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Electronic Supplementary Information

A Combination of Automated Solid-Phase and Enzymatic Oligosaccharide Synthesis Provides Access to α(2,3)-Sialylated Glycans

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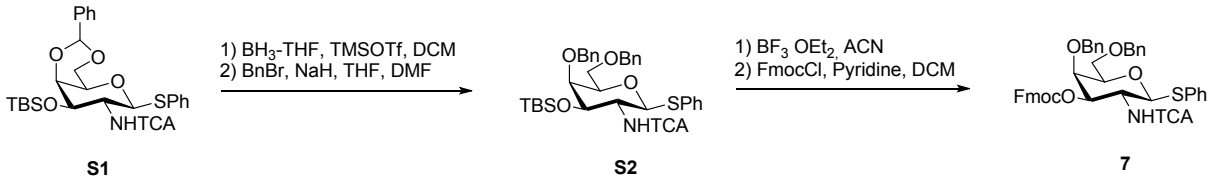
1. General Materials and Methods

All chemicals used were reagent grade and used as supplied unless otherwise noted. All reactions were performed in oven-dried glassware under an inert atmosphere unless noted otherwise. All solvents used on the automated synthesizer were dried by using a dry still. α-2,3-Sialyltransferase from *Pasteurella multocida*, recombinant expressed in *E. coli* BL21 (EC 2.4.99.4), was purchased from Sigma-Aldrich. Calf-intestinal alkaline phosphatase (EC 3.1.3.1) was purchased from New England Biolabs. Cytidine-5'-monophospho-N-acetylneuraminic acid disodium salt (CMP-Neu5Ac) was purchased from Nacalai Tesque (Japan). Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in a p-anisaldehyde (PAA) solution. Flash column chromatography was carried out by using forced flow of the indicated solvent on Fluka Kieselgel 60 M (0.04 – 0.063 mm). Analysis and purification by normal phase HPLC was performed by using an Agilent 1200 series equipped with Luna silica columns (length 250 mm, 4.6 mm i.d., flow 1 mL/min) or (length 250 mm, 10 mm i.d., flow 5 mL/min) respectively unless. Analysis and purification by reverse phase HPLC was performed by using an Agilent 1200 series equipped with Hypercarb columns (length 150 mm, 4.6 mm i.d., flow 0.7 mL/min) or (length 150 mm, 10 mm i.d., flow 3.6 mL/min) respectively unless noted otherwise. Products were lyophilized using a Christ Alpha 2-4 LD plus freeze dryer. ^{1}H, ^{13}C, COSY, TOCSY, HMQC, and HSQC NMR spectra were recorded on a Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), or Bruker Biospin AVANCE700 (700 MHz) spectrometer. Spectra were recorded in $CDCl_3$ by using the solvent residual peak chemical shift as the internal standard ($CDCl_3$: 7.26 ppm ^{1}H, 77.0 ppm ^{13}C) or in D_2O using the solvent as the internal standard in ^{1}H NMR (D_2O: 4.79 ppm ^{1}H) and a D_6-Acetone spike as the internal standard in ^{13}C NMR (Acetone in D_2O: 30.89 ppm ^{13}C) unless otherwise stated. NMR chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. High-resolution mass spectral (HRMS) analyses were performed by the MS-service at the MS-service at Department of Organic Chemistry at Free University Berlin. High-resolution MALDI and ESI mass spectra were run on IonSpec Ultima instruments. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured by using a Perkin-Elmer 241 and Unipol L1000 polarimeter.

2. Synthesis of Building Blocks and Merrifield Supported Linker

2.1 Synthesis of Phenyl 4,6-*O*-dibenzyl-3-*O*-fluorenylmethoxycarbonyl-2-deoxy-2-*N*-trichloroacetamido-thio-β-D-galactopyranoside, 7



S1 **S2** **7**

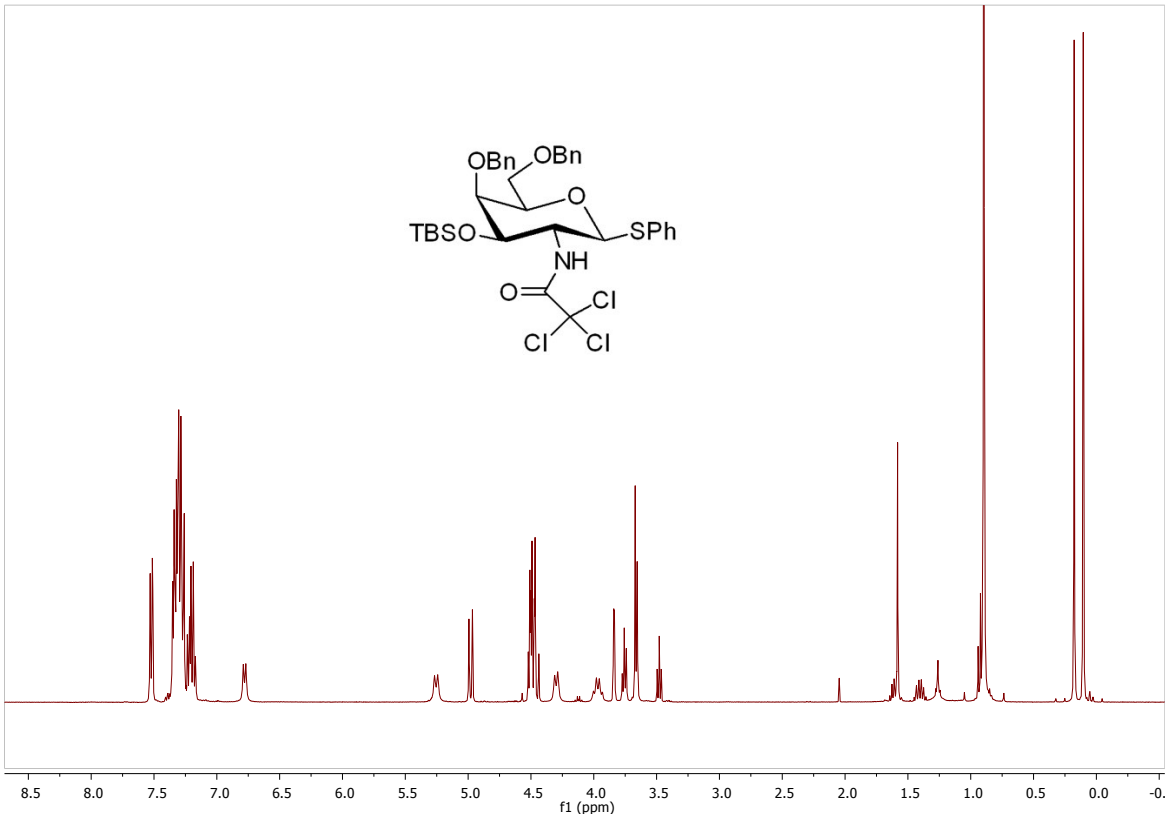
S1 was made according to previously establish procedures.[S1]

To a solution of **S1** (4.76 g, 7.66 mmol) in DCM (47.4 mL) was added a 1 M solution of BH_3 in terahydrofuran (30.6 mL, 30.64 mmol). The solution was cooled to 0 °C and after 10 min trimethylsilyl triflate (0.69 mL, 3.83 mmol) was added. The reaction was at 0 °C until complete conversion of the starting material (4 h). The solution was quenched by addition of a saturated

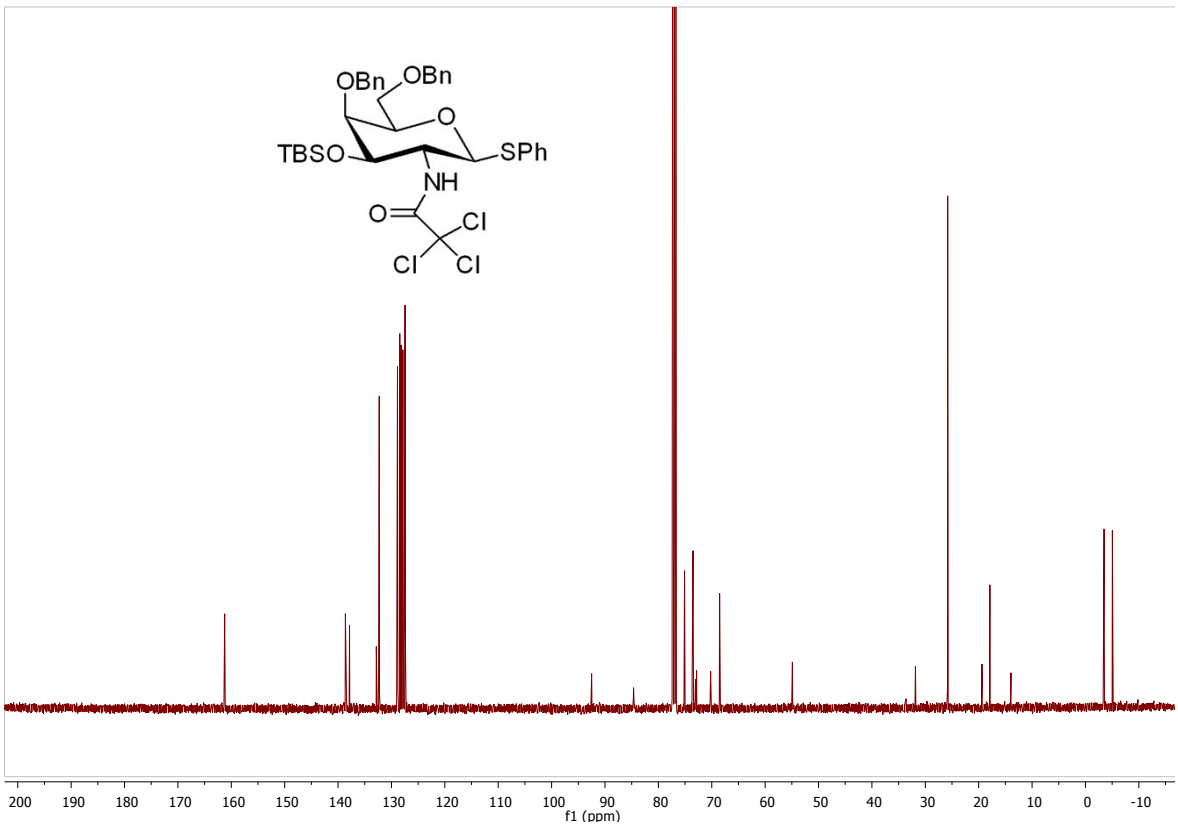
solution of $NaHCO_3$. It was then diluted with DCM and extracted with saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo* using a cool (20 °C) bath. The remaining residue was dissolved in anhydrous DMF (3.55 mL) and THF (34.81 mL). To this solution was added NaH (60%, 920 mg, 22.98 mmol) at 0 °C. This was stirred for 30 min and then BnBr (2.73 mL, 22.98 mmol) was added dropwise. The solution was kept at 0 °C for the duration of the reaction (2 h) during which a large amount of white precipitate formed. Aqueous NH_4Cl was added to quench. DCM was added and then the reaction was extracted three times with water. The organics were dried over $MgSO_4$. The product was purified by column chromatography (3 Hexane: 1 EtOAc) to give a clear gel. This was coevaporated with ACN to yield **S2** (5.18 g, 7.28 mmol) as a white foamy solid in 95% yield. R_f = 0.60 (hexanes/ethyl acetate, 3:1); $[\alpha]_D^{20}$ -1.92 (c 1, $CHCl_3$); IR (neat) vmax = 1689, 1531 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.50 (m, 2H), 7.36 – 7.25 (m, 10H), 7.24 – 7.16 (m, 3H), 6.78 (d, J = 8.0 Hz, 1H), 5.26 (d, J = 10.0 Hz, 1H), 4.97 (d, J = 11.6 Hz, 1H), 4.53 – 4.44 (m, 3H), 4.30 (d, J = 9.6 Hz, 1H), 4.00 – 3.93 (m, 1H), 3.84 (d, J = 2.4 Hz, 1H), 3.77 – 3.74 (m, 1H), 3.67 - 3.65 (m, 1H), 0.90 (s, 9H), 0.18 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.38, 138.77, 137.99, 132.98, 132.43, 129.01, 128.58, 128.47, 128.33, 128.00, 127.94, 127.81, 127.74, 127.59, 127.57, 92.64, 84.79, 77.36, 76.84, 73.65, 73.19, 72.99, 68.67, 55.04, 31.99, 25.94, -3.35, -4.90; ESI HR-MS: m/z [M+Na]+ calcd. for $C_{34}H_{42}Cl_3NNaO_5SSi$: 732.1516; Found: 732.1526.

S2 (5.18 mg, 7.28 mmol) was dissolved in 86.8 mL anhydrous ACN and cooled to 0 °C. BF_3 OEt_2 (1.11 mL, 8.74 mmol) was added. The reaction was stirred at 0 °C until completion (35 min), then a saturated solution of $NaHCO_3$ was added to quench. DCM was added and this was extracted twice with saturated $NaHCO_3$ and once with H_2O. The organics were dried with $MgSO_4$ and rotovaped. The remaining solid was coevaporated with pyridine and then dissolved in 24.3 mL DCM. Pyridine (1.77 mL, 21.84 mmol) was added and then Fmoc-Cl (3.77 g, 14.56 mmol). The yellow solution was stirred at room temperature until completion (4 h). The reaction was diluted in DCM and extracted with 1 M HCl, with saturated $NaHCO_3$, and with H_2O. The organics were dried over $MgSO_4$ and rotovaped. The product is purified by column chromatography (3 Hexane: 1 EtOAc) to give **7** (5.00 g, 6.11 mmol) as a white solid in 84% yield over two steps. R_f = 0.65 (hexanes/ethyl acetate, 2:1); $[\alpha]_D^{20}$ +9.00 (c 1, $CHCl_3$); IR (neat) vmax = 1746, 1702, 1266 cm-1; ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 – 7.71 (m, 2H), 7.58 – 7.53 (m, 4H), 7.45 – 7.22 (m, 17H), 6.88 (d, J = 8.4 Hz, 1H), 5.31 (dd, J = 10.8, 2.8 Hz, 1H), 5.14 (d, J = 10.4 Hz, 1H), 4.75 (d, J = 11.6 Hz, 1H), 4.55 – 4.36 (m, 4H), 4.34 – 4.26 (m, 1H), 4.21 (t, J = 7.2 Hz, 1H) 4.11 (d, J = 2.4 Hz, 1H), 3.85 (t, J = 6.4 Hz, 1H), 3.72-3.69 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.67, 154.68, 143.15, 142.92, 141.29, 137.87, 137.73, 132.53, 132.47, 129.02, 128.48, 128.33, 128.03, 128.01, 127.89, 127.78, 127.65, 127.27, 127.23, 125.09, 124.99, 120.15, 120.13, 120.07, 92.40, 85.61, 77.12, 76.80, 74.99, 73.54, 73.42, 70.35, 68.15, 52.15, 46.63; ESI HR-MS: m/z [M+Na]+ calcd. for $C_{43}H_{38}Cl_3NNaO_7S$: 840.1332; Found: 840.1342.

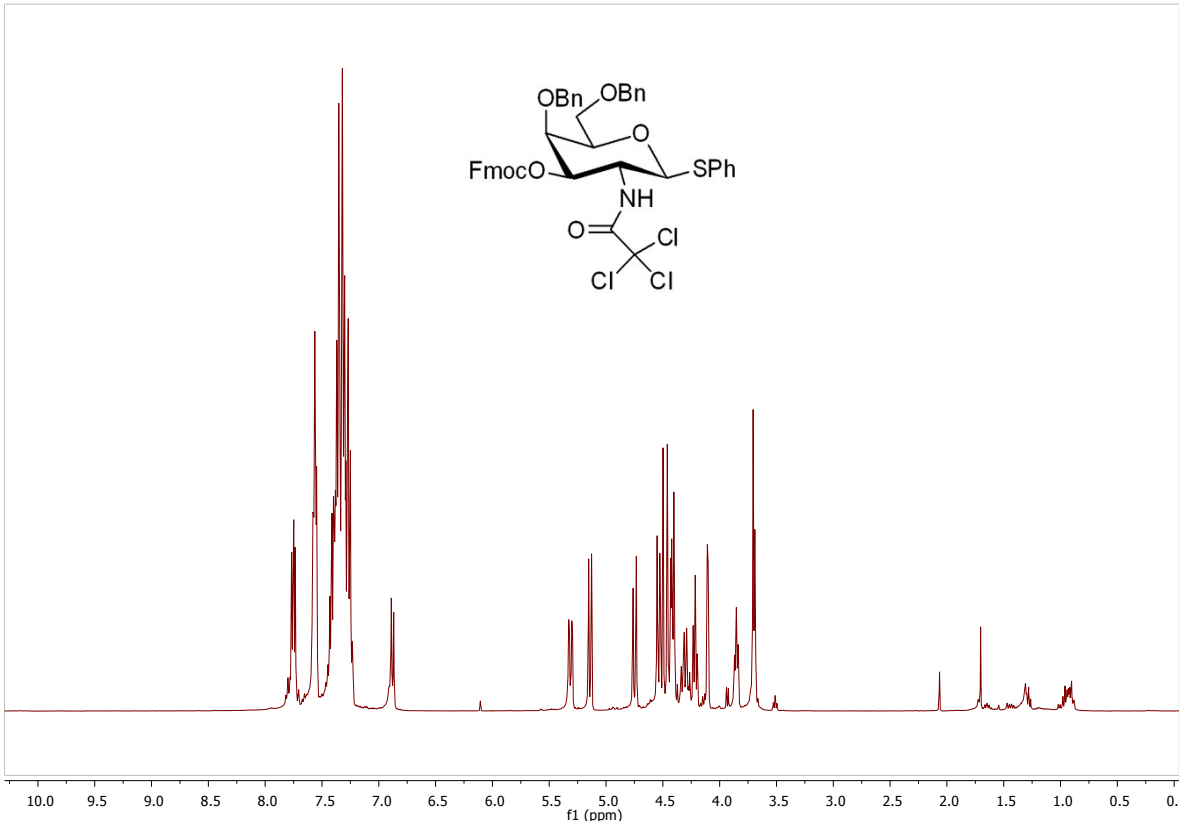
^{1}H NMR of Compound S2



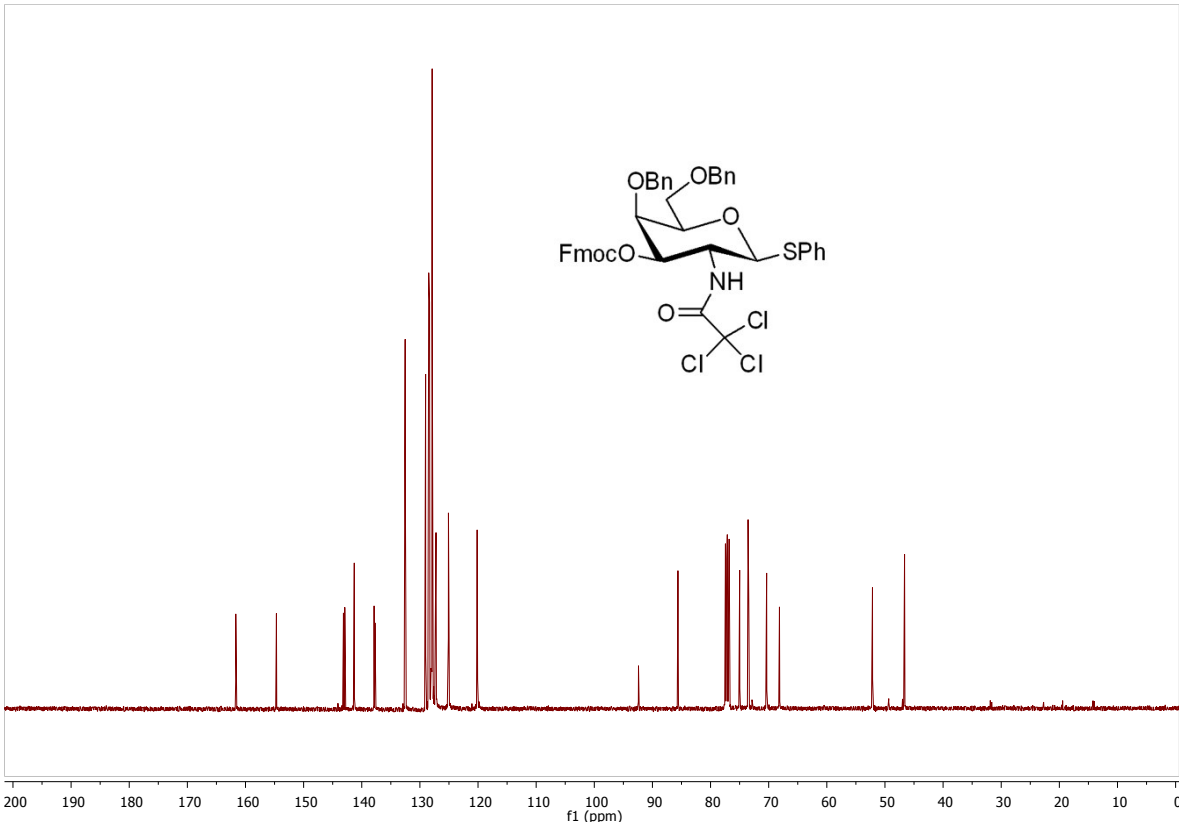
^{13}C NMR of Compound S2



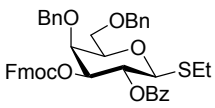
^{1}H NMR of Compound 7



^{13}C NMR of Compound 7



2.2 Synthesis of Other Building Blocks and Merrifield Supported Linker



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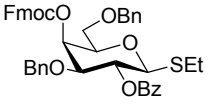

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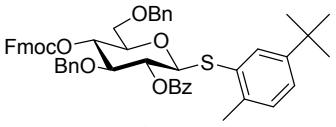

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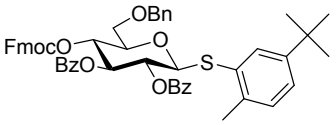
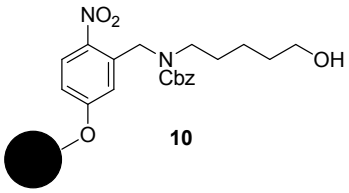


S3



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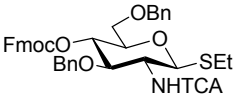
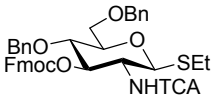


S4



S5

Building blocks **6**, **8**, **9**, **S3 – S5**, and the photo-cleavable linker (**10**) were made using previously established procedures. [31, 33-35]

3. Automated Synthesis of Protected Oligosaccharides

3.1 General Materials and Methods

All solvents used were taken from an anhydrous solvent system (jcmeyer-solvent systems). The building blocks were coevaporated three times with $CHCl_3$ and dried for 3 h on high vacuum before use. Activator, deprotection, acidic wash and building block solutions were freshly prepared and kept under argon during the automation run. Modules were adopted from a previous publication.[31]

3.2 Preparation of Stock Solutions

Building Block Solution: 0.25 mmol of building block was dissolved in 2 mL of DCM.

Activator Solution: 1.35 g of recrystallized NIS was dissolved in 40 mL of a 2:1 mixture of anhydrous DCM and anhydrous dioxane. Then 55 µL of triflic acid was added. The solution is kept at 0 °C for the duration of the automation run.

Fmoc Deprotection Solution: A solution of 20% TEA in DMF (v/v) was prepared.

Acidic Wash Solution: 0.9 mL of TMSOTf was added to 40 mL DCM.

3.3 Modules for Automated Synthesis

3.3.1 Module A: Preparation of the Resin for Synthesis:

For all compounds, automated synthesis reaction was carried out on a 0.0285 mmol scale using Merrifield supported photo-cleavable linker (**10**) (resin loading: 0.339 mmol/g). The resin **10** was loaded into the reaction vessel of the synthesizer and swollen in 2 mL DCM for at least 30 min. To start the synthesis sequence, the resin is washed consecutively with DMF, THF, and DCM (three times each with 2 mL for 25 s). In all reactions, the resin was stirred by Ar bubbling from the bottom of the reaction vessel.

3.3.2 Module B: Acidic Wash with TMSOTf Solution:

The resin was swollen in 2 mL DCM and the temperature of the reaction vessel was adjusted to -20 °C. Upon the low temperature was reached, 350 µL of acidic wash solution was added drop wise to the reaction vessel. After bubbling for 1 min, the acidic solution was drained and the resin was washed with 2 mL DCM for 25 s.

3.3.3 Module C: Thioglycoside Glycosylation:

The glycosylation reaction was performed after acidic wash. The DCM was drained and the thioglycoside building block solution (4.4 eq. or 5 eq. for SLPG (**5**) synthesis in 1.0 mL) was delivered to the reaction vessel. After the set temperature was reached, the reaction was started by drop wise addition of the activator solution (1.0 mL, 5 eq.). The glycosylation was performed under conditions A followed by conditions B (See Table S1), which are building block dependent. After completion of the reaction, the solution is drained and the resin was washed with DCM (three times each with 2 mL for 25 s). This procedure was repeated twice.

Table S1. Glycosylation Modules for Automated Synthesis

	A		B	
Module	Temperature [a]	Time[b]	Temperature [a]	Time[b]
C1	-15	5	-10	20
C2	-20	30	-15	30
C3	-20	20	-15	5
C4	-30	5	-10	20
C5	-40	5	-20	30
C6	-30	5	-10	30

[a] Temperature in °C. [b] Time in minutes.

3.3.4 Module D: Fmoc Deprotection:

The resin was washed with DMF (six times with 2 mL for 25 s), swollen in 2 mL DMF and the temperature of the reaction vessel was adjusted to 25 °C. DMF was drained and then 2 mL of Fmoc deprotection solution was delivered into the reaction vessel. After 5 min, the reaction solution was collected in the fraction collector of the oligosaccharide synthesizer. This procedure was repeated three times. The resin was then washed with DMF (three times with 3 mL for 25 s), THF, and DCM (three times each with 2 mL for 25 s).

3.4 Post-Synthesizer Manipulations

3.4.1 Cleavage from Solid Support

The resin was swollen in 2 mL DCM and taken up in a 20 mL glass syringe. Photo-reactor FEP tubing was washed with 15 mL DCM. The UV light source was a medium pressure Hg lamp with arc lengths of 27.9 cm and power of 450 W, surrounded by a Pyrex UV filter with 50% transmittance at 305 nm. For the cleavage reaction, the resin was slowly injected from the 20 mL glass syringe into the reactor and pushed through the tubing with 20 mL DCM (flow rate: 0.5 mL per min). To slowly react and

wash out remaining resin in the tube, the resin was pushed with 10 mL DCM (flow rate: 2 mL per minute). The suspension leaving the reactor is directed into a filter (resin is filtered off). The entire procedure was performed twice to ensure the complete of cleavage, and finally the tube was washed with 20 mL DCM.

3.4.2 Purification

Solvent is evaporated in *vacuo* and the crude products were analyzed/ purified using analytical/preparative HPLC (Agilent 1200 Series spectrometer).

3.5 Automated Synthesis of Protected Disaccharide S6

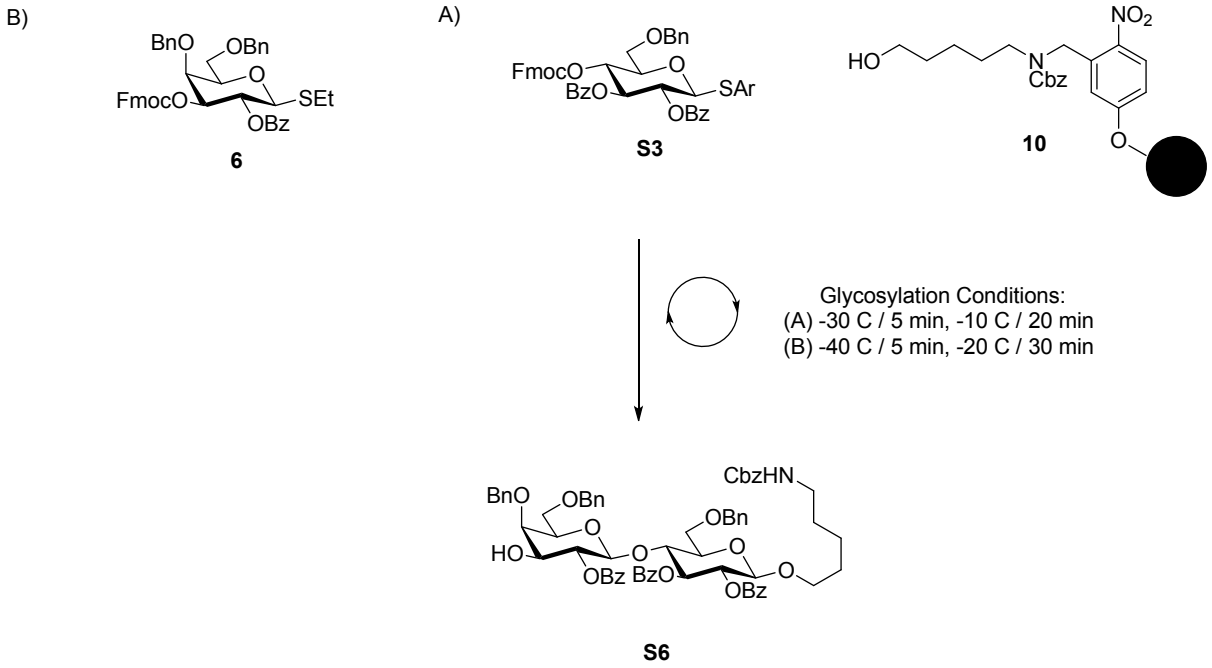
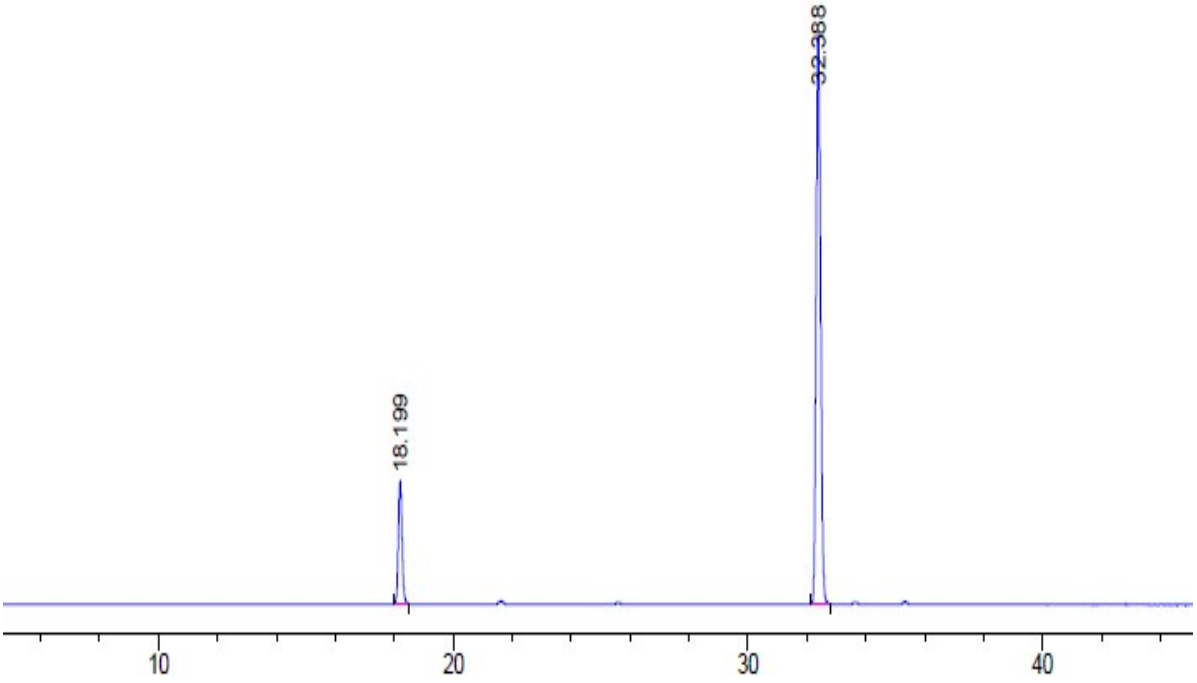


Table S2. Automated Synthesis Program Protocol for Disaccharide **S6**		
Steps	Automation Process	Module
1	Preparation of Resin for Synthesis	A
2	Acidic Wash	B
3	Thioglycoside Glycosylation: BB **S3**	C4
4	Fmoc Deprotection	D
5	Acidic Wash	B
6	Thioglycoside Glycosylation: BB **6**	C5
7	Fmoc Deprotection	D

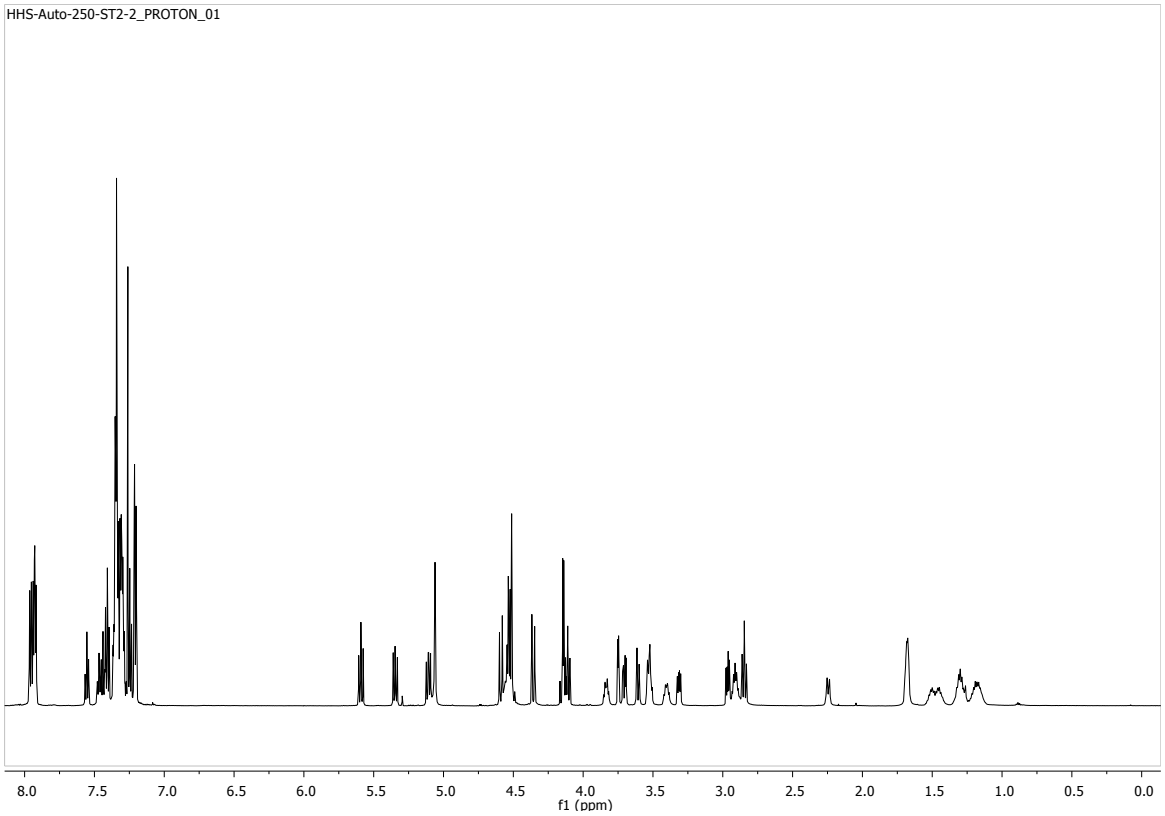
Cleavage, Analysis and Purification: Disaccharide **S6** was cleaved from the solid support as described in Post-Synthesizer Manipulations. The crude product was analyzed using normal phase analytical HPLC (Luna silica; length 250 mm, 4.6 mm i.d., flow 1 mL/min; Linear gradient: EtOAc (3% DCM)/Hexane (3% DCM); 20% EtOAc (3% DCM) for 5 min, 20% to 55% EtOAc (3% DCM) in 35 min) and purified using preparative HPLC (Luna silica; length 250 mm, 10 mm i.d., flow 5 mL/min, Linear gradient: EtOAc (3% DCM)/Hexane (3% DCM); 20% EtOAc (3% DCM) for 5 min, 20% to 55% EtOAc (3% DCM) in 35 min) to obtain compound **S6** (12.0 mg, 42% overall yield based on the resin loading. Compound **S6** elutes at 32 minutes.

*Analytical data for disaccharide **S6**:* ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 – 7.89 (m, 6H), 7.58 – 7.53 (m, 1H), 7.49 – 7.38 (m, 4H), 7.37 – 7.27 (m, 18H), 7.23 (dt, J = 8.3, 5.3 Hz, 6H), 5.59 (t, J = 9.4 Hz, 1H), 5.35 (dd, J = 9.8, 8.0 Hz, 1H), 5.11 (dd, J = 10.0, 7.9 Hz, 1H), 5.06 (s, 2H), 4.59 (d, J = 12.2 Hz, 1H), 4.57 – 4.49 (m, 5H), 4.36 (d, J = 12.2 Hz, 1H), 4.17 – 4.08 (m, 3H), 3.86 – 3.80 (m, 1H), 3.75 (d, J = 3.4 Hz, 1H), 3.71 (dd, J = 10.9, 3.9 Hz, 1H), 3.61 (dd, J = 10.9, 1.6 Hz, 1H), 3.56 – 3.49 (m, 2H), 3.40 (dd, J = 15.5, 6.8 Hz, 1H), 3.31 (dd, J = 9.3, 5.2 Hz, 1H), 2.96 (dd, J = 9.0, 4.9 Hz, 1H), 2.91 (td, J = 12.7, 6.3 Hz, 2H), 2.85 (t, J = 9.2 Hz, 1H), 2.24 (d, J = 10.4 Hz, 1H), 1.48 (ddd, J = 20.5, 12.9, 6.5 Hz, 2H), 1.34 – 1.24 (m, 2H), 1.23 – 1.10 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.23, 165.34, 165.31, 156.34, 138.22, 138.16, 137.73, 136.76, 133.32, 133.25, 132.77, 130.45, 129.98, 129.91, 129.84, 129.77, 129.57, 128.61, 128.58, 128.57, 128.51, 128.47, 128.22, 128.18, 127.99, 127.94, 127.87, 127.77, 127.67, 101.15, 100.63, 76.02, 75.61, 75.14, 74.72, 74.29, 73.63, 73.54, 73.21, 72.93, 72.74, 72.03, 69.86, 67.82 , 66.89, 66.60, 40.88, 29.47, 28.97, 23.15. MS ESI HRMS: *m/z* $[M+Na]^+$ calcd for $C_{67}H_{69}O_{16}NNa$ 1166.4509, found 1166.1548.

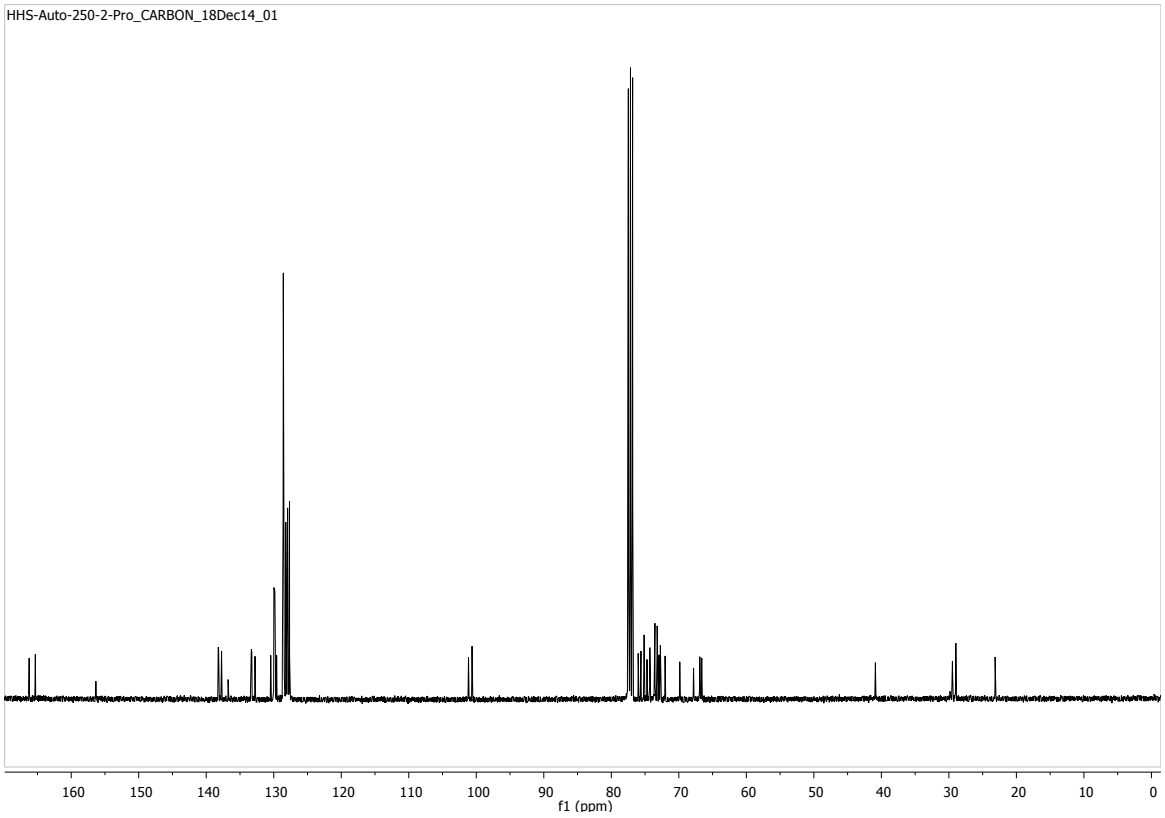
Analytical NP-HPLC Luna Silica of Crude Disaccharide S6 (ELSD trace)



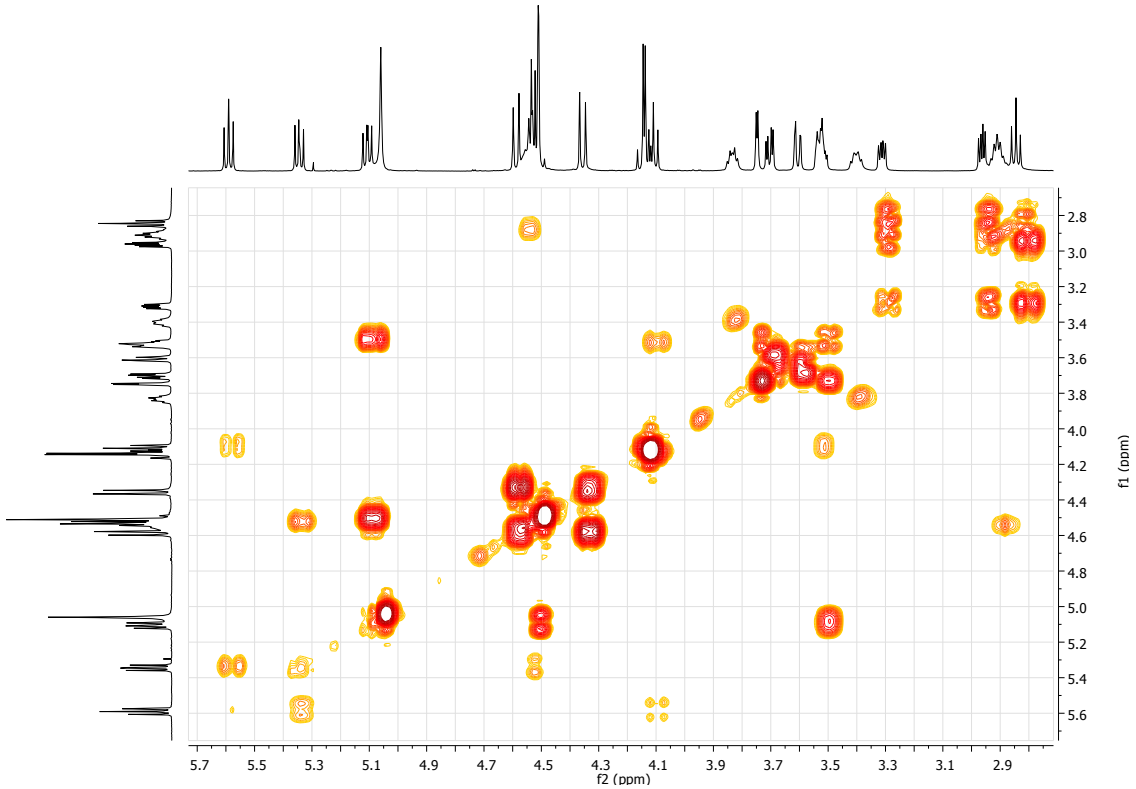
^{1}H NMR of Compound Disaccharide S6



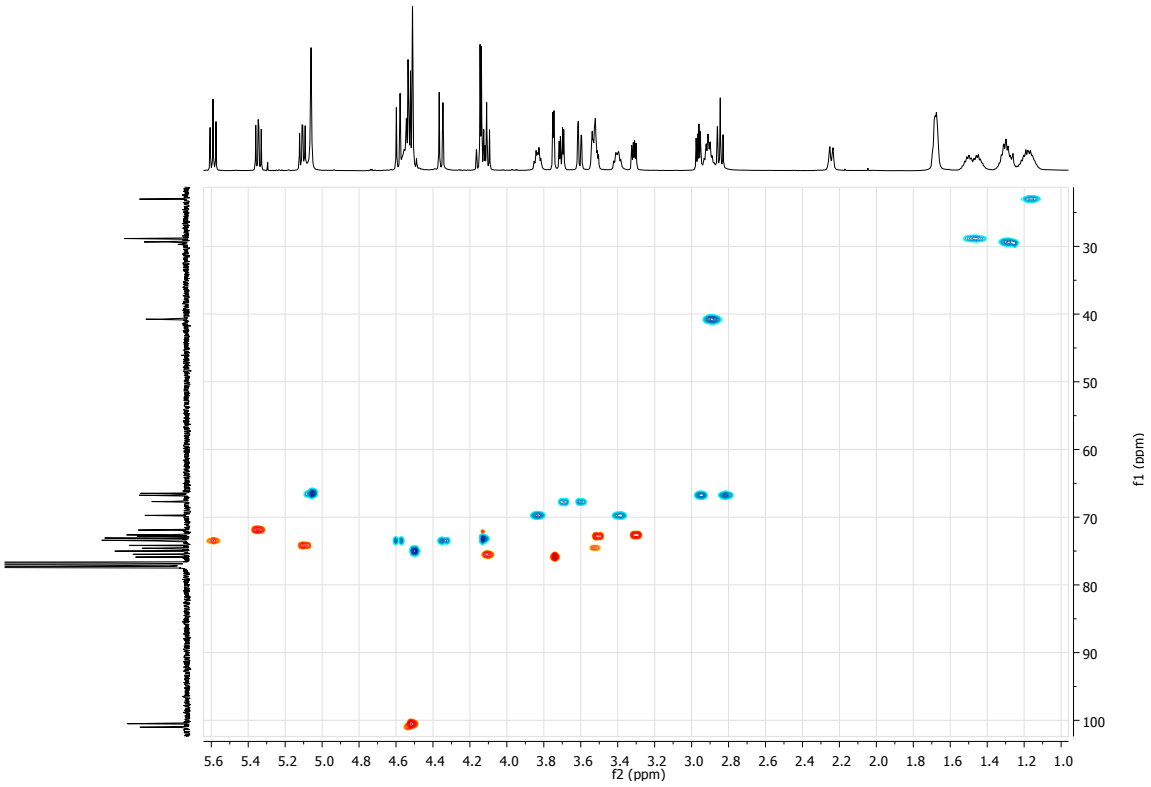
^{13}C NMR of Compound Disaccharide S6



COSY NMR of Compound Disaccharide S6



HSQC NMR of Compound Disaccharide S6



3.6 Automated Synthesis of Protected Tetrasaccharide S7

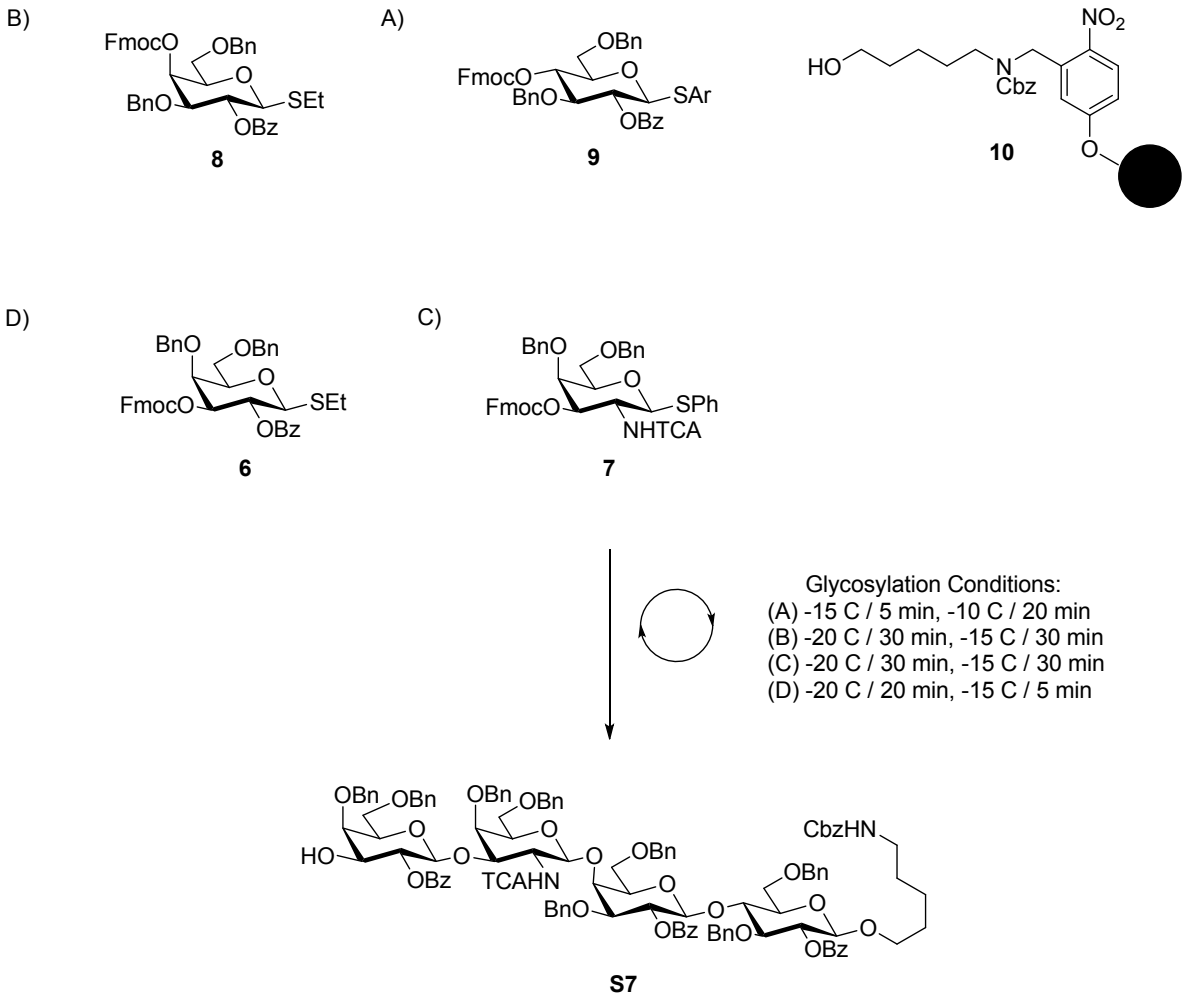
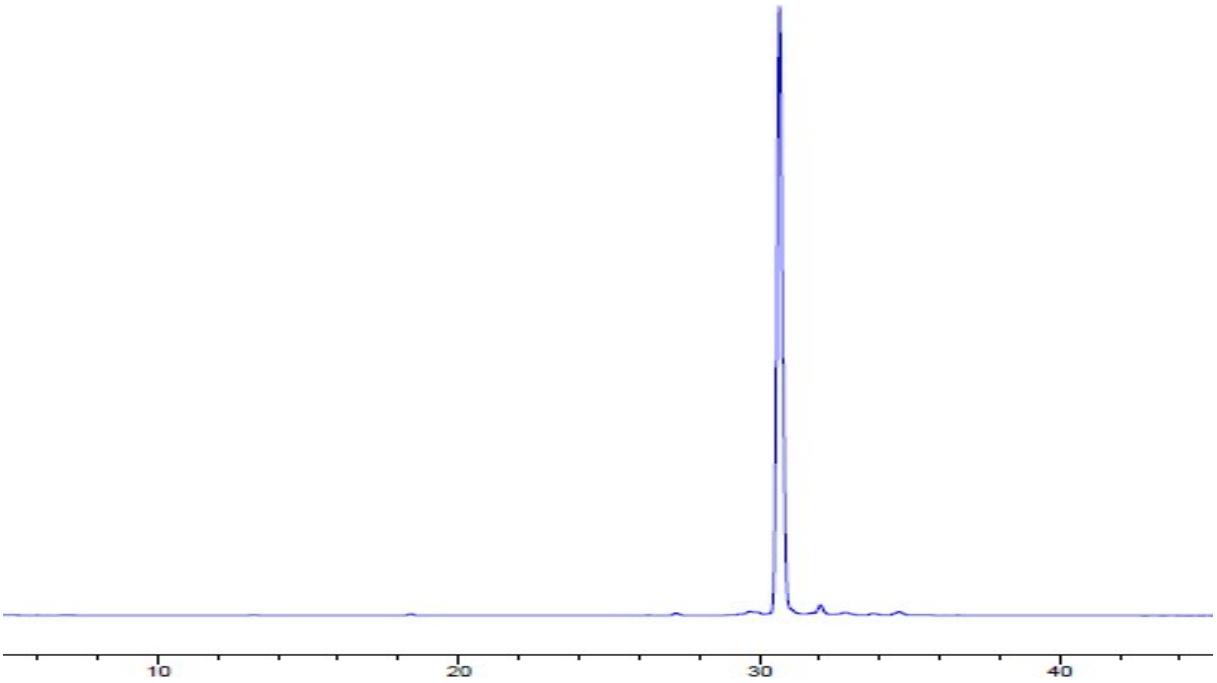


Table S3. Automated Synthesis Program Protocol for Tetrasaccharide **S7**		
Steps	Automation Process	Module
1	Preparation of Resin for Synthesis	A
2	Acidic Wash	B
3	Thioglycoside Glycosylation: BB **9**	C1
4	Fmoc Deprotection	D
5	Acidic Wash	B
6	Thioglycoside Glycosylation: BB **8**	C2
7	Fmoc Deprotection	D
8	Acidic Wash	B
9	Thioglycoside Glycosylation: BB **7**	C2
10	Fmoc Deprotection	D
11	Acidic Wash	B
12	Thioglycoside Glycosylation: BB **6**	C3
13	Fmoc Deprotection	D

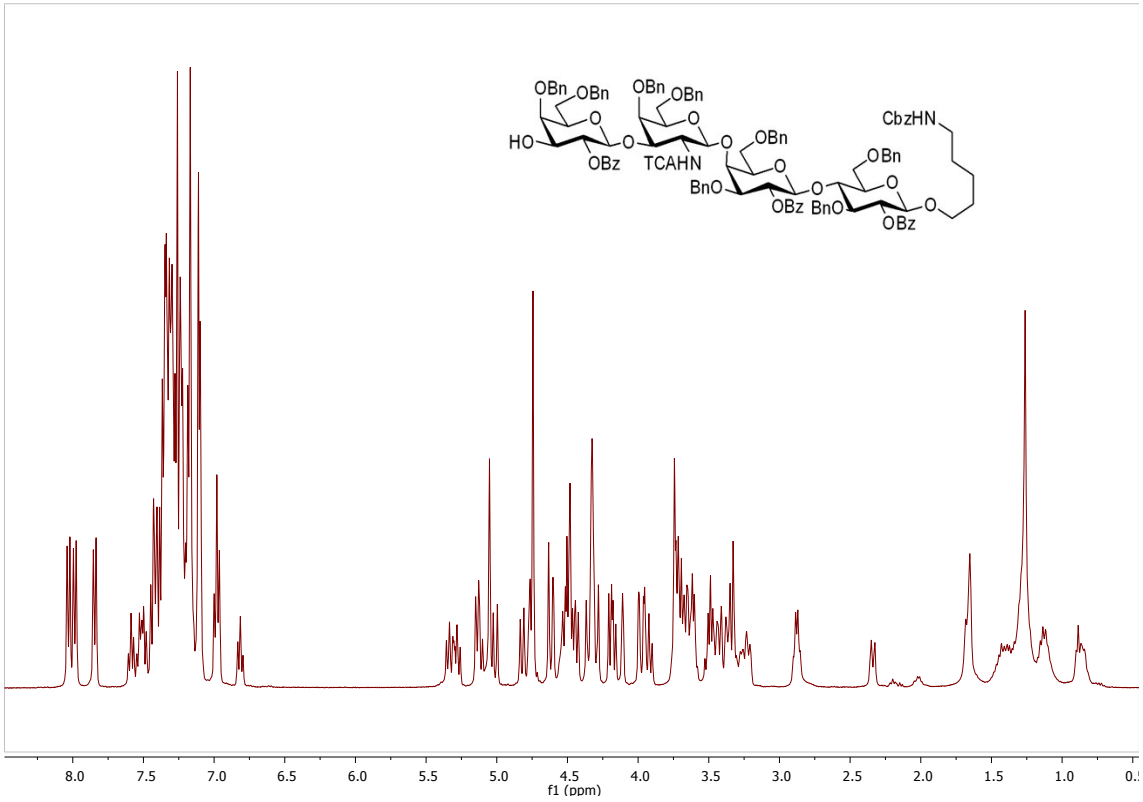
Cleavage, Analysis and Purification: Tetrasaccharide **S7** was cleaved from the solid support as described in Post-Synthesizer Manipulations. The crude product was analyzed using normal phase analytical HPLC (Luna silica; length 250 mm, 4.6 mm i.d., flow 1 mL/min; Linear gradient: EtOAc (3% DCM)/Hexane (3% DCM); 20% EtOAc (3% DCM) for 5 min, 20% to 55% EtOAc (3% DCM) in 35 min) and purified using preparative HPLC (Luna silica; length 250 mm, 10 mm i.d., flow 5 mL/min, Linear gradient: EtOAc (3% DCM)/Hexane (3% DCM); 20% EtOAc (3% DCM) for 5 min, 20% to 55% EtOAc (3% DCM) in 35 min) to obtain compound **S7** (25.5 mg, 46% overall yield based on the resin loading). Compound **S7** elutes at 30 minutes.

*Analytical data for tetrasaccharide **S7**:* ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (dd, J = 8.3, 1.2 Hz, 2H), 7.98 (dd, J = 8.3, 1.2 Hz, 2H), 7.85 (dd, J = 8.3, 1.2 Hz, 2H), 7.60 – 7.56 (m, 1H), 7.55 – 7.47 (m, 2H), 7.45 – 7.07 (m, 50H), 7.00 – 6.95 (m, 2H), 6.82 (t, J = 7.4 Hz, 1H), 5.34 (dd, J = 10.1, 7.8 Hz, 1H), 5.29 (dd, J = 10.1, 7.8 Hz, 1H), 5.16 – 5.10 (m, 2H), 5.04 (s, 2H), 5.01 (d, J = 11.4 Hz, 1H), 4.82 (d, J = 10.5 Hz, 1H), 4.78 – 4.71 (m, 4H), 4.62 (d, J = 4.6 Hz, 1H), 4.60 (d, J = 6.4 Hz, 1H), 4.56 – 4.43 (m, 5H), 4.38 – 4.28 (m, 5H), 4.21 – 4.16 (m, 2H), 4.12 – 4.10 (m, 1H), 3.99 (d, J = 2.7 Hz, 1H), 3.96 (d, J = 3.6 Hz, 1H), 3.92 (t, J = 9.0 Hz, 1H), 3.77 – 3.58 (m, 9H), 3.53 – 3.43 (m, 3H), 3.43 – 3.30 (m, 4H), 3.27 (dd, J = 15.3, 7.1 Hz, 1H), 3.25 – 3.20 (m, 1H), 2.88 (dd, J = 12.9, 6.4 Hz, 2H), 2.35 (d, J = 10.0 Hz, 1H), 1.48 – 1.34 (m, 2H), 1.31 – 1.22 (m, 2H), 1.19 – 1.07 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.91, 165.13, 164.78, 162.25, 156.38 (5 C=O), 139.00, 138.72, 138.28, 138.26, 138.22, 138.16, 137.90, 137.80, 136.86, 133.25, 133.20, 133.10, 130.27, 130.17, 130.00, 129.92, 129.87, 129.79, 128.86, 128.68, 128.65, 128.61, 128.53, 128.48, 128.43, 128.39, 128.23, 128.15, 128.10, 128.04, 128.00, 127.95, 127.90, 127.85, 127.83, 127.81, 127.77, 127.65, 127.57, 127.44, 127.39, 101.26, 100.96, 100.80, 98.57, 92.61, 80.78, 78.04, 76.80, 76.11, 75.67, 75.59, 75.05, 75.00, 74.72, 74.61, 74.28, 74.14, 73.73, 73.71, 73.54, 73.50, 73.45, 73.42, 73.32, 73.12, 72.32, 70.98, 70.86, 69.41, 69.22, 69.16, 68.15, 68.05, 66.59, 56.82, 40.93, 29.47, 28.98, 23.19. MS ESI HRMS: *m/z* $[M+Na]^+$ calcd for $C_{116}H_{119}Cl_3N_2NaO_{26}$ 2083.7014, found 2083.6961.

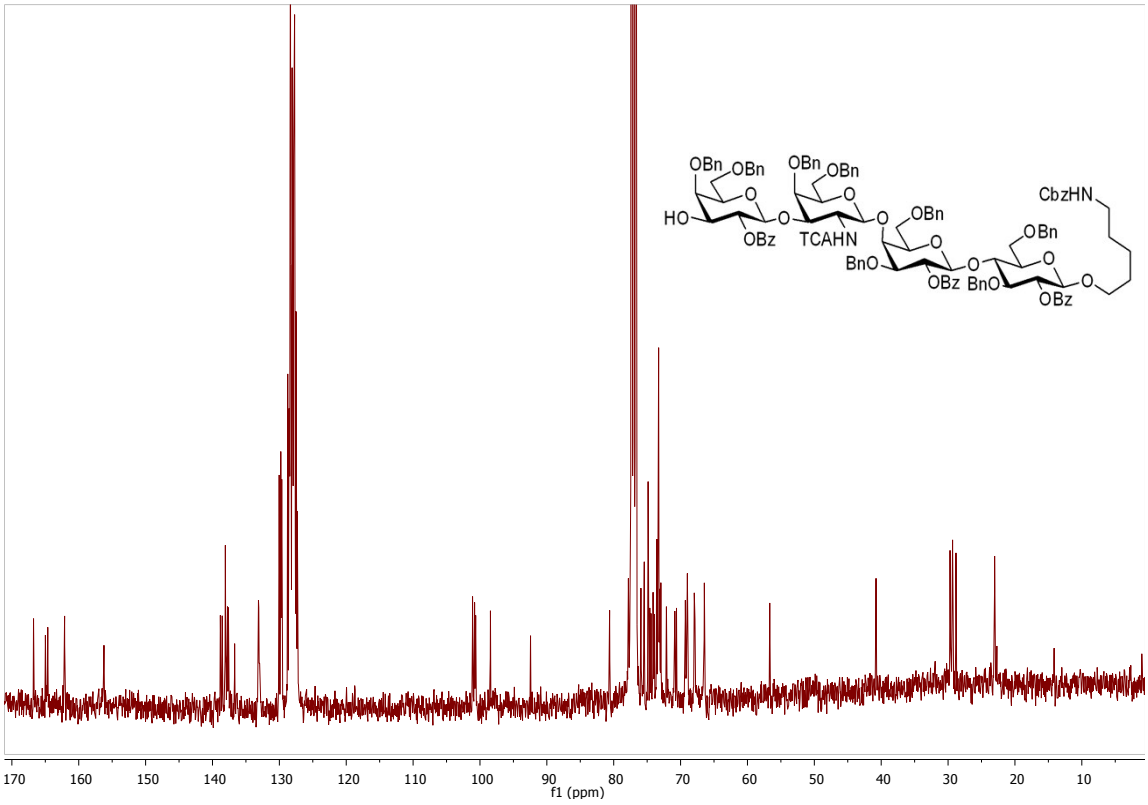
Analytical NP-HPLC Luna Silica of Crude Tetrasaccharide S7 (ELSD trace)



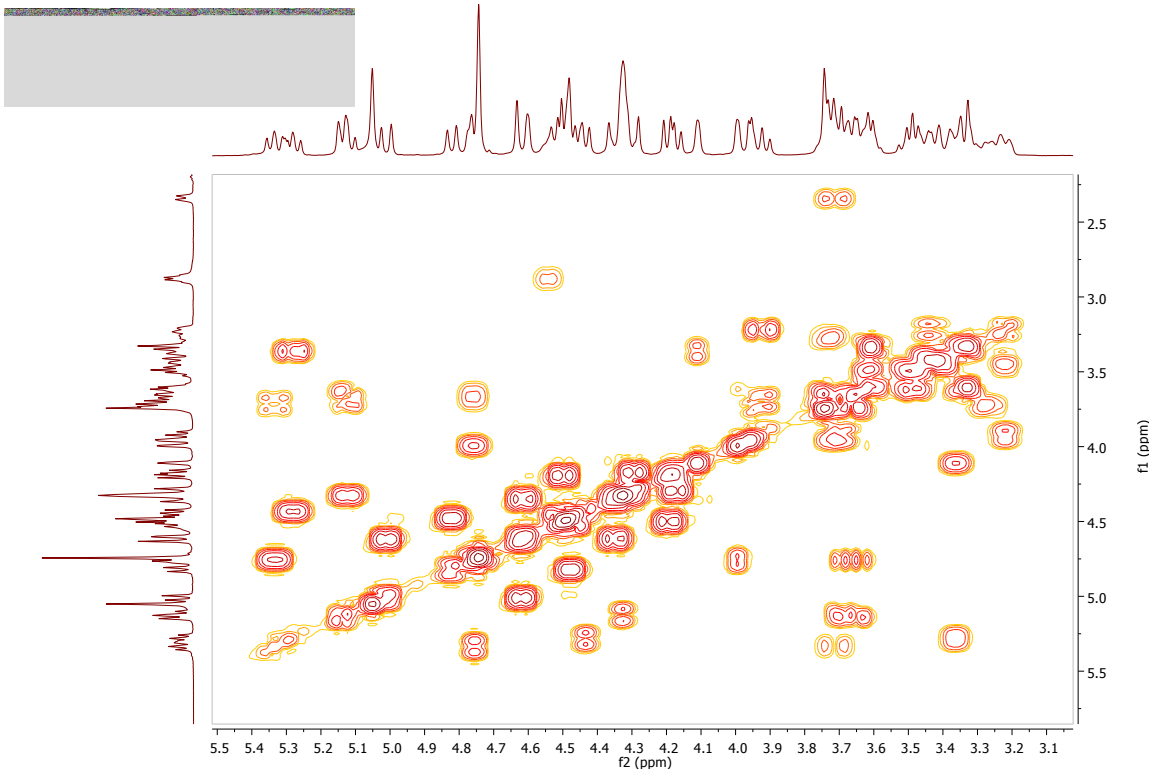
^{1}H NMR of Compound Tetrasaccharide S7



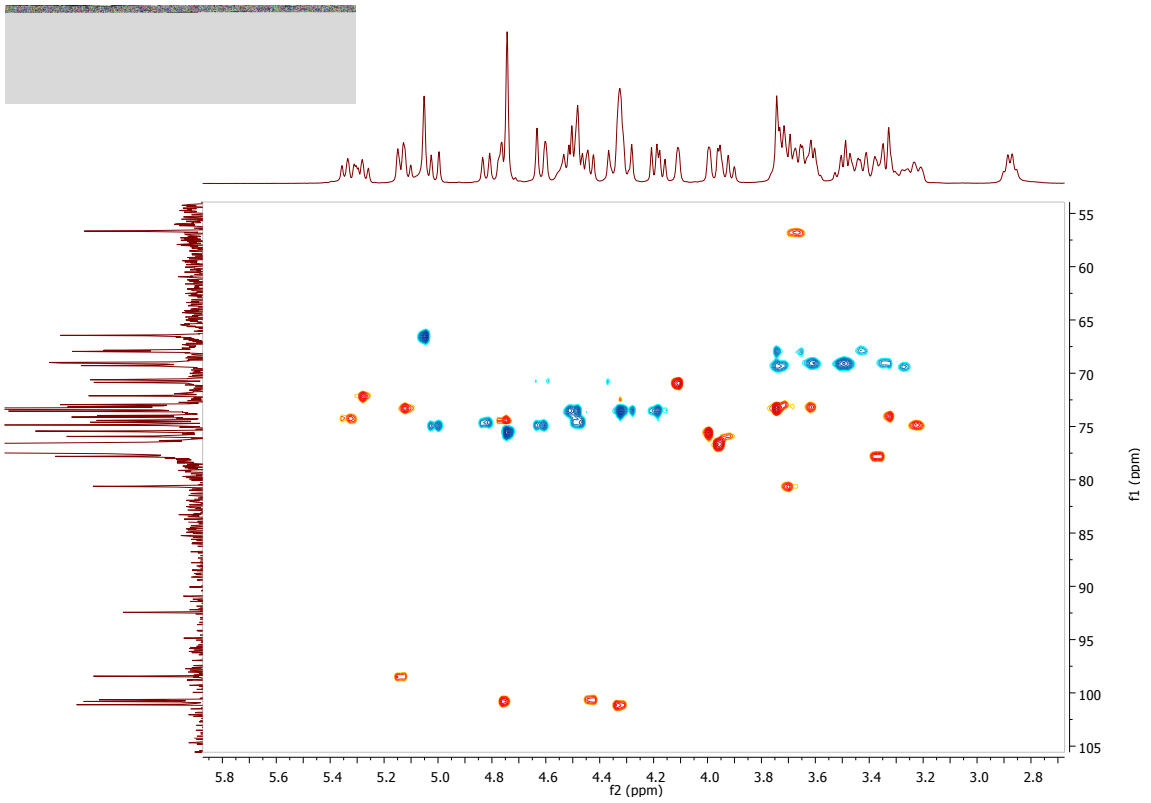
^{13}C NMR of Compound Tetrasaccharide S7



COSY NMR of Compound Tetrasaccharide S7



HSQC NMR of Compound Tetrasaccharide S7



3.7 Automated Synthesis of Protected Tetrasaccharide S8

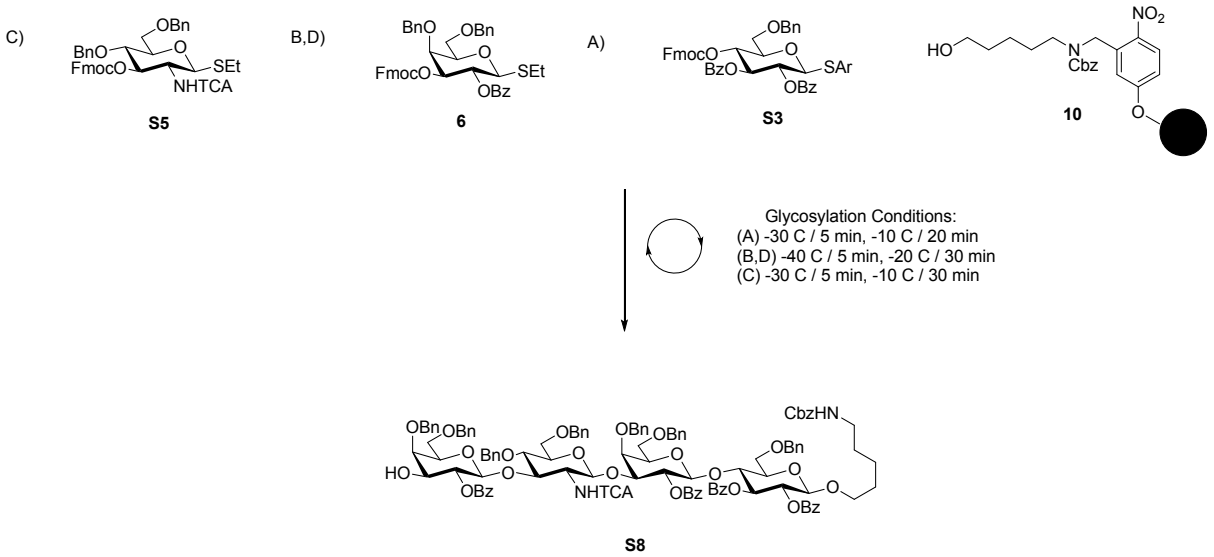


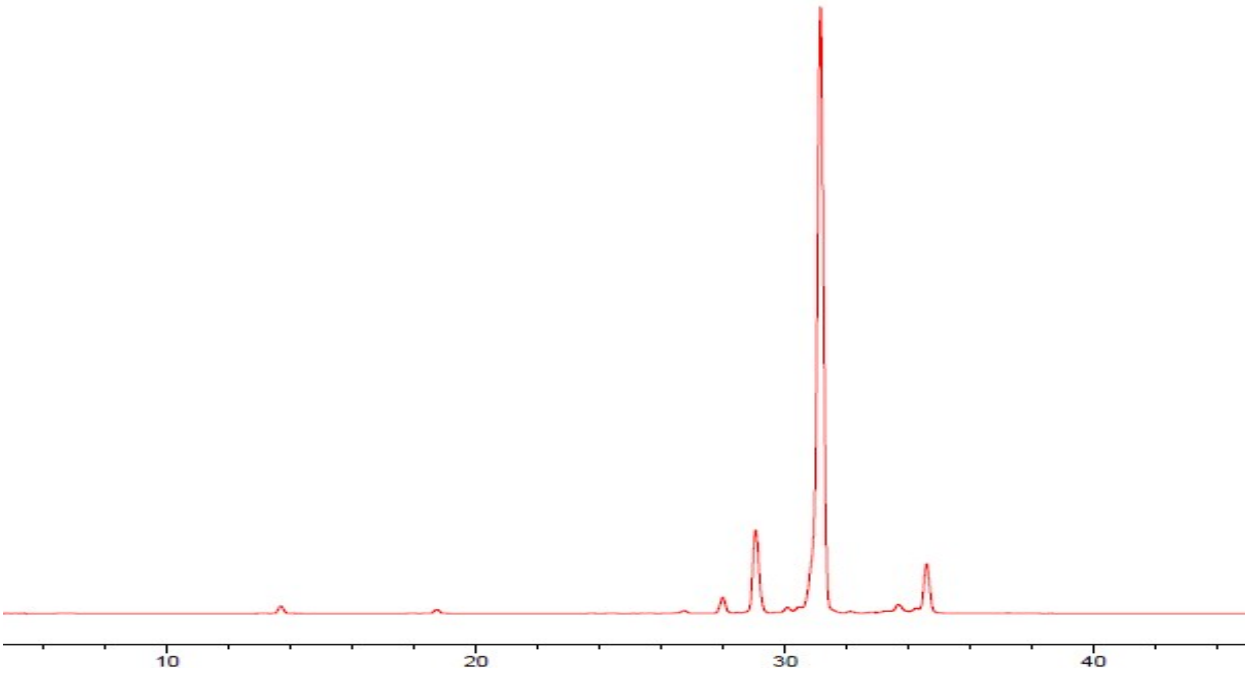
Table S4. Automated Synthesis Program Protocol for Tetrasaccharide **S8**		
Steps	Automation Process	Module
1	Preparation of Resin for Synthesis	A
2	Acidic Wash	B
3	Thioglycoside Glycosylation: BB **S3**	C4
4	Fmoc Deprotection	D
5	Acidic Wash	B
6	Thioglycoside Glycosylation: BB **6**	C5
7	Fmoc Deprotection	D
8	Acidic Wash	B
9	Thioglycoside Glycosylation: BB **S5**	C6
10	Fmoc Deprotection	D
11	Acidic Wash	B
12	Thioglycoside Glycosylation: BB **6**	C5
13	Fmoc Deprotection	D

Cleavage, Analysis and Purification: Tetrasaccharide **S8** was cleaved from the solid support as described in Post-Synthesizer Manipulations. The crude product was analyzed using normal phase analytical HPLC (Luna silica; length 250 mm, 4.6 mm i.d., flow 1 mL/min; Linear gradient: EtOAc (3% DCM)/Hexane (3% DCM); 20% EtOAc (3% DCM) for 5 min, 20% to 55% EtOAc (3% DCM) in 35 min) and purified using preparative HPLC (Luna silica; length 250 mm, 10 mm i.d., flow 5 mL/min, Linear gradient: EtOAc (3% DCM)/Hexane (3% DCM); 20% EtOAc (3% DCM) for 5 min, 20% to 55% EtOAc (3% DCM) in 35 min) to obtain compound **S8** (21.3 mg, 36% overall yield based on the resin loading). Compound **S8** elutes at 31 minutes.

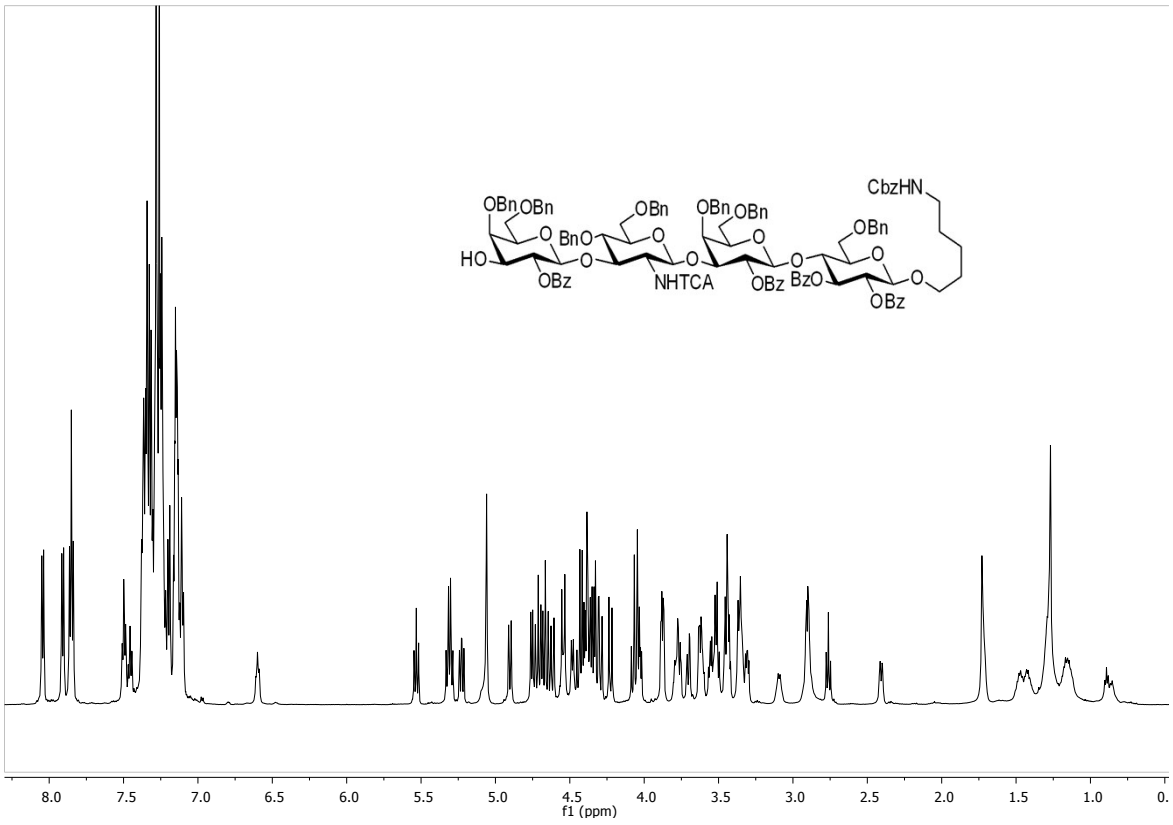
*Analytical data for tetrasaccharide **S8**:* ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.2 Hz, 2H), 7.91 (d, *J* = 7.3 Hz, 2H), 7.85 (t, *J* = 7.8 Hz, 3H), 7.52 - 7.48 (m, 2H), 7.45 (t, *J* = 12.2 Hz, 1H), 7.40 – 7.08 (m, 51H),

6.62 – 6.58 (m, 1H), 5.53 (t, *J* = 9.4 Hz, 1H), 5.33 – 5.27 (m, 2H), 5.23 (dd, *J* = 10.0, 8.0 Hz, 1H), 5.06 (s, 2H), 4.90 (d, *J* = 10.4 Hz, 1H), 4.77 – 4.59 (m, 5H), 4.54 (d, *J* = 12.3 Hz, 2H), 4.49 – 4.27 (m, 8H), 4.23 (d, *J* = 12.3 Hz, 1H), 4.10 – 4.00 (m, 3H), 3.88 (dd, *J* = 8.0, 3.0 Hz, 2H), 3.81 – 3.74 (m, 2H), 3.73 – 3.68 (m, 1H), 3.65 – 3.59 (m, 2H), 3.59 – 3.48 (m, 3H), 3.48 – 3.41 (m, 3H), 3.40 – 3.33 (m, 3H), 3.31 (dd, *J* = 8.4, 5.1 Hz, 1H), 3.11 – 3.06 (m, 1H), 2.93 – 2.86 (m, 3H), 2.76 (t, *J* = 8.7 Hz, 1H), 2.41 (d, *J* = 7.8 Hz, 1H), 1.52 – 1.38 (m, 2H), 1.33 – 1.22 (m, 2H), 1.21 – 1.10 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.10, 165.38, 165.31, 164.66, 161.28, 156.36, 139.31, 138.21, 138.10, 138.06, 137.75, 136.84, 133.58, 133.30, 133.16, 132.49, 130.59, 130.03, 130.00, 129.97, 129.90, 129.81, 129.70, 129.62, 128.65, 128.61, 128.59, 128.57, 128.55, 128.51, 128.50, 128.47, 128.43, 128.24, 128.20, 128.17, 128.06, 127.98, 127.96, 127.93, 127.88, 127.79, 127.77, 127.70, 127.17, 101.05, 100.83, 99.72, 98.95, 92.37, 77.81, 76.70, 76.41, 76.35, 75.68, 75.08, 74.86, 74.77, 74.73, 74.68, 74.49, 73.61, 73.59, 73.58, 73.48, 73.42, 73.28, 73.16, 73.01, 72.67, 72.08, 69.73, 69.32, 67.83, 67.53, 67.32, 66.60, 59.37, 40.91, 29.47, 28.97, 23.15. MS ESI HRMS: *m/z* $[M+Na]^+$ calcd for $C_{116}H_{117}Cl_3N_2NaO_{27}$ 2097.6807, found 2097.6773.

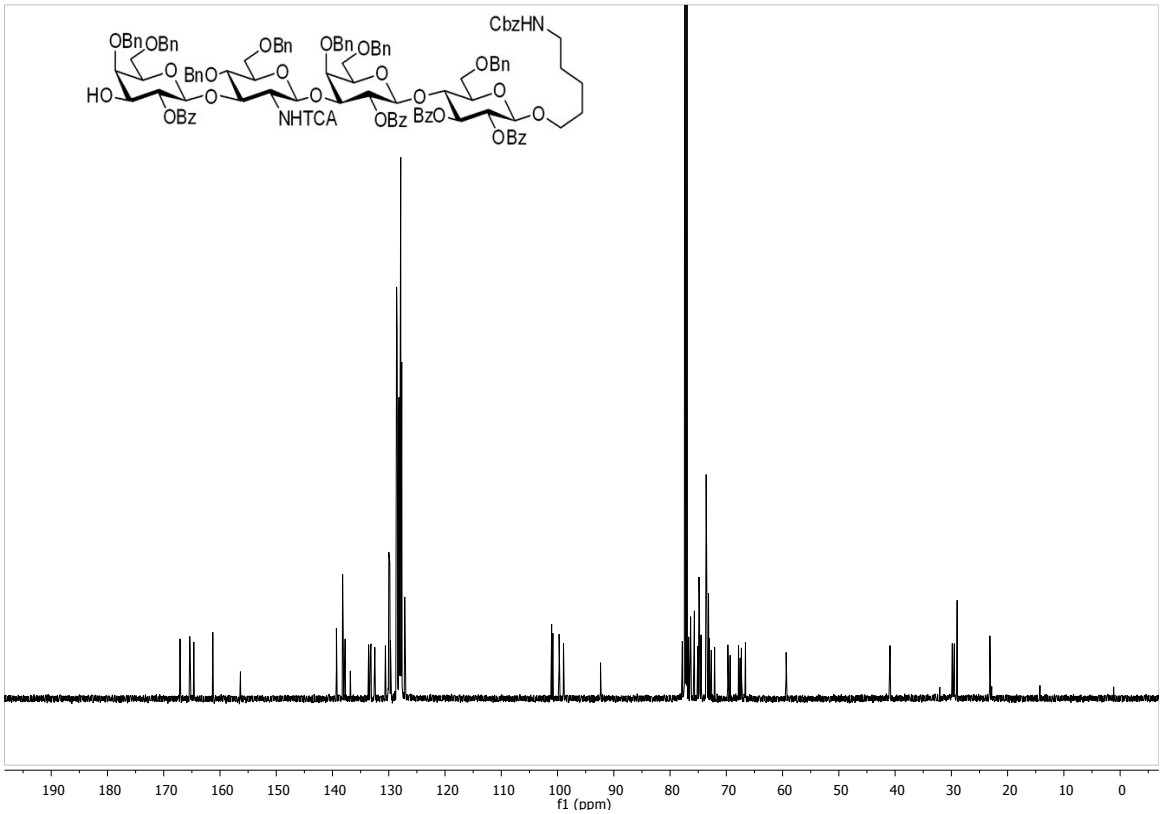
Analytical NP-HPLC Luna Silica of Crude Tetrasaccharide S8 (ELSD trace)



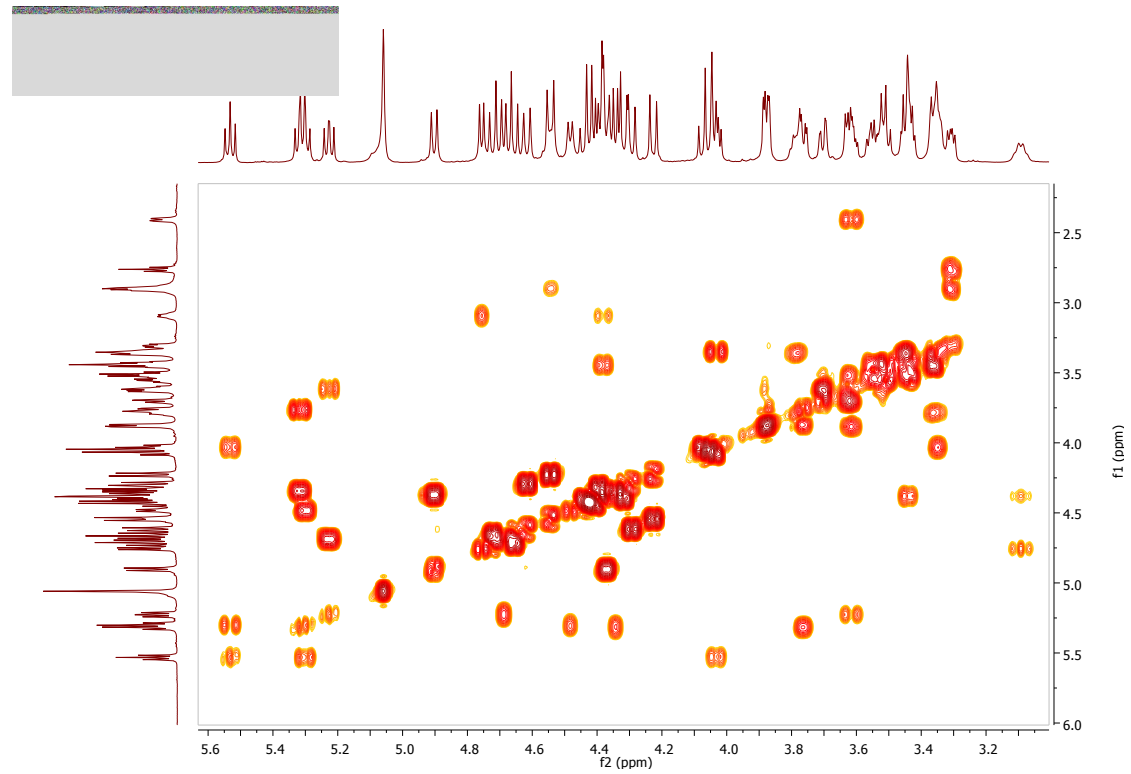
^{1}H NMR of Compound Tetrasaccharide S8



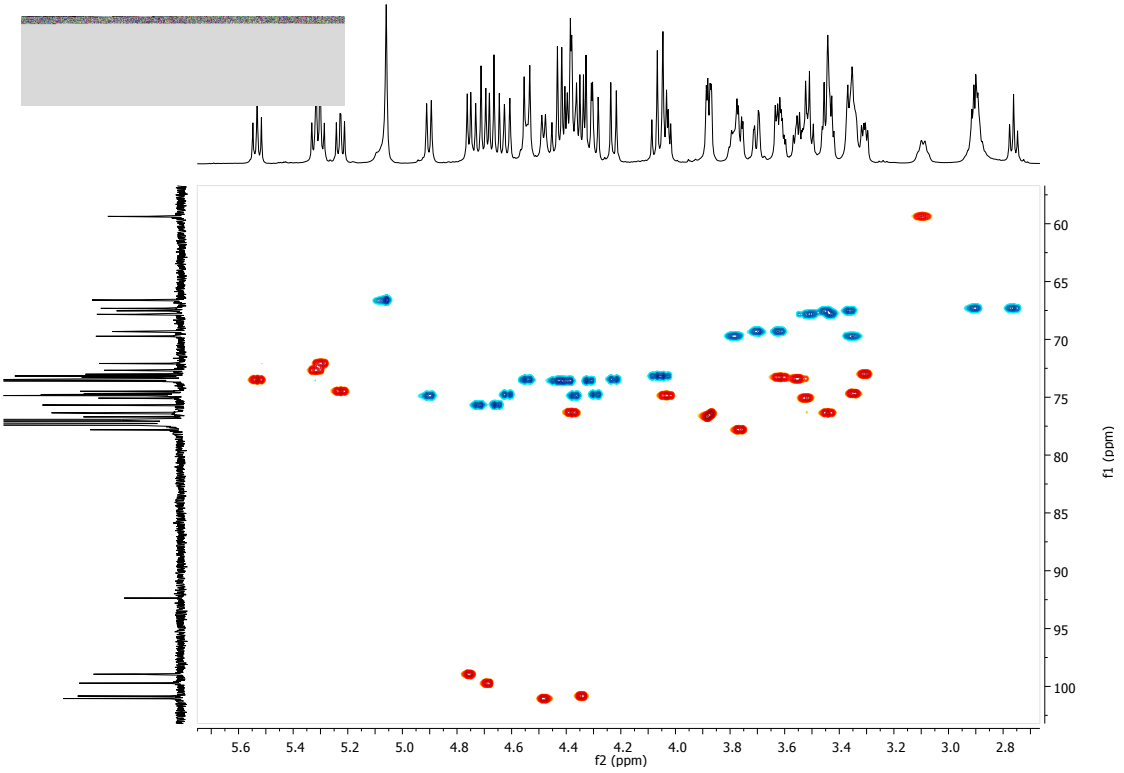
^{13}C NMR of Compound Tetrasaccharide S8



COSY NMR of Compound Tetrasaccharide S8



HSQC NMR of Compound Tetrasaccharide S8



3.8 Automated Synthesis of Protected Tetrasaccharide S9

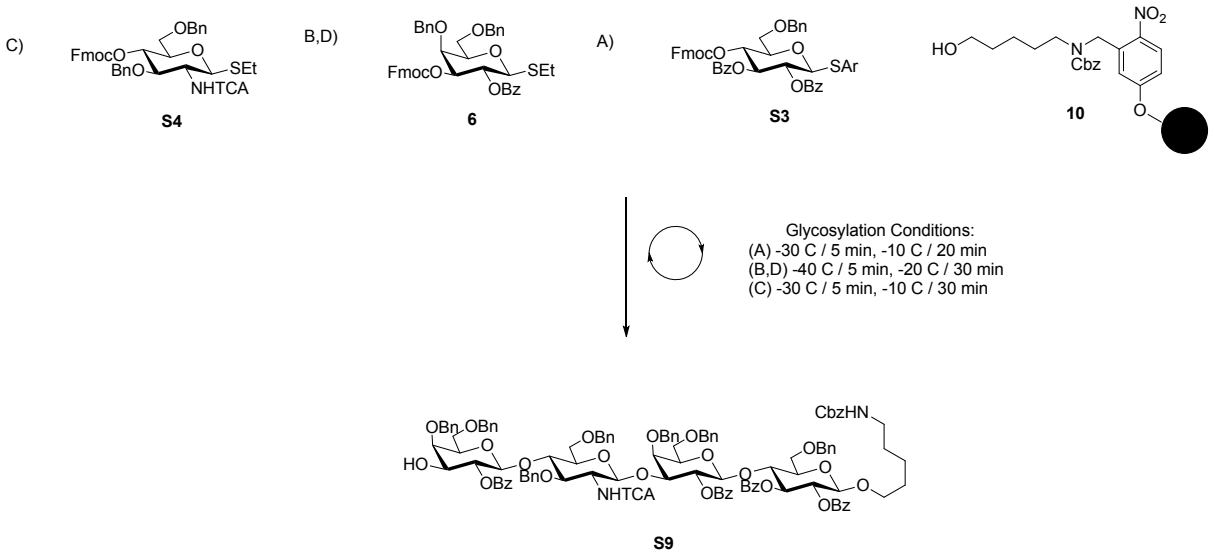
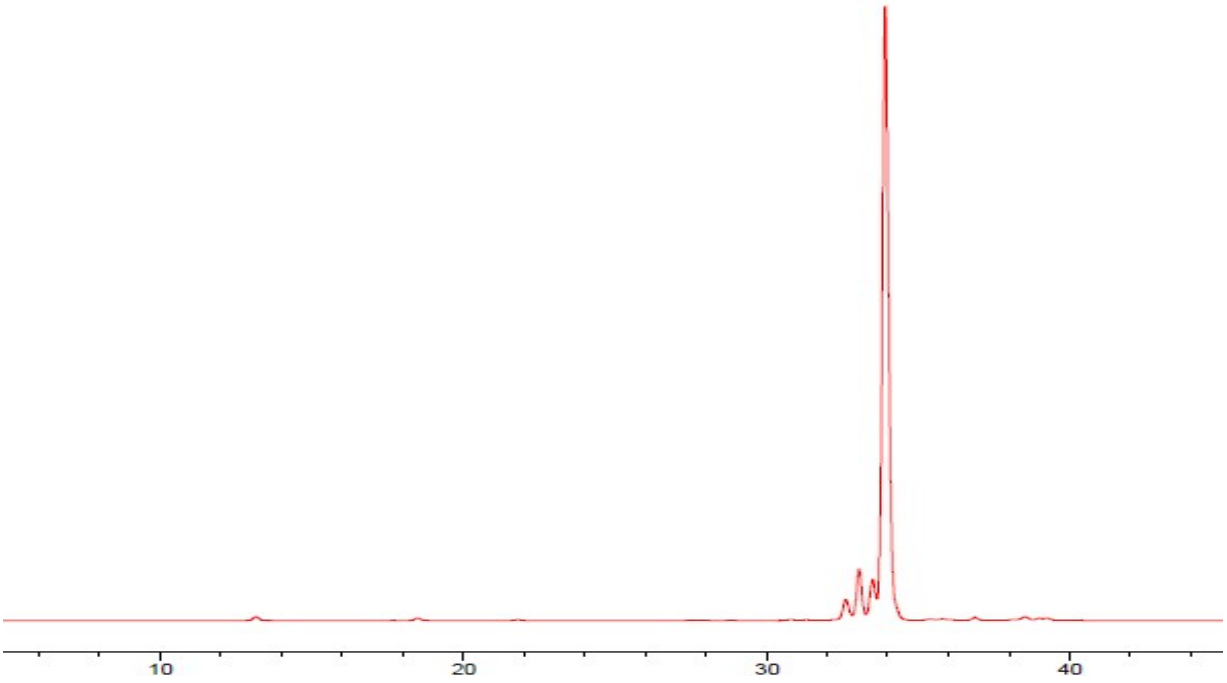


Table S5. Automated Synthesis Program Protocol for Tetrasaccharide **S9**		
Steps	Automation Process	Module
1	Preparation of Resin for Synthesis	A
2	Acidic Wash	B
3	Thioglycoside Glycosylation: BB **S3**	C4
4	Fmoc Deprotection	D
5	Acidic Wash	B
6	Thioglycoside Glycosylation: BB **6**	C5
7	Fmoc Deprotection	D
8	Acidic Wash	B
9	Thioglycoside Glycosylation: BB **S4**	C6
10	Fmoc Deprotection	D
11	Acidic Wash	B
12	Thioglycoside Glycosylation: BB **6**	C5
13	Fmoc Deprotection	D

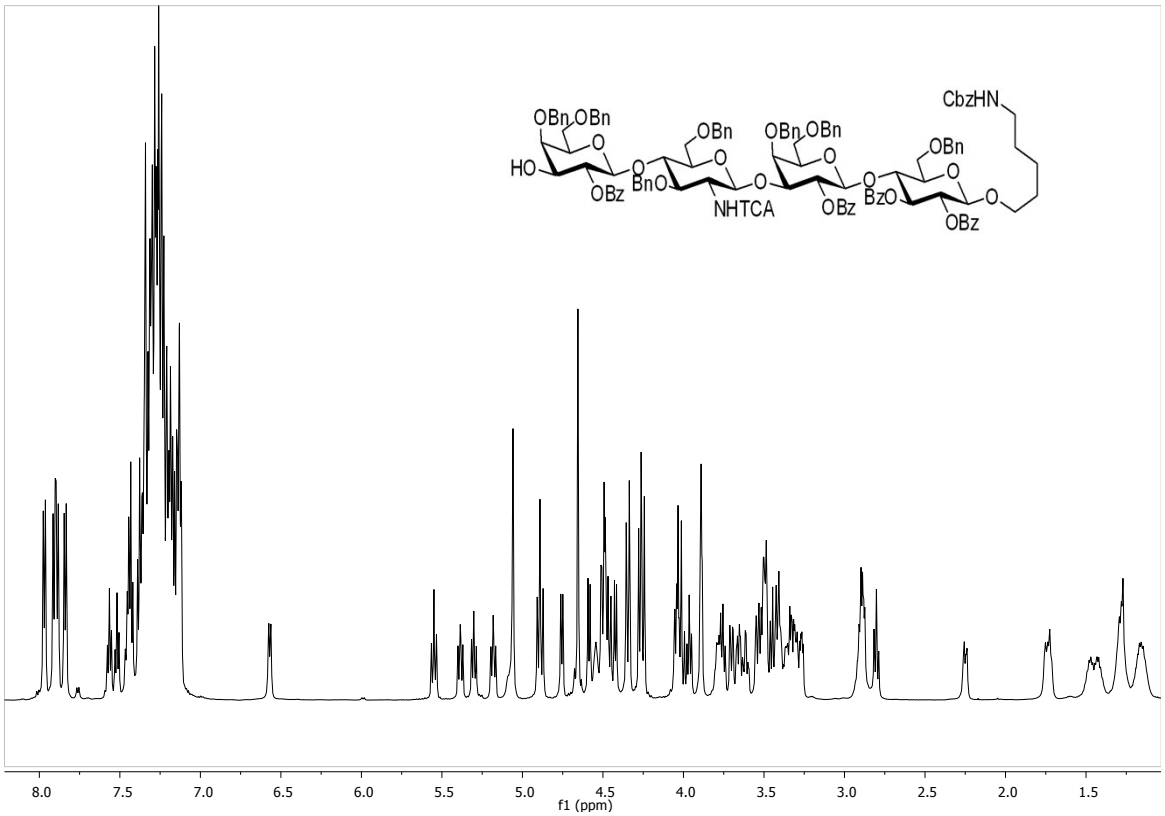
Cleavage, Analysis and Purification: Tetrasaccharide **S9** was cleaved from the solid support as described in Post-Synthesizer Manipulations. The crude product was analyzed using normal phase analytical HPLC (Luna silica; length 250 mm, 4.6 mm i.d., flow 1 mL/min; Linear gradient: EtOAc (3% DCM)/Hexane (3% DCM); 20% EtOAc (3% DCM) for 5 min, 20% to 55% EtOAc (3% DCM) in 35 min) and purified using preparative HPLC (Luna silica; length 250 mm, 10 mm i.d., flow 5 mL/min, Linear gradient: EtOAc (3% DCM)/Hexane (3% DCM); 20% EtOAc (3% DCM) for 5 min, 20% to 55% EtOAc (3% DCM) in 35 min) to obtain compound **S9** (23.8 mg, 44% overall yield based on the resin loading). Compound **S9** elutes at 33 min.

*Analytical data for tetrasaccharide **S9**:* ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.8 Hz, 2H), 7.90 (dd, *J* = 11.5, 8.0 Hz, 4H), 7.84 (d, *J* = 7.7 Hz, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.4 Hz, 1H), 7.47 – 7.42 (m, 3H), 7.41 – 7.08 (m, 47H), 6.57 (d, *J* = 7.8 Hz, 1H), 5.55 (t, *J* = 9.3 Hz, 1H), 5.39 (t, J = 11.6 Hz, 1H), 5.30 (t, *J* = 11.7, 1H), 5.18 (t, J = 9.3 Hz, 1H), 5.06 (s, 2H), 4.89 (t, *J* = 10.9 Hz, 2H), 4.76 (d, *J* = 7.7 Hz, 1H), 4.65 (s, 2H), 4.59 (d, *J* = 7.9 Hz, 1H), 4.57 – 4.44 (m, 5H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.35 (d, *J* = 11.4 Hz, 2H), 4.30 – 4.22 (m, 2H), 4.08 – 3.99 (m, 3H), 3.96 (t, *J* = 8.4 Hz, 1H), 3.89 (s, 2H), 3.82 – 3.73 (m, 2H), 3.70 (dd, *J* = 10.2, 2.4 Hz, 1H), 3.66 (dd, *J* = 10.5, 3.4 Hz, 1H), 3.61 (td, *J* = 10.1, 3.2 Hz, 1H), 3.57 – 3.24 (m, 11H), 2.94 – 2.86 (m, 3H), 2.80 (t, *J* = 8.7 Hz, 1H), 2.28 – 2.22 (m, 1H), 1.52 – 1.38 (m, 2H), 1.33 – 1.23 (m, 2H), 1.19 – 1.12 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.28, 165.33, 165.31, 164.65, 161.69, 156.35, 139.23, 138.42, 138.31, 138.15, 138.13, 137.96, 137.74, 136.83, 133.44, 133.35, 133.16, 132.49, 130.56, 129.99, 129.93, 129.87, 129.81, 129.77, 129.68, 128.69, 128.65, 128.60, 128.57, 128.45, 128.43, 128.19, 128.15, 128.11, 128.09, 128.06, 128.04, 128.00, 127.91, 127.87, 127.82, 127.79, 127.77, 127.73, 127.66, 127.36, 127.18, 101.04, 100.97, 100.39, 100.15, 92.14, 79.19, 77.66, 76.51, 76.47, 76.00, 75.58, 75.18, 74.93, 74.80, 74.72, 74.52, 74.33, 73.58, 73.52, 73.44, 73.37, 73.13, 72.96, 72.85, 72.46, 72.13, 69.72, 68.20, 67.72, 67.63, 67.32, 66.59, 57.99, 40.90, 29.47, 28.97, 23.15. MS ESI HRMS: *m/z* $[M+Na]^+$ calcd for $C_{116}H_{117}Cl_3N_2NaO_{27}$ 2097.6807, found 2097.6781.

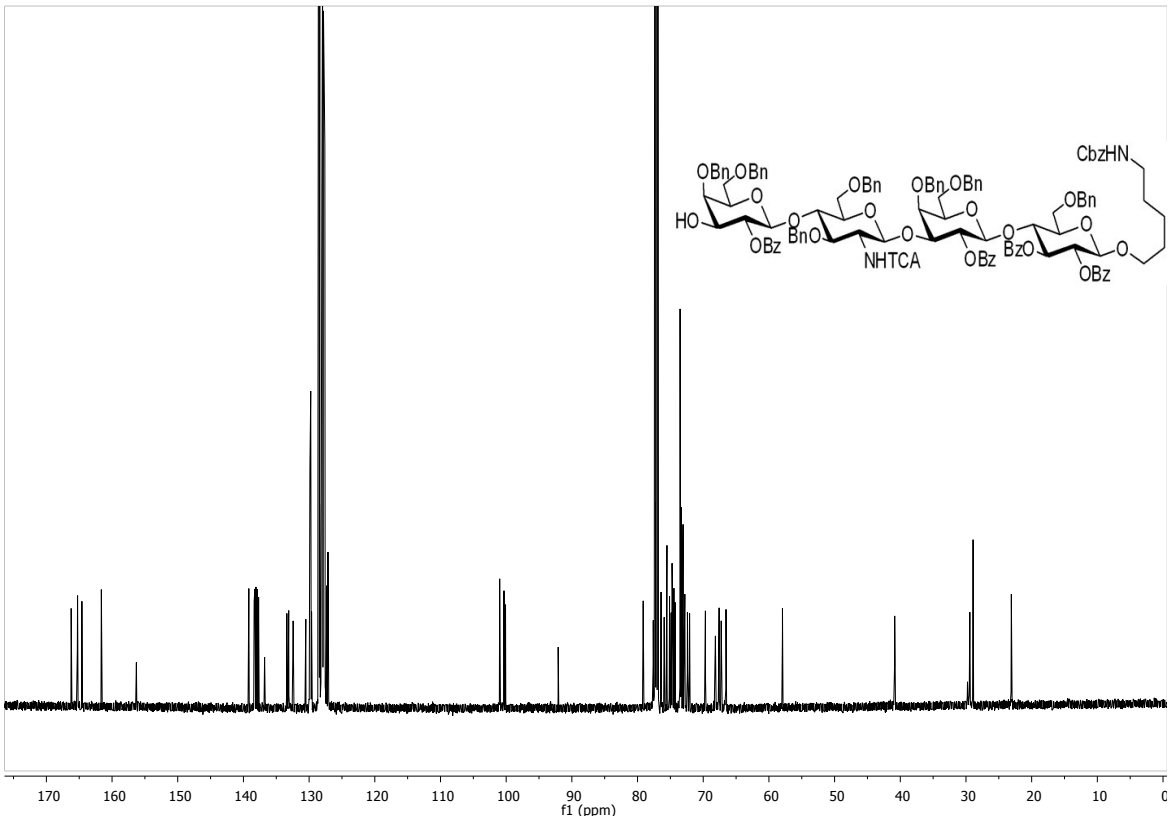
Analytical NP-HPLC Luna Silica of Crude Tetrasaccharide S9 (ELSD trace)



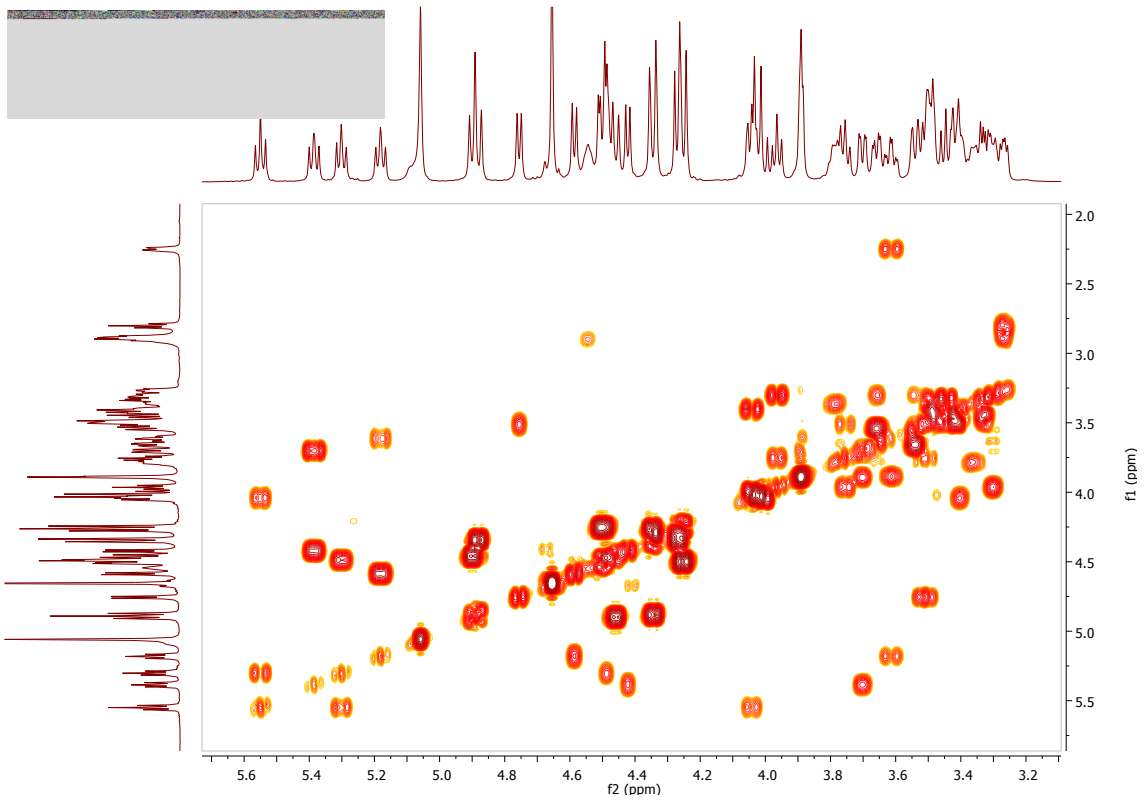
^{1}H NMR of Compound Tetrasaccharide S9



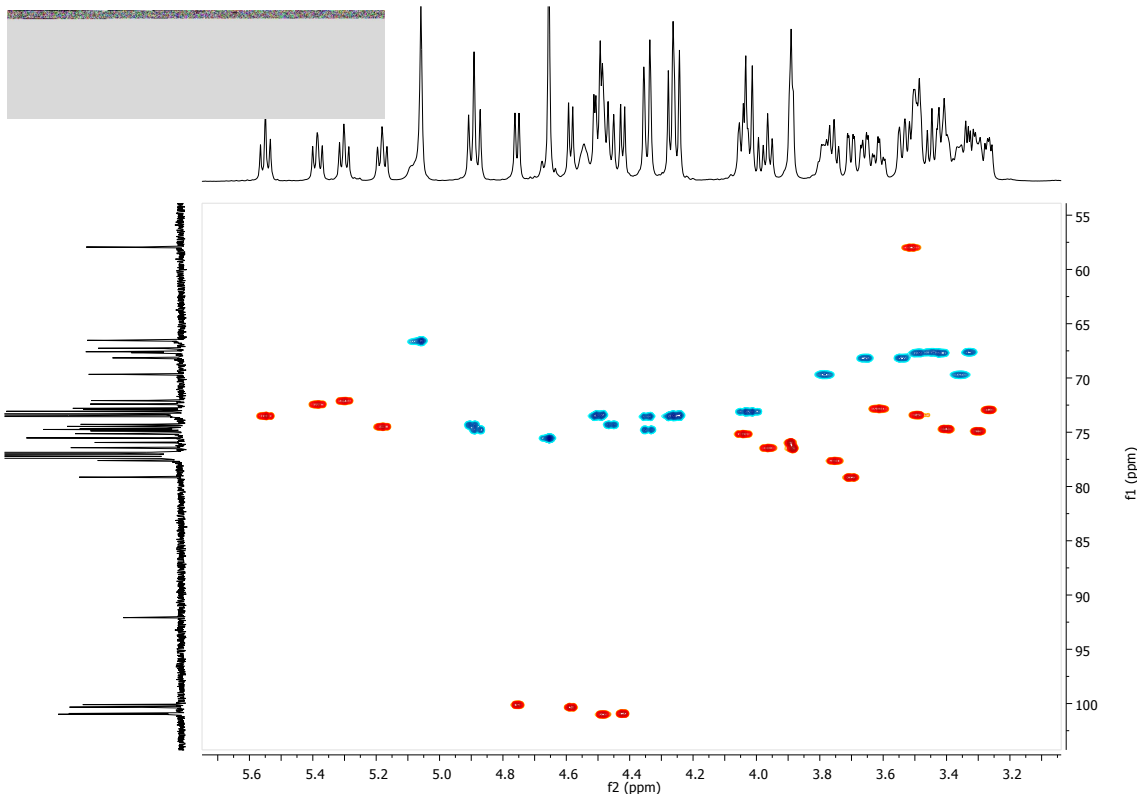
^{13}C NMR of Compound Tetrasaccharide S9



COSY NMR of Compound Tetrasaccharide S9



HSQC NMR of Compound Tetrasaccharide S9



3.9 Automated Synthesis of Protected Hexasaccharide S10

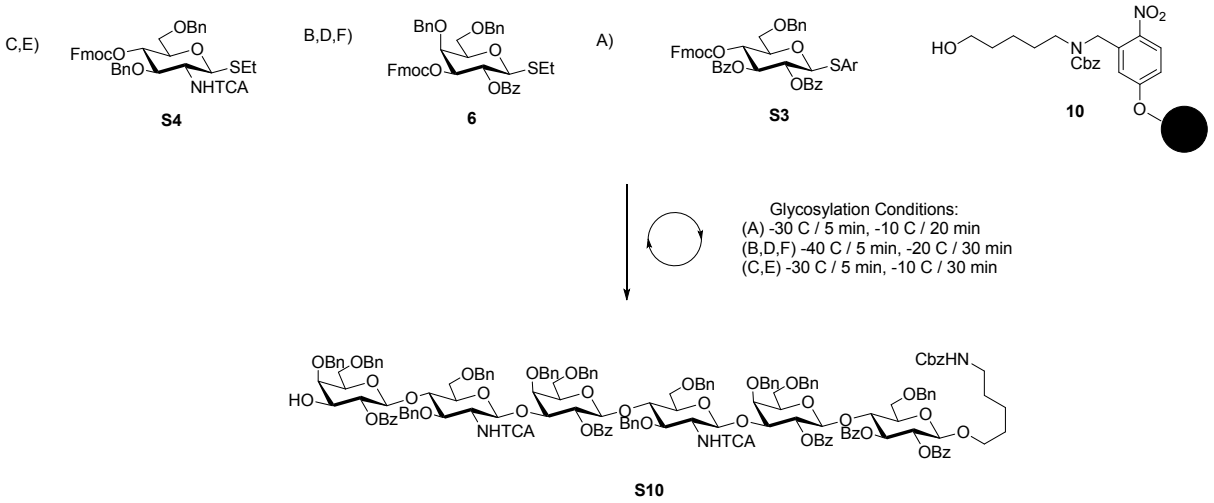


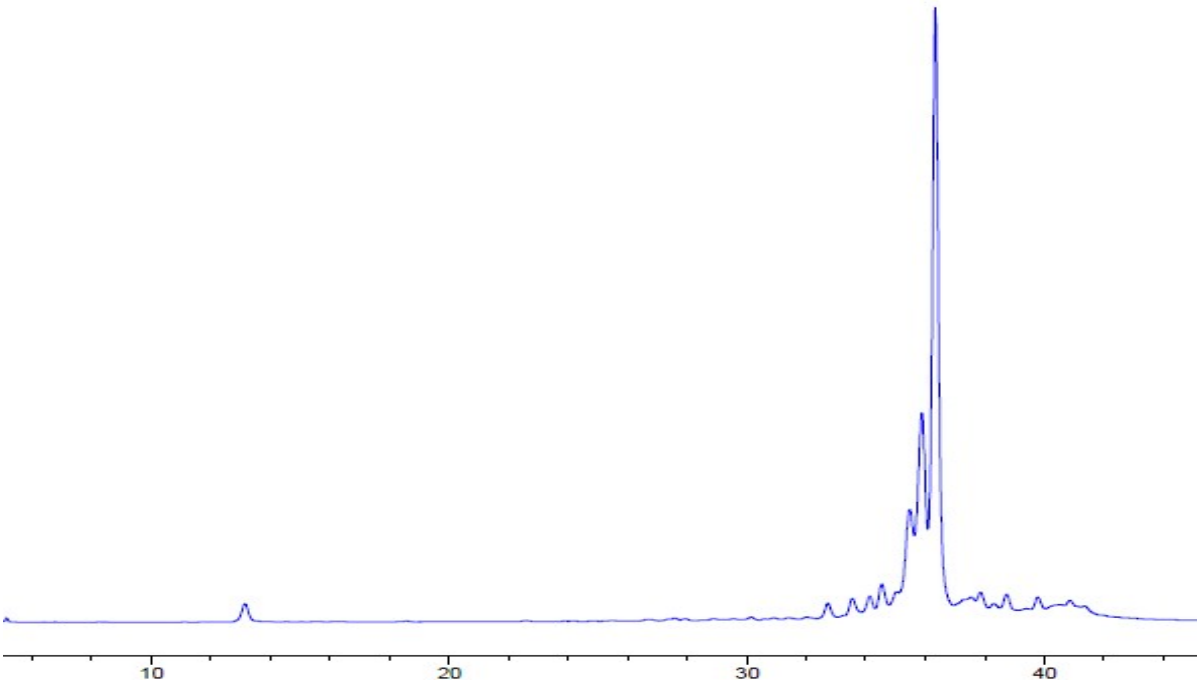
Table S6. Automated Synthesis Program Protocol for Hexasaccharide **S10**		
Steps	Automation Process	Module
1	Preparation of Resin for Synthesis	A
2	Acidic Wash	B
3	Thioglycoside Glycosylation: BB **S3**	C4
4	Fmoc Deprotection	D
5	Acidic Wash	B
6	Thioglycoside Glycosylation: BB **6**	C5
7	Fmoc Deprotection	D
8	Acidic Wash	B
9	Thioglycoside Glycosylation: BB **S4**	C6
10	Fmoc Deprotection	D
11	Acidic Wash	B
12	Thioglycoside Glycosylation: BB **6**	C5
13	Fmoc Deprotection	D
14	Acidic Wash	B
15	Thioglycoside Glycosylation: BB **S4**	C6
16	Fmoc Deprotection	D
17	Acidic Wash	B
18	Thioglycoside Glycosylation: BB **6**	C5
19	Fmoc Deprotection	D

Cleavage, Analysis and Purification: Hexasaccharide **S10** was cleaved from the solid support as described in Post-Synthesizer Manipulations. The crude product was analyzed using normal phase analytical HPLC (Luna silica; length 250 mm, 4.6 mm i.d., flow 1 mL/min; Linear gradient: EtOAc (3% DCM)/Hexane (3% DCM); 20% EtOAc (3% DCM) for 5 min, 20% to 55% EtOAc (3% DCM) in 35 min) and purified using preparative HPLC (Luna silica; length 250 mm, 10 mm i.d., flow 5 mL/min, Linear gradient: EtOAc (3% DCM)/Hexane (3% DCM); 20% EtOAc (3% DCM) for 5 min, 20% to 55% EtOAc

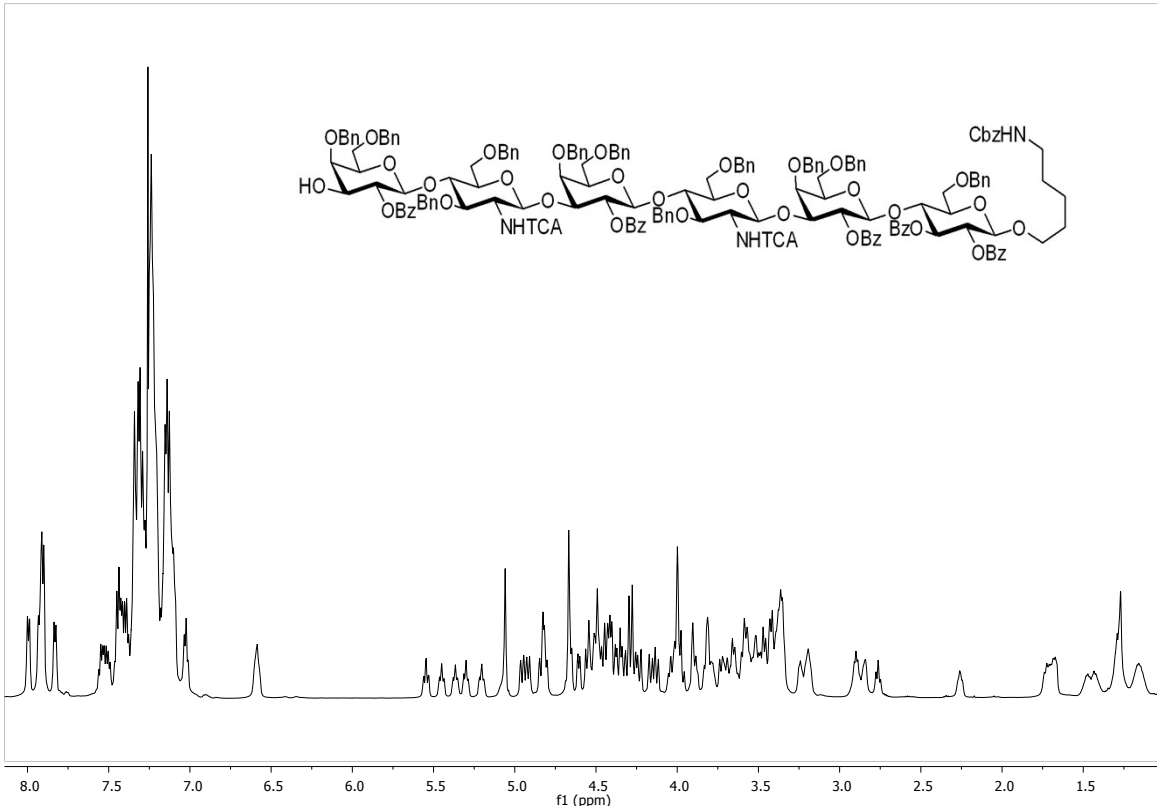
(3% DCM) in 35 min) to obtain compound **S10** (27.6 mg, 37% overall yield based on the resin loading). Compound **S10** elutes at 36 min.

*Analytical data for hexasaccharide **S10**:* ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01 – 7.98 (m, 2H), 7.95 – 7.88 (m, 6H), 7.85 – 7.81 (m, 2H), 7.57 – 7.48 (m, 3H), 7.47 – 7.07 (m, 71H), 7.05 – 7.00 (m, 2H), 6.59 (br s, 2H), 5.54 (t, *J* = 8.6 Hz, 1H), 5.45 (t, *J* = 8.1 Hz, 1H), 5.37 (t, *J* = 8.0 Hz, 1H), 5.30 (t, *J* = 7.9 Hz, 1H), 5.20 (t, *J* = 7.3 Hz, 1H), 5.05 (s, 2H), 4.95 (d, *J* = 11.1 Hz, 1H), 4.92 (d, *J* = 10.5 Hz, 1H), 4.86 – 4.78 (m, 3H), 4.70 – 4.63 (m, 3H), 4.61 (d, *J* = 7.6 Hz, 1H), 4.58 – 4.20 (m, 14H), 4.20 – 4.10 (m, 2H), 4.07 – 3.94 (m, 5H), 3.93 – 3.86 (m, 2H), 3.86 – 3.75 (m, 3H), 3.75 – 3.30 (m, 21H), 3.27 – 3.15 (m, 3H), 2.95 – 2.81 (m, 3H), 2.76 (t, *J* = 8.6 Hz, 1H), 2.29 – 2.22 (m, 1H), 1.52 – 1.38 (m, 2H), 1.34 – 1.22 (m, 2H), 1.22 – 1.09 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.31, 165.34, 165.32, 164.94, 164.62, 161.70, 161.66, 156.36, 139.21, 138.96, 138.34, 138.30, 138.18, 138.11, 138.08, 138.02, 137.92, 137.71, 136.82, 133.49, 133.35, 133.16, 132.47, 130.56, 129.98, 129.93, 129.87, 129.81, 129.77, 129.67, 128.76, 128.70, 128.65, 128.60, 128.58, 128.50, 128.43, 128.40, 128.23, 128.18, 128.15, 128.11, 128.09, 128.06, 128.02, 127.94, 127.89, 127.83, 127.79, 127.75, 127.68, 127.61, 127.45, 127.22, 127.12, 101.04, 100.99, 100.76, 100.46, 92.14, 92.10, 79.44, 79.26, 77.81, 77.68, 76.50, 76.36, 75.91, 75.76, 75.60, 75.27, 75.19, 75.12, 75.08, 74.77, 74.67, 74.52, 74.31, 74.14, 73.68, 73.63, 73.60, 73.50, 73.41, 73.39, 73.05, 72.93, 72.81, 72.46, 72.27, 72.13, 69.72, 68.32, 68.23, 68.17, 67.65, 67.35, 66.58, 57.95, 57.44, 40.90, 29.46, 28.97, 23.15. MS ESI HRMS: *m/z* $[M+Na]^+$ calcd for $C_{165}H_{165}Cl_6N_3NaO_{38}$ 3028.9100, found 3028.9145.

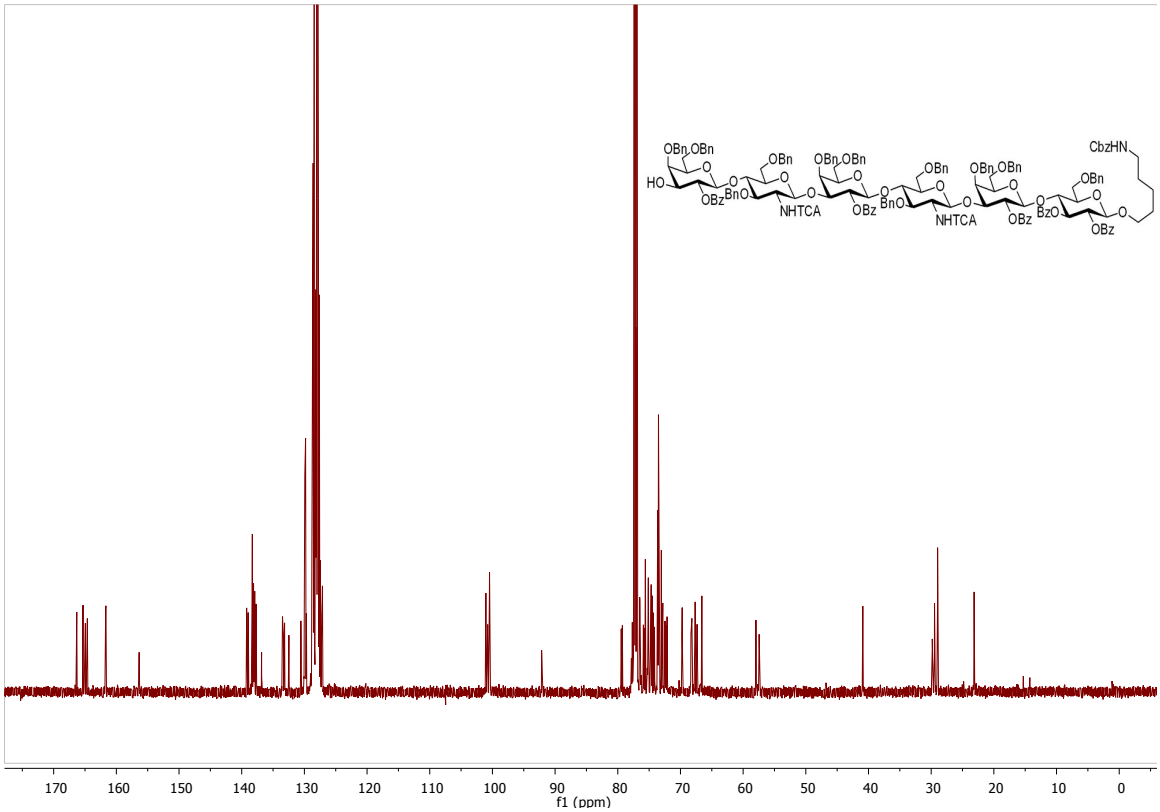
Analytical NP-HPLC Luna Silica of Crude Hexasaccharide S10 (ELSD trace)



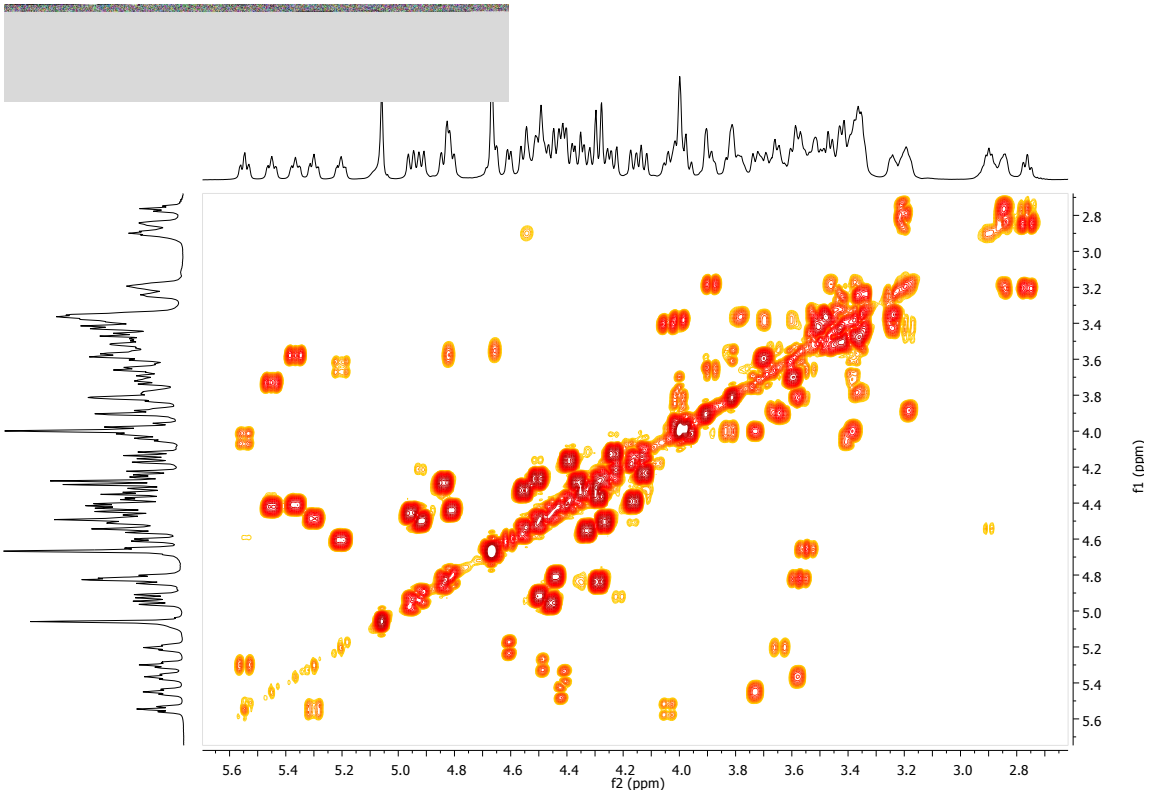
^{1}H NMR of Compound Hexasaccharide S10



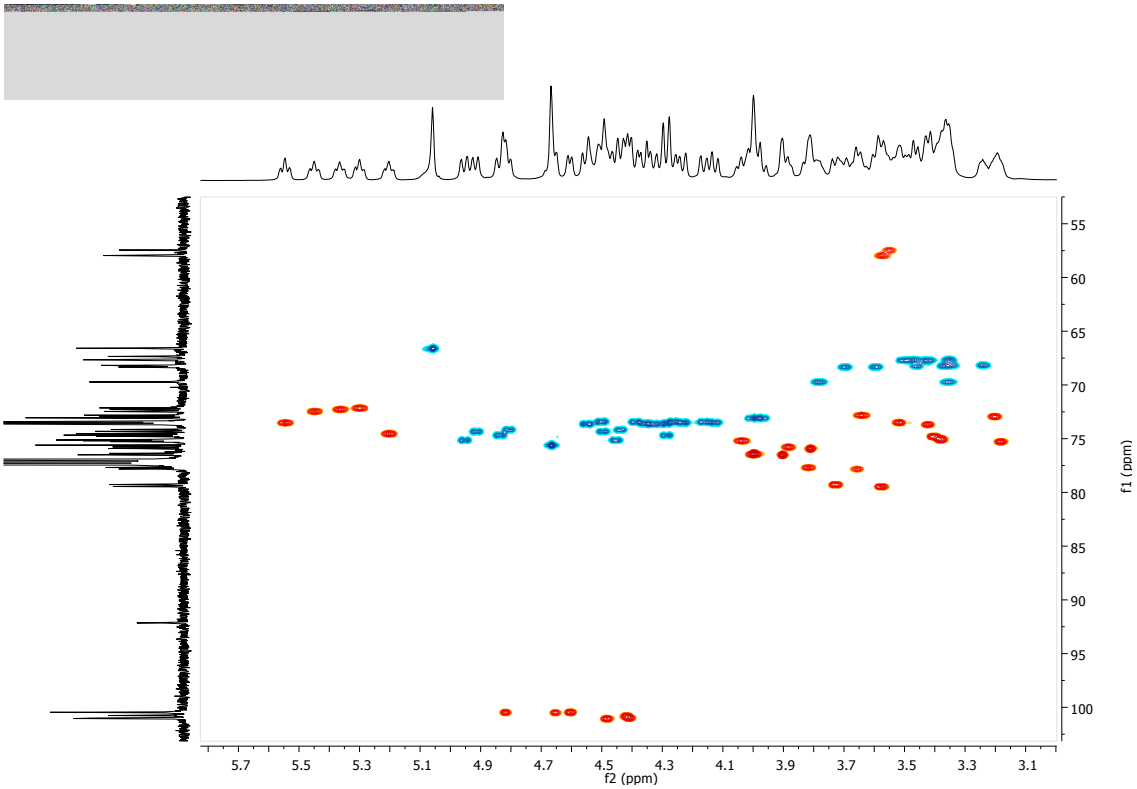
^{13}C NMR of Compound Hexasaccharide S10



COSY NMR of Compound Hexasaccharide S10



HSQC NMR of Compound Hexasaccharide S10



4. Oligosaccharide Deprotection

4.1 Methanolysis General Procedure:

Protected oligosaccharides (**S6** – **S10**) were put into 10 mL round bottom flasks and dissolved in 1 mL methanol and 0.2 mL DCM. 10 eq. NaOMe were added and the vessels were sealed with glass caps. The reactions were heated to 40 °C and stirred for 18 h. The reactions were cooled to room temperature and neutralized with Amberlite IR-120 (H^+ form) resin. The resin was filtered and the reactions were concentrated *in vacuo*. The remaining crude clear gels were taken forward without further purification. (TLC = 10% methanol in DCM)

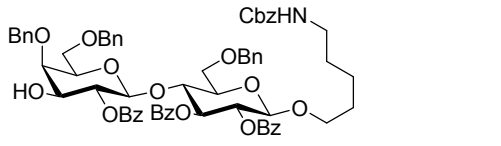
4.2 Hydrogenolysis General Procedure:

The products of the methanolysis reactions were dissolved in 2 mL methanol, 50 μL H_2O, and 50 μL acetic acid in 10 mL round bottom flasks. 60% by weight Pd/C (10%) was added. The reaction was purged by bubbling H_2 for 20 min. The reactions were equipped with H_2 balloons and stirred for 48 hours. The reactions were filtered through celite, washing with a 1 methanol : 1 H_2O solution, H_2O, and 10 mM aqueous ammonium bicarbonate. The filtrates were concentrated *in vacuo*. The partially deprotected products were redissolved in 5 mL H_2O, 0.1 mL MeOH, and 0.1 mL acetic acid and transferred to cylindrical vials. The vials were put inside a H_2 bomb and subjected to 45 psi H_2 for 24 hours (or 48 hours for **S10**). The reactions were filtered through celite, washing with a 1 methanol : 1 H_2O solution, H_2O, and 10 mM aqueous ammonium bicarbonate. The filtrates were concentrated *in vacuo*.

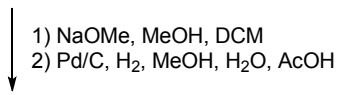
4.3 Purification

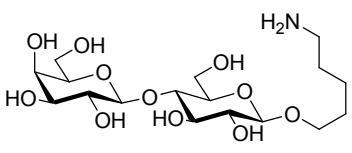
Following both methanolysis and hydrogenolysis, crude products were analyzed/ purified using analytical/preparative HPLC (Agilent 1200 Series spectrometer). Following purification all products were lyophilized on a Christ Alpha 2-4 LD plus freeze dryer prior to characterization.

4.4 Synthesis of Deprotected Disaccharide S11



S6



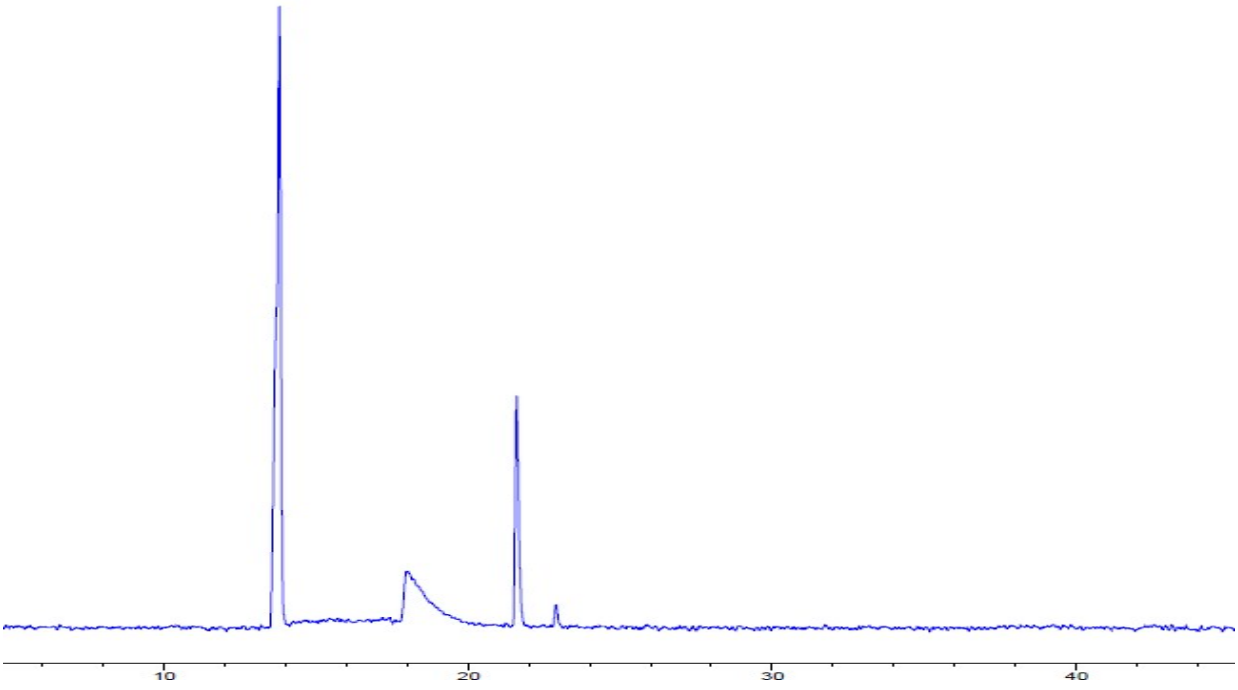


S11

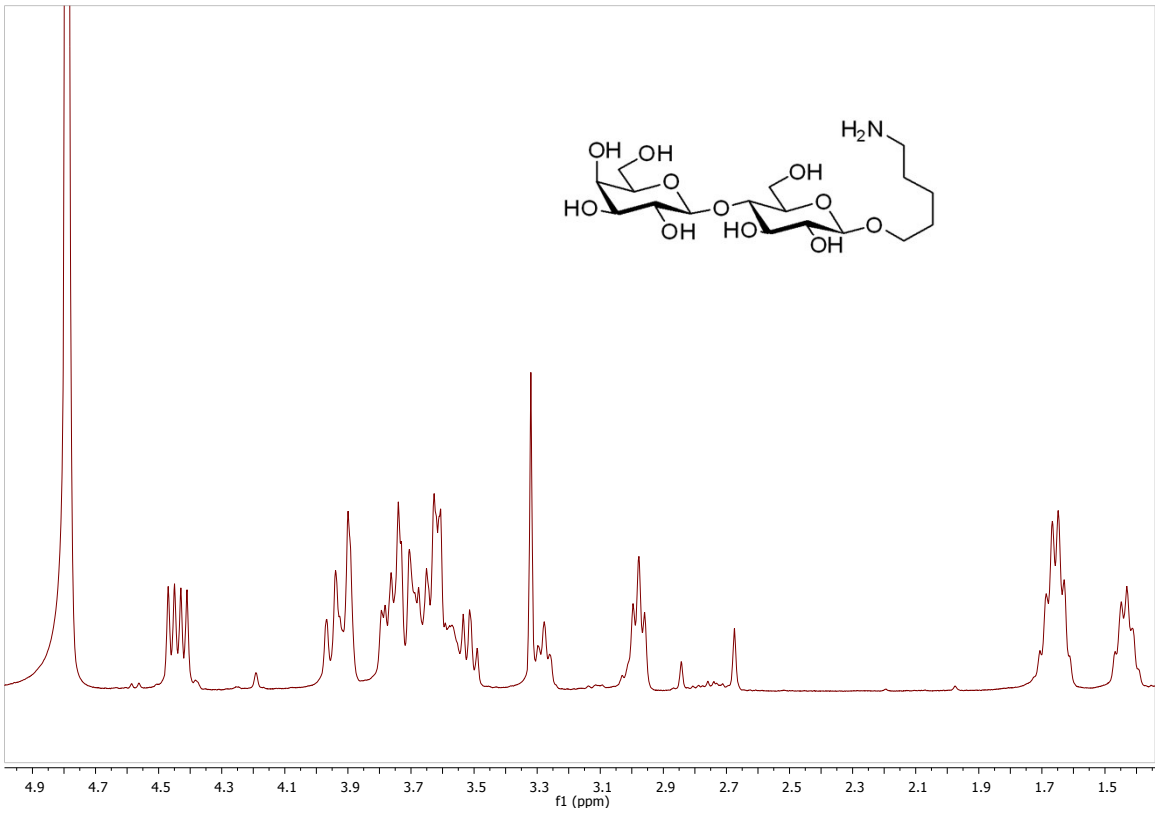
Deprotection, Analysis and Purification: Disaccharide **S6** (34 mg, 0.034 mmol) was deprotected as described in Methanolysis General Procedure and Hydrogenolysis General Procedure. The crude product was analyzed using reverse phase analytical HPLC (Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min; Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) and purified using preparative HPLC (Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min, Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) to obtain compound **S11** (11.8 mg, 0.028 mmol, 82% overall yield). Compound **S11** elutes at 21 min.

*Analytical data for disaccharide **S11***: ^{1}H NMR (400 MHz, D_2O) d 4.46 (d, *J* = 8.0 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.00 – 3.86 (m, 3H), 3.82 – 3.47 (m, 10H), 3.28 (t, *J* = 7.5 Hz, 1H), 2.98 (t, *J* = 7.3 Hz, 2H), 1.73 – 1.58 (m, 4H), 1.49 – 1.37 (m, 2H). ^{13}C NMR (101 MHz, D_2O) d 104.51, 103.59, 79.83, 76.96, 76.37, 76.05, 74.41, 74.10, 72.54, 71.68, 70.12, 62.55, 61.66, 41.03, 29.70, 28.00, 23.68. MS ESI HRMS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{34}NO_{11}$ 428.2132, found 428.2132.

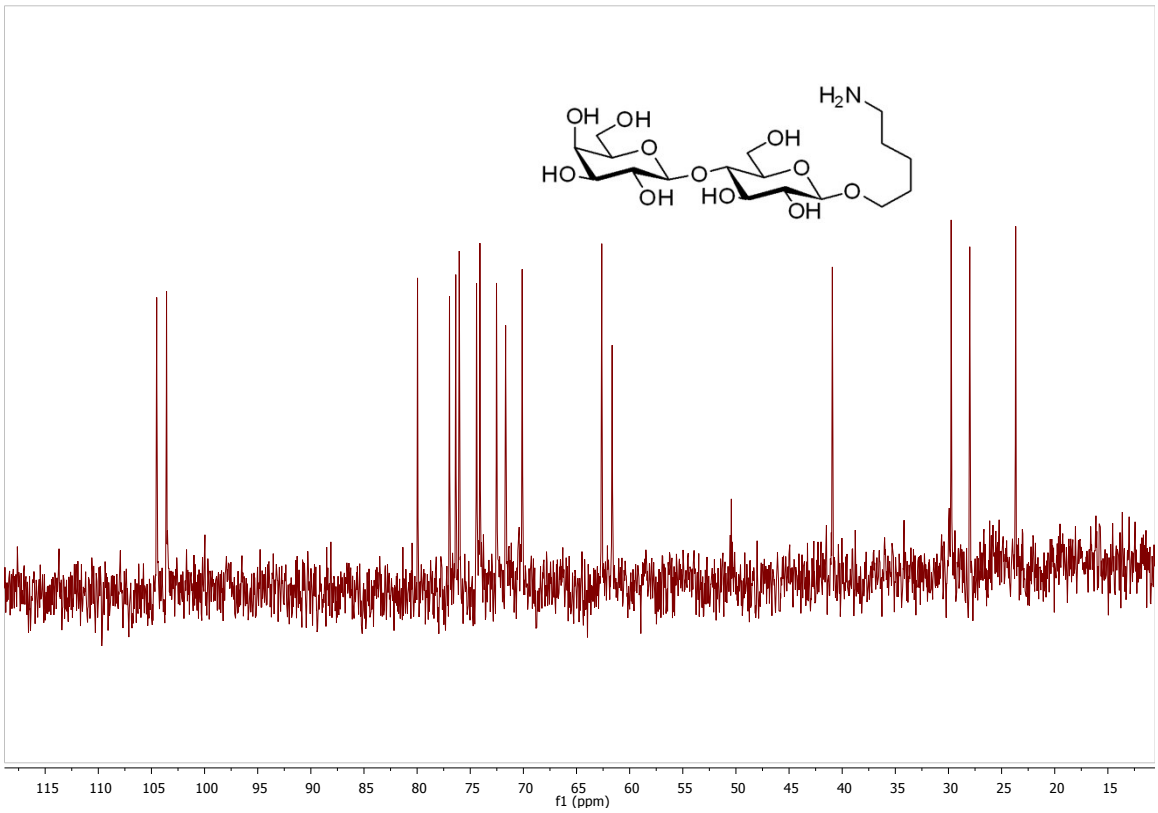
Analytical RP-HPLC Hypercarb of Crude Disaccharide S11 (ELSD trace)



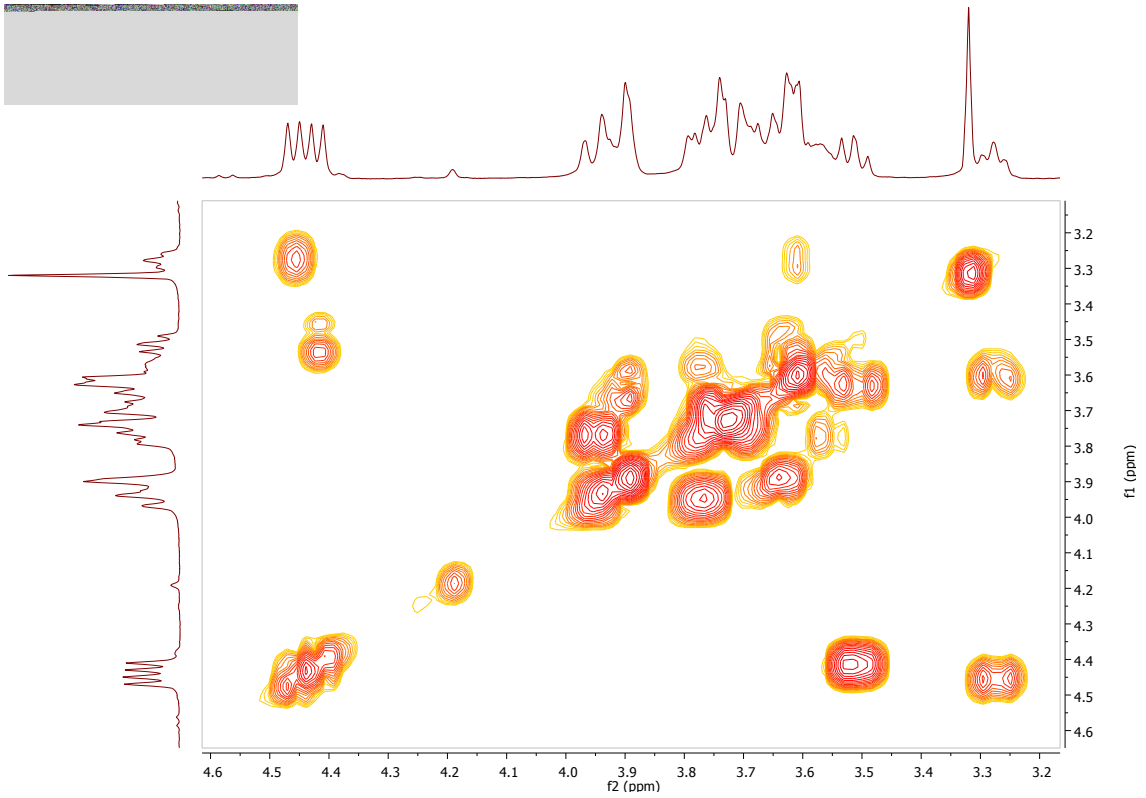
^{1}H NMR of Compound Disaccharide S11



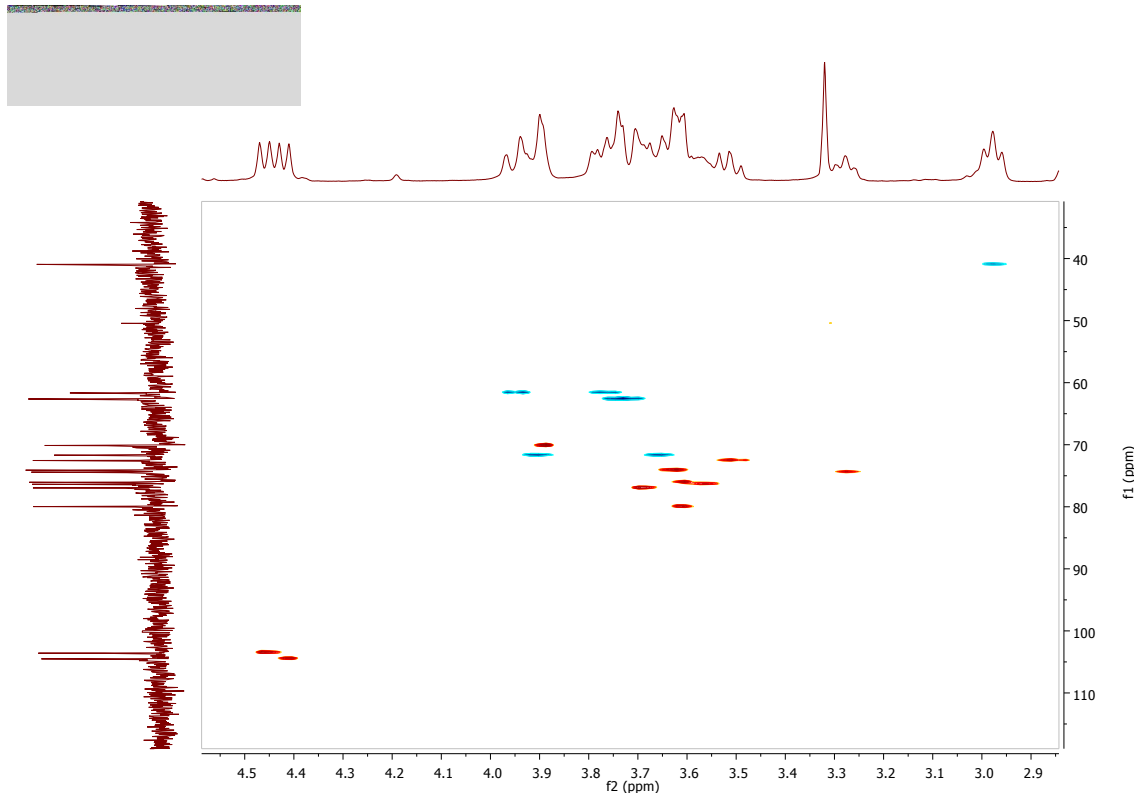
^{13}C NMR of Compound Disaccharide S11



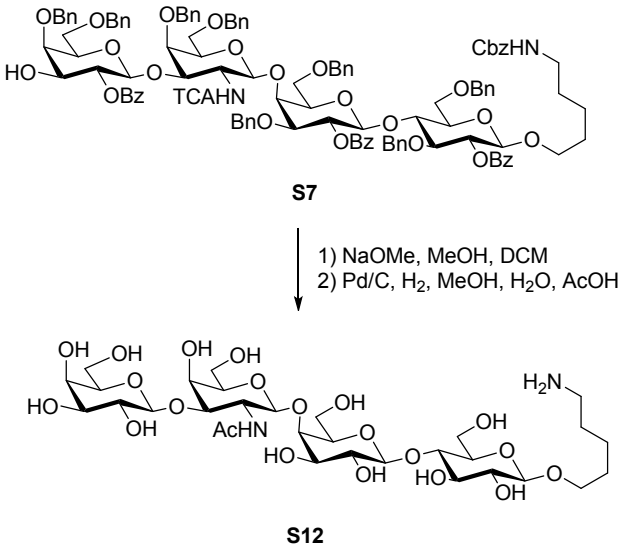
COSY NMR of Compound Disaccharide S11



HSQC NMR of Compound Disaccharide S11



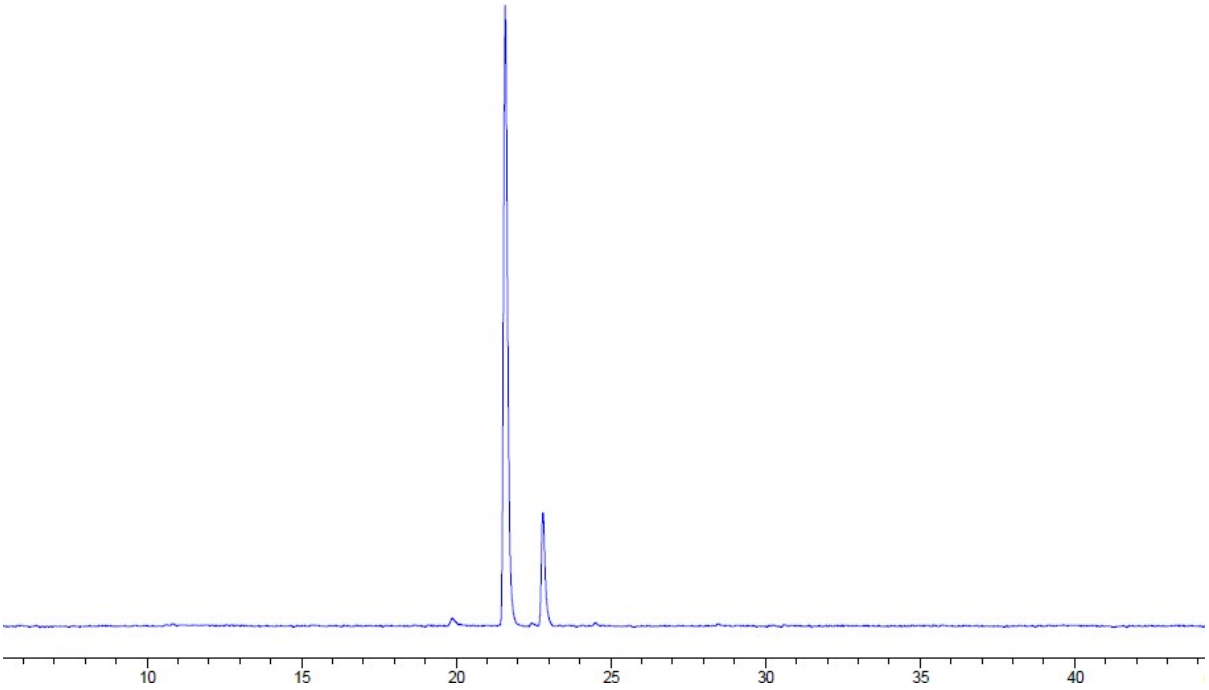
4.5 Synthesis of Deprotected Tetrasaccharide S12



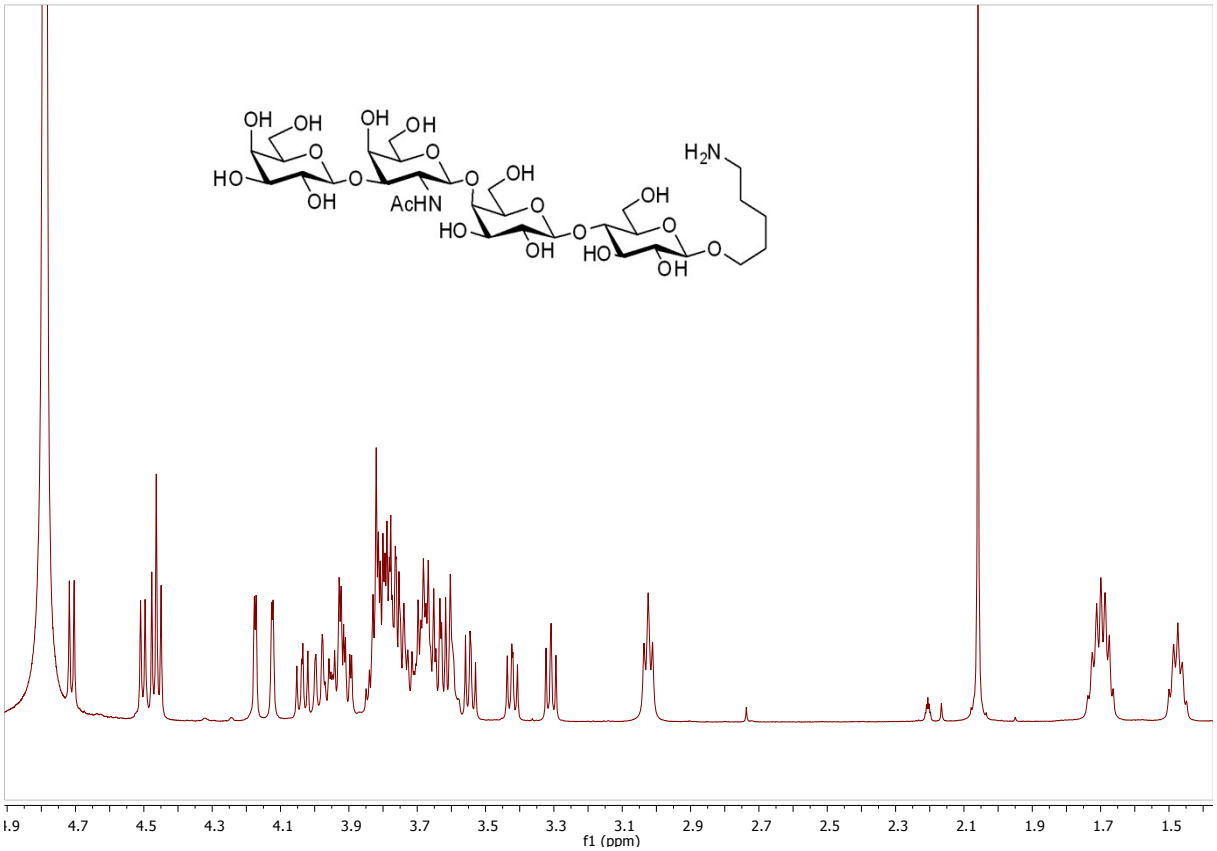
Deprotection, Analysis and Purification: Tetrasaccharide **S7** (35.4 mg, 0.017 mmol) was deprotected as described in Methanolysis General Procedure and Hydrogenolysis General Procedure. The crude product was analyzed using reverse phase analytical HPLC (Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min; Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) and purified using preparative HPLC (Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min, Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) to obtain compound **S12** (12.3 mg, 0.015 mmol, 91% overall yield). Compound **S12** elutes at 21 min.

*Analytical data for tetrasaccharide **S12**:* ^{1}H NMR (600 MHz, D_2O) δ 4.71 (d, *J* = 8.5 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.49 – 4.43 (m, 2H), 4.17 (d, *J* = 3.0 Hz, 1H), 4.12 (d, *J* = 2.6 Hz, 1H), 4.06 – 4.01 (m, 1H), 4.01 – 3.88 (m, 4H), 3.86 – 3.57 (m, 16H), 3.54 (dd, *J* = 9.9, 7.8 Hz, 1H), 3.42 (dd, *J* = 9.8, 8.0 Hz, 1H), 3.31 (t, *J* = 8.4 Hz, 1H), 3.02 (t, *J* = 7.4 Hz, 2H), 2.06 (s, 3H), 1.75 – 1.64 (m, 4H), 1.51 – 1.43 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 172.05, 106.51, 104.70, 104.09, 103.70, 81.46, 80.25, 77.79, 76.72, 76.51, 76.25, 76.13, 75.98, 74.48, 74.17, 72.79, 72.34, 71.79, 70.31, 69.75, 62.73, 62.47, 61.78, 53.23, 41.09, 29.87, 28.12, 24.15, 23.80. MS ESI HRMS: m/z $[M+Na]^+$ calcd for $C_{31}H_{56}N_2NaO_{21}$ 815.3273, found 815.3260.

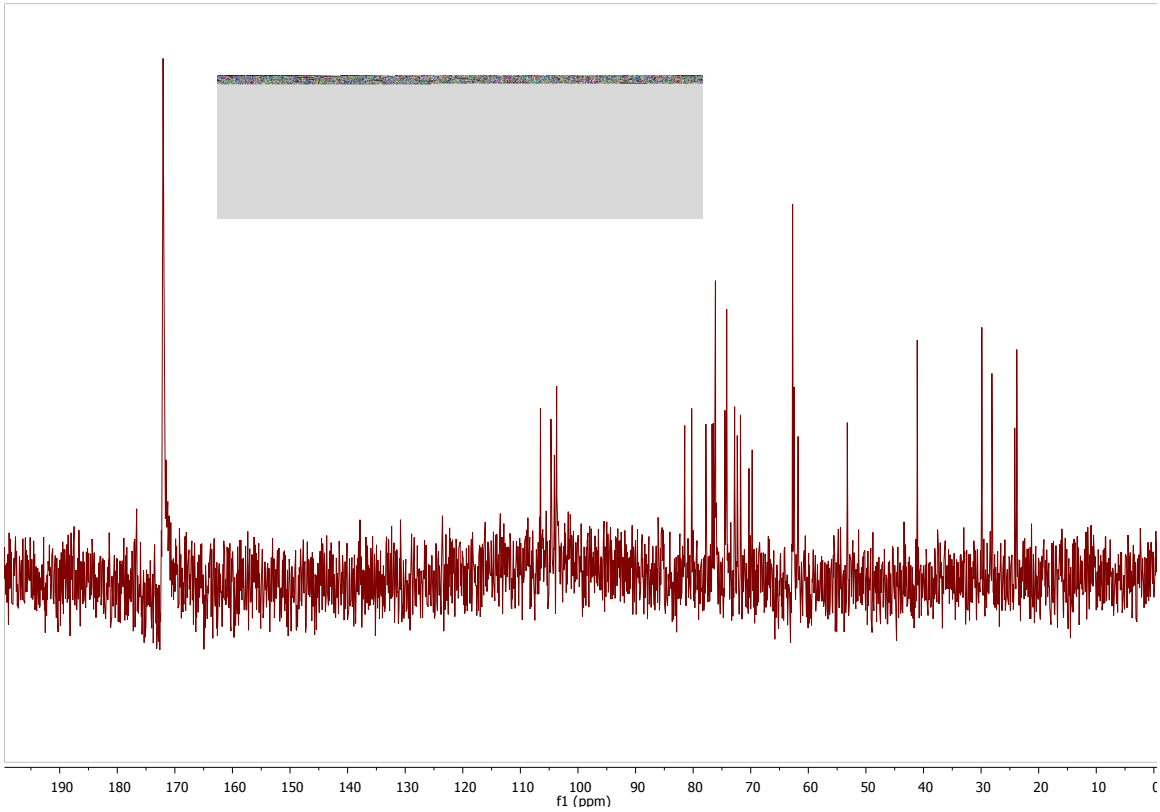
Analytical RP-HPLC Hypercarb of Crude Tetrasaccharide S12 (ELSD trace)



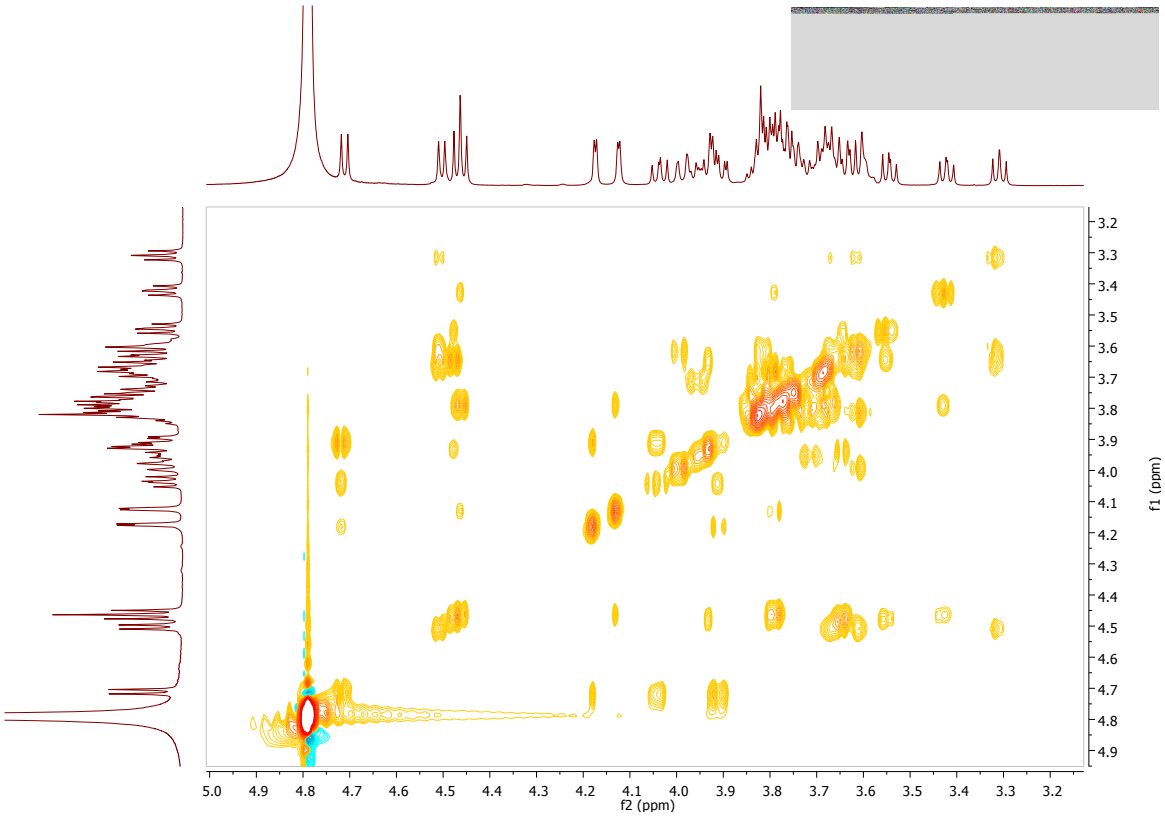
^{1}H NMR of Compound Tetrasaccharide S12



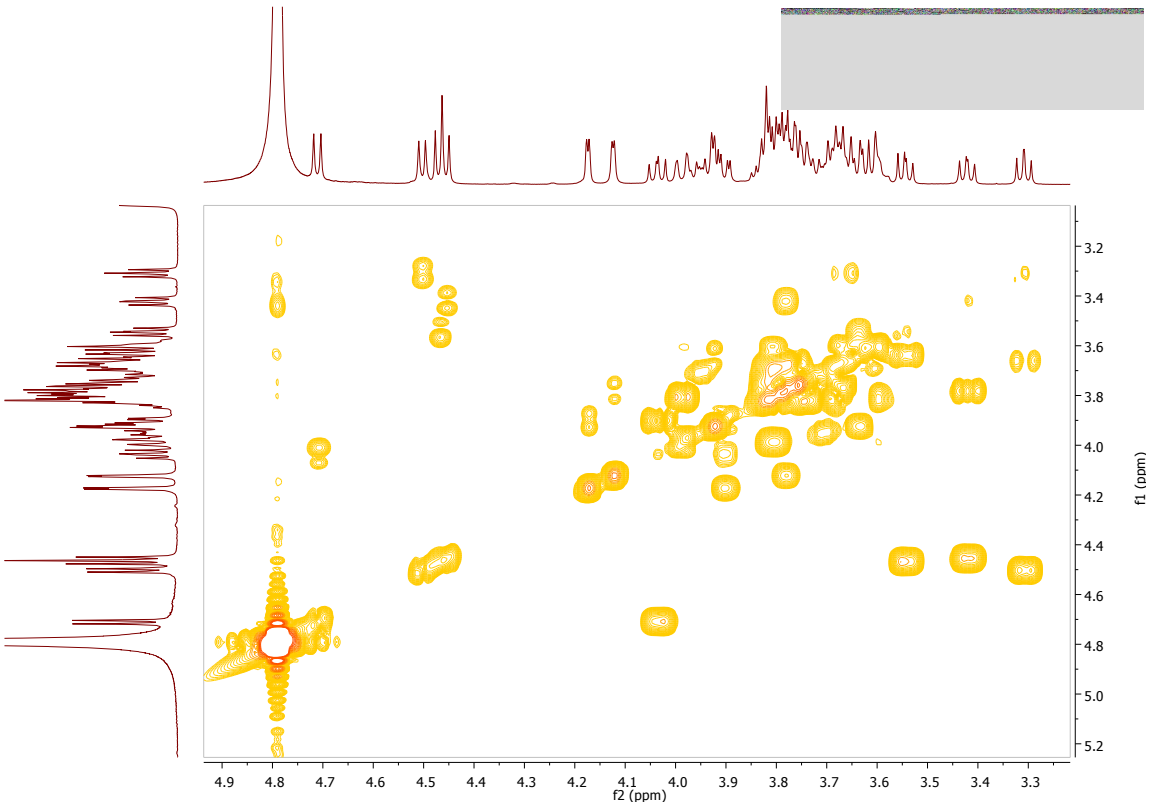
^{13}C NMR of Compound Tetrasaccharide S12



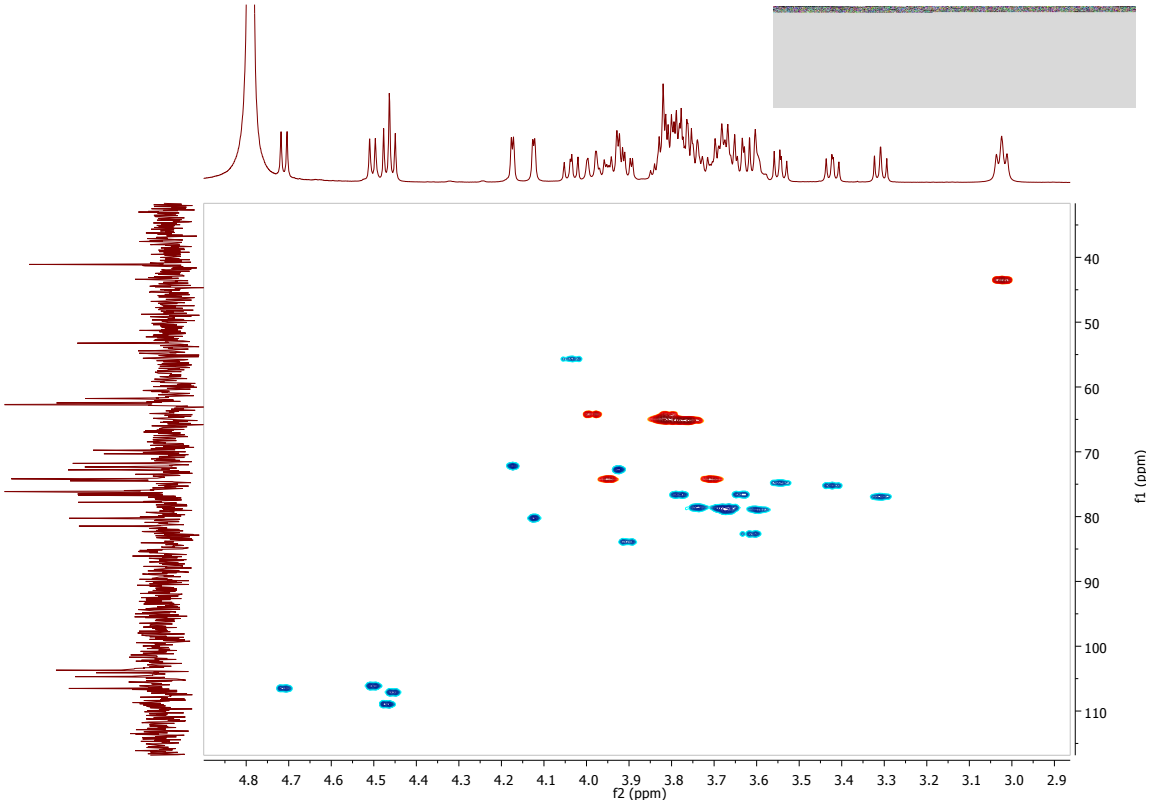
TOCSY NMR of Compound Tetrasaccharide S12



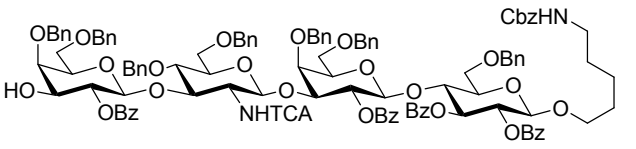
COSY NMR of Compound Tetrasaccharide S12



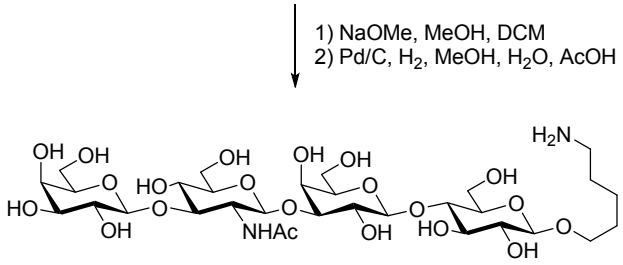
HSQC NMR of Compound Tetrasaccharide S12



4.6 Synthesis of Deprotected Tetrasaccharide S13



S8

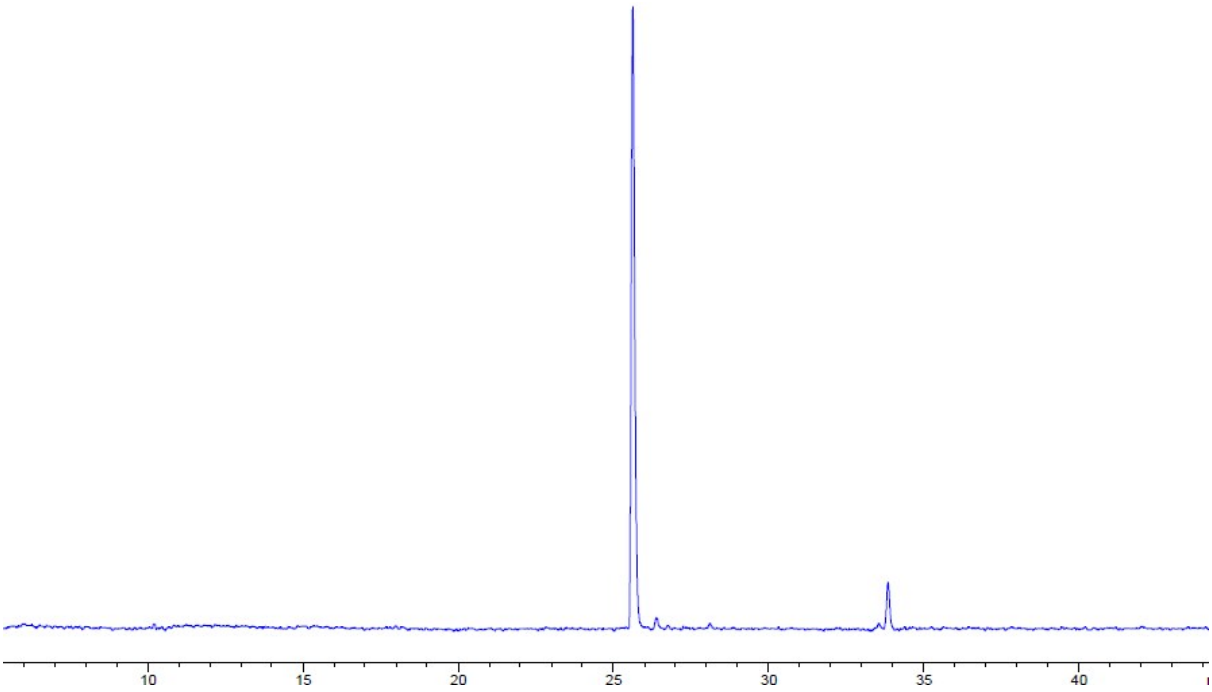


S13

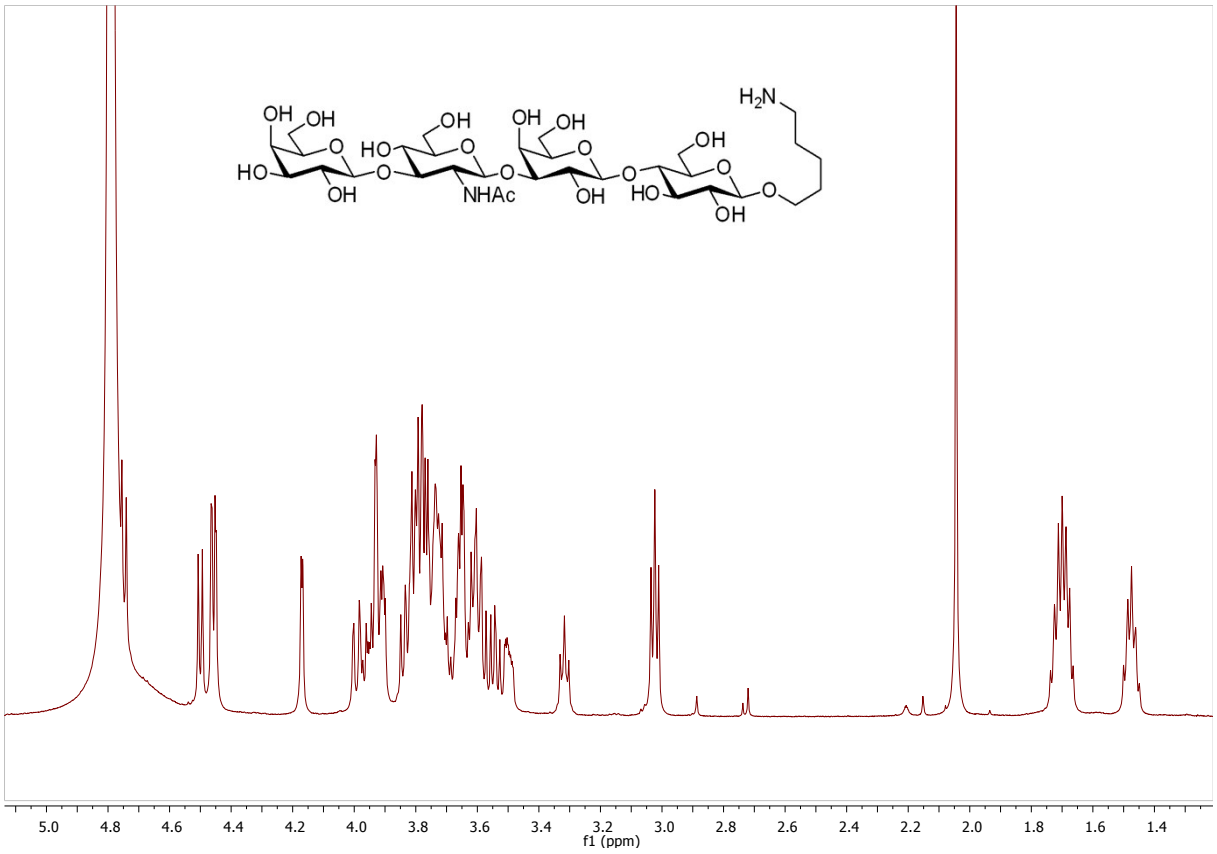
Deprotection, Analysis and Purification: Tetrasaccharide **S8** (43.8 mg, 0.021 mmol) was deprotected as described in Methanolysis General Procedure and Hydrogenolysis General Procedure. The crude product was analyzed using reverse phase analytical HPLC (Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min; Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) and purified using preparative HPLC (Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min, Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) to obtain compound **S13** (13.8 mg, 0.017 mmol, 83% overall yield). Compound **S13** elutes at 26 min.

*Analytical data for tetrasaccharide **S13**:* ^{1}H NMR (600 MHz, D_2O) δ 4.75 (d, *J* = 8.5 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.48 – 4.43 (m, 2H), 4.17 (d, *J* = 3.1 Hz, 1H), 4.03 – 3.87 (m, 5H), 3.87 – 3.46 (m, 19H), 3.32 (t, *J* = 8.5 Hz, 1H), 3.02 (t, *J* = 7.8 Hz, 2H), 2.04 (s, 3H), 1.76 – 1.64 (m, 4H), 1.52 – 1.43 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 172.08, 104.88, 104.33, 103.93, 103.39, 83.49, 83.40, 79.83, 76.67, 76.59, 76.30, 76.17, 75.85, 74.20, 73.87, 72.08, 71.46, 71.39, 69.92, 69.86, 69.69, 62.42, 62.35, 61.90, 61.50, 56.10, 40.75, 29.55, 27.80, 23.63, 23.48. MS ESI HRMS: m/z $[M+Na]^+$ calcd for $C_{31}H_{56}N_2NaO_{21}$ 815.3273, found 815.3285.

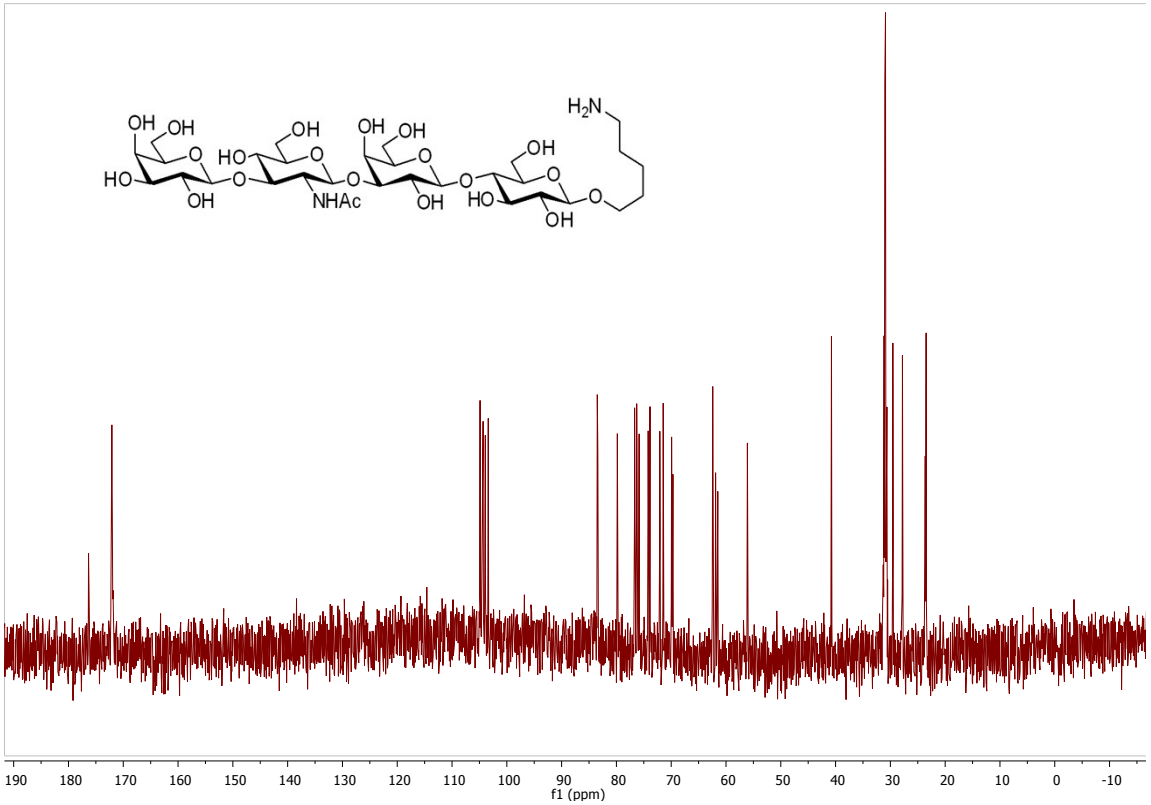
Analytical RP-HPLC Hypercarb of Crude Tetrasaccharide S13 (ELSD trace)



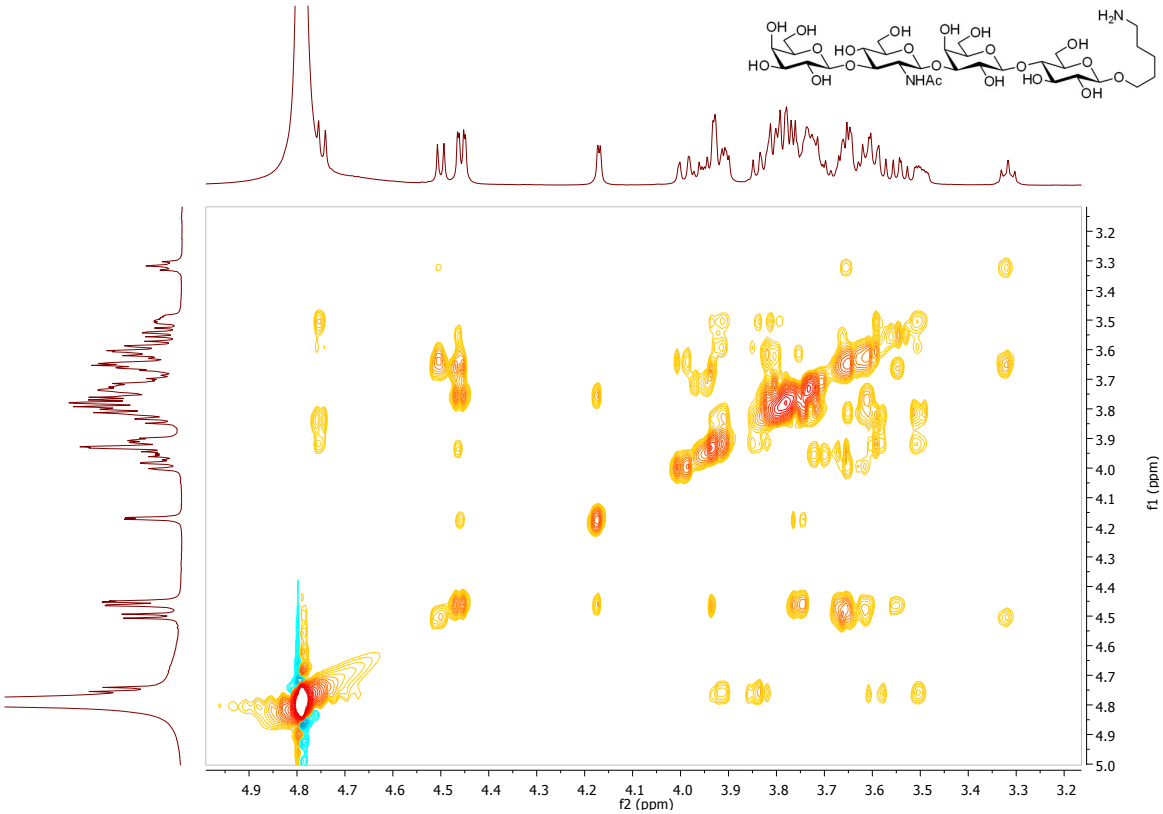
^{1}H NMR of Compound Tetrasaccharide S13



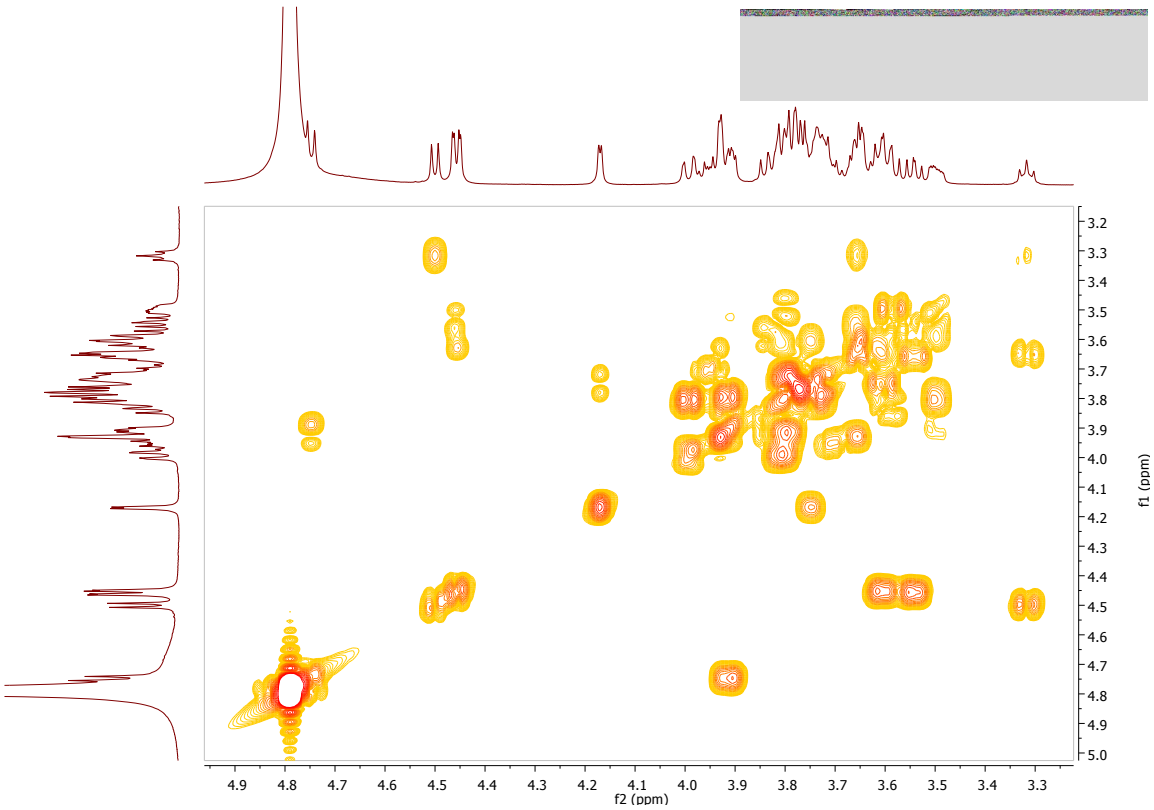
^{13}C NMR of Compound Tetrasaccharide S13



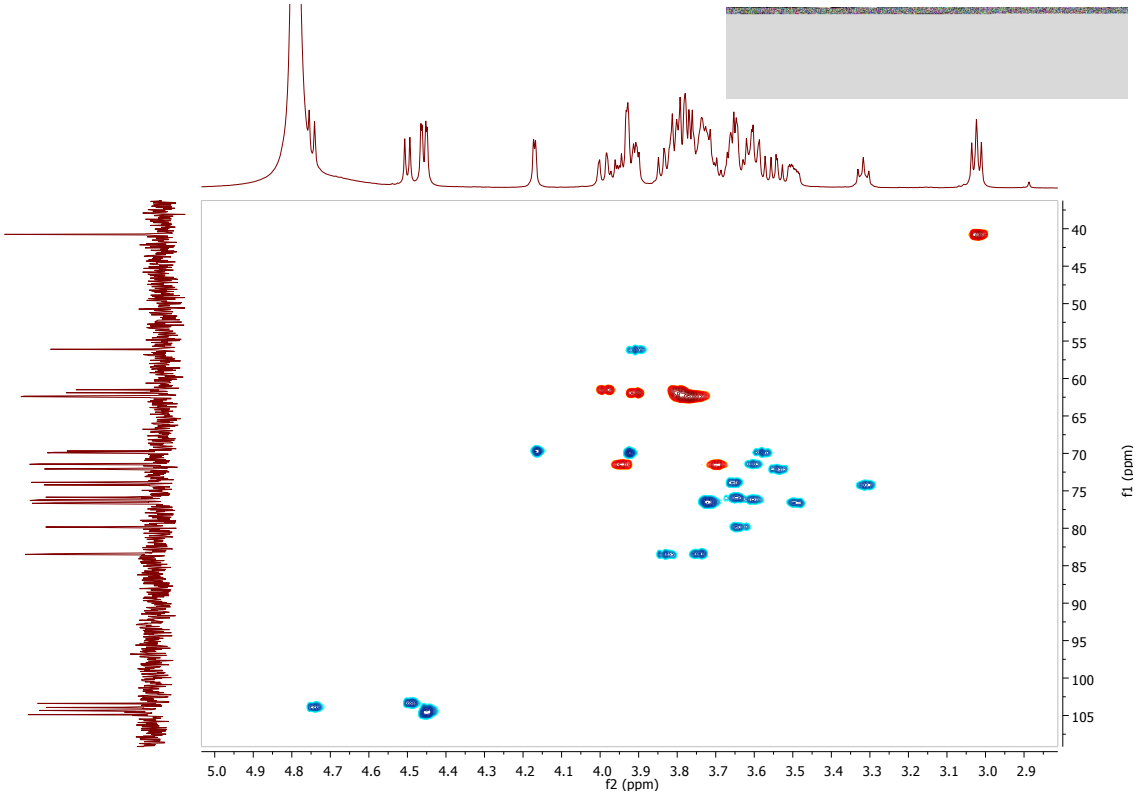
TOCSY NMR of Compound Tetrasaccharide S13



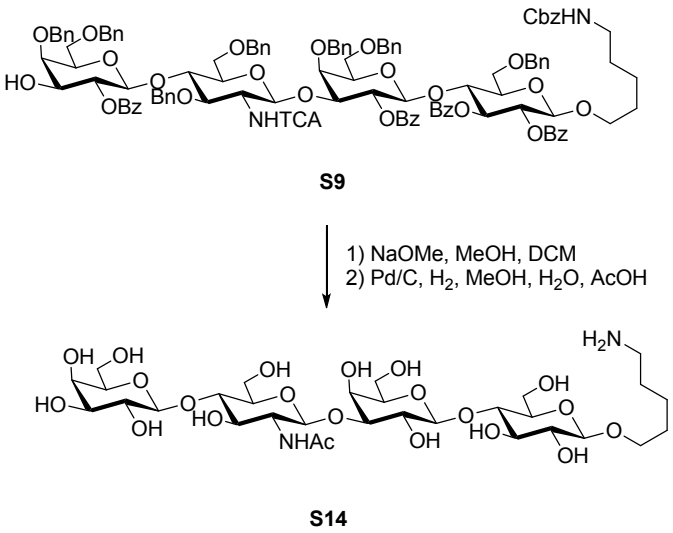
COSY NMR of Compound Tetrasaccharide S13



HSQC NMR of Compound Tetrasaccharide S13



4.7 Synthesis of Deprotected Tetrasaccharide S14



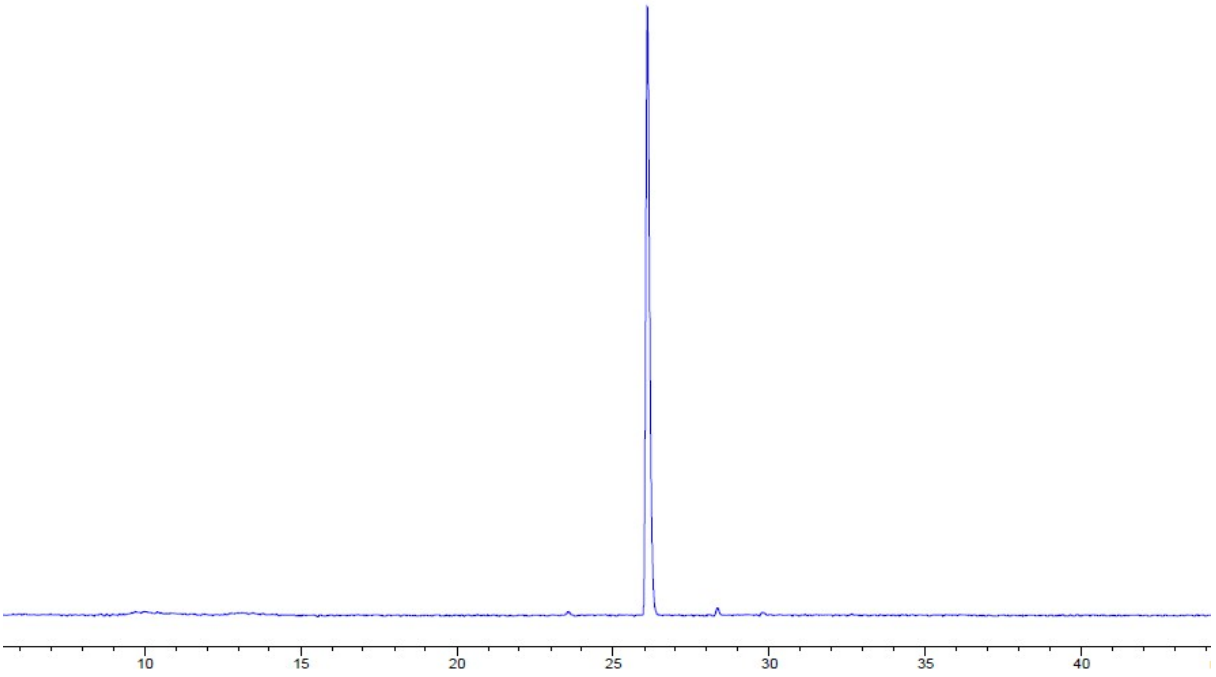
S9

S14

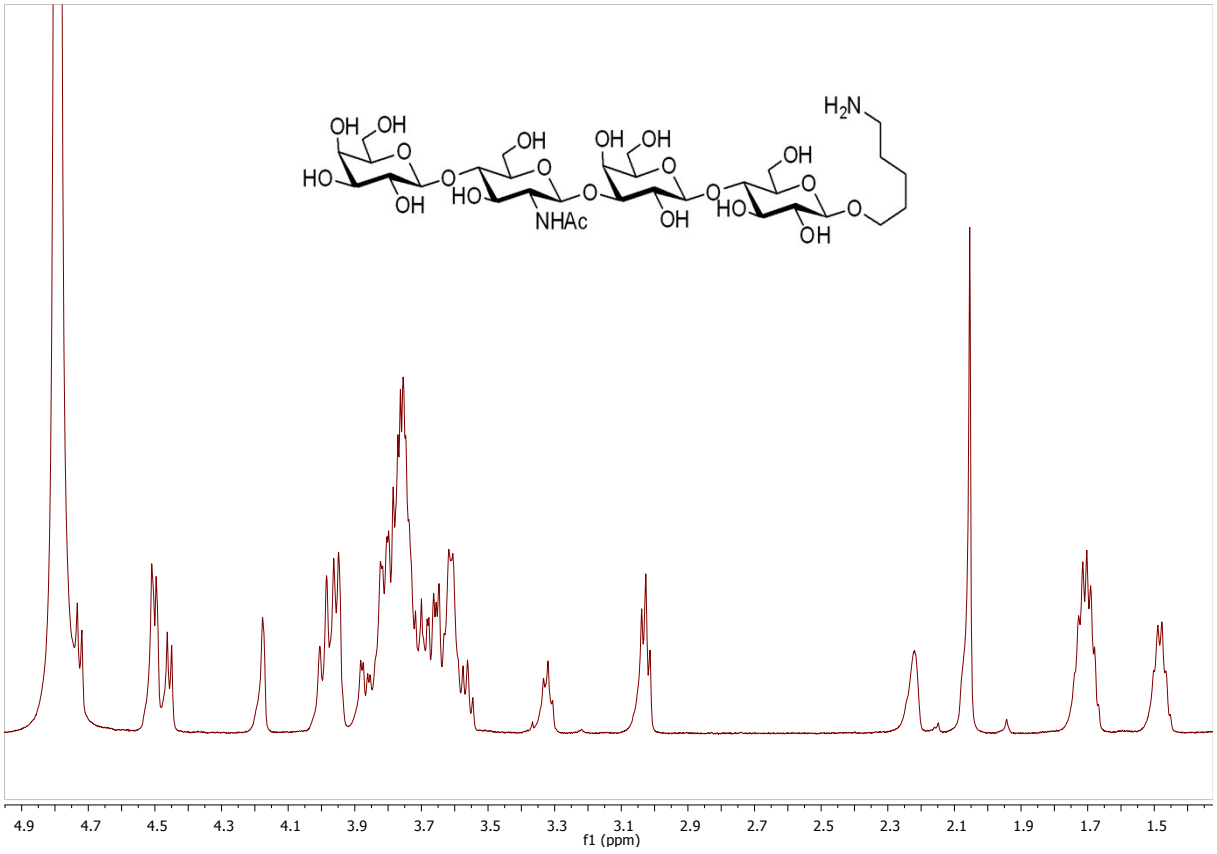
Deprotection, Analysis and Purification: Tetrasaccharide **S9** (51.8 mg, 0.024 mmol) was deprotected as described in Methanolysis General Procedure and Hydrogenolysis General Procedure. The crude product was analyzed using reverse phase analytical HPLC (Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min; Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) and purified using preparative HPLC (Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min, Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) to obtain compound **S14** (16.4 mg, 0.021 mmol, 86% overall yield). Compound **S14** elutes at 26 min.

*Analytical data for tetrasaccharide **S14**:* ^{1}H NMR (600 MHz, D_2O) δ 4.73 (d, J = 8.3 Hz, 1H), 4.55 – 4.49 (m, 2H), 4.46 (d, J = 7.8 Hz, 1H), 4.18 (br s, 1H), 4.04 – 3.92 (m, 4H), 3.91 – 3.53 (m, 20H), 3.37 – 3.29 (m, 1H), 3.03 (t, J = 7.4 Hz, 2H), 2.05 (s, 3H), 1.76 – 1.65 (m, 4H), 1.53 – 1.44 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 172.29, 104.32, 104.26, 104.10, 103.37, 83.44, 79.82, 79.61, 76.74, 76.27, 76.16, 75.94, 75.86, 74.20, 73.91, 73.57, 72.35, 71.45, 71.35, 69.94, 69.70, 62.40, 62.34, 61.48, 61.27, 56.59, 40.75, 29.54, 27.79, 23.58, 23.46. MS ESI HRMS: m/z $[M+Na]^+$ calcd for $C_{31}H_{56}N_2NaO_{21}$ 815.3273, found 815.3297.

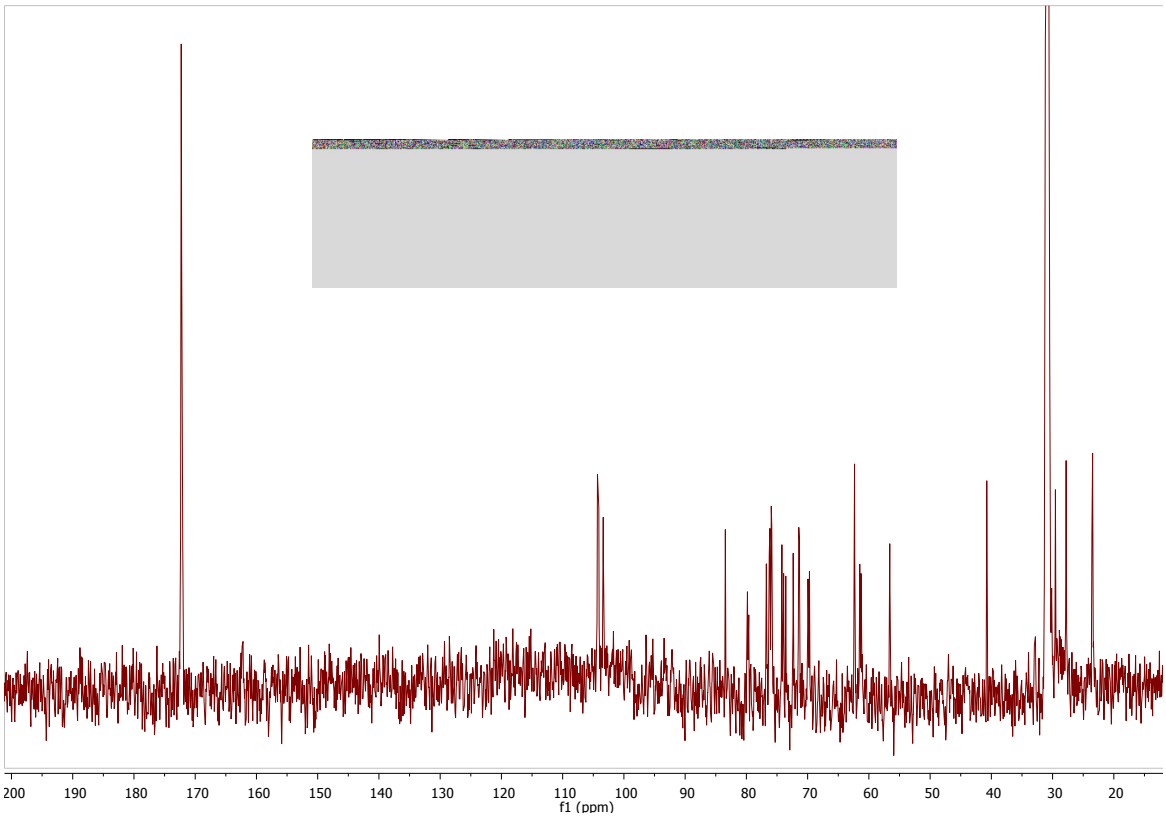
Analytical RP-HPLC Hypercarb of Crude Tetrasaccharide S14 (ELSD trace)



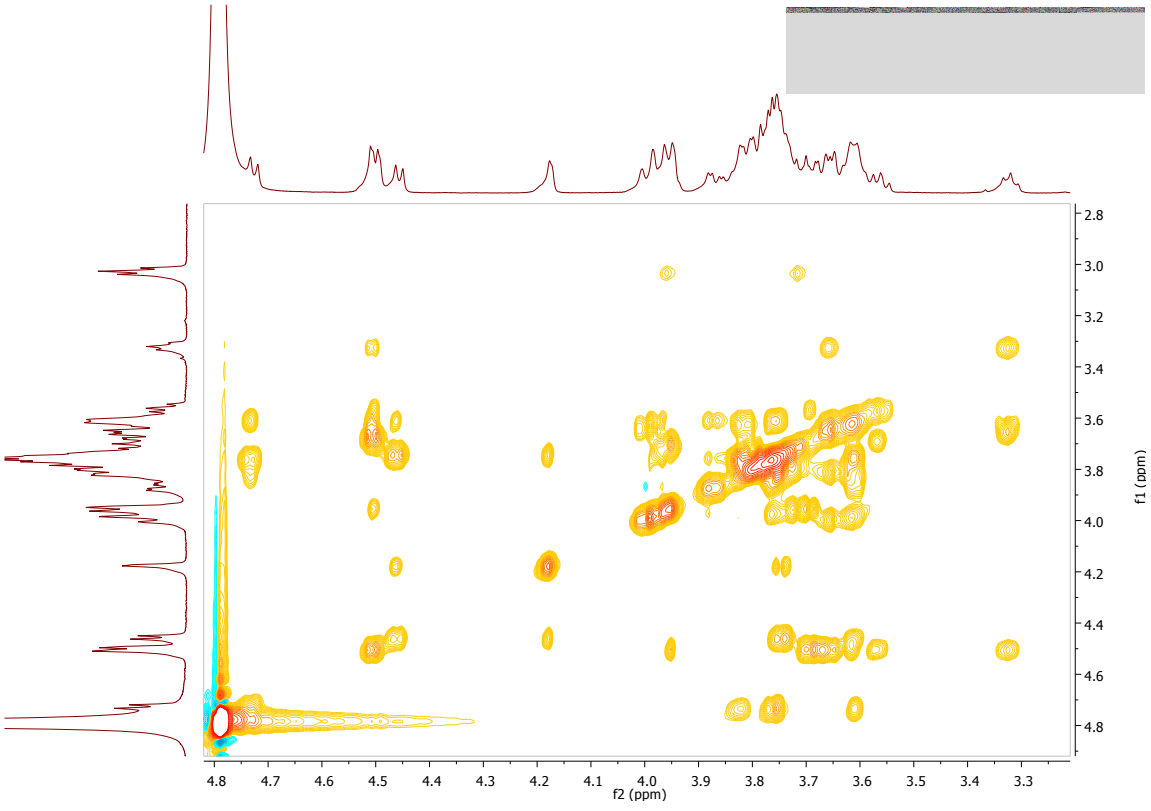
^{1}H NMR of Compound Tetrasaccharide S14



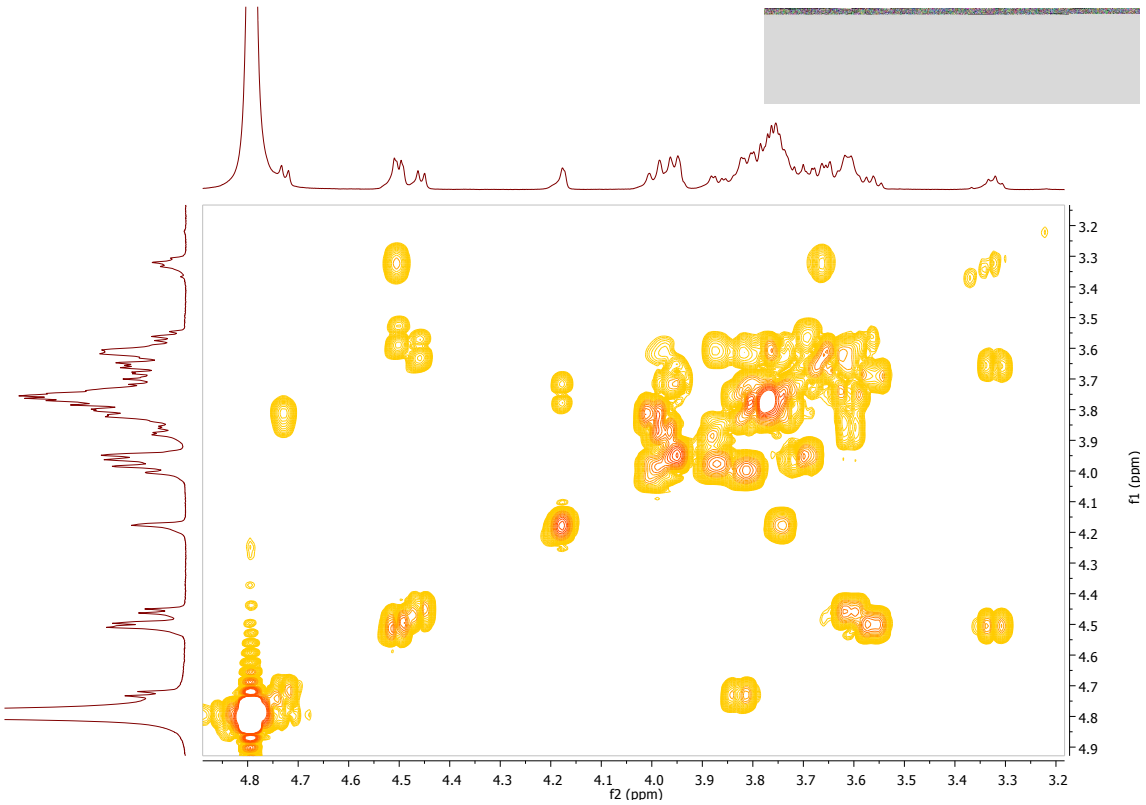
^{13}C NMR of Compound Tetrasaccharide S14



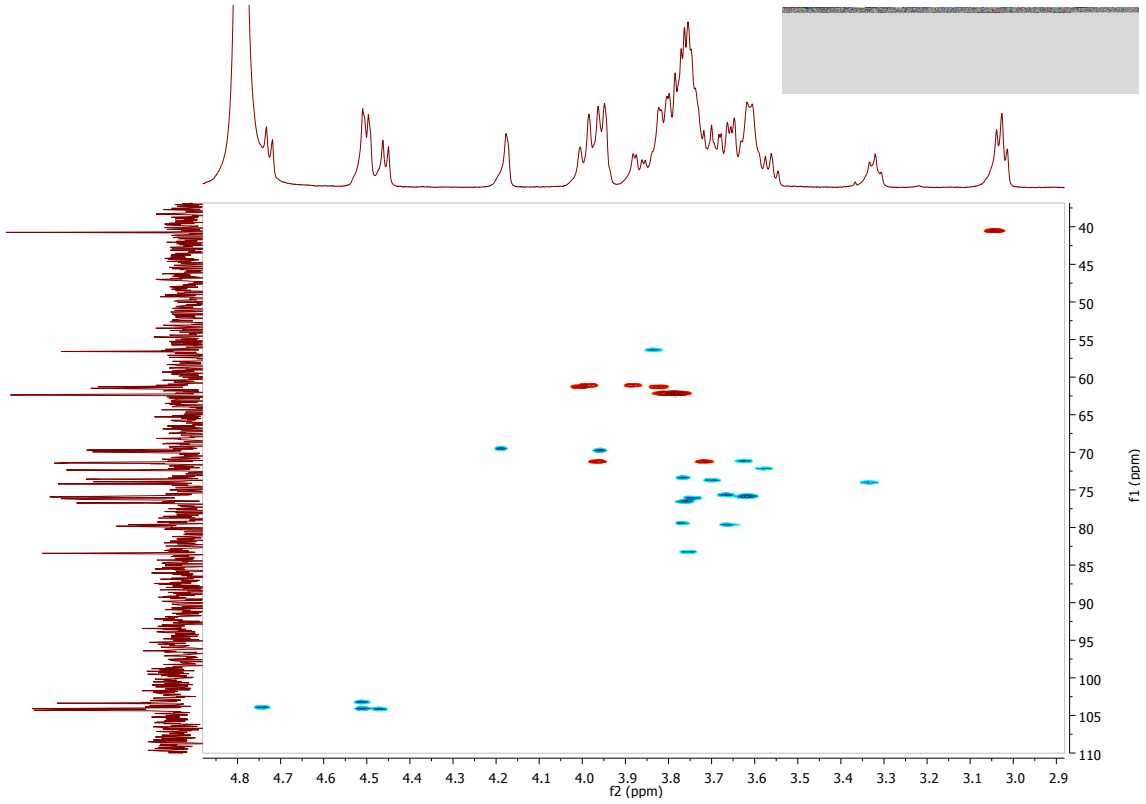
TOCSY NMR of Compound Tetrasaccharide S14



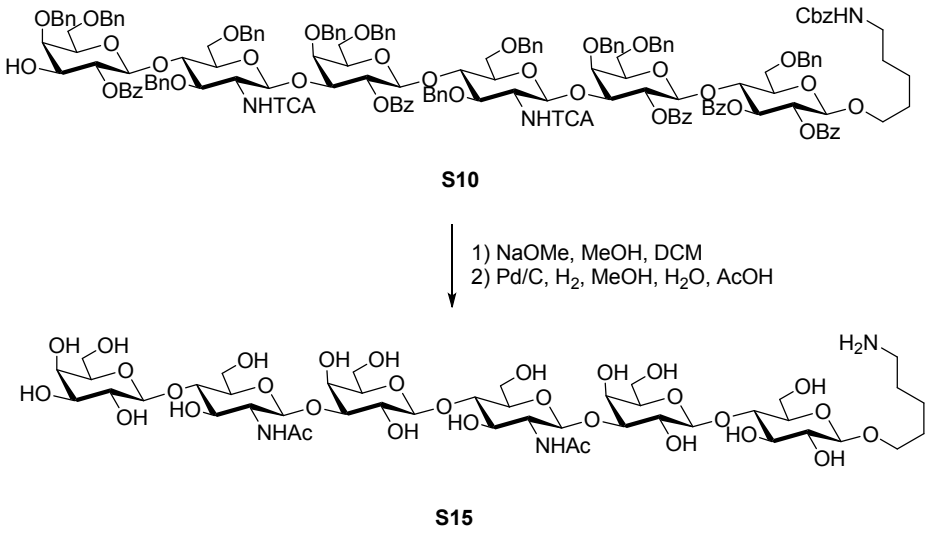
COSY NMR of Compound Tetrasaccharide S14



HSQC NMR of Compound Tetrasaccharide S14



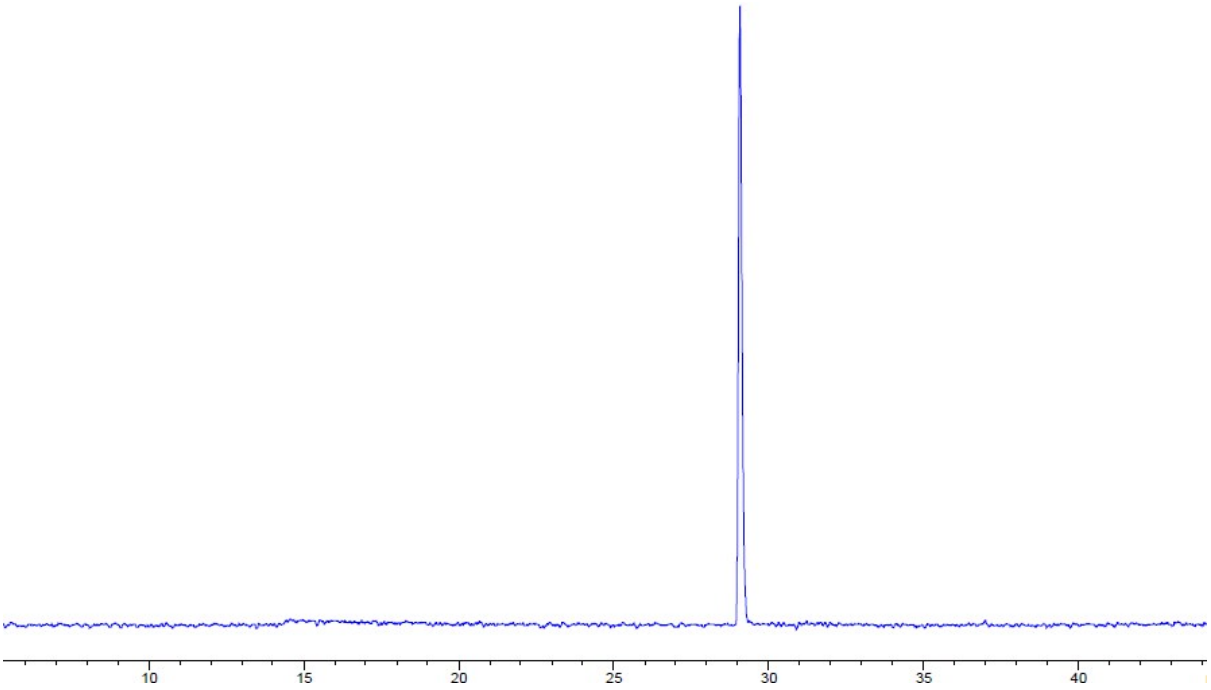
4.8 Synthesis of Deprotected Hexasaccharide S15



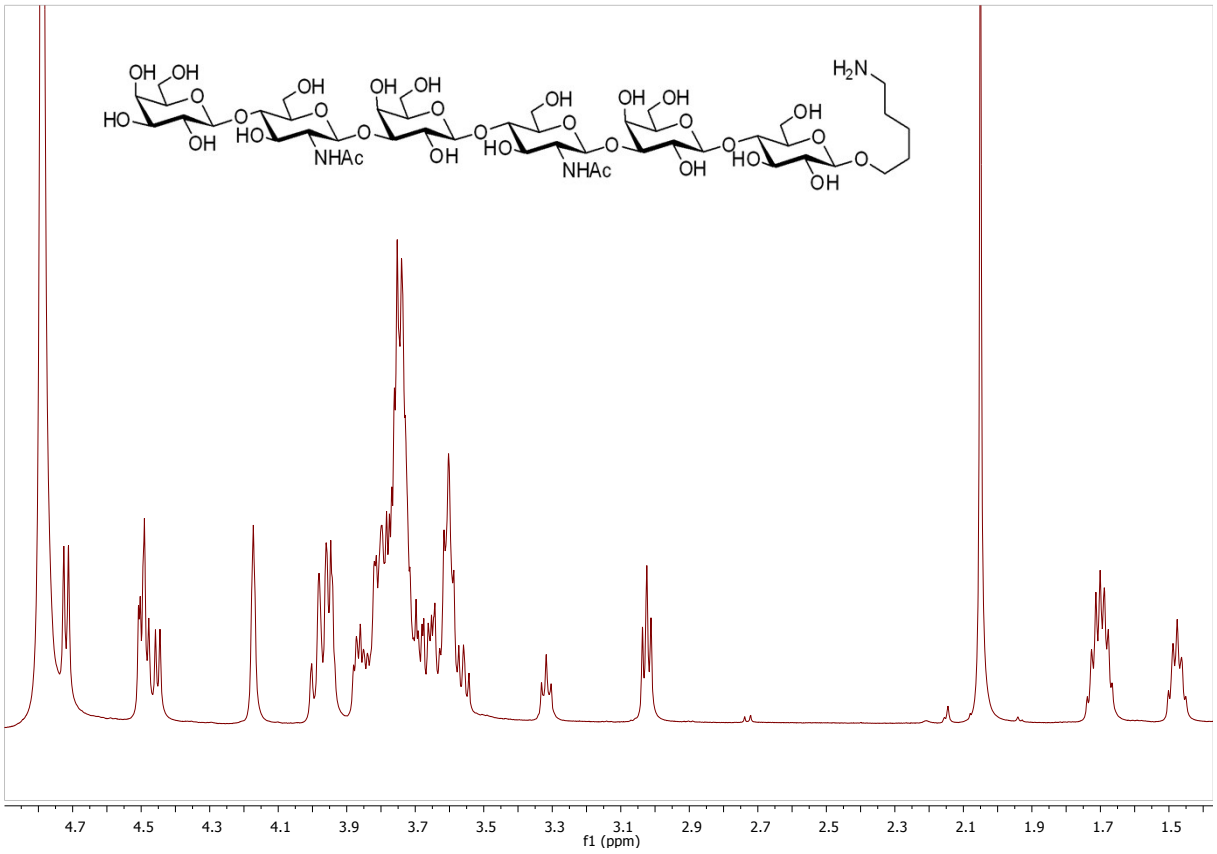
Deprotection, Analysis and Purification: Hexasaccharide **S10** (61.8 mg, 0.021 mmol) was deprotected as described in Methanolysis General Procedure and Hydrogenolysis General Procedure. The crude product was analyzed using reverse phase analytical HPLC (Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min; Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) and purified using preparative HPLC (Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min, Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) to obtain compound **S15** (18.5 mg, 0.016 mmol, 78% overall yield). Compound **S15** elutes at 29 min.

*Analytical data for hexasaccharide **S15**:* ^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.3 Hz, 2H), 4.52 – 4.47 (m, 3H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.17 (br s, 2H), 4.04 – 3.90 (m, 5H), 3.90 – 3.52 (m, 30H), 3.32 (t, *J* = 8.4 Hz, 1H), 3.02 (t, *J* = 7.5 Hz, 2H), 2.05 (s, 6H), 1.75 – 1.66 (m, 4H), 1.51 – 1.44 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ ^{13}C NMR (151 MHz, d_2o) δ 176.26, 172.36, 104.34, 104.27, 104.12, 103.38, 83.45, 79.82, 79.59, 76.75, 76.27, 76.17, 75.95, 75.85, 74.20, 73.91, 73.57, 72.36, 71.46, 71.35, 69.94, 69.70, 62.41, 62.34, 61.48, 61.26, 56.55, 40.75, 29.54, 27.80, 23.57, 23.47. MS ESI HRMS: m/z $[M+Na]^+$ calcd for $C_{45}H_{79}N_3NaO_{31}$ 1180.4595, found 1180.4583.

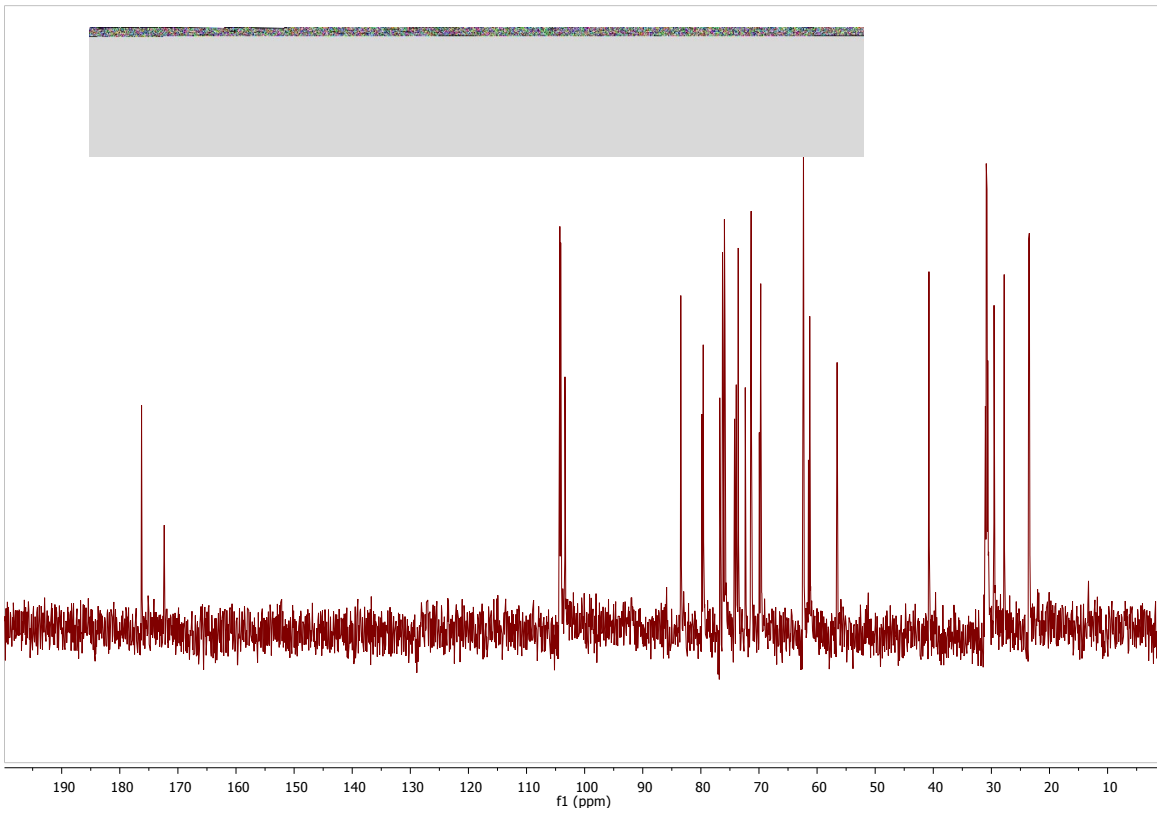
Analytical RP-HPLC Hypercarb of Crude Hexasaccharide S15 (ELSD trace)



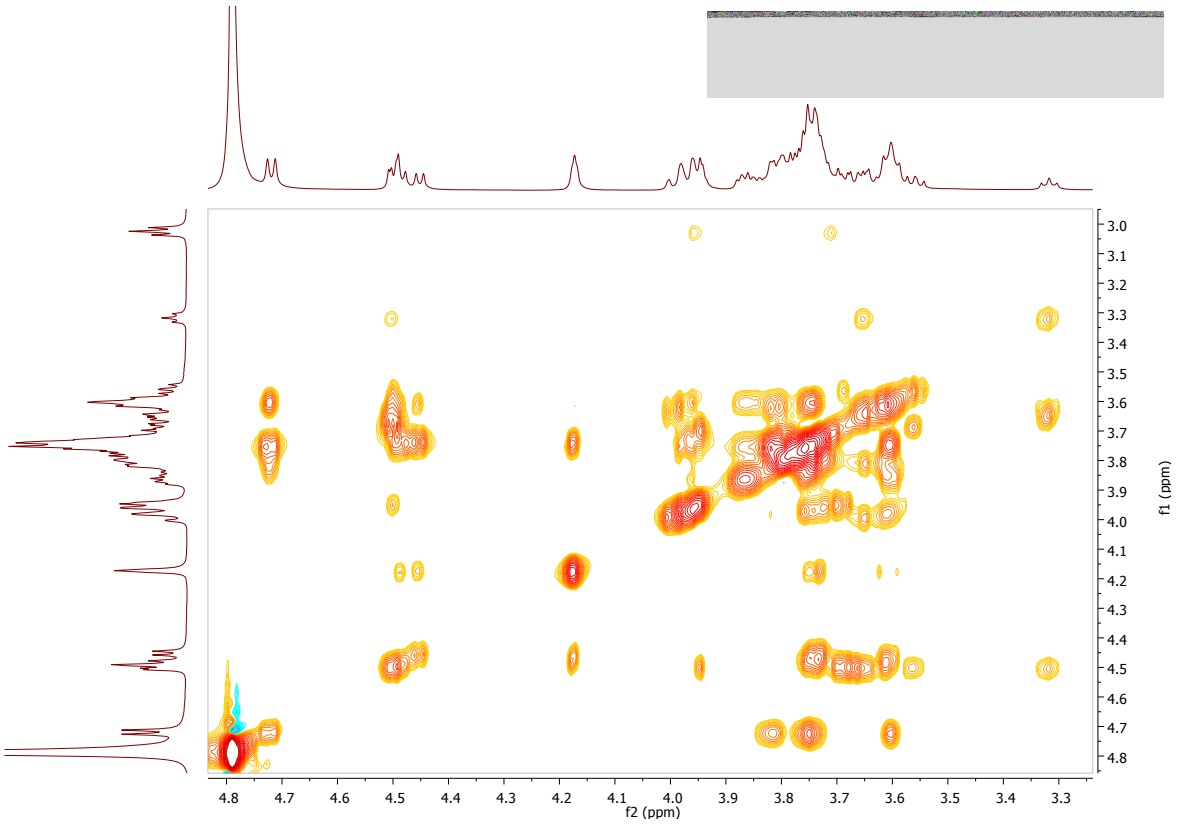
^{1}H NMR of Compound Hexasaccharide S15



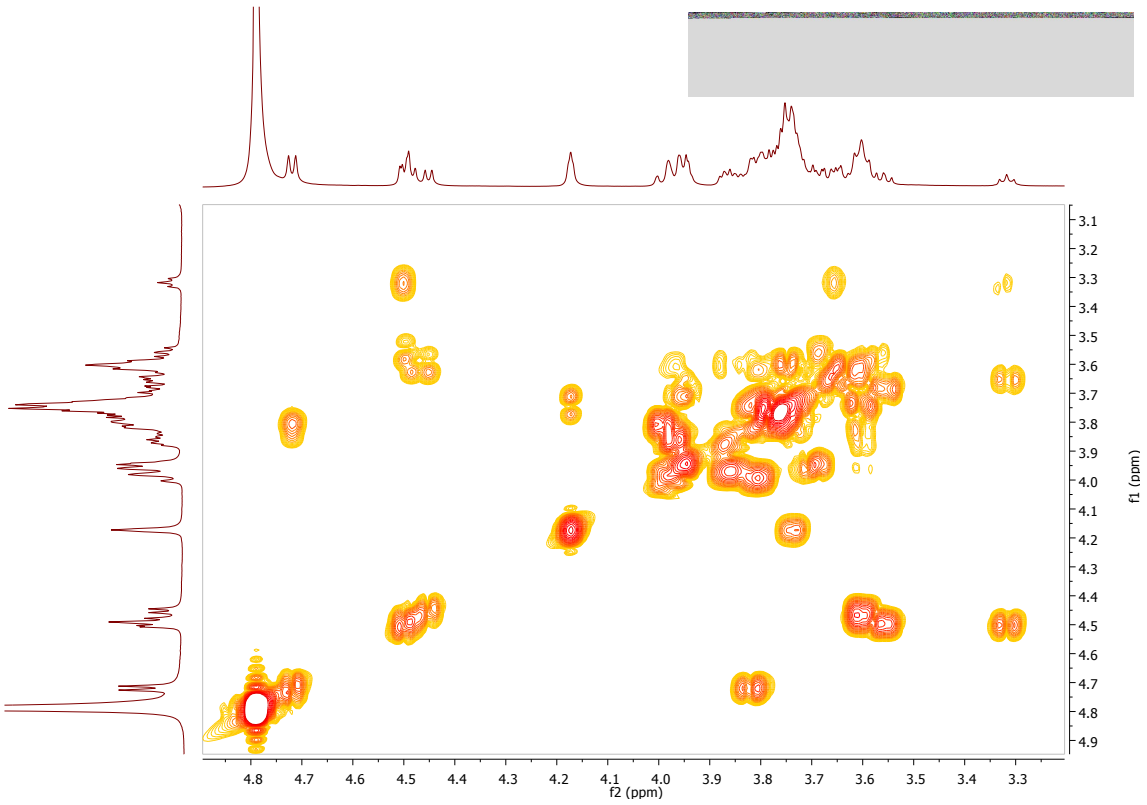
^{13}C NMR of Compound Hexasaccharide S15



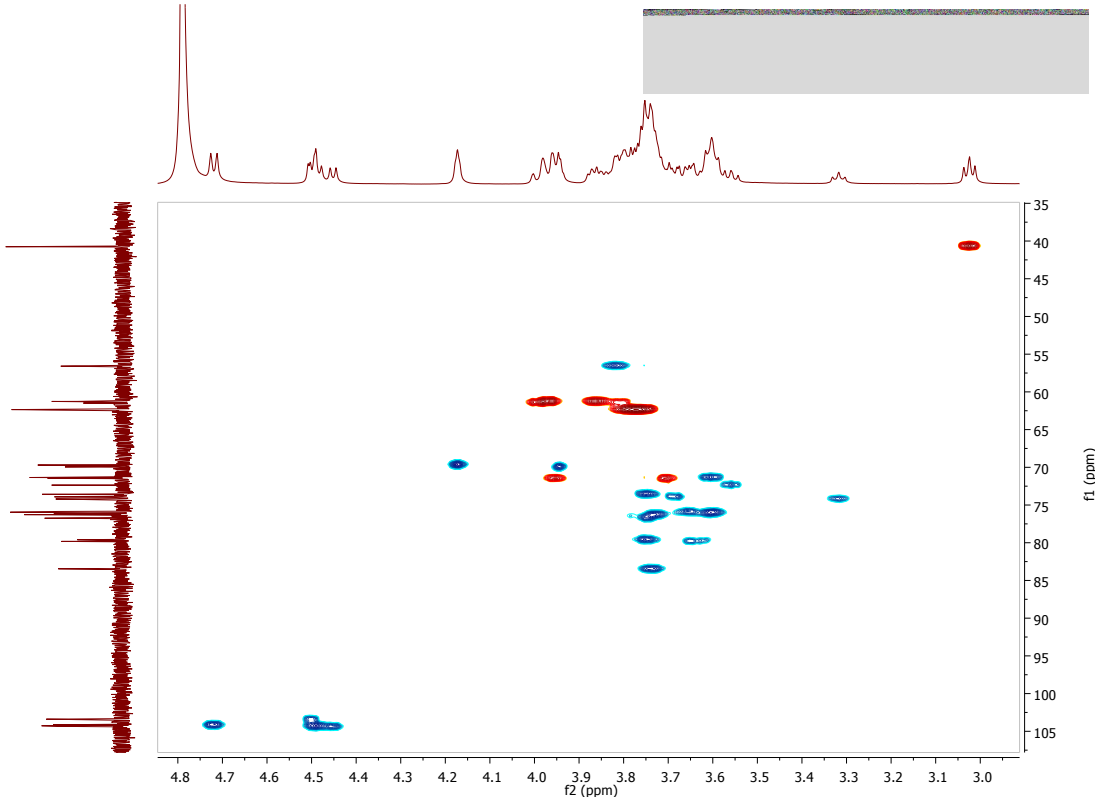
TOCSY NMR of Compound Hexasaccharide S15



COSY NMR of Compound Hexasaccharide S15



HSQC NMR of Compound Hexasaccharide S15

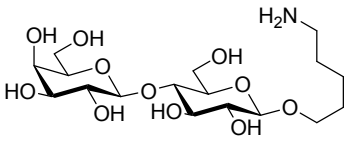


5. Oligosaccharide Sialylation

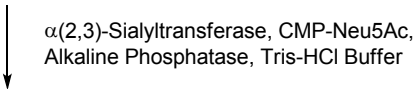
5.1 Purification

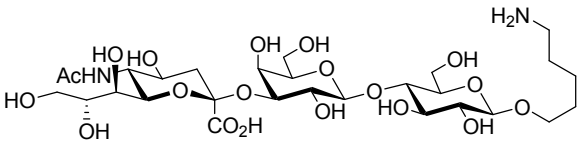
Crude products were analyzed/ purified using analytical/preparative HPLC (Agilent 1200 Series spectrometer). Following purification all products were lyophilized on a Christ Alpha 2-4 LD plus freeze dryer prior to characterization.

5.2 Synthesis of Sialyl Trisaccharide 1 (GM3)



S11





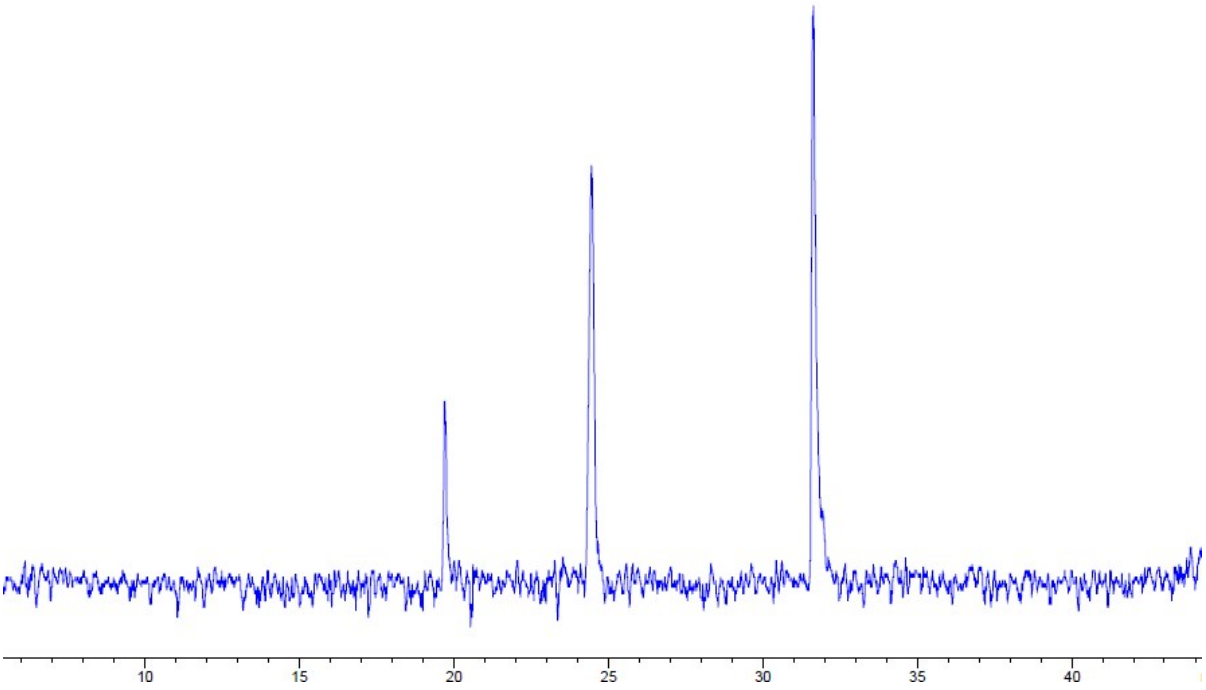
GM3 (**1**)

Sialylation, Analysis and Purification: Disaccharide **S11** (2.7 mg, 6.4 μmol) was dissolved in 0.62 mL of 100 mM Tris buffer (pH 8.0, containing 20 mM $MgCl_2$ and 15 mM NaCl) in a 1.5 mL Eppendorf tube. CMP-Neu5Ac (4.5 mg, 6.8 μmol, 1.07 eq), 47.8 μL 2,3-sialyltransferase from *Pasturella multocida* (2.0 mU/μL: 116.6 mU), and 9.56 uL alkaline phosphatase (10 U/μL: 116.6 U) were added. The reaction was incubated at 37 °C for 15.5 h. The reaction was quenched by addition of 2.64 mL ethanol followed by vortexing for 2 min. The reaction was centrifuged and the supernatant was pipetted off. The solution was diluted with MilliQ water, frozen, and lyophilized. The crude product was analyzed using reverse phase analytical HPLC (Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min; Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) and purified using preparative HPLC (Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min, Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) to obtain compound **1** (4.1 mg, 5.6 μmol, 87% overall yield). Compound S11 elutes at 20 min, cytidine elutes at 24 min, and Compound **1** elutes at 31 min.

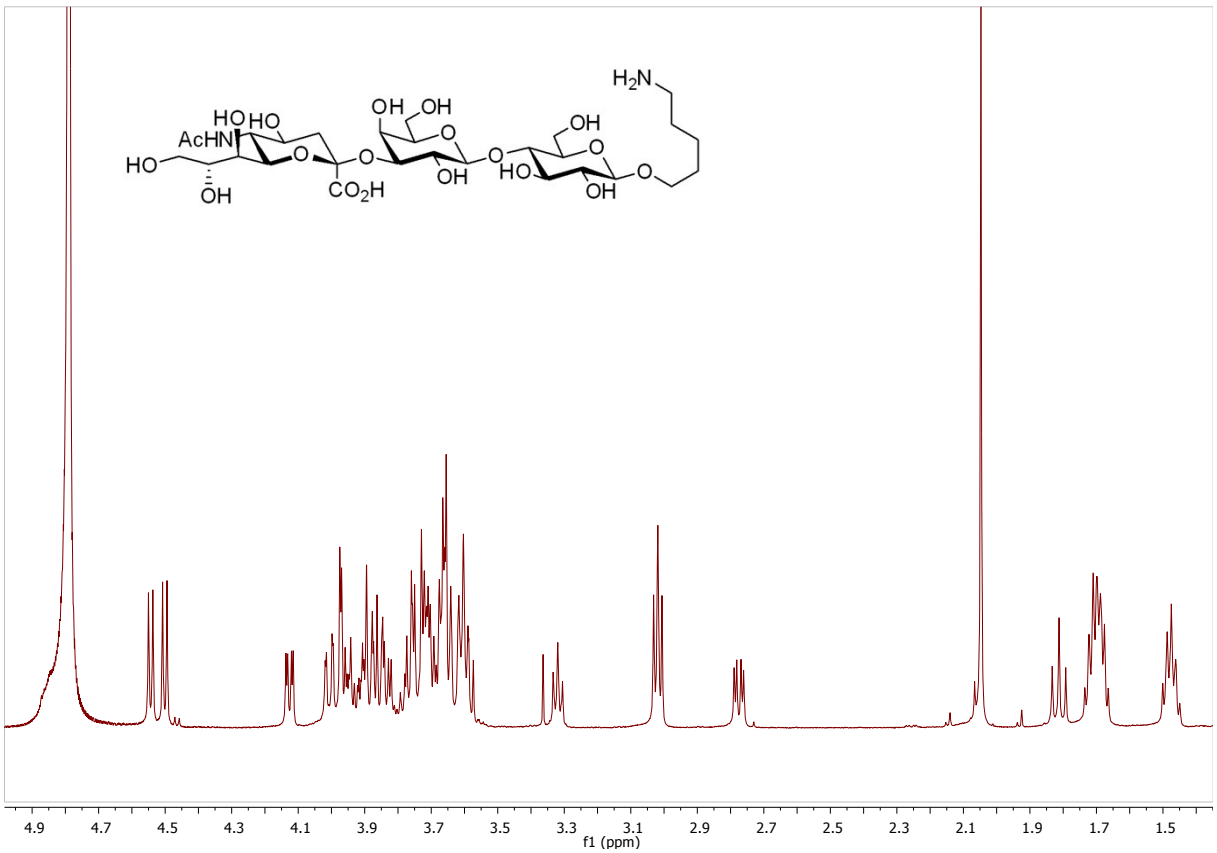
*Analytical data for trisaccharide **1**:* ^{1}H NMR (600 MHz, D_2O) δ 4.54 (d, *J* = 7.9 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.13 (dd, *J* = 9.9, 3.1 Hz, 1H), 4.01 (dd, *J* = 12.2, 2.0 Hz, 1H), 3.98 – 3.57 (m, 18H), 3.32 (t, *J* = 8.5 Hz, 1H), 3.02 (t, *J* = 7.2 Hz, 2H), 2.78 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.05 (s, 3H), 1.81 (t, *J* = 12.1 Hz, 1H), 1.74 – 1.64 (m, 4H), 1.51 – 1.43 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 176.43, 175.25, 104.05, 103.42, 101.20, 79.71, 76.91, 76.59, 76.18, 75.84, 74.23, 73.18, 71.46, 70.77, 69.73, 69.51, 68.86, 64.00,

62.43, 61.48, 53.09, 41.05, 40.77, 29.54, 27.80, 23.46. MS ESI HRMS: m/z $[M-H]^-$ calcd for $C_{28}H_{49}N_2O_{19}$ 717.2930, found 717.2892.

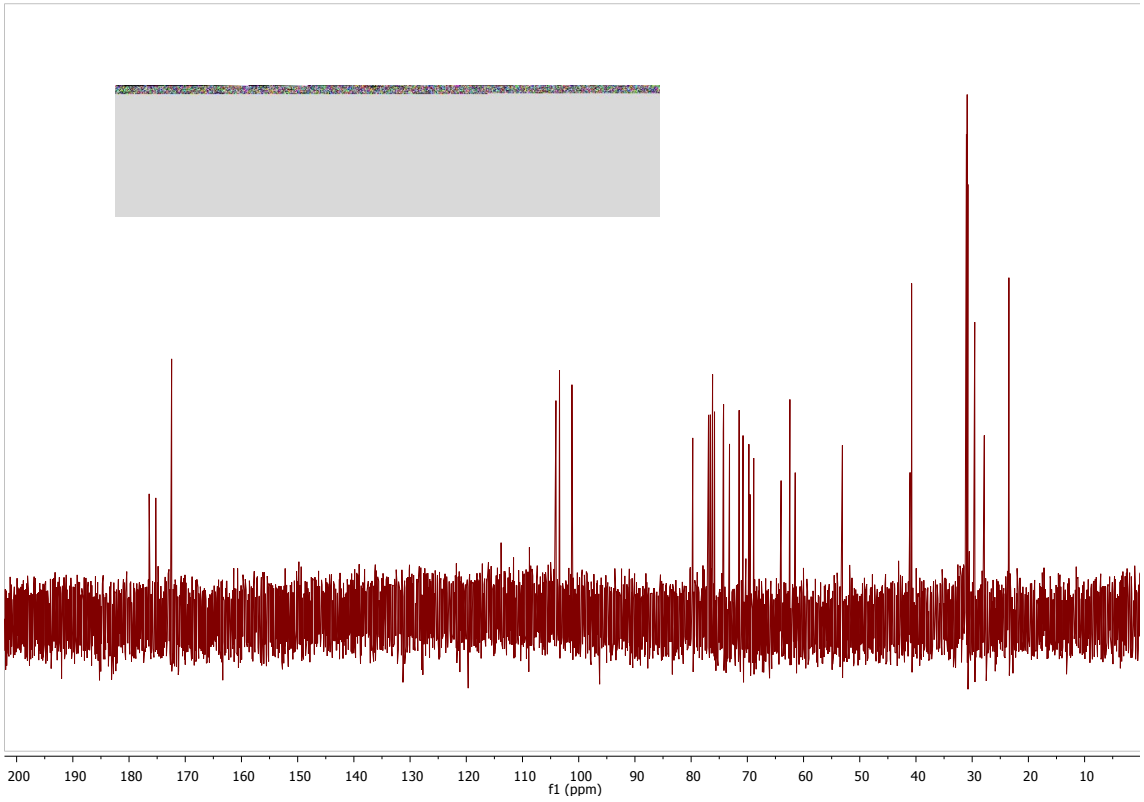
Analytical RP-HPLC Hypercarb of Crude Trisaccharide (GM3) 1 (ELSD trace)



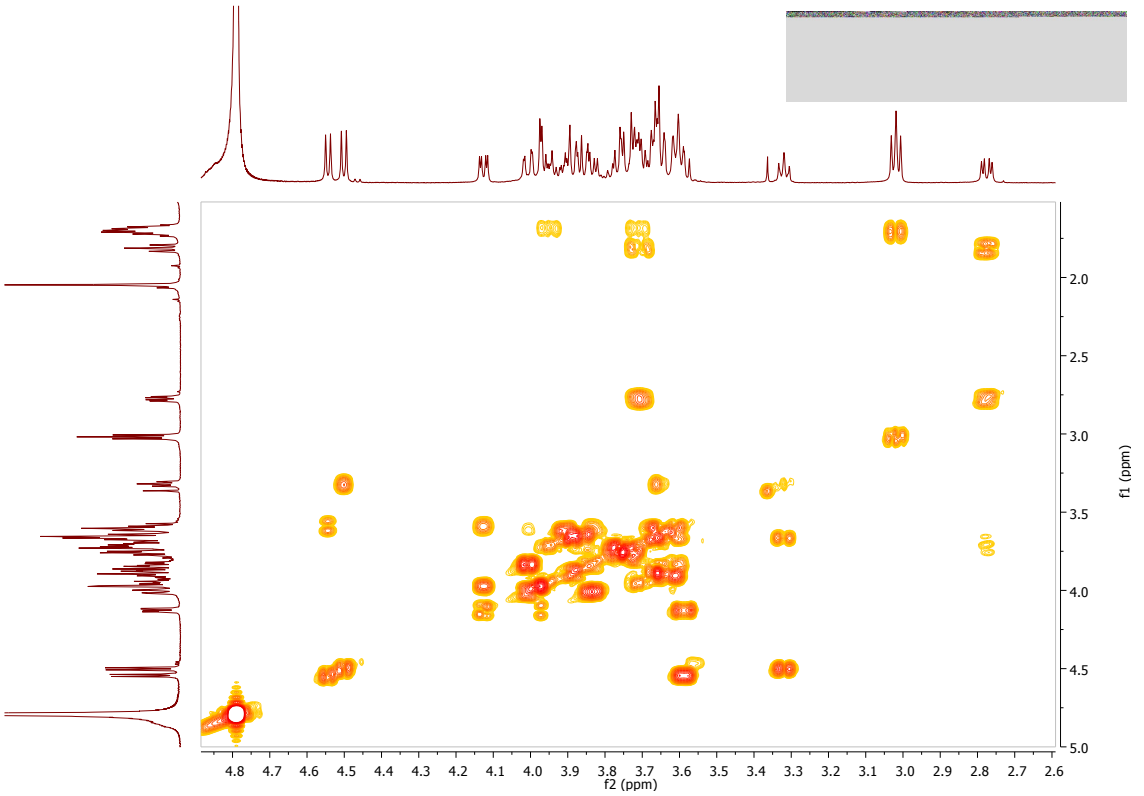
^{1}H NMR of Compound Trisaccharide (GM3) 1



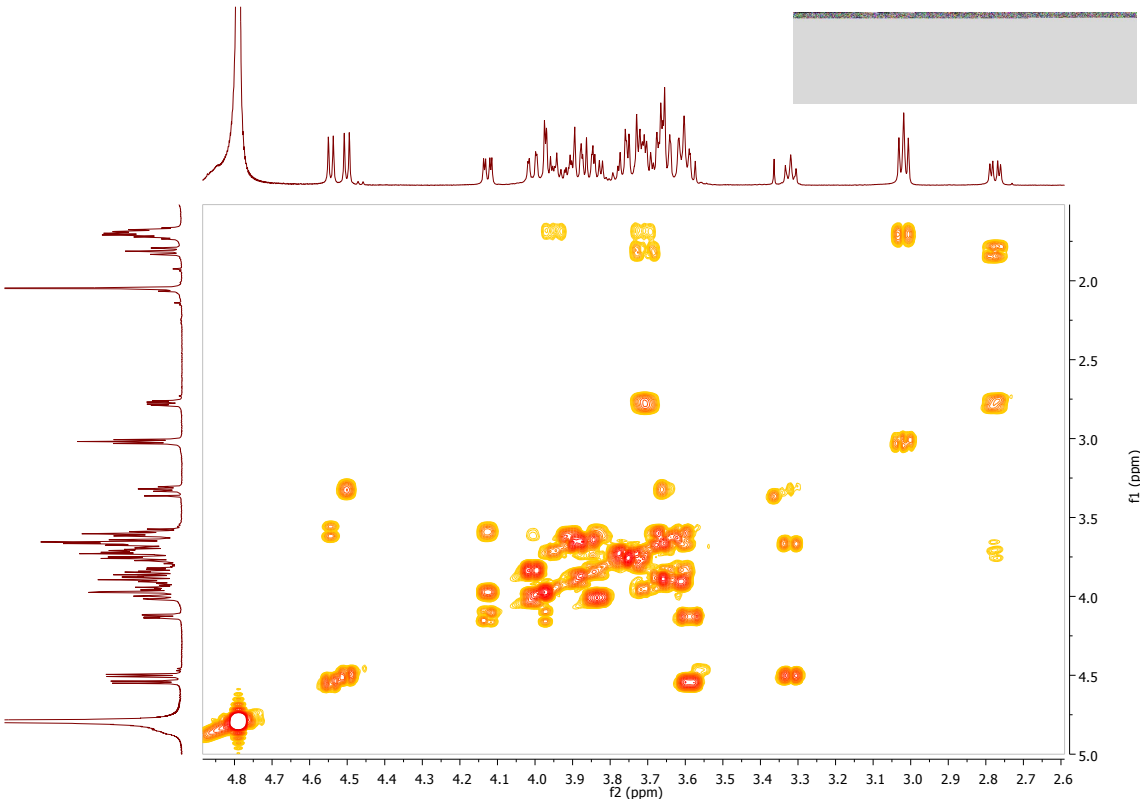
^{13}C NMR of Compound Trisaccharide (GM3) 1



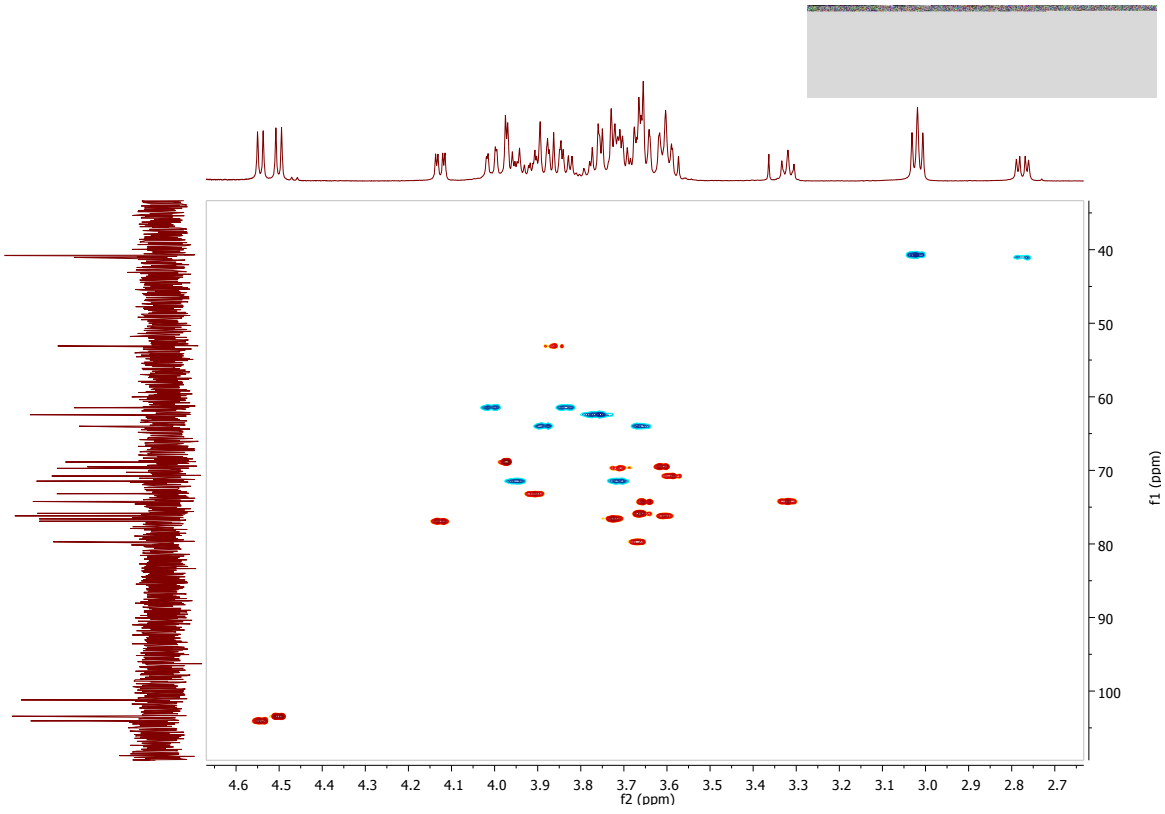
TOCSY NMR of Compound Trisaccharide (GM3) 1



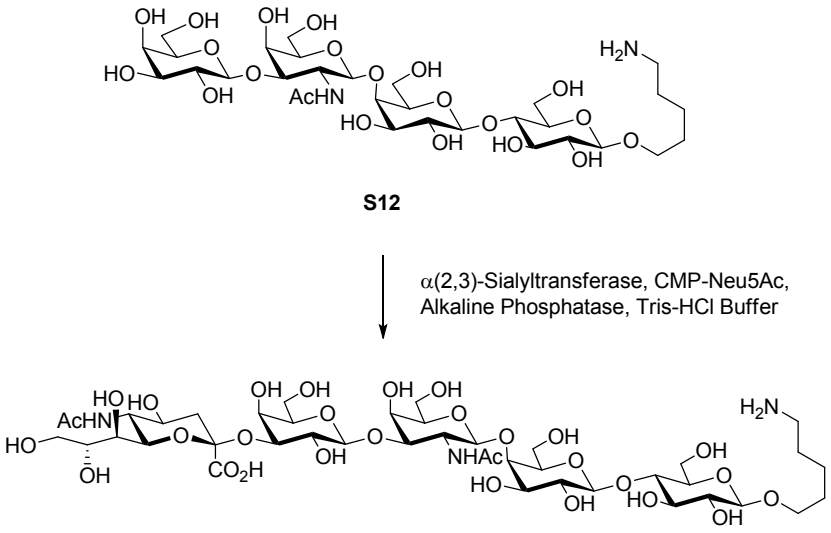
COSY NMR of Compound Trisaccharide (GM3) 1



HSQC NMR of Compound Trisaccharide (GM3) 1



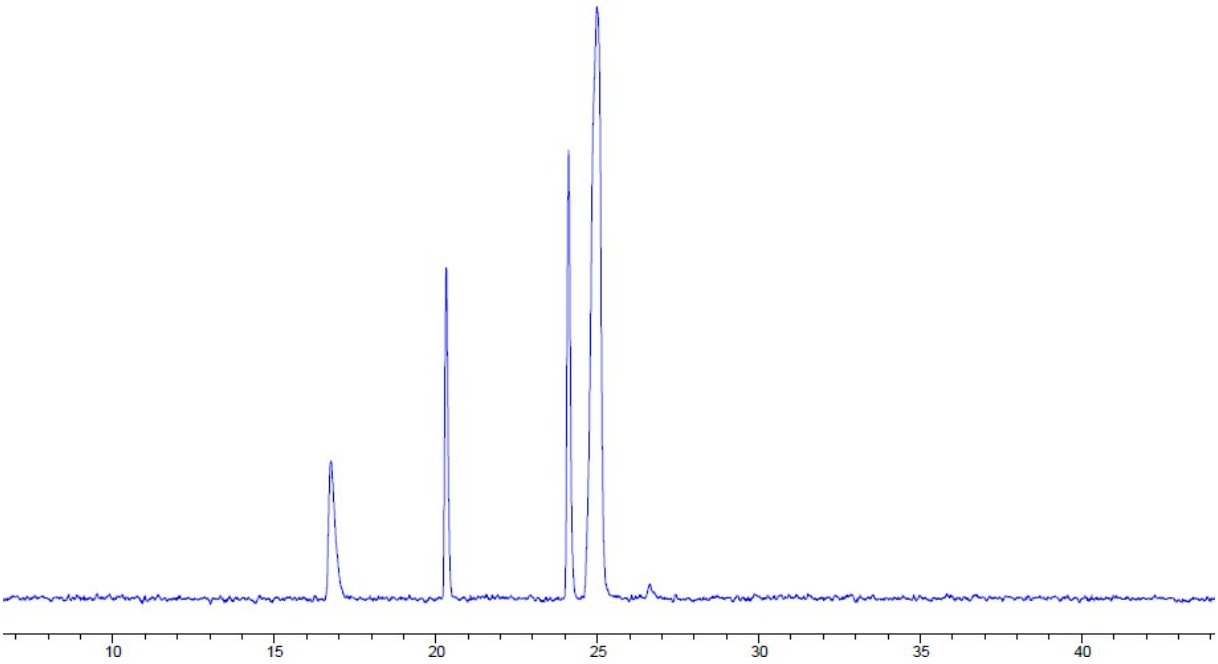
5.3 Synthesis of Sialyl Pentsaccharide 2 (GM1b)



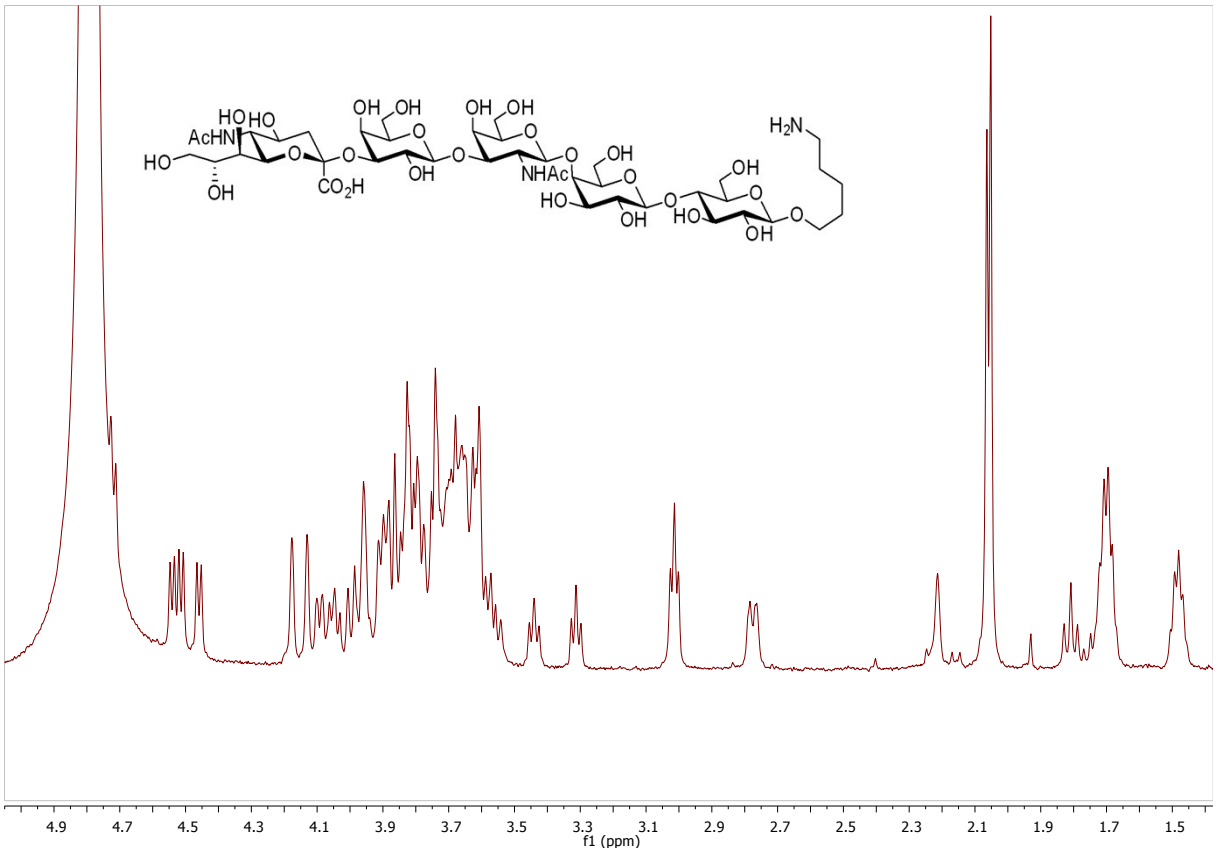
Sialylation, Analysis and Purification: Tetrasaccharide **S12** (1.8 mg, 2.3 μmol) was dissolved in 36.9 μL H_2O and pipetted into a 1.5 mL Eppendorf tube. CMP-Neu5Ac (4.6 mg, 7.0 μmol, 3 eq) in 91.8 μL H_2O was added. 224 μL of 100 mM Tris buffer (pH 8.0, containing 20 mM $MgCl_2$ and 15 mM NaCl) was added, followed by 139 uL 2,3-sialyltransferase from *Pasturella multocida* (2.0 mU/μL: 278 mU) and 27.8 μL alkaline phosphatase (10 U/μL: 278 U). The reaction was incubated at 37 °C for 24 h. The reaction was quenched by addition of 160 μL ethanol followed by vortexing for 2 min. The reaction was centrifuged and the supernatant was pipetted off. The solution was diluted with MilliQ water, frozen, and lyophilized. The crude product was analyzed using reverse phase analytical HPLC (Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min; Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) and purified using preparative HPLC (Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min, Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) to obtain compound **2** (2.0 mg, 1.8 μmol, 78% overall yield). CMP-Neu5Ac elutes at 16 min, cytidine elutes at 24 min, compound **S12** elutes at 20 min, and Compound **1** elutes at 25 min.

*Analytical data for pentasaccharide **2**:* ^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.8 Hz, 1H), 4.54 (d, *J* = 7.8 Hz, 1H), 4.51 (d, *J* = 7.9 Hz, 1H), 4.46 (d, *J* = 7.8 Hz, 1H), 4.18 (br s, 1H), 4.14 – 3.53 (m, 30H), 3.44 (t, *J* = 9.0 Hz, 1H), 3.31 (t, *J* = 8.4 Hz, 1H), 3.01 (t, *J* = 7.2 Hz, 2H), 2.77 (dd, *J* = 12.2 Hz, 3.2 Hz, 1H), 2.06 (s, 3H), 2.05 (s, 3H), 1.81 (t, *J* = 12.3 Hz, 1H), 1.75 – 1.66 (m, 4H), 1.52 – 1.45 (m, 2H). ^{13}C NMR (175 MHz, D_2O) δ 175.95, 175.01, 172.15, 104.55, 102.99, 102.36, 101.97, 100.81, 79.90, 78.55, 75.99, 75.50, 74.76, 74.50, 74.42, 72.76, 72.46, 71.81, 71.04, 70.03, 69.00, 68.36, 68.12, 67.87, 67.35, 62.50, 61.02, 60.71, 60.05, 51.73, 51.39, 39.76, 39.41, 28.09, 26.48, 22.47, 22.06. MS ESI HRMS: m/z $[M+Na]^+$ calcd for $C_{42}H_{73}N_3NaO_{29}$ 1106.4227, found 1106.4214.

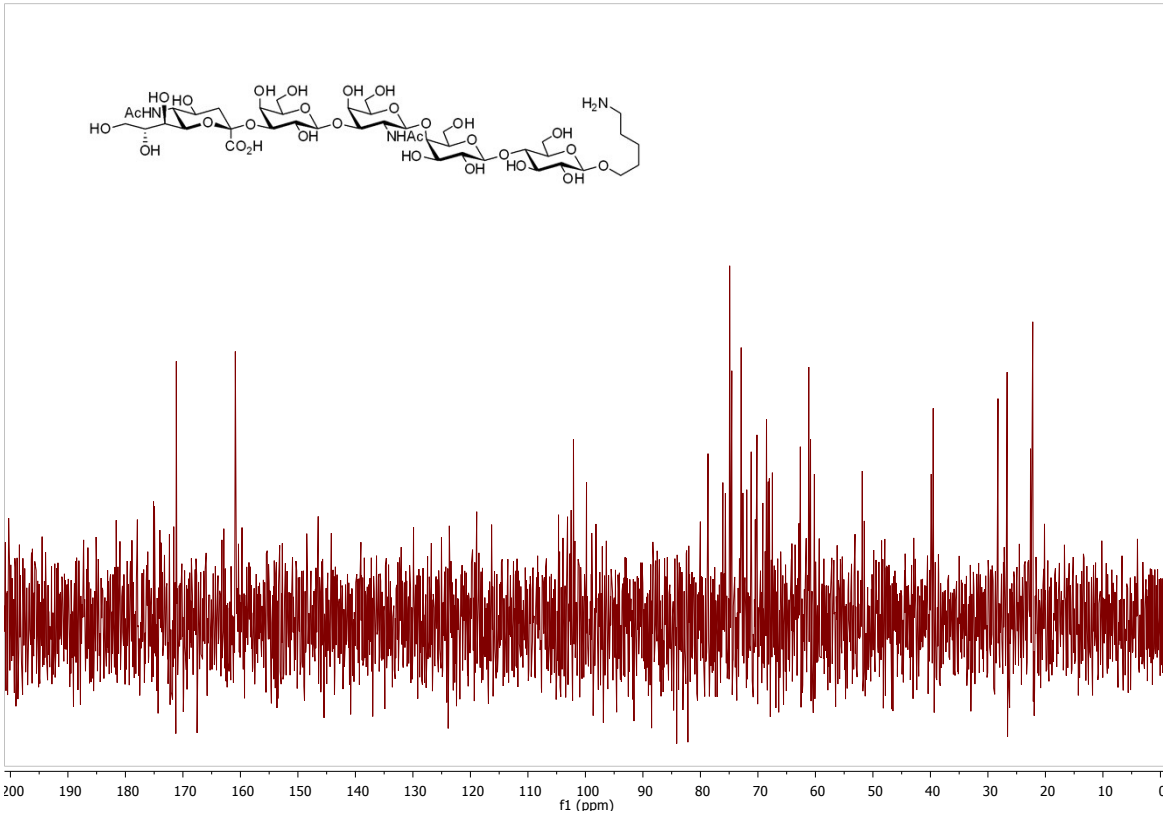
Analytical RP-HPLC Hypercarb of Crude Pentasaccharide (GM1b) 2 (ELSD trace)



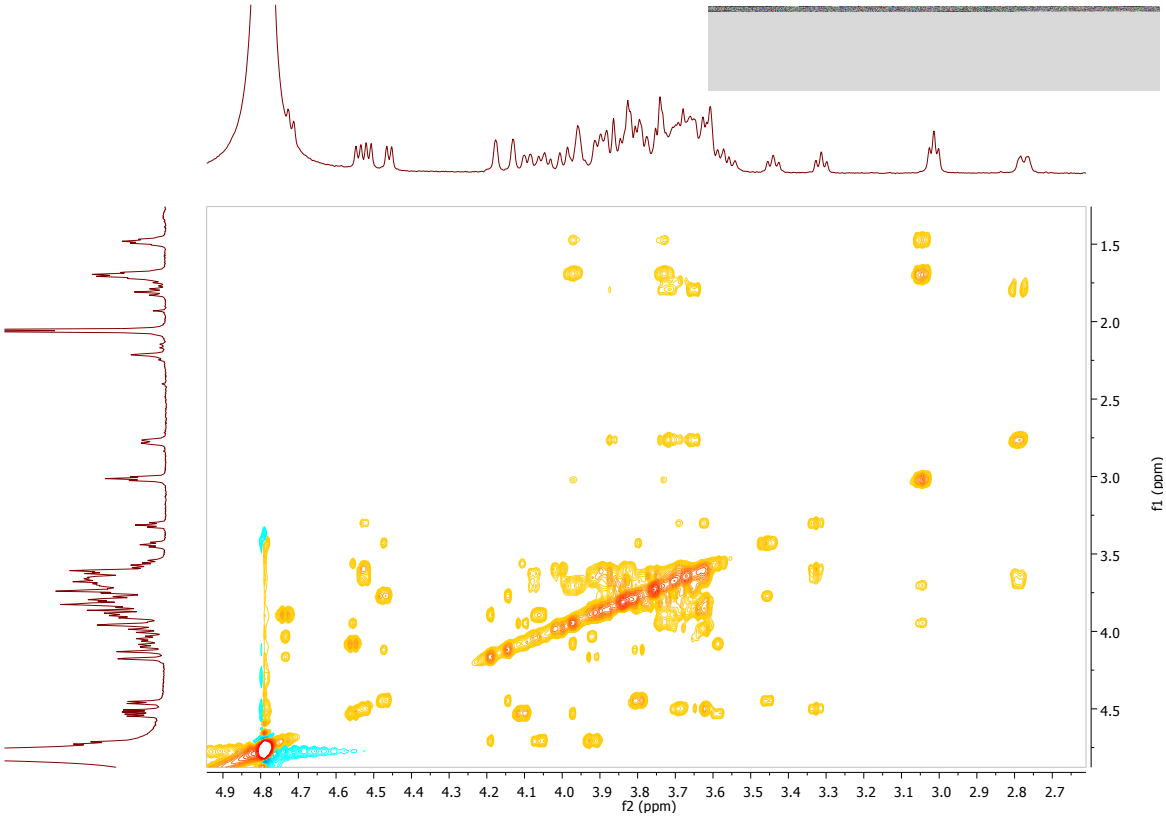
^{1}H NMR of Compound Pentasaccharide (GM1b) 2



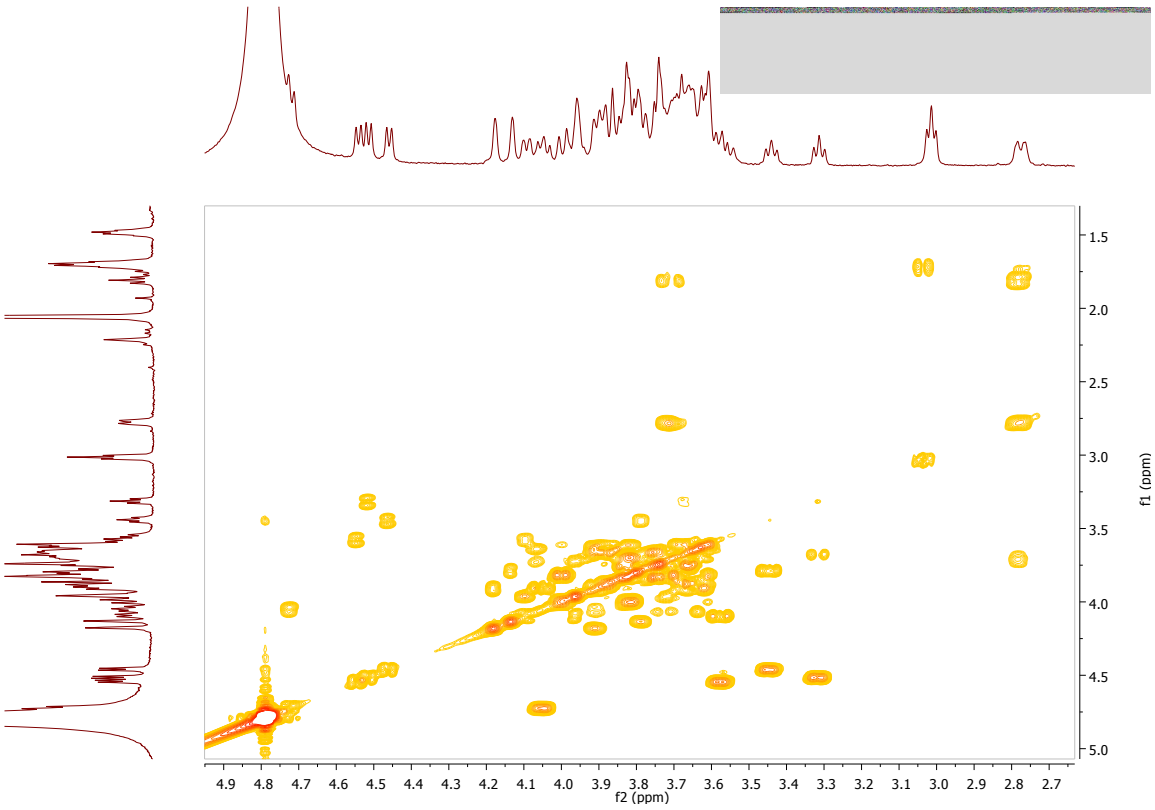
^{13}C NMR of Compound Pentasaccharide (GM1b) 2



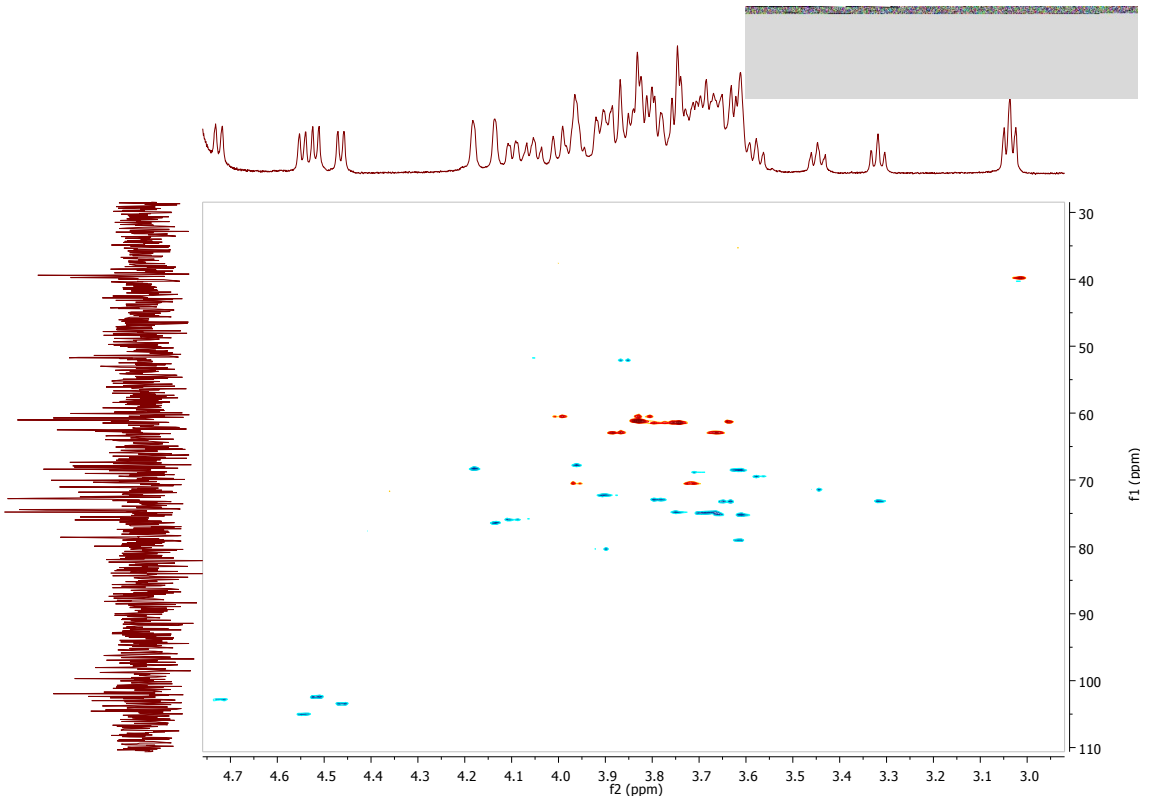
TOCSY NMR of Compound Pentasaccharide (GM1b) 2



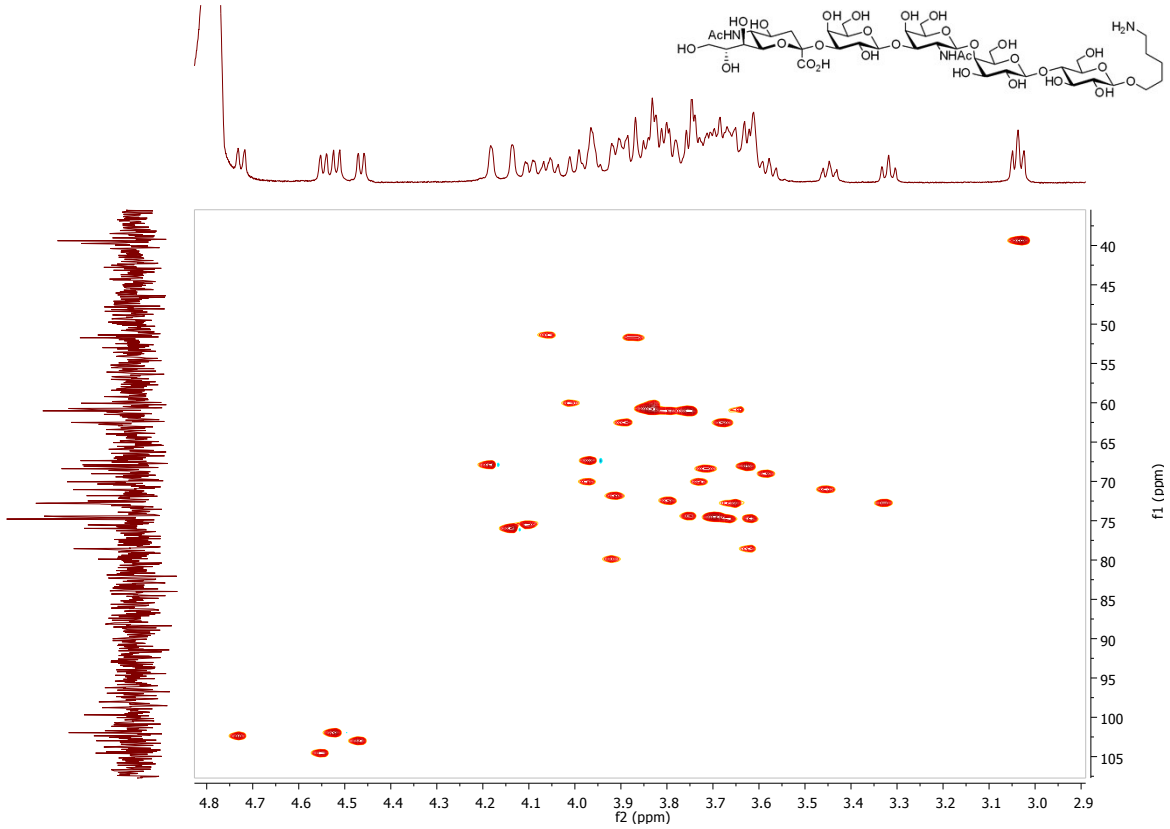
COSY NMR of Compound Pentasaccharide (GM1b) 2



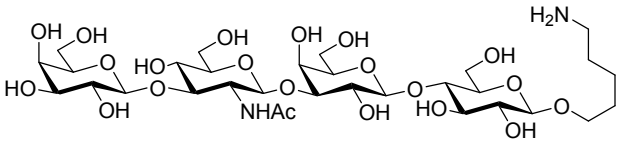
HSQC NMR of Compound Pentasaccharide (GM1b) 2



HMQC NMR of Compound Pentasaccharide (GM1b) 2

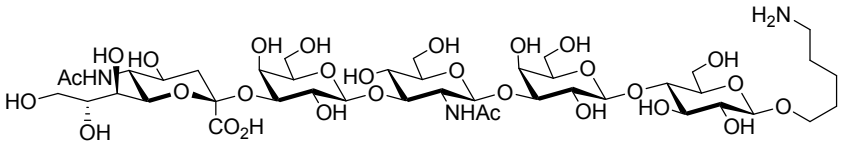


5.4 Synthesis of Sialyl Pentsaccharide 3 (sLC4)



S13

α(2,3)-Sialyltransferase, CMP-Neu5Ac,
Alkaline Phosphatase, Tris-HCl Buffer



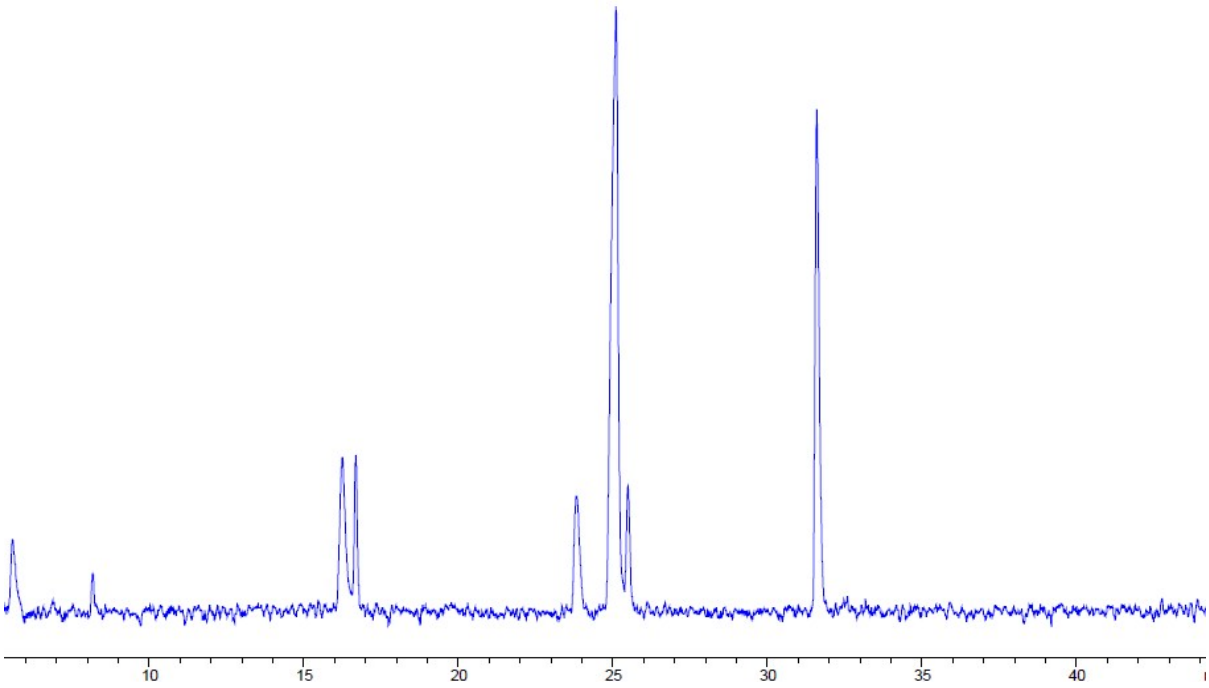
sLC4 (**3**)

Sialylation, Analysis and Purification: Tetrasaccharide **S13** (1.5 mg, 1.9 μmol) was dissolved in 30 μL H_2O and pipetted into a 1.5 mL Eppendorf tube. CMP-Neu5Ac (1.9 mg, 2.9 μmol, 1.5 eq) in 38 μL H_2O was added. 183 μL of 100 mM Tris buffer (pH 8.0, containing 20 mM $MgCl_2$ and 15 mM NaCl) was

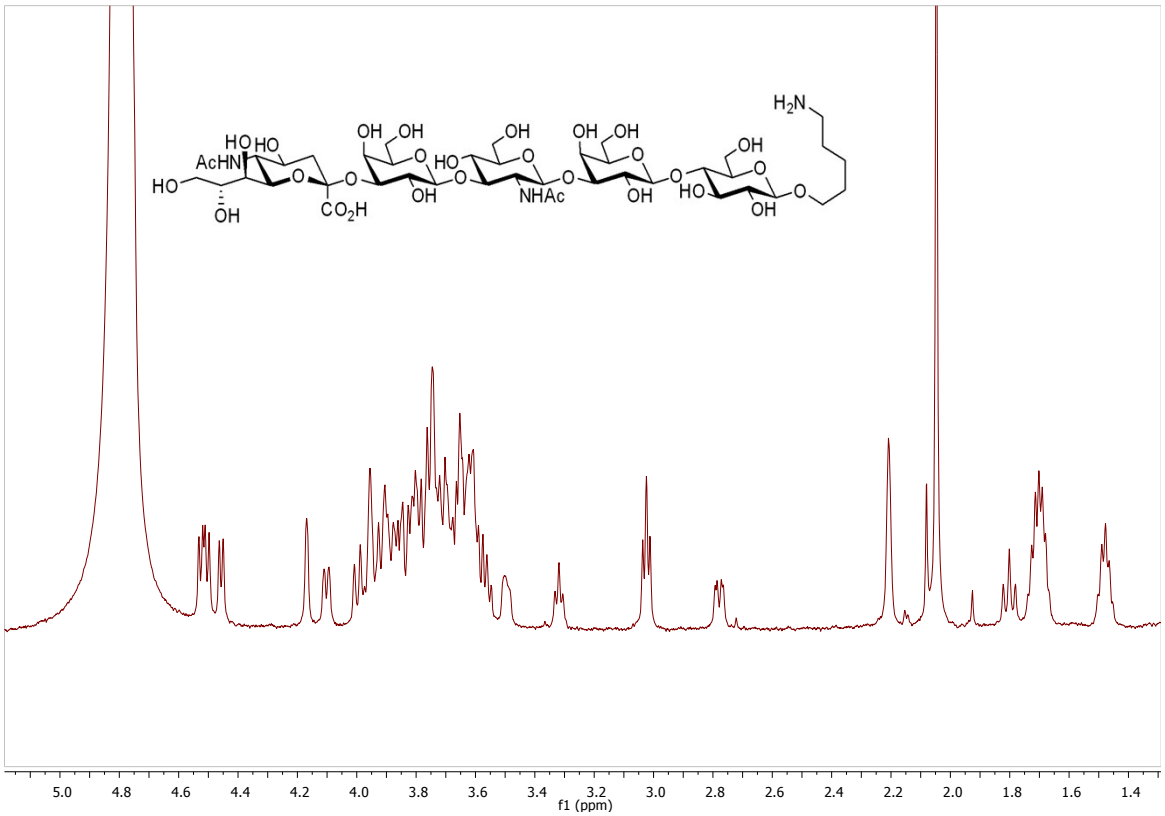
added, followed by 113 uL 2,3-sialyltransferase from *Pasturella multocida* (2.0 mU/μL: 226 mU) and 22.6 μL alkaline phosphatase (10 U/μL: 226 U). The reaction was incubated at 37 °C for 24 h. The reaction was quenched by addition of 160 μL ethanol followed by vortexing for 2 min. The reaction was centrifuged and the supernatant was pipetted off. The solution was diluted with MilliQ water, frozen, and lyophilized. The crude product was analyzed using reverse phase analytical HPLC (Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min; Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) and purified using preparative HPLC (Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min, Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) to obtain compound **3** (1.6 mg, 1.5 μmol, 79% overall yield). CMP-Neu5Ac elutes at 16 min, cytidine elutes at 25 min, compound **S13** elutes at 26 min, and Compound **3** elutes at 31 min.

*Analytical data for pentasaccharide **3**:* ^{1}H NMR (600 MHz, D_2O) δ 4.75 (d, 1H), 4.53 (d, *J* = 7.9 Hz, 1H), 4.50 (d, *J* = 8.1 Hz, 1H), 4.46 (d, *J* = 7.9 Hz, 1H), 4.17 (br s, 1H), 4.10 (dd, *J* = 9.8, 2.9 Hz, 1H), 4.02 – 3.46 (m, 30H), 3.32 (t, *J* = 7.8 Hz, 1H), 3.02 (t, *J* = 7.2 Hz, 2H), 2.78 (dd, *J* = 12.3, 3.7 Hz, 1H), 2.05 (s, 6H), 1.80 (t, *J* = 12.0 Hz, 1H), 1.75 – 1.66 (m, 4H), 1.51 – 1.44 (m, 2H). ^{13}C NMR (175 MHz, D_2O) δ 176.43, 175.41, 172.97, 104.79, 104.27, 103.85, 103.39, 101.51, 83.54, 83.38, 79.82, 76.94, 76.62, 76.45, 76.21, 76.14, 75.98, 74.30, 74.26, 73.19, 71.58, 71.54, 70.43, 69.81, 69.66, 69.55, 68.82, 63.87, 62.69, 62.08, 61.54, 55.96, 52.85, 41.23, 40.87, 29.97, 28.23, 23.91, 23.87. MS ESI HRMS: m/z $[M+Na]^+$ calcd for $C_{42}H_{73}N_3NaO_{29}$ 1106.4227, found 1106.4256.

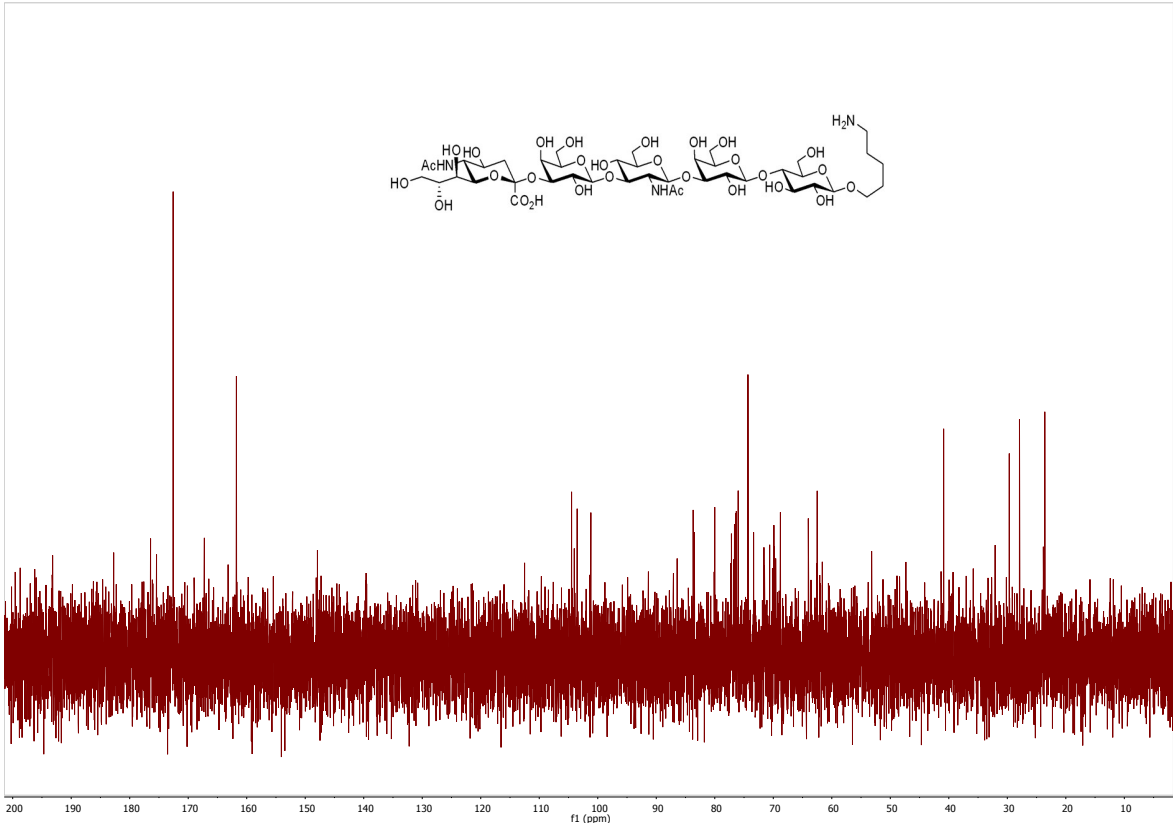
Analytical RP-HPLC Hypercarb of Crude Pentasaccharide (sLC4) 3 (ELSD trace)



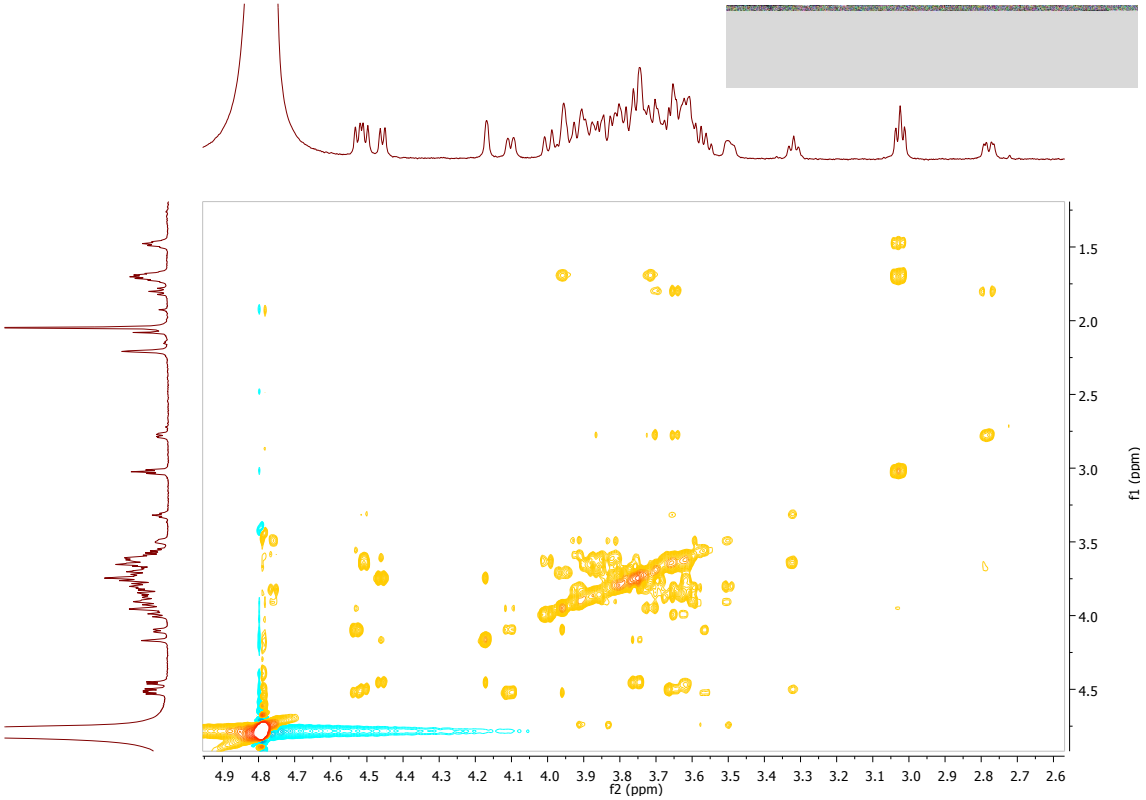
^{1}H NMR of Compound Pentasaccharide (sLC4) 3



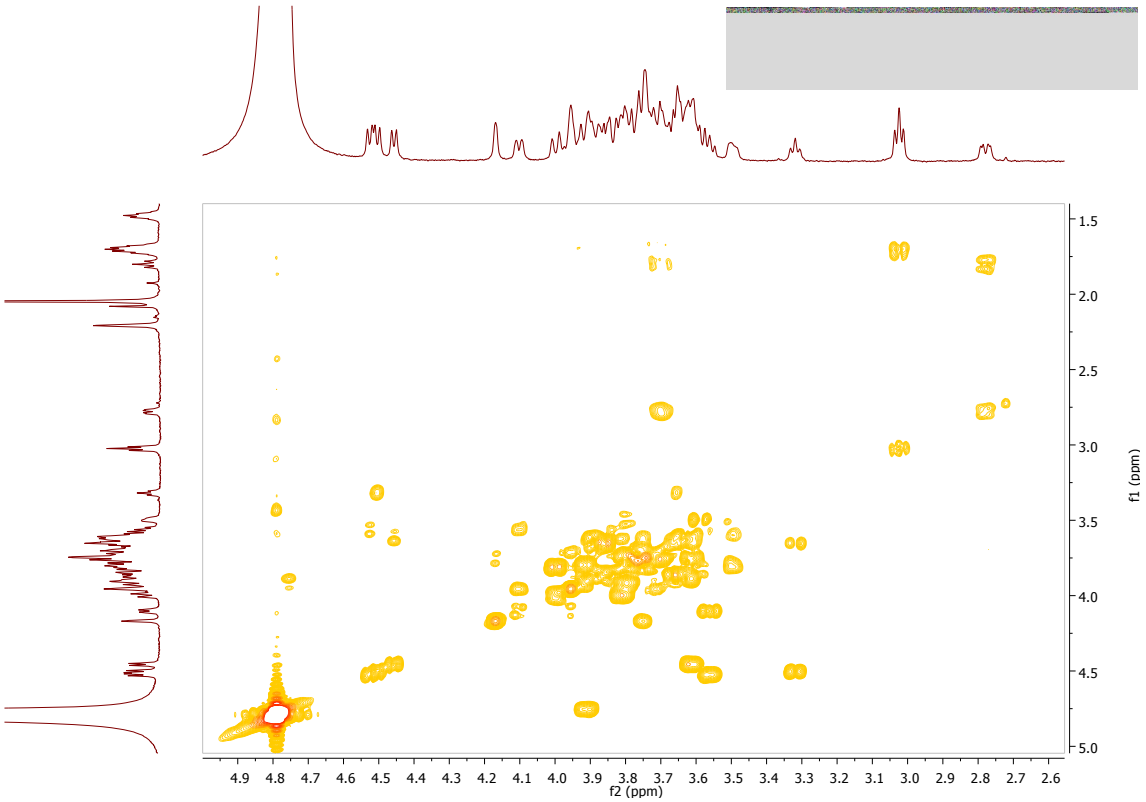
^{13}C NMR of Compound Pentasaccharide (sLC4) 3



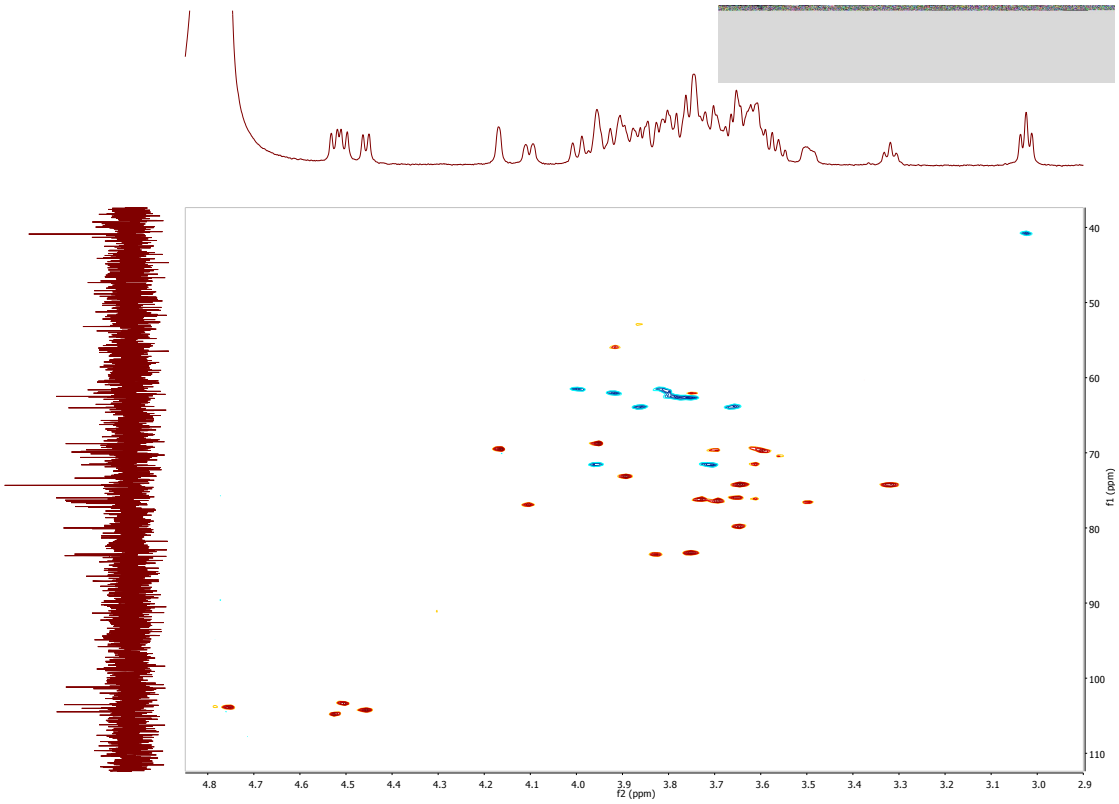
TOCSY NMR of Compound Pentasaccharide (sLC4) 3



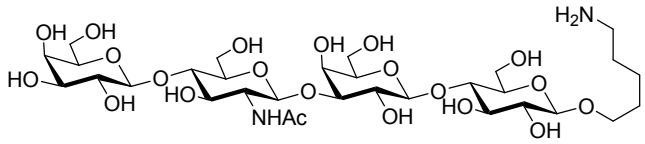
COSY NMR of Compound Pentasaccharide (sLC4) 3



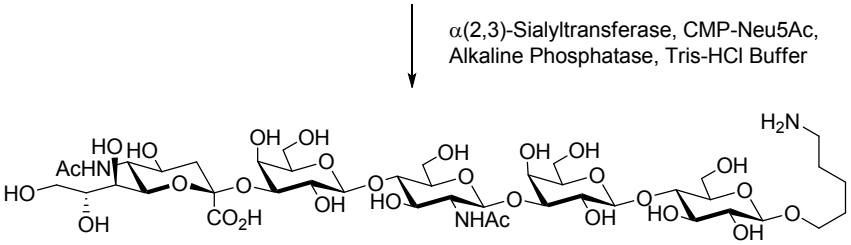
HSQC NMR of Compound Pentasaccharide (sLC4) 3



5.5 Synthesis of Sialyl Pentsaccharide 4 (SPG)



S14



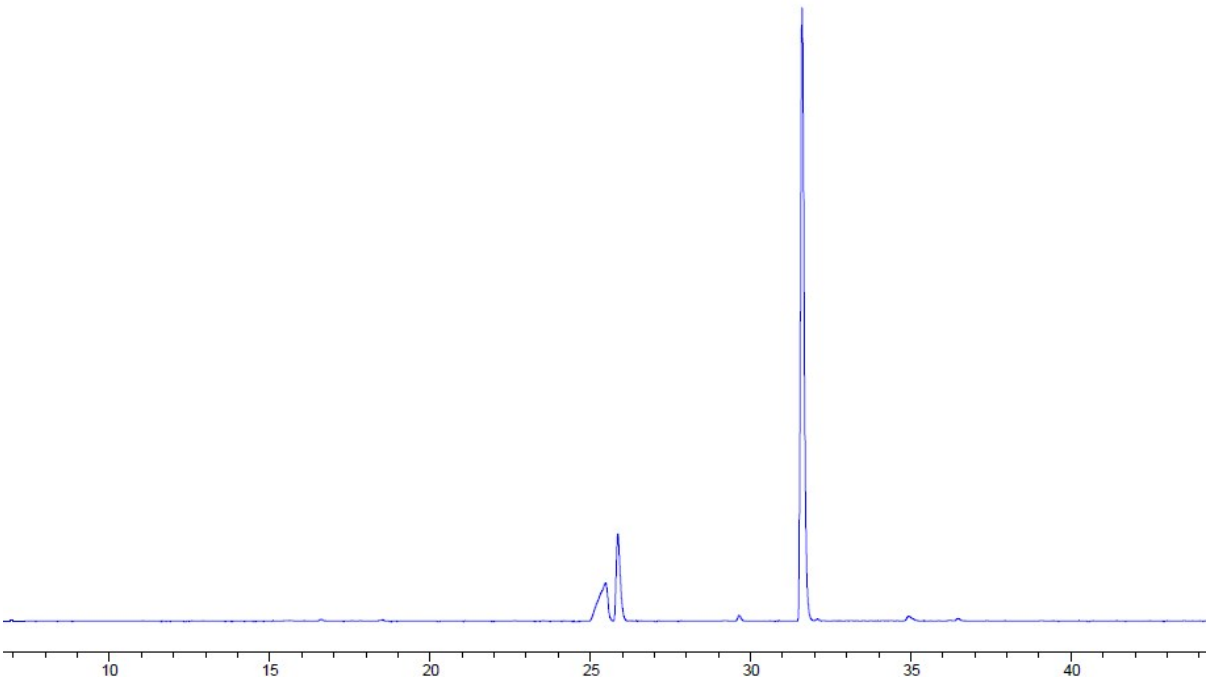
SPG (**4**)

Sialylation, Analysis and Purification: Tetrasaccharide **S14** (2.3 mg, 2.8 μmol) was dissolved in 45 μL H_2O and pipetted into a 1.5 mL Eppendorf tube. CMP-Neu5Ac (2.1 mg, 3.2 μmol, 1.1 eq) in 42 μL H_2O was added. 275 μL of 100 mM Tris buffer (pH 8.0, containing 20 mM $MgCl_2$ and 15 mM NaCl) was

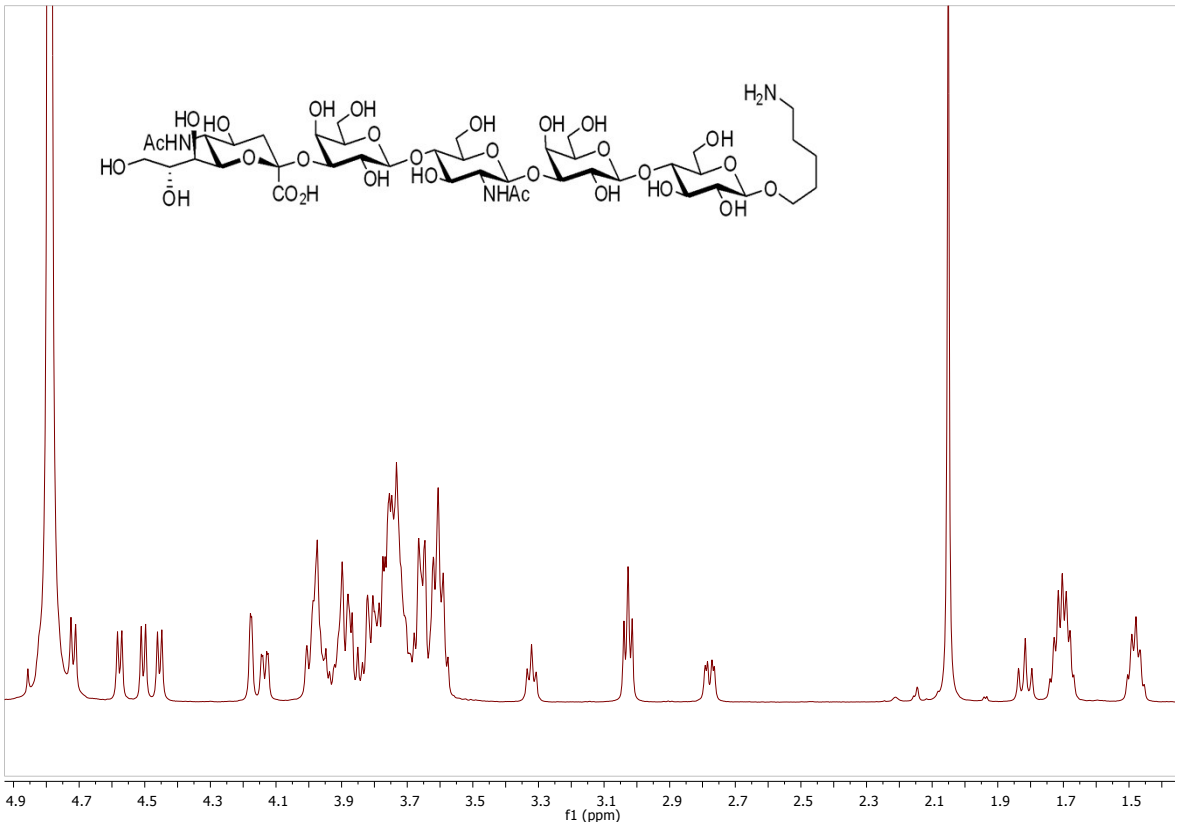
added, followed by 42.5 μL 2,3-sialyltransferase from *Pasturella multocida* (2.0 mU/μL: 85 mU) and 8.5 μL alkaline phosphatase (10 U/μL: 85 U). The reaction was incubated at 37 °C for 18 h. The reaction was quenched by addition of 160 μL ethanol followed by vortexing for 2 min. The reaction was centrifuged and the supernatant was pipetted off. The solution was diluted with MilliQ water, frozen, and lyophilized. The crude product was analyzed using reverse phase analytical HPLC (Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min; Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) and purified using preparative HPLC (Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min, Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) to obtain compound **4** (2.7 mg, 2.4 μmol, 87% overall yield). Cytidine elutes at 25 min, compound **S14** elutes at 26 min, and Compound **4** elutes at 31 min.

*Analytical data for pentasaccharide **4**:* ^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.3 Hz, 1H), 4.58 (d, *J* = 7.8 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.18 (br s, 1H), 4.13 (dd, *J* = 9.8, 2.5 Hz, 1H), 4.03 – 3.56 (m, 30H), 3.32 (t, *J* = 8.3 Hz, 1H), 3.03 (t, *J* = 7.5 Hz, 2H), 2.78 (dd, *J* = 12.3, 4.2 Hz, 1H), 2.05 (s, 6H), 1.82 (t, *J* = 12.1 Hz, 1H), 1.75 – 1.66 (m, 4H), 1.51 – 1.45 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 176.27, 175.23, 172.40, 104.34, 104.17, 103.95, 103.38, 101.21, 83.44, 79.84, 79.43, 76.90, 76.58, 76.31, 76.18, 75.96, 75.85, 74.29, 74.19, 73.54, 73.16, 71.46, 71.35, 70.78, 69.70, 69.49, 68.87, 63.99, 62.43, 62.37, 61.49, 61.25, 56.58, 53.08, 41.04, 40.75, 29.54, 27.79, 23.57, 23.47. MS ESI HRMS: m/z $[M+Na]^+$ calcd for $C_{42}H_{73}N_3NaO_{29}$ 1106.4227, found 1106.4229.

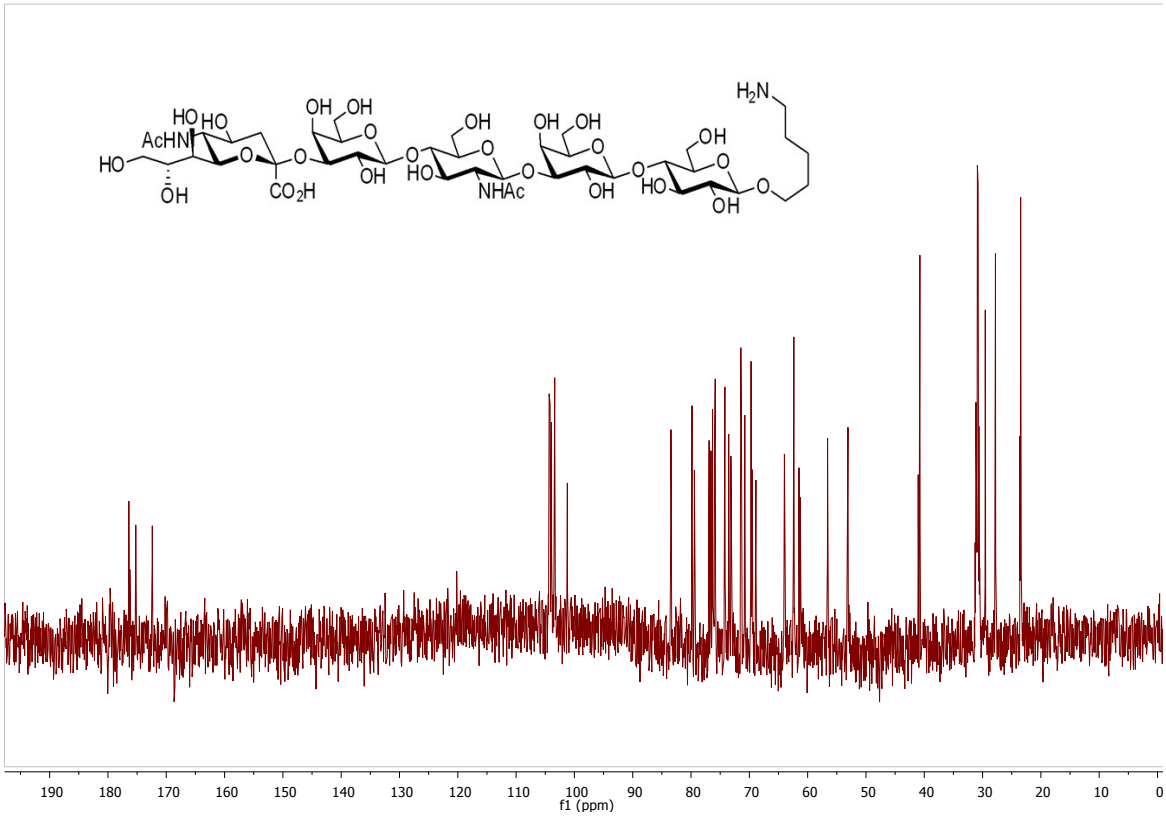
Analytical RP-HPLC Hypercarb of Crude Pentasaccharide (SPG) 4 (ELSD trace)



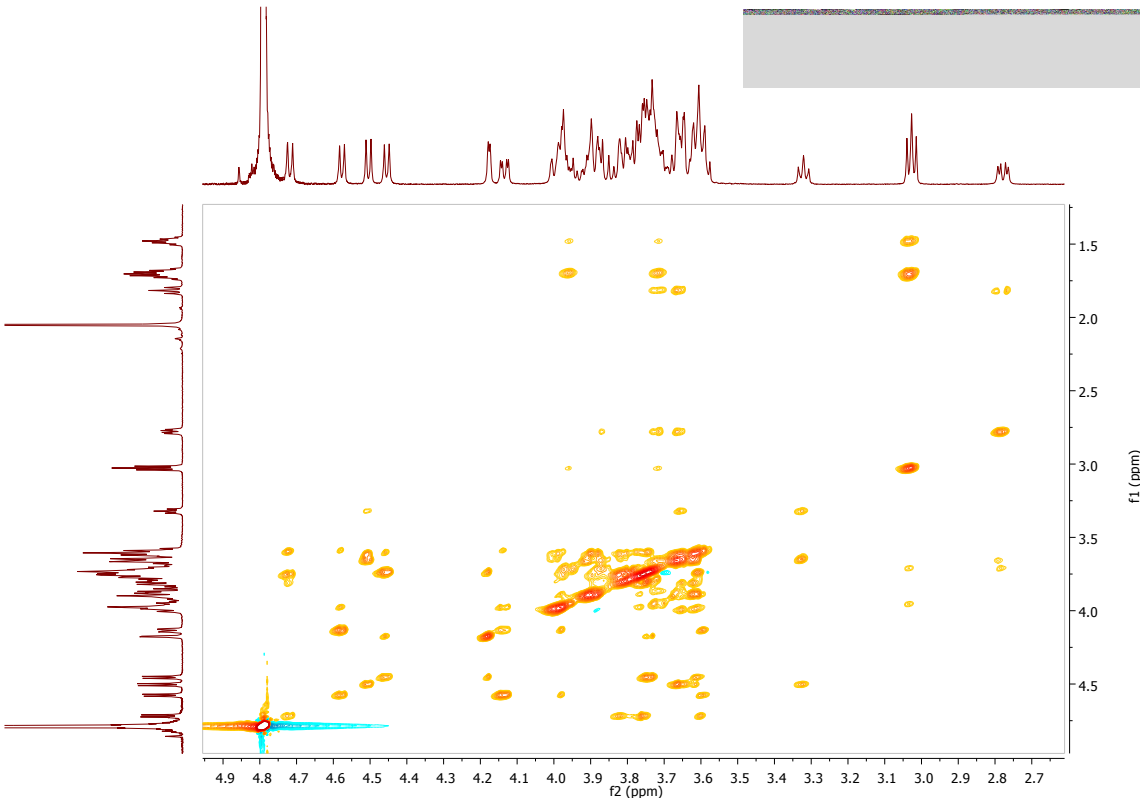
^{1}H NMR of Compound Pentasaccharide (SPG) 4



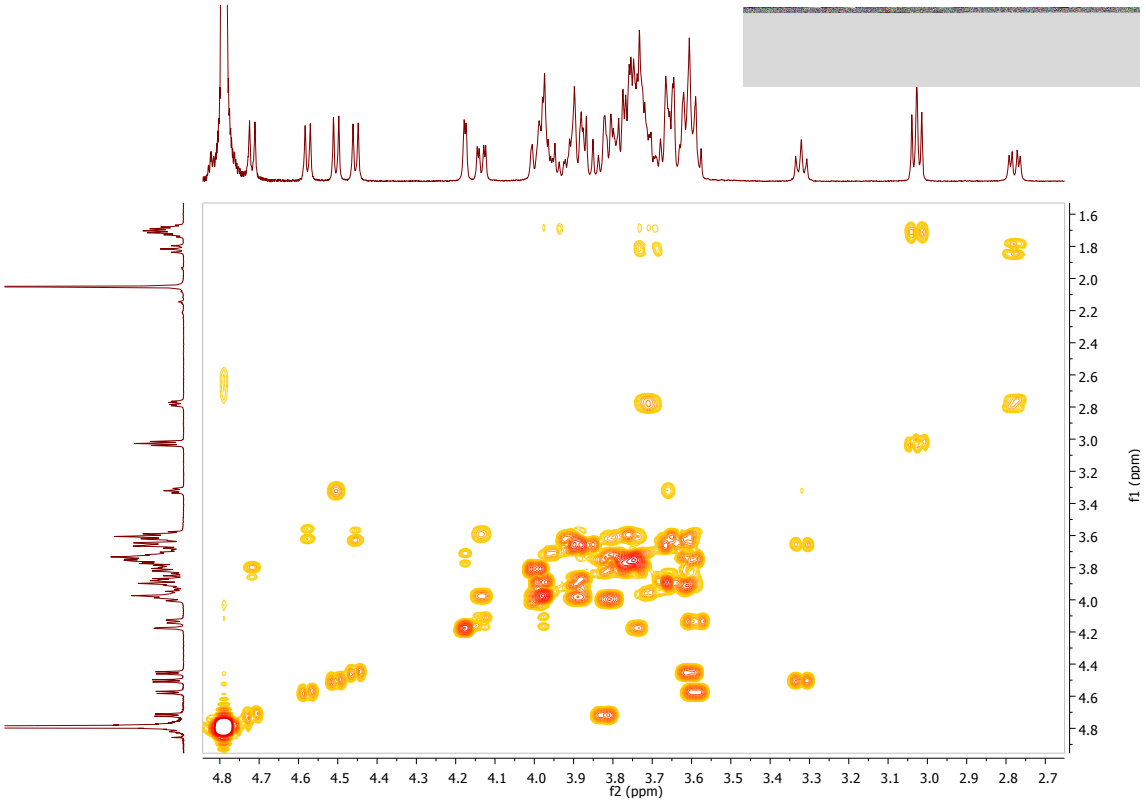
^{13}C NMR of Compound Pentasaccharide (SPG) 4



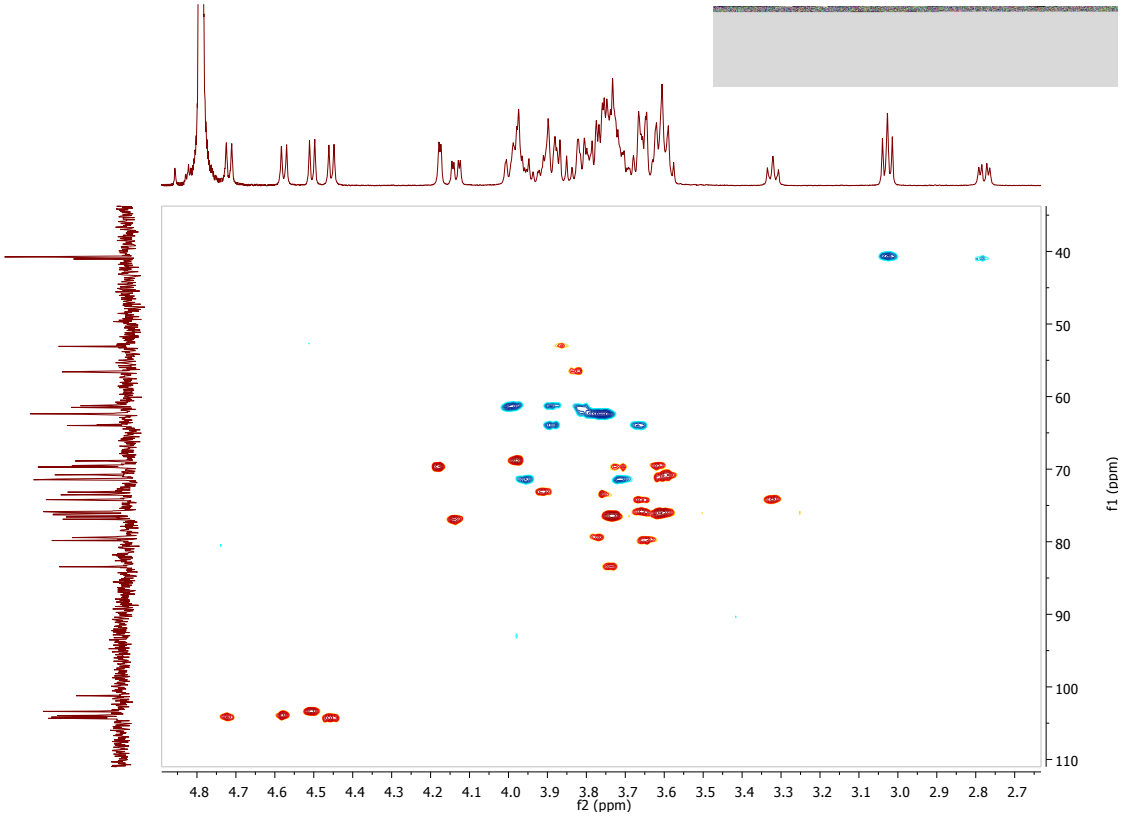
TOCSY NMR of Compound Pentasaccharide (SPG) 4



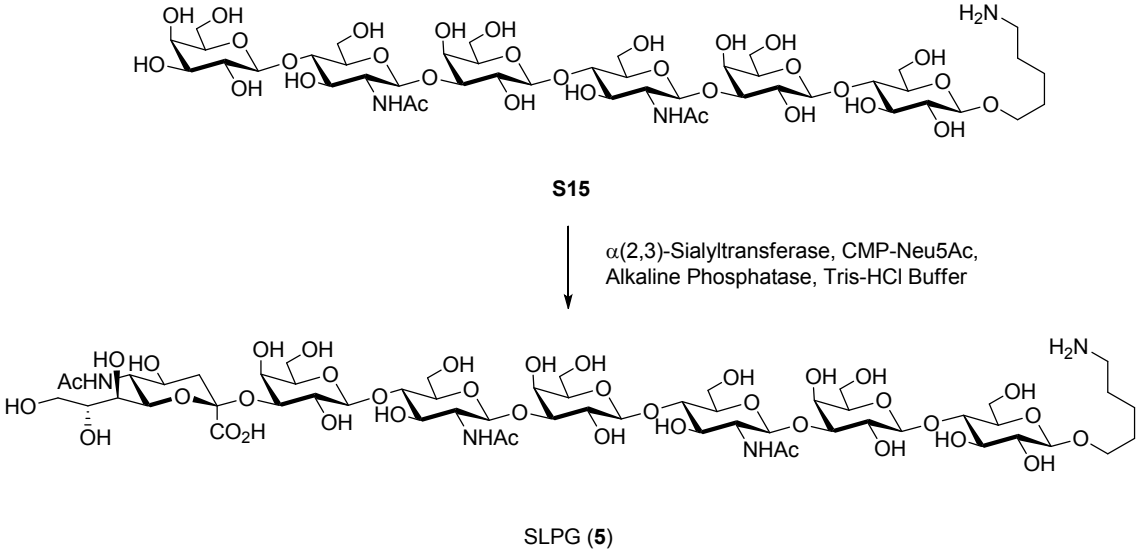
COSY NMR of Compound Pentasaccharide (SPG) 4



HSQC NMR of Compound Pentasaccharide (SPG) 4



5.6 Synthesis of Sialyl Heptaccharide 5 (SLPG)

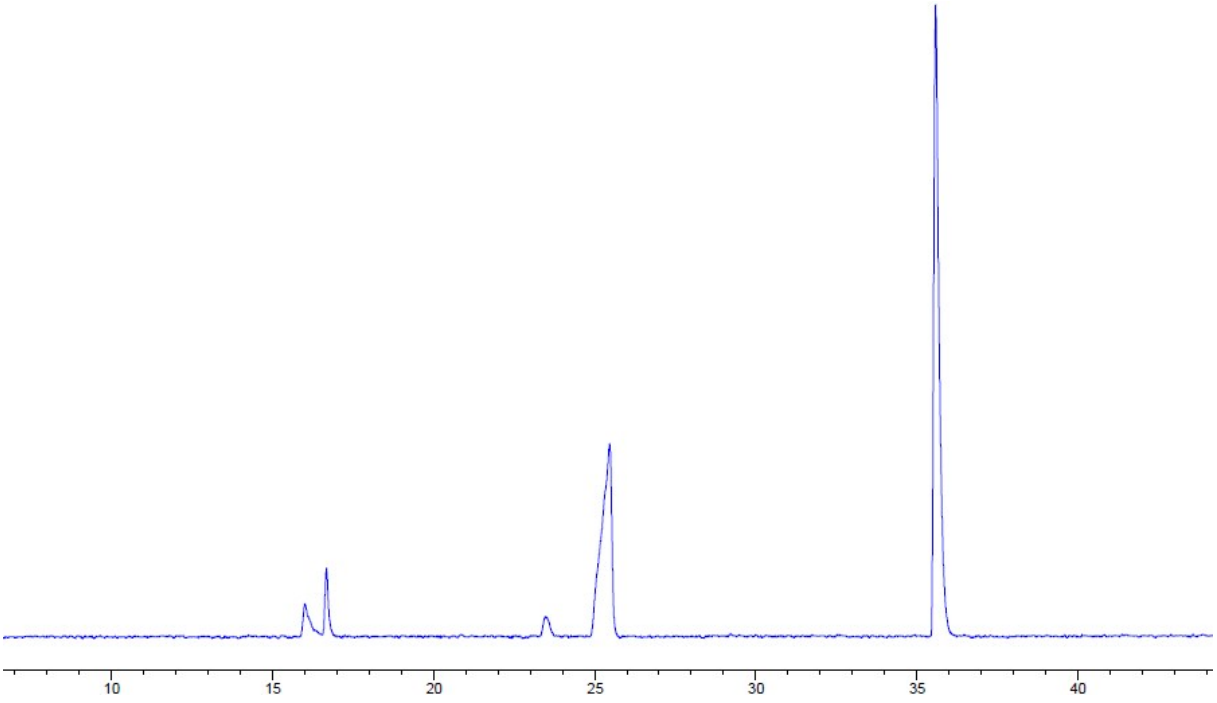


Sialylation, Analysis and Purification: Hexasaccharide **S15** (3.0 mg, 2.6 µmol) was dissolved in 60 µL H_2O and pipetted into a 1.5 mL Eppendorf tube. CMP-Neu5Ac (2.0 mg, 2.9 µmol, 1.1 eq) in 56 µL H_2O was added. 252 µL of 100 mM Tris buffer (pH 8.0, containing 20 mM $MgCl_2$ and 15 mM NaCl) was

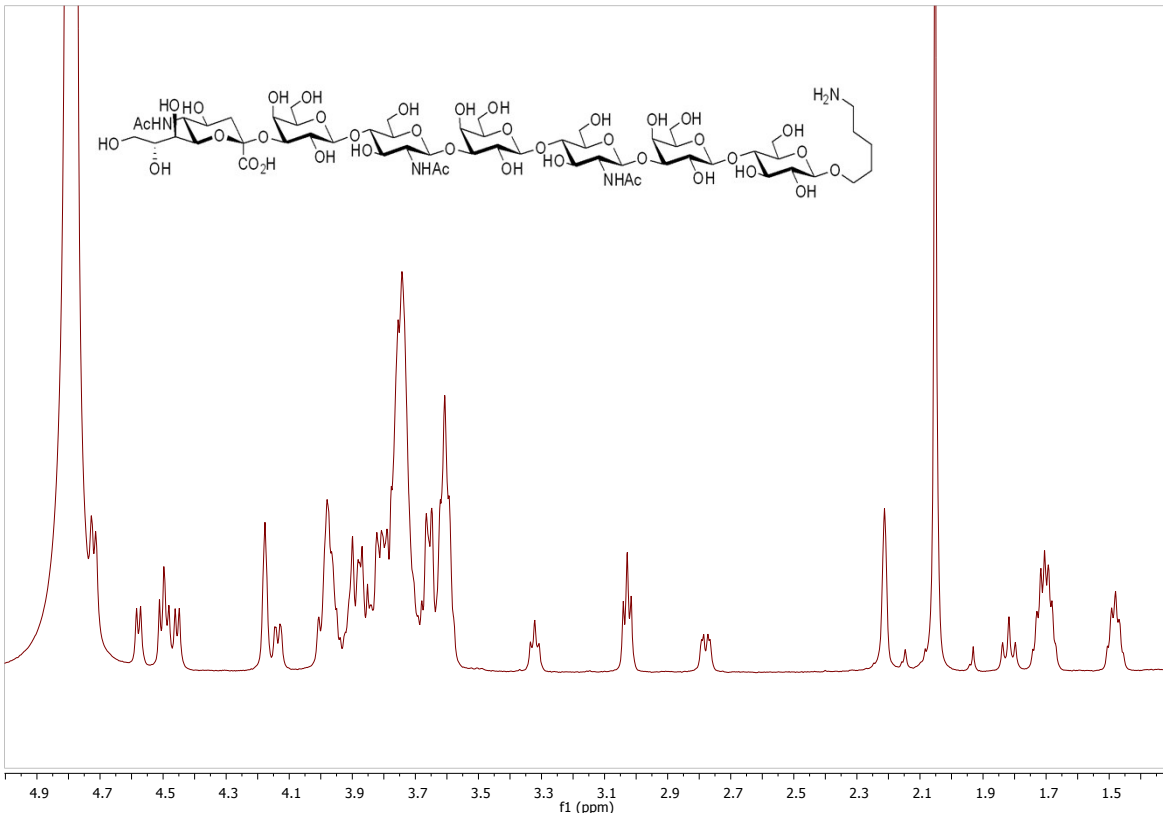
added, followed by 39 μL 2,3-sialyltransferase from *Pasturella multocida* (2.0 mU/μL: 78 mU) and 7.8 μL alkaline phosphatase (10 U/μL: 78 U). The reaction was incubated at 37 °C for 18 h. The reaction was quenched by addition of 160 μL ethanol followed by vortexing for 2 min. The reaction was centrifuged and the supernatant was pipetted off. The solution was diluted with MilliQ water, frozen, and lyophilized. The crude product was analyzed using reverse phase analytical HPLC (Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min; Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) and purified using preparative HPLC (Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min, Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 0% ACN (0.1% formic acid) for 5 min, 0% to 40% ACN (0.1% formic acid) in 45 min) to obtain compound **5** (3.3 mg, 2.3 μmol, 89% overall yield). CMP-Neu5Ac elutes at 16 min, cytidine elutes at 25 min, and Compound **5** elutes at 35 min.

*Analytical data for heptasaccharide **5**:* ^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.1 Hz, 2H), 4.58 (d, *J* = 7.7 Hz, 1H), 4.52 – 4.47 (m, 2H), 4.46 (d, *J* = 7.7 Hz, 1H), 4.18 (br s, 2H), 4.14 (dd, *J* = 9.7, 2.4 Hz, 1H), 4.03 – 3.55 (m, 34H), 3.32 (t, *J* = 7.8 Hz, 1H), 3.03 (t, *J* = 7.2 Hz, 2H), 2.78 (dd, *J* = 12.1, 4.1 Hz, 1H), 2.05 (s, 9H), 1.82 (t, *J* = 12.0 Hz, 1H), 1.75 – 1.66 (m, 4H), 1.51 – 1.45 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 176.41, 176.27, 175.23, 172.41, 104.34, 104.28, 104.18, 104.12, 103.95, 103.39, 101.21, 83.48, 83.44, 79.83, 79.63, 79.43, 76.90, 76.58, 76.29, 76.18, 75.96, 75.86, 74.29, 74.20, 73.57, 73.16, 71.49, 71.36, 70.78, 69.70, 69.50, 68.88, 63.99, 63.88, 62.43, 62.36, 61.49, 61.25, 56.59, 53.09, 41.05, 40.80, 29.56, 28.04, 23.58, 23.49, 23.43. MS ESI HRMS: m/z $[M+2Na]^{2+}$ calcd for $C_{56}H_{96}N_4Na_2O_{39}$ 747.6722, found 747.7846.

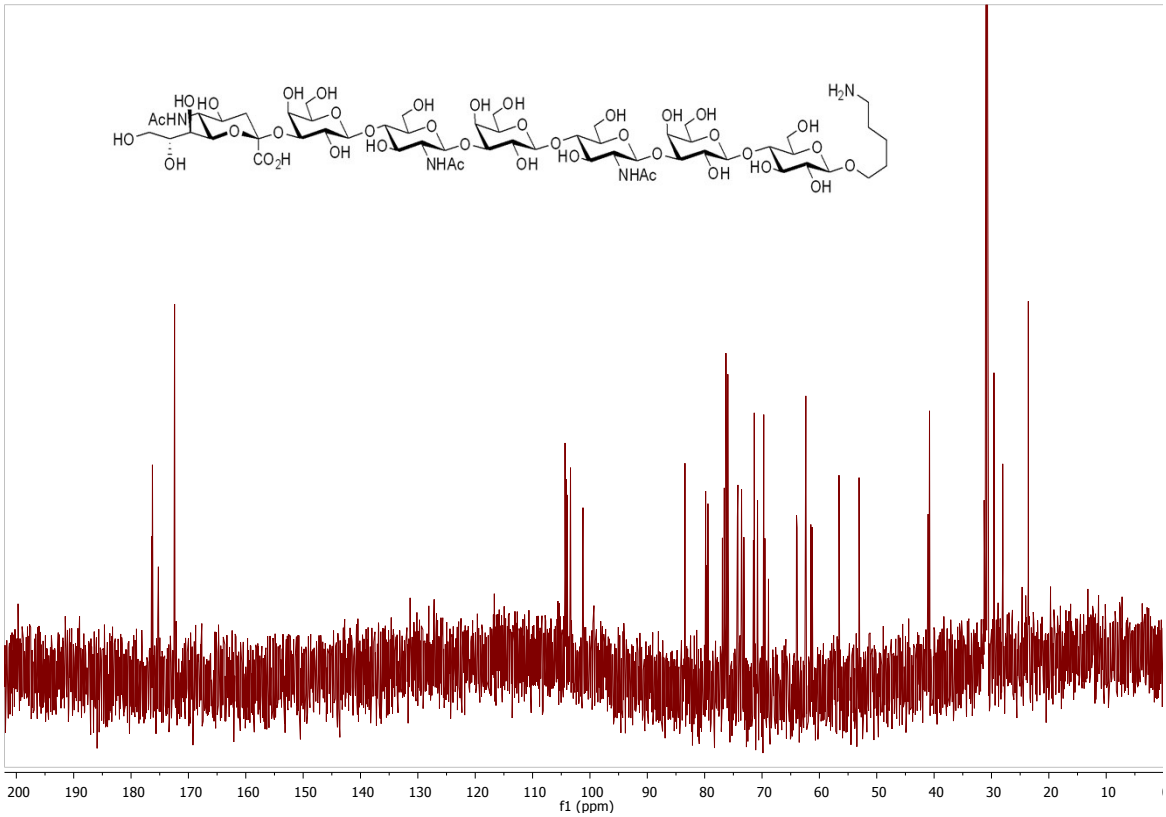
Analytical RP-HPLC Hypercarb of Crude Heptasaccharide (SLPG) 5 (ELSD trace)



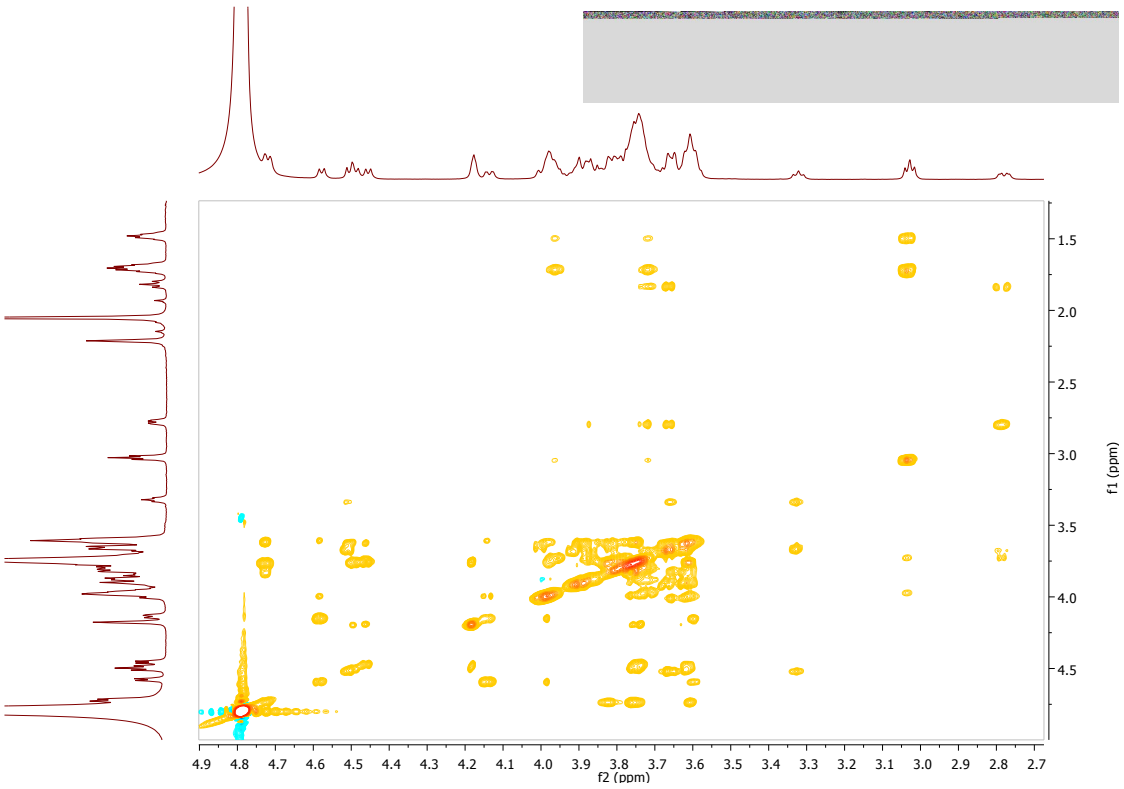
^{1}H NMR of Compound Heptasaccharide (SLPG) 5



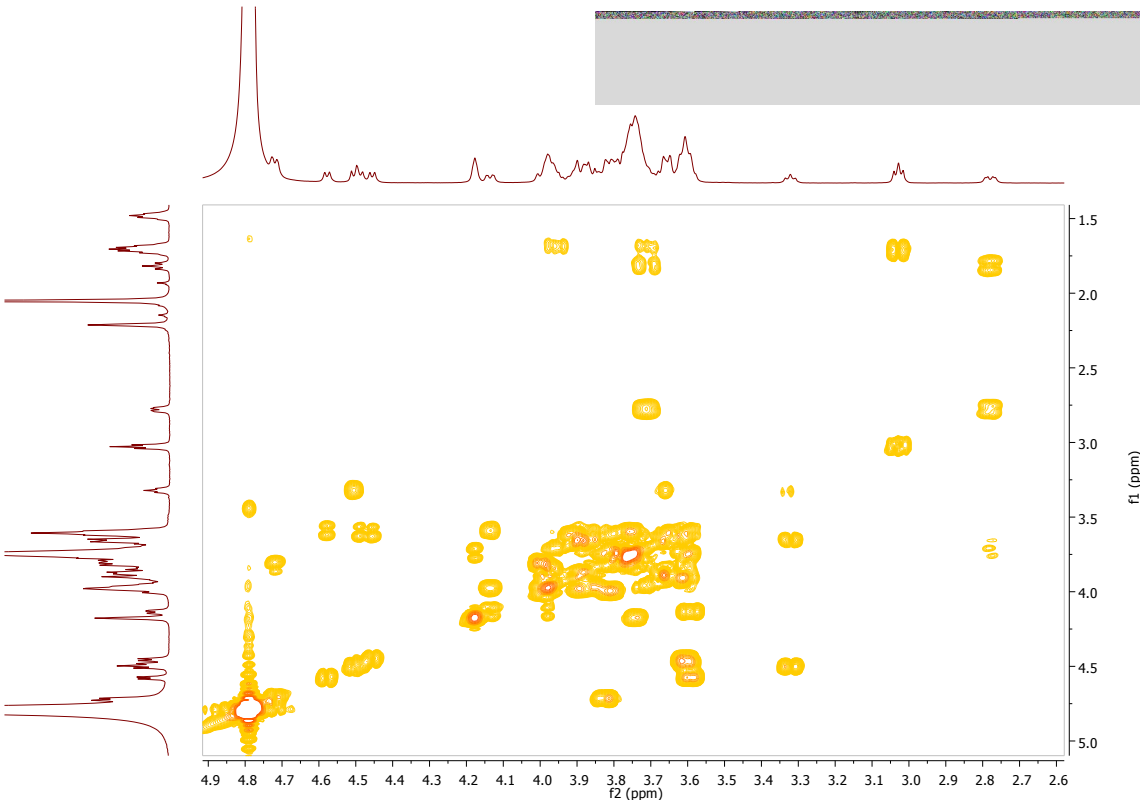
^{13}C NMR of Compound Heptasaccharide (SLPG) 5



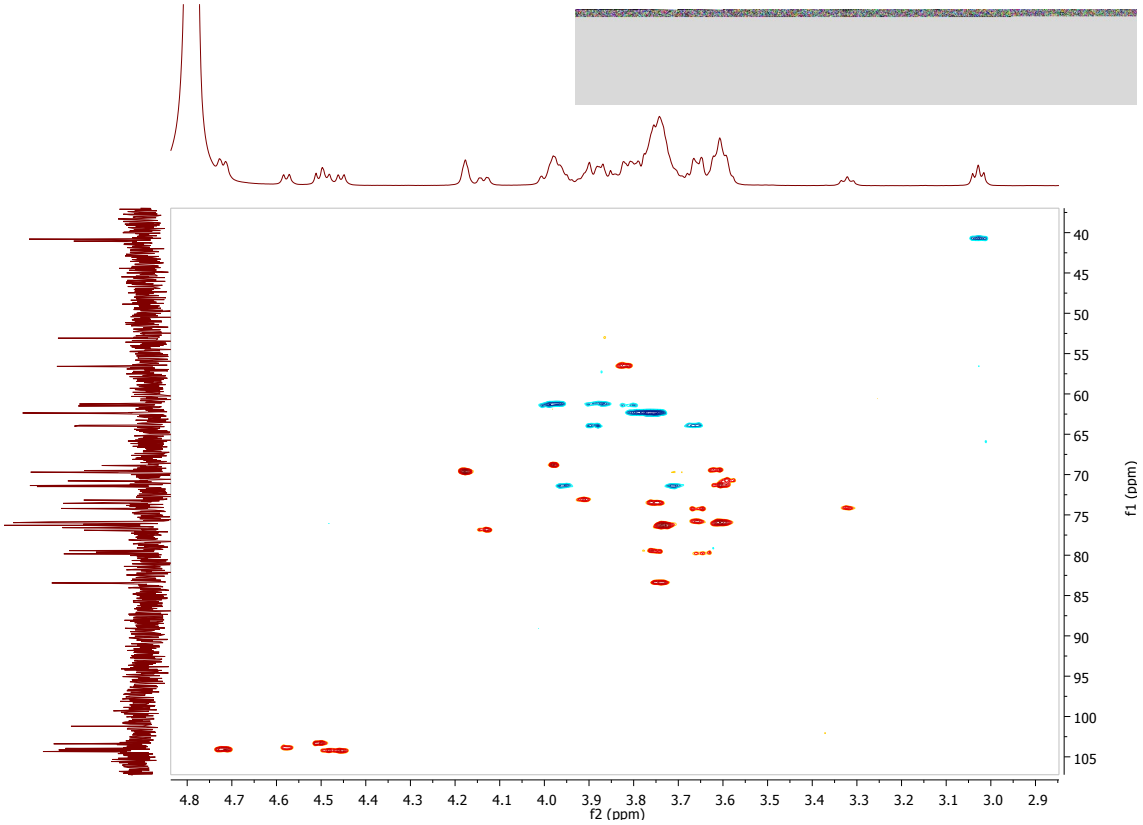
TOCSY NMR of Compound Heptasaccharide (SLPG) 5



COSY NMR of Compound Heptasaccharide (SLPG) 5



HSQC NMR of Compound Heptasaccharide (SLPG) 5



References

S1. F. Belot and J. C. Jacquinet, *Carbohydr. Res.,* 2000, **325**, 93 – 106.