SI – PAPER TITLE

1. General materials and methods

All reagents and solvents were acquired from commercial sources, unless stated otherwise. All the building blocks were obtained from GlycoUniverse stock. The resin equipped with a photocleavable linker was also obtained from GlycoUniverse stock and had a loading of 0.40 mmol/g. Anhydrous solvents were obtained from a Solvent Dispensing System (J.C. Meyer). Amberlite IR-120 (Across Organics) protonic exchange resin was rinsed with THF, water, methanol and dichloromethane before use. NMR spectra were obtained using Ascend 400 (Bruker) and Agilent 400 MHz NMR Magnet (Agilent Technologies) spectrometers at 400 MHz (^{1}H) and 100 MHz (^{13}C) or a Varian 600 (Agilent) at 600 MHz (^{1}H) and 150 MHz (^{13}C), or a Ascend 700 (Bruker) at 700 MHz (^{1}H) and 176 MHz (^{13}C). $CDCl_3$ or D_2O were used as solvents and chemical shifts (δ) referenced to residual non-deuterated solvent peak unless stated otherwise. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad singlet for 1 H-NMR data. NMR chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. Assignments were supported by COSY and HSQC experiments and compared with literature data when available. MALDI-TOF spectra were obtained with a Daltonics Autoflex Speed spectrometer (Bruker) using 2,5-dihydroxybenzoic acid (DHB) as matrix. All MALDI measurements were done in reflectron mode except for the protected octamers whose data was acquired using the linear mode. ESI-HRMS were performed with a Xevo G2-XS Q-Tof (Waters). HPLCs were performed on Agilent 1200 Series systems.

2. Automated glycan assembly using the Glyconeer

General procedure for setting up the Glyconeer

All solvents were loaded in the respective bottle and attached in the corresponding position of the Glyconeer prior to the synthesis. For each synthesis, the building blocks were co-evaporated with toluene, dried under high vacuum for 2h, dissolved in anhydrous DCM (1 mL) and transferred to oven-dried building block vials. The vials were then placed in the appropriate position in the building block carousel. The reagent solutions were prepared as described below. A polystyrene base resin functionalized with 0.40 mmol/g of a photocleavable linker was used. The resin (12.5 µmol of hydroxyl groups, unless otherwise stated) was weighed and transferred into the reaction vessel and the synthesis programme was initiated.

Preparation of reagent solutions:

TMSOTf solution: 40 mL of anhydrous DCM were transferred into an oven-dried reagent bottle under Ar. TMSOTf (0.45 mL) was added and the bottle was placed in the predetermined acid-wash position and attached to the Glyconeer.

Activation solution: N-iodosuccinimide (NIS, 1.35 g) was added to an oven-dried reagent bottle. Anhydrous dioxane (13 mL) and anhydrous DCM (26 mL) were added and the mixture gently stirred. Triflic acid (TfOH, 55 µL) was added and the bottle placed in the cooling block, in the predetermined position, and attached to the Glyconeer.

Pyridine solution: 5 mL of pyridine were diluted in 45 mL of DMF and the resulting solution was transferred into a reagent bottle. The bottle was placed in the predetermined position and attached to the Glyconeer.

Capping solution: anhydrous DCM (36 mL) was transferred into an oven-dried reagent bottle under Ar. Ac_2O (4 mL) was added followed by MsOH (0.8 mL). The bottle was placed in the predetermined position and attached to the Glyconeer.

Piperidine solution: 50 mL of piperidine were diluted with 200 mL of DMF and the resulting solution was transferred into the reagent bottle. The bottle was placed in the predetermined position and attached to the Glyconeer.

Hydrazine solution: hydrazine acetate (550 mg) was transferred into a reagent bottle and pyridine (32 mL) was added. AcOH (8 mL) was carefully added followed by H_2O (2 mL). The resulting mixture was gently stirred for a few minutes and the bottle was then placed in the predetermined position and attached to the Glyconeer.

All experiments in the Glyconeer start with a “Resin swelling” module. During this module, DCM (2 mL) is delivered in the reaction vessel and the resin is incubated at 25 °C for 30 min. During the incubation time, the machine rinses the manifolds. Afterwards syntheses were programmed by combining the pre-defined modules described below. For each monosaccharide to be added to the target glycan, the cycle Acid Wash, Glycosylation, Capping, Deprotection was repeated.

Module 1 – Acid Wash: the temperature is set to -20 °C and the resin is washed with the TMSOTf solution (1 mL).

Module 2 – Glycosylation: The temperature is adjusted to the addition temperature T1 (-20 °C unless otherwise stated), the building block solution (1 mL) is delivered and then the activator solution (1 mL). An incubation period t1 = 5 min is followed. The temperature is then adjusted to T2 (0 °C unless otherwise stated) and an incubation period t2 = 20 min is followed. The resin is then washed with a 1:1 solution of DCM/Dioxane, and then with DCM. When a double coupling was required, the procedure was repeated. When a quadruple couple was required, the procedure was repeated to a total of four times.

Module 3 – Capping: The temperature is set to 25 °C. The resin is washed with DMF, then with the pyridine solution (2 mL), then with DCM. The capping solution is added (4 mL) and resin is incubated for 20 min. The resin is finally washed with DCM.

Module 4 – Fmoc Deprotection: The temperature is set to 25 °C. The resin is washed with DMF and then the piperidine solution (2 mL) is added. The resin is incubated for 5 min and afterwards washed with DMF and DCM.

Module 5 – Lev Deprotection: The temperature is set to 25 °C. The resin is washed with DCM and drained. DCM (1.3 mL) is added followed by the hydrazine solution (0.8 mL). The resin is incubated for 30 min and the procedure is repeated another two times. The resin is finally washed with DMF and DCM.

25 µmol experiments

The resin (25 µmol of hydroxyl groups) was weighed and transferred into the reaction vessel. The building blocks were prepared as described above dissolved in 2 mL of anhydrous DCM instead of 1 mL. The parameters of the synthesis were adjusted for the glycosylation, Fmoc deprotection, and Lev deprotection modules, as follows:

Module 2 – Glycosylation: The temperature is adjusted to the addition temperature T1 (-20 °C unless otherwise stated), the building block solution (2 mL) is delivered and then the activator solution (2 mL). An incubation period t1 = 5 min is followed. The temperature is then adjusted to T2 (0 °C unless otherwise stated) and an incubation period t2 = 20 min is followed. The resin is then washed with a 1:1 solution of DCM/Dioxane, and then with DCM. When a double coupling was required, the procedure was repeated. When a quadruple couple was required, the procedure was repeated to a total of four times.

Module 4 – Fmoc Deprotection: The temperature is set to 25 °C. The resin is washed with DMF and then the piperidine solution (4 mL) is added. The resin is incubated for 5 min and afterwards washed with DMF and DCM.

Module 5 – Lev Deprotection: The temperature is set to 25 °C. The resin is washed with DCM and drained. DCM (2.5 mL) is added followed by the hydrazine solution (1.5 mL). The resin is incubated for 30 min and the procedure is repeated another two times. The resin is finally washed with DMF and DCM.

50 µmol experiment

The resin (50 µmol of hydroxyl groups) was weighed and transferred into the reaction vessel. The building block were prepared as described above and dissolved in 2 mL of DCM mL. The parameters of the synthesis were adjusted for the glycosylation, Fmoc deprotection, and Lev deprotection modules, as follows:

Module 2 – Glycosylation: The temperature is adjusted to the addition temperature T1 (-20 °C unless otherwise stated), the building block solution (2 mL) is delivered and then the activator solution (4 mL). An incubation period t1 = 5 min is followed. The temperature is then adjusted to T2 (0 °C unless otherwise stated) and an incubation period t2 = 20 min is followed. The resin is then washed with a 1:1 solution of DCM/Dioxane, and then with DCM. When a double coupling was required, the procedure was repeated. When a quadruple couple was required, the procedure was repeated to a total of four times.

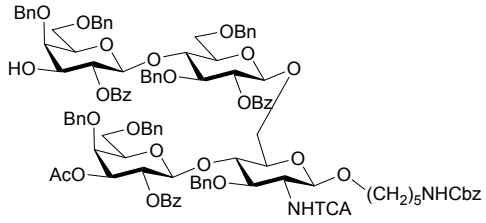
Module 4 – Fmoc Deprotection: The temperature is set to 25 °C. The resin is washed with DMF and then the piperidine solution (8 mL) is added. The resin is incubated for 5 min and afterwards washed with DMF and DCM.

Module 5 – Lev Deprotection: The temperature is set to 25 °C. The resin is washed with DCM and drained. DCM (5 mL) is added followed by the hydrazine solution (3 mL). The resin is incubated for 30 min and the procedure is repeated another two times. The resin is finally washed with DMF and DCM

General procedure for cleavage from the solid support and purification

After automated synthesis in the Glyconeer, the solid-support was suspended in DCM and injected into a continuous-flow photoreactor as described previously[1] to release the generated oligosaccharides. The resulting crude material was analysed by analytical HPLC using a YMC-Diol-300 column (150 x 4.6 mm) and the following elution method: flow rate 1 mL/min, elution started with 20% EtOAc in hexane for 5 min (isocratic), then linear gradient to 55% EtOAc in hexane for 35 min, then linear gradient to 100% EtOAc for 5 min, then 100% EtOAc for 5 min (isocratic). The target oligosaccharide was purified using a preparative HPLC using a YMC-Diol-300 column (150 x 20 mm) and the following elution method: flow rate 15 mL/min, elution started with 20% EtOAc in hexane for 5 min (isocratic), then linear gradient to 55% EtOAc in hexane for 35 min, then linear gradient to 100% EtOAc for 5 min, then 100% EtOAc for 5 min (isocratic). The product fractions were collected, evaporated under reduced pressure and dried under high vacuum overnight.

Compound 9



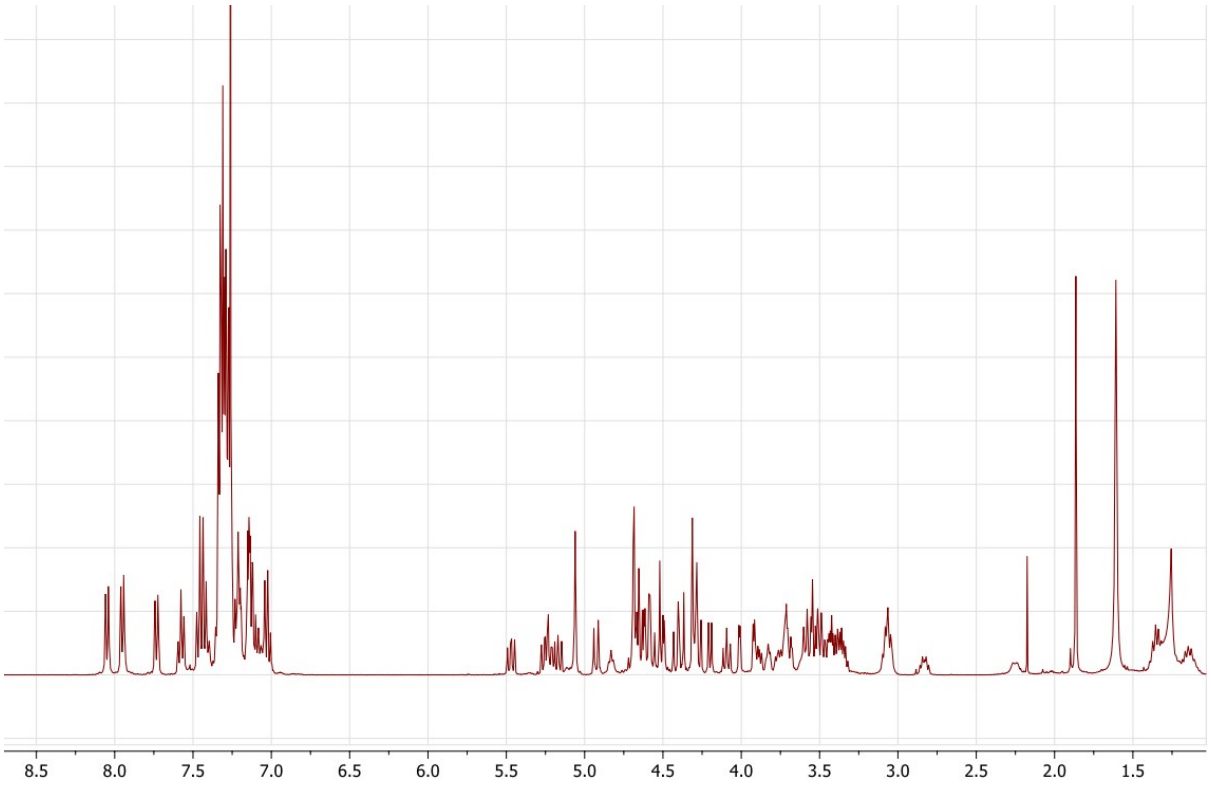
9

^{1}H NMR (400 MHz, Chloroform-d) δ 8.08 – 8.01 (m, 2H), 7.99 – 7.92 (m, 2H), 7.77 – 7.70 (m, 2H), 7.63 – 7.53 (m, 2H), 7.45 (q, J = 8.0 Hz, 5H), 7.38 – 7.17 (m, 33H), 7.18 – 6.97 (m, 10H), 5.47 (dd, J = 10.5, 7.8 Hz, 1H), 5.28 – 5.20 (m, 2H), 5.17 (dd, J = 9.5, 7.9 Hz, 1H), 5.06 (s, 2H), 4.93 (d, J = 11.2 Hz, 1H), 4.83 (t, J = 6.0 Hz, 1H), 4.73 – 4.48 (m, 10H), 4.42 (d, J = 11.9 Hz, 1H), 4.38 (d, J = 11.9 Hz, 1H), 4.32 – 4.24 (m, 4H), 4.20 (d, J = 7.9 Hz, 1H), 4.09 (t, J = 9.2 Hz, 1H), 4.01 (d, J = 3.2 Hz, 1H), 3.93 – 3.87 (m, 2H), 3.82 (d, J = 5.4 Hz, 1H), 3.79 – 3.67 (m, 4H), 3.63 – 3.31 (m, 11H), 3.13 – 3.01 (m, 3H), 2.83 (dt, J = 9.8, 6.2 Hz, 1H), 2.25 (d, J = 10.0 Hz, 1H, OH), 1.86 (s, 3H, COCH_3), 1.42 – 1.20 (m, 4H, pentanylene chain), 1.15 (p, J = 8.4, 7.7 Hz, 2H, pentanylene chain).

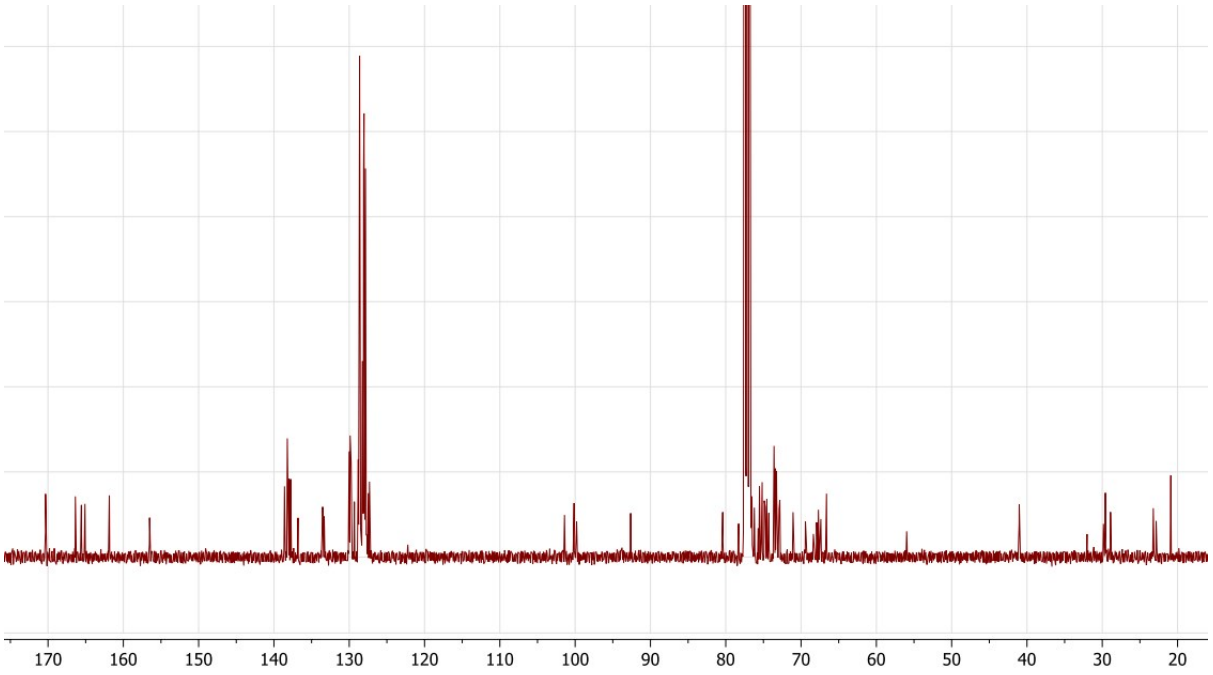
^{13}C NMR (101 MHz, CDCl 3) δ 170.33, 166.37, 165.57, 165.09, 161.86, 156.51, 138.60, 138.23, 138.22, 138.21, 138.14, 137.99, 137.78, 136.80, 133.67, 133.53, 133.32, 130.01, 129.98, 129.88, 129.75, 129.66, 129.32, 128.80, 128.72, 128.71, 128.67, 128.63, 128.56, 128.45, 128.25, 128.21, 128.17, 128.09, 128.08, 128.05, 128.04, 128.02, 128.00, 127.87, 127.84, 127.80, 127.47, 127.31, 101.41, 100.15, 100.02, 99.80, 92.63, 80.40, 78.29, 77.48, 77.36, 77.16, 76.84, 76.53, 76.21, 75.70, 75.52, 75.17, 74.88, 74.76, 74.55, 74.28, 73.61, 73.59, 73.41, 73.27, 73.23, 73.17, 72.91, 72.82, 71.06, 69.41, 68.36, 67.93, 67.70, 67.38, 66.62, 55.97, 41.03, 29.59, 28.90, 23.22, 20.90.

MALDI: calcd for $C_{111}H_{115}Cl_3N_2NaO_{27}$ [M+Na] 2035.7. Found 2035.2.

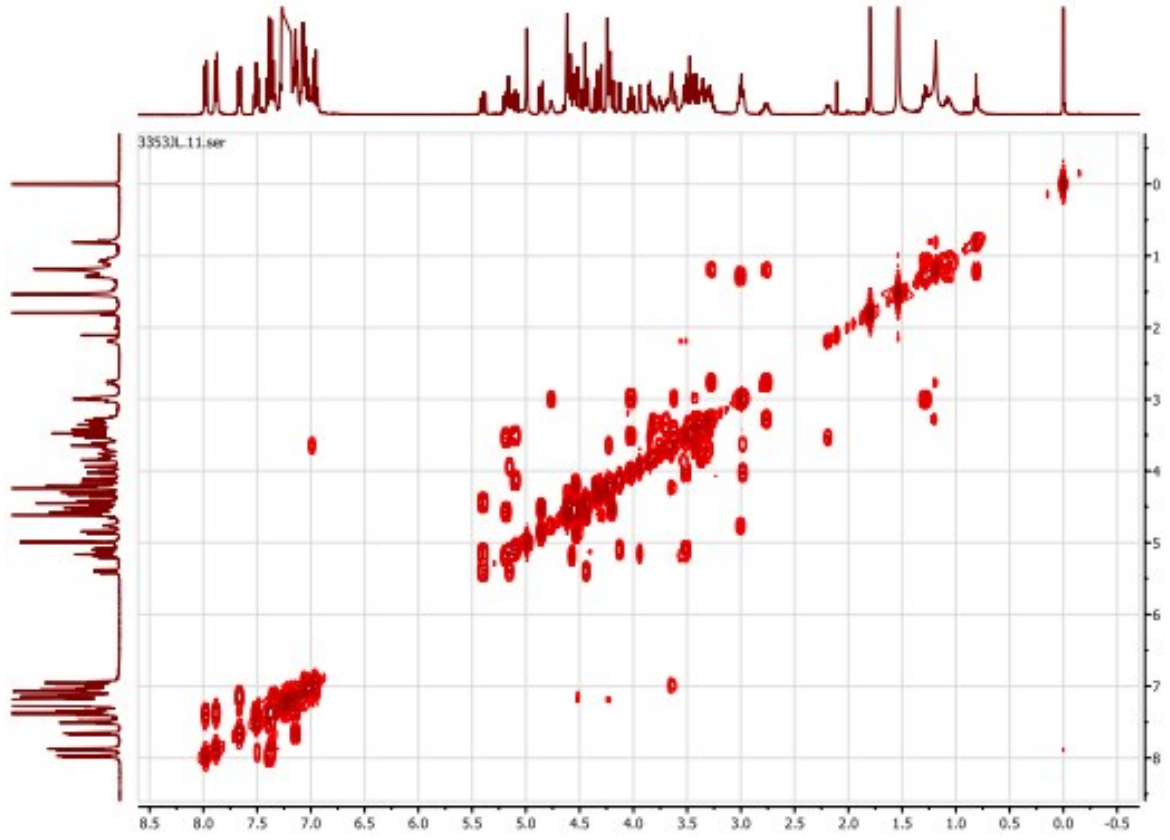
^{1}H NMR of compound **9**



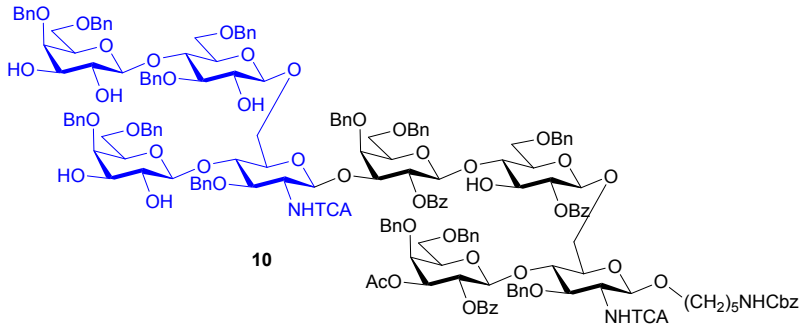
^{13}C NMR of compound **9**



COSY of compound **9**



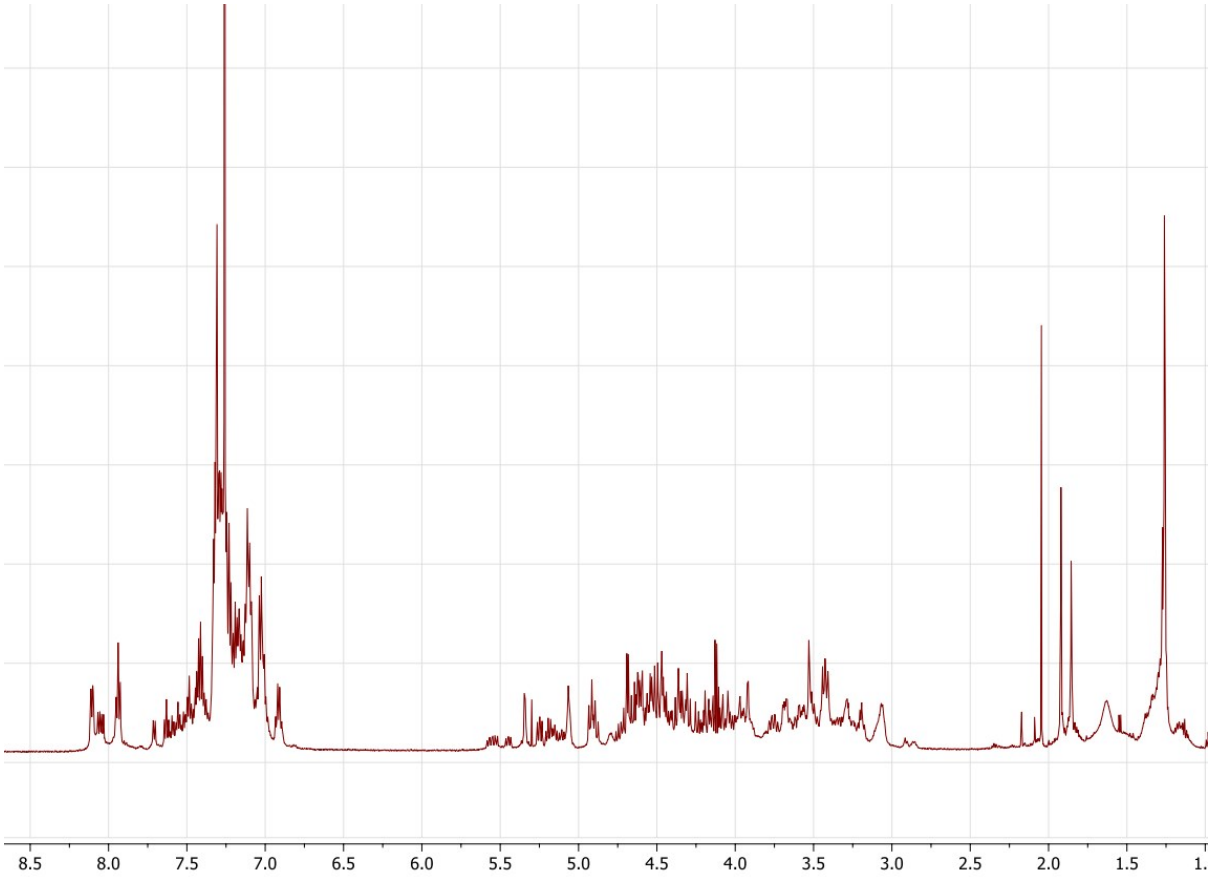
Compound 10



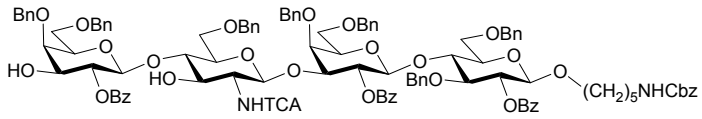
10

^{1}H NMR (600 MHz, Chloroform-d) δ 8.13 – 8.09 (m, 2H), 8.08 – 8.02 (m, 2H), 7.96 – 7.92 (m, 3H), 7.73 – 7.69 (m, 1H), 7.65 – 6.97 (m, 97H), 6.94 – 6.87 (m, 2H), 5.57 (dd, J = 10.2, 7.8 Hz, 1H), 5.53 (dd, J = 10.2, 8.0 Hz, 1H), 5.45 (dd, J = 10.5, 8.0 Hz, 1H), 5.37 – 5.31 (m, 2H), 5.25 (dd, J = 10.0, 7.9 Hz, 1H), 5.21 – 5.04 (m, 5H), 4.96 – 4.87 (m, 3H), 4.82 – 3.87 (m, 40H), 3.81 – 3.02 (m, 35H), 2.94 – 2.82 (m, 2H), 1.92 (s, 3H), 1.85 (s, 3H), 1.42 – 1.27 (m, 4H), 1.20 – 1.10 (m, 2H).

MALDI: calcd for $C_{209}H_{211}Cl_6N_3NaO_{51}$ [M+Na] 3811.2. Found 3813.0.



Compound 11



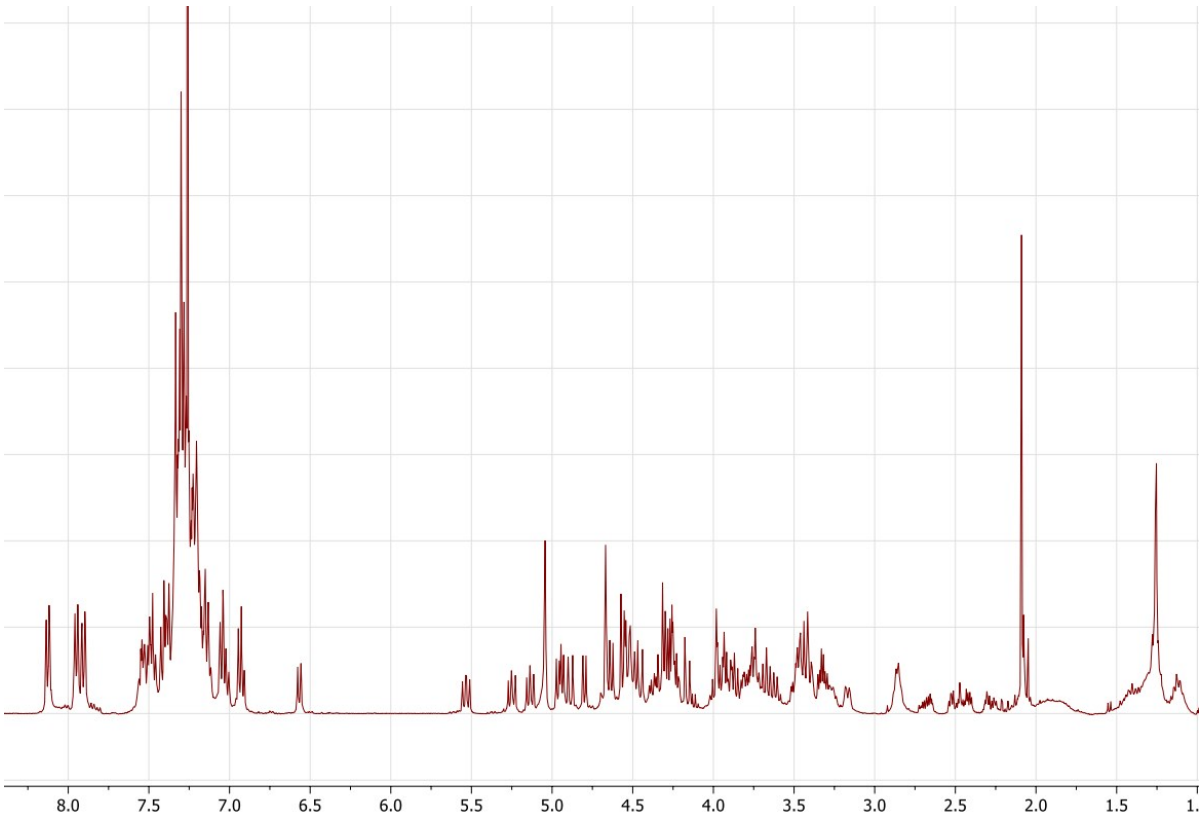
11

^{1}H NMR (400 MHz, Chloroform-d) δ 8.13 (dd, J = 8.4, 1.4 Hz, 2H), 7.95 (dd, J = 8.4, 1.4 Hz, 2H), 7.91 (dd, J = 8.4, 1.3 Hz, 2H), 7.59 – 7.09 (m, 44H), 7.08 – 6.98 (m, 3H), 6.97 – 6.89 (m, 2H), 6.57 (d, J = 8.2 Hz, 1H, N*H*TCA), 5.53 (dd, J = 10.2, 7.9 Hz, 1H), 5.25 (dd, J = 9.8, 7.9 Hz, 1H), 5.14 (dd, J = 9.4, 8.1 Hz, 1H), 5.04 (s, 2H), 4.96 (d, J = 11.6 Hz, 1H), 4.94 (d, J = 10.9 Hz, 1H), 4.89 (d, J = 11.1 Hz, 1H), 4.80 (d, J = 7.7 Hz, 1H), 4.69 – 4.64 (m, 2H), 4.63 (d, J = 7.8 Hz, 1H), 4.56 (d, J = 7.8 Hz, 1H), 4.57 – 4.42 (m, 5H), 4.38 (dd, J = 12.0, 4.5 Hz, 1H), 4.37 – 4.19 (m, 5H), 4.16 (d, J = 11.6 Hz, 7H), 4.03 – 3.58 (m, 9H), 3.52 – 3.23 (m, 7H), 3.20 – 3.14 (m, 1H), 2.86 (d, J = 6.6 Hz, 2H), 2.75 – 2.62 (m, 1H), 2.56 – 2.38 (m, 2H), 2.35 – 2.22 (m, 1H), 2.09 (s, 3H), 1.48 – 1.19 (m, 4H, pentanylene chain), 1.19 – 1.06 (m, 2H, pentanylene chain).

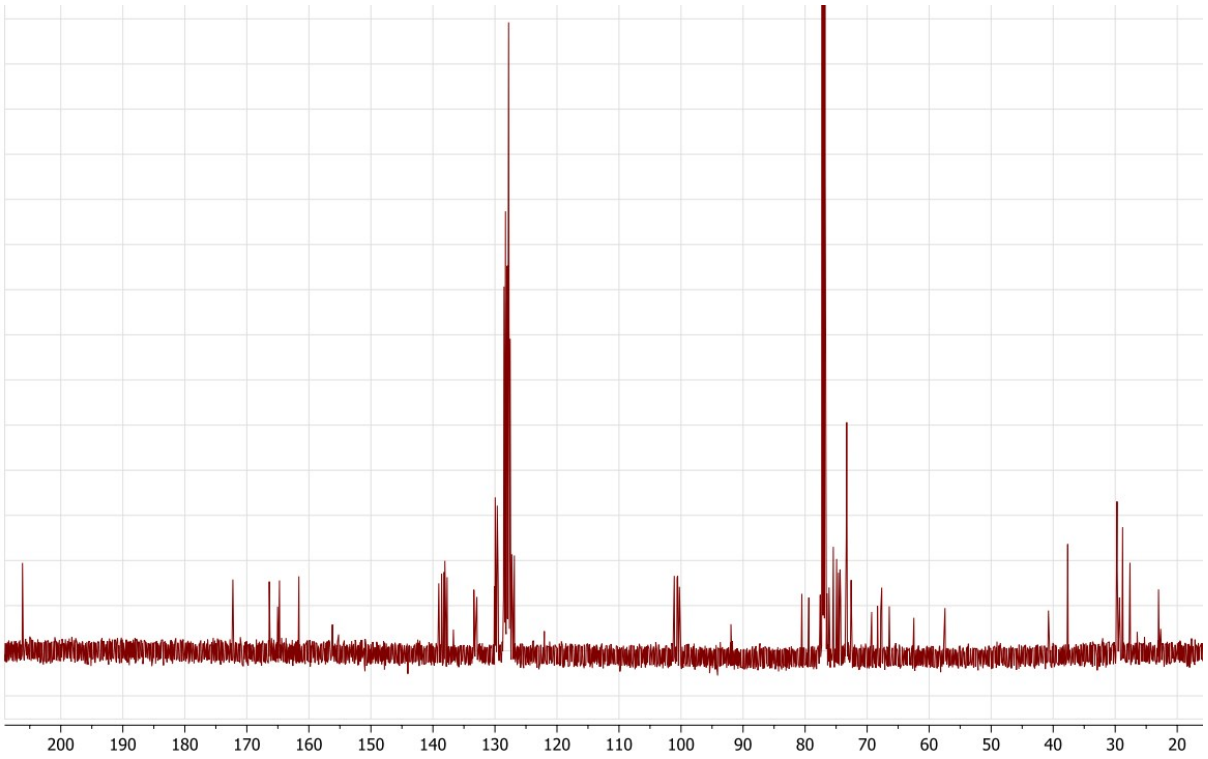
^{13}C NMR (151 MHz, Chloroform-d) δ 206.17, 172.25, 166.38, 165.04, 164.74, 161.61, 156.21, 139.05, 138.60, 138.25, 138.19, 138.16, 138.08, 137.71, 136.70, 133.40, 133.33, 132.92, 130.09, 129.96, 129.82, 129.62, 129.59, 129.47, 128.61, 128.55, 128.53, 128.52, 128.46, 128.40, 128.34, 128.30, 128.10, 128.05, 128.01, 127.92, 127.90, 127.88, 127.80, 127.78, 127.76, 127.72, 127.64, 127.60, 127.55, 127.53, 127.28, 127.25, 126.87, 126.85, 101.09, 100.64, 100.54, 100.26, 91.95, 80.52, 79.39, 77.56, 76.59, 76.44, 76.13, 75.45, 74.92, 74.88, 74.61, 74.37, 74.26, 73.49, 73.35, 73.34, 72.57, 72.55, 69.27, 68.32, 67.81, 67.64, 66.42, 62.46, 57.45, 40.77, 37.66, 29.71, 29.31, 28.81, 27.62, 23.01.

MALDI: calcd for $C_{114}H_{119}Cl_3N_2NaO_{28}$ [M+Na] 2091.6. Found 2091.5.

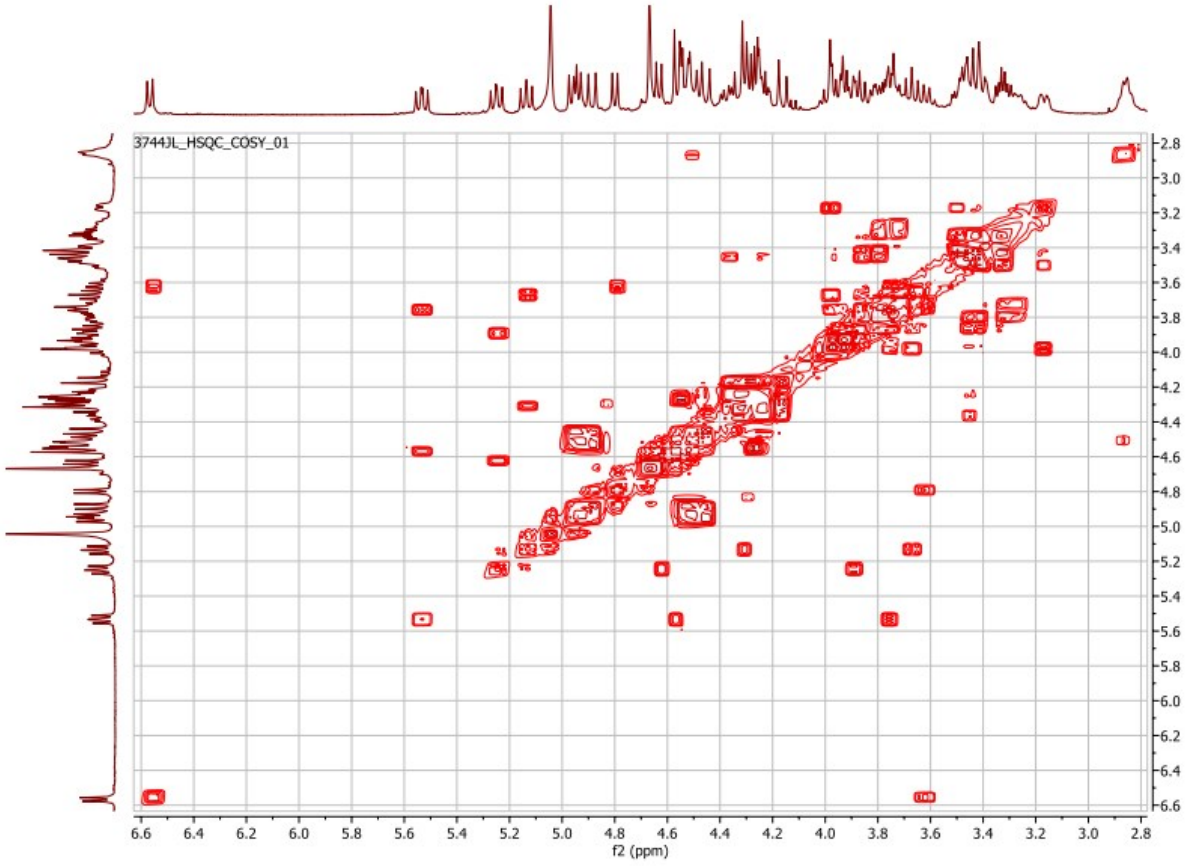
^{1}H NMR of compound **9**



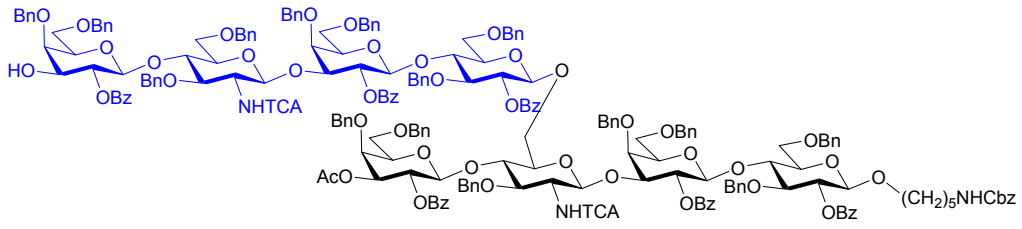
^{13}C NMR of compound **9**



COSY of compound **11**



Compound 12



12

MALDI: calcd for $C_{212}H_{215}Cl_6N_3KO_{52}$ $[M+K]^+$ 3883.2. Found 3886.4

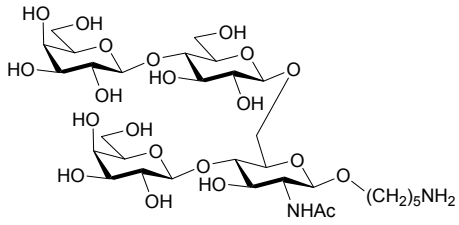
3. Oligosaccharide deprotection

General procedure for deprotection and purification

The purified protected oligosaccharide was dissolved in a 1:1 mixture of MeOH/DCM (anhydrous) and NaOMe (0.5 M in MeOH) was added. The resulting solution was stirred at room temperature until completion of reaction (monitored by MALDI). The reaction was neutralized with Amberlyte IR 120, filtered and concentrated under reduced pressure. The resulting material was dissolved in EtOAc/t-BuOH/H_2O 2:1:1 and one drop of acetic acid. The resulting mixture was purged with H_2 for ca. 15 min. The reaction vessel was equipped with two H_2 balloons and the mixture was stirred at room temperature for 24h. Then the mixture was filtered through a filter syringe equipped with a reverse cellulose syringe filter (pore size: 0.45 µm). The reaction vessel and the catalyst were washed with EtOAc (1 mL), t-BuOH (2x 1 mL) and H_2O (4x 1 mL). The combined filtrates were concentrated under reduced pressure and lyophilised.

The crude material was purified using a pre-packed 6 mL C-18 CHROMABOND column. To the crude material was added H_2O (10 mL, first fraction) and the mixture was passed through the cartridge. Then 10 mL fractions of H_2O, 10% MeOH in H_2O, 50% MeOH in H_2O and MeOH were successively passed through the cartridge and analysed by MALDI. The fractions containing the product were gathered and dried in the lyophilizer. When needed, the product was further purified by HPLC using a Synergi Hydro RP18 column (250 x 10 mm) using a flow rate of 4.0 mL/min and the following elution method: H_2O (0.1% formic acid) for 5 min (isocratic), then linear gradient to 10% ACN 30 min, then linear gradient to 100% ACN for 5 min. The product fractions were collected and lyophilised to obtain the pure product as formate salt. Analytical HPLC was done with a Synergi Hydro RP18 column (250 x 4.6 mm) using a flow rate of 1.0 mL/min and the following elution method: H_2O (0.1% formic acid) for 5 min (isocratic), then linear gradient to 10% ACN 30 min, then linear gradient to 100% ACN for 5 min. Alternatively, analytical HPLC was done using a Hypercarb column (150 x 4.6 mm) using a flow rate of 0.7 mL/min and the following elution method: H_2O (0.1% formic acid) for 5 min (isocratic), then linear gradient to 30% ACN 30 min, then linear gradient to 100% ACN for 5 min.

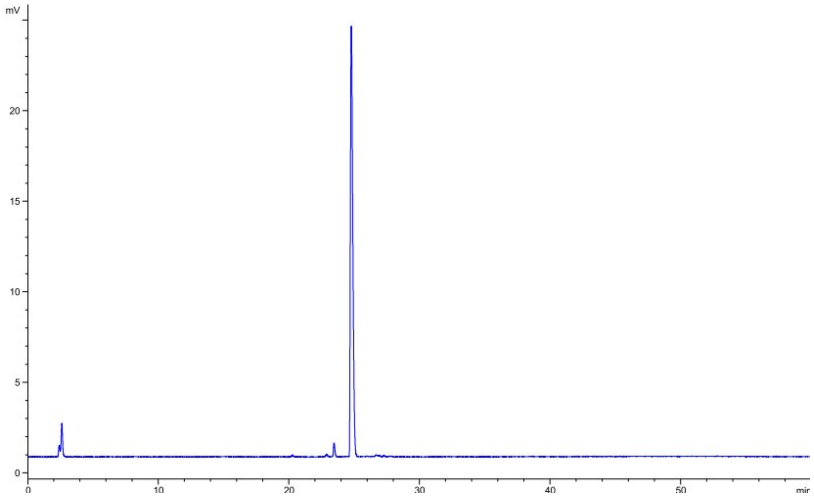
Compound 1



1

Protected tetrasaccharide **9** (14 mg) was subjected to methanolysis with NaOMe (final concentration 25 mM) for 24 h, according to the general procedure. The resulting crude material was subjected to hydrogenation according to the general procedure. Purification by C18 cartridge gave compound **1** (5.0 mg, 93%).

Analytical HPLC of pure product (Hypercarb column):

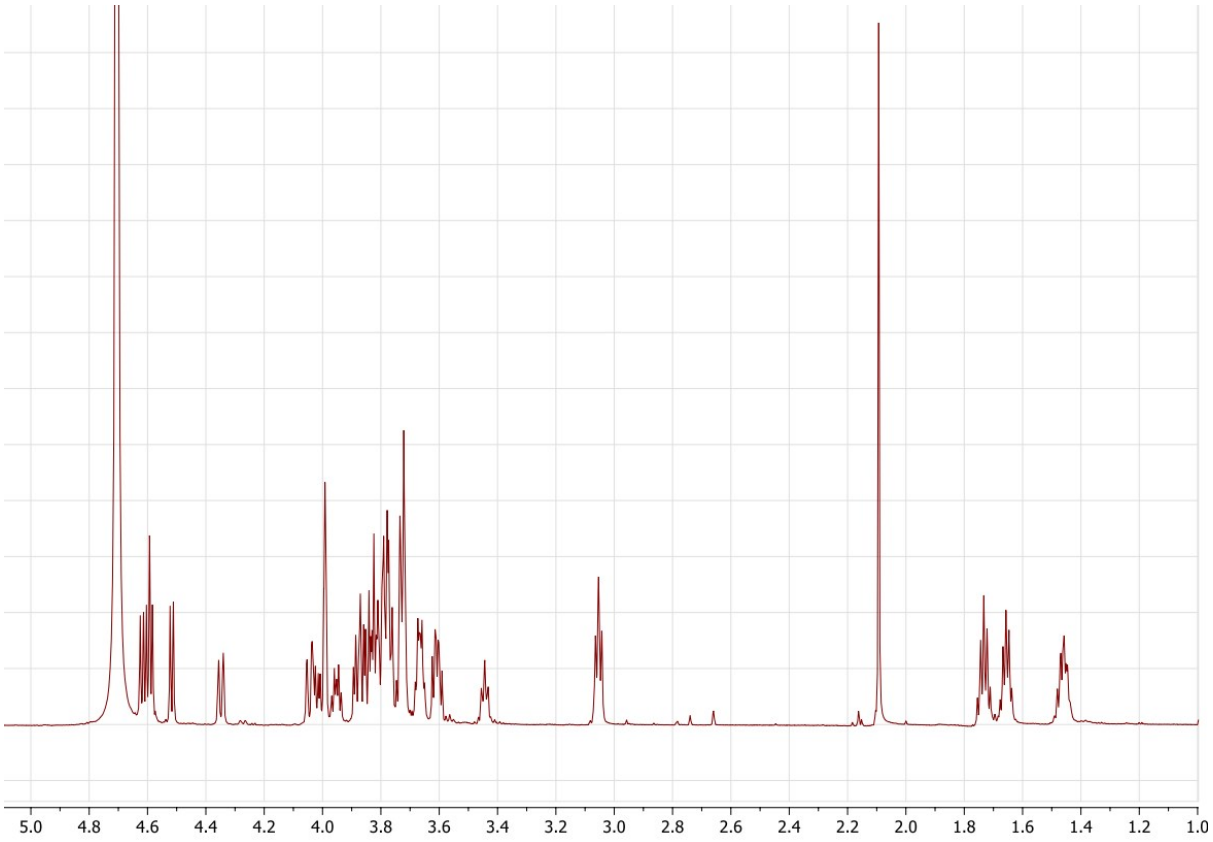


NMR data in agreement with the literature[2].

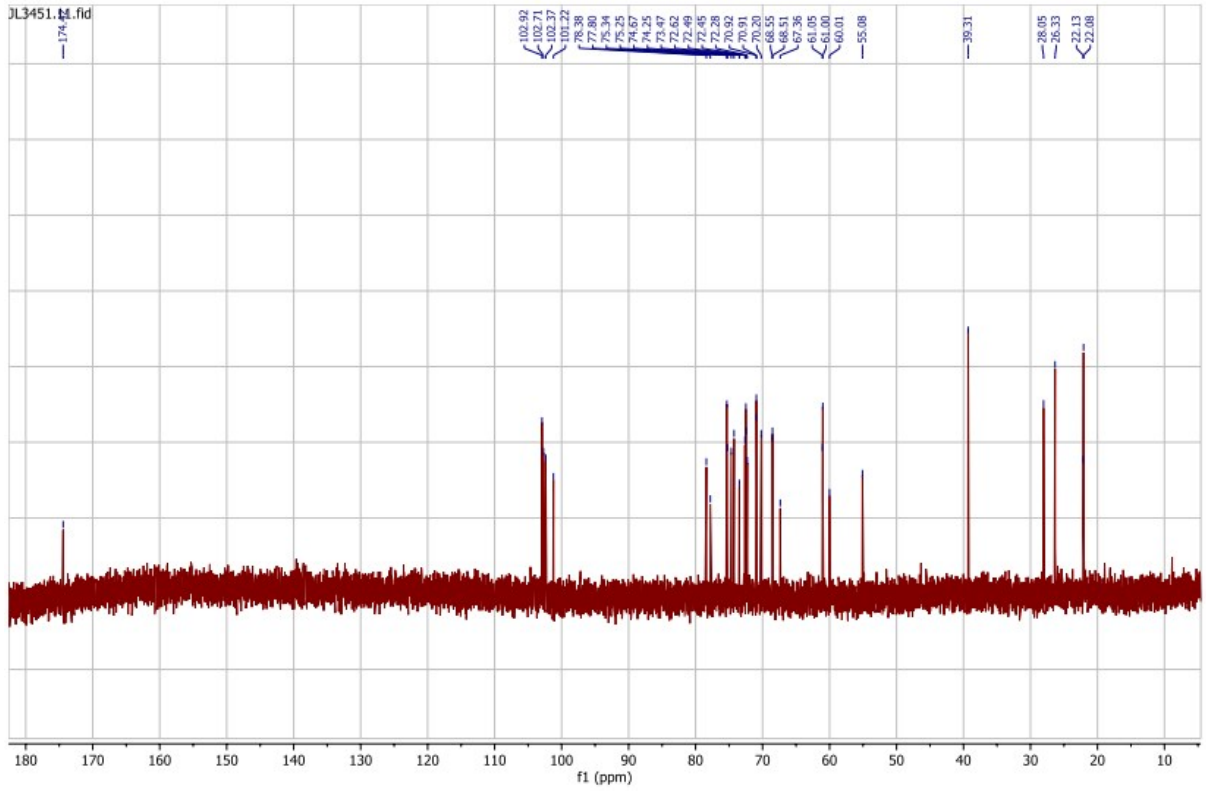
^{1}H NMR (700 MHz, Deuterium Oxide) δ 4.62 (d, J = 7.9 Hz, 1H, GlcNAc H-1), 4.62 – 4.57 (m, 2H, Glc H-1, Gal_b H-1), 4.52 (d, J = 7.8 Hz, 1H, Gal_a H-1), 4.35 (d, J = 11.2 Hz, 1H, GlcNAc H-6a), 4.05 (dd, J = 12.3, 2.2 Hz, 1H, Glc H-6a), 4.02 (dd, J = 11.4, 4.7 Hz, 1H, GlcNAc H-6b), 4.00 – 3.98 (m, 2H, Gal_a H-4, Gal_b H-4), 3.95 (dt, J = 10.6, 6.2 Hz, 1H, pentanylene chain, OC*H*H), 3.90 – 3.71 (m, 15H), 3.67 (dt, J = 10.2, 6.2 Hz, 1H, pentanylene chain, OCH*H*), 3.61 (dt, J = 9.4, 6.5 Hz, 2H, Gal_a H-2, Gal_b H-2), 3.44 (t, J = 8.4 Hz, 1H, Glc H-2), 3.05 (t, J = 7.6 Hz, 2H, pentanylene chain, CH_2NH$_2$), 2.09 (s, 3H, NHCOCH_3), 1.73 (p, J = 7.7 Hz, 2H, pentanylene chain), 1.66 (p, J = 6.7 Hz, 2H, pentanylene chain), 1.51 – 1.42 (m, 2H, pentanylene chain, CH_2NH$_2$). Gal_a refers to Gal-β-1,4-Glc. Gal_b refers to Gal-β-1,4-GlcNAc.

HRMS (ESI-QTOF): calcd for $C_{31}H_{57}N_2O_{21}$ $[M+H]^+$ 793.3448. Found: 793.3448.

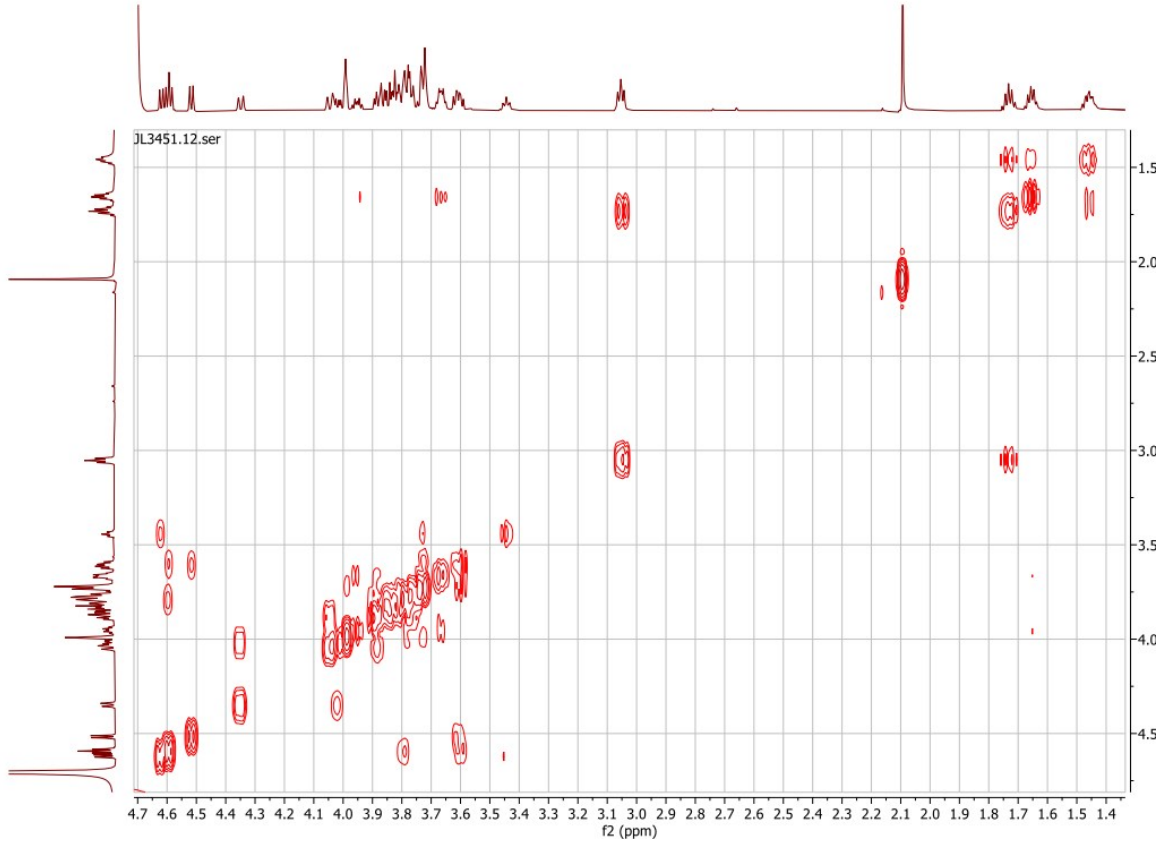
^{1}H NMR of compound **1**:



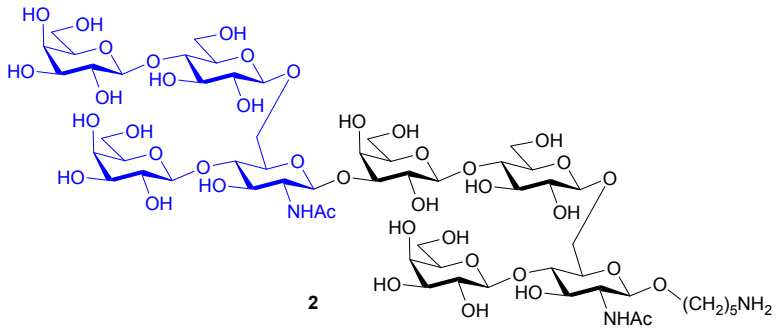
^{13}C NMR of compound **9**



COSY of compound **9**



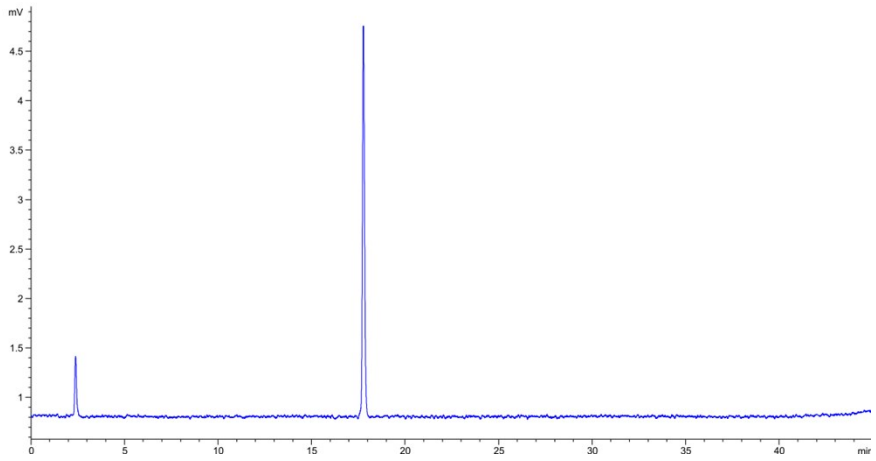
Compound 2



2

Protected octasaccharide **10** (8 mg) was subjected to methanolysis with NaOMe (final concentration 25 mM) for 24 h, according to the general procedure. The resulting crude material was subjected to hydrogenation according to the general procedure. The mixture was neutralized with Et_3N (1.5 µL). After RP-HPLC purification, compound **2** was isolated as the formate salt (0.7 mg, 22%), containing traces of triethylammonium formate.

Analytical HPLC of purified product (Synergi column):

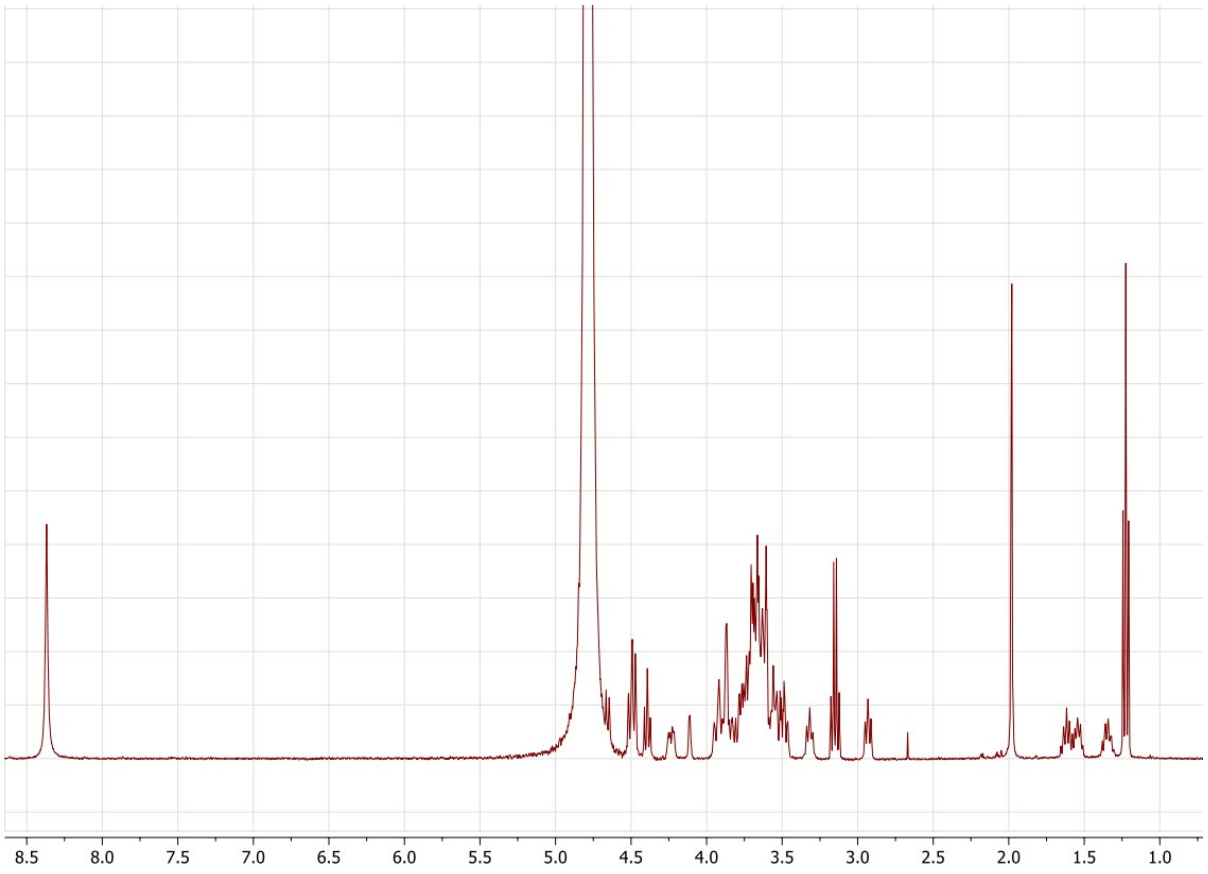


NMR data in agreement with the literature[3].

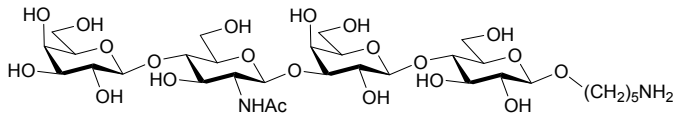
^{1}H NMR (400 MHz, Deuterium Oxide) δ 4.66 (d, J = 8.3 Hz, 1H, H-1), 4.54 – 4.45 (m, 5H, 5x H-1), 4.39 (m, 2H, 2x H-1), 4.23 (dd, J = 10.2, 4.6 Hz, 2H, 2x GlcNAc H-6a), 4.11 (d, J = 3.3 Hz, 1H), 3.96 – 3.46 (m, 45H), 3.32 (t, J = 8.1 Hz, 2H, 2x Glc H-2), 2.93 (t, J = 7.7 Hz, 2H, pentanylene chain, C*H*$_2$NH$_2$), 1.98 (s, 3H, NHCOC*H*$_3$), 1.98 (s, 3H, NHCOC*H*$_3$), 1.66 – 1.50 (m, 4H, pentanylene chain), 1.40 – 1.30 (m, 2H, pentanylene chain).

HRMS (ESI-QTOF): calcd for $C_{57}H_{100}N_3O_{41}$ $[M+H]^+$ 1482.5827. Found: 1482.5850.

^{1}H NMR of compound **2**:



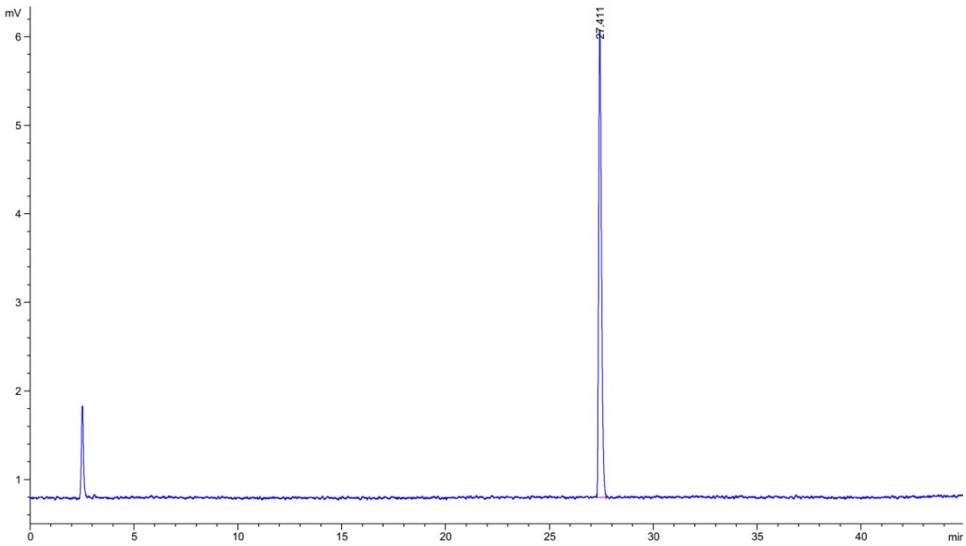
Compound 3



3

Protected tetrasaccharide **12** (6 mg) was subjected to methanolysis with NaOMe (final concentration 50 mM). After 24h, another portion of NaOMe was added (final concentration 100 mM) and after another 24h the conversion was complete, and the reaction was worked up according to the general procedure. The resulting crude material was subjected to hydrogenation according to the general procedure. After RP-HPLC purification, compound **3** was isolated as the formate salt (1.6 mg, 71%).

Analytical HPLC of pure product:

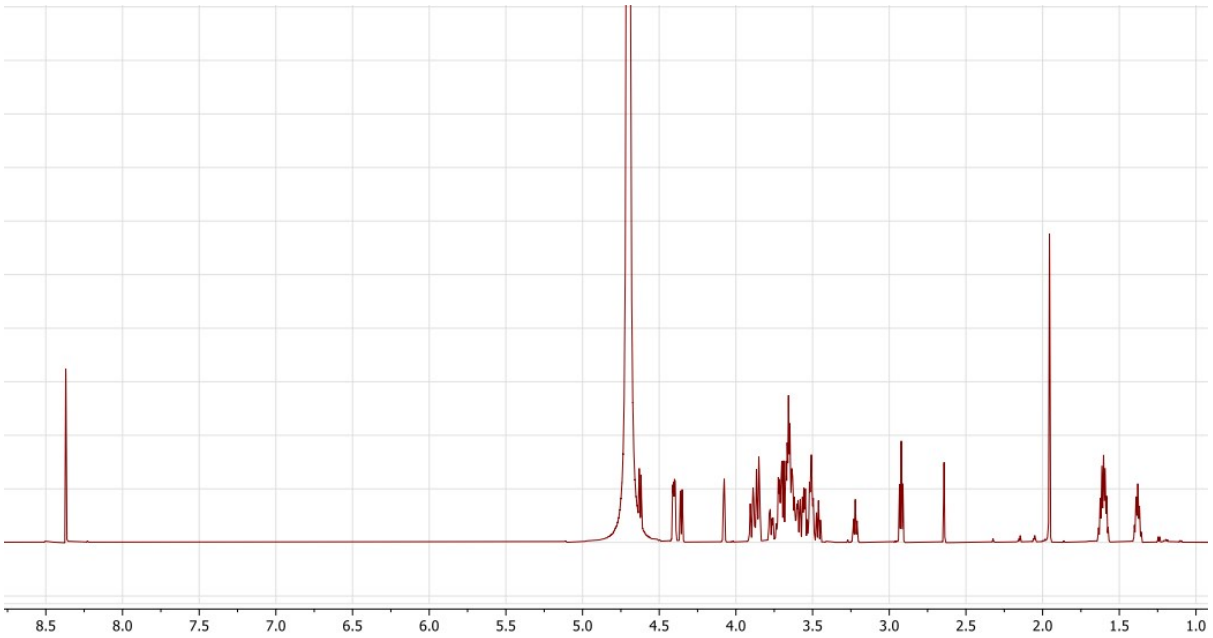


NMR in agreement with the literature[4].

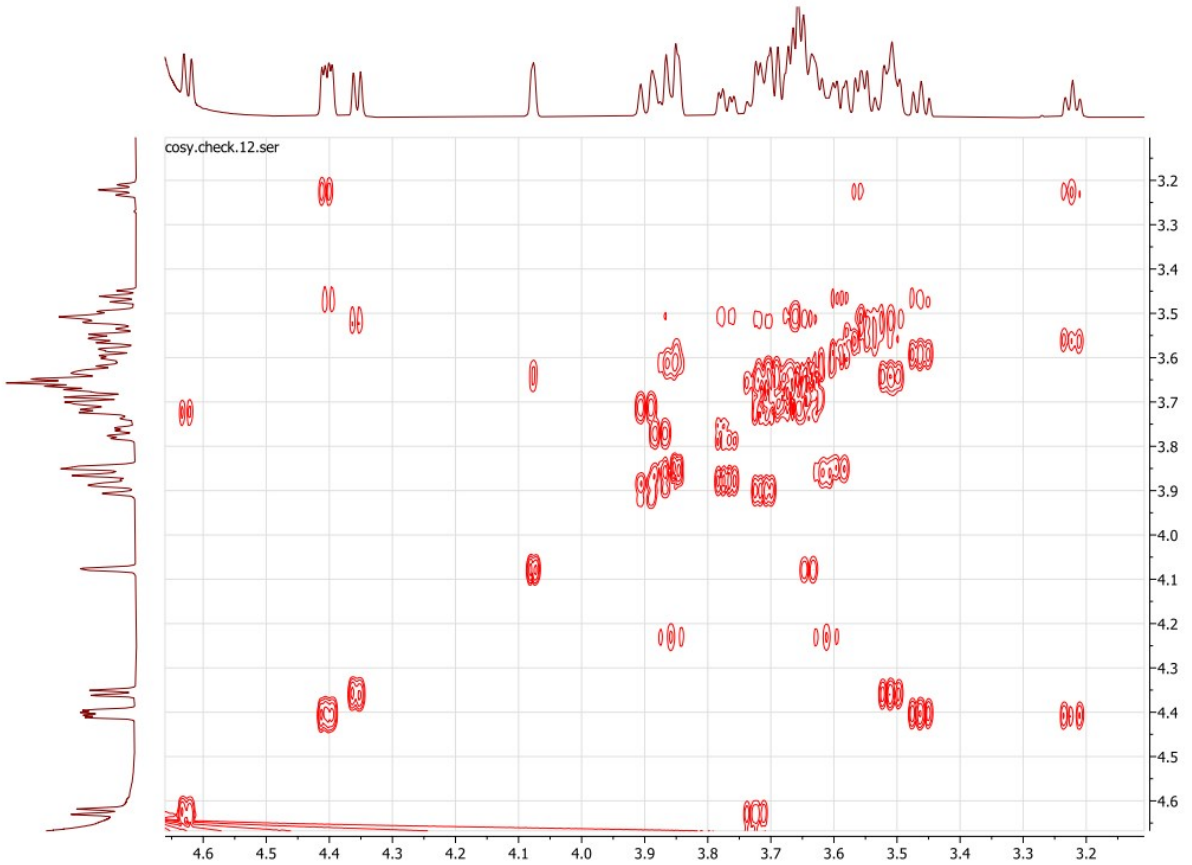
^{1}H NMR (600 MHz, Deuterium Oxide) δ 4.73 (d, J = 8.3 Hz, 1H, H-1), 4.51 (d, J = 8.0 Hz, 1H, H-1), 4.50 (d, J = 7.8 Hz, 1H, H-1), 4.46 (d, J = 7.9 Hz, 1H, H-1), 4.18 (d, J = 3.3 Hz, 1H), 4.02 – 3.94 (m, 4H), 3.87 (dd, J = 12.3, 4.7 Hz, 1H), 3.84 – 3.59 (m, 18H), 3.56 (dd, J = 9.9, 7.8 Hz, 1H), 3.32 (t, J = 8.4 Hz, 1H), 3.03 (t, J = 7.6 Hz, 2H, pentanylene chain, C*H*$_2$NH$_2$), 2.06 (s, 3H, NHCOC*H*$_3$), 1.71 (dp, J = 13.8, 7.2, 6.7 Hz, 5H, pentanylene chain), 1.48 (p, J = 7.7 Hz, 2H, pentanylene chain).

HRMS (ESI-QTOF): calcd for $C_{31}H_{57}N_2O_{21}$ $[M+H]^+$ 793.3448. Found: 793.3449.

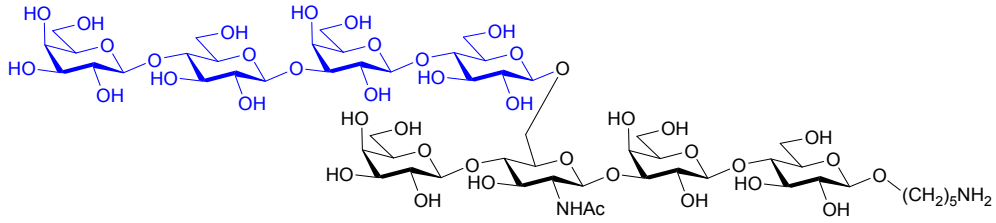
^{1}H NMR of Compound **3**



COSY of Compound **3**



Compound 4



4

Protected octasaccharide **12** (6 mg) was subjected to methanolysis with NaOMe (final concentration 50 mM). After 24h, another portion of NaOMe was added (final concentration 100 mM) and after another 24h the conversion was complete, and the reaction was worked up according to the general procedure. The resulting crude material was subjected to hydrogenation according to the general procedure. Purification by C18 cartridge gave compound **4** (0.5 mg, 22%).

MALDI: calcd for $C_{57}H_{99}N_3O_{41}Na$ $[M+Na]^+$ 1504.6. Found: 1504.9.

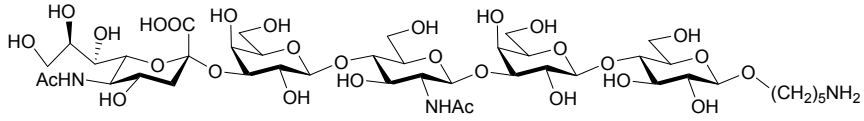
4. Enzymatic sialylation

Tetrasaccharide **3** (1.0 mg, 1.3 µmol) was dissolved in 781 µL of H_2O and combined with:

- 12.6 µL of CIAP (calf-intestine alkaline phosphatase purchased from Promega, 20 u/µL)
- 25 µL of CIAP buffer (buffer supplied with the CIAP, when diluted 1:10, it has a composition of 50mM Tris-HCl (pH 9.3 at 25°C), 1mM $MgCl_2$, 0.1mM $ZnCl_2$ and 1mM spermidine)
- 12.6 µL of $MgCl_2$ 10 mM
- 75.6 µL of CMP-NANA 3 mM (3 equiv)
- 126 µL of α(2,3)-sialyltransferase from *Pasteurella multocida* (PmST1)[5]

The resulting mixture was incubated at 37 °C for 18 h. Then the mixture was centrifuged, and the supernatant was filtered through a polyethersulfone Vivaspin 500 membrane (MWCO 10 kDa). The resulting material was analysed and purified using preparative reverse-phase HPLC with a Hypercarb column (150 x 4.6 mm), flow 0.7 mL/min, starting with H_2O (0.1% formic acid) isocratic for 5 min, then linear gradient of 0 to 30% ACN for 15 min, then linear gradient of 30 to 90% ACN for 15 min. The purified fractions were lyophilized to give the pure compound **15** (0.4 mg, 30%).

Compound 15



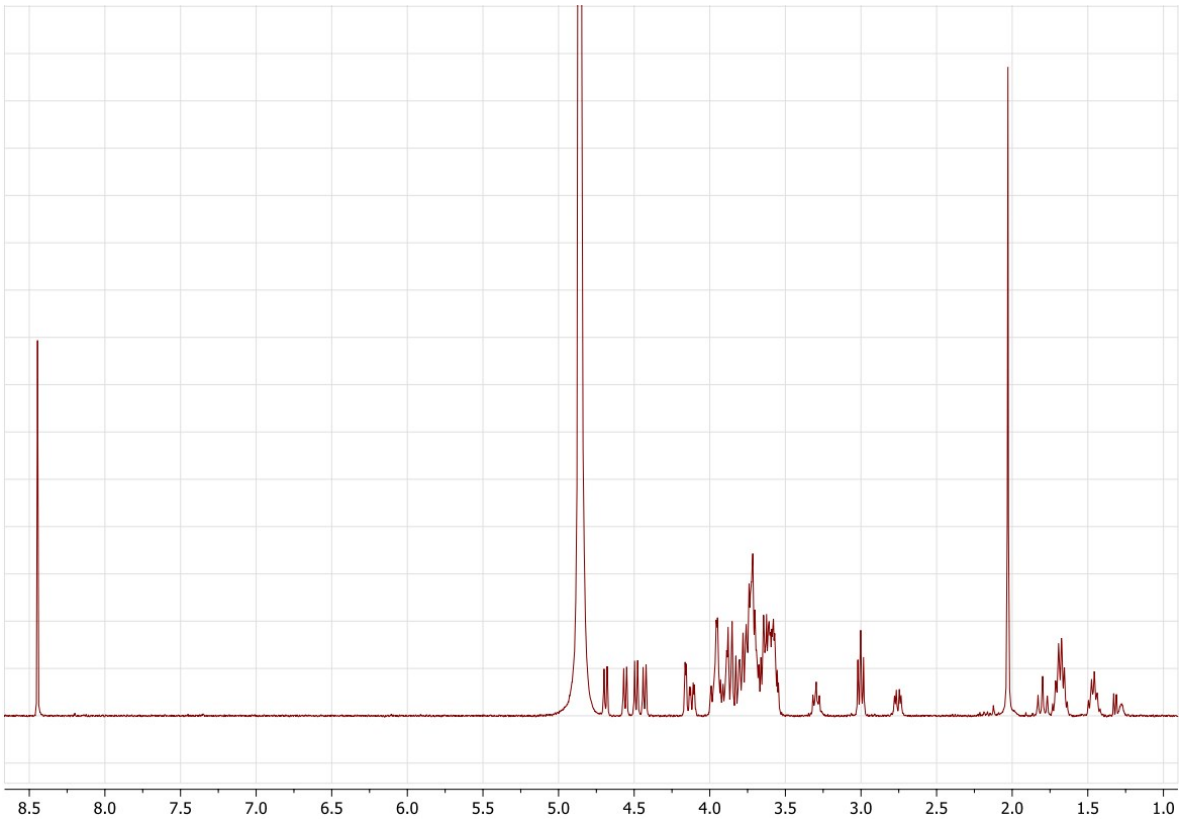
15

NMR in agreement with the literature[6].

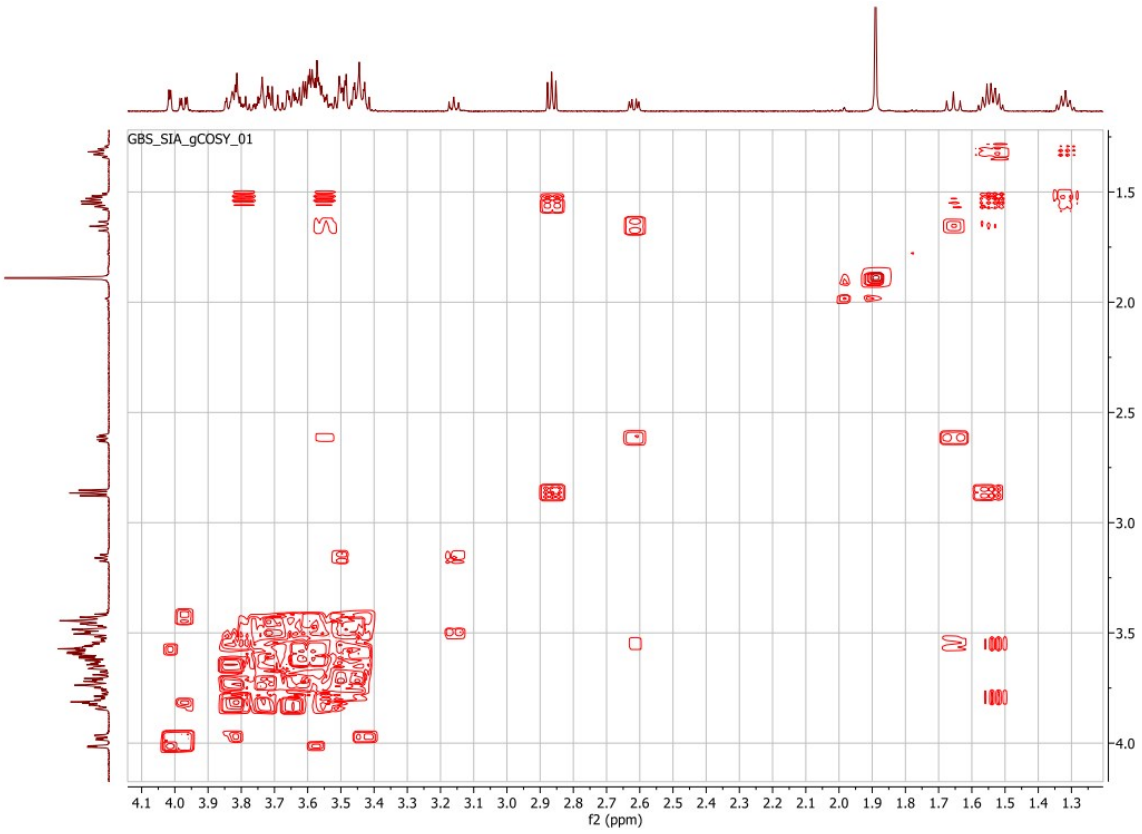
^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.45 (s, 1H, formate), 4.69 (d, J = 8.2 Hz, 1H, GlcNAc H-1), 4.56 (d, J = 7.9 Hz, 1H, Gal_s H-1), 4.49 (d, J = 8.0 Hz, 1H, Glc H-1), 4.43 (d, J = 7.9 Hz, 1H, Gal H-1), 4.16 (d, J = 3.3 Hz, 1H, Gal H-4), 4.12 (dd, J = 9.9, 3.1 Hz, 1H, Gal_s H-3), 4.00 – 3.54 (m, 30H), 3.30 (t, J = 8.4 Hz, 1H, Glc H-2), 3.00 (t, J = 7.5 Hz, 2H, pentanylene chain, C*H*$_2$NH$_2$), 2.76 (dd, J = 12.5, 4.6 Hz, 1H, Neu5Ac H-3e), 2.03 (s, 6H, 2x Ac), 1.80 (t, J = 12.2 Hz, 1H, Neu5Ac H-3a), 1.68 (m, 4H, pentanylene chain), 1.46 (p, J = 7.9 Hz, 2H, pentanylene chain). (Gal_s refers to the galactose residue linked to Neu5Ac.)

HRMS (ESI-QTOF): calcd for $C_{31}H_{57}N_2O_{21}$ $[M+H]^+$ 1084.4402. Found: 1084.4414.

^{1}H NMR of compound **15**



COSY of compound **15**



5. Competitive ELISA

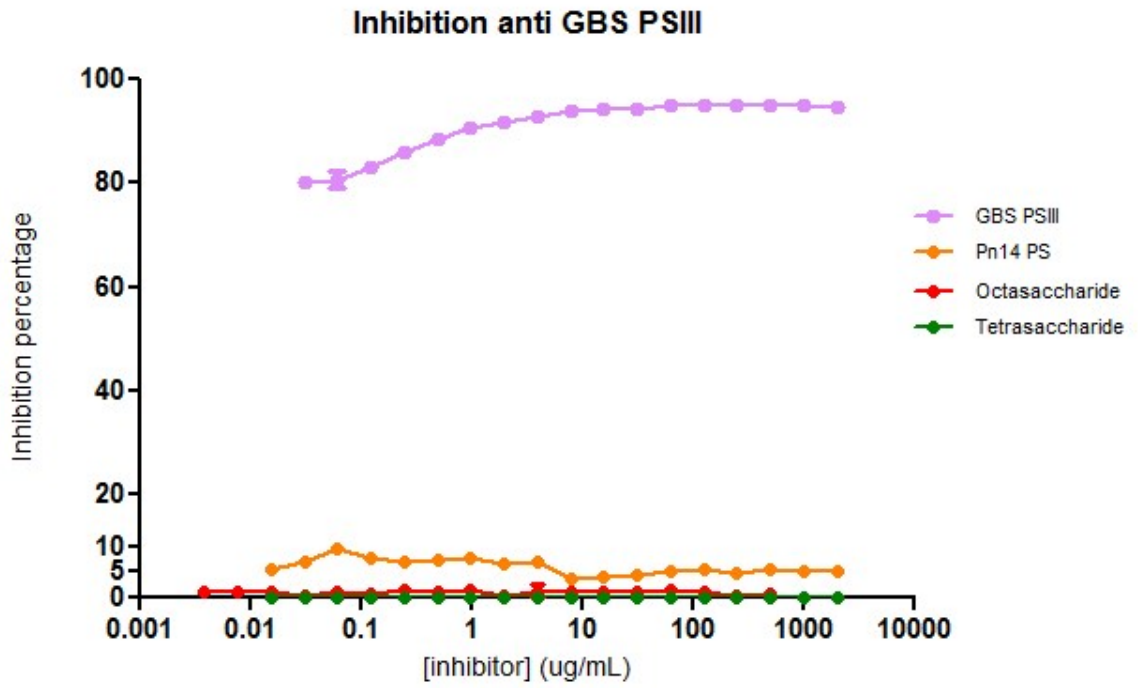


Figura S1. Inhibition percentage of Pn14 tetrasaccharide **1** and octasaccharide **2** fragments and Pn14 capsular polysaccharide against an anti GBS PSIII rabbit monoclonal antibody. GBS III capsular polysaccharide was the positive control. Microtiter plates (96 wells, NUNC, Maxisorp) were coated with 100 mL of 1 mg/mL of GBS PSIII-HSA conjugate and incubated overnight at 2-8°C. The oligosaccharides and the polysaccharides were used as inhibitors according to the protocol described in the experimental part. An anti-rabbit IgG-alkaline phosphatase (Sigma) diluted 1:2000 was used as secondary antibody.

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