Electronic Supplementary Material (ESI)

Comparative Analysis of Sulfated and Sulfonated Disaccharide Analogs as TLR4 Modulators and Heparanase Inhibitors

Jamal El-Abid,[a] Dindet Steve-Evanes K.L.C. Koffi Teki ,[a] Abed Bil,[a] Agnès Denys,[b] Aurélie Vallin,[a] Corentin Lefebvre,[a] Fabrice Allain,[b] Vincent Chagnault*[,a] and José Kovensky*[,a]

[a] *Laboratoire de Glycochimie et des Agroressources d'Amiens (LG2A), UR 7378, Université de Picardie Jules Verne, 33 rue Saint Leu, F-80039 Amiens Cedex, France Email: vincent.chagnault@u-picardie.fr*
[b] *Univ. Lille, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, F-59000 Lille, France*

I. Materials and Methods

1. General informations

All reagent-grade chemicals were obtained from commercial suppliers and were used as received. Cobalt (II) chloride purum p.a., anhydrous (98.0%) was purchased from Sigma-Aldrich (CAS # 7646-79-9), Hydrogen peroxide solution 30 % in H_2O, contains stabilizer (CAS # 7722-84-1), and was used as received without further purification process. Characterizations of known compounds were in accordance with literature. Optical rotations were recorded in MeOH, DCM and H_2O solutions, using a MCP 100 from Anton Paar® equipped with an automatic Peltier temperature regulator (20 and 25°C). FTIR spectra were obtained using IRAffinity-1S (IR) et MIRacle 10 (ATR) from Shimadzu® and are reported in cm^{-1}. ^{1}H NMR (400 and 600 MHz) and ^{13}C NMR (101 and 151 MHz) spectra were recorded in CD_3CN, D_2O, $CDCl_3$, MeOD. The proton and carbon signal assignments were determined from decoupling experiments, COSY, HSQC, HMBC spectra. TLC were performed on Silica F254 and detection by UV light at 254 nm or by charring with cerium molybdate reagent. Column chromatography was performed on Silica Gel 60 (230 mesh). High-resolution electrospray mass spectra in the positive ion mode were obtained on a Q-TOF *Ultima Global* (Waters-Micromass) hybrid quadrupole/time-of-flight instrument, equipped with a pneumatically assisted electrospray (Z-spray) ion source and an additional sprayer (Lock Spray) for the reference compound. The source and desolvation temperatures were kept at 80 and 150 °C, respectively. Nitrogen was used as the drying and nebulizing gas at flow rates of 350 and 50 L/h, respectively. The capillary voltage was 3.5 kV, the cone voltage 50 V and the RF lens1 energy was optimized for each sample (50 V). Lock mass correction, using appropriate cluster ions of sodium iodide $(NaI)_nNa^+$, was applied for accurate mass

measurements. The mass range was typically 50-2050 Da and spectra were recorded at 2 s/scan in the profile mode at a resolution of 10000 (FWMH).

2. Cellular Models

HEK-Blue™/hTLR4 cell line (termed here HEK-TLR4) was obtained from InvivoGen. This cell line is stably transfected with a reporter gene encoding SEAP, for which transcriptional activation is under the control of a TLR4-inducible gene promoter. In the current study, HEK-Blue™/Null1 (HEK-Null) (InvivoGen) was also used as a negative control, as this cell line is transfected with the reporter gene encoding SEAP but does not express any TLR. According to the instructions of the manufacturer, HEK-Blue cell lines were cultured in DMEM medium (Lonza) supplemented with 10 % heat inactivated foetal calf serum (FCS), 2 mM L-glutamine, 0.5 % penicillin/streptomycin. In addition, each cell line was cultured with a specific antibiotic mixture (InvivoGen): 0.4 % HEK-Blue Selection™ and 0.2 % Normocin™ for HEK-TLR4 cells; 0.2 % Normocin™ and 0.1 % Zeocin™ for HEK-Null cells. For cell stimulation, HEK-TLR4 and HEK-Null cells were seeded into 96-well culture plates in culture medium without antibiotics (180 µL, 50×103 cells per well). Compounds 9 to 16 were diluted in culture medium, after which 20 µL of each solution were added into the wells to obtain final concentrations of 10, 30 and 100 µM. After 16 h of incubation at 37°C, 50 µL of the supernatants were collected and mixed with 150 µL of Quanti-Blue™ mixture, which contains a chromogenic substrate of SEAP (InvivoGen). After 1 h-incubation at 37°C, the absorbance was measured at 620 nm. In parallel experiments, HEK-TLR4 cells were stimulated with LPS (E. coli 055B5, Sigma-Aldrich) at 10 ng/mL, and the cellular response to this potent TLR4 agonist was considered as the maximal level of response of the cell line.

3. TR-FRET Heparanase Inhibition Assay

In a 96-well microplate (Cisbio, white polystyrene, half area) were added in triplicates:

- For the sample: 2 µL of our inhibitor solutions in Milli-Q water at different concentrations and 3 µL of 300 ng/mL heparanase (R&D system) solution in Tris-HCl 50 mM pH 7.4 buffer with NaCl 150 mM, 0.1% CHAPS, 0.1% BSA.
- For the positive control: 2 µL of Milli-Q water and 300 ng/mL heparanase (R&D system) solution in Tris-HCl 50 mM pH 7.4 buffer with NaCl 150 mM, 0.1% CHAPS, 0.1% BSA.
- For the negative control and the background: 2 µL of Milli-Q water and 3 µL of Tris-HCl 50 mM pH 7.4 buffer with NaCl 150 mM, 0.1% CHAPS, 0.1% BSA.

The plate was then preincubated at 37°C for 10 min. Thereafter, 5 µL of 0.8 µg/mL biotin-heparan sulfate-Eu cryptate (cisbio) solution in Na-acetate buffer 200 mM pH 5.5 were added and the plate was incubated in the dark at 37°C for 30 min.

The reaction was then stopped by adding:

- For the sample, positive and negative control: 10 µL of 1 µg/mL Streptavidin-Xlent! (Cisbio) solution in PPI detection buffer from Cisbio.
- For background: 10 µL of PPI detection buffer from Cisbio.

After 15 min at room temperature in the dark, HTRF emissions at 620 and 665 nm were measured by exciting at 340 nm using Tecan infinite M1000. The positive control corresponds to the fluorescence with heparanase, biotin-heparan sulfate-Eu cryptate, Streptavidin-Xlent! solution and without inhibitor solutions. This value is the minimum fluorescence. The negative control corresponds to the fluorescence with biotin-heparan sulfate-Eu cryptate, Streptavidin-Xlent! solution and without heparanase, inhibitor solutions. This value is the maximum fluorescence. The background

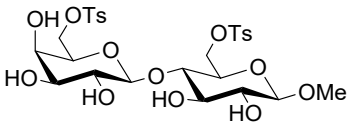
corresponds to the fluorescence with Streptavidin-Xlent! solution and without biotin-heparan sulfate-Eu cryptate, heparanase, inhibitor solutions

4. Synthetic procedures

Methyl 6,6'-di-*O*-tosyl-D-maltoside (2)

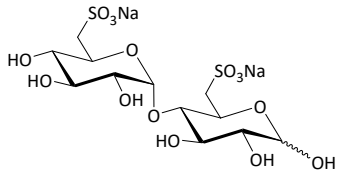
To a solution of methyl-β-maltoside[1] (0.38 g, 1.07 mmol) in dry pyridine (10.9 ml), anhydrous cobalt (II) chloride (83 mg, 0.642 mmol), was added and the reaction mixture was cooled to 0°C. Then p-toluenesulfonyl chloride (816 mg, 4.28 mmol), and anhydrous triethylamine (0.43 ml, 3.21 mmol) were slowly added and the reaction mixture stirred at room temperature for 4 h, The reaction was then quenched using methanol (50 mL). After stirring for 5 min., the mixture was concentrated to dryness *in vacuo*. The crude reaction mixture was purified over silica gel column chromatography (gradient EtOAc/Methanol (8/2; v/v). The desired product **2** was isolated as a white foam (0.29 g, 40.1%). $[\alpha]_D^{20}$ +40.9 (c 0.137, Methanol); ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.84 – 7.74 (m, CH-Ar, 4H), 7.47 – 7.38 (m, CH-Ar, 4H), 4.94 (d, J = 3.8 Hz, H1', 1H), 4.35 – 4.26 (m, H6, H6', 2H), 4.22 – 4.12 (m, H6, H6', 2H), 4.10 (d, J = 7.8 Hz, H1, 1H), 3.70 (ddd, J = 10.1, 4.5, 2.0 Hz, H5', 1H), 3.56 – 3.48 (m, H3, H3', H5, 3H), 3.42 (s, OMe, 3H), 3.36 – 3.21 (m, H2', H4, H4', 3H), 3.11 (dd, J = 9.5, 7.8 Hz, H2, 1H), 2.45 (s, CH_3, 3H), 2.44 (s, CH_3, 3H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 146.5 (C-Ar), 146.5 (C-Ar), 134.4 (C-Ar), 134.3 (C-Ar), 131.1 (C-Ar), 131.0 (C-Ar), 129.2 (C-Ar), 129.1 (C-Ar), 105.0 (C1), 102.8 (C1'), 81.2 (C4), 77.4 (C3), 74.8 – 73.5 (C2, C3', C4, C5), 72.2 (C5'), 70.5 (C6, C6'), 70.4 (C4'), 57.3 (OMe); IR (ATR) *v* = 3500, 1599, 1356, 1174, 1072, 993, 975, 927, 815 cm^{-1}. HRMS [M-Na$^+$]: calcd. For $C_{27}H_{36}O_{15}NaS_2$, 687.1393; found, 687.1397.

Methyl 6,6'-di-*O*-tosyl-D-lactoside (4)



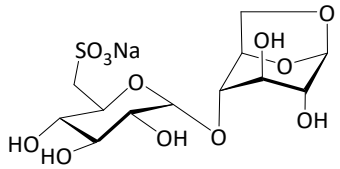
To a solution of methyl-β-lactoside[2] (2.5 g, 7.27 mmol) in dry pyridine (75 ml), anhydrous cobalt (II) chloride (0.5 g, 4.2 mmol), was added and the reaction mixture was cooled to 0°C. Then p-toluenesulfonyl chloride (4.3 g, 23 mmol), and anhydrous triethylamine (3.2 ml, 23 mmol) were slowly added and the reaction mixture stirred at room temperature for 45 min, The reaction was then quenched using methanol (150 mL). After stirring for 5 min., the mixture was concentrated to dryness *in vacuo*. The crude reaction mixture was purified over silica gel column chromatography (gradient EtOAc/Methanol (8/2; v/v). The desired product **4** was isolated as a white solid (1.78 g, 38%). MP: 81-85°C; $[\alpha]_D^{25}$ -8 (c 0.15, Methanol); ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.91 (dd, *J* = 15.7, 8.4 Hz, CH-Ar, 4H), 7.51 (t, *J* = 8.5 Hz, CHAr, 4H), 4.57 (dd, *J* = 11.0, 2.0 Hz, H6, 1H), 4.41 (dd, *J* = 11.0, 5.2 Hz, H6, 1H), 4.36 – 4.27 (m, H1', H6', 2H), 4.26 – 4.20 (m, H1, H6', 2H), 3.90 – 3.84 (m, H4', H5', 2H), 3.69 (ddd, *J* = 9.7, 5.3, 2.0 Hz, H5, 1H), 3.61 – 3.38 (m, H3, H4, H2', H3', OCH_3, 7H), 3.27 (dd, *J* = 9.1, 7.8 Hz, H2, 1H), 2.52 (s, Ar-CH_3, 6H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 145.4 (C-Ar), 145.1 (C-Ar), 133.0 (C-Ar), 132.6 (C-Ar), 129.9 (CH-Ar), 129.8 (CH-Ar), 127.9 (CH-Ar), 127.8 (CH-Ar), 103.5 (C1), 103.0 (C1'), 78.5 (C4), 74.4, 73.1, 72.8, 72.6, 72.1, 70.4 (C2, C3, C5, C2', C3', C5'), 68.9 (C6, C6'), 68.3 (C4'), 56.0 (OCH_3), 20.42 (Ar-CH_3); IR (ATR) *v* = 3419, 2891, 1355, 1174, 1074, 975, 815 cm^{-1}; HRMS [M-Na$^+$]: calcd. For $C_{27}H_{36}O_{15}NaS_2$, 687.1393; found, 687.1389.

6,6'-dideoxy-6,6'-disulfonato-D-maltose disodium salt (7).



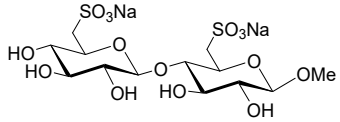
To a solution of compound **11** (0.18 g, 0.25 mmol) and sodium acetate (3 equiv., 62 mg, 0.75 mmol) in acetic acid (0.22 mL), 30% (w/w) aqueous hydrogen peroxide (9.4 equiv., 0.24 mL, 2.35 mmol) was added. The reaction mixture was heated at 60°C for 4h. The solution was then concentrated under reduced pressure. The crude product was dissolved in dry methanol (5 mL), and a methanolic solution of NaOMe (4.5 M) was added dropwise over a period of 15 min until a basic pH was reached (pH≈13). The solution was stirred for 3h at room temperature, and then concentrated *in vacuo* to remove acetic acid and traces of hydrogen peroxide. The crude product was purified on Sephadex LH20 (water), and lyophilized to afford compound **7** (22 mg, 66%), as a white powder. MP: 221-223°C; ^{1}H NMR (400 MHz, D_2O) δ 5.74-5.72 (m, H1'), 5.24 (d, *J* = 3.9 Hz, H1α), 4.70 (d, *J* = 8.0 Hz, H1β), 4.47 – 4.27 (m, H5'), 4.10 – 3.92 (m, H5β, H3'), 3.85 – 3.69 (m, H3β, H4'), 3.65 – 3.57 (m, H2α, H4β, H2'), 3.52 – 3.46 (m, H6'), 3.44 (dd, *J* = 14.8, 2.0 Hz, H6), 3.35-3.31 (m, H2β), 3.28 – 3.19 (m, H6), 3.16 (dd, *J* = 14.9, 9.0 Hz, H6); ^{13}C NMR (101 MHz, D_2O) δ 96.8 (C1'), 96.7 (C1'), 95.8 (C1β), 91.6 (C1β), 76.4-69.1 (C2, C3, C4, C5, C2', C3', C4'), 66.1 (C5'), 52.5 (C6'), 52.0 (C6); IR (ATR) ν = 3346, 1616, 1419, 1170, 1039 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{12}H_{20}O_{15}Na_3S_2$, 536.9937; found, 536.9943.

1,6-anhydro-6'-deoxy-6'-sulfonatomaltose sodium salt (8).



To a solution of **1**[3] (0.2 g, 0.307 mmol) in water (2.5 ml) was added sodium sulfite (0.310 g, 2.46 mmol). The reaction mixture was then heated at reflux for 6h30. Then, the mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The crude product was purified on Sephadex LH20 (water), and lyophilized to afford compound **8** (75 mg, 60%), as a white powder. MP: 279-281°C; $[\alpha]_D^{20}$ +15 (c 0.01, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.51 (t, *J* = 1.7 Hz, 1H, H1), 5.15 (d, *J* = 3.9 Hz, 1H, H1'), 4.90 – 4.83 (m, 1H, H4), 4.21 – 4.11 (m, 2H, H6a, H5'), 3.91 – 3.78 (m, 4H, H3, H3', H5, H6b), 3.64 (dd, *J* = 9.9, 3.9 Hz, 1H, H2'), 3.60 (d, *J* = 1.6 Hz, 1H, , H2), 3.43 (dd, *J* = 14.7, 1.7 Hz, 1H, H6a'), 3.33 (dd, *J* = 10.0, 9.1 Hz, 1H, H4'), 3.12 (dd, *J* = 14.7, 10.1 Hz, 1H, H6b'). ^{13}C NMR (101 MHz, D_2O) δ 101.1 (C1), 96.5 (C1'), 75.5 (C4), 74.0 (C3), 73.0 (C3'), 72.4 (C4'), 71.3 (C2'), 69.5 (C2), 69.2 (C5), 68.7 (C5'), 65.4 (C6), 52.0 (C6'); IR (ATR) *v* = 3541, 1635, 1442, 1139, 1031, 966 cm^{-1}; HRMS [M-Na$^-$]: calcd. For $C_{12}H_{19}O_{12}S$, 387.0597; found, 387.0598.

Methyl 6,6'-dideoxy-6,6'-disulfonato-β-cellobioside disodium salt (9).



<u>Method using H_2O_2:</u>

This compound was obtained as previously described for compound **7**, using compound **13** (95 mg, 0.14 mmol)**,** sodium acetate (34 mg, 0.41 mmol), acetic acid (0.12 mL) and 30% (w/w) aqueous

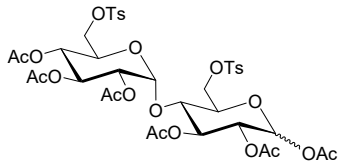
hydrogen peroxide (0.16 mL, 1.57 mmol). The reaction was stirred at 60°C for 12h and concentrated under vacuum to remove acetic acid and traces of hydrogen peroxide. Purification on Sephadex LH20 (water) led, after lyophilization, to compound **9** (45 mg, 61%) as a white powder.

Method using Na_2SO_3:

To a solution of **3** (0.2 g, 0.301 mmol) in water (2.5 ml) was added sodium sulfite (8 equiv., 0.303 g, 2.408 mmol). The reaction mixture was then heated at reflux for 6h. Then, the mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The crude product was purified on Sephadex LH20 (water), and lyophilized to afford compound **9** (88 mg, 55%), as a white powder.

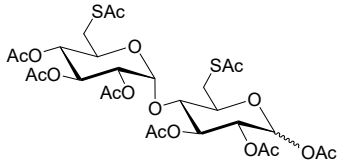
MP: 232-235°C; ^{1}H NMR (400 MHz, D_2O) δ 4.59 (d, *J* = 8.0 Hz, H1', 1H), 4.42 (d, *J* = 8.1 Hz, H1, 1H), 3.95 (td, *J* = 9.6, 1.6 Hz, H5, 1H), 3.85 (td, *J* = 9.7, 1.8 Hz, H5', 1H), 3.67 – 3.60 (m, H3, H6b, 2H), 3.59 (s, OCH_3, 3H), 3.58 – 3.51 (m, H4, H3', 2H), 3.45 (dd, *J* = 14.8, 1.8 Hz, H6b', 1H), 3.40 – 3.30 (m, H2, H2', H4', 3H), 3.18-3.11 (m, H6a, H6a', 2H); ^{13}C NMR (101 MHz, D_2O) δ 103.1 (C1'), 102.7 (C1), 81.9 (C4), 75.2 (C3'), 74.7 (C3), 73.1, 73.0, 72.1 (C2, C2', C4'), 72.1 (C5'), 70.9 (C5), 57.1 (OCH_3), 51.8 (C6'), 51.7 (C6); $[\alpha]_D^{20}$= +7 (c 0.28, H_2O); IR (ATR) ν = 3412, 1618, 1408, 1172, 1039 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{13}H_{22}O_{15}Na_3S_2$, 551.0093; found, 551.0108.

1,2,2',3,3',4'-hexa-*O*-acetyl-6,6'-di-*O*-tosyl-D-maltose (10).[4,5]



To a solution of 6,6'-di-*O*-tosyl-D-maltose[3] (1 g, 1.43 mmol) in dry pyridine (1.8 ml), was added anhydride acetic (4.4 ml). After stirring overnight at room temperature under argon, the reaction was quenched with methanol dropwise at 0°C. The resulting mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate and was washed with 1M HCl, saturated $NaHCO_3$ and water successively. The organic phase was then dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using EtOAc/cyclohexane (1/1; v/v). The desired product **10** was isolated as a white powder (1.2 g, 98%). MP: 107-109°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84 – 7.72 (m, CH-Ar), 7.40 – 7.30 (m, CH-Ar), 6.00 (d, J = 3.7 Hz, H1), 5.59 (d, J = 7.9 Hz, H1), 5.40 (dd, J = 10.2, 8.8 Hz, H3), 5.35 – 5.25 (m, H1'), 5.16 (t, J = 8.9 Hz, H3), 5.04 (dt, J = 13.8, 9.9 Hz, H2', H3'), 4.80 – 4.69 (m, H2, H2', H3'), 4.64 (dd, J = 10.2, 3.7 Hz, H2), 4.36 – 4.08 (m, H6, H6'), 4.05 – 3.57 (m, H4 , H5, H4', H5'), 2.54 – 2.37 (m, CH_3-Ar), 2.21 – 1.89 (m, CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4 - 168.7 (CO), 145.6 - 132.3 (C-Ar), 130.1 - 127.9 (CHAr), 95.5 (C1'), 95.3 (C1'), 91.1 (C1), 88.6 (C1), 74.8– 67.8 (C2, C3, C4, C5, C2', C3', C4', C5'), 67.6 - 67.1 (C6, C6'), 21.8 - 21.7 (CH_3-Ar), 21.0 - 20.4 (m, CH_3); IR (ATR) ν = 2983, 1755, 1365, 1215, 1176, 1041, 939, 817 cm^{-1}; HRMS [M+NH_4^+]: calcd. For $C_{38}H_{50}NO_{21}S_2$, 920.2317; found, 920.2322.

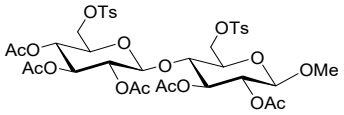
1,2,2',3,3',4'-hexa-*O*-acetyl-6,6'-di-*S*-acetyl-D-maltose (11).



Potassium thioacetate (0.25 g, 2.21 mmol) was added to a solution of compound **10** (0.5 g, 0.55 mmol) in dry DMF (3 mL) and the reaction was stirred at 60 °C overnight. The reaction was then

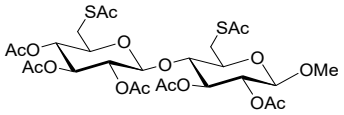
cooled to room temperature, diluted with ethyl acetate (50 mL) and washed with brine (3×50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness in *vacuo*. The crude product was purified by silica gel column chromatography using EtOAc/cyclohexane (1/1; v/v). Pure compound **11** (0.33 g, 84%) was obtained as a brown powder. MP: 78-80°C. ^{1}H NMR (600 MHz, CD_3OD) δ 6.17 (d, J = 3.7 Hz, H1), 5.76 (d, J = 8.1 Hz, H1), 5.46 – 5.30 (m, H3, H1', H2'), 5.01 – 4.84 (m, H2, H3', H4'), 4.26-4.22 (m, H5'), 4.01 – 3.78 (m, H4, H5), 3.62 (ddd, J = 13.9, 2.9, 1.7 Hz, H6), 3.30 – 3.14 (m, H6'), 2.99 (ddd, J = 13.9, 8.5, 4.2 Hz, H6), 2.38 – 2.36 (m, CH_3CO-S), 2.34 – 2.31 (m, CH_3CO-S), 2.10 – 1.94 (m, CH_3CO); ^{13}C NMR (151 MHz, CD_3OD) δ 196.3, 196.0, 196.0 (CO-S), 171.9-170.3 (CO), 97.4 (C1'), 97.3 (C1'), 92.5 (C1), 90.0 (C1), 77.6 - 70.z8 (C2, C3, C4, C5, C2', C3', C4', C5'), 32.4 (C6), 32.3 (C6), 30.7 (C6'), 30.4 (CH_3CO-S), 21.1 - 20.3 (CH_3CO); IR (ATR) ν = 1753, 1697, 1369, 1217, 1134, 1074, 1037 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{28}H_{38}O_{17}NaS_2$, 733.1448; found, 733.1454.

Methyl 2,2',3,3',4'-penta-*O*-acetyl-6,6'-di-*O*-tosyl-β-cellobioside (12).



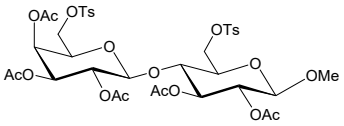
This compound was obtained as previously described for compound **10**, using compound **3**[3] (2 g, 3 mmol), dry pyridine (3.6 mL) and anhydride acetic (7 mL). Purification by silica gel column chromatography using EtOAc/cyclohexane (1/1; v/v) led to compound **12** as a white powder (2.22 g, 98%). Analytical data were in agreement with those previously reported.[2] MP: 92-94°C; $[\alpha]_D^{20}$ = +0.6 (c 0.5, DCM); IR (ATR) ν = 3660, 2983, 2902, 1755, 1365, 1240, 1219, 1176, 1051, 815 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{37}H_{46}O_{20}NaS_2$, 897.1922; found, 897.1930.

Methyl-2,2',3,3',4'-penta-*O*-acetyl-6,6'-dithioacetyl-β-cellobioside (13).



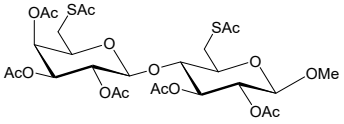
This compound was obtained as previously described for compound **11,** using compound **12** (0.2 g, 0.23 mmol), dry DMF (4.5 mL) and potassium thioacetate (0.12 g, 1.02 mmol). The reaction was stirred at 110 °C for 12 h. Purification by silica gel column chromatography using EtOAc/cyclohexane (1/1; v/v) led to compound **13** (0.09 g, 58%) as a brown solid. Analytical data were in agreement with those previously reported.[2] MP: 180-182°C; ^{1}H NMR (600 MHz, CD_3CN) δ 5.16 (t, *J* = 9.6 Hz, H3', 1H), 5.07 (dd, *J* = 9.9, 9.1 Hz, H3, 1H), 4.90 (t, *J* = 9.7 Hz, H4', 1H), 4.76 (ddd, *J* = 21.1, 9.8, 8.1 Hz, H2, H2', 2H), 4.69 (d, *J* = 8.1 Hz, H1', 1H), 4.41 (d, *J* = 8.1 Hz, H1, 1H), 3.71 (ddd, *J* = 9.9, 7.0, 3.1 Hz, H5', 1H), 3.66 (t, *J* = 9.4 Hz, H4, 1H), 3.57 (ddd, *J* = 9.6, 8.9, 2.8 Hz, H5, 1H), 3.49 (dd, *J* = 13.7, 2.8 Hz, H6, 1H), 3.20 (dd, *J* = 14.3, 3.0 Hz, H6', 1H), 3.02 (ddd, *J* = 13.6, 8.0, 5.9 Hz, H6, H6', 2H), 2.33 (s, $SCOCH_3$, 3H), 2.32 (s, $SCOCH_3$, 3H), 2.02 (s, $OCOCH_3$, 3H), 2.00 (s, $OCOCH_3$, 3H), 2.00 (s, $OCOCH_3$, 3H), 1.97 (s, $OCOCH_3$, 3H), 1.92 (s, $OCOCH_3$, 3H); ^{13}C NMR (151 MHz, CD_3CN) δ 195.7 ($SCOCH_3$), 195.4 ($SCOCH_3$), 170.8 - 170.3 ($OCOCH_3$), 101.9 (C1), 100.9 (C1'), 79.7 (C4), 74.0 (C5), 73.6 (C5'), 73.4 (C3'), 72.9 (C3), 72.3, 72.1 (C2, C2'), 71.3 (C4'), 57.2 (OCH_3), 31.0 (C6), 30.7 ($SCOCH_3$), 30.6 ($SCOCH_3$), 30.6 (C6'), 21.2 - 20.8 ($OCOCH_3$); $[\alpha]_D^{20}$ = -5.6 (c 0.11, DCM); IR (ATR) ν = 2933, 1747, 1695, 1371, 1222, 1045 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{27}H_{38}O_{16}NaS_2$, 705.1499; found, 705.1506.

Methyl 2,2',3,3',4'-penta-*O*-acetyl-6,6'-di-*O*-tosyl-β-lactoside (14).



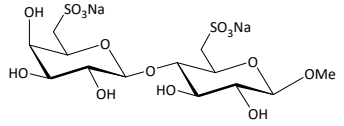
This compound was obtained as previously described for compound **10**, using compound **4** (1.73 g, 2.6 mmol), dry pyridine (3.14 ml) and anhydride acetic (6.15 ml, 64 mmol). Purification by silica gel column chromatography using EtOAc: cyclohexane (1:1; v: v). afforded compound **14** as white powder (2.22 g, 98%). Analytical data were in agreement with those previously reported.[2] MP: 86-91°C. $[\alpha]_D^{25}$ = -9 (c 0.15, DCM); IR (ATR) ν = 1751, 1346, 1234, 1215, 1172, 1058, 975, 923, 827 cm^{-1}; HRMS [M+Na^+]: calcd. For $C_{37}H_{46}O_{20}NaS_2$, 897.1922; found, 897.1952.

Methyl 2,2',3,3',4'-penta-*O*-acetyl-6,6'-dideoxy-6,6'-di-*S*-acetyl-β-lactoside (15).



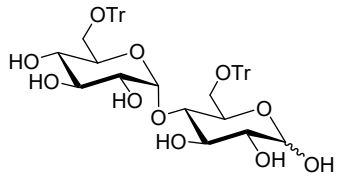
This compound was obtained as previously described for compound **11,** using compound **14** (0.5 g, 0.57 mmol), dry DMF (6 mL) and potassium thioacetate (0.52 g, 4.57 mmol). The reaction was stirred at 110 °C for 20 h. Purification by silica gel column chromatography using EtOAc/cyclohexane (1/1; v/v) led to pure compound **15** (0.23 g, 51%) as a brown solid. Analytical data were in agreement with those previously reported.[2] MP: 100-105°C. $[\alpha]_D^{25}$ = +28 (c 0.1, DCM); IR (ATR) ν = 2983, 1749, 1693, 1369, 1238, 1220, 1055 cm^{-1}; HRMS [M+Na^+]: calcd. For $C_{27}H_{38}O_{16}NaS_2$, 705.1499; found, 705.1506;

Methyl 6,6'-dideoxy-6,6'-disulfonato-β-lactoside disodium salt (16).



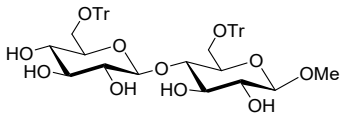
This compound was obtained as previously described for compound **7**, using compound **15** (0.14 g, 0.21 mmol), sodium acetate (50 mg, 0.61 mmol), acetic acid (0.17 mL) and 30% (w/w) aqueous hydrogen peroxide (0.23 mL, 2.25 mmol). The reaction was stirred at 60°C for 12h and concentrated under vacuum to remove acetic acid and traces of hydrogen peroxide. Purification on Sephadex LH20 (water), led to compound **16** (58 mg, 55%) as a white powder after lyophilization. MP: 241-243°C; ^{1}H NMR (400 MHz, D_2O) δ 4.53 (d, *J* = 7.9 Hz, H1', 1H), 4.42 (d, *J* = 8.1 Hz, H1, 1H), 4.12 (ddd, *J* = 7.1, 4.8, 1.1 Hz, H5', 1H), 4.01 – 3.91 (m, H5, H4', 2H), 3.79 – 3.71 (m, H3', 1H), 3.69 – 3.60 (m, H3, H6b, 2H), 3.60 – 3.53 (m, H4, OCH_3, H2', 5H), 3.35 (dd, *J* = 9.3, 8.1 Hz, H2, 1H), 3.29 – 3.24 (m, H6', 2H), 3.15 (dd, *J* = 14.6, 9.8 Hz, H6a, 1H); ^{13}C NMR (101 MHz, D_2O) δ 103.4 (C1'), 102.8 (C1), 81.5 (C4), 74.6 (C3), 73.0 (C2), 72.3 (C3'), 71.2 (C5'), 71.0, 70.6, 70.3 (C5, C2', C4'), 57.1 (OCH_3), 51.8 (C6'), 51.6 (C6); $[\alpha]_D^{20}$ = +30 (c 0.04, H_2O); IR (ATR) ν = 3429, 1608, 1431, 1172, 1122, 1041 cm^{-1}; HRMS [M+Na^+]: calcd. For $C_{13}H_{22}O_{15}Na_3S_2$, 551.0093; found, 551.096.

6,6'-di-*O*-Trityl-D-maltose (17).



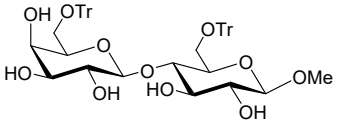
This compound was obtained as previously described for compound **19**, using commercial D-maltose monohydrate (2 g, 5.8 mmol), dry pyridine (60 mL) and triphenylmethyl chloride (6 equiv., 9 g, 35 mmol) for 24h at room temperature. Purification by silica gel column chromatography using EtOAc/MeOH (9/1; v/v) led to compound **17** as a white solid (3.14 g, 65 %). MP: 143-145°C; ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.48 – 7.40 (m, 11H), 7.39 – 7.22 (m, 24H), 7.15 – 7.02 (m, 15H), 5.21-5.19 (m, H1α, H1', 1.8H), 5.13 (d, *J* = 3.7 Hz, H1', 1H), 4.59 (d, *J* = 7.8 Hz, H1β, 1H), 4.20 – 4.13 (m, H4', 1.9H), 3.94 (dd, *J* = 9.8, H3', 1H), 3.69 – 3.12 (m, H2, H3, H4, H5, H2', H5', H6', methanol peak included, 23H), 3.00 – 2.85 (m, H6, 3,3H); ^{13}C NMR (101 MHz, CD_3OD) δ 145.6 (CAr), 145.3 (CAr), 145.2 (CAr), 130.0 - 127.9 (CHAr), 102.2 (C1'), 98.0 (C1β), 93.5 (C1α), 87.8 (CPh_3), 87.7 (CPh_3), 87.5 (CPh_3), 87.4 (CPh_3), 81.8 - 71.1 (C2, C3, C4, C5, C2', C3', C5'), 70.7 (C4'), 65.3 (C6'), 65.1 (C6'), 63.9 (C6), 63.6 (C6); IR (ATR) *v* = 1489, 1448, 1045, 1033, 900, 775, 765, 707 cm^{-1}**;** HRMS [M+Na$^+$]: calcd. For $C_{50}H_{50}O_{11}Na$, 849.3251; found, 849.3280.

Methyl 6,6'-di-*O*-trityl-β-cellobioside (18).



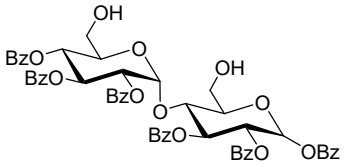
This compound was obtained as previously described for compound **19**, using methyl β-cellobioside[2] (2 g, 5.61 mmol), dry pyridine (56 mL) and triphenylmethyl chloride (6 equiv., 10 g, 33.6 mmol) for 48h at room temperature. Purification by silica gel column chromatography using EtOAc/MeOH (9/1; v/v) led to compound **18** as a white solid (2.9 g, 61%). MP: 141-144°C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.57-7.53 (m, CHAr, 12H), 7.33 (dd, *J* = 8.4, 6.7 Hz, CHAr, 6H), 7.29 – 7.17 (m, CHAr, 12H), 4.39 – 4.35 (m, H1', 1H), 4.32 – 4.23 (m, H1, H4, 2H), 3.69 – 3.50 (m, H2, H3, H5, H6, H6', OCH3, 8H), 3.39 – 3.29 (m, H6, H5', 2H), 3.26 – 3.11 (m, H2', H3', H4', H6', 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 145.3 (CAr), 145.3 (CAr), 130.0 - 128.0 (CHAr), 104.9 (C1), 102.4 (C1'), 87.8 (CPh_3), 87.6 (CPh_3), 77.8 (C4), 77.6-74.8 (C2, C3, C5, C2', C3', C5'), 72.0 (C4'), 64.3 (C6'), 63.1 (C6), 56.9 (OCH_3); $[\alpha]_D^{25}$= +3 (c 0.1, MeOH); IR (ATR) *v* = 3454, 2881, 1732, 1490, 1448, 1244, 1045, 765, 707 cm^{-1}**;** HRMS [M+Na$^+$]: calcd. For $C_{51}H_{52}O_{11}Na$, 863.3407; found, 863.3401.

Methyl 6,6'-di-*O*-trityl-β-lactoside (19).



To a solution of methyl β-lactoside[2] (1 g, 2.8 mmol) in dry pyridine (28 mL), triphenylmethyl chloride (6 equiv., 5 g, 16.8 mmol) was added and the reaction mixture was stirred at room temperature for 24h. The reaction was then quenched using methanol. After stirring for 5 min, the mixture was evaporated under reduced pressure. The crude was purified by silica gel column chromatography using EtOAc/MeOH (9/1; v/v). The desired product **19** was isolated as a white solid (1.7g, 70 %). MP: 153-157 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.54-7.50 (m, CHAr, 10H), 7.37 – 7.18 (m, CHAr, 20H), 4.28 (d, *J* = 7.7 Hz, H1, 1H), 4.21 (d, *J* = 7.7 Hz, H1', 1H), 4.15-4.09 (m, H4, 1H), 3.73 (d, *J* = 3.3 Hz, H4', 1H), 3.68 – 3.12 (m, H2, H3, H5, H6, H2', H3', H5', H6', OCH_3, 13H); ^{13}C NMR (101 MHz, CD_3OD) δ 145.4 (CO), 145.3 (CO), 129.9 (CHAr), 129.9 (CHAr), 129.8 (CHAr), 128.8 (CHAr), 128.8 (CHAr), 128.7 (CHAr), 128.1 (CHAr), 128.0 (CHAr), 105.0 (C1), 103.2 (C1'), 88.0 (CPh_3), 87.62 (CPh_3), 77.4 - 70.2 (C2, C3, C4, C5, C2', C3', C4', C5'), 63.5, 63.1 (C6, C6'), 56.9 (OCH_3); $[\alpha]_D^{25}$= +29 (c 0.11, MeOH); IR (ATR) v = 3425, 3072, 1649, 1448, 1047, 761, 704 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{51}H_{52}O_{11}Na$, 863.3407; found, 863.3392.

1,2,3,2',3',4'-hexa-*O*-benzoyl-D-maltose (20).

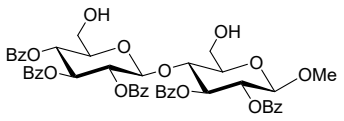


To a solution of compound **17** (0.2 g, 0.241 mmol) in dry pyridine (3.3 mL), benzoyl chloride (30 equiv., 0.85 mL, 7.3 mmol) was added. After stirring overnight at room temperature, the reaction was quenched with methanol. The resulting mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate and was washed with 1M HCl, saturated $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. To the crude dissolved in dry DCM (43 mL), Iron (III) chloride hexahydrate (4 equiv., 0.262 g, 0.97 mmol) was added, and the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate and was washed with water (3x50 ml), dried over anhydrous Na_2SO_4, filtered and evaporated. The crude was purified over silica gel column chromatography using EtOAc/cyclohexane (6/4; v/v), leading to the α-anomer **20α** as a white solid (0.107 g, 46 %) and the β-anomer **20β** as a white solid (0.076 g, 33%).

β-anomer: MP: 140-142°C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 – 7.21 (m, CHAr, 30H), 6.33 (d, *J* = 8.1 Hz, H1, 1H), 6.09 (dd, *J* = 10.6, 9.5 Hz, H3', 1H), 6.01 (t, *J* = 9.4 Hz, H3, 1H), 5.83 (d, *J* = 3.9 Hz, H1', 1H), 5.67 – 5.56 (m, H2, H4', 2H), 5.31 (dd, *J* = 10.5, 3.9 Hz, H2', 1H), 4.70 (t, *J* = 9.0 Hz, H4, 1H), 4.40 (ddd, *J* = 10.2, 4.8, 2.3 Hz, H5', 1H), 4.21 – 4.08 (m, H5, H6, 4H), 3.92 – 3.70 (m, H6', 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 167.1 (CO) , 166.7(CO) , 166.7 (CO) , 166.6 (CO), 166.5 (CO), 165.8 (CO) , 134.9 - 130.4 (10xCHAr), 130.3 - 129.9 (6xCAr), 129.6 - 129.1 (5xCHAr), 98.0 (C1'), 93.8 (C1), 77.4 (C5), 76.6 (C3), 73.2 (C4), 73.0 (C2), 72.7 (C5'), 72.3 (C2'), 71.8 (C3'), 70.4 (C4'), 61.6 (C6'), 61.5 (C6); $[\alpha]_D^{20}$= +20 (c 0.05, DCM); IR (ATR) *v* = 2983, 1728, 1452, 1267, 1093, 1068, 1026, 707 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{54}H_{46}O_{17}Na$, 989.2633; found, 989.2651.

α-anomer: MP: 137-149°C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.13 – 8.06 (m, CHAr, 2H), 7.97 – 7.91 (m, CHAr, 2H), 7.75 – 7.64 (m, CHAr, 8H), 7.53 – 7.13 (m, CHAr, 18H), 6.82 (d, *J* = 3.6 Hz, H1, 1H), 6.25 (t, *J* = 9.7 Hz, H3, 1H), 6.14 (dd, *J* = 10.5, 9.5 Hz, H3', 1H), 5.91 (d, *J* = 3.9 Hz, H1', 1H), 5.64 (t, *J* = 9.9 Hz, H4', 1H), 5.50 (dd, *J* = 10.2, 3.6 Hz, H2, 1H), 5.38 (dd, *J* = 10.5, 3.9 Hz, H2', 1H), 4.77 (t, *J* = 9.5 Hz, H4, 1H), 4.44 (ddd, *J* = 10.3, 4.9, 2.3 Hz, H5', 1H), 4.37 (dt, *J* = 9.8, 2.5 Hz, H5, 1H), 4.05-4.22 (m, H6, 2H), 3.94 – 3.72 (m, H6', 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 167.1 (CO), 166.7 (2xCO), 166.6 (CO), 166.6 (CO), 166.0 (CO), 134.9 - 130.4 (CHAr), 130.2 (CAr), 130.2 (CAr), 130.1 (CAr), 130.1 (CAr), 129.8 - 129.1 (CHAr), 98.3 (C1'), 91.4 (C1), 75.3 (C5), 74.1 (C3), 73.3 (C4), 72.8 (C5'), 72.5 (C2), 72.3 (C2'), 71.8 (C3'), 70.4 (C4'), 61.7 (C6'), 61.5 (C6); $[\alpha]_D^{20}$= +22 (c 0.05, DCM); IR (ATR) *v* = 2954, 1730, 1263, 1093, 1068, 1026,707 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{54}H_{46}O_{17}Na$, 989.2633; found, 989.2670.

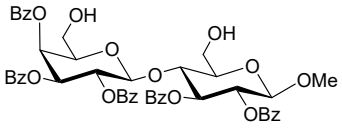
Methyl 2,2',3,3',4'-penta-*O*-benzoyl-β-cellobioside (21).



To a solution of compound **18** (0.34 g, 0.4 mmol) in dry pyridine (20 mL), benzoyl chloride (35 equiv., 1.6 mL, 14 mmol) was added. After stirring overnight at room temperature, the reaction was quenched with methanol. The resulting mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate and was washed with 1M HCl, saturated $NaHCO_3$ and water,

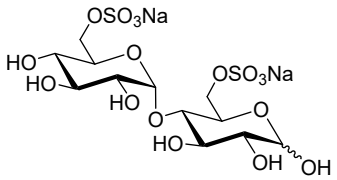
dried over anhydrous Na_2SO_4, filtered and the solvent evaporated. To a solution of the crude in dry DCM (72 ml), Iron (III) chloride hexahydrate (4 equiv., 0.435 g, 1.61 mmol) was added. The reaction mixture was stirred at room temperature for 2.5 h. The resulting mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate and the solution was washed with water (3x50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated. The crude was purified by silica gel column chromatography (EtOAc/cyclohexane; 6/4; v/v). The desired product **21** was isolated as a white solid (0.276 g, 78 %). MP: 136-138°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 – 8.05 (m, CH-Ar, 2H), 8.03 – 7.98 (m, CH-Ar, 2H), 7.98 – 7.94 (m, CH-Ar, 2H), 7.89 – 7.84 (m, CH-Ar, 2H), 7.82 – 7.77 (m, CH-Ar, 2H), 7.59 – 7.21 (m, CH-Ar, 15H), 5.86 (t, J = 9.7 Hz, H3', 1H), 5.74 (t, J = 9.5 Hz, H3, 1H), 5.50 (dd, J = 9.9, 7.9 Hz, H2', 1H), 5.45 – 5.27 (m, H2, H4', 2H), 5.07 (d, J = 8.0 Hz, H1', 1H), 4.61 (d, J = 7.9 Hz, H1, 1H), 4.32 (t, J = 9.5 Hz, H4, 1H), 3.89 – 3.77 (m, H6, 2H), 3.69 (ddd, J = 10.1, 5.1, 2.2 Hz, H5', 1H), 3.54 – 3.42 (m, H5, OCH_3, 4H), 3.36 (dd, J = 12.7, 2.1 Hz, H6', 1H), 3.10 (dd, J = 12.7, 5.1 Hz, H6', 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7 - 165.3 (CO), 164.8 - 128.3 (CHAr), 101.9 (C1), 100.8 (C1'), 75.3 (C4), 75.1 (C5), 74.8 (C5'), 73.4 (C3), 73.1 (C3'), 72.1 (C2'), 71.9 (C2), 68.9 (C4'), 61.1 (C6'), 60.3 (C6), 57.3 (OCH_3); $[\alpha]_D^{25}$= -17 (c 0.08, DCM); IR (ATR) *v* = 3495, 2943, 1728, 1261, 1091, 1070, 1028, 707 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{48}H_{44}O_{16}Na$, 899.2527; found, 899.2545.

Methyl 2,2',3,3',4'-penta-*O*-benzoyl-β-lactoside (22).



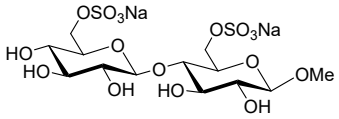
To a solution of compound **19** (0.2 g, 0.237 mmol) in dry pyridine (3 mL), benzoyl chloride (36 equiv., 1 mL, 8.6 mmol) was added. After stirring overnight at room temperature, the reaction was quenched with methanol. The resulting mixture was concentrated under reduced pressure and the residue was diluted with ethyl acetate. The organic phase was washed with 1M HCl, saturated $NaHCO_3$ and water, then dried over anhydrous Na_2SO_4, filtered and evaporated. To a solution of the crude in dry DCM (43 mL), was added Iron (III) chloride hexahydrate (4 equiv., 0.256 g, 0.95 mmol), and the reaction was stirred at room temperature for 3 h and concentrated under reduced pressure. The residue was diluted with ethyl acetate and the organic phase was washed with water (3x50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated. The crude was purified by silica gel column chromatography (EtOAc/cyclohexane, 6/4, v/v). The desired product **22** was isolated as a white solid (0.177 g, 85 %). MP: 156-160°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 – 7.18 (m, CHAr, 25H), 5.96 – 5.77 (m, H3, H2', H4', 3H), 5.67 (dd, J = 10.4, 3.3 Hz, H3', 1H), 5.57 (dd, J = 9.9, 8.0 Hz, H2, 1H), 5.18 (d, J = 7.9 Hz, H1', 1H), 4.73 (d, J = 7.9 Hz, H1, 1H), 4.47 (t, J = 9.5 Hz, H4, 1H), 4.06 – 3.89 (m, H6, H5', 3H), 3.64 (dt, J = 9.8, 2.5 Hz, H5, 1H), 3.58 (s, OMe, 3H), 3.14 (dd, J = 11.9, 6.6 Hz, H6', 1H), 2.96 (dd, J = 11.9, 6.7 Hz, H6', 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3 - 164.9 (CO), 133.7 – 129.5 (CHAr), 129.3 – 128.7 (CAr), 128.6 (CHAr), 128.5 (CAr), 128.3 (CHAr), 128.3 (CHAr), 128.1 (CHAr), 102.0 (C1), 100.7 (C1'), 75.2 (C4), 74.7 (C5'), 73.8 (C5), 73.3 (C3), 72.0 (C3'), 71.7 (C2), 70.4 (C2'), 68.5 (C4'), 60.2 (C6), 59.8 (C6'), 57.2 (OMe); $[\alpha]_D^{25}$= +47 (c 0.09, DCM); IR (ATR) *v* = 3587, 1726, 1452, 1269, 1070, 1028, 709 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{48}H_{44}O_{16}Na$, 899.2527; found, 899.2523.

6,6'-disulfato-D-maltose (23).



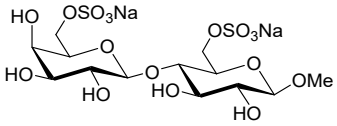
Compound **20** (0.15 g, 0.155 mmol) and SO_3.pyridine complex (50% wt) (30 equiv., 0.74 g, 4.6 mmol) were mixed and then dried under high reduced pressure. After 12h, dry DMF (3.5 mL) was added and the reaction mixture was stirred at 60 °C for 5h, and then quenched with methanol. The resulting mixture was concentrated under reduced pressure. The crude was dissolved in dry methanol (5 ml), and a methanolic solution of NaOMe (4.5 M) was added dropwise over a period of 15 min until a basic pH was reached (pH≈13). The solution was stirred for 3h at room temperature, and then evaporated. After purification over Sephadex LH20 (water), the desired product **23** was isolated as a white solid (38 mg, 45%). MP: 210-212°C; ^{1}H NMR (400 MHz, D_2O) δ 5.55-5.53 (m, H1', 1.7H), 5.26 (d, *J* = 3.8 Hz, H1α, 0.9H), 4.71 (d, *J* = 8.0 Hz, H1β, 1H), 4.45 – 4.15 (m, H6, H6', 8.6H), 4.06 – 3.68 (m, H3β, H4, H5, H4', H5', 8.6H), 3.67 – 3.54 (m, H2α, H3α, H2', H3', 5.73H), 3.33 (dd, *J* = 9.4, 8.0 Hz, H2β, 1H); ^{13}C NMR (101 MHz, D_2O) δ 99.0 (C1'), 95.8 (C1β), 91.9 (C1α), 76.2, 75.5, 75.5, 73.9, 73.3, 72.5, 72.4, 71.5, 71.4, 71.1, 70.5, 68.5, 68.0 (C2, C3, C4, C5, C2', C3', C4', C5'), 67.2, 66.4 (C6, C6'); IR (ATR) *v* = 3213, 1591, 1421, 1224, 1058, 1002, 603 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{12}H_{20}O_{17}Na_3S_2$, 568.9835; found, 568.9838.

Methyl 6,6'-disulfato-β-cellobioside (24).



This compound was obtained as previously described for compound **23**, using compound **21** (0.11 g, 0.112 mmol), dry DMF (2.11 mL), SO_3.pyridine complex (22 equiv., 0.4 g, 2.5 mmol) for 12h at 60°C. Purification on Sephadex LH20 (water) led to the desired product **24** isolated as a white solid (30 mg, 49%). MP: 255-257°C; ^{1}H NMR (400 MHz, D_2O) δ 4.61 (d, *J* = 7.9 Hz, H1', 1H), 4.48 – 4.22 (m, H1, H6, H6', 5H), 3.85 (ddd, *J* = 6.6, 4.5, 2.3 Hz, H5, 1H), 3.77 – 3.64 (m, H4, H3', H5', 3H), 3.60 (s, OCH_3, 3H), 3.54 (m, H3, H4', 2H), 3.35 (m, H2, H2', 2H); ^{13}C NMR (101 MHz, D_2O) δ 103.0 (C1), 102.5 (C1'), 78.5 (C4), 75.2 (C3), 74.2, 73.6 (C3', C5'), 73.0, 72.6 (C2, C2'), 72.4 (C5), 69.1 (C4'), 66.9, 66.2 (C6, C6'), 57.2 (OCH_3); $[\alpha]_D^{25}$= -9 (c 0.13, H_2O); IR (ATR) *v* = 3253, 1616, 1435, 1213, 1166, 1043, 1008, 698, 605 cm^{-1}; HRMS [M+Na$^+$]: calcd. For $C_{13}H_{22}O_{17}Na_3S_2$, 582.9991; found, 583.0016.

Methyl 6,6'-disulfato-β-lactoside (25).



This compound was obtained as previously described for compound **23**, using compound **22** (0.15 g, 0.171 mmol), dry DMF (2.8 mL), SO_3.pyridine complex (20 equiv., 0.544 g, 3.4 mmol) for 12h at 60°C. Purification over Sephadex LH20 (water) led to the desired product **25** isolated as a white solid (60 mg, 63%). MP: 271-273°C; ^{1}H NMR (400 MHz, D_2O) δ 4.56 (d, *J* = 7.9 Hz, H1', 1H), 4.49 – 4.41 (m, H1, H6, 2H), 4.34 (dd, *J* = 11.1, 4.7 Hz, H6, 1H), 4.25 (d, *J* = 6.1 Hz, H6', 2H), 4.06 – 3.99 (m, H4', H5', 2H), 3.87 (ddd, *J* = 9.4, 4.7, 2.0 Hz, H5, 1H), 3.76 – 3.66 (m, H3, H4, H3', 3H), 3.64 – 3.54 (m, OCH_3, H2', 5H), 3.43 – 3.33 (m, H2, 1H); ^{13}C NMR (101 MHz, D_2O) δ 103.0 (C1), 102.9 (C1'), 78.7 (C4), 74.3

(C3/C3'), 72.8 (C4'/C5'), 72.6 (C2), 72.4 (C5), 72.3 (C3/C3'), 70.7 (C2'), 68.2 (C4'/C5'), 67.2 (C6'), 66.4 (C6), 57.3 (OCH_3); $[\alpha]_D^{25}$= +32 (c 0.16, H_2O); IR (ATR) *v* = 3439, 1631, 1452, 1222, 1124, 997, 615 cm^{-1}; HRMS [M+Na^+]: calcd. For $C_{13}H_{22}O_{17}Na_3S_2$, 582.9991; found, 582.9993.

5. Computational details

Conformers research has been carried out using XTB 6.6.1 and its CREST 2.12 submodule in water at the GFN2-xTB[ALPB] level.[6,7] The conformer-rotamers ensemble (CRE) has been refined using the CENSO 1.2.0,[8] ORCA 5.0.4[9] and Gaussian 16 rev B.01[10] programs according to the following procedure:

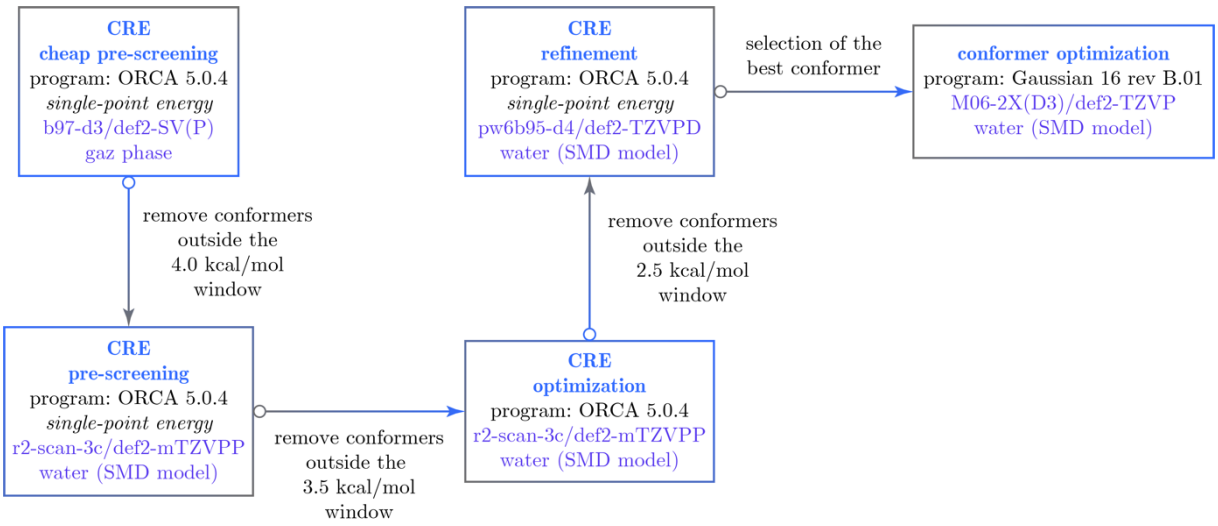


Figure S1 – Procedure for the determination of the most stable conformer.

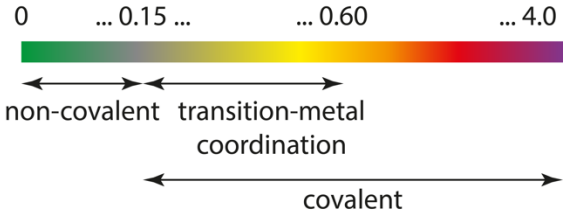
Harmonic frequency analysis has been performed on the optimized conformers in order to confirm that the obtained structures correspond to a minimum (no imaginary frequencies). Solvation Gibbs energies $\Delta G^{0}_{\text{solv}}$ in water for the two molecules have been determined *via* the difference with the Gibbs energy resulting from the optimization in gas phase through the solvation model based on density (SMD).

$$\Delta G^{0}_{\text{solv}} = G^{0}_{\text{solv}} - G^{0}_{\text{gas}}.$$

The intramolecular interactions between the two glycosyl fragments have been investigated with the use of the IGMPlot 3.08 software[11] at quantum mechanics level *via* the gradient-based partition.[12,13] The hydrogen bonds have been characterized with the help of the intrinsic bond strength index (IBSI)[14] for which the expression is given by the following equation:

$$IBSI = \frac{d_{H_2}^2}{d^2} \cdot \frac{\int_V \delta g^{pair}\, dV}{\int_V \delta g^{H_2}\, dV}$$

with d the distance between the two considered atoms and H_2 molecule playing the role of a reference. Within the IGM approach, δg measures the electron sharing between two fragments (two atoms, two fragments, two molecules). δg^{pair} corresponds to the IGM-descriptor associated to a given atom pair (the studied bond). A typical scale for IBSI is given as follows:



NBO 7.0 program has been used to quantify the hydrogen-bond interaction *via* a second-order perturbation theory analysis.[15] The stabilization energy, $E^{(2)}$ is given as follows:

$$E^{(2)} = \Delta E_{ij} = q_i \frac{F(i;j)^2}{\varepsilon_j - \varepsilon_i}$$

with q_i the occupation of the natural orbital *i*, ε the natural orbital energy and $F(i;j)$ the off-diagonal elements of the Fock matrix in the NBO formalism. The proportion of each conformer has been evaluated using the Boltzmann equation:

$$\frac{n_{conf_1}}{n_{conf_i}} = \exp\left\{\frac{\Delta H_{conf_1/conf_i}}{k_B T}\right\}$$

Assuming that $k_B = 1.986 \cdot 10^{-3}\ \text{kcal} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ and *T* is the room temperature (298.15 K) with $\sum_{i=1}^{3} n_{conf_i} = 1$. VMD 1.9.4[16] and IQMol have been used for graphical outputs.

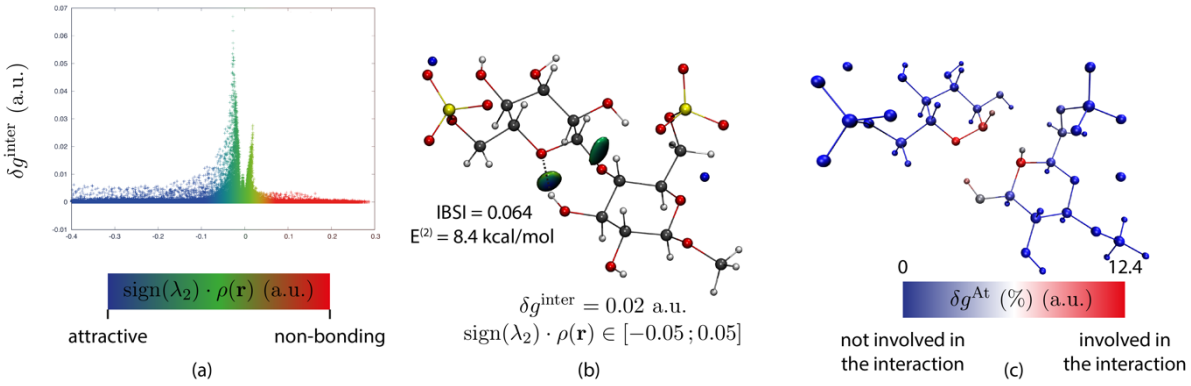


Figure S2 – 2D plot (a) and 3D plot (b) of molecular interactions between the two glycosyls fragments in the case of the best conformer in the sulfate functionalized disaccharide. IBSI and $E^{(2)}$ is given in (b) for the intra-fragment hydrogen bond. (c) Atomic decomposition of the molecular interaction in (b).

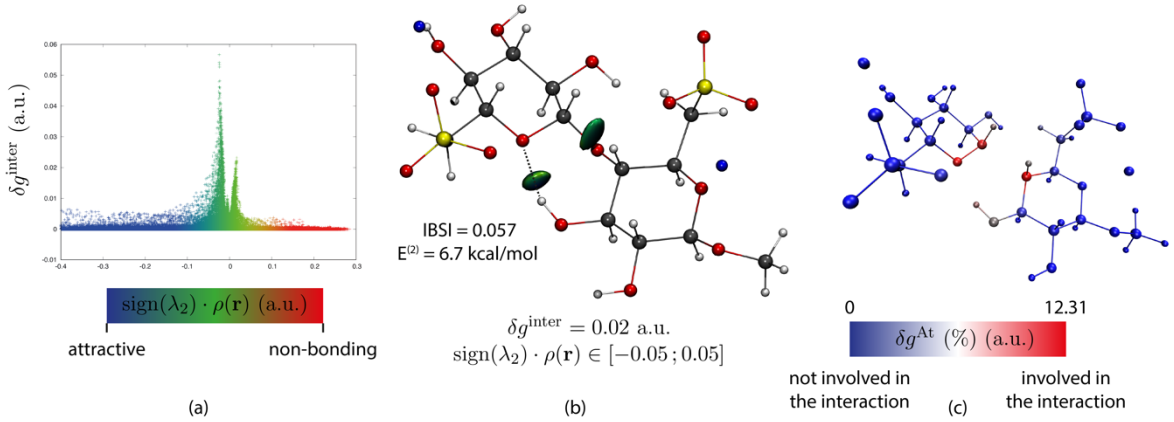
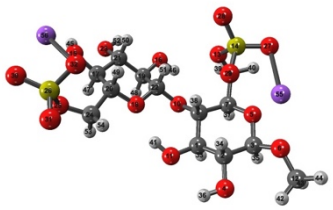


Figure S3 – 2D plot (a) and 3D plot (b) of molecular interactions between the two glycosyls fragments in the case of the best conformer in the sulfonate functionalized disaccharide. IBSI and $E^{(2)}$ is given in (b) for the inter-fragment hydrogen bond. (c) Atomic decomposition of the molecular interaction in (b).

Sulfate conformer 1 – gas phase



M06-2X(D3) Def2-TZVP

Charge = 0, Multiplicity = 1, Point group = C1

Electronic Energy = -2908.599502 Hartree

Number of imaginary frequencies = 0

Sum of electronic and zero-point Energies = -2908.182804 Hartree

Sum of electronic and thermal Energies = -2908.148526 Hartree

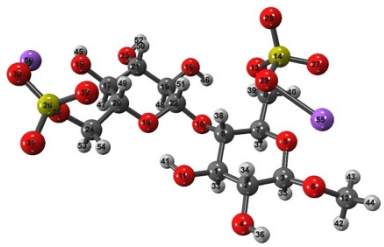
Sum of electronic and thermal Enthalpies = -2908.147582 Hartree

Sum of electronic and thermal Free Energies = -2908.251121 Hartree

Atoms	Cartesian Coordinates			Atoms	Cartesian Coordinates		
	X	*Y*	*Z*		*X*	*Y*	*Z*
C	1.920170	1.182881	1.795484	C	2.970741	1.973315	1.033192
C	4.263715	1.182754	0.919035	O	3.260644	3.186143	1.683708
O	4.029722	-0.085764	0.362680	O	5.147078	1.758991	-0.008390
C	3.019737	-0.883965	0.970187	C	1.699450	-0.123569	1.062392
C	2.885491	-2.174346	0.173693	O	0.780020	-0.952526	1.763708
O	0.781669	2.002364	1.862819	C	6.028599	2.744716	0.518548

O	1.989725	-2.091848	-0.919065	S	2.453807	-1.364678	-2.295303
O	-4.446351	-1.370793	0.270203	O	-0.493640	-3.516486	1.675744
C	-3.288269	-1.143229	1.076433	O	-1.202037	0.023154	1.264617
C	-1.167002	-2.286268	1.789286	C	-2.441748	0.025199	0.559543
C	-2.486052	-2.435147	1.067006	C	-0.427167	-1.149112	1.115224
O	-3.313535	-3.422760	1.645239	C	-3.012279	1.404560	0.883203
O	-4.365609	1.546042	0.510419	S	-4.641097	2.088287	-0.999775
O	3.876300	-1.678269	-2.423927	O	1.573334	-1.924533	-3.260181
O	2.294407	0.069180	-2.042463	O	-6.060455	1.795750	-1.119184
O	-4.236049	3.453232	-1.041562	O	-3.857993	1.167833	-1.830186
H	2.288912	0.967441	2.809910	H	2.593216	2.146862	0.016299
H	4.751040	1.098666	1.902547	H	2.414636	3.605027	1.887652
H	3.344294	-1.145346	1.987095	H	1.330627	0.085950	0.055445
H	2.464642	-2.942440	0.820333	H	3.871107	-2.496095	-0.166616
H	-0.015936	1.452400	1.867388	H	5.474935	3.617380	0.859050
H	6.605581	2.326424	1.346764	H	6.703689	3.019689	-0.287695
H	-4.794444	-2.217470	0.584166	H	0.352073	-3.442556	2.129577
H	-3.604179	-0.926777	2.103813	H	-1.334455	-2.014403	2.838591
H	-2.269366	-0.085659	-0.515645	H	-2.265990	-2.695336	0.021780
H	-0.263193	-1.360902	0.049247	H	-2.813889	-4.246528	1.683692
H	-2.973131	1.536464	1.966088	H	-2.390076	2.171372	0.414951
Na	4.537715	0.403080	-1.836546	Na	-5.476222	-0.368201	-1.489451

Sulfate conformer 1 - water



M06-2X(D3) Def2-TZVP

Charge = 0, Multiplicity = 1, Point group = C1

Electronic Energy = -2908.703938 Hartree

Number of imaginary frequencies = 0

Sum of electronic and zero-point Energies = -2908.289433 Hartree

Sum of electronic and thermal Energies = -2908.253923 Hartree

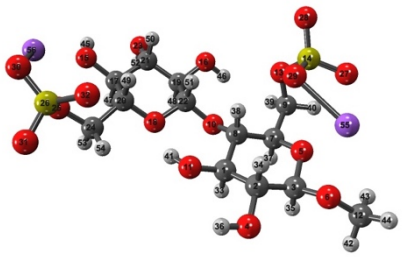
Sum of electronic and thermal Enthalpies = -2908.252979 Hartree

Sum of electronic and thermal Free Energies = -2908.360139 Hartree

Atoms	Cartesian Coordinates			Atoms	Cartesian Coordinates		
	X	*Y*	*Z*		*X*	*Y*	*Z*
C	-1.562293	-1.387638	1.596914	C	-2.684271	-2.215033	0.997452
C	-4.008967	-1.481149	1.118740	O	-2.746773	-3.446123	1.686088
O	-3.926003	-0.204266	0.509894	O	-4.971870	-2.182161	0.395890
C	-2.942603	0.647631	1.081371	C	-1.564122	0.000911	0.969835
C	-3.019015	1.997039	0.402210	O	-0.652973	0.865692	1.640993
O	-0.354000	-2.080035	1.352902	C	-6.303692	-2.014955	0.895575
O	-2.336569	2.064354	-0.861252	S	-2.965033	1.417023	-2.176682
O	4.588468	1.652246	0.349910	O	0.290006	3.535171	1.125022
C	3.375994	1.456143	1.066575	O	1.441589	0.085001	1.283960
C	1.111015	2.445480	1.490811	C	2.676192	0.185432	0.591112
C	2.470307	2.657536	0.848652	C	0.536417	1.135203	0.981231
O	3.143429	3.778232	1.391877	C	3.409646	-1.099744	0.928691
O	4.602424	-1.212121	0.145686	S	4.583268	-2.131822	-1.184941
O	-4.413069	1.538419	-2.048243	O	-2.370666	2.197742	-3.229016
O	-2.573506	0.010670	-2.185203	O	5.761611	-1.590810	-1.850523
O	4.723648	-3.496263	-0.738609	O	3.335884	-1.851817	-1.856984
H	-1.734896	-1.283789	2.676284	H	-2.473603	-2.373261	-0.068318
H	-4.301522	-1.374587	2.170955	H	-3.376558	-4.017320	1.227969
H	-3.178386	0.803801	2.141310	H	-1.282995	-0.090084	-0.082483
H	-2.518582	2.739270	1.017407	H	-4.061443	2.285555	0.273279
H	0.391657	-1.469841	1.479444	H	-6.380657	-2.451508	1.891733
H	-6.573118	-0.957713	0.934870	H	-6.964993	-2.539685	0.211092
H	4.871215	2.562582	0.523983	H	-0.496439	3.529281	1.684530
H	3.589950	1.358016	2.137189	H	1.220221	2.379318	2.578546
H	2.500912	0.242047	-0.489845	H	2.326295	2.792383	-0.230593
H	0.379760	1.179658	-0.104728	H	2.728862	4.581964	1.055267
H	3.718340	-1.103788	1.973281	H	2.750715	-1.950132	0.747797
Na	-4.705784	-0.772326	-1.590477	Na	6.130585	0.347054	-0.652900

$$\boxed{\Delta G_{\mathrm{solv}}^{0} = -68.4\ \mathrm{kcal \cdot mol^{-1}}}.$$

Sulfate conformer 2 - water



M06-2X(D3) Def2-TZVP

Charge = 0, Multiplicity = 1, Point group = C1

Electronic Energy = -2908.703706 Hartree

Number of imaginary frequencies = 0

Sum of electronic and zero-point Energies = -2908.287756 Hartree

Sum of electronic and thermal Energies = -2908.252954 Hartree

Sum of electronic and thermal Enthalpies = -2908.252010 Hartree

Sum of electronic and thermal Free Energies = -2908.356617 Hartree

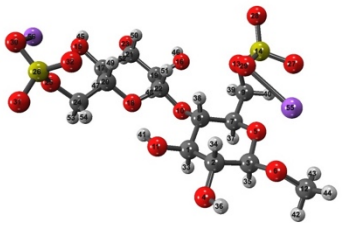
Atoms	Cartesian Coordinates			Atoms	Cartesian Coordinates		
	X	*Y*	*Z*		*X*	*Y*	*Z*
C	-1.313758	-1.546001	1.209666	C	-2.428459	-2.394109	0.616368
C	-3.780333	-1.844331	1.023268	O	-2.359225	-3.726910	1.077788
O	-3.881817	-0.483627	0.638084	O	-4.780021	-2.507073	0.315650
C	-2.910855	0.375642	1.219987	C	-1.508914	-0.084701	0.829051
C	-3.211602	1.795266	0.792649	O	-0.605840	0.772533	1.520160
O	-0.104425	-2.081373	0.707872	C	-6.046820	-2.509399	0.983875
O	-2.716790	2.136608	-0.512846	S	-3.462107	1.642974	-1.834251
O	4.607083	1.820047	0.311088	O	0.167445	3.453312	0.886664
C	3.389964	1.586517	1.006815	O	1.546615	0.103716	1.258788
C	1.057622	2.451096	1.328629	C	2.784189	0.254065	0.578455
C	2.419717	2.724924	0.714304	C	0.584644	1.077167	0.878384
O	2.969129	3.956922	1.142262	C	3.604090	-0.961429	0.974120
O	4.776430	-1.049814	0.157372	S	4.768753	-2.040170	-1.123076
O	-4.884211	1.590619	-1.513075	O	-3.083062	2.638766	-2.801382
O	-2.960354	0.304597	-2.130133	O	5.851445	-1.432696	-1.886498
O	5.063324	-3.358488	-0.617762	O	3.463947	-1.913071	-1.728970
H	-1.346990	-1.630342	2.303603	H	-2.354103	-2.355770	-0.478651
H	-3.932176	-1.938598	2.105988	H	-1.475675	-4.063993	0.880188
H	-3.008928	0.336308	2.311736	H	-1.370521	0.025597	-0.249537
H	-2.711340	2.488207	1.463359	H	-4.285398	1.972808	0.837673
H	0.632165	-1.527836	1.016750	H	-5.973985	-3.079136	1.910736

H	-6.372235	-1.490448	1.203640	H	-6.755386	-2.987320	0.312475
H	4.838169	2.750258	0.453391	H	-0.651507	3.376467	1.391813
H	3.582245	1.548926	2.086341	H	1.137296	2.447196	2.422419
H	2.620739	0.253095	-0.506151	H	2.300701	2.810006	-0.370314
H	0.455820	1.055779	-0.211981	H	3.037962	3.944546	2.107557
H	3.945788	-0.880621	2.005234	H	2.996996	-1.861482	0.869175
Na	-4.920404	-0.777681	-1.403179	Na	6.137653	0.579562	-0.786105

Sulfate conformer 3 - water

M06-2X(D3) Def2-TZVP

Charge = 0, Multiplicity = 1, Point group = C1



Electronic Energy = -2908.703163 Hartree

Number of imaginary frequencies = 0

Sum of electronic and zero-point Energies = -2908.287540 Hartree

Sum of electronic and thermal Energies = -2908.252666 Hartree

Sum of electronic and thermal Enthalpies = -2908.251722 Hartree

Sum of electronic and thermal Free Energies = -2908.356031 Hartree

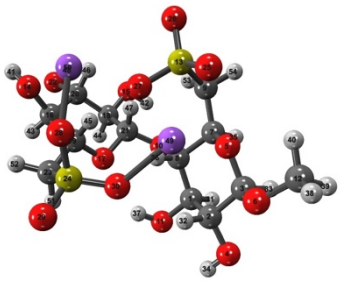
	Cartesian Coordinates			Atoms	Cartesian Coordinates		
Atoms	***X***	***Y***	***Z***		***X***	***Y***	***Z***
C	1.467511	1.536827	1.389731	C	2.600349	2.341835	0.780530
C	3.935389	1.665645	1.047717	O	2.587958	3.631636	1.355337
O	3.925313	0.334083	0.562981	O	4.922601	2.325315	0.318849
C	2.934695	-0.490133	1.161167	C	1.548672	0.094645	0.905480
C	3.083330	-1.898908	0.631555	O	0.630542	-0.737363	1.605354
O	0.256343	2.157602	1.006293	C	6.221163	2.264046	0.920184
O	2.496894	-2.108678	-0.664557	S	3.208957	-1.587714	-1.992701
O	-4.593051	-1.726828	0.355483	O	-0.133115	-3.383982	1.090586
C	-3.378318	-1.485657	1.053850	O	-1.491611	-0.046424	1.247875
C	-1.066843	-2.388677	1.451761	C	-2.728781	-0.193066	0.566428
C	-2.424063	-2.648174	0.814893	C	-0.544311	-1.058890	0.945520

O	-2.986730	-3.867243	1.265215	C	-3.500231	1.069395	0.904529
O	-4.709325	1.133255	0.141433	S	-4.742512	2.040999	-1.197275
O	4.645695	-1.649835	-1.746731	O	2.716080	-2.495780	-2.994444
O	2.786780	-0.203634	-2.184422	O	-5.919914	1.463028	-1.832722
O	-4.908814	3.405258	-0.759666	O	-3.502563	1.787149	-1.893200
H	1.574688	1.544985	2.482389	H	2.450163	2.395666	-0.305329
H	4.165026	1.669675	2.120731	H	3.225259	4.180996	0.881454
H	3.111313	-0.531994	2.243118	H	1.330662	0.070513	-0.165375
H	2.545194	-2.583472	1.281475	H	4.136461	-2.175784	0.608258
H	-0.481685	1.564612	1.225208	H	6.216677	2.811969	1.862773
H	6.518027	1.228384	1.097264	H	6.912497	2.733002	0.224871
H	-4.828585	-2.653466	0.512898	H	-0.520316	-4.245249	1.296175
H	-3.579449	-1.393587	2.128373	H	-1.175340	-2.326506	2.541979
H	-2.561767	-0.250864	-0.515767	H	-2.285199	-2.765306	-0.264366
H	-0.385118	-1.099138	-0.140113	H	-3.097429	-3.822903	2.226143
H	-3.790867	1.072508	1.954315	H	-2.875136	1.940996	0.704721
Na	4.860542	0.694975	-1.514092	Na	-6.200929	-0.477830	-0.612358

	conformer 1	conformer 2	conformer 3
ΔH (0 K, $\mathrm{kcal \cdot mol^{-1}}$)	**0.00**	**1.05**	**1.19**
n_{conf_i} (%)	**76.7**	**13.0**	**10.3**

Table S1. Evaluation of the proportion of each conformer in the sulfate case (in water, 298.15 K).

Sulfonate conformer 1 - gas



M06-2X(D3) Def2-TZVP

Charge = 0, Multiplicity = 1, Point group = C1

Electronic Energy = -2758.149712 Hartree

Number of imaginary frequencies = 0

Sum of electronic and zero-point Energies = -2757.742807 Hartree

Sum of electronic and thermal Energies = -2757.710080 Hartree

Sum of electronic and thermal Enthalpies = -2757.709136 Hartree

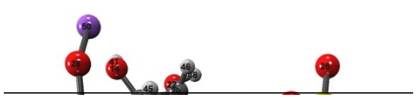
Sum of electronic and thermal Free Energies = -2757.804856 Hartree

Atoms	Cartesian Coordinates	Atoms	Cartesian Coordinates

	X	Y	Z		X	Y	Z
C	-1.839520	-0.780043	-2.298017	C	-2.965668	0.110188	-1.771070
C	-3.633111	-0.491772	-0.548363	O	-3.949725	0.299349	-2.753618
O	-2.658351	-0.906702	0.401021	O	-4.379121	0.481397	0.081639
C	-1.714595	-1.858501	-0.059023	C	-0.916526	-1.147970	-1.152566
C	-0.929398	-2.385380	1.142624	O	0.210611	-1.880502	-1.631518
O	-1.168592	-0.102531	-3.334551	C	-5.232048	-0.025703	1.097790
S	-0.427746	-1.221188	2.425146	O	4.004053	0.914367	0.677833
O	2.724945	-3.278169	-1.005416	C	3.546613	0.349749	-0.553090
O	1.439545	0.044878	-1.652222	C	2.671793	-1.927893	-1.405311
C	2.111826	0.844161	-0.704159	C	3.634940	-1.179821	-0.476328
C	1.342514	-1.268557	-1.125211	O	4.985024	-1.519461	-0.687735
C	1.938931	2.311870	-1.036964	S	0.578209	2.991444	-0.078756
O	-1.639137	-0.637413	2.977639	O	0.393943	-1.981014	3.325910
O	0.336324	-0.150885	1.724710	O	1.014586	2.827363	1.320583
O	0.409414	4.350729	-0.496047	O	-0.570710	2.110181	-0.349731
H	-2.271605	-1.683213	-2.743246	H	-2.517404	1.066655	-1.467300
H	-4.253728	-1.356825	-0.830019	H	-3.499714	0.622327	-3.543341
H	-2.240137	-2.717177	-0.499519	H	-0.568028	-0.205612	-0.724062
H	-0.458248	0.426152	-2.944558	H	-5.792692	0.820165	1.486533
H	-5.928045	-0.756604	0.677199	H	-4.652710	-0.489406	1.899034
H	4.931690	0.678129	0.791904	H	2.102427	-3.783123	-1.538989
H	4.168799	0.695569	-1.384892	H	2.947321	-1.800617	-2.457183
H	1.619160	0.659300	0.248238	H	3.331001	-1.468482	0.541158
H	1.231293	-1.194929	-0.038670	H	5.068681	-2.476013	-0.599916
Na	-1.673972	1.123818	1.357642	Na	2.045258	1.186274	2.323863
H	1.718579	2.454787	-2.093979	H	2.814730	2.899906	-0.764031
H	-0.022904	-2.892836	0.809618	H	-1.537011	-3.107907	1.687390

Sulfonate conformer 1 - water

M06-2X(D3) Def2-TZVP



Charge = 0, Multiplicity = 1, Point group = C1

Electronic Energy = -2758.232935 Hartree

Number of imaginary frequencies = 0

Sum of electronic and zero-point Energies = -2757.828573 Hartree

Sum of electronic and thermal Energies = -2757.794763 Hartree

Sum of electronic and thermal Enthalpies = -2757.793818 Hartree

Sum of electronic and thermal Free Energies = -2757.896020 Hartree

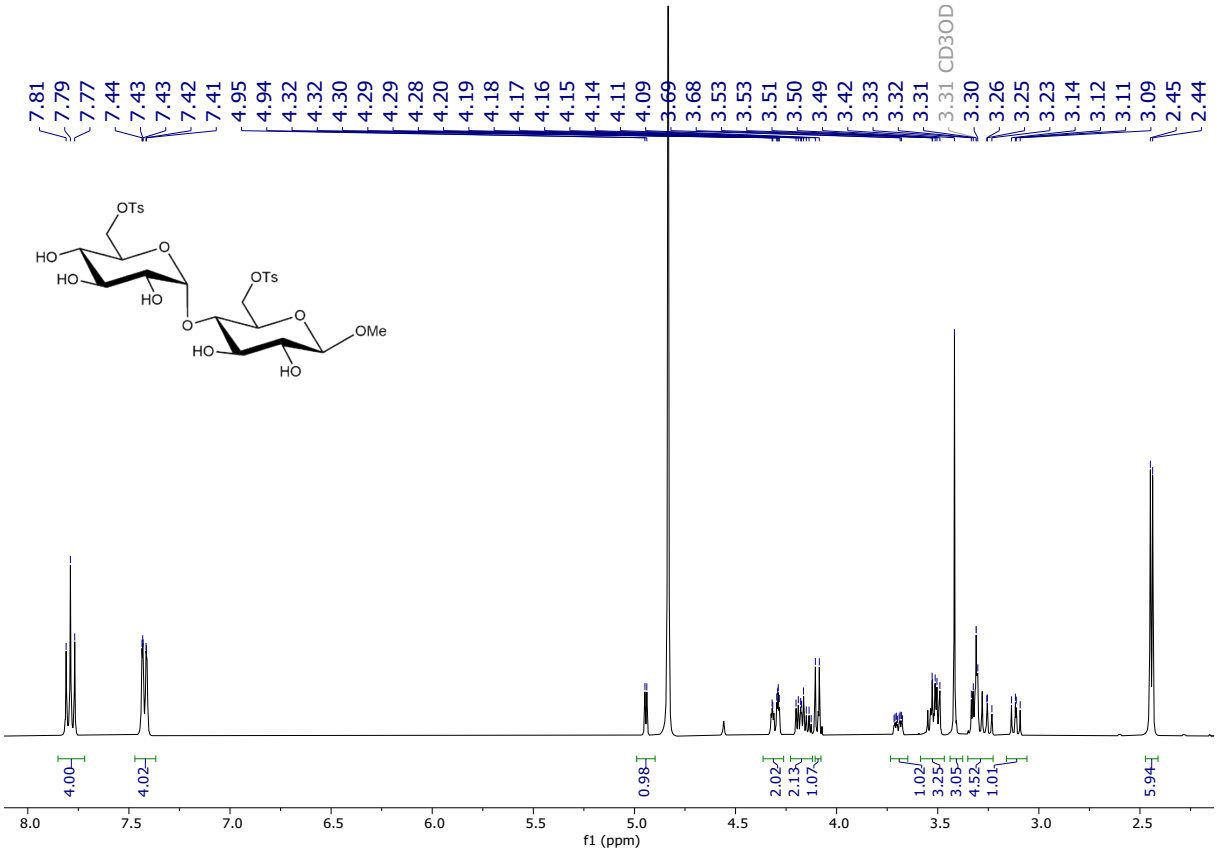
	Cartesian Coordinates			Atoms	Cartesian Coordinates		
Atoms	***X***	***Y***	***Z***		***X***	***Y***	***Z***
C	-1.415347	1.368090	-1.536350	C	-2.573373	2.118935	-0.899581
C	-3.840011	1.288195	-0.964770	O	-2.831154	3.334798	-1.569680
O	-3.622437	0.019596	-0.372028	O	-4.817366	1.892804	-0.179176
C	-2.573011	-0.762674	-0.920983	C	-1.253849	0.009361	-0.876373
C	-2.509396	-2.082955	-0.164807	O	-0.299713	-0.770741	-1.591595
O	-0.268920	2.183723	-1.385505	C	-6.149262	1.499154	-0.523963
S	-2.760236	-1.959152	1.603545	O	4.901573	-1.202463	-0.097120
O	0.857502	-3.383766	-1.383738	C	3.752518	-1.094370	-0.926814
O	1.701439	0.145379	-1.089283	C	1.596736	-2.194574	-1.570250
C	2.926207	0.063859	-0.381646	C	2.947945	-2.382587	-0.898455
C	0.895433	-1.008960	-0.931148	O	3.722233	-3.375459	-1.543829
C	3.634038	1.402552	-0.532683	S	3.521802	2.373078	0.951068
O	-4.210058	-1.827149	1.808685	O	-2.211148	-3.180067	2.165415
O	-2.084525	-0.738424	2.063450	O	4.267068	1.600261	1.962864
O	4.152725	3.650997	0.660995	O	2.106191	2.473532	1.280976
H	-1.635603	1.213026	-2.600820	H	-2.332449	2.302185	0.156295
H	-4.179314	1.166490	-2.001005	H	-2.029122	3.871869	-1.530857
H	-2.793869	-0.980455	-1.972047	H	-0.927183	0.146766	0.157407
H	0.527686	1.630250	-1.436738	H	-6.815083	1.998647	0.174774
H	-6.376817	1.816310	-1.542521	H	-6.270495	0.417103	-0.441247
H	5.467306	-1.907618	-0.440141	H	0.042677	-3.321452	-1.899119
H	4.054186	-0.876408	-1.956734	H	1.737648	-1.980035	-2.635474
H	2.707534	-0.150094	0.673894	H	2.774498	-2.658143	0.149419

H	0.722445	-1.193053	0.138717	H	3.313693	-4.235110	-1.383548
Na	-4.166255	0.496283	1.838684	Na	5.368450	-0.291846	1.920948
H	3.177371	1.976313	-1.339281	H	4.696010	1.282950	-0.747807
H	-1.534077	-2.545528	-0.314386	H	-3.275292	-2.773951	-0.515027

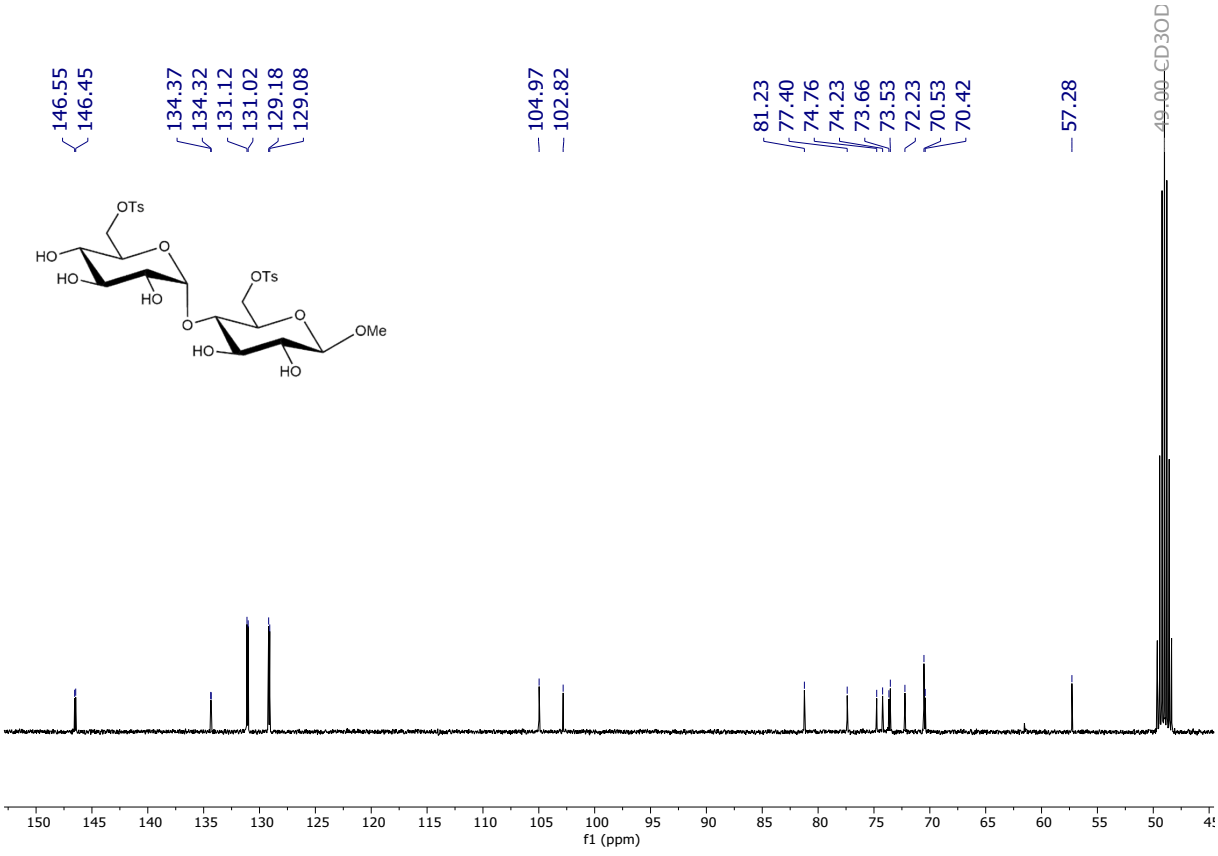
$$\boxed{\Delta G_{\mathrm{solv}}^{0} = -57.2\ \mathrm{kcal \cdot mol^{-1}}}.$$

6. NMR spectra

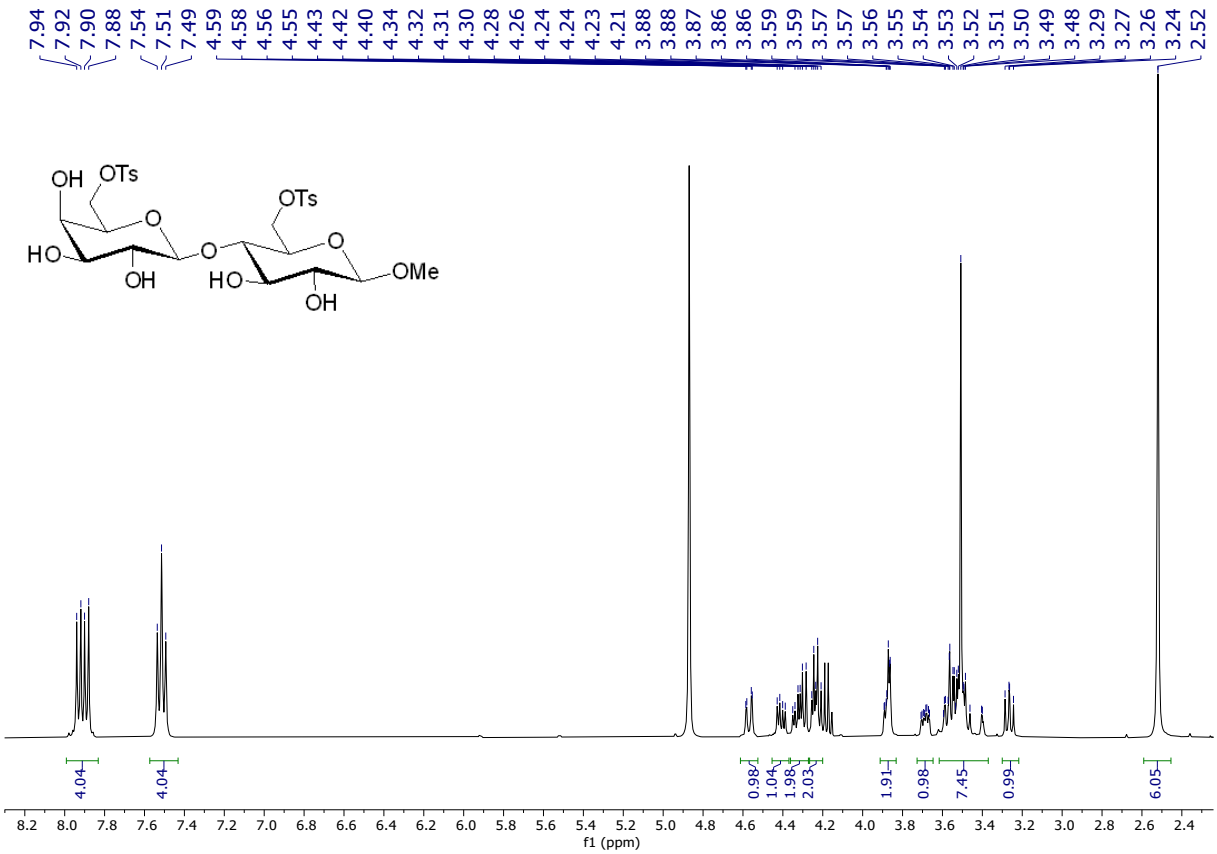
^{1}H NMR spectrum of compound 2 (400 MHz, Methanol-d_4)



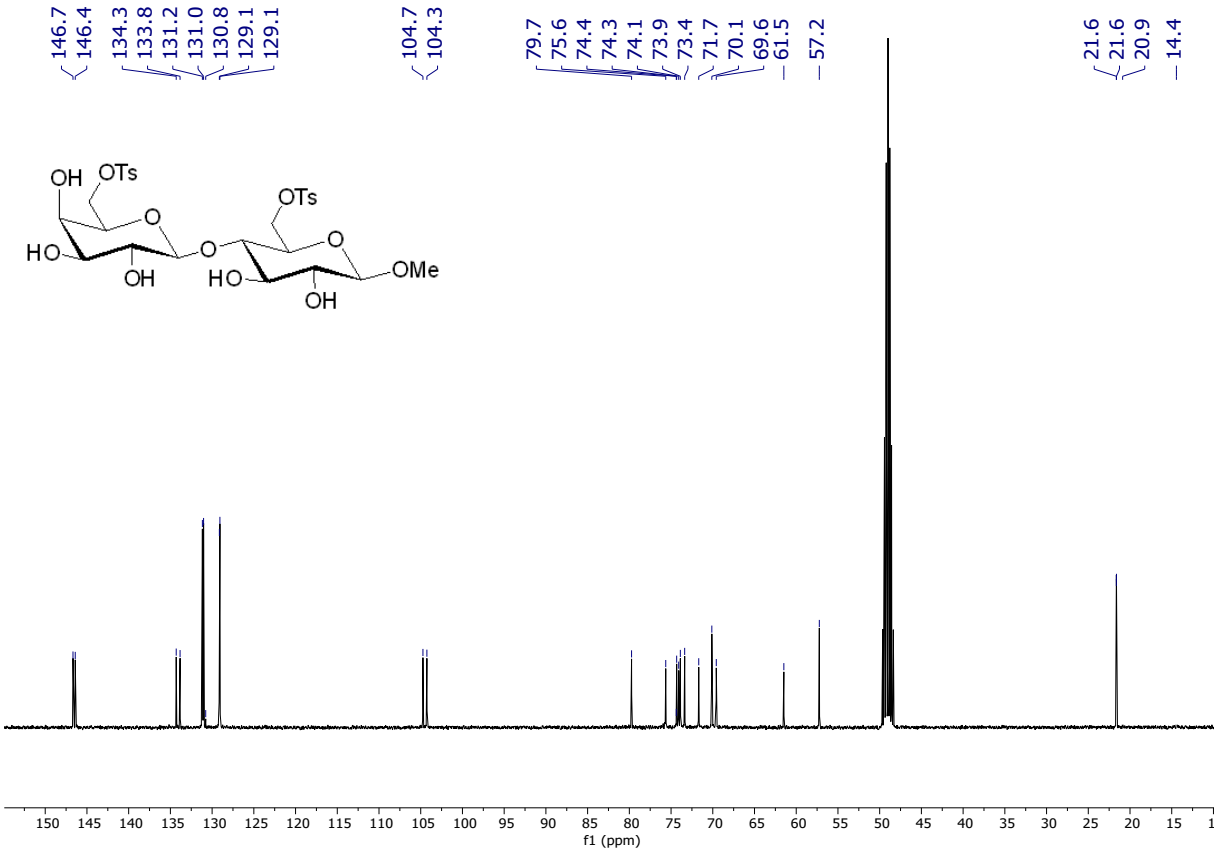
^{13}C NMR spectrum of compound 2 (101 MHz, Methanol-d_4)



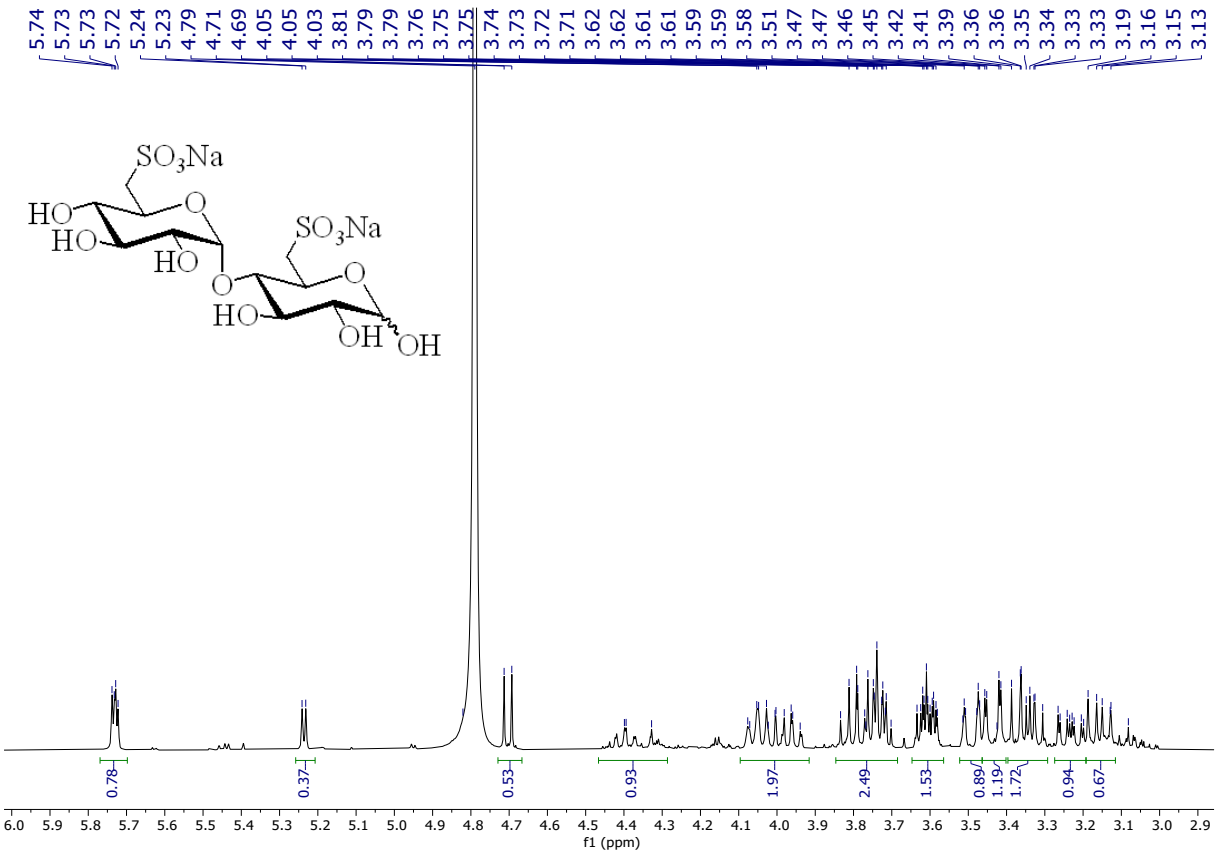
^{1}H NMR spectrum of compound 4 (400 MHz, Methanol-d_4)



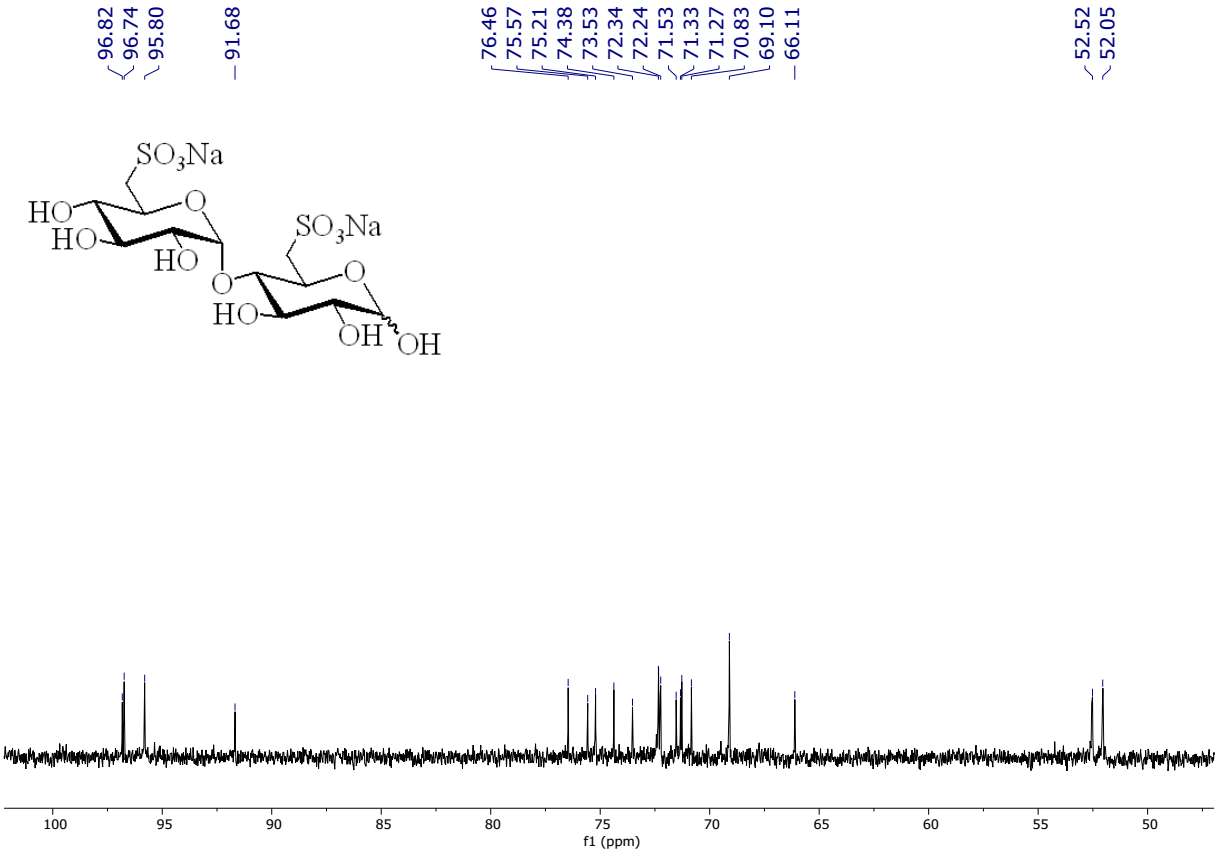
^{13}C NMR spectrum of compound 4 (101 MHz, Methanol-d_4)



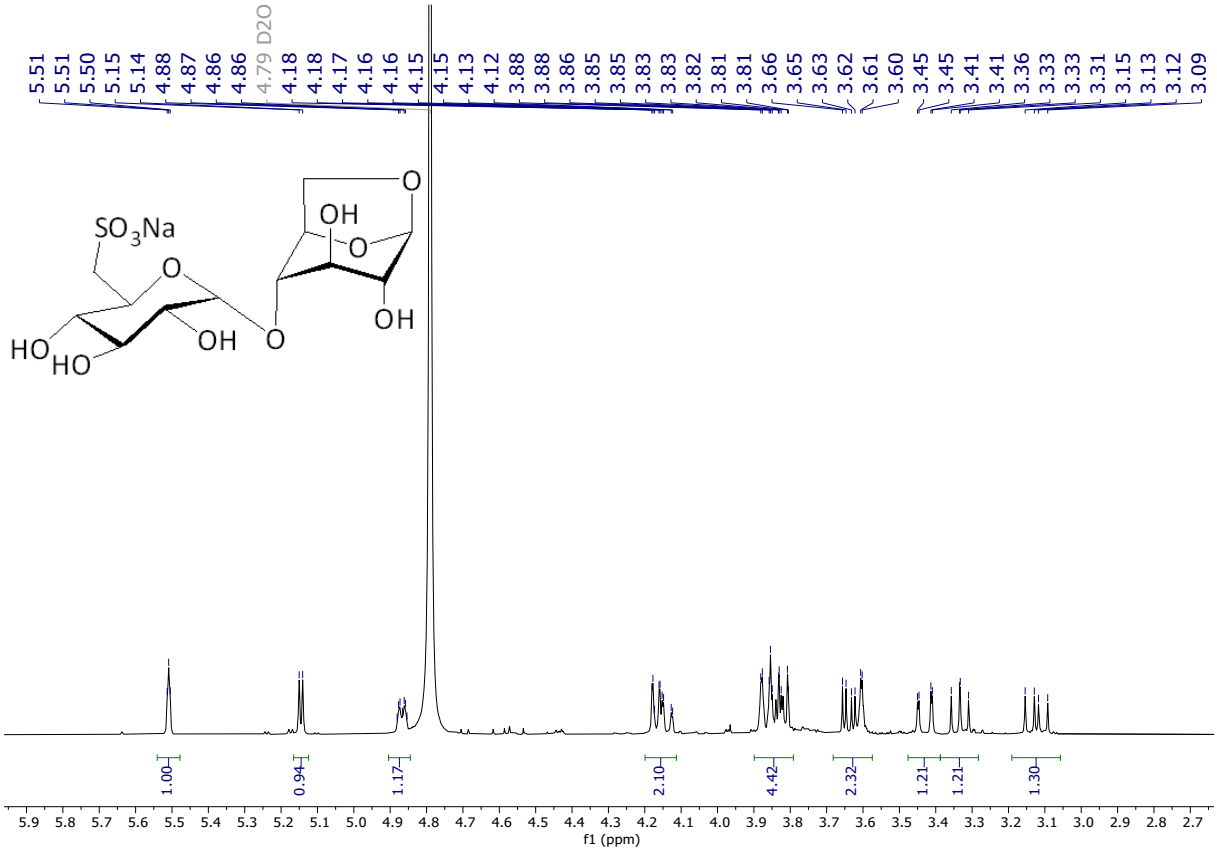
^{1}H NMR spectrum of compound 7 (400 MHz, D_2O)



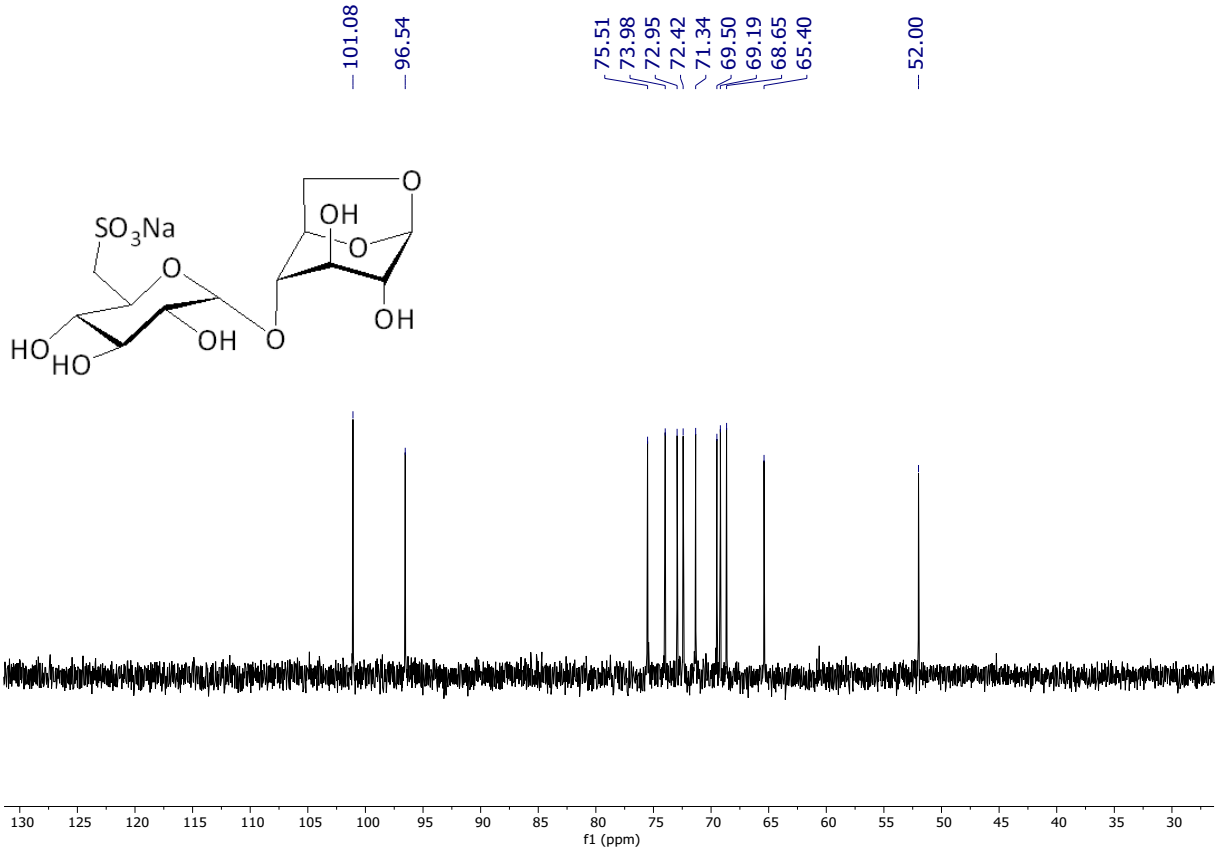
^{13}C NMR spectrum of compound 7 (101 MHz, D_2O)



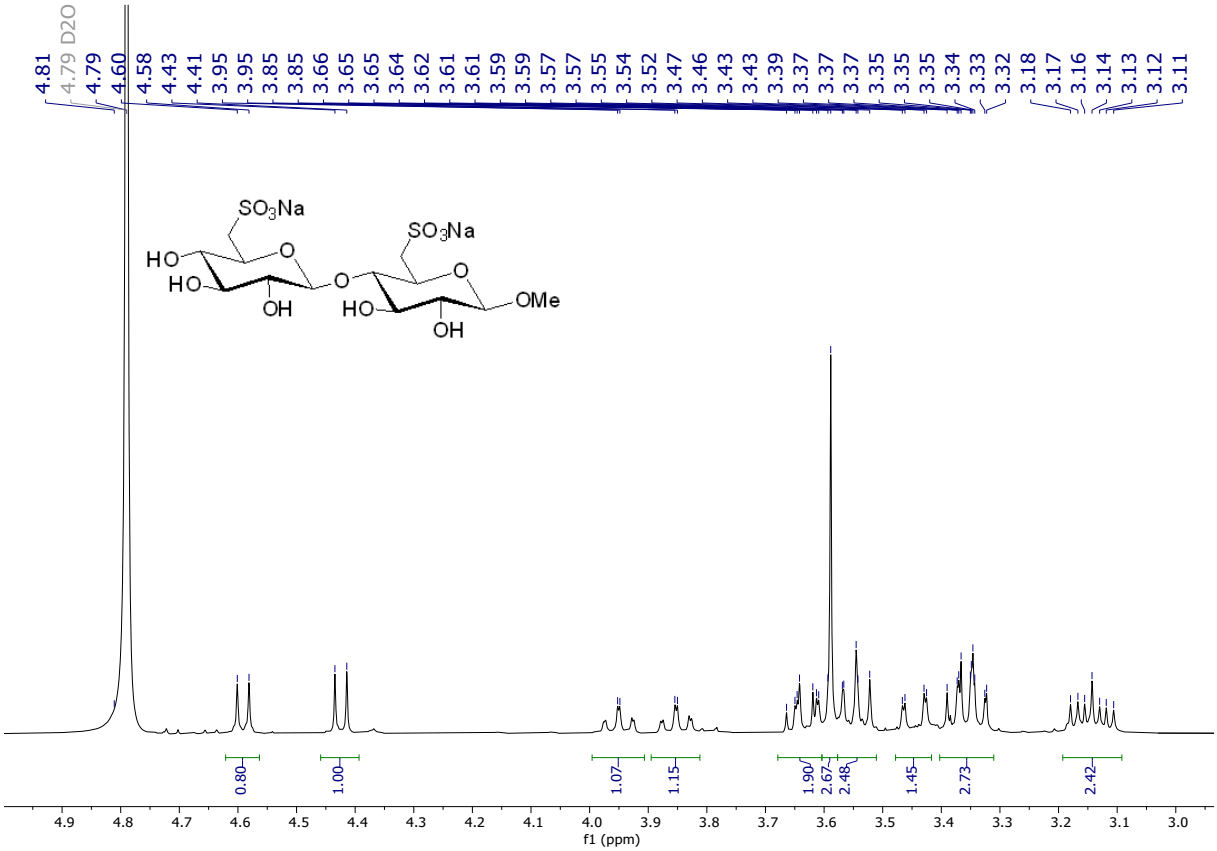
^{1}H NMR spectrum of compound 8 (400 MHz, D_2O)



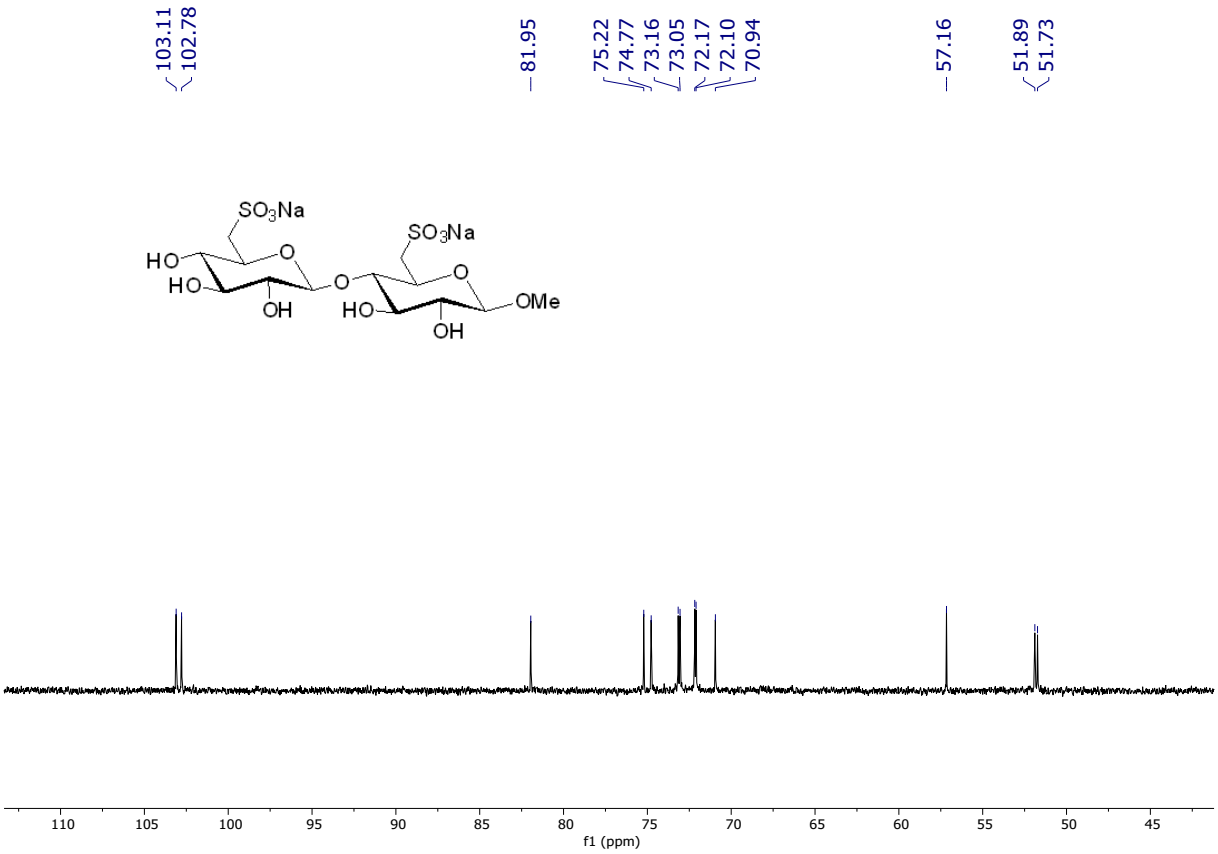
^{13}C NMR spectrum of compound 8 (101 MHz, D_2O)



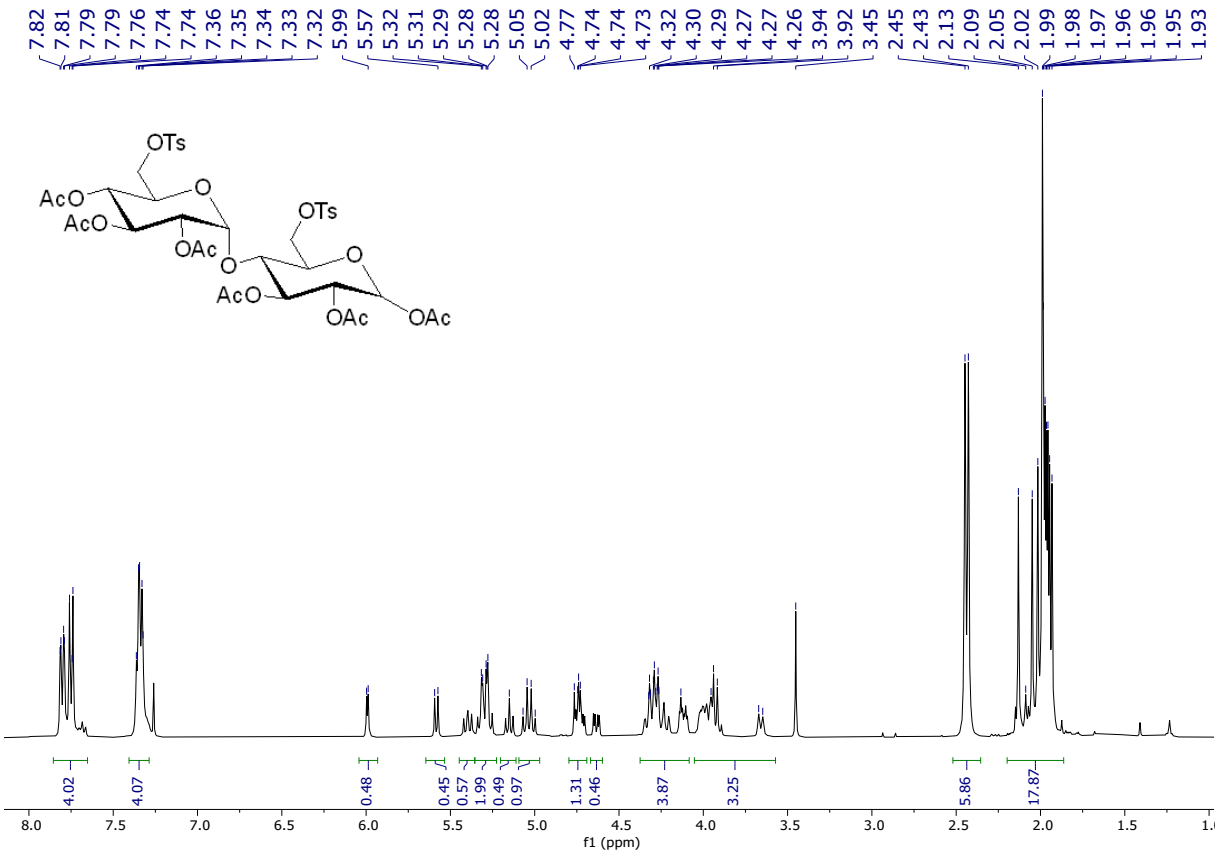
^{1}H NMR spectrum of compound 9 (400 MHz, D_2O)



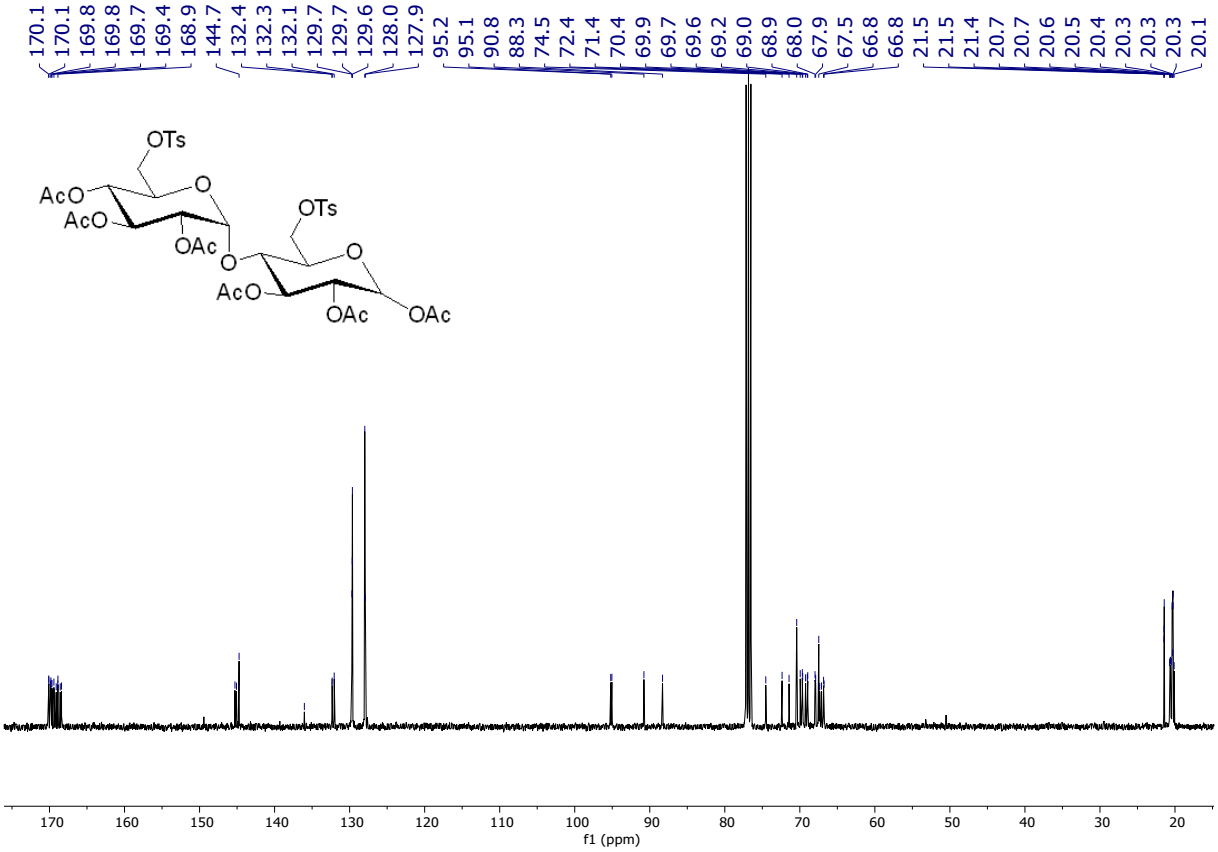
^{13}C NMR spectrum of compound 9 (101 MHz, D_2O)



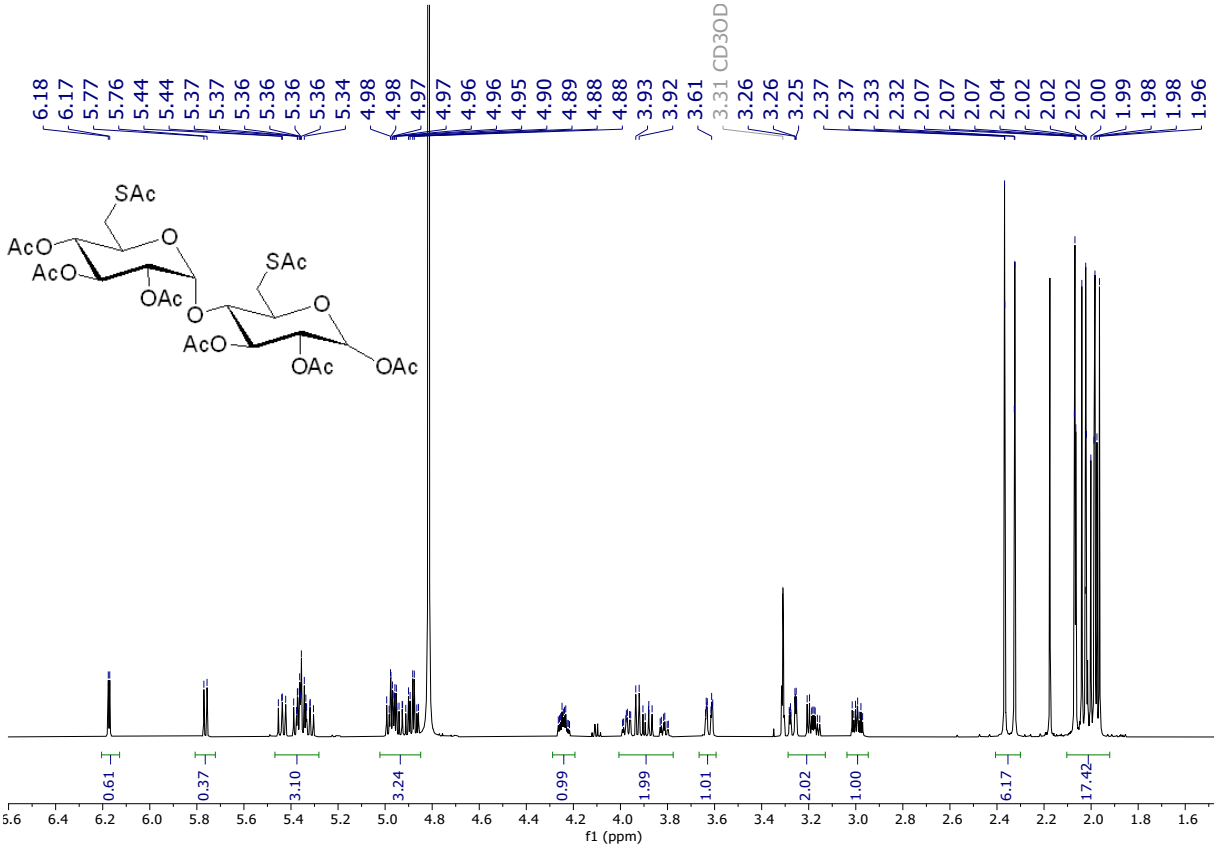
^{1}H NMR spectrum of compound 10 (600 MHz, $CDCl_3$)



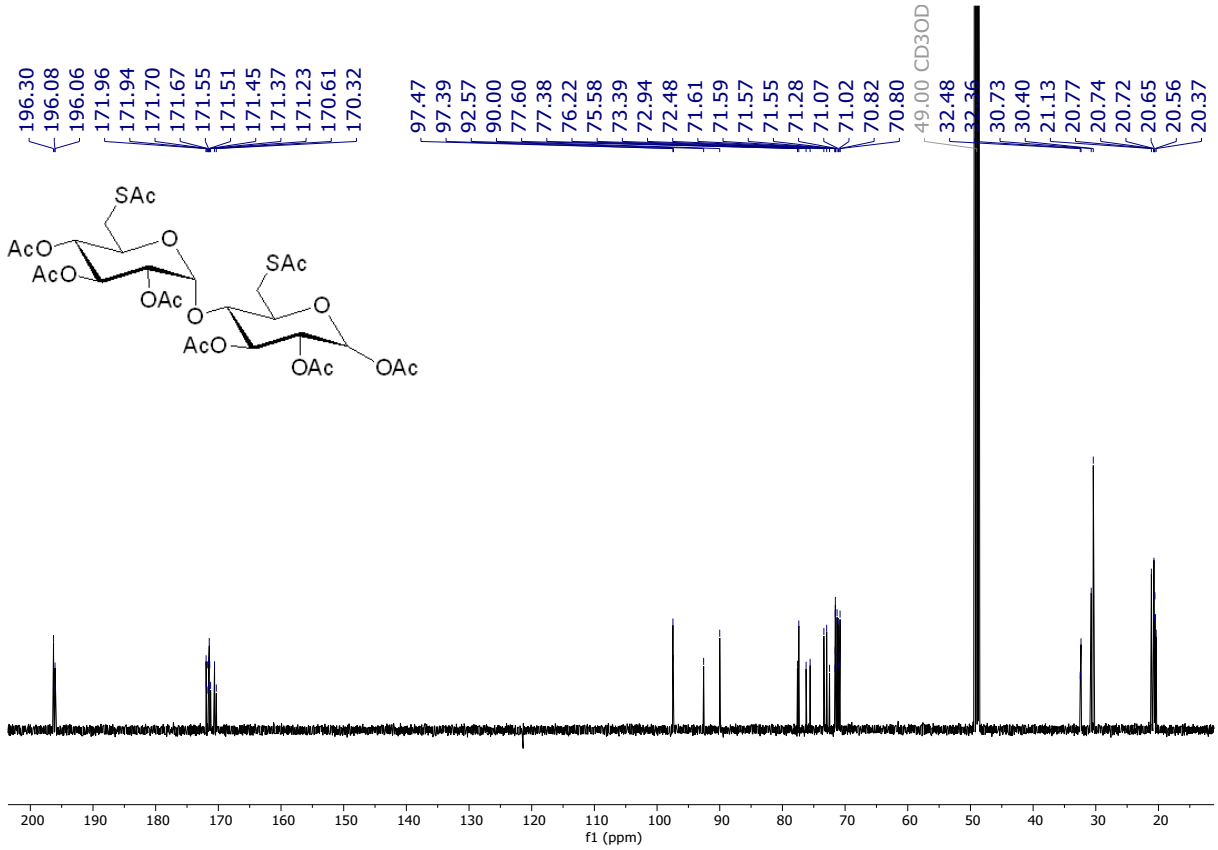
^{13}C NMR spectrum of compound 10 (151 MHz, $CDCl_3$)



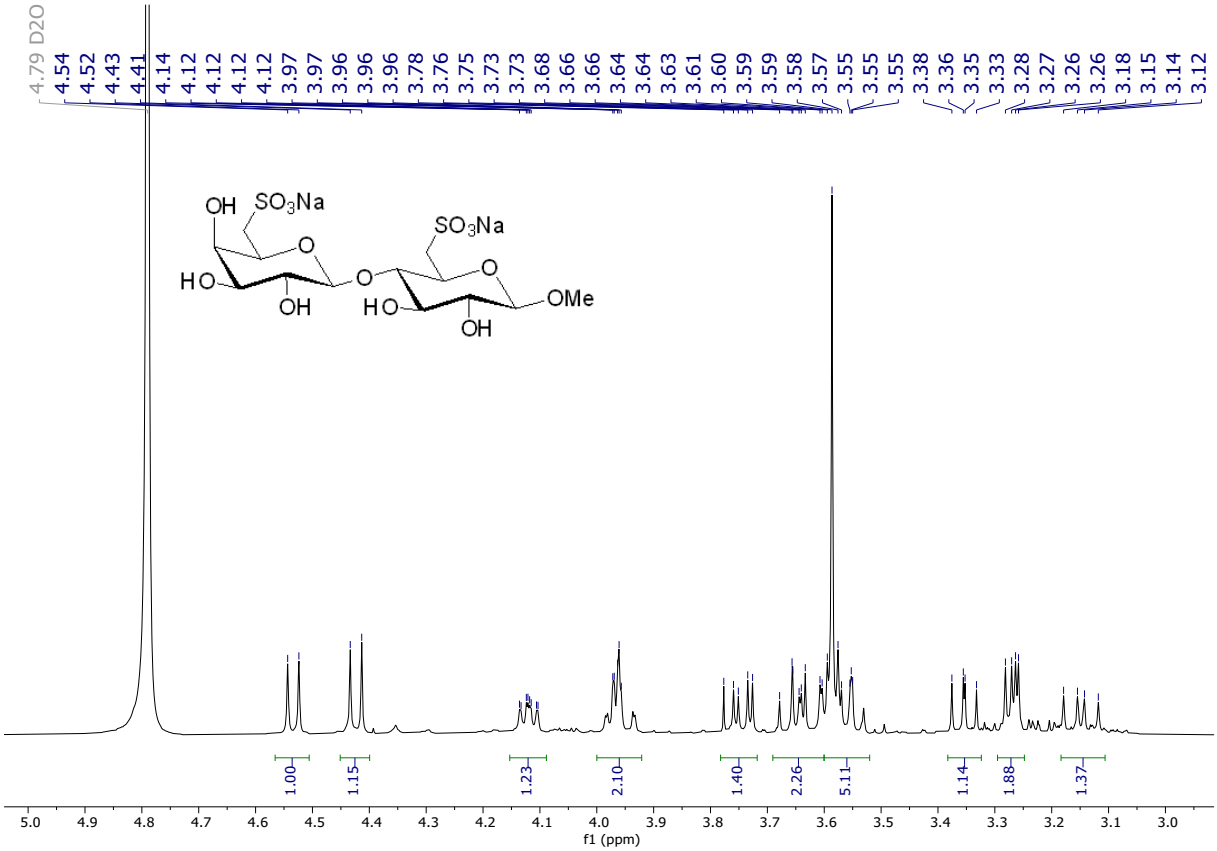
^{1}H NMR spectrum of compound 11 (600 MHz, Methanol-d_4)



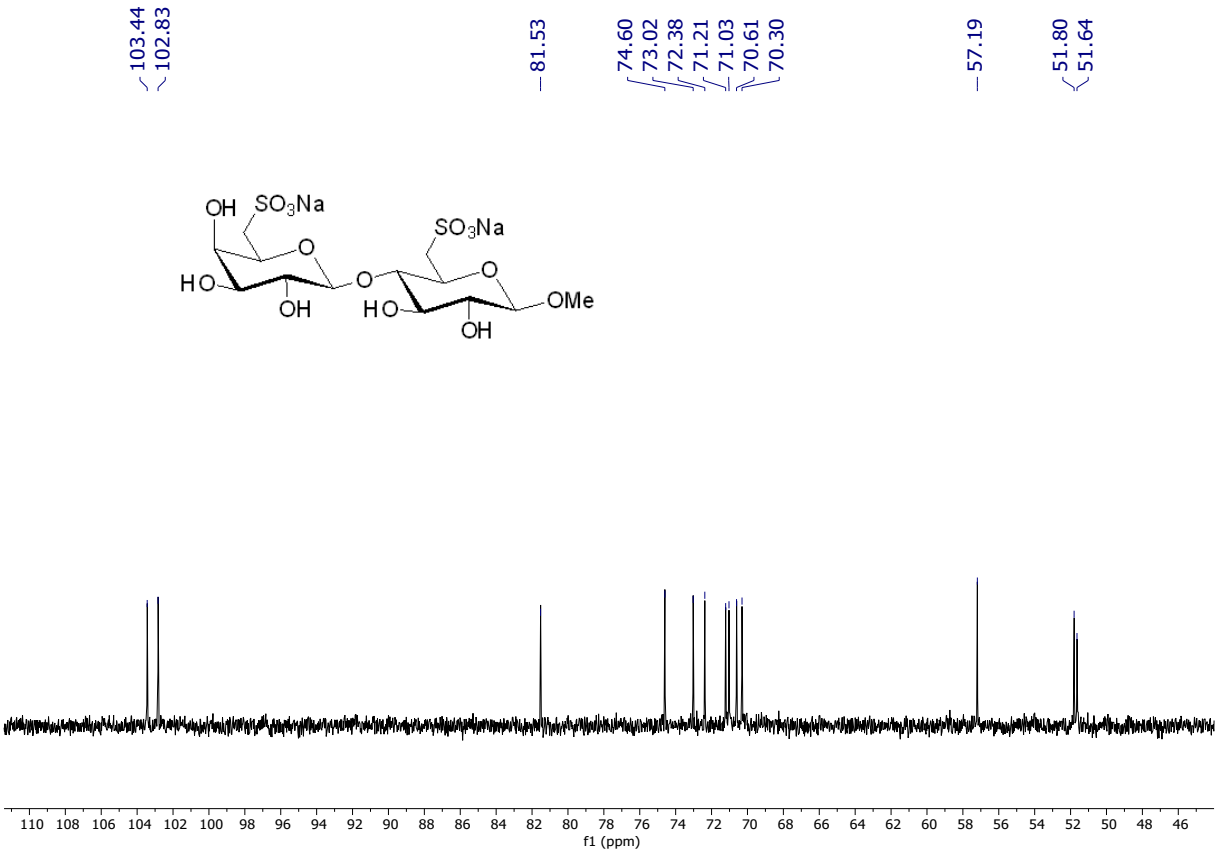
^{13}C NMR spectrum of compound 11 (151 MHz, Methanol-d_4)



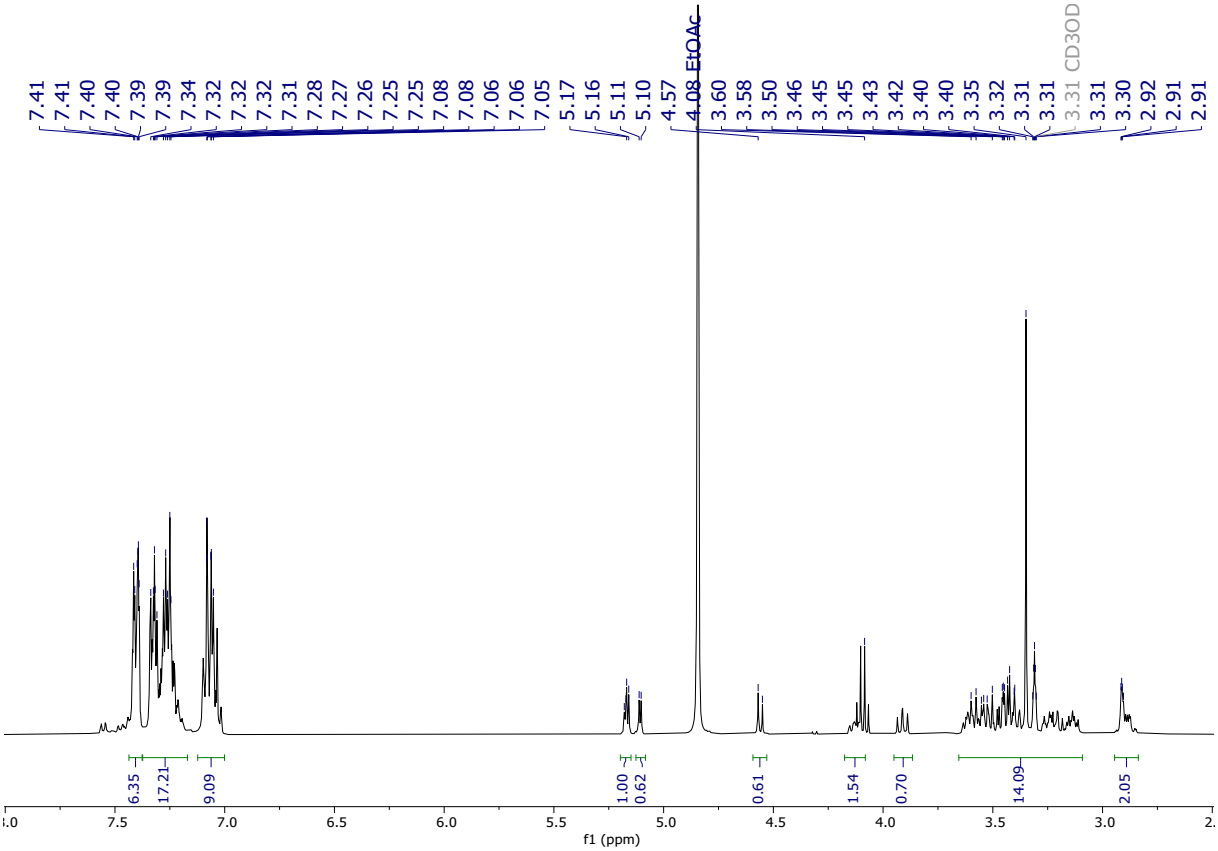
^{1}H NMR spectrum of compound 16 (400 MHz, D_2O)



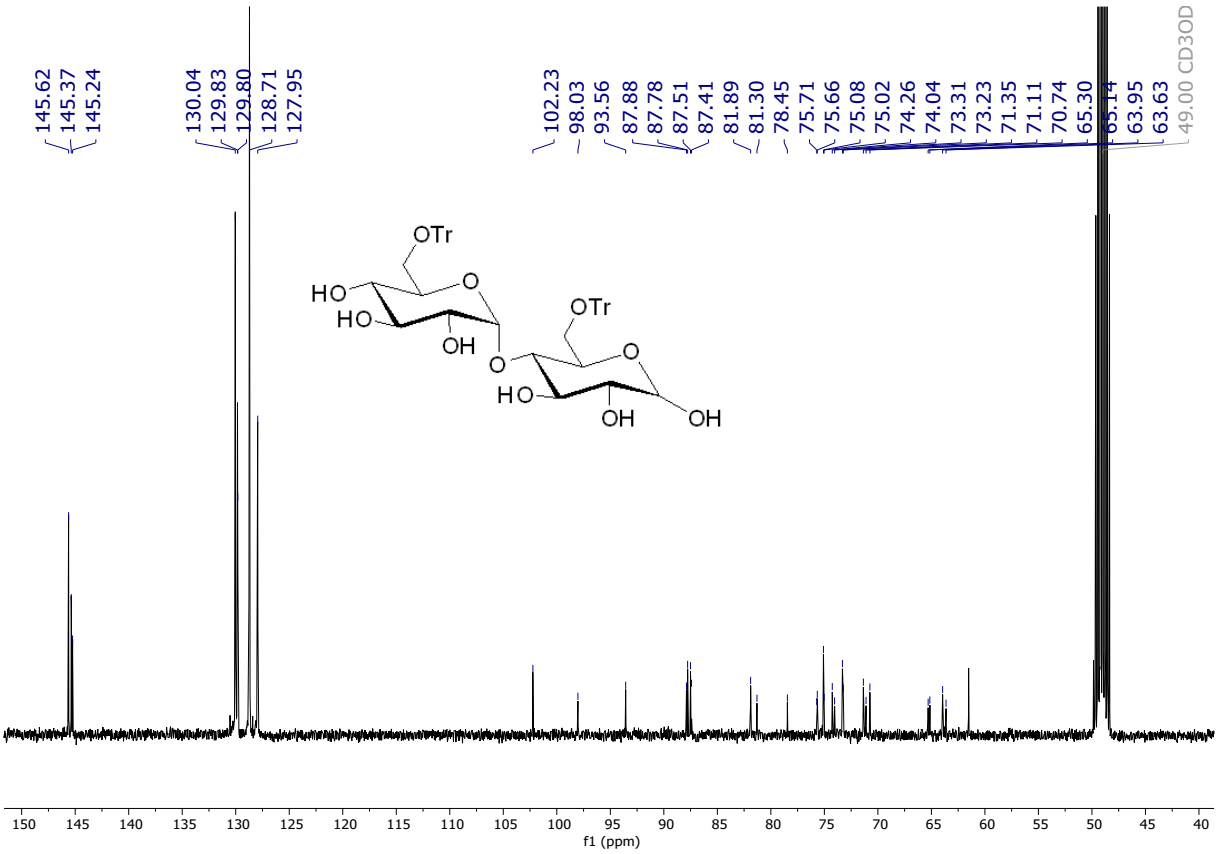
^{13}C NMR spectrum of compound 16 (101 MHz, D_2O)



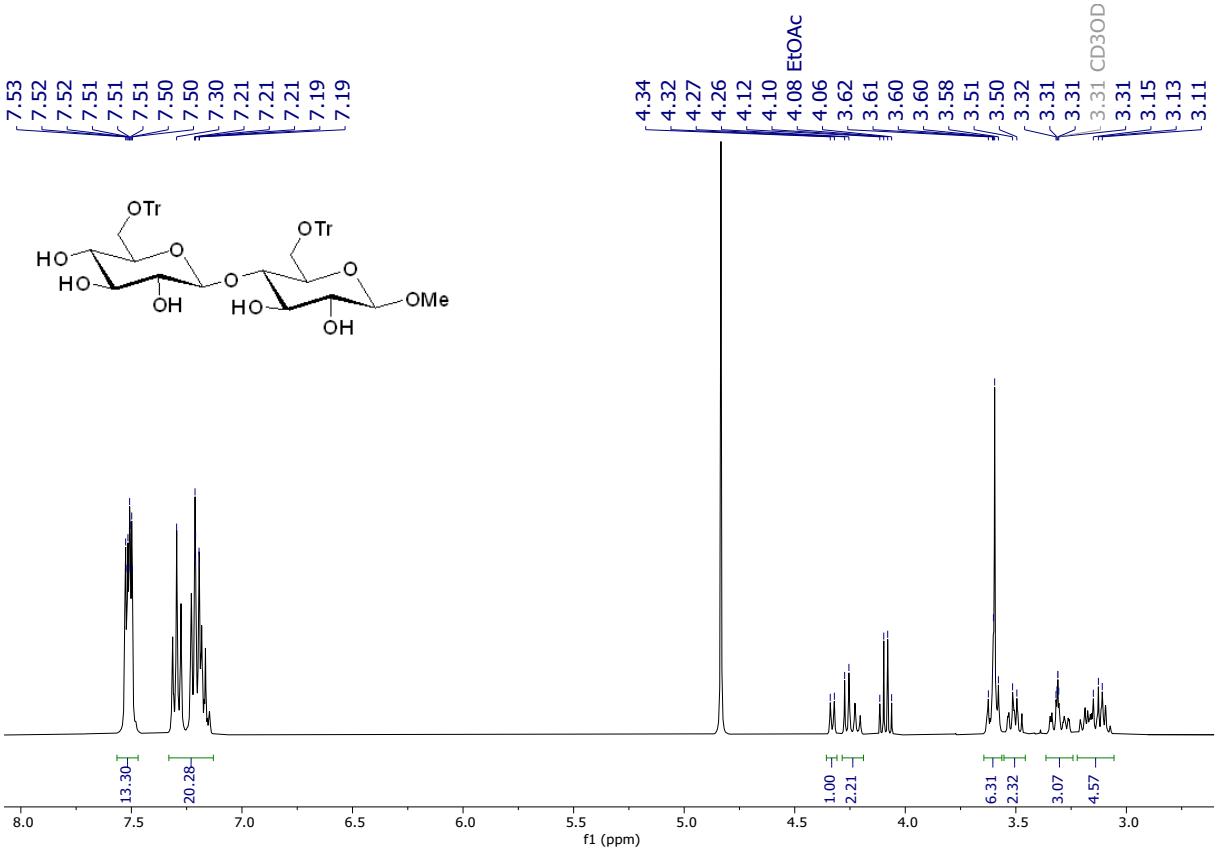
^{1}H NMR spectrum of compound 17 (400 MHz, Methanol-d_4)



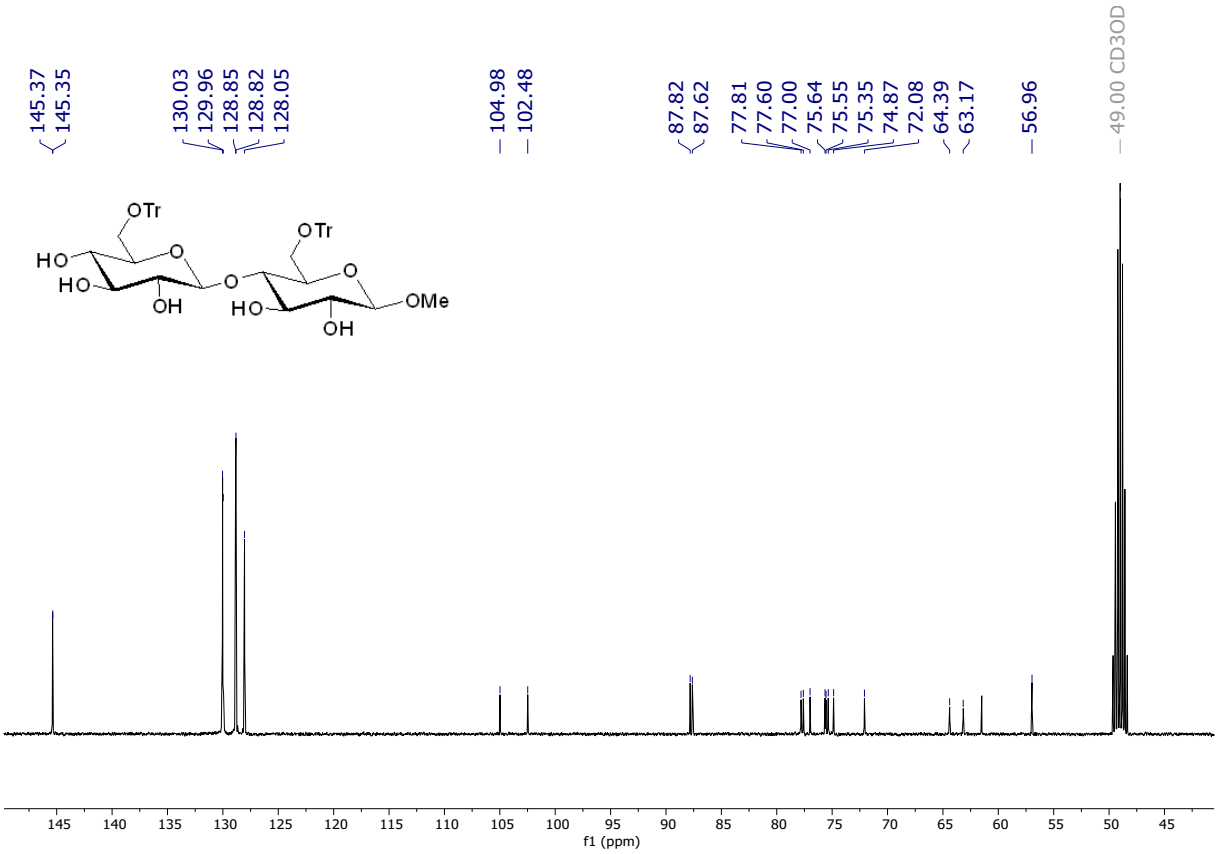
^{13}C NMR spectrum of compound 17 (101 MHz, Methanol-d_4)



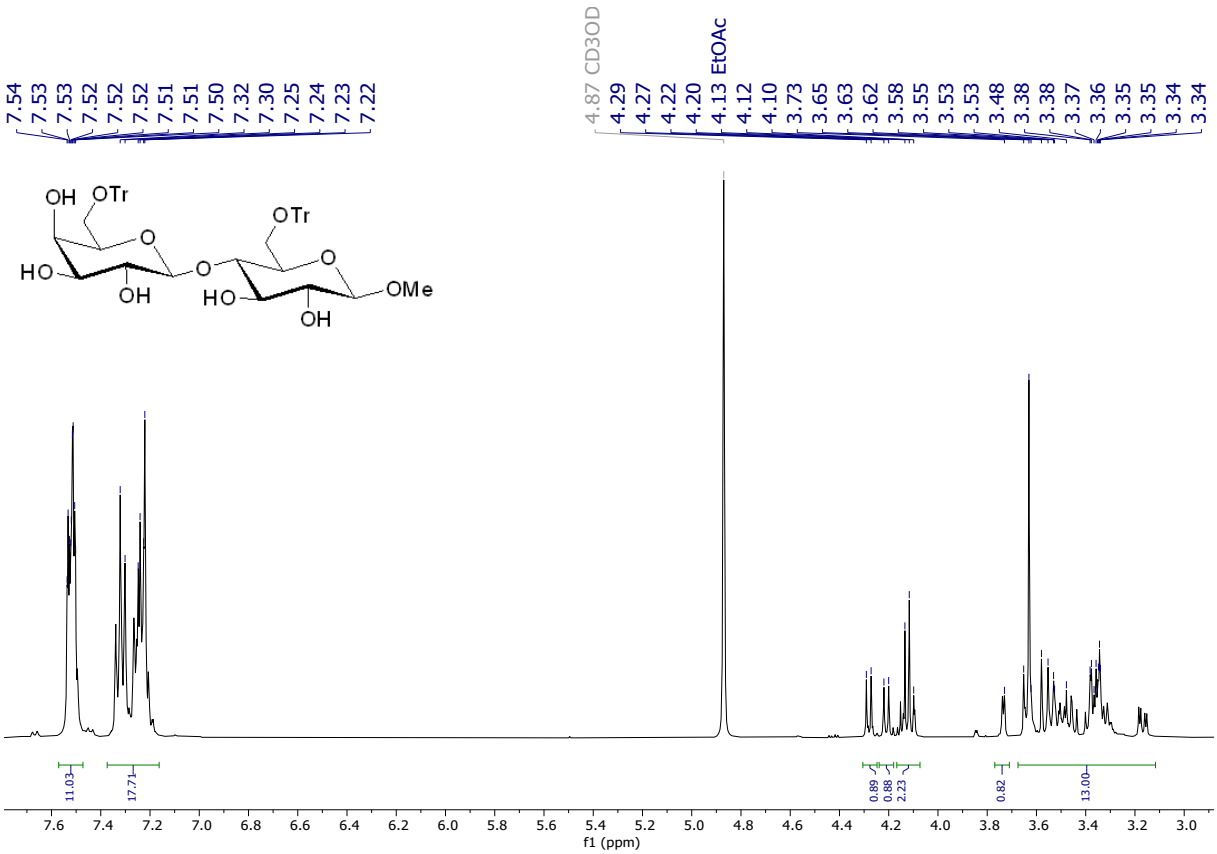
^{1}H NMR spectrum of compound 18 (400 MHz, Methanol-d_4)



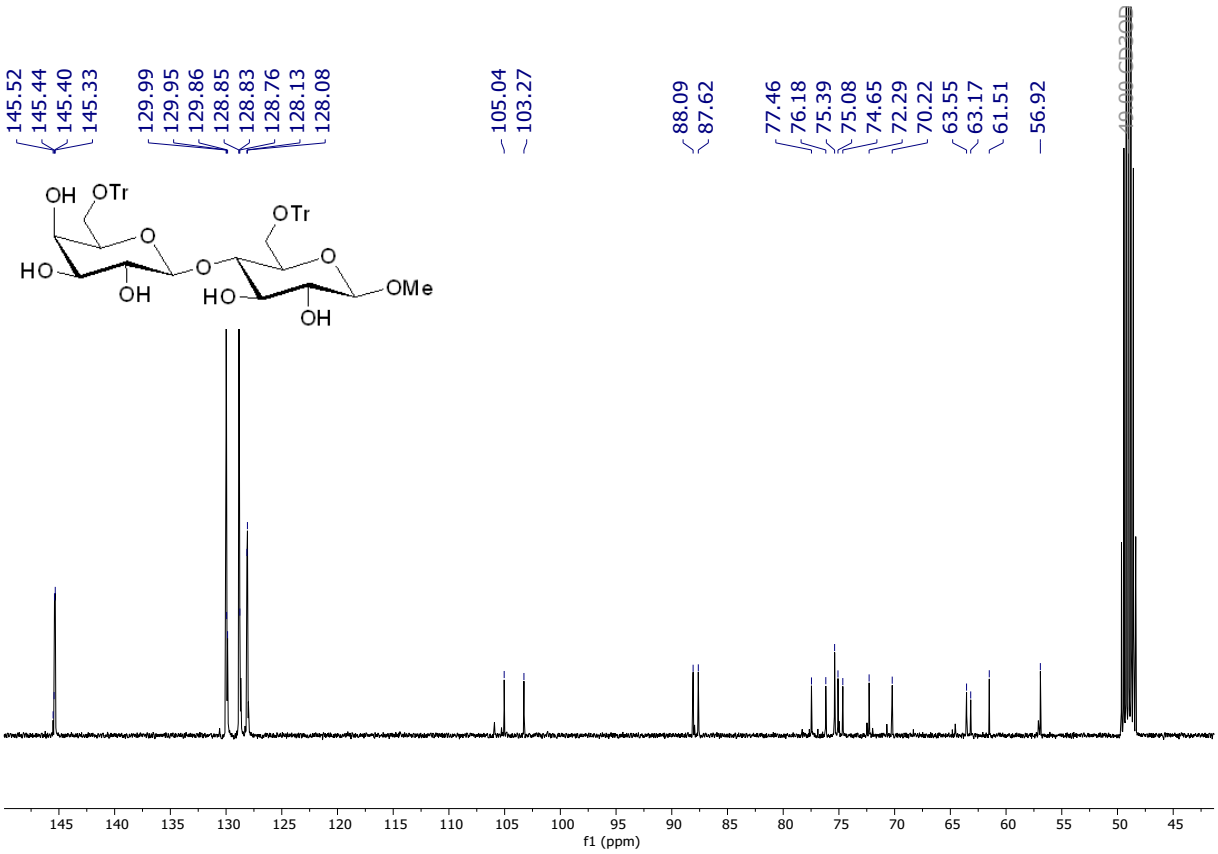
^{13}C NMR spectrum of compound 18 (101 MHz, Methanol-d_4)



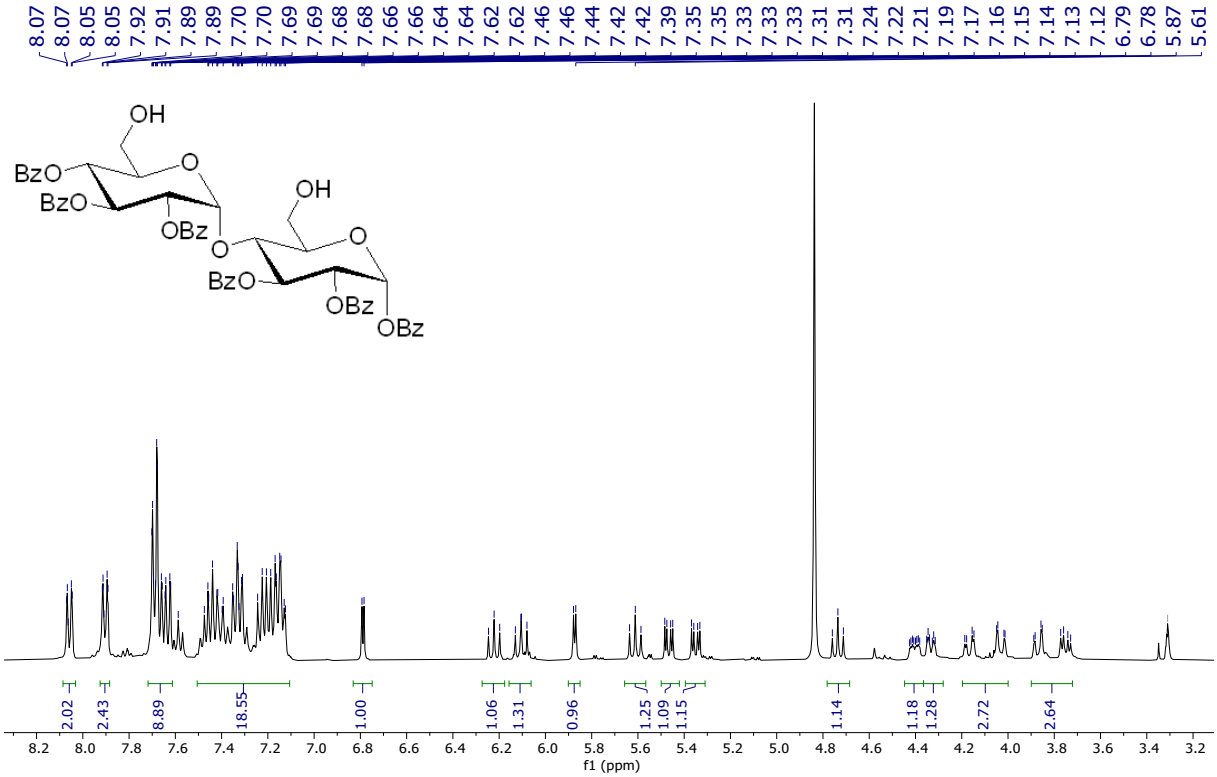
^{1}H NMR spectrum of compound 19 (400 MHz, Methanol-d_4)



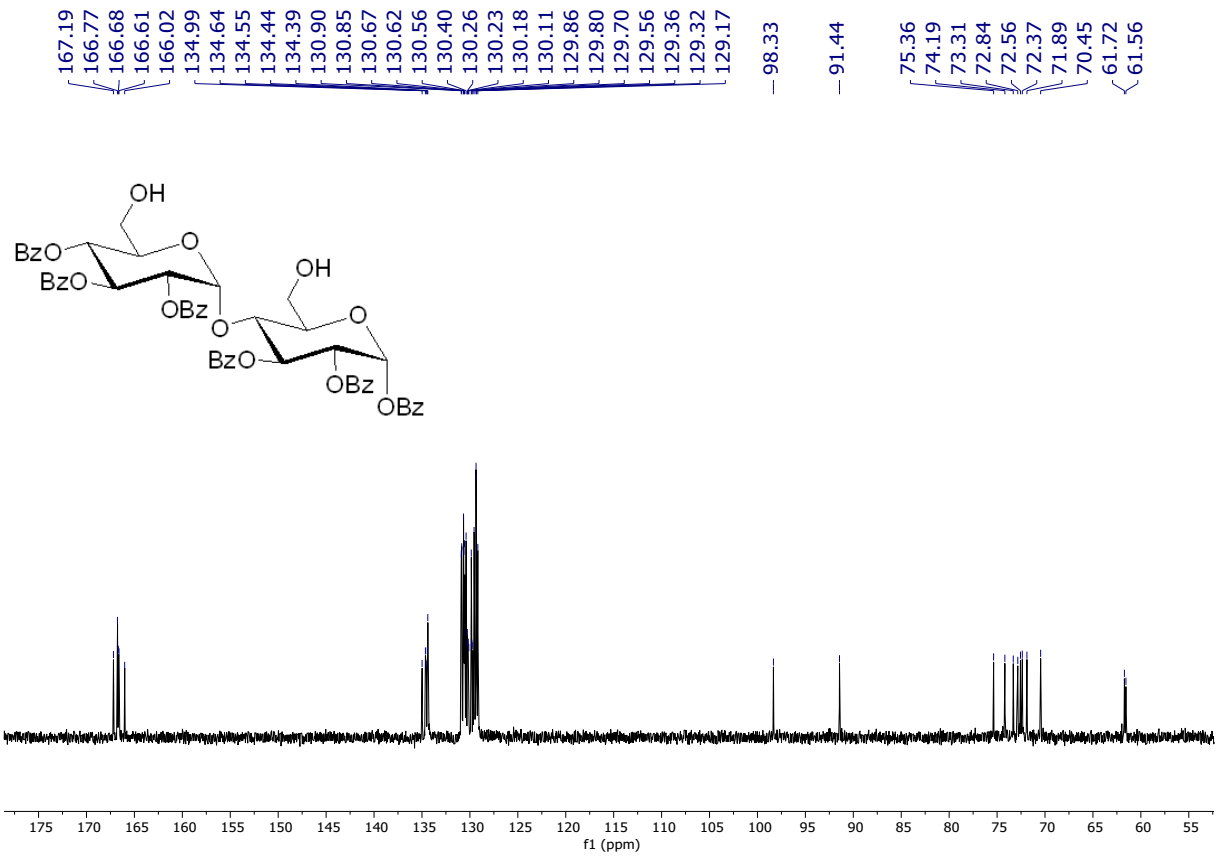
^{13}C NMR spectrum of compound 19 (101 MHz, Methanol-d_4)



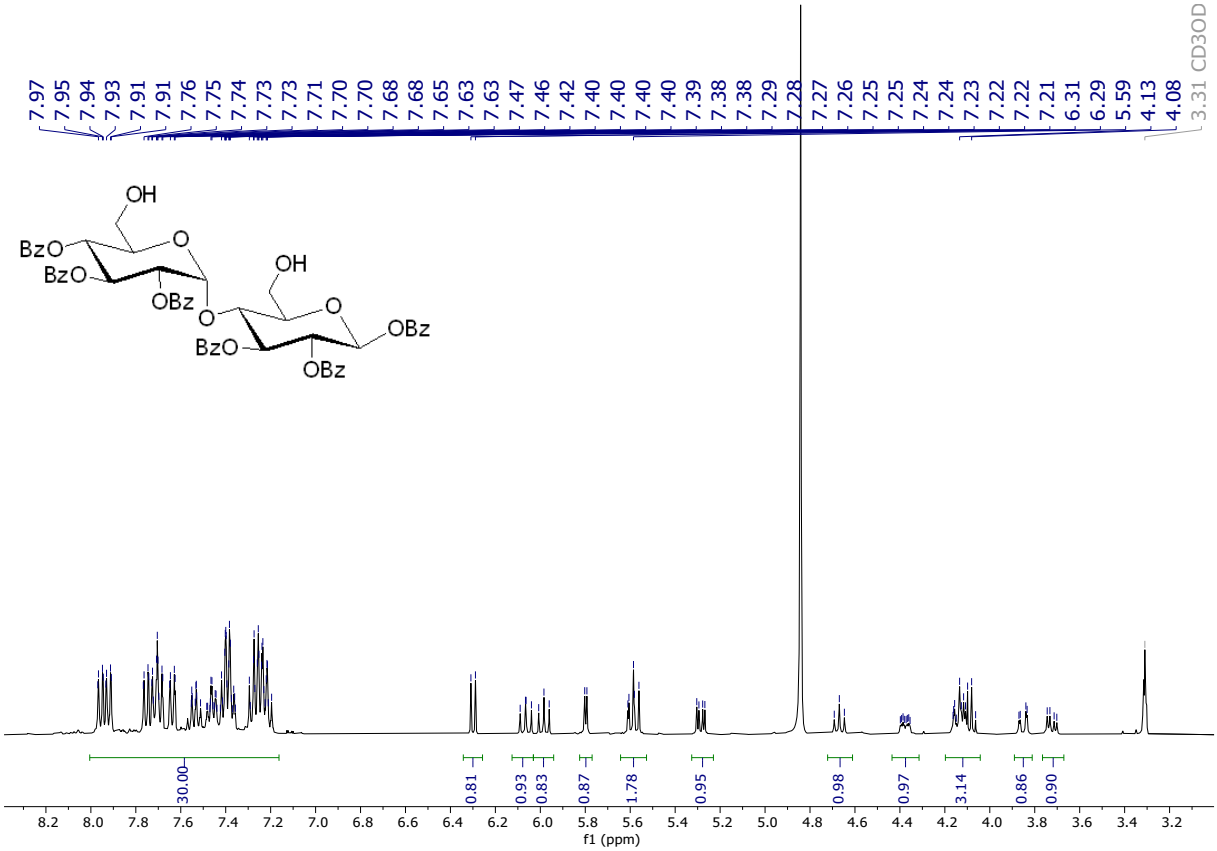
^{1}H NMR spectrum of compound 20α (400 MHz, Methanol-d_4)



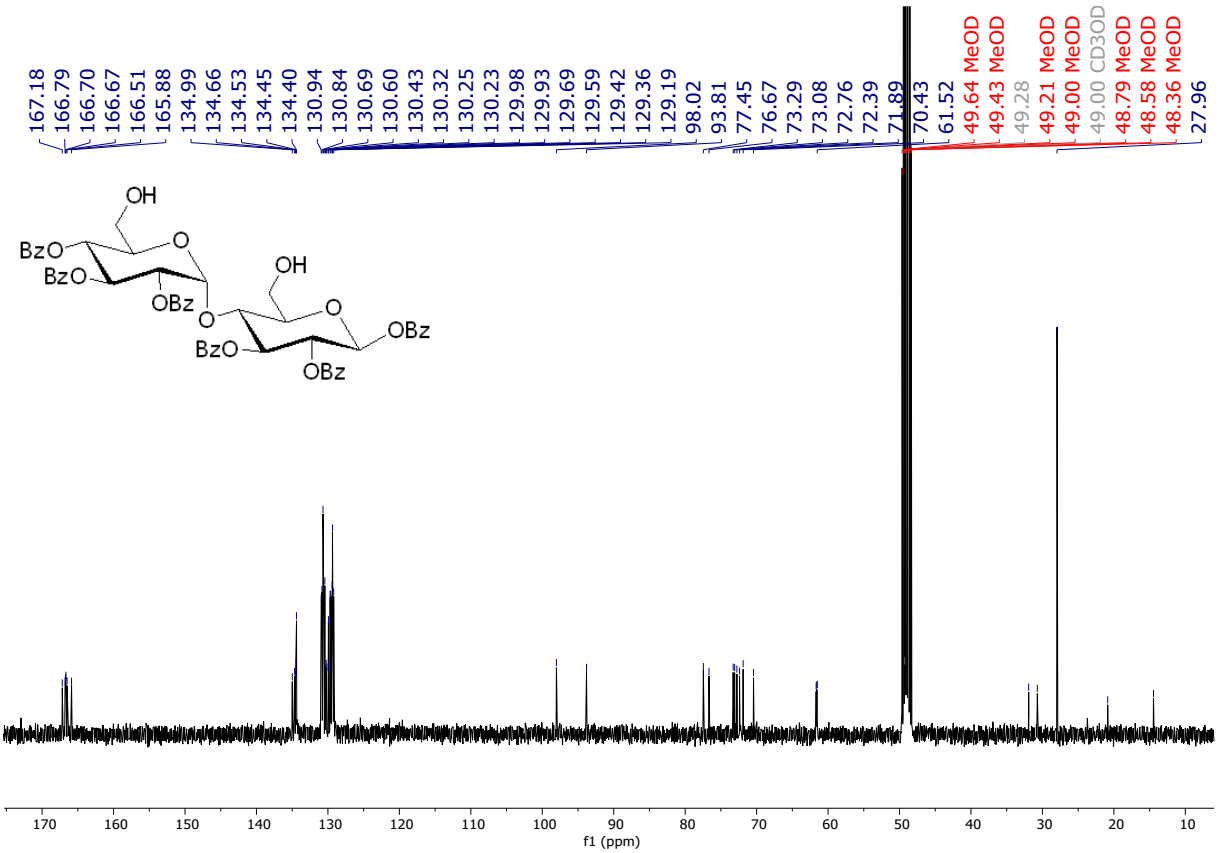
^{13}C NMR spectrum of compound 20α (101 MHz, Methanol-d_4)



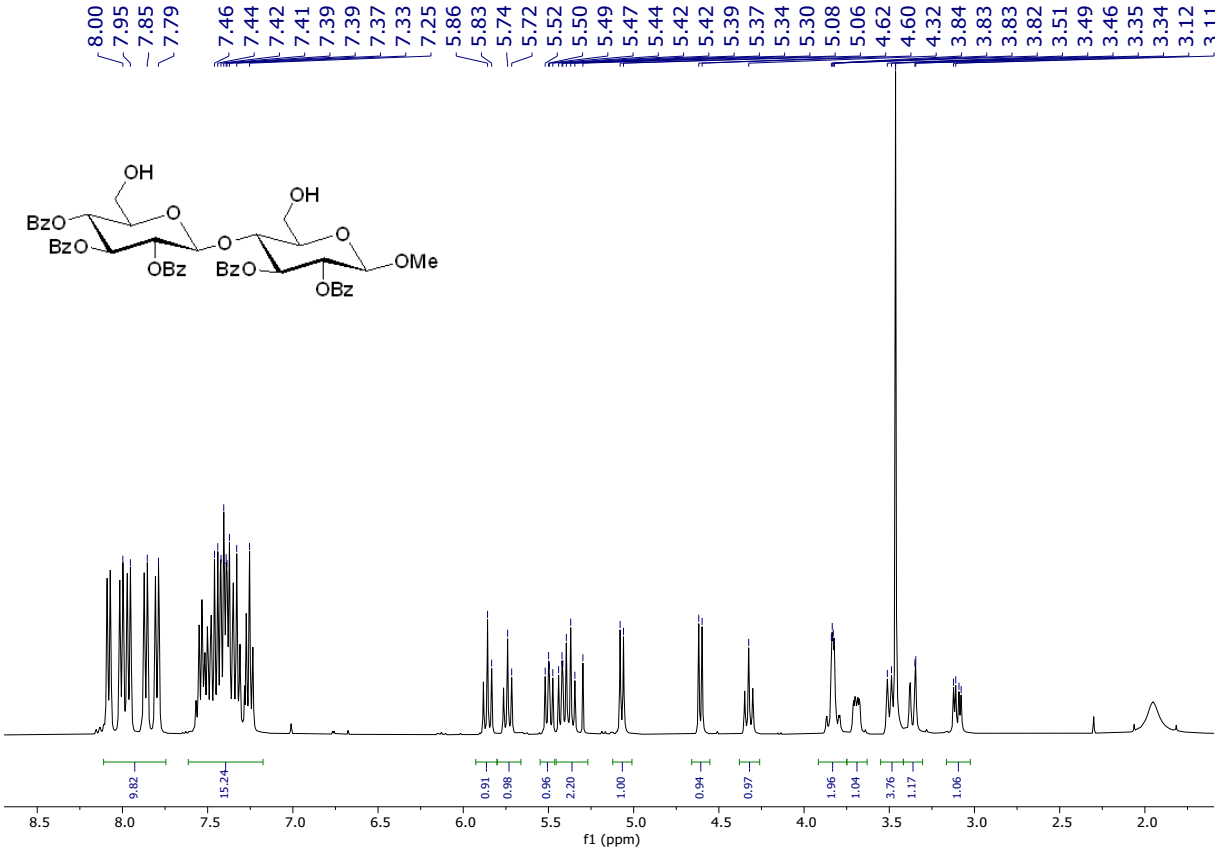
^{1}H NMR spectrum of compound 20β (400 MHz, Methanol-d_4)



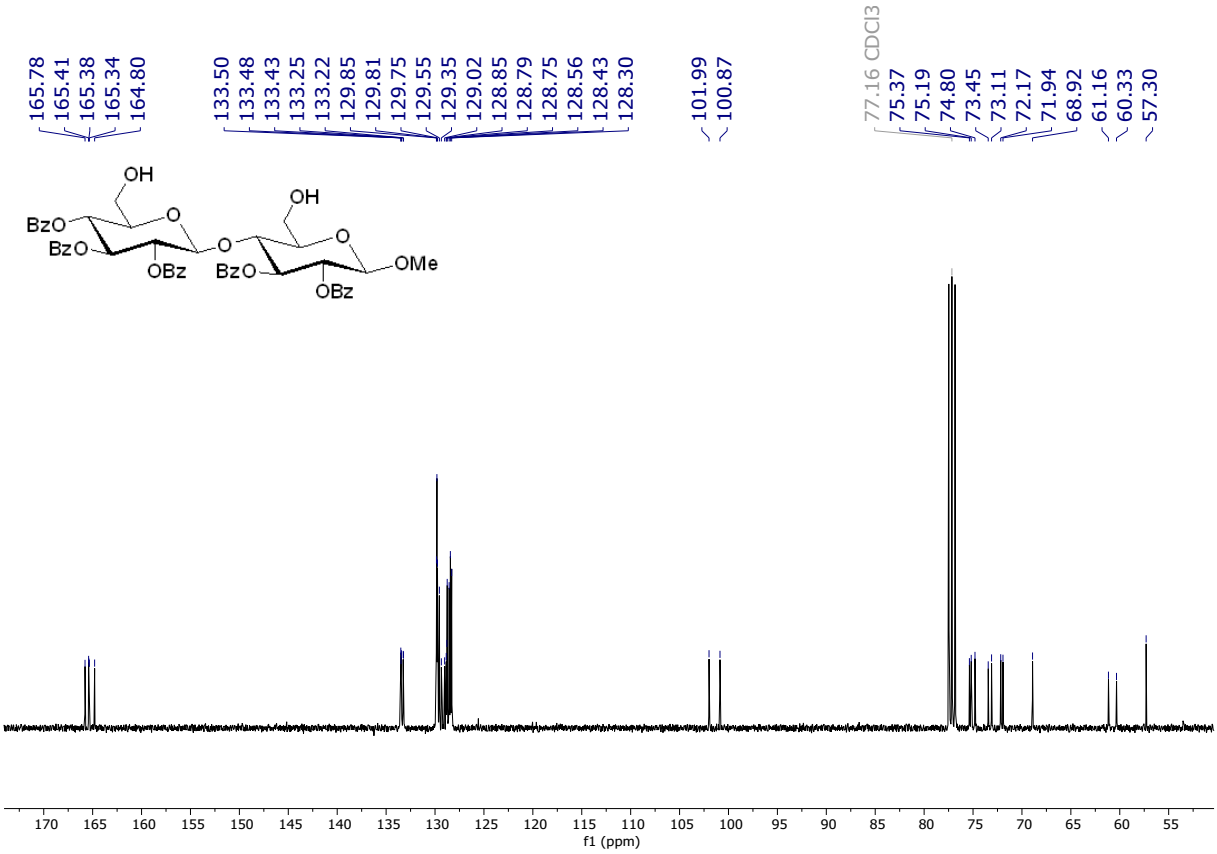
^{13}C NMR spectrum of compound 20β (101 MHz, Methanol-d_4)



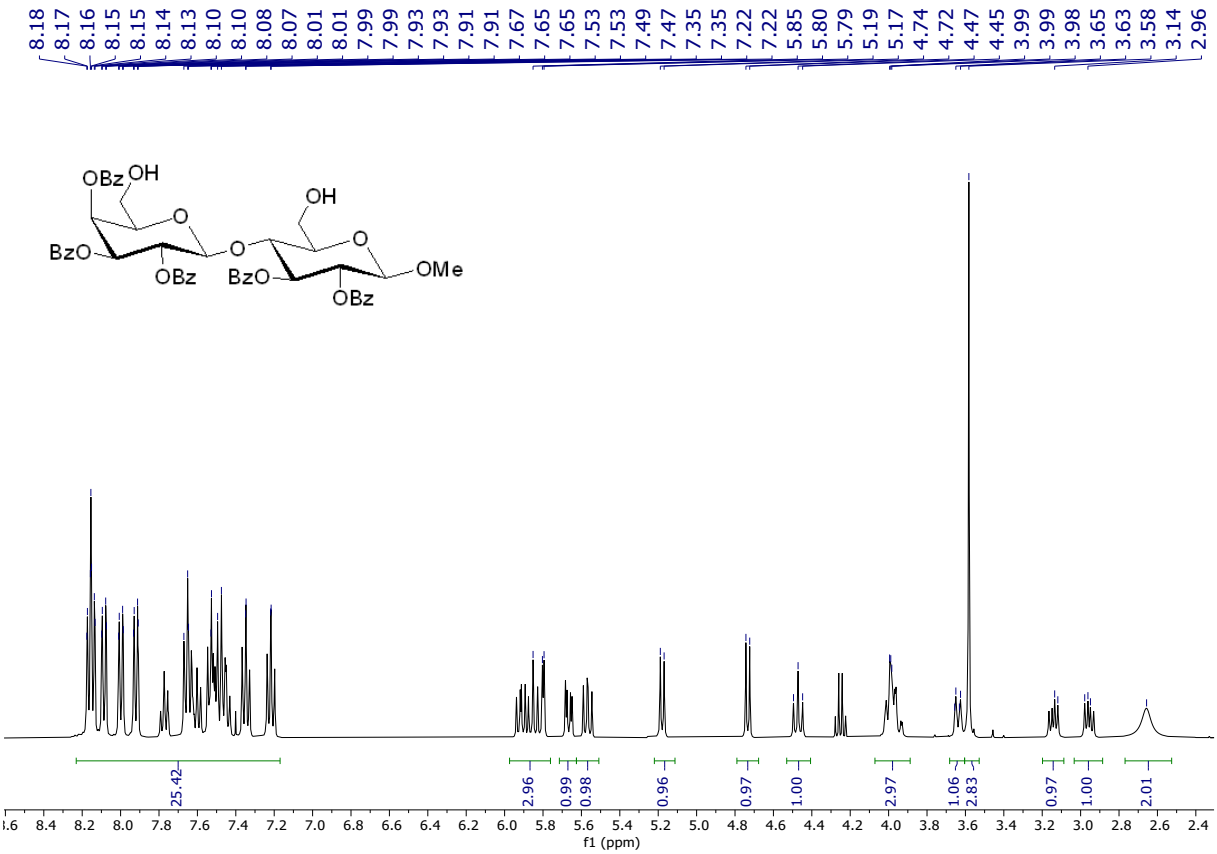
^{1}H NMR spectrum of compound 21 (400 MHz, $CDCl_3$)



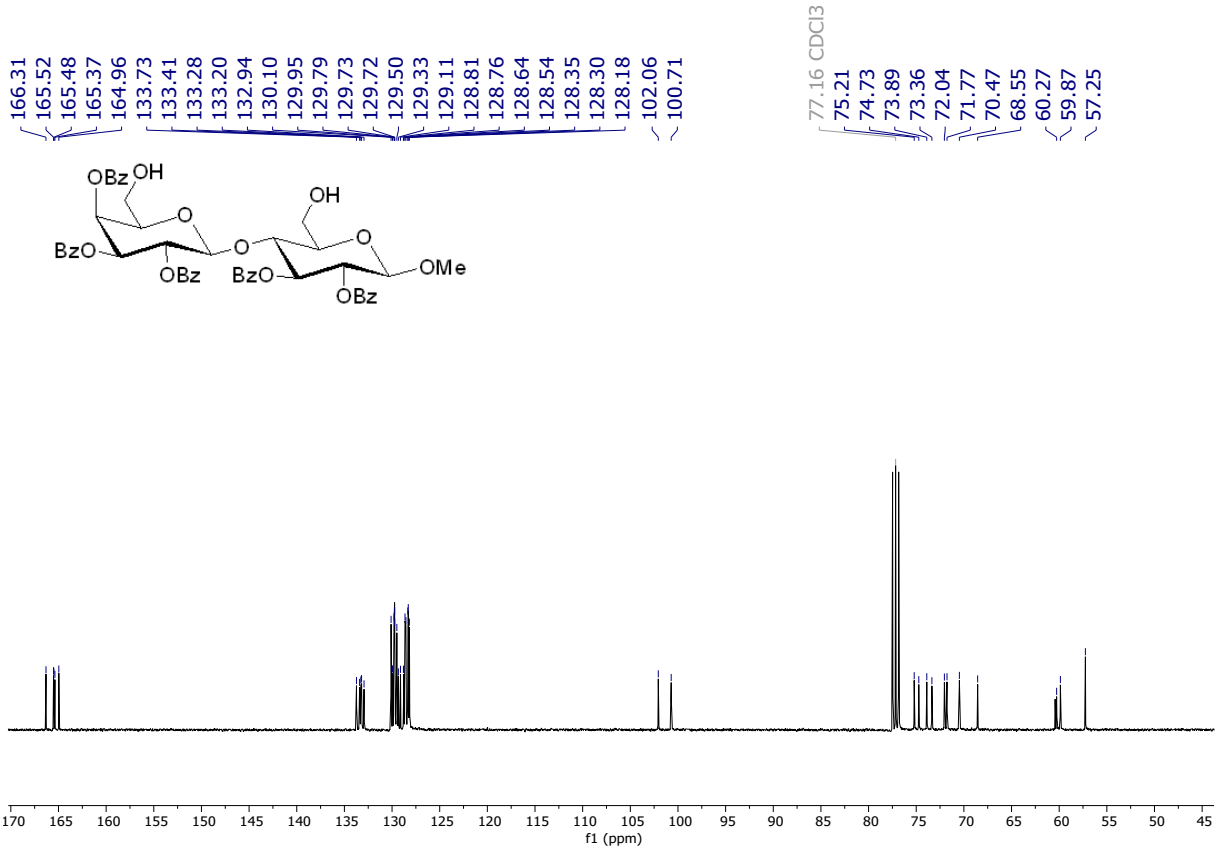
^{13}C NMR spectrum of compound 21 (101 MHz, $CDCl_3$)



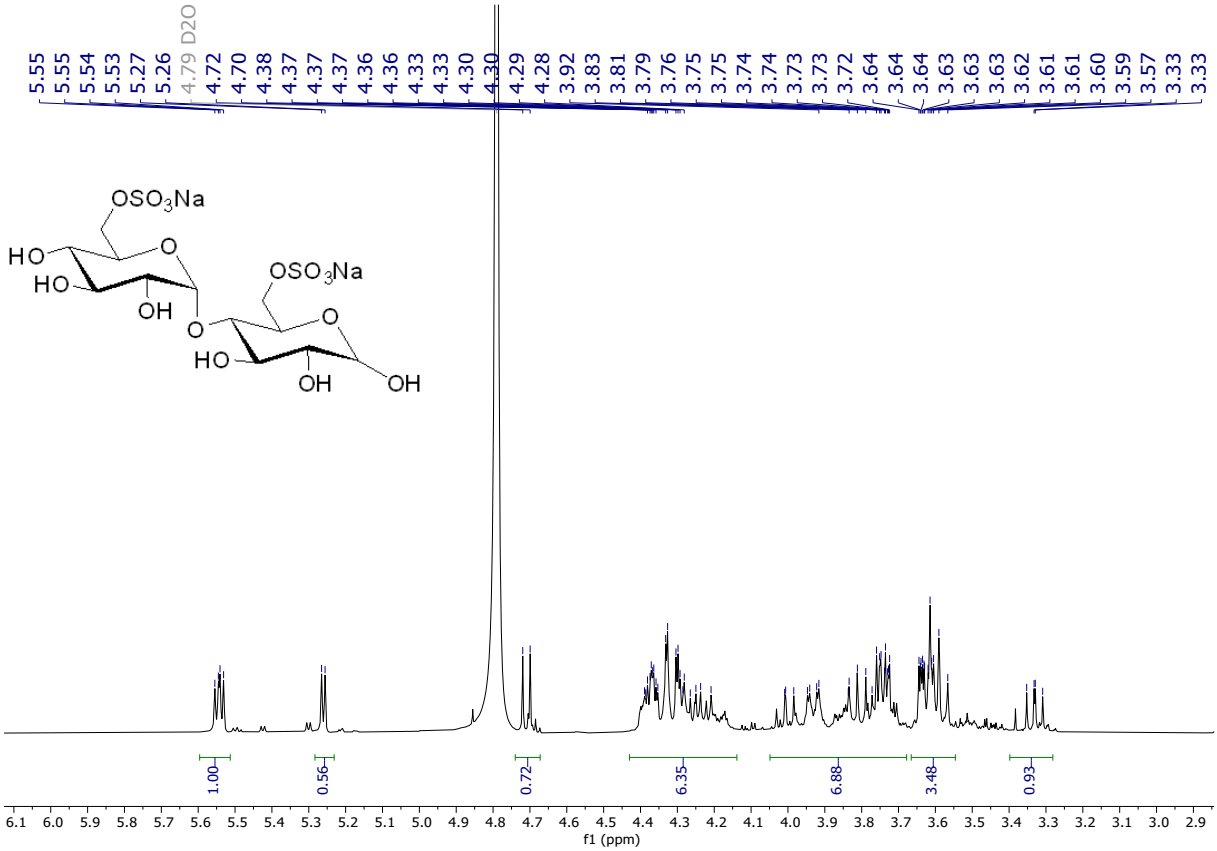
^{1}H NMR spectrum of compound 22 (400 MHz, $CDCl_3$)



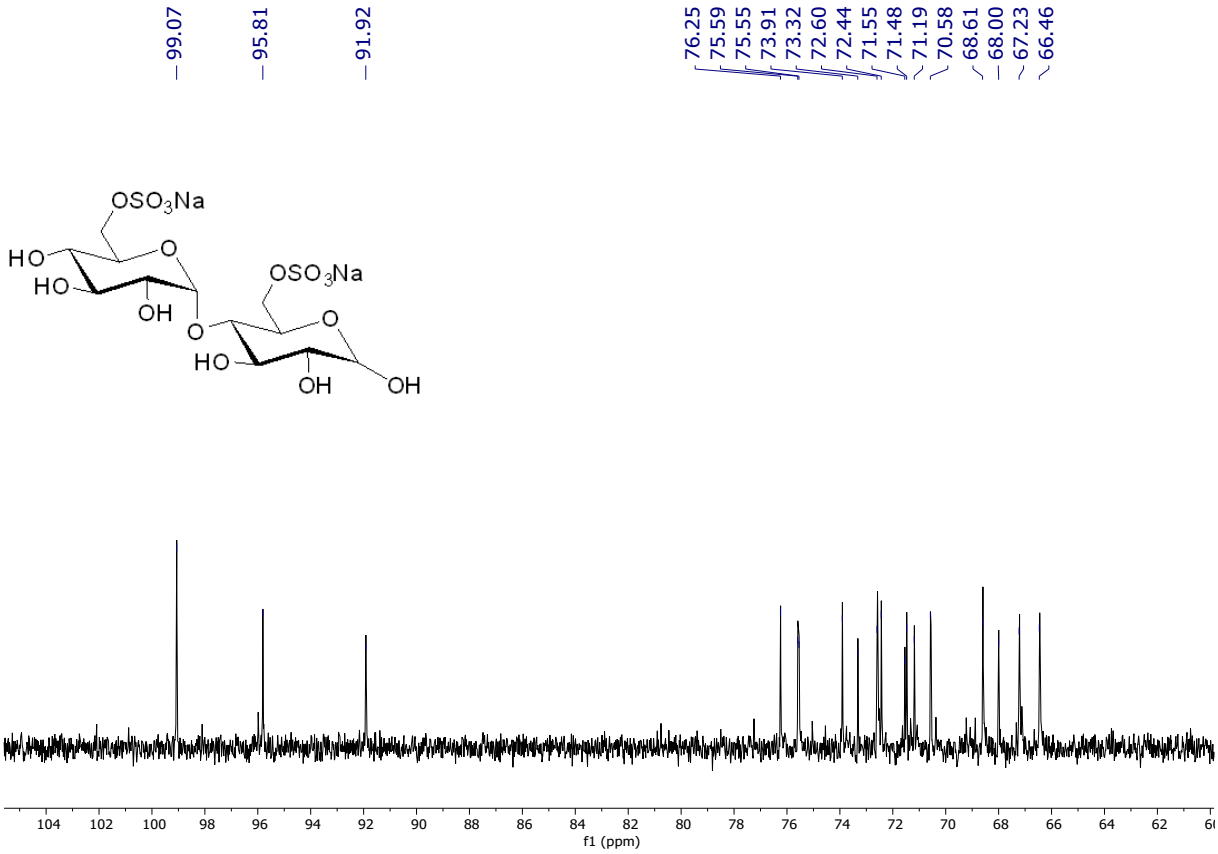
^{13}C NMR spectrum of compound 22 (101 MHz, $CDCl_3$)



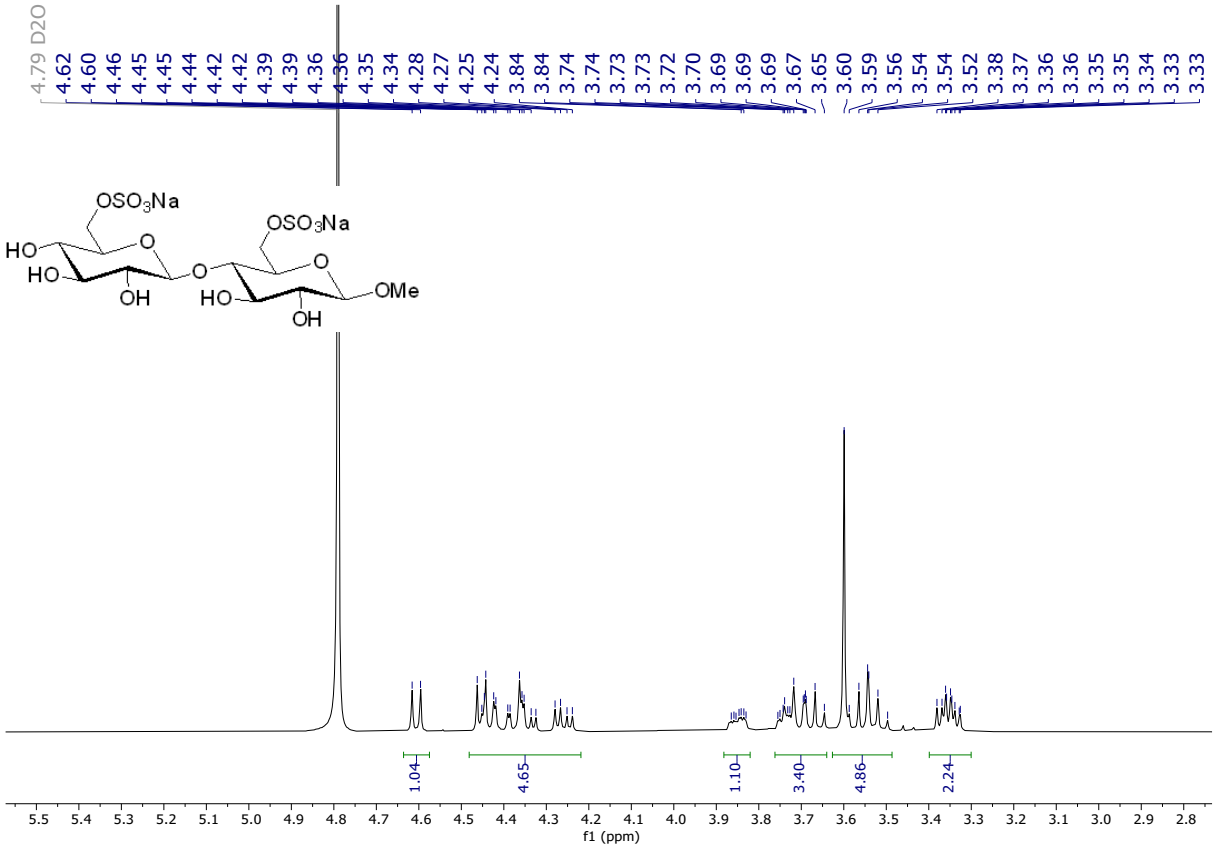
^{1}H NMR spectrum of compound 23 (400 MHz, D_2O)



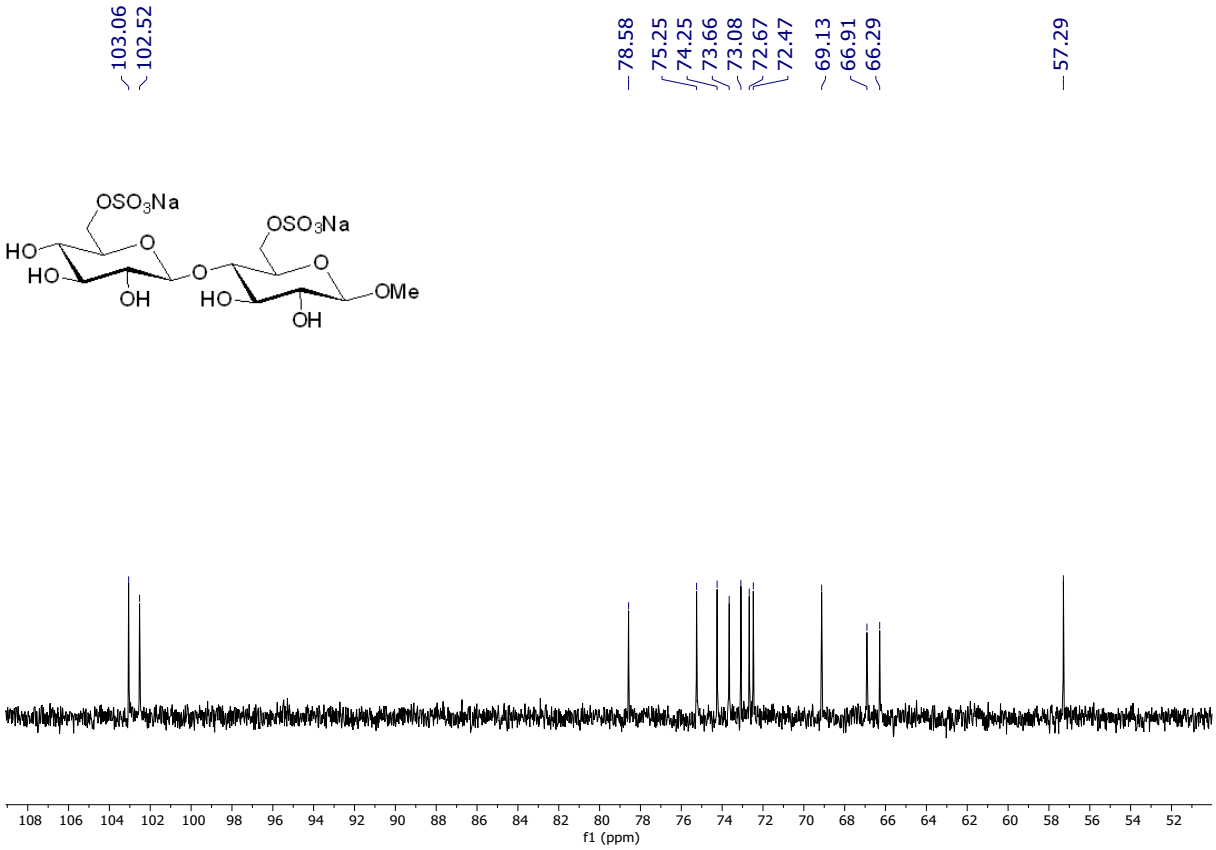
^{13}C NMR spectrum of compound 23 (101 MHz, D_2O)



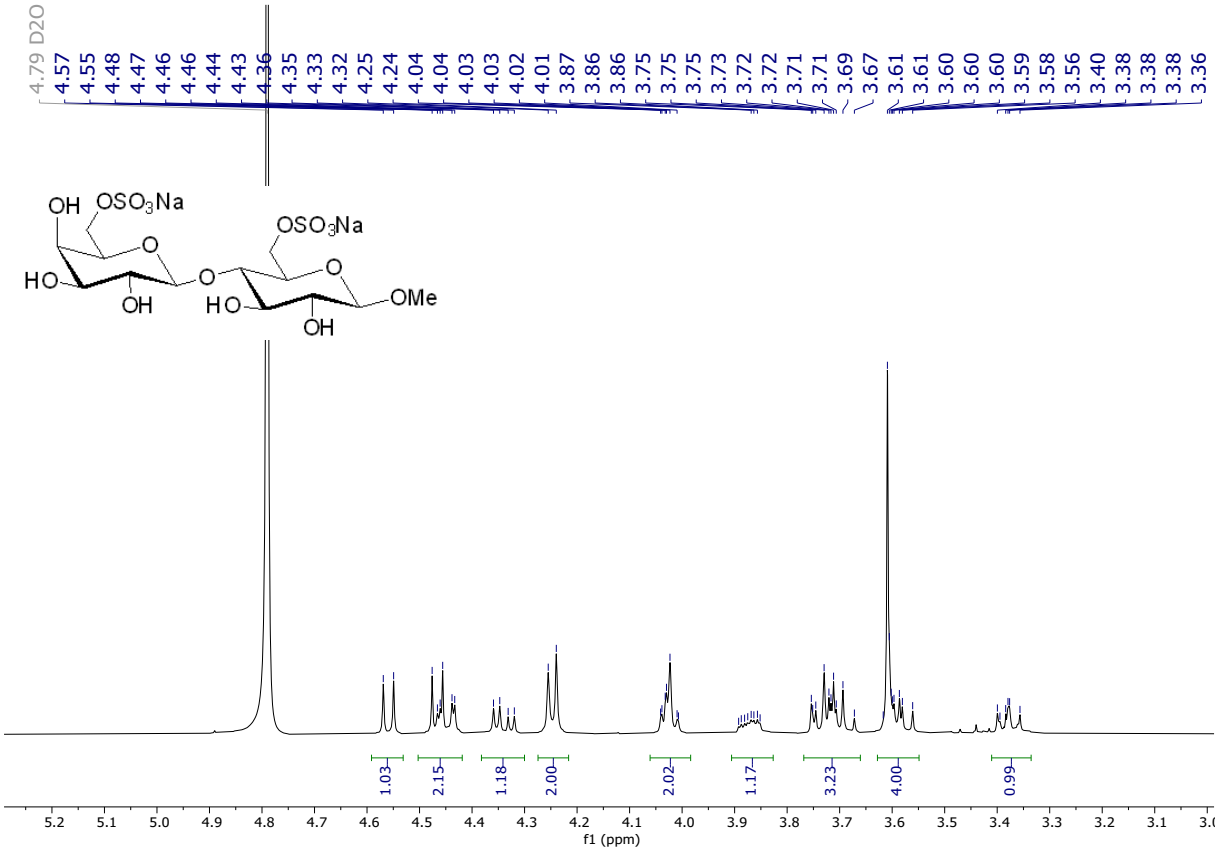
^{1}H NMR spectrum of compound 24 (400 MHz, D_2O)



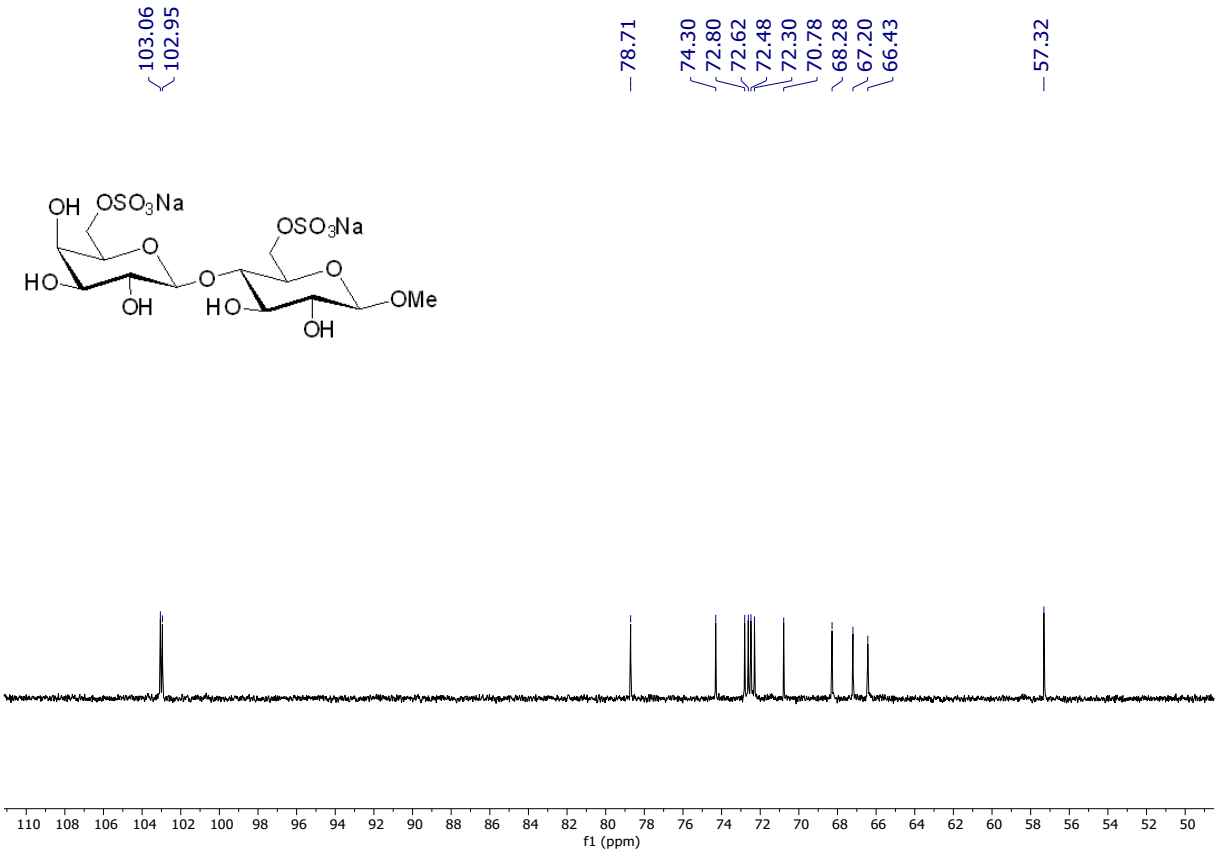
^{13}C NMR spectrum of compound 24 (101 MHz, D_2O)



^{1}H NMR spectrum of compound 25 (400 MHz, D_2O)



^{13}C NMR spectrum of compound 25 (101 MHz, D_2O)



7. References

1 N.m.r. and conformational studies of some 1,4-linked disaccharides, I. Backman, B. Erbing, P. E. Jansson and L. Kenne, *J. Chem. Soc. Perkin Trans. 1*, 1988, 889–898.

2 Dithiolsaccharide mucolytic agents and uses thereof., The Regents of the University of California, USA; University College Dublin ., WO2020055916A1, *PCT Int. Appl.*, 2020, 229pp.

3 Effects of CoCl2 on the regioselective tosylation of oligosaccharides, J. El-Abid, V. Moreau, J. Kovensky and V. Chagnault, *J. Mol. Struct.*, 2021, **1241**, 130609.

4 Synthése totale d'α-disaccharidyles de tridésoxy-2,5,6-streptamine apparentés aux aminosides à partir du maltose et du lactose, N. Rolland, G. Vass, J. Cleophax, A. -M Sepulchre, S. D. Gero and A. Cier, *Helv. Chim. Acta*, 1982, **65**, 1627–1636.

5 Studies of Aminosugars. XIV. Syntheses of 6,6'-Diamino-6,6'-dideoxymaltosylamine, 1',6,6'-Triamino-,1',6,6'-trideoxysucrose and 6,6'-Diamino-6,6'-dideoxytrehalose, S. Umezawa, T. Tsuchiya, S. Nakada and K. Tatsuta, *Bull. Chem. Soc. Jpn.*, 1967, **40**, 395–401.

6 Extended tight-binding quantum chemistry methods, C. Bannwarth, E. Caldeweyher, S. Ehlert, A. Hansen, P. Pracht, J. Seibert, S. Spicher and S. Grimme, *Wiley Interdiscip. Rev. Comput. Mol. Sci.*, 2021, **11**, e1493.

7 Automated exploration of the low-energy chemical space with fast quantum chemical methods, P. Pracht, F. Bohle and S. Grimme, *Phys. Chem. Chem. Phys.*, 2020, **22**, 7169–7192.

8 Efficient Quantum Chemical Calculation of Structure Ensembles and Free Energies for Nonrigid Molecules, S. Grimme, F. Bohle, A. Hansen, P. Pracht, S. Spicher and M. Stahn, *J. Phys. Chem. A*, 2021, **125**, 4039–4054.

9 The ORCA quantum chemistry program package, F. Neese, F. Wennmohs, U. Becker and C. Riplinger, *J. Chem. Phys.*, 2020, **152**, 224108.

10 Gaussian 16 Rev. C.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V Ortiz, A. F. Izmaylov, J. L. Sonnenberg, Williams, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman and D. J. Fox, 2016.

11 IGMPlot: A program to identify, characterize, and quantify molecular interactions, C. Lefebvre, J. Klein, H. Khartabil, J.-C. Boisson and E. Hénon, *J. Comput. Chem.*, 2023, **44**, 1750–1766.

12 The Independent Gradient Model: A New Approach for Probing Strong and Weak Interactions in Molecules from Wave Function Calculations, C. Lefebvre, H. Khartabil, J.-C. Boisson, J. Contreras-García, J.-P. Piquemal and E. Hénon, *ChemPhysChem*, 2018, **19**, 724–735.

13 Atomic Decomposition Scheme of Noncovalent Interactions Applied to Host–Guest Assemblies, M. Ponce-Vargas, C. Lefebvre, J.-C. Boisson and E. Hénon, *J. Chem. Inf. Model.*, 2020, **60**, 268–278.

14 New Way for Probing Bond Strength, J. Klein, H. Khartabil, J.-C. Boisson, J. Contreras-García, J.-

P. Piquemal and E. Hénon, *J. Phys. Chem. A*, 2020, **124**, 1850–1860.

15 NBO 6.0: Natural bond orbital analysis program, E. D. Glendening, C. R. Landis and F. Weinhold, *J. Comput. Chem.*, 2013, **34**, 1429–1437.

16 VMD: Visual molecular dynamics, W. Humphrey, A. Dalke and K. Schulten, *J. Mol. Graph.*, 1996, **14**, 33–38.