Supporting Information

HPLC-assisted automated oligosaccharide synthesis: the implementation of the two-way split valve as a mode of complete automation

Matteo Panza, Keith J. Stine, and Alexei V. Demchenko*

Department of Chemistry and Biochemistry, University of Missouri – St. Louis, One University Boulevard, St. Louis, Missouri 63121, USA
demchenkoa@umsl.edu

Contents

General Methods

The reactions were performed using commercial reagents and the ACS grade solvents were purified and dried according to standard procedures. HPLC grade solvents used for automation were utilized without purification. Column chromatography was performed on silica gel 60 (70−230 mesh) or using flash purification system Biotage Isolera One, reactions were monitored by TLC on Kieselgel 60 F254. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2 was distilled from CaH_2 directly prior to application. Amberlite IR20 (H^+) was washed three times with MeOH and stored under MeOH. Optical rotations were measured using a Jasco 'P-2000' polarimeter. ^{1}H NMR spectra were recorded at 300 or 600 MHz, ^{13}C NMR spectra were recorded at 75 or 150 MHz. The ^{1}H chemical shifts are referenced

to the signal of the residual $CHCl_3$ (δH = 7.24 ppm). The ^{13}C chemical shifts are referenced to the central signal of $CDCl_3$ (δC = 77.23 ppm). HRMS determinations were made with the use of a mass spectrometer with FAB ionization and ion-trap detection. Agilent 1260 infinity II HPLC System and Agilent 1260 Variable Wavelength UV−vis Detector were used to assemble the automated synthesizer.

Set up of the HPLC-A synthesizer

The HPLC based synthesizer has been assembled using

- 1260 Agilent Infinity II series Quaternary Pump
- Variable Wavelength Detector with dual-wavelenght mode
- The Autosampler is the preparative module from 1260 Infinity series. The autosampler is equipped a 900 µL preparative loop and two trays holding 15 x 6 mL vials each. The wells of the left tray are numbered from 1 to 15 while the ones of the right tray are numbered from 101 to 115.
- The valve is a 2-way 6-port Quick Change Valve.
- The column utilized is an Omnifit Solvent Plus 50 mm.

The synthesis sequences are programmed using Chemstation software and the autosampler programming option.

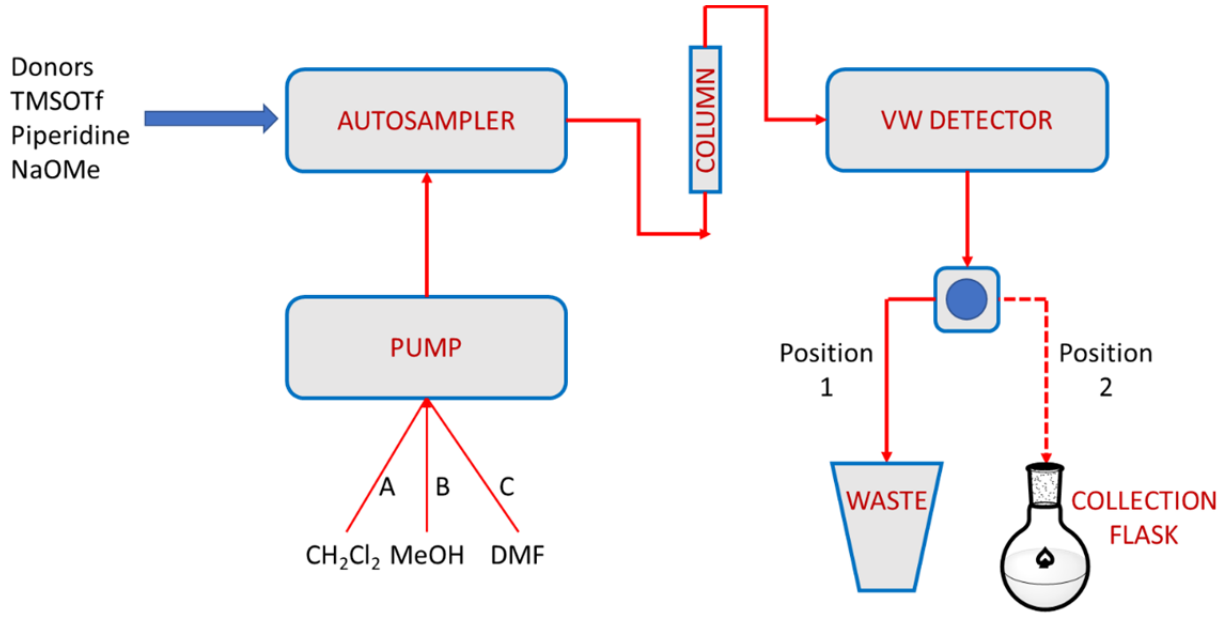
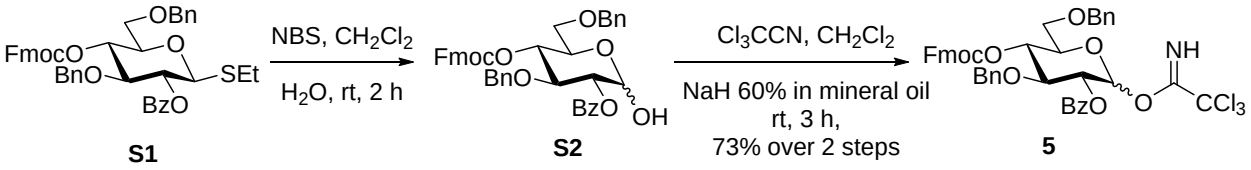


Figure S1. The synthesizer set up with the two channels of the valve

Synthesis of Glycosyl Donors and Glycosyl Acceptors

2,3,4-Tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucopyranosyl trichloroacetimidate (4) was obtained in accordance with the reported procedure and its analytical data were in accordance with that previously described.[1]

2-*O*-Benzoyl-3,6-di-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucopyranosyl trichloroacetimidate (5).

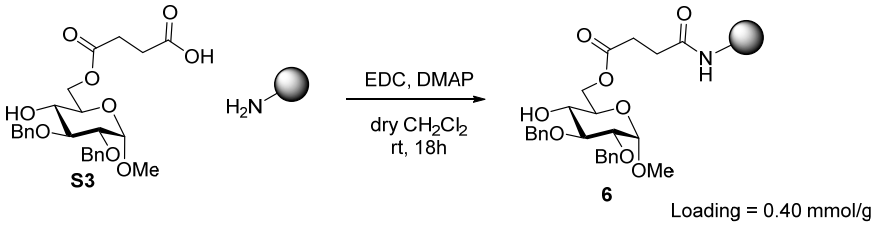


Water (8.0 mL) and *N*-bromosuccinimide (NBS, 1.25 g, 7.03 mmol) were added to a solution of ethyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-glucopyranoside[2] (**S1**, 4.50 g, 6.39 mmol) in methylene chloride (80 mL) and the resulting mixture was stirred vigorously for 2 h at rt. The reaction was quenched with 10% aq. $Na_2S_2O_3$ and diluted with methylene chloride (~120 mL). The organic phase was separated and washed with brine (2 x 30 mL), dried with $MgSO_4$ and concentrated in *vacuo*. The residue was filtered through a pad of silica gel (hexanes - ethyl acetate isocratic elution, 7/3, v/v) to afford 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-D-glucopyranose (**S2**). Crude residue containing hemiacetal **S2** (3.53 g, 5.14 mmol) was dissolved in anhydrous methylene chloride (80 mL). Trichloroacetonitrile (7.73 mL, 77.10 mmol) and NaH (60% suspension in mineral oil, 0.025 g, 0.62 mmol) were added and the resulting mixture was stirred under argon for 3 h at rt. The volatiles were removed under the reduced pressure, and the residue was purified using a Biotage Isolera One (50 g SNAP ULTRA cartidge, hexane - ethyl acetate elution) to afford the title compound as an amorphous solid (3.90 g, 73% over two steps, α/β = 12.5/1).

Selected analytical data for **5α**: R_f = 0.54 (hexane/ethyl acetate, 7/3, v/v); ^{1}H NMR (300 MHz, CDCl3): δ, 8.54 (s, 1H), 8.02-7.93 (m, 2H, aromatic), 7.77 (dd, 2H, *J* = 7.4, 2.7 Hz, aromatic), 7.68-7.51 (m, 4H, aromatic), 7.48-7.05 (m, 15H, aromatic), 6.67 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 5.41 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 5.24 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.9 Hz, H-4), 4.69 (s, 2H, C*H*$_2$Ph), 4.61-4.52 (m, 2H, C*H*$_2$Ph), 4.42-4.21 (m, 4H, H-3, 5, 6a, CH/Fmoc), 4.15 (dd, 1H, $J_{5,6a} = J_{6a,6b}$ = 6.9 Hz, H-6b), 3.77-3.60 (m, 2H, CH_2/Fmoc) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 165.36, 160.50, 154.25, 143.34, 143.21, 141.41, 137.75, 137.51, 133.58, 129.93, 129.16, 128.56, 128.44, 128.34, 128.04, 128.02, 127.88, 127.79, 127.32, 125.18, 125.10, 120.22, 93.62, 90.96, 76.60, 74.99, 74.54, 73.71, 72.28, 71.54, 70.21, 68.52, 46.80; HR-TOF MS [M+Na+] calcd 854.1486 (100% abundance) found 854.1480, calcd 852.1504 (91% abundance) found 852.1530.

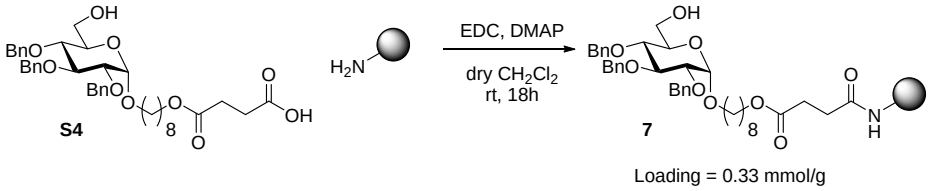
Selected analytical data for **5β**: R_f = 0.50 (hexane/ethyl acetate, 7/3, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 5.97 (d, 1H, H-1), 5.61 (m, 1H, H-2) ppm.

Conjugate 6



The title conjugate was obtained from methyl 2,3-di-*O*-benzyl-6-*O*-(3-hydroxycarbonylpropanoyl)-α-D-glucopyranoside (**S3**)[3] and JandaJel in accordance with the reported procedure.[4] The acceptor loading of 0.40 mmol/g for conjugate **6** was determined by direct cleavage from the solid support (50 mg of resin) and quantifying on HPLC to mimic conditions for the subsequent reactions.

Conjugate 7



The title conjugate was obtained by reaction of 8-(3-carboxypropanoyloxy)oct-1-yl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (**S4**)[4] with JandaJel performed in accordance with the reported procedure.[4] The acceptor loading of 0.33 mmol/g for conjugate **7** was determined by direct cleavage from the solid support (50 mg of resin) and quantifying on HPLC to mimic conditions for the subsequent reactions.

Preparation of the reagent vials

All the solutions were freshly prepared using the HPLC grade solvents and kept at room temperature for the duration of the synthesis.
Donor: a solution of glycosyl donor (0.08-0.2 mmol) in CH_2Cl_2 (3.5 mL).
Promoter: a solution of TMSOTf (0.04-0.16 mmol) in CH_2Cl_2 (2.0 mL
Reagents for Fmoc removal: a solution of piperidine in DMF (5.0 mL, 2/3, v/v).
Reagents for cleaving off the resin: a solution prepared from 1M NaOMe in MeOH (1.5 mL), CH_2Cl_2 (1.0 mL) and MeOH (1.0 mL).
Washing solutions and blanks: methylene chloride (6 mL).

The Automated Assembly of Oligosaccharides

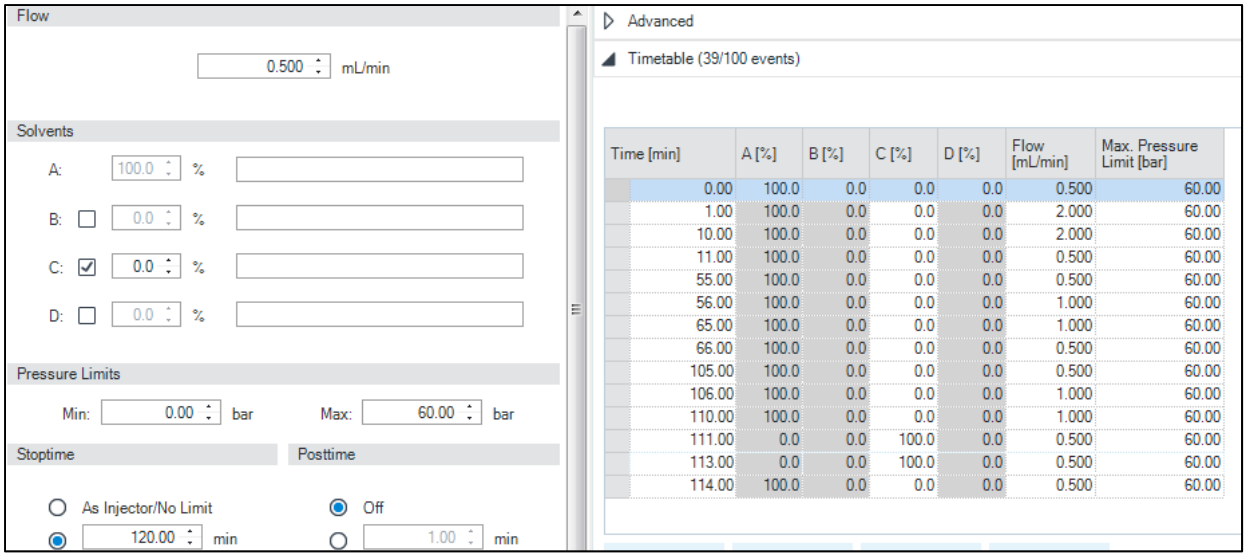
All reactions were carried out using 50 mg of preloaded resin. The acceptor is loaded on the resin prior automation, therefore the loading depended on the batch of the resin. Syntheses with acceptors **6** and **7** were performed with 0.02 and 0.0165 mmol, respectively.

Glycosylations. The sequences are comprehensive of washings of the resin pre and after glycosylation steps.The glycosylations are split in two interations of 5 injections each. The donor and the promoter are drawn from the vial, mixed in the needle seat and the injected. Once the activated donor has passed through the column, the same operation is repeated. The resin is washed with CH_2Cl_2 and DMF and 5 more injections are performed. The volume of solvent in the vials is constant, hence the concentration of donor and promoter varies depending on the equivalents required to successfully glycosylate the acceptor.
In Figure S3 the programming of the components of the synthesizer is reported. In figure 3A the pump timetable, in 3B the autosampler programming and in 3C the variable wave-length detector (VWD). The valve was on position 1 during the whole sequence.

Fmoc removal. The reaction is monitored at λ = 250 and λ = 301 nm. The dibenzofulvene absorbance at 301 nm is a clear indication of the reaction completion. The removal is completed within 1 hour, including the washings and the resin is reacidified with TMSOTf as a last step. Even in this case, the valve was left in postion 1 (waste) for the duration of the sequence.
In Figure S4 A, B and C the settings of the pump, autosampler and VWD, respectively, are reported.

Final cleavage. The cleavage is performed in Zemplen conditions, with the solution prepared as reported above. The valve after the 30 seconds of the sequence switches from position 1 to position 2 and divert the flow to the collection flask. The reaction is monitored at both 250 and 301 nm and the sequence consists of 4 consecutive injections of the cleaving solution.
In Figure S5 A, B and C the settings of the modules are depicted.

Detection. Representative UV traces for each sequence are depicted in Figure S6 A, B and C. The absorbance of the donor at λ = 301 nm is significantly lower compared to 250 nm, therefore the signals are not saturated and in some cases is easier to understand the proceeding of the reaction.
The glycosylation trace **A** shows a progressive increase in the concentration of the donor with the numver of injections, indication of the lower availability of free acceptor that can still be glycosylated.
The Fmoc removal trace **B** clearly indicates the majority of the temporary protecting group being removed in the first injection of the piperidine solution, with only minor amounts cleaved in the following injections.
The final cleavage trace **C** shows high absorbance during the injections, corresponding the the prograssive cleavage of the oligosaccharides from the resin and the Bz and Fmoc groups from the glycans. The absorbance decreases progressively during the washings.

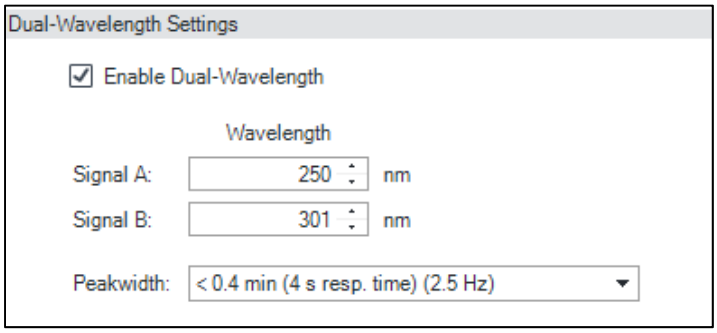


Time [min]	A [%]	B [%]	C [%]	D [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	100.0	0.0	0.0	0.0	0.500	60.00
1.00	100.0	0.0	0.0	0.0	2.000	60.00
10.00	100.0	0.0	0.0	0.0	2.000	60.00
11.00	100.0	0.0	0.0	0.0	0.500	60.00
55.00	100.0	0.0	0.0	0.0	0.500	60.00
56.00	100.0	0.0	0.0	0.0	1.000	60.00
65.00	100.0	0.0	0.0	0.0	1.000	60.00
66.00	100.0	0.0	0.0	0.0	0.500	60.00
105.00	100.0	0.0	0.0	0.0	0.500	60.00
106.00	100.0	0.0	0.0	0.0	1.000	60.00
110.00	100.0	0.0	0.0	0.0	1.000	60.00
111.00	0.0	0.0	100.0	0.0	0.500	60.00
113.00	0.0	0.0	100.0	0.0	0.500	60.00
114.00	100.0	0.0	0.0	0.0	0.500	60.00

A

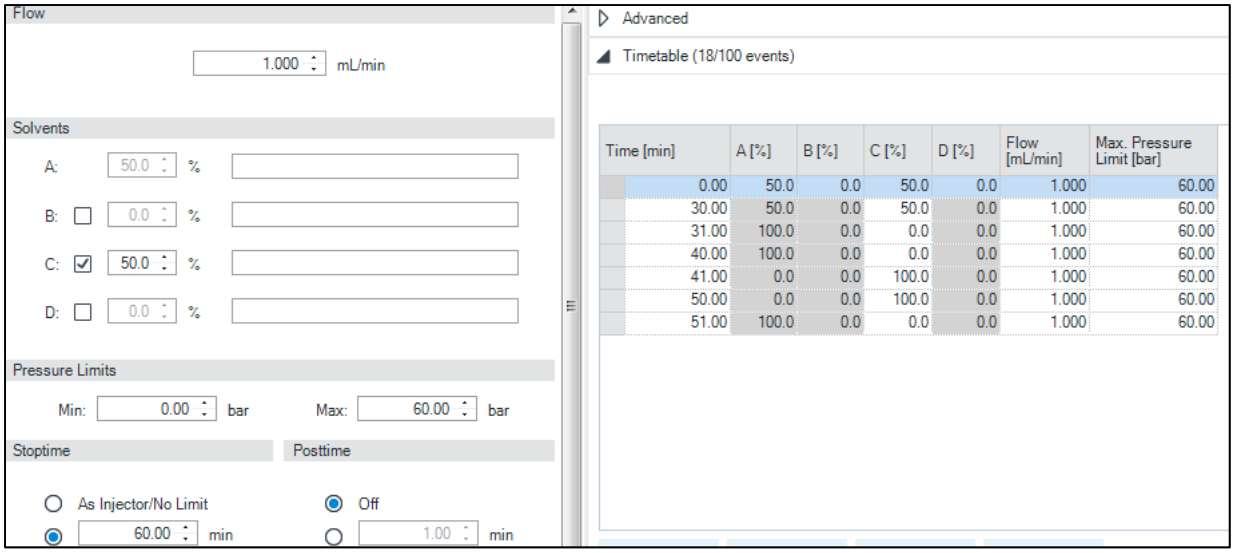
Function	Parameter
Draw	Draw 100 µL from location "111" with default speed using default offset
Inject	Inject
Wait	Wait 8 min
Repeat	Repeat 5 time(s)
Draw	Draw 500 µL from sample with maximum speed using default offset
Draw	Draw 100 µL from vial+ 1 with maximum speed using default offset
Wash	Wash needle in location "112" 1 times
Mix	Mix 600 µL from seat with maximum speed for 1 times
Inject	Inject
Wait	Wait 3 min
Valve	Switch valve to "Bypass"
Eject	Eject maximum volume to seat with maximum speed using default offset
End Repeat	End Repeat
Wait	Wait 35 min
Repeat	Repeat 5 time(s)
Draw	Draw 500 µL from sample with maximum speed using default offset
Draw	Draw 100 µL from vial+ 1 with maximum speed using default offset
Wash	Wash needle in location "112" 1 times
Mix	Mix 600 µL from seat with maximum speed for 1 times
Inject	Inject

B

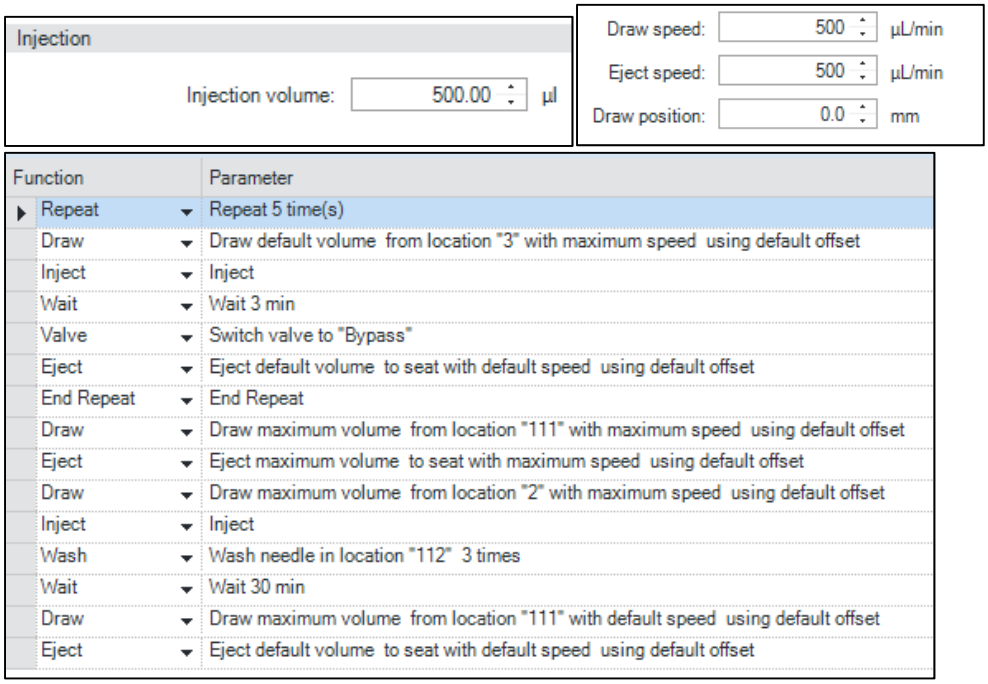


C

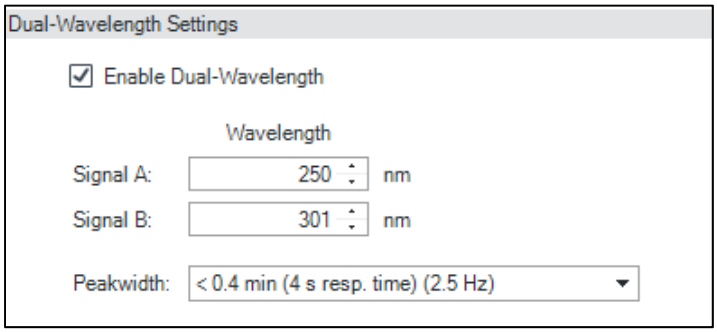
Figure S2. Settings of the components of the HPLC synthesizer during glycosylation.



A

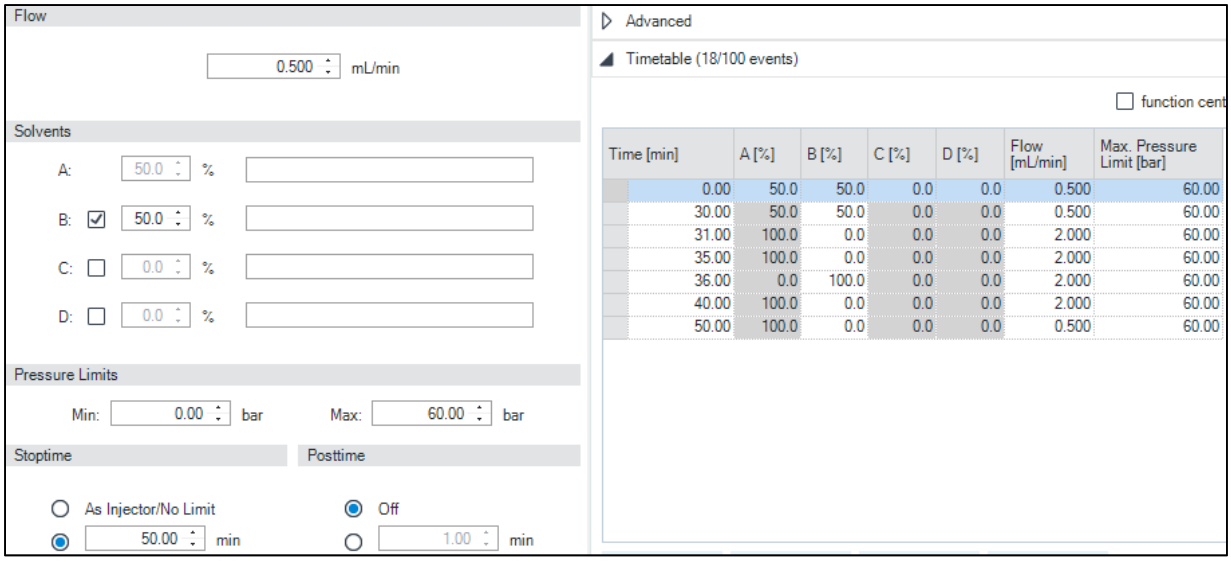


B

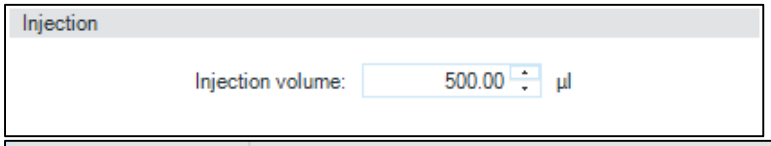


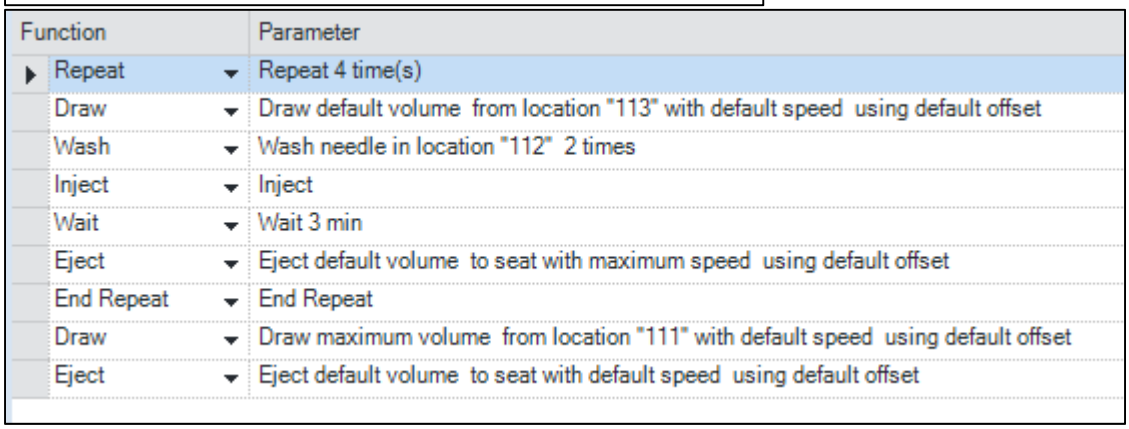
C

Figure S3. Settings of the components of the HPLC synthesizer during Fmoc removal.

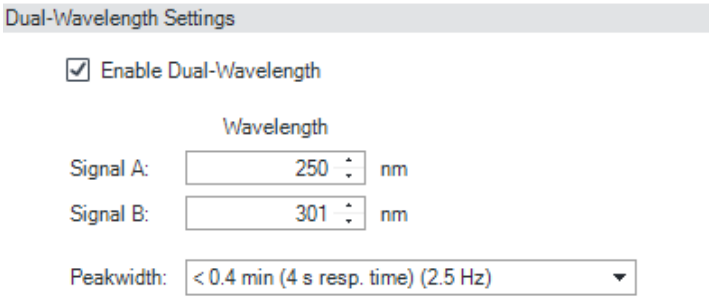


A



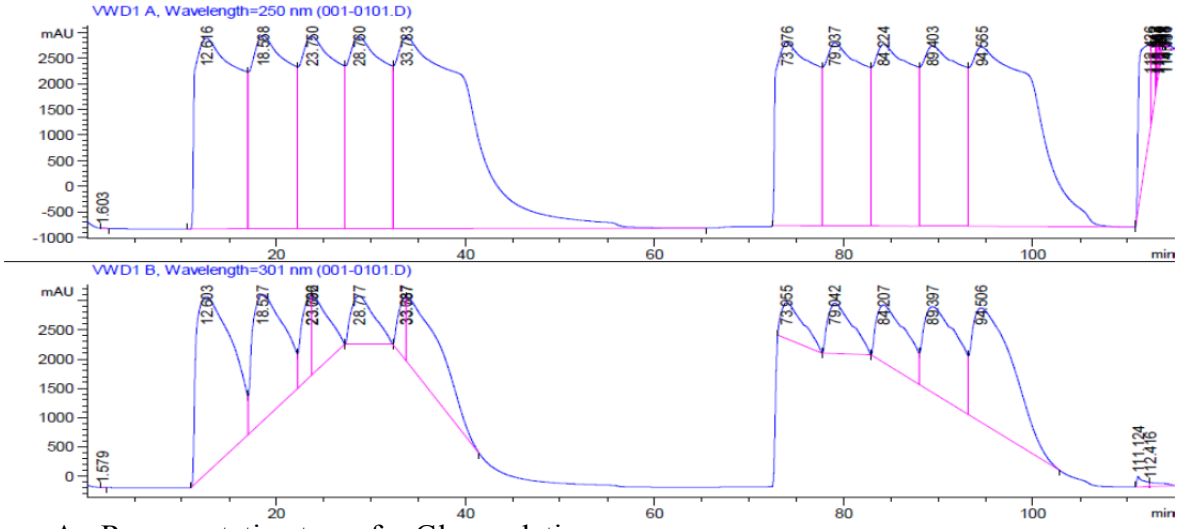


B

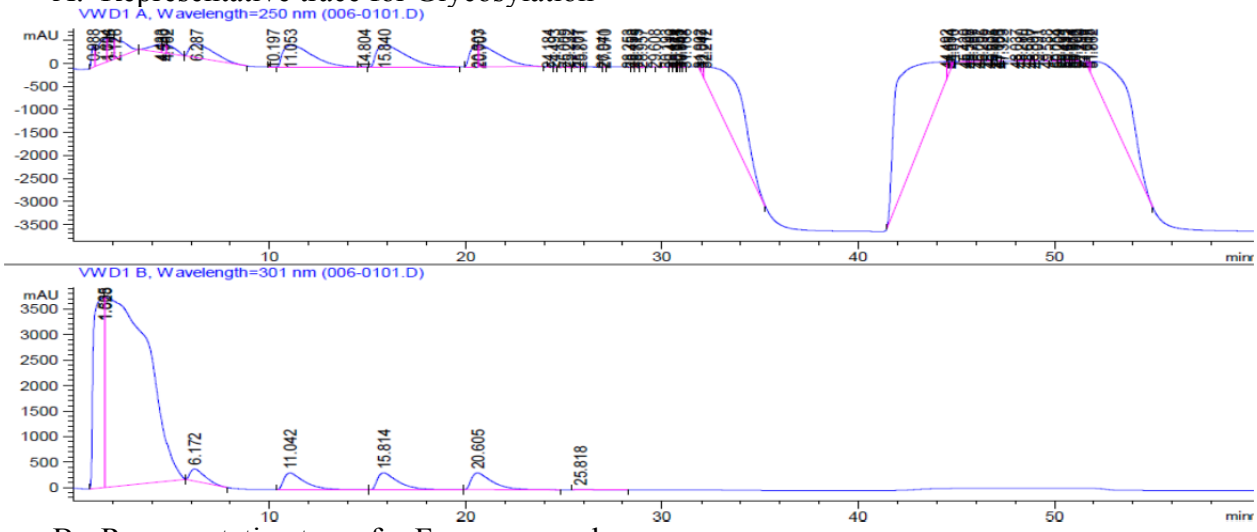


C

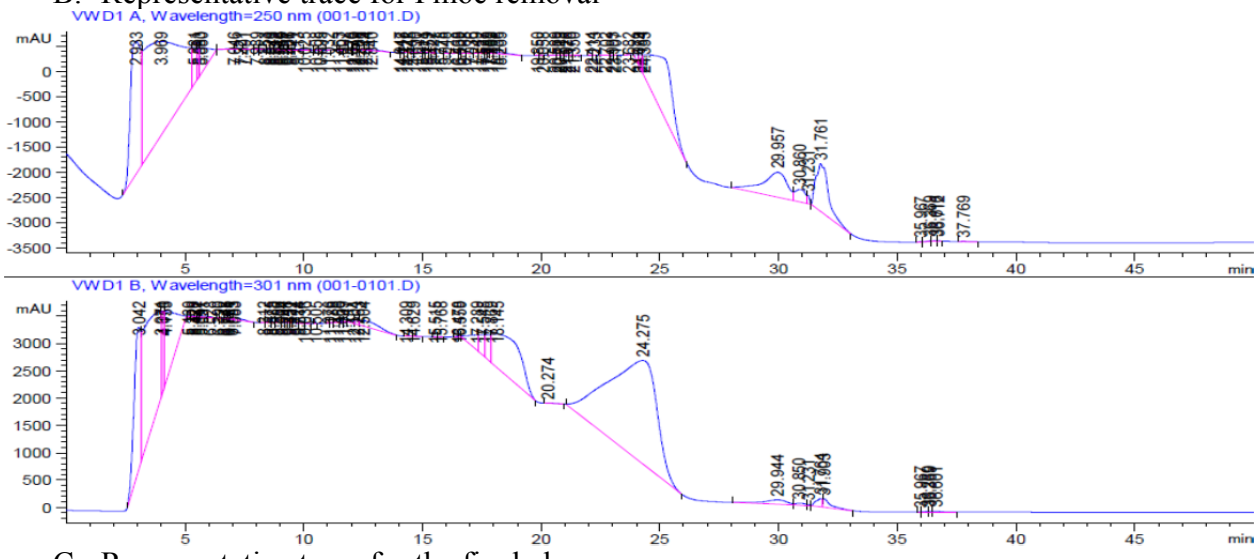
Figure S4. Settings of the components of the HPLC synthesizer during the final cleavage.



A. Representative trace for Glycosylation



B. Representative trace for Fmoc removal



C. Representative trace for the final cleavage

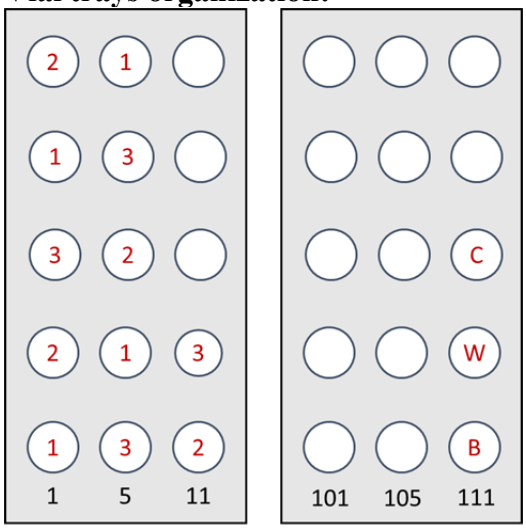
Figure S5. UV traces for the synthetic steps

Synthesis of pentasaccharide 1

8-Acetyloxyoct-1-yl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (1).

Automation modules: The Omnifit column is filled with resin (50 mg) preloaded with acceptor **7** (0.016 mmol). The vials were prepared according to the general methods and organized as depicted below. Vial 1 contains donor **4** (71 mg, 0.08 mmol, 5.0 equiv), vial 2 contains of TMSOTf (14 µL, 0.08 mmol, 5.0 equiv), and vial 3 contains a piperidine-DMF solution prepared as indicated in the general methods. Vials B, W and C are blank, washing, and final off-resin cleavage, respectively, were prepared as described in the general methods.

Vial trays organization:



Automation Sequence:
Glycosylation 120 min
Fmoc removal 60 min
Glycosylation 120 min
Fmoc removal 60 min
Glycosylation 120 min
Fmoc removal 60 min
Glycosylation 120 min
Final Cleavage 50 min

Post Automation: The reaction mixture collected from cleavage step was evaporated under reduced pressure, then diluted with MeOH (2.0 mL) and quenched with Amberlite IR20 H^+ form. The resin was filtrated and washed with MeOH, the solvent was evaporated and dried in *vacuo* for 1 h. The crude was dissolved in pyridine (2.0 mL) and acetic anhydride (0.5 mL) was added. The resulting mixture was stirred under argon at room temperature for 16 h. The reaction was quenched with MeOH, then the solvent was evaporated and co-evaporated with toluene. The

residue was purified by column chromatography on silica gel (hexanes-acetone gradient elution) to afford compound **1** in 33% (10.0 mg, 0.0055 mmol) yield.

Analytical data for 1: R_f = 0.50 (hexane/acetone, 1/1, v/v); $[\alpha]_D^{21}$ -2.7 (*c* 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ, 7.39 – 7.21 (m, 15H), 5.23 – 5.05 (m, 6H), 5.02 – 4.83 (m, 9H), 4.80 – 4.74 (m, 2H), 4.71 (d, *J* = 3.2 Hz, 1H), 4.62 (d, *J* = 12.0 Hz, 1H), 4.58 – 4.47 (m, 5H), 4.27 (dd, *J* = 12.3, 4.9 Hz, 1H), 4.15 – 4.02 (m, 5H), 4.01 – 3.84 (m, 5H), 3.76 – 3.47 (m, 13H), 3.33 (dd, J = 16.0, 6.5 Hz, 1H), 2.14 – 1.87 (m, 42H), 1.67 – 1.53 (m, 8H), 1.31 (s, 8H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 171.41, 170.78, 170.43, 170.32, 170.27, 170.24, 169.81, 169.74, 169.66, 169.61, 169.59, 169.53, 169.32, 169.19, 138.97, 138.40, 138.27, 128.75, 128.56, 128.50, 128.15, 128.09, 127.99, 127.97, 127.69, 101.00, 100.77, 100.63, 96.80, 81.98, 80.17, 77.54, 75.76, 75.13, 73.29, 73.27, 73.17, 73.10, 72.92, 72.85, 72.78, 72.10, 71.51, 71.49, 71.24, 71.12, 71.10, 69.75, 69.24, 69.16, 69.10, 68.49, 68.43, 68.16, 68.15, 68.12, 68.07, 64.77, 62.05, 62.02, 61.95, 29.85, 29.60, 29.51, 29.49, 29.36, 28.74, 26.32, 26.08, 21.20, 20.92, 20.89, 20.87, 20.85, 20.82, 20.80, 20.78, 20.76 ppm; HR-TOF MS [M+Na^+] calcd 1837.6728 found 1837.6672

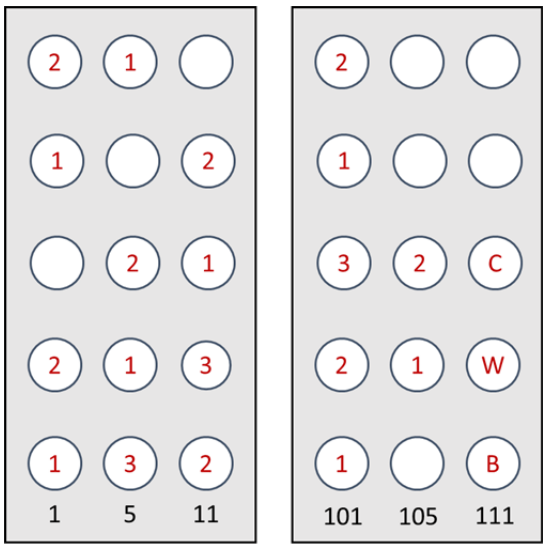
Deletion sequences were isolated and characterized by HR-TOF MS: tetrasaccharide 5.5 mg (22%, 0.0036 mmol) [M+Na^+] calcd 1549.5883 found 1549.5878, trisaccharide 2.0 mg (9%, 0.0016 mmol) [M+Na^+] calcd 1261.5037 found 1261.5010.

Synthesis of pentasaccharide 2

Methyl *O*-(2,4-di-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-6-*O*-benzoyl-2,3-di-*O*-benzyl-α-D-glucopyranoside (2).

Automation modules: The Omnifit column is filled with resin (50 mg) preloaded with acceptor **6** (0.02 mmol). The vials were prepared according to the general methods and organized as depicted below. Vial 1 contains donor **5** (160 mg, 0.20 mmol, 10.0 equiv), vial 2 contains of TMSOTf (5 μL, 0.03 mmol, 1.5 equiv), and vial 3 contains a piperidine-DMF solution prepared as indicated in the general methods. Vials B, W and C are blank, washing, and final off-resin cleavage, respectively, were prepared as described in the general methods.

Vial trays organization:



Automation Sequence:
Glycosylation 120 min
Glycosylation 120 min
Fmoc removal 60 min
Glycosylation 120 min
Glycosylation 120 min
Fmoc removal 60 min
Glycosylation 120 min
Glycosylation 120 min
Fmoc removal 60 min
Glycosylation 120 min
Glycosylation 120 min
Final Cleavage 50 min

Post Automation: The reaction mixture collected from cleavage step was evaporated under reduced pressure, then diluted with MeOH (2.0 mL) and quenched with Amberlite IR20 H^+ form. The resin was filtrated and washed with MeOH, the solvent was evaporated and dried *in vacuo* for 1 h. The crude was dissolved in pyridine (2.0 mL) and benzoyl chloride (0.5 mL) was added. The resulting mixture was stirred under argon at room temperature for 16 h. The reaction was quenched with MeOH, then the solvent was evaporated and co-evaporated with toluene. The residue was purified by column chromatography on silica gel (hexanes-ethyl acetate gradient elution) to afford compound **2** in 20% (10.3 mg, 0.0044 mmol) yield.

Analytical data for 2: R_f = 0.20 (hexane/EtOAc, 7/3, v/v); $[\alpha]_D^{21}$ +25.7 (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 8.07 – 7.68 (m, 38H), 7.68 – 6.59 (m, 196H), 5.40 – 5.31 (m, 6H), 5.20 – 5.08 (m, 7H), 5.05 – 4.94 (m, 7H), 4.94 – 4.78 (m, 12H), 4.73 (dd, *J* = 9.8 Hz, 8H), 4.63 – 4.28 (m, 42H), 4.28 – 4.02 (m, 22H), 4.02 – 3.72 (m, 26H), 3.68 (s, 5H), 3.65 – 3.42 (m, 22H), 3.42 – 3.15 (m, 31H), 2.87 (d, *J* = 8.8 Hz, 1H), 2.75 (d, *J* = 10.1 Hz, 3H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 165.87, 165.34, 165.13, 165.08, 165.02, 164.90, 164.86, 164.79, 138.90, 138.76,

138.38, 138.16, 137.81, 137.54, 133.59, 133.38, 133.09, 132.96, 129.84, 129.60, 129.00, 128.65, 128.57, 128.45, 128.27, 128.12, 127.93, 127.68, 127.39, 127.30, 127.12, 127.02, 101.31, 100.16, 100.03, 99.97, 97.91, 80.41, 80.28, 80.06, 79.88, 79.45, 78.29, 77.37, 77.16, 76.95, 76.44, 76.13, 75.18, 74.90, 74.72, 74.48, 74.00, 73.88, 73.78, 73.56, 73.44, 72.09, 69.95, 68.43, 67.45, 67.16, 62.84, 55.31, 32.08, 29.85, 29.51, 29.28, 22.85, 14.28 ppm; HR-TOF MS [M+Na^+] calcd 2390.9097 found 2390.9045.

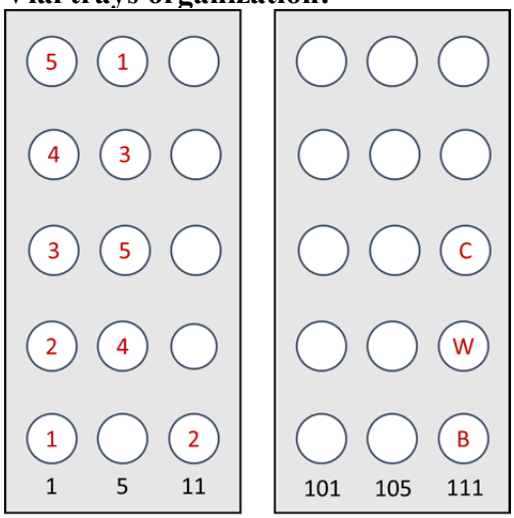
Deletion sequences were isolated and characterized by HR-TOF MS: tetrasaccharide 2.6 mg (6.5%, 0.0013 mmol) [M+Na^+] calcd 1943.7334 found 1943.7323, traces of trisaccharide not isolated, but confirmed by [M+Na^+] calcd 1497.5605 found 1497.5627.

Synthesis of tetrasaccharide 3

Methyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-6-*O*-benzoyl-2,3-di-*O*-benzyl-α-D-glucopyranoside (3).

Automation modules: The Omnifit column is filled with resin (50 mg) preloaded with acceptor **6** (0.02 mmol). The vials were prepared according to the general methods and organized as depicted below. Vial 1 contains donor **4** (130 mg, 0.15 mmol, 7.5 equiv), vial 2 contains of TMSOTf (10 µL, 0.05 mmol, 2.5 equiv), and vial 3 contains a piperidine-DMF solution prepared as indicated in the general methods. Vial 4 contains donor **5** (83 mg, 0.10 mmol, 5.0 equiv) and vial 2 contains of TMSOTf (7.5 µL, 0.04 mmol, 2.0 equiv). Vials B, W and C are blank, washing, and final off-resin cleavage, respectively, were prepared as described in the general methods.

Vial trays organization:



Automation Sequence:
Glycosylation 120 min
Fmoc removal 60 min
Glycosylation 120 min
Glycosylation 120 min
Fmoc removal 60 min
Glycosylation 120 min
Final Cleavage 50 min

Post Automation: The reaction mixture collected from cleavage step was evaporated under reduced pressure, then diluted with MeOH (2.0 mL) and quenched with Amberlite IR20 H^+ form. The resin was filtrated and washed with MeOH, the solvent was evaporated and dried *in vacuo* for 1 h. The crude was dissolved in pyridine (2.0 mL) and benzoyl chloride (0.5 mL) was added. The resulting mixture was stirred under argon at room temperature for 16 h. The reaction was quenched with MeOH, then the solvent was evaporated and co-evaporated with toluene. The residue was purified by column chromatography on silica gel (hexanes-ethyl acetate gradient elution) to afford compound **3** in 30% (13 mg, 0.0066 mmol) yield.

Analytical data for 3: R_f = 0.15 (hexane/EtOAc, 7/3, v/v); $[\alpha]_D^{21}$ +1.8 (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 7.98 – 7.71 (m, 26H), 7.61 – 7.12 (m, 66H), 7.05 – 6.87 (m, 9H), 5.66 (m, 2H), 5.53 – 5.46 (m, 2H), 5.43 – 5.39 (m, 1H), 5.33 – 5.26 (m, 2H), 5.17 – 5.12 (m, 1H), 4.91 (dd, *J* = 11.5, 6.2 Hz, 4H), 4.77 (dd, *J* = 14.6, 9.6 Hz, 2H), 4.69 (d, *J* = 12.0 Hz, 1H), 4.63 – 4.58 (m, 2H), 4.55 (d, *J* = 12.5 Hz, 1H), 4.41 (dd, *J* = 20.2, 7.6 Hz, 3H), 4.33 (d, *J* = 12.1 Hz, 1H), 4.07 (ddd, *J* = 18.1, 10.4, 6.3 Hz, 4H), 3.89 – 3.85 (m, 1H), 3.81 – 3.68 (m, 7H), 3.61 (dd, *J* = 12.4, 7.2 Hz, 1H), 3.50 – 3.44 (m, 3H), 3.39 – 3.34 (m, 1H), 3.33 – 3.22 (m, 4H), 3.14 – 3.07 (m, 1H), 2.76 (d, *J* = 9.6 Hz, 1H), 1.26 (s, 17H), 0.94 – 0.77 (m, 5H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 166.16, 166.08, 165.84, 165.79, 165.63, 165.50, 165.32, 165.26, 165.20, 164.82, 139.23, 138.49, 138.41, 138.24, 138.16, 133.76, 133.54, 133.49, 133.44, 133.31, 133.25, 133.15, 133.06, 129.97, 129.91, 129.84, 129.69, 129.61, 129.16, 129.09, 128.95, 128.89, 128.80, 128.66, 128.52, 128.48, 128.42, 128.37, 128.35, 128.22, 128.20, 128.11, 128.05, 127.98, 127.85, 127.50, 127.44, 127.22, 100.66, 100.59, 100.51, 98.16, 80.71, 80.63, 80.60, 77.83, 77.76, 76.21, 74.86, 74.26, 74.21, 73.67, 73.26, 73.05, 72.47, 72.20, 72.11, 69.94, 69.78, 68.55, 67.22, 66.82, 63.12, 63.08, 55.37, 32.08, 31.10, 29.85, 29.52, 22.85 ppm; HR-TOF MS [$M+NH_4^+$] calcd 1995.6984 found 1995.7010.

Deletion sequences were isolated and characterized by HR-TOF MS: trisaccharide isolated in <1mg [$M+Na^+$] calcd 1525.5190 found 1525.5159; 5 mg of unreacted acceptor were recovered (38%, 0.0075 mmol).

References:

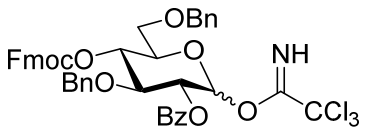
(1) Vijaya Ganesh, N.; Fujikawa, K.; Tan, Y. H.; Stine, K. J.; Demchenko, A. V. HPLC-assisted automated oligosaccharide synthesis. *Org. Lett.* **2012**, *14*, 3036-3039.

(2) Delbianco, M.; Kononov, A.; Poveda, A.; Yu, Y.; Diercks, T.; Jimenez-Barbero, J.; Seeberger, P. H. Well-defined oligo- and polysaccharides as ideal probes for structural studies. *J. Am. Chem. Soc.* **2018**, *140*, 5421-5426.

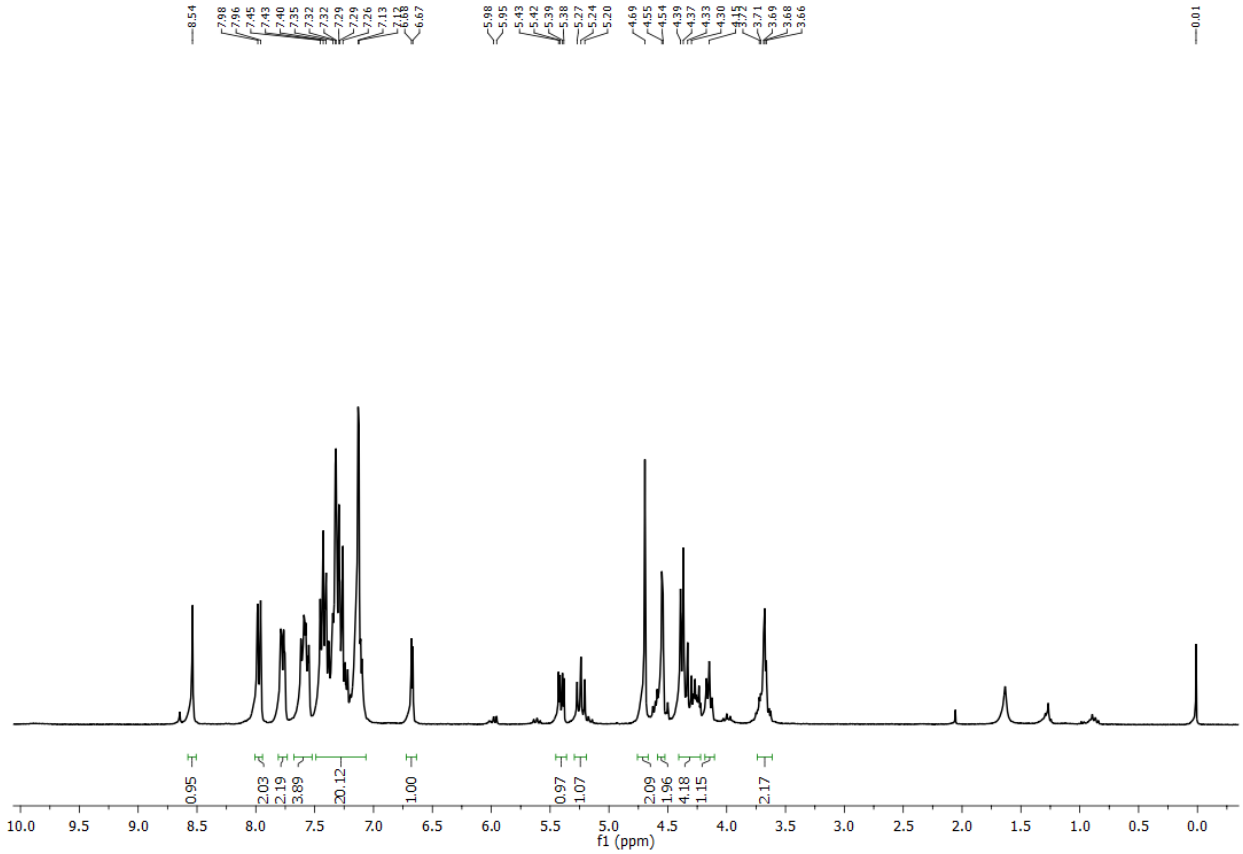
(3) Pornsuriyasak, P.; Jia, X. G.; Kaeothip, S.; Demchenko, A. V. Templated oligosaccharide synthesis: the linker effect on the stereoselectivity of glycosylation. *Org. Lett.* **2016**, *18*, 2316-2319.

(4) Pistorio, S. G.; Nigudkar, S. S.; Stine, K. J.; Demchenko, A. V. HPLC-assisted automated oligosaccharide synthesis: the implementation of the autosampler as a mode of the reagent delivery. *J. Org. Chem.* **2016**, *81*, 8796-8805.

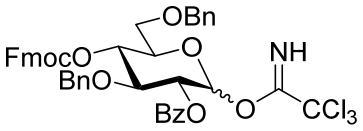
NMR spectra



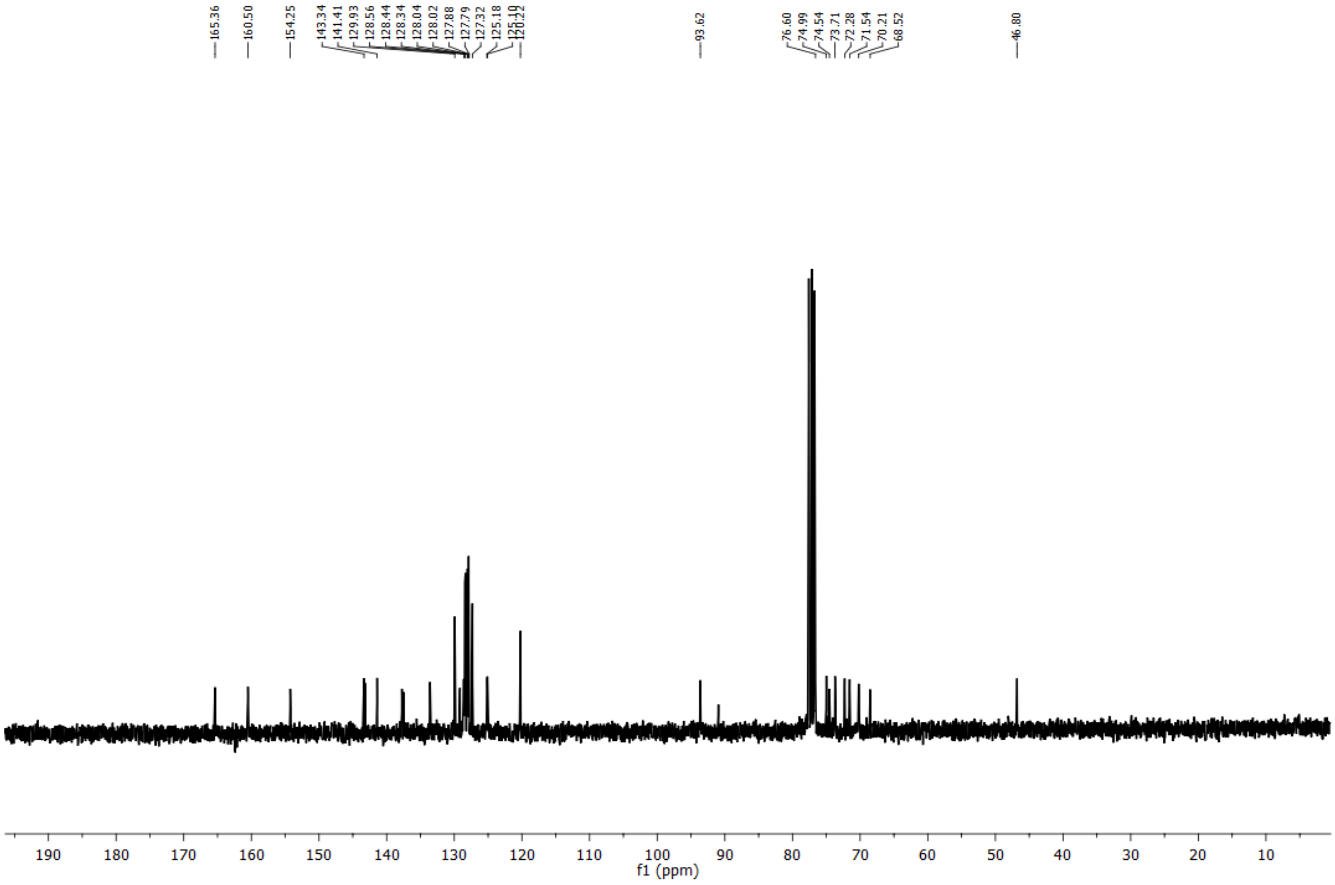
5



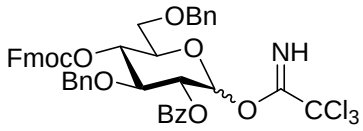
^{1}H NMR ($CDCl_3$, 300 MHz)



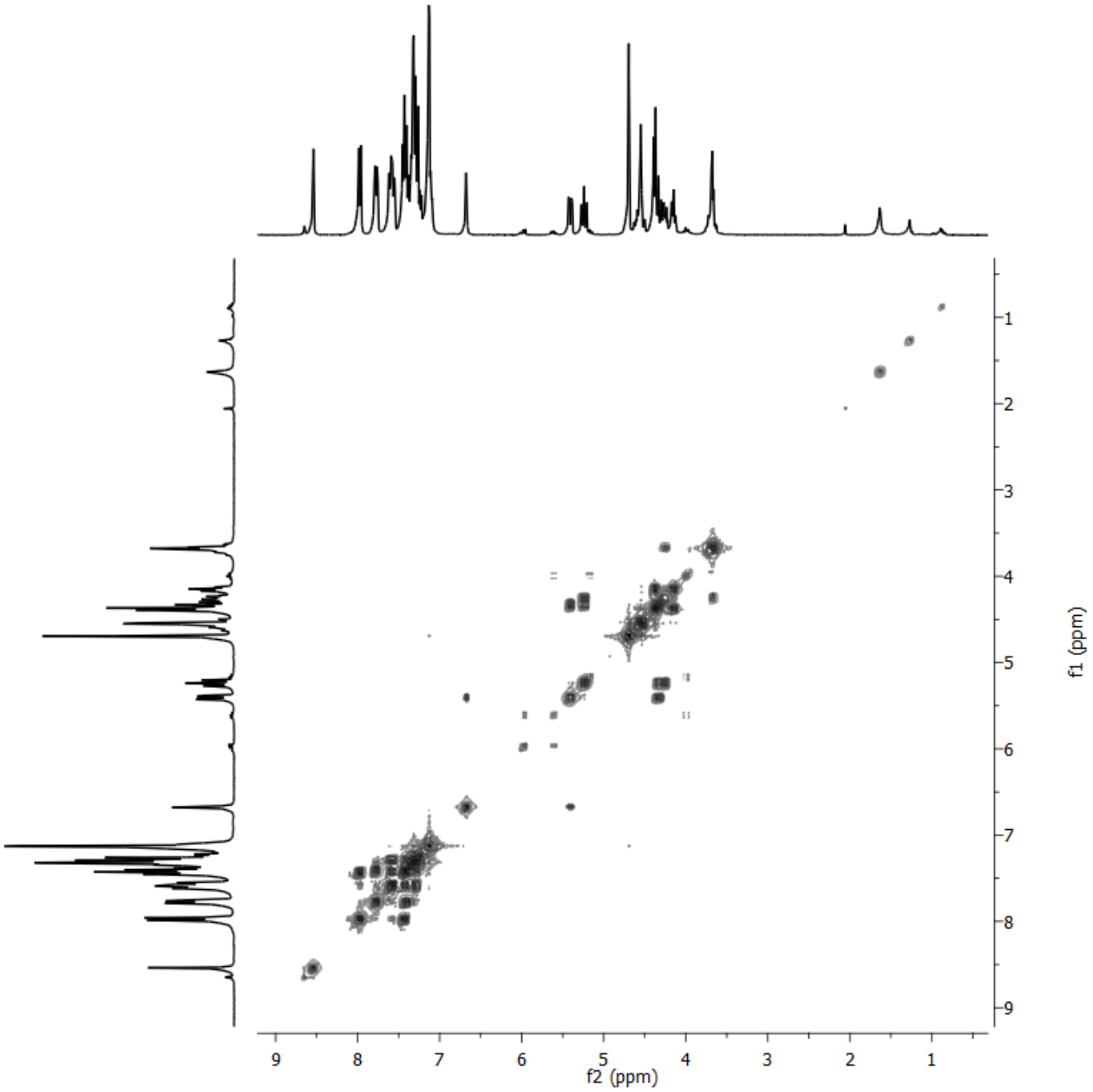
5



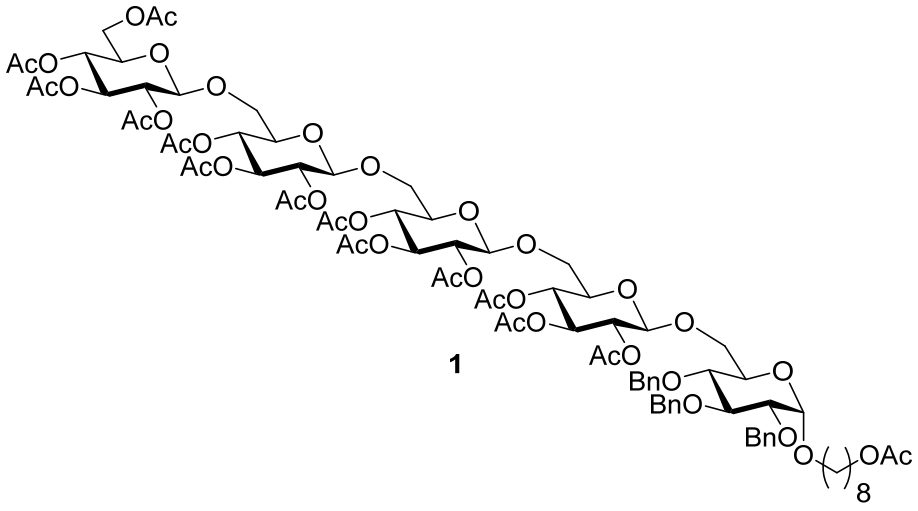
^{13}C NMR ($CDCl_3$, 75 MHz)



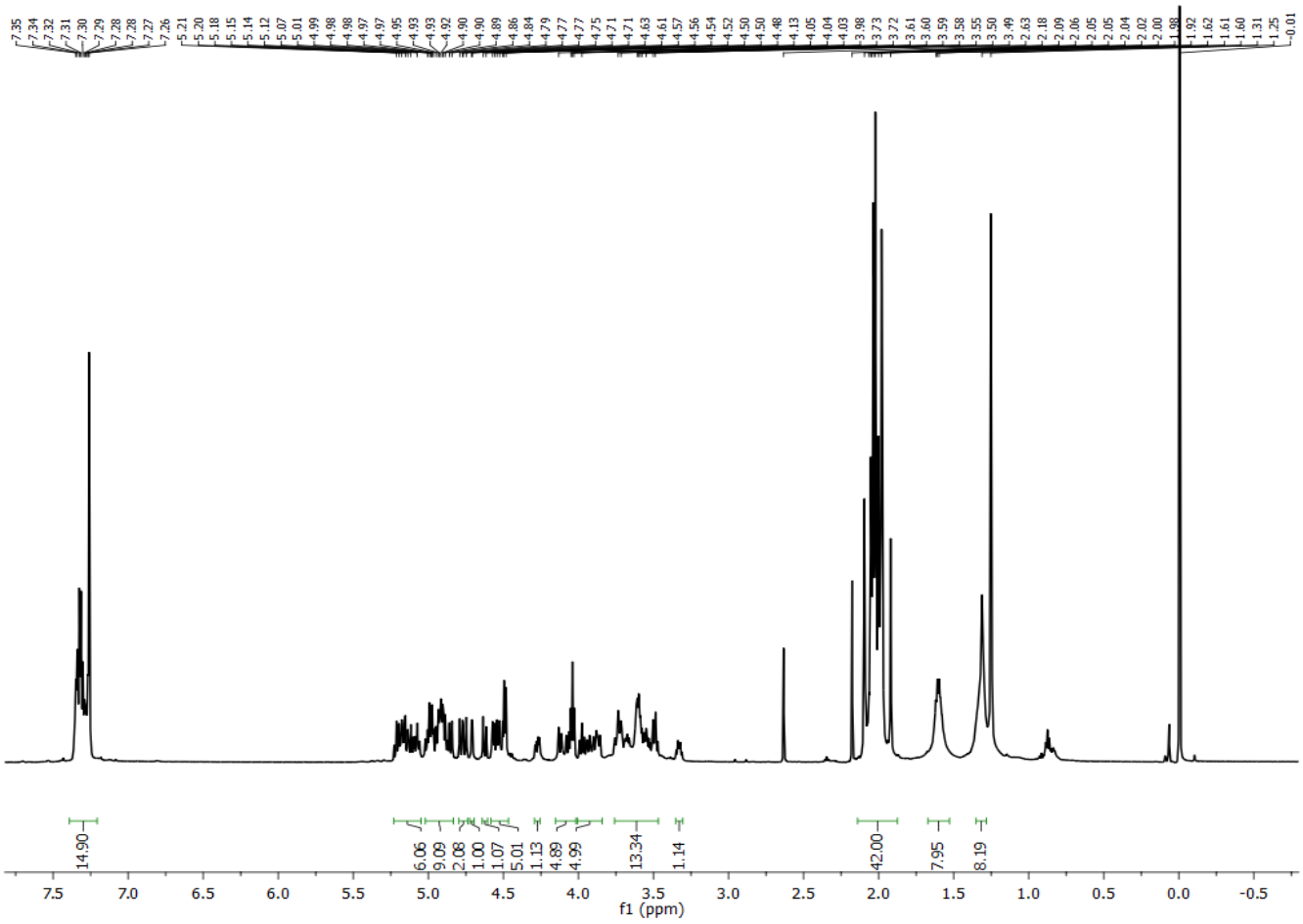
5



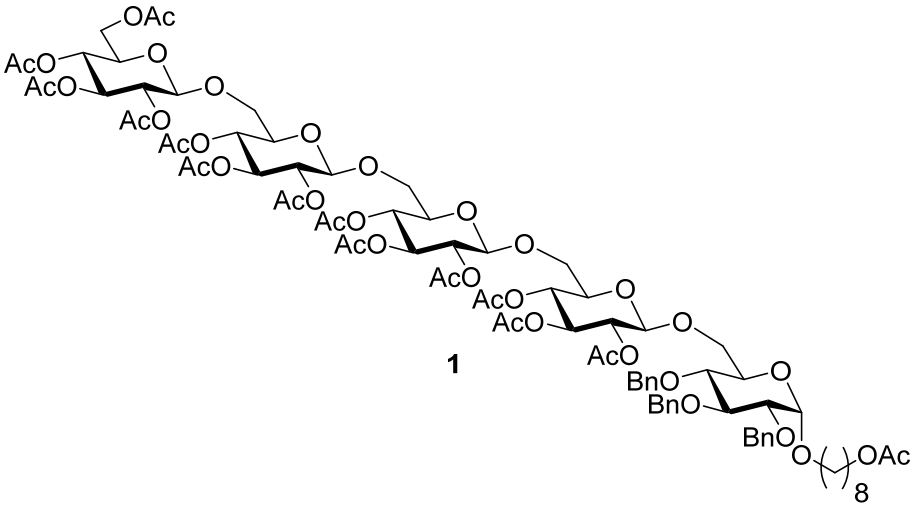
COSY NMR ($CDCl_3$, 300 MHz)



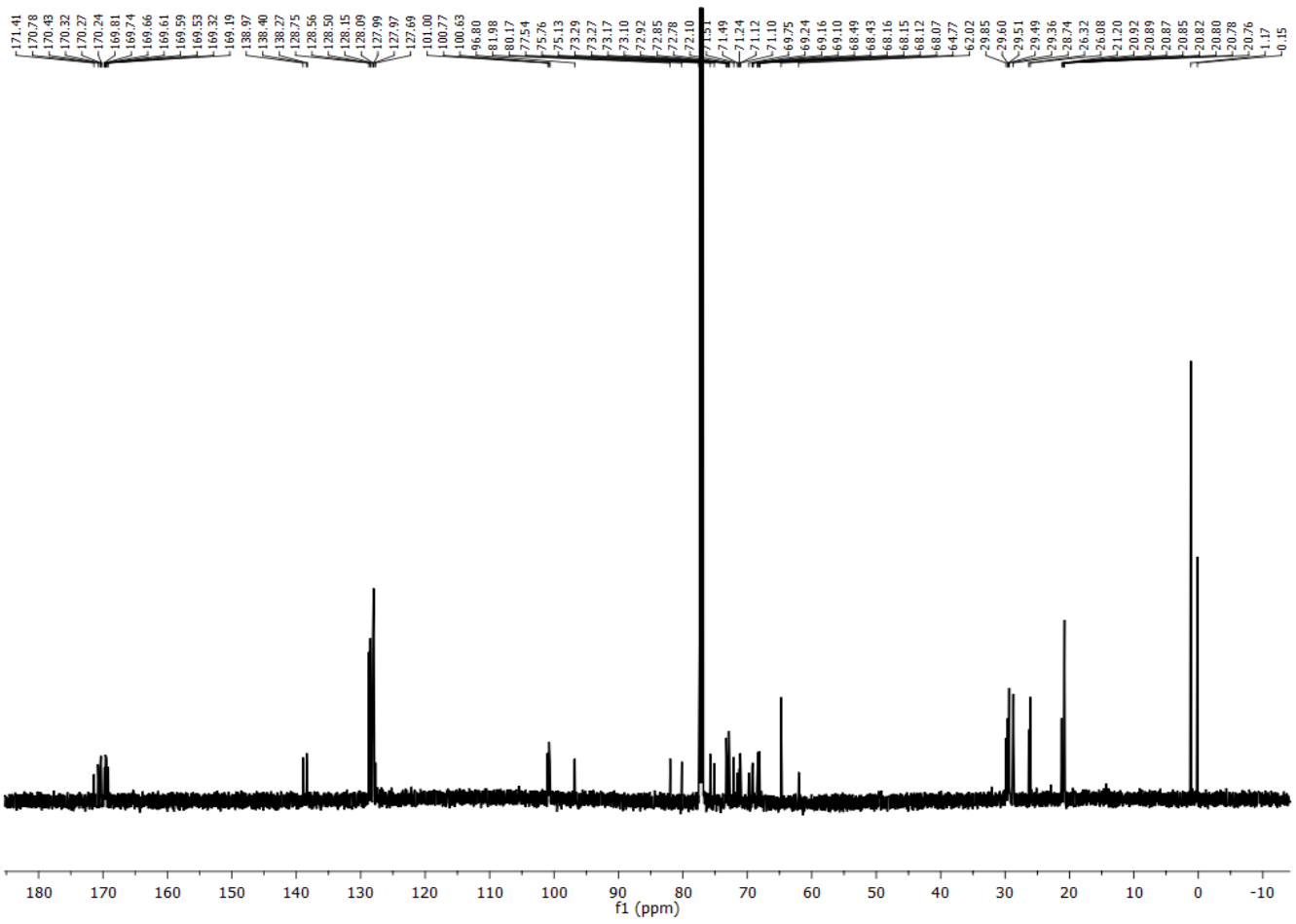
1



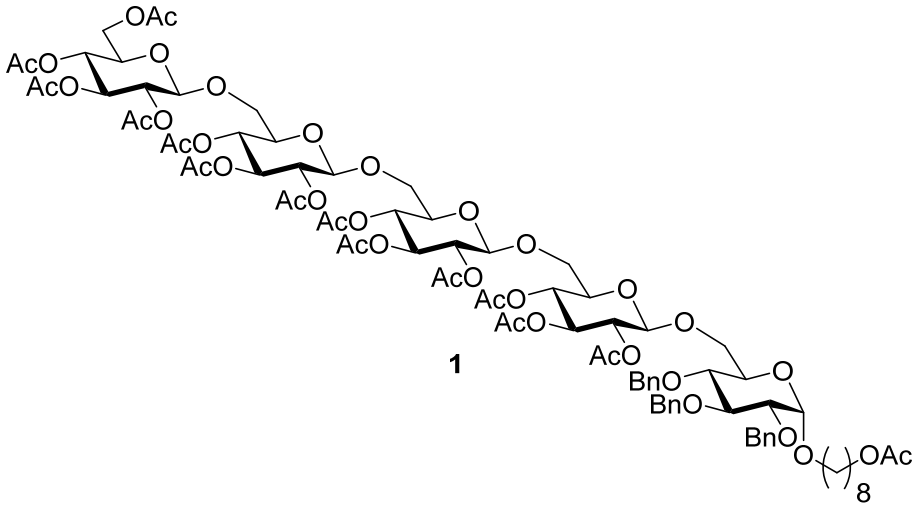
^{1}H NMR ($CDCl_3$, 600 MHz)



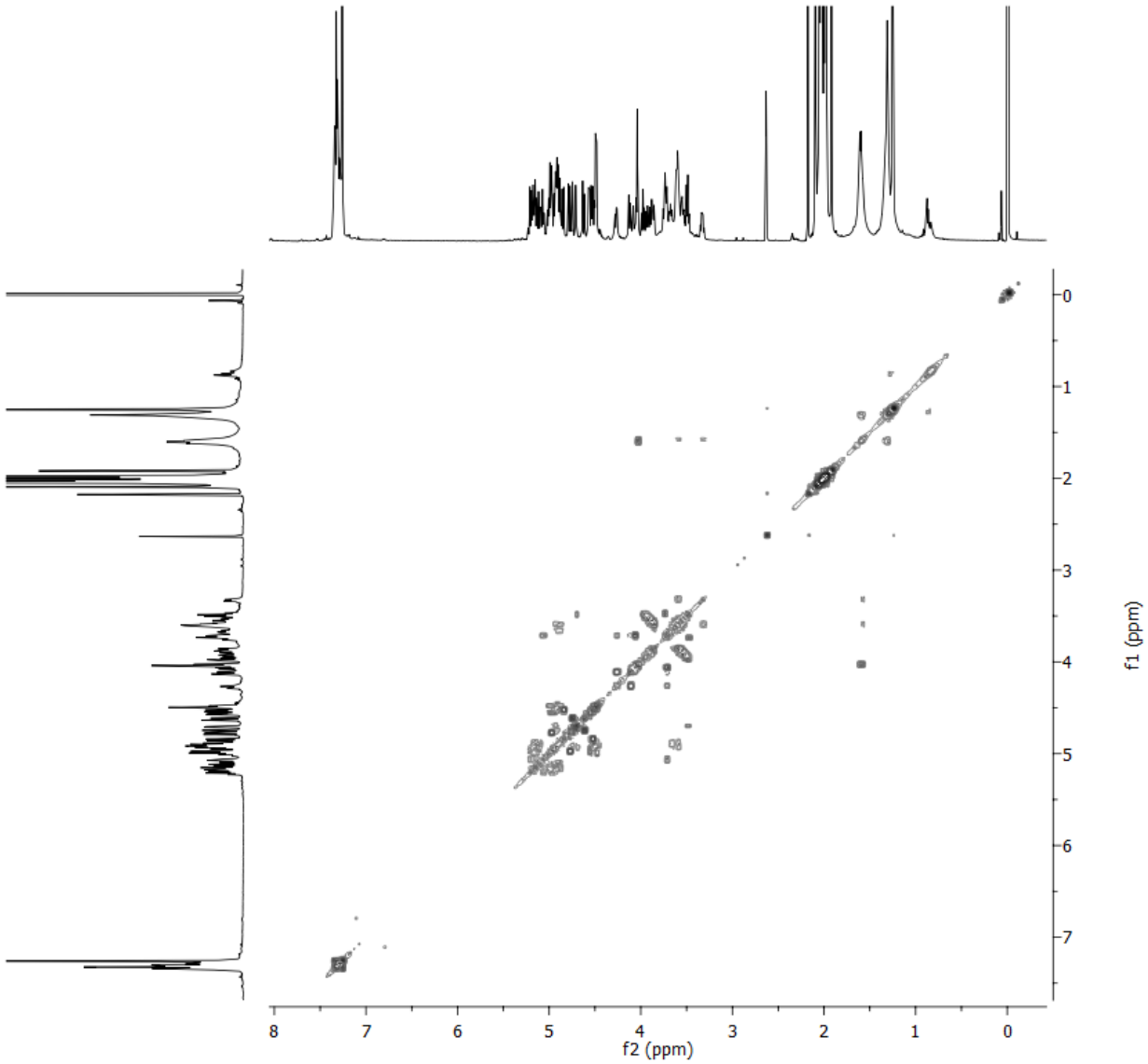
1



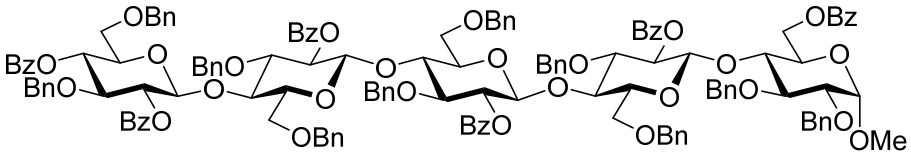
^{13}C NMR ($CDCl_3$, 151 MHz)



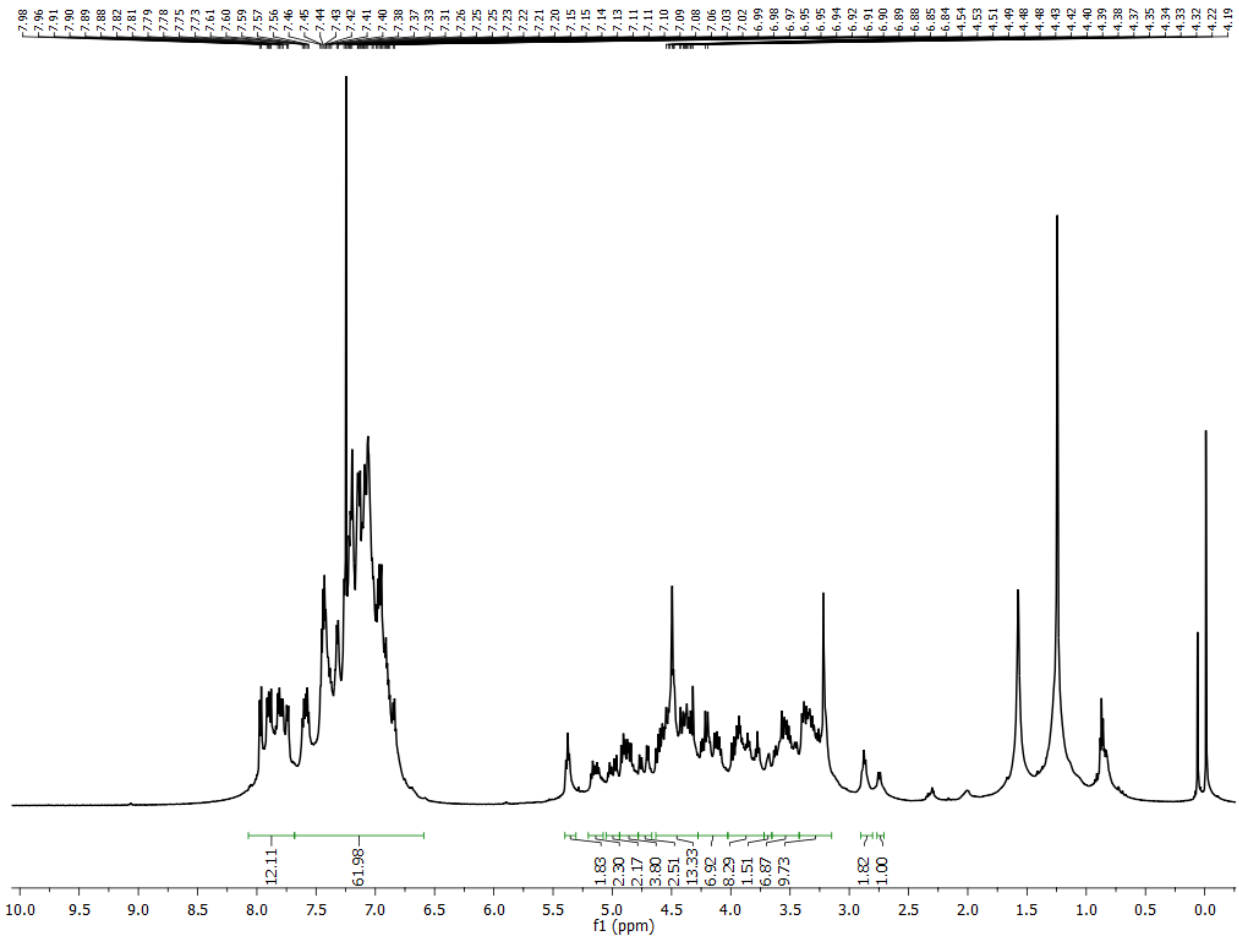
1



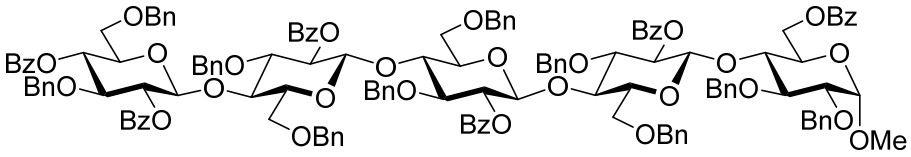
COSY NMR ($CDCl_3$, 600 MHz)



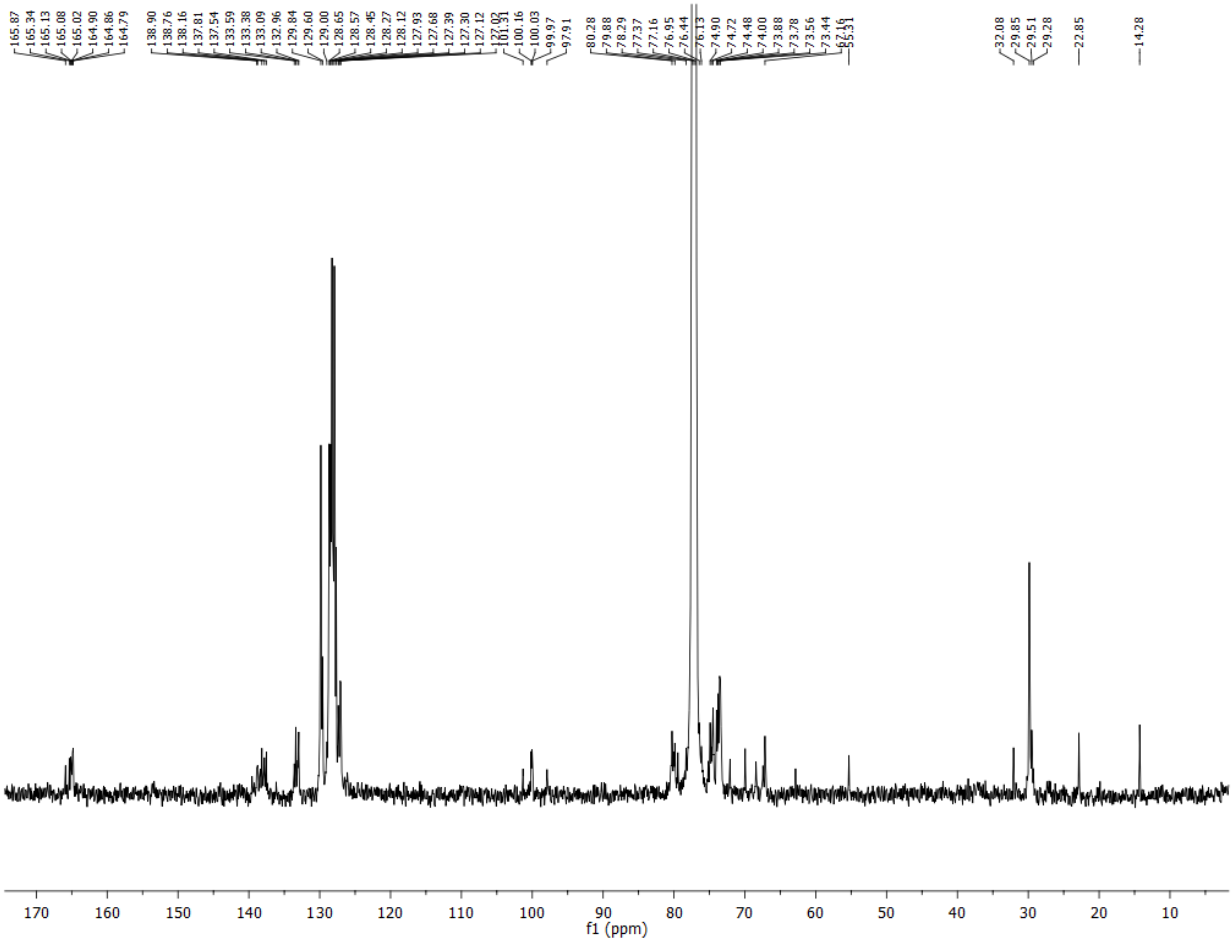
2



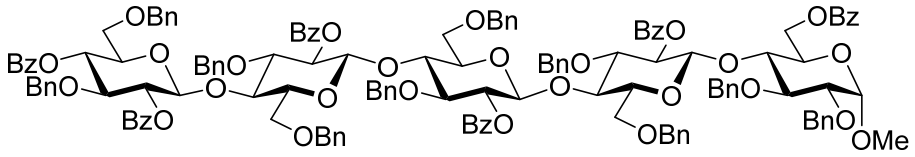
^{1}H NMR ($CDCl_3$, 600 MHz)



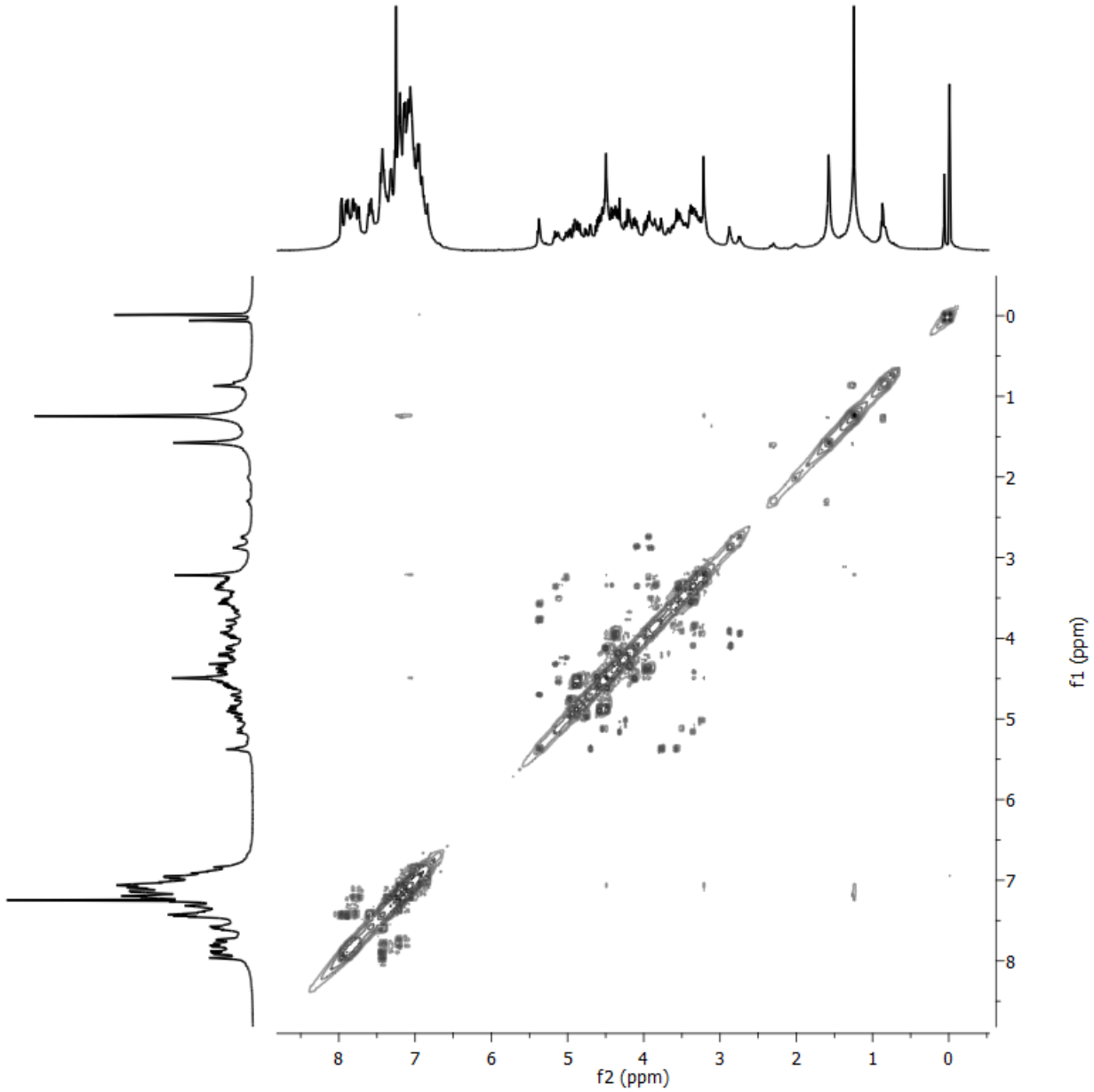
2



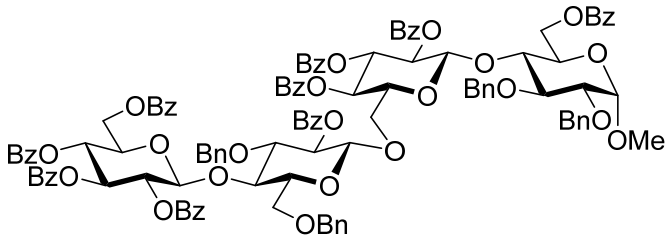
^{13}C NMR ($CDCl_3$, 151 MHz)



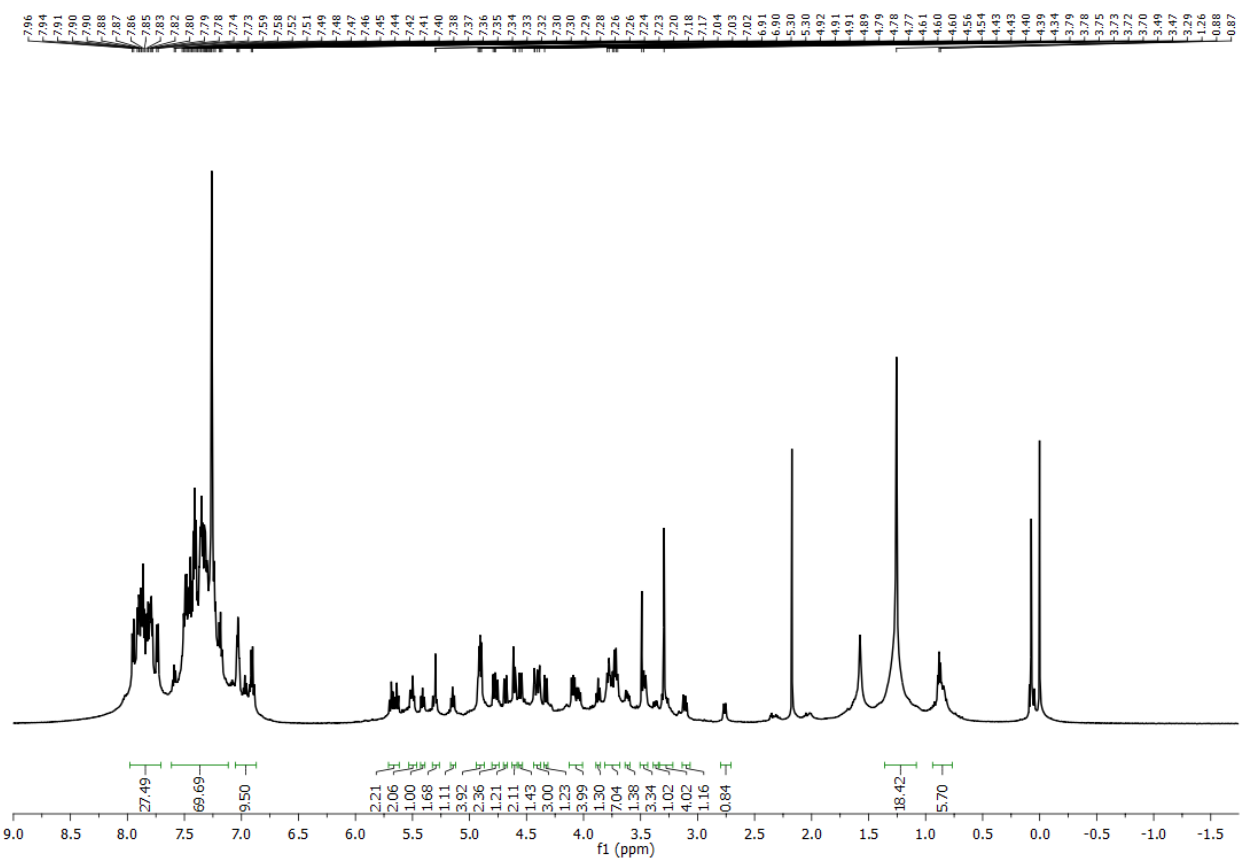
2



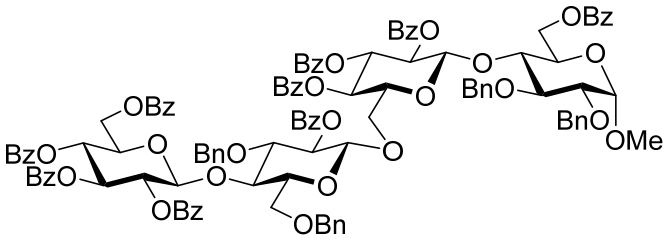
COSY NMR ($CDCl_3$, 600 MHz)



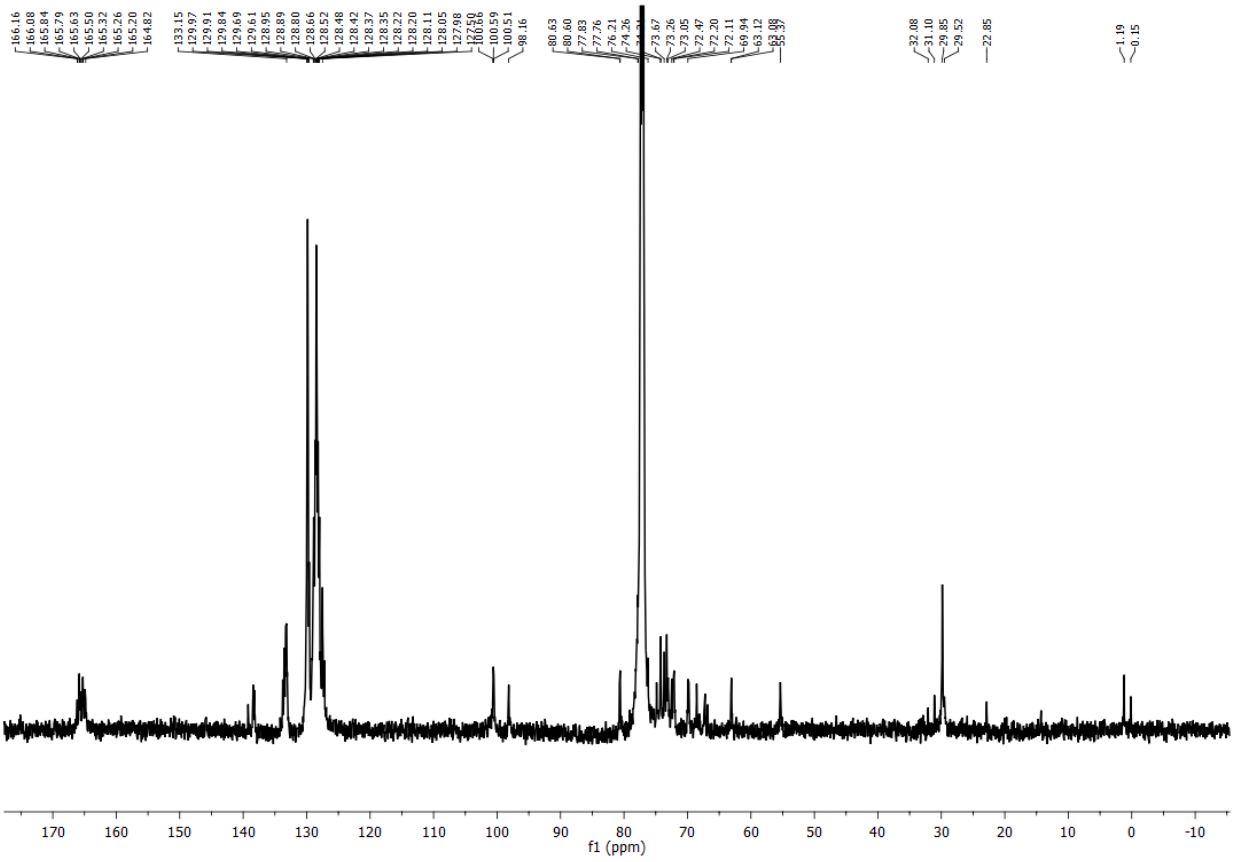
3



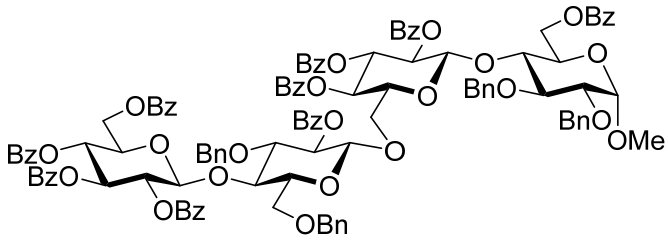
^{1}H NMR ($CDCl_3$, 600 MHz)



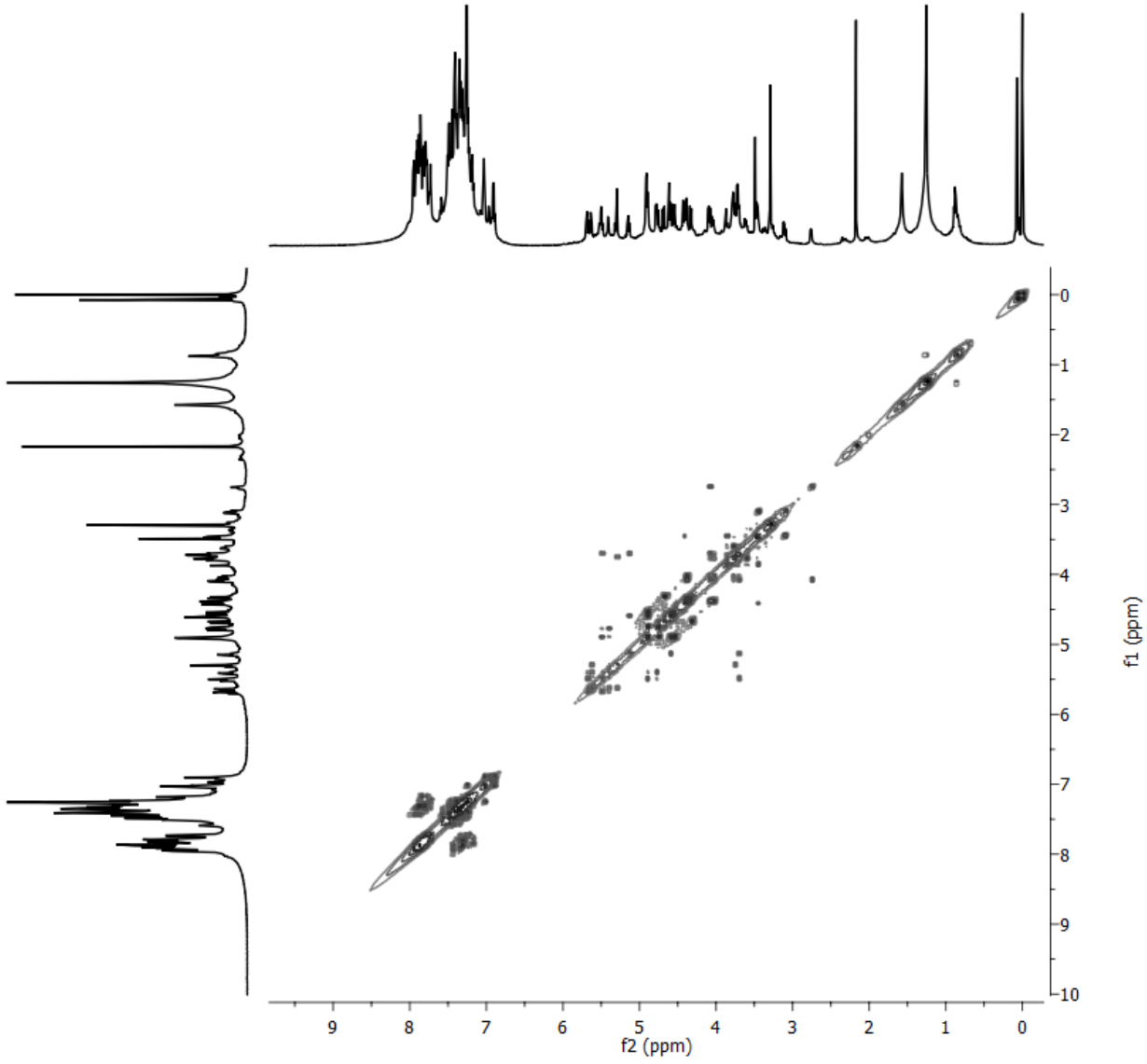
3



^{13}C NMR ($CDCl_3$, 151 MHz)



3



COSY NMR ($CDCl_3$, 600 MHz)