{\beta\text{-}Man}$), 4.61 (d, *J* = 7.7 Hz, 1H), 4.55 (d, *J* = 7.6 Hz, 1H, H-$1_{\beta Gal}$), 4.51 (d, *J* = 8.0 Hz, 1H), 4.48 (d, *J* = 7.8 Hz, 1H), 4.46 (d, *J* = 7.7 Hz, 1H), 4.26 (d, *J* = 2.8 Hz, 1H, H-2_{Man}), 4.18-4.17 (m, 1H, H-2_{Man}), 4.04-3.98 (m, 2H), 3.94-3.43 (m, 37H), 3.40-3.36 (m, 1H) 3.26 (t, *J* = 11.4 Hz, 1H), 2.95 (t, *J* = 7.4 Hz, 2H, linker), 2.08, 2.06, 2.04 (3xs, 9H,-NH*Ac*), 1.70-1.56 (m, 4H, linker)1.44-1.37 (m, 2H, linker); **^{13}C NMR (126 MHz, D_2O) δ** 105.0, 102.9, 101.3, 101.0, 100.2, 99.6, 99.2, 79.1, 78.5, 77.2, 76.5, 76.4, 75.3, 75.3, 74.7, 74.6, 74.5, 73.7, 73.3, 72.4, 71.9, 71.0, 70.2, 70.2, 70.1, 69.4, 68.5, 67.2, 66.5, 65.0, 64.9, 61.8, 61.8, 60.9, 60.3, 60.3, 60.2, 59.9, 59.9,58.3, 55.1, 54.9, 39.4, 28.0, 26.8, 22.2, 22.1; **HRMS (ESI) *m/z*** $[M+Na]^+$ calculated for $C_{52}H_{90}N_4O_{35}Na$ 1353.5278, found 1353.5264.

Octasaccharide G15: Following the general procedure C, compound **G15** (0.64 mg, 94.5%) was prepared from **G5** (0.6 mg, 0.450 µmol) applying using 94 mU of bovine milk β-1,4-galactosyltransferase and, UDP-Gal (0.59 mg, 0.98 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.16 (s, 1H, H-$1_{\alpha Man}$), 4.93 (s, 1H, H-$1_{\alpha Man}$), 4.88 (s, 1H, H-$1_{\beta Man}$), 4.61 (d, *J* = 7.7 Hz, 1H), 4.55 (d, *J* = 7.7 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 7.7 Hz, 1H), 4.27 (d, *J* = 2.8 Hz, 1H, H-2_{Man}), 4.18-4.17 (m, 1H, H-2_{Man}), 4.04-3.43(m, 46H), 3.26 (t, *J*=11.3 Hz, 1H), 2.96 (t, 2H, *J*=7.5 Hz, linker), 2.09,2.06, 2.04 (3xs, 9H, -NH*Ac*), 1.67-1.59 (m, 4H, linker), 1.43-1.39 (m, 2H, linker); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ:** 107.8, 105.5, 104.0, 103.7, 103.2, 102.3, 102.2, 101.8, 82.1, 82.0, 81.9, 81.1, 79.7, 79.1, 78.0, 77.9, 77.1, 77.1, 77.0, 76.3, 75.3, 75.0, 74.5, 73.8, 73.1, 72.8, 72.4, 71.9, 71.2, 69.8, 69.4, 69.3, 68.0, 67.6, 67.6, 64.4, 64.4, 63.6, 63.5, 62.6, 62.6, 62.6, 60.8, 57.6, 57.6, 57.5, 42.1, 30.7, 29.5, 24.9, 24.8, 24.7, 24.7; **HRMS (ESI) *m/z*** $[M+Na+H]^{2+}$ calculated for $C_{58}H_{101}N_4O_{40}Na$ 758.2939 found 758.2911.

Octasaccharide G16: Following the general procedure C, compound G16 (0.65 mg, 99 %) was prepared from **G4** (0.582 mg, 0.437 µmol) using 62.5 mU of bovine milk β-1,4-galactosyltransferase and UDP-Gal (Uridine 5′-diphosphogalactose disodium salt) (0.306 mg, 0.502 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.14 (s, 1H, H-$1_{\alpha Man}$), 4.92 (s, 1H, H-$1_{\alpha Man}$), 4.89 (s, 1H, H-$1_{\beta Man}$), 4.61 (d, *J* = 8.4 Hz, 1H), 4.59 (d, *J* = 7.90Hz, 1H), 4.51 (d *J* = 7.9 Hz, 1H), 4.49 (d, *J* = 7.9 Hz, 1H), 4.46 (d, *J* = 7.6 Hz, 1H), 4.29-4.27 (m, 1H, H-2_{Man}), 4.13-4.11 (m, 1H, H-2_{Man}), 4.06-4.05 (m, 1H, H-2_{Man}), 4.02-3.50 (m, 45H), 3.45 (t, *J* = 9.5 Hz, 1H), 3.41-3.36 (m,1H), 3.26 (t, *J* = 11.1 Hz, 1H), 3.00 (t, *J*=7.6 Hz, 2H, linker), 2.09, 2.06, 2.04 (3xs, 9H, NH*Ac*), 1.71– 1.65 (m, 2H, linker), 1.63-1.58 (m, 2H, linker), 1.44-1.38 (m, 2H, linker); **^{13}C NMR (126 MHz, D_2O) δ** 105.1, 102.9, 102.2, 101.4, 101.0, 100.6, 99.4, 96.9, 79.7, 79.4, 79.2, 78.5, 78.3, 77.3, 76.2, 75.3, 74.5, 74.4, 73.6, 73.3, 73.1, 72.9, 72.6, 72.3, 72.0, 70.9, 70.2, 70.09, 69.8, 69.9, 69.7, 69.6, 69.5, 69.2, 68.5, 66.6, 66.3, 65.5, 65.1, 65.0, 61.5, 61.4, 61.1, 60.1, 60.0, 60.0, 55.0, 54.9, 39.3, 28.0, 26.3, 22.2, 22.1, 22.1; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{58}H_{101}N_4O_{40}$ 1493.5987, found 1493.6013.

Decasaccharide G17: Following the general procedure C, compound **G17** (0.7 mg, 99%) was prepared from **G4** (0.470 mg, 0.306 µmol) using 88mU of bovine milk β-1,4-galactosyltransferase and, UDP-Gal (0.55 mg, 0.91 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.14 (s, 1H, H-$1_{\alpha Man}$), 4.91 (s, 1H, H-$1_{\alpha Man}$), 4.87 (s, 1H, H-$1_{\beta Man}$), 4.59 (dd, *J* = 8.6, 7.9 Hz, 2H), 4.54 (d, *J* = 7.9 Hz, 1H), 4.49 (d, *J* = 7.9 Hz, 1H), 4.47 (dd, *J* = 7.8, 4.7 Hz, 2H), 4.44 (d, *J* = 7.7 Hz, 1H), 4.26-4.25 (d, *J* = 3.2 Hz, 1H, H-2_{Man}), 4.16-4.15 (m, 1H, H-2_{Man}), 4.11-4.10 (m, 1H, H-2_{Man}), 4.02-3.97 (m, 4H), 3.93-3.48 (m, 51H), 3.45-3.42 (td, *J* = 9.2, 2.3 Hz, 1H), 3.38-3.36 (m, 1H), 3.25 (t, *J* = 11.3 Hz, 1H), 2.98 (t, 2H, *J* =7.7 Hz, linker), 2.08, 2.05, 2.04, 2.03 (4xs, 12H, NH*Ac*), 1.69-1.65 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.40-1.38 (m, 2H, linker); **^{13}C NMR(201 MHz, D2O) δ:** 107.7, 105.6, 105.6, 104.0, 103.7, 103.2, 102.2, 102.1, 101.7, 99.7, 82.1, 82.05, 81.9, 81.1, 79.7, 79.1, 78.9, 78.0, 77.9, 77.9, 77.4, 77.4, 77.1, 77.0, 76.3, 76.2, 75.9, 75.5, 75.1, 74.7, 74.6, 74.4, 73.6, 73.5, 72.8, 72.7, 72.2, 72.1, 71.9, 71.9, 71.4, 71.2, 69.8, 69.1, 68.0, 67.9, 67.6, 67.6, 64.3, 64.2, 63.6, 62.9, 62.7, 62.6, 62.6, 62.6, 62.6, 62.5, 57.6, 57.6, 57.5, 41.9, 30.7, 29.0, 24.9, 24.9, 24.8, 24.7; **HRMS (MALDI-tof) *m/z*** $[M+Na]^+$ calculated for $C_{72}H_{123}N_5O_{50}Na$ 1880.7128, found 1880.7042.

Heptasaccharide G18: Following the general procedure E, compound **G18** (0.68 mg, 99%) was prepared from **G3** (0.59 mg, 0.50 µmol) using concentrated *P. pastoris* extract containing GalNAcT (40 µL) and UDP-GalNAc (0.39 mg, 0.60 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.15 (s, 1H, H-$1_{\alpha Man}$), 4.86 (s, 1H, H-$1_{\beta Man}$), 4.61 (d, *J* = 7.5 Hz, 1H, H-$1_{\beta GlcNAc}$), 4.54-4.50 (m, 3H), 4.46 (d, *J* = 7.5 Hz, 1H, H-$1_{\beta Xyl}$), 4.26-4.24 (m, 1H, H-2_{Man}), 4.18-4.16 (m, 1H, H-2_{Man}), 4.05-3.99 (dd, *J* = 11.4, 5.5 Hz, 1H), 3.96-3.43 (m, 38H), 3.40-3.36 (m, 1H), 3.26 (t, *J* = 11.1 Hz, 1H), 2.98 (t, *J* = 7.7 Hz, 2H, linker), 2.08, 2.06, 2.04 (3xs, 9H, NH*Ac*), 1.71-1.65 (m, 2H, linker), 1.62-1.58 (m, 2H, linker), 1.44-1.39 (m, 2H, linker); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ:**107.6, 104.4, 104.0, 103.7, 102.7, 102.2, 101.8, 81.9, 81.8, 81.7, 81.3, 79.8, 79.1, 78.9, 78.0, 77.9, 77.2, 77.1, 76.3, 75.9, 75.0, 74.6, 74.4, 73.3, 72.8, 72.8, 72.1, 72.0, 70.2, 69.8, 69.1, 67.6, 64.4, 64.4, 63.6, 63.0, 62.7, 62.7, 62.6, 57.8, 57.6, 57.2, 55.1, 41.9, 30.7, 29.0, 24.8, 24.7, 24.7, 24.7**;** **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{54}H_{94}N_5O_{35}$ 1372.5724, found 1372.5740.

Octasaccharide G19: Following the general procedure E, compound **G19** (0.5 mg, 99%) was prepared from **G4** (0.450 mg, 0.338 µmol), using concentrated *P. pastoris* extract containing GalNAcT (75 µL) and UDP-GalNAc (0.27 mg, 0.41 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.14 (s, 1H, H-$1_{\alpha Man}$), 4.92 (s, 1H, H-$1_{\alpha Man}$), 4.87 (s, 1H, H-$1_{\beta Man}$), 4.59 (d, *J* = 8.3 Hz, 1H), 4.52-4.50 (m, 2H), 4.49 (d, *J* = 7.9 Hz, 1H), 4.44 (d, *J* = 7.6 Hz, 1H), 4.25 (d, *J* = 3.3 Hz, 1H, H-2_{Man}), 4.14 (m, 1H, H-2_{Man}), 4.01 (dd, *J* = 11.7, 5.6 Hz, 1H), 3.99-3.98 (m, 1H), 3.97-3.54 (m, 39H), 3.52-3.49 (m, 3H), 3.43 (t, *J* = 9.2 Hz, 1H), 3.37 (dd , *J* = 9.3, 7.6 Hz, 1H), 3.25 (t, *J* = 11.1 Hz, 1H), 2.98 (t, *J* = 7.7 Hz, 2H, linker), 2.08, 2.07, 2.04, 2.03 (4xs, 12H, NH*Ac*), 1.69-1.64 (m, 2H, linker), 1.62-1.57 (m, 2H, linker), 1.40-1.37 (m, 2H); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment)**105.0, 101.6, 101.2, 101.0, 100.4, 99.5, 99.4, 98.8, 82.2, 81.9, 81.9, 81.8, 79.7, 79.1, 77.9, 77.9, 77.1, 77.0, 77.0, 76.3, 75.9, 75.3, 75.1, 74.6, 74.4, 73.3, 73.1, 72.7, 72.7, 72.4, 72.2, 72.0, 71.9, 70.2, 69.8, 69.3, 68.0, 64.4, 64.4, 63.5, 63.5, 62.9, 62.7, 62.7, 57.6, 57.6, 57.2, 55.1**,** 41.9, 30.7, 29.0, 24.9, 24.8, 24.8, 24.8, 24.7; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{60}H_{104}N_5O_{40}$ 1534.6252, found 1534.6299.

Octasaccharide G20: Following the general procedure E, compound G20 (0.7 mg, 99 %) was prepared from **G4** (0.61 mg, 0.458 µmol) using concentrated *P. pastoris* extract containing GalNAcT (120 µL) and UDP-GalNAc (Uridine 5′-diphospho-N-acetylgalactosamine disodium salt) (0.752 mg, 1.15 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.14 (s, 1H, H-$1_{\alpha Man}$), 4.91 (s, 1H, H-$1_{\alpha Man}$), 4.89 (s, 1H, H-$1_{\beta Man}$), 4.61 (d, *J* = 7.9 Hz, 1H), 4.58-4.55 (d, *J* = 7.8 Hz, 1H), 4.52 (d, *J* = 8.3 Hz, 1H), 4.51 (d, *J* = 7.8 Hz, 1H), 4.46 (d, *J* = 7.6 Hz, 1H), 4.28 (d, *J* = 3.0 Hz, 1H, H-2_{Man}), 4.12-4.10 (m, 1H, H-2_{Man}), 4.05 (dd, *J* = 3.2, 1.4 Hz, 1H, H-2_{Man}), 4.03-3.58 (m, 40H), 3.53-3.49 (m, 3H), 3.45 (t, *J* = 9.26 Hz, 1H), 3.40-3.37 (m, 1H), 3.26 (t, *J* = 11.1 Hz, 1H), 3.00 (t, *J* = 7.7 Hz, 2H, linker), 2.10, 2.09, 2.05, 2.04 (4xs, 12H, NH*Ac*), 1.71-1.65 (m, 2H, linker), 1.62-1.58 (m, 2H, linker), 1.44-1.38 (m, 2H, linker); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ:**107.6, 104.8, 104.4, 104.0, 103.7, 103.3, 102.1, 99.5, 82.3, 82.2, 81.9, 79.9, 78.8, 78.0, 78.0, 77.1, 77.0, 76.2, 75.9, 75.5, 75.0, 74.7, 74.3, 73.3, 72.9, 72.8, 72.7, 72.6, 72.2, 72.2, 71.9, 70.2, 69.9, 69.4, 69.0, 68.0, 67.6, 67.5, 64.2, 63.8, 63.7, 62.8, 62.7, 62.7, 57.6, 57.6, 57.3, 55.1, 41.9, 30.7, 29.0, 24.9, 24.9, 24.8, 24.8, 24.7; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{60}H_{104}N_5O_{40}$ 1534.6252, found 1534.6201.

Decasaccharide G21: Following the general procedure E, compound **G21** (0.70 mg, 99%) was prepared from **G6** (0.57 mg, 0.37 µmol), using concentrated *P. pastoris* extract containing GalNAcT (150 µL) and UDP-GalNAc (0.96 mg, 1.48 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.15 (s, 1H, H-$1_{\alpha Man}$), 4.91 (s, 1H, H-$1_{\alpha Man}$), 4.88 (s, 1H, H-$1_{\beta Man}$), 4.61 (d, *J* = 8.0 Hz, 1H), 4.58-4.50 (m, 5H), 4.45 (d, *J* = 8.1 Hz, 1H), 4.28-4.26 (m, 1H, H-2_{Man}), 4.16-4.14 (m, 1H, H-2_{Man}), 4.01-3.42 (m, 56H), 3.38 (s, 2H), 3.28-3.23 (m, 1H), 2.97 (t, *J* = 7.4 Hz, 2H), 2.10-2.07 (m, 9H, NH*Ac*), 2.06-2.04 (m, 9H, NH*Ac*), 1.69-1.63 (m, 2H, linker), 1.62-1.57 (m, 2H, linker), 1.42-1.38 (m, 2H, linker); **HRMS (MALDI-tof) *m/z*** $[M+Na]^+$ calculated for $C_{76}H_{129}N_7O_{50}Na$ 1962.7659, found 1962.7672.

Octasaccharide G22: Following the general procedure D, compound **G22** (0.65 mg, 99%) was prepared from **G7** (0.47 mg, 0.36 µmol) using core α-1,3-fucosyltransferase AtFucTA (25µL, 0.25 mg/mL), and GDP-Fuc (267 µg, 0.42 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.16 (s, 1H, H-$1_{\alpha Man}$), 5.14 (d, *J* = 4.1 Hz, 1H, H-$_{1Fuc\ \alpha1,3}$), 4.94 (d, *J* = 4.1 Hz, 1H, H-$_{1Fuc\ \alpha1,6}$), 4.84 (s, 1H, H-$1_{\beta Man}$), 4.74-4.72 (m, 1H, H-$5_{Fuc\ \alpha1,3),}$ 4.69 (d, *J* = 7.9 Hz, 1H), 4.52 (d, *J* = 8.5 Hz, 1H), 4.49 (t, *J* = 8.9Hz, 2H), 4.25 (d, *J* = 3.17 Hz, 1H, H-2_{Man}), 4.18-4.16 (m, 2H, H-2_{Man} ,H-$5_{Fuc\ \alpha1,6}$), 4.03-3.46 (m, 39H), 3.40-3.35 (m, 1H), 3.27 (t, *J* = 11.3 Hz, 1H), 2.99 (t, *J* = 7.7 Hz, 2H, linker), 2.06, 2.05, 2.03 (3xs, 9H, NH*Ac*), 1.71-1.65 (m, 2H, linker), 1.62-1.57(m, 2H, linker), 1.41-1.36 (m, 2H, linker), 1.28 (d, *J* = 6.8 Hz, 3H, C$H_{3Fuc\ \alpha1,3}$), 1.24 (d, *J* = 6.7 Hz, 3H, C$H_{3Fuc\ \alpha1,6}$); **^{13}C NMR(201 MHz, D2O) δ** 107.6, 103.6, 102.7, 102.69, 102.4, 101.8, 101.6, 100.9, 82.7, 81.8, 79.7, 79.2, 78.8, 78.5, 77.9, 77.7, 77.3, 76.6, 76.3, 76.0,75.9, 75.9, 74.8, 74.4, 72.7, 72.5, 72.1, 72.0, 71.9, 71.8, 70.7, 70.4, 69.8, 69.4, 69.4, 69.1, 67.6, 67.5, 65.3, 64.4, 64.3, 63.2, 63.0, 58.3, 57.9, 57.9, 42.0, 30.7, 29.2, 24.8, 24.8, 24.8, 24.8, 18.1, 18.0; **HRMS (ESI)** *m/z* [M+Na]$^+$ calculated for $C_{58}H_{100}N_4O_{38}$ Na 1483.5908 found 1483.5907.

Nonasaccharide G23: Following the general procedure D, compound **G23** (0.34 mg, 91.5%) was prepared from **G8** (0.34 mg, 0.23 µmol) using core α-1,3-fucosyltransferase AtFucTA (25µL, 0.25 mg/mL), and GDP-Fuc (228 µg, 0.36 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.16 (s, 1H, H-$1_{\alpha Man}$), 5.14 (d, *J* = 3.9 Hz, 1H, H-$_{1Fuc\ \alpha1,3}$), 4.94 (d, *J* = 3.9 Hz, 1H, H-$_{1Fuc\ \alpha1,6}$), 4.9 (d, *J* = 1.6 Hz, 1H, H-$1_{\alpha Man}$), 4.87 (s, 1H, H-$1_{\beta Man}$), 4.74-4.72 (m, 1H, H-$5_{Fuc\ \alpha1,3}$), 4.70 (d, *J* = 7.9 Hz, 1H), 4.53 (d, *J* = 8.6 Hz, 1H), 4.50 (d, *J* = 8.2 Hz, 1H), 4.48 (d, *J* = 7.8 Hz, 1H), 4.27 (d, *J* = 3.1 Hz, 1H, H-2_{Man}), 4.18-4.15 (m, 2H, H-2_{Man}, H-$5_{Fuc\ \alpha1,6}$), 4.05-3.45 (m, 42H), 3.41-3.36 (m, 1H), 3.28 (t, *J* = 11.3 Hz, 1H), 3.00 (t, *J* = 7.5 Hz, 2H, linker), 2.07, 2.06 ,2.03 (3xs, 12H, NH*Ac*), 1.71-1.64 (m, 2H, linker), 1.63-1.58 (m, 2H, linker), 1.43-1.38 (m, 2H, linker), 1.29 (d, *J* = 6.5 Hz, 3H, C$H_{3Fuc\ \alpha1,3}$), 1.24(d, *J* = 6.7 Hz, 3H, C$H_{3Fuc\ \alpha1,6}$); **^{13}C NMR (201 MHz, D2O) δ** 107.8, 103.6, 103.2, 102.6, 102.4, 102.3, 101.8, 101.6, 100.9 , 83.6, 82.05, 79.6, 79.2, 78.4, 77.9, 77.3, 77.1, 77.0, 76.6, 76.3, 76.0, 75.9, 75.8, 75.3, 75.00, 74.5, 73.1, 72.7, 72.4, 72.3, 72.1, 72.0, 71.9, 71.8, 70.7, 70.4, 70.0, 69.4, 69.4, 69.3, 69.2, 68.0, 68.1, 67.6, 67.5, 64.4, 64.3, 63.5, 63.2, 58.3, 57.9, 57.7, 42.0, 30.7, 29.3, 24.9, 24.8, 24.8, 24.8, 18.1, 18.0; **HRMS (ESI)** ***m/z*** [M+Na]$^+$ calculated for $C_{64}H_{110}N_4O_{43}$Na 1645.6436 found 1645.6415.

Decasaccharide G24: Following the general procedure D, compound **G24** (1.64 mg, 93 %) was prepared from **G9** (1.62 mg, 0.96 µmol) using core α-1,3-fucosyltransferase AtFucTA (30 µL, 0.25 mg/mL), and GDP-Fuc (728 µg, 1.15 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.15 (s, 1H, H-$1_{\alpha Man}$), 5.13 (d, *J* = 3.6 Hz, 1H, H-$1_{Fuc\alpha1\text{-}3}$), 4.94 (d, *J* = 3.6 Hz, 1H, H-$1_{Fuc\alpha1\text{-}6}$), 4.90 (s, 1H, H-$1_{\alpha Man}$), 4.86 (s, 1H, H-$1_{\beta Man}$), 4.68 (d, *J* = 8.3 Hz, 1H), 4.56 (d, *J* = 8.6 Hz, 1H), 4.51 (d, *J* = 8.6 Hz, 1H), 4.49-4.48 (m, 1H), 4.46 (d, *J* = 7.6 Hz, 1H), 4.26 (d, *J* = 3.2 Hz, 1H, H-2_{Man}), 4.16-4.15(m, 2H, H-$2_{Man,}$ H-$5_{Fuc\alpha1\text{-}6}$), 4.11-4.10 (m, 1H), 4.03-3.42 (m, 51H), 3.38 (dt, *J*=9.8, 1.8 Hz, 1H), 3.27 (t, *J* = 11.3 Hz, 1H), 2.97-2.94 (m, 2H, linker), 2.06 (s, 3H, NH*Ac*), 2.05 (m, 6H, NH*Ac*), 2.02 (s, 3H, NH*Ac*), 1.67-1.63 (m, 2H, linker), 1.60-1.57 (m, 2H, linker), 1.40-1.37 (m, 2H, linker), 1.27 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha1\text{-}3}$), 1.23 (d, *J* = 6.5 Hz, 3H, $CH_{3Fuc\alpha1\text{-}6}$); **^{13}C NMR(201 MHz, D2O) δ:** 106.6, 102.4, 102.0, 101.4, 101.3, 100.9, 100.5, 100.4, 99.7, 98.5, 82.3, 80.8, 78.4, 77.9, 77.5, 77.2, 76.6, 76.0, 75.9, 75.8, 75.3, 75.0, 74.8, 74.7, 74.7, 74.2, 73.8, 73.3, 73.1, 71.4, 71.3, 70.9, 70.8, 70.6, 70.5, 69.9, 69.5, 69.1, 68.6, 68.2, 68.1, 67.8, 67.4, 67.4, 66.9, 66.4, 66.3, 63.1, 63.1, 62.9,

62.5, 62.4, 62.0, 62.0, 61.7, 57.0, 56.7, 56.4, 40.8, 29.5, 28.1, 23.7, 23.6, 23.6, 23.6, 23.5, 16.9, 16.8; **HRMS (ESI) *m/z*** $[M+Na+H]^{2+}$ calculated for $C_{72}H_{123}N_5O_{48}$ 924.8651, found 924.8627.

Octasaccharide G25: Following the general procedure E, compound **G25** (0.37 mg, 99%) was prepared from **G11** (0.32 mg, 0.24 µmol) using concentrated *P. pastoris* extract containing GalNacT (50 µL) and UDP-GalNAc (189 µg, 0.291 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.14-5.13 (m, 2H, H-$1_{\alpha Man,}$ H-1_{Fuc}), 4.83 (s, 1H, H-$1_{\beta Man}$), 4.71-4.69 (m, 1H, H-5_{Fuc}), 4.54 (d, 1H, *J* =8.5 Hz), 4.52-4.50 (m, 2H), 4.48 (d, *J* = 8.2 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.25-4.23 (m, 1H, H-2_{Man}), 4.15-4.14 (m, 1H, H-2_{Man}), 4.00 (dd, *J* = 11.4, 5.6 Hz, 1H), 3.97 (dd, *J* = 10.5, 3.4 Hz, 1H), 3.94-3.43 (m, 40H), 3.37 (dd, *J* = 10.0, 7.8 Hz, 1H), 3.25 (t, *J* = 11.0 Hz, 1H), 2.98 (t, *J* = 7.9 Hz, 2H, linker), 2.07, 2.04, 2.04, 2.02 (4xs, 12H), 1.69-1.65 (m, 2H, linker), 1.60-1.57 (m, 2H, linker), 1.41-1.38 (m, 2H, linker), 1.26 (d, *J* = 6.6 Hz, 3H, CH_{3Fuc}); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ:** 107.6, 104.4, 103.7, 103.1, 102.7, 102.2, 101.7, 100.9, 82.5, 81.8, 81.7, 79.7, 79.0, 78.9, 78.0, 78.0, 77.9, 77.1, 76.4, 76.4, 75.9, 74.8, 74.7, 74.4, 73.3, 72.8, 72.7, 72.1, 72.0, 71.9, 70.4, 70.2, 69.8, 69.4, 69.1, 67.6, 67.5, 64.4, 64.4, 63.6, 63.6, 63.5, 62.9, 62.7, 62.7, 62.7, 58.2, 57.9, 57.2, 55.2, 30.7, 29.0, 24.9, 24.8, 24.8, 24.7, 18.1; **HRMS (MALDI-tof) *m/z*** $[M+Na]^+$ calculated for 1540.6122 $C_{60}H_{103}N_5O_{39}$, found 1540.6124.

Octasaccharide G26: Following the general procedure C, compound **G26** (0.83mg, 89%) was prepared from **G7** (0.830 mg, 0.631 µmol) using 87 mU of bovine milk β-1,4-galactosyltransferase and UDP-Gal (464 µg, 0.76 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.14 (s, 1H, H-$1_{\alpha Man}$), 4.91 (s, 1H), 4.85 (s, 1H), 4.66 (d, *J* = 8.3 Hz, 1H), 4.54 (d, *J* = 7.6 Hz, 1H), 4.50 (d, *J* = 8.6 Hz, 1H), 4.45 (dd, *J* = 7.9, 7.7 Hz, 2H), 4.24 (s, 1H, H-2_{Man}), 4.16 (s, 1H, H-2_{Man}), 4.14-4.12 (m, 1H, H-5_{Fuc}), 4.02-3.97 (m, 2H), 3.92-3.44 (m, 40H), 3.37 (t, *J* = 8.5 Hz, 1H), 3.24 (t, *J* = 11.0 Hz, 1H), 2.98 (t, *J* = 7.9 Hz, 2H), 2.08, 2.05, 2.03 (3xs, 9H, NH*Ac*), 1.68-1.65 (m, 2H, linker), 1.62-1.58 (m, 2H, linker), 1.41-1.37 (m, 2H, linker), 1.23 (d, *J* = 6.6 Hz, 3H, CH_{3Fuc}); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ:** 106.4, 104.3, 102.5, 102.5, 101.5, 101.0, 100.8, 100.5, 80.5, 79.8, 78.5, 77.9, 77.7, 76.7, 76.6, 76.1, 75.9, 75.0, 74.9, 74.7, 73.3, 73.1, 72.9, 72.3, 71.4, 70.9, 70.8, 70.7, 69.9, 69.5, 68.6, 68.2, 67.9, 67.8, 66.3, 66.3, 63.2, 63.1, 62.4, 61.6, 61.4, 61.3, 61.3, 61.3, 56.6, 56.5, 56.4, 40.7, 29.5, 27.81, 23.7, 23.6, 23.5, 23.5, 16.8; **HRMS (MALDI-tof) *m/z*** $[M+Na]^+$ calculated for $C_{58}H_{100}N_4O_{39}Na$ 1499.5857, found 1499.5786.

Octasaccharide G27: Following the general procedure D, compound **G27** (0.38 mg, 99%) was prepared from **G14** (0.35 mg, 0.26 µmol) using core α-1,3-fucosyltransferase CeFUT6 (20 µL, 1.07 mg/mL) and GDP-Fuc (202 µg, 0.32 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.13 (s, 1H, H-$1_{\alpha Man}$), 5.13 (d, *J* = 4.1 Hz, 1H, H-1_{Fuc}), 4.85(s, 1H, H-$1_{\beta Man}$), 4.84-4.82 (m, 1H, H-5_{Fuc}), 4.59 (d, *J* = 8.1 Hz, 1H), 4.55-4.53 (m, 1H), 4.49 (d, *J* = 7.7 Hz, 1H), 4.44 (d, *J*=8.2 Hz, 2H), 4.24 (d, *J* = 2.8 Hz, 1H, H-2_{Man}), 4.16-4.15 (m, 1H, H-2_{Man}), 4.03-3.99 (m, 2H), 3.96-3.84 (m, 14H), 3.80-3.56 (m, 21H), 3.52-3.46 (m, 4H), 3.43 (t, *J* = 9.3 Hz, 1H), 3.37 (dd, *J* = 9.5, 7.7 Hz, 1H), 3.24 (t, *J* = 11.1 Hz, 1H), 2.98 (t, *J* = 7.7 Hz, 2H, linker), 2.06, 2.04, 2.03 (3xs, 9H, NH*Ac*), 1.69-1.64 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.42-1.37 (m, 2H, linker), 1.17 (d, *J* = 6.6 Hz, 3H, CH_{3Fuc}); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ:** 106.4, 103.2, 102.8, 102.4, 101.3, 100.8, 100.5, 99.93, 80.7, 80.5, 80.0, 78.5, 77.8, 77.7, 76.3, 75.9, 75.8, 75.1, 74.7, 74.6, 73.8, 73.3, 73.0, 72.5, 71.5, 70.8, 70.7, 70.6, 69.8, 69.1, 68.6, 68.1, 67.8, 66.4, 66.3, 63.2, 63.1, 62.9, 61.7, 61.6, 61.5, 61.4,

61.3, 61.1, 57.1, 56.6, 56.4, 40.7, 29.4, 27.8, 23.8, 23.6, 23.5, 23.4, 16.7; **HRMS (ESI) *m/z*** [M+H] calculated for $C_{58}H_{101}N_4O_{39}$ 1477.6037, found 1477.6034.

Nonasaccharide G28: Following the general procedure D, compound **G28** (0.38 mg, 99%) was prepared from **G15** (0.325 mg, 0.217 µmol) using α-1,3-fucosyltransferase CeFUT6 (15 µL, 1.07 mg/mL) and GDP-Fuc (164 µg, 0.26 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.13 (s, 1H, H-$1_{\alpha Man}$), 5.12(d, *J* = 3.9 Hz, 1H, H-1_{Fuc}), 4.92 (s, 1H, H-$1_{\alpha Man}$), 4.87 (s, 1H, H-$1_{\beta Man}$), 4.60 (d, *J* = 8.0 Hz, 1H), 4.56-4.54 (m, 1H), 4.5 (d, *J* = 7.8 Hz, 1H), 4.45-4.43 (m, 2H), 4.26 (d, *J* = 2.7 Hz, 1H, H-2_{Man}), 4.16-4.15 (m, 1H, H-2_{Man}), 4.13-4.09 (m, 1H), 4.02-3.48 (m, 48H), 3.44 (t, *J* = 9.3 Hz, 1H), 3.37 (dd, *J* = 9.6, 8.0 Hz, 1H), 3.25 (t, *J* = 11.0 Hz, 1H), 2.98 (t, *J* = 7.8 Hz, 2H, linker), 2.08, 2.04, 2.03 (3xs, 9H, NH*Ac*), 1.68-1.65 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.41-1.38 (m, 2H, linker), 1.18 (d, *J* = 6.5 Hz, 3H, CH_{3Fuc}); **^{13}C NMR (201 MHz, D2O, peaks from HSQC experiment) δ** 107.8, 104.4, 104.0, 103.7, 103.2, 102.3, 101.9, 101.7, 101.2, 82.1, 82.0, 81.9, 79.7, 79.0, 77.9, 77.9, 77.6, 77.2, 77.1, 77.0, 76.3, 75.9, 75.9, 75.3, 75.0, 75.0, 74.6, 74.6, 74.4, 73.7, 73.4, 72.7, 72.7, 72.4, 72.0, 71.9, 71.7, 70.9, 70.3, 69.8, 69.5, 69.3, 69.2, 68.2, 67.6, 67.5, 64.8, 64.1, 63.5, 62.9, 63.0, 62.3, 57.6, 57.6, 30.7, 29.1, 25.0, 24.8, 24.8, 24.7, 17.9; **HRMS (ESI) *m/z*** $[M+Na+H]^{2+}$ calculated for $C_{64}H_{110}N_4O_{44}$ 831.3229, found 831.3253.

Nonasaccharide G29: Following the general procedure D, compound **G29** (0.26 mg, 74%) was prepared from **G16** (0.32 mg, 0.22 µmol) using core α-1,3-fucosyltransferase CeFUT6 (15 µL, 1.07 mg/mL) and GDP-Fuc (164 µg, 0.26 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.14-5.12 (m, 2H, H-$1_{\alpha Man,}$ H-$1_{Fuc\alpha\text{-}1,3}$), 4.90 (s, 1H, H-$1_{\alpha Man}$), 4.88 (s, 1H, H-$1_{\beta Man}$), 4.59-4.58 (m, 2H), 4.49 (d, *J* = 7.62 Hz, 1H), 4.44 (dd, *J* = 7.91, 2.8 Hz, 2H), 4.27 (d, *J* = 2.8 Hz, 1H, H-2_{Man}), 4.11-4.10 (m, 1H), 4.04-3.54 (m, 45H), 3.51-3.47 (m, 3H), 3.44 (t, *J* = 9.4 Hz, 1H), 3.39-3.36 (m, 1H), 3.25 (t, *J* = 11.1 Hz, 1H), 2.98 (t, *J* = 7.7 Hz, 2H, linker), 2.08, 2.04, 2.03 (3xs, 9H, NH*Ac*), 1.68-1.63 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.40-1.38 (m, 2H, linker), 1.17 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha\text{-}1,3}$); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ:**107.7, 104.8, 104.4, 104.0, 103.7 ,103.3, 101.8, 101.2, 99.4, 82.2, 81.9, 81.9, 79.9, 78.7, 77.9, 77.9, 77.6, 77.3, 77.1, 77.0, 76.2, 75.9, 75.9, 75.5, 75.1, 75.1, 74.7, 74.6, 74.3, 73.7, 72.9, 72.7, 72.6, 72.2, 71.8, 71.1, 71.0, 70.3, 69.9, 69.4, 69.3, 69.1, 67.5, 64.1, 63.8, 63.0, 62.7, 62.7, 62.3, 62.1, 58.3, 57.6, 57.6, 41.9, 30.7, 28.9, 25.0, 24.8, 24.8, 22.6, 17.9; **HRMS (ESI) *m/z*** $[M+Na+H]^{2+}$ calculated for $C_{64}H_{111}N_4O_{44}Na$ 831.3229, found 831.3199.

Dodecasaccharide G30: Following the general procedure D, compound **G30** (0.4 mg, 99%) was prepared from **G17** (0.36 mg, 0.19 µmol) using α-1,3-fucosyltransferase CeFUT6 (15 µL, 1.07 mg/mL) and GDP-Fuc (291 µg, 0.46 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.13-5.11 (m, 3H, H-$1_{\alpha Man,}$ H-$1_{Fuc\alpha\text{-}1,3,}$ H-$1_{Fuc\alpha\text{-}1,3}$), 4.89 (s, 1H, H-$1_{\alpha Man}$), 4.87(s, 1H, H-$1_{\beta Man}$), 4.84 (m, 1H H-$5_{Fuc\alpha\text{-}1,3}$), 4.60-4.58 (m, 2H), 4.55-4.53 (m, 1H), 4.48 (d, *J* = 8.1 Hz, 1H), 4.45-4.43 (m, 3H), 4.25 (d, *J* = 3.1 Hz, 1H, H-2_{Man}), 4.16-4.15 (m, 1H, H-2_{Man}), 4.11-4.10 (m, 1H, H-2_{Man}), 4.02-3.55 (m, 57H), 3.50-3.47 (m, 5H), 3.44-3.42 (m, 1H), 3.39-3.36 (m, 1H), 3.24 (t, *J* = 11.0 Hz, 1H), 2.98 (t, *J* = 7.7 Hz, 2H, linker), 2.08, 2.04, 2.04, 2.03 (4xs, 12H, NH*Ac*), 1.68-1.64 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.41-1.38 (m, 2H, linker), 1.17 (m, 6H, $CH_{3Fuc\alpha\text{-}1,3,}$ $CH_{3Fuc\alpha\text{-}1,3}$); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ:**108.0**,** 104.5**,** 104.4**,** 103.9**,** 103.7**,** 103.2**,** 101.9**,** 101.8**,** 101.7**,** 101.2**,** 101.2**,** 99.5, 82.2, 82.0, 81.9, 79.9, 79.0, 78.7, 77.9, 77.9, 77.6, 77.2, 77.1, 76.9, 76.3, 75.9, 75.9, 75.5, 75.1, 75.0, 75.0, 74.7, 74.5, 74.4, 73.7, 73.7, 72.7, 72.2, 72.2, 72.2, 72.1, 71.9, 71.8, 71.7,

71.0, 70.3, 70.3, 69.8, 69.3, 69.3, 69.1, 68.3, 68.1, 67.6, 67.6, 64.4, 64.3, 64.2, 64.1, 63.0, 62.8, 62.7, 62.7, 62.6, 62.4, 62.3, 58.3, 58.3, 57.6, 57.6, 41.9, 30.7, 29.0, 25.0, 25.0, 24.8, 24.8, 24.7, 17.9, 17.9; **HRMS (ESI) *m/z*** $[M+K]^+$ calculated for $C_{84}H_{143}N_5O_{57}K$ 2172.8286, found 2172.8364.

Nonasaccharide G31: Following the general procedure D, compound **G31** (0.42 mg, 79%) was prepared from **G26** (0.480 mg, 0.324 µmol) using α-1,3-fucosyltransferase CeFUT6 (30 µL, 1.07 mg/mL) and GDP-Fuc (297 µg, 0.47 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.13 (s, 1H, H-$1_{\alpha Man}$), 5.12 (d, *J* = 4.1 Hz, 1H, H-$1_{Fuc\alpha\text{-}1,3}$), 4.90 (d, *J* = 3.9 Hz, 1H, H-$1_{Fuc\alpha\text{-}1,6}$), 4.85 (s, 1H, H-$1_{\beta Man}$), 4.83-4.81 (m, 1H, H-$5_{Fuc\alpha\text{-}1,3}$), 4.65 (d, *J* = 8.0 Hz, 1H), 4.55-4.53 (m, 1H), 4.50 (d, *J* = 8.5, 1H), 4.44 (dd, *J* = 7.4, 3.9 Hz, 2H), 4.24 (d, *J* = 2.94 Hz, 1H, H-$2_{Man\beta}$), 4.16-4.15 (m, 1H, H-$2_{Man\alpha}$), 4.12 (q, *J* = 6.7 Hz, 1H, H-$5_{Fuc\alpha\text{-}1,6}$), 4.02-3.99 (m, 2H), 3.96-3.56 (m, 39H), 3.52-3.48 (m, 3H), 3.45 (t, *J* = 9.2 Hz, 1H), 3.38-3.36 (m, 1H), 3.25 (t, *J* = 11.0 Hz, 1H), 2.98 (t, *J* = 7.7 Hz, 2H, linker), 2.08, 2.04, 2.03 (3xs, 9H, NH*Ac*), 1.69-1.65 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.41-1.37 (m, 2H, linker), 1.22 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha\text{-}1,3}$), 1.17 (d, *J* = 6.5 Hz, 3H, $CH_{3Fuc\alpha\text{-}1,6}$); **^{13}C NMR(201 MHz, D2O) δ:** 106.4, 103.2, 102.5, 102.4, 101.5, 100.8, 100.7, 100.4, 99.9, 80.6, 80.1, 78.5, 77.8, 77.7, 76.7, 76.6, 76.0, 75.9, 75.1, 74.9, 74.7, 74.6, 73.8, 73.4, 73.3, 73.1, 72.5, 71.4, 71.4, 70.8, 70.8, 70.6, 69.7, 69.5, 69.1, 68.6, 68.2, 68.1, 67.9, 67.8, 66.3, 66.3, 63.1, 62.9, 61.6, 61.6, 61.5, 61.1, 61.0, 57.1, 56.5, 56.4, 40.6, 29.4, 27.8, 23.7, 23.6, 23.5, 23.5, 16.7, 16.7; **HRMS (ESI) *m/z*** $[M+Na]^+$ calculated for $C_{64}H_{110}N_4O_{43}Na$ 1645.6436, found 1645.6499.

Dodecasaccharide G32: Following the general procedure E, compound **G32** (0.62 mg, 63%) was prepared from **G24** (0.900 mg, 0.493 µmol) using concentrated *P. pastoris* extract containing GalNAcT (210 µL) and UDP-GalNAc (0.32 mg, 1.97 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.15-5.13 (m, 2H, H-$1_{\alpha Man}$, H-$1_{Fuc\alpha 1\text{-}3}$), 4.95, (m, 1H, H-$1_{Fuc\alpha 1\text{-}6}$), 4.90 (m, 1H, H-$1_{\alpha Man}$), 4.86 (s, 1H, H-$1_{\beta Man}$), 4.69 (m, 1H), 4.57-4.45 (m, 6H), 4.26-4.25 (m, 1H, H-2_{Man}), 4.16-4.15 (m, 2H, H-$2_{Man,}$ H-$5_{Fuc\alpha 1\text{-}6}$), 4.10-4.09 (m, 2H), 4.03-3.34 (m, 69H), 2.98 (m, 2H, linker), 2.08, 2.07, 2.05, 2.02 (4xs, 12H, NH*Ac*), 1.69-1.64 (m, 2H, linker), 1.62-1.55 (m, 2H, linker), 1.42-1.36 (m, 2H, linker), 1.28 (d, *J* = 6.7 Hz, 3H, $CH_{3Fuc\alpha 1\text{-}3}$), 1.24 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha 1\text{-}6}$); **HRMS (ESI) *m/z*** $[M+Na]^+$ calculated for $C_{88}H_{149}N_7O_{58}$ 2254.8817, found 2254.885.

Tetradecasaccharide G33: Following the general procedure B, compound **G24** (1.18mg, 0.689mmol) was first galactosylated employing bovine GalT (35 µL) and UDP-Gal. The galactosylated intermediate (0.714 mg, 0.331 µmol) was purified and fucosylated using α-1,3-fucosyltransferase CeFUT6 (75 µL, 1.07 mg/mL) and GDP-Fuc (633 µg, 1.0 µmol) to arrive at compound **G33** (0.8 mg, 99%); **^{1}H NMR (500 MHz, D_2O)** δ 5.16-5.13 (m, 4H, H-$1_{\alpha Man,}$ H-$1_{Fuc\alpha\text{-}1,3,}$ H-$1_{Fuc\alpha\text{-}1,3,}$ H-$1_{Fuc\alpha\text{-}1,3}$), 4.95 (d, *J* = 3.61 Hz, 1H), 4.91 (s, 1H, H-$1_{\alpha Man,}$), 4.87-4.83 (m, 3H, H-$1_{\beta Man,}$ 2xH-$5_{Fuc\alpha 1,3/LewisX,}$), 4.73-4.69 (m, 1H, H-$5_{Fuc\alpha 1,3}$), 4.69 (d, *J* = 8.0 Hz, 1H), 4.60 (d, *J* = 8.4 Hz, 1H), 4.56 (d, *J* = 7.6 Hz, 1H), 4.50 (d, *J* =8.4 Hz, 1H), 4.48-4.45 (m, 3H), 4.27-4.26 (m, 1H, H-2_{Man}), 4.17-4.16 (m, 1H, H-$2_{Man,}$), 4.11-4.10 (m, 1H, H-$2_{Man,}$), 4.04 –3.47 (m, 70H), 3.42-3.37 (m, 1H), 3.28 (t, *J* = 10.8 Hz, 1H), 3.01 (t, *J* = 7.7 Hz, 2H, linker), 2.08, 2.05, 2.03 (3xs, 12H, NH*Ac*), 1.70-1.57 (m, 4H, linker), 1.43-1.38 (m, 2H,linker), 1.29 (d, *J* = 6.54 Hz, 3H, $CH_{3Fuc\alpha 1,3}$), 1.24 (d, *J* = 6.54 Hz, 3H, $CH_{3Fuc\alpha 1,6}$), 1.17 (m, 6H, 2x$CH_{3Fuc/LewisX}$); **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{96}H_{164}N_5O_{66}$ 1232.9759, found 1232.9803.

Hexasaccharide G34: Following the general procedure F, compound G34 (0.67 mg, 99 %) was prepared from **G6** (0.924 mg, 0.602µmol) using 1.4 U of Jack bean β-N-acetylglucosaminidase; ^{1}H NMR (800 MHz, D_2O) δ 5.12 (d, *J* = 1.6 Hz, 1H, H-$1_{\alpha Man}$), 4.91 (d, *J* = 1.8 Hz, 1H, H-$1_{\alpha Man}$), 4.87 (s, 1H, H-$1_{\beta Man}$), 4.60 (d, *J* = 8.2 Hz, 1H, H-1_{GlcNAc}), 4.50 (d, *J* = 8.0 Hz, 1H, H-1_{GlcNAc}), 4.45 (d, *J* = 7.7, 1H, H-1_{Xyl}), 4.27 (d, *J* = 3.1 Hz, 1H, H-$2_{Man\beta}$), 4.04 (dd, *J* = 3.38 Hz, *J* =1.6 Hz, 1H, H-$2_{Man\alpha}$), 4.01 (dd, *J* = 11.62 Hz, *J* =5.4 Hz, 1H), 4.00-3.94 (m, 3H), 3.92-3.58 (m, 27H), 3.51-3.49 (m, 1H), 3.44 (td, *J* = 9.2Hz, *J* =2.3 Hz, 1H), 3.39-3.37 (m, 1H), 3.25 (t, *J* = 11.0 Hz, 1H), 2.98 (t, *J*=7.9Hz, 2H, linker), 2.08, 2.03 (2xs, 6H, NH*Ac*), 1.69-1.65 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.41-1.38 (m, 2H, linker); ^{13}C NMR(201 MHz, D2O) δ 107.7, 104.8, 103.9, 103.7, 103.2, 102.2, 82.2, 81.9, 81.9, 79.9, 77.9, 77.2, 77.1, 76.1, 75.9, 75.2, 75.0, 74.7, 73.1, 72.6, 72.4, 72.2, 71.9, 69.1, 68.0, 67.6, 67.5, 63.9, 63.8, 63.5, 63.5, 62.8, 62.7, 62.7, 57.6, 57.6, 41.9, 30.7, 28.9, 24.8, 24.7, 24.7; HRMS (MALDI-tof) *m/z* $[M+Na]^+$ calculated for $C_{44}H_{77}N_3O_{30}Na$ 1150.4484, found 1150.4433.

Hexasaccharide G35: Following the general procedure F, compound G35 (1 mg, 90%) was prepared from G7 (1.3 mg, 0.99 µmol) using 0.5 U of jack bean β-N-acetylglucosaminidase; ^{1}H NMR (500 MHz, D_2O) δ 5.14 (s, 1H, H-$1_{\alpha Man}$), 4.92 (d, *J* = 3.9 Hz, 1H, H-$1_{\alpha Fuc}$), 4.87 (s, 1H, H-$1_{\beta Man}$), 4.67 (d, *J* = 7.7 Hz, 1H), 4.51 (d, *J* = 7.7 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.25 (d, *J* = 3.1 Hz, 1H, H-2_{Man}), 4.14 (dd, *J*=13.4 Hz, *J* = 6.22 Hz, 1H, H-5_{Fuc}), 4.06 (dd, *J* = 3.3, 1.5 Hz, 1H, H-2_{Man}), 4.04-3.99 (m, 2H), 3.94-3.58 (m, 20H), 3.51-3.45 (m, 2H), 3.41-3.36 (m, 1H), 3.26 (t, *J* = 11.4 Hz, 1H), 3.00 (t, *J* = 7.6 Hz, 2H, linker), 2.09, 2.05 (2xs, 6H, NH*Ac*), 1.71-1.67 (m, 2H, linker), 1.64-1.58 (m, 2H, linker), 1.44-1.39 (m, 2H, linker), 1.24 (d, *J* = 6.7 Hz, 3H, CH_{3Fuc}); ^{13}C NMR (126 MHz, D_2O) δ 104.9, 102.2, 101.2, 101.1, 100.1, *99.4*, 79.1, 78.7, 77.3, 75.4, 74.5, 73.5, 73.3, 72.4, 72.3, 72.0, 71.8, 71.7, 70.3, 70.0, 69.9, 69.6, 69.1, 68.1, 66.8, 66.7, 66.5, 66.43, 64.90, 64.87, 61.7, 60.2, 60.2, 55.1, 55.1, 39.3, 28.1, 26.4, 22.2, 22.1, 22.1, 15.3; HRMS (ESI) *m/z* $[M+H]^+$ calculated for $C_{45}H_{77}N_3O_{29}$ 1112.4716, found 1112.4758**.**

Heptasaccharide G37: Following the general procedure D, compound **G37** (0.43 mg, 99%) was prepared from **G34** (0.34 mg, 0.30 µmol) using core α-1,3-fucosyltransferase CeFUT1 (20 µL, 0.9 mg/mL), and GDP-Fuc (272 µg, 0.43 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.13 (d, *J* = 3.7 Hz, 1H, H-1_{Fuc}), 5.12 (s, 1H, H-$1_{\alpha Man}$), 4.91 (s, 1H, H-$1_{\alpha Man}$), 4.85 (s, 1H, H-$1_{\beta Man}$), 4.72-4.70 (m, 1H, H-5_{Fuc}), 4.54 (d, *J* = 8.3 Hz, 1H), 4.49-4.46 (m, 2H), 4.26 (d, *J* = 3.1 Hz, 1H, H-$2_{Man\beta}$), 4.04-4.03 (m, 1H, H-$2_{Man\alpha}$), 4.03-3.44 (m, 36H), 3.39-3.37 (m, 1H), 3.26 (t, *J* = 11.0 Hz, 1H), 2.98 (t, *J* = 7.8 Hz, 2H, linker), 2.05, 2.02 (2xs, 6H, NH*Ac*), 1.69-1.65 (m, 2H, linker), 1.59-1.57 (m, 2H, linker), 1.41-1.38 (m, 2H, linker), 1.27 (d, *J* = 6.7 Hz, 3H, CH_{3Fuc}); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ:** 106.4, 103.7, 102.4, 101.9, 101.8, 101.1, 99.7, 82.1, 80.6, 78.7, 76.7, 76.7, 75.9, 75.9, 75.7, 75.2, 74.9, 74.7, 74.0, 73.7, 73.4, 73.1, 71.7, 71.7, 71.3, 71.2, 70.7, 70.5, 69.1, 68.2, 68.1, 67.9, 66.6, 66.4, 62.6, 62.4, 62.3, 62.3, 61.7, 57.0, 56.5, 40.7, 29.5, 27.8, 23.6, 23.5, 23.6, 16.8; **HRMS (MALDI-tof) *m/z*** $[M+Na]^+$ calculated for $C_{50}H_{87}N_3O_{34}$ 1296.5063, found 1296.512.

Heptasaccharide G38: Following the general procedure D, compound **G38** (0.57 mg, 99%) was prepared from **G35** (0.46 mg, 0.41 µmol) using core α-1,3-fucosyltransferase CeFUT1 (20 µL, 0.9 mg/mL) and GDP-Fuc (310 µg, 0.49 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.13 (d, *J* = 3.8 Hz, 1H, H-$1_{Fucα1,3}$), 5.12 (s, 1H, H-$1_{αMan}$), 4.93 (d, *J* = 3.8 Hz, 1H, H-$1_{Fucα1,6}$), 4.91 (s, 1H, H-$1_{αMan}$), 4.85 (s, 1H. H-$1_{βMan}$), 4.73-4.71 (m, 1H, H-$5_{Fucα1,3}$), 4.69 (d, *J* = 8.6 Hz, 1H), 4.48 (d, *J* = 8.9 Hz, 1H), 4.47 (d, *J* = 7.6 Hz, 1H), 4.26 (d, *J* = 3.1 Hz, 1H, H-$2_{Manβ}$), 4.15 (q, *J* = 6.8 Hz, 1H, H-$5_{Fucα1,6}$), 4.04 (dd, *J* = 3.4, 1.4 Hz, 1H, H-$2_{Manα}$), 3.99-3.55 (m, 37H), 3.49 (t, *J* = 9.2 Hz, 1H), 3.46 (t, *J* = 8.9 Hz, 1H), 3.38 (dd, *J* = 9.4, 7.5 Hz, 1H), 3.27 (t, *J* = 11.3 Hz, 1H), 2.98 (t, *J* = 7.9 Hz, 2H, linker), 2.06, 2.02 (2xs, 6H, NH*Ac*), 1.68-1.64 (m, 2H, linker), 1.60-1.57 (m, 2H, linker), 1.40-1.36 (m, 2H, linker), 1.28 (d, *J* = 6.6 Hz, 3H, $CH_{3Fucα1,3}$), 1.23 (d, *J* = 6.6 Hz, 3H, $CH_{3Fucα1,6}$); **^{13}C NMR(201 MHz, D2O, peaks from HSQC experiment) δ:** 104.5, 101.9, 100.6, 99.6, 99.6, 98.0, 97.9, 79.6, 78.7, 76.8, 75.8, 74.8, 74.3, 74.2, 73.6, 73.0, 72.9, 71.5, 71.3, 69.8, 69.6, 69.5, 69.5, 69.1, 68.7, 67.6, 66.5, 66.4, 65.7, 65.3, 64.9, 64.4, 64.4, 60.7, 59.9, 59.9, 58.7, 56.7, 55.3, 55.0, 38.9, 27.7, 26.1, 21.8, 21.7, 21.7, 15.1, 14.9; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{50}H_{88}N_3O_{33}$ 1258.5295, found 1258.5330.

Octasaccharide G39: Following the general procedure D, compound **G39** (0.36 mg, 99%) was prepared from **G36** (0.32 mg, 0.26 µmol) using core α-1,3-fucosyltransferase CeFUT1 (20 µL, 0.9 mg/mL) and GDP-Fuc (190 µg, 0.31 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.13 (d, *J* = 3.8 Hz, 1H, H-$1_{Fucα-1,3}$), 5.12 (s, 1H, H-$1_{αMan}$), 4.93 (d, *J* = 3.8 Hz, 1H, H-$1_{Fucα1,6}$), 4.91 (s, 1H, H-$1_{αMan}$), 4.85 (s, 1H. H-$1_{βMan}$), 4.73-4.71 (m, 1H, H-$5_{Fuc1,3}$), 4.69 (d, *J* = 8.6 Hz, 1H), 4.48 (d, *J* = 8.9 Hz, 1H), 4.47 (d, *J* = 7.6 Hz, 1H), 4.26 (d, *J* = 3.1 Hz, 1H, H-$2_{Manβ}$), 4.15 (q, *J* = 6.8 Hz, 1H, H-$5_{Fucα1,6}$), 4.04 (dd, *J* = 3.4, 1.4 Hz, 1H, H-$2_{Manα}$), 3.99-3.55(m, 37H), 3.49 (t, *J* = 9.2 Hz, 1H), 3.46 (t, *J* = 8.9 Hz, 1H), 3.38 (dd, *J* = 9.4, 7.5 Hz, 1H), 3.27 (t, *J* = 11.3 Hz, 1H), 2.98 (t, *J* = 7.9 Hz, 2H, linker), 2.06, 2.02 (2xs, 6H, NH*Ac*), 1.68-1.64 (m, 2H, linker), 1.60-1.57 (m, 2H, linker), 1.40-1.36 (m, 2H, linker), 1.28 (d, *J* = 6.6 Hz, 3H, $CH_{3Fucα1,3}$), 1.23 (d, *J* = 6.6 Hz, 3H, $CH_{3Fucα1,6}$); **^{13}C NMR(201 MHz, D_2O) δ:** 106.5, 103.6, 102.4, 102.0, 101.4, 101.1, 100.34, 99.71, 82.3, 80.8, 78.6, 76.6, 76.0, 75.9, 75.8, 75.4, 74.9, 74.8, 74.7, 74.1, 73.7, 73.3, 71.8, 71.3, 71.3, 71.2, 70.9, 70.9, 70.6, 70.5, 69.4, 68.2, 68.2, 68.1, 67.8, 67.5, 66.8, 66.8, 66.3, 62.6, 62.6, 62.4, 62.3, 62.3, 56.9, 56.4, 40.7, 29.5, 27.8, 23.6, 23.6, 23.5, 16.9, 16.7; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{56}H_{98}N_3O_{38}$ 1420.5823, found 1420.5786.

Nonasaccharide G63: Following the general procedure E, compound **G63** (1.30 mg, 99%) was prepared from **G50** (1.07 mg, 0.76 µmol) using concentrated extract of *P. pastoris* containing GalNacT (260 µL) and UDP-GalNAc (1.88 mg, 2.89 µmol); **^{1}H NMR (800 MHz, D_2O) δ** 5.11 (s, 1H, H-$1_{αMan}$), 4.92 (s, 1H, H-$1_{αMan}$), 4.76 (s, 1H, H-$1_{βMan}$), 4.60 (d, *J* = 8.2 Hz, 1H), 4.57-4.55 (m, 2H), 4.52 (dd, *J* = 8.9, 6.7 Hz, 2H), 4.50 (d, *J* = 7.9 Hz, 1H), 4.24 (d, *J* = 2.9 Hz, 1H, H-2_{Man}), 4.18 (dd, *J* = 3.5, 1.6 Hz, 1H, H-2_{Man}), 4.09 (dd, *J* = 3.5, 1.6 Hz, 1H), 3.95-3.57 (m, 47H), 3.51-3.46 (m, 6H), 2.98 (t, *J* =7.7 Hz, 2H, linker), 2.08, 2.07, 2.07, 2.05, 2.04, 2.03 (6xs, 18H, NH*Ac*), 1.69-1.65 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.40-1.38 (m, 2H, linker); **^{13}C NMR (201 MHz, D_2O) δ:** 104.4, 104.4, 104.0, 103.7, 103.1, 102.2, 102.1, 102.0 99.7, 83.1, 82.1, 82.0, 81.9, 81.9, 79.0, 79.0, 78.0, 77.9, 77.5, 77.1, 77.0, 76.2, 75.4, 75.2, 75.0, 74.7, 73.3, 73.2, 73.0, 72.8, 72.8, 72.7, 72.7, 72.1, 72.1, 70.2, 69.6, 69.5, 68.4, 68.3, 64.3, 64.3, 63.7, 63.6, 63.6, 62.7, 62.7, 62.6, 62.6, 57.6, 57.6, 57.2, 57.2, 55.2, 55.1, 41.9, 30.7, 29.0, 24.9, 24.9, 24.8, 24.8, 24.8, 24.8, 24.7; **HRMS (ESI) *m/z*** $[M+H]^+$ calculated for $C_{71}H_{122}N_7O_{46}$ 1808.7417, found 1808.7428.

Hexasaccharide G64: Following the general procedure F, compound **G64** (300 µg, 93%) was prepared from **G66** (377 µg, 0.28 µmol) using 50 mU of jack bean β-N-acetylglucosaminidase; **^{1}H NMR (500 MHz, D_2O) δ** 5.12 (d, *J* = 1.7 Hz, 1H), 4.93 (d, *J* = 1.6 Hz, 1H), 4.91 (d, *J* = 4.0 Hz, 1H), 4.68 (d, *J* = 7.9 Hz, 1H), 4.51 (d, *J* = 8.1 Hz, 1H), 4.27 (d, *J* = 1.9 Hz, 1H), 4.14 (q, *J* = 6.8 Hz, 1H), 4.08 (dd, *J* = 3.4, 1.5 Hz, 1H), 3.98 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.97-3.56 (m, 30H), 3.05-2.94 (m, 2H, linker), 2.11 (s, 3H, NH*Ac*), 2.04 (s, 3H, NH*Ac*), 1.73-1.55 (m, 4H, linker), 1.47-1.35 (m, 2H, linker), 1.24 (d, *J* = 6.6 Hz, 3H); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{45}H_{79}N_3NaO_{30}$ 1164.4646, found 1164.4664.

Heptasaccharide G66: Following the general procedure D, compound **G66** (580 µg, 86%) was prepared from **G49** (700 µg, 0.58 µmol) using core α-1,6-fucosyltransferase AgFUT6 (16.5 µg, 1.05 mg/mL) and GDP-Fuc (380 µg, 0.60 µmol)**; ^{1}H NMR (500 MHz, D_2O) δ** 5.12 (s, 1H), 4.92 (d, *J* = 1.5 Hz, 1H), 4.90 (d, *J* = 3.7 Hz, 1H), 4.67 (d, *J* = 8.0 Hz, 1H), 4.56 (d, *J* = 8.5 Hz, 1H), 4.50 (d, *J* = 7.9 Hz, 1H), 4.26 (d, *J* = 1.9 Hz, 1H), 4.19 (dd, *J* = 3.3, 1.4 Hz, 1H), 4.13 (q, *J* = 6.6 Hz, 1H), 3.98 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.96-3.40 (m), 3.02-2.93 (m, 2H, linker), 2.10 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 1.74-1.53 (m, 4H), 1.47-1.34 (m, 2H), 1.23 (d, *J* = 6.6 Hz, 3H); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{53}H_{92}N_4NaO_{35}$ 1367.5440, found 1367.5493.

Octasaccharide G68: Following the general procedure D, compound **G68** (750 µg, 79%) was prepared from **G57** (860 µg, 0.63 µmol) using core α-1,6-fucosyltransferase AgFUT6 (16.5 µg, 1.05 mg/mL) and GDP-Fuc (437 µg, 0.69 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.12 (s, 1H), 4.95-4.88 (m, 2H), 4.67 (d, *J* = 8.1 Hz, 1H), 4.56 (d, *J* = 8.4 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.26 (s, 1H), 4.20 (d, *J* = 3.1 Hz, 1H), 4.12 (q, *J* = 6.8 Hz, 1H), 4.06-3.41 (m, 16H), 3.05-2.91 (m, 2H, linker), 2.09 (s, 3H, NH*Ac*), 2.06 (s, 3H, NH*Ac*), 2.03 (s, 3H, NH*Ac*), 1.74-1.51 (m, 4H, linker), 1.47-1.31 (m, 2H, linker), 1.23 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc1\alpha,6}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{59}H_{102}N_4NaO_{40}$ 1529.5968, found 1529.5904.

Octasaccharide G69: Following the general procedure D, compound **G69** (800 µg, 91%) was prepared from **G56** (788 µg, 0.58 µmol) using core α-1,6-fucosyltransferase AgFUT6 (16.5 µg, 1.05 mg/mL) and GDP-Fuc (405 µg, 0.64 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.13 (d, *J* = 0.6 Hz, 1H), 5.11 (d, *J* = 1.2 Hz, 1H), 4.95-4.87 (m, 2H), 4.67 (d, *J* = 8.1 Hz, 1H), 4.57 (d, *J* = 8.4 Hz, 1H), 4.50 (d, *J* = 7.9 Hz, 1H), 4.26 (d, *J* = 0.6 Hz, 1H), 4.20 (s, 1H), 4.15 (dd, *J* = 3.1, 1.9 Hz, 1H), 4.08 (dd, *J* = 3.4, 1.4 Hz, 1H), 4.04-3.39 (m, 21H), 3.02-2.96 (m, 2H, linker), 2.09 (s, 3H, NH*Ac*), 2.07 (s, 3H, NH*Ac*), 2.04 (s, 3H, NH*Ac*), 1.75-1.52 (m, 4H, linker), 1.47-1.34 (m, 2H, linker), 1.24 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha1,6}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{59}H_{102}N_4NaO_{40}$ 1529.5968, found 1529.5942.

Heptasaccharide G71: Following the general procedure D, compound **G71** (650 µg, 99%) was prepared from **G47** (600 µg, 0.48 µmol) using core α-1,6-fucosyltransferase AgFUT6 (16.5 µg, 1.05 mg/mL) and GDP-Fuc (336 µg, 0.53 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.13 (s, 1H), 4.92 (d, *J* = 3.5 Hz, 1H), 4.67 (d, *J* = 7.5 Hz, 3H), 4.57-4.48 (m, 3H), 4.25-4.19 (m, 2H), 4.13 (q, *J* = 6.4 Hz, 1H), 4.05 (dd, *J* = 9.2, 3.2 Hz, 1H), 3.98-3.39 (m, 45H), 3.04-2.94 (m, 2H, linker), 2.09 (s, 3H), 2.09 (s, 3H, NH*Ac*), 2.06 (s, 3H, NH*Ac*), 2.04 (s, 3H, NH*Ac*), 1.71-1.56 (m, 4H, linker), 1.47-1.36 (m, 2H, linker), 1.24 (d, *J* = 6.5 Hz, 3H, $CH_{3Fuc\alpha1,6}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{55}H_{95}N_5NaO_{35}$ 1408.5705, found 1408.5769.

Pentasaccharide G72: Following the general procedure D, compound **G72** (1.44 mg, 1.47 µmol, 98%) was prepared from **G41** (1.27 mg, 1.50 µmol) using core α-1,3-fucosyltransferase CeFUT1 (27 µg), and GDP-Fuc (1.0 mg, 1.7 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.11 (d, *J* = 3.5 Hz, 1H), 5.08 (s, 1H), 4.77 (s, 1H), 4.72 (dd, *J* = 10.9, 5.2 Hz, 1H), 4.58 (d, *J* = 7.1 Hz, 1H), 4.53 (d, *J* = 7.0 Hz, 1H), 4.30 (d, *J* = 2.6 Hz, 1H), 4.16 (dd, *J* = 2.9, 1.4 Hz, 1H), 4.10-3.58 (m), 3.23-3.15 (m, 2H), 2.36 (s, 3H, NH*Ac*), 2.34 (s, 3H, NH*Ac*), 2.09-1.87 (m, 4H, linker), 1.84-1.72 (m, 2H, linker), 1.67 (d, *J* = 5.9 Hz, 3H, $CH_{3Fuc\alpha1\text{-}3}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{39}H_{69}N_3NaO_{25}$ 1002.4118, found 1002.4063.

Pentasaccharide G73: Following the general procedure D, compound **G73** (365 µg, 0.32 µmol, 99%) was prepared from **G42** (320 µg, 0.32 µmol) using core α-1,3-fucosyltransferase (CeFUT1, 15 µg) and GDP-Fuc (222 µg, 0.35 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.14 (d, *J* = 4.0 Hz, 1H), 5.10 (d, *J* = 1.0 Hz, 1H), 4.54 (d, *J* = 7.6 Hz, 1H), 4.49 (d, *J* = 7.9 Hz, 2H), 4.26 (d, *J* = 1.4 Hz, 1H), 4.07 (s, 1H), 4.02-3.41 (m), 3.05-2.92 (m, 2H, linker), 2.06 (s, 3H, NH*Ac*), 2.02 (s, 3H, NH*Ac*), 1.74-1.51 (m, 4H, linker), 1.48-1.35 (m, 2H, linker), 1.28 (d, *J* = 6.3 Hz, 3H, $CH_{3Fuc\alpha1\text{-}3}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{45}H_{79}N_3NaO_{30}$ 1164.4646, found 1164.4701.

Nonasaccharide G74: Following the general procedure F, compound **G74** (340 µg, 84%) was prepared from **G77** (453 µg, 0.25 µmol) using 50 mU of jack bean β-N-acetylglucosaminidase; **^{1}H NMR (500 MHz, D_2O) δ** 5.15 (d, *J* = 3.7 Hz, 1H), 5.11 (s, 1H), 5.08 (s, 1H), 4.94 (d, *J* = 3.8 Hz, 1H), 4.92 (s, 1H), 4.88 (s, 2H), 4.67 (d, *J* = 8.0 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.27 (d, *J* = 2.7 Hz, 1H), 4.20-4.13 (m, 1H), 4.12-3.53 (m, 50H), 3.04-2.95 (m, 2H, linker), 2.07 (s, 3H, NH*Ac*), 2.03 (s, 3H, NH*Ac*), 1.73-1.55 (m, 4H, linker), 1.45-1.36 (m, 2H, linker), 1.30 (d, *J* = 6.7 Hz, 3H, $CH_{3Fuc\alpha1,3}$), 1.24 (d, *J* = 6.5 Hz, 3H, $CH_{3Fuc\alpha1,6}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{63}H_{109}N_3NaO_{44}$ 1634.6282, found 1634.6301.

Heptasaccharide G75: Following the general procedure D, compound **G75** (190 µg, 99%)was prepared from **G64** (171 µg, 0.15 µmol) using core α-1,3-fucosyltransferase (CeFUT1, 18 µg) and GDP-Fuc (101 µg, 0.16 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.14 (d, *J* = 3.8 Hz, 1H), 5.11 (s, 1H), 4.97-4.91 (m, 2H), 4.55 (d, *J* = 7.3 Hz, 1H), 4.49 (d, *J* = 8.1 Hz, 1H), 4.26 (d, *J* = 0.8 Hz, 1H), 4.15 (dd, *J* = 13.0, 6.6 Hz, 1H), 4.09-4.06 (m, 1H), 4.05-3.53 (m, 40H), 3.48-3.42 (m, 1H), 2.07 (s, 3H, NH*Ac*), 2.02 (s, 3H, NH*Ac*), 1.75-1.52 (m, 4H, linker), 1.48-1.35 (m, 2H, linker), 1.29 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha1,3}$), 1.23 (d, *J* = 6.7 Hz, 3H, $CH_{3Fuc\alpha1,6}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{51}H_{89}N_3NaO_{34}$ 1310.5225, found 1310.5254.

Nonasaccharide G76: Following the general procedure D, compound **G76** (410 µg, 98%) was prepared from **G68** (376 µg, 0.25 µmol) using core α-1,3-fucosyltransferase AtFucTA (11 µg) and GDP-Fuc (171 µg, 0.27 µmol) ; **^{1}H NMR (500 MHz, D_2O) δ** 5.15-5.13 (m, 2H), 4.94 (d, *J* = 3.5 Hz, 1H), 4.92 (s, 1H), 4.91 (s, 1H), 4.69 (d, *J* = 7.8 Hz, 1H), 4.57 (d, *J* = 8.2 Hz, 1H), 4.50 (d, *J* = 7.5 Hz, 1H), 4.27 (s, 1H), 4.24-4.19 (q, *J* = 6.6 Hz, 1H), 4.19-4.12 (m, 1H), 4.07-3.41 (m, 26H), 3.07-2.93 (m, 2H, linker), 2.08 (s, 3H, NH*Ac*), 2.07 (s, 3H, NH*Ac*), 2.03 (s, 3H, NH*Ac*), 1.73-1.54 (m, 4H, linker), 1.46-1.36 (m, 4H, linker), 1.29 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha1,3}$), 1.24 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha1,6}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{65}H_{112}N_4NaO_{44}$ 1675.6547, found 1675.6625.

Decasaccharide G77: Following the general procedure D, compound **G77** (640 µg, 77%) was prepared from **G55** (700 µg, 0.46 µmol) using core α-1,6-fucosyltransferase AgFUT6 (16.5 µg, 1.05 mg/mL), and GDP-Fuc (316 µg, 0.50 µmol). After complete conversion of starting material GDP-Fuc (316 µg, 0.50 µmol) and AtFucTA (16 µg, 0.37 mg/mL) were added and the resulting mixture was incubated at room temperature for 48 hours; **^{1}H NMR (500 MHz, D_2O) δ** 5.14 (d, *J* = 4.1 Hz, 1H), 5.12 (s, 1H), 5.07 (d, *J* = 1.2 Hz, 1H), 4.93 (d, *J* = 3.8 Hz, 1H), 4.91 (s, 1H), 4.87 (s, 1H), 4.66 (d, *J* = 7.0 Hz, 1H), 4.57-4.55 (m, 2H), 4.49 (d, *J* = 7.9 Hz, 1H), 4.25-4.24 (m, 1H), 4.21-4.20 (m, 2H), 4.15-4.14 (m, 2H), 4.30-3.33 (m, 78H), 3.00-2.96 (m, 2H), 2.06 (s, 3H, NH*Ac*), 2.06 (s, 3H, NH*Ac*), 2.02 (s, 3H, NH*Ac*), 1.75-1.53 (m, 4H, linker), 1.44-1.36 (m, 2H, linker), 1.29 (d, *J* = 6.5 Hz, 3H, $CH_{3Fuc\alpha1\text{-}3}$), 1.23 (d, *J* = 6.5 Hz, 3H, $CH_{3Fuc\alpha1\text{-}6}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{71}H_{122}N_4NaO_{49}$ 1837.7075, found 1837.7139.

Nonasaccharide G78: Following the general procedure D, compound **G78** (400 µg, 92%) was prepared from **G69** (376 µg, 0.25 µmol) using core α-1,3-fucosyltransferase AtFucTA (11 µg) and GDP-Fuc (171 µg, 0.27 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.15-5.12 (m, 2H), 5.09 (s, 1H), 4.94 (d, *J* = 3.7 Hz, 1H), 4.90 (s, 1H), 4.67 (d, *J* = 8.0 Hz, 1H), 4.57 (d, *J* = 8.2 Hz, 1H), 4.50 (d, *J* = 7.8 Hz, 1H), 4.26 (d, *J* = 2.6 Hz, 1H), 4.21 (d, *J* = 2.4 Hz, 1H), 4.17-4.13 (m, 2H), 4.11-3.42 (m, 74H), 3.05-2.95 (m, 2H, linker), 2.07 (s, 6H, NH*Ac*), 2.03 (s, 3H, NH*Ac*), 1.77-1.51 (m, 4H, linker), 1.49-1.34 (m, 2H, linker), 1.30 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha1,3}$), 1.24 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha1,6}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{65}H_{112}N_4NaO_{44}$ 1675.6547, found 1675.6541.

Octasaccharide G79: Following the general procedure D, compound **G79** (355 µg, 92%) was prepared from **G71** (346 µg, 0.25 µmol) using core α-1,3-fucosyltransferase (AtFucTA, 11 µg) and GDP-Fuc (171 µg, 0.27 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.16-5.10 (m, 2H), 4.95 (d, *J* = 3.8 Hz, 1H), 4.68 (d, *J* = 8.3 Hz, 1H), 4.57-4.52 (m, 2H), 4.50 (d, *J* = 7.8 Hz, 1H), 4.24-4.19 (m, 2H), 4.19-4.13 (m, 1H), 4.09-3.42 (m, 19H), 3.05-2.96 (m, 2H), 2.10 (s, 3H, NH*Ac*), 2.07 (s, 3H, NH*Ac*), 2.06 (s, 3H, NH*Ac*), 2.03 (s, 3H, NH*Ac*), 1.73-1.54 (m, 4H, linker), 1.46-1.33 (m, 2H, linker), 1.28 (d, *J* = 6.6 Hz, 3H, $CH_{3Fu\alpha c1,3}$), 1.24 (d, *J* = 6.5 Hz, 3H, $CH_{3Fuc\alpha1,6}$); **HRMS (MALDI-tof)** ***m/z*** $[M+Na]^+$ calculated for $C_{61}H_{105}N_5NaO_{39}$ 1554.6284, found 1554.6315.

Pentasaccharide G81: Following the general procedure D, compound **G81** (950 µg, 91%) was prepared from **G41** (894 µg, 1.07 µmol) using core α-1,3-fucosyltransferase (CeFUT6, 24 µg) and GDP-Fuc (760 µg, 1.20 µmol)**; ^{1}H NMR (500 MHz, D_2O) δ** 5.22 (d, *J* = 3.9 Hz, 1H), 5.09 (d, *J* = 1.2 Hz, 1H), 4.71 (s, 1H), 4.66 (q, *J* = 6.6 Hz, 1H), 4.62 (d, *J* = 7.9 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.21 (d, *J* = 3.3 Hz, 1H), 4.08 (dd, *J* = 3.4, 1.6 Hz, 1H), 4.06-3.56 (m, 10H), 3.54-3.48 (m, 1H), 3.44-3.37 (m, 1H), 3.04-2.95 (m, 2H, linker), 2.07 (s, 3H, NH*Ac*), 2.04 (s, 3H, NH*Ac*), 1.75-1.53 (m, 4H, linker), 1.45-1.35 (m, 2H, linker), 1.17 (d, *J* = 6.5 Hz, 3H, $CH_{3Fuc\alpha1,3}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{39}H_{69}N_3NaO_{25}$ 1002.4118, found 1002.4061.

Hexasaccharide G84: Following the general procedure D, compound **G84** (510 µg, 96%) was prepared from **G72** (461 µg, 0.47 µmol) using α-1,3-fucosyltransferase (CeFUT6, 16 µg) and GDP-Fuc (317 µg, 0.50 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.23 (d, *J* = 3.8 Hz, 1H), 5.16 (d, *J* = 3.9 Hz, 1H), 5.09 (s, 1H), 4.69 (s, 3H), 4.66 (dd, *J* = 13.8, 6.5 Hz, 3H), 4.53 (d, *J* = 8.1 Hz, 1H), 4.50 (d, *J* = 7.7 Hz, 1H), 4.21 (d, *J* = 3.1 Hz, 1H), 4.13-3.48 (m, 28H), 3.45-3.36 (m, 1H), 3.04-2.93 (m, 2H, linker), 2.05 (s, 3H, NH*Ac*), 2.03 (s, 3H, NH*Ac*), 1.76-1.50 (m, 4H, linker), 1.47-1.33 (m, 2H,

linker), 1.28 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha1,3}$), 1.17 (d, *J* = 6.6 Hz, 3H, $CH_{3Fuc\alpha1,6}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{45}H_{79}N_3NaO_{29}$ 1148.4697, found 1148.4689.

Dodecasaccharide G87: Following the general procedure C, compound **G87** (1.25 mg, 89%) was prepared from **G80** (1.18 mg, 0.67 µmol) using 0.72U of bovine milk β-1,4-galactosyltransferase and UDP-Gal (1.63 mg, 2.67 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.15 (s, 1H, H-$1_{\alpha Man}$), 5.13 (d, *J* = 3.3 Hz, 1H, H-$1_{Fuc\alpha1-3}$), 4.94 (d, *J* = 4.0 Hz, 1H, H-$1_{Fuc\alpha1-6}$), 4.92 (s, 1H, H-$1_{\alpha Man}$), 4.76-4.73 (m, 2H, H-$1_{\beta Man}$, H-$5_{Fuc\alpha1-3}$), 4.70-4.66 (d, *J* = 8.2 Hz, 1H), 4.60-4.58 (m, 2H), 4.51-4.46 (m, 3H), 4.26-4.25 (d, *J* = 2.8 Hz, 1H), 4.21-4.20 (m, 1H), 4.15-4.14 (m, 1H, H-$5_{Fuc1\alpha-6}$), 4.13-4.11 (m, 1H), 4.04-3.46 (m, 55H), 2.99 (t, *J*=7.7 Hz, 2H, linker), 2.09-2.07 (s, 4H), 2.08, 2.06, 2.06, 2.03 (4xs, 12H, NH*Ac*), 1.71-1.65 (m, 2H, linker), 1.61-1.56 (m, 2H, linker), 1.43-1.38 (m, linker), 1.29 (d, *J* = 6.7 Hz, 3H, $CH_{3Fuc\alpha1-3}$), 1.24 (d, *J* = 6.7 Hz, 3H, $CH_{3Fuc\alpha1-6}$); **^{13}C NMR (126 MHz, D_2O) δ** 15.6, 15.7, 22.3, 28.1, 26.5, 22.6, 22.3, 22.4, 22.3, 39.2, 80.8, 67.4, 74.4, 70.9, 74.6, 70.0, 74.8, 72.9, 72.9, 61.5, 72.4, 60.7, 69.5, 67.6, 78.6, 75.3, 78.4, 72.0, 54.9, 80.4, 54.9, 73.5, 66.3, 61.1, 67.9, 71.9, 59.9, 74.4, 55.7, 61.6, 68.5, 61.5, 69.2, 61.6, 61.3, 69.2, 68.8, 66.0, 60.0, 73.3, 76.2, 66.8, 76.3, 70.2, 102.8, 102.8, 101.2, 99.3, 99.3, 100.1, 66.7, 100.4, 96.9, 98.8, 99.5, 98.4; **HRMS (MALDI-tof)** ***m/z*** $[M+Na]^+$ calculated for $C_{79}H_{135}N_5O_{54}$ 2240.7864, found 2240.7828.

Dodecasaccharide G88: Following the general procedure C, compound **G88** (0.79 mg, 81%) was prepared from the G67 (0.54 mg, 0.348 µmol) using 0.15U of bovine milk β-1,4-galactosyltransferase and UDP-Gal (0.506 mg, 0.83 µmol). After complete conversion of starting material, GDP-Fuc (696 µg, 1.1 µmol) and α-1,3-fucosyltransferase CeFUT6 (70 µL, 1.07 mg/mL) were added. The resulting mixture was incubated at room temperature for 3 days**; ^{1}H NMR (500 MHz, D_2O) δ** 5.14 (m, 2H), 5.12 (s, 1H), 4.92-4.91 (m, 2H), 4.68– 4.67 (m, 1H), 4.61-4.59 (m, 2H), 4.50 (d, *J*=7.8 Hz, 1H), 4.46 (dd, *J*=7.4 Hz, *J*=2.3 Hz, 2H), 4.27 (broad s, 1H), 4.20-4.19 (m, 1H), 4.14-4.11 (m, 2H), 4.02-3.47 (m), 2.99 (t, *J*=7.7 Hz, 2H, linker), 2.11, 2.06, 2.04 (3xs, 12H, NH*Ac*), 1.71-1.65 (m, 4H, linker), 1.62-1.58 (m, 2H, linker), 1.43-1.38 (m, 2H, linker), 1.24 (d, *J* = 6.6 Hz, 3H, CH_{3Fuc}), 1.18 (2xd, *J*=6.88 Hz, *J*=2.42 Hz, 6H, 2xCH_{3Fuc}); **^{13}C NMR (126 MHz, D_2O) δ** 101.8, 101.8, 101.0, 101.0, 100.6, 99.5, 99.5, 99.2, 99.1, 98.6, 98.6, 97.4, 80.3, 79.8, 76.3, 76.2, 75.1, 74.8, 74.5, 74.4, 73.7, 73.4, 73.2, 72.6, 72.1, 71.8, 71.1, 70.2, 70.1, 70.0, 69.7, 69.2, 69.1, 68.0, 67.7, 67.4, 67.1, 66.7, 66.7, 66.2, 66.1, 62.6, 61.6, 61.6, 61.5, 59.8, 59.8, 55.8, 55.7, 55.1, 54.9, 39.3, 27.8, 26.4, 22.4, 22.3, 22.2, 22.1, 15.4, 15.3, 15.3; **HRMS (MALDI-tof)** ***m/z*** $[M+Na]^+$ calculated for $C_{85}H_{145}N_5O_{58}Na$ 2186.8443, found 2186.8404.

Undecasaccharide G89: Following the general procedure D, compound **G89** (670 µg, 97%) was prepared from **G59** (590 µg, 0.34 µmol) using α-1,3-fucosyltransferase (CeFUT6, 48 µg) and GDP-Fuc (667 µg, 1.05 µmol); **^{1}H NMR (500 MHz, D_2O) δ** 5.10-5.00 (m, 3H), 4.84 (s, 1H), 4.56-4.49 (m, 2H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.40-4.34 (m, 2H), 4.15 (s, 1H), 4.08 (s, 1H), 4.00 (s, 1H), 3.97-3.36 (m), 2.95-2.86 (m, 2H, linker), 2.01 (s, 3H, NH*Ac*), 1.97 (s, 6H, NH*Ac*), 1.95 (s, 3H, NH*Ac*), 1.66-1.44 (m, 4H, linker), 1.39-1.25 (m, 2H, linker), 1.15-1.04 (m, 6H, 2x$CH_{3Fuc\alpha1,3}$); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{79}H_{135}N_5NaO_{54}$ 2040.7869, found 2040.7877.

Undecasaccharide G90: Following the general procedure D, compound **G90** (200 µg, 25%) was prepared from **G63** (670 µg, 0.37 µmol) using core α-1,3-fucosyltransferase CeFUT6 (170 µL, 1.07 mg/mL) and GDP-Fuc (128.9 µL, 2.57 µmol); **HRMS (MALDI-tof)** *m/z* $[M+Na]^+$ calculated for $C_{83}H_{141}N_7O_{54}$ 2122.8395, found 2122.8315. Amount of final product was not enough for a NMR analysis.

6. Microarray Fabrication and analysis

6.1 Immobilization of the ligands and test for biofunctionality of the printed ligands

Ligand solutions were prepared from stock solutions (1 mM in water) by dilution with sodium phosphate buffer (300 mM, pH 8.4, 0.005% Tween 20) to a final concentration of 50 µM. Total amount of 40 µl of each glycans solutions were placed into a 384 well source plate (Scienion, Berlin, Germany) which was stored at -20ºC and reused if necessary. These solutions (1.25 nL, 5 drops of 250 pL) were spatially arrayed onto NHS functionalized glass slides. After printing, the slides were placed in a 75 % humidity chamber (saturated NaCl solution) at 25 ºC for 18 hours. The unreacted NHS groups were quenched by placing the slides in a 50 mM solution of ethanolamine in sodium borate buffer 50 mM, pH 9.0, for 1h. The standard washing of the slides was performed with PBST (PBS solution containing 0.5% Tween 20), PBS and water. The slides were dried in a slide spinner.

<u>18x18 array:</u>

27 N-glycans (Figure S-1) were spotted with a distance of 350 µm. Ligands were printed in 6 replicates (3 different N-glycans/row), establishing the complete 18x18 array which was printed in 14 copies into each slide.

The array was used to predict possible enzymatic elongations of xylosylated compounds (G1-G6) and to compare enzyme specificities with corresponding, non-xylosylated structures.

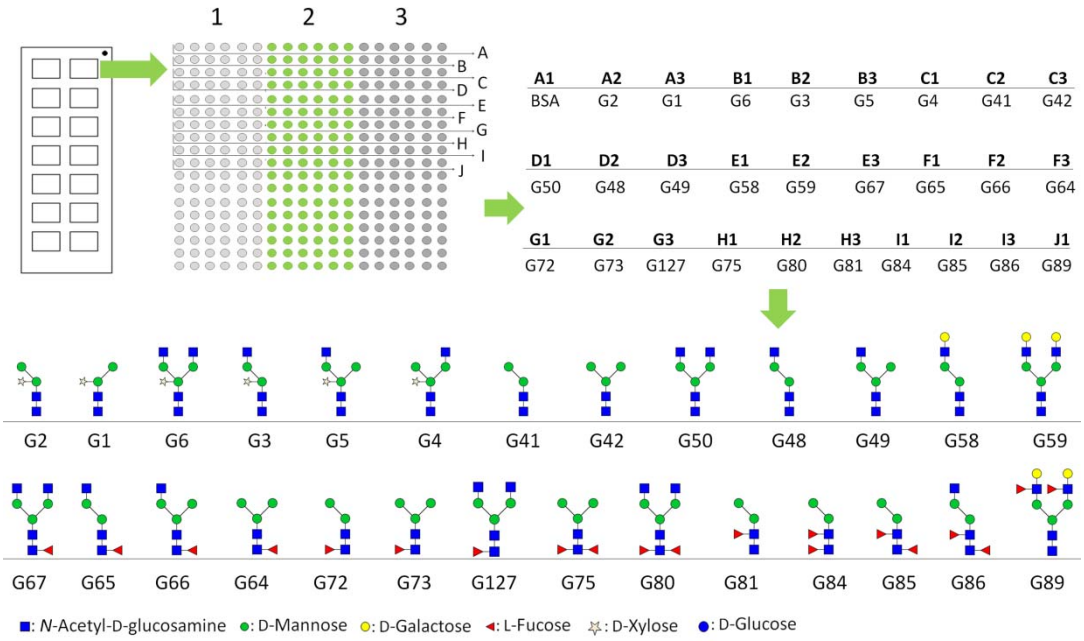


Figure S-1. 27 ligands printed on the NHS activated glycan array (18x18) used for the evaluation of enzyme specificity.

The immobilized glycans were probed with solutions of fluorescently labelled lectins. Solutions of *Aleuria aurantia* lectin, fucose recognition (AAL-555, 50 µg/mL) and *Ricinus communis* agglutinin, galactose recognition (RCA-120, 10 µg/mL) were prepared in PBS, 2 mM $CaCl_2$, 2 mM $MgCl_2$ containing 0.01% Tween-20 (Figure S-2). For incubations, 100 µL of each lectin solution was applied to each microarray using using 16 well Fast Frame® incubation chambers for 1h in the dark at room temperature. The slides were washed under standard conditions, dried with argon and the emitted fluorescence was analyzed with a microarray scanner.

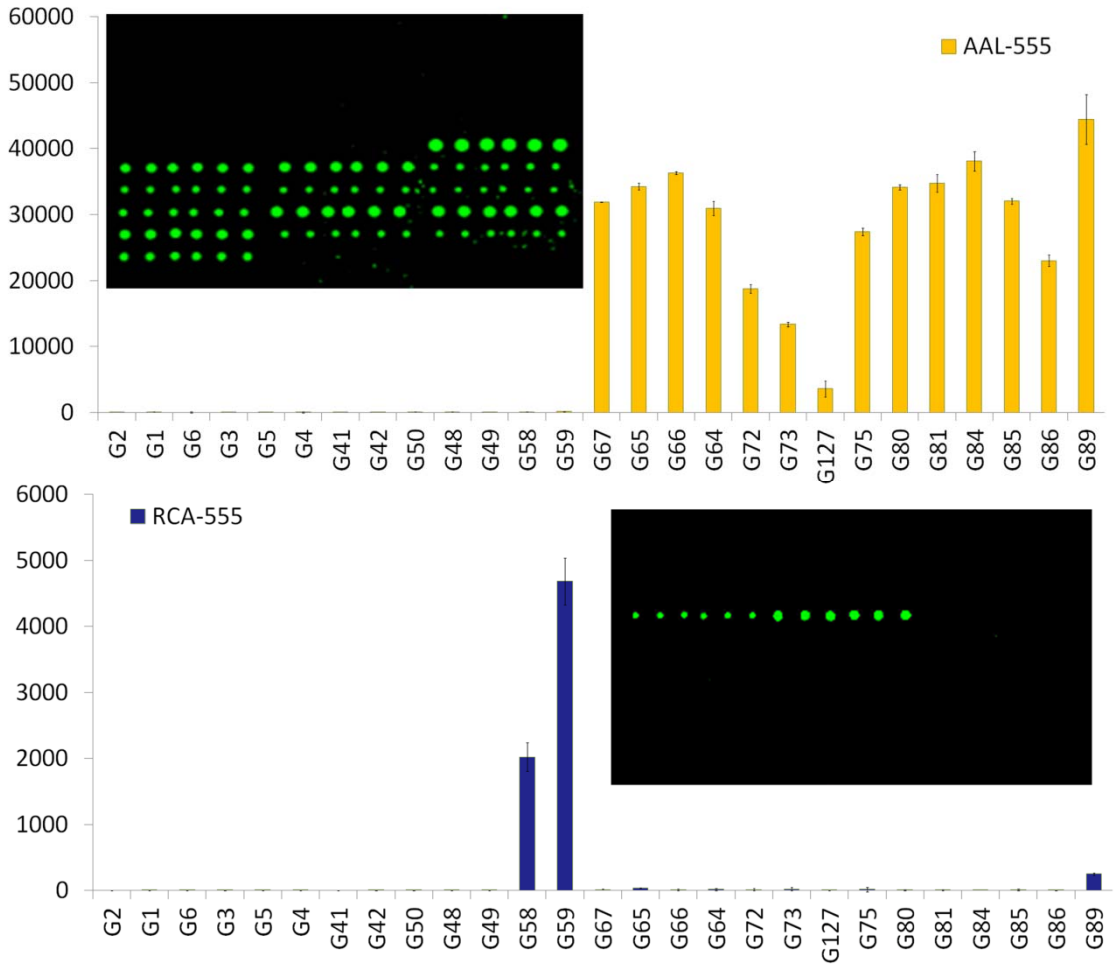


*Figure S-2. Binding profiles for **AAL-555** (orange) and **RCA-555** (blue) on the 27 ligand array.*

6.2 Data analysis

Quantification of spots was performed using an adaptive circle with minimum diameter of 50 µm and maximum diameter of 200 µm. Histograms represents average of mean fluorescence of spots with local background substraction. Protein incubations and enzymatic screening reactions were performed using 16 well Fast Frame® incubation chambers from Whatman, Kent, UK and 8 Well ProPlate Module 7 x 16 mm Well from Electron Microscopy Sciences, Hatfield, UK.

6.3 Enzymatic Extension in well format

The 18x18 glycan microarrays were compartmentalized using 16 well Fast Frame® incubation chambers from Whatman® and treated with the corresponding enzyme containing solutions .

Fucosylation: A solution (100 µL) containing a fucosyltransferase (either AtFucTA, CeFUT6, CeFUT8 or CeFUT1, 10 µL), GDP-Fuc (2 mM), $MnCl_2$ (20 mM) in MES buffer (80 mM, pH 6.5) was added o each well and the slide was incubated at room temperature for 24 hours. The slide was then washed with PBS and water. To test efficient fucosylation one well was incubated with *Aleuria aurantia* lectin (AAL-555, 20 µg/mL) in PBS, 5mM $CaCl_2$, 5 mM $MgCl_2$ containing 0.1% Tween-20, for 1h at room temperature. The slide was washed with PBST, PBS and water, dried under a stream of Ar and analyzed (Figure S-3).

Galactosylation: A solution (100 µL) containing of bovine β-1,4-GalT (10 mU), UDP-Gal (2 mM), $MnCl_2$ (10 mM), and alkaline phosphatase (50 mU) in HEPES buffer (50 mM, pH 7.4) was added into each well and the slide incuabted at 37°C for 24 hours and the slide subsequently washed with PBS and water. Galactosylation was qualitatively evaluated by incubating a well with fluoresecnetly tagged *Ricinus communis* agglutinin (RCA120-555, 1 µg/mL) lectin in PBS, 5mM $CaCl_2$, 5 mM $MgCl_2$ containing 0.1% Tween-20, for 1h at room temperature. The slide was washed with PBST, PBS and water, dried under a stream of Ar and analyzed (Figure S-4)

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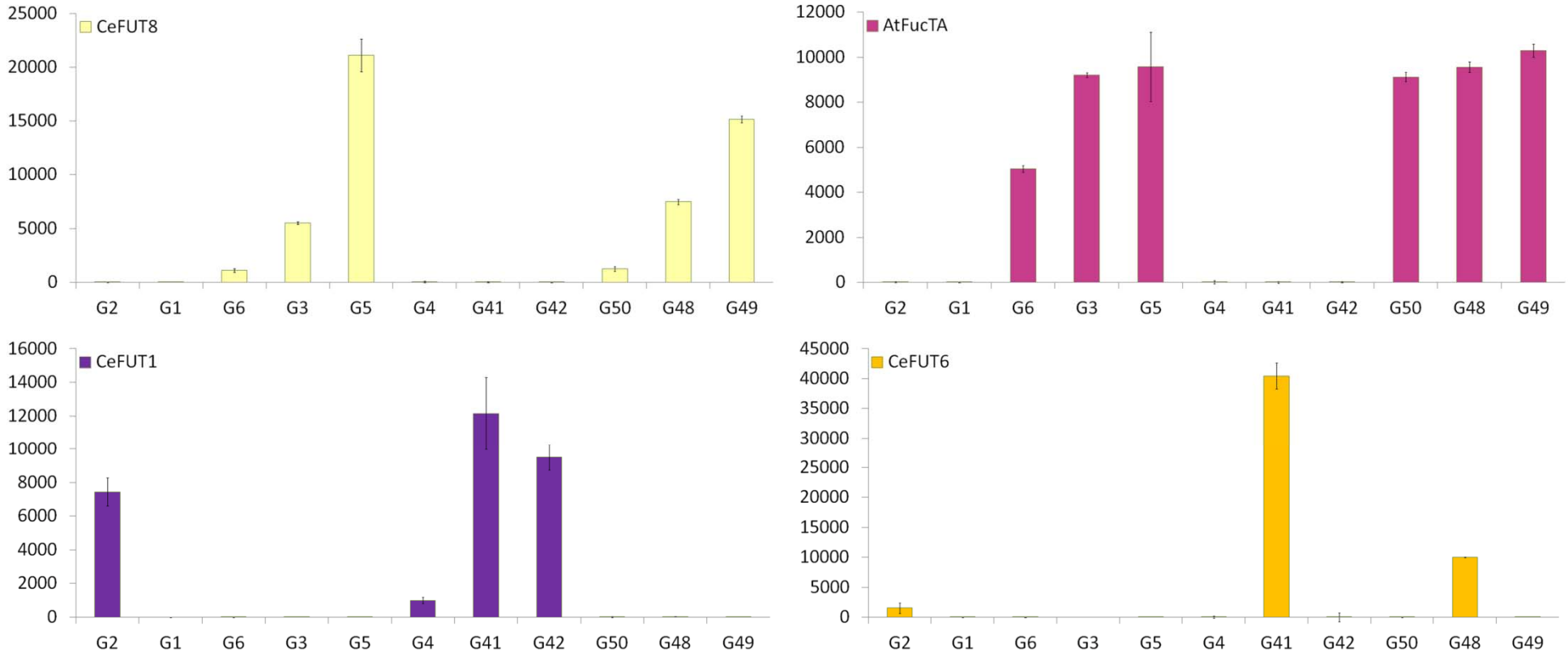


Figure S-3. ***AAL-555*** *binding profile after on chip enzymatic extension (24h of incubation) with four different fucosyltransferases: α-1,6-FucT (yellow bars-CeFUT8) and α-1,3-FucT (pink-AtFucTA, violet-CeFUT1, orange-CeFUT6); each histogram shows the average fluorescence from 6 spots including standard deviation.*

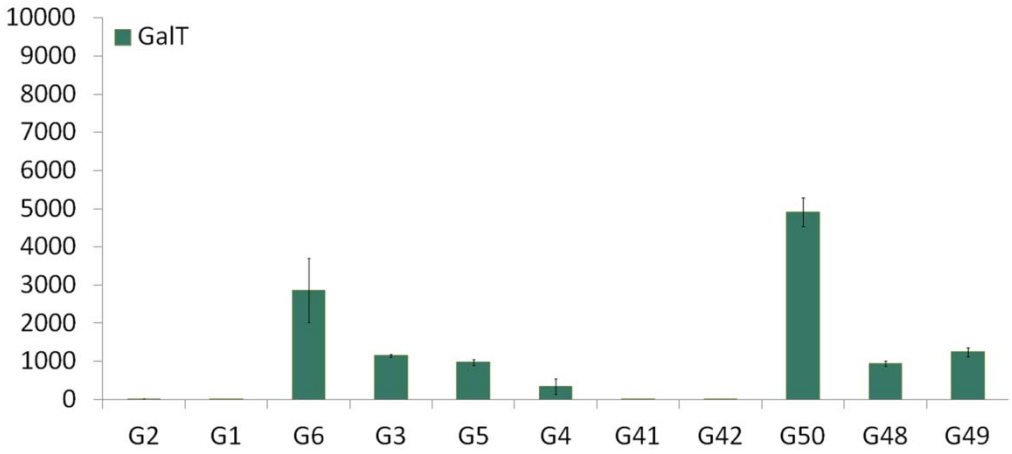


Figure S-4. ***RCA120-555*** *binding profile after on chip enzymatic extension (24 h incubation) with β-1,4-galactosyltransferase; each histogram shows the average fluorescence from 6 spots including standard deviation*

Reaction with β-1,4-N-acetylgalactosaminyltransferase: A solution (100 µL) containing of β-1,4-GalNAcT (20 µL), UDP-GalNAc (2 mM), $MnCl_2$ (20 mM) in HEPES buffer (50 mM, pH 7.4) was added to each well and the slide left at room temperature for 24 hours. The slide was then washed with PBS and water. Incubation of a single well with *Wisteria floribunda* lectin (WFL-555, 10 µg/mL) in in PBS, 5mM $CaCl_2$, 5 mM $MgCl_2$ containing 0.1% Tween-20 for 1h at room temperature, washing and subsequent scanning of the slide showed the successful introduction of GalNAc residues on selected ligands (Figure S-5).

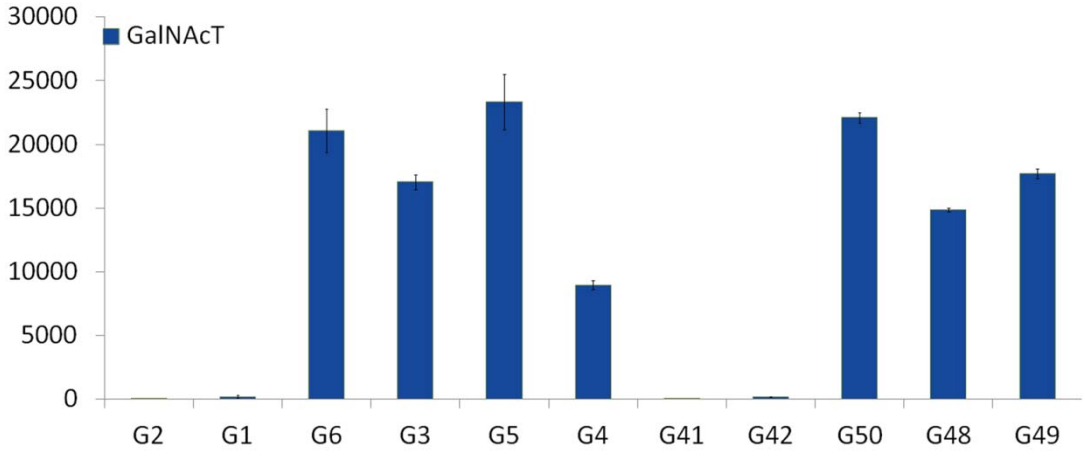


Figure S-5. ***WFL-555*** *binding profile after on chip enzymatic extensions (24h incubation) with β-1,4-N-acetylgalactosaminyltransferase; each histogram shows the average of fluorescence from 6 spots including standard deviation.*

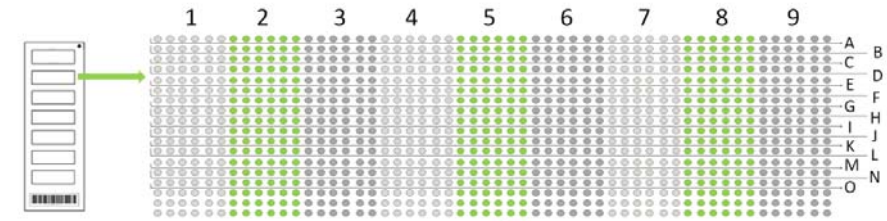
7 Glycan array Screening Experiments

7.1 Interaction with plant lectins

<u>36x18 array:</u>

Volumes (1.25 nL, 5 drops of 250 pL) of the buffered solutions were spotted with a distance of 400 µm. 127 N-glycans were spotted in 4 replicates (9 different N-glycans/row), establishing the complete 36x18 array which was printed in 7 copies into each slide (Figure S-6).

The immobilized glycans were probed with solutions of fluorescently labelled lectins (Figure S-4). Solutions of *Galanthus nivalis* agglutinin (GNA-555, 22 µg/mL), *ConcanavalinA* (ConA-647, 22 µg/mL) (Figure S-7), *Aleuria aurantia* lectin (AAL-555, 20 µg/mL), *Bandeiraea simplicifolia* lectin (BSL-II-647, 24 µg/mL) (Figure S-8),, *Wisteria floribunda* lectin (WFL-555, 10 µg/mL) and *Ricinus communis* agglutinin (RCA-120-555*,* 1 µg/mL) (Figure S-9), were prepared in PBS, 2 mM $CaCl_2$, 2 mM $MgCl_2$ containing 0.01% Tween-20. For incubations, 200 µL of each lectin solution was applied to each microarray using 8 Well ProPlate Module incubation chambers for 1h in the dark at room temperature. The slides were washed under standard conditions, dried with Ar and their fluorescence was analyzed with a microarray scanner.



A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	B4	B5	B6	B7	B8	B9
BSA	G1	G2	G3	G4	G5	G6	G34	G10	G37	G39	G11	G12	G13	G35	G7	G8	G9
C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**
G38	G22	G23	G24	G14	G16	G15	G17	G18	G20	G19	G21	G25	G26	G31	G27	G29	G28
E1	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**	**E8**	**E9**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
G30	G32	G33	G40	G41	G42	G43	G44	G45	G46	G94	G47	G50	G48	G51	G52	G53	G54
G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**	**G9**	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
G55	G49	G56	G57	G58	G59	G60	G61	G62	G63	G67	G70	G65	G66	G68	G69	G64	G71
I1	**I2**	**I3**	**I4**	**I5**	**I6**	**I7**	**I8**	**I9**	**J1**	**J2**	**J3**	**J4**	**J5**	**J6**	**J7**	**J8**	**J9**
G73	G77	G74	G75	G76	G78	G79	G80	G87	G92	G88	G81	G82	G83	G84	G85	G86	G101
K1	**K2**	**K3**	**K4**	**K5**	**K6**	**K7**	**K8**	**K9**	**L1**	**L2**	**L3**	**L4**	**L5**	**L6**	**L7**	**L8**	**L9**
G72	G100	G89	G102	G103	G91	G93	G104	G105	G99	G95	G96	G97	G98	G106	G107	G108	G109
M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**N1**	**N2**	**N3**	**N4**	**N5**	**N6**	**N7**	**N8**	**N9**
G110	G111	G112	G113	G114	G115	G116	G117	G118	G119	G120	G121	G122	G123	G124	G125	G126	G127

O1
G90

Figure S-6. 127 ligands printed on the NHS activated glycan array (36x18) used for screening of GBP's.

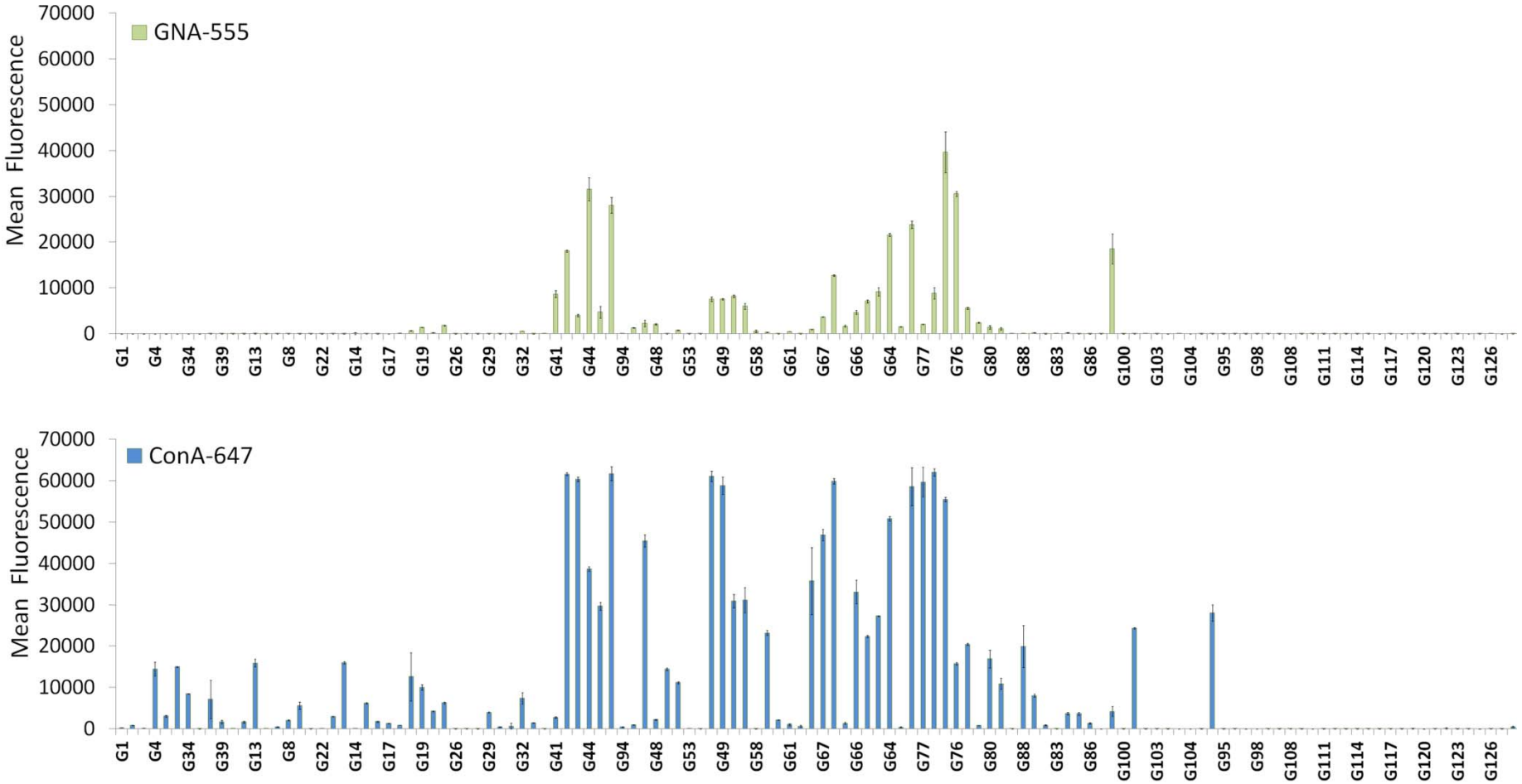


Figure S-7. Fluorescence intensities for binding of mannose recognizing plant lectins: ***GNA-555*** *(green) and* ***ConA-647*** *(blue) on large glycan array.*

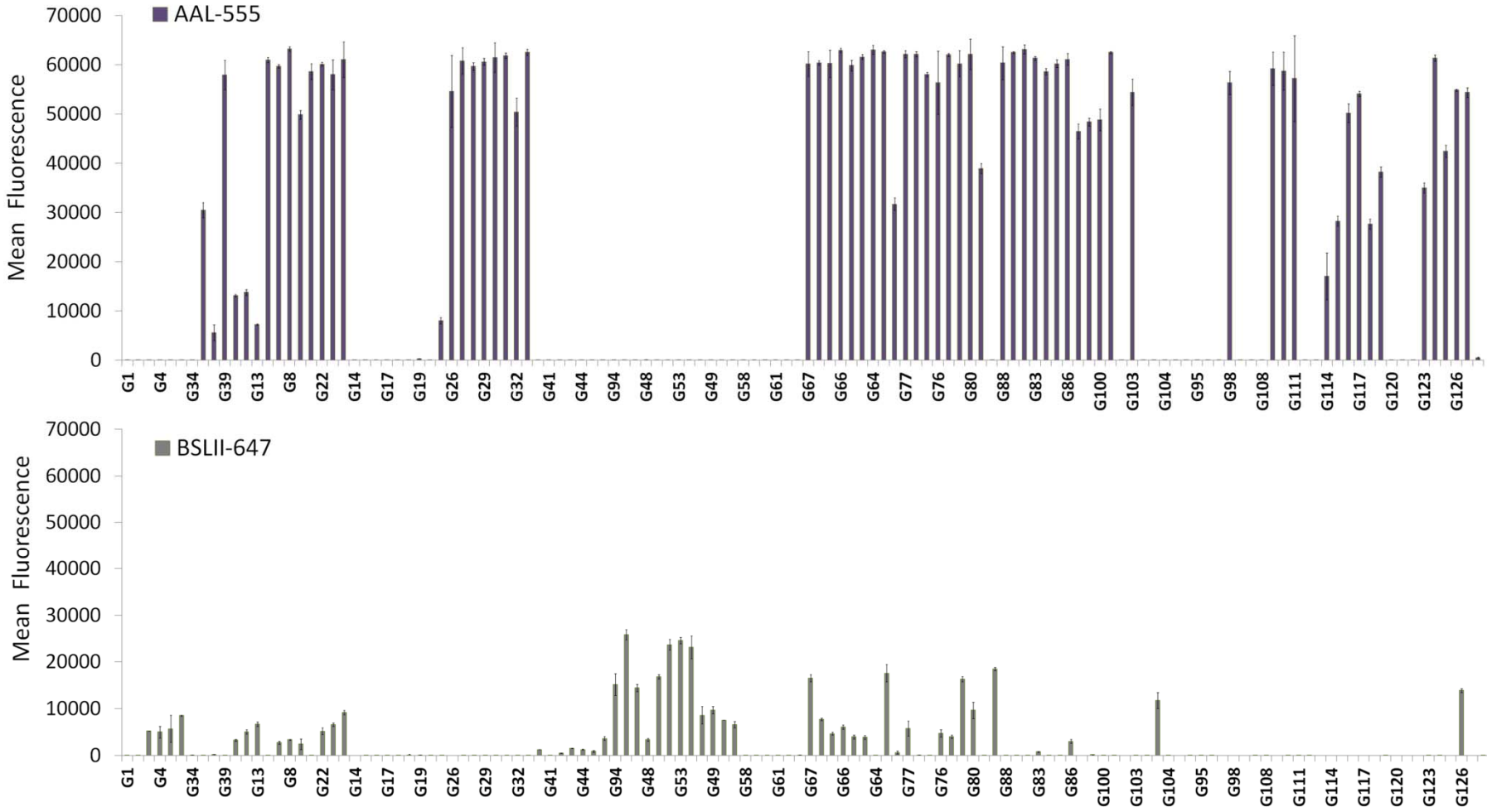


*Figure S-8. Large (18x36) glycan array probed with fucose specific lectin **AAL-555** (violet) and **BSLII-647** (gray) recognizing terminal, non-reducing α- or β-linked GlcNAc.*

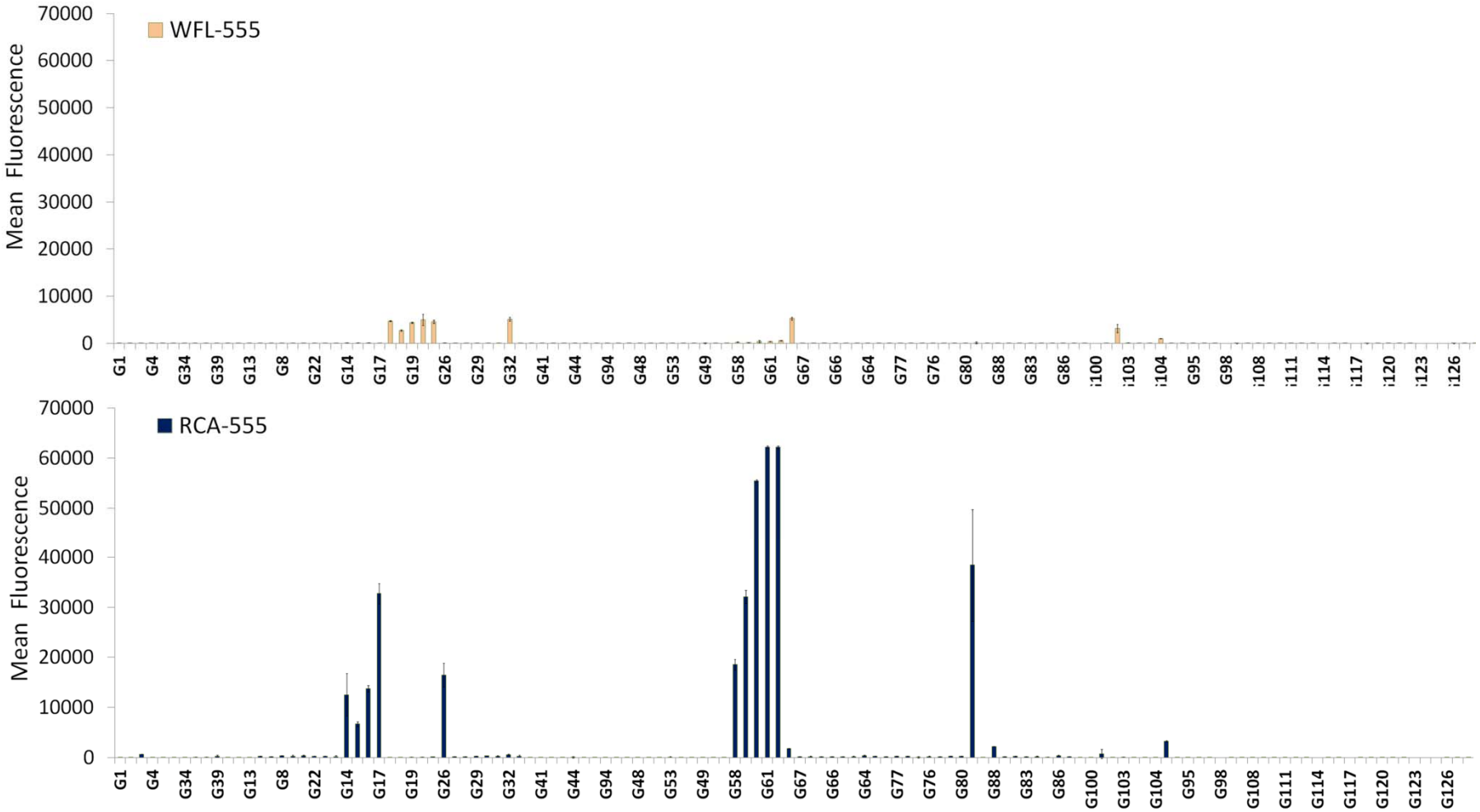


Figure S-9. Large (18x36) glycan array probed with WFL-555 (orange) specific for GalNAc residues and RCA-555 (blue) recognizing terminal β-linked galactose.

7.2 Glycan binding analysis of a DC-SIGN-Fc fusion protein

DC-SIGN/Fc solutions were prepared from stock solution (0.25 mg/mL in PBS) by dilution with TSMT binding buffer pH=7.5 (20 mM Tris·HCl, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1% BSA and 0.005% Tween-20) to a final concentration of 10 µg/mL (Figure 9a). These solutions (200 µL) were placed into subarrays and incubated overnight at 0°C with gently shaking. Solutions containing protein were removed and each subarray was washed with binding buffer, followed by 1h incubation with anti-human IgG (Fc specific)-Cy3 in TSM binding buffer (1:1000). The washing of slides was performed with TSM_T (TSM containing 0.005% Tween-20), TSM and water. Slides were dried in a slide spinner. The fluorescence was analysed with a microarray scanner (Figure S-10).

7.3 Glycan binding analysis of anti-carbohydrate polyclonal antibodies in sera from *S. mansoni* infected patients

Previously collected selected sera of schistosomiasis patients were pooled into two age-groups: children (21 individuals, age between 5 to 11 years) and adults (20 individuals from 20 to 46 years)[9]. In addition, sera from 3 healthy Europeans were analyzed and used as negative controls for the study (Figure S-11, pooled binding data). In both groups the ratio female/male ratio was 3:2. Sera were diluted 1:100 with PBS containing 1% BSA and 0.01% Tween-20, and aliquots (180 µL per array) used to incubate individual wells on a glycan array slide at room temperature for 1h. The slides were washed with PBST (PBS containing 0.05% Tween-20) followed by PBS. Slides were then incubated with anti-human IgG(Fc specific)-Cy3 (Figure S-12) and anti human-IgM(µ specific)-Alexa fluor 647 (Figure S-13) in PBS containing 1% BSA and 0.01% Tween-20 for 30 minutes in the dark. Slides were washed subsequently with PBST, PBS and H_2O and dried in a slide spinner prior to scanning the fluorescence emission in a microarray scanner.

[9] van Diepen, A., Smit, C.H, van Egmond, L., Kabatereine, N.B, de Moira, A. P, Dunne, D.W, Hokke, C.H. *PLOS NTD*, **2012**,*6*, 1922.

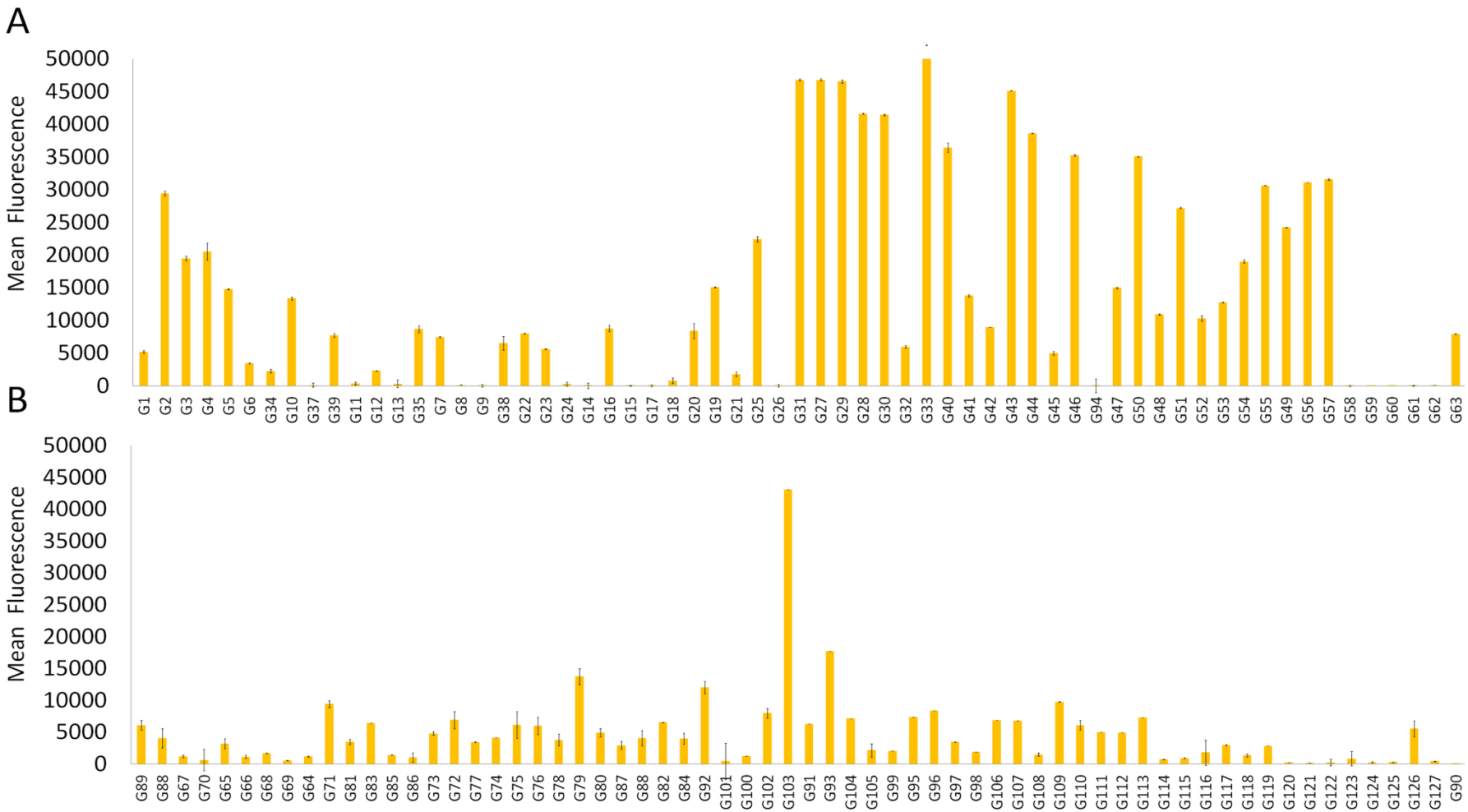


Figure S-10. DC-SIGN-Fc binding profile on a large (18x36) glycan array. Mean fluorescence after incubation with DC-SIGN-Fc (10 µg/mL) followed incubation with secondary cnetly labeled anti-human antibody. Each bar in the histogram shows the average of fluorescence from 4 spots and the standard deviation as error bar.

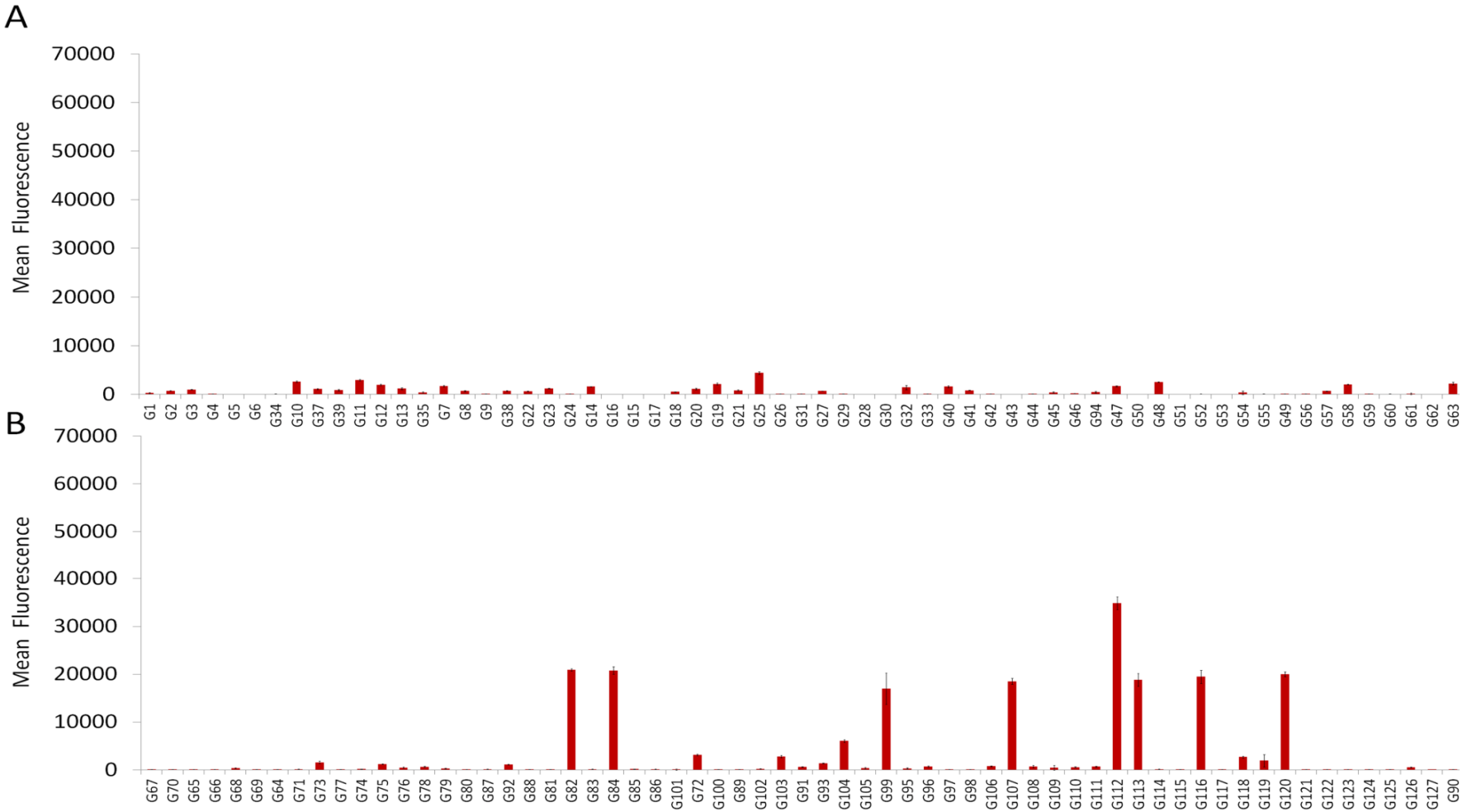


Figure S-11. Glycan array screening sera of healthy Europeans followed by incubation with secondary anti human-IgG-(Fc specific)-Cy3; ***A.*** *Mean fluorescence for IgG binding to glycans* ***G1-G63****;* ***B.*** *Mean fluorescence for IgG binding to glycans* ***G89-G90****; Histogram shows pooled binding data of 3 individuals. Fluorescence values are averaged from 4 replicate spots.*

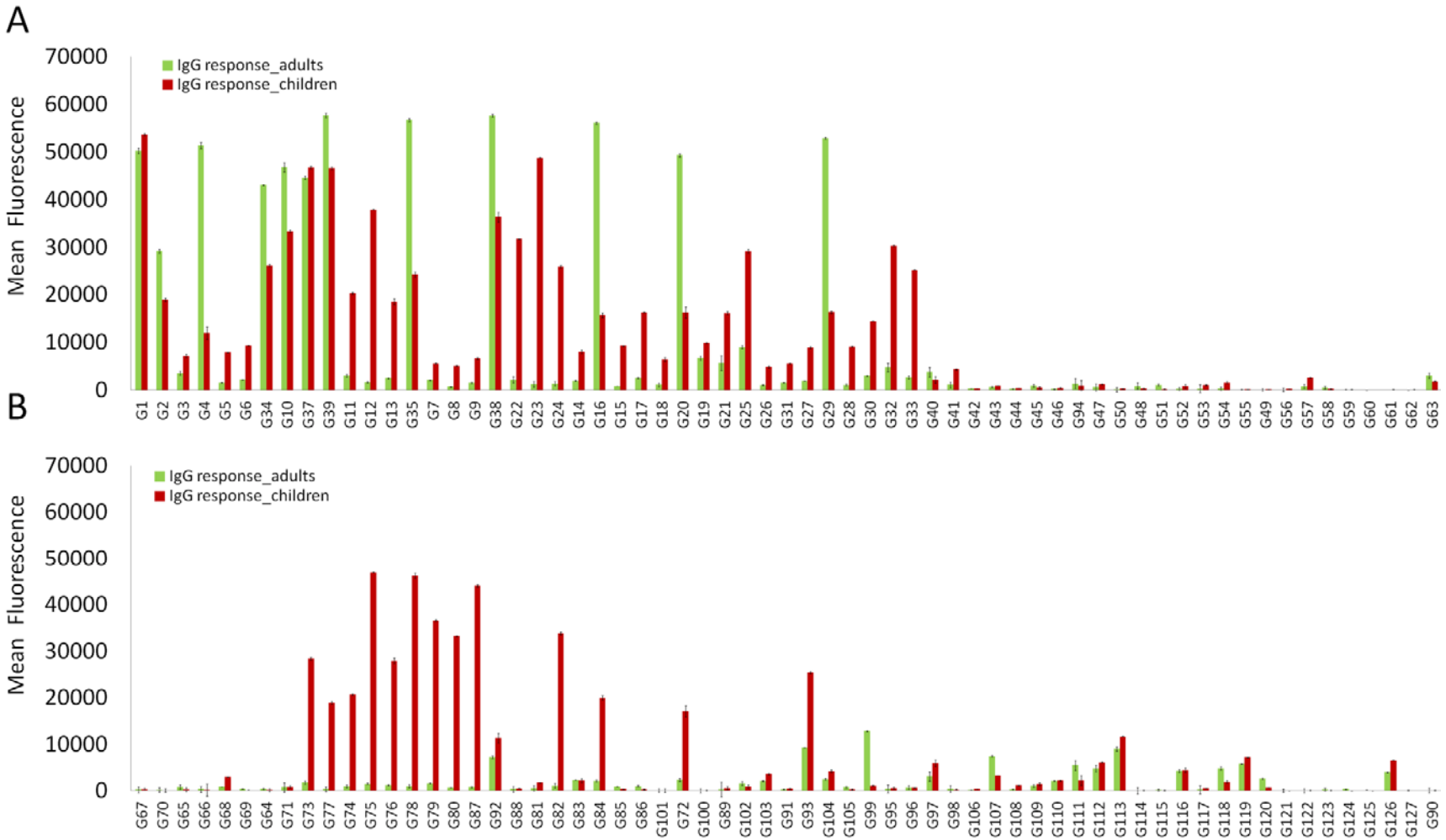


Figure S-12. Glycan array screening of schistosomiasis infected sera followed by incubation with anti human-IgG-(Fc specific)-Cy3; **A.** IgG response observed in pooled sera from children and adults against glycans **G1-G63**; **B.** IgG response observed in pooled sera from children and adults against glycans **G89-G90;** Error bars show the standard deviation of mean fluorescence for 4 replicate spots quantified for each ligand

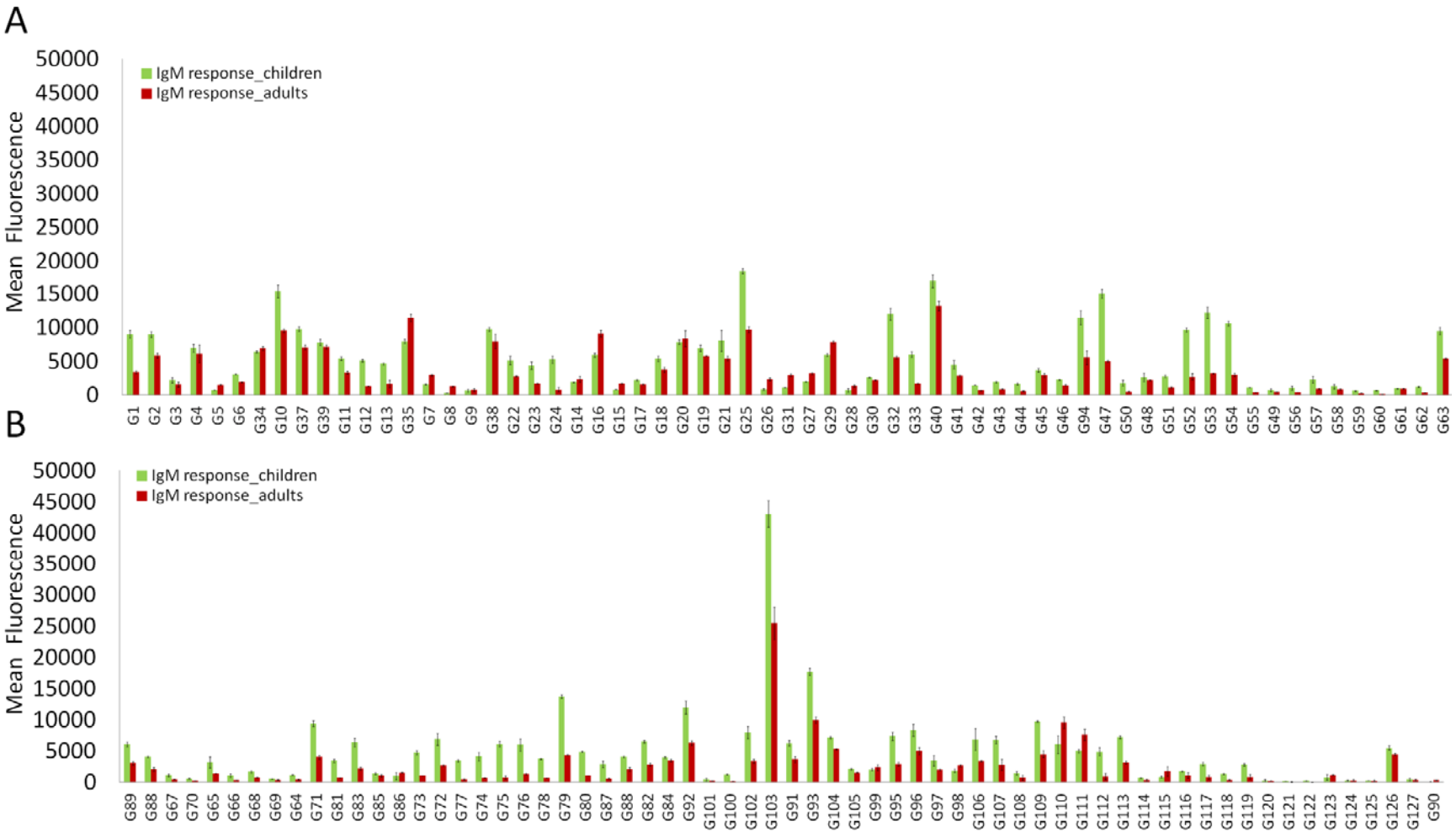
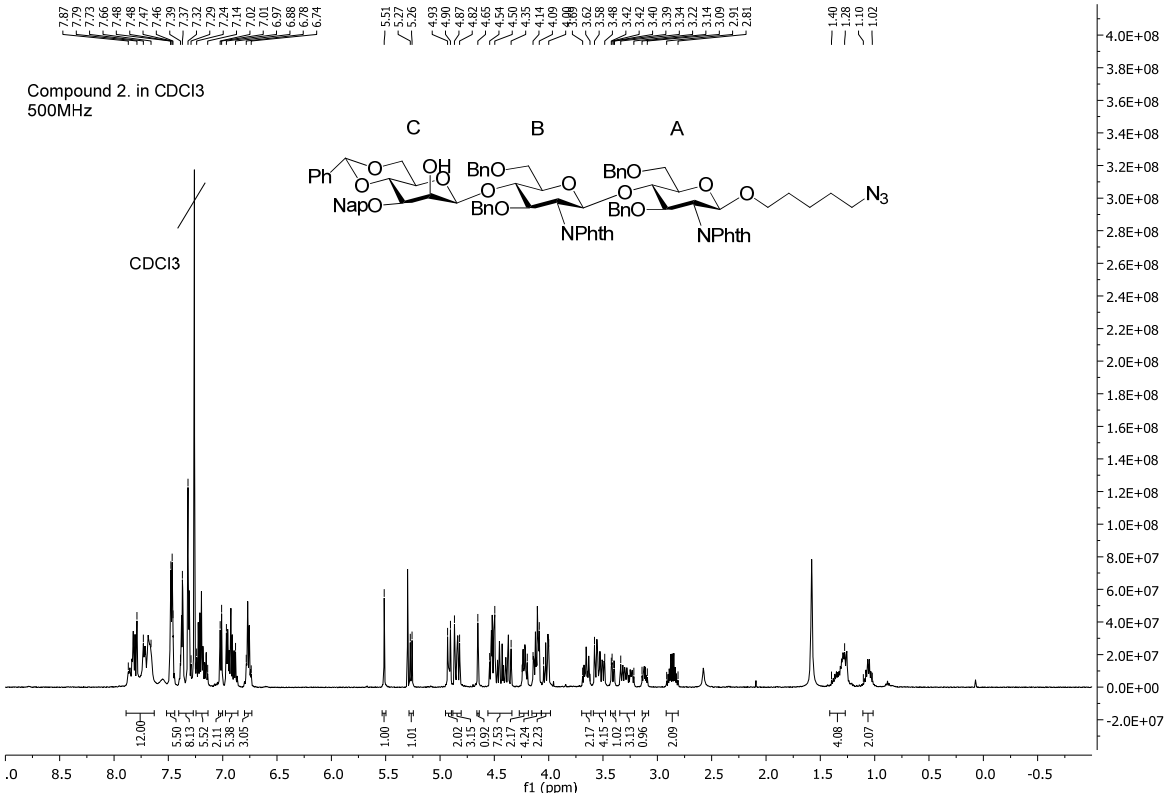


Figure S-13. Glycan array screening of schistosomiasis infected sera followed by incubation with anti human-IgM-647; **A.** IgM from pooled sera of children (green) and adults (red) binding to glycans **G1-G63**; **B.** IgM from pooled sera of children (green) and adults (red) binding to glycans **G89-G90**; Error bars show the standard deviation of mean fluorescence for 4 replicate spots quantified for each ligand

8. NMR spectra

2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Ph
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N3
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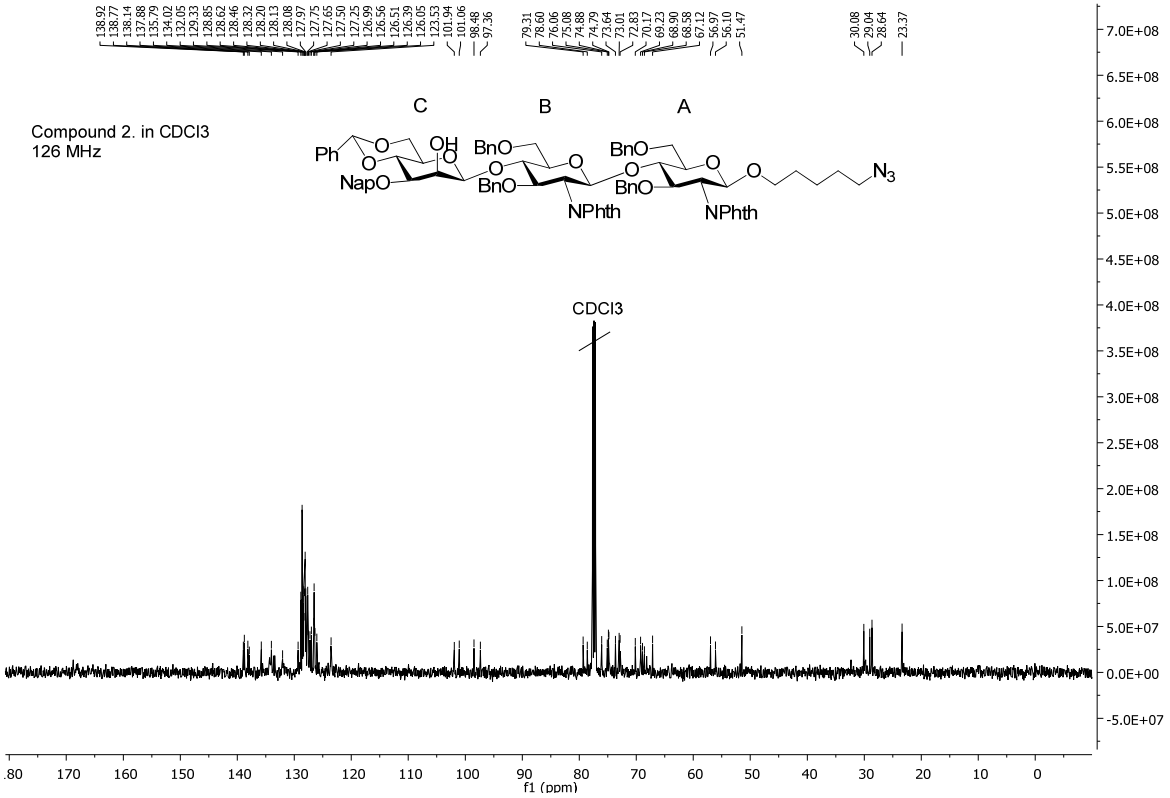
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Ph
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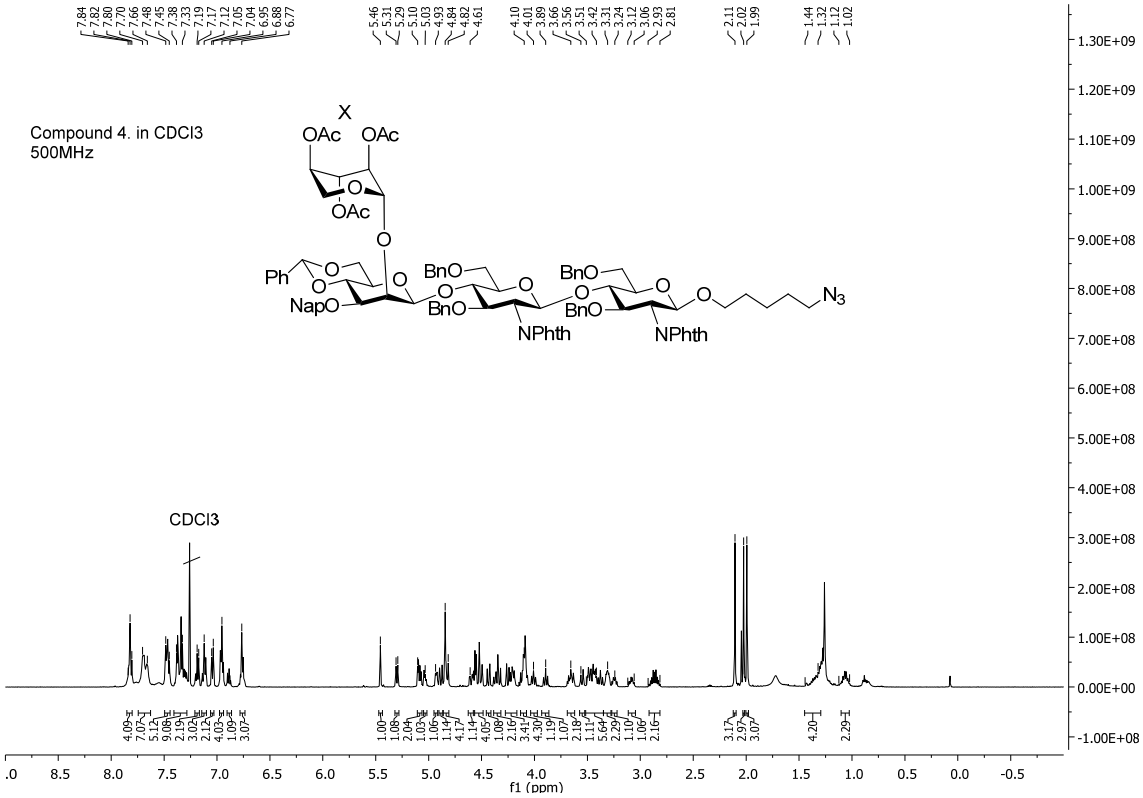
4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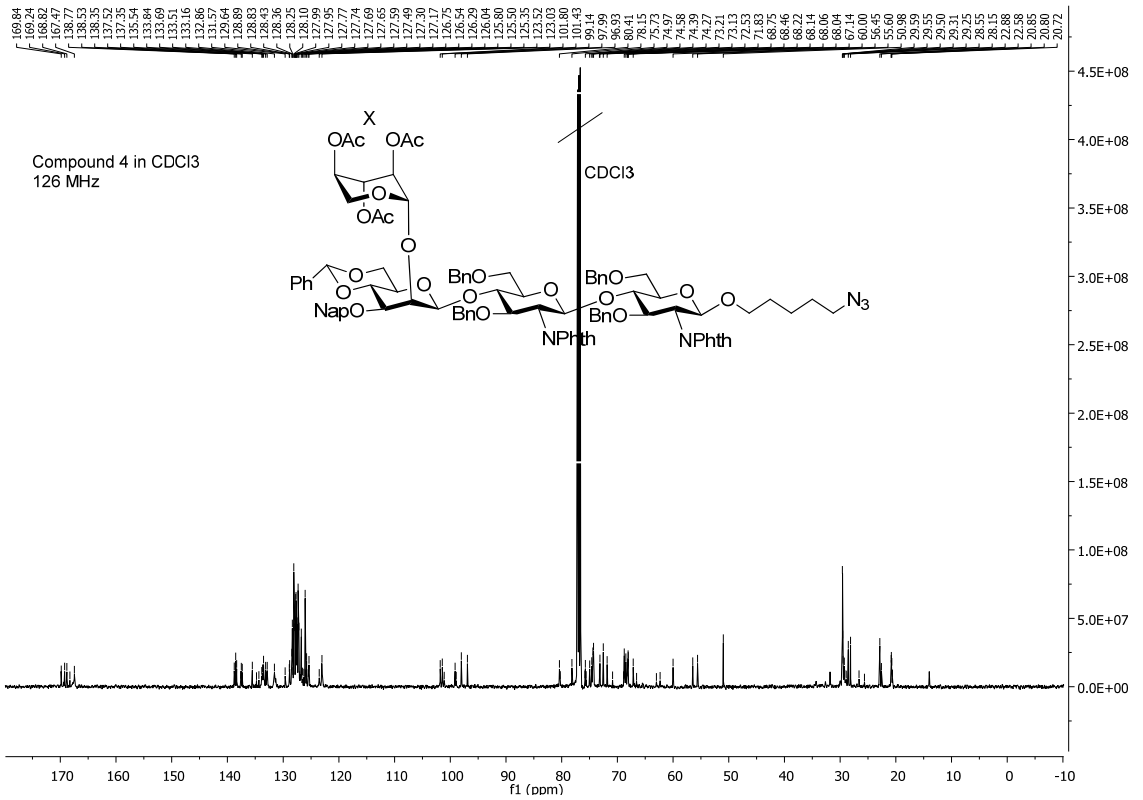
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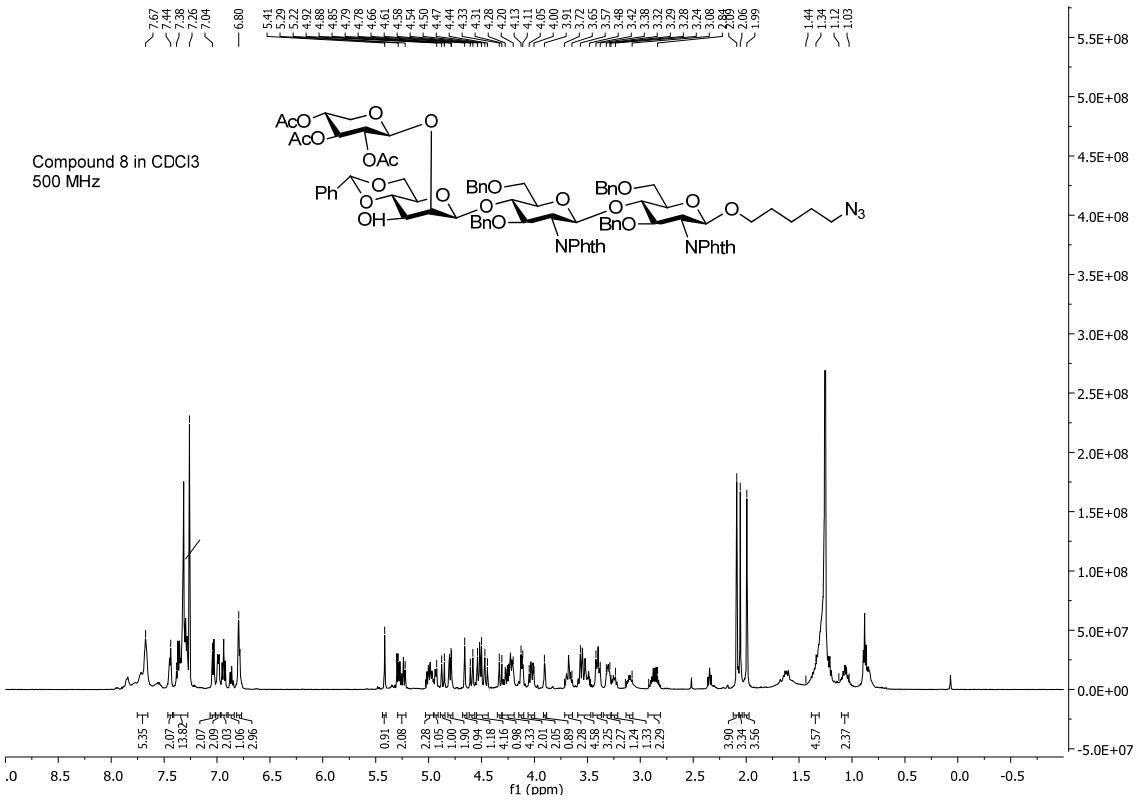
8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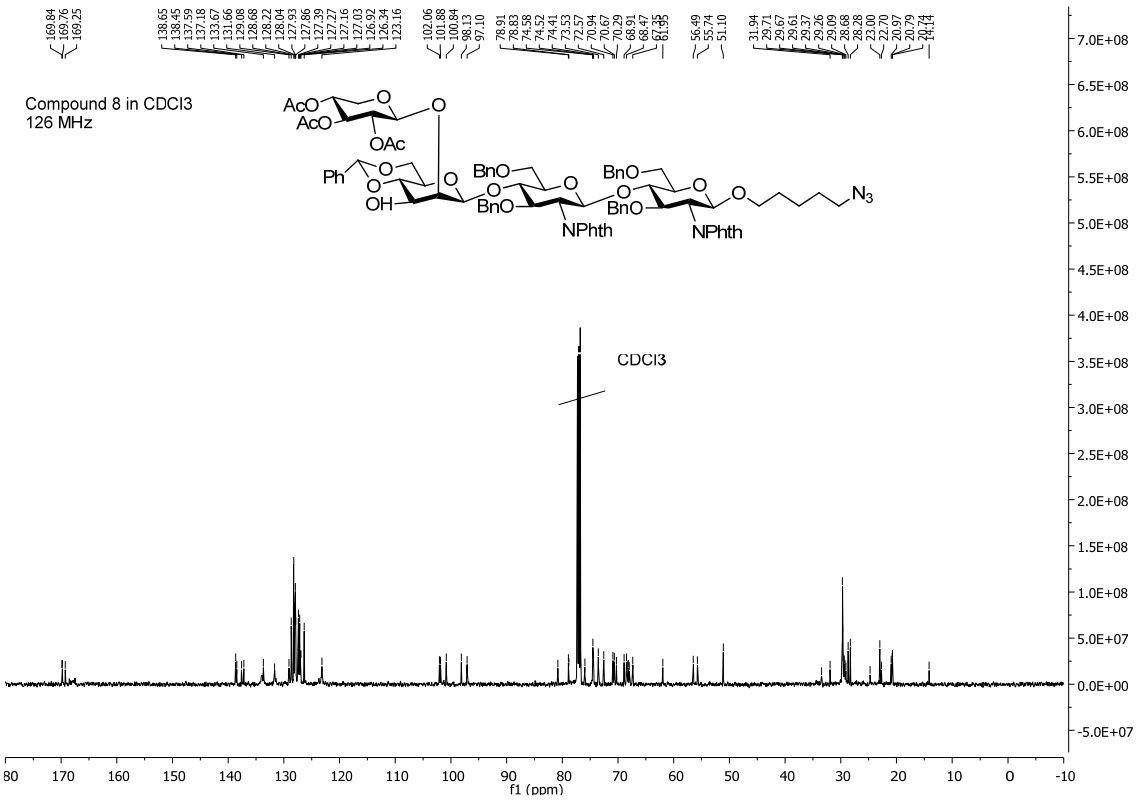
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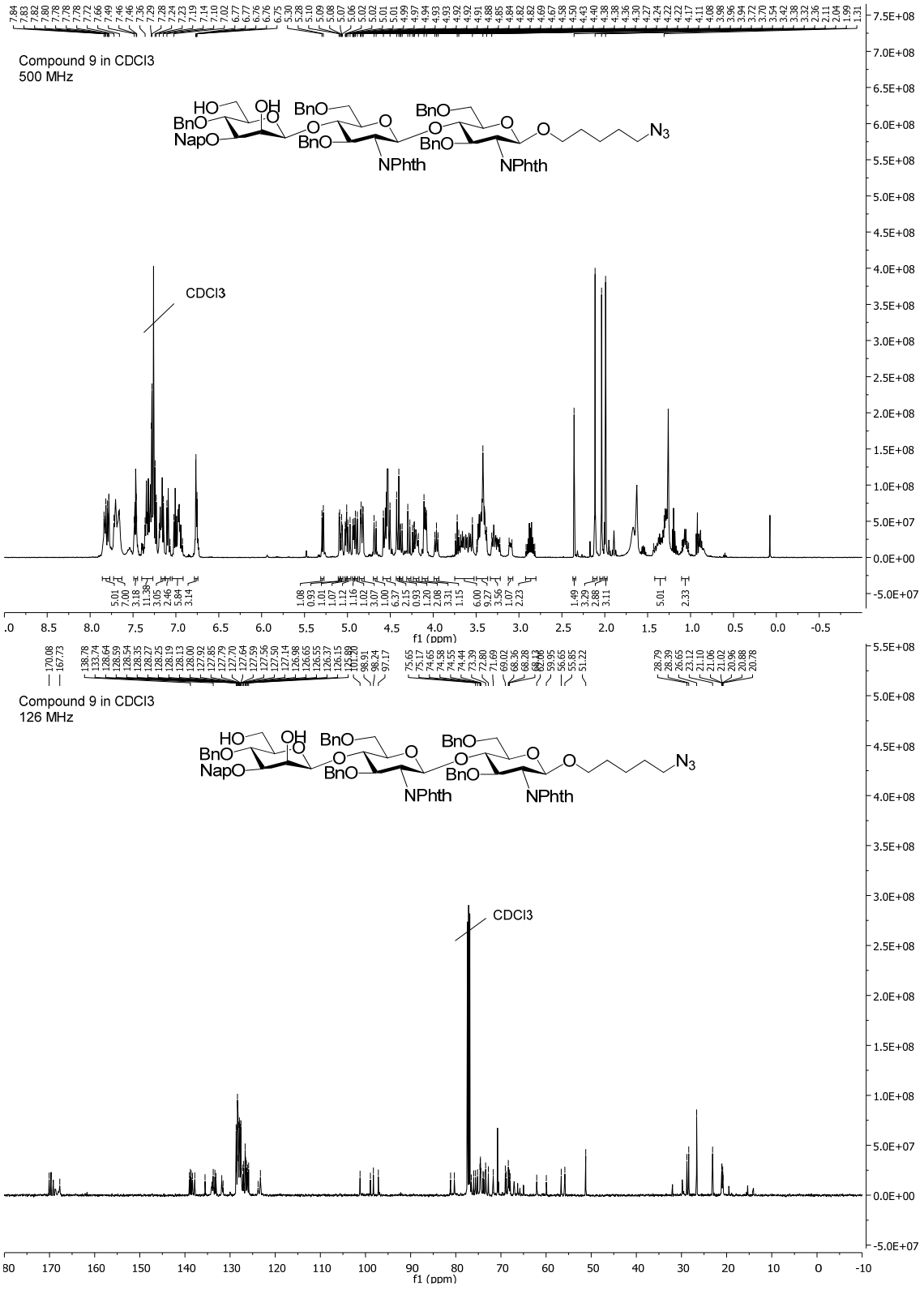
Compound 9 in CDCl3
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OH
BnO
NapO
O
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N3
CDCl3
f1 (ppm)
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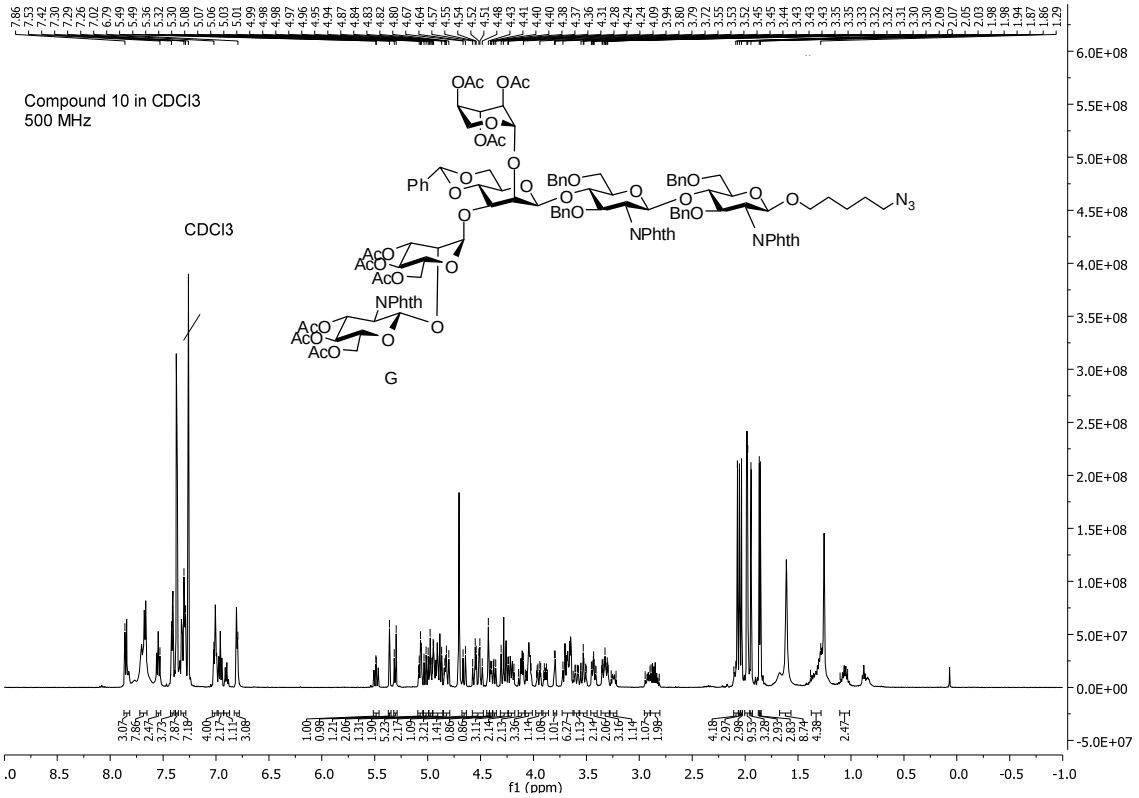
CDCl3
f1 (ppm)

10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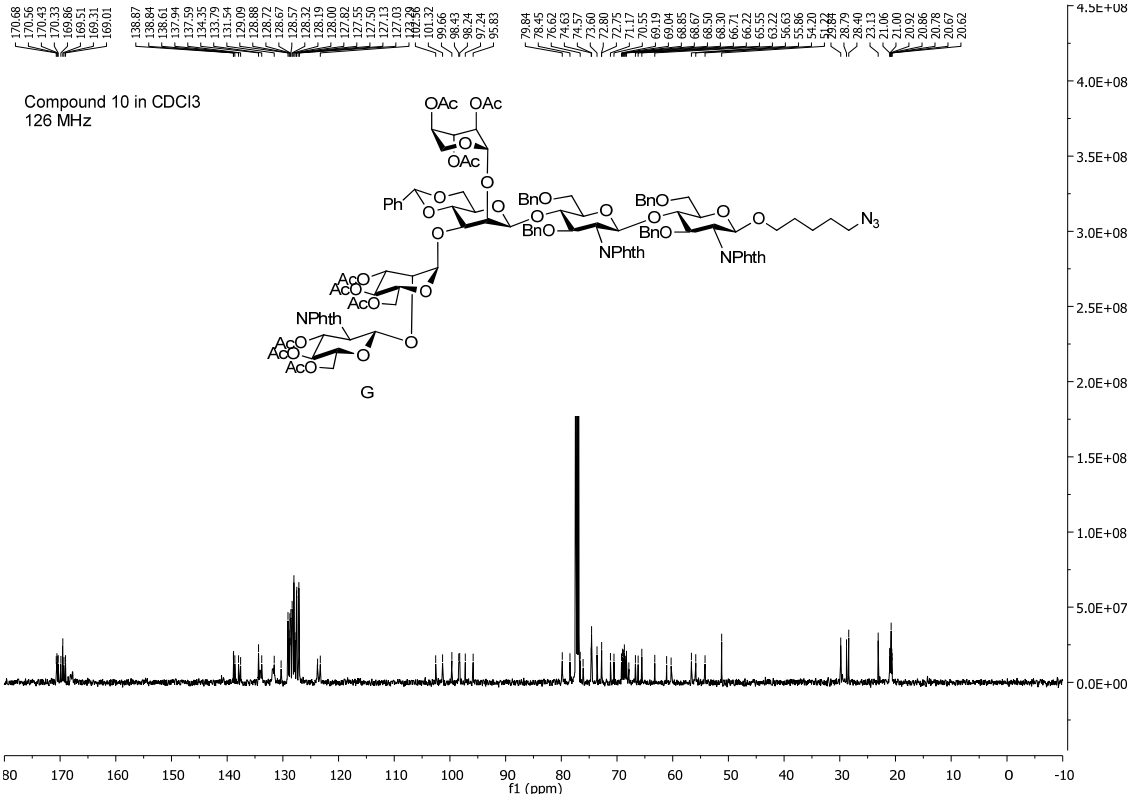
CDCl3
G
f1 (ppm)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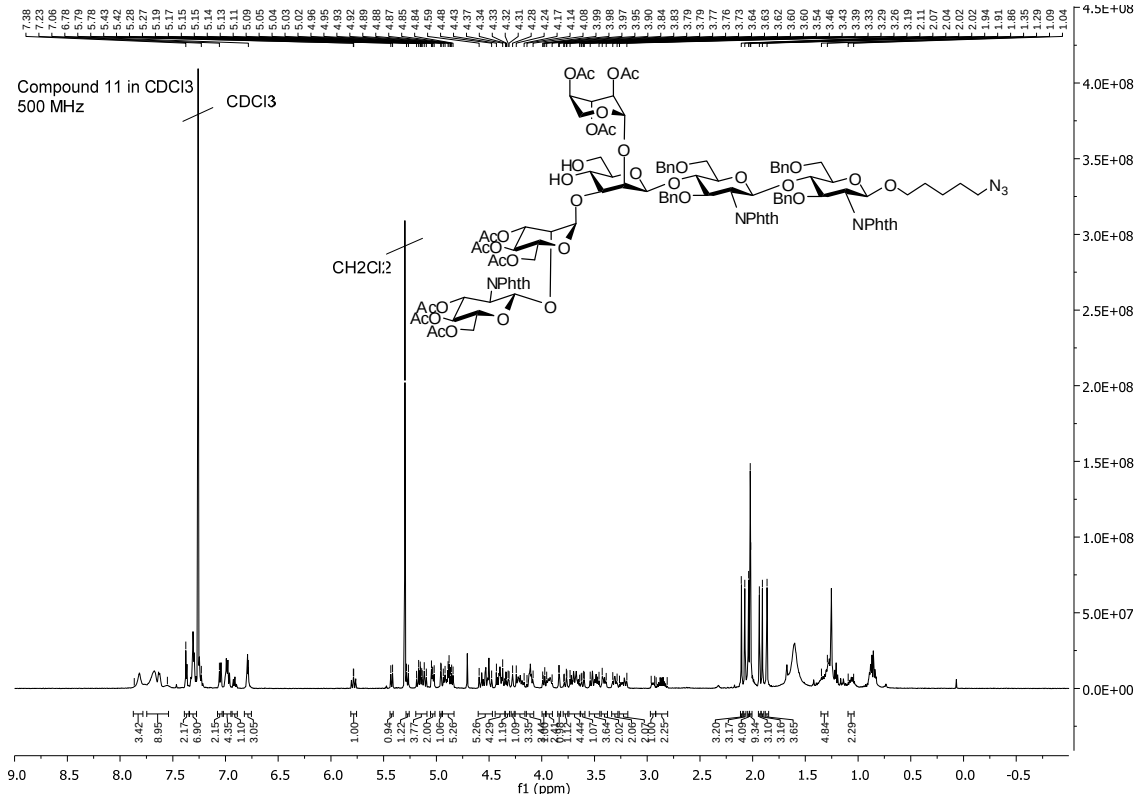
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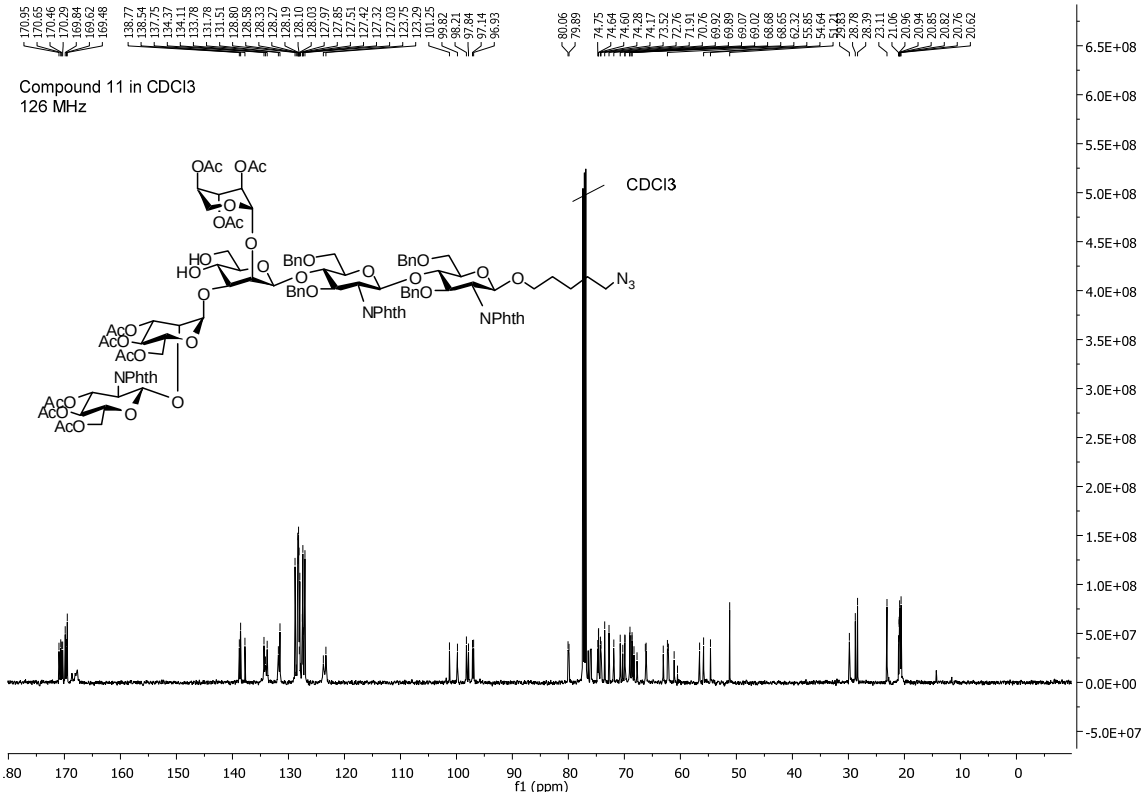
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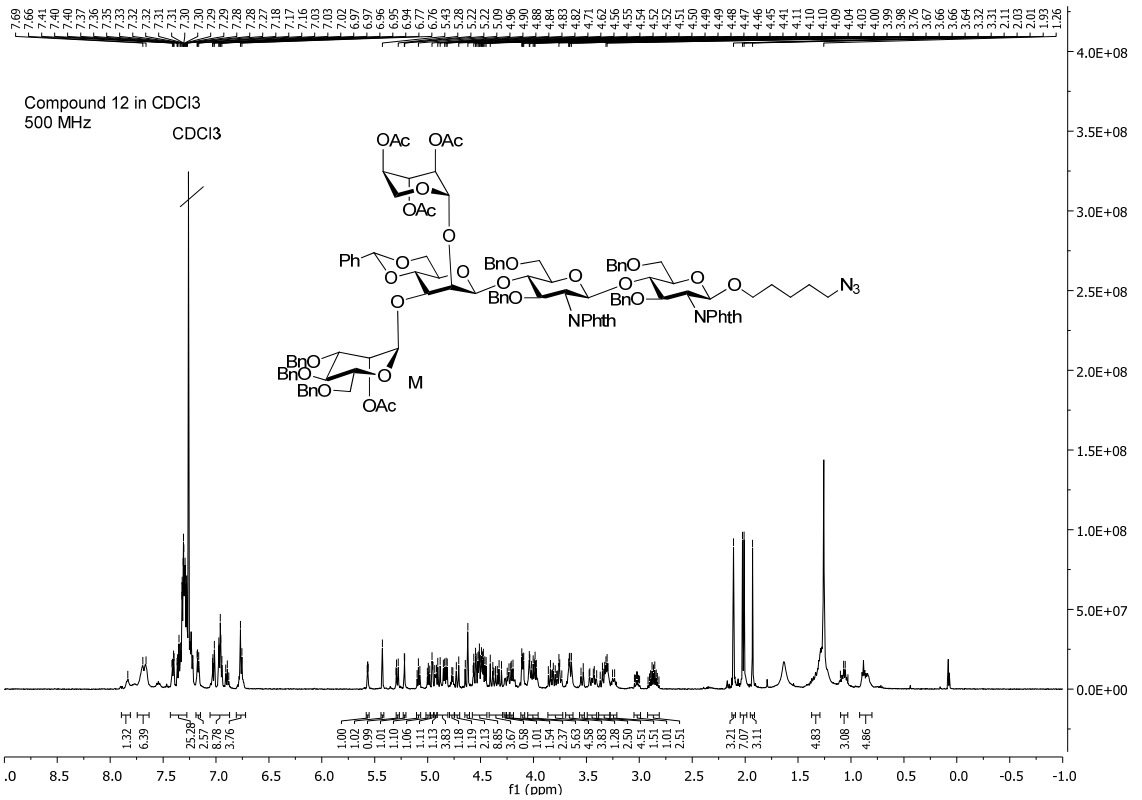
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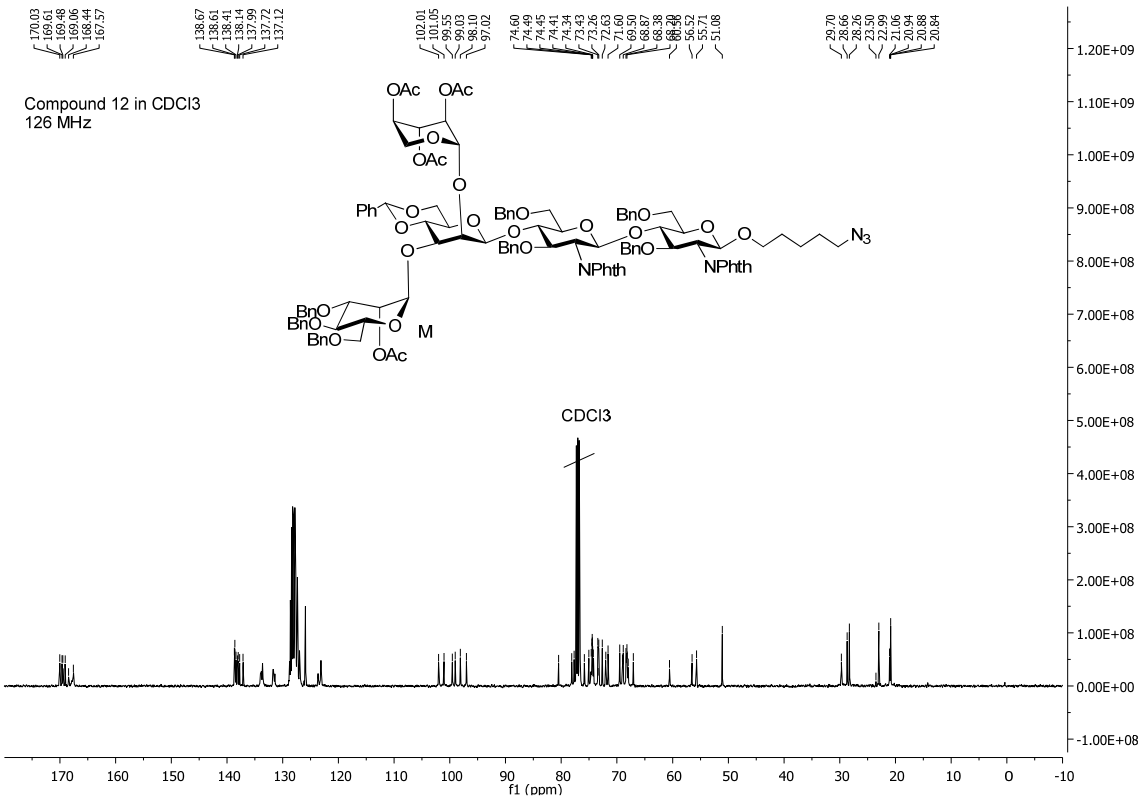
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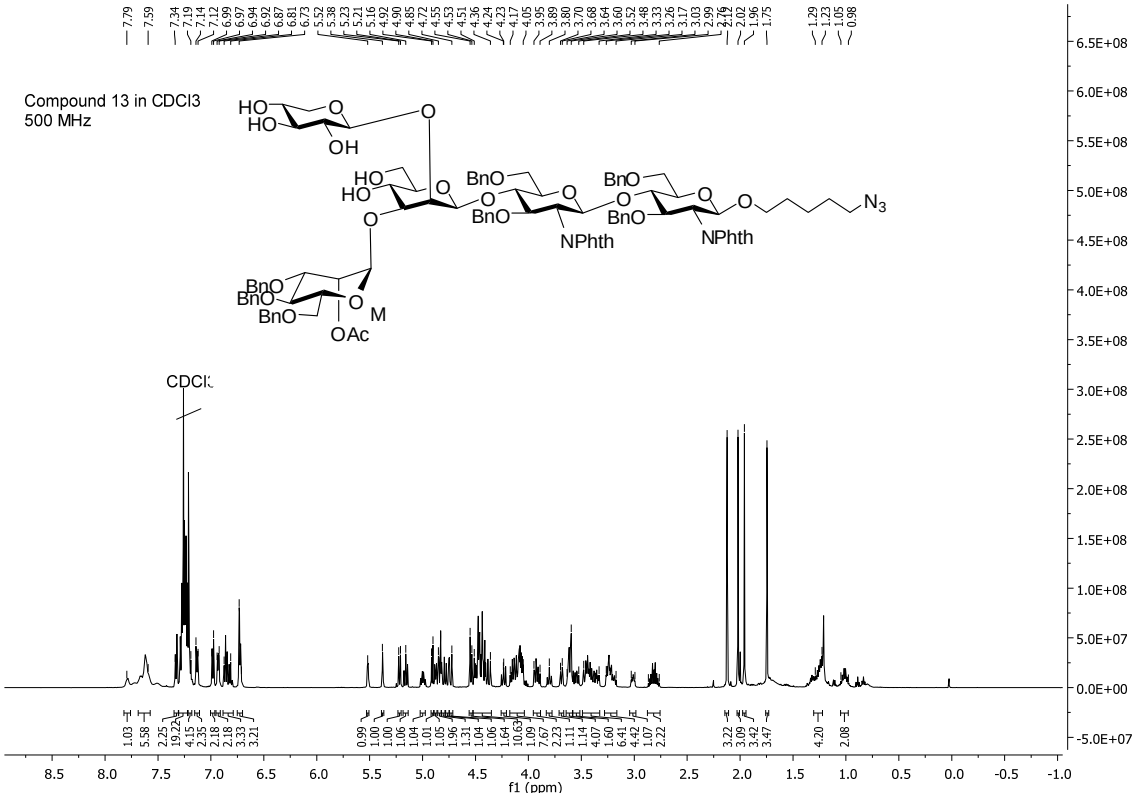
f1 (ppm)

13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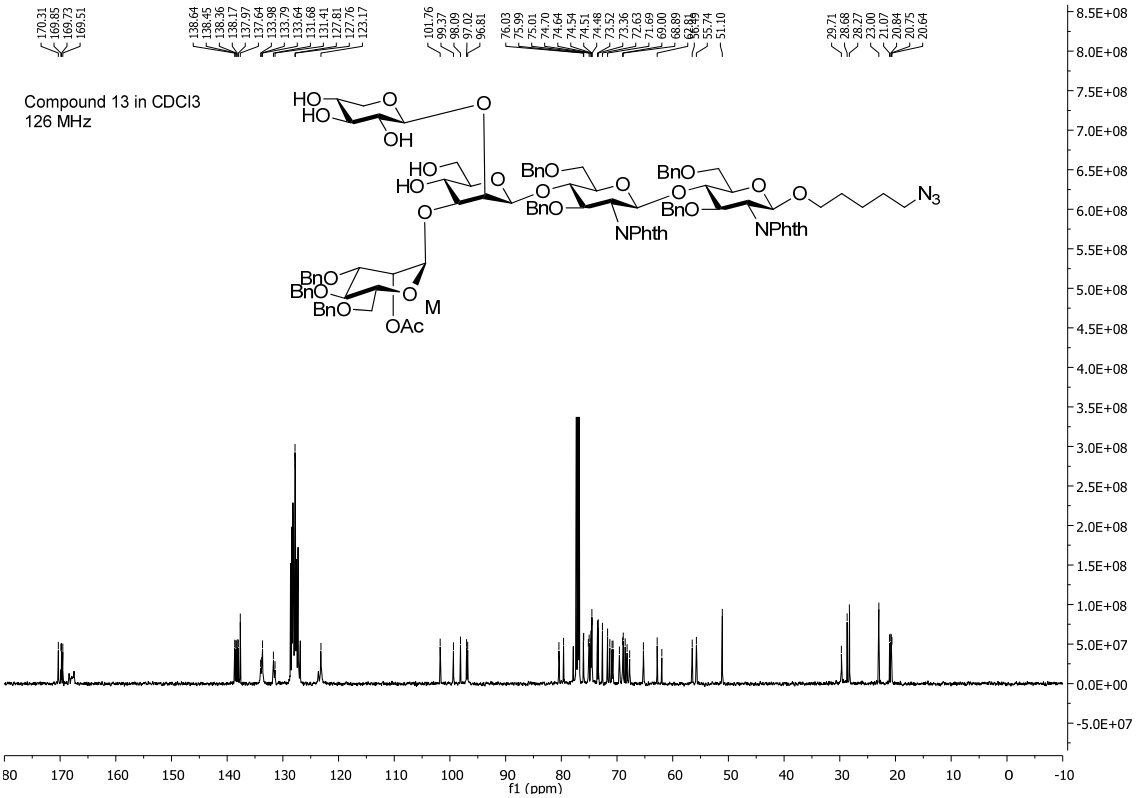
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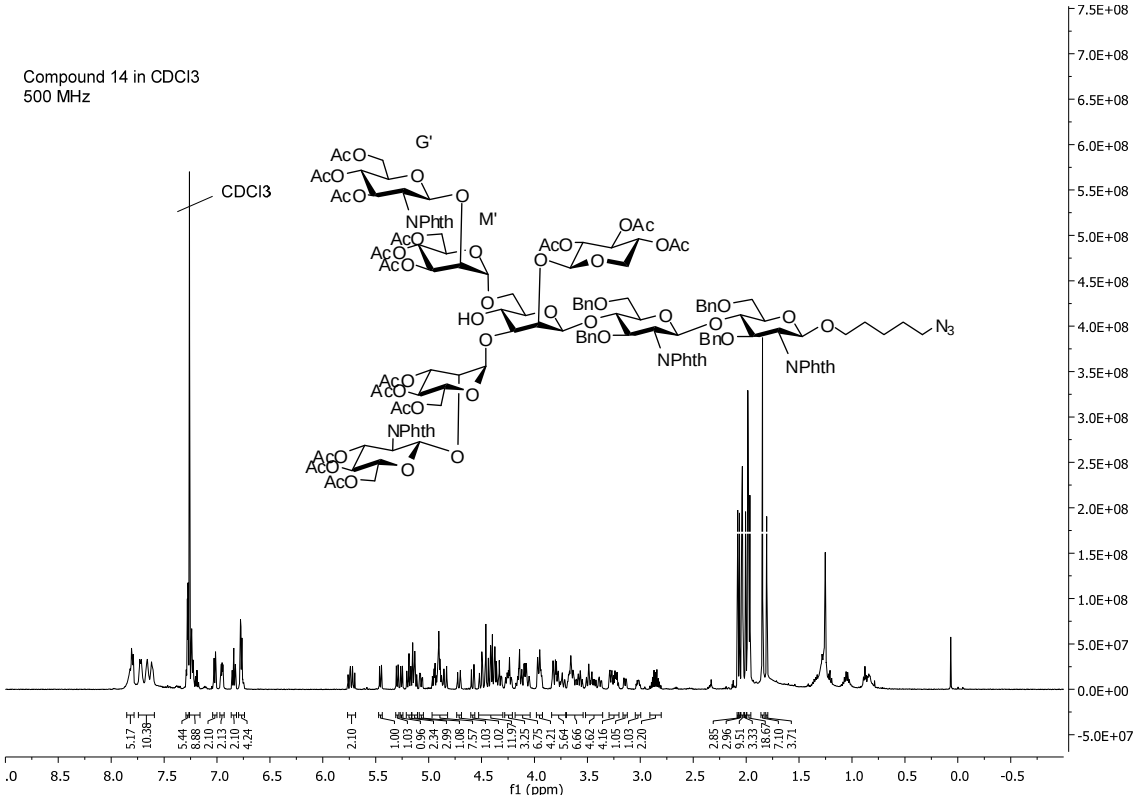
f1 (ppm)

14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M'
NPhth
OAc
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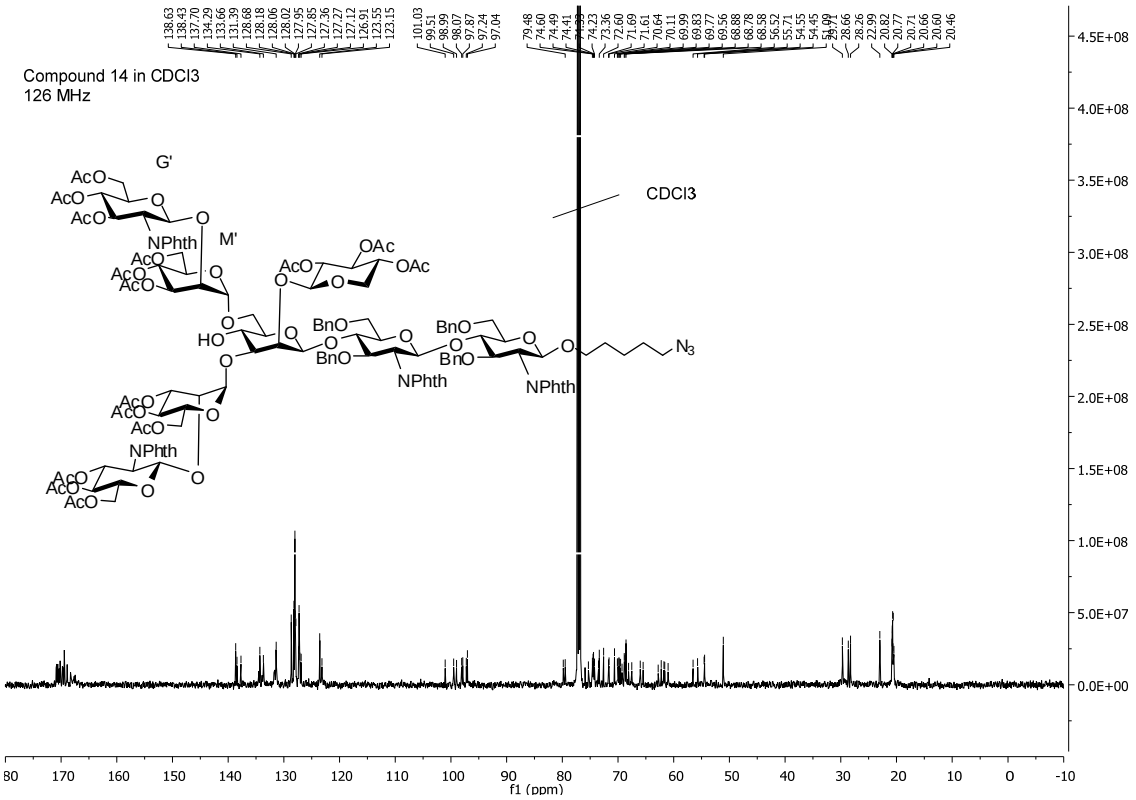
HO
N3
f1 (ppm)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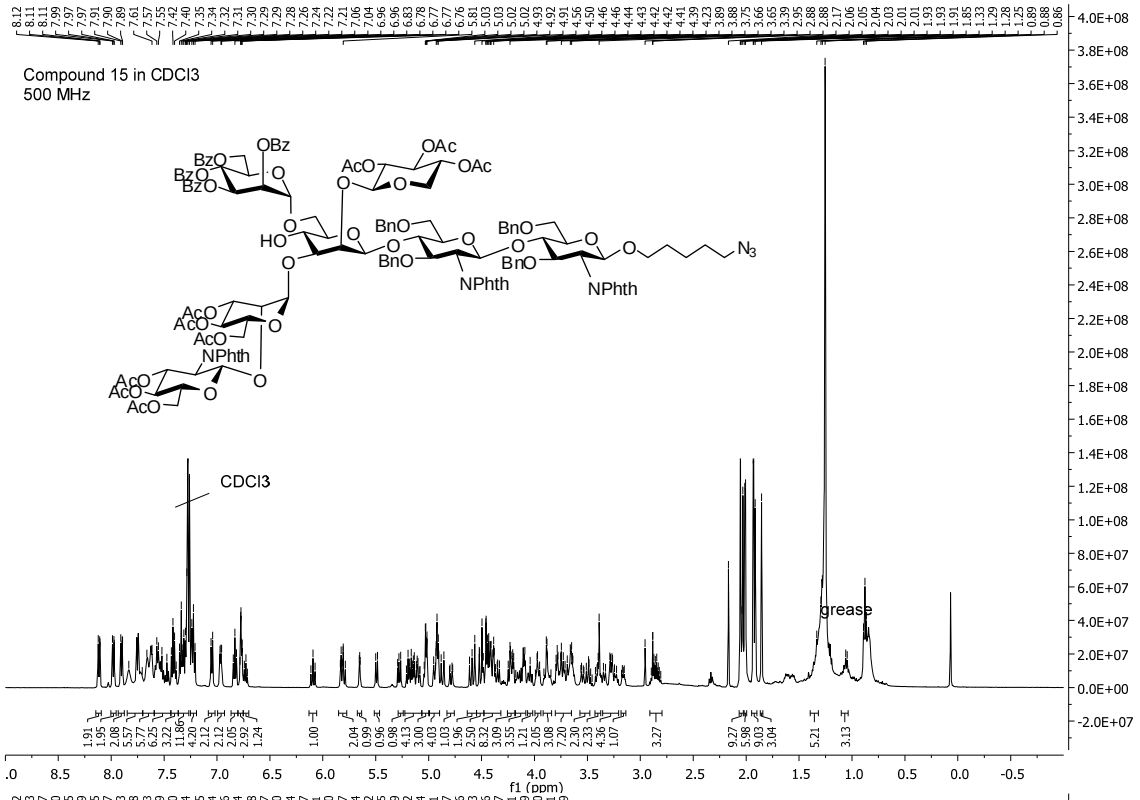
N3
f1 (ppm)

15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grease
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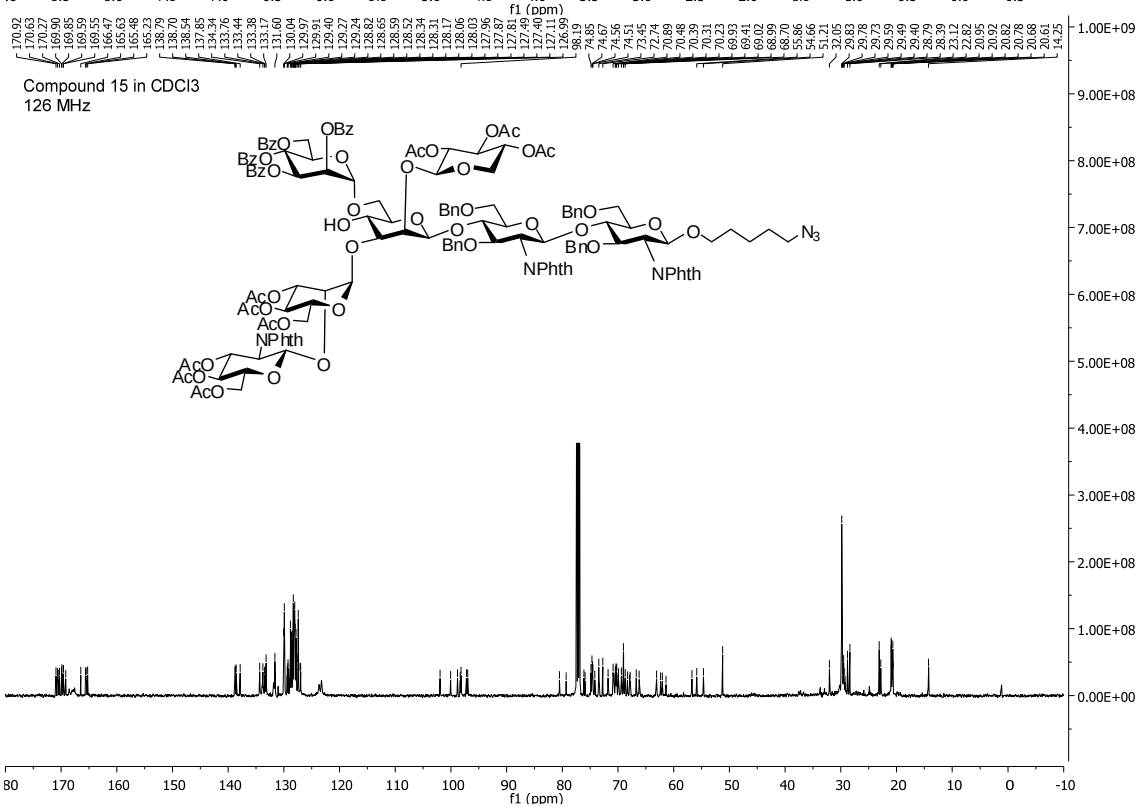
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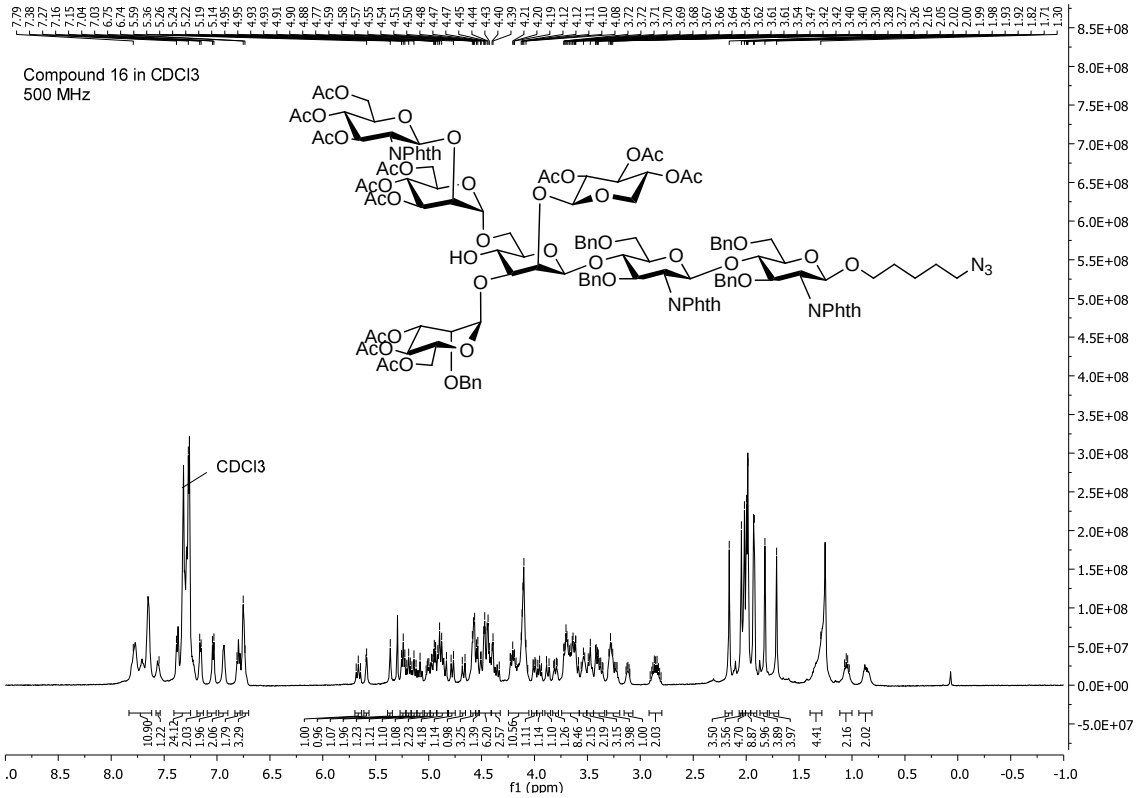
16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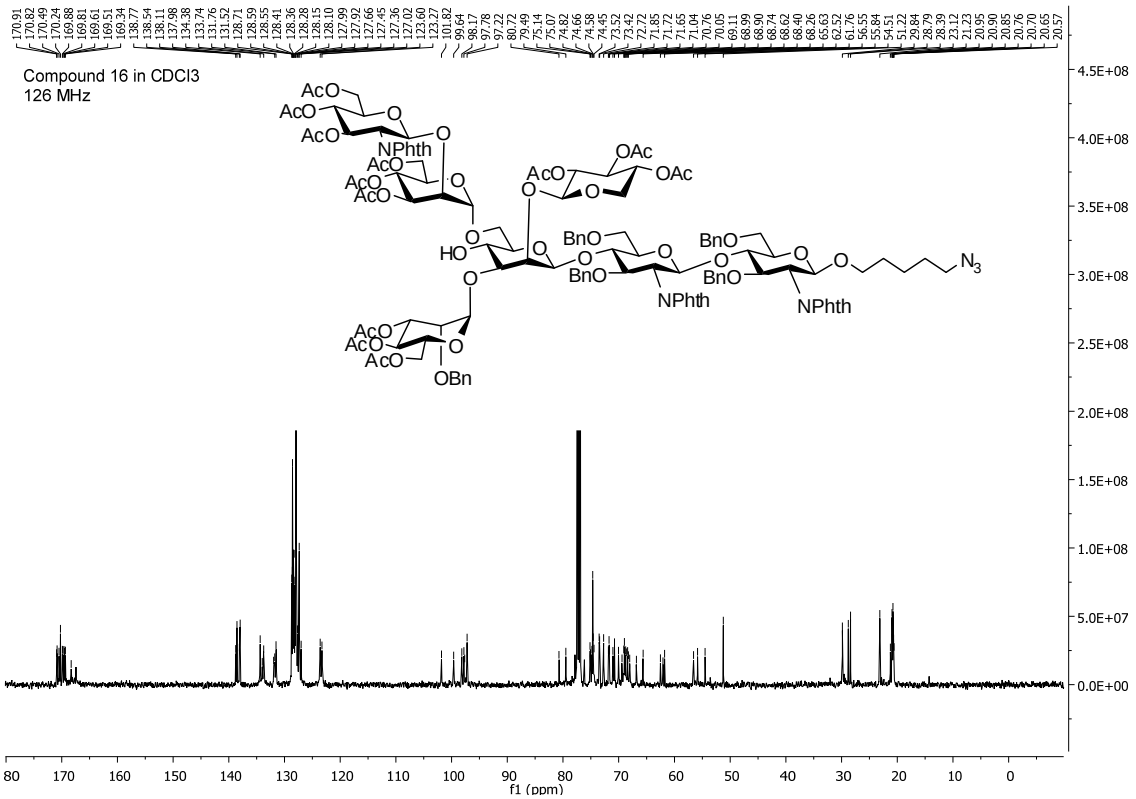
CDCl3
f1 (ppm)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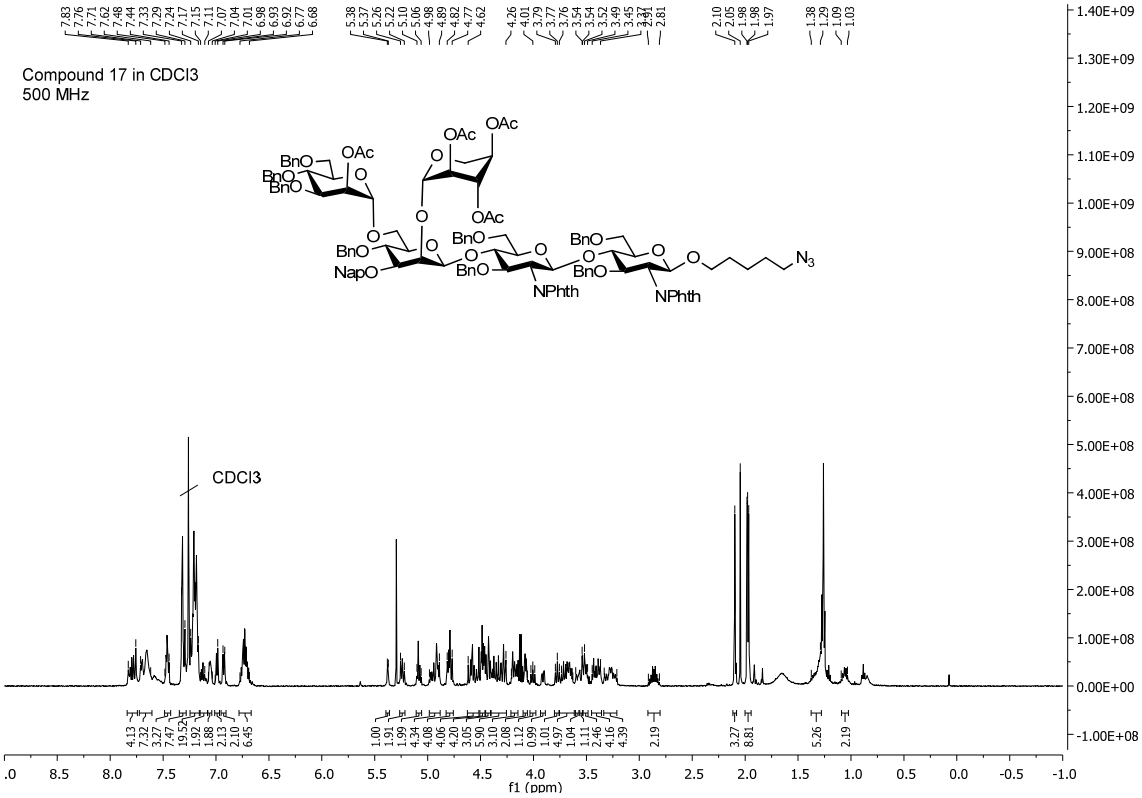
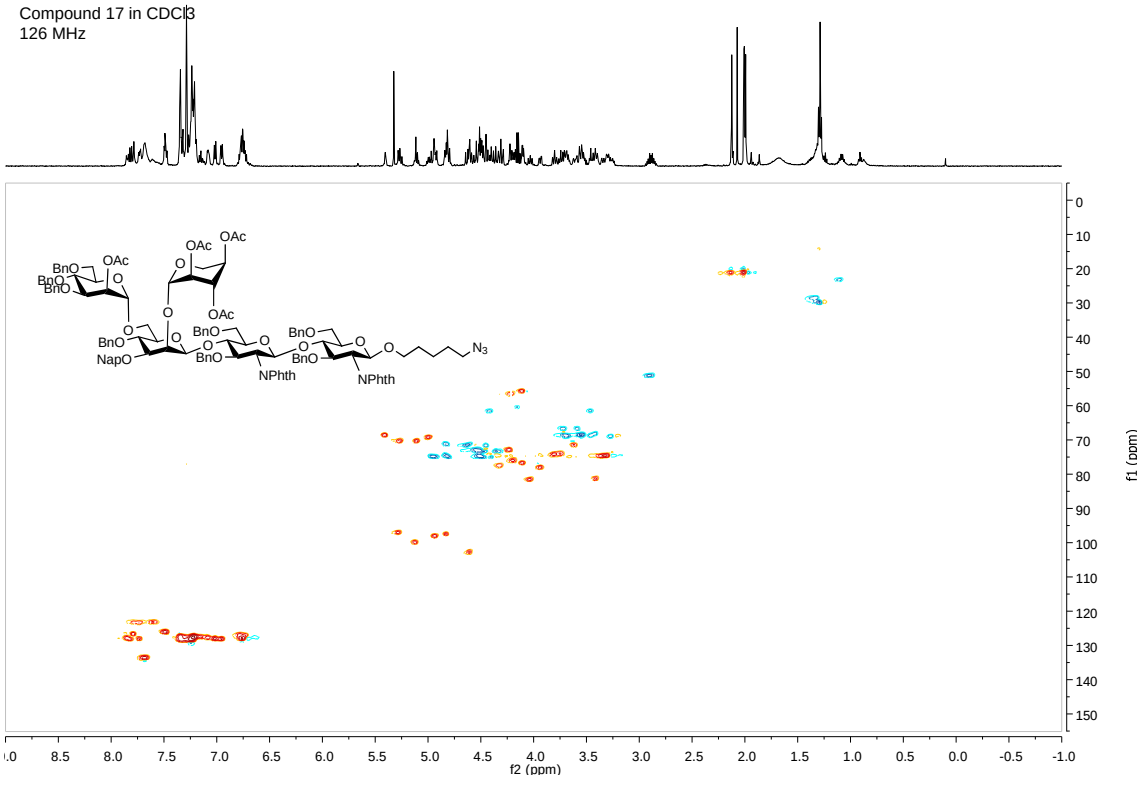
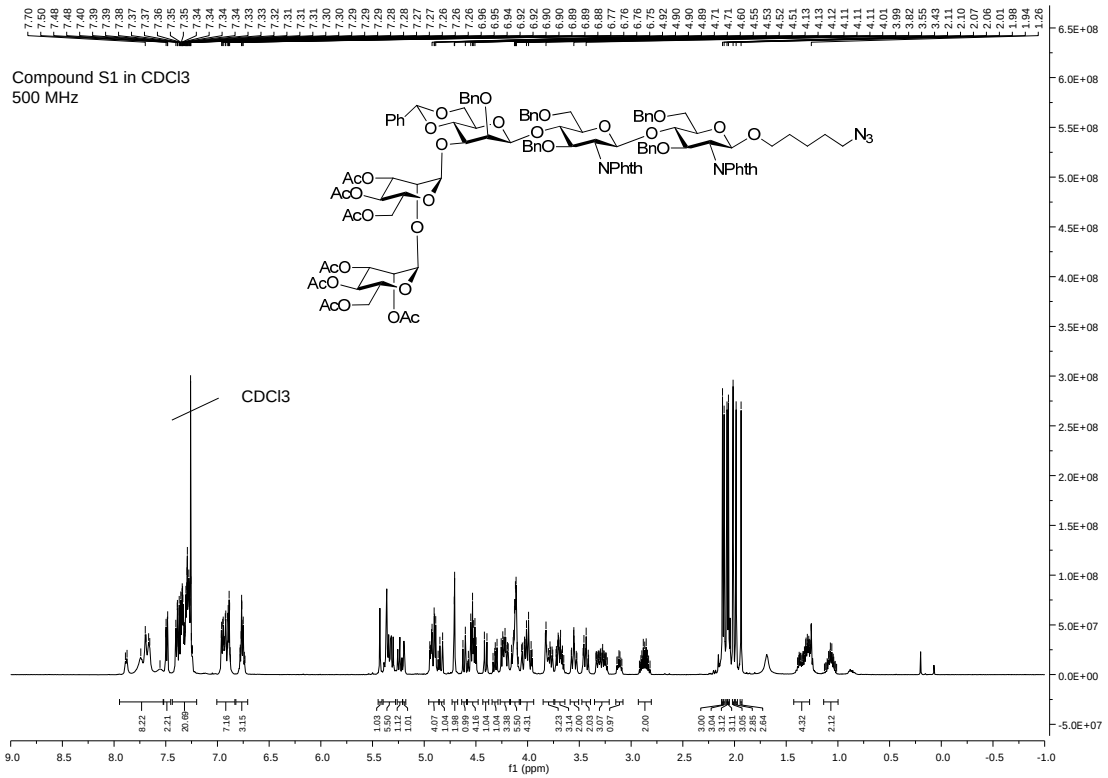
f1 (ppm)

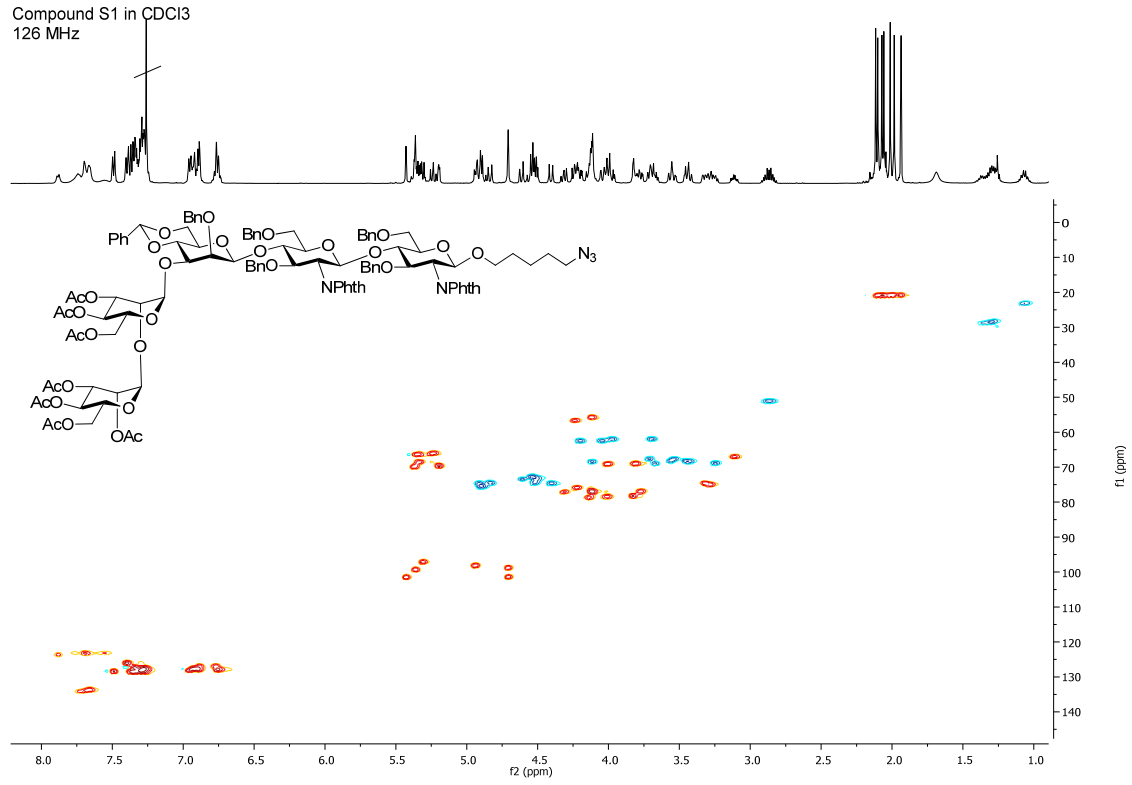
17


Compound 17 in CDCl3
500 MHz
CDCl3
f1 (ppm)

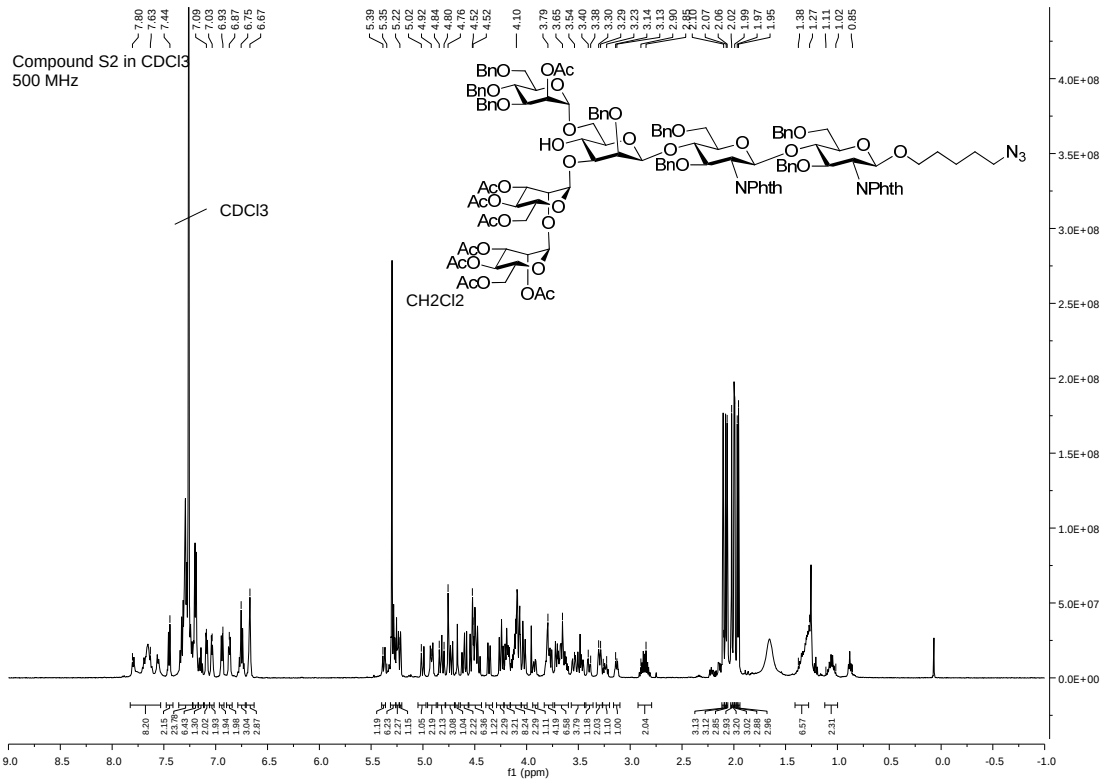

Compound 17 in CDCl3
126 MHz
f2 (ppm)
f1 (ppm)

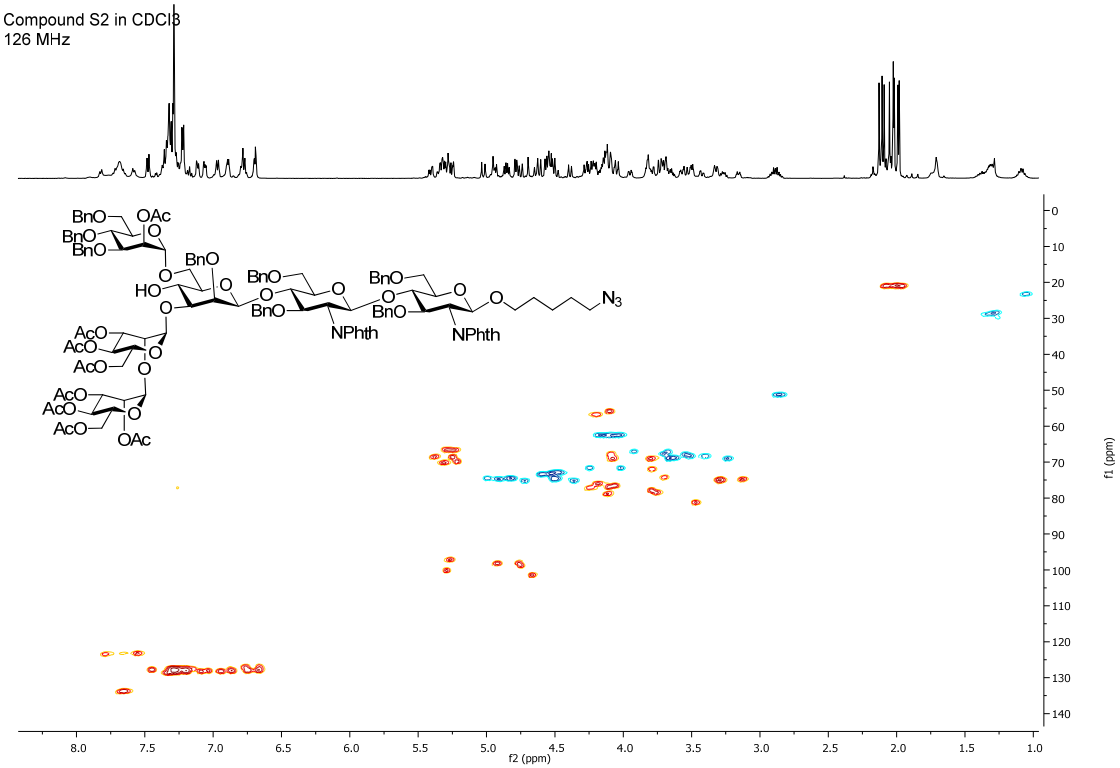
S1


Compound S1 in CDCl3
500 MHz
CDCl3
f1 (ppm)

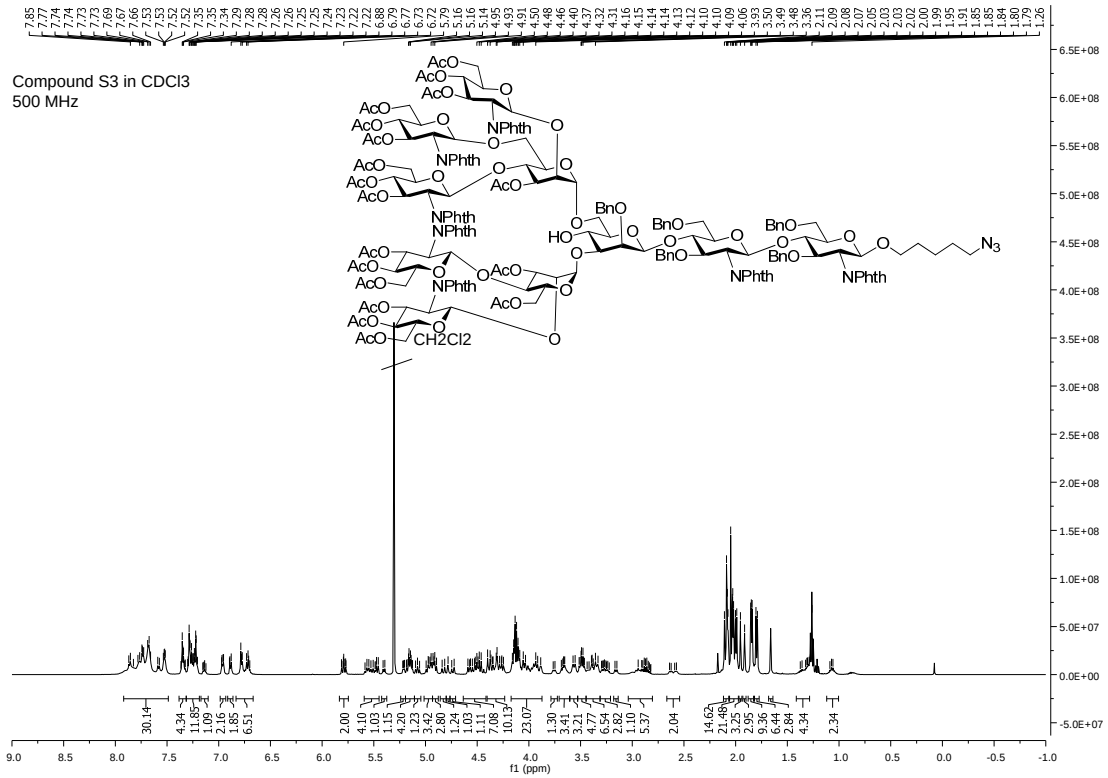

Compound S1 in CDCl3
126 MHz
f2 (ppm)
f1 (ppm)

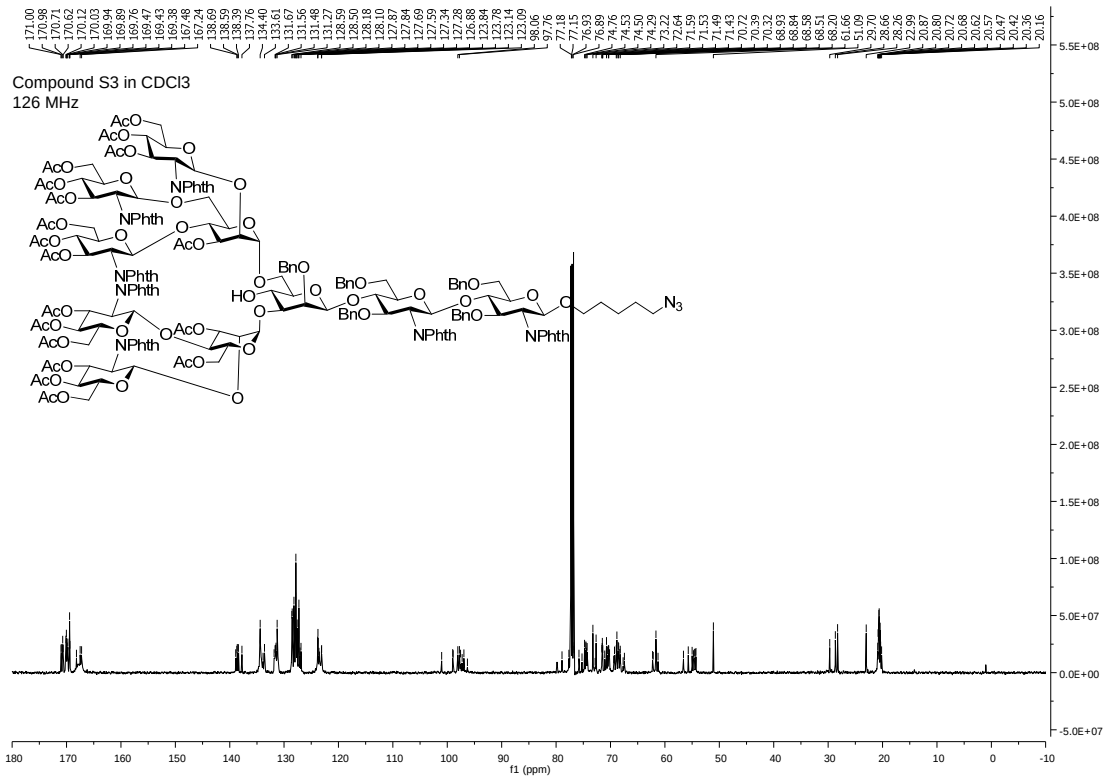
S2


Compound S2 in CDCl3
500 MHz
CDCl3
CH2Cl2
f1 (ppm)

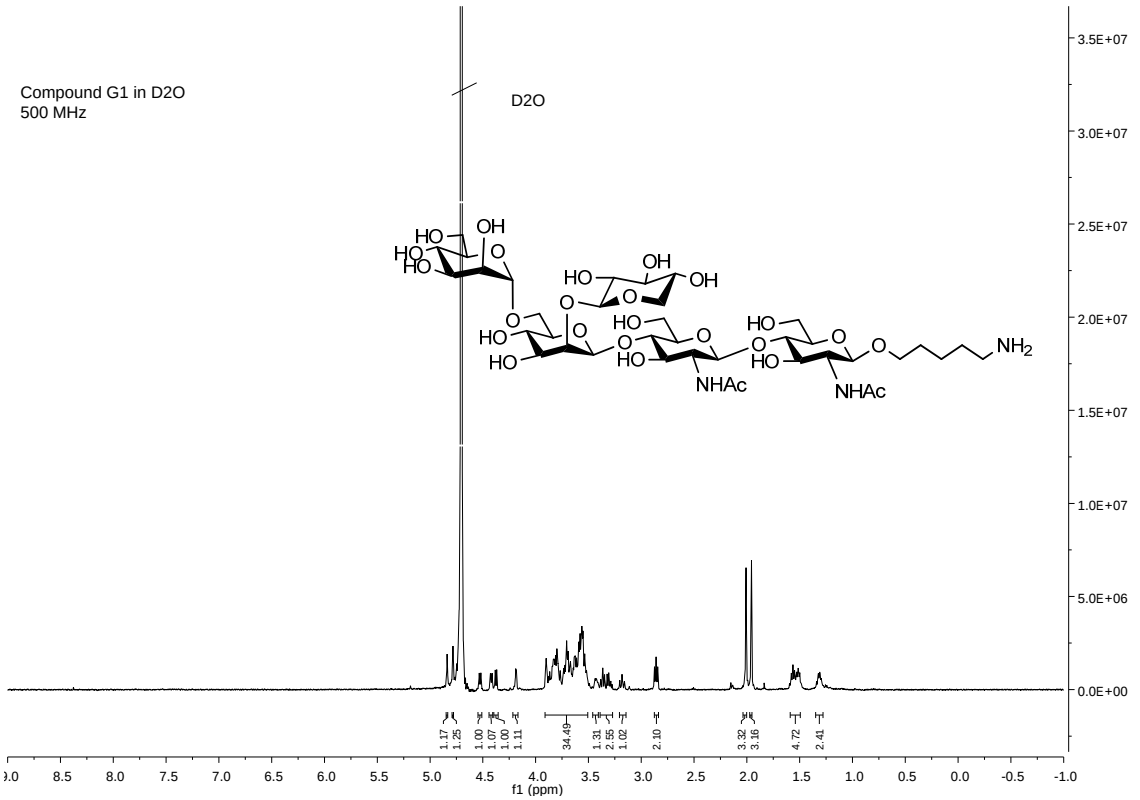

Compound S2 in CDCl3
126 MHz
f2 (ppm)
f1 (ppm)

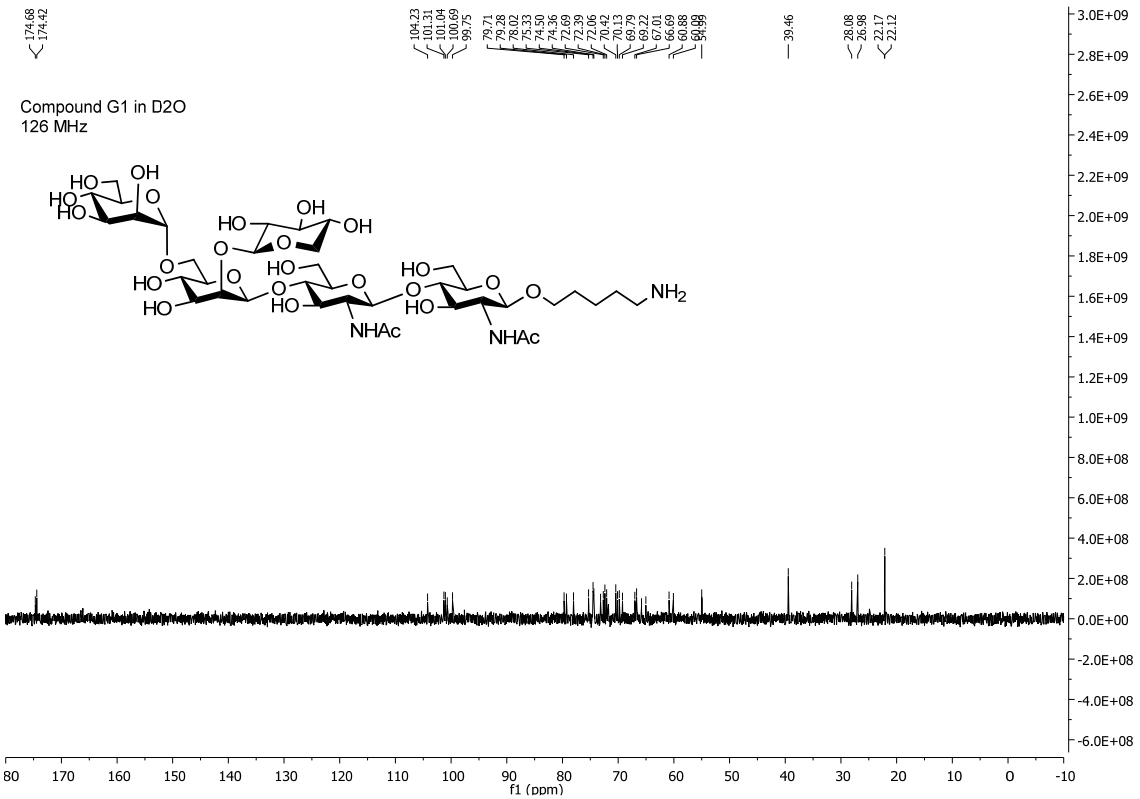
S3


Compound S3 in CDCl3
500 MHz
CH2Cl2
f1 (ppm)

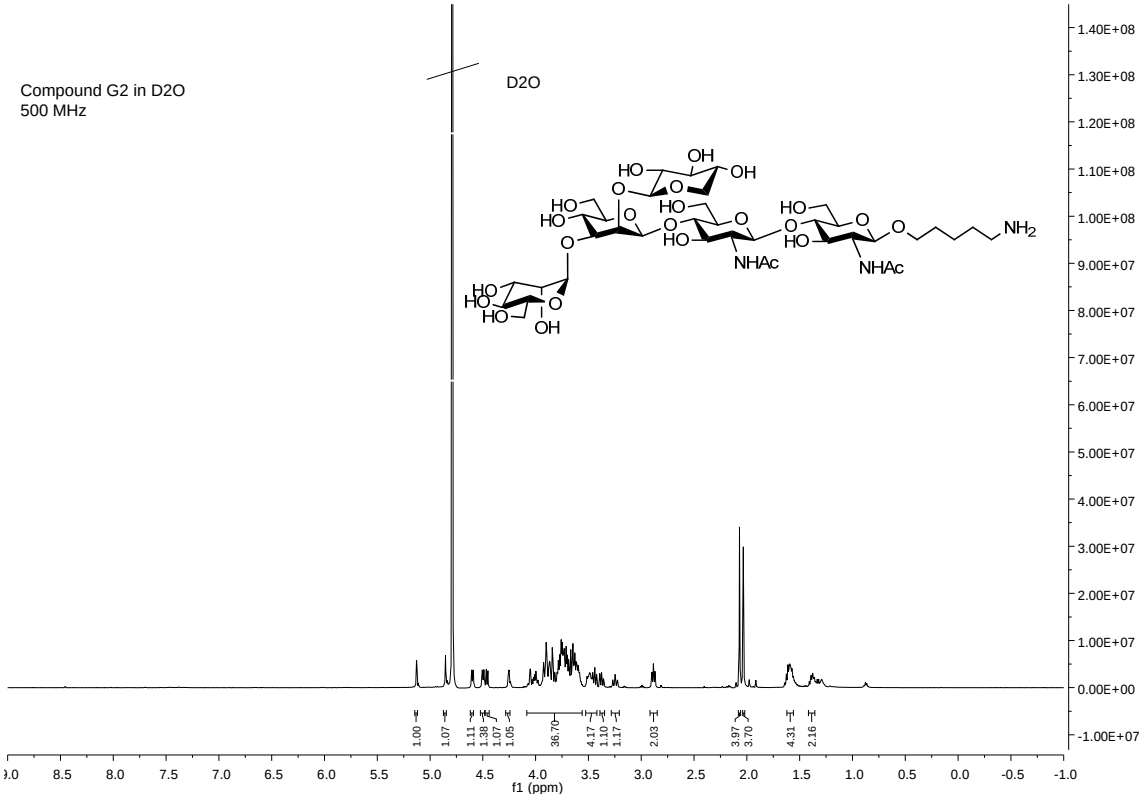

Compound S3 in CDCl3
126 MHz
f1 (ppm)

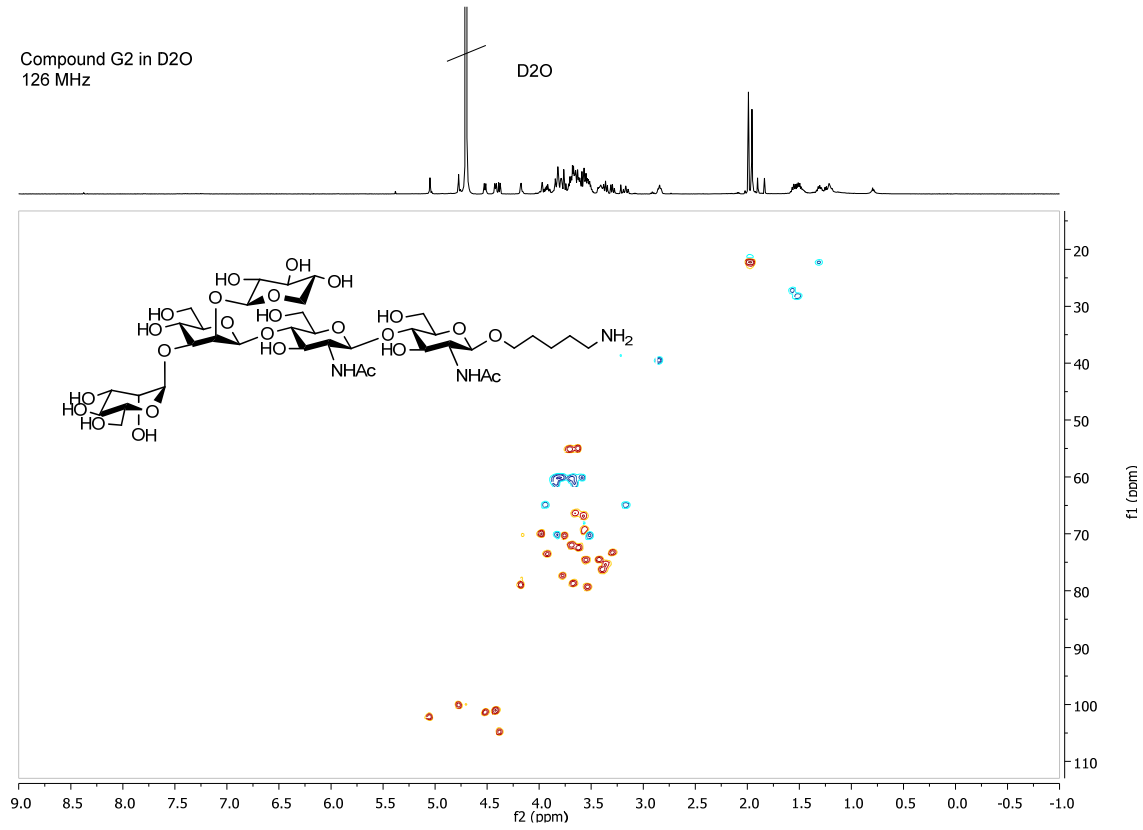
G1


Compound G1 in D2O
500 MHz
D2O
f1 (ppm)

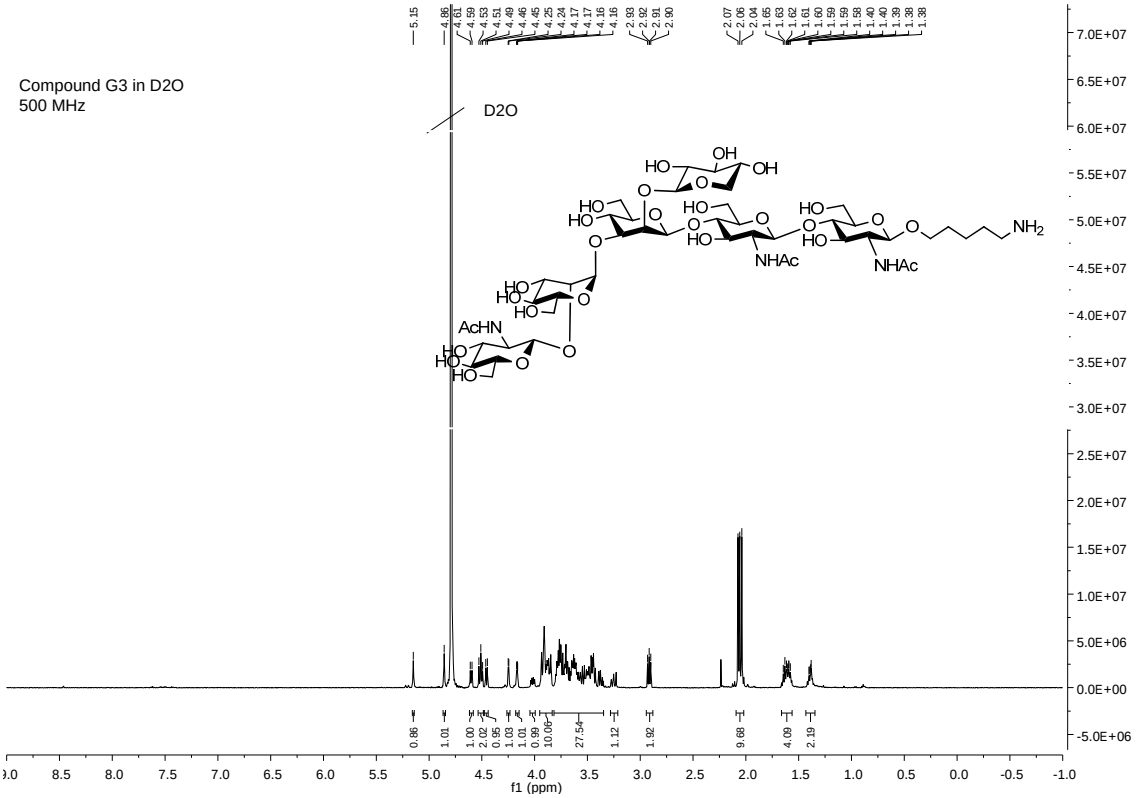

Compound G1 in D2O
126 MHz
f1 (ppm)

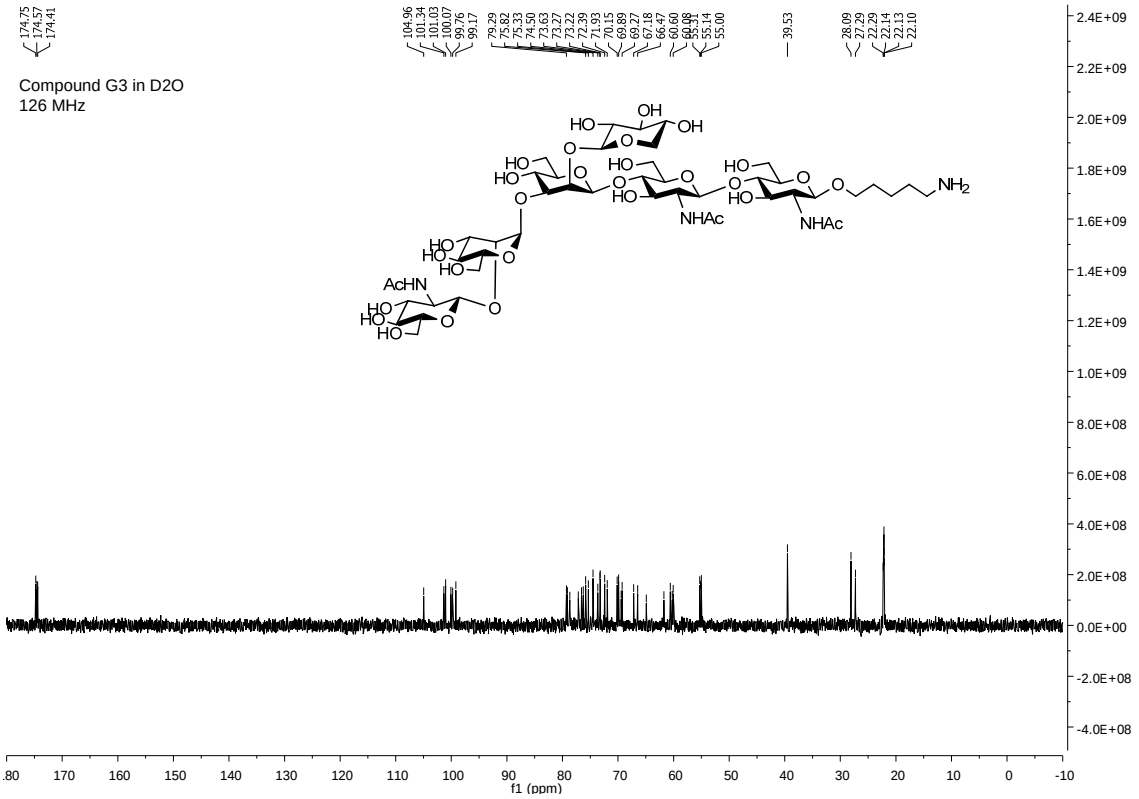
G2


Compound G2 in D2O
500 MHz
D2O
NHAc
NHAc
NH2
1.00
1.07
1.11
1.38
1.07
1.05
36.70
4.17
1.10
1.17
2.03
3.97
3.70
4.31
2.16
f1 (ppm)

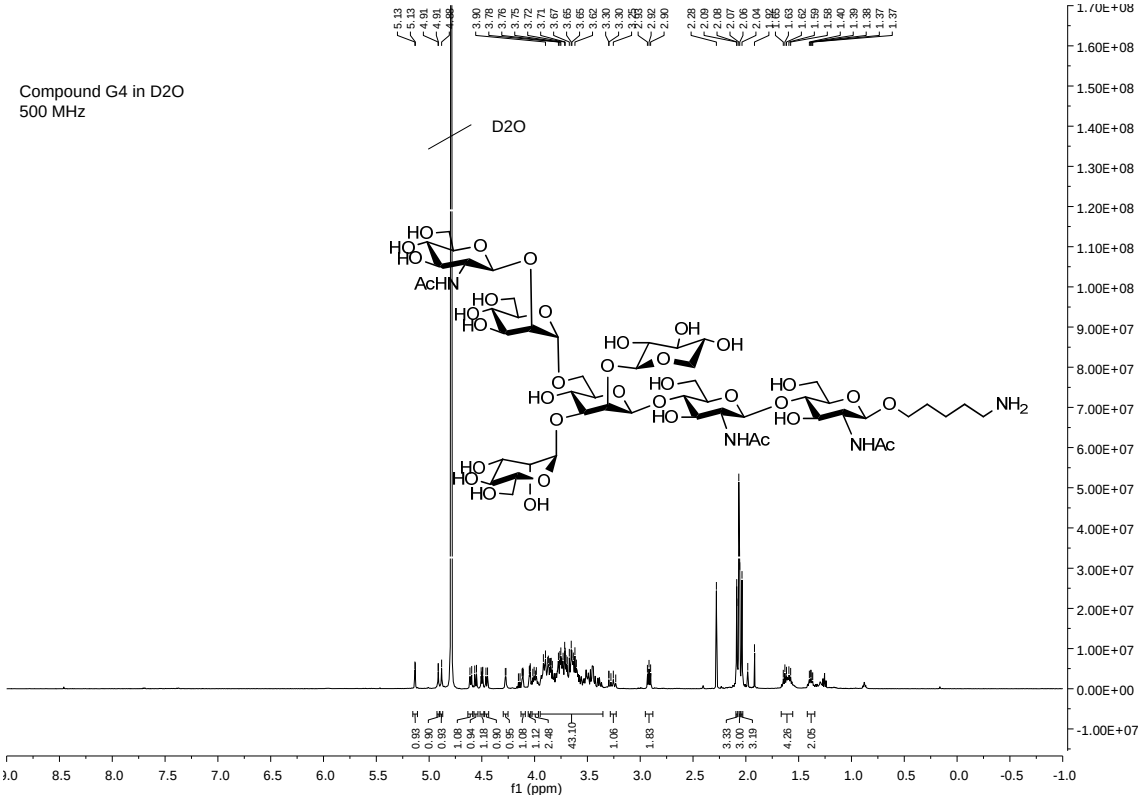

Compound G2 in D2O
126 MHz
D2O
NHAc
NHAc
NH2
f2 (ppm)
f1 (ppm)

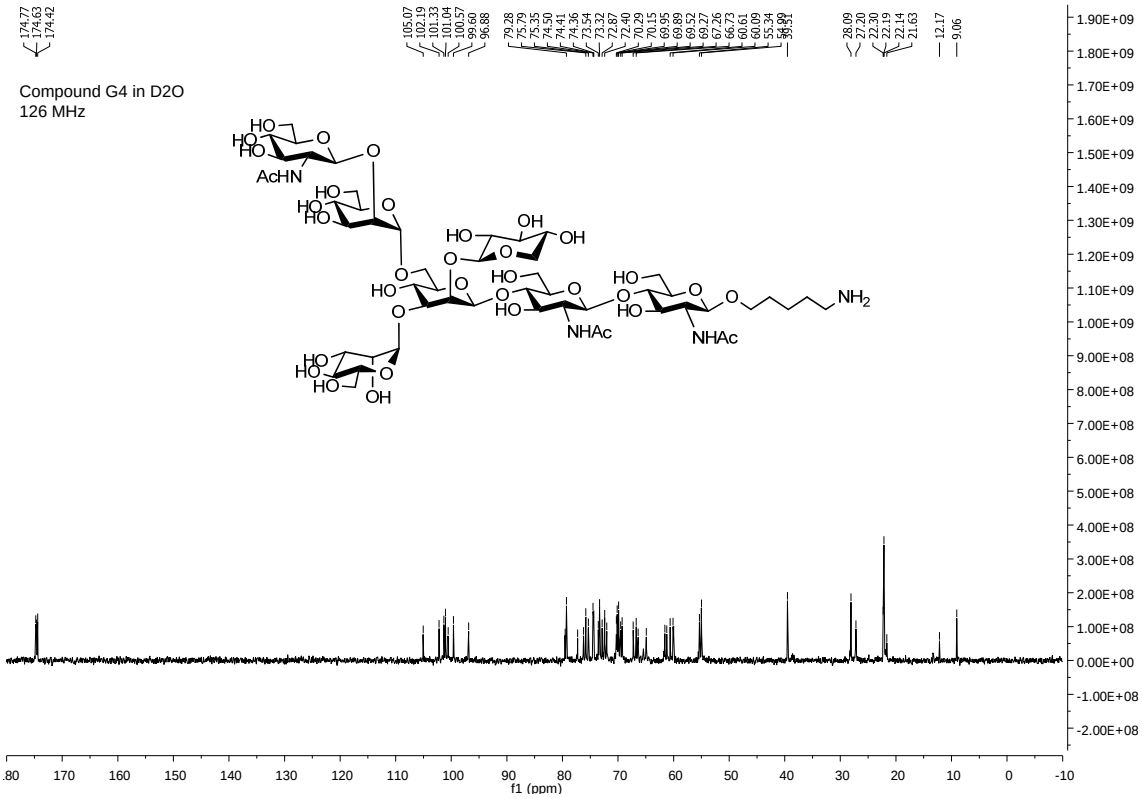
G3


Compound G3 in D2O
500 MHz
D2O
f1 (ppm)

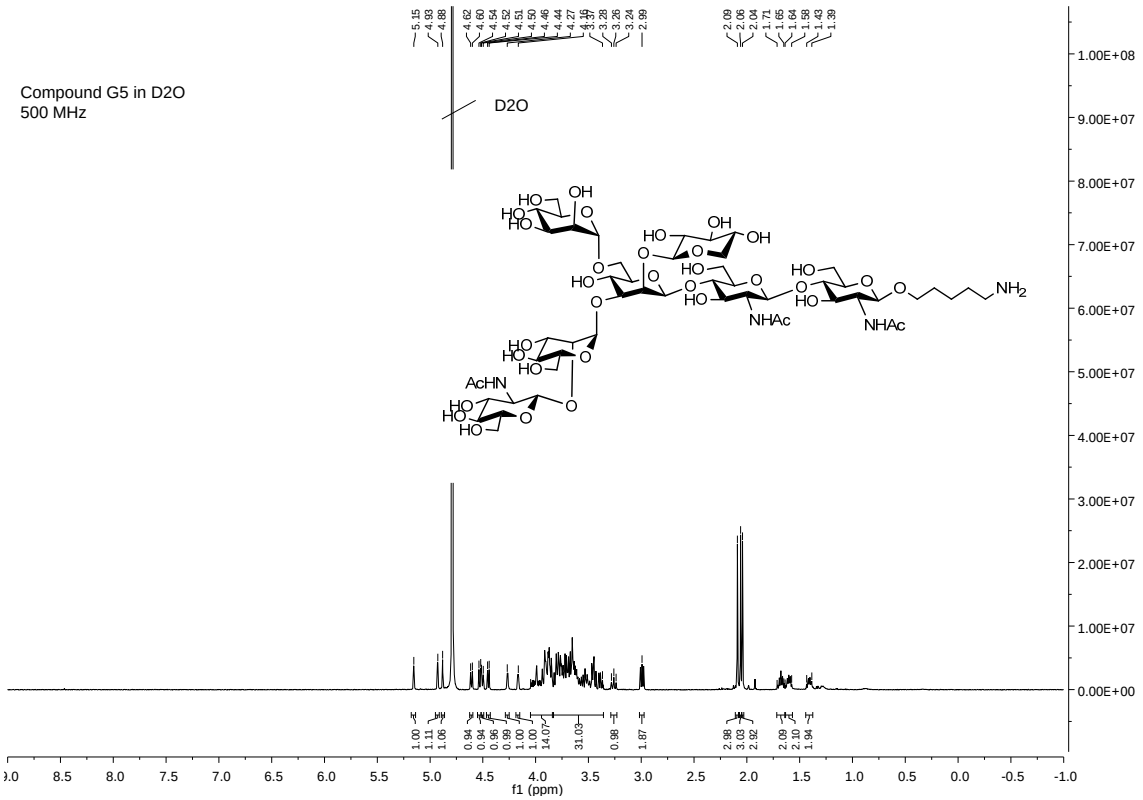

Compound G3 in D2O
126 MHz
f1 (ppm)

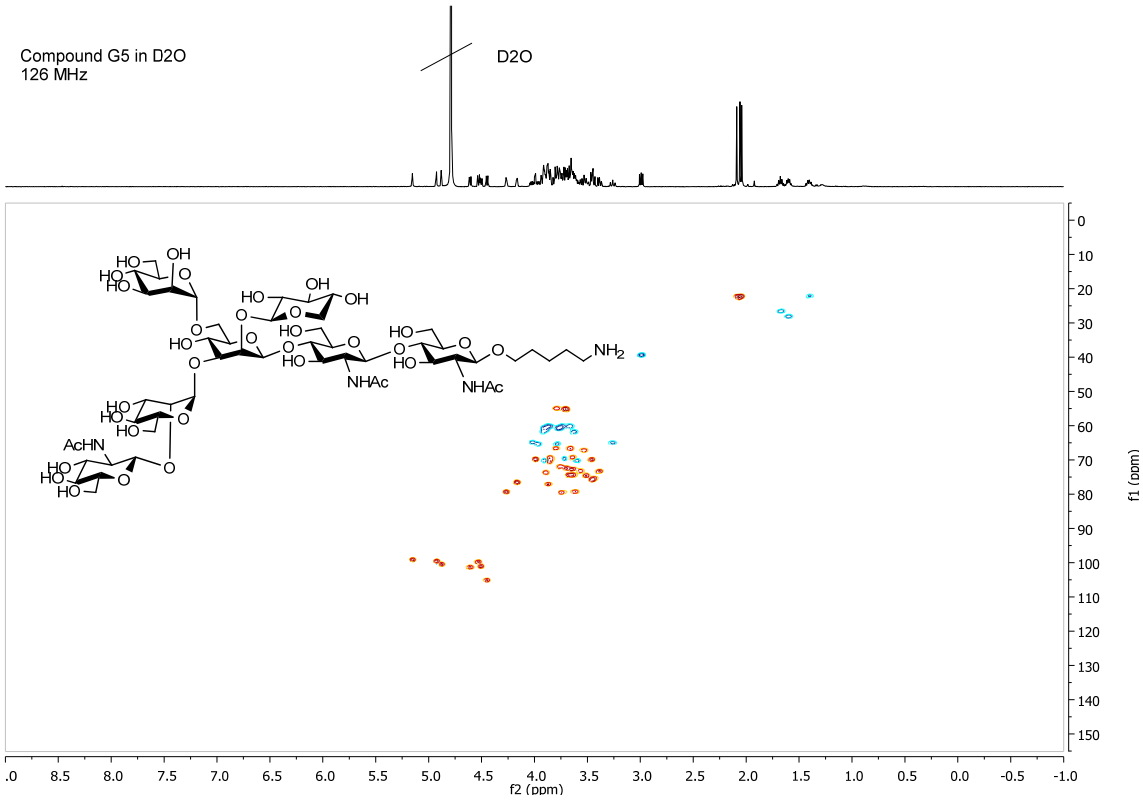
G4


Compound G4 in D2O
500 MHz
D2O
f1 (ppm)

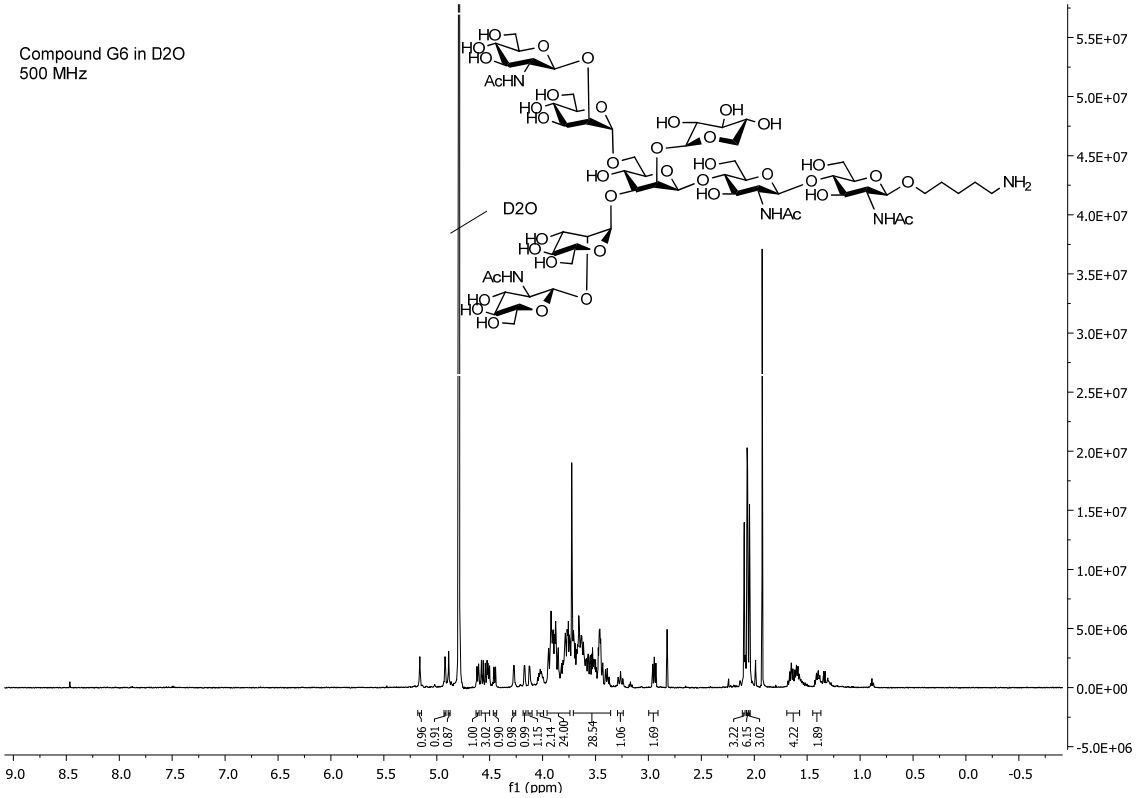

Compound G4 in D2O
126 MHz
f1 (ppm)

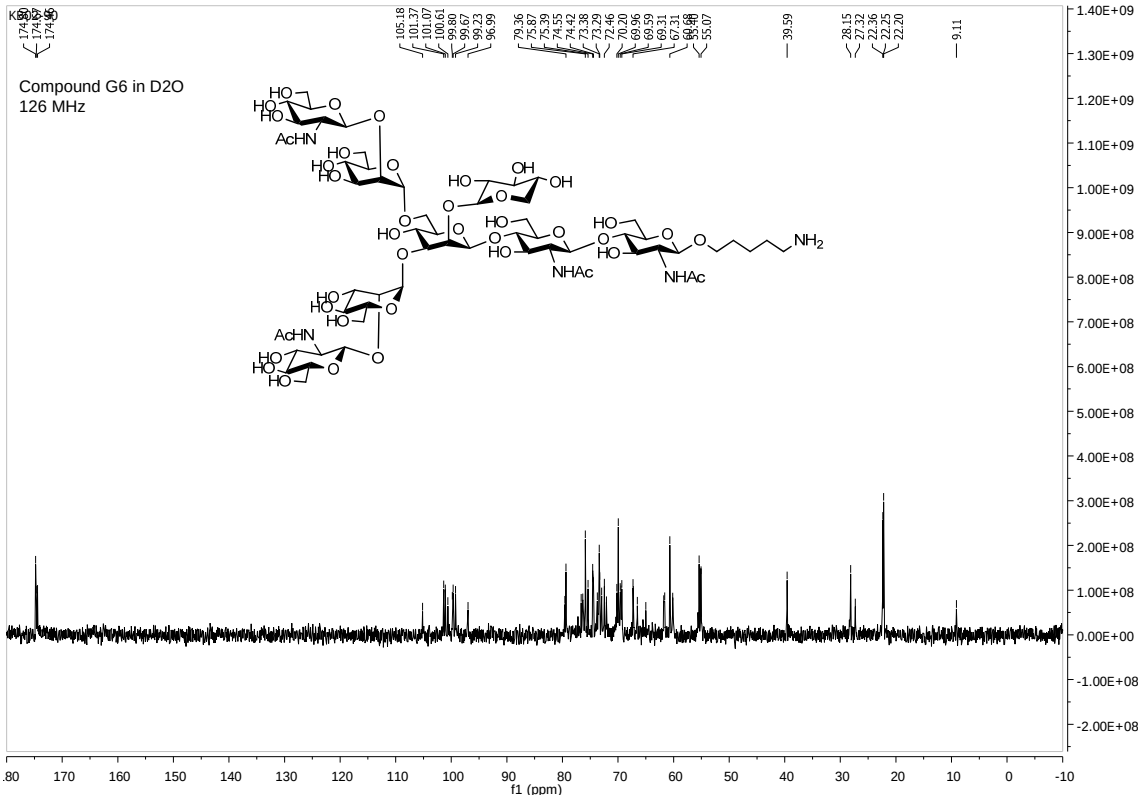
G5


Compound G5 in D2O
500 MHz
D2O
f1 (ppm)

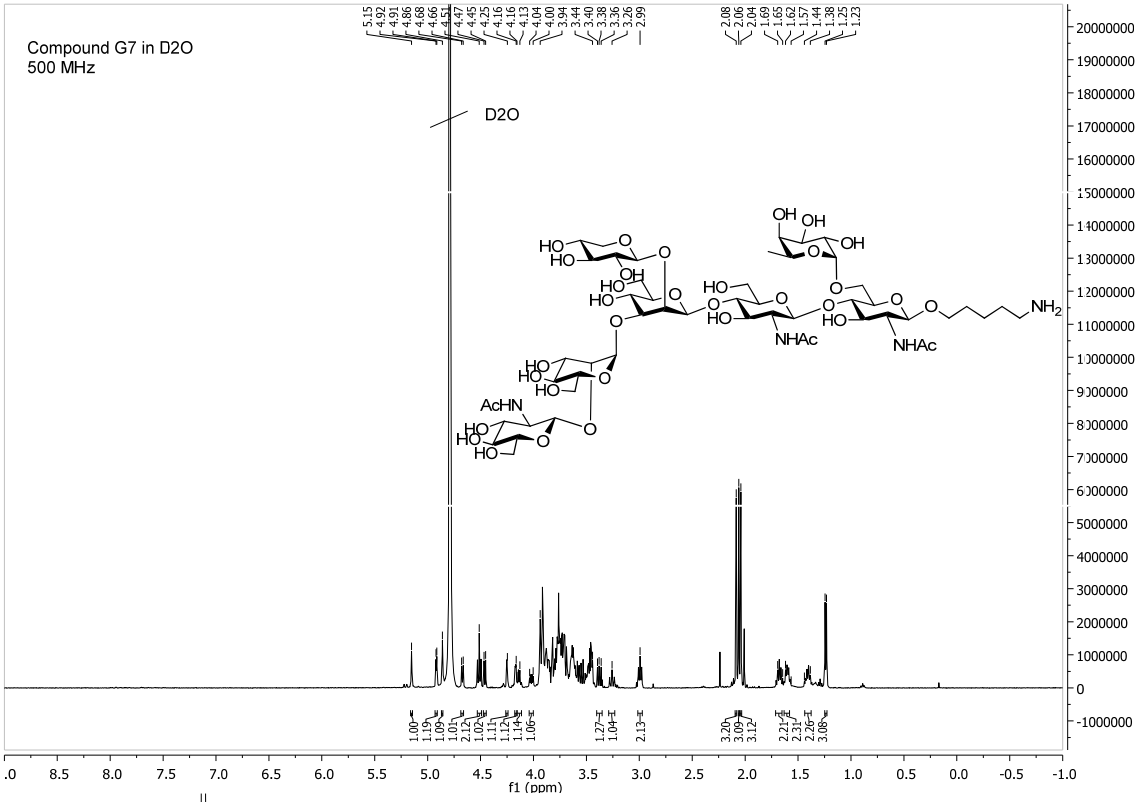

Compound G5 in D2O
126 MHz
D2O
f2 (ppm)
f1 (ppm)

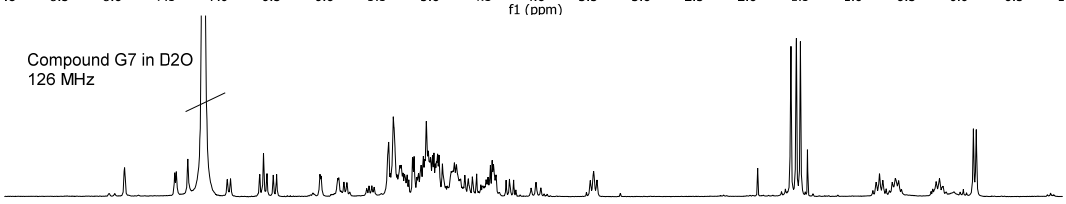
G6

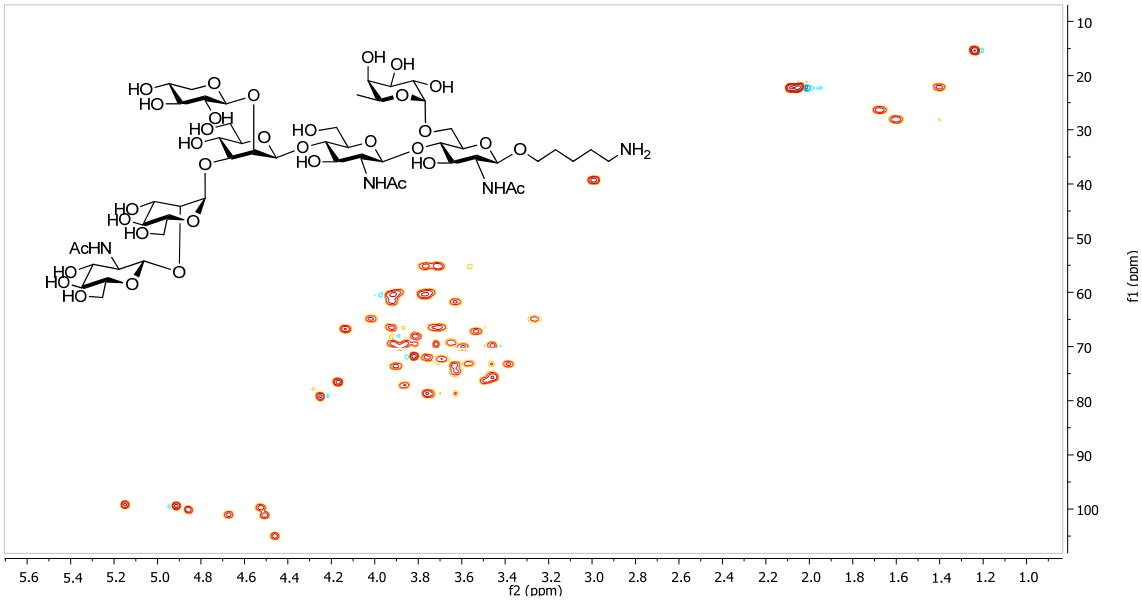

Compound G6 in D2O
500 MHz
D2O
NHAc
NHAc
NH2
f1 (ppm)


Compound G6 in D2O
126 MHz
NHAc
NHAc
NH2
f1 (ppm)

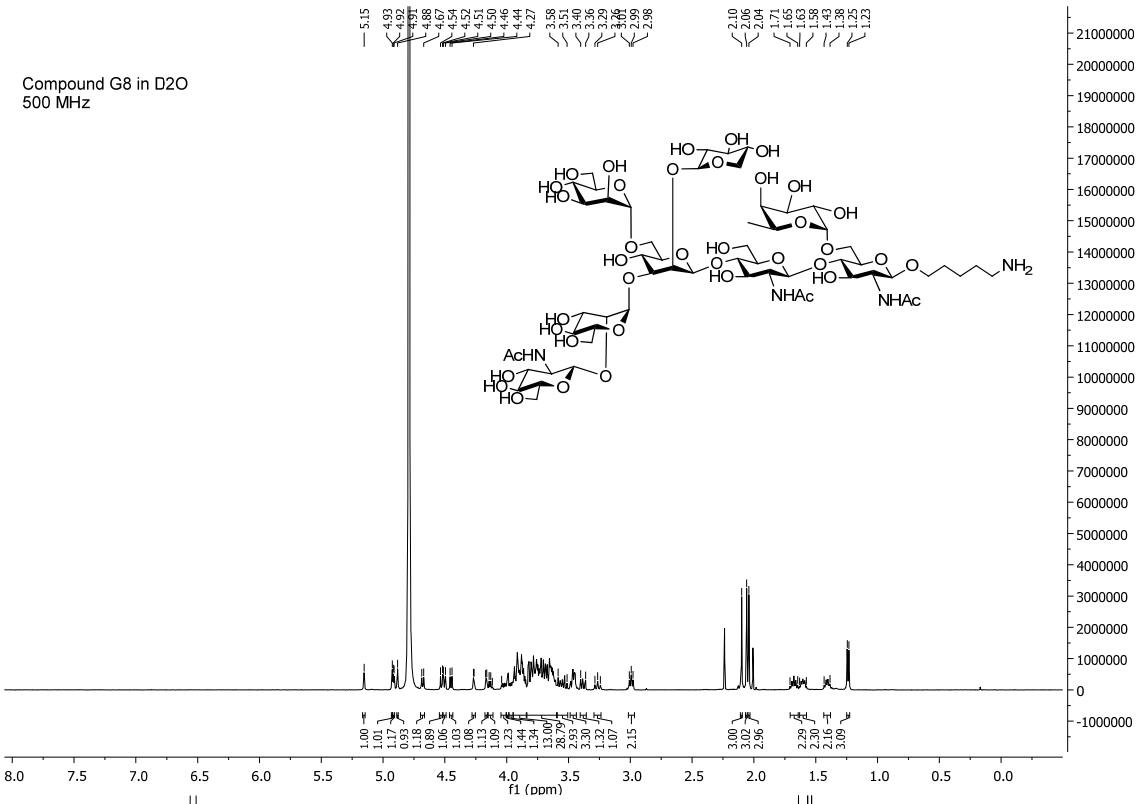
G7

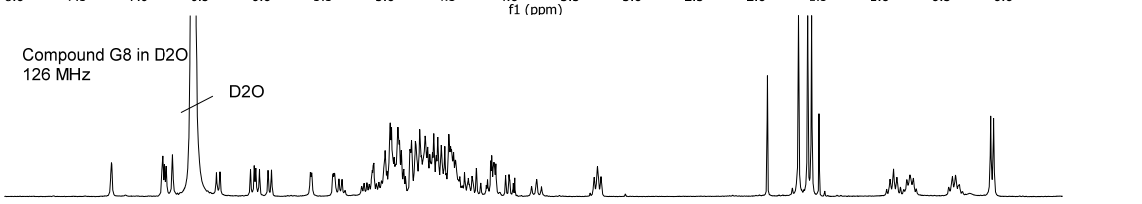

Compound G7 in D2O
500 MHz
D2O
AcHN
NHAc
NHAc
NH2
f1 (ppm)

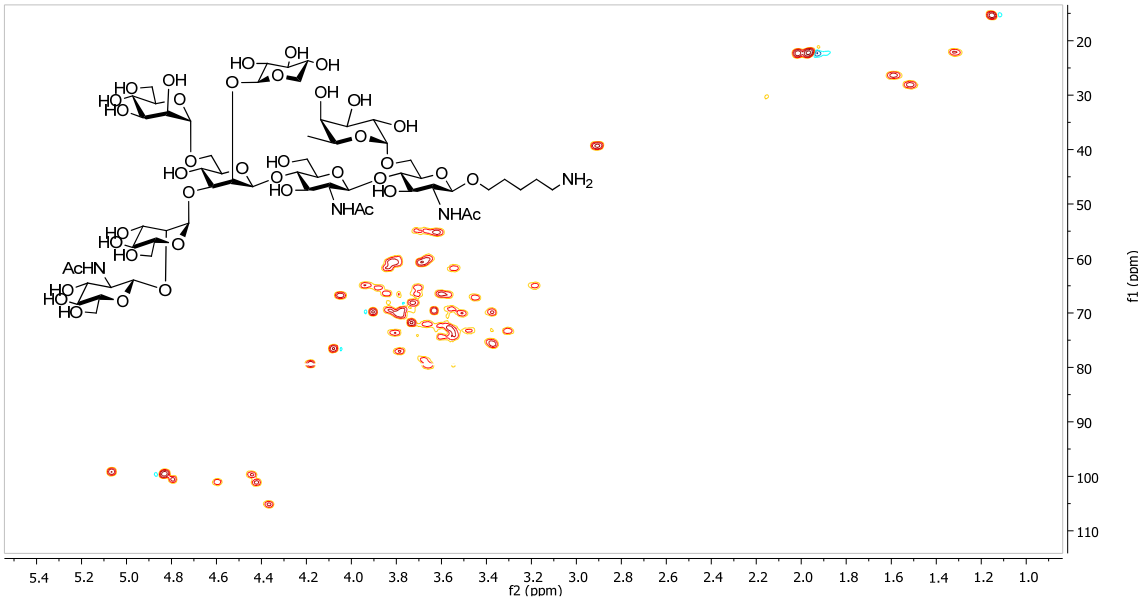

Compound G7 in D2O
126 MHz


AcHN
NHAc
NHAc
NH2
f2 (ppm)
f1 (ppm)

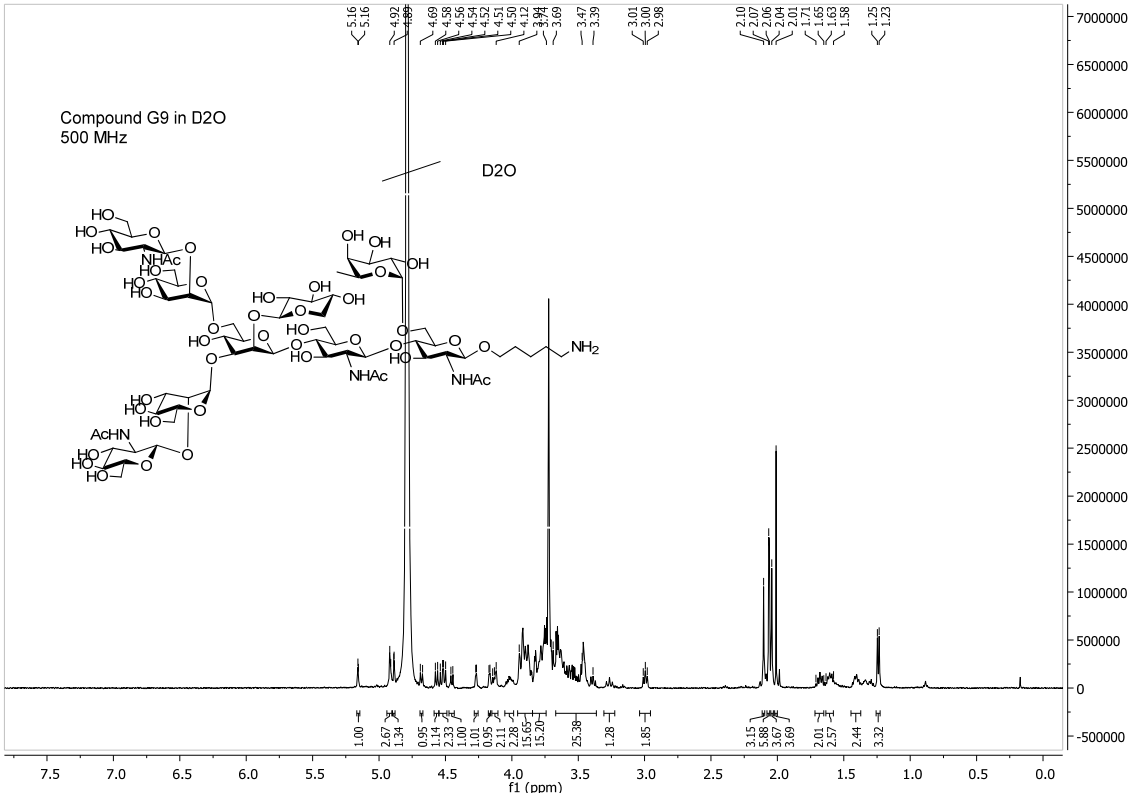
G8

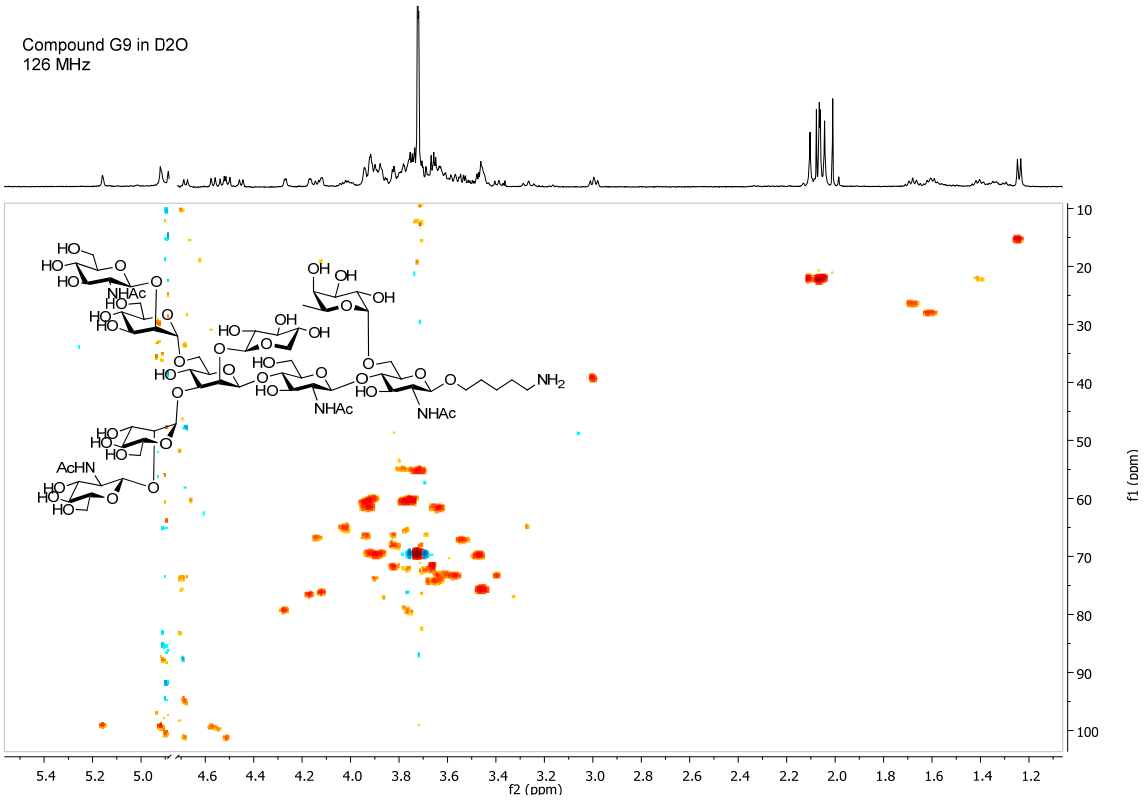

Compound G8 in D2O
500 MHz
f1 (ppm)


Compound G8 in D2O
126 MHz
D2O

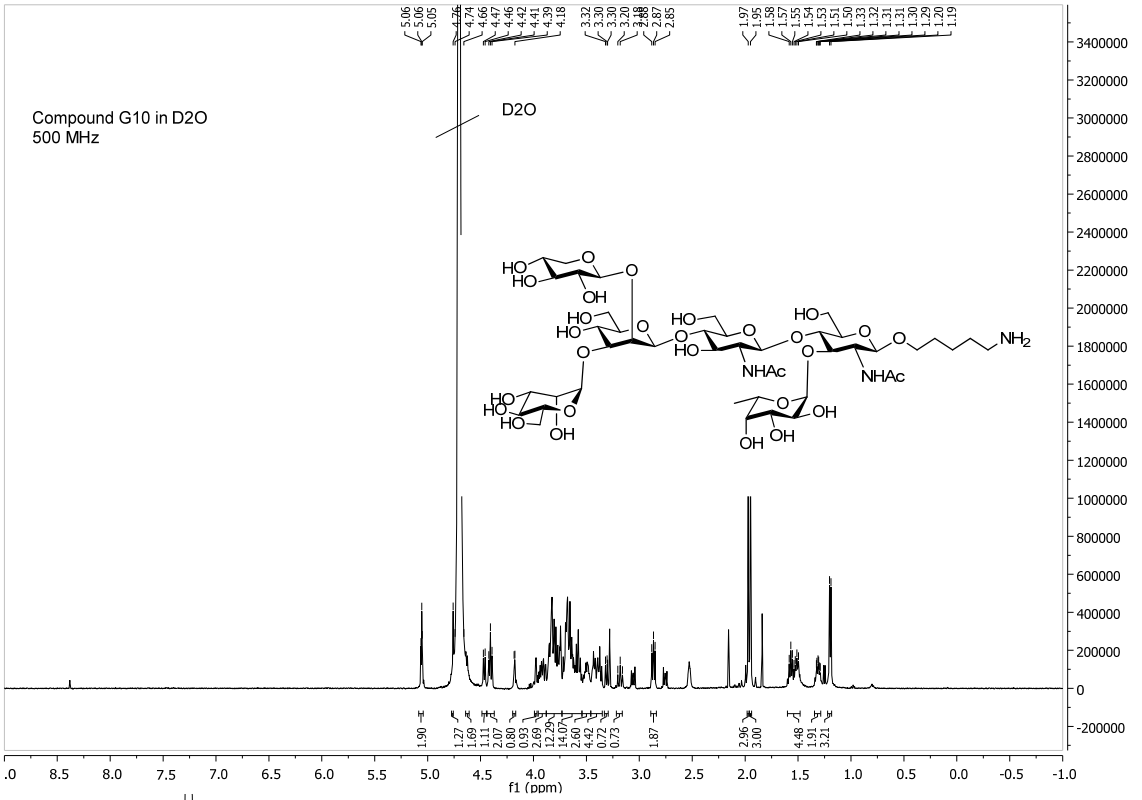

f2 (ppm)
f1 (ppm)

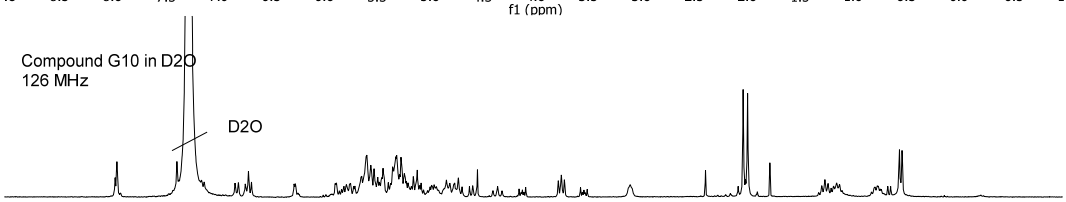
G9

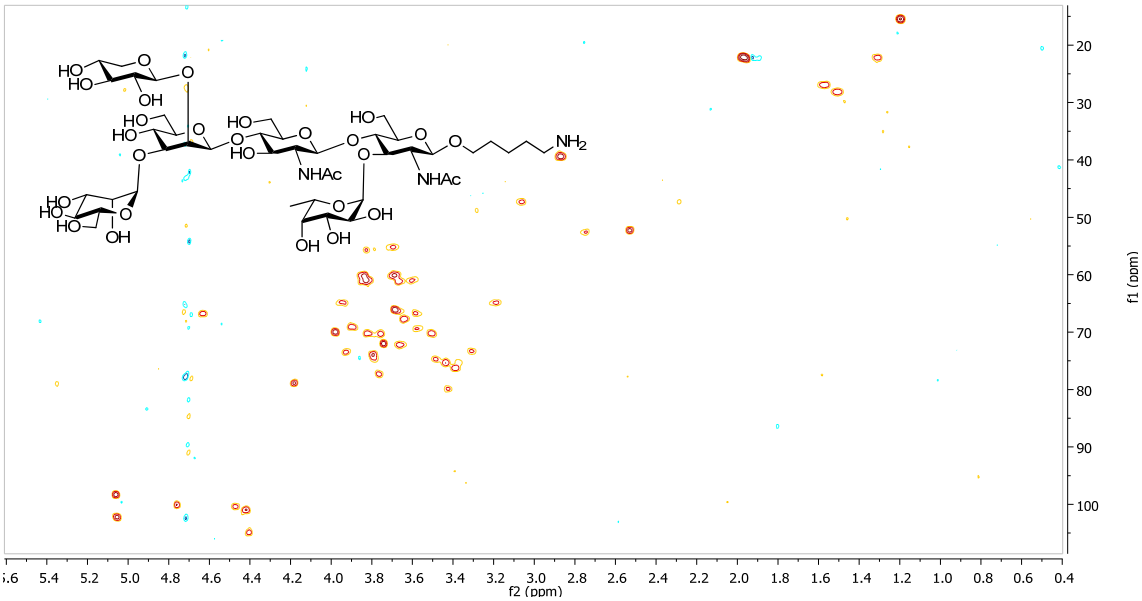

Compound G9 in D2O
500 MHz
D2O
f1 (ppm)


Compound G9 in D2O
126 MHz
f2 (ppm)
f1 (ppm)

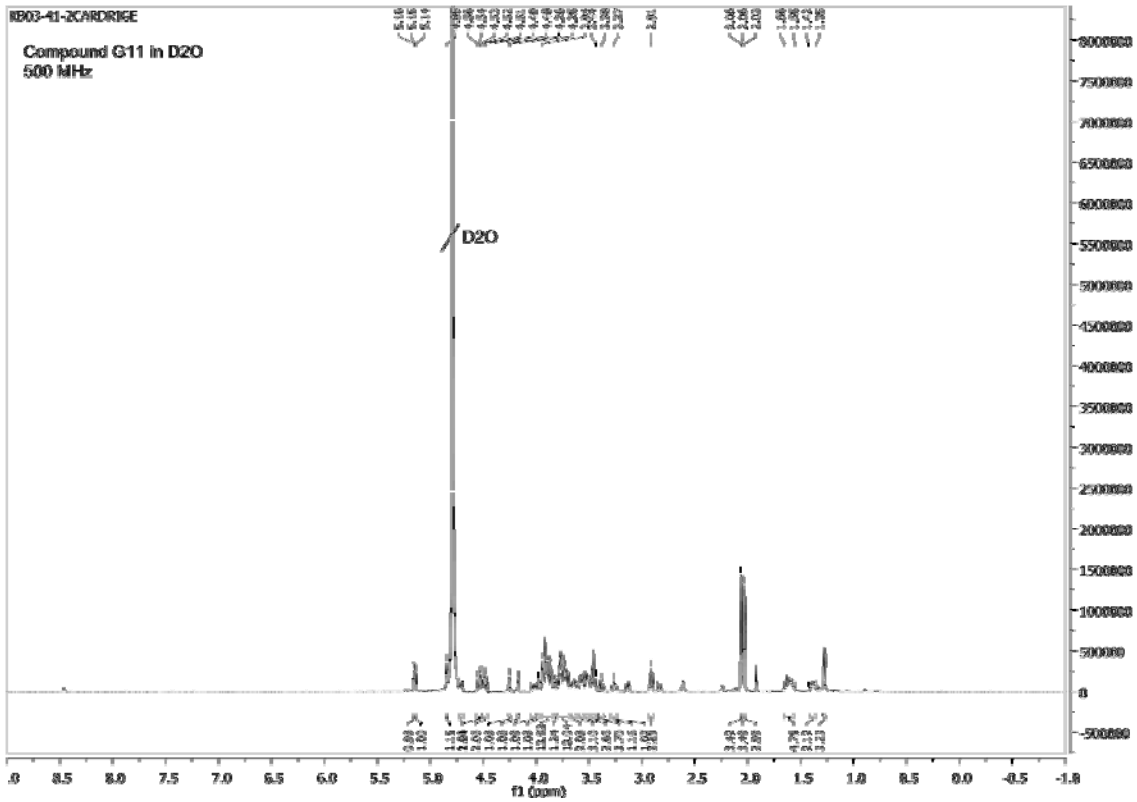
G10

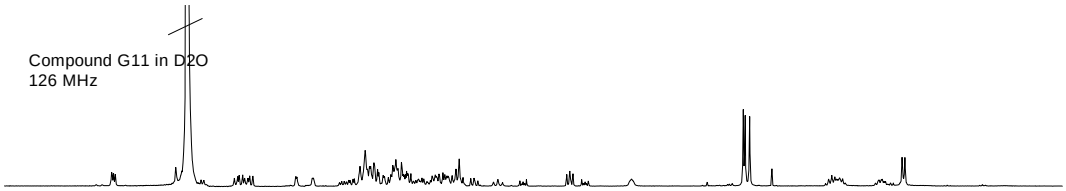

Compound G10 in D2O
500 MHz
D2O
f1 (ppm)

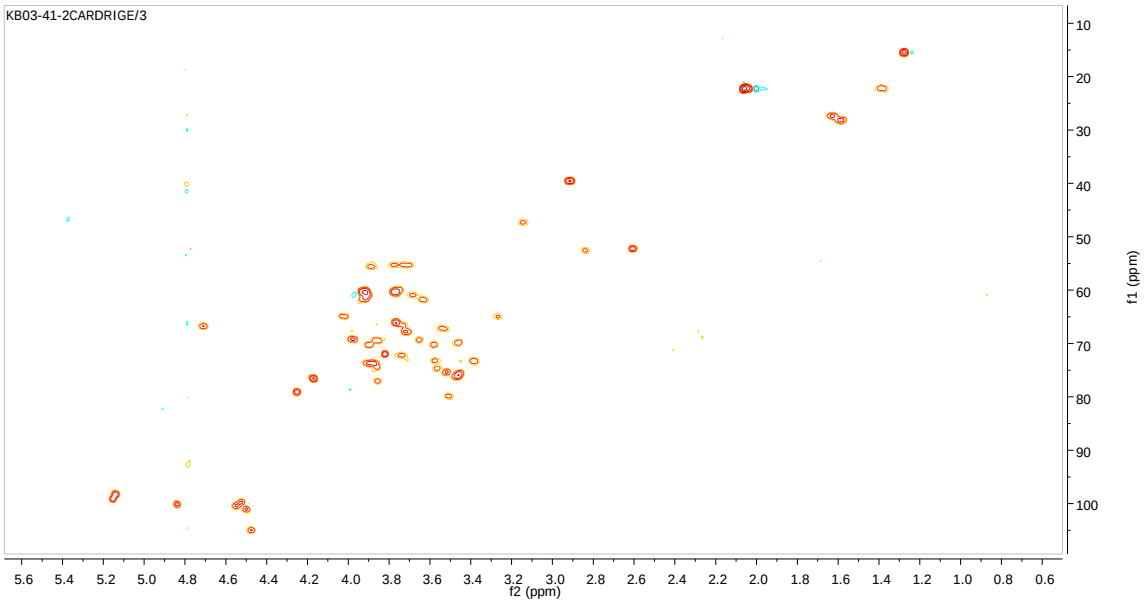

Compound G10 in D2O
126 MHz
D2O


f2 (ppm)
f1 (ppm)

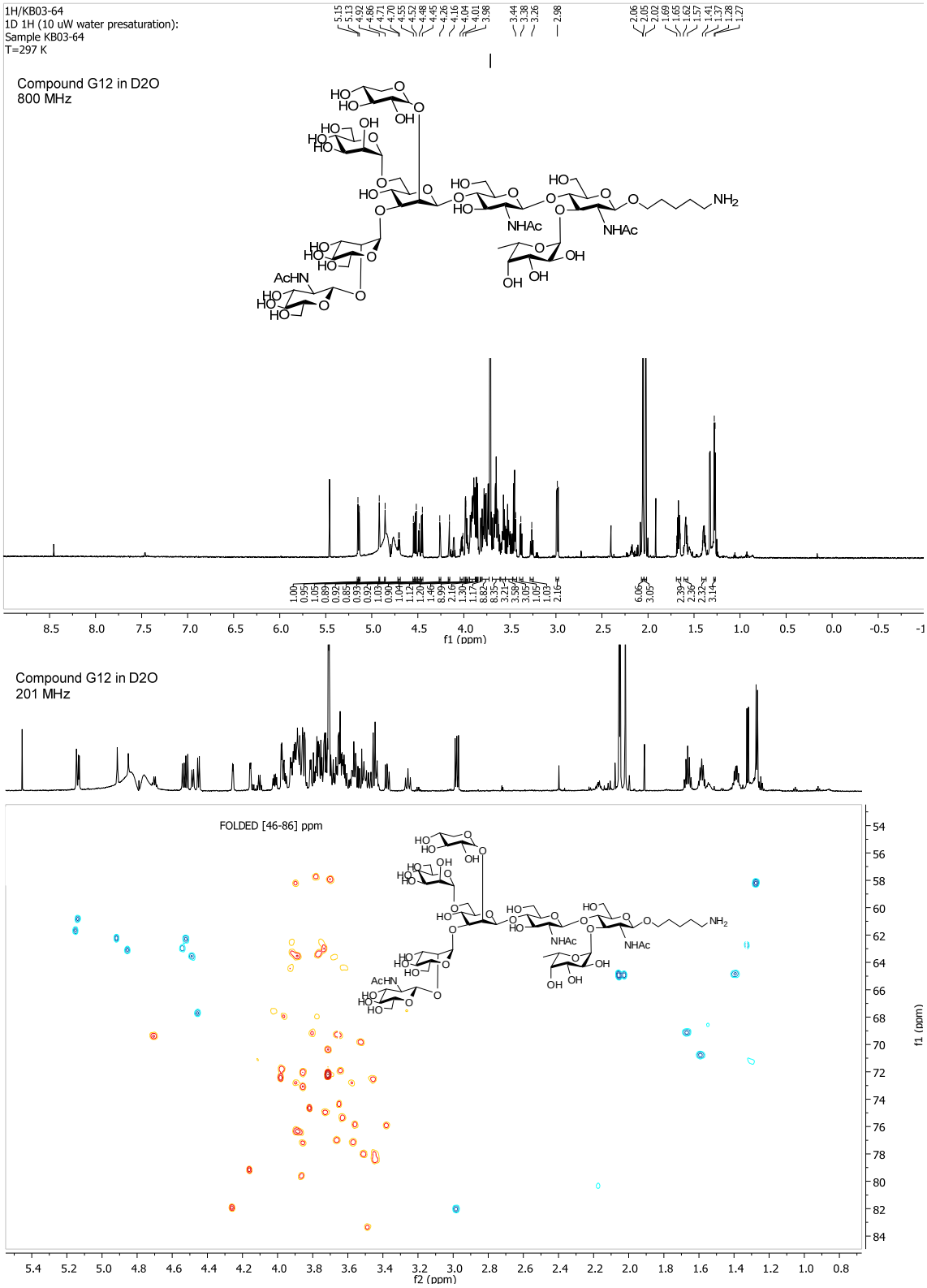
G11


KB03-41-2CARDRIGE
Compound G11 in D2O
500 MHz
D2O
f1 (ppm)

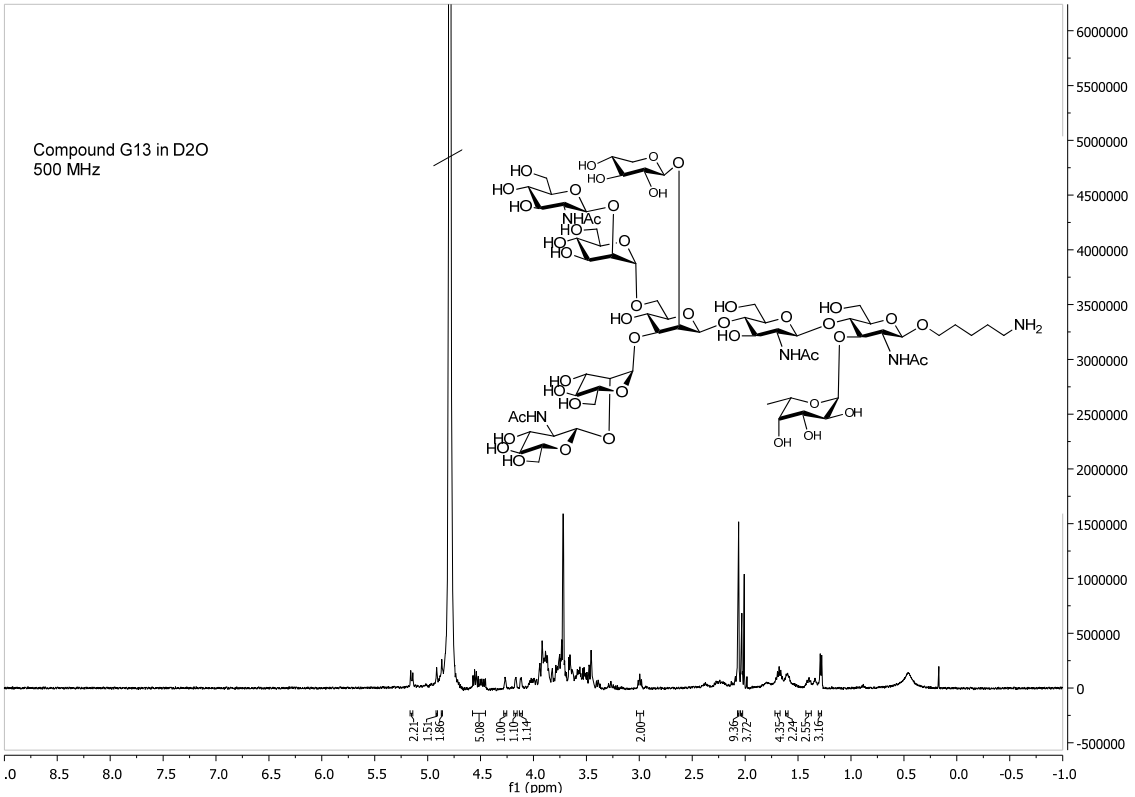

Compound G11 in D2O
126 MHz

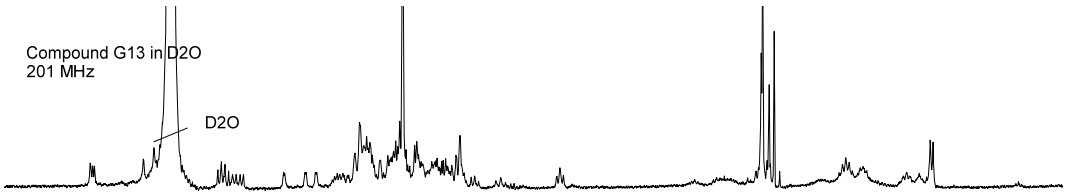

KB03-41-2CARDRIGE/3
f2 (ppm)
f1 (ppm)

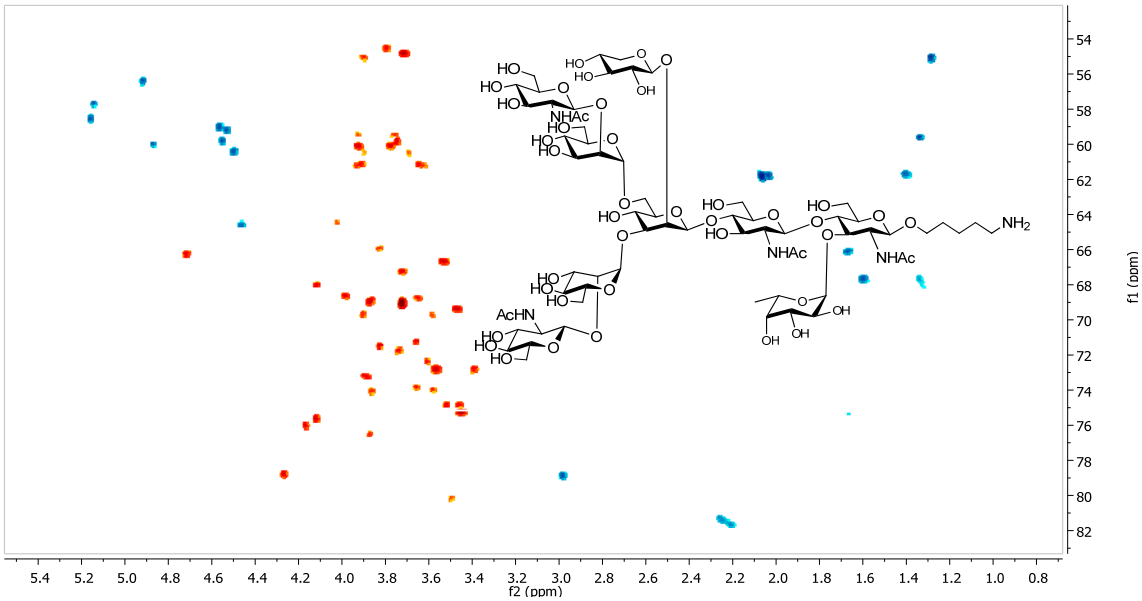
G12


1H/KB03-64
1D 1H (10 uW water presaturation):
Sample KB03-64
T=297 K
Compound G12 in D2O
800 MHz
f1 (ppm)
Compound G12 in D2O
201 MHz
FOLDED [46-86] ppm
f2 (ppm)
f1 (ppm)

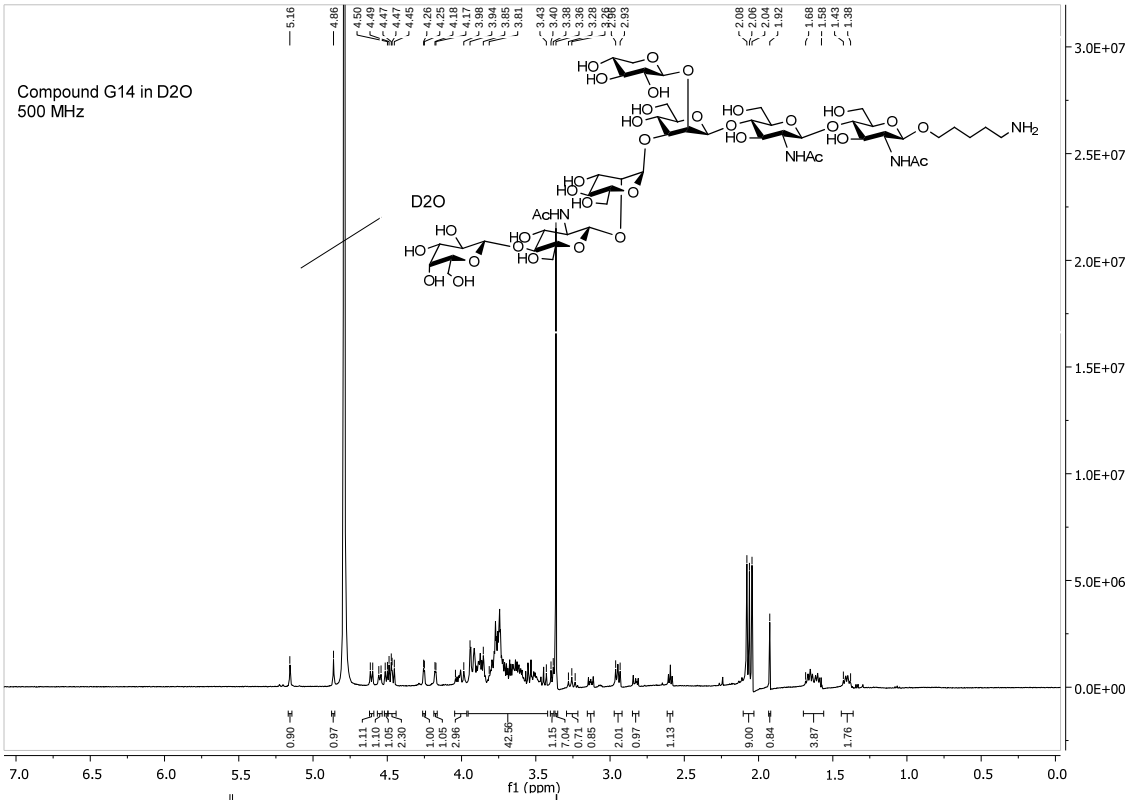
G13

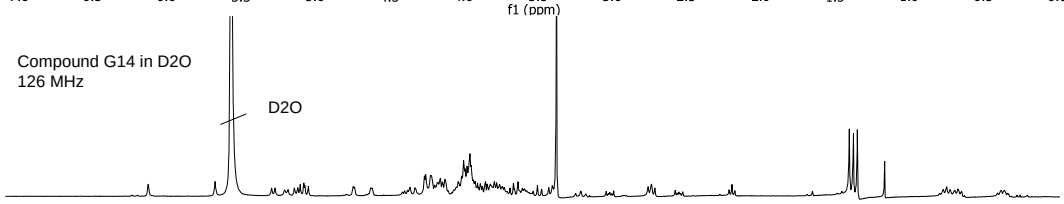

Compound G13 in D2O
500 MHz
NHAc
AcHN
NH2
f1 (ppm)

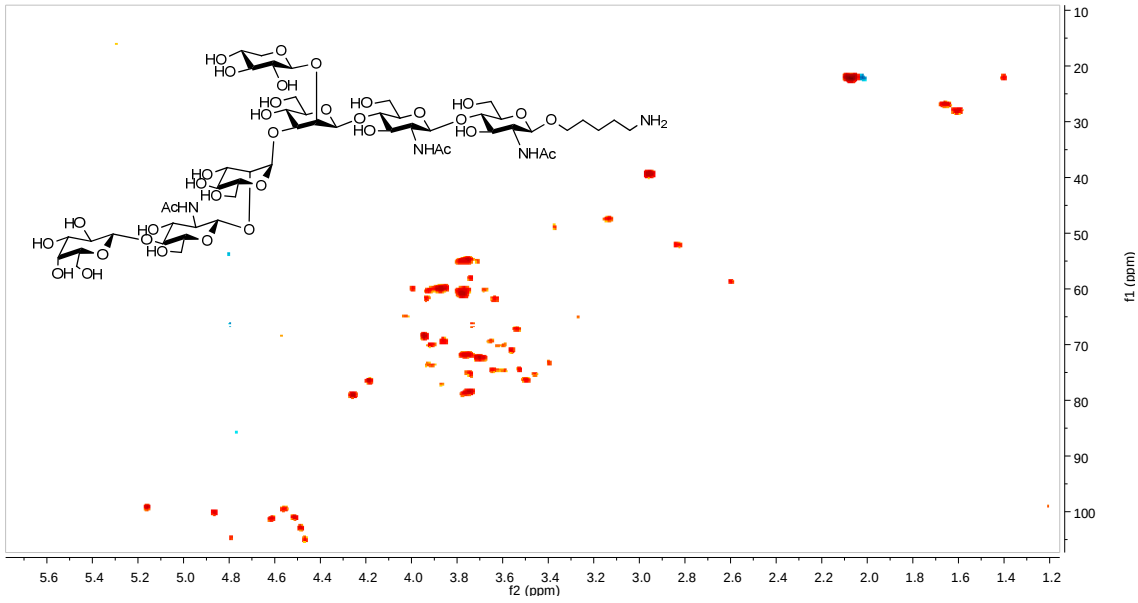

Compound G13 in D2O
201 MHz
D2O


NHAc
AcHN
NH2
f2 (ppm)
f1 (ppm)

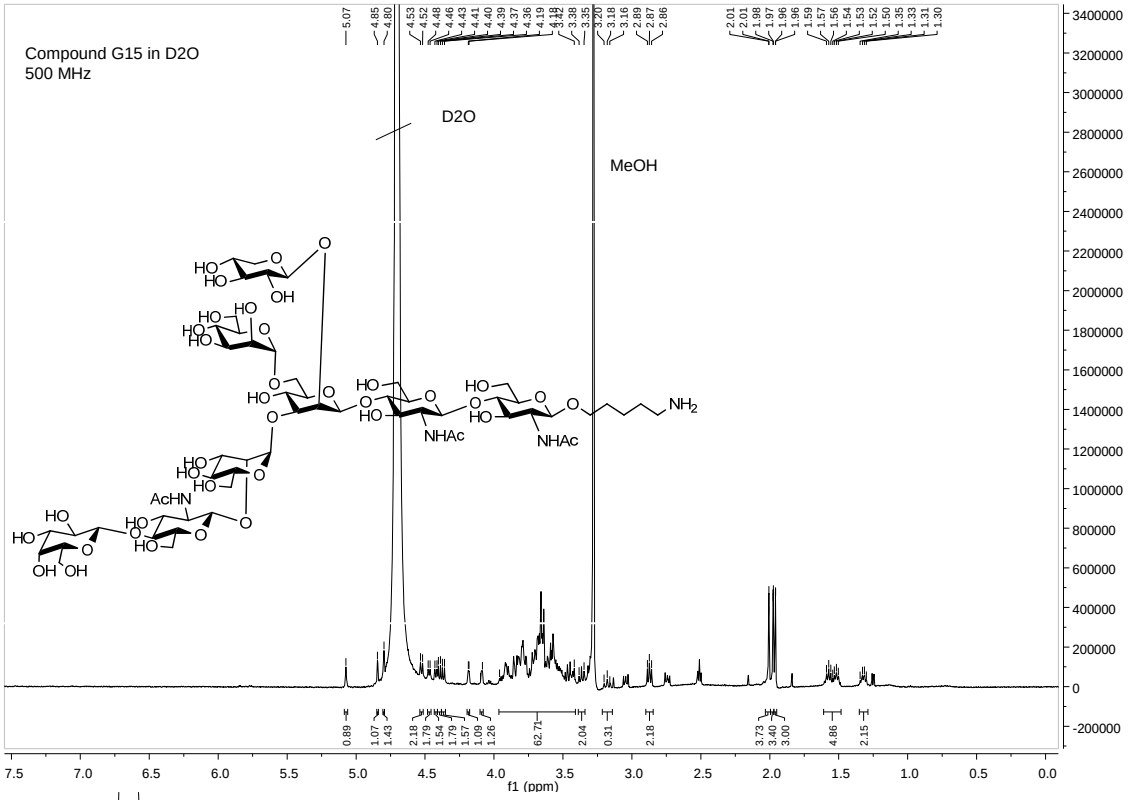
G14

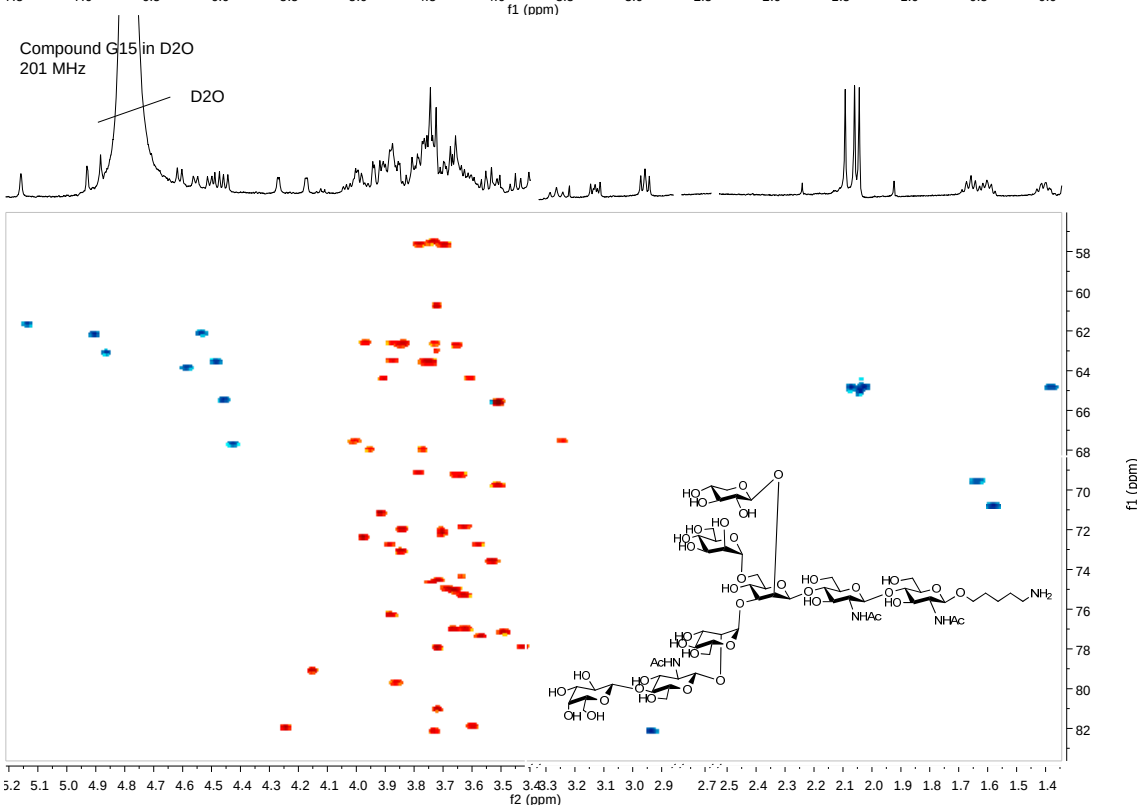

Compound G14 in D2O
500 MHz
D2O
f1 (ppm)


Compound G14 in D2O
126 MHz
D2O

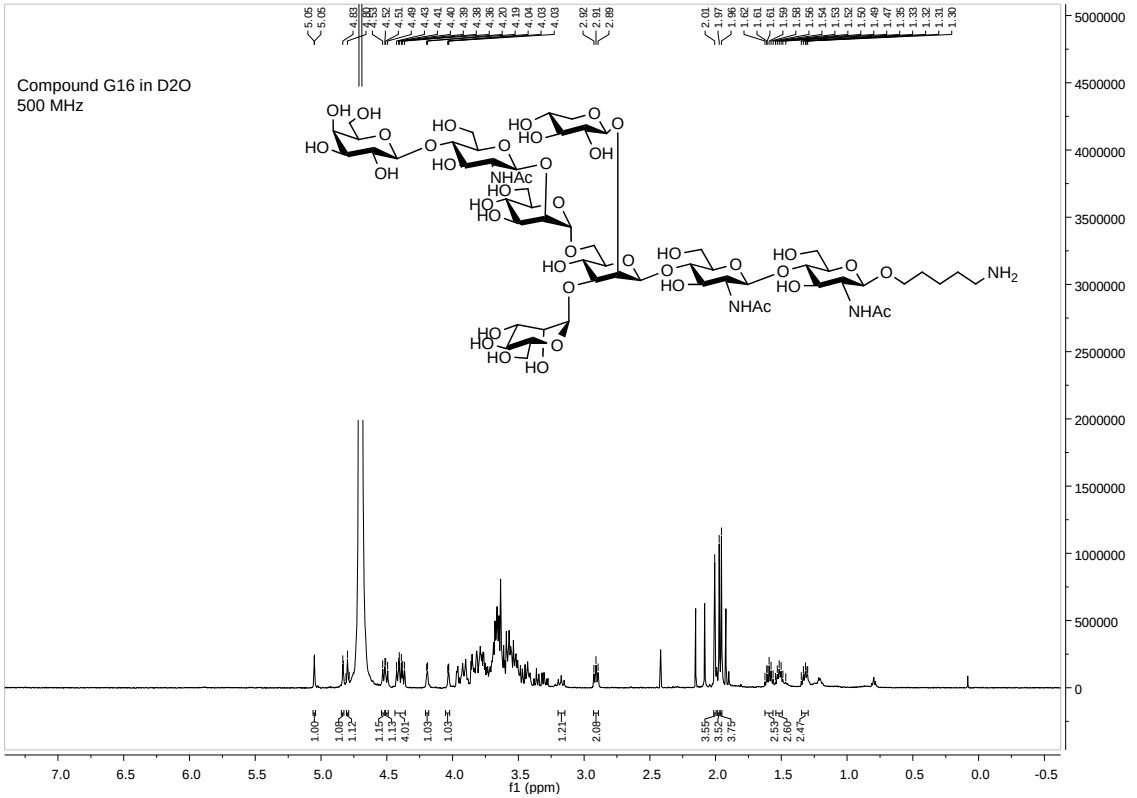

f2 (ppm)
f1 (ppm)

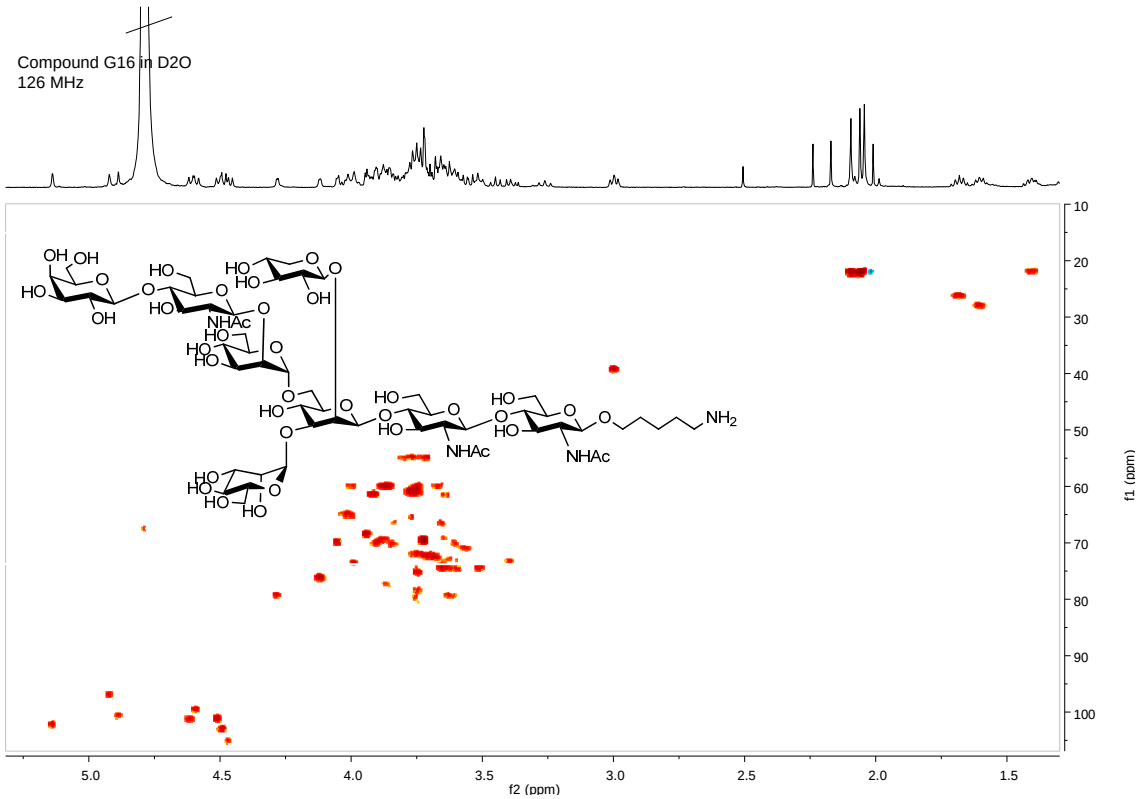
G15


Compound G15 in D2O
500 MHz
D2O
MeOH
NHAc
NHAc
AcHN
NH2
f1 (ppm)

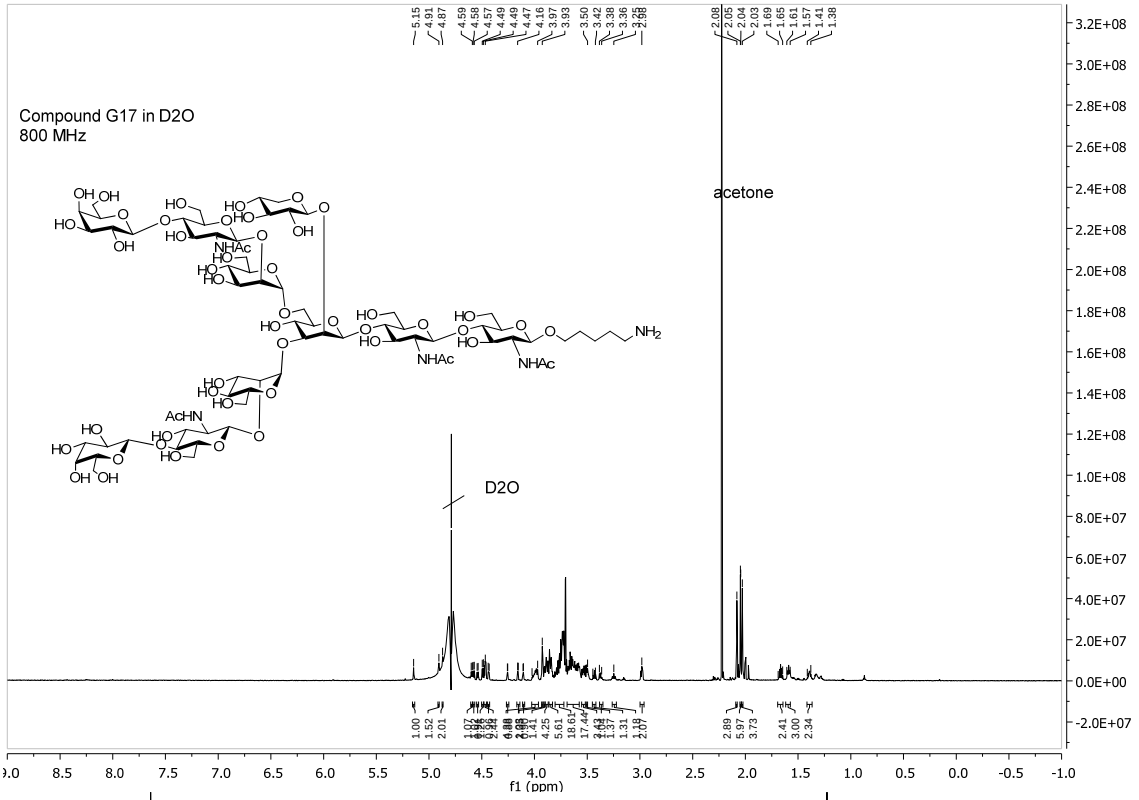

Compound G15 in D2O
201 MHz
D2O
NHAc
NHAc
AcHN
NH2
f2 (ppm)
f1 (ppm)

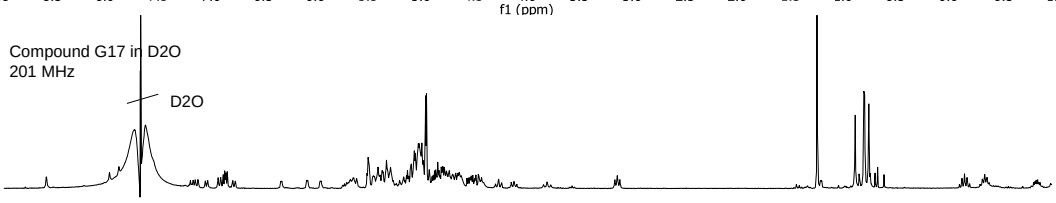
G16

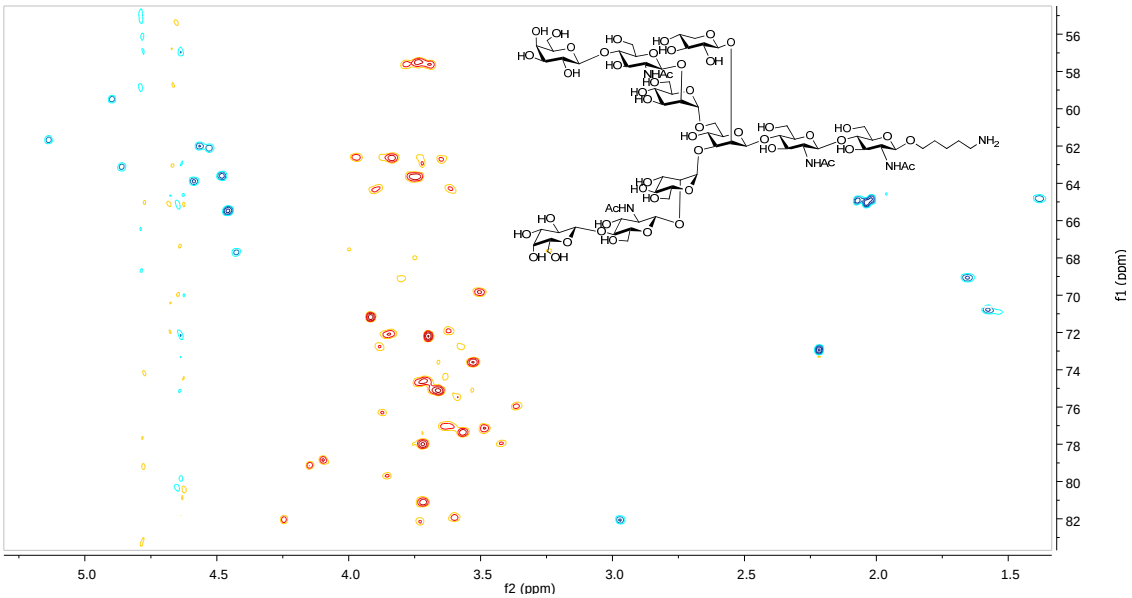

Compound G16 in D2O
500 MHz
f1 (ppm)


Compound G16 in D2O
126 MHz
f2 (ppm)
f1 (ppm)

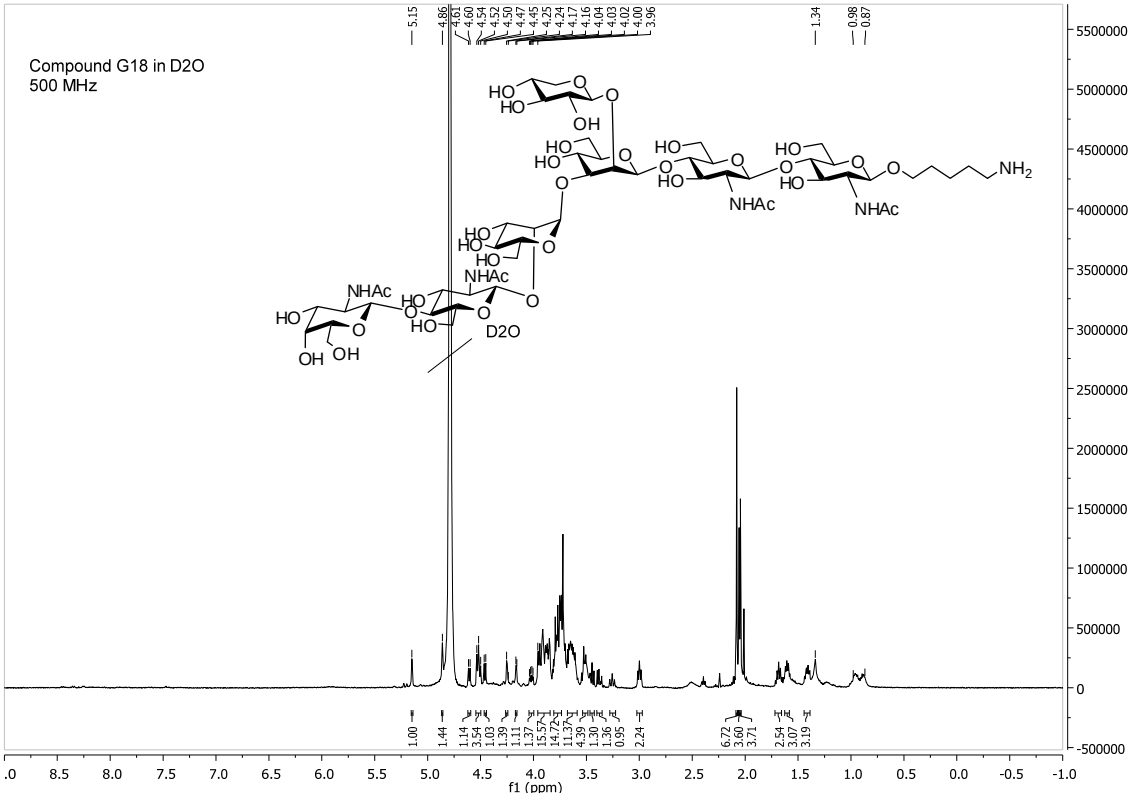
G17

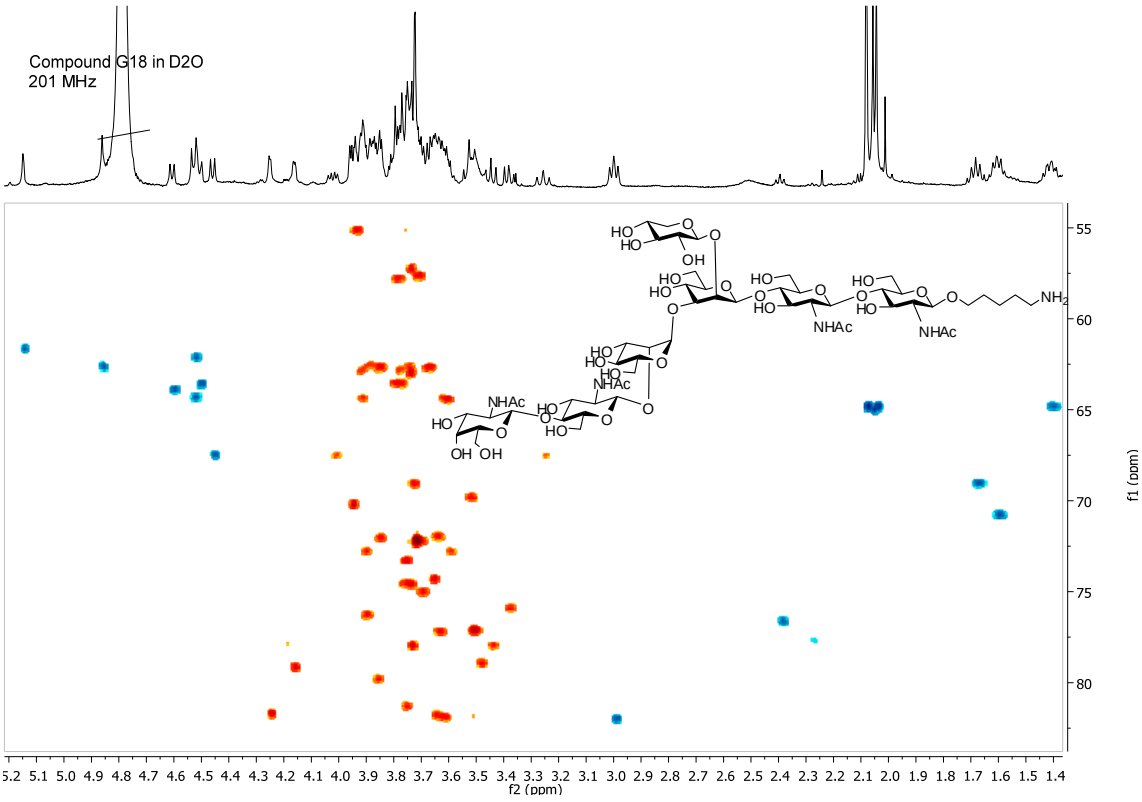

Compound G17 in D2O
800 MHz
acetone
D2O
f1 (ppm)


Compound G17 in D2O
201 MHz
D2O

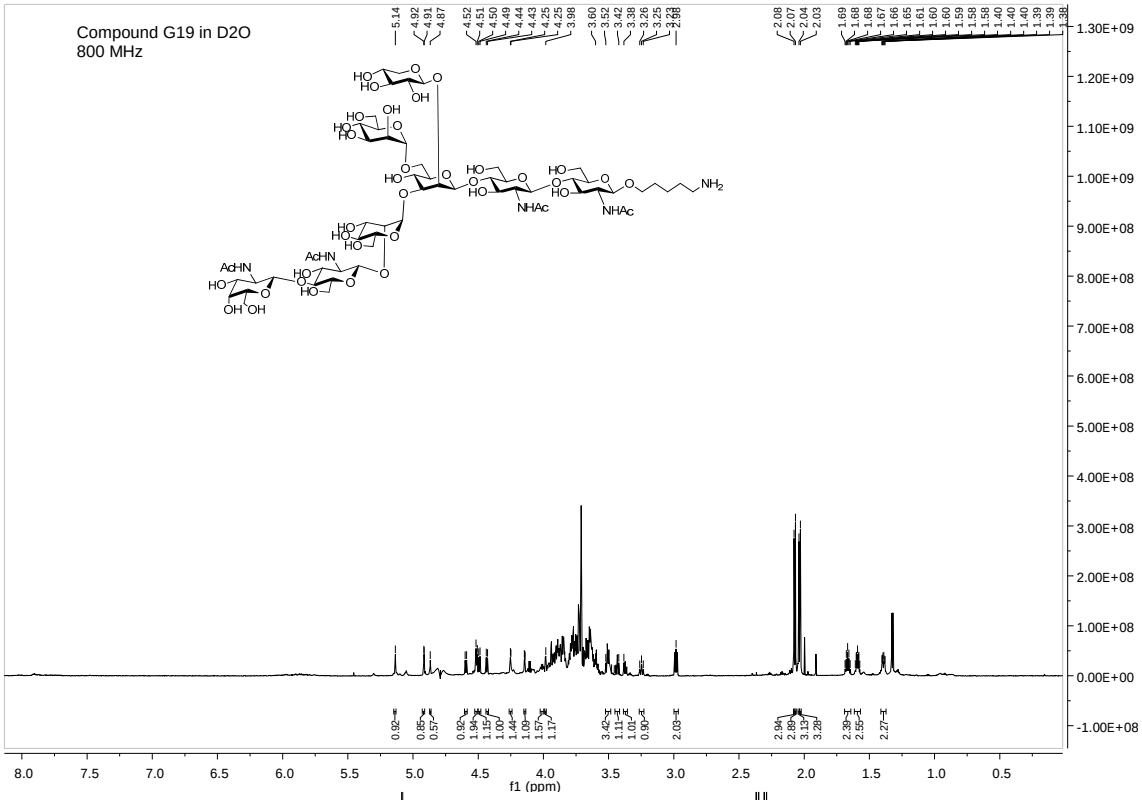

f2 (ppm)
f1 (ppm)

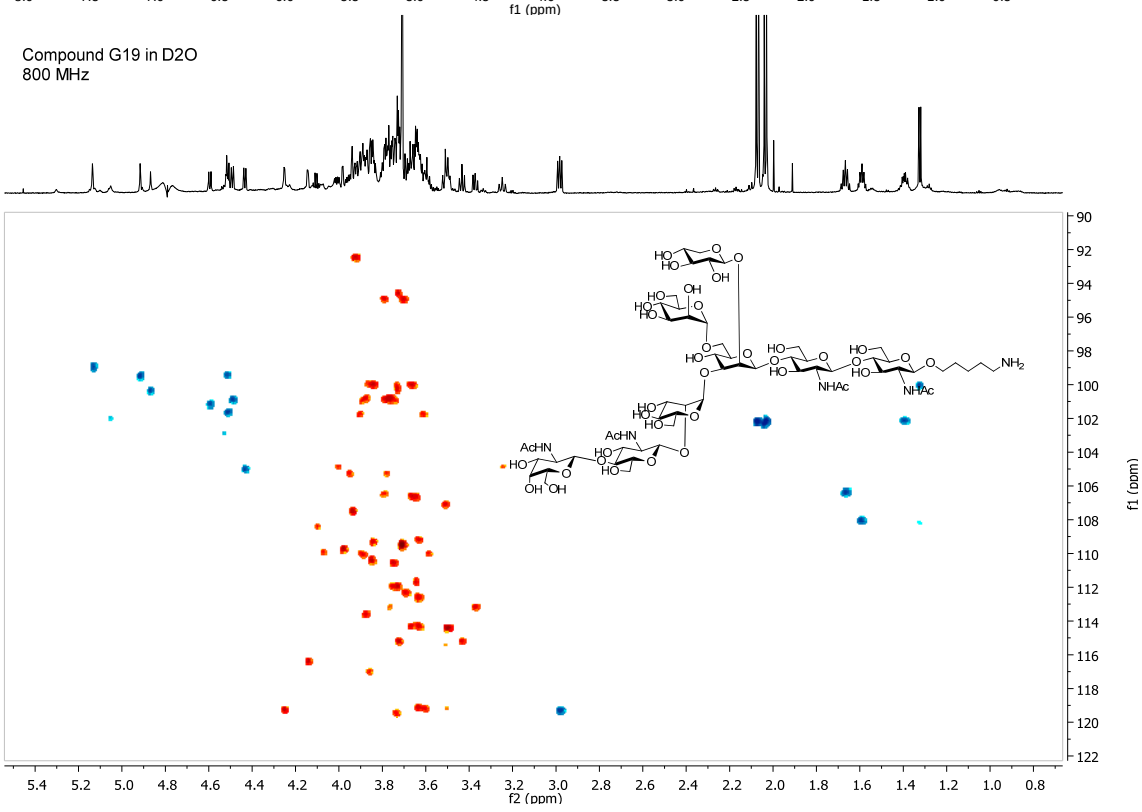
G18


Compound G18 in D2O
500 MHz
D2O
f1 (ppm)

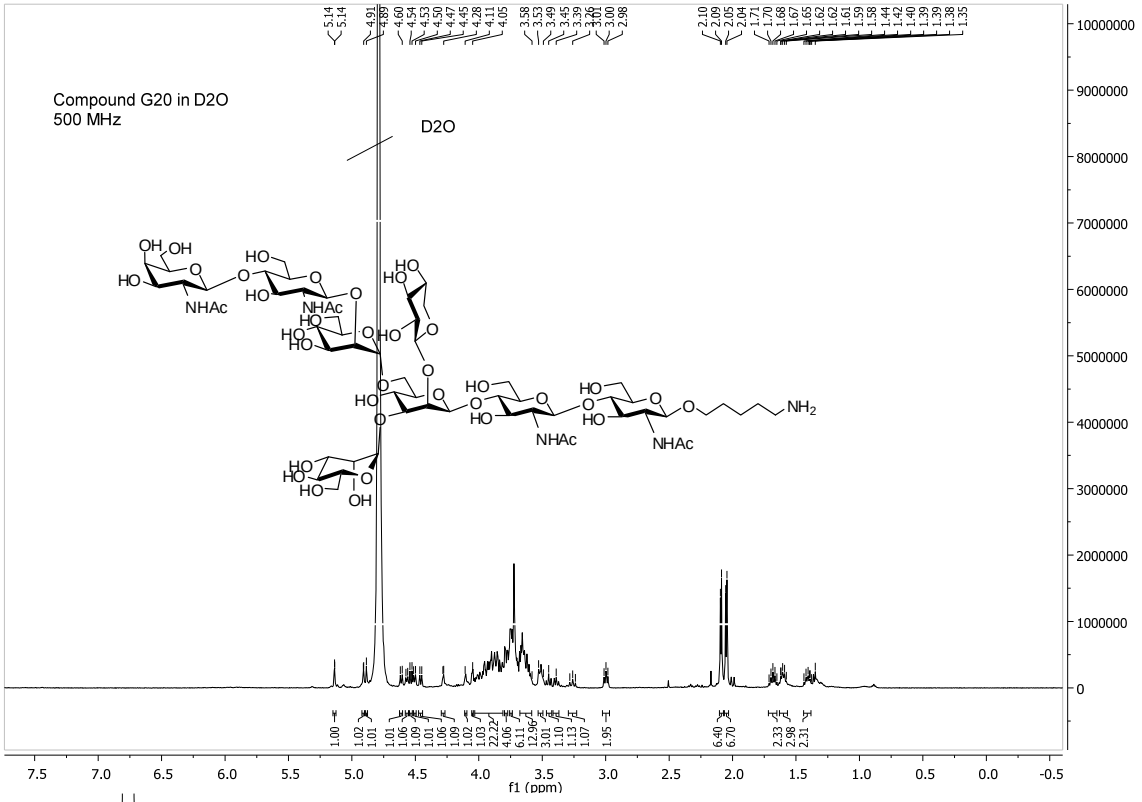

Compound G18 in D2O
201 MHz
f2 (ppm)
f1 (ppm)

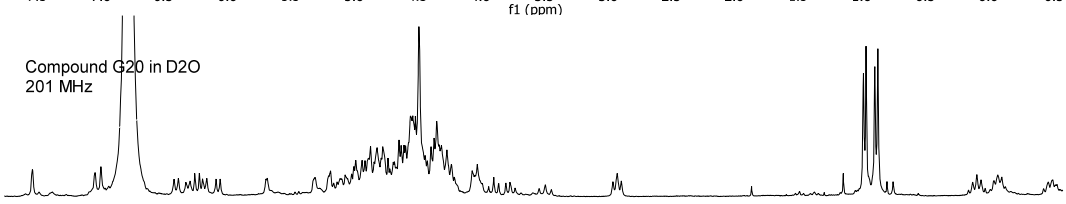
G19

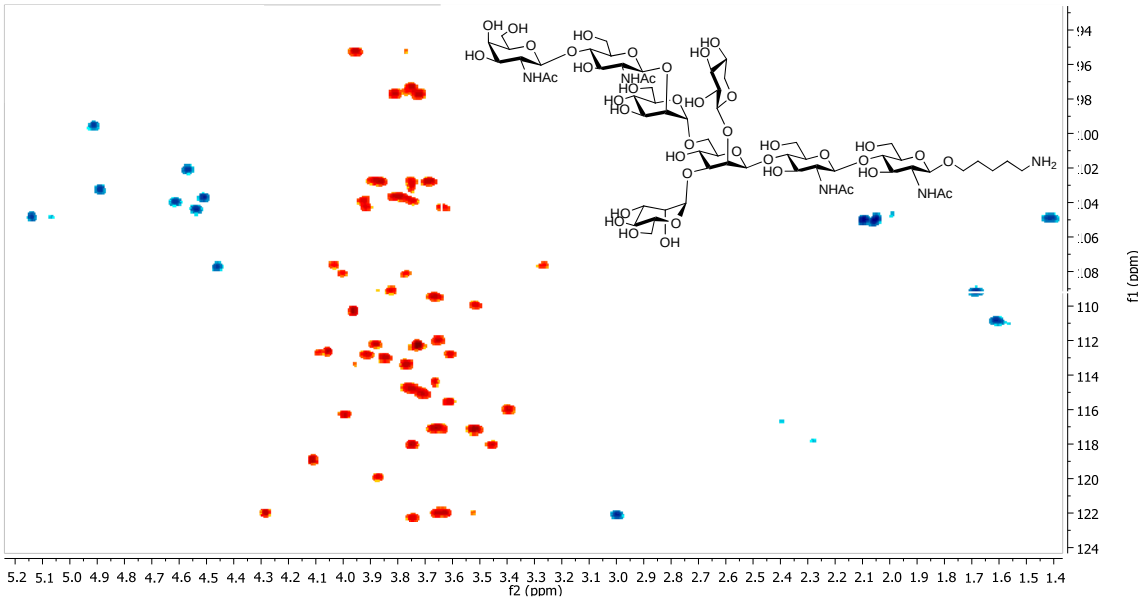

Compound G19 in D2O
800 MHz
f1 (ppm)


Compound G19 in D2O
800 MHz
f2 (ppm)
f1 (ppm)

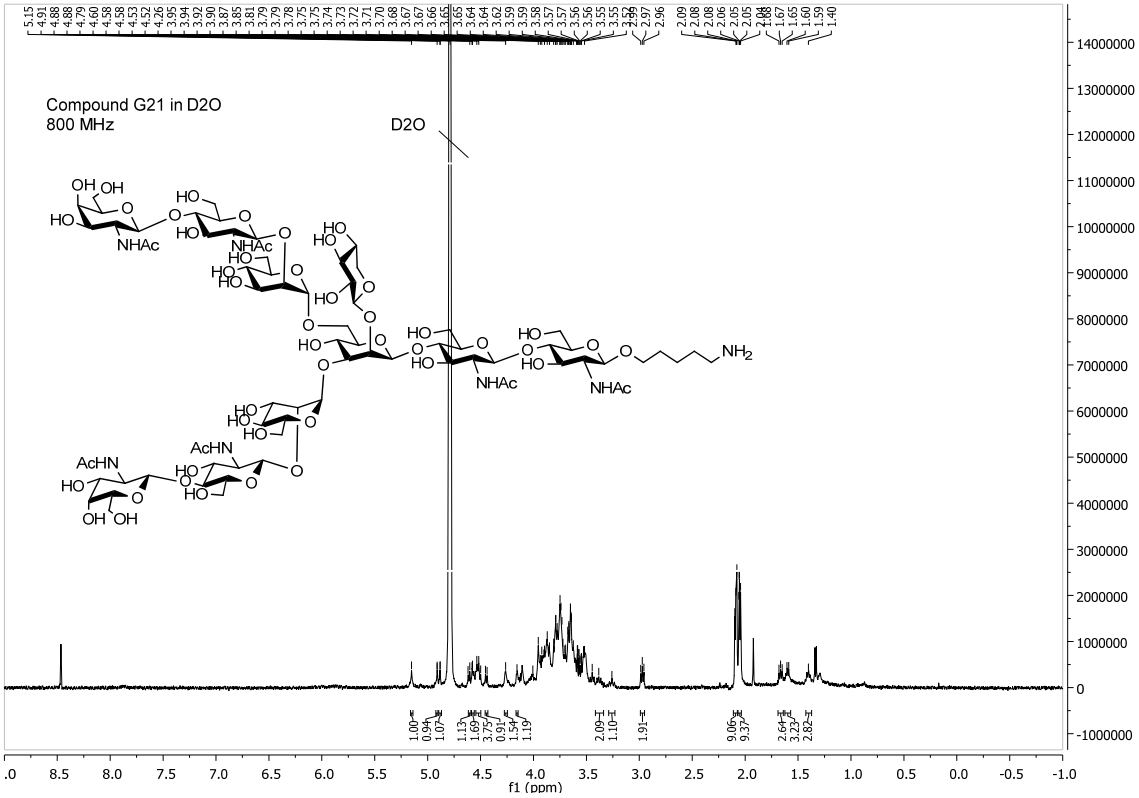
G20


Compound G20 in D2O
500 MHz
D2O
f1 (ppm)

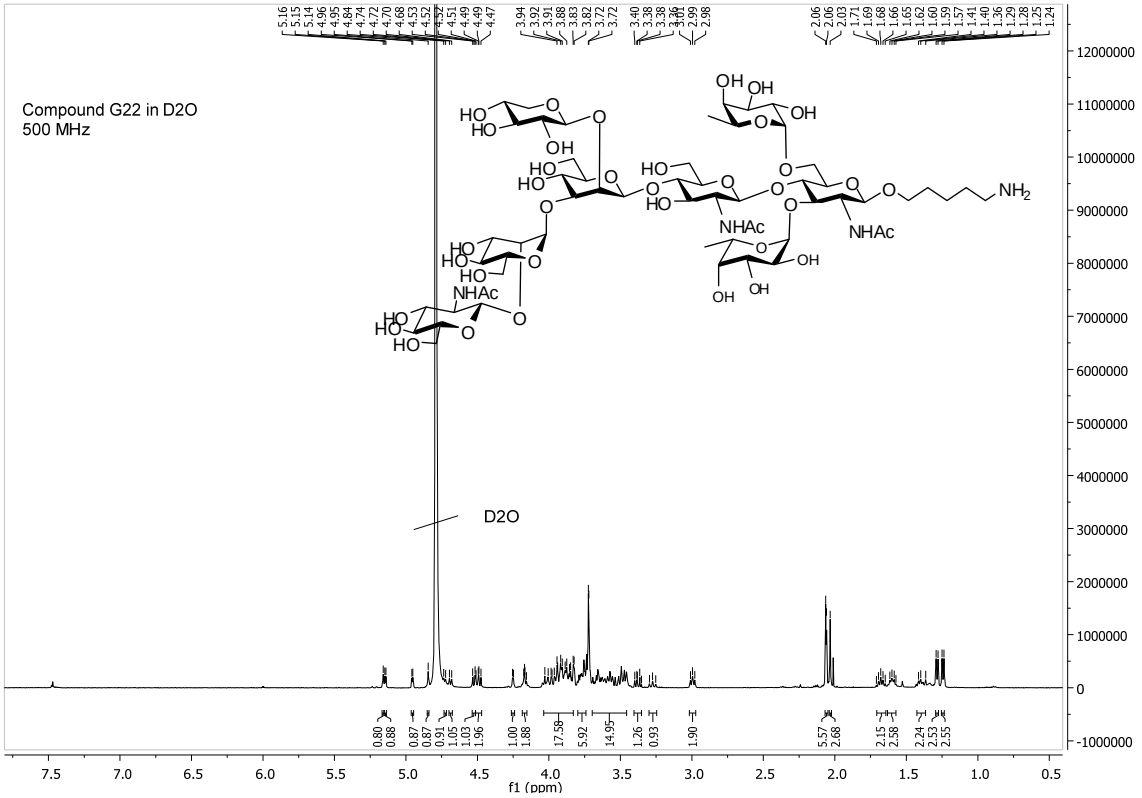

Compound G20 in D2O
201 MHz

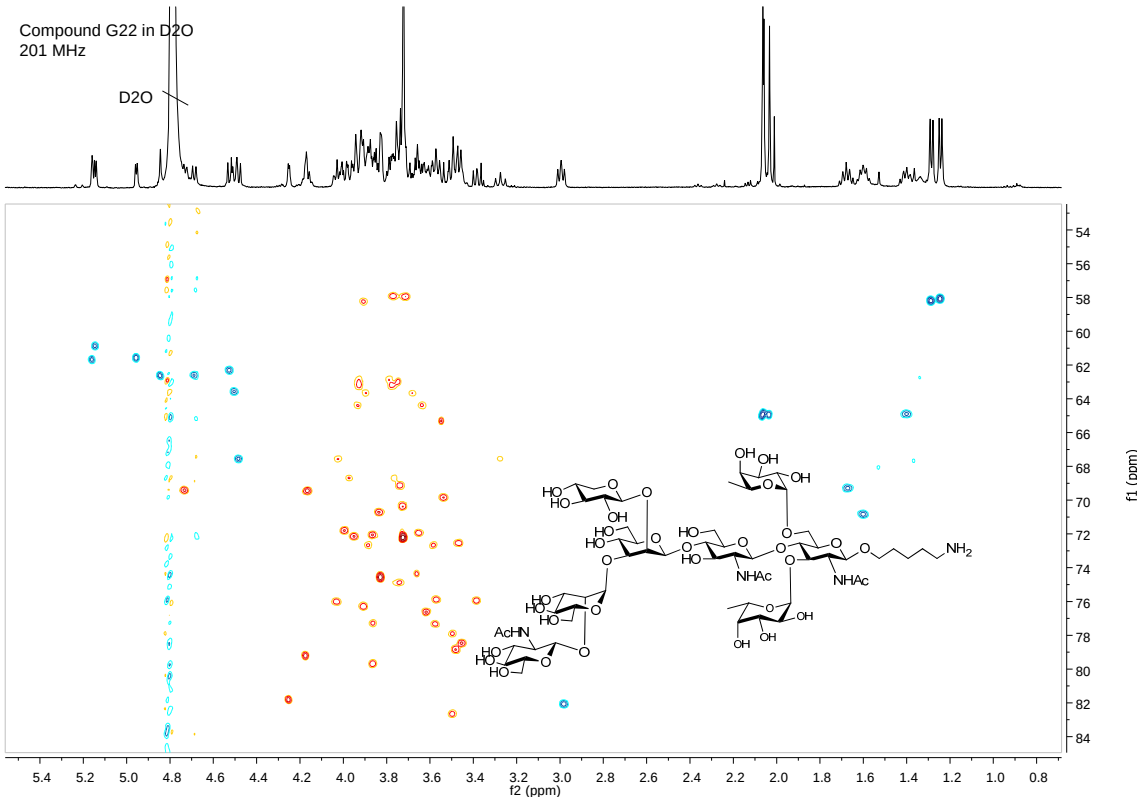

f2 (ppm)
f1 (ppm)

G21

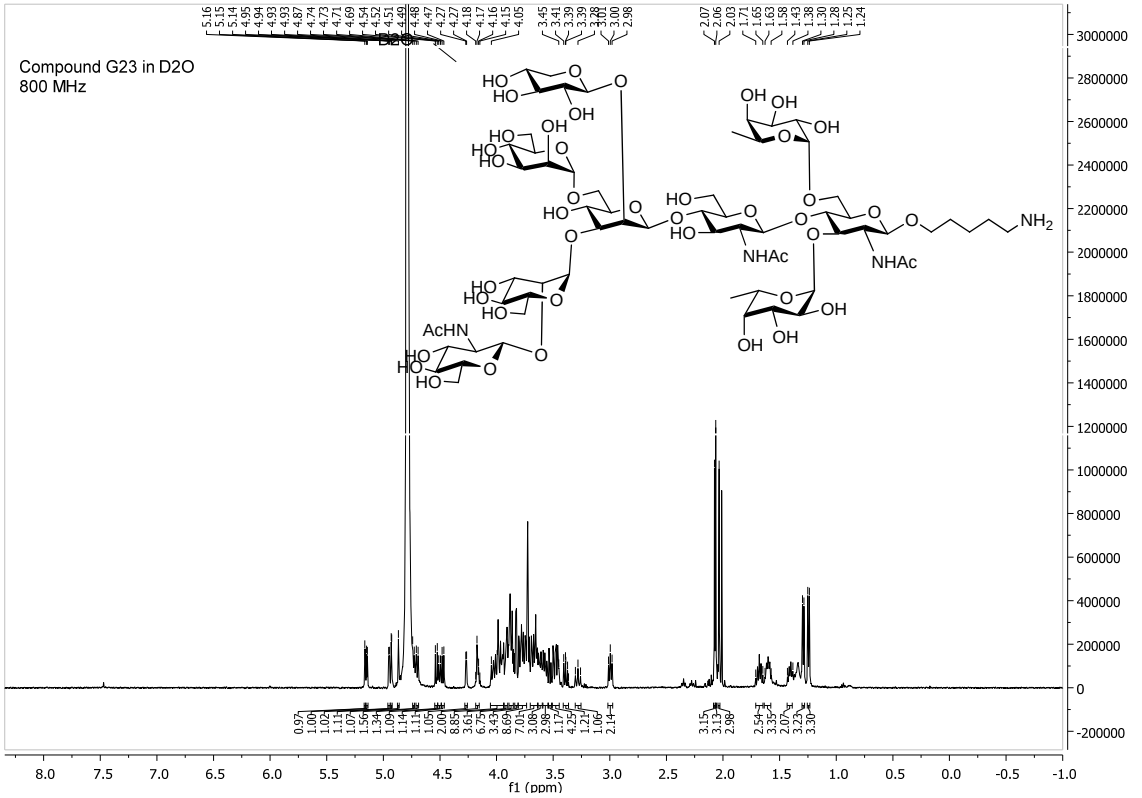


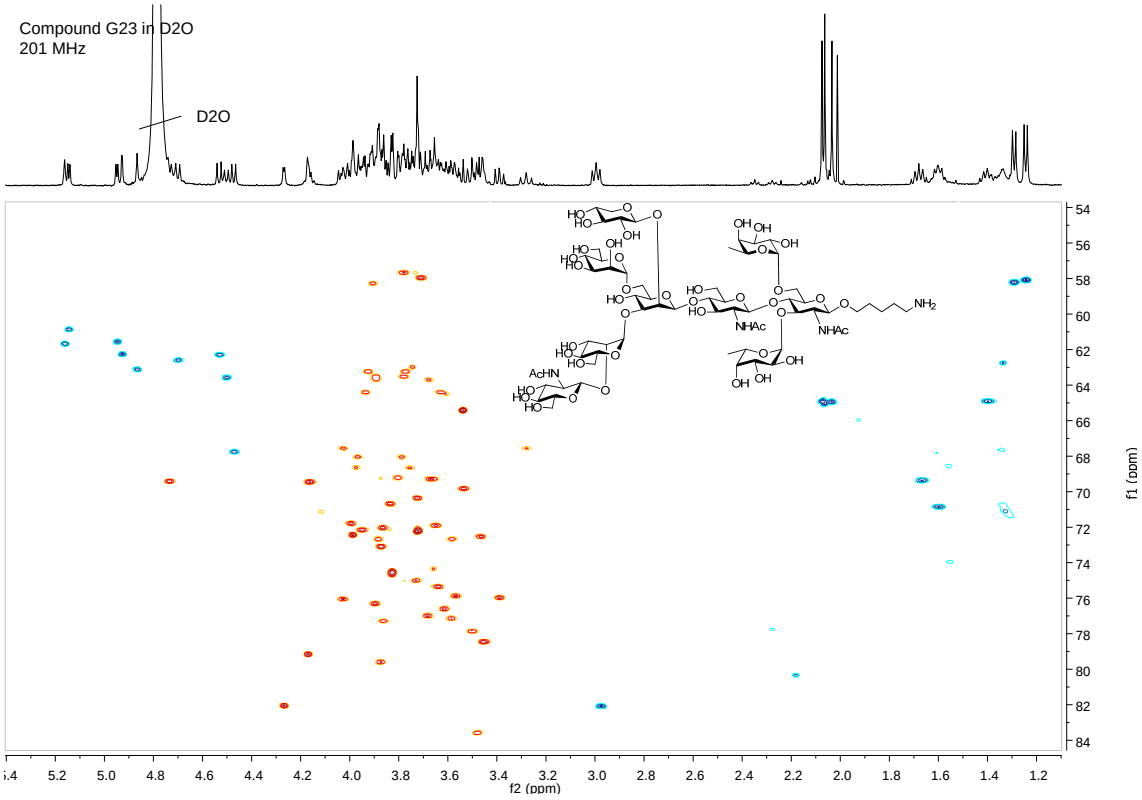
G22


Compound G22 in D2O
500 MHz
D2O
f1 (ppm)

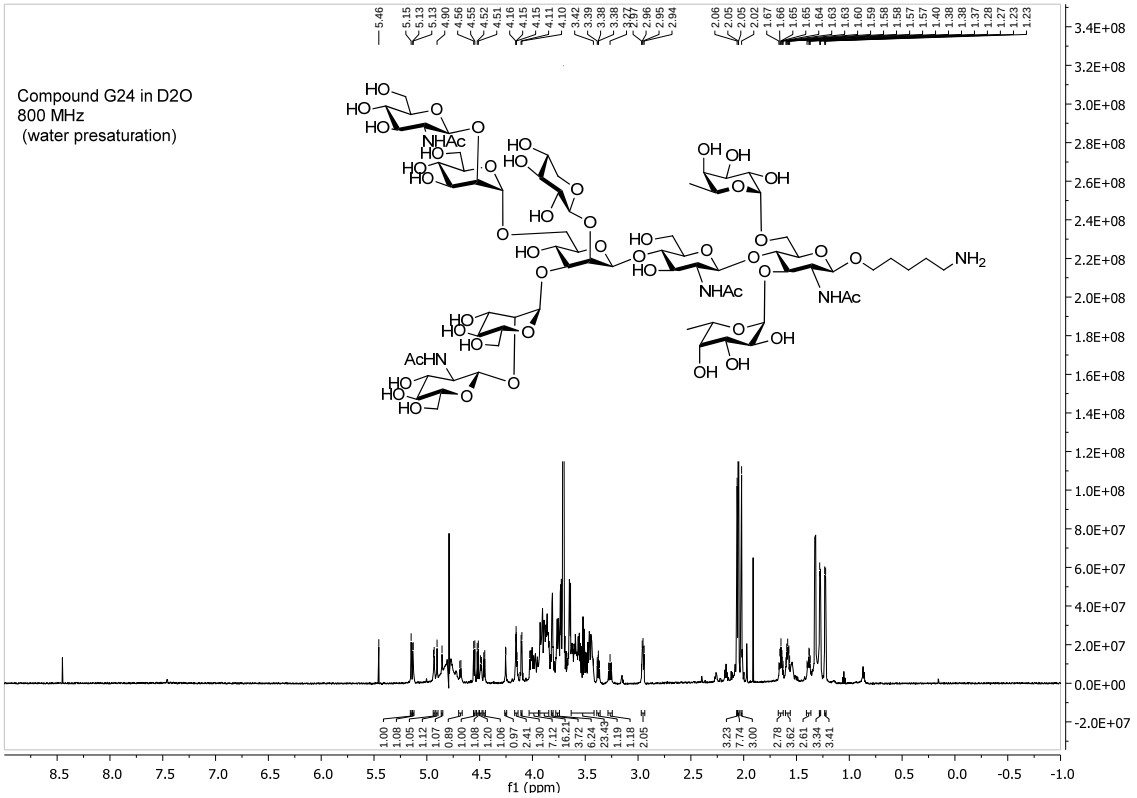

Compound G22 in D2O
201 MHz
D2O
f2 (ppm)
f1 (ppm)

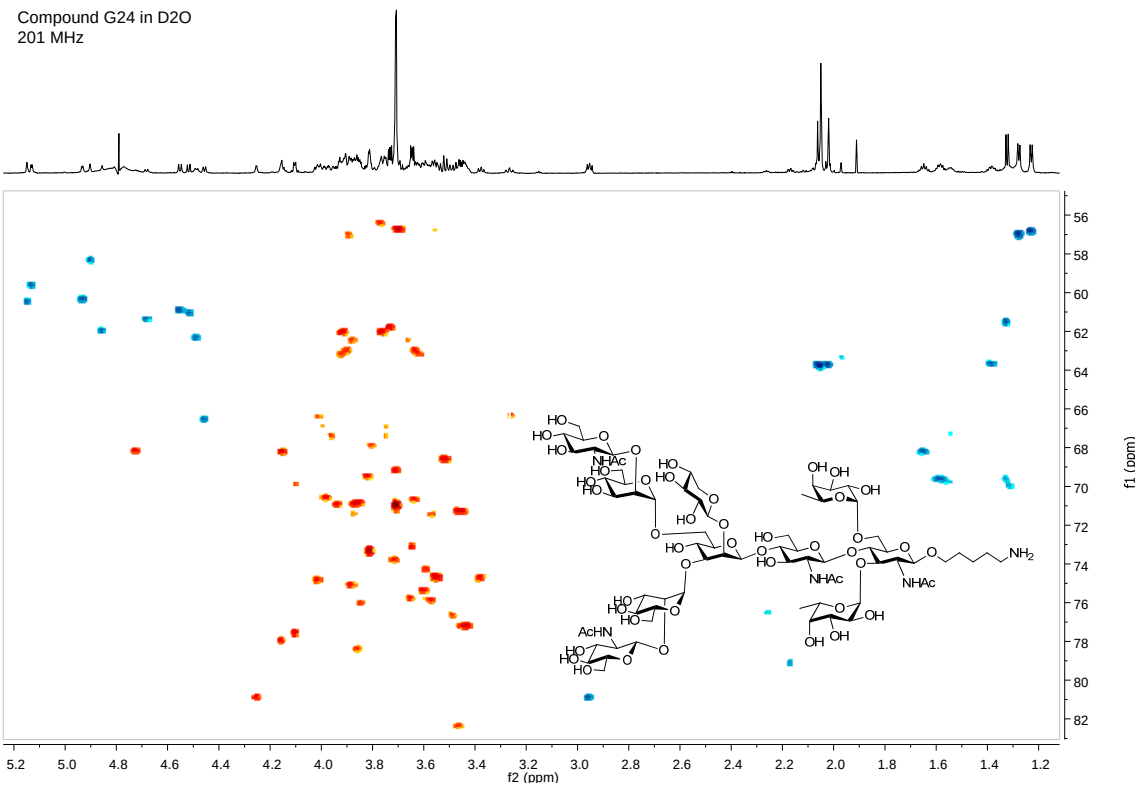
G23


Compound G23 in D2O
800 MHz
f1 (ppm)

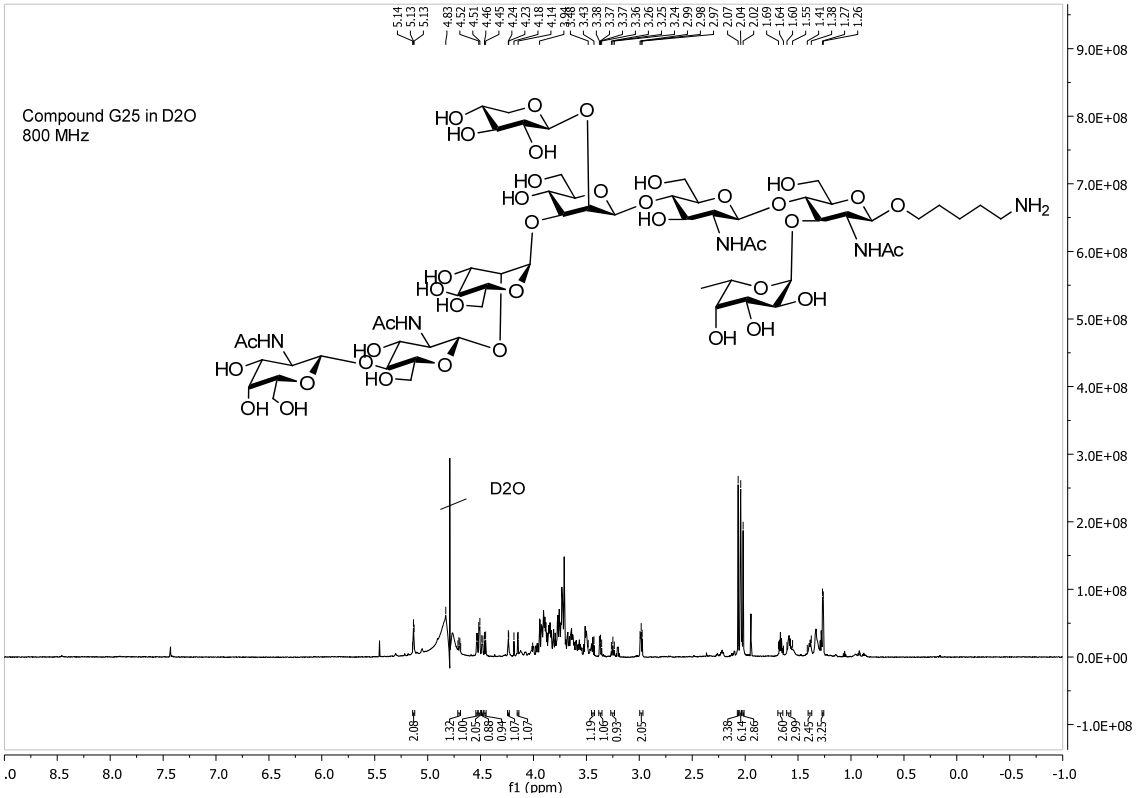

Compound G23 in D2O
201 MHz
D2O
f2 (ppm)
f1 (ppm)

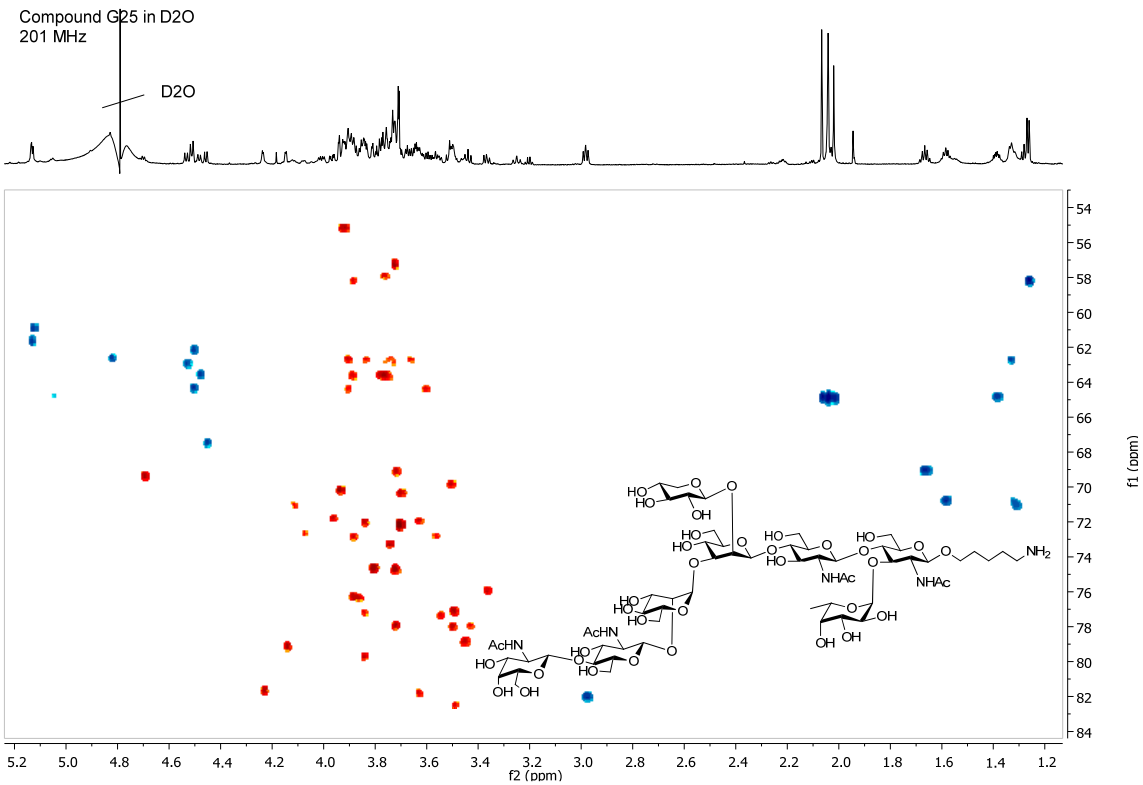
G24


Compound G24 in D2O
800 MHz
(water presaturation)
f1 (ppm)

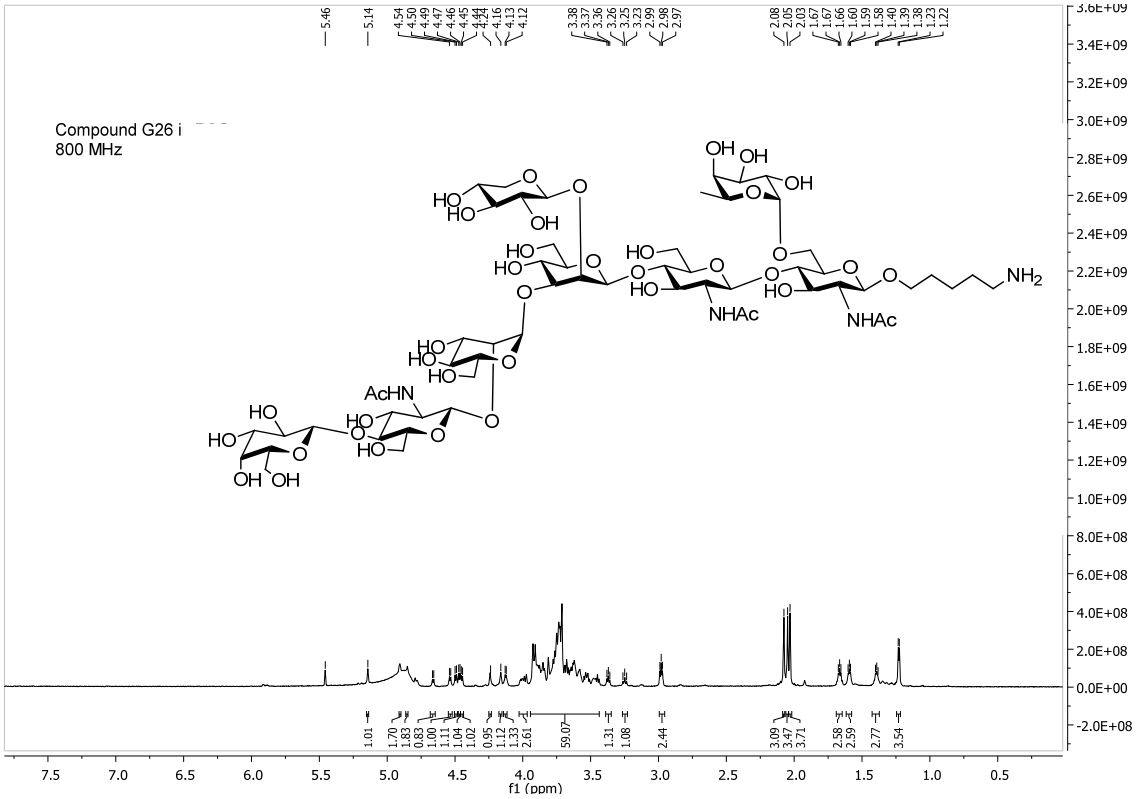

Compound G24 in D2O
201 MHz
f2 (ppm)
f1 (ppm)

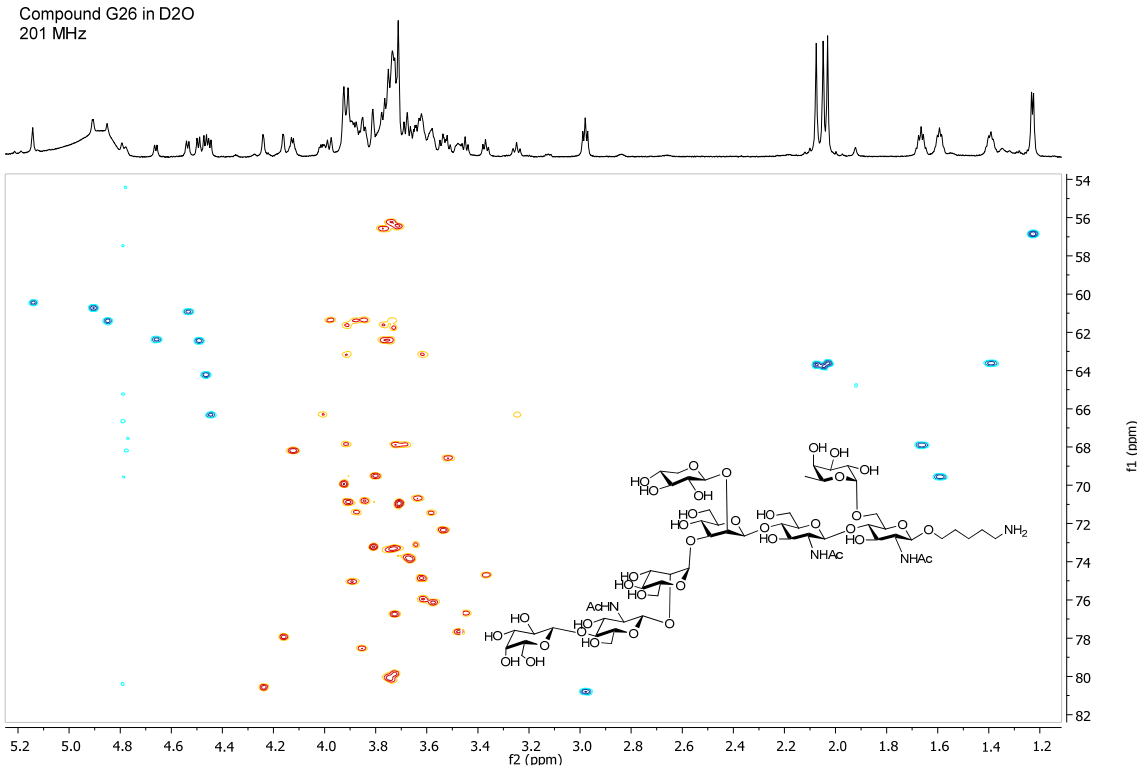
G25


Compound G25 in D2O
800 MHz
D2O
f1 (ppm)

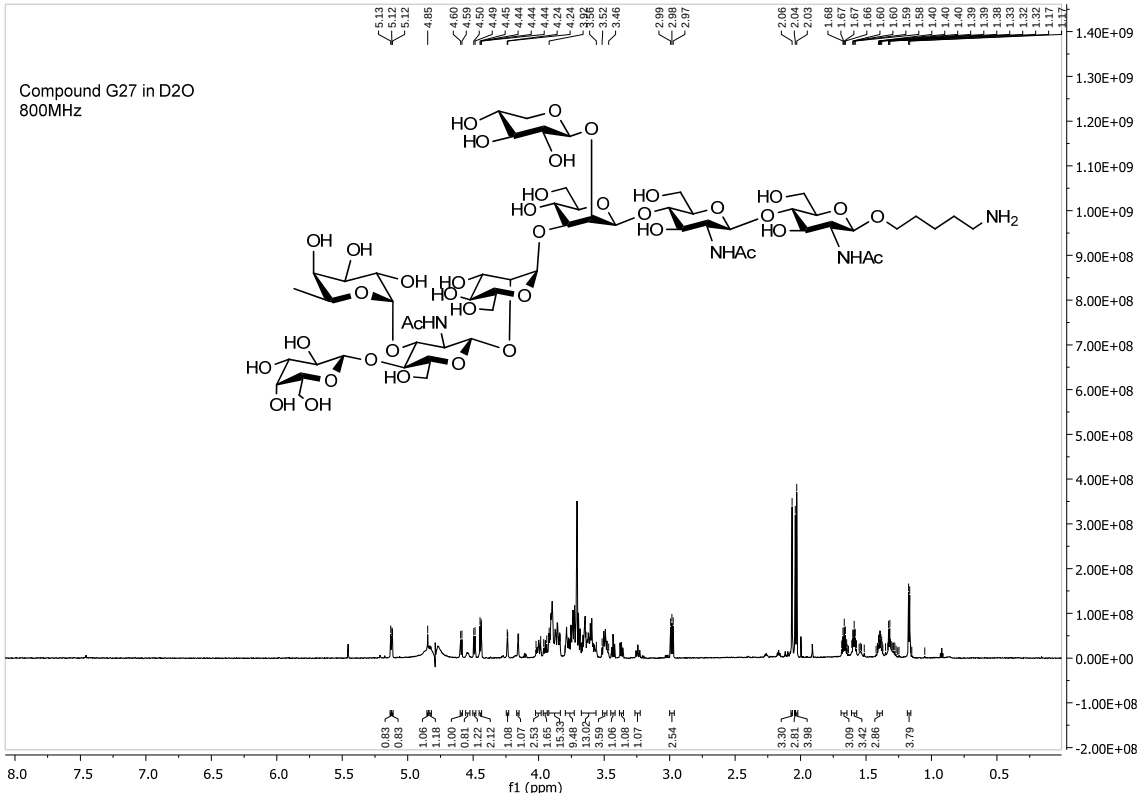

Compound G25 in D2O
201 MHz
D2O
f2 (ppm)
f1 (ppm)

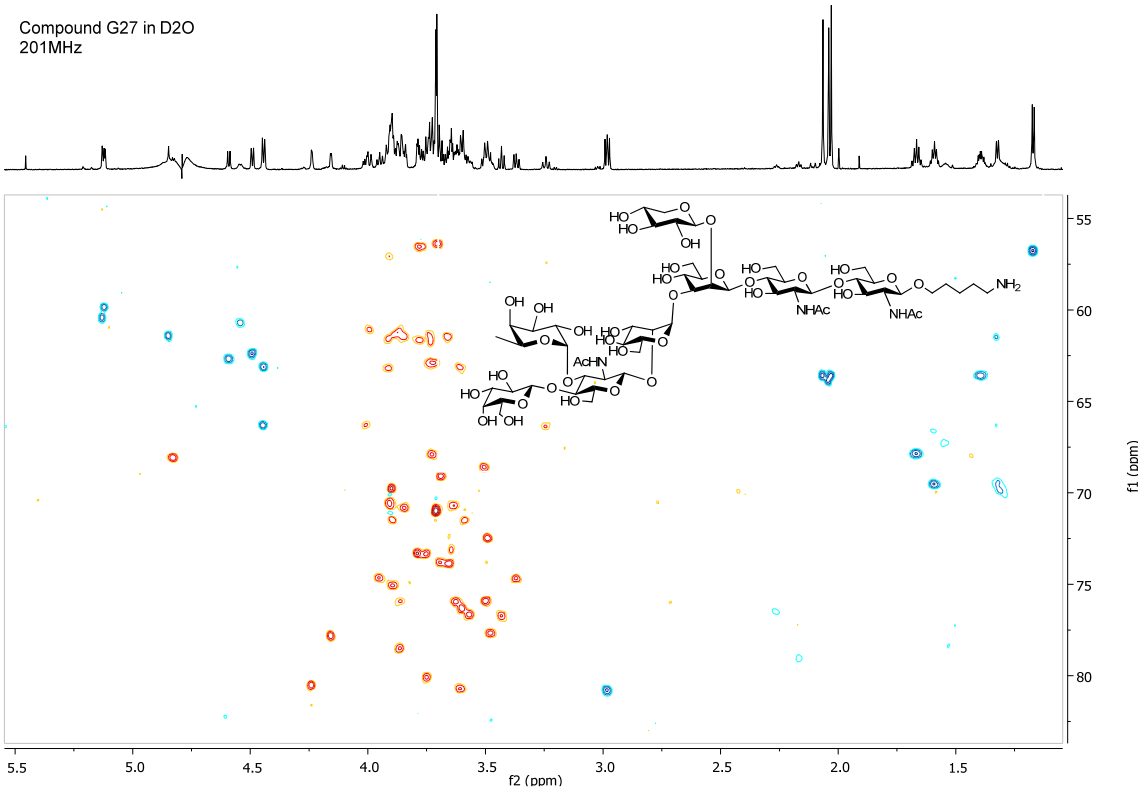
G26


Compound G26 i
800 MHz
f1 (ppm)

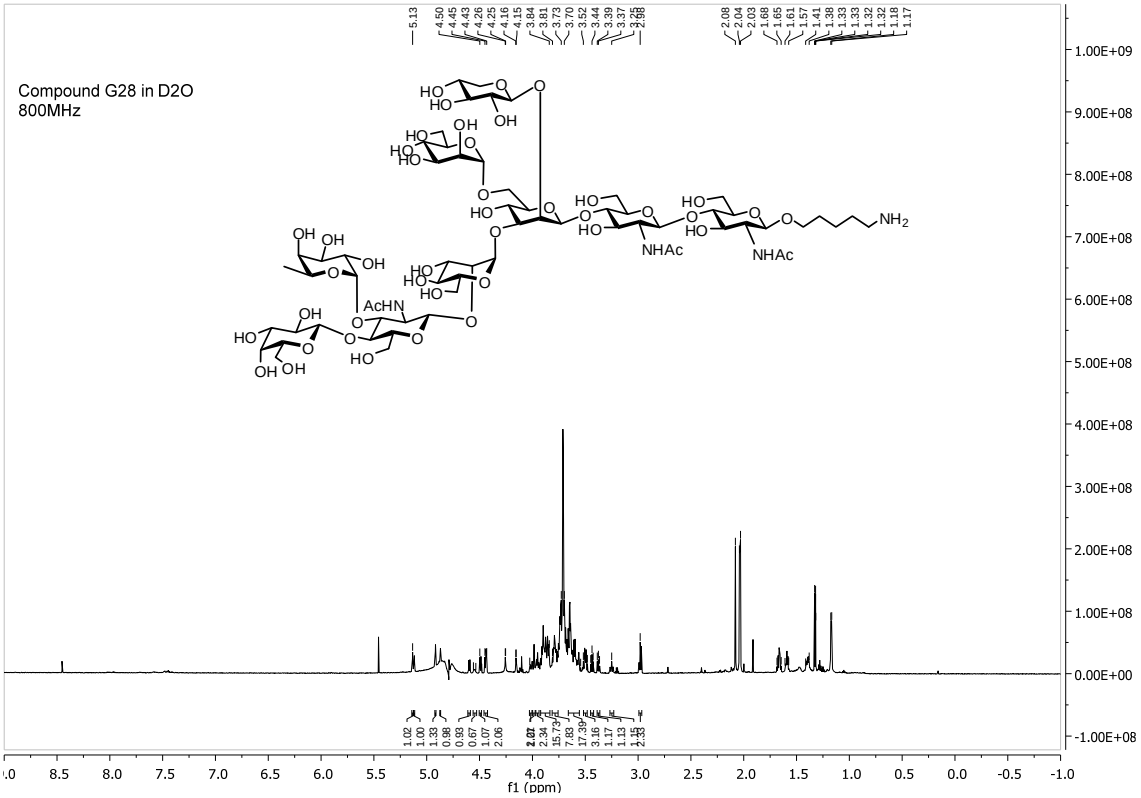

Compound G26 in D2O
201 MHz
f2 (ppm)
f1 (ppm)

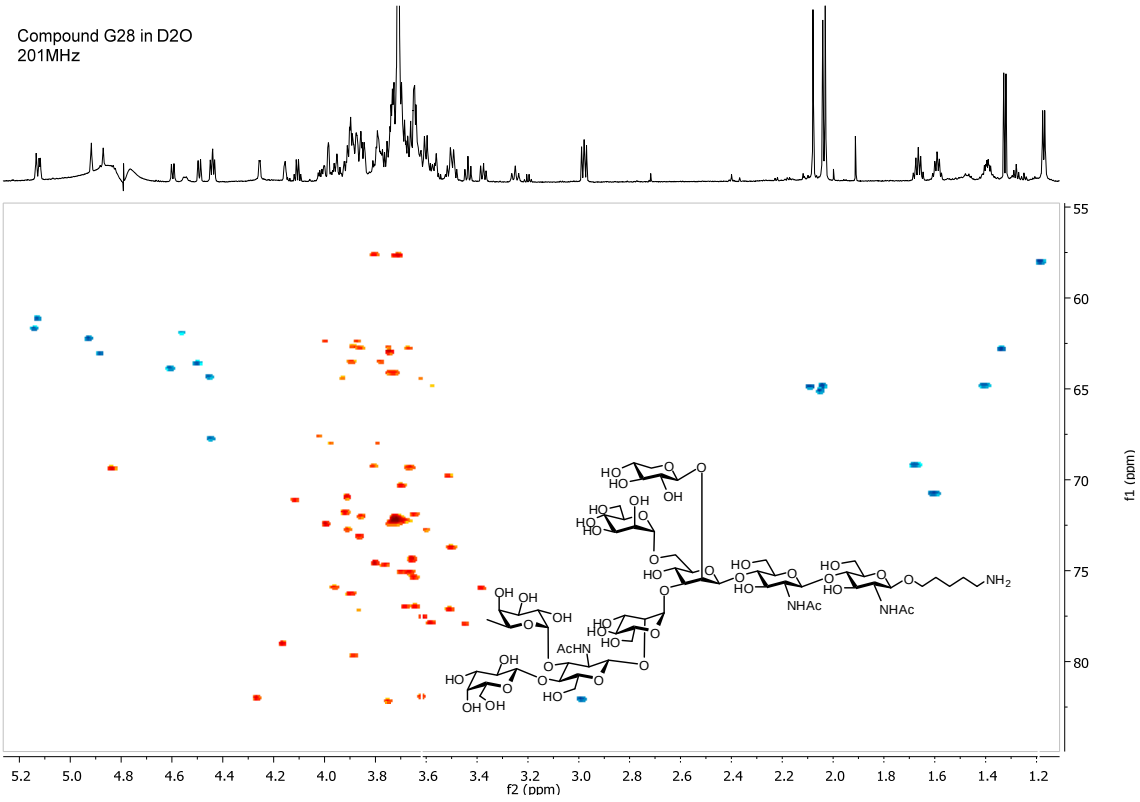
G27


Compound G27 in D2O
800MHz
f1 (ppm)

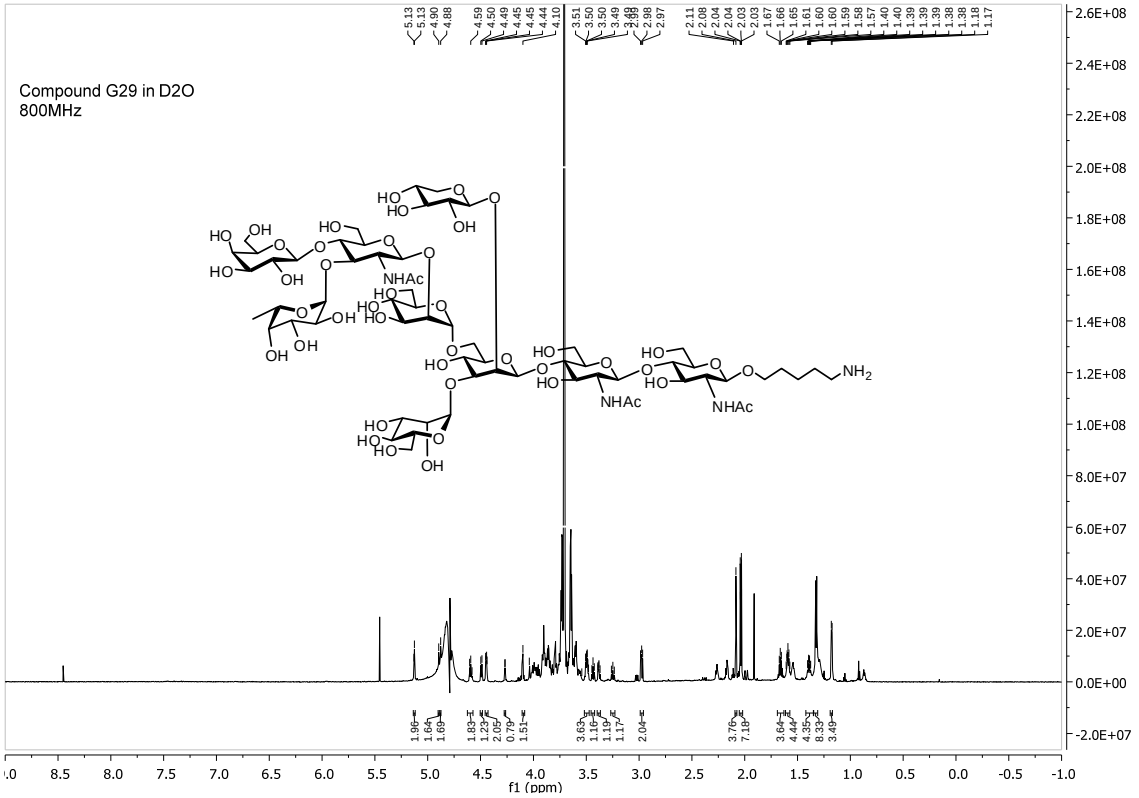

Compound G27 in D2O
201MHz
f2 (ppm)
f1 (ppm)

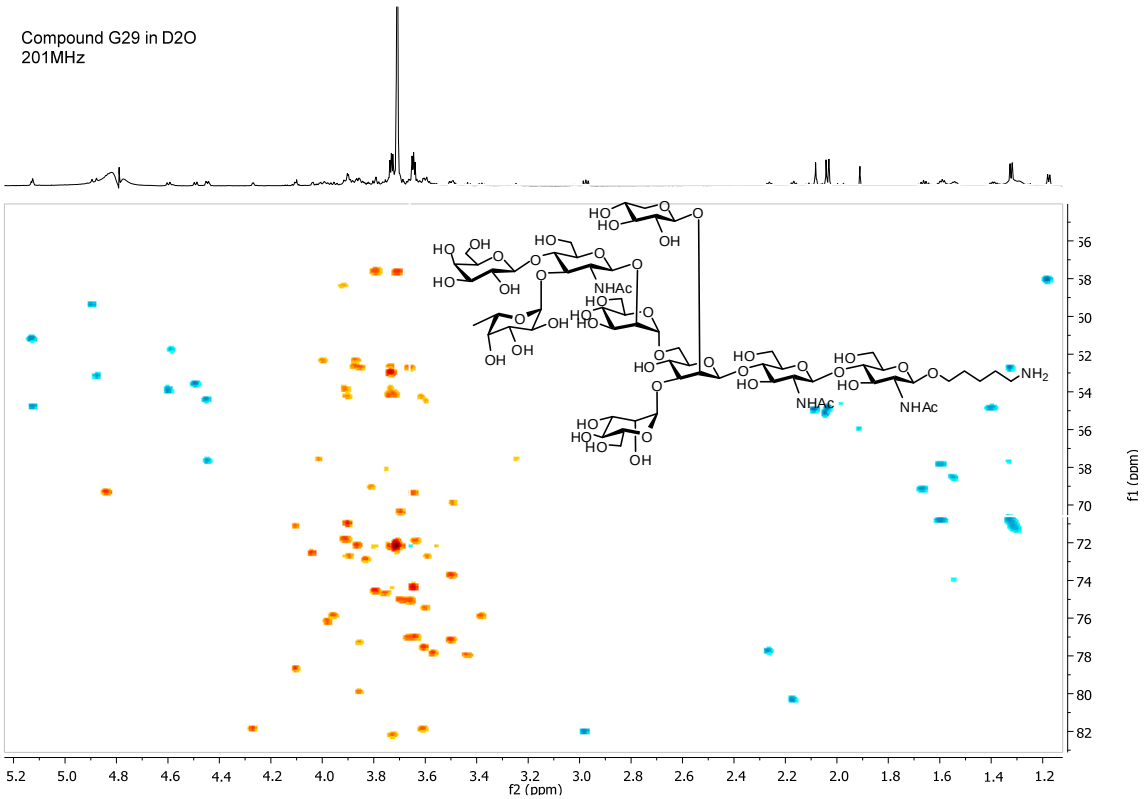
G28


Compound G28 in D2O
800MHz
f1 (ppm)

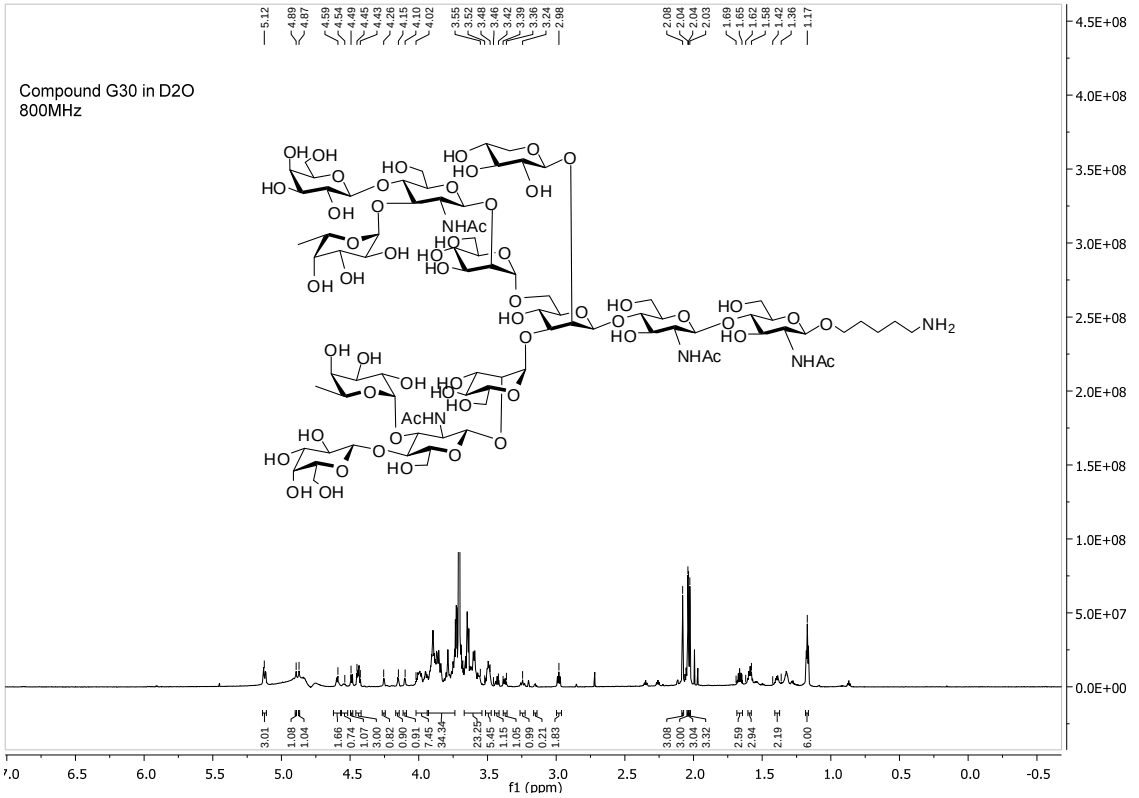

Compound G28 in D2O
201MHz
f2 (ppm)
f1 (ppm)

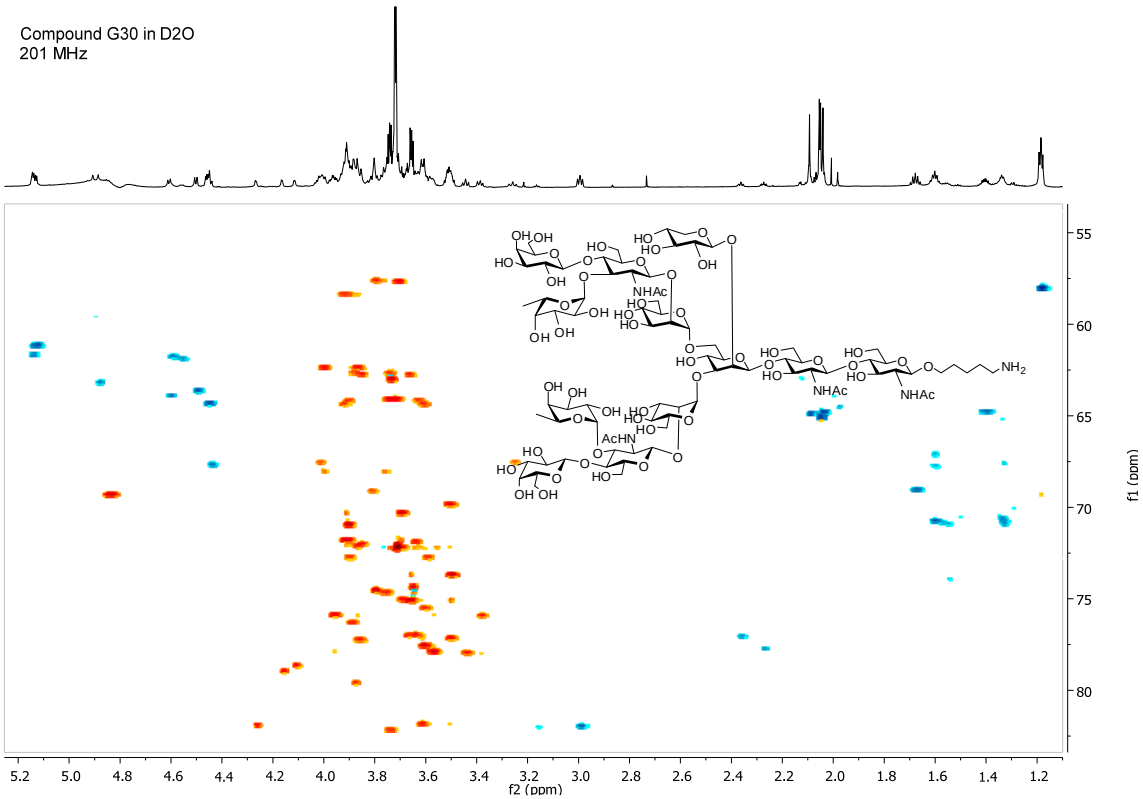
G29


Compound G29 in D2O
800MHz
f1 (ppm)

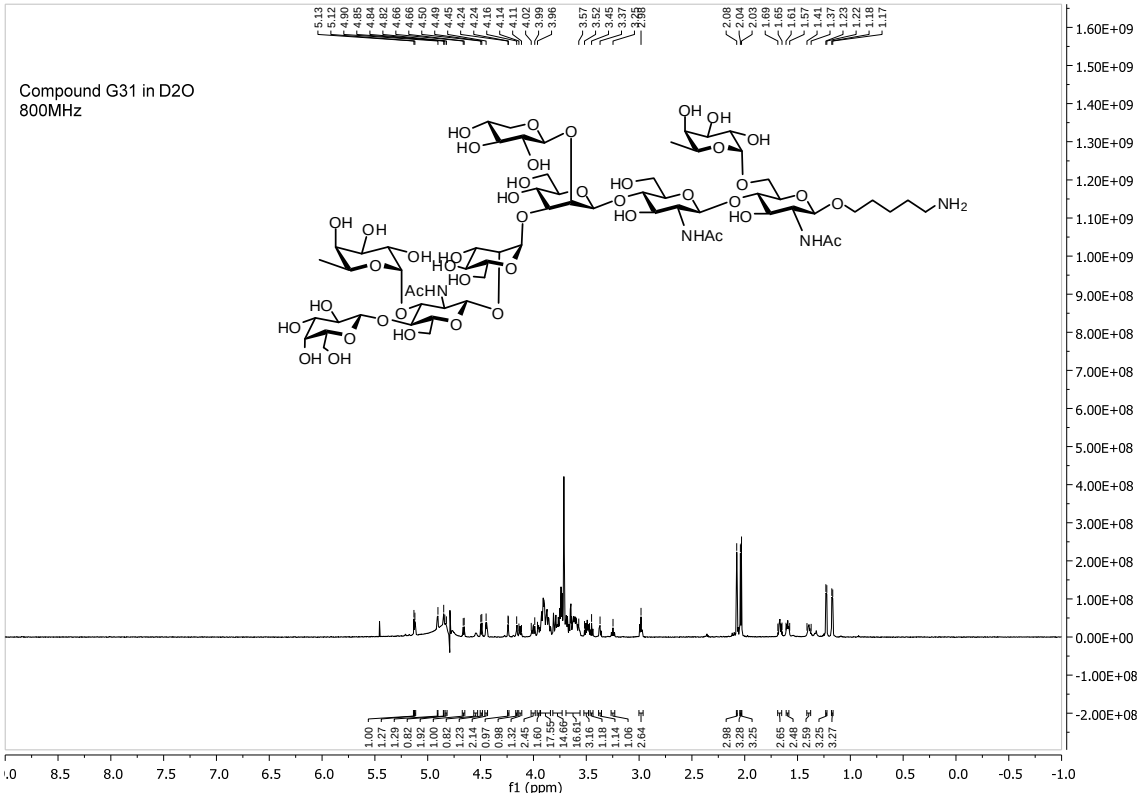

Compound G29 in D2O
201MHz
f2 (ppm)
f1 (ppm)

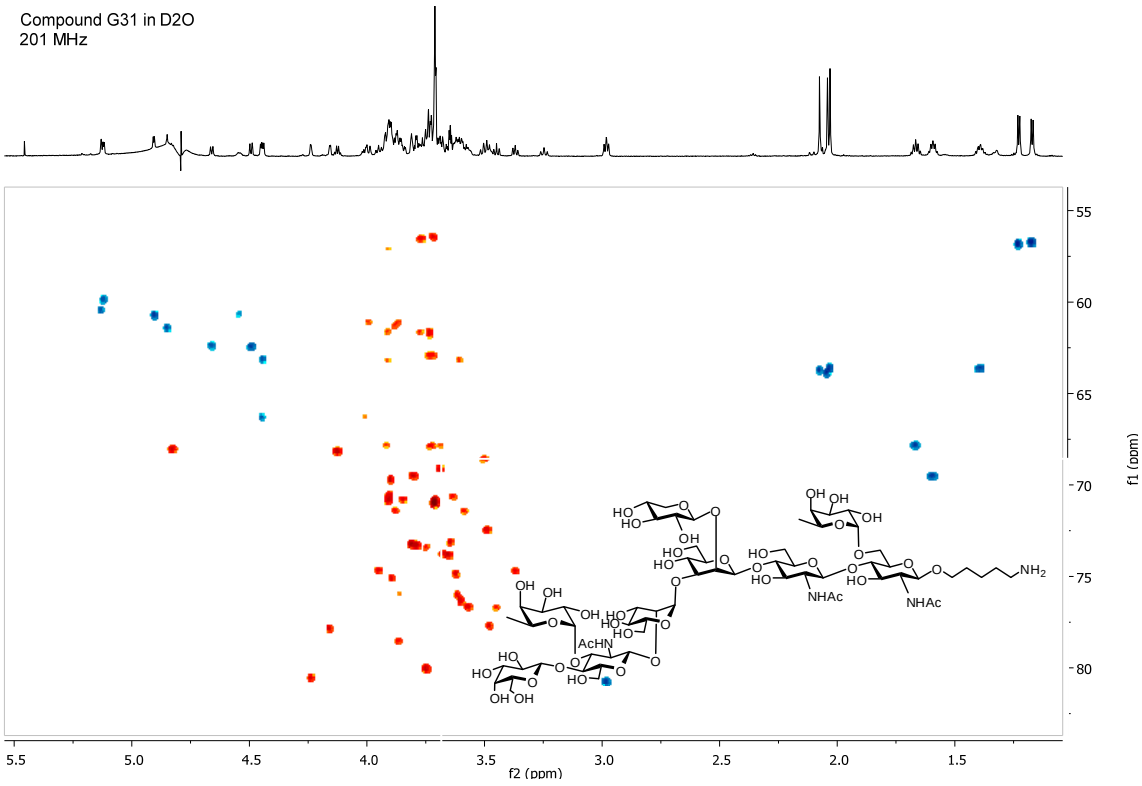
G30


Compound G30 in D2O
800MHz
f1 (ppm)

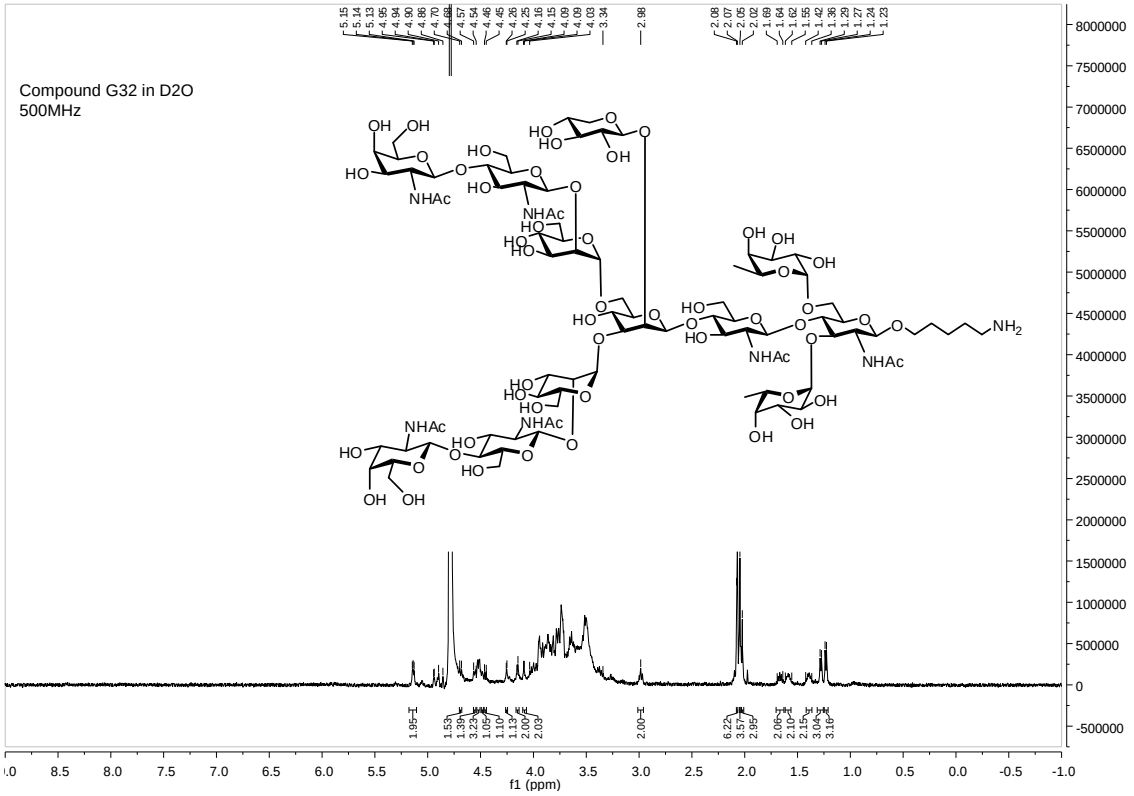

Compound G30 in D2O
201 MHz
f2 (ppm)
f1 (ppm)

G31

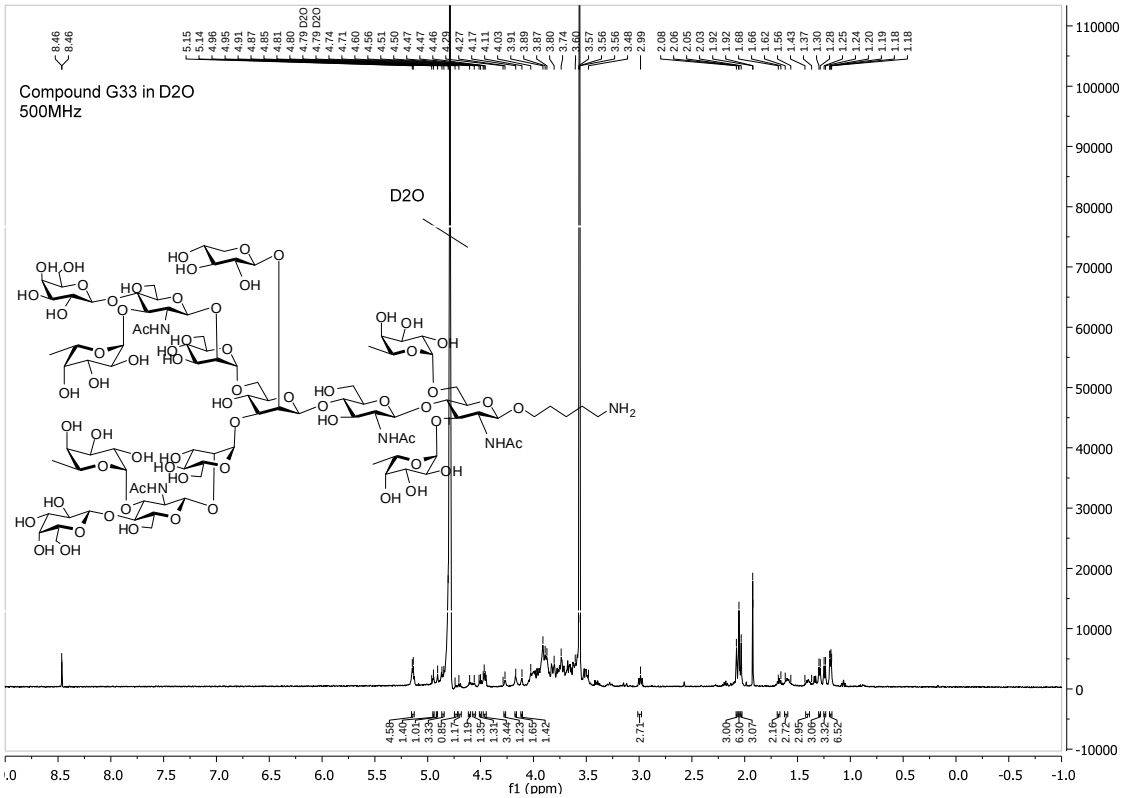

Compound G31 in D2O
800MHz
f1 (ppm)


Compound G31 in D2O
201 MHz
f2 (ppm)
f1 (ppm)

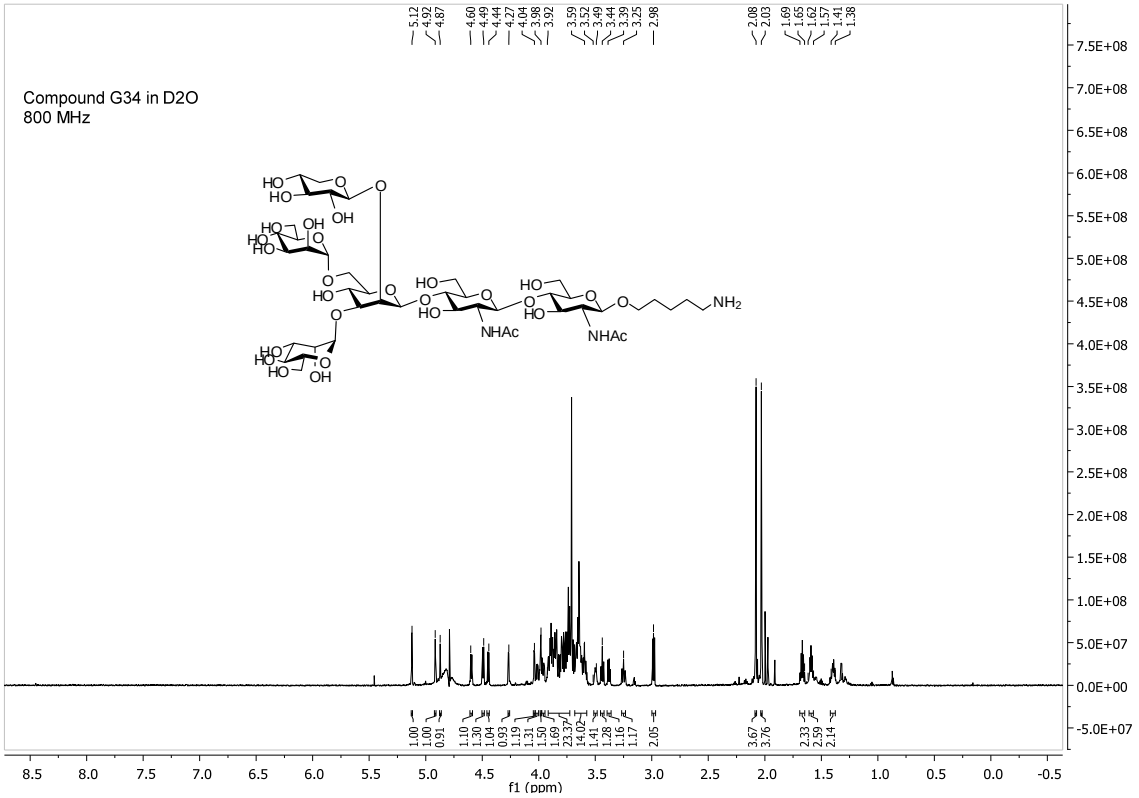
G32

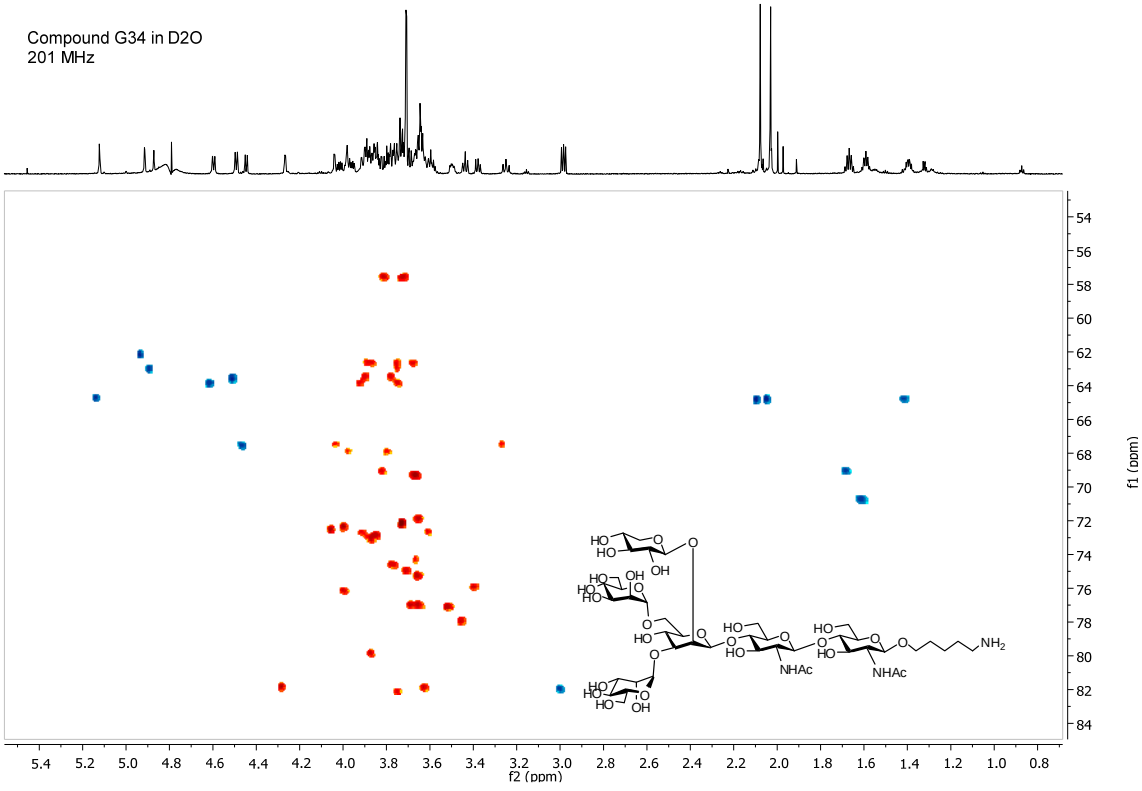

Compound G32 in D2O
500MHz
f1 (ppm)

G33

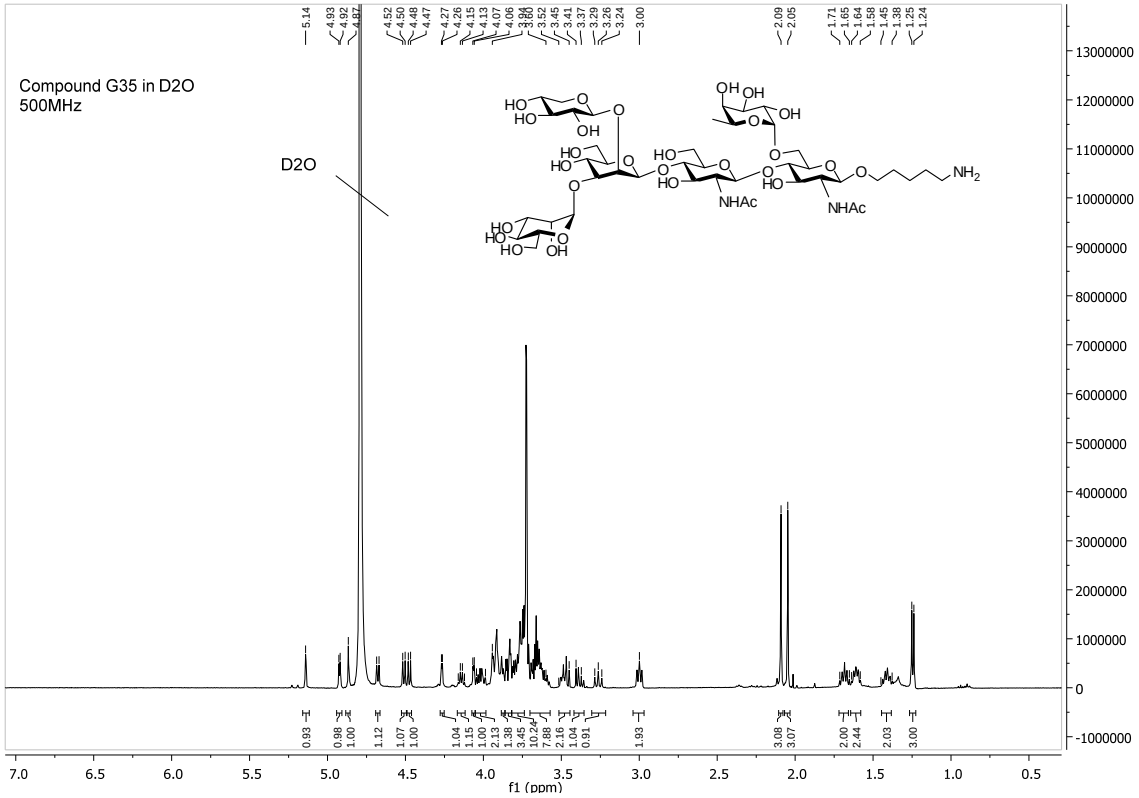

Compound G33 in D2O
500MHz
D2O
f1 (ppm)

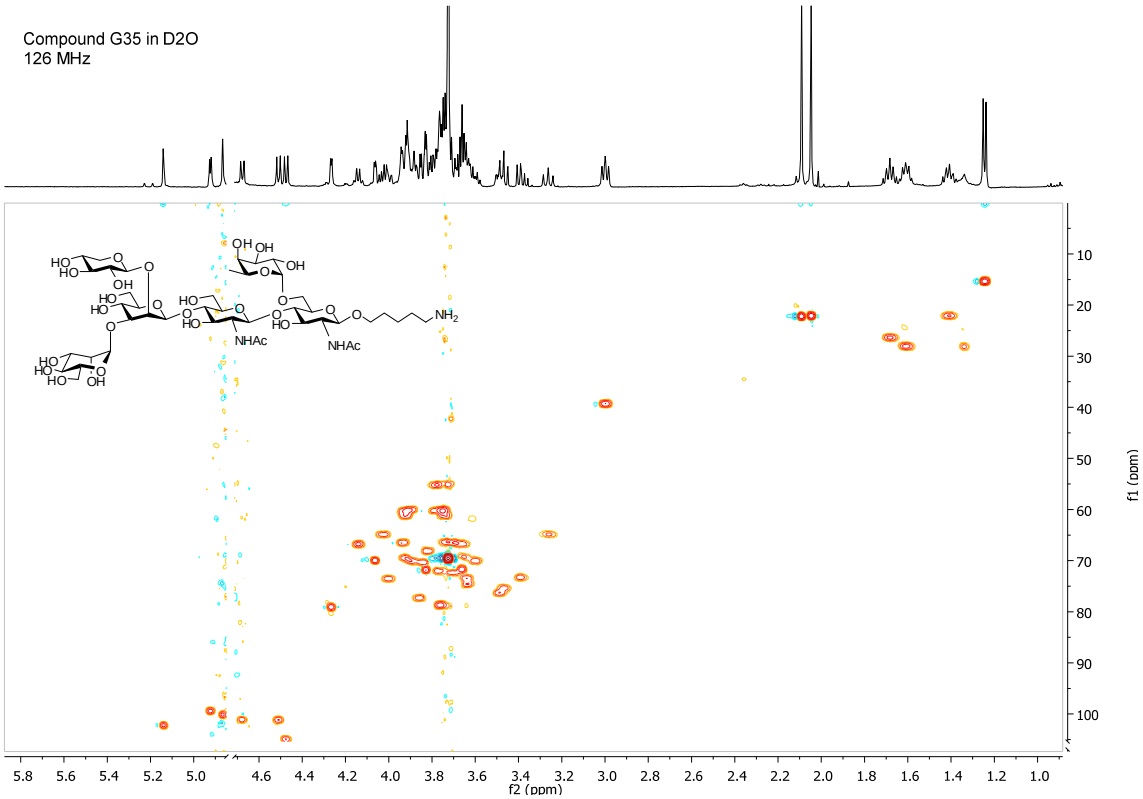
G34


Compound G34 in D2O
800 MHz
f1 (ppm)

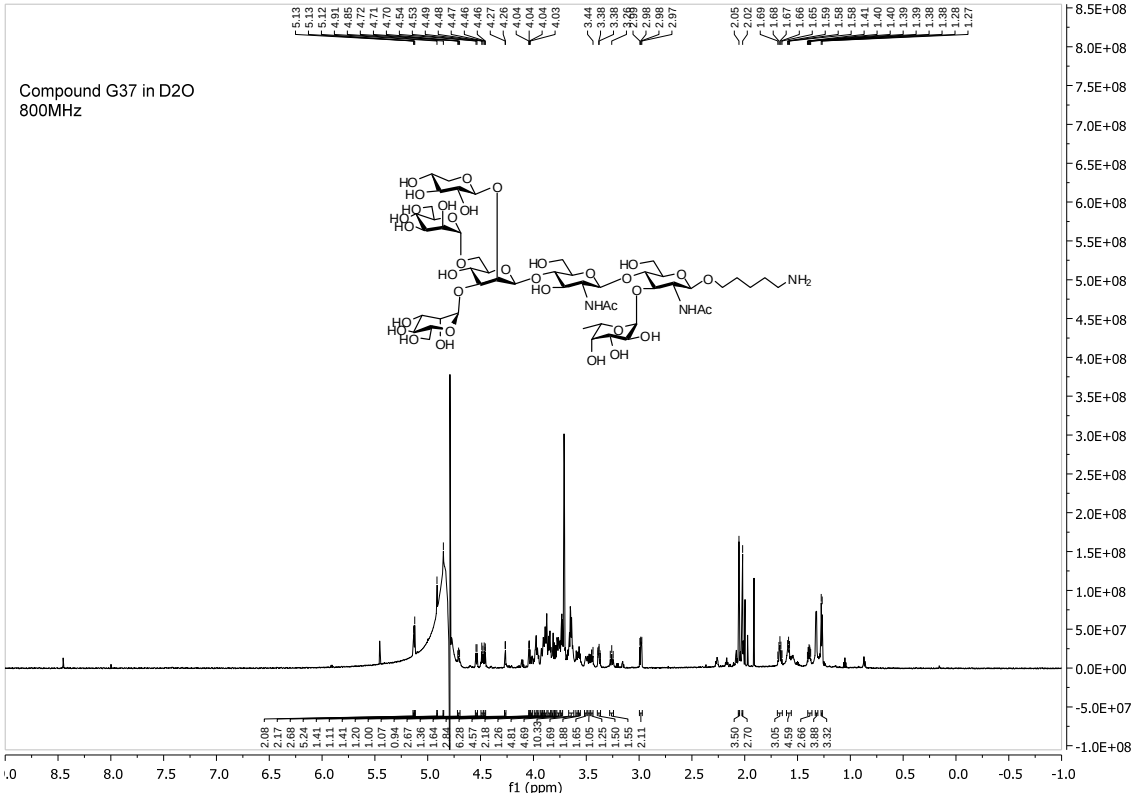

Compound G34 in D2O
201 MHz
f2 (ppm)
f1 (ppm)

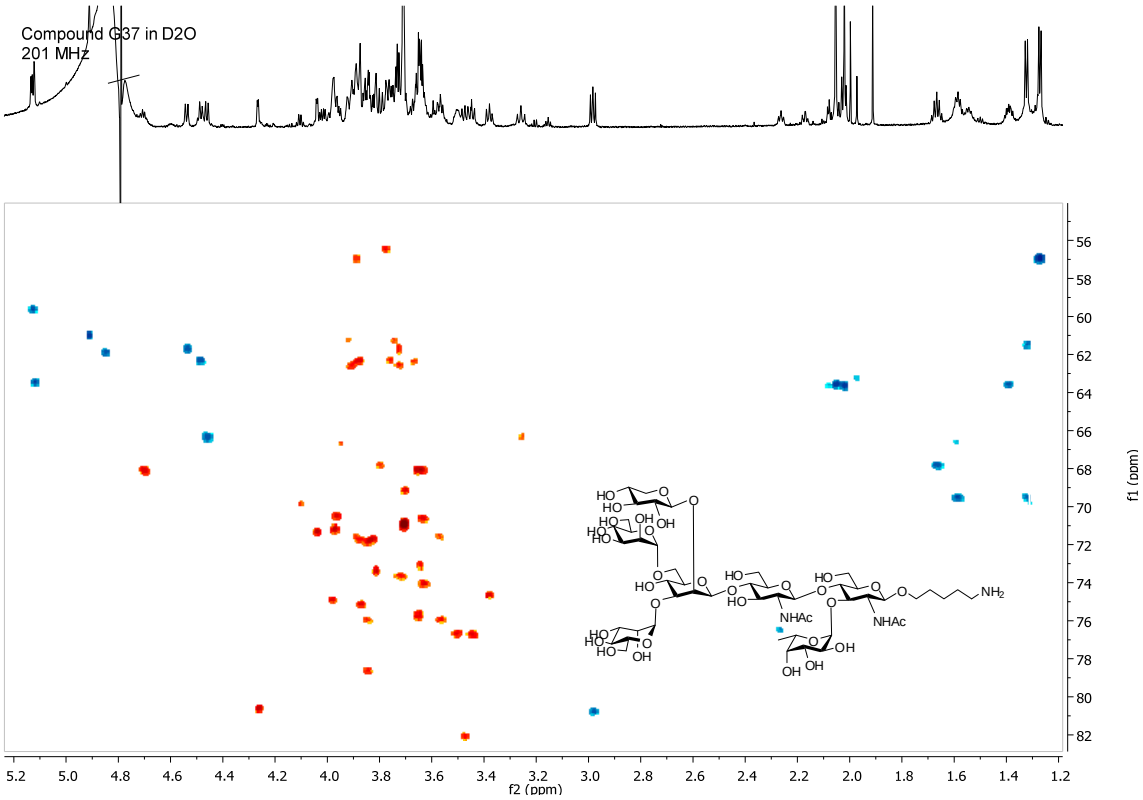
G35


Compound G35 in D2O
500MHz
D2O
NHAc
NHAc
NH2
f1 (ppm)

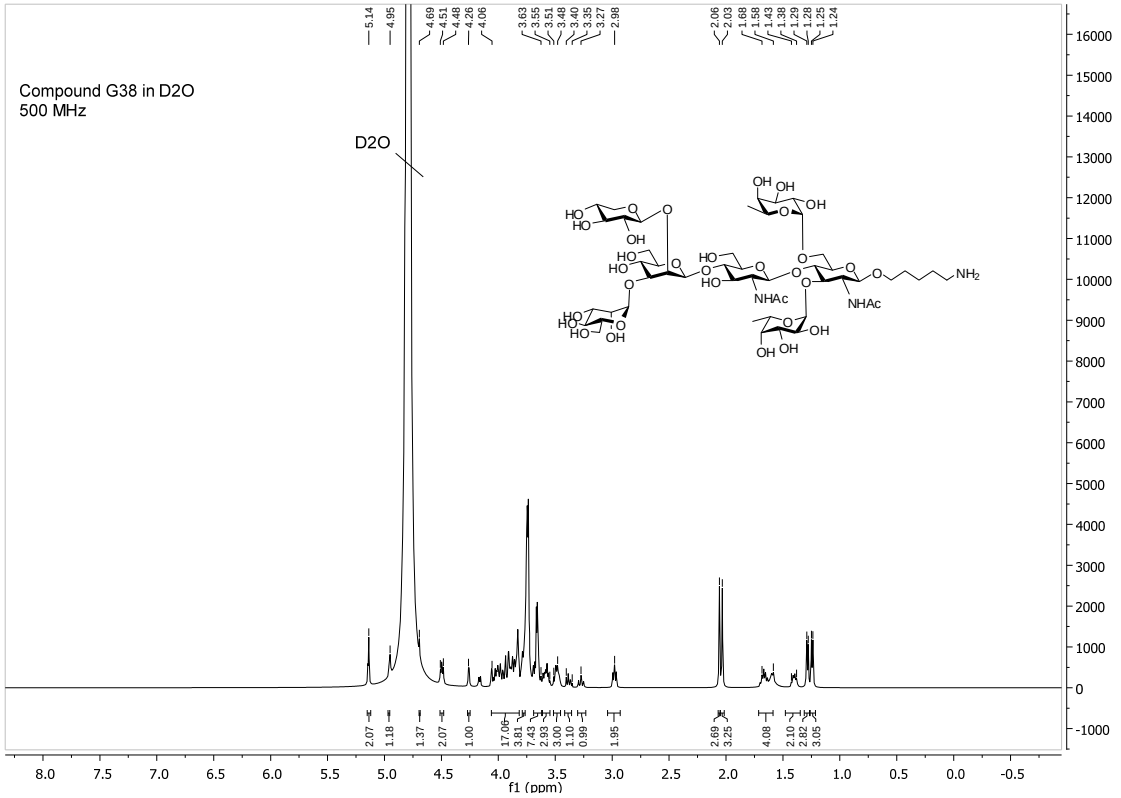

Compound G35 in D2O
126 MHz
NHAc
NHAc
NH2
f2 (ppm)
f1 (ppm)

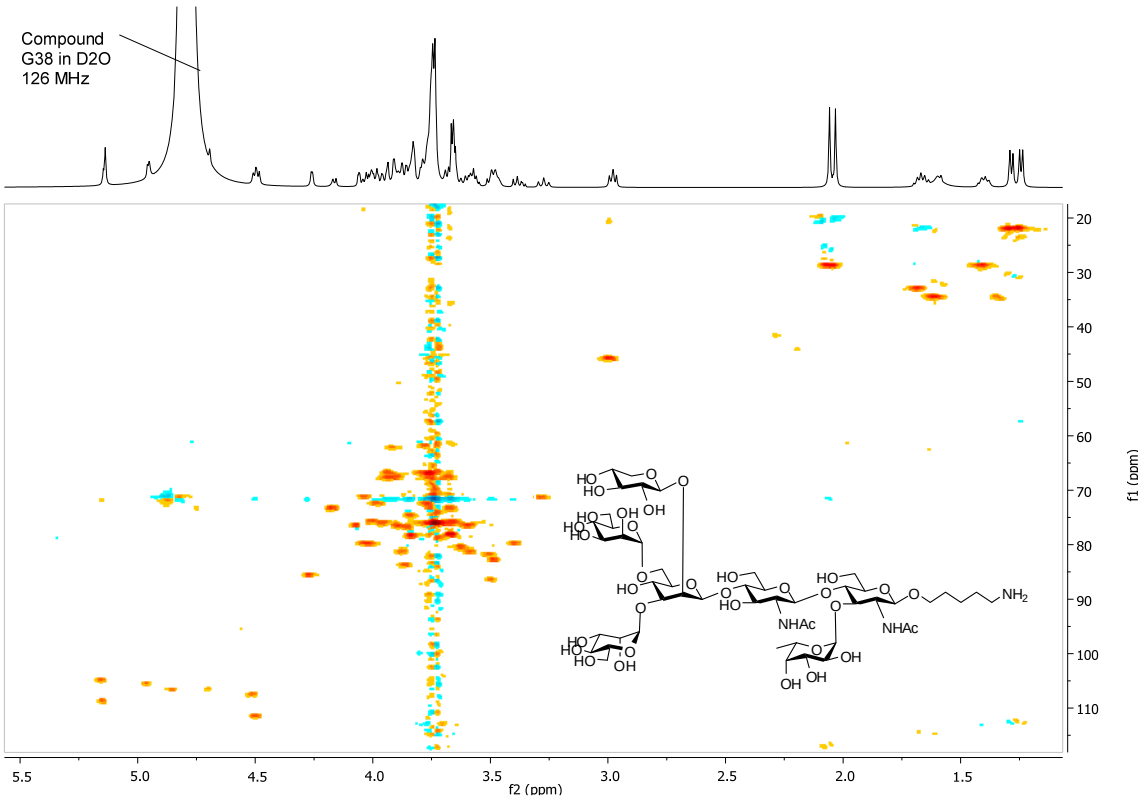
G37


Compound G37 in D2O
800MHz
f1 (ppm)

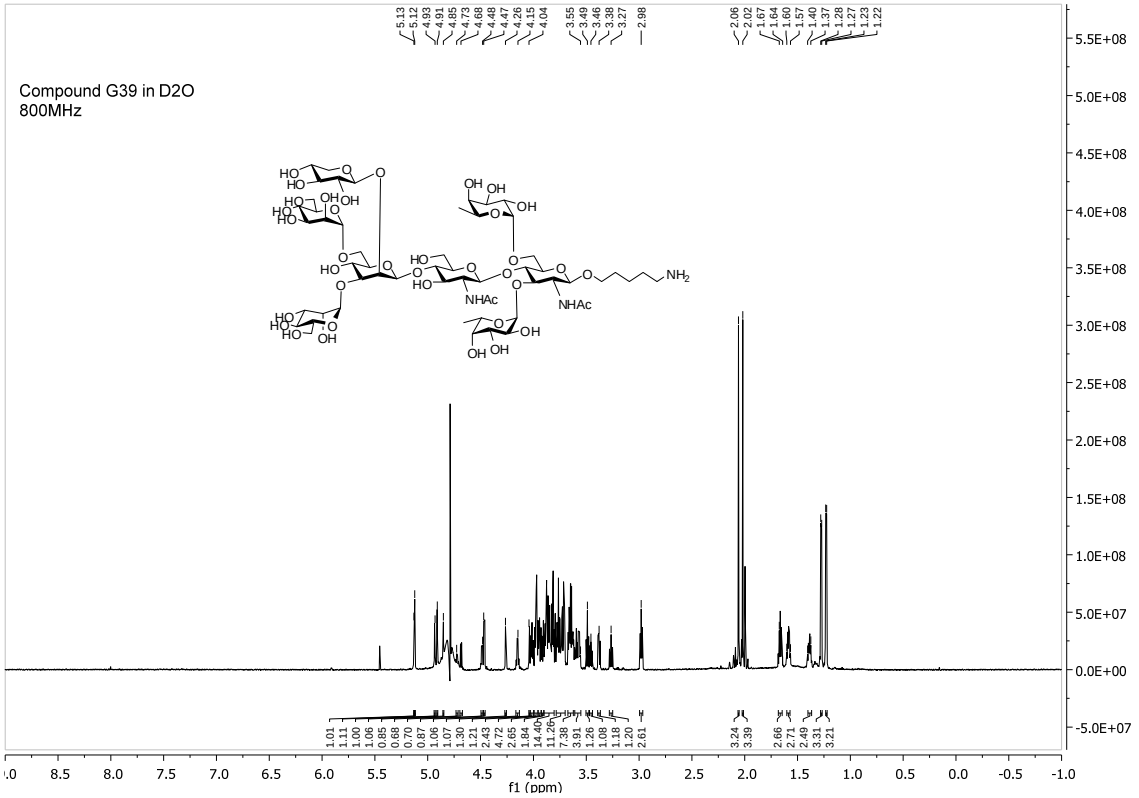

Compound G37 in D2O
201 MHz
f2 (ppm)
f1 (ppm)

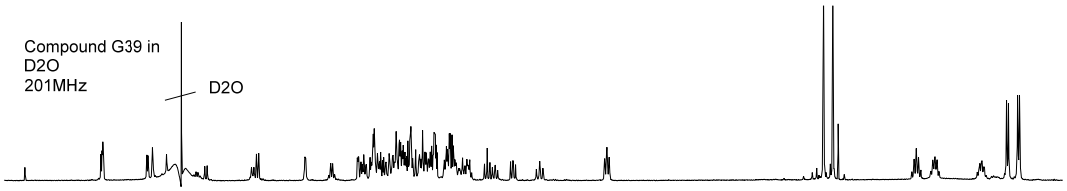
G38

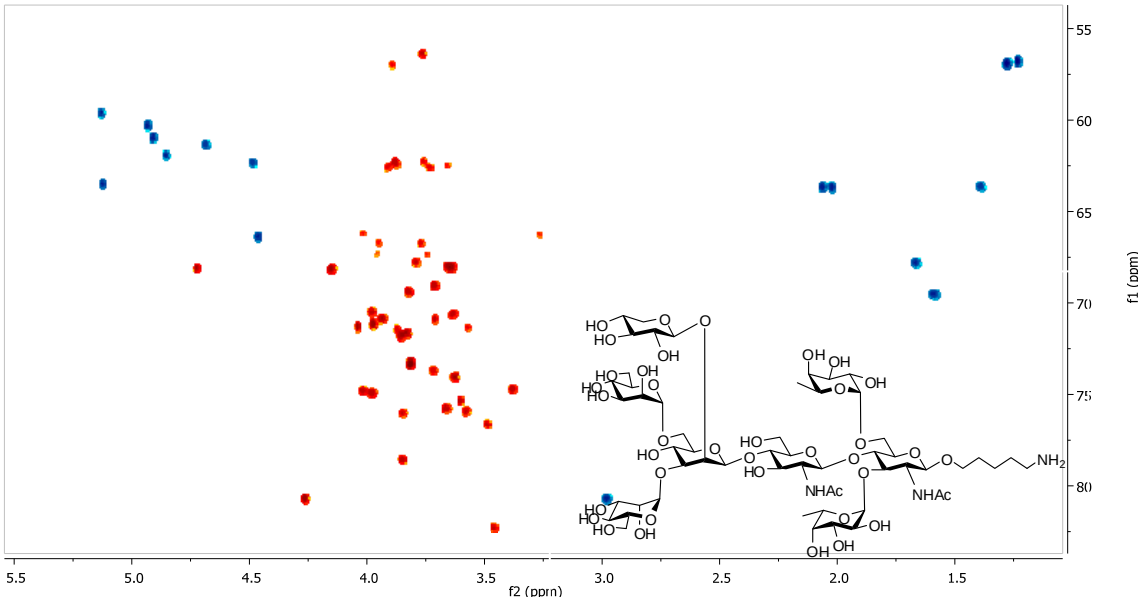

Compound G38 in D2O
500 MHz
D2O
f1 (ppm)


Compound
G38 in D2O
126 MHz
f2 (ppm)
f1 (ppm)

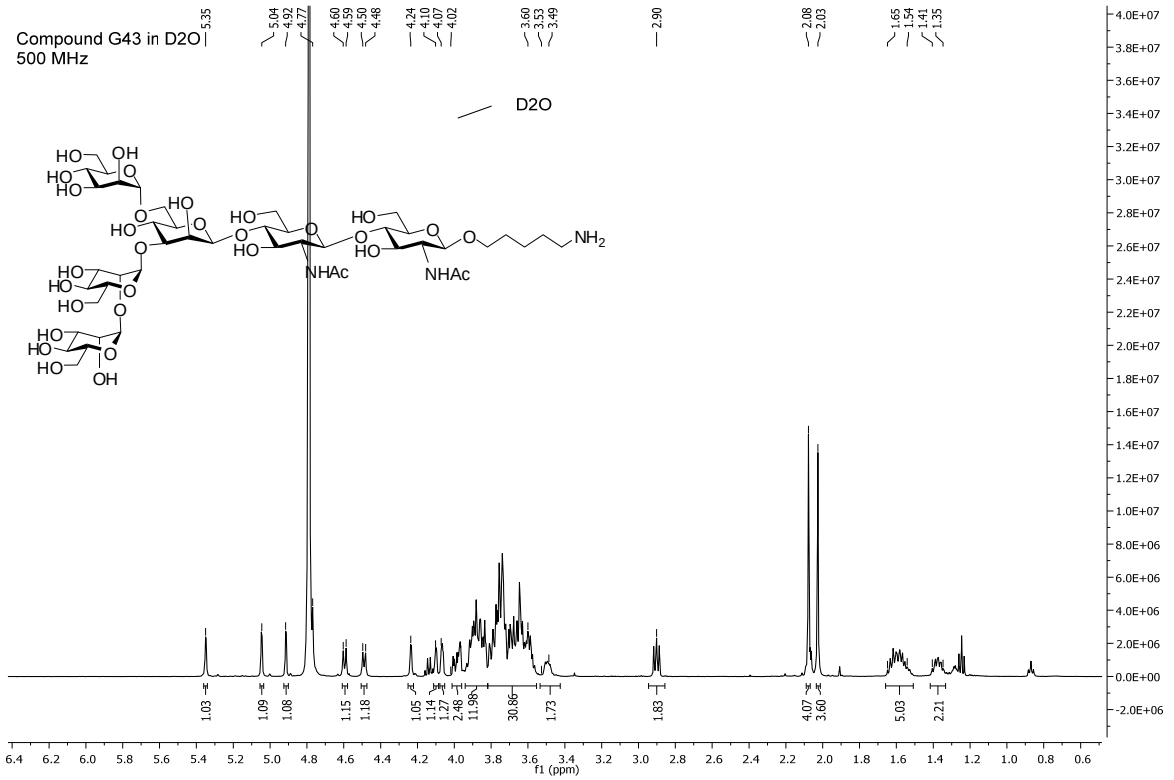
G39

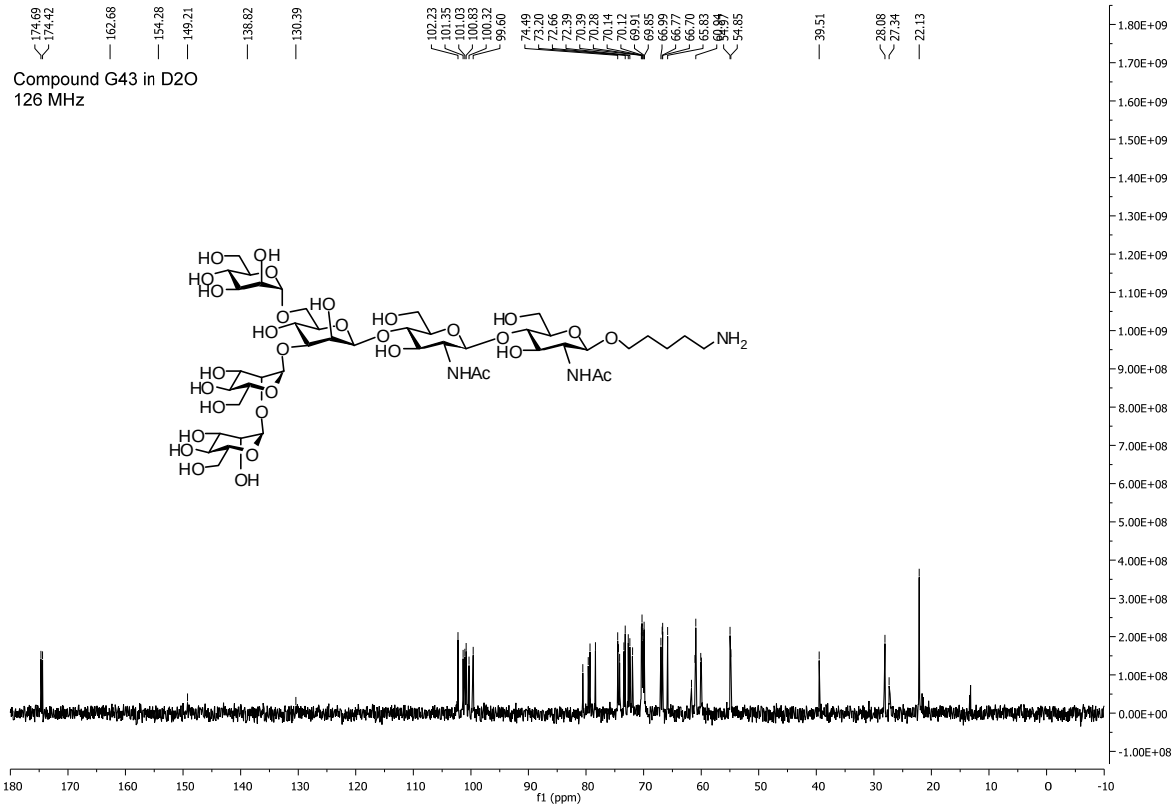

Compound G39 in D2O
800MHz
NHAc
NHAc
NH2
f1 (ppm)


Compound G39 in
D2O
201MHz
D2O

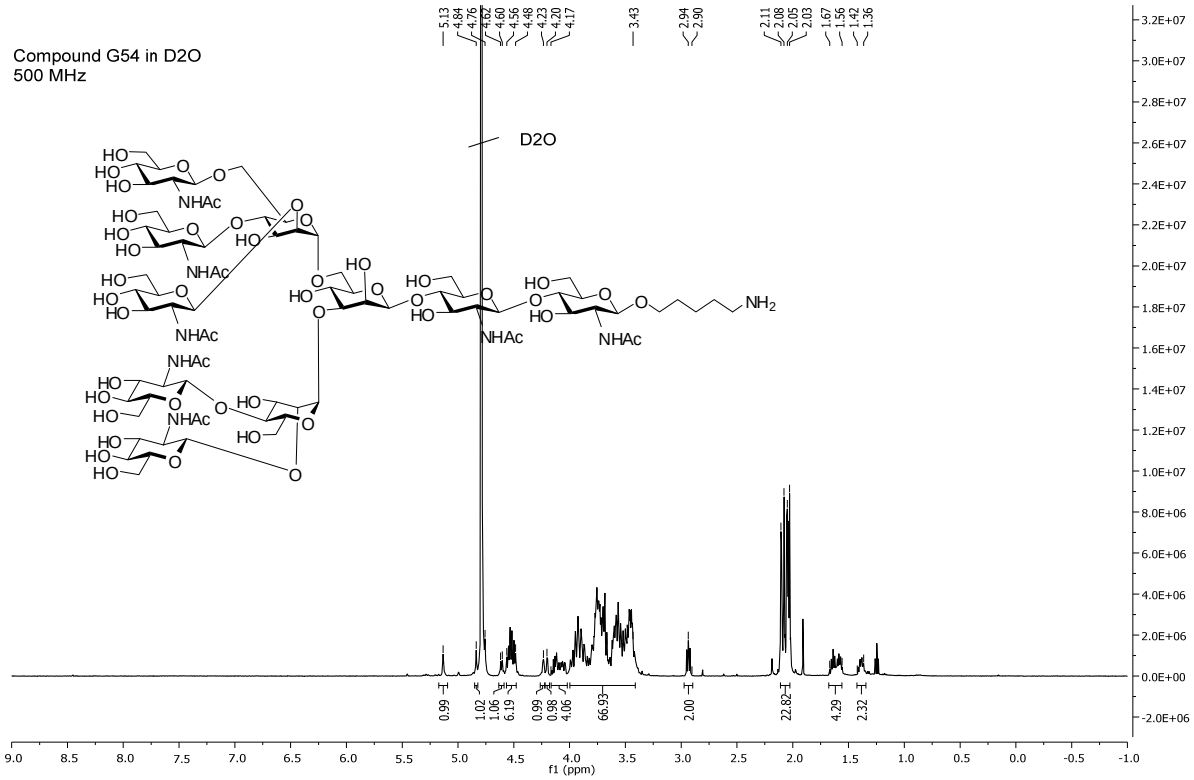

NHAc
NHAc
NH2
f2 (ppm)
f1 (ppm)

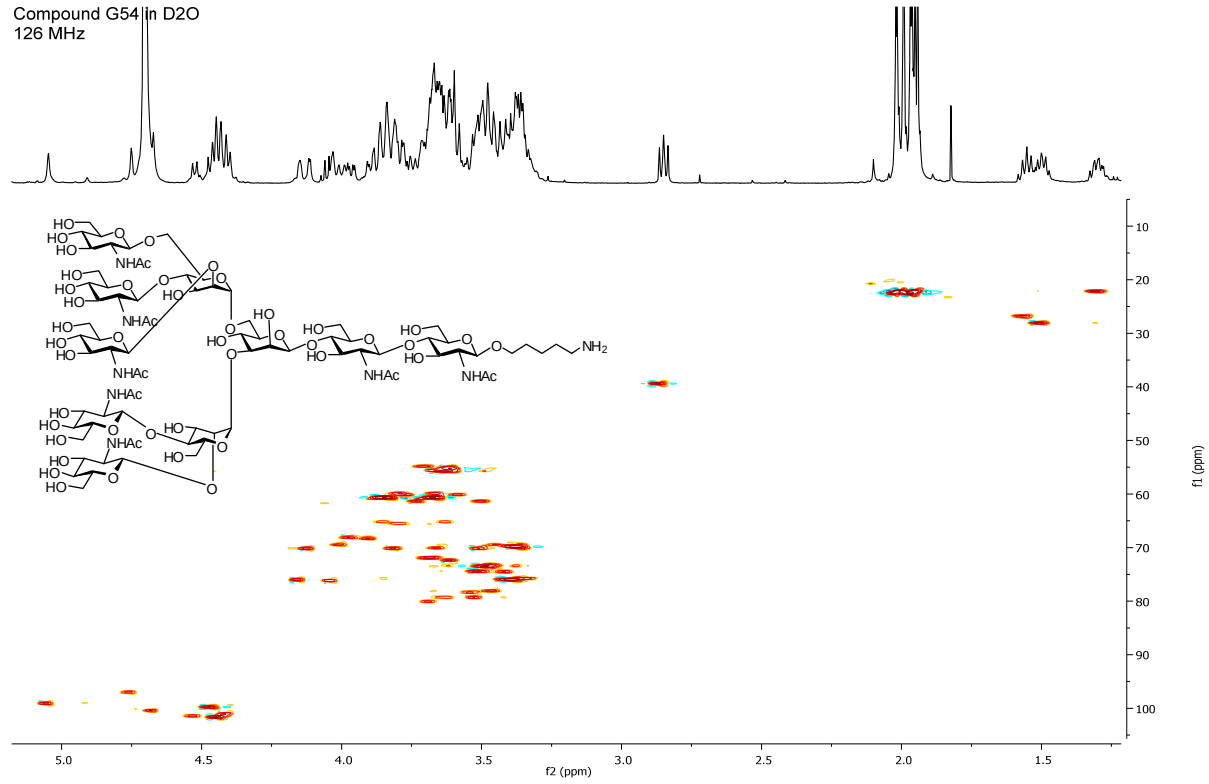
G43


Compound G43 in D2O
500 MHz
D2O
NHAc
NHAc
NH2
f1 (ppm)

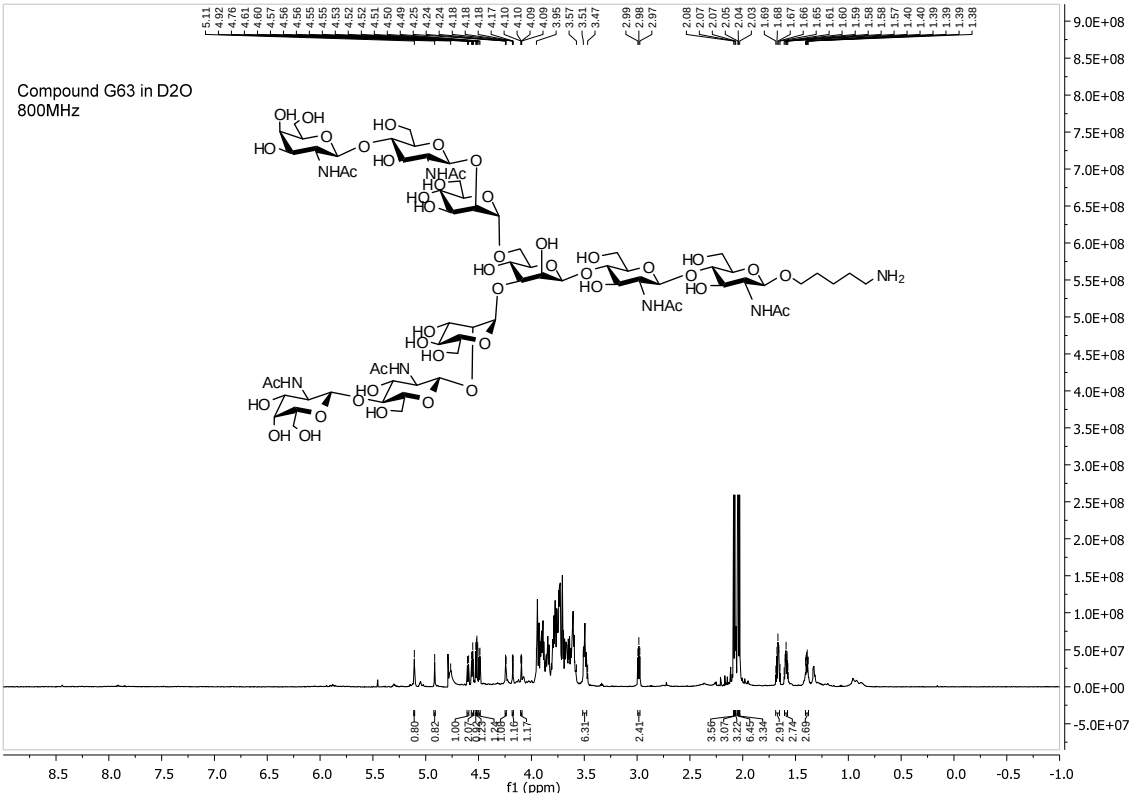

Compound G43 in D2O
126 MHz
NHAc
NHAc
NH2
f1 (ppm)

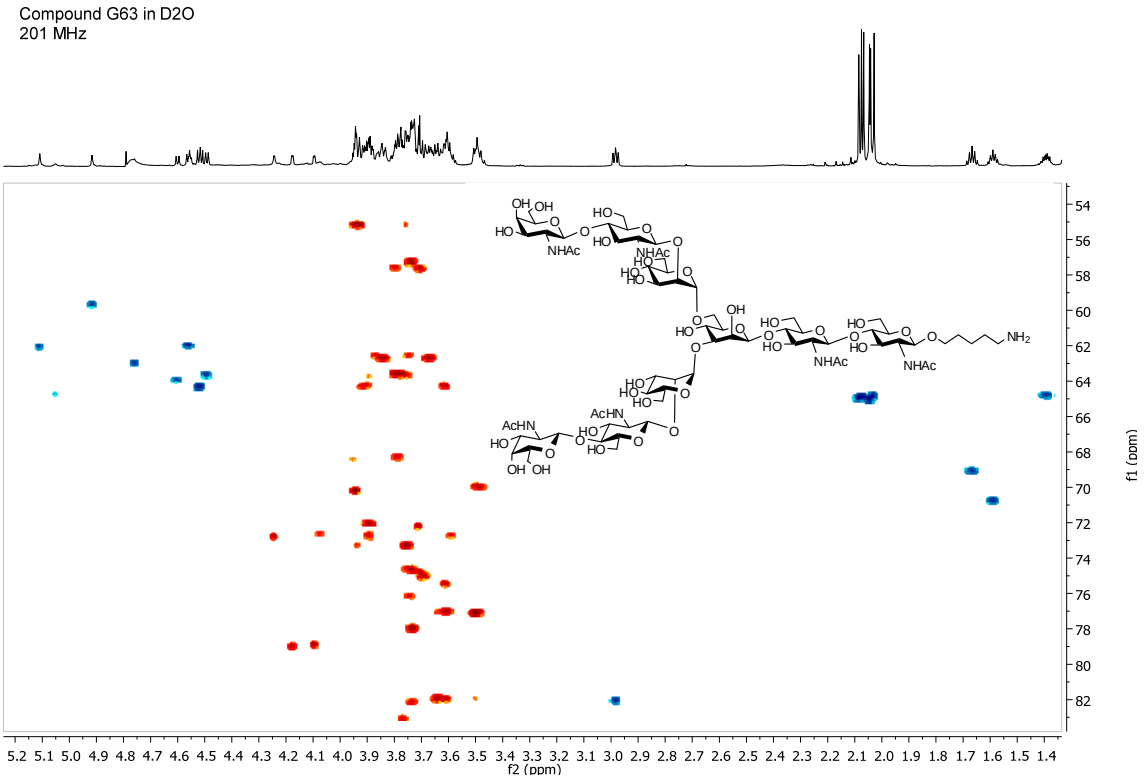
G54


Compound G54 in D2O
500 MHz
D2O
f1 (ppm)

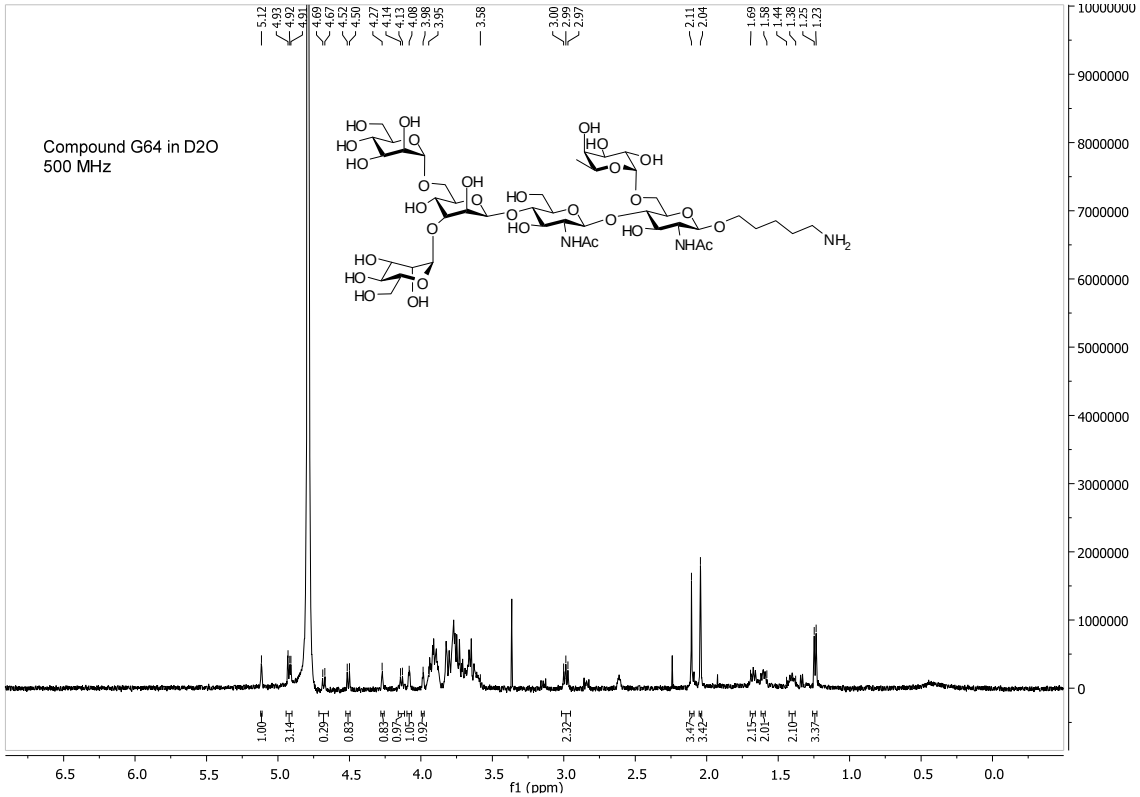

Compound G54 in D2O
126 MHz
f2 (ppm)
f1 (ppm)

G63

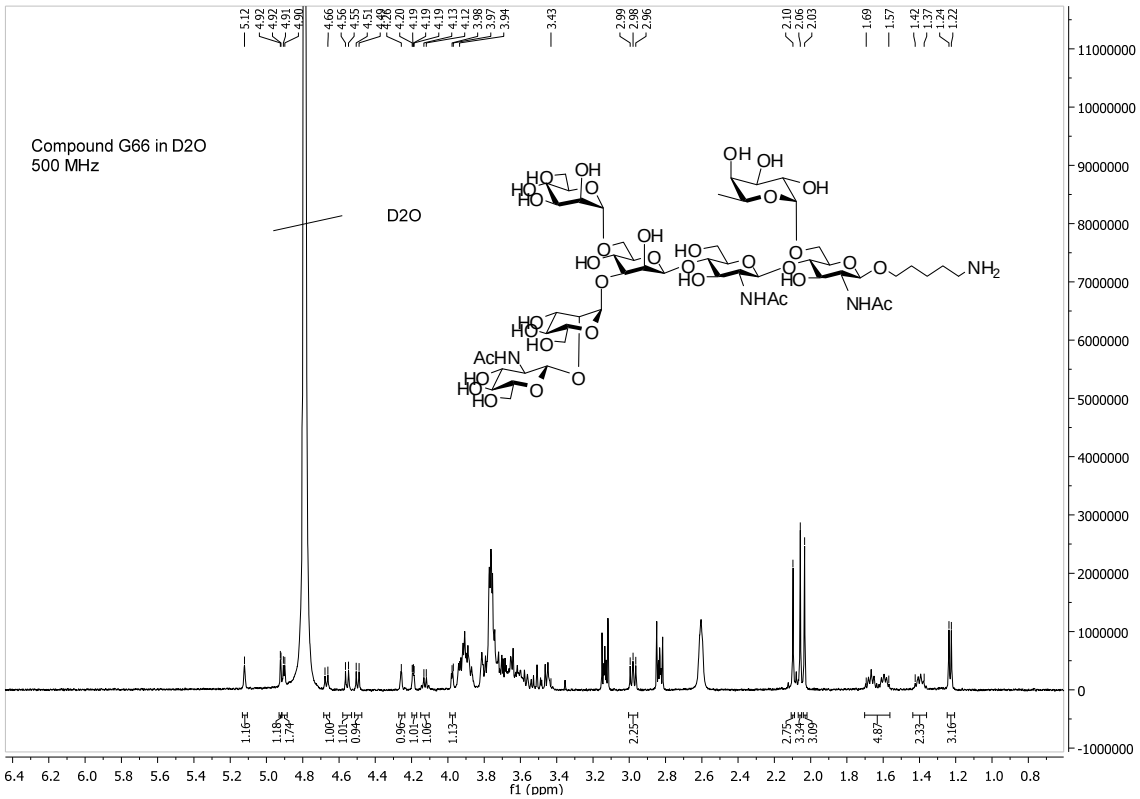

Compound G63 in D2O
800MHz
f1 (ppm)


Compound G63 in D2O
201 MHz
f2 (ppm)
f1 (ppm)

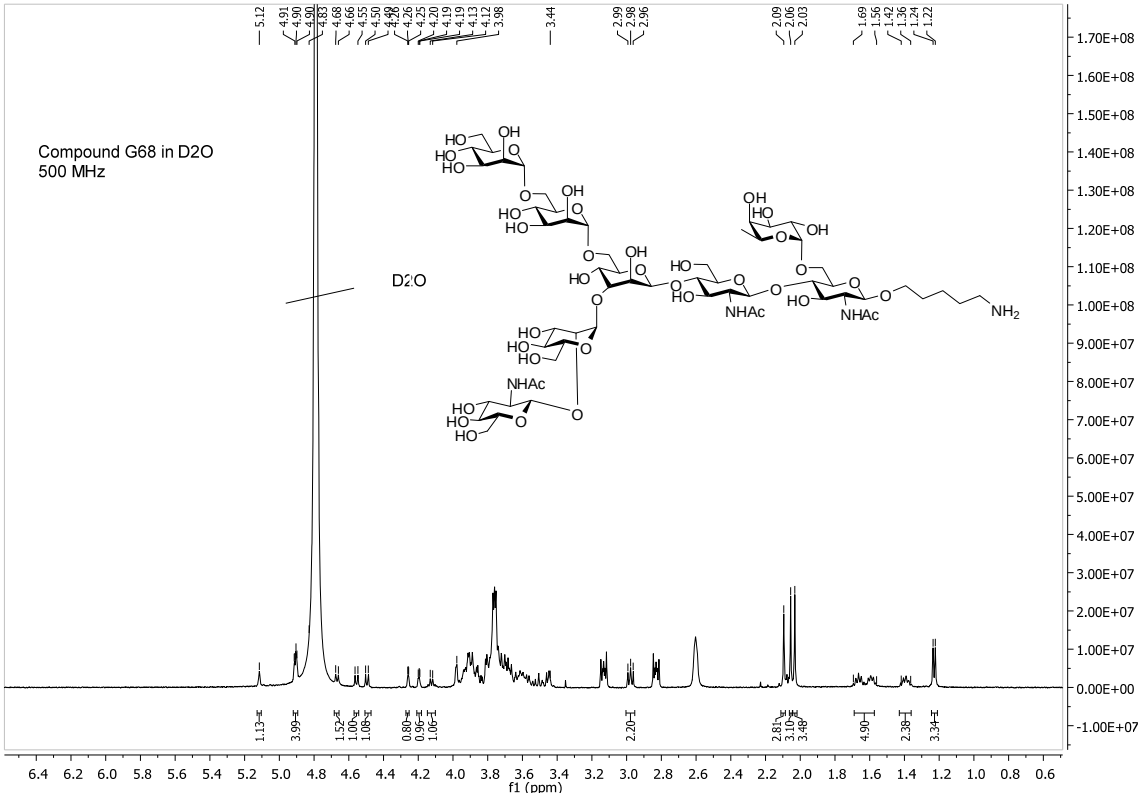
G64


Compound G64 in D2O
500 MHz
f1 (ppm)

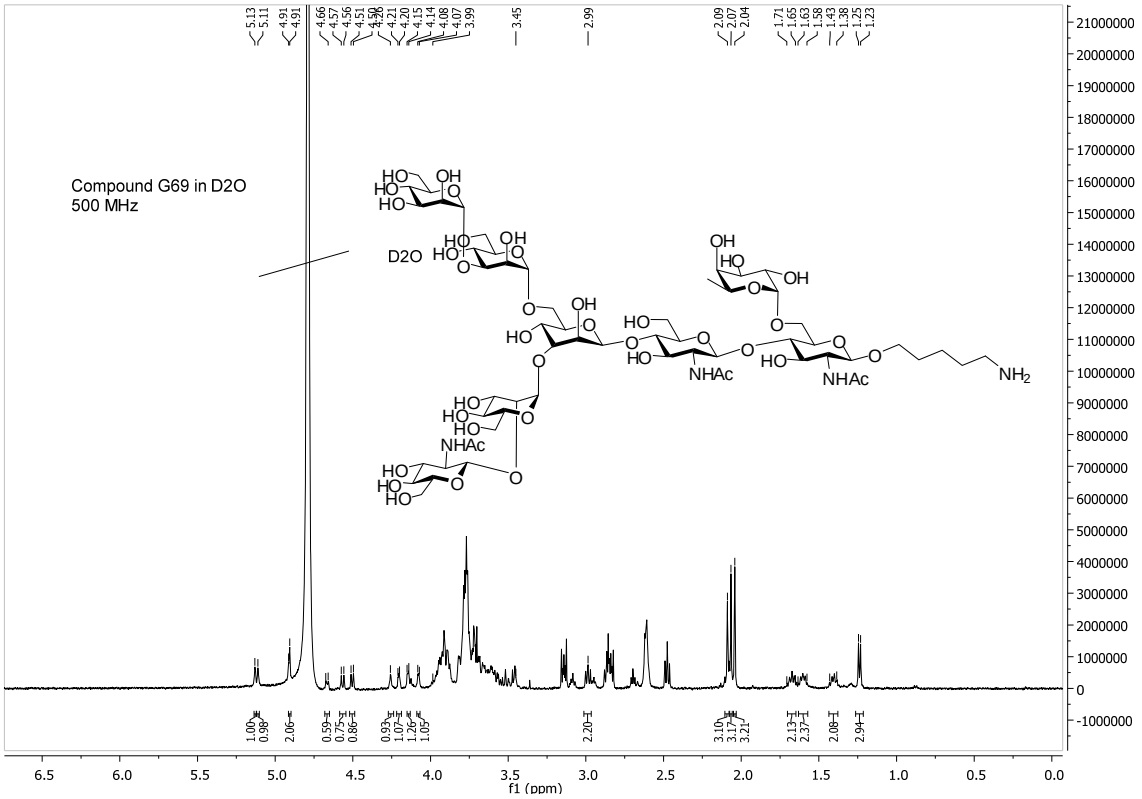
G66


Compound G66 in D2O
500 MHz
D2O
f1 (ppm)

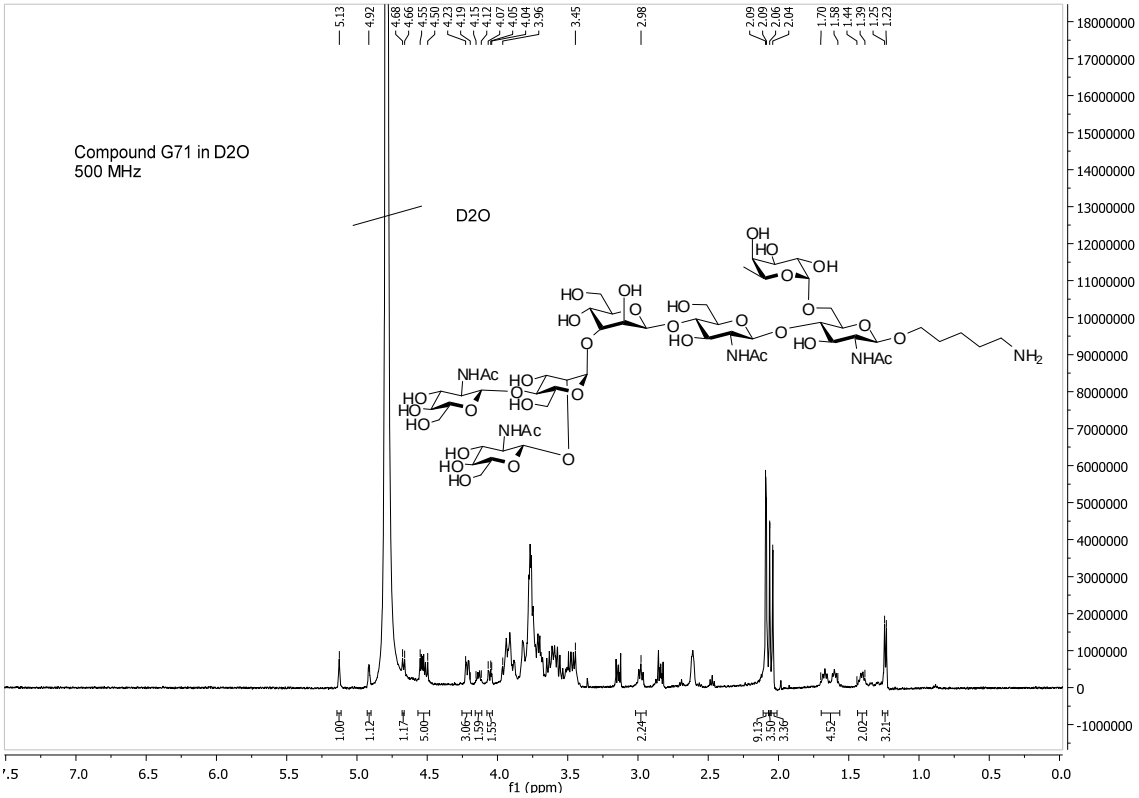
G68


Compound G68 in D2O
500 MHz
D2O
NHAc
NHAc
NH2
f1 (ppm)

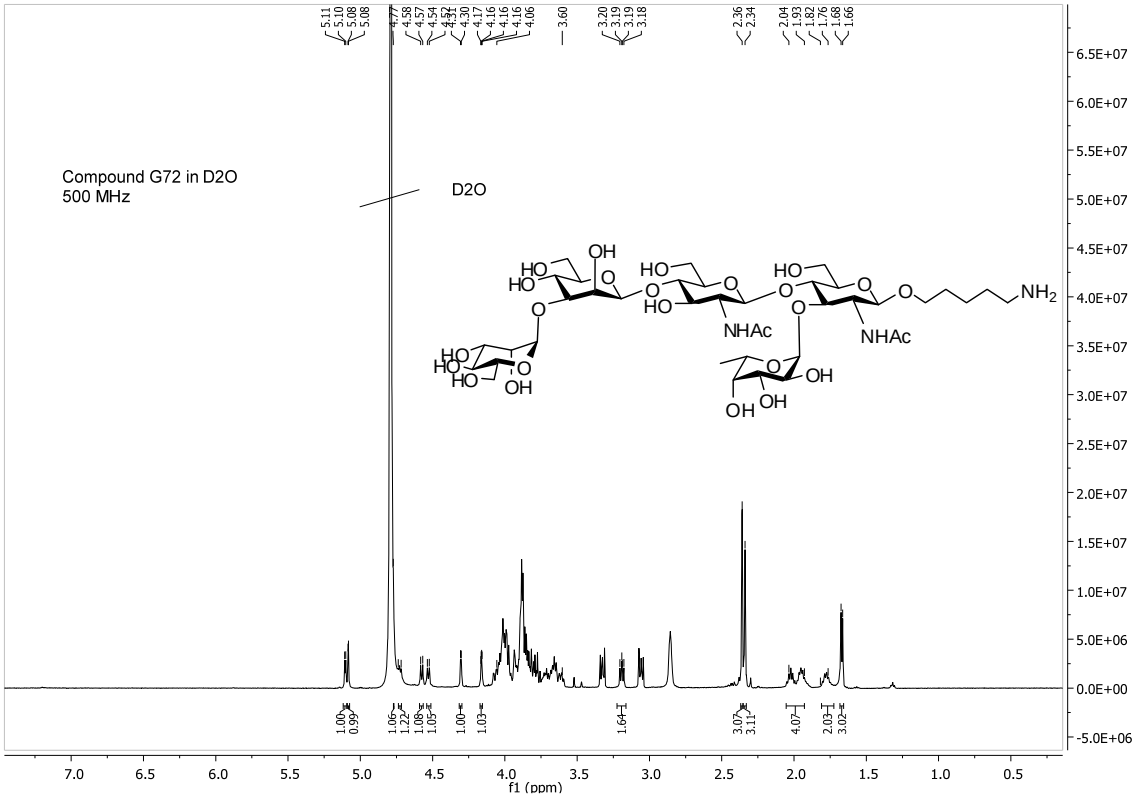
G69


Compound G69 in D2O
500 MHz
D2O
NHAc
NHAc
NH2
f1 (ppm)

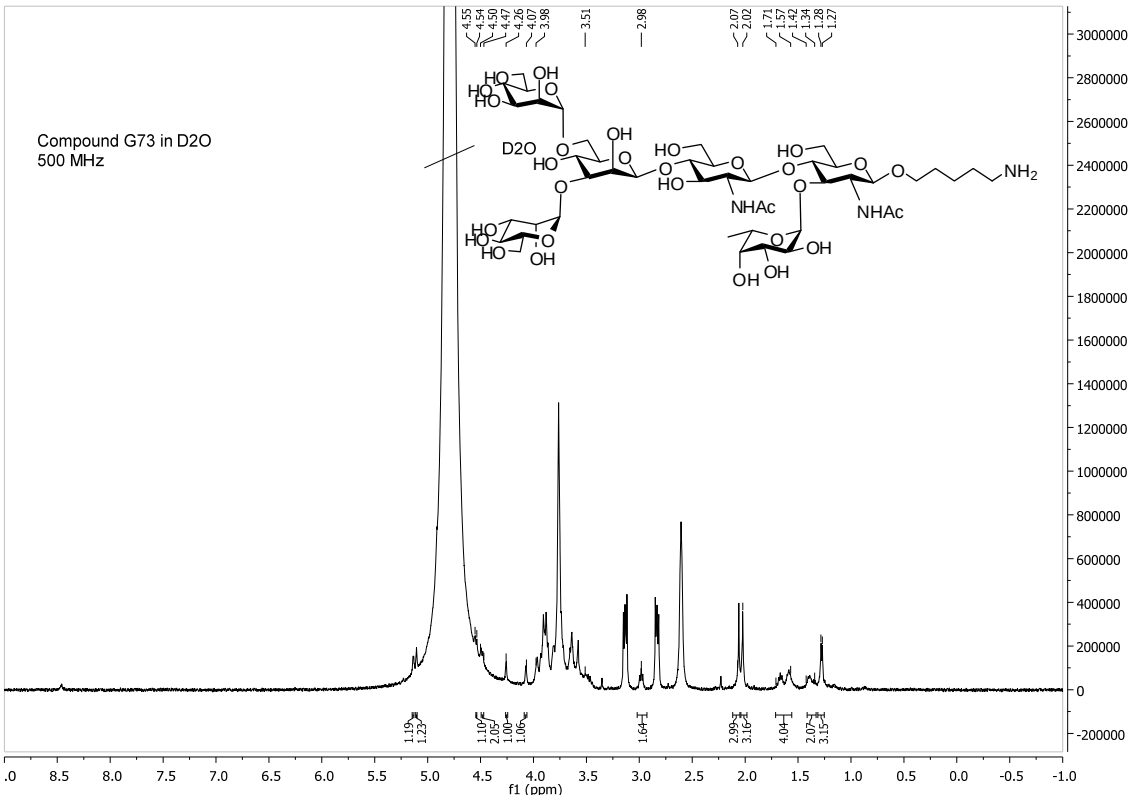
G71


Compound G71 in D2O
500 MHz
D2O
NHAc
NHAc
NHAc
NH2
f1 (ppm)

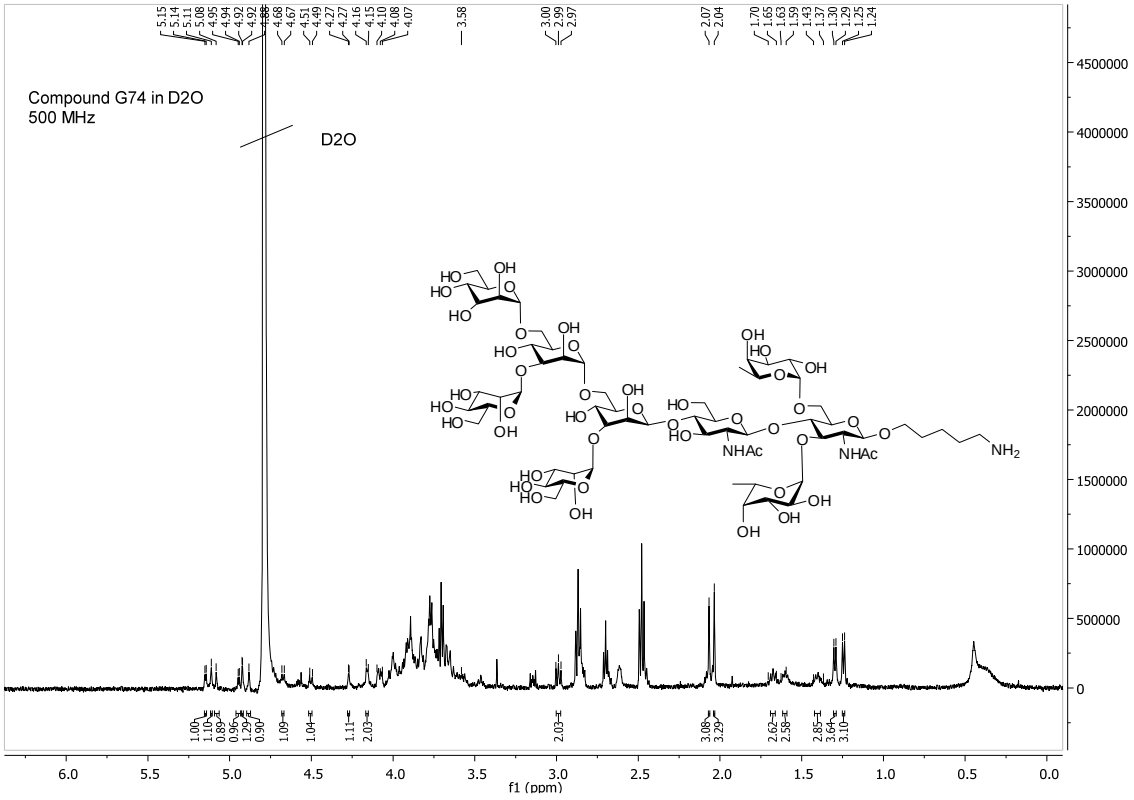
G72


Compound G72 in D2O
500 MHz
D2O
NHAc
NHAc
NH2
f1 (ppm)

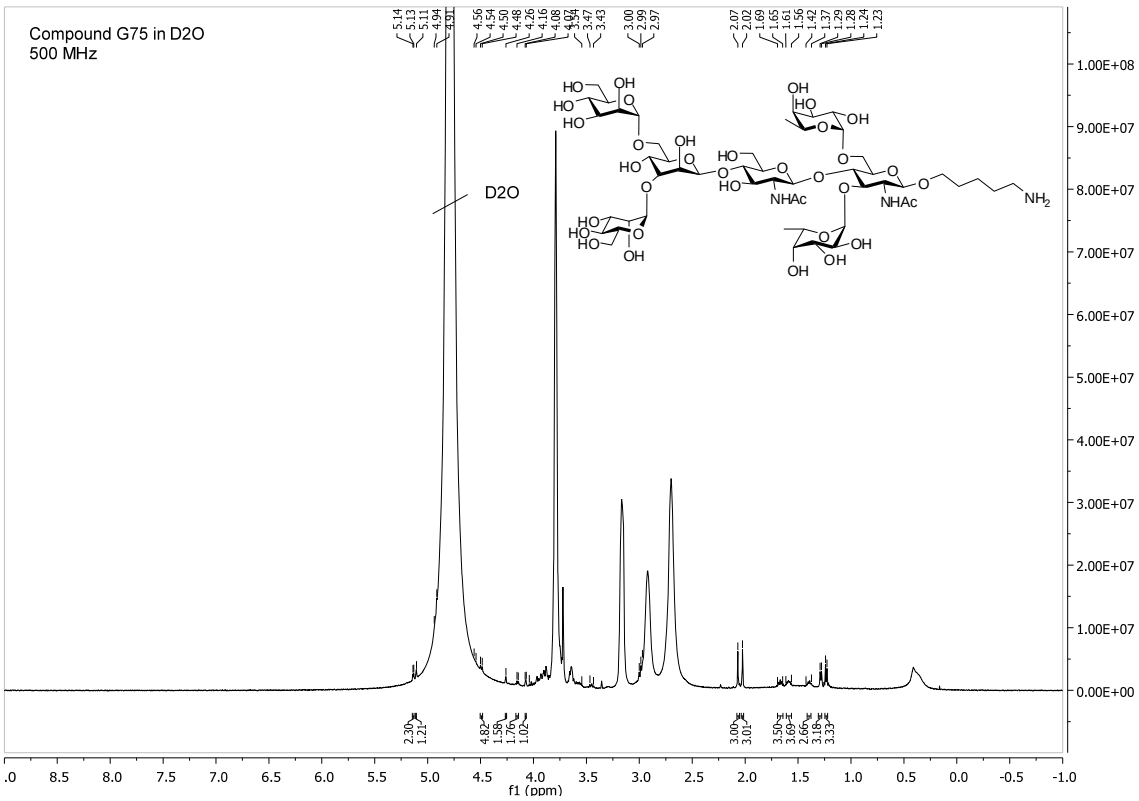
G73


Compound G73 in D2O
500 MHz
D2O
NHAc
NHAc
NH2
f1 (ppm)

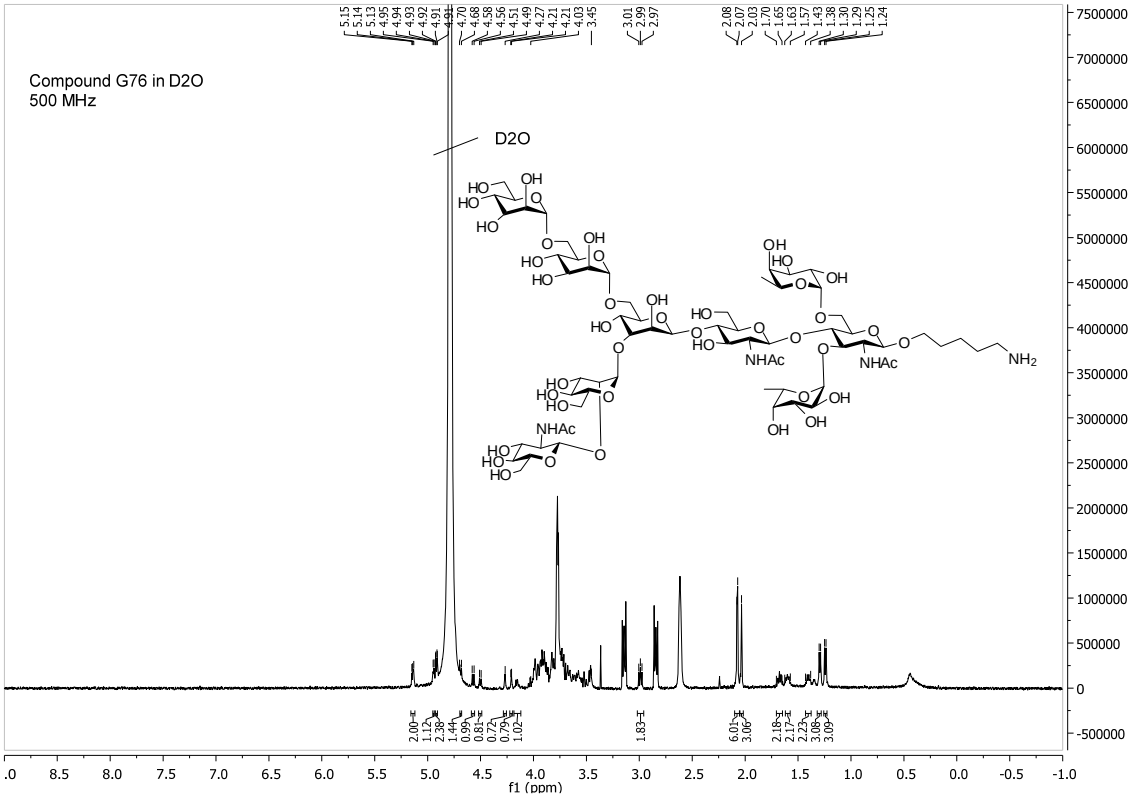
G74


Compound G74 in D2O
500 MHz
D2O
NHAc
NHAc
NH2
f1 (ppm)

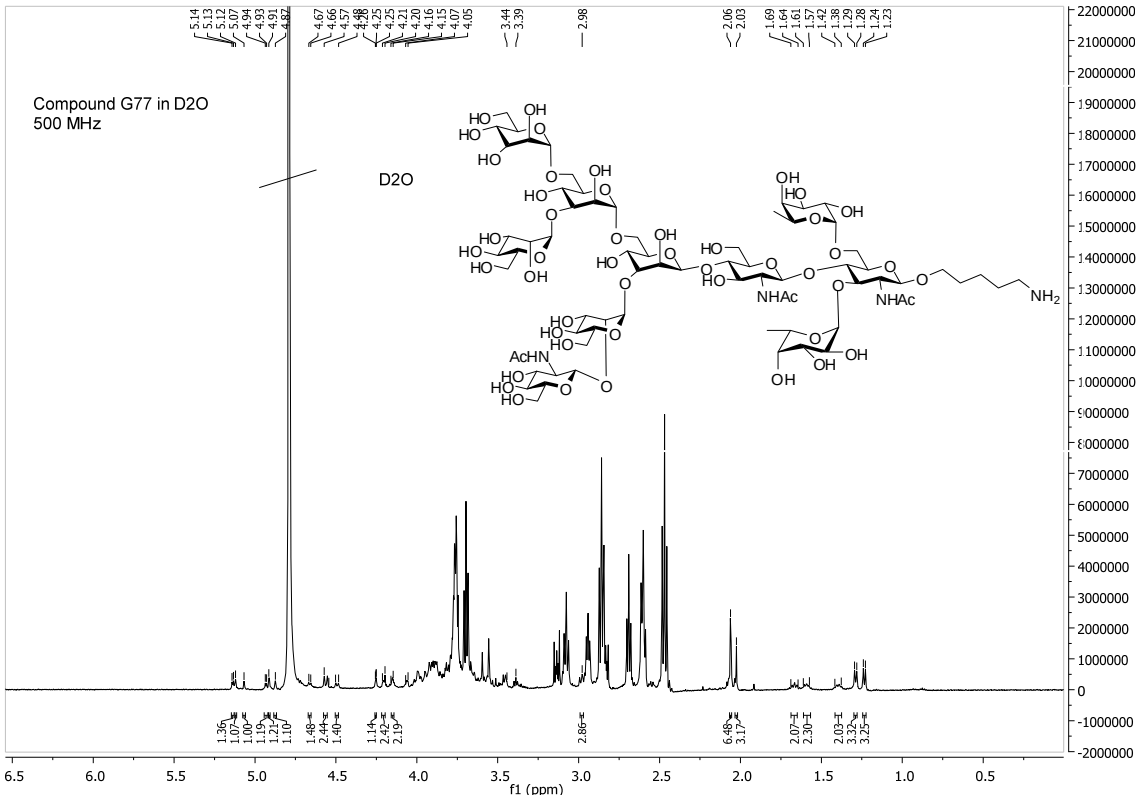
G75


Compound G75 in D2O
500 MHz
D2O
NHAc
NHAc
NH2
f1 (ppm)

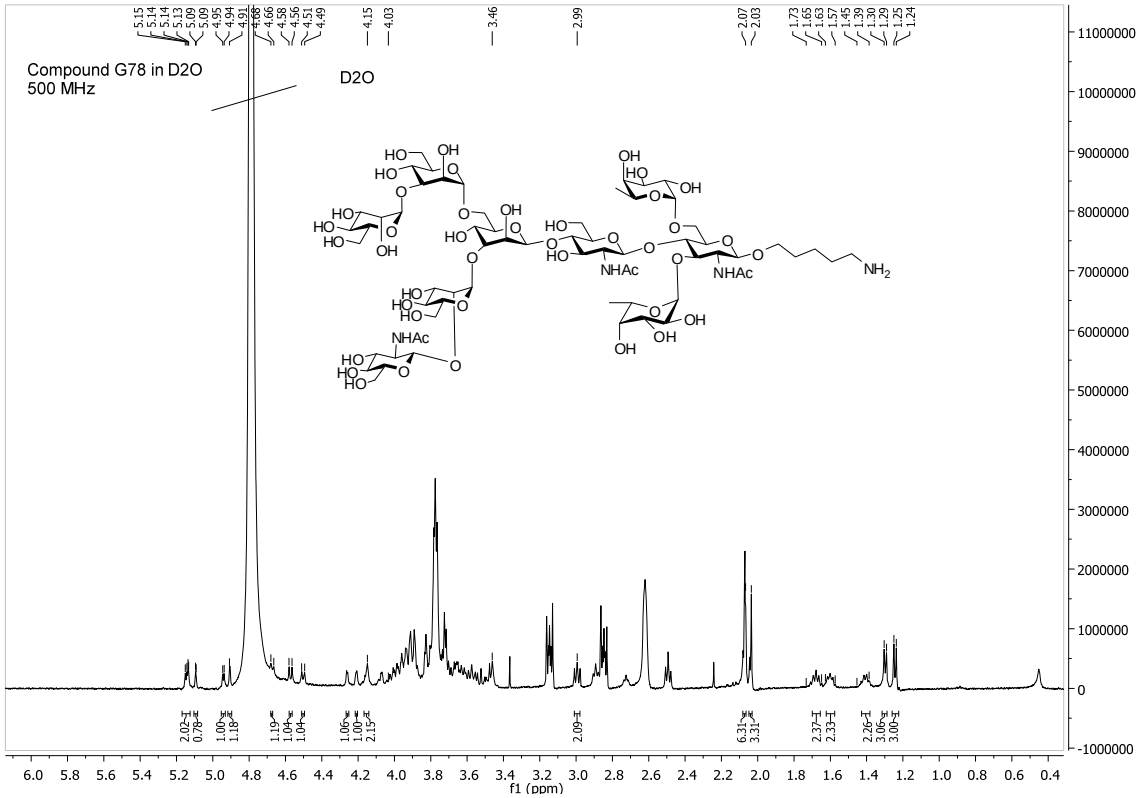
G76


Compound G76 in D2O
500 MHz
D2O
NHAc
NHAc
NHAc
NH2
f1 (ppm)

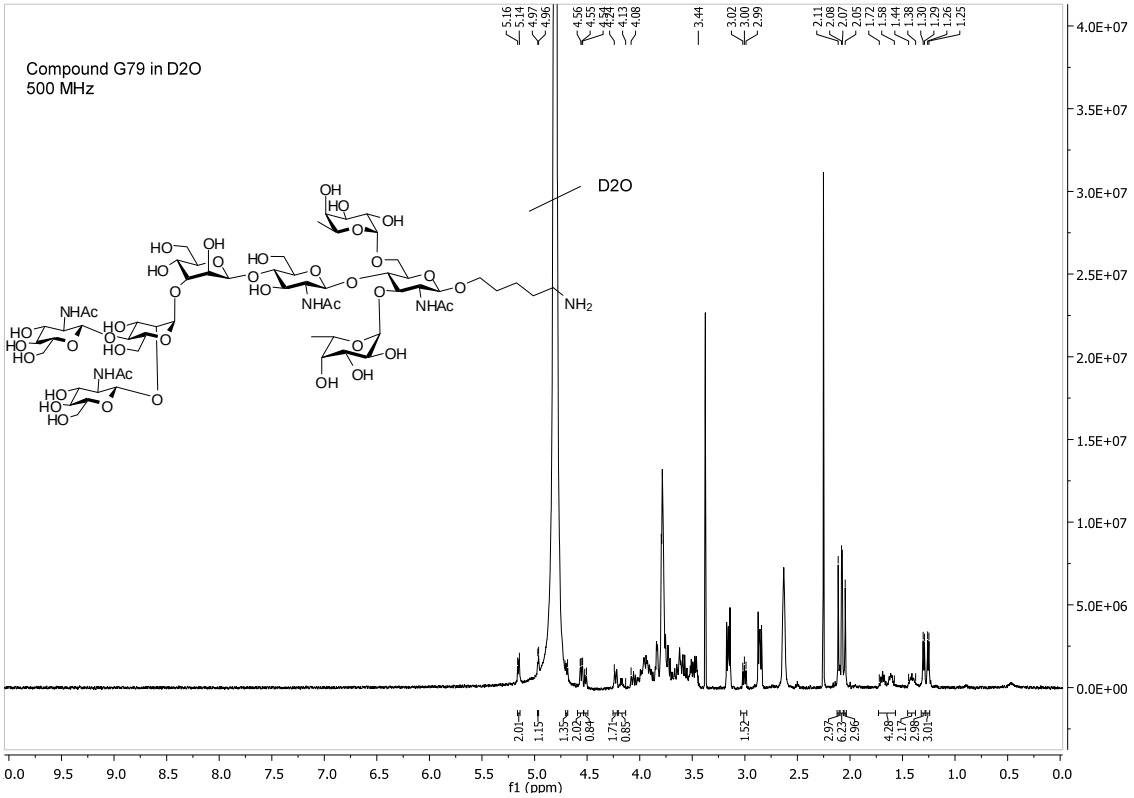
G77


Compound G77 in D2O
500 MHz
D2O
f1 (ppm)

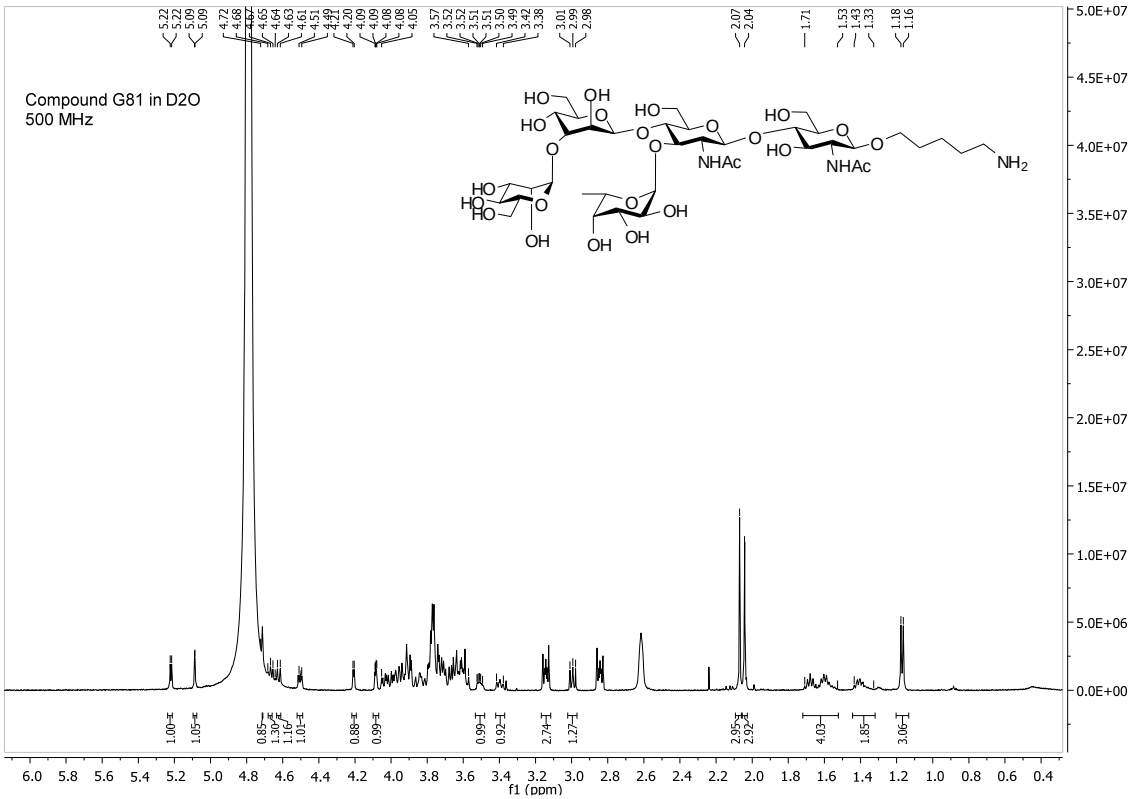
G78


Compound G78 in D2O
500 MHz
D2O
f1 (ppm)

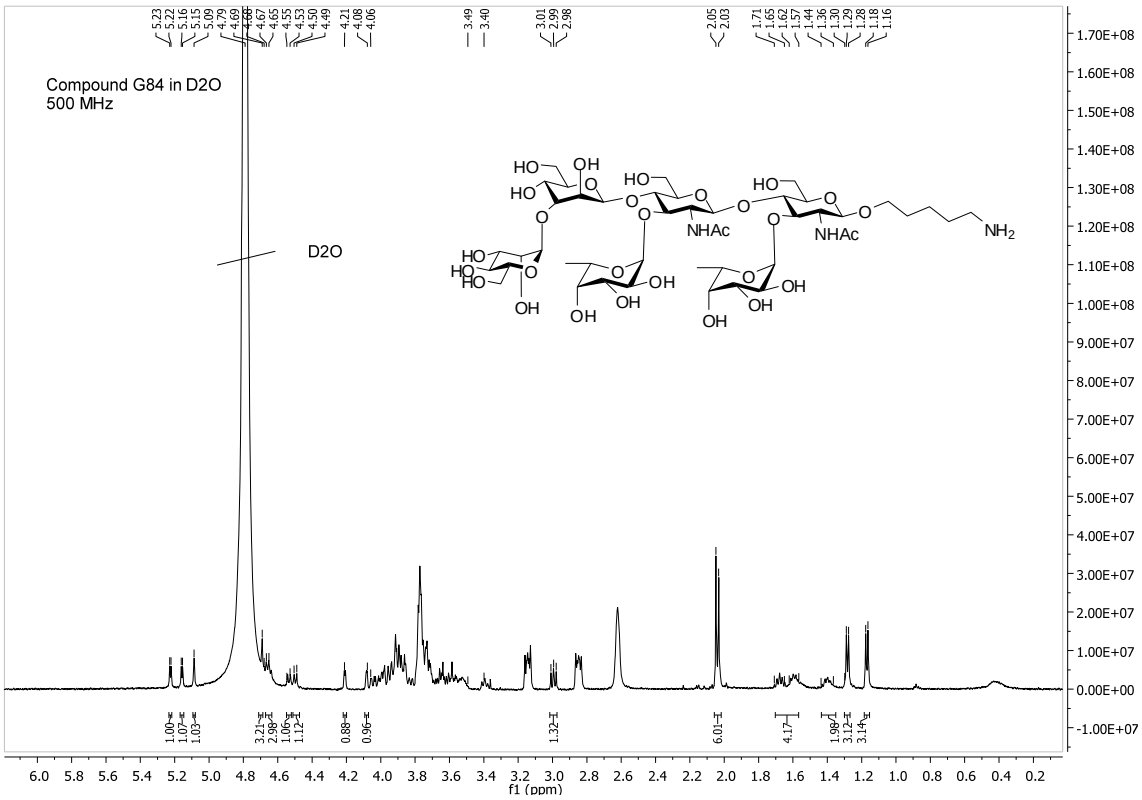
G79


Compound G79 in D2O
500 MHz
D2O
NHAc
NH2
f1 (ppm)

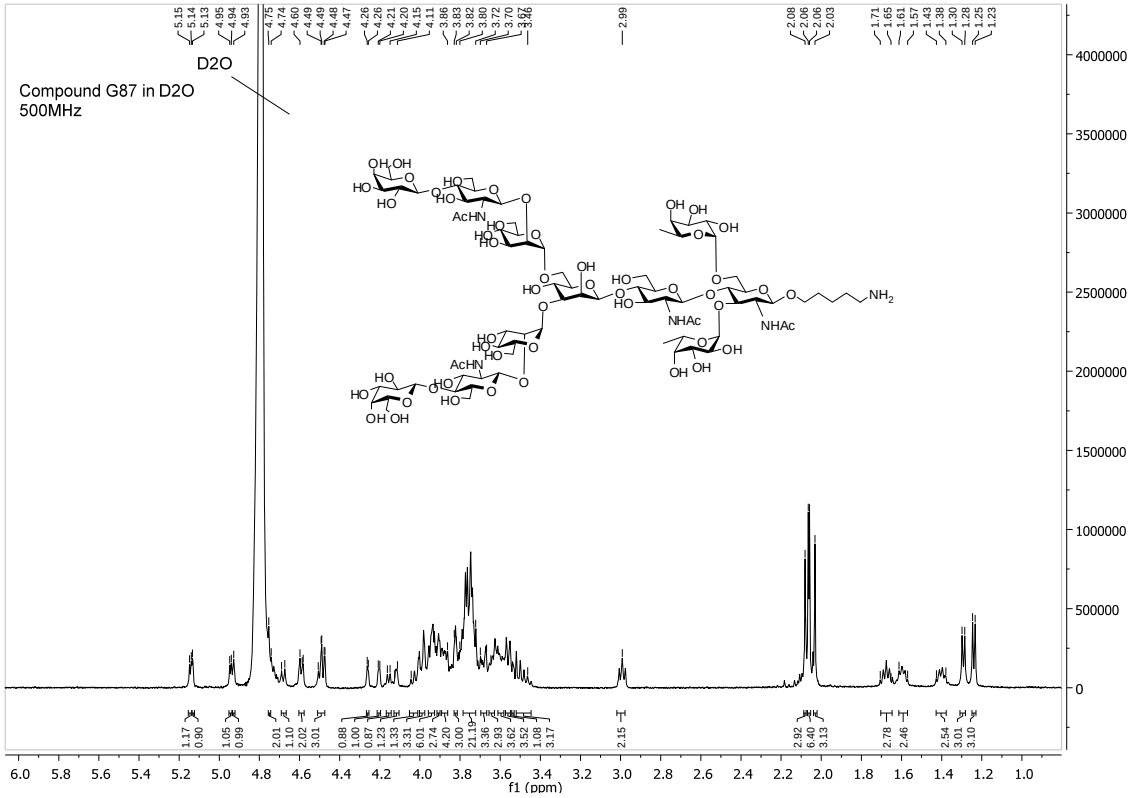
G81

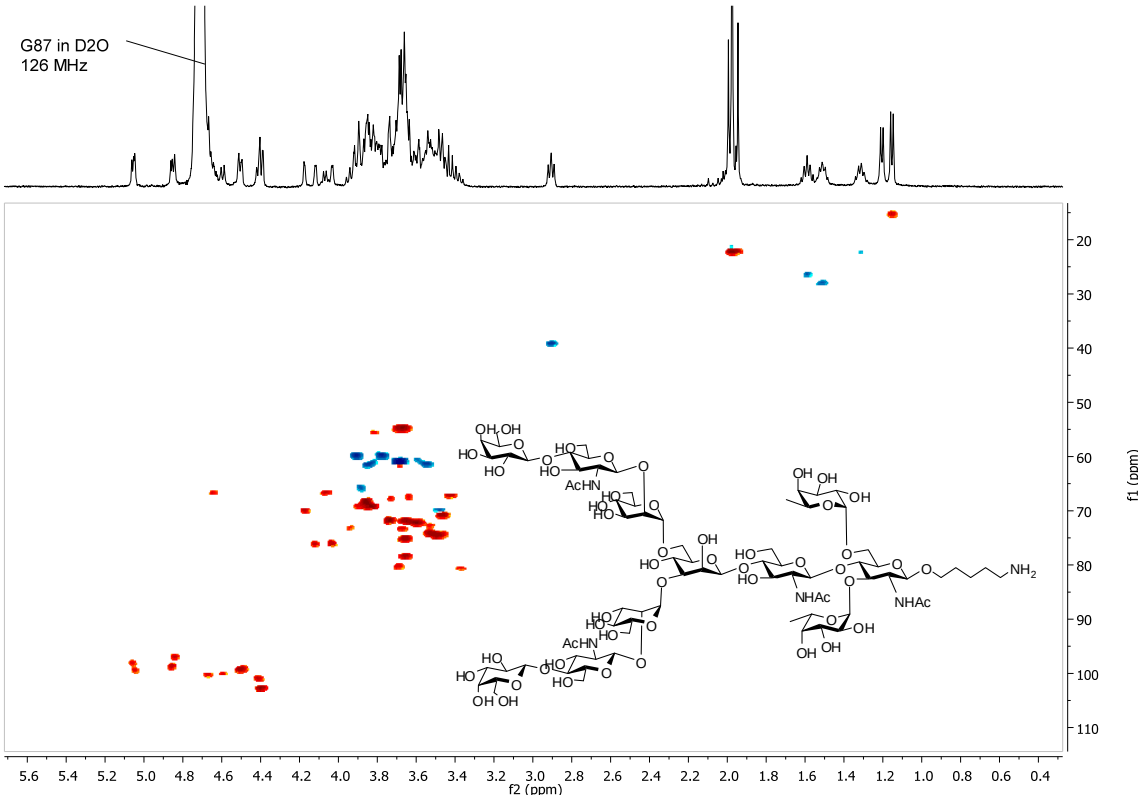

Compound G81 in D2O
500 MHz
NHAc
NH2
f1 (ppm)

G84

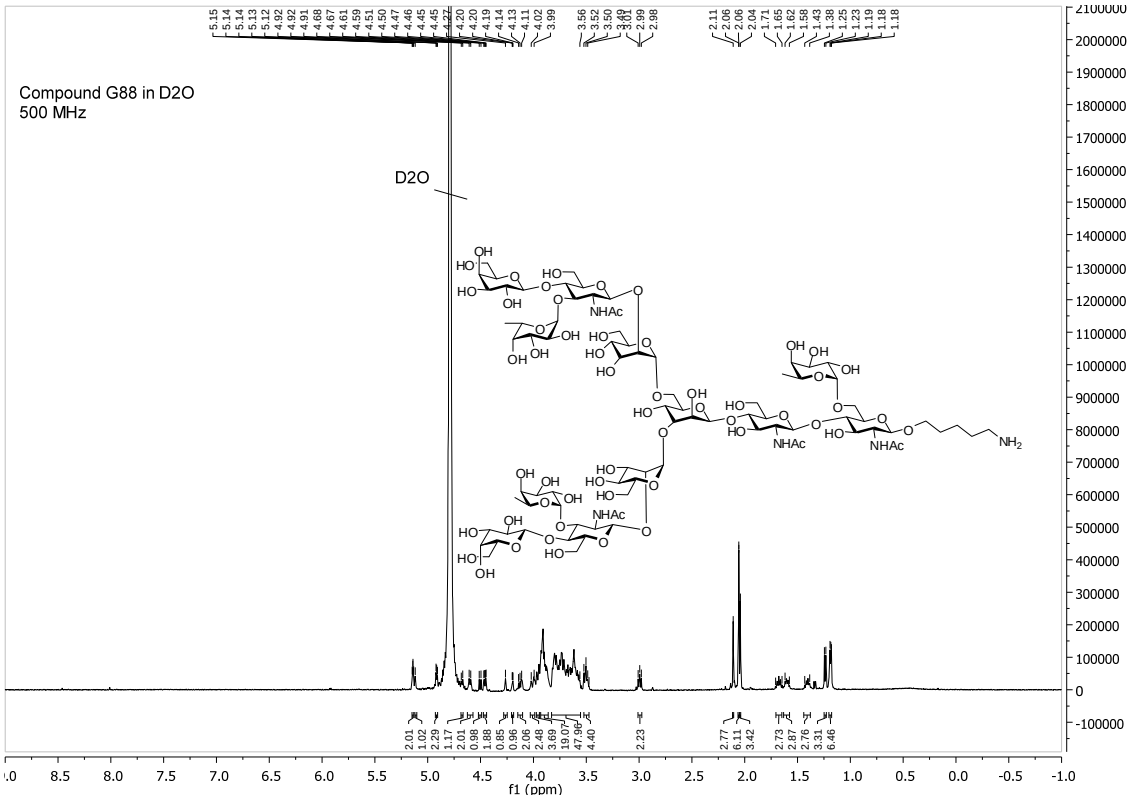


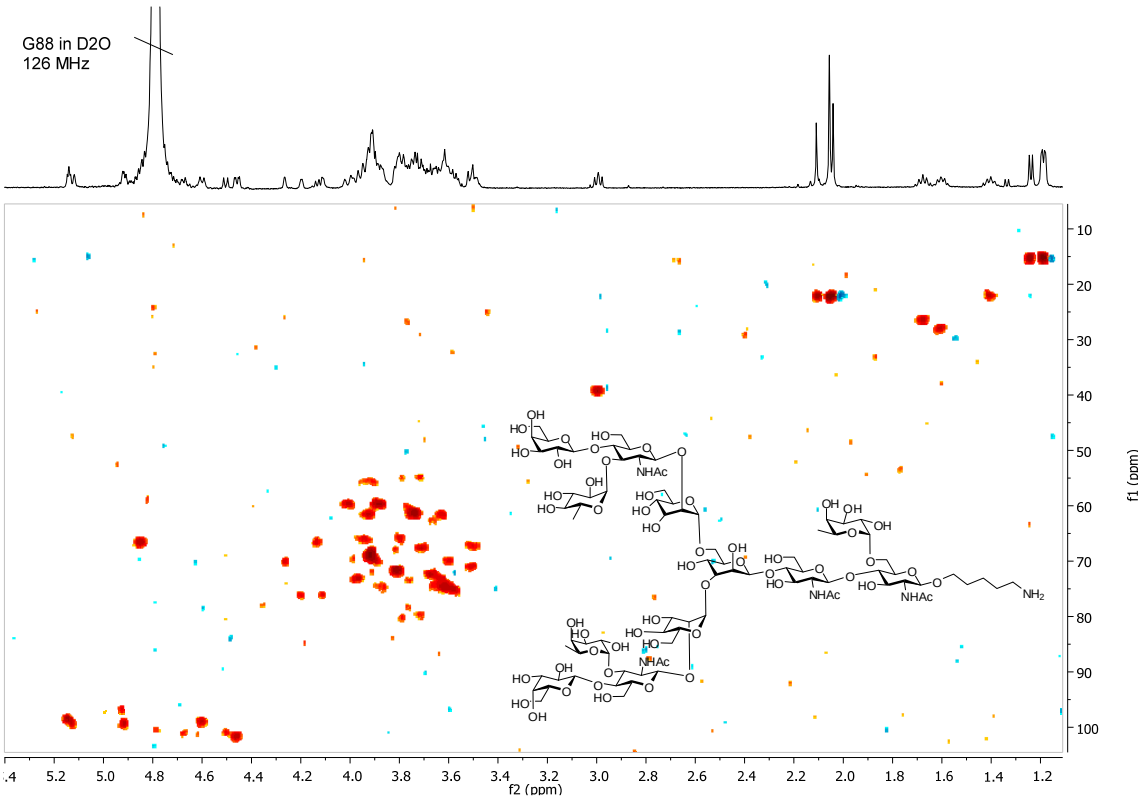
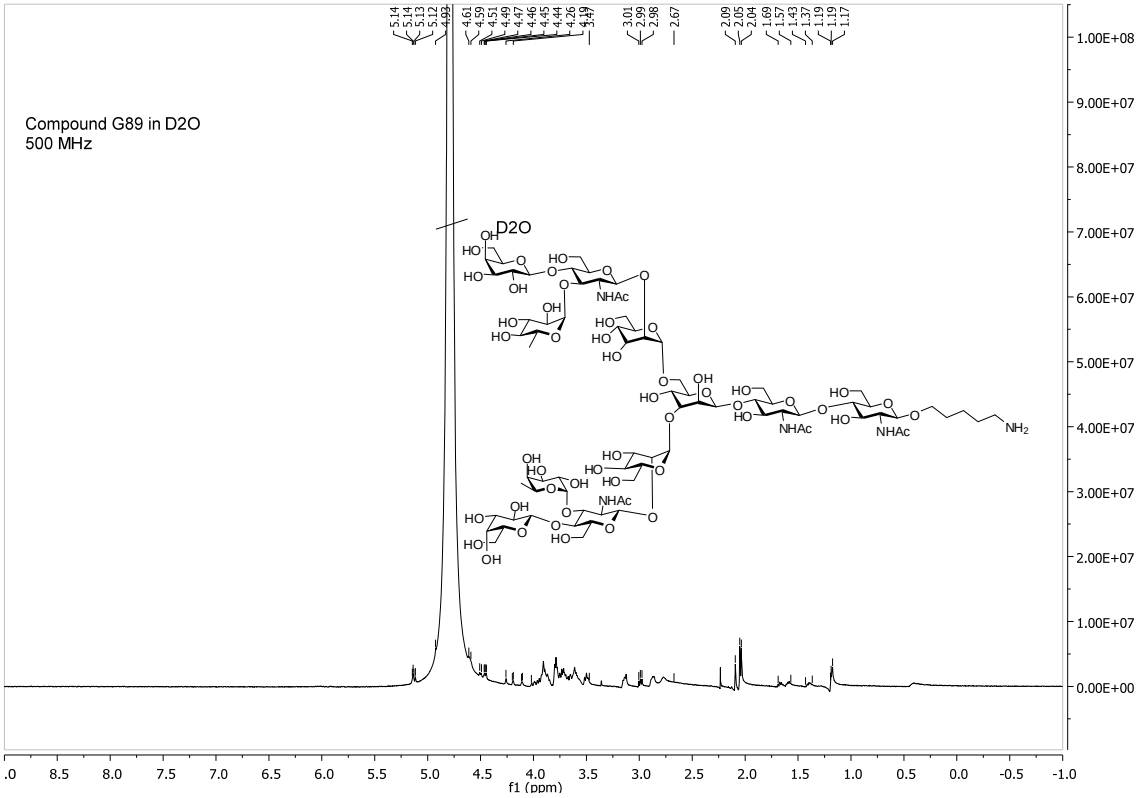
G87


D2O
Compound G87 in D2O
500MHz
f1 (ppm)


G87 in D2O
126 MHz
f2 (ppm)
f1 (ppm)

G88


Compound G88 in D2O
500 MHz
D2O
f1 (ppm)


G88 in D2O
126 MHz
f2 (ppm)
f1 (ppm)

G89