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Supporting Information

The development of a dedicated polymer support for the solid-phase oligosaccharide synthesis

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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 any further purification. Column chromatography was performed on silica gel 60 (70−230 mesh) or on 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. Pyridine was dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3 Å). Amberlite IR20 (H^+) was washed three times with MeOH and stored under MeOH. Optical rotations were measured using a Jasco 'P-2000' polarimeter. IR spectra were recoreded using an Agilent Cary 630 FTIR or a Thermo Nicolet Avatar 360 FTIR. ^{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 CDCl3 (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.
The surface murphlogy of the polymer was characterized using scanning electron microscopy using JEOL JSM-6320F field emission scanning electron microscope (JEOL USA Inc. California USA). The surface area and the total pore volume were calculated from the N_2 adsorption/desorption isotherms, obtained using automated surface area analyser (BECKman Coulter SA-3100 Gas Adsorption Surface Area and Pore Size Analyzer, Beckman Coulter, Inc, California,USA). Thermogravimetric analysis was done using Q500 Thermogravimetric analyzer (TA instruments,Delaware, USA).

Set up of the HPLC-A synthesizer

The HPLC based synthesizer has been assembled using

- 1260 Agilent Infinity I series Quaternary Pump
- Variable Wavelength Detector with single-wavelenght mode
- The Autosampler is the analytical module from 1260 Infinity series. The autosampler is equipped a 900 µL loop and one tray holding 100 x 2 mL vials.
- 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.

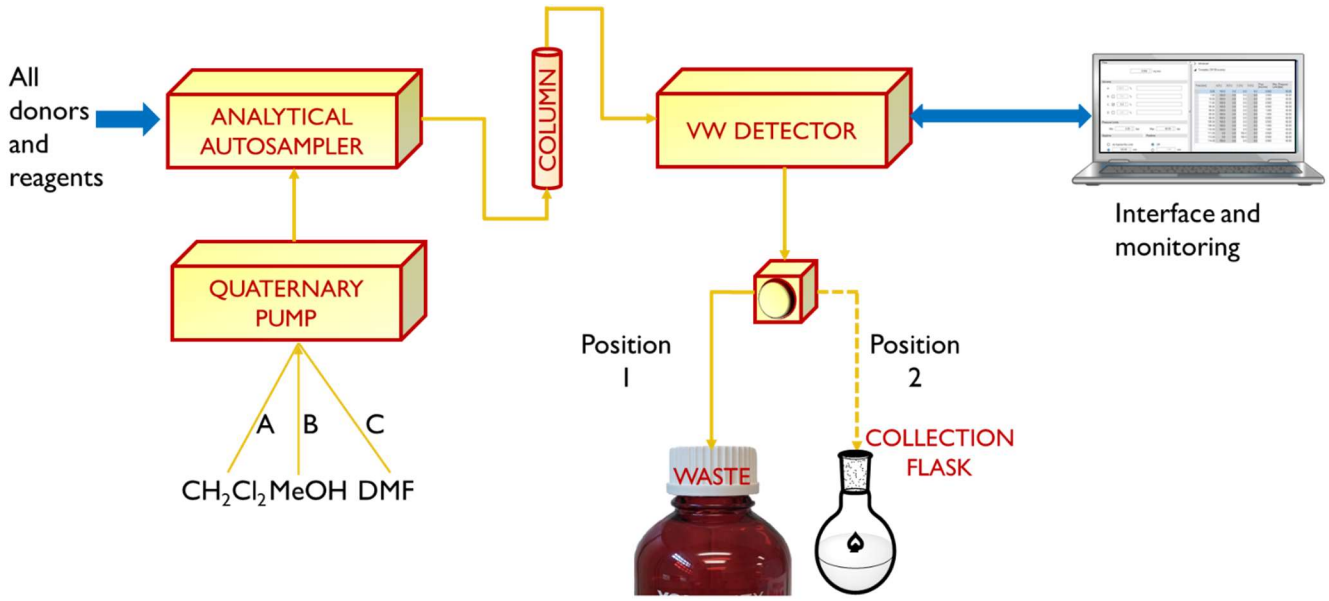


Figure S1. The HPLC-A synthesizer set up

Synthesis of Crosslinker and Panzagel resin

Di (ethylene glycol) bis (4-vinylbenzyl) ether (2) was obtained in accordance with the reported procedure and its analytical data were in accordance with that previously described.[1]
Selected analytical data for **2**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, J = 8.2 Hz, 4H, aromatic), 7.30 (d, J = 8.2 Hz, 4H, aromatic), 6.71 (dd, J = 17.6, 10.9 Hz, 2H, C*H*CH_2), 5.74 (dd, J = 17.6, 0.8 Hz, 2H, CHC*H*$_2$-*trans*), 5.23 (dd, J = 10.9, 0.7 Hz, 2H, CHC*H*$_2$-*cis*), 4.56 (s, 4H, PhC*H*$_2$), 3.66 (m 16H, OC*H*$_2$C*H*$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.94 (*C*CH_2), 136.98 (*C*CH), 136.59 (C*C*HCH_2), 128.02 (*C*H aromatic), 126.28 (*C*H aromatic), 113.83 (*C*H_2CH), 73.02 (Ph*C*H_2), 70.77 (*C*H_2O), 69.46 (*C*H_2O).

Panzagel-Cl Resin (6) was obtained in accordance with the reported procedure.[2] The polymer was analyzed using solid state FT-IR.

Panzagel-NH_2 Resin (7)

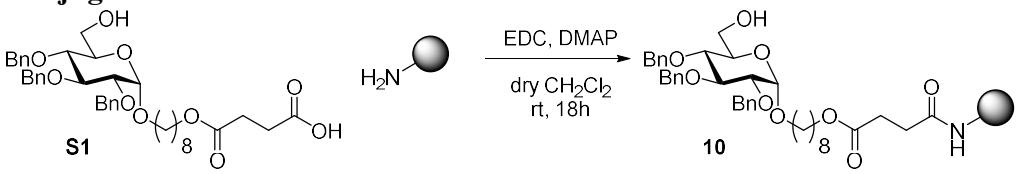
To Panzagel-Cl beads (**6**) (1 g) in a round bottom flask equipped with a mechanical stirred, dimethylformamide (15 mL) was added. After swelling the polymer for 1 h, Phthalimide Potassium salt (1 g) was added and the mixture was stirred for 16 h at 55 °C. The resin was then washed with CH_2Cl_2 (3 x 30 mL), MeOH (3 x 30 mL), acetone (3 x 30 mL) and water (3 x3 0 mL) and dried at 60 °C under vacuum. The obtained polymer was swelled with MeOH:$CHCl_3$ = 3:7 for an hour in a flask equipped with a mechanical stirrer and $H_2NNH_2{\cdot}H_2O$ (3 mL) was added. The mixture was stirred at rt for 48 h. The resin was then washed with CH_2Cl_2 (3 x 30 mL), MeOH (3 x3 0 mL), acetone (3 x 30 mL) and water (3 x 30 mL) and dried at 60 °C under vacuum.
The polymer was characterized using FT-IR, SEM, BET and the presence of NH_2 groups was confirmed using the Kaiser test.

Synthesis of Glycosyl Donors and Glycosyl Acceptors

2,3,4-Tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)- α/β-D-glucopyranosyl trichloroacetimidate (11) was obtained in accordance with the reported procedure and its analytical data were in accordance with that previously described.[3]

8-(3-Carboxypropanoyloxy)oct-1-yl 2,3,4-tri-O-benzyl-6-O-triphenylmethyl-α-D-glucopyranoside (S1) was performed in accordance with the reported procedure and its analytical data were in accordance with that previously described.[4]

Conjugate 10



Loading = 0.33 mmol/g

The title conjugate was obtained by reaction of **S1** with JandaJel performed in accordance with the reported procedure.[4] The loading of 0.33 mmol/g for **10** was determined by direct cleavage from the solid support (50 mg) on HPLC to mimic conditions for the subsequent reactions.

Preparation of the reagent vials

All the solutions were freshly prepared using the ACS-grade solvents and kept at room temperature for the duration of the synthesis.
Donor: a solution of glycosyl donor **11** (103 mg, 0.12 mmol, 7.2 equiv) in CH_2Cl_2 (2.7 mL), split in two vials containing 1.6 mL and 1.1 mL of solution.
Promoter: a solution of TMSOTf (22 µL, 0.124 mmol, 7.5 equiv) in CH_2Cl_2 (500 µL). Both the amount were doubled to prepare an excess of acidic solution to acidify the resin after Fmoc removal.
Reagents for Fmoc removal: a solution of piperidine in DMF (2.5 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), split equally in 2 vials.
Washing solutions and blanks: methylene chloride in W_1, MeOH in W_2 and methylene chloride in B (2 mL).

The Automated Assembly of Oligosaccharides

All reactions were carried 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 acceptor **10** were performed with 0.0165 mmol.

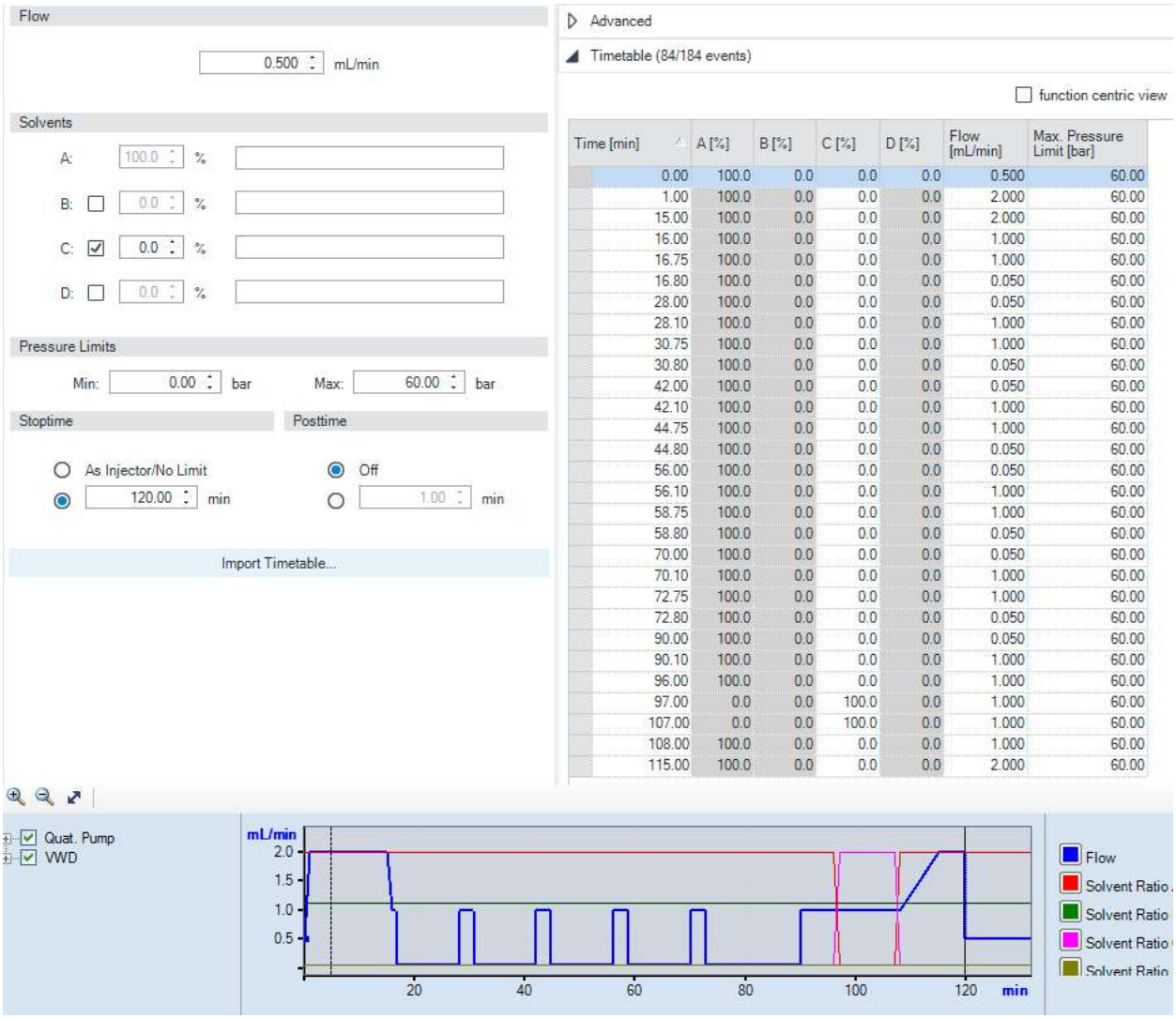
Glycosylations. The sequences are comprehensive of washings of the resin pre and after glycosylation steps.The glycosylations are split in 5 iterations of donor injections. The donor and the promoter are drawn from the vial, mixed in the needle seat and the injected. The flowrate is

lowered to maximize the permanence of the donor in the column. Once the activated donor has passed through the column, the same operation is repeated. At the end of the glycosylation the resin is washed with CH_2Cl_2 and DMF.
In figure S3 the programming of the components of the synthesizer is reported. In figure 3A the pump timetable, in 3B the autosampler programmin. The valve was on position 1 during the whole sequence.

Fmoc removal. The reaction is monitored at 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. Default volume is 500 µL.

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 nm and the sequence consists of 4 consecutive injections of the cleaving solution.
In figure S5 A, B and C are depicted the settings of the modules.

Detection. Representative UV traces for each sequence are depicted in Figure S6A, 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 Fmoc removal trace **A** 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.

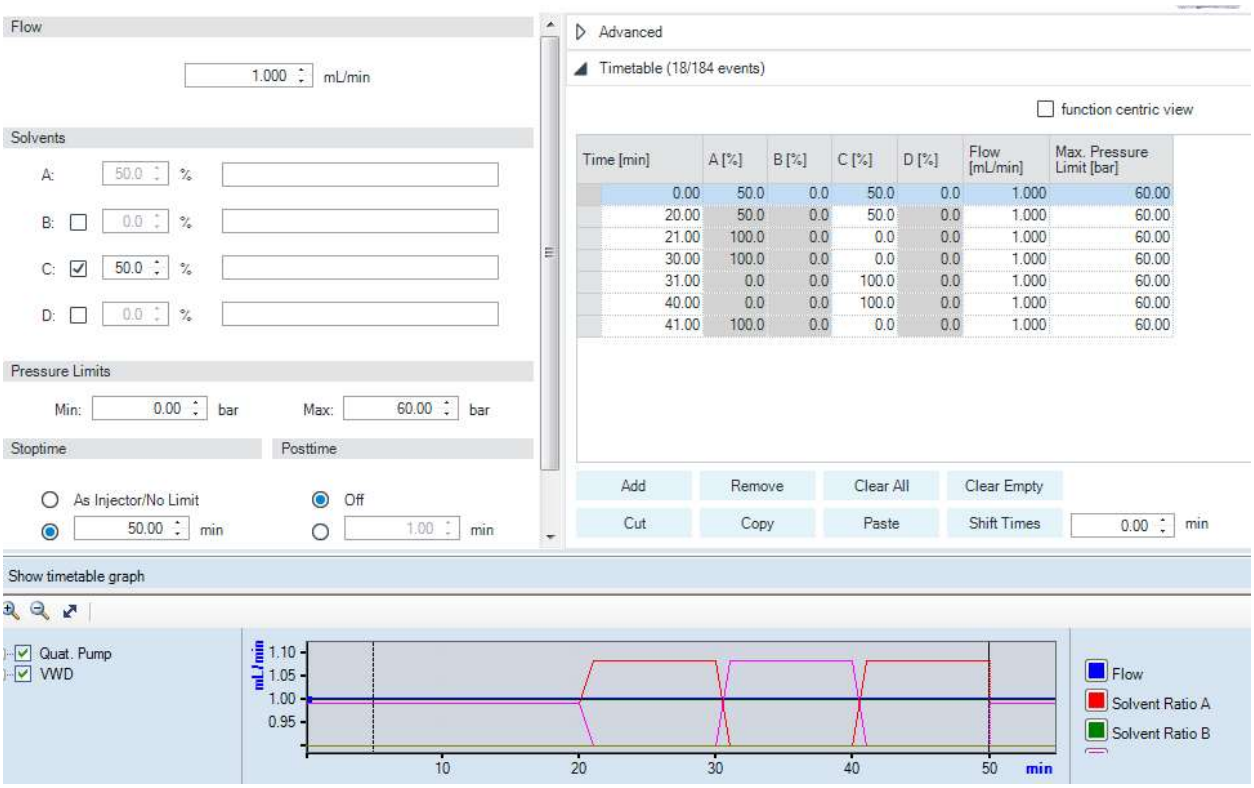

Flow
0.500 mL/min
Solvents
A: 100.0 %
B: 0.0 %
C: 0.0 %
D: 0.0 %
Pressure Limits
Min: 0.00 bar
Max: 60.00 bar
Stoptime
As Injector/No Limit
120.00 min
Posttime
Off
1.00 min
Import Timetable...
Advanced
Timetable (84/184 events)
function centric view
Time [min] | A [%] | B [%] | C [%] | D [%] | Flow [mL/min] | 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
15.00 100.0 0.0 0.0 0.0 2.000 60.00
16.00 100.0 0.0 0.0 0.0 1.000 60.00
16.75 100.0 0.0 0.0 0.0 1.000 60.00
16.80 100.0 0.0 0.0 0.0 0.050 60.00
28.00 100.0 0.0 0.0 0.0 0.050 60.00
28.10 100.0 0.0 0.0 0.0 1.000 60.00
30.75 100.0 0.0 0.0 0.0 1.000 60.00
30.80 100.0 0.0 0.0 0.0 0.050 60.00
42.00 100.0 0.0 0.0 0.0 0.050 60.00
42.10 100.0 0.0 0.0 0.0 1.000 60.00
44.75 100.0 0.0 0.0 0.0 1.000 60.00
44.80 100.0 0.0 0.0 0.0 0.050 60.00
56.00 100.0 0.0 0.0 0.0 0.050 60.00
56.10 100.0 0.0 0.0 0.0 1.000 60.00
58.75 100.0 0.0 0.0 0.0 1.000 60.00
58.80 100.0 0.0 0.0 0.0 0.050 60.00
70.00 100.0 0.0 0.0 0.0 0.050 60.00
70.10 100.0 0.0 0.0 0.0 1.000 60.00
72.75 100.0 0.0 0.0 0.0 1.000 60.00
72.80 100.0 0.0 0.0 0.0 0.050 60.00
90.00 100.0 0.0 0.0 0.0 0.050 60.00
90.10 100.0 0.0 0.0 0.0 1.000 60.00
96.00 100.0 0.0 0.0 0.0 1.000 60.00
97.00 0.0 0.0 100.0 0.0 1.000 60.00
107.00 0.0 0.0 100.0 0.0 1.000 60.00
108.00 100.0 0.0 0.0 0.0 1.000 60.00
115.00 100.0 0.0 0.0 0.0 2.000 60.00
Quat. Pump
VWD
mL/min
2.0
1.5
1.0
0.5
20
40
60
80
100
120
min
Flow
Solvent Ratio
Solvent Ratio
Solvent Ratio
Solvent Ratio

A

Function	Parameter
Draw	Draw 100 µL from vial+ 1 with default speed using default offset
Inject	Inject
Wait	Wait 13 min
Repeat	Repeat 3 time(s)
Draw	Draw 500 µL from sample with 700 µL/min using default offset
Draw	Draw 100 µL from vial+ 1 with 200 µL/min using default offset
Wash	Wash needle in location "97" 1 times
Mix	Mix 600 µL from seat with maximum speed for 2 times
Inject	Inject
Wait	Wait 0.8 min
Valve	Switch valve to "Bypass"
Wait	Wait 10 min
Eject	Eject maximum volume to seat with maximum speed using default offset
End Repeat	End Repeat
Repeat	Repeat 2 time(s)
Draw	Draw 500 µL from vial+ 2 with 700 µL/min using default offset
Draw	Draw 100 µL from vial+ 1 with 200 µL/min using default offset
Wash	Wash needle in location "97" 1 times
Mix	Mix 600 µL from seat with maximum speed for 2 times
Inject	Inject
Wait	Wait 0.8 min
Valve	Switch valve to "Bypass"
Wait	Wait 10 min
Eject	Eject maximum volume to seat with maximum speed using default offset
End Repeat	End Repeat

B

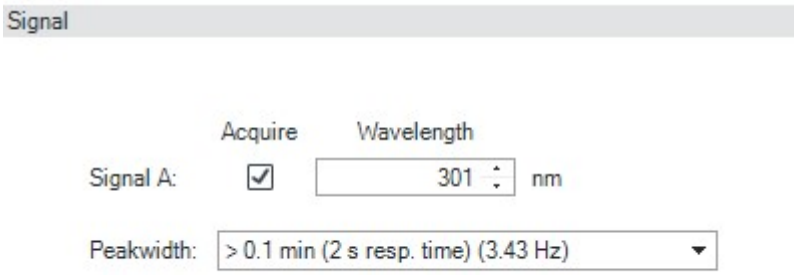
Figure S2. Settings of the components of the HPLC synthesizer during glycosylation.



A

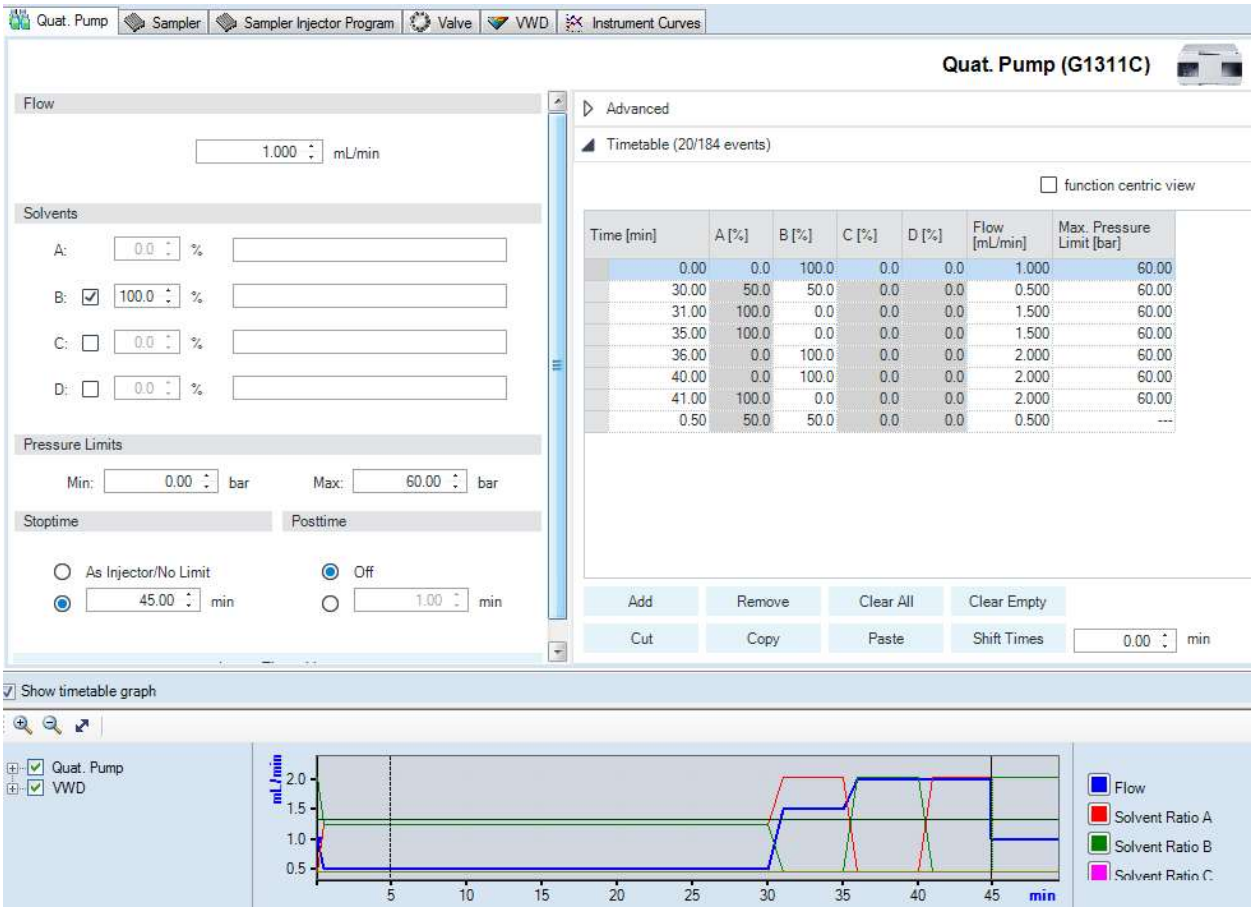
Function	Parameter
Repeat	Repeat 3 time(s)
Draw	Draw default volume from sample with default speed using default offset
Inject	Inject
Wait	Wait 3 min
Valve	Switch valve to "Bypass"
Eject	Eject default volume to seat with default speed using default offset
End Repeat	End Repeat
Draw	Draw maximum volume from location "96" with maximum speed using default offset
Eject	Eject maximum volume to seat with maximum speed using default offset
Draw	Draw 100 µL from vial+ -2 with 300 µL/min using default offset
Inject	Inject
Wash	Wash needle in location "97" 3 times
Wait	Wait 30 min
Draw	Draw 100 µL from location "96" with default speed using default offset
Eject	Eject default volume to seat with default speed using default offset

B

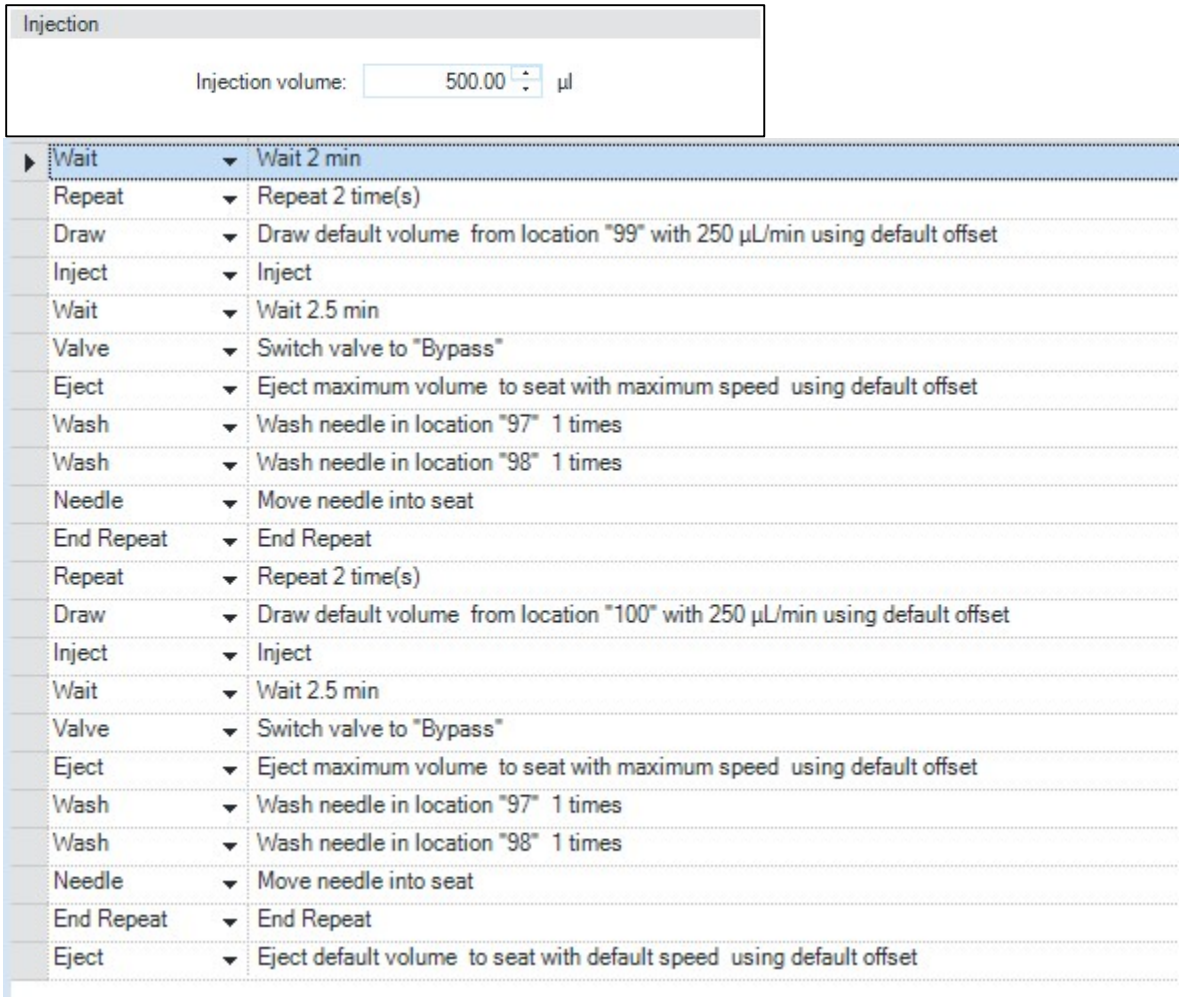


C

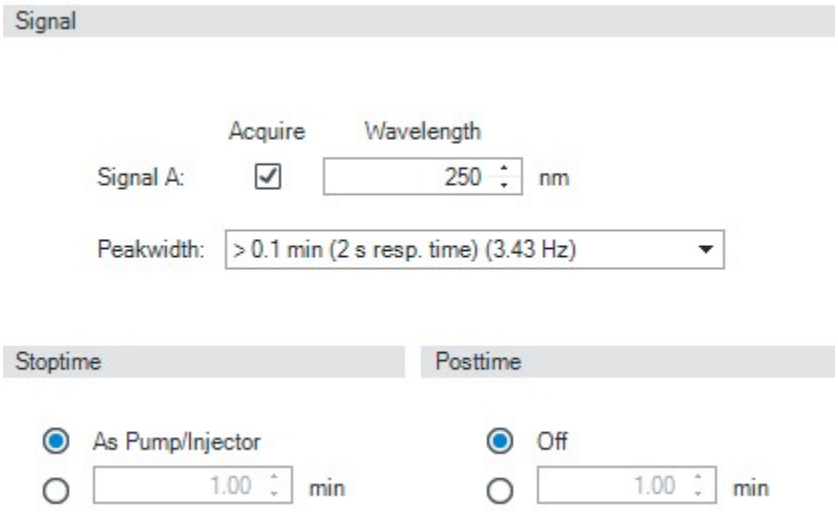
Figure S3. Settings of the components of the HPLC synthesizer during Fmoc removal.


Quat. Pump
Sampler
Sampler Injector Program
Valve
VWD
Instrument Curves
Quat. Pump (G1311C)
Flow
1.000 mL/min
Solvents
A: 0.0 %
B: 100.0 %
C: 0.0 %
D: 0.0 %
Pressure Limits
Min: 0.00 bar
Max: 60.00 bar
Stoptime
As Injector/No Limit
45.00 min
Posttime
Off
1.00 min
Advanced
Timetable (20/184 events)
function centric view
Time [min] A [%] B [%] C [%] D [%] Flow [mL/min] Max. Pressure Limit [bar]
0.00 0.0 100.0 0.0 0.0 1.000 60.00
30.00 50.0 50.0 0.0 0.0 0.500 60.00
31.00 100.0 0.0 0.0 0.0 1.500 60.00
35.00 100.0 0.0 0.0 0.0 1.500 60.00
36.00 0.0 100.0 0.0 0.0 2.000 60.00
40.00 0.0 100.0 0.0 0.0 2.000 60.00
41.00 100.0 0.0 0.0 0.0 2.000 60.00
0.50 50.0 50.0 0.0 0.0 0.500 ---
Add
Remove
Clear All
Clear Empty
Cut
Copy
Paste
Shift Times
0.00 min
Show timetable graph
Quat. Pump
VWD
mL/min
2.0
1.5
1.0
0.5
5
10
15
20
25
30
35
40
45
min
Flow
Solvent Ratio A
Solvent Ratio B
Solvent Ratio C

A

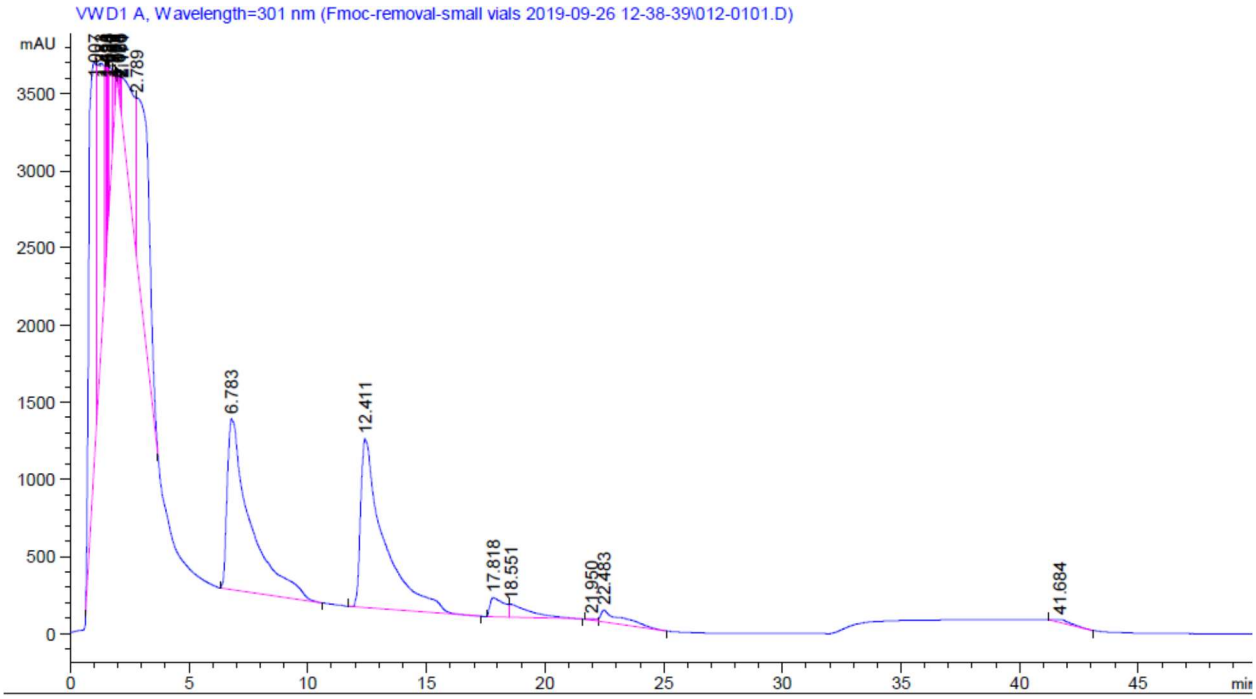


B

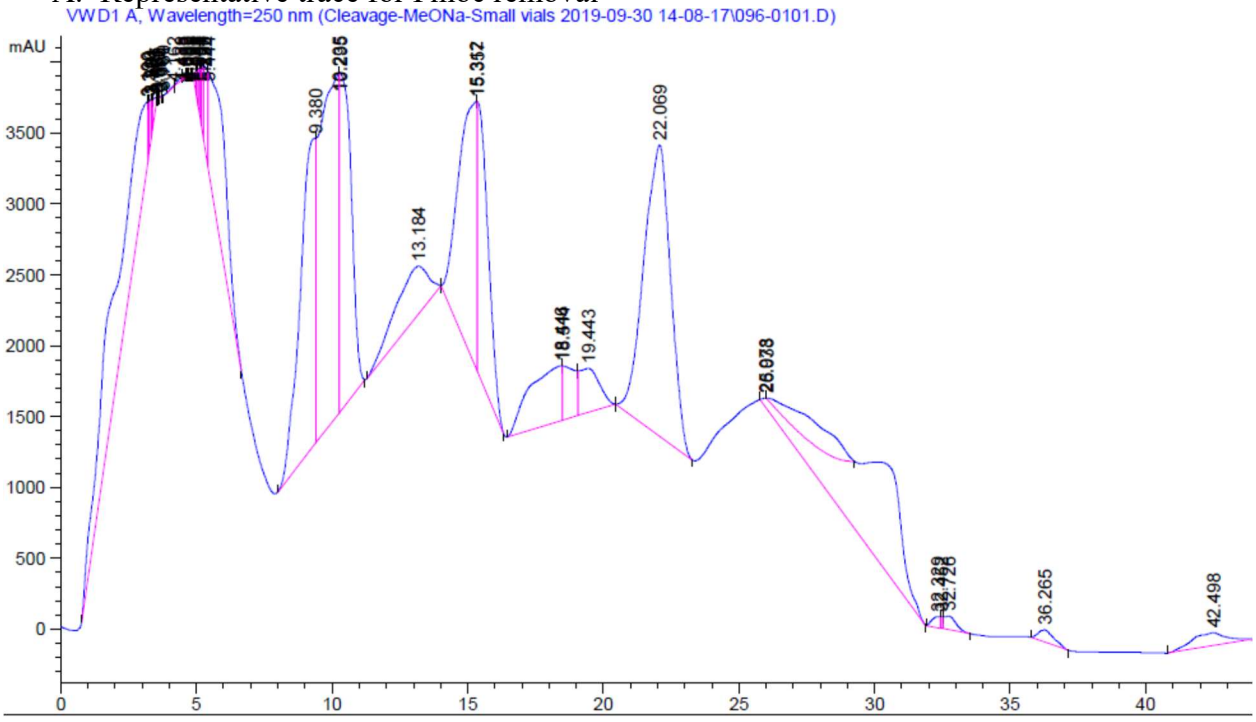


C

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



A. Representative trace for Fmoc removal



B. Representative trace for the final cleavage

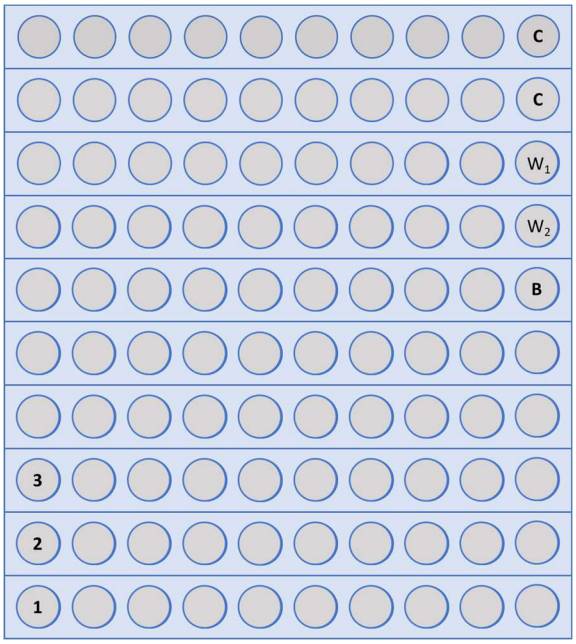
Figure S5. UV traces for the synthetic steps

Synthesis of disaccharide 8

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

Automation modules: The Omnifit column is filled with resin (50 mg) preloaded with acceptor **10** (0.0165 mmol). The vials were prepared according to the general methods and organized as depicted below. Vials 1 and 3 contain donor **11** (103 mg, 0.12 mmol, 7.2 equiv) split as described in the general methods, vial 2 contains of TMSOTf (22 µL, 0.124 mmol, 7.5 equiv), and vial 3 contains a piperine-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 tray organization:



Automation Sequence:
Glycosylation 120 min
Fmoc removal 60 min

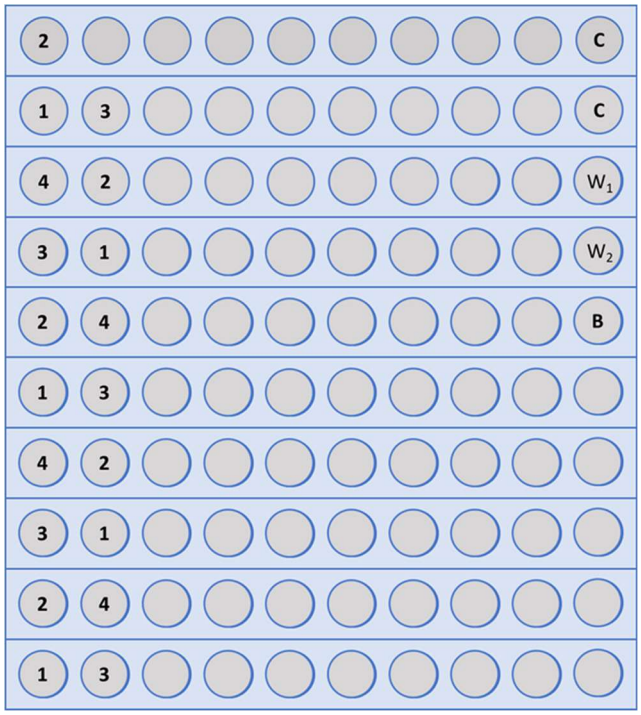
Post Automation: The reaction mixture collected from cleavage step was evaporated under reduced pressure, then diluted with MeOH (2.0 mL) and quenched with Amberlite IR20 H^+ form. The resin was filtrated and washed with MeOH, the solvent was evaporated and dried *in vacuo* for 1h. The crude was dissolved in pyridine (2.0 mL) and acetic anhydride (0.5 mL) was added. The resulting mixture was stirred under argon at room temperature for 16h. 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 **8** in 80% yield (12.2 mg, 0.0128 mmol). Analytical data matched what has been previously reported.[4]

Synthesis of pentasaccharide 9

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 (9).

Automation modules: The Omnifit column is filled with resin (50 mg) preloaded with acceptor **10** (0.0165 mmol).the vials were prepared according to the general methods and organized as depicted below. Vials 1 and 3 contain donor **11** (103 mg, 0.12 mmol, 7.2 equiv) split as described in the general methods, vial 2 contains of TMSOTf (22 µL, 0.124 mmol, 7.5 equiv), and vial 3 contains a piperine-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 tray 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 1h. 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 16h. 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 **9** in 30% yield (8.7 mg, 0.0048 mmol). Analytical data matched what has been previously reported.[5]

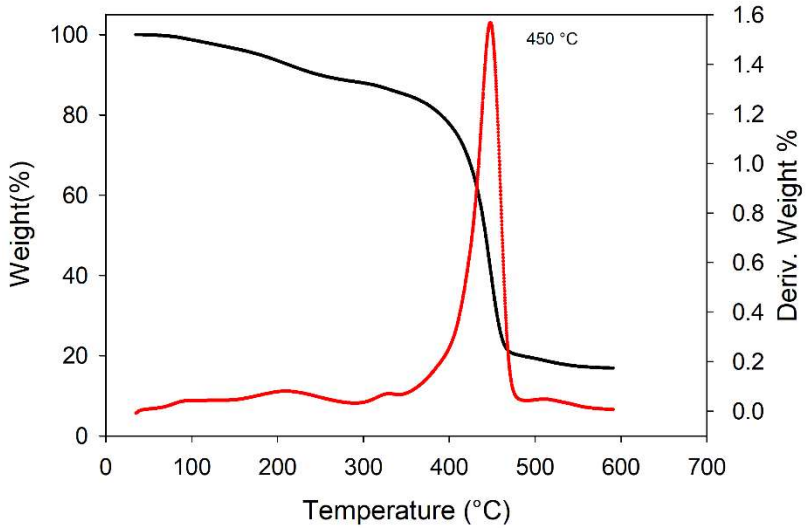


Figure S6. Thermogravimetric analysis (TGA) plots (black line) and Weight derivatives curves (red line) of Panzagel-TMSOTf

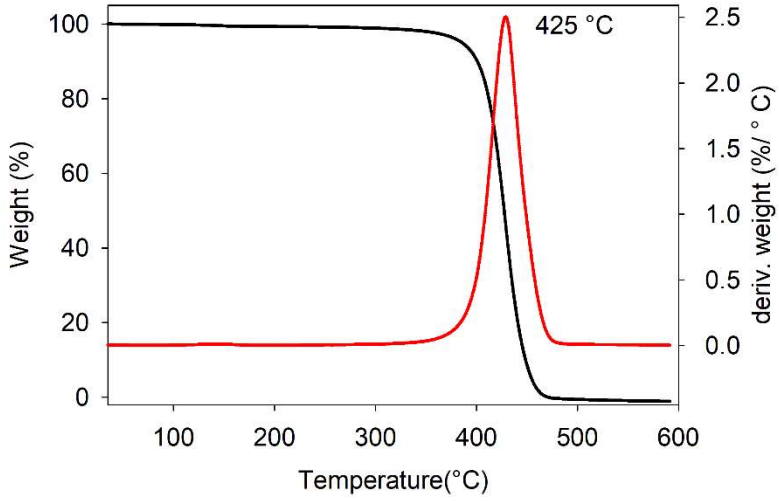


Figure S7. Thermogravimetric analysis (TGA) plots (black line) and Weight derivatives curves (red line) of Panzagel

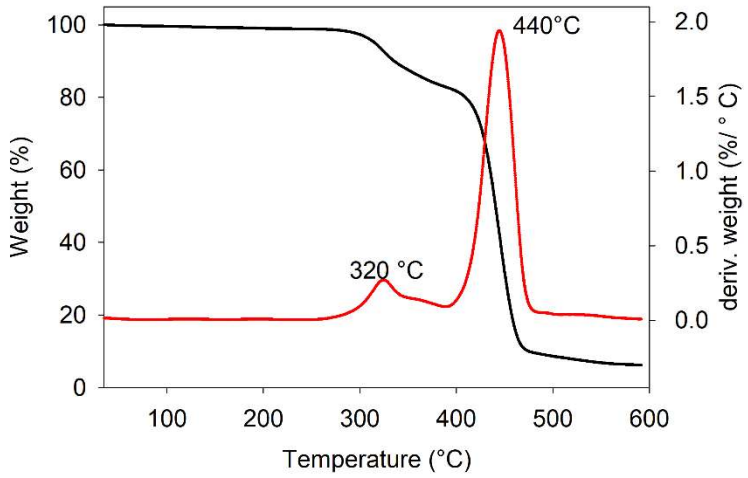


Figure S8. Thermogravimetric analysis (TGA) plots (black line) and Weight derivatives curves (red line) of Panzagel-TMSOTf

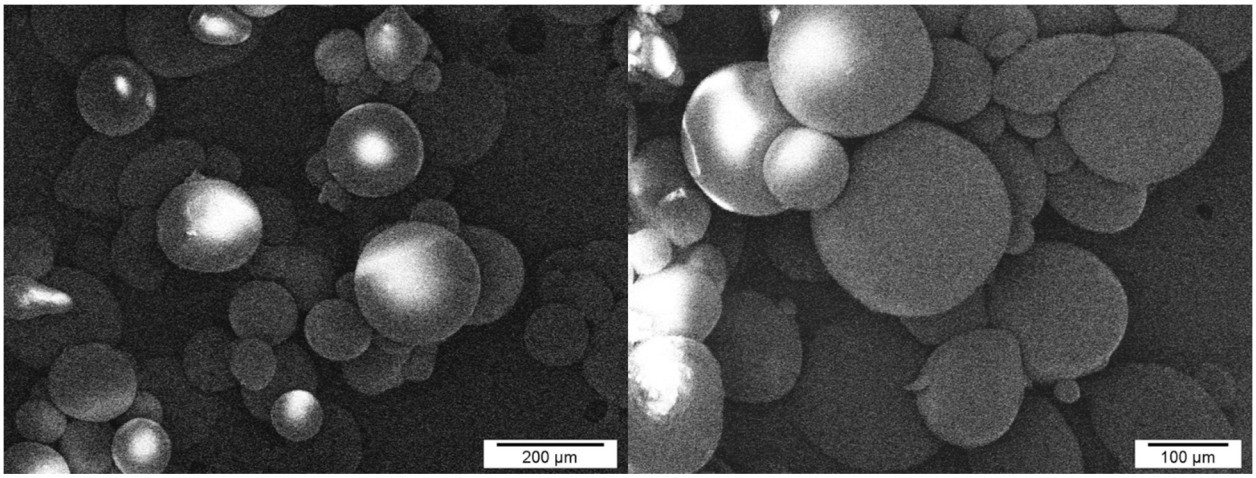


Figure S9. SEM images of Panzagel-TMSOTf polymer at a)100x and b) 150x magnifications

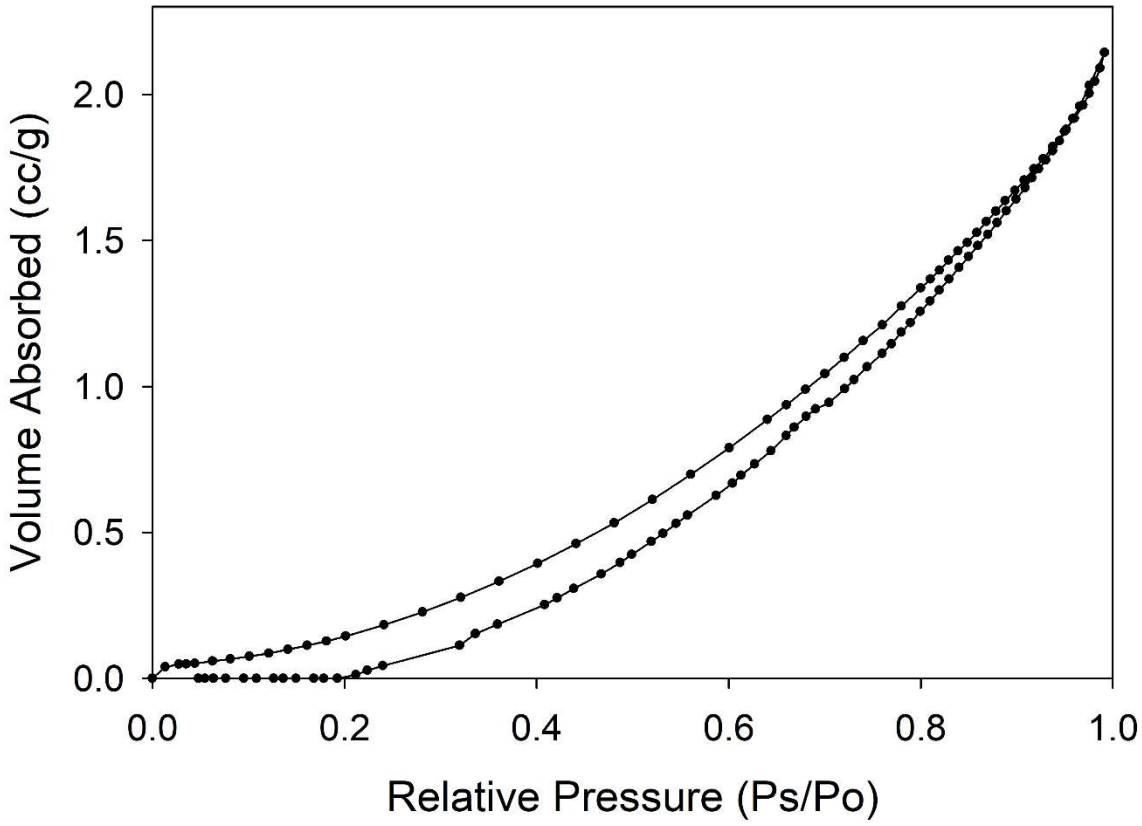


Figure S10. BET adsorption-desorption curves of the Panzagel

References:

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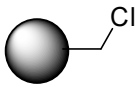
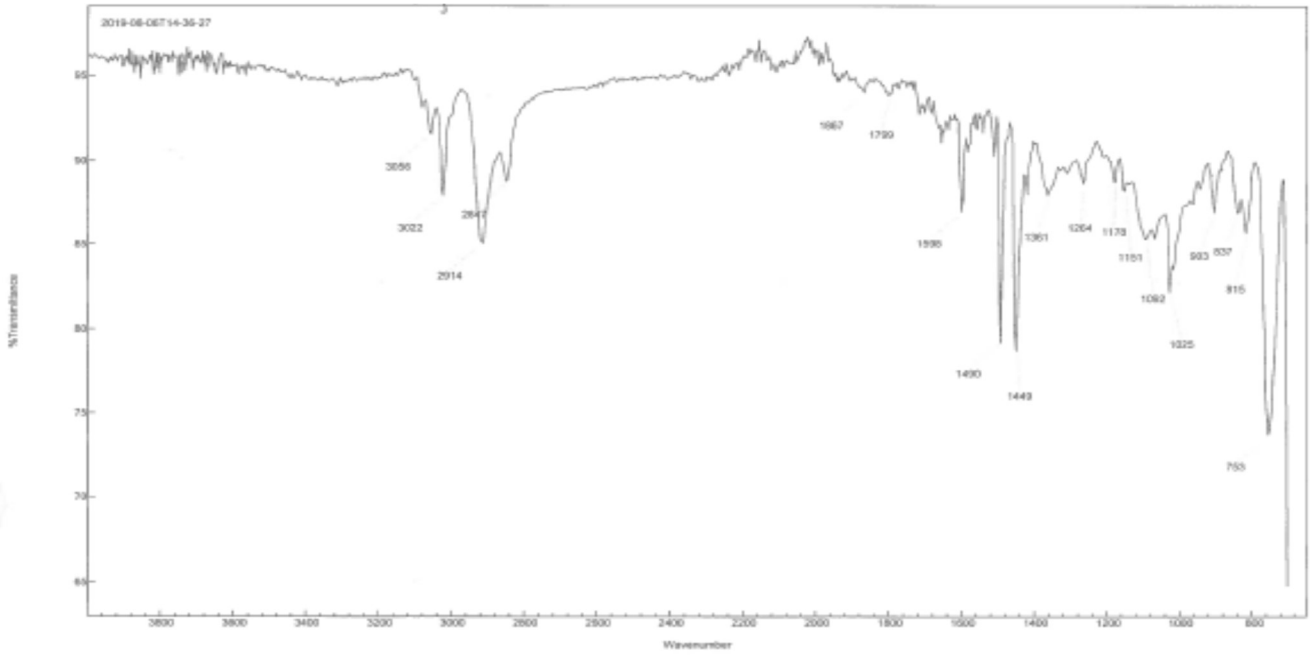
IR Spectra


Cl

6


2019-08-06T14-36-27
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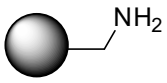
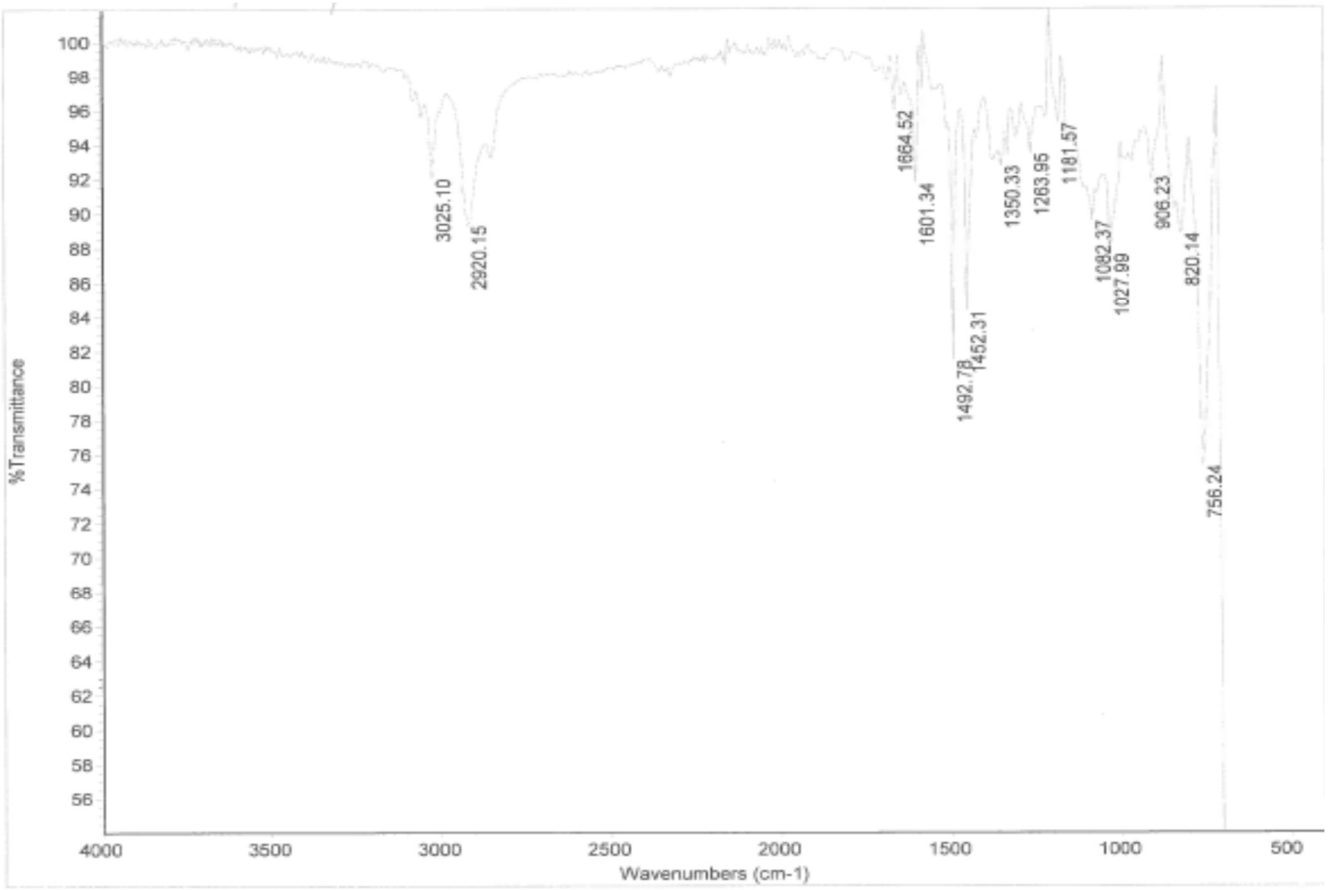
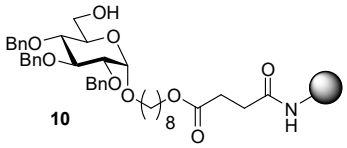
Wavenumber
3056
3022
2914
1667
1599
1490
1443
1361
1264
1178
1151
1092
1025
993
937
815
755


NH2

7


%Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
500
3025.10
2920.15
1664.52
1601.34
1492.78
1452.31
1350.33
1263.95
1181.57
1082.37
1027.99
906.23
820.14
756.24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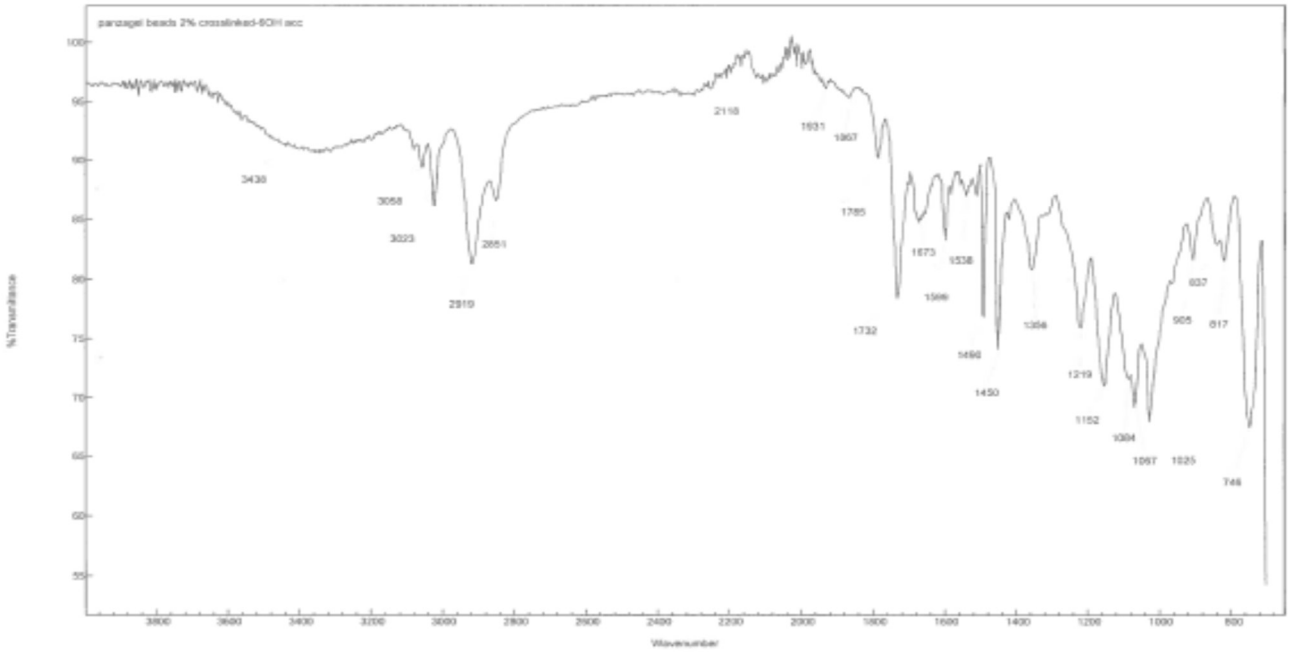
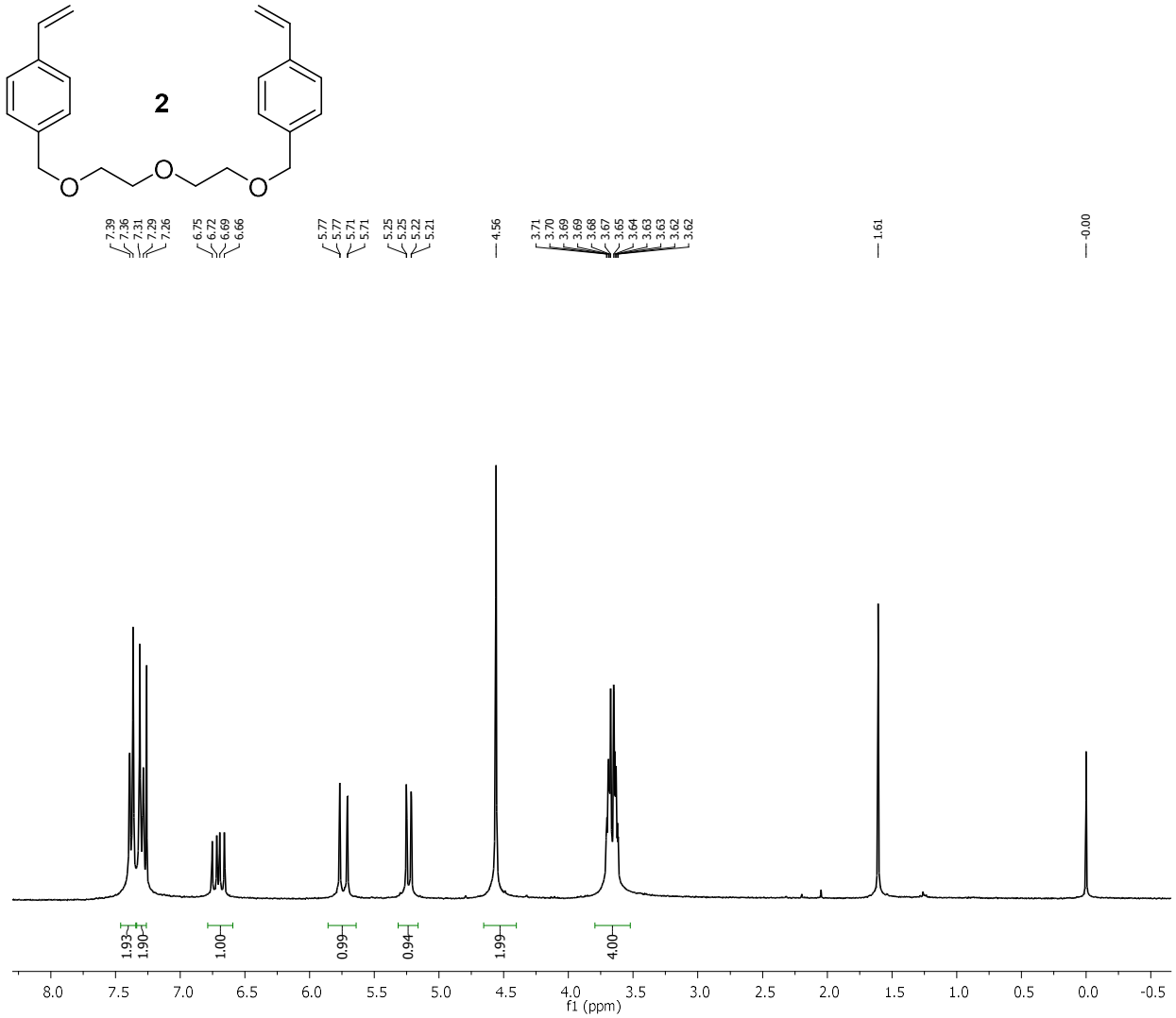
BnO
BnO
BnO
O
O
8
O
O
N
H

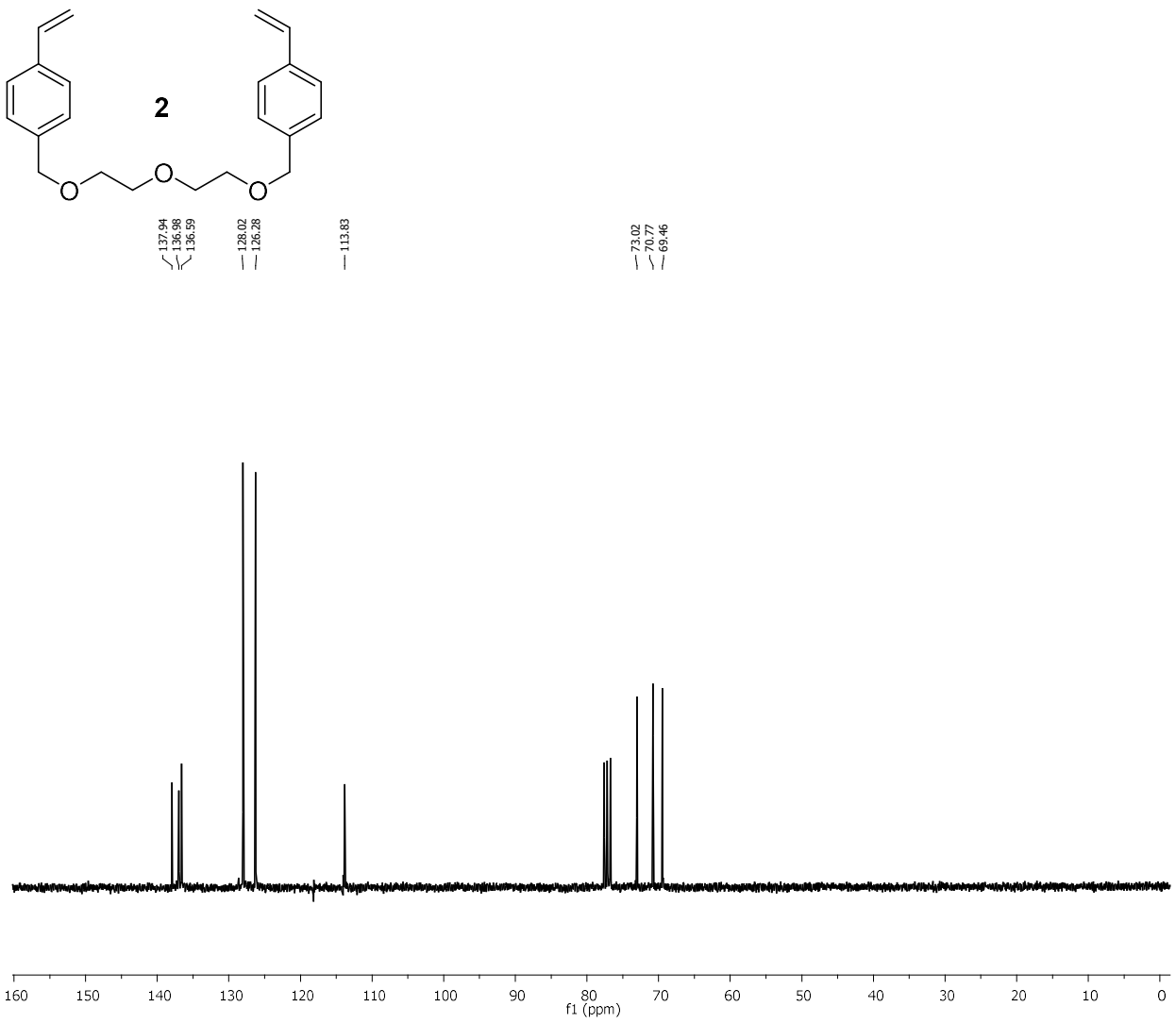
10


panzagel beads 2% crosslinked-ROH acc
%Transmittance
Wavenumber
3439
3058
3023
2851
2819
2118
1931
1867
1785
1732
1673
1598
1538
1490
1450
1384
1219
1152
1094
1067
1025
937
905
817
746

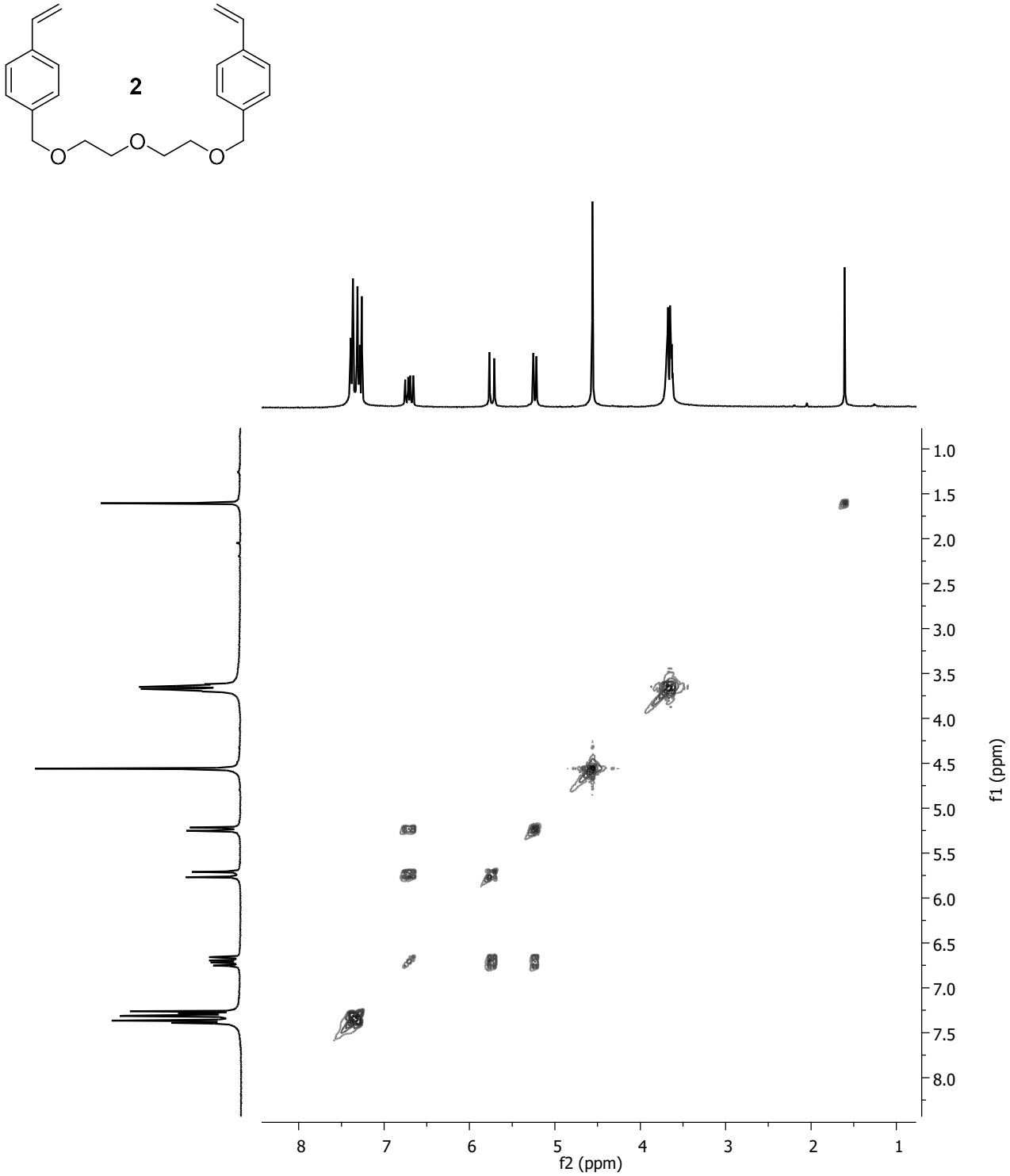
NMR spectra



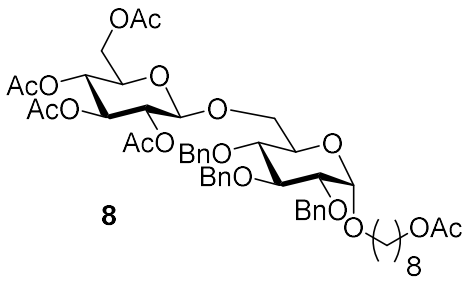
^{1}H NMR ($CDCl_3$, 300 MHz)



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



COSY NMR ($CDCl_3$, 300 MHz)



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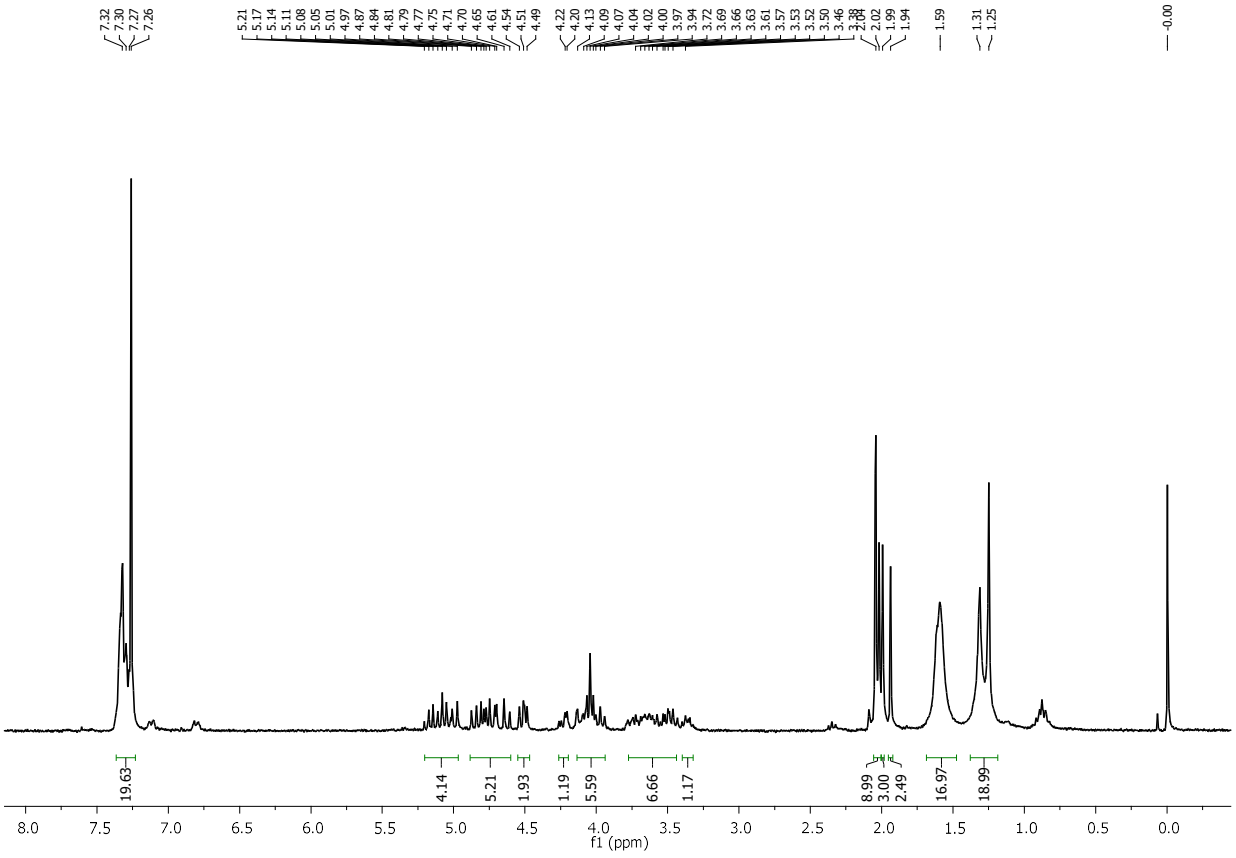
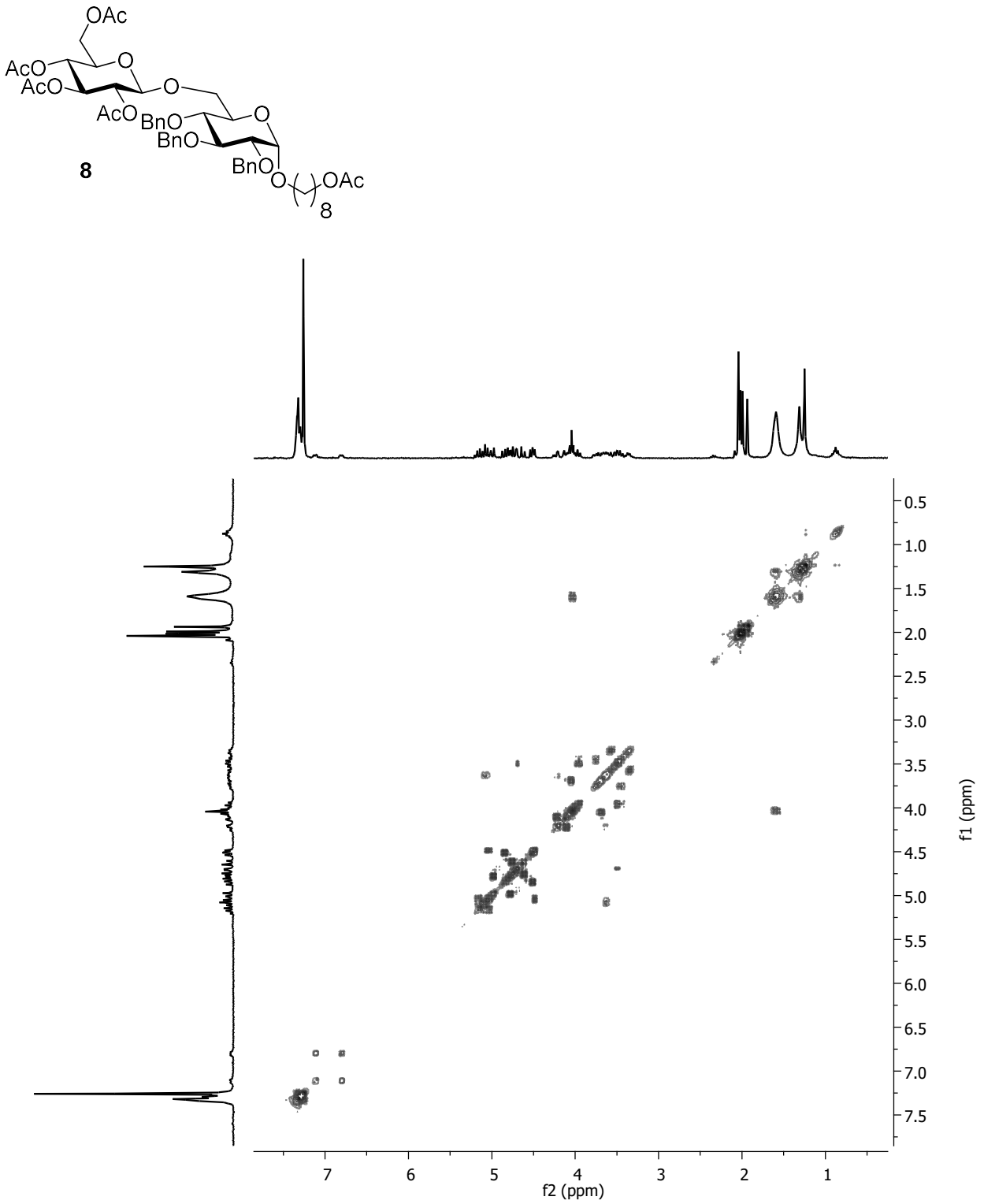
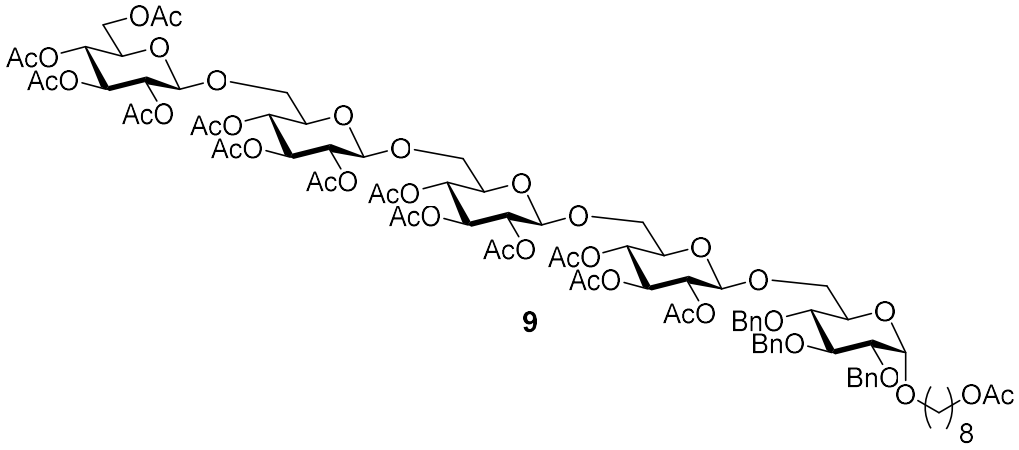


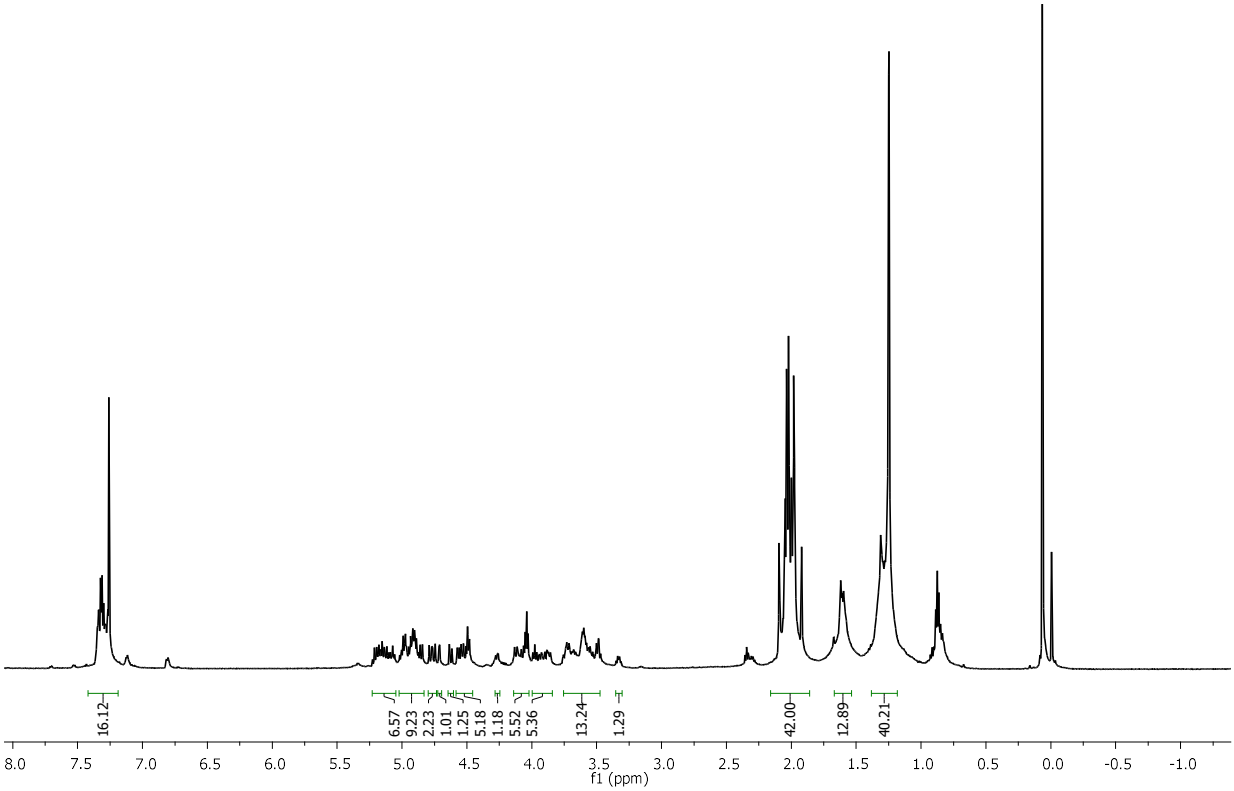
^{1}H NMR ($CDCl_3$, 300 MHz)



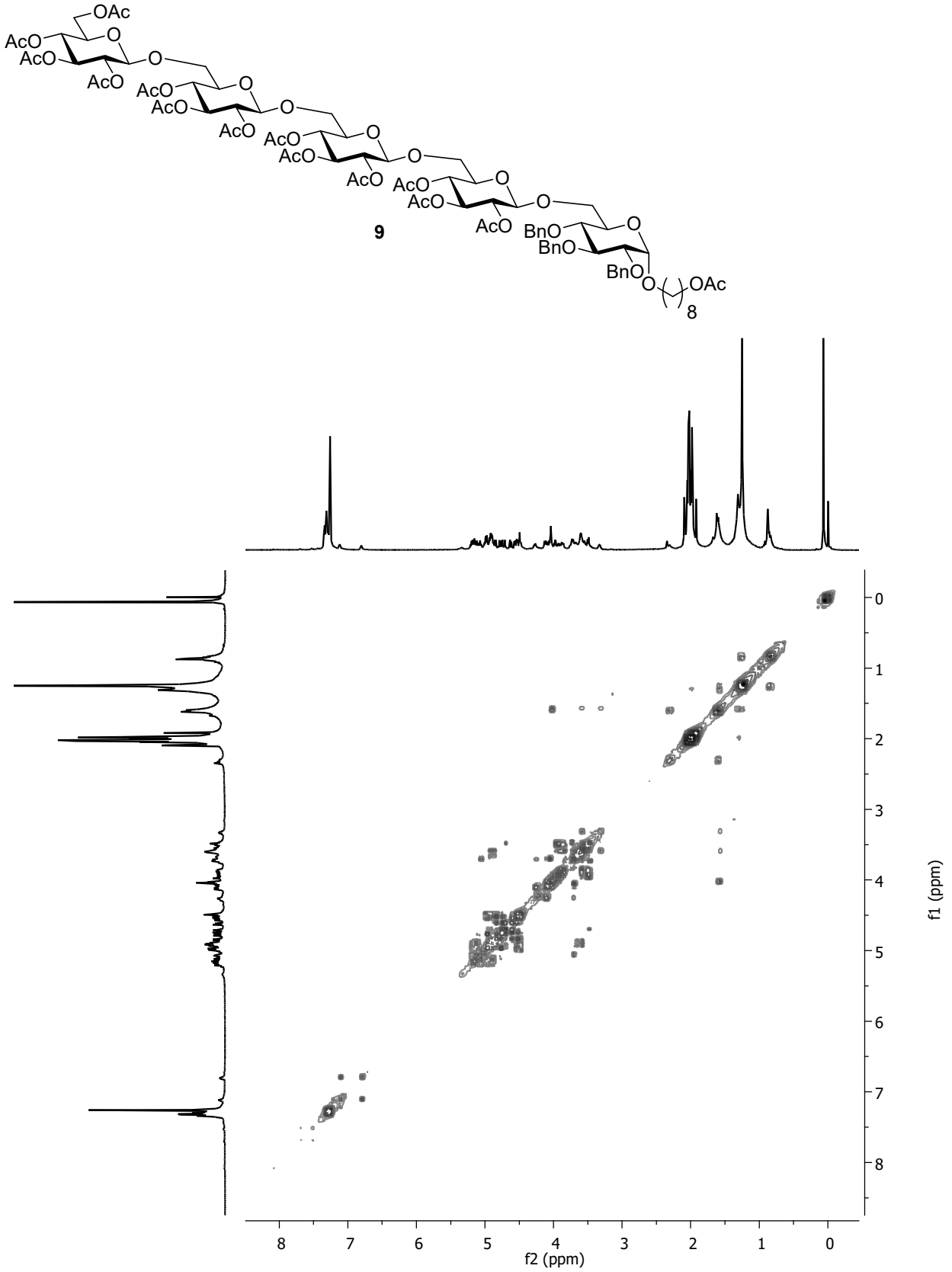
COSY NMR ($CDCl_3$, 300 MHz)



9



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



COSY NMR ($CDCl_3$, 600 MHz)