Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Synthesis and evaluation of inhibitors for *E. coli* PgaB, a polysaccharide de-*N*-acetylase involved in biofilm formation

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Supplementary Materials

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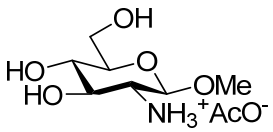
General Procedures

Reagents were obtained from Sigma-Aldrich or Acros Organics and were used without further purification. If appropriate, reactions were carried out under an inert atmosphere of nitrogen. Standard syringe techniques were applied for the transfer of dry solvents and air- or moisture-sensitive reagents. Reactions were monitored by TLC using Silica Gel 60 F254 (EMD Science) with detection by quenching of fluorescence and/or by visualization with phosphomolybdic acid in ethanol (0.5% w/v), methanolic H_2SO_4 (10% v/v), or ninhydrin in ethanol (0.2% w/v). ^1H and ^{13}C NMR spectra were recorded at 25 °C with a Mercury 300 MHz or a Varian 400 MHz (75 MHz or 100 MHz for ^{13}C, respectively) spectrometer. ^1H-NMR chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, or a residual proton peak of the solvent: δ = 7.26 ppm for $CDCl_3$, δ = 4.81 ppm for D_2O, δ = 3.31 ppm for CD_3OD. Multiplicities are reported as: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), or m (multiplet). Broad peaks are indicated by br. Coupling constants are reported as *J*-values in Hz. The number of protons (n) for a given resonance is indicated as nH, and is based on spectral integration values. ^{13}C-NMR chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, or a carbon peak of the solvent: δ = 77.0 for $CDCl_3$, δ = 49.0 for CD_3OD. Multiplicities in carbon represent different rotamers and are reported as: d (doublet). Flash chromatography was performed on Silica-P Flash Silica Gel 60 (40–63 *μ*m particle size, Silicycle). High-resolution mass spectra were obtained from an ABI/Sciex QStar mass spectrometer (ESI). Absorption spectra were measured on a Schimadzu UV-2401PC spectrophotometer. Fluorescence measurements were obtained on a TECAN Safire2 plate reader. HPLC was performed on a Waters 1525 binary HPLC pump with a Waters 2487 dual k absorbance detector or a Gilson 321 HPLC pump with a Gilson UV–Vis 156 dual k absorbance detector.

SYNTHESIS

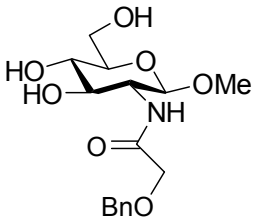
Methyl 2-ammonium-2-deoxy-β-D-glucopyranoside acetate[1, 2] (2)



N-Acetylglucosamine (5 g, 25 mmol) and tosylhydrazide (5.5 g, 25 mmol) in a mixture of DMF (20 mL), water (4.5 mL) and acetic acid (0.5 mL) was stirred at 37 °C for 24 h. The solution was then poured into diethyl ether (1 L) and stirred vigorously to promote formation of a white precipitate. Vacuum filtration of the suspension yielded the glycosyltosylhydrazide donor (10.2 g, 92%). The glycosyl donor (5 g, 12 mmol) was then dissolved in DMF (150 mL) containing MeOH (10 mL, 240 mmol). *N*-Bromosuccinimide (2.2 g, 26 mmol) was then added to the solution portion-wise over 5 min 0 °C. Upon completion of the addition, the solution was allowed to stir for 30 min at room temperature. The solution was then evaporated under reduced pressure, and the resulting residue was dissolved in hot EtOH (100 mL), and chilled to -20 °C to allow crystallization of the succinimide. Filtration and subsequent evaporation of the EtOH filtrate followed by flash chromatography (10% MeOH in DCM) of the resulting residue yielded **1** (1.8 g 62%) as a white solid. **1** (1.8 g, 8 mmol) was then dissolved in

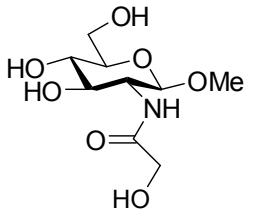
hydrazine monohydrate (10 mL) and allowed to stir at 110 °C for 48 h. The solution was then evaporate under reduced pressure, followed by the addition of 1M AcOH (5 mL) and a subsequent evaporation under reduced pressure to form the acetate salt. Recrystalization of the resulting residue with MeOH/EtOAc yielded **2** (1.85 g, 96%).

Methyl 2-(2-benzyloxy)acetamido-2-deoxy-β-D-glucopyranoside (3)



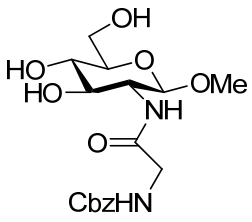
The title compound was prepared by dissolving compound **2** (200 mg, 0.93 mmol), benzyloxyacetic acid (220 mg, 0.93 mmol) and triethylamine (55 µL, 1 mmol) in DMF. HBTU (355 mg, 0.93 mmol) was then added portion wise to the mixture at 0 °C over 30 min and allowed to stir overnight at room temperature. The solution was then evaporated under reduced pressure and the resulting residue was purified by flash chromatography (8% MeOH in DCM) to afford **3** (198 mg, 84%) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 7.56 – 7.41 (m, 5H, Ar-H), 4.69 (s, 2H, CH_2), 4.54 (d, *J* = 8.5 Hz, 1H, H-1), 4.14 (d, *J* = 5.3 Hz, 2H, C**H$_2$**Ar), 3.97 (dd, J = 12.1 Hz, 1.8 Hz, 1H, H-6a), 3.84 – 3.73 (m, 2H, H-6b, H-5), 3.64 (dd, *J* = 10.3 Hz, 8.7 Hz, 1H, H-2), 3.54 (s, 3H, CH_3), 3.52 - 3.47 (m, 2H, H-3, H-4). ^{13}C NMR (75 MHz, D_2O) δ 173.37, 136.72, 128.99, 128.79, 128.76, 101.93, 76.07, 73.80, 73.37, 70.18, 68.60, 60.90, 57.28, 55.32. HRMS *m*/*z* calcd. for $C_{16}H_{24}NO_7$ ($M+H^+$) 342.1536, found 342.1547.

Methyl 2-(2-hydroxy)acetamido-2-deoxy-β-D-glucopyranoside (4)



The title compound was prepared by dissolving compound **3** (100 mg, 0.41 mmol) in MeOH (5 mL), followed by the addition of a catalytic amount of 10% Pd/C. The resulting suspension was stirred vigorously under H_2 gas (1 atm) for 12 h. The suspension was filtered through celite, and the resulting filtrate evaporated under reduced pressure to afford **4** (82 mg, 89%) as a colourless oil. ^{1}H NMR (400 MHz, D_2O) δ 4.57 (d, *J* = 8.4 Hz, 1H, H-1), 4.16 (s, 2H, CH_2), 3.98 (dd, *J* = 12.3 Hz, 1.9 Hz, 1H, H-6a), 3.84 – 3.76 (m, 2H, H-6b, H-5), 3.67 (dd, *J* = 10.3 Hz, 8.6 Hz, 1H, H-2), 3.55 (s, 3H, CH_3), 3.53 – 3.45 (m, 2H, H-3, H-4). ^{13}C NMR (75 MHz, D_2O) δ 175.77, 101.93, 76.08, 73.86, 70.15, 61.17, 60.91, 57.24, 55.30. HRMS *m*/*z* calcd. for $C_9H_{16}NO_7Na$ ($M+Na^+$) 274.0906, found 274.0921

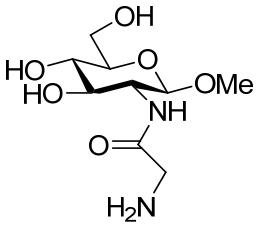
Methyl 2-(2-benzylcarboxyamino)acetamido-2-deoxy-β-D-glucopyranoside (5)



The title compound was prepared by dissolving compound **2** (200 mg, 0.93 mmol)**,** Cbz-Gly-OH (180 mg, 0.93 mmol) and triethylamine (55 µL, 1 mmol) in DMF. HBTU (355 mg, 0.93 mmol) was then added portion wise to the mixture at 0 °C over 30 min and allowed to stir overnight at room temperature. The solution was then evaporated under reduced pressure and the resulting residue was purified by flash chromatography (8% MeOH in DCM) to afford **5** (178 mg, 76%) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 7.58 – 7.39 (m, 5H, Ar-H), 4.52 (d, *J* = 8.4 Hz, 1H, H-1), 4.22 (d, *J* = 5.3 Hz, 2H, C**H$_2$**Ar), 3.97 (dd, J = 12.0 Hz, 1.9 Hz, 1H, H-6a), 3.90 – 3.70 (m, 4H, H-6b, H-5, CH_2), 3.66 – 3.58 (m, 1H, H-

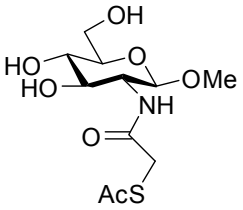
2), 3.58 – 3.42 (m, 5H, H-3, H-4, CH_3). ^{13}C NMR (75 MHz, D_2O) δ 175.74, 160.93, 137.29, 130.29, 129.38, 129.17, 104.55, 84.41, 78.61, 76.49, 72.70, 70.12, 63.48, 59.92, 58.30. HRMS *m*/*z* calcd for $C_{17}H_{25}N_2O_8$ ($M+H^+$) 385.1554, found 385.1561

Methyl 2-(2-amino)acetamido-2-deoxy-β-D-glucopyranoside (6)



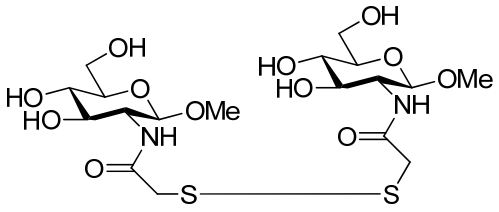
The title compound was prepared by dissolving compound **5** (100 mg, 0.40 mmol) in MeOH (5 mL), followed by the addition of a catalytic amount of 10% Pd/C. The resulting suspension was stirred vigorously under H_2 gas (1 atm) for 12 h. The suspension was filtered through celite, and the resulting filtrate evaporated under reduced pressure to afford **6** (72 mg, 81%) as a colourless oil. ^{1}H NMR (400 MHz, D_2O) δ 4.57 (d, *J* = 8.4 Hz, 1H, H-1), 3.98 (dd, *J* = 12.3 Hz, 1.9 Hz, 1H, H-6a), 3.91 (d, J = 2.8 Hz, 2H, CH_2), 3.84 – 3.76 (m, 2H, H-6b, H-5), 3.67 (dd, *J* = 10.3 Hz, 8.6 Hz, 1H, H-3), 3.55 (s, 3H, CH_3), 3.53 – 3.45 (m, 2H, H-4, H-2).^{13}C NMR (75 MHz, D_2O) δ 175.77, 101.93, 76.08, 73.86, 70.15, 61.17, 60.91, 57.24, 55.30. HRMS *m*/*z* calcd. For $C_9H_{18}N_2O_6Na$ ($M+Na^+$) 273.0900, found 273.0918

Methyl 2-(2-thioacetyl)acetamido-2-deoxy-β-D-glucopyranoside (7)



The title compound was prepared by dissolving compound **2** (200 mg, 0.93 mmol) and triethylamine (55 µL, 1 mmol) in DMF. Pentafluorophenyl 2-(acetylthio)acetate (310 mg, 0.93 mmol) was then added to the mixture and allowed to stir overnight at room temperature. The solution was evaporated under reduced pressure and the resulting residue was purified by flash chromatography (8% MeOH in DCM) to afford **7** (160 mg, 78%) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 4.49 (d, *J* = 8.3 Hz, 1H, H-1), 3.96 (dd, *J* = 12.2 Hz, 1.6 Hz, 1H, H-6a), 3.74 - 3.64 (m, 4H, H-6b, H-2, CH_2), 3.60 (dd, J = 9.8 Hz, J = 8.6 Hz, 1H, H-3), 3.52 (s, 3H, OCH_3), 3.51 – 3.44 (m, 2H, H-3, H-4), 2.45 (s, 3H, CH_3). ^{13}C NMR (100 MHz, D_2O) δ 199.48, 171.71, 102.14, 76.04, 73.77, 70.16, 60.90, 57.40, 56.12, 33.24, 29.67. HRMS *m*/*z* calcd. for $C_{11}H_{19}NO_7SNa$ ($M+Na^+$) 332.0778, found 332.0774

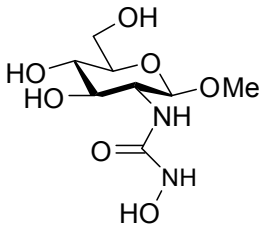
2,2'-disulfanediylbis(*N*-(Methyl-2-deoxy-β-D-glucopyranos-2-yl)acetamide) (8)



The title compound was prepared by dissolving compound **7** (80 mg, 0.3 mmol) in MeOH (5 mL) containing a catalytic amount of NaOMe and the solution was stirred for 10 min. Amberlite resin (H^+) was added to the mixture until the solution was neutral. Filtration and evaporation under reduced pressure yielded the symmetrical disulfide **8** (65 mg, 92%) as a white powder. ^{1}H NMR (400 MHz, D_2O) δ 4.54 (d, *J* = 8.4 Hz, 1H, H-1), 3.98 (d, *J* = 11.9 Hz, 1H, H-6a), 3.78 (d, *J* = 9.1 Hz, 2H, CH_2), 3.68 – 3.43 (m, 8H, H-6b, H-5, H-3, H-4, OCH3, H-2). ^{13}C NMR (100 MHz, D_2O) δ 175.23, 100.27, 76.34,

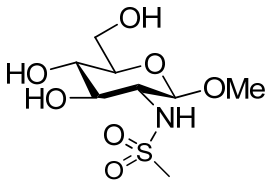
72.38, 69.93, 60.60, 57.58, 55.94, 23.34. $C_{18}H_{33}N_2O_{14}S_2$ ($M+H^+$) 533.1484, found 533.1469

Methyl 2-(3-hydroxy)ureayl-2-deoxy-β-D-glucopyranoside (9)



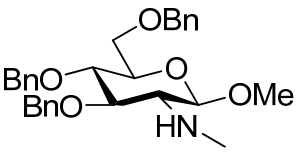
The title compound was prepared by dissolving compound **2** (200 mg, 0.93 mmol) in MeOH with triethylamine (55 µL, 1 mmol). 4-nitrophenyl carboxamate (385 mg, 1.5 mmol),[4] was subsequently added portion wise over 30 min at 0 °C and the mixture was allowed to stir overnight. Following complete consumption of the glycosyl starting material, as judged by TLC, the reaction mixture was evaporated under reduced pressure, and the resulting residue was dissolved in 1M AcOH (25 mL) and washed with 3x50 mL EtOAc. Collection and evaporation of the aqueous layer under reduced pressure, followed by flash chromatography of the resulting residue (8% MeOH in DCM) yielded **9** (110 mg, 54%) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 4.54 (d, *J* = 8.1 Hz, 1H, H-1), 3.98 (dd, *J* = 12.3 Hz, 1.8 Hz, 1H, H-6a), 3.79 (dd, *J* = 12.3 Hz, 5.6 Hz, 1H, H-6b), 3.62 – 3.42 (m, 7H, H-2, H-3, CH_3, H-4, H-5). ^{13}C NMR (75 MHz, D_2O) δ 163.57, 100.43, 76.05, 74.14, 70.20, 60.95, 57.30, 56.00. HRMS *m*/*z* calcd. for $C_8H_{16}N_2O_7Na$ ($M+Na^+$) 275.0855, found 275.0849

Methyl 2-methylsulfonamido-2-deoxy-β-D-glucopyranoside (10)



The title compound was prepared by dissolving compound **2** (200 mg, 0.93 mmol) and triethylamine (110 uL, 2 mmol) in DMF. Mesyl chloride (65 uL, 1 mmol) was then added to the mixture slowly and allowed to stir overnight at room temperature. The mixture was evaporated under reduced pressure and the resulting residue was purified by flash chromatography (8% MeOH in DCM) to afford **10** (112 mg, 58%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 4.37 (d, *J* = 8.4 Hz, 1H, H-1), 3.89 (dd, *J* = 12.3 Hz, 1.9 Hz, 1H, H-6a), 3.69 (dd, *J* = 12.4 Hz, 5.8 Hz, 1H, H-6b), 3.54 (s, 3H, OCH_3), 3.46 – 3.34 (m, 4H, H-2, H-3, H-4, H-5), 3.11 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, D_2O) δ 100.27, 76.34, 72.38, 69.93, 60.60, 57.58, 55.94, 39.12 HRMS *m*/*z* calcd. for $C_8H_{17}NO_7SNa$ ($M+Na^+$) 294.0711, found 294.0713

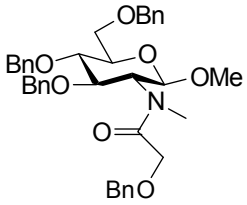
Methyl 2-methylamino-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (14)



The title compound was synthesized in 3 steps following a literature procedure.[3] Methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (5 g, 10 mmol) was dissolved in THF (100 mL). Boc Anhydride (4.4 g, 20 mmol) and DMAP (0.5 g, 0.5 mmol) were then added and the solution was then refluxed for 16 h. The solution was then evaporated to dryness under reduced pressure. The resulting residue was then dissolved in MeOH (100 mL) containing a catalytic amount of NaOMe for 4 h, at which point the Boc protected compound precipitated. Vacuum filtration of resulting solid yielded methyl 2-tert-butylcarboxamido-2-deoxy-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (3.8 g, 7.2 mmol). The resulting sugar (**13**) was

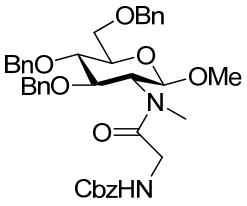
then dissolved in THF (100 mL) and to the solution, on ice, was slowly added $LiAlH_4$ (21.6 mmol) a 1M solution in THF. Following complete addition of $LiAlH_4$, the solution was refluxed for 12 h. The solution was then neutralized with water, washed consecutively with a 200 mL of a 0.1 M solution of sodium potassium tartrate, water, brine, and the resulting organics were dried over magnesium sulphate. Evaporation under reduced pressure, followed by flash chromatography (1:1 EtOAc/Pentane) afforded **14** (2.1 g, 4.8 mmol) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.12 (m, 15H, ArH), 4.95 (d, *J* = 11.5 Hz, 1H, Ar-C**H$_a$**H$_b$), 4.79 (d, *J* = 10.8 Hz, 1H, Ar-CH$_a$**H$_b$**), 4.74 – 4.55 (m, 4H, Ar-CH_2, Ar-CH_2), 4.16 (d, *J* = 7.9 Hz, 1H, H-1), 3.81 – 3.65 (m, 3H, H-6a, H-6b, H5), 3.57 – 3.44 (m, 5H, H-4, H-3, OCH_3), 2.51 – 2.43 (m, 4H, H-2, NCH_3), 1.26 (s, 1H, N-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.53, 138.26, 137.86, 128.64, 128.59, 128.56, 128.49, 128.36, 128.08, 127.93, 127.75, 100.22, 100.08, 79.63, 79.39, 79.26, 78.54, 75.17, 74.96, 73.63, 68.82, 61.49, 57.01, 42.73, 29.84, 28.56, 28.51. HRMS *m*/*z* calcd. for $C_{29}H_{36}NO_5$ (M+H$^+$) 478.2596, found 478.2588.

Methyl 2-N-methyl-(2-benzyloxy)acetamido-2-deoxy-3,4,6-tri-O-benzyl-β-D-glucopyranoside (15)



The title compound was prepared by dissolving **14** (250 mg, 0.55 mmol)**,** benzyloxyacetic acid (120 mg, 0.6 mmol) and triethylamine (30 uL, 0.58 mmol) in a 50:50 EtOAc/DCM solution. DCC (140 mg, 0.66 mmol) was then added portion wise to the mixture at 0°C over 30 min and allowed to stir overnight at room temperature. The mixture was then filtered through celite and evaporated under reduced pressure. The resulting residue was then dissolved in EtOAc, washed consecutively with 1M $NaOH_{(aq)}$, water, and brine, and dried with magnesium sulphate. Evaporation under reduced pressure, followed by flash chromatography (1:1 EtOAc/Pentane) afforded **15** (260 mg 89%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$; mixture of rotamers) δ 7.40 – 7.04 (m, 20H, Ar-H), 4.81 – 4.63 (m, 2H, Ar-CH_2), 4.60 – 4.37 (m, 7H, Ar-CH_2, H-1), 4.32 – 4.15 (m, 1.4H, $COCH_2$), 3.97 (d, *J* = 13.8 Hz, 0.6H, $COCH_2$), 3.73 – 3.55 (m, 5H, H-6a, H-6b, H-5, H-2, H-4), 3.48 – 3.30 (m, 4H, H-3, OCH_3), 2.98 - 2.79 (br s, 2.2H, NCH_3), 2.68 (s, 1.8H NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.07, 138.65, 138.30, 137.98, 137.96, 137.85, 137.57, 128.66, 128.61, 128.56, 128.52, 128.49, 128.44, 128.39, 128.33, 128.17, 128.15, 128.08, 128.04, 128.00, 127.96, 127.88, 127.78, 127.68, 100.43 (d), 79.05, 78.98, 75.14, 75.07, 74.96, 74.82, 73.72, 73.56, 73.10, 69.67, 68.89, 68.53, 61.31, 57.13, 34.05, 27.83, 25.07. HRMS *m*/*z* calcd. for $C_{38}H_{43}NO_7Na$ (M+Na$^+$) 648.2938, found 648.2931.

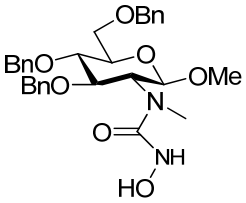
Methyl 2-N-methyl-(2-benzylcarboxyamino)acetamido-2-deoxy-3,4,6-tri-O-benzyl-β-D-glucopyranoside (16)



The title compound was prepared by dissolving **14** (250 mg, 0.55 mmol)**,** Cbz-Gly-OH (120 mg, 0.6 mmol) and triethylamine (30 µL, 0.58 mmol) in a 50:50 EtOAc/DCM solution. DCC (140 mg, 0.66 mmol) was then added portion wise to the mixture at 0°C over 30 min and allowed to stir overnight at room temperature. The mixture was then filtered through celite and evaporated under reduced

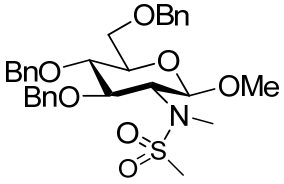
pressure. The resulting residue was then dissolved in EtOAc, washed consecutively with 1M $NaOH_{(aq)}$, water, and brine, dried with magnesium sulphate. Evaporation under reduced pressure, followed by flash chromatography (1:1 EtOAc/Pentane) afforded **16** (268 mg, 92%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$; mixture of rotamers) δ 7.45 – 7.00 (m, 20H, Ar-H), 4.79 – 4.59 (m, 2H, Ar-CH_2), 4.57 – 4.40 (m, 7H, Ar-CH2, H-1), 4.35 – 4.15 (m, 1.5H, $COCH_2$), 3.98 (d, *J* = 11.9 Hz, 0.5H, $COCH_2$), 3.73 – 3.50 (m, 5H, H-6a, H-6b, H-5, H-2, H-4), 3.45 – 3.26 (m, 4H, H-3, OCH_3), 2.95 (br s, 0.9H, NCH_3), 2.71 (s, 2.1H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$; mixture of rotamers) δ 170.64, 160.38, 138.22, 137.87, 137.56, 137.54, 137.43, 137.15, 128.23, 128.18, 128.14, 128.10, 128.07, 128.02, 127.97, 127.90, 127.75, 127.72, 127.66, 127.62, 127.58, 127.54, 127.45, 127.36, 127.26, 100.00 (d), 78.62 (d), 74.72, 74.65, 74.54, 74.40 (d), 73.13, 72.68, 69.25, 68.46, 68.11 (d), 56.70, 33.63, 27.40. HRMS *m*/*z* calcd. for $C_{38}H_{43}NO_7Na$ ($M+Na^+$) 691.8276, found 691.8270.

Methyl 2-N-methyl-(3-hydroxy)ureayl-2-deoxy-3,4,6-tri-O-benzyl-β-D-glucopyranoside (17)



The title compound was prepared by dissolving **14** (250 mg, 0.55 mmol) and triethylamine (30 µL, 0.58 mmol) in DCM. 4-nitrophenyl carboxamate (210 mg, 0.66 mmol) was then added slowly to the mixture at 0 °C over 30 min and allowed to stir overnight at room temperature. The mixture was washed consecutively with 1M $NaOH_{(aq)}$, water, and brine, dried with magnesium sulphate. Evaporation under reduced pressure, followed by flash chromatography (2:1 EtOAc/Pentane) afforded **17** (204 mg, 72%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.01 (br s, 1H, OH) 7.62 – 6.91 (m, 15H, Ar-H), 4.95 (d, *J* = 11.3 Hz, 1H, Ar-C**H$_a$**H$_b$), 4.84 – 4.73 (m, 2H, H-1, CH$_a$**H$_b$**), 4.69 – 4.52 (m, 4H, Ar-CH_2, Ar-CH_2), 4.31 (d, *J* = 7.7 Hz, 1H, N-H), 3.79 – 3.42 (m, 10H, H-6a, H-6b, H-5, H-4, H-2, H-3, OCH_3), 2.54 (s, 3H, N-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.03, 138.65, 138.40, 135.86, 129.55, 129.49, 129.42, 129.34, 129.33, 129.27, 129.19, 129.01, 128.84, 128.80, 128.63, 128.59. 99.88, 79.82, 77.38, 77.11, 73.51, 71.60, 67.66, 58.40, 54.76, 53.46, 26.10. HRMS *m*/*z* calcd. for $C_{30}H_{36}N_2O_7Na$ ($M+Na^+$) 559.6523, found 559.6531.

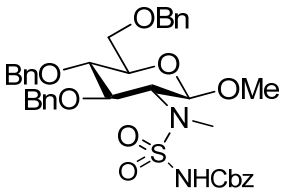
Methyl 2-N-methyl-(methylsulfonamido)-2-deoxy-3,4,6-tri-O-benzyl-β-D-glucopyranoside (18)



The title compound was prepared by dissolving **14** (250 mg, 0.55 mmol) and triethylamine (60 µL, 1.15 mmol) in DCM. MsCl (50 µL, 0.82 mmol) was then added slowly to the mixture at 0 °C over 30 min and allowed to stir overnight at room temperature. The mixture was then washed consecutively with 1M $NaOH_{(aq)}$, water, and brine, dried with magnesium sulphate. Evaporation under reduced pressure, followed by flash chromatography (1:1 EtOAc/Pentane) afforded **18** (232 mg, 88%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.10 (m, 15H, ArH), 4.87 – 4.65 (m, 4H, ArCH_2, ArCH_2), 4.50 – 4.35 (m, 3H, H-1, ArCH_2), 3.65 – 3.53 (m, 6H, H-6a, H-6b, H-5, H-3, H-4, H-2), 3.32 (s, 3H, OCH_3), 2.98 (s, 3H, SCH_3), 2.58 (s, 3H, NCH_3). ^{13}C NMR (75 MHz,

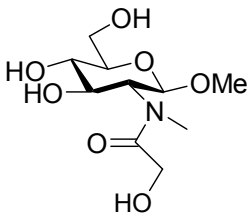
$CDCl_3$) δ 137.61, 137.51, 134.72, 128.41, 128.35, 128.28, 128.27, 128.23, 128.18, 128.13, 128.05, 127.94, 127.86, 127.69, 127.66, 127.62, 127.49, 127.44, 99.78, 78.48, 74.86, 74.75, 74.62, 73.21, 68.16, 67.77, 66.67, 56.41, 31.06, 21.72. HRMS *m*/*z* calcd. for $C_{30}H_{37}NO_7SNa$ ($M+Na^+$) 578.1120, found 578.1131.

Methyl 2-N-methyl-*N*-benzylcarboxyaminosulfonamido-2-deoxy-3,4,6-tri-O-benzyl-β-D-glucopyranoside (19)



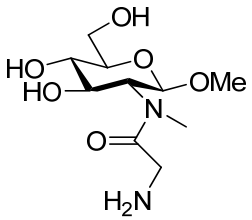
The title compound was prepared by first dissolving chlorosufonyl isocyanate (0.80 µL, 0.75 mmol) and benzyl alcohol (95 µL, 0.75 mmol) in DCM, and allowed to stir for 30 min at 0 °C.[4] Following this, the DCM solution was combined with a DCM solution containing **14** (250 mg, 0.55 mmol) and triethylamine (60 µL, 1.2 mmol), and allowed to stir for 3 h. The mixture was then washed consecutively with 1M $NaOH_{(aq)}$, water, and brine, dried with magnesium sulphate. Evaporation under reduced pressure, followed by flash chromatography (1:1 EtOAc/Pentane) afforded **19** (232 mg, 88%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 6.99 (m, 20H, ArH), 5.07 (s, 2H, $ArCH_2$), 4.77 – 4.68 (m, 4H, $ArCH_2$, $ArCH_2$), 4.55 – 4.41 (m, 3H, H-1, $ArCH_2$), 4.26 (br s, 1H, NH), 3.69 – 3.56 (m, 5H, H-6a, H-6b, H-5, H-3, H-4), 3.32 (m, 4H, OCH_3. H-2), 2.74 (s, 3H, NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.55, 137.98, 137.88, 137.63, 136.32, 135.09, 128.78, 128.72, 128.65, 128.64, 128.60, 128.56, 128.50, 128.42, 128.31, 128.24, 128.07, 128.03, 127.99, 127.86, 127.82, 100.16, 78.86, 75.23, 75.13, 75.00, 73.58, 68.54, 68.14, 67.04, 56.78, 22.86. HRMS *m*/*z* calcd. for $C_{37}H_{46}N_3O_9S$ ($M+NH_4^+$) 708.2922, found 708.2928.

Methyl 2-N-methyl-(2-hydroxy)acetamido-2-deoxy-β-D-glucopyranoside (20)



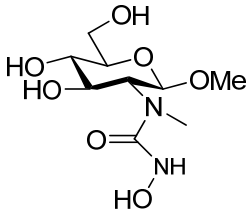
The title compound was prepared by dissolving **15** (200 mg, 0.41 mmol) in MeOH (5 mL), followed by the addition of a catalytic amount of 10% Pd/C. The resulting suspension was stirred vigorously under H_2 gas (1 atm) for 12 h. Upon reaction completion, the suspension was filtered through celite, and the resulting filtrate was evaporated under reduced pressure to afford **20** (68 mg, 82%) as a white powder. ^{1}H NMR (400 MHz, D_2O; mixture of rotamers) δ 4.89 (d, J = 7.9 Hz, 1H, H-1) 4.39 (br d, *J* = 5.2 Hz, 2H, CH_2), 4.03 – 3.71 (m, 3H, H-6a, H-6b, H-5), 3.58 – 3.48 (br m, 5H, H-4, H-3, CH_3), 3.34 – 3.23 (br m, 1H, H-2), 2.95 (br s, 3H, NCH_3).^{13}C NMR (100 MHz, D_2O; mixture of rotamers) δ 175.00, 99.86, 76.03 - 75.98 (d), 70.69 - 70.58 (d), 70.36, 61.74, 60.93-60.87 (d), 60.24 - 60.14 (d) 57.48 – 57.36 (d), 27.97. HRMS *m*/*z* calcd. For $C_{10}H_{19}NO_7Na$ ($M+Na^+$) 288.1223, found 288.1222

Methyl 2-N-methyl-(2-amino)acetamido-2-deoxy-β-D-glucopyranoside (21)



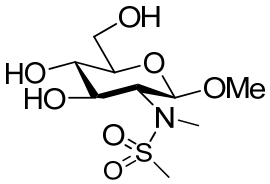
The title compound was prepared by dissolving **16** (200 mg, 0.41 mmol) in MeOH (5 mL), followed by the addition of a catalytic amount of 10% Pd/C. The resulting suspension was stirred vigorously under H_2 gas (1 atm) for 12 h. Upon reaction completion, the suspension was filtered through celite, and the resulting filtrate evaporated under reduced pressure to afford **21** (64 mg, 78%) as a white powder. ^{1}H NMR (400 MHz, CD_3OD; mixture of rotamers) δ 4.50 (br d, *J* = 7.5 Hz, 1H, H-1), 4.00 – 3.76 (br m, 3H, H-6a, H-6b, H-5), 3.63 (br m, 2H, CH_2), 3.52 – 3.18 (br m, 6H, OCH_3, H-4. H-2, H-3), 2.95 (br s, 1.2H, NCH_3), 2.80 (br s, 1.8H, NCH_3). ^{13}C NMR (100 MHz, CD_3OD; mixture of rotamers) δ 172.47, 101.07 - 100.99 (d), 77.69 – 77.56 (d), 72.13, 63.37, 62.50 – 62.42 (d), 57.04, 43.10, 28.09. HRMS *m*/*z* calcd. For $C_{10}H_{21}N_2O_6$ ($M+H^+$) 265.1393, found 265.1394

Methyl 2-N-methyl-(3-hydroxy)ureayl-2-deoxy-β-D-glucopyranoside (22)



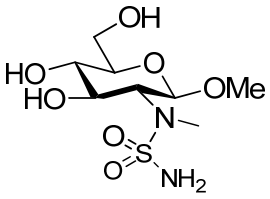
The title compound was prepared by dissolving **17** (200 mg, 0.38 mmol) in MeOH (5 mL), followed by the addition of a catalytic amount of 10% Pd/C. The resulting suspension was stirred vigorously under H_2 gas (1 atm) for 12 h. Upon reaction completion, the suspension was filtered through celite, and the resulting filtrate evaporated under reduced pressure to afford **22** (54 mg, 75%) as a white powder. ^{1}H NMR (400 MHz, D_2O) δ 4.75 (d, *J* = 7.3 Hz, 1H, H-1), 3.93 (dd, *J* = 11.5 Hz, 4.3 Hz, 1H. H-6a), 3.86 (m, 1H, H-5), 3.74 (dd, *J* = 11.1 Hz, 7.1 Hz, 1H, H-6b), 3.60 (s, 3H, OCH_3), 3.50 – 3.40 (m, 3H, H-4, H-2, H-3), 2.62 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, D_2O) δ 161.43, 100.33, 73.97, 72.05, 68.12, 58.86, 55.21, 53.91, 25.26. HRMS *m*/*z* calcd. for $C_8H_{16}N_2O_7Na$ ($M+Na^+$) 289.0735, found 289.0747

Methyl 2-N-methyl-(methylsulfonamido)-2-deoxy-β-D-glucopyranoside (23)



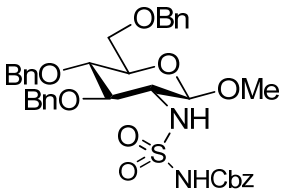
The title compound was prepared by dissolving **18** (200 mg, 0.38 mmol) in MeOH (5 mL), followed by the addition of a catalytic amount of 10% Pd/C. The resulting suspension was stirred vigorously under H_2 gas (1 atm) for 12 h. Upon reaction completion, the suspension was filtered through celite, and the resulting filtrate evaporated under reduced pressure to afford **23** (71 mg, 75%) as a colourless paste. ^{1}H NMR (400 MHz, D_2O) δ 4.67 (d, *J* = 8.4 Hz, 1H, H-1), 3.92 (dd, *J* = 11.3 Hz, J =1.9 Hz, 1H, H-6a), 3.81 (m, 1H, H-5) 3.69 (dd, *J* = 11.4, J = 5.8 Hz, 1H, H-6b), 3.59 (s, 3H, OCH_3), 3.46 – 3.34 (m, 3H, H-2, H-3, H-4), 3.11 (s, 3H, SCH_3), 2.74 (s, 3H, NCH_3). ^{13}C NMR (100 MHz, D_2O) δ 101.98, 76.34, 72.58, 69.72, 60.24, 57.58, 55.12, 40.21, 23.31. HRMS *m*/*z* calcd. for $C_8H_{17}NO_7SNa$ ($M+Na^+$) 289.0859, found 289.0851

Methyl 2-N-methyl-*N*-aminosulfonamido-2-deoxy-β-D-glucopyranoside (24)



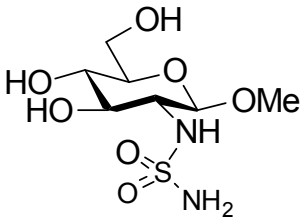
The title compound was prepared by dissolving **19** (200 mg, 0.42 mmol) in MeOH (5 mL), followed by the addition of a catylitic amount of 10% Pd/C. The resulting suspension was stirred vigorously under H_2 gas (1 atm) for 12 h. Upon reaction completion, the suspension was filtered through celite, and the resulting filtrate evaporated under reduced pressure to afford **24** (74 mg, 75%) as a colourless oil. ^{1}H NMR (400 MHz, D_2O) δ 4.61 (d, *J* = 8.1 Hz, 1H, H-1) 3.93 (dd, *J* = 7.8 Hz, 4.6 Hz, 1H, H-6a), 3.88 – 3.84 (m, 1H, H-5), 3.78 – 3.71 (m, 1H, H-6b), 3.60 (s, 3H, OCH_3), 3.48 – 3.44 (m, 3H, H-4, H-2, H-3), 2.80 (s, 3H, NCH_3). ^{13}C NMR (100 MHz, D_2O) δ 99.69, 75.60, 70.51, 70.32, 63.12, 60.63, 56.76, 21.46. HRMS *m*/*z* calcd. for $C_8H_{18}N_2O_7NaS$ ($M+Na^+$) 309.0726, found 309.0726

Methyl 2-benzylcarboxyaminosulfonamido-2-deoxy-3,4,6-tri-O-benzyl-β-D-glucopyranoside (26)



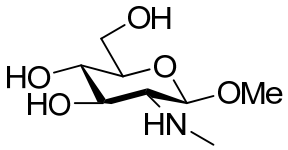
The title compound was prepared by first dissolving chlorosufonyl isocyanate (0.80 µL, 0.75 mmol) and benzyl alcohol (95 µL, 0.75 mmol) in DCM, and allowed to stir for 30 min at 0 °C. Following this, the reaction mixture was combined with a DCM solution containing **25** (240 mg, 0.55 mmol) and triethylamine (60 uL, 1.2 mmol), and allowed to stir for 3 h. The mixture was then washed consecutively with 1M $NaOH_{(aq)}$, water, and brine, dried with magnesium sulphate. Evaporation under reduced pressure, followed by flash chromatography (1:1 EtOAc/Pentane) afforded **26** (232 mg, 88%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.04 (m, 20H), 4.88 – 4.61 (m, 3H), 4.60 – 4.37 (m, 6H), 4.35 – 4.15 (m, 2H), 3.73 – 3.55 (m, 3H), 3.48 – 3.30 (m, 4H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.78, 151.43, 137.87, 137.77, 137.52, 136.20, 134.97, 128.67, 128.61, 128.54, 128.53, 128.48, 128.44, 128.38, 128.30, 128.20, 128.12, 127.95, 127.91, 127.88, 127.74, 127.70, 100.04, 78.74, 75.12, 75.01, 74.88, 73.46, 68.42, 68.02, 66.92, 56.66. HRMS *m*/*z* calcd. for $C_{37}H_{42}N_2O_9S$ ($M+Na^+$) 699.2385, found 694.2388.

Methyl 2-aminosulfonamido-2-deoxy-β-D-glucopyranoside (27)



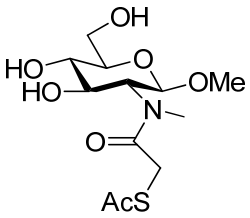
The title compound was prepared by dissolving **26** (200 mg, 0.42 mmol) in MeOH (5 mL), followed by the addition of a catalytic amount of 10% Pd/C. The resulting suspension was stirred vigorously under H_2 gas (1 atm) for 12 h. Upon reaction completion, the suspension was filtered through celite, and the resulting filtrate evaporated under reduced pressure to afford **27** (74 mg, 75%) as a colourless oil. ^{1}H NMR (400 MHz, D_2O) δ 4.53 (d, *J* = 8.4 Hz, 1H, H-1), 3.78 (dd, *J* = 12.3 Hz, J = 2.0 Hz, 1H H-6a), 3.59 - 3.54 (m, 2H H-6b, H-5), 3.47 – 3.25 (m, 6H, H-4, H-2, H-3, CH_3) ^{13}C NMR (100 MHz, D_2O) δ 102.65, 76.60, 74.51, 70.32, 60.12, 57.63, 56.76. HRMS *m*/*z* calcd. for $C_8H_{18}N_2O_7NaS$ ($M+Na^+$) 294.0825, found 294.0820

Methyl 2-methylamino 2-deoxy-β-D-glucopyranoside (28)



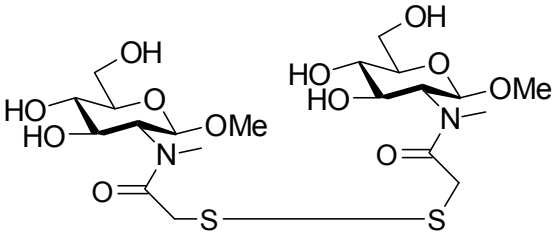
The title compound was prepared by dissolving **14** (200 mg, 0.41 mmol) in aldehyde free MeOH (5 mL) followed by the addition of a catalytic amount of 10% Pd/C. The resulting suspension was stirred vigorously under H_2 gas (1 atm) for 12 h. Upon reaction completion, the suspension was filtered through celite, and the resulting filtrate evaporated under reduced pressure to afford **28** (64 mg, 78%) as a colorless film. ^{1}H NMR (400 MHz, D_2O) δ 4.47 (d, *J* = 8.5 Hz, 1H, H-1), 3.77 (dd, *J* = 12.6 Hz, 4.3 Hz, 1H, H-6a), 3.60 (dd, *J* = 12.4 Hz, 6.4 Hz, 1H, H-6b), 3.50 (dd, *J* = 10.6, 8.4 Hz, 1H, H-3), 3.43 (s, 3H, OCH_3), 3.37 – 3.27 (m, 2H, H-5, H-4), 2.84 (dd, *J* = 10.6 Hz, 8.5 Hz, 1H, H-2), 2.51 (s, 3H, NCH_3). ^{13}C NMR (100 MHz, D_2O) δ 100.27, 76.34, 72.38, 69.93, 60.60, 57.58, 55.94, 23.34. HRMS *m*/*z* calcd. for $C_8H_{19}NO_5$ ($M+H^+$) 206.1014, found 206.1022

Methyl 2-N-methyl-(2-thioacetyl)acetamido-2-deoxy-β-D-glucopyranoside (29)



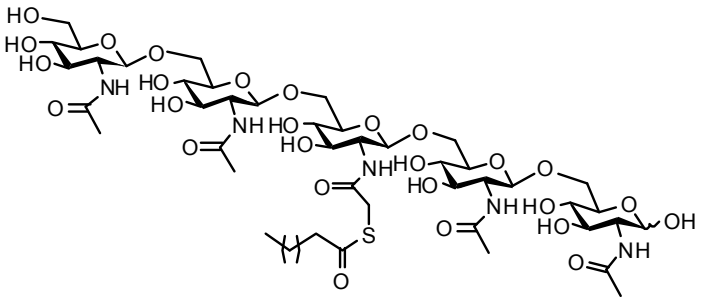
The title compound was prepared by dissolving compound **28** (50 mg, 0.23 mmol) and triethylamine (12 µL, 0.25 mmol) in DMF. Pentafluorophenyl 2-(acetylthio)acetate (75 mg, 0.23 mmol) was then added to the mixture and allowed to stir overnight at room temperature. The mixture was evaporated under reduced pressure. The resulting residue was then purified by flash chromatography (8% MeOH in DCM) to afford **20** (35 mg, 69%) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 4.72 (d, *J* = 8.3 Hz, 1H, H-1), 3.94 (dd, *J* = 12.2 Hz, *J* = 1.6 Hz, 1H, H-6a), 3.74 – 3.44 (m, 9H, H-6b, H-5, H-2, CH_2, H-4, H-3, OCH_3), 2.78 (s, 3H, N-CH_3), 2.32 (s, 3H, CH_3). ^{13}C NMR (100 MHz, D_2O) δ 197.04, 169.27, 99.70, 73.61, 71.34, 67.72, 58.47, 54.97, 53.68, 30.80, 29.09, 27.23. HRMS *m*/*z* calcd. for $C_{11}H_{19}NO_7SNa$ ($M+Na^+$) 323.0978, found 323.0981

2,2'-disulfanediylbis (*N*-methyl-*N*-(methyl-2--deoxy-β-D-glucopyranos-2-yl)acetamide (30)



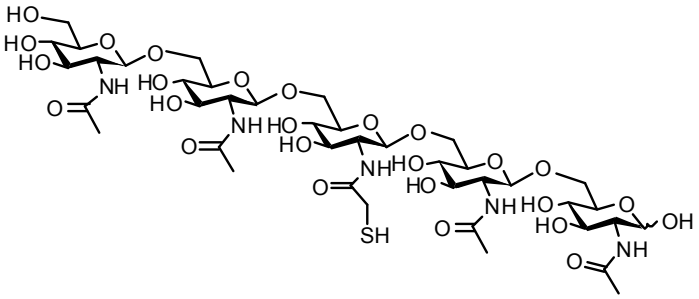
The title compound was prepared by dissolving **29** (40 mg, 0.32 mmol) in MeOH (5 mL) containing a catylatic amount of NaOMe and stirred for 10 min. Following complete consumption of the starting material as judged by TLC, amberlite resin (H^+) was added to the mixture until the pH was neutral. Filtration and evaporation under reduced pressure yielded the symmetrical disulfide **30** (23 mg 64%) as a white powder. ^{1}H NMR (400 MHz, D_2O) δ 4.68 (d, *J* = 8.4 Hz, 1H, H-1), 3.98 (d, *J* = 11.9 Hz, 1H, H-6a), 3.78 (d, *J* = 9.1 Hz, 2H, CH_2), 3.68 – 3.43 (m, 8H, H-6b, H-5, H-3, H-4, OCH3, H-2), 2.72 (s, 3H, NCH_3),. ^{13}C NMR (100 MHz, D_2O) δ 175.23, 100.27, 76.34, 72.38, 69.93, 60.60, 57.58, 55.94, 23.34. $C_{18}H_{33}N_2O_{14}S_2$ ($M+H^+$) 551.1524, found 551.1521

GlcNAc-GlcNAc-GlcNAcSOc-GlcNAc-GlcNAc (31)



The title compound was prepared by dissolving GlcNAc pentasaccharide (50 mg, 0.045 mmol) in HEPES buffer (50 mM, pH 7.5) to a final concentration of 10 mM, followed by the addition of MBP-PgaB to a final concentration of 50 μM. The solution was then incubated at 37 °C for 48 h. Desalting of the de-*N*-acetylated pentasaccharide on HPLC p2 size exclusion column yielded a 1:1 mixture of product and starting material (38 mg), which was used without further purification. The obtained powder (38 mg) was then dissolved in water (0.5 mL), and N-hydroxysuccinidyl 2-(octoylthio)acetate (100 mg, 0.55 mmol), dissolved in MeOH (0.5 mL), was then added to the solution and allowed to mix for 2 h. Upon complete consumption of the starting material, as judged by MALDI mass spectrometry, the reaction mixture was then evaporated under reduced pressure, redissolved in water, washed with EtOAc, and the aqueous layer was then subjected to reverse phase HPLC to isolate **31** (7.2 mg, 0.007 mmol) as a white foam. ^{1}H NMR (400 MHz, D_2O) δ 5.17 (d, *J* = 3.5 Hz, 0.4H. H-1α), 4.62 – 4.51 (m, 4H, H-1', H-1'', H-1''', H-1''''), 4.25 – 3.37 (m, 34H), 2.17 – 1.99 (m, 12H), 1.58 - 1.52 (m, 2H), 1.30 - 1.22 (m, 8H), 0.86 – 0.78 (m, 3H) $CH_3C_{18}H_{33}N_2O_{14}S_2$ ($M+Na^+$) 1214.8043, found 1214.8051

GlcNAc-GlcNAc-GlcNAcSH-GlcNAc-GlcNAc (32)



The title compound was prepared by dissolving **31** (7.2 mg, 0.007 mmol) in MeOH (0.5 mL) containing NaOMe (1 μM) for 1 hr. Upon complete consumption of the starting material, as judged by MALDI mass spectrometry, amberlite resin (H^+) was added to the mixture until the pH was neutral, and the solution was evaporated under reduced pressure. The residue was then redissolved in AcOH (0.5 M, 0.5 mL), and washed 3 times with EtOAc (0.5 mL). The aqueous phase was then freeze-dried, yielding **32** (4.6 mg, 0.005 mmol) as a white foam. ^{1}H NMR (500 MHz, D_2O) δ 5.19 (d, *J* = 3.5 Hz, 0.4H. H-1α), 4.70 (d, *J* = 8.6 Hz, 0.6H, H-1β), 4.60 – 4.53 (m, 4H, H-1', H-1'', H-1''', H-1''''), 4.25 – 3.37 (m, 32H), 2.17 – 1.99 (m, 12H, CH_3). $C_{18}H_{33}N_2O_{14}S_2$ ($M+Na^+$) 1088.1461, found 1088.1462

Enzyme Assays

MBP-PgaB Expression and Purification

MBP-PgaB was expressed in *E. coli* BL21(DE3) cells as previously described. [5] Briefly, a 1L culture containing kanamycin (100 mg/L) was grown at 37 °C to an OD_{600} of 0.3. Nickel (II) sulfate (0.26 g, 100 mmol) was then added to the culture and the temperature was reduced to 10 °C. Isopropyl 1-thio-β-D-galactopyranoside (0.123 g, 0.5 mmol) was then added to the culture 20 min after cooling. The culture was then allowed to incubate for 16 h at 10 °C. The culture was then centrifuged (3750 x g, 70 min) and the cell pellet resuspended in 10 mL buffer containing HEPES (50 mM, pH 7.5), NaCl (300 mM) and a Complete Mini protease inhibitor cocktail tablet (Roche) prior to cell lysis by sonication. The cell debris was pelleted by centrifugation (16 000 x g, 50 min). The resulting soluble fraction was purified using an amylose resin column (Sigma-Aldrich). PgaB was eluted from the column with a buffer containing HEPES (50 mM, pH 7.5). The fractions containing MBP-PgaB were concentrated using centrifugal filter units (Millipore, 30K cutoff) and stored at 4 °C. Under these conditions, the resulting fractions were stable for approximately 1 month.

Enzyme Substrate Analysis

Enzyme reactions were carried out in 50 µL solutions containing HEPES (100 mM, pH 7.5), PgaB (10 µM), and varying concentrations of pNPAc, AMC, 5-AMQ, 7-AMQ, and ACC. (0.05–1 mM for pNPAc, AMC, 0.05-10m M for 5-AMQ, 7-AMQ, ACC) at 25 °C in a 96 well plate. Substrates were all predissolved in DMSO and added as such. Final DMSO concentration was 10% (v/v) in all cases. Due to substrate solubility constraints and background absorbance, 1-10 mM was used as a maximum concentration of substrate. Reactions were monitored in real time using the increase of either absorbance signal or fluorescence signal at the wavelengths found in Table 1, resulting from the hydrolysis and release of acetate over 10 min. Background hydrolysis rate were also monitored and subtracted from the enzyme-catalyzed reaction.

Table 1. Absorbance / Excitation and emission values for compounds tested as enzyme substrates.

Substrate	Absorbance / Excitation (nm)	Fluorescence Emission (nm)
pNPAc	405	na
AMC	354	445
5-AMQ	460	na
7-AMQ	405	na
ACC	386	446

Determining Michaelis-Menten Parameters of ACC

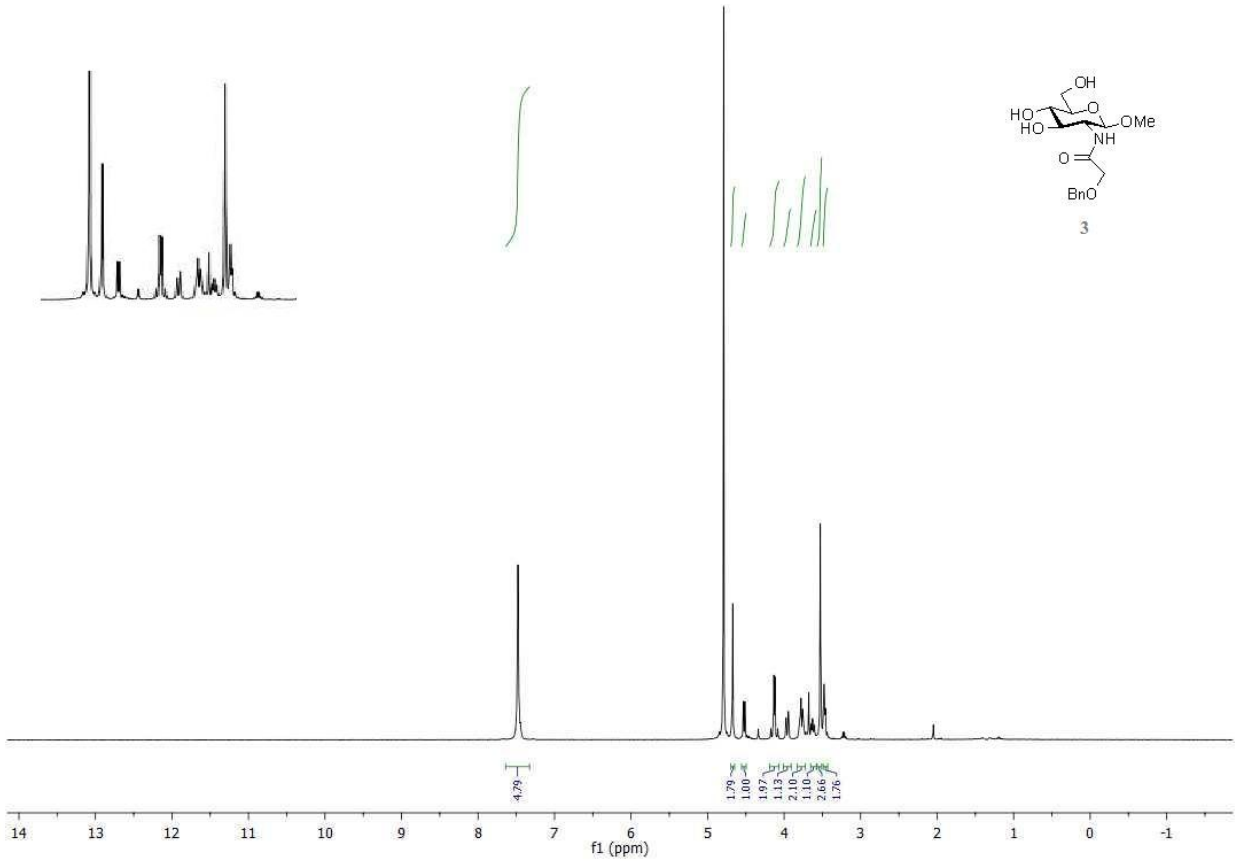
Enzyme reactions were carried out in 50 µL solutions containing HEPES (100 mM, pH 7.5), PgaB (10 µM), and varying concentrations of **ACC** (0.05 –10 mM) at 25 °C in a 96 well plate. **ACC** was predissolved in DMSO and added as such. Final DMSO concentration was 10% (v/v) in all cases. Reactions were monitored in real time using the increase of fluorescence signal at 446 nm resulting from the hydrolysis of the acetyl moiety and excitation at 386 nm over a 20 min period. Michealis-Menten kinetic parameters were obtained from direct curve fitting of the data. A slow but measurable background hydrolysis rate was also monitored and subtracted from the enzyme-catalyzed reaction. A control experiment replacing PgaB with bovine serum albumin (BSA, 10 *µ*M) showed no increase in reaction rate above background. All assays were performed in triplicate. A calibration curve for **ACC** was obtained under the reaction conditions and used to calculate reaction rate.

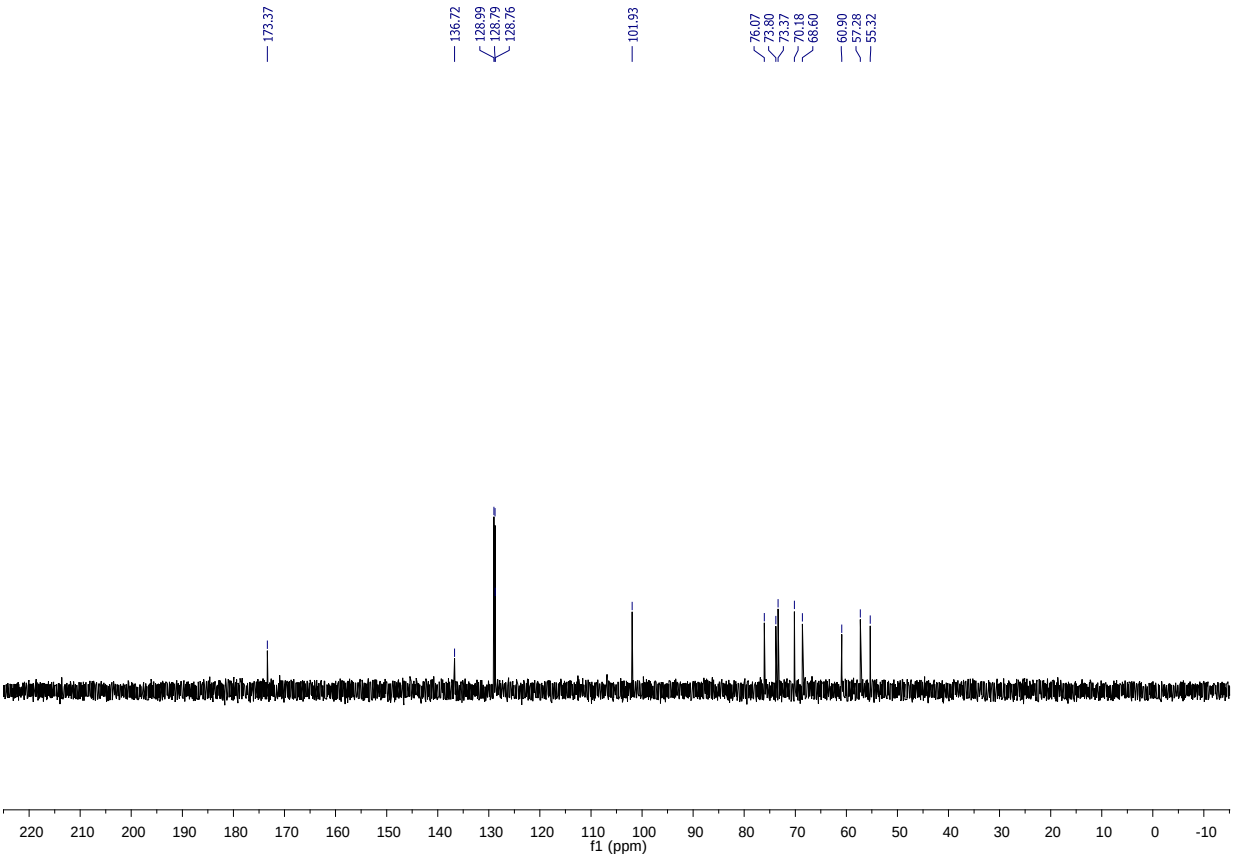
Obtaining Inhibitory Activity

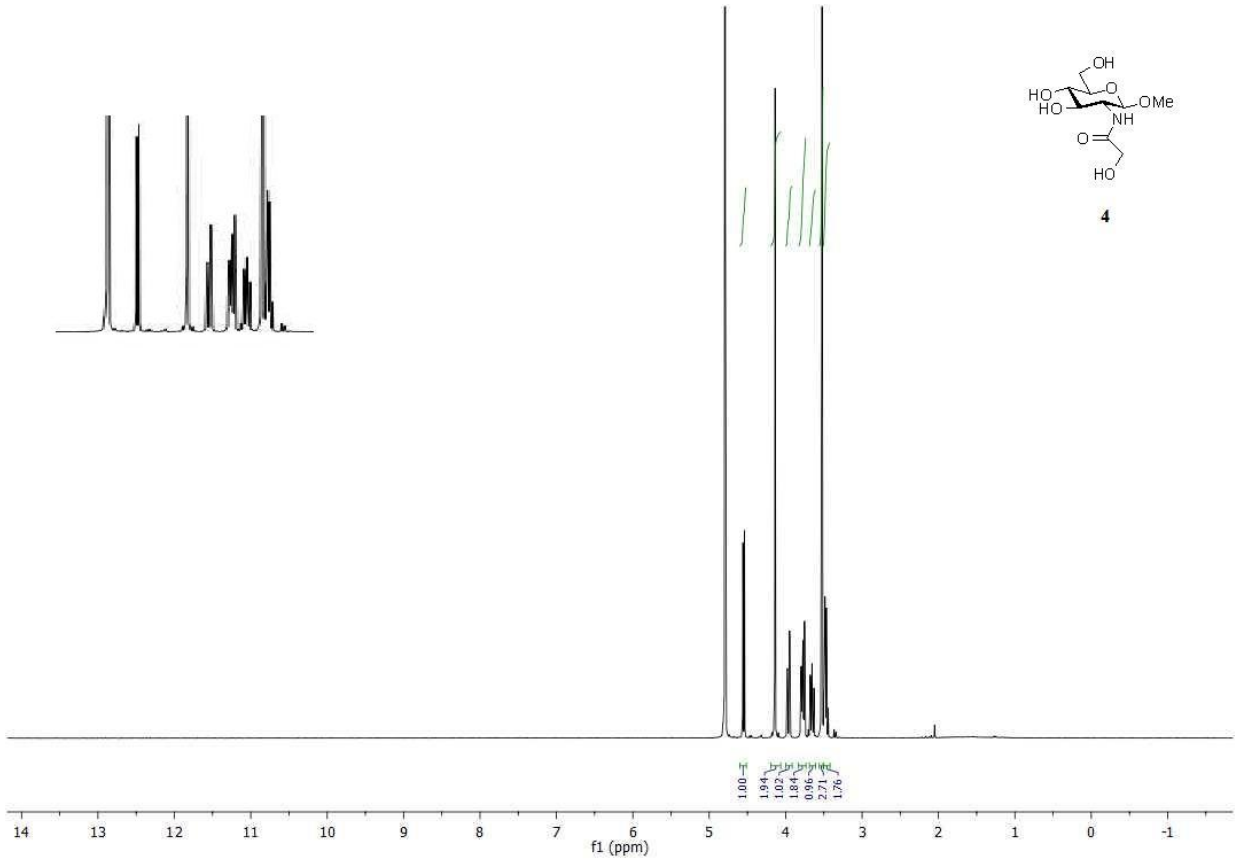
Enzyme reactions were carried out in 50 µL solutions containing HEPES (100 mM, pH 7.5), PgaB (10 µM), and **ACC** (5 mM) at 25 °C in a 96 well plate in the presence of inhibitors **4, 6, 8**, **10, 20-24, 27 30** (10 mM) and **6** and **21** (1 mM). **ACC** was predissolved in DMSO and added as such. Final DMSO concentration was 10% (v/v) in all assays. Reactions containing a free thiol (**8, 30**) were pre-incubated with 1.1 equivalents of DTT for 5 min before assay onset. Reactions were monitored in real time using the increase of fluorescence signal at 446 nm resulting from the hydrolysis of the acetyl moiety over a 20 min period. Percent activity was measured as the relative difference of **ACC** acetolysis rate in the presence and absence of each inhibitor. Samples were corrected for background hydrolysis lacking only PgaB enzyme.

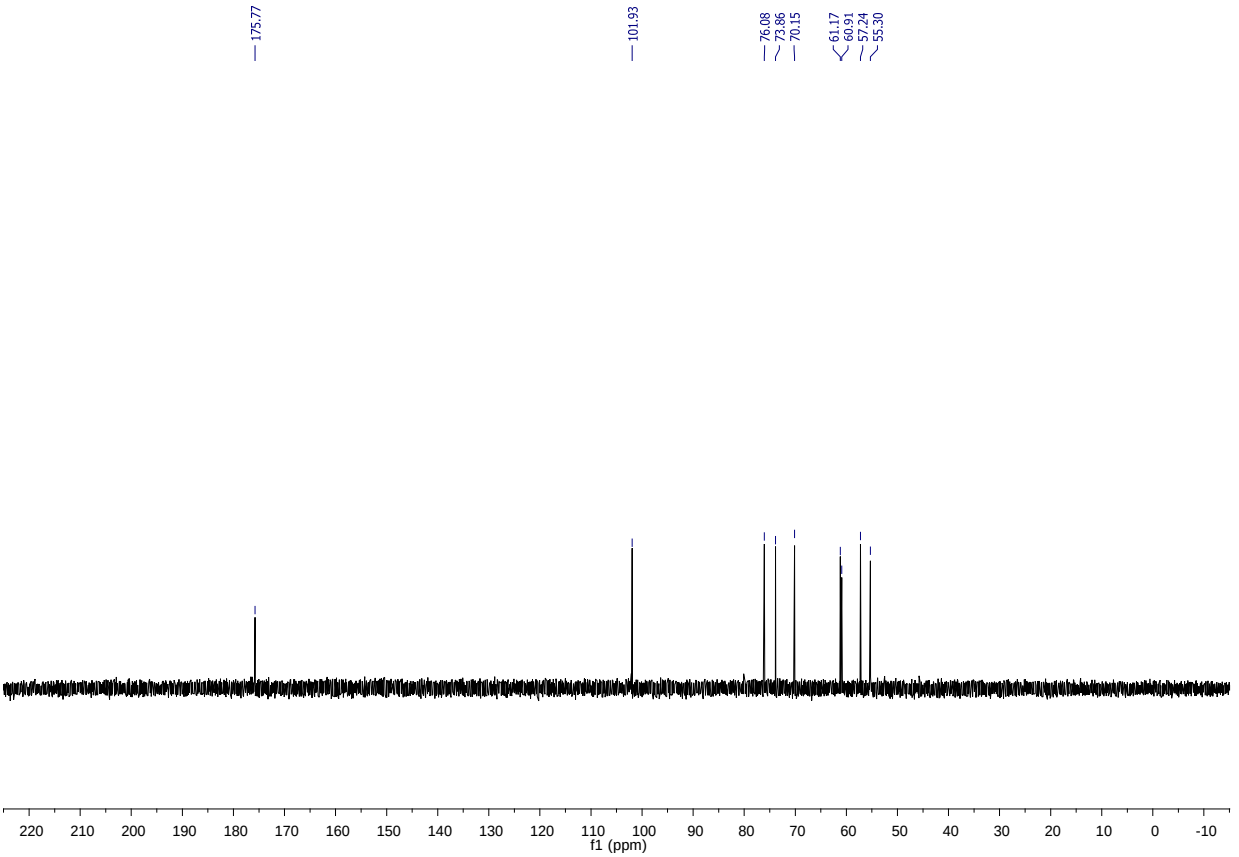
Determining Inhibition Constants

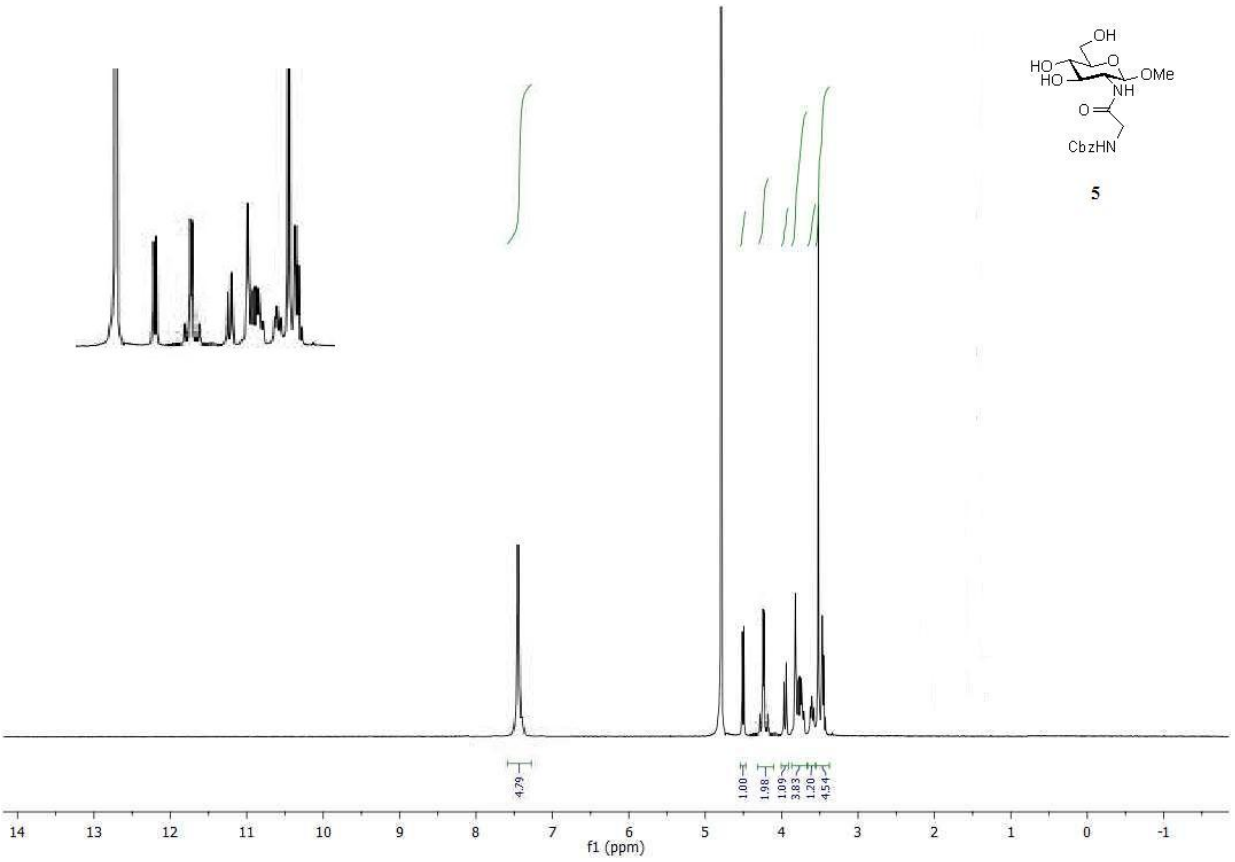
Enzyme reactions were carried out in 50 µL solutions containing HEPES (100 mM, pH 7.5) PgaB (10 µM) and varying concentrations of **ACC** (0.05 – 10mM) predissolved in DMSO at 25°C in a 96 well plate in the presence of 3 different concentrations (1 mM, 5 mM, 10 mM or 0.1 mM, 0.5 mM 1 mM for **32**) for inhibitors **8**, **20**, **23**, **24, 30** ,**32**. Final DMSO concentration was 10% in every assay. Reactions containing a free thiol (**8, 32**) were pre-incubated with 1.1 equivalents of DTT for 5 min before assay onset. Reactions were monitored in real time over a 20 min period using the increase of fluorescence signal at 446 nm resulting from the hydrolysis of the acetyl moiety. K_i values were obtained for each inhibitor by use of line fitting the double reciprocal (1/rate vs 1/[**ACC**]) of each curve to obtain an α coefficient at each concentration, representing the change in slope. From the derived α values, K_i values could be derived from the following formula ($\alpha = 1 + [I]/K_i$) and the average K_i value (n = 3) is reported. A slow but measurable background hydrolysis rate was also monitored and subtracted from the enzyme-catalyzed reaction. All assays were performed in duplicate.

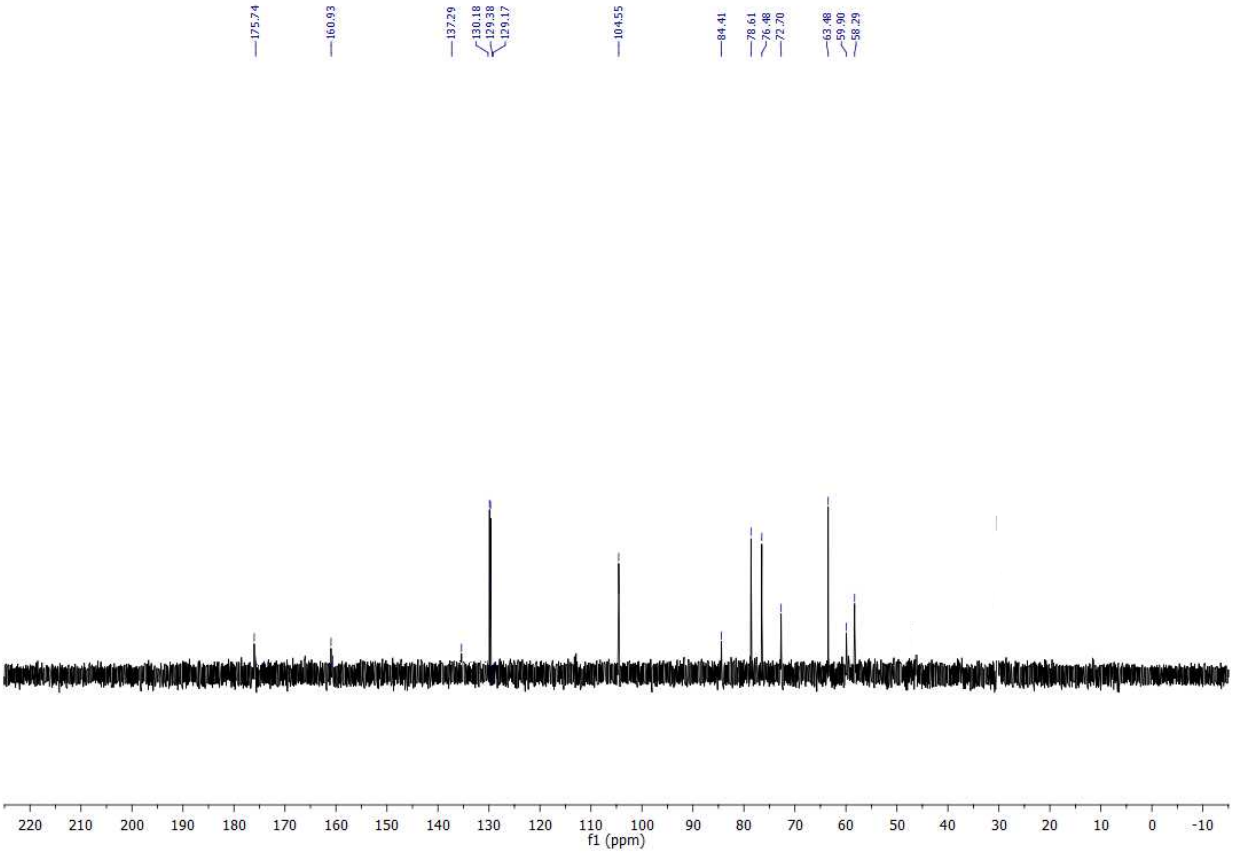


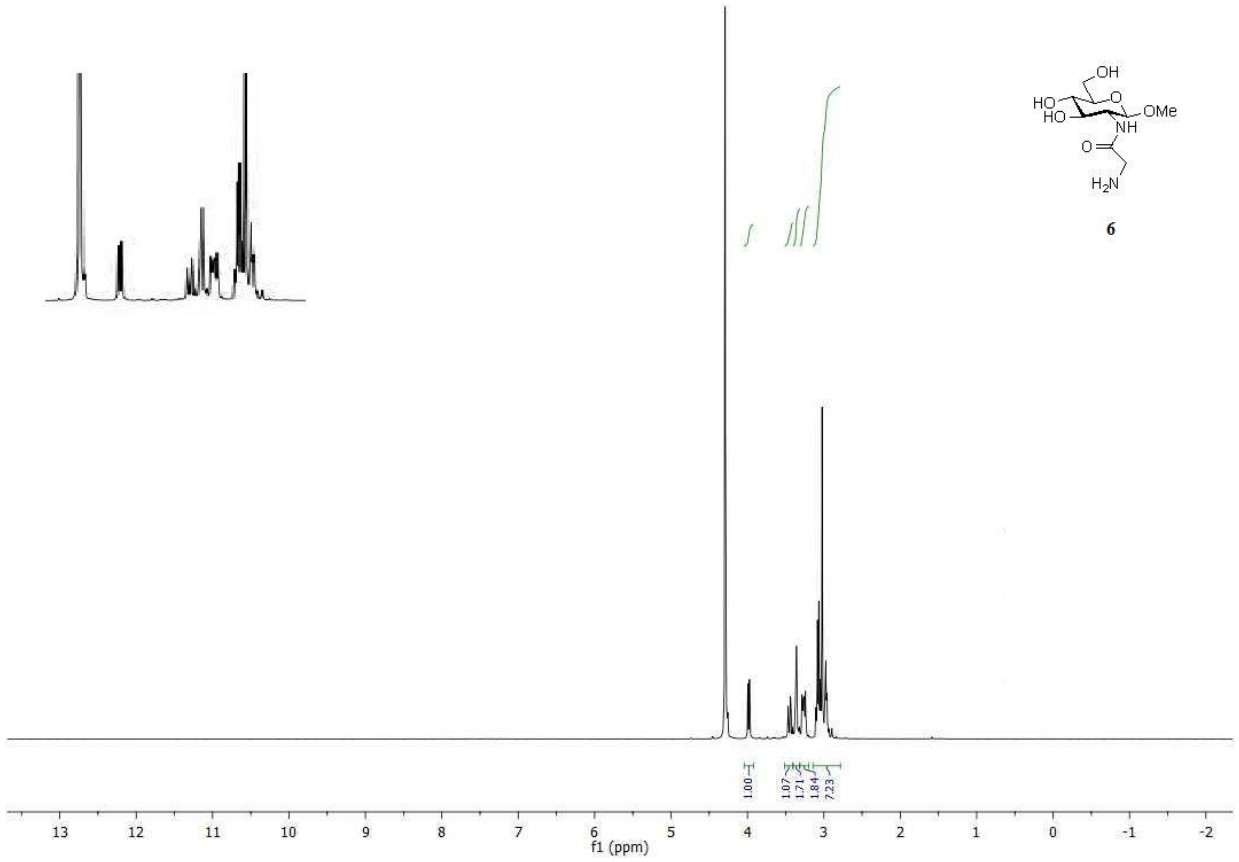


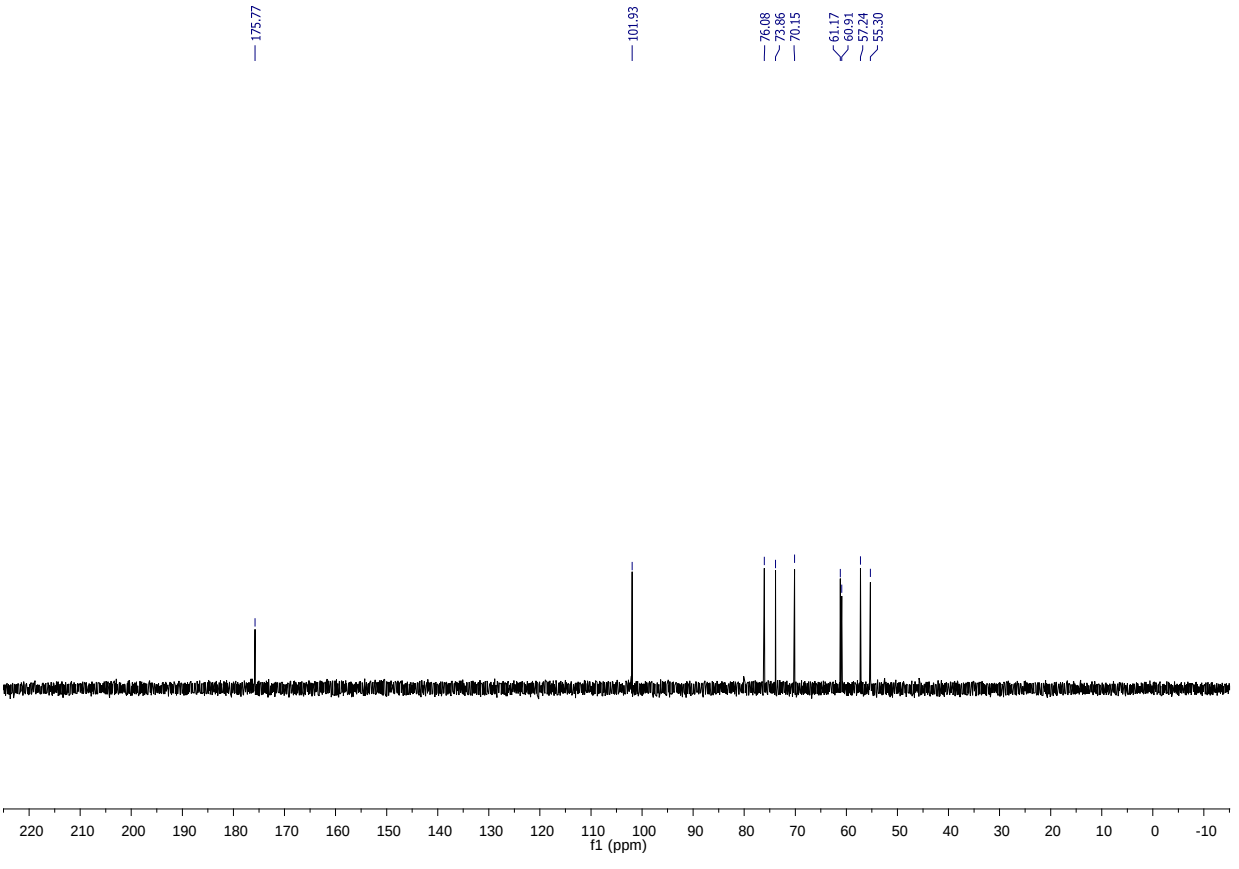


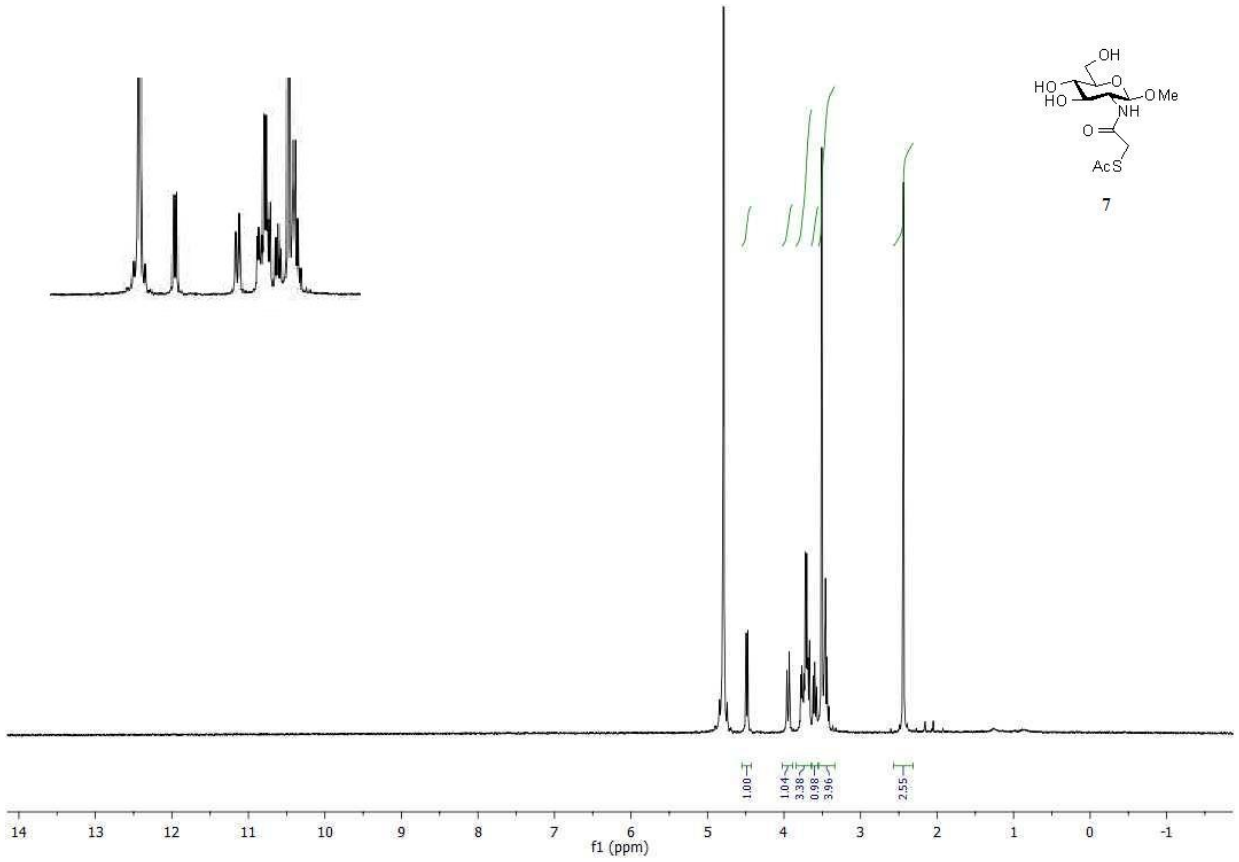








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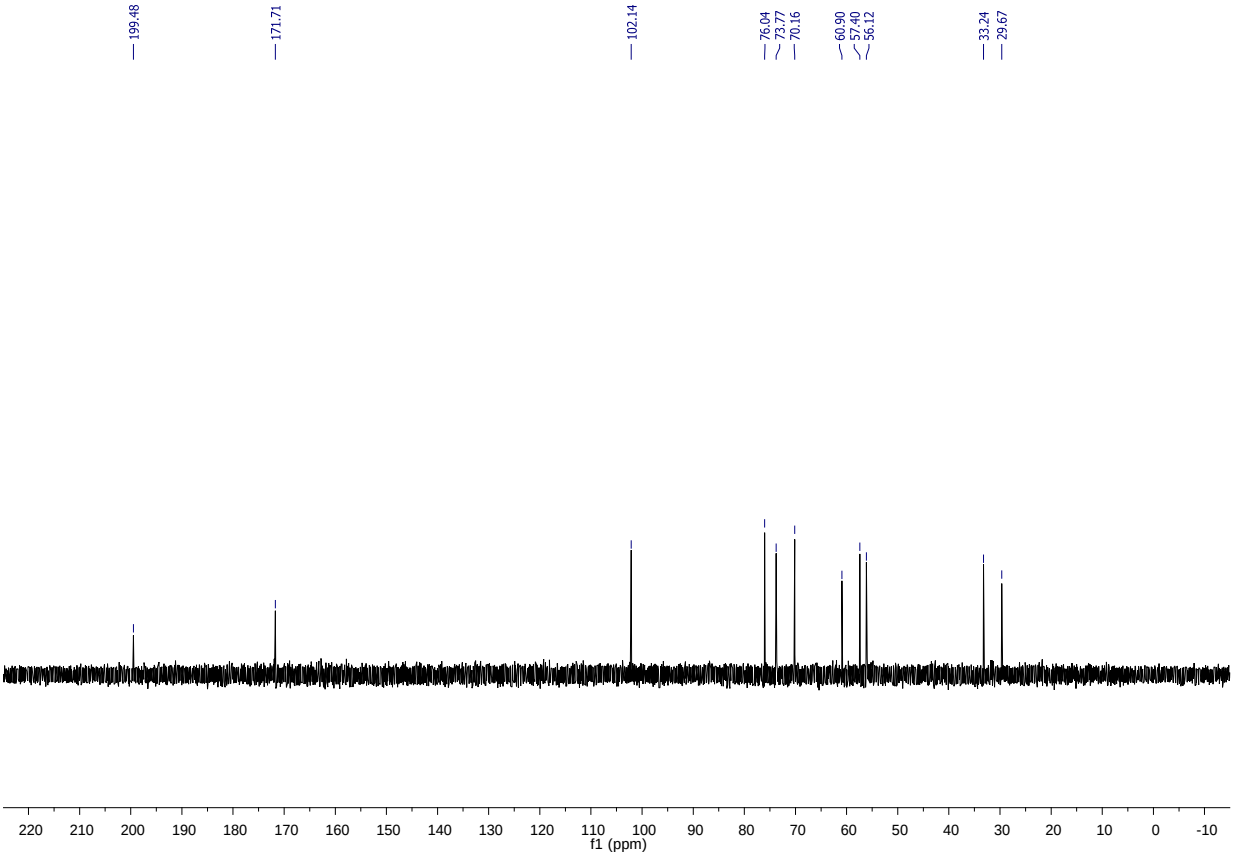
f1 (ppm)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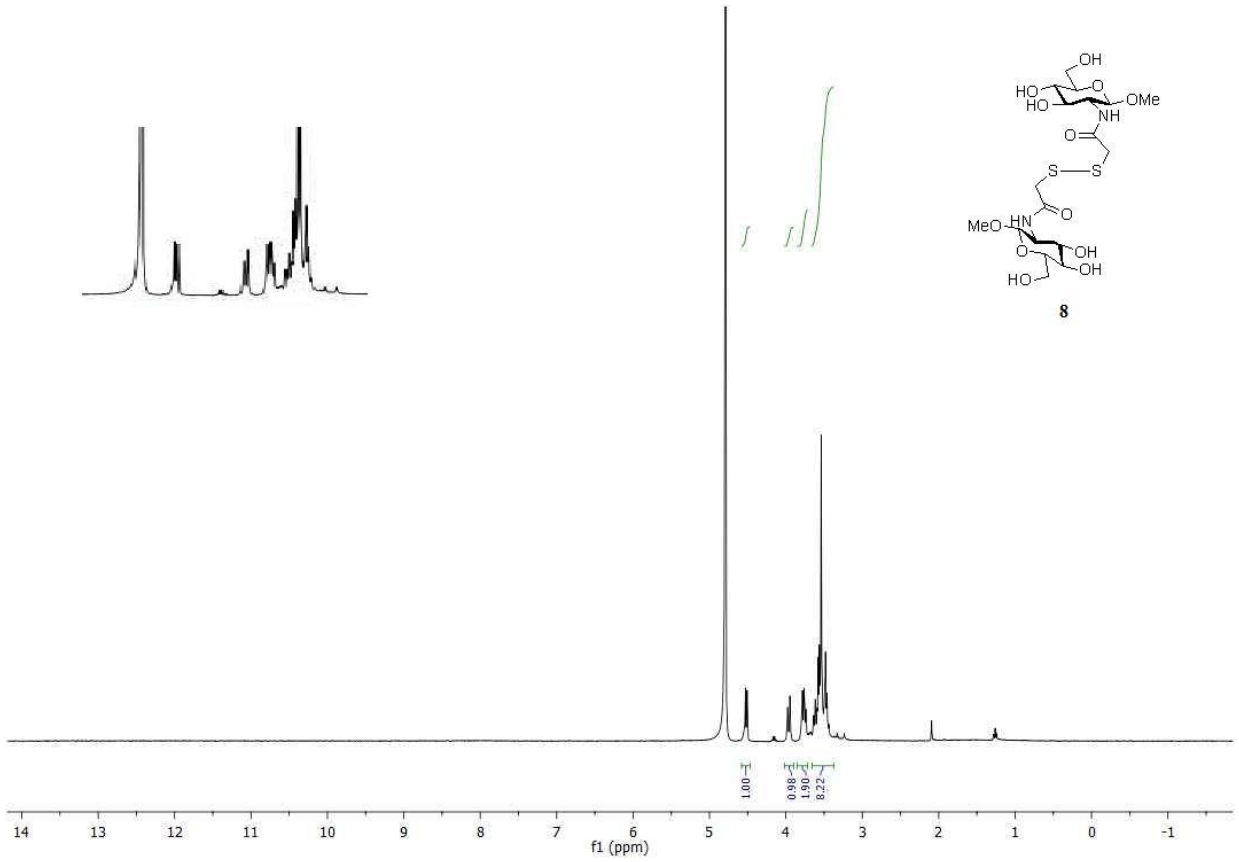
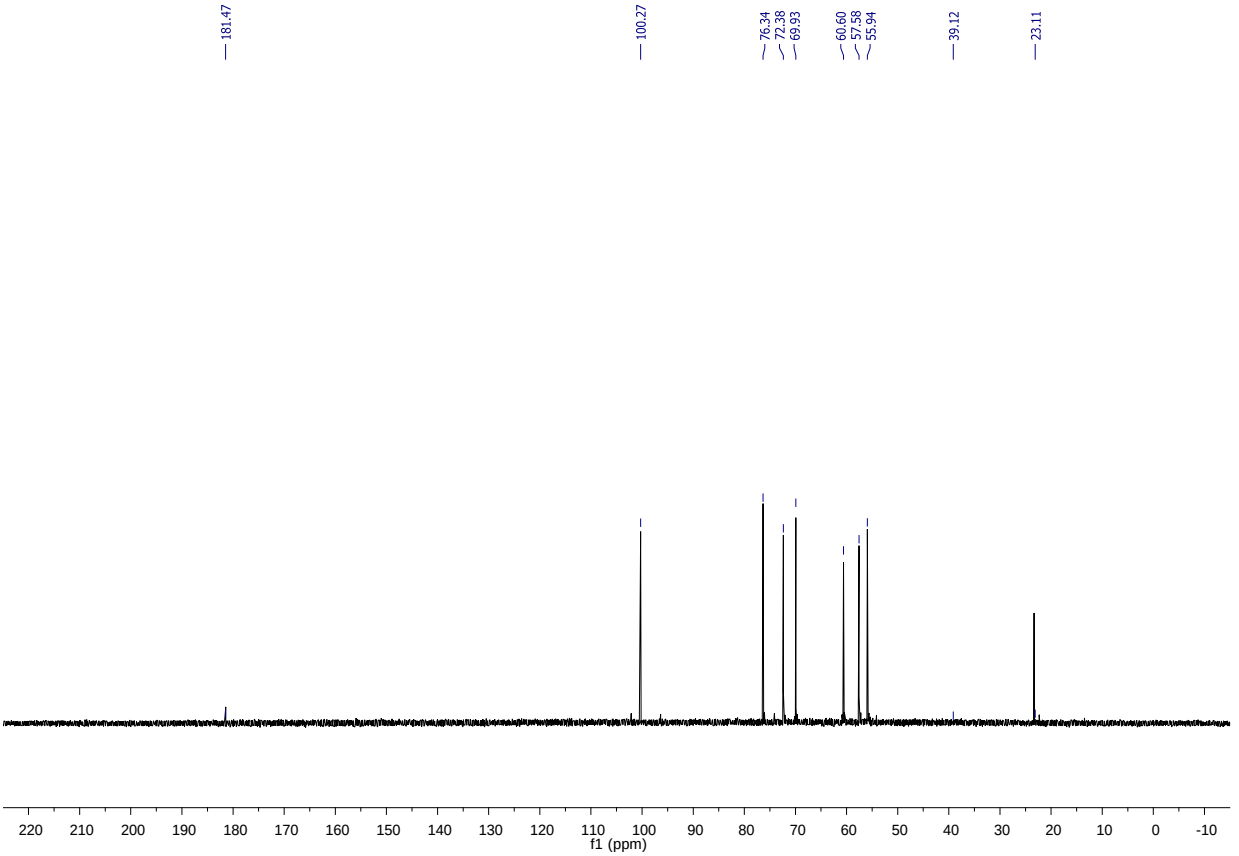
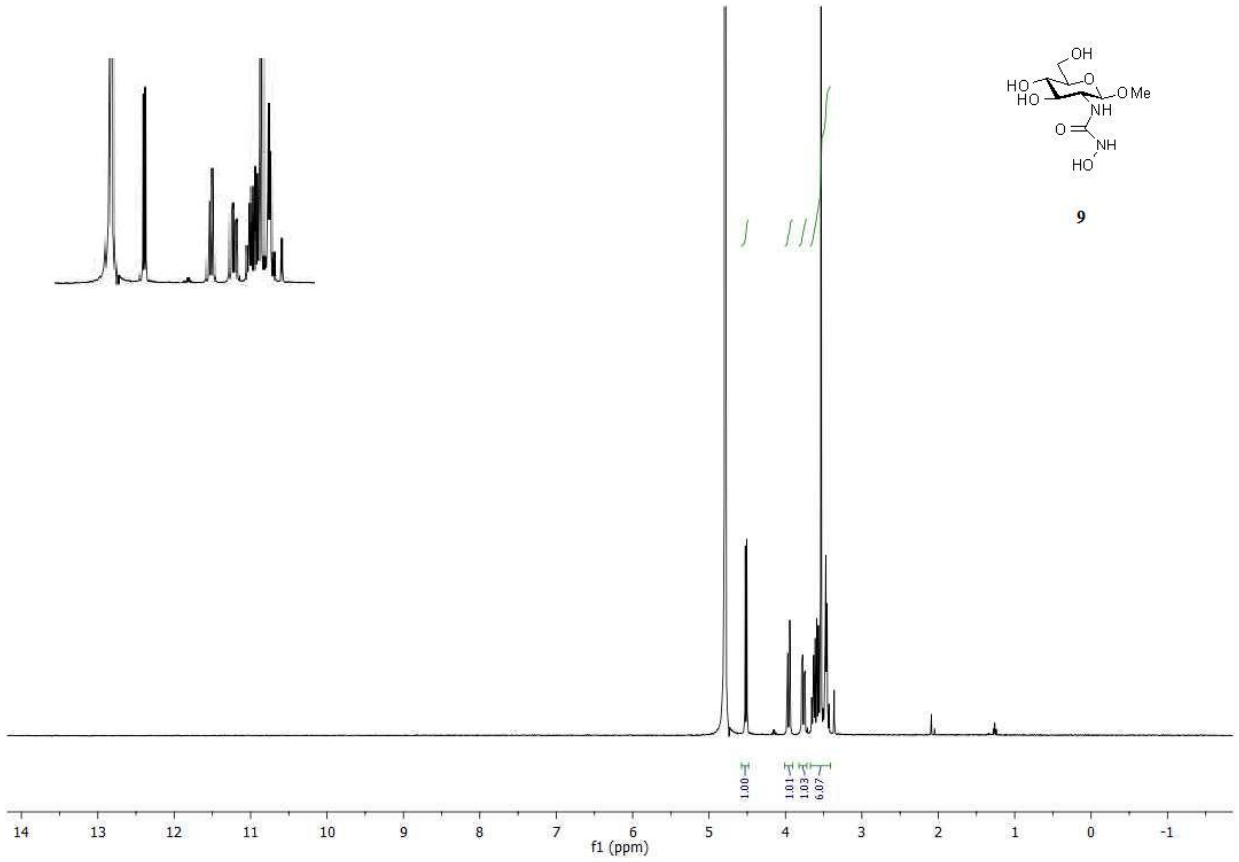
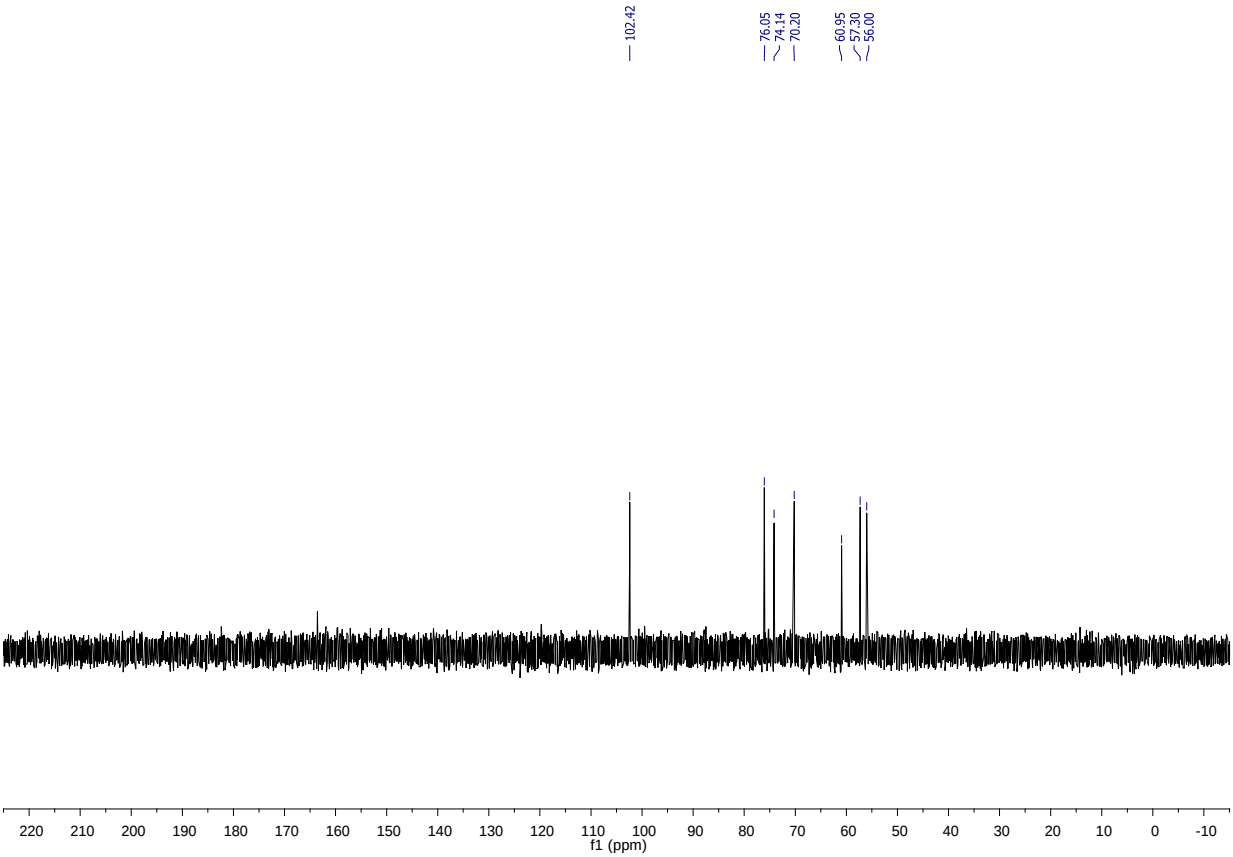
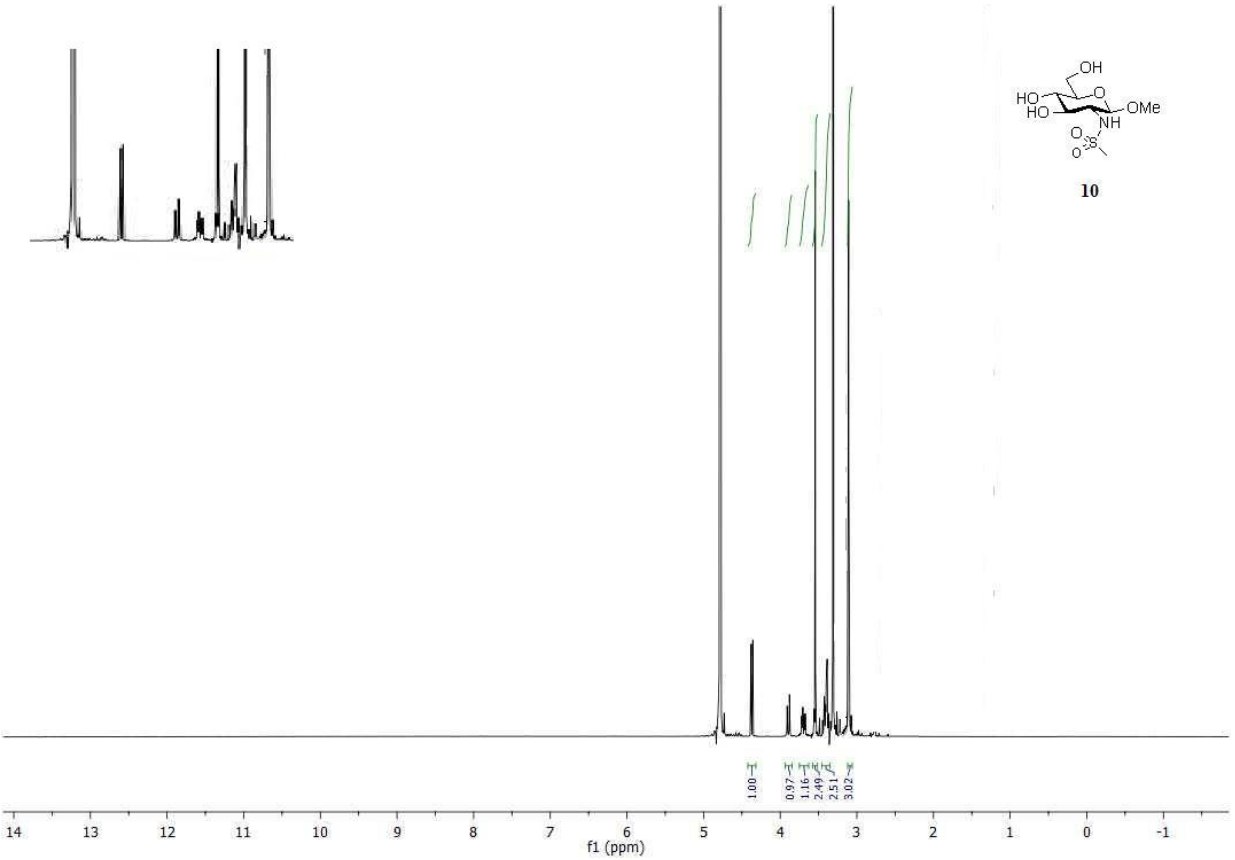
f1 (ppm)







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10
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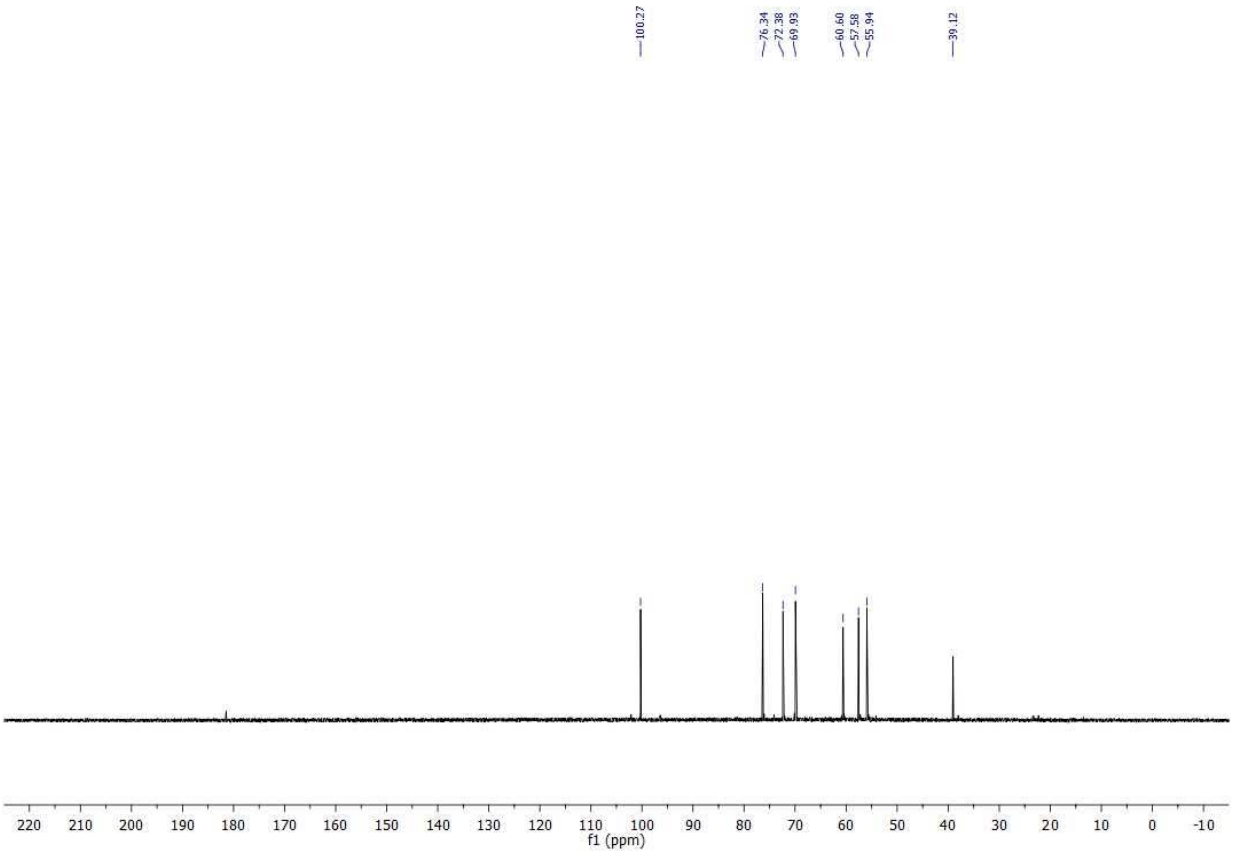
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

10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30
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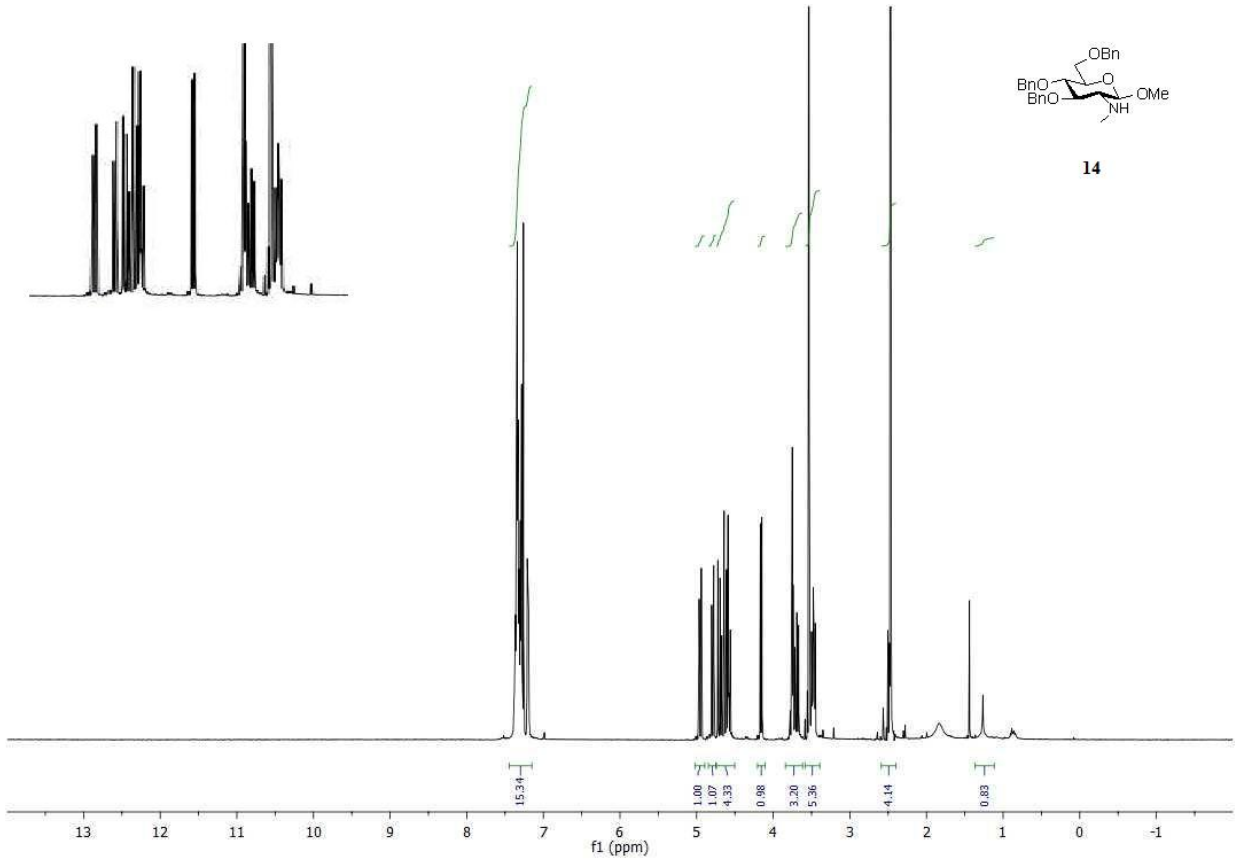
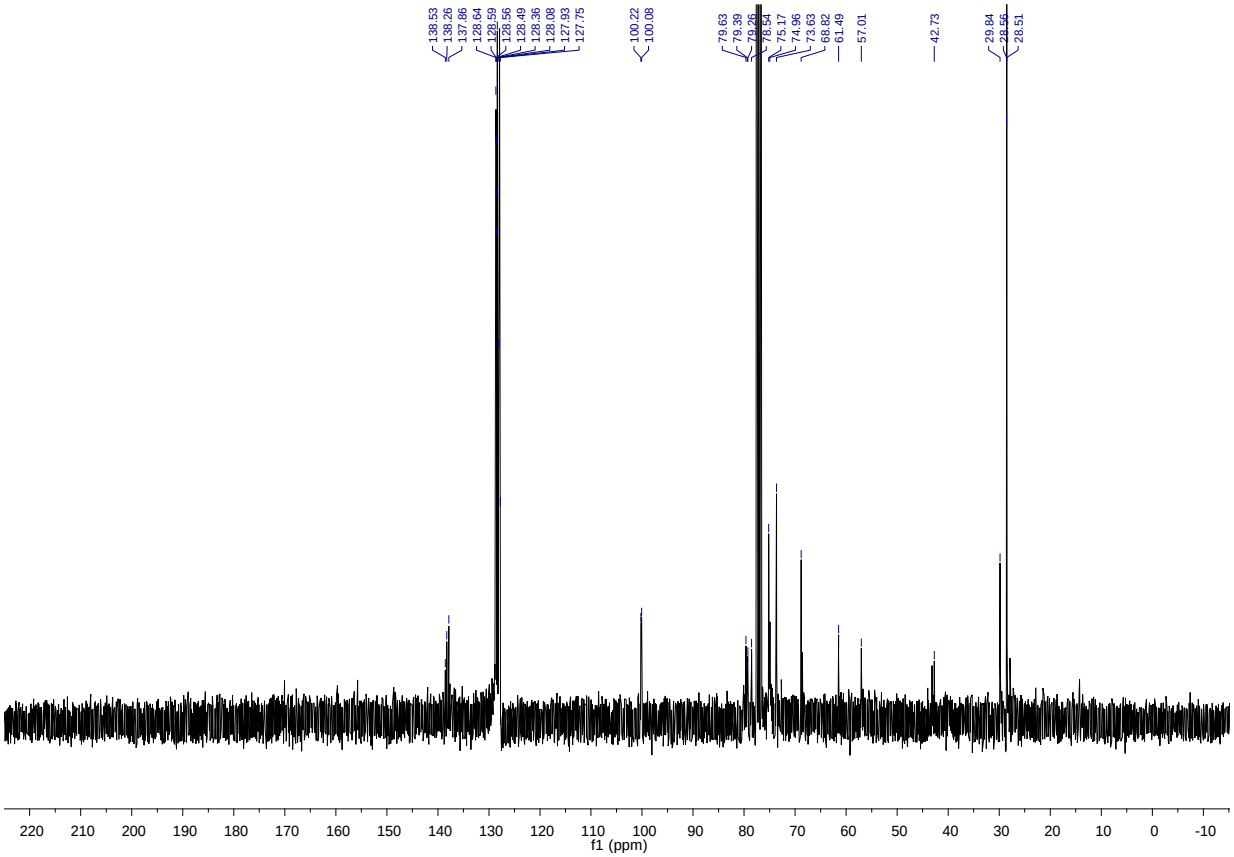
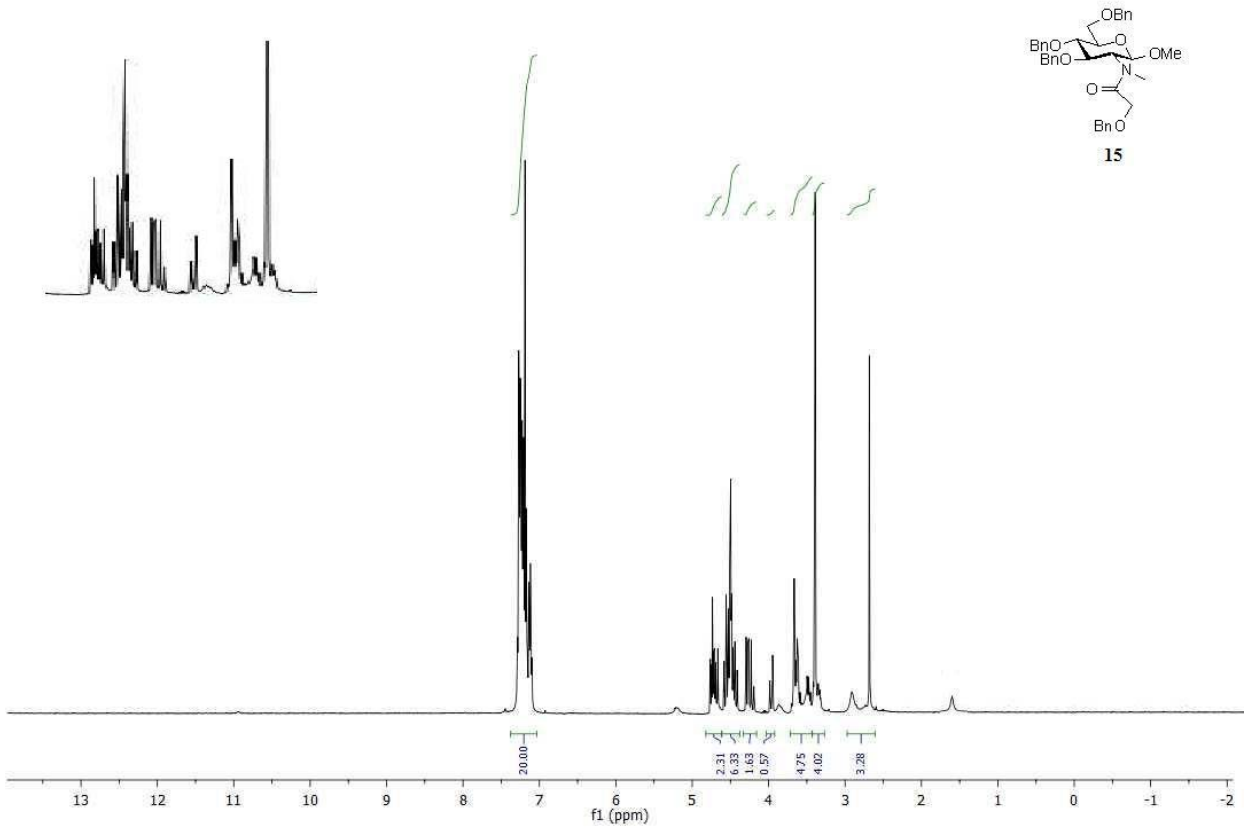
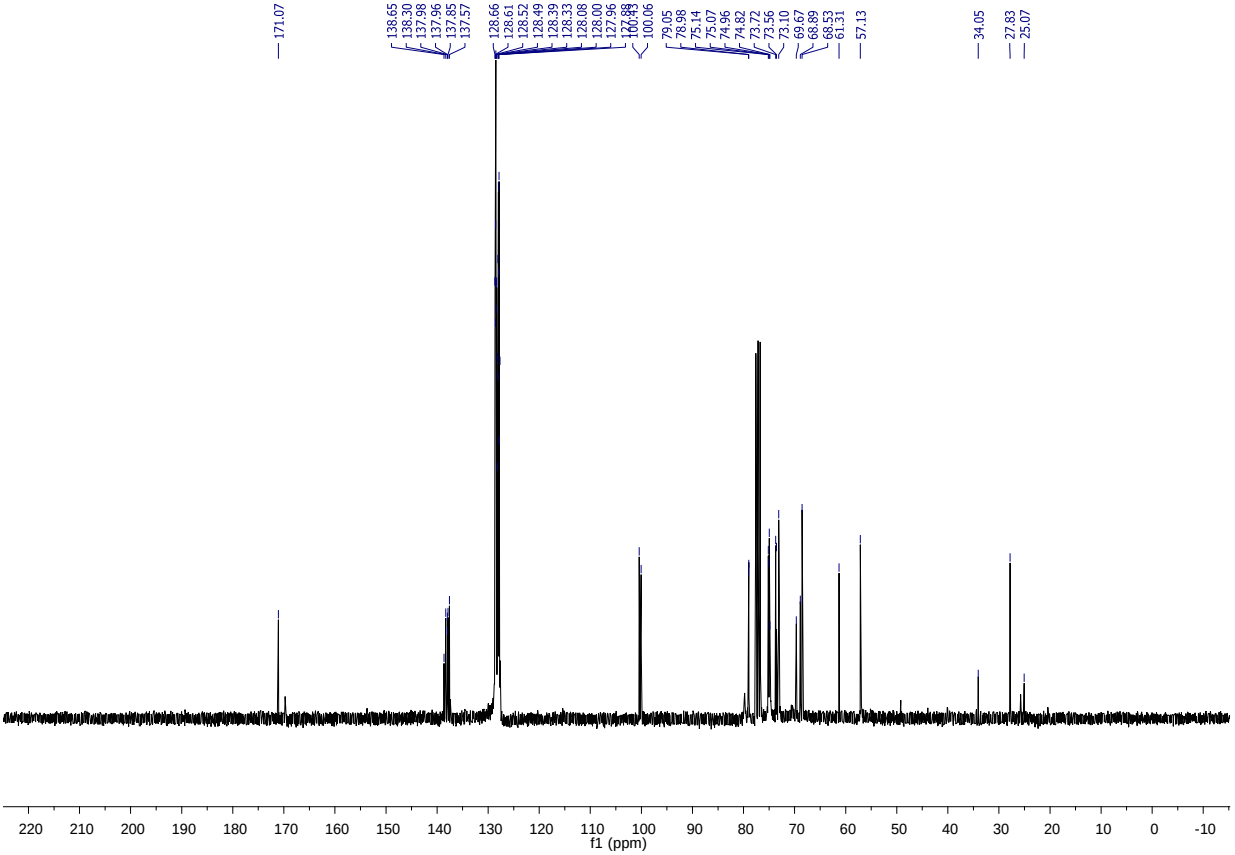
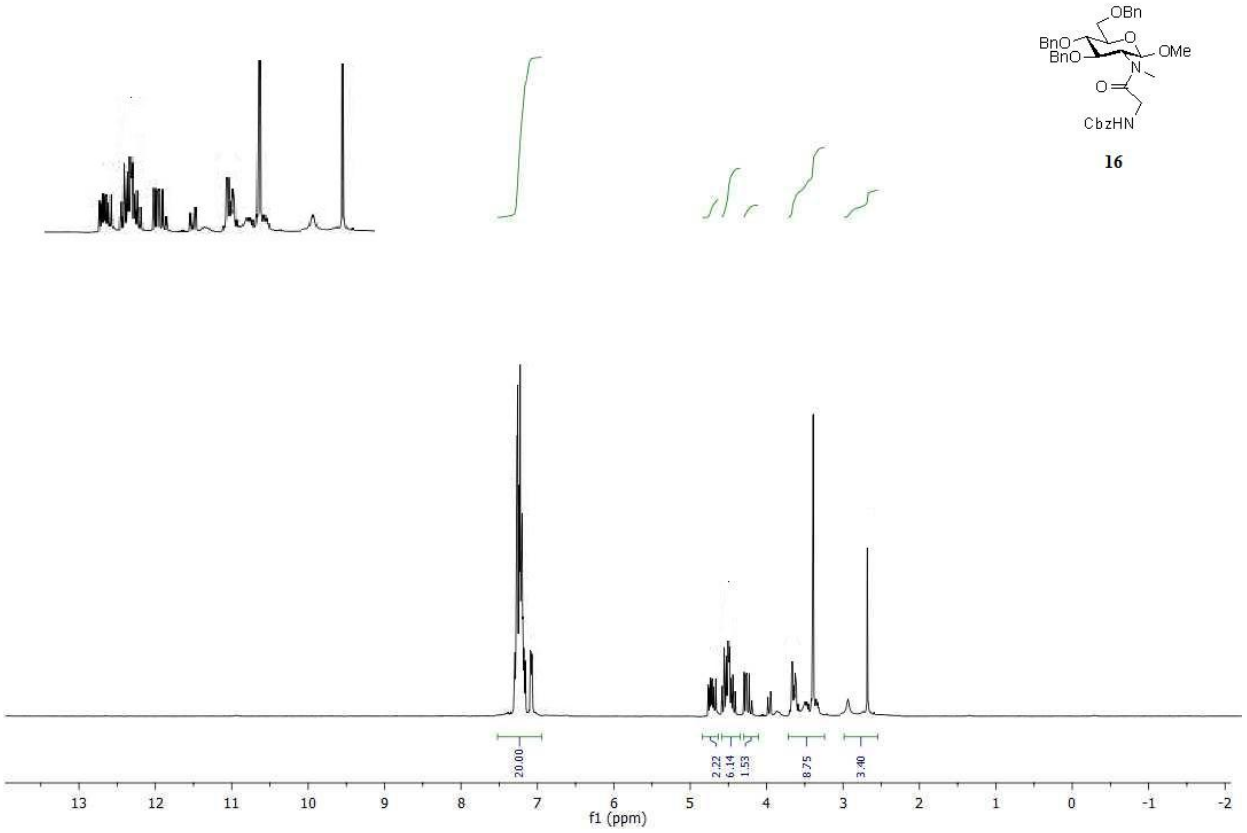
f1 (ppm)

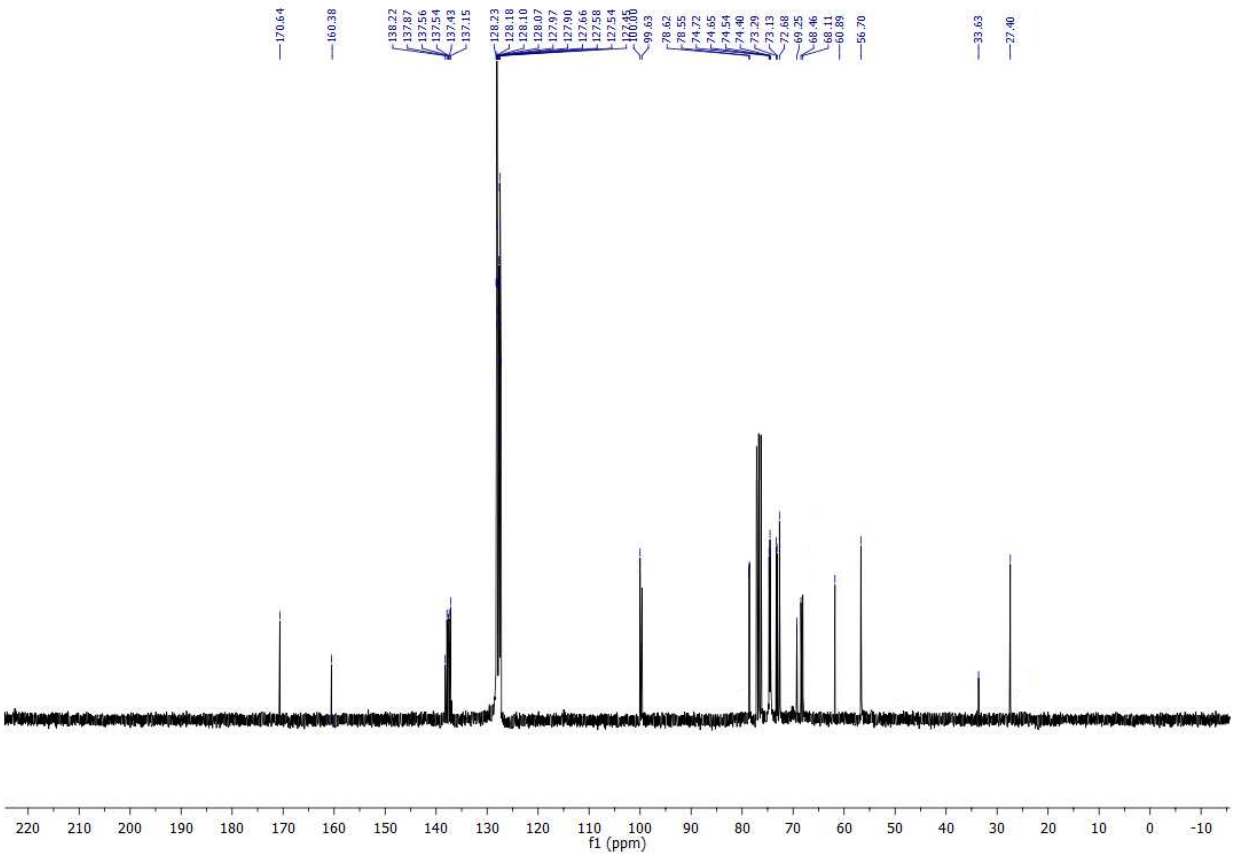


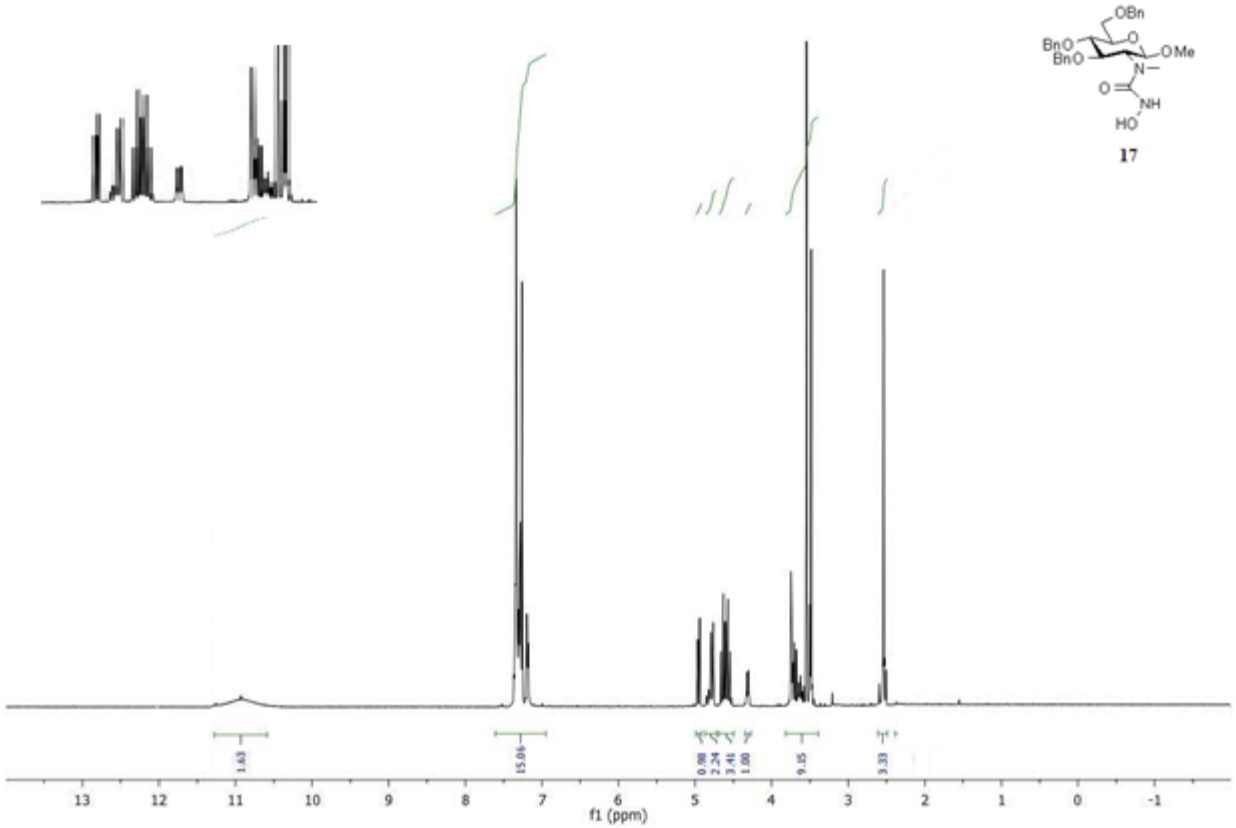


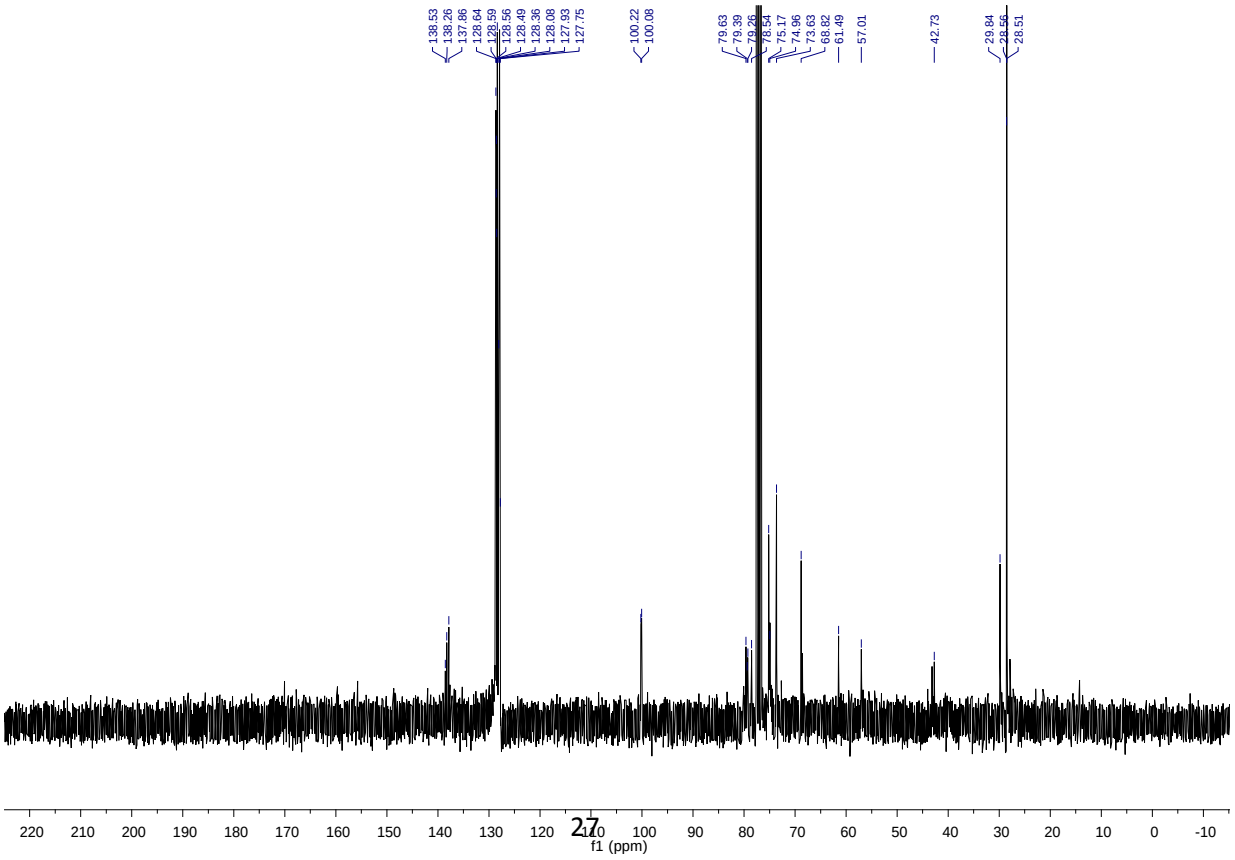


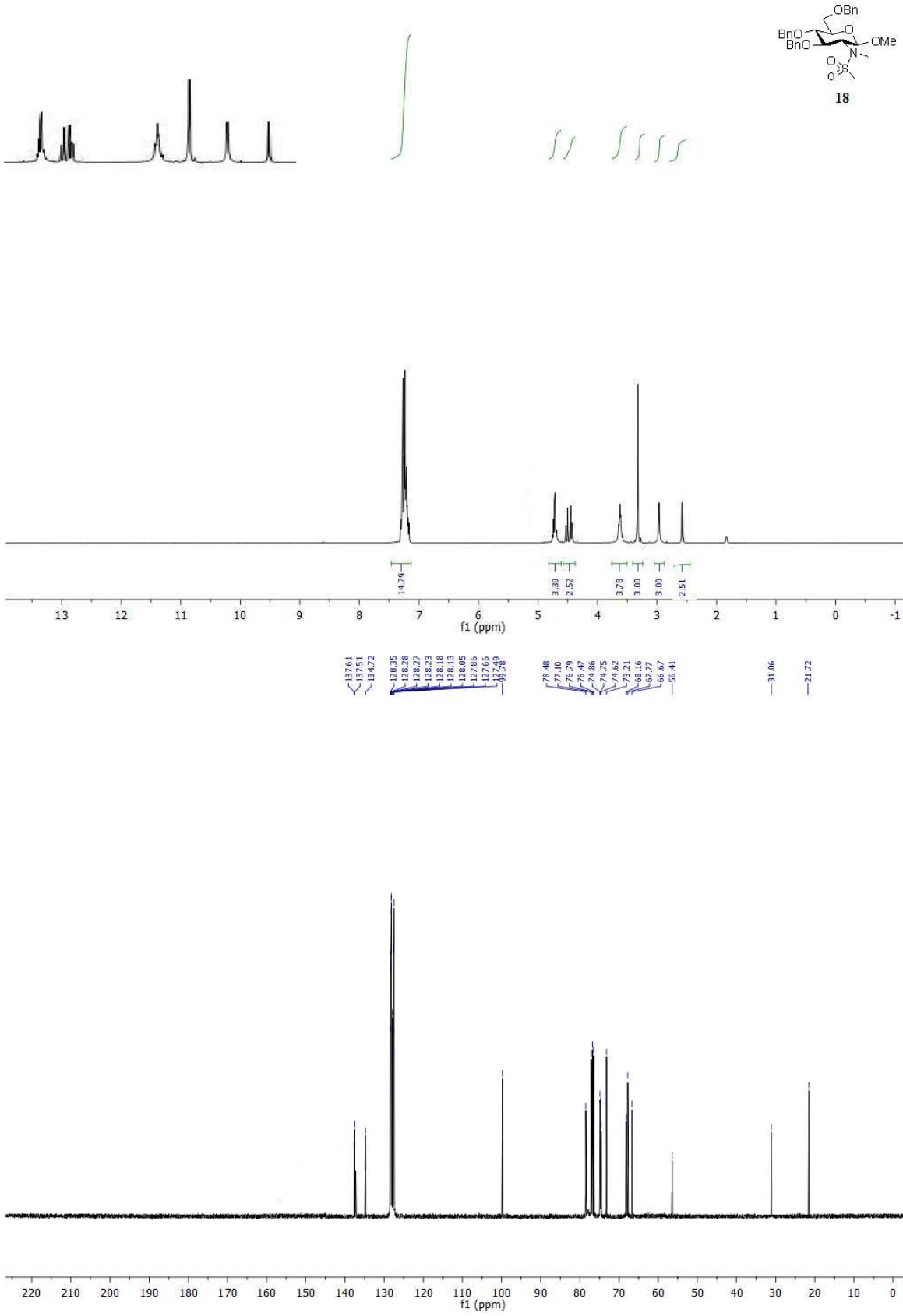


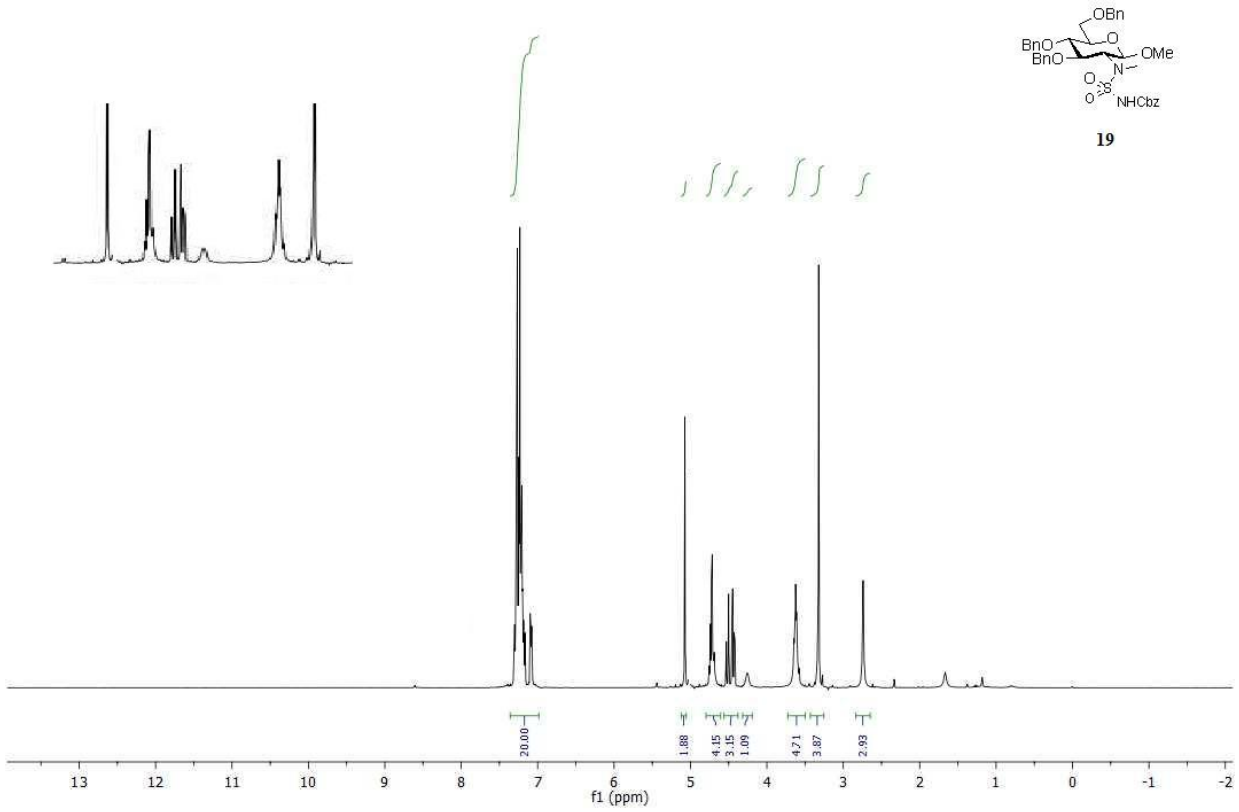








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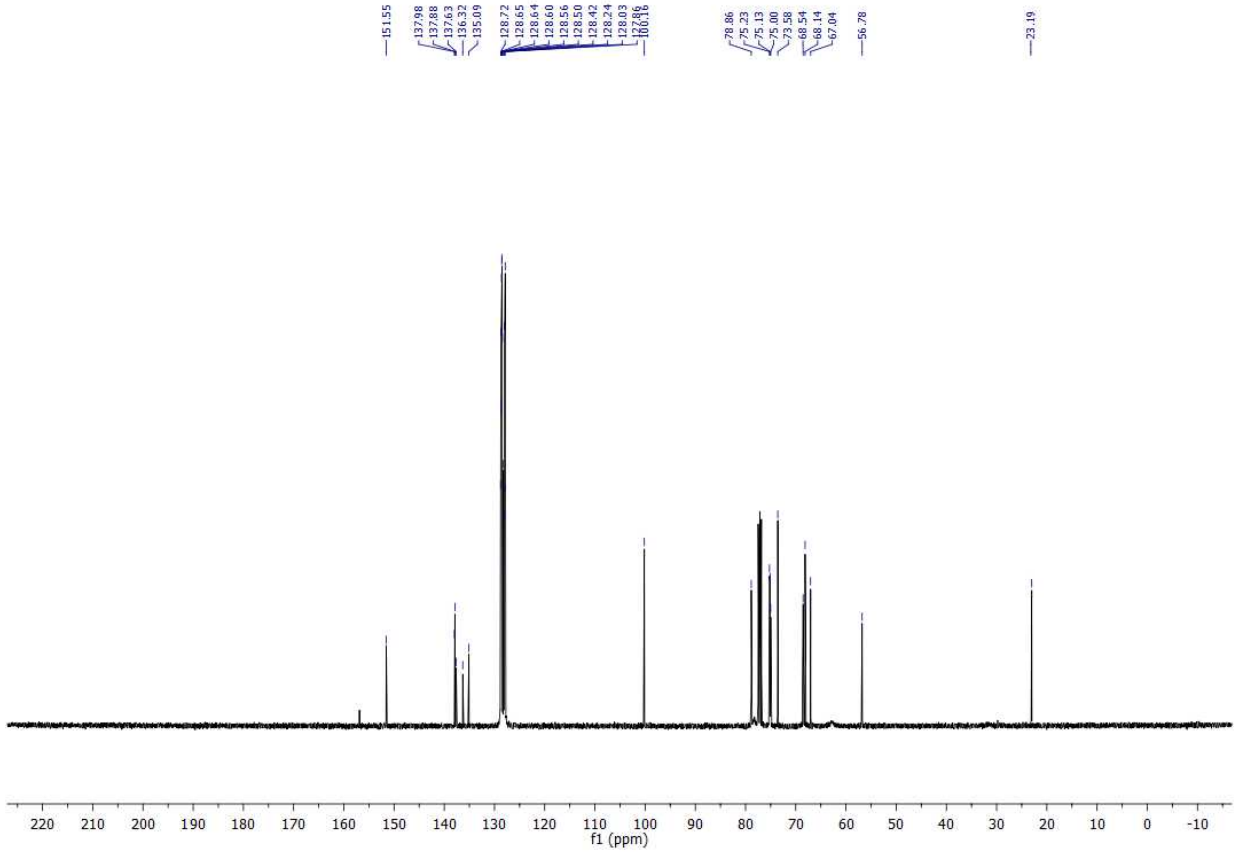
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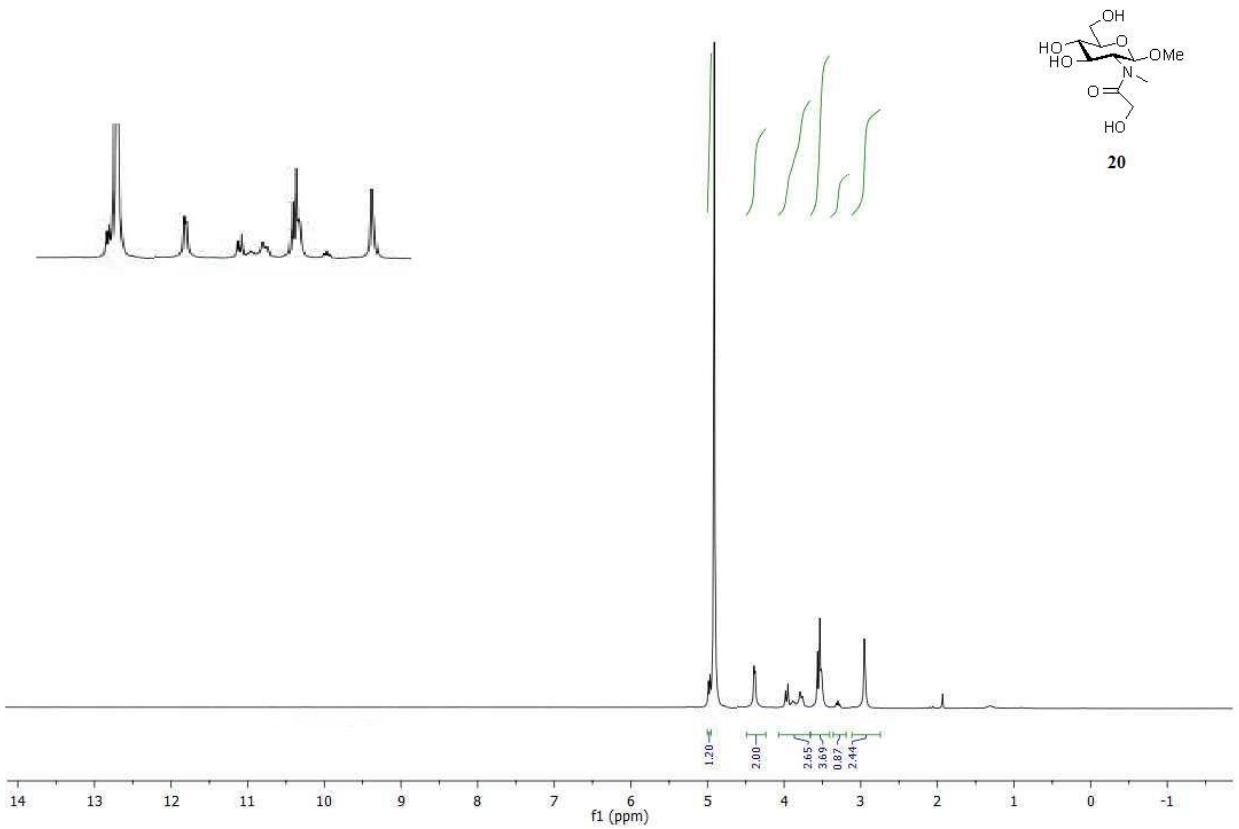
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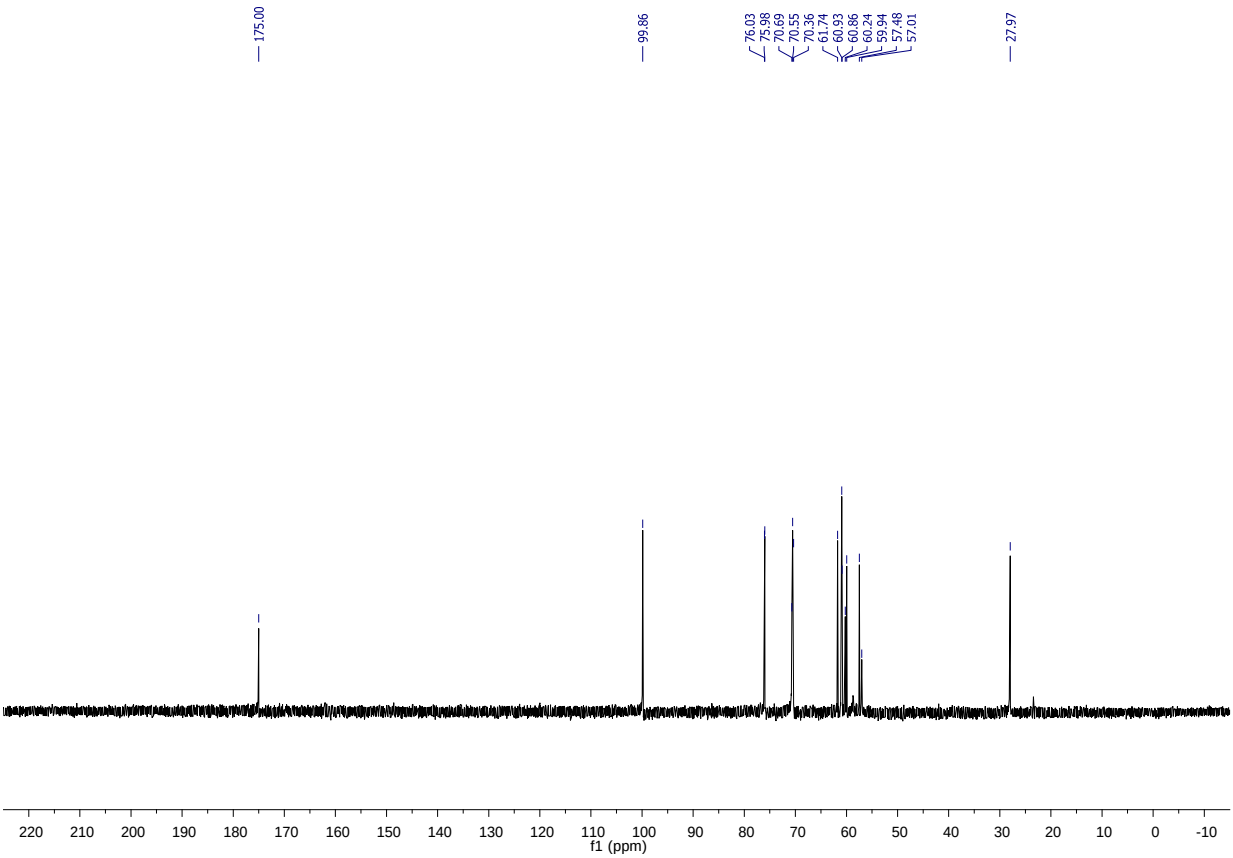
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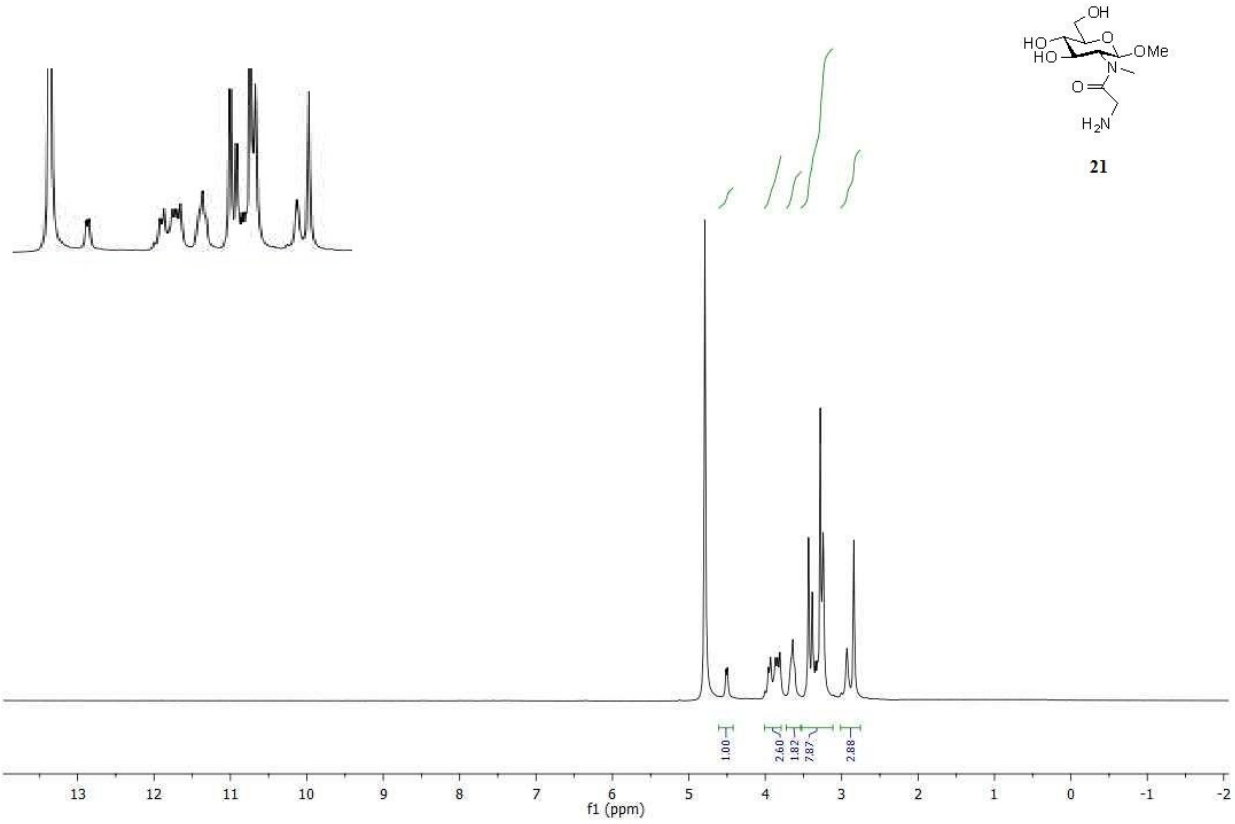

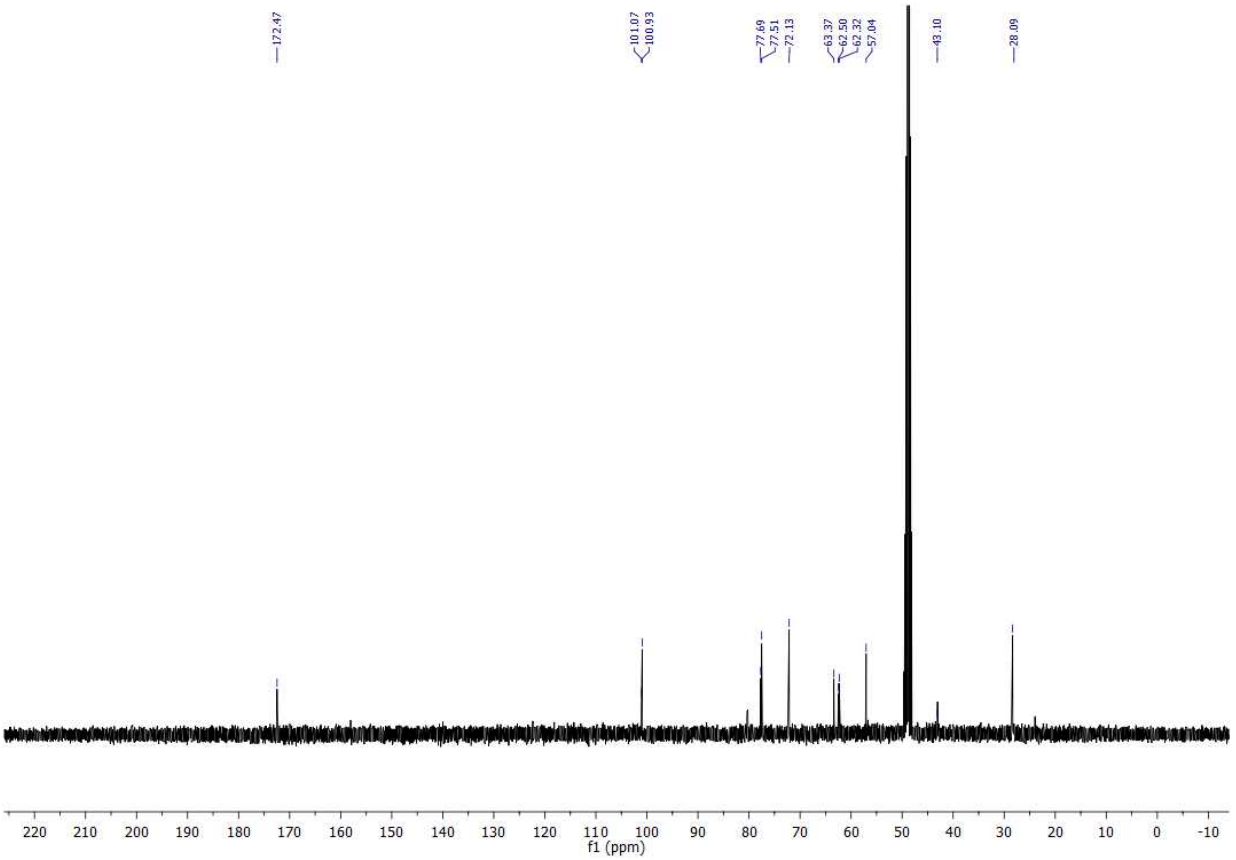

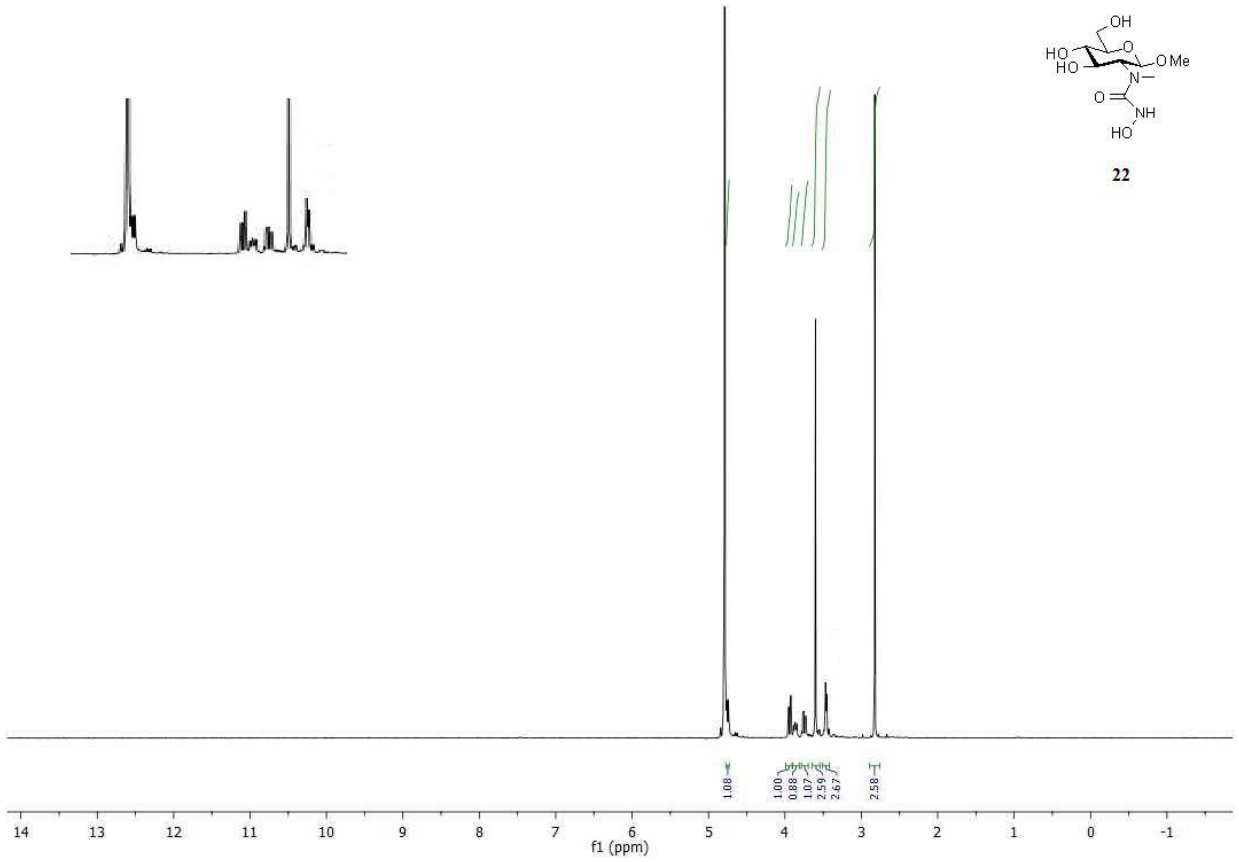
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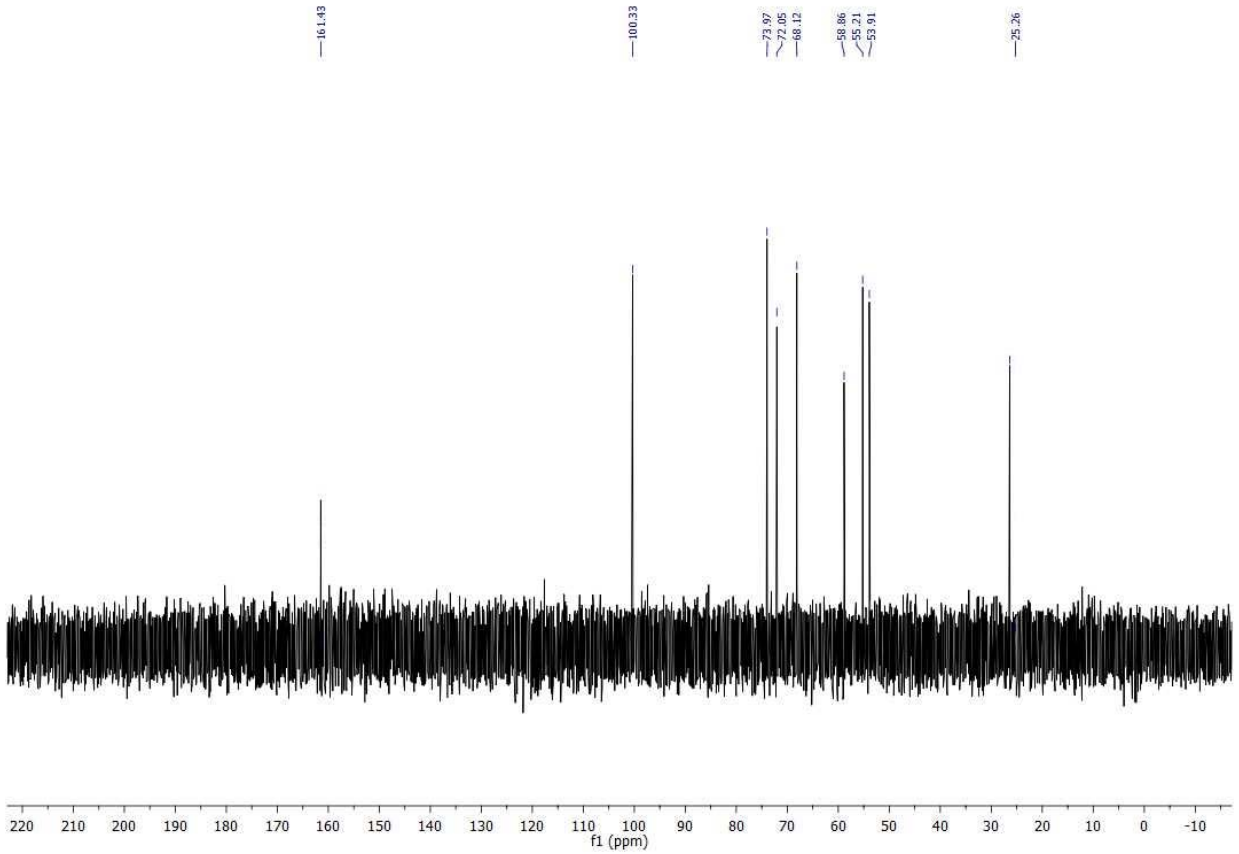
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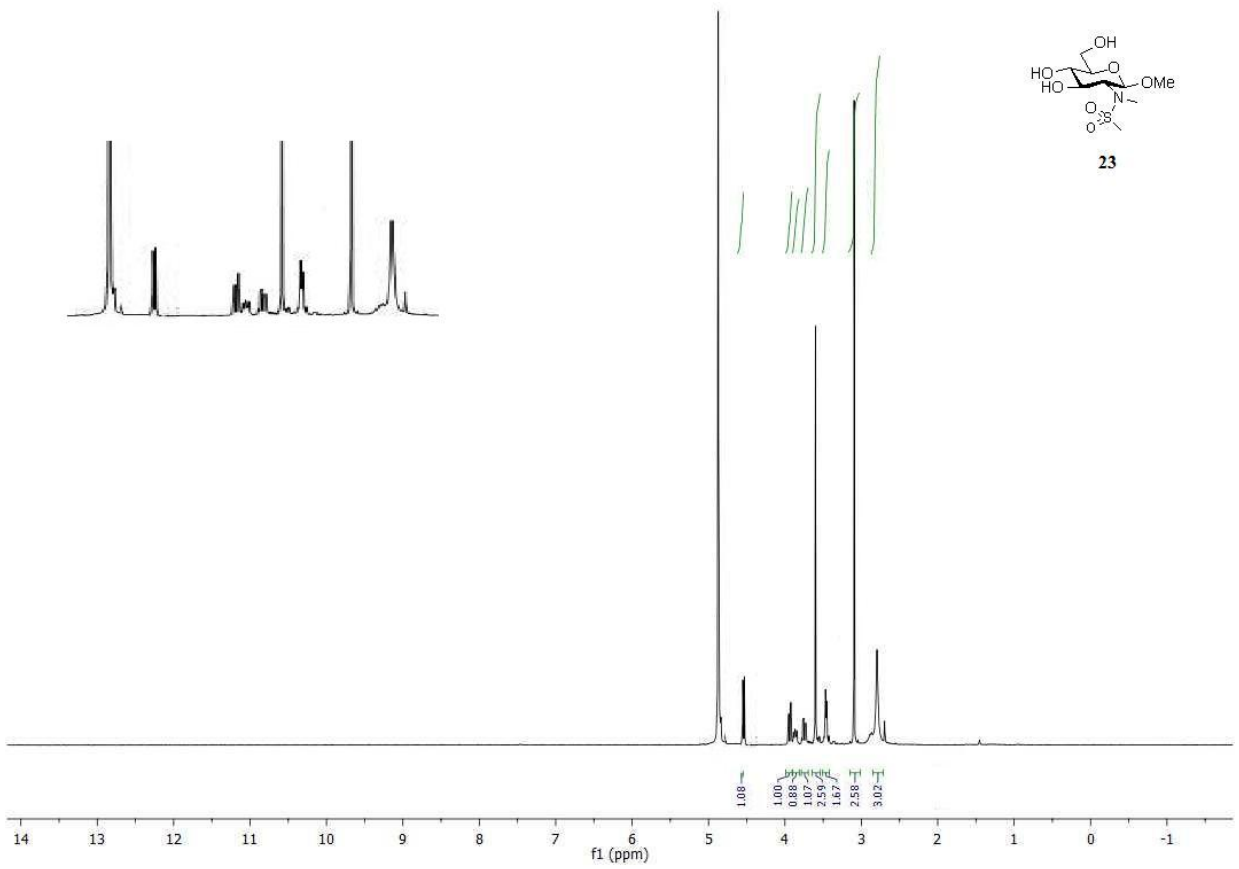
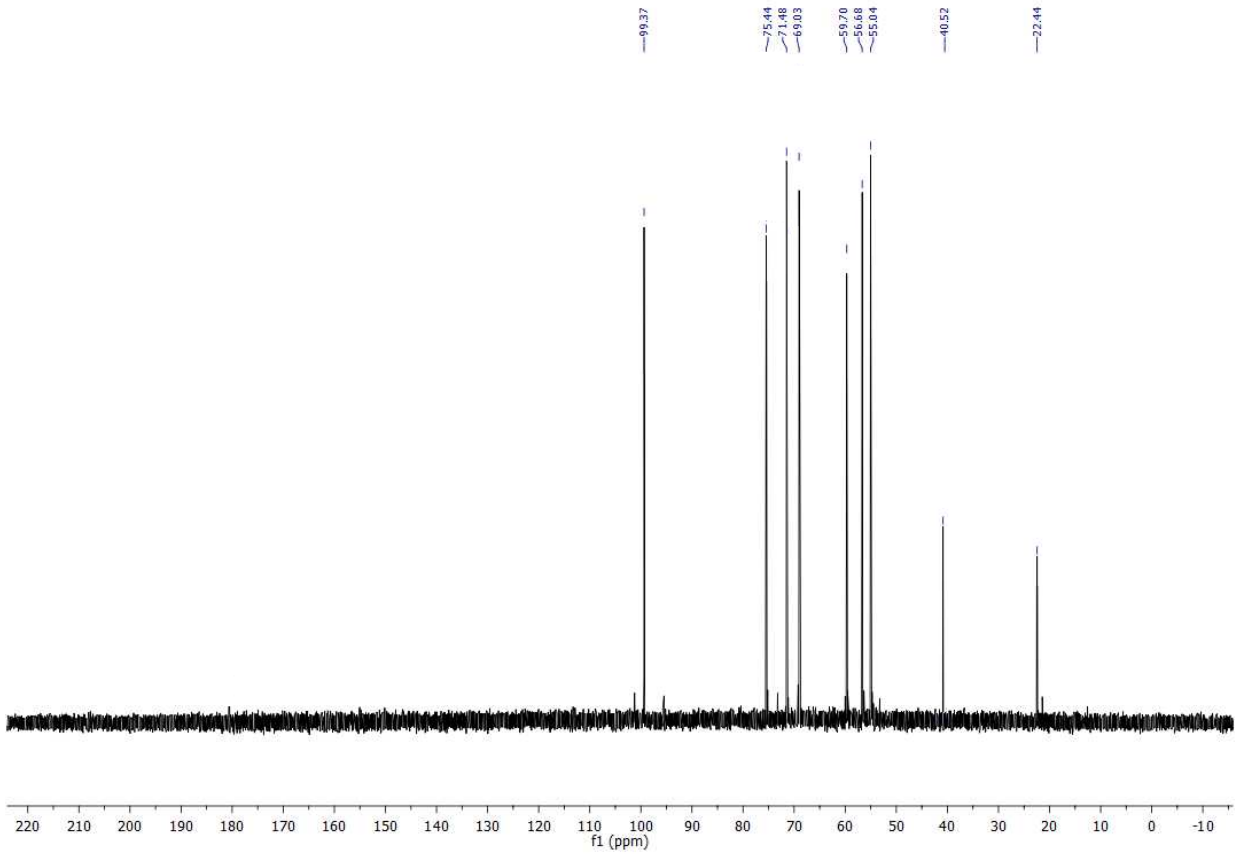
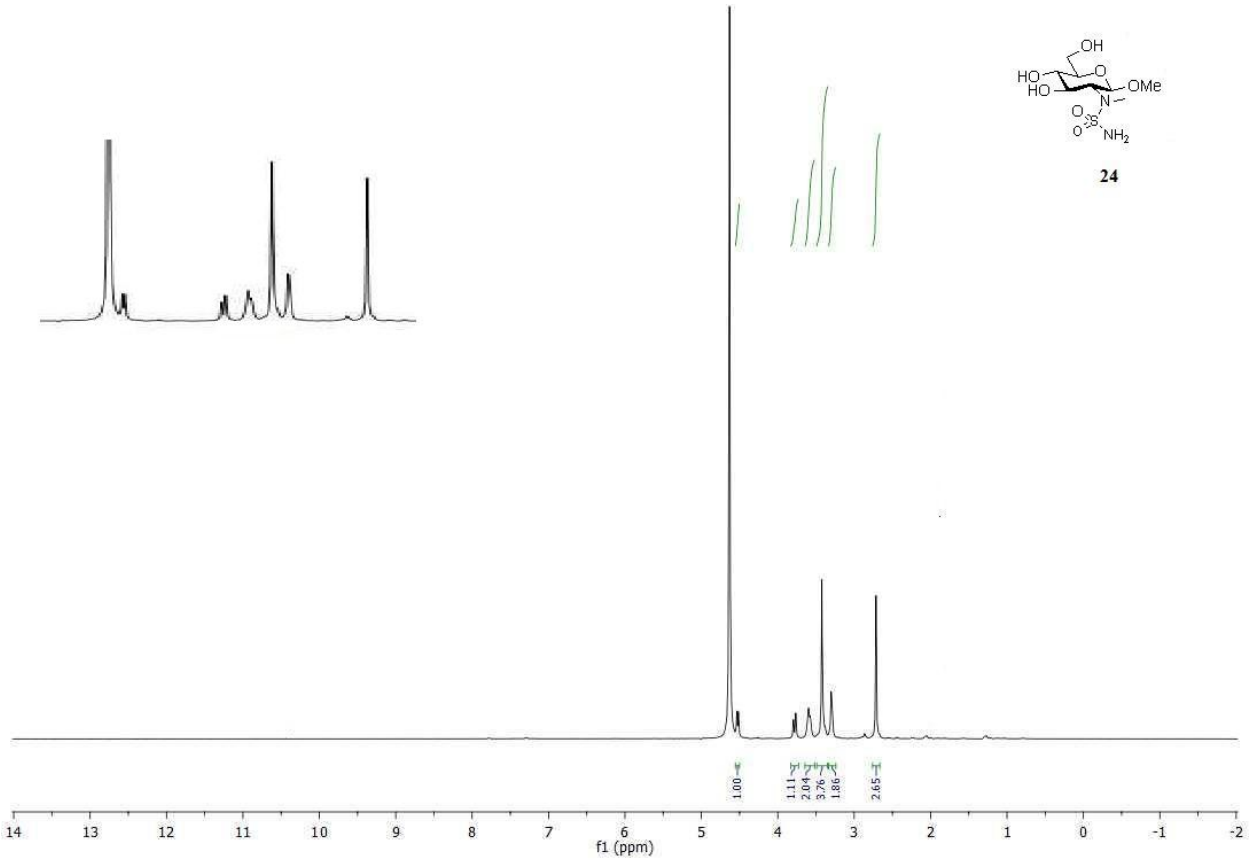
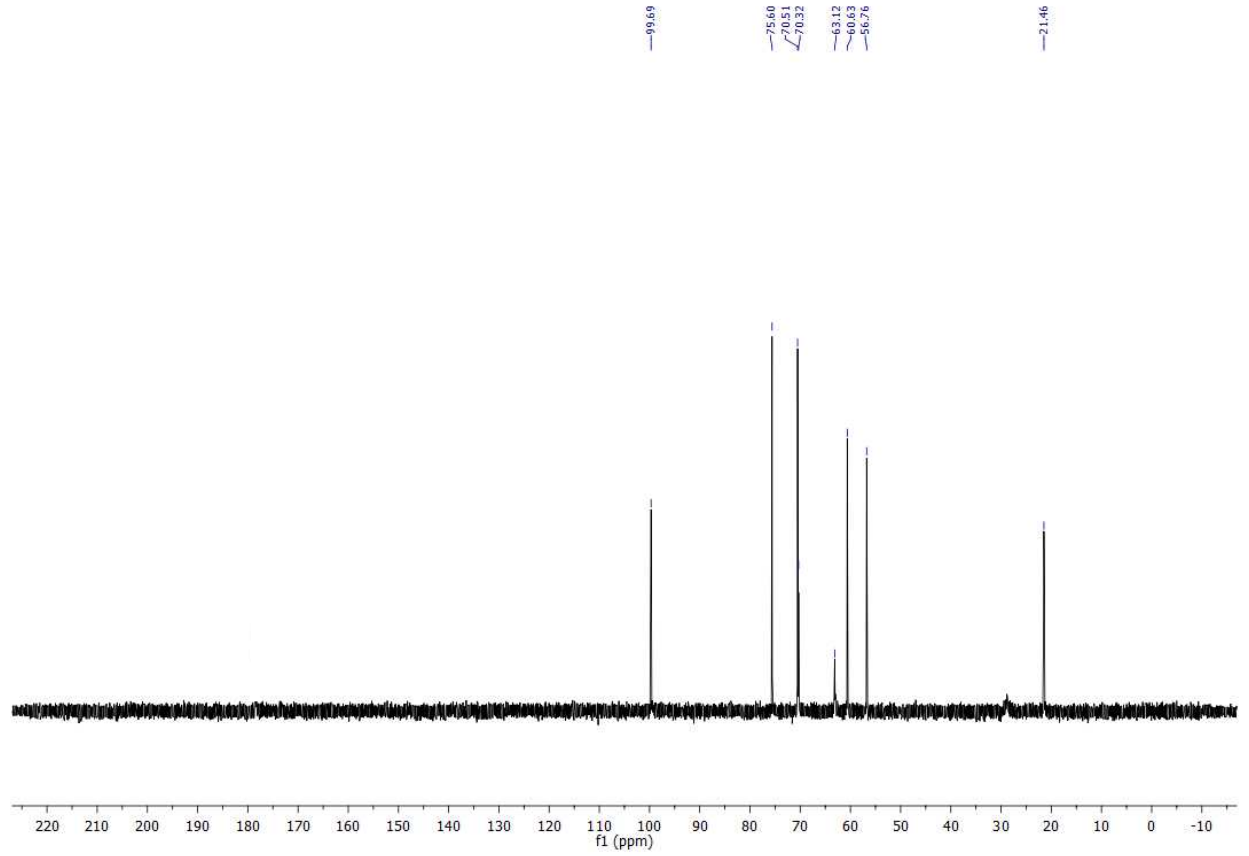
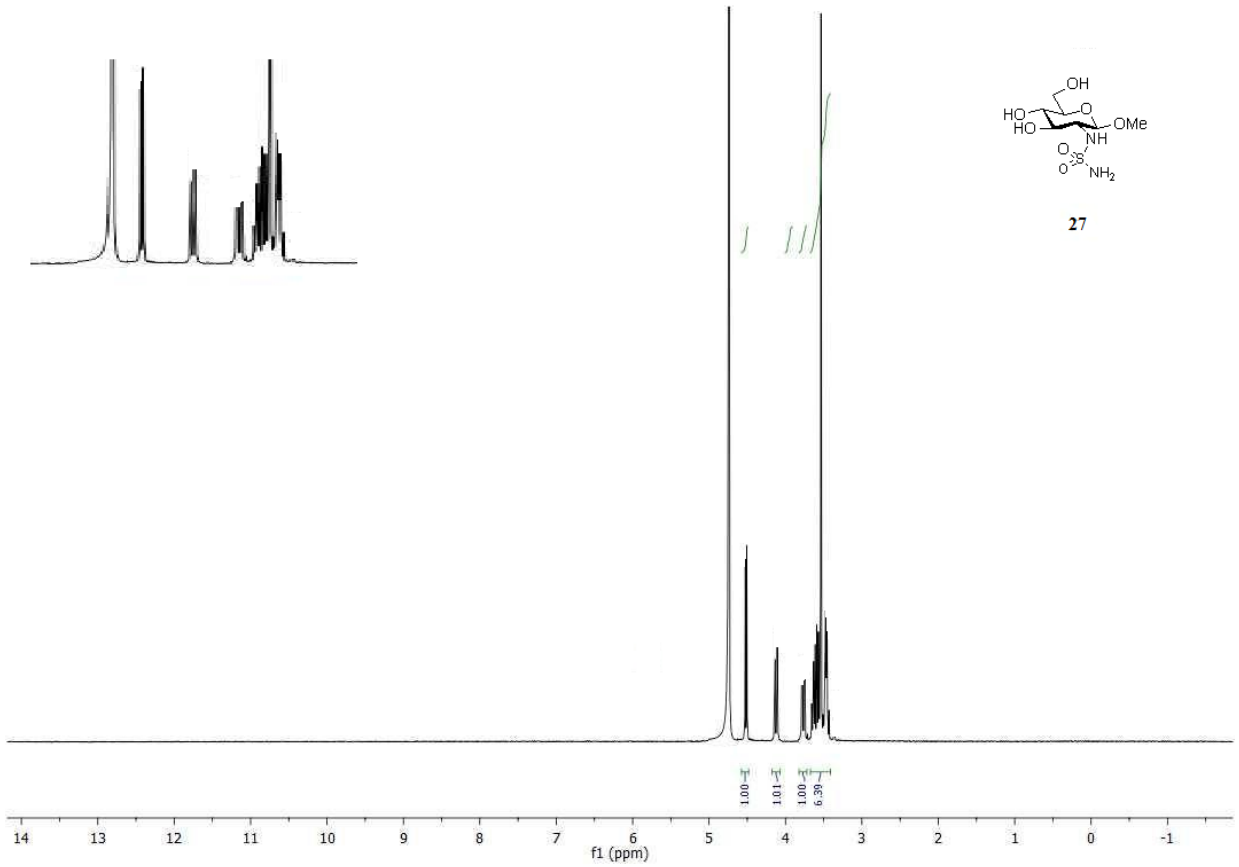
f1 (ppm)

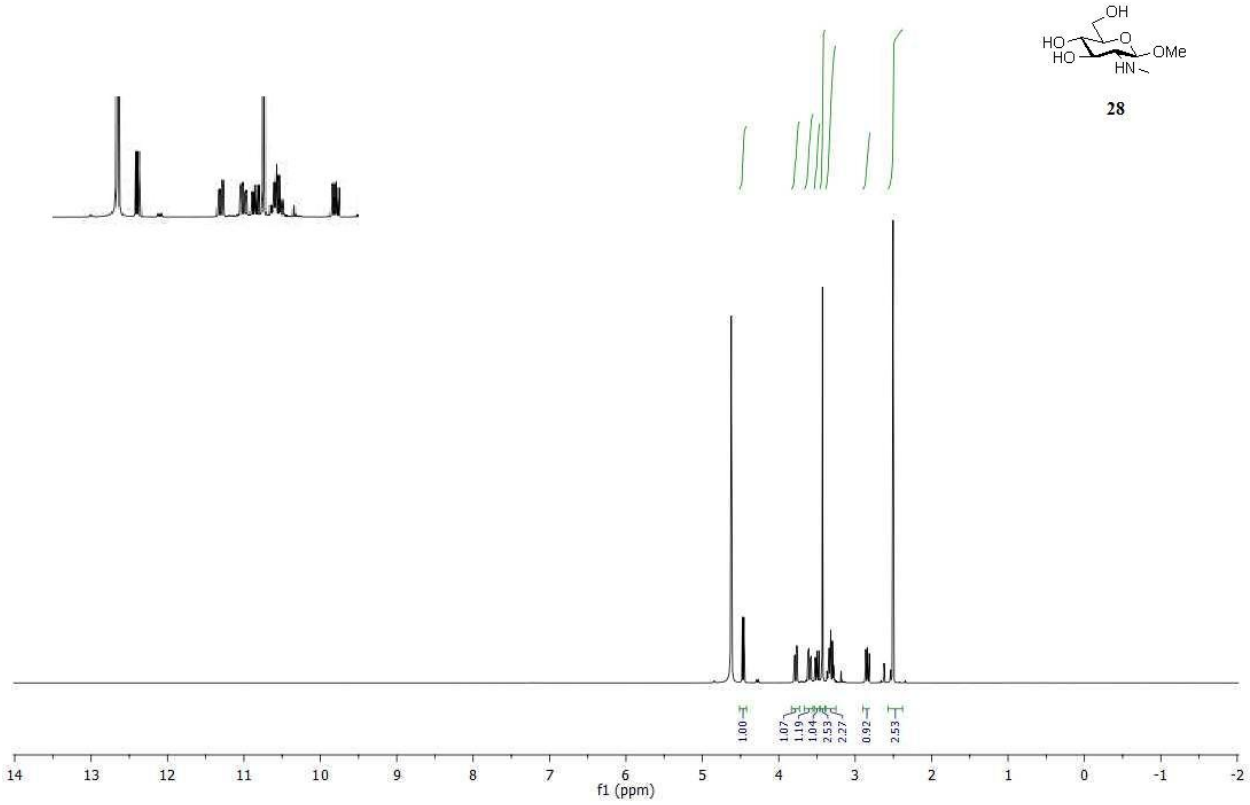


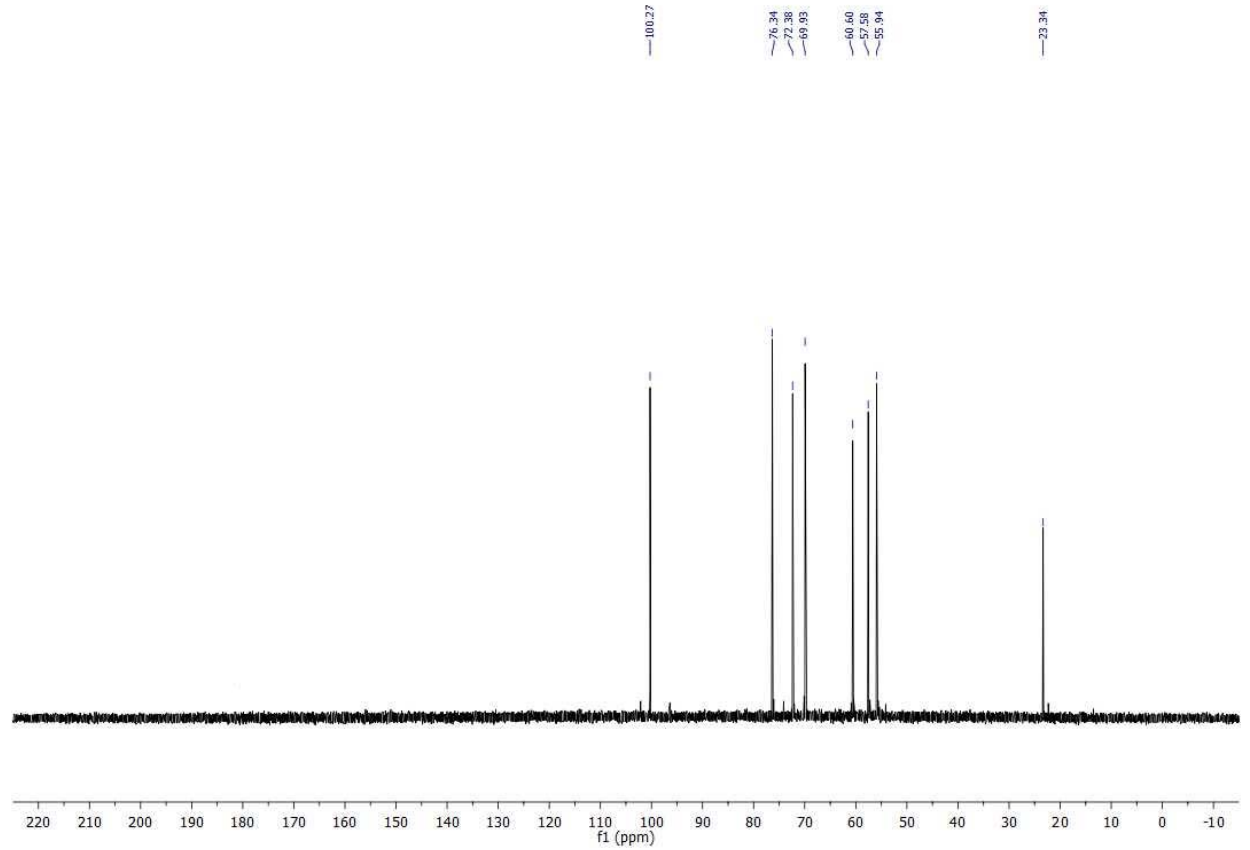


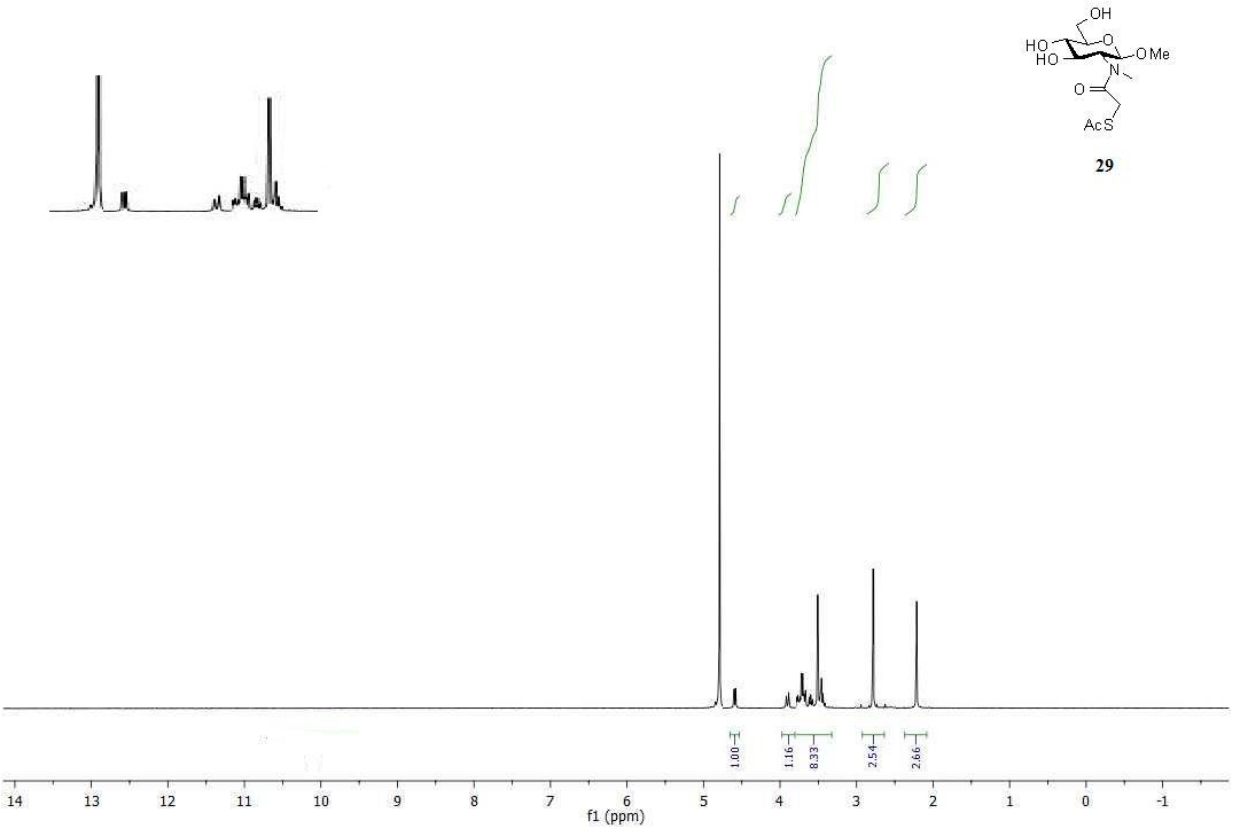


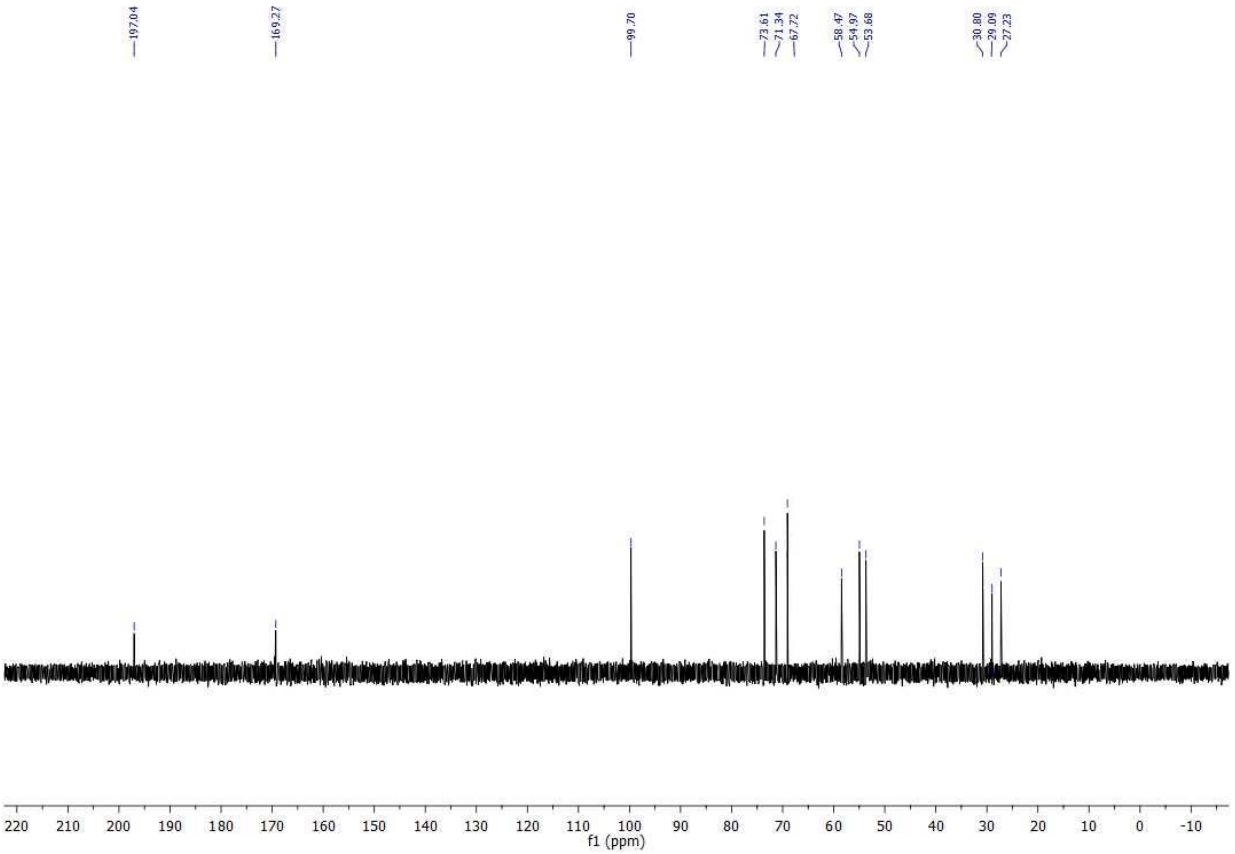


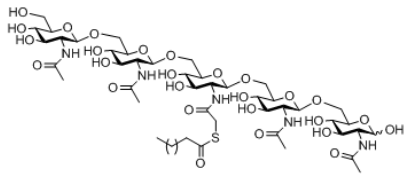



OH
HO
HO
O
OMe
HN
28
1.00
1.07
1.19
1.04
2.53
2.27
0.92
2.53
f1 (ppm)

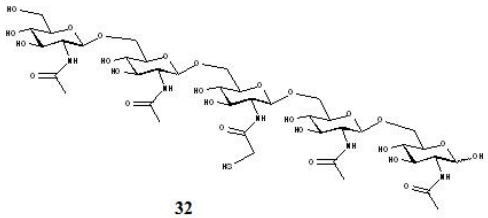

100.27
76.34
72.38
69.93
60.60
57.58
55.94
23.34
f1 (ppm)


OH
HO
HO
O
OMe
N
O
AcS
29
1.00
1.16
8.33
2.54
2.66
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f1 (ppm)

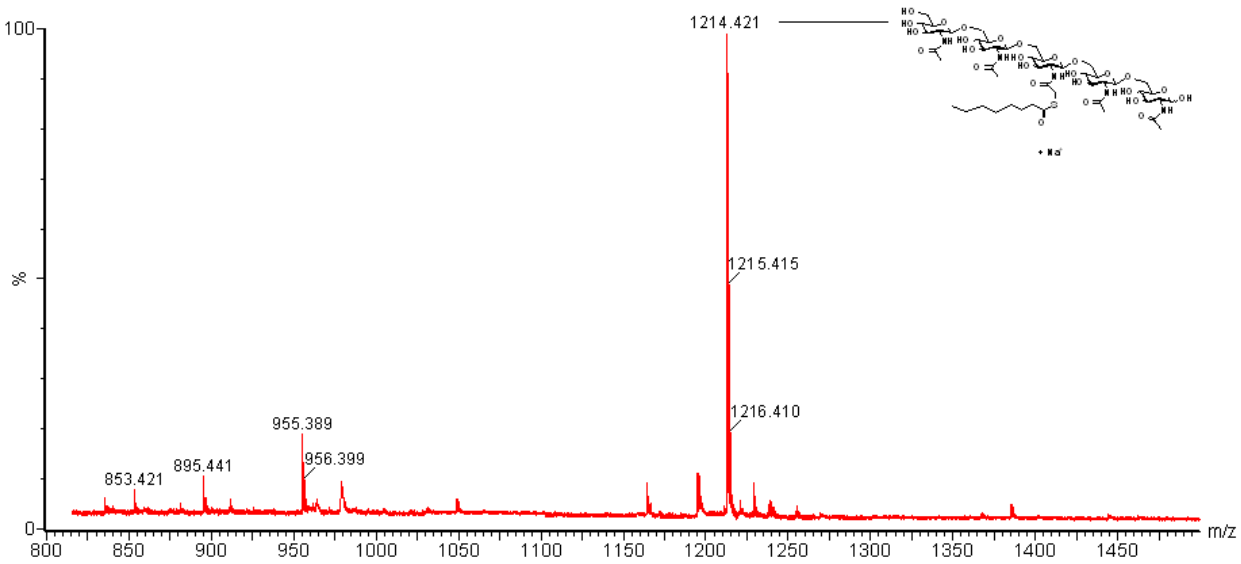

197.04
169.27
99.70
73.61
71.34
67.72
58.47
54.97
53.68
30.80
29.09
27.23
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

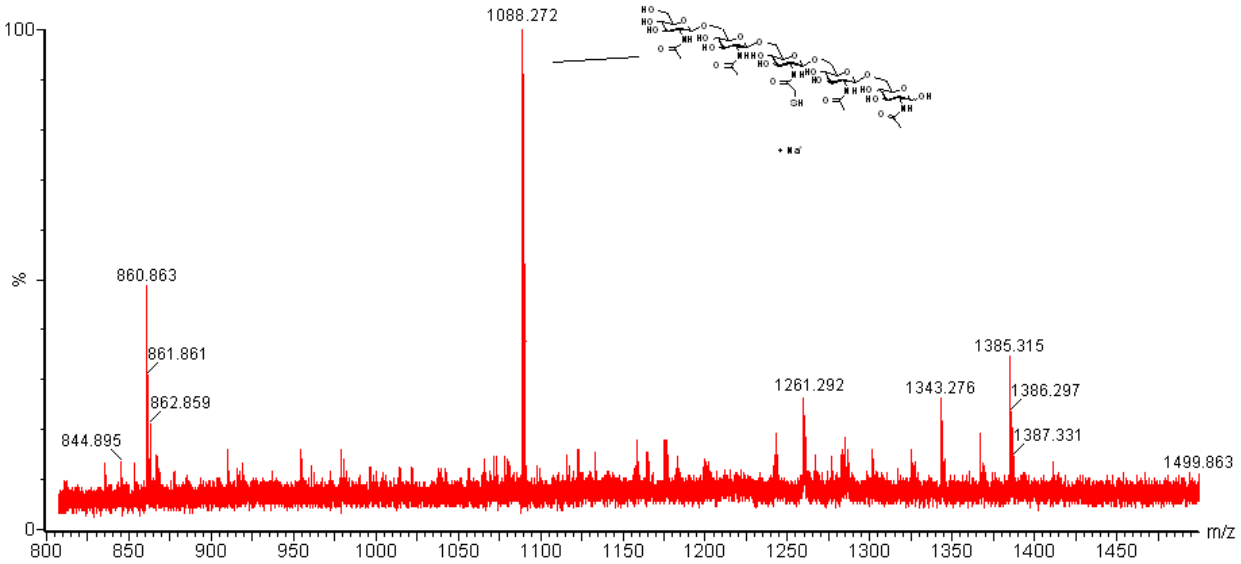


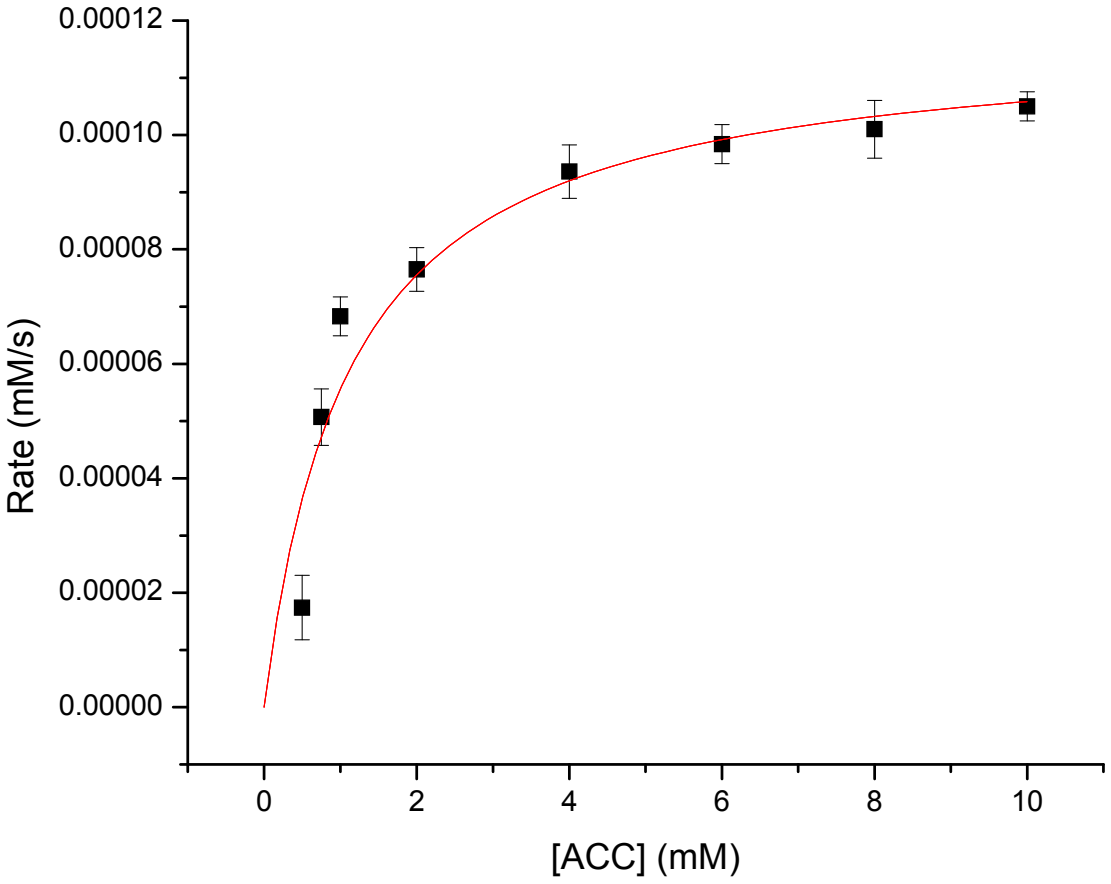
31



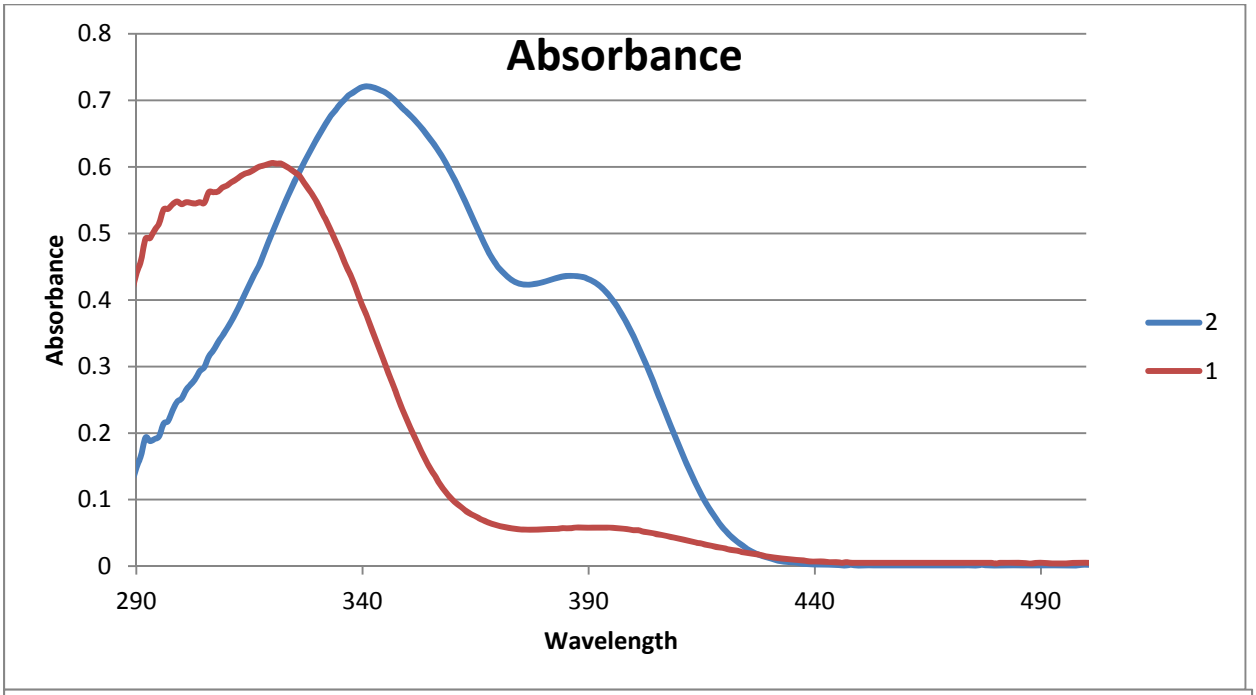
32

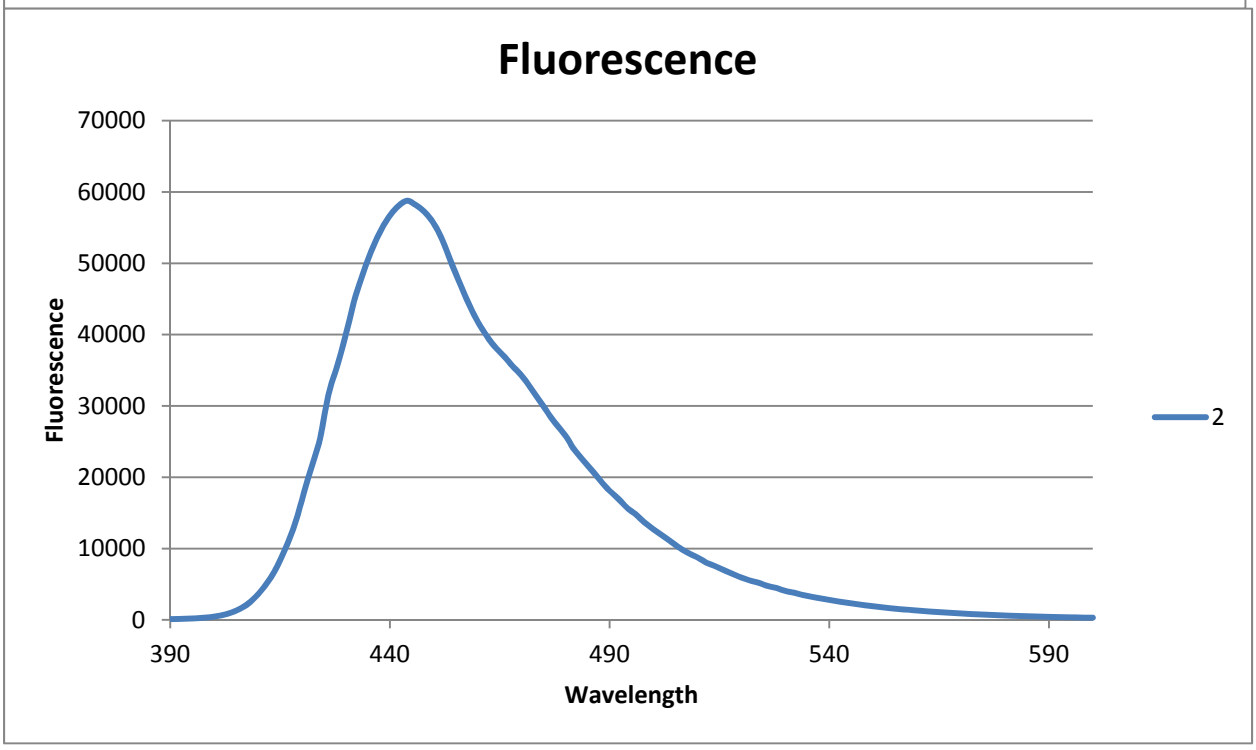

100
%
0
1214.421
1215.415
1216.410
955.389
956.399
895.441
853.421
800
850
900
950
1000
1050
1100
1150
1200
1250
1300
1350
1400
1450
m/z


100
%
0
1088.272
860.863
861.861
862.859
844.895
1261.292
1343.276
1385.315
1386.297
1387.331
1499.863
800
850
900
950
1000
1050
1100
1150
1200
1250
1300
1350
1400
1450
m/z

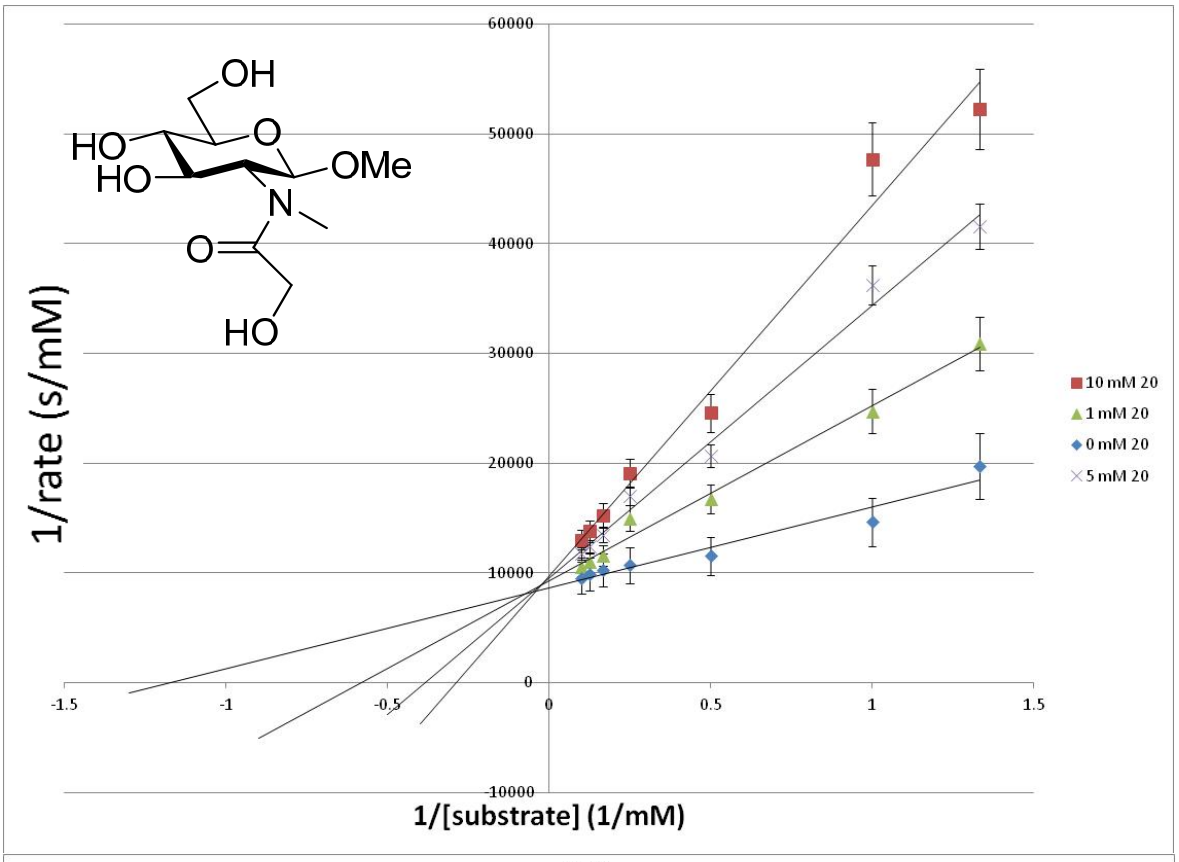


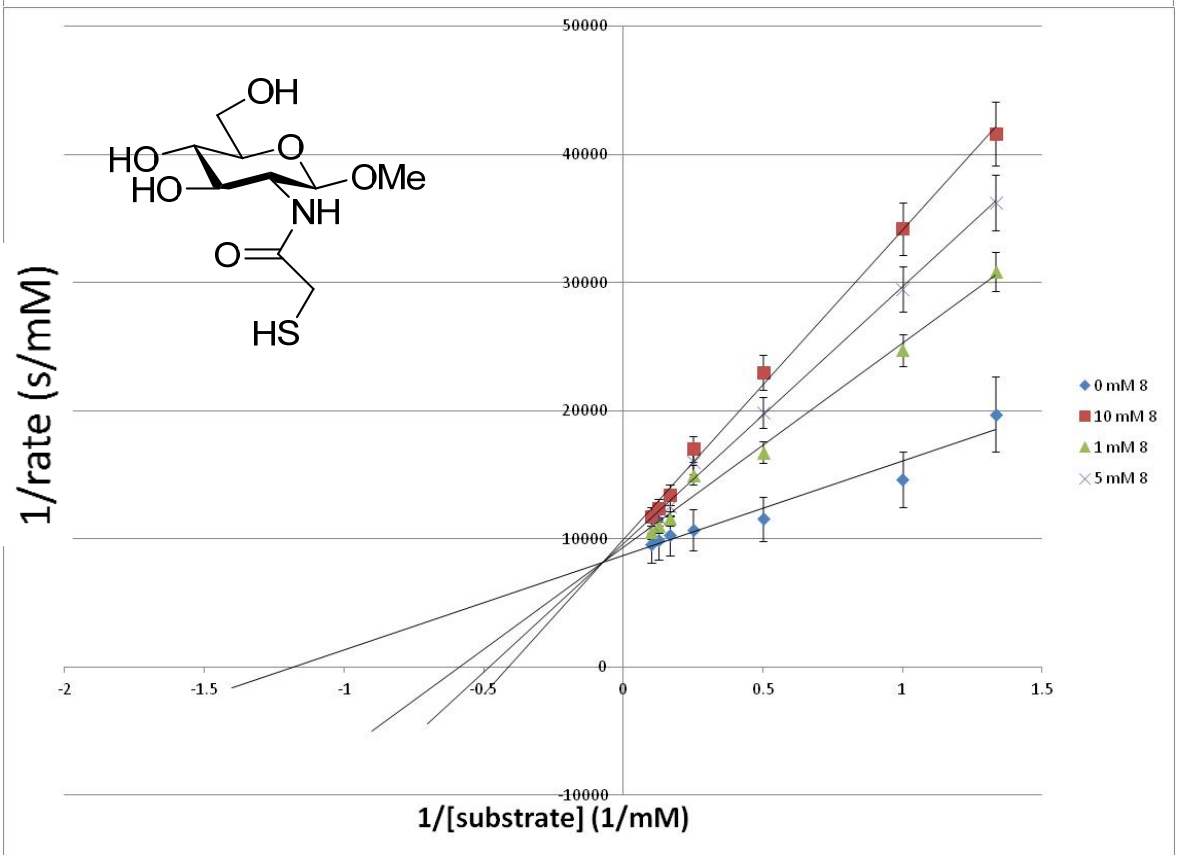
PgaB **ACC** deacetylation rates at various **ACC** concentrations. Conditions were as follows: HEPES buffer containing 10% (v/v) DMSO (100 mM, pH 7.5), 10 uM PgaB, ACC (0.5 mM – 10 mM). Assays were done in triplicate. Rates were obtained by measuring the increase of fluorescence emission at 446 nM resulting from the acetylosis of ACC. V_{max}, K_M and k_{cat} were determined from direct curve fitting of the data.

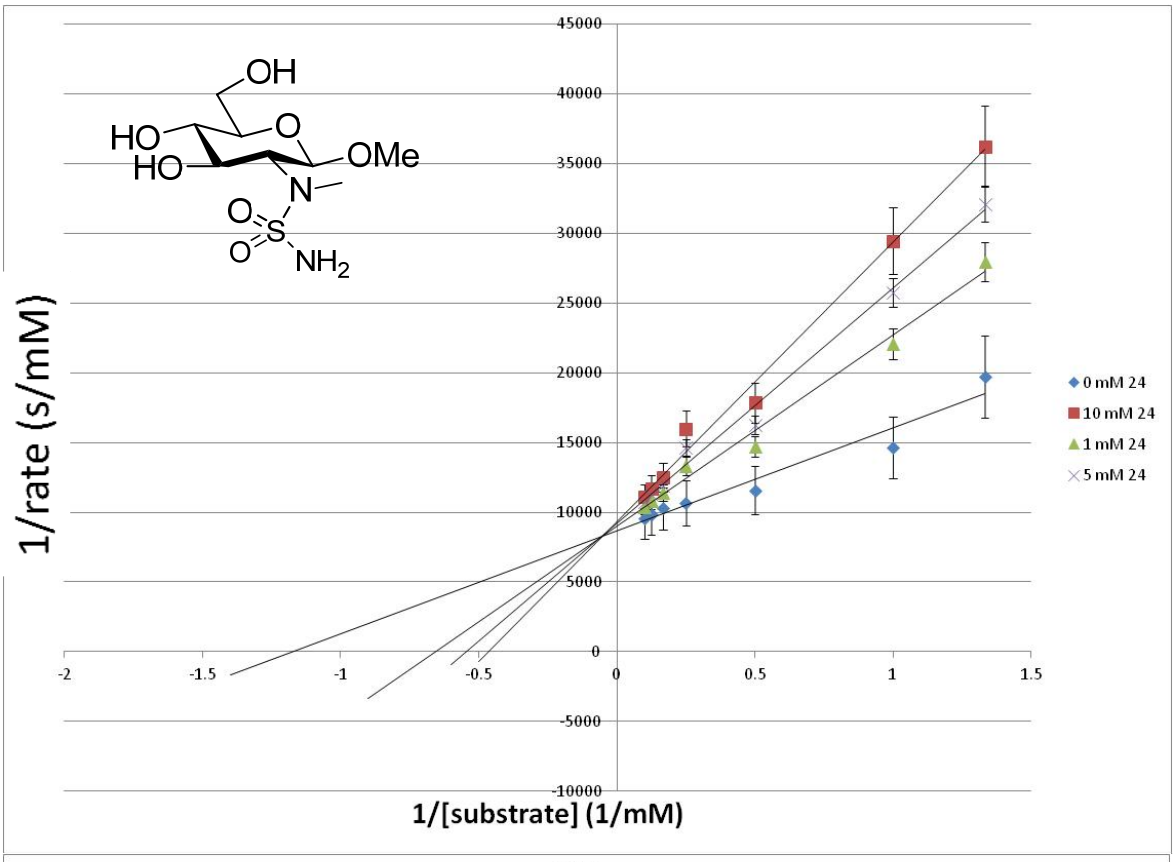


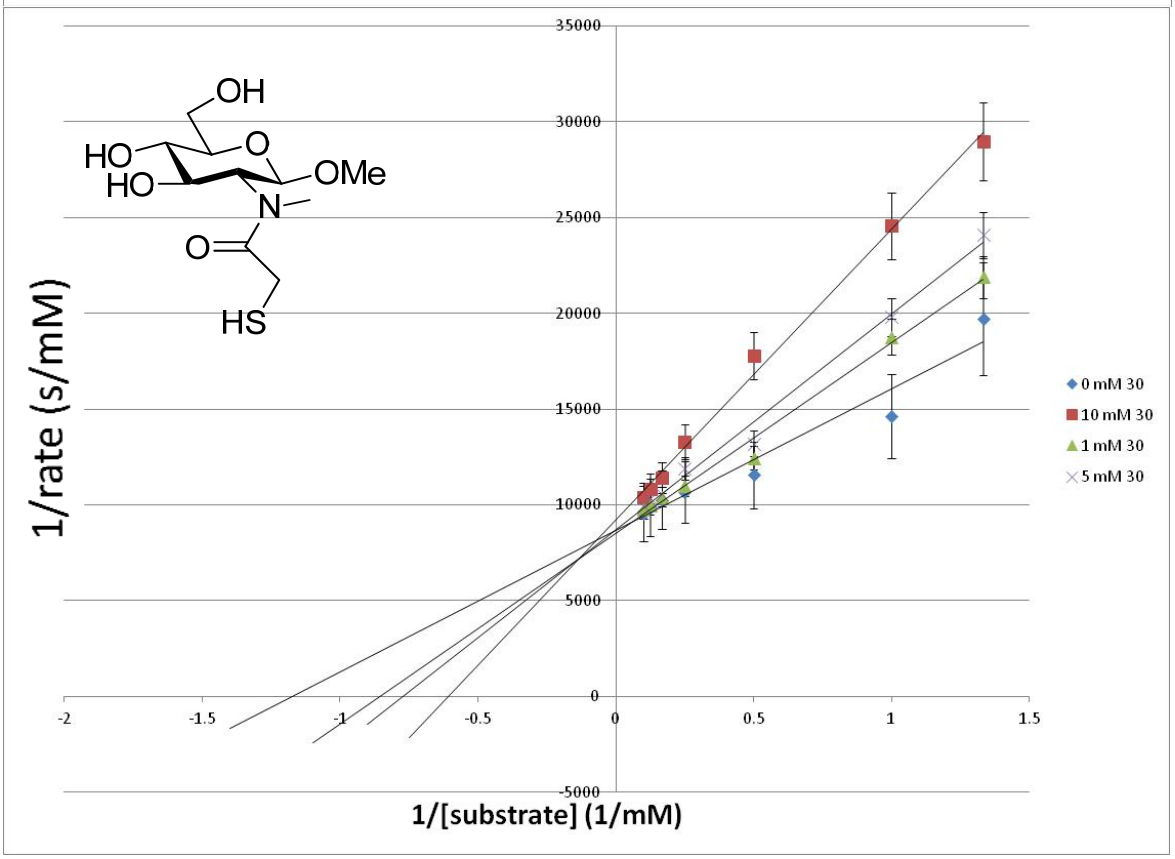


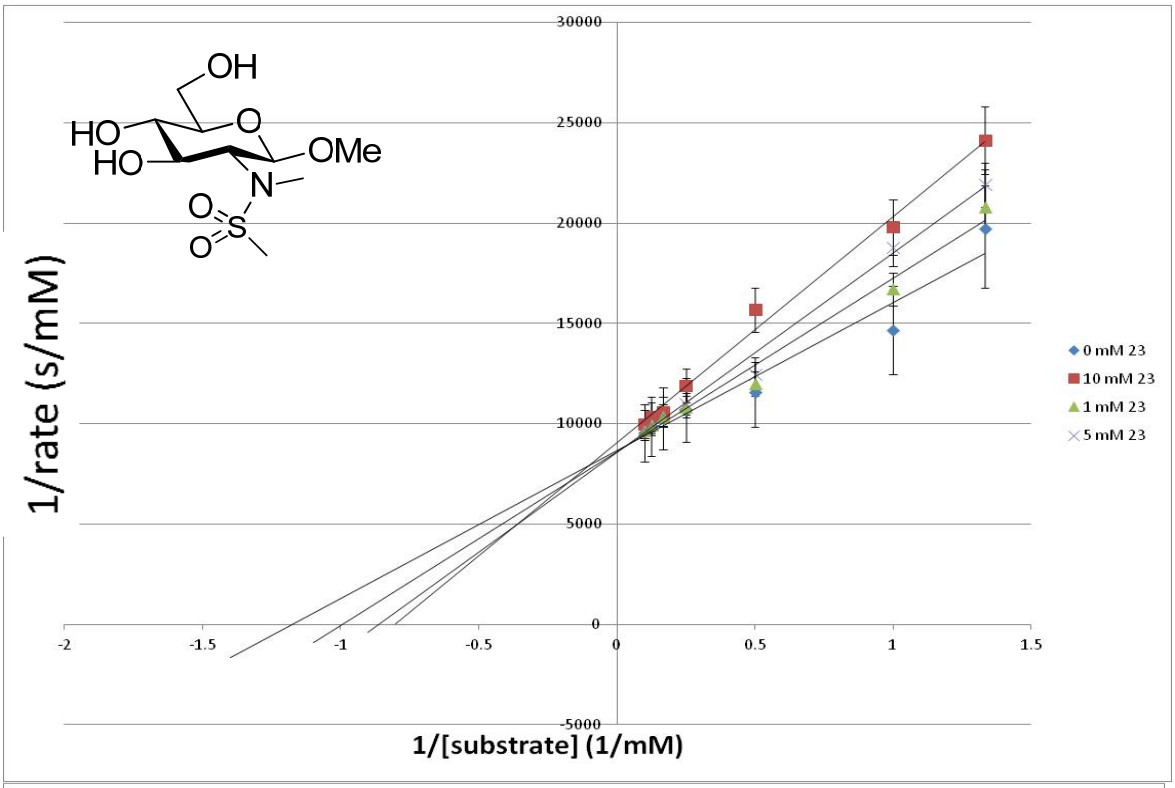
Top – Absorbance spectra of 1 (**ACC**) and 2 (Deacetylated **ACC**) at 5mM in 100mM HEPES containing 10% (v/v) DMSO. Bottom – Fluorescence spectra of 2 when excited at either 346 or 388.

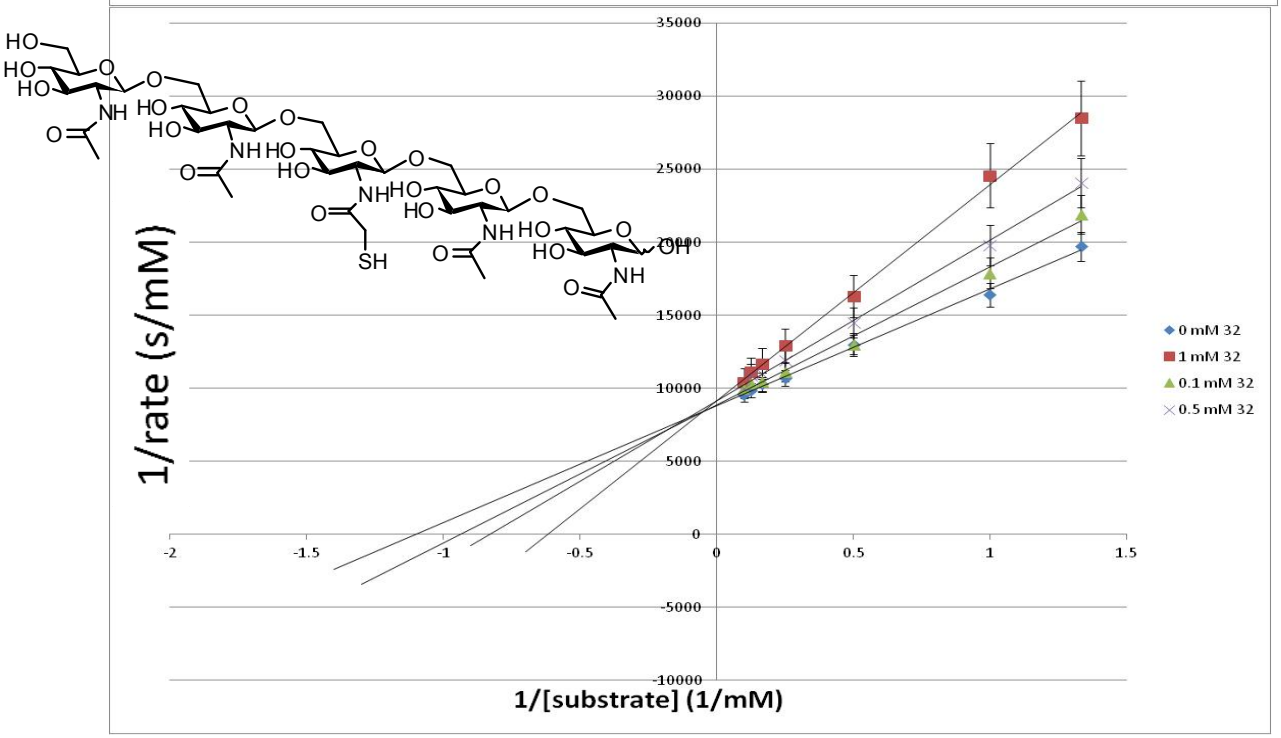












Lineweaver Burke plots – All inhibitors showed competitive enzyme inhibition. The value of α was assessed as the factor increase of the slope in the presence of different inhibitor concentrations (slope = $\alpha K_m/V_{max}$). Using each obtained α coefficient, a mean K_i value was derived from the following formula ($\alpha = 1 + [I]/K_i$).

References:

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