Supporting Information

Protecting-group-free *S*-glycosylation towards thioglycosides and thioglycopeptides in water

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1. General methods

All chemical reagents were purchased from Sigma-Aldrich or Acros and were used without further purification. Solvents, for example, methanol (MeOH), *N*,*N*-dimethyl formamide (DMF), acetone and pyridine were dried according to standard procedures prior to use. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 plates (Merck) using *p*-anisaldehyde sugar staining solution. Flash chromatography was carried out with Biotage. All protected amino acids and Wang resin was obtained from Novabiochem. High performance liquid chromatography (HPLC) was performed on a Shimadzu UFLC equipped with a SPD-20A UV detector. Analyses were carried out at C18 column (3.6 μm PEPTIDE XB-C18 100 Å, LC Column 250 × 4.6 mm, Merck, Darmstadt, Germany) with UV monitoring at 220 nm using a linear A–B gradient (Solvent A: 0.1% CF_3CO_2H in water; Solvent B: 0.1% CF_3CO_2H in acetonitrile).

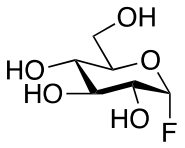
^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR, ^{1}H coupled ^{13}C NMR, DEPT-135 NMR and ^{1}H-^{13}C HSQC spectra were recorded on a Bruker AVANCE 400 MHz NMR or 600 MHz NMR spectrometer at room temperature or 40 ^{o}C in $CDCl_3$, CD_3OD, or D_2O. All chemical shifts in ^{1}H NMR were assigned in reference to $CDCl_3$ with TMS (δ = 0 ppm) as internal standard, CD_3OD (δ = 3.31 ppm) or D_2O (δ = 4.79 ppm). ^{13}C NMR spectra were reported relative to the signal of $CDCl_3$ (δ = 77.26 ppm) and CD_3OD (δ = 49.00 ppm). Coupling constants (*J*) are reported in hertz (Hz). Splitting patterns are described using the following abbreviations: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, a doublet of doublets of doublets; m, multiplet. High resolution electrospray ionization (ESI) mass spectra were obtained using Thermo LTQ-Orbitrap Elite. High resolution MALDI mass spectra were recorded on Bruker ultraflextreme MALDI-TOF/TOF mass spectrometer using 2,5-dihydroxybenzoic acid (DHB) matrix.

2. Synthetic procedures

All the glycopyranosyl fluoride donors and thiol monosaccharide acceptors are known compounds. They were synthesized according to the reported procedure.

2.1. Synthesis of fluoride donors[1]

Fluoride α-D-glucopyranoside 1

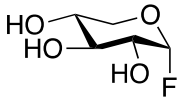


1

Peracetyl glucose (5 g, 12.8 mmol, 1 equiv.) was added to a polytetrafluoroethylene (PTFE) bottle in an ice-water bath and then a cold solution of 70% HF·pyridine (8 mL, 64.1 mmol, 5 equiv.) was added to the solid while stirring at 0 ^{o}C. This suspension was warmed to r.t. and stirred overnight. Then the reaction mixture was diluted with ice-cold water followed by CH_2Cl_2, and stirred for 5 min. The aqueous phase was extracted with CH_2Cl_2, and the combined organic layers were washed with sat. aq. $NaHCO_3$ followed by brine washing, dried over Na_2SO_4, filtered, evaporated to dryness and directly used for the next step.

Then the crude peracetylated glucosyl fluoride was dissolved in MeOH (50 mL) and cooled to 0 ^{o}C. NaOMe (138 mg, 2.56 mmol, 0.2 equiv.) was added and stirred at r.t. for 2 h. The mixture was neutralized with Amberlite IR120 (H^+) resin, filtered and concentrated *in vacuo*. Finally, the residue was purified by the column chromatography (10% MeOH in EtOAc) to obtain the product **1** as an amorphous solid (2.19 g, 94% over two steps). ^{1}H NMR (400 MHz, D_2O) δ 5.68 (dd, *J* = 53.5, 2.8 Hz, 1H, H-1), 3.88-3.70 (m, 4H), 3.65-3.47 (m, 2H); ^{19}F NMR (376 MHz, D_2O) δ –150.34 ppm; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_6H_{11}FNaO_5^+$: 205.0483, found: 205.0481.

Fluoride α-D-xylopyranoside 8

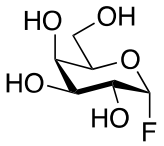


8

Peracetyl xylose (9.5 g, 29.8 mmol, 1 equiv.) was added to a polytetrafluoroethylene (PTFE) bottle in an ice-water bath and then a cold solution of 70% HF·pyridine (19 mL, 149 mmol, 5 equiv.) was added to the solid while stirring at 0 ^{o}C. This suspension was warmed to r.t. and stirred overnight. Then the reaction mixture was diluted with ice-cold water followed by CH_2Cl_2, and stirred for 5 min. The aqueous phase was extracted with CH_2Cl_2, and the combined organic layers were

washed with sat. aq. $NaHCO_3$ followed by brine washing, dried over Na_2SO_4, filtered, evaporated to dryness and directly used for the next step.
Then the crude peracetylated xylosyl fluoride was dissolved in MeOH (100 mL) and cooled to 0 ^oC. NaOMe (302 mg, 5.6 mmol, 0.2 equiv.) was added and stirred at r.t. for 2 h. The mixture was neutralized with Amberlite IR120 (H^+) resin, filtered and concentrated *in vacuo*. Finally, the residue was purified by the column chromatography (10% MeOH in EtOAc) to obtain the product **8** as an amorphous solid (4.17 g, 92% over two steps). ^1H NMR (400 MHz, D_2O) δ 5.62 (dd, *J* = 53.3, 2.6 Hz, 1H, H-1), 3.87-3.81 (m, 1H), 3.73-3.50 (m, 4H); ^{13}C NMR (101 MHz, D_2O) δ 108.58 (C-1), 106.37 (C-1), 72.61, 71.19, 70.94, 68.32, 63.19, 63.15; HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_5H_9FNaO_4^+$: 175.0377, found: 175.0375.

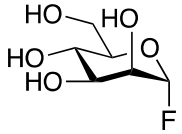
Fluoride α-D-galactopyranoside 13



13

Peracetyl galactose (4.0 g, 10.2 mmol, 1 equiv.) was added to a polytetrafluoroethylene (PTFE) bottle in an ice-water bath and then a cold solution of 70% HF·pyridine (6.6 mL, 51.0 mmol, 5 equiv.) was added to the solid while stirring at 0 ^oC. This suspension was warmed to r.t. and stirred overnight. Then the reaction mixture was diluted with ice-cold water followed by CH_2Cl_2, and stirred for 5 min. The aqueous phase was extracted with CH_2Cl_2, and the combined organic layers were washed with sat. aq. $NaHCO_3$ followed by brine washing, dried over Na_2SO_4, filtered, and evaporated to dryness and directly used for the next step.
Then the crude peracetylated galactosyl fluoride was dissolved in MeOH and cooled to 0 ^oC. NaOMe (110 mg, 2.04 mmol, 0.2 equiv.) was added and stirred at r.t. for 2 h. The mixture was neutralized with Amberlite IR120 (H^+) resin, filtered and concentrated *in vacuo*. Finally, the residue was purified by the column chromatography (10% MeOH in EtOAc) to obtain the product **13** as an amorphous solid (1.50 g, 81% over two steps). ^1H NMR (400 MHz, D_2O) δ 5.71 (dd, *J* = 53.7, 2.6 Hz, 1H, H-1), 4.09 (dd, *J* = 7.2, 5.2 Hz, 1H), 4.04-4.03 (m, 1H), 3.92-3.72 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 110.38 (C-1), 108.16 (C-1), 74.84, 74.81, 70.93, 70.53, 69.94, 69.70, 62.44; ^{19}F NMR (376 MHz, CD_3OD) δ -153.74.

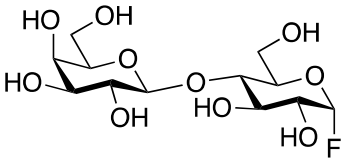
Fluoride α-D-mannopyranoside 16



16

Peracetyl mannose (3.9 g, 10.0 mmol, 1 equiv.) was added to a polytetrafluoroethylene (PTFE) bottle in an ice-water bath and then a cold solution of 70% HF·pyridine (7 mL, 50.0 mmol, 5 equiv.) was added to the solid while stirring at 0 °C. This suspension was warmed to r.t. and stirred overnight. Then the reaction mixture was diluted with ice-cold water followed by CH_2Cl_2, and stirred for 5 min. The aqueous phase was extracted with CH_2Cl_2, and the combined organic layers were washed with sat. aq. $NaHCO_3$ followed by brine washing, dried over Na_2SO_4, filtered, and evaporated to dryness and directly used for the next step.
Then the crude peracetylated mannosyl fluoride was dissolved in MeOH and cooled to 0 °C. NaOMe (108 mg, 2.0 mmol, 0.2 equiv.) was added and stirred at r.t. for 2 h. The mixture was neutralized with Amberlite IR120 (H^+) resin, filtered and concentrated *in vacuo*. Finally, the residue was purified by the column chromatography (10% MeOH in EtOAc) to obtain the product **16** as an amorphous solid (1.45 g, 80% over two steps). NMR spectral data are consistent with the literature.[1]

Fluoride α-D-lactopyranoside 18

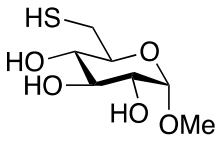


18

Peracetyl lactose (5.0 g, 7.4 mmol, 1 equiv.) was added to a polytetrafluoroethylene (PTFE) bottle in an ice-water bath and then a cold solution of 70% HF·pyridine (4.7 mL, 36.8 mmol, 5 equiv.) was added to the solid while stirring at 0 °C. This suspension was warmed to r.t. and stirred overnight. Then the reaction mixture was diluted with ice-cold water followed by CH_2Cl_2, and stirred for 5 min. The aqueous phase was extracted with CH_2Cl_2, and the combined organic layers were washed with sat. aq. $NaHCO_3$ followed by brine washing, dried over Na_2SO_4, filtered, evaporated to dryness and directly used for the next step.
Then the crude peracetylated lactosyl fluoride was dissolved in MeOH and cooled to 0 °C. NaOMe (80 mg, 1.48 mmol, 0.2 equiv.) was added and stirred at r.t. for 2 h. The mixture was neutralized with Amberlite IR120 (H^+) resin, filtered and concentrated *in vacuo*. Finally, the residue was purified by the column chromatography (10% MeOH in EtOAc) to obtain the product **18** as an amorphous solid (2.24 g, 88% over two steps). ^{1}H NMR (400 MHz, D_2O) δ 5.67 (dd, *J* = 53.5, 2.8 Hz, 1H, H-1), 4.44 (d, *J* = 7.8 Hz, 1H, H-1'), 3.96-3.60 (m, 11H), 3.53 (dd, *J* = 10.0, 7.7 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 108.09 (C-1), 105.87 (C-1), 102.80 (C-1'), 76.96, 75.32, 72.82, 72.79, 72.45, 71.03, 70.89, 70.84, 70.60, 68.51, 61.03, 59.40; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{12}H_{22}FO_{10}^+$: 345.1192, found: 345.1179.

2.2. Synthesis of 6-thiol acceptors[2]

Methyl α-D-6-SH-glucopyranoside 2



2

To the flask containing free glucose (5.0 g, 27.8 mmol), MeOH (100 mL) was added followed by the addition of Amberlite IR120 (H^+) resin (8 g). The reaction mixture was stirred under refluxing (65 °C) for 24 h, then cooled to room temperature, and filtered to remove the resin. The filtrate was evaporated to dryness and directly used for the next step.

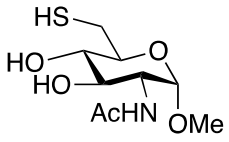
To a stirred solution of the crude mixture in pyridine (50 mL) was added TosCl (5.83 g, 30.6 mmol, 1.1 equiv.) slowly at 0 °C. The reaction was stirred at 0 °C for 8 h. After the consumption of the starting material monitored by TLC, the reaction mixture was evaporated to remove pyridine, diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The combined organic phase was washed with brine, dried over Na_2SO_4, concentrated under reduced pressure and directly used for the next step.

To the solution of the crude compound in pyridine (100 mL) was added acetic anhydride (40 mL, 0.42 mol, 15 equiv) and DMAP (170 mg, 1.39 mmol, 0.05 euqiv.). The reaction mixture was stirred at r.t. overnight. Then most of the pyridine and acetic acid were removed by evaporation. The crude product was diluted with EtOAc and washed with 1N HCl and sat. aq. $NaHCO_3$ sequentially. The combined organic phase was washed with brine, dried over Na_2SO_4, concentrated under reduced pressure and directly used for the next step.

To the crude compound in dry DMF (60 mL) was added potassium thioacetate (9.5 g, 83.4 mmol, 3 equiv.) was added in the solution. The reaction mixture was stirred at 70 °C for 24 h. Then the reaction mixture was diluted with EtOAc and washed with water and the combined organic phase was washed with brine, dried with Na_2SO_4, concentrated under reduced pressure and directly used for the next step.

To the solution of the crude compound in MeOH (50 mL) was added NaOMe (600 mg, 11.12 mmol, 0.4 equiv.). The reaction mixture was stirred at room temperature for 2 h and monitored with TLC. After the consumption of the starting material, the Amberlite IR120 (H^+) resin was added to neutralize the solution and then filtered. The filtrate was concentrated and purified by flash column chromatography (10% MeOH in EtOAc) to give compound **2** as an amorphous solid (2.5 g, 43% for five steps). ^{1}H NMR (400 MHz, D_2O) δ 4.70 (s, 1H, H-1), 3.64-3.43 (m, 3H), 3.34 (s, 3H), 3.30 (t, *J* = 9.3 Hz, 1H), 2.90 (dd, *J* = 14.4, 2.7 Hz, 1H), 2.64 (dd, *J* = 14.4, 7.4 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 99.24 (C-1), 72.86, 71.70, 71.66, 71.24, 55.09, 25.04; HRMS (ESI): m/z $[M + NH_4]^+$ calcd for $C_7H_{18}NO_5S^+$: 228.0900, found: 228.0892.

Methyl α-D-2-acetamido-2-deoxy-6-SH-glucopyranoside 4



4

To the flask containing free *N*-acetylglucosamine (5.0 g, 22.6 mmol), MeOH (100 mL) was added followed by the addition of Amberlite IR120 (H^+) resin (8 g). The reaction mixture was stirred under refluxing (65 ^oC) for 24 h, then cooled to room temperature, and filtered to remove the resin. The filtrate was evaporated to dryness and directly used for the next step.

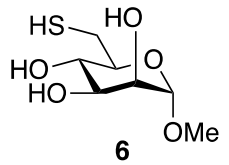
To a stirred solution of the crude mixture in pyridine was added TosCl (4.74 g, 24.9 mmol, 1.1 equiv.) slowly at 0 ^oC. The reaction was stirred at 0 ^oC for 10 h. After the consumption of the starting material monitored by TLC, the reaction mixture was evaporated to remove pyridine, diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The combined organic phase was washed with brine, dried over Na_2SO_4, concentrated under reduced pressure and directly used for the next step.

To the solution of the crude compound in pyridine (80 mL) was added acetic anhydride (32 mL, 0.34 mol, 15 equiv) and DMAP (138 mg, 1.13 mmol, 0.05 euqiv.). The reaction mixture was stirred at r.t. overnight. Then most of the pyridine and acetic acid were removed by evaporation. The crude product was diluted with EtOAc and washed with 1N HCl and sat. aq. $NaHCO_3$ sequentially. The combined organic phase was washed with brine, dried over Na_2SO_4, concentrated under reduced pressure and directly used for the next step.

To the crude compound in dry DMF (60 mL) was added potassium thioacetate (7.74 g, 67.8 mmol, 3 equiv.) was added in the solution. The reaction mixture was stirred at 70 ^oC for 24 h. Then the reaction mixture was diluted with EtOAc and washed with water and the combined organic phase was washed with brine, dried with Na_2SO_4, concentrated under reduced pressure and directly used for the next step.

To the solution of the crude compound in MeOH (40 mL) was added NaOMe (488 mg, 9.04 mmol, 0.4 equiv.). The reaction mixture was stirred at room temperature for 2 h and monitored with TLC. After the consumption of the starting material, the Amberlite IR120 (H^+) resin was added to neutralize the solution and then filtered. The filtrate was concentrated and purified by flash column chromatography (10% MeOH in EtOAc) to give compound **4** as an amorphous solid (2.2 g, 39% for five steps). ^1H NMR (400 MHz, D_2O) δ 4.74 (d, *J* = 3.7 Hz, 1H, H-1), 3.93 (dd, *J* = 10.6, 3.7 Hz, 1H), 3.73-3.66 (m, 2H), 3.47 (t, *J* = 9.4 Hz, 1H), 3.40 (s, 3H), 3.00 (dd, *J* = 14.4, 2.6 Hz, 1H), 2.75 (dd, *J* = 14.3, 7.3 Hz, 1H), 2.02 (s, 3H); ^{13}C NMR (101 MHz, D_2O) δ 174.40, 98.03 (C-1), 72.15, 71.77, 70.97, 55.18, 53.61, 25.05, 21.83; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_9H_{18}NO_5S^+$: 252.0900, found: 252.0893.

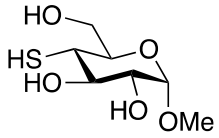
Methyl α-D-6-SH-mannopyranoside 6



6

To the flask containing free mannose (5.0 g, 27.8 mmol), MeOH (100 mL) was added followed by the addition of Amberlite IR120 (H^+) resin (8 g). The reaction mixture was stirred under refluxing (65 ^oC) for 24 h, then cooled to room temperature, and filtered to remove the resin. The filtrate was evaporated to dryness and directly used for the next step.
To a stirred solution of the crude mixture in pyridine (50 mL) was added TosCl (5.83 g, 30.6 mmol, 1.1 equiv.) slowly at 0 ^oC. The reaction was stirred at 0 ^oC for 8 h. After the consumption of the starting material monitored by TLC, the reaction mixture was evaporated to remove pyridine, diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The combined organic phase was washed with brine, dried over Na_2SO_4, concentrated under reduced pressure and directly used for the next step.
To the solution of the crude compound in pyridine (100 mL) was added acetic anhydride (40 mL, 0.42 mol, 15 equiv) and DMAP (170 mg, 1.39 mmol, 0.05 euqiv.). The reaction mixture was stirred at r.t. overnight. Then most of the pyridine and acetic acid were removed by evaporation. The crude product was diluted with EtOAc and washed with 1N HCl and sat. aq. $NaHCO_3$ sequentially. The combined organic phase was washed with brine, dried over Na_2SO_4, concentrated under reduced pressure and directly used for the next step.
To the crude compound in dry DMF (60 mL) was added potassium thioacetate (9.5 g, 83.4 mmol, 3 equiv.) was added in the solution. The reaction mixture was stirred at 70 ^oC for 24 h. Then the reaction mixture was diluted with EtOAc and washed with water and the combined organic phase was washed with brine, dried with Na_2SO_4, concentrated under reduced pressure and directly used for the next step.
To the solution of the crude compound in MeOH (50 mL) was added NaOMe (600 mg, 11.12 mmol, 0.4 equiv.). The reaction mixture was stirred at room temperature for 2 h and monitored with TLC. After the consumption of the starting material, the Amberlite IR120 (H^+) resin was added to neutralize the solution and then filtered. The filtrate was concentrated and purified by flash column chromatography (10% MeOH in EtOAc) to give compound **6** as an amorphous solid (2.7 g, 47% for five steps). ^1H NMR (400 MHz, D_2O) δ 4.66 (d, *J* = 1.6 Hz, 1H, H-1), 3.84 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.65 (dd, *J* = 9.4, 3.3 Hz, 1H), 3.58-3.51 (m, 2H), 3.34 (s, 3H), 2.92 (dd, *J* = 14.2, 1.9 Hz, 1H), 2.67-2.62 (m, 1H); ^{13}C NMR (101 MHz, D_2O) δ 100.94 (C-1), 72.83, 70.36, 69.85, 69.01, 54.78, 25.14; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_7H_{14}NaO_5S^+$: 233.0454, found: 233.0444.

2.3. Synthesis of methyl 4-SH-α-D-glucopyranoside 11[3]



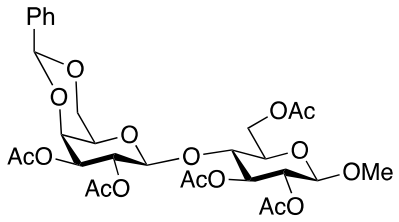
11

To a solution of methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (commerically available in Carbosynth, 1 g, 1.97 mmol, 1 equiv.) in pyridine (10 mL) at 0 ^oC was added Tf_2O (0.66 mL, 3.95 mmol, 2 equiv.) dropwise. The reaction mixture was allowed to warm gradually to r.t. with stirring for 3 h, then the mixture was diluted with CH_2Cl_2, washed with 1 M HCl, sat. aq. $NaHCO_3$ and

brine, dried over Na_2SO_4, filtered, evaporated and dried under reduced pressure to give a colorless foam. Then to this crude mixture in DMF (8 mL) was added KSAc (675 mg, 5.91 mmol, 3 equiv.). The mixture was stirred at r.t. for 5 h, then diluted with EtOAc, washed with H_2O, dried over Na_2SO_4, filtered, concentrated to dryness and directly used for the next step.
To the solution of the crude mixture in dry MeOH (15 mL) was added NaOMe (106 mg, 1.97 mmol, 1 equiv.). The reaction mixture was stirred at r.t. for 4 h, neutralized with Amberlite IR120 (H^+) ion exchange resin, filtered, washed with MeOH and concentrated. The residue was purified by flash chromatography (10% MeOH in EtOAc) to give compound **11** (294 mg, 71%) as a colorless foam. ^{1}H NMR (400 MHz, D_2O) δ 4.86 (d, *J* = 2.9 Hz, 1H, H-1), 3.93-3.92 (m, 2H), 3.72 (dt, *J* = 10.9, 3.5 Hz, 1H), 3.59-3.53 (m, 2H), 3.40 (s, 3H), 2.74-2.69 (m, 1H); ^{13}C NMR (101 MHz, D_2O) δ 99.43 (C-1), 73.73, 73.47, 72.11, 61.33, 55.01, 41.63; HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_7H_{14}NaO_5S^+$: 233.0454, found: 233.0446.

2.4. Synthesis of methyl 6'-SH-β-D-lactoside 21

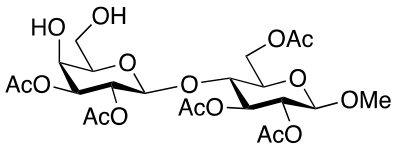
Methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside 29



29

To a solution of methyl β-D-lactoside[4] (0.5 g, 1.4 mmol, 1 equiv.) in anhydrous DMF (10 mL) was added benzaldehyde dimethyl acetal (0.42 g, 2.8 mmol, 2 equiv.) and CSA (camphorsulfonic acid, 65 mg, 0.28 mmol, 0.2 equiv.) at r.t. and stirred at 60 °C over 12 h. Then the reaction mixture was concentrated to dryness under reduced pressure and dissolved in anhydrous pyridine (10 mL). To the above reaction mixture, acetic anhydride (5 mL) was added at 0 °C and allowed to r.t. and stirred over 12 h. The reaction mixture was concentrated under reduced pressure and dissolved in EtOAc (100 mL). The organic layer was washed with 1 N HCl (100 mL), sat. aq. $NaHCO_3$ (100 mL) and water (100 mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified over silica gel using 50% EtOAc in hexane to obtain compound **29** (0.75 g, 82%) as a foamy solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48-7.36 (m, 5H), 5.47 (s, 1H, PhC*H*), 5.29-5.19 (m, 2H), 4.93-4.86 (m, 2H), 4.53 (dd, *J* = 11.9, 2.1 Hz, 1H), 4.48 (d, *J* = 7.9 Hz, 1H, H-1), 4.40 (d, *J* = 7.9 Hz, 1H, H-1), 4.33 (dd, *J* = 3.7, 1.0 Hz, 1H), 4.29 (dd, *J* = 12.5, 1.6 Hz, 1H), 4.16-4.10 (m, 1H), 4.04 (dd, *J* = 12.5, 1.8 Hz, 1H), 3.80 (t, *J* = 9.5 Hz, 1H), 3.61 (ddd, *J* = 10.0, 5.0, 2.1 Hz, 1H), 3.48 (s, 3H), 3.46 (t, *J* = 1.4 Hz, 1H), 2.12 (s, 3H), 2.05-2.03 (m, 12H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.86, 170.55, 170.34, 169.91, 169.04, 137.62, 129.37, 128.41, 126.66, 101.71 (PhC*H*), 101.49 (C-1), 101.21 (C-1), 76.18, 73.29, 73.00, 72.59, 72.25, 71.63, 69.17, 68.59, 66.60, 62.15, 57.19, 21.04, 21.00, 20.92, 20.83; HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{30}H_{38}NaO_{16}^+$: 677.2052, found: 677.1956.

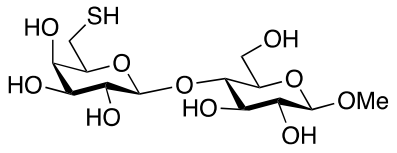
Methyl 2,3-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside 30



30

To a solution of compound **29** (0.5 g, 0.76 mmol, 1 equiv.) in anhydrous methanol (10 mL) was added PTSA·H_2O (p-toluenesulfonic acid, 29 mg, 0.15 mmol, 0.2 equiv.) and stirred at 30 °C until completion. The reaction mixture was quenched with triethyl amine (0.1 mL) and concentrated under reduced pressure. The crude was purified over silica gel using 70% EtOAc in hexane to obtain compound **30** (75%, 0.32 g) as a foamy solid. ^{1}H NMR (600 MHz, CDCl3) δ 5.22-5.17 (m, 2H), 4.91-4.86 (m, 2H), 4.53-4.49 (m, 2H, H-1), 4.41 (d, *J* = 7.9 Hz, 1H, H-1), 4.14-4.12 (m, 1H), 4.10 (dd, *J* = 11.9, 5.4 Hz, 1H), 3.89 (ddd, *J* = 9.5, 7.0, 4.0 Hz, 1H), 3.86-3.81 (m, 2H), 3.65 (ddd, *J* = 9.9, 5.3, 2.3 Hz, 1H), 3.59-3.56 (m, 1H), 3.48 (s, 3H), 3.33-3.30 (m, 1H), 2.80 (s, 1H), 2.12 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.05 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.88, 170.77, 170.48, 170.00, 169.75, 101.50 (C-1), 101.32 (C-1), 76.58, 74.64, 73.77, 73.48, 72.80, 71.82, 69.90, 67.88, 62.45, 62.19, 57.19, 21.13, 21.08, 21.02, 20.96, 20.89; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{23}H_{34}NaO_{16}^+$: 589.1739, found: 589.1697.

Methyl 6-thiol-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 21



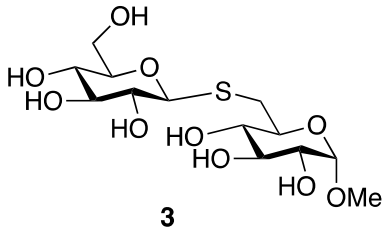
21

To a solution of compound **30** (0.3 g, 0.53 mmol, 1 equiv.) in anhydrous pyridine (10 mL) was added TosCl (0.2 g, 1.0 mmol, 2 equiv.) at 0 °C and stirred over 12 h at room temperature. The reaction mixture was concentrated under pressure to dryness and dissolved in anhydrous acetone (20 mL). KSAc (0.3 g, 2.65 mmol, 5 equiv.) was added and refluxed at 70 °C over 48 h. The reaction mixture was filtered over Celite 545 and concentrated under reduced pressure. The crude mixture was dissolved in EtOAc (100 mL). The organic layer was washed with 1 N HCl (100 mL), sat. aq. $NaHCO_3$ (100 mL) and water (100 mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude mixture was dried over 12 h under high vacuum and dissolved in anhydrous methanol (10 mL). Solid NaOMe was added slowly until the pH reached 10 and stirred over 12 h under inert atmosphere. The reaction mixture was neutralized with acidic resin and filtered over Celite 545. The filtrate was

concentrated and purified over C18 reverse phase column using 30% acetonitrile (0.1% TFA) in H_2O to obtain compound **21** (0.1 g, 52%) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ 4.48 (d, *J* = 7.9 Hz, 1H, H-1), 4.40 (d, *J* = 8.0 Hz, 1H, H-1), 4.02-3.98 (m, 2H), 3.96 (d, *J* = 3.4 Hz, 1H), 3.81 (dd, *J* = 12.5, 3.8 Hz, 1H), 3.71 (dd, *J* = 10.0, 3.4 Hz, 1H), 3.69-3.65 (m, 1H), 3.63-3.60 (m, 2H), 3.57 (s, 3H), 3.54 (dd, *J* = 10.0, 7.9 Hz, 1H), 3.36-3.31 (m, 1H), 3.02-3.01 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 103.30 (C-1), 103.26 (C-1), 79.57, 74.85, 74.60, 72.98, 72.69, 70.90, 69.87, 60.44, 57.39, 38.14; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{13}H_{24}NaO_{10}S^+$: 395.0982, found: 395.0965.

2.5. Synthesis of *S*-linked oligosaccharides

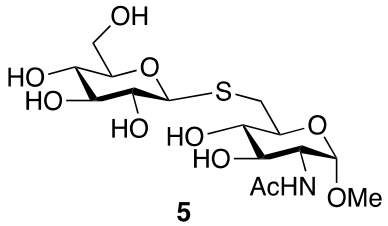
Methyl β-D-glucopyranosyl-(1→6)-*S*-6-thio-α-D-glucopyranoside 3



3

To the solution of α-D-glucopyranosyl fluoride **1** (99 mg, 0.54 mmol, 3 equiv.) and methyl-6-thiol-α-D-glucopyranoside **2** (38 mg, 0.18 mmol, 1 equiv.) in H_2O (0.5 mL) was added $Ca(OH)_2$ (40 mg, 0.54 mmol, 3 equiv.) and stirred at r.t. for 0.5 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (50% MeOH in H_2O) to yield **3** as the colorless foam (64 mg, 96%). ^{1}H NMR (400 MHz, D_2O) δ 4.78 (brs, 1H, H-1), 4.61 (d, *J* = 9.9 Hz, 1H, H-1'), 3.94-3.85 (m, 1H), 3.85-3.77 (m, 1H), 3.71 (dd, *J* = 12.4, 5.2 Hz, 1H), 3.65-3.55 (m, 2H), 3.52-3.27 (m, 8H), 3.21 (dd, *J* = 14.3, 2.4 Hz, 1H), 2.93 (dd, *J* = 14.2, 8.2 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 99.17 (C-1), 86.01 (C-1'), 79.75, 77.12, 72.89, 72.40, 71.48, 71.18, 69.40, 60.82, 55.22, 31.33; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{13}H_{24}NaO_{10}S^+$: 395.0982, found: 395.0964.

Methyl β-D-glucopyranosyl-(1→6)-*S*-6-thio-2-acetamido-2-deoxy-α-D-glucopyranoside 5

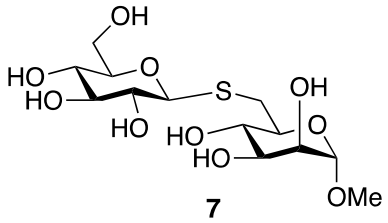


5

To the solution of α-D-glucopyranosyl fluoride **1** (65 mg, 0.36 mmol, 3 equiv.) and methyl-2-acetamido-2-deoxy-6-thiol-α-D-glucopyranoside **4** (30 mg, 0.12 mmol, 1 equiv.) in H_2O (0.3 mL) was added $Ca(OH)_2$ (27 mg, 0.36 mmol, 3 equiv.) and stirred at r.t. for 0.5 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (10% MeOH in H_2O) to yield **5** as the colorless foam (46 mg, 94%). ^{1}H NMR (400 MHz, D_2O) δ 4.72

(d, *J* = 3.7 Hz, 1H, H-1), 4.61 (d, *J* = 9.8 Hz, 1H, H-1'), 3.95-3.82 (m, 3H), 3.73-3.64 (m, 2H), 3.50-3.38 (m, 7H), 3.31 (t, *J* = 9.2 Hz, 1H), 3.22 (dd, *J* = 14.2, 2.5 Hz, 1H), 2.96 (dd, *J* = 14.2, 8.2 Hz, 1H), 2.02 (s, 3H); ^{13}C NMR (101 MHz, D_2O) δ 174.38, 97.98 (C-1), 86.01 (C-1'), 79.76, 77.12, 72.82, 72.42, 71.58, 70.98, 69.41, 60.82, 55.32, 53.57, 31.35, 21.85; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{15}H_{28}NO_{10}S^+$: 414.1428, found: 414.1416.

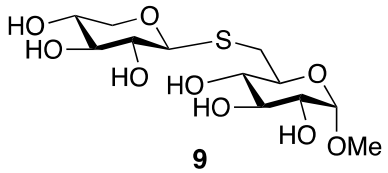
Methyl β-D-glucopyranosyl-(1→6)-*S*-6-thio-α-D-mannopyranoside 7



7

To a stirred the solution of α-D-glucopyranosyl fluoride **1** (120 mg, 0.66 mmol, 3 equiv.) and methyl-6-thiol-α-D-mannopyranoside **6** (46 mg, 0.22 mmol, 1 equiv.) in H_2O (0.4 mL) was added $Ca(OH)_2$ (49 mg, 0.66 mmol, 3 equiv.). The reaction mixture was stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (10% MeOH in H_2O) to yield **7** as a colorless foam (75 mg, 92%). ^{1}H NMR (400 MHz, D_2O) δ 4.69 (d, *J* = 1.6 Hz, 1H, H-1), 4.60 (d, *J* = 9.8 Hz, 1H, H-1'), 3.90-3.85 (m, 2H), 3.77-3.66 (m, 3H), 3.56 (t, *J* = 9.6 Hz, 1H), 3.48-3.36 (m, 6H), 3.31-3.27 (m, 1H), 3.20 (dd, *J* = 14.1, 2.4 Hz, 1H), 2.92 (dd, *J* = 14.1, 8.6 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 100.84 (C-1), 86.11 (C-1'), 79.73, 77.09, 72.43, 72.37, 70.32, 69.80, 69.57, 69.40, 60.83, 54.94, 31.49; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{13}H_{24}NaO_{10}S^+$: 395.0982, found: 395.0967.

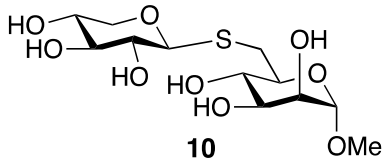
Methyl β-D-xylopyranosyl-(1→6)-*S*-6-thio-α-D-glucopyranoside 9



9

To the solution of α-D-xylopyranosyl fluoride **8** (64 mg, 0.42 mmol, 3 equiv.) and methyl-6-thiol-α-D-glucopyranoside **2** (30 mg, 0.14 mmol, 1 equiv.) in H_2O (0.3 mL) was added $Ca(OH)_2$ (31 mg, 0.42 mmol, 3 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (5% MeOH in H_2O) to yield **9** as the colorless foam (45 mg, 94%). ^{1}H NMR (400 MHz, D_2O) δ 4.77 (bs, 1H, H-1), 4.56 (d, *J* = 9.7 Hz, 1H, H-1'), 4.00 (dd, *J* = 11.4, 5.4 Hz, 1H), 3.79 (ddd, *J* = 10.3, 8.6, 2.5 Hz, 1H), 3.66-3.55 (m, 3H), 3.45-3.41 (m, 4H), 3.37-3.26 (m, 3H), 3.19 (dd, *J* = 14.2, 2.5 Hz, 1H), 2.90 (dd, *J* = 14.2, 8.4 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 99.20 (C-1), 86.90 (C-1'), 77.12, 72.86, 72.47, 72.36, 71.54, 71.19, 69.09, 68.74, 55.16, 31.54; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{12}H_{22}NaO_9S^+$: 365.0877, found: 365.0860.

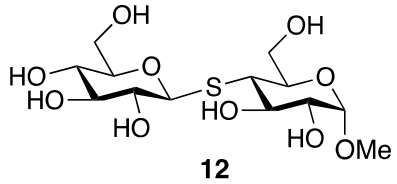
Methyl β-D-xylopyranosyl-(1→6)-*S*-6-thio-α-D-mannopyranoside 10



10

To the solution of α-D-xylopyranosyl fluoride **8** (64 mg, 0.42 mmol, 3 equiv.) and methyl-6-thiol-α-D-mannopyranoside **6** (30 mg, 0.14 mmol, 1 equiv.) in H_2O (0.3 mL) was added $Ca(OH)_2$ (31 mg, 0.42 mmol, 3 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (10% MeOH in H_2O) to yield **10** as the colorless foam (43 mg, 90%). ^{1}H NMR (400 MHz, D_2O) δ 4.72 (d, *J* = 1.6 Hz, 1H, H-1), 4.57 (d, *J* = 9.7 Hz, 1H, H-1'), 4.00 (dd, *J* = 11.4, 5.4 Hz, 1H), 3.92 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.76-3.71 (m, 2H), 3.65-3.56 (m, 2H), 3.45-3.41 (m, 4H), 3.36-3.28 (m, 2H), 3.21 (dd, *J* = 14.2, 2.3 Hz, 1H), 2.91 (dd, *J* = 14.1, 8.7 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 100.86 (C-1), 87.00 (C-1'), 77.09, 72.44, 72.32, 70.30, 69.82, 69.63, 69.09, 68.72, 54.85, 31.66; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{12}H_{22}NaO_9S^+$: 365.0877, found: 395.0869.

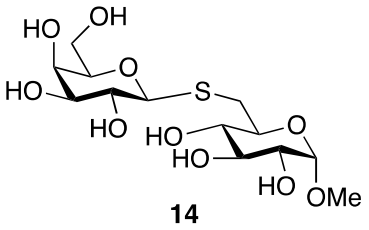
Methyl β-D-glucopyranosyl-(1→4)-*S*-4-thio-α-D-glucopyranoside 12



12

To the solution of α-D-glucopyranosyl fluoride **1** (130 mg, 0.71 mmol, 3 equiv.) and methyl-4-thiol-α-D-glucopyranoside **11** (50 mg, 0.24 mmol, 1 equiv.) in H_2O (0.4 mL) was added $Ca(OH)_2$ (53 mg, 0.71 mmol, 3 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (5% MeOH in H_2O) to yield **12** as the colorless foam (81 mg, 91%). ^{1}H NMR (400 MHz, D_2O) δ 4.86 (d, *J* = 3.7 Hz, 1H, H-1), 4.65 (d, *J* = 9.8 Hz, 1H, H-1'), 4.05-3.31 (m, 14H), 2.87 (t, *J* = 10.8 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 99.26 (C-1), 83.59 (C-1'), 79.86, 77.06, 72.35, 71.88, 69.44, 69.33, 61.28, 60.71, 54.99, 46.88; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{13}H_{24}NaO_{10}S^+$: 395.0982, found: 395.0963.

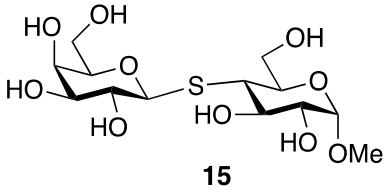
Methyl β-D-galactopyranosyl-(1→6)-*S*-6-thio-α-D-glucopyranoside 14



14

To the solution of α-D-galactopyranosyl fluoride **13** (52 mg, 0.29 mmol, 3 equiv.) and methyl-6-thiol-α-D-glucopyranoside **2** (20 mg, 0.095 mmol, 1 equiv.) in H_2O (0.3 mL) was added $Ca(OH)_2$ (21 mg, 0.29 mmol, 3 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (10% MeOH in H_2O) to yield **14** as the colorless foam (22 mg, 62%). ^{1}H NMR (400 MHz, D_2O) δ 4.81 (d, *J* = 3.6 Hz, 1H, H-1), 4.59 (d, *J* = 9.7 Hz, 1H, H-1'), 4.00 (d, *J* = 3.3 Hz, 1H), 3.90-3.57 (m, 8H), 3.47 (s, 3H), 3.40 (dd, *J* = 9.9, 8.6 Hz, 1H), 3.26 (dd, *J* = 14.2, 2.6 Hz, 1H), 2.98 (dd, *J* = 14.2, 8.1 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 99.23 (C-1), 86.54 (C-1'), 78.89, 73.89, 72.92, 72.44, 71.45, 71.24, 69.77, 68.80, 61.08, 55.27, 31.40; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{13}H_{24}NaO_{10}S^+$: 395.0982, found: 395.0975.

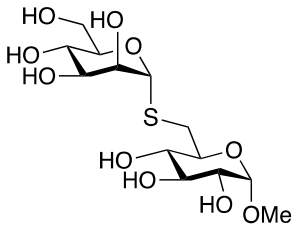
Methyl β-D-galactopyranosyl-(1→4)-*S*-4-thio-α-D-glucopyranoside 15



15

To the solution of α-D-galactopyranosyl fluoride **13** (52 mg, 0.29 mmol, 3 equiv.) and methyl-4-thiol-α-D-glucopyranoside **11** (20 mg, 0.095 mmol, 1 equiv.) in H_2O (0.3 mL) was added $Ca(OH)_2$ (21 mg, 0.29 mmol, 3 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet, rinsing the reaction flash with H_2O, and purified via reversed phase chromatography (10% MeOH in H_2O) to yield **15** as the colorless foam (19 mg, 55%). ^{1}H NMR (400 MHz, D_2O) δ 4.89 (d, *J* = 3.7 Hz, 1H, H-1), 4.62 (d, *J* = 9.7 Hz, 1H, H-1'), 4.10-3.91 (m, 4H), 3.83-3.57 (m, 7H), 3.43 (s, 3H), 2.89 (t, *J* = 10.8 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 99.29 (C-1), 84.13 (C-1'), 79.12, 73.82, 72.35, 71.92, 69.75, 69.66, 68.77, 61.41, 61.26, 55.03, 47.15; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{13}H_{24}NaO_{10}S^+$: 395.0982, found: 395.0976.

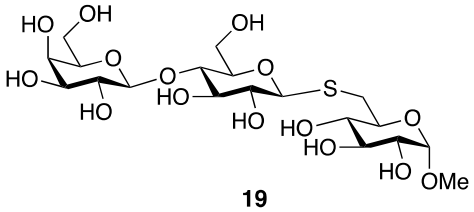
Methyl α-D-mannopyranosyl-(1→6)-*S*-6-thio-α-D-glucopyranoside 17



17

To the solution of α-D-mannopyranosyl fluoride **16** (160 mg, 0.87 mmol, 3 equiv.) and methyl-6-thiol-α-D-glucopyranoside **2** (61 mg, 0.29 mmol, 1 equiv.) in H_2O (0.5 mL) was added $Ca(OH)_2$ (64 mg, 0.87 mmol, 3 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (5% MeOH in H_2O) to yield **17** as the colorless foam (89 mg, 83%). ^{1}H NMR (600 MHz, D_2O, recorded at 40 °C) δ 5.52 (d, *J* = 1.5 Hz, 1H, H-1'), 4.95 (d, *J* = 3.8 Hz, 1H, H-1), 4.24 (dd, *J* = 3.4, 1.6 Hz, 1H), 4.15 (ddd, *J* = 9.9, 6.0, 2.3 Hz, 1H), 4.06 (dd, *J* = 12.3, 2.3 Hz, 1H), 4.00 – 3.94 (m, 3H), 3.89 – 3.80 (m, 2H), 3.75 (dd, *J* = 9.8, 3.8 Hz, 1H), 3.60 (s, 3H), 3.58 – 3.53 (m, 1H), 3.35 (dd, *J* = 14.0, 2.7 Hz, 1H), 2.99 (dd, *J* = 14.0, 7.8 Hz, 1H); ^{13}C NMR (151 MHz, D_2O, recorded at 40 °C) δ 99.44 (C-1), 84.76 (C-1'), 73.49, 73.17, 72.47, 71.87, 71.47, 71.24, 70.17, 67.25, 61.05, 55.32, 31.81; ^{1}H coupled ^{13}C NMR (101 MHz, D_2O) δ 100.03, 98.34, 85.28, 83.62 (J_{CH} = 168 Hz), 73.88, 73.59, 72.89, 72.43, 72.15, 71.90, 71.67, 71.46, 70.85, 70.53, 70.24, 69.16, 67.69, 66.25, 62.19, 60.77, 59.34, 57.20, 55.77, 54.34, 52.92, 32.84, 31.44, 30.04; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{13}H_{24}NaO_{10}S^+$: 395.0982, found: 395.0960.

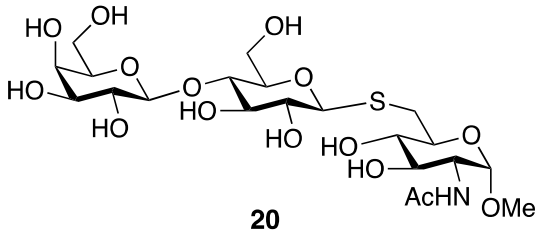
Methyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-*S*-6-thio-α-D-glucopyranoside 19



19

To the solution of α-D-lactopyranosyl fluoride **18** (197 mg, 0.57 mmol, 3 equiv.) and methyl-6-thiol-α-D-glucopyranoside **2** (40 mg, 0.19 mmol, 1 equiv.) in H_2O (0.5 mL) was added $Ca(OH)_2$ (42 mg, 0.57 mmol, 3 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (5% MeOH in H_2O) to yield **19** as the colorless foam (94 mg, 87%). ^{1}H NMR (400 MHz, D_2O) δ 4.79 (brs, 1H, H-1), 4.65 (d, *J* = 9.9 H z, 1H, H-1'), 4.46 (d, *J* = 7.8 Hz, 1H, H-1''), 3.97 (dd, *J* = 12.4, 2.1 Hz, 1H), 3.92 (d, *J* = 3.4 Hz, 1H), 3.86-3.52 (m, 12H), 3.45 (s, 3H), 3.40-3.34 (m, 2H), 3.22 (dd, *J* = 14.3, 2.5 Hz, 1H), 2.94 (dd, *J* = 14.2, 8.2 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 102.84 (C-1''), 99.20 (C-1), 85.91 (C-1'), 78.58, 78.04, 75.70, 75.33, 72.90, 72.50, 72.42, 72.16, 71.50, 71.20, 70.93, 68.53, 61.01, 60.17, 55.25, 31.35; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{19}H_{34}NaO_{15}S^+$: 557.1511, found: 557.1502.

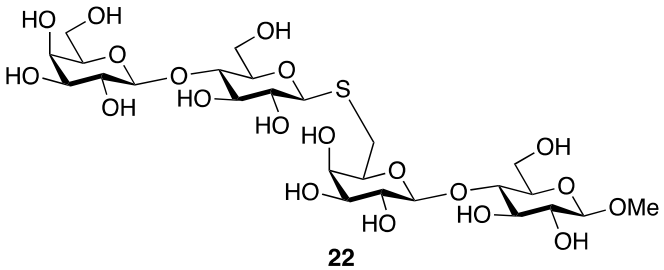
Methyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-*S*-6-thio-2-acetamido-2-deoxy-α-D-glucopyranoside 20



20

To the solution of α-D-lactopyranosyl fluoride **18** (124 mg, 0.36 mmol, 3 equiv.) and methyl-2-acetamido-2-deoxy-6-thiol-α-D-glucopyranoside **4** (30 mg, 0.12 mmol, 1 equiv.) in H_2O (0.4 mL), was added $Ca(OH)_2$ (27 mg, 0.36 mmol, 3 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (5% MeOH in H_2O) to yield **20** as the colorless foam (63 mg, 91%). ^{1}H NMR (400 MHz, D_2O) δ 4.75 (d, *J* = 3.7 Hz, 1H, H-1), 4.67 (d, *J* = 9.9 Hz, 1H, H-1'), 4.47 (d, *J* = 7.8 Hz, 1H, H-1''), 4.01-3.93 (m, 3H), 3.90-3.54 (m, 11H), 3.48-3.37 (m, 5H), 3.25 (dd, *J* = 14.2, 2.5 Hz, 1H), 2.99 (dd, *J* = 14.2, 8.2 Hz, 1H), 2.05 (s, 3H); ^{13}C NMR (101 MHz, D_2O) δ 174.41, 102.84 (C-1''), 98.00 (C-1), 85.90 (C-1'), 78.59, 78.04, 75.70, 75.33, 72.84, 72.50, 72.17, 71.61, 70.99, 70.93, 68.54, 61.01, 60.17, 55.35, 53.59, 31.36, 21.85; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{21}H_{37}NNaO_{15}S^+$: 598.1776, found: 598.1751.

Methyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-*S*-6-thio-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 22

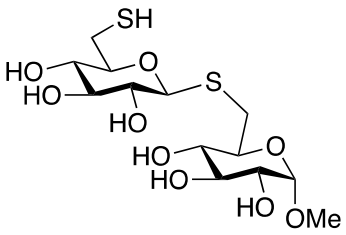


22

To the solution of α-D-lactopyranosyl fluoride **18** (56 mg, 0.162 mmol, 3 equiv.) and thiol acceptor **21** (20 mg, 0.054 mmol, 1 equiv.) in H_2O (0.3 mL) was added $Ca(OH)_2$ (12 mg, 0.162 mmol, 3 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (10% CH_3CN in H_2O) to yield **22** as the white solid (30 mg, 80%). 1H NMR (400 MHz, D_2O) δ 4.67 (d, *J* = 9.9 Hz, 1H, H-1), 4.45-4.42 (m, 2H, H-1x2), 4.39 (d, *J* = 8.0 Hz, 1H, H-1), 4.00-3.94 (m, 3H), 3.91 (d, *J* = 3.4 Hz, 1H), 3.85-3.58 (m, 14H), 3.56 (s, 3H), 3.53-3.48 (m, 2H), 3.41-3.29 (m, 2H), 3.08 (dd, *J* = 14.3, 8.5 Hz, 1H), 2.90 (dd, *J* = 14.3, 5.1 Hz,

1H); ^{13}C NMR (101 MHz, D_2O) δ 103.04 (C-1), 102.89 (C-1), 102.80 (C-1), 85.73 (C-1), 78.45, 78.05, 75.60, 75.29, 74.73, 74.69, 74.33, 72.77, 72.45, 72.06, 70.90, 70.75, 69.32, 68.50, 60.98, 60.19, 60.03, 57.17, 30.43; HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{25}H_{44}NaO_{20}S^+$: 719.2039, found: 719.1952.

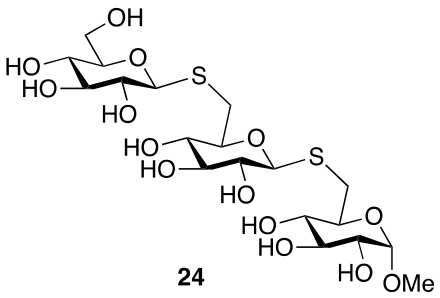
Methyl 6-thiol-β-D-glucopyranosyl-(1→6)-*S*-6-thio-α-D-glucopyranoside 23



23

To a solution of compound **3** (0.5 g, 1.34 mmol, 1 equiv.) in 5 mL of anhydrous pyridine was added TosCl (0.26 g, 1.34 mmol, 1 equiv.) at 0 °C. The reaction mixture was slowly allowed to reach room temperature and stirred over 12 h. To the above reaction mixture, 3 mL of acetic anhydride was added at 0 °C. The reaction mixture was slowly allowed to reach room temperature and stirred over 2h. The solvents were evaporated under reduced pressure and coevaporated twice with anhydrous toluene (2×10 mL). The crude mixture was dissolved in 20 mL of anhydrous acetone and KSAC (228 mg, 2.0 mmol, 1.5 equiv.) was added and stirred at 60 °C over 12 h. The reaction mixture was cooled to room temperature and the salt precipitate was filtered over celite and concentrated under reduced pressure. The crude was dried under vacuum for 2 h and dissolved in 5 mL of anhydrous methanol and sodium methoxide was added until the pH was 9. The reaction mixture was stirred at the same pH for 6 h. The reaction was neutralized with Amberlite IR120 (H^+) resin and filtered over celite. The solvent was evaporated, and the crude mixture was purified over C18 column (10% CH_3CN in H_2O). The tubes containing the compound **23** was lyophilized to obtain it as a white solid (416 mg, 80% overall yield). ^{1}H NMR (600 MHz, D_2O) δ 4.76 (d, *J* = 3.8 Hz, 1H, H-1), 4.64 (d, *J* = 10.0 Hz, 1H, H-1'), 3.85 (ddd, *J* = 10.5, 8.7, 2.4 Hz, 1H), 3.63-3.54 (m, 2H), 3.48-3.43 (m, 2H), 3.43 (s, 3H), 3.40-3.35 (m, 1H), 3.30 (dt, *J* = 15.5, 9.4 Hz, 2H), 3.21 (dd, *J* = 14.3, 2.4 Hz, 1H), 2.98 (dd, *J* = 14.4, 2.5 Hz, 1H), 2.93 (dd, *J* = 14.4, 8.6 Hz, 1H), 2.68 (dd, *J* = 14.4, 7.7 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 99.17 (C-1), 86.60 (C-1'), 80.02, 76.87, 72.86, 72.65, 72.51, 71.78, 71.73, 71.19, 55.25, 32.10, 25.37; HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{13}H_{24}NaO_9S_2^+$: 411.0754, found: 411.0743.

Methyl β-D-glucopyranosyl-(1→6)-*S*-6-thio-β-D-glucopyranosyl-(1→6)-*S*-6-thio-α-D-glucopyranoside 24

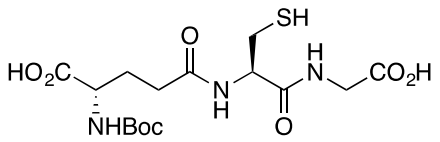


24

To the solution of fluoride donor **1** (53 mg, 0.29 mmol, 3 equiv.) and thiol acceptor **23** (38 mg, 0.098 mmol, 1 equiv.) in H_2O (0.4 mL) was added $Ca(OH)_2$ (21 mg, 0.29 mmol, 3 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (10% CH_3CN in H_2O) to yield **24** as the white solid (50 mg, 92%). ^{1}H NMR (600 MHz, CD_3OD/D_2O = 9/1) δ 4.66 (d, *J* = 3.8 Hz, 1H, H-1), 4.55 (d, *J* = 9.8 Hz, 2H, H-1', H-1''), 3.88 (dd, *J* = 12.1, 1.5 Hz, 1H), 3.79 (td, *J* = 9.2, 2.3 Hz, 1H), 3.70-3.65 (m, 1H), 3.62 (t, *J* = 9.3 Hz, 1H), 3.51 (td, *J* = 7.8, 4.0 Hz, 1H), 3.46-3.42 (m, 4H), 3.41-3.32 (m, 4H), 3.29-3.13 (m, 6H), 2.87 (ddd, *J* = 14.5, 8.4, 6.1 Hz, 2H); ^{13}C NMR (151 MHz, CD_3OD/D_2O = 9/1) δ 100.92 (C-1), 88.06 (C-1'), 87.44 (C-1''), 81.82, 79.31, 79.22, 74.87, 74.78, 74.61, 74.41, 74.21, 73.50, 73.46, 71.31, 62.76, 55.82, 33.24, 32.57; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{19}H_{34}NaO_{14}S_2^+$: 573.1282, found: 573.1306.

2.6. Synthesis of *S*-Glucopyranosyl peptides

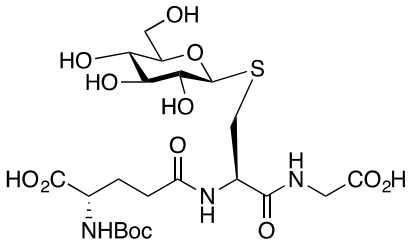
N-^{t}Boc L-Glutathione (GSH) 25[5]



25

To the solution of L-glutathione (commercially available, 800 mg, 2.6 mmol, 1 equiv.) in THF/H_2O (4/11, 15 mL) were added $NaHCO_3$ (480 mg, 5.2 mmol, 2.2 equiv.) and *t*Boc_2O (568 mg, 2.6 mmol, 1 equiv.) and stirred at r.t. for 15 h. Then the solution was concentrated under vacuum and purified by column chromatography (2% MeOH in H_2O) to give an amorphous solid **25** (678 mg, 64%). ^{1}H NMR (400 MHz, D_2O) δ 4.56 (dd, *J* = 7.1, 5.2 Hz, 1H), 3.92-3.72 (m, 3H), 2.99-2.89 (m, 2H), 2.44-2.40 (m, 2H), 2.17-2.06 (m, 1H), 1.93-1.84 (m, 1H), 1.43 (s, 9H); ^{13}C NMR (101 MHz, D_2O) δ 179.15, 176.28, 175.90, 171.69, 157.44, 81.02, 55.58, 55.50, 43.32, 32.08, 27.69, 25.60; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{15}H_{25}N_3NaO_8S^+$: 430.1255, found: 430.1234.

S-Glucopyranosyl tripeptide 26



26

To the solution of α-D-glucopyranosyl fluoride **1** (38 mg, 0.21 mmol, 3 equiv.) and *N*-^{t}Boc-glutathione **25** (28 mg, 0.07 mmol, 1 equiv.) in H_2O (0.2 mL) was added $Ca(OH)_2$ (15 mg, 0.21 mmol, 3.0 equiv.) and stirred at r.t. for 1 h. Then the resulting emulsion was loaded onto a Biotage samplet and purified via reversed phase chromatography (2% MeOH in H_2O) to yield **26** as the colorless foam (35 mg, 90%). ^{1}H NMR (400 MHz, D_2O) δ 4.62 (dd, *J* = 8.3, 5.6 Hz, 1H), 4.55 (d, *J* = 9.7 Hz, 1H, H-1), 3.94-3.25 (m, 10H), 2.96 (td, *J* = 15.1, 14.7, 8.6 Hz, 1H), 2.44-2.36 (m, 2H), 2.11-2.06 (m, 1H), 1.87 (dq, *J* = 15.0, 7.9 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR (151 MHz, D_2O) δ 179.23, 176.23, 175.83, 171.76, 157.52, 85.27 (C-1), 81.03, 79.89, 77.07, 72.10, 69.43, 60.83, 55.57, 55.50, 53.54, 43.37, 32.13, 31.25, 27.71; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{21}H_{35}N_3NaO_{13}S^+$: 592.1783, found: 592.1760.

Synthesis of thioglycopentapeptide 27 and thioglycoheptapeptide 28

Both the penta and heptapeptides were synthesized on a CEM multipep 2 automated peptide synthesizer employing Fmoc protected Val and Gly on Wang resin respectively and other standard Fmoc amino acids in a 25 μmol scale by using the following method.

Prepare

1	Memo	25 μmol HBTU on MultiPep
2	RinseNeedle	1000 / 2000 μL
3	PrimeManifold	10000 μL, DMF
4	WashResin	4800 μL, DMF, 3x

Cycle 1. -> 5/7(5 for penta and 7 for heptapeptide respectively)

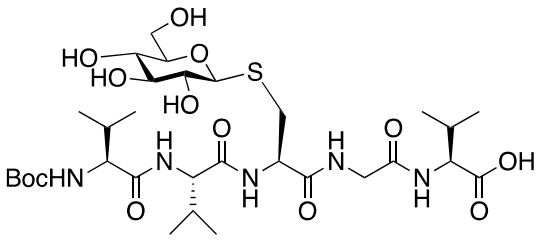
5	Deprotection	450 μL, Piperidine->Peptides
6	Deprotection	450 μL, Piperidine->Peptides
7	RinseNeedle	1000 / 2500 μL
8	SetTempReg to : 50 °C, <1min	
9	WashResin	3600 μL, DMF, 4x
10	Coupling	200+150+5+210, Peptides <-Cpl.Type
11	Coupling	200+150+5+210, Peptides <-Cpl.Type
12	Capping	400 μL, CapMixture->Peptides
13	RinseNeedle	1000 / 2500 μL
14	WashResin	3600 μL, DMF, 5x

Final

15 Deprotection 450 µL, Piperidine->Peptides
16 Deprotection 450 µL, Piperidine->Peptides
17 SetTempReg to : OFF
18 RinseNeedle 500 / 2500 µL
19 WashResin 3600 µL, DMF, 6x
20 Extract 60 s
21 PrimeManifold 10000 µL, EtOH
22 WashResin 3600 µL, EtOH, 4x
23 PrimeManifold 5000 µL, DMF
24 Extract 300 s
25 RinseNeedle 1000 / 2500 µL

The oligopeptide on the resin was taken in a flask and treated with 3 mL of TFA/TIS/DODT/H_2O (92.5/2.5/2.5/2.5) solution for 3 h and filtered. The resin was washed with 2 mL of the above solvent mixture and concentrated to 1 mL under reduced pressure. 50 mL of cold diethyl ether was added to precipitate the crude peptide which was collected by centrifugation. The crude peptide was dried under vacuum for 10 min and used in the next step without further purification. The crude peptide was dissolved in 1/1.5 mL of THF/Water and 5 equiv. of $NaHCO_3$ was added followed by 3 equiv. of *t*Boc_2O and stirred over 3 h at room temperature. After complete conversion (confirmed by MALDI-TOF), the solution was concentrated and used in the next reaction as purification resulted in the disulfide formation. The crude peptide was dissolved in water (0.1 M) and 3 equiv. of glucopyranosyl fluoride was added and stirred at room temperature for 1 h. The crude solution was centrifuged, and the supernatant was purified by C18 flash chromatography to obtain the glycopeptide as a white solid (8.7 mg, 44% overall yield for pentathioglycopeptide **27** and 8.1 mg, 39% overall yield for heptathioglycopeptide **28**).

S-Glucopyranosyl pentapeptide 27

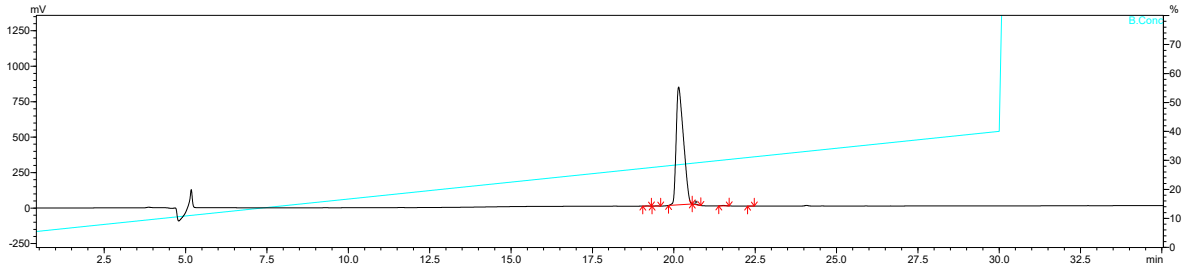


27

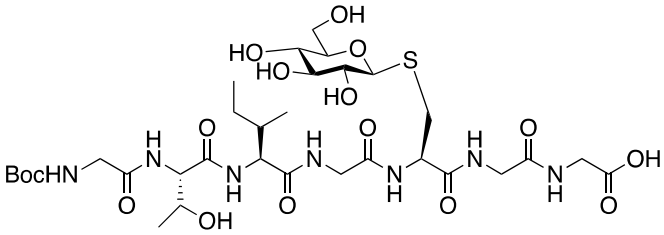
^{1}H NMR (600 MHz, D_2O) δ 4.65 (t, *J* = 7.6 Hz, 1H), 4.60 (d, *J* = 10.0 Hz, 1H, H-1), 4.31 (d, *J* = 6.0 Hz, 1H), 4.16 (d, *J* = 8.1 Hz, 1H), 4.08-3.93 (m, 3H), 3.88 (d, *J* = 7.6 Hz, 1H), 3.71 (dd, *J* = 12.4, 6.3 Hz, 1H), 3.54-3.49 (m, 2H), 3.41 (t, *J* = 9.5 Hz, 1H), 3.34 (t, *J* = 9.4 Hz, 1H), 3.19 (dd, *J* = 14.1, 7.7 Hz, 1H), 3.06 (dd, *J* = 14.0, 7.0 Hz, 1H), 2.21 (dt, *J* = 13.5, 6.8 Hz, 1H), 2.07-2.01 (m, 2H), 1.44 (s, 9H), 1.00 -0.89 (m, 18H); 13C NMR (150 MHz, D_2O) δ 175.31, 173.23, 172.18, 170.95, 163.14, 117.37, 115.43, 85.94 (C-1), 81.33, 79.76, 77.09, 72.17, 69.60, 60.99, 60.47, 59.30, 53.87, 42.49, 31.45,

30.04, 29.58, 27.59, 18.54, 18.36, 17.39; MALDI-MS m/z [M + Na]$^+$ calcd for $C_{31}H_{55}N_5NaO_{13}S^+$: 760.3409, found: 760.3181.

The purity of the pentaglycothiopeptide **27** was also verified via RP-HPLC using a gradient from 5% to 40% (Solvent A: 0.1% CF_3CO_2H in water; Solvent B: 0.1% CF_3CO_2H in acetonitrile, flowrate: 1 mL/min) over 30 min.



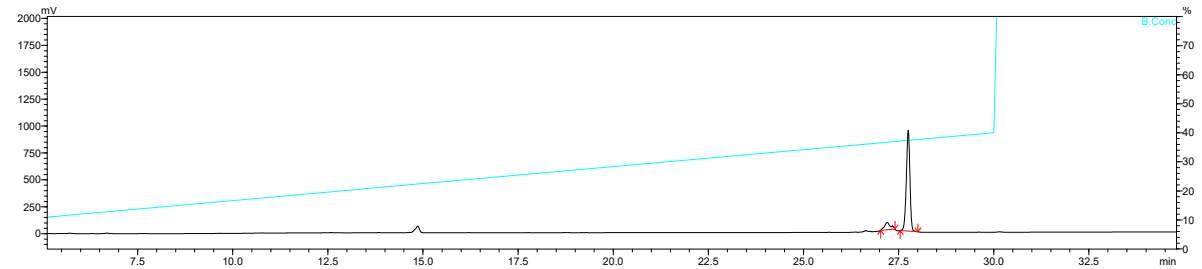
S-Glucopyranosyl heptapeptide 28



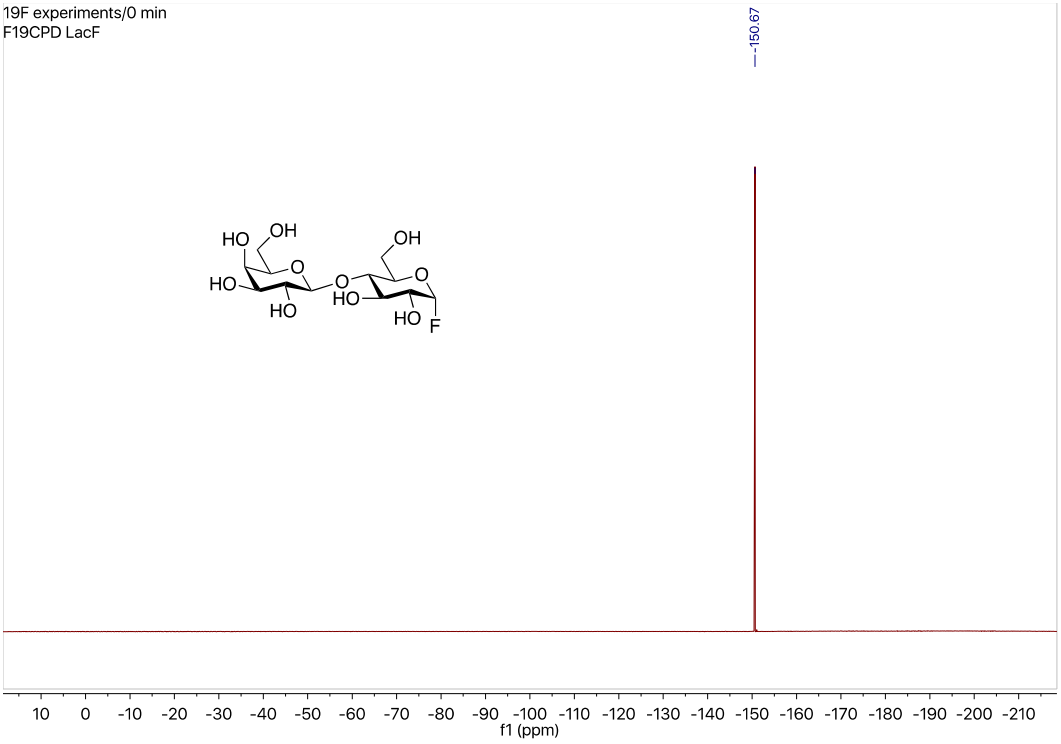
28

^{1}H NMR (600 MHz, D_2O) δ 4.82 (d, *J* = 3.3 Hz, 1H), 4.76-4.57 (m, 2H, H-1), 4.45-4.36 (m, 1H), 4.24-4.15 (m, 2H), 4.06-3.93 (m, 7H), 3.85 (brs, 1H), 3.70 (dd, *J* = 12.4, 6.5 Hz, 1H), 3.55-3.49 (m, 1H), 3.43-3.38 (m, 1H), 3.33 (t, *J* = 9.4 Hz, 1H), 3.26-3.22 (m, 1H), 3.07-3.03 (m, 1H), 1.89 (brs, 1H), 1.51 (brs, 1H), 1.44 (s, 9H), 1.24-1.18 (m, 5H), 0.94 (d, *J* = 6 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, D_2O) δ 173.96, 173.05, 172.45, 172.07, 171.86, 171.58, 163.21, 85.54 (C-1), 81.69, 79.84, 77.05, 72.13, 69.59, 67.20, 60.94, 59.03, 58.63, 53.77, 43.36, 42.39, 42.31, 40.95, 40.39, 35.91, 31.26, 29.57, 27.54, 24.63, 18.81, 14.59, 10.04; MALDI-MS m/z [M + Na]$^+$ calcd for $C_{32}H_{55}N_7NaO_{16}S^+$: 848.3318, found: 848.4687.

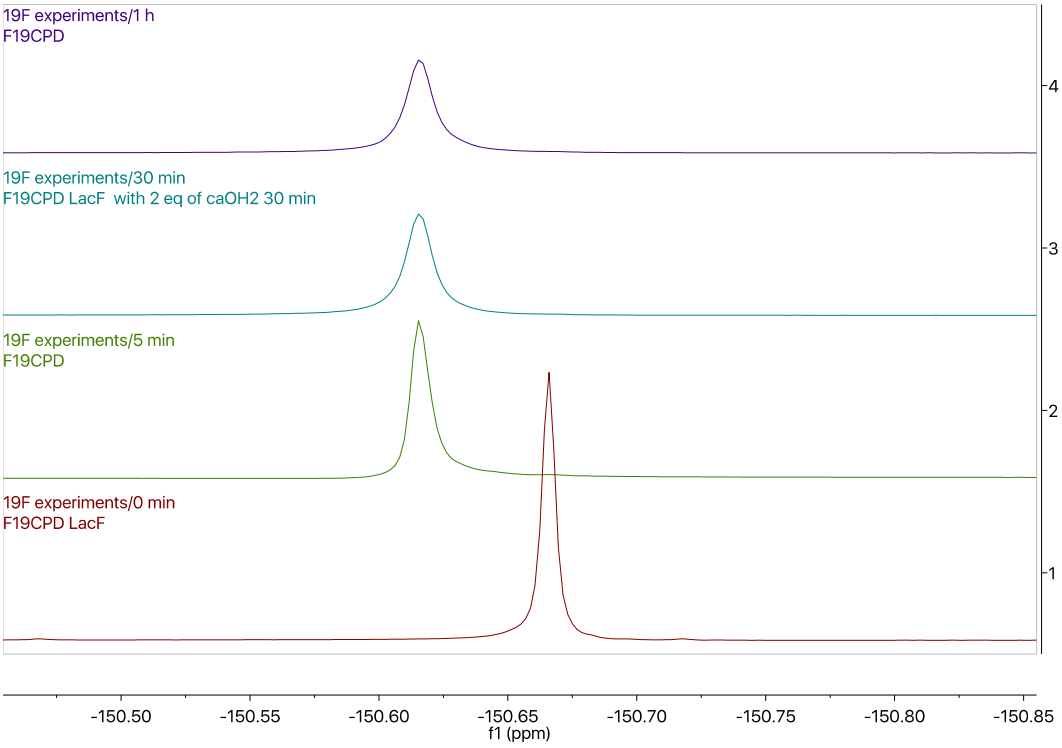
The purity of the heptaglycothiopeptide **28** was also verified via RP-HPLC using a gradient from 5% to 40% (Solvent A: 0.1% CF_3CO_2H in water; Solvent B: 0.1% CF_3CO_2H in acetonitrile, flowrate: 0.8 mL/min) over 30 min.



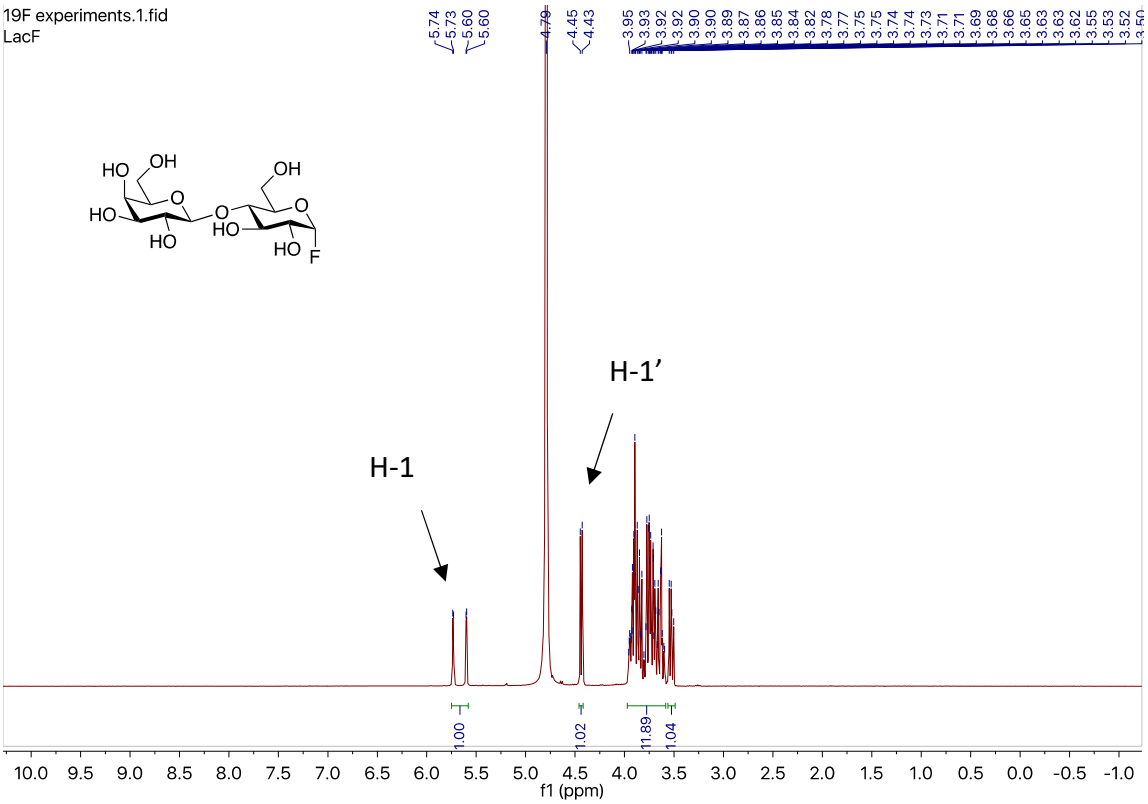
3. NMR Spectra



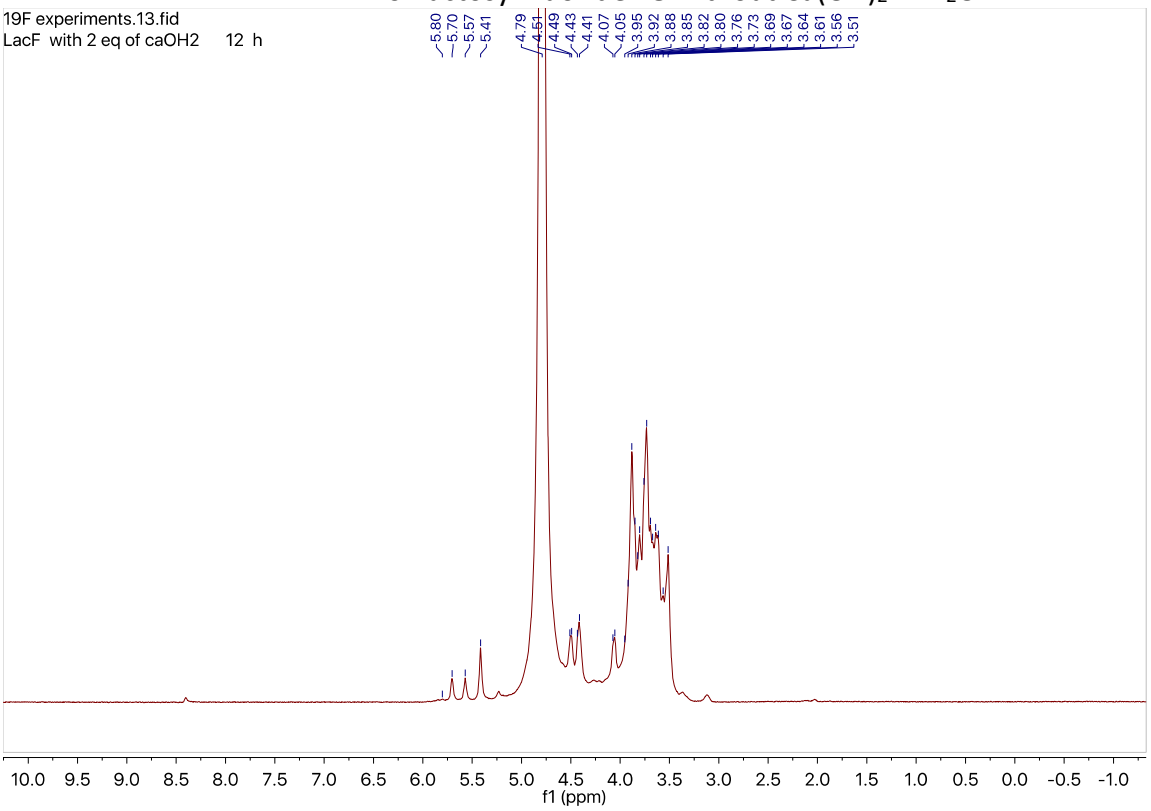
^{19}F NMR of lactosyl fluoride **18** without $Ca(OH)_2$ in D_2O



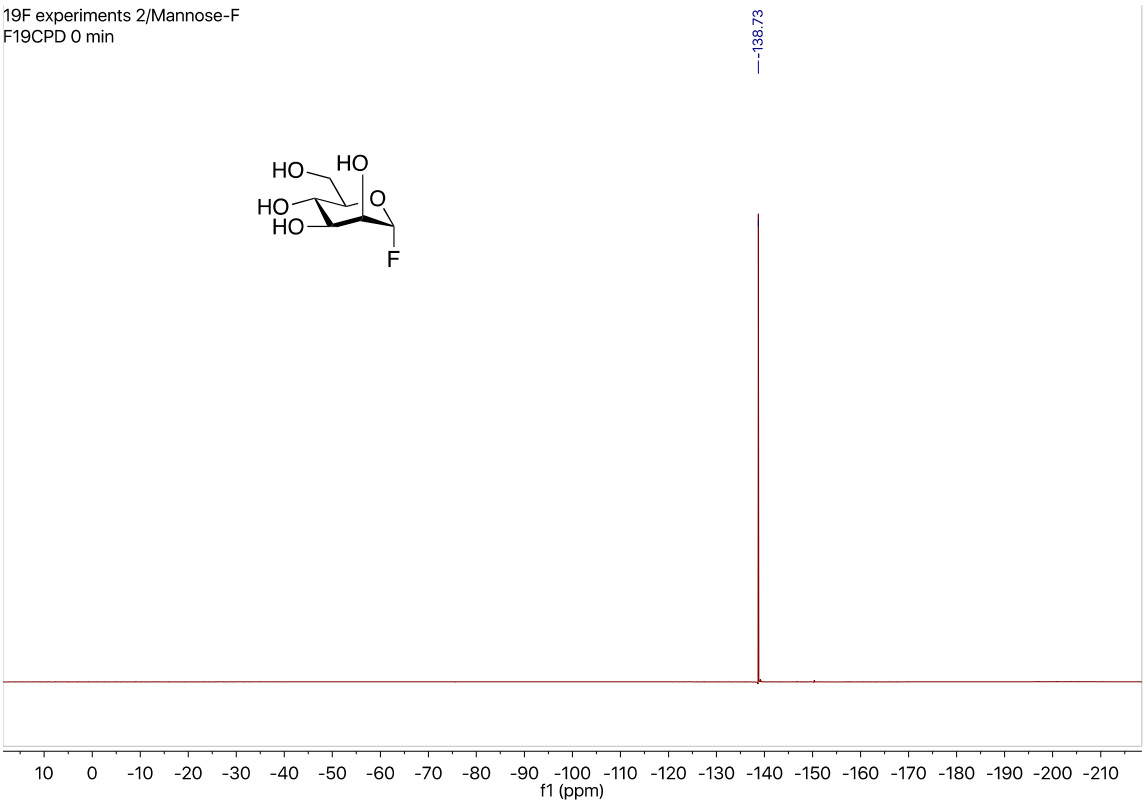
^{19}F NMR of lactosyl fluoride **18** without $Ca(OH)_2$ (0 min) and with 2 equiv. of $Ca(OH)_2$ in D_2O at 5, 30, 60 min



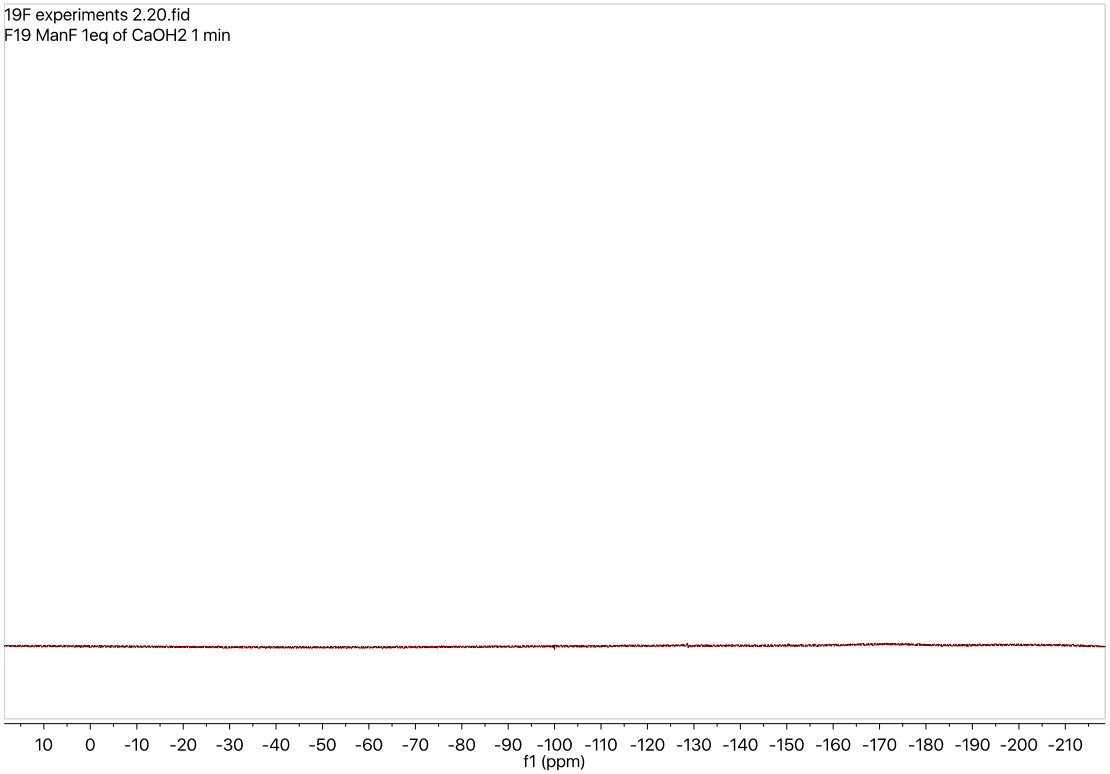
^{1}H NMR of lactosyl fluoride **18** without $Ca(OH)_2$ in D_2O



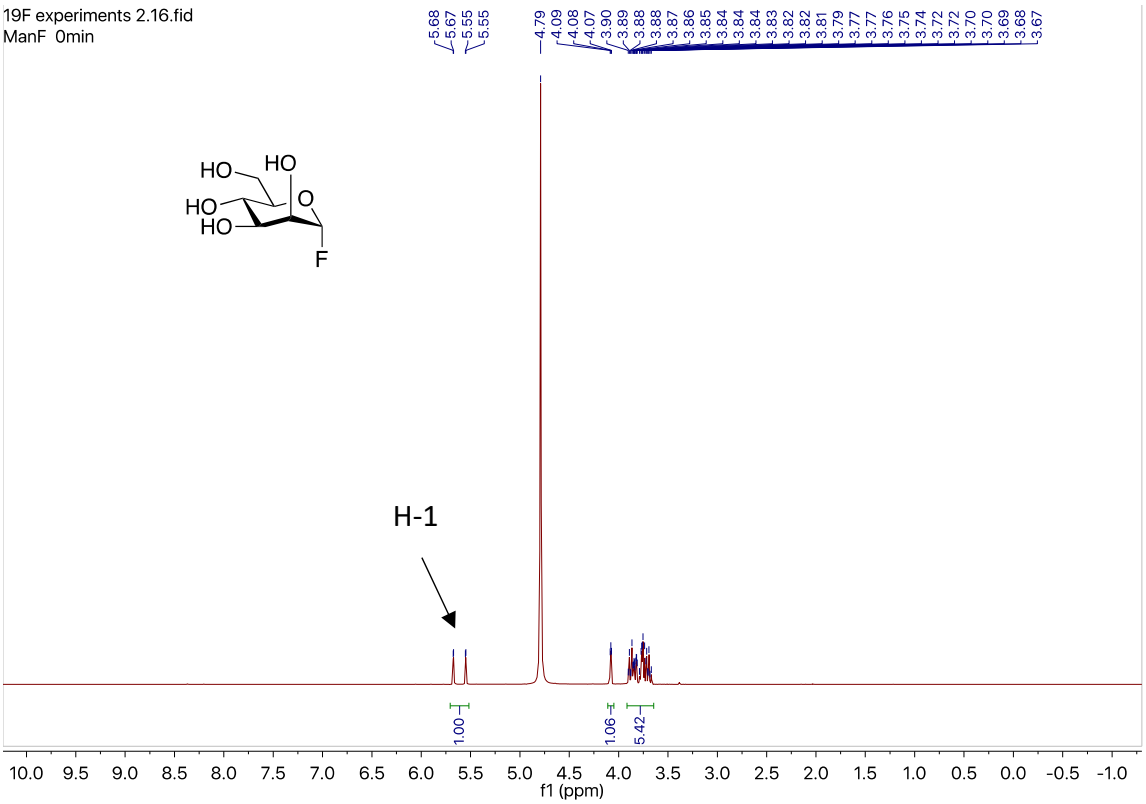
^{1}H NMR of lactosyl fluoride **18** with $Ca(OH)_2$ in D_2O, 12 h



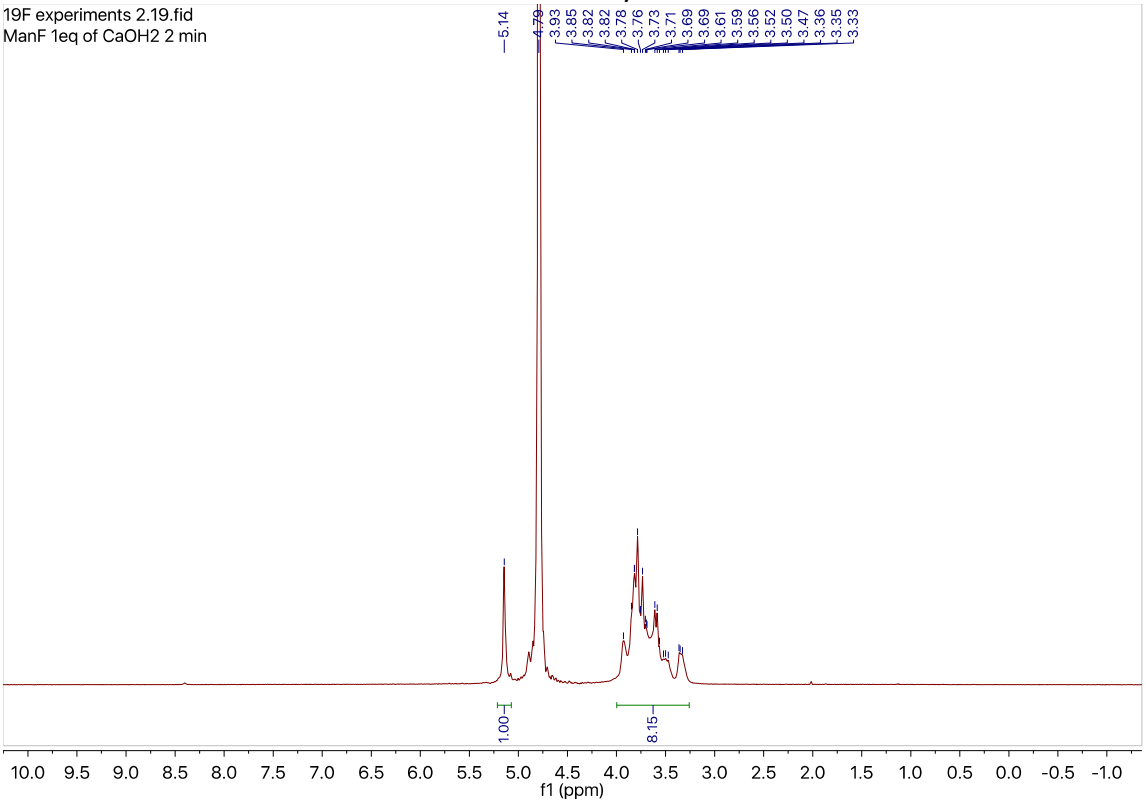
^{19}F NMR of mannosyl fluoride **16** in D_2O



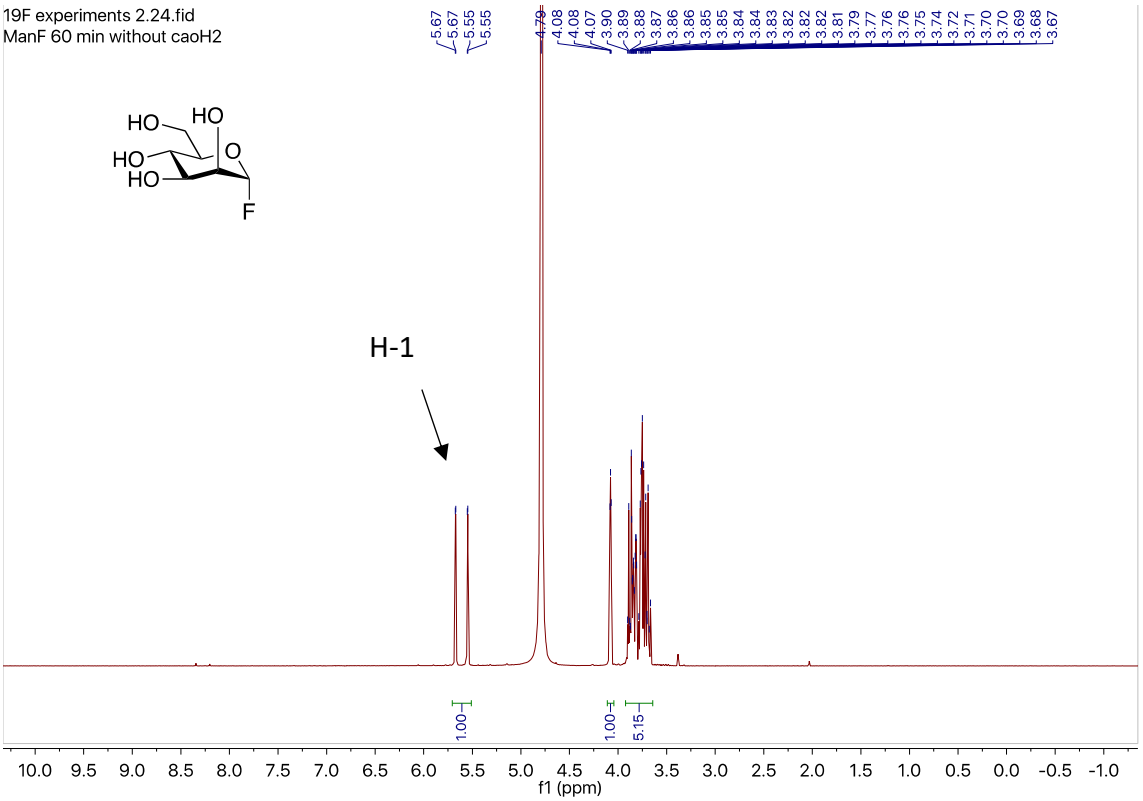
^{19}F NMR of mannosyl fluoride **16** with 1 equiv. of $Ca(OH)_2$ in D_2O, 1 min



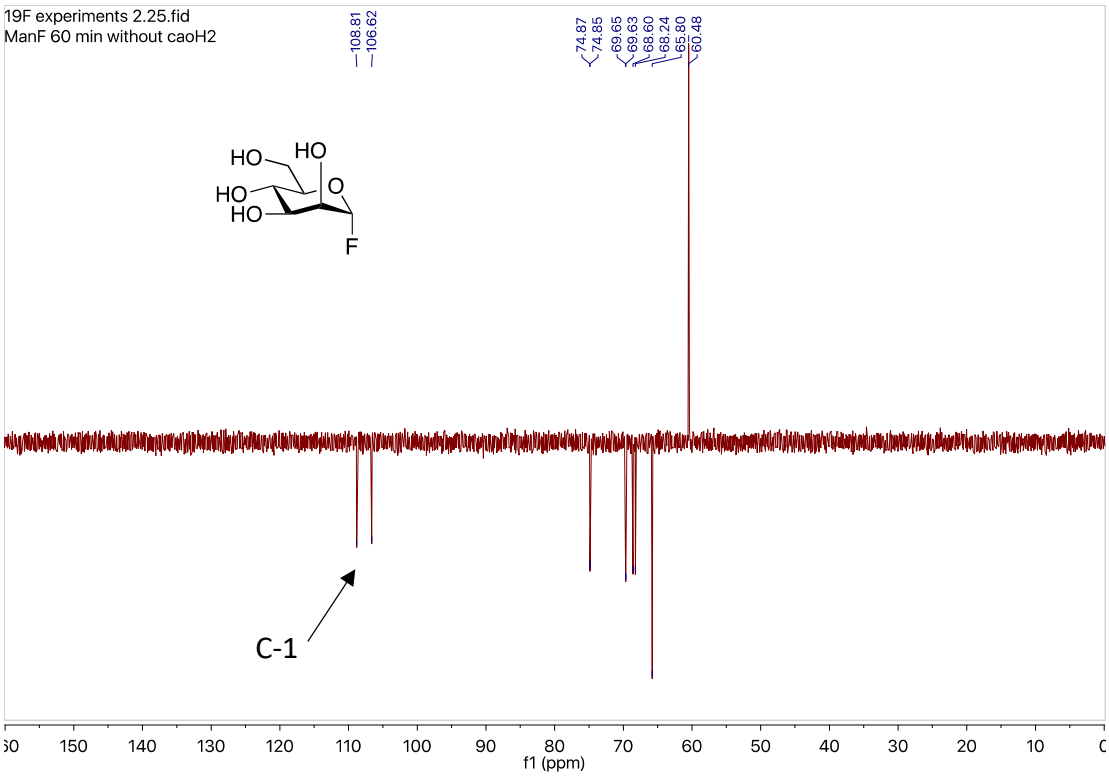
^{1}H NMR of mannosyl fluoride **16** in D_2O



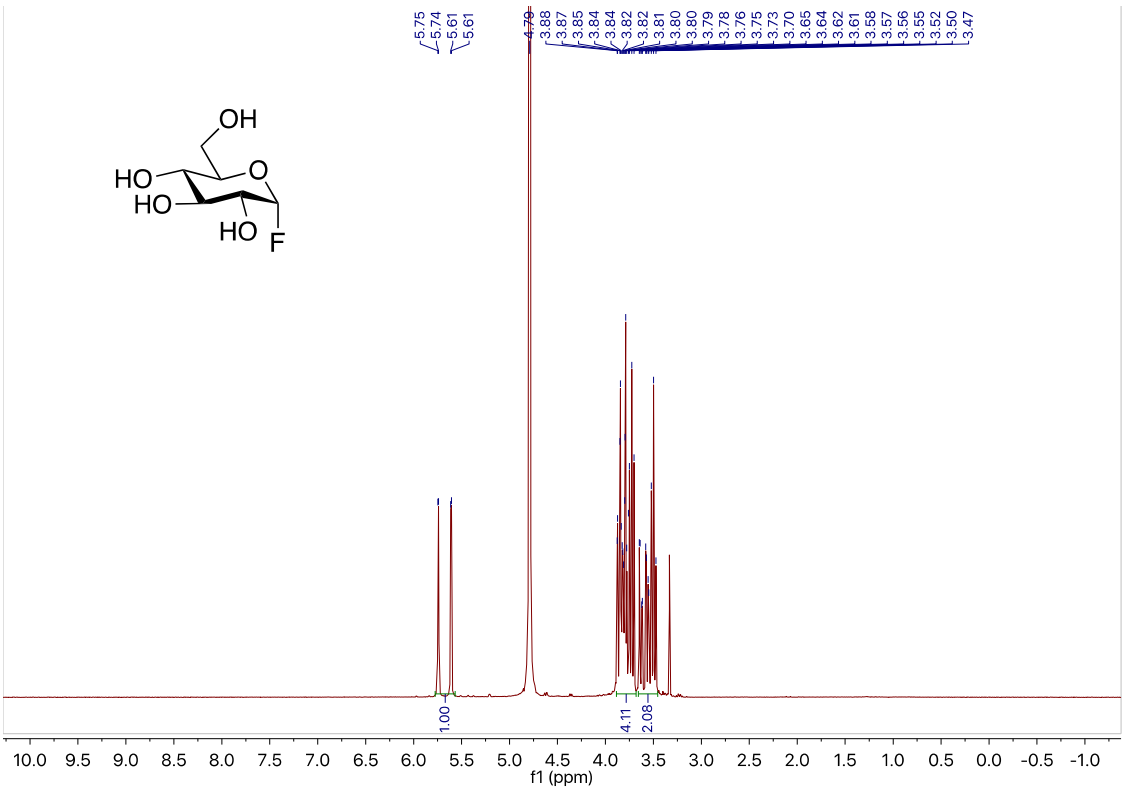
^{1}H NMR of mannosyl fluoride **16** with 1 equiv. of $Ca(OH)_2$ in D_2O, 2 min



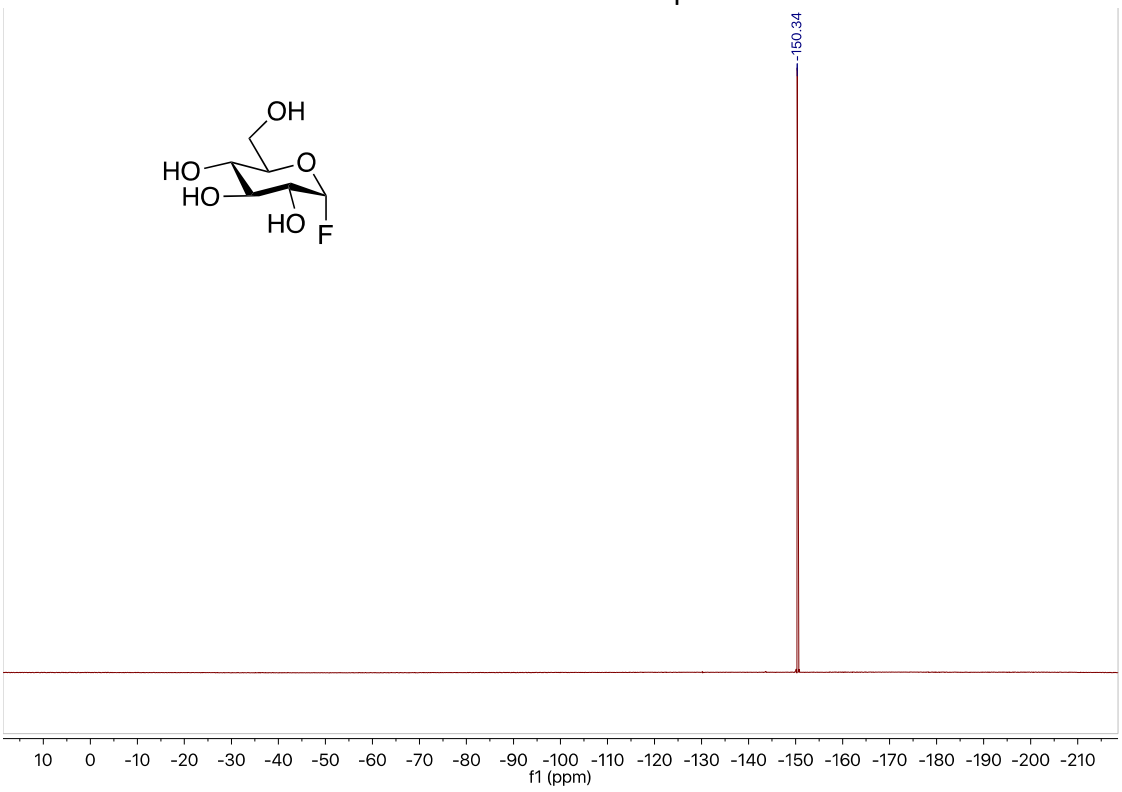
^{1}H NMR of mannosyl fluoride **16** without $Ca(OH)_2$ in D_2O, 60 min



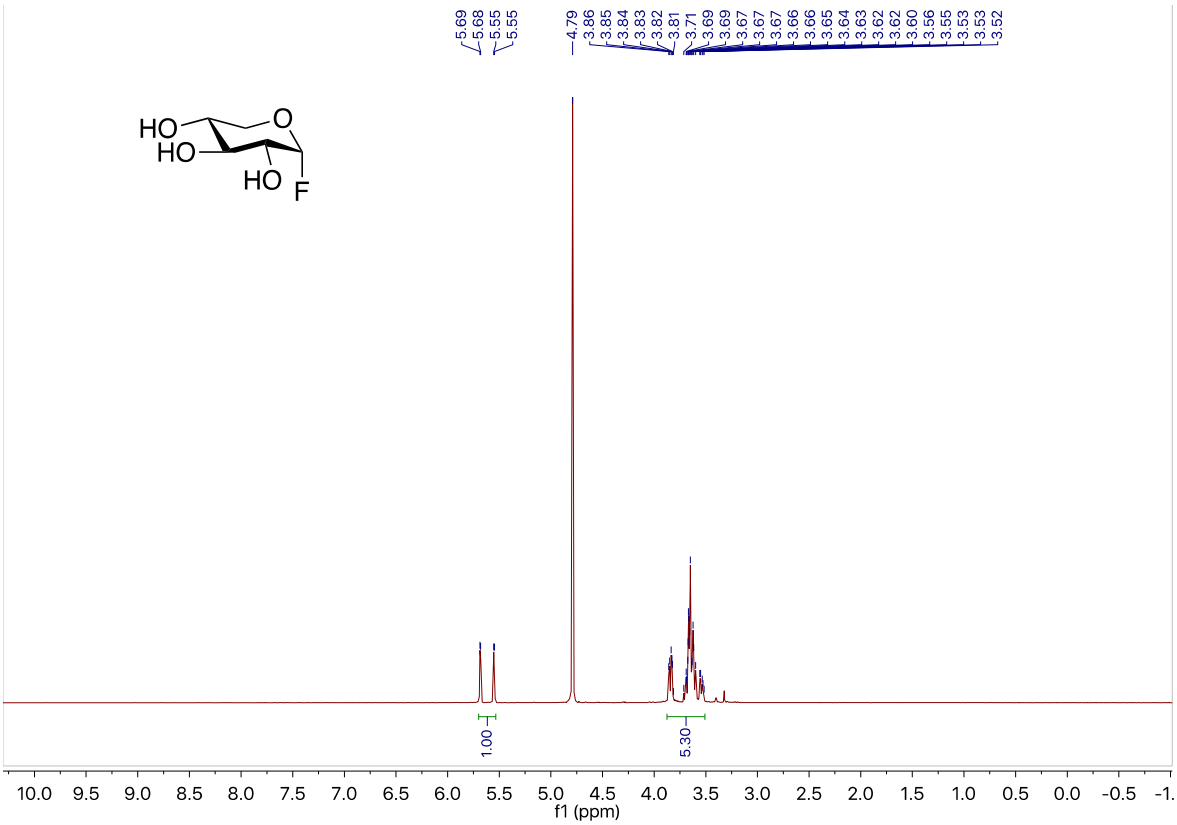
DEPT-135 NMR of mannosyl fluoride **16** without $Ca(OH)_2$ in D_2O, 60 min



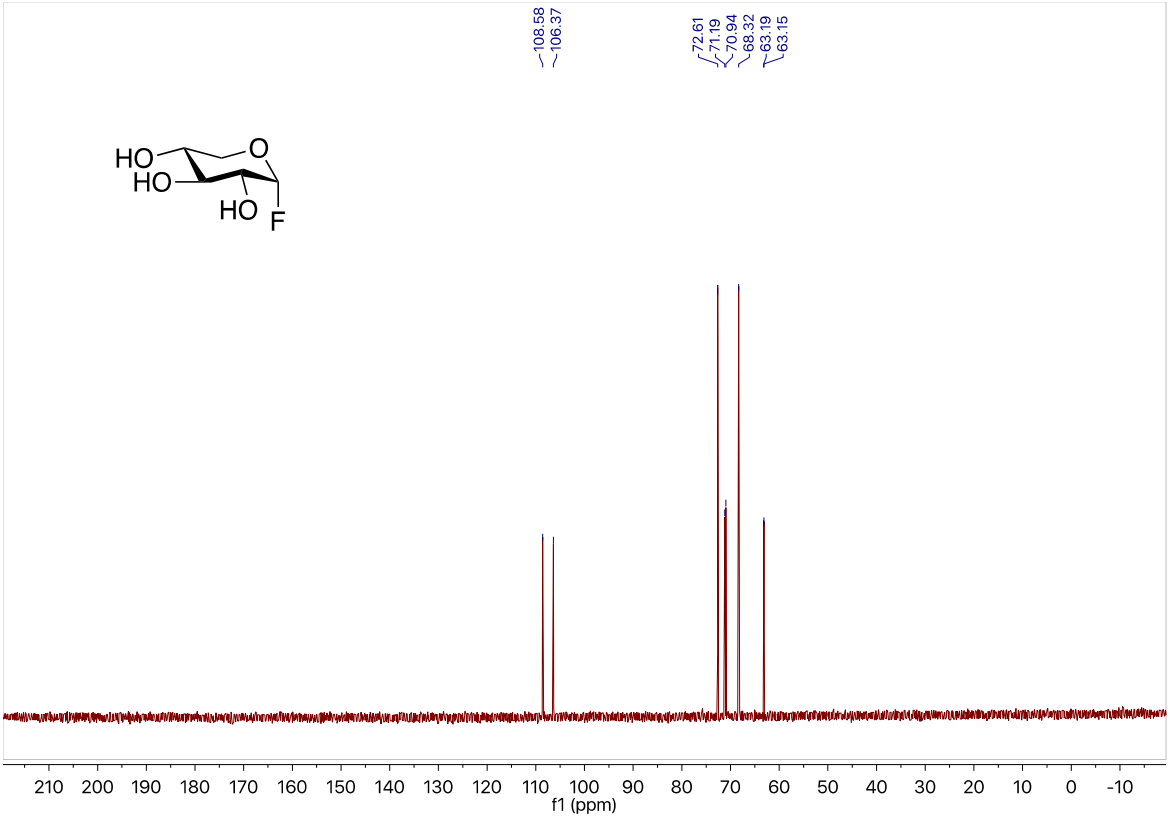
^{1}H NMR of compound **1**



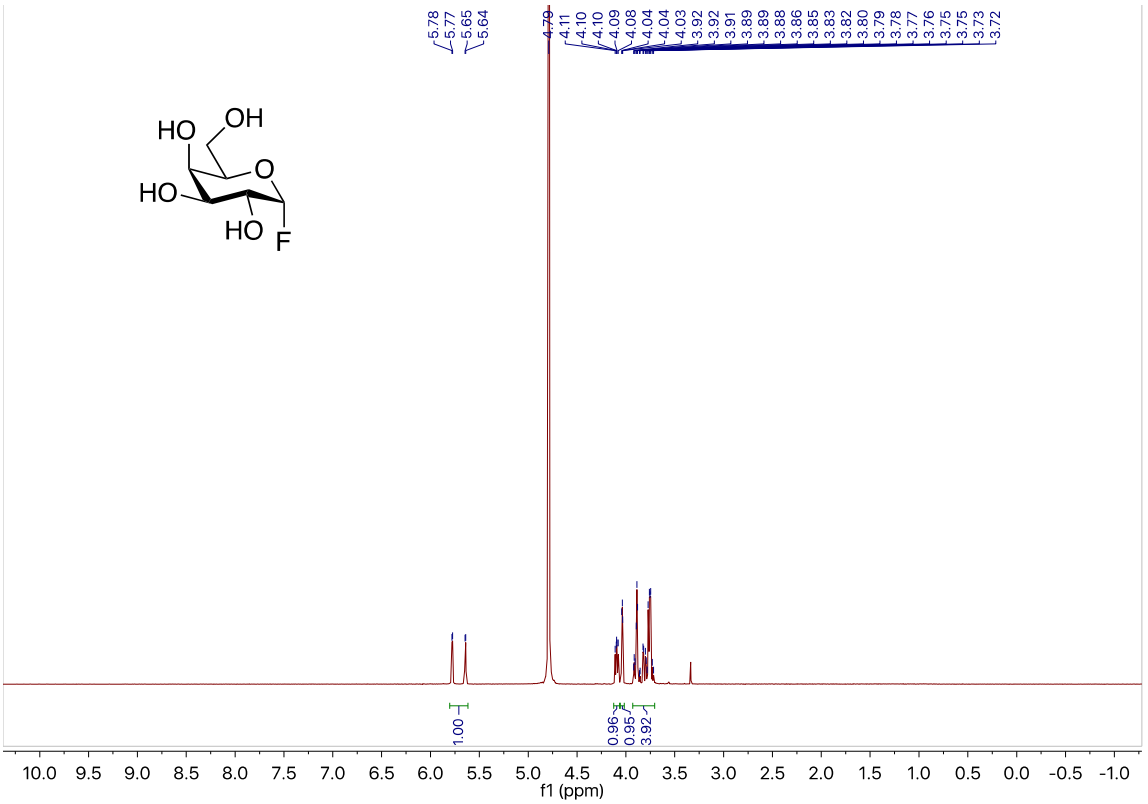
^{19}F NMR of compound **1**



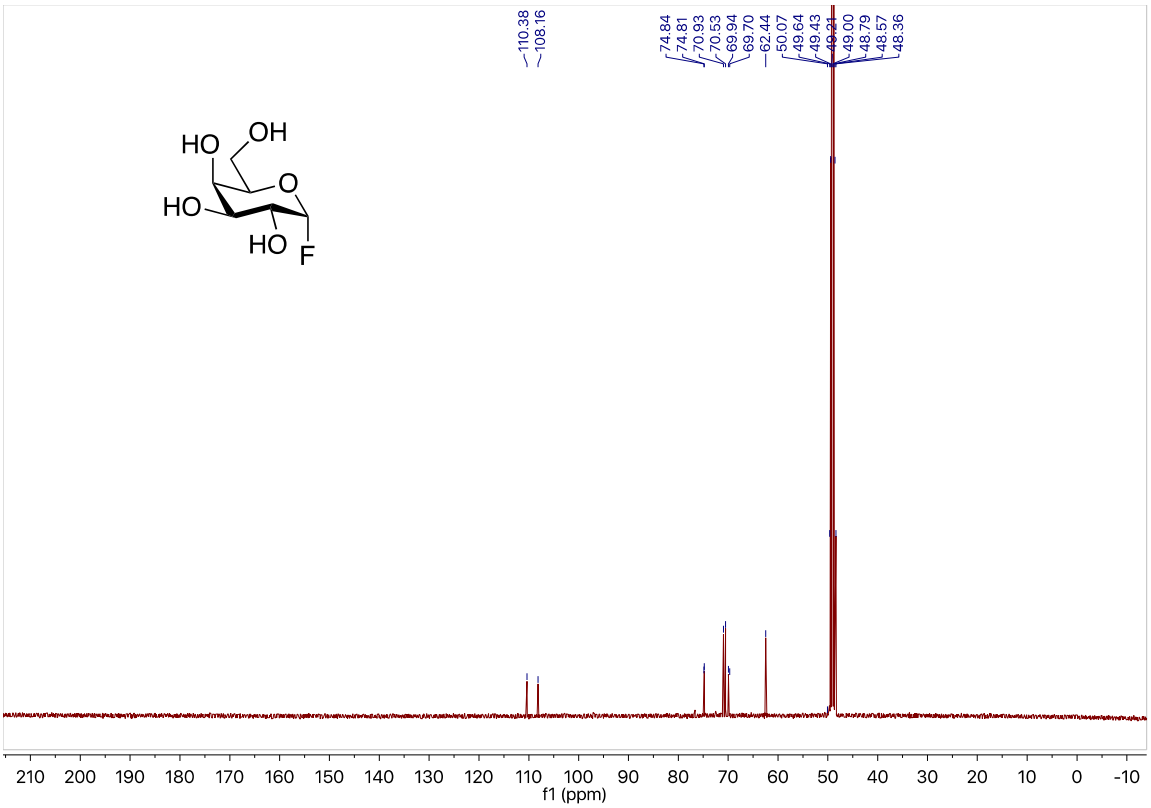
^{1}H NMR of compound **8**



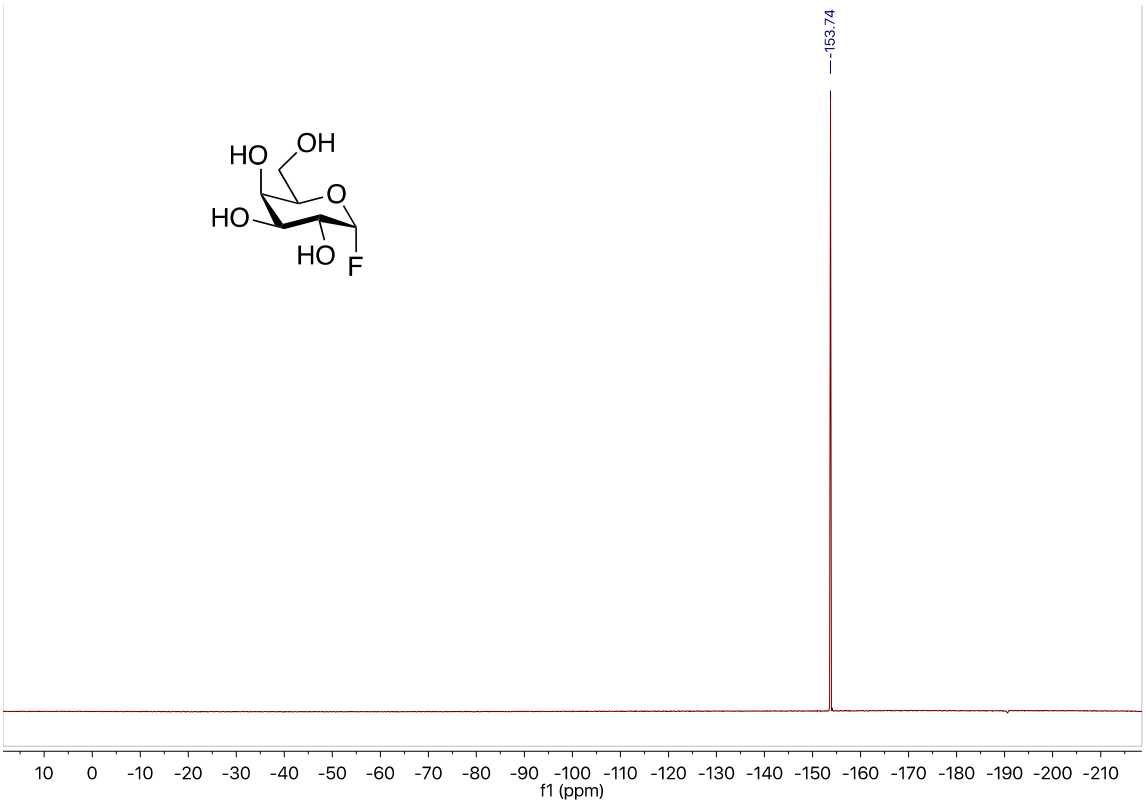
^{13}C NMR of compound **8**



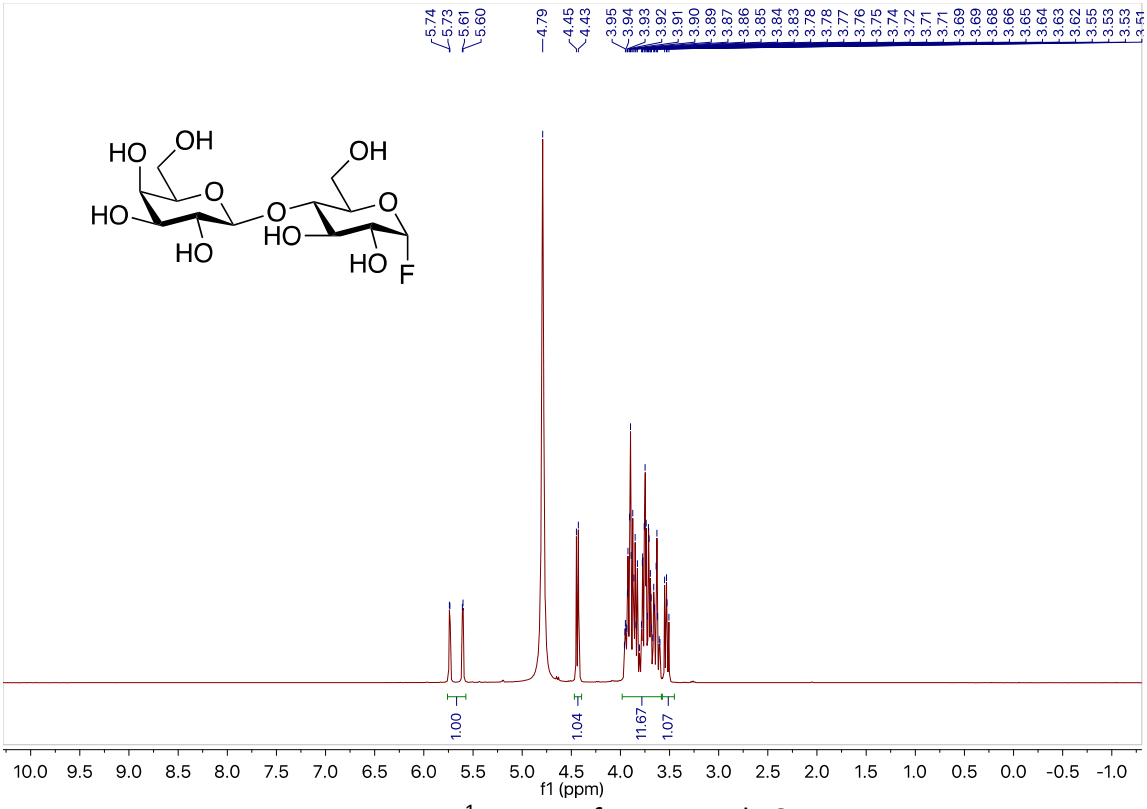
^{1}H NMR of compound **13**



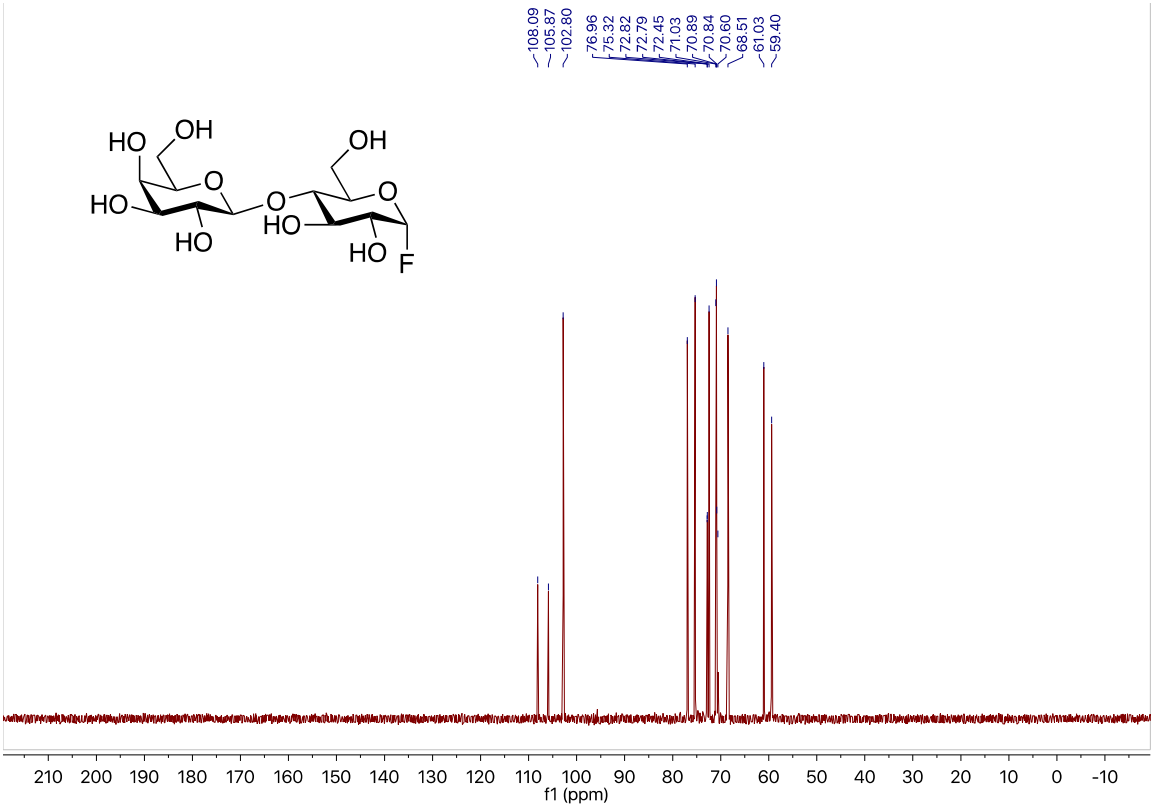
^{13}C NMR of compound **13**



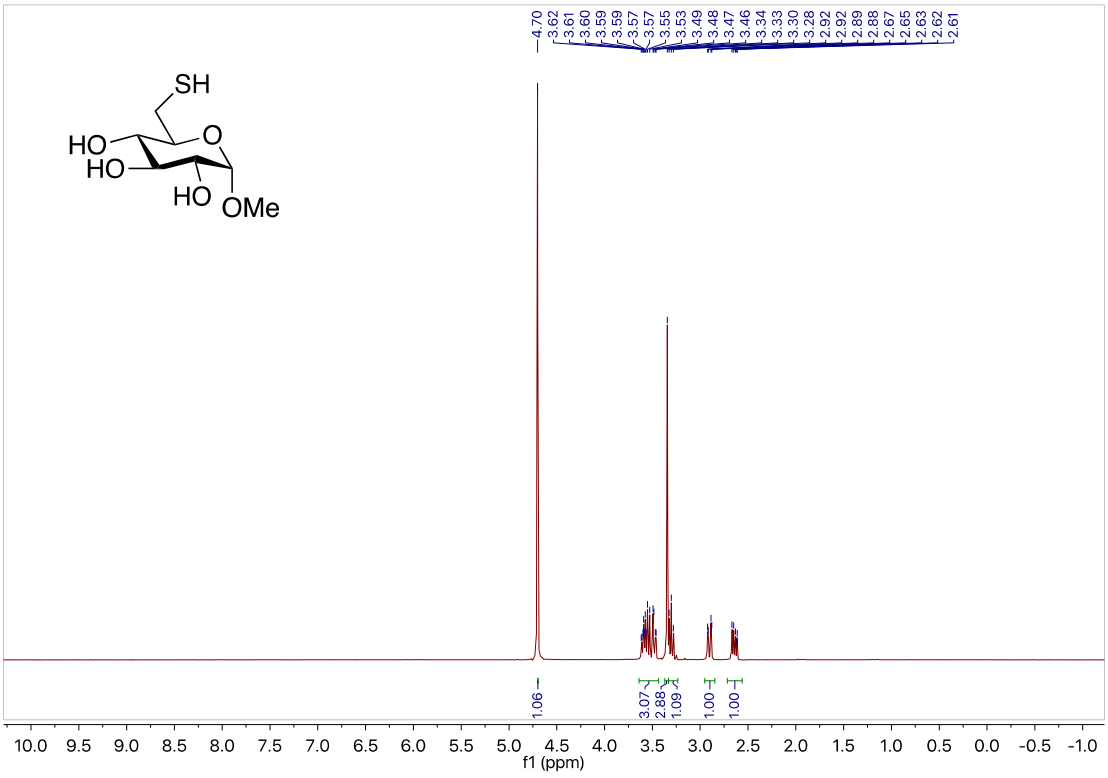
^{19}F NMR of compound **13**



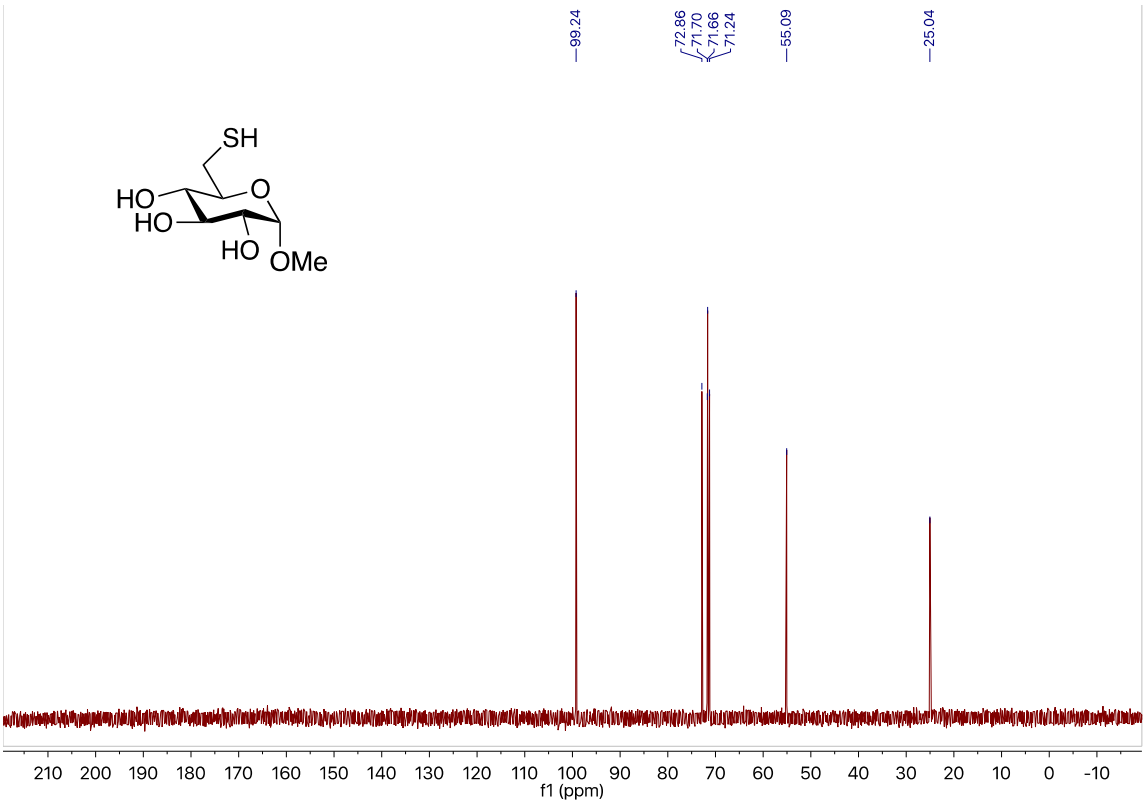
^{1}H NMR of compound **18**



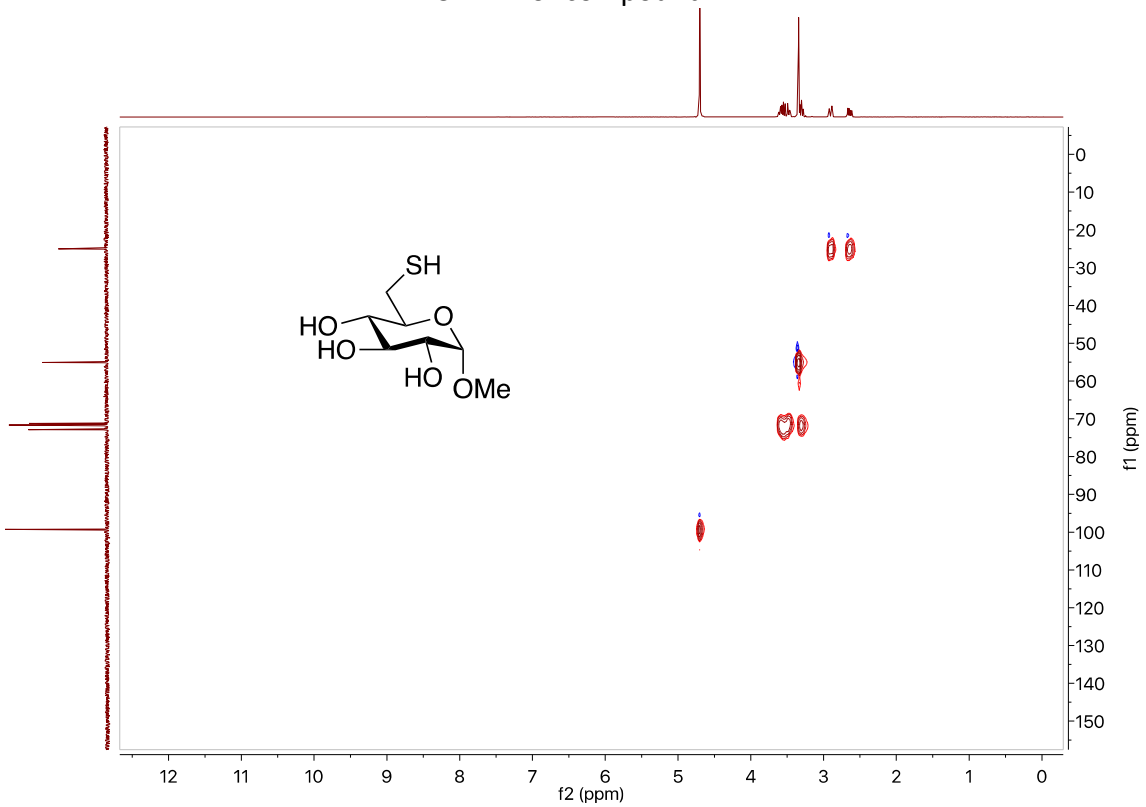
^{13}C NMR of compound **18**



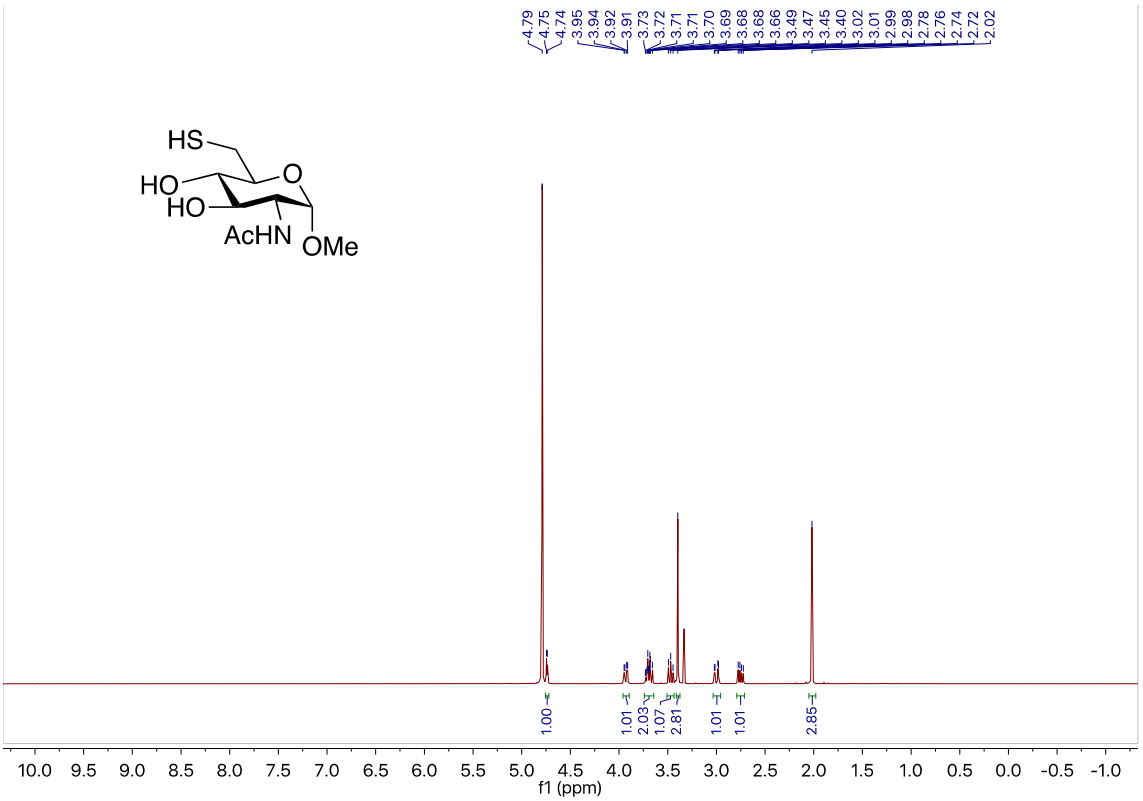
^{1}H NMR of compound **2**



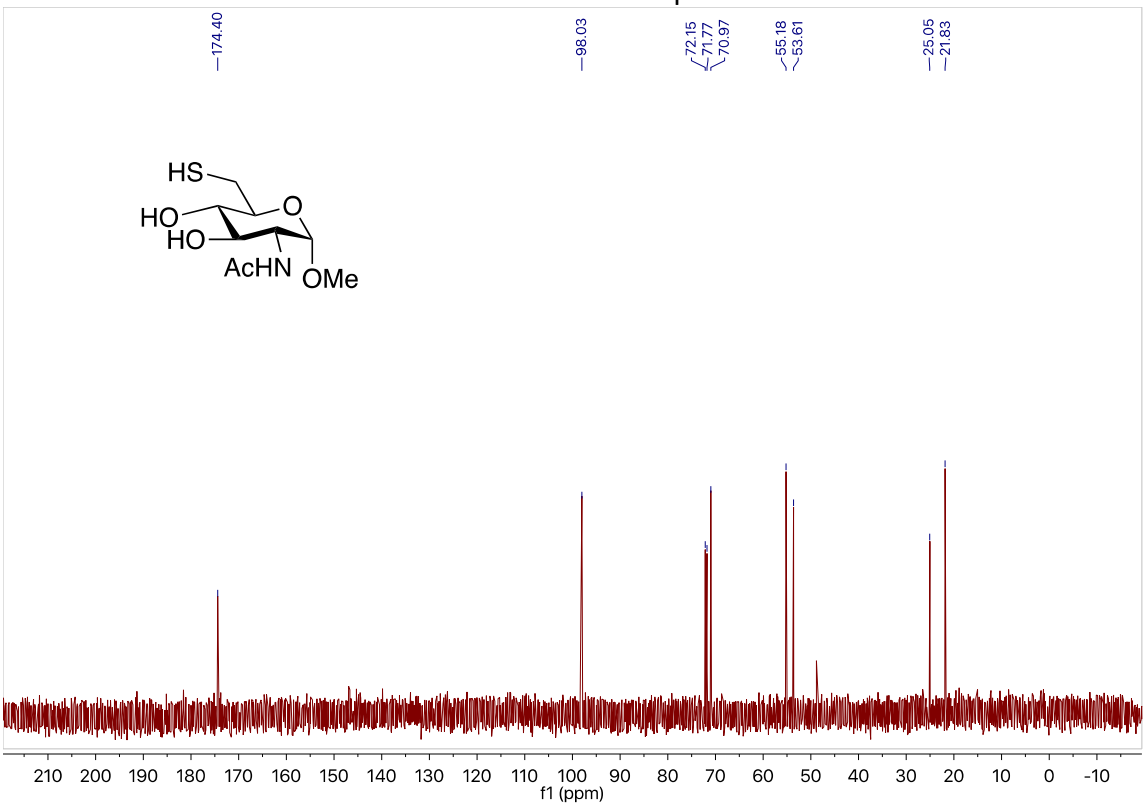
^{13}C NMR of compound **2**



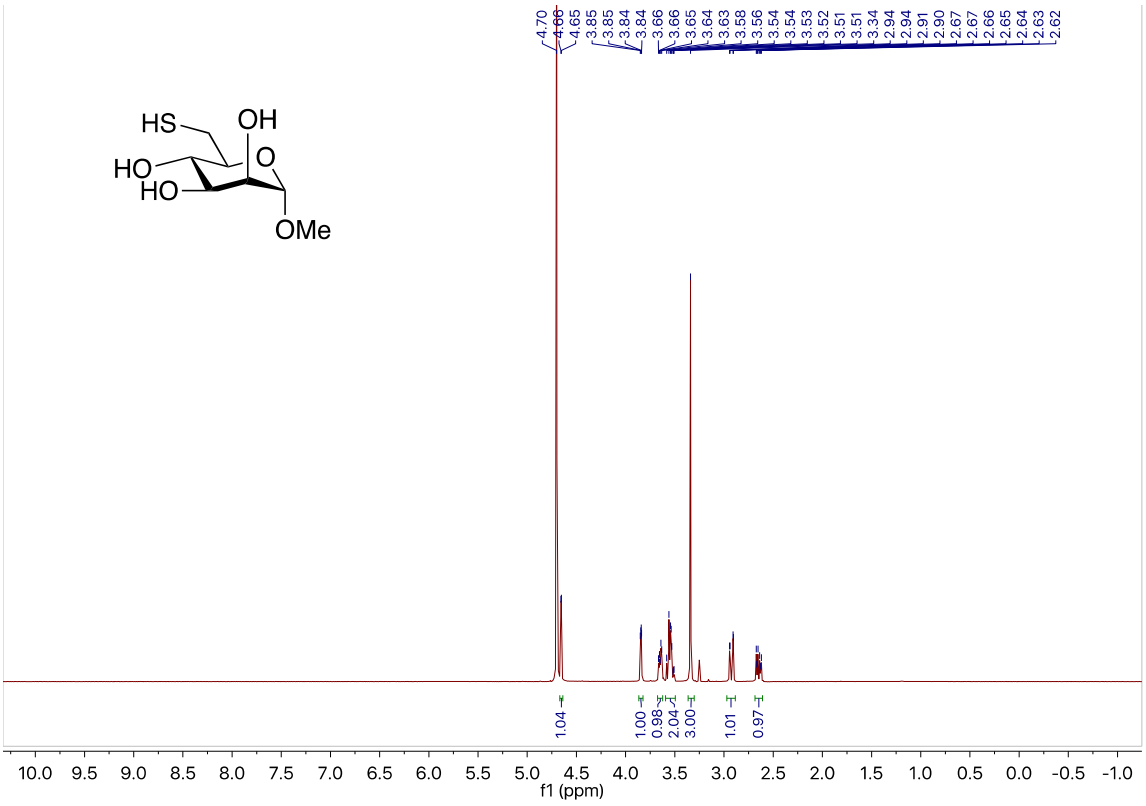
HSQC NMR of compound **2**



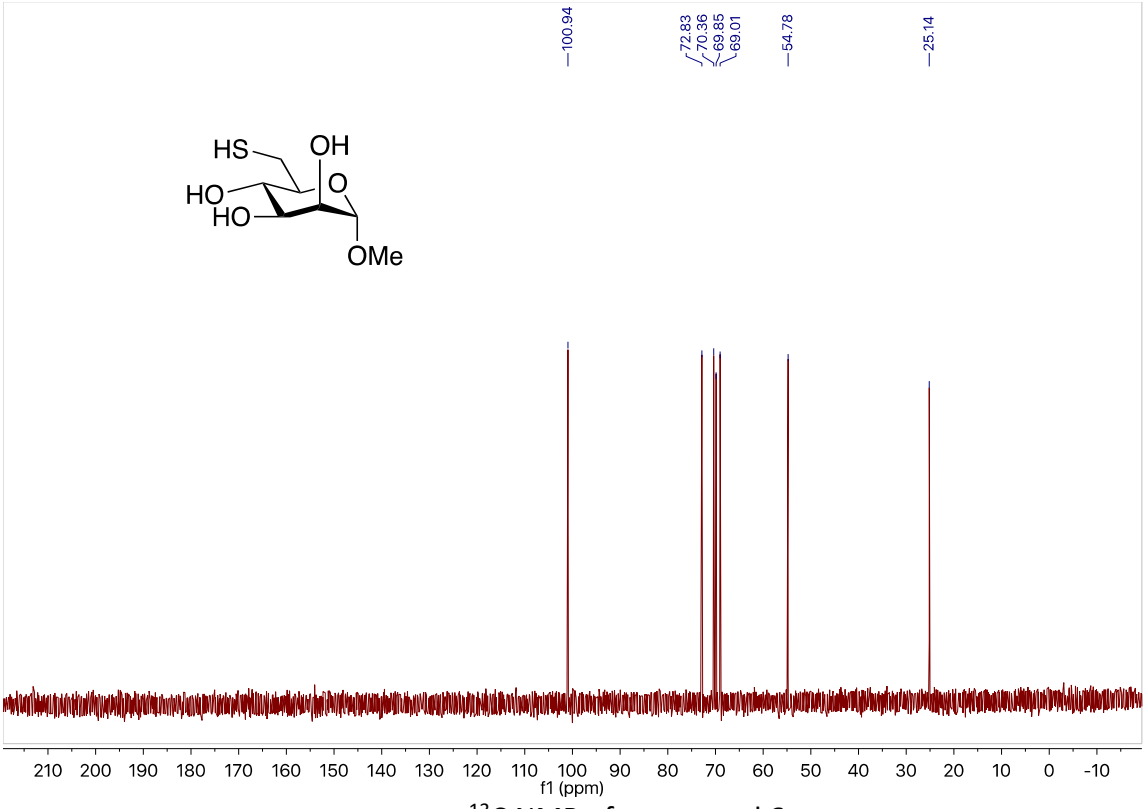
^{1}H NMR of compound **4**



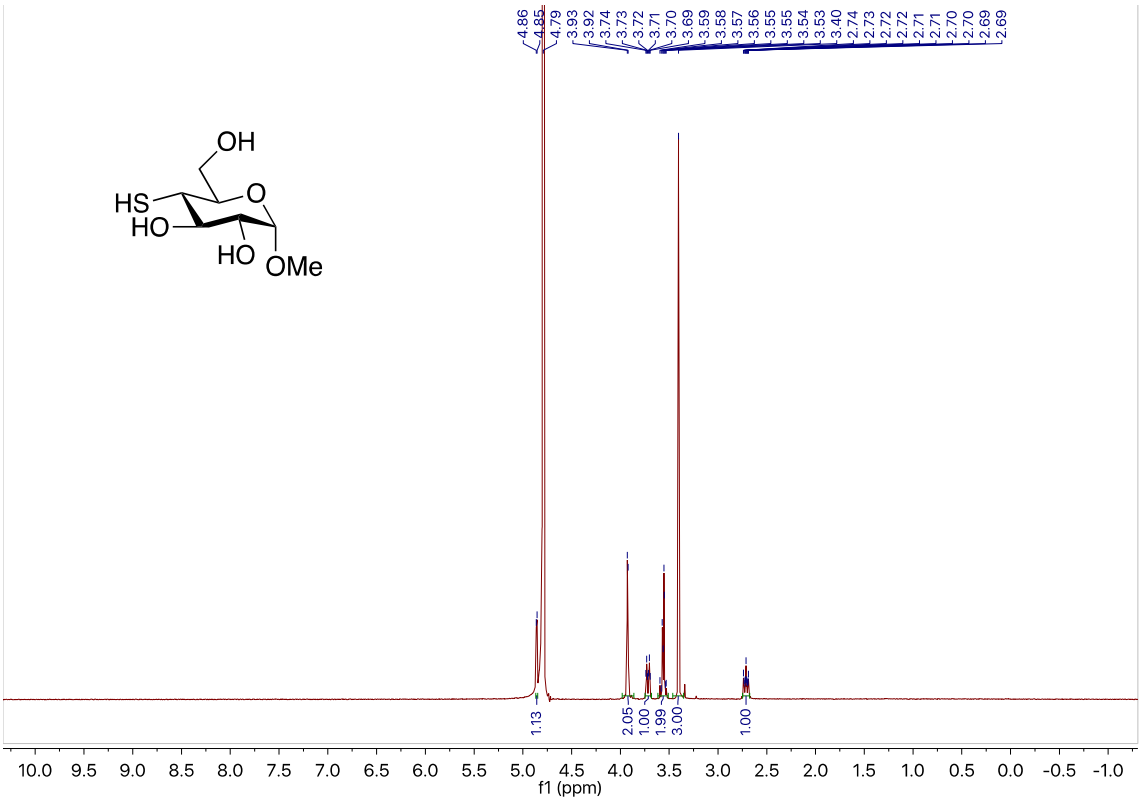
^{13}C NMR of compound **4**



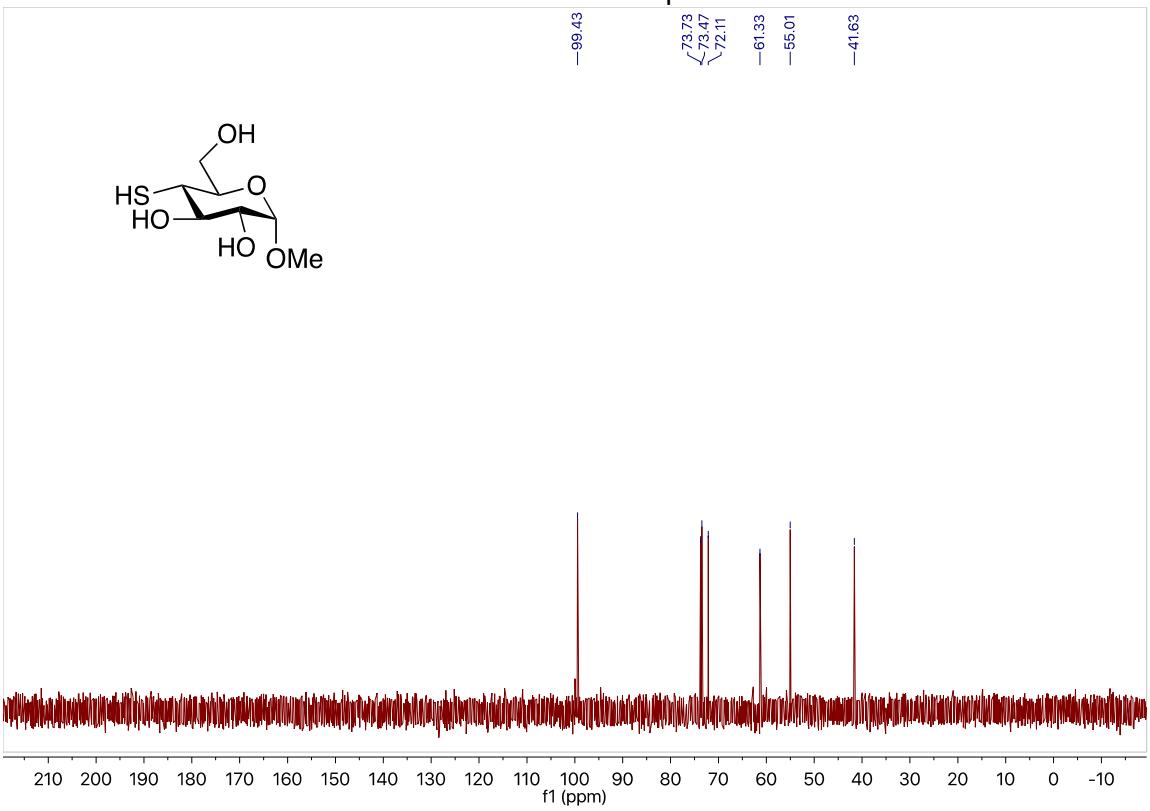
^{1}H NMR of compound **6**



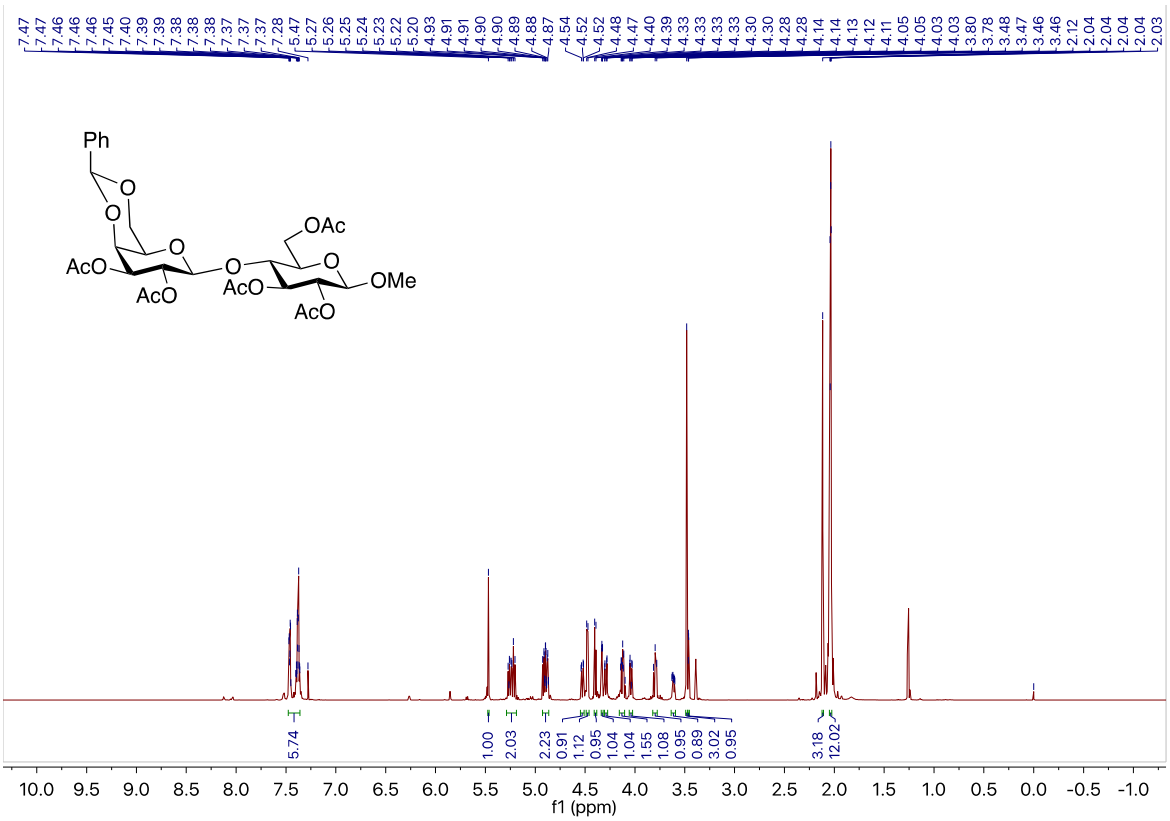
^{13}C NMR of compound **6**



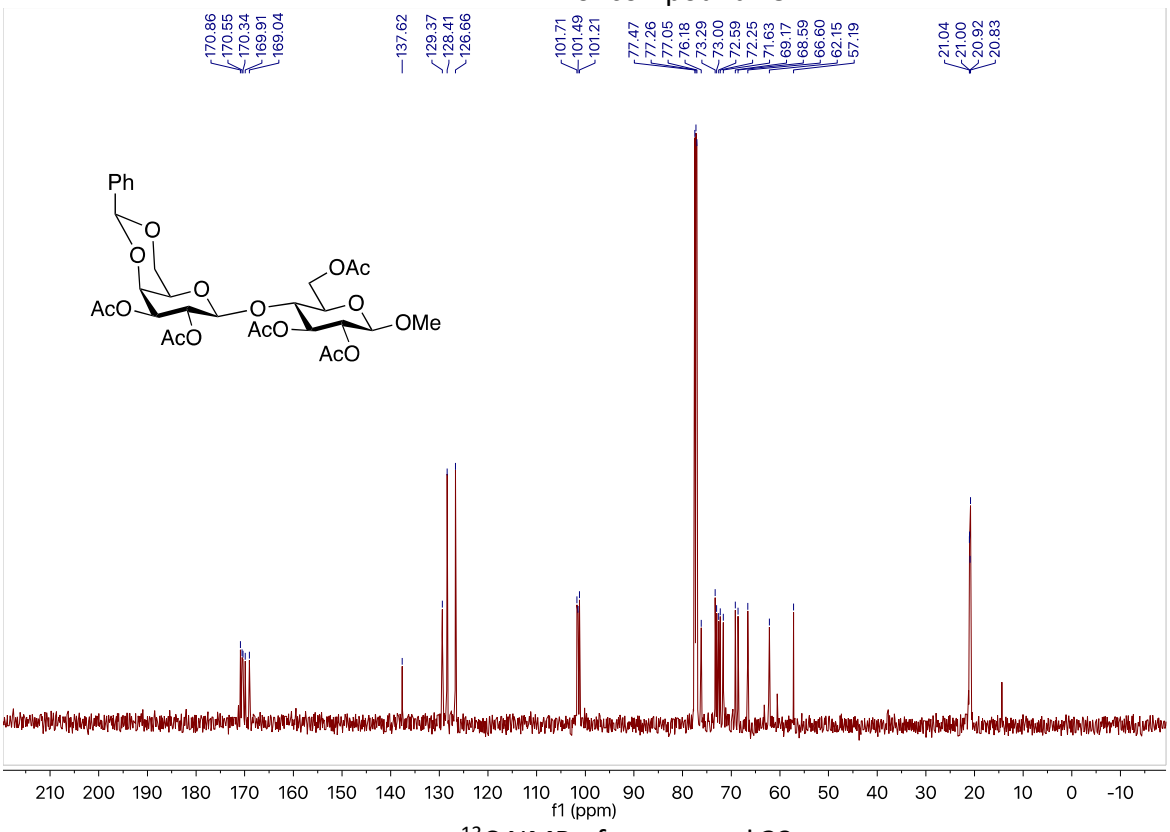
^{1}H NMR of compound **11**



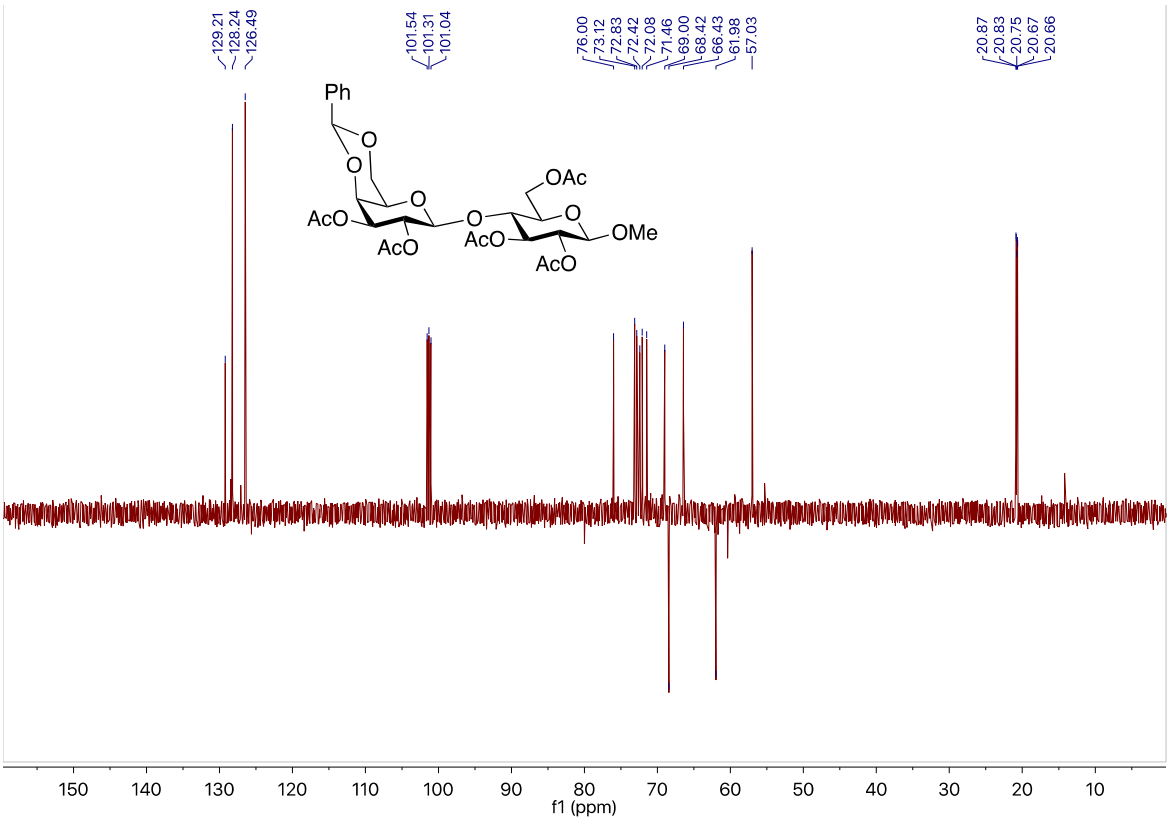
^{13}C NMR of compound **11**



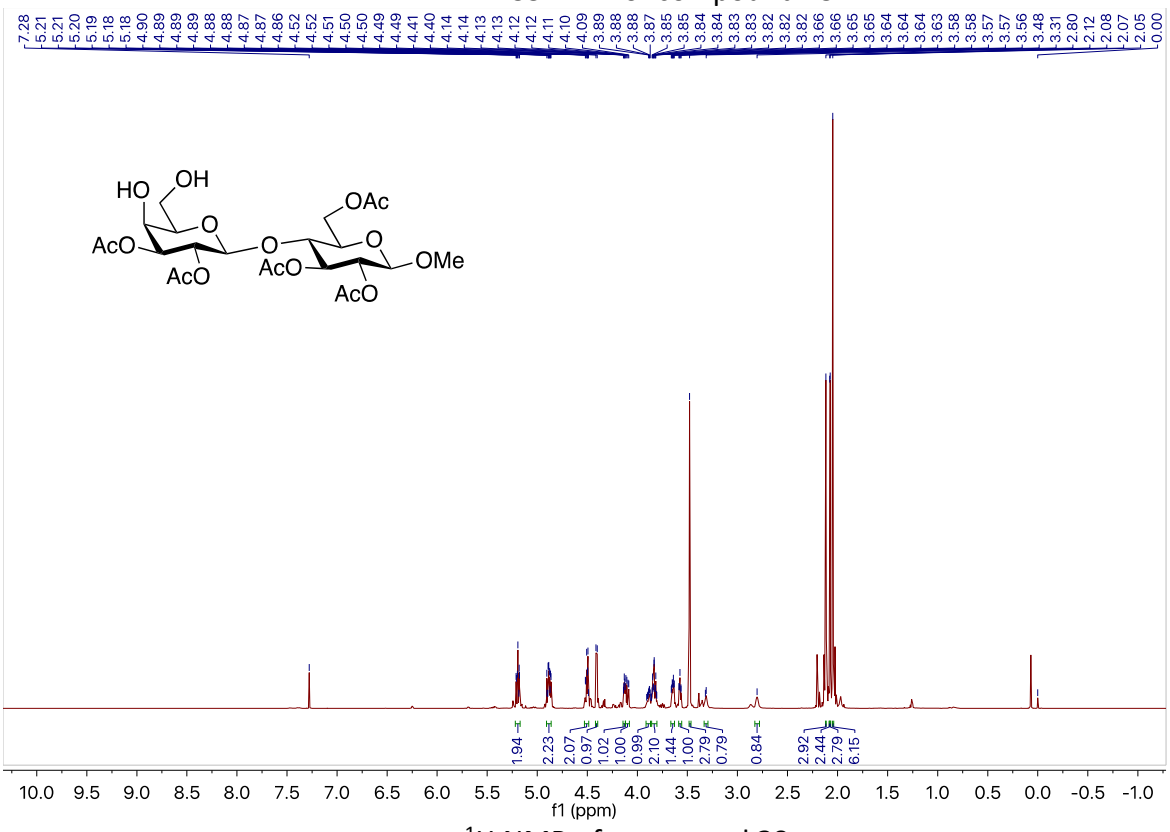
^{1}H NMR of compound **29**



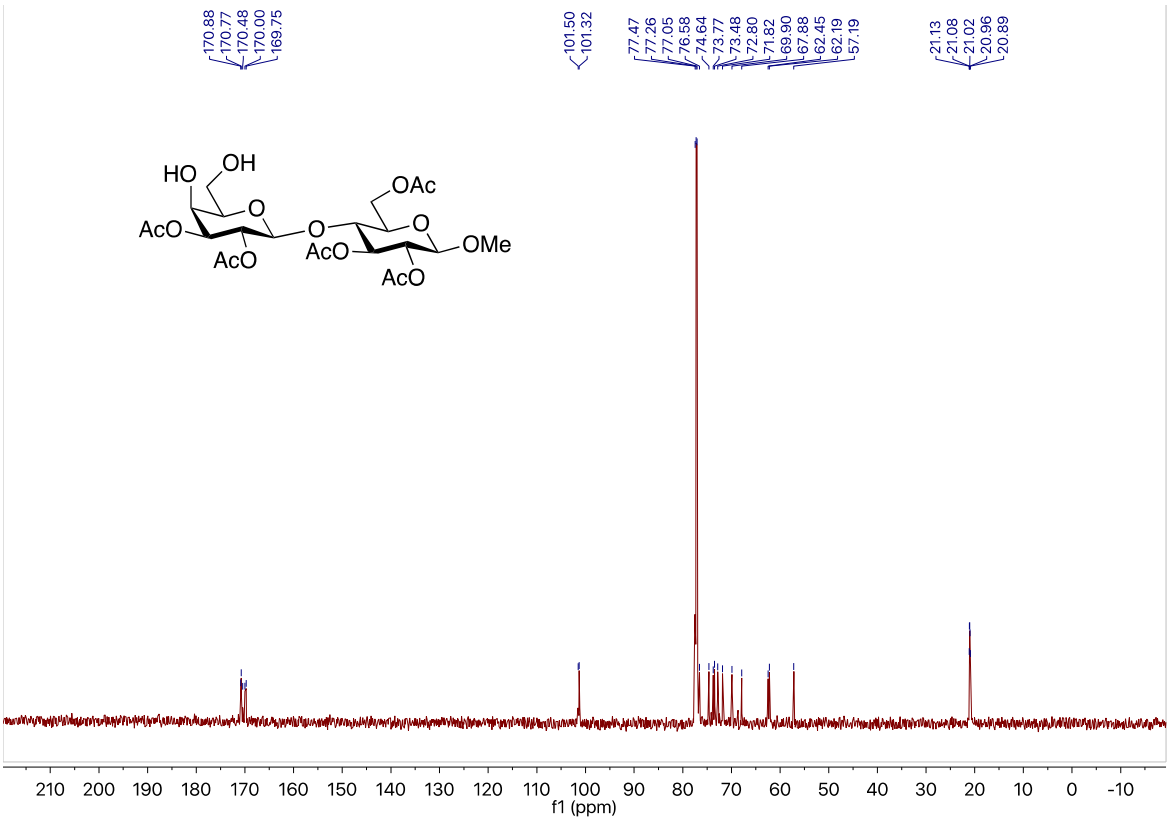
^{13}C NMR of compound **29**



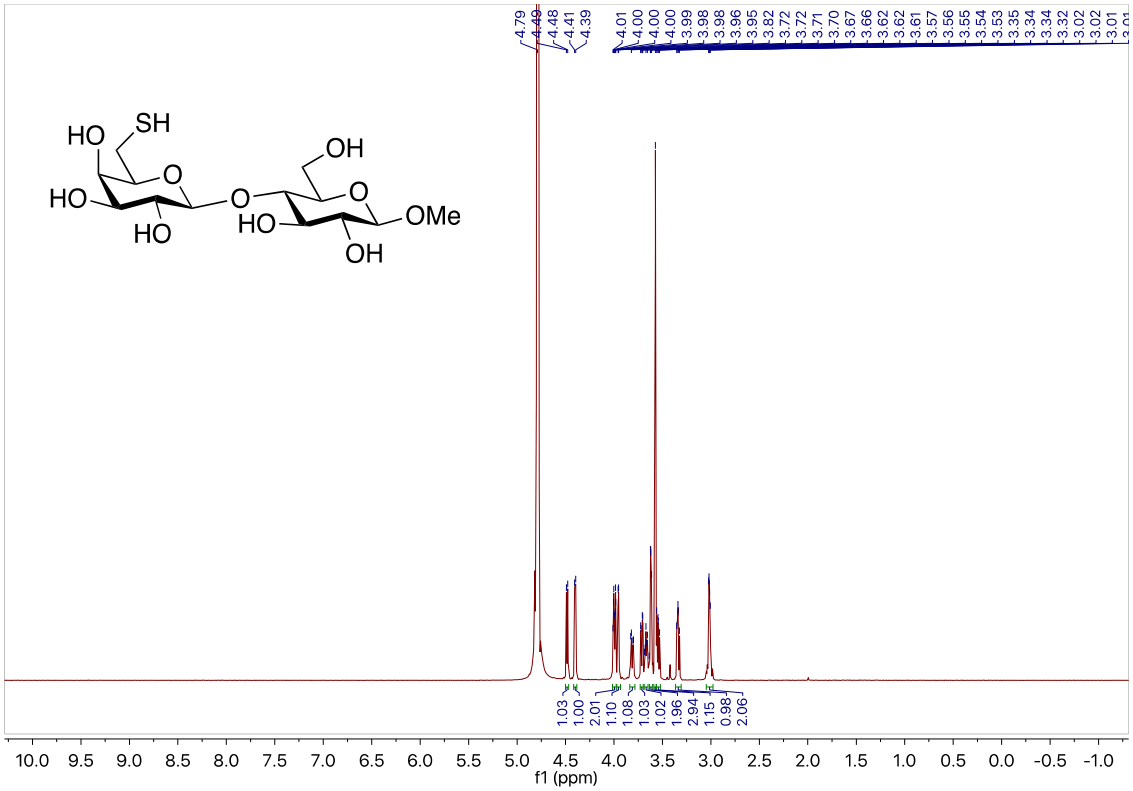
DEPT-135 NMR of compound **29**



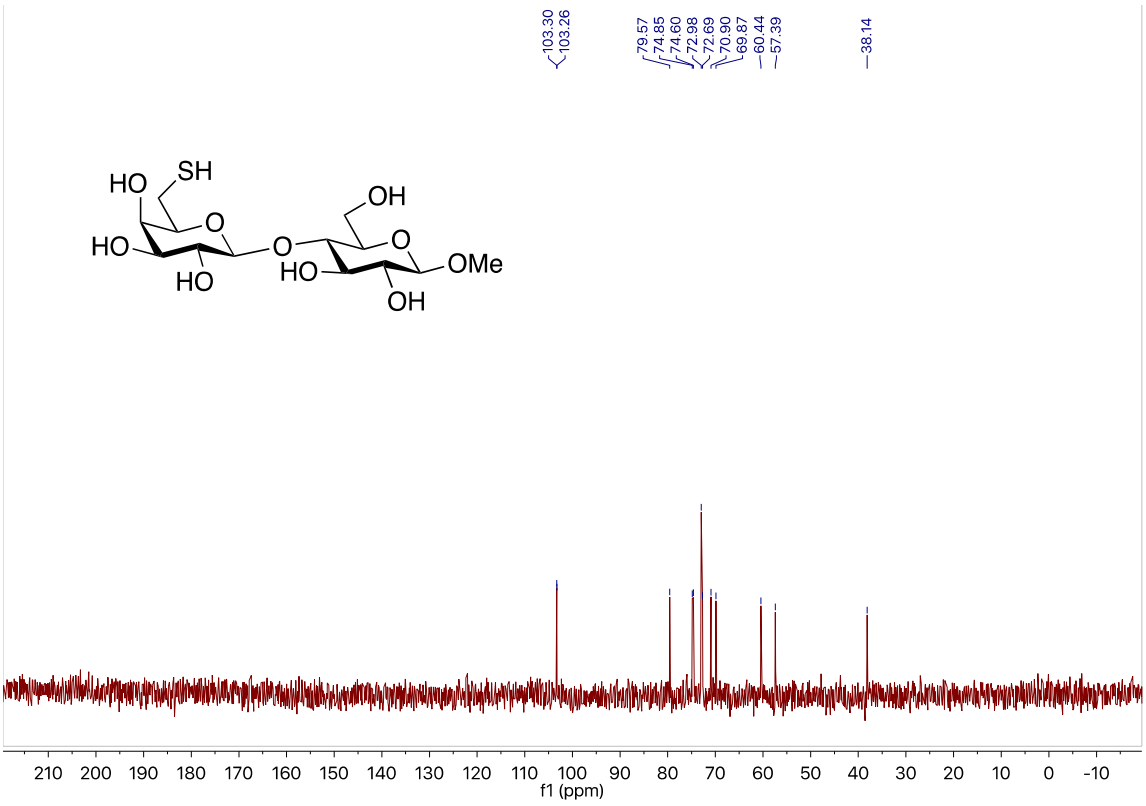
^{1}H NMR of compound **30**



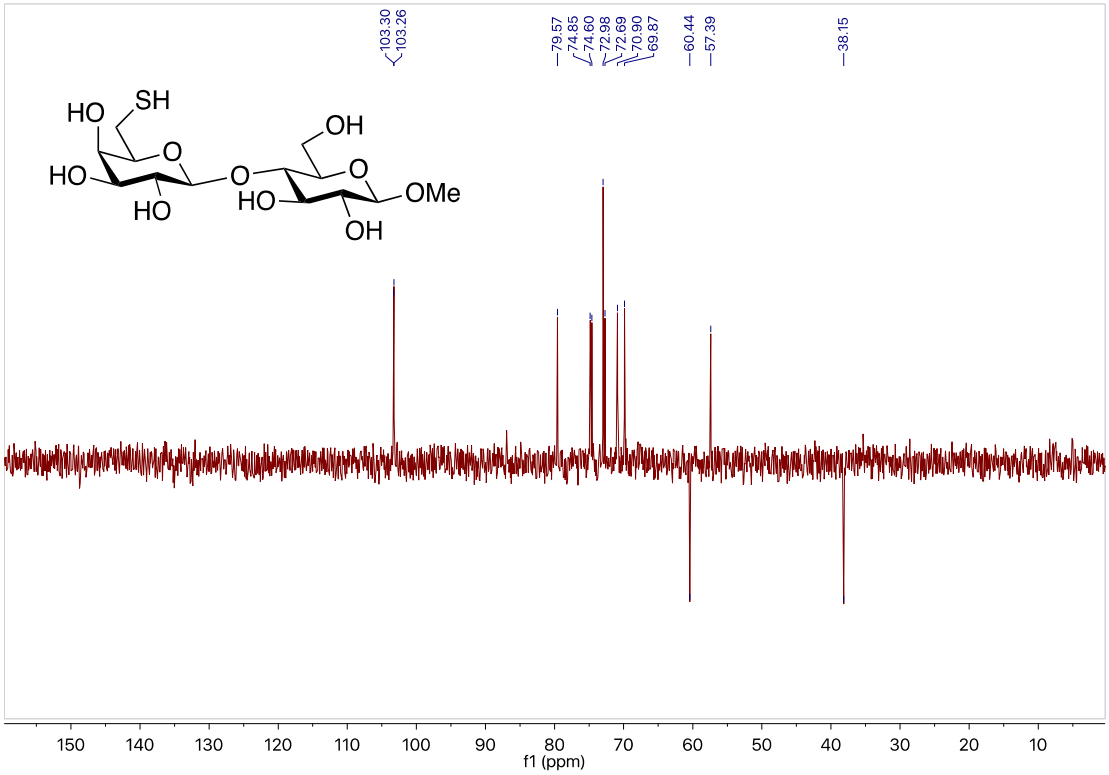
^{13}C NMR of compound **30**



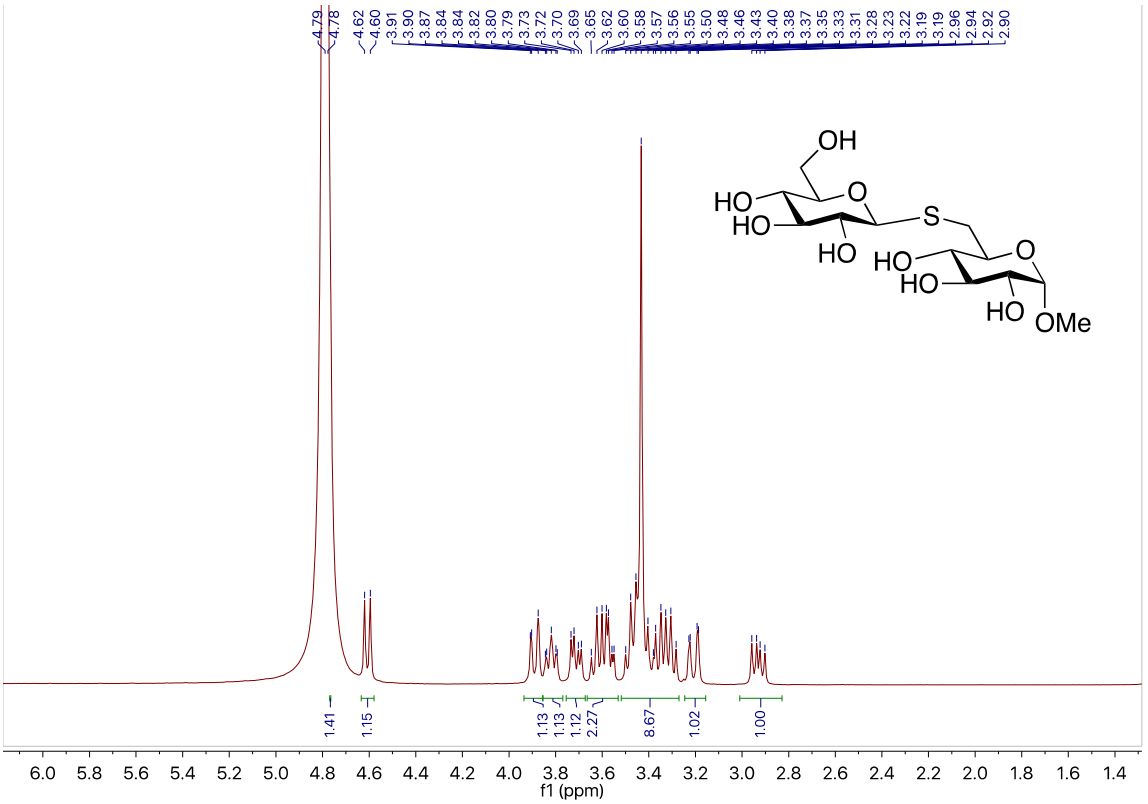
^{1}H NMR of compound **21**



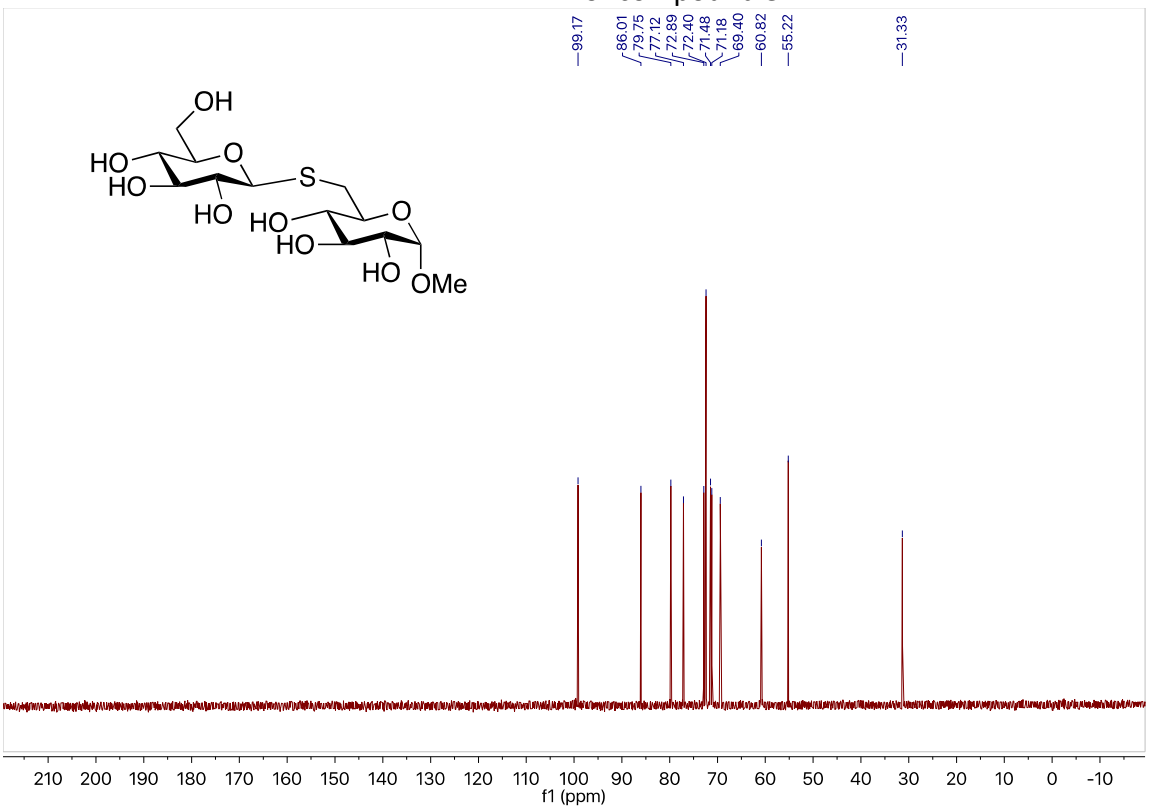
^{13}C NMR of compound **21**



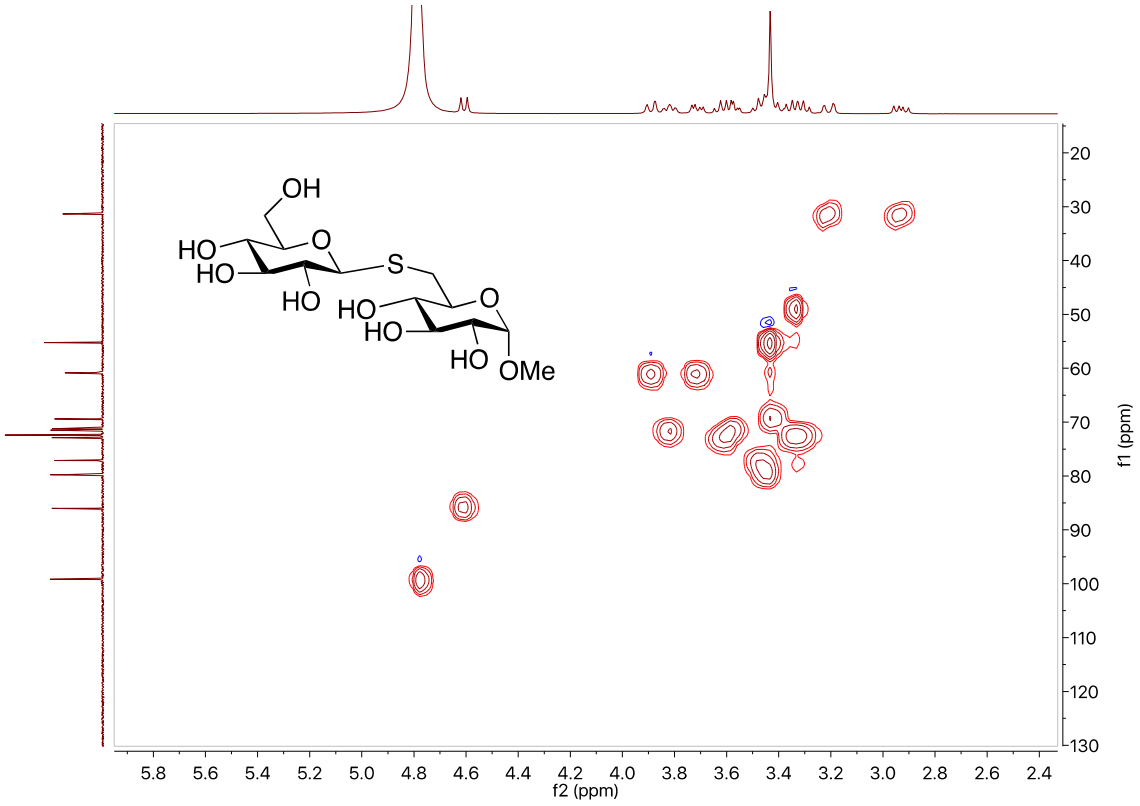
DEPT-135 NMR of compound **21**



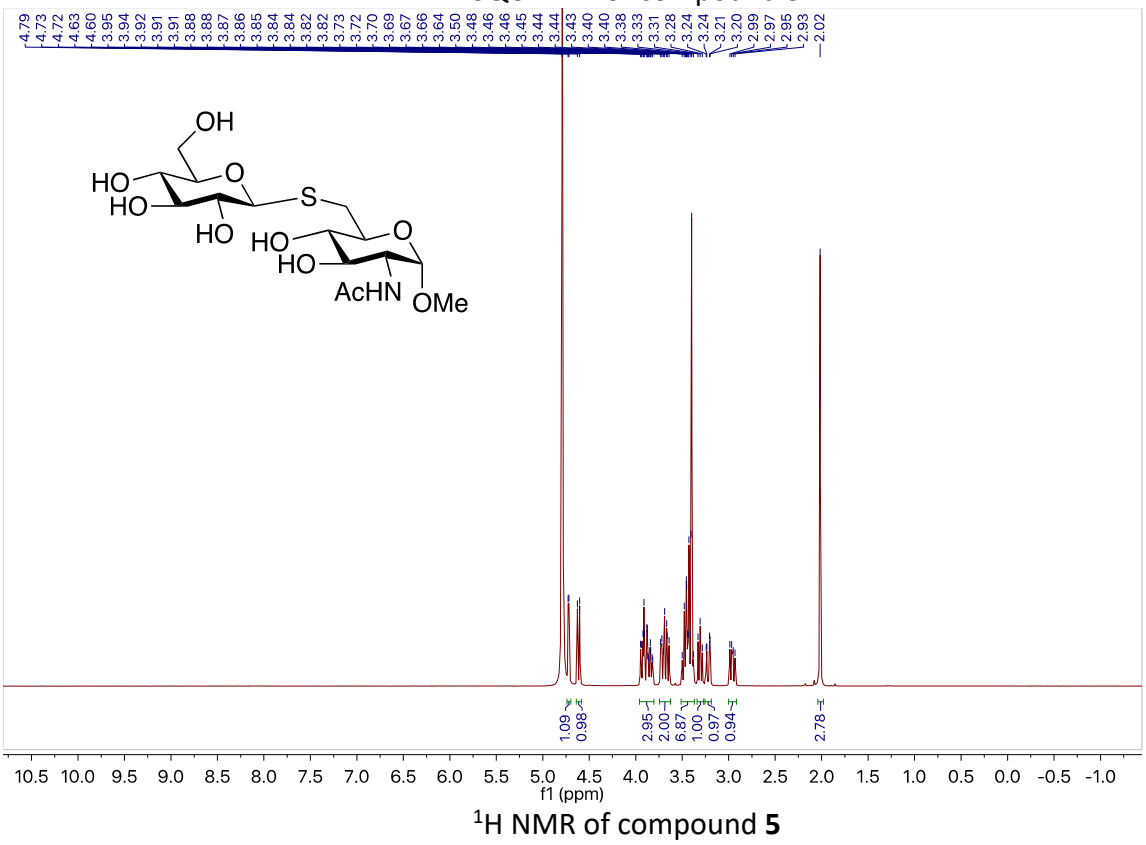
^{1}H NMR of compound **3**



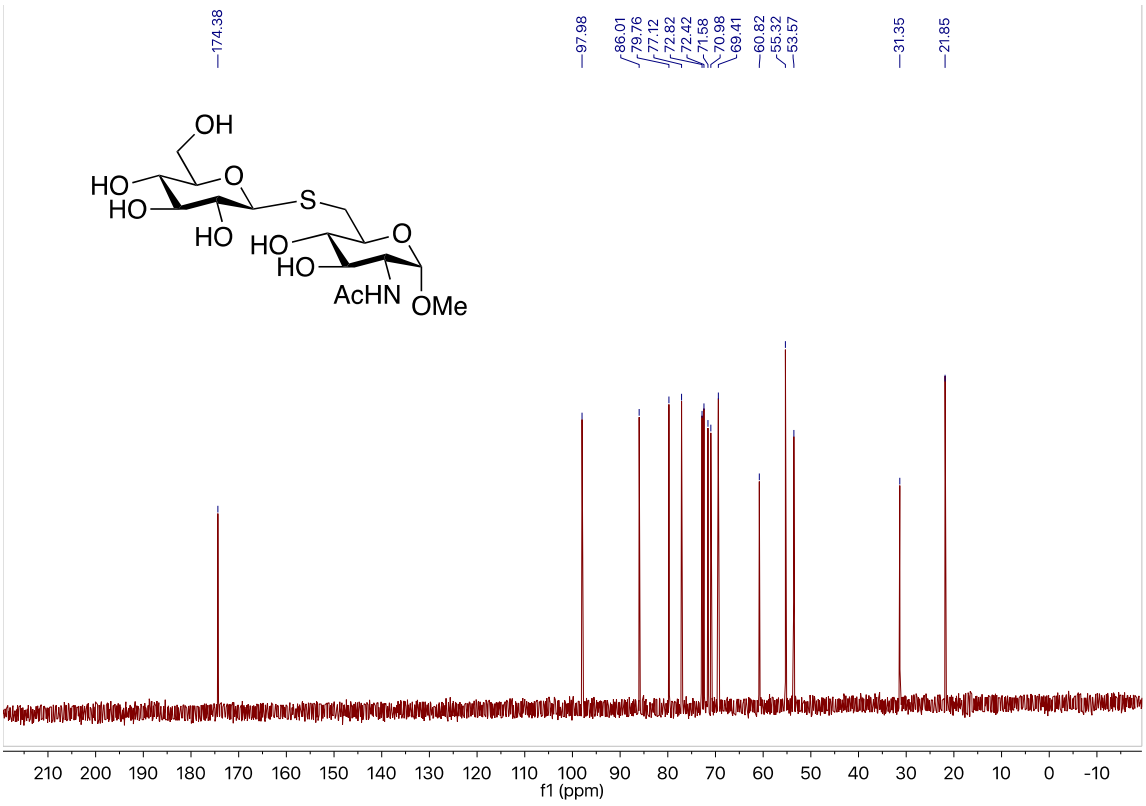
^{13}C NMR of compound **3**



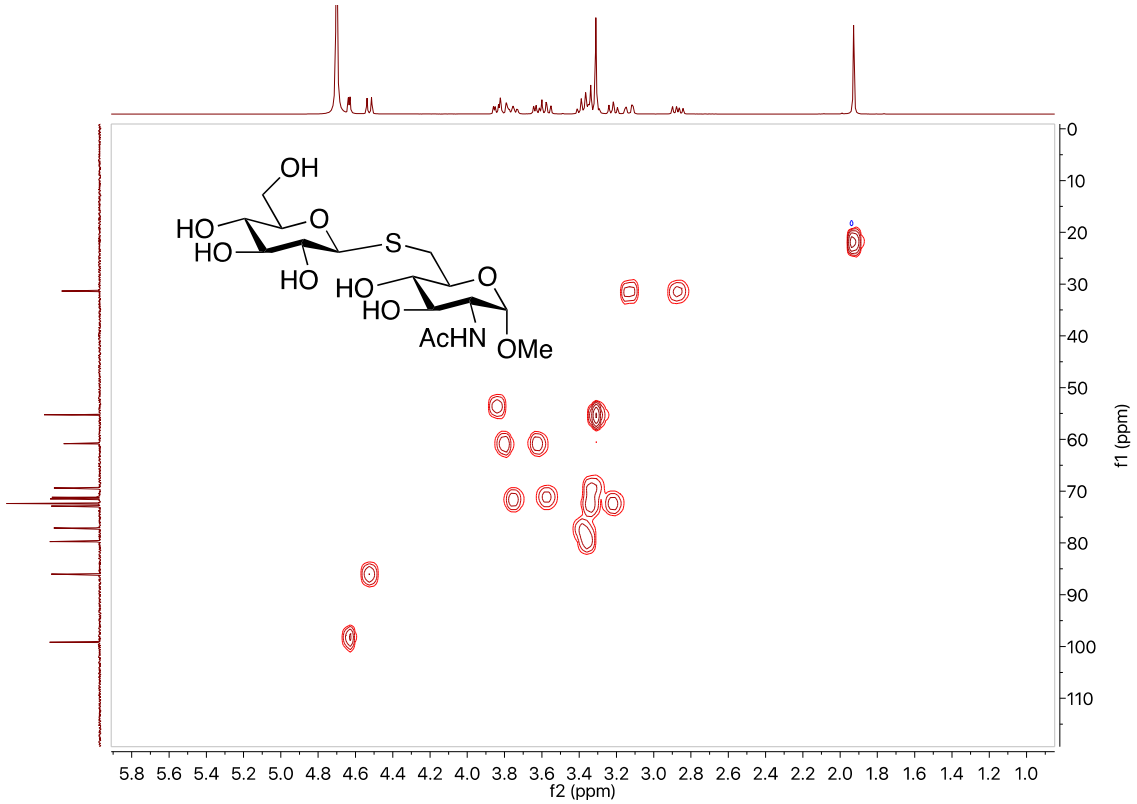
HSQC NMR of compound **3**



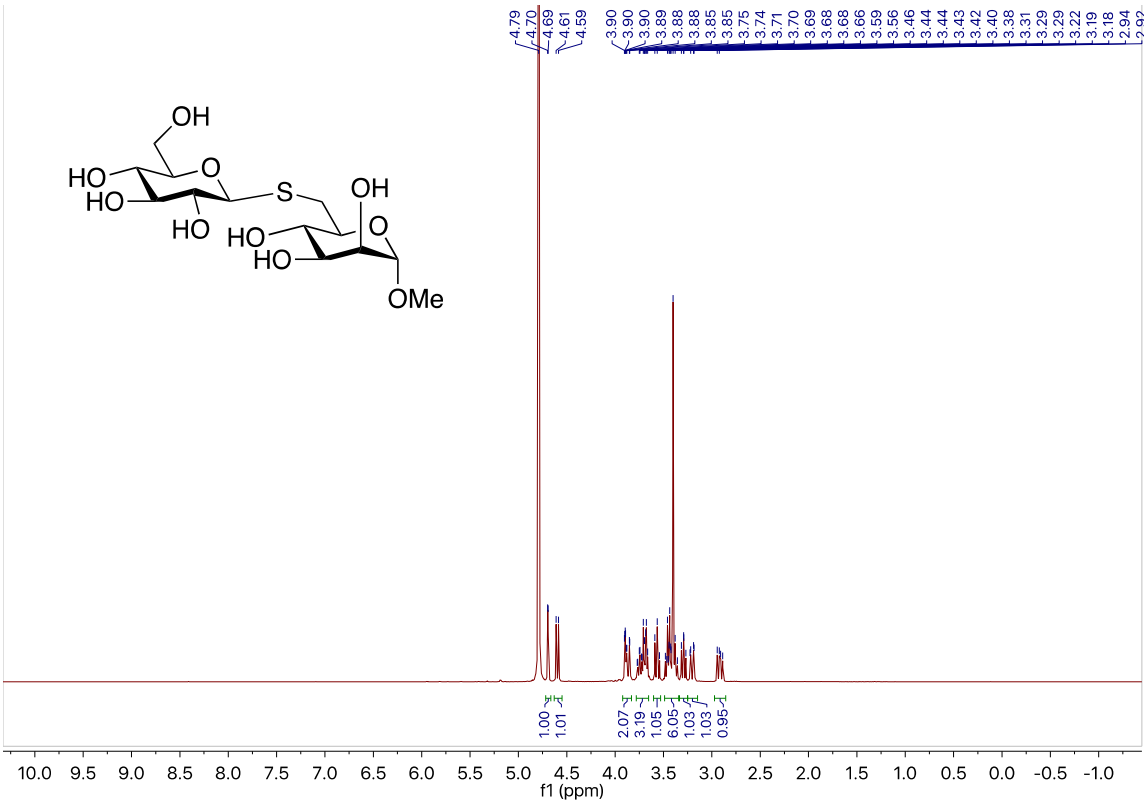
^{1}H NMR of compound **5**



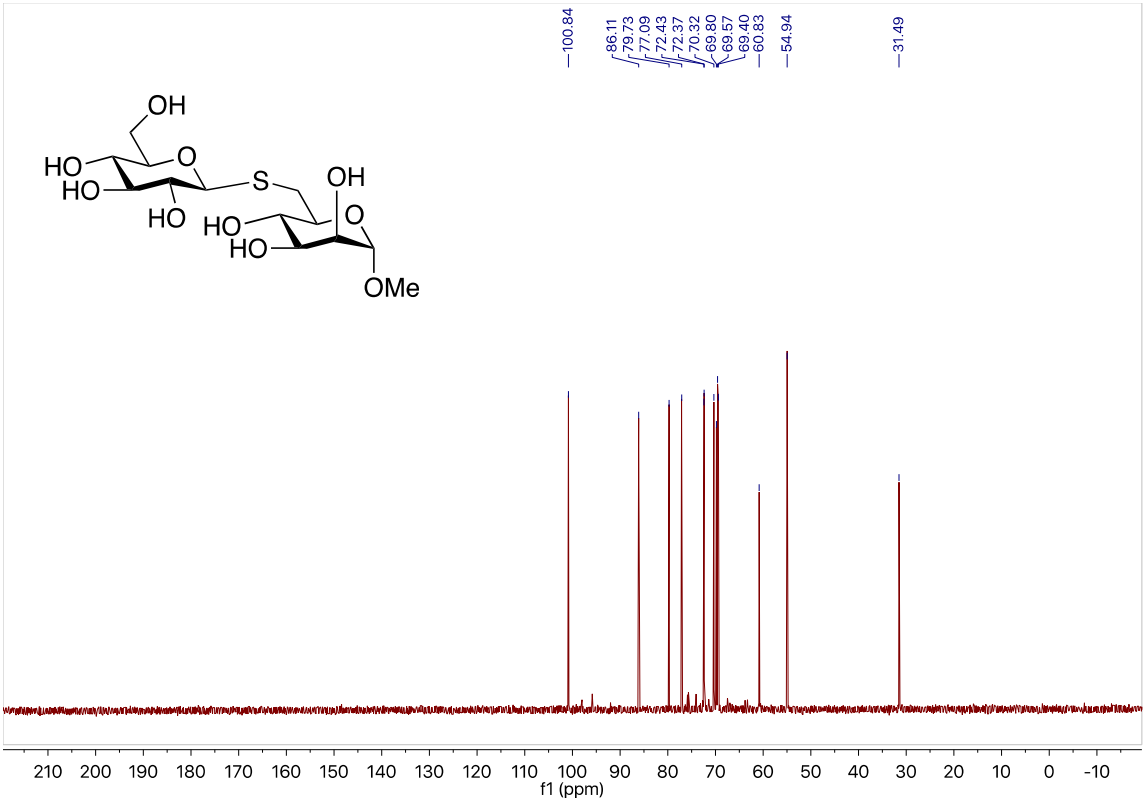
^{13}C NMR of compound **5**



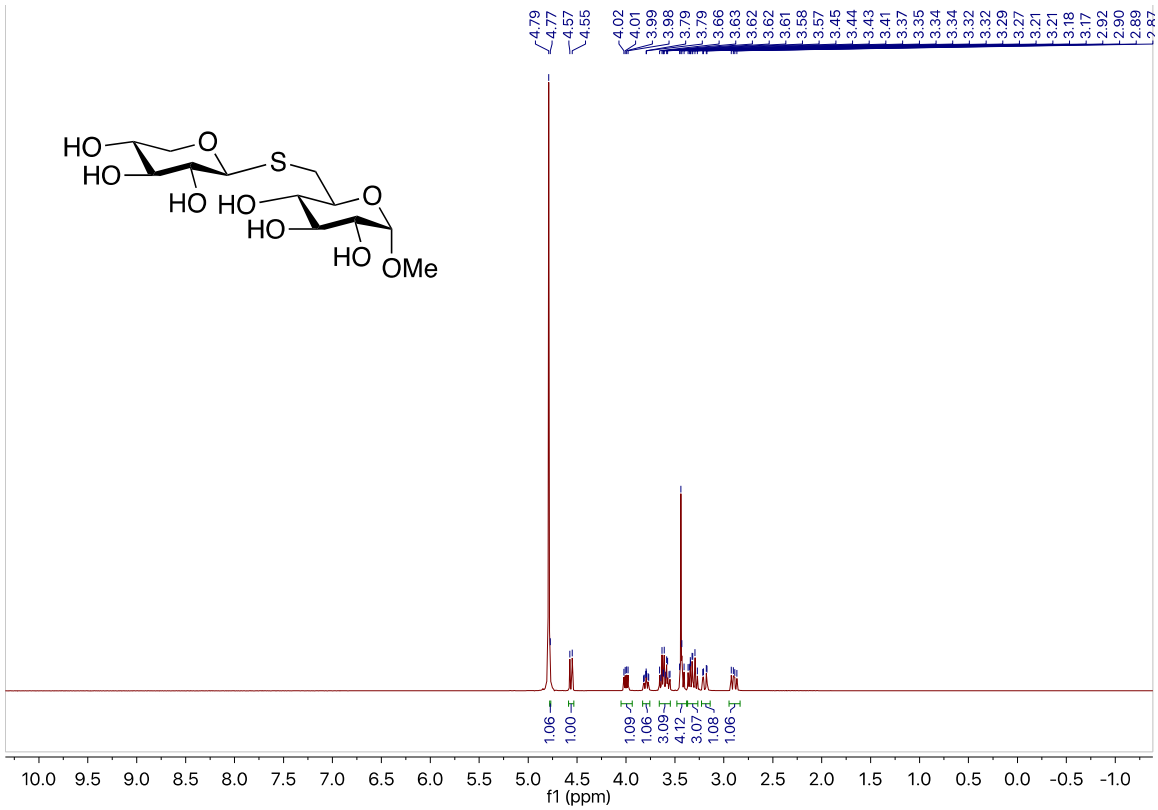
HSQC NMR of compound **5**



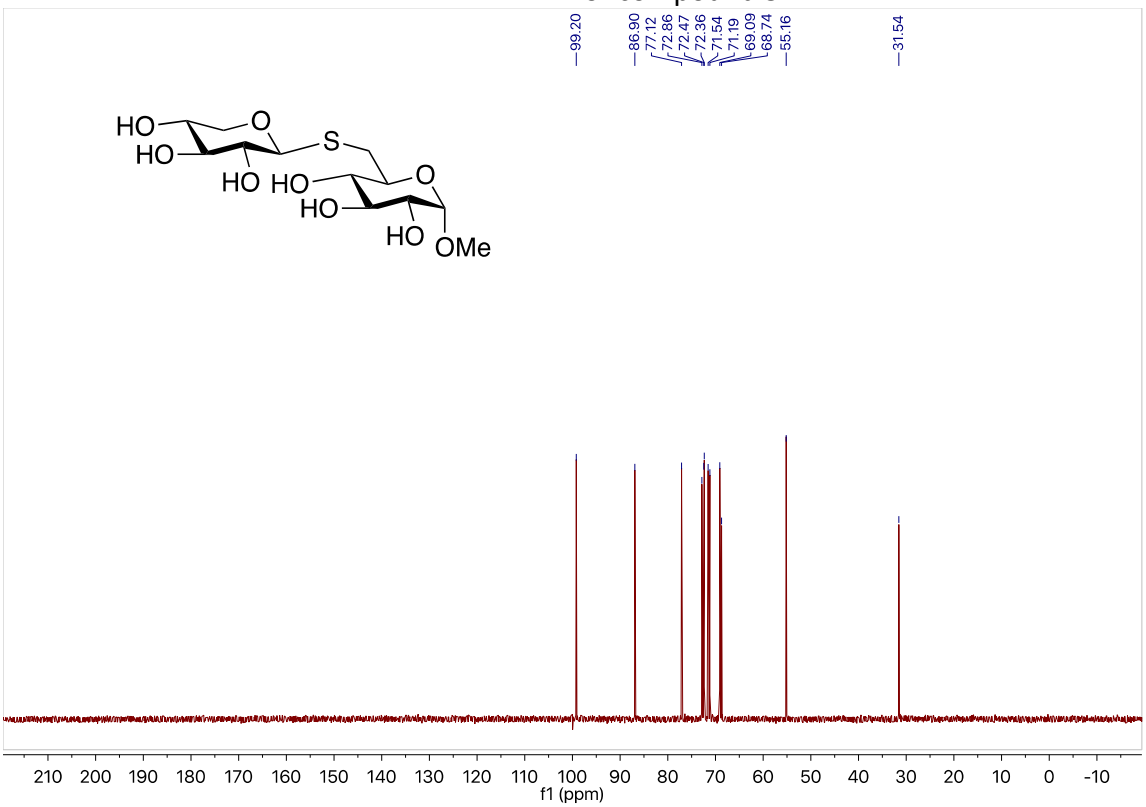
^{1}H NMR of compound **7**



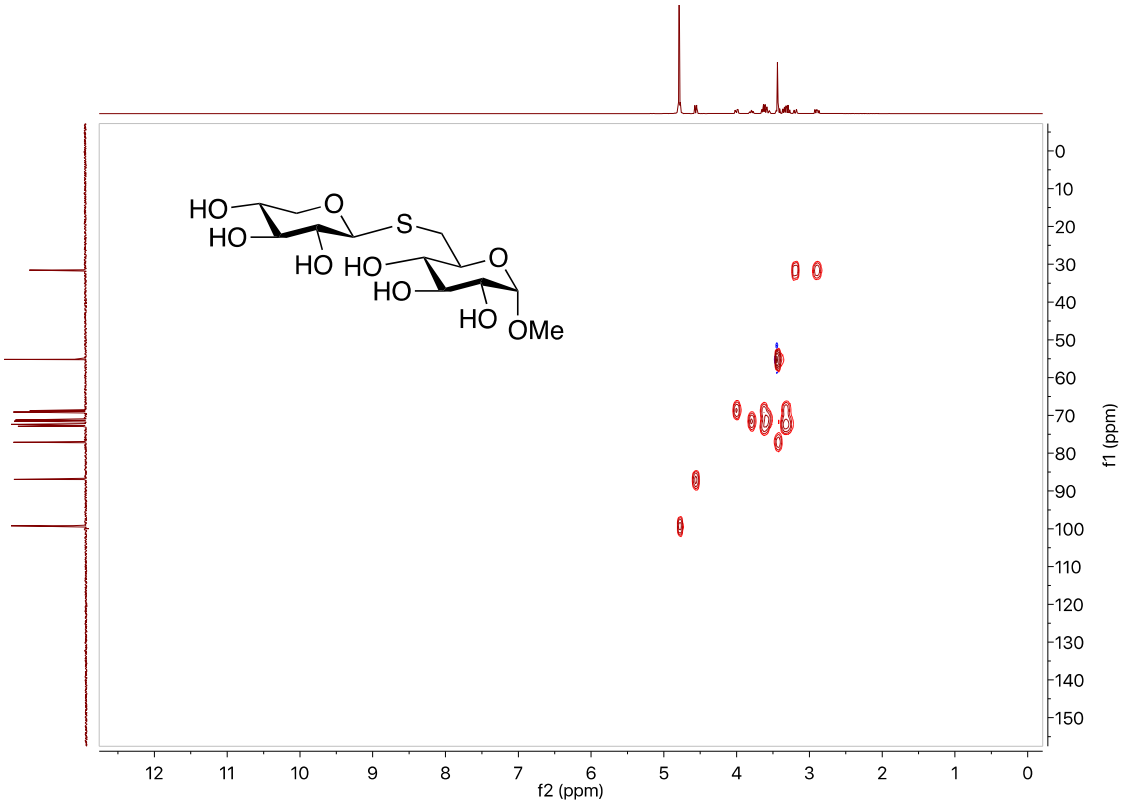
^{13}C NMR of compound **7**



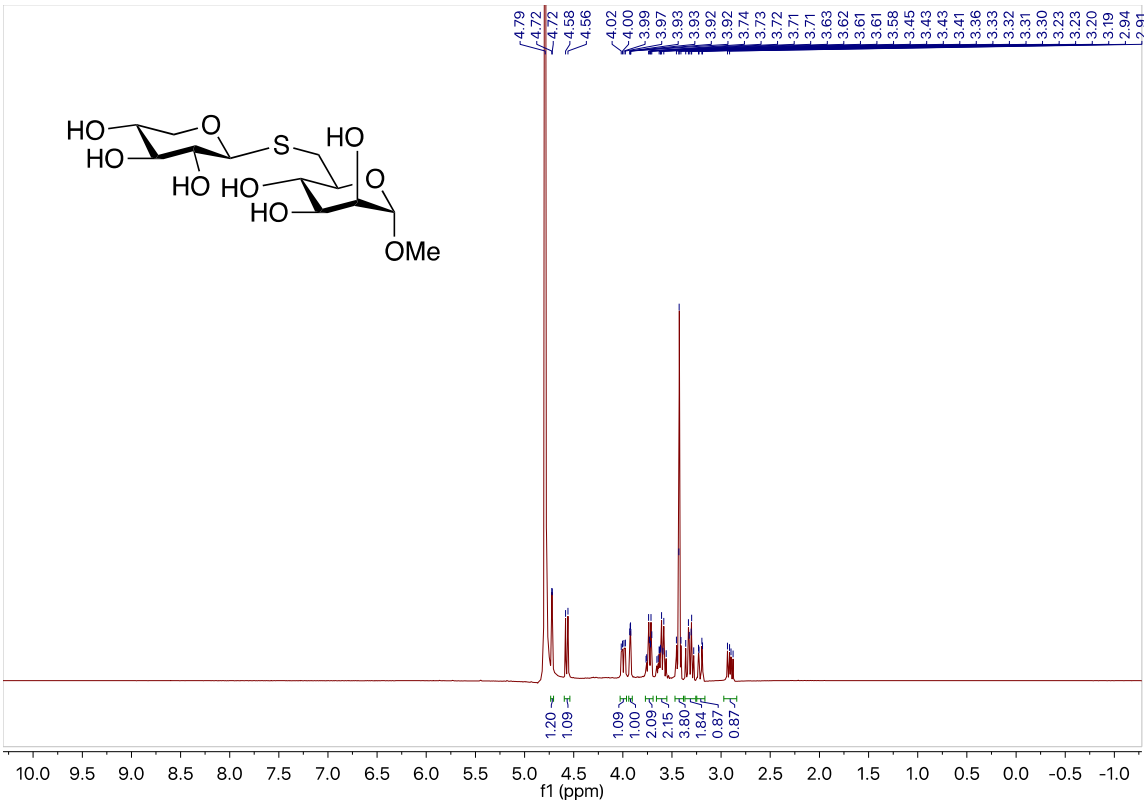
^{1}H NMR of compound **9**



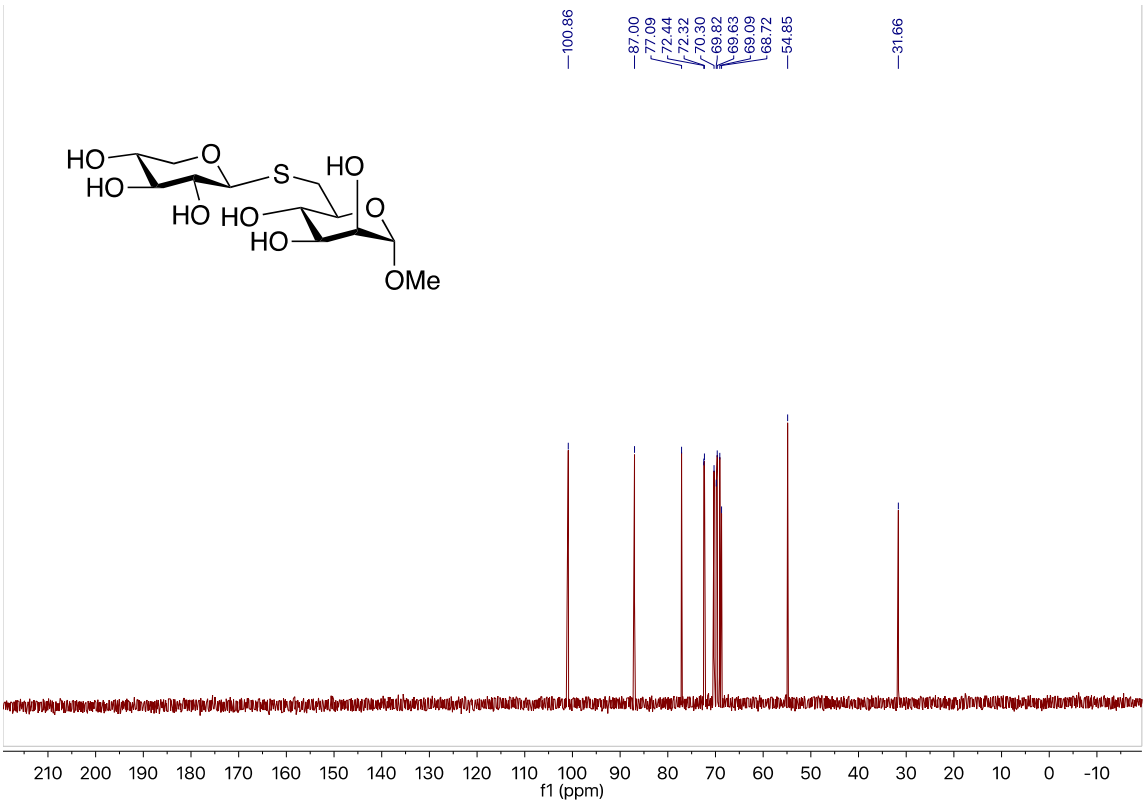
^{13}C NMR of compound **9**



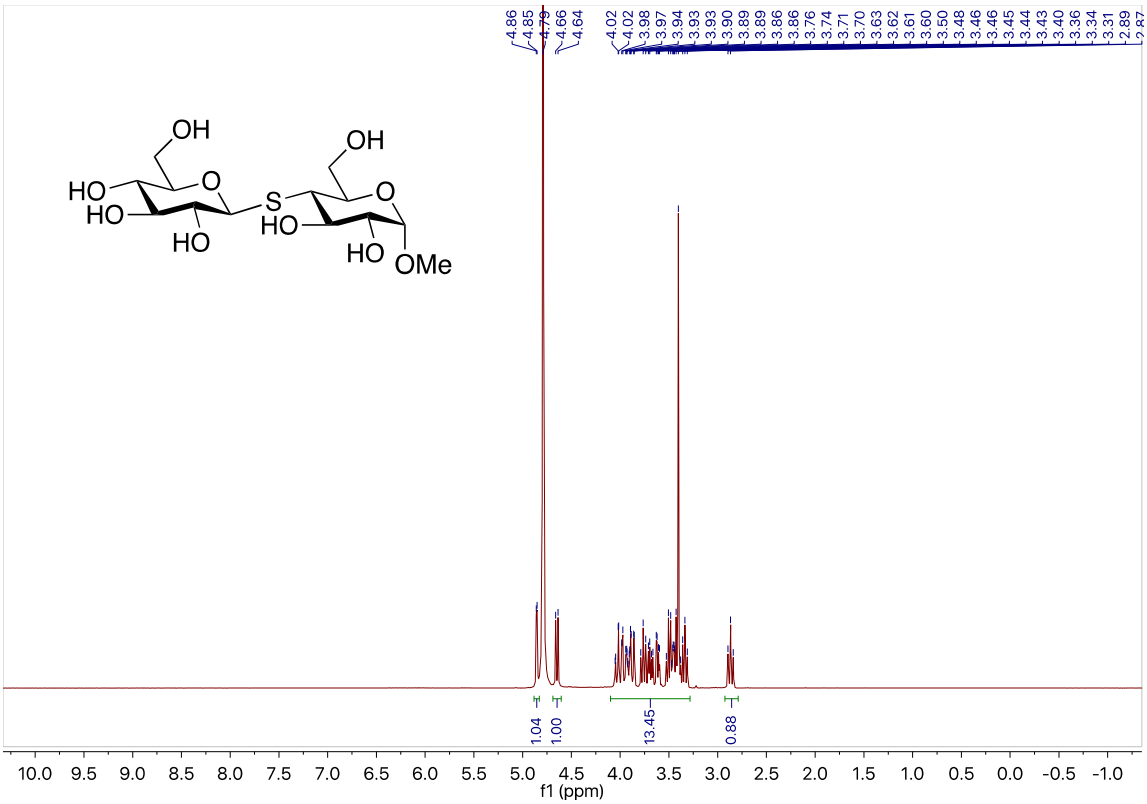
HSQC NMR of compound **9**



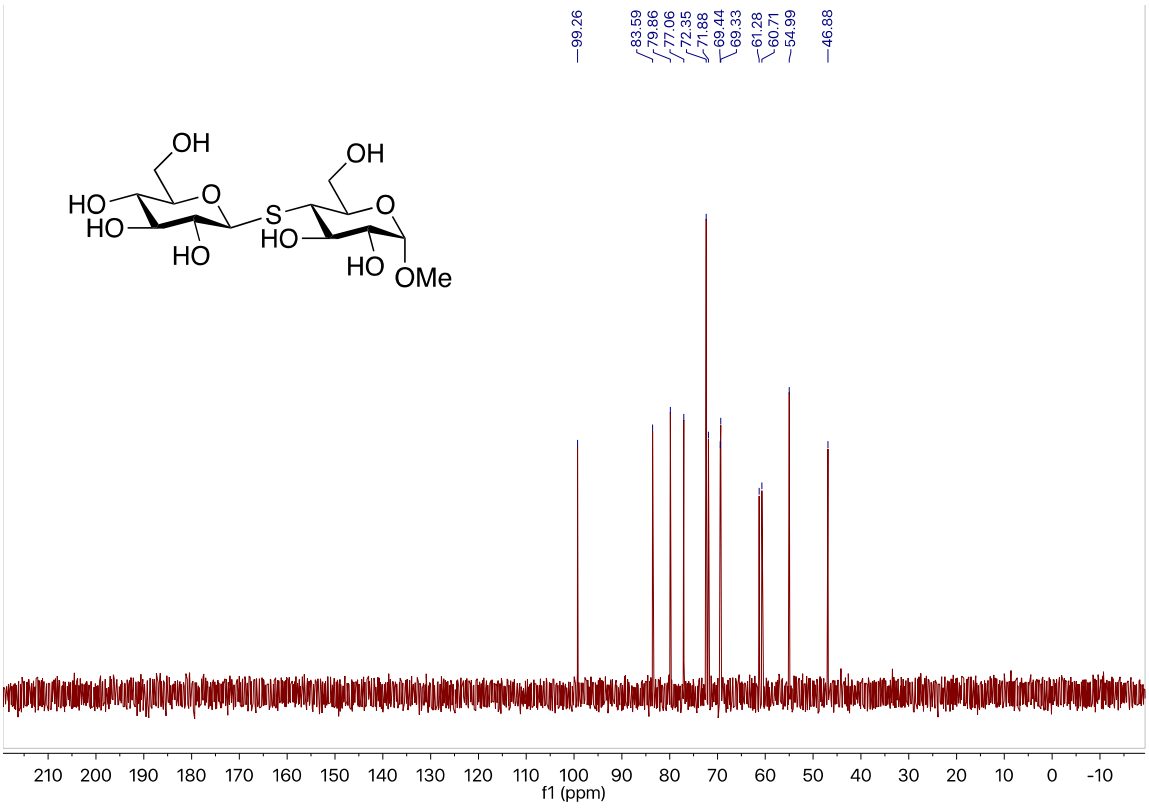
^{1}H NMR of compound **10**



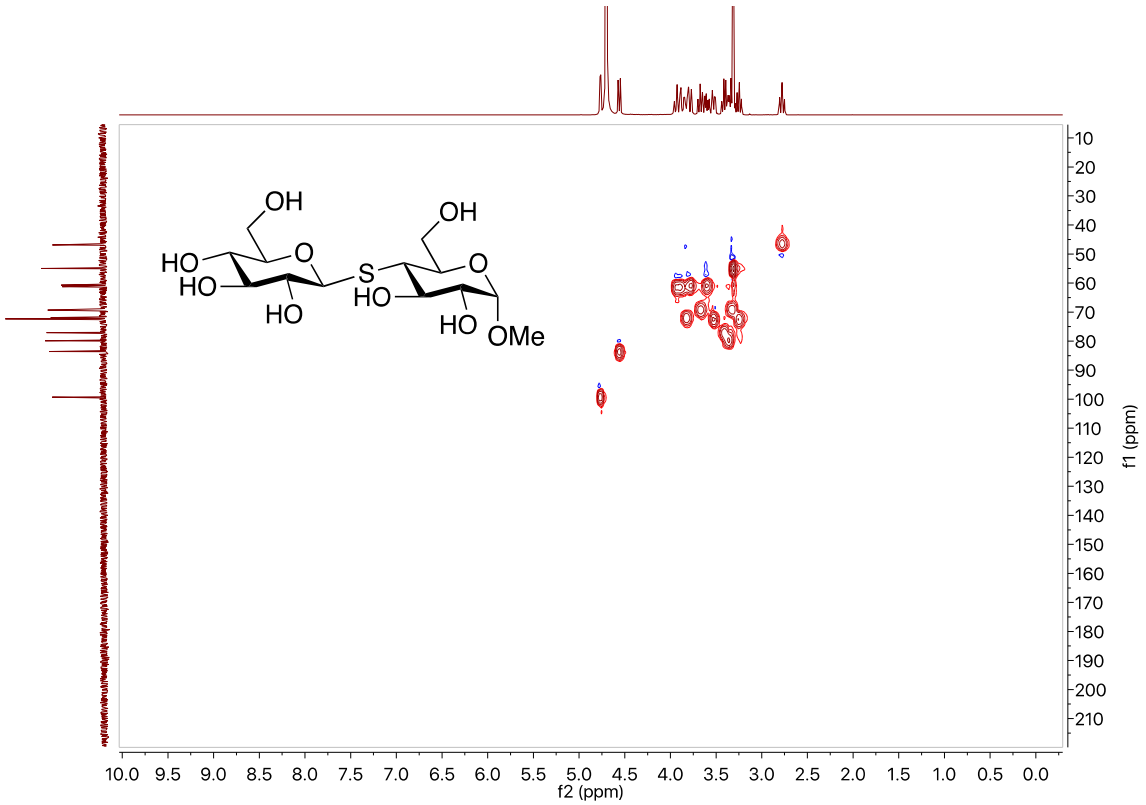
^{13}C NMR of compound **10**



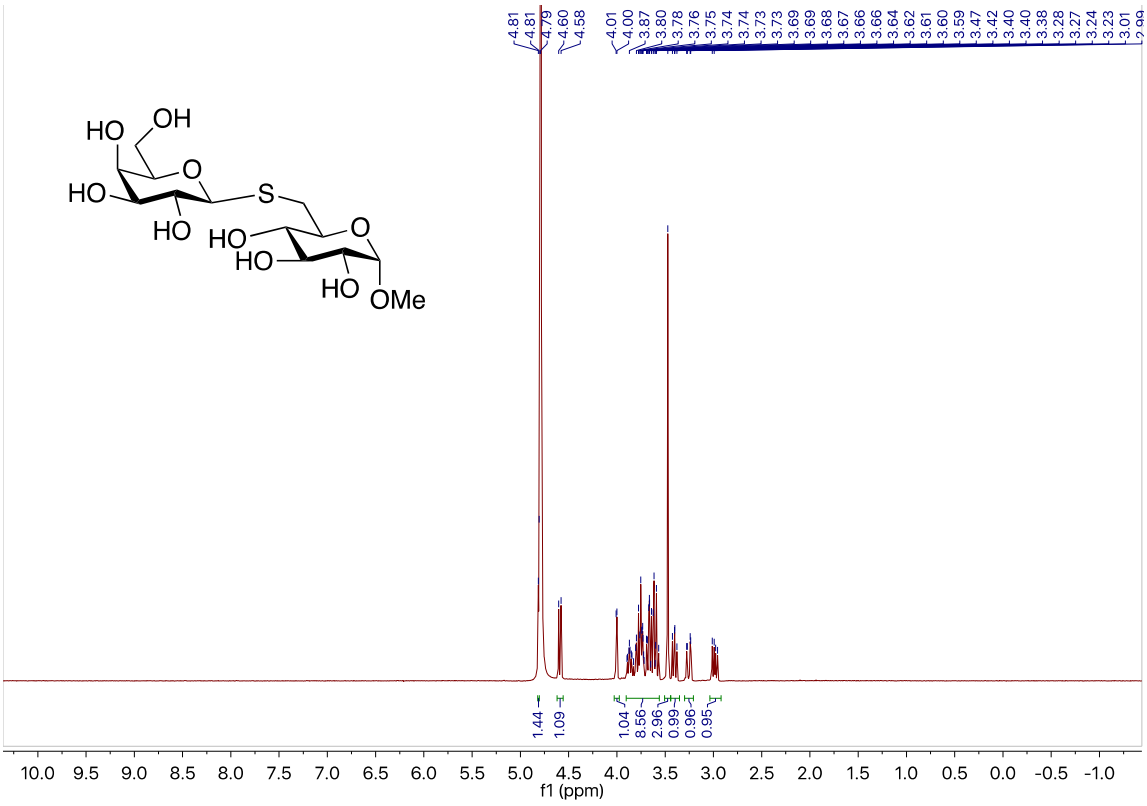
^{1}H NMR of compound **12**



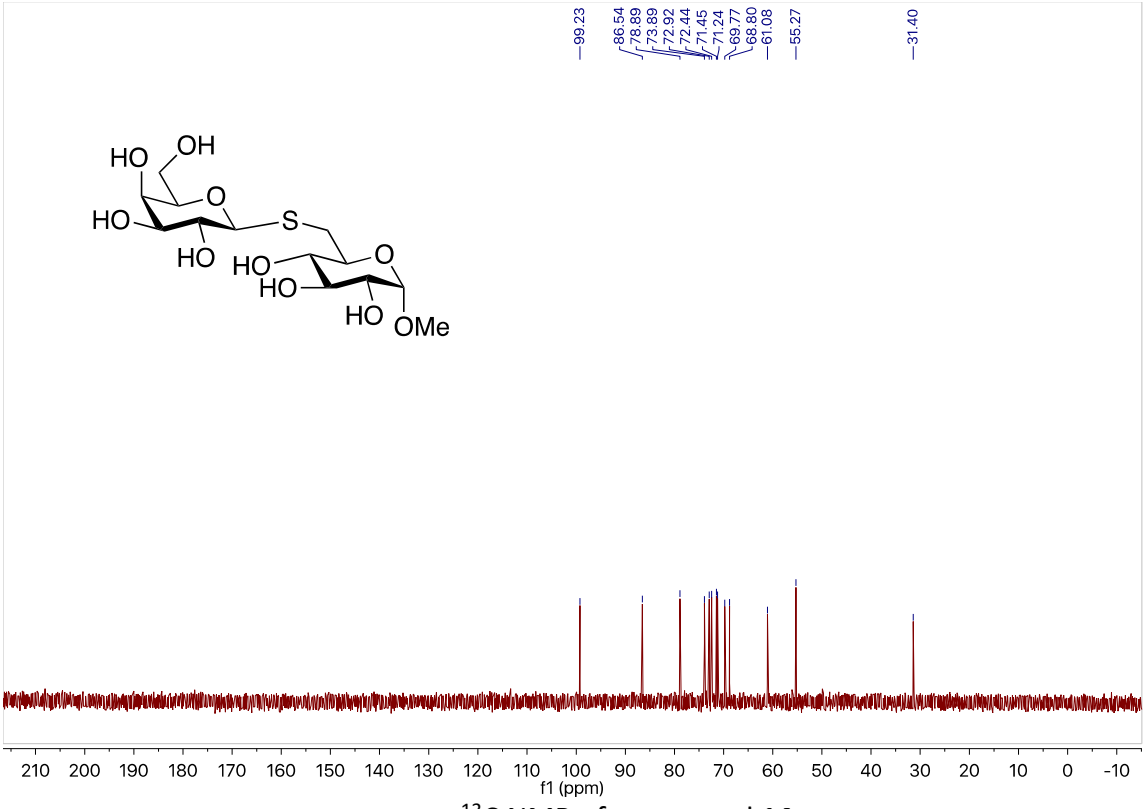
^{13}C NMR of compound **12**



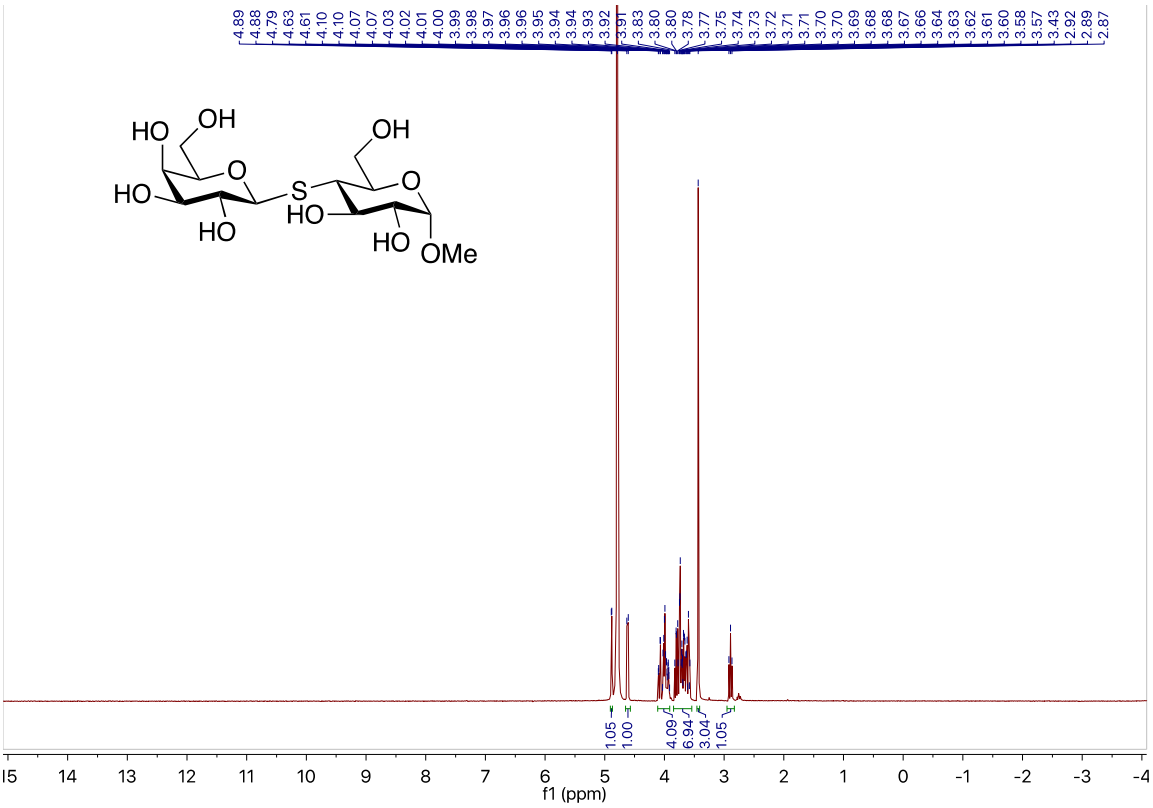
HSQC NMR of compound **12**



^{1}H NMR of compound **14**



^{13}C NMR of compound **14**



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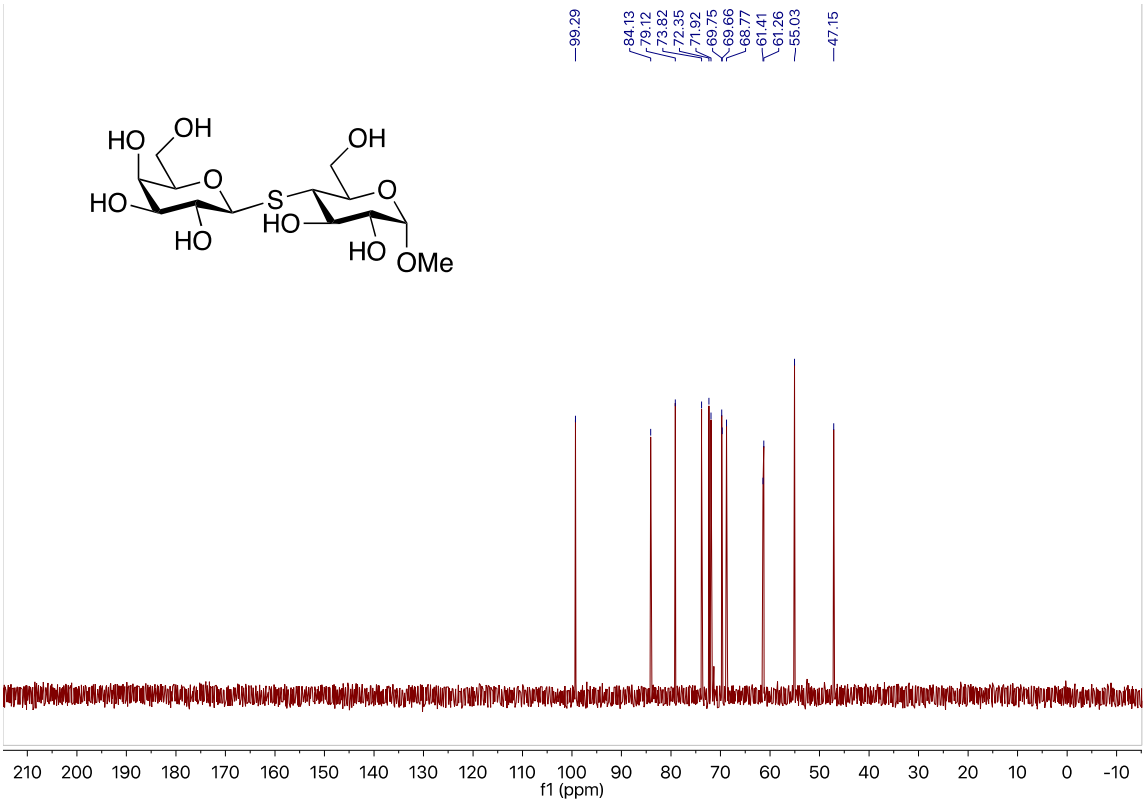
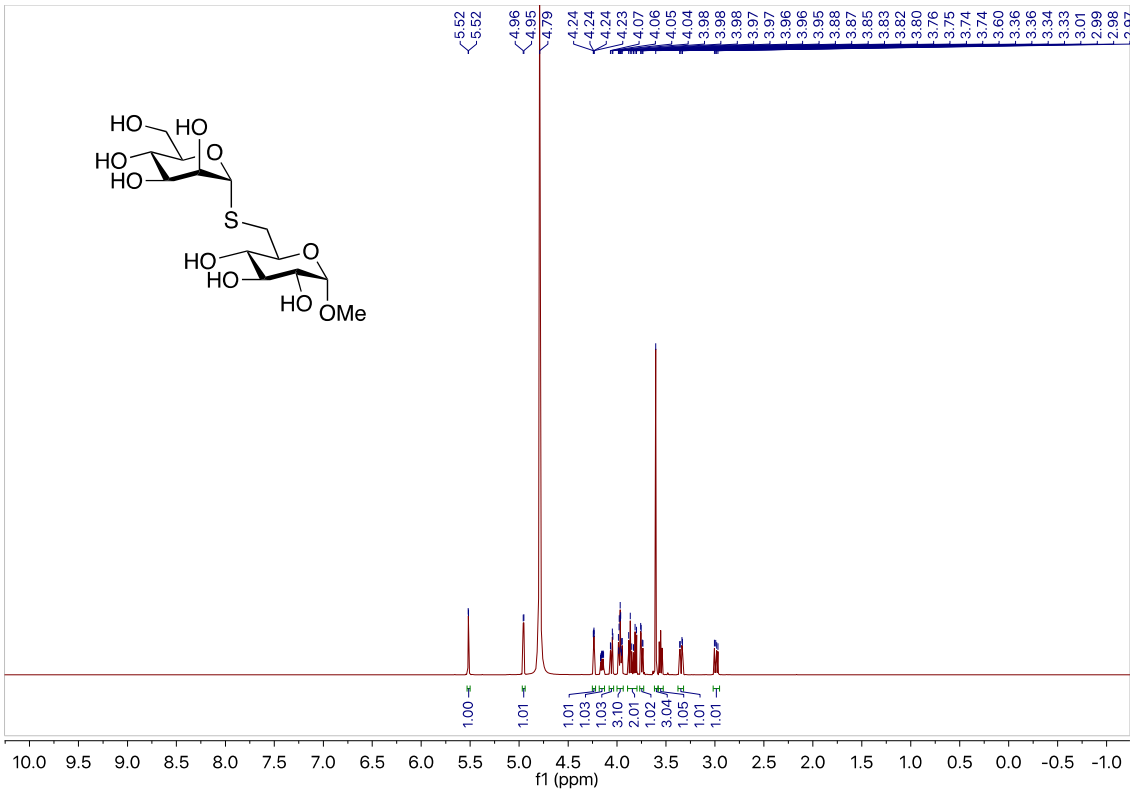
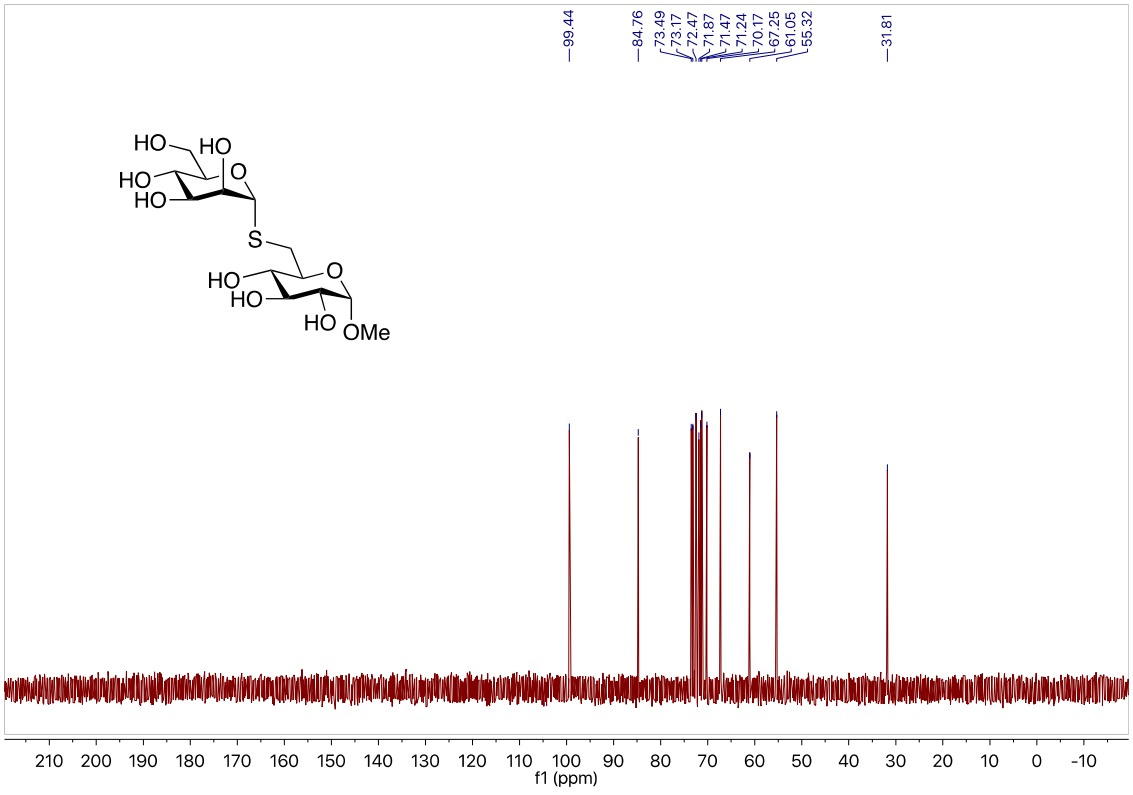


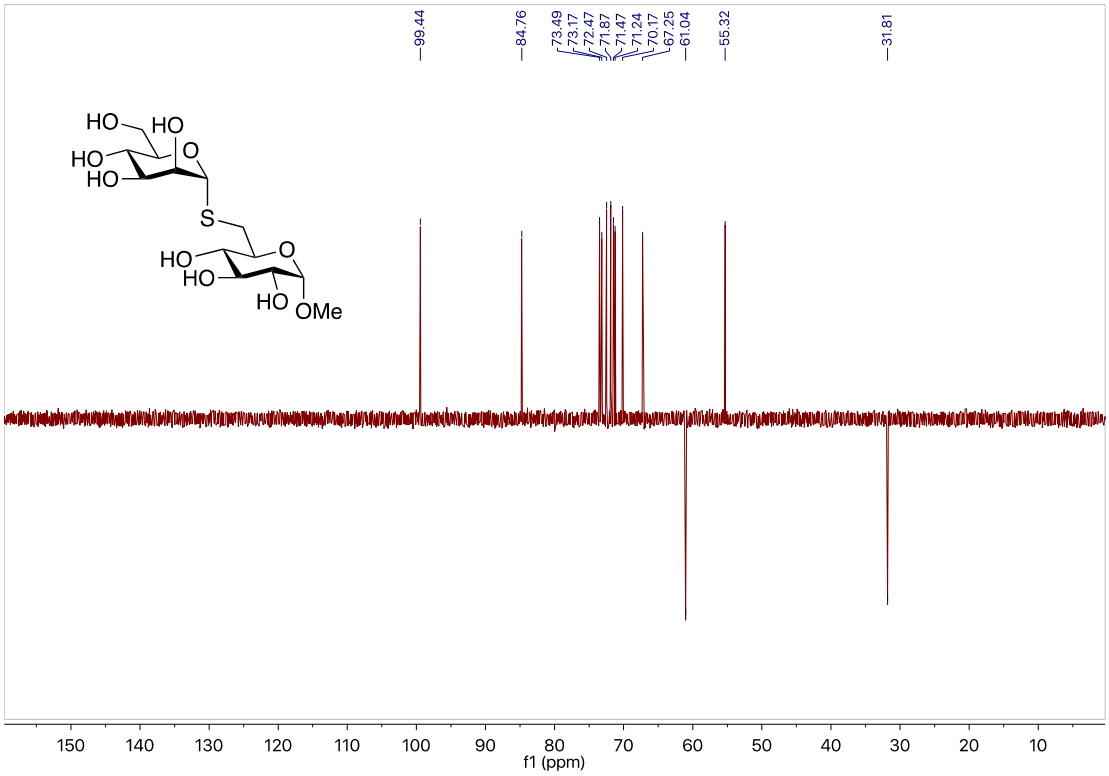
^{13}C NMR of compound **15**



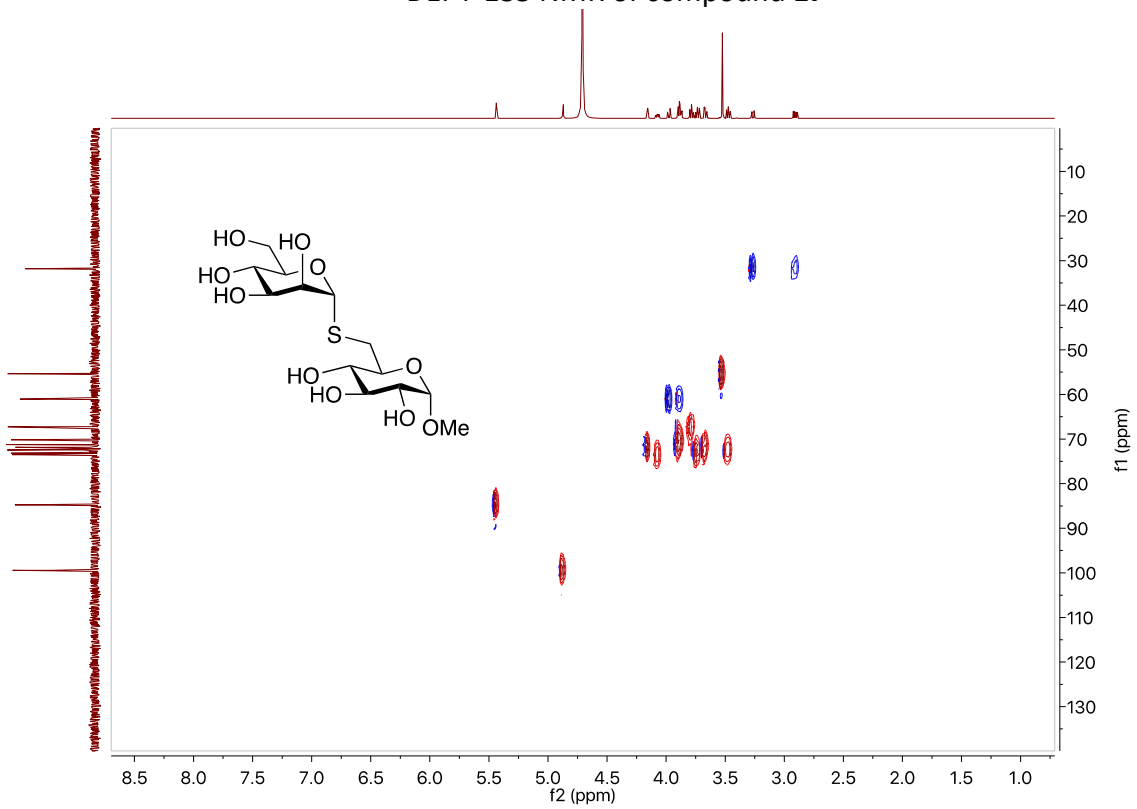
^{1}H NMR of compound **17**



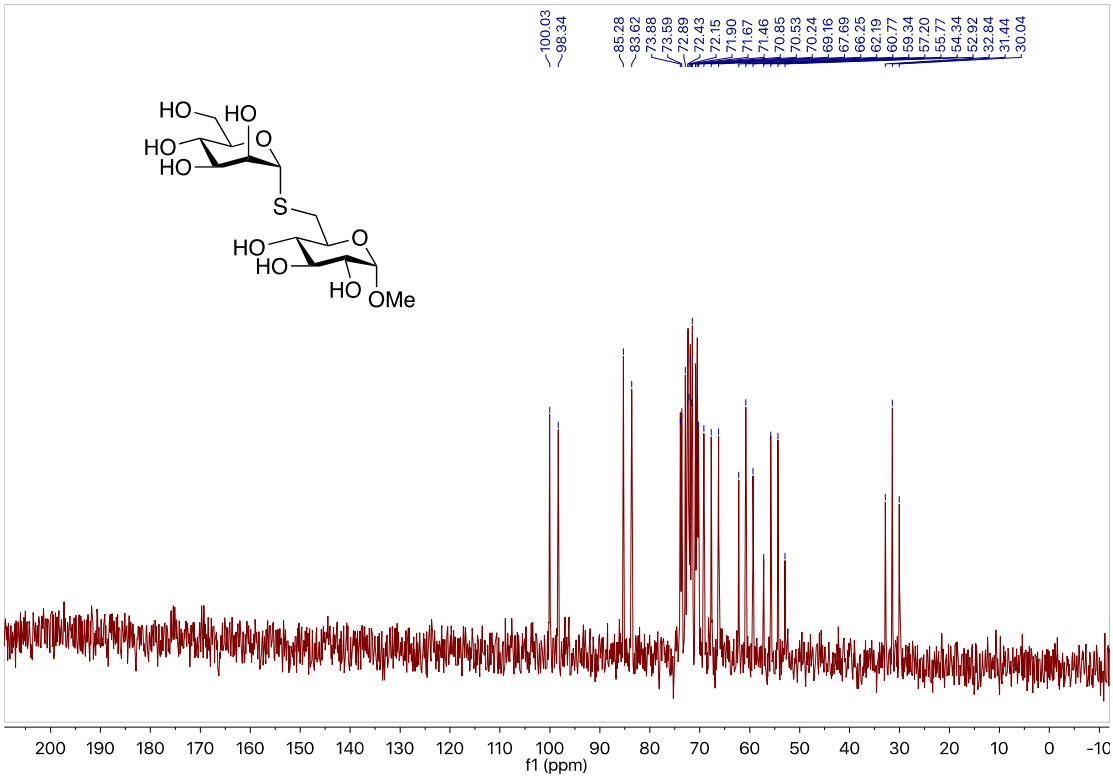
^{13}C NMR of compound **17**



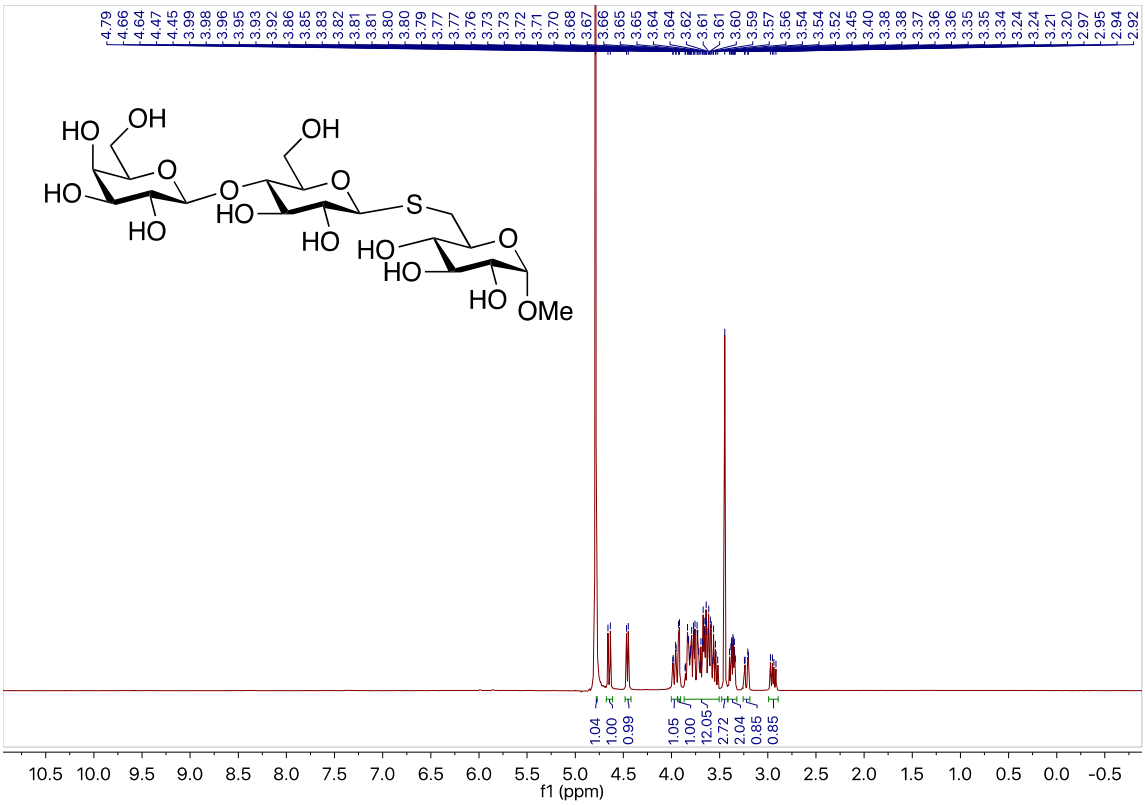
DEPT-135 NMR of compound **17**



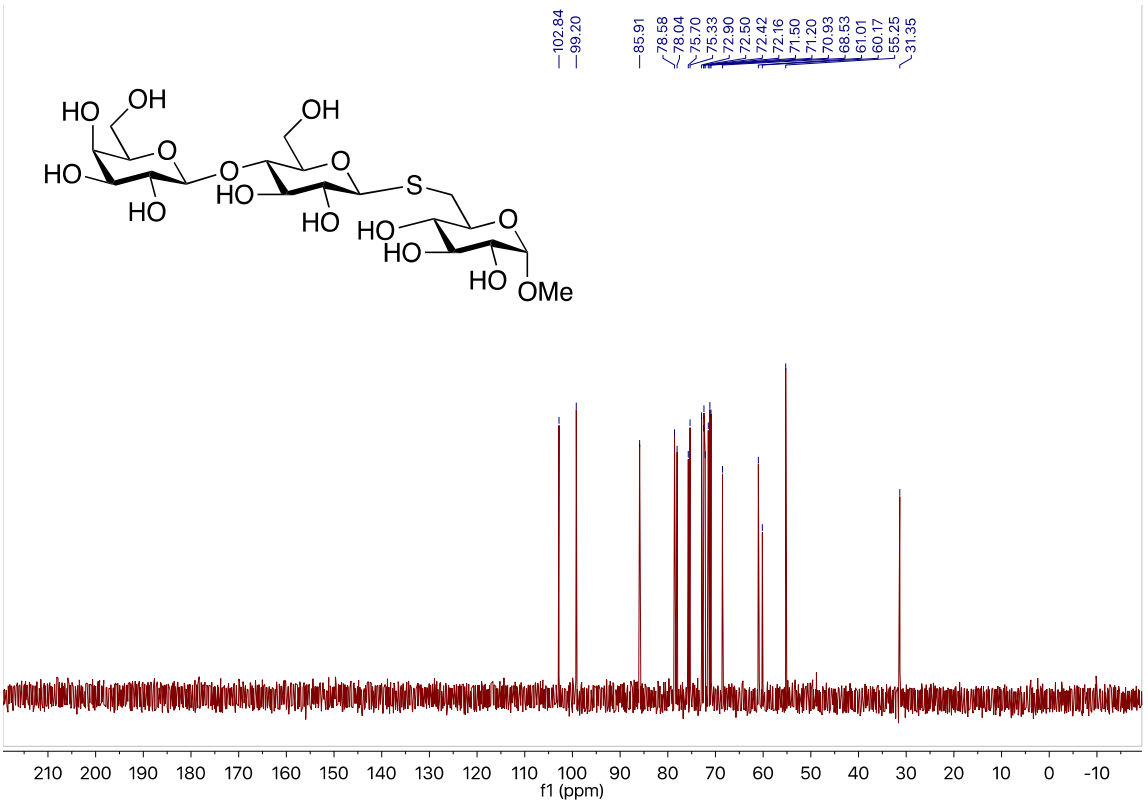
HSQC NMR of compound **17**



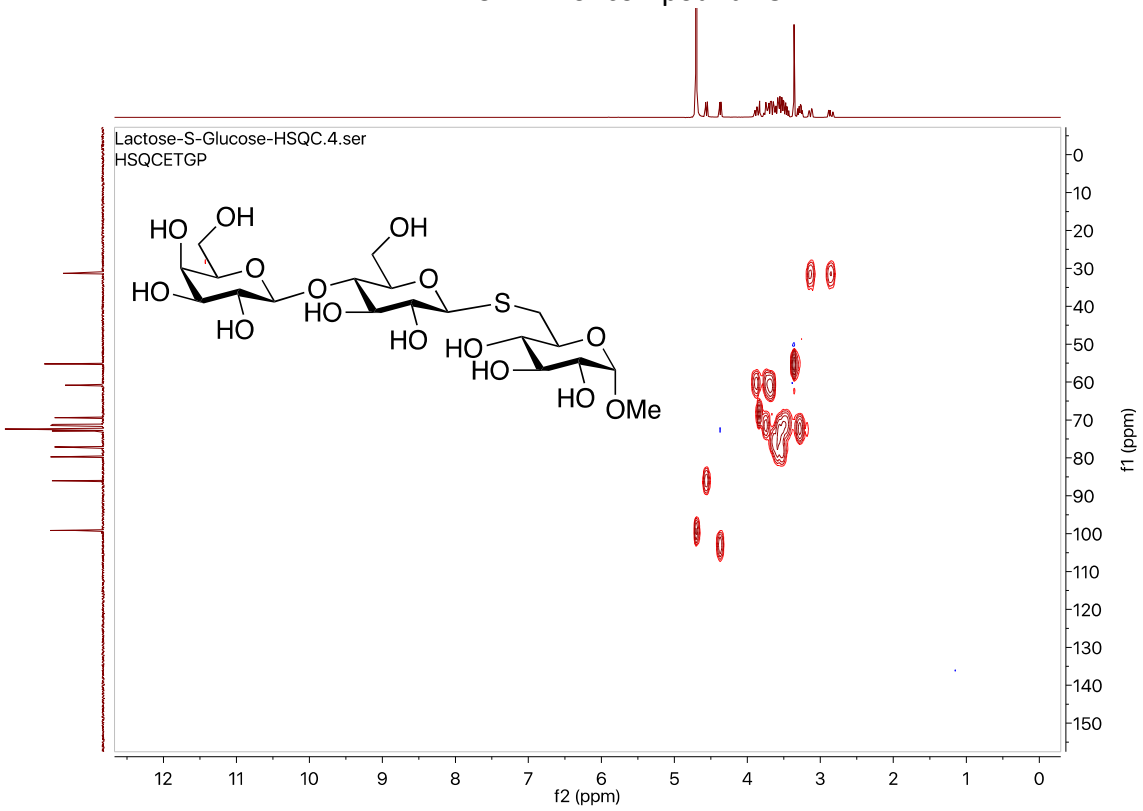
^{1}H coupled ^{13}C NMR of compound **17**



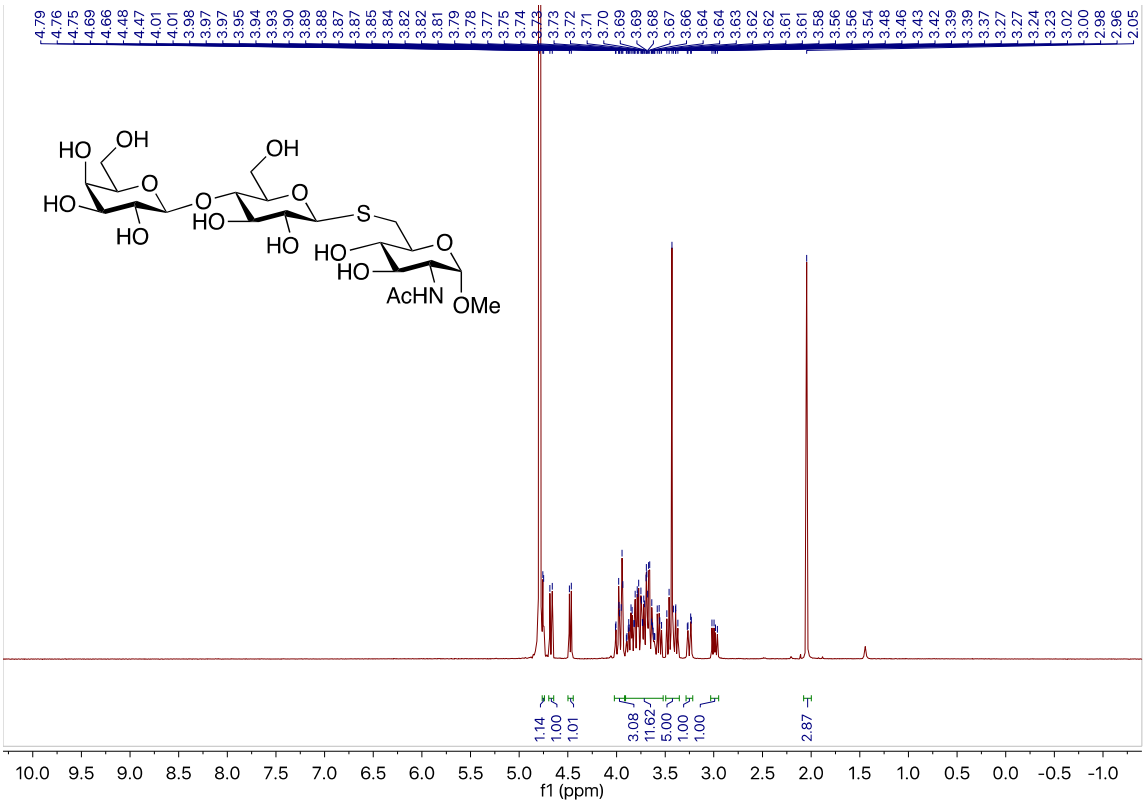
^{1}H NMR of compound **19**



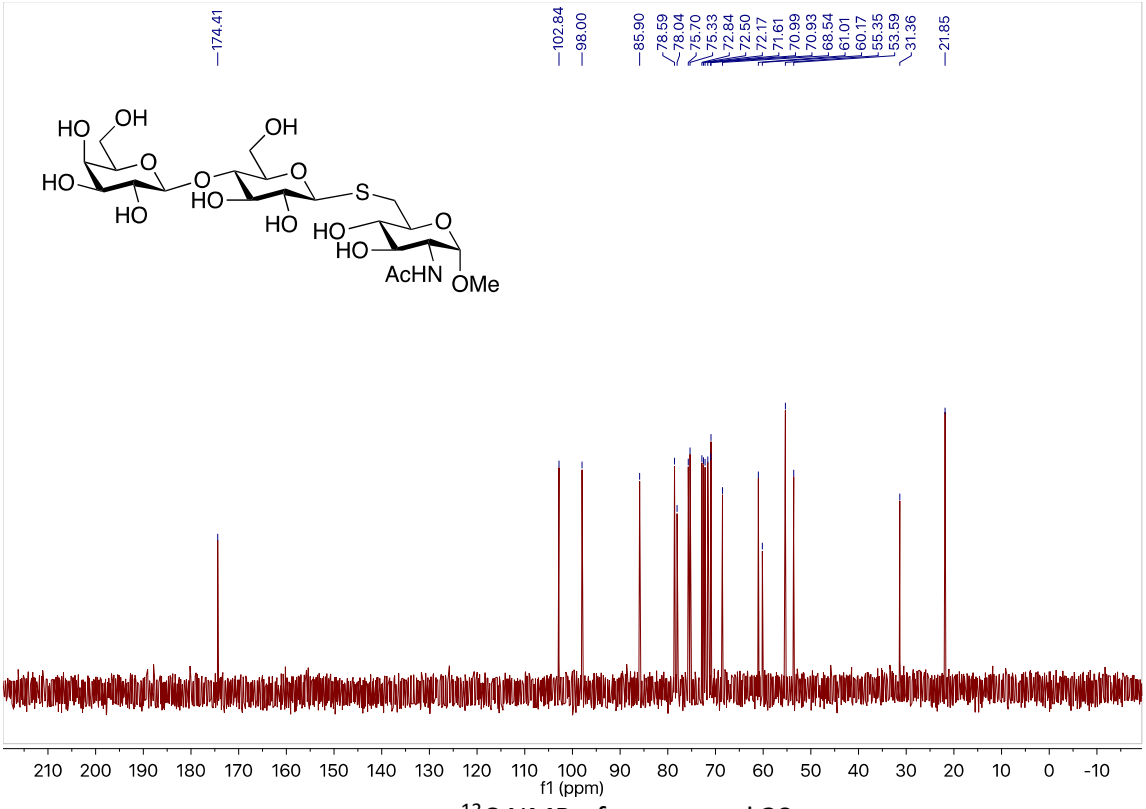
^{13}C NMR of compound **19**



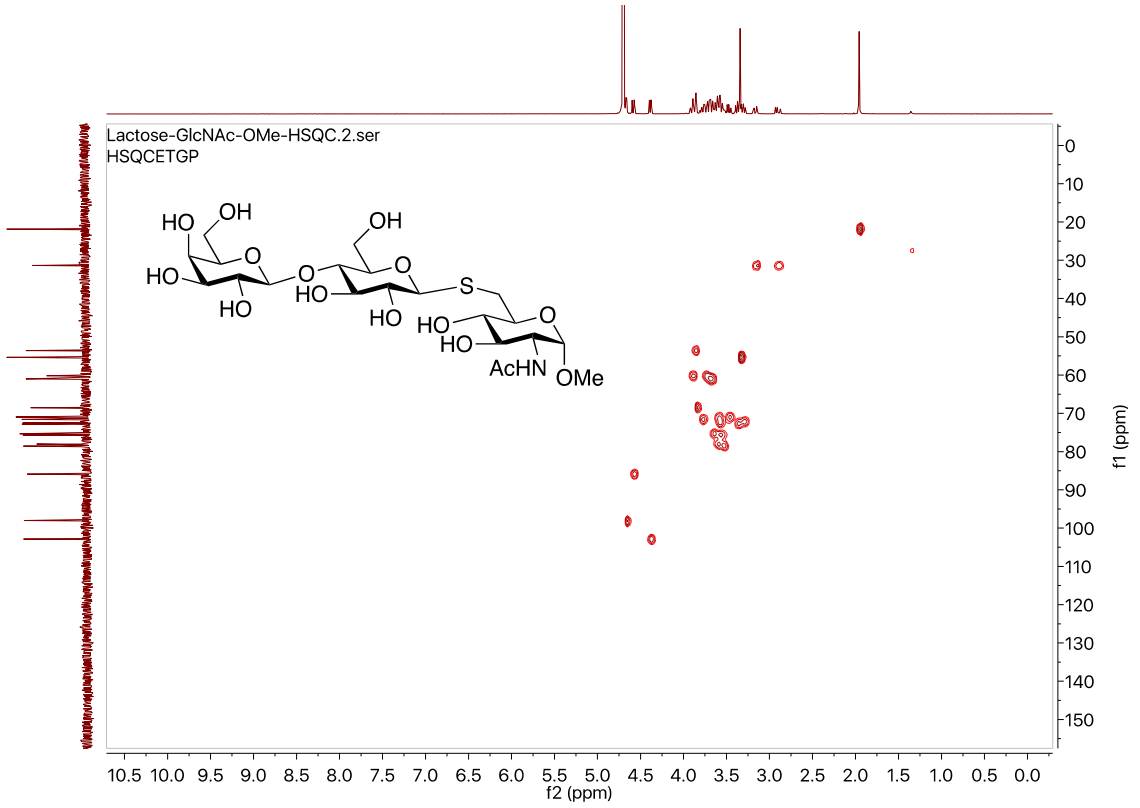
HSQC NMR of compound **19**



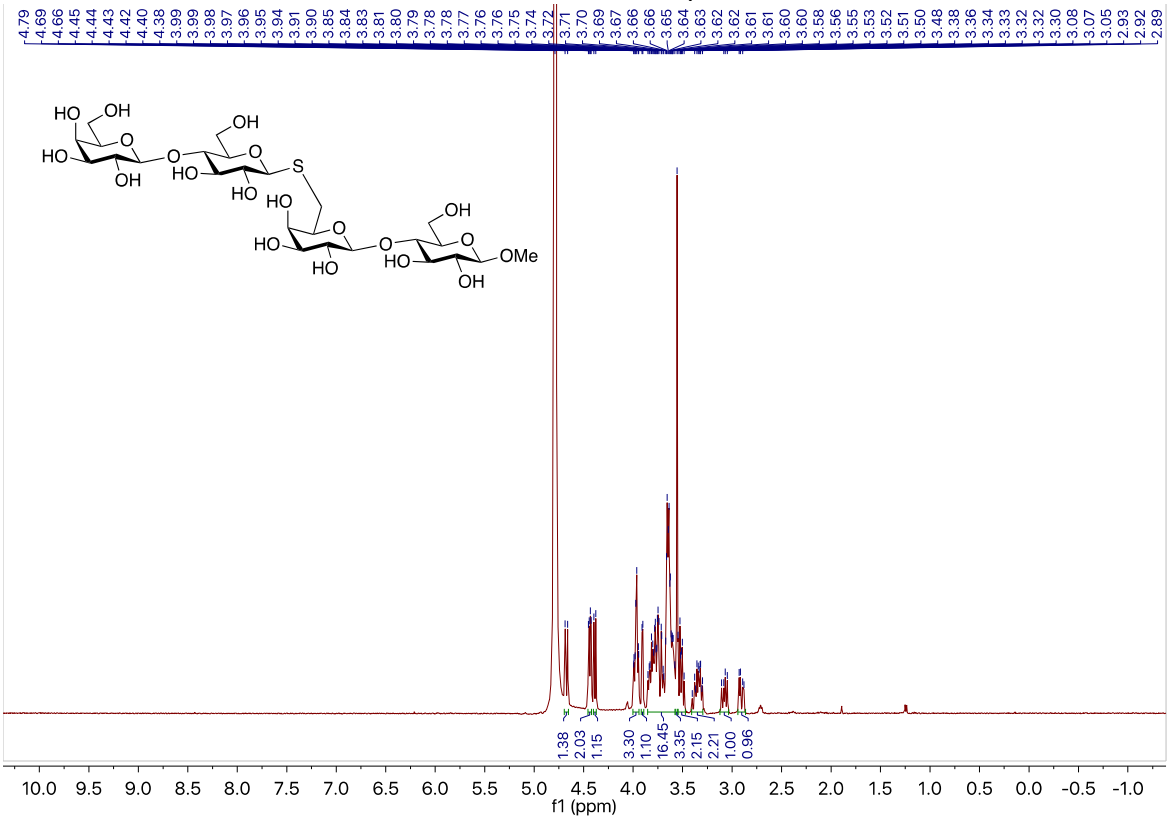
^{1}H NMR of compound **20**



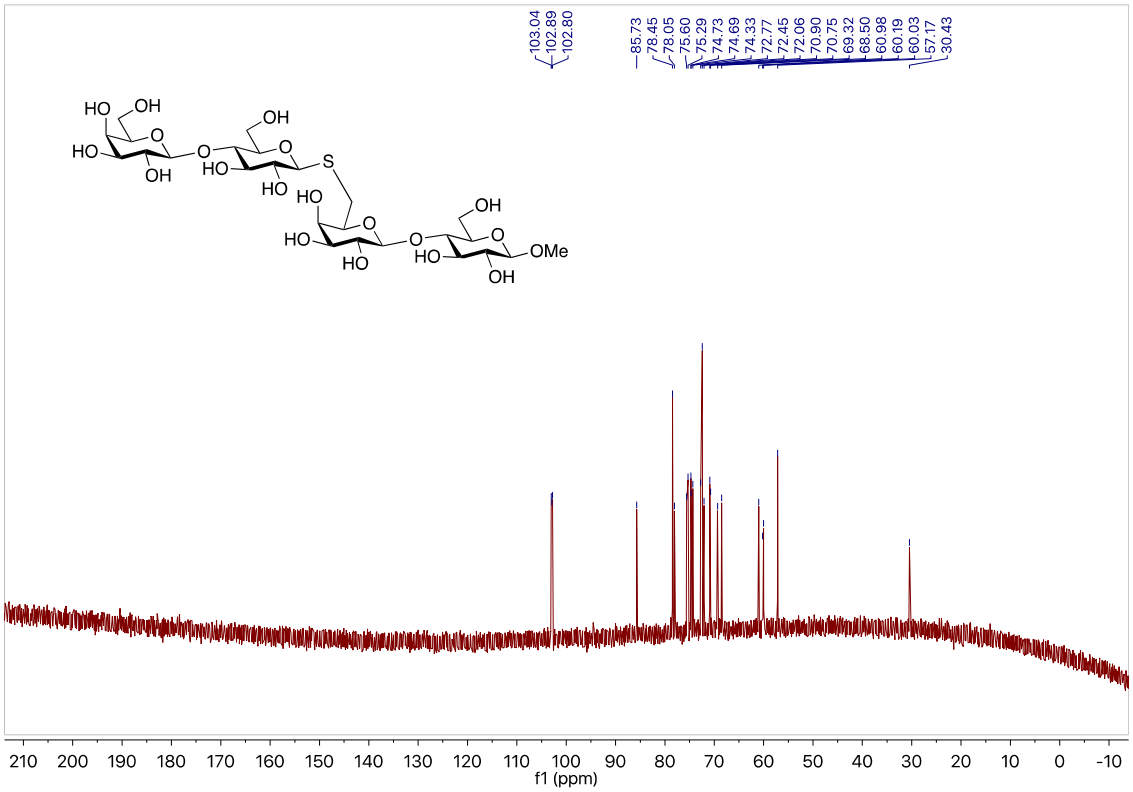
^{13}C NMR of compound **20**



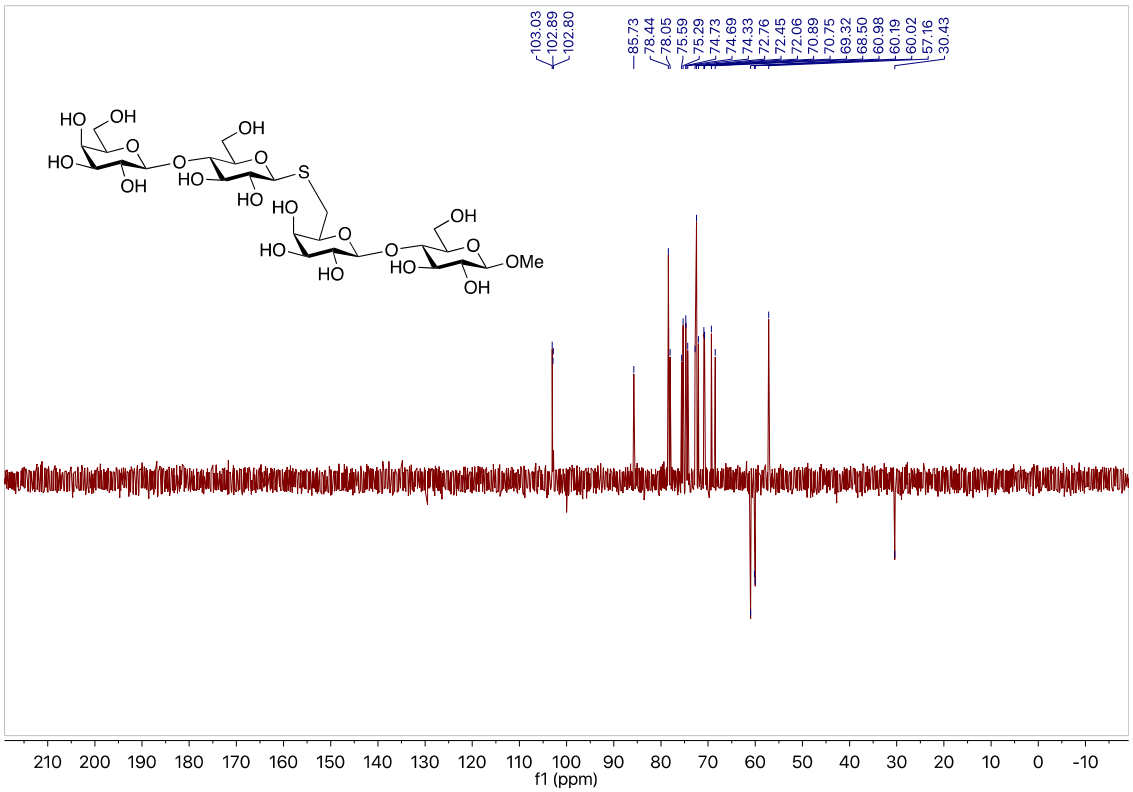
HSQC NMR of compound **20**



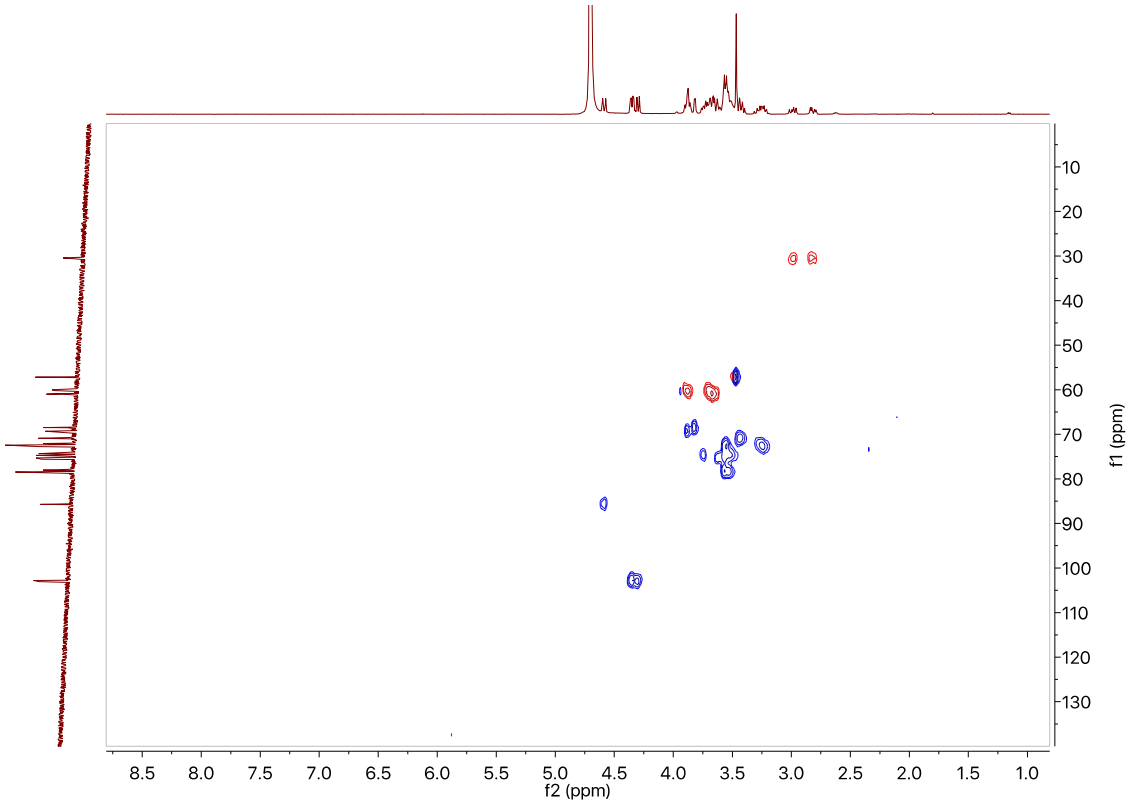
^{1}H NMR of compound **22**



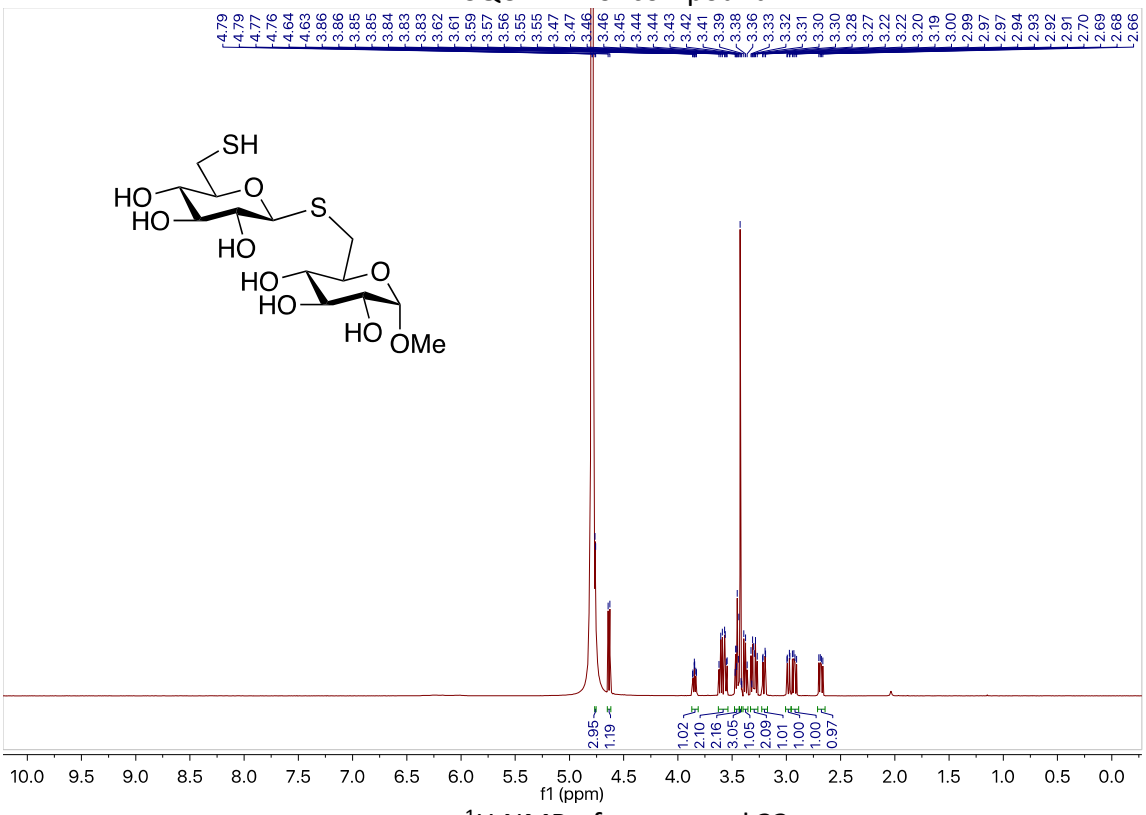
^{13}C NMR of compound **22**



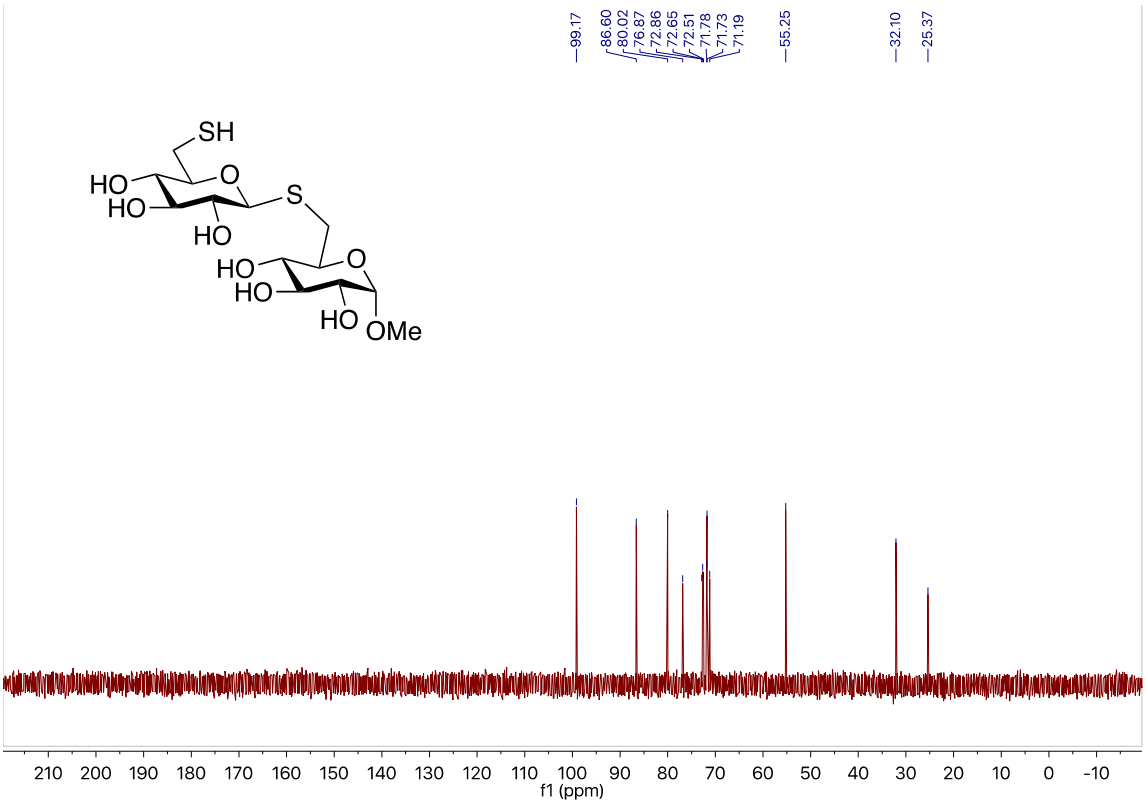
DEPT-135 NMR of compound **22**



HSQC NMR of compound **22**



^{1}H NMR of compound **23**



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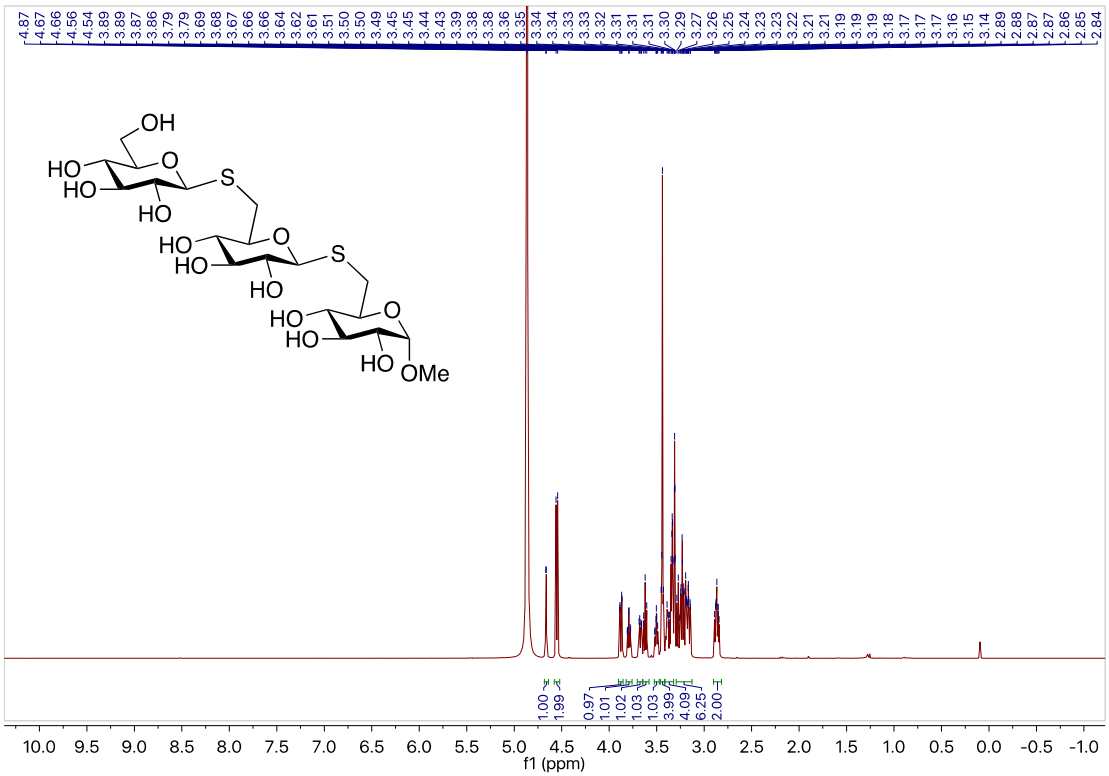
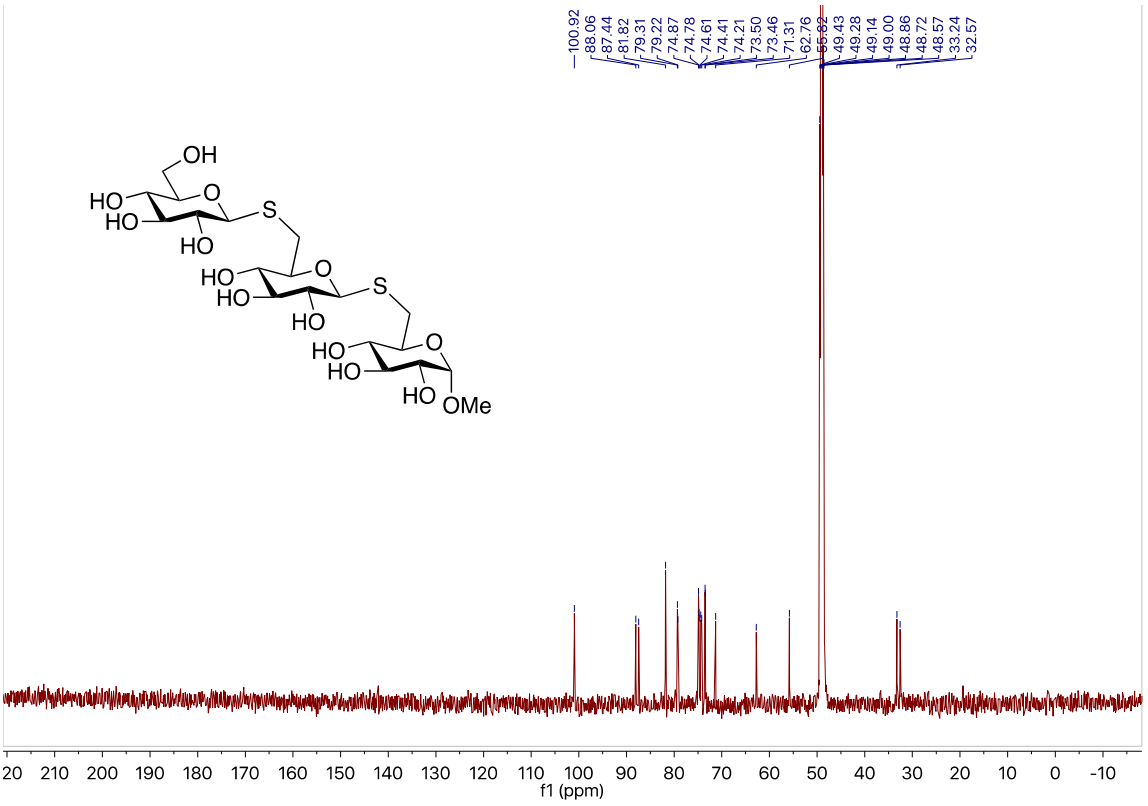


^{1}H NMR of compound **24**



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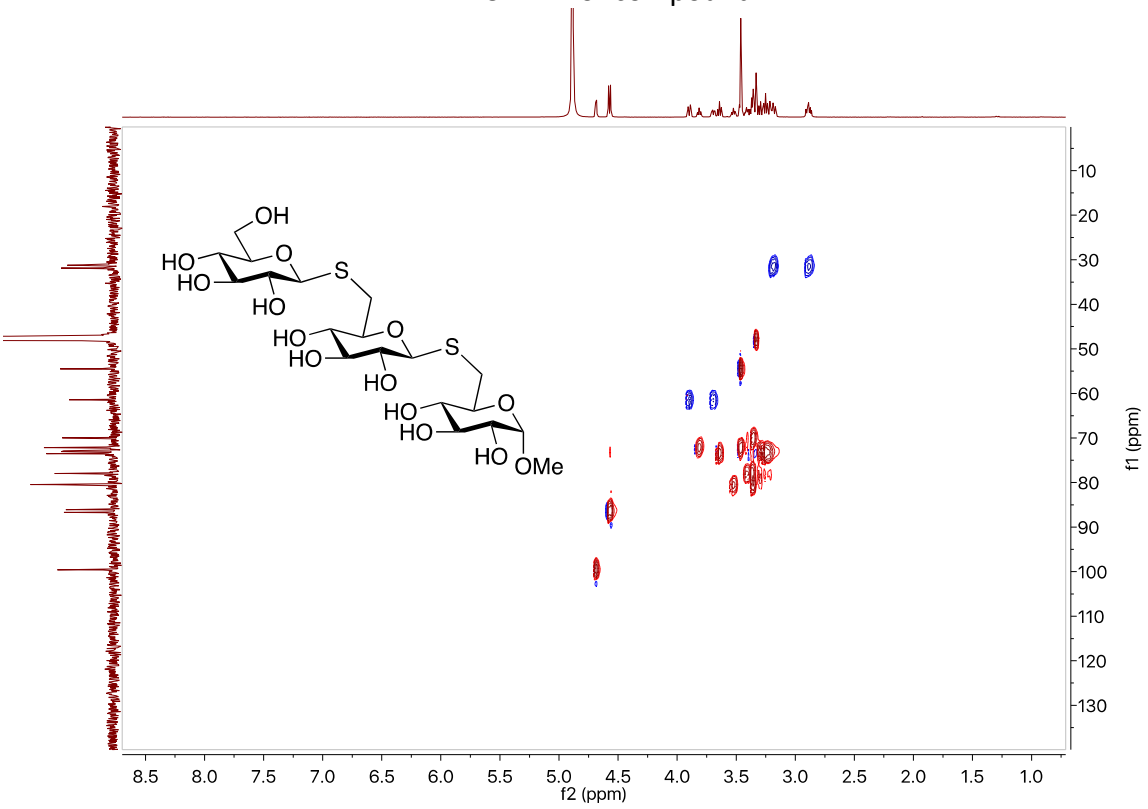
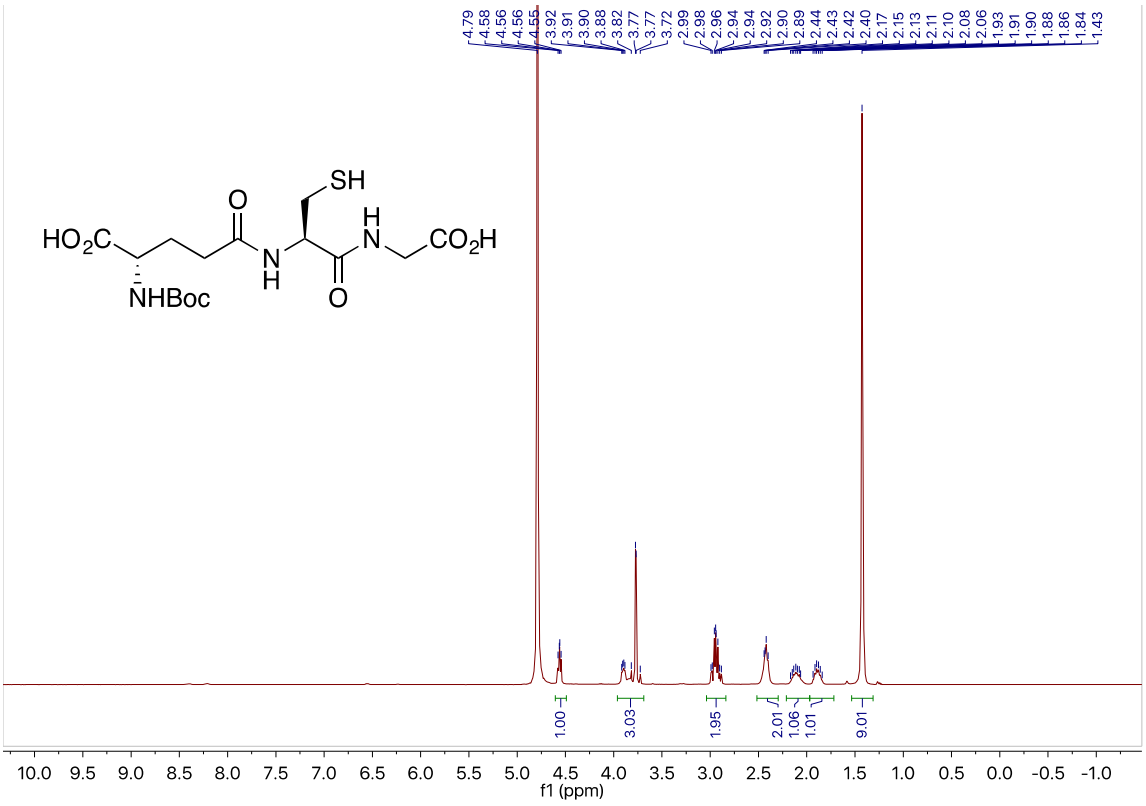
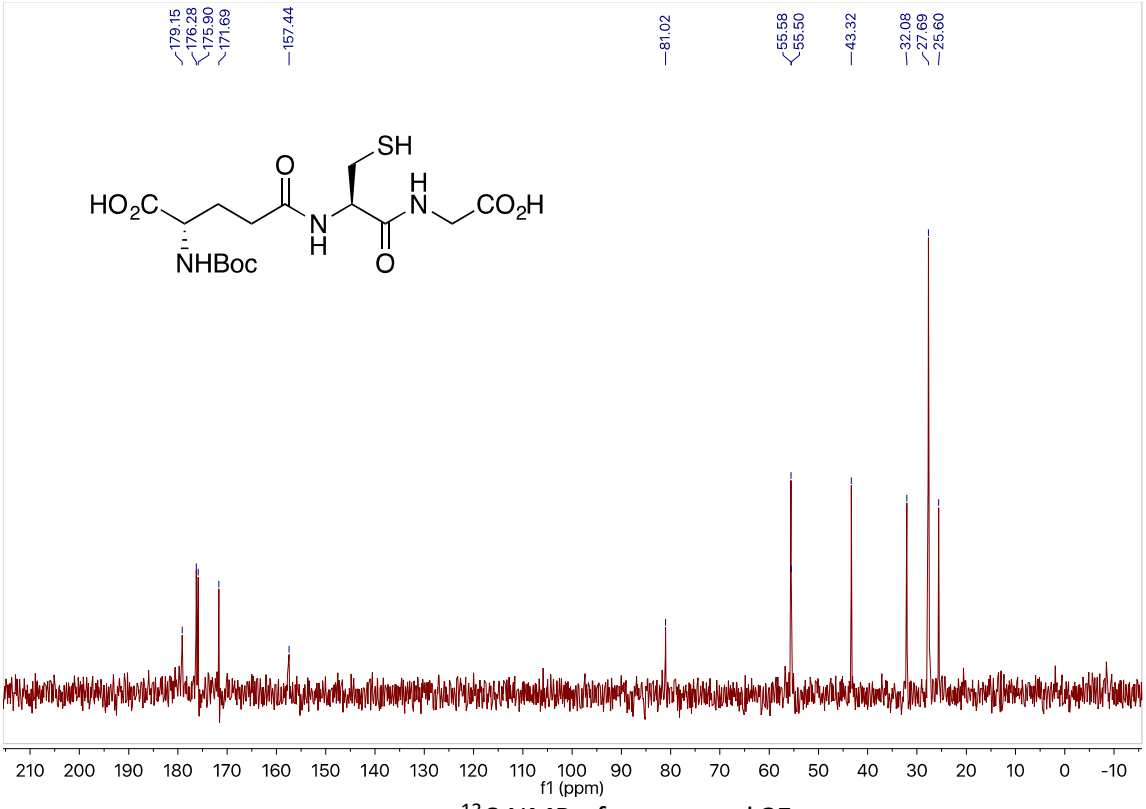


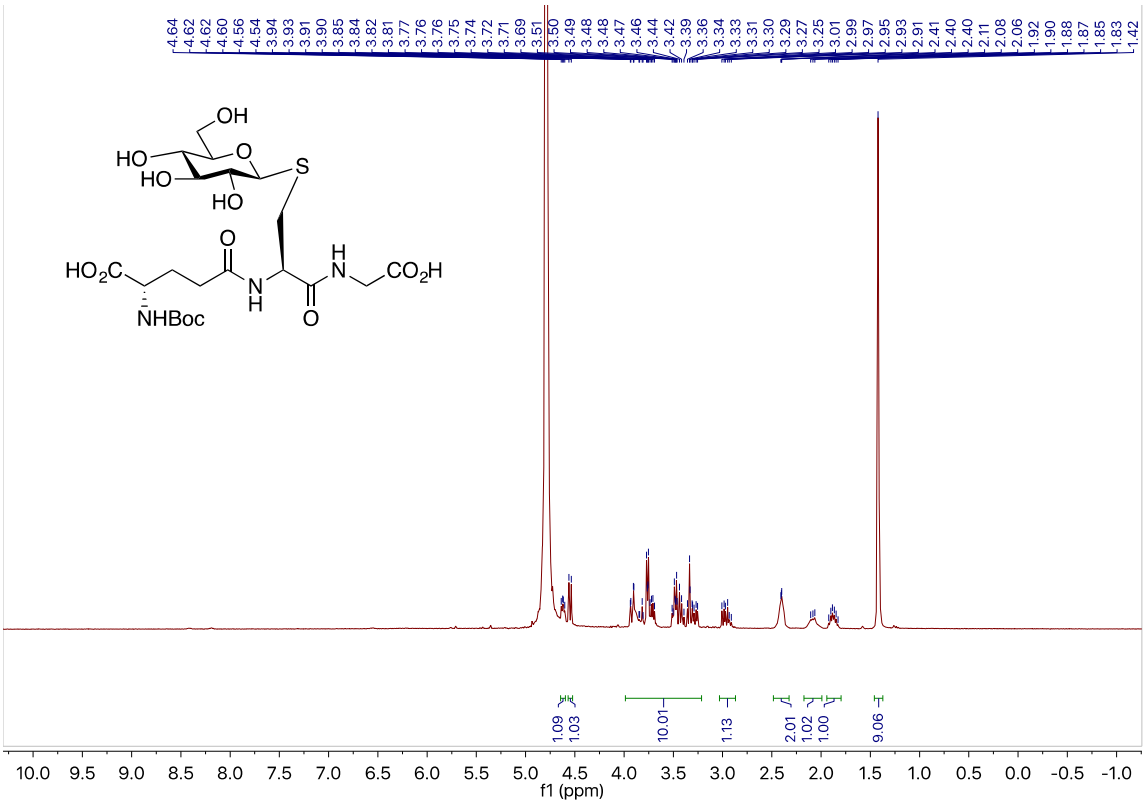
HSQC NMR of compound **24**



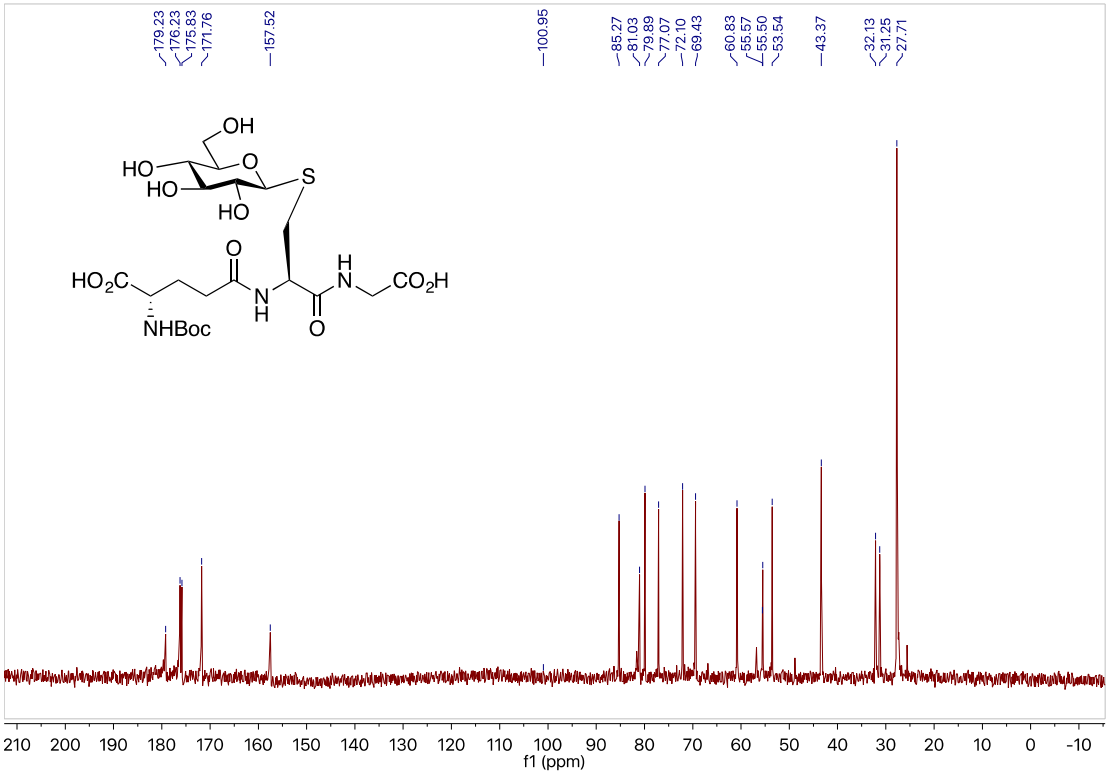
^{1}H NMR of compound **25**



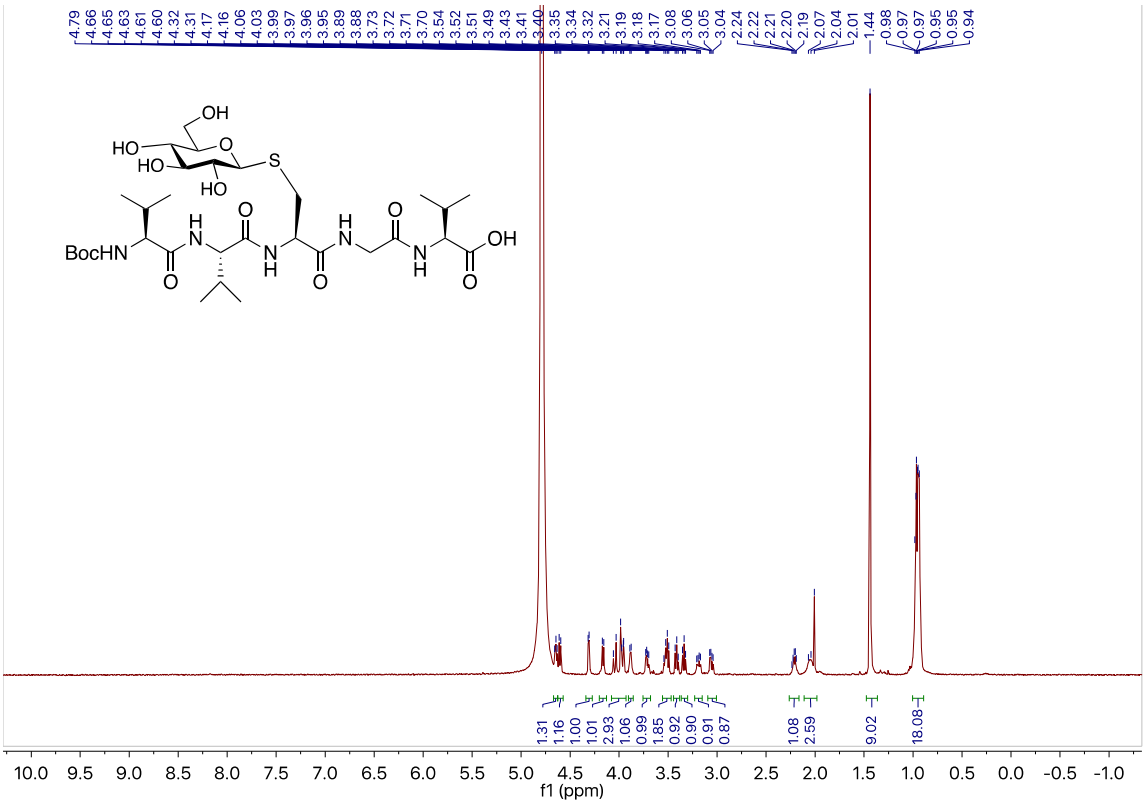
^{13}C NMR of compound **25**



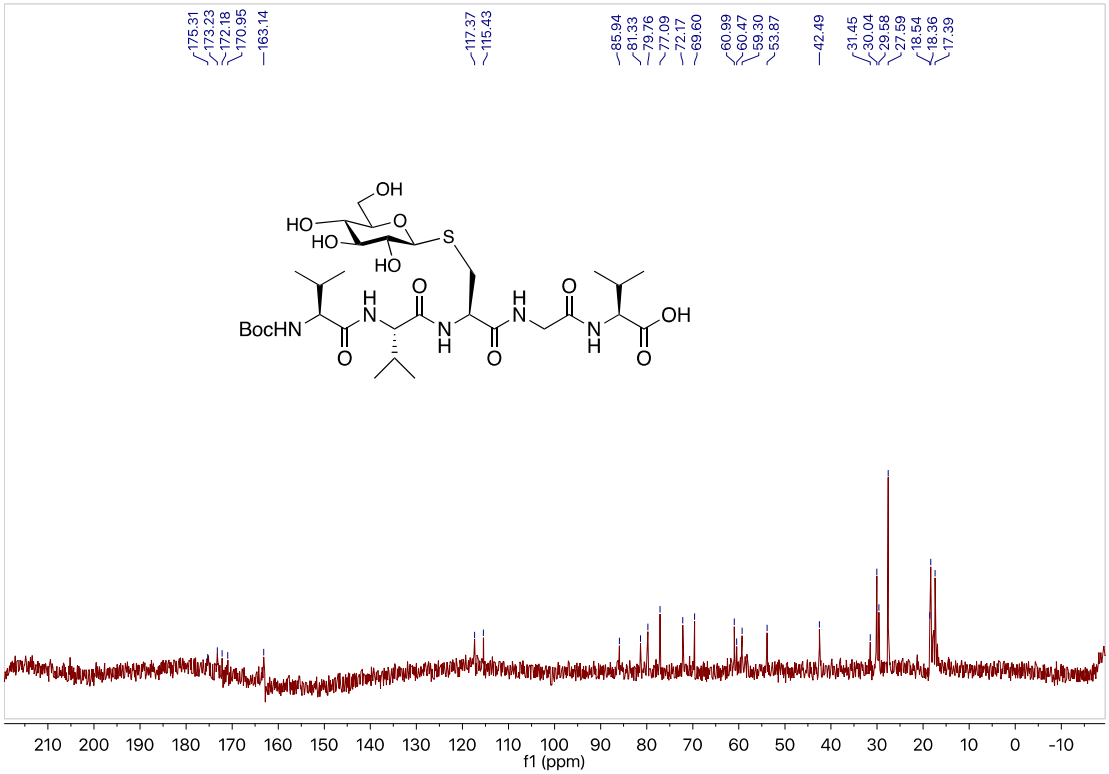
^{1}H NMR of compound **26**



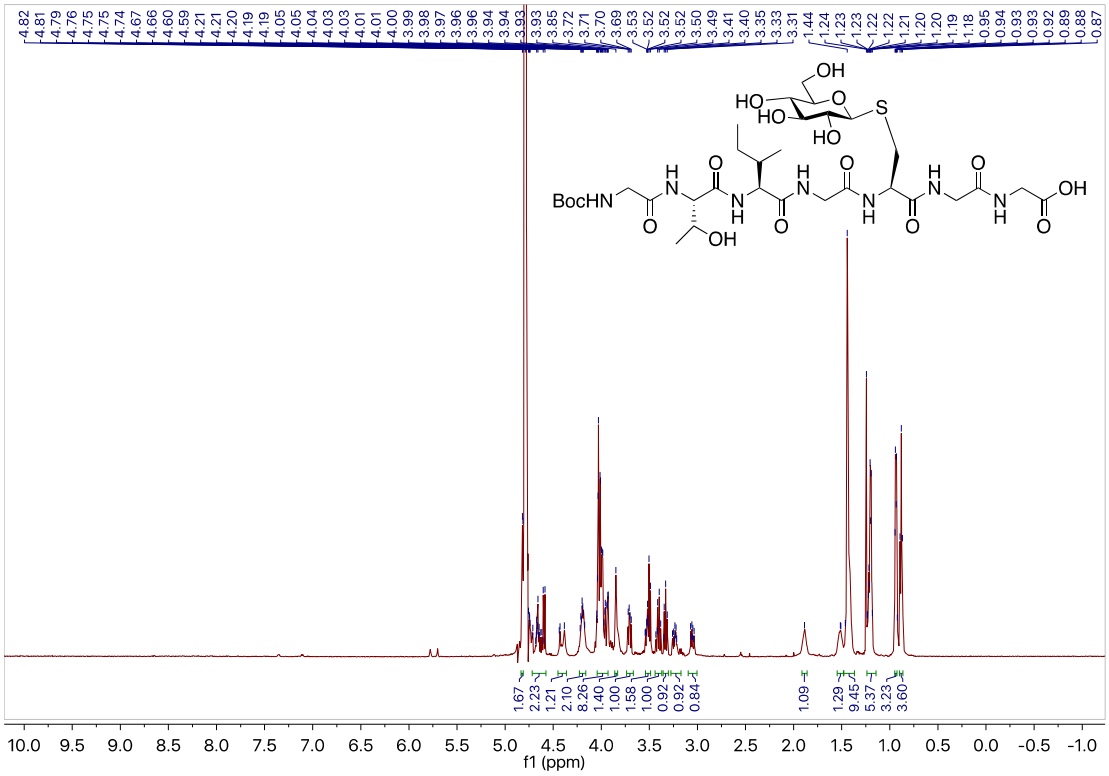
^{13}C NMR of compound **26**



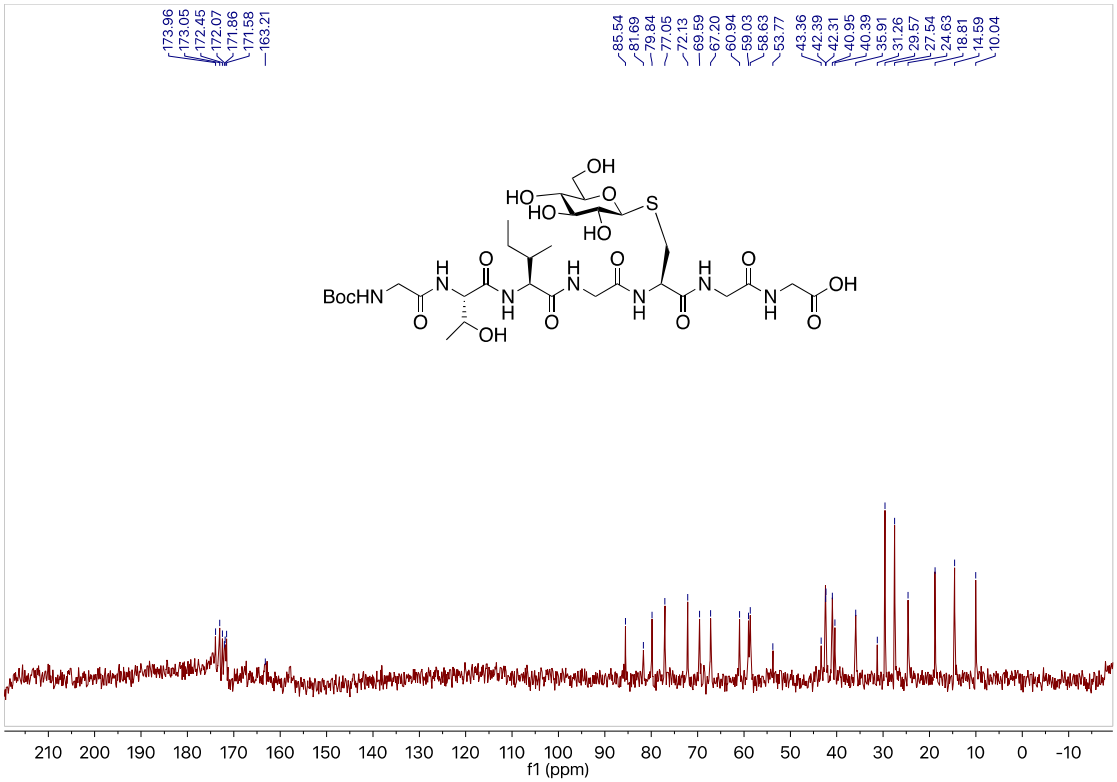
^{1}H NMR of compound **27**



^{13}C NMR of compound **27**



^{1}H NMR of compound **28**



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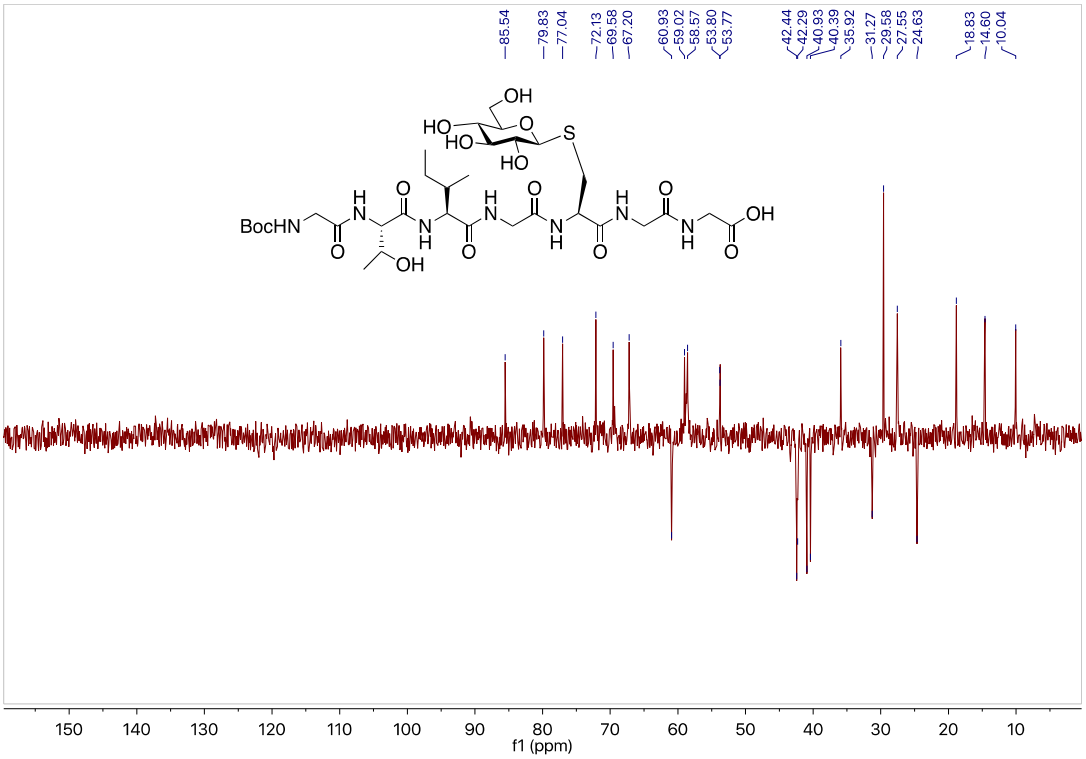
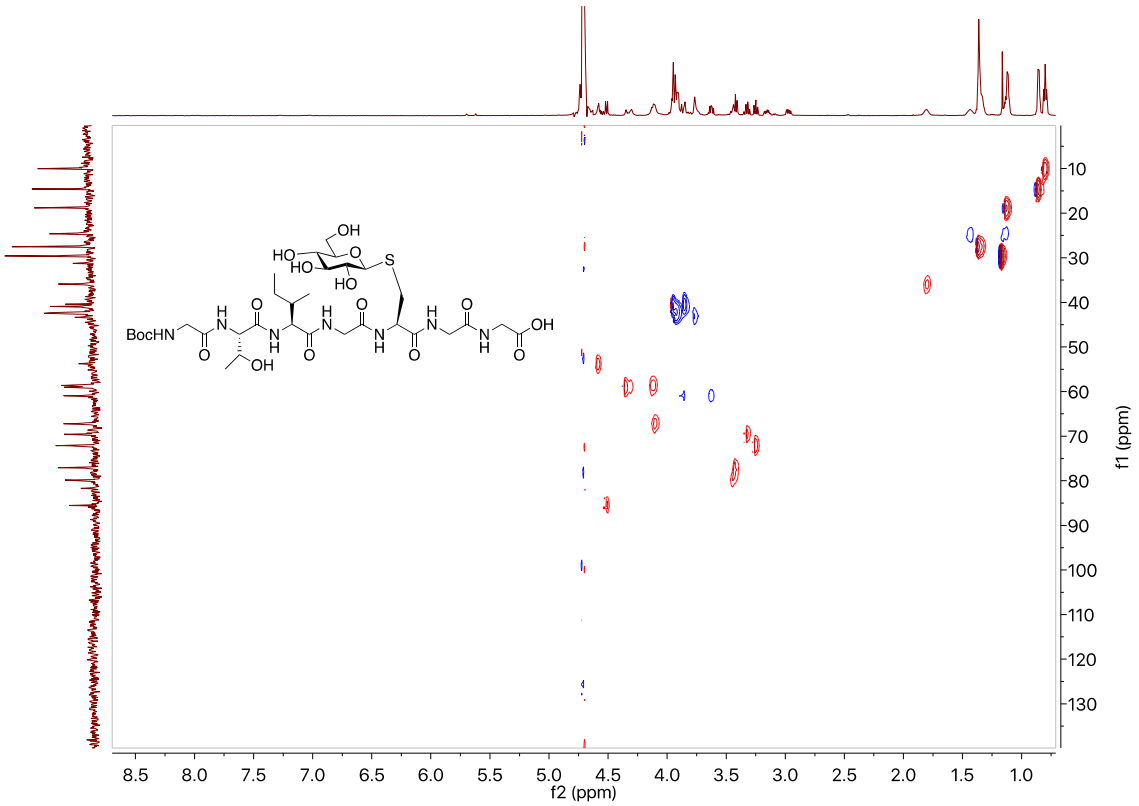


DEPT-135 NMR of compound **28**



HSQC NMR of compound **28**

4. References

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