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Electronic Supplementary Information

Using Automated Glycan Assembly (AGA) for the Practical Synthesis of Heparan Sulfate Oligosaccharide Precursors

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Contents

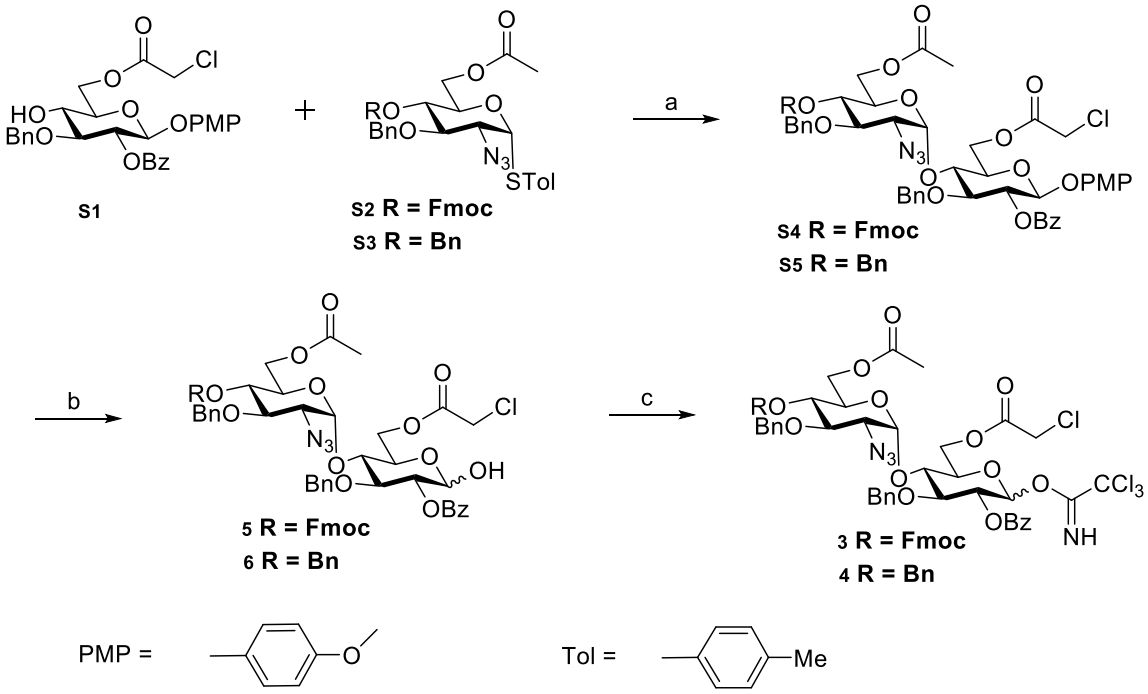
1. General Materials and Methods

All chemicals used were obtained from commercial sources and used as received, unless specified otherwise. Except where stated, all experimental procedures were carried out under an atmosphere of nitrogen. The anhydrous solvents were obtained from an Innovative Technology Inc. PureSolv solvent purification system. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel $60F_{254}$ pre-coated aluminium foil sheets. The reaction TLC was visualised using UV light (254 nm) and stained with 10% ethanolic sulphuric acid solution. Flash column chromatography was performed using slurry packed Fluka silica gel 60 Å, pore size 200-400 mesh, under a light positive pressure, eluting with a specified system. ^{1}H, ^{13}C NMR spectra were recorded on a Bruker 500-MR spectrometer, operating at 500 MHz and 125 MHz respectively with Me_4Si as the internal standard. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) are quoted in Hertz (Hz) to the nearest 0.1 Hz. NMR abbreviations are given as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), pentet (p) or multiplet (m). Signal

assignment was achieved by analysis of ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and DEPT experiments where required. TopSpin 3.5pl7 and MestReNova were primarily used for processing the spectral data. MALDI data was recorded on a Bruker Solarix XR 9.4 T instrument. Small-molecule HRMS data were obtained on a Bruker Daltonics microTOF at room temperature. Fourier transform infrared (FTIR) spectra were recorded on a PerkinElmer UATR 2 spectrometer by attenuated total reflectance (ATR) technique.

2. Synthesis of Building Blocks:

The disaccharide building block was synthesized according to the following scheme **(Scheme S1)**:



Reagents and conditions: a) NIS, AgOTf, DCM/toluene, 71-79%; b) $Ce(NH_4)_2NO_3$, aq. CH_3CN; c) Cl_3CCN, NaH, DCM, 81-85% yield in two steps.

Scheme S1: Synthesis of disaccharide building blocks

2.1 General method for synthesis of disaccharide building block

Thioglycoside donor (1.5 eq) and alcohol acceptor (1.0 eq) were dissolved in a mixture of anhydrous toluene and anhydrous dichloromethane (25 mL per mmol acceptor), cooled to -15 °C and powdered dry molecular sieves (4Å) were added. After 10 min *N*-iodosuccinimide (1.7 eq) and silver trifluoromethanesulfonate (0.4 eq) were added. The reaction mixture was allowed to warm to room temperature over 1 h. The mixture was diluted with ethyl acetate and filtered through celite. The filtrate was washed with a 1:1 mixture of saturated aq. sodium bicarbonate and aq. thiosulfate (30%), washed with saturated aq. sodium chloride, dried over magnesium sulphate and concentrated. The residue was purified by flash chromatography or crystallisation.

2.1.1 Synthesis of 4-methoxyphenyl 4-*O*-[6-*O*-acetyl-2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(9-fluorenylmethyloxycarbonyl)-α-D-glucopyranosyl]-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-β-D-glucopyranoside (S4)

Compound S4[1] was prepared from reported donor S2 and acceptor S1 according to general procedure (71 % crystalline α-anomer), crystallised from hot toluene after addition of petroleum ether. TLC (toluene:ethyl acetate, 19:1 v/v): R_f = 0.33; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.08 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.75 (dd, *J* = 7.6, 4.1 Hz, 2H), 7.62 – 7.53 (m, 3H), 7.46 (t, *J* = 7.8 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.30 – 7.21 (m, 12H), 6.91 (d, *J* = 9.1 Hz, 2H), 6.78 (d, *J* = 9.1 Hz, 2H), 5.60 – 5.53 (m, 2H), 5.12 (d, *J* = 7.0 Hz, 1H), 4.89 – 4.84 (m, 1H), 4.82 – 4.76 (m, 2H), 4.72 (d, *J* = 10.9 Hz, 1H), 4.66 – 4.60 (m, 2H), 4.49 (dd, *J* = 10.5, 6.7 Hz, 1H), 4.39 (dd, *J* = 11.8, 6.1 Hz, 1H), 4.33 (dd, *J* = 10.5, 7.3 Hz, 1H), 4.27 (dd, *J* = 12.4, 4.9 Hz, 1H), 4.19 (t, *J* = 6.9 Hz, 1H), 4.11 – 4.04 (m, 3H), 4.03 (d, *J* = 2.8 Hz, 2H), 4.01 – 3.97 (m, 1H), 3.97 – 3.91 (m, 2H), 3.75 (s, 3H), 3.37 (dd, *J* = 10.3, 3.9 Hz, 1H), 2.06 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5, 166.9, 165.0, 155.6, 154.1, 150.9, 143.2, 142.9, 141.3, 141.2, 137.2, 137.0, 133.5, 129.8, 129.4, 128.6, 128.4, 128.3, 127.9, 127.9, 127.8, 127.8, 127.6, 127.2, 127.2, 125.1, 124.9, 120.1, 120.0, 118.6, 114.5, 99.9, 97.8, 82.4, 77.4, 75.1, 74.7, 74.6, 74.2, 73.4, 72.3, 70.3, 68.7, 64.8, 62.5, 61.9, 55.6, 46.6, 40.5, 20.6; HRMS (ESI) calculated for $C_{59}H_{56}N_3ClO_{16}Na$ $[M + Na]^+$ *m/z* 1120.3247, found 1120.3249.

2.1.2 Synthesis of 4-methoxyphenyl 4-*O*-(6-*O*-acetyl-2-deoxy-2-azido-3,4-di-*O*-benzyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-β-D-glucopyranoside (S5)

Compound S5 was prepared from reported donor S3 and acceptor S1 according to general procedure (79 % crystalline α-anomer), crystallised from toluene after addition of petroleum ether. TLC (toluene:ethyl acetate, 19:1 v/v): R_f = 0.41; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.07 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 2H), 7.39 – 7.29 (m, 8H), 7.28 (d, *J* = 1.7 Hz, 1H), 7.27 (s, 1H), 7.25 (s, 1H), 7.22 (dd, *J* = 6.6, 3.4 Hz, 4H), 6.89 (d, *J* = 9.1 Hz, 2H), 6.76 (d, *J* = 9.1 Hz, 2H), 5.58 – 5.52 (m, 2H), 5.09 (d, *J* = 7.2 Hz, 1H), 4.90 – 4.77 (m, 5H), 4.62 – 4.54 (m, 2H), 4.38 (dd, *J* = 11.8, 6.2 Hz, 1H), 4.27 (dd, *J* = 12.1, 2.2 Hz, 1H), 4.21 (dd, *J* = 12.1, 4.7 Hz, 1H), 4.09 (t, *J* = 8.1 Hz, 1H), 4.06 – 3.99 (m, 3H), 3.94 – 3.88 (m, 2H), 3.85 (ddd, *J* = 10.0, 4.6, 2.2 Hz, 1H), 3.74 (s, 3H), 3.53 – 3.46 (m, 1H), 3.32 (dd, *J* = 10.4, 3.9 Hz, 1H), 2.04 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5, 166.9, 165.0, 155.6, 150.9, 137.5, 137.3, 137.1, 133.4, 129.8, 129.4, 128.6, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 118.6, 114.5, 100.0, 98.0, 82.6, 80.1, 77.8, 75.5, 75.2, 74.6, 74.2, 73.6, 72.4, 70.3, 64.9, 63.1, 62.6, 55.6, 40.5, 20.7; HRMS (ESI) calculated for $C_{51}H_{52}N_3ClO_{14}Na$ $[M + Na]^+$ *m/z* 988.3036, found 1120.3040.

2.2 General method for anomeric deprotection and trichloroacetamidate installation

Ammonium cerium (IV) nitrate (1.3 eq) was added to a solution of the starting *p*-methoxyphenylglycoside (1 eq) in a mixture of 7:1 acetonitrile and water (12.5 mL per mmol). The mixture was stirred at ambient temperature until TLC (toluene/ethyl acetate 4:1 v/v) indicated complete consumption of the starting material (2-6 hours). The reaction mixture was diluted with ethyl acetate and washed with successively with water (2 x), saturated aq. sodium chloride followed by drying over magnesium sulfate and concentrated. The residue was purified by flash chromatography (stepwise gradient 4%, 10%, 20% ethyl acetate in toluene) to give the hemiacetal product as a foam. This material was dissolved in trichloroacetonitrile (20 eq) and the same volume of anhydrous dichloromethane. The solution was cooled in an ice-bath and sodium hydride (60 % in mineral oil) (0.05 eq) was added. After 5 min the ice-bath was removed and the reaction was allowed to warm to ambient temperature over 1 hour, until TLC (toluene/ethyl acetate 4:1 v/v) indicated completion. The reaction was quenched by addition of silica (~15 cm^3), evaporated to dryness and purified by flash chromatography to yield the trichloroacetimidate donor.

2.2.1 Synthesis of trichloroacetamidate donor (3)

Compound 3 was prepared from compound S4 following general procedure described in section 2.2. The crude donor was purified by flash chromatography (5% ethyl acetate in toluene, dry-loaded) to

furnish the pure donor (83%, 2 anomers in the ratio of α:β = 1:0.3) as an off-white foam. TLC (toluene/ethyl acetate, 5:1 v/v): R_f = 0.6/0.7; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.64 (s, 0.3H), 8.60 (s, 1H), 8.04 (dd, *J* = 8.4, 1.4 Hz, 0.6H), 8.0 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.76 (dt, *J* = 7.6, 3.4 Hz, 2.8H), 7.62 – 7.53 (m, 4H), 7.44 – 7.37 (m, 5H), 7.30 – 7.26 (m, 3H), 7.25 – 7.20 (m, 7.6H), 7.20 – 7.16 (m, 4.4H), 6.58 (d, *J* = 3.6 Hz, 1H), 6.1 (d, *J* = 6.1 Hz, 0.3H), 5.66 (d, *J* = 3.9 Hz, 1H), 5.60 (t, *J* = 6.2 Hz, 0.3H), 5.45 (d, *J* = 3.9 Hz, 0.3H), 5.41 (dd, *J* = 9.7, 3.6 Hz, 1H), 4.92 – 4.75 (m, 5.3H), 4.68 (d, *J* = 10.9 Hz, 1.3H), 4.62 (dd, *J* = 11.9, 2.4 Hz, 1.3H), 4.55 – 4.45 (m, 2H), 4.45 – 4.40 (m, 1H), 4.40 – 4.35 (m, 1.3H), 4.32 – 4.26 (m, 2.3H), 4.24 – 4.21 (m, 1H), 4.20 – 4.17 (m, 1.3H), 4.15 – 4.12 (m, 2.6H), 4.09 (dd, *J* = 5.8, 2.5 Hz, 1.3H), 4.06 (d, *J* = 2.8 Hz, 0.6H), 4.05 – 3.96 (m, 3.6H), 3.89 (t, *J* = 9.6 Hz, 0.3H), 3.35 (ddd, *J* = 10.3, 3.9, 2.2 Hz, 1.3H), 2.06 (s, 1H), 2.05 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5, 170.4, 167, 166.9, 165.2, 164.8, 160.4, 154.1, 154.1, 143.2, 142.9, 141.3, 141.2, 137.4, 137.0, 133.6, 133.5, 129.8, 129.7, 129.2 128.9, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.3, 127.2, 127.2, 125.1, 125.0, 124.9, 124.8, 120.1, 120.0, 98.4, 98.2, 95.6, 93.1, 90.8, 90.4, 80.8, 79.9, 77.4, 75.1, 75.0, 74.7, 74.5, 72.8 72.7, 71.0, 70.5, 70.4, 70.3, 68.8, 68.7, 64.7, 64.3, 62.6, 62.5, 61.9, 61.8, 46.7, 46.6, 40.7, 40.6, 20.7, 20.6; HRMS (ESI) calculated for $C_{54}H_{50}N_4Cl_4O_{15}Na$ $[M + Na]^+$ *m/z* 1157.1924, found 1157.1929.

2.2.2 Synthesis of trichloroacetamidate donor (4)

Compound 4 was prepared from compound S5 following general procedure described in section 2.2. The crude donor was purified by flash chromatography (5% ethyl acetate in toluene, dry-loaded) to furnish the pure donor (85%, 2 anomers in the ratio of α:β = 3:2) as off-white foam. TLC (toluene/ethyl acetate, 9:1 v/v): R_f = 0.45/0.5; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.62 (s, 0.4H), 8.58 (s, 0.6H), 8.02 (d, *J* = 8.5 Hz, 0.8H), 8.00 (d, *J* = 8.5 Hz, 1.2H), 7.57-7.52 (m, 1H), 7.42-7.12 (m, 17H), 6.57 (d, *J* = 3.6 Hz, 0.6H) 6.08 (d, *J* = 6.3 Hz, 0.4H), 5.60 (d, *J* = 3.8 Hz, 0.6H), 5.59 (dd, *J* = 6.3 Hz, *J* = 6.3 Hz, 0.4H), 5.44 (d, *J* = 3.8 Hz, 0.4H), 5.39 (dd, *J* = 3.7 Hz, *J* = 9.7 Hz, 0.6H), 4.96-4.75 (m, 5H), 4.63-4.55 (m, 2H), 4.41-4.31 (m, 1.6H), 4.29-4.17 (m, 2.6H), 4.13-4.05 (m, 3.2H), 4.01 (dd, *J* = 8.6 Hz, *J* = 10.0 Hz, 0.6H), 3.97 (dd, *J* = 8.8 Hz, *J* = 10.4 Hz, 0.6H), 3.90-3.81 (m, 1.4H), 3.52 (dd, *J* = 9.0 Hz, *J* = 9.9 Hz, 0.6H), 3.49 (dd, *J* = 9.0 Hz, *J* = 9.9 Hz, 0.4H), 3.32-3.27 (m, 1H), 2.03 (s, 1.2H), 2.02 (s, 1.8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5, 170.4, 167.0, 165.3, 164.8, 137.6, 137.2, 133.6, 133.5, 129.8, 129.7, 129.3, 129.1, 129.0, 128.6, 128.5, 128.4, 128.25, 128.2, 128.15, 128.1, 128.0, 127.8, 127.7, 127.4, 125.3, 98.7, 98.3, 95.7, 93.2, 81.4, 80.1, 79.9, 77.9, 77.8, 75.6, 75.5, 75.2, 75.0, 74.6, 74.5, 73.8, 73.0, 72.9, 64.8, 64.4, 63.3, 63.1, 62.6, 62.4, 40.7, 40.6, 20.8; HRMS (ESI) calculated for $C_{46}H_{46}N_4Cl_4O_{13}Na$ $[M + Na]^+$ *m/z* 1025.1713, found 1025.1718.

3. Automated synthesis of heparan sulfate protected oligosaccharide

3.1 General methods:

All anhydrous solvents from Innovative Technology Inc. PureSolv solvent purification system were used and kept under argon. The building blocks were coevapporated with toluene thrice and dried overnight in a high vacuum prior to use. The building block, activator and deprotection solutions were freshly prepared and kept under argon during the automation run.

3.2 Preparation of stock solutions[2]

Building block solution: 0.2 mmol of building block in 3 mL of DCM (for 10 equivalents) and 0.1 mmol in 1.5 mL of DCM (for 5 equivalents)

Activator Solution: 108 µL TMSOTf was dissolved in 30 mL DCM

TMSOTf acid wash solution: 500 µL TMSOTf in 40 mL DCM

Fmoc deprotection solution: 5% (v/v) piperidine in DMF

3.3 Modules for automated synthesis

3.3.1 Resin swelling

The functionalised Merrifield resin was purchased from GlycoUniverse GmbH & Co KGaA. The resin (50 mg, loading 0.4 mmol g^{-1}, 0.02 mmol) was loaded into the reaction vessel (RV) upon the synthesizer and washed with DMF, THF, DCM (3x with 2 mL for 10 s). After washing, the resin was swollen in 2 mL of DCM and incubated for 30 min with occasional argon mixing. After the resin is swollen, the DCM is drained to waste.

3.3.2 TMSOTf acidic wash

To the swollen resin, 2 mL DCM was added and the temperature was adjusted to -20 °C. To the RV, 1 mL of the TMSOTf acid wash solution was delivered at -20 °C, incubated for 1 min and then drained to waste. The acid wash is performed prior to a glycosylation cycle to ensure neutralization of any basic residues present on the resin from previous synthesizer cycle. After the acid wash, the resin is swollen in 2 mL of DCM.

3.3.3 Glycosylation module

3.3.3.1 Module 1:

The temperature of the RV was adjusted to -15 °C (T_1) whilst the DCM present was drained and a solution of trichloacetamidate disaccharide building block (10 eq. in 3 mL DCM, 0.2 mmol) was delivered to the RV. After the T_1 was reached, the glycosylation commenced by the addition of 1 mL of TMSOTf (2 eq. in 1 mL DCM, 0.04 mmol) solution. The glycosylation mixture was activated for 10 min. (t_1) at T_1 with continuous argon mixing. The temperature was then linearly ramped to incubation temperature 0 °C (T_2) and the glycosylation was continued at T_2 for 40 min (t_2). After completion of the reaction, the excess sugar was drained to fraction collector. The hydrolysed sugar was subjected to trichloroacetamidate installation and reused as a building block for glycosylation. The resin was washed with DCM (3x with 2 mL, for 25 s) and the temperature of the RV was adjusted to 25 °C.

3.3.3.2 Module 2:[3]

Conditions as described for module 1, differing in the amount of building block (5 eq. in 1.5 mL DCM, 0.1 mmol) and activating reagent, TMSOTf (1 eq. in 1 mL DCM, 0.02 mmol) solution.

For each glycosidic bond formation, two glycosylation cycles were performed. During the attachment of the first building block to the resin, module 1 was used in the first cycle to ensure maximum loading, followed by module 2. For further automated glycan assembly, two cycles of module 2 were used.

3.3.4 Fmoc deprotection module

The resin was washed with DMF (6x with 2 mL, for 25 s), swollen in 2 mL DMF. After the set temperature (25 °C) was reached, the DMF was drained and 2 mL of a solution of 5% piperidine in DMF was delivered to the RV. The reaction was incubated for 5 min with argon mixing and then drained to waste via the UV detector. A sharp decrease in UV transmittance was observed,

confirming Fmoc cleavage. The procedure was repeated for three times. For the next glycosylation, the resin is washed with DMF, THF and DCM (6x with 2 mL for 25 s).

3.3.5 End of synthesis process

After the completion of synthesis, the temperature of the RV was adjusted to 25 °C. The resin functionalised with oligosaccharide was subsequently washed with DMF and DCM (6x with 2 mL for 25 s). All the solvents were discharged to waste, leaving the resin in a free-flowing condition.

4. Resin cleavage

The oligosaccharide was cleaved from solid support using an in-house constructed continuous flow UV reactor.[4] The continuous UV flow reactor was designed to comprise five parts; a 400W mercury UV lamp surrounded by a cooling jacket, fluorinated ethylene propylene (FEP) tubing wound around the cooling jacket, a peristaltic pump, a resin injection point and a cartridge fitted with a frit to filter the cleaved resin. A diagram of this set-up is depicted below (**Figure S1**)

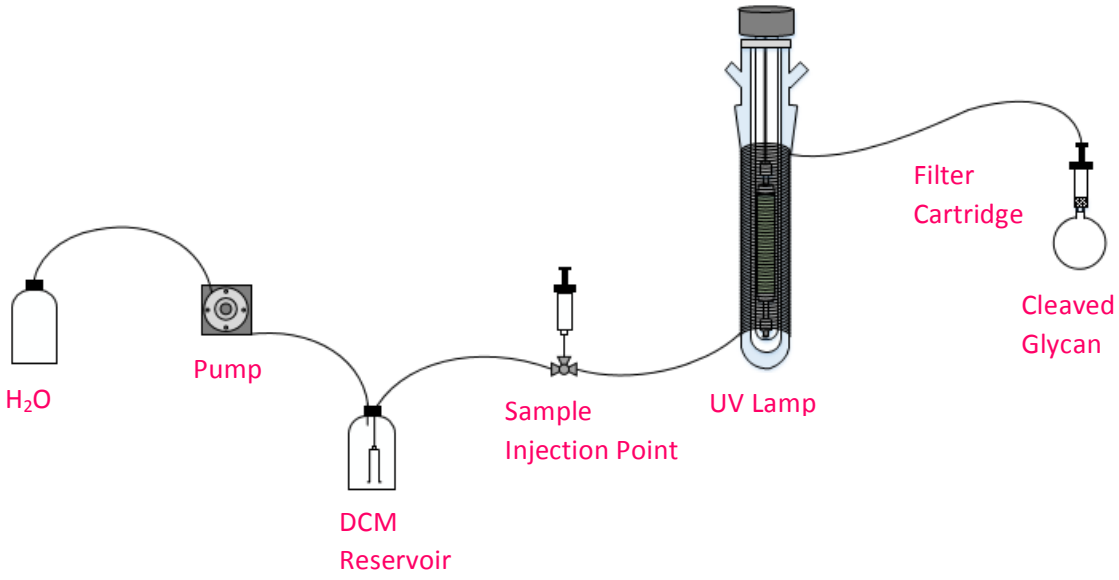
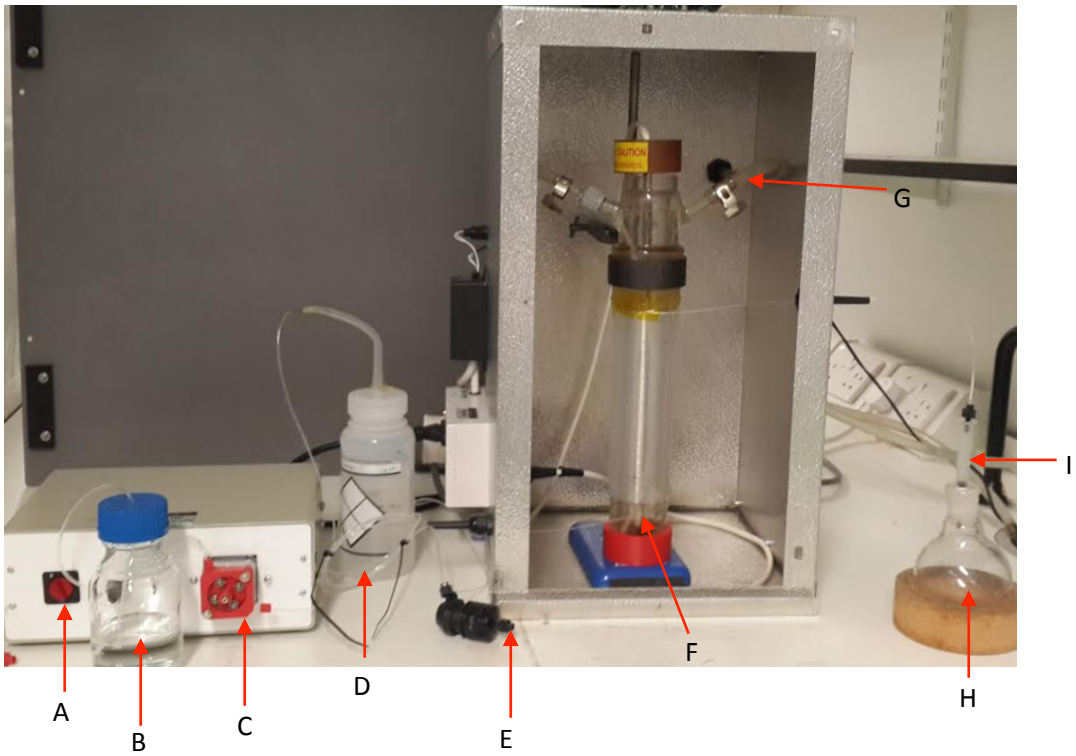


Figure S1: Set up for sample treatment with UV reactor

In order to ensure safe operation of the flow reactor, solvent and UV resistant FEP tubing was utilised (0.75 mm ID, 1.59 mm OD). The FEP tubing was wrapped around the cooling jacket 118 times giving a total surface area per unit volume of 11,289 m^2 m^{-3} for UV exposure. To prevent UV exposure to operators, a metal box with safety lock inter-switches was constructed to encase the UV lamp (**Figure S2**). Initially, a syringe pump design was proposed, however, due to cost benefits and the desire to have controlled and steady flow, a continuous peristaltic pump design was favoured. The pump was designed using Autodesk Inventor software and printed with acrylonitrile butadiene styrene (ABS) using a 3D printer.



[A = On/Off switch, B = H_2O, C = Peristaltic pump, D = DCM reservoir, E = Sample Injection Point, F = 400W mercury UV lamp, G = Cooling jacket, H = UV treated glycan, I = Filter cartridge]

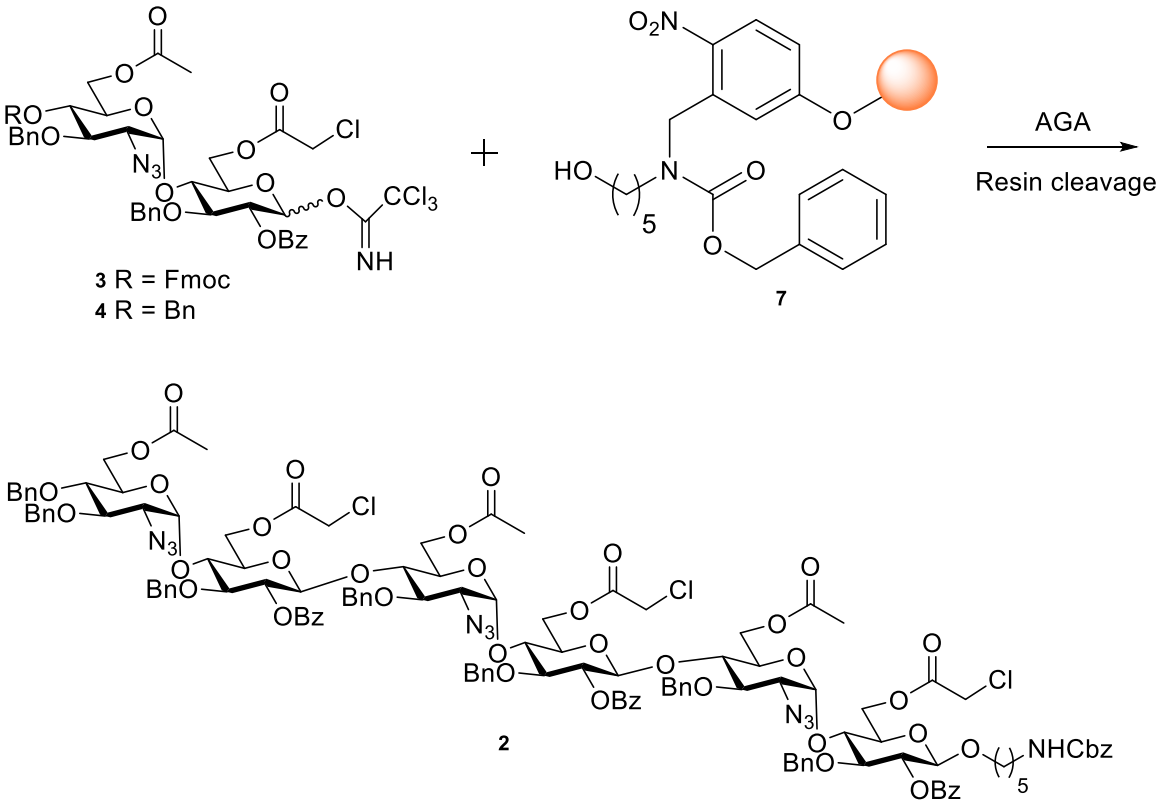
Figure S2: The UV reactor system to cleave glycan from resin

The glycan containing resin was swollen in 2 mL DCM and taken up in a glass syringe. After turning on the UV lamp and equilibrating the tubing with 20 mL DCM, the resin was injected into the flow reactor and pushed through with DCM at a speed of 850 µL min^{-1}. Approximately, 10 mL of DCM was pushed through at this flow rate, affording a minimum irradiation time of 11.30 minutes per pass. To ensure all of the resin was pushed through, another 10 mL of DCM was passed through at the same flow rate. Finally 20 mL of DCM: MeOH (1:1) was pumped by a glass syringe through the reactor. The reactor outlet was directed into a filter of the cartridge and the cleaved oligosaccharide was collected and concentrated *in vacuo*.

This system therefore allows for the user to control single-pass irradiation time between 10 to 20 minutes (based on flow rates ranging from 0.3-0.9 mL min^{-1}). In addition, the system is safe, user-friendly and provides a high surface area of exposure per sample volume (>11,000 m^2 m^{-3}).

5. Automated synthesis of protected hexasaccharide

Schematic diagram of the synthesis of the HS hexasaccharide precursor **2** is illustrated in **Scheme S2**.



Scheme S2: Automated synthesis of HS hexasaccharide precursor

Functionalised resin (50 mg, 0.4 mmol/g, 0.02 mmol) was subjected to glycosylation and deprotection modules as described in **Table S1.**

Steps	Automated Process	Protocol Section
1	Resin swelling	3.3.1
2	TMSOTf acidic wash	3.3.2
3	Glycosylation: Donor **3**	3.3.3.1 + 3.3.3.2
4	Fmoc deprotection	3.3.4
5	TmsOTf acidic wash	3.3.2
6	Glycosylation Donor **3**	3.3.3.2 + 3.3.3.2
7	Fmoc deprotection	3.3.4
8	TMSOTf acidic wash	3.3.2
9	Glycosylation donor **3**	3.3.3.2 + 3.3.3.2
10	Fmoc deprotection	3.3.4
11	TMSOTf acidic wash	3.3.2
12	Glycosylation donor **4**	3.3.3.2 + 3.3.3.2
13	End of synthesis process	3.3.5

Table S1: Automated synthesis protocol for hexasccharide **2**

Cleavage, Analysis and Purification:
The oligosaccharide was cleaved from the resin as described in **Section 4**. The crude product was dissolved in DCM and assayed for MALDI on a Solarix XR instrument, where 1 µL of the sample along with 2,5-dihydroxybenzoic acid (DHB) matrix in 50% MeCN/ 0.1 & CF_3COOH (1 µL), was spotted

directly on the plate. MALDI indicated formation of hexasaccharide with a minor amount of tetrasaccharide by-product (**Figure 3**).

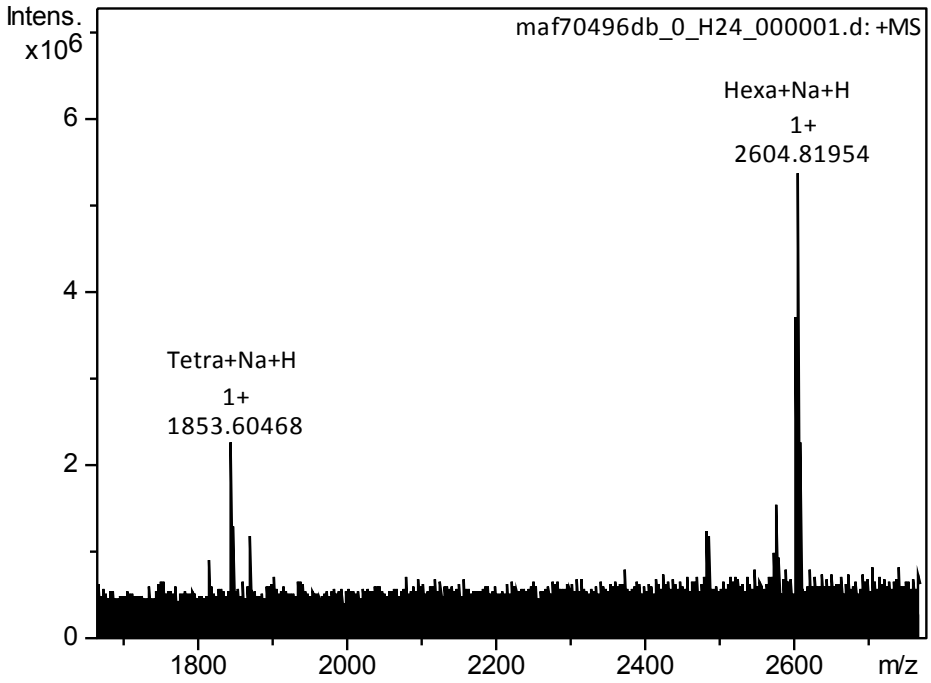


Figure S3: MALDI analysis of crude hexasaccharide **2**

TLC (DCM: MEOH, 99:1 v/v) supported the formation of hexasaccharide as major product

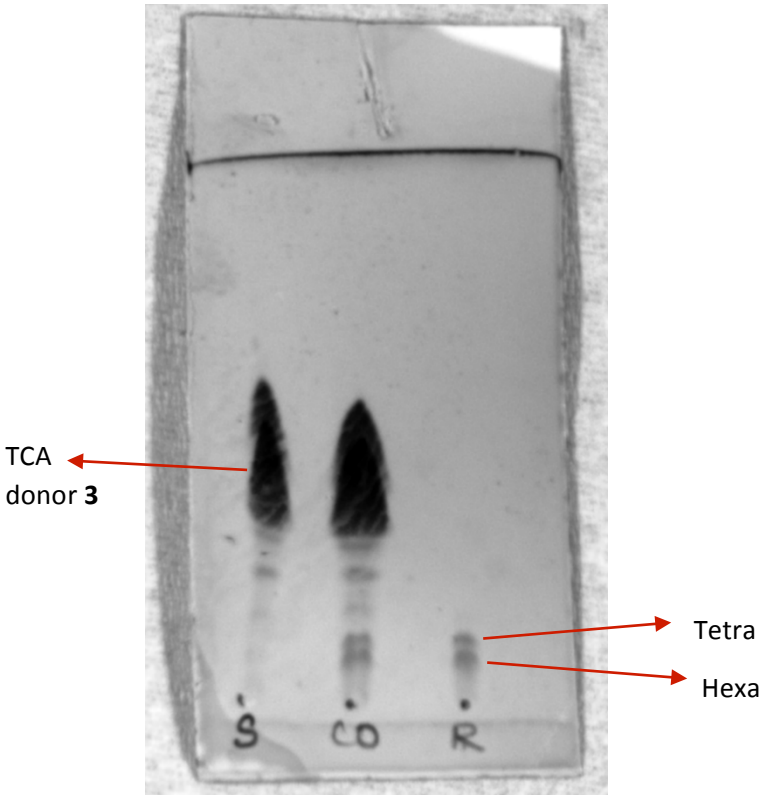
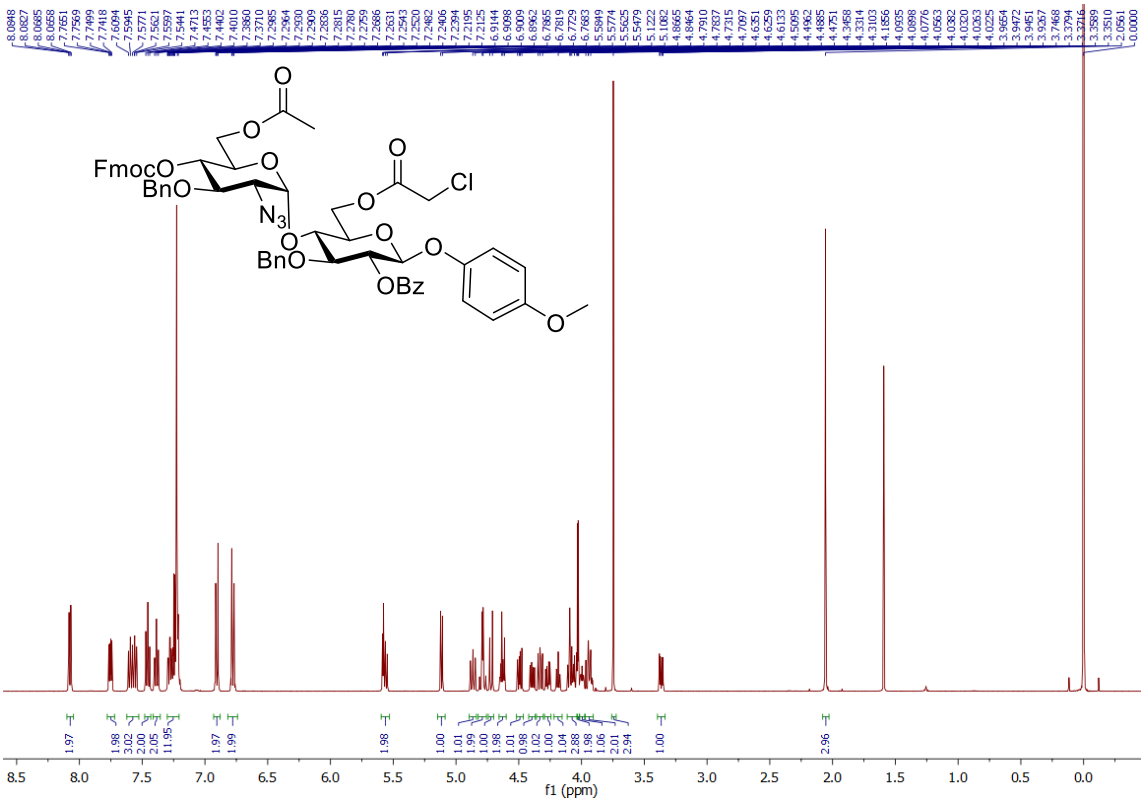


Figure S4: TLC after resin cleavage

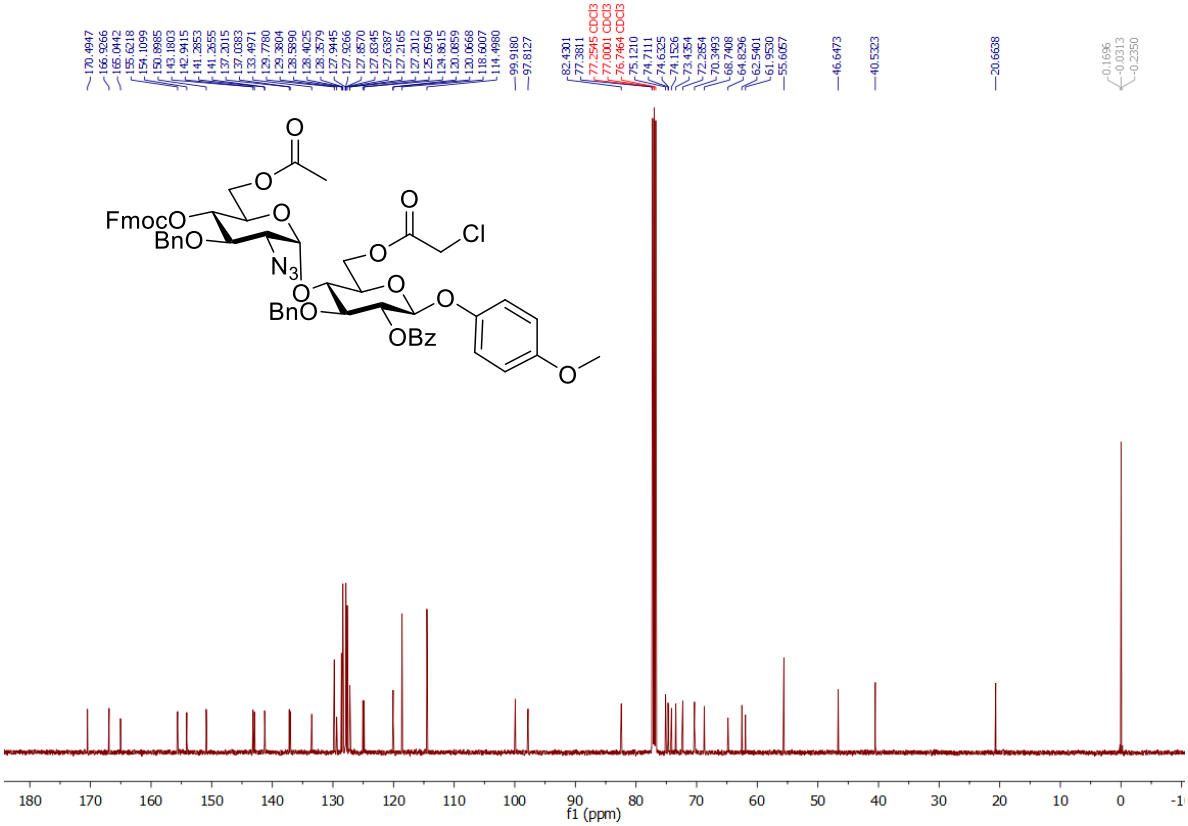
The hexasaccharide was purified by flash chromatography (1% MeOH in DCM) and the fractions containing the product were concentrated to yield the hexasaccharide **2** as a white foam (15.4 mg, 30% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.03 (m, 5H), 7.62-7.56 (m, 2H), 7.53 (dd, *J* = 30.9, 5.8 Hz, 1H), 7.50 – 7.38 (m, 6H), 7.38 – 7.31 (m, 16H), 7.29 (dt, *J* = 7.1, 1.5 Hz, 3H), 7.27 (d, *J* = 7.7 Hz, 7H), 7.24 – 7.21 (m, 2H), 7.20 – 7.10 (m, 11H), 7.10 – 7.06 (m, 2H), 5.46 (m, 2H), 5.41-5.32 (m, 2H), 5.21 (dd, *J* = 9.0, 7.7 Hz, 1H), 5.11 (dd, *J* = 8.4, 3.2 Hz, 1H), 5.11 (dd, *J* = 8.4, 3.2 Hz, 1H), 5.07 (bs, 2H), 5.04 – 4.98 (m, 1H), 4.87 (bs, 2H), 4.80 (dd, *J* = 14.2, 10.6 Hz, 2H), 4.74 – 4.61 (m, 8H), 4.60 – 4.54 (m, 2H), 4.52 – 4.46 (m, 1H), 4.40 – 4.35 (m, 1H), 4.35 – 4.30 (m, 1H), 4.25 – 4.03 (m, 9H), 4.01 – 3.81 (m, 10H), 3.80 – 3.65 (m, 10H), 3.62 – 3.52 (m, 3H), 3.51 – 3.47 (m, 2H), 3.39 (td, *J* = 8.9, 3.8 Hz, 1H), 3.30 (ddd, *J* = 10.4, 3.9, 1.7 Hz, 1H), 3.22 (m, 2H), 2.94 (m, 2H), 2.01, 2.0, 1.99 (3s, 9H), 1.51 – 1.41 (m, 2H), 1.34 – 1.28 (m, 2H), 1.21 – 1.11 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5, 170.4, 166.9, 166.4, 166.3, 165.0, 165.0, 164.9, 156.3, 138.2, 138.1, 137.4, 137.3, 137.3, 137.2, 137.2, 137.2, 137.1, 136.7, 133.8, 133.7, 133.6, 133.4, 129.8, 129.7, 129.6, 129.5, 128.9, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2,128.2, 128.1, 128.1, 128.0, 128.0, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.4, 127.3, 100.9, 100.8, 100.7, 98.0, 97.8, 97.6, 82.9, 82.8, 80.1, 77.7, 77.6, 77.5, 77.5, 77.4, 75.6, 75.4, 75.2, 75.1, 75.0, 74.9, 74.7, 74.6, 74.5, 74.4, 74.2, 74.1, 73.9, 72.3, 72.2, 70.3, 69.7, 69.6, 69.5, 66.5, 64.9, 64.3, 64.1, 63.1, 62.6, 62.5, 62.3, 40.8, 40.5, 40.4, 40.3, 29.3, 28.8, 23.0, 20.8, 20.7, 20.7; HRMS (MALDI) calculated for $C_{131}H_{139}Cl_3N_{10}O_{39}Na$ $[M+Na]^+$ *m/z* 2603.815868, found 2603.816301.

6. NMR spectra:

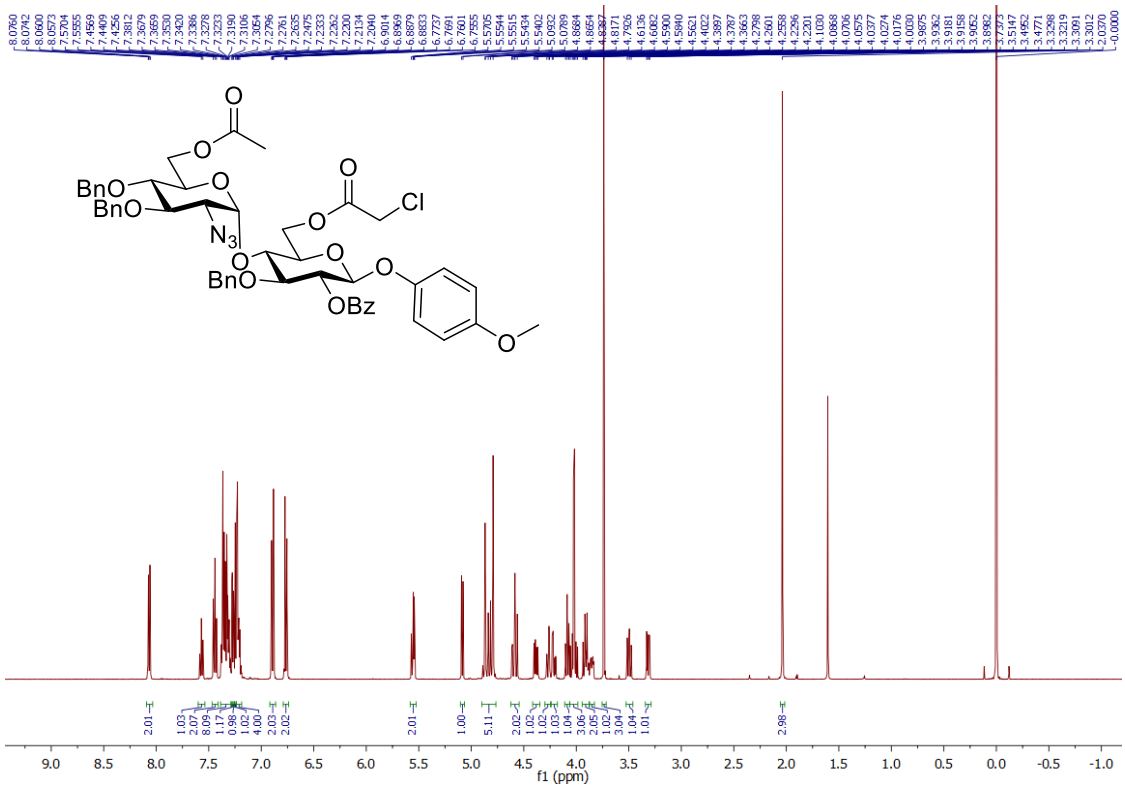
^{1}H NMR spectra of disaccharide S4:



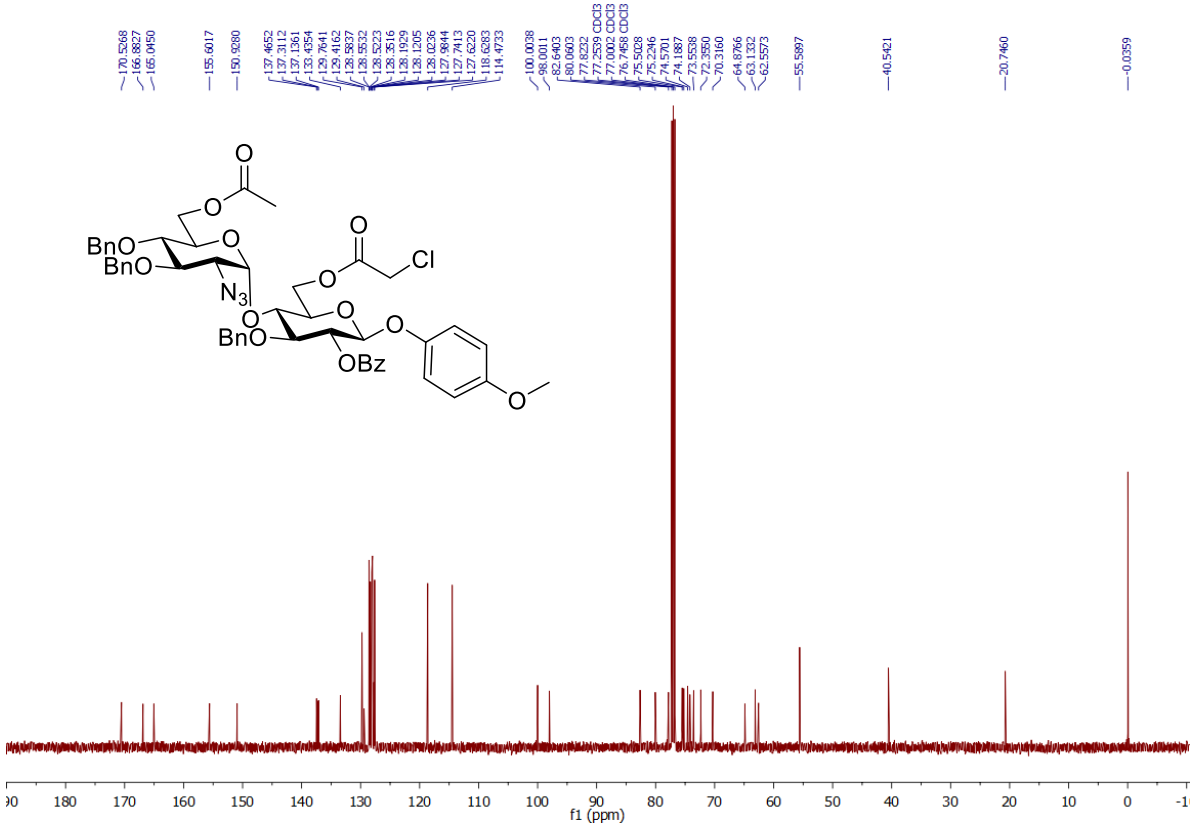
^{13}C NMR of disaccharide S4:



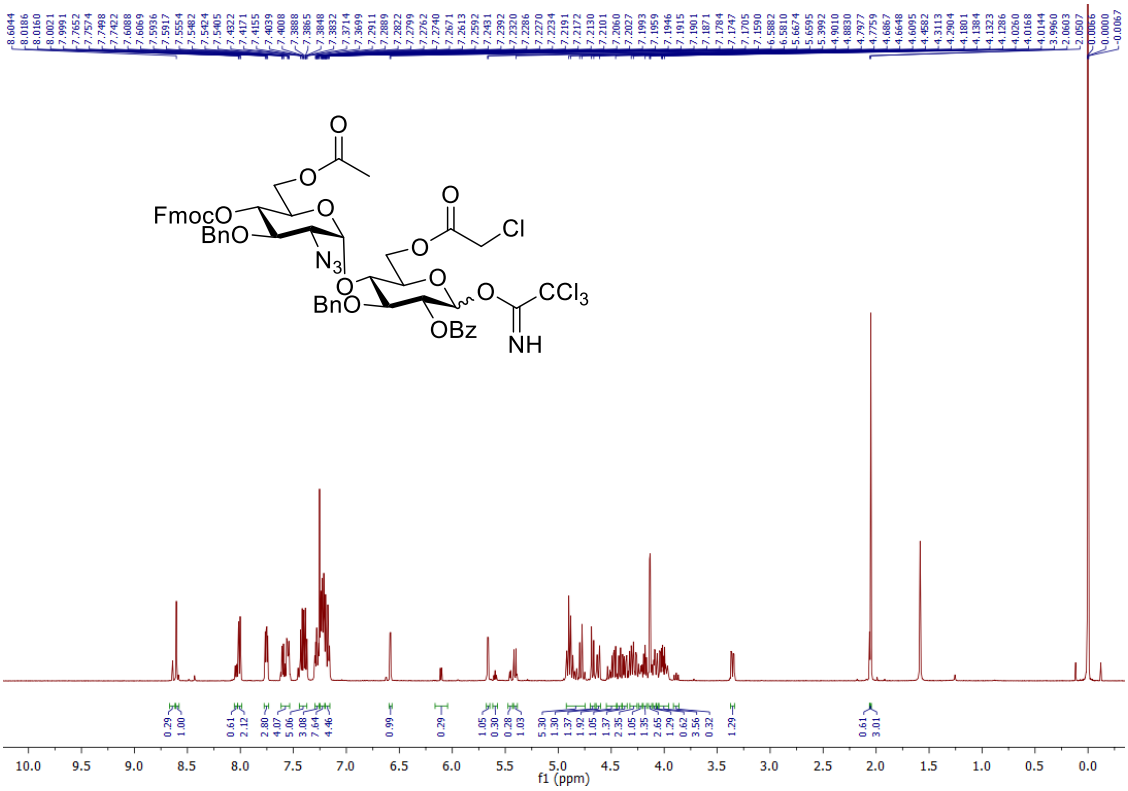
^{1}H NMR spectra of disaccharide S5:



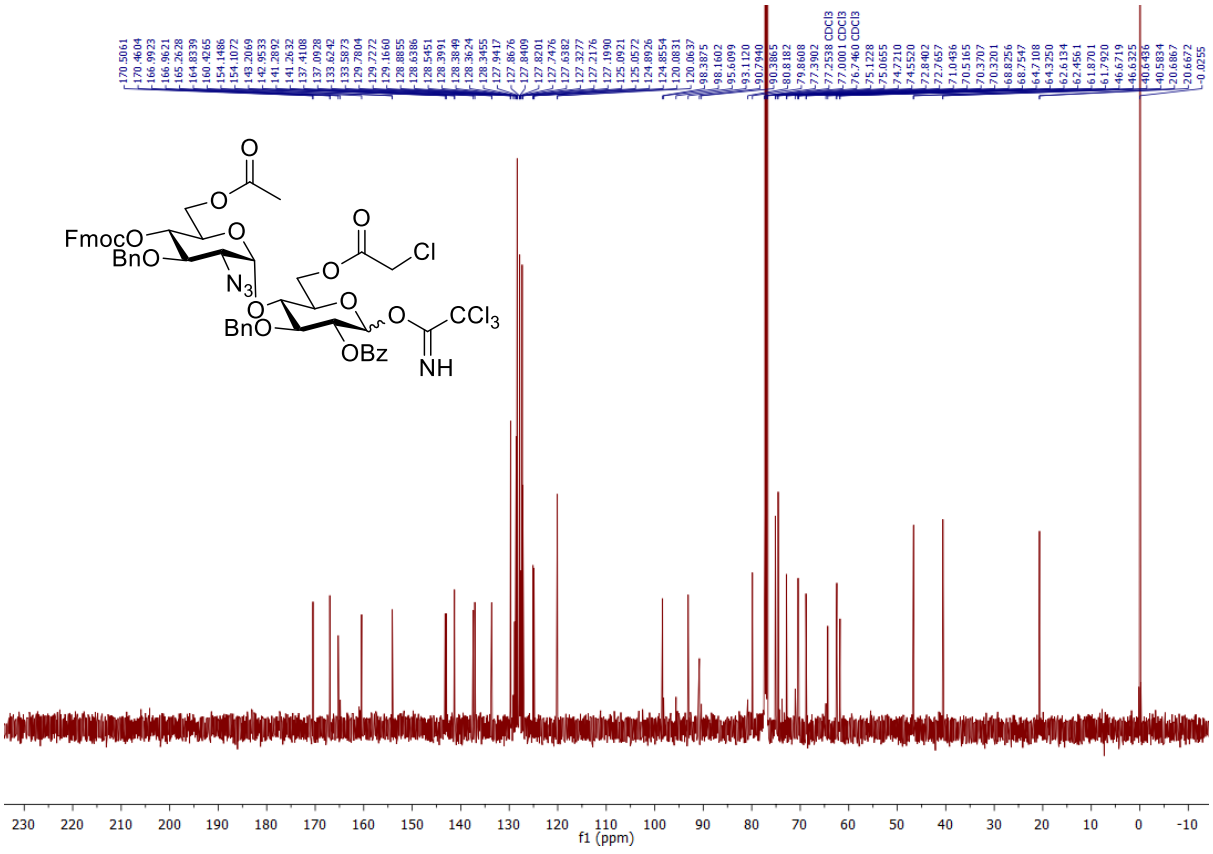
^{13}C NMR of disaccharide S5:



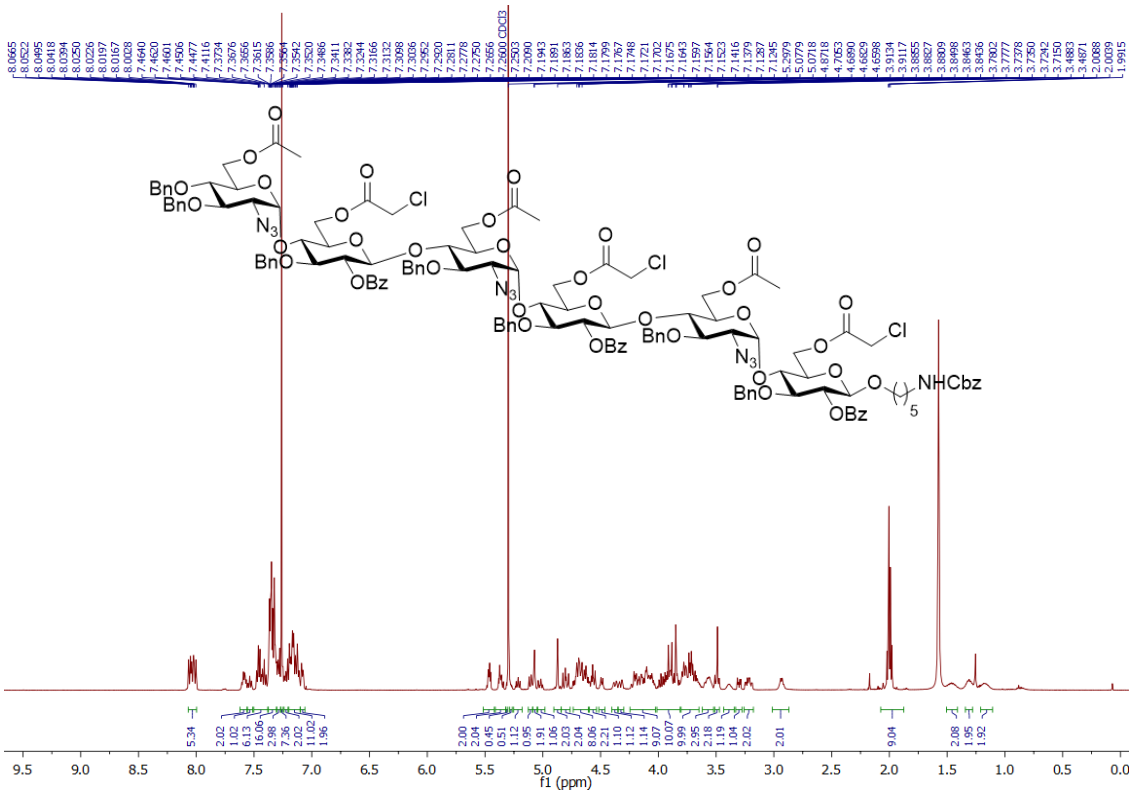
^{1}H NMR spectra of disaccharide 3:



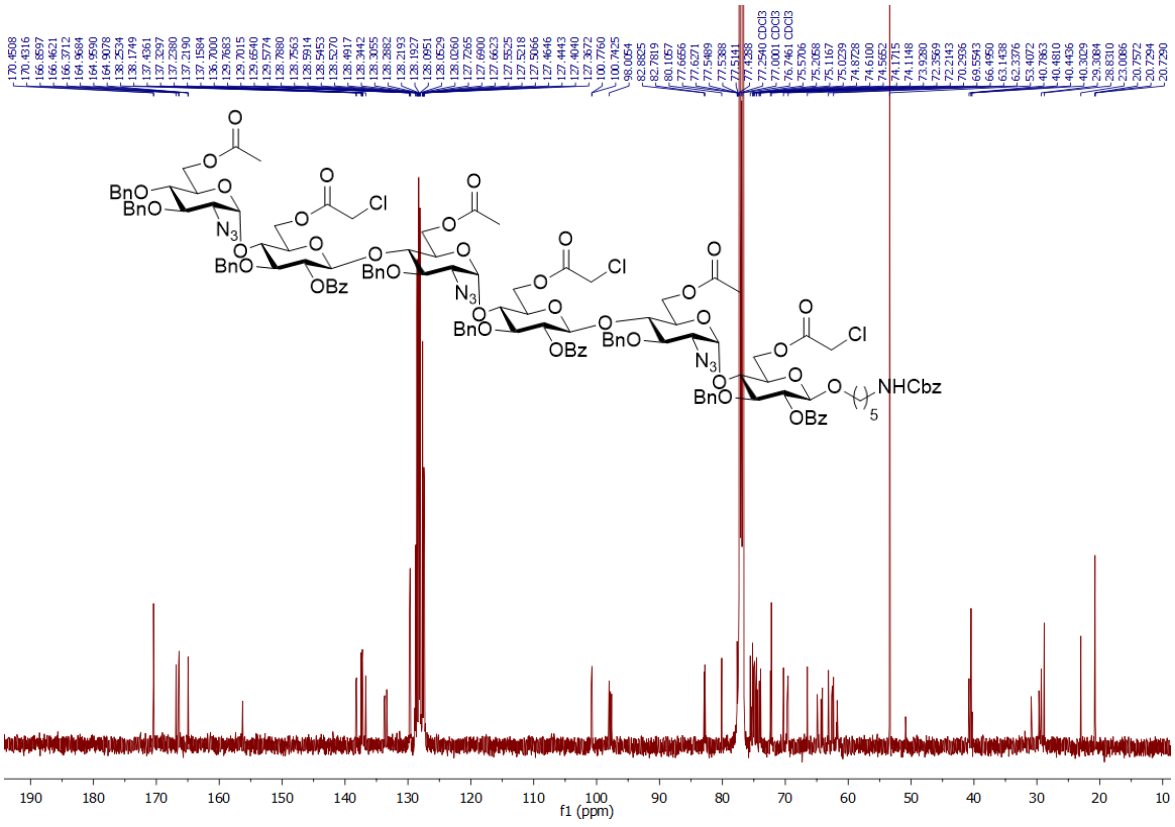
^{13}C NMR of disaccharide 3:



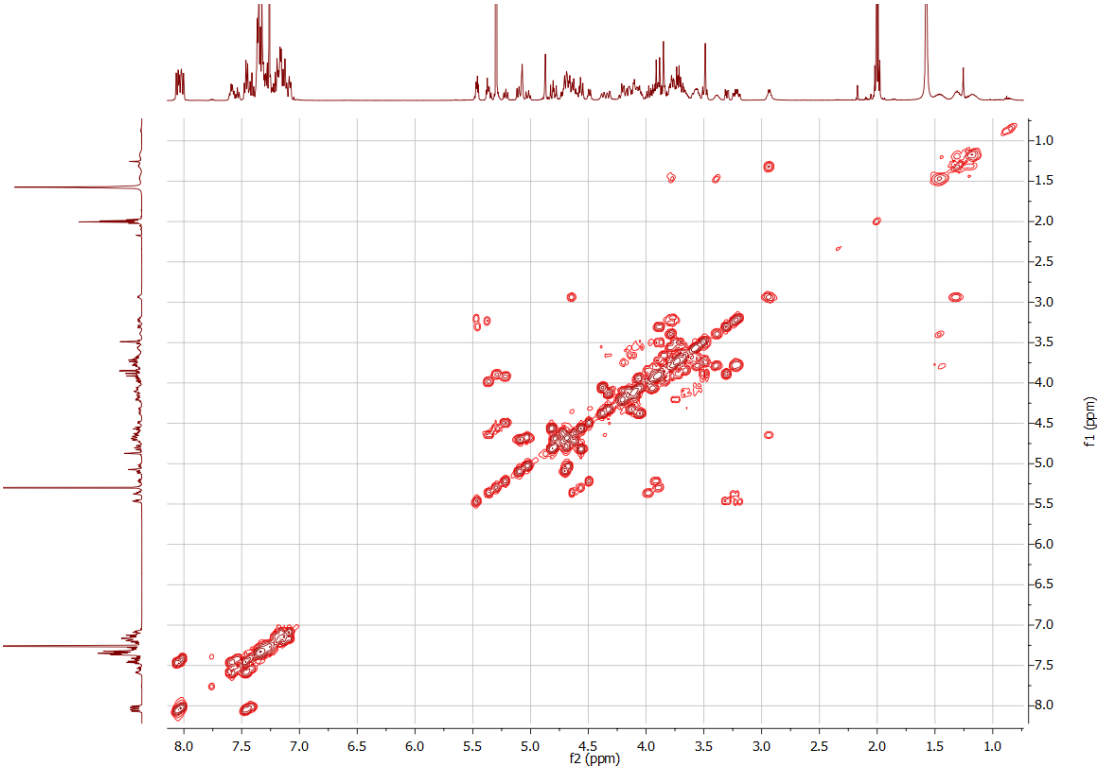
^{1}H NMR spectra of hexasaccharide 2:



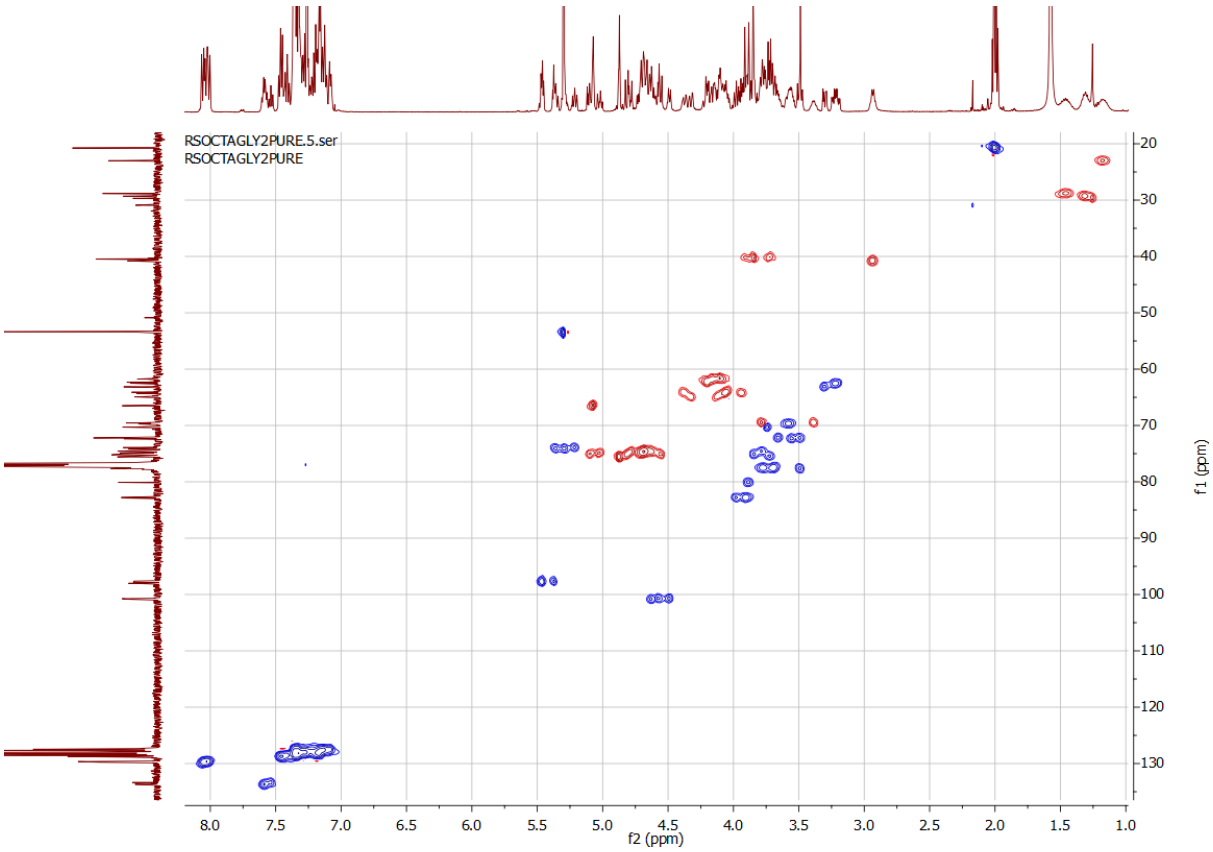
^{13}C NMR of hexasaccharide 2:

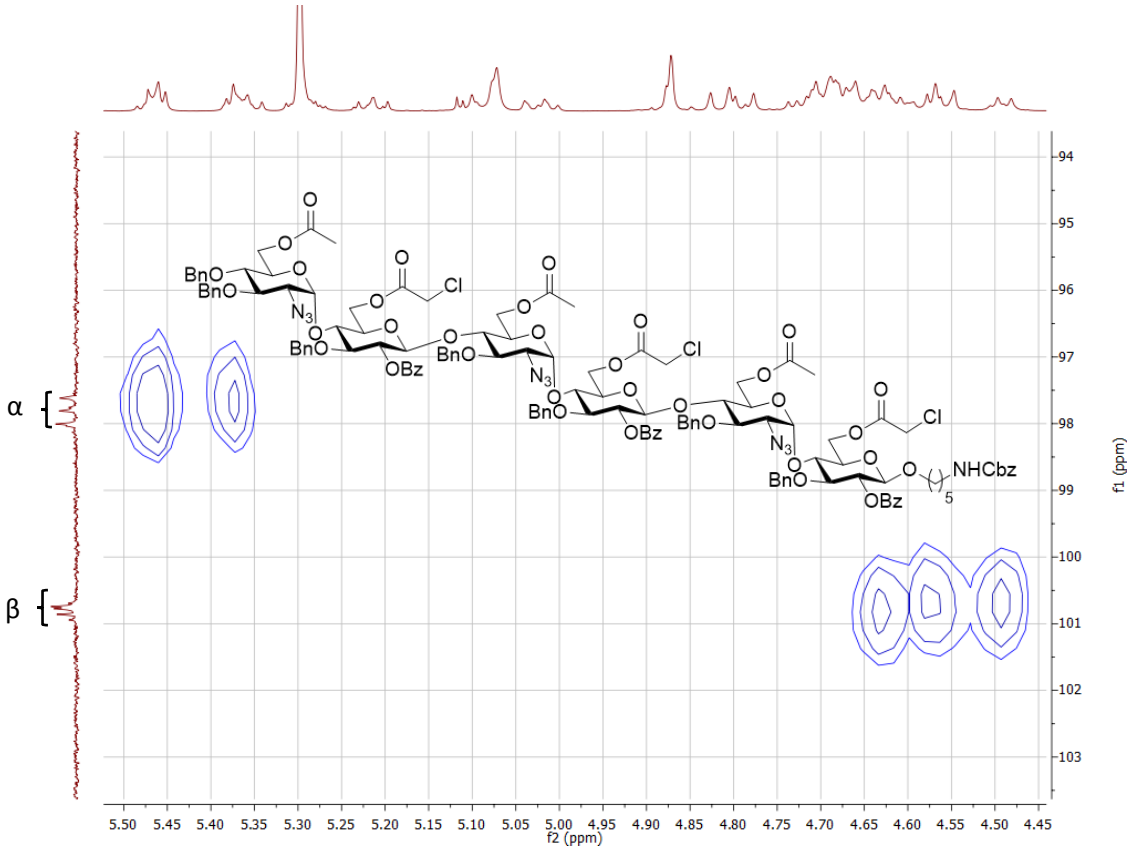


^{1}H-^{1}H COSY Spectra of hexasaccharide 2:



^{1}H-^{13}C HSQC Spectra of hexasaccharide 2:





7. References

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