Sulfonated, sulfated thioglycosides and multivalence in heparanase inhibition

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Supporting information

Experimental part

General Information.

All reagent-grade chemicals were obtained from commercial suppliers and were used as received. All solvents used for the anhydrous reactions were either taken from the INERT PureSolv MD5 solvent purifier or freshly distilled on CaH_2 under argon atmosphere. Distilled water and ultrapure water were obtained from Millipore purification systems. Characterizations of known compounds were in accordance with literature. Optical rotations were recorded in a $CHCl_3$, MeOH or H_2O solution with an Anton Paar MPC 100 polarimeter. The instrument is equipped with an automatic Peltier temperature controller (20 and 25 °C as desired). FTIR spectra were obtained using ATR and are reported in cm-1. 1H NMR (600, 400 MHz) and 13C NMR (151, 101 MHz) spectra were recorded in D_2O, CD_3OD or $CDCl_3$. The proton and carbon signal assignments were determined from decoupling experiments, COSY, HSQC and 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). The melting points were measured using a KOFLER bench. Microwave irradiation was carried out in a CEM Discover instrument, at 70 °C (power max. 300 W). High-resolution electrospray mass spectra in the positive ion mode were obtained on a Q-TOF Ultima Global 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 100 V and the RF lens1 energy was optimized for each sample (40 V). For collision-induced dissociation (CID) experiments, argon was used as collision gas at an indicated analyser pressure of 5.10-5 Torr and the collision energy was optimized for each parent ion (50-110 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).

TR-FRET Heparanase Inhibition Assay.[1]

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 nM, 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 610 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.

Synthesis of compound 2.

To a solution of compound **1**[2] (100 mg, 0.13 mmol) in MeOH/DCM (230 µL, 1/1, v/v), was added acetyl chloride (1.1 equiv., 10 µL, 0.14 mmol). The mixture was stirred 13 h at room temperature under an argon atmosphere. DCM (10 mL) was then added and the mixture was washed with a saturated solution of $NaHCO_3$, a saturated solution of NaCl and water. The organic layer was dried over sodium sulfate, filtered, the solvent evaporated under reduced pressure and then well dried. To the dry residue (0.07 g, 0.115 mmol) in anhydrous pyridine (0.5 mL) at -20°C and under argon, a solution of tosyl chloride (0.049 g, 0.259 mmol) in anhydrous pyridine (0.5 mL) was slowly added. After 2.5 h at room temperature, the excess of tosyl chloride was neutralized by the addition of methanol (2 mL) and the solution was diluted with ethyl acetate (20 mL). The organic layer was washed with a saturated solution of sodium chloride (3 x 20 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. To the obtained crude mixture, pyridine (0.5 mL) and acetic anhydride (0.25 mL) were added. The mixture was stirred at room temperature overnight. The excess of acetic anhydride was neutralized at 0°C by adding methanol (2 mL) and the solution was then concentrated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) and washed with a saturated solution of $NaHCO_3$ and then water. The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated. Flash chromatography (4:1 to 3:2 cyclohexane/ethyl acetate) afforded **2** as white crystals (0.072 g; 63%). Rf: 0.32 (3:2 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$: +27.0 (c = 0.5 $CHCl_3$); IR (ATR): 3748, 3672, 3021, 1754, 1730, 1601, 1452, 1366, 1275, 1244, 1219, 1179, 1096, 1069, 1038, 980, 925, 713 cm^{-1}; HRMS: Calcd. for [$C_{41}H_{48}O_{18}NS_2$]: m/z 906.2313 $[M+NH_4]^+$, found 906.2292 $[M+NH_4]^+$; ^{1}HNMR (600 MHz, $CDCl_3$) δ 7.95 (ddt, J = 10.1, 6.8, 1.3 Hz, 4H, H_{ar}), 7.86 – 7.81 (m, 2H, H_{ar}), 7.55 – 7.46 (m, 2H, H_{ar}), 7.44 – 7.31 (m, 6H, H_{ar}), 5.90 (dd, *J* = 11.2, 9.5 Hz, 1H, H_3), 5.27 (t, *J* = 9.2 Hz, 1H, $H_{3'}$), 5.17 (t, *J* = 9.7 Hz, 1H, $H_{4'}$), 5.14 – 5.05 (m, 2H, H_2, H_1), 5.00 (d, *J* = 10.0 Hz, 1H, $H_{1'}$), 4.80 (dd, *J* = 10.1, 9.1 Hz, 1H, $H_{2'}$), 4.56 (dd, *J* = 10.8, 4.0 Hz, 1H, H_{6a}), 4.46 – 4.40 (m, 1H, H_{6b}), 4.34 (ddd, *J* = 11.1, 4.0, 1.8 Hz, 1H, H_5), 4.11 (d, *J* = 10.1 Hz, 1H, $H_{5'}$), 3.75 (s, 3H, -$COOCH_3$), 3.33 (s, 3H, -OCH_3), 3.14 (t, *J* = 11.1 Hz, 1H, H_4), 2.48 (s, 3H, -$PhCH_3$), 2.02 (s, 3H, CH_3CO-), 1.97 (s, 3H, CH_3CO-), 1.54 (s, 3H, CH_3CO-); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 169.5, 169.3 (3 x $CH_3\underline{C}O$-), 166.6 (-$\underline{C}OOCH_3$), 165.8 (2x –$O\underline{C}OPh$), 145.2 (Cq tosyl), 133.6 – 128.3 (17C_{ar}), 97.2 (C_1), 81.3 ($C_{1'}$), 75.3 ($C_{5'}$), 73.3 ($C_{3'}$), 73.1 (C_2), 69.6 ($C_{2'}$), 69.3 (C_6), 69.0 ($C_{4'}$), 68.9 (C_5), 67.0 ($C_{3'}$), 55.6 (-OCH_3), 53.1 (-$COO\underline{C}H_3$), 45.7 (C_4), 21.8 (-$Ph\underline{C}H_3$), 20.7 – 20.1 (3 x $\underline{C}H_3CO$-).

Synthesis of compound 6.

To a solution of compound **5**[2] (0.3 g, 0.326 mmol) in anhydrous pyridine (1 mL) at -20 °C and under argon atmosphere, was added slowly a solution of tosyl chloride (0.139 g, 0.733 mmol) in a mixture of anhydrous pyridine (1 mL) and anhydrous triethylamine (0.102 mL, 0.733 mmol). The mixture was stirred 3 h at room temperature then the excess of tosyl chloride was neutralized by addition of methanol (1 mL). The resulting solution was diluted with ethyl acetate (50 mL) and washed with a saturated solution of NaCl (3 x 50 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography (7:3 cyclohexane/ethyl acetate) to give **6** as white crystals (0.331 g, 95%). Rf: 0.72 (1:1 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$: +23.0 (c = 0.5 $CHCl_3$); IR (ATR): 3667, 3005, 1728, 1601, 1452, 1364, 1273, 1260, 1179, 1094, 1071, 1028, 928, 856, 708, 667, 542, 521, 448 cm^{-1}; HRMS: Calcd. for [$C_{56}H_{50}O_{18}S_2Na$]: m/z 1097.2336 $[M+Na]^+$, found 1097.2361 $[M+Na]^+$; ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.03 – 7.01 (m, 29H, H_{ar}), 6.02 – 5.89 (m, 2H, H_3, $H_{3'}$), 5.64 (t, *J* = 9.7 Hz, 1H, $H_{4'}$), 5.43 (d, *J* = 9.9 Hz, 1H, $H_{1'}$), 5.36 (dd, *J* = 10.0, 8.9 Hz, 1H, $H_{2'}$), 5.12 (dd, *J* = 9.5, 3.5 Hz, 1H, H_2), 5.09 (d, *J* = 3.5 Hz, 1H, H_1), 4.67 (dd, *J* = 10.8, 3.6 Hz, 1H, H_{6a}), 4.46 (m, 3H, H_5, $H_{5'}$, H_{6b}), 3.69 (s, 3H, -$COOCH_3$), 3.32 (s, 3H, -OCH_3), 3.20 (t, *J* = 11.1 Hz, 1H, H_4), 2.49 (s, 3H, -$PhCH_3$) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (-$\underline{C}OOCH_3$) , 166.1 – 164.9 (5x-$O\underline{C}OBz$) , 145.3 (Cq tosyl) , 133.6 –128.3 (35 C_{ar}) , 97.2 (C_1) , 81.6 ($C_{1'}$) , 75.7 ($C_{5'}$) , 73.6 ($C_{3'}$) , 73.2 (C_2) , 70.3 ($C_{2'}$) , 69.8 ($C_{4'}$) , 69.3 (C_6) , 69.1 (C_5) , 66.9 (C_3) , 55.5 (-OCH_3) , 53.0 (-$COO\underline{C}H_3$) , 45.6 (C_4) , 21.8 (Ph-$\underline{C}H_3$).

Synthesis of compound 7.

To a solution of tosyl **2** (64.1 mg, 0.07 mmol) in anhydrous DMF (2 mL) under argon, potassium thioacetate (2 equiv., 16.48 mg, 0.144 mmol) was added. The reaction mixture was stirred at 60 °C for 3 h. The solvent was then evaporated under reduced pressure and the residue was dissolved in dichloromethane (10 mL). The mixture was washed several times with a saturated solution of NaCl and water. The organic layer was then dried over sodium sulfate, filtered and the solvent evaporated under reduced pressure to afford **7** as yellow crystals (51.2 mg, 99 %). Rf: 0.36 (3:2 cyclohexane/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 – 7.88 (m, 10H, H_{ar}), 5.93 (dd, *J* = 11.0, 9.7 Hz, 1H, H_3), 5.32 – 5.27 (m, 1H, $H_{3'}$), 5.22 (t, *J* = 9.6 Hz, 1H, $H_{4'}$), 5.16 (dd, *J* = 9.7, 3.5 Hz, 1H, H_2), 5.09 (d, *J* = 3.5 Hz, 1H, H_1), 5.01 (d, *J* = 10.1 Hz, 1H, $H_{1'}$), 4.93 (dd, *J* = 10.1, 8.7 Hz, 1H, $H_{2'}$), 4.29 (ddd, *J* = 10.4, 7.3, 2.8 Hz, 1H, H_5), 4.12 (d, *J* = 9.9 Hz, 1H, $H_{5'}$), 3.74 (s, 3H, -$COOCH_3$), 3.37 (s, 3H, -OCH_3), 3.03 (t, *J* = 10.9 Hz, 1H, H_4), 2.36 (s, 3H, -$SCOCH_3$), 2.00 (s, 3H, CH_3CO-), 1.96 (s, 3H, CH_3CO-), 1.57 (s, 3H, CH_3CO-); ^{13}C NMR (101 MHz, CDCl3) δ 194.4 (-$S\underline{C}OCH_3$), 170.0, 169.4, 169.4 (3 x $CH_3\underline{C}O$-), 166.5 (-$\underline{C}OOCH_3$), 165.8, 165.7 (2x –$O\underline{C}OPh$), 133.4– 128.5 (12C_{ar}), , 97.0 (C_1), 81.9 ($C_{1'}$), 75.6 ($C_{5'}$), 73.3($C_{3'}$, C_2), 69.6 (C_5), 69.5 ($C_{2'}$), 69.1 ($C_{4'}$), 67.3 (C_3), 55.4 (-OCH_3), 52.9 (-$COO\underline{C}H_3$), 49.7 (C_4) , 31.5 (C_6), 30.7 (-$SCO\underline{C}H_3$), 20.6 – 20.0 (3 x $\underline{C}H_3CO$-).

Synthesis of compounds 8a et 8b.

To a solution of compound **7** (54.1 mg, 0.07 mmol) in acetic acid (0.5 mL), sodium acetate (8.4 mg, 0.102 mmol) and aqueous hydrogen peroxide (30%, w/w, 10.4 µL, 0.341 mmol) were added. The reaction mixture was stirred at 60 °C for 20 h. Water (2 mL) was added and the mixture was stirred for 3 hours. The solvent was evaporated under reduced pressure and the crude product was purified by reverse flash chromatography C18 (7:3 acetonitrile/water) to afford a mixture of compounds **8a** and **8b** (17.4 mg, 38%). Compound **8b** was obtained in a relatively pure fraction, allowing partial characterizations : Compound **8b**: Rf: 0.30 (7:3 acetonitrile/water); ^{1}H NMR

(400 MHz, CD_3OD) δ 8.02 – 7.45 (m, 10H, H_{ar}), 6.26 (t, *J* = 9.6 Hz, 1H, H_3), 5.54 (t, *J* = 9.2 Hz, 1H, $H_{3'}$), 5.39 (t, *J* = 9.1 Hz, 1H, $H_{2'}$), 5.32 – 5.27 (m, 2H, H_2 , $H_{1'}$), 5.16 – 5.10 (m, 2H, $H_{4'}$, H_1), 4.85 (m, 1H, H_4), 4.78 (m, *J* = 10.0, 5.9, 3.6 Hz, 1H, H_5), 4.57 (d, *J* = 9.9 Hz, 1H, $H_{5'}$), 3.78 (s, 3H, -$COOCH_3$), 3.66 – 3.61 (m, 1H, H_{6a}), 3.54 – 3.47 (m, 4H, H_{6b} , -OCH_3), 2.06 (s, 3H, CH_3CO-), 1.97 (s, 3H, CH_3CO-), 1.93 (s, 3H, CH_3CO-); ^{13}C NMR (101 MHz, CD_3OD) δ 171.3, 171.2, 170.7 (3 x $CH_3\underline{C}O$-), 168.2 (-$\underline{C}OOCH_3$), 167.2, 166.9 (2x –O$\underline{C}$OPh), 134.6 – 129.5 (12C_{ar}), 97.6 (C_1), 88.1 ($C_{1'}$), 76.3 ($C_{5'}$), 74.0, 73.9 (C_2 , $C_{3'}$), 70.0 ($C_{4'}$), 67.5 ($C_{2'}$), 66.5 (C_3), 65.7 (C_5), 62.1 (C_4), 56.6 (-OCH_3), 54.1 (C_6), 53.5 (-COO$\underline{C}H_3$) , 20.5, 20.4 (3 x $\underline{C}H_3CO$-).

Synthesis of compound 9.

To a solution of compound **2** (0.281 g, 0.316 mmol) in butanone (15 mL), sodium iodide (0.474 g, 3.16 mmol) was added. The reaction mixture was refluxed for 15 h, then cooled to room temperature and concentrated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (50 mL) and washed with water. After evaporation, the residue was purified by flash chromatography (7:3 cyclohexane/ethyl acetate). Compound **9** was obtained as beige crystals of the desired product (0.200 g; 75%). Rf: 0.58 (1:1 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$ **:** +35.2 (c = 0.5 $CHCl_3$); IR (ATR): 3673, 3069, 1735, 1728, 1603, 1452, 1441, 1370, 1275, 1246, 1219, 1096, 1069, 1034, 936, 889, 856, 781, 712 cm$^{-1;}$ HRMS: Calcd. for [$C_{34}H_{41}NO_{15}IS$]: m/z 862.1242 $[M+NH_4]^+$, found 862.1248 $[M+NH_4]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (td, *J* = 7.8, 7.1, 1.4 Hz, 4H, H_{ar}), 7.58 – 7.44 (m, 2H, H_{ar}), 7.38 (td, *J* = 7.8, 1.6 Hz, 4H, H_{ar}), 6.00 (dd, *J* = 11.0, 9.5 Hz, 1H, H_3), 5.32 (t, *J* = 9.2 Hz, 1H, $H_{3'}$), 5.26 – 5.15 (m, 3H, $H_{2,}$ $H_{1,}$ $H_{4'}$), 5.08 (d, *J* = 10.1 Hz, 1H, $H_{1'}$), 4.93 (dd, *J* = 10.1, 9.0 Hz, 1H, $H_{2'}$), 4.17 (d, *J* = 9.9 Hz, 1H, $H_{5'}$), 3.93 (ddd, *J* = 10.5, 5.8, 2.6 Hz, 1H, H_5), 3.83 (dd, *J* = 10.8, 2.6 Hz, 1H, H_{6a}), 3.77 (s, 3H, -$COOCH_3$), 3.68 (dd, *J* = 10.8, 5.8 Hz, 1H, H_{6b}), 3.44 (s, 3H, -OCH_3), 3.08 (t, *J* = 10.8 Hz, 1H, H_4), 2.03 (s, 3H, CH_3CO-), 1.98 (s, 3H, CH_3CO-), 1.54 (s, 3H, CH_3CO-) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.1, 169.5, 169.4 (3 x $CH_3\underline{C}O$-), 166.6 (-$\underline{C}OOCH_3$), 165.8 (2x –O$\underline{C}$OPh), 133.5 – 128.6 (12C_{ar}) , 97.3 (C_1), 81.4 ($C_{1'}$), 75.7 ($C_{5'}$), 73.4 (C_2), 73.3 ($C_{3'}$), 69.6 ($C_{2'}$), 69.2 ($C_{4'}$,C_5), 66.8 (C_3), 55.7 (-OCH_3), 53.2 (-COO$\underline{C}H_3$), 50.9 (C_4), 20.7 – 20.1 (3 x $\underline{C}H_3CO$-), 8.84 (C_6).

Synthesis of compound 10.

To a solution of compound **6** (0.246 g, 0.229 mmol) in butanone (15 mL), sodium iodide (0.343 g, 2.29 mmol) was added. The reaction mixture was refluxed for 8 h, then cooled to room temperature and concentrated to dryness under reduced pressure. The residue was dissolved in dichloromethane (30 mL) and washed with water. After evaporation, the residue was purified by flash chromatography (4:1 cyclohexane/ethyl acetate) to give **10** as beige crystals (0.232 g; 99%). Rf: 0.67 (3:2 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$: +22.6 (c = 0.5 $CHCl_3$); IR (ATR): 2972, 1728, 1601, 1452, 1316, 1273, 1260, 1094, 1071, 1028, 802, 708, 567, 527, 467, 434 cm$^{-1;}$ HRMS: Calcd. for [$C_{49}H_{43}O_{15}NaSI$]: m/z 1053.1265 $[M+Na]^+$, found 1053.1278 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 – 7.06 (m, 25H, H_{aro}), 6.06 (dd, *J* = 11.1, 9.6 Hz, 1H, H_3), 6.00 (dt, *J* = 9.3, 4.5 Hz, 1H, $H_{3'}$), 5.70 (t, *J* = 9.7 Hz, 1H, $H_{4'}$), 5.50 (m, 2H, $H_{1',}$ $H_{2'}$), 5.21 (dd, *J* = 9.5, 3.5 Hz, 1H, H_2), 5.17 (d, *J* = 3.5 Hz, 1H, H_1), 4.51 (d, *J* = 9.9 Hz, 1H, $H_{5'}$), 4.03 (ddd, *J* = 10.4, 5.1, 2.7 Hz, 1H, H_5), 3.84 (dd, *J* = 10.9, 2.7 Hz, 1H, H_{6a}), 3.77 (dd, *J* = 10.9, 5.2 Hz, 1H, H_{6b}), 3.71 (s, 3H, -$COOCH_3$), 3.42 (s, 3H, -OCH_3), 3.13 (t, *J* = 10.8 Hz, 1H, H_4) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (-$\underline{C}OOCH_3$), 166.0 – 165.1 (5x-O$\underline{C}$OBz), 133.6 –128.3 (35 C_{ar}), 97.3 (C_1), 81.9 ($C_{1'}$), 76.1 ($C_{5'}$), 73.5 ($C_{3'}$), 73.4 (C_2), 70.4 ($C_{2'}$), 70.0 ($C_{4'}$), 69.1 (C_5), 66.8 (C_3), 55.7 (-OCH_3), 53.1 (-COO$\underline{C}H_3$), 51.1 (C_4), 9.4 (C_6).

Synthesis of compound 11.

To a solution of compound **9** (0.120 g, 0.149 mmol) in methanol (3 mL) sodium methanolate (0.092 g, 0.171 mmol) was added. The reaction mixture was stirred 15 h at room temperature. IR 120 H^+ resin was added in order to obtain a pH = 3-4. The mixture was filtered and the solvent was concentrated under reduced pressure. To a solution of the residue in water (2 mL), sodium sulfite (23 mg, 0.185 mmol) was added. The mixture was refluxed until total conversion of the substrate (24 h). After cooling to room temperature, a solution of NaOH (0.5 M) was added in order to obtain a pH = 8 (using a pH-meter). After concentration, the residue was purified over Sephadex™ LH-20. After freeze-drying, the desired product **11** was obtained as beige crystals (53 mg; 76%). Compound **11** can be obtained under the same conditions from compound **10** in 85% yield. $[\alpha]_D^{25}$: +57.6 (c = 0.5 H_2O); IR (ATR): 3298, 1613, 1422, 1136, 1111; 1049, 791, 691, 617, 488, 475, 420.5 cm$^{-1;}$ HRMS: Calcd. for [$C_{13}H_{21}O_{13}Na_2S_2$]: m/z 495.0219 $[M+Na]^+$, found 495.0219 $[M+Na]^+$; ^{1}H NMR (600 MHz, D_2O) δ 4.85 (d, *J* = 3.7 Hz, 1H, H_1), 4.59 (d, *J* = 9.9 Hz, 1H, $H_{1'}$), 4.19 (ddd, *J* = 11.1, 9.3, 1.8 Hz, 1H, H_5), 3.82 (dd, *J* = 14.7, 1.8 Hz, 1H, H_{6a}), 3.79 – 3.76 (m, 1H, $H_{4'}$), 3.73 (t, *J* = 9.9 Hz, 1H, H_3), 3.66 (dd, *J* = 9.5, 3.7 Hz, 1H, H_2), 3.58 – 3.52 (m, 2H, $H_{3',}$ H_5), 3.48 (s, 3H, -O$\underline{C}H_3$), 3.44 – 3.39 (m, 1H, $H_{2'}$), 3.23 (dd, *J* = 14.7, 9.3 Hz, 1H, H_{6b}), 2.89 (t, *J* = 10.7 Hz, 1H, H_4) ; ^{13}C NMR (151 MHz, D_2O) δ 175.4 (-$\underline{C}OONa$), 98.9 (C_1), 85.8 ($C_{1'}$), 79.8 ($C_{4'}$), 76.7 ($C_{5'}$), 72.4 (C_2), 72.0 ($C_{2'}$), 71.6 ($C_{3'}$), 70.8 (C_3), 68.2 (C_5), 55.3(-OCH_3), 52.7 (C_6), 51.7 (C_4).

Synthesis of compound 13.

Compound **6** (0.30 g, 0.187 mmol) was added in a round bottom flask placed in an ice bath. Acetic anhydride (9.19 mL, 96.54 mmol), sulfuric acid (0.91 mL, 16.98 mmol) and acetic acid (4.04 mL, 70.75 mmol) were successively added. The reaction mixture was stirred at 0 °C for 10 min followed by 2.5 h at room temperature. Ethyl acetate (100 mL) was then added and the mixture was washed with ice-cold water (2 x 100 mL), 3 times with a saturated solution of $NaHCO_3$ (3 x 100 mL) and with a saturated solution of NaCl (100 mL). The organic phase was then dried over sodium sulfate, filtered and concentrated under reduced pressure. After silica gel chromatography (7:3 to 3:2 cyclohexane/ethyl acetate), the desired compound **13** was obtained as white crystals (0.320 g, 99 %). Rf: 0.30 (7:3 cyclohexane/ethyl acetate); IR (ATR): 1730, 1603, 1452, 1368, 1271, 1258, 1150, 1109; 1071, 1045, 1022, 708, 687, 471, 430 cm$^{-1;}$ HRMS: Calcd. for [$C_{52}H_{46}O_{18}NaS$]: m/z 1013.2303 $[M+Na]^+$, Found 1013.2290 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.99 – 7.06

(m, H_{ar}), 6.54 (d, *J* = 3.6 Hz, 1H, $H_{1\alpha}$), 6.03 – 5.95 (m, 3H, $H_{3'\alpha}$, $H_{3\alpha}$, $H_{2\beta}$), 5.93 (d, *J* = 8.4 Hz, 1H, $H_{1\beta}$), 5.70 (t, *J* = 9.7 Hz, 1H, $H_{4'\alpha}$, m, 2H, $H_{3\beta}$, $H_{4'\beta}$), 5.55 – 5.37 (m, 6H, $H_{3'\beta}$, $H_{2'\alpha}$, $H_{2\alpha}$, $H_{1'\alpha}$, $H_{2'\alpha}$, $H_{1'\beta}$), 4.73 – 4.49 (m, 5H, $H_{6(a,b)\alpha}$, $H_{6(a,b)\beta}$, $H_{5\alpha}$), 4.47 (d, *J* = 9.9 Hz, 1H, $H_{5'\alpha}$), 4.32 (ddd, *J* = 10.7, 3.9, 2.0 Hz, 1H, $H_{5\beta}$), 3.75 (s, 3H, -COOC$\underline{H}_{3\beta}$), 3.69 (s, 3H, -COOC$\underline{H}_{3\alpha}$), 3.38 (t, *J* = 11.1 Hz, 1H, $H_{4\alpha}$), 3.31 (d, *J* = 10.9 Hz,1H, $H_{4\beta}$), 2.14 (s, 3H, C$\underline{H}_3$CO-CH_2-), 2.13 (s, 3H, C$\underline{H}_3$CO- α), 2.03 (s, 3H, C$\underline{H}_3$CO- β); ^{13}C NMR (101 MHz, $CDCl_3$) : δ 170.5 ($CH_3\underline{C}O$-CH_2-), 168.7 ($CH_3\underline{C}O$-CH-), 166.6 (-$\underline{C}OOCH_3$), 165.6 –165.0 (5 x –O$\underline{C}$OBz), 133.6 –128,3 (30 C_{aro}), 89.7 ($C_{1\alpha}$), 82.1 ($C_{1'\alpha}$) 76.2 ($C_{5'\alpha}$), 73.4 ($C_{3'\alpha}$), 71.9 ($C_{2\alpha}$), 71.7 ($C_{5\alpha}$), 70.5 ($C_{2'\alpha}$), 69.9 ($C_{4'\alpha}$), 67.1 ($C_{3\alpha}$), 63.2 ($C_{6\alpha}$), 53.1 (-COO$\underline{C}H_3$), 45.7 ($C_{4\alpha}$), 21.0 – 20.7 ($\underline{C}H_3$CO- α, $\underline{C}H_3$CO-CH_2-).

Synthesis of compound 14.

To a solution of compound **13** (300 mg, 0.302 mmol) in anhydrous DCM (16 mL), HBr (33% in acetic acid; 1.48 mL, 3.732 mmol) was added at 0°C. After stirring for 2 h at 0°C, crushed ice was added to the reaction mixture. The organic phase was separated and washed with a saturated solution of $NaHCO_3$ (3 x 50 mL) and water (50 mL), dried over sodium sulfate, filtered and the solvent evaporated. Compound **14** was obtained as white crystals (297 mg, 97 %). Rf: 0.51 (3:2 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$: +32.6 (c = 0.5 $CHCl_3$); HRMS: Calcd. for [$C_{50}H_{43}O_{16}NaSBr$]: m/z 1033.1353 $[M+Na]^+$; Found 1033.1388 $[M+Na]^+$; IR (ATR): 3075, 1730, 1603, 1452, 1315, 1258, 1179, 1096, 1070, 1026, 708, 687, 552, 442 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (ddd, *J* = 8.8, 7.6, 1.4 Hz, 4H, H_{ar}), 7.81 (dd, *J* = 8.4, 1.3 Hz, 2H, H_{ar}), 7.71 (ddd, *J* = 8.5, 2.4, 1.3 Hz, 4H, H_{ar}), 7.54 (dddd, *J* = 10.6, 4.8, 2.5, 1.4 Hz, 2H, H_{ar}), 7.47 – 7.03 (m, 15H, H_{ar}), 6.80 (d, *J* = 3.8 Hz, 1H, H_1), 6.15 (dd, *J* = 11.2, 9.3 Hz, 1H, H_3), 6.03 (dd, *J* = 9.5, 8.3 Hz, 1H, $H_{3'}$), 5.72 (t, *J* = 9.7 Hz, 1H, $H_{4'}$), 5.58 – 5.47 (m, 2H, $H_{2'}$, $H_{1'}$), 5.23 (dd, *J* = 9.3, 3.8 Hz, 1H, H_2), 4.95 (dt, *J* = 11.1, 2.7 Hz, 1H, H_5), 4.78 (dd, *J* = 12.6, 3.3 Hz, 1H, H_{6a}), 4.61 (dd, *J* = 12.5, 2.0 Hz, 1H, H_{6b}), 4.55 (d, *J* = 9.9 Hz, 1H, $H_{5'}$), 3.71 (s, 3H, -$COOCH_3$), 3.37 (t, *J* = 11.2 Hz, 1H, H_4), 2.15 (s, 3H, CH_3CO-); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.4 ($CH_3\underline{C}O$-), 166.6 (–$\underline{C}OOCH_3$), 165.9 – 165.1 (5 x –O$\underline{C}$OPh), 133.9 – 128.3 (30 C_{ar}), 88.5 (C_1), 81.7 ($C_{1'}$), 74.8 ($C_{5'}$), 73.5 (C_5), 72.5 ($C_{3'}$), 70.4 (C_2), 69.9 ($C_{2'}$), 67.3 ($C_{4'}$), 62.8 (C_6), 53.1 (-COO$\underline{C}H_3$), 45.0 (C_4), 21.0 ($\underline{C}H_3$CO-).

Synthesis of compound 15.

Method 1:

To a solution of compound **14** (1.27 g, 1.225 mmol) in dry DCM (30 mL) at -15 °C under argon, silver trifluoromethanesulfonate (0.354 g, 1.38 mmol) and propargyl alcohol (0.74 mL, 12.55 mmol) were successively added. After stirring at room temperature for 1 h, the reaction mixture was filtered over celite, washed with water and concentrated under reduced pressure. Silica gel chromatography (4:1 to 7:3 cyclohexane/ethyl acetate) afforded **15** as white crystals (0.7g, 57 %).

Method 2.

To a solution of compound **17** (0.511 g, 0.467 mmol) in dry DCM (14 mL) under argon, propargyl alcohol (0.093 mL, 1.495 mmol) was added. The reaction mixture was stirred at room temperature for 15 min and then cooled to -15 °C. TMSOTf (0.093 mL, 0.093 mmol) was added and the mixture was gradually warmed up to room temperature. After 1.5 h of stirring, DCM (20 mL) was added and the mixture was washed with water (30 mL), a saturated solution of $NaHCO_3$ (2 x 30 mL) and water again. The organic phase was dried over sodium sulfate, filtered and concentrated under reduced pressure. After silica gel chromatography (4:1 to 7:3 cyclohexane/ethyl acetate), **15** was obtained as white crystals (0.436 g, 95 %). Rf: 0.43 (3:2 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$: - 32 (c = 0.5 $CHCl_3$); IR (ATR): 2970, 1768, 1726, 1603, 1452, 1370, 1263, 1227, 1177, 1148, 1096, 1069, 1028, 708, 687, 554, 529, 498, 413 cm^{-1}; HRMS: Calcd. for [$C_{53}H_{46}O_{17}NaS$]: m/z 1009.2353 $[M+Na]^+$; Found 1009.2330 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.98 – 7.08 (m, 25H, H_{aro}), 5.96 (t, *J* = 9.3 Hz, 1H, $H_{3'}$), 5.75 – 5.64 (m, 2H, H_3, $H_{2'}$), 5.47 (dd, *J* = 9.9, 8.9 Hz, 1H, $H_{4'}$), 5.43 – 5.35 (m, 2H, $H_{1'}$, H_2), 4.94 (d, *J* = 8.0 Hz, 1H, H_1), 4.67 (d, *J* = 3.0 Hz, 2H, $H_{6a,b}$), 4.49 (d, *J* = 9.9 Hz, 1H, $H_{5'}$), 4.41 (dd, *J* = 16.0, 2.4 Hz, 1H, $H_{1''a}$), 4.35 (dd, *J* = 16.0, 2.4 Hz, 1H, $H_{1''b}$), 4.16 (dt, *J* = 10.7, 3.0 Hz, 1H, H_5), 3.72 (s, 3H, -$COOCH_3$), 3.31 (t, *J* = 10.9 Hz, 1H, H_4), 2.38 (t, *J* = 2.4 Hz, 1H, –C≡CH), 2.14 (s, 3H, CH_3CO-); ^{13}C NMR (101 MHz, $CDCl_3$) : δ 170.6 (-$\underline{C}OOCH_3$), 166.7 ($CH_3\underline{C}O$-), 166.1– 165.0 (5 x -O$\underline{C}$OBz), 133.6 – 128.3 (30 C_{ar}), 98.4 (C_1), 81.7 ($C_{1'}$), 78.4 (–$\underline{C}$≡CH), 75.9 ($C_{5'}$), 75.5 (–C≡$\underline{C}$H), 74.4 (C_5), 73.4 ($C_{3'}$), 73.1 (C_2), 70.6 ($C_{4'}$), 70.2 (C_3), 69.8 ($C_{2'}$), 63.4 (C_6), 55.9 ($C_{1''}$), 53.1 (-COO$\underline{C}H_3$), 46.2 (C_4), 21.1 ($\underline{C}H_3$CO-).

Synthesis of compound 16.

To a solution of **13** (1.17 g, 1.18 mmol) in dry DMF (14 ml), hydrazine acetate (0.124 g, 1.345 mmol) was added under argon. After stirring at room temperature for 2 h, ethyl acetate was added (100 mL) and the mixture was washed with a saturated solution of $NaHCO_3$ (3 x 100 mL) and with a saturated solution of NaCl (100 mL). The organic phase was then dried over sodium sulfate, filtered and concentrated. The desired product **16** was obtained as a 3:2 mixture of α and β anomers (NMR ratio) and as white crystals (1.11 g, 99 %). Rf: 0.39, 0.6 (1:1 cyclohexane/ethyl acetate); IR (ATR): 3455, 2926, 2857, 1730, 1601, 1452, 1375, 1273, 1223, 1258, 1092, 1069, 1028, 708, 611, 540, 488 cm^{-1}; HRMS: Calcd. for [$C_{50}H_{44}O_{17}NaS$]: m/z 971.2197 $[M+Na]^+$; Found 971.2206 $[M+Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) : δ 8.01 – 7.08 (m, H_{ar}), 6.09 (dd, *J* = 11.2, 9.7 Hz, 1H, $H_{3\alpha}$), 6.02 – 5.92 (m, 2H, $H_{3'\alpha}$, $H_{3'\beta}$), 5.78 – 5.63 (m, 4H, $H_{3\beta}$, $H_{1\alpha}$, $H_{4'\alpha}$, $H_{4'\beta}$), 5.53 – 5.42 (m, 3H, $H_{1'\alpha}$, $H_{2'\alpha}$, $H_{2'\beta}$), 5.37 (d, *J* = 9.8 Hz, 1H, $H_{1'\beta}$), 5.26 – 5.18 (m, 2H, $H_{2\alpha}$, $H_{2\beta}$), 4.90 (d, *J* = 8.1 Hz, 1H, $H_{1\beta}$), 4.75 (dt, *J* = 11.0, 2.7 Hz, 1H, $H_{5\alpha}$), 4.71 – 4.58 (m, 4H, $H_{6(a,b)\beta}$, $H_{6(a,b)\alpha}$), 4.46 (d, *J* = 9.9 Hz, 1H, $H_{5'\alpha}$), 4.45 (d, *J* = 8 Hz, 1H, $H_{5'\beta}$), 4.19 – 4.14 (m, 1H, $H_{5\beta}$), 3.69 (s, 3H, -$COOCH_{3\beta}$), 3.65 (s, 3H, -$COOCH_{3\alpha}$), 3.29 (m, 2H, $H_{4\alpha}$, $H_{4\beta}$), 2.13 (s, 3H, C$\underline{H}_3$CO-CH_2- α), 2.13 (s, 3H, C$\underline{H}_3$CO-CH_2- β); ^{13}C NMR (101 MHz, $CDCl_3$) : δ 170.8 ($CH_3\underline{C}O$-CH_2- α), 170.7 ($CH_3\underline{C}O$-CH_2- β), 166.8 – 165.1 (–O$\underline{C}$OBz), 133.6 – 128.3 (C_{ar}), 95.9 ($C_{1\beta}$), 90.7 ($C_{1\alpha}$), 81.8 ($C_{1'\alpha}$), 81.7 ($C_{1'\beta}$); 75.9 ($C_{2\beta}$), 75.9 ($C_{2\alpha}$, $C_{5'\alpha}$, $C_{5'\beta}$), 74.5 ($C_{5\beta}$), 73.7 ($C_{2\alpha}$), 73.5 ($C_{3'\alpha}$); 73.4 ($C_{3'\beta}$), 70.6 ($C_{2'\beta}$), 70.5 ($C_{2'\alpha}$), 69.9 ($C_{4'\alpha}$), 69.7 ($C_{4'\beta}$); 69.6 ($C_{3\beta}$), 69.4 ($C_{5\alpha}$), 67.0 ($C_{3\alpha}$), 63.6 ($C_{6\,(a,b)\,\alpha,\beta}$), 53.1 (-COO$\underline{C}H_3$ β), 53.0 (-COO$\underline{C}H_3$ α), 46.2 ($C_{4\beta}$,$C_{4\alpha}$), 21.1 ($\underline{C}H_3$CO-CH_2- α,β).

Synthesis of compound 17.

To a solution of compound **16** (0.5 g, 0.527 mmol) in anhydrous DCM (15 mL), trichloroacetonitrile (0.528 mL; 5.274 mmol) was added. The reaction mixture was stirred at room temperature for 15 min, and DBU (0.014 mL; 0.105 mmol) was added at 0 °C. The reaction mixture was then stirred at room temperature for 2 h under argon. The solvent was evaporated and the residue was purified by silica gel chromatography (3:2 cyclohexane/ethyl acetate) to afford the trichloroacetimidate **17** (0.533 g; 93%) as white crystals. Rf: 0.48 (3:2 cyclohexane / ethyl acetate); IR (ATR): 2936, 1730, 1680, 1601, 1452, 1253.7, 1094, 1071, 1026, 972, 708, 604, 588, 538, 480, 430 cm^{-1}; HRMS: Calcd. for $[C_{52}H_{44}NO_{17}NaSCl_3]$: m/z 1114.1293 $[M+Na]^+$; Found 1114.1318 $[M+Na]^+$; ^1H NMR (400 MHz, $CDCl_3$) : δ 8.64 (s, 1H, N$\underline{H}_\beta$), 8.56 (s, 1H, N$\underline{H}_\alpha$), 8.16 – 7.04 (m, H_{ar}), 6.78 (d, *J* = 3.6 Hz, 1H, $H_{1\alpha}$), 6.22 (dd, *J* = 11.2, 9.8 Hz, 1H, $H_{3\beta}$), 6.16 – 5.93 (m, 3H, $H_{3\alpha,}$ $H_{3'\alpha,}$ $H_{3'\beta}$), 5.62 (t, *J* = 9.7 Hz, 1H, $H_{4'\alpha}$), 5.58 – 5.53 (m, 1H, $H_{4'\beta}$), 5.52 – 5.39 (m, 3H, $H_{2\alpha,}$ $H_{2'\alpha,}$ $H_{1'\alpha}$), 4.79 – 4.62 (dt, *J* = 12.2, 2.5 Hz, 1H, $H_{6a\alpha,}$ dd, *J* = 12.2, 1.9 Hz, 1H, $H_{6b\alpha,}$ m, $H_{5\alpha}$), 4.35 (d, *J* = 9.8 Hz, 1H, $H_{5'\alpha}$), 3.78 (s, 3H, -$COOCH_3$ α), 3.64 (s, 3H, -$COOCH_3$ β), 3.50 – 3.46 (m, 1H, H_4 β), 3.41 (t, *J* = 11.2 Hz, 1H, H_4 α), 2.14 (s, 3H, CH_3CO- α,), 2.12 (s, 3H, CH_3CO- β); ^{13}C NMR (101 MHz, $CDCl_3$) : δ 170.5 (-$\underline{C}OOCH_3$), 166.5 ($CH_3\underline{C}O$-), 165.9 – 165.1 (5 x –O$\underline{C}$OBz), 160.5 (-C=NH), 133.6 – 128.3 (30 C_{ar}), 93.8 (C_1 α), 90.9 (-$\underline{C}Cl_3$), 81.5 ($C_{1'}\alpha$), 75.8 ($C_{5'}\alpha$), 73.2 ($C_{3'}\alpha$), 72.5 ($C_5\alpha$), 72.2 ($C_2\alpha$), 70.3 ($C_{2'}\alpha$), 69.9 ($C_{4'}\alpha$), 66.8 ($C_3\alpha$), 63.2 ($C_6\alpha$), 53.0 (-COO$\underline{C}H_3$) 45.7 ($C_4\alpha$), 21.0 ($\underline{C}H_3CO$-).

Synthesis of compound 18.

To a solution of compound **15** (0.365 g, 0.369 mmol) in a mixture of 1:1 DCM/MeOH (7 mL), acetyl chloride (2 equiv., 0.052 mL, 0.739 mmol) was added. The mixture was stirred at room temperature for 15 h under argon. DCM (10 mL) was then added and the mixture was washed with a saturated solution of $NaHCO_3$, a saturated solution of NaCl and water. The organic layer was dried over sodium sulfate, filtered and the solvent evaporated under reduced pressure to afford **18** as white crystals (0.345 g, 98%). Rf: 0.29 (3:2 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$: -0.2 (c = 0.5 $CHCl_3$); IR (ATR): 2949, 1767, 1728, 1603, 1453, 1316, 1260, 1094, 1069, 1028, 804, 708, 687 cm^{-1}; HRMS: Calcd. for $[C_{51}H_{44}O_{16}NaS]$: m/z 967.2248 $[M+Na]^+$, found 967.2226 $[M+Na]^+$; ^1H NMR (400 MHz, $CDCl_3$) : δ 8.01 – 7.87 (m, 4H, H_{ar}), 7.78 (m, 6H, H_{ar}), 7.59 – 7.15 (m, 15H, H_{ar}), 5.97 (t, *J* = 9.3 Hz, 1H, $H_{3'}$), 5.71 – 5.57 (m, 2H, $H_{3,}$ $H_{4'}$), 5.46 (dd, *J* = 10.0, 9.1 Hz, 1H, $H_{2'}$), 5.39 – 5.31 (m, 2H, $H_{1',}$ H_2), 4.95 (d, *J* = 8.0 Hz, 1H, H_1), 4.44 (d, *J* = 9.8 Hz, 1H, , $H_{5'}$), 4.40 (dd, *J* = 16.0, 2.5 Hz, 1H, $H_{1''a}$), 4.34 (dd, *J* = 16.0, 2.5 Hz, 1H, $H_{1''b}$), 4.17 (dd, *J* = 12.4, 2.1 Hz, 1H, H_{6a}), 4.06 (dd, *J* = 12.3, 3.7 Hz, 1H, H_{6b}), 3.82 (ddd, *J* = 10.7, 3.6, 2.1 Hz, 1H, H_5), 3.68 (s, 3H, -$COOCH_3$), 3.39 (t, *J* = 11.0 Hz, 1H, H_4), 2.37 (t, *J* = 2.4 Hz, 1H, –C≡CH); ^{13}C NMR (101 MHz, $CDCl_3$) : δ 167.0 (-$\underline{C}OOCH_3$), 166.1 – 165.0 (-O$\underline{C}$OBz), 133.6 –128.3 (30 C_{aro}), 98.8 (C_1), 82.6 ($C_{1'}$), 78.6 (–$\underline{C}$≡CH), 77.3 (C_5), 76.0 ($C_{5'}$), 75.5 (–C≡$\underline{C}$H), 73.4 ($C_{3'}$), 73.2 (C_2), 70.8 ($C_{4'}$), 70.7 ($C_{2'}$), 70.0 (C_3), 62.2 (C_6), 56.3 ($C_{1''}$), 53.1 (-COO$\underline{C}H_3$), 45.8 (C_4).

Synthesis of compound 19.

Compound **18** (0.178 mg, 0.188 mmol) and SO_3.Py complex (50 %, 2 equiv., 119.89 mg, 0.376 mmol) in a round bottom flask were dried for 5 hours under high reduced pressure. Anhydrous DMF (4 mL) was then added and the mixture was stirred at room temperature under argon. After 15 hours, ethyl acetate (30 mL) was added and the mixture was then washed with a saturated solution of $NaHCO_3$ and with a saturated solution of NaCl. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel (95:5 DCM/MeOH) to afford **19** as white crystals (0.195 g, 99%). Rf: 0.45 (9:1 DCM/MeOH); $[\alpha]_D^{25}$: +3.6 (c = 0.5 MeOH); IR (ATR): 3628, 2340, 1730, 1584, 1452, 1271, 1092, 1069, 1026, 999, 708, 575, 482, 434 cm^{-1}; HRMS: Calcd. for $[C_{51}H_{43}O_{19}Na_2S_2]$: m/z 1069.1635 $[M+Na]^+$, found 1069.1642 $[M+Na]^+$; ^1H NMR (400 MHz, CD_3OD) δ 7.99 – 7.84 (m, 6H, H_{ar}), 7.82 – 7.77 (m, 2H, H_{ar}), 7.76 – 7.68 (m, 2H, H_{ar}), 7.62 – 7.18 (m, 15H, H_{ar}), 6.04 (t, *J* = 9.4 Hz, 1H, $H_{3'}$), 5.71 (d, *J* = 7.9 Hz, 1H, H_3), 5.70 – 5.69 (d, *J* = 10.1 Hz, 1H, $H_{1'}$), 5.58 (t, *J* = 9.8 Hz, 1H, $H_{4'}$), 5.38 (dd, *J* = 10.0, 9.3 Hz, 1H, $H_{2'}$), 5.24 (dd, *J* = 9.3, 8.0 Hz, 1H, H_2), 5.07 (d, *J* = 8.0 Hz, 1H, H_1), 4.79 (d, *J* = 10.0 Hz, 1H, $H_{5'}$), 4.71 (dd, *J* = 10.7, 3.0 Hz, 1H, H_{6a}), 4.44 (dd, *J* = 10.6, 1.8 Hz, 1H, H_{6b}), 4.38 (dd, *J* = 4.5, 2.4 Hz, 2H, $H_{1''a,b}$), 4.10 (dt, *J* = 10.6, 2.4 Hz, 1H, H_5), 3.66 (s, 3H, -$COOCH_3$), 3.56 (t, *J* = 11.0 Hz, 1H, H_4), 2.81 (t, *J* = 2.4 Hz, 1H, –C≡CH); ^{13}C NMR (101 MHz, CD_3OD) δ 168.9 (-$\underline{C}OOCH_3$), 167.4 – 166.4 (5 x -O$\underline{C}$OBz), 134.7 –129.4 (30 C_{ar}), 99.5 (C_1), 82.8 ($C_{1'}$), 79.6 (–$\underline{C}$≡CH), 76.6 (–C≡$\underline{C}$H), 76.4 ($C_{5'}$), 76.1 (C_5), 75.1 ($C_{3'}$), 74.7 (C_2), 72.6 (C_3), 72 ($C_{2'}$), 71.5 ($C_{4'}$), 67.6 (C_6) , 56.8 ($C_{1''}$), 53.3 (-COO$\underline{C}H_3$), 46.2 (C_4).

Synthesis of compound 20.

To a solution of compound **19** (0.100 g, 0.095 mmol) in 5:1 MeOH/H_2O (6 mL) a solution of NaOH (3 M, 2 mL, 6 mmol) was slowly added at 0 °C. The reaction mixture was stirred at 0 °C for 10 min and then at room temperature for 15 h. 1M HCl was slowly added to pH = 8 (using a pH-meter). The reaction mixture was then concentrated under reduced pressure and the desired product was purified over Sephadex™ LH-20. Compound **20** was obtained as white crystals after freeze-drying (0.045 g, 88%). $[\alpha]_D^{25}$: -75.6 (c = 0.25 H_2O); HRMS: Calcd. for $[C_{15}H_{21}O_{14}S_2]$: m/z 489.0373 $[M-2Na+H]^-$, found 489.0395 $[M-2Na+H]^-$; ^1H NMR (400 MHz, D_2O) δ 4.77 (d, *J* = 6.9 Hz, 1H, $H_{1'}$), 4.64 (d, *J* = 8.1 Hz, 1H, H_1), 4.46 – 4.40 (m, 4H, H_{6a} , H_{6a} , $H_{1''}$), 4.06 (d, *J* = 9.3 Hz, 1H, $H_{5'}$), 3.92 – 3.84 (m, 1H, H_5), 3.64 – 3.49 (m, 3H, H_3 , $H_{4'}$, $H_{3'}$), 3.44 – 3.29 (m, 2H, $H_{2'}$, H_2), 2.98 (t, *J* = 10.8 Hz, 1H, H_4), 2.90 (t, *J* = 2.4 Hz, 1H, –C≡C$\underline{H}$); ^{13}C NMR (101 MHz, D_2O) δ 171.9 (-COONa), 100.3 (C_1) , 84.0 ($C_{1'}$), 78.7 (–$\underline{C}$≡CH), 77.5 ($C_{5'}$) , 76.5 ($C_{3'}$), 76.3 ((–C≡$\underline{C}$H), 74.4 (C_5), 73.9 (C_2), 72.7 (C_3), 72.2 ($C_{2'}$), 71.1 ($C_{4'}$), 67.4 (C_6), 56.5 ($C_{1''}$), 46.7 (C_4).

Synthesis of compound 21.

To a solution of compound **18** (0.799 g, 0.847 mmol) in anhydrous pyridine (2 mL) at -20°C under argon, a solution of tosyl chloride (0.363 g, 1.905 mmol) in a mixture of anhydrous pyridine (1 mL) and anhydrous triethylamine (0.265 mL; 1.905 mmol) were slowly

added. After stirring at room temperature for 3 h, methanol (3 mL) was added. The resulting solution was diluted with ethyl acetate (50 mL) and washed with a saturated solution of sodium chloride (3 x 50 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. Flash chromatography (7:3 cyclohexane/ethyl acetate) afforded **21** as white crystals (0.874 g, 94%). Rf: 0.51 (3:2 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$: -7.0 (c = 0.5 $CHCl_3$); IR (ATR)**:** 1730, 1601, 1452, 1362, 1273, 1260, 1177, 1094, 1070, 1026, 980, 912, 708 cm^{-1}; HRMS: Calcd. for [$C_{58}H_{50}O_{18}S_2Na$]: m/z 1121.2336 $[M+Na]^+$, found 1121.2349 $[M+Na]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.09 (m, 29H, H_{ar}), 5.92 (t, *J* = 9.2 Hz, 1H, $H_{3'}$), 5.69 – 5.52 (m, 2H, $H_{4'}$, $H_{3'}$), 5.42 – 5.23 (m, 3H, $H_{1'}$, $H_{2'}$, H_2), 4.88 (d, *J* = 8.0 Hz, 1H, H_1), 4.65 (dd, *J* = 10.8, 3.9 Hz, 1H, H_{6a}), 4.56 (dd, *J* = 10.9, 1.8 Hz, 1H, H_{6b}), 4.45 (d, *J* = 9.9 Hz, 1H, $H_{5'}$), 4.29 (d, *J* = 2.4 Hz, 2H, $H_{1''}$), 4.18 – 4.04 (m, 1H, H_5), 3.73 (s, 3H, -$COOCH_3$), 3.19 (t, *J* = 10.9 Hz, 1H, H_4), 2.50 (s, 3H, -Ph$C\underline{H}_3$), 2.37 (t, *J* = 2.4 Hz, 1H, -C≡CH) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (-$\underline{C}OOCH_3$), 166.1 – 164.9 (5x-O$\underline{C}$OBz), 145.3 145.3 (Cq tosyl), 133.6 –128.3 (35 C_{ar}), 98.2 (C_1), 81.4 ($C_{1'}$), 78.3 (-$\underline{C}$≡CH), 75.7 ($C_{5'}$), 75.5 (-C≡$\underline{C}$H), 74.1 (C_5), 73.4 ($C_{3'}$), 72.9 (C_2), 70.5 ($C_{2'}$), 70.0 (C_3), 69.6 ($C_{4'}$), 69.0 (C_6), 55.7 ($C_{1''}$), 53.1 (-COO$\underline{C}H_3$), 45.7 (C_4), 21.8 (Ph-$\underline{C}H_3$).

Synthesis of compound 22.

To a solution of compound **21** (0.780 g, 0.709 mmol) in butanone (50 mL), sodium iodide (1.06 g, 7.096 mmol) was added. The reaction mixture was refluxed for 15 h, cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in dichloromethane (30 mL) and washed with water. The residue was purified by flash chromatography (7:3 cyclohexane/ethyl acetate) to give **22** as beige crystals (0.711 g; 95%). Rf: 0.4 (7:3 cyclohexane/ ethyl acetate); $[\alpha]_D^{25}$: -16.8 (c = 0.5 $CHCl_3$); IR (ATR) : 1769, 1728, 1601, 1452, 1315, 1260, 1177, 1093, 1067, 1028, 804, 708, 687 cm^{-1}; HRMS: Calcd. for [$C_{51}H_{43}O_{15}NaSI$]: m/z 1077.1265 $[M+Na]^+$, found 1077.1263 $[M+Na]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 – 6.99 (m, 25H, H_{ar}), 5.97 (dd, *J* = 9.5, 8.4 Hz, 1H, $H_{3'}$), 5.76 – 5.65 (m, 2H, $H_{4'}$, H_3), 5.52 – 5.44 (m, 2H, $H_{2'}$, $H_{1'}$), 5.43 – 5.36 (m, 1H, H_2), 5.01 (d, *J* = 8.0 Hz, 1H, H_1), 4.54 – 4.44 (m, 2H, $H_{5'}$, $H_{1''}$), 4.39 (dd, *J* = 16.0, 2.5 Hz, 1H, H_{6a}), 3.93 – 3.85 (m, 1H, H_{6a}), 3.75 (s, 3H, -$COOCH_3$), 3.74 – 3.61 (m, 2H, H_{6b}, H_5), 3.23 – 2.97 (m, 1H, H_4), 2.40 (t, *J* = 2.4 Hz, 1H, -C≡CH) ; ^{13}C NMR (101 MHz, $CDCl_3$) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (-$\underline{C}OOCH_3$), 166.0 – 165.0 (5x-O$\underline{C}$OBz), 133.6 –128.3 (30 C_{ar}), 98.0 (C_1), 81.8 ($C_{1'}$), 78.4 (-$\underline{C}$≡CH), 76.1 ($C_{5'}$), 75.5 (-C≡$\underline{C}$H), 74.5 (C_5), 73.4 ($C_{3'}$), 73.1 (C_2), 70.5 ($C_{2'}$), 70.0 (C_3), 69.6 ($C_{4'}$), 56.0 ($C_{1''}$), 53.3 (-COO$\underline{C}H_3$), 50.8 (C_4), 7.8 (C_6).

Synthesis of compound 23.

To a solution of compound **22** (0.132 g, 0.125 mmol) in 1:4 DCM/methanol (2.5 mL) at 0 °C, a solution of 25% wt sodium methanolate (0.034 mL g, 0.150 mmol) was added. The reaction mixture was stirred at room temperature for 3 h. IR 120 H^+ resin was added in order to obtain a pH = 7. The mixture was filtered and the solvent was evaporated under reduced pressure. To a solution of the residue in water (2 mL), sodium sulfite (0.075 g, 0.595 mmol) was added. The mixture was refluxed until total conversion of the substrate (24 hours). The mixture was cooled to room temperature and then washed with ethyl acetate and diethyl ether. The aqueous phase was concentrated and purified over Sephadex™ LH-20. After freeze-drying, the desired product **23** was obtained as white crystals (0.053 g; 81%). $[\alpha]_D^{25}$: -20.5 (c = 0.19 H_2O); IR (ATR): 1622, 1132, 1107, 997, 617, 478, 424 $cm^{-1;}$ HRMS: Calcd. for [$C_{15}H_{21}O_{13}S_2$]$^{2-}$: m/z 473.0424 $[M-2Na]^{2-}$, found 473.0437 $[M-2Na]^{2-}$; ^1H NMR (600 MHz, D_2O) δ 4.67 (d, *J* = 8.1 Hz, 1H, H_1), 4.61 (d, *J* = 9.8 Hz, 1H, $H_{1'}$), 4.55 – 4.45 (m, 2H, $H_{1''}$), 4.02 – 3.96 (m, 1H, H_5), 3.88 (dd, *J* = 14.8, 1.8 Hz, 1H, H_{6a}), 3.79 (dd, *J* = 6.9, 2.8 Hz, 1H, $H_{4'}$), 3.62 (dd, *J* = 10.5, 9.1 Hz, 1H, H_3), 3.59 - 3.57 (m, 2H, $H_{5'}$, $H_{3'}$), 3.46 – 3.38 (m, 2H, $H_{2'}$, H_2), 3.23 (dd, *J* = 14.7, 9.4 Hz, 1H, H_{6b}), 2.97 (t, *J* = 2.4 Hz, 1H, -C≡C$\underline{H}$), 2.92 (t, *J* = 10.7 Hz, 1H, H_4) ; ^{13}C NMR (151 MHz, D_2O) δ 175.6 (-$\underline{C}$OONa) , 100.0 (C_1), 86.01 ($C_{1'}$), 79.9 ($C_{4'}$), 78.9 (-$\underline{C}$≡CH) , 76.8 ($C_{5'}$), 76.4 (-C≡$\underline{C}$H) , 74.2 (C_3), 73.8 (C_2), 72.8 (C_5), 72.5 ($C_{2'}$), 71.6 ($C_{3'}$), 56.4 ($C_{1''}$), 52.8 (C_6), 51.8 (C_4).

Synthesis of compound 24.

To a solution of compound **15** (0.26 g, 0.26 mmol) in 5:1 MeOH/H_2O (12 mL) a solution of NaOH (3 M, 3.6 mL, 10.9 mmol) was slowly added at 0 °C. The reaction mixture was stirred at 0 °C for 10 min and then at room temperature for 15 h. 1M HCl was slowly added to a pH = 8 (using a pH-meter). The reaction mixture was then concentrated under reduced pressure and the desired product purified over Sephadex™ LH-20 and freeze-dried. White crystals of **24** were obtained (0.091 g, 80%). $[\alpha]_D^{25}$: -95.4 (c = 0.5 $CHCl_3$); HRMS: Calcd. for [$C_{15}H_{23}O_{14}S$]: m/z 433.0781 $[M+H]^+$, found 433.0783; ^1H NMR (400 MHz, D_2O) δ 4.57 (d, *J* = 9.7 Hz, 1H, $H_{1'}$), 4.55 (d, *J* = 8.0 Hz, 1H, H_1), 4.39 (m, 2H, $H_{1''}$), 4.03 (dd, 1H, *J* = 12.4, *J* = 2.1 Hz, H_{6a}), 3.87 (dd, 1H, *J* = 12.4, *J* = 4.9 Hz, H_{6b}), 3.67 – 3.64 (d, *J* = 9.3 Hz, 1H, $H_{3'}$,$H_{5'}$), 3.61 (ddd, 1H, *J* = 11.0, *J* = 4.9, *J* = 2.1 Hz, H_5), 3.50 (dd, 1H, *J* = 10.7, *J* = 9.0 Hz, H_3), 3.46 – 3.42 (m, 2H, $H_{2'}$, $H_{4'}$), 3.26 (dd, 1H, *J* = 9.0, *J* = 8.0 Hz, H_2), 2.93 (t, *J* = 2.5 Hz, 1H, –C≡CH), 2.89 (t, *J* = 10.7 Hz, 1H, H_4); ^{13}C NMR (101 MHz, D_2O) δ 175.9 (-COONa), 100.2 (C_1) , 83.7 ($C_{1'}$), 78.8 ($C_{5'}$) , 78.8 (–$\underline{C}$≡CH), 76.8 ($C_{3'}$), 76.6 (–C≡$\underline{C}$H), 76.2 (C_5), 74.0 (C_2), 73.0 (C_3), 72.1 ($C_{2'}$), 71.6 ($C_{4'}$), 61.1 (C_6), 56.4 ($C_{1''}$), 46.9 (C_4).

Synthesis of compound 26.

To a solution of compound **25**[3] (3.25 g, 6.27 mmol) and sodium azide (13.83 g, 230.502 mmol) in dry DMF (60 mL) under argon, triphenylphosphine (12.1 g, 46.13 mmol) was added. The reaction mixture was stirred for 5 min and then a solution of tetrabromomethane (15.3 g, 46.133 mmol) in dry DMF (20 mL) was added dropwise. After stirring at room temperature for 60 h, methanol (40 mL) was added. The mixture was filtered through celite® and concentrated under reduced pressure. The residue was then dissolved in a 1:1 mixture of water/toluene (320 mL) under vigorous agitation. Ethyl acetate (1 L) was added and the mixture was stirred until the aqueous phase begin clear. The aqueous phase was recovered, washed several times with diethyl ether and concentrated to a syrup. The residue was dissolved in dry pyridine (120 mL) under argon and benzoyl chloride (130 mL) was dropwise added at 0°C. After stirring at room temperature for 5 h, methanol (100 mL) was added at 0°C. The mixture was concentrated and co-

evaporated several times with toluene. The residue was dissolved in ethyl acetate and washed with a saturated NaCl solution and water. The organic phase was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. Flash chromatography on silica gel (4:1 cyclohexane/ethyl acetate) afforded **26** as light-yellow crystals (3.625 g; 44%). Rf: 0.18 (4:1 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$: +84.8 (c = 0.5 $CHCl_3$); IR (ATR): 2104, 1730, 1603, 1452, 1314, 1271, 1094, 1069, 1028; 1003, 708, 602 cm^{-1}; HRMS: Calcd. for $[C_{68}H_{59}O_{20}N_9Na]$: m/z 1344.3774 $[M+Na]^+$; Found 1344.3810 $[M+Na]^+$; ^1H NMR (400 MHz, $CDCl_3$) : δ 8.00 – 7.02 (m, 35H, H_{ar}), 6.03 (dd, *J* = 10.5, 9.5 Hz, 1H, H^{III}_3), 5.80 (dd, *J* = 10.2, 8.9 Hz, 1H, H^{II}_3), 5.73 (d, *J* = 3.9 Hz, 1H, H^{III}_1), 5.65 (t, *J* = 9.6 Hz 1H, H^{I}_3), 5.62 (d, *J* = 1.4 Hz, 1H, H^{II}_1), 5.50 (t, *J* = 9.8 Hz, 1H, H^{III}_4), 5.32 (dd, *J* = 9.6, 7.7 Hz, 1H, H^{I}_2), 5.21 (dd, *J* = 8.9, 4.0 Hz 1H, H^{III}_2), 5.03 (dd, *J* = 10.1, 4.0 Hz, 1H, H^{II}_2), 4.67 (d, *J* = 7.7 Hz, 1H, H^{I}_1), 4.40 (t, *J* = 9.3 Hz, 1H, H^{I}_4), 4.32 (t, *J* = 9.3 Hz, 1H, H^{II}_4), 4.21 – 4.15 (m, 1H, H^{III}_5), 4.14 – 4.07 (m, 1H, H^{II}_5), 3.95 – 3.46 (m, 7H, H^{I}_5, $H^{I\text{-}III}_6$), 3.53 (s, 3H, -OCH_3); ^{13}C NMR (101 MHz, $CDCl_3$) : 165.8 – 165.0 (7x -O<u>C</u>OBz), 133.8 – 128.1 (C_{ar}), 101.5 (C^{I}_1), 96.3 (C^{III}_1), 95.9 (C^{II}_1), 74.9 (C^{I}_3), 74.2 (C^{I}_5), 72.9 (C^{II}_4), 72.5 (C^{I}_4), 72.1 (C^{I}_2), 71.8 (C^{II}_3), 71.0 (C^{II}_5), 70.9 (C^{II}_2, C^{III}_2), 70.3 (C^{III}_5), 69.8 (C^{III}_4, C^{III}_3), 56.7 (-OCH_3), 51.5 – 51.20 ($C^{I\text{-}III}_6$).

Synthesis of compound 28.

To a solution of heptakis (6-azido-6-deoxy)-cyclomaltoheptaose[4] (100 mg, 0.076 mmol) in dry pyridine (1 mL) under argon benzoyl chloride (0.29 mL, 2.543 mmol) was dropwise added at 0 °C. The mixture is stirred at 40 °C for 24 h, then methanol (1 mL) was added at 0 °C. The mixture was concentrated and co-evaporated several times with toluene. The residue was dissolved in ethyl acetate (50 mL), washed with a saturated NaCl solution and water. The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in a minimum amount of ethyl acetate and crystallized by addition of methanol. Compound **28** was obtained as white crystals (0.2 mg; 95%). MP: 175-177 °C; $[\alpha]_D^{25}$: +86.667 (c = 0.21 $CHCl_3$); IR (ATR): 2104, 1728, 1452, 1314, 1273, 1175, 1094, 1069, 1028, 706 cm^{-1}; HRMS: Calcd. for $[C_{140}H_{119}N_{21}O_{42}Na]$: m/z 2788.7719 $[M+Na]^+$; Found 2788.7837 $[M+Na]^+$; ^1H NMR (400 MHz, $CDCl_3$) : δ 7.45 (ddd, *J* = 19.0, 8.3, 1.4 Hz, 28H, H_{aro}), 7.33 – 7.15 (m, 15H, H_{aro}), 6.97 (dt, *J* = 20.1, 7.7 Hz, 27H, H_{aro}), 5.92 (dd, *J* = 10.5, 8.9 Hz, 7H, H_3), 5.49 (d, *J* = 3.8 Hz, 7H, H_1), 5.05 (dd, *J* = 10.4, 3.7 Hz, 7H, H_2), 4.45 (ddd, *J* = 9.9, 4.8, 2.1 Hz, 7H, H_5), 4.09 (t, *J* = 9.3 Hz, 7H, H_4), 4.04 – 3.89 (m, 14H, $H_{6\,a,b}$) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.1 (7 x –O<u>C</u>OBz), 164.7 (7 x –O<u>C</u>OBz), 132.8 - 127.8 (84 x C_{ar}), 97.2 (C_1), 77.5 (C_4), 71.6 (C_2), 71.3 (C_5), 71.2 (C_3), 52.0 (C_6).

Synthesis of compound 29.

To a solution of compounds **26** (187 mg, 0.178 mmol) and **27** (71.40 mg, 0.054 mmol) in a 4:1 mixture of dioxane/water (2.5 mL), copper(II) sulfate pentahydrate (8.38 mg, 0.032 mmol) and sodium ascorbate (12.79 mg, 0.064 mmol) were added. The mixture was stirred at 70 °C in a microwave reactor. After 60 min, the reaction mixture was poured into a 1:1 solution of H_2O/NH_4Cl (30 mL), and extracted with ethyl acetate (4 x 30 mL). The combined organic phases were dried over sodium sulfate, filtered and concentrated under reduced pressure. After purification by silica gel chromatography (9:1 dichloromethane/methanol), the protected glycocluster was dissolved in 5:1 methanol/water (6 mL). 3M NaOH (2 mL, 6 mmol) was dropwise added at 0 °C. The reaction mixture was stirred 10 min at 0 °C and 7 h at room temperature. 1M HCl was added in order to obtain a pH = 8 (using a pH-meter). The mixture was concentrated and the residue purified on Sephadex™ LH-20. The desired product **29** was obtained after freeze-drying as a cottony white solid (94.64 mg, 86%). $[\alpha]_D^{25}$: -35.6 (c = 0.5 H_2O); IR (ATR): 1649.1, 1612.5, 1417.7, 1275, 1230.6, 1058.9, 1003, 727.2, 692.4, 599.9, 559.4, 530.4, 482.2 cm^{-1}; HRMS: Calcd. for $[C_{64}H_{91}N_9O_{55}Na_6S_6]$: m/z 1120.6053 $[M+2Na]^{2+}$; Found 1120.6036 $[M+2Na]^{2+}$; ^1H NMR (600 MHz, D_2O) δ 8.14 (s, 1H, -CH-triazole), 8.09 (s, 1H, -CH-triazole), 8.03 (s, 1H, -CH-triazole), 5.43 (d, *J* = 4.0 Hz, 1H, H^{III}_1), 5.32 (d, *J* = 3.9 Hz, 1H, H^{II}_1), 5.02 – 4.39 (m, 24H, 6x $H^{I\text{-}III}_6$, 3x $H_{1''a}$, 3x $H_{1'}$, 3x H_1, 6x H_6, 3x $H_{1''b}$), 4.24 (d, *J* = 7.9 Hz, 1H, H^{I}_1), 4.22 – 3.29 (m, 40H, $H^{I\text{-}III}_2$, $H^{I\text{-}III}_3$, $H^{I\text{-}III}_4$, $H^{I\text{-}III}_5$, 3x$H_{2'}$, 3x$H_{3'}$, 3x$H_{4'}$, 3x$H_{5'}$, 3xH_2, 3xH_3, 3xH_5), 3.33 (s, 3H, -O<u>C</u>H_3), 3.02 (m, 3H, 3x H_4). ^{13}C NMR (151 MHz, D_2O) δ 175.5 (<u>C</u>OONa), 143.9 – 143.6 (Cq triazole), 127.0 – 126.3 (CH-triazole), 102.7 (C^{I}_1), 101.8 – 101.5 (C_1), 100.8, 100.4 (C^{II}_1, C^{III}_1), 84.7 – 84.6 ($C_{1'}$), 80.5, 80.3 ($C^{I,II}_4$), 79.5 ($C_{5'}$), 76.8 ($C_{3'}$), 74.4 (C_5), 74.1 (C_2), 73.4 (C_3), 72.7 ($C_{2'}$), , 71.7 ($C_{4'}$), 75.7– 69.8 ($C^{I\text{-}III}_2$, $C^{I\text{-}III}_3$, $C^{I\text{-}III}_5$, C^{III}_4 maltotriose), 67.6 – 67.5 (C_6), 62.2 –61.7 ($C_{1''}$) , 56.9 (OCH_3), 51.3 – 50.2 ($C^{I\text{-}III}_6$), 47.4 – 47.2 (C_4).

Synthesis of compound 30.

Compound **30** was synthesized from compound **15** and **26** by CuAAC through the same protocol used for compound **31**, and was obtained as a colourless oil (0.060 g, 50%). $[\alpha]_D^{25}$: -37.8 (c = 0.5 H_2O) ; IR (ATR): 3385.1, 2848.9, 1608.6, 1413.8, 1151.5, 1103.3, 1055.1, 1016.5, 788.9, 698.2, 607.6, 540.1, 476.4, 438.8 cm^{-1}; HRMS: Calcd. for $[C_{64}H_{97}N_9O_{46}Na_2S_3]$: m/z 934.7237 $[M+2Na]^{2+}$; Found 934.7243 $[M+2Na]^{2+}$; ^1H NMR (600 MHz, D_2O) δ 8.15, 8.03, 7.94 (3s, 3H, -CH-triazole), 5.39 (d, *J* = 3.4 Hz, 1H, H^{III}_1), 5.27 (d, *J* = 3.9 Hz, 1H, H^{II}_1), 5.02 – 4.39 (m, 18H, 6x $H^{I\text{-}III}_6$, 3x $H_{1''a}$, 3x $H_{1'}$, 3x H_1 , 3x $H_{1''b}$), 4.64 (d, *J* = 7.9 Hz, 1H, H^{I}_1), 4.25 – 3.10 (m, 46H, $H^{I\text{-}III}_2$, $H^{I\text{-}III}_3$, $H^{I\text{-}III}_4$, $H^{I\text{-}III}_5$, 3x$H_{2'}$, 3x$H_{3'}$, 3x$H_{4'}$, 3x$H_{5'}$, 3xH_2 , 3xH_3 , 3xH_5, 6x H_6 ,), 3.33 (s, 3H, -OCH_3), 2.92 – 2.85 (m, 3H, 3x H_4). ^{13}C NMR (151 MHz, D_2O) δ 175.9 (<u>C</u>OONa), 144.0 – 143.3 (Cq triazole), 126.6 – 126.1 (CH-triazole), 103.3, 103.0, 102.83, 101.5 – 101.1 (C_1, C^{I}_1, C^{II}_1 , C^{III}_1), 84.7 – 84.6 ($C_{1'}$), 79.7, 79.5 ($C^{I,II}_4$, $C_{5'}$), 76.6, 76.5 ($C_{3'}$), 74.4 (C_5), 74.0 (C_2), 73.7 (C_3), 72.6 ($C_{2'}$), , 71.7 ($C_{4'}$), 75.7– 69.8 ($C^{I\text{-}III}_2$, $C^{I\text{-}III}_3$, $C^{I\text{-}III}_5$, C^{III}_4 maltotriose), 61.6 – 61.2 (C_6, $C_{1''}$) , 56.8 (OCH_3), 51.8 – 50.9 ($C^{I\text{-}III}_6$), 47.1 – 46.9 (C_4).

Synthesis of compound 31.

To a solution of compounds **19** (100 mg, 0.0954 mmol) and **28** (30.99 mg, 0.011 mmol) in a anhydrous DMF (0.5 mL), copper(I) iodide (1.47 mg, 0.0077 mmol) and *N,N*-diisopropylethylamine (DIPEA) (0.01 mL, 0.077 mmol) were added. The mixture was stirred at 70 °C in a microwave reactor. After 90 min, the reaction mixture was poured into a solution of 1/1 H_2O/NH_4Cl (30 mL), and extracted with

ethyl acetate (4 x 30 mL). The combined organic phases were dried over sodium sulfate, filtered and concentrated under reduced pressure. After purification by silica gel chromatography (100:0 to 96:4 DCM/MeOH), the protected glycocluster was dissolved in 5:1 methanol/water (3.5 mL). 3M NaOH (0.25 mL, 0.706 mmol) was dropwise added at 0 °C. After addition of DMF (0.5 mL), the reaction mixture was stirred 10 min at 0 °C and 24 h at room temperature. Water (0.5 mL) and 1M HCl were added in order to obtain a pH = 8 (using a pH-meter). The mixture was concentrated and the residue was purified over Sephadex™ LH-20. The desired product **31** was obtained after freeze-drying as a cottony white solid (30 mg, 78% yield). $[\alpha]_D^{25}$: -30.455 (c = 0.22 H_2O); IR (ATR): 3350, 1620, 1429, 1269, 1227, 1053, 1001, 692, 606 cm^{-1}; HRMS: Calcd. for $[C_{147}H_{209}N_{21}O_{126}S_{14}Na_4]$: m/z 1205.6552 $[M\text{-}10Na+6H]^{4-}$, Found 1205.6490 $[M\text{-}10Na+6H]^{4-}$; ^1H NMR (600 MHz, D_2O) δ 8.32 – 7.92 (m, 7H, CH-triazole), 5.21 (m, 7H, $H^{I\text{-}VII}_1$), 4.76 (m, 7H, $H_{1'}$), 4.61 – 4.13 (m, 56H, H_6, $H_{1''}$, $H^{I\text{-}VII}_2$, $H^{I\text{-}VII}_6$, H_1), 4.10 – 3.09 (m, 42H, $H_{2'}$, H_3, H_5, $H_{3'}$, $H^{I\text{-}VII}_3$, $H^{I\text{-}VII}_5$), 3.06 – 2.78 (m, 7H, H_4); ^{13}C NMR (151 MHz, D_2O) δ 173.3 (COONa), 140.0 (Cq triazole), 102.3 – 101.8 ($C^{I\text{-}VII}_1$, C_1), 84.4 ($C_{1'}$), 82.7 ($C^{I\text{-}VII}_4$), 78.3 ($C_{5'}$), 76.9 ($C_{3'}$), 74.6 – 71.6 ($C_{2'}$, C_3, C_5, $C_{3'}$, $C^{I\text{-}VII}_3$, $C^{I\text{-}VII}_5$), 70.1 ($C^{I\text{-}VII}_2$), 67.7 (C_6), 62.3 ($C_{1''}$), 50.7 ($C^{I\text{-}VII}_6$), 47.3 – 46.6 (C_4).

Notes and references

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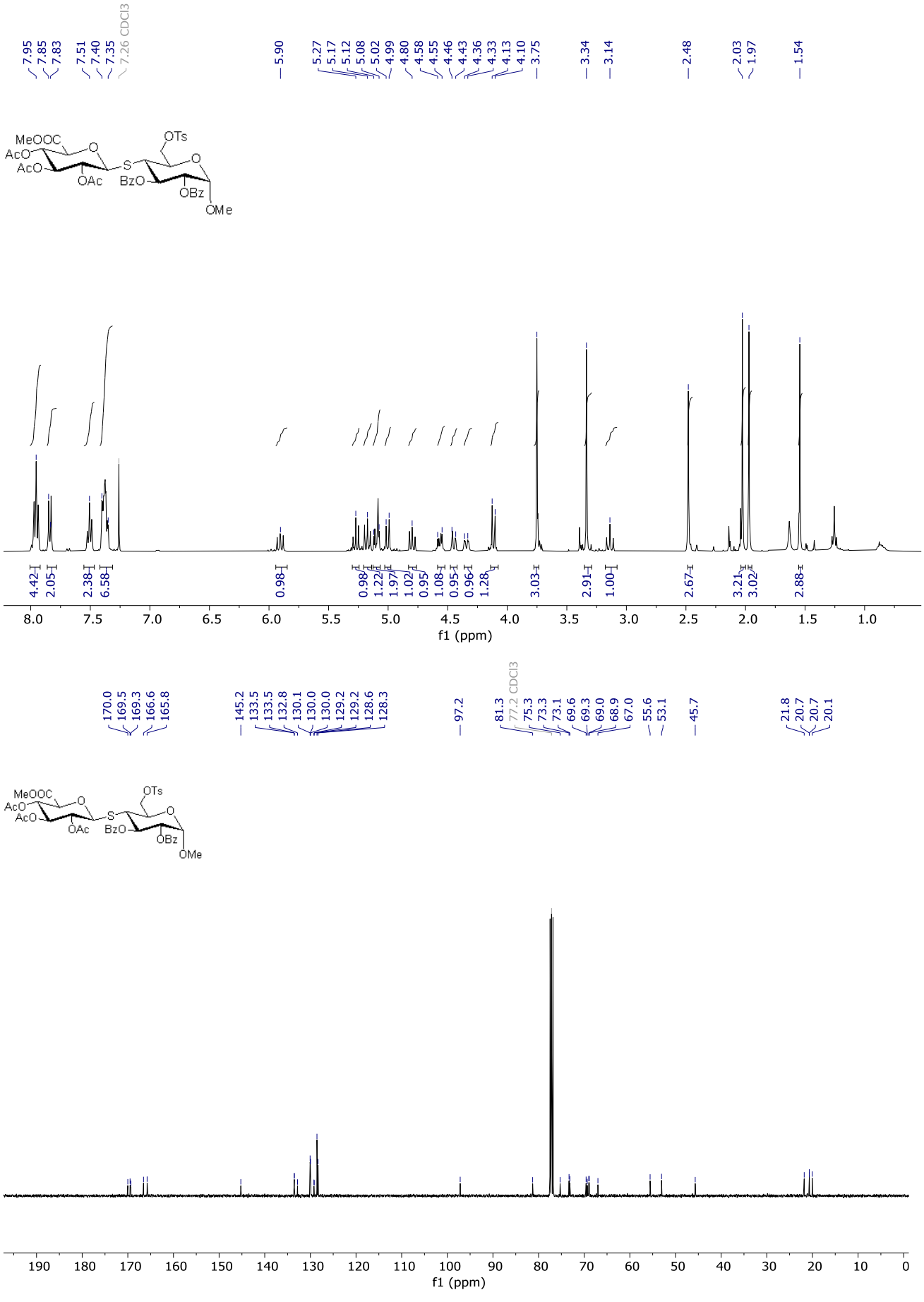
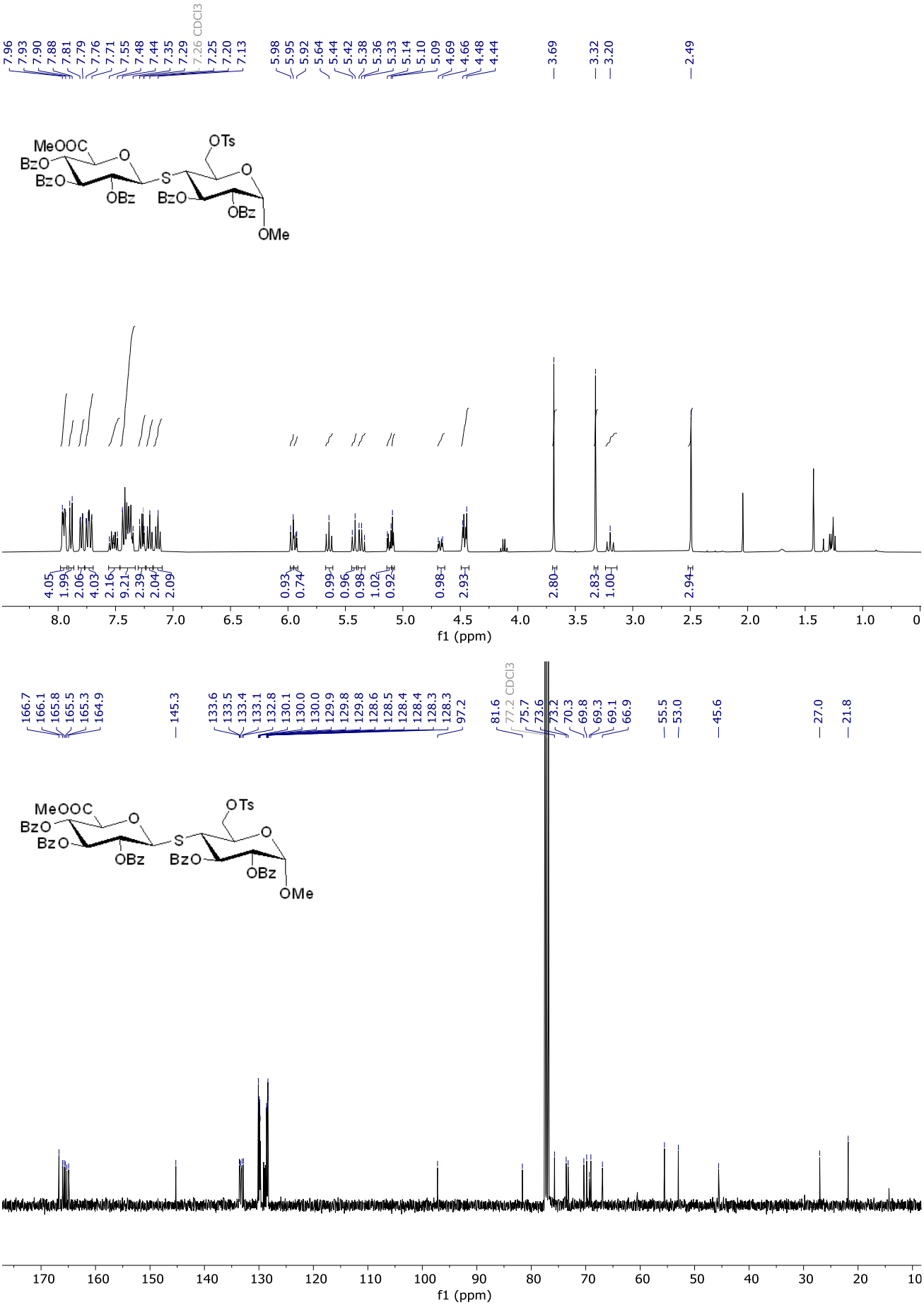
4 U. Alali, A. Vallin, A. Bil, T. Khanchouche, D. Mathiron, C. Przybylski, R. Beaulieu, J. Kovensky, M. Benazza and V. Bonnet, The uncommon strong inhibition of α-glucosidase by multivalent glycoclusters based on cyclodextrin scaffolds, *Org. Biomol. Chem.*, 2019, **17**, 7228–7237.

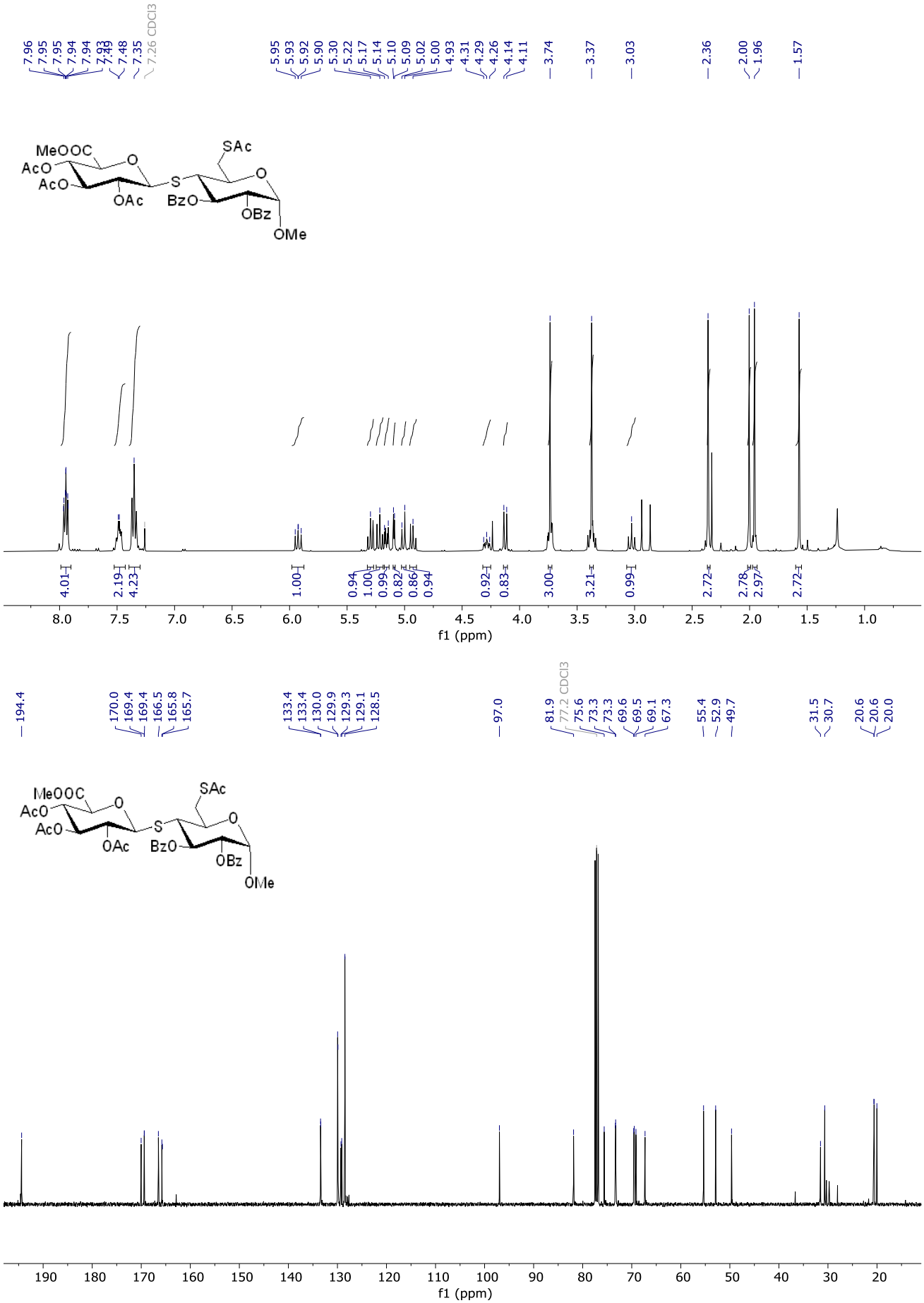
^{1}H (top) and ^{13}C (bottom) NMR spectra of **2**



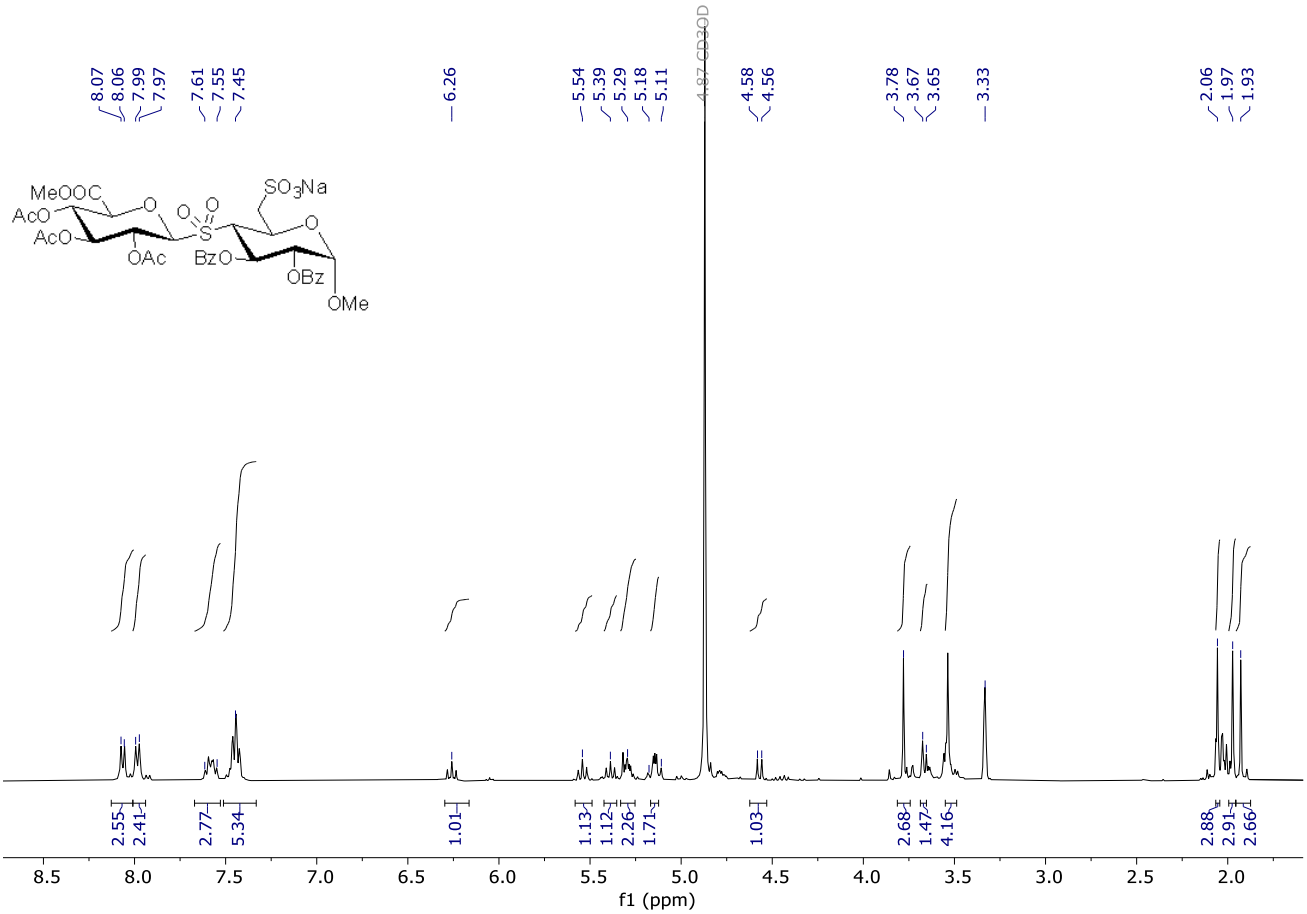
^{1}H (top) and ^{13}C (bottom) NMR spectra of **6**

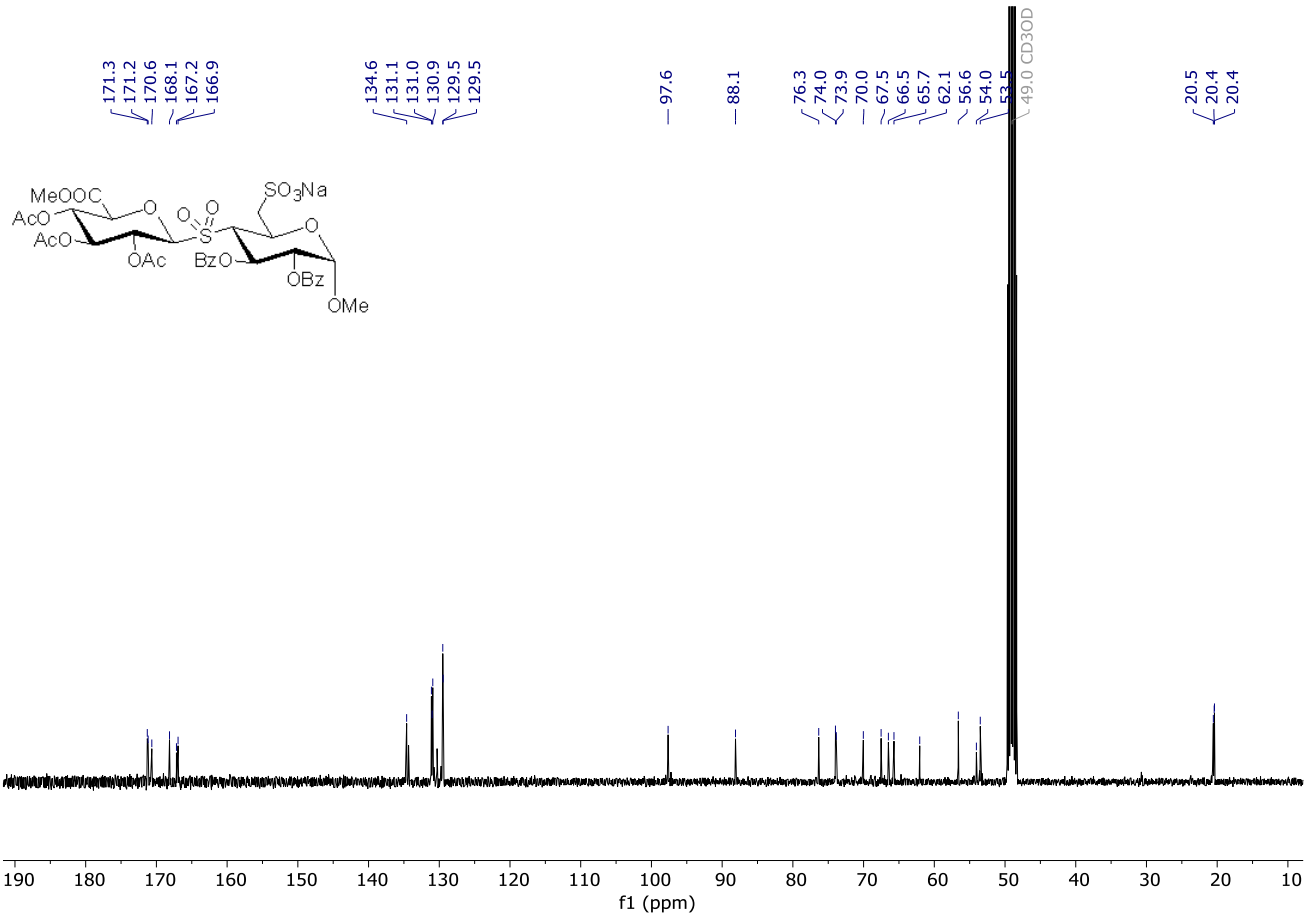


^{1}H (top) and ^{13}C (bottom) NMR spectra of **7**

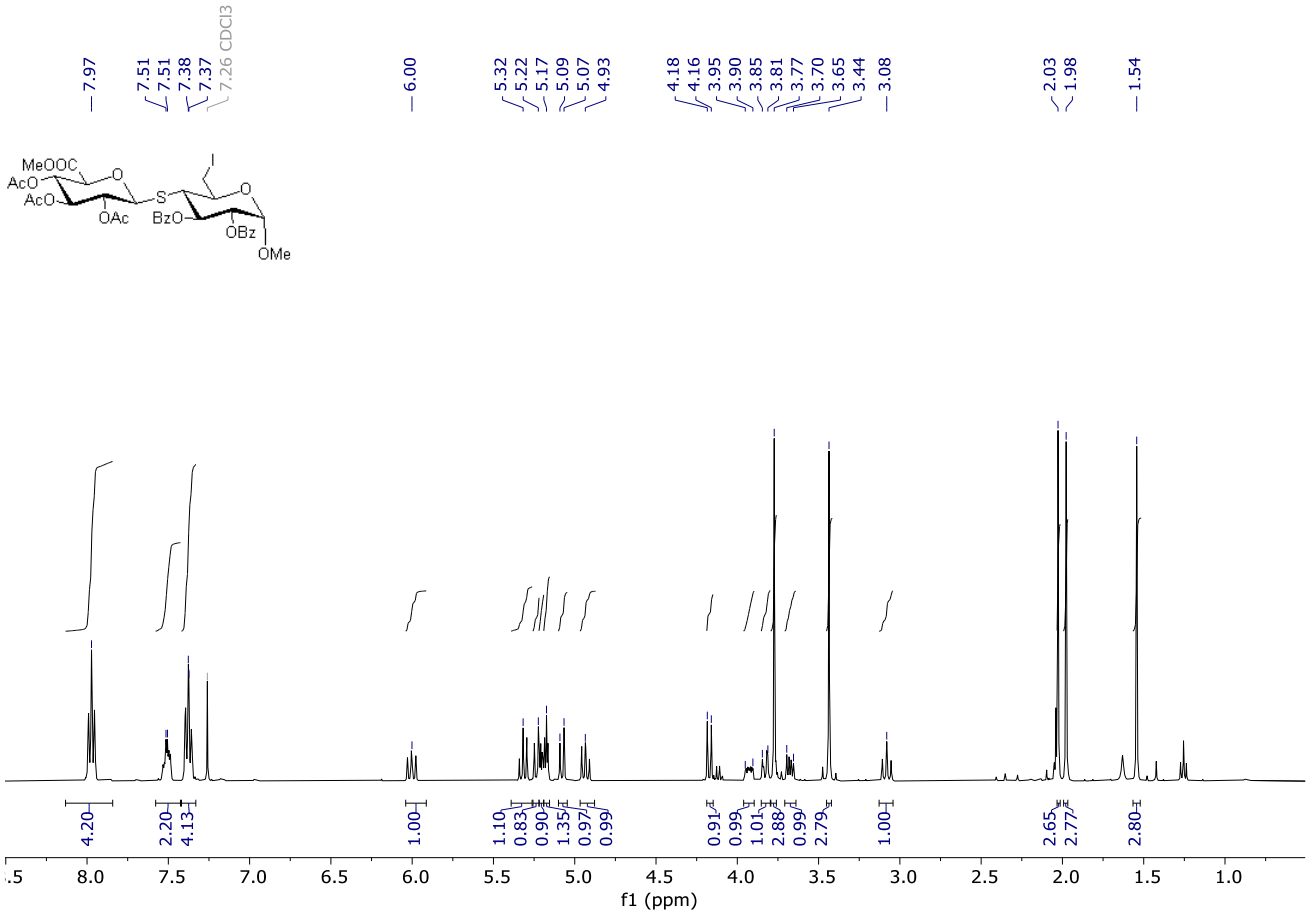


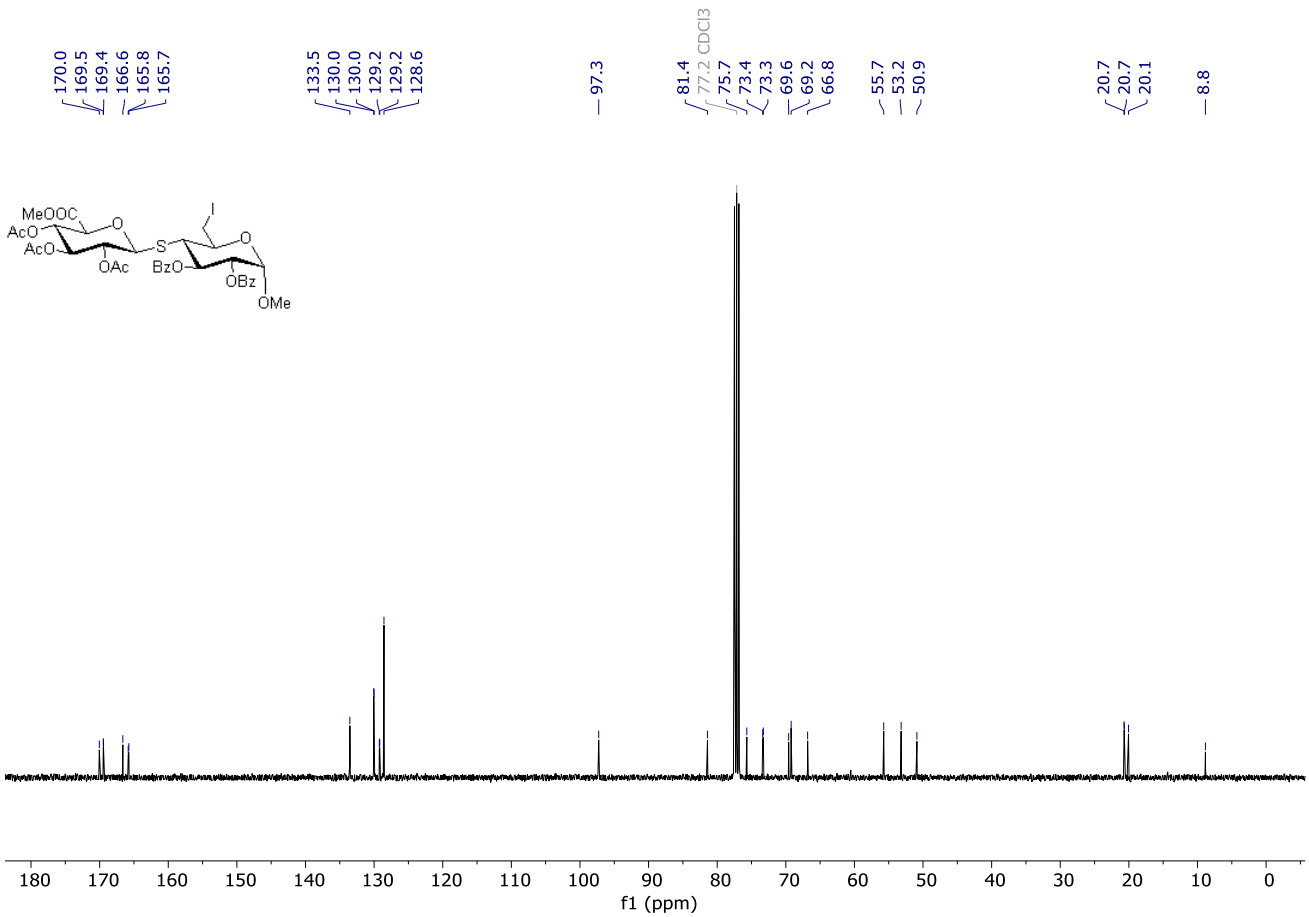
^{1}H (top) and ^{13}C (bottom) NMR spectra of **8b**



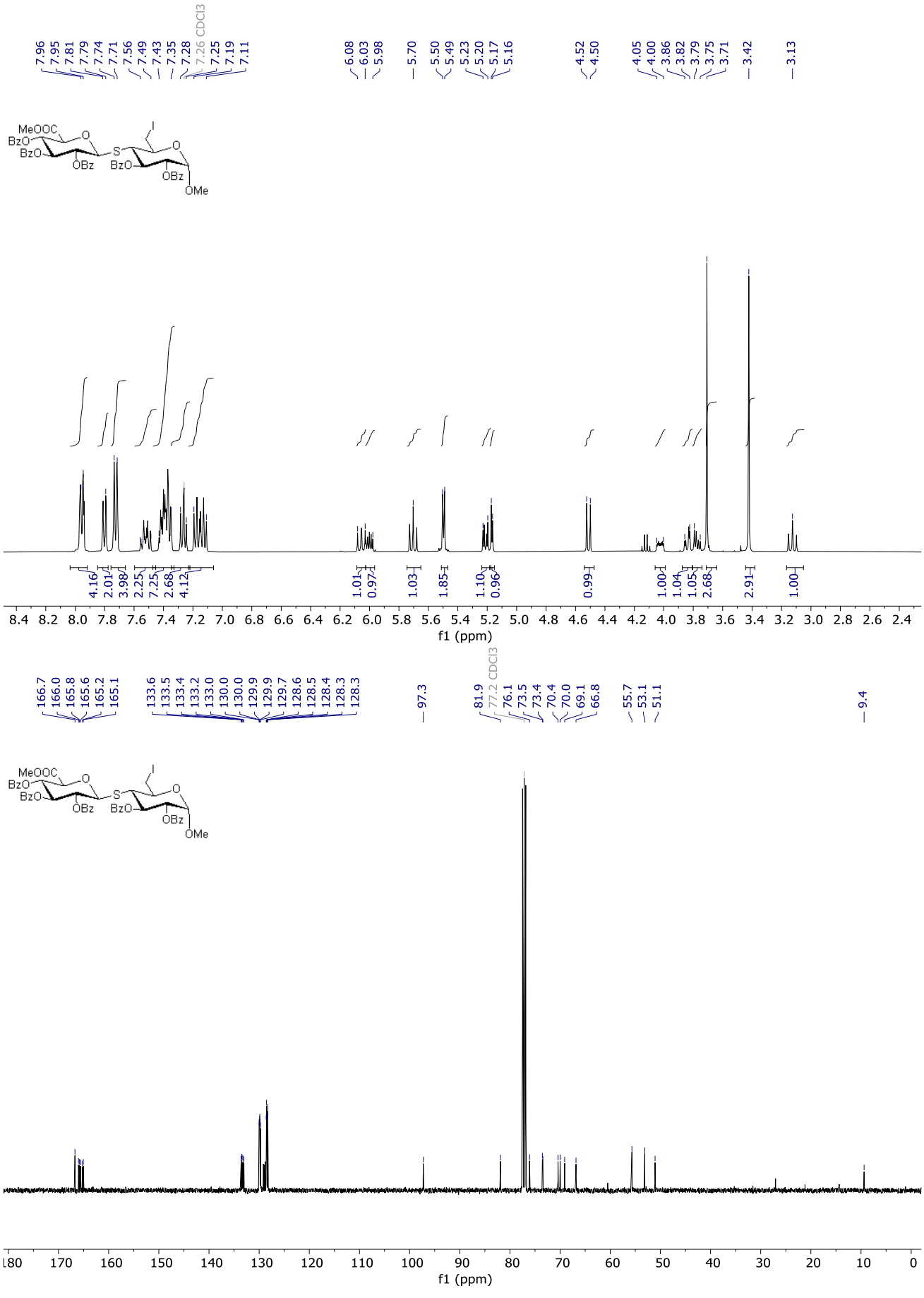


^{1}H (top) and ^{13}C (bottom) NMR spectra of **9**

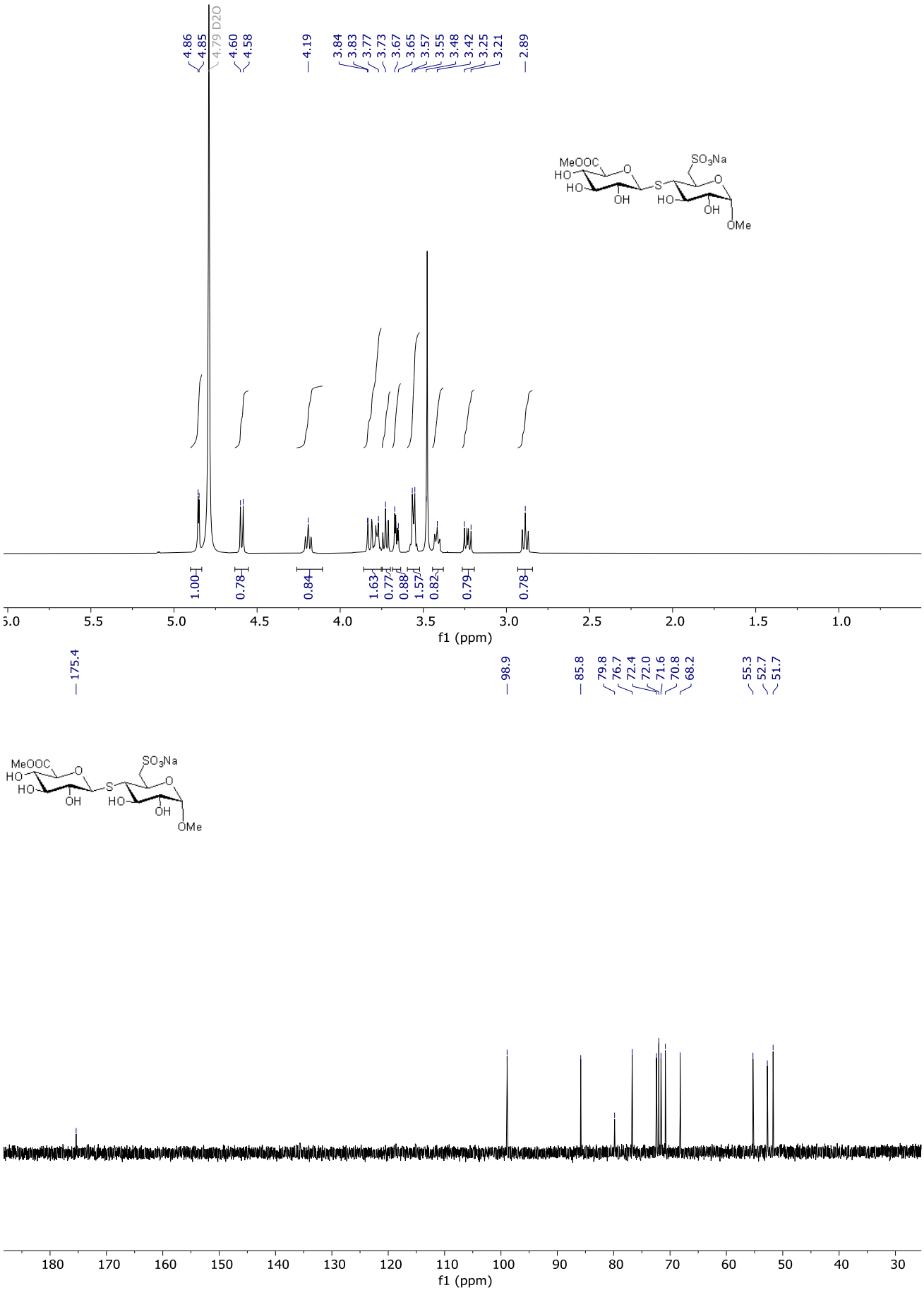




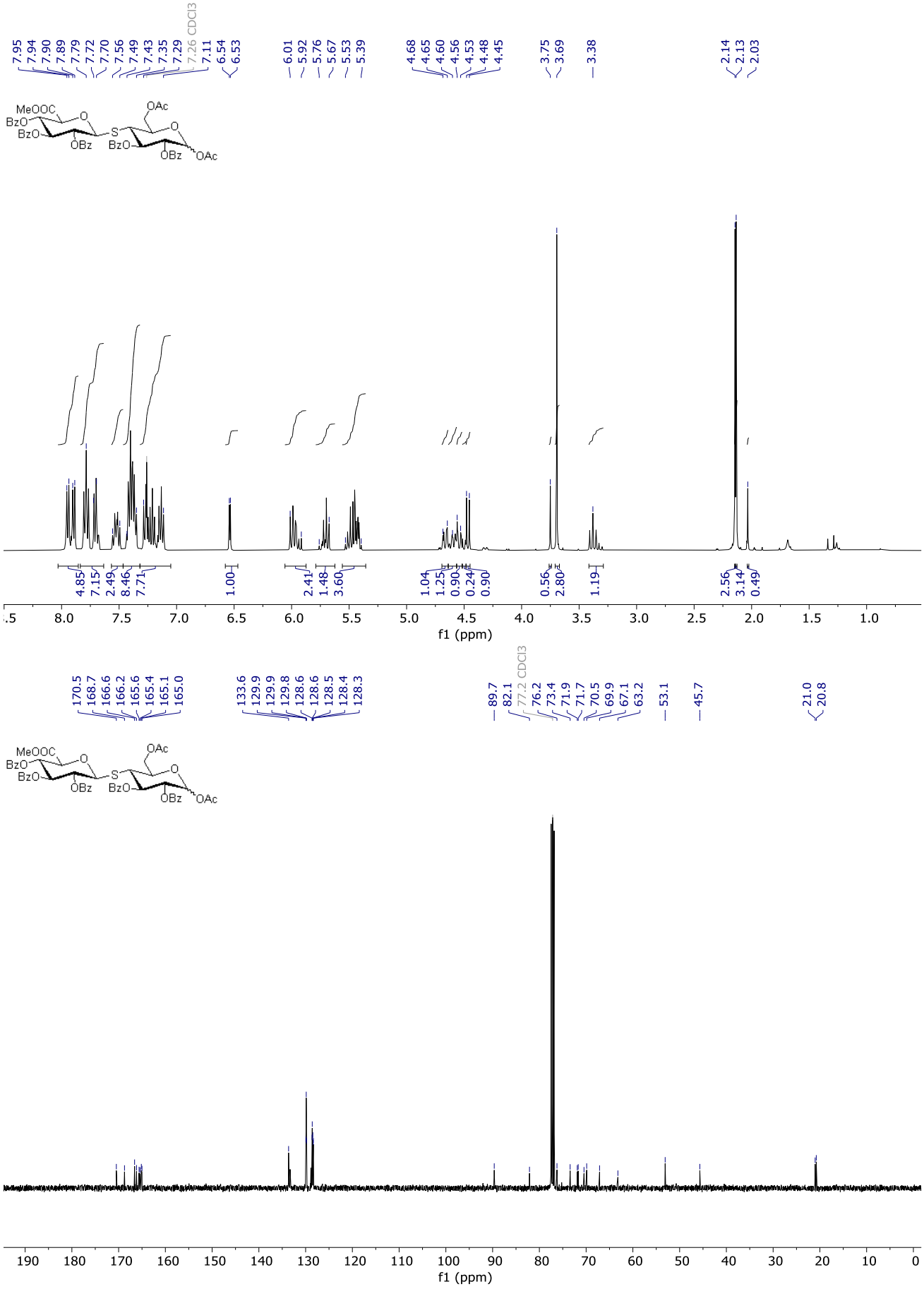
^{1}H (top) and ^{13}C (bottom) NMR spectra of **10**



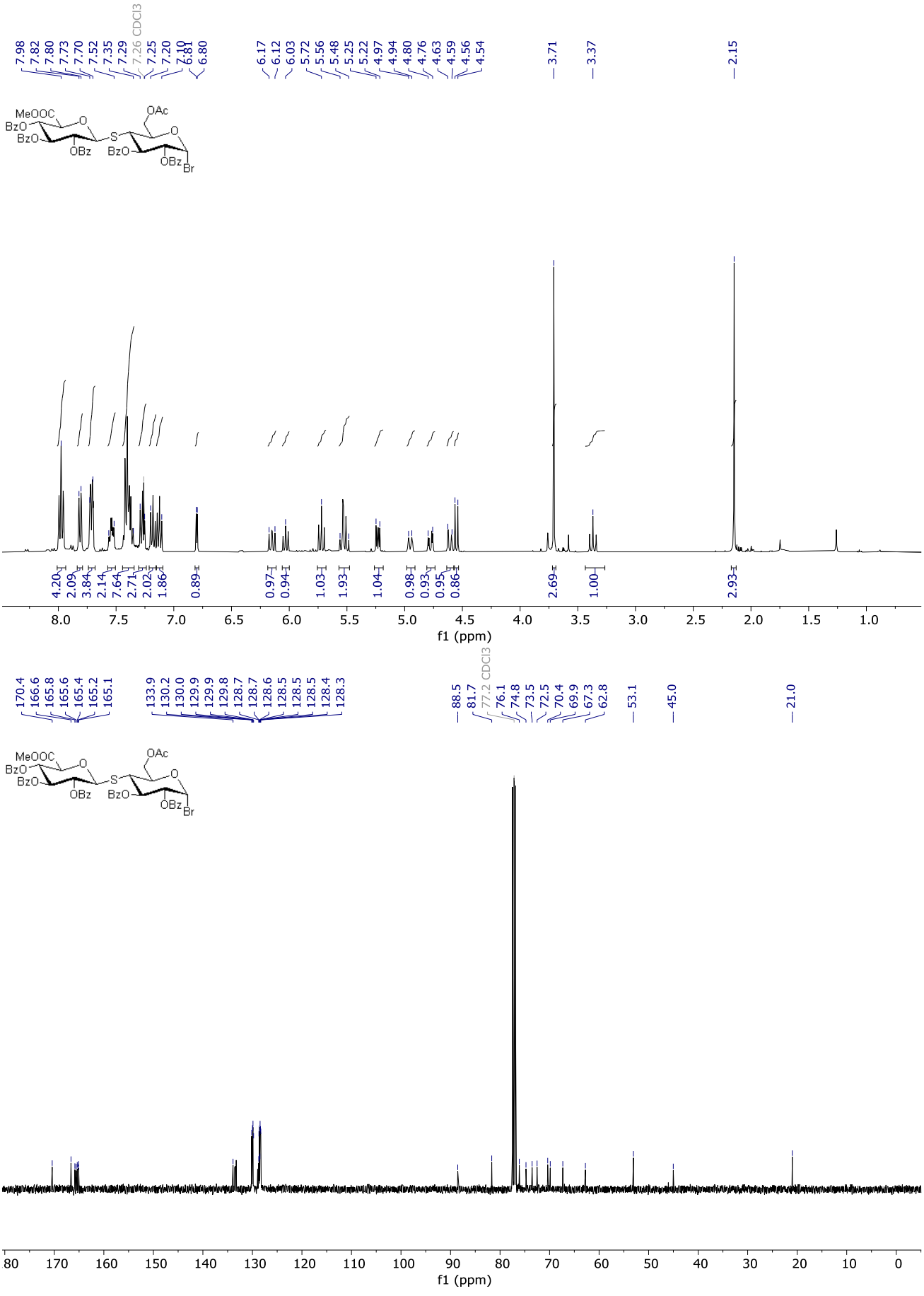
^{1}H (top) and ^{13}C (bottom) NMR spectra of **11**



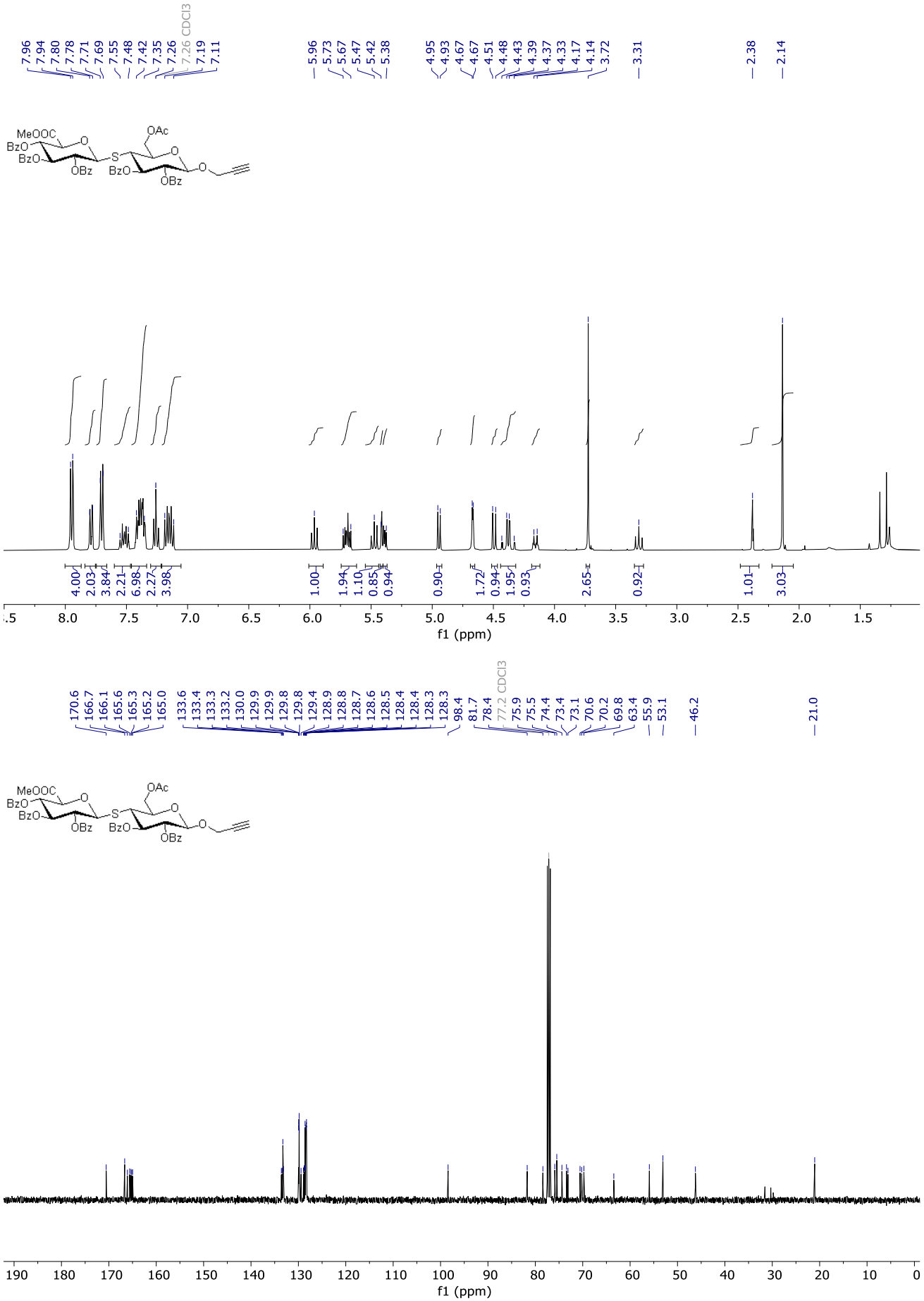
^{1}H (top) and ^{13}C (bottom) NMR spectra of **13**



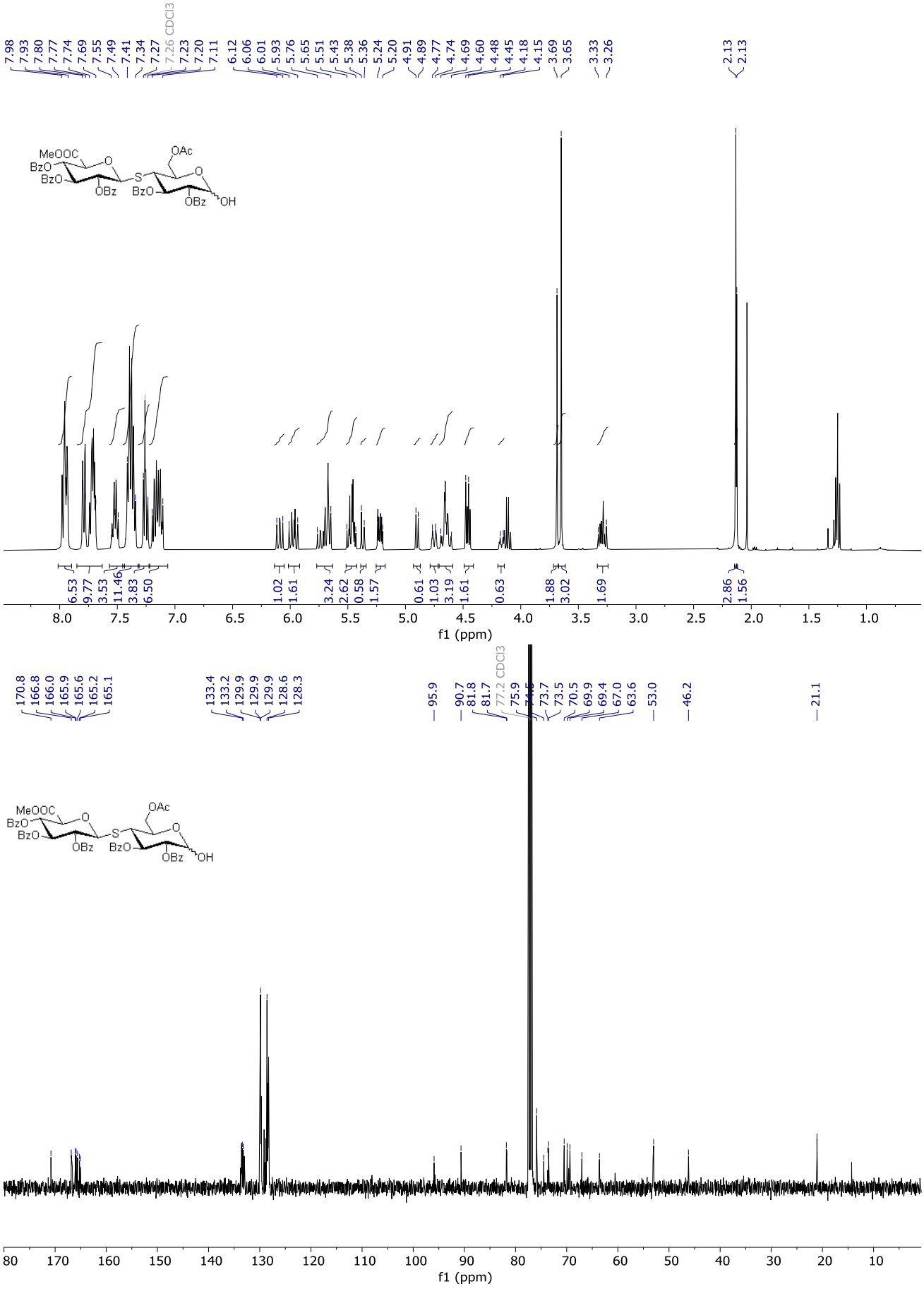
^{1}H (top) and ^{13}C (bottom) NMR spectra of **14**



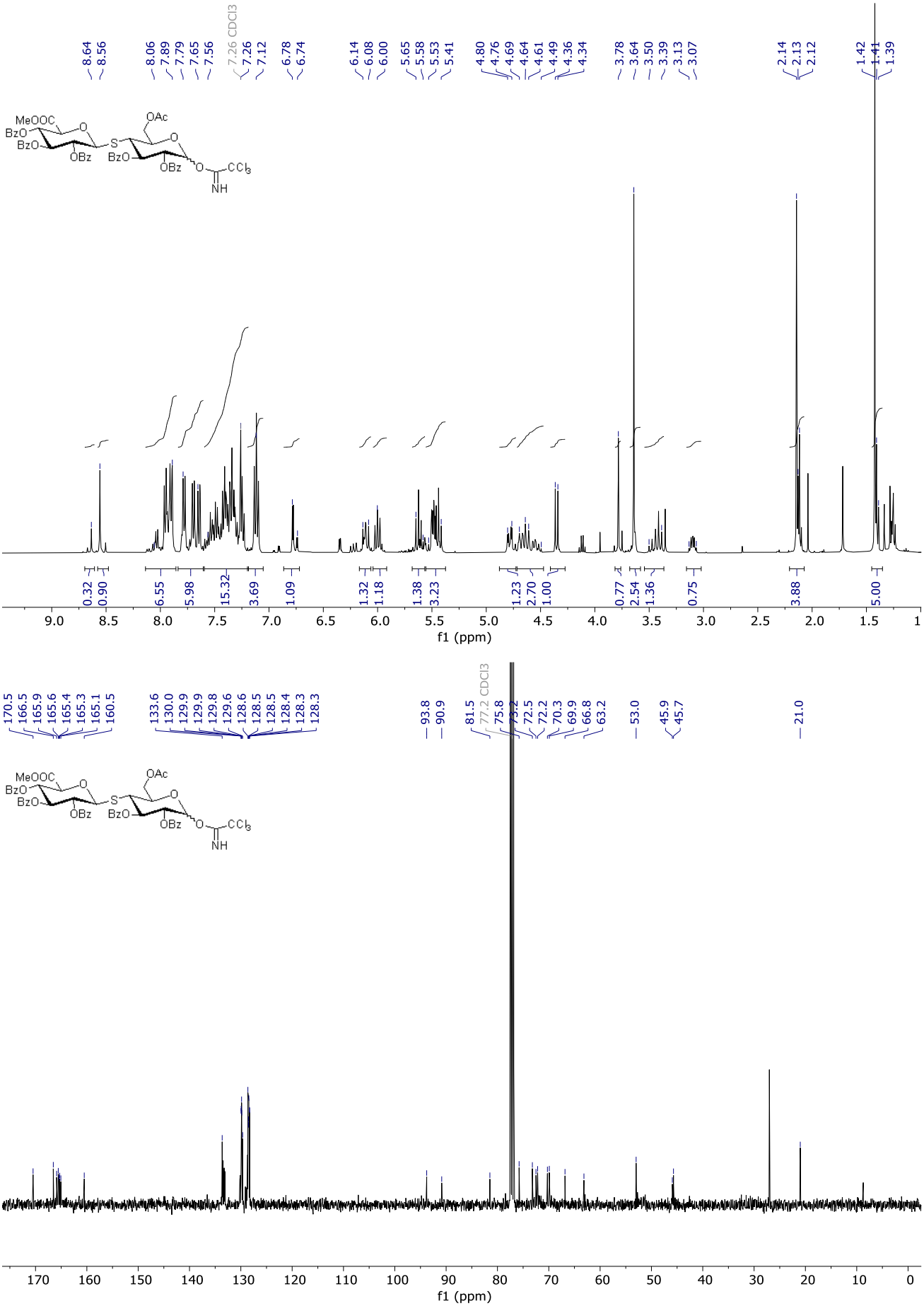
^{1}H (top) and ^{13}C (bottom) NMR spectra of **15**



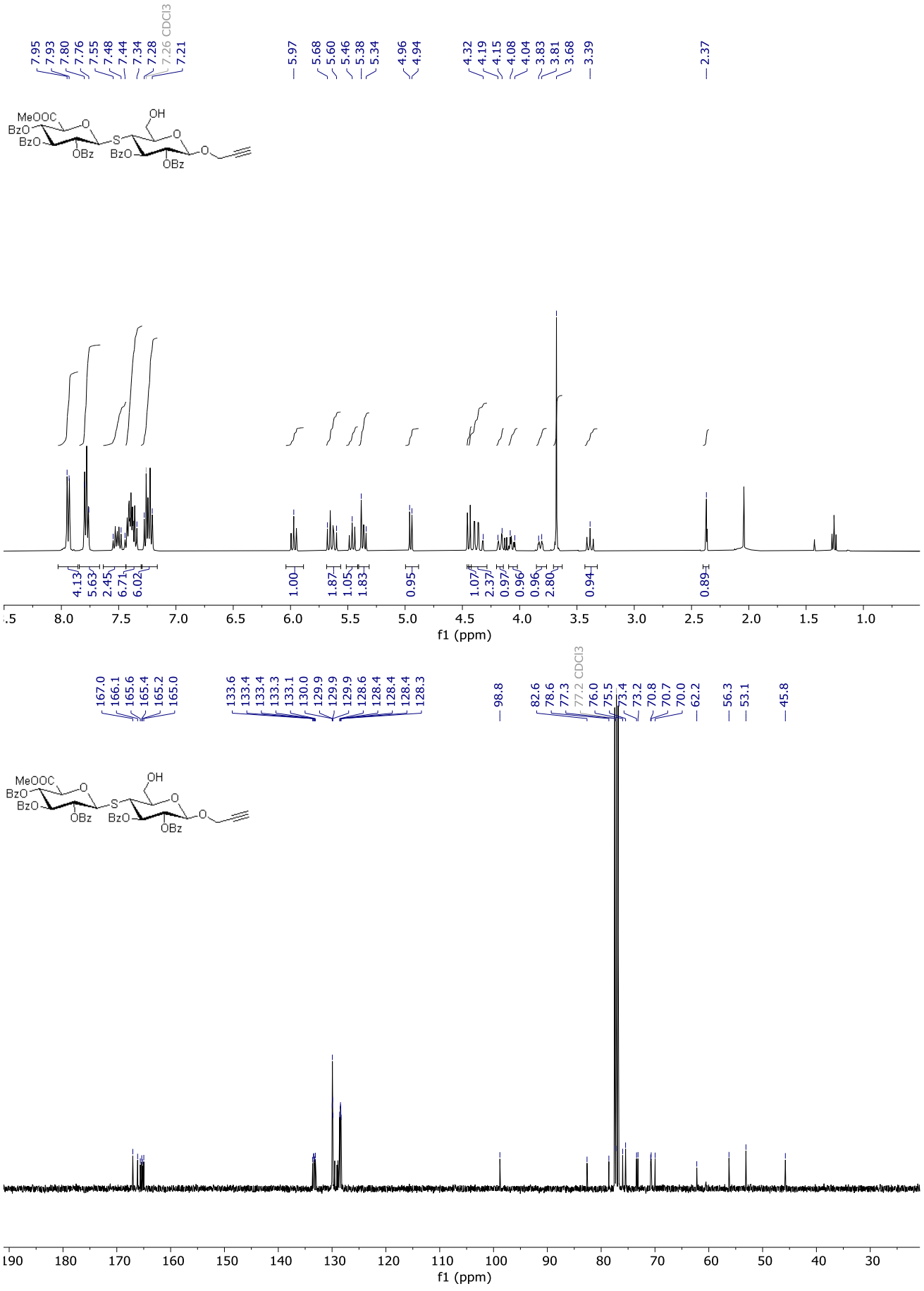
^{1}H (top) and ^{13}C (bottom) NMR spectra of **16**



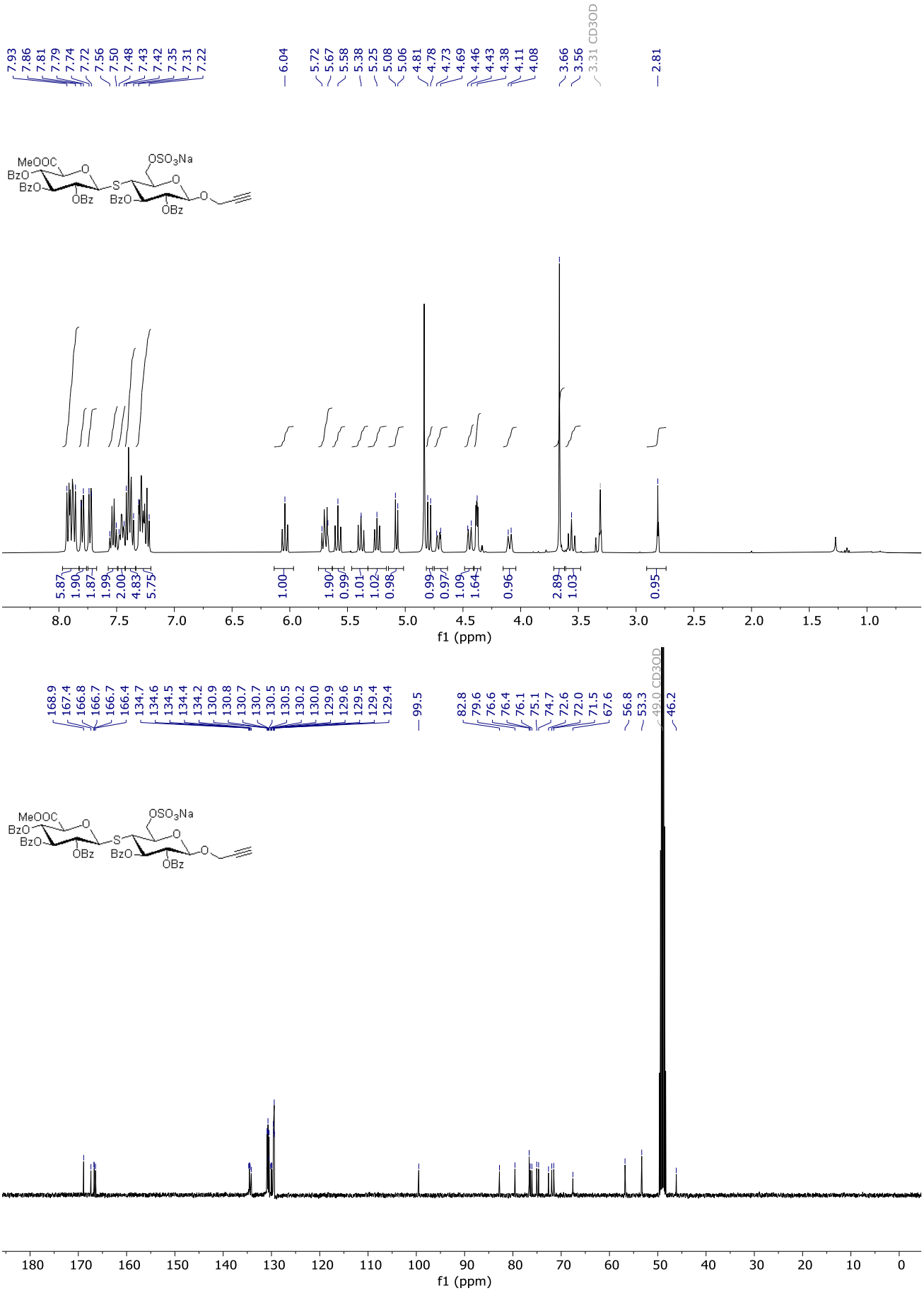
^{1}H (top) and ^{13}C (bottom) NMR spectra of **17**



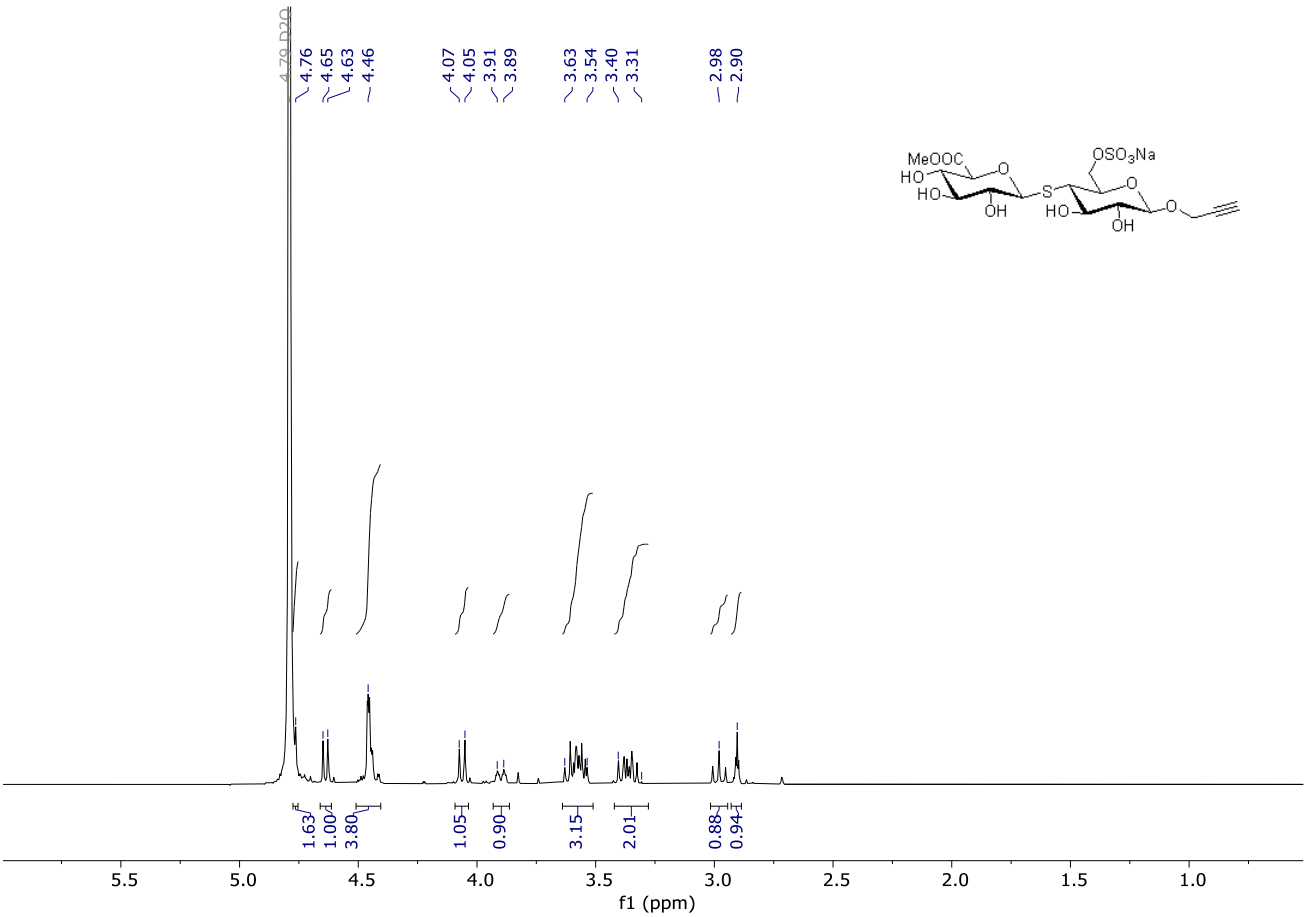
^{1}H (top) and ^{13}C (bottom) NMR spectra of **18**

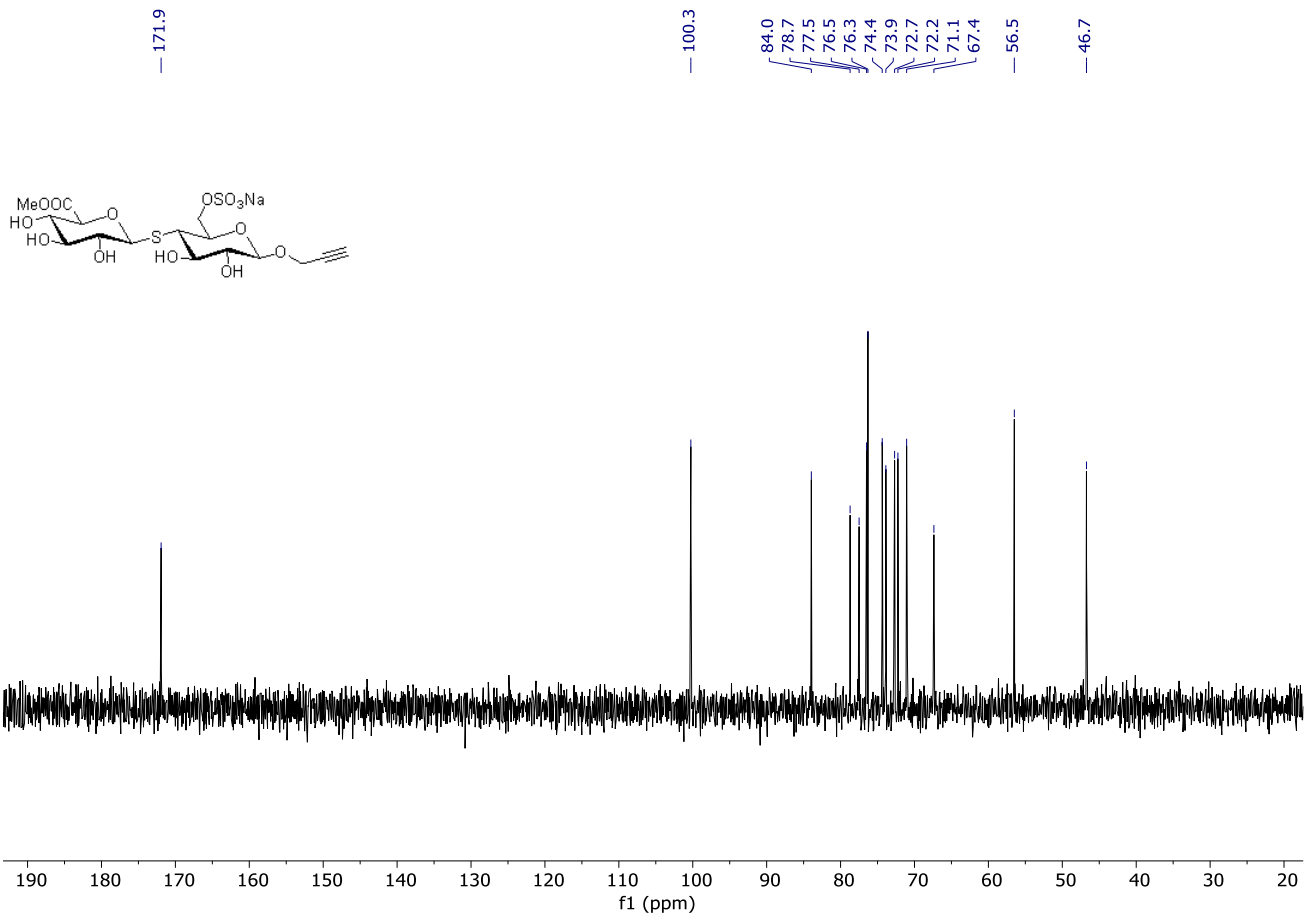


^{1}H (top) and ^{13}C (bottom) NMR spectra of **19**

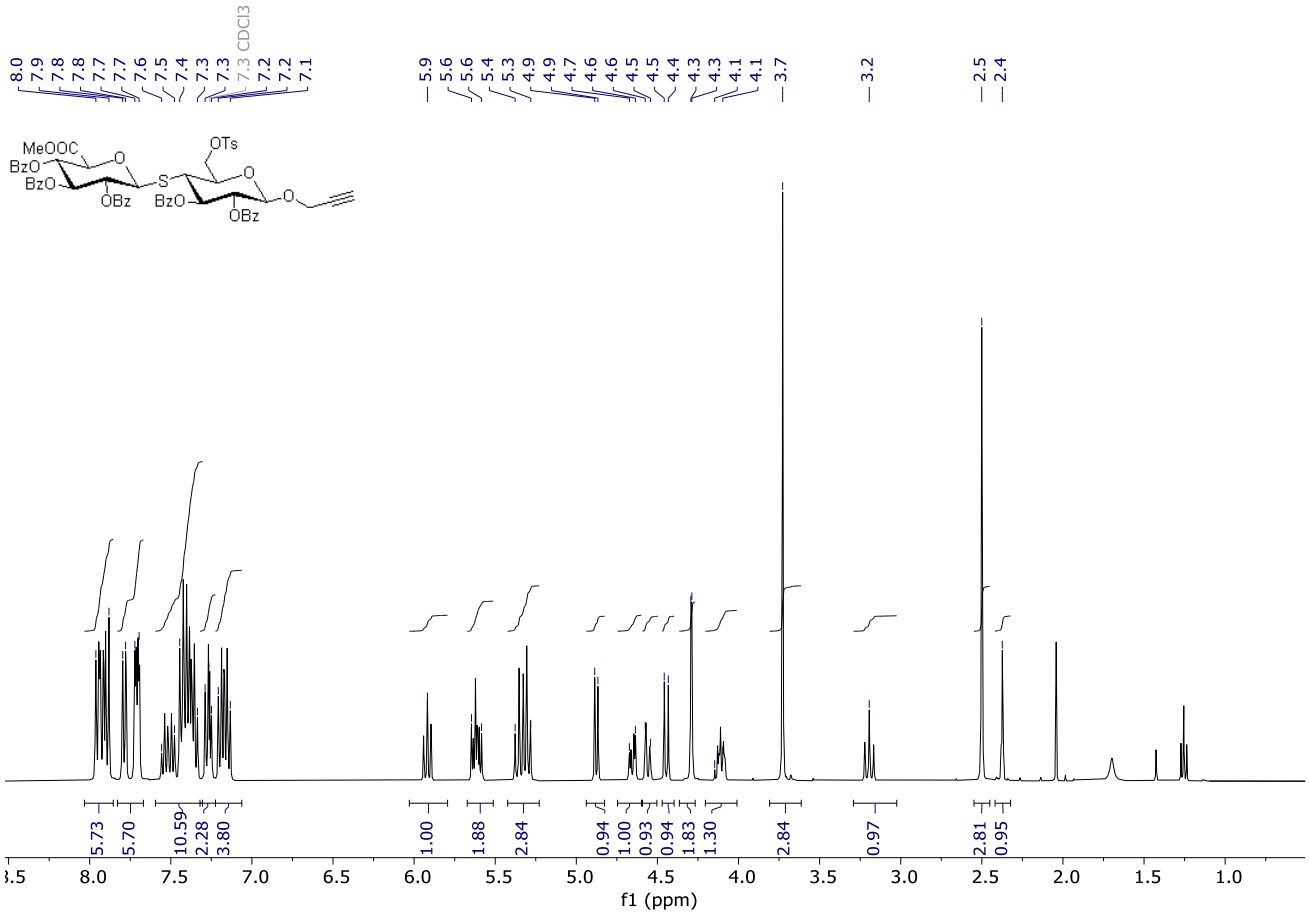


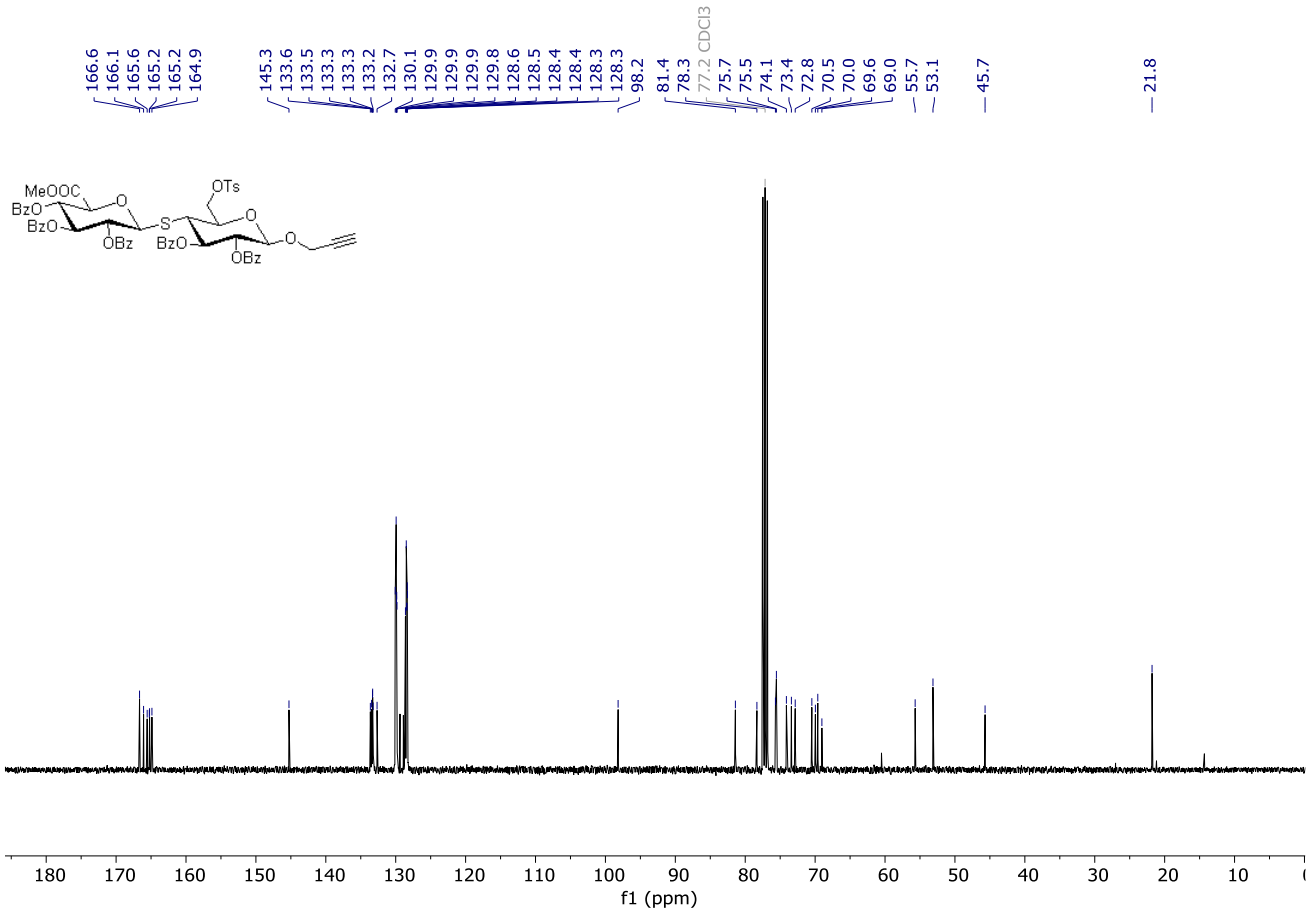
^{1}H (top) and ^{13}C (bottom) NMR spectra of **20**



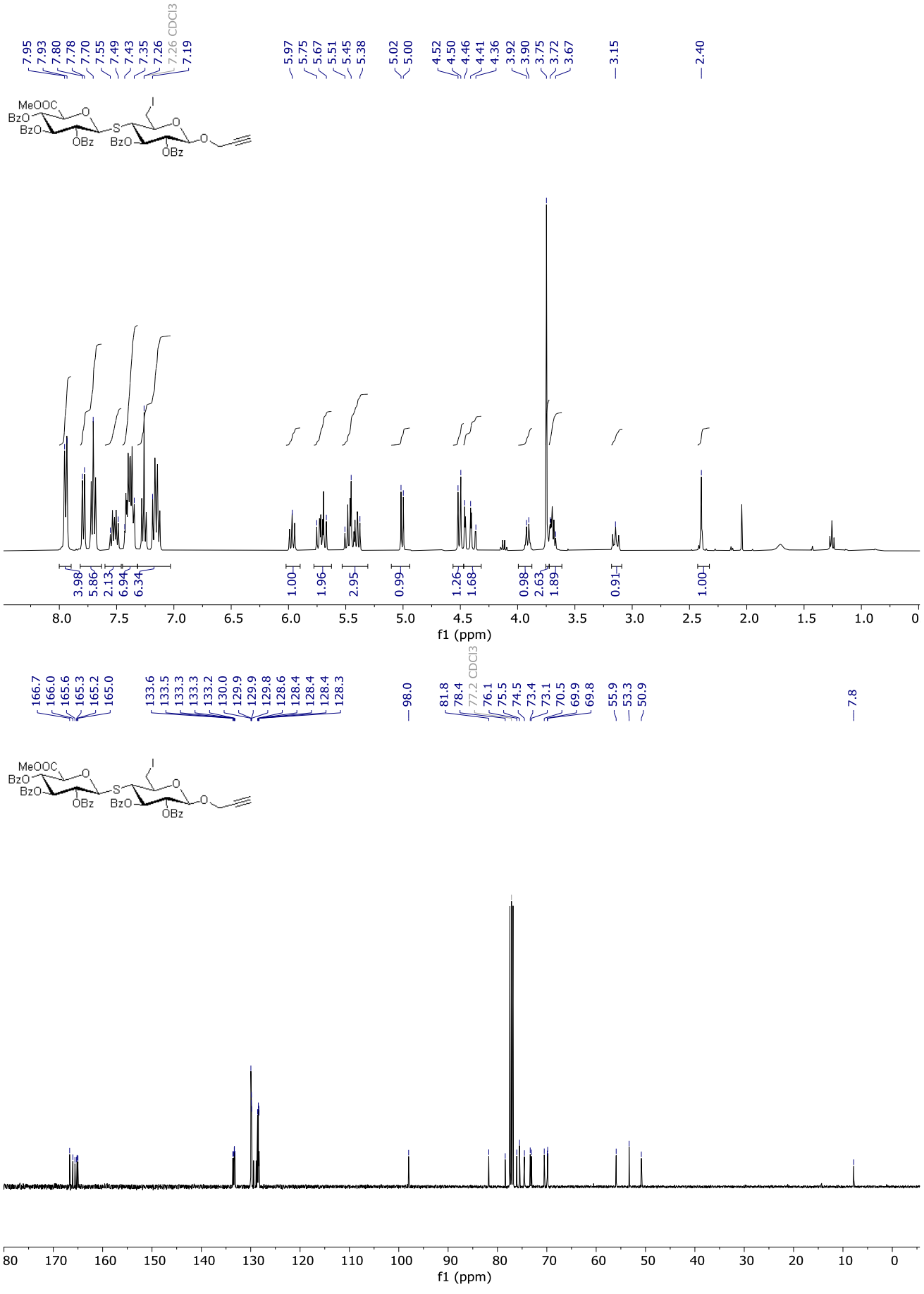


^{1}H (top) and ^{13}C (bottom) NMR spectra of **21**

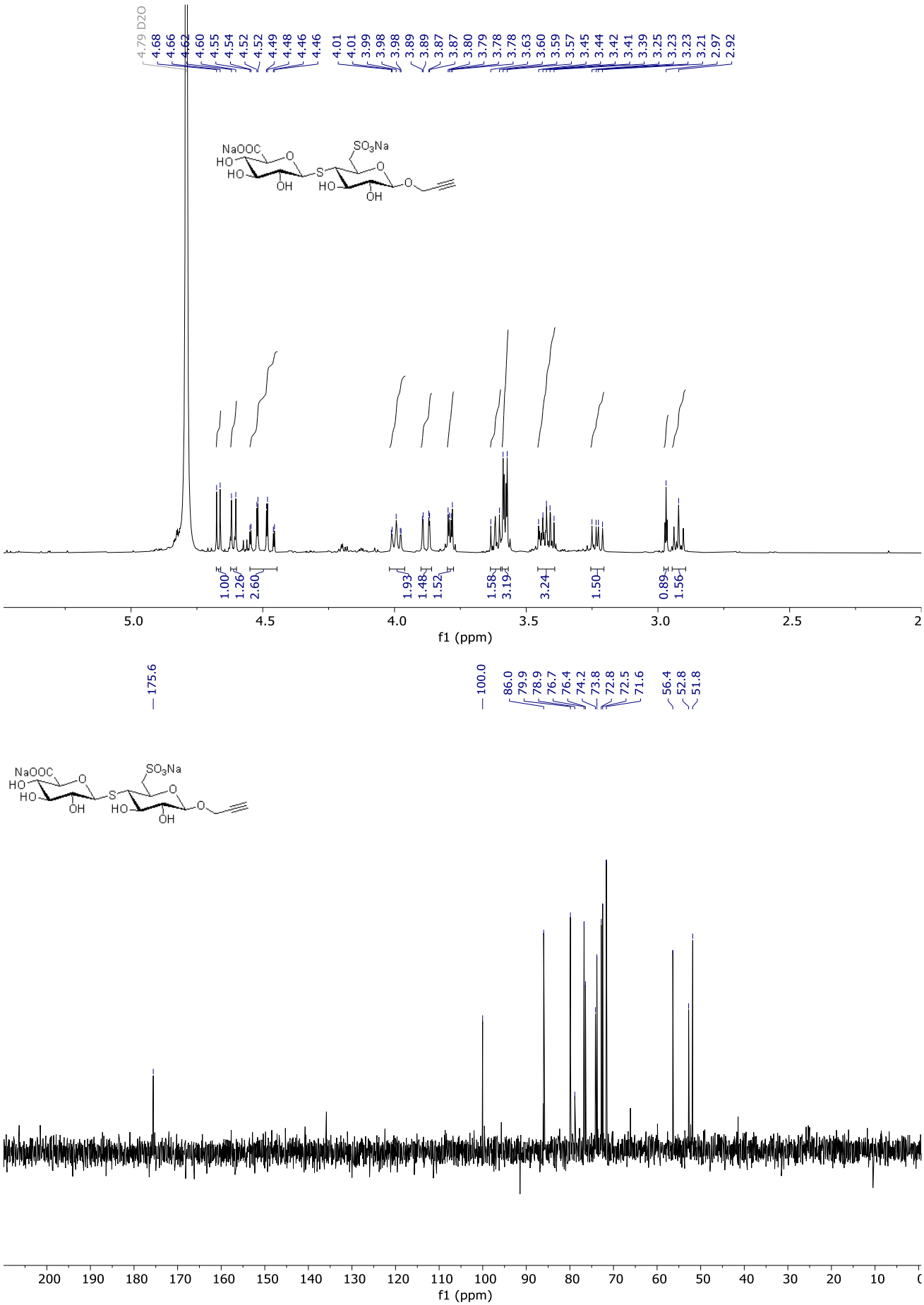




^{1}H (top) and ^{13}C (bottom) NMR spectra of **22**



^{1}H (top) and ^{13}C (bottom) NMR spectra of **23**



^{1}H (top) and ^{13}C (bottom) NMR spectra of **24**

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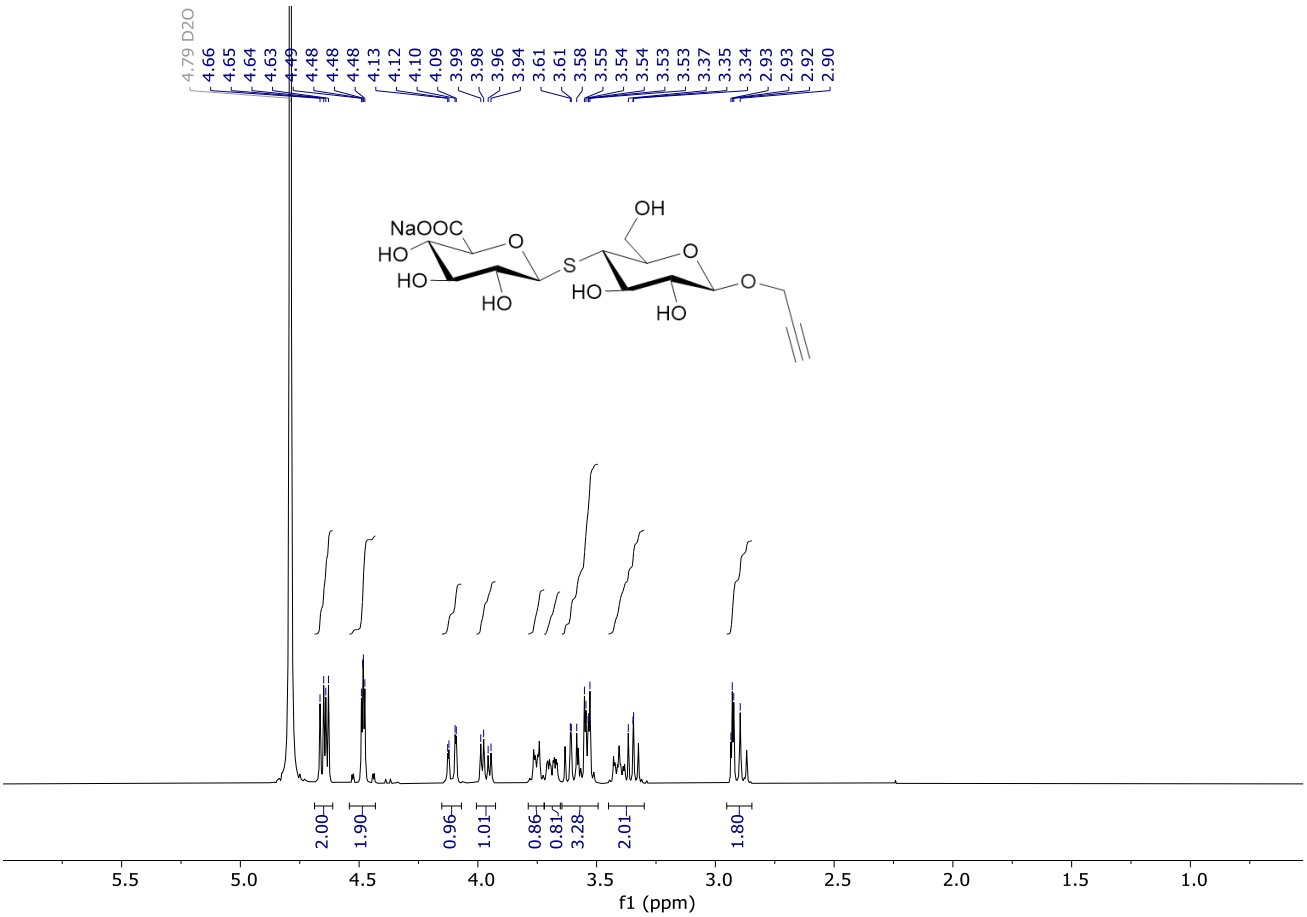
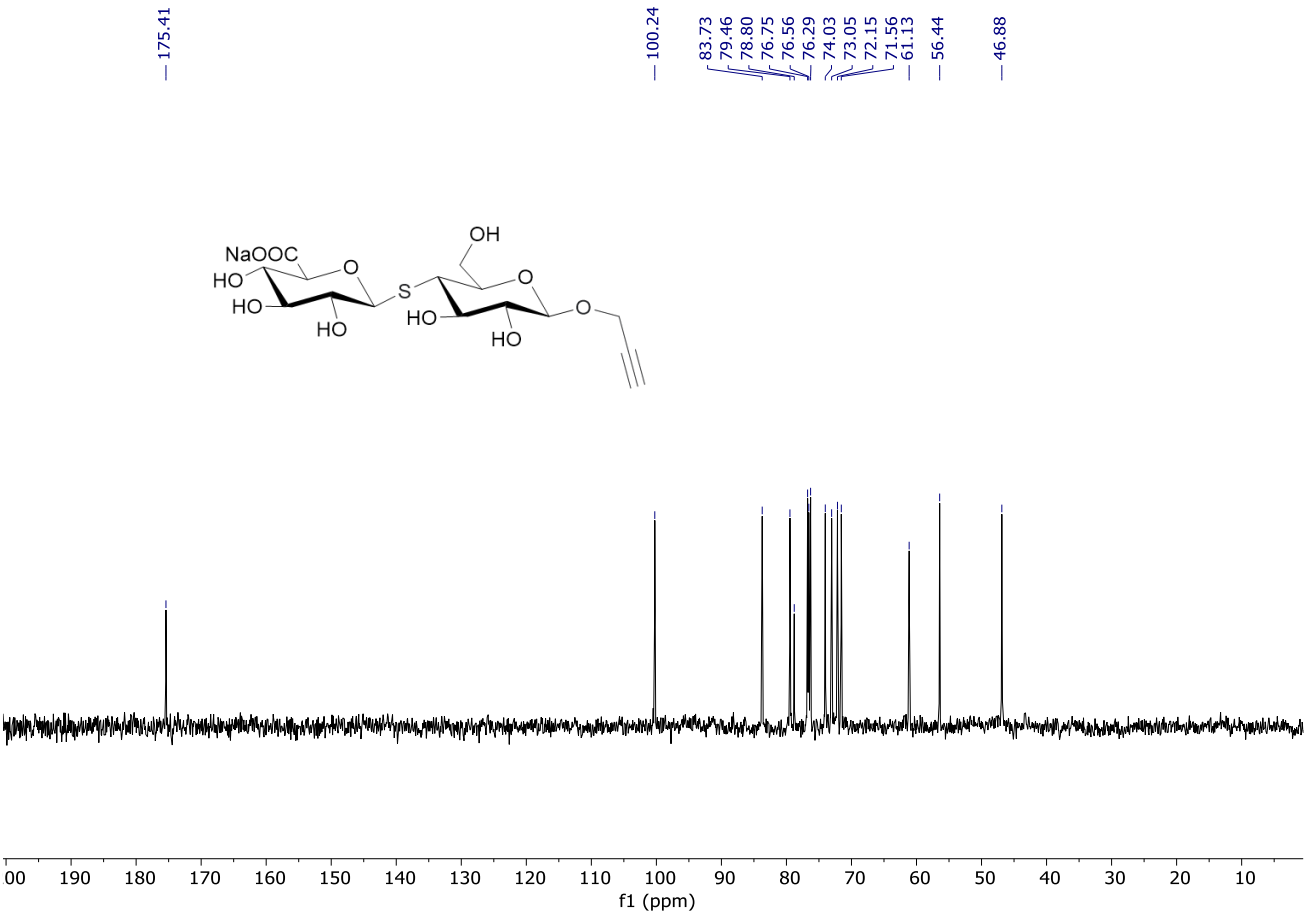
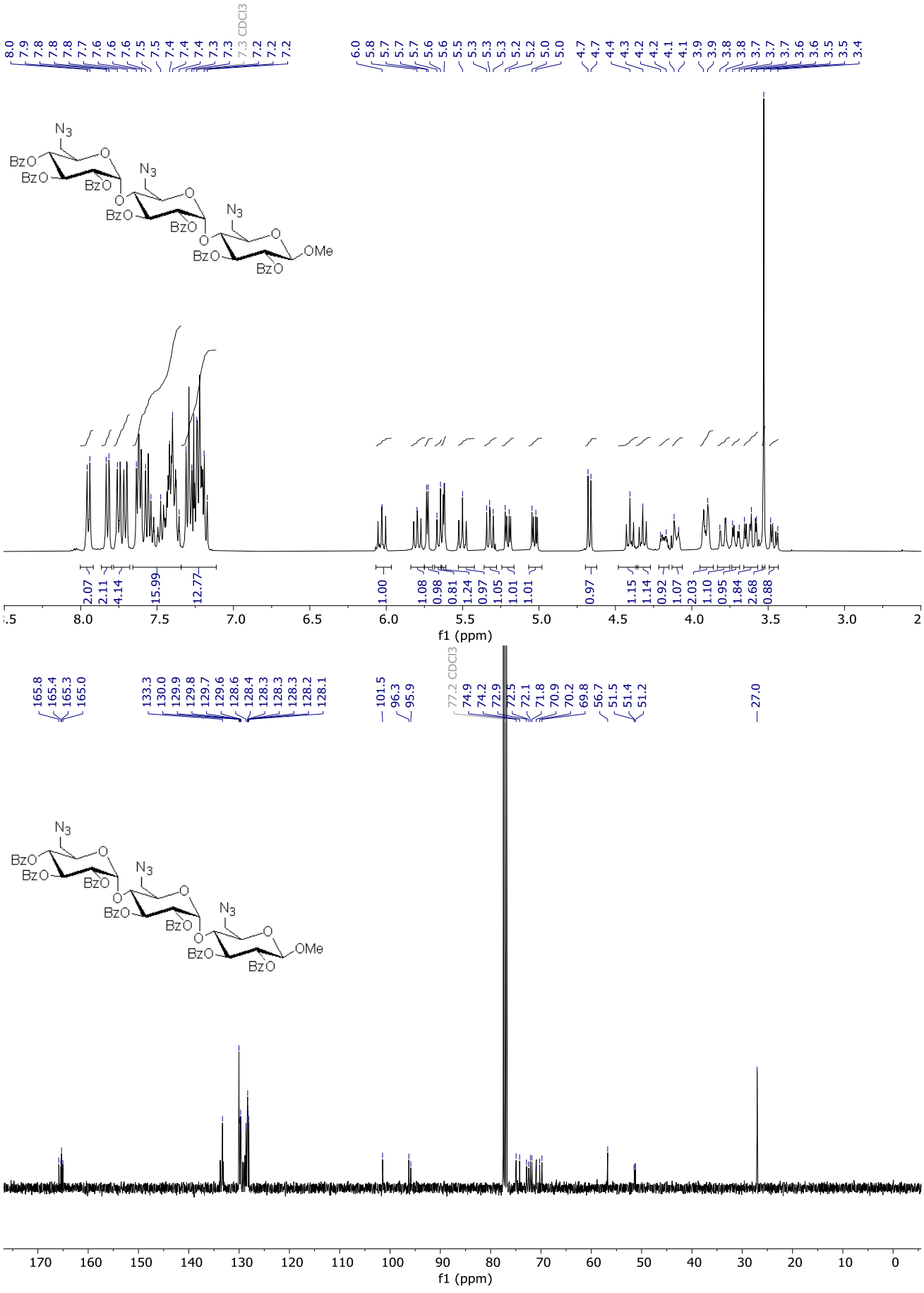
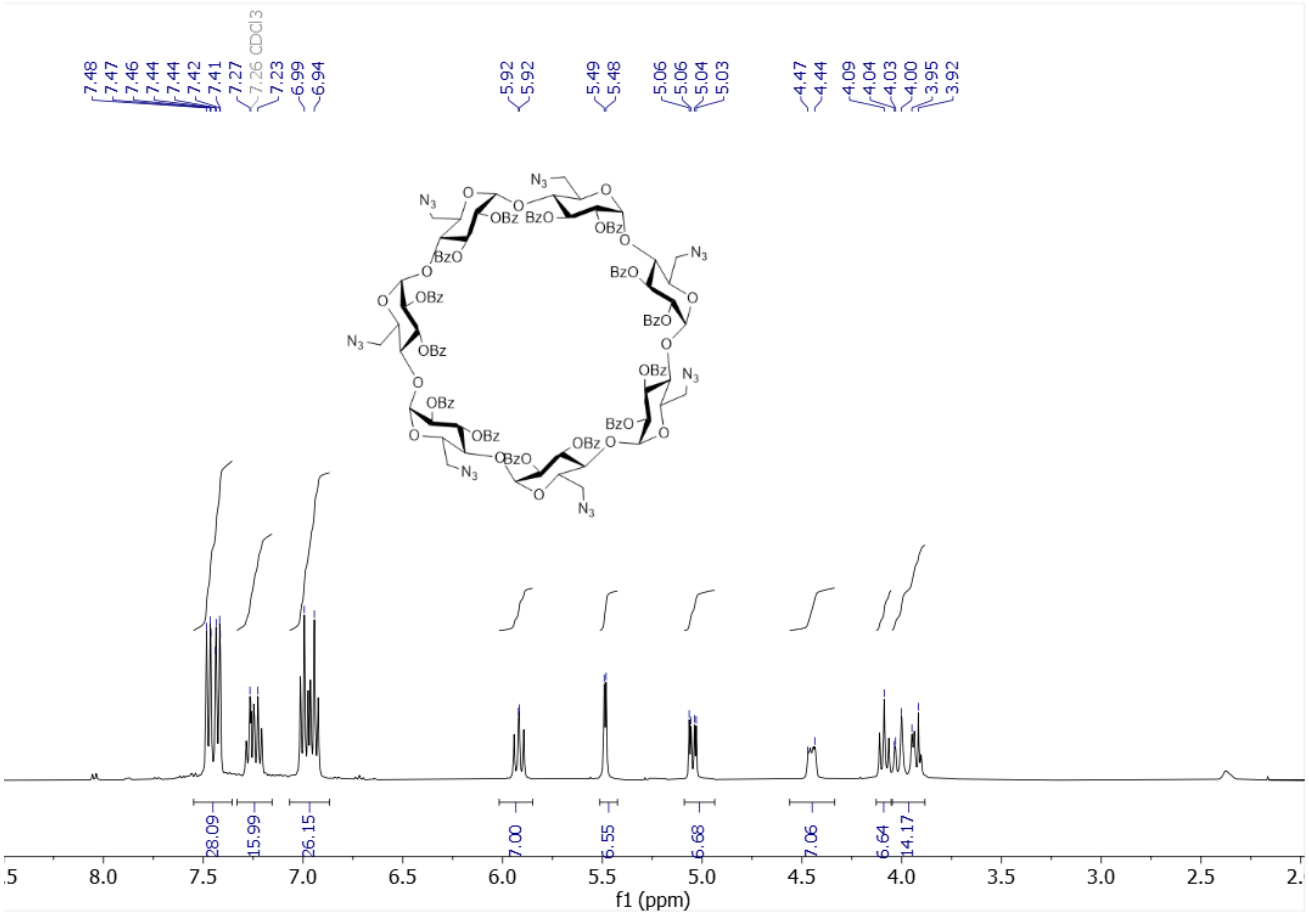


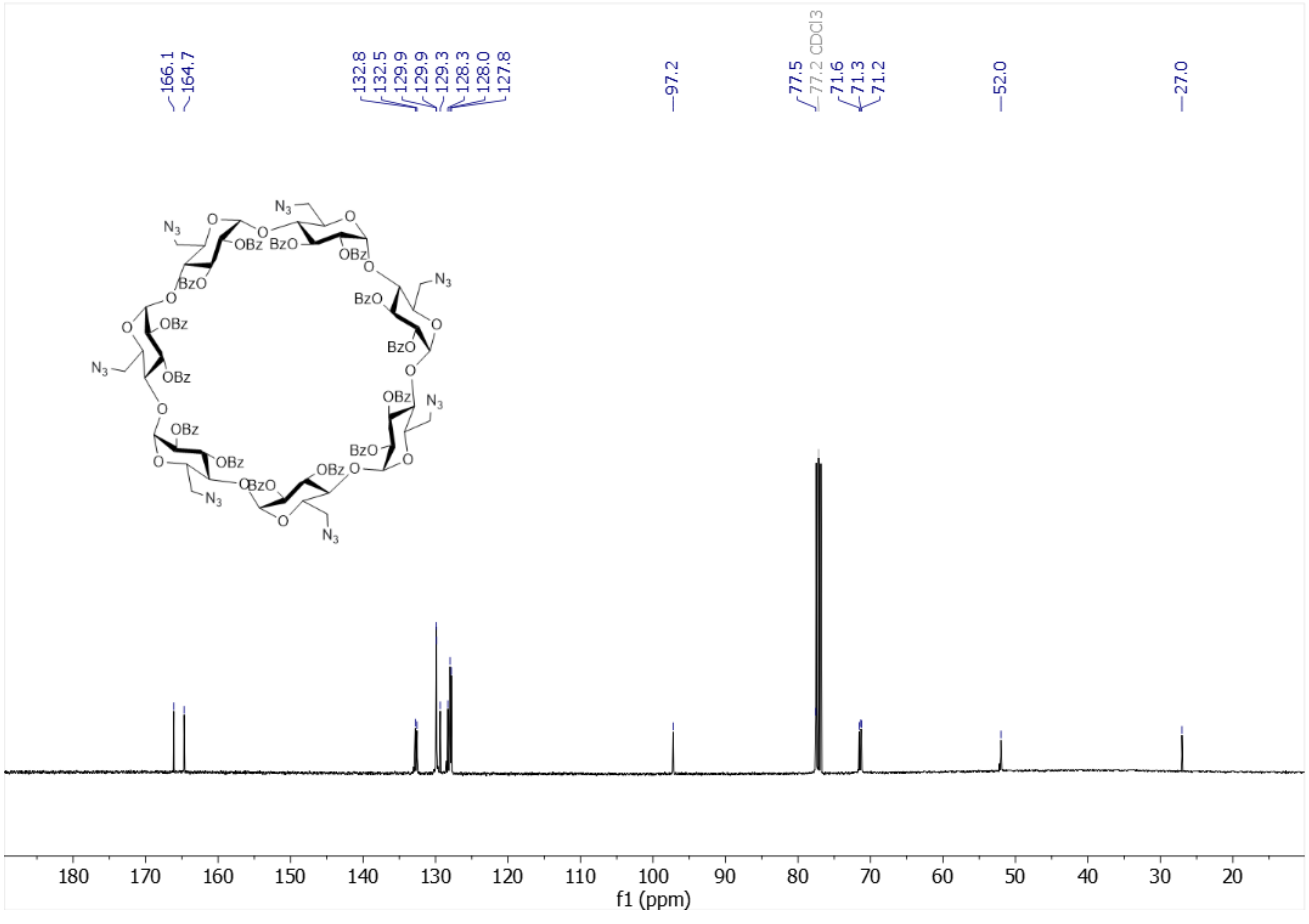


^{1}H (top) and ^{13}C (bottom) NMR spectra of **26**

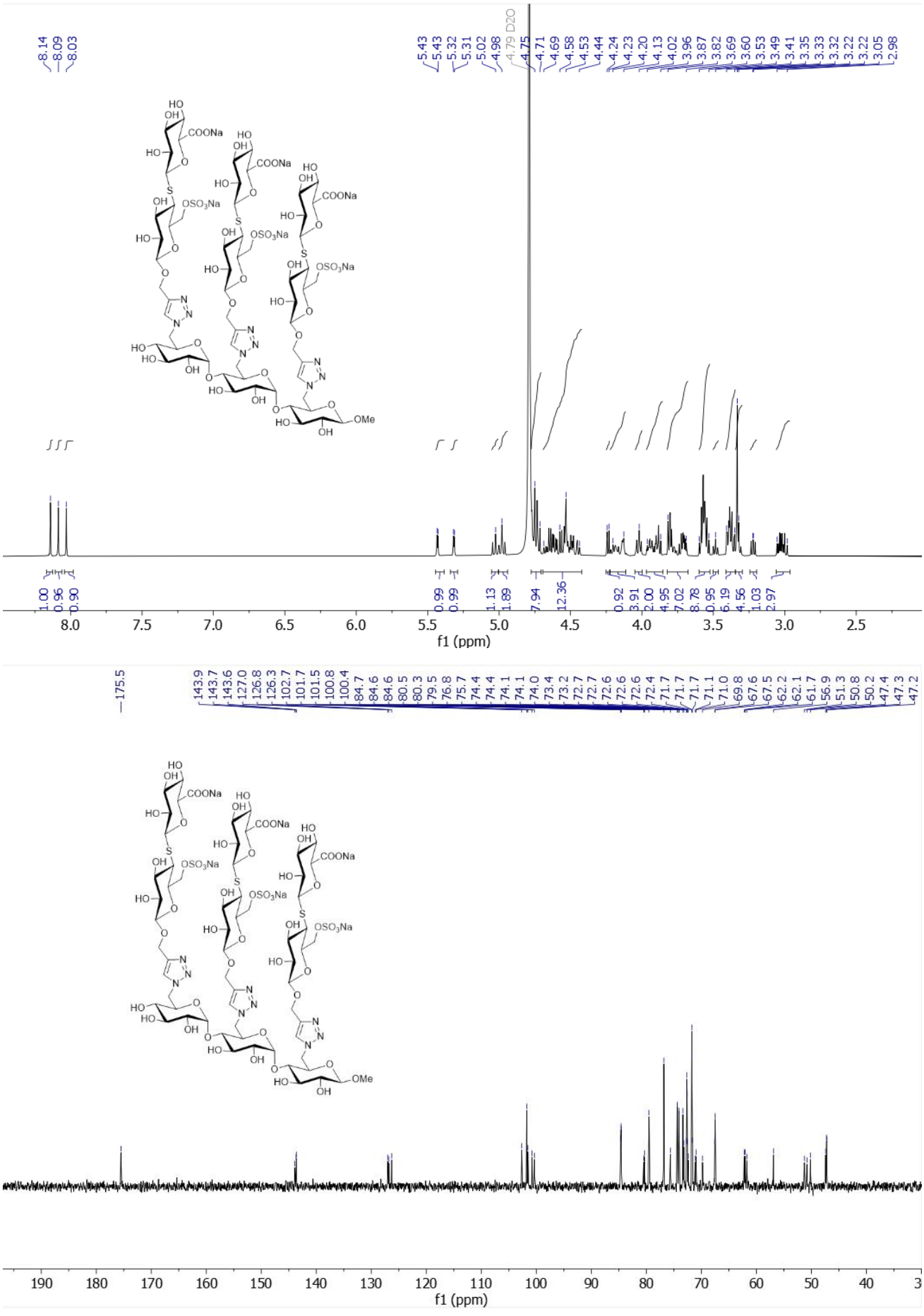


^{1}H (top) and ^{13}C (bottom) NMR spectra of **28**

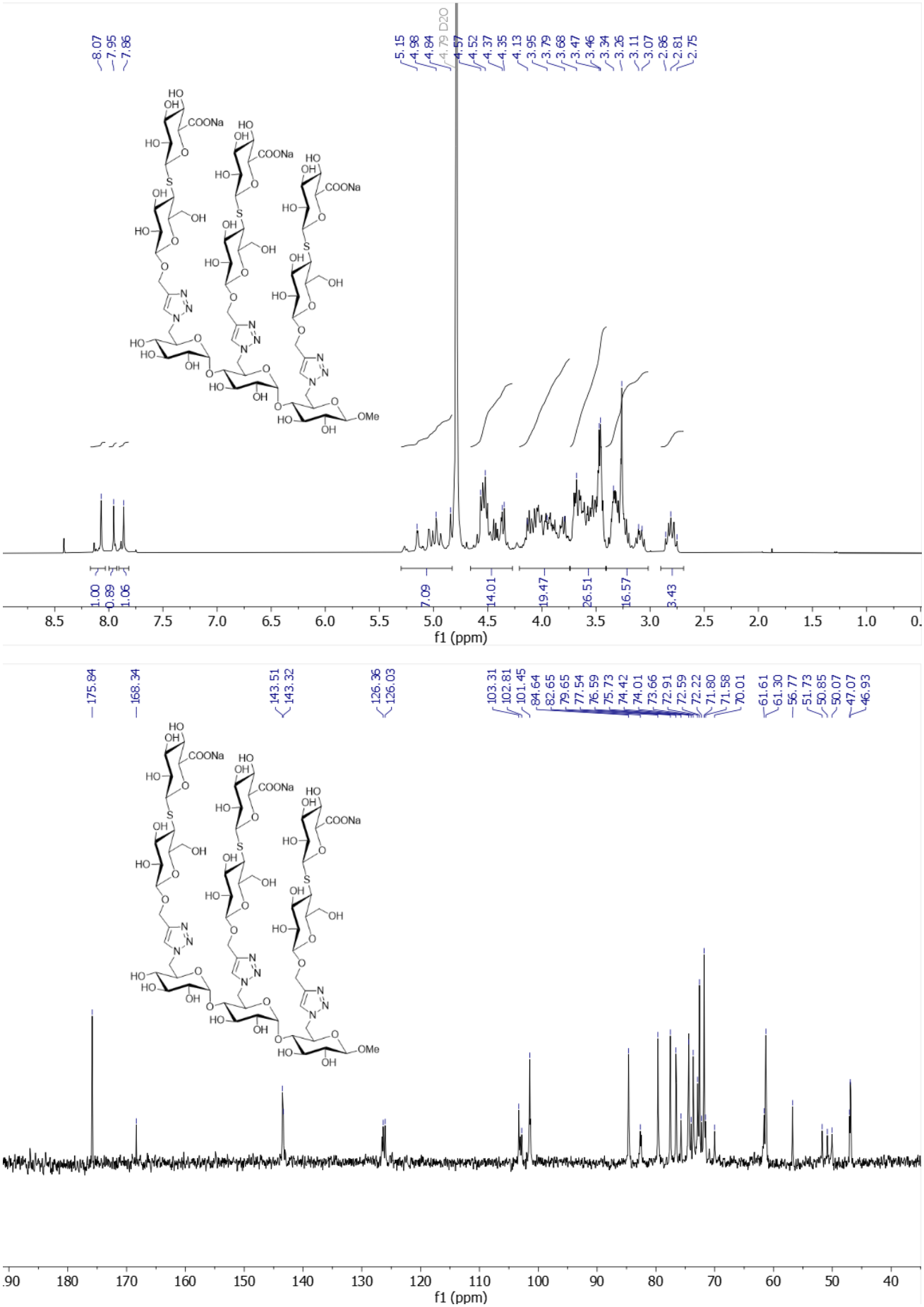




^{1}H (top) and ^{13}C (bottom) NMR spectra of **29**



^{1}H (top) and ^{13}C (bottom) NMR spectra of **30**



^{1}H (top) and ^{13}C (bottom) NMR spectra of **31**