Supporting Information

Pushing the Limits of Automated Glycan Assembly: Synthesis of a 50mer Polymannoside

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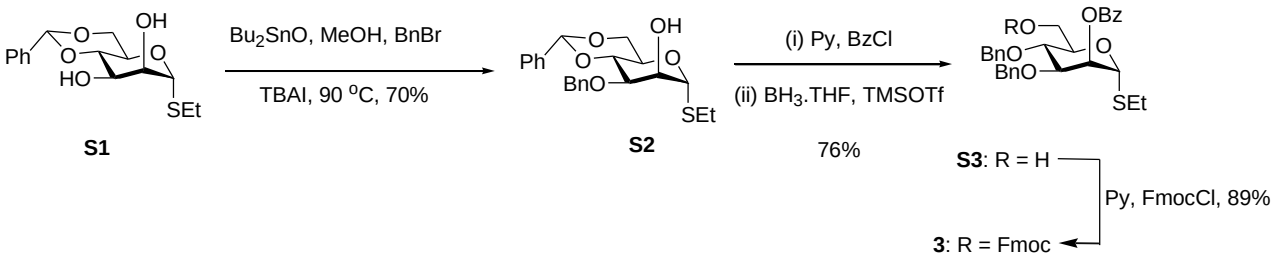
1. General Materials and Methods

All chemicals used were reagent grade and used as supplied except where noted. Anhydrous solvents used were taken from a dry solvent system (jcmeyer-solvent systems). 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 cerium sulfate-ammonium molybdate (CAM) / *p*-anisaldehyde (PAA) solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (0.04-0.063 mm). Analysis and purification by normal phase HPLC was performed by using an Agilent 1200 series equipped with Luna silica and YMC diol columns. Analysis and purification by reverse phase HPLC was performed by using an Agilent 1200 series equipped with Hypercarb columns. ^{1}H and ^{13}C 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 $CDCl_3$ (δ 7.24) or D_2O (δ 4.79). NMR chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. High-resolution mass spectrometric (HRMS) analyses were performed by staff at the MS core facility at Freie Universität Berlin. MALDI-TOF MS measurements were performed using an Autoflex Speed mass spectrometer (Bruker). IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured using a Perkin-Elmer 241 and Unipol L1000 polarimeter.

2. Synthesis of Building Block **3**

Scheme S1



To a solution of thiomannoside **S1**[1] (30 g, 96 mmol**)** in methanol (750 mL), was added dibutyltin oxide (27 g, 108 mmol) and the solution was refluxed at 90 °C for 5 h. The solvent was removed *in vacuo* and to the dibutyltin complex was added toluene, which was removed by evaporation. The crude dibutyltin complex was redissolved in toluene (600 mL) and benzyl bromide (12.8 mL, 108 mmol) and tertiary butyl ammonium iodide (7.2 g, 19.6mmol) were added. The solution was refluxed at 70 °C for 12 h. TLC analysis showed the presence of starting material. Benzyl bromide (8.3 mL) was added to the reaction mixture and stirred for another 31 h at 90 °C. The reaction mixture was quenched with methanol, concentrated *in vacuo* and purified (hexanes:ethyl acetate = 4:1) to afford **S2** as a solid. Yield (27 g, 70%); $[\alpha]_D^{20}$ +134.7 (c 0.87, $CHCl_3$); IR (neat) ν_{max} = 3465, 2929, 1455, 1375, 1092 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.44 (m, 2H), 7.42 – 6.94 (m, 8H), 5.61 (s, 1H), 5.36 (s, 1H), 4.84 (d, *J* = 11.8 Hz, 1H), 4.69 (d, *J* = 11.8 Hz, 1H), 4.28 – 4.19 (m, 2H), 4.17 – 4.08 (m, 2H), 3.92 – 3.84 (m, 2H), 2.89 (s, 1H), 2.76 – 2.42 (m, 2H), 1.28 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.68, 137.40, 128.86, 128.40, 128.13, 127.91, 127.79, 125.98, 101.49, 84.07, 79.03, 75.80, 73.02, 71.30, 68.56, 63.76, 24.84, 14.78; ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{22}H_{26}NaO_5S$: 425.1399; found: 425.1407.

To a cooled solution of **S2** (24 g, 59.6 mmol) in pyridine (10 mL, 120 mmol) and CH_2Cl_2 (190 mL), benzoyl chloride (8.3 mL, 72 mmol) in CH_2Cl_2 (10 mL) was added slowly, over 30 min. The mixture was stirred at room temperature for 24 h, diluted with CH_2Cl_2 and washed with aq. HCl, a saturated aq. $NaHCO_3$ solution, and water. The organic phase was dried over Na_2SO_4, filtered and the filtrate was concentrated. To a solution of crude residue in anhydrous THF (250 mL), borane (1M solution in THF, 120 mL) and TMSOTf (10.8 mL, 60 mmol) were added at 0°C and stirred for 5 h at the same temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with a saturated aq. $NaHCO_3$ solution and water. The organic phase was dried over Na_2SO_4, filtered and

the filtrate was concentrated and purified (hexanes:ethyl acetate 4:1) to afford **S3** as a solid. Yield (23 g, 76%); $[\alpha]_D^{20}$ +78.3 (c 1, $CHCl_3$); IR (neat) ν_{max} = 3492, 2929, 1721, 1453, 1267, 1094 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 – 8.06 (m, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.47 (t, *J* = 7.4 Hz, 2H), 7.38 – 7.22 (m, 9H), 5.68 (s, 1H), 5.38 (d, *J* = 1.2 Hz, 1H), 4.93 (d, *J* = 10.9 Hz, 1H), 4.76 (d, *J* = 11.4 Hz, 1H), 4.67 (d, *J* = 10.9 Hz, 1H), 4.57 (d, *J* = 11.4 Hz, 1H), 4.11-4.02 (m, 3H), 3.86-3.85 (m, 2H), 2.73 – 2.48 (m, 2H), 1.92 (s, 1H), 1.28 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.56, 138.10, 137.59, 133.26, 129.82, 129.74, 128.45, 128.35, 128.30, 128.07, 128.00, 127.76, 127.68, 82.48, 78.46, 75.18, 74.04, 72.23, 71.52, 70.89, 61.98, 25.56, 14.84; ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{29}H_{32}NaO_6S$: 531.1817; found: 531.1818.

To a cooled solution of **S3** (18 g, 35 mmol) in pyridine (9 mL) and CH_2Cl_2 (250mL), FmocCl (14.5 g, 56 mmol) was added slowly. The mixture was stirred at room temperature for 36 h, diluted with CH_2Cl_2 and washed with aq. HCl, a saturated aq. $NaHCO_3$ solution, and water. The organic phase was dried over Na_2SO_4, filtered, and the filtrate was concentrated and purified (hexanes:ethyl acetate 9:1) to afford **3** as a solid. Yield (23 g, 89%); $[\alpha]_D^{20}$ +42.3 (c 1, $CHCl_3$); IR (neat) ν_{max} = 2962, 1748, 1722, 1451, 1254, 1094 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.26 – 8.19 (m, 2H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.74-7.68 (m, 2H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.53 – 7.30 (m, 16H), 5.82 (d, *J* = 1.8 Hz, 1H), 5.52 (s, 1H), 5.02 (d, *J* = 10.9 Hz, 1H), 4.87 (d, *J* = 11.3 Hz, 1H), 4.72 (d, *J* = 10.9 Hz, 1H), 4.65 (d, *J* = 11.3 Hz, 1H).4.58-4.57 (m, 2H), 4.51 – 4.38 (m, 3H), 4.33 (t, *J* = 7.5 Hz, 1H), 4.19 – 4.04 (m, 2H), 2.79-2.69 (m, 2H), 1.37 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.45, 155.02, 143.31, 143.14, 141.14, 137.81, 137.40, 133.19, 129.82, 129.72, 128.38, 128.31, 128.29, 128.08, 127.80, 127.74, 127.72, 127.06, 125.10, 125.05, 119.96, 82.45, 78.53, 75.10, 73.86, 71.48, 70.49, 70.06, 69.88, 66.68, 46.63, 25.59, 14.88; ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{44}H_{42}NaO_8S$: 753.2498; found: 753.2485.

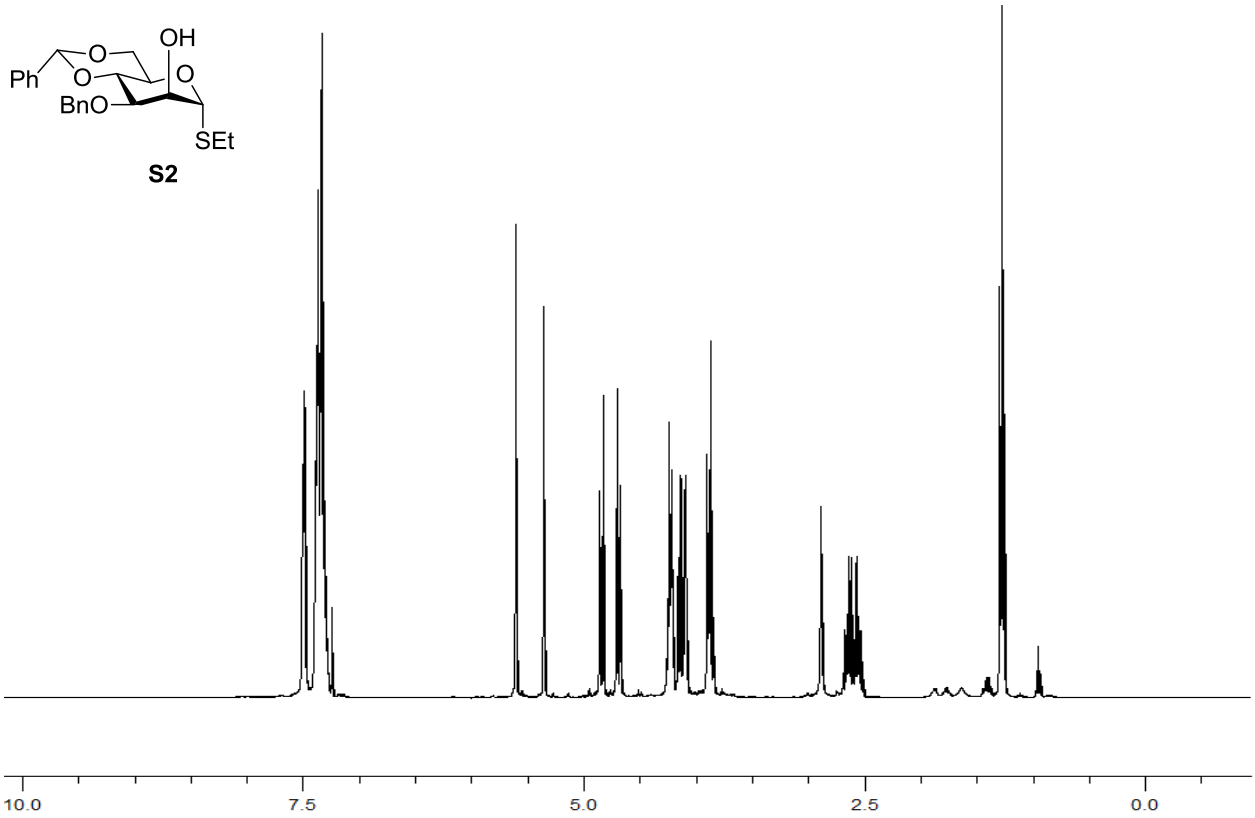


Figure S1. ^{1}H NMR of **S2**.

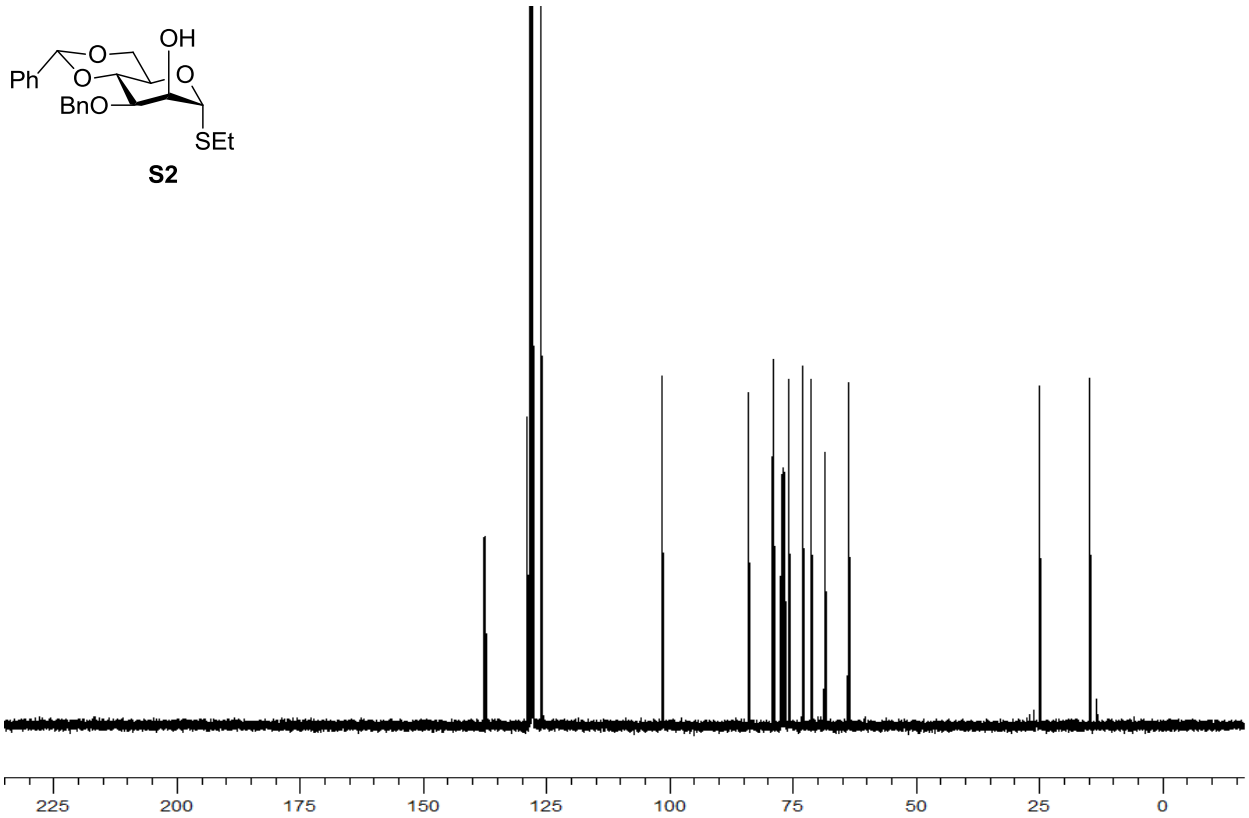


Figure S2. ^{13}C NMR of **S2**.

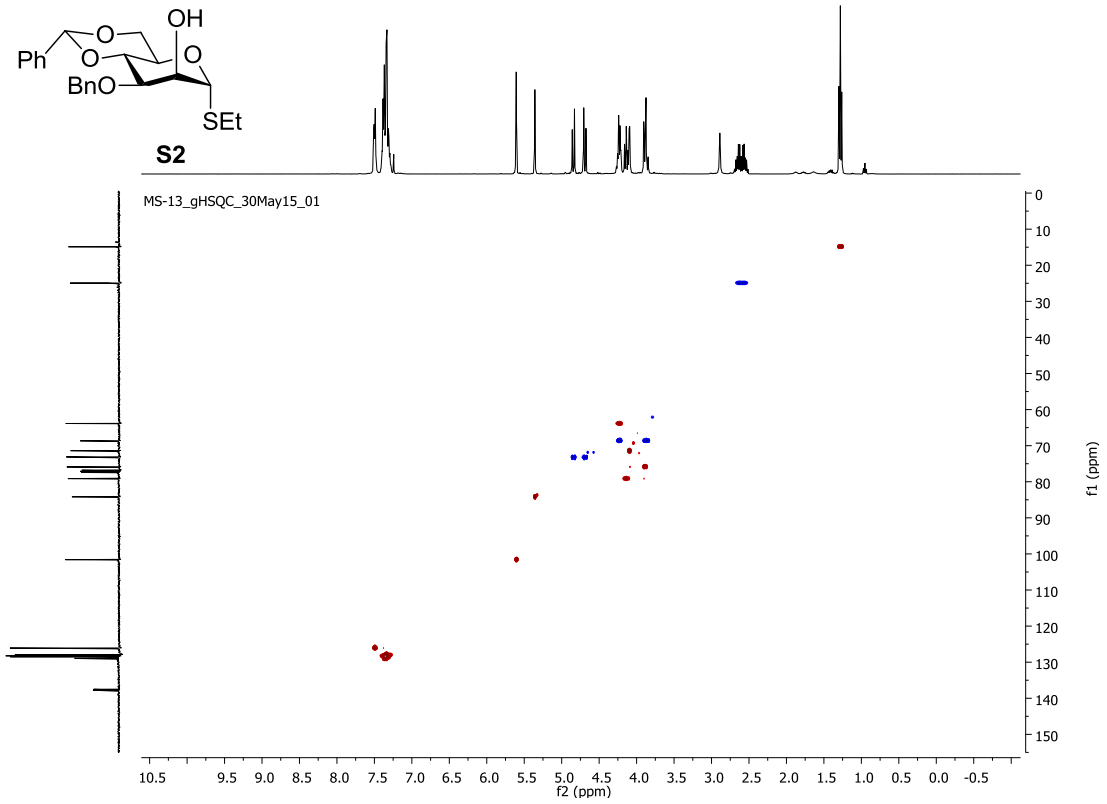


Figure S3. HSQC spectrum of **S2**.

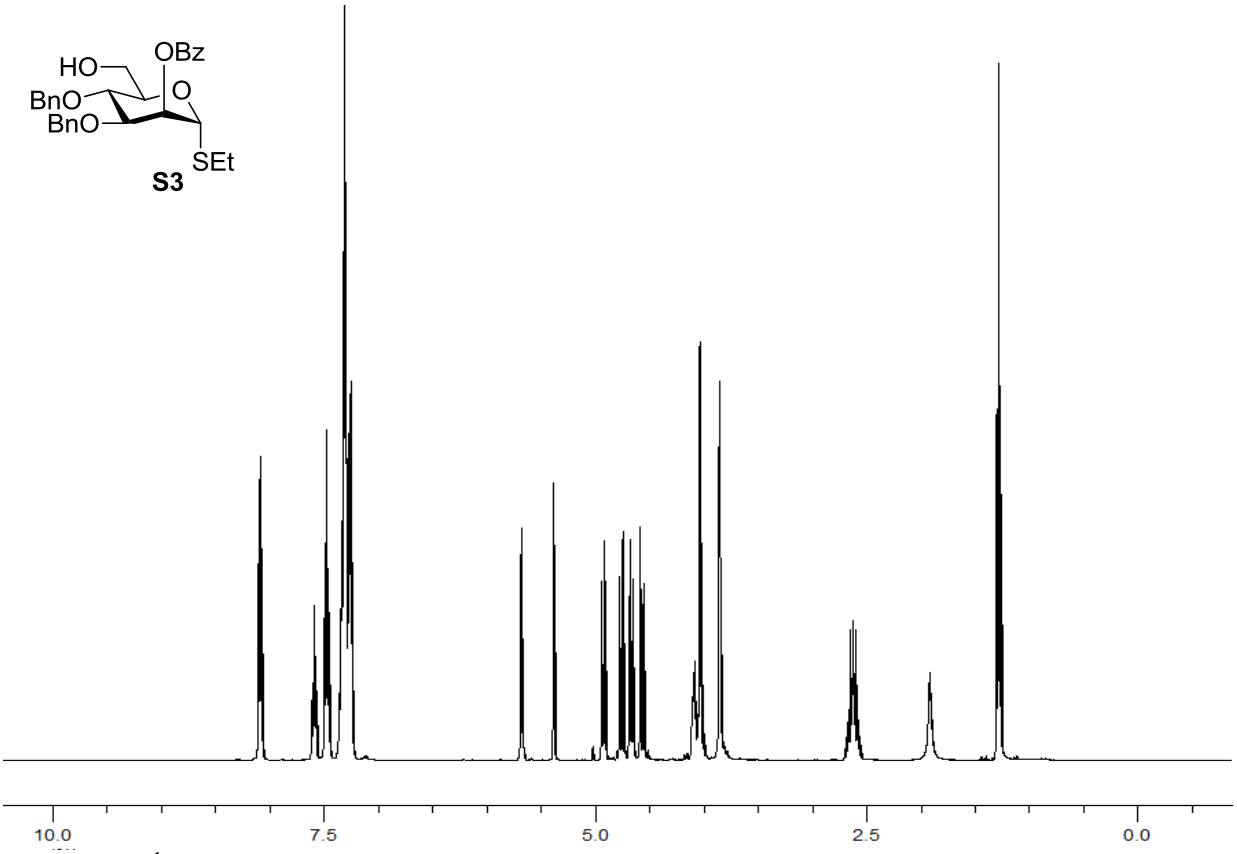


Figure S4. ^{1}H NMR of **S3**.

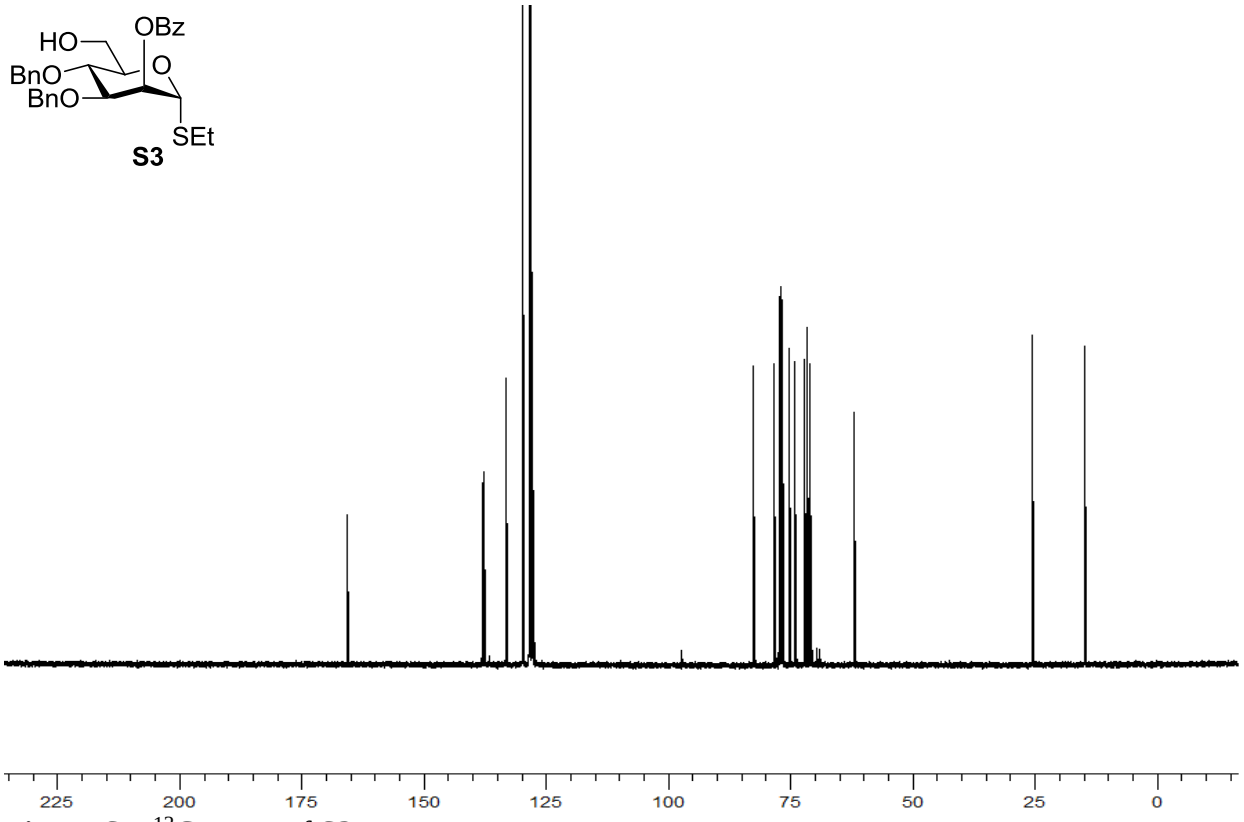


Figure S5. ^{13}C NMR of **S3**.

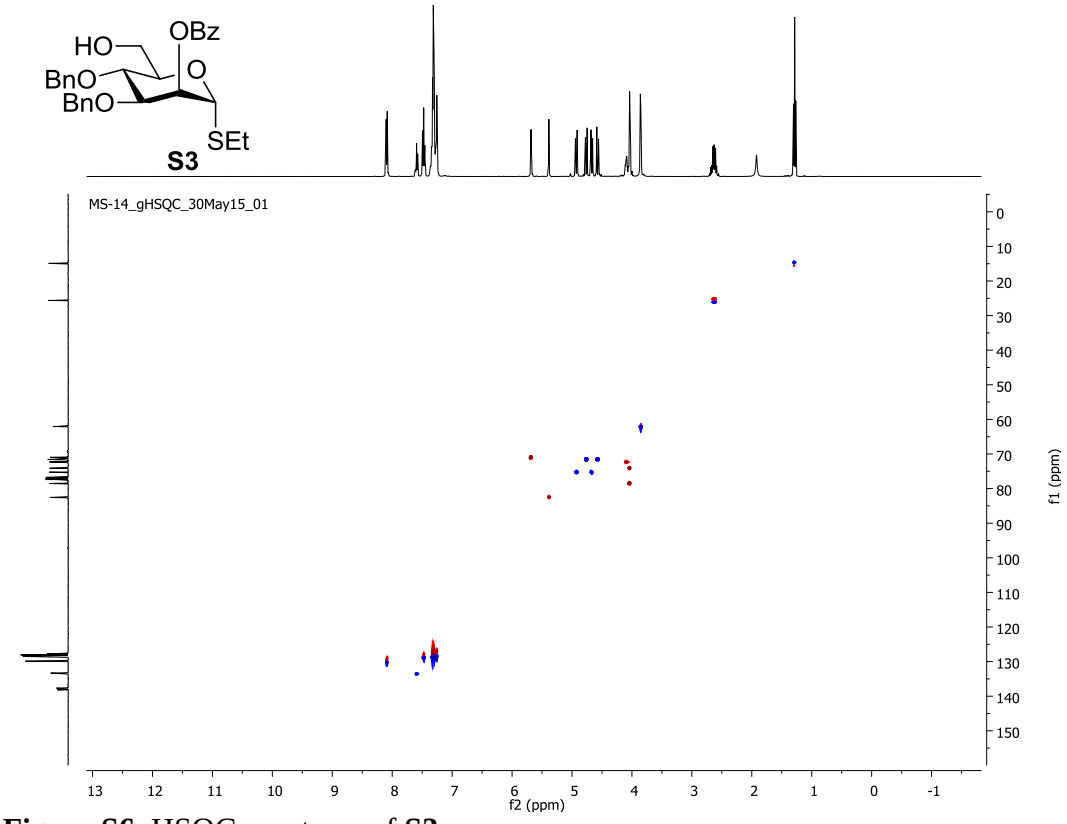


Figure S6. HSQC spectrum of **S3**.

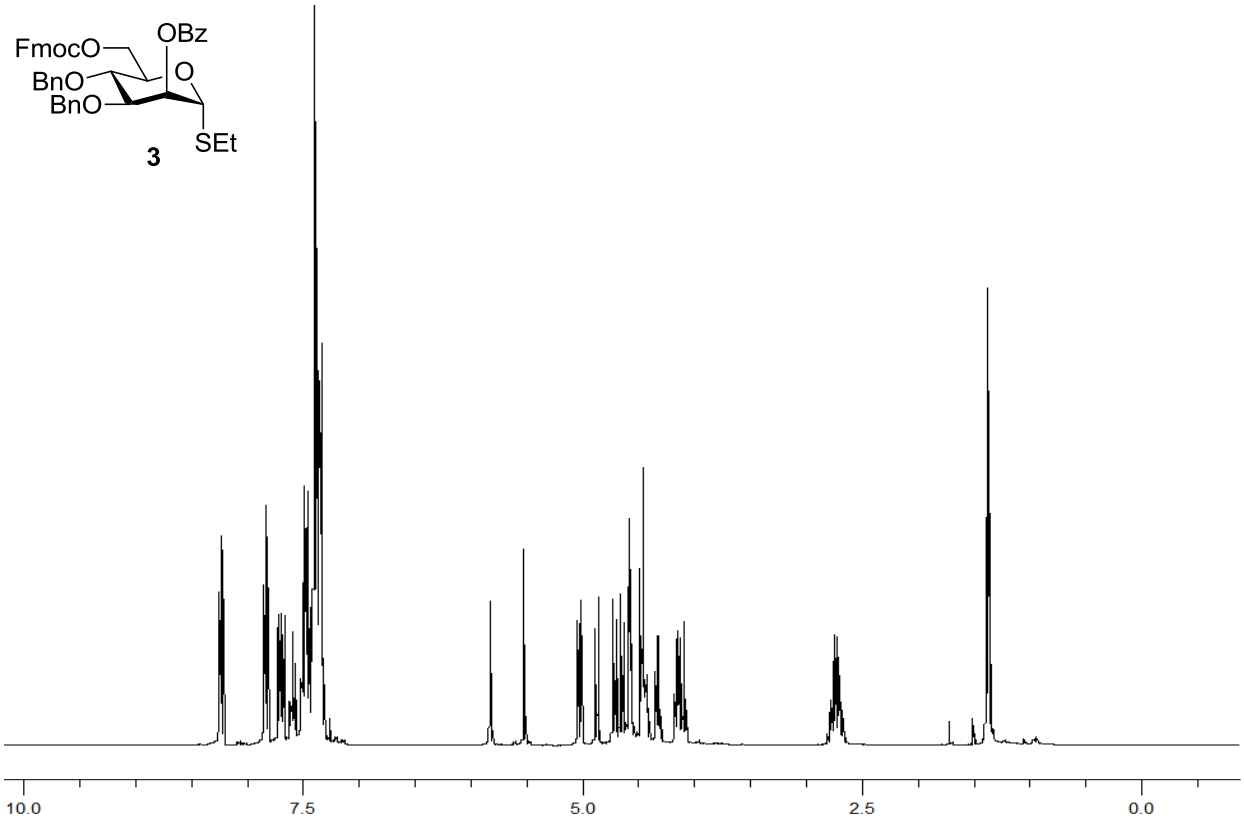


Figure S7. ^{1}H NMR of **3**.

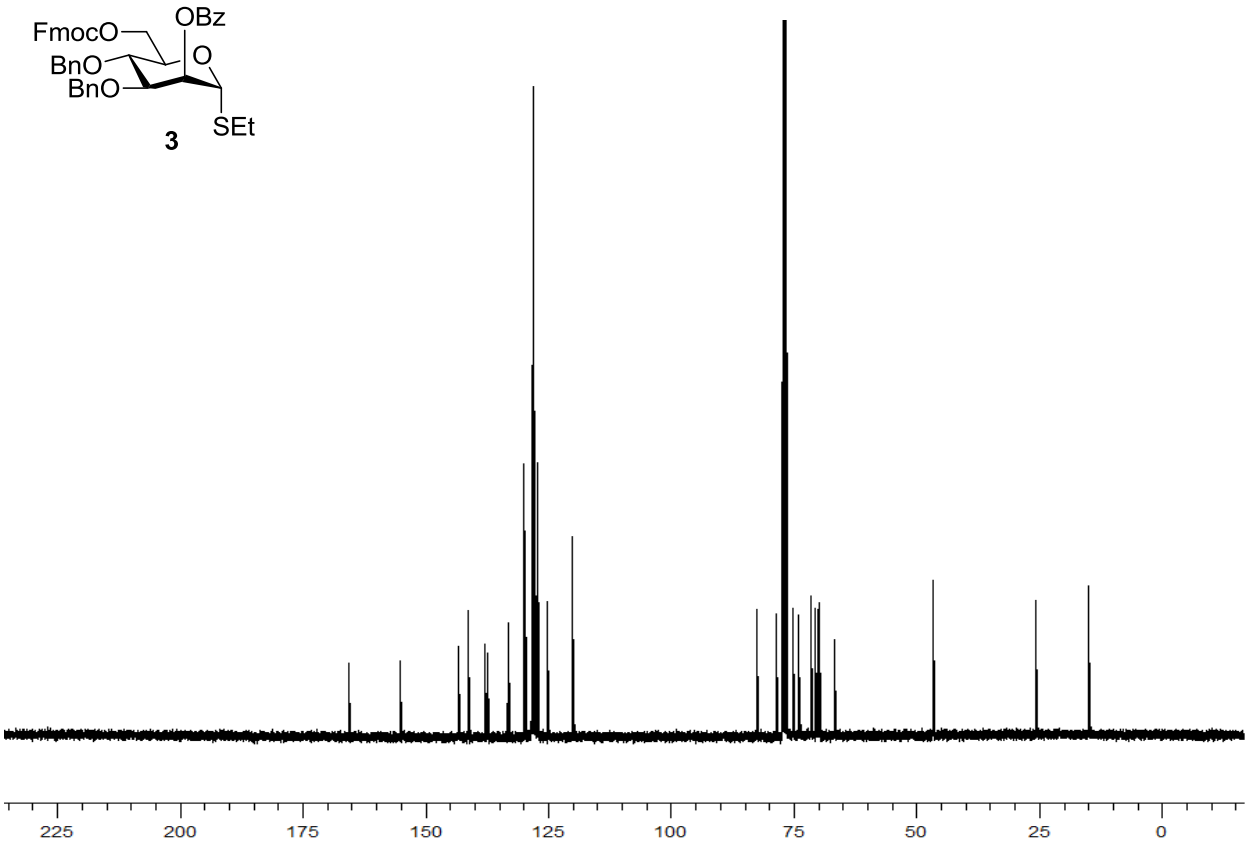


Figure S8. ^{13}C NMR of **3**.

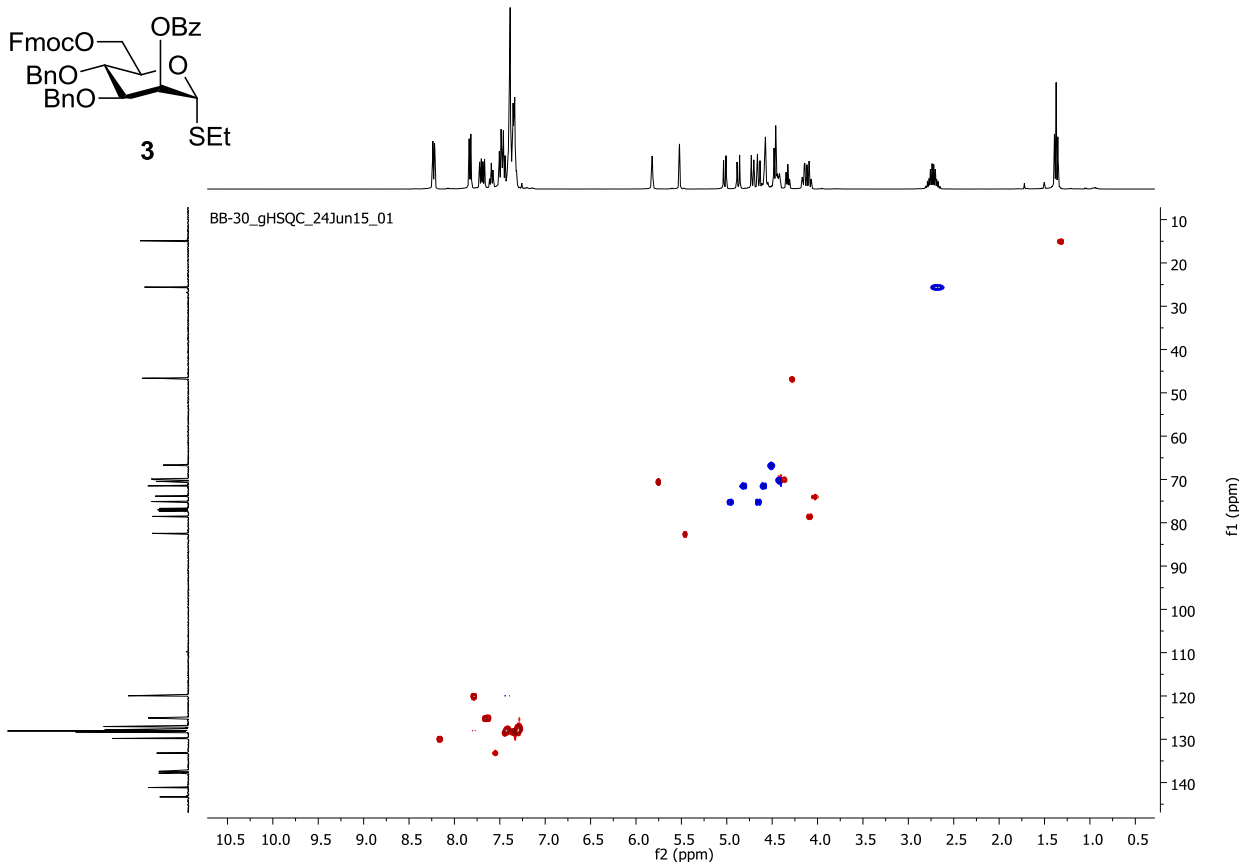


Figure S9. HSQC spectrum of **3**.

3. AGA of Protected Mannose Oligo/Polysaccharides

3.1 General Procedures. Activator, deprotection, acid wash and building block solutions were freshly prepared and kept under argon during AGA. The modules described below were adopted from a previous publication.[2] In all reactions, the resin was bubbled with Ar from the bottom of the reaction vessel.

3.2 Stock Solutions

3.2.1 Building Block Solution. 0.075 mmol of building blocks (**1**, **2**, **3** and **9**) were dissolved in 1 mL of DCM

3.2.2 Activator Solution. NIS (0.675 g) was dissolved in 40 mL of a 2:1 mixture of anhydrous DCM and anhydrous dioxane. Then, triflic acid (28μL) was added. The solution was kept at 0°C for the duration of AGA. The solution can be used for up to five days without compromising reactivity.

3.2.3 Fmoc Deprotection Solution. A solution of 20% Et_3N in DMF (v/v) was prepared.

3.2.4 Acid Wash Solution. TMSOTf (0.9 mL) was added to 40 mL DCM. The solution was kept at room temperature for the duration of AGA.

3.3 AGA Modules

3.3.1 *Module A*: Preparation of resin

All automated syntheses were performed on a 9.7 μmol scale based on resin capacity calculated by Fmoc loading as reported.[2] Resin was placed in the reaction vessel and allowed to swell in DCM (2-3 mL) for 30 min at room temperature prior to synthesis. Prior to the first glycosylation step, the resin was washed with the DMF, THF, and DCM (three times each with 2 mL for 25 s).

3.3.2 *Module B*: Acid wash

The acid wash step was performed at -20 °C. Once the target temperature was reached, 350 μL of acidic wash solution (TMSOTf in DCM) was added dropwise to the reaction vessel. After bubbling for 1 min, the acidic wash solution was drained and the resin was washed with 2 mL DCM for 25 s.

3.3.3 *Module C*: Glycosylation

Glycosylations were performed following the acid wash. Thioglycoside building block solution (5 eq. in 1.0 mL CH_2Cl_2) was delivered to the reaction vessel and the temperature was adjusted to -40 °C. After the set temperature was reached, the reaction was started by the addition of NIS (5 eq.) and TfOH (0.5 eq.) in DCM:dioxane (2:1, 1mL). The glycosylation was performed by first immediately ramping up the temperature from -40 °C to -20 °C, then incubating at the target temperature for 5 min (trisaccharide syntheses for discovery of optimal building block) or 20 min (all other syntheses). Subsequently the solution was drained and the resin was washed three times with DCM.

3.3.4 *Module D*: Fmoc deprotection

The resin was washed with DMF, allowed to swell in 2 mL DMF and the temperature of the reaction vessel was adjusted to 25 °C. Prior to the deprotection step, the DMF was drained and the resin was washed with DMF three times. For Fmoc deprotection, 2 mL of a solution of 20% Et_3N in DMF was delivered to the reaction vessel. After 5 min, the solution was drained and the whole procedure was repeated two more times. After Fmoc deprotection, the resin was washed with DMF, THF and DCM.

3.3.5 *Module E*: Capping

The temperature was adjusted to 25 °C and the resin was washed with pyridine (2 mL) three times. For capping, pyridine (1 mL) and acetic anhydride (1 mL) were delivered to the reaction vessel. After 30 min the reaction solution was drained and the whole procedure was repeated another two times. After capping, the resin was washed three times with pyridine and DCM.

4. Post-AGA Steps

4.1 Cleavage

After automated synthesis, oligosaccharides were cleaved from the solid support using a continuous-flow photoreactor as described previously.[2]

4.2 Purification

After photo cleavage, the solvent was evaporated *in vacuo* and the crude products were analyzed/purified using analytical/preparative HPLC.

4.2.1 Yield calculation

All yields of products obtained by AGA were calculated on the basis of resin loading.[3] Resin loading was determined by performing one glycosylation (Module C) with 10 eq of building block **3** followed by DBU promoted Fmoc-cleavage and determination of dibenzofulvene production by measuring its UV absorbance.

4.2.2 Column specifications

Analytical Luna silica: length 250 mm, 4.6 mm i.d., flow 1 mL/min;
Analytical YMC diol: length 250 mm, 4.6 mm i.d., flow 1 mL/min;
Preparative Luna silica: length 250 mm, 10 mm i.d., flow 5 mL/min;
Preparative YMC diol: length 250 mm, 20 mm i.d., flow 15 mL/min;
Analytical Hypercarb; length 150 mm, 4.6 mm i.d., flow 0.7 mL/min;
Preparative Hypercarb; length 150 mm, 10 mm i.d., flow 3.6 mL/min;

4.3 Deprotection

4.3.1 Zemplén Methanolysis

To a solution of protected oligosaccharide in methanol:CH_2Cl_2 (1:1), sodium methoxide in methanol (0.5 M, pH ~ 13) was added and stirred at room temperature for 12 h, neutralized with Amberlite ion exchange (H^+) resin, filtered and concentrated *in vacuo* and carried forward directly into hydrogenolysis without purification. During the synthesis of oligosaccharide **13**, after the removal of the esters, the methyl ester group was subjected to hydrolysis under basic conditions: to a solution of crude residue from the Zemplén methanolysis in methanol, NaOH (10 eq) in water was added and stirred at room temperature for 12 h, neutralized with Amberlite ion exchange (H^+) resin, filtered and concentrated *in vacuo.*

4.3.2 Hydrogenolysis

Hydrogenolysis was carried out in a hydrogen bomb with 60 psi pressure. The products of Zemplén methanolysis were dissolved in methanol:THF:AcOH (9:1:0.1) and transferred to cylindrical vials. Pd-C (10%) (100 weight %) was added. The reaction mixture was stirred under an atmosphere of hydrogen for 72 to 96 h. The reactions were filtered through Celite and washed with methanol and water. The filtrates were concentrated *in vacuo* and purified on Sephadex LH20. For **13**, the amine group was protected with Cbz prior to purification. To a solution of amine in water, Et_3N (10 μL) and CbzCl (10 μL) were added at 0 $^{\circ}$C and stirred at room temperature for 18 h and concentrated *in vacuo*.

4.3.3 Sephadex LH20 purification

The crude products of hydrogenolysis were fractionated between water and ether. The water layer was washed with ether to remove any hydrophobic organic impurities. The crude oligosaccharides were dialyzed against 1 KD membranes to eliminate smaller molecular weight impurities. The oligosaccharides were then subjected to size exclusion chromatography using Sephadex LH20. The product fractions were identified using *p*-anisaldehyde, combined and lyophilized to obtain the oligomers.

5. Trisaccharide Synthesis to Identify Optimal Building Block

To identify the best building block in terms of reactivity and purification, trisaccharides were synthesized employing the three different building blocks **1**-**3** (Scheme **S2**).

Scheme S2

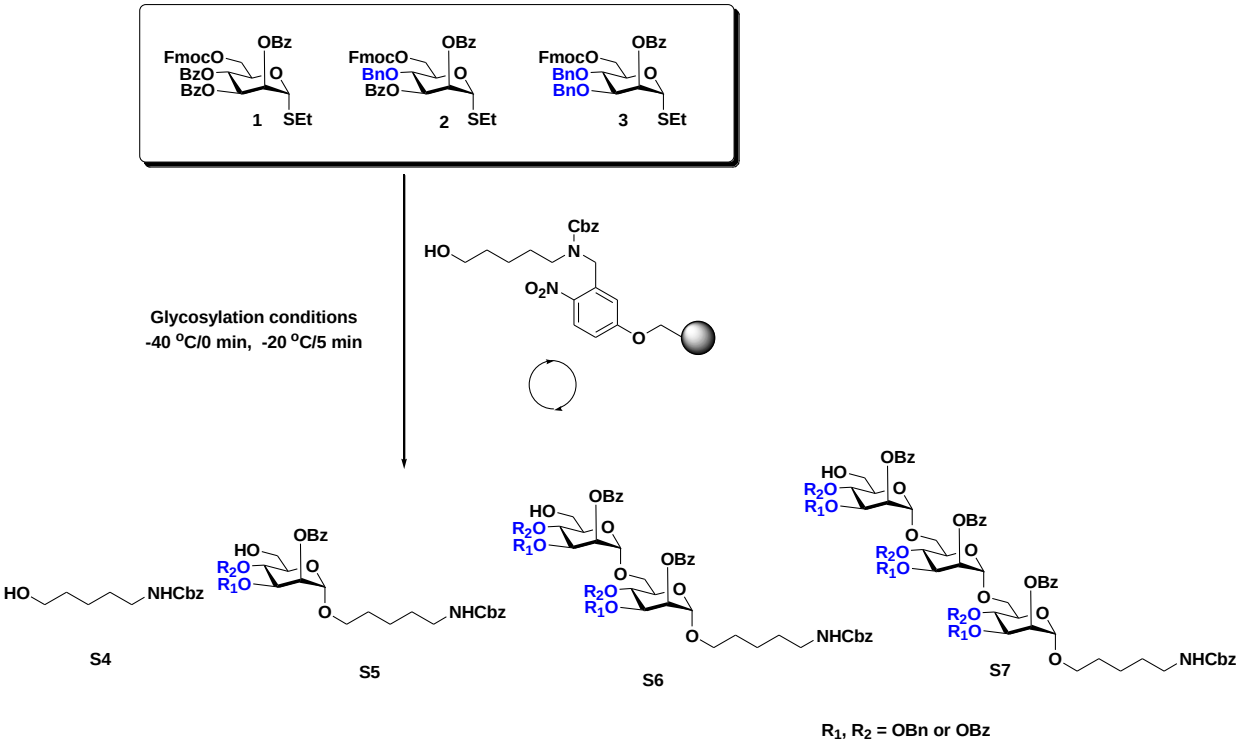


Table S1. AGA protocol for trisaccharide synthesis using building blocks **1**-**3**.

Step	Automation process	Module
1	Preparation of resin	**A**
2	Acid wash	**B**
3	Glycosylation with building blocks **1, 2, or 3**	**C** (-40 °C for 0 min, -20 °C for 5 min)
4	Fmoc deprotection	**D**
5	Chain elongation	Repeat **B**-**D** (twice)

5.1 Cleavage, Analysis and Purification

The products were cleaved from the solid support as described in "Post-AGA Steps". The crude products were analyzed and purified using normal phase analytical HPLC with a Luna silica column. Linear gradient: EtOAc (2% DCM)/Hexane (2% DCM); 20% EtOAc (2% DCM) for 5 min, 20% to 60% EtOAc (2% DCM) in 40 min, 60 to 100% EtOAc (2% DCM) for 5 min. In the case of products from building block **1**, the normal phase HPLC was not successful for the purification. The products were analyzed/purified after the removal of benzoyl protecting groups (Zemplen methanolysis) using reverse phase HPLC with a Hypercarb column. Linear gradient: ACN (0.1% formic acid)/H_2O (0.1% formic acid); 20% ACN (0.1% formic acid) for 5 min, 20% to 60% ACN (0.1% formic acid) in 45 min, 60% to 100% ACN (0.1% formic acid) in 5 min.

5.2 Product Analysis

Table S2. Analysis of the products from automation synthesis **Scheme S2**

Building block	Products (yield of the trisaccharide)
1	**S4, S5, S6, S7** (9% after deprotection)
2	**S4, S5, S6, S7** (16%)
3	**S6, S7** (27%)

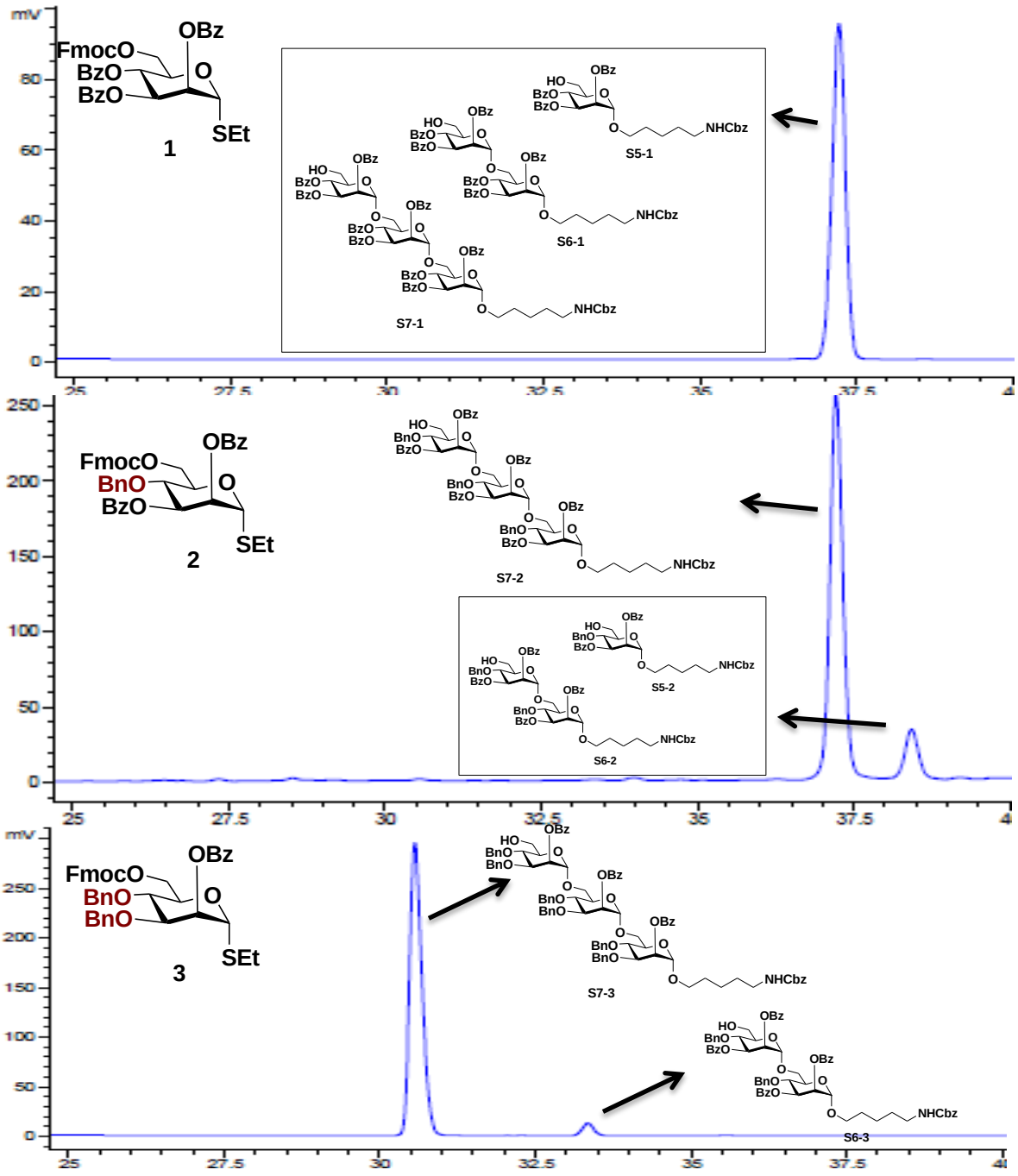


Figure S10. Separation products in analytical normal phase HPLC (**Scheme S2**) from the building blocks **1**-**3** (similar gradient and scale). Note: product **S4** was also detected by mass spec in syntheses using building blocks **1** and **2**, but is not drawn here for space reasons.

6. Synthesis of **5** (6mer)

Scheme S3

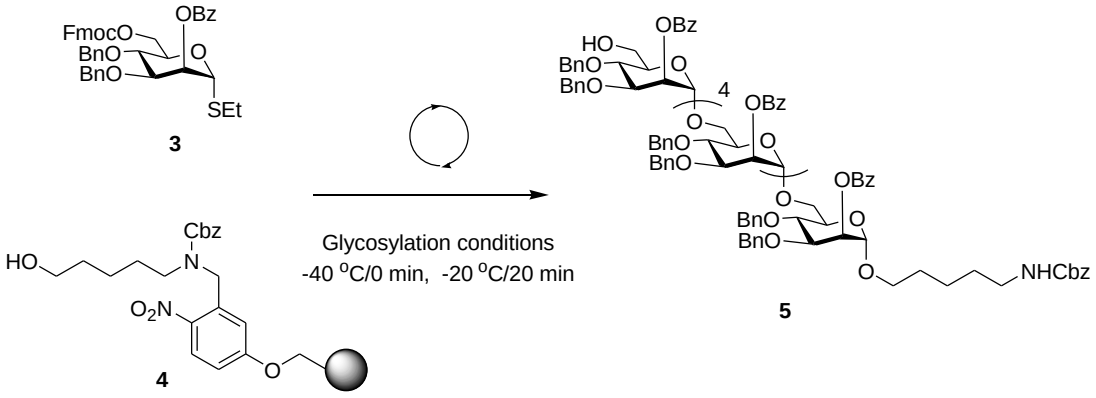


Table S3. AGA protocol for the assembly of hexasaccharide **5** using building block **3**.

Step	Automation process	Module
1	Preparation of resin	**A**
2	Acid wash	**B**
3	Glycosylation with building block **3**	**C** (-40 °C for 0 min, -20 °C for 20 min)
4	Fmoc deprotection	**D**
5	Chain elongation	Repeat **B**-**D** (five times)

The products were cleaved from the solid support as described in "Post-AGA Steps". The crude products were analyzed and purified using normal phase analytical HPLC with a Luna silica column. Linear gradient: EtOAc (2% DCM)/Hexane (2% DCM); 20% EtOAc (2% DCM) for 5 min, 20% to 60% EtOAc (2% DCM) in 40 min, 60 to 100% EtOAc (2% DCM) for 5 min. Hexasaccharide **5** eluted at 32.1 minutes.

Analytical data for hexasaccharide **5**:Yield: 14.5 mg (50 %); (^{1}H NMR (600 MHz, $CDCl_3$) δ 8.19 – 8.05 (m, 13H), 7.62 – 7.56 (m, 1H), 7.54 – 7.41 (m, 18H), 7.37 – 7.03 (m, 63H), 5.83 (dd, *J* = 4.7, 2.6 Hz, 2H), 5.81 (dd, *J* = 2.9, 2.0 Hz, 1H), 5.80 (dd, *J* = 3.0, 1.9 Hz, 1H), 5.76 (dd, *J* = 2.9, 2.0 Hz, 1H), 5.62 (dd, *J* = 3.2, 1.8 Hz, 1H), 5.10-5.04 (m, 6H), 4.91 – 4.75 (m, 12H), 4.72 (d, *J* = 11.4 Hz, 1H), 4.60-4.54 (m, 2H), 4.49-4.33 (m, 10H), 4.10-4.02 (m, 6H), 3.98-3.83 (m, 10H), 3.81 – 3.70 (m, 5H), 3.69-3.58 (m, 7H), 3.55-3.50 (m, 2H), 3.46 (d, *J* = 11.9 Hz, 2H), 3.42-3.39 (m, 1H), 3.20 – 3.13 (m, 2H). 1.58–1.45 (m, 4H), 1.4 – 1.30 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.83, 165.59, 165.51, 165.49, 165.39, 156.35, 138.51, 138.50, 138.45, 138.29, 138.24, 137.92, 137.63, 137.56, 137.53, 137.51, 136.66, 133.27, 133.23, 129.98, 129.93, 129.88, 129.85, 129.84, 129.81, 128.62, 128.55, 128.46, 128.32, 128.31, 128.27, 128.24, 128.19, 128.14, 128.13, 128.12, 128.11, 128.07, 128.01, 127.98, 127.66, 127.61, 127.59, 127.39, 127.35, 127.34, 127.29, 127.22, 127.13, 127.10, 98.46, 98.38, 98.13, 97.86, 78.57, 78.29, 78.23, 78.20, 78.15, 77.67, 75.15, 75.07, 75.02, 74.98, 74.19, 73.90, 73.81, 73.75, 73.70, 72.08, 71.62, 71.40, 71.32, 71.30, 71.18, 70.98, 70.94, 70.91, 70.73, 69.06, 68.56, 68.43, 68.38, 67.75, 66.53, 66.10, 65.81, 65.74, 65.45, 61.82, 40.94, 29.76, 29.02, 23.41. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{175}H_{175}NNaO_{39}$: 2937.1639; found: 2937.1557.

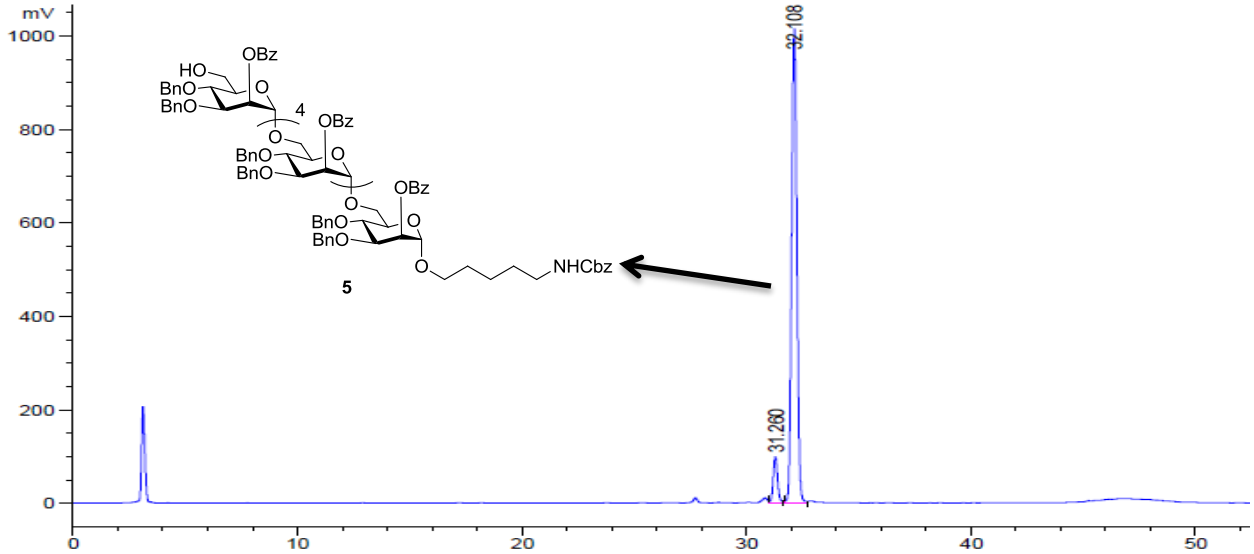


Figure S11. Analytical NP-HPLC Luna silica trace of crude hexasaccharide **5** (ELSD trace).

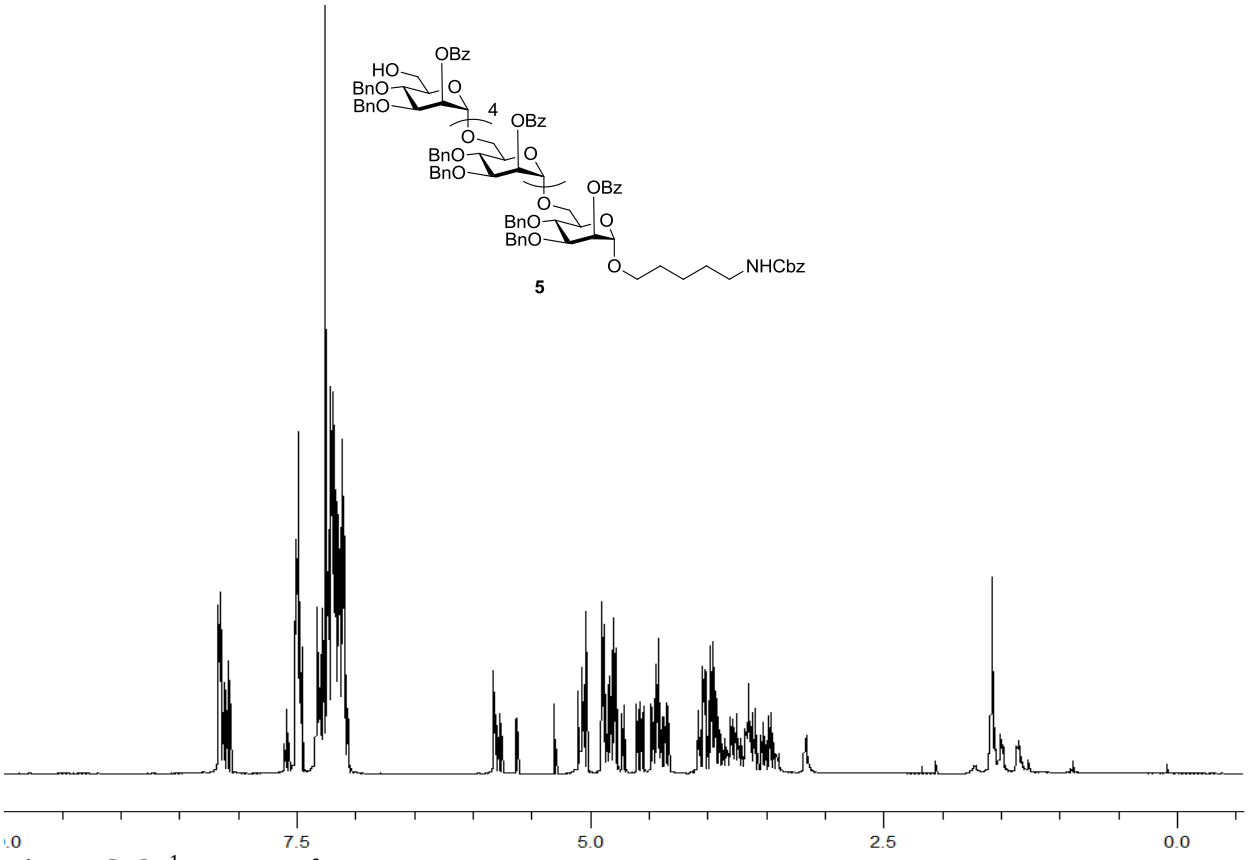


Figure S12. ^{1}H NMR of **5**.

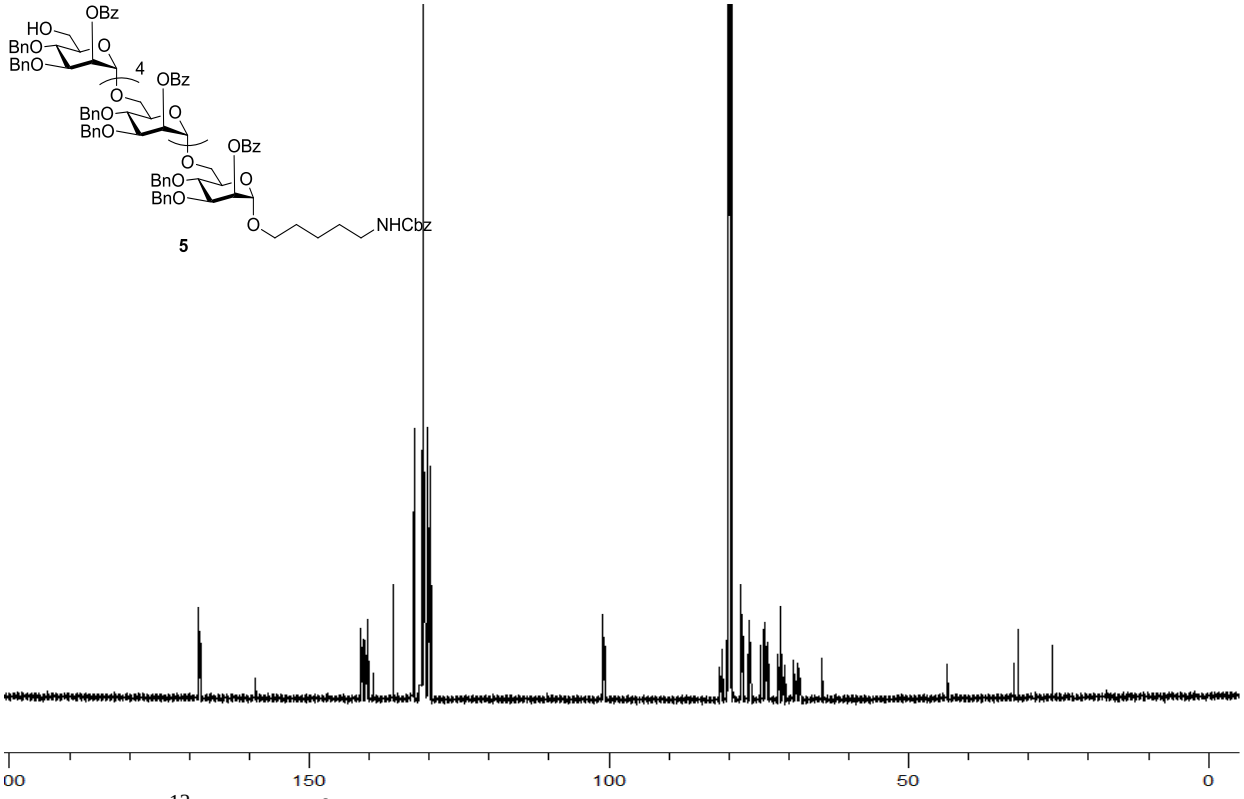


Figure S13. ^{13}C NMR of **5**.

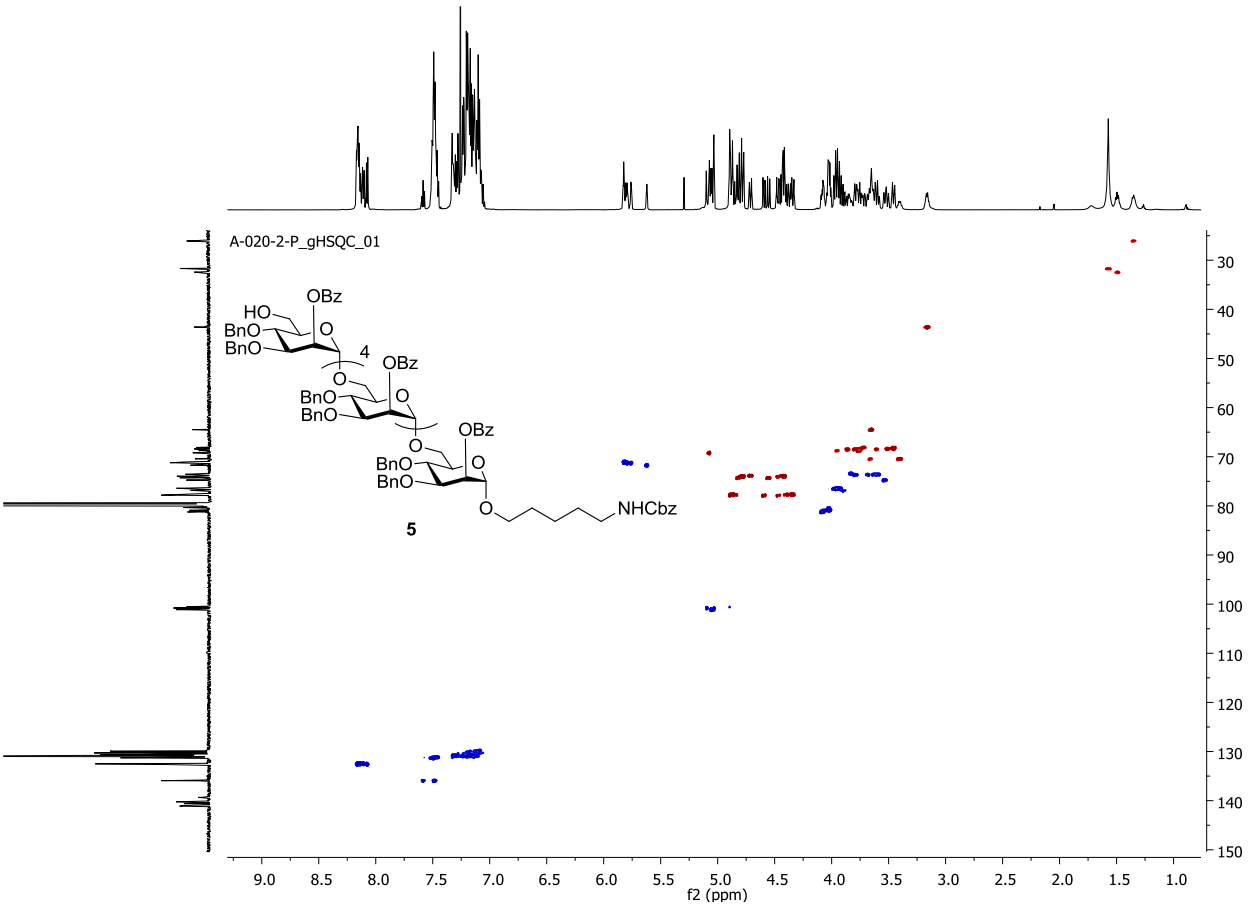


Figure S14. HSQC spectrum of **5**.

7. Synthesis of **6** (12mer)

Scheme S4

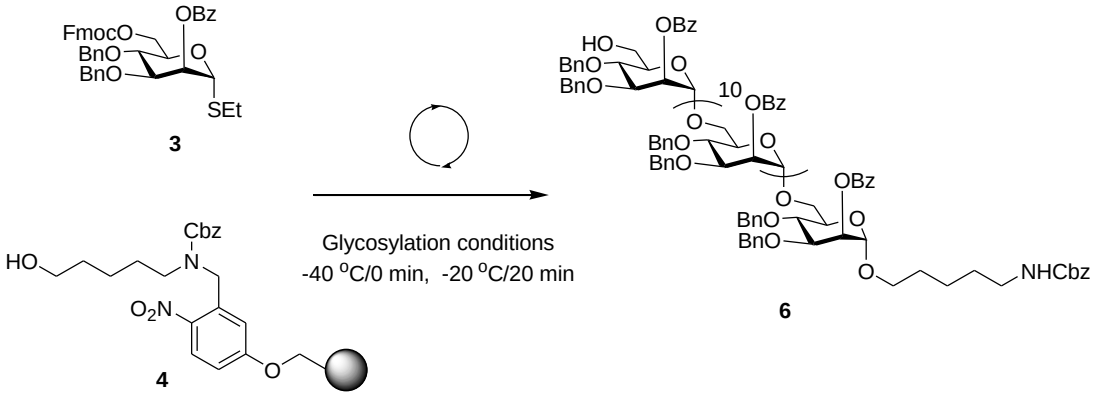


Table S4. AGA protocol for the assembly of dodecasaccharide **6** using building block **3**.

Step	Automation process	Module
1	Preparation of resin	**A**
2	Acid wash	**B**
3	Glycosylation with building block **3**	**C** (-40 °C for 0 min, -20 °C for 20 min)
4	Fmoc deprotection	**D**
5	Chain elongation	Repeat **B**-**D** (11 times)

The products were cleaved from the solid support as described in "Post-AGA Steps". The crude products were analyzed and purified using normal phase analytical HPLC with a Luna silica column. Linear gradient: EtOAc (2% DCM)/Hexane (2% DCM); 20% EtOAc (2% DCM) for 5 min, 20% to 60% EtOAc (2% DCM) over 40 min, 60 to 100% EtOAc (2% DCM) over 5 min. Dodecasaccharide **6** eluted at 36.2 minutes.

Analytical data for dodecasaccharide **6**: Yield: 21 mg (38 %); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.28 – 7.76 (m, 24H), 7.62 – 7.34 (m, 30H), 7.39 – 6.82 (m, 131H), 5.88 – 5.74 (m, 10H), 5.65 – 5.61 (m, 1H), 5.10 – 5.04 (m, 12H), 4.89 – 4.75 (m, 22H), 4.72 (d, *J* = 11.4 Hz, 2H), 4.54-4.60 (m, 4H), 4.50 – 4.33 (m, 21H), 4.12 – 3.83 (m, 26H), 3.80-3.57 (m, 26H), 3.55 – 3.36 (m, 12H), 3.17-3.6 (m, 2H), 1.59 – 1.53 (m, 4H), 1.51 – 1.44 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.83, 165.59, 165.50, 165.39, 156.35, 138.50, 138.45, 138.41, 138.28, 138.23, 137.92, 137.63, 137.54, 137.51, 137.48, 136.66, 133.28, 129.98, 129.93, 129.88, 129.83, 128.62, 128.54, 128.45, 128.33, 128.30, 128.27, 128.23, 128.19, 128.15, 128.11, 128.01, 128.00, 127.98, 127.65, 127.60, 127.39, 127.34, 127.27, 127.21, 127.08, 127.06, 127.04, 127.01, 98.52, 98.47, 98.38, 98.13, 98.10, 97.85, 78.57, 78.28, 78.17, 77.67, 75.15, 75.07, 74.97, 74.19, 73.89, 73.79, 73.70, 72.08, 71.62, 71.39, 71.27, 71.17, 70.97, 70.88, 70.72, 69.06, 68.55, 68.36, 67.75, 66.52, 66.09, 65.71, 65.43, 61.81, 40.93, 29.69, 29.01, 23.41.

MALDI-MS ($C_{337}H_{331}NNaO_{75}$): calcd for $[M+Na]^+$ 5617.2, found 5618.6.

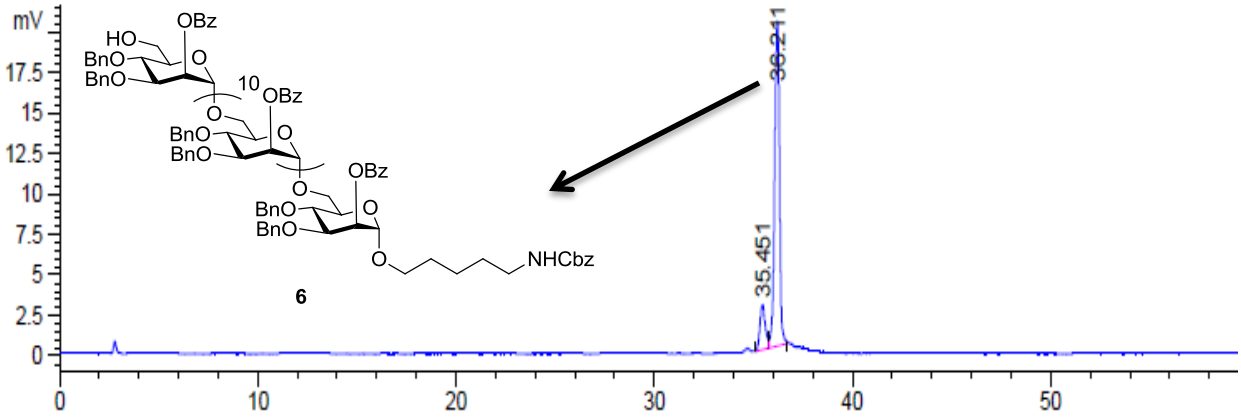


Figure S15. Analytical NP-HPLC Luna silica trace of crude **6** (ELSD trace).

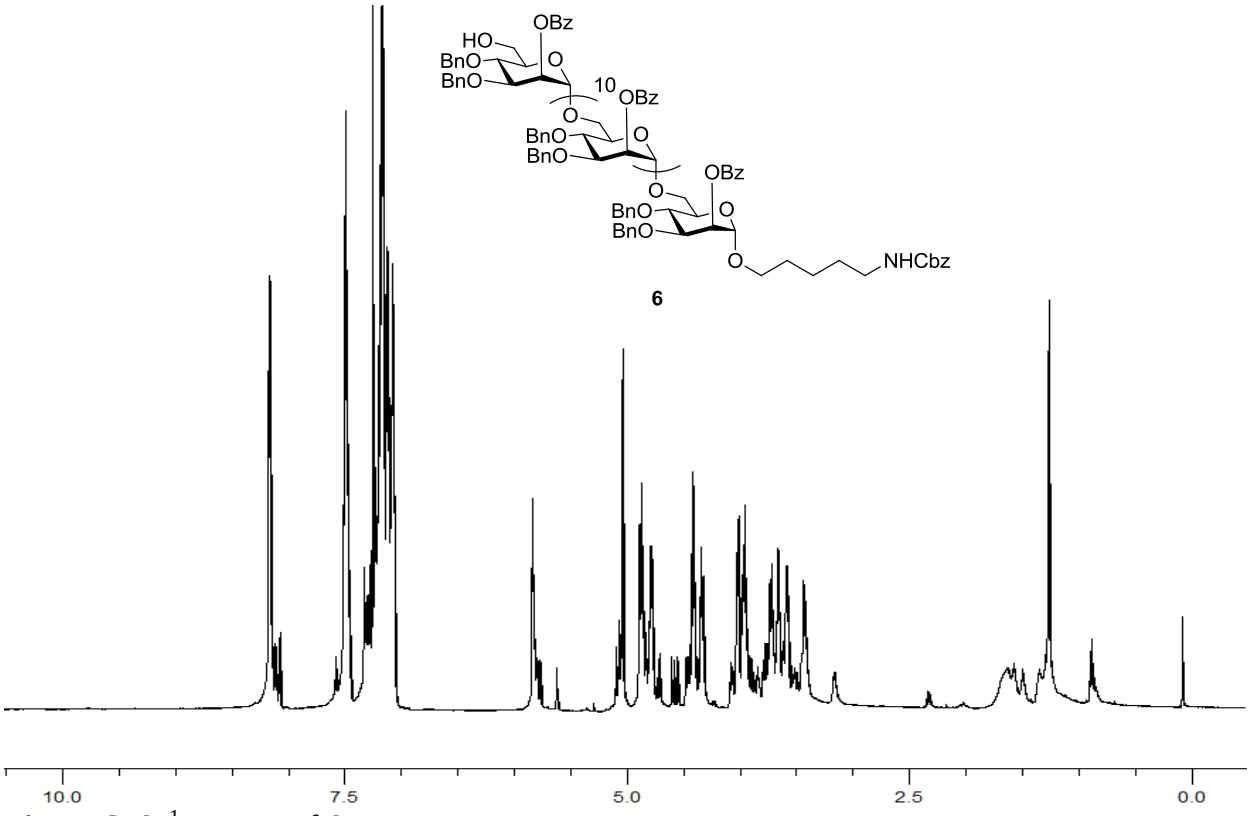


Figure S16. ^{1}H NMR of **6**.

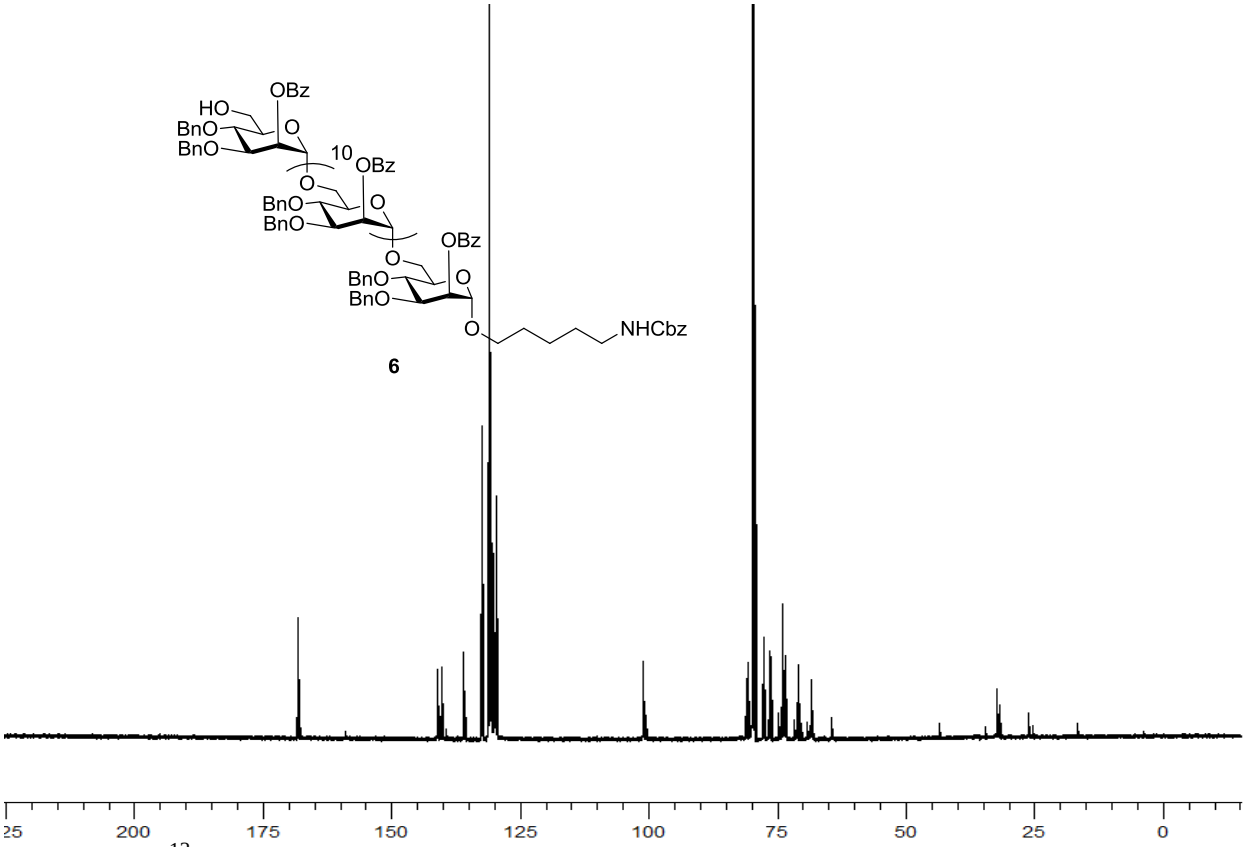


Figure S17. ^{13}C NMR of **6**.

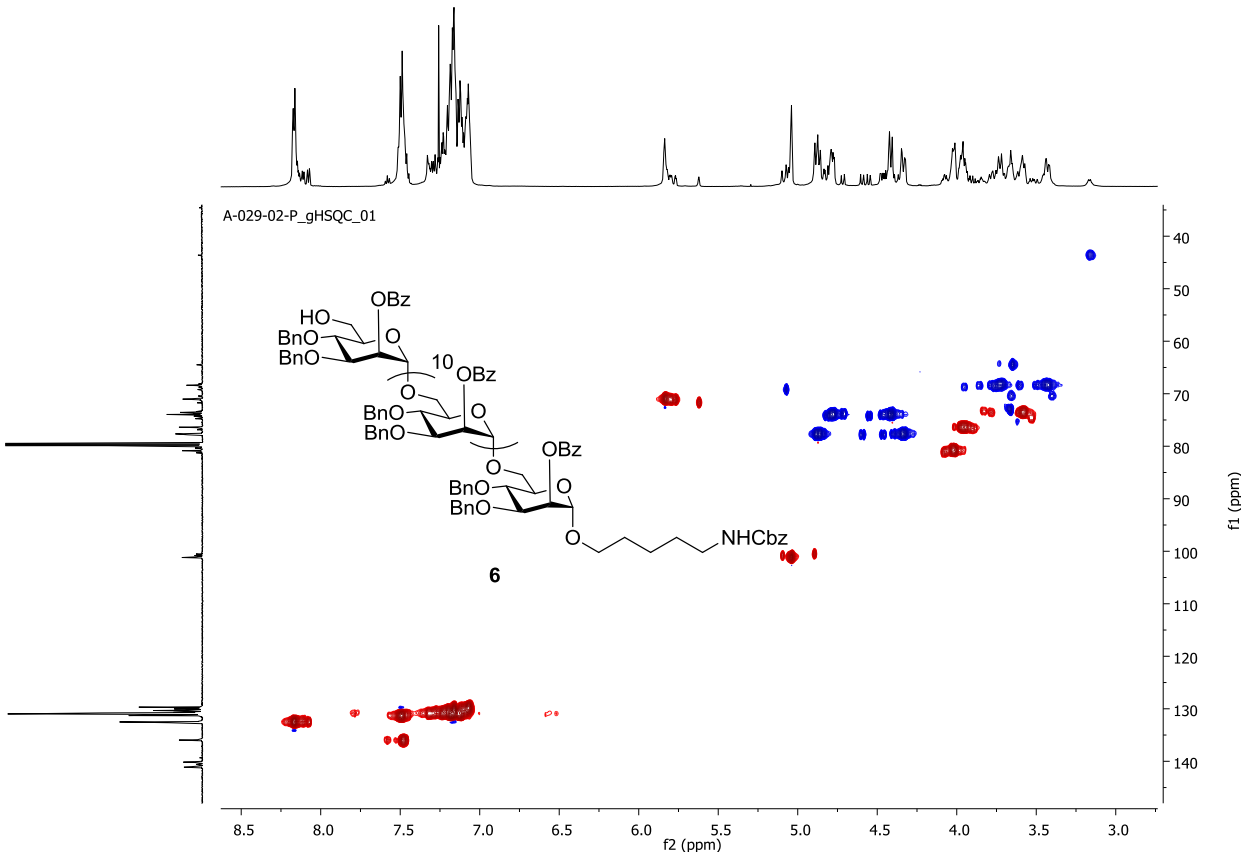


Figure S18. HSQC spectrum of **6**.

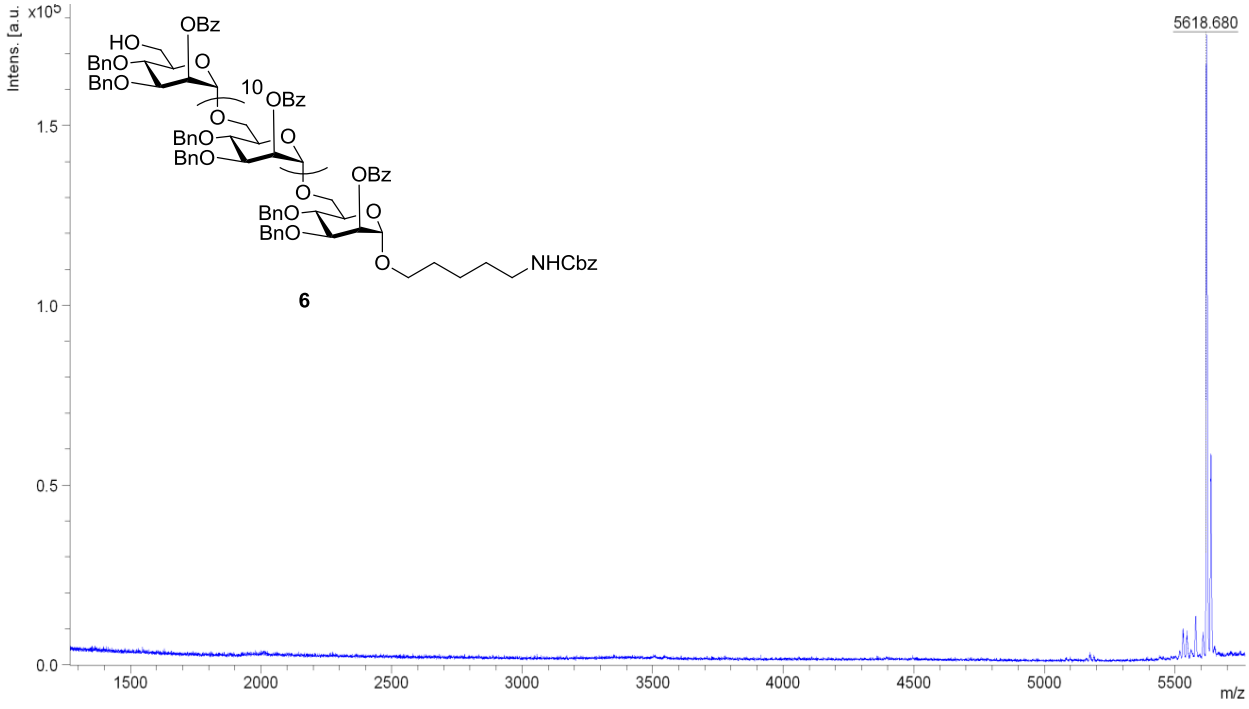


Figure S19. MALDI-TOF spectrum of **6**.

8. Syntheses of **7** and **8** (37mer)

Scheme S5

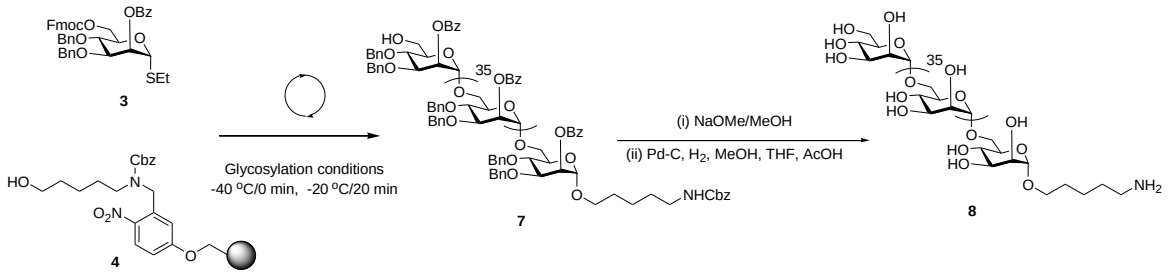


Table S5. AGA protocol for the assembly of 37mer **7** using building block **3**.

Step	Automation process	Module
1	Preparation of resin	**A**
2	Acidic wash	**B**
3	Glycosylation with building block **3**	**C** (-40 °C for 0 min, -20 °C for 20 min)
4	Fmoc deprotection	**D**
5	Chain elongation	Repeat **B**-**D** (36 times)

A small quantity of resin was subjected to photo-cleavage to release the oligosaccharides from the solid support after assembling a 24-mer to assess the progress of the reaction by using normal phase analytical HPLC (YMC diol column) and MALDI. Linear gradient: EtOAc (2% DCM)/Hexane (2% DCM); 10% EtOAc (2% DCM) for 5 min, 10% to 60% EtOAc (2% DCM) over 40 min, 60 to 100% EtOAc (2% DCM) over 5 min. The 24-mer **S8** eluted at 38.8 minutes.

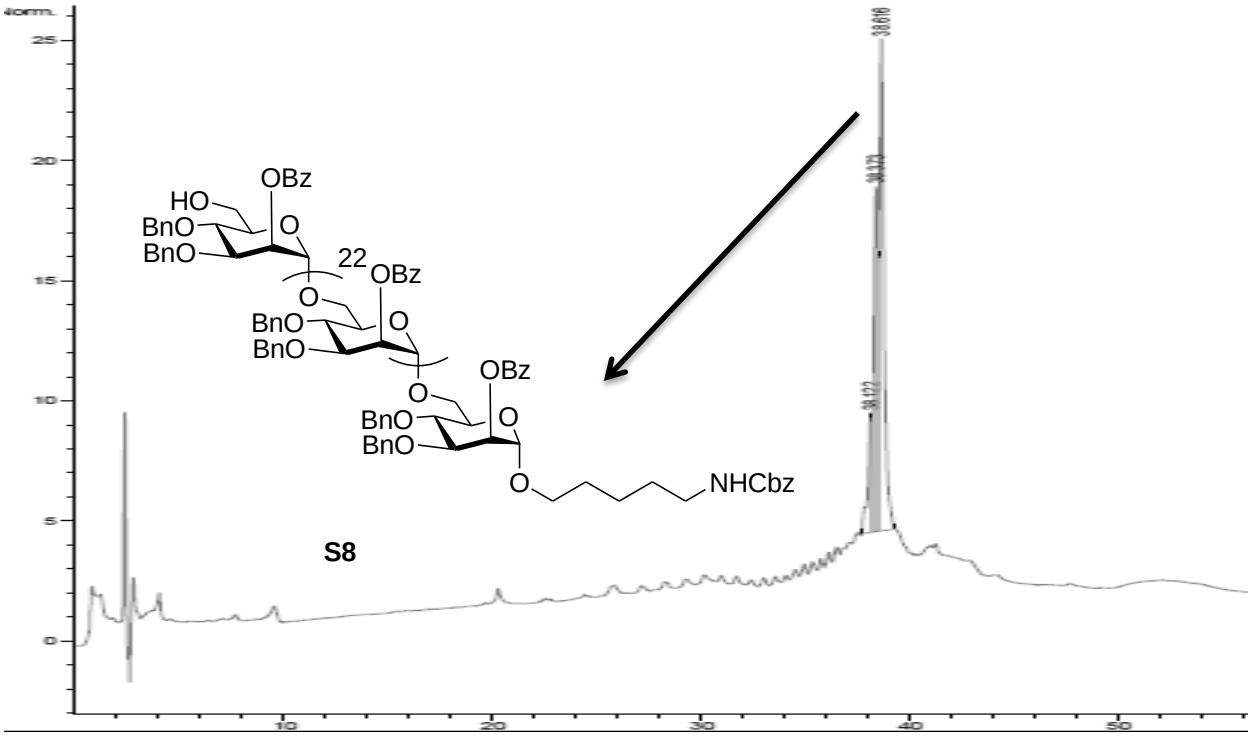


Figure S20. Analytical NP-HPLC YMC diol trace of crude 24mer **S8** (UV-280 nm trace).

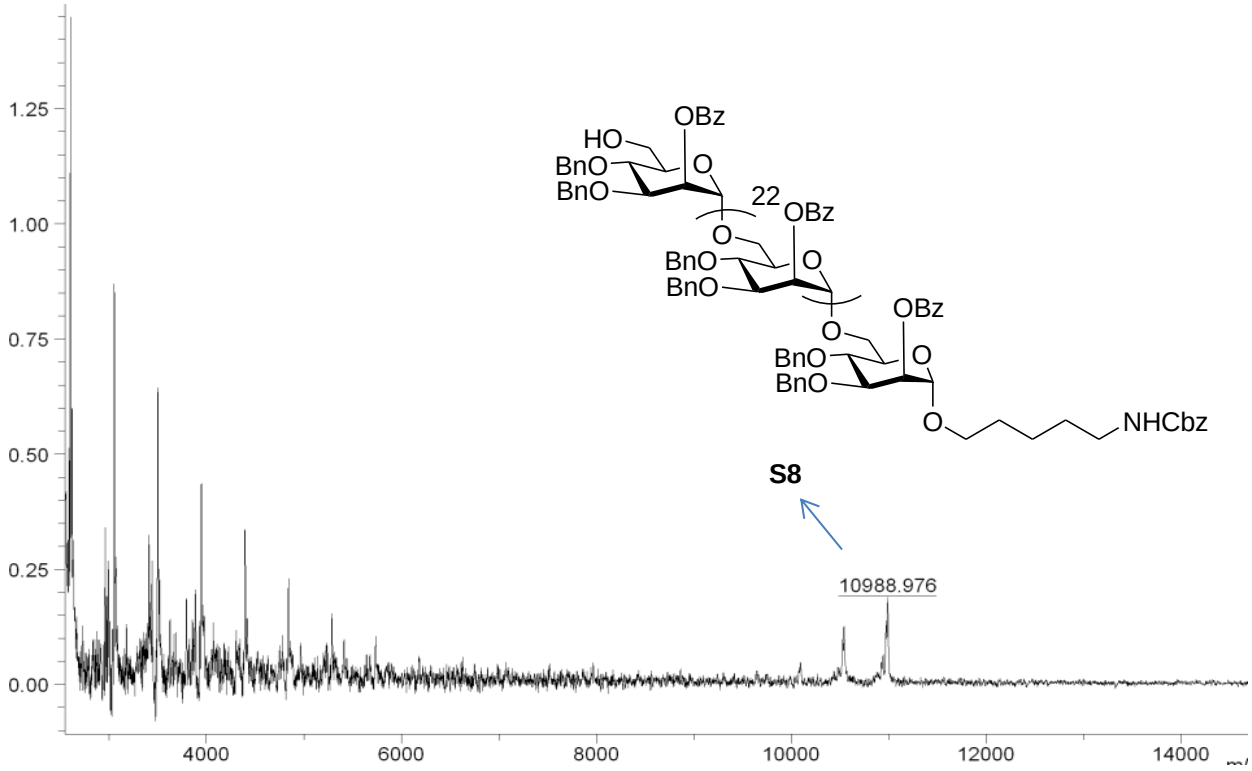


Figure S21. MALDI-TOF spectrum of crude 24mer **S8**.

After 37 cycles, the products from 0.011 mmol resin (based on Fmoc loading) were cleaved from the solid support as described in "Post-AGA Steps". The crude products were analyzed and purified using normal phase analytical HPLC with a YMC diol column. Linear gradient: EtOAc (2% DCM)/Hexane (2% DCM); 20% EtOAc (2% DCM) for 5 min, 20% to 60% EtOAc (2% DCM) over 40 min, 60 to 100% EtOAc (2% DCM) for 5 min. Mannose 37mer **7** eluted together with 36mer at 39 minutes.

Analytical data for 37mer (plus 36mer in 1:1 ratio) **7**: Yield: 27 mg (18%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (d, *J* = 6.9 Hz, 74H), 7.57 – 7.39 (m, 92H), 7.21 – 6.98 (m, 394H), 5.83 (s, 37H), 5.30 (s, 2H), 5.03 (s, 37H), 4.90 – 4.81 (m, 37H), 4.77 (d, *J* = 10.8 Hz, 37H), 4.31-4.41 (m, 37H), 4.33-4.31 (m, 37H), 4.08 – 3.88 (m, 76H), 3.72-3.70 (m, 37H), 3.57-3.55 (m, 37H), 3.42-3.40 (m, 37H), 3.16-3.15 (m, 2H), 1.51 – 1.48 (m, 4H), 1.39 – 1.33 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.51, 138.47, 137.51, 133.28, 130.01, 129.84, 128.63, 128.46, 128.33, 128.31, 128.12, 128.02, 127.99, 127.65, 127.36, 127.28, 127.08, 127.01, 98.55, 78.19, 74.98, 73.72, 71.29, 70.91, 68.39, 65.73. MALDI-MS ($C_{1012}H_{981}NNaO_{225}$): calcd for $[M+Na]^+$ 16768.5, found 16774.5

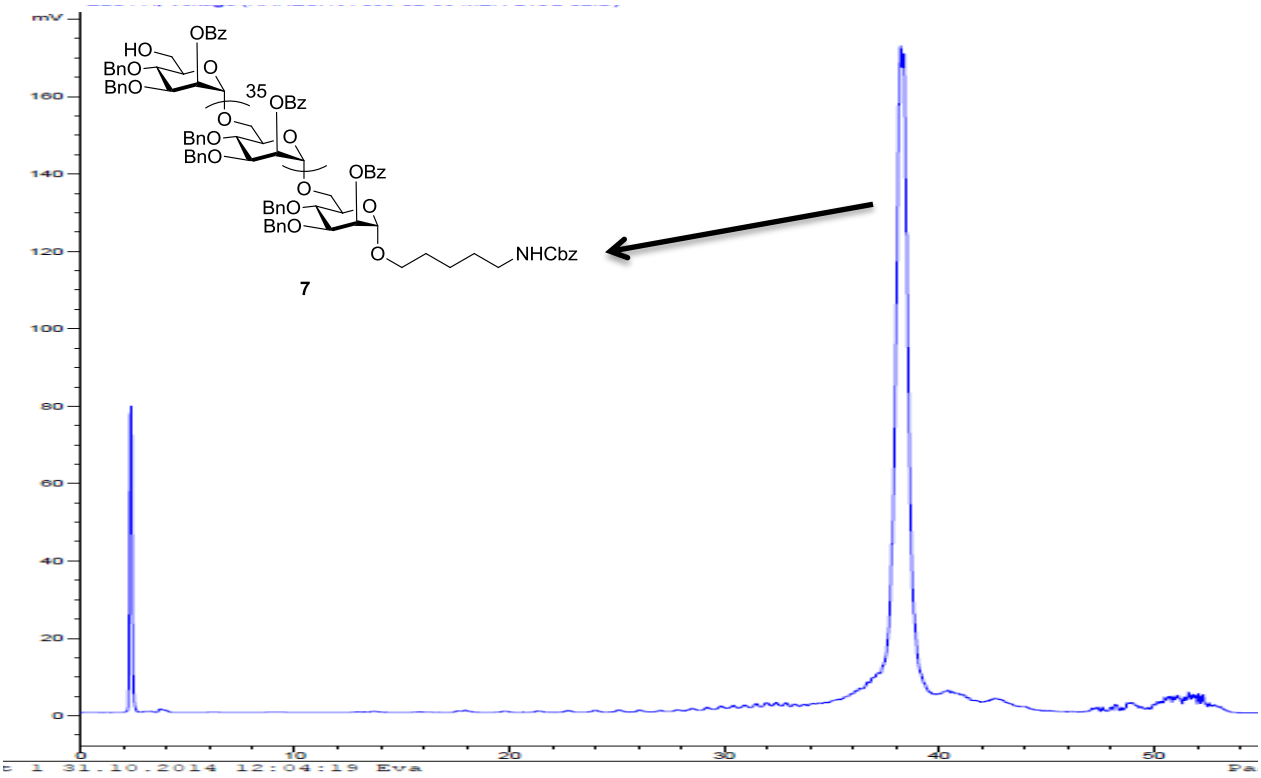


Figure S22. Analytical NP-HPLC YMC diol trace of crude 37mer **7** and 36mer impurity (ELSD trace).

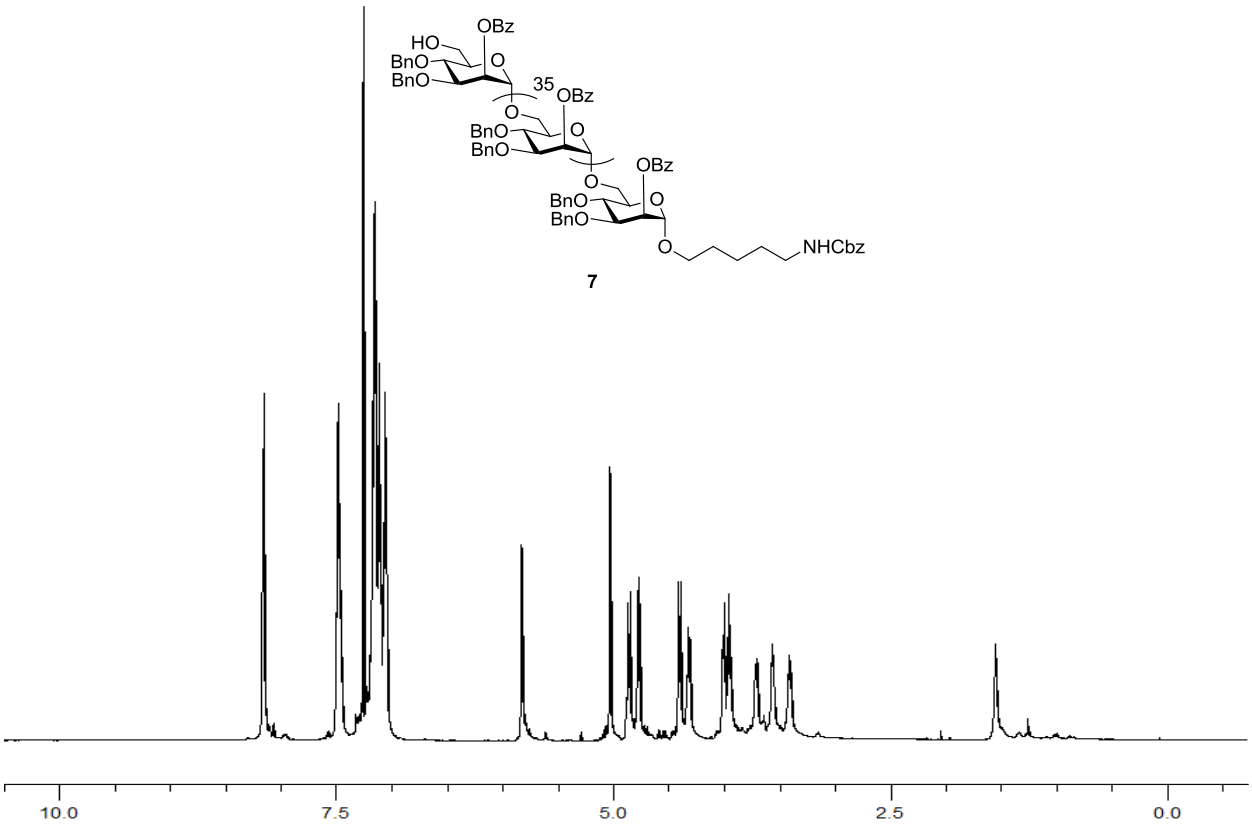


Figure S23. ^{1}H NMR of **7** and 36mer impurity.

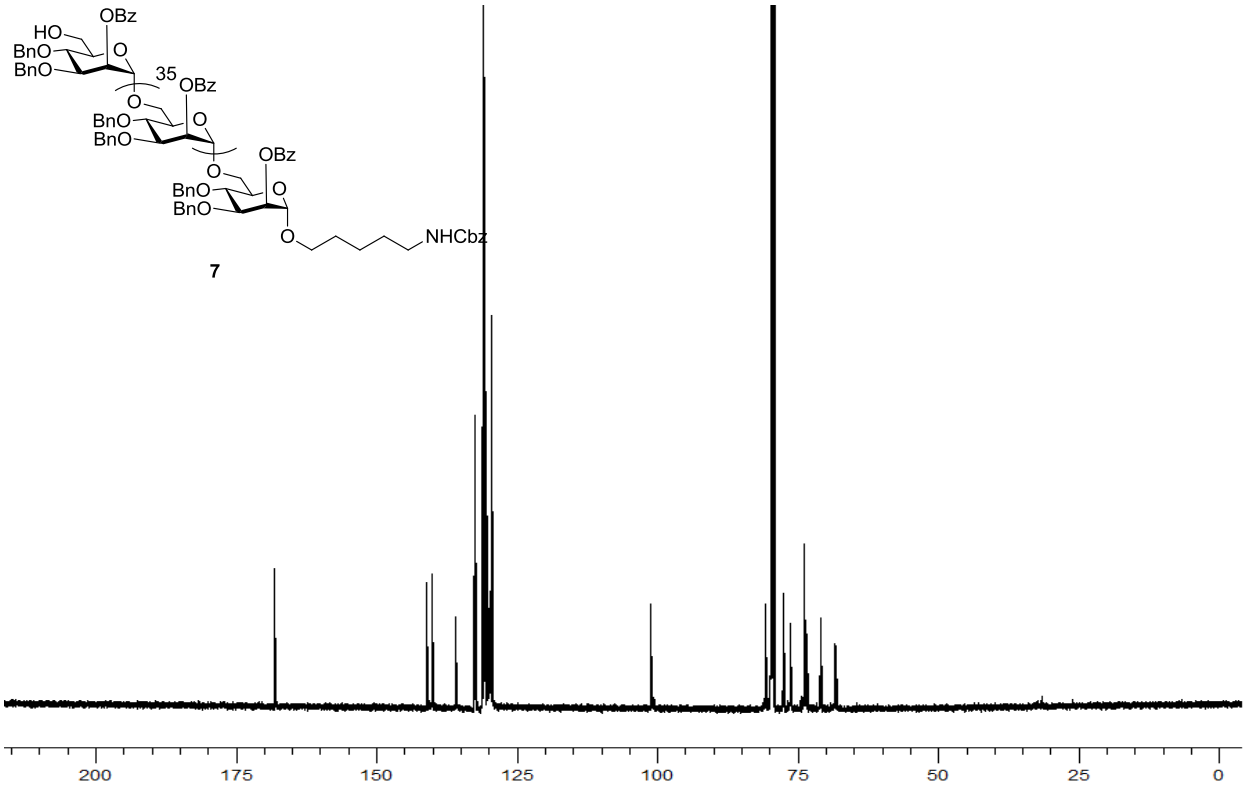


Figure S24. ^{13}C NMR of **7** and 36mer impurity.

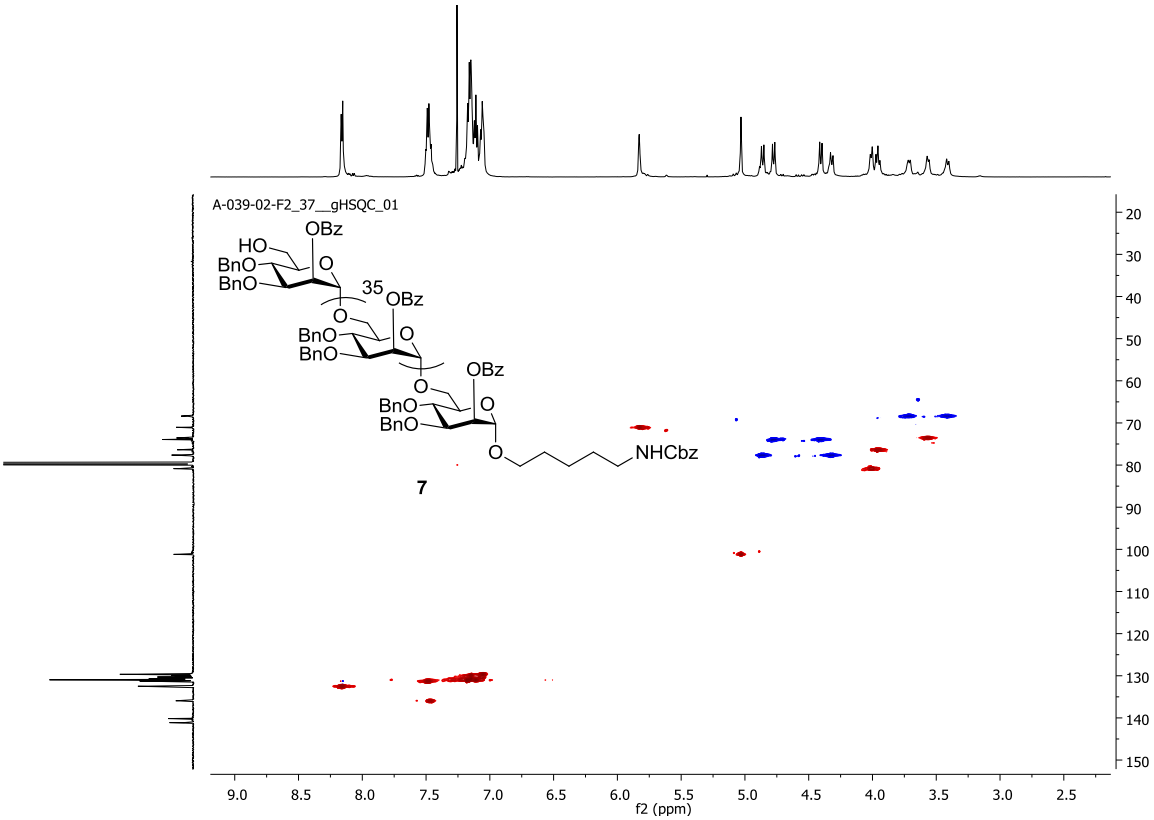


Figure S25. HSQC spectrum of **7** and 36mer impurity.

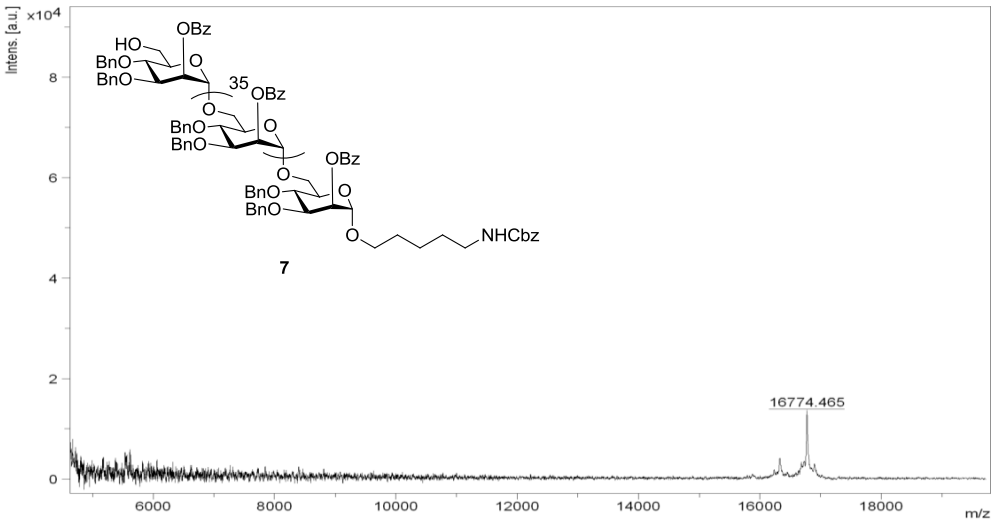


Figure S26. MALDI-TOF spectrum of **7** and 36mer impurity.

Mannose 37mer **7** along with 36mer impurity (22 mg, 1.3 μmol) was deprotected and purified to afford **8** (without contaminating n-1 product) as described in section 4.3.1 (Zemplen Methanolysis), 4.3.2 (Hydrogenolysis) and 4.3.3 (Sephadex LH20 Purification). Yield: 4.3 mg (54%); ^{1}H NMR (700 MHz, D_2O) δ 4.94-4.93 (m, 37H), 4.02 (s, 37H), 3.96-3.95 (m, 37H), 3.91 – 3.72 (m, 148H). ^{13}C NMR (176 MHz, D_2O) δ 99.34, 70.82, 70.69, 69.97, 66.60, 65.52. MALDI-TOF analysis: M-(linker $C_5H_{12}NO$), calcd: 5997.9; found 5991.6; M-(2 sugar residues $C_{12}H_{21}O_{10}$), calcd: 5774.9; found 5783.3

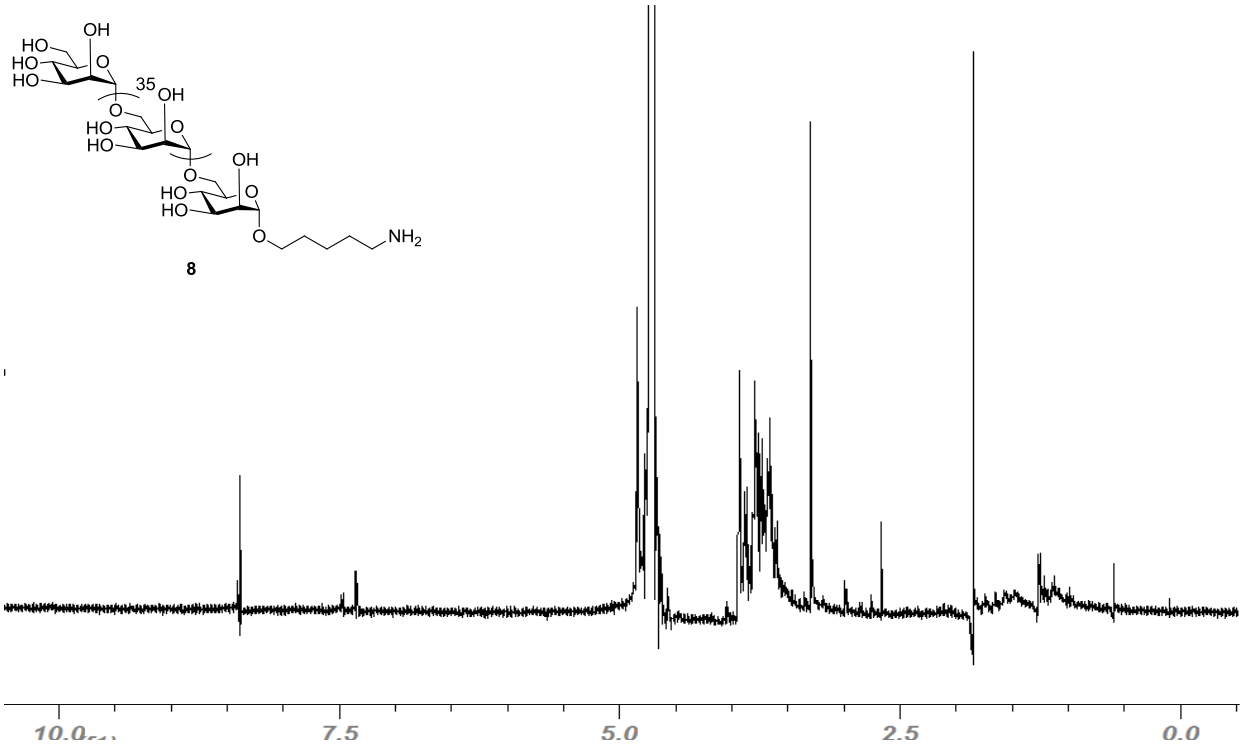


Figure S27. ^{1}H NMR of **8**.

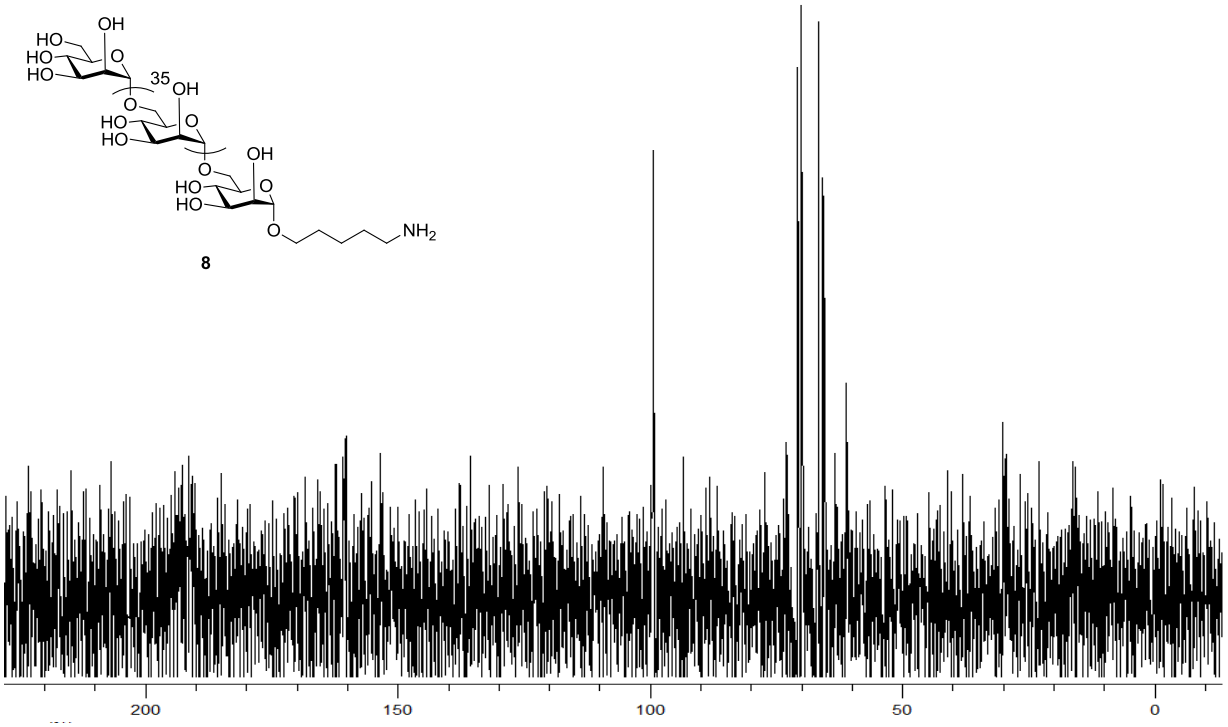


Figure S28. ^{13}C NMR of **8**.

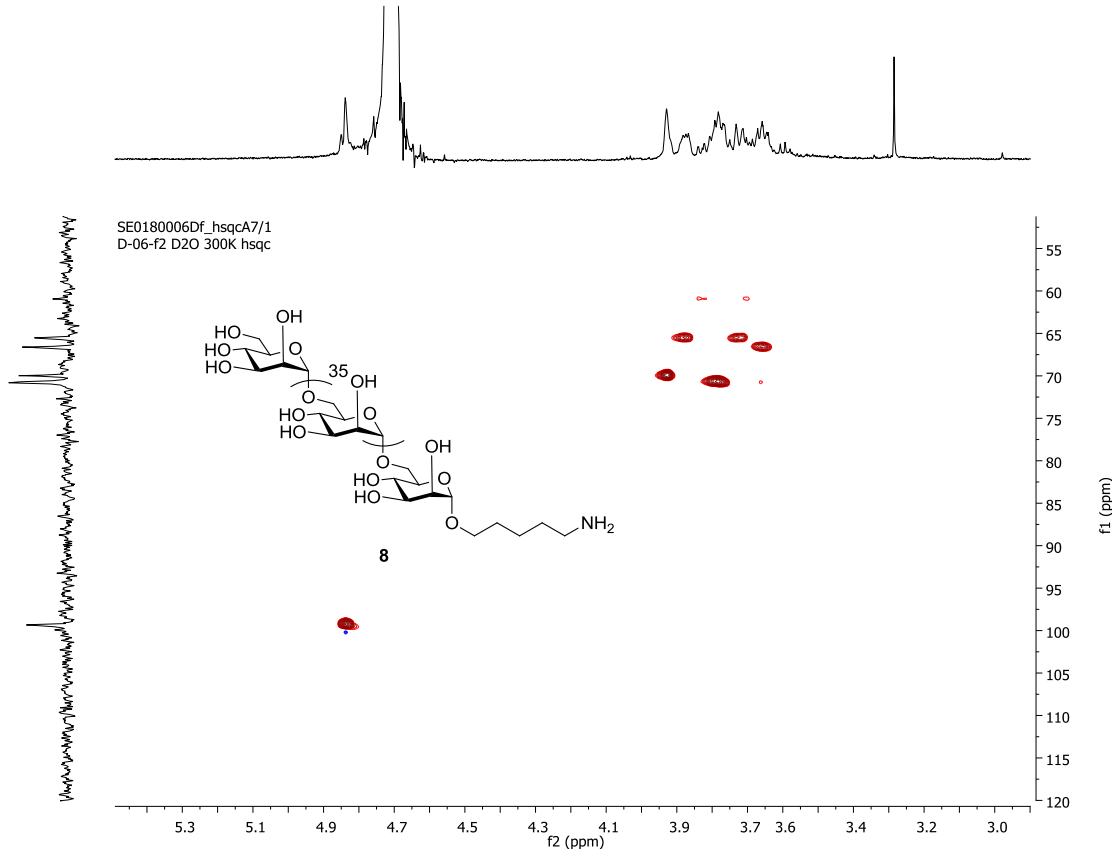


Figure S29. HSQC spectrum of **8**.

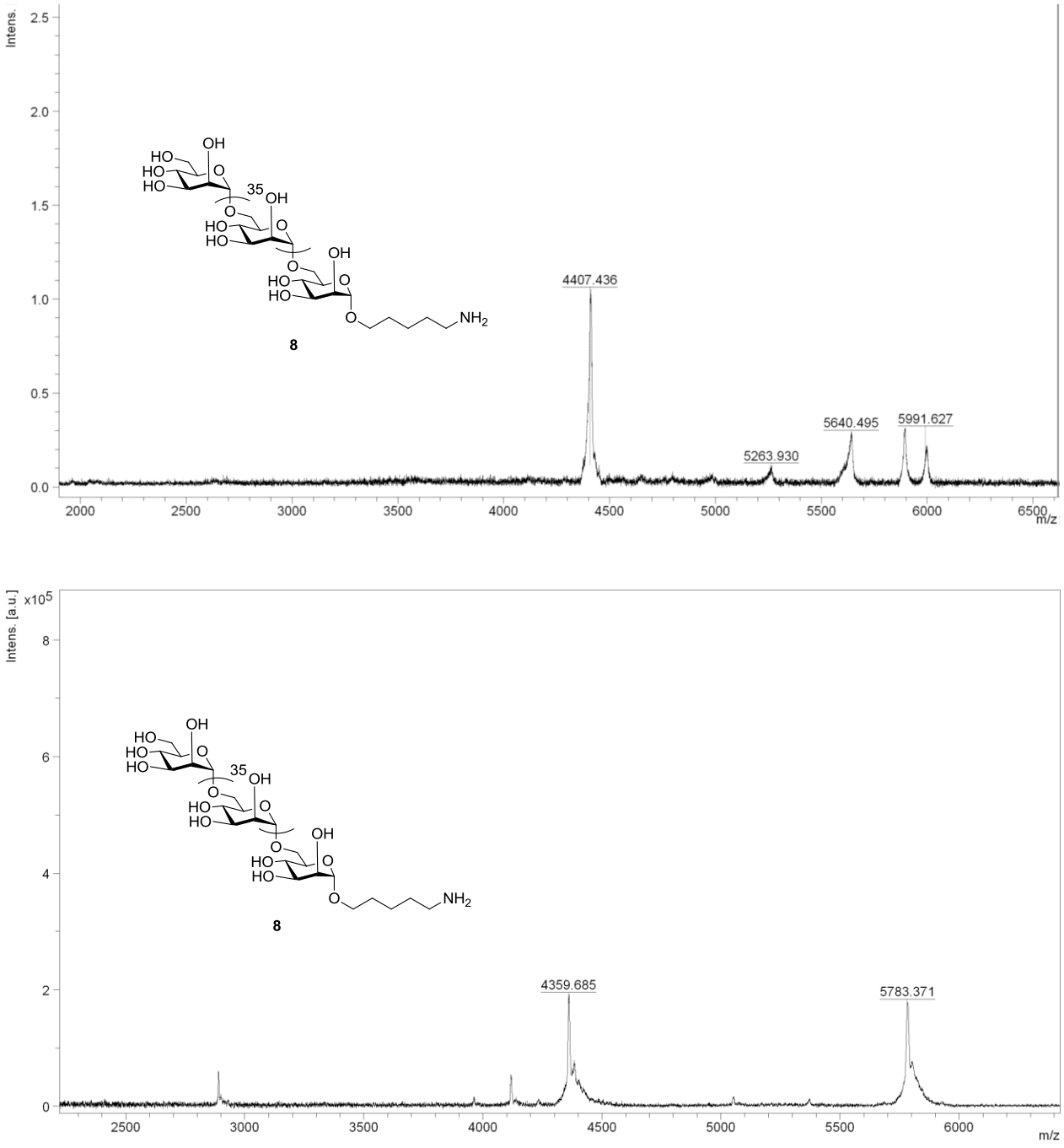


Figure S30. MALDI-TOF spectra of **8;** fragments top: M-(linker $C_5H_{12}NO$), calcd: 5997.9; found 5991.6 and bottom: M-(2 sugar residues $C_{12}H_{21}O_{10}$), calcd: 5774.9; found 5783.3

9. Syntheses of **10** and **12** (50mer)

Scheme S6

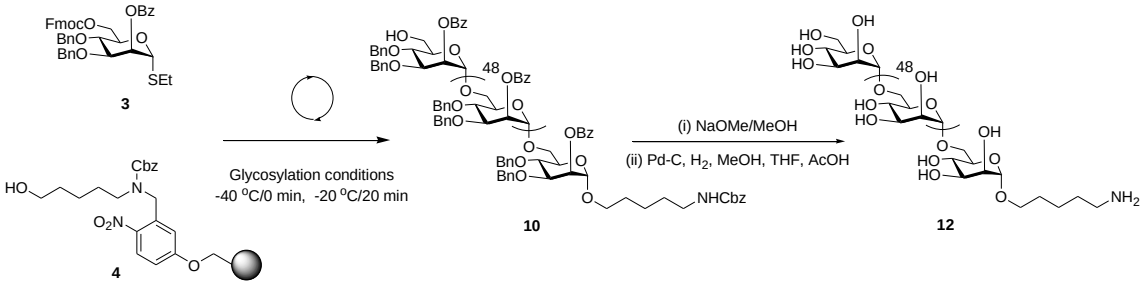


Table S6. AGA protocol for the assembly of 50mer **10** using building block **3**.

Step	Automation process	Module
1	Preparation of resin	**A**
2	Acid wash	**B**
3	Glycosylation with building block **3**	**C** (-40 °C for 0 min, -20 °C for 20 min)
4	Capping with Ac_2O	**E**
5	Fmoc deprotection	**D**
6	Chain elongation to 45mer	Repeat **B**, **C**, **E**, **D** (44 times)
7	Acid wash	**B**
8	Glycosylation with building block **3**	**C** (-40 °C for 0 min, -20 °C for 20 min)
9	Glycosylation with building block **3**	**C** (-40 °C for 0 min, -20 °C for 20 min)
10	Capping with Ac_2O	**E**
11	Fmoc deprotection	**D**
12	Chain elongation from 46mer to 50mer	Repeat **B**, **C**, **C**, **E**, **D** (five times)

The products of a 10 μmol (based on Fmoc loading) scale were cleaved from the resin as described in "Post-AGA Steps". The crude products were analyzed and purified using normal phase analytical HPLC with a YMC diol column. Linear gradient: EtOAc (2% DCM)/Hexane (2% DCM); 20% EtOAc (2% DCM) for 5 min, 20% to 60% EtOAc (2% DCM) over 40 min, 60 to 100% EtOAc (2% DCM) over 5 min. 50mer **10** eluted at 48.2 minutes.

Analytical data for 50mer **10**: Yield: 8.9 mg (5 %); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (d, *J* = 6.8 Hz, 100H), 7.58 – 7.37 (m, 125H), 7.23 – 6.88 (m, 530H), 5.83 (s, 50H), 5.37 (s, 2H), 5.03 (s, 50H), 4.93 – 4.82 (m, 50H), 4.77 (d, *J* = 10.8 Hz, 50H), 4.40 (d, *J* = 10.8 Hz, 50H), 4.32 (d, *J* = 11.6 Hz, 50H), 4.08 – 3.85 (m, 102H), 3.72-3.70 (m, 50H), 3.567-3.55 (m, 50H), 3.42-3.40 (m, 50H), 3.16-3.15 (m, 2H), 1.51 – 1.48 (m, 4H), 1.39 – 1.33 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.50, 138.46, 137.48, 133.29, 129.98, 129.84, 128.63, 128.31, 128.11, 127.66, 127.27, 126.98, 98.53, 78.18, 74.98, 73.68, 71.27, 70.87, 68.38, 65.70. MALDI-MS ($C_{1363}H_{1319}NNaO_{303}$): calcd. for $[M+Na]^+$ 22568.7; found 22570.4.

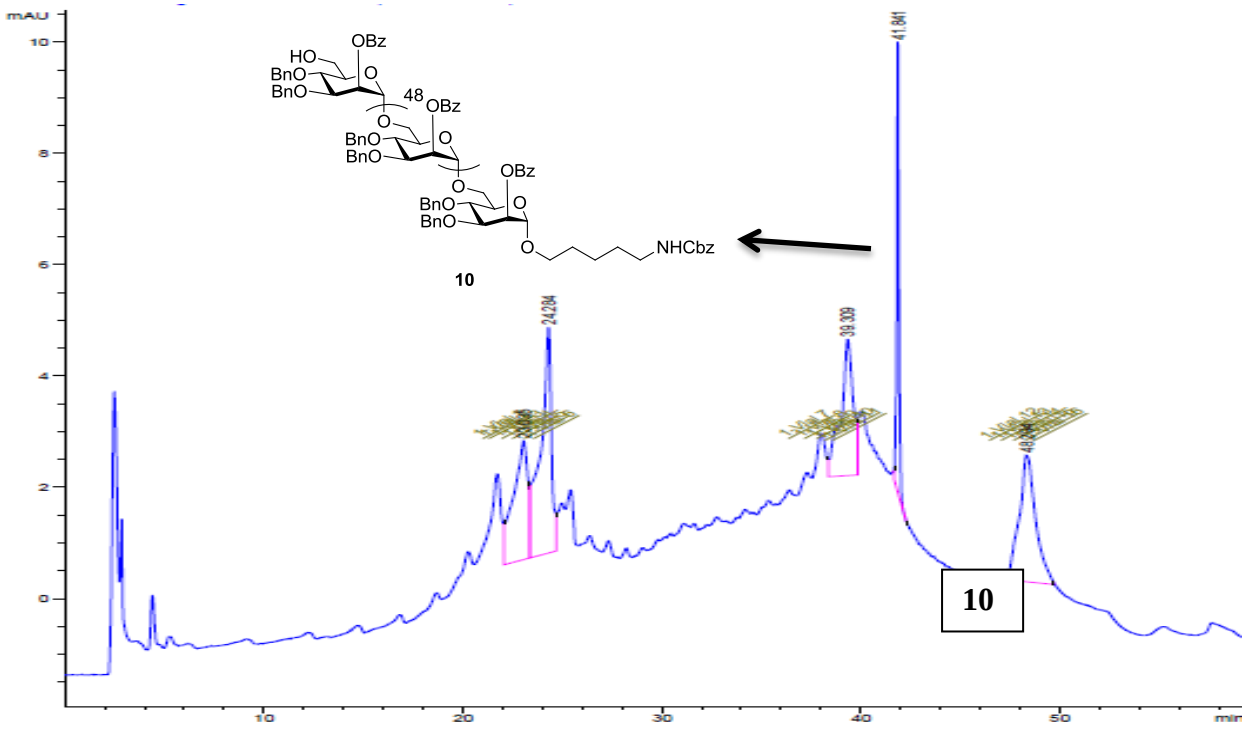


Figure S31. Analytical NP-HPLC YMC diol trace of crude 50mer **10** (UV-280 nm trace).

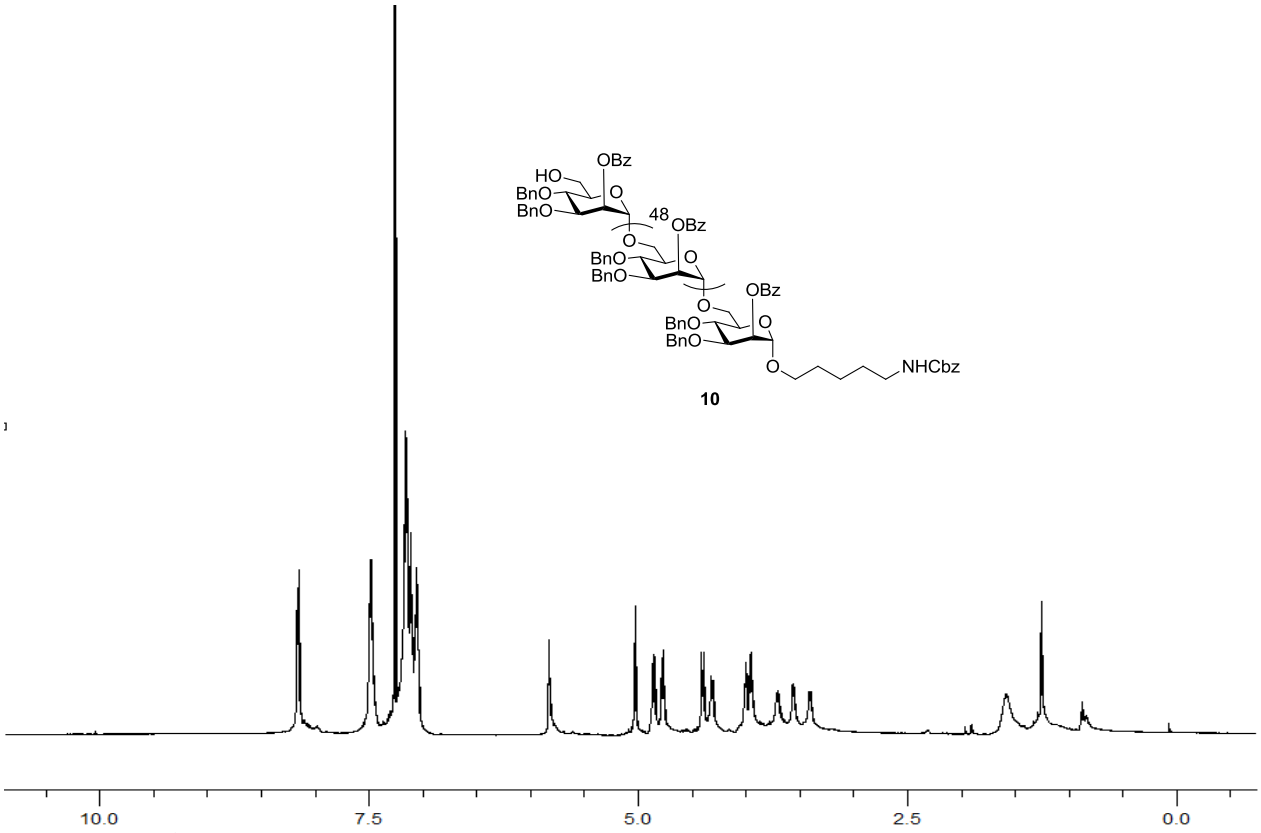


Figure S32. ^{1}H NMR of **10**.

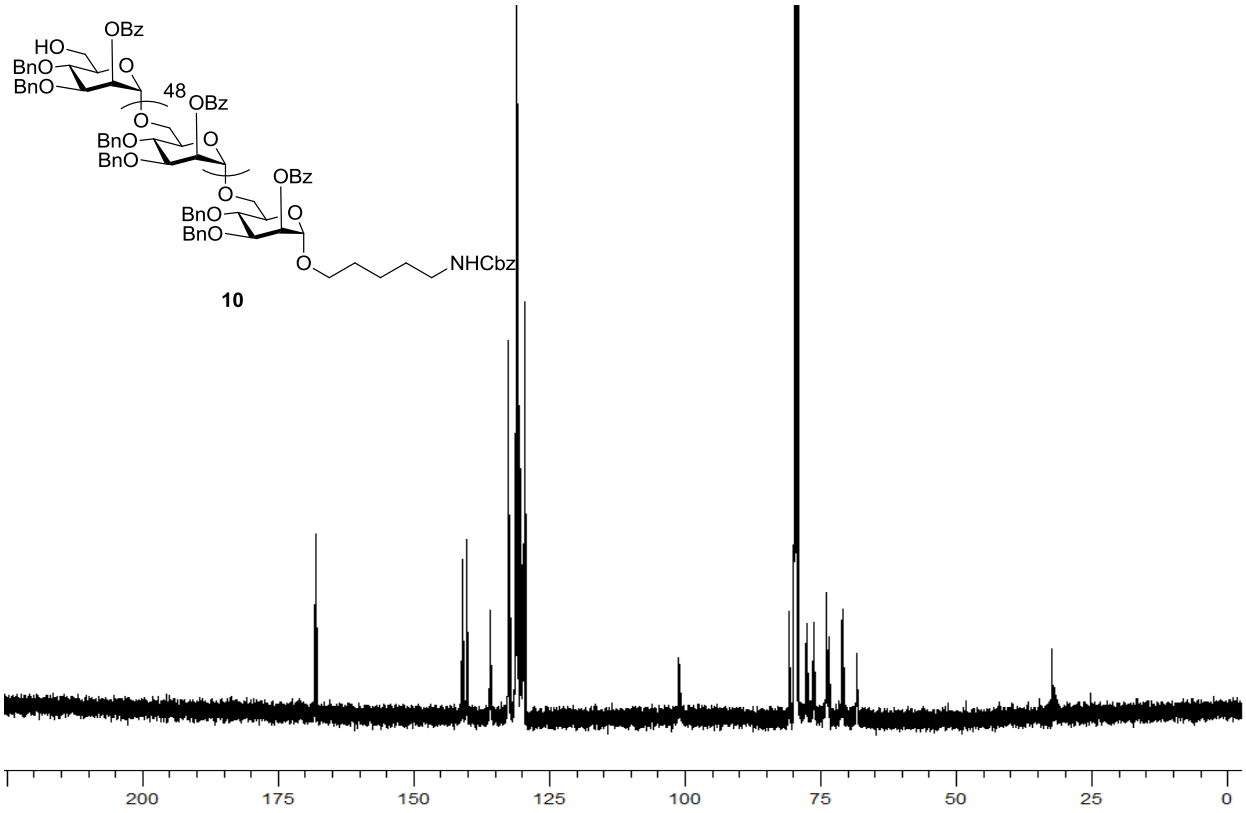


Figure S33. ^{13}C NMR of **10**.

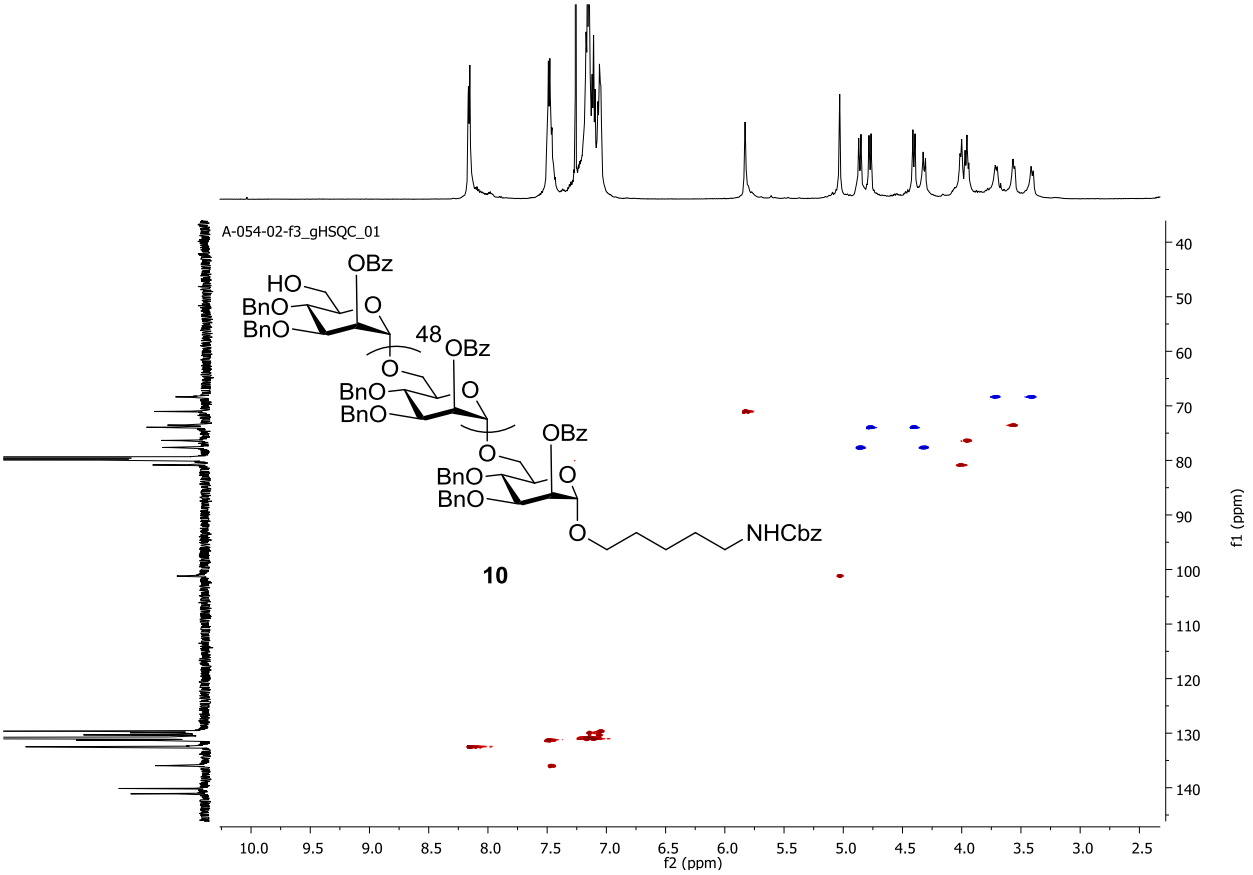


Figure S34. HSQC spectrum of **10**.

Mannose 50mer **10** (8.9 mg, 0.4μmol) was deprotected and purified to afford **12** following the general procedures for Zemplén methanolysis, hydrogenolysis and Sephadex LH20 purification. Yield: (1.3 mg, 39%); ^{1}H NMR (700 MHz, D_2O) 4.93 (br s, 25H), 4.02 (s, 50H), 3.98-3.95 (m, 50H), 3.93 – 3.71 (m, 200H). ^{13}C NMR (176 MHz, D_2O) δ 99.34, 70.83, 69.97, 67.89, 66.60, 65.52. MALDI-MS ($C_{305}H_{514}NNaO_{251}$): calcd for $[M+H]^+$ 8206.7, found 8212.7.

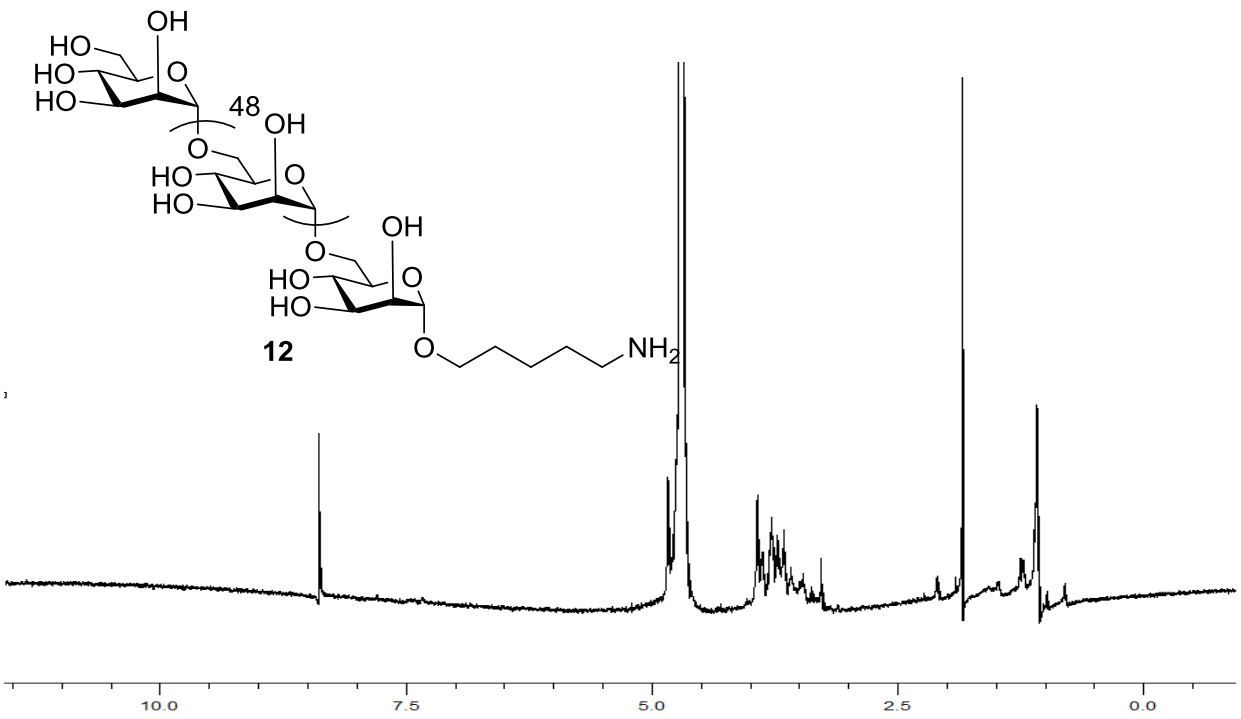


Figure S35. ^{1}H NMR of **12**.

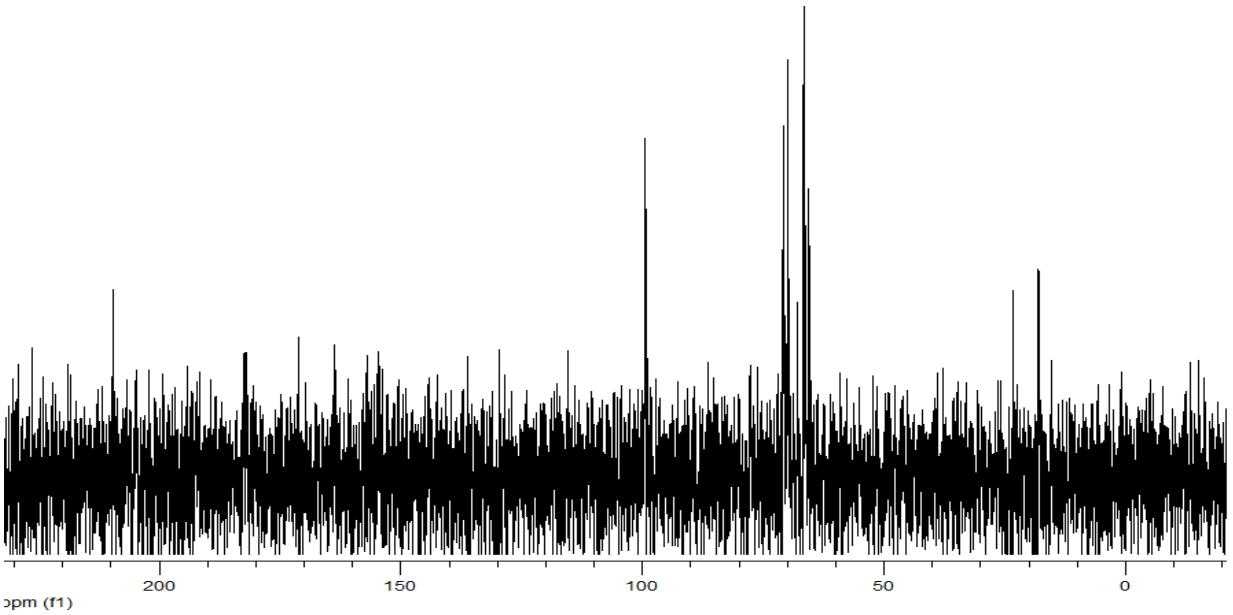


Figure S36. ^{13}C NMR of **12**.

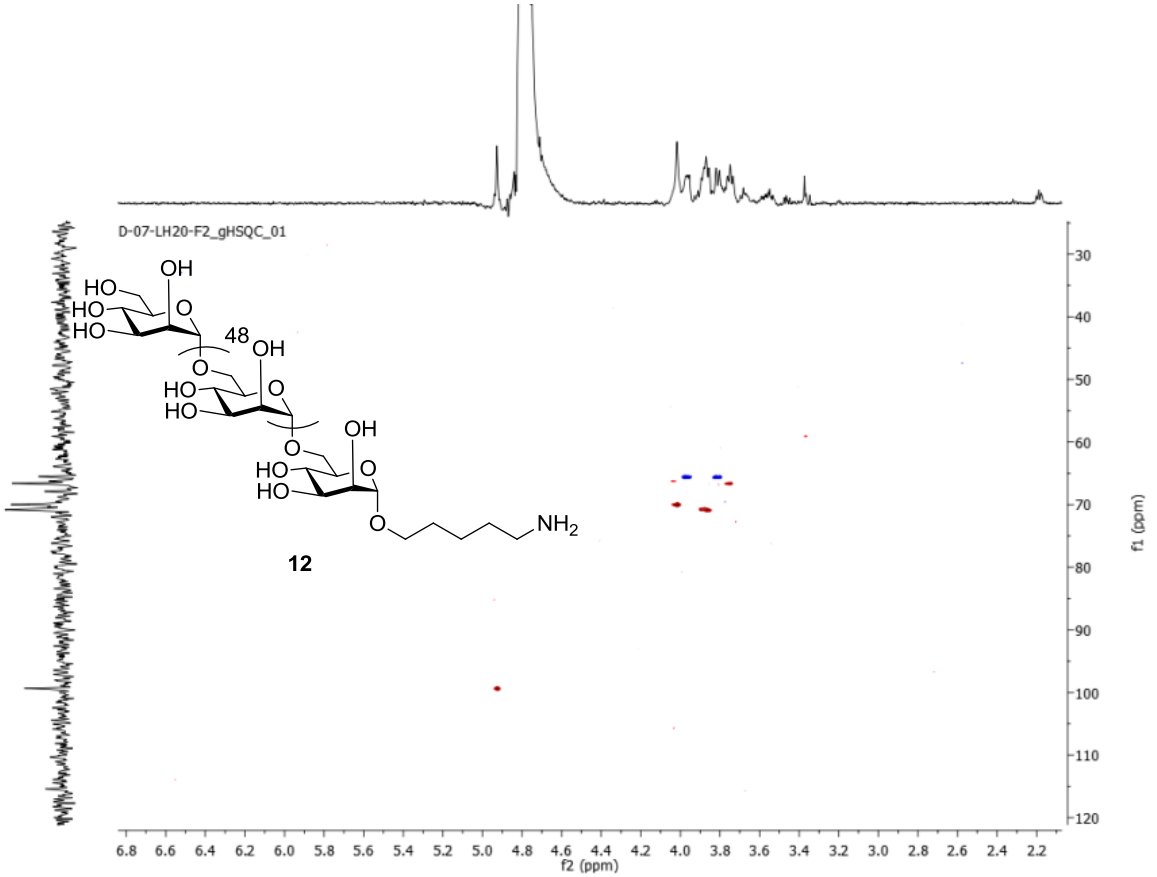
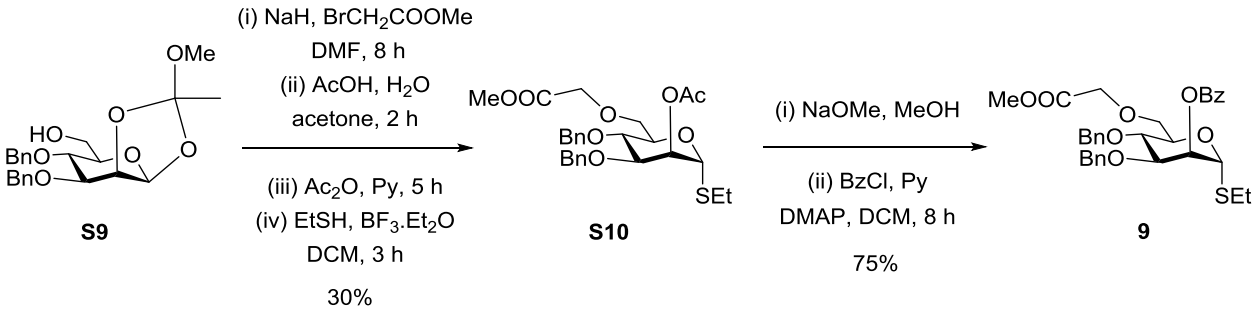


Figure S37. HSQC spectrum of **12**.

10. Synthesis of Building Block **9**

Scheme S7



Mannose orthoester **S9**[3] (8 g, 19 mmol) dissolved in DMF (5 mL) was added to a suspension of NaH (1.91 g, 60% in mineral oil, 48 mmol) in DMF (75 mL). After stirring for 30 min at 0 ºC, methyl bromoacetate (4.4 mL, 48 mmol) was added slowly and the mixture was stirred at room temperature for 24 h. TLC analysis showed the presence of starting material. Excess NaH (3.8 g,

60% in mineral oil, 96 mmol) and methyl bromoacetate (8.9 mL, 96 mmol) were added and stirred at the room temperature for another 24 h. The reaction mixture was quenched with methanol and concentrated *in vacuo* and purified. The orthoester (5.65 g, 12 mmol) was dissolved in AcOH:H_2O:Acetone (6:3:1, 100 mL) and stirred for 2 h at rt. The solvent was removed *in vacuo* and the residue co-evaporated with toluene. Acetic anhydride (25 mL) was added to a solution of the crude residue in pyridine (25 mL) at 0 °C and stirred at room temperature for 12 h. The solvent was removed *in vacuo* and the residue co-evaporated with toluene and purified. $BF_3.OEt_2$ (2 mL, 16 mmol) was added dropwise at 0 °C to a solution of diacetate (5.4 g, 10 mmol) and ethanethiol (2 mL, 26 mmol) in CH_2Cl_2. After stirring for 12 h at rt, the reaction mixture was diluted with CH_2Cl_2 and washed with water, aq. $NaHCO_3$ solution and with water. The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and purified (Hexanes: EtOAc 4:1) to afford **S10** as a solid. Yield: (2.95 g, 30% over four steps). $[\alpha]_D^{20}$ +69.3 (c 1, $CHCl_3$); IR (neat) ν_{max} = 2956, 1743,1455, 1373, 1233, 1103 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.45 – 6.94 (m, 10H), 5.40 (s, 1H), 5.26 (s, 1H), 4.88 (d, *J* = 10.8 Hz, 1H), 4.74 – 4.57 (m, 2H), 4.50 (d, *J* = 11.3 Hz, 1H), 4.21-4.06 (m, 3H), 3.93 (dd, *J* = 10.8, 4.0 Hz, 1H), 3.90 – 3.85 (m, 1H), 3.77 – 3.63 (m, 5H), 2.65 – 2.49 (m, 2H), 2.13 (s, 3H), 1.24 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.51, 170.24, 138.36, 137.53, 128.28, 128.22, 127.97, 127.80, 127.68, 127.52, 82.30, 78.36, 74.95, 73.99, 71.66, 71.59, 70.32, 70.03, 68.56, 51.61, 25.36, 21.03, 14.73. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{27}H_{34}NaO_8S$: 541.1872; found: 541.1875.

To a solution of **S10** (2.85 g, 5.5 mmol) in methanol, sodium methoxide in methanol (0.5 M, pH ~13) was added and stirred at room temperature for 12 h, neutralized with Amberlite ion-exchange (H^+) resin, filtered and concentrated *in vacuo*. To a cooled solution of crude residue, pyridine (1.7 mL, 22 mmol) and DMAP (0.13 g, 1 mmol) in CH_2Cl_2, benzoyl chloride (1.27 mL, 11 mmol) was added slowly. The mixture was stirred at room temperature for 24 h, diluted with CH_2Cl_2 and washed with aq. HCl, a saturated aq. $NaHCO_3$ solution, and water. The organic phase was dried over sodium sulfate, filtered, and the filtrate was concentrated and purified (hexanes: EtOAc 85:15) to afford **9** as a solid. Yield: (2.4 g, 75% over two steps). $[\alpha]_D^{20}$ +55.8 (c 1, $CHCl_3$); IR (neat) ν_{max} = 2875, 1756, 1721, 1453, 1267, 1095 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 7.3 Hz, 2H), 7.59 (d, *J* = 7.3 Hz, 1H), 7.49 (t, *J* = 7.3 Hz, 2H), 7.41 – 7.22 (m, 10H), 5.70 (d, *J* = 1.9 Hz, 1H), 5.43 (s, 1H), 4.93 (d, *J* = 10.8 Hz, 1H), 4.79-4.70 (m, 2H), 4.58 (d, *J* = 11.4 Hz, 1H), 4.30-

4.26 (m, 1H), 4.23-4.21 (m, 1H), 4.18 – 4.05 (m, 2H), 4.05 – 3.97 (m, 2H), 3.81-3.78 (m, 1H), 3.74 (s, 3H), 2.68-2.63 (m, 2H), 1.30 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.55, 165.64, 138.38, 137.65, 133.15, 129.93, 129.83, 128.38, 128.27, 127.99, 127.64, 127.59, 82.49, 78.53, 75.07, 74.10, 71.89, 71.51, 70.82, 70.16, 68.79, 51.68, 25.58, 14.88. ESI HR-MS: m/z $[M+Na]^+$calcd. for $C_{32}H_{36}NaO_8S$: 603.2029; found: 603.2025.

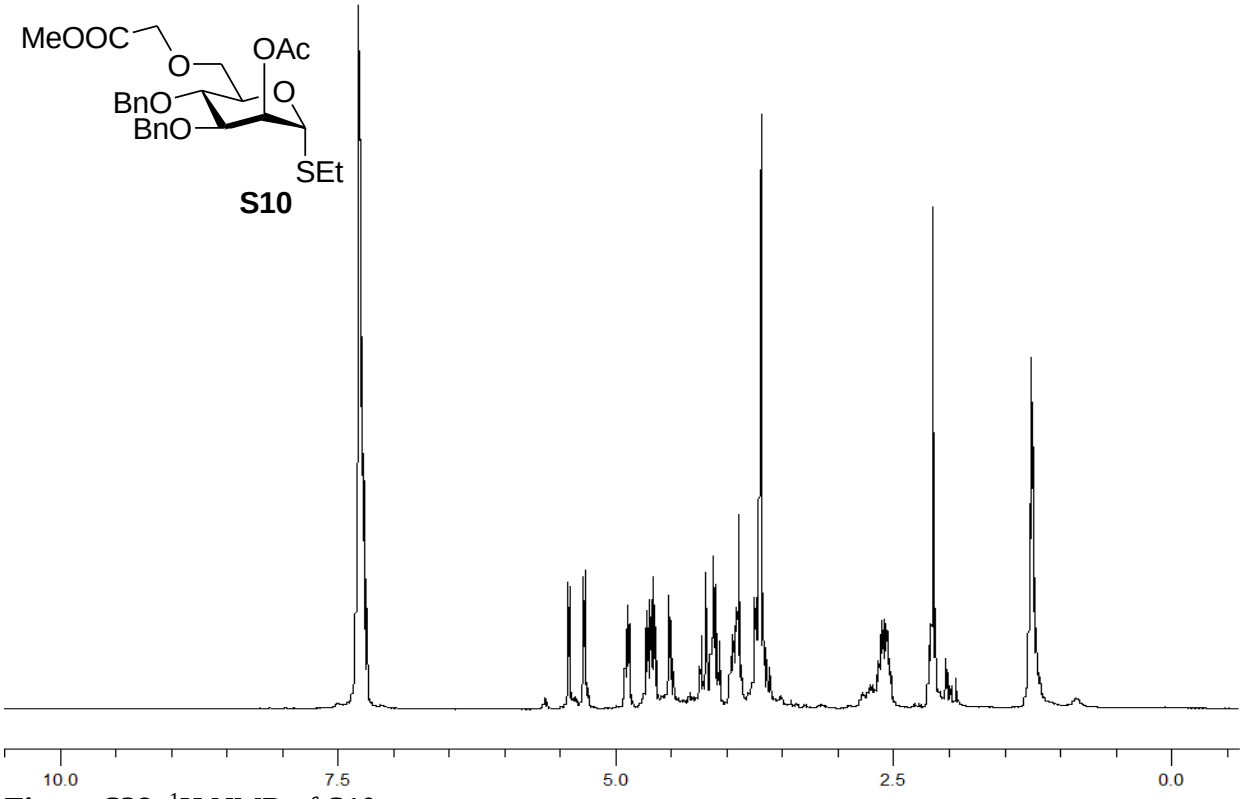


Figure S38. ^{1}H NMR of **S10**.

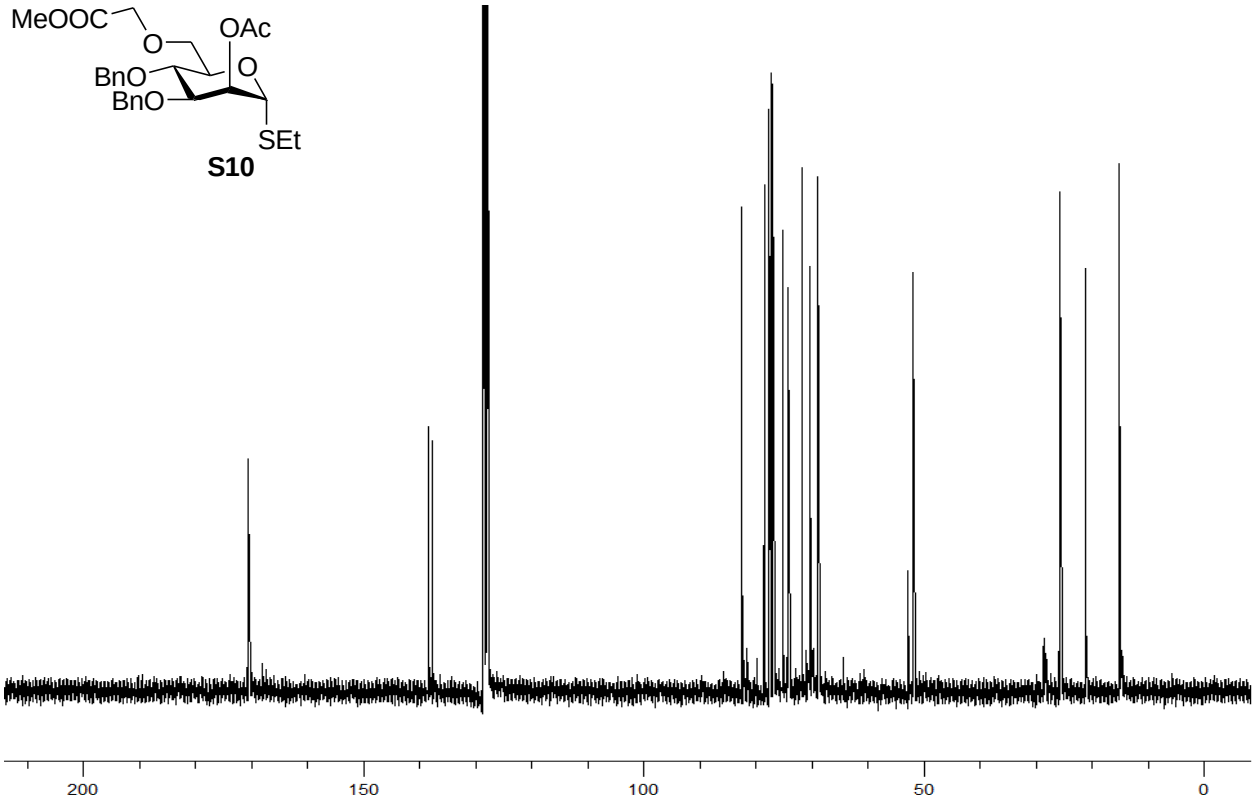


Figure S39. ^{13}C NMR of **S10**.

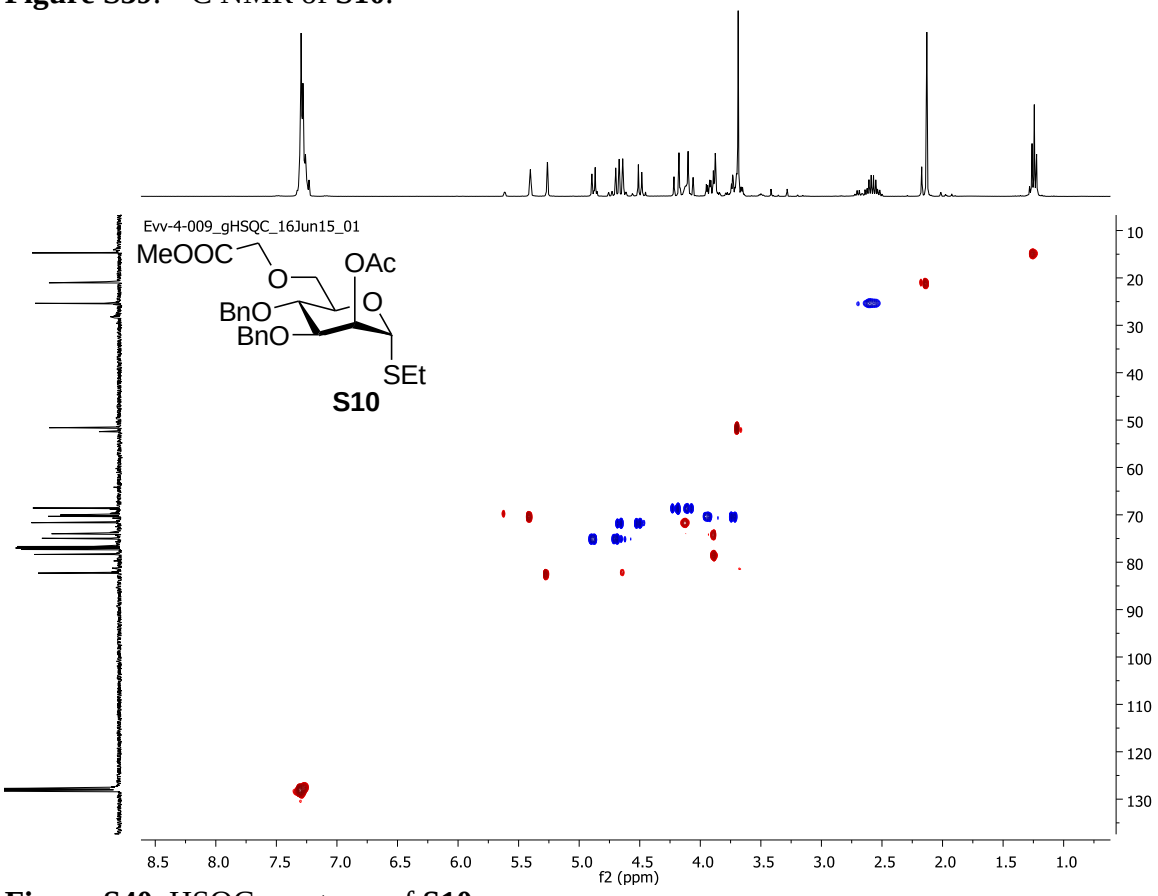


Figure S40. HSQC spectrum of **S10**

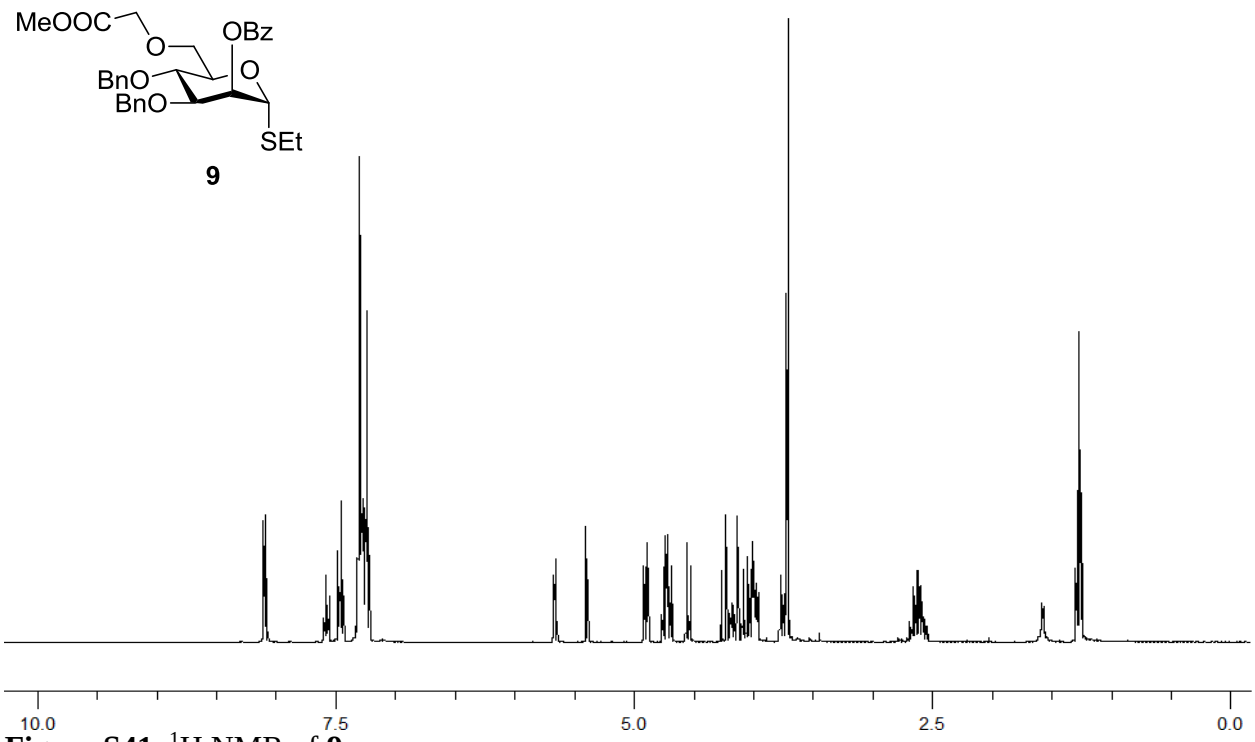


Figure S41. ^{1}H NMR of **9**.

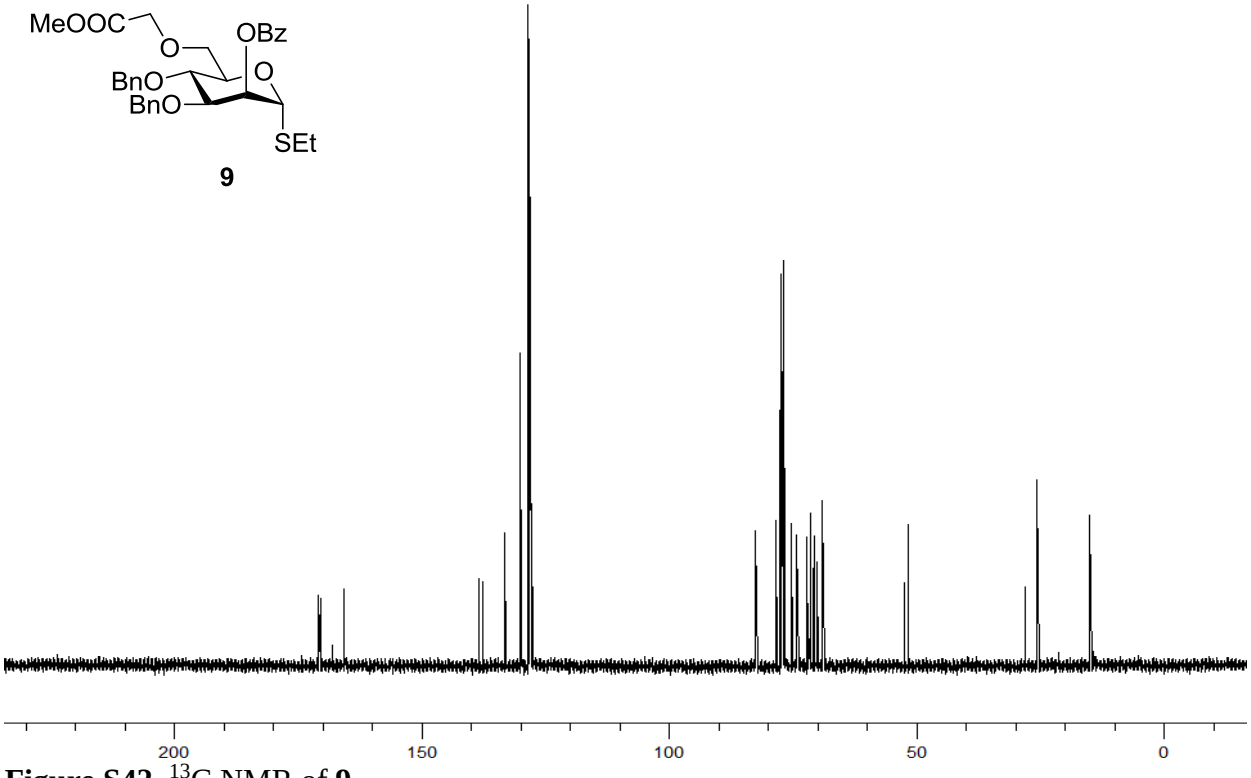


Figure S42. ^{13}C NMR of **9**.

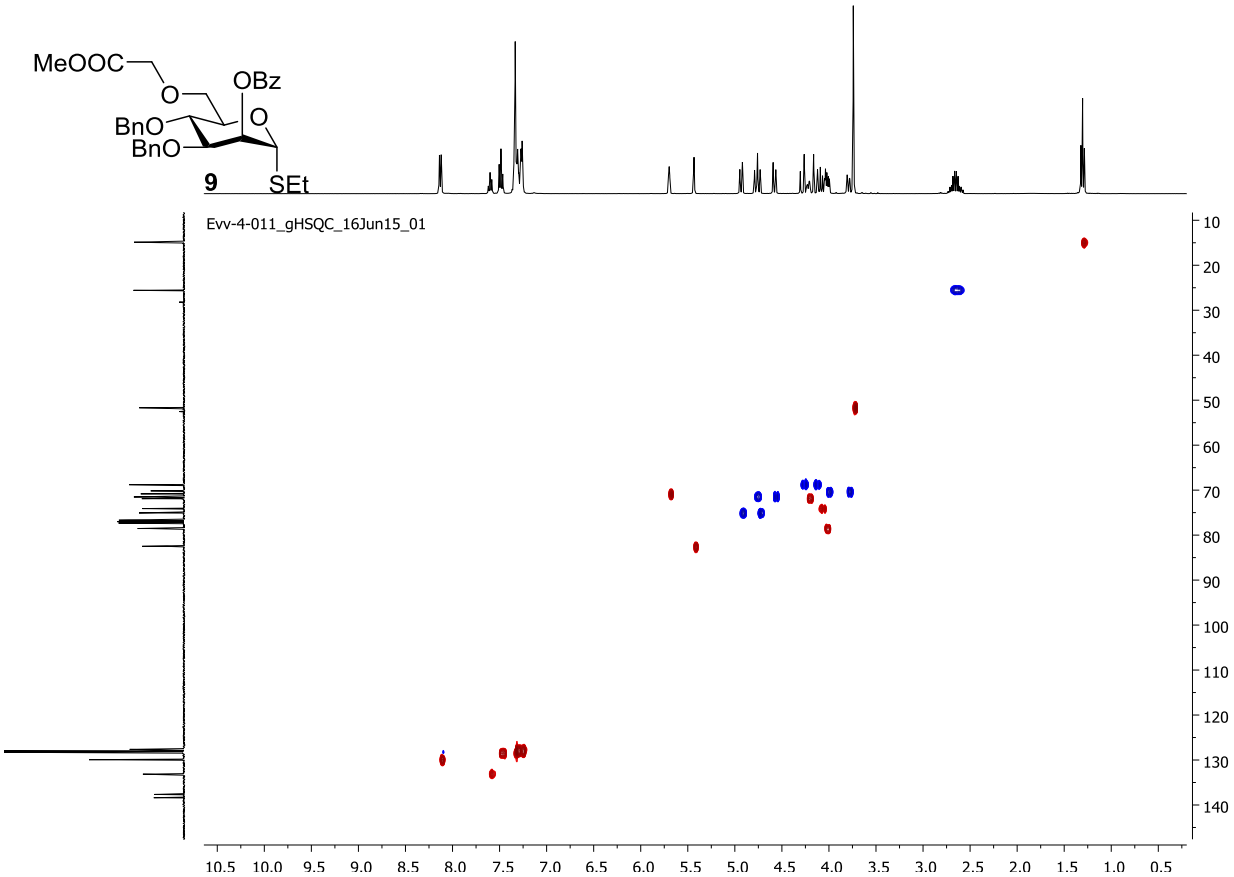


Figure S43. HSQC spectrum of **9**.

11. Syntheses of **11** and **13** (25mer)

Scheme S8

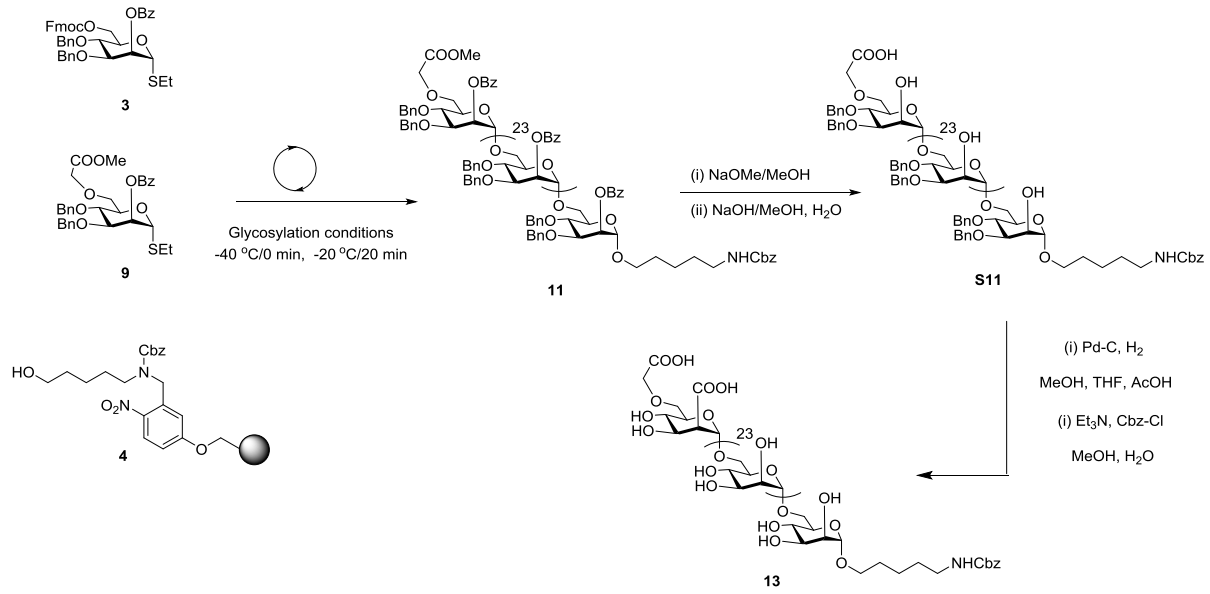


Table S7. AGA protocol for the assembly of 25mer **11** using building blocks **3** and **9**.

Step	Automation process	Module
1	Preparation of resin	**A**
2	Acid wash	**B**
3	Glycosylation with building block **3**	**C** (-40 °C for 0 min, -20 °C for 20 min)
4	Fmoc deprotection	**D**
5	Chain elongation to 20mer	Repeat **B**, **C**, **D** (19 times)
6	Acid wash	**B**
7	Glycosylation with building block **3**	**C** (-40 °C for 0 min, -20 °C for 20 min)
8	Glycosylation with building block **3**	**C** (-40 °C for 0 min, -20 °C for 20 min)
9	Capping with Ac_2O	**E**
10	Fmoc deprotection	**D**
11	Chain elongation from 21mer to 25mer	Repeat **B**, **C**, **C**, **E**, **D** (four times)
12	Acid wash	**B**
13	Glycosylation with building block **9**	**C** (-40 °C for 0 min, -20 °C for 20 min)
14	Glycosylation with building block **9**	**C** (-40 °C for 0 min, -20 °C for 20 min)
15	Capping with Ac_2O	**E**

The products were cleaved from the solid support as described in "Post-AGA Steps". The crude products were analyzed and purified using normal phase analytical HPLC with a YMC diol column. Linear gradient: EtOAc (2% DCM)/Hexane (2% DCM); 20% EtOAc (2% DCM) for 5 min, 20% to 60% EtOAc (2% DCM) over 40 min, 60 to 100% EtOAc (2% DCM) over 5 min. 25mer **11** eluted at 25.3 minutes.

Analytical data for 25mer **11**: Yield: 31 mg (28 %); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.22 – 7.95 (m, 50H), 7.62 – 7.38 (m, 62H), 7.36 – 6.92 (m, 268H), 5.88 – 5.73 (m, 24H), 5.62 (s, 2H), 5.48 (s, 1H), 5.12 – 5.01 (m, 24H), 4.92 – 4.74 (m, 48H), 4.72 (d, *J* = 11.3 Hz, 2H), 4.66 (d, *J* = 11.3 Hz, 2H), 4.56 (d, *J* = 11.0 Hz, 2H), 4.50 – 4.25 (m, 48H), 4.22-4.19 (m, 3H), 4.12 – 3.86 (m, 49H), 3.81 – 3.64 (m, 29H), 3.60-3.56 (m, 24H), 3.44-3.41 (m, 25H), 3.1-3.16 (m, 4H), 1.51 – 1.48 (m, 4H), 1.39 – 1.33 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.56, 165.83, 165.50, 138.57, 138.46, 138.24, 137.93, 137.60, 137.49, 133.29, 129.99, 129.93, 129.84, 129.68, 128.62, 128.55, 128.46, 128.40, 128.34, 128.31, 128.27, 128.26, 128.23, 128.20, 128.11, 128.02, 128.00, 127.89, 127.66, 127.61, 127.55, 127.44, 127.40, 127.31, 127.27, 127.21, 127.08, 126.99, 98.53, 98.25, 97.86, 78.58, 78.18, 77.72, 75.16, 74.98, 73.69, 71.89, 71.62, 71.40, 71.28, 71.15, 70.88, 69.06, 68.84, 68.36, 65.70, 51.64, 29.69, 29.02, 23.42. MALDI-MS ($C_{691}H_{674}NO_{155}$): calcd for $[M+H]^{+}$11464.4, found 11468.5.

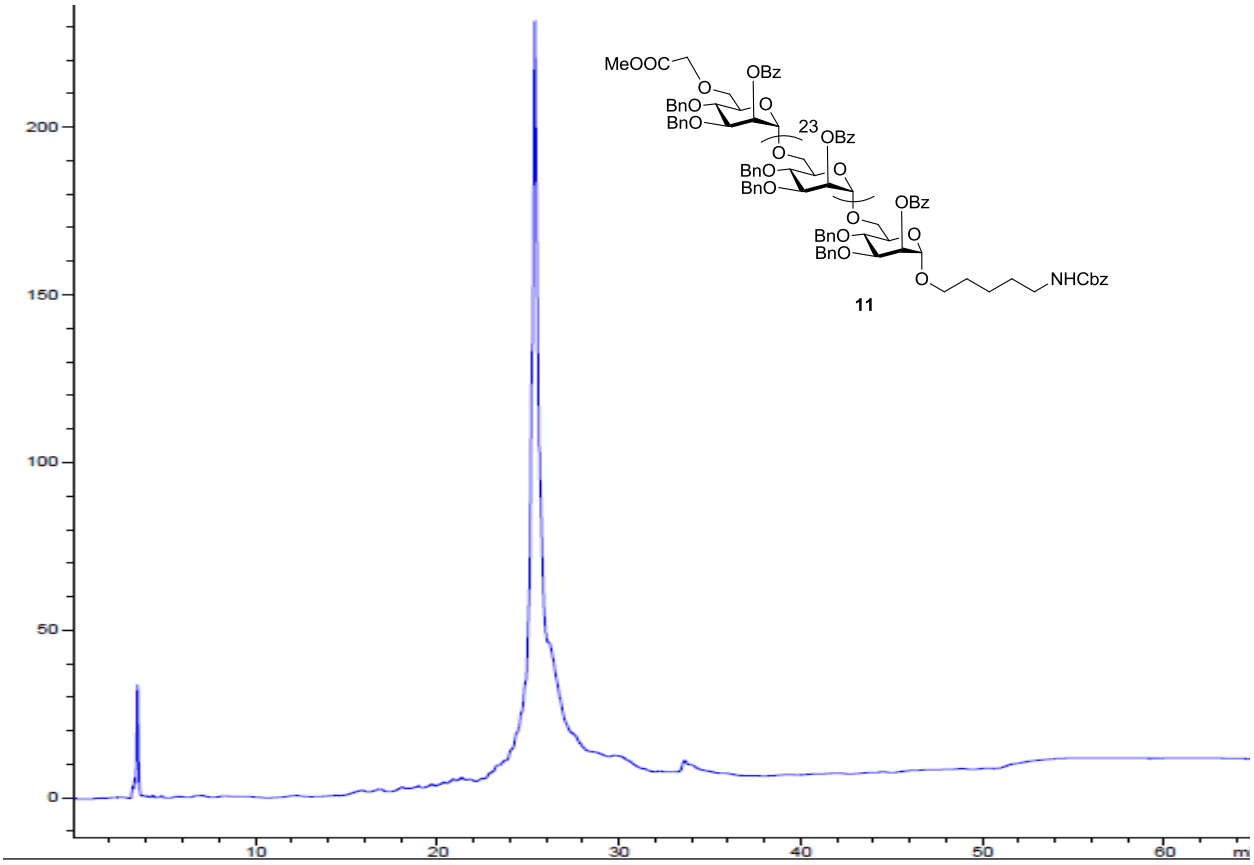


Figure S44. Analytical NP-HPLC YMC diol trace of crude 25mer **11** (UV-280 nm trace).

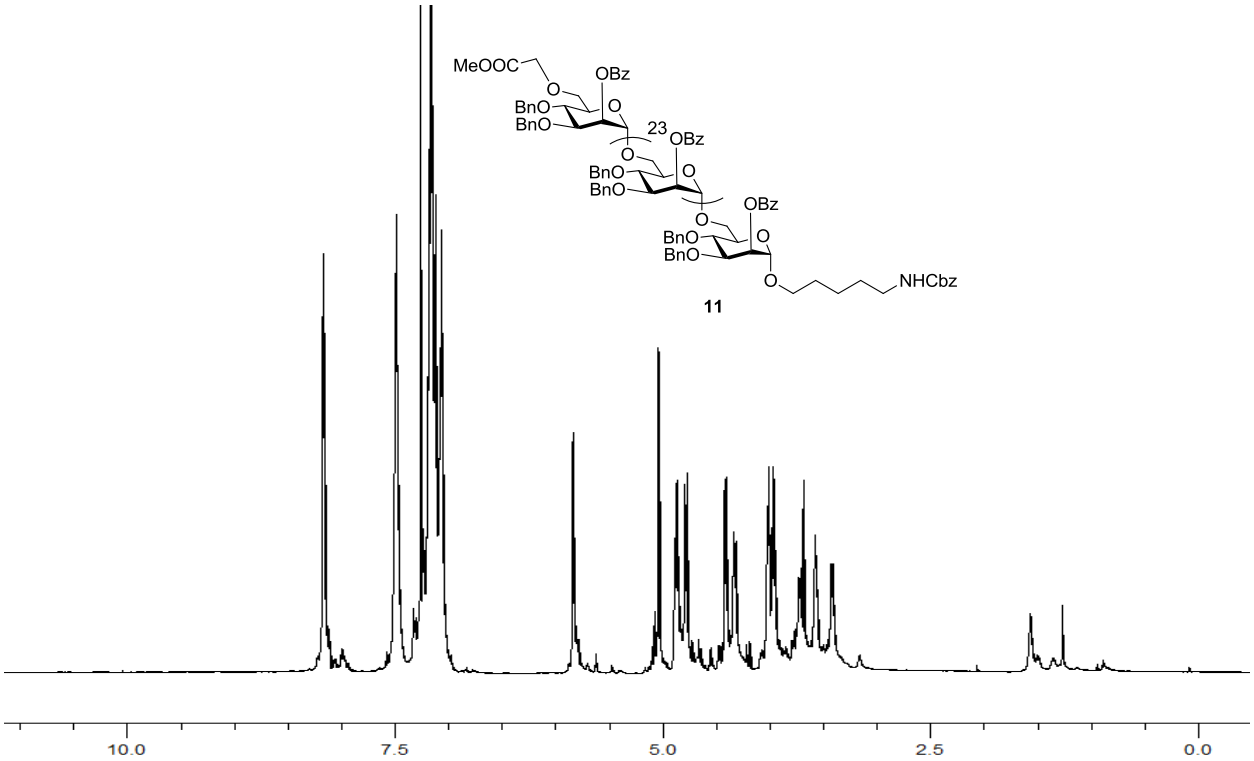


Figure S45. ^{1}H NMR of **11**.

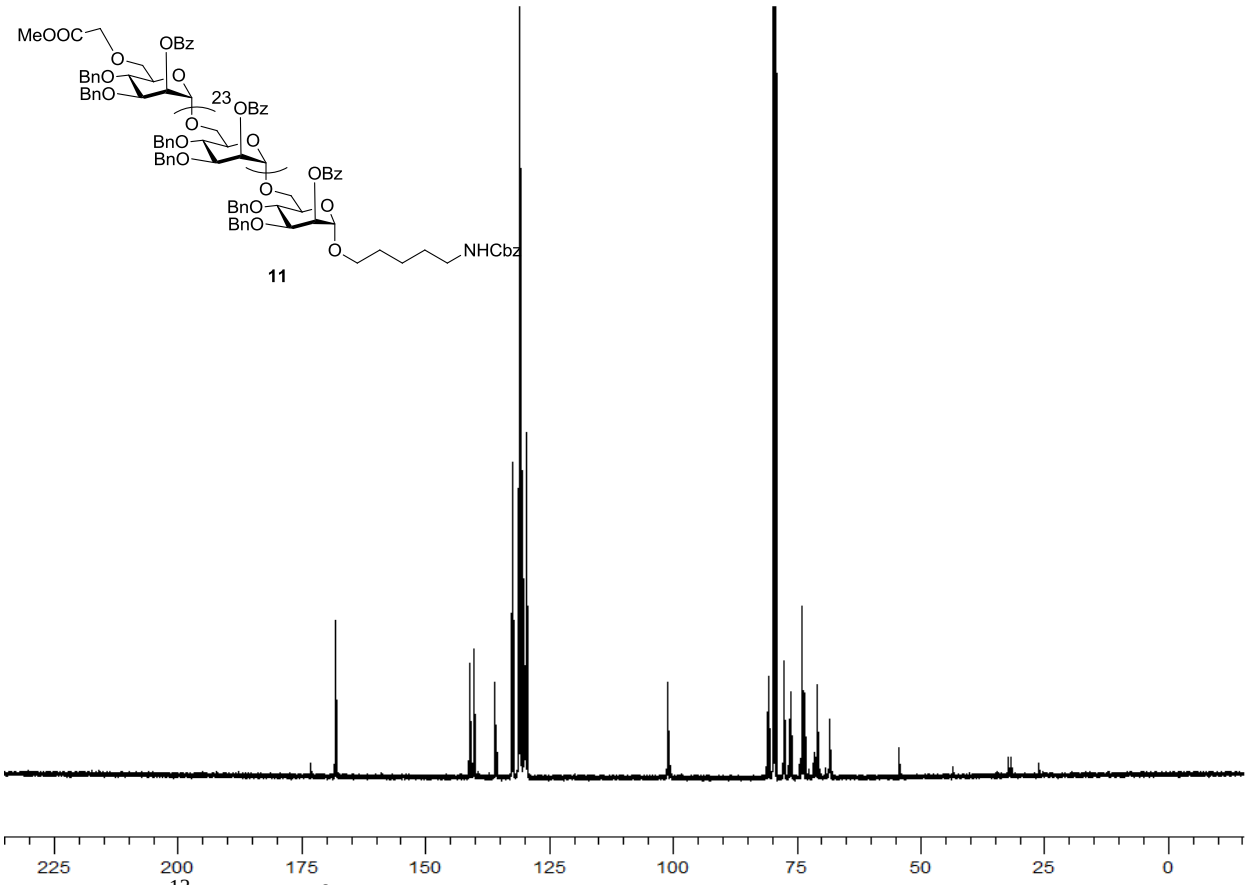


Figure S46. ^{13}C NMR of **11**.

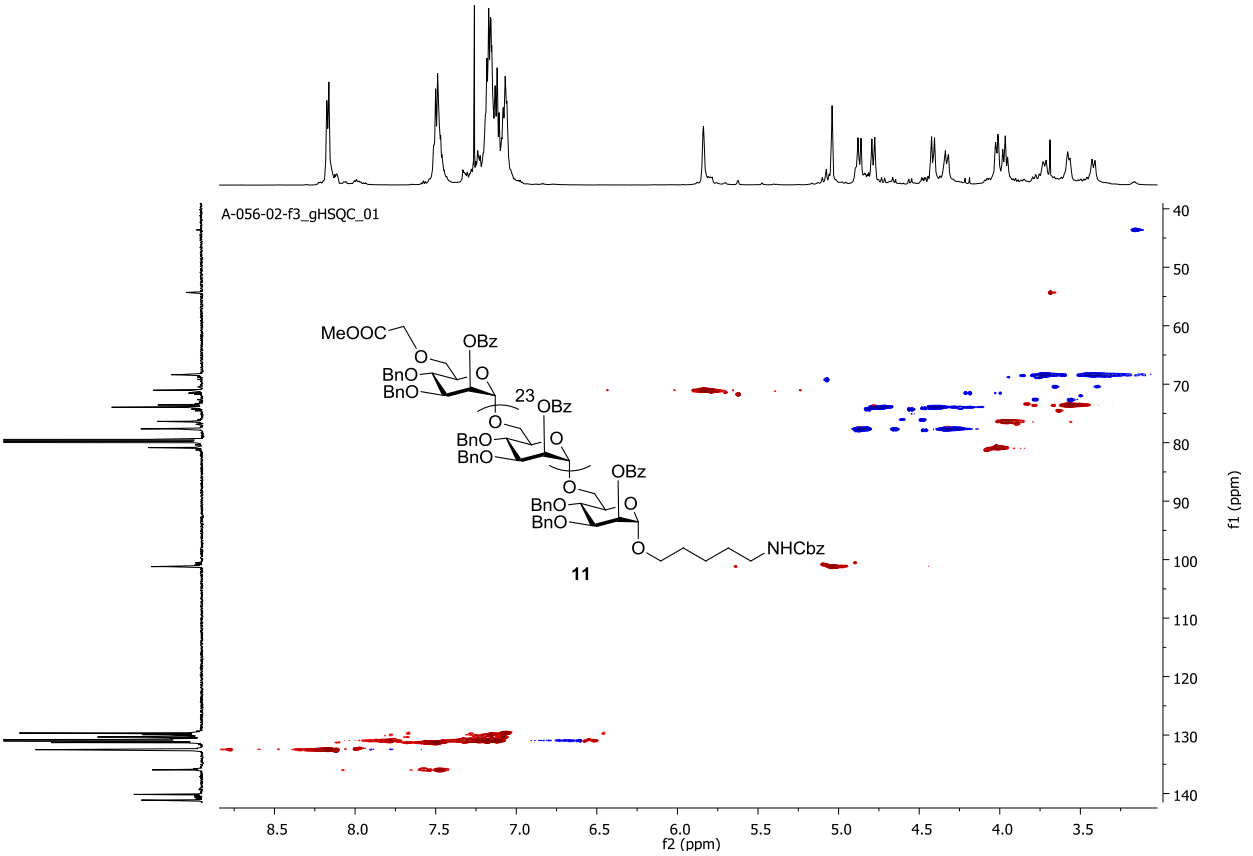


Figure S47. HSQC spectrum of **11**.

Mannose 25mer **11** (18mg, 1.5µmol) was deprotected and purified to afford **13**. After the removal of the esters to afford **S11**, the methyl ester group was subjected to hydrolysis under basic conditions: to a solution of crude residue from the Zemplén methanolysis in methanol, NaOH (10 eq) in water was added and stirred at room temperature for 12 h, neutralized with Amberlite ion exchange (H^+) resin, filtered and concentrated *in vacuo.* The amine group was protected with CBz prior to purification. To a solution of amine in water, Et_3N (10 µL) and CbzCl (10 µL) were added at 0 °C and stirred at room temperature for 18 h and concentrated *in vacuo*.

Analytical data for **13**: Yield: 4.9 mg (75%); ^{1}H NMR (700 MHz, D_2O) δ 4.93 (br s, 25H), 4.02 (s, 25H), 3.98-3.97 (m, 25H), 3.93 – 3.71 (m, 100H). ^{13}C NMR (176 MHz, D_2O) δ 99.33, 70.82, 70.68, 69.97, 66.59, 65.50. MALDI-MS ($C_{165}H_{271}NNaO_{130}$): calcd for $[M+Na]^+$ 4370.5, found 4372.7.

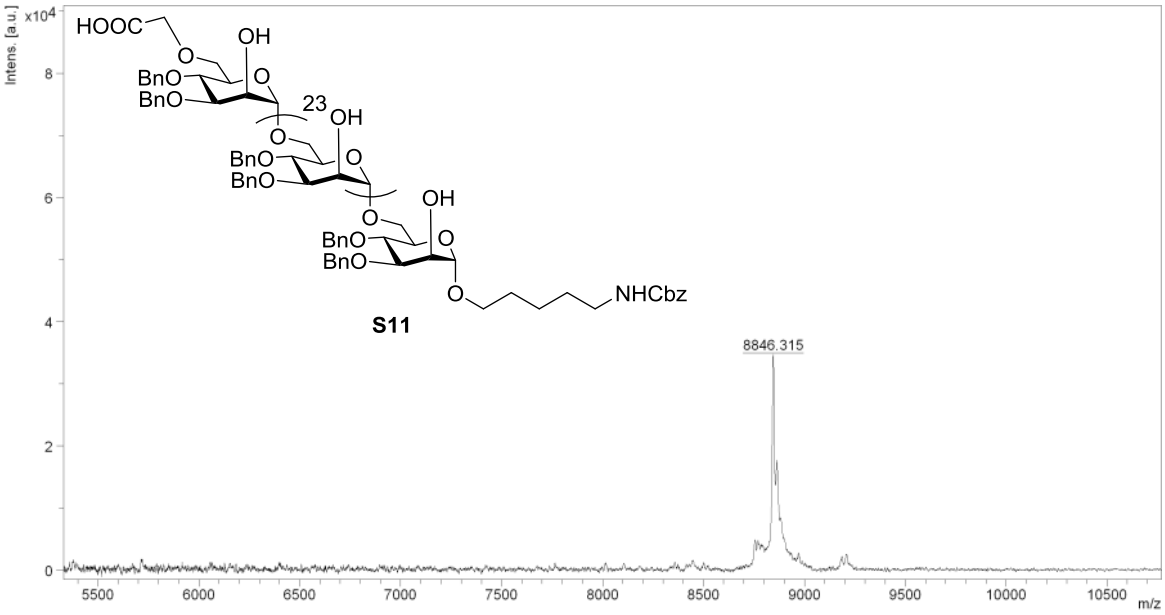


Figure S48. MALDI-TOF spectrum of **S11**.

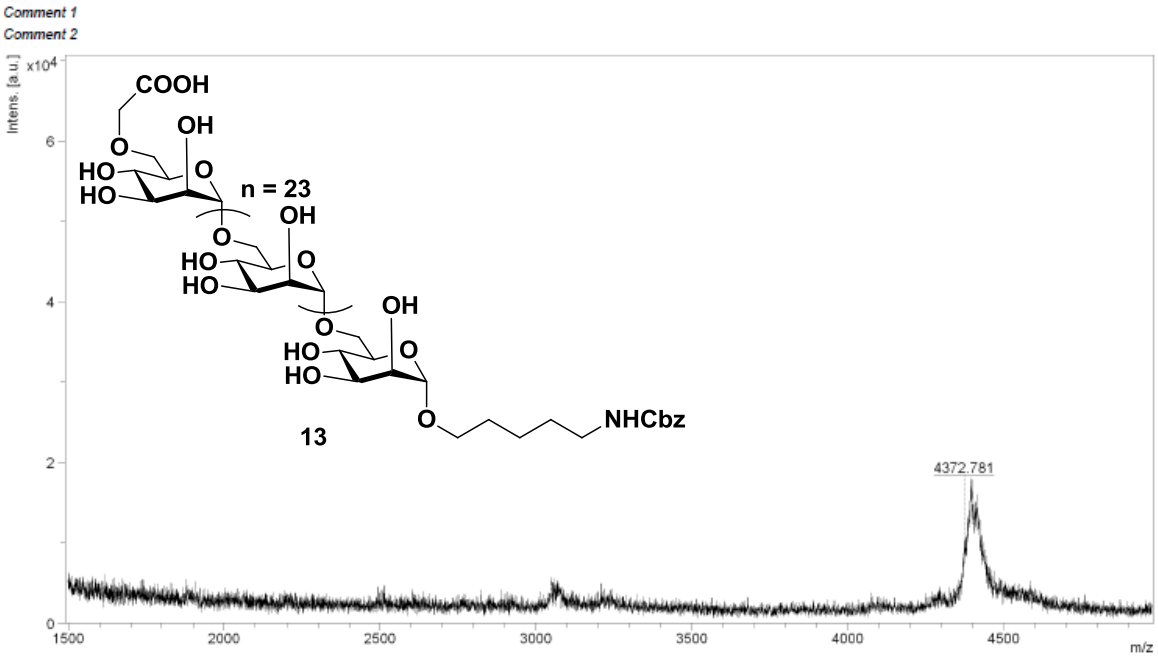


Figure S49. MALDI-TOF spectrum of **13**.

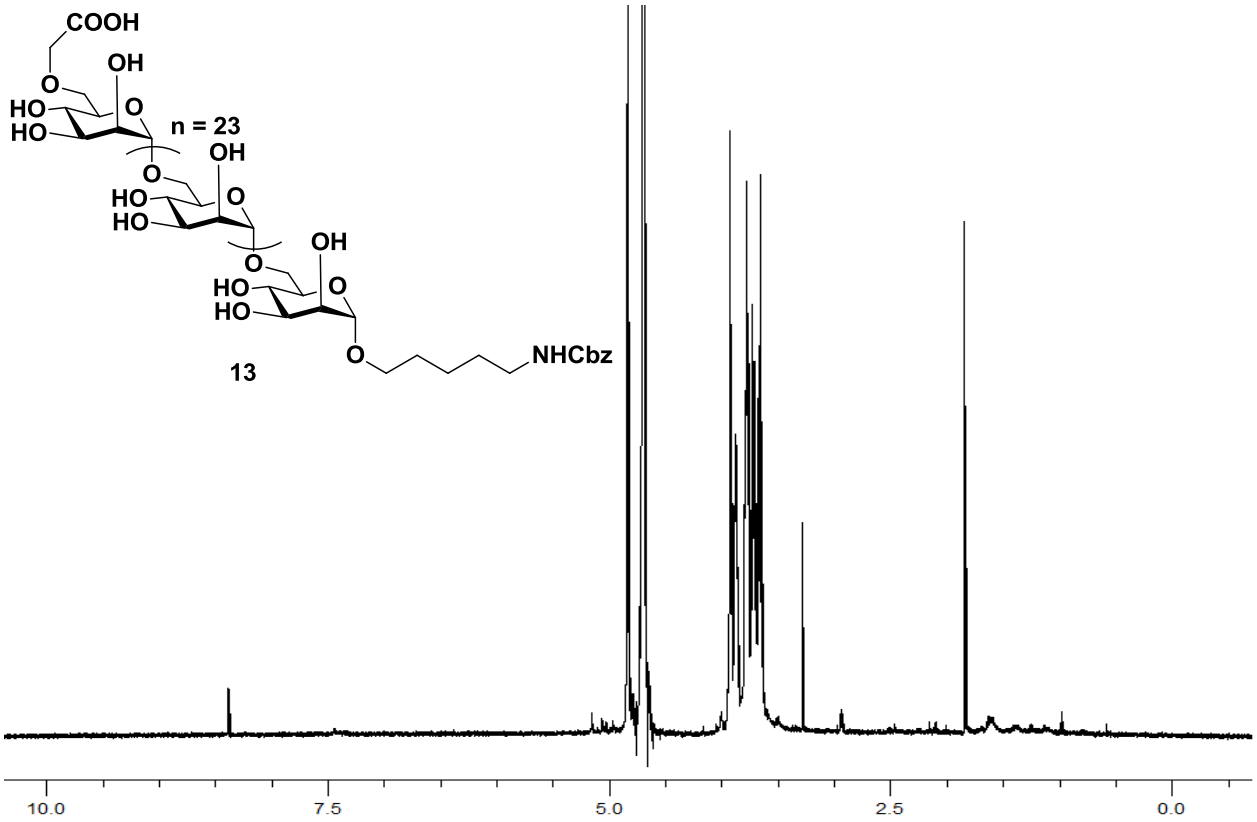


Figure S50. ^{1}H NMR of **13**.

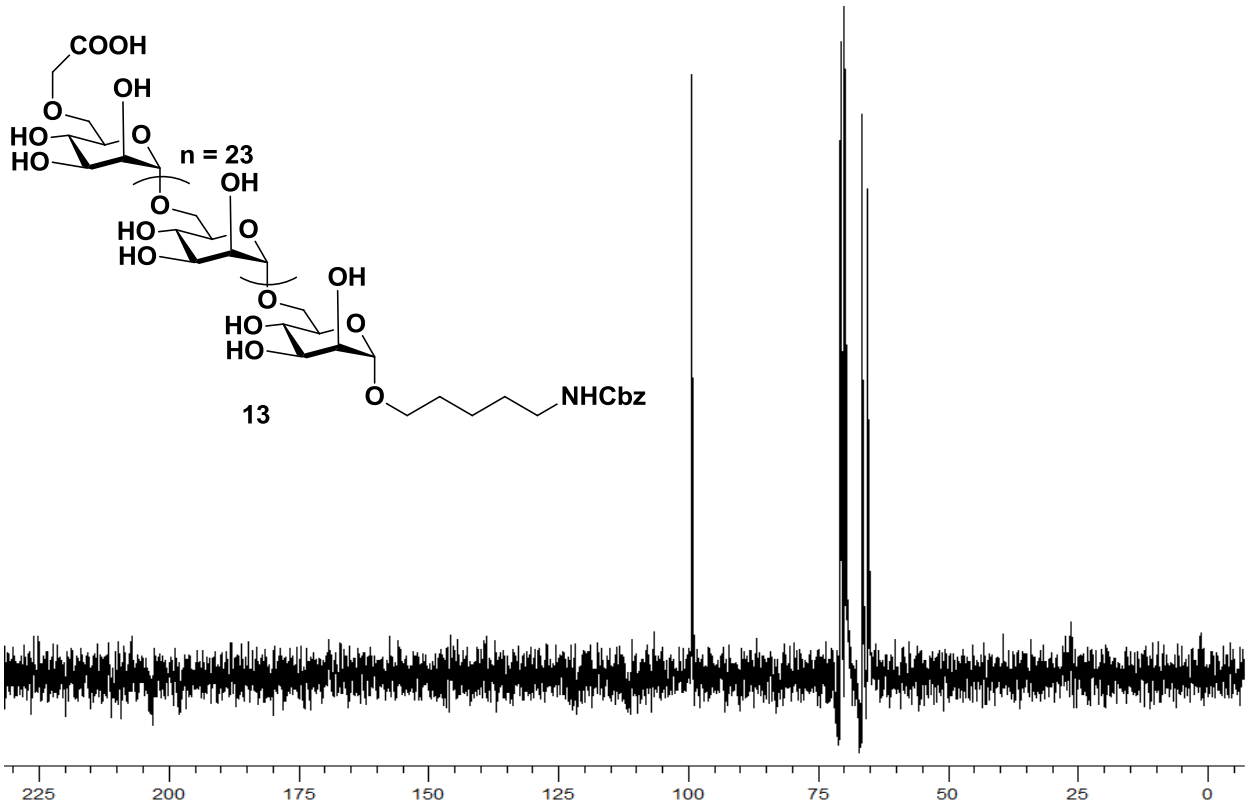


Figure S51. ^{13}C NMR of **13**.

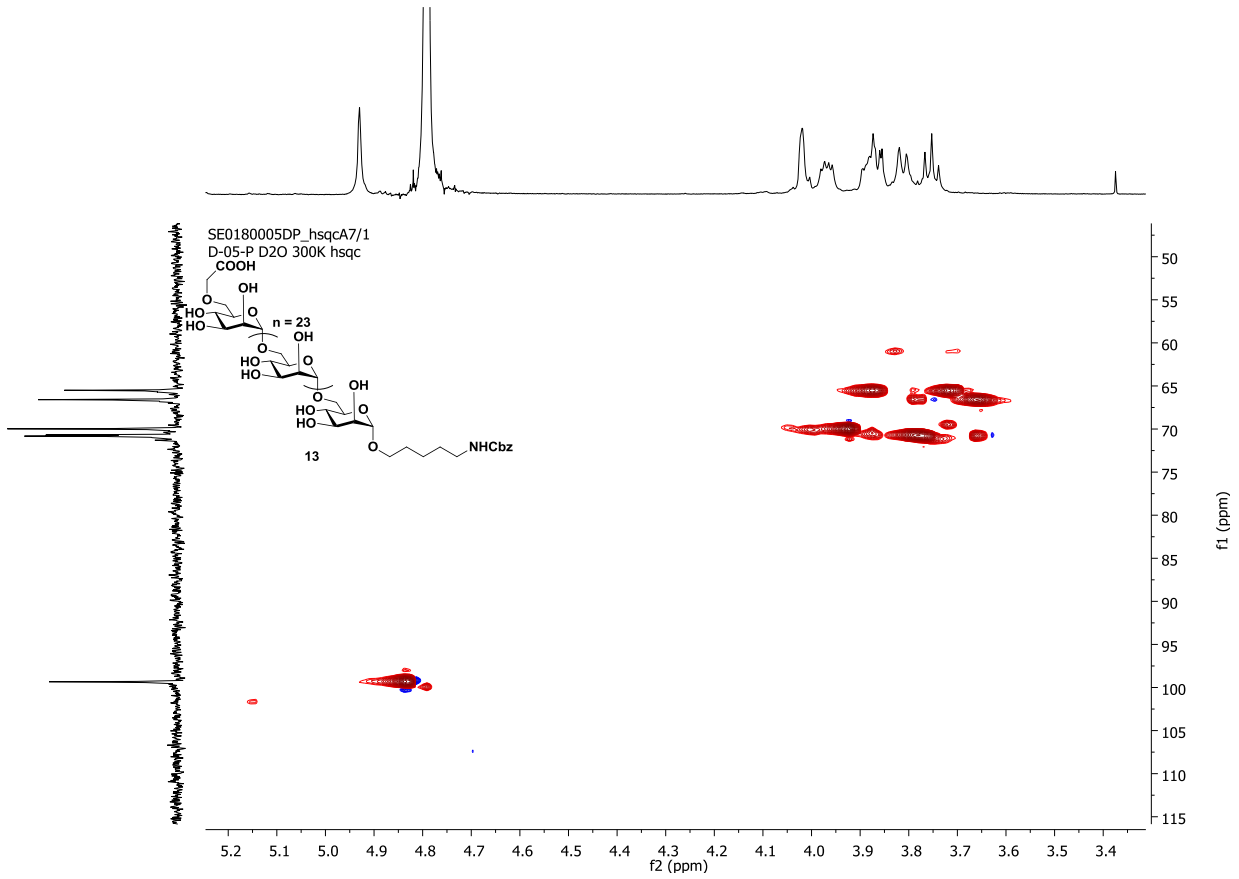


Figure S52. HSQC spectrum of **13**.

12. Aligned NMR Spectra of **6**, **11**, **7**, and **10**; and **13**, **8**, and **12**

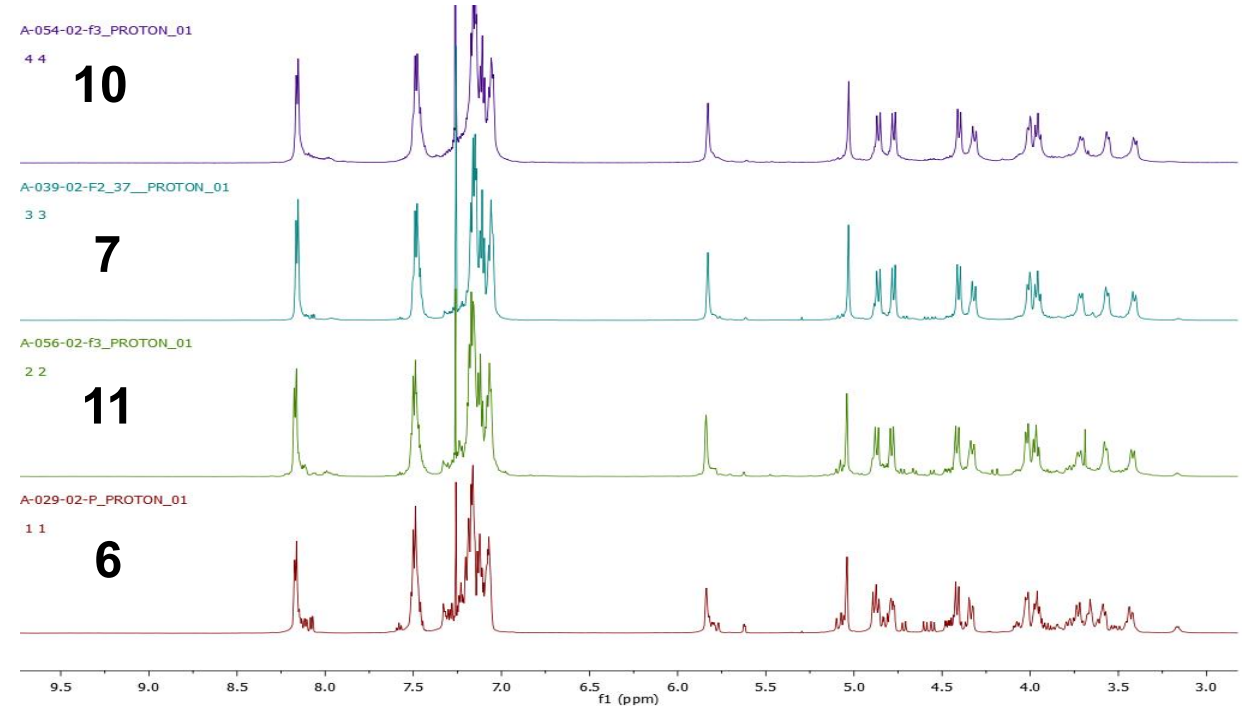


Figure S53. Overlaid ^{1}H NMR spectra of protected **6** (12mer), **11** (25mer), **7** (37mer) and **10** (50mer).

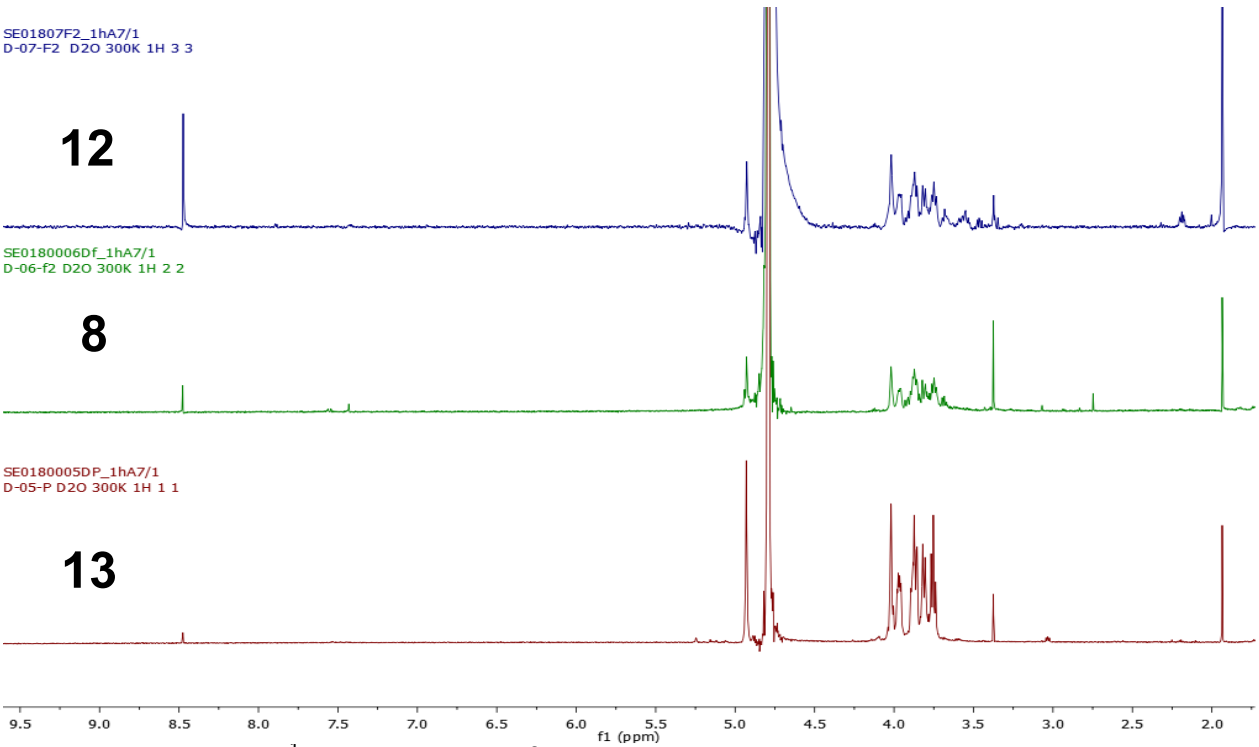


Figure S54. Overlaid ^{1}H NMR spectra of deprotected **13** (25mer), **8** (37mer) and **12** (50mer).

SE01807F2_13cA7/2
D-07-F2 D2O 300K 13C 3 3

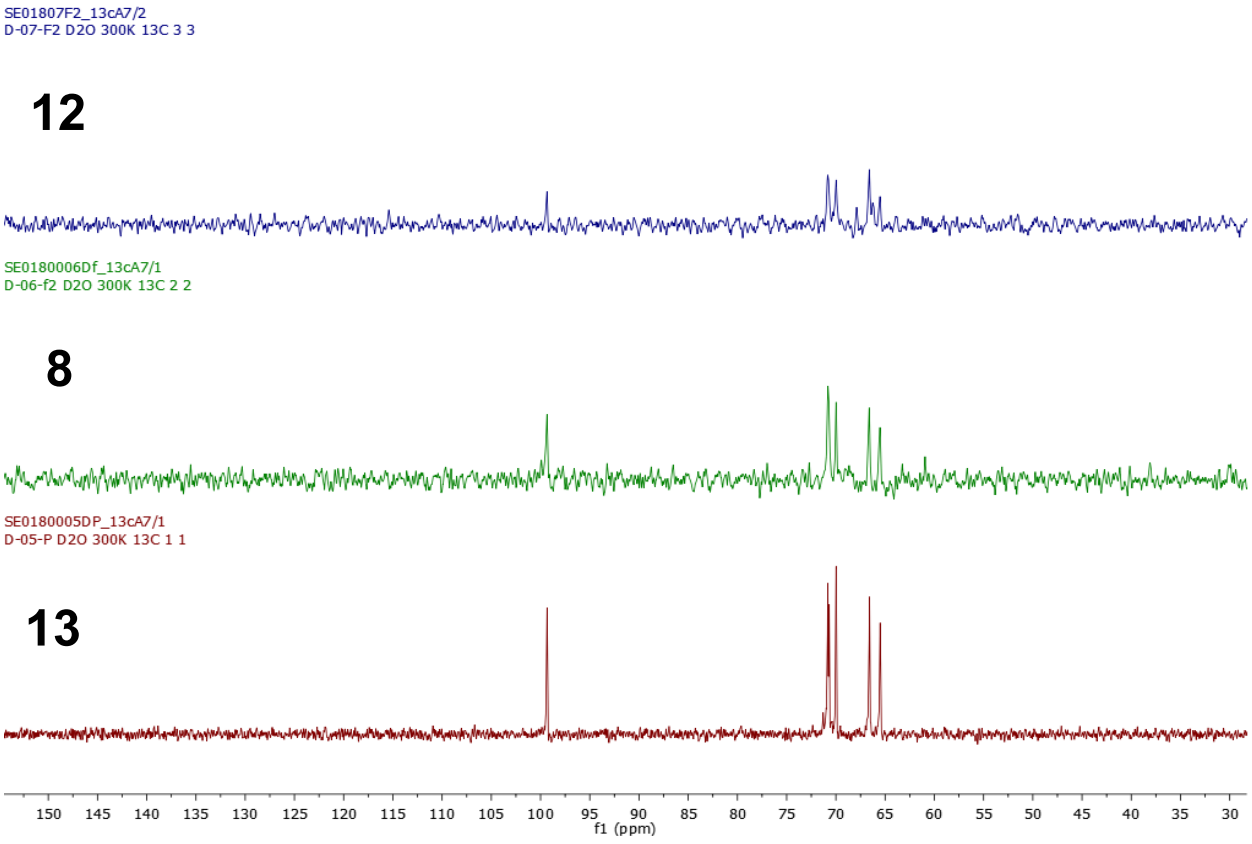


Figure S55. Overlaid ^{13}C NMR spectra of deprotected **13** (25mer), **8** (37mer) and **12** (50mer).

13. References

1. M. Yun, S. Yoon, Y. Shin, K. H. Chun, J. E. Nam Shin, *Arch. Pharm. Res.*, 2004, **27**, 143.

2. S. Eller, M. Collot, J. Yin, H. S. Hahm and P. H. Seeberger, *Angew. Chem. Int. Ed.*, 2013, **52**, 5858.

3. T. G. Mayer, R. R. Schmidt, *Eur. J. Org. Chem.*, 1999, 1153.