Supplementary information

Oligosaccharides self-assemble and show intrinsic optical properties

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This PDF file includes:

Materials and Methods
Figs. S1 to S16
Captions for Movie S1

Other Supplementary Materials for this manuscript include the following:

Movie S1

Contents

1. General Materials and Methods

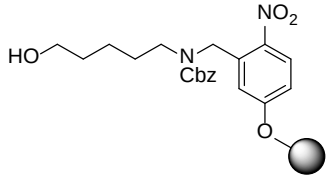
All chemicals used were reagent grade and used as supplied unless otherwise noted. The automated syntheses were performed on a home-built synthesizer developed at the Max Planck Institute of Colloids and Interfaces. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in a p-anisaldehyde (PAA) solution. Flash column chromatography was carried out by using forced flow of the indicated solvent on Fluka Kieselgel 60 M (0.04 – 0.063 mm). Analysis and purification by normal and reverse phase HPLC was performed by using an Agilent 1200 series. Products were lyophilized using a Christ Alpha 2-4 LD plus freeze dryer. ^{1}H, ^{13}C and HSQC NMR spectra were recorded on a Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), or Bruker Biospin AVANCE700 (700 MHz) spectrometer. Spectra were recorded in $CDCl_3$ by using the solvent residual peak chemical shift as the internal standard ($CDCl_3$: 7.26 ppm ^{1}H, 77.0 ppm ^{13}C) or in D_2O using the solvent as the internal standard in ^{1}H NMR (D_2O: 4.79 ppm ^{1}H) and a D_6-acetone spike as the internal standard in ^{13}C NMR (acetone in D_2O: 30.89 ppm ^{13}C) unless otherwise stated. High resolution mass spectra were obtained using a 6210 ESI-TOF mass spectrometer (Agilent) and a MALDI-TOF autoflex^{TM} (Bruker). MALDI and ESI mass spectra were run on IonSpec Ultima instruments. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured by using a Perkin-Elmer 241 and Unipol L1000 polarimeter. Transmission electron microscopy (TEM) images were obtained on carbon-coated copper grids with a Zeiss EM 912Ω instrument at 120 kV. Samples were stained with 2% uranyl acetate solution and dried after removal of the excess staining solution. For scanning electron microscopy (SEM), samples were prepared on glass substrates and coated with Au/Pd. The SEM measurement was done with a Gemini SEM, LEO 1550 system with cold field emission gun operation at 3 kV. JEOL JSM 7500 F was used to obtain cryogenic scanning electron microscopy (cryo-SEM) images with frozen and Pt-coated droplet samples. Atomic force microscopy investigation was performed with a Multimode Nanoscope IIIa AFM in tapping mode. Fluorescence and bright filed images were acquired at 4 different excitation/emission wavelengths (405/415-498, 488/498-566, 561/571-628, 633/643-780 nm) with a LEICA DMi8 Confocal Laser Microscope (63X water and 20X dry objective). Fluorescence spectra and fluorescence quantum yield were measured with a Jasco FP-8300 spectrofluorometer. Excitation was performed with a Xe lamp. Emission spectra for the quantum yield measurements were collected with excitation at 360 nm and detection from 350 to 900 nm with an integrating sphere setup. The emission quantum yield was calculated considering two regions: (1) 350-372 nm (excitation beam) and (2) 380-700 nm (sample fluorescence). Absorption spectra were collected with a SHIMADZU UV-vis spectrophotometer (UV-2600). Polarized optical microscope images were obtained with an Olympus BX41 (40X) system. For XRD measurements, a Bruker D8 Advanced X-ray diffractometer with Cu Kα radiation was used.

2. General procedure for automated glycan assembly

2.1. General materials and methods

Solvents used for dissolving building block and making activator, TMSOTf and capping solutions were taken from an anhydrous solvent system (jcmeyer-solvent systems). Other solvents were HPLC grade. The building blocks were co-evaporated three times with chloroform and dried for 1 h on high vacuum before use. Activator, deprotection, acidic wash, capping and building block solutions were freshly prepared and kept under argon during the automation run. All yields of

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



The photo-cleavable linker was prepared according to previously established procedures.[1]

2.2. Preparation of stock solutions

- **Building block**: 0.08 mmol of building block was dissolved in 1 mL of dichloromethane (DCM).
- **Activator solution**: 1.35 g of recrystallized NIS was dissolved in 40 mL of a 2:1 mixture of anhydrous DCM and anhydrous dioxane. Then triflic acid (55 µL) was added. The solution is kept at 0°C for the duration of the automation run.
- **Fmoc deprotection solution**: A solution of 20% piperidine in dimethylformamide (DMF) (v/v) was prepared.
- **TMSOTf solution**: Trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.45 mL) was added to DCM (40 mL).
- **Capping solution**: A solution of 10% acetic anhydride (Ac_2O) and 2% methanesulfunic acid (MsOH) in DCM (v/v) was prepared.

2.3. Modules for automated synthesis

Module A: Resin Preparation for Synthesis (20 min)

All automated syntheses were performed on 0.0125 mmol scale. Resin was placed in the reaction vessel and swollen in DCM for 20 min at room temperature prior to synthesis. During this time, all reagent lines needed for the synthesis were washed and primed. Before the first glycosylation, the resin was washed with the DMF, tetrahydrofuran (THF), and DCM (three times each with 2 mL for 25 s).

Module B: Acidic Wash with TMSOTf Solution (20 min)

The resin was swollen in 2 mL DCM and the temperature of the reaction vessel was adjusted to -20 °C. Upon reaching the low temperature, TMSOTf solution (1 mL) was added drop wise to the reaction vessel. After bubbling for 3 min, the acidic solution was drained and the resin was washed with 2 mL DCM for 25 s.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Cooling	-	-	-	-20	(15 min)*
Deliver	1	DCM	2 mL	-20	-
Deliver	1	TMSOTf solution	1 mL	-20	3 min

Wash	1	DCM	2 mL	-20	25 s

*Time required to reach the desired temperature.

Module C: Thioglycoside Glycosylation (35 min)

The building block solution (0.08 mmol of BB in 1 mL of DCM per glycosylation) was delivered to the reaction vessel. After the set temperature was reached, the reaction was started by dropwise addition of the activator solution (1.0 mL, excess). The glycosylation conditions are building block dependent (we report the most common set of conditions). After completion of the reaction, the solution is drained and the resin was washed with DCM, DCM:dioxane (1:2, 3 mL for 20 s) and DCM (two times, each with 2 mL for 25 s). The temperature of the reaction vessel is increased to 25 °C for the next module.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Cooling	-	-	-	-20	-
Deliver	1	BB solution	1 mL	-20	-
Deliver	1	Activator solution	1 mL	-20	-
Reaction time (BB dependent)	1			-20 to 0	5 min 20 min
Wash	1	DCM	2 mL	0	5 s
Wash	1	DCM : Dioxane (1:2)	2 mL	0	20 s
Heating	-	-	-	25	-
Wash	2	DCM	2 mL	> 0	25 s

Module D: Capping (30 min)

The resin was washed with DMF (two times with 2 mL for 25 s) and the temperature of the reaction vessel was adjusted to 25 °C. 2 mL of Pyridine solution (10% in DMF) was delivered into the reaction vessel. After 1 min, the reaction solution was drained and the resin washed with DCM (three times with 3 mL for 25 s). 4 mL of capping solution was delivered into the reaction vessel. After 20 min, the reaction solution was drained and the resin washed with DCM (three times with 3 mL for 25 s).

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Heating	-	-	-	25	(5 min)*
Wash	2	DMF	2 mL	25	25 s
Deliver	1	10% Pyridine in DMF	2 mL	25	1 min
Wash	3	DCM	2 mL	25	25 s
Deliver	1	Capping Solution	4 mL	25	20 min
Wash	3	DCM	2 mL	25	25 s

*Time required to reach the desired temperature.

Module E: Fmoc Deprotection (9 min)
The resin was washed with DMF (three times with 2 mL for 25 s) and the temperature of the reaction vessel was adjusted to 25 °C. 2 mL of Fmoc deprotection solution was delivered into the reaction vessel. After 5 min, the reaction solution was drained and the resin washed with DMF (three times with 3 mL for 25 s) and DCM (five times each with 2 mL for 25 s). The temperature of the reaction vessel is decreased to -20 °C for the next module.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Heating	-	-	-	25	
Wash	3	DMF	2 mL	25	25 s
Deliver	1	Fmoc depr. solution	2 mL	25	5 min
Wash	1	DMF	2 mL		
Cooling	-	-	-	-20	-
Wash	3	DMF	2 mL	< 25	25 s
Wash	5	DCM	2 mL	< 25	25 s

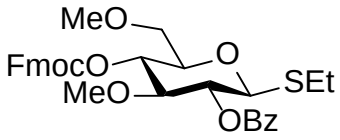
2.4. Post-synthesizer manipulations

Cleavage from Solid Support
After automated synthesis, the oligosaccharides were cleaved from the solid support using a continuous-flow photoreactor as described previously.[1]

Purification
Solvent is evaporated *in vacuo* and the crude products were dissolved in 1:1 mixture of hexane and ethyl acetate and analyzed using analytical HPLC (DAD1F, 280 nm). Pure compounds were afforded by preparative HPLC (Agilent 1200 Series spectrometer).

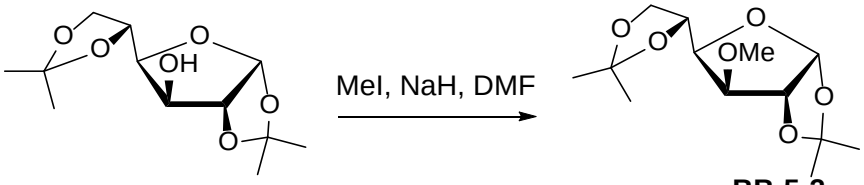
- Method A: (YMC-Diol-300 column, 150 x 4.6 mm) flow rate of 1.0 mL / min with Hex – 10% EtOAc as eluent [isocratic 10% EtOAc (5 min), linear gradient to 50% EtOAc (20 min), linear gradient to 100% EtOAc (5 min)].
- Method B: (YMC-Diol-300 column, 150 x 20 mm) flow rate of 15 mL / min with Hex – 20% EtOAc as eluents [isocratic 20% EtOAc (5 min), linear gradient to 55% EtOAc (35 min), linear gradient to 100% EtOAc (5 min)].

3. Synthesis of building block BB-5



BB-5

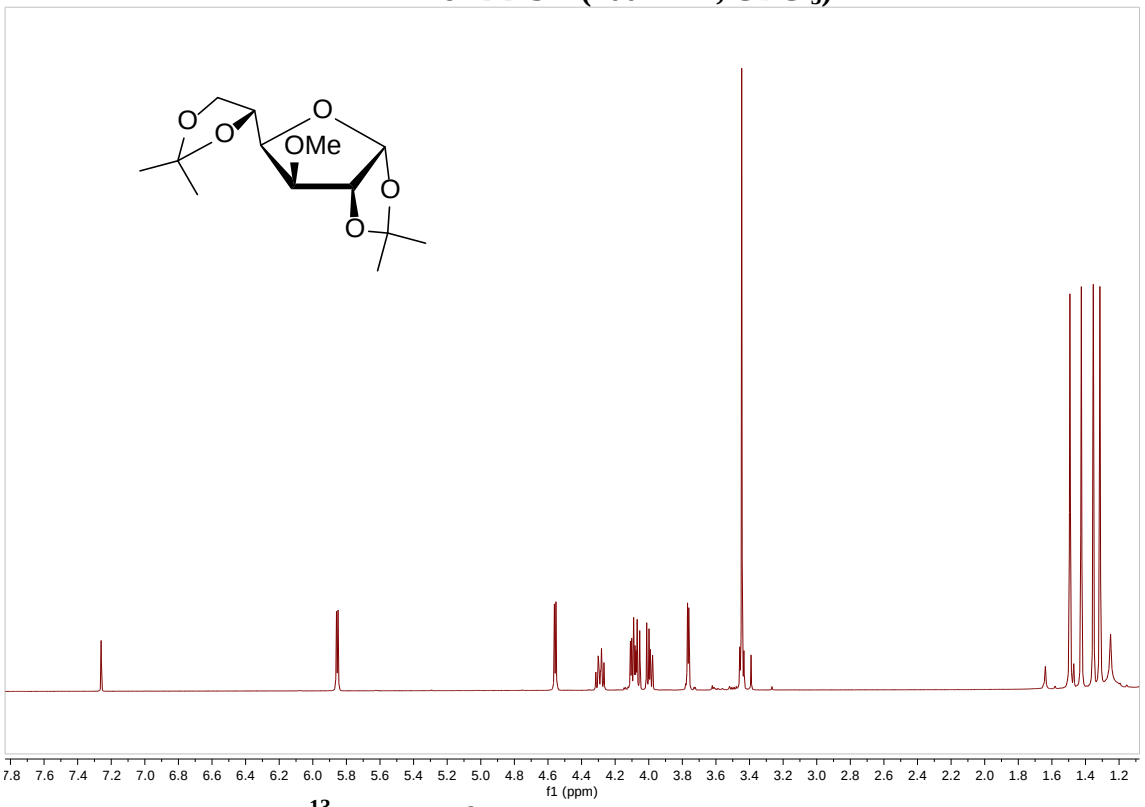
Synthesis of BB-5-2



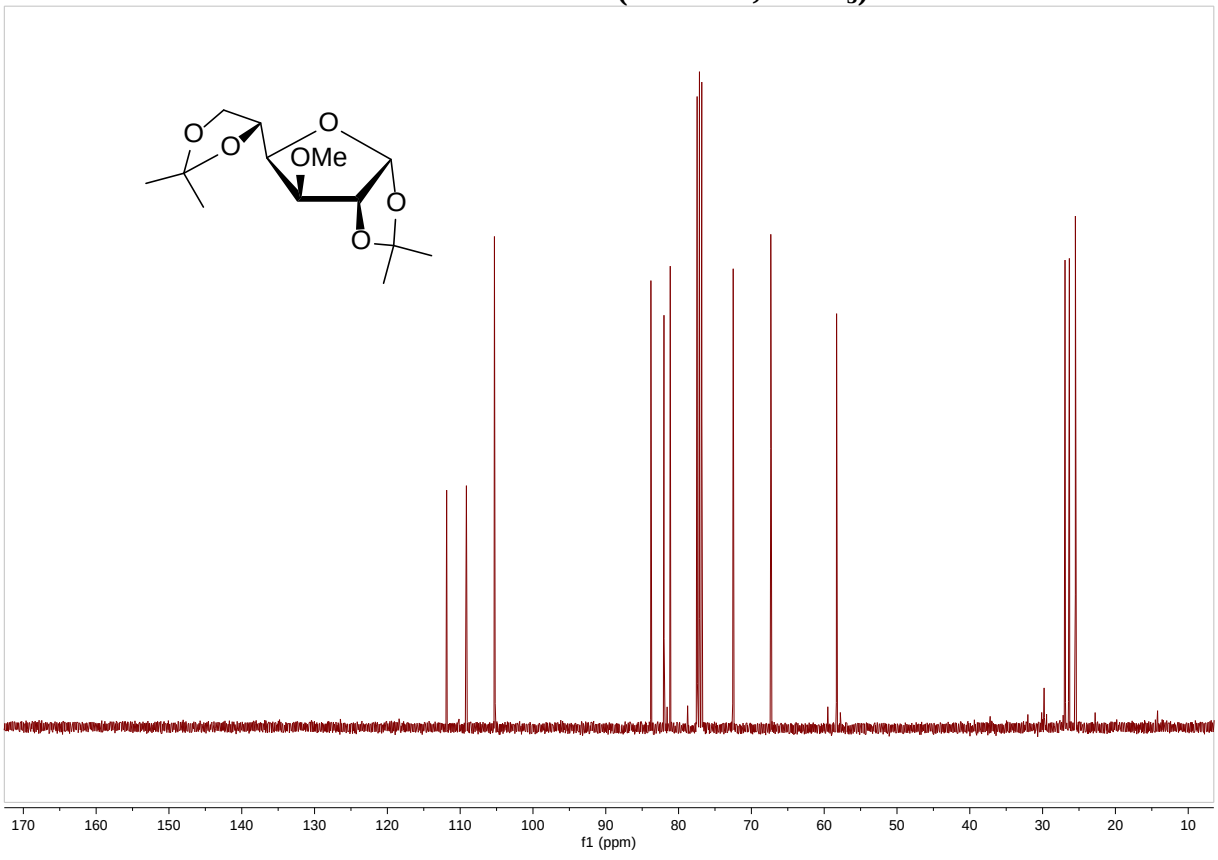
BB-5-1 **BB-5-2**

BB-5-1 (53 g, 204 mmol) was dissolved in 1 L anhydrous DMF. The solution was cooled to 0 °C and NaH (60% by weight in mineral oil, 12.2 g, 305 mmol) was slowly added portion-wise with vigorous stirring. Evolved H_2 was periodically vented during NaH addition. After the NaH addition was complete, the reaction was stirred for 1 h at 0 °C. MeI (25 mL, 407 mmol) was added drop-wise. After MeI addition was complete, the reaction was stirred for 15 min. at 0 °C. The reaction was allowed to warm to rt, during which time a white precipitate formed. The reaction was stirred for 1.5 h at rt and then cooled back to 0 °C. Saturated aq. NH_4Cl was slowly added to quench the reaction. When all H_2 appeared to have been evolved an additional 50 mL saturated aq. NH_4Cl was added and the reaction was stirred for additional 15 min. at 0 °C. The reaction was concentrated to < 200 mL under reduced pressure and 1 L DCM was added. The organic layer was separated and extracted twice with H_2O. The organics were dried over $MgSO_4$, filtered, and concentrated to give **BB-5-2** as a light yellow oil (56 g, quantitative). $[\alpha]_D^{20}$ -100.07 (c 1, $CHCl_3$); IR (neat) νmax = 1373, 1072 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.85 (d, *J* = 3.7 Hz, 1H), 4.56 (d, *J* = 3.7 Hz, 1H), 4.31 – 4.25 (m, 1H), 4.11 – 4.04 (m, 2H), 3.99 (dd, *J* = 8.6, 5.4 Hz, 1H), 3.76 (d, *J* = 3.0 Hz, 1H), 3.45 (s, 3H), 1.49 (s, 3H), 1.42 (s, 3H), 1.35 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 111.88, 109.16, 105.31, 83.83, 82.03, 81.17, 72.53, 67.37, 58.33, 27.01, 26.96, 26.37, 25.54; m/z (HRMS+) 297.1330 $[M + Na]^+$ ($C_{13}H_{22}O_6Na$ requires 297.1309).

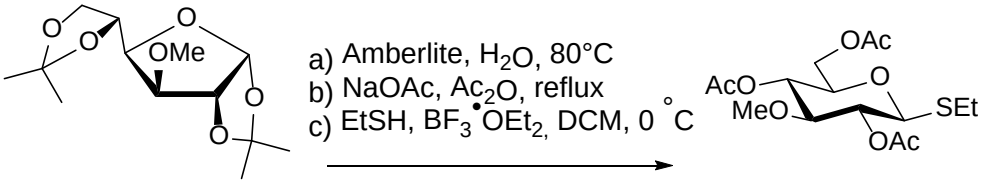
^{1}H NMR of BB-5-2 (400 MHz, $CDCl_3$)



^{13}C NMR of BB-5-2 (101 MHz, $CDCl_3$)



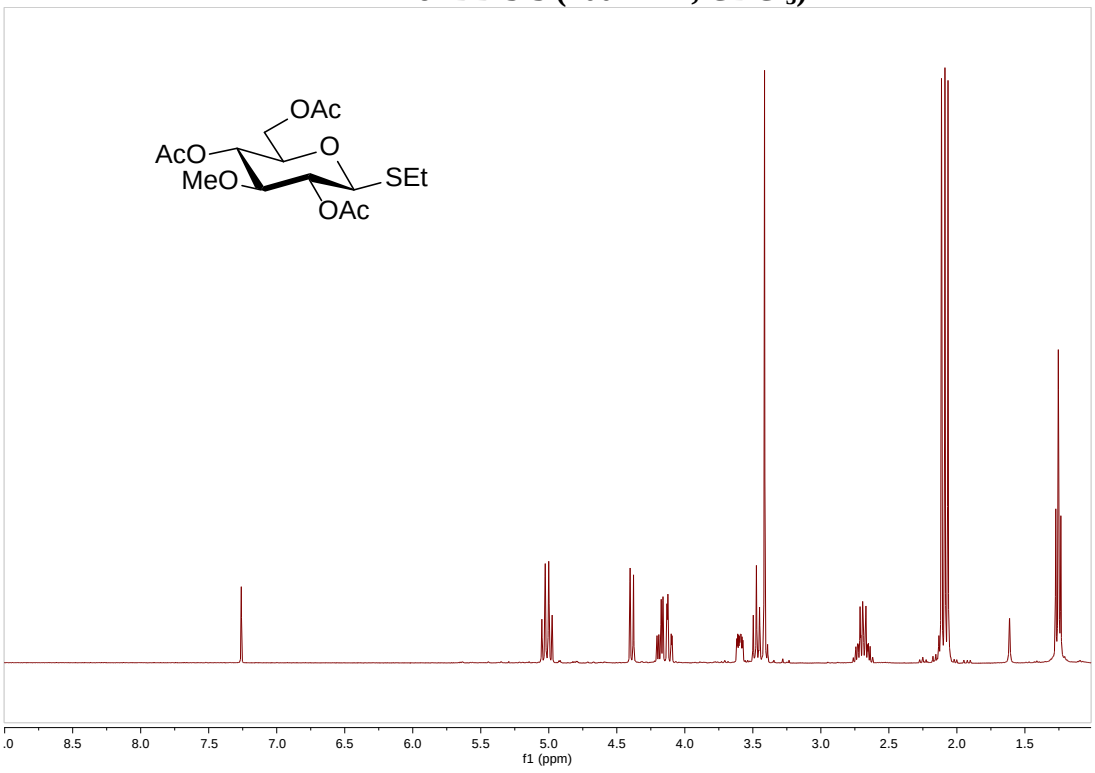
Synthesis of BB-5-3



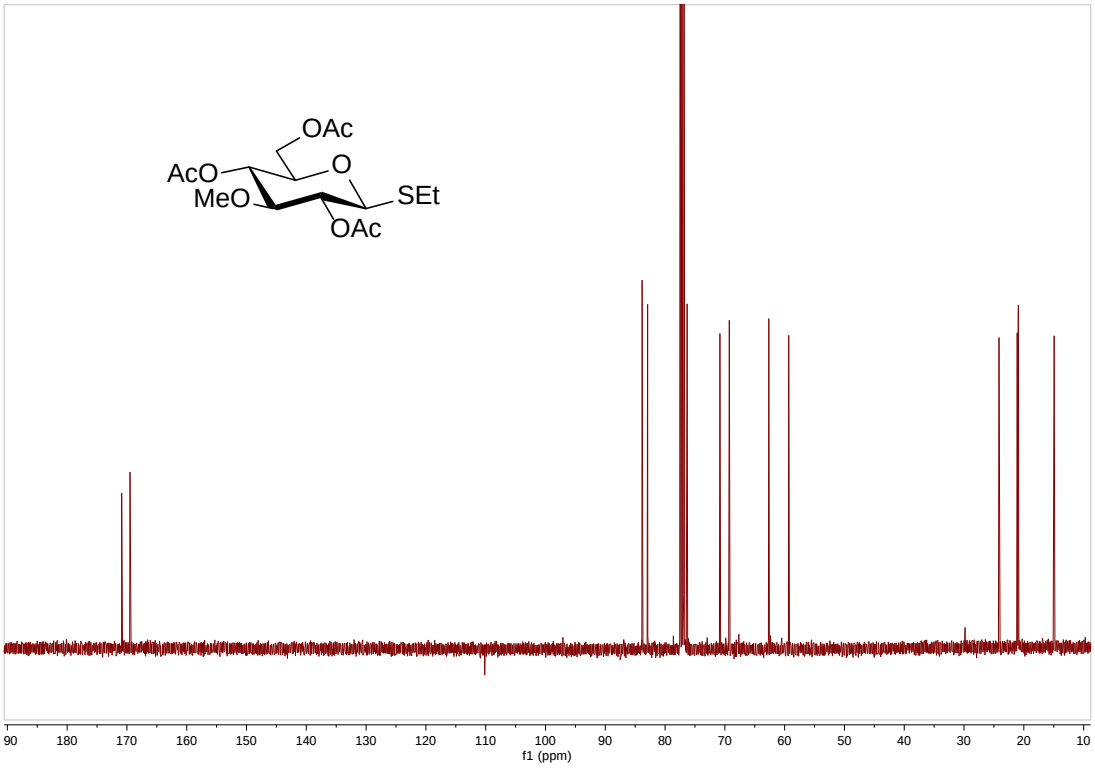
BB-5-2 **BB-5-3**

BB-5-2 (56 g, 204 mmol) was suspended in 400 mL H_2O. 75 g Amberlite IR-120 (H^+ form) was added. The reaction mixture was heated to 80 °C and stirred vigorously for 16 h. The reaction was cooled to rt and the solid material was filtered off. The reaction was concentrated under reduced pressure. The residue was suspended in ACN and then the solvent was removed under reduced pressure to give a pale yellow powder. NaOAc (8.4 g, 102 mmol) was added to the solid sample followed by slow qaddition of Ac_2O (192 mL, 2.04 mol). The mixture was refluxed for 45 min, during which time it became orange. The reaction was cooled to room temperature, and then quenched with ice. 700 mL DCM were added and the organic layers were washed twice with H_2O. The organics were dried over $MgSO_4$, filtered, and concentrated to give a crude orange oil. This oil was dissolved in 1.3 L DCM. Ethanethiol (38 mL, 510 mmol) was added and the solution was cooled to 0 °C. $BF_3 \bullet OEt_2$ (39 mL, 306 mmol) was slowly added and the reaction was stirred at 0 °C for 5 h. The pink solution was quenched by slow addition of saturated aq. $NaHCO_3$. 700 mL DCM were added and the organic layers washed with saturated aq. $NaHCO_3$ and H_2O. The organics were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (2 hexanes: 1 EtOAc) to give **BB-5-3** as a yellow gel (56.7 g, 76%); R_f = 0.35 (hexanes/EtOAc, 2:1); $[\alpha]_D^{20}$ -56.58 (c 1, $CHCl_3$); IR (neat) νmax = 1743, 1218 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.01 (dd, *J* = 20.2, 10.0 Hz, 2H), 4.39 (d, *J* = 10.0 Hz, 1H), 4.22 – 4.07 (m, 2H), 3.64 – 3.55 (m, 1H), 3.47 (t, *J* = 9.2 Hz, 1H), 3.41 (s, 3H), 2.78 – 2.61 (m, 2H), 2.11 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 1.25 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.88, 169.49, 169.47, 83.84, 82.92, 77.48, 77.16, 76.84, 76.31, 70.83, 69.26, 62.65, 59.32, 24.15, 21.11, 20.95, 20.91, 14.93; m/z (HRMS+) 387.1093 $[M + Na]^+$ ($C_{15}H_{24}O_8SNa$ requires 387.1084).

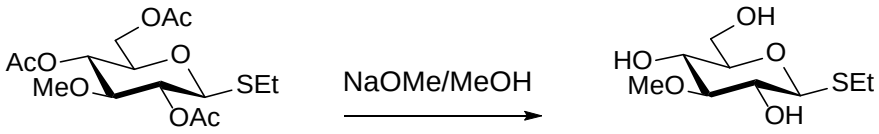
^{1}H NMR of BB-5-3 (400 MHz, $CDCl_3$)



^{13}C NMR of BB-5-3 (101 MHz, $CDCl_3$)



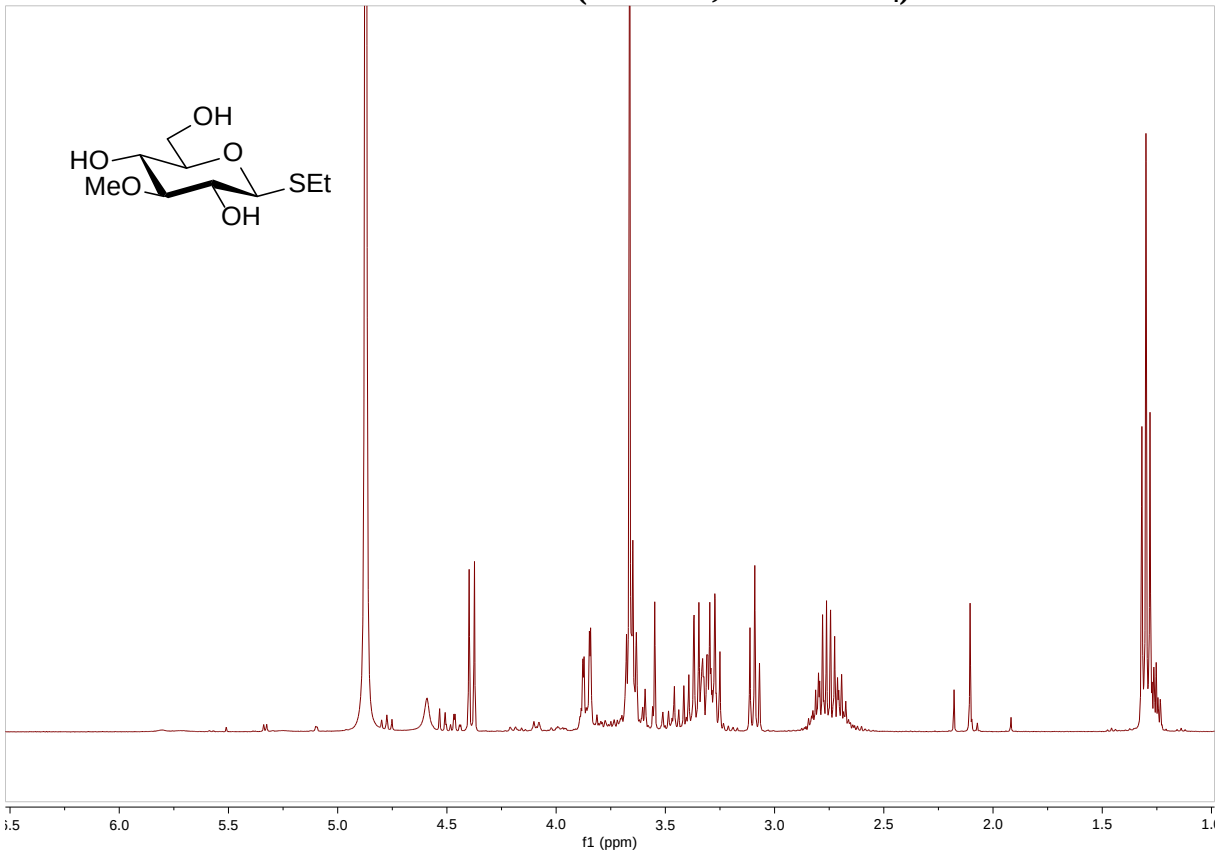
Synthesis of BB-5-4



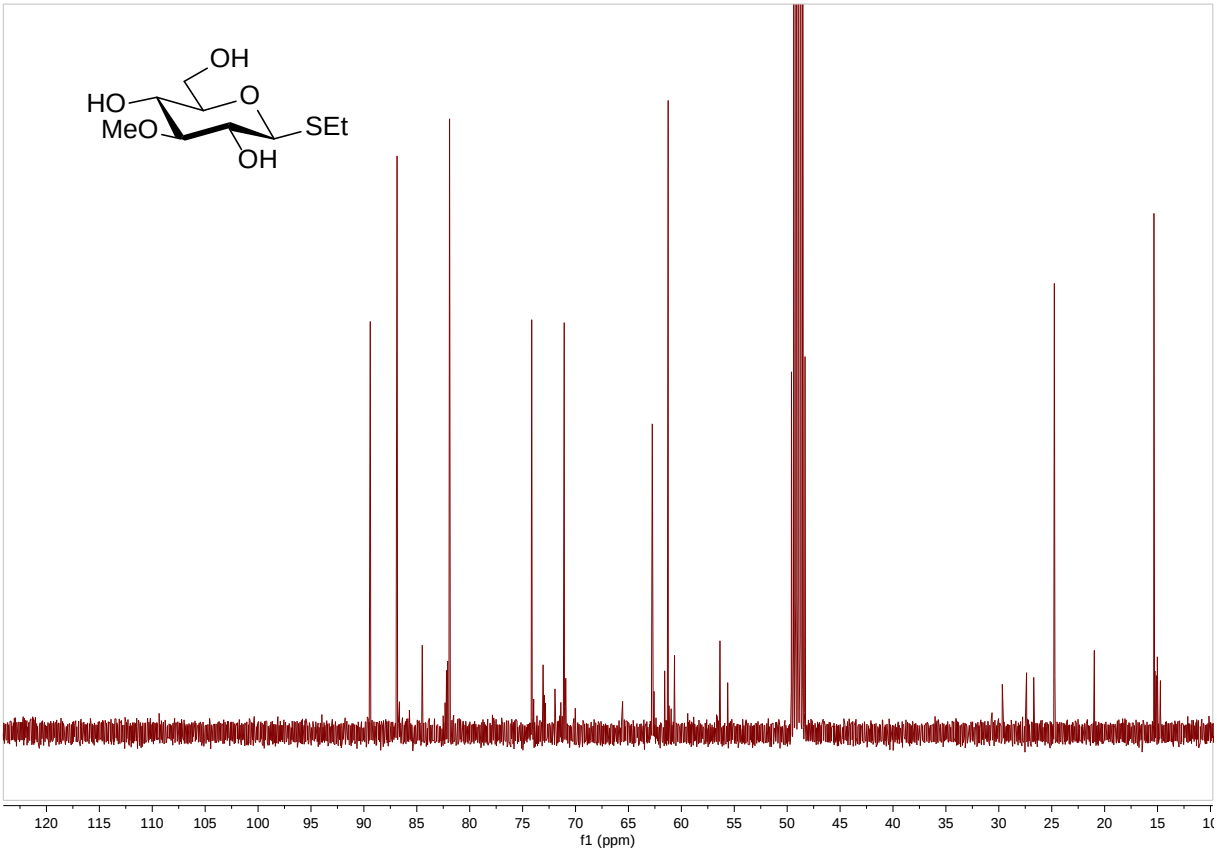
BB-5-3 **BB-5-4**

BB-5-3 (56.7 g, 156 mmol) was dissolved in 310 mL of MeOH and the solution was cooled to 0 °C. NaOMe (840 mg, 15.6 mmol) was added. The reaction was allowed to warm to rt and stirred for 14 h. The reaction was neutralized with Amberlite IR-120 (H^+ form) and the solid was filtered off. The solvent was removed under reduced pressure to give **BB-5-4** as a yellow gel (35.7 g, 96%); $[\alpha]_D^{20}$ +13.10 (c 1, $CHCl_3$); IR (neat) νmax = 3389, 1035 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 4.39 (d, *J* = 9.8 Hz, 1H), 3.86 (dd, *J* = 12.0, 2.2 Hz, 1H), 3.69 – 3.61 (m, 4H), 3.39 – 3.23 (m, 3H), 3.09 (t, *J* = 8.7 Hz, 1H), 2.84 – 2.65 (m, 2H), 1.30 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 89.42, 86.89, 81.93, 74.17, 71.10, 62.78, 61.28, 24.81, 15.40; m/z (HRMS+) 261.0791 $[M + Na]^+$ ($C_9H_{18}O_5SNa$ requires 261.0767).

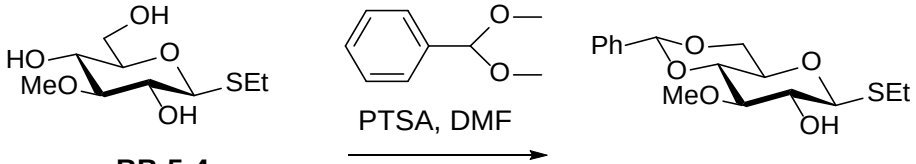
^1H NMR of BB-5-4 (400 MHz, Methanol-d_4)



^{13}C NMR of BB-5-4 (101 MHz, Methanol-d_4)

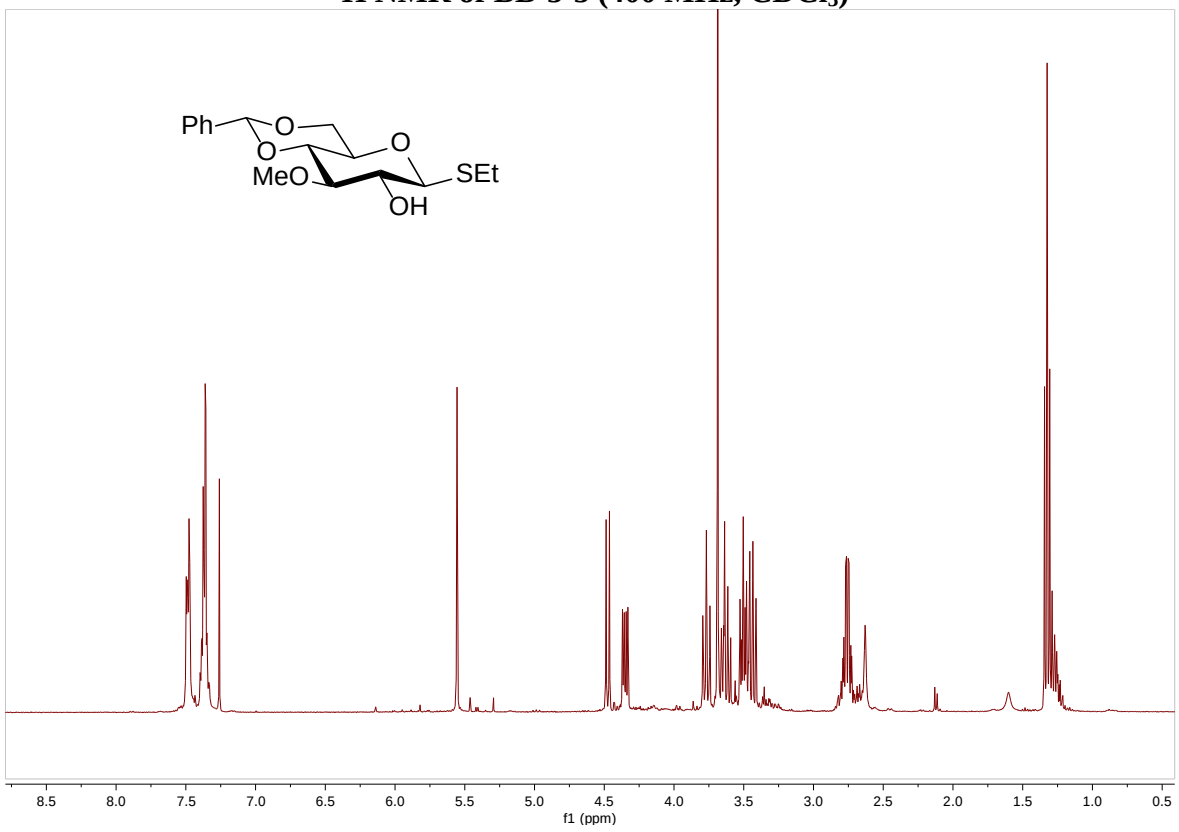


Synthesis of BB-5-5

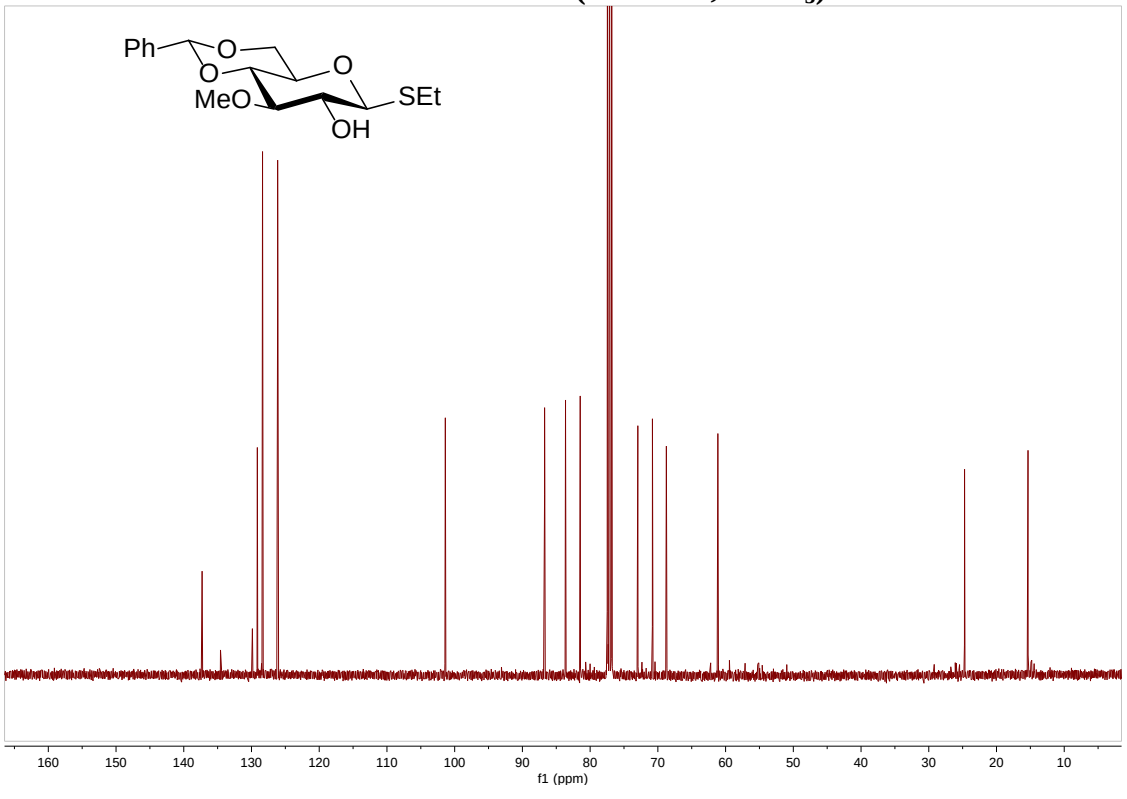


BB-5-4 (35.7 g, 150 mmol) was dissolved in 500 mL of anhydrous DMF. p-Toluenesulfonic acid (PTSA) (4.3 g, 22.5 mmol) was added and then benzaldehyde dimethyl acetal (45 mL, 300 mmol) was slowly added using a dropping funnel. Following benzaldehyde dimethyl acetal addition the reaction was heated to 45°C and stirred for 18 h. The reaction was cooled to 0°C and quenched by addition of TEA (7 mL). The reaction was concentrated under reduced pressure and the crude product was purified by column chromatography (1 hexanes: 1 EtOAc) to give **BB-5-5** as a white powder (30.0 g, 61 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ7.55 – 7.43 (m, 2H), 7.41 – 7.30 (m, 3H), 5.56 (s, 1H), 4.47 (d, *J* = 9.5 Hz, 1H), 4.35 (dd, *J* = 10.5, 4.9 Hz, 1H), 3.77 (t, *J* = 10.3 Hz, 1H), 3.69 (s, 3H), 3.67 – 3.58 (m, 2H), 3.54 – 3.47 (m, 2H), 3.47 – 3.40 (m, 1H), 2.80 – 2.72 (m, 2H), 1.32 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.30, 134.59, 129.88, 129.15, 128.38, 126.15, 101.40, 86.75, 83.66, 81.49, 72.99, 70.83, 68.77, 61.17, 24.76, 15.39; m/z (HRMS+) 349.1106 $[M + Na]^+$ ($C_{16}H_{22}O_5SNa$ requires 349.1080).

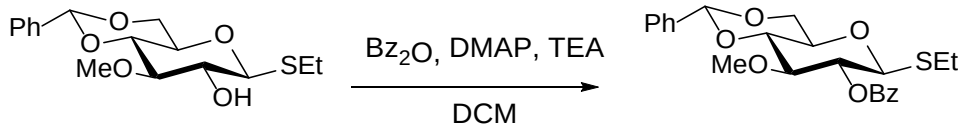
^{1}H NMR of BB-5-5 (400 MHz, $CDCl_3$)



^{13}C NMR of BB-5-5 (101 MHz, $CDCl_3$)



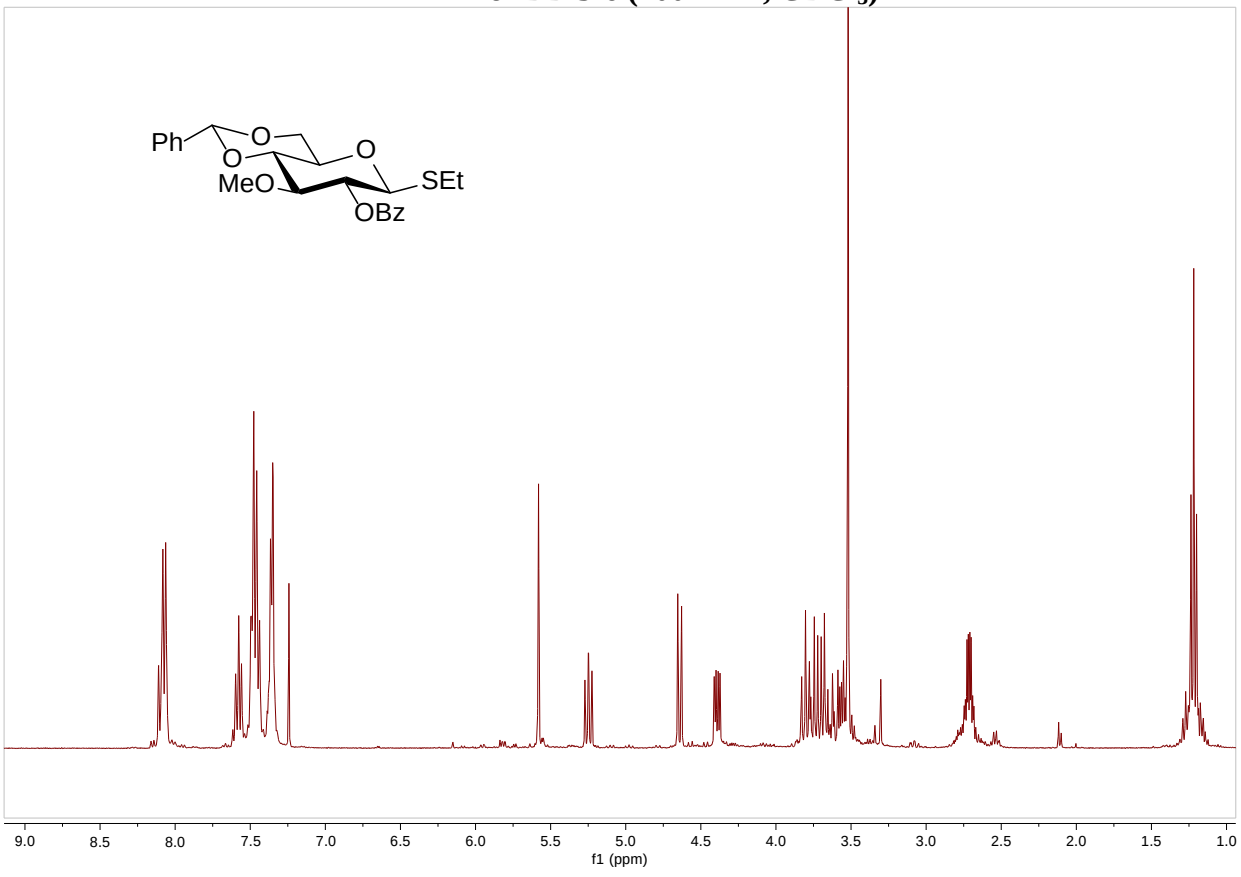
Synthesis of BB-5-6



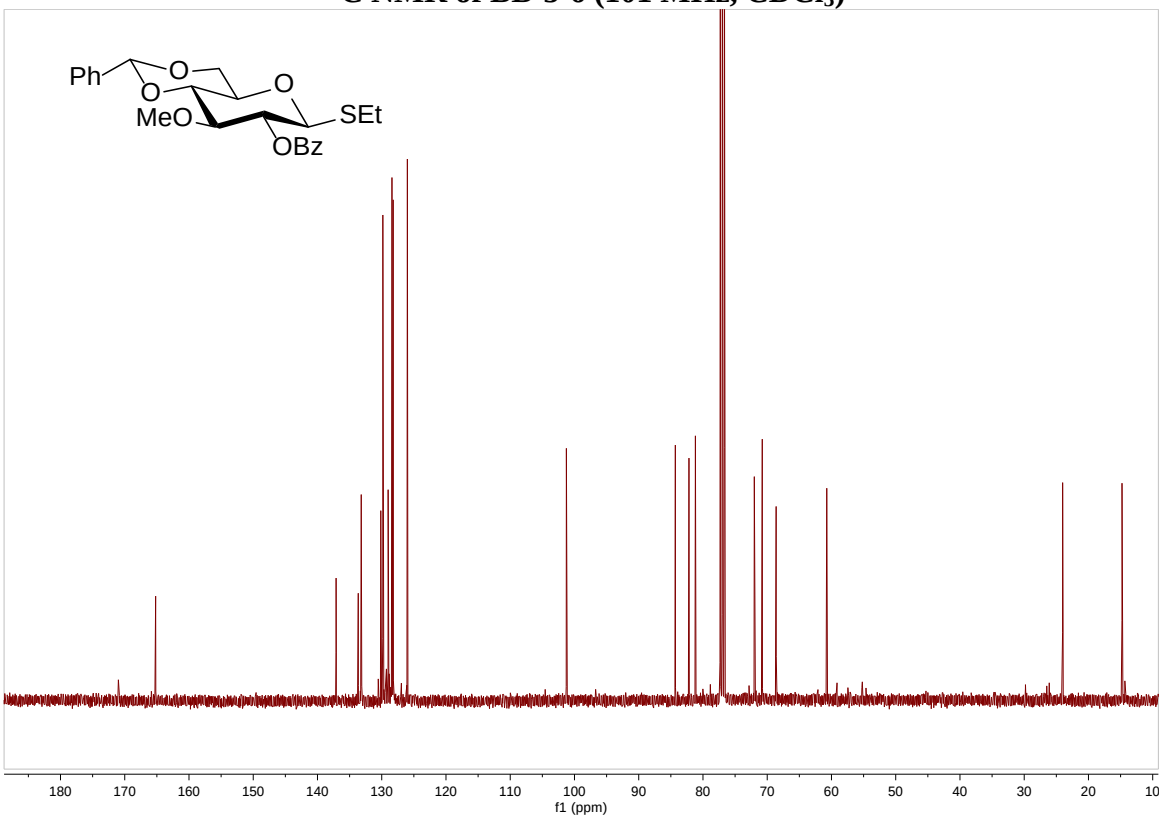
BB-5-5 **BB-5-6**

BB-5-5 (30.0 g, 92 mmol) was dissolved in 310 mL of anhydrous DCM. The solution was cooled to 0°C and Bz_2O (41.6 g, 184 mmol), 4-Dimethylaminopyridine (DMAP) (5.6 g, 46 mmol), and trimethylamine (TEA) (38 mL, 276 mmol) were successively added. The reaction was allowed to warm to rt and stirred for 12 h, during which time it became yellow and a white precipitate formed. 300 mL DCM was added and the organic layers were washed with saturated aq. $NaHCO_3$ and brine. The organics were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (7 hexanes: 2 EtOAc) to give **BB-5-6** as a pale yellow solid (33.1 g, 84%); $[\alpha]_D^{20}$ +8.07 (c 1, $CHCl_3$); IR (neat) νmax = 3442, 1071 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 – 8.06 (m, 2H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.53 – 7.44 (m, 4H), 7.41 – 7.33 (m, 3H), 5.60 (s, 1H), 5.27 (t, *J* = 10.2 Hz, 1H), 4.66 (d, *J* = 10.1 Hz, 1H), 4.41 (dd, *J* = 10.5, 4.9 Hz, 1H), 3.82 (t, *J* = 10.3 Hz, 1H), 3.79 – 3.61 (m, 2H), 3.59 (dd, *J* = 9.8, 5.2 Hz, 1H), 3.54 (s, 3H), 2.73 (qd, *J* = 7.4, 3.4 Hz, 2H), 1.24 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.36, 137.27, 133.82, 133.36, 130.31, 130.00, 129.95, 129.17, 128.60, 128.58, 128.39, 126.18, 101.43, 84.47, 82.35, 81.35, 72.18, 70.94, 68.78, 60.90, 24.19, 14.94; m/z (HRMS+) 431.1529 $[M + Na]^+$ ($C_{23}H_{26}O_6SNa$ requires 431.1523).

^1H NMR of BB-5-6 (400 MHz, $CDCl_3$)



^{13}C NMR of BB-5-6 (101 MHz, $CDCl_3$)

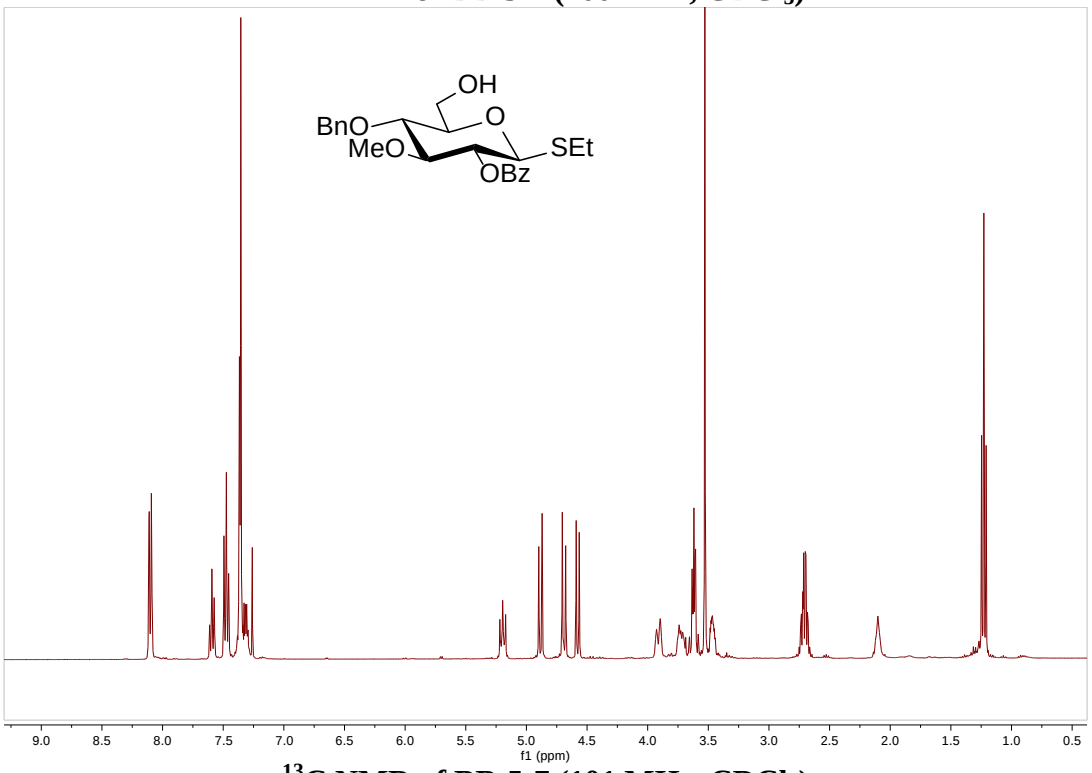


Synthesis of BB-5-7

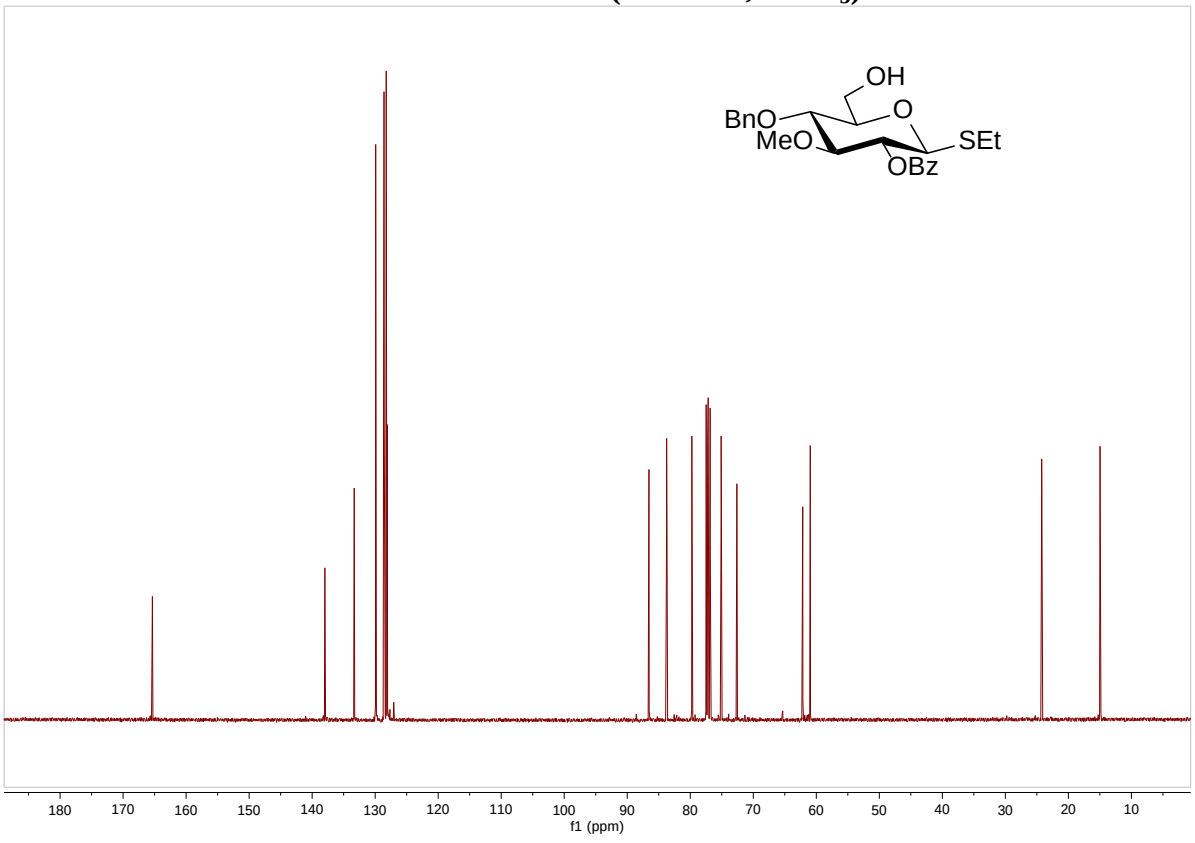
BB-5-6 **BB-5-7**

BB-5-6 (19.4 g, 45.1 mmol) was dissolved in 78 mL of anhydrous DCM. The solution was cooled to 0°C and a 1 M solution of BH_3 in THF (0.1 M, 180 mL, 180 mmol) was added followed by TMSOTf (4.1 mL, 22.5 mmol). The reaction was allowed to warm to rt and stirred for 4 h. The reaction was then cooled to 0 °C and quenched by addition of saturated aq. $NaHCO_3$. The reaction was then diluted with DCM and the aqueous layer was separated. The organic layer was extracted with saturated aq. $NaHCO_3$ and H_2O. The organics were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (4 hexanes: 1 EtOAc) to give **BB-5-7** as a fluffy white solid (16.0 g, 82%); $[\alpha]_D^{20}$ +152.44 (c 1, $CHCl_3$); IR (neat) νmax = 3484, 1725, 1069 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 7.1 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.40 – 7.28 (m, 5H), 5.24 – 5.14 (m, 1H), 4.88 (d, *J* = 11.0 Hz, 1H), 4.69 (d, *J* = 11.0 Hz, 1H), 4.58 (d, *J* = 10.1 Hz, 1H), 3.91 (d, *J* = 12.0 Hz, 1H), 3.72 (dd, *J* = 14.0, 7.8 Hz, 1H), 3.65 – 3.59 (m, 2H), 3.53 (s, 3H), 3.50 – 3.41 (m, 1H), 2.71 (qd, *J* = 7.4, 2.4 Hz, 2H), 1.23 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.35, 137.99, 133.33, 129.92, 128.60, 128.56, 128.25, 128.07, 86.55, 83.75, 79.75, 77.25, 75.10, 72.60, 62.15, 60.96, 24.24, 14.98; m/z (HRMS+) 455.1497 $[M + Na]^+$ ($C_{23}H_{28}O_6SNa$ requires 455.1499).

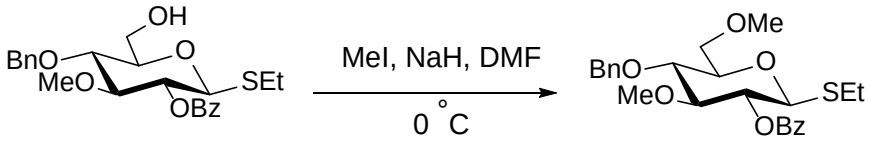
^{1}H NMR of BB-5-7 (400 MHz, $CDCl_3$)



^{13}C NMR of BB-5-7 (101 MHz, $CDCl_3$)



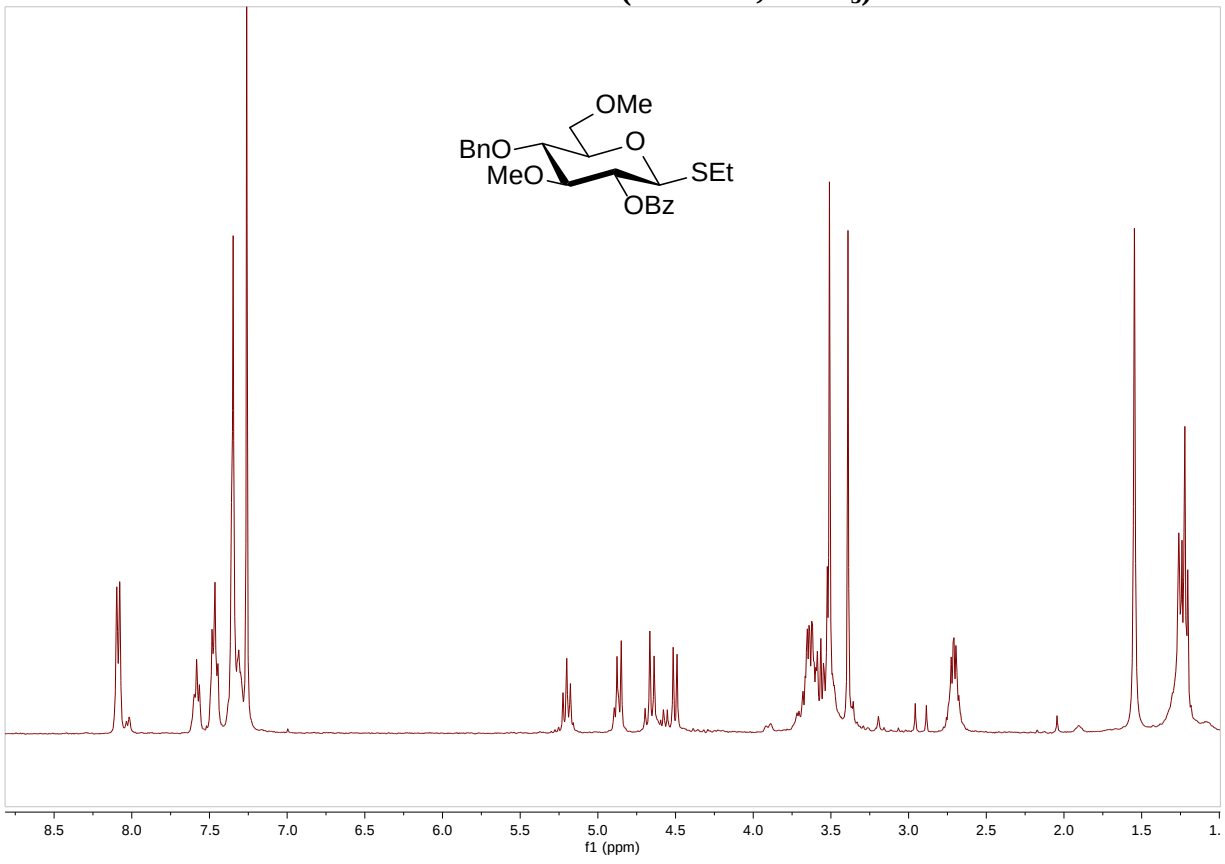
Synthesis of BB-5-8



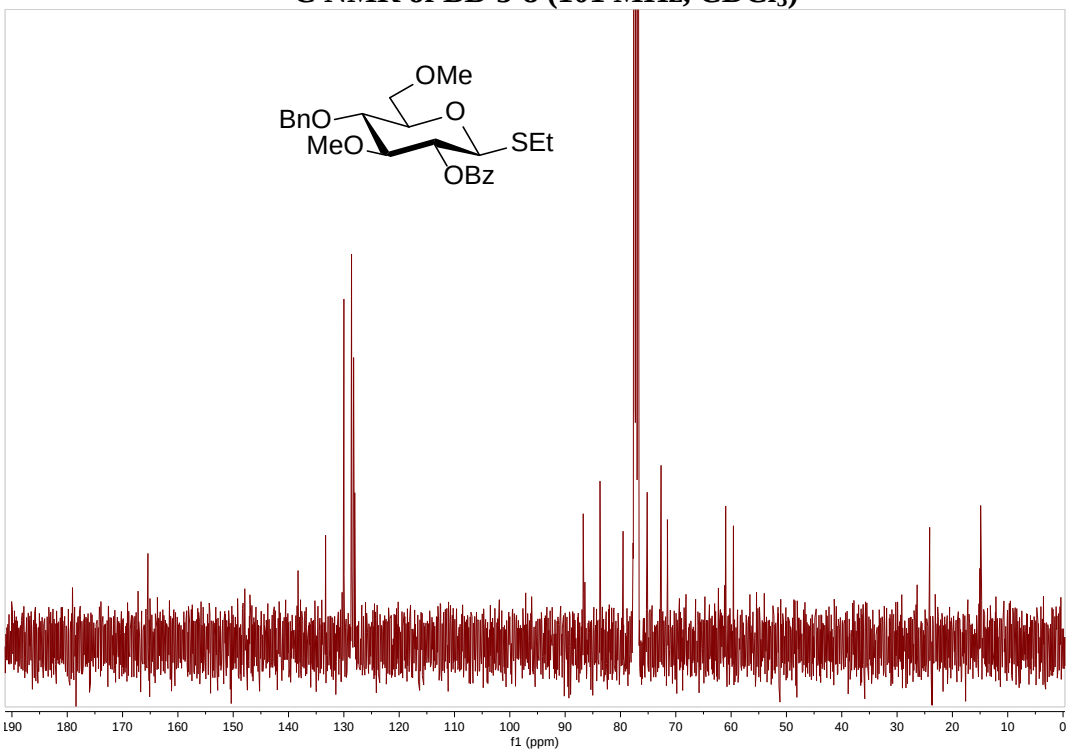
BB-5-7 **BB-5-8**

BB-5-7 (9.25 g, 21.4 mmol) was dissolved in 110 mL anhydrous DMF. The solution was cooled to 0 °C and NaH (60% by weight in mineral oil, 2.14 g, 53.5 mmol) was slowly added. After the NaH addition was complete, the reaction was stirred for 30 min at 0 °C. MeI (4.0 mL, 64.2 mmol) was added drop-wise. The reaction was allowed to warm to rt, during which time a white precipitate formed. The reaction was stirred for 1 hour at rt and then cooled back to 0 °C. Saturated aq. NH_4Cl was slowly added to quench and the reaction. The reaction was concentrated to < 30 mL under reduced pressure and 200 mL DCM was added. The organic layer was separated and extracted twice with H_2O. The organics were dried over $MgSO_4$, filtered, and concentrated to give **BB-5-8** as a light yellow gel (9.55 g, quantitative); $[\alpha]_D^{20}$ +11.61 (c 1, $CHCl_3$); IR (neat) νmax = 1728, 1094, 1071 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 7.4 Hz, 2H), 7.58 (t, J = 6.8 Hz, 1H), 7.47 (t, J = 7.7 Hz, 2H), 7.37 – 7.28 (m, 5H), 5.20 (t, J = 9.4 Hz, 1H), 4.86 (d, J = 11.1 Hz, 1H), 4.65 (d, J = 11.0 Hz, 1H), 4.50 (d, J = 10.1 Hz, 1H), 3.69 – 3.53 (m, 5H), 3.51 (s, 3H), 3.39 (s, 3H), 2.76 – 2.65 (m, 2H), 1.20 (t, J = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.42, 138.28, 133.30, 129.99, 128.62, 128.24, 128.03, 86.74, 83.70, 79.53, 77.48, 75.17, 72.66, 71.51, 60.98, 59.58, 24.11, 14.89; m/z (HRMS+) 469.1666 $[M + Na]^+$ ($C_{24}H_{30}O_6SNa$ requires 469.1655).

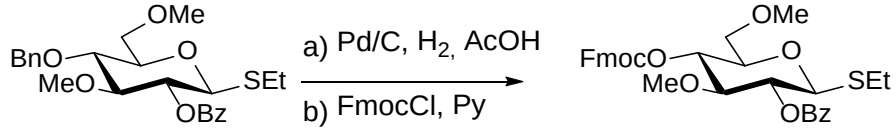
^1H NMR of BB-5-8 (400 MHz, $CDCl_3$)



^{13}C NMR of BB-5-8 (101 MHz, $CDCl_3$)



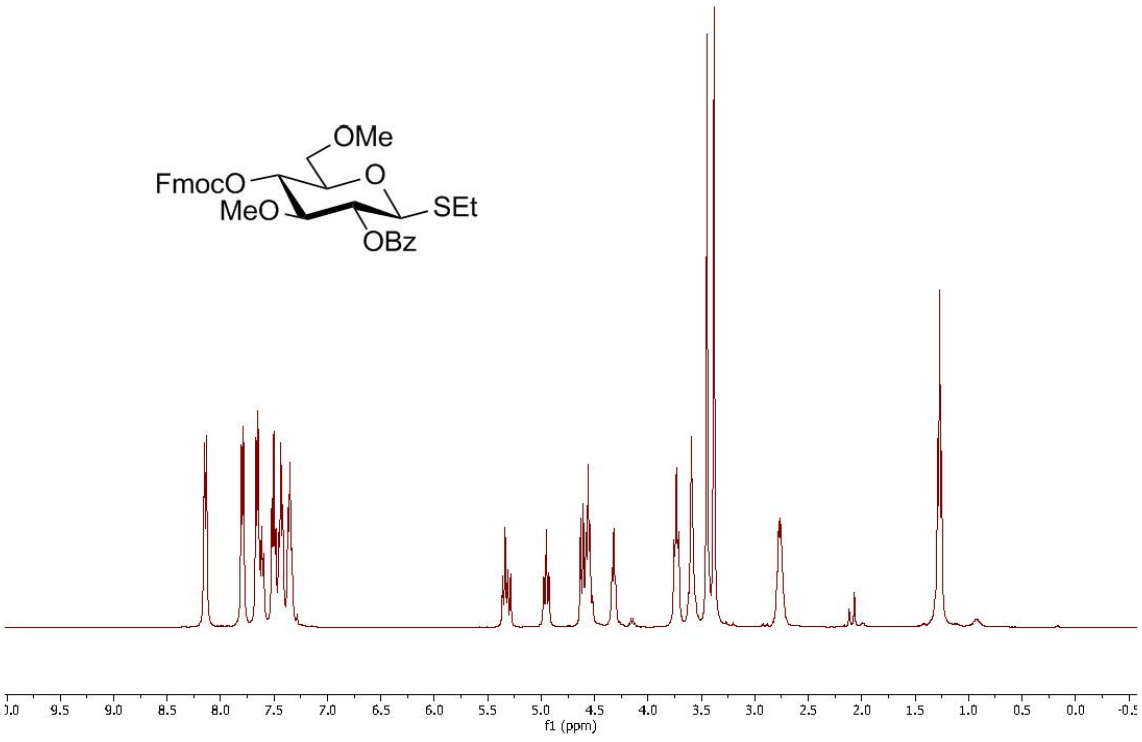
Synthesis of BB-5



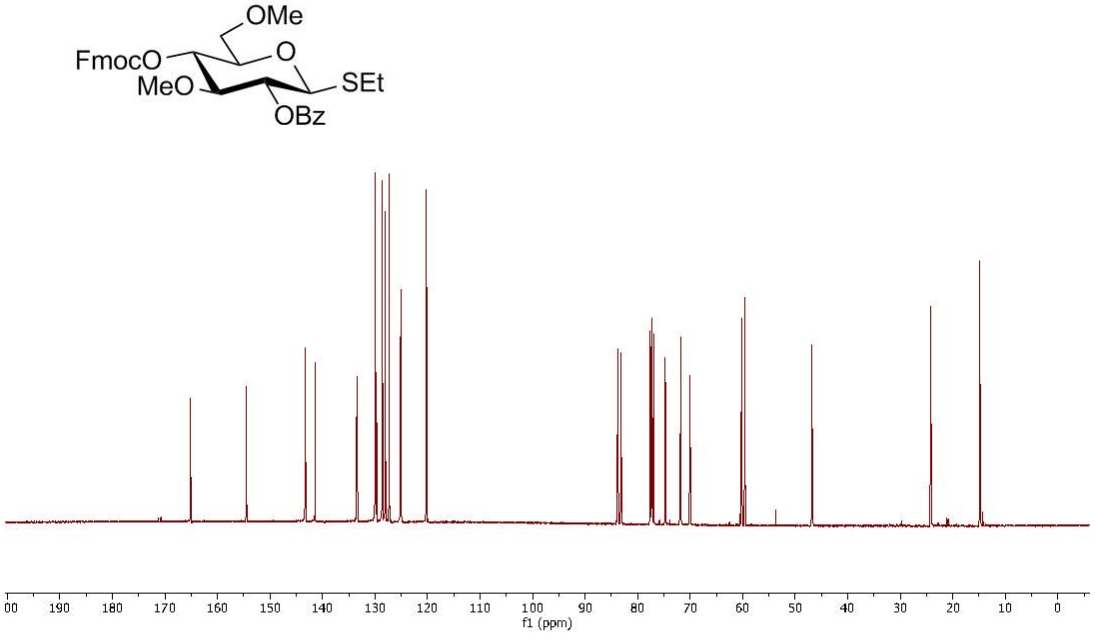
BB-5-8 **BB-5**

BB-5-8 (7.36 g, 17.9 mmol) was dissolved in 40 mL of AcOH. 3.2 g Pd/C (40% of reactant by weight) was added. The mixture was stirred in H_2 atmosphere at 40 psi for 3 days. The crude material was filtered through celite and the filtrate was concentrated. The crude product was purified by column chromatography (3 hexanes : 1 EtOAc - 1 hexanes : 1 EtOAc) to give a clear gel. The gel was then co-evaporated with pyridine and dissolved in 22 mL of DCM. Pyridine (2 mL, 19.8 mmol) was added followed by Fmoc-Cl (3.45 g, 13.2 mmol). The yellow solution was stirred at rt until completion (3 h). The reaction was diluted with DCM and extracted with 1 M HCl, sat. aq. $NaHCO_3$, and H_2O. The organics were dried over $MgSO_4$ and concentrated. The product was purified by column chromatography (4 Hexane: 1 EtOAc) to give **BB-5** as a flaky white solid (3.8 g, 40% over two steps); $[\alpha]_D^{20}$ +12.75 (c 1, $CHCl_3$); IR (neat) νmax = 1752, 1729, 1247 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 5.1, 3.3 Hz, 2H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.64 – 7.56 (m, 3H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.31 (tdd, *J* = 7.5, 2.3, 1.2 Hz, 2H), 5.26 (t, *J* = 9.6 Hz, 1H), 4.87 (t, *J* = 9.6 Hz, 1H), 4.58 – 4.43 (m, 3H), 4.28 (t, *J* = 7.0 Hz, 1H), 3.69 (ddd, *J* = 12.6, 5.8, 3.0 Hz, 2H), 3.58 – 3.52 (m, 2H), 3.40 (s, 3H), 3.35 (s, 3H), 2.78 – 2.66 (m, 2H), 1.23 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.18, 154.52, 143.32, 141.47, 133.45, 130.01, 129.81, 128.63, 128.08, 127.32, 125.17, 120.25, 83.82, 83.23, 77.48, 74.81, 71.99, 71.84, 70.19, 60.21, 59.74, 46.90, 24.22, 14.87; m/z (HRMS+) 601.1870 $[M + Na]^+$ ($C_{32}H_{34}O_8SNa$ requires 601.1867)

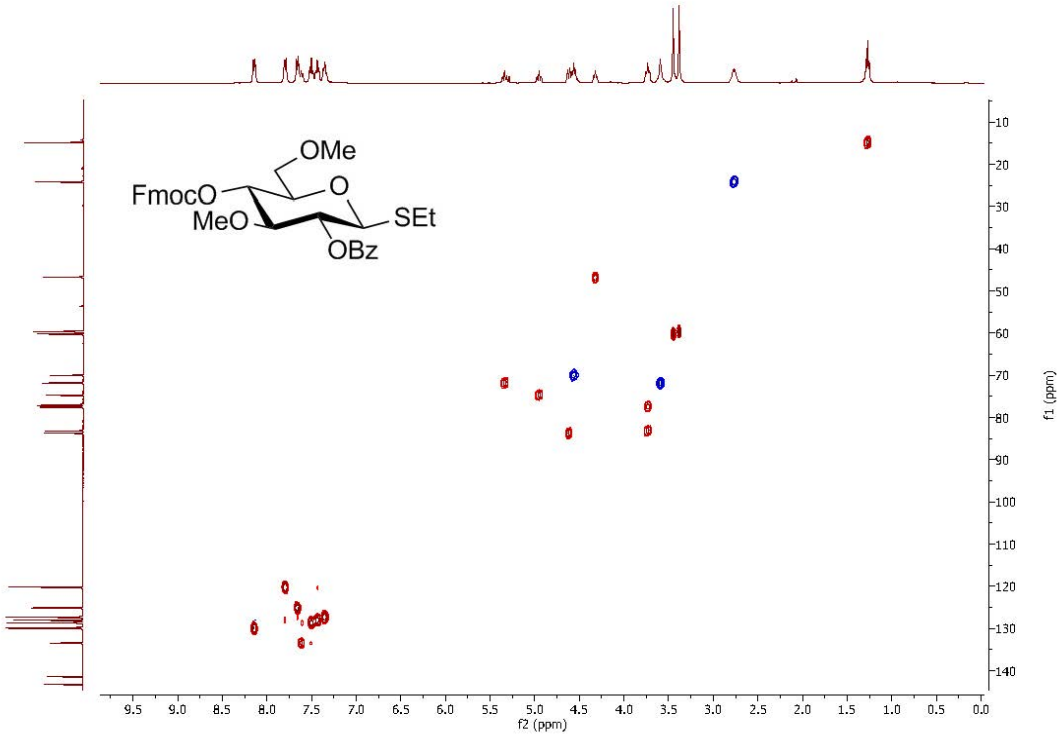
^{1}H NMR of BB-5 (400 MHz, $CDCl_3$)



^{13}C NMR of BB-5 (101 MHz, $CDCl_3$)



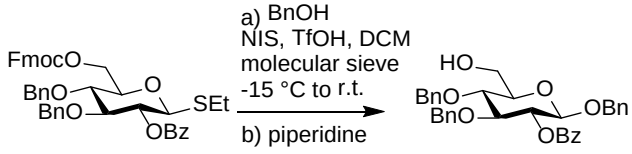
HSQC NMR of BB-5 ($CDCl_3$)



4. Synthesis of partially protected dimers

4.1. Synthesis of 1

Synthesis of benzyl 2-O-benzoyl-3,4-O-benzyl- β-D-glucopyranoside, S4

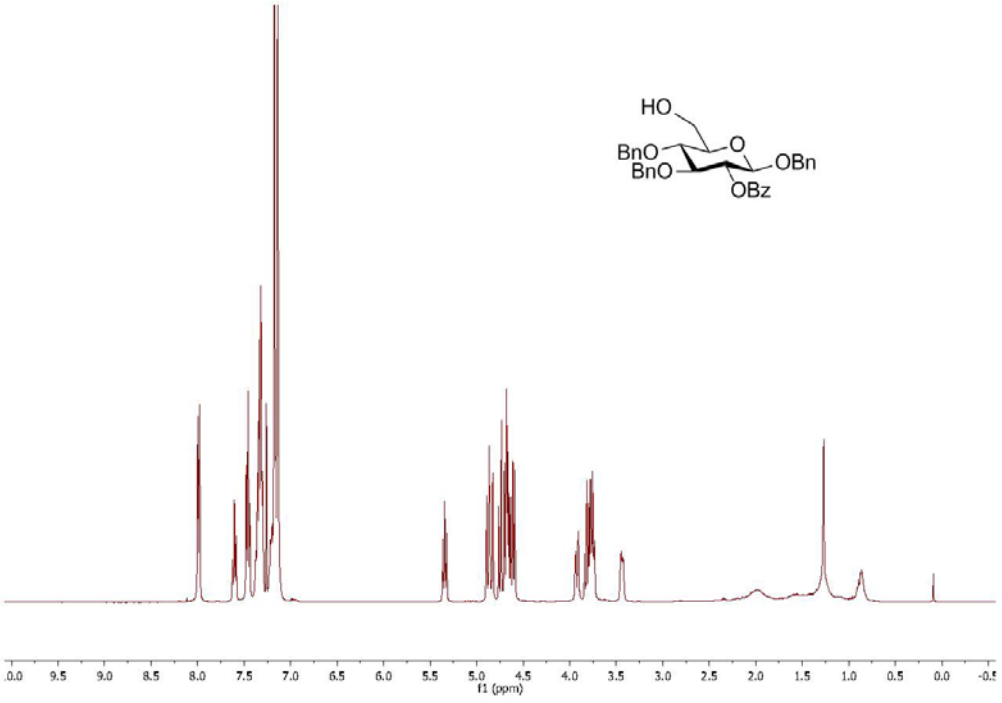


BB-1 **S4**

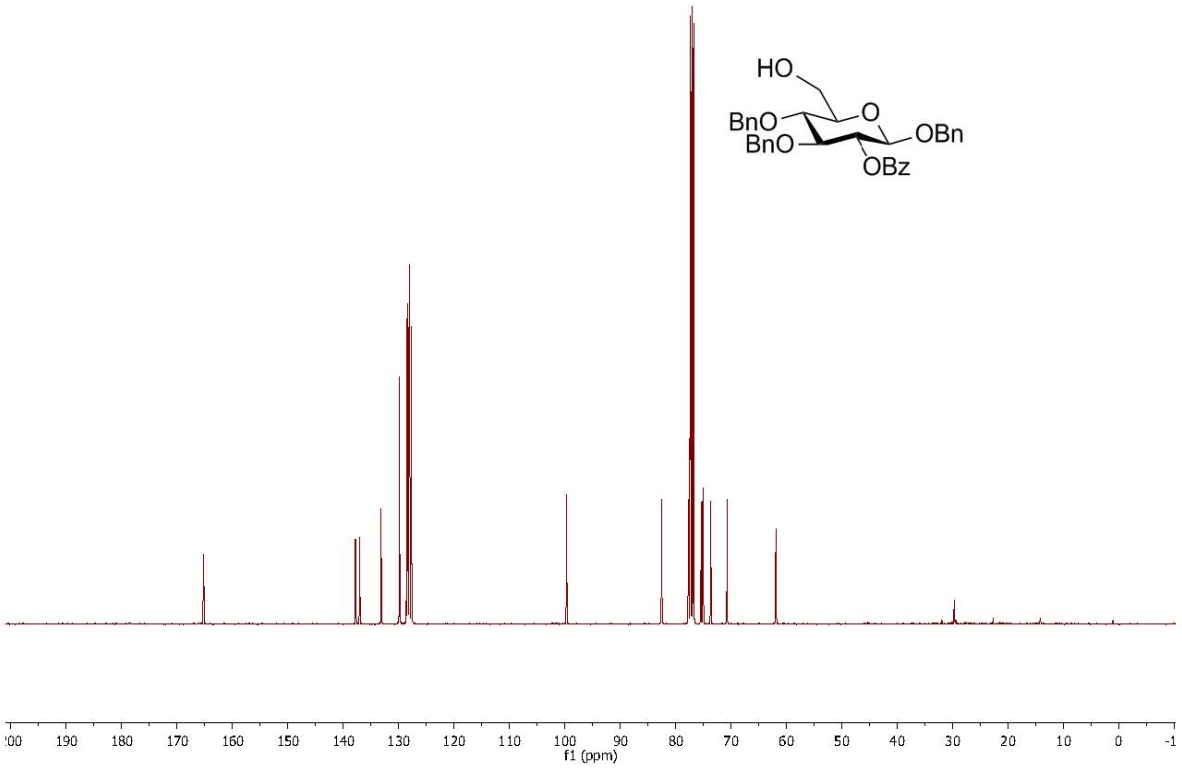
BB-1 was prepared following previously established procedures.[2]
BB-1 (20.0 mg, 0.027 mmol), *N*-iodosuccinimide (7.4 mg, 0.0239 mmol) and benzyl alcohol (5.9 mg, 0.055 mmol) were dissolved in anhydrous DCM (2 mL). The solution was then stirred with molecular sieve for 1h at room temperature under nitrogen atmosphere and then cooled to -15 °C. A 1% solution of TfOH in DCM (10 µL) was added and the reaction was stirred for 30 min at -15 °C before the removal of cooling bath to raise the temperature to room temperature. After TLC indicated the disappearance of **S1**, piperidine (0.1 mL) was added and the yellow solution was stirred at room temperature for additional 30 min. The reaction was diluted with DCM and washed with H_2O, then saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: EtOAc = 3:1) to give **S4** as white solid (13.5 mg, 89%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.02 – 7.95

(m, 2H), 7.65 – 7.56 (m, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.41 – 7.27 (m, 5H), 7.24 – 7.10 (m, 10H), 5.39 – 5.30 (m, 1H), 4.86 (dd, *J* = 14.6, 11.8 Hz, 2H), 4.75 (d, *J* = 11.2 Hz, 1H), 4.72 – 4.62 (m, 3H), 4.60 (d, *J* = 8.0 Hz, 1H), 3.92 (dd, *J* = 12.1, 2.5 Hz, 1H), 3.86 – 3.71 (m, 3H), 3.44 (ddd, *J* = 9.6, 4.5, 2.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.24, 137.80, 137.71, 137.02, 133.18, 129.89, 129.85, 128.57, 128.39, 128.35, 128.30, 128.13, 128.06, 128.04, 127.79, 127.71, 127.67, 99.69, 82.50, 77.67, 75.40, 75.14, 75.10, 73.70, 70.68, 61.90; m/z (HRMS+) 577.2203 $[M + Na]^+$ ($C_{34}H_{34}O_7Na$ requires 577.2197).

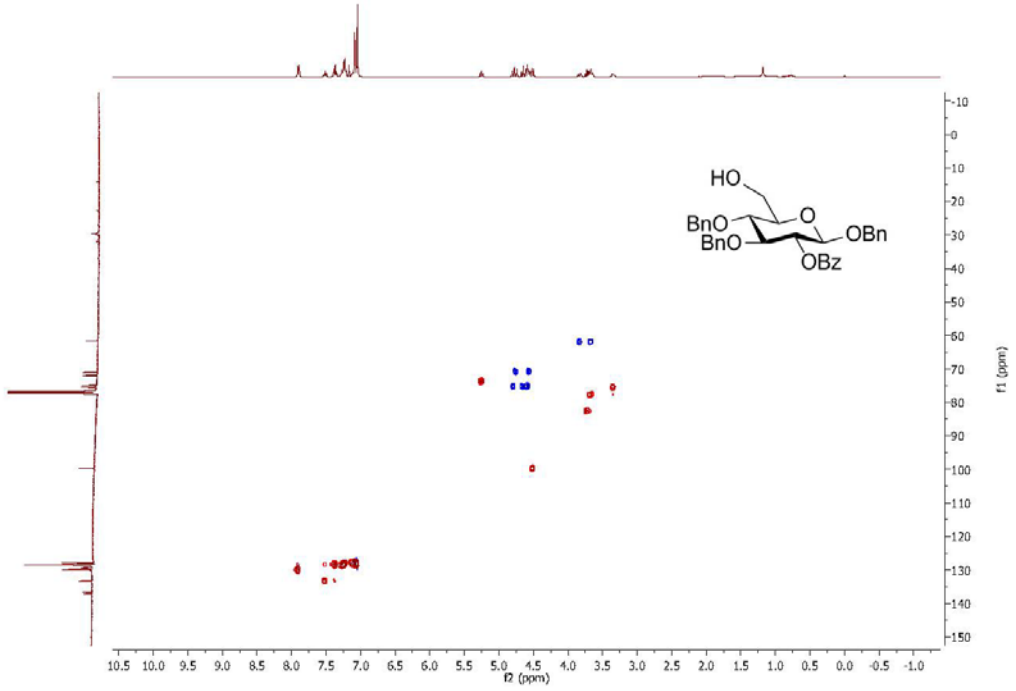
^{1}H NMR of S4 (400 MHz, $CDCl_3$)



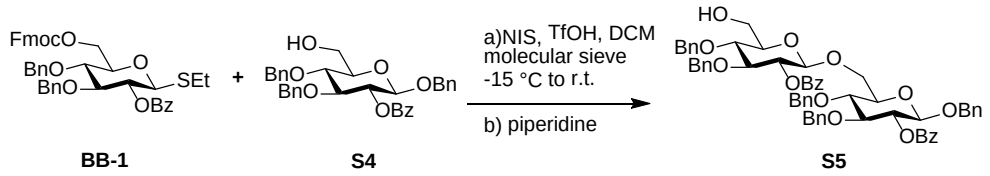
^{13}C NMR of S4 (101 MHz, $CDCl_3$)



HSQC NMR of S4 ($CDCl_3$)

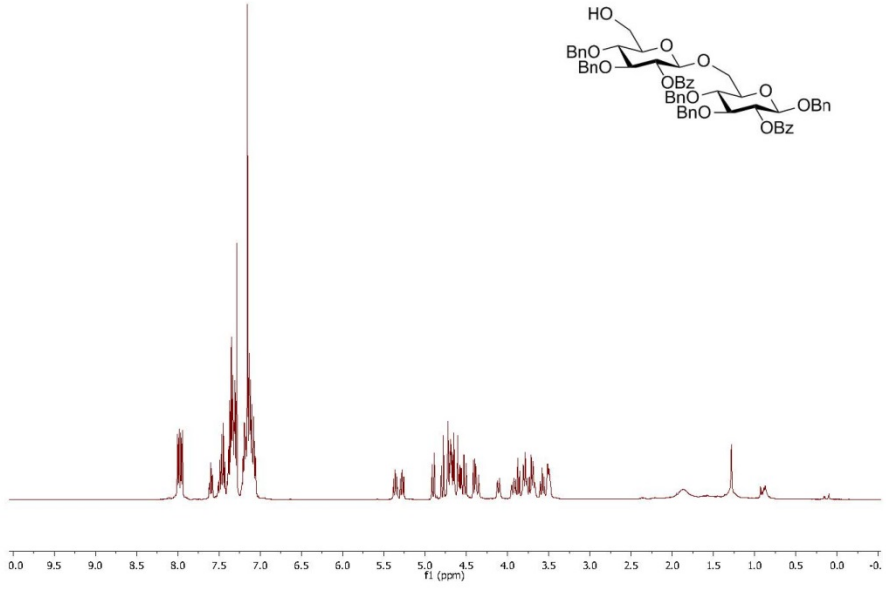


Synthesis of S5

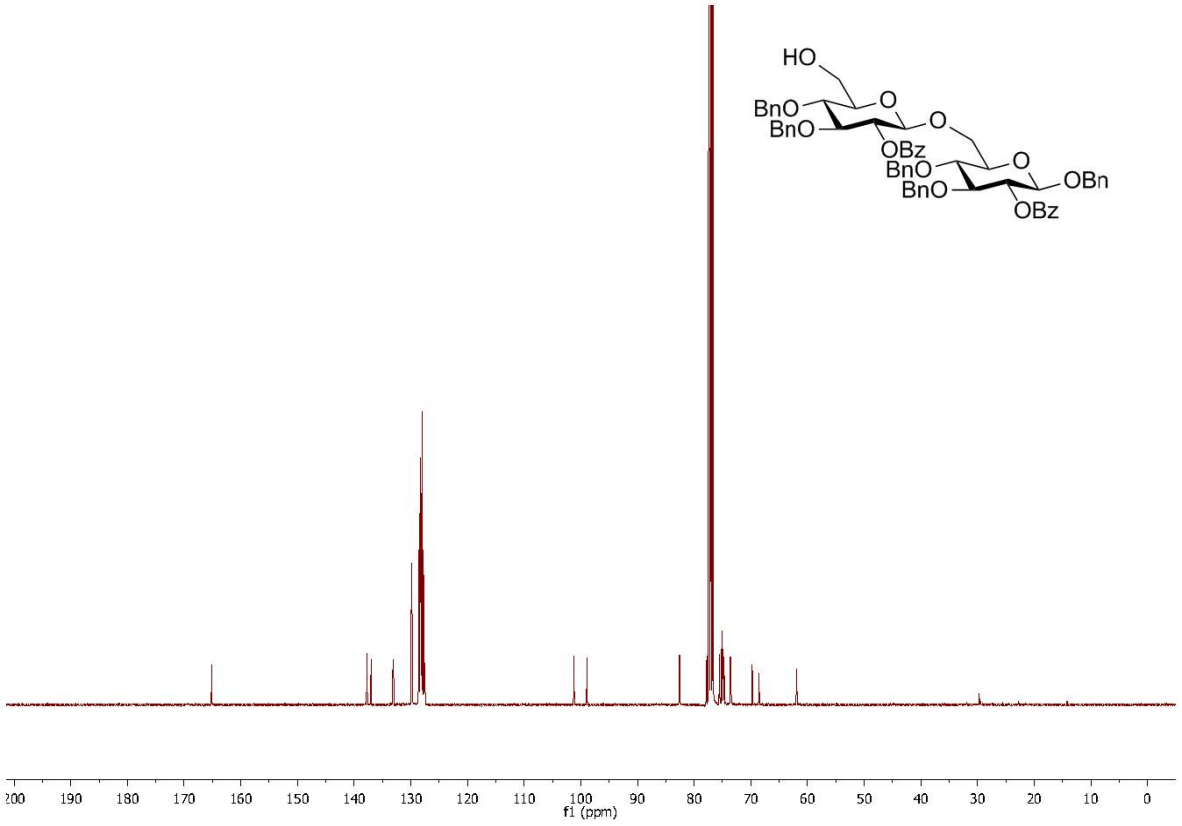


S4 (13.5 mg, 0.0244 mmol), **BB-1** (21.3 mg, 0.0292 mmol) and *N*-iodosuccinimide (6.6 mg, 0.0292 mmol) were dissolved in anhydrous DCM (2 mL). The solution was stirred with molecular sieve for 1h at room temperature under nitrogen atmosphere and then cooled to -15 °C. A 1% solution of TfOH in DCM (10 µL) was added and the reaction was stirred for 30 min at -15 °C before the removal of cooling bath to raise the temperature to room temperature. After TLC indicated the disappearance of **S4**, 0.1 mL piperidine was added and the yellow solution was stirred at room temperature for additional 30 min. The reaction was diluted with DCM and washed with H_2O, then saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: EtOAc = 3:1) to give **S5** as white solid (20.1 mg, 82%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.97 (ddd, *J* = 16.2, 8.3, 1.4 Hz, 4H), 7.65 – 7.56 (m, 1H), 7.47 (dt, *J* = 16.8, 7.5 Hz, 3H), 7.41 – 7.29 (m, 10H), 7.25 – 7.01 (m, 17H), 5.36 (dd, *J* = 9.3, 7.8 Hz, 1H), 5.28 (dd, *J* = 9.4, 7.9 Hz, 1H), 4.90 (d, *J* = 10.9 Hz, 1H), 4.79 (d, *J* = 11.1 Hz, 1H), 4.75 – 4.63 (m, 5H), 4.63 – 4.54 (m, 2H), 4.51 (d, *J* = 11.1 Hz, 1H), 4.44 – 4.32 (m, 2H), 4.11 (d, *J* = 10.8 Hz, 1H), 3.98 – 3.84 (m, 2H), 3.84 – 3.62 (m, 4H), 3.62 – 3.52 (m, 1H), 3.49 (dd, *J* = 10.1, 5.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.13, 165.11, 137.82, 137.78, 137.70, 137.67, 136.99, 133.14, 133.08, 129.86, 129.78, 129.72, 128.58, 128.44, 128.41, 128.30, 128.23, 128.21, 128.15, 128.06, 128.02, 127.90, 127.87, 127.75, 127.71, 127.64, 127.58, 101.19, 98.91, 82.64, 82.58, 77.87, 77.67, 75.49, 75.13, 75.12, 74.94, 74.82, 74.71, 73.64, 73.51, 69.75, 68.55, 61.94; m/z (HRMS+) 1023.387 $[M + Na]^+$ ($C_{61}H_{60}O_{13}Na$ requires 1023.393).

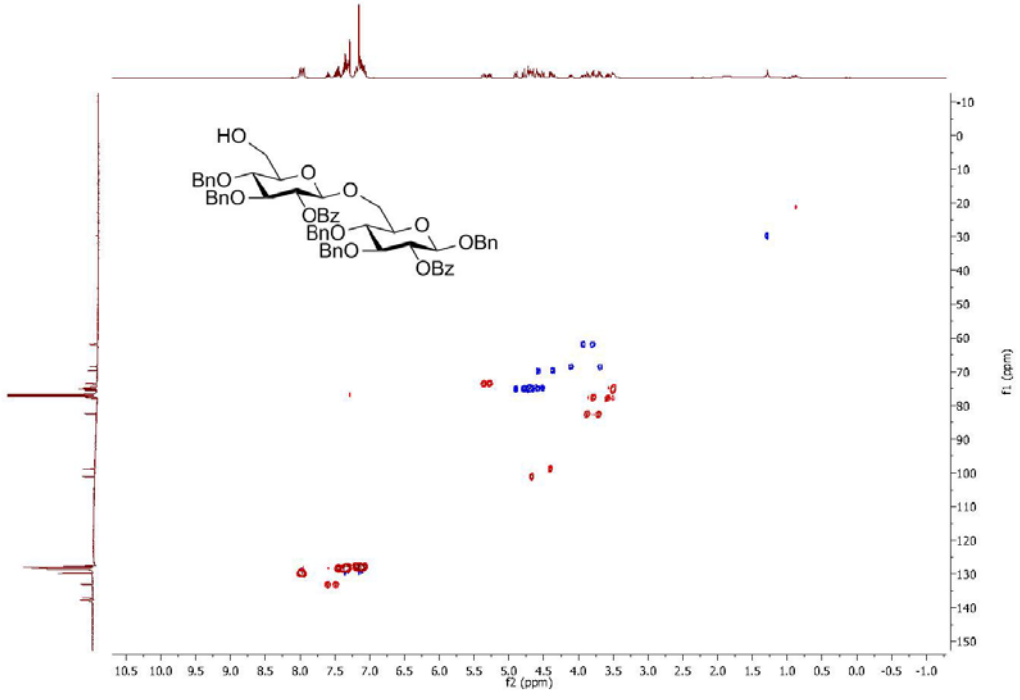
^{1}H NMR of S5 (400 MHz, $CDCl_3$)



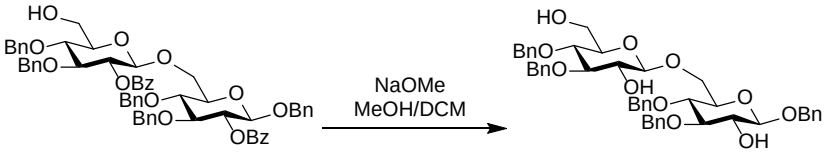
^{13}C NMR of S5 (101 MHz, $CDCl_3$)



HSQC NMR of S5 ($CDCl_3$)



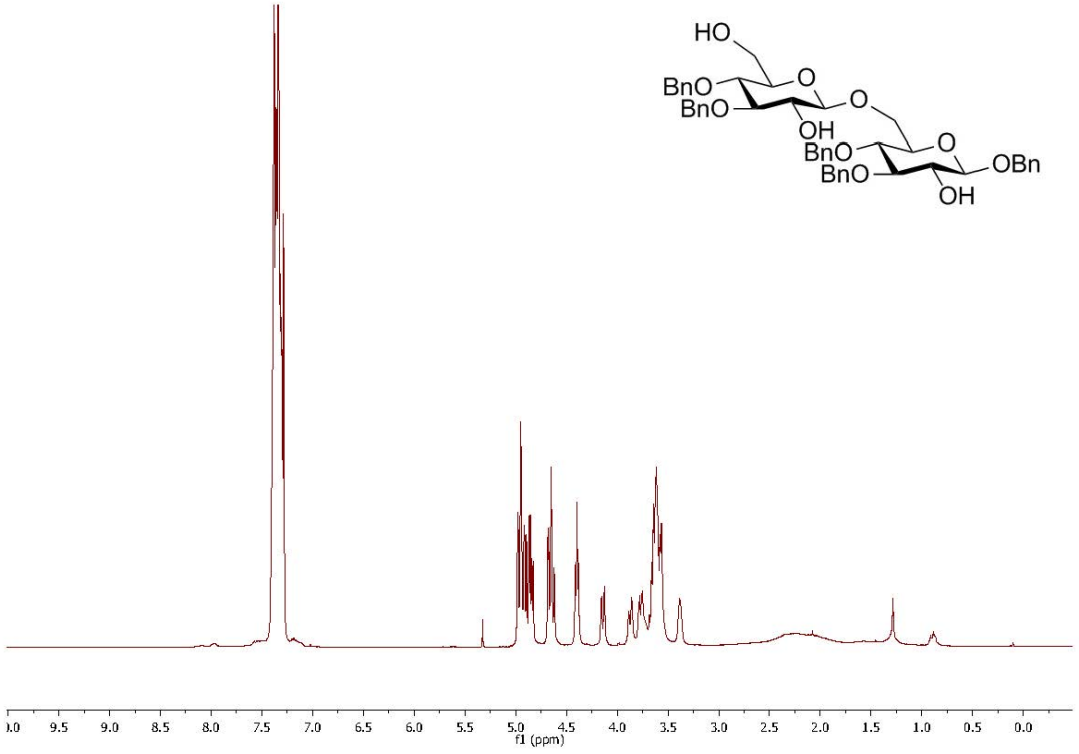
Synthesis of 1



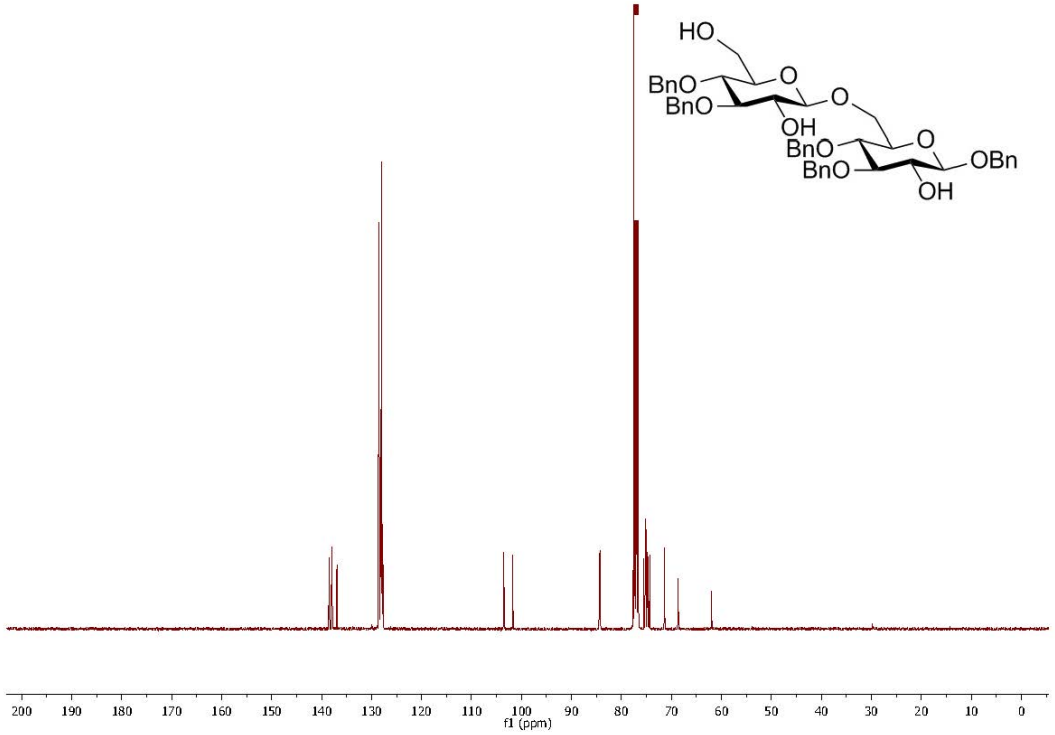
S5 **1**

S5 (20.1 mg, 0.020 mmol) was dissolved in MeOH: DCM (1.5 mL,1:1). NaOMe in MeOH (0.5 M, 3 equiv. per benzoyl ester) was added and the solution was stirred at room temperature for 12 h, neutralized with Amberlite IR-120 (H^+ form) resin, filtered and concentrated *in vacuo*. The resulting yellow oil was purified by column chromatography (hexane: acetone = 3:1) to give **1** as white solid (13.1 mg, 83%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.27 (m, 25H), 4.95 (ddd, *J* = 14.9, 11.4, 3.5 Hz, 4H), 4.90 – 4.78 (m, 3H), 4.71 – 4.57 (m, 3H), 4.40 (t, *J* = 6.5 Hz, 2H), 4.14 (d, *J* = 11.5 Hz, 1H), 3.87 (d, *J* = 11.6 Hz, 1H), 3.76 (dt, *J* = 16.8, 8.4 Hz, 2H), 3.62 (ddt, *J* = 15.7, 12.1, 7.5 Hz, 7H), 3.39 (d, *J* = 5.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.54, 138.48, 137.97, 137.96, 136.92, 129.95, 128.57, 128.53, 128.51, 128.48, 128.18, 128.11, 128.10, 128.02, 127.99, 127.96, 127.84, 127.77, 103.56, 101.75, 84.39, 84.22, 77.62, 77.26, 75.51, 75.29, 75.15, 75.11, 75.09, 74.83, 74.69, 74.36, 71.36, 68.63, 61.96; m/z (HRMS+) 815.3407 $[M + Na]^+$ ($C_{47}H_{52}O_{11}Na$ requires 815.3402).

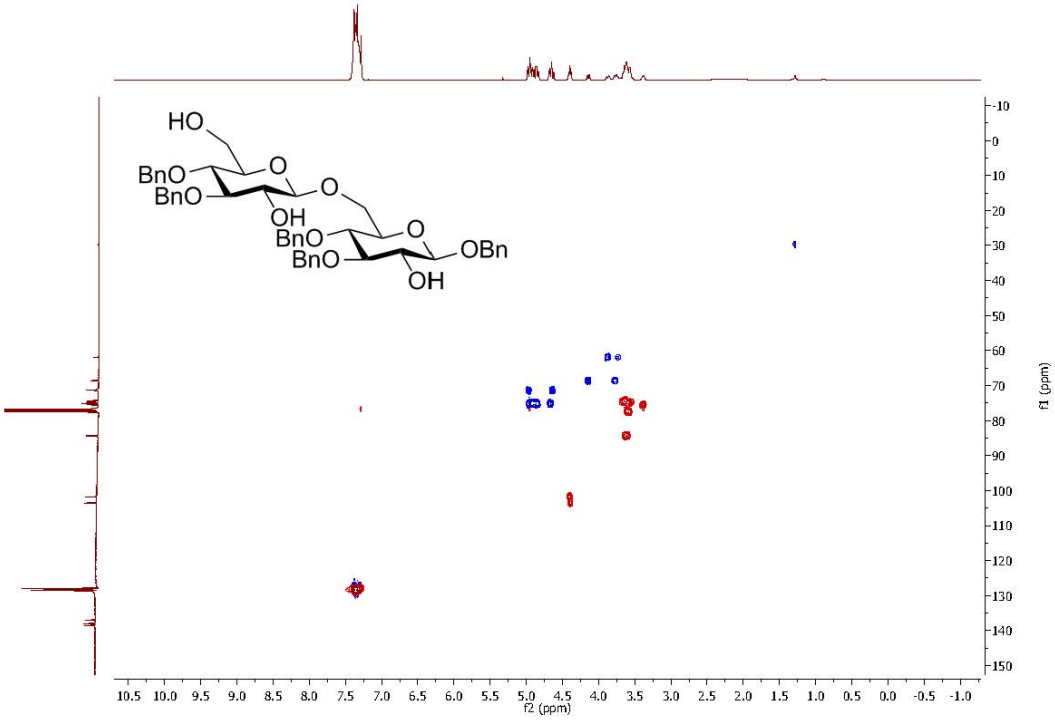
^{1}H NMR of 1 (400 MHz, $CDCl_3$)



^{13}C NMR of 1 (101 MHz, $CDCl_3$)

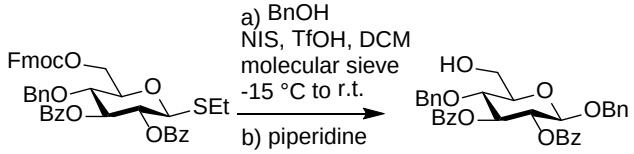


HSQC NMR of 1 ($CDCl_3$)



4.2.Synthesis of 2

Synthesis of benzyl 2,3-O-dibenzoyl-4-O-benzyl- β-D-glucopyranoside S6

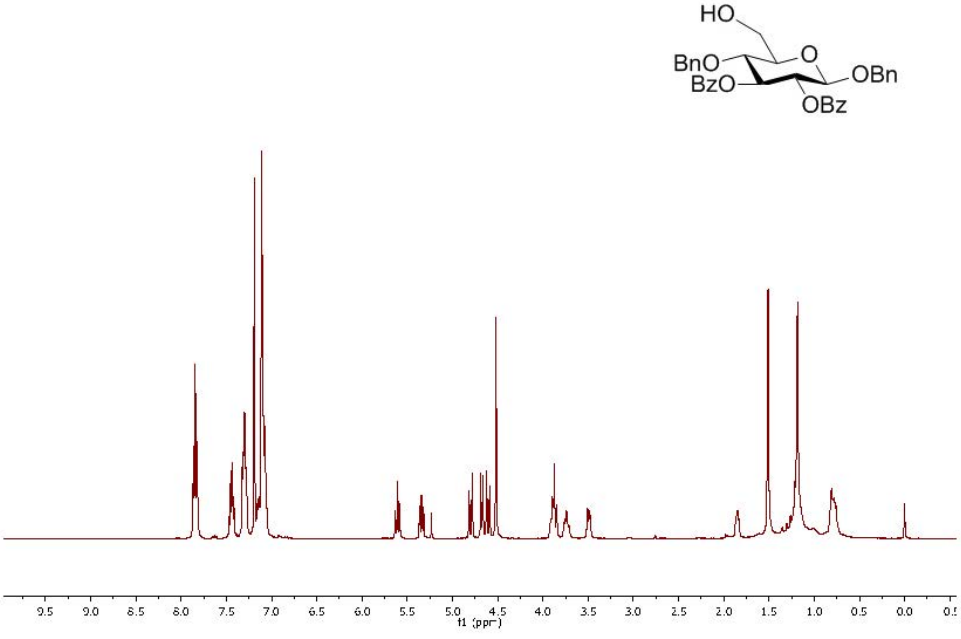


BB-2 **S6**

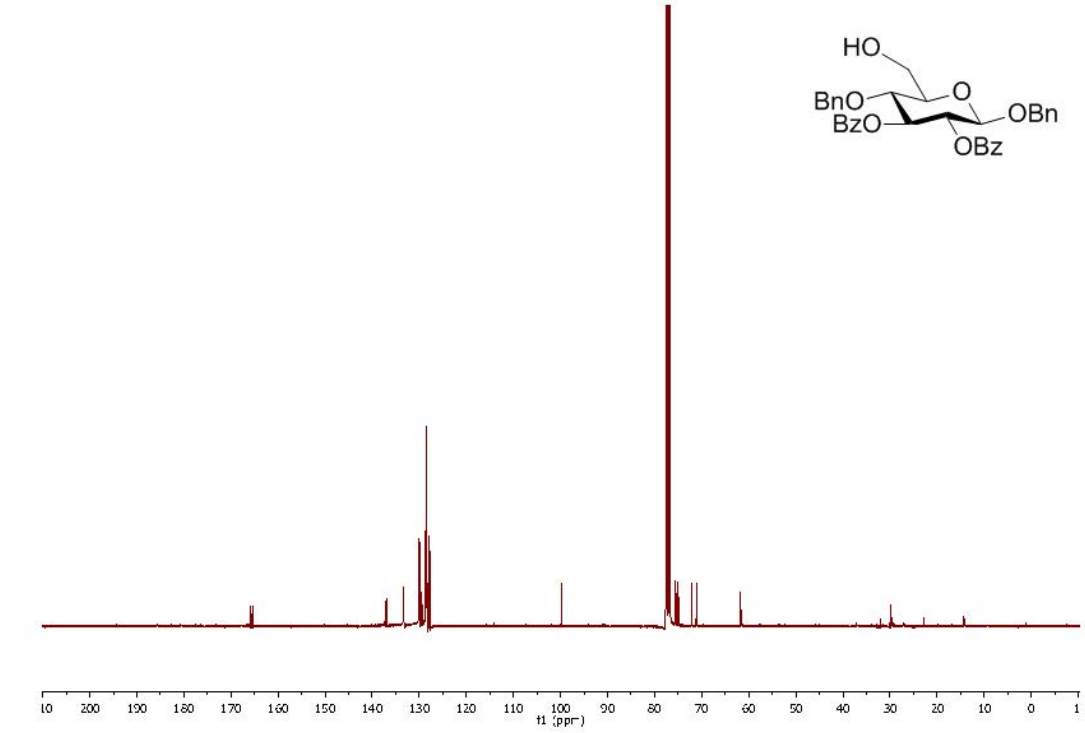
BB-2 was prepared following previously established procedures.[3]

BB-2 (204 mg, 0.274 mmol), *N*-iodosuccinimide (74 mg, 0.33 mmol) and benzyl alcohol (59.3 mg, 0.055 mmol) were dissolved in anhydrous DCM (2 mL). The solution was then stirred with molecular sieve for 1h at room temperature under nitrogen atmosphere and cooled to -15 °C. A 1% solution of TfOH in DCM (100 µL) was added and the reaction was stirred for 30 min at -15 °C before the removal of cooling bath to raise the temperature to room temperature. After TLC indicated the disappearance of **S2**, 1 mL piperidine was added and the yellow solution was stirred at room temperature for additional 30 min. The reaction was diluted with DCM and washed with H_2O, then saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: EtOAc = 3:1) to give **S6** as white solid (132 mg, 85%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.89 – 7.80 (m, 4H), 7.44 (q, *J* = 7.2 Hz, 2H), 7.30 (td, *J* = 7.7, 3.9 Hz, 4H), 7.16 – 7.03 (m, 10H), 5.61 (t, *J* = 9.6 Hz, 1H), 5.34 (dd, *J* = 9.9, 7.9 Hz, 1H), 4.80 (d, *J* = 12.6 Hz, 1H), 4.68 (d, *J* = 8.0 Hz, 1H), 4.61 (d, *J* = 12.6 Hz, 1H), 4.52 (s, 2H), 3.95 – 3.82 (m, 2H), 3.74 (ddd, *J* = 12.2, 8.4, 3.9 Hz, 1H), 3.49 (dt, *J* = 9.7, 3.2 Hz, 1H), 1.85 (dd, *J* = 8.4, 5.3 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.81, 165.45, 137.24, 136.87, 133.34, 133.29, 130.00, 129.89, 129.46, 128.52, 128.50, 128.43, 128.34, 128.13, 128.04, 127.81, 114.22, 99.77, 77.36, 75.61, 75.53, 75.02, 74.94, 72.09, 71.09, 61.73; m/z (HRMS+) 591.1990 $[M + H]^+$ ($C_{34}H_{32}O_8Na$ requires 591.1989);

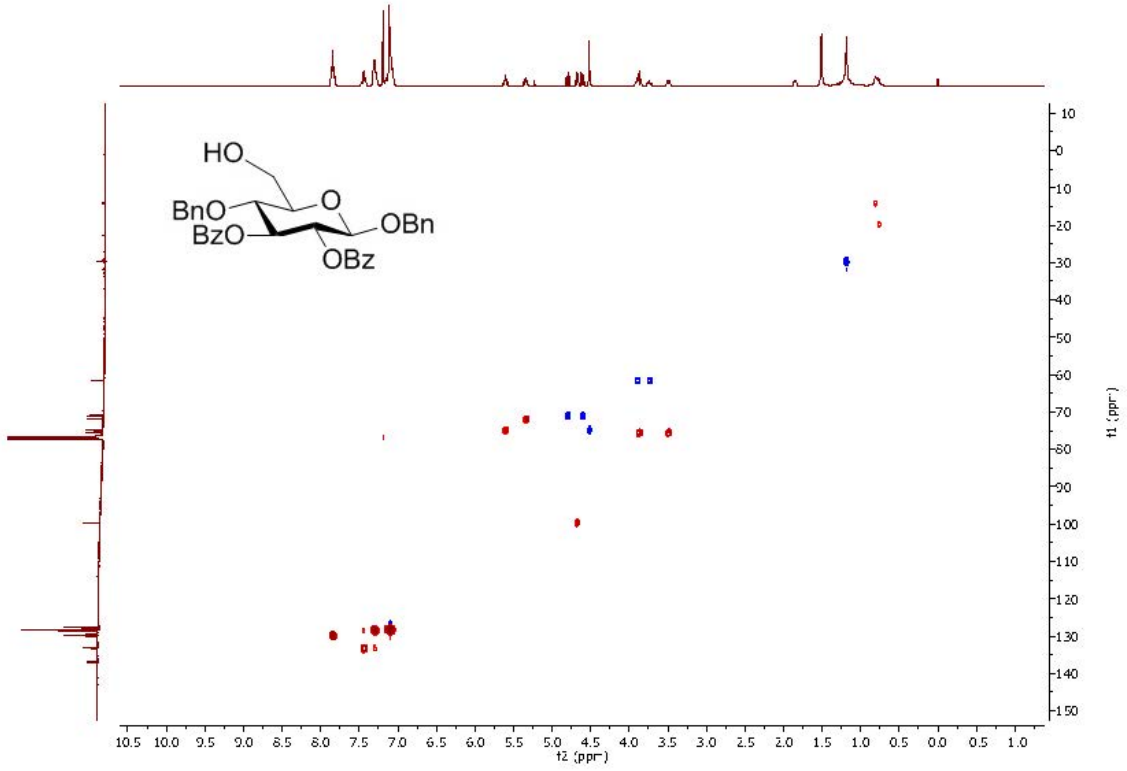
^{1}H NMR of S6 (400 MHz, $CDCl_3$)



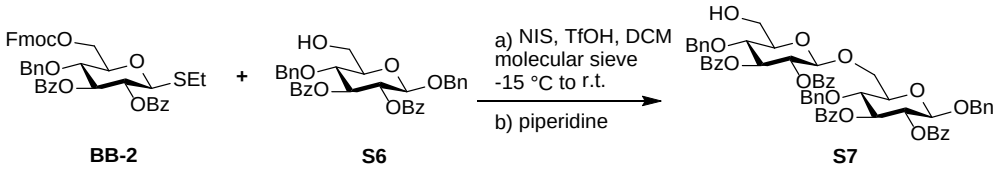
^{13}C NMR of S6 (101 MHz, $CDCl_3$)



HSQC NMR of S6 ($CDCl_3$)



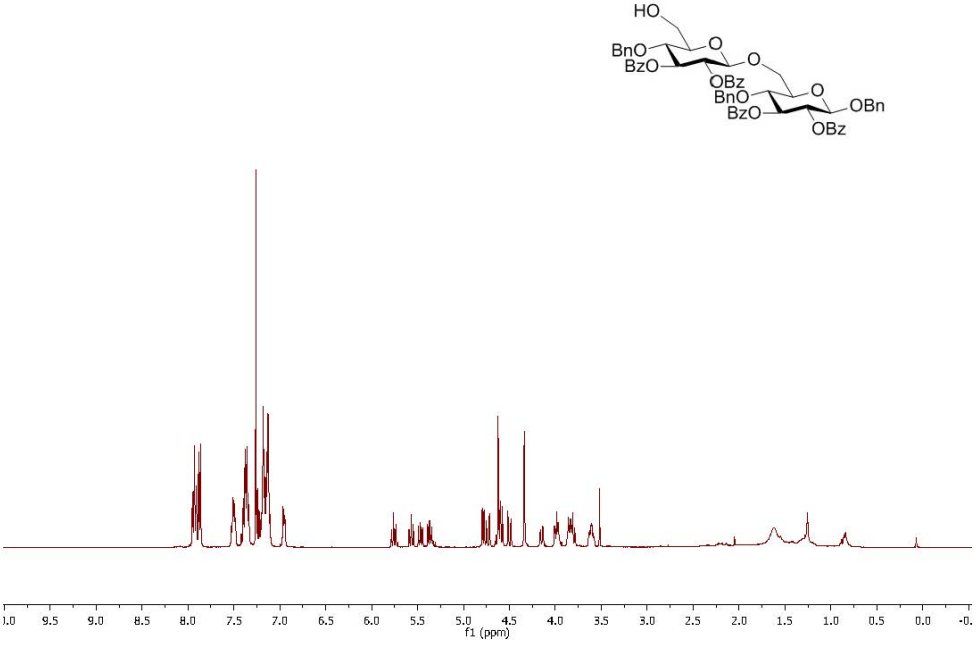
Synthesis of S7



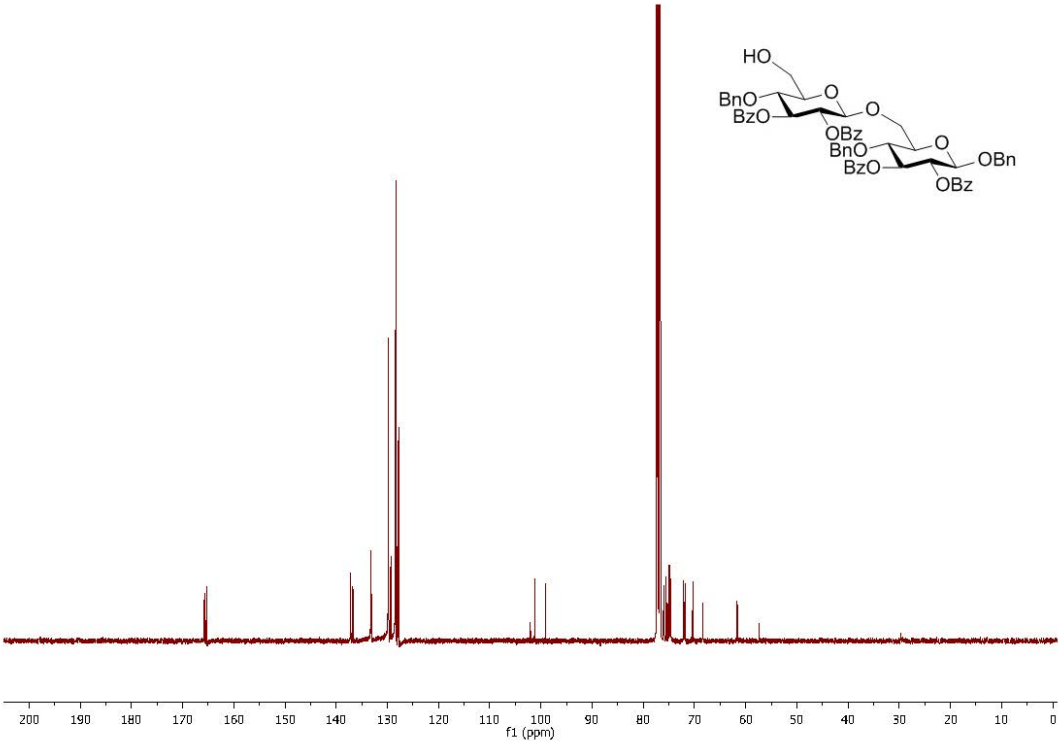
BB-2 **S6** **S7**

S6 (132 mg, 0.232 mmol), (**BB-2** (207 mg, 0.278 mmol) and *N*-iodosuccinimide (62.6 mg, 0.278 mmol) were dissolved in anhydrous DCM (2 mL). The solution was then stirred with molecular sieve for 1h at room temperature under nitrogen atmosphere and cooled to -15 °C. A 1% solution of TfOH in DCM (100 µL) was added and the reaction was stirred for 30 min at -15 °C before the removal of cooling bath to raise the temperature to room temperature. After TLC indicated the disappearance of **S6**, 1 mL piperidine was added and the yellow solution was stirred at room temperature for additional 30 min. The reaction was diluted with DCM and washed with H_2O, then saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: EtOAc = 3:1) to give **S7** as white solid (196 mg, 82%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.97 – 7.83 (m, 8H), 7.54 – 7.45 (m, 3H), 7.37 (tdt, *J* = 7.5, 5.9, 5.0 Hz, 8H), 7.25 – 7.07 (m, 14H), 7.01 – 6.92 (m, 2H), 5.75 (td, *J* = 9.7, 7.9 Hz, 1H), 5.57 (t, *J* = 9.5 Hz, 1H), 5.47 (dd, *J* = 9.8, 7.8 Hz, 1H), 5.35 (ddd, *J* = 14.6, 9.9, 7.9 Hz, 1H), 4.79 (d, *J* = 7.8 Hz, 1H), 4.73 (d, *J* = 12.7 Hz, 1H), 4.66 – 4.55 (m, 3H), 4.50 (d, *J* = 12.7 Hz, 1H), 4.34 (s, 2H), 4.19 – 4.09 (m, 1H), 4.04 – 3.91 (m, 2H), 3.89 – 3.76 (m, 3H), 3.61 (tdd, *J* = 9.8, 4.5, 2.0 Hz, 2H), 3.52 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.74, 165.59, 165.26, 165.24, 137.15, 137.14, 136.74, 133.26, 133.23, 133.19, 133.10, 129.88, 129.85, 129.76, 129.38, 129.32, 129.24, 128.44, 128.43, 128.41, 128.35, 128.33, 128.31, 128.26, 128.03, 127.86, 127.83, 127.77, 101.22, 99.14, 75.99, 75.59, 75.51, 75.09, 74.92, 74.84, 74.69, 74.60, 72.13, 71.86, 70.30, 68.41, 61.66; m/z (HRMS+) 1051.345 $[M + Na]^+$ ($C_{61}H_{56}O_{15}Na$ requires 1051.351).

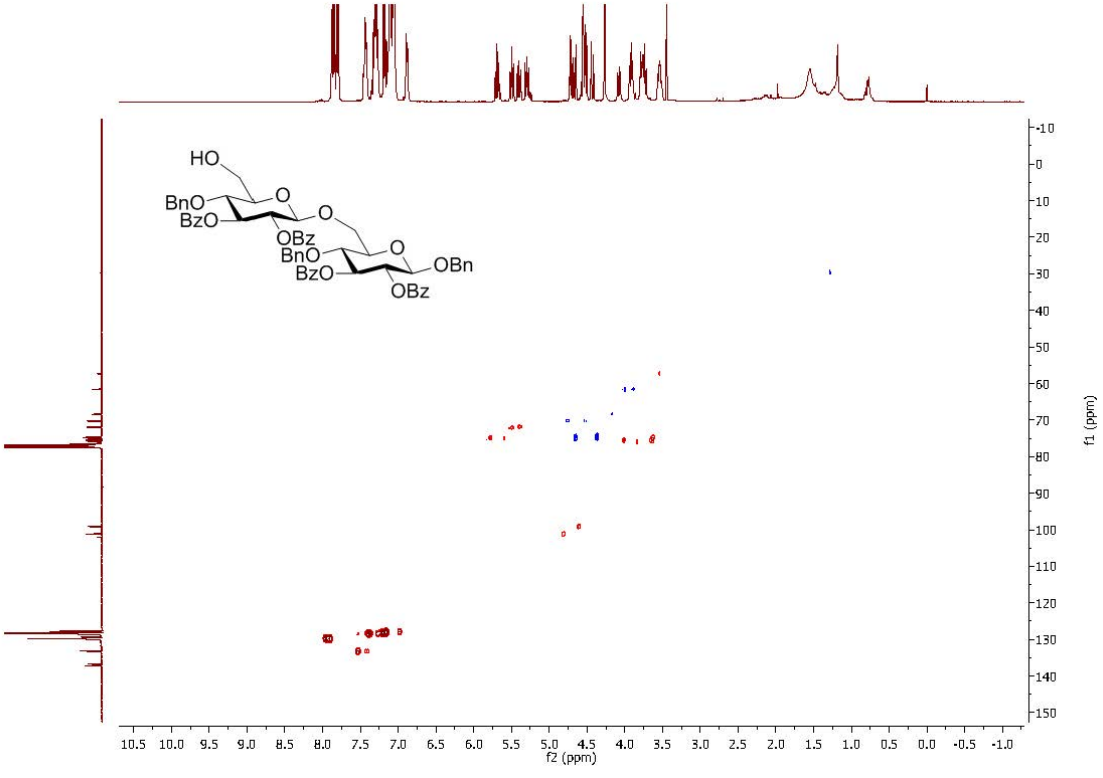
^{1}H NMR of S7 (400 MHz, $CDCl_3$)



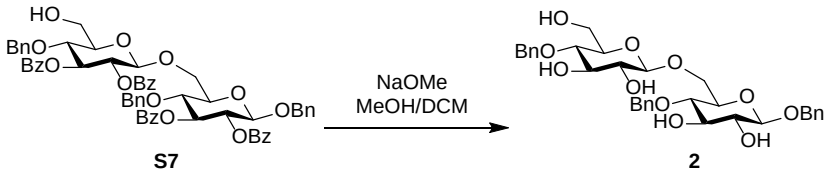
^{13}C NMR of S7 (101 MHz, $CDCl_3$)



HSQC NMR of S7 ($CDCl_3$)



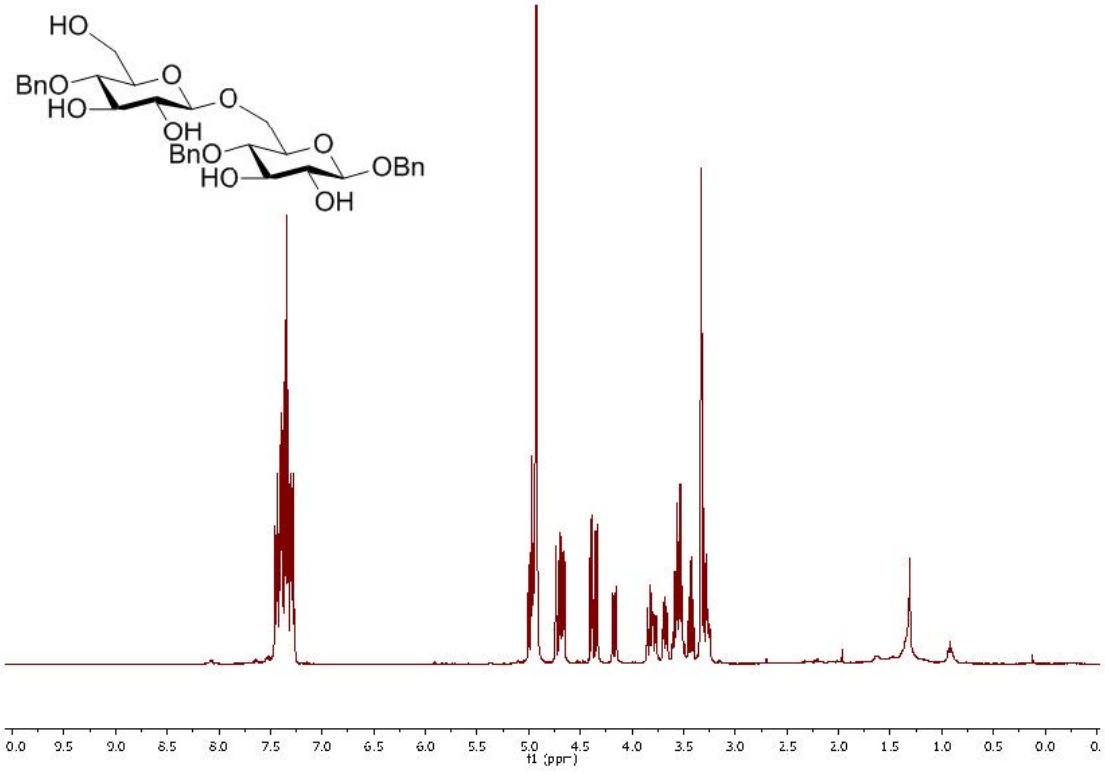
Synthesis of 2



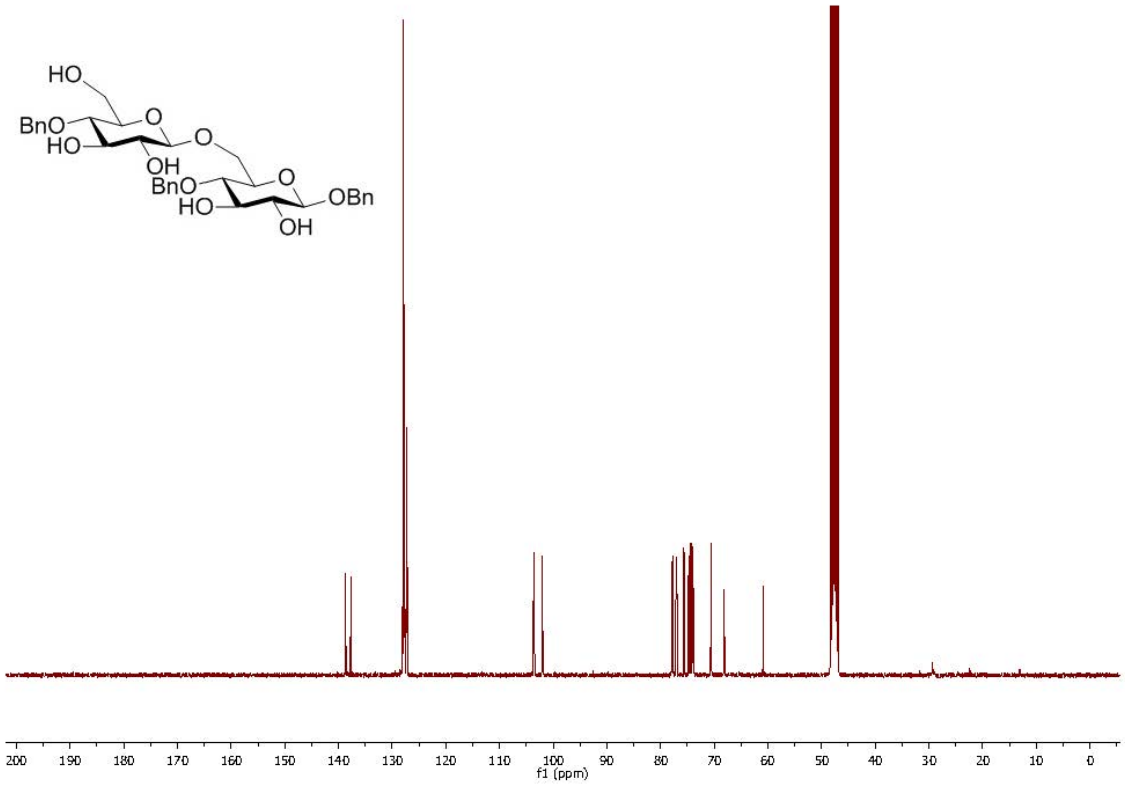
S7 2

S7 (196 mg, 0.191 mmol) was dissolved in MeOH: DCM (15 mL,1:1). NaOMe in MeOH (0.5 M, 3equiv. per benzoyl ester) was added and the solution was stirred at room temperature for 12 h, neutralized with Amberlite IR-120 (H^+ form) resin, filtered and concentrated *in vacuo*. The resulting yellow oil was purified by column chromatography (DCM: MeOH = 15:1) to give **2** as white solid (115 mg, 98%); ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.46 – 7.26 (m, 15H), 5.02 – 4.93 (m, 4H), 4.76 – 4.63 (m, 3H), 4.37 (dd, *J* = 20.3, 7.8 Hz, 2H), 4.17 (dd, *J* = 11.5, 1.4 Hz, 1H), 3.81 (ddd, *J* = 21.8, 11.8, 3.3 Hz, 2H), 3.68 (dd, *J* = 12.0, 4.9 Hz, 1H), 3.61 – 3.47 (m, 4H), 3.43 (t, *J* = 9.3 Hz, 1H), 3.31 – 3.24 (m, 2H); ^{13}C NMR (101 MHz, Methanol-d_4) δ 138.69, 138.63, 137.69, 127.91, 127.89, 127.76, 127.73, 127.69, 127.29, 127.26, 103.60, 102.00, 77.91, 77.70, 77.10, 76.96, 75.63, 74.75, 74.35, 74.29, 73.95, 73.90, 70.56, 68.13, 60.88; m/z (HRMS+) 635.2457 [M + Na]$^+$ ($C_{33}H_{40}O_{11}Na$ requires 635.2462).

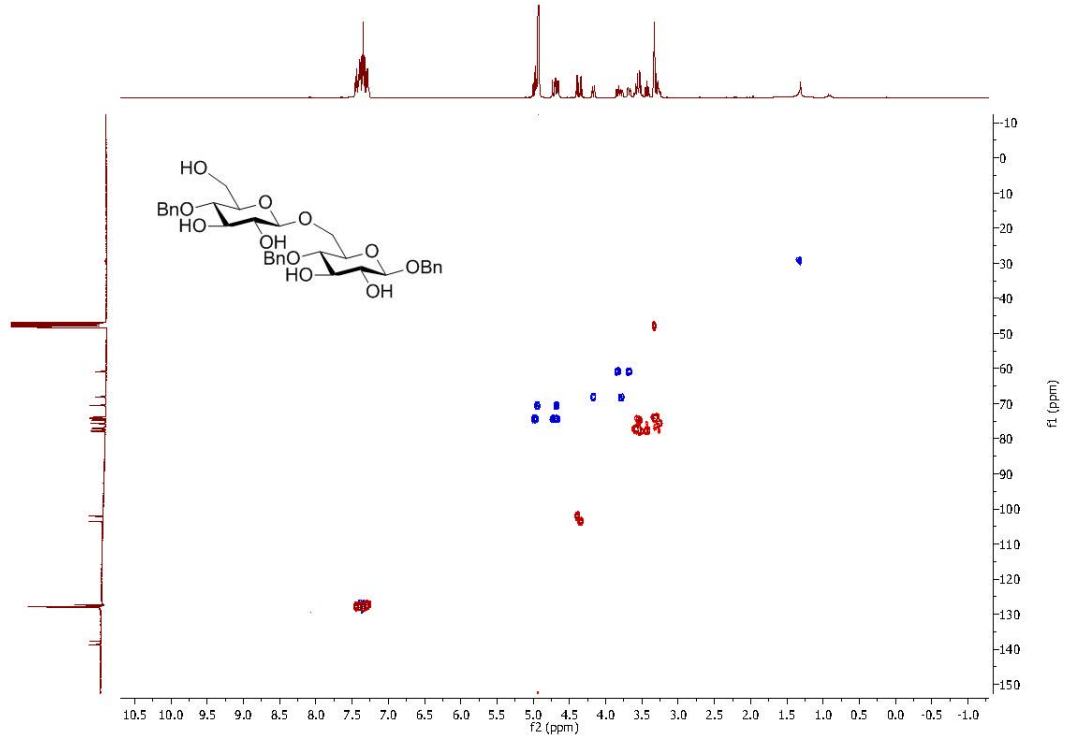
^{1}H NMR of 2 (400 MHz, Methanol-d$_4$)



^{13}C NMR of 2 (101 MHz, Methanol-d_4)

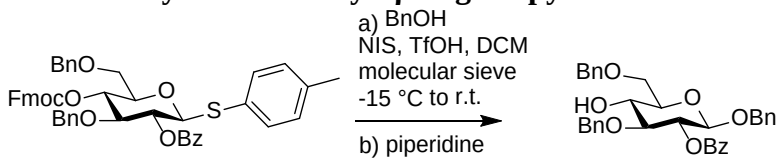


HSQC NMR of 2 (Methanol-d_4)



4.3.Synthesis of 3

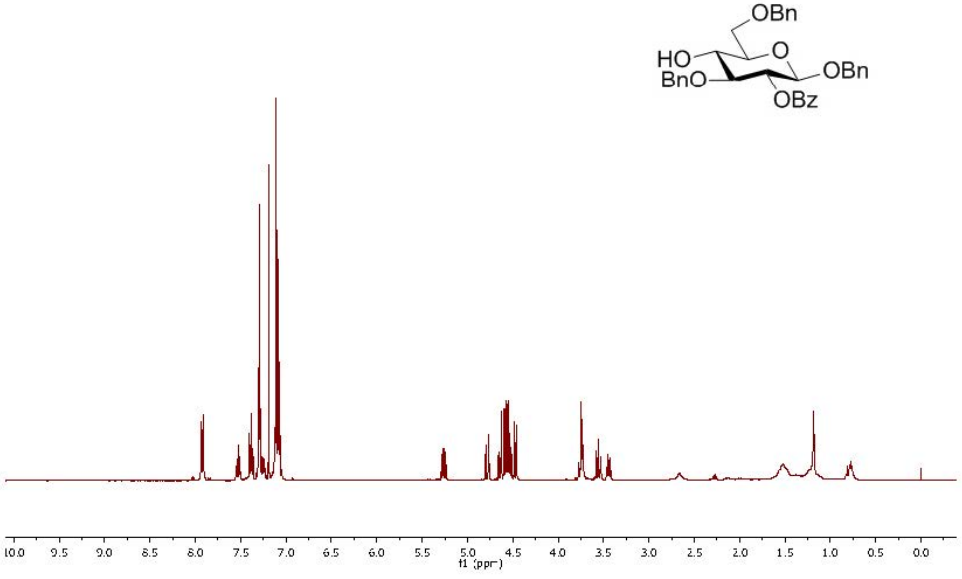
Synthesis of benzyl 2-O-benzoyl-3-6-O-benzyl- β-D-glucopyranoside S8



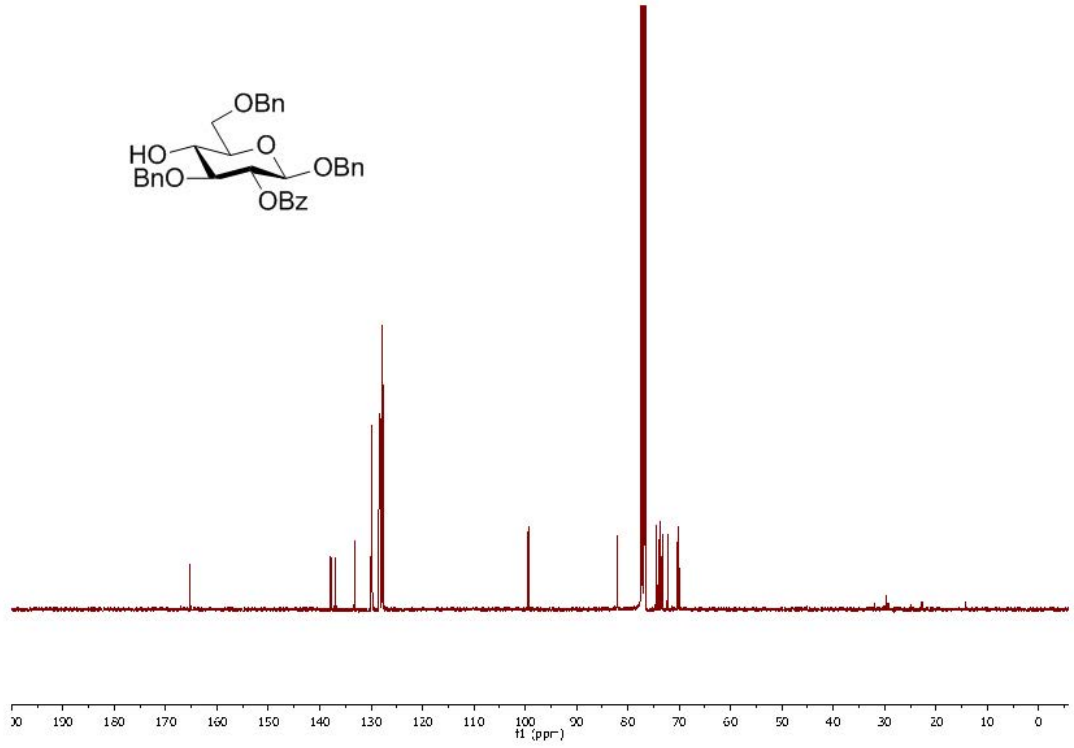
BB-3 **S8**

BB-3 (28.9mg, 0.0274 mmol, commercially available from GlycoUniverse), *N*-iodosuccinimide (7.4 mg, 0.024 mmol) and benzyl alcohol (5.9 mg, 0.055 mmol) were dissolved in anhydrous DCM (2 mL). The solution was then stirred with molecular sieve for 1h at room temperature under nitrogen atmosphere and cooled to -15 °C. A 1% solution of TfOH in DCM (10 µL) was added and the reaction was stirred for 30 min at -15 °C before the removal of cooling bath to raise the temperature to room temperature. After TLC indicated the disappearance of **BB-3**, 0.1 mL piperidine was added and the yellow solution was stirred at room temperature for additional 30 min. The reaction was diluted with DCM and washed with H_2O, then saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: EtOAc = 3:1) to give **S8** as white solid (12.3 mg, 81%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.96 – 7.86 (m, 2H), 7.57 – 7.48 (m, 1H), 7.41 – 7.35 (m, 2H), 7.32 – 7.21 (m, 5H), 7.18 – 7.02 (m, 10H), 5.32 – 5.21 (m, 1H), 4.78 (d, *J* = 12.7 Hz, 1H), 4.67 – 4.50 (m, 5H), 4.47 (d, *J* = 7.9 Hz, 1H), 3.79 – 3.68 (m, 3H), 3.62 – 3.51 (m, 1H), 3.44 (dt, *J* = 9.5, 4.8 Hz, 1H), 2.66 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.21, 137.93, 137.72, 137.04, 133.15, 129.89, 128.52, 128.42, 128.37, 128.27, 128.09, 128.03, 127.89, 127.81, 127.68, 127.65, 99.39, 82.09, 74.40, 74.08, 73.80, 73.33, 72.21, 70.35, 70.11; m/z (HRMS+) 577.2198 $[M + Na]^+$ ($C_{34}H_{34}O_7Na$ requires 577.2197).

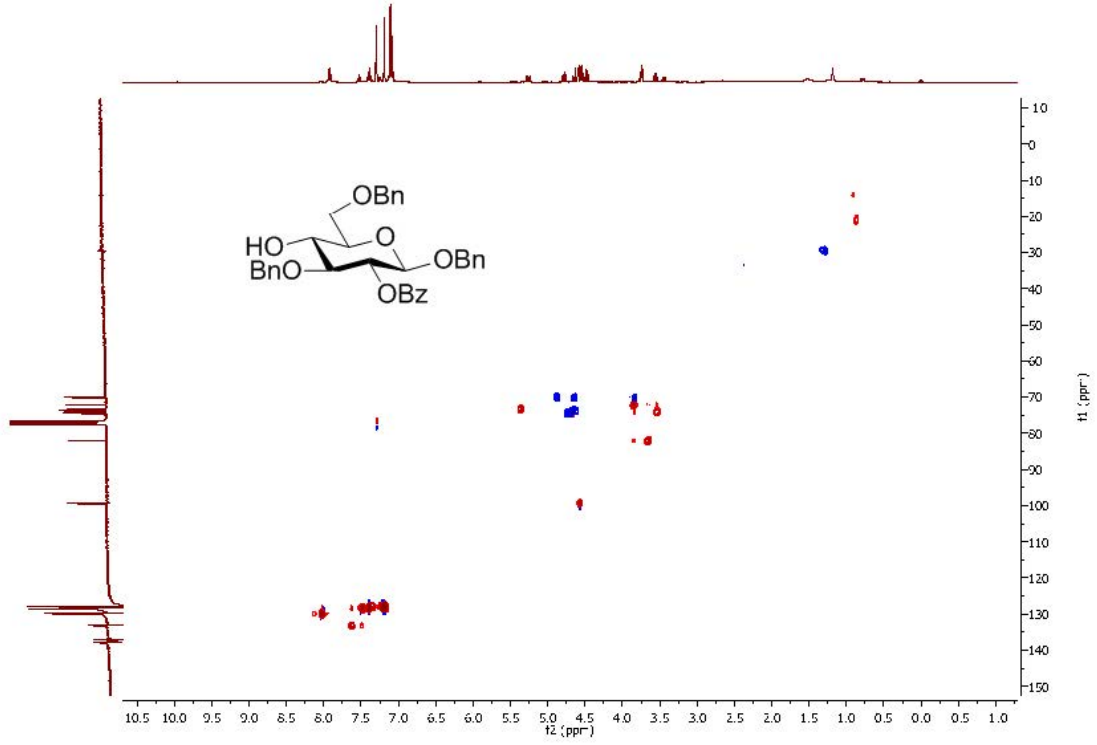
^{1}H NMR of S8 (400 MHz, $CDCl_3$)



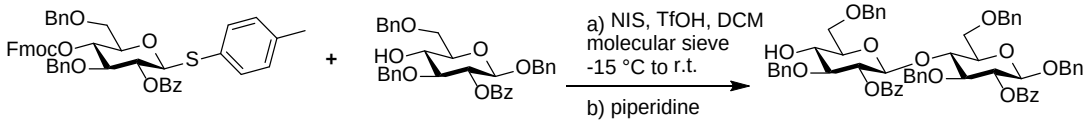
^{13}C NMR of S8 (101 MHz, $CDCl_3$)



HSQC NMR of S8 ($CDCl_3$)



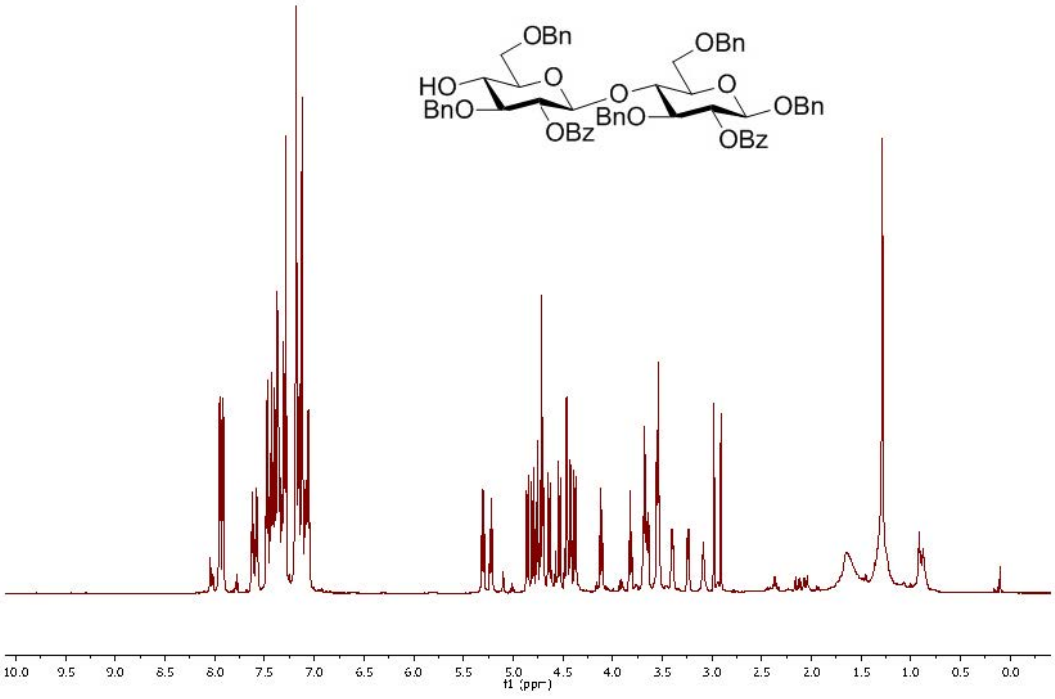
Synthesis of S9



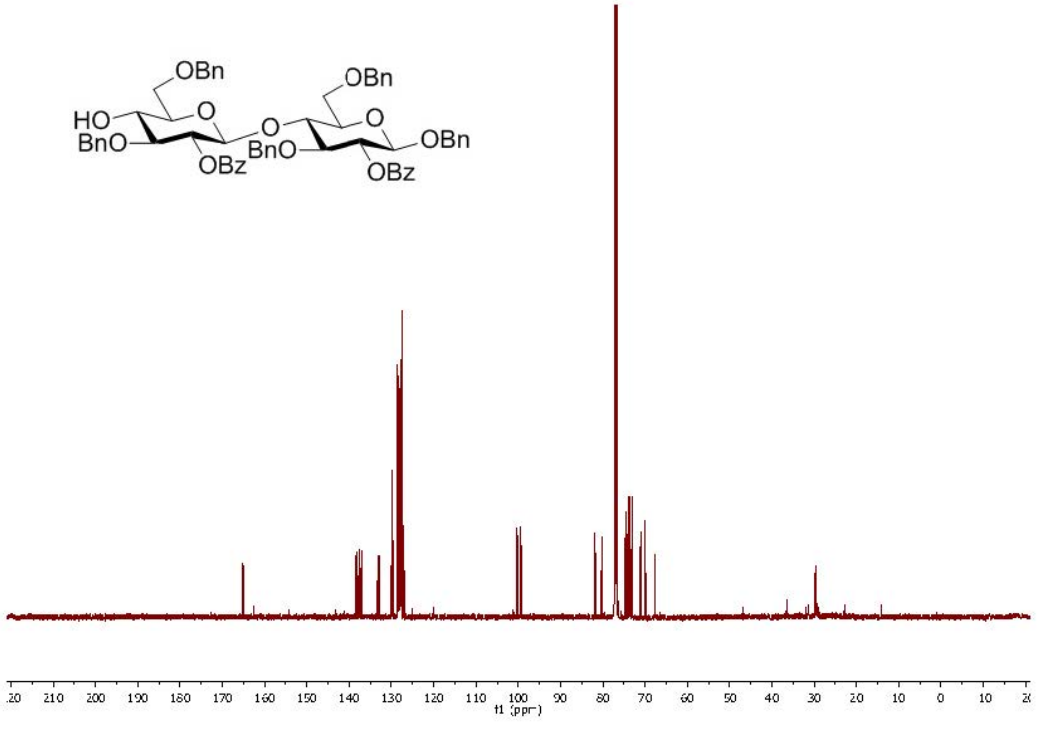
BB-3 **S8** **S9**

S8 (12.3 mg, 0.0222 mmol), **BB-3** (21.1 mg, 0.0266 mmol) and *N*-iodosuccinimide (6.0 mg, 0.027 mmol) were dissolved in anhydrous DCM (2 mL). The solution was then stirred with molecular sieve for 1h at room temperature under nitrogen atmosphere and cooled to -15 °C. A 1% solution of TfOH in DCM (10 µL) was added and the reaction was stirred for 30 min at -15 °C before the removal of cooling bath to raise the temperature to room temperature. After TLC indicated the disappearance of **S8**, 0.1 mL piperidine was added and the yellow solution was stirred at room temperature for additional 30 min. The reaction was diluted with DCM and washed with H_2O, then saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: EtOAc = 3:1) to give **S9** as white solid (18.2 mg, 82%); ^{1}H NMR (700 MHz, Chloroform-*d*) δ 7.94 (dd, *J* = 22.2, 7.7 Hz, 4H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.58 (q, *J* = 6.8, 6.2 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.37 (dddd, *J* = 45.9, 30.2, 13.5, 7.5 Hz, 14H), 7.22 – 7.17 (m, 6H), 7.12 (d, *J* = 4.5 Hz, 4H), 7.07 (dt, *J* = 14.2, 6.9 Hz, 3H), 5.30 (t, *J* = 8.6 Hz, 1H), 5.22 (t, *J* = 8.8 Hz, 1H), 4.86 (d, *J* = 11.7 Hz, 1H), 4.81 (d, *J* = 12.7 Hz, 1H), 4.76 (d, *J* = 11.6 Hz, 1H), 4.74 – 4.68 (m, 3H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.53 (d, *J* = 12.7 Hz, 1H), 4.50 – 4.44 (m, 2H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.38 (d, *J* = 12.2 Hz, 1H), 4.12 (t, *J* = 9.2 Hz, 1H), 3.82 (t, *J* = 9.1 Hz, 1H), 3.72 – 3.62 (m, 3H), 3.57 – 3.51 (m, 3H), 3.40 (dt, *J* = 10.2, 5.3 Hz, 1H), 3.24 (dq, *J* = 9.0, 3.6, 3.0 Hz, 1H), 3.09 (s, 1H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 165.12, 164.90, 138.55, 138.13, 138.10, 137.50, 137.11, 133.24, 132.90, 130.02, 129.84, 129.78, 129.68, 128.55, 128.52, 128.49, 128.29, 128.21, 128.19, 128.10, 127.96, 127.94, 127.88, 127.79, 127.71, 127.61, 127.55, 127.10, 100.26, 99.38, 81.82, 80.28, 76.49, 74.77, 74.39, 74.35, 73.95, 73.78, 73.64, 73.59, 73.10, 71.08, 70.01, 67.63; m/z (HRMS+) 1023.391 $[M + Na]^+$ ($C_{61}H_{60}O_{13}Na$ requires 1023.393).

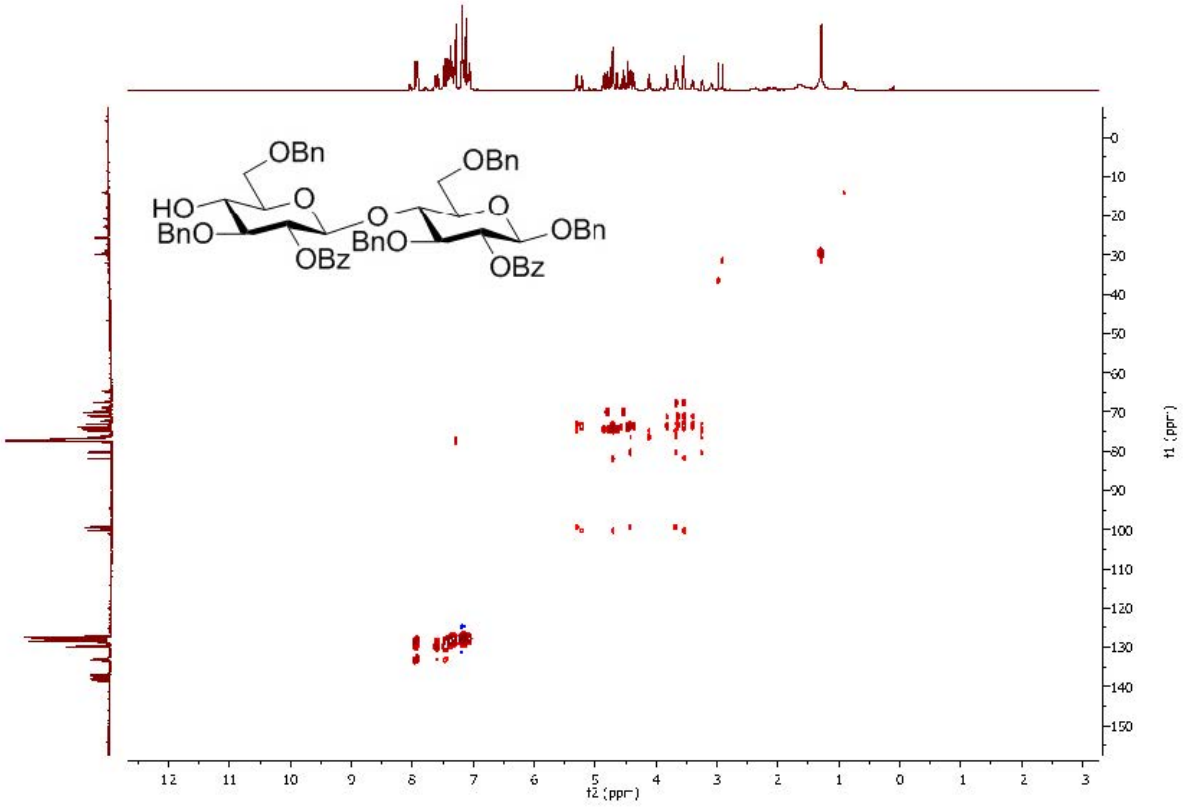
^{1}H NMR of S9 (400 MHz, $CDCl_3$)



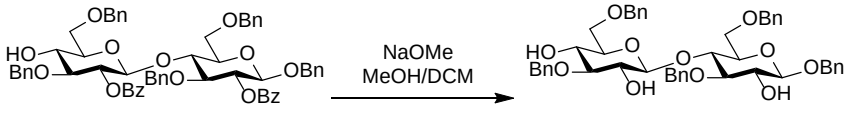
^{13}C NMR of S9 (101 MHz, $CDCl_3$)



HSQC NMR of S9 ($CDCl_3$)



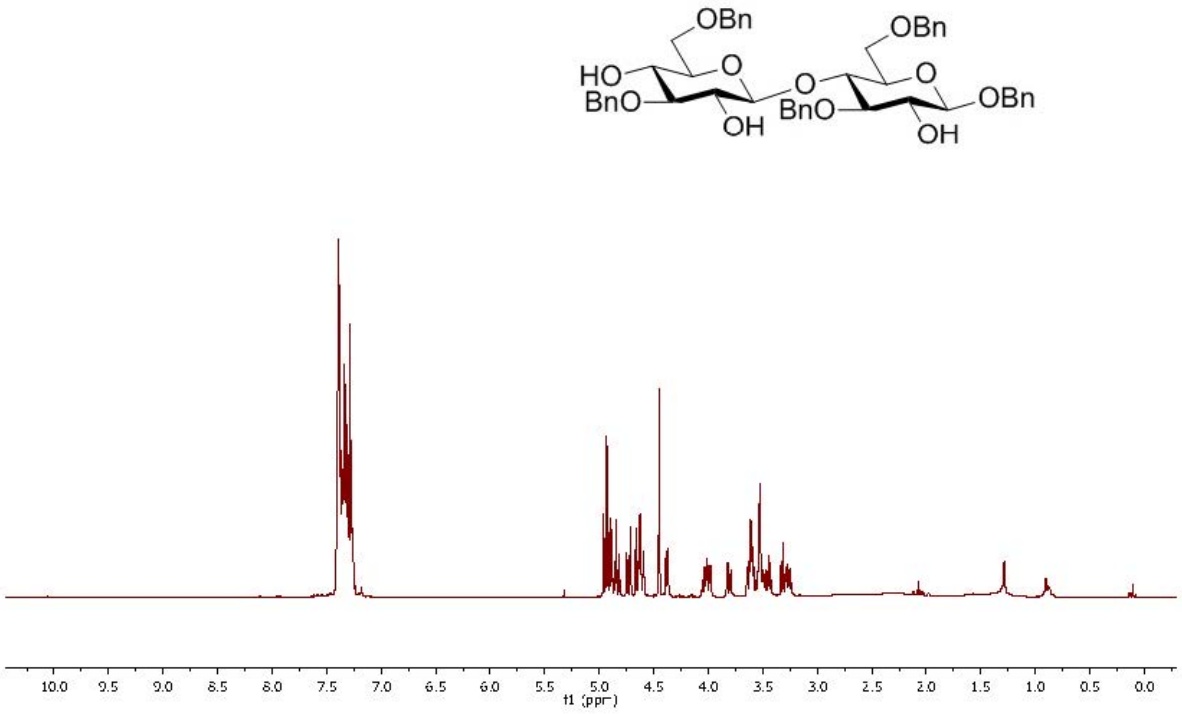
Synthesis of 3



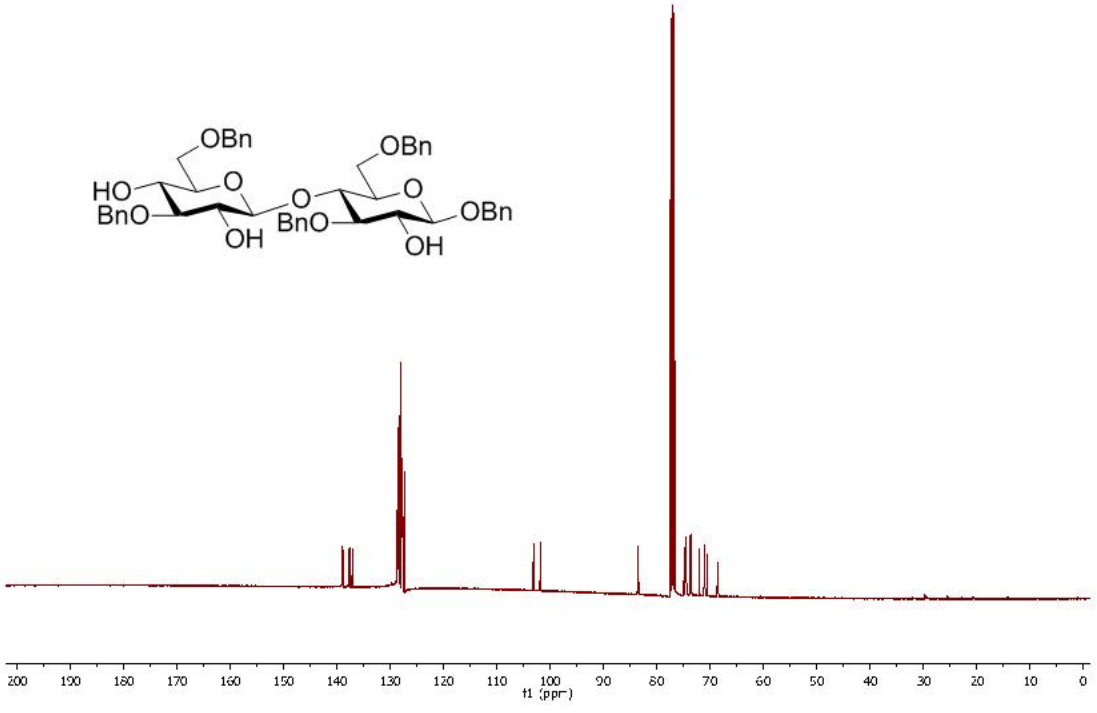
S9 **3**

S9 (18.2 mg, 0.0182 mmol) was dissolved in MeOH: DCM (1.5 mL,1:1). NaOMe in MeOH (0.5 M, 3eq per benzoyl ester) was added and the solution was stirred at room temperature for 12 h and concentrated *in vacuo*. The resulting yellow oil was purified by column chromatography (hexane: acetone = 3:1) to give **3** as white solid (12.5 mg, 87%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.45 – 7.24 (m, 25H), 5.01 – 4.79 (m, 5H), 4.73 (d, *J* = 12.1 Hz, 1H), 4.67 – 4.53 (m, 3H), 4.45 (s, 2H), 4.38 (d, *J* = 7.0 Hz, 1H), 4.08 – 3.94 (m, 2H), 3.81 (dd, *J* = 11.5, 2.2 Hz, 1H), 3.67 – 3.55 (m, 3H), 3.55 – 3.41 (m, 4H), 3.36 – 3.21 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.95, 138.70, 137.67, 137.53, 137.07, 128.56, 128.53, 128.46, 128.42, 128.33, 128.11, 128.06, 128.03, 127.93, 127.85, 127.77, 127.70, 127.45, 127.30, 103.11, 101.82, 83.55, 83.40, 77.26, 74.88, 74.68, 74.60, 74.57, 74.49, 73.70, 73.65, 73.60, 72.03, 71.10, 70.60, 68.58; m/z (HRMS+) 815.3397 $[M + Na]^+$ ($C_{47}H_{52}O_{11}Na$ requires 815.3402).

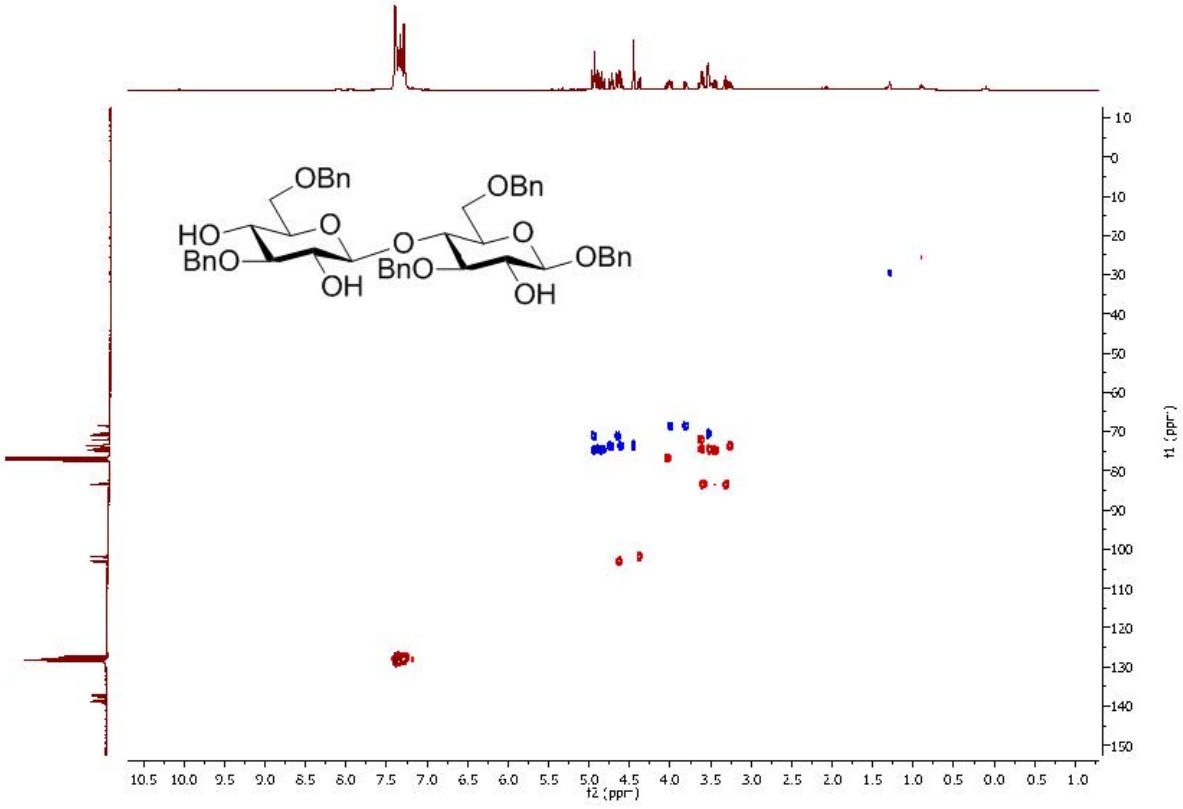
^{1}H NMR of 3 (400 MHz, $CDCl_3$)



^{13}C NMR of 3 (101 MHz, $CDCl_3$)

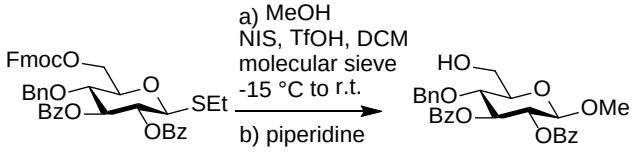


HSQC NMR of 3 ($CDCl_3$)



4.4.Synthesis of 7

Synthesis of methyl 2-O-benzoyl-3,4-O-benzyl- β-D-glucopyranoside, S10



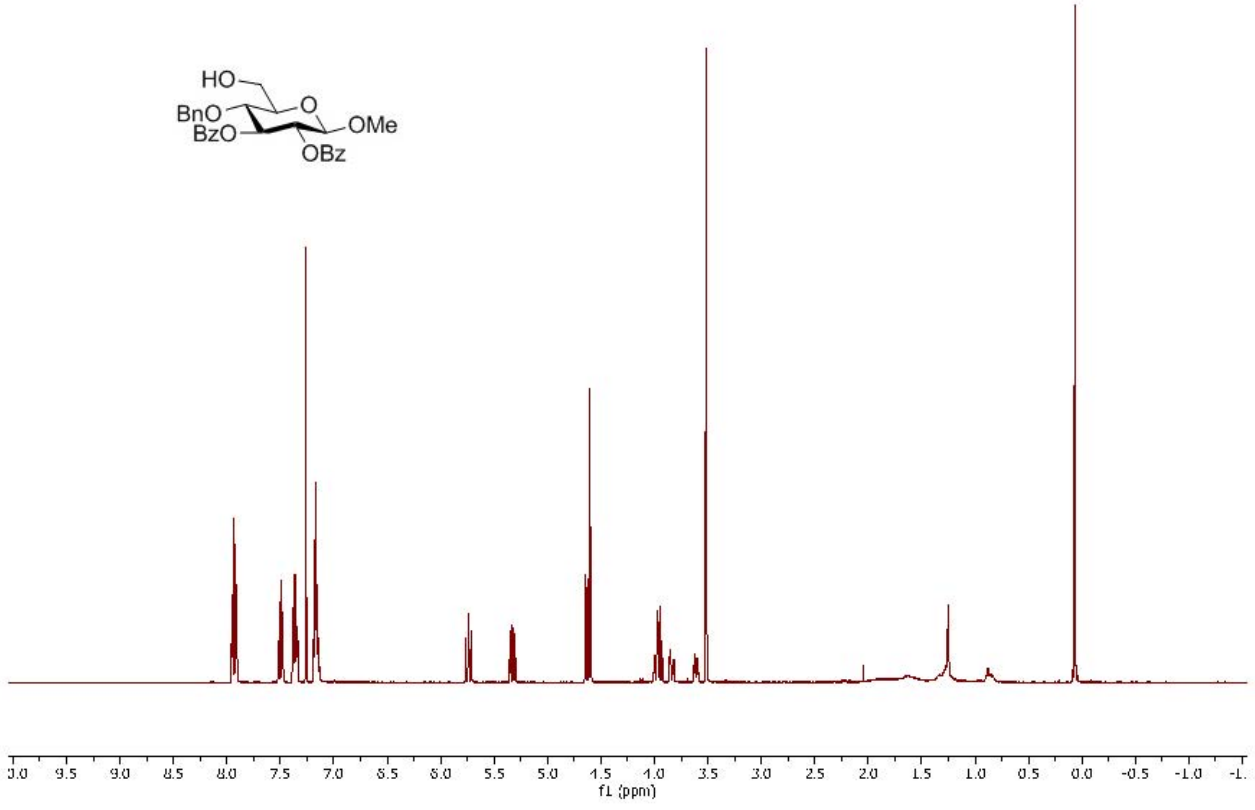
BB-4 **S10**

BB-4 was prepared following previously established procedures.[3]

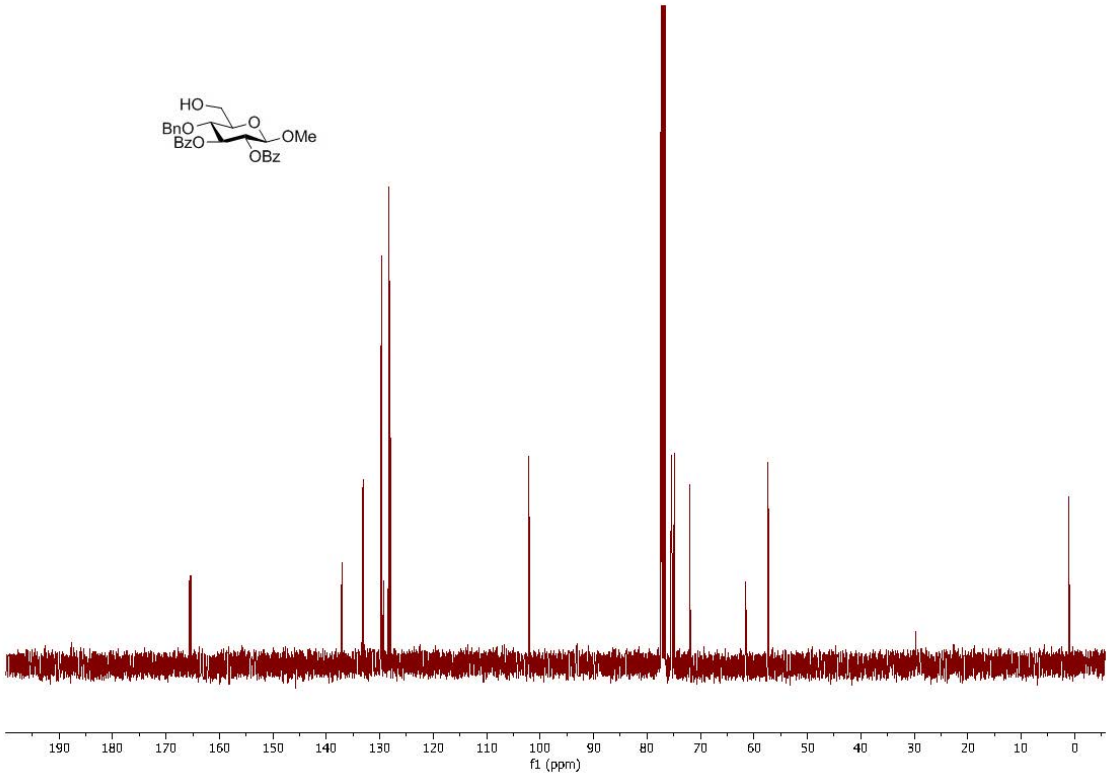
BB-4 (200 mg, 0.27 mmol), *N*-iodosuccinimide (74.4 mg, 0.239 mmol) and MeOH (18 mg, 0.55 mmol) were dissolved in anhydrous DCM (20 mL). The solution was then stirred with molecular sieve for 1h at room temperature under nitrogen atmosphere and then cooled to -15 °C. A 1% solution of TfOH in DCM (100 µL) was added and the reaction was stirred for 30 min at -15 °C before the removal of cooling bath to raise the temperature to room temperature. After TLC indicated the disappearance of **S1**, piperidine (1 mL) was added and the yellow solution was stirred at room temperature for additional 30 min. The reaction was diluted with DCM and washed with H_2O, then saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: EtOAc = 2:1) to give **S10** as colorless oil (119 mg, 91%); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.93 (ddt, *J* = 12.2, 8.3, 1.1 Hz, 4H), 7.52 – 7.46 (m, 2H), 7.36 (td, *J* = 7.7, 3.8 Hz, 4H), 7.22 – 7.06 (m, 5H),

5.74 (t, *J* = 9.6 Hz, 1H), 5.36 – 5.30 (m, 1H), 4.66 – 4.62 (m, 1H), 4.61 (s, 2H), 4.01 – 3.93 (m, 2H), 3.84 (dd, *J* = 12.1, 3.8 Hz, 1H), 3.61 (dt, *J* = 9.7, 3.1 Hz, 1H), 3.52 (s, 3H); ^{13}C NMR (151 MHz, cdcl$_3$) δ 165.84, 165.52, 137.30, 133.31, 133.25, 129.96, 129.86, 129.53, 129.52, 128.52, 128.51, 128.44, 128.34, 128.33, 128.12, 102.21, 75.67, 75.56, 75.11, 74.98, 72.18, 61.68, 57.43; m/z (HRMS+) 515.1687 $[M + Na]^+$ ($C_{28}H_{28}O_8Na$ requires 515.1676).

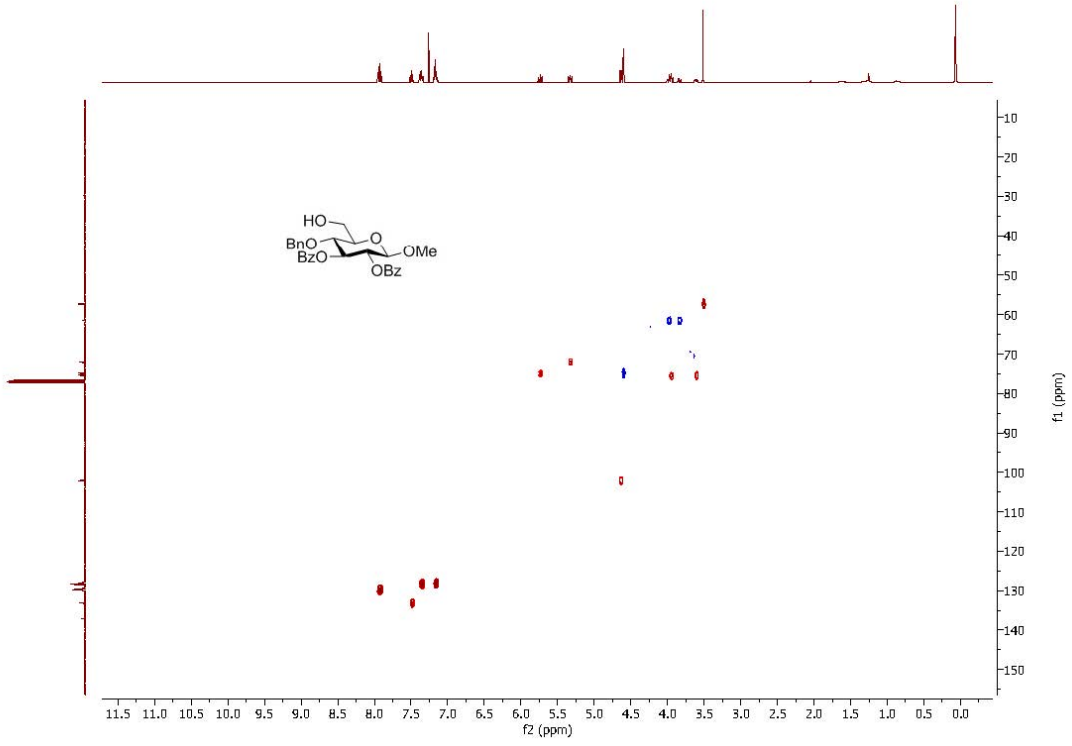
^{1}H NMR of S10 (400 MHz, $CDCl_3$)



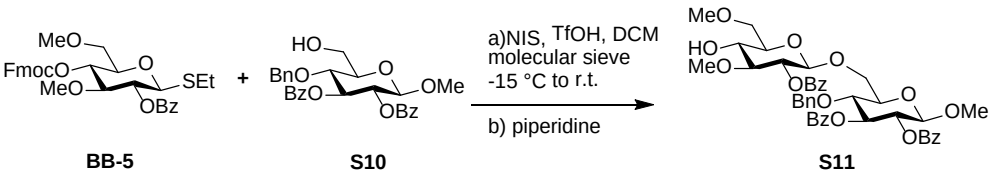
^{13}C NMR of S10 (101 MHz, $CDCl_3$)



HSQC NMR of S4 ($CDCl_3$)

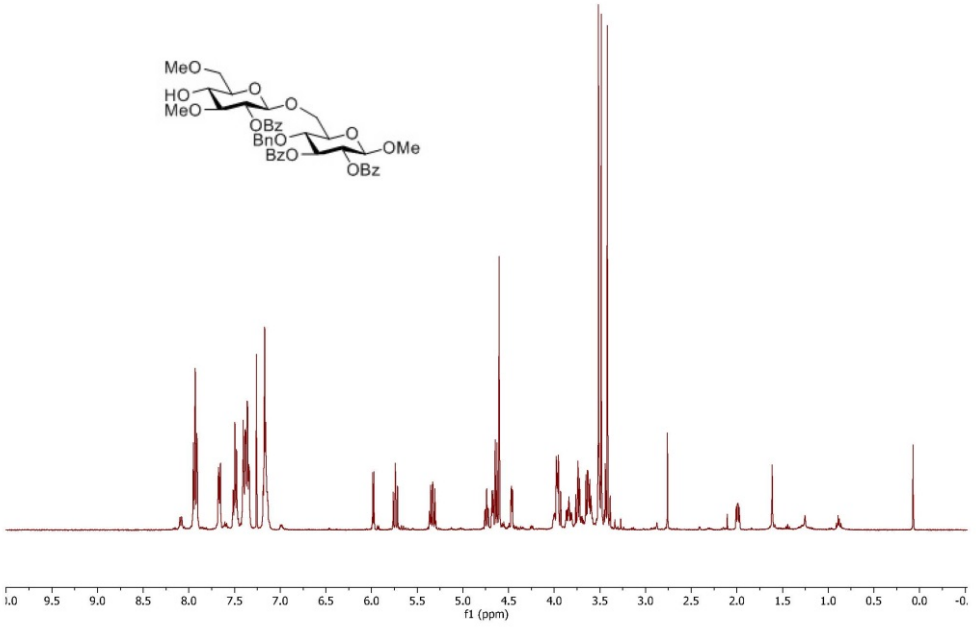


Synthesis of S11

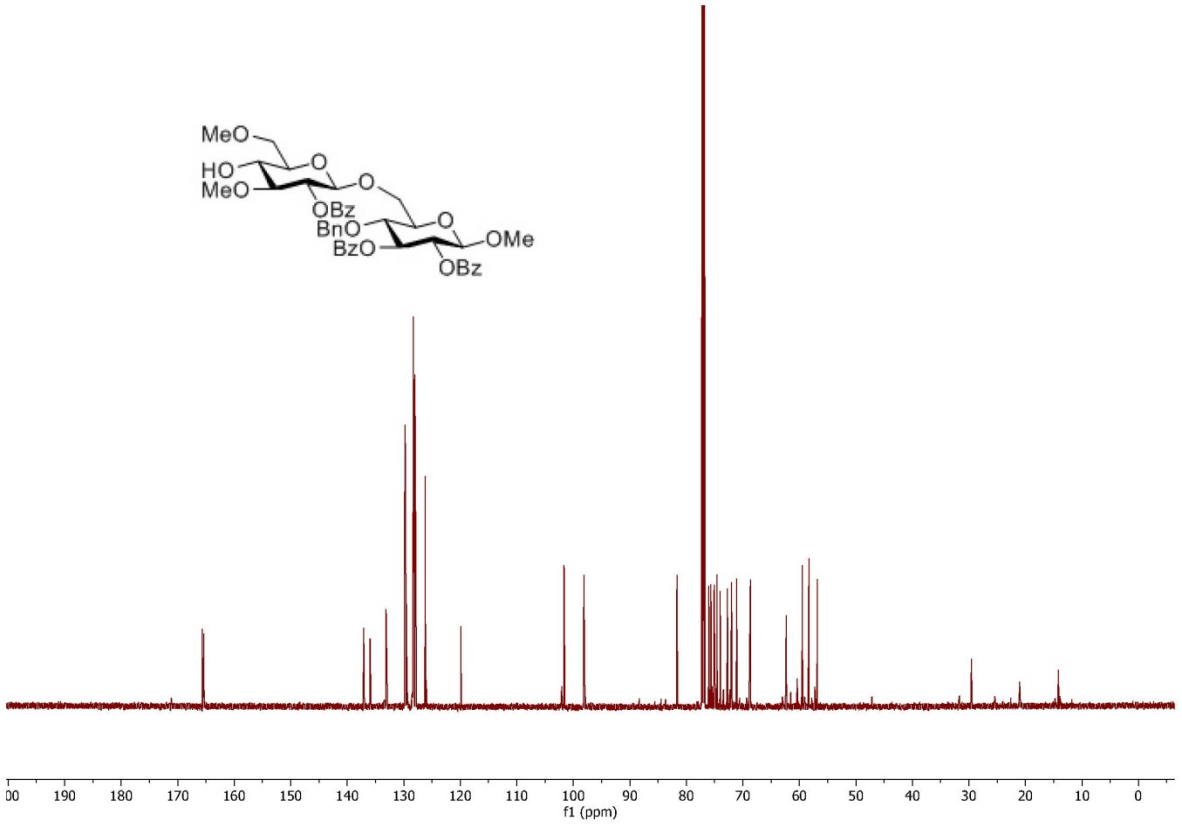


S10 (90.0 mg, 0.188 mmol), **BB-5** (131.6 mg, 0.226 mmol) and *N*-iodosuccinimide (51.9 mg, 0.226 mmol) were dissolved in anhydrous DCM (20 mL). The solution was stirred with molecular sieve for 1h at room temperature under nitrogen atmosphere and then cooled to -15 °C. A 1% solution of TfOH in DCM (90 µL) was added and the reaction was stirred for 30 min at -15 °C before the removal of cooling bath to raise the temperature to room temperature. After TLC indicated the disappearance of **S10**, 1 mL piperidine was added and the yellow solution was stirred at room temperature for additional 30 min. The reaction was diluted with DCM and washed with H_2O, then saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: EtOAc = 1:1.5) to give **S11** as white solid (125 mg, 78%).^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.92 (ddd, *J* = 13.7, 8.3, 1.4 Hz, 4H), 7.71 – 7.65 (m, 2H), 7.48 (ddt, *J* = 7.4, 5.7, 1.7 Hz, 2H), 7.43 – 7.38 (m, 3H), 7.35 (ddd, *J* = 8.2, 4.1, 2.9 Hz, 4H), 7.18 – 7.09 (m, 3H), 7.00 (dd, *J* = 7.8, 1.7 Hz, 2H), 5.93 (d, *J* = 5.2 Hz, 1H), 5.70 – 5.65 (m, 1H), 5.32 (dd, *J* = 9.8, 7.9 Hz, 1H), 4.59 – 4.54 (m, 2H), 4.52 – 4.44 (m, 2H), 3.84 (t, *J* = 9.4 Hz, 1H), 3.77 (dd, *J* = 10.5, 2.0 Hz, 1H), 3.70 (td, *J* = 4.3, 2.8 Hz, 2H), 3.67 – 3.62 (m, 2H), 3.58 (ddd, *J* = 10.0, 7.5, 4.2 Hz, 2H), 3.51 (q, *J* = 1.6 Hz, 1H), 3.50 (s, 3H), 3.49 (s, 3H), 3.39 (s, 3H), 2.75 (s, 1H); ^{13}C NMR (151 MHz, cdcl$_3$) δ 165.79, 165.53, 165.51, 137.24, 136.11, 133.28, 133.18, 129.96, 129.86, 129.85, 129.66, 129.60, 129.54, 128.56, 128.52, 128.51, 128.50, 128.44, 128.41, 128.19, 127.99, 126.39, 120.05, 101.79, 98.28, 81.82, 76.17, 75.80, 75.24, 74.77, 74.16, 72.89, 72.16, 71.26, 68.82, 62.51, 59.63, 58.46, 56.98; m/z (HRMS+) 809.2792 $[M + Na]^+$ ($C_{43}H_{46}O_{14}Na$ requires 809.2780).

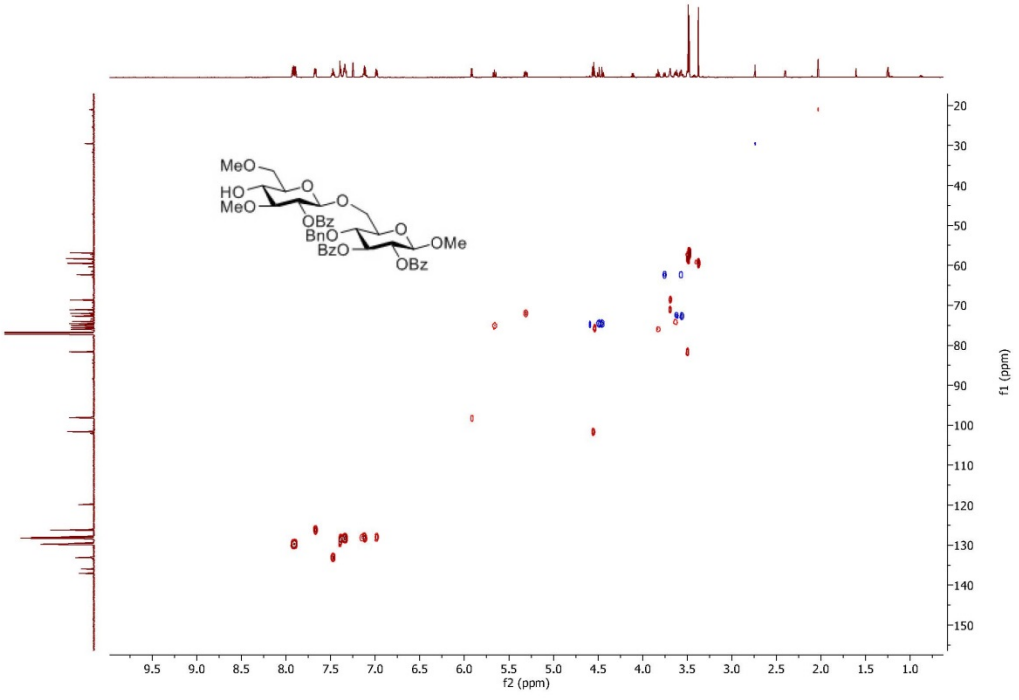
^{1}H NMR of S11 (400 MHz, $CDCl_3$)



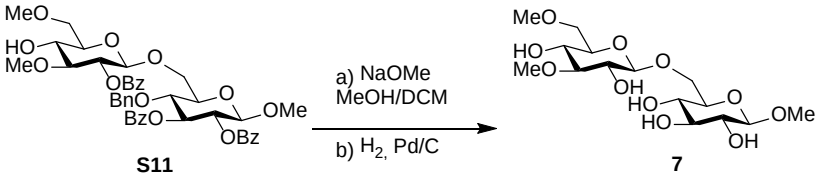
^{13}C NMR of S5 (101 MHz, $CDCl_3$)



HSQC NMR of S5 ($CDCl_3$)



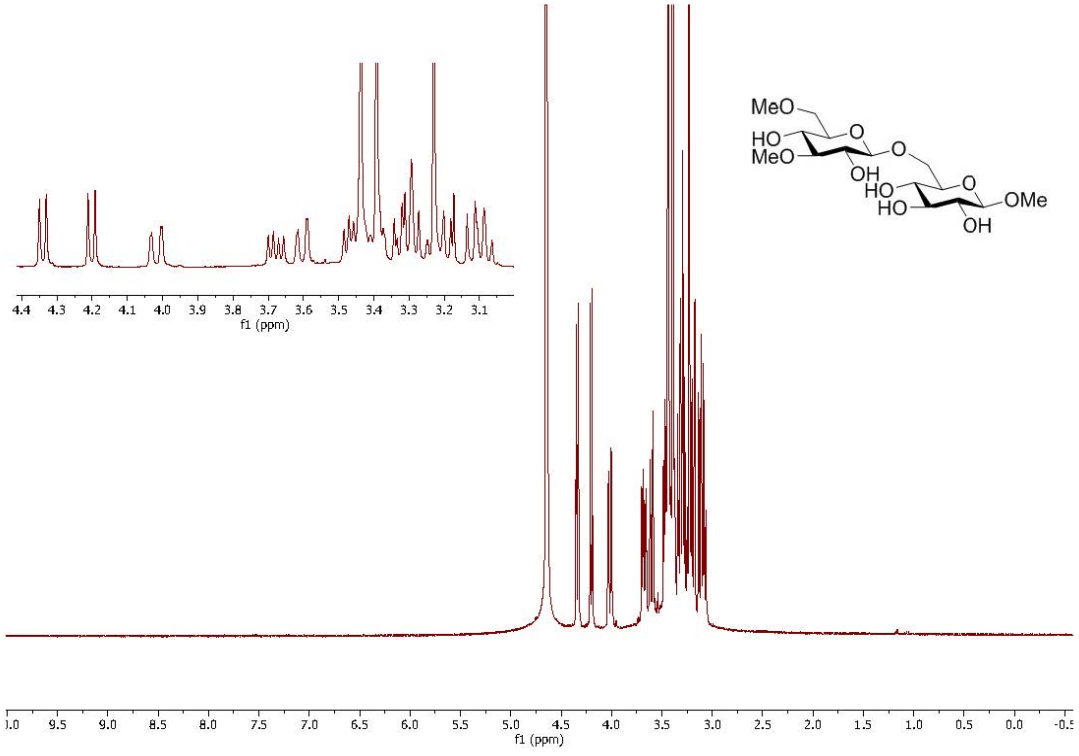
Synthesis of 7



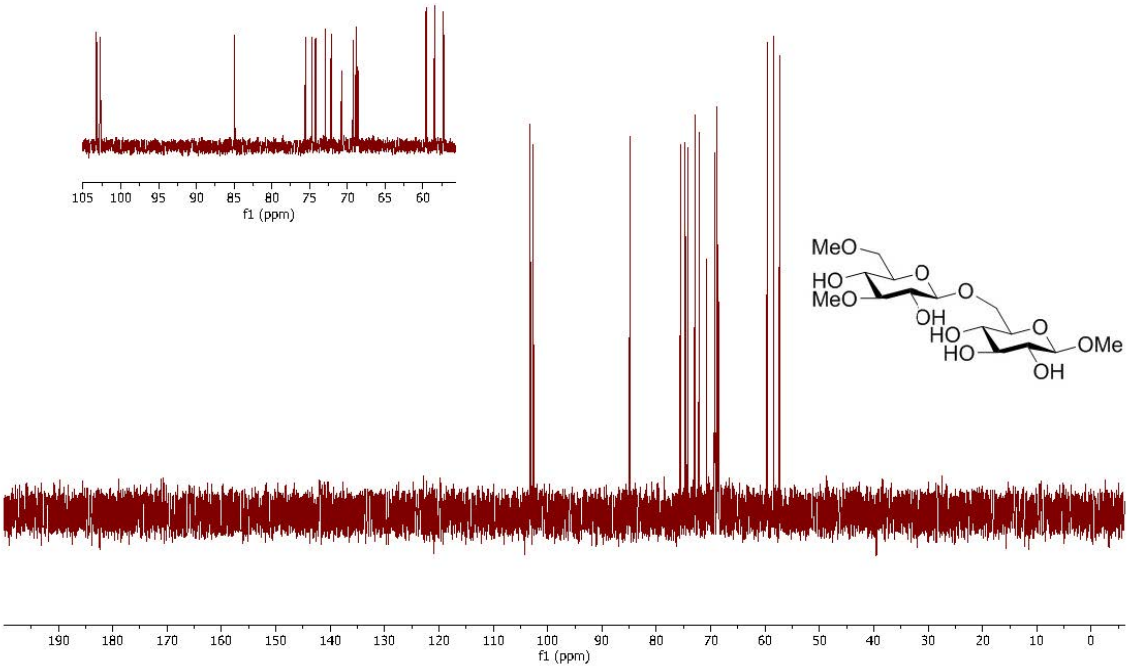
S11 **7**

S11 (125 mg, 0.106 mmol) was dissolved in MeOH: DCM (1.5 mL,1:1). NaOMe in MeOH (0.5 M, 3 equiv. per benzoyl ester) was added and the solution was stirred at room temperature for 12 h, neutralized with Amberlite IR-120 (H^+ form) resin, filtered and concentrated *in vacuo*. The crude compound was dissolved in 2 mL of *t*BuOH:H_2O (1:1). 100% by weight Pd-C (10%) was added and the reaction was stirred in H_2 bomb with 60 psi pressure for 10 minutes. The reactions were filtered through celite, washed with MeOH. The filtrates were concentrated *in vacuo.* The resulting yellow oil was purified by C18 silica column chromatography (H_2O: MeOH = 10:1) to give **7** as white solid (45.8 mg, 75%); ^{1}H NMR (400 MHz, Deuterium Oxide) δ 4.34 (d, *J* = 7.8 Hz, 1H), 4.20 (d, *J* = 8.0 Hz, 1H), 4.02 (dd, *J* = 11.7, 2.0 Hz, 1H), 3.68 (dd, *J* = 11.7, 5.8 Hz, 1H), 3.60 (dd, *J* = 11.1, 1.9 Hz, 1H), 3.49 – 3.45 (m, 1H), 3.44 (s, 3H), 3.41 (d, *J* = 1.9 Hz, 1H), 3.39 (s, 3H), 3.37 (d, *J* = 1.8 Hz, 1H), 3.35 – 3.25 (m, 3H), 3.23 (s, 3H), 3.19 (dd, *J* = 10.4, 2.5 Hz, 1H), 3.15 – 3.05 (m, 2H); ^{13}C NMR (101 MHz, d_2o) δ 103.23, 102.69, 84.92, 75.53, 74.71, 74.22, 72.91, 72.16, 70.76, 69.27, 68.87, 68.61, 59.58, 58.46, 57.27; m/z (HRMS+) 407.1528 $[M + Na]^+$ ($C_{15}H_{28}O_{11}Na$ requires 407.1524).

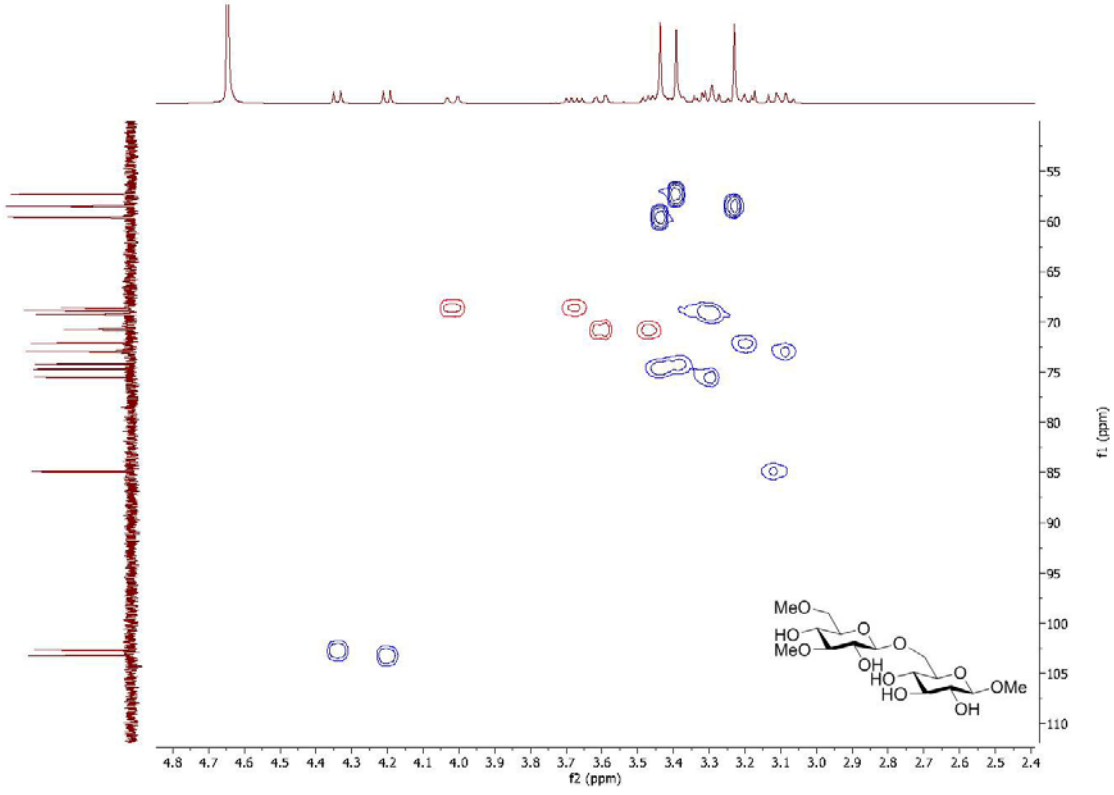
^{1}H NMR of 7 (400 MHz, D_2O)



^{13}C NMR of 7 (101 MHz, D_2O)

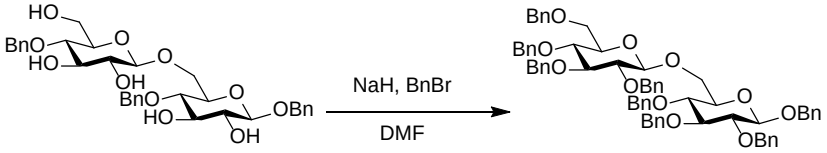


HSQC NMR of 7 (D_2O)



5. Synthesis of fully functionalized dimers

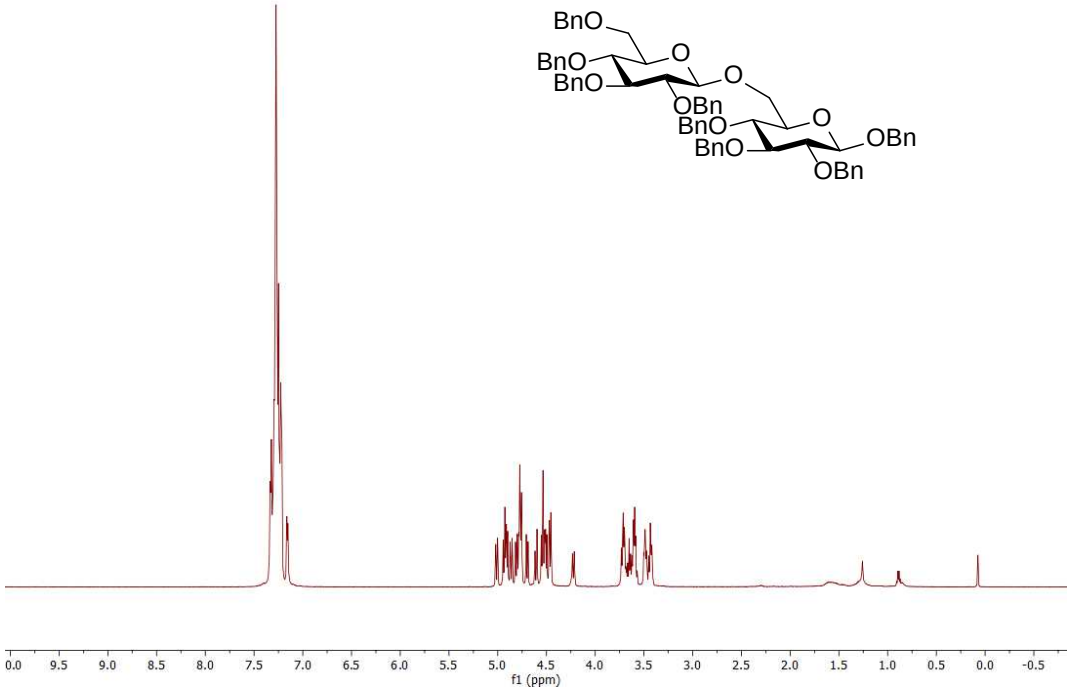
5.1. Synthesis of 8



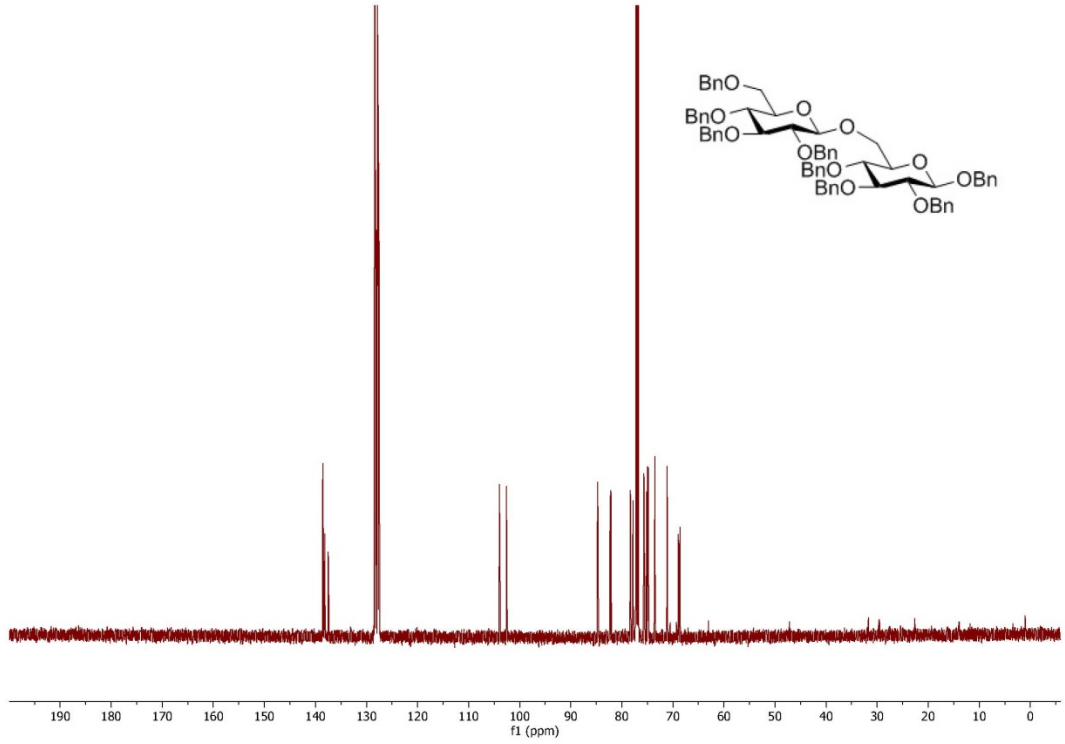
2 **8**

2 (25.0 mg, 0.0408 mmol) and benzyl bromide (52.0 mg, 0.306 mmol) were dissolved in 2 mL DMF. NaH (7.3 mg, 0.306 mmol) was added and the solution was stirred at room temperature for 12 h. The reaction was then quenched with 0.1 mL of MeOH, diluted with DCM and washed with H_2O and saturated aqueous NaCl. The organic layers were dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: EtOAc = 4:1) to give **8** as white solid (30.5 mg, 70%); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.35 – 7.19 (m, 38H), 7.16 (d, *J* = 6.9 Hz, 2H), 5.01 (d, *J* = 11.1 Hz, 1H), 4.96 – 4.88 (m, 3H), 4.86 (d, *J* = 12.0 Hz, 1H), 4.81 (d, *J* = 10.8 Hz, 1H), 4.77 (dd, *J* = 10.9, 6.7 Hz, 4H), 4.70 (d, *J* = 10.9 Hz, 1H), 4.61 (d, *J* = 12.2 Hz, 1H), 4.57 – 4.48 (m, 4H), 4.48 – 4.43 (m, 2H), 4.22 (d, *J* = 11.3 Hz, 1H), 3.70 (ddd, *J* = 21.0, 11.1, 7.4 Hz, 3H), 3.66 – 3.55 (m, 4H), 3.49 (td, *J* = 8.4, 3.2 Hz, 2H), 3.44 (t, *J* = 9.2 Hz, 2H); ^{13}C NMR (151 MHz, cdcl$_3$) δ 138.56, 138.47, 138.37, 138.17, 138.01, 137.49, 128.40, 128.36, 128.34, 128.32, 128.14, 128.06, 127.92, 127.86, 127.76, 127.70, 127.63, 127.61, 127.59, 127.57, 103.97, 102.59, 84.75, 84.70, 82.30, 82.14, 78.31, 77.82, 75.69, 75.67, 75.16, 74.95, 74.93, 74.87, 74.84, 74.77, 73.51, 71.13, 68.92, 68.62; m/z (HRMS+) 1085.4802 $[M + Na]^+$ ($C_{68}H_{70}O_{11}Na$ requires 1085.4810).

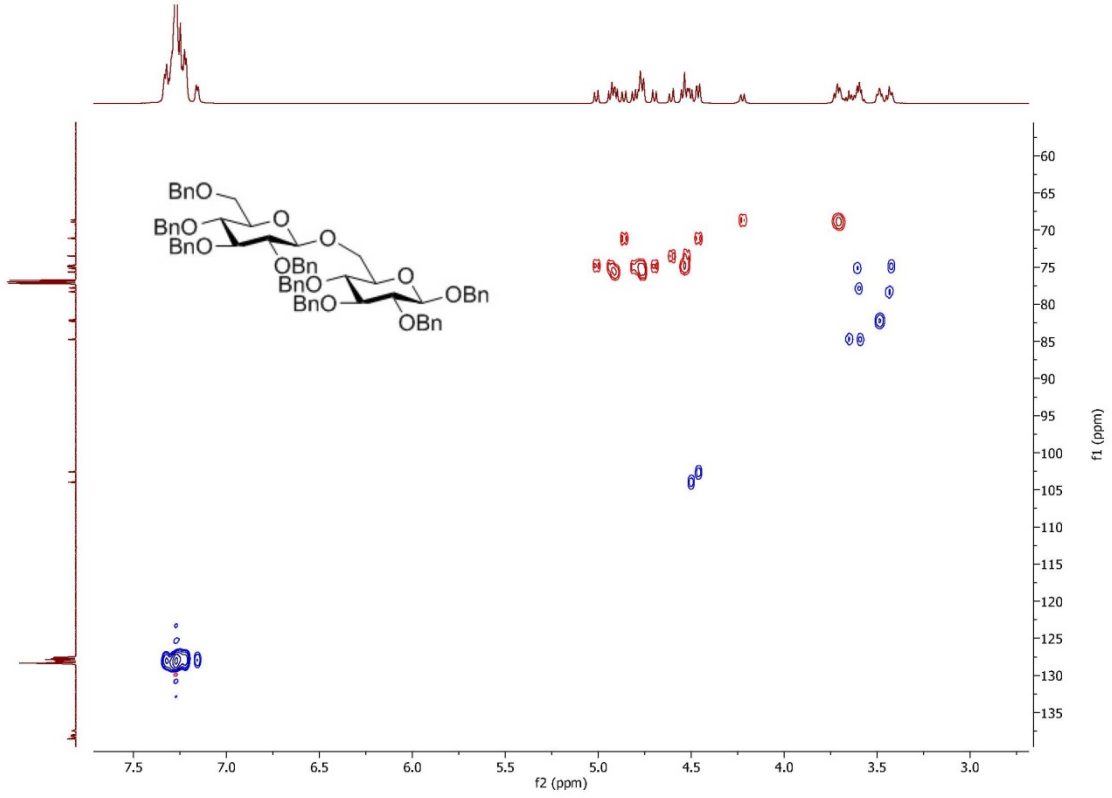
^{1}H NMR of 8 (400 MHz, $CDCl_3$)



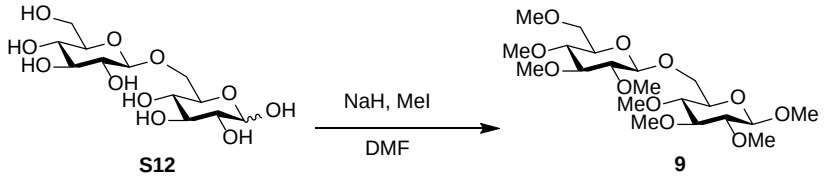
^{13}C NMR of 8 (101 MHz, $CDCl_3$)



HSQC NMR of 8 ($CDCl_3$)



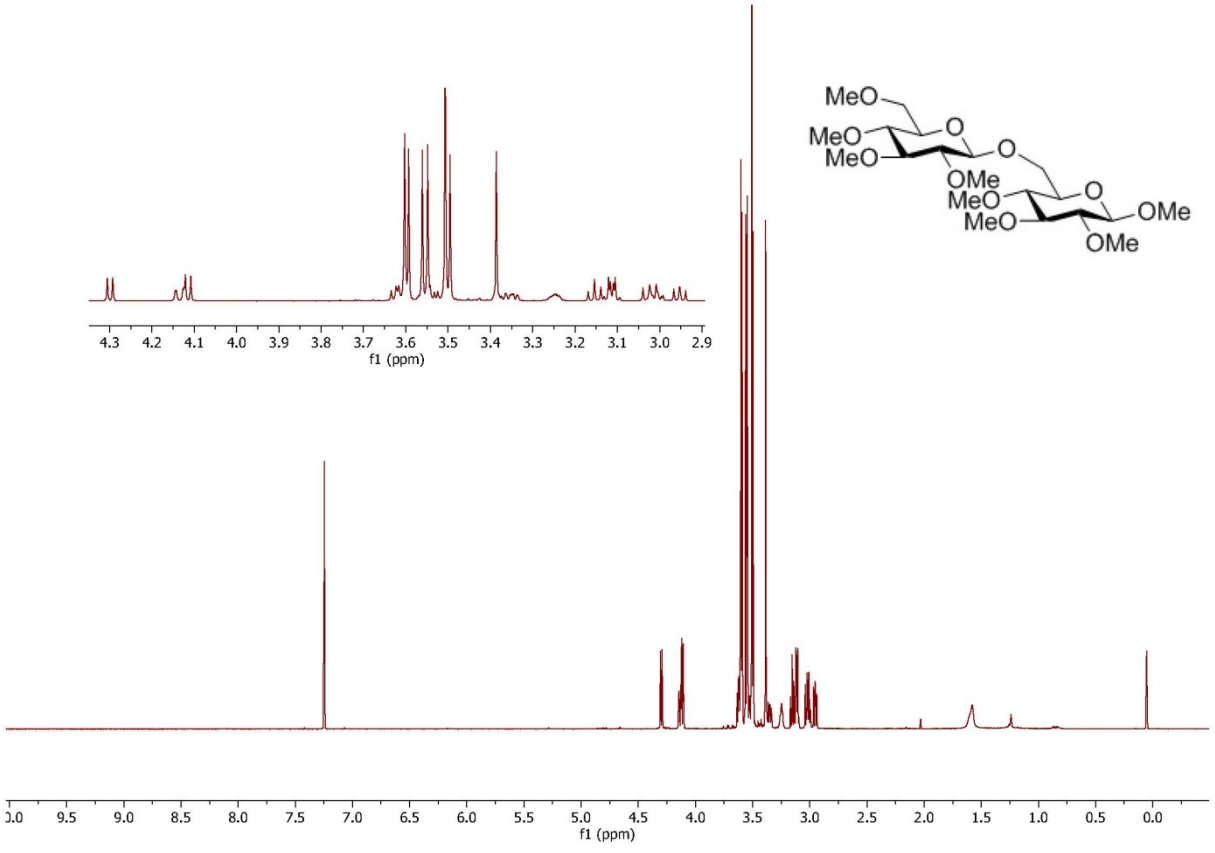
5.2. Synthesis of 9



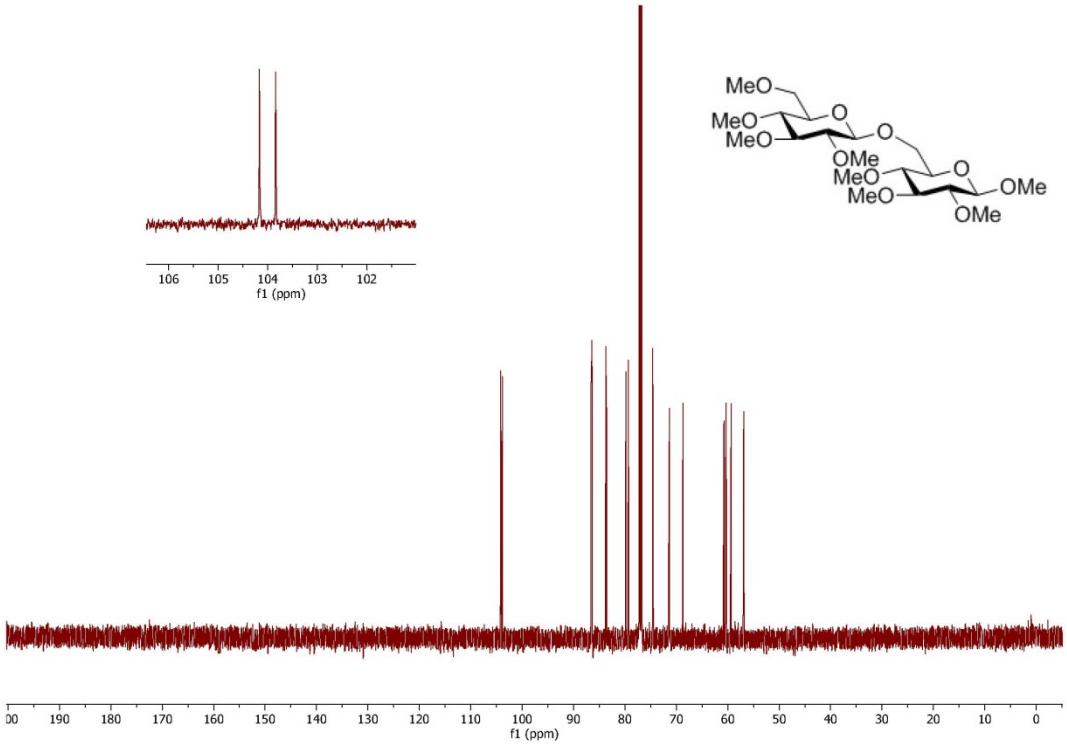
S12 **9**

S12 (25.0 mg, 0.0731 mmol) and methyl iodide (125 mg, 0.877 mmol) were dissolved in 2 mL of DMF. NaH (21 mg, 0.877 mmol) was added and the solution was stirred at room temperature for 12 h. The reaction was then quenched by 0.1 mL MeOH, diluted with DCM and washed with H_2O and saturated aqueous NaCl. The organic layers were dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: acetone: DCM = 9:3:1) to give **9** as white solid (10.5 mg, 31%); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 4.31 (d, *J* = 7.8 Hz, 1H), 4.15 (dd, *J* = 10.9, 1.9 Hz, 1H), 4.13 (d, *J* = 7.7 Hz, 1H), 3.65 – 3.62 (m, 2H), 3.62 (s, 3H), 3.61 (s, 3H), 3.57 (s, 3H), 3.56 (s, 3H), 3.56 – 3.53 (m, 1H), 3.52 (s, 6H), 3.51 (s, 3H), 3.40 (s, 3H), 3.36 (ddd, *J* = 10.0, 6.4, 1.9 Hz, 1H), 3.26 (ddt, *J* = 7.0, 4.9, 2.2 Hz, 1H), 3.17 (t, *J* = 8.9 Hz, 1H), 3.13 (dd, *J* = 6.8, 2.6 Hz, 2H), 3.06 – 3.00 (m, 2H), 2.97 (dd, *J* = 9.1, 7.7 Hz, 1H); ^{13}C NMR (151 MHz, cdcl$_3$) δ 104.17, 103.84, 86.59, 86.40, 83.73, 83.59, 79.82, 79.33, 74.60, 74.59, 71.36, 68.72, 60.77, 60.74, 60.43, 60.40, 60.35, 60.30, 59.34, 56.91; m/z (HRMS+) 477.2307 $[M + Na]^+$ ($C_{68}H_{70}O_{11}Na$ requires 477.2306).

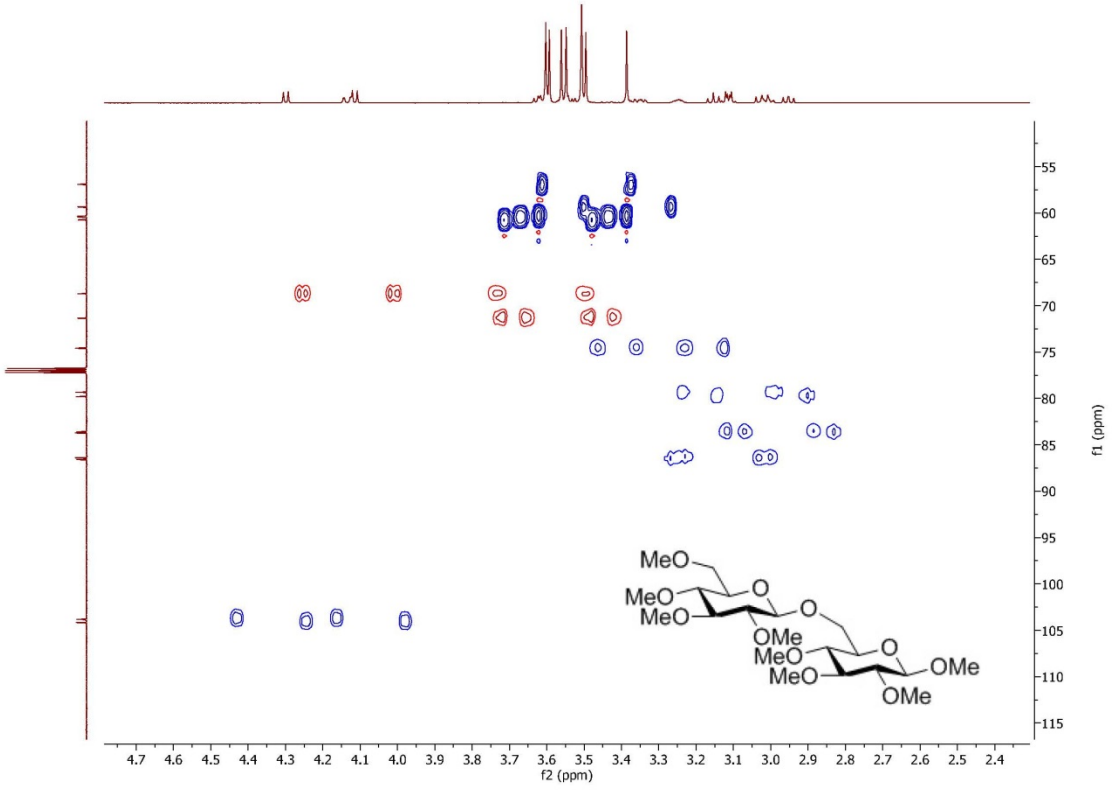
^{1}H NMR of 9 (400 MHz, $CDCl_3$)



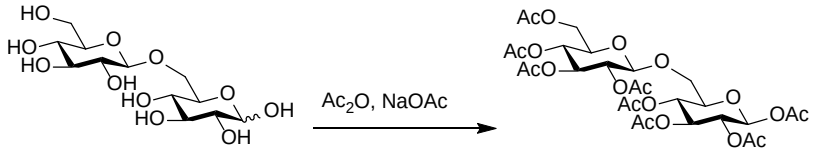
^{13}C NMR of 9 (101 MHz, $CDCl_3$)



Coupled HSQC NMR of 9 ($CDCl_3$)



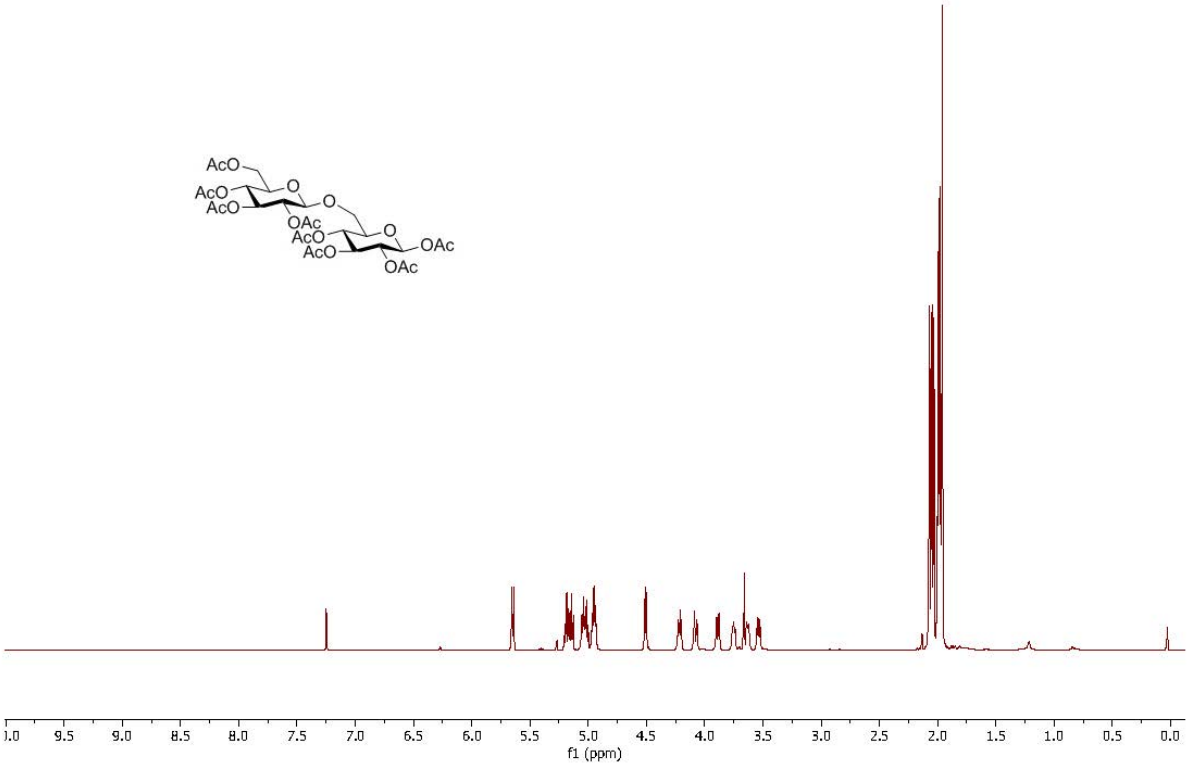
5.3.Synthesis of 10



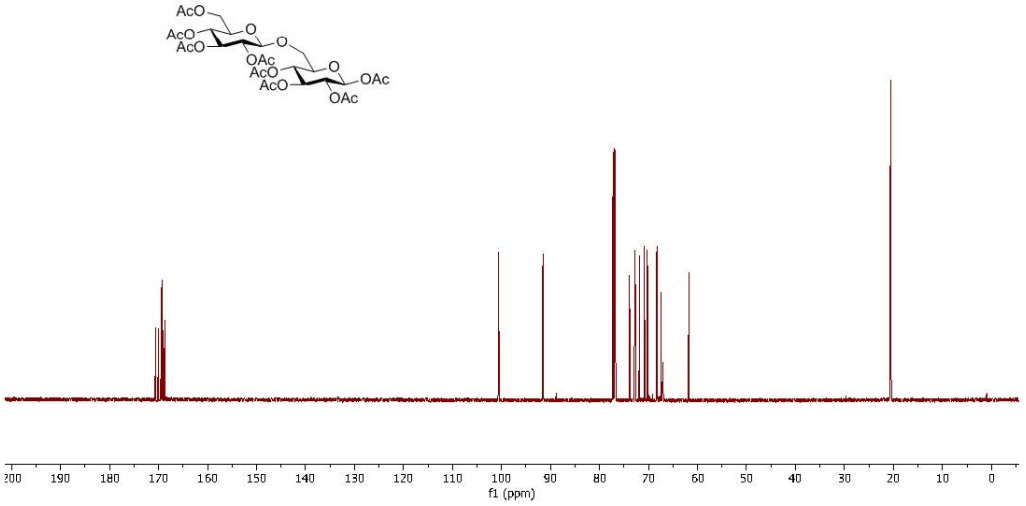
S12 **10**

A suspension of **S12** (50.0 mg, 0.146 mmol) in 2 mL of acetic anhydride was heated to 90°C. NaOAc (201 mg, 2.33 mmol) was added and the solution was stirred at the same temperature for 12 h. The reaction was then cooled down to room temperature and evaporated. The crude product was suspended in DCM and washed with H_2O and saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting yellow oil was purified by column chromatography (hexane: EtOAc = 3:1) and recrystallized to give **10** as white solid (45.0 mg, 45%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.67 (d, *J* = 8.2 Hz, 1H), 5.19 (dt, *J* = 15.4, 9.5 Hz, 2H), 5.11 – 4.92 (m, 4H), 4.53 (d, *J* = 7.9 Hz, 1H), 4.24 (dd, *J* = 12.4, 4.7 Hz, 1H), 4.10 (dd, *J* = 12.3, 2.4 Hz, 1H), 3.97 – 3.87 (m, 1H), 3.81 – 3.72 (m, 1H), 3.65 (dt, *J* = 9.6, 3.3 Hz, 1H), 3.55 (dd, *J* = 11.4, 5.8 Hz, 1H), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 6H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 170.58, 170.17, 170.02, 169.46, 169.34, 169.33, 169.16, 168.75, 100.58, 91.56, 73.85, 72.81, 72.70, 71.87, 70.86, 70.22, 68.39, 68.30, 67.45, 61.80, 20.73, 20.68, 20.55, 20.53, 20.51, 20.49; m/z (HRMS+) 701.1893 $[M + Na]^+$ ($C_{68}H_{70}O_{11}Na$ requires 701.1899).

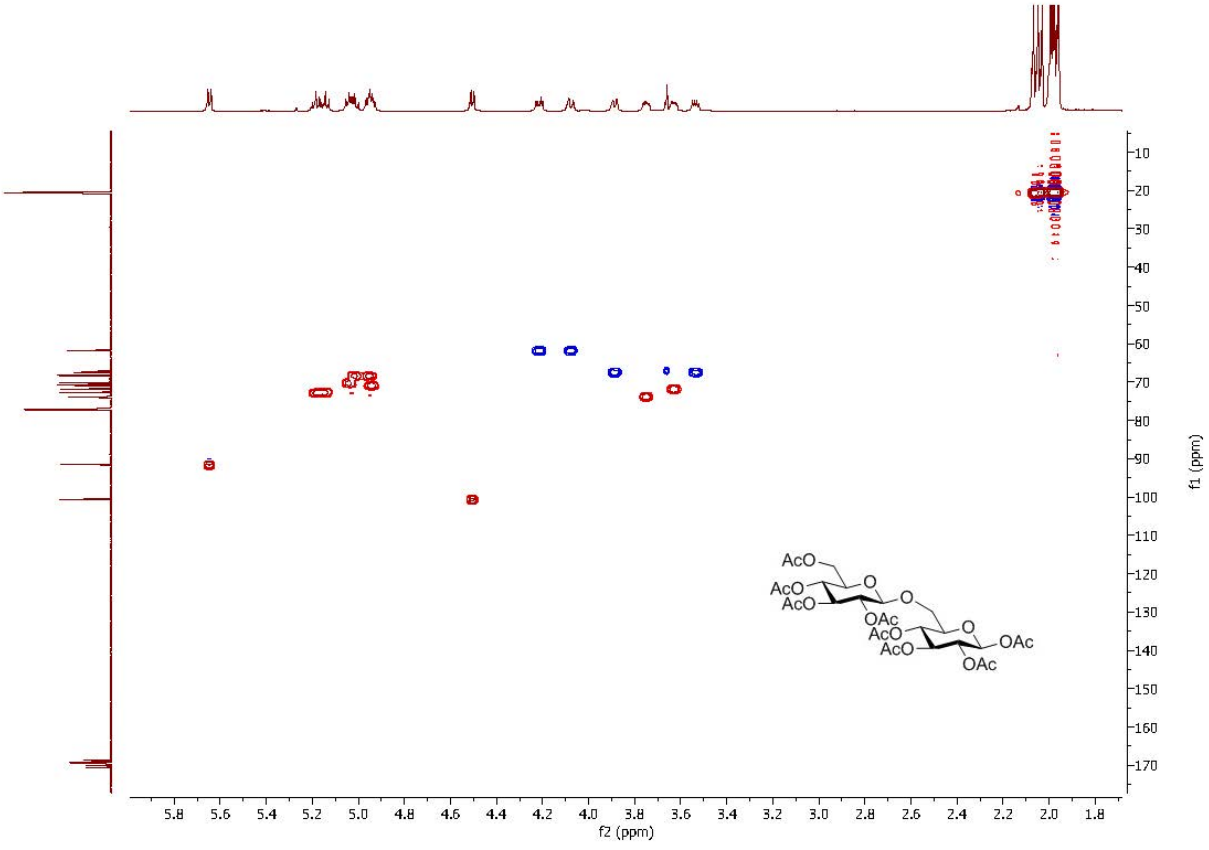
^{1}H NMR of 10 (400 MHz, $CDCl_3$)



^{13}C NMR of 10 (101 MHz, $CDCl_3$)

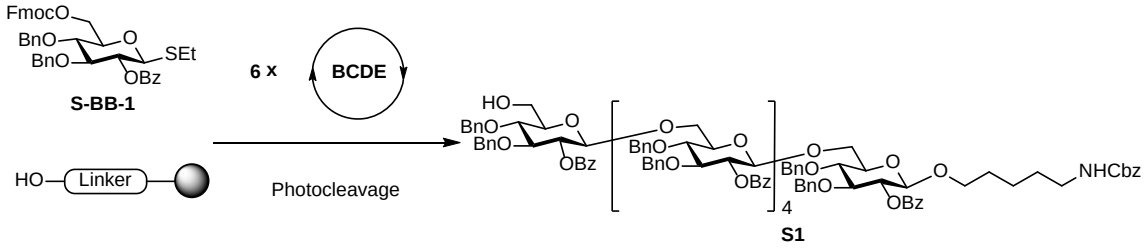


HSQC NMR of 10 ($CDCl_3$)



6. Synthesis of partially deprotected hexamers

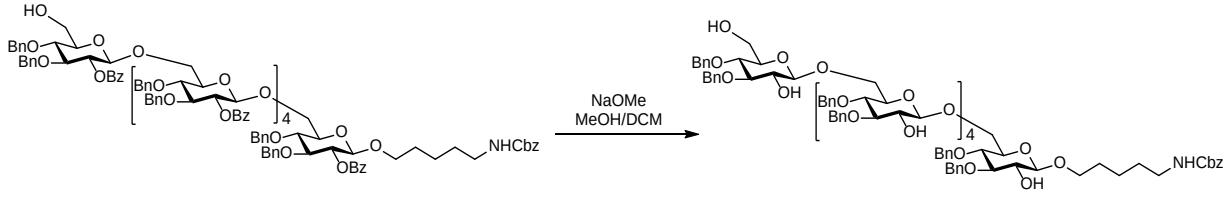
6.1. Synthesis of 4



Module		Conditions
A: Resin Preparation for Synthesis		
6	**B**: Acidic Wash	
	C: Thioglycoside Glycosylation	**BB-1** 6.5 eq (-20° for 5 min, 0° for 20 min)
	D: Capping	
	E: Fmoc Deprotection	

Cleavage from the solid support as described in *Post-synthesizer manipulation* followed by purification using preparative HPLC afforded compound **S1** (27.0 mg, 74%).

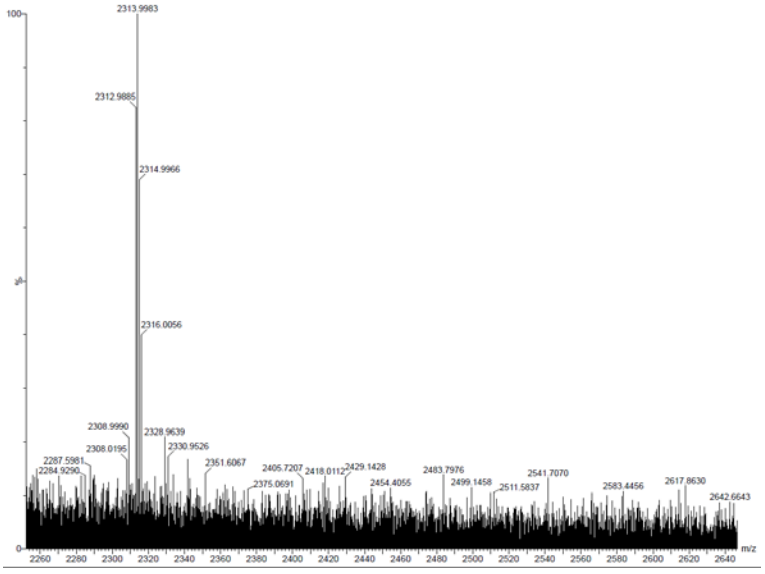
Analytical data for **S1:** ^{1}H NMR (700 MHz, Chloroform-*d*) δ 8.24 (d, *J* = 7.8 Hz, 2H), 8.22 – 8.15 (m, 6H), 8.08 (t, *J* = 8.4 Hz, 4H), 7.56 (t, *J* = 7.5 Hz, 2H), 7.44 (ddd, *J* = 31.3, 16.2, 7.9 Hz, 8H), 7.36 – 7.26 (m, 18H), 7.22 (d, *J* = 7.4 Hz, 2H), 7.17 – 7.13 (m, 5H), 7.12 – 7.06 (m, 5H), 7.06 – 6.98 (m, 28H), 6.97 – 6.93 (m, 10H), 6.88 – 6.79 (m, 5H), 5.50 (t, *J* = 8.7 Hz, 1H), 5.43 (p, *J* = 9.3 Hz, 3H), 5.33 (t, *J* = 8.7 Hz, 1H), 5.24 (t, *J* = 8.7 Hz, 1H), 5.12 – 4.99 (m, 2H), 4.85 (t, *J* = 9.5 Hz, 2H), 4.78 (t, *J* = 10.1 Hz, 3H), 4.75 – 4.71 (m, 2H), 4.71 – 4.61 (m, 11H), 4.53 (d, *J* = 7.9 Hz, 1H), 4.51 – 4.44 (m, 3H), 4.39 (ddd, *J* = 21.8, 10.3, 5.1 Hz, 5H), 4.27 (d, *J* = 10.0 Hz, 1H), 4.17 (s, 1H), 4.12 (d, *J* = 11.6 Hz, 1H), 4.09 – 4.01 (m, 4H), 3.97 (t, *J* = 9.1 Hz, 1H), 3.91 (t, *J* = 9.0 Hz, 3H), 3.87 (q, *J* = 8.5 Hz, 4H), 3.80 (q, *J* = 9.7, 9.3 Hz, 2H), 3.75 (dd, *J* = 12.1, 7.3 Hz, 6H), 3.72 – 3.63 (m, 4H), 3.58 (t, *J* = 11.0 Hz, 3H), 3.39 (dtt, *J* = 25.8, 17.0, 7.1 Hz, 6H), 3.30 (t, *J* = 9.4 Hz, 1H), 2.88 (q, *J* = 6.7 Hz, 2H), 1.45 (d, *J* = 7.0 Hz, 1H), 1.37 (dt, *J* = 13.3, 7.0 Hz, 1H), 1.33 – 1.27 (m, 2H), 1.22 (t, *J* = 7.2 Hz, 1H), 1.12 (d, *J* = 12.9 Hz, 1H). NMR data were in good agreement with those previously reported.[2]



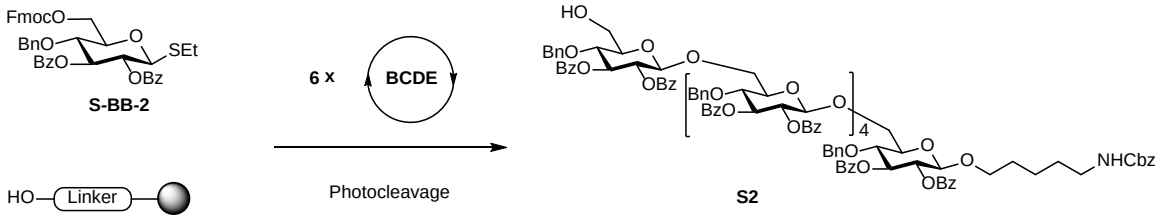
S1 (27.0 mg, 9.27 μmol) was dissolved in MeOH: DCM (1.5 mL, 1:1). NaOMe in MeOH (0.5 M, 3 equiv. per benzoyl ester) was added and the solution was stirred at room temperature for 12 h and then filtered. The resulting solid compound was dispersed in Milli-Q water and sonicated for 1h.

The white suspension was then centrifuged at 7000 rcf for 10 min followed by removal of supernatant. The sonication and centrifugation was repeated twice. The solid was then dried *in vacuo* overnight to give **4** as white powder (13.5 mg, 63%); m/z (HRMS+) 2312.999 $[M + Na]^+$ ($C_{133}H_{151}NO_{33}Na$ requires 2313.006).

HRMS of 4



6.2. Synthesis of 5

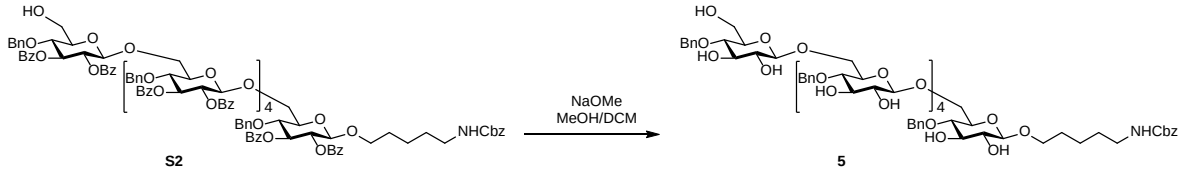


Module	Conditions
A: Resin Preparation for Synthesis	
6 — **B**: Acidic Wash **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-2** 6.5 eq (-20° for 5 min, 0° for 20 min)

Cleavage from the solid support as described in *Post-synthesizer manipulation* followed by purification using preparative HPLC afforded **S2** (23.0 mg, 61%).

Analytical data for **S2** (1-6): ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.29 – 8.22 (m, 6H), 8.20 (d, *J* = 7.7 Hz, 2H), 8.14 – 8.08 (m, 4H), 8.07 (d, *J* = 7.9 Hz, 2H), 8.03 (d, *J* = 8.0 Hz, 1H), 8.01 – 7.92 (m, 6H), 7.90 – 7.86 (m, 2H), 7.53 – 7.43 (m, 4H), 7.42 – 7.20 (m, 22H), 7.20 – 6.99 (m, 21H), 6.99 –

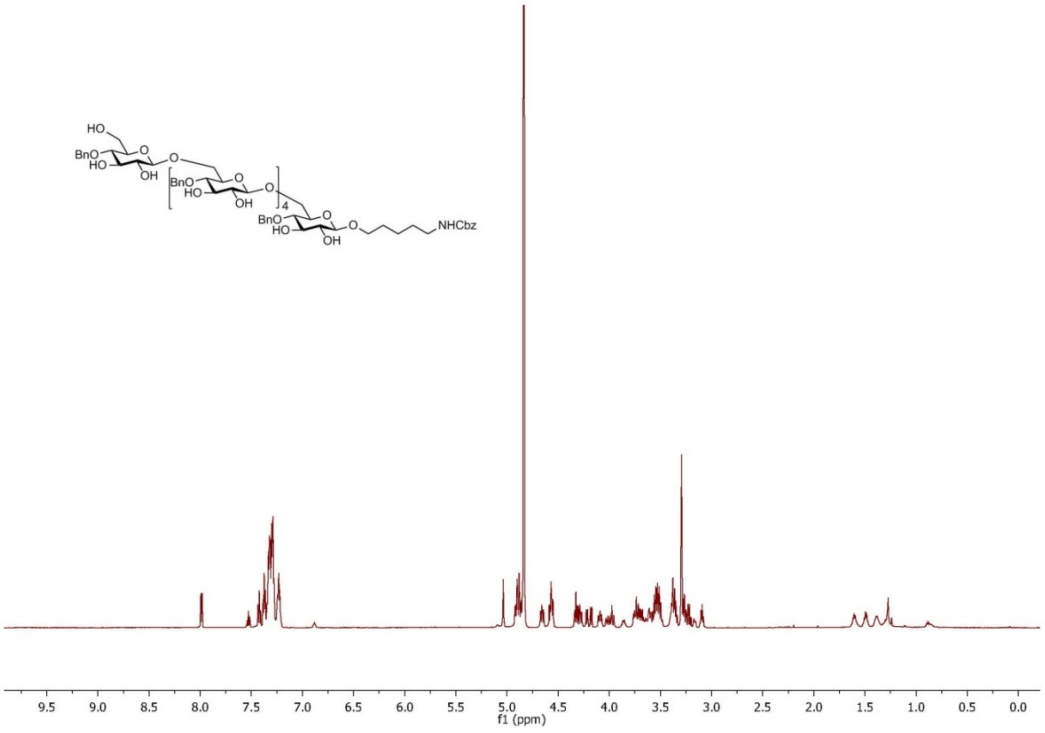
6.94 (m, 2H), 6.93 – 6.85 (m, 5H), 6.85 – 6.73 (m, 4H), 6.73 – 6.64 (m, 5H), 6.61 (t, *J* = 7.4 Hz, 1H), 6.53 (t, *J* = 7.5 Hz, 2H), 6.50 – 6.44 (m, 4H), 6.41 (t, *J* = 7.5 Hz, 2H), 6.02 (t, *J* = 9.6 Hz, 2H), 5.95 (td, *J* = 9.6, 7.1 Hz, 2H), 5.89 – 5.78 (m, 4H), 5.72 – 5.60 (m, 3H), 5.53 – 5.43 (m, 3H), 5.31 (d, *J* = 8.0 Hz, 1H), 5.27 (d, *J* = 8.1 Hz, 1H), 5.11 (t, *J* = 10.5 Hz, 1H), 5.07 – 4.95 (m, 3H), 4.95 – 4.88 (m, 1H), 4.78 (br, 1H), 4.69 – 4.60 (m, 4H), 4.42 – 4.22 (m, 11H), 4.22 – 4.12 (m, 4H), 4.09 – 3.92 (m, 7H), 3.85 – 3.72 (m, 3H), 3.71 – 3.65 (m, 2H), 3.63 (d, *J* = 9.6 Hz, 1H), 3.59 – 3.53 (m, 2H), 3.51 (t, *J* = 9.5 Hz, 1H), 3.28 – 3.24 (m, 1H), 3.21 (dd, *J* = 13.4, 2.6 Hz, 1H), 2.93 (dq, *J* = 12.5, 6.3 Hz, 2H), 1.68 – 1.59 (m, 2H), 1.47 – 1.35 (m, 2H), 1.34 – 1.27 (m, 2H). NMR data were in good agreement with those previously reported.[3]



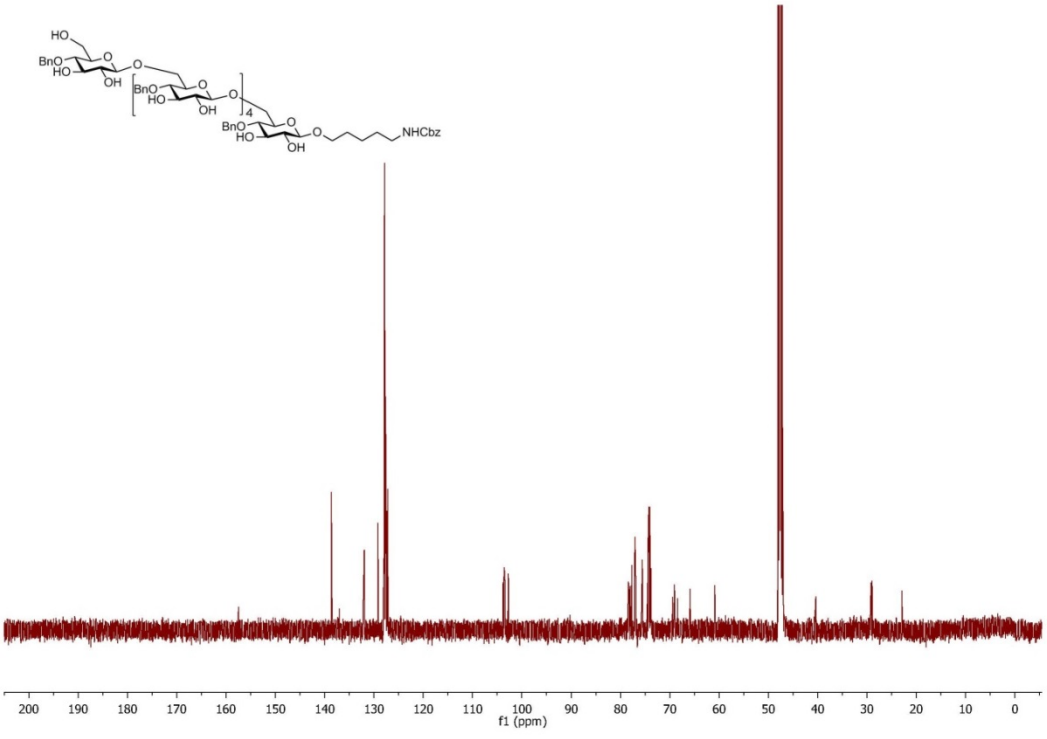
S2 **5**

S2 (23.0 mg, 7.67 μmol) was dissolved in MeOH: DCM (1.5 mL, 1:1). NaOMe in MeOH (0.5 M, 3 equiv. per benzoyl ester) was added and the solution was stirred at room temperature for 12 h, neutralized with Amberlite IR-120 (H^+ form) resin, filtered and concentrated *in vacuo*. The resulting yellow oil was purified by column chromatography (DCM: MeOH = 10:1) to give **5** as white solid (10.2 mg, 76%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.01 – 7.96 (m, 2H), 7.55 – 7.51 (m, 1H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.37 (t, *J* = 6.6 Hz, 4H), 7.35 – 7.26 (m, 20H), 7.23 (qt, *J* = 7.2, 4.2 Hz, 6H), 6.88 (dd, *J* = 6.9, 4.5 Hz, 1H), 5.04 (s, 2H), 4.92 (d, *J* = 4.6 Hz, 1H), 4.91 – 4.86 (m, 5H), 4.66 (dd, *J* = 11.1, 6.7 Hz, 2H), 4.60 – 4.54 (m, 4H), 4.34 – 4.30 (m, 2H), 4.29 – 4.26 (m, 1H), 4.22 (d, *J* = 7.8 Hz, 1H), 4.18 (d, *J* = 7.9 Hz, 1H), 4.09 (ddd, *J* = 11.9, 7.5, 2.1 Hz, 2H), 4.04 – 3.94 (m, 3H), 3.86 (dt, *J* = 9.7, 6.7 Hz, 1H), 3.77 – 3.70 (m, 4H), 3.70 – 3.58 (m, 5H), 3.57 – 3.47 (m, 8H), 3.37 (pd, *J* = 9.2, 5.6 Hz, 7H), 3.28 – 3.20 (m, 4H), 3.16 (ddd, *J* = 10.1, 4.8, 2.2 Hz, 1H), 3.10 (q, *J* = 6.7 Hz, 2H), 1.60 (q, *J* = 7.7, 7.3 Hz, 2H), 1.49 (q, *J* = 7.3 Hz, 2H), 1.39 (s, 2H), 1.27 (s, 2H), 0.87 (dt, *J* = 18.7, 6.4 Hz, 2H); ^{13}C NMR (151 MHz, cd_3od) δ 157.46, 138.66, 138.64, 138.62, 131.97, 129.18, 128.03, 127.96, 127.95, 127.92, 127.92, 127.88, 127.85, 127.81, 127.71, 127.66, 127.63, 127.50, 127.37, 127.30, 127.29, 127.25, 127.23, 127.20, 103.81, 103.61, 103.58, 103.52, 103.45, 102.72, 78.44, 78.41, 78.29, 78.07, 77.71, 77.14, 77.09, 77.04, 76.94, 75.57, 74.54, 74.42, 74.38, 74.30, 74.26, 74.13, 74.03, 73.92, 73.84, 73.80, 69.44, 69.05, 68.44, 65.90, 60.88, 48.13, 47.99, 47.84, 47.70, 47.56, 47.42, 47.28, 47.14, 40.38, 29.17, 28.97, 22.88; m/z (HRMS+) 1172.721 $[M + Na]^+$ ($C_{91}H_{111}NNaO_{33}Na$ requires 1772.724).

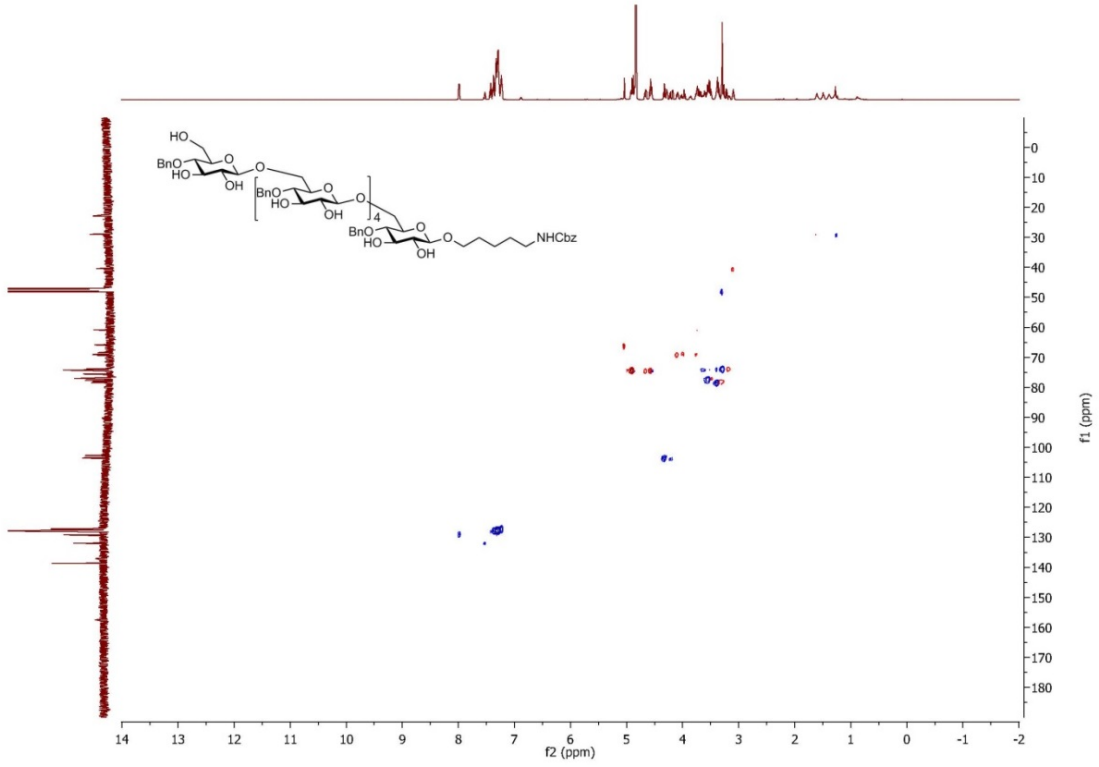
^{1}H NMR of 5 (400 MHz, Methanol-d_4)



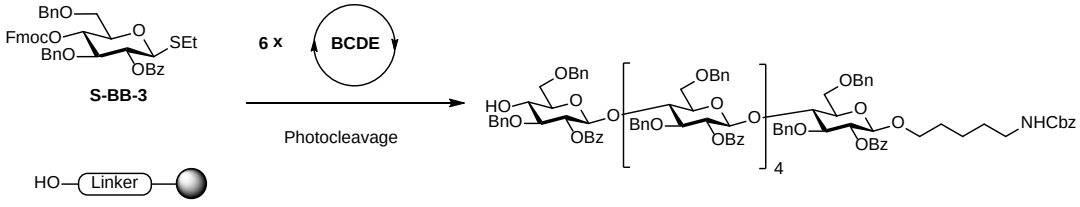
^{13}C NMR of 5 (101 MHz, Methanol-d_4)



HSQC NMR of 5 (Methanol-d_4)



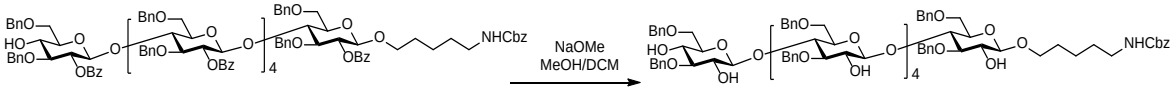
6.3. Synthesis of 6



Module	Conditions
A: Resin Preparation for Synthesis	
6 — **B**: Acidic Wash **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-3** 6.5 eq (-20° for 5 min, 0° for 20 min)

Post-synthesizer manipulation followed by purification using preparative HPLC afforded compound **S3** (17.0 mg, 47%).

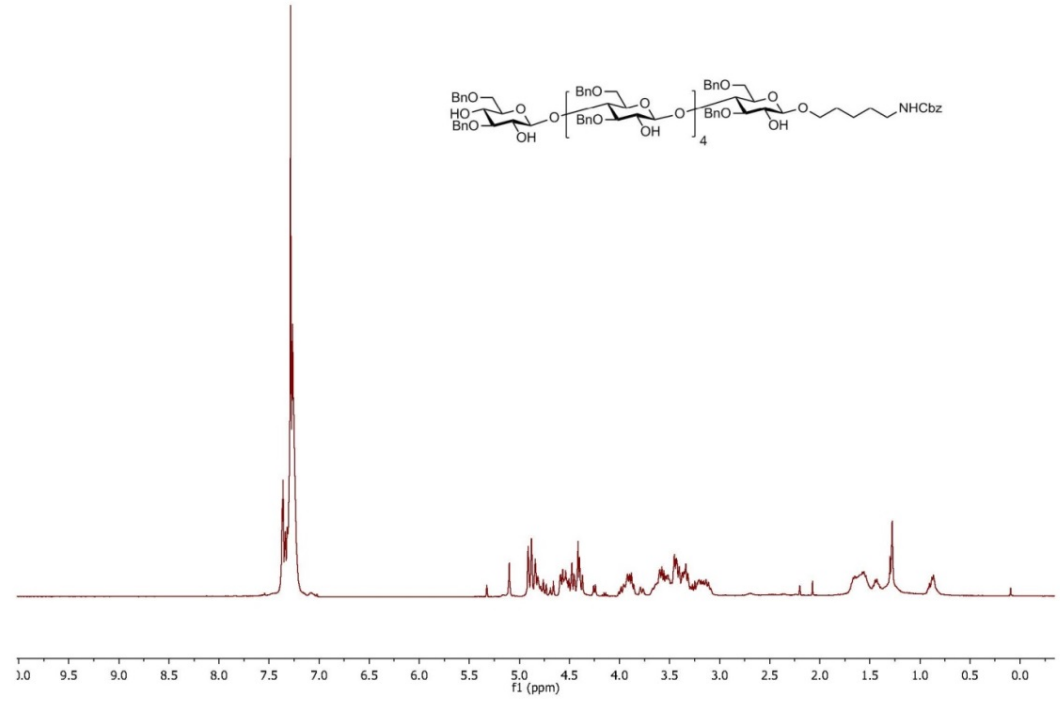
Analytical data for **S3:** ^{1}H NMR (700 MHz, Chloroform-*d*) δ 7.89 (dd, *J* = 17.6, 7.7 Hz, 4H), 7.82 (q, *J* = 8.6 Hz, 8H), 7.59 (dq, *J* = 16.0, 7.9 Hz, 4H), 7.54 (t, *J* = 7.5 Hz, 1H), 7.48 (t, *J* = 7.4 Hz, 1H), 7.47 – 7.43 (m, 4H), 7.43 – 7.37 (m, 6H), 7.33 (p, *J* = 7.7 Hz, 10H), 7.27 (d, *J* = 8.7 Hz, 4H), 7.24 (d, *J* = 7.5 Hz, 3H), 7.18 – 7.01 (m, 38H), 6.99 – 6.86 (m, 12H), 5.23 (t, *J* = 8.8 Hz, 1H), 5.17 (t, *J* = 8.8 Hz, 1H), 5.13 – 5.00 (m, 6H), 4.94 – 4.80 (m, 5H), 4.73 (d, *J* = 11.6 Hz, 1H), 4.67 (d, *J* = 11.6 Hz, 1H), 4.63 (d, *J* = 8.1 Hz, 1H), 4.54 (dtd, *J* = 28.5, 16.3, 14.1, 9.6 Hz, 8H), 4.45 – 4.33 (m, 6H), 4.32 – 4.22 (m, 4H), 4.15 (dd, *J* = 19.8, 12.0 Hz, 2H), 4.08 (t, *J* = 9.3 Hz, 1H), 4.05 – 3.87 (m, 7H), 3.79 (t, *J* = 9.1 Hz, 1H), 3.70 (dd, *J* = 10.7, 5.5 Hz, 1H), 3.63 – 3.55 (m, 2H), 3.55 – 3.46 (m, 5H), 3.46 – 3.38 (m, 4H), 3.31 (ddt, *J* = 36.1, 18.2, 9.1 Hz, 8H), 3.23 (p, *J* = 8.1, 7.3 Hz, 1H), 3.10 (d, *J* = 9.7 Hz, 1H), 3.05 (s, 1H), 2.88 (d, *J* = 9.9 Hz, 1H), 2.84 (d, *J* = 6.0 Hz, 2H), 2.78 (q, *J* = 10.4, 9.6 Hz, 3H), 1.40 (q, *J* = 9.1, 7.0 Hz, 1H), 1.35 – 1.31 (m, 1H), 1.23 – 1.17 (m, 2H), 1.13 – 1.03 (m, 2H). NMR data were in good agreement with those previously reported.[3]



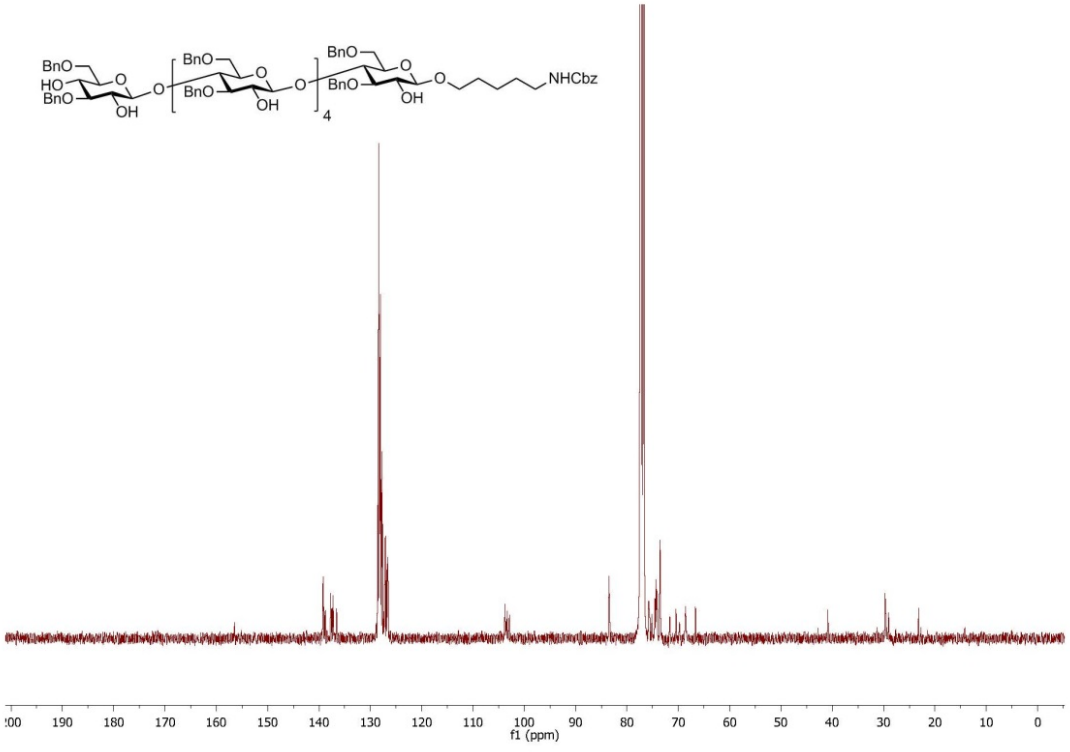
S3 **6**

S3 (17.0 mg, 5.83 μmol) was dissolved in MeOH: DCM (1.5 mL, 1:1). NaOMe in MeOH (0.5 M, 3 equiv. per benzoyl ester) was added and the solution was stirred at room temperature for 12 h, neutralized with Amberlite IR-120 (H^+ form) resin, filtered and concentrated *in vacuo*. The resulting yellow oil was purified by column chromatography (hexane: acetone = 3:1) to give **6** as white oil (10.2 mg, 76%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.18 (m, 65H), 5.10 (s, 1H), 4.94 – 4.85 (m, 6H), 4.85 – 4.64 (m, 6H), 4.62 – 4.32 (m, 14H), 4.25 (d, *J* = 7.4 Hz, 1H), 4.01 – 3.83 (m, 6H), 3.78 (d, *J* = 10.9 Hz, 1H), 3.69 – 3.48 (m, 11H), 3.42 (ddd, *J* = 21.0, 12.1, 3.4 Hz, 10H), 3.36 – 3.06 (m, 13H), 1.66 (q, *J* = 6.7 Hz, 4H), 1.59 – 1.52 (m, 4H), 1.43 (t, *J* = 7.8 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.44, 139.21, 139.19, 139.14, 139.11, 139.00, 138.75, 137.74, 137.44, 137.26, 137.21, 136.56, 128.54, 128.48, 128.41, 128.38, 128.36, 128.29, 128.27, 128.20, 128.15, 128.04, 127.91, 127.88, 127.75, 127.69, 127.38, 127.28, 127.22, 127.09, 126.84, 126.67, 126.57, 126.53, 103.83, 103.79, 103.69, 103.57, 103.35, 102.90, 83.52, 83.47, 83.38, 75.77, 75.51, 75.17, 74.57, 74.38, 74.22, 73.64, 73.60, 73.55, 71.65, 70.44, 69.76, 68.58, 66.64, 40.85, 29.74, 29.58, 29.03, 23.17; m/z (HRMS+) 2328.977 $[M + K]^+$ ($C_{133}H_{151}NO_{33}K$ requires 2328.980);

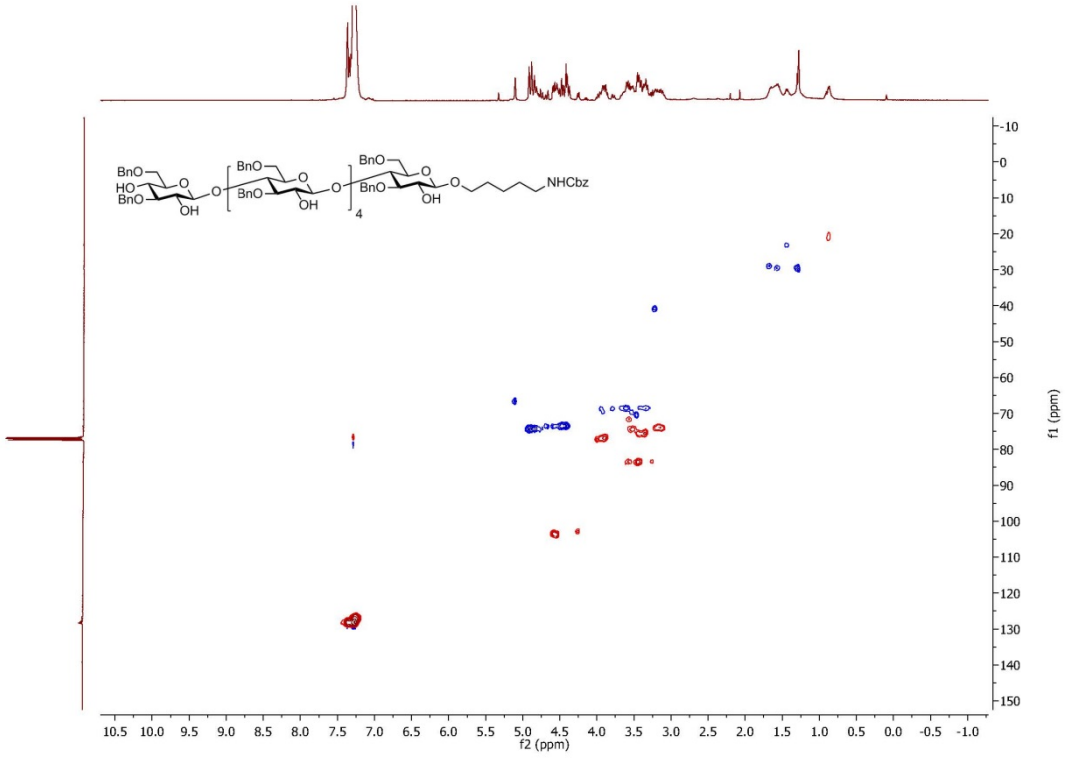
^{1}H NMR of 6 (400 MHz, $CDCl_3$)



^{13}C NMR of 6 (101 MHz, $CDCl_3$)



HSQC NMR of 6 ($CDCl_3$)



7. Oligosaccharide self-assembly

a) Dialysis method: The oligosaccharide was dissolved in 1 mL of DMAc and sonicated for 10 minutes. The mixture was diluted with 1 mL of ultrapure water and sonicated for additional 10 minutes. The final solutions with concentration of 0.01, 0.1 and 2 mg mL^{-1} were prepared by extensive dialysis (3 days) with 500 Da and 1 kDa dialysis tube, for dimers and hexamers, respectively.

Sample	Compound	Preparation method	Organic Solvent	Concentration (mg mL^{-1})	Temperature
1-D	1	Dialysis	DMAc	0.1	R.T.
2-D	2			0.1	
2-D-high				2.0	
3-D	3			0.1	
4-D	4			0.01*	
5-D	5			0.1	
6-D	6			0.01*	

*0.01 mg mL−1 due to poor solubility of the starting material.

b) Solvent-switch method: Stock solutions of the oligosaccharide (5, 10 and 100 mg mL^{-1}) in HFIP, isopropyl alcohol, acetone and DMAc were prepared. Ultrapure water was added to give a final concentration of 0.1, 2 and 20 mg mL^{-1}.

Sample	Compound	Preparation method	Solvent	Organic solvent content (%)	Concentration (mg mL^{-1})	Temperature
1-S-HFIP	1	Solvent-switch	HFIP	2	2.0	R.T.
1-S-HFIP-low			HFIP	2	0.1	
2-S-HFIP	2		HFIP	2	2.0	
2-S-HFIP-20%			HFIP	20		
2-S-iPrOH-20%			iPrOH	20		
2-S-Ace-20%			Ace	20		
2-S-Ace-20%-high					20.0	
2-S-DMAc			DMAc	2	2.0	
3-S-HFIP	3		HFIP	2		
4-S-DMAc	4		DMAc	2		
5-S-HFIP	5		HFIP	2		
6-S-DMAc	6		DMAc	2		

c) Film-forming method: The oligosaccharide was dissolved in a proper solvent (10 mg mL^{-1}) and dried on the slide glass.

Sample	Compound	Preparation method	Organic Solvent	Concentration (mg mL^{-1})	Temperature
1-F-HFIP	1	Film-forming	HFIP	10.0	R.T.
2-F-HFIP	2		HFIP		
2-F-iPrOH			iPrOH		
2-F-Ace			Ace		
2-F-DCM			DCM		
3-F-HFIP	3		HFIP		
4-F-HFIP	4				
5-F-HFIP	5				
6-F-HFIP	6				
7-F-HFIP	7				
8-F-HFIP	8				
9-F-HFIP	9				
10-F-HFIP	10				
11-F-water	11		water		

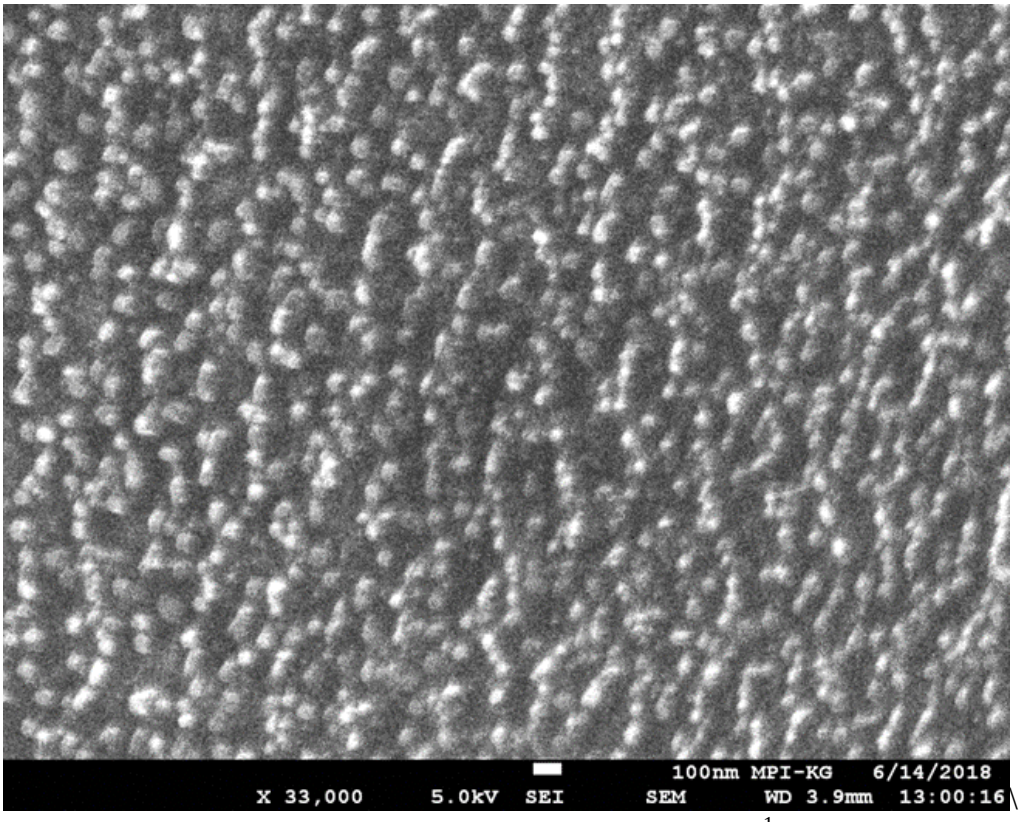


Fig. S1. Cryo SEM image of **2-D** with a concentration of 0.1 mg mL^{-1} confirming the presence of the particles in solution.

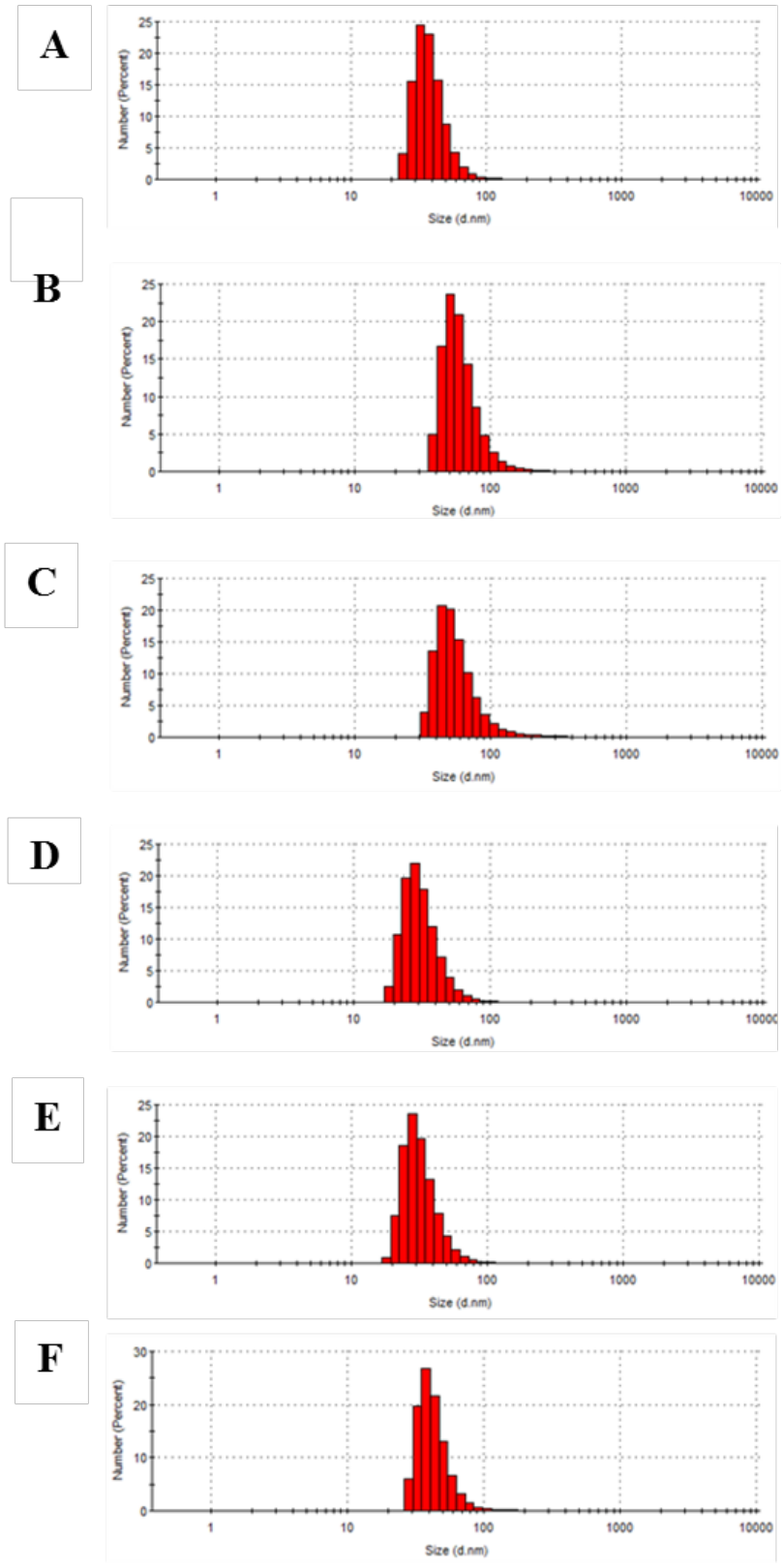


Fig. S2. DLS analysis of (A) **1-D**, (B) **2-D**, (C) **3-D**, (D) **4-D**, (E) **5-D**, and (F) **6-D**.

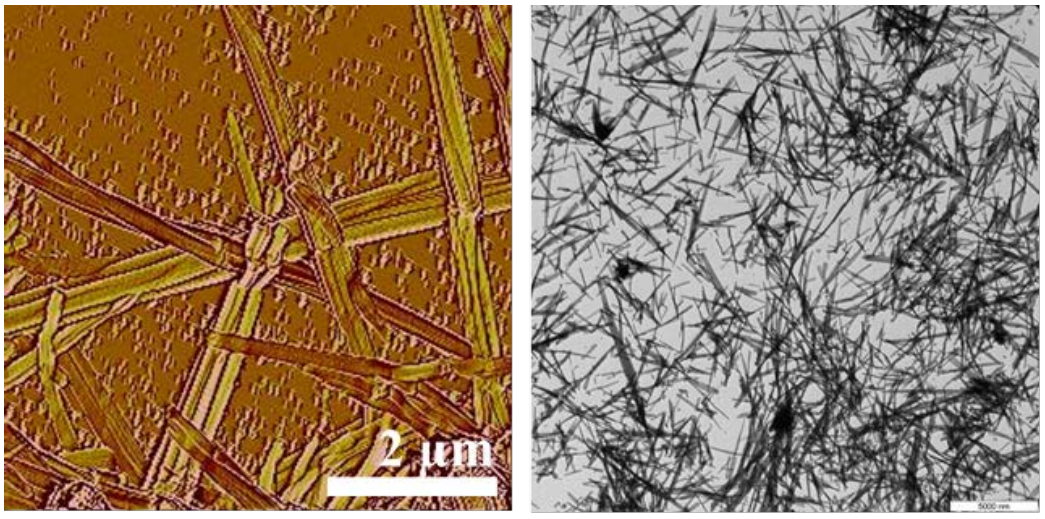


Fig. S3. AFM (left) and TEM (right) images of **2-S-HFIP**.

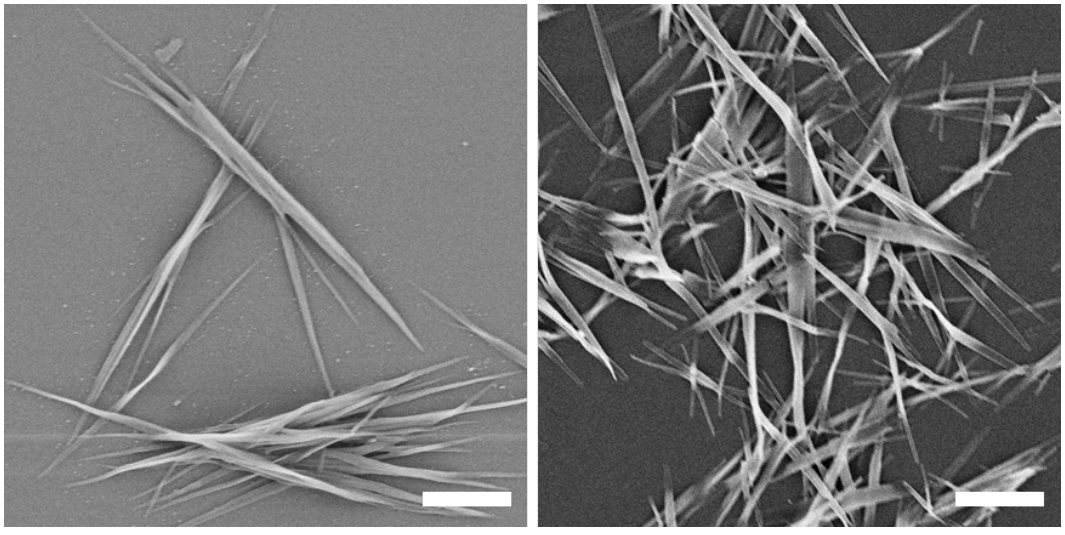


Fig. S4. SEM images of **2-S-HFIP** for time 0 (left) and after one month upon dilution (right) (scale bars: 2 µm).

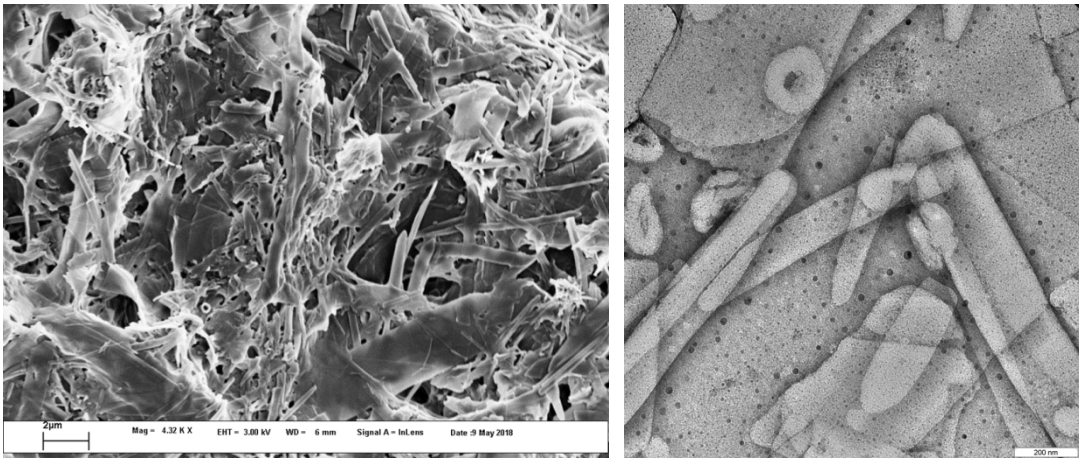


Fig. S5. SEM (left) and TEM (right) images of **1-S-HFIP**.

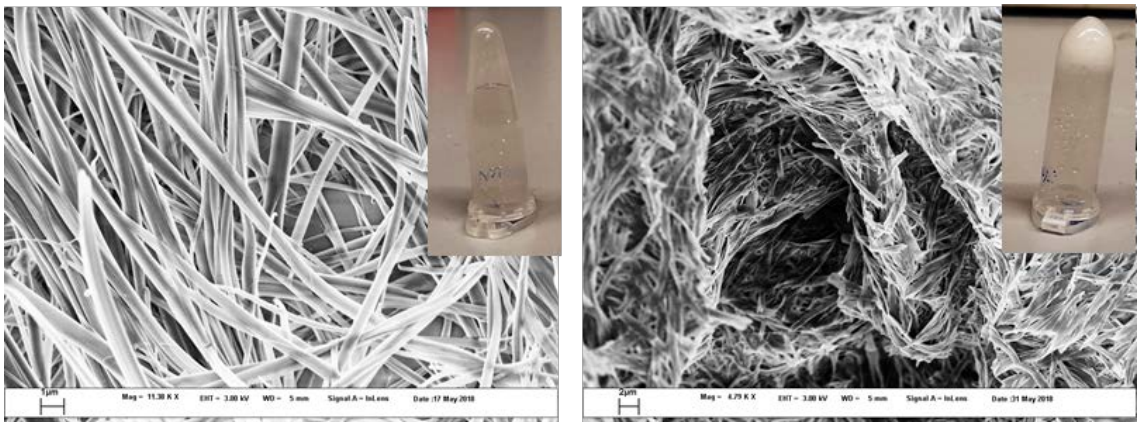


Fig. S6. SEM images and photographs (inset) of **2-S-Ace-20%** with 2 mg mL^{-1} (left) and **2-S-Ace-20%-high** with 20 mg mL^{-1} (right).

8. Photophysical characterization

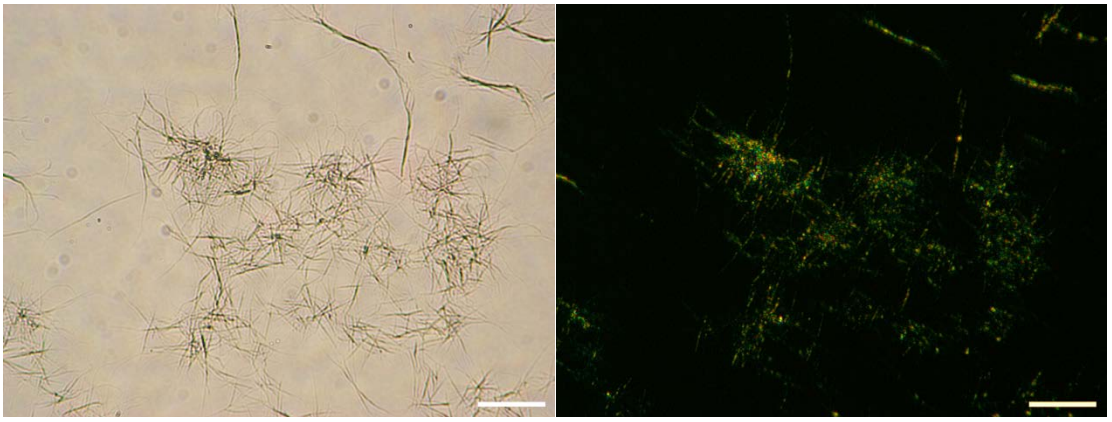


Fig. S7. Polarized microscopy images of **2-S-HFIP** with parallel (left) and crossed polarizer (right) (scale bars: 20 μm).

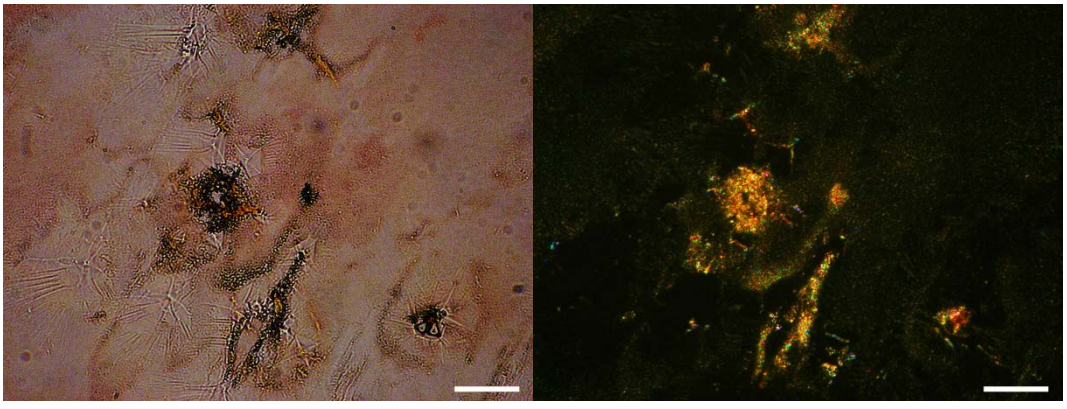


Fig. S8. Congo red birefringence assay of **2-S-HFIP** with parallel (left) and crossed polarizer (right) (scale bars: 20 μm). The detailed method was previously reported.[4]

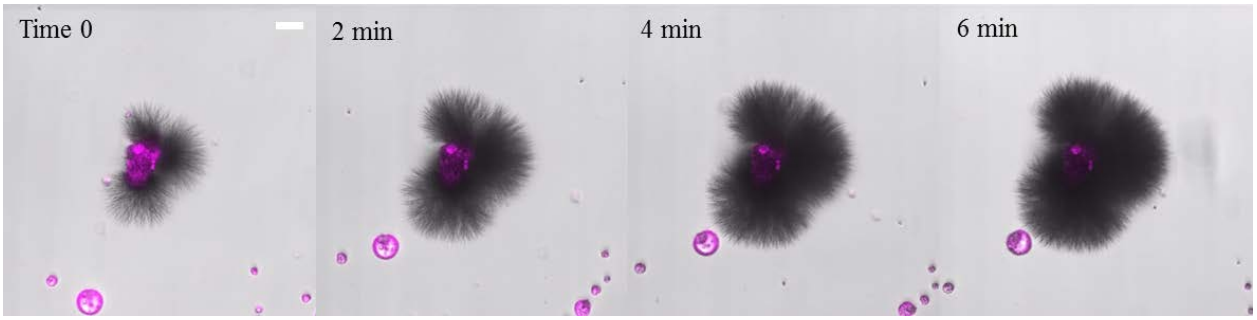


Fig. S9. Real-time merged bright-field (scale of gray) and fluorescence (magenta) images of self-assembly process for **2-S-HFIP** with excitation wavelength at 405 nm and detection range 410-676 nm (scale bar: 20 μm). Compound **2** was dissolved in HFIP with a 100 mg mL^{-1} concentration. After addition of ultrapure water (final concentration of 2 mg mL^{-1}), the solution was transferred to a cell counting slide (EVE™ slide from NanoEnTek) and observed with a confocal microscope.

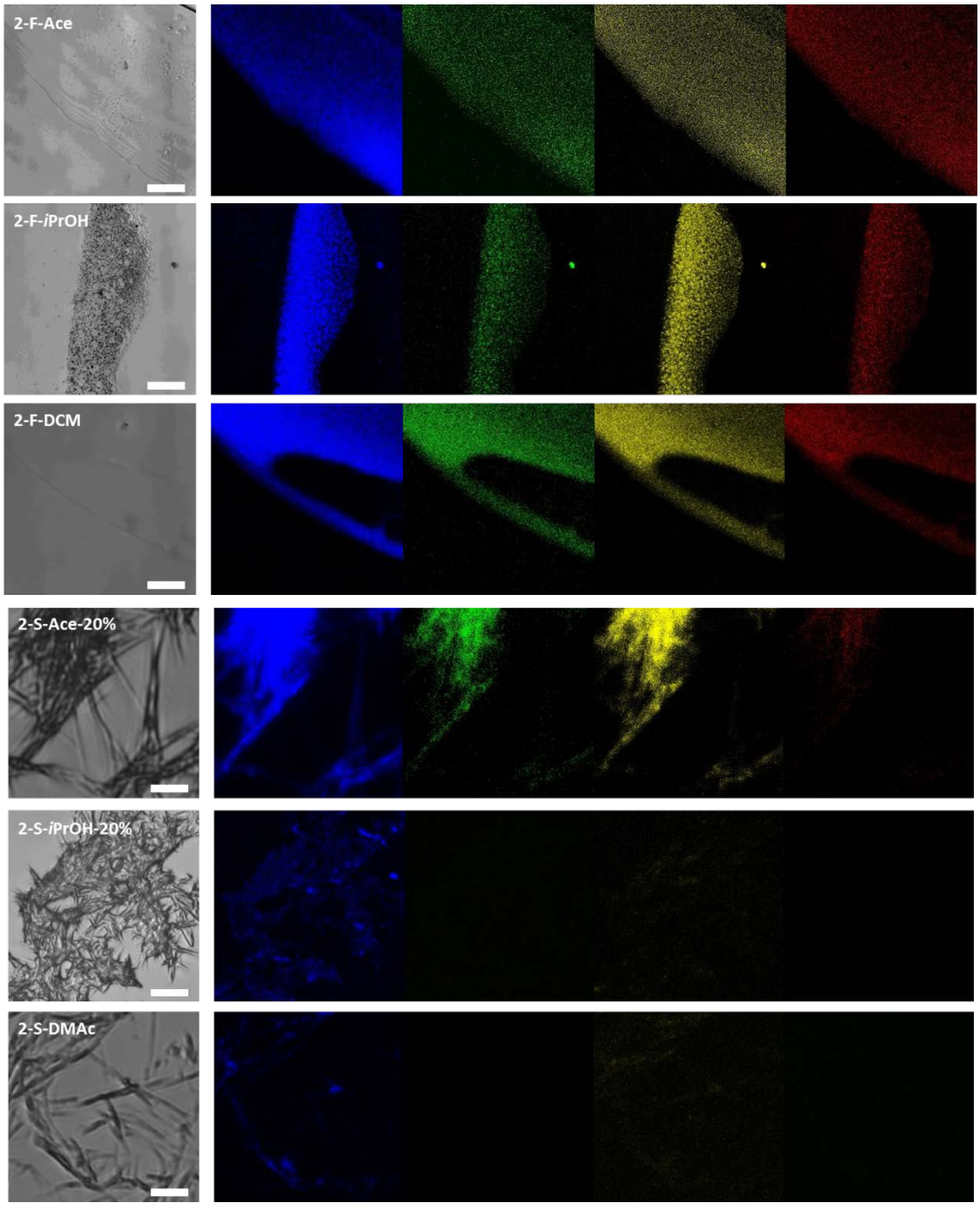


Fig. S10. Confocal microscopy images of **2** prepared with different solvent with the film forming method **F** (top, scale bar: 100 μm) and the solvent switch method **S** (bottom, scale bar: 10 μm) in four different channels (blue(ex/em): 405/451 nm, green: 488/529 nm, yellow: 561/597 nm, and red: 633/709 nm).

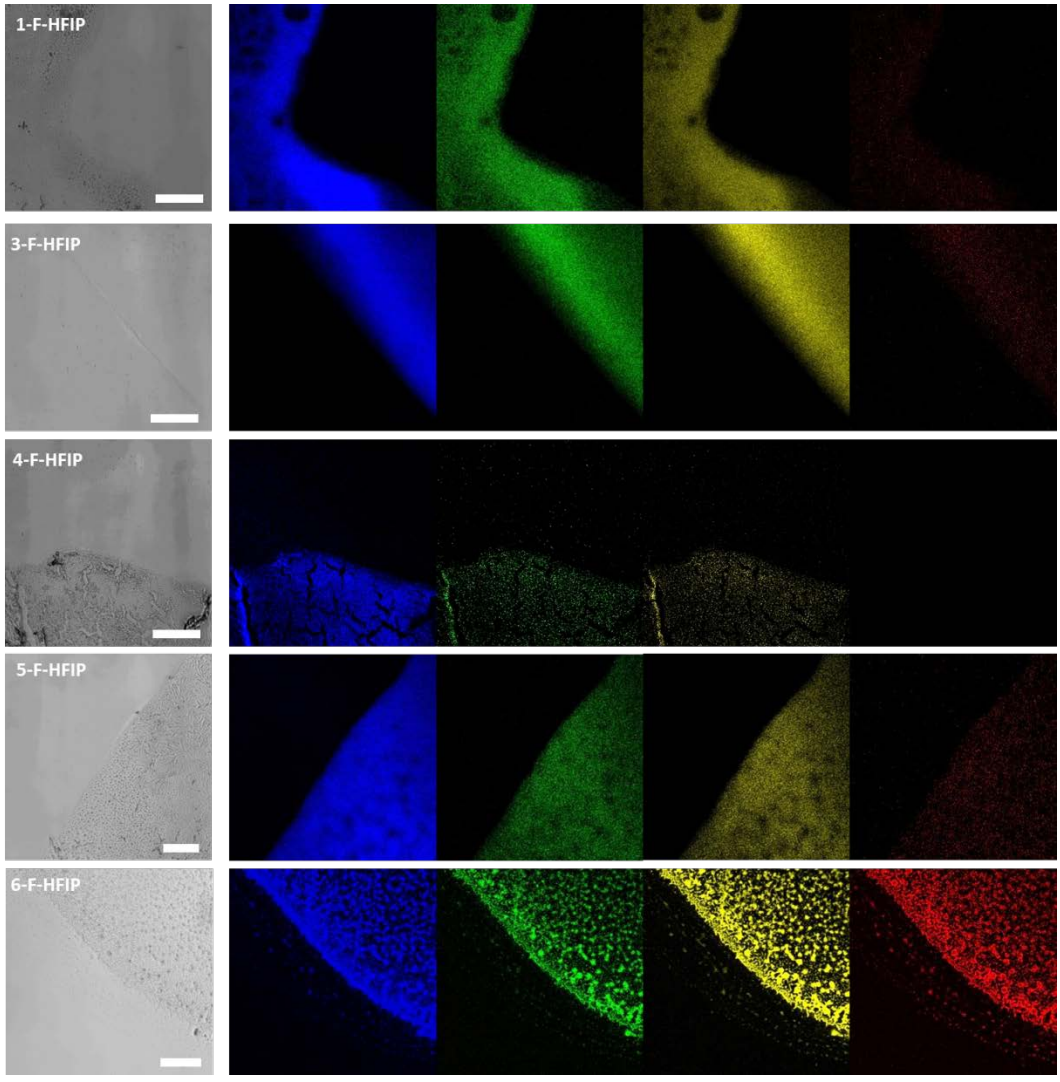


Fig. S11. Confocal microscopy images of the four oligosaccharides prepared by film forming method **F** in four different channels (blue(ex/em): 405/451 nm, green: 488/529 nm, yellow: 561/597 nm, and red: 633/709 nm). Scale bar: 100 μm.

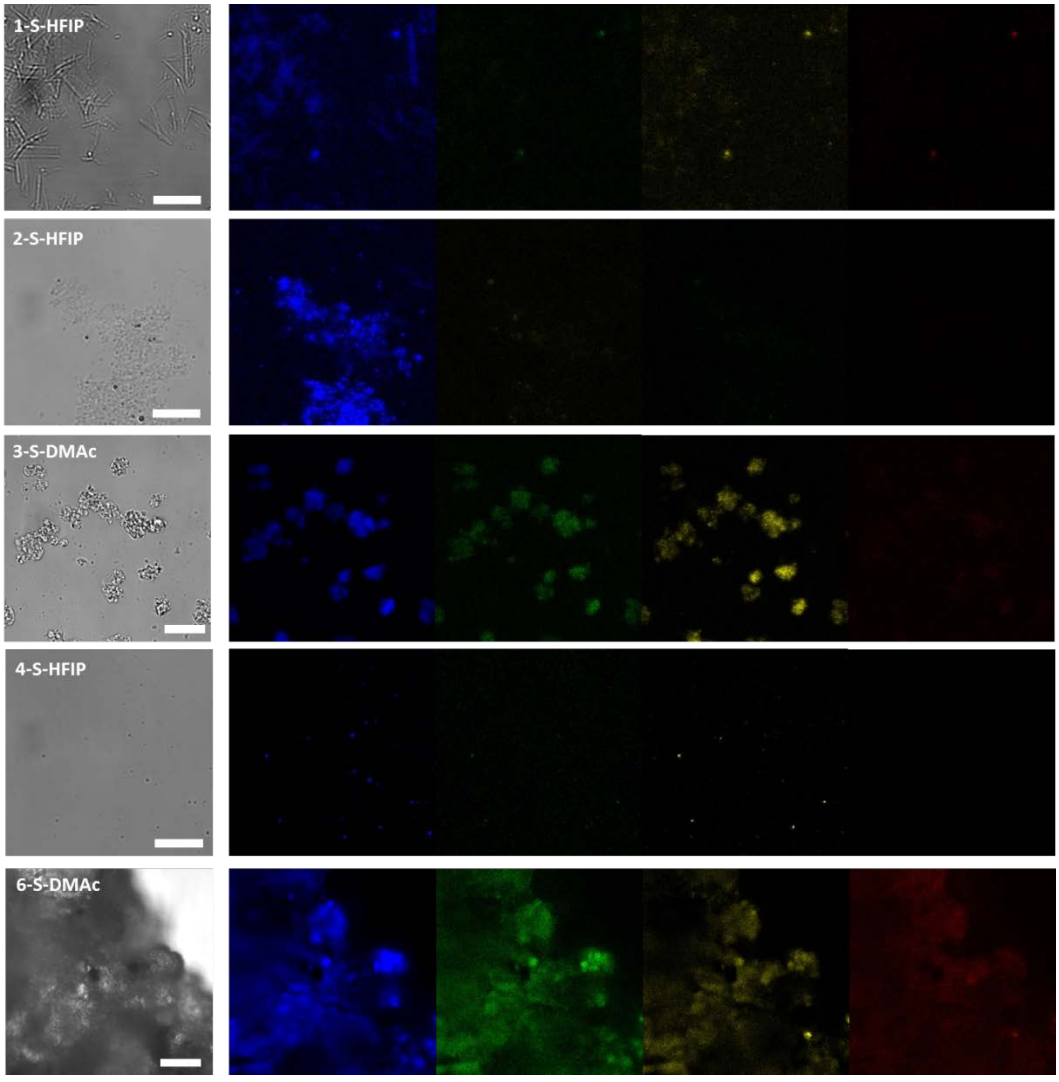


Fig. S12. Confocal microscopy images of the four oligosaccharides prepared by solvent switch method **S** in four different channels (blue(ex/em): 405/451 nm, green: 488/529 nm, yellow: 561/597 nm, and red: 633/709 nm). Scale bar: 10 μm.

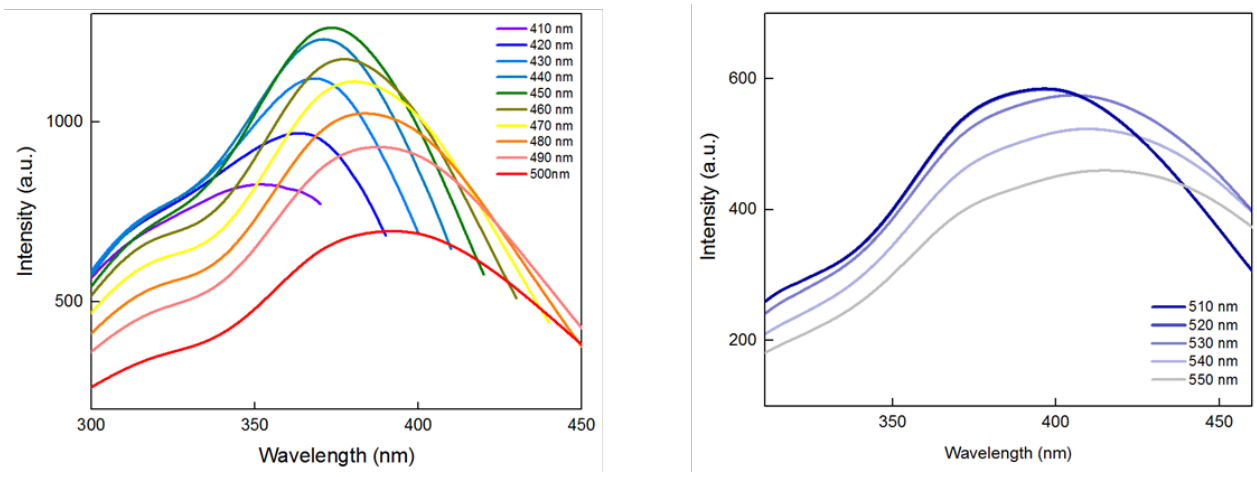


Fig. S13. Excitation spectra for **2-F-HFIP** at emission wavelengths of 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540 and 550 nm.

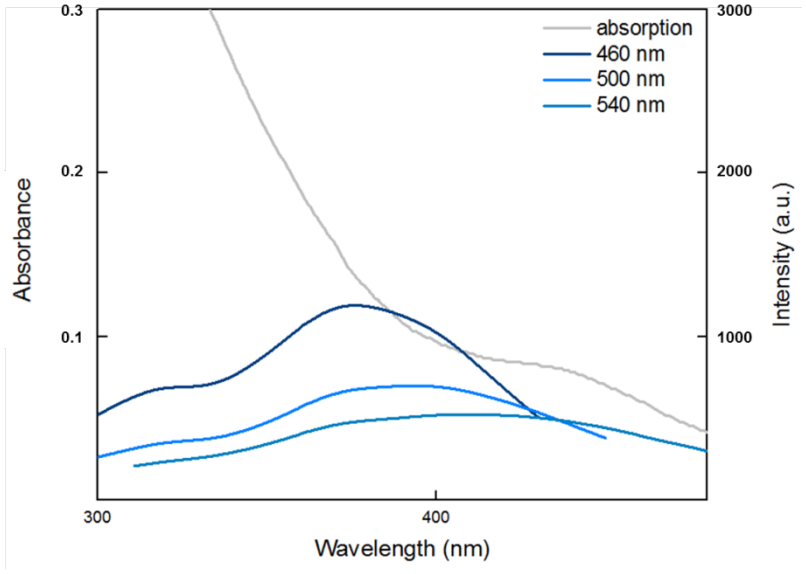


Fig. S14. Absorption (grey) and excitation (scale of blue) spectra for **2-F-HFIP.** Excitation spectra were recorded for emission wavelengths of 460, 500, and 540 nm.

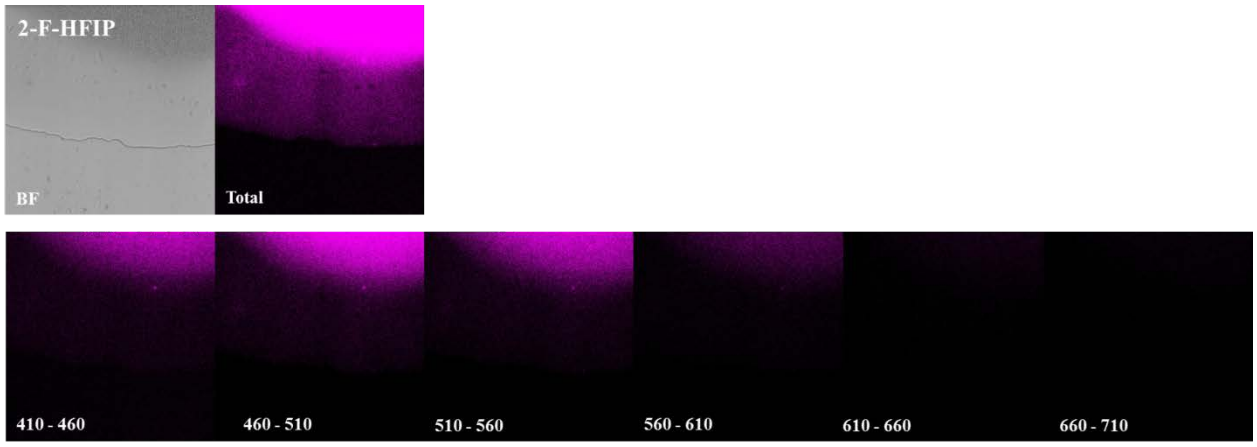


Fig. S15. Fluorescence emission images of **2-F-HFIP** collected at different spectral windows with excitation wavelength at 405 nm.

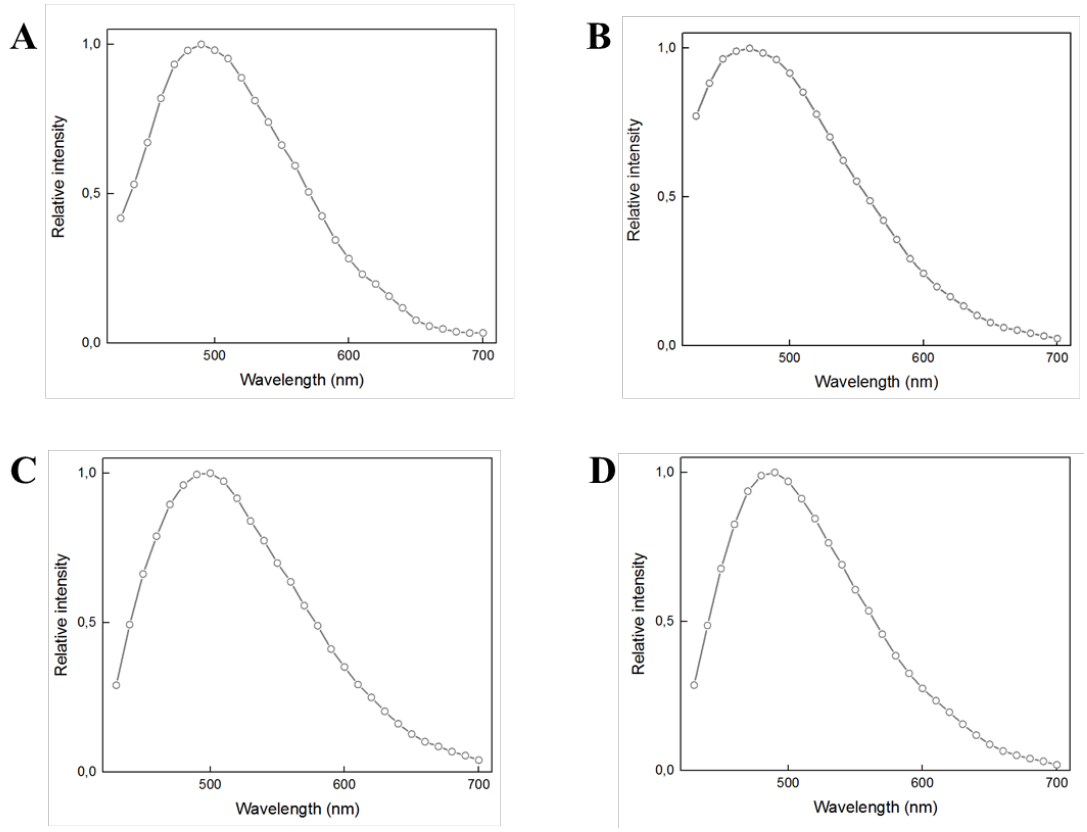


Fig. S16. Emission spectra of (A) **2-F-HFIP**, (B) **7-F-HFIP**, (C) **8-F-HFIP**, and (D) **9-F-HFIP** from confocal microscopy with excitation wavelength at 405 nm.

Movie S1. Video of self-assembly process for **2-S-HFIP**.

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