Supporting Information

Automated Solid Phase Assisted Synthesis of a Heparan Sulfate Disaccharide Library

Sherif Ramadan,[a,b] Guowei Su,[c] Kedar Baryal,[a] Linda C. Hsieh-Wilson,[d] Jian Liu,[e] Xuefei Huang[a,f,g]*

[a]Department of Chemistry, Michigan State University, 578 S Shaw Lane, East Lansing, Michigan 48824, USA

[b]Chemistry Department, Faculty of Science, Benha University, Benha, Qaliobiya 13518, Egypt

[c]Glycan Therapeutics, 617 Hutton Street, Raleigh, North Carolina 27606, USA.

[d]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, USA

[e]Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, USA

[f]Institute for Quantitative Health Science and Engineering, [g]Department of Biomedical Engineering, Michigan State University, East Lansing, Michigan 48824, USA

Email: huangxu2@msu.edu

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List of abbreviations:

Name	Abbreviation
Acetic anhydride	Ac_2O
Dichloromethane	DCM
4-Dimethylaminopyridine	DMAP
N,N'-Diisopropylcarbodiimide	DIC
N,N-Diisopropylethylamine	DIPEA
Ethyl acetate	EtOAc
Equivalence	Equiv.
Fluorenylmethyloxycarbonyl chloride	FmocCl
Levulinic acid	LevOH
Molecular sieve	MS
Pyridine	Py.
Tetrabutylammonium fluoride	TBAF
tert-Butyl(chloro)diphenylsilane	TBDPSCl
Thin layer chromatography	TLC
***p*-toluenesulfonic acid**	pTSA
Triethylamine	Et_3N
Trifluoromethanesulfonic acid	TfOH
Silver trifluoromethanesulfonate	AgOTf

1. Experimental Section:

1.1. General experimental procedures:

All reactions were carried out under nitrogen with anhydrous solvents in flame-dried glassware, unless otherwise noted. Glycosylation reactions were performed in the presence of molecular sieves, which were flame–dried right before the reaction under high vacuum. Glycosylation solvents were dried using a solvent purification system and used directly without further drying. Chemicals used were reagent grade as supplied except where noted. Analytical thin-layer chromatography was performed using silica gel 60 F254 glass plates. Compounds were visualized by UV light (254 nm) and by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}$ $4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL). Flash column chromatography was performed on silica gel 60 (230-400 Mesh). Optical rotations were recorded on a Perkin Elmer 341 Polarimeter (λ= 589 nm, 1 dm cell).

1.2. Mass spectrometry (MS) analysis:

ESI-MS measurements were performed according to the published procedures[1] on a Q-TOF Ultima API LC-MS instrument with Waters 2795 Separation Module (Waters Corporation, Milford, MA).

1.3. Nuclear magnetic resonance analysis:

Proton and carbon nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on an Agilent-500MHz spectrometer at ambient temperature with $CDCl_3$ as the solvent unless otherwise stated. Chemical shifts are reported in parts per million (ppm) relative to residual protic solvent internal standard $CHCl_3$ for ^{1}H NMR at δ 7.26 ppm and $CDCl_3$^{13}C NMR at δ 77.36 ppm. All ^{13}C NMR spectra were recorded with broad band proton decoupling. Peak and coupling constants assignments are based on ^{1}H-NMR, ^{1}H-^{1}H gCOSY and (or) ^{1}H-^{13}C gHMQC and ^{1}H-^{13}C gHMBC experiments.

1.4. Characterization of anomeric stereochemistry:

The stereochemistry of the newly formed glycosidic linkages in the oligosaccharide and intermediates are determined by $^3J_{H1,H2}$ through ^{1}H-NMR and/or $^1J_{C1,H1}$ through gHMQC 2-D NMR (without ^{1}H decoupling). The smaller coupling constants of $^3J_{H1,H2}$ (around 3 Hz) indicate α linkages and larger coupling constants $^3J_{H1,H2}$ (7.2 Hz or larger) indicate β linkages for glucosamine and glucuronic acid linkages. $^1J_{C1,H1}$ around 170 Hz suggests α linkages and 160 Hz suggests β linkages.[2]

Screening of the Hycron linker for immobilizing disaccharides onto the Synphase lanterns

Our approach for linker development started with using the Hycron linker **A** bearing the amino-oxy moiety as a first-generation linker (**Figure S1**). However, we observed that the N-O bond was cleaved during catalytic hydrogenation reaction to remove the benzyl groups. Subsequently, we investigated the ester linked Hycron **B**, which was found to be labile and was prematurely cleaved when the derivatized lantern was treated with a base. To enhance the linker stability, we prepared the Hycron linker **C**, which was connected with the glycan through a glycolic amide linkage. Interestingly, the glycolic amide also turned out to be labile to base. These results prompted us to design the linker **19**, which has two additional methylene units between the reducing end oxygen atom and the amide as compared to the glycolic amide linker **C**. **19** was found stable under a wide range of typical reactions for functionalizing HS as described in the manuscript.

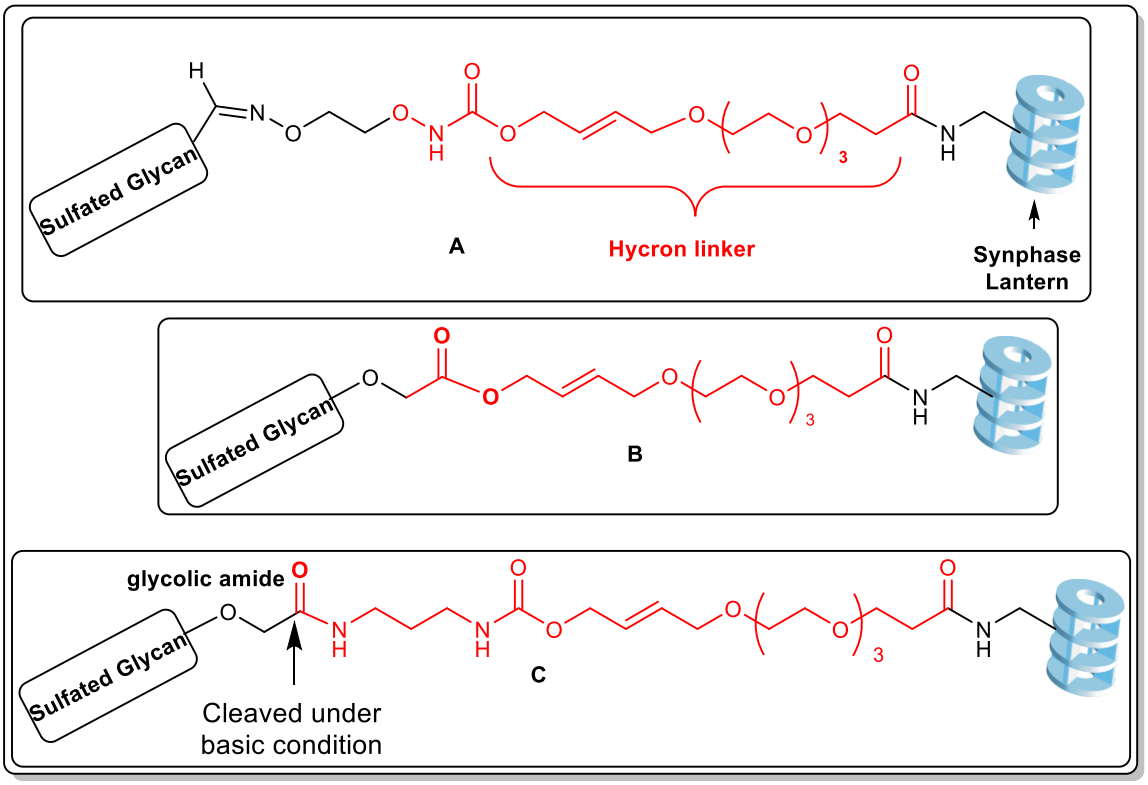
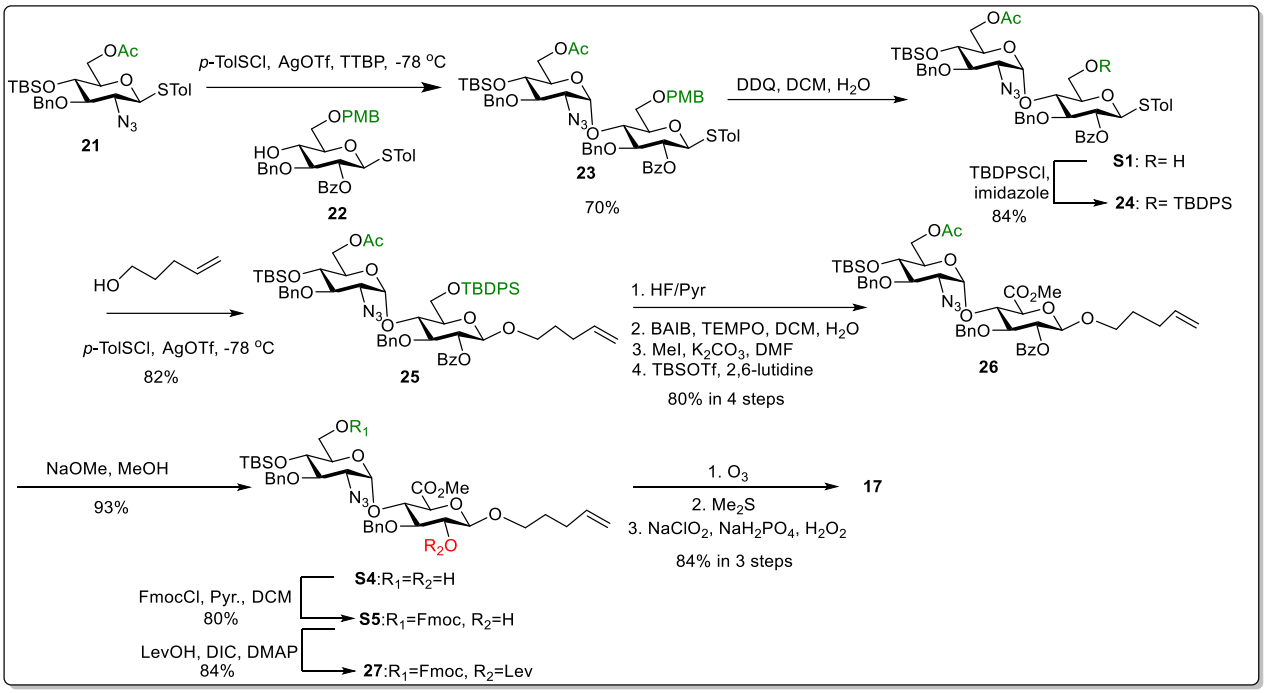
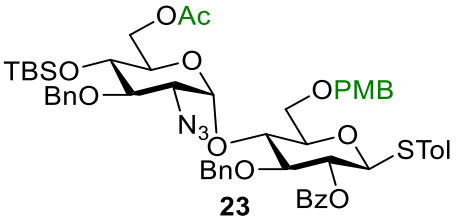


Figure S1: Structures of Hycron linkers A, B, and C investigated, which were not suitable for HS synthesis on the Lantern.



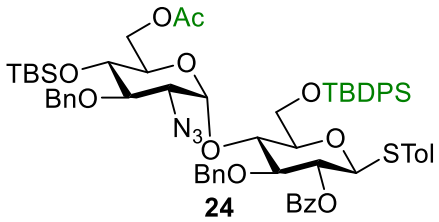
_p_-Tolyl 6-_O_-acetyl-2-azido-3-_O_-benzyl-4-_O_-tert-butyldimethylsilyl-2-deoxy-_α_-D-glucopyranosyl-(1→4)-2-_O_-benzoyl-3-_O_-benzyl-6-_O_-_p_-methoxybenzyl-1-thio-_β_-D-glucopyranoside 23:



A mixture of donor **21** (2.79 g, 5 mmol) and freshly activated molecular sieve (MS) 4Å in DCM (15 mL) was stirred for 30 min. under rt and cooled to -78°C. A solution of silver trifluoromethanesulfonate (AgOTf) (3.53 g, 13.75 mol) in anhydrous acetonitrile (2 mL) was added to the reaction solution without touching the wall of the flask. Then, the orange colored *p*-TolSCl (715 µL, 4.95 mmol) was added to the reaction mixture through a microsyringe. After complete donor activation as indicated by TLS analysis, a solution of acceptor **22** (2.5 g, 4.2 mmol) and TTBP (1.24 g, 5 mmol) in DCM (10 mL) was added to the reaction via a syringe. The reaction was stirred for 2 h at -78 °C then warmed up to -20 °C and stirred for another 1 h. Upon reaction completion, the reaction mixture was diluted with DCM, quenched by triethyl amine (Et_3N), filtered over celite, and washed with DCM. The filtrate was washed with aqueous $NaHCO_3$ and NaCl solutions. The organic layer was collected, dried over Na_2SO_4, concentrated and purified by

silica gel chromatography (hexane: EtOAc, 8:1 to 3:1) to afford disaccharide **23**[3] (3.02 g, 2.9 mmol, 70%). ESI-MS: $C_{56}H_{67}N_3O_{12}SSi$ $[M+NH_4]^{+1}$ calcd: 1051.4553, obsd: 1051.4571.

***p*-Tolyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-1-thio-*β*-D-glucopyranoside 24:**



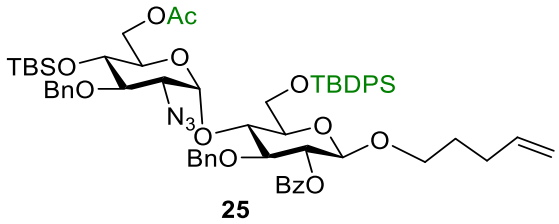
24

Compound **23** (2.51 g, 2.4 mmol) was dissolved in DCM/H_2O (10:1, 30 mL) followed by the addition of DDQ (1.1 g, 4.8 mmol). The reaction mixture was stirred at room temperature and after completion (≈3 h), it was quenched with saturated aqueous $NaHCO_3$ solution and diluted with DCM. The organic phase was washed with H_2O until the solution became colorless. The solvent was dried over Na_2SO_4, concentrated *in vacuo* and the residue was purified by silica gel column chromatography (hexane: EtOAc, 6:1 to 1:2) to afford *p*-tolyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-1-thio-*β*-D-glucopyranoside **S1** as white solid (1.77 g, 1.9 mmol, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 – 8.12 (m, 2H), 7.62 – 7.57 (m, 1H), 7.48 (t, *J* = 7.8 Hz, 2H), 7.44 – 7.36 (m, 7H), 7.33 – 7.29 (m, 1H), 7.24 – 7.19 (m, 4H), 7.13 (d, *J* = 8.0 Hz, 2H), 5.68 (d, *J* = 4.0 Hz, 1H, H-1-GlcN), 5.40 – 5.35 (m, 1H, H-2-Glc), 4.93 – 4.86 (m, 3H, H-1-Glc), 4.82 – 4.74 (m, 2H), 4.43 (dd, *J* = 12.0, 2.2 Hz, 1H, H-3-Glc), 4.16 – 4.08 (m, 2H, H-2-Glc), 4.06-4.00 (m, 2H), 3.88 – 3.83 (m, 2H, H-4- GlcN, H-4-Glc), 3.75 (dd, *J* = 10.2, 8.4 Hz, 1H, H-3- GlcN), 3.69 – 3.63 (m, 2H, H-4- GlcN, H-5-Glc), 3.28 (dd, *J* = 10.2, 4.0 Hz, 1H, H-2-GlcN), 2.35 (s, 3H, CH_3(STol)), 2.12 (s, 3H, CH_3CO), 0.96 (s, 9H, $(CH_3)_3CSi$), 0.07 (s, 3H, CH_3Si), 0.04 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 165.2, 138.5, 137.9, 137.4, 133.46, 133.37, 129.9, 129.8, 129.7, 128.6, 128.44, 128.36, 128.35, 127.8, 127.7, 127.6, 127.3, 97.9 (C-1-GlcN), 86.3 (C-1-Glc), 84.9, 80.2, 79.0, 75.1, 74.7, 73.0, 72.9, 71.5, 71.3, 63.5, 63.2, 62.3, 26.0, 25.9, 21.2, 20.9, 18.1, -3.7, -4.8. ESI-MS: $C_{48}H_{59}N_3O_{11}SSi$ $[M+NH_4]^{+1}$ calcd: 931.3978, obsd: 931.3998.

Compound **S1** (1.77 g, 1.9 mmol) was dissolved in DCM (25 mL) followed by the addition of imidazole (0.198 g, 2.9 mmol) and TBDPSCl (0.604 mL, 2.3 mmol). The resulting mixture was

stirred at room temperature until the reaction was completed. Then, the reaction was diluted with DCM, washed with 10% HCl, saturated aqueous $NaHCO_3$ solution and dried over Na_2SO_4. After concentration, silica gel column purification afforded **24** as white solid (1.87 g, 1.6 mmol, 84%). $[\alpha]_D^{20}$= +55.9 (C =1.28, DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 – 8.15 (m, 2H), 7.85 – 7.82 (m, 4H), 7.65 – 7.61 (m, 1H), 7.53 – 7.45 (m, 12H), 7.44 – 7.40 (m, 2H), 7.37 – 7.33 (m, 1H), 7.27 – 7.21 (m, 5H), 7.04 (d, *J* = 7.9 Hz, 2H), 5.71 (d, *J* = 4.0 Hz, 1H, H-1-GlcN), 5.48 – 5.43 (m, 1H, , H-2-Glc), 4.97 – 4.78 (m, 5H, H-1-Glc), 4.20 (dd, *J* = 11.2, 2.1 Hz, 1H), 4.14 (t, *J* = 8.6 Hz, 1H, , H-3-Glc), 4.08 – 4.02 (m, 2H), 3.95 (dd, *J* = 9.6, 8.5 Hz, 1H), 3.90 (dd, *J* = 12.0, 5.3 Hz, 1H), 3.83 – 3.78 (m, 1H), 3.67 – 3.57 (m, 3H, H-3-GlcN), 3.24 (dd, *J* = 10.0, 4.0 Hz, 1H, H-2-GlcN), 2.34 (s, 3H, CH_3(STol)), 1.93 (s, 3H, CH_3CO), 1.21 (s, 9H, $(CH_3)_3CSi$), 0.95 (s, 9H, $(CH_3)_3CSi$), 0.06 (s, 3H, CH_3Si), 0.04 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6, 165.3, 138.0, 137.7, 137.4, 135.8, 135.7, 133.44, 133.39, 133.3, 132.1, 130.4, 129.94, 129.93, 129.92, 129.81, 129.76, 128.6, 128.4, 127.94, 127.90, 127.87, 127.76, 127.66, 127.3, 97.8 (C-1-GlcN), 87.3 (C-1-Glc), 85.0, 80.1, 80.0, 75.2, 74.6, 74.2, 73.1, 71.33, 71.27, 64.5, 63.6, 63.0, 27.1, 26.1, 21.2, 20.8, 19.4, 18.1, -3.8, -4.8. ESI-MS: $C_{64}H_{77}N_3O_{11}SSi_2$ $[M+NH_4]^{+1}$ calcd: 1169.5156, obsd: 1169.5107.

Pent-4-enyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-1-*β*-D-glucopyranoside 25:



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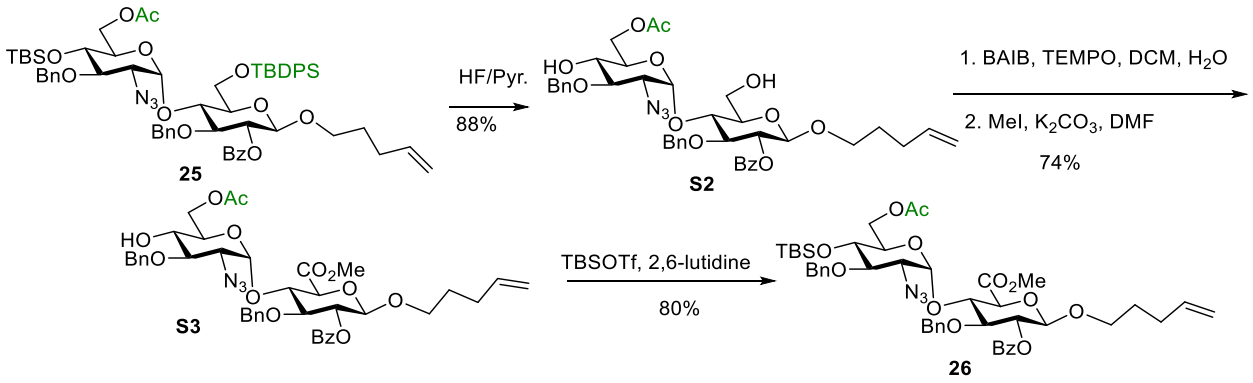
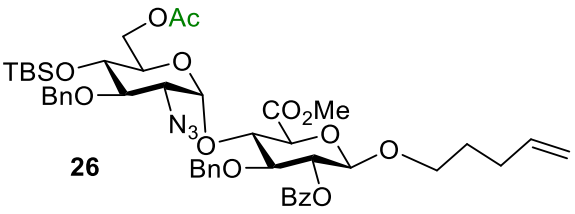
A mixture of donor **24** (1 g, 0.87 mmol), 4-penten-1-ol (0.108 mL, 1.05 mmol) and freshly activated molecular sieves 4 Å molecular sieves (800 mg) in DCM (15 mL) was stirred at room temperature for 30 min. and then cooled to -78 ^{o}C, which was followed by the addition of AgOTf (0.74 g, 2.9 mmol) dissolved in acetonitrile (0.5 mL) without touching the wall of the flask. After 5 minutes, orange colored *p*-TolSCl (150 µL, 1.04 mmol) was added through a microsyringe directly into the reaction mixture to prevent it from freezing on the flask wall. The reaction mixture was stirred for 1.5 h at the same temperature, then raised to 0 ^{o}C. Upon completion as monitored by TLC, the reaction was quenched with triethylamine. The reaction mixture was diluted with

DCM (30 mL) and filtered through Celite. The Celite was washed extensively with DCM until TLC showed no products in the filtrate. The filtrate was combined, concentrated and purified by silica column chromatography (hexane/EtOAc: 6:1 to 1:1) to give disaccharide **25** (0.79 g, 0.71 mmol, 82%). $[\alpha]_D^{20}$= -1.5 (C =0.2, DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 – 8.10 (m, 2H), 7.80 – 7.77 (m, 4H), 7.62 – 7.58 (m, 1H), 7.53 – 7.37 (m, 13H), 7.36 – 7.31 (m, 1H), 7.27 – 7.19 (m, 5H), 5.74 – 5.65 (m, 2H, H-1-GlcN), 5.40 (dd, *J* = 9.2, 7.9 Hz, 1H, H-2-Glc), 4.92 – 4.83 (m, 4H), 4.83 – 4.77 (m, 2H), 4.63 (d, *J* = 7.9 Hz, 1H, H-1-Glc), 4.17 – 4.08 (m, 3H, H-3-Glc), 4.02 – 3.90 (m, 4H, H-4-Glc), 3.75 – 3.57 (m, 4H, H-3-GlcN, H-5-Glc), 3.55 – 3.48 (m, 1H), 3.24 (dd, *J* = 10.1, 4.0 Hz, 1H, H-2-GlcN), 2.05 – 1.99 (m, 2H), 1.91 (s, 3H, CH_3CO), 1.74 – 1.60 (m, 2H), 1.17 (s, 9H, $(CH_3)_3CSi$), 0.92 (s, 9H, $(CH_3)_3CSi$), 0.04 (s, 3H, CH_3Si), 0.03 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6, 165.2, 137.9, 137.6, 135.72, 135.65, 133.5, 133.4, 133.3, 129.91, 129.88, 129.87, 129.77, 128.5, 128.4, 127.6, 127.8, 127.81, 127.7, 127.6, 127.3, 114.8, 100.9 (C-1-Glc), 97.8 (C-1-GlcN), 83.6, 80.2, 75.8, 75.2, 74.6, 74.4, 74.2, 71.3, 71.2, 68.9, 64.3, 63.6, 63.1, 29.9, 28.6, 27.0, 26.0, 20.7, 19.4, 18.0, -3.8, -4.8. ESI-MS: $C_{62}H_{79}N_3O_{12}Si_2$ $[M+NH_4]^{+1}$ calcd: 1131.5541, obsd: 1131.5501.



Pent-4-enyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl-2-*O*-benzoyl-3-*O*-benzyl-1-*β*-D-glucopyranosyluronate 26:



Compound **25** (0.79 g, 0.71 mmol) was dissolved in pyridine (6 mL) in a plastic flask and cooled down to 0 °C, then followed by the addition of 65–70% HF.pyridine solution (3 mL). The

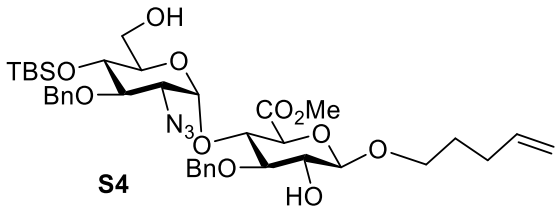
reaction was stirred at rt. till completion. Then, the reaction mixture was diluted with EtOAc (100 mL) and washed with 1 M HCl solution, saturated aqueous $NaHCO_3$ solution (3×50 mL), drying over Na_2SO_4 and concentrated. The obtained residue was purified by flash column chromatography (hexane/EtOAc: 3:1 to 1:2) to give the desired diol **S2** (0.48 g, 0.62 mmol, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.60 – 7.55 (m, 1H), 7.48 – 7.15 (m, 12H), 5.68 – 5.59 (m, 2H, H-1-GlcN), 5.33 (t, *J* = 8.4 Hz, 1H, H-2-Glc), 4.91 (d, *J* = 2.5 Hz, 2H), 4.85 – 4.72 (m, 4H), 4.61 (d, *J* = 7.9 Hz, 1H, H-1-Glc), 4.54 (dd, *J* = 12.4, 4.1 Hz, 1H, H-4-GlcN), 4.20 (dd, *J* = 12.4, 2.1 Hz, 1H, H-5-GlcN), 4.11 – 4.01 (m, 2H, H-6a-GlcN, H-3-Glc), 3.97 – 3.79 (m, 5H, H-3-GlcN, H-6aa-GlcN), 3.55 (ddd, *J* = 9.4, 4.1, 2.3 Hz, 1H), 3.51 – 3.42 (m, 2H, H-4-Glc, , H-6a-Glc), 3.26 – 3.20 (m, 1H, H-2-GlcN), 3.15 (dd, *J* = 4.1, 1.7 Hz, 1H, H-5-Glc), 2.22 (t, *J* = 6.6 Hz, 1H), 2.10 (s, 3H, CH_3CO), 2.01 – 1.90 (m, 2H), 1.66 – 1.52 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.2, 165.1, 137.9, 137.8, 137.4, 133.3, 129.72, 129.68, 128.6, 128.5, 128.3, 128.2, 128.1, 127.8, 127.7, 114.9, 101.2 (C-1-Glc), 97.7 (C-1-GlcN), 83.5, 79.2, 75.4, 74.8, 74.6, 74.2, 72.3, 71.0, 70.7, 69.3, 63.1, 62.6, 61.8, 29.8, 28.5, 20.9. ESI-MS: $C_{40}H_{47}N_3O_{12}$ $[M+NH_4]^{+1}$ calcd: 779.3498, obsd: 779.3453.

Compound **S2** (0.48 g, 0.62 mmol) was dissolved in DCM/H_2O (4:1, 5 mL), followed by the addition of TEMPO (29.5 mg, 0.19 mmol) and BAIB (0.406 g, 1.3 mmol). The resulting mixture was stirred at room temperature until all starting material was consumed as indicated by TLC analysis (≈4 h). Then it was diluted with EtOAc, washed with H_2O, dried over Na_2SO_4. After concentration, the crude mixture was dissolved in dry DMF (3 mL), followed by addition of MeI (39.2 µL, 0.63 mmol) and K_2CO_3 (0.435 g, 3.2 mmol). The resulting mixture was stirred under rt for 4 h, then diluted with EtOAc, washed with $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated. The crude was purified with silica gel column chromatography (hexane/EtOAc: 5:1 to 1:1) to afford **S3** (0.368 g, 0.47 mmol, 74%). ESI-MS: $C_{41}H_{47}N_3O_{13}$ $[M+NH_4]^{+1}$ calcd: 807.3447, obsd: 807.3451.

To a solution of compound **S3** (0.368 g, 0.47 mmol) in DCM (10 mL), 2,6-lutidine (0.108 mL, 0.93 mmol) and TBSOTf (0.139 mL, 0.6 mmol) were added at -40 °C. The reaction was stirred at the same temperature for 1h, then warmed up to 0 °C slowly until starting material was consumed. Then, the mixture was diluted with DCM (50 mL) and washed with saturated $NaHCO_3$ solution. The organic phase was collected and dried over Na_2SO_4 followed by separation by flash column chromatography (hexane/EtOAc: 5:1 to 1:1) to give **26** as a white solid (0.42 g, 0.37 mmol,

80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 – 8.05 (m, 2H), 7.61 – 7.55 (m, 1H), 7.48 – 7.16 (m, 13H), 5.72 – 5.58 (m, 2H, H-1-GlcN), 5.39 (dd, *J* = 8.7, 7.3 Hz, 1H, H-2-GlcA), 4.93 – 4.75 (m, 6H), 4.68 (d, *J* = 7.3 Hz, 1H, H-1-GlcA), 4.43 – 4.32 (m, 2H, H-4-GlcA, H-5-GlcA), 4.16 – 4.05 (m, 3H, H-3-GlcA, H-6a-GlcA, H-6aa-GlcA), 3.91 (m, 1H, H-6a-GlcN), 3.83 (s, 3H, $COOCH_3$), 3.73 – 3.65 (m, 2H, H-3-GlcN), 3.59 – 3.54 (m, 1H, H-4-GlcN), 3.51 – 3.45 (m, 1H, H-5-GlcN), 3.29 (dd, *J* = 10.0, 3.6 Hz, 1H, H-2-GlcN), 2.11 (s, 3H), 2.03 – 1.91 (m, 2H), 1.68 – 1.53 (m, 2H), 0.92 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 168.7, 165.0, 137.9, 137.8, 137.3, 133.4, 129.7, 129.6, 128.5, 128.34, 128.31, 127.9, 127.8, 127.6, 127.2, 114.9, 101.2 (C-1-GlcA), 97.5 (C-1-GlcN), 82.5, 80.1, 77.4, 75.1, 74.44, 74.35, 73.6, 71.0, 70.8, 69.3, 63.7, 62.4, 52.7, 29.8, 28.5, 25.9, 20.9, 18.0, -3.7, -5.0. ESI-MS: $C_{47}H_{61}N_3O_{13}Si$ $[M+NH_4]^{+1}$ calcd: 921.4312, obsd: 921.4300.

Pent-4-enyl 2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl-3-*O*-benzyl-1-*β*-D-glucopyranosyluronate S4:

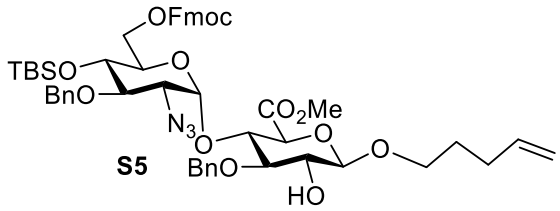


S4

Compound **26** (0.42 g, 0.37 mmol) was dissolved in MeOH/DCM (3/2) (10 mL), then NaOMe solution (25 wt. %. in MeOH) was added until pH≈14. The reaction mixture was stirred till completion, then neutralized with Amberlite resin until the pH was around 7. After filtration, the filtrate was concentrated, dried, and purified by silica column chromatography (hexane/EtOAc: 3:1 to 1:2) to give **S4** (0.327 g, 0.43 mmol, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.26 (m, 10H), 5.88 – 5.75 (m, 1H), 5.62 (d, *J* = 3.7 Hz, 1H, H-1-GlcN), 5.08 – 4.97 (m, 3H), 4.88 – 4.80 (m, 3H), 4.33 (dd, *J* = 7.7, 1.1 Hz, 1H, H-1-GlcA), 4.13 (t, *J* = 9.1 Hz, 1H, H-5-GlcN), 4.00 (dd, *J* = 9.6, 1.1 Hz, 1H, H-6a-GlcN), 3.97 – 3.89 (m, 1H, H-5-GlcA), 3.82 (s, 3H, $COOCH_3$), 3.80 – 3.74 (m, 2H, H-4-GlcN), 3.68 – 3.51 (m, 5H, H-3-GlcN, H-2-GlcA, H-4-Glc, H-6-GlcA), 3.37 – 3.31 (m, 1H, H-3-GlcA), 3.22 (dd, *J* = 9.9, 3.7 Hz, 1H, H-2-GlcN), 2.45 (t, *J* = 1.9 Hz, 1H), 2.18 – 2.08 (m, 2H), 1.91 (dd, *J* = 8.0, 4.9 Hz, 1H), 1.78 – 1.67 (m, 2H), 0.90 (s, 9H, $(CH_3)CSi$), 0.09 (s, 3H, CH_3Si), 0.00 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.1, 138.2, 137.99, 137.97, 129.0, 128.6, 128.5, 128.3, 127.9, 127.8, 127.5, 127.3, 115.1, 103.0 (C-1-GlcA), 97.5 (C-1-GlcN),

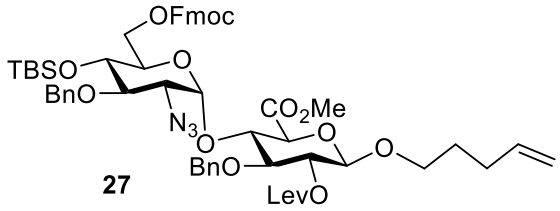
83.8, 80.0, 75.1, 74.9, 74.7, 74.6, 74.4, 73.2, 70.6, 69.8, 63.7, 61.0, 52.8, 30.1, 28.6, 25.9, 18.0, -3.8, -4.8. ESI-MS: $C_{38}H_{55}N_3O_{11}Si$ $[M+NH_4]^{+1}$ calcd: 775.3944, obsd: 775.3953.

Pent-4-enyl 2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-6-*O*-fluorenylmethoxycarbonyl-2-deoxy-*α*-D-glucopyranosyl-(1→ 4)-methyl-3-*O*-benzyl-1-*β*-D-glucopyranosyluronate S5:



To a solution of **S4** (0.327 g, 0.43 mmol) in DCM (5 mL), FmocCl (0.134 g, 0.5 mmol) and pyridine (70 µL, 0.9 mmol) were added, and the reaction was stirred overnight. Upon completion, the reaction was diluted with EtOAc, washed with 1 M HCl solution, saturated Na_2CO_3 solution, saturated NaCl solution, dried over Na_2SO_4, concentrated, and purified with silica column chromatography (hexane/EtOAc: 3:1 to 1:2) to afford **S5** (0.338 g, 0.34 mmol, 80%). ESI-MS: $C_{53}H_{65}N_3O_{13}Si$ $[M+NH_4]^{+1}$ calcd: 997.4625, obsd: 997.4601.

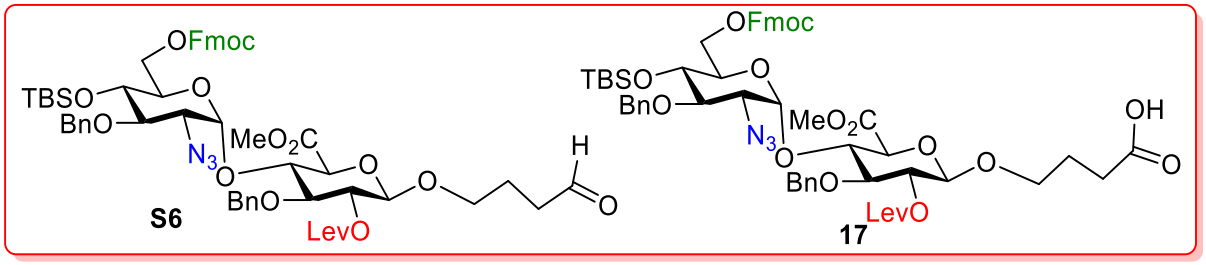
Pent-4-enyl 2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-6-*O*-fluorenylmethoxycarbonyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl-3-*O*-benzyl-2-*O*-levulinyl-1-*β*-D-glucopyranosyluronate 27:



To a solution of **S5** (0.338 g, 0.34 mmol) in DCM (5 mL), LevOH (0.177 mL, 1.7 mmol), DMAP (4 mg, 0.03 mmol) and *N*,*N*'-diisopropylcarbodiimide (DIC) (0.27 mL, 1.7 mmol) were added. The resulting solution was stirred at rt for 5 h. After completion, the reaction was diluted with DCM, washed with 10% HCl solution, saturated $NaHCO_3$ solution, dried over Na_2SO_4, concentrated and purified with silica gel column (hexane/EtOAc, 8:1 to 3:1) to afford compound **27** as a white solid (0.359 g, 0.33 mmol, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, J = 7.6 Hz, 2H), 7.63 – 7.58 (m, 2H), 7.41 – 7.34 (m, 7H), 7.32 – 7.29 (m, 6H), 7.18 (d, J = 7.0 Hz, 1H), 5.83 – 5.73 (m, 1H), 5.54 (d, J = 3.7 Hz, 1H, H-1-GlcN), 5.08 (dd, J = 8.7, 7.3 Hz, 1H), 5.03 – 4.98 (m,

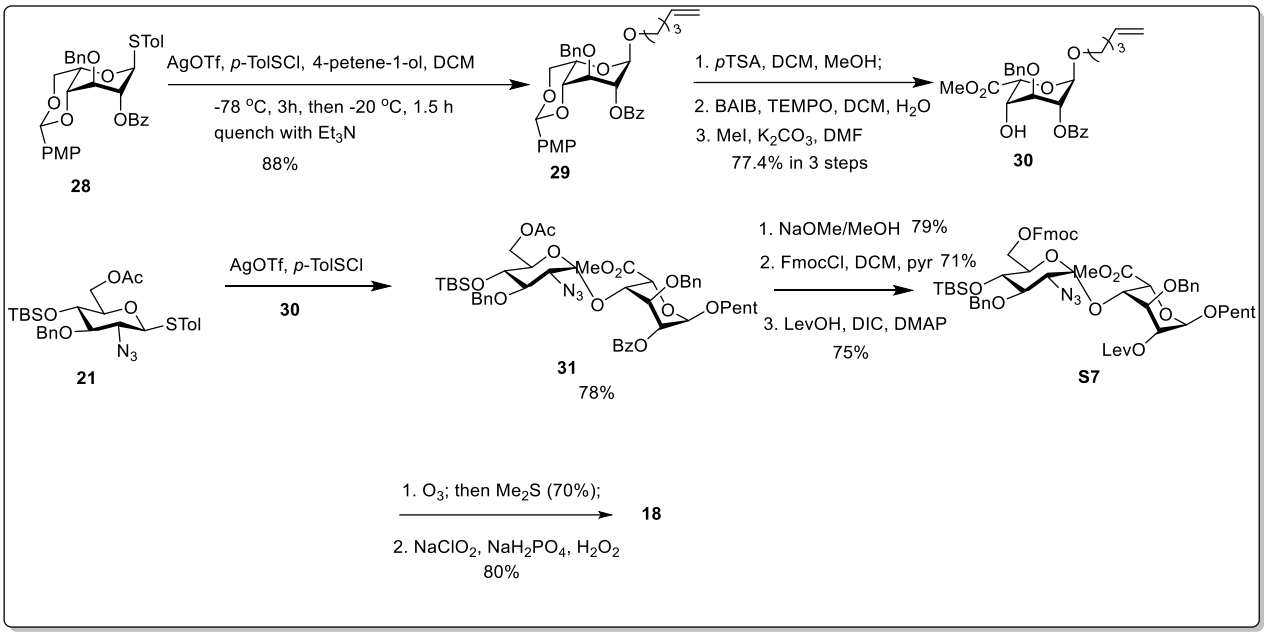
1H), 5.03 – 4.98 (m, 1H), 4.89 (d, J = 11.2 Hz, 1H), 4.82 – 4.78 (m, 2H), 4.74 (d, J = 10.9 Hz, 1H), 4.48 (d, J = 7.3 Hz, 1H, H-1-GlcA), 4.42 – 4.38 (m, 3H), 4.26 – 4.20 (m, 3H), 4.02 (d, J = 9.4 Hz, 1H), 3.89 – 3.83 (m, 2H), 3.78 (s, 3H, $COOCH_3$), 3.74 (d, J = 9.1 Hz, 1H), 3.65 (dd, J = 10.3, 8.5 Hz, 1H), 3.52 (d, J = 9.5 Hz, 1H), 3.45 (dd, J = 9.7, 6.8 Hz, 1H), 3.30 (dd, J = 10.3, 3.7 Hz, 1H), 2.71 – 2.66 (m, 2H), 2.54 – 2.44 (m, 2H), 2.14 (s, 3H, CH_2COCH_3), 2.10 – 2.04 (m, 2H), 1.68 – 1.61 (m, 2H), 0.87 (s, 9H, $(CH_3)_3CSi$), 0.001 (s, 3H, CH_3Si), -0.002 (s, 3H, CH_3Si). ESI-MS: $C_{58}H_{71}N_3O_{15}Si$ $[M+NH_4]^{+1}$ calcd: 1095.4993, obsd: 1095.5004.

Carboxypropyl azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy-6-*O*-fluorenylmethoxycarbonyl-*α*-D-glucopyranosyl-(1→4)-methyl-3-*O*-benzyl-2-*O*-levulinyl-1-*β*-D-glucopyranosyluronate 17:

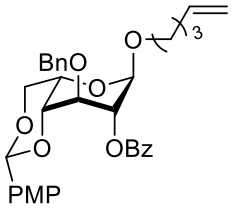


To a solution of **27** (0.645 g, 0.6 mmol) in DCM (7 mL) at -78 °C, O_3 was passed through the solution via a glass pipette till the blue color persisted (in about 10-15 min). Then, Me_2S (0.245 mL, 3.3 mmol) was added, and the reaction was stirred at rt overnight. The mixture was concentrated and purified with silica gel chromatography (toluene/acetone, 8:1 to 2:1) to afford the aldehyde **S6** (0.53 g, 0.49 mmol, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.75 (d, *J* = 1.4 Hz, 1H, CHO), 7.77 (dt, *J* = 7.6, 0.9 Hz, 2H), 7.66 – 7.59 (m, 2H), 7.43 – 7.25 (m, 15H), 5.55 (d, *J* = 3.7 Hz, 1H, H-1-GlcN), 5.09 (dd, *J* = 8.6, 7.3 Hz, 1H, H-2-GlcA), 4.92 (d, *J* = 11.2 Hz, 1H), 4.86 – 4.81 (m, 2H), 4.76 (d, *J* = 10.9 Hz, 1H), 4.50 (d, *J* = 7.3 Hz, 1H, H-1-GlcA), 4.46 – 4.42 (m, 3H, H-5-GlcA), 4.31 – 4.22 (m, 3H, H-4-GlcA), 4.05 (d, *J* = 9.4 Hz, 1H), 3.93 – 3.86 (m, 2H, H-5-GlcN, H-2-GlcA), 3.81 (s, 3H, $COOCH_3$), 3.77 (dd, *J* = 9.6, 8.5 Hz, 1H, H-6a-GlcN), 3.68 (dd, *J* = 10.2, 8.5 Hz, 1H, H-3-GlcN), 3.55 – 3.48 (m, 2H, H-4-GlcN, H-6aa-GlcN), 3.32 (dd, J = 10.3, 3.7 Hz, 1H, H-2-GlcN), 2.78 – 2.56 (m, 3H), 2.55 – 2.51 (m, 2H), 2.47 – 2.40 (m, 1H), 2.16 (s, 3H, CH_2COCH_3), 1.94 – 1.86 (m, 2H), 0.90 (s, 9H, $(CH_3)_3CSi$), 0.032 (s, 3H, CH_3Si), 0.027 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.1, 202.3, 171.3, 168.7, 154.9, 143.5, 143.3, 141.31, 141.27, 137.9, 137.6, 128.5, 128.3, 127.89, 127.87, 127.78, 127.60, 127.58, 127.24,

127.19, 127.17, 125.3, 125.1, 120.05, 120.02, 100.9 (C-1-GlcA), 97.6 (C-1-GlcN), 82.4, 80.1, 75.1, 74.4, 74.3, 73.2, 71.0, 70.4, 69.9, 68.6, 65.5, 63.6, 52.8, 46.7, 40.4, 37.7, 29.8, 27.8, 25.9, 22.1, 18.0, -3.7, -5.1. ESI-MS: $C_{57}H_{69}N_3O_{16}Si$ $[M+Na]^{+1}$ calcd: 1102.4339, obsd: 1102.4201. The aldehyde **S6** (0.53 g, 0.49 mmol) was dissolved in acetonitrile (20 mL), cooled to 0 °C, which was followed by the addition of NaH_2PO_4 (95 mg, 0.69 mmol, in 10 mL H_2O) and H_2O_2 (120 µL). Then $NaClO_2$ (0.183 g, 1.6 mmol, in 10 mL H_2O) was added. The reaction was stirred at room temperature overnight. Then the solvent was removed, and the residue was dissolved in EtOAc, washed with brine, dried over Na_2SO_4, concentrated, and purified by silica gel chromatography (DCM/MeOH, 20:1 to 10:1) to give **17** (0.45 g, 0.41 mmol, 84%). $[\alpha]_D^{20}$= +36.3 (C =0.16, DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (dt, *J* = 7.6, 1.0 Hz, 2H), 7.64 – 7.60 (m, 2H), 7.42 – 7.27 (m, 15H), 5.55 (d, *J* = 3.7 Hz, 1H, H-1-GlcN), 5.10 (dd, *J* = 8.8, 7.4 Hz, 1H, 1H, H-2-GlcA), 4.91 (d, *J* = 11.2 Hz, 1H), 4.85 – 4.81 (m, 2H), 4.75 (d, *J* = 11.0 Hz, 1H), 4.50 (d, *J* = 7.4 Hz, 1H, H-1-GlcA), 4.45 – 4.41 (m, 3H, 1H, H-5-GlcA), 4.29 – 4.22 (m, 3H, H-6aa-GlcN, 1H, H-4-GlcA, 1H, H-6aa-GlcA), 4.04 (d, *J* = 9.5 Hz, 1H), 3.97 – 3.91 (m, 1H, H-6a-GlcN), 3.88 (t, *J* = 8.8 Hz, 1H, 1H, H-3-GlcA), 3.80 (s, 3H, $COOCH_3$), 3.77 (dd, *J* = 9.6, 8.5 Hz, 1H, H-4-GlcN), 3.67 (dd, *J* = 10.3, 8.5 Hz, 1H, H-3-GlcN), 3.55 – 3.48 (m, 2H, H-5-GlcN, 1H, H-6a-GlcA), 3.32 (dd, *J* = 10.3, 3.7 Hz, 1H, H-2-GlcN), 2.79 – 2.72 (m, 1H), 2.68 – 2.59 (m, 2H), 2.46 – 2.37 (m, 3H), 2.18 (s, 3H, CH_2COCH_3), 1.93 – 1.88 (m, 2H), 0.90 (s, 9H, $(CH_3)_3CSi$), 0.024 (s, 3H, CH_3Si) , 0.021 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.3, 178.0, 171.3, 168.6, 154.9, 143.5, 143.2, 141.31, 141.27, 137.9, 137.6, 128.5, 128.3, 127.89, 127.86, 127.76, 127.57, 127.55, 127.24, 127.18, 127.15, 125.3, 125.1, 120.04, 120.01, 100.9 (C-1-GlcA), 97.6 (C-1-GlcN), 82.4, 80.1, 77.3, 75.1, 74.5, 74.4, 74.3, 73.2, 71.0, 70.3, 69.9, 68.5, 65.1, 63.7, 52.8, 46.7, 37.7, 30.5, 29.9, 27.8, 25.9, 24.4, 18.0, -3.8, -5.2. ESI-MS: $C_{57}H_{69}N_3O_{17}Si$ $[M+Na]^{+1}$ calcd: 1118.4288, obsd: 1118.4240.



Pent-4-enyl 2-*O*-benzoyl-4,6-*O*-(*p*-methoxybenzylidene)-3-*O*-benzyl-*α*-L-idopyranoside 29:

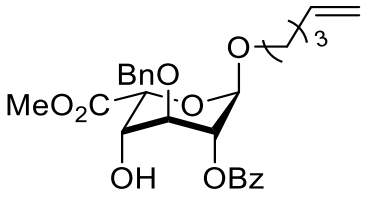


29

A mixture of donor **28** (4.29 g, 7.2 mmol), 4-penten-1-ol (888 µL, 8.6 mmol) and freshly activated molecular sieves 4 Å molecular sieves (800 mg) in DCM (25 mL) was stirred at room temperature for 30 min. and then cooled to -78 °C, which was followed by the addition of AgOTf (4.4 g, 17.1 mmol) dissolved in acetonitrile (2 mL) without touching the wall of the flask. After 5 minutes, orange colored *p*-TolSCl (1.03 mL, 7.2 mmol) was added through a microsyringe directly into the reaction mixture to prevent it from freezing on the flask wall. The reaction mixture was stirred for 1.5 h at the same temperature, then warmed up to 0 °C. Upon completion as judged by TLC, the reaction was quenched with triethylamine. The reaction mixture was diluted with DCM (30 mL) and filtered through Celite. The Celite was washed extensively with DCM until TLC showed no products in the filtrate. The filtrate was combined, concentrated and purified by silica column chromatography (hexanes/EtOAc: 6:1 to 1:1) to give **29** (3.54 g, 6.3 mmol, 88%). $[\alpha]_D^{20}$ = -52.6 (C =0.58, DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 – 7.99 (m, 2H), 7.55 – 7.50 (m, 1H), 7.46 (d, *J* = 8.7 Hz, 2H), 7.42 – 7.38 (m, 2H), 7.36 – 7.28 (m, 5H), 6.86 – 6.81 (m, 2H), 5.87 –

5.79 (m, 1H, CH=), 5.54 (s, 1H, CHPMP), 5.30 (dd, *J* = 3.1, 1.5 Hz, 1H, H-2), 5.10 (d, *J* = 1.8 Hz, 1H, H-1), 5.05 – 4.96 (m, 2H), 4.91 (d, *J* = 11.7 Hz, 1H), 4.67 (d, *J* = 11.7 Hz, 1H), 4.34 (dd, *J* = 12.5, 1.6 Hz, 1H), 4.14 (dd, *J* = 12.5, 1.9 Hz, 1H), 4.09 (t, *J* = 1.9 Hz, 1H), 4.05 – 4.01 (m, 1H, H-6aa), 3.87 – 3.85 (m, 1H, H-5), 3.84 – 3.80 (m, 4H, OCH_3, H-4), 3.56 – 3.50 (m, 1H, H-6a), 2.21 – 2.13 (m, 2H), 1.80 – 1.73 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.7, 160.0, 138.2, 137.8, 133.1, 130.6, 130.1, 129.74, 129.70, 128.7, 128.40, 128.37, 128.2, 127.82, 127.76, 127.73, 114.9, 113.4, 101.0, 98.5, 74.3, 73.6, 71.9, 71.8, 69.8, 68.4, 68.0, 67.5, 66.1, 59.7, 55.3, 30.3, 28.7. ESI-MS: $C_{33}H_{36}O_8$ $[M+NH_4]^{+1}$ calcd: 578.2748, obsd: 578.2742.

Pent-4-enyl methyl-2-*O*-benzoyl-3-*O*-benzyl-1-*α*-L-idopyranosyluronate 30:

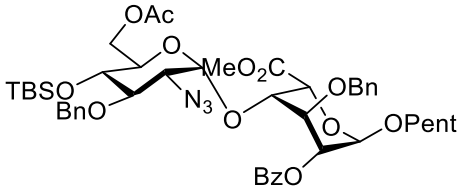


30

Compound **29** (3.54 g, 6.3 mmol) was dissolved in DCM/MeOH (1:1, 10 mL) and then *p*-toluenesulfonic acid (*p*-TSA) (0.6 g, 3.2 mmol) was added. The reaction was stirred for 6h. Upon completion, the reaction was concentrated and, then purified with silica gel chromatography to give diol (2.51 g, 5.7 mmol, 90%). The diol (2.51 g, 5.7 mmol) was dissolved in DCM/H_2O (4:1, 25 mL), followed by the addition of TEMPO (0.266 g, 1.7 mmol) and BAIB (3.65 g, 11.3 mmol)). The resulting mixture was stirred at room temperature until all starting material was consumed as indicated by TLC analysis (≈4 h). Then it was diluted with EtOAc, washed with H_2O, dried over Na_2SO_4. After concentration, the crude was dissolved in dry DMF (10 mL), followed by the addition of MeI (3.5 mL, 56.7 mmol) and K_2CO_3 (3.92 g, 28.4 mmol). The resulting mixture was stirred under rt for 4 h, then diluted with EtOAc, washed with $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated. The crude was purified with silica gel column chromatography (hexane/EtOAc: 5:1 to 1:1) to afford **30** (2.3 g, 4.9 mmol, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 – 7.99 (m, 2H), 7.63 – 7.57 (m, 1H), 7.49 – 7.43 (m, 2H), 7.42 – 7.28 (m, 5H), 5.88 – 5.74 (m, 1H, CH=), 5.26 – 5.23 (m, 1H, H-2), 5.12 (d, *J* = 1.4 Hz, 1H, H-1), 5.00 – 4.93 (m, 2H), 4.87 (d, *J* = 11.6 Hz, 1H), 4.67 (d, *J* = 11.6 Hz, 1H), 4.13 (dd, *J* = 9.1, 6.3 Hz, 1H, H-4), 3.91 – 3.87 (m, 1H, H-3), 3.84 (s, 3H, $COOCH_3$), 3.58 – 3.52 (m, 1H), 2.79 (d, *J* = 11.8 Hz, 1H, H-5), 2.21 – 2.11 (m, 2H), 1.81 – 1.71 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.1, 165.0, 137.9, 137.6, 133.7, 129.8, 129.0,

128.7, 128.4, 127.9, 127.7, 115.1, 98.7 (C-1), 74.4, 71.9, 68.2, 68.2, 67.6, 67.2, 52.4, 30.2, 28.6. ESI-MS: $C_{26}H_{30}O_8$ $[M+NH_4]^{+1}$ calcd: 488.2279, obsd: 488.22802.

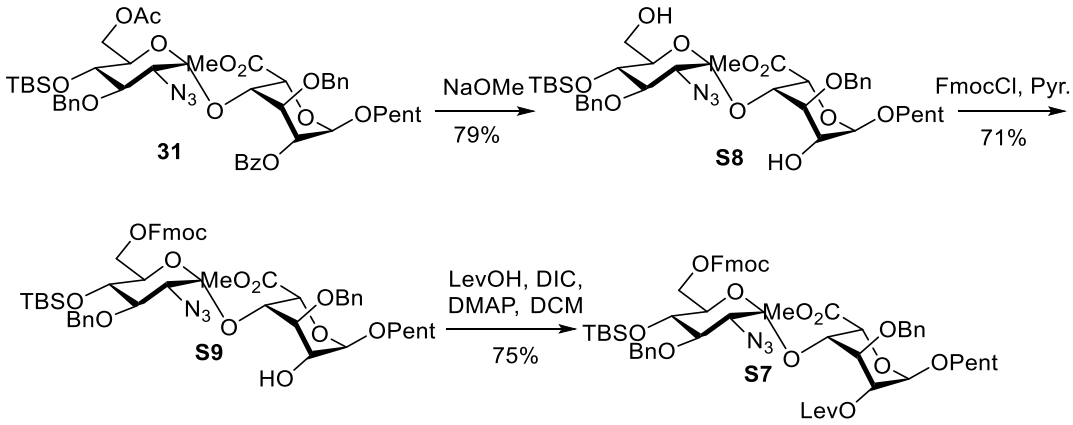
Pent-4-enyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl-2-*O*-benzoyl-3-*O*-benzyl-1-*α*-L-idopyranosyluronate 31:



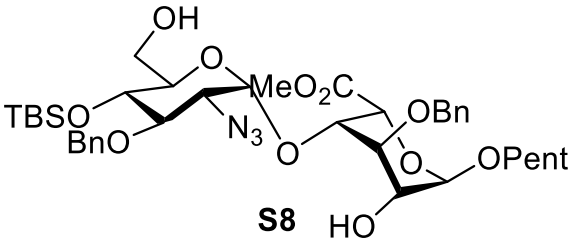
31

A mixture of donor **21** (1.42, 2.55 mmol) and acceptor **30** (1 g, 2.1mmol) was dissolved and co-evaporated with anhydrous toluene (2× 20 mL) and dried in high vacuum. Then the mixture was dissolved in anhydrous DCM (0.01M donor concentration). 4 Å molecular sieves was added to the reaction mixture, which was stirred at room temperature for 30 min. and then cooled to -78°C. A solution of AgOTf (1.8 g, 3 mmol) in anhydrous acetonitrile (0.5 mL) was added to the reaction solution without touching the wall of the flask. After 5 min., orange colored *p*-TolSCl (367.8 µL, 2.55 mmol) was added to the reaction mixture through a microsyringe. The reaction was stirred for 1 h at -78°C then warmed up to 0°C. Upon reaction completion, the reaction mixture was diluted with DCM, quenched by triethylamine and filtered over Celite with DCM. The DCM solution was concentrated, and the residue was subjected for purification by silica gel chromatography (toluene/acetone: 10:1 to 3:1) to afford the desired product **31** (1.5 g, 1.66 mmol, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 – 8.13 (m, 2H), 7.54 – 7.48 (m, 1H), 7.44 – 7.39 (m, 4H), 7.34 (t, *J* = 7.4 Hz, 2H), 7.31 – 7.24 (m, 5H), 7.18 – 7.14 (m, 2H), 5.84 – 5.75 (m, 1H, CH=), 5.24 (s, 1H, H-1-GlcN), 5.14 (t, *J* = 2.3 Hz, 1H, H-2-GlcN), 5.02 – 4.93 (m, 3H), 4.89 (d, *J* = 2.5 Hz, 1H), 4.81 – 4.75 (m, 2H, H-1-IdoA), 4.45 (dd, *J* = 12.2, 2.2 Hz, 1H, H-5-GlcN), 4.23 – 4.14 (m, 3H, H-6-IdoA, H-3-GlcN), 4.09 – 4.05 (m, 2H, H-4-GlcN, H-6-IdoA), 3.85 – 3.78 (m, 5H, H-5-IdoA, H-6-GlcN, $COOCH_3$), 3.61 – 3.53 (m, 2H, H-4-IdoA), 3.38 (dd, *J* = 10.2, 8.6 Hz, 1H, H-3-IdoA), 3.24 (dd, *J* = 10.2, 3.6 Hz, 1H, H-2-IdoA), 2.17 – 2.11 (m, 2H), 2.07 (s, 3H, CH_3CO), 1.77 – 1.70 (m, 2H), 0.89 (s, 9H, $(CH_3)_3CSi$), -0.02 (s, 3H, CH_3Si), -0.09 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7, 169.7, 165.7, 138.0, 137.9, 137.6, 133.3, 129.9, 129.8, 128.6, 128.4, 128.1, 127.91, 127.85, 127.3, 127.0, 115.0, 99.1 (C-1-GlcN), 98.9 (C-1-IdoA), 80.3, 75.5, 74.4,

72.7, 72.4, 71.2, 70.5, 68.3, 68.1, 67.6, 64.4, 62.4, 52.3, 30.2, 28.5, 25.9, 20.9, 18.0, -3.7, -5.2. ESI-MS: $C_{47}H_{61}N_3O_{13}Si$ $[M+NH_4]^{+1}$ calcd: 1131.5541, obsd: 1131.5501.

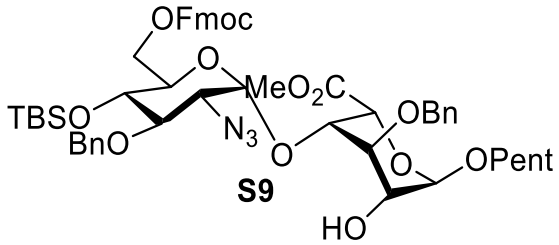


Pent-4-enyl 2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl-3-*O*-benzyl-1-*α*-L-idopyranosyluronate S8:



Compound **31** (1.5 g, 1.66 mmol) was dissolved in MeOH/DCM (3/2) (20 mL), then NaOMe solution (25 wt. % in MeOH) was added until pH≈14. The reaction mixture was stirred till completion, then neutralized with Amberlite resin until the pH is around 7. After filtration, the filtrate was concentrated and dried, purified by silica column chromatography (hexane/EtOAc: 3:1 to 1:2) to give **S8** (0.993 g, 1.3 mmol, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.30 (m, 10H), 5.87 – 5.75 (m, 1H, CH=), 5.07 (d, *J* = 1.4 Hz, 1H, H-1-GlcN), 5.04 – 5.01 (m, 1H, H-3-GlcN), 4.98 (h, *J* = 1.4 Hz, 1H, H-2-GlcN), 4.93 (d, *J* = 1.7 Hz, 1H), 4.86 – 4.78 (m, 3H), 4.76 (d, *J* = 8.8 Hz, 1H), 4.56 (d, *J* = 11.4 Hz, 1H), 4.22 – 4.17 (m, 1H), 3.89 (d, *J* = 3.5 Hz, 1H), 3.84 (s, 3H, $COOCH_3$), 3.83 – 3.77 (m, 3H), 3.68 – 3.59 (m, 4H, H-5-GlcN), 3.56 – 3.52 (m, 2H, H-4-GlcN), 3.41 (d, *J* = 6.3 Hz, 1H), 2.18 – 2.11 (m, 2H), 1.80 – 1.72 (m, 3H), 0.88 (s, 9H, $(CH_3)_3CSi$), 0.09 (s, 3H, CH_3Si), -0.02 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.5, 138.0, 128.48, 128.46, 128.3, 128.0, 127.9, 127.8, 127.5, 127.1, 115.0, 101.9 (C-1), 101.8 (C-1), 94.9, 81.1, 75.7, 73.5, 72.0, 71.9, 71.8, 70.3, 68.0, 66.8, 65.9, 64.5, 61.0, 52.6, 30.3, 30.1, 28.6, 25.8, 17.9, -3.8, -4.8. ESI-MS: $C_{38}H_{55}N_3O_{11}Si$ $[M+NH_4]^{+1}$ calcd: 775.3944, obsd: 775.3953.

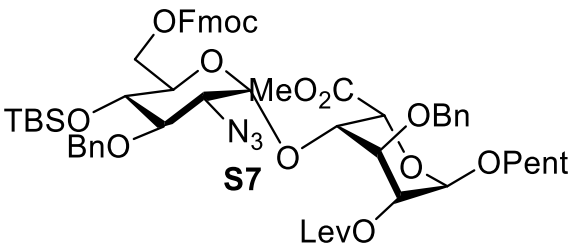
Pent-4-enyl 2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-6-*O*-fluorenylmethoxycarbonyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl-3-*O*-benzyl-1-*α*-L-idopyranosyluronate S9:



S9

To a solution of **S8** (0.993 g, 1.3 mmol) in DCM (5 mL), FmocCl (0.407 g, 1.57 mmol) and pyridine (211.7 μL, 2.6 mmol) were added, and the reaction was stirred overnight. Upon completion, the reaction was diluted with EtOAc, washed with 1 M HCl solution, saturated Na_2CO_3 solution, saturated NaCl solution, dried over Na_2SO_4, concentrated, and purified with silica column chromatography (hexane/EtOAc: 3:1 to 1:2) to afford **S9** (0.912 g, 0.93 mmol, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.66 – 7.60 (m, 2H), 7.43 – 7.29 (m, 14H), 5.86 – 5.76 (m, 1H, CH=), 5.07 (d, *J* = 3.2 Hz, 2H, H-1-GlcN, H-1-IdoA), 5.02 – 4.95 (m, 2H), 4.93 (d, *J* = 1.7 Hz, 1H, H-2-GlcN), 4.88 (d, *J* = 11.4 Hz, 1H, H-3-GlcN), 4.81 (d, *J* = 11.4 Hz, 1H), 4.76 (d, *J* = 11.4 Hz, 1H, H-4-GlcN), 4.57 (d, *J* = 11.4 Hz, 1H, H-5-GlcN), 4.47 – 4.42 (m, 3H, H-6-GlcN), 4.29 – 4.22 (m, 3H), 3.92 – 3.89 (m, 1H), 3.86 – 3.74 (m, 6H, $COOCH_3$), 3.65 – 3.51 (m, 5H, H-2-IdoA, H-3-IdoA), 2.17 – 2.10 (m, 2H), 1.79 – 1.70 (m, 2H), 0.88 (s, 9H, $(CH_3)_3CSi$), 0.03 (s, 3H, CH_3Si), 0.01 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.2, 155.0, 143.4, 143.2, 141.32, 141.29, 138.0, 137.8, 137.4, 128.5, 128.3, 127.94, 127.90, 127.8, 127.5, 127.2, 127.0, 125.2, 125.1, 120.07, 120.05, 115.0, 101.7 (C-1-GlcN), 94.9 (C-1-IdoA), 81.0, 75.7, 71.9, 71.8, 71.6, 71.3, 70.1, 70.0, 68.0, 66.8, 65.9, 65.5, 64.4, 52.5, 46.7, 30.3, 28.6, 25.8, 17.9, -3.8, -5.1. ESI-MS: $C_{53}H_{65}N_3O_{13}Si$ $[M+NH_4]^{+1}$ calcd: 997.4625, obsd: 997.4601.

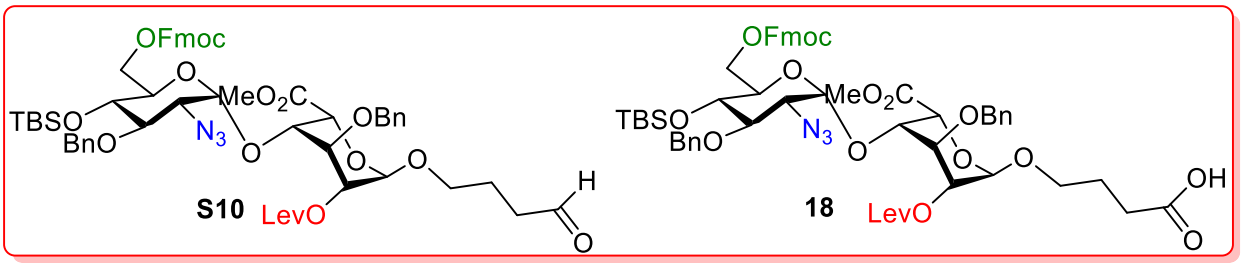
Pent-4-enyl 2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-6-*O*-fluorenylmethoxycarbonyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl-3-*O*-benzyl-2-*O*-levulinyl-1-*α*-L-idopyranosyluronate S7:



S7

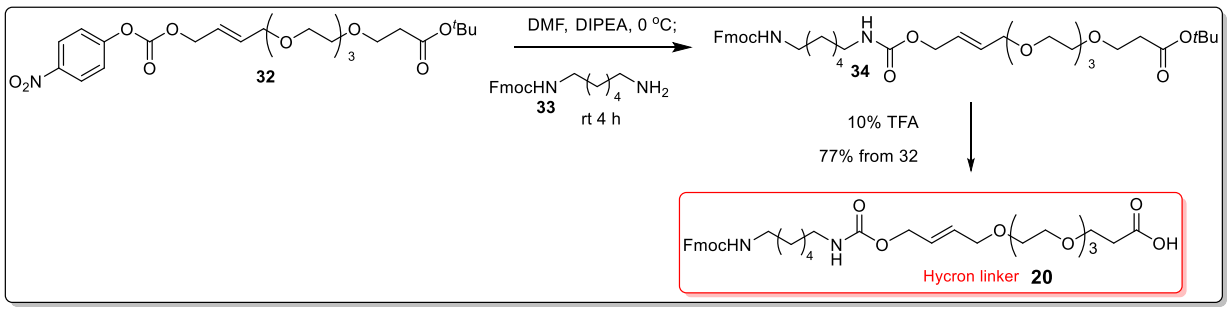
To a solution of **S9** (0.5 g, 0.34 mmol) in DCM (5 mL), LevOH (0.262 mL, 2.55 mmol), DMAP (6.2 mg, 0.05 mmol) and DIC (0.399 mL, 2.55 mmol) were added. The resulting solution was stirred at rt for 5 h. After completion, the reaction was diluted with DCM, washed with 10% HCl solution, saturated $NaHCO_3$ solution, dried over Na_2SO_4, concentrated and purified with silica gel column (hexane/EtOAc, 8:1 to 3:1) to afford compound **S7** as a white solid (0.41 g, 0.38 mmol, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.65 – 7.58 (m, 2H), 7.44 – 7.28 (m, 14H), 5.84 – 5.74 (m, 1H, CH=), 5.10 (d, *J* = 3.6 Hz, 1H, H-1-GlcN), 5.05 – 5.03 (m, 1H), 5.02 – 4.94 (m, 3H), 4.89 – 4.77 (m, 4H), 4.66 (d, *J* = 11.6 Hz, 1H), 4.50 (dd, *J* = 11.7, 2.1 Hz, 1H), 4.41 (d, *J* = 7.4 Hz, 2H), 4.26 – 4.21 (m, 2H), 4.19 – 4.15 (m, 1H), 3.99 – 3.96 (m, 1H), 3.82 (m, 4H, $COOCH_3$), 3.80 – 3.75 (m, 2H), 3.67 (dd, *J* = 10.2, 8.1 Hz, 1H), 3.54 – 3.48 (m, 1H), 3.34 (dd, *J* = 10.2, 3.5 Hz, 1H), 2.82 – 2.67 (m, 3H), 2.62 – 2.56 (m, 1H), 2.17 (s, 3H, CH_2COCH_3), 2.15 – 2.08 (m, 2H), 1.73 – 1.67 (m, 2H), 0.88 (s, 9H, $(CH_3)_3CSi$), 0.03 (s, 3H, CH_3Si), 0.01 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.1, 172.1, 169.7, 155.0, 143.4, 143.2, 141.29, 141.25, 138.02, 137.95, 137.5, 128.34, 128.27, 128.24, 127.9, 127.79, 127.75, 127.5, 127.2, 127.1, 125.2, 125.1, 120.04, 120.02, 114.9, 98.5 (C-1-GlcN), 96.5 (C-1-IdoA), 80.1, 74.9, 72.3, 72.0, 71.7, 70.9, 70.4, 70.0, 68.1, 67.6, 67.6, 65.9, 63.8, 52.3, 46.7, 37.8, 30.2, 29.8, 28.6, 27.7, 25.9, 18.0, -3.8, -5.1. ESI-MS: $C_{58}H_{71}N_3O_{15}Si$ $[M+NH_4]^{+1}$ calcd: 1095.4993, obsd: 1095.5004.

Carboxypropyl-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-6-*O*-fluorenylmethoxycarbonyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl-3-*O*-benzyl-2-*O*-levulinyl-1-*α*-L-idopyranosyluronate 18:



To a solution of **S7** (0.41 g, 0.38 mmol) in DCM (7 mL) at -78 °C, O_3 was passed through the solution via a glass pipette till the blue color persisted (takes about 10-15 min). Then, Me_2S (0.245 mL, 3.3 mmol) was added, and the reaction was stirred at rt overnight. The mixture was concentrated and purified with silica gel chromatography (toluene/acetone, 8:1 to 2:1) to afford the aldehyde **S10** (0.288 g, 0.27 mmol, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.68 (d, *J* = 1.6 Hz, 1H, CHO), 7.77 (d, *J* = 7.8 Hz, 2H), 7.62 (dd, *J* = 11.0, 7.5 Hz, 2H), 7.47 – 7.28 (m, 14H), 5.11

(d, *J* = 3.6 Hz, 1H), 5.05 (s, 1H), 4.94 (s, 1H), 4.87 (d, *J* = 11.1 Hz, 1H), 4.83 – 4.77 (m, 3H), 4.68 (d, *J* = 11.5 Hz, 1H), 4.52 – 4.47 (m, 1H), 4.42 (d, *J* = 7.4 Hz, 2H), 4.26 – 4.21 (m, 2H), 4.18 (s, 1H), 3.98 (s, 1H), 3.82 (d, *J* = 9.0 Hz, 4H, $COOCH_3$), 3.77 (t, *J* = 8.8 Hz, 1H), 3.66 (dd, *J* = 10.3, 8.3 Hz, 1H), 3.55 – 3.49 (m, 1H), 3.33 (dd, *J* = 10.3, 3.5 Hz, 1H), 2.81 – 2.68 (m, 3H), 2.63 – 2.56 (m, 1H), 2.49 – 2.45 (m, 1H), 2.18 (s, 3H, CH_2COCH_3), 1.91 (p, *J* = 6.5 Hz, 2H), 1.30 – 1.16 (m, 2H), 0.89 (s, 9H, $(CH_3)_3CSi$), 0.04 (s, 3H, CH_3Si), 0.01 (s, 3H, CH_3Si). The aldehyde **S10** (0.288 g, 0.27 mmol, 70%) was dissolved in acetonitrile (15 mL), cooled to 0 °C, followed by addition of NaH_2PO_4 (51.5 mg, 0.37 mmol, in 7 mL H_2O) and H_2O_2 (75 µL). Then $NaClO_2$ (99.5 mg, 0.88 mmol, in 7 mL H_2O) was added. The reaction was stirred at room temperature overnight. Then the solvent was removed, and the residue was dissolved in EtOAc, washed with brine, dried over Na_2SO_4, concentrated, and purified by silica gel chromatography (DCM/MeOH, 20:1 to 10:1) to give **18** (0.234 g, 0.41 mmol, 80%). $[\alpha]_D^{20}$= -50.0 (C =0.1, DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.64 – 7.59 (m, 2H), 7.43 – 7.29 (m, 14H), 5.11 (d, *J* = 3.6 Hz, 1H, H-1-IdoA), 5.07 (d, *J* = 2.6 Hz, 1H, H-1-GlcN), 4.98 – 4.95 (m, 1H), 4.87 (d, *J* = 11.1 Hz, 1H), 4.83 (d, *J* = 3.1 Hz, 1H), 4.79 (d, *J* = 11.1 Hz, 2H), 4.69 (d, *J* = 11.6 Hz, 1H), 4.50 (dd, *J* = 11.8, 2.2 Hz, 1H), 4.42 (d, *J* = 7.4 Hz, 2H), 4.26 – 4.21 (m, 2H), 4.17 (t, *J* = 3.7 Hz, 1H), 3.99 (t, *J* = 4.0 Hz, 1H), 3.86 – 3.81 (m, 5H, $COOCH_3$), 3.76 (dd, *J* = 9.7, 8.3 Hz, 1H), 3.65 (dd, *J* = 10.2, 8.3 Hz, 1H), 3.58 – 3.52 (m, 1H), 3.33 (dd, *J* = 10.2, 3.5 Hz, 1H), 2.75 (t, *J* = 6.7 Hz, 2H), 2.66 – 2.58 (m, 2H), 2.43 (t, *J* = 7.2 Hz, 2H), 2.19 (s, 3H, CH_2COCH_3), 1.98 – 1.90 (m, 2H), 0.89 (s, 9H, $(CH_3)_3CSi$), 0.04 (s, 3H, CH_3Si), 0.01 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.8, 172.1, 169.7, 155.0, 143.4, 143.2, 141.31, 141.26, 137.9, 137.5, 128.4, 128.3, 127.9, 127.8, 127.5, 127.21, 127.15, 125.2, 125.1, 120.1, 120.0, 98.4 (C-1-GlcN), 96.8 (C-1-IdoA), 80.1, 74.9, 72.7, 72.5, 72.2, 71.0, 70.5, 70.0, 68.5, 68.4, 67.5, 65.9, 63.7, 52.3, 46.7, 37.8, 30.5, 29.8, 27.7, 25.9, 25.6, 24.6, 18.0, -3.8, -5.1. ESI-MS: $C_{57}H_{69}N_3O_{17}Si$ $[M+Na]^{+1}$ calcd: 1118.4288, obsd: 1118.4240.



To a solution of **32** (2.6 g, 5 mmol) in DMF (0.1 M), DIPEA (1.9 mL, 11 mmol) and amine **33** (2.2 g, 5 mmol) were added respectively at 0°C. The reaction was stirred for 4 h at rt. Upon completion, the mixture was diluted with EtOAc, washed with 1M HCl, water, and brine solution, then dried over Na_2SO_4, concentrated, and purified with silica gel chromatography to afford **34** (4.5 g, 4.5 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, *J* = 9.2 Hz, 1H), 7.77 (dt, *J* = 7.6, 1.0 Hz, 2H), 7.60 (dq, *J* = 7.5, 1.0 Hz, 2H), 7.40 (tt, *J* = 7.5, 0.9 Hz, 2H), 7.31 (td, *J* = 7.4, 1.1 Hz, 2H), 6.93 (d, *J* = 9.2 Hz, 1H), 5.84 –5.80 (m, 2H), 4.57 – 4.53 (m, 2H), 4.40 (d, *J* = 6.9 Hz, 2H), 4.03 – 4.01 (m, 2H), 3.71 (td, *J* = 6.6, 2.1 Hz, 3H), 3.66 – 3.64 (m, 6H), 3.63 – 3.58 (m, 8H), 3.22 – 3.14 (m, 4H), 1.53 – 1.47 (m, 4H), 1.44 (s, 9H), 1.36 – 1.31 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0, 144.0, 141.3, 130.2, 127.7, 127.57, 127.55, 127.0, 126.1, 125.0, 120.0, 115.7, 80.6, 71.0, 70.61, 70.58, 70.57, 70.5, 70.4, 69.6, 66.9, 66.5, 64.5, 47.3, 40.8, 36.2, 29.9, 28.1, 26.3, 26.2. ESI-MS: $C_{39}H_{56}N_2O_{10}$ $[M+NH_4]^{+1}$ calcd: 730.4273, obsd: 730.4260.

To a solution of **34** (4.423 g, 4.5 mmol) in DCM (90 mL), trifluoroacetic acid (TFA) (10 mL) was added. The reaction was stirred at rt till completion. Then the mixture was concentrated and purified with column chromatography (DCM:MeOH, 20:1 to 10:1) to provide **20** (2.53 g, 3.9 mmol, 77% from **32**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 – 7.75 (m, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.32 (td, *J* = 7.5, 1.2 Hz, 2H), 7.20 – 7.16 (m, 1H), 5.83 (q, *J* = 3.7 Hz, 2H), 4.93 – 4.88 (m, 1H), 4.59 – 4.54 (m, 2H), 4.44 – 4.38 (m, 2H), 4.22 (t, *J* = 6.9 Hz, 1H), 4.06 – 4.02 (m, 2H), 3.78 (t, *J* = 6.1 Hz, 2H), 3.67 – 3.59 (m, 13H), 3.18 (q, *J* = 6.6 Hz, 4H), 2.62 (t, *J* = 6.2 Hz, 2H), 1.55 – 1.47 (m, 4H), 1.37 – 1.29 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.0, 141.3, 130.0, 129.0, 128.2, 127.7, 127.0, 125.3, 125.0, 120.0, 77.2, 70.9, 70.6, 70.6, 70.5, 70.2, 69.5, 66.6, 66.5, 64.5, 47.3, 40.8, 35.0, 29.8, 26.2. ESI-MS: $C_{35}H_{48}N_2O_{10}$ $[M+NH_4]^{+1}$ calcd: 674.3647, obsd: 674.3653.

1.5. Reagent solutions for solid phase synthesis:

1.5.1. Lev deprotection solution: To a solution of Pyr./AcOH (3:1, 15 mL), $N_2H_4.H_2O$ (0.5 mL) was added to prepare 0.56 M hydrazine hydrate solution.

1.5.2. Fmoc deprotection solution: The solution was 20% (v/v) piperidine in DMF.

1.5.3. Staudinger azide reduction solution: To a solution of THF/H_2O (10:1) (25 mL), trimethylphosphine in THF (1M) (1 mL) was added to prepare 0.04 M of trimethylphosphine in THF.

1.5.4. TBS deprotection solution: In case of manual deprotection; a solution of Pyr./HF.Pyr. (2:1) was prepared.

For automated synthesis, to a solution of THF (10 mL), tetrabutylammonium fluoride (TBAF) (1M) in THF (0.4 mL) was added to prepare 0.04 M of TBAF in THF.

1.5.5. *O*-Acetylation solution: a solution of pyridine/acetic anhydride (Ac_2O) (2:1, 15 mL) was prepared.

1.5.6. *N*-Acetylation solution: a solution of MeOH/Ac_2O/Et_3N (3:1:1, 20 mL) was prepared.

1.5.7. *O*-Sulfation solution: 0.5 M of sulfur trioxide triethylamine ($SO_3.Et_3N$) complex in DMF/Et_3N (0.5 mL). A stock solution of $SO_3.Et_3N$ complex (1.8 g) in anhydrous DMF (20 mL) / Et_3N (1 mL) was prepared.

1.5.8. *N*-Sulfation solution: 0.5 M of sulfur trioxide pyridine (SO_3.Pyr.) complex in MeOH/Et_3N (0.5 mL). A stock solution of SO_3.Pyr. complex (1.6 g) in MeOH (20 mL) / Et_3N (1 mL) was prepared. Then an aqueous solution of NaOH (2M) was added till pH= 8.5.

1.5.9. Cleavage cocktail solution: A solution of tetrakis (triphenylphosphine)palladium (0) (5 equiv.) and borane dimethylamine complex (10 equiv.) in DCM/DMF (1:1, 5 mL) was prepared.

1.5.10. Coupling solution: To a solution of Hycron linker (788.2 mg, 2 equiv.), HATU (456.3 mg, 2equiv.), HOAt (163.3 mg, 2 equiv.) and DIPEA (418 µL, 2.4 equiv.) in DMF (10 mL) in the reaction vessel, 75 lanterns were added. The reactor was placed on CEM Liberty Blue automated peptide synthesizer and heated under microwave irradiation at temperature (60 ^oC), power (w) (35), time (3h), and Delta T ^oC (2). After finishing the coupling, the lanterns were washed with DMF (10 mL X 2) and DCM (10 mL X 2).

1.6. Quantification of Fmoc cleavage product: Upon completion of lantern loading, one of lanterns was subjected for Fmoc cleavage in 20% piperidine in DMF (1 mL). After 30 min, we made different concentrations of that solution, measured the absorbance values at λ=301nm using a UV-vis spectrophotometer, and then used the following equation to quantify the amount of Fmoc being cleaved (Q):

$$Q=A_{301}\times dilution\times V(total)\div 7800$$

We found the loading of the linker is 6.4 μmol per lantern.

1.7. Coupling of disaccharides 17 and 18 on lantern:

1.7.1. For loading of disaccharide 17: To 50 lanterns following Fmoc cleavage placed in the reaction vessel on the synthesizer, a solution of disaccharide **17** (245.6 mg, 0.7 equiv.), HATU (97.3 mg, 0.8 equiv.), HOAt (34.8 mg, 0.8 equiv.) and DIPEA (64.1μL, 1.6 equiv.) in DMF (8 mL) was added. The mixture was heated under microwave irradiation on CEM Liberty Blue automated peptide synthesizer at temperature (60 ^{o}C), power (w) (35), time (3h), and Delta T ^{o}C (2). After the reaction finished, the lanterns were washed with DMF (10 mL X 2) and DCM (10 mL X 2).

Note: For glycan coupling, the coupling was set up with no drain cycle, so that we could recover the unreacted sugar for the reaction mixture without any loss.

1.7.2. For loading of disaccharide 18: Similar to the procedure for disaccharide **17**, to 50 lanterns (with amine loading of 5.3 μmol/lantern) placed on the reactor, a solution of disaccharide **18** (203.4 mg, 0.7 equiv.), HATU (80.6 mg, 0.8 equiv.), HOAt (28.9 mg, 0.8 equiv.) and DIPEA (40.7 μL, 1.6 equiv.) in DMF (8 mL) was added. The mixture was heated under microwave irradiation at 50 ºC for 3 h on CEM Liberty Blue automated peptide synthesizer. After the reaction finished, the lanterns were washed with DMF (10 mL X 2) and DCM (10 mL X 2).

Quantification of disaccharide loading: following the general protocol for quantification of Fmoc cleavage product.

- Loading for lantern loaded with sugar **18** was 4.2 µmol with 93.8% yield.
- Loading for lantern loaded with sugar **17** was 3.2 µmol with 86.3% yield.

1.8. Microwave methods:

- ***O*-Acetylation method:** Temperature (60 ^{o}C), power (w) (25), time (2h), Delta T ^{o}C (2).
- **Lev deprotection method:** Temperature (40 ^{o}C), power (w) (25), time (30 min), Delta T ^{o}C (2).
- **Sulfation method:** Temperature (60 ^{o}C), power (w) (5), time (6h), Delta T ^{o}C (5).
- **Azide reduction method:** Temperature (45 ^{o}C), power (w) (25), time (1h), Delta T ^{o}C (2).
- **LiOH hydrolysis method:** Temperature (30 ^{o}C), power (w) (5), time (18h), Delta T ^{o}C (2).
- **TBS deprotection method:** Temperature (35 ^{o}C), power (w) (5), time (18h), Delta T ^{o}C (2).
- ***N*-Acetylation method:** Temperature (50 ^{o}C), power (w) (25), time (2h), Delta T ^{o}C (2).
- **Cleavage from lantern:** Temperature (35 ^{o}C), power (w) (5), time (18h), Delta T ^{o}C (2).

1.9. Automation protocols:

***O*-Acetylation method:**

1- Add reagent: *O*-Acetylation solution (7 mL).
2- Microwave method: *O*-Acetylation method.
3- Drain.
4- Wash: DMF (5 mL).
5- Wash: DMF (5 mL).
6- Add reagent: *O*-Acetylation solution (7 mL).
7- Microwave method: *O*-Acetylation method.
8- Wash: DMF (5 mL).
9- Wash: DMF (5 mL).
10- Wash: DMF (5 mL).

***N*-Acetylation method:**

1- Add reagent: *N*-Acetylation solution (7 mL).
2- Microwave method: *N*-Acetylation method.
3- Drain.
4- Wash: DMF (5 mL).
5- Wash: DMF (5 mL).

***O*-Sulfation method:**

1- Add reagent: *O*-Sulfation solution (7 mL).
2- Microwave method: Sulfation deprotection method.
3- Drain.
4- Wash: DMF (5 mL).
5- Wash: DMF (5 mL).
6- Add reagent: *O*-Sulfation solution (7 mL).
7- Microwave method: Sulfation deprotection method.
8- Wash: DMF (5 mL).
9- Wash: DMF (5 mL).
10- Wash: DMF (5 mL).

***N*-Sulfation method:**

1- Add reagent: *N*-Sulfation solution (7 mL).
2- Microwave method: Sulfation deprotection method.
3- Drain.
4- Add reagent: MeOH (5 mL).
5- Wait (5 min).
6- Drain.
7- Wash: DMF (5 mL).
8- Add reagent: MeOH (5 mL).
9- Wait (5 min).
10- Drain.
11- Add reagent: *N*-Sulfation solution (7 mL).
12- Microwave method: Sulfation deprotection method.
13- Drain.

14- Add reagent: MeOH (5 mL).

15- Wait (5 min).

16- Drain.

17- Wash: DMF (5 mL).

18- Add reagent: MeOH (5 mL).

19- Wait (5 min).

20- Drain.

21- Add reagent: *N*-Sulfation solution (7 mL).

22- Microwave method: Sulfation deprotection method.

23- Drain.

24- Add reagent: MeOH (5 mL).

25- Wait (5 min).

26- Drain.

27- Wash: DMF (5 mL).

28- Wash: DMF (5 mL).

TBS deprotection method:

1- Add reagent: TBS deprotection solution (TBAF) (5 mL).

2- Microwave method: TBS deprotection method.

3- Drain.

4- Add reagent: MeOH (5 mL).

5- Wait (5 min).

6- Drain.

7- Add reagent: MeOH (5 mL).

8- Wait (5 min).

9- Drain.

10- Wash: DMF (5 mL).

11- Wash: DMF (5 mL).

Lev deprotection method:

1- Add reagent: Lev deprotection solution (8 mL).

2- Microwave method: Lev deprotection method.

3- Drain.
4- Wash: DMF (5 mL).
5- Wash: DMF (5 mL).
6- Add reagent: Lev deprotection solution (8 mL).
7- Microwave method: Lev deprotection method.
8- Drain.
9- Wash: DMF (5 mL).
10- Wash: DMF (5 mL).
11- Add reagent: Lev deprotection solution (8 mL).
12- Microwave method: Lev deprotection method.
13- Drain.
14- Wash: DMF (5 mL).
15- Wash: DMF (5 mL).
16- Wash: DMF (5 mL).

Azide reduction method:

1- Add reagent: Staudinger azide reduction solution (8 mL).
2- Microwave method: Azide reduction method.
3- Drain.
4- Add reagent: MeOH (5 mL).
5- Wait (5 min).
6- Drain.
7- Add reagent: Staudinger azide reduction solution (8 mL).
8- Microwave method: Azide reduction method.
9- Drain.
10- Add reagent: MeOH (5 mL).
11- Wait (5 min).
12- Drain.
13- Add reagent: Staudinger azide reduction solution (8 mL).
14- Microwave method: Azide reduction method.
15- Drain.

16- Add reagent: MeOH (5 mL).
17- Wait (5 min).
18- Drain.
19- Wash: DMF (5 mL).
20- Wash: DMF (5 mL).
21- Wash: DMF (5 mL).

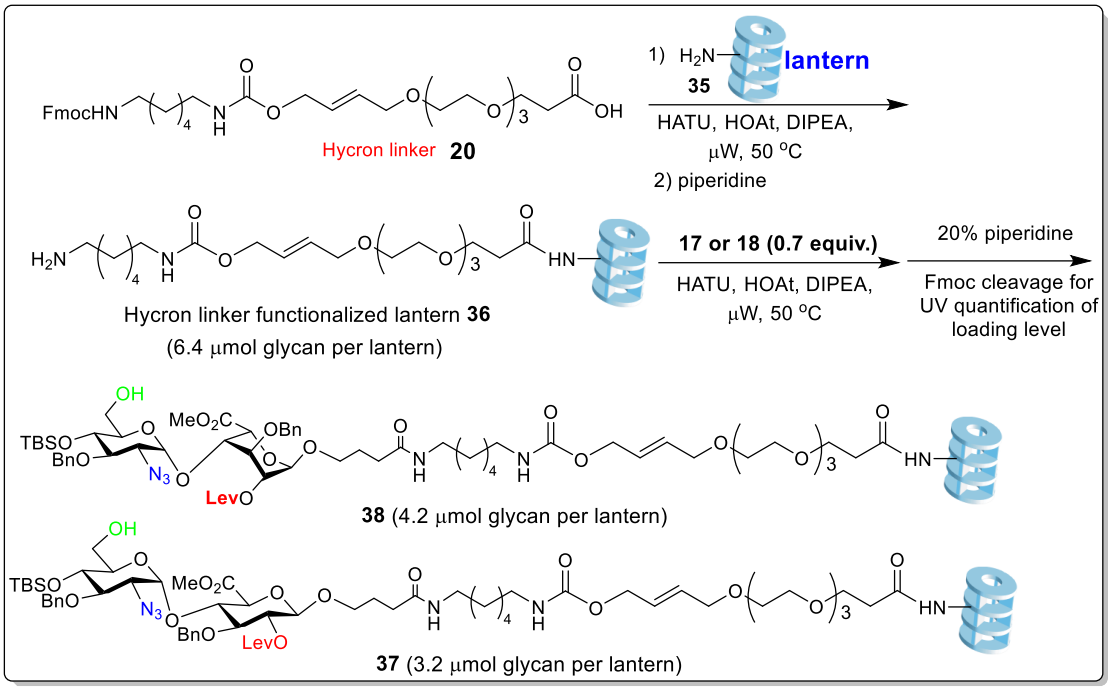
LiOH hydrolysis method:

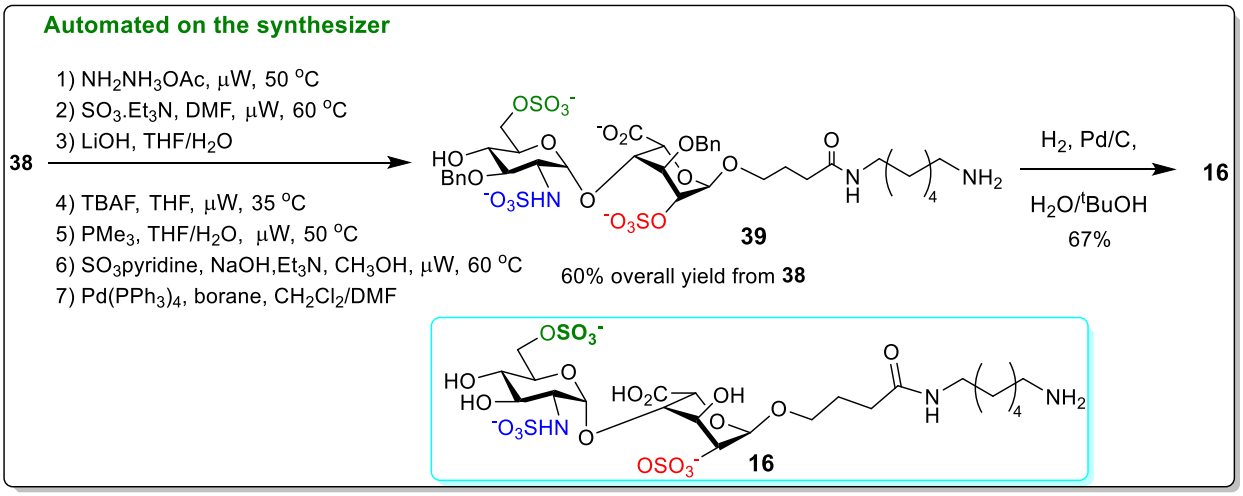
1- Add reagent: LiOH hydrolysis solution (5 mL).
2- Microwave method: LiOH hydrolysis method.
3- Drain.
4- Add reagent: MeOH (5 mL).
5- Wait (5 min).
6- Drain.
7- Add reagent: LiOH hydrolysis solution (5 mL).
8- Microwave method: LiOH hydrolysis method.
9- Drain.
10- Add reagent: MeOH (5 mL).
11- Wait (5 min).
12- Drain.
13- Wash: DMF (5 mL).
14- Wash: DMF (5 mL).

Cleavage from lantern:

1- Add reagent: Cleavage cocktail solution (5 mL).
2- Microwave method: Cleavage of lantern method.
3- No drain and collect the filtrate.

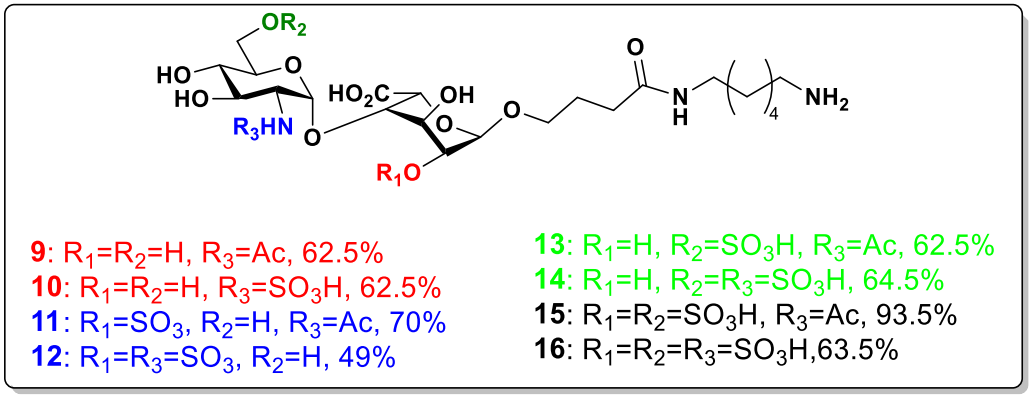
Then, the filtrate was concentrated and subjected to LH-20 for purification to afford the product.

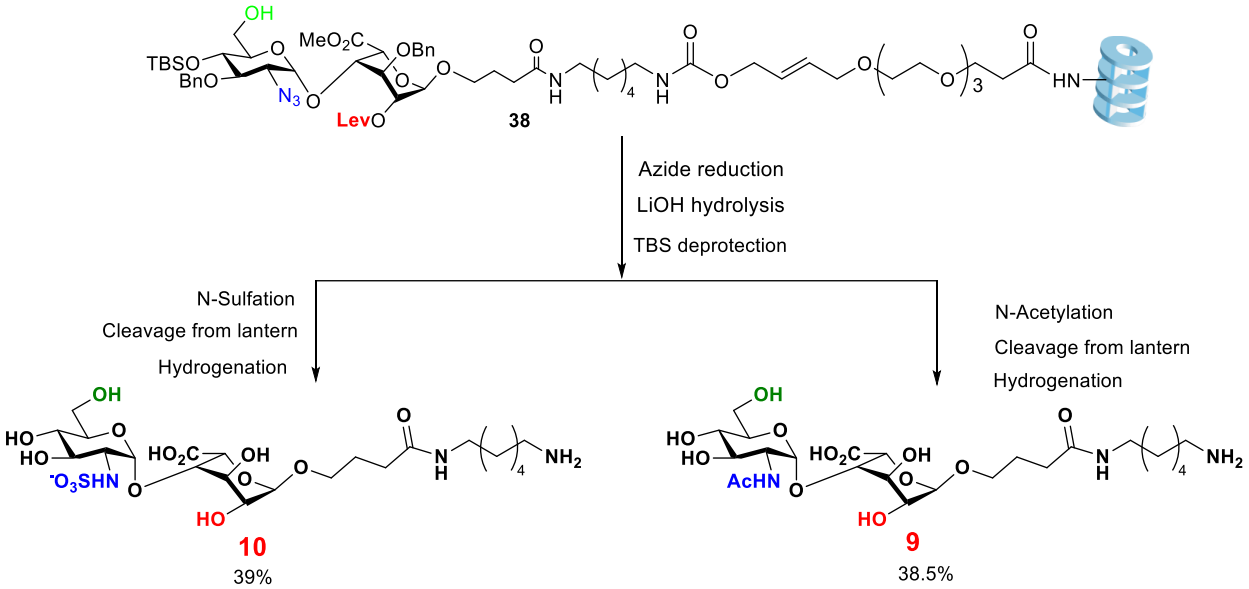




Compound **39**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.77 – 7.09 (m, 10H), 5.44 (d, *J* = 3.4 Hz, 1H), 5.35 (d, *J* = 3.0 Hz, 1H), 5.23 (s, 1H), 5.03 (d, *J* = 11.1 Hz, 1H), 4.85 – 4.75 (m, 3H), 4.56 – 4.51 (m, 2H), 4.35 – 4.22 (m, 4H), 4.07 – 4.02 (m, 1H), 3.72 – 3.61 (m, 5H), 3.51 – 3.43 (m, 2H), 3.32 – 3.29 (m, 1H), 3.17 – 3.10 (m, 2H), 2.95 (t, *J* = 7.5 Hz, 1H), 2.30 – 2.23 (m, 2H), 2.15 (t, *J* = 7.6 Hz, 4H), 2.06 – 2.01 (m, 2H), 1.95 – 1.90 (m, 2H), 1.73 – 1.69 (m, 2H), 1.62 – 1.58 (m, 4H). ^{13}C NMR (126 MHz, CD_3OD) δ 127.7, 127.5, 127.1, 99.6, 97.8, 75.0, 74.2, 73.6, 72.4, 71.8, 71.6, 70.1, 68.1, 66.6, 66.4, 47.8, 38.8, 37.8, 32.0, 26.5, 26.3, 25.3. ESI-MS: $C_{36}H_{53}N_3O_{21}S_3$ $[M-H]^{-1}$ calcd: 958.2261, obsd: 958.2219.

Automation Using CEM Liberty Blue Synthesizer:

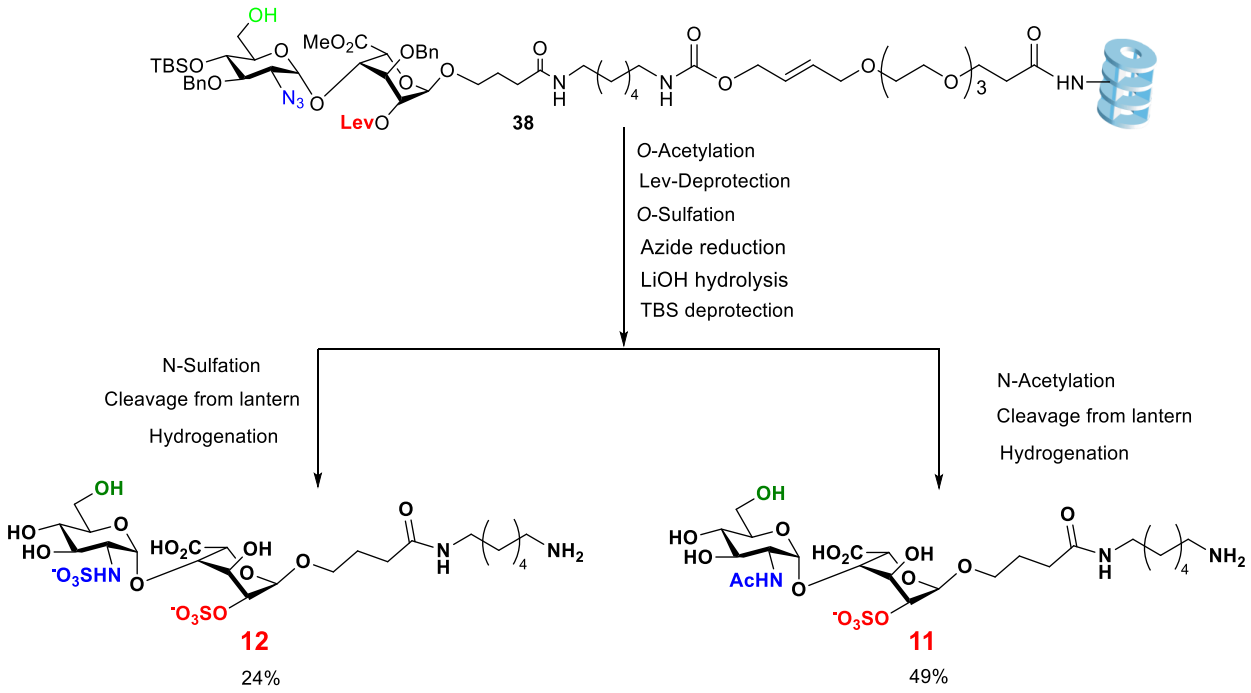




9: $[\alpha]_D^{20}$= +41.1 (C =0.09, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.98 (d, *J* = 3.7 Hz, 1H, H-1-GlcN), 4.69 (d, *J* = 2.9 Hz, 1H, H-1-IdoA), 4.39 (d, *J* = 2.8 Hz, 1H), 3.88 (t, *J* = 3.2 Hz, 1H), 3.79 – 3.74 (m, 2H), 3.64 (d, *J* = 3.2 Hz, 2H), 3.61 – 3.51 (m, 4H), 3.46 (dd, *J* = 5.1, 2.8 Hz, 1H), 3.43 – 3.37 (m, 2H), 3.34 – 3.29 (m, 1H), 3.00 (t, *J* = 6.9 Hz, 2H), 2.81 (t, *J* = 7.7 Hz, 1H), 2.14 (t, *J* = 7.2 Hz, 2H), 1.84 (s, 3H), 1.73 (p, *J* = 6.8 Hz, 2H), 1.48 (p, *J* = 7.5 Hz, 2H), 1.34 (p, *J* = 7.2 Hz, 2H), 1.24 – 1.15 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 100.8 (C-1-IdoA), 94.3 (C-1-GlcN), 73.4, 71.8, 71.1, 69.5, 69.2, 68.9, 68.2, 67.6, 60.2, 53.4, 39.5, 39.2, 38.9, 32.5, 27.9, 26.7, 25.4, 25.0, 21.8. ESI-MS: $C_{24}H_{43}N_3O_{13}$ $[M-H]^{-1}$ calcd: 580.2723, obsd: 580.2738.

10: $[\alpha]_D^{20}$= +36.9 (C =0.16, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.17 (d, *J* = 3.7 Hz, 1H, H-1-GlcN), 4.74 (t, *J* = 3.0 Hz, 1H, H-1-IdoA), 4.61 (d, *J* = 2.3 Hz, 1H), 4.01 (t, *J* = 3.5 Hz, 1H), 3.90 (d, *J* = 2.8 Hz, 1H), 3.66 (dd, *J* = 12.4, 3.5 Hz, 1H), 3.60 – 3.52 (m, 3H), 3.43 – 3.32 (m, 5H), 3.06 – 2.91

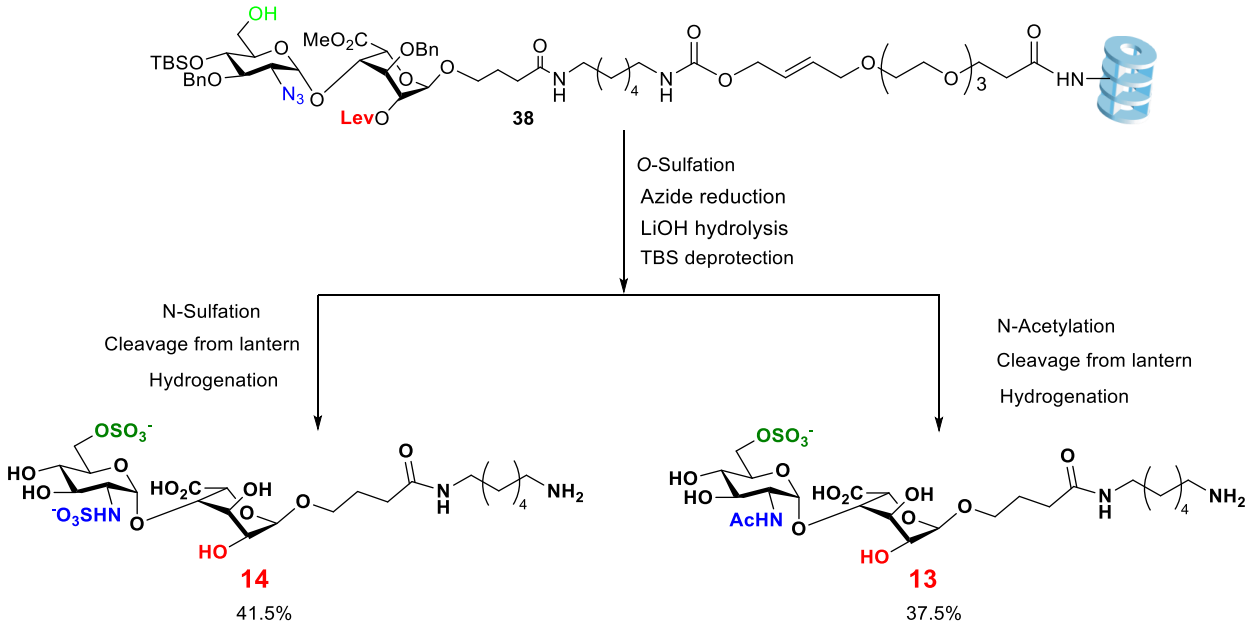
(m, 4H), 2.90 – 2.79 (m, 2H), 2.69 (s, 1H), 2.17 – 2.12 (m, 2H), 1.73 (p, *J* = 7.1 Hz, 2H), 1.452 – 1.45 (m, 2H), 1.34 (p, *J* = 6.8 Hz, 2H), 1.23 – 1.15 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 175.9, 173.1, 100.8 (C-1-IdoA), 96.2 (C-1-GlcN), 74.1, 72.1, 71.0, 69.2, 67.8, 67.5, 66.6, 59.9, 57.6, 39.3, 39.0, 32.3, 27.9, 26.5, 25.3, 25.0, 24.9. ESI-MS: $C_{22}H_{41}N_3O_{15}S$ $[M-H]^{-1}$ calcd: 618.2186, obsd: 618.2179.



11: $[\alpha]_D^{20}$= +8 (C =0.3, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.93 (d, *J* = 3.5 Hz, 1H, H-1-GlcN), 4.89 (s, 1H, H-1-IdoA), 4.35 (d, *J* = 2.1 Hz, 1H), 4.09 (d, *J* = 3.0 Hz, 1H), 4.06 – 4.03 (m, 1H), 3.83 (d, *J* = 2.7 Hz, 1H), 3.78 (dd, *J* = 10.5, 3.5 Hz, 1H), 3.68 – 3.64 (m, 1H), 3.63 – 3.58 (m, 2H), 3.55 – 3.47 (m, 2H), 3.39 – 3.34 (m, 1H), 3.27 (dd, *J* = 10.7, 8.3 Hz, 1H), 3.02 – 2.95 (m, 2H), 2.85 – 2.77 (m, 2H), 2.17 – 2.10 (m, 2H), 1.86 (s, 3H), 1.74 – 1.69 (m, 2H), 1.50 – 1.44 (m 2H), 1.36 – 1.30 (m, 2H), 1.22 – 1.13 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 98.9 (C-1-IdoA), 93.4 (C-1-GlcN), 73.5, 72.3, 71.1, 70.4, 67.5, 67.3, 63.3, 60.1, 53.2, 39.2, 38.9, 32.8, 26.4, 25.2, 25.0, 22.0. ESI-MS: $C_{24}H_{43}N_3O_{16}S$ $[M-H]^{-1}$ calcd: 660.2291, obsd: 660.2272.

12: $[\alpha]_D^{20}$= -20 (C =0.08, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.21 (d, *J* = 3.6 Hz, 1H, H-1-GlcN), 4.91 (d, *J* = 3.2 Hz, 1H, H-1-IdoA), 4.31 (d, *J* = 2.8 Hz, 1H), 4.10 – 4.06 (m, 1H), 4.05 – 4.02 (m, 1H), 3.89 (t, *J* = 3.3 Hz, 1H), 3.67 – 3.56 (m, 4H), 3.46 (dd, *J* = 10.4, 8.9 Hz, 1H), 3.37 – 3.26 (m,

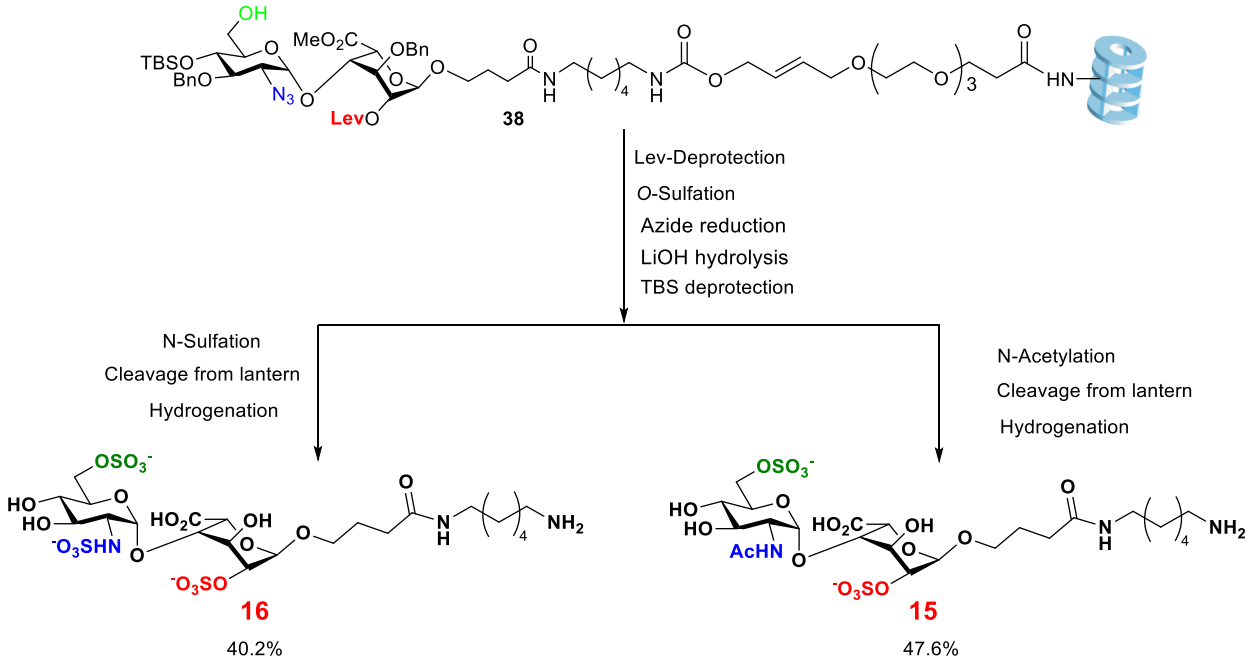
2H), 3.07 – 2.96 (m, 3H), 2.84 – 2.81 (m, 1H), 2.22 – 2.10 (m, 2H), 1.73 – 1.67 (m, 2H), 1.50 (p, *J* = 7.4 Hz, 2H), 1.36 (p, *J* = 7.1 Hz, 2H), 1.26 – 1.14 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 98.9 (C-1-IdoA), 97.0 (C-1-GlcN), 76.1, 71.9, 71.2, 69.9, 69.0, 68.6, 60.3, 58.3, 39.3, 39.0, 32.5, 28.0, 25.4, 25.1. ESI-MS: $C_{22}H_{41}N_3O_{18}S_2$ $[M-H]^{-1}$ calcd: 698.1754, obsd: 698.1763.



13: $[\alpha]_D^{20}$= +6 (C =0.2, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.99 (d, *J* = 3.7 Hz, 1H, H-1-GlcN), 4.70-4.66 (m, 1H, H-1-IdoA), 4.33 (t, *J* = 2.9 Hz, 1H), 4.19 – 4.14 (m, 1H), 4.05 – 4.00 (m, 1H), 3.89 (t, *J* = 3.3 Hz, 1H), 3.83 – 3.74 (m, 3H), 3.58 – 3.52 (m, 3H), 3.46 – 3.36 (m, 3H), 3.03 – 2.93 (m, 3H), 2.84 – 2.76 (m, 1H), 2.17 – 2.10 (m, 2H), 1.84 (s, 3H), 1.76 – 1.70 (m, 2H), 1.54 (p, *J* = 7.5 Hz, 2H), 1.37 – 1.03 (m, 3H), 1.23 – 1.17 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 100.6 (C-1-IdoA), 94.2 (C-1-GlcN), 73.2, 70.9, 70.0, 69.0, 68.4, 68.0, 66.1, 61.3, 59.7, 57.4, 53.2, 42.3, 41.3, 39.0, 33.9, 32.3, 28.1, 27.8, 26.5, 25.2, 21.6. ESI-MS: $C_{24}H_{43}N_3O_{16}S$ $[M-H]^{-1}$ calcd: 660.2291, obsd: 660.2272.

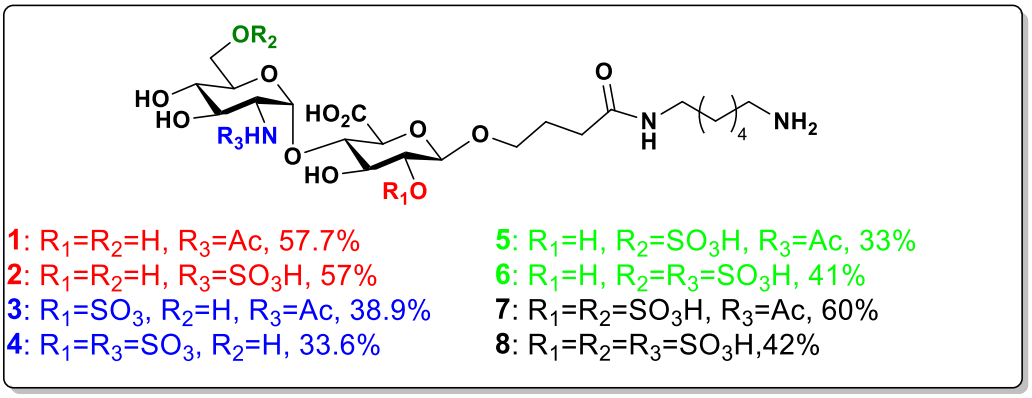
14: $[\alpha]_D^{20}$= +39.2 (C =0.12, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.19 (d, *J* = 3.6 Hz, 1H, H-1-GlcN), 4.71-4.62 (m, 1H, H-1-IdoA), 4.31 (d, *J* = 2.5 Hz, 1H), 4.16 (dd, *J* = 11.2, 3.0 Hz, 1H), 4.00 (d, *J* = 11.3 Hz, 1H), 3.95 – 3.91 (m, 1H), 3.88 (s, 1H), 3.77 (d, *J* = 10.1 Hz, 1H), 3.56 – 3.34 (m, 6H), 3.08 – 2.97 (m, 3H), 2.82 (t, *J* = 7.6 Hz, 2H), 2.14 (d, *J* = 6.9 Hz, 2H), 1.77 – 1.69 (m, 2H), 1.52 – 1.45 (m, 2H), 1.37 – 1.31 (m, 2H), 1.24 – 1.17 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 100.8 (C-1-

IdoA), 95.6 (C-1-GlcN), 74.4, 69.8, 69.2, 68.6, 68.2, 67.9, 67.6, 66.6, 66.3, 57.6, 39.2, 32.5, 27.9, 26.7, 25.0. ESI-MS: $C_{22}H_{41}N_3O_{18}S_2$ $[M-2H]^{-2}$ calcd: 348.5840, obsd: 348.5853.



15: $[\alpha]_D^{20}$= +6.3 (C =0.27, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.96 (d, *J* = 3.3 Hz, 2H, H-1-GlcN, H-1-IdoA), 4.54 (d, *J* = 16.3 Hz, 1H), 4.17 – 4.06 (m, 4H), 3.91 (s, 1H), 3.85 (dd, *J* = 10.5, 3.6 Hz, 1H), 3.72 (t, *J* = 11.0 Hz, 1H), 3.61 – 3.47 (m, 3H), 3.43 – 3.36 (m, 2H), 3.05 – 2.97 (m, 2H), 2.91 – 2.80 (m, 2H), 2.72 (d, *J* = 13.4 Hz, 1H), 2.55 (s, 1H), 2.17 (t, *J* = 7.0 Hz, 2H), 1.90 (s, 3H), 1.78 – 1.71 (m, 2H), 1.50 (p, *J* = 8.3, 7.8 Hz, 2H), 1.36 (p, *J* = 7.2 Hz, 2H), 1.24 – 1.15 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 98.5 (C-1-IdoA), 93.7 (C-1-GlcN), 73.7, 71.1, 70.8, 70.2, 69.8, 69.5, 69.2, 67.6, 66.9, 66.6, 63.7, 63.4, 53.1, 42.5, 39.2, 34.4, 32.5, 27.9, 26.7, 25.4, 25.1, 22.1. ESI-MS: $C_{24}H_{43}N_3O_{19}S_2$ $[M-H]^{-1}$ calcd: 740.1859, obsd: 740.1899.

16: $[\alpha]_D^{20}$= +10.6 (C =0.16, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.20 (d, *J* = 3.6 Hz, 1H, H-1-GlcN), 4.90 (d, *J* = 3.0 Hz, 1H, H-1-IdoA), 4.30 (d, *J* = 2.8 Hz, 1H), 4.15 (dd, *J* = 11.0, 2.8 Hz, 1H), 4.07 (dd, *J* = 5.7, 2.9 Hz, 1H), 4.03 – 3.99 (m, 2H), 3.88 (t, *J* = 3.3 Hz, 1H), 3.78 (d, *J* = 10.0 Hz, 1H), 3.59 – 3.50 (m, 2H), 3.46 – 3.42 (m, 1H), 3.39 – 3.31 (m, 2H), 3.08 – 2.94 (m, 4H), 2.83 – 2.77 (m, 2H), 2.12 – 2.09 (m, 3H), 1.73 – 1.68 (m, 2H), 1.51 – 1.45 (m, 2H), 1.37 – 1.31 (m, 2H), 1.23 – 1.15 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 98.9 (C-1-IdoA), 96.6 (C-1-GlcN), 76.3, 76.0, 75.6, 70.9, 69.8, 69.4, 69.1, 69.0, 68.6, 68.5, 67.1, 67.1, 66.3, 57.8, 39.2, 38.9, 32.2, 27.8, 26.4, 25.1, 25.0. ESI-MS: $C_{22}H_{41}N_3O_{21}S_3$ $[M-H]^{-1}$ calcd: 778.1322, obsd: 778.1273.



1: $[\alpha]_D^{20}$= +18.6 (C =0.29, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.21 (d, *J* = 3.8 Hz, 1H, H-1-GlcN), 4.24 (d, *J* = 8.0 Hz, 1H, H-1-GlcA), 3.71 – 3.46 (m, 10H), 3.29 (t, *J* = 9.6 Hz, 1H), 3.10 (t, *J* = 8.5 Hz, 1H), 3.03 – 2.92 (m, 3H), 2.89 – 2.76 (m, 2H), 2.18 – 2.057 (m, 2H), 1.85 (s, 3H), 1.73 – 1.66 (m, 2H), 1.46 (p, *J* = 7.5 Hz, 2H), 1.31 (p, *J* = 7.0 Hz, 2H), 1.22 – 1.12 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 101.5 (C-1-GlcA), 96.3 (C-1-GlcN), 76.0, 75.2, 72.8, 71.3, 70.0, 68.9, 68.5, 59.4, 53.0, 46.0, 38.8, 38.5, 31.7, 27.4, 26.0, 24.8, 24.7, 21.3. ESI-MS: $C_{24}H_{43}N_3O_{13}$ $[M-H]^{-1}$ calcd: 580.2723, obsd: 580.2710.

2: $[\alpha]_D^{20}$= +24.6 (C =0.26, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.42 (d, *J* = 3.7 Hz, 1H, H-1-GlcN), 4.30 (d, *J* = 8.0 Hz, 1H, H-1-GlcA), 3.81 – 3.73 (m, 1H), 3.69 – 3.55 (m, 5H), 3.52 – 3.45 (m, 1H), 3.44 – 3.29 (m, 3H), 3.16 (d, *J* = 9.7 Hz, 2H), 3.05 (dd, *J* = 10.2, 3.7 Hz, 1H), 3.02 – 2.93 (m, 2H), 2.79 (t, *J* = 7.6 Hz, 2H), 2.19 – 5.07 (m, 2H), 1.76 – 1.65 (m, 2H), 1.46 (p, *J* = 7.4 Hz, 2H), 1.35 – 1.28 (m, 2H), 1.22 – 1.11 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 102.0 (C-1-GlcA), 97.4 (C-1-GlcN), 75.7, 75.0, 72.5, 71.7, 71.0, 69.2, 69.1, 59.8, 57.8, 48.7, 39.2, 38.9, 32.2, 27.9, 26.4, 25.2, 25.1. ESI-MS: $C_{22}H_{41}N_3O_{15}S$ $[M-H]^{-1}$ calcd: 618.2186, obsd: 618.2192.

3: $[\alpha]_D^{20}$= +6.7 (C =0.12, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.24 (d, *J* = 3.8 Hz, 1H, H-1-GlcN), 4.41 (d, *J* = 7.7 Hz, 1H, H-1-GlcA), 3.91 (t, *J* = 8.4 Hz, 1H), 3.74 – 3.69 (m, 3H), 3.64 – 3.61 (m, 3H), 3.56 (d, *J* = 9.7 Hz, 2H), 3.42 (d, *J* = 9.7 Hz, 2H), 3.32 (t, *J* = 9.6 Hz, 1H), 3.04 – 2.96 (m, 3H), 2.86 – 2.79 (m, 1H), 2.23 – 2.16 (m, 2H), 1.87 (s, 3H), 1.76 – 1.66 (m, 2H), 1.54 – 1.46 (m, 2H), 1.37 – 1.31 (m, 3H), 1.24 – 1.16 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 100.2 (C-1-GlcA), 97.0 (C-1-GlcN), 80.6, 76.7, 75.4, 69.6, 60.2, 53.8, 39.3, 32.2, 28.0, 26.7, 25.4, 21.9. ESI-MS: $C_{24}H_{43}N_3O_{16}S$ $[M-H]^{-1}$ calcd: 660.2291, obsd: 660.2272.

4: $[\alpha]_D^{20}$= -4.3 (C =0.14, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.53 – 5.46 (m, 1H, H-1-GlcN), 4.37 (d, *J* = 7.6 Hz, 1H, H-1-GlcA), 3.91 (d, *J* = 7.1 Hz, 1H), 3.81 – 3.74 (m, 1H), 3.73 – 3.57 (m, 5H), 3.51 – 3.44 (m, 1H), 3.42 – 3.32 (m, 2H), 3.31 – 3.24 (m, 1H), 3.05 – 2.93 (m, 3H), 2.83 – 2.77 (m, 2H), 2.24 – 2.12 (m, 2H), 1.73 – 1.64 (m, 2H), 1.52 – 1.44 (m, 2H), 1.36 – 1.29 (m, 2H), 1.24 – 1.14 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 100.6 (C-1-GlcA), 97.0 (C-1-GlcN), 80.9, 76.7, 75.1, 71.25, 69.6, 68.7, 60.3, 58.0, 39.3, 31.9, 28.1, 26.8, 25.5, 25.2. ESI-MS: $C_{22}H_{41}N_3O_{18}S_2$ $[M-H]^{-1}$ calcd: 698.1754, obsd: 698.1777.

5: $[\alpha]_D^{20}$= +20 (C =0.12, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.25 (d, *J* = 3.9 Hz, 1H, H-1-GlcN), 4.26 (d, *J* = 8.0 Hz, 1H, H-1-GlcA), 4.18 (d, *J* = 11.3 Hz, 1H), 4.00 (d, *J* = 11.0 Hz, 1H), 3.78 – 3.71 (m, 2H), 3.60 – 3.57 (m, 2H), 3.55 – 3.49 (m, 3H), 3.42 – 3.34 (m, 2H), 3.18 – 3.09 (m, 2H), 3.06 – 2.96 (m, 4H), 2.92 – 2.87 (m, 1H), 2.83 – 2.79 (m, 1H), 2.21 – 2.09 (m, 2H), 1.88 (s, 3H), 1.77 – 1.70 (m, 2H), 1.51 – 1.44 (m, 2H), 1.38 – 1.31 (m, 2H), 1.24 – 1.16 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 102.1 (C-1-GlcA), 97.0 (C-1-GlcN), 76.7, 73.4, 69.6, 68.6, 66.4, 65.7, 53.5, 39.3, 37.3, 32.5, 28.3, 28.0, 25.4, 21.9. ESI-MS: $C_{24}H_{43}N_3O_{16}S$ $[M-H]^{-1}$ calcd: 660.2291, obsd: 660.2282.

6: $[\alpha]_D^{20}$= +21.1 (C =0.09, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.48 (d, *J* = 3.8 Hz, 1H, H-1-GlcN), 4.28 (d, *J* = 8.0 Hz, 1H, H-1-GlcA), 4.17 (dd, *J* = 11.0, 2.7 Hz, 1H), 3.99 (dd, *J* = 11.1, 2.2 Hz, 1H), 3.72 – 3.59 (m, 5H), 3.54 – 3.49 (m, 2H), 3.44 – 3.37 (m, 2H), 3.18 – 3.12 (m, 1H), 3.09 (dd, *J* = 9.9, 4.0 Hz, 1H), 3.04 – 2.96 (m, 2H), 2.84 – 2.80 (m, 1H), 2.22 – 2.09 (m, 2H), 1.78 – 1.69 (m, 2H), 1.49 (p, *J* = 7.5 Hz, 2H), 1.34 (p, *J* = 7.0 Hz, 2H), 1.24 – 1.14 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 102.0 (C-1-GlcA), 97.1 (C-1-GlcN), 76.2, 72.7, 69.6, 69.4, 68.9, 66.3, 66.2, 57.7, 39.3, 39.1, 32.3, 27.9, 26.4, 25.3. ESI-MS: $C_{22}H_{41}N_3O_{18}S_2$ $[M-H]^{-1}$ calcd: 698.1754, obsd: 698.1732.

7: $[\alpha]_D^{20}$= +37.7 (C =0.13, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.23 (d, *J* = 3.9 Hz, 1H, H-1-GlcN), 4.41 (d, *J* = 7.9 Hz, 1H, H-1-GlcA), 4.18 (dd, *J* = 11.1, 2.5 Hz, 1H), 3.99 (dd, *J* = 11.0, 2.1 Hz, 1H), 3.94 – 3.89 (m, 1H), 3.75 – 3.51 (m, 8H), 3.47 – 3.38 (m, 2H), 3.06 – 2.95 (m, 2H), 2.86 – 2.78 (m, 2H), 2.24 – 2.15 (m, 2H), 1.86 (s, 3H), 1.75 – 1.66 (m, 2H), 1.52 – 1.46 (m, 2H), 1.36 – 1.32 (m, 2H), 1.23 – 1.17 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 100.4 (C-1-GlcA), 97.5 (C-1-GlcN), 80.8, 76.9, 75.6, 76.6, 70.8, 70.4, 69.8, 68.8, 66.6, 53.7, 39.5, 39.2, 32.4, 28.2, 26.9, 25.6, 25.3, 22.1. ESI-MS: $C_{24}H_{43}N_3O_{19}S_2$ $[M-H]^{-1}$ calcd: 740.1859, obsd: 740.1879.

8: $[\alpha]_D^{20}$= +6.7 (C =0.15, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.50 (d, *J* = 3.6 Hz, 1H, H-1-GlcN), 4.39 (d, *J* = 7.8 Hz, 1H, H-1-GlcA), 4.15 (dd, *J* = 11.0, 2.7 Hz, 1H), 4.00 – 3.88 (m, 2H), 3.78 (t, *J* = 8.9 Hz, 1H), 3.74 – 3.63 (m, 3H), 3.61 – 3.57 (m, 1H), 3.52 – 3.47 (m, 1H), 3.42 – 3.35 (m, 3H), 3.07 – 2.96 (m, 3H), 2.83 – 2.78 (m, 2H), 2.23 – 2.15 (m, 2H), 1.73 – 1.65 (m, 2H), 1.49 (p, *J* = 7.4 Hz, 2H), 1.36 – 1.30 (m, 2H), 1.23 – 1.16 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 100.5 (C-1-GlcA), 97.2 (C-1-GlcN), 80.2, 76.3, 75.0, 71.1, 69.8, 69.5, 66.9, 66.3, 57.9, 39.2, 37.6, 31.8, 28.6, 27.9, 26.0, 25.0, 24.4. ESI-MS: $C_{22}H_{41}N_3O_{21}S_3$ $[M-H]^{-1}$ calcd: 778.1322, obsd: 778.1338.

Procedures for microarray studies

Immobilization of disaccharides on the array slides.

All 16 disaccharides were dissolved in sodium phosphate buffer (pH 8.5, 50 mM) in concentrations of 1000 μM, 500 uM and 250 uM. The solution was spatially arrayed onto NHS-activated slides (Nexterion® Slide H from SCHOTT, Jena, Germany) under ~50% relative humidity at 20^{O}C. The robotic arrayer SX (from Scienion, Berlin, Germany) delivered 419 pL of the solution containing disaccharides to the array slide. The array spots had an average diameter of about 70 μm with a distance of 400 μm between the centers of adjacent spots. The slides were incubated overnight in a saturated $(NH_4)_2SO_4$ chamber (81% relative humidity). The slides were then washed with water to remove the unreacted oligosaccharides from the surface. The remaining *N*-hydroxysuccinimidyl groups were blocked by placing slides in a solution that contained 50 mM ethanolamine in PBST (137 mM NaCl, 13.2 mM Na_2HPO_4, 1.56 mM NaH_2PO_4, 2.68 mM KCl, 0.01% Tween 20) at 50°C for at least 1.5 h. Slides were rinsed several times with deionized water, and the residual liquid was dried by centrifugation.

Preparation of fluorescently labeled FGF-2

Fluorescently labeled FGF-2 was prepared by direct labeling with Alexa Fluor 488 NHS Ester (Thermo Scientific, Waltham, MA, USA). FGF-2 (100 μg) was mixed with three equivalents of heparin and 100 mM $NaHCO_3$. The mixture was allowed to equilibrate for 15 min at room temperature followed by the addition of 3–10 equivalents of Alexa Fluor 488 NHS Ester. The reaction was left to proceed at room temperature for 1 h. The reaction mixture was purified by heparin column and buffer exchanged [phosphate-buffered saline (PBS)] using a centrifugal filter to get Alexa Fluor 488 labeled FGF-2.

Hybridization of array slides with Alexa Fluor 488 labeled FGF-2.

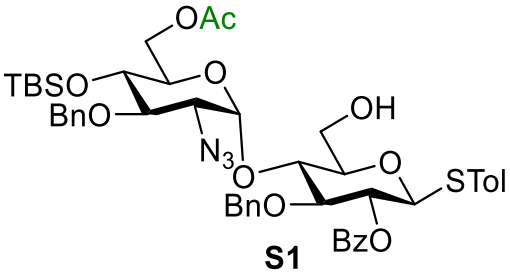
The hybridization solution contained 10 μg mL^{-1} of Alexa Fluor 488 labeled FGF-2 PBST (137mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, 0.05% Tween 20), 20 mM Tris (pH 7.5) and 10% bovine serum albumin (BSA). The solution was placed between array slide and cover slip and incubated for 1 h at room temperature in a saturated $(NH_4)_2SO_4$ chamber (81% relative humidity). The slide was then washed with 45 mL of PBST solution containing BSA (1%) and Tris (20 mM) for 30 min in a clean 50 mL conical tube. Slides were rinsed several times with deionized water, and the residual liquid was dried by centrifugation before analyzing the slide with the array scanner.

Array data analysis

The array slides were scanned by a GenePix 4400 scanner (Molecular Dynamics). Scanning wavelengths were 488 nm. Resolution was set at 5 μm. The array images were analyzed by GenePix Pro 7.2.29.002 software. Spots were automatically found, and spot deviations were manually fit to correct. Mean median fluorescence intensities of arrays were obtained by Array Quality Control of software. Some thresholds were listed as follows: median signal-to-background, >10; mean of median background, < 500; median signal-to-noise, > 10; The intensity data are the mean value ± S.D. of 36 individual spots.

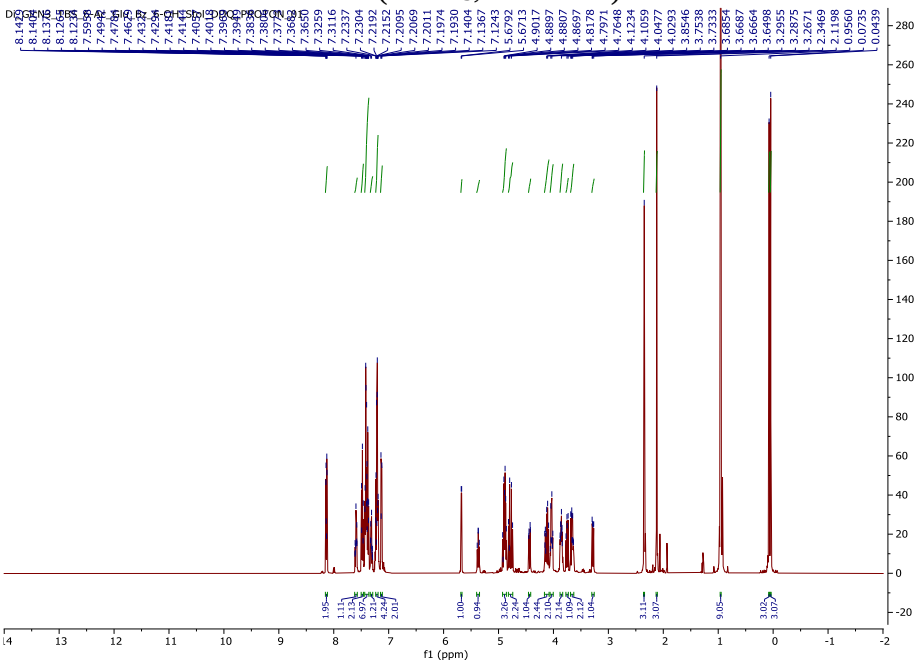
References:

1. Doneanu, C. E.; Chen, W.; Gebler, J. C., Analysis of oligosaccharides derived from heparin by ion-pair reversed-phase chromatography/mass spectrometry. *Anal. Chem.* **2009,** *81*, 3485-3499.
2. Bock, K.; Pedersen, C., A Study of ^{13}CH Coupling Constants in Hexopyranoses. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293-297.
3. Wang, Z.; Xu, Y.; Yang, B.; Tiruchinapally, G.; Sun, B.; Liu, R.; Dulaney, S.; Liu, J.; Huang, X., Preactivation-based One-pot Combinatorial Synthesis of Heparin-like Hexasaccharides for the Analysis of Heparin-protein Interactions. *Chem. Eur. J.* **2010,** *16*, 8365-8375.

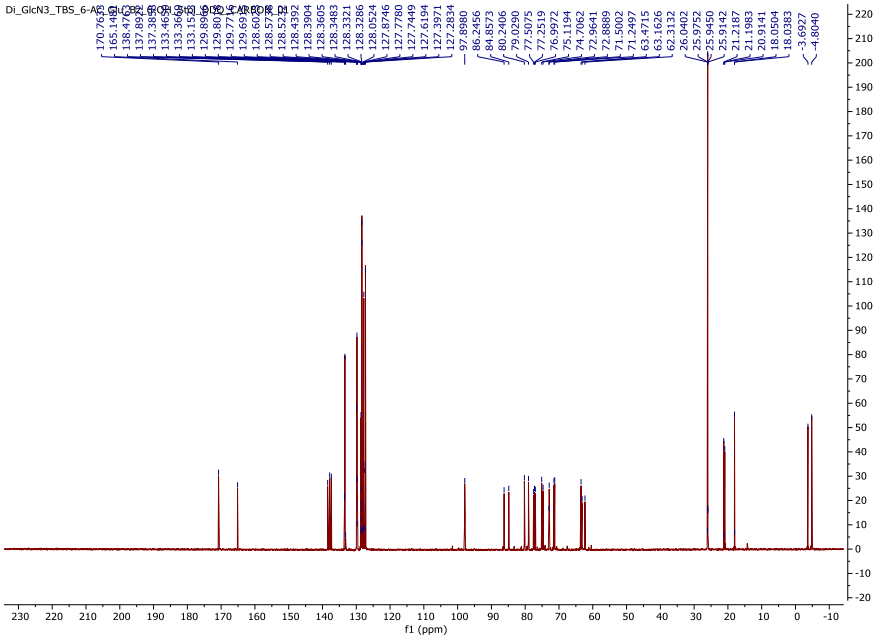


S1

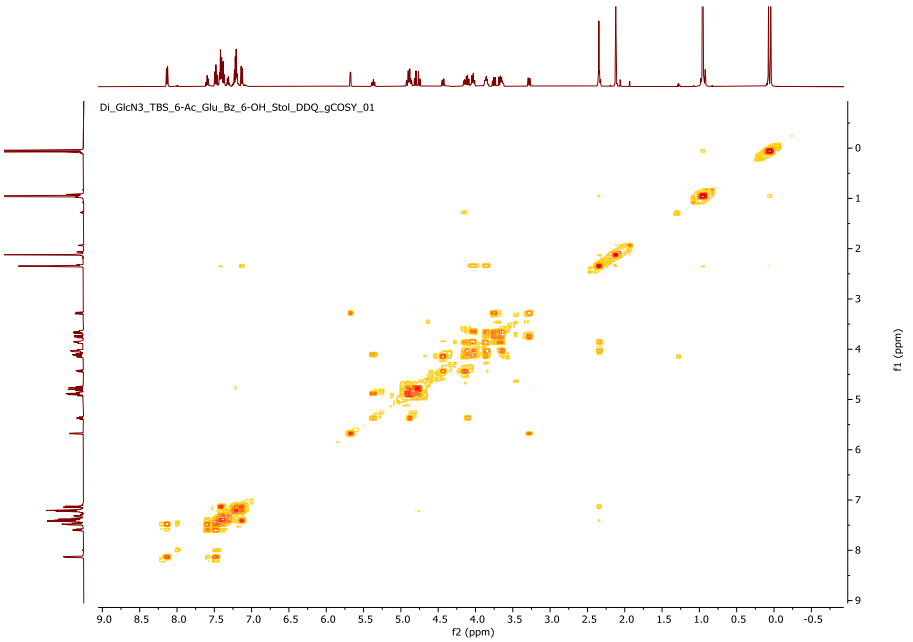
^{1}H-NMR ($CDCl_3$, 500 MHz) of **S1**



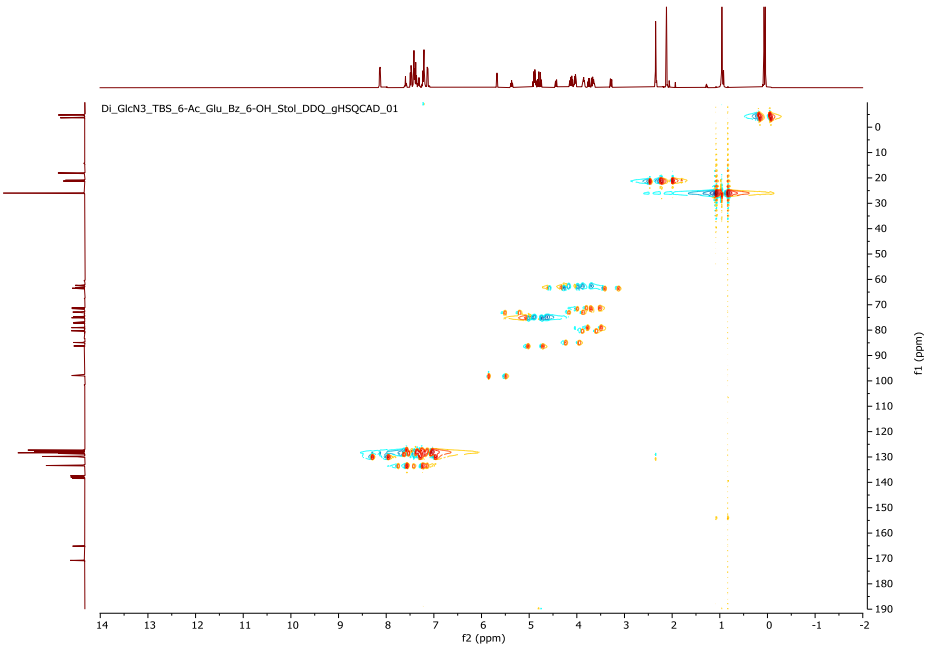
^{13}C-NMR ($CDCl_3$, 126 MHz) of **S1**

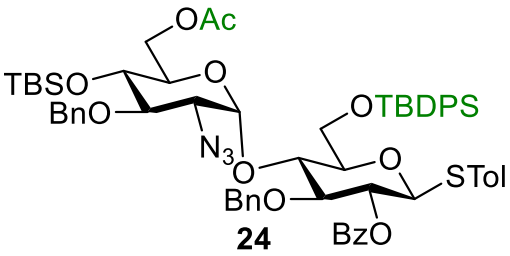


gCOSY ($CDCl_3$, 500 MHz) of **S1**



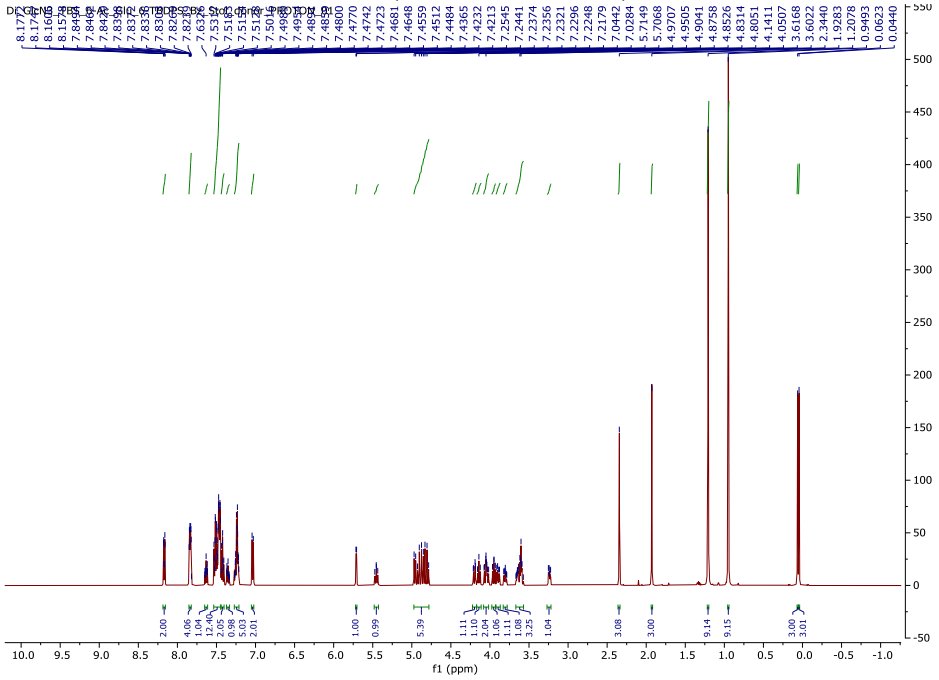
Coupled-gHSQC ($CDCl_3$, 500 MHz) of **S1**



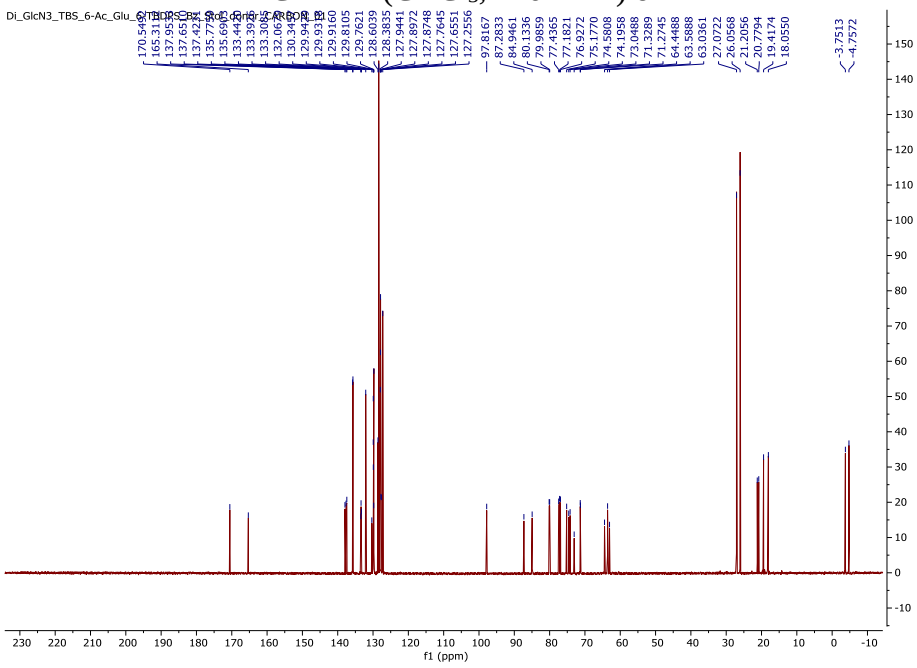


24

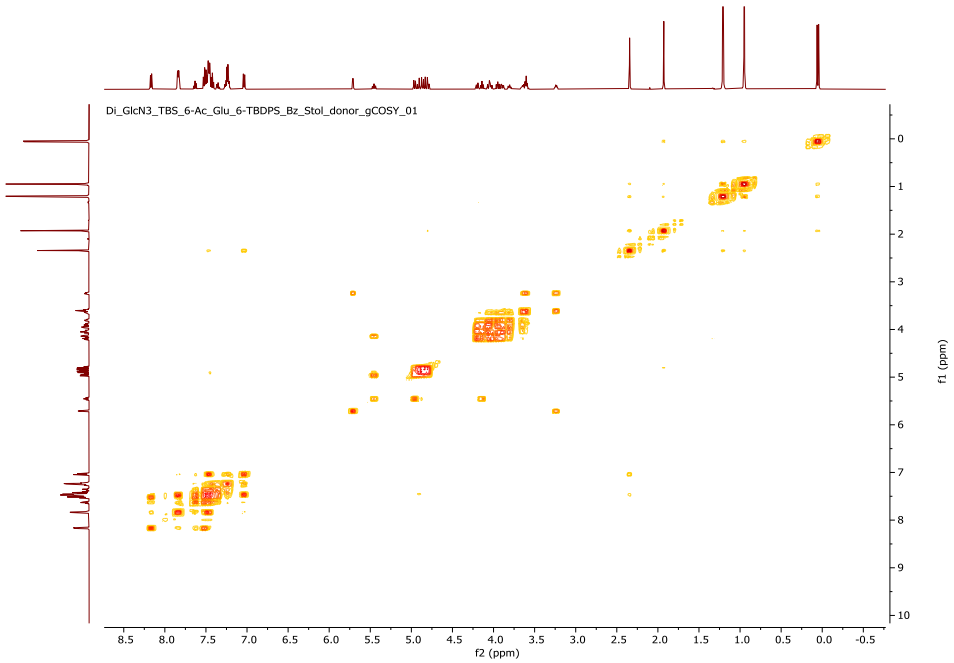
^{1}H-NMR ($CDCl_3$, 500 MHz) of **24**

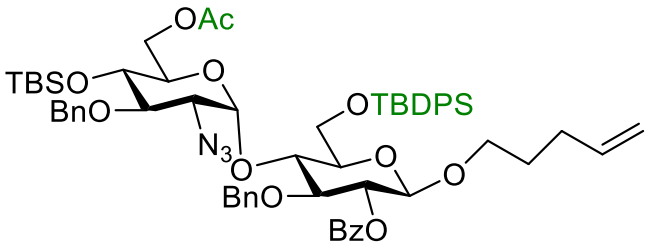


^{13}C-NMR ($CDCl_3$, 126 MHz) of **24**



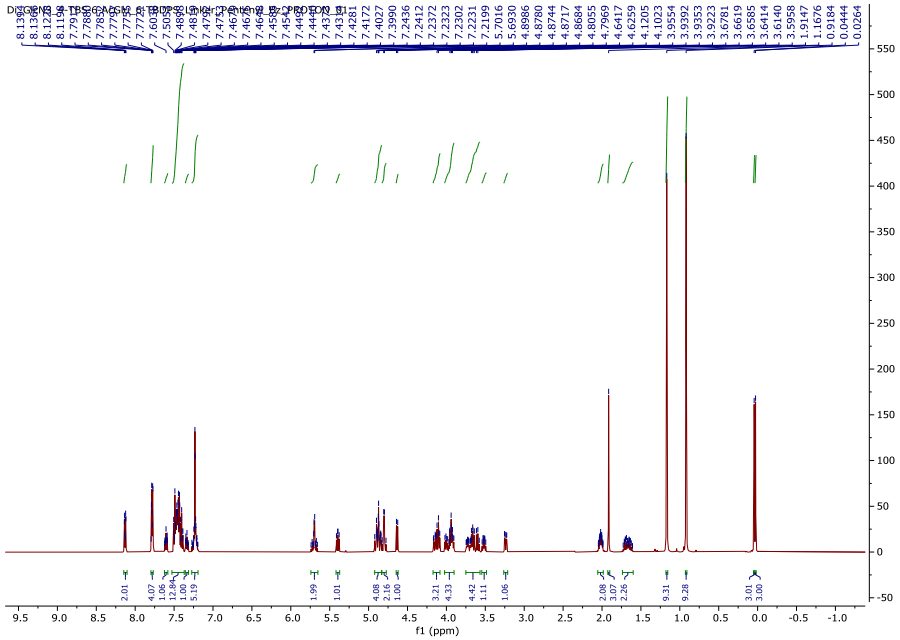
gCOSY ($CDCl_3$, 500 MHz) of **24**



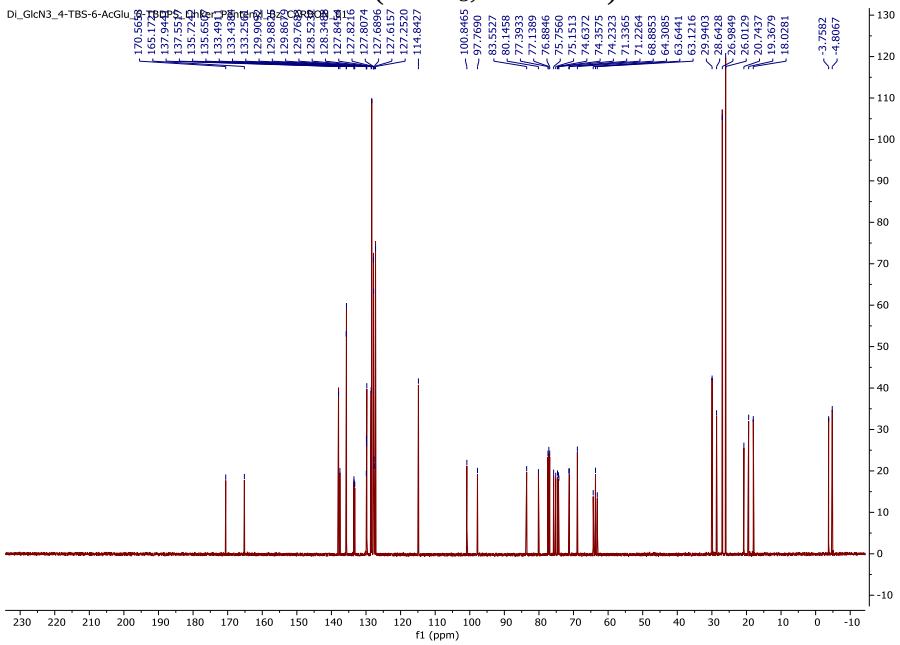


25

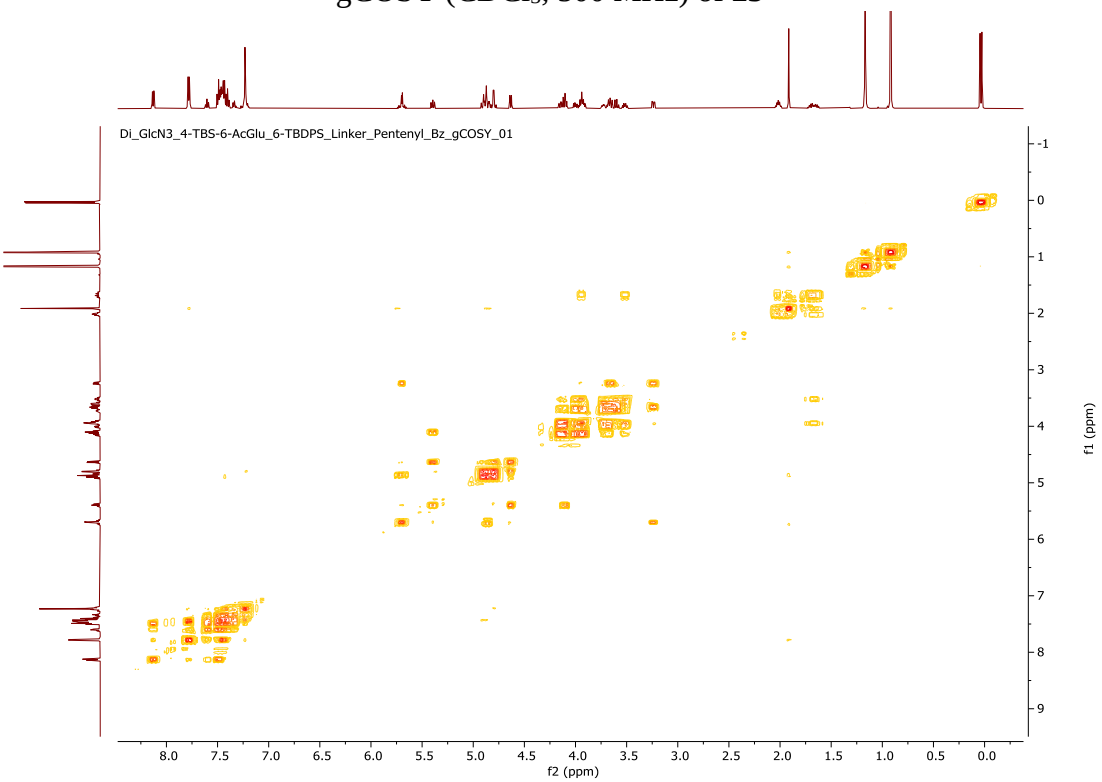
^{1}H-NMR ($CDCl_3$, 500 MHz) of **25**



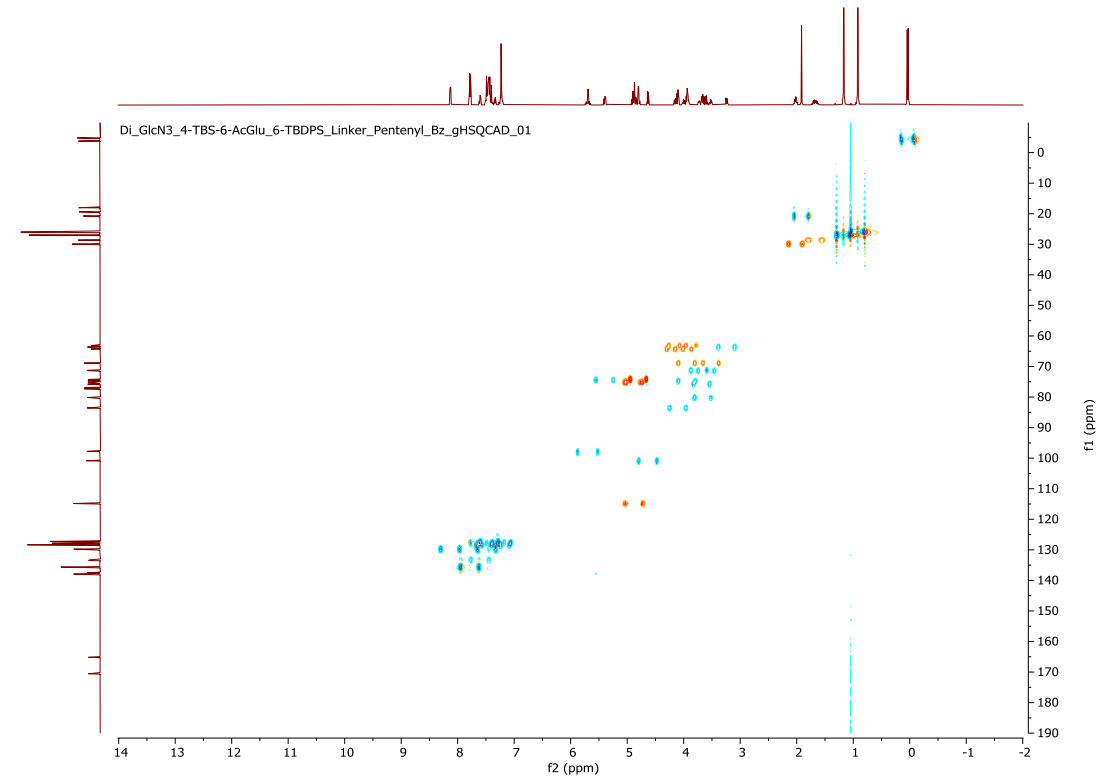
^{13}C-NMR ($CDCl_3$, 126 MHz) of **25**

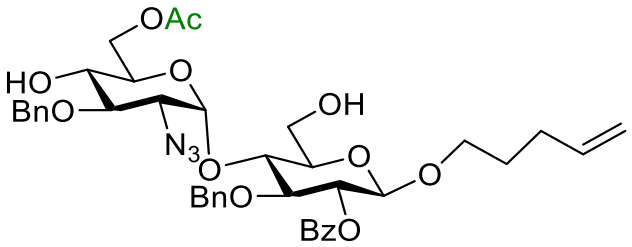


gCOSY ($CDCl_3$, 500 MHz) of **25**



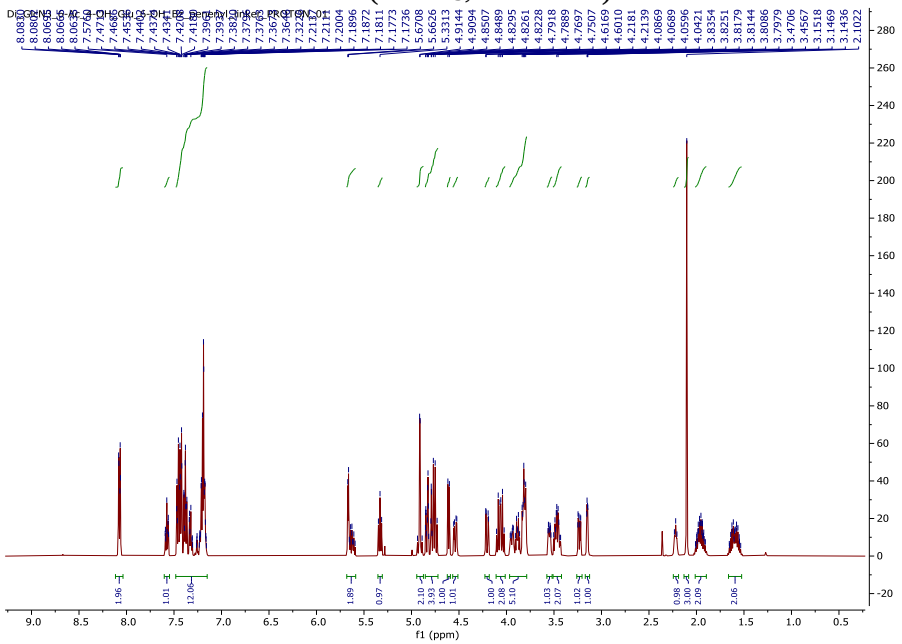
Coupled-gHSQC ($CDCl_3$, 500 MHz) of **25**



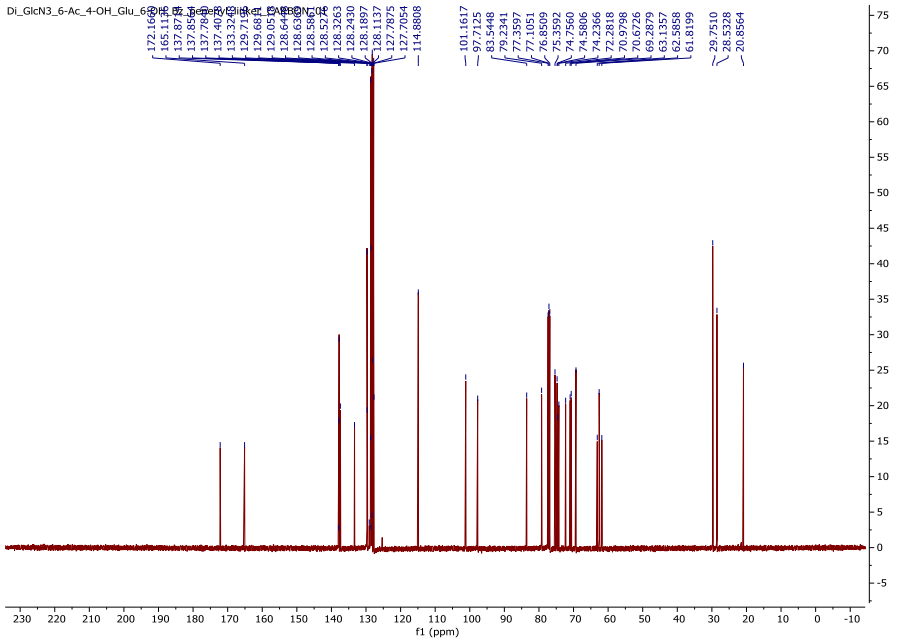


S2

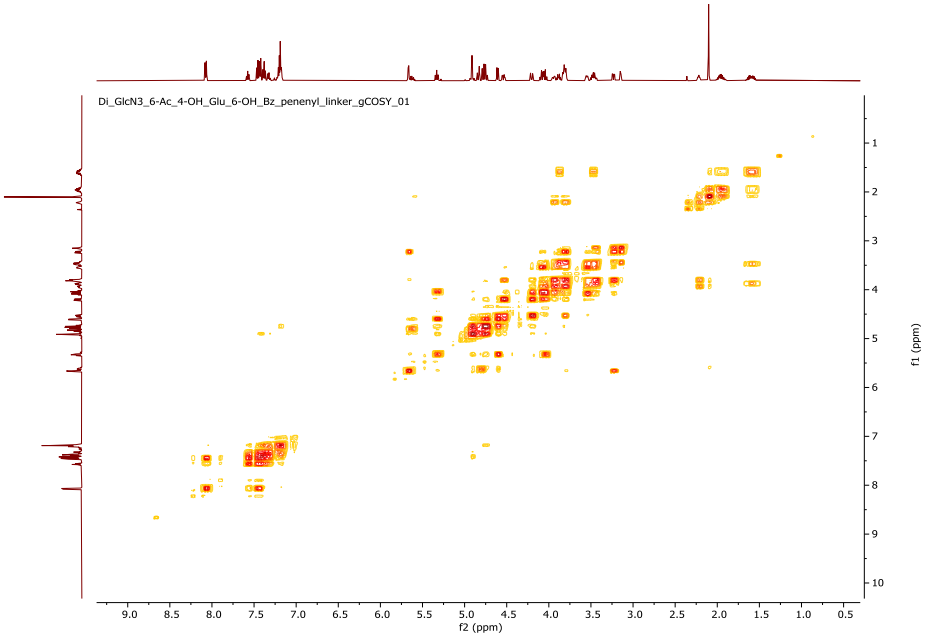
^{1}H-NMR ($CDCl_3$, 500 MHz) of **S2**

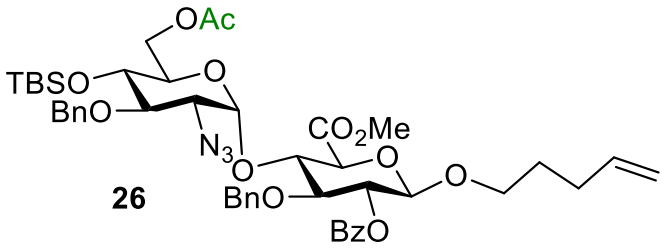


^{13}C-NMR ($CDCl_3$, 126 MHz) of **S2**



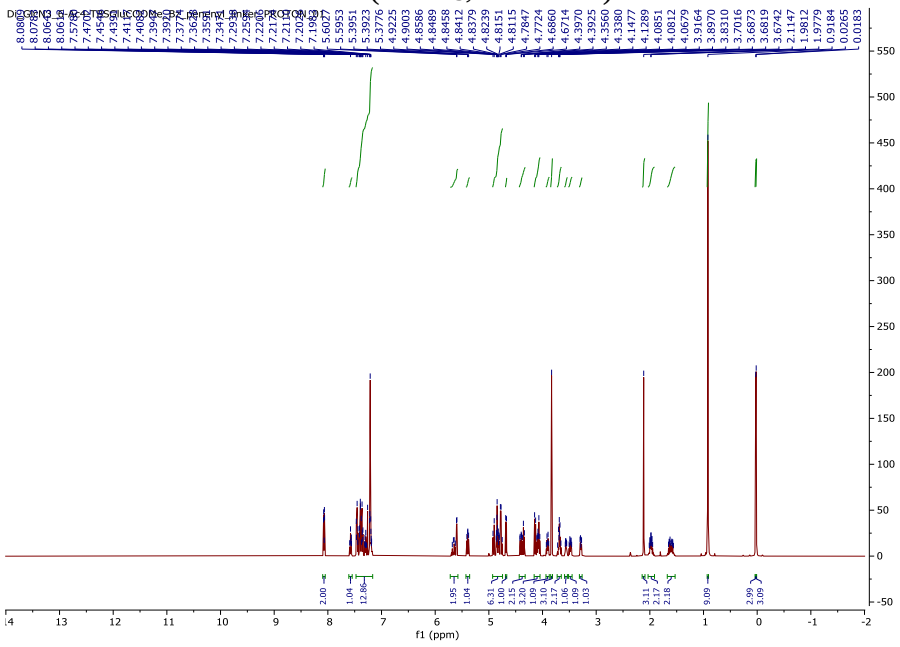
gCOSY ($CDCl_3$, 500 MHz) of **S2**



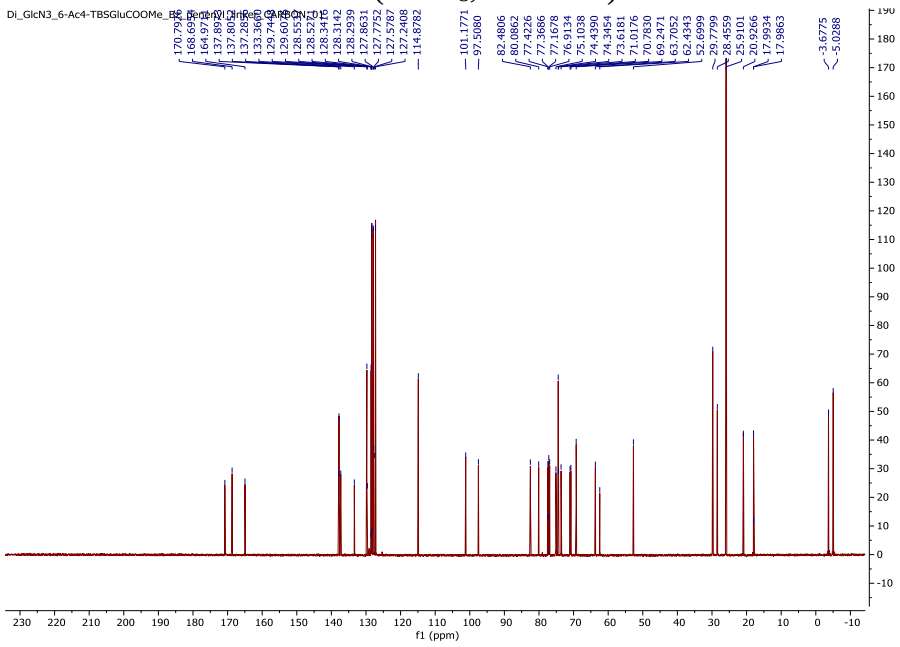


26

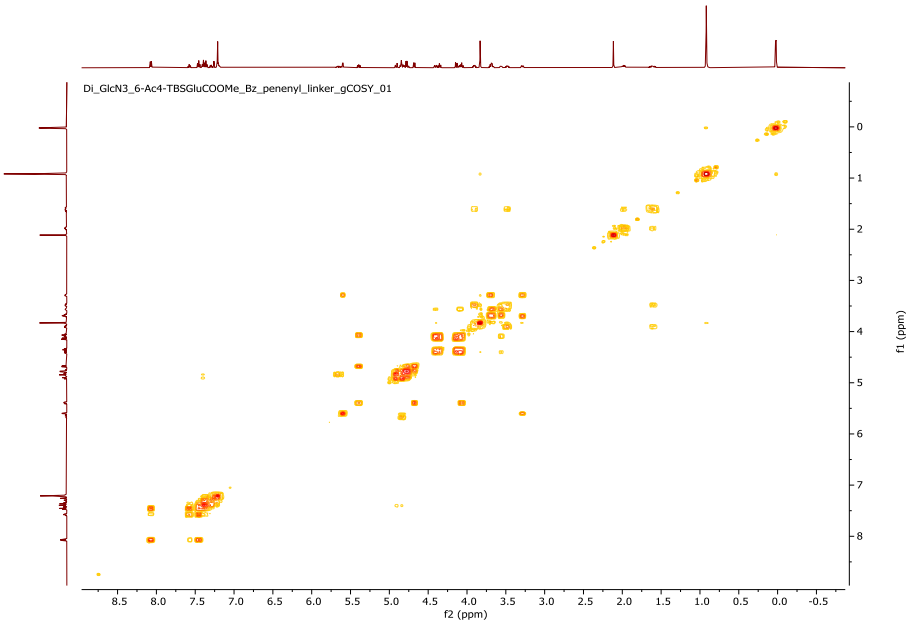
^{1}H-NMR ($CDCl_3$, 500 MHz) of **26**

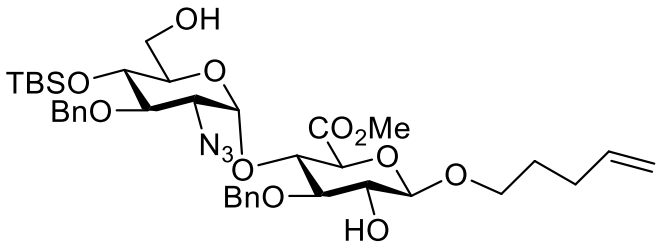


^{13}C-NMR ($CDCl_3$, 126 MHz) of **26**



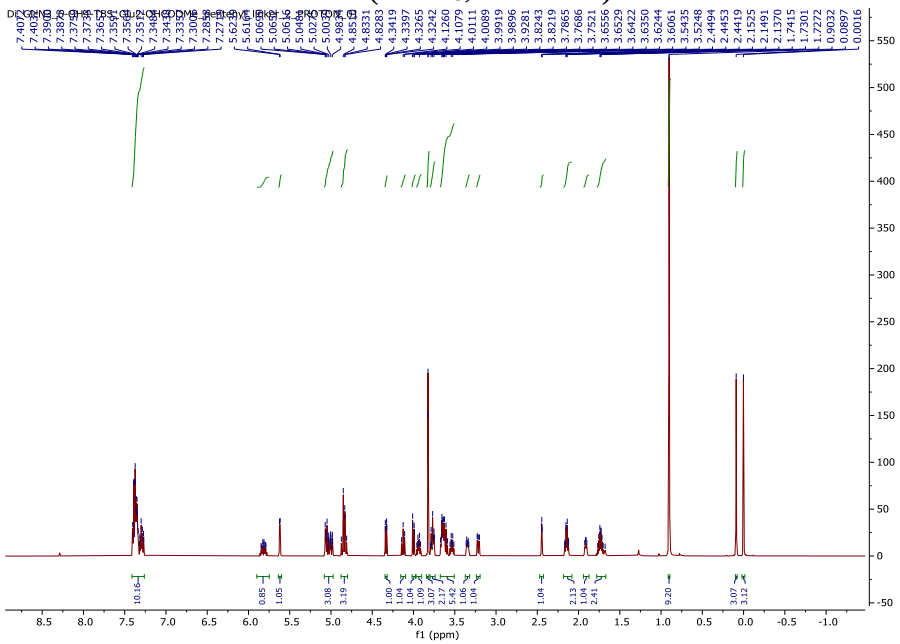
gCOSY ($CDCl_3$, 500 MHz) of **26**



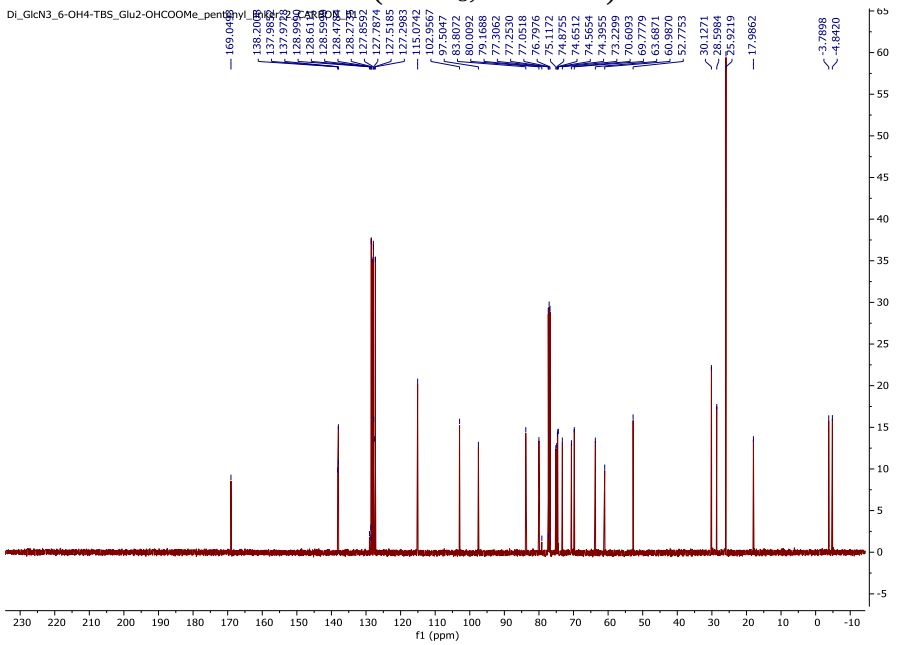


S4

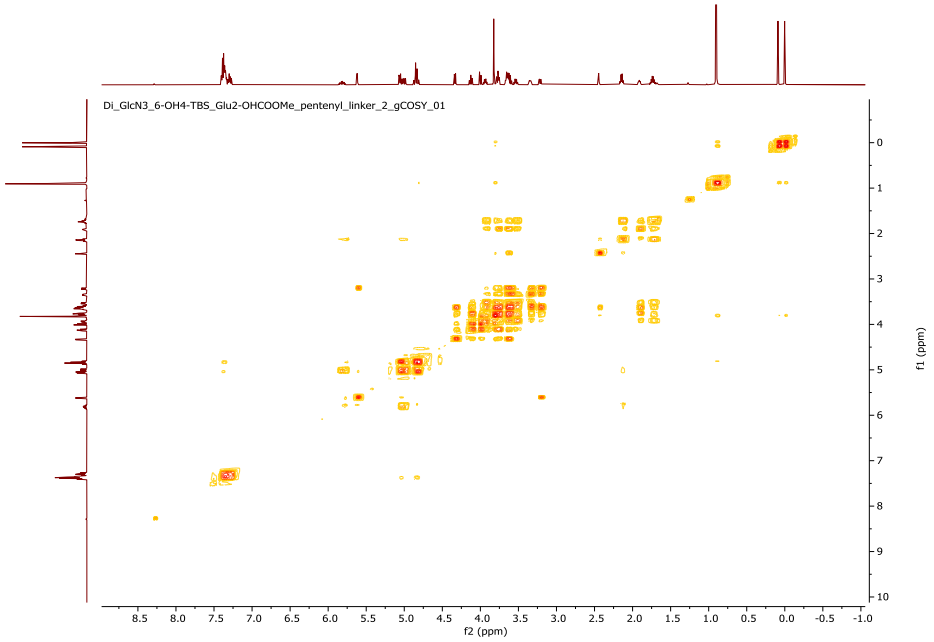
^{1}H-NMR ($CDCl_3$, 500 MHz) of **S4**

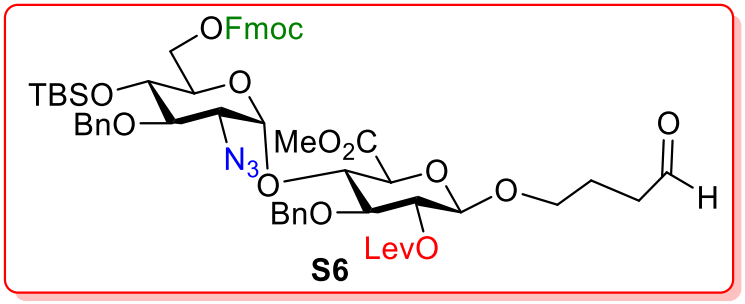


^{13}C-NMR ($CDCl_3$, 126 MHz) of **S4**



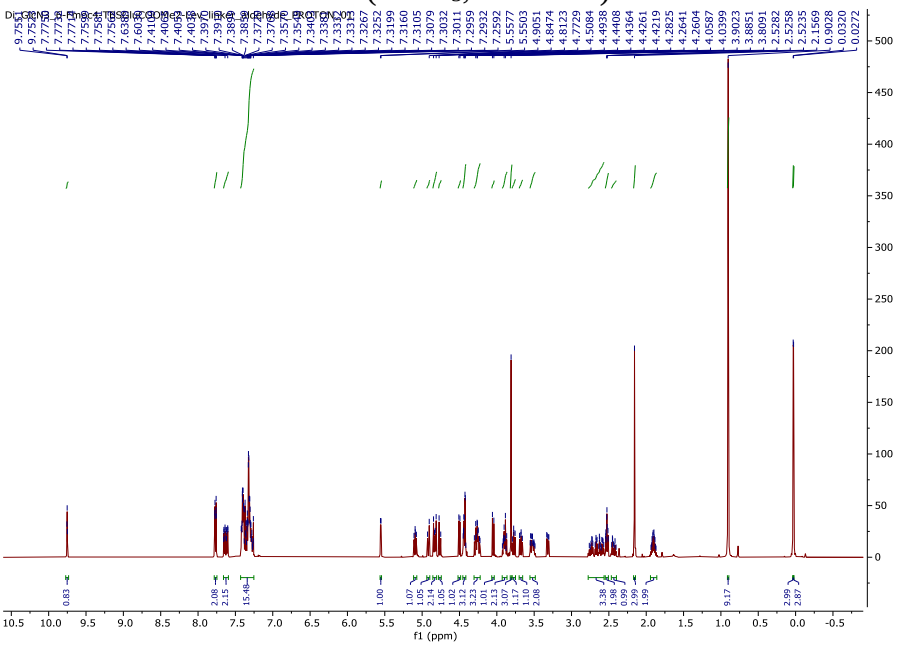
gCOSY ($CDCl_3$, 500 MHz) of **S4**



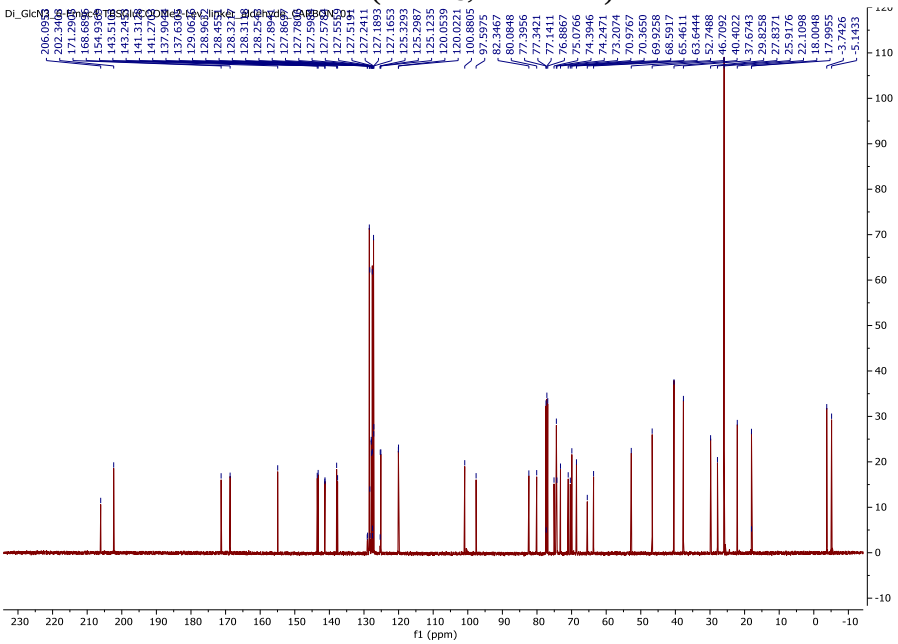


S6

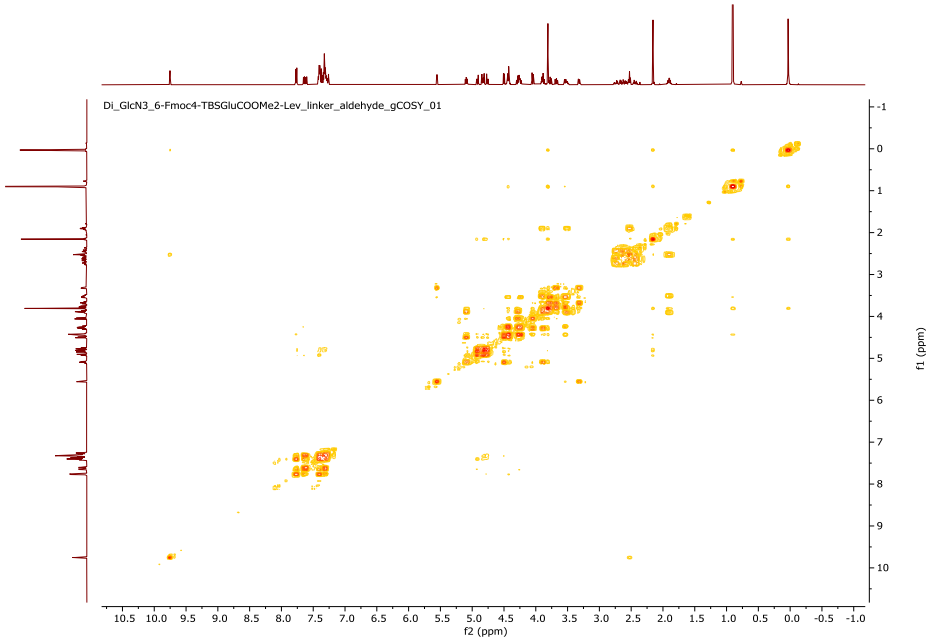
^{1}H-NMR ($CDCl_3$, 500 MHz) of **S6**

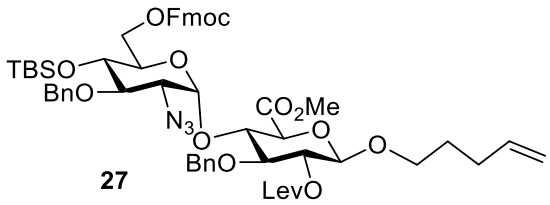


^{13}C-NMR ($CDCl_3$, 126 MHz) of **S6**



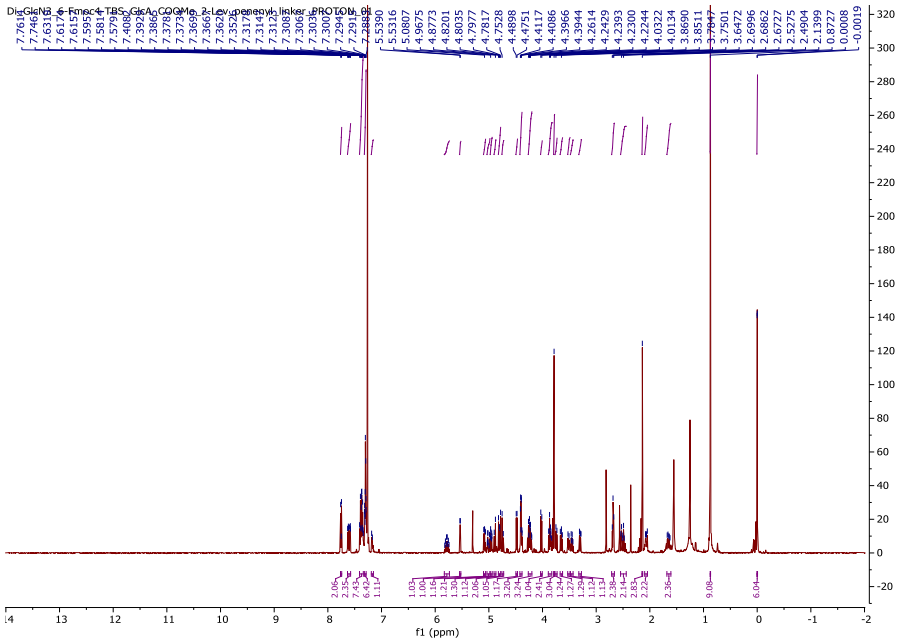
gCOSY ($CDCl_3$, 500 MHz) of **S6**



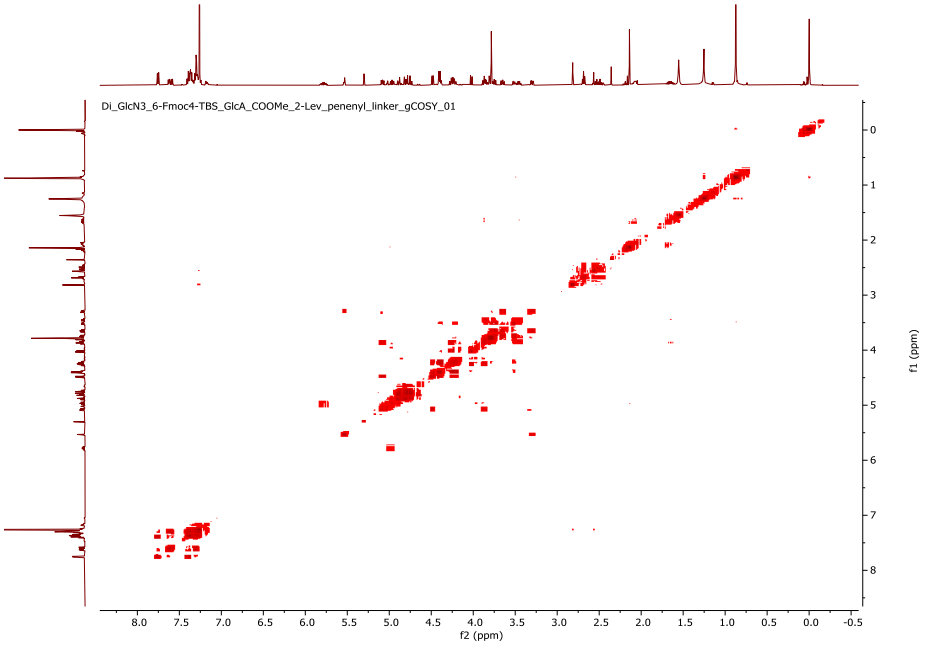


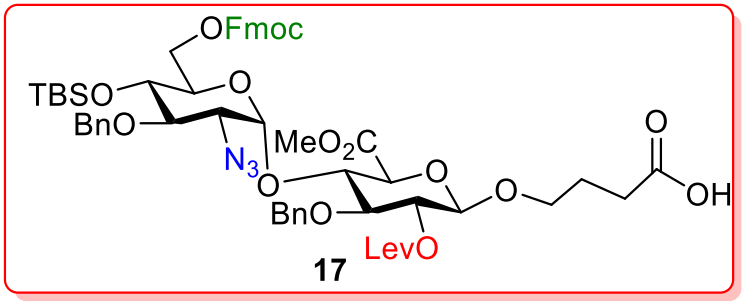
27

^{1}H-NMR ($CDCl_3$, 500 MHz) of **27**



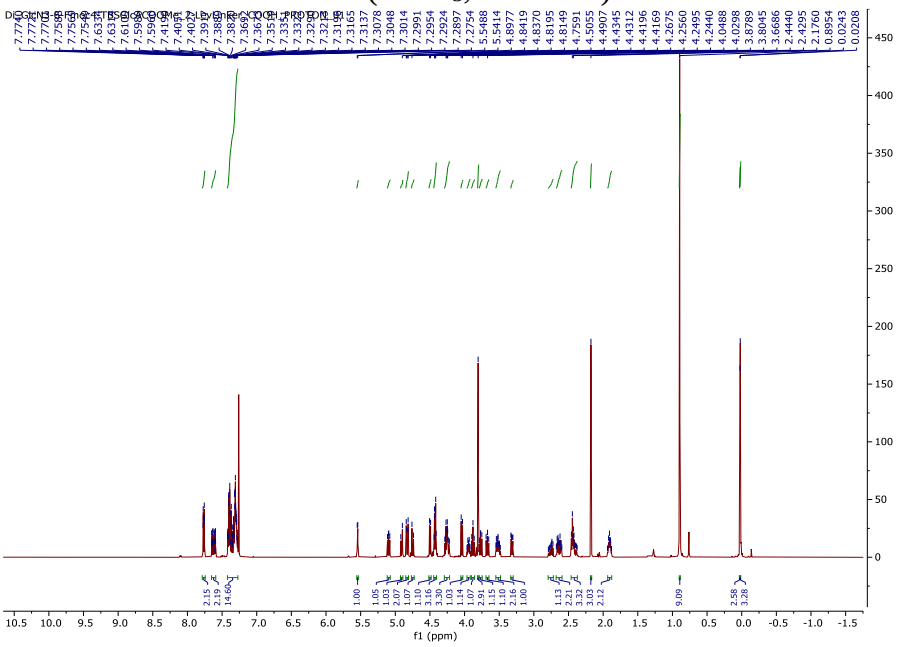
gCOSY ($CDCl_3$, 500 MHz) of **27**



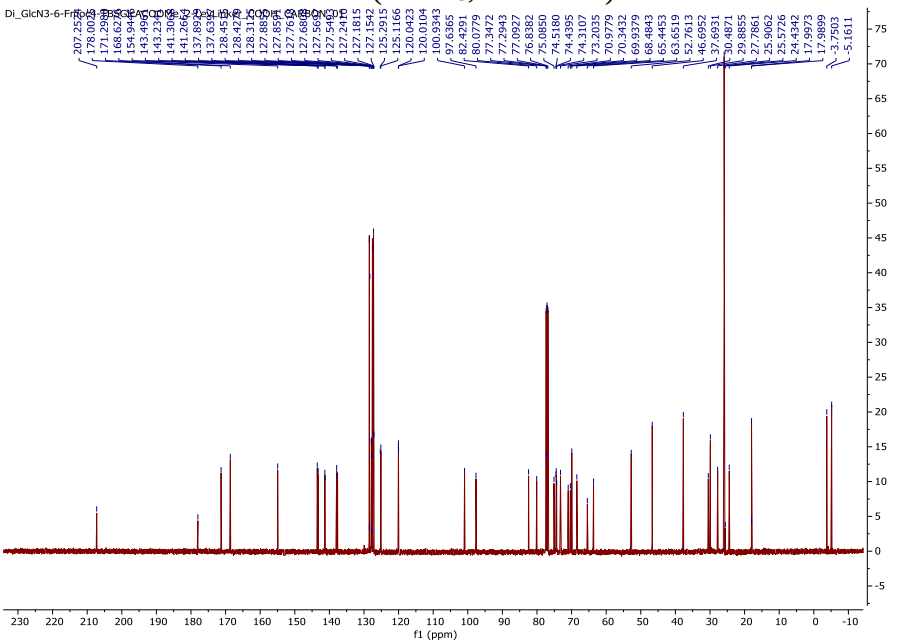


17

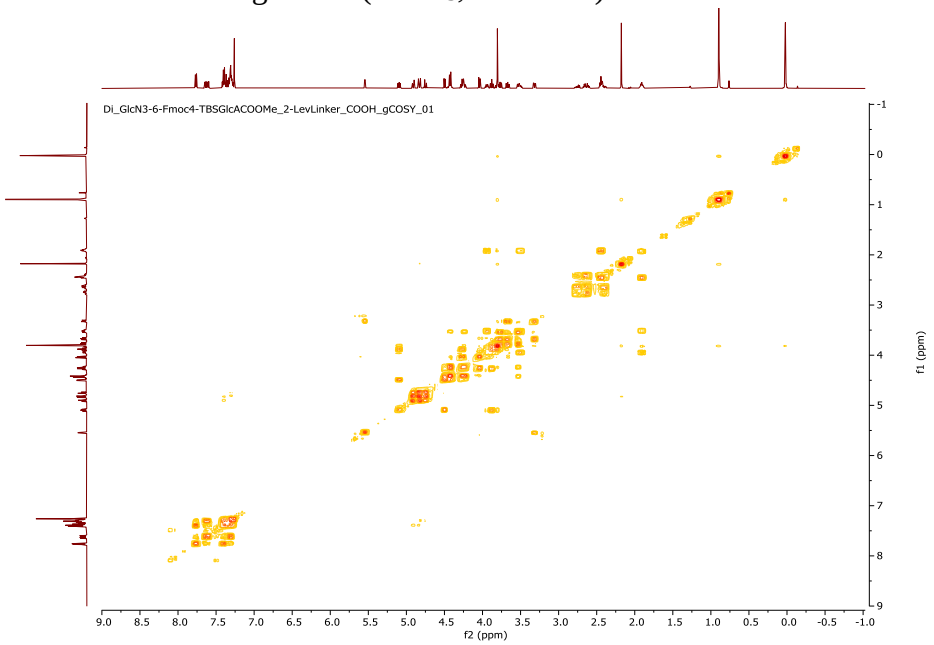
^{1}H-NMR ($CDCl_3$, 500 MHz) of **17**



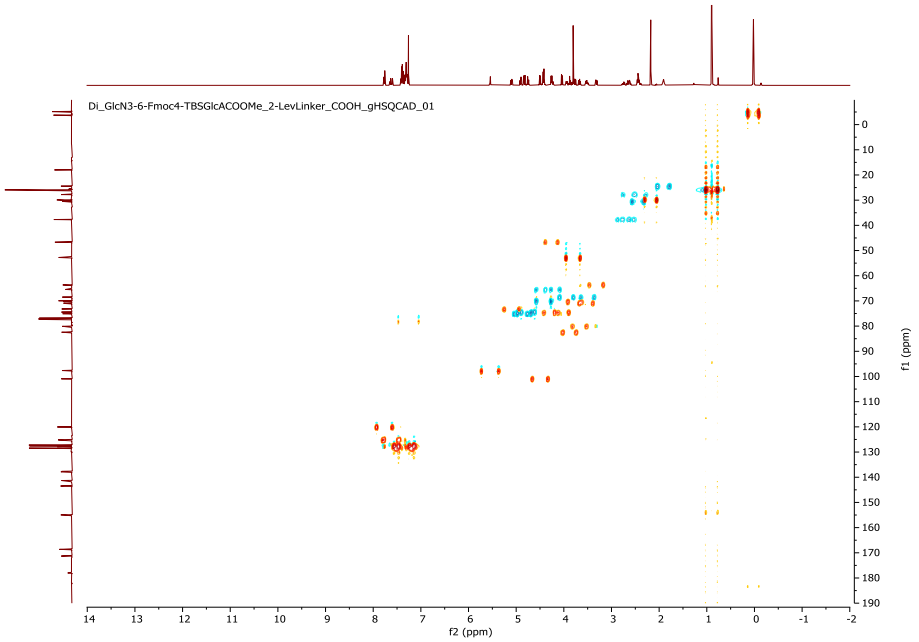
^{13}C-NMR ($CDCl_3$, 126 MHz) of **17**

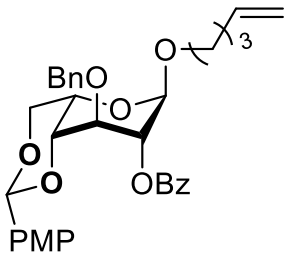


gCOSY ($CDCl_3$, 500 MHz) of **17**



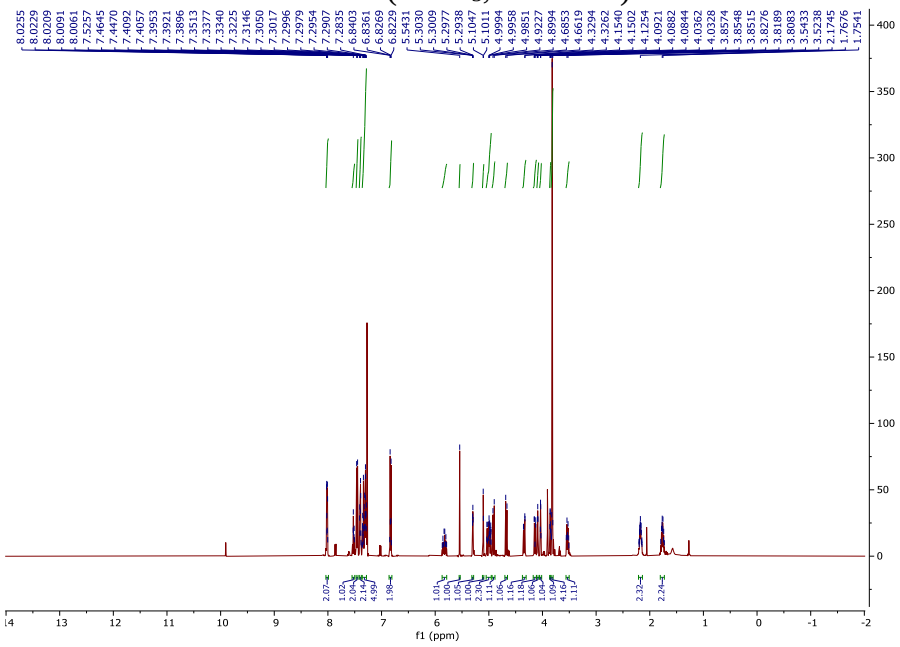
Coupled-gHSQC ($CDCl_3$, 500 MHz) of **17**



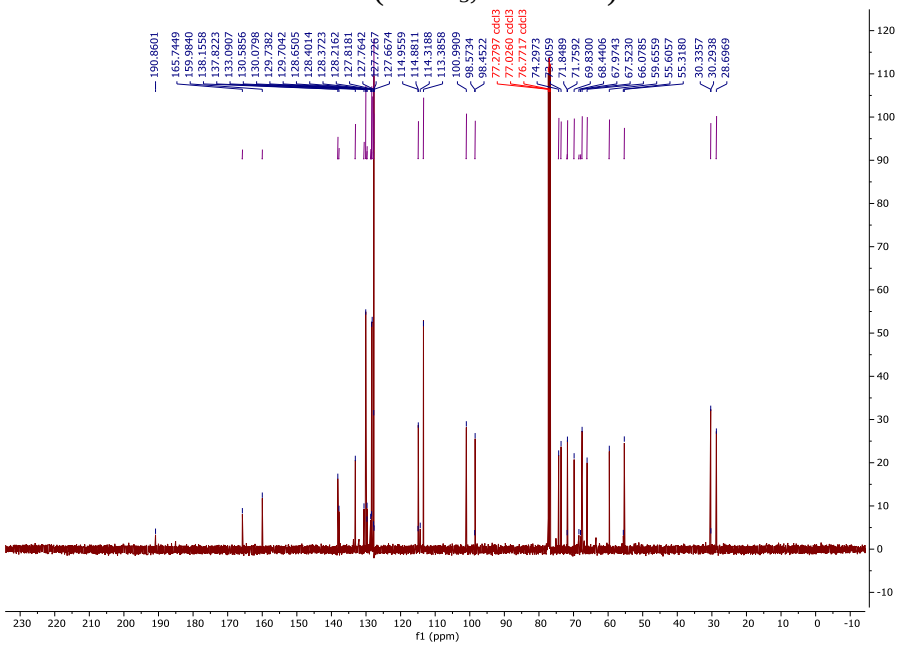


29

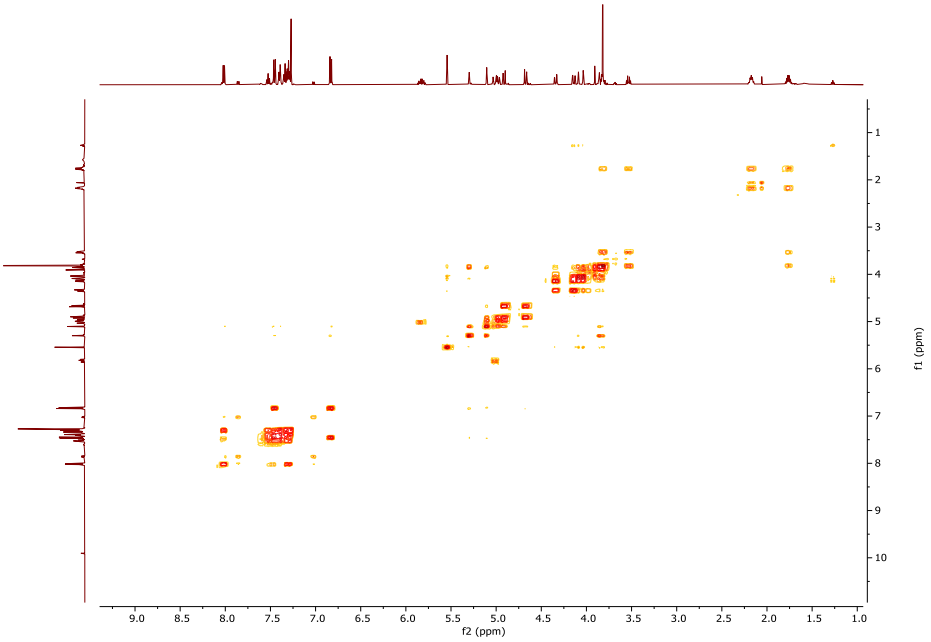
^{1}H-NMR ($CDCl_3$, 500 MHz) **29**



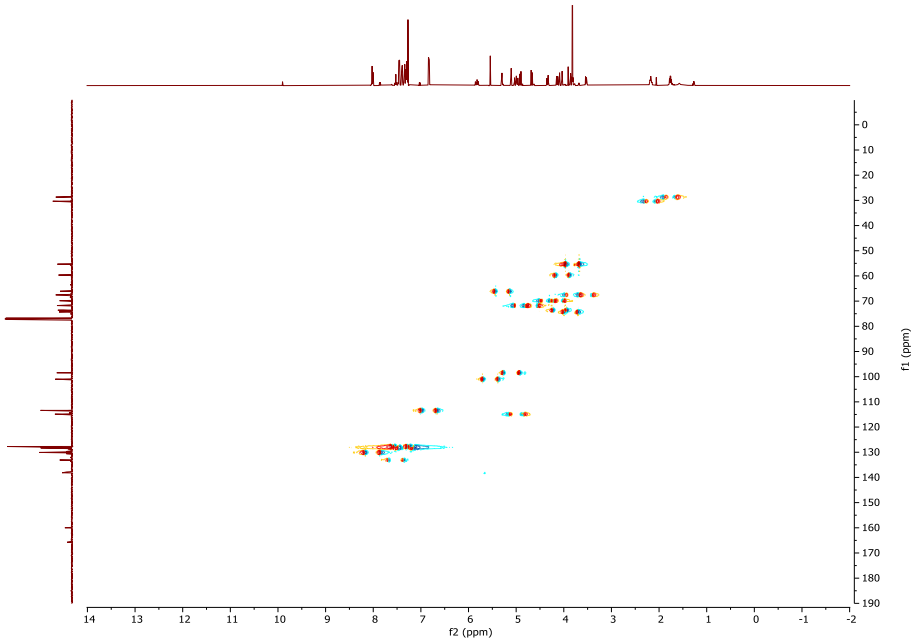
^{13}C-NMR ($CDCl_3$, 126 MHz) of **29**

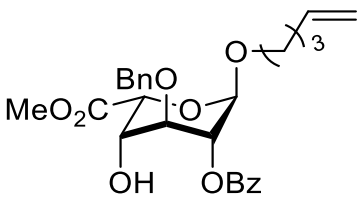


gCOSY ($CDCl_3$, 500 MHz) of **29**



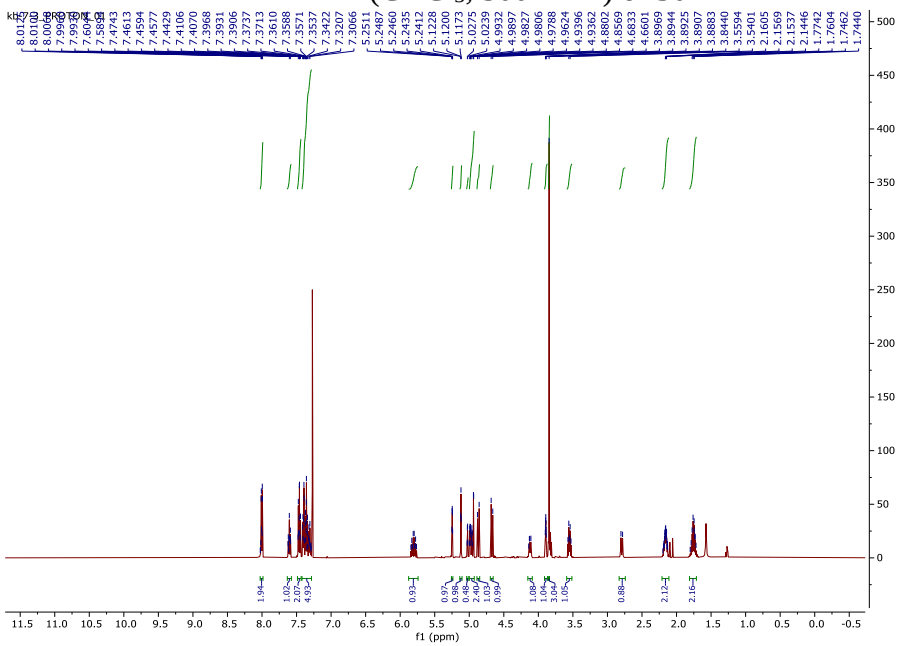
Coupled-gHSQC ($CDCl_3$, 500 MHz) of **29**



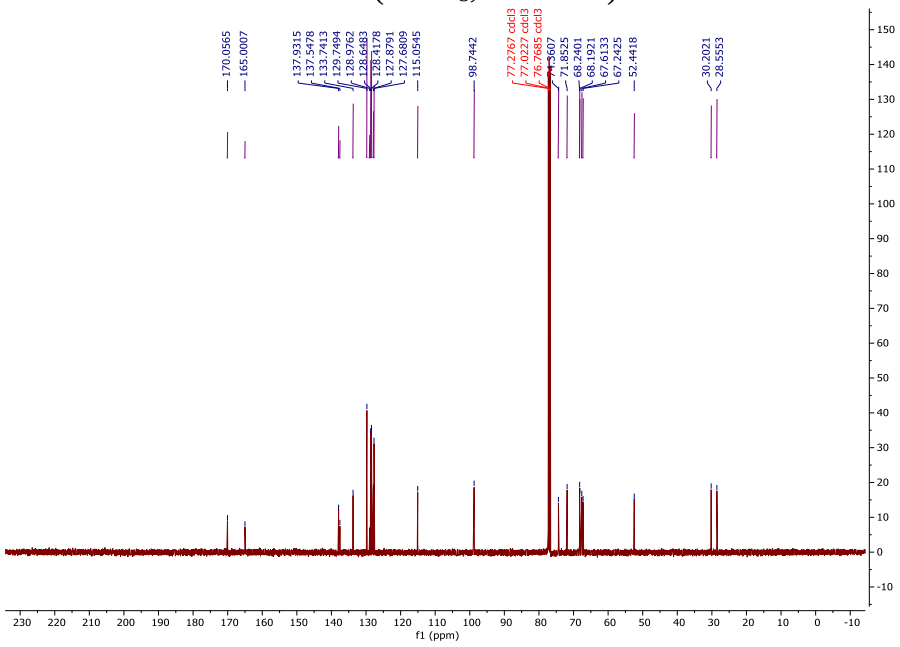


30

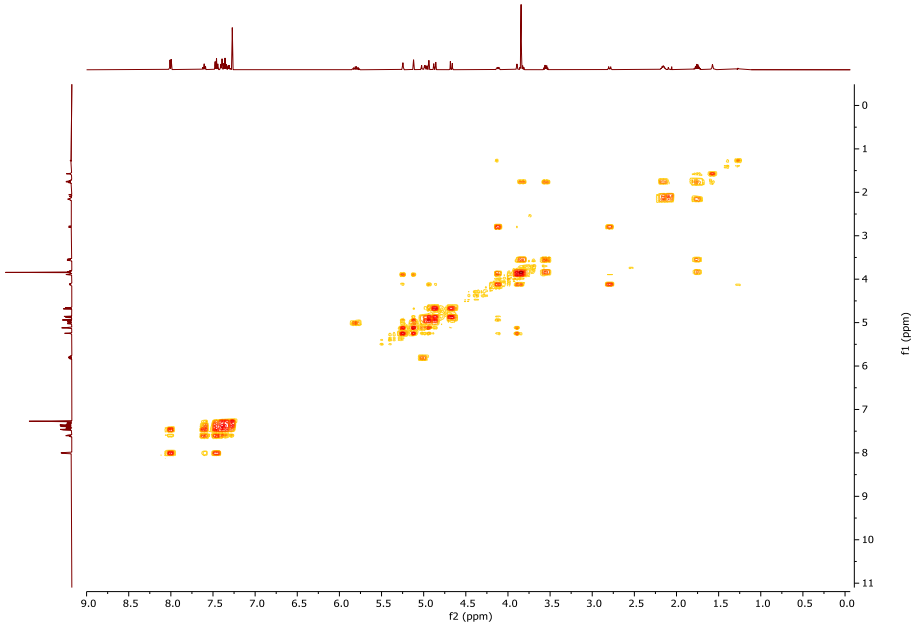
^{1}H-NMR ($CDCl_3$, 500 MHz) of **30**



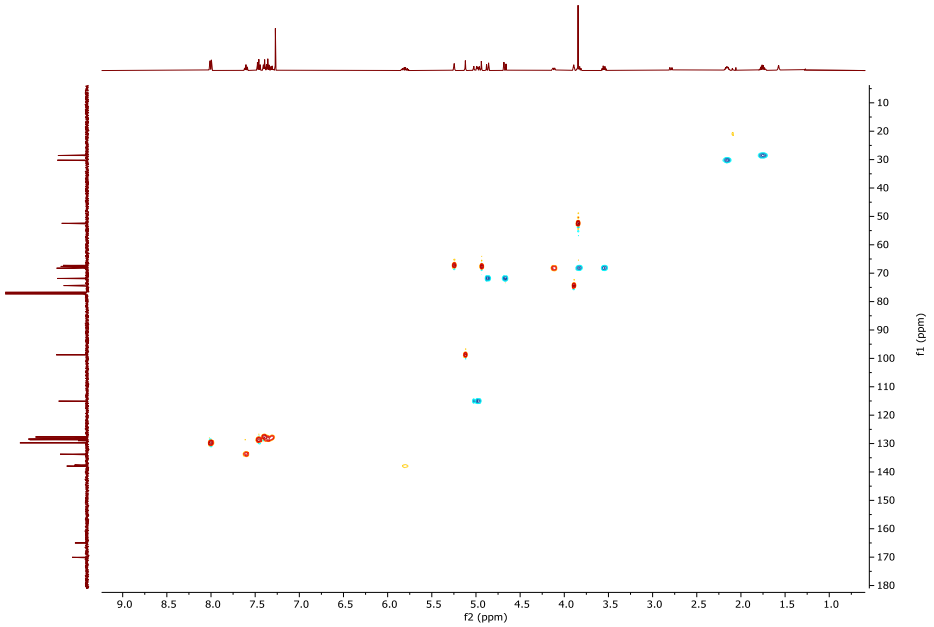
^{13}C-NMR ($CDCl_3$, 126 MHz) of **30**

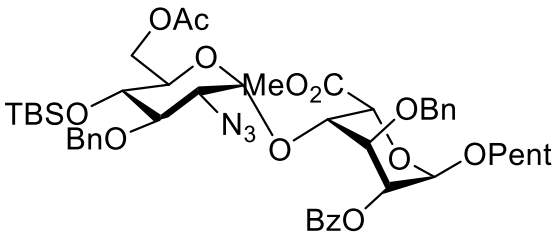


gCOSY ($CDCl_3$, 500 MHz) of **30**



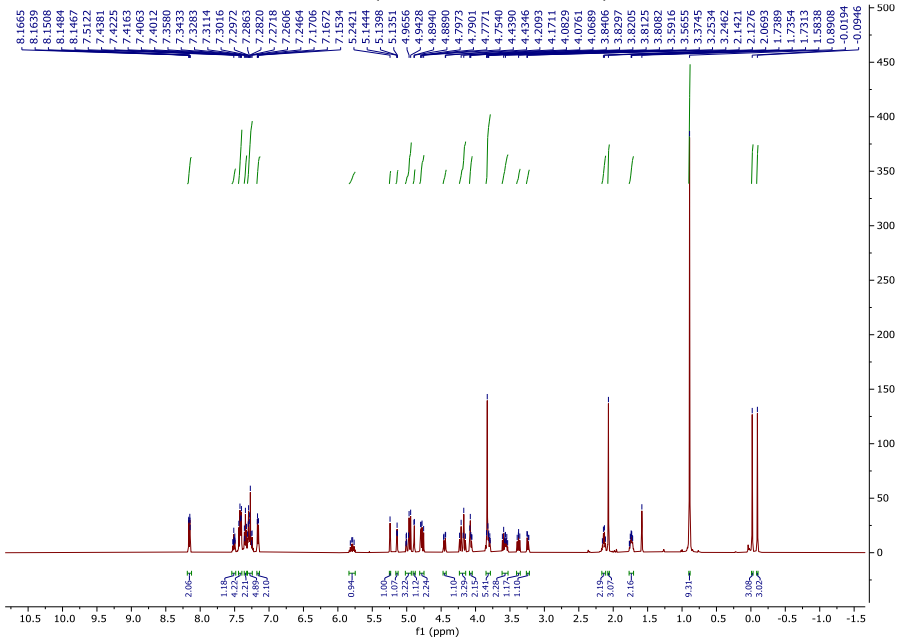
gHSQC ($CDCl_3$, 500 MHz) of **30**



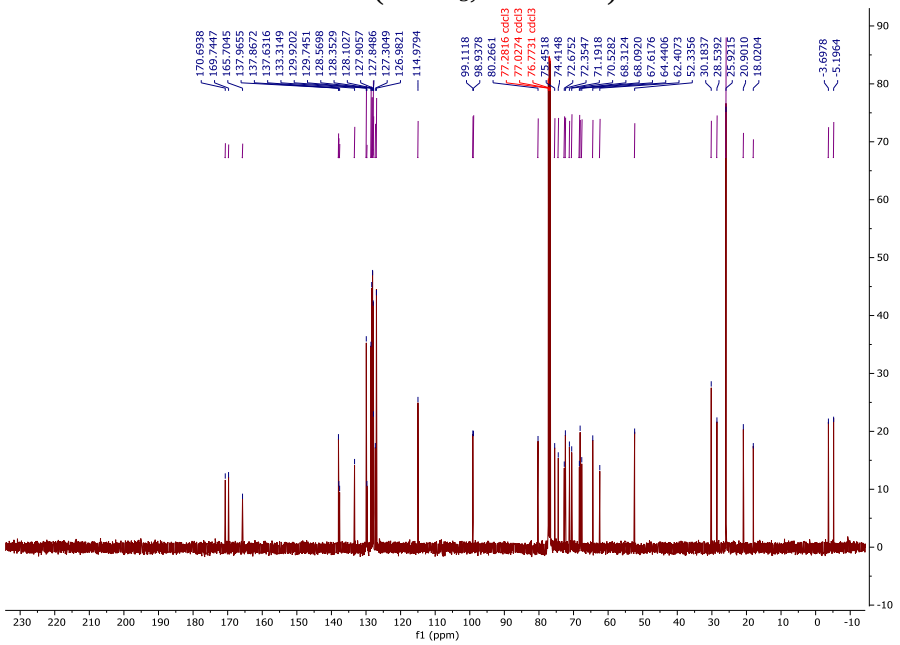


31

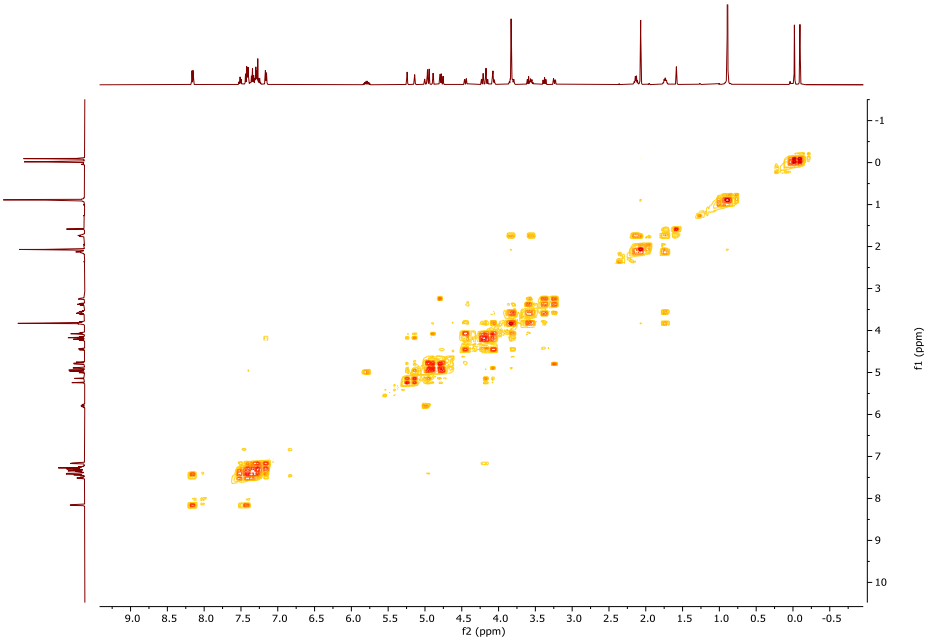
^{1}H-NMR ($CDCl_3$, 500 MHz) of **31**



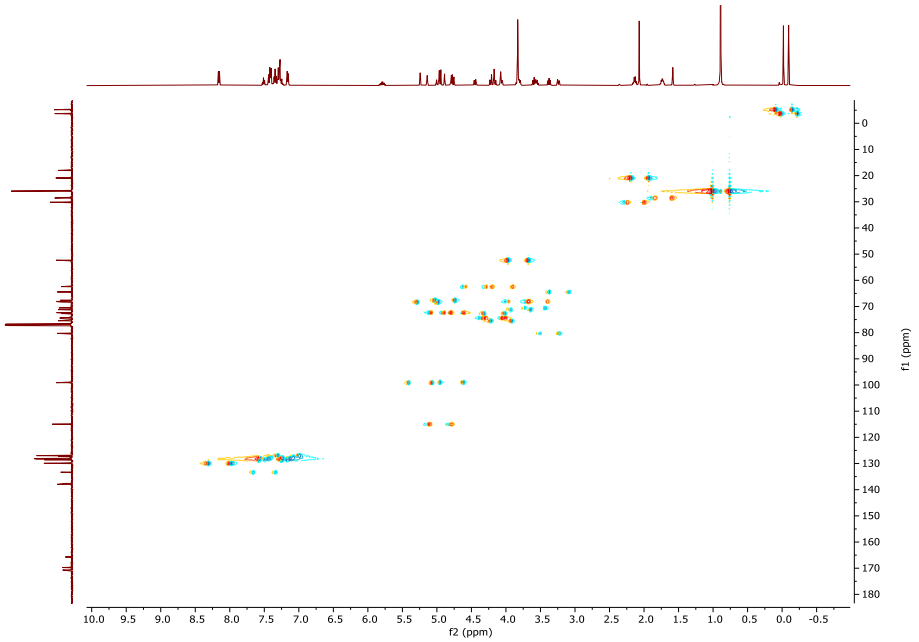
^{13}C-NMR ($CDCl_3$, 126 MHz) of **31**

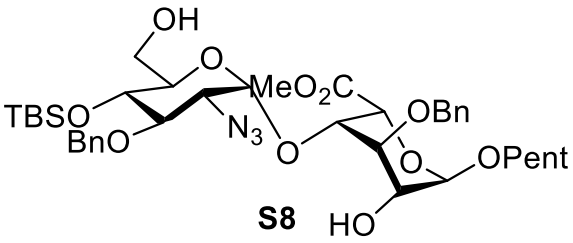


gCOSY ($CDCl_3$, 500 MHz) of **31**



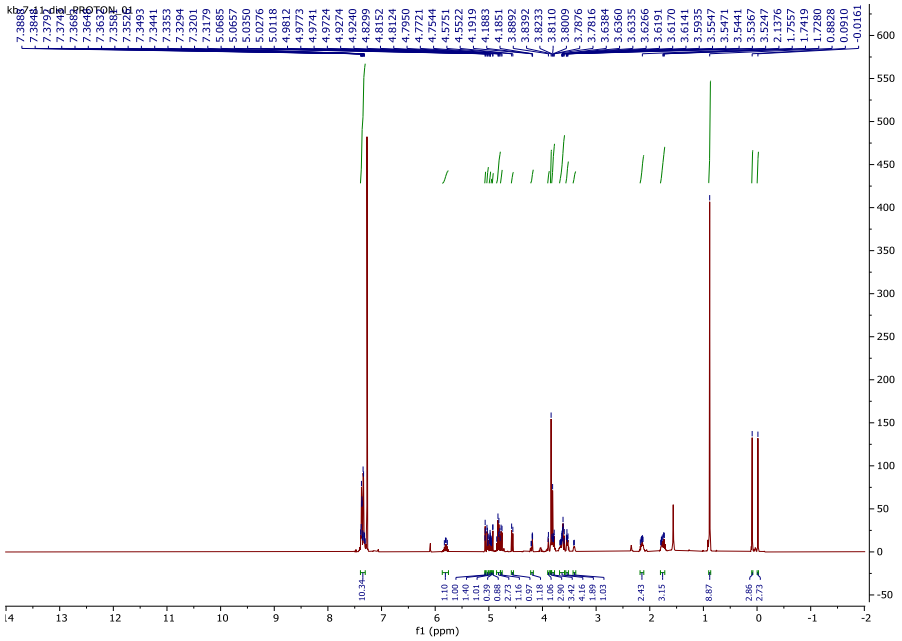
Coupled-gHSQC ($CDCl_3$, 500 MHz) of **31**



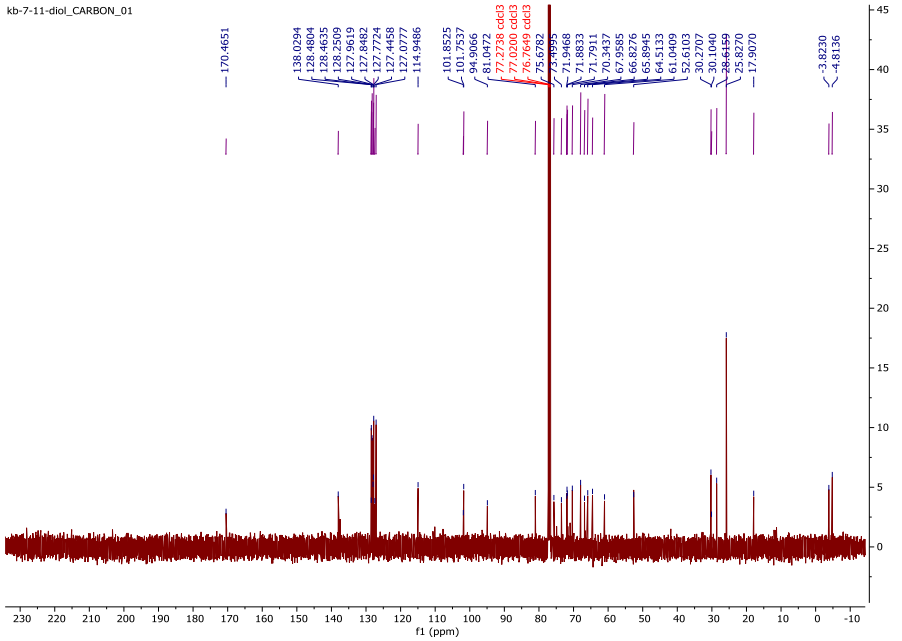


S8

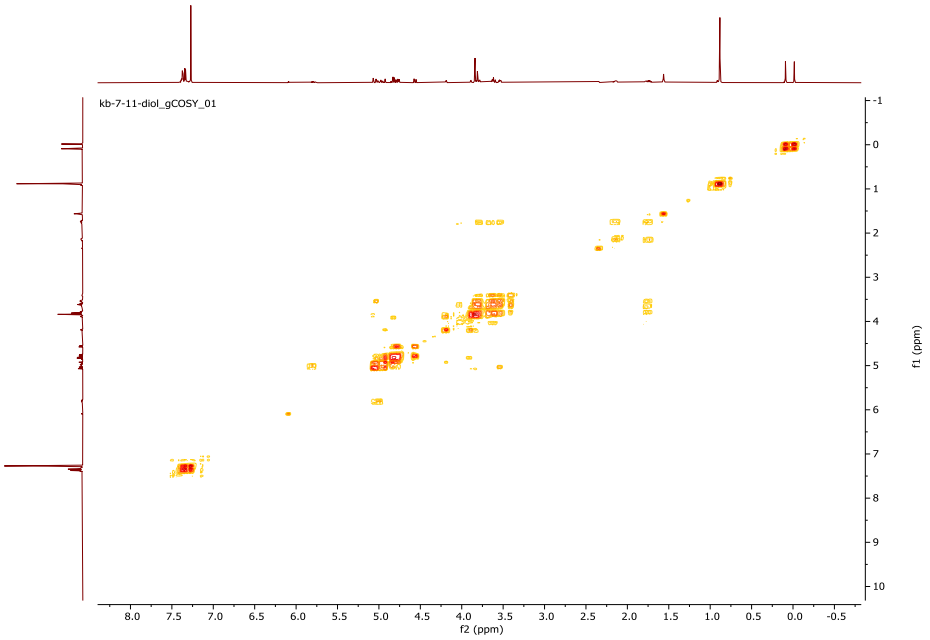
^{1}H-NMR ($CDCl_3$, 500 MHz) of **S8**

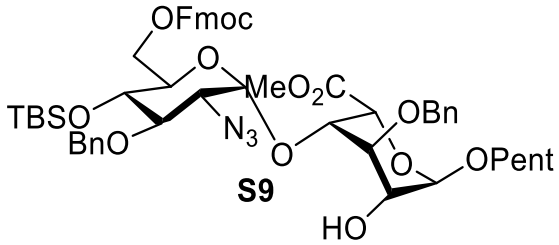


^{13}C-NMR ($CDCl_3$, 126 MHz) of **S8**



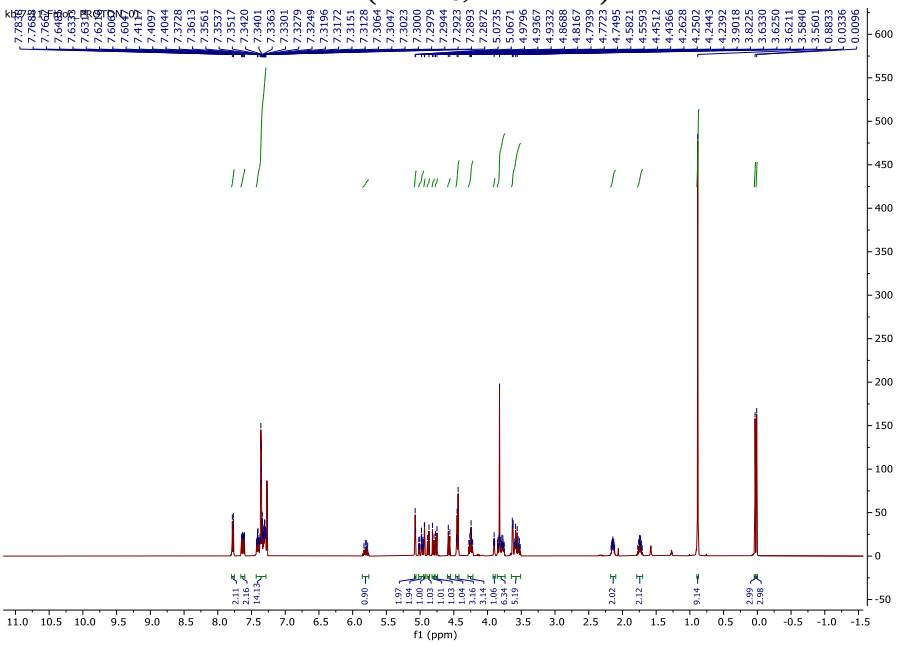
gCOSY ($CDCl_3$, 500 MHz) of **S8**



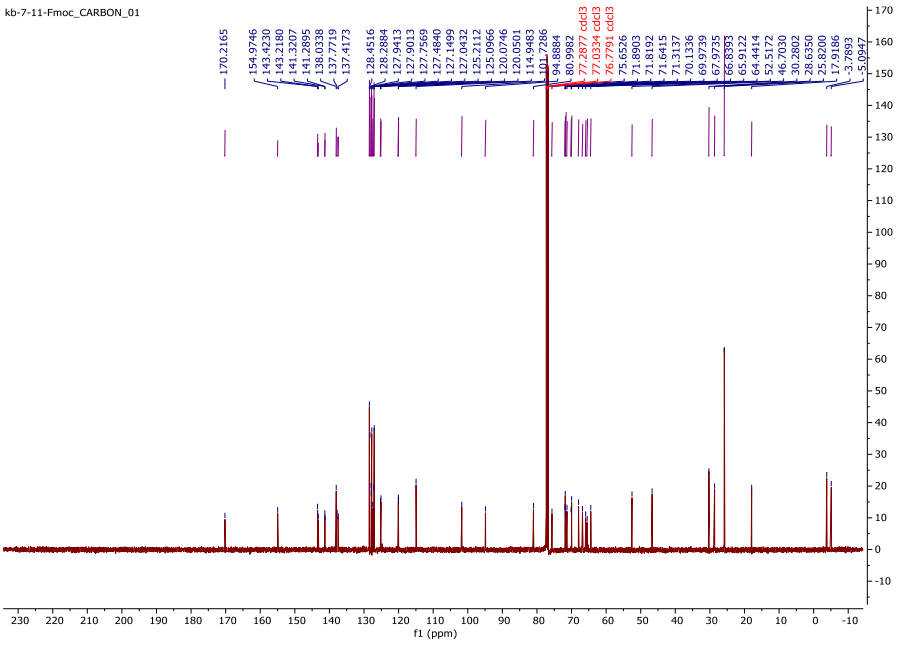


S9

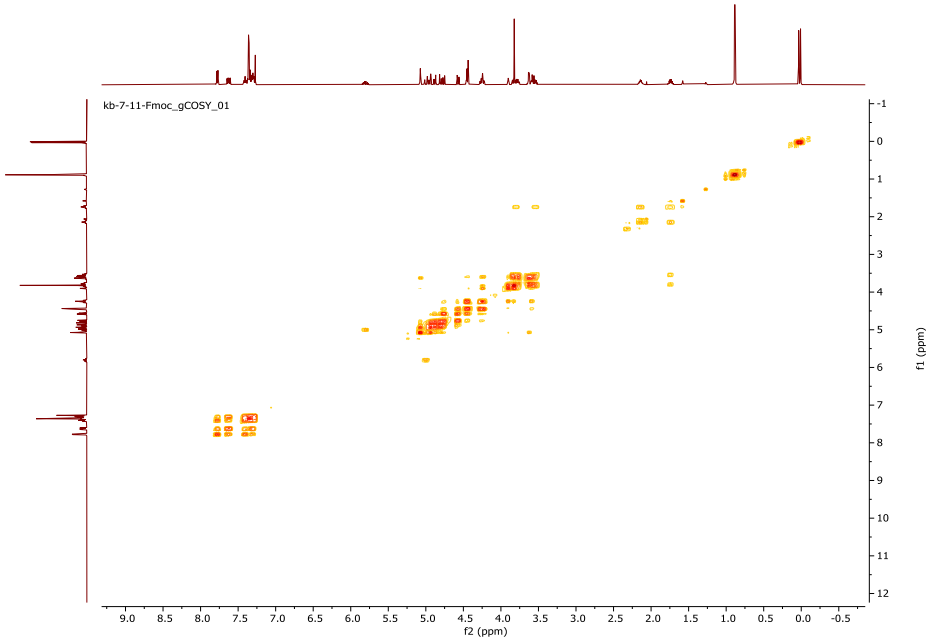
^{1}H-NMR ($CDCl_3$, 500 MHz) of **S9**



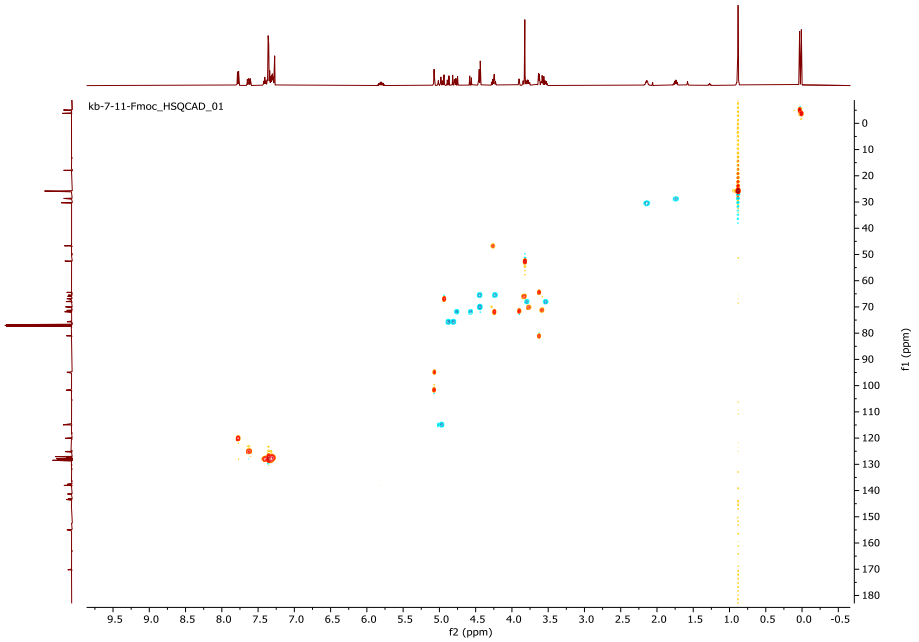
^{13}C-NMR ($CDCl_3$, 126 MHz) of **S9**

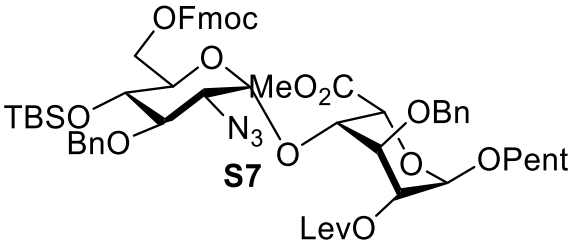


gCOSY ($CDCl_3$, 500 MHz) of **S9**



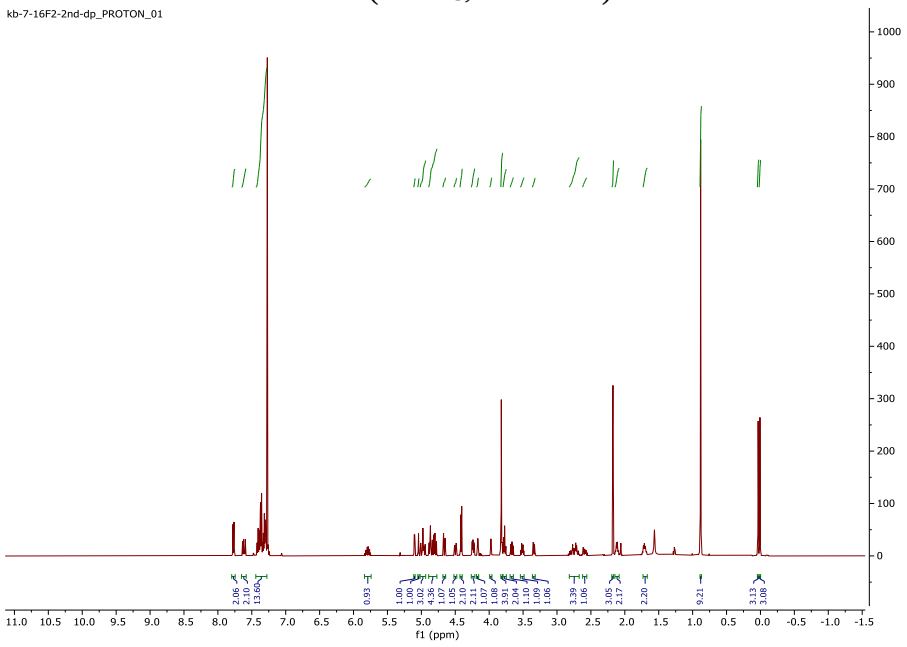
gHSQC ($CDCl_3$, 500 MHz) of **S9**



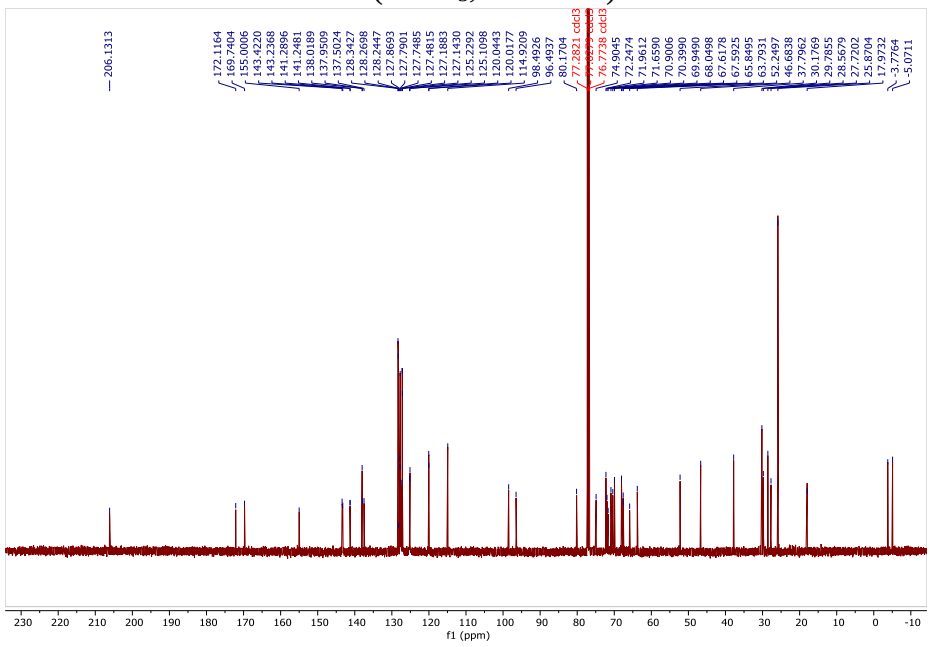


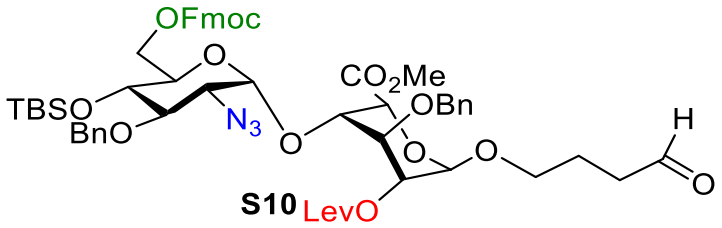
S7

^{1}H-NMR ($CDCl_3$, 500 MHz) of **S7**

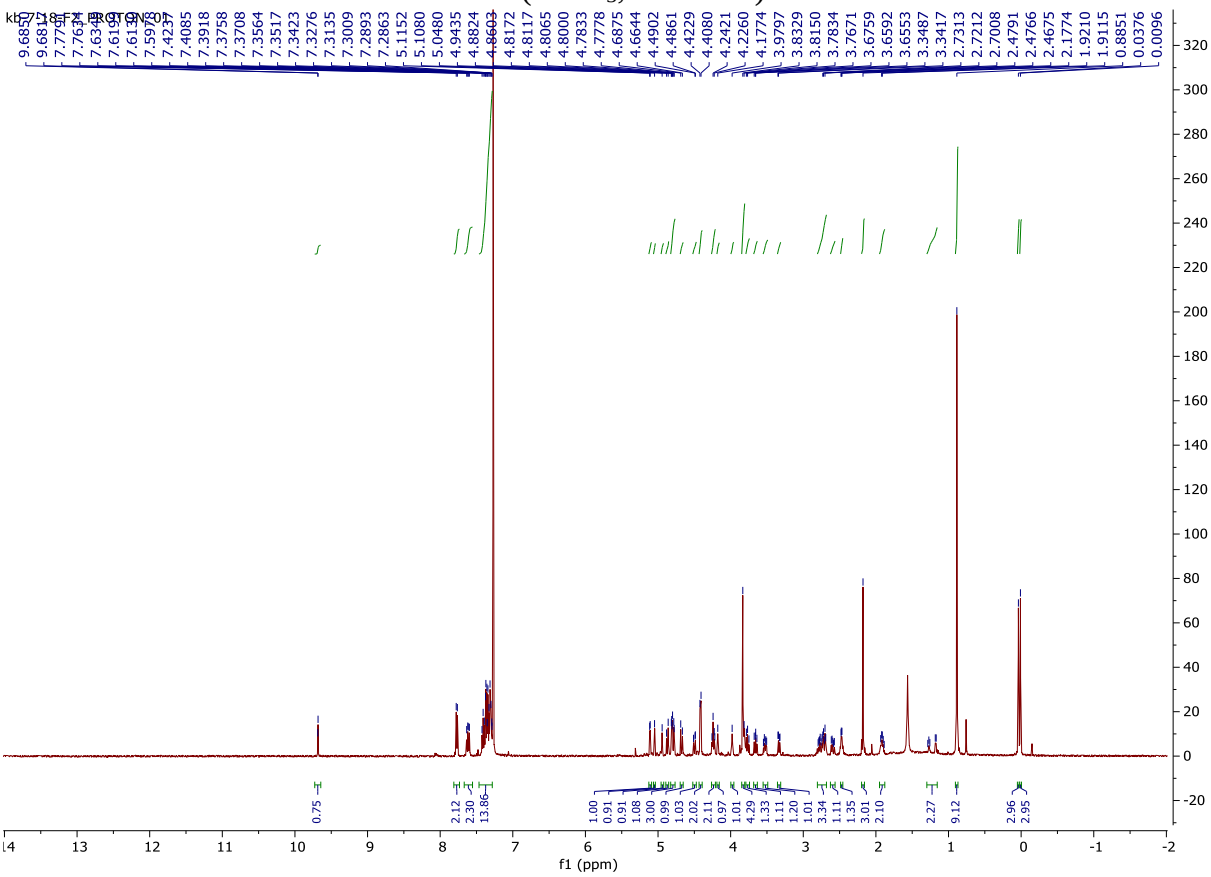


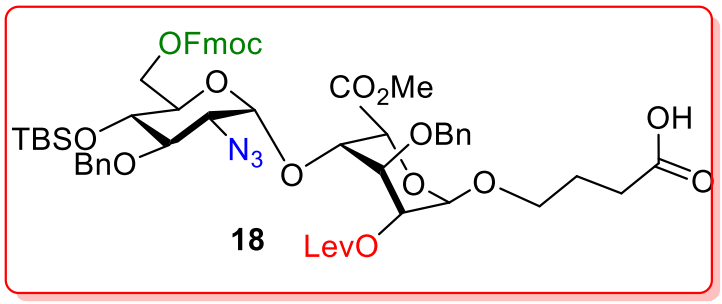
^{13}C-NMR ($CDCl_3$, 126 MHz) of **S7**



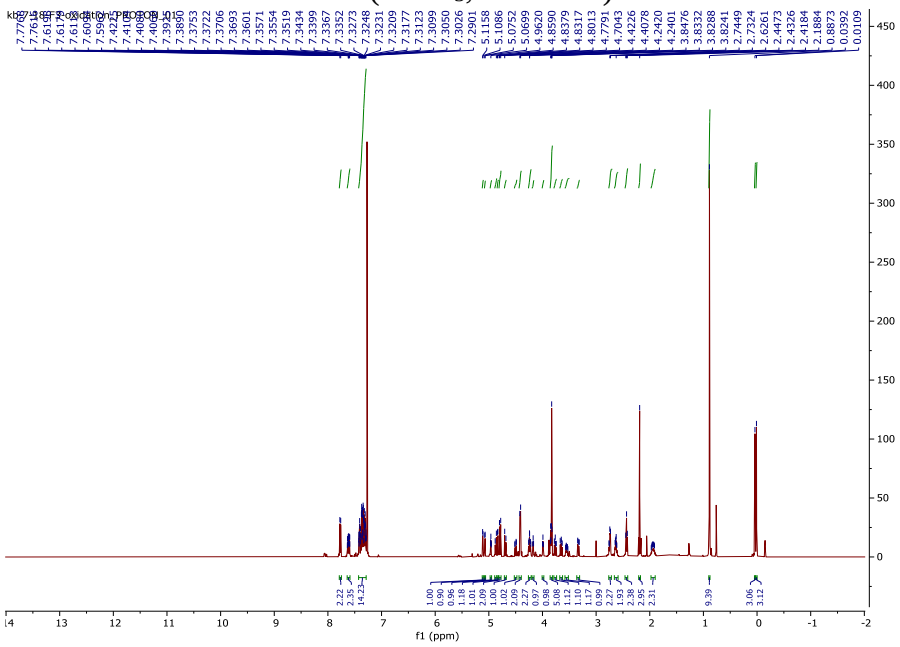


^{1}H-NMR ($CDCl_3$, 500 MHz) of **S10**

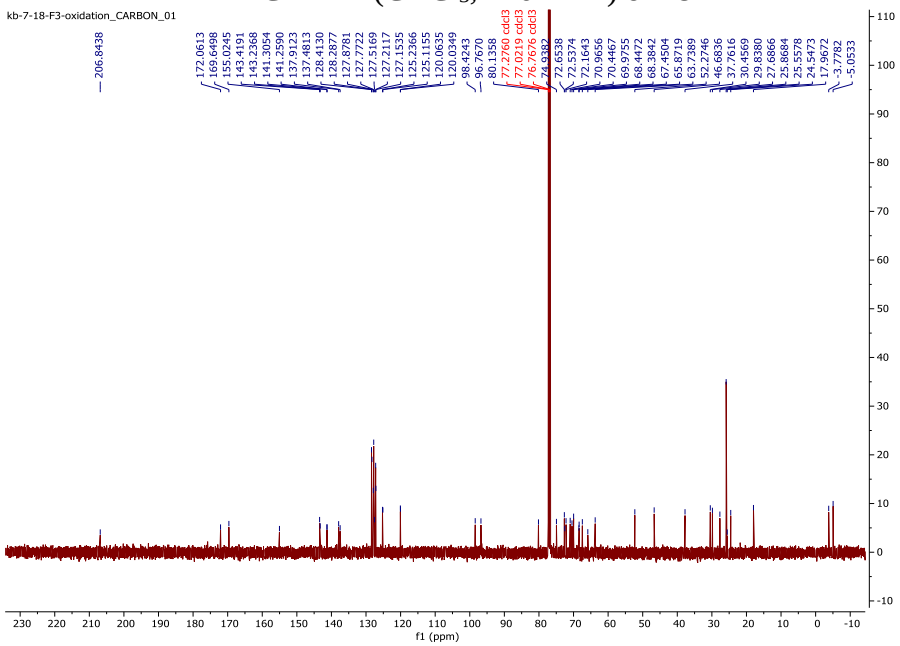




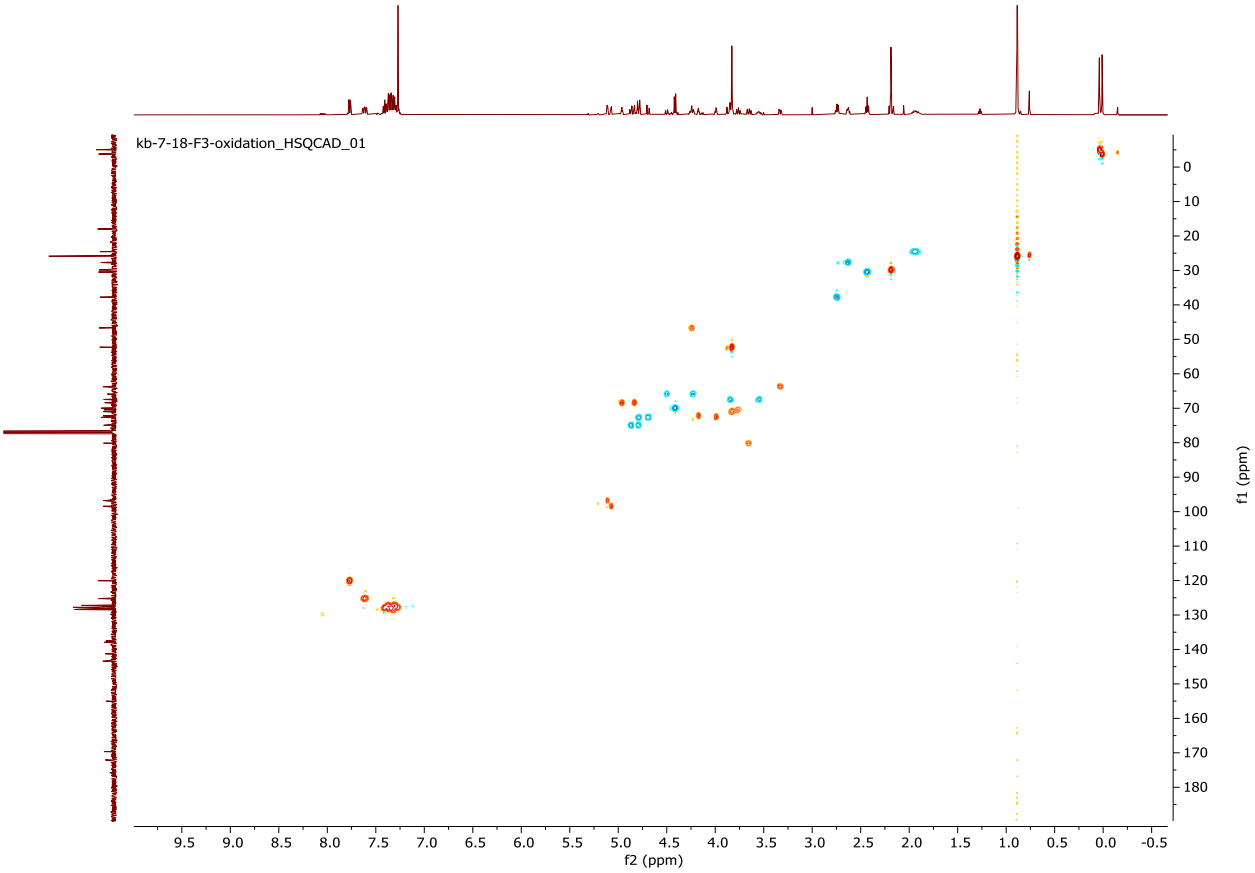
^{1}H-NMR ($CDCl_3$, 500 MHz) of **18**

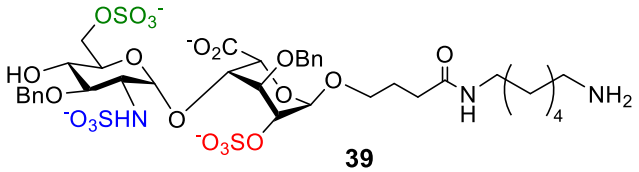


^{13}C-NMR ($CDCl_3$, 126 MHz) of **18**



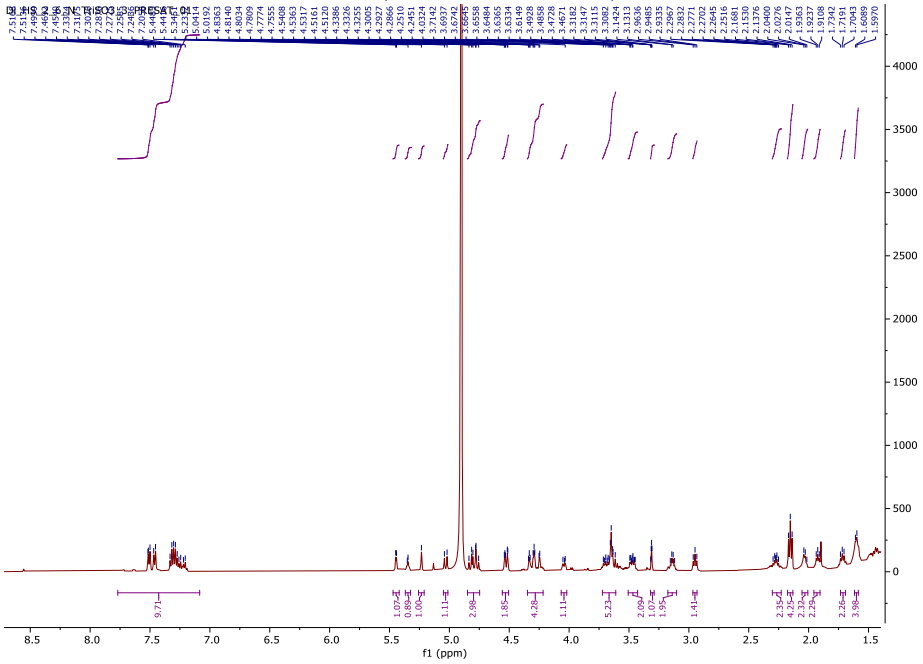
gHSQC ($CDCl_3$, 500 MHz) of **18**



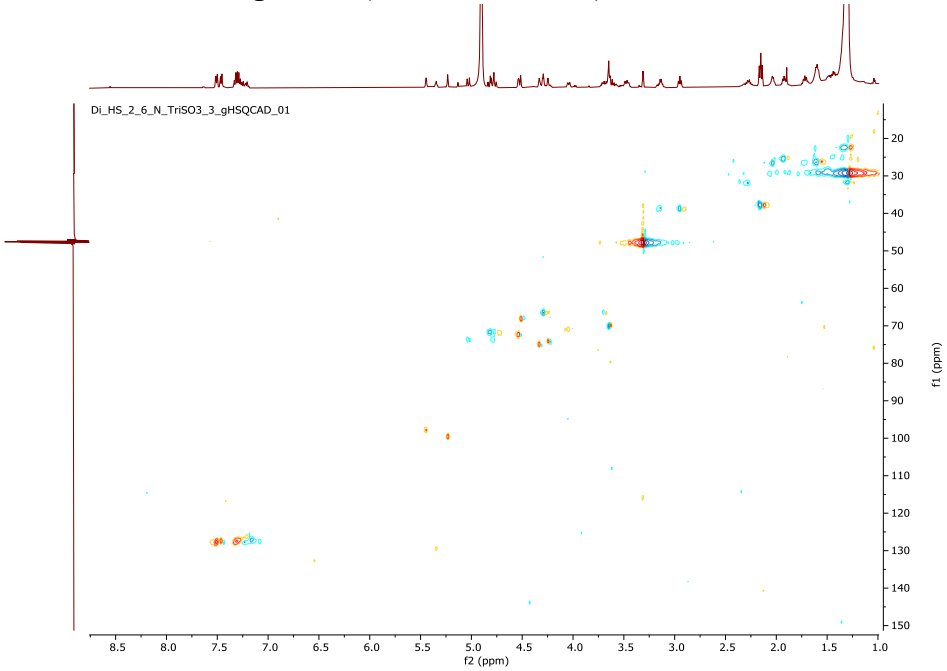


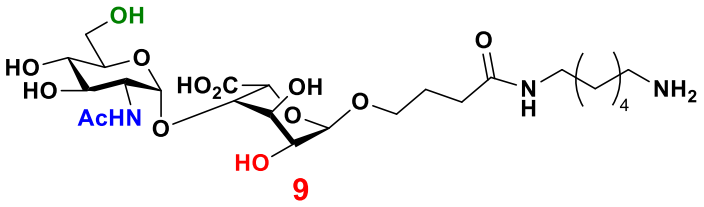
39

^{1}H-NMR ($CDCl_3$, 500 MHz) of **39**



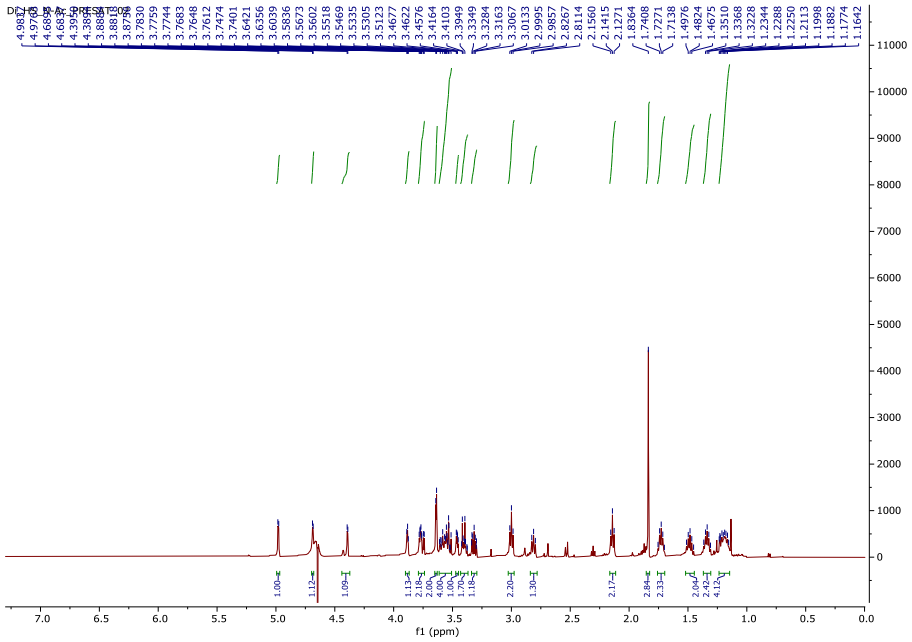
gHSQC ($CDCl_3$, 500 MHz) of **39**



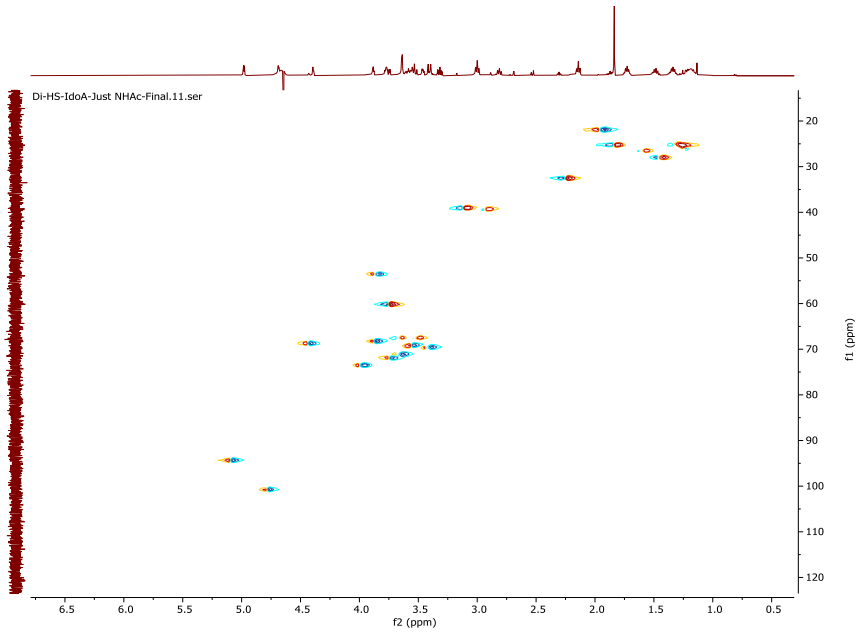


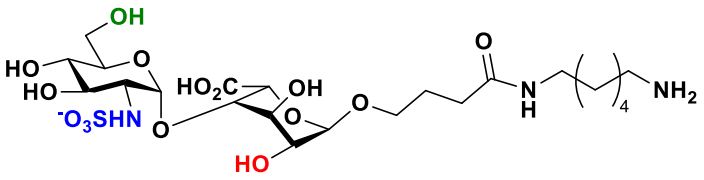
9

^{1}H-NMR (D_2O, 500 MHz) of **9**



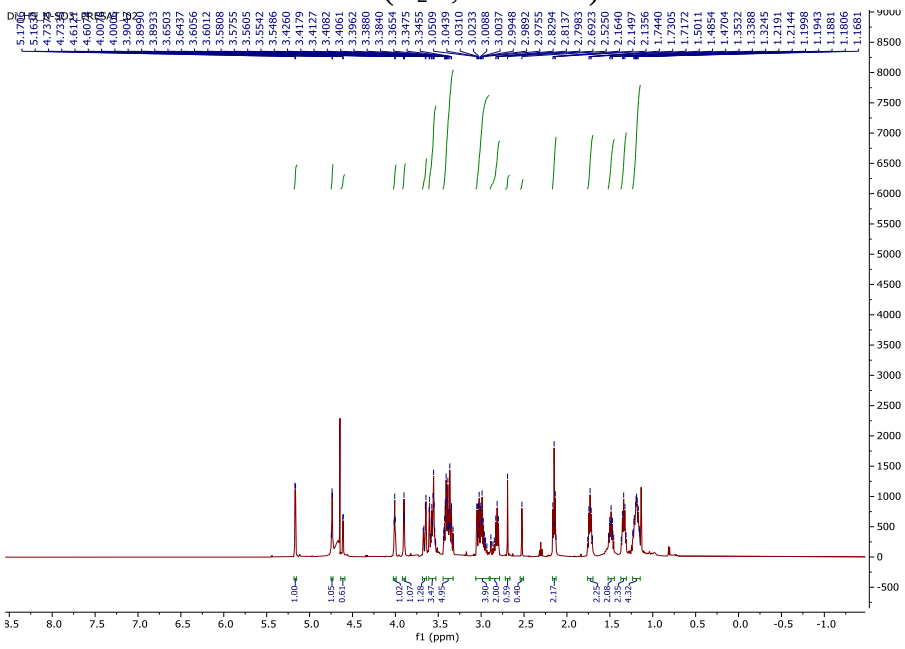
gHSQC (D_2O, 500 MHz) of **9**



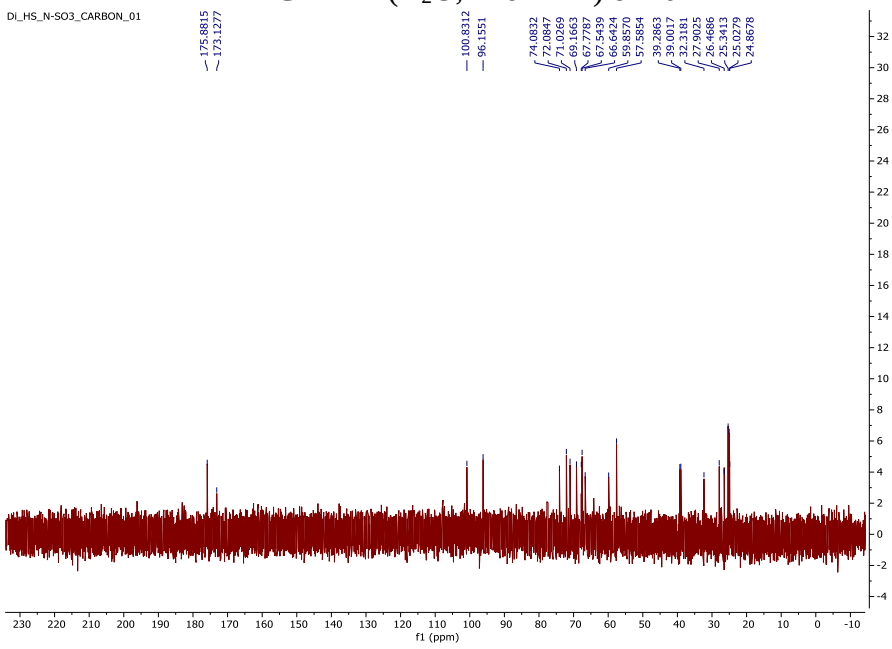


10

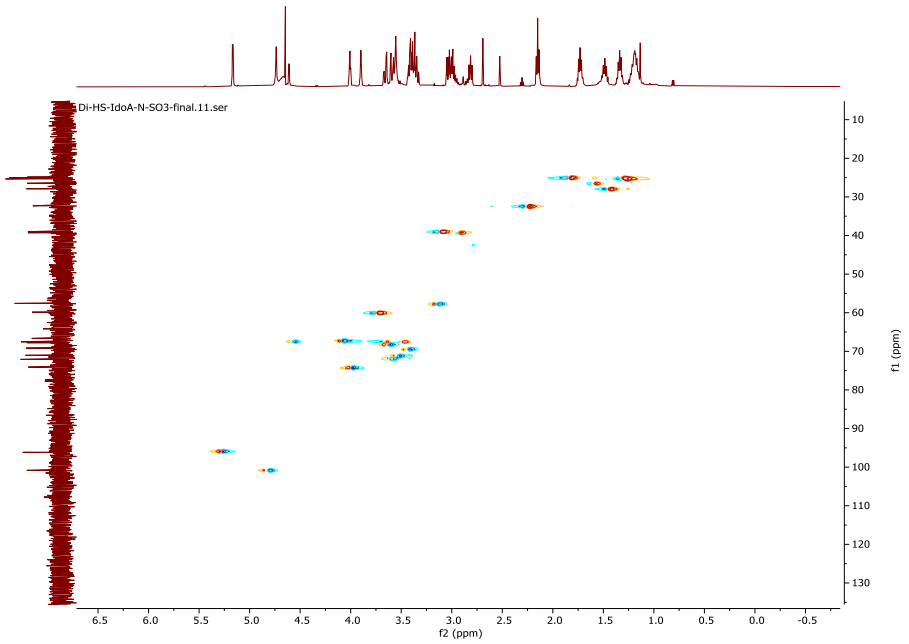
^{1}H-NMR (D_2O, 500 MHz) of **10**

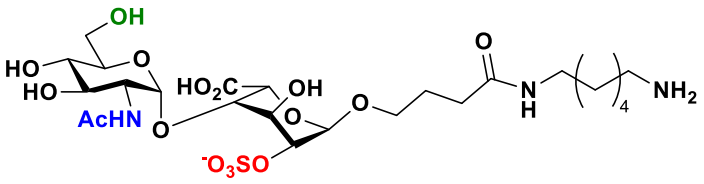


^{13}C-NMR (D_2O, 126 MHz) of **10**

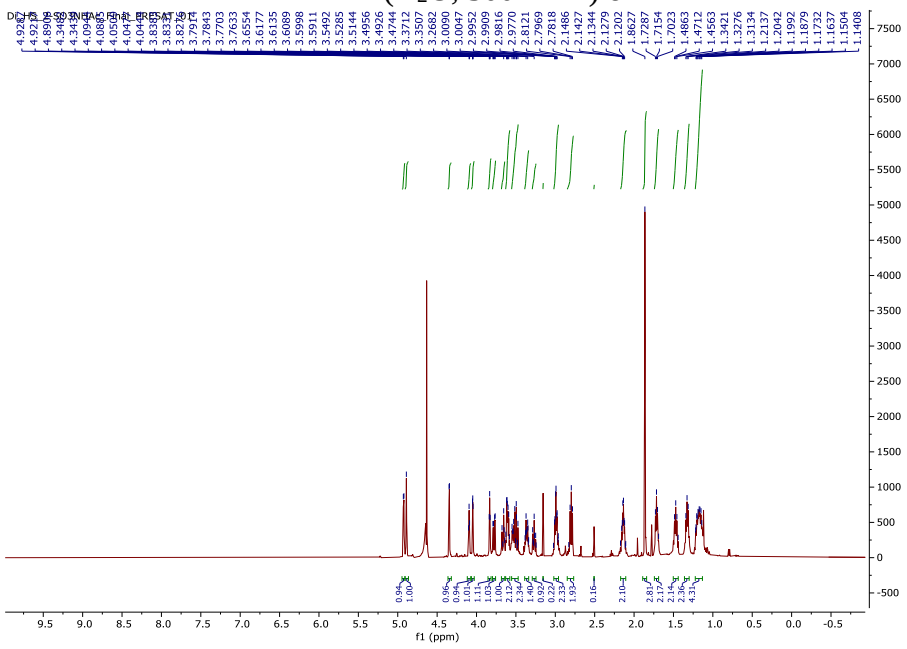


gHSQC (D_2O, 500 MHz) of **10**

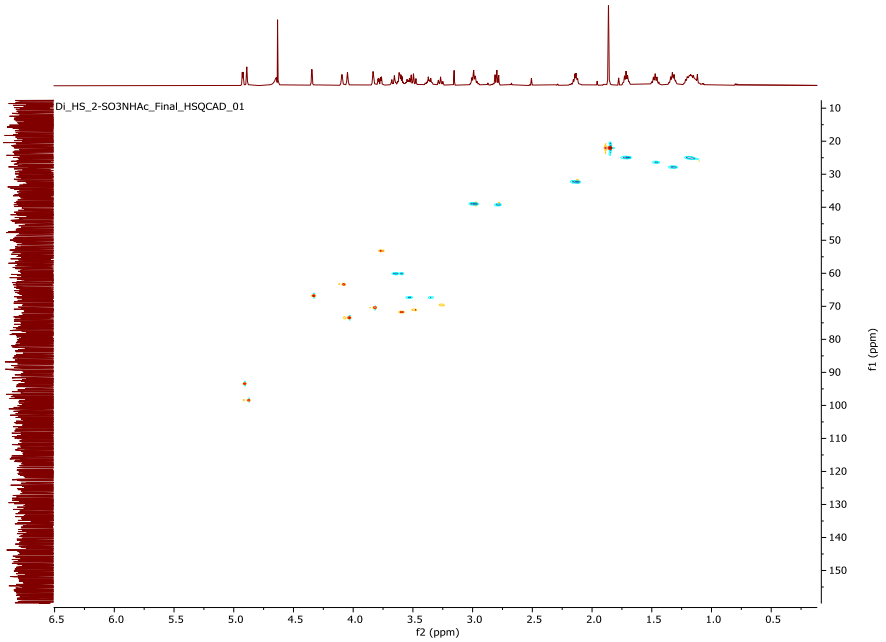

Di-HS-IdoA-N-SO3-final.11.ser
f2 (ppm)
f1 (ppm)



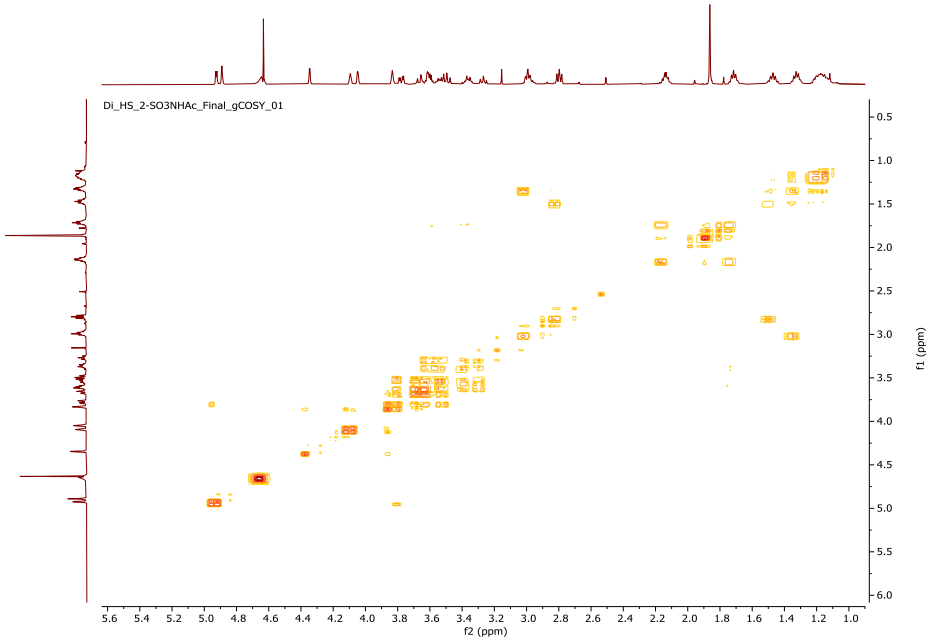
^{1}H-NMR (D_2O, 500 MHz) of **11**

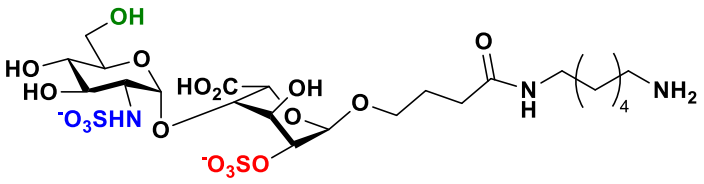


gHSQC (D_2O, 500 MHz) of **11**



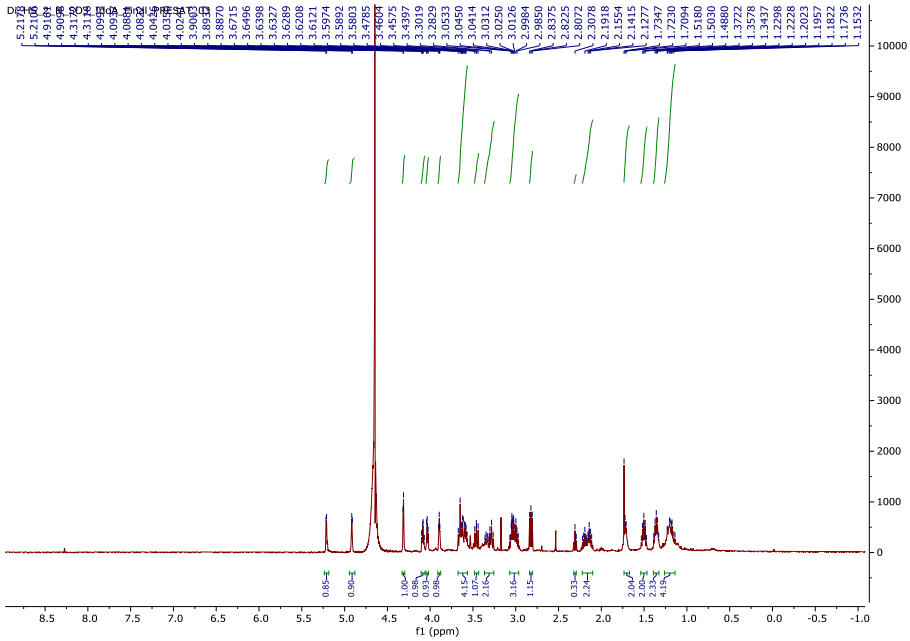
gCOSY (D_2O, 500 MHz) of **11**



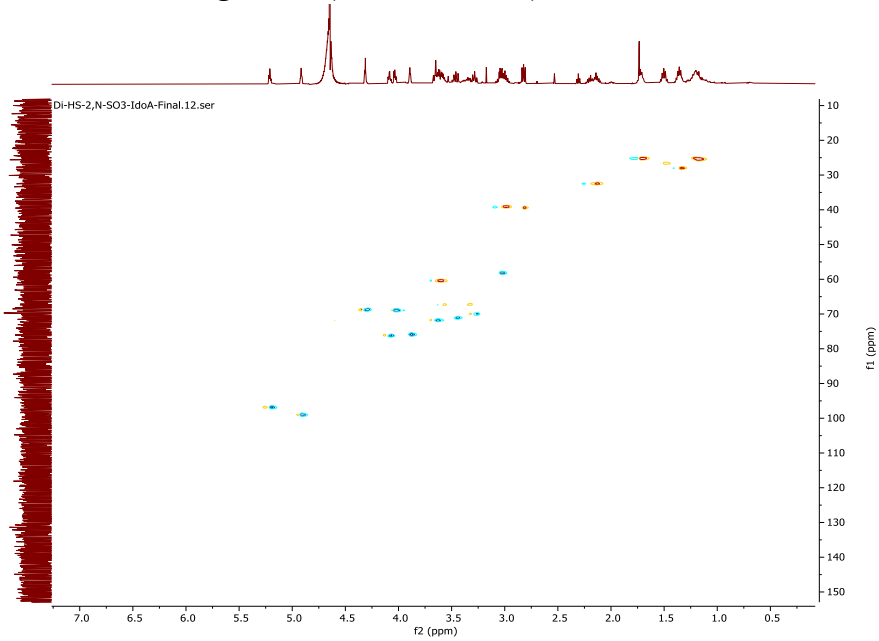


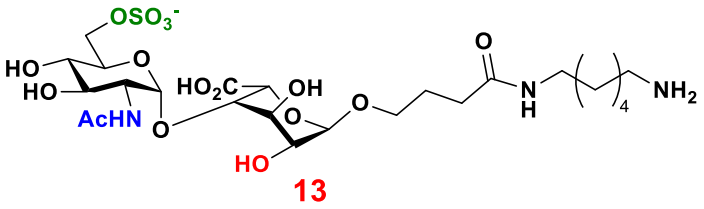
12

^{1}H-NMR (D_2O, 500 MHz) of **12**

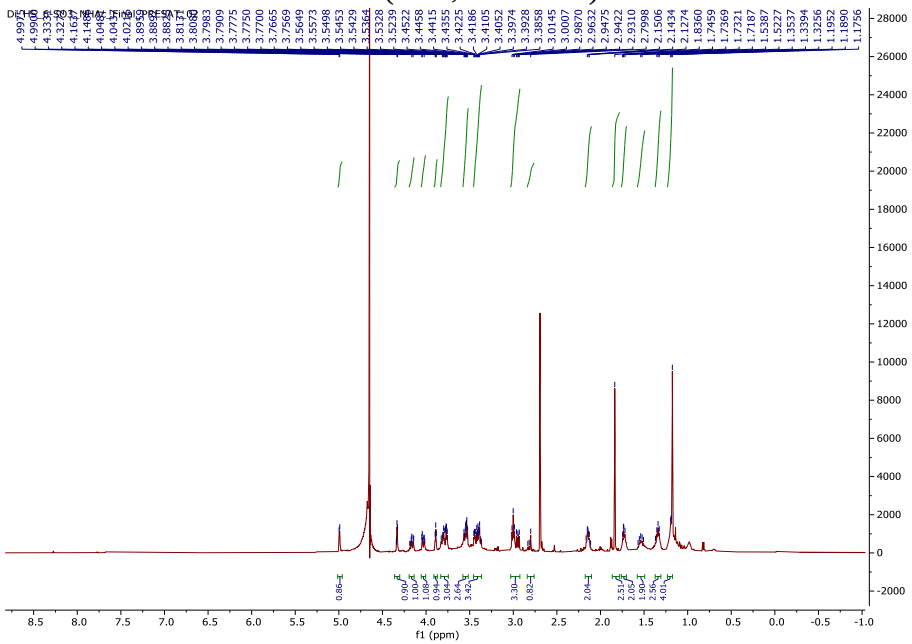


gHSQC (D_2O, 500 MHz) of **12**

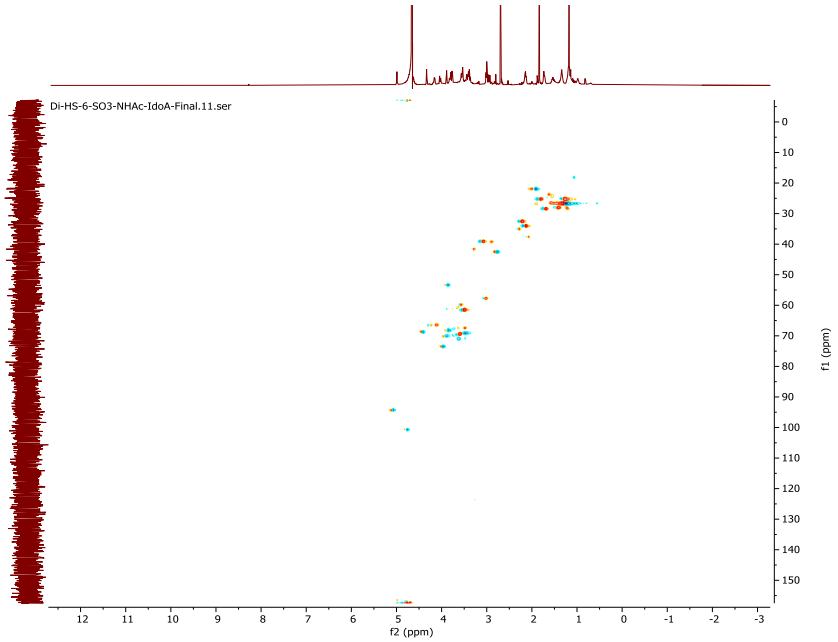


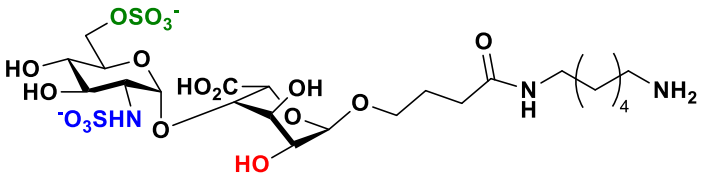


^{1}H-NMR (D_2O, 500 MHz) of **13**



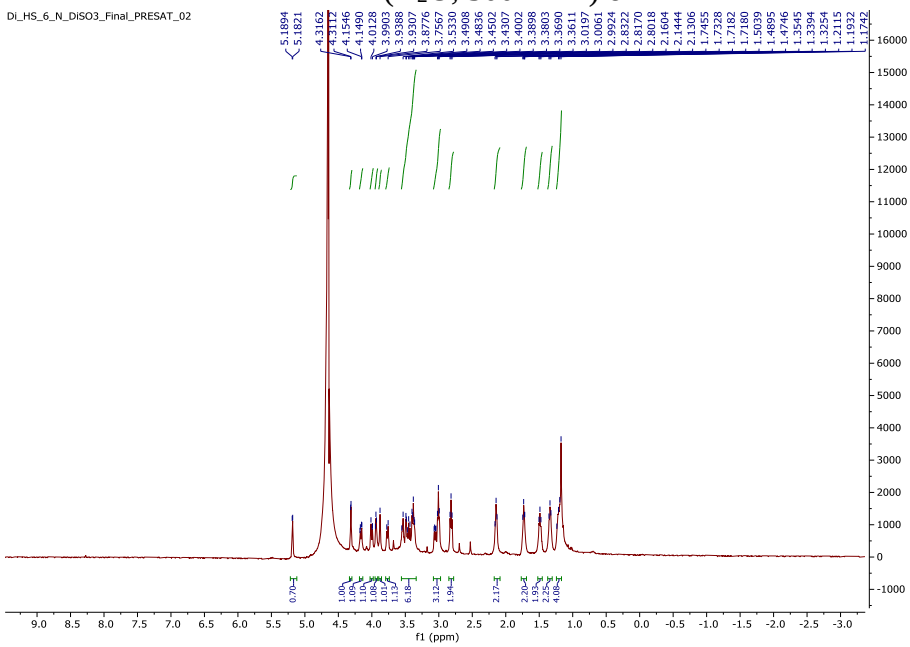
gHSQC (D_2O, 500 MHz) of **13**



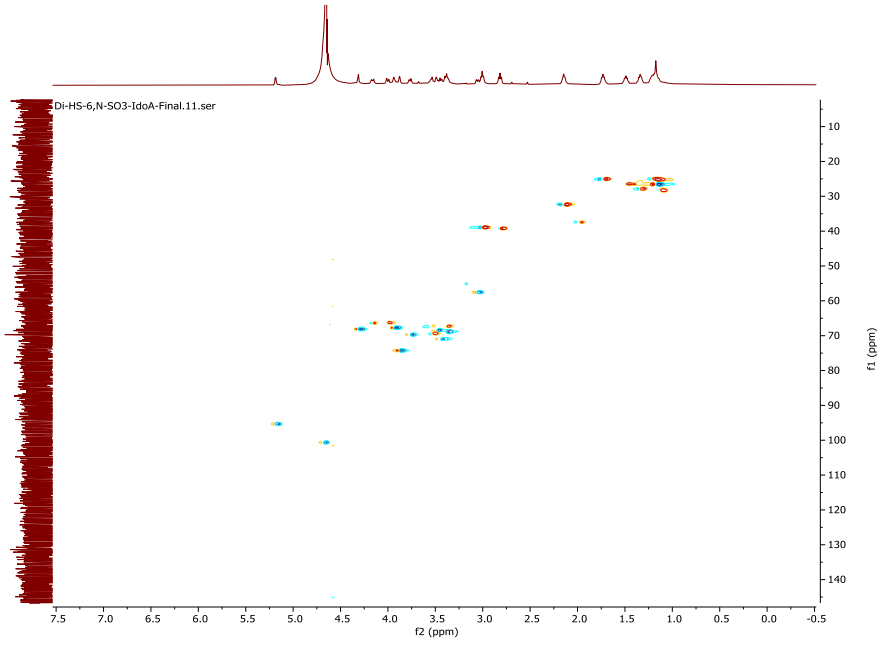


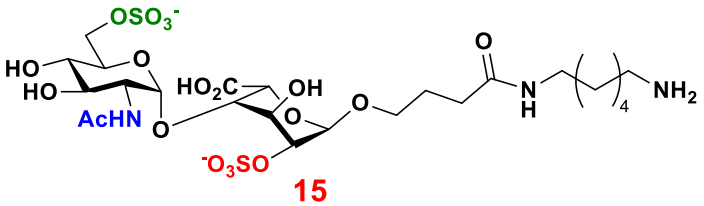
14

^{1}H-NMR (D_2O, 500 MHz) of **14**



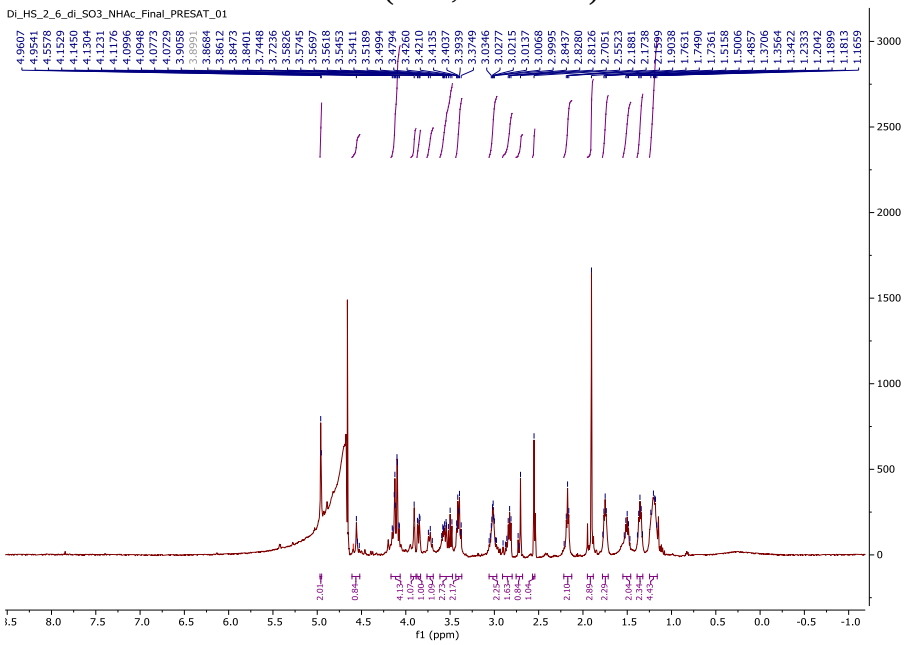
gHSQC (D_2O, 500 MHz) of **14**



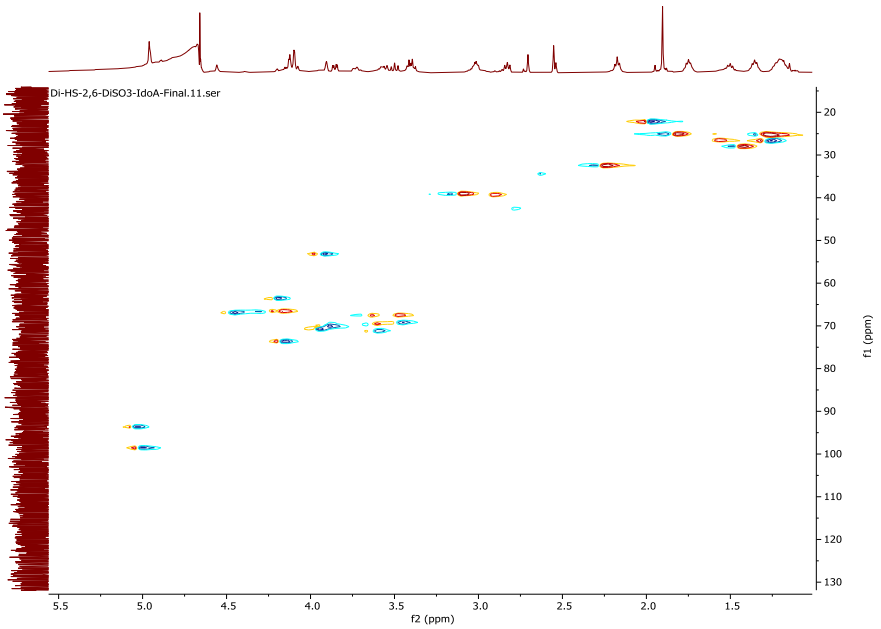


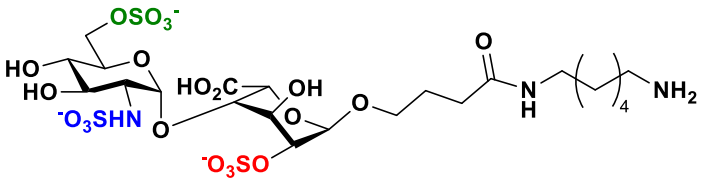
15

^{1}H-NMR (D_2O, 500 MHz) of **15**



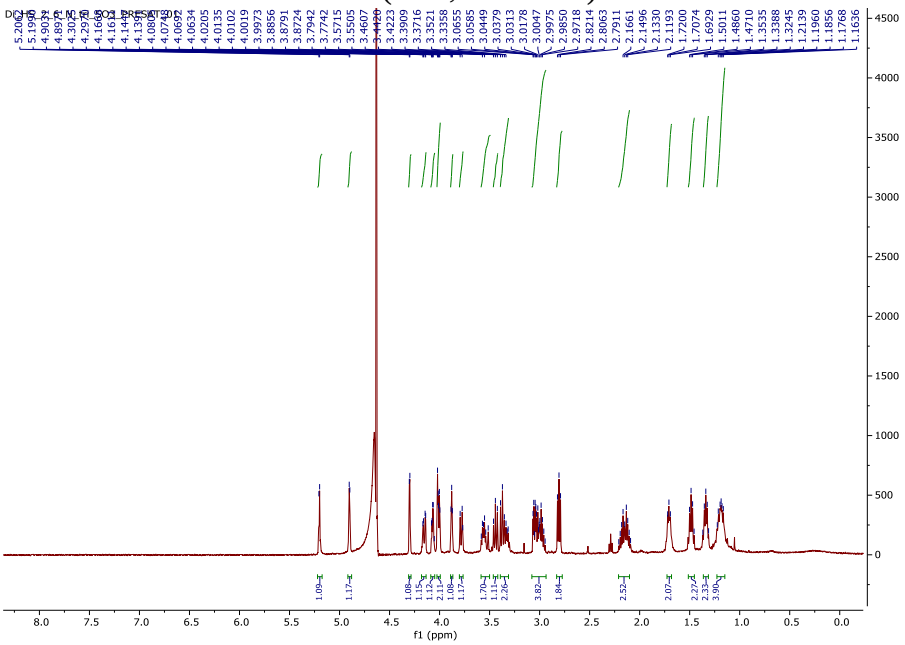
gHSQC (D_2O, 500 MHz) of **15**



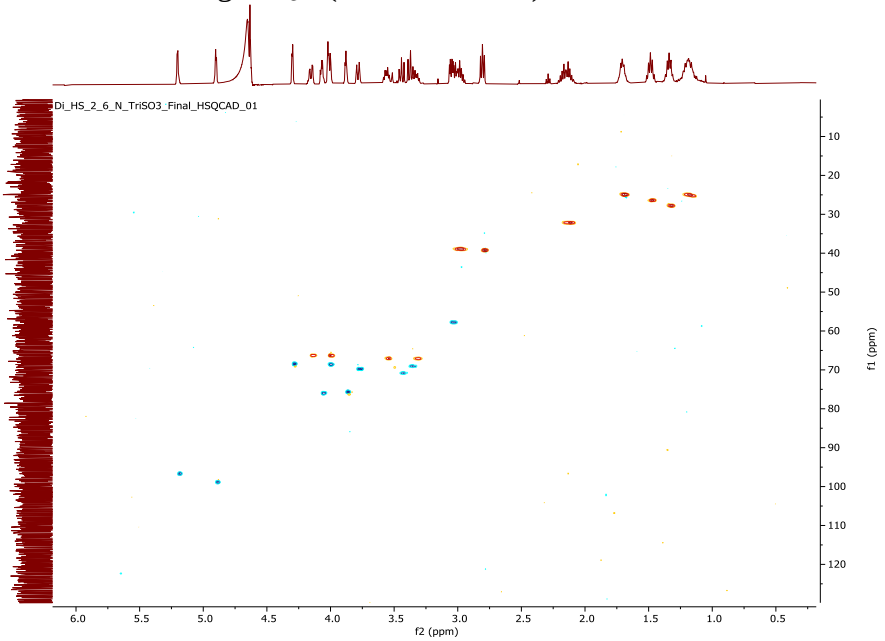


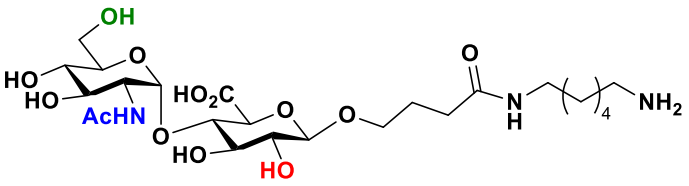
16

^{1}H-NMR (D_2O, 500 MHz) of **16**



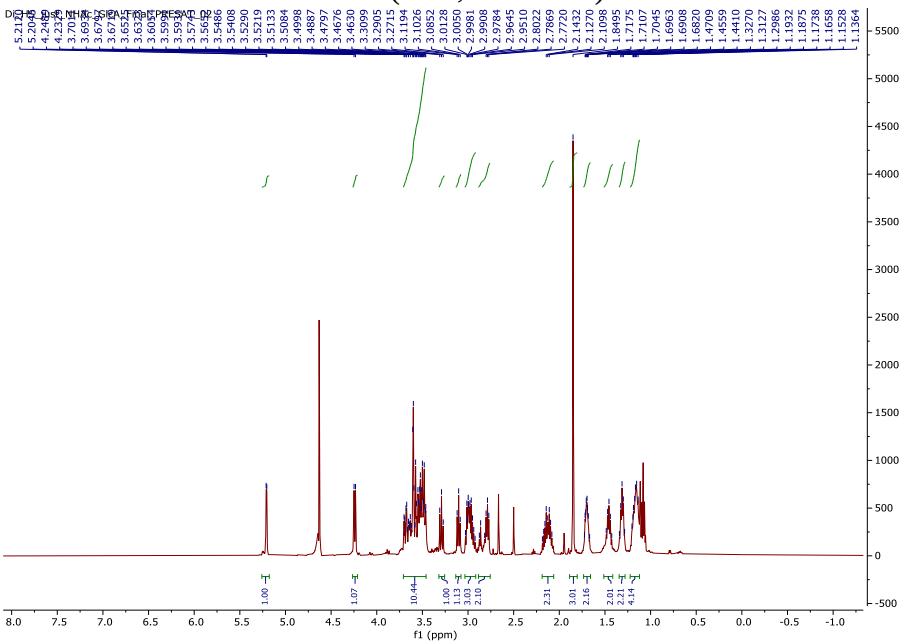
gHSQC (D_2O, 500 MHz) of **16**



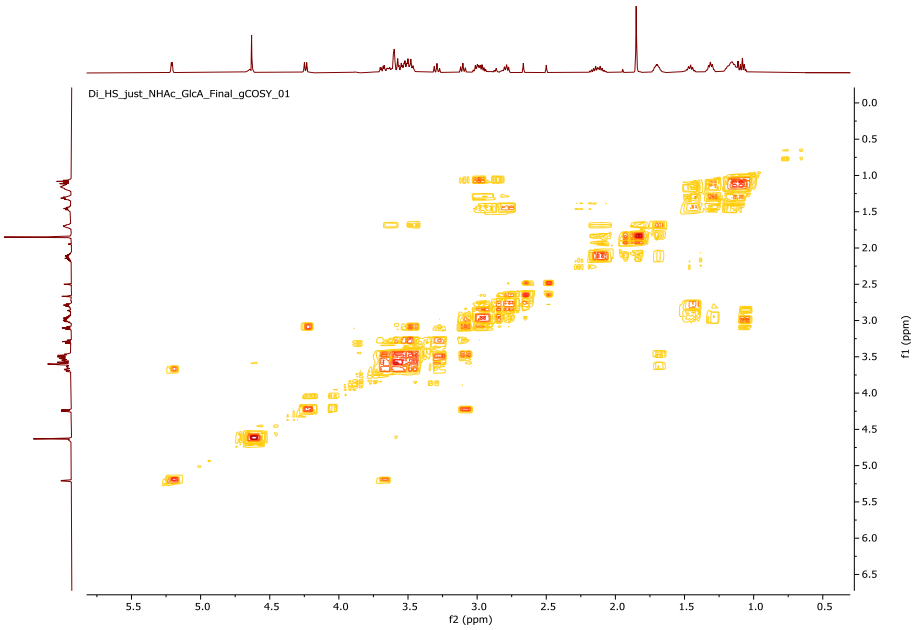


1

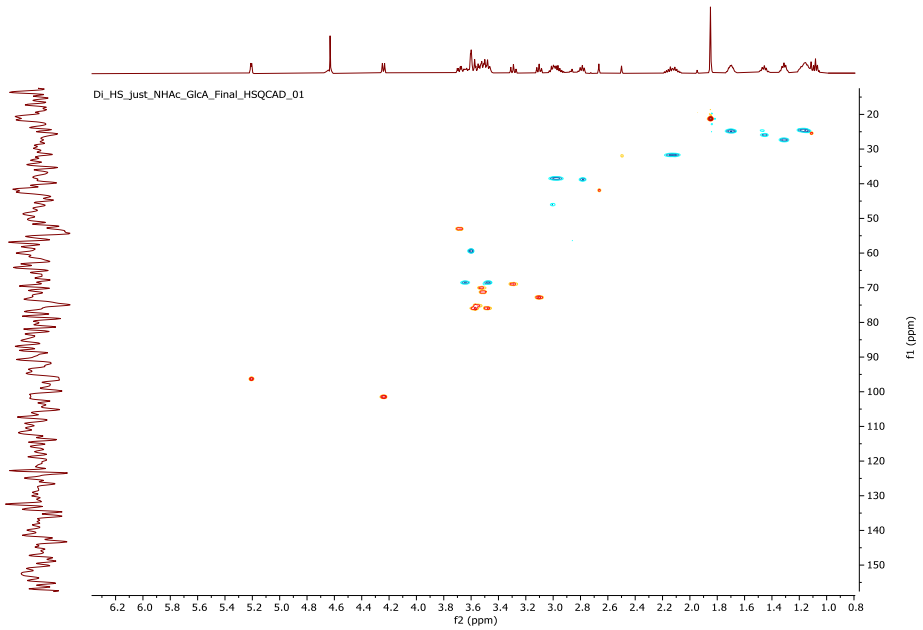
^{1}H-NMR (D_2O, 500 MHz) of **1**

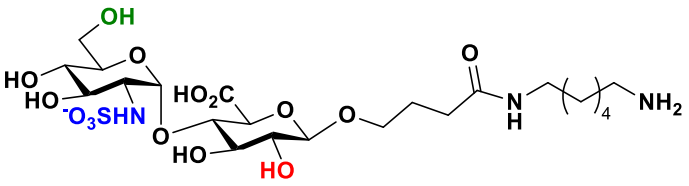


gCOSY (D_2O, 500 MHz) of **1**



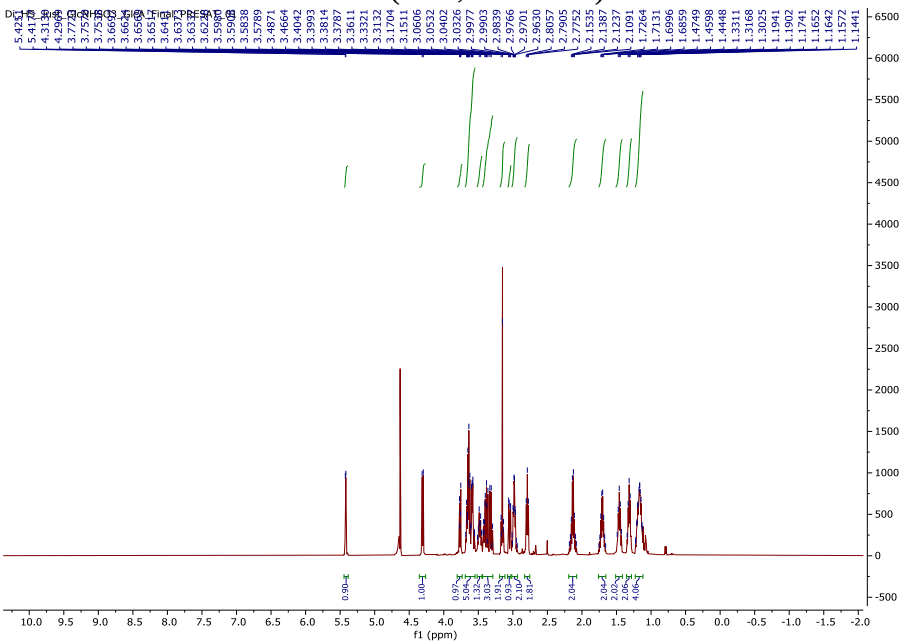
gHSQC (D_2O, 500 MHz) of **1**



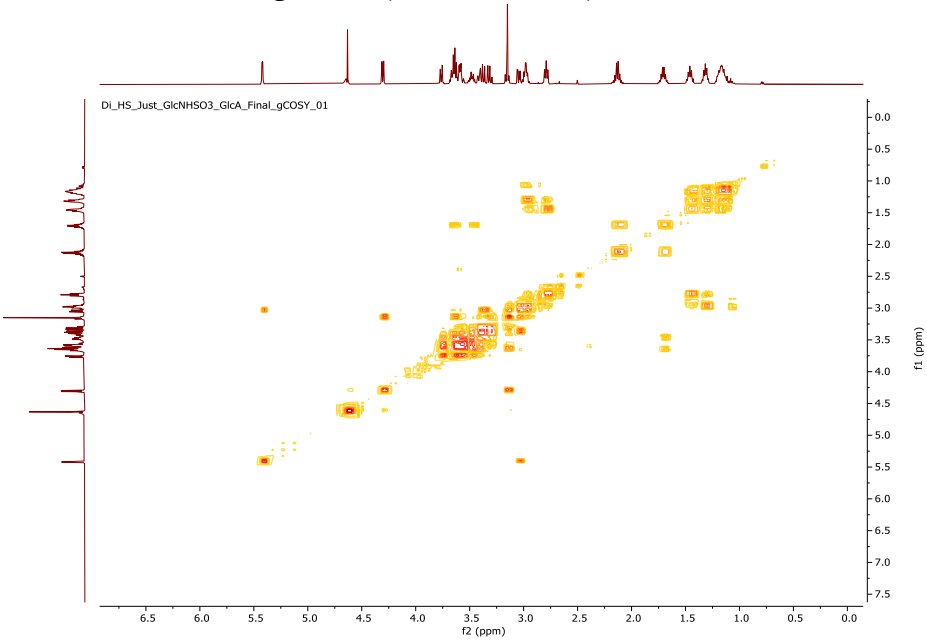


2

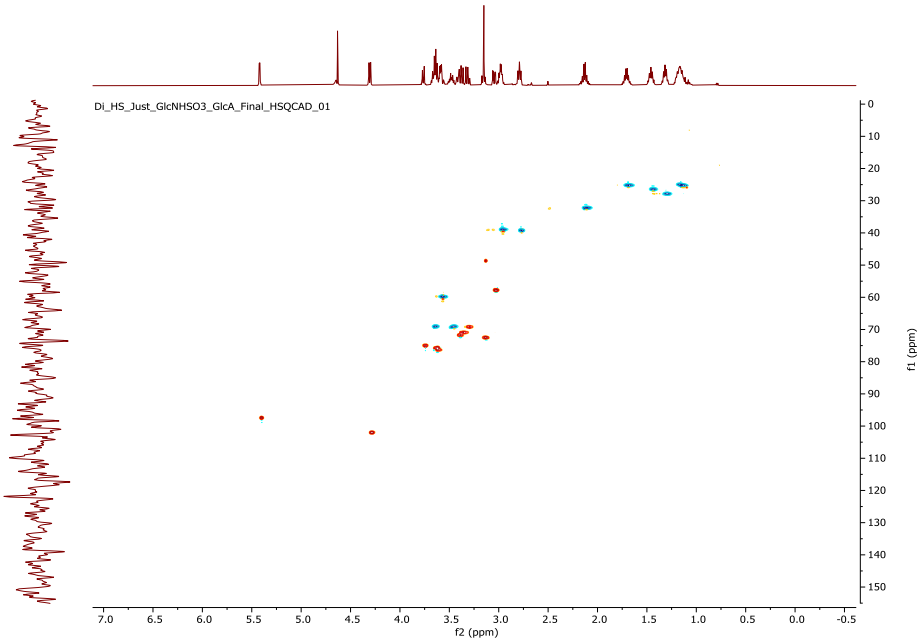
^{1}H-NMR (D_2O, 500 MHz) of **2**

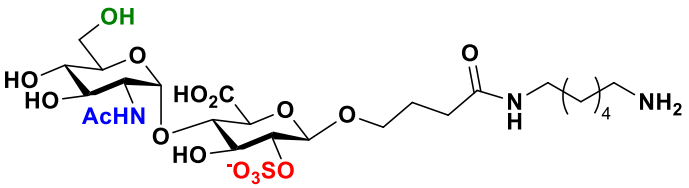


gCOSY (D_2O, 500 MHz) of **2**



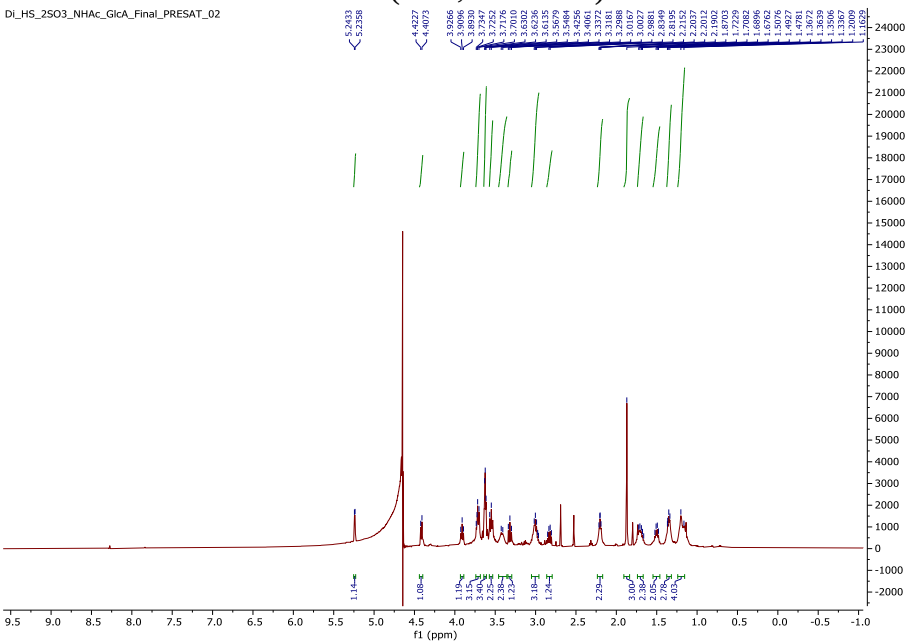
gHSQC (D_2O, 500 MHz) of **2**



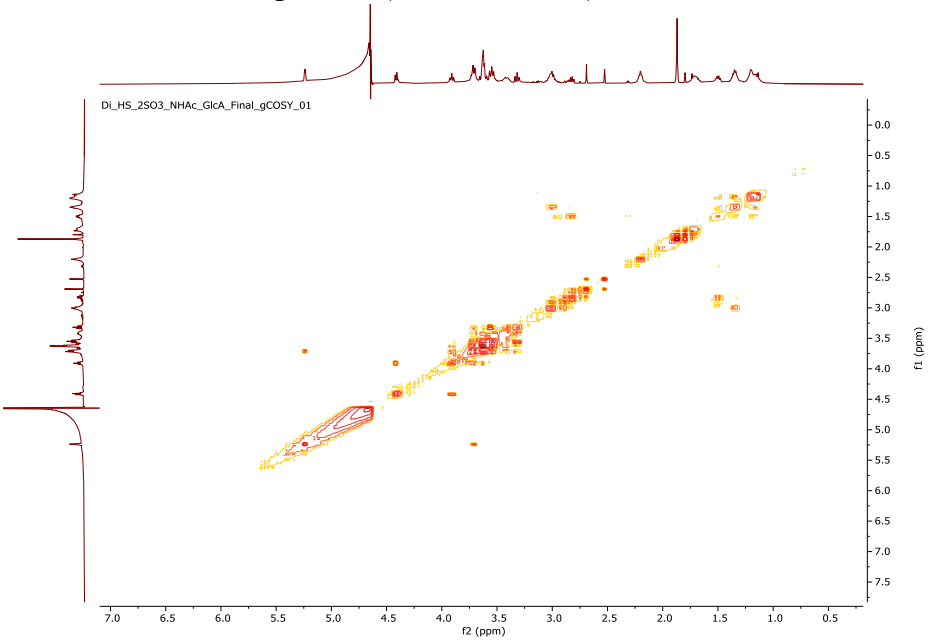


3

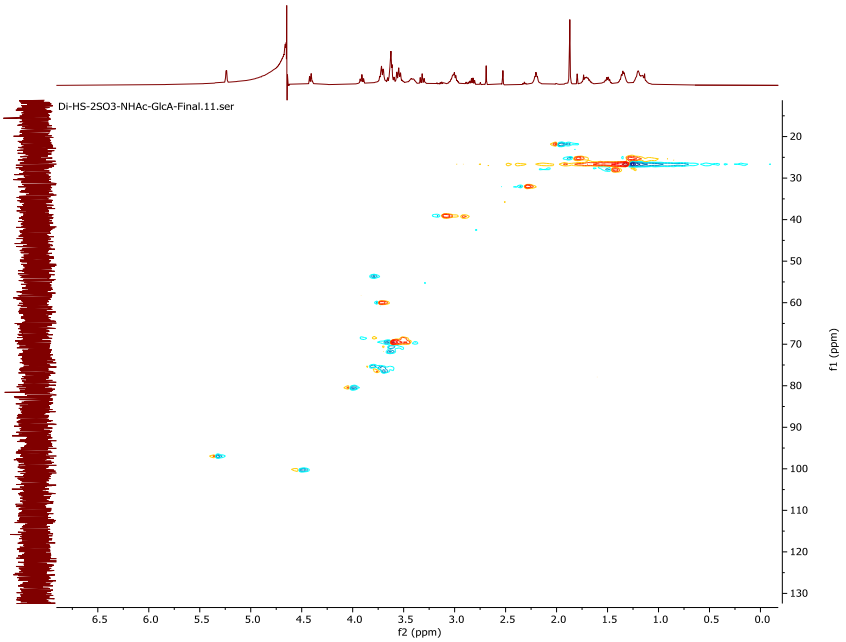
^{1}H-NMR (D_2O, 500 MHz) of **3**

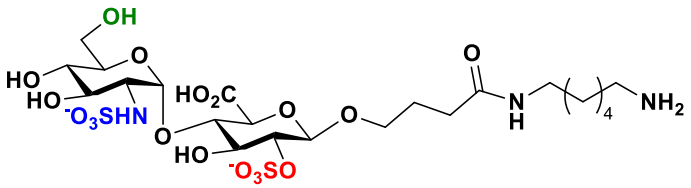


gCOSY (D_2O, 500 MHz) of **3**

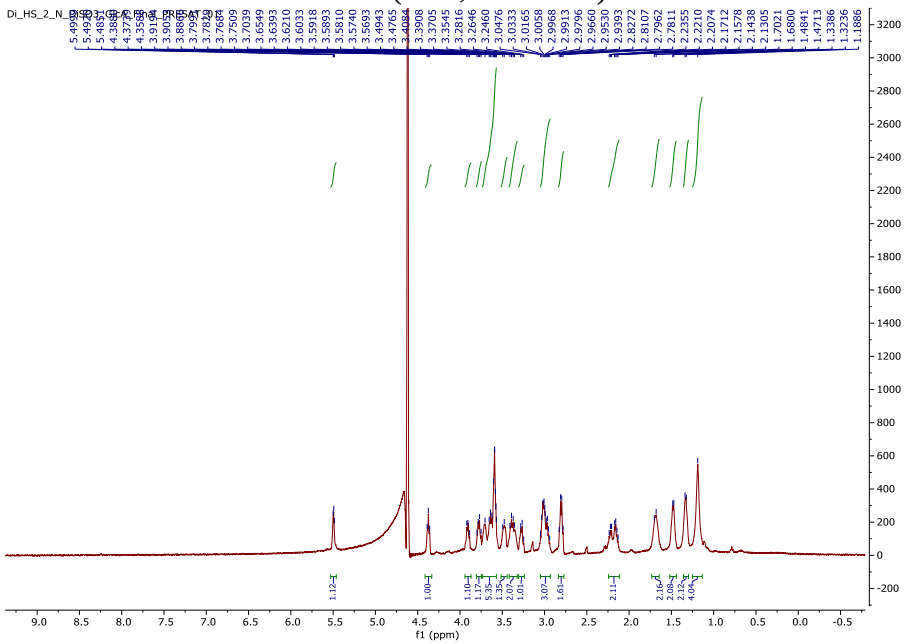


gHSQC (D_2O, 500 MHz) of **3**

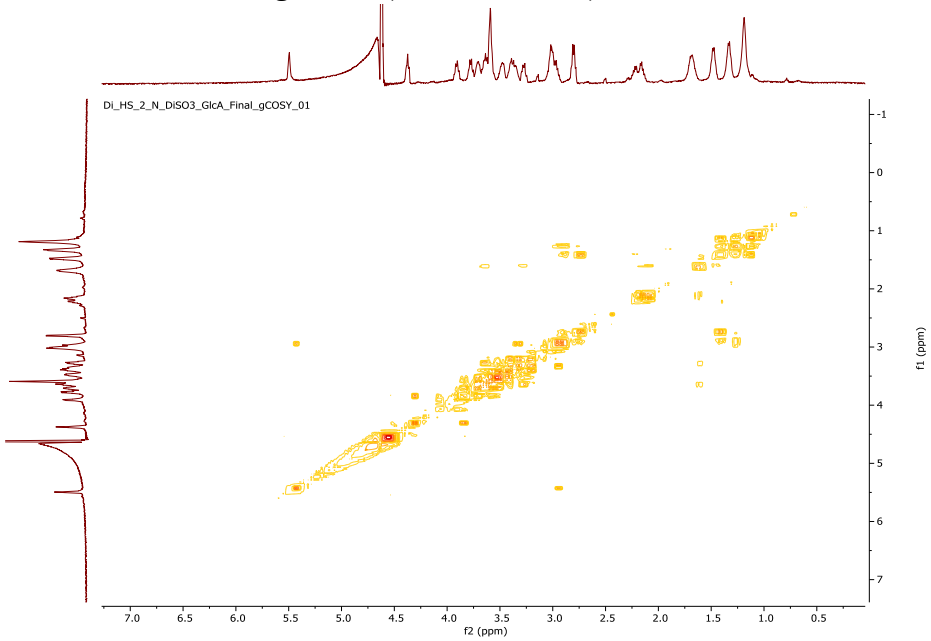




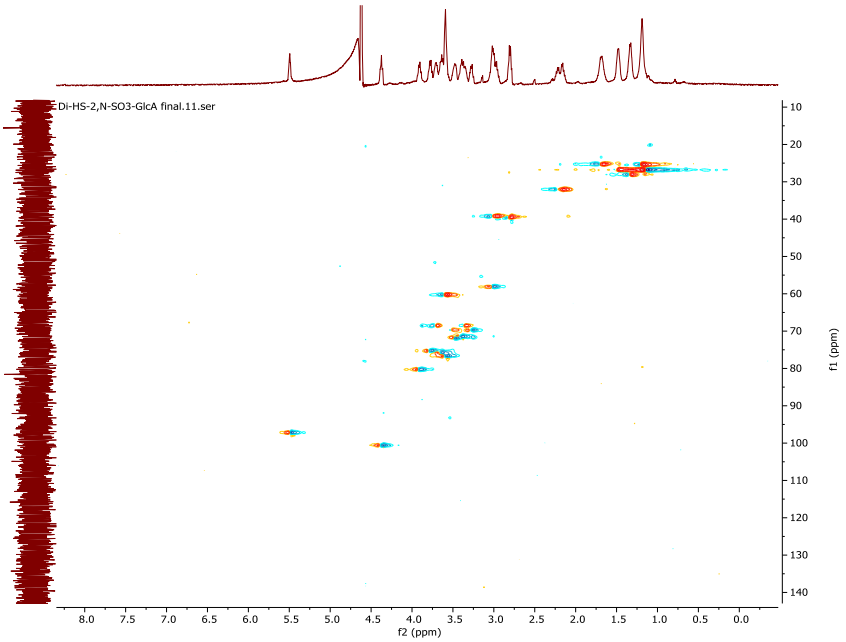
^{1}H-NMR (D_2O, 500 MHz) of **4**

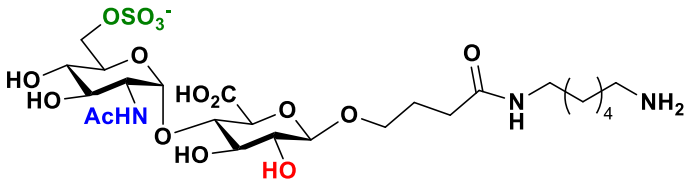


gCOSY (D_2O, 500 MHz) of **4**



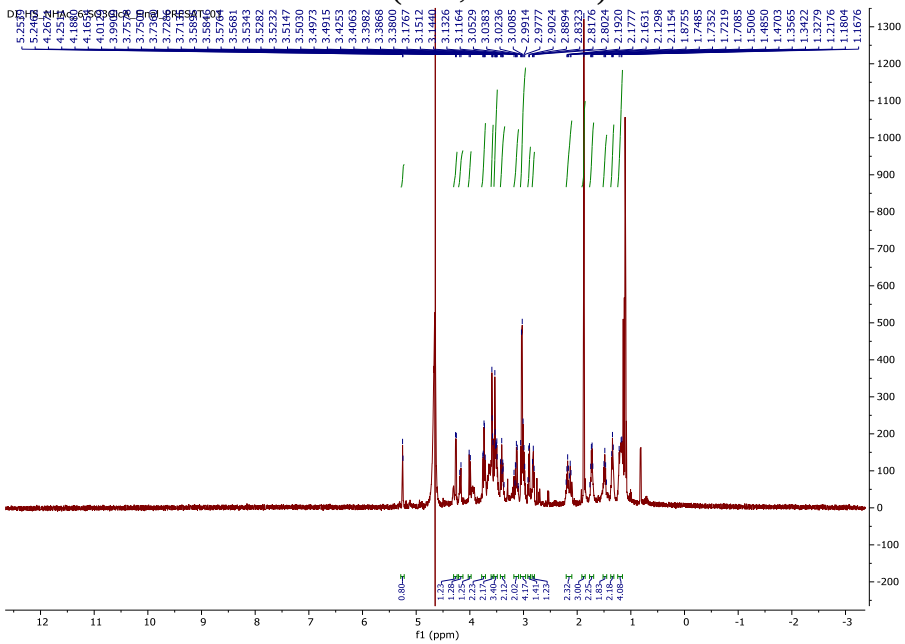
gHSQC (D_2O, 500 MHz) of **4**



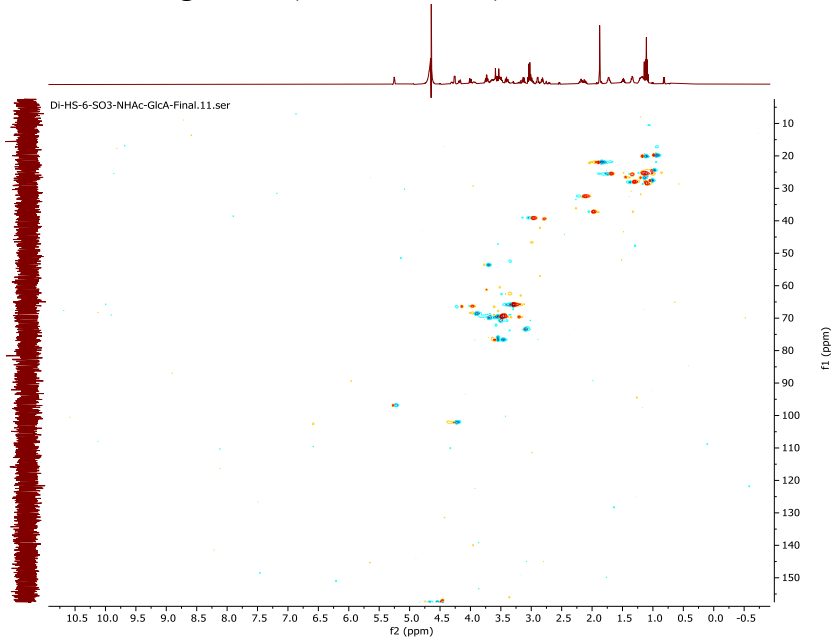


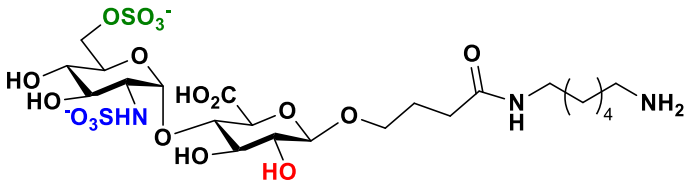
5

^{1}H-NMR (D_2O, 500 MHz) of **5**



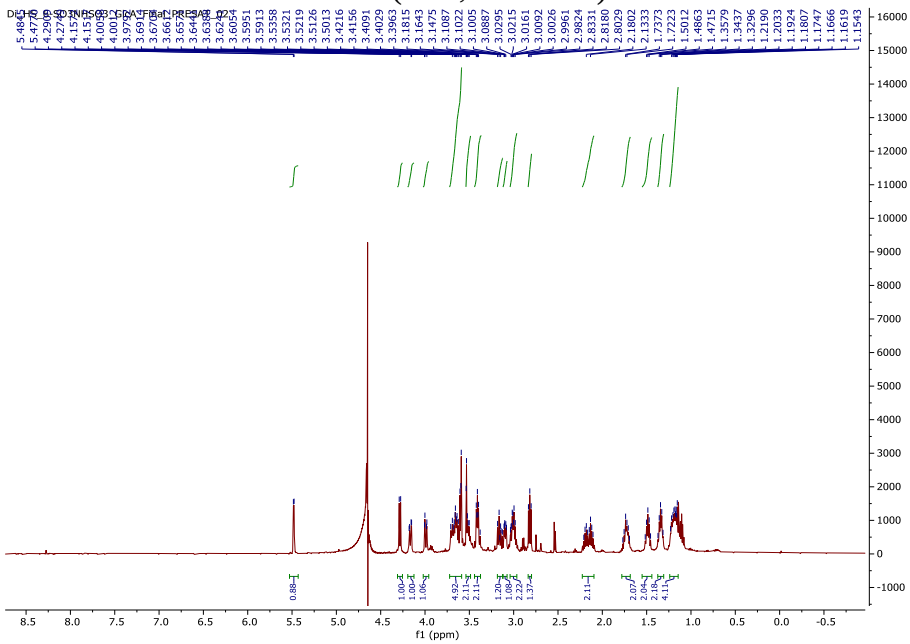
gHSQC (D_2O, 500 MHz) of **5**



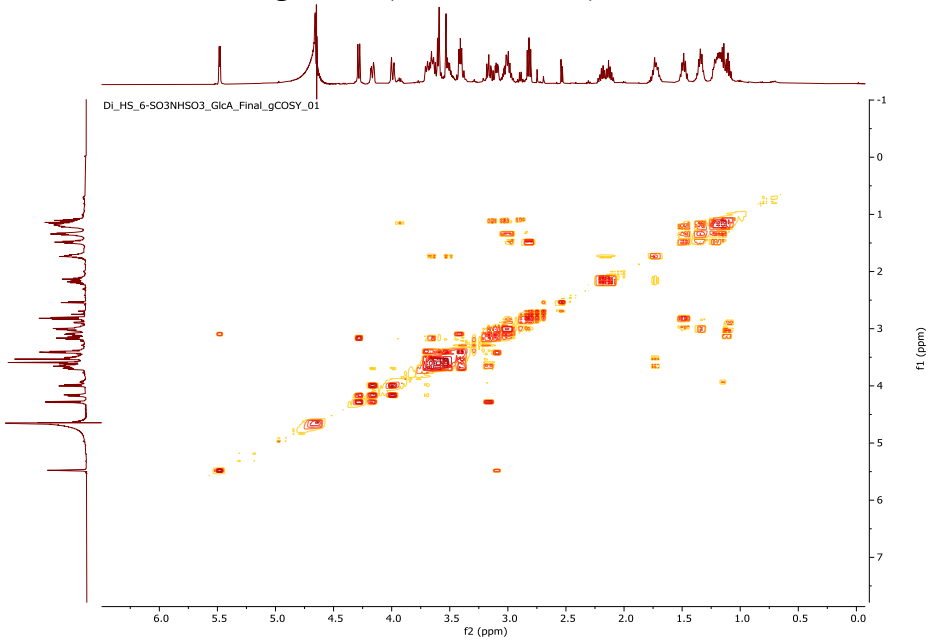


6

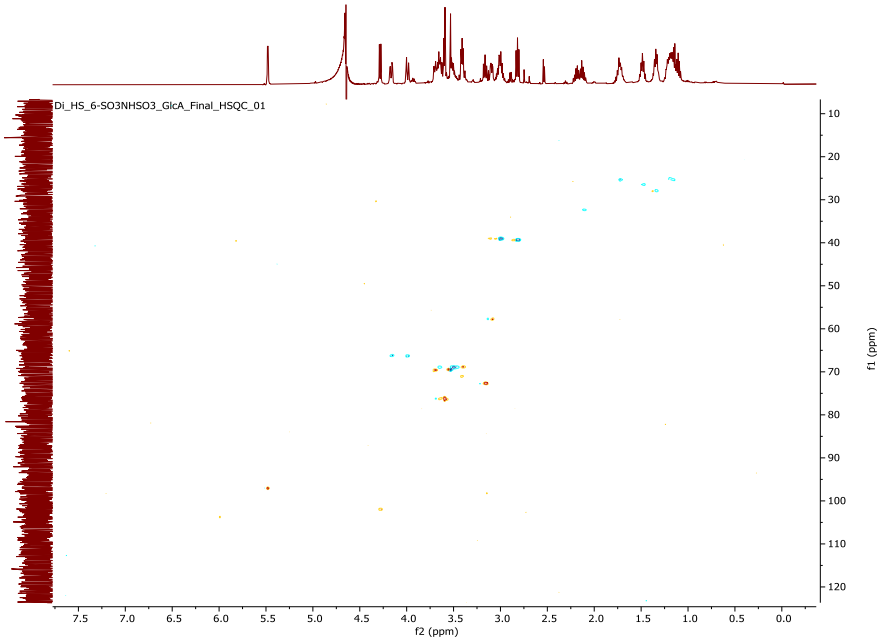
^{1}H-NMR (D_2O, 500 MHz) of **6**

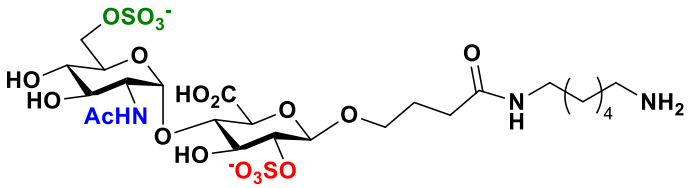


gCOSY (D_2O, 500 MHz) of **6**



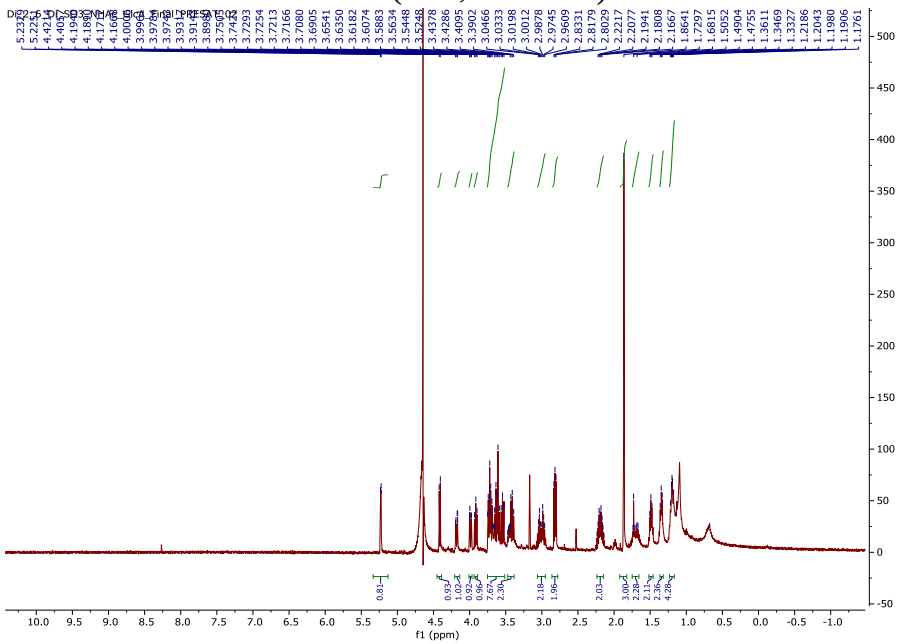
gHSQC (D_2O, 500 MHz) of **6**



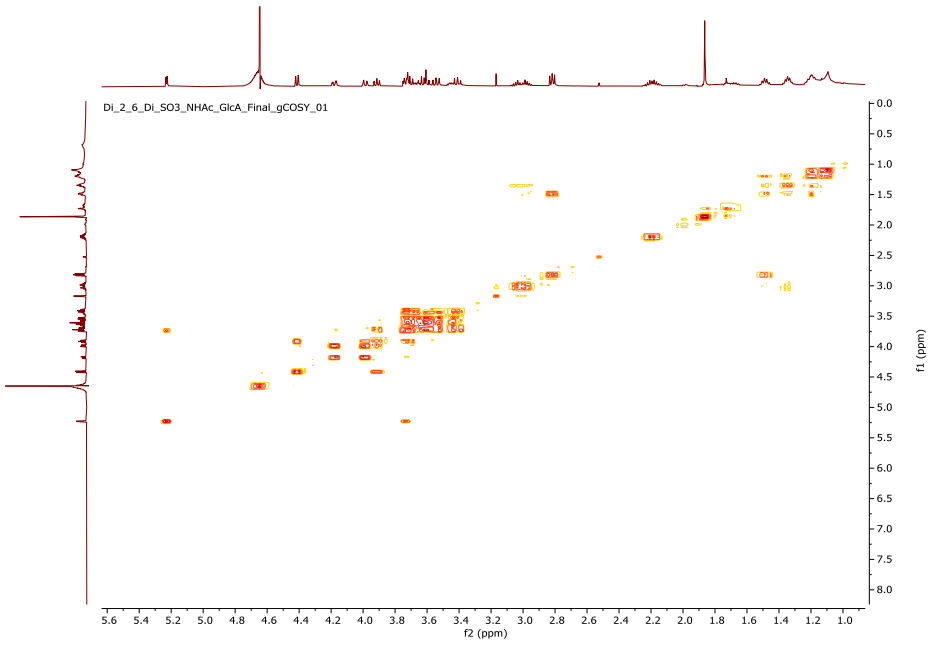


7

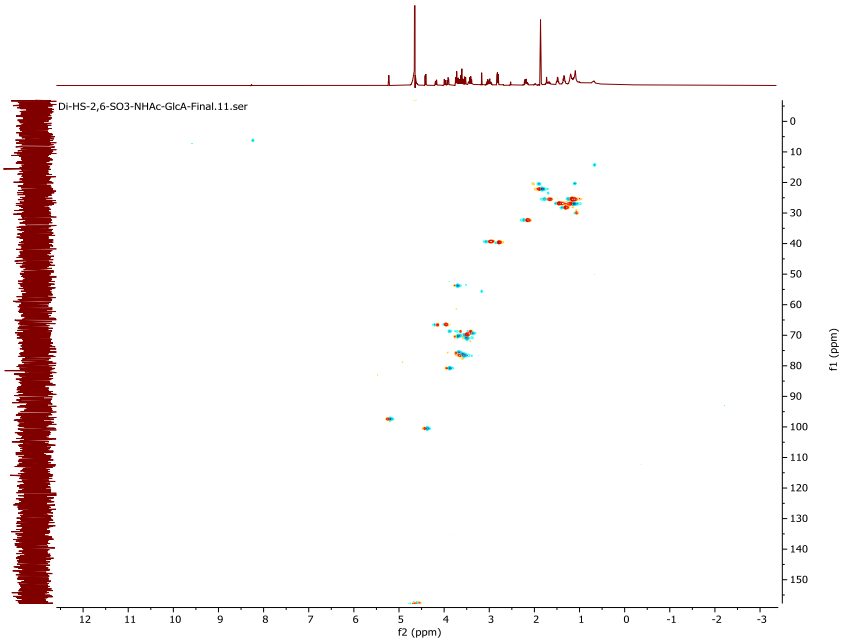
^{1}H-NMR (D_2O, 500 MHz) of **7**

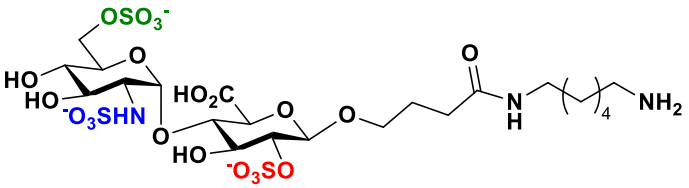


gCOSY (D_2O, 500 MHz) of **7**



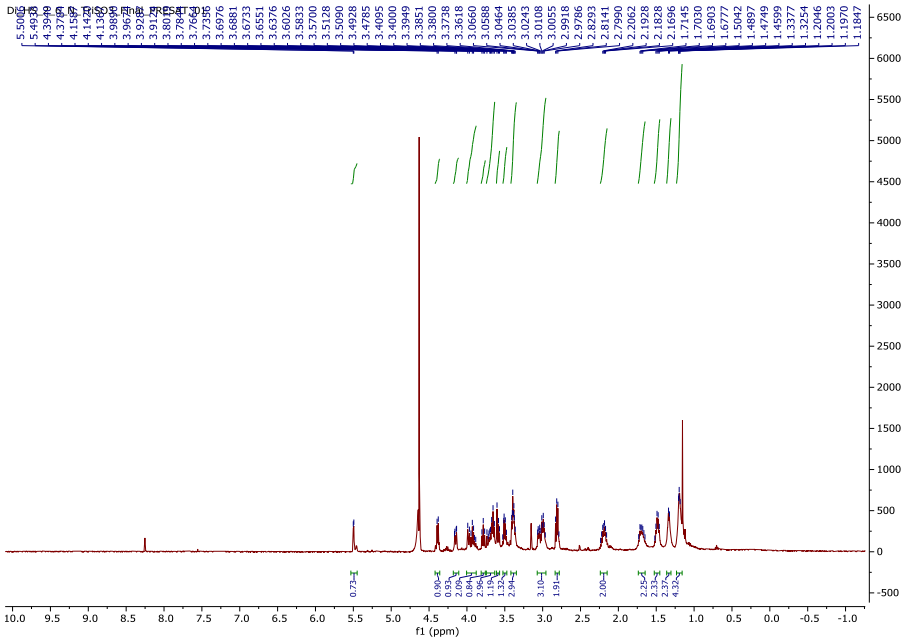
gHSQC (D_2O, 500 MHz) of **7**





8

^{1}H-NMR (D_2O, 500 MHz) of **8**



gHSQC (D_2O, 500 MHz) of **8**