Supporting Information

Protecting group free synthesis of glycosyl thiols from reducing sugars in water; application to the production of *N*-glycan glycoconjugates.

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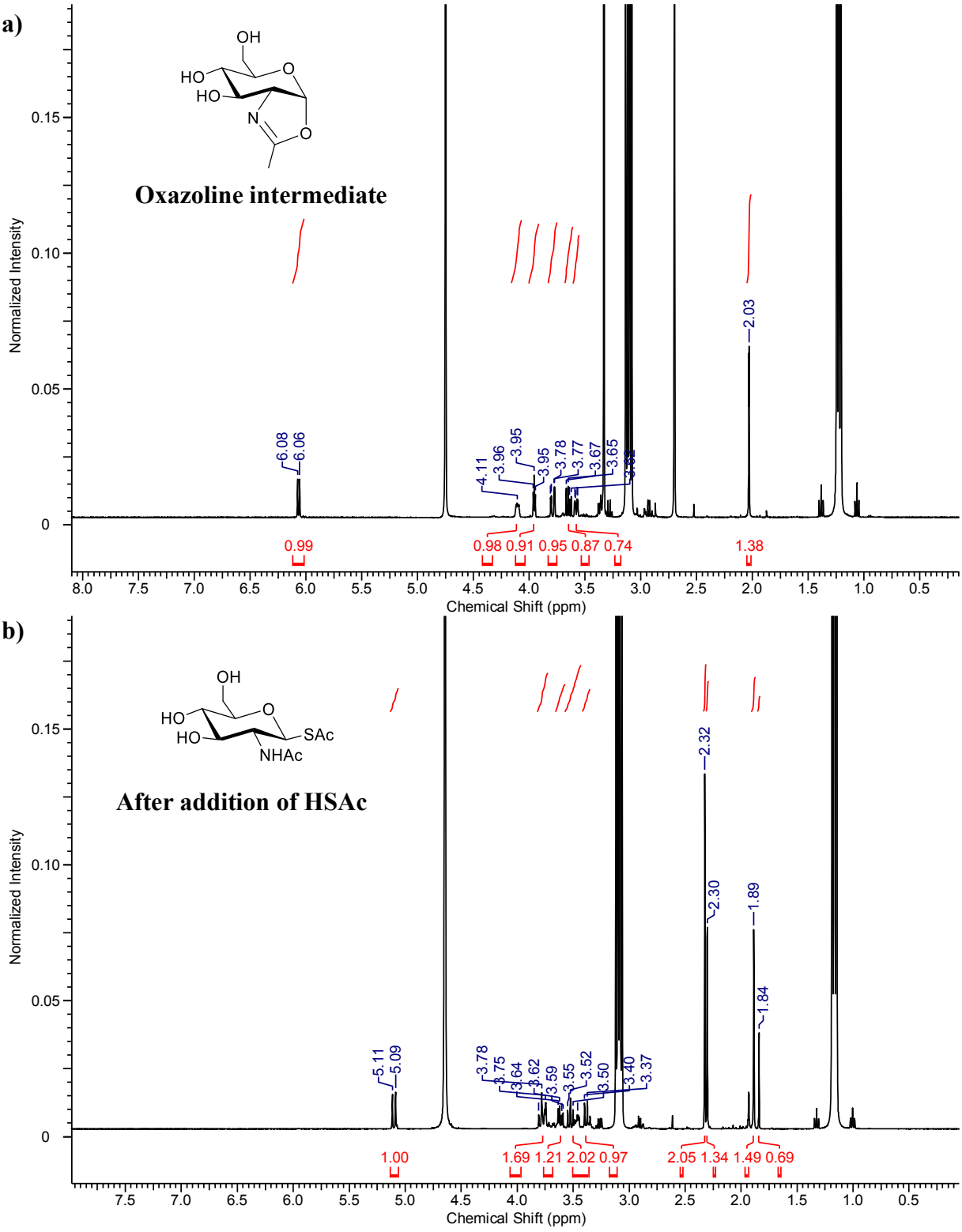
1. **Figure S1**: NMR study of the formation of glycosyl thioacetate product *via* an intermediate oxazoline: a) crude NMR spectrum taken 30 min after the addition of DMC showing characteristic anomeric proton of oxazoline at 6.07ppm; b) crude NMR spectrum then taken after the addition of thioacetic acid to the reaction mixture, showing the formation of the corresponding glycosyl thioacetate.



2. Experimental procedures and characterizations of compounds

General Experimental: All reactions involving moisture sensitive reagents were performed under an atmosphere of argon or nitrogen *via* standard vacuum Schlenk line techniques. All glassware for such reactions was flame-dried and cooled under an atmosphere of argon. Reactions conducted at -78 °C were cooled by means of an acetone/dry ice bath; those conducted at 0 °C were cooled by means of an ice bath. Solvent was removed under reduced pressure using a Buchi^{TM} rotary evaporator. HPLC–grade solvents were used for reactions and in case of moisture–sensitive reactions; solvents were dried by literature procedures and freshly distilled as required. Petroleum ether (Petrol) refers to the fraction of light petroleum ether boiling in the range 40-60 °C. Reagents were used as supplied without further purification unless otherwise stated. Thin Layer Chromatography (t.l.c.) was carried out on Merck Silica Gel $60F_{254}$ aluminium-backed plates. Visualisation of the plates was achieved using a UV lamp (λ_{max} = 254 or 365 nm), and/or ammonium molybdate (5% in 2 M H_2SO_4). Flash column chromatography was carried out using Sorbsil C60 40/60 silica. Melting points were recorded on an Electrothermal melting point apparatus and are uncorrected. Proton and carbon nuclear magnetic resonance (^1H, ^{13}C) spectra were recorded on Agilent 400–MR instrument operating for ^1H NMR at 400 MHz, and at 100 MHz, for ^{13}C NMR. All chemical shifts are quoted on the δ-scale in ppm using residual solvent as an internal standard. 1H and 13C spectra were assigned using COSY, DEPT, HSQC, HMBC, TOCSY and DPFGSE-TOCSY. High resolution mass spectra were recorded by on either a DIONEX Ultimate 3000 or Bruker MaXis 4G spectrometer, operated in high resolution positive ion electrospray mode. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a water-jacketed 1 cm^3 cell with a path length of 1 dm, and are quoted in units of $°.cm^2.g^{-1}$. Concentrations (*c*) are given in g / 100 cm^3, solvent and temperature are recorded. Infrared spectra were recorded on a Perkin–Elmer Spectrum One FTIR instrument operating in diffuse reflectance mode with samples prepared as KBr pellets (KBr) or on a Bruker FTIR spectrometer with Alpha's Platinum ATR single reflection diamond where the neat samples were recorded.

Solvents for reversed phase-high performance liquid chromatography (RP-HPLC) were used as RP-HPLC grade and used without further purification. 6-Chloro-1-hydroxybenzotriazole (6-Cl-HOBt) was purchased from Aapptec (Louisville, Kentucky). *O*-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N'*,*N''*-tetramethyluronium hexafluorophosphate (HCTU), *N,N*-dimethylformamide

(DMF) (AR grade), acetonitrile (CH_3CN) [high-performance liquid chromatography (HPLC) grade], *N,N'*-diisopropylethylamine (iPr$_2$EtN), piperazine, triisopropylsilane (iPr$_3$SiH), and *N,N'*-diisopropylcarbodiimide (DIC) were purchased from Sigma–Aldrich (Sydney, Australia). Aminomethyl polystyrene resin was synthesised "in house" as previously described in the literature.[1] Dichloromethane (CH_2Cl_2) was purchased from ECP Limited (Auckland, New Zealand). Fmoc-Tyr(tBu)-O-CH_2-Phi-OCH_2-CH_2-COOH was purchased from PolyPeptide Laboratories Group (Strasbourg, France). Diethyl ether was purchased from Avantor Performance Materials. Trifluoroacetic acid (TFA) was purchased from Halocarbon (River Edge, USA).

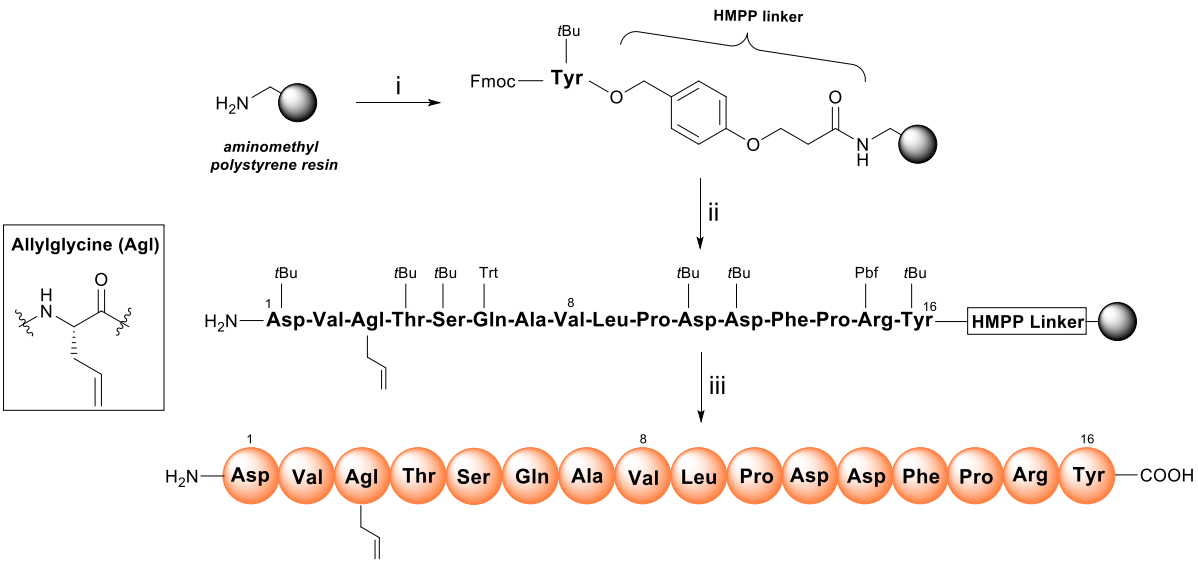
The following Fmoc-amino acids were purchased from GL Biochem: Fmoc-allylglycine-OH (Fmoc-AgI), Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-Phe-OH, Fmoc-Val-OH, Fmoc-Ala-OH, and Fmoc protected amino acids with the following side chain protection: Fmoc-Asp(tBu)-OH (tBu = *tert*-butyl), Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gln(Trt)-OH (Trt = triphenylmethyl), Fmoc-Arg(Pbf)-OH (Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl).

RP-HPLC and LC-MS: Analytical RP-HPLC spectrum was acquired on a Dionex (California, USA) Ultimate 3000 System equipped with a two-channel UV detector using an analytical column (Phenomenex Gemini C_{18}, 250 x 4.6 mm; 5 μm) and a linear gradient of 5% solvent B to 65% (*ca.* 2%B per minute) at a flow rate of 1 mL min^{-1} (where solvent A was 0.1% TFA in water and solvent B was 0.1% TFA in CH3CN). The ratio of product was determined by integration of spectrum recorded at 210 nm. Peptide mass was confirmed by a Hewlett Packard (HP) 1100 series mass spectrometer (California, USA) using direct flow injection at 0.3 mL min^{-1} into an ESI source in the positive mode.

Semi-preparative RP-HPLC was performed on a Waters (Massachusetts, USA) 600E system using a semi-preparative column (Waters XTerrra® C_{18}, 300 mm x 19 mm, 10 μm) at a flow rate of 10 mL min^{-1} and eluted using a one-step slow gradient protocol with a detection at 210 nm.[1] Fractions were collected, analysed by RP-HPLC and ESI-MS, pooled, and lyophilised.

Peptide Synthesis - Peptide 20a

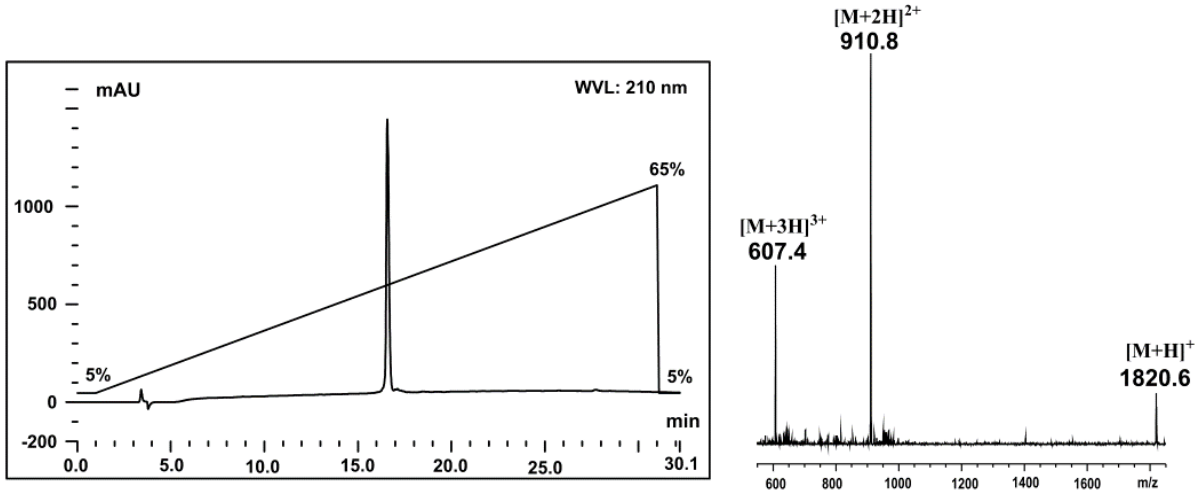
Peptide **20a** used in this work corresponds to residues 1-16 of the rat pancreatic hormone preptin, in which Ser(3) was changed to the non-proteinogenic allylglycine. Synthesis of the 16-residue peptide was performed following a procedure described previously for other structurally-related peptides.[2]



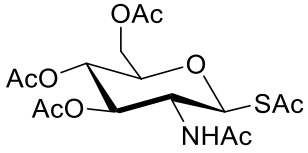
Scheme S1. Synthesis of [Agl(3)]preptin (1-16). Reagents and conditions: (i) Fmoc-Tyr(*t*Bu)-O-CH_2-phi-OCH_2-CH_2-COOH, DIC, CH_2Cl_2, DMF, rt, 4 h; (ii) Fmoc-SPPS (Fmoc deprotection: 5% piperazine, 0.1 M 6-Cl-HOBt, DMF, 62 W, 75 °C, 1 x 0.5 min + 1 x 3 min; coupling: Fmoc-amino acid, HCTU, *i*Pr_2EtN, DMF, 25 W, 75 °C, 5 min (except Fmoc-Arg(Pbf)-OH, rt, 25 min then 25 W, 72 °C, 5 min)); (iii) TFA, *i*Pr_3SiH, H_2O, rt, 2 h, 24%.

A solution of Fmoc-Tyr(*t*Bu)-O-CH_2-phi-OCH_2-CH_2-COOH (127.5 mg, 0.2 mmol) and DIC (31 µL, 0.2 mmol) in CH_2Cl_2/DMF (*v/v*; 2:1, 3 mL) was added to pre-swollen (CH_2Cl_2, 3 mL, 20 min) aminomethyl polystyrene resin (110.0 mg, 0.1 mmol, loading 0.91 mmol g^{-1}) and the mixture gently agitated for 4 h, at room temperature, filtered and washed with DMF (4 x 3 mL). Extension of the *C*-terminal amino acid was performed *via* Fmoc-SPPS on a Liberty Microwave Peptide Synthesiser (CEM Corporation, Mathews, NC). All amino acid couplings were performed as single coupling cycles, with the exception of Fmoc-Arg(Pbf)-OH where a double coupling cycle was performed as part of a synthetic protocol recommended by CEM Microwave Technology. Couplings were performed using a mixture of Fmoc-amino acid (0.4 mmol, 0.2 M), HCTU (0.36 mmol, 0.45 M) and *i*Pr_2EtN (0.8 mmol, 2M) in DMF for 5 min, at 25 W and a maximum temperature of 75 °C, except Fmoc-Arg(Pbf)-OH that was initially coupled for 25 min at room temperature which was followed by the second coupling for 5 min, at 25 W and a maximum temperature of 72 °C. The Fmoc group was removed using 5% (*w/v*) solution of piperazine with 0.1 M 6-Cl-HOBt in DMF (1 x 30 sec then 1 x 3 min). A 30 sec deprotection cycle was followed by a second deprotection for 3 min using microwave conditions of 62 W and a maximum temperature of 75 °C.

The resulting peptide was released from the resin with concomitant removal of the side chain protecting groups by treatment with TFA/iPr$_3$SiH/H$_2$O (*v/v/v*; 95:2.5:2.5, 5 mL) at room temperature for 2 h. The resin was removed by filtration, washed with TFA (2 x 3 mL) and the combined filtrates were concentrated. The crude peptides were recovered by precipitation with cold diethyl ether, isolated by centrifugation (2 x 10 min; 3000 rpm), re-suspended with H$_2$O/CH$_3$CN (*v/v*; 1:1), and lyophilised. RP-HPLC purification afforded the desired peptide **20a** as an amorphous solid (43.7 mg, 24% yield, 98% purity); R_t 16.4; *m/z* (ESI-MS) 1820.6 ([M+H]$^+$ requires 1819.9).



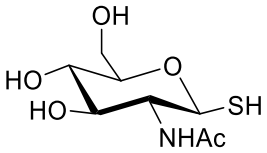
1-Thioacetyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranoside 11a



N-Acetyl-D-glucosamine (500 mg, 2.26 mmol) and triethylamine (3.1 mL, 22.6 mmol) were stirred in water (10 mL), and cooled to 0 °C. 2-Chloro-1,3-dimethylimidazolinium chloride **1** (1.2 g, 6.78 mmol) was added. After 0.5 h, thioacetic acid (2.4 mL, 33.9 mmol) was added dropwise, and the reaction mixture was allowed to stir for an additional 0.5 h at 0 °C. The reaction mixture was then diluted with water (10 mL) and washed with DCM (5 x 20 mL). The aqueous layer was concentrated *in vacuo* and the solid suspended in pyridine (7 mL) under an atmosphere of nitrogen. The mixture was stirred at RT and acetic anhydride (7 mL, 74.1 mmol) was added. The reaction was stirred for 12 h, after which time t.l.c. (petrol:ethyl

acetate 2:1) indicated the formation of a single major product (R_f 0.8). Water (10 mL) was added, and the mixture was then washed with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were washed with aqueous HCl (1 M, 100 mL), $NaHCO_3$ (sat. aqueous soln., 100 mL), water (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was recrystallised (CH_2Cl_2/petrol) to afford 1-thioacetyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranoside **11a** (0.807 g, 88%) as a colourless crystalline solid; m.p. 190-191 °C (CH_2Cl_2/petrol) [lit. 186-188 °C (CH_2Cl_2/petrol)][3]; $[\alpha]_D^{20}$ +20 (*c*, 1.0 in $CHCl_3$) [lit. $[\alpha]_D^{20}$ +12.1 (*c*, 2.2 in $CHCl_3$)][4]; υ_{max} (neat) 1739 cm^{-1} (OC=O), 1699 cm^{-1} (SC=O), 1661 cm^{-1} (NHC=O); δ_H (400 MHz, $CDCl_3$) 1.92 (3H, s, NHC(O)C$\underline{H}_3$), 2.03 (6H, s, 2 x C(O)C$\underline{H}_3$), 2.08 (3H, s, C(O)C$\underline{H}_3$), 2.37 (3H, s, SC(O)C$\underline{H}_3$), 3.75 - 3.81 (1H, m, H-5), 4.09 (1H, dd, $J_{6,6'}$ 11.7 Hz, H-6), 4.23 (1H, dd, $J_{6,6'}$ 12.7 Hz, $J_{5,6'}$ 4.5 Hz, H-6'), 4.35 (1H, aq, *J* 9.9 Hz, H-2), 5.05 - 5.18 (3H, m, H-1, H-3 & H-4), 5.65 (1H, d, $J_{NH,2}$ 9.8 Hz, N$\underline{H}$C(O)CH_3); δ_C (100.5 MHz, $CDCl_3$) 20.6 (q, C(O)$\underline{C}H_3$), 20.6 (q, C(O)$\underline{C}H_3$), 20.7 (q, C(O)$\underline{C}H_3$), 23.1 (q, NHC(O)$\underline{C}H_3$), 30.8 (q, SC(O)$\underline{C}H_3$), 52.3 (d, C-2), 61.8 (t, C-6), 67.7 (d, C-3), 74.0 (d, C-4), 76.6 (d, C-5), 81.6 (d, C-1), 169.2 (s, $\underline{C}$(O)CH_3), 170.0 (s, $\underline{C}$(O)CH_3), 170.7 (s, $\underline{C}$(O)CH_3), 171.3 (s, $\underline{C}$(O)CH_3), 193.7 (s, S$\underline{C}$(O)CH_3); HRMS (ESI-TOF): calcd. for $C_{16}H_{24}NO_9S^+$: 406.1172. Found: 406.1166 (MH^+).

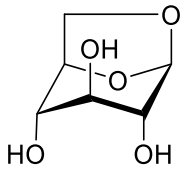
1-Thio-2-acetamido-2-deoxy-β-D-glucopyranose 11b



Sodium metal (25.4 mg, 1.1 mmol) was added to MeOH (5 mL) and stirred. 1-Thioacetyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranoside **11a** (203 mg, 0.50 mmol) was added and the reaction was stirred for 0.5 h, at which point t.l.c. (ethyl acetate:petrol 2:1) indicated complete consumption of starting material (R_f 0.2) and formation of a single product (R_f 0). Dowex® 50WX8 (H^+) ion exchange resin was added portion-wise until the reaction reached neutral pH. The mixture was then filtered and concentrated *in vacuo* to afford 1-thio-2-acetamido-2-deoxy-β-D-glucopyranose **11b** (122 mg, quant.) as a white solid; m.p. 174-175 °C (ethyl acetate/MeOH) [lit. 177-179 °C (ethyl acetate/MeOH)][5]; $[\alpha]_D^{20}$ -6 (*c*,

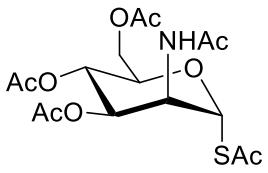
1.0 in MeOH) [lit. $[\alpha]_D^{22}$ -10.4 (*c*, 1.0 in MeOH)][6]; υ_{max} (neat) 3286 cm^{-1} (OH, NH), 2531 cm^{-1} (SH), 1634 cm^{-1} (NHC=O); δ_H (400 MHz, D_2O) 1.96 (3H, s, NHC(O)C<u>H</u>$_3$), 3.35 - 3.48 (3H, m, H-3, H-4 & H-5), 3.59 - 3.70 (2H, m, H-2 & H-6), 3.78 - 3.84 (1H, m, H-6'), 4.60 (1H, d, $J_{1,2}$ 9.8 Hz, H-1); δ_C (100.5 MHz, D_2O) 22.2 (q, NHC(O)<u>C</u>H$_3$), 58.0 (d, C-2), 60.8 (t, C-6), 69.7 (d, C-4), 75.0 (d, C-3), 79.1 (d, C-1), 80.1 (d, C-5), 174.6 (s, NH<u>C</u>(O)CH$_3$); HRMS (ESI-TOF): calcd. for $C_8H_{16}NO_5SNa^+$: 260.0563. Found: 260.0560 (MNa^+).

1,6-Anhydro-β-D-glucose 13



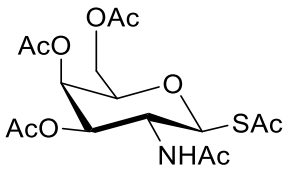
Glucose (180 mg, 1.0 mmol) and triethylamine (1.37 mL, 10 mmol) were stirred in water (4.4 mL) and cooled to 0 °C. 2-Chloro-1,3-dimethylimidazolinium chloride **29** (504 mg, 3.0 mmol) was added. After 0.5 h, thioacetic acid (1.06 mL, 15 mmol) was added dropwise and the reaction mixture was stirred for an additional 0.5 h at 0 °C. The reaction mixture was then diluted with water (5 mL) and washed with DCM (5 x 10 mL). The aqueous layer was filtered through a column of Amberlite® IR-120 (Na^+ form, produced by treating the H^+ form with 1 M aqueous NaOH solution and then washing with water until pH 7). The solution was concentrated *in vacuo*, dissolved in the minimum amount of water, and purified by flash column chromatography ($CHCl_3$:MeOH 5:1) to afford 1,6-anhydro-β-D-glucose **80** (65 mg, 40%) as a colourless oil; δ_H (400 MHz, D_2O) 3.50 (1H, s, H-2), 3.63 - 3.68 (2H, m, H-3 & H-4), 3.73 (1H, dd, $J_{5,6}$ 5.9 Hz, $J_{6,6'}$ 7.8 Hz, H-6), 4.07 (1H, d, $J_{6,6'}$ 7.8 Hz, H-6'), 4.61 (1H, d, $J_{5,6}$ 5.9 Hz, H-5), 5.43 (1H, s, H-1); δ_C (100.5 MHz, D_2O) 65.1 (t, C-6), 70.1 (d, C-2), 70.8 (d, C-4), 72.4 (d, C-3), 76.2 (d, C-5), 101.4 (d, C-1); HRMS (ESI-TOF): calcd. for $C_6H_{10}O_5Na^+$: 185.0420. Found: 185.0417 (MNa^+).

1-Thioacetyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-α-D-mannopyranoside 14a



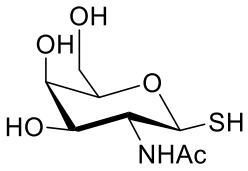
N-Acetyl-D-mannosamine (500 mg, 2.26 mmol) and triethylamine (3.1 mL, 22.6 mmol) were stirred in D_2O (10 mL) and the mixture was cooled to 0 °C. 2-Chloro-1,3-dimethylimidazolinium chloride **1** (1.2 g, 6.78 mmol) was added. After 0.5 h, thioacetic acid (2.4 mL, 33.9 mmol) was added dropwise, and the reaction mixture was allowed to stir at 0 °C for an additional 0.5 h. The reaction mixture was then diluted with water (10 mL) and washed with DCM (5 x 20 mL). The aqueous layer was concentrated *in vacuo* and the residue stirred in pyridine (7 mL) under an atmosphere of nitrogen. Acetic anhydride (7 mL, 74.1 mmol) was added and the mixture was stirred for 12 h, after which time t.l.c. (petrol:ethyl acetate, 2:1) indicated the formation of a single major product (R_f 0.8). Water (10 mL) was added, and the mixture was then washed with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were washed with aqueous HCl (1 M, 100 mL), $NaHCO_3$ (sat. aqueous soln., 100 mL), water (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:petrol, 3:2) to afford 1-thioacetyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-α-D-mannopyranoside **14a** (0.807 g, 88%) as a colourless oil; $[\alpha]_D^{20}$ +55 (*c*, 1.0 in $CHCl_3$); υ_{max} (neat) 1740 cm^{-1} (OC=O), 1711 cm^{-1} (SC=O), 1684 cm^{-1} (NHC=O); δ_H (400 MHz, $CDCl_3$) 1.99 (3H, s, C(O)C$\underline{H}_3$), 2.05 (3H, s, C(O)C$\underline{H}_3$), 2.06 (3H, s, C(O)C$\underline{H}_3$), 2.08 (3H, s, C(O)C$\underline{H}_3$), 2.41 (3H, s, SC(O)C$\underline{H}_3$), 3.96 - 4.06 (2H, m, H-5 & H-6), 4.28 (1H, dd, $J_{5,6'}$ 4.7 Hz, $J_{6,6'}$ 12.1, H-6'), 4.65 - 4.70 (1H, m, H-2), 5.04 (1H, dd, $J_{3,4}$ 9.8 Hz, $J_{2,3}$ 3.9 Hz, H-3), 5.15 (1H, t, *J* 9.8 Hz, H-4), 5.85 (1H, d, $J_{1,2}$ 1.6 Hz, H-1), 6.02 (1H, d, *J* 8.6 Hz, N$\underline{H}$C(O)CH_3); δ_C (100.5 MHz, $CDCl_3$) 20.6 (q, C(O)$\underline{C}H_3$), 20.6 (q, C(O)$\underline{C}H_3$), 20.7 (q, C(O)$\underline{C}H_3$), 23.2 (q, C(O)$\underline{C}H_3$), 31.2 (q, SC(O)$\underline{C}H_3$), 51.4 (d, C-2), 62.1 (t, C-6), 65.7 (d, C-4), 70.1 (d, C-3), 72.0 (d, C-5), 81.1 (d, C-1), 169.7 (s, $\underline{C}$(O)CH_3), 169.9 (s, $\underline{C}$(O)CH_3), 170.0 (s, $\underline{C}$(O)CH_3), 170.5 (s, $\underline{C}$(O)CH_3), 190.8 (s, S$\underline{C}$(O)CH_3); HRMS (ESI-TOF): calcd. for $C_{16}H_{24}NO_9S^+$: 406.1172. Found: 406.1152 (MH^+).

1-Thioacetyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranoside 15a



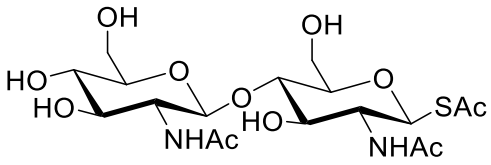
N-Acetyl-D-galactosamine (500 mg, 2.26 mmol) and triethylamine (3.1 mL, 22.6 mmol) were stirred in water (10 mL) and the mixture was cooled to 0 °C. 2-Chloro-1,3-dimethylimidazolinium chloride **1** (1.2 g, 6.78 mmol) was added. After 0.5 h, thioacetic acid (2.4 mL, 33.9 mmol) was added dropwise and the reaction mixture was allowed to stir at 0 °C for an additional 0.5 h. The reaction mixture was then diluted with water (10 mL) and washed with DCM (5 x 20 mL). The aqueous layer was concentrated *in vacuo* and the solid stirred in pyridine (7 mL) under an atmosphere of nitrogen. Acetic anhydride (7 mL, 74.1 mmol) was added and the mixture was stirred for 12 h, after which time t.l.c. (ethyl acetate:petrol, 2:1) indicated the formation of a single product (R_f 0.1). Water (10 mL) was added, and the mixture was then washed with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were washed with aqueous HCl (1 M, 100 mL), $NaHCO_3$ (sat. aqueous soln., 100 mL), water (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was pre-adsorbed onto silica and purified by flash column chromatography (ethyl acetate:petrol, 2:1) to afford 1-thioacetyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranoside **15a** (511 mg, 56%) as a colourless solid; m.p. 185-187 °C (CH_2Cl_2/Et_2O) [lit. 197-198 °C (CH_2Cl_2/Et_2O)];[5] $[\alpha]_D^{20}$ +20 (*c*, 1.0 in $CHCl_3$) [lit. +11.3 (*c*, 1.0 in $CHCl_3$)][6]; υ_{max} (neat) 1738 cm^{-1} (OC=O), 1701 cm^{-1} (SC=O), 1645 cm^{-1} (NHC=O); δ_H (400 MHz, $CDCl_3$)[7] 1.92 (3H, s, C(O)C$\underline{H}_3$), 2.01 (3H, s, C(O)C$\underline{H}_3$), 2.04 (3H, s, C(O)C$\underline{H}_3$), 2.16 (3H, s, C(O)C$\underline{H}_3$), 2.38 (3H, s, SC(O)C$\underline{H}_3$), 3.98 - 4.16 (3H, m, H-5, H-6 & H-6'), 4.52 (1H, q, *J* 10.6 Hz, H-2), 5.05 (1H, dd, $J_{2,3}$ 10.8 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.15 (1H, d, $J_{1,2}$ 10.6 Hz, H-1), 5.36 - 5.45 (2H, m, N$\underline{H}$C(O)CH_3 & H-4); δ_C (100.5 MHz, $CDCl_3$) 20.6 (q, C(O)$\underline{C}H_3$), 20.7 (q, C(O)$\underline{C}H_3$), 20.7 (q, C(O)$\underline{C}H_3$), 23.2 (q, C(O)$\underline{C}H_3$), 30.8 (q, SC(O)$\underline{C}H_3$), 48.7 (d, C-2), 61.4 (t, C-6), 66.7 (d, C-4), 71.6 (d, C-3), 75.3 (d, C-5), 82.1 (d, C-1), 170.1 (s, $\underline{C}$(O)CH_3), 170.2 (s, $\underline{C}$(O)CH_3), 170.4 (s, $\underline{C}$(O)CH_3), 170.8 (s, $\underline{C}$(O)CH_3), 193.7 (s, S$\underline{C}$(O)CH_3); HRMS (ESI-TOF): calcd. for $C_{16}H_{24}NO_9S^+$: 406.1172. Found: 406.1165 (MH^+).

1-Thio-2-acetamido-2-deoxy-β-D-galactopyranose 15b



Sodium metal (25.4 mg, 1.1 mmol) was added to MeOH (5 mL) and stirred. 1-Thioacetyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranoside **15a** (179 mg, 0.44 mmol) was added and the mixture was stirred for 0.5 h, at which point t.l.c. (ethyl acetate:petrol, 2:1) indicated complete consumption of starting material (R_f 0.1) and formation of a single product (R_f 0). Dowex® 50WX8 (H^+) ion exchange resin was added portion-wise until the reaction reached neutral pH. The reaction was then filtered and concentrated *in vacuo* to afford 1-thio-2-acetamido-2-deoxy-β-D-galactopyranose **15b** (108 mg, quant.) as a white amorphous solid; $[\alpha]_D^{20}$ +29 (*c*, 0.9 in MeOH); υ_{max} (neat) 3275 cm^{-1} (b, OH, NH), 2545 cm^{-1} (SH), 1625 cm^{-1} (NHC=O); δ_H (400 MHz, D_2O) 1.94 (3H, s, C(O)C$\underline{H}_3$), 3.56 - 3.67 (4H, m, H-3, H-5, H-6 & H-6'), 3.80 - 3.88 (2H, m, H-2 & H-4), 4.50 (1H, d, $J_{1,2}$ 10.2 Hz, H-1); δ_C (100.5 MHz, D_2O) 22.2 (q, C(O)$\underline{C}H_3$), 54.6 (d, C-2), 61.1 (t, C-6), 67.8 (d, C-4), 71.8 (d, C-3), 79.3 (d, C-1), 79.3 (d, C-5), 174.8 (s, $\underline{C}$(O)CH_3); HRMS (ESI-TOF): calcd. for $C_8H_{15}NO_5S^+$: 238.0744. Found: 238.0745 (MH^+).

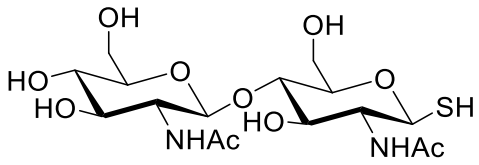
1-Thioacetyl-*N,N'*-diacetylchitobiose 16a



N,N'-Diacetylchitobiose (9.8 mg, 0.023 mmol) and triethylamine (32 µL, 0.23 mmol) were stirred in D_2O (92 µL) and cooled to 0 °C. 2-Chloro-1,3-dimethylimidazolinium chloride **1** (11.8 mg, 0.069 mmol) was added. After 0.5 h, thioacetic acid (24 µL, 0.345 mmol) was added dropwise and the reaction mixture was allowed to stir for an additional 1 h at 0 °C. The reaction was purified by HPLC (column: Luna 5u C18 (100 Å) column (Phenomenex); gradient: 100% water for 15 min, followed by an increase to 100% MeOH over 5 min; column oven: 40 °C; flow rate: 2 mL/min; detection: UV 210 nm) to afford 1-thioacetyl-

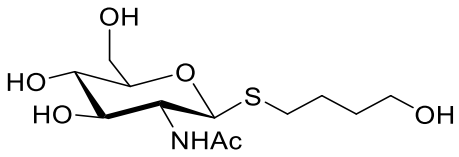
N,N'-diacetylchitobiose **16a** (10.3 mg, 93%) as a white powder; υ_{max} (neat) 3270 cm^{-1} (OH), 1697 cm^{-1} (SC=O), 1649 cm^{-1} (NHC=O); $[\alpha]_D$ +0.1 (*c*, 0.5 in water); δ_H (400 MHz, D_2O) 1.97 (3H, s, NHC(O)C$\underline{H}_3$), 2.05 (3H, s, NHC(O)C$\underline{H}_3$), 2.39 (3H, s, SC(O)C$\underline{H}_3$), 3.42 - 3.84, 3.86 - 3.96 (12H, 2 x m), 4.57 (1H, d, *J* 8.6 Hz, H-1_b), 5.18 (1H, d, *J* 11.0 Hz, H-1_a); δ_C (100.5 MHz, D_2O) 21.9 (q, NHC(O)$\underline{C}H_3$), 22.0 (q, NHC(O)$\underline{C}H_3$), 30.1 (q, SC(O)$\underline{C}H_3$), 52.8, 55.5 (2 x d), 59.9 , 60.5 (2 x t, 2 x C-6), 69.6, 73.4, 73.5, 75.8, 78.6, 78.9 (6 x d), 80.8 (d, C-1_a), 101.3 (d, C-1_b), 174.5 (s, 2 x NH$\underline{C}$(O)$\underline{C}H_3$), 197.4 (s, S$\underline{C}$(O)CH_3); HRMS (ESI-TOF): calcd. for $C_{18}H_{31}N_2O_{11}S^+$: 483.1643. Found: 483.1655 (MH^+).

1-Thio-*N,N'*-diacetylchitobiose 16b



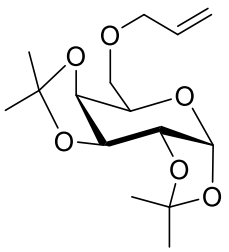
1-Thioacetyl-*N,N'*-diacetylchitobiose **16a** (2.2 mg, 4.6 μmol) was dissolved in aqueous NaOH (1 M, 1.5 mL) and stirred for 1 h. Amberlite® IR-120 (H^+) was then added until pH 7 was reached. The solution was then filtered and lyophilised to afford 1-thio-*N,N'*-diacetylchitobiose **16b** (2.0 mg, quant.) as a white powder; $[\alpha]_D^{20}$ -5 (*c*, 0.28 in water); δ_H (400 MHz, D_2O)[7] 2.02 (3H, s, NHC(O)C$\underline{H}_3$), 2.04 (3H, s, NHC(O)C$\underline{H}_3$), 3.42 - 3.57, 3.58 - 3.78 (10H, 2 x m), 3.81 (1H, dd, $J_{5,6}$ 1.2 Hz, $J_{6,6'}$ 12.1 Hz, H-6_a'), 3.89 (1H, dd, $J_{5,6'}$ 12.3 Hz, $J_{6,6'}$ 1.6 Hz, H-6_b'), 4.56 (1H, d, $J_{1b,2b}$ 8.2 Hz, H-1_b), 4.65 (1H, d, $J_{1a,2a}$ 9.8 Hz, H-1_a); δ_C (100.5 MHz, D_2O) 22.0 (q, NHC(O)$\underline{C}H_3$), 22.1 (q, NHC(O)$\underline{C}H_3$), 55.5, 57.2 (2 x d), 60.1 (t, C-6), 60.5 (t, C-6), 69.6, 73.4, 73.5, 75.8, 78.6 (5 x d), 78.8 (d, H-1_a), 79.0 (d), 101.3 (d, H-1_b), 174.5 (s, 2 x q, NH$\underline{C}$(O)CH_3); HRMS (ESI-TOF): calcd. for $C_{16}H_{29}N_2O_{10}S^+$: 441.1537. Found: 441.1544 (MH^+).

4-Hydroxybutyl 1-thio-2-deoxy-2-acetamido-β-D-glucopyranoside 17b



1-Thio-2-deoxy-2-acetamido-β-D-glucopyranoside **11b** (200 mg, 0.84 mmol) and 3-buten-1-ol **17a** 65 μL, 0.76 mmol) were dissolved in water (7 mL). 2,2-Dimethoxy-2-phenyl acetophenone (19.6 mg, 0.076 mmol) was added, and the reaction was stirred and irradiated in a Rayonet photoreactor (wavelength: 254 nm) for 2 h. The reaction was then concentrated *in vacuo* and the residue purified by flash column chromatography ($CHCl_3$:MeOH 5:1) to afford 4-hydroxybutyl 1-thio-2-deoxy-2-acetamido-β-D-glucopyranoside **17b** (142.4 mg, 61%) as a colourless solid; $[\alpha]_D^{20}$ -29 (*c*, 1.0 in MeOH); υ_{max} (neat) 1620 cm^{-1} (C=O); δ_H (400 MHz, D_2O) 1.43 - 1.58 (4H, m, 2 x C$\underline{H}_2$), 1.90 (3H, s, C(O)C$\underline{H}_3$) 2.51 - 2.71 (2H, m, C$\underline{H}_2$S), 3.29-3.34 (2H, m, H-4 & H-5), 3.36 - 3.44 (1H, m, H-3), 3.47 (2H, t, *J* 6.1 Hz, C$\underline{H}_2$OH), 3.55 - 3.65 (2H, m, H-2 & H-6), 3.77 (1H, d, $J_{6,6'}$ 12.5 Hz, H-6'), 4.47 (1H, d, $J_{1,2}$ 10.2 Hz, H-1); δ_C (100.5 MHz, D_2O) 22.1 (t, C(O)$\underline{C}H_3$), 25.5 (t, $\underline{C}H_2CH_2OH$), 29.9 (t, $\underline{C}H_2S$), 30.3 (t, $SCH_2\underline{C}H_2$), 54.7 (d, C-2), 60.8 (t, C-6), 61.1 (t, $\underline{C}H_2OH$), 69.7 (d, C-4), 75.1 (d, C-3), 79.8 (d, C-5), 84.2 (d, C-1), 174.3 (s, $\underline{C}(O)CH_3$); HRMS (ESI-TOF): calcd. for $C_{12}H_{23}NO_6SNa^+$: 332.1138. Found: 332.1146 (MNa^+).

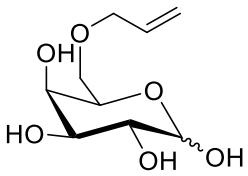
6-*O*-Allyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside[8]



1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranoside (1.185 g, 4.55 mmol) and allyl bromide (0.86 mL, 10 mmol) were dissolved in dry MeCN (5 mL) under an atmosphere of nitrogen. Potassium hydroxide (0.56 g, 10 mmol) was suspended in the reaction mixture and the reaction was stirred for 21 h, at which point t.l.c. (petrol:ethyl acetate 3:1) indicated complete

consumption of starting material (R_f 0.1) and formation of a single product (R_f 0.6). The reaction was concentrated *in vacuo*, the residue dissolved in CH_2Cl_2 (15 mL), and washed with water (15 mL). The aqueous phase was washed with CH_2Cl_2 (3 x 15 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The product was purified by flash column chromatography (petrol:ethyl acetate 3:1) to afford 6-*O*-allyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside (1.28 g, 94%) as a colourless oil; $[\alpha]_D^{20}$ -69 (*c*, 1.0 in $CHCl_3$) [lit. $[\alpha]_D$ -71.8 (*c*, 1.0 in $CHCl_3$)][9]; δ_H (400 MHz, $CDCl_3$)[10] 1.31 (3H, s, C$\underline{H}_3$), 1.32 (3H, s, C$\underline{H}_3$), 1.43 (3H, s, C$\underline{H}_3$), 1.52 (3H, s, C$\underline{H}_3$), 3.53 - 3.67 (2H, m, C$\underline{H}_2$CH=CH$_2$), 3.93 - 3.99 (1H, m, H-5), 4.00 - 4.05 (2H, m, H-6 & H-6'), 4.25 (1H, dd, $J_{3,4}$ 8.0, $J_{4,5}$ 1.8 Hz, H-4), 4.29 (1H, dd, $J_{1,2}$ 4.9 Hz, $J_{2,3}$ 2.5 Hz, H-2), 4.58 (1H, dd, $J_{2,3}$ 2.3 Hz, $J_{3,4}$ 7.8 Hz, H-3), 5.13 - 5.18 (1H, m, CH$_2$CH=C$\underline{H}_a$H$_b$), 5.26 (1H, dq, *J* 17.2 Hz, *J* 1.6 Hz, CH$_2$CH=CH$_a\underline{H}_b$), 5.52 (1H, d, $J_{1,2}$ 4.7 Hz, H-1), 5.90 (1H, ddt, *J* 17.2 Hz, *J* 10.4 Hz, *J* 5.8 Hz, CH$_2$C$\underline{H}$=CH$_2$); δ_C (100.5 MHz, $CDCl_3$) 24.4 (q, $\underline{C}H_3$), 24.9 (q, $\underline{C}H_3$), 25.9 (q, $\underline{C}H_3$), 26.0 (q, $\underline{C}H_3$), 66.8 (d, C-5), 68.8 (t, $\underline{C}H_2$CH=CH$_2$), 70.5 (d, C-2), 70.6 (d, C-3), 71.2 (d, C-4), 72.3 (t, C-6), 96.3 (d, C-1), 108.5 (s, $\underline{C}(CH_3)_2$), 109.2 (s, $\underline{C}(CH_3)_2$), 117.0 (t, CH$_2$CH=$\underline{C}$H$_2$), 134.8 (d, CH$_2\underline{C}$H=CH$_2$); HRMS (ESI-TOF): calcd. for $C_{15}H_{24}O_6Na^+$: 323.1465. Found: 323.1469 (MNa^+).

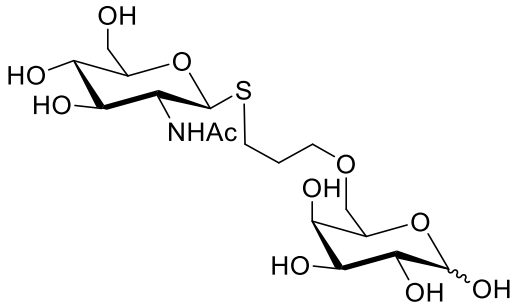
6-*O*-Allyl-D-galactopyranoside 18a[6]



6-*O*-Allyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside (1.12 g, 3.73 mmol) was dissolved in water (50 mL). Amberlite® IR-120 (H^+, 4.0 g) was added, and the reaction was heated to 80 °C and stirred for 16 h. The reaction was then cooled to RT and lyophilised to afford 6-*O*-allyl-D-galactopyranoside **18a** (0.84 g, quant.) as a colourless oil (1:1.3 α:β); δ_H (400 MHz, D_2O) 3.46 (1H, dd, $J_{1,2}$ 7.8 Hz, $J_{3,4}$ 9.8 Hz, H-2β), 3.62 (1H, dd, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 3.5 Hz, H-3β), 3.64 - 3.67 (3.5H, m, H-6α, H-6'α, H-6β & H-6'β), 3.75 - 3.85 (2.5H, m, H-2α, H-3α & H-5β), 3.88 (1H, d, $J_{3,4}$ 3.5 Hz, H-4β), 3.94 (0.8H, d, $J_{3,4}$ 3.1 Hz, H-4α), 4.03 - 4.09 (3.6H, m, 2 x C$\underline{H}_2$CH=CH$_2$), 4.19 (0.8H, t, *J* 6.3 Hz, H-5α), 4.55 (1H, d, $J_{1,2}$ 7.8 Hz, H-1β), 5.23 (0.8H, d, $J_{1,2}$ 3.9 Hz, H-1α), 5.26 (1.8H, d, *J* 10.6 Hz, 2 x CH$_2$CH=C$\underline{H}_a$H$_b$), 5.32

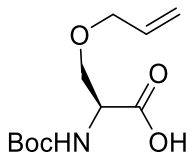
(1.8H, d, *J* 17.2 Hz, 2 x $CH_2CH=CH_a\underline{H}_b$), 5.86 - 6.01 (1.8H, m, 2 x $CH_2\underline{C}H=CH_2$); δ_C (100.5 MHz, D_2O) 68.2 (d, C-2α), 68.6 (d, C-3α), 68.9 (d, C-5α), 69.0 (d, C-4β), 69.2, 69.4 (2 x t, C-6α & C-6β), 69.5 (d, C-4α), 71.7 (d, C-2β), 71.9, 72.0 (2 x t, $\underline{C}H_2CH=CH_2\alpha$ & $\underline{C}H_2CH=CH_2\beta$), 72.6 (d, C-3β), 73.3 (d, C-5β), 92.2 (d, C-1α), 96.3 (d, C-1β), 118.5 (t, $CH_2CH=\underline{C}H_2\alpha$ & $CH_2CH=\underline{C}H_2\beta$), 133.5, 133.5 (2 x d, $CH_2\underline{C}H=CH_2\alpha$ & $CH_2\underline{C}H=CH_2\beta$); HRMS (ESI-TOF): calcd. for $C_9H_{16}O_6Na^+$: 243.0839. Found: 243.0840 (MNa^+).

6-*O*-(2-[1-Thio-2-deoxy-2-acetamido-β-D-glucopyranoside]-ethyl)-D-galactopyranoside 18b



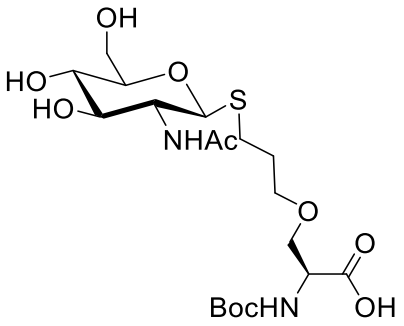
1-Thio-2-deoxy-2-acetamido-β-D-glucopyranoside **11b** (104 mg, 0.44 mmol) and 6-*O*-allyl-D-galactopyranoside **18a** (88 mg, 0.4 mmol) were dissolved in water (4 mL). 2,2-Dimethoxy-2-phenyl acetophenone (10.3 mg, 0.04 mmol) was added, and the reaction was stirred and irradiated in a Rayonet photoreactor (wavelength: 254 nm) for 2 h. The reaction was then concentrated *in vacuo* and the residue purified by flash column chromatography ($CHCl_3$:MeOH 5:1) to afford 6-*O*-(2-[1-thio-2-deoxy-2-acetamido-β-D-glucopyranoside]-ethyl)-D-galactopyranoside **18b** (133.1 mg, 73 %) as a colourless solid (α:β, 1:1.7); υ_{max} (neat) 3265 cm^{-1} (br. s, OH), 1637 cm^{-1} (C=O); δ_H (400 MHz, D_2O) 1.82 - 1.95 (5.4H, m, 2 x $SCH_2\underline{C}H_2CH_2O$), 2.02 (8.1H, s, 2 x $C(O)C\underline{H}_3$), 2.62 - 2.86 (5.4H, m, 2 x $SC\underline{H}_2CH_2CH_2O$), 3.41 - 3.96 (20.4H, m, H-$2_1\alpha$, H-$3_1\alpha$, H-$4_1\alpha$, H-$6_1\alpha$, H-$6'_1\alpha$, H-$2_1\beta$, H-$3_1\beta$, H-$4_1\beta$, H-$5_1\beta$, H-$6_1\beta$, H-$6'_1\beta$, H-2_2, H-3_2, H-4_2, H-5_2, H-6_2, H-$6'_2$ & 2 x $SCH_2CH_2C\underline{H}_2O$), 4.19 (1H, t, *J* 5.5 Hz, H-$5_1\alpha$), 4.55 (1H, d, $J_{1,2}$ 7.8 Hz, H-$1_1\beta$), 4.60 (1.6H, d, $J_{1,2}$ 10.6 Hz, 2 x H-1_2), 5.23 (0.6H, d, $J_{1,2}$ 3.5 Hz, H-$1_1\alpha$); δ_C (100.5 MHz, D_2O) 22.1 (q, $C(O)\underline{C}H_3$), 26.9 (t, $S\underline{C}H_2CH_2CH_2O$), 28.9 (t, $SCH_2\underline{C}H_2CH_2O$), 54.7 (d, C-$2_2$), 60.8 (t, C-$6_2$), 68.2, 68.5, 68.9, 69.0, 69.4, 69.5, 69.6, 69.7, 69.8, 71.7, 72.7, 73.2, 75.1, 79.8 (d, C-5_2), 84.3, 84.4 (2 x d, 2 x C-1_2), 92.2 (d, C-$1_1\alpha$), 96.3 (d, C-$1_1\beta$), 174.3 (s, $\underline{C}(O)CH_3$); HRMS (ESI-TOF): calcd. for $C_{17}H_{32}NO_{11}S^+$: 458.1691. Found: 458.1689 (MH^+).

***N-tert*-Butoxycarbonyl-*O*-allyl-L-serine 19a**



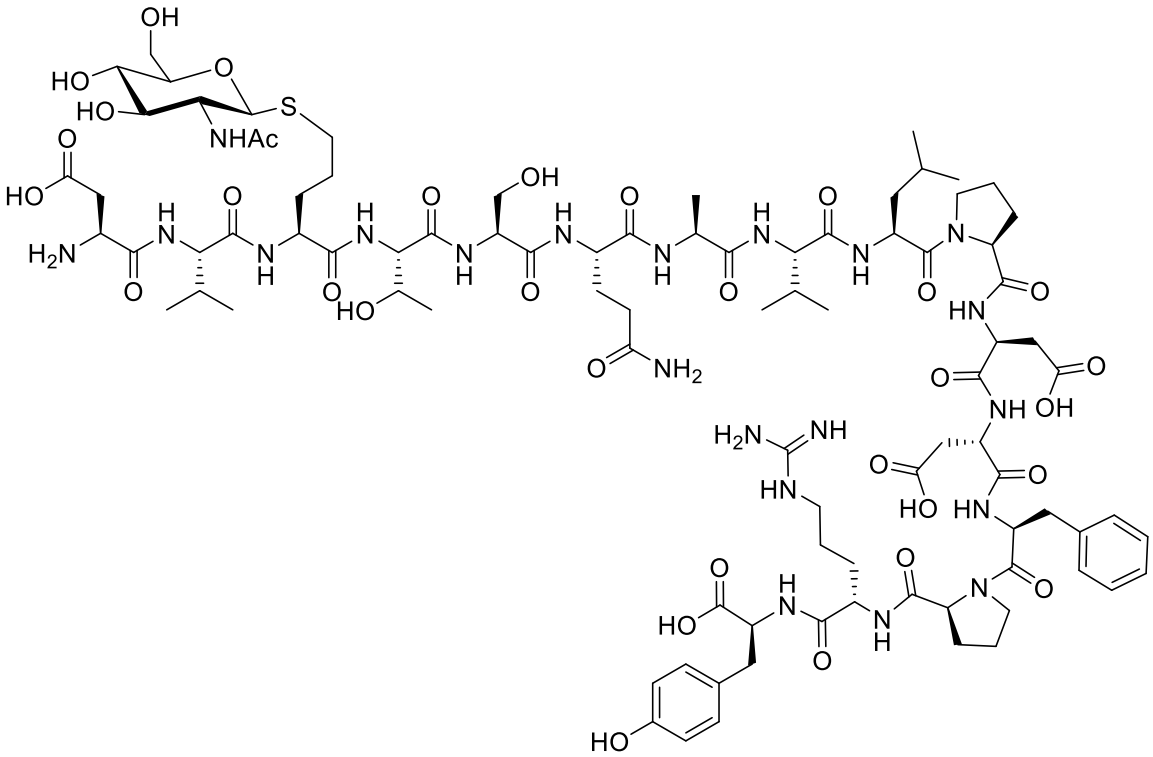
L-Serine (500 mg, 4.76 mmol) was dissolved in a mixture of 1,4-dioxane (12 mL) and aqueous NaOH (1 M, 5.7 mL) at 0 °C. Di-*tert*-butyl dicarbonate (1.25 g, 5.71 mmol) was added portionwise to the reaction, and the reaction was then warmed to RT and stirred for 24 h. The reaction was concentrated *in vacuo* to remove the 1,4-dioxane and the aqueous layer was washed with Et_2O (10 mL), acidified to pH 2-3 with H_2SO_4 (1 M, 10 mL), and extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was dissolved in anhydrous DMF (30 mL) and NaH (60% dispersion in mineral oil, 460 mg, 11.4 mmol) was added at 0 °C under an atmosphere of nitrogen. Allyl bromide (0.49 mL, 5.71 mmol) was added dropwise to the mixture and the reaction was stirred at RT for 5 h. The reaction was concentrated *in vacuo* and the residue was partitioned between ethyl acetate (50 mL) and water (50 mL). The aqueous layer was acidified with HCl (1 M, 20 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with water (50 mL), followed by brine (50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford *N-tert*-butoxycarbonyl-*O*-allyl-L-serine **19a** (0.982 g, 84%) as a colourless oil; $[\alpha]_D^{20}$ +16 (*c*, 1.0 in $CHCl_3$) [lit. $[\alpha]_D$ +9.2 (*c*, 1.1 in CH_2Cl_2)][11]; υ_{max} (neat) 1712 cm^{-1} (C=O); δ_H (400 MHz, $CDCl_3$)[9] 1.44 (9H, s, 3 x C$\underline{H}_3$), 3.67 (1H, dd, *J* 9.4 Hz, *J* 3.1 Hz, CHC$\underline{H}$H'), 3.89 (1H, d, *J* 8.2 Hz, CHCH$\underline{H}$'), 4.00 (2H, d, *J* 5.5 Hz, OC$\underline{H}_2$CH=CH_2), 4.44 (1H, br. s., C$\underline{H}$$CH_2$), 5.18 (1H, d, *J* 10.6 Hz, $OCH_2CH=C\underline{H}_ZH_E$), 5.25 (1H, d, *J* 17.2 Hz, $OCH_2CH=CH_Z\underline{H}_E$), 5.41 (1H, d, *J* 7.4 Hz, N$\underline{H}$), 5.76 - 5.93 (1H, m, $OCH_2C\underline{H}=CH_2$); δ_C (100.5 MHz, $CDCl_3$)[9] 28.3 (q, 3 x $\underline{C}H_3$), 53.8 (d, $\underline{C}HCH_2$), 69.6 (t, $CH\underline{C}H_2$), 72.4 (t, $O\underline{C}H_2CH=CH_2$), 80.3 (s, $\underline{C}(CH_3)_3$), 117.7 (t, $CH_2CH=\underline{C}H_2$), 133.9 (d, $CH_2\underline{C}H=CH_2$), 155.7 (s, O$\underline{C}$(O)NH), 175.1 (s, $\underline{C}$(O)OH); HRMS (ESI-TOF): calcd. for $C_{11}H_{19}NO_5Na^+$: 268.1155. Found: 268.1160 (MNa^+).

***N-tert*-Butoxycarbonyl-*O*-(3-[1-thio-2-deoxy-2-acetamido-β-D-glucopyranoside]-propyl)-L-serine 19b**



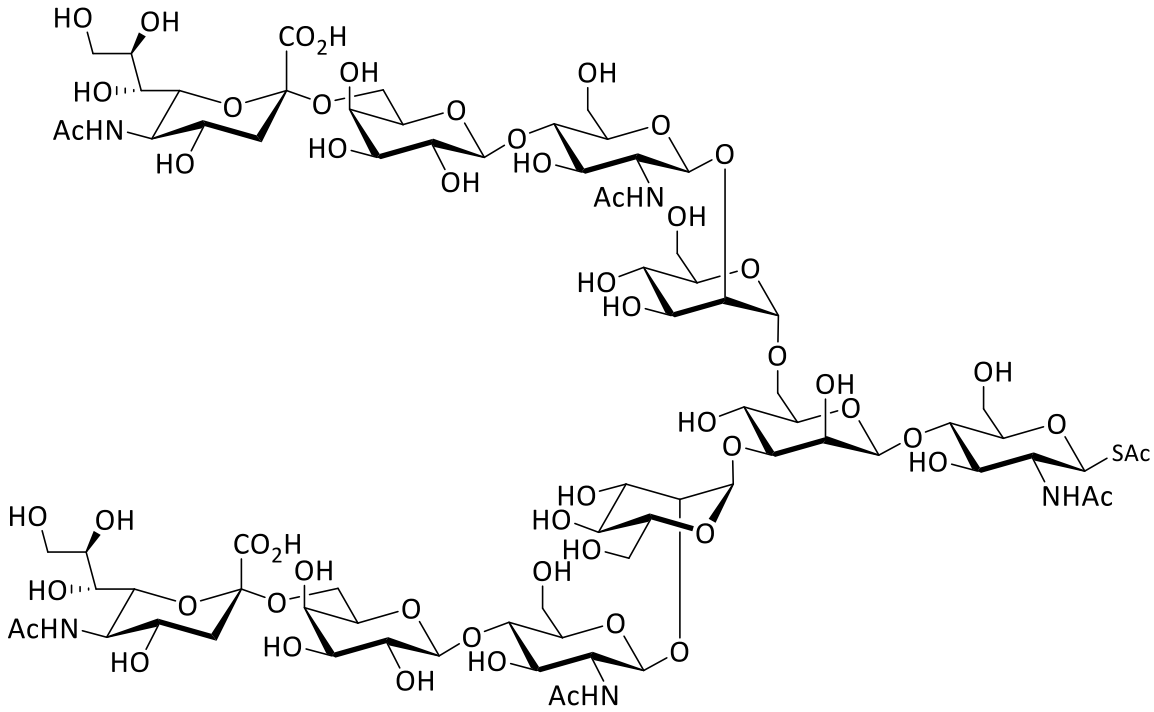
1-Thio-2-deoxy-2-acetamido-β-D-glucopyranoside **11b** (52 mg, 0.22 mmol) and *N-tert*-butoxycarbonyl-*O*-allyl-L-serine **19a** (49 mg, 0.2 mmol) were dissolved in a 1:1 mixture of water and MeOH (2 mL). 2,2-Dimethoxy-2-phenyl acetophenone (5.1 mg, 0.02 mmol) was added, and the reaction was stirred and irradiated in a Rayonet photoreactor (wavelength: 254 nm) for 2 h. The reaction was then concentrated *in vacuo* and the residue purified by flash column chromatography ($CHCl_3$:MeOH 5:1) to afford *N-tert*-butoxycarbonyl-*O*-(2-[1-thio-2-deoxy-2-acetamido-β-D-glucopyranoside]-ethyl)-L-serine **19b** (88 mg, 91%) as a colourless oil; $[\alpha]_D^{20}$ 0 (*c*, 1.0 in $CHCl_3$); υ_{max} (neat) 3306 cm^{-1} (OH), 1737 cm^{-1} (HOC=O), 1648 cm^{-1} (NHC=O); δ_H (400 MHz, CD_3OD) 1.44 (9H, s, $C(C\underline{H}_3)_3$), 1.78 - 1.89 (2H, m, $SCH_2C\underline{H}_2CH_2O$), 1.98 (3H, s, $NHC(O)C\underline{H}_3$), 2.64 - 2.84 (2H, m, $SC\underline{H}_2CH_2CH_2O$), 3.26-3.37 (2H, m, H-4 & H-5), 3.45 (1H, t, *J* 8.4 Hz, H-3), 3.50 - 3.59 (2H, m, $SCH_2CH_2C\underline{H}_2O$), 3.63 - 3.81 (4H, m, H-2, H-6 & $CHC\underline{H}_2$), 3.87 (1H, d, $J_{6,6'}$ 12.1 Hz, H-6'), 4.21 (1H, br. s, $C\underline{H}CH_2$) 4.48 (1H, d, *J* 10.2 Hz, H-1); δ_C (100.5 MHz, CD_3OD) 21.6 (q, $NHC(O)\underline{C}H_3$), 26.3 (t, $S\underline{C}H_2CH_2CH_2O$), 27.3 (q, $C(\underline{C}H_3)_3$), 29.4 (t, $SCH_2\underline{C}H_2CH_2O$), 54.5 (d, $\underline{C}HCH_2$), 54.8 (d, C-2), 61.5 (t, C-6), 69.3 (t, $SCH_2CH_2\underline{C}H_2O$), 70.4 (t, $CH\underline{C}H_2$), 70.6 (d, C-4), 76.0 (d, C-3), 80.6 (d, C-5), 84.4 (d, C-1); HRMS (ESI-TOF): calcd. for $C_{19}H_{35}N_2O_{10}S^+$: 483.2007. Found: 483.2012 (MH^+).

Neoglycopeptide 20b



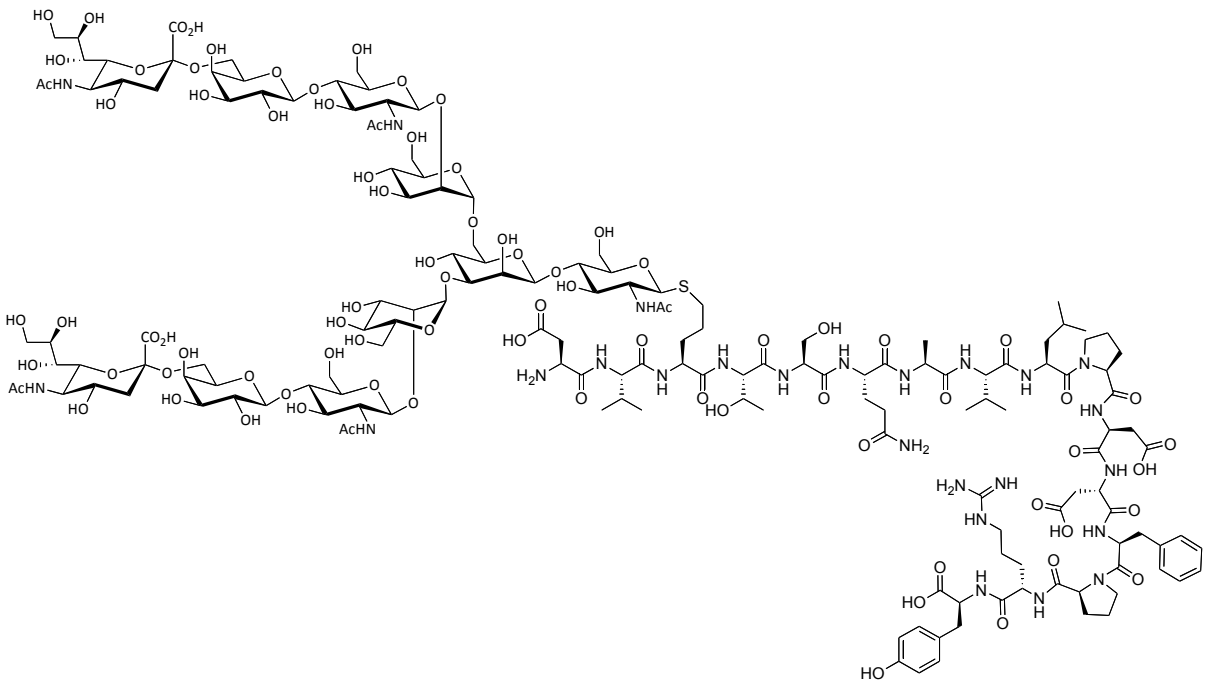
Peptide **20a** (1.0 mg, 0.55 μmol) and 1-thio-2-acetamido-2-deoxy-β-D-glucopyranose **11b** (6.5 mg, 27 μmol) were dissolved in DMF (100 μL). 2,2-Dimethoxy-2-phenyl acetophenone (0.7 mg, 2.8 μmol) was added, and the reaction was stirred and irradiated in a Rayonet photoreactor (wavelength: 254 nm) for 2 h. The reaction was analysed by LCMS (column: Jupiter 5u C18 (300 Å) column (Phenomenex); gradient: 5% MeCN in water for 15 min, followed by an increase to 75% MeCN over 30 min; column oven: 40 °C; flow rate: 0.5 mL/min; detection: UV 210 nm) to reveal the presence of neoglycopeptide **20b** (>95%); t_R = 33.2 min; HRMS (ESI-TOF): calcd. for $C_{90}H_{138}N_{21}O_{32}S^+$: 2056.9532. Found: 2056.9590 (MH^+).

Sialoglycan thioacetate 22a



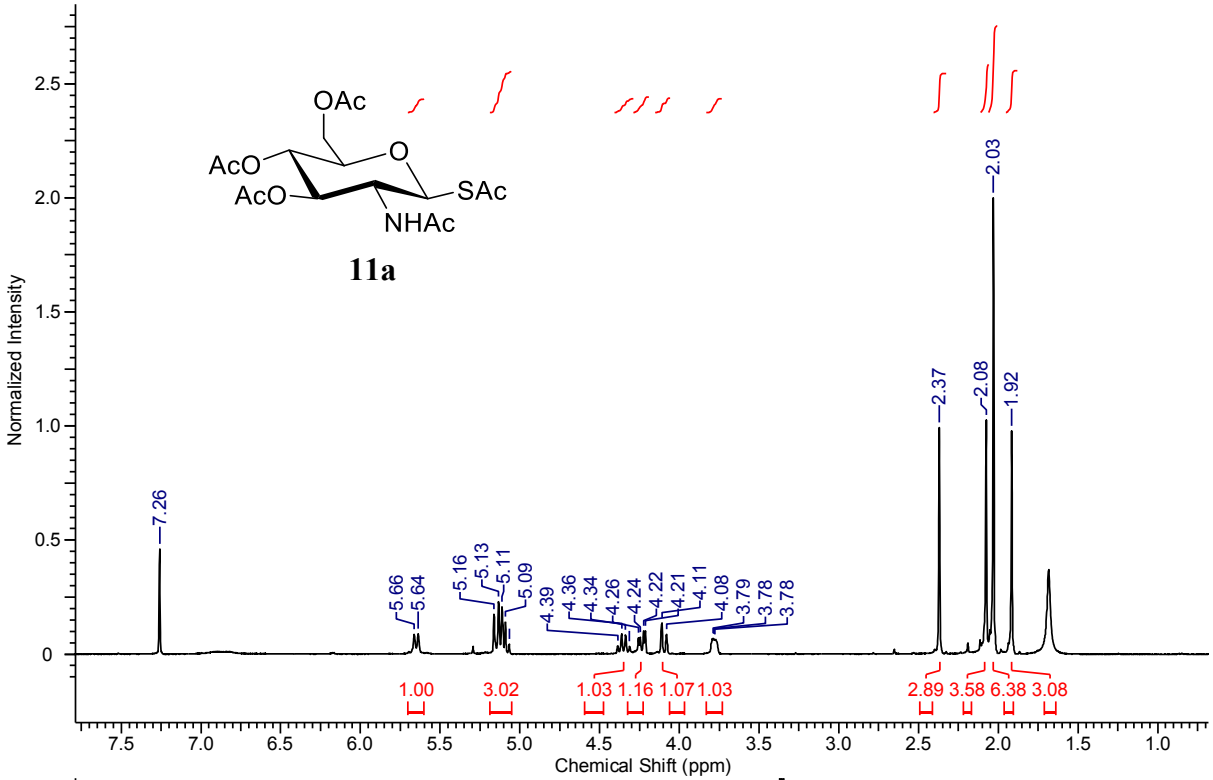
Sialoglycan **21** (5.1 mg, 2.5 µmol) was dissolved in D_2O (100 µL). Triethylamine (35.0 µL, 250 µmol) was added and the reaction was cooled to 0 °C and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride **1** (12.6 mg, 75 µmol) was dissolved in D_2O (50 µL) and the resulting solution was added dropwise. The reaction was stirred at 0 °C for 2 h. Thioacetic acid (26.4 µL, 375 µmol) was dissolved in MeCN (75 µL) and the resulting solution was added dropwise to the reaction mixture. The reaction was stirred for 1 h and then purified by HPLC (t_R = 14.8 min; column: XBridge® Glycan BEH Amide 3.5µm (130 Å) column; gradient: 10% water in MeCN for 7 min, followed by an increase to 40% water over 5 min, followed by 40% water in MeCN for 5 min; column oven: 40 °C; flow rate: 1 mL/min; detection: UV 210 nm) to afford sialoglycan thioacetate **22a** (3.1 mg, 60%) as a white powder; δ_H (400 MHz, D_2O) 2.35 (3H, s, SC(O)$\underline{H}_3$), 5.17 (1H, d, $J_{1,2}$ 11.0 Hz, H-1_a); δ_C (100.5 MHz, D_2O) 30.0 (q, SC(O)$\underline{C}H_3$), 80.8 (d, C-1_a); HRMS (ESI-TOF): calcd. for $C_{78}H_{128}N_5O_{57}S^+$: 2078.6986. Found: 2078.7007 (MH^+).

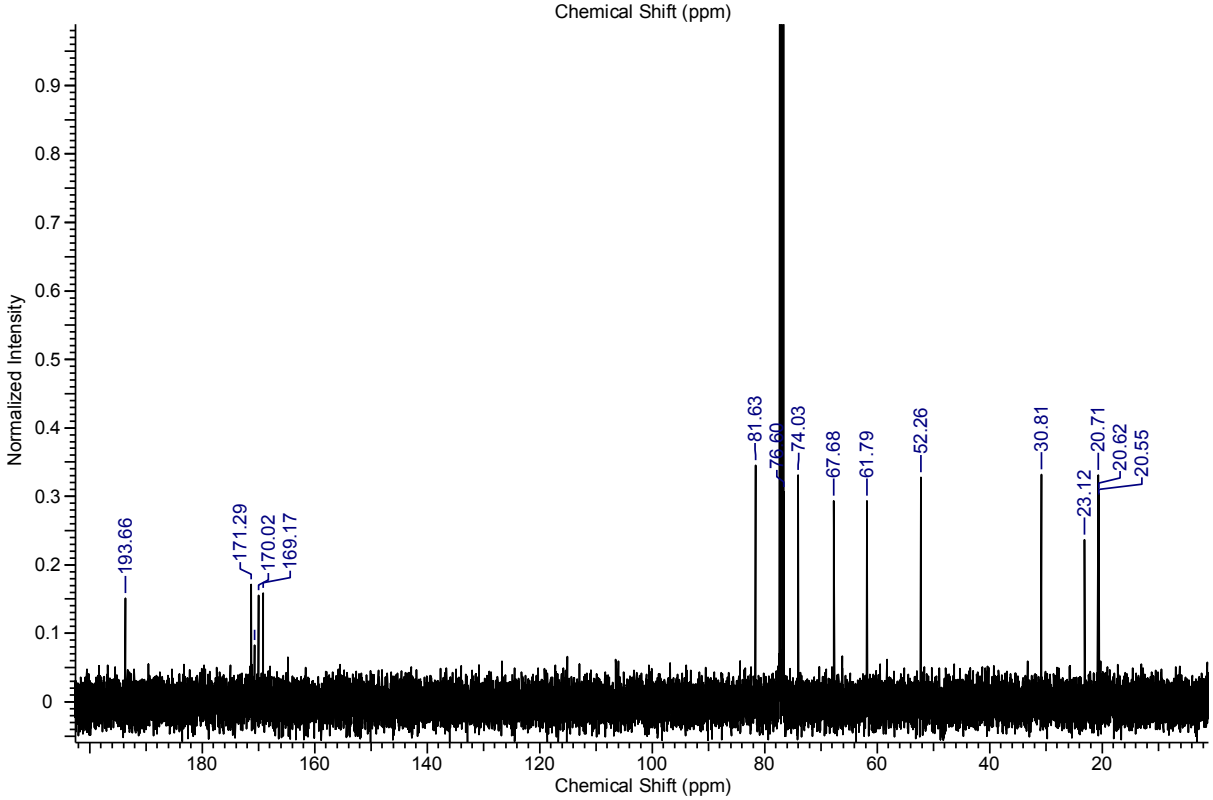
Sialoglycan neoglycopeptide 23

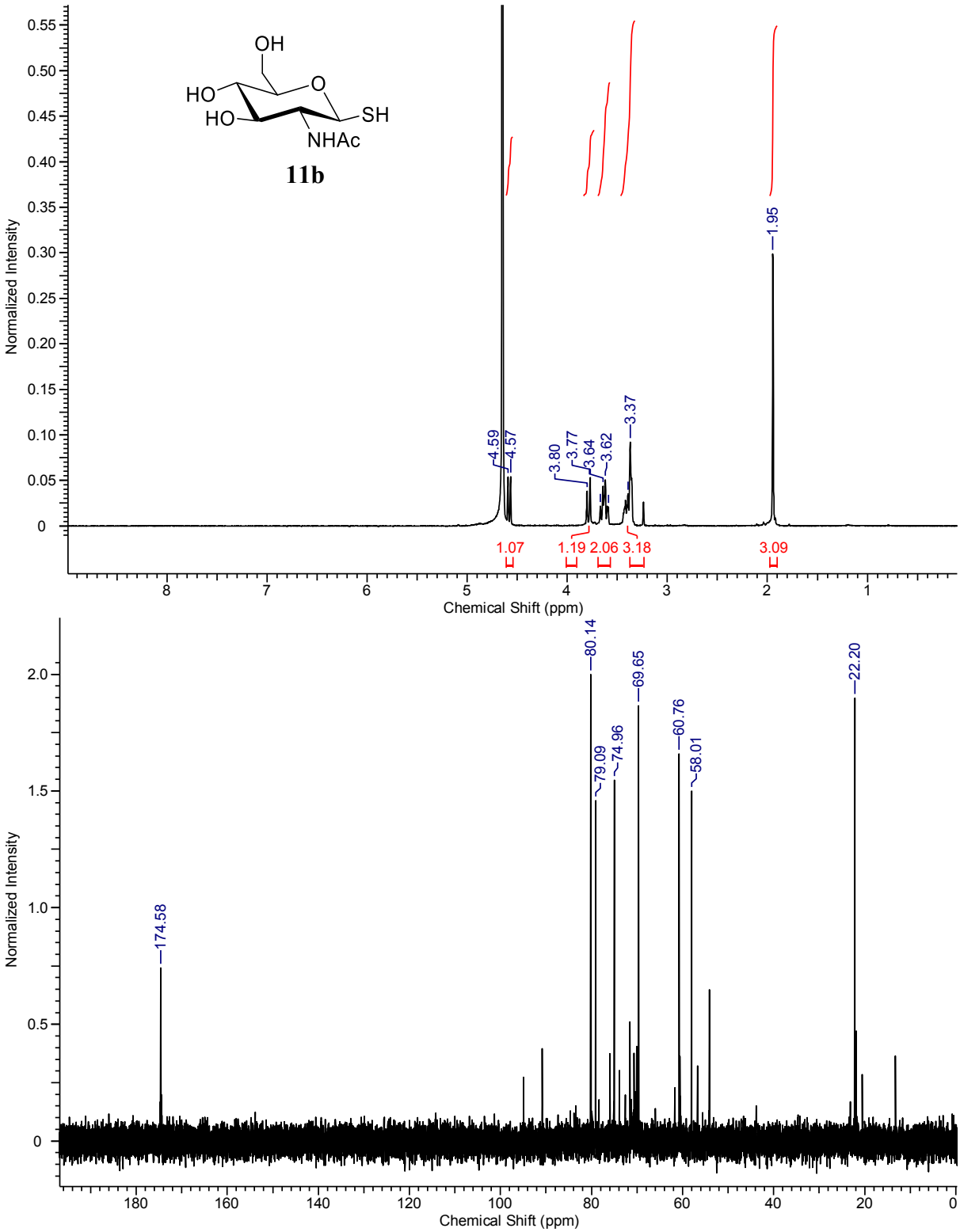


Sialoglycan thioacetate **22a** (1.5 mg, 0.75 μmol) was dissolved in aqueous NaOH (1 M, 5 mL) and stirred for 2 h. Amberlite® IR-120 (H^+) was added until pH 7 was reached, and the reaction was then filtered and concentrated *in vacuo*. The resulting colourless solid **22b** was dissolved in water (40 μL). Peptide **20a** (0.5 mg, 0.28 μmol) was dissolved in DMF (100 μL), and 10 μL of the resulting solution was dissolved in water (150 μL). 5 μL of the diluted peptide solution was added to the sialoglycan solution. 2,2-Dimethoxy-2-phenyl acetophenone (1 mg, 3.9 μmol) was dissolved in DMF (200 μL), and 1 μL of this solution was added to the reaction mixture. The reaction was stirred and irradiated in a Rayonet photoreactor (wavelength: 254 nm) for 4 h, and then analysed by LCMS (column: Jupiter 5u C18 (300 Å) column (Phenomenex); gradient: 5% MeCN in water for 15 min, followed by an increase to 75% MeCN over 30 min; column oven: 40 °C; flow rate: 0.5 mL/min; detection: UV 235 nm) to reveal the presence of sialoglycan neoglycopeptide **23** (>95%); t_R = 32.1 min; HRMS (ESI-TOF): calcd. for $C_{158}H_{248}N_{25}O_{83}S^+$: 385.5669. Found: 385.5333 (MH^+).

3. Spectra of compounds






OH
HO
O
HO
SH
NHAc
11b
Normalized Intensity
Chemical Shift (ppm)
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4.57
3.80
3.77
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3.62
3.37
1.95
1.07
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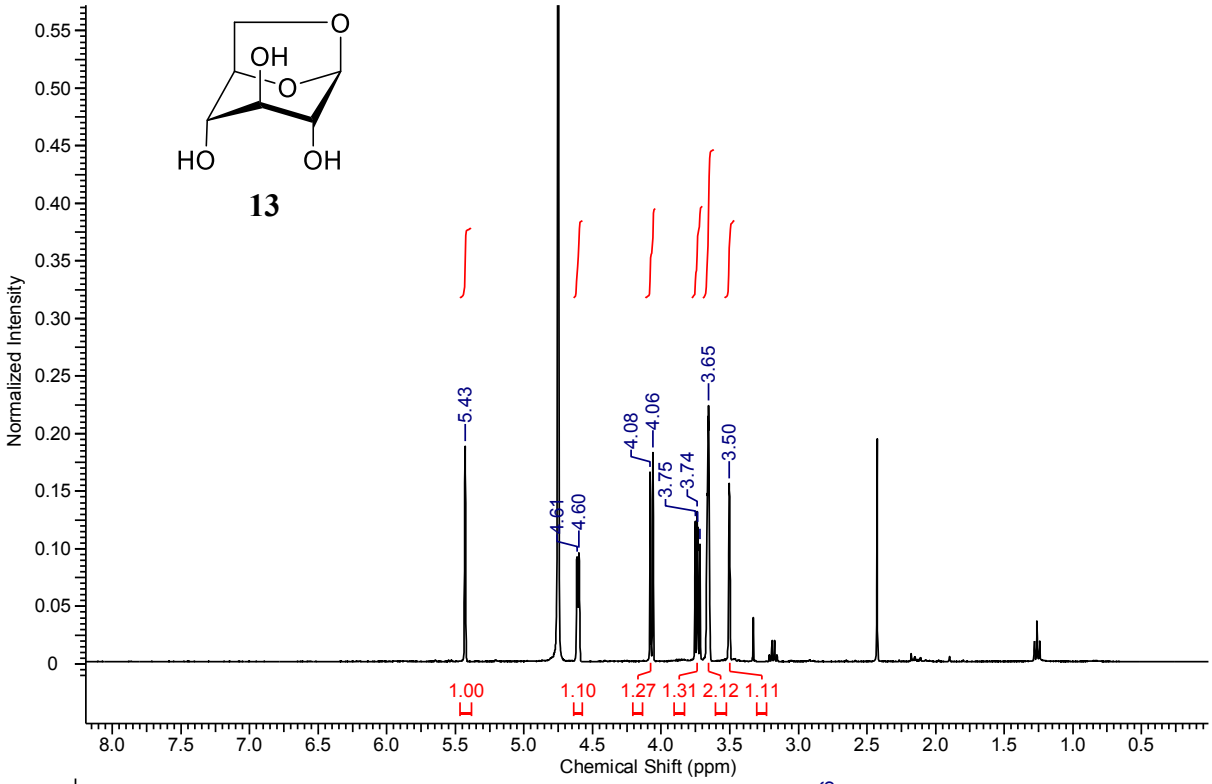
79.09
74.96
69.65
60.76
58.01
22.20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O
HO
OH
O
13
Normalized Intensity
Chemical Shift (ppm)
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3.50
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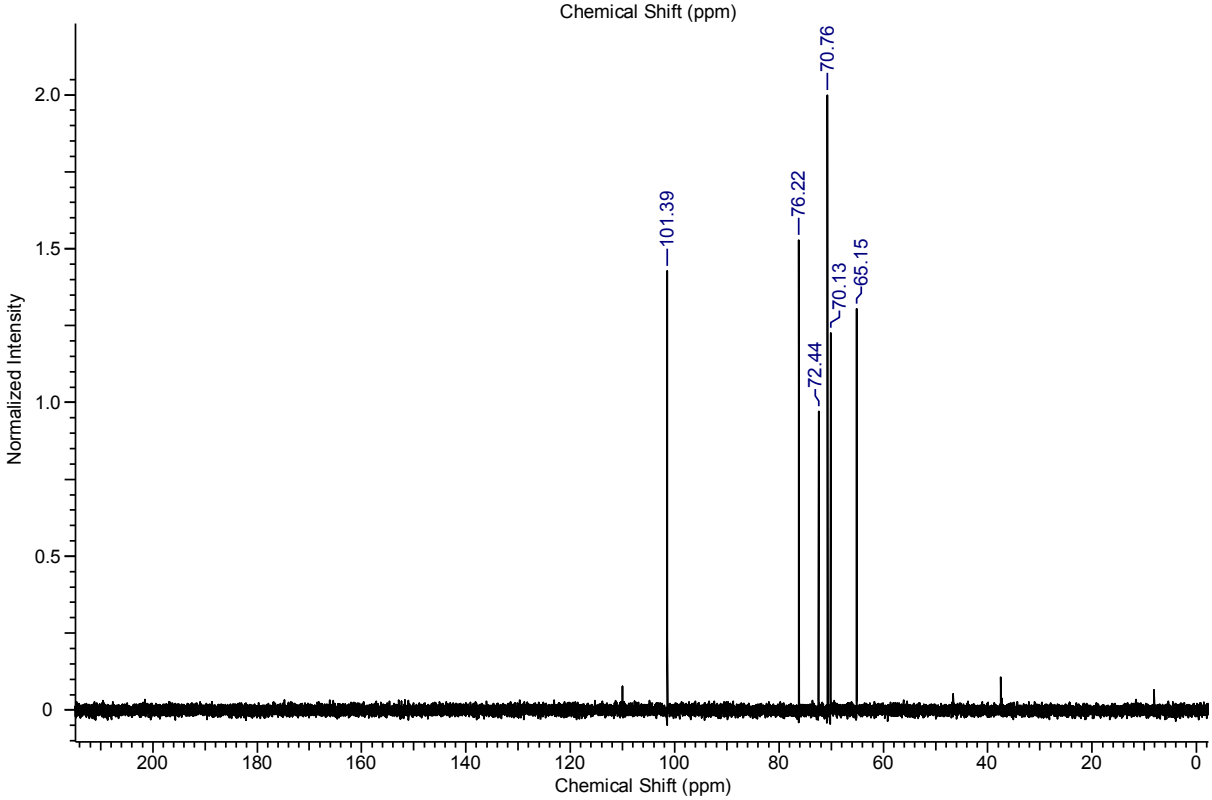
1.10
1.27
1.31
2.12
1.11


Normalized Intensity
Chemical Shift (ppm)
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70.76
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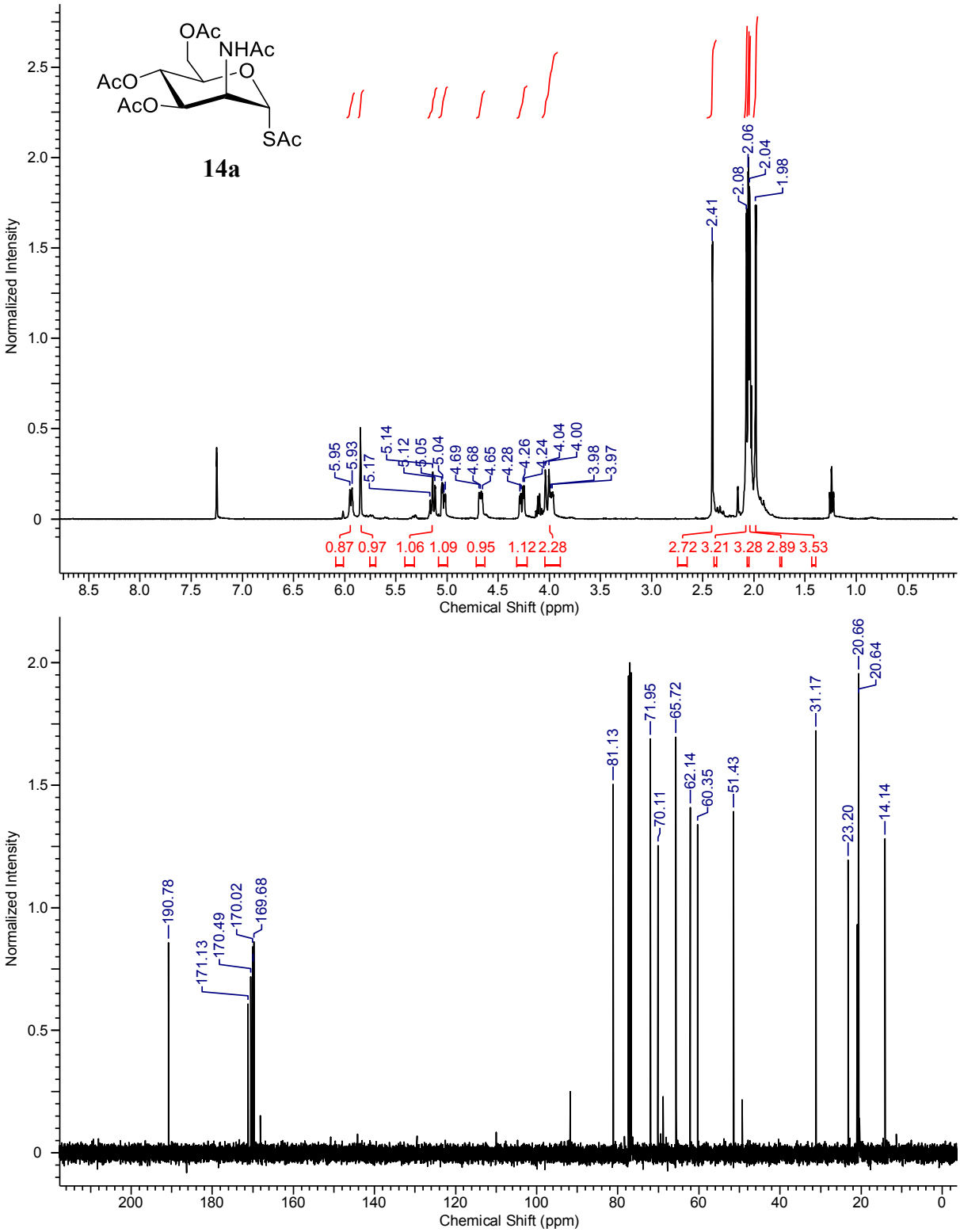
65.15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NHAc
AcO
O
AcO
SAc
14a
Normalized Intensity
Chemical Shift (ppm)
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5.93
5.17
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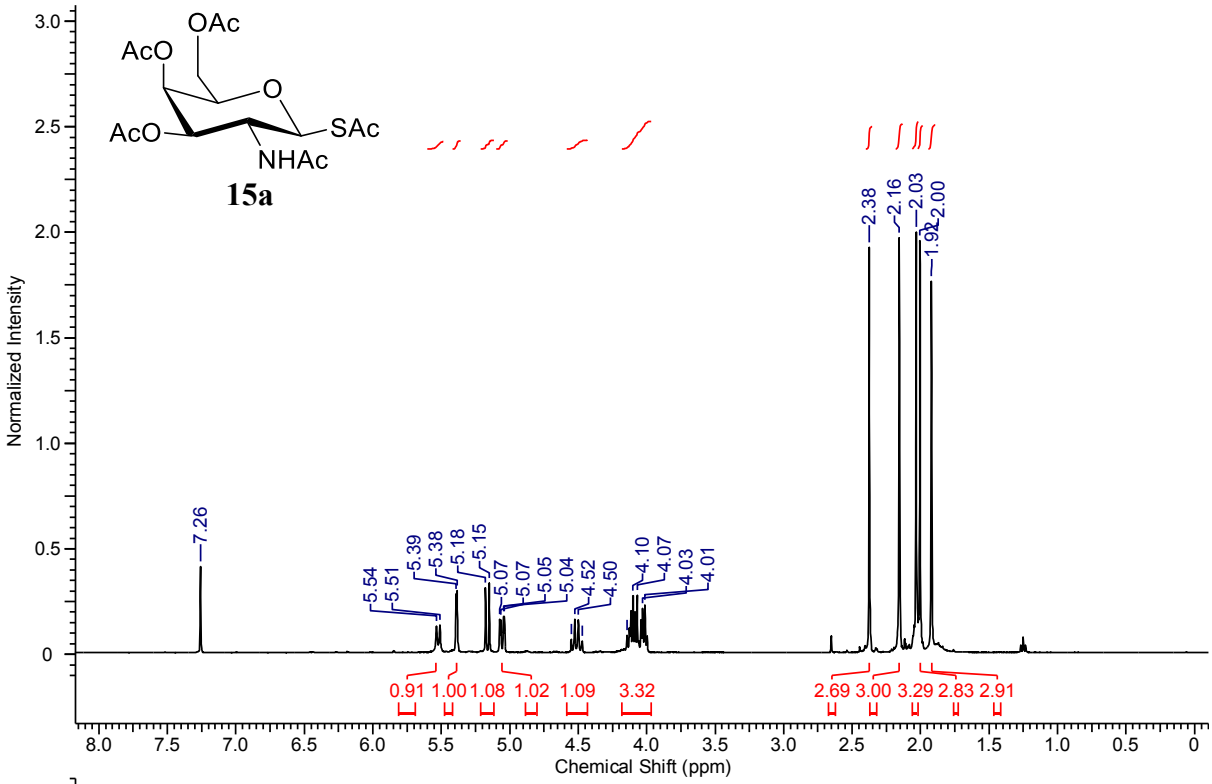
4.28
4.26
4.24
4.04
4.00
3.98
3.97
2.41
2.08
2.06
2.04
1.98
0.87 0.97 1.06 1.09 0.95 1.12 2.28
2.72 3.21 3.28 2.89 3.53
190.78
171.13
170.49
170.02
169.68
81.13
71.95
70.11
65.72
62.14
60.35
51.43
31.17
23.20
20.66
20.64
14.14


OAc
AcO
O
AcO
SAc
NHAc
15a
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5.38
5.18
5.15
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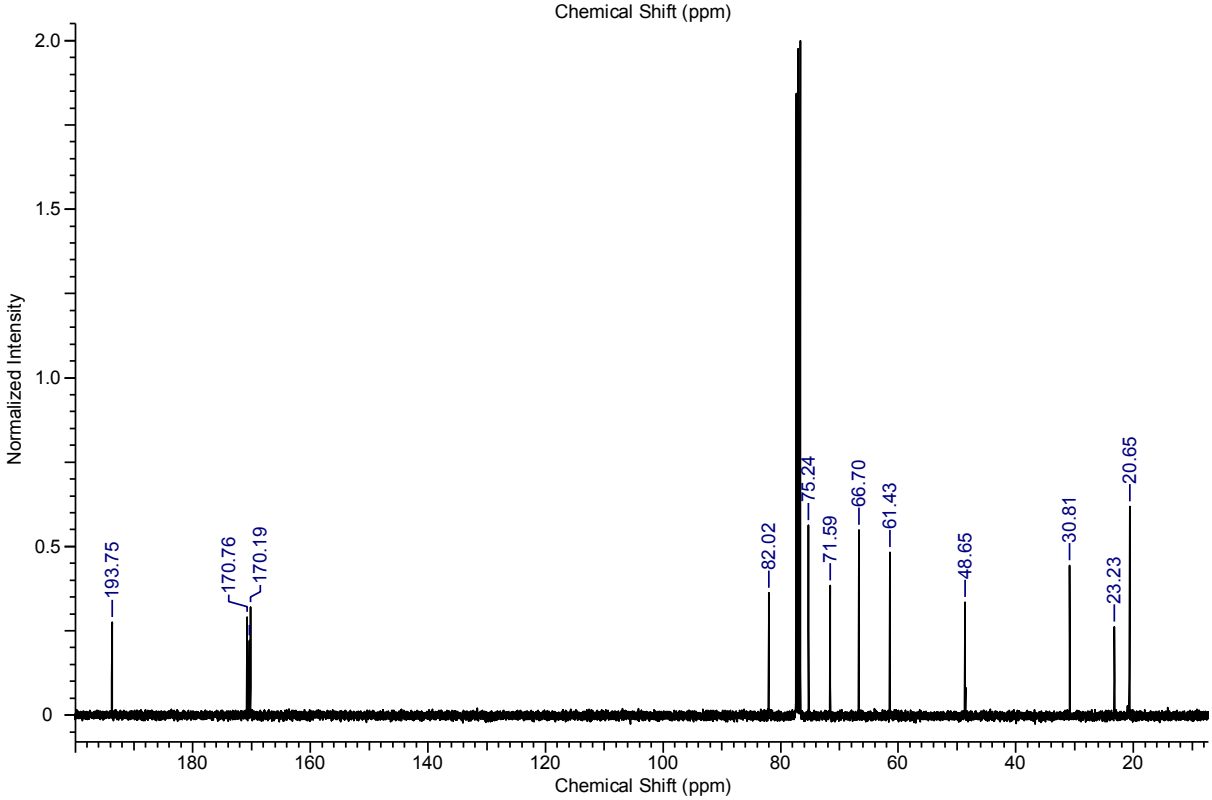
5.07
5.05
5.04
4.52
4.50
4.10
4.07
4.03
4.01
2.38
2.16
2.03
2.00
1.92
0.91 1.00 1.08 1.02 1.09 3.32
2.69 3.00 3.29 2.83 2.91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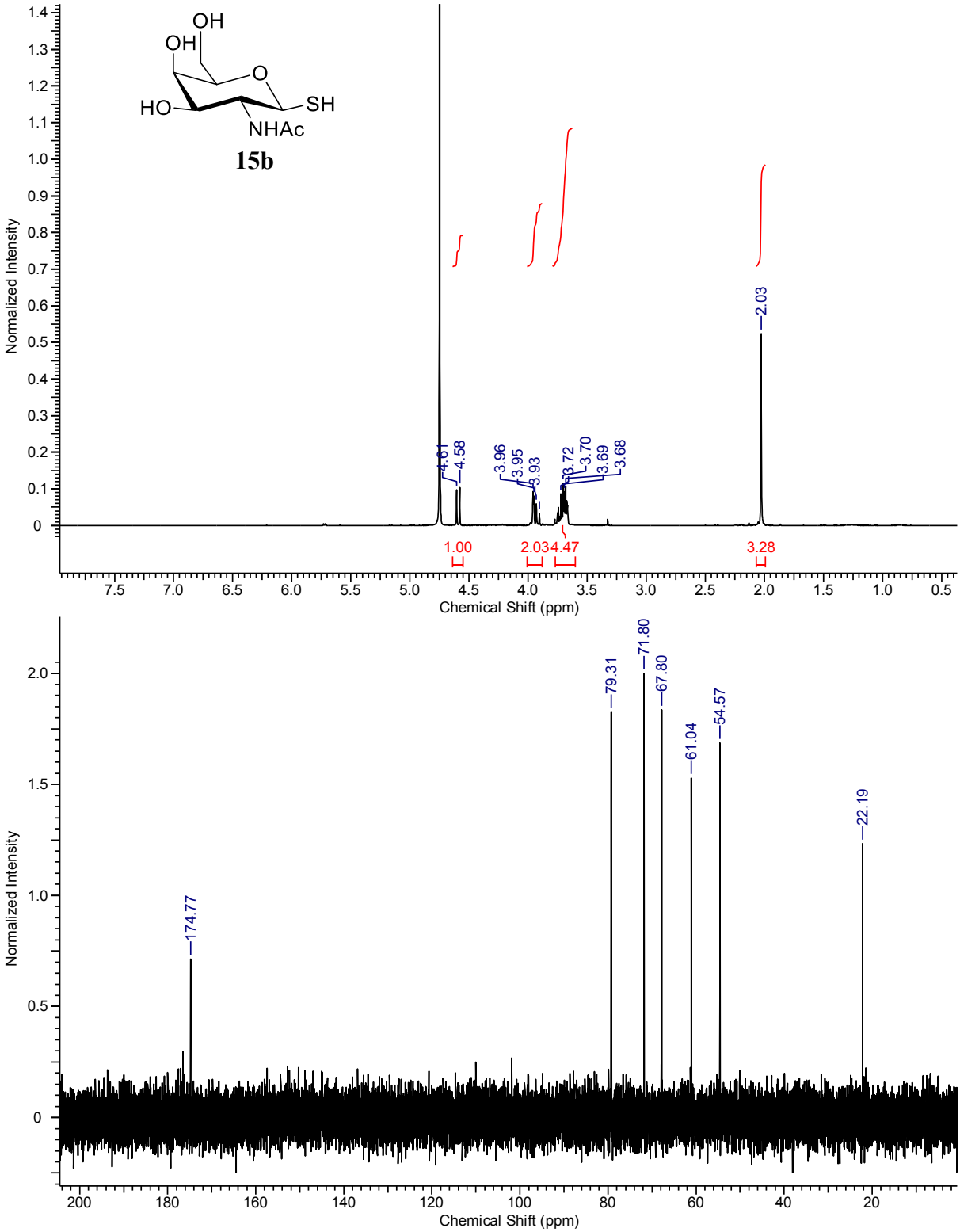
30.81
23.23
20.65


OH
OH
O
HO
SH
NHAc
15b
Normalized Intensity
Chemical Shift (ppm)
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3.93
3.72
3.70
3.69
3.68
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67.80
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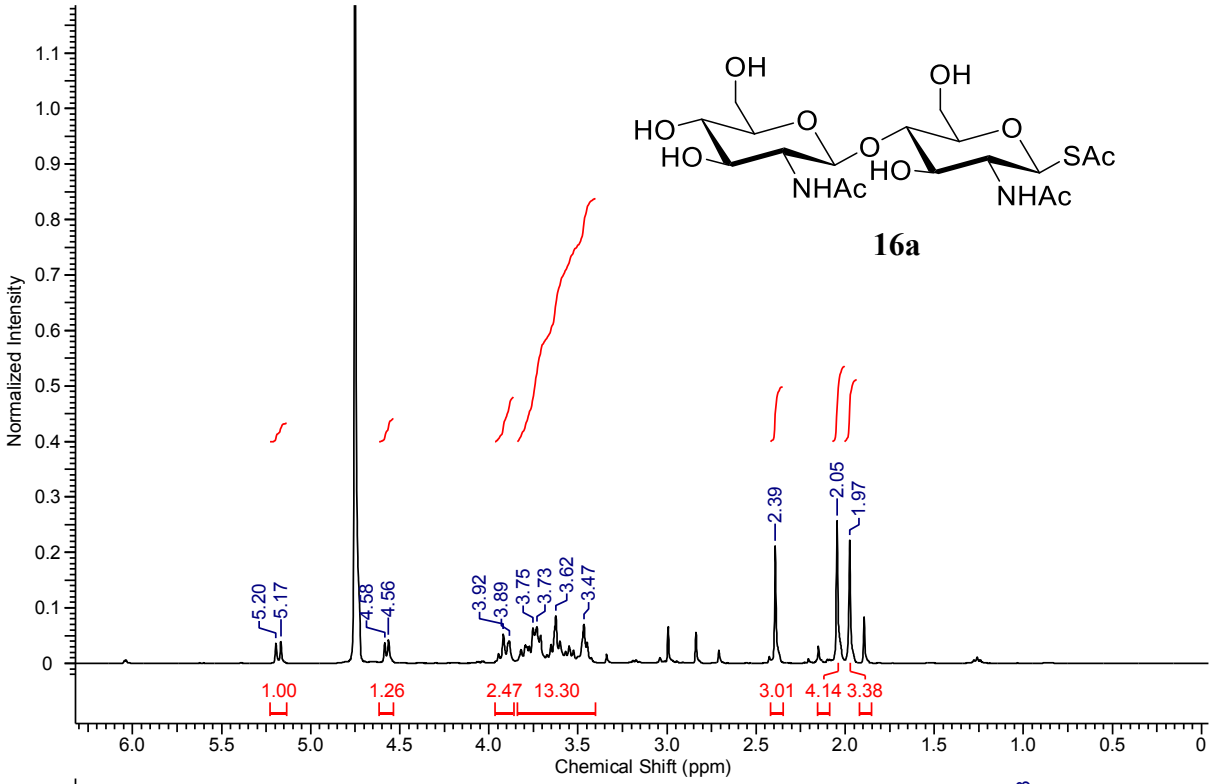
54.57
22.19
174.77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HO
HO
O
O
NHAc
OH
HO
O
SAc
NHAc
Normalized Intensity
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3.92
3.89
3.75
3.73
3.62
3.47
2.39
2.05
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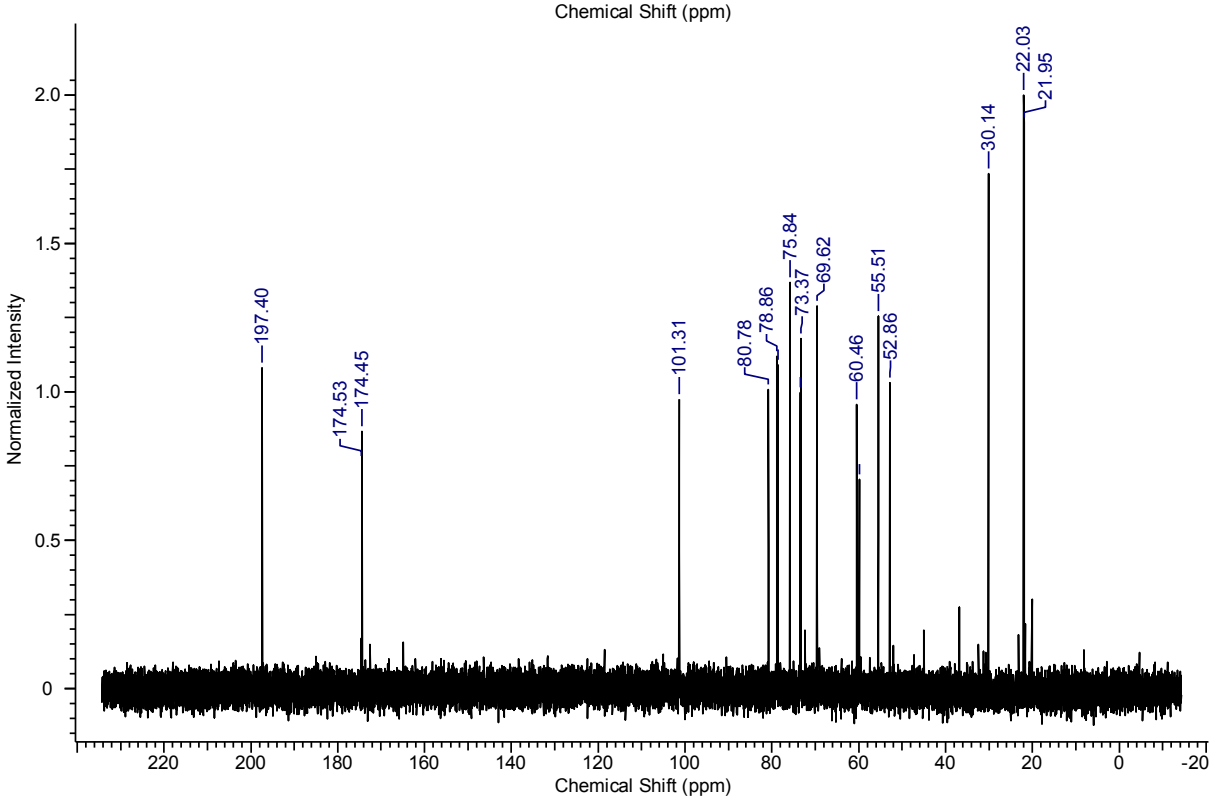
1.00
1.26
2.47
13.30
3.01
4.14
3.38
Chemical Shift (ppm)

16a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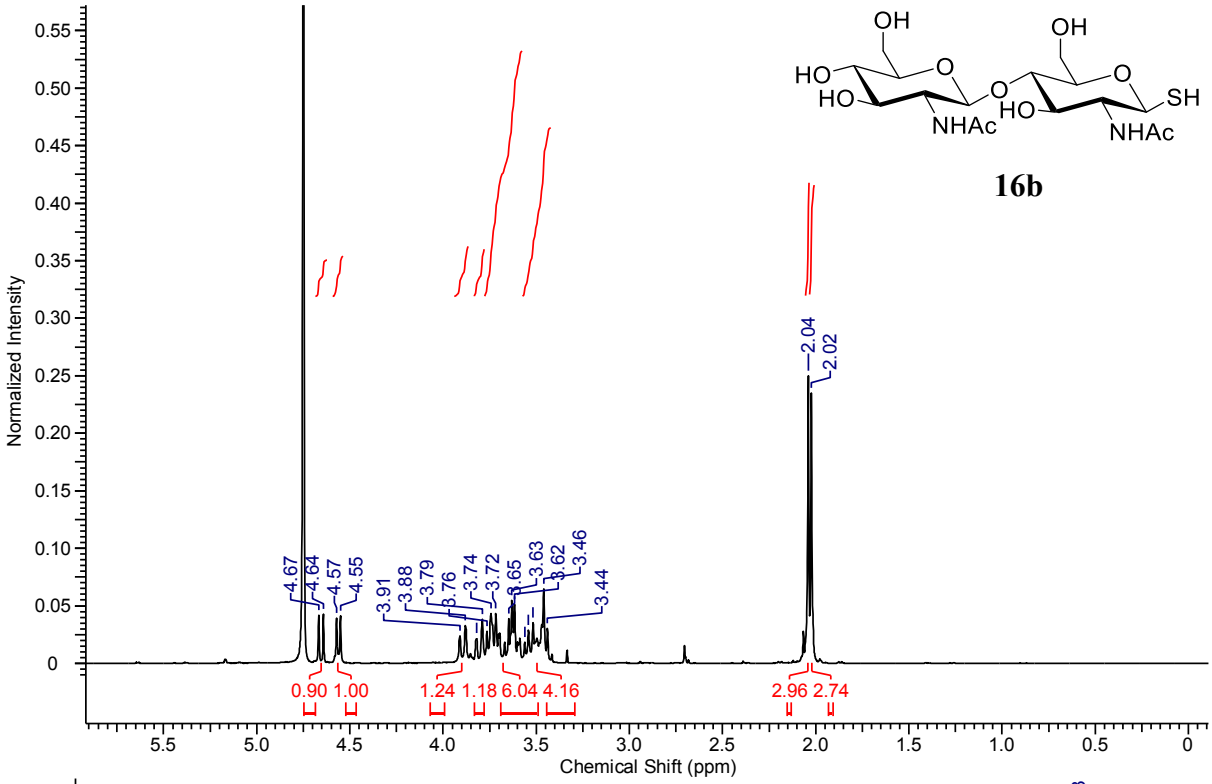
30.14
22.03
21.95
Chemical Shift (ppm)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3.62
3.46
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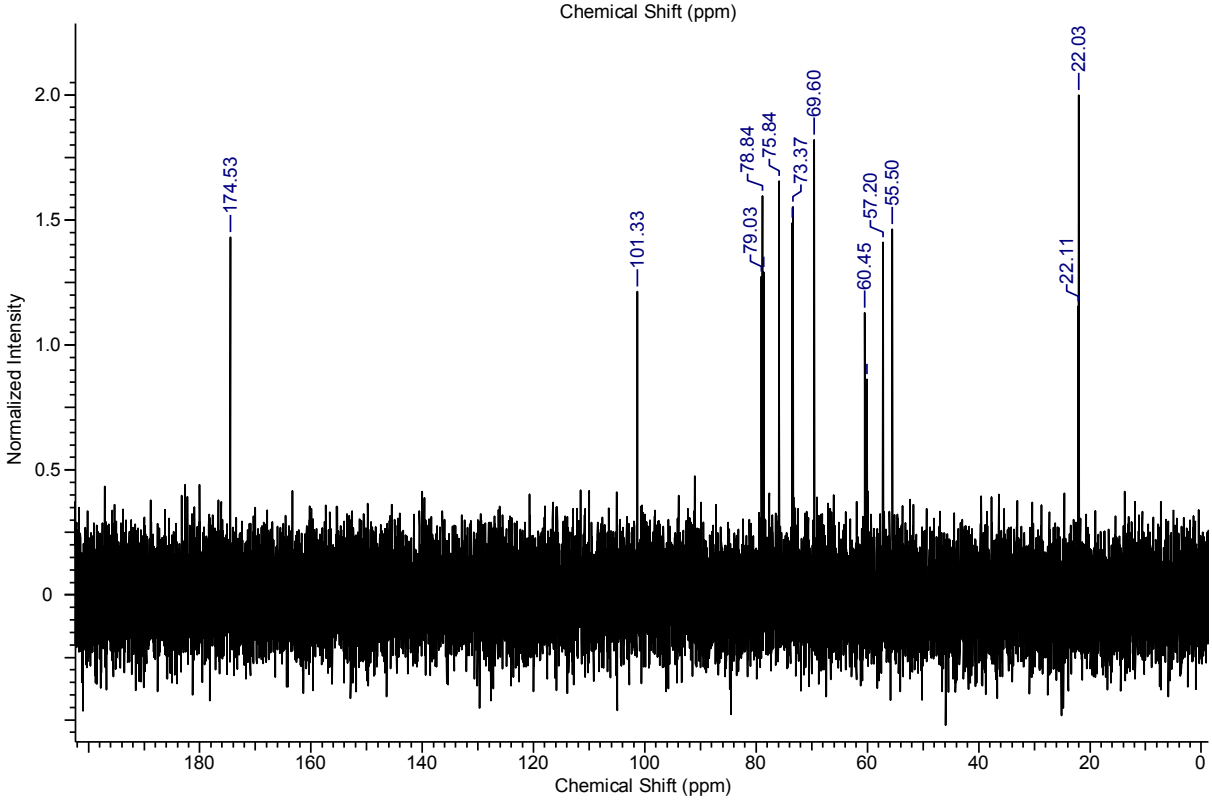
OH
HO
HO
O
NHAc
O
OH
HO
O
SH
NHAc

16b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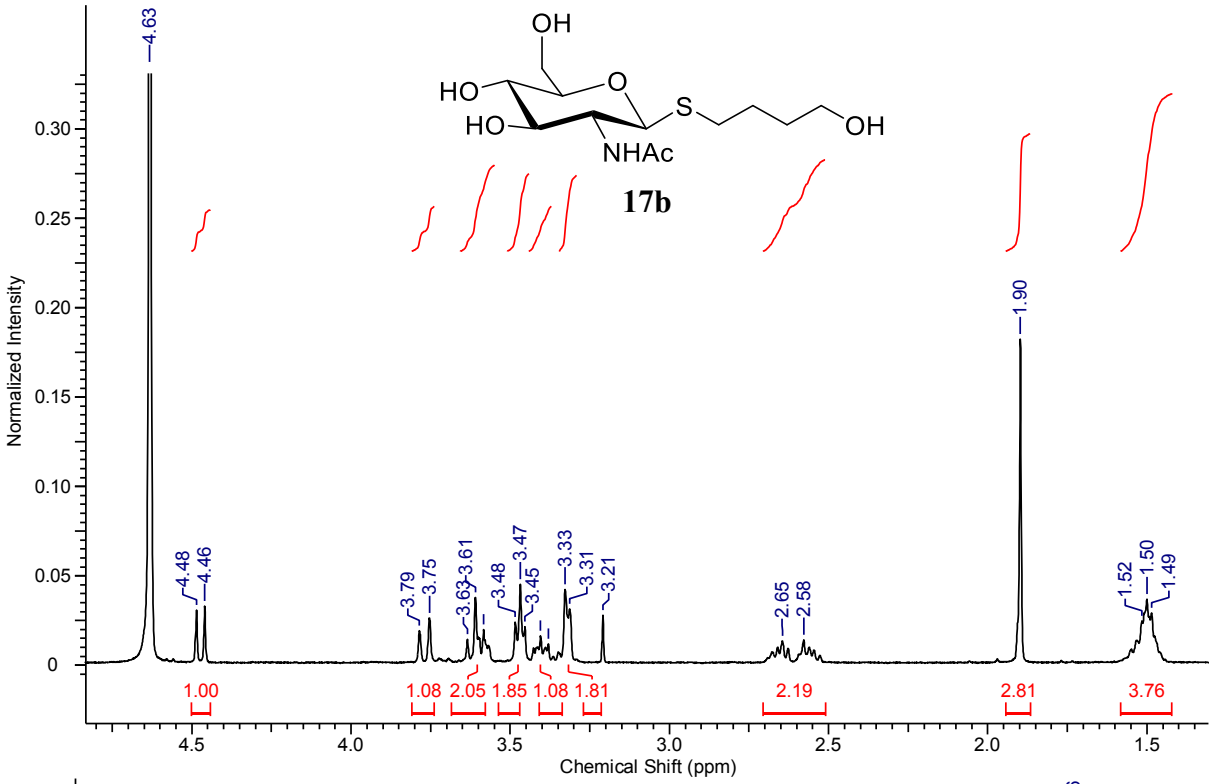
22.11
22.03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HO
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17b
Normalized Intensity
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1.08
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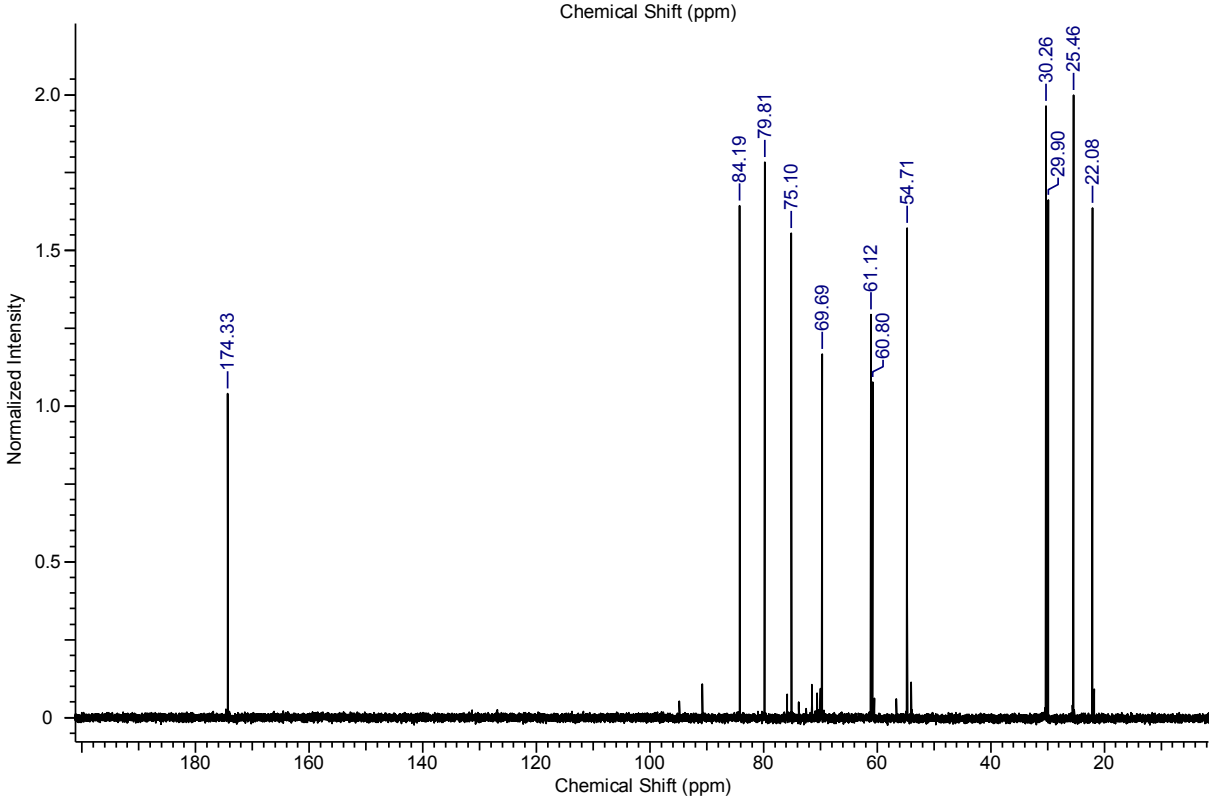
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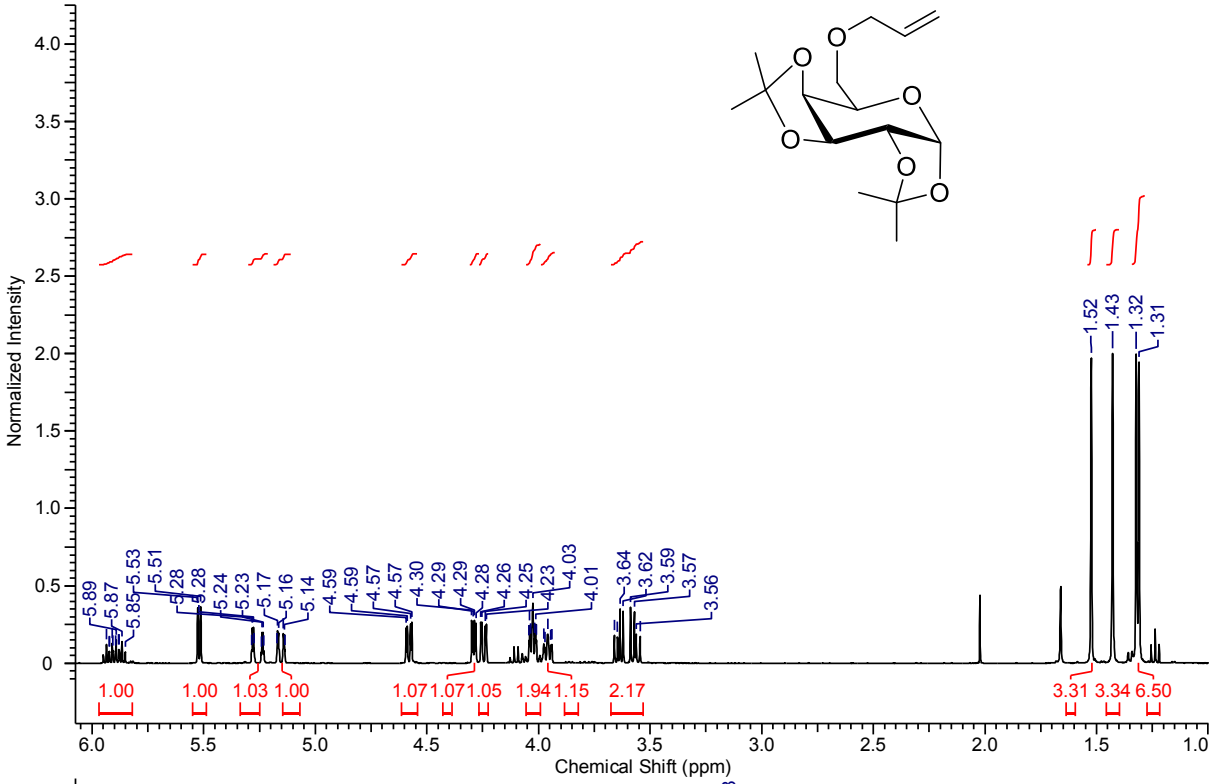
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4.57
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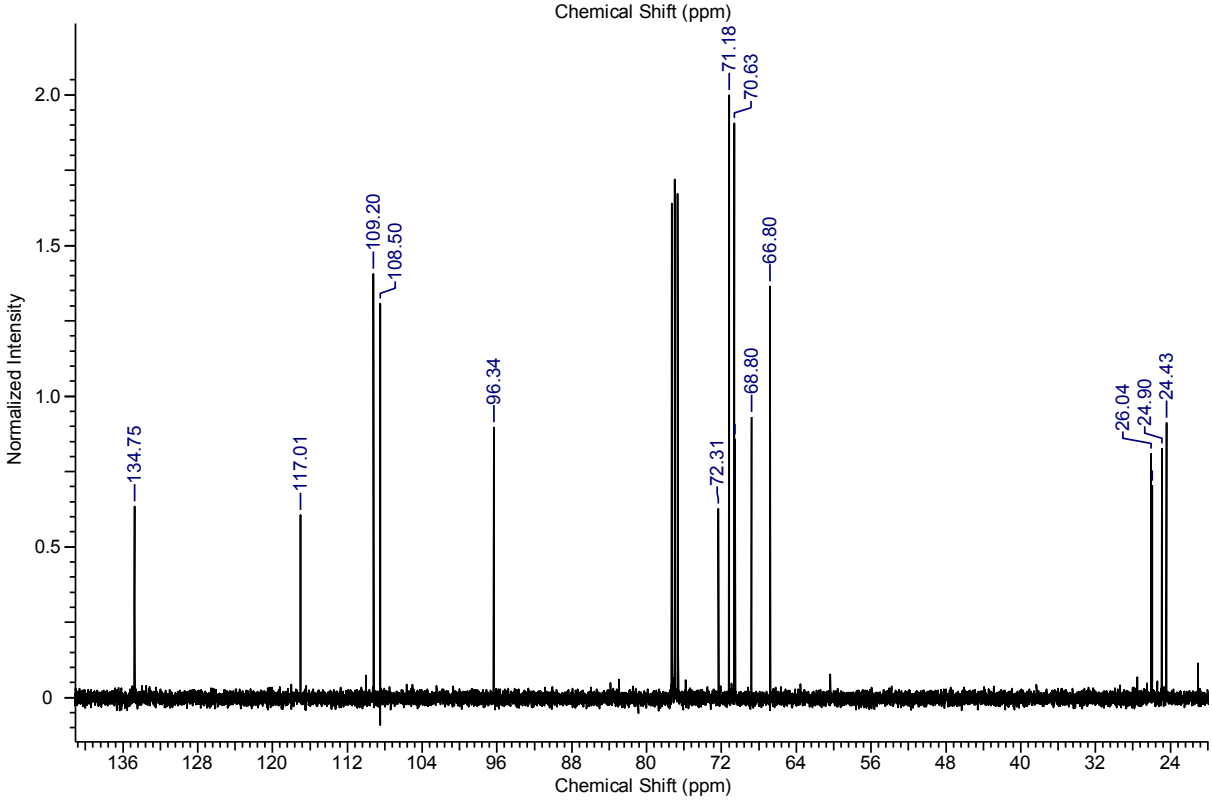
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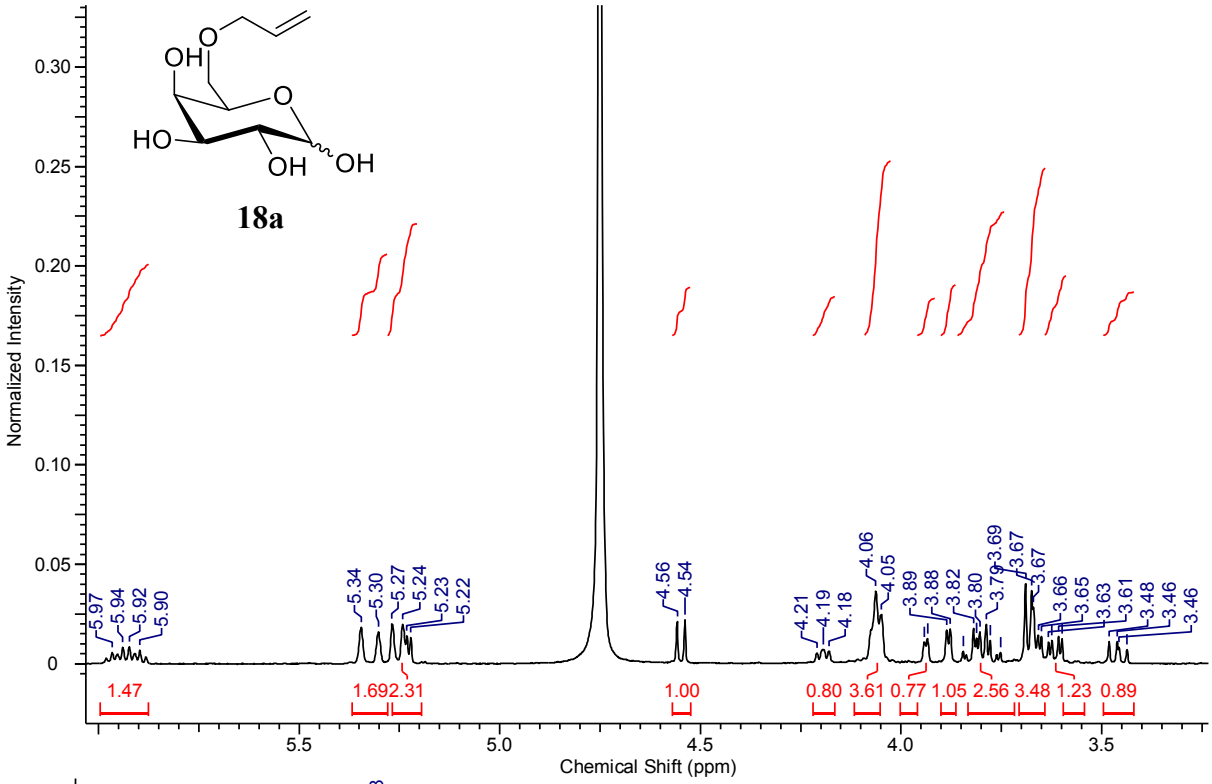
24.43


OH
O
O
HO
OH
OH
18a
Normalized Intensity
Chemical Shift (ppm)
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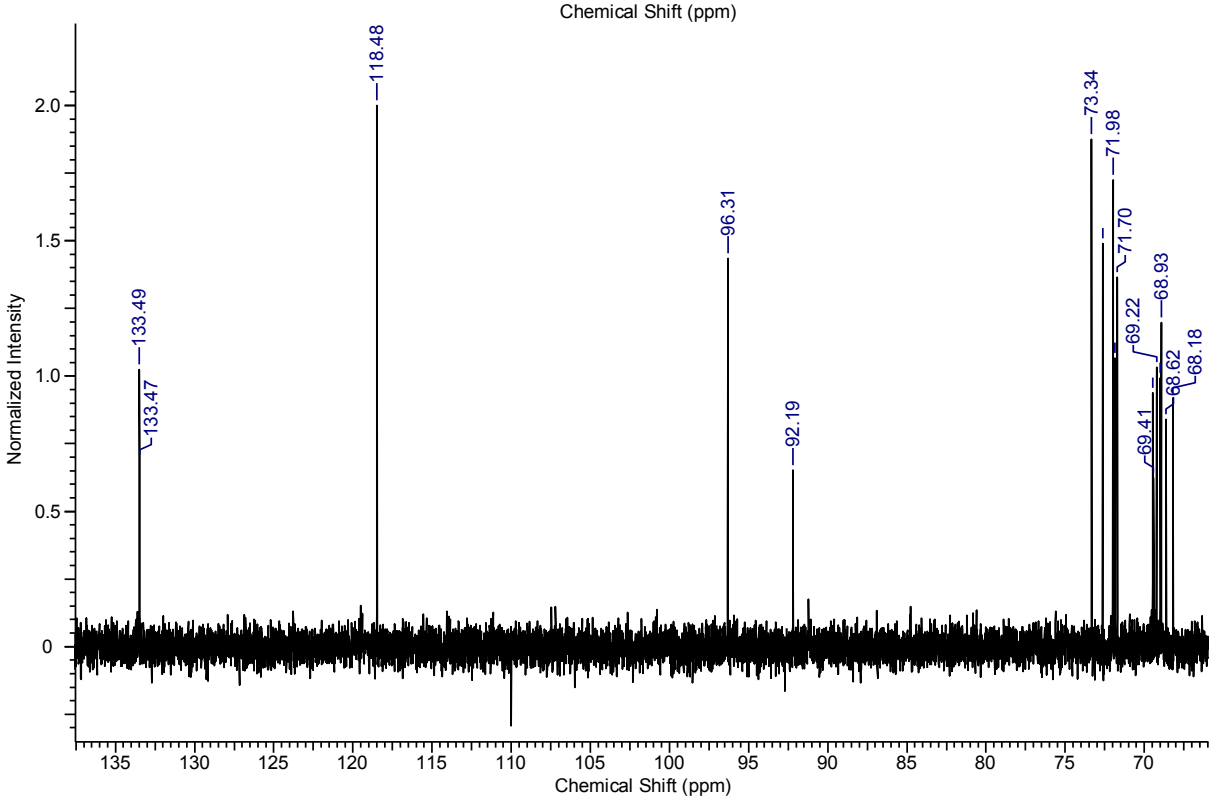
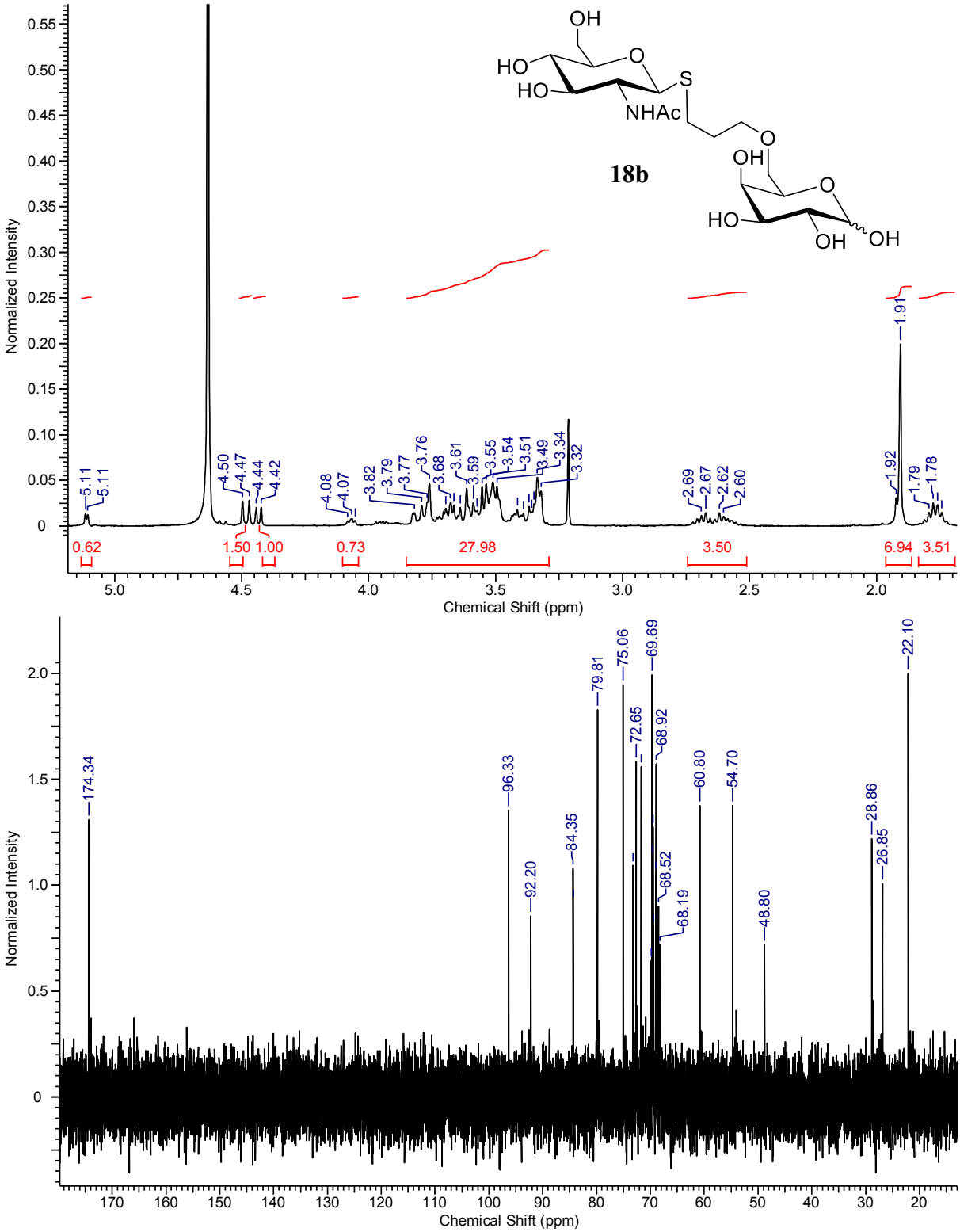
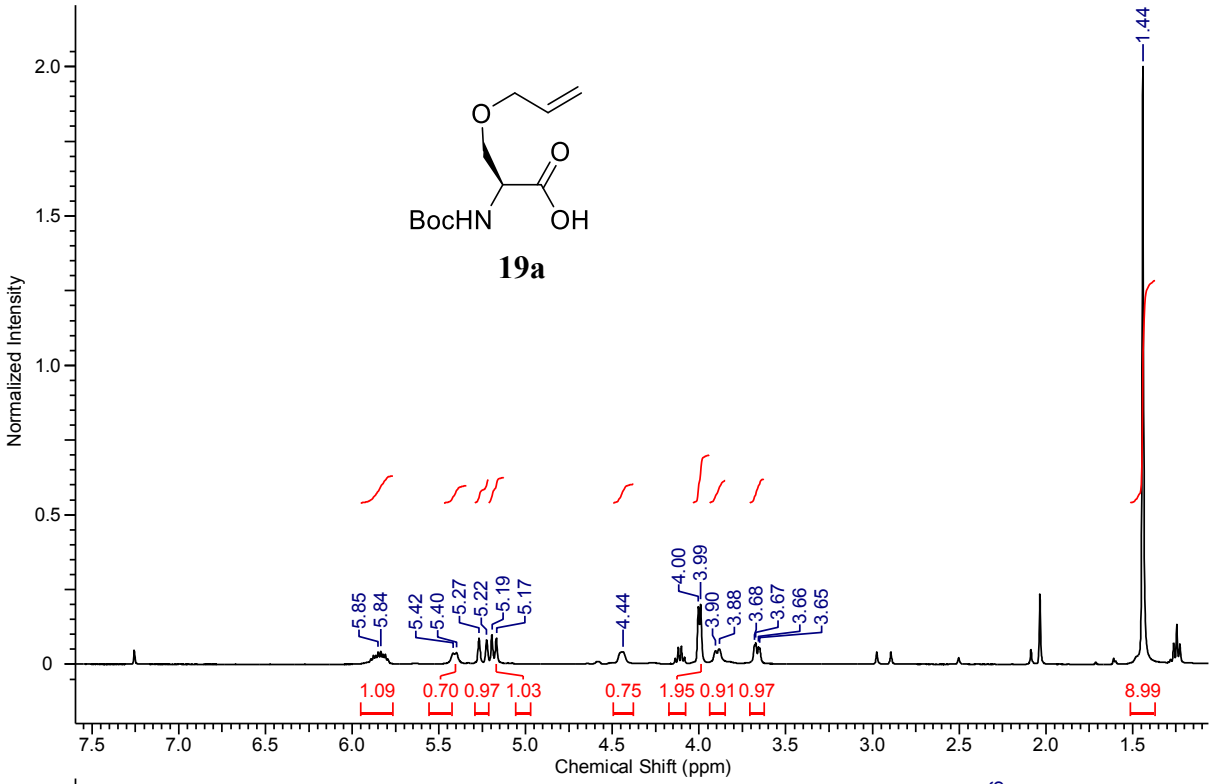
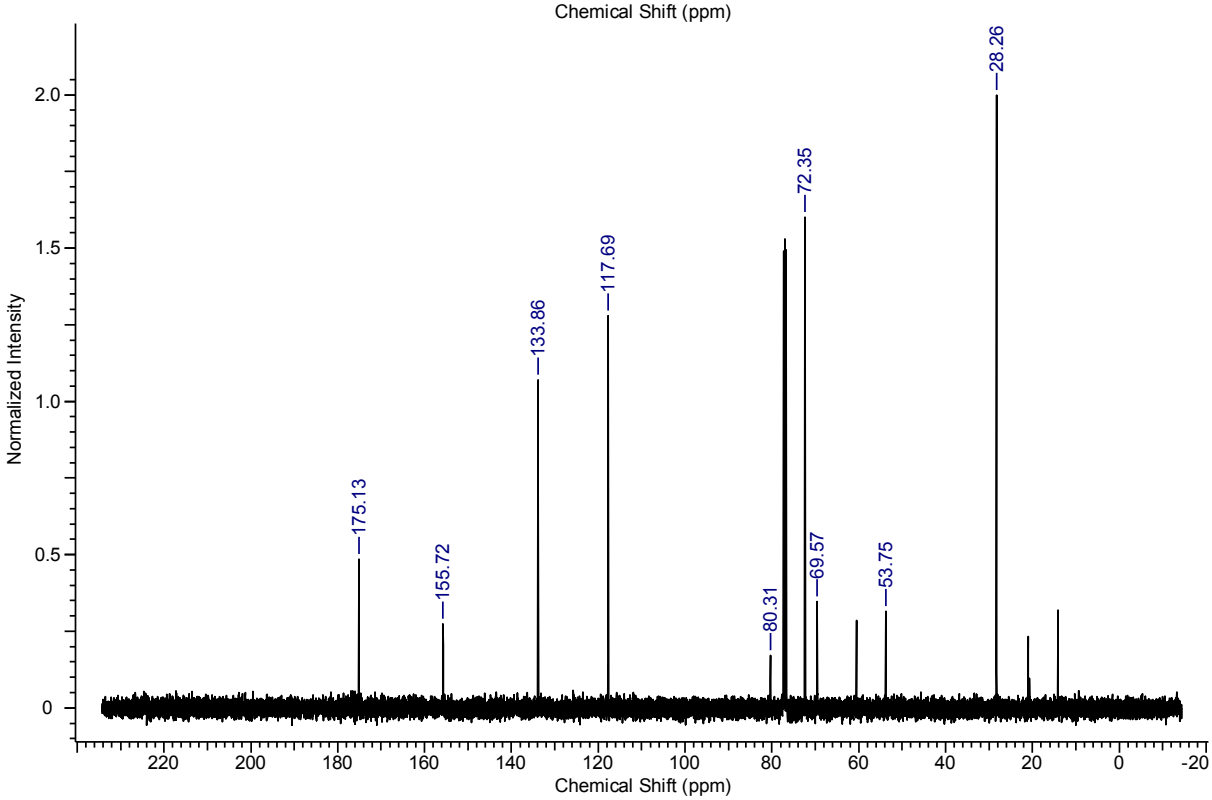
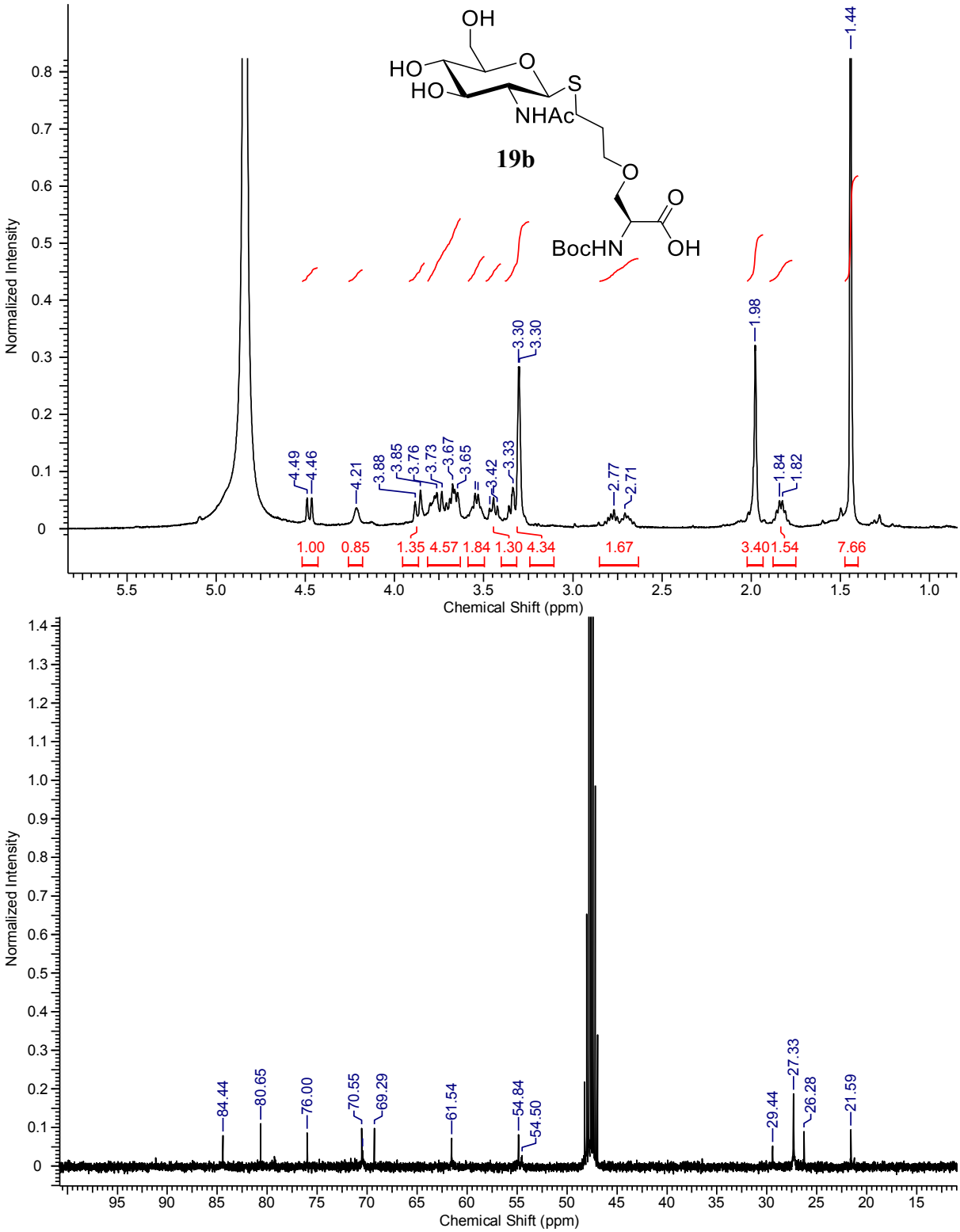
4.54
4.21
4.19
4.18
4.06
4.05
3.89
3.88
3.82
3.80
3.79
3.69
3.67
3.67
3.66
3.65
3.63
3.61
3.48
3.46
3.46
1.47
1.69
2.31
1.00
0.80
3.61
0.77
1.05
2.56
3.48
1.23
0.89


Normalized Intensity
Chemical Shift (ppm)
118.48
133.49
133.47
96.31
92.19
73.34
71.98
71.70
69.22
68.93
68.62
69.41
68.18

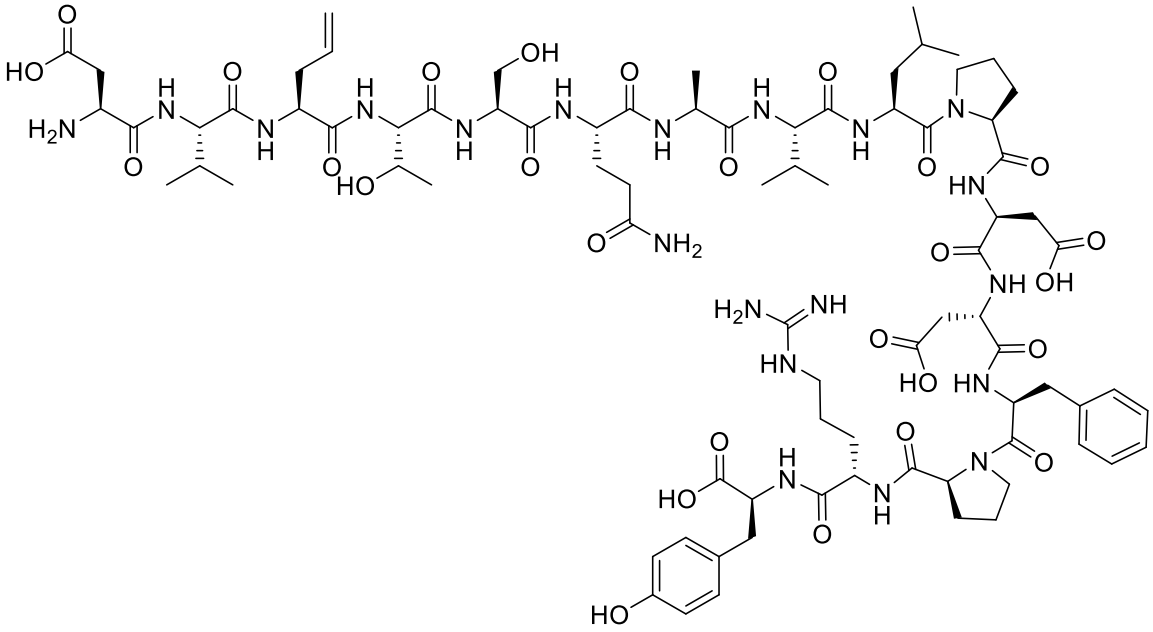

OH
HO
O
HO
S
NHAc
O
OH
O
HO
OH
OH
18b
Normalized Intensity
Chemical Shift (ppm)
5.11
5.11
4.50
4.47
4.44
4.42
4.08
4.07
3.82
3.79
3.77
3.76
3.68
3.61
3.59
3.55
3.54
3.51
3.49
3.34
3.32
2.69
2.67
2.62
2.60
1.92
1.91
1.79
1.78
0.62
1.50
1.00
0.73
27.98
3.50
6.94
3.51
174.34
96.33
92.20
84.35
79.81
75.06
72.65
69.69
68.92
68.52
68.19
60.80
54.70
48.80
28.86
26.85
22.10


BocHN
OH
O
O
19a
Normalized Intensity
Chemical Shift (ppm)
1.44
5.85
5.84
5.42
5.40
5.27
5.22
5.19
5.17
4.44
4.00
3.99
3.90
3.88
3.68
3.67
3.66
3.65
1.09
0.70
0.97
1.03
0.75
1.95
0.91
0.97
8.99

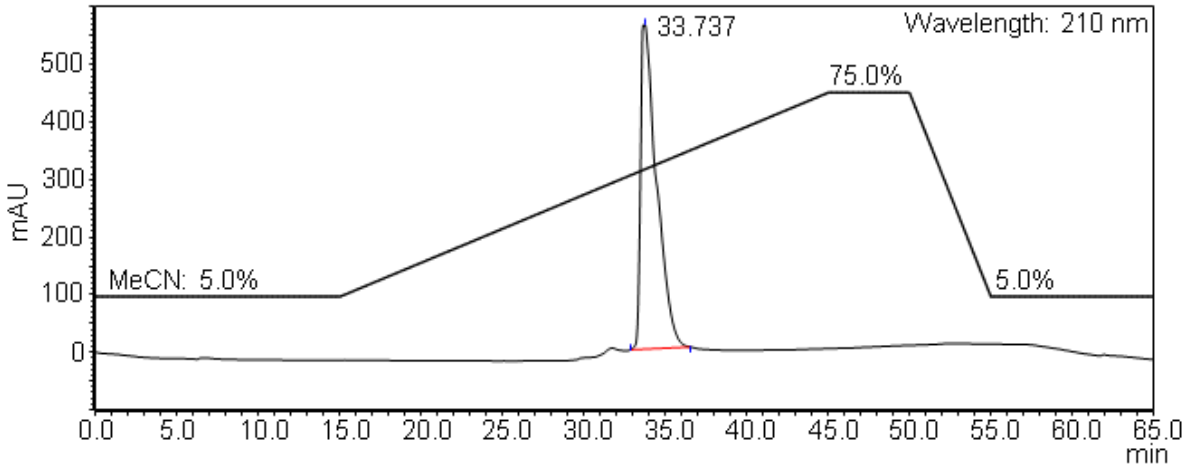

Normalized Intensity
Chemical Shift (ppm)
28.26
72.35
117.69
133.86
175.13
155.72
80.31
69.57
53.75

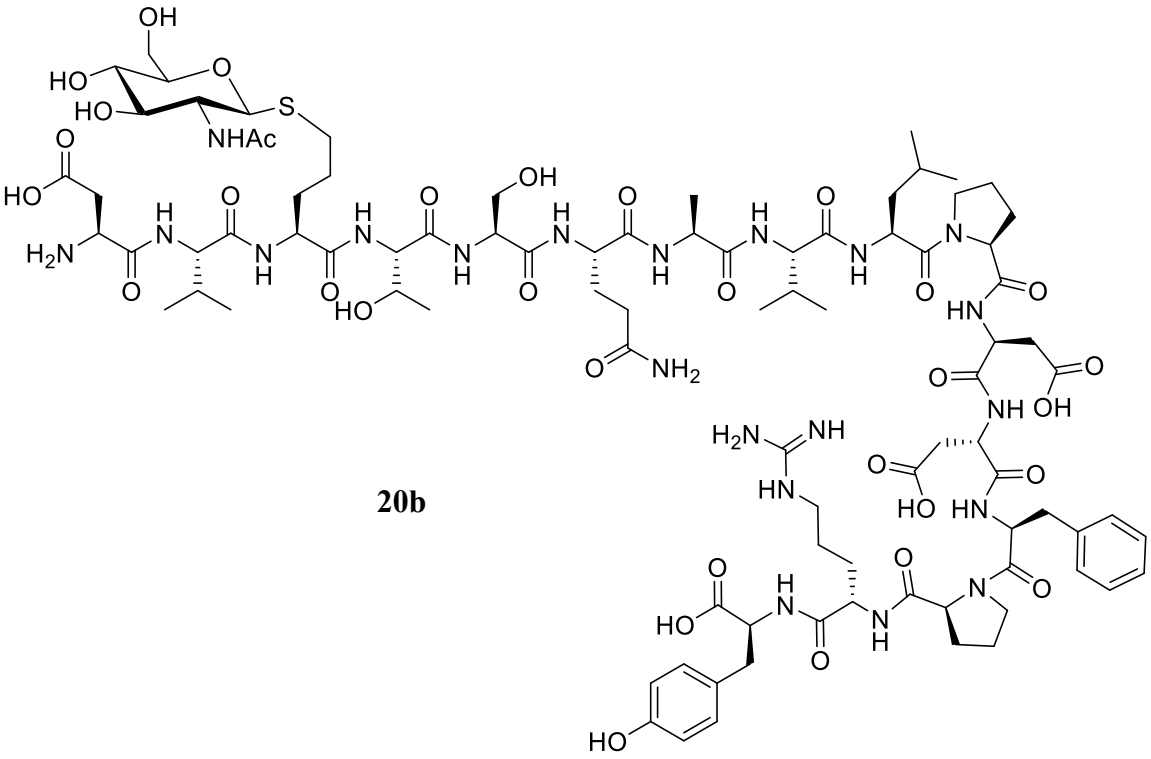

OH
HO
HO
O
S
NHAc
19b
O
O
BocHN
OH
Normalized Intensity
Chemical Shift (ppm)
1.44
1.98
3.30
3.30
4.49
4.46
4.21
3.88
3.85
3.76
3.73
3.67
3.65
3.42
3.33
2.77
2.71
1.84
1.82
1.00
0.85
1.35
4.57
1.84
1.30
4.34
1.67
3.40
1.54
7.66
84.44
80.65
76.00
70.55
69.29
61.54
54.84
54.50
29.44
27.33
26.28
21.59

Peptide 20a



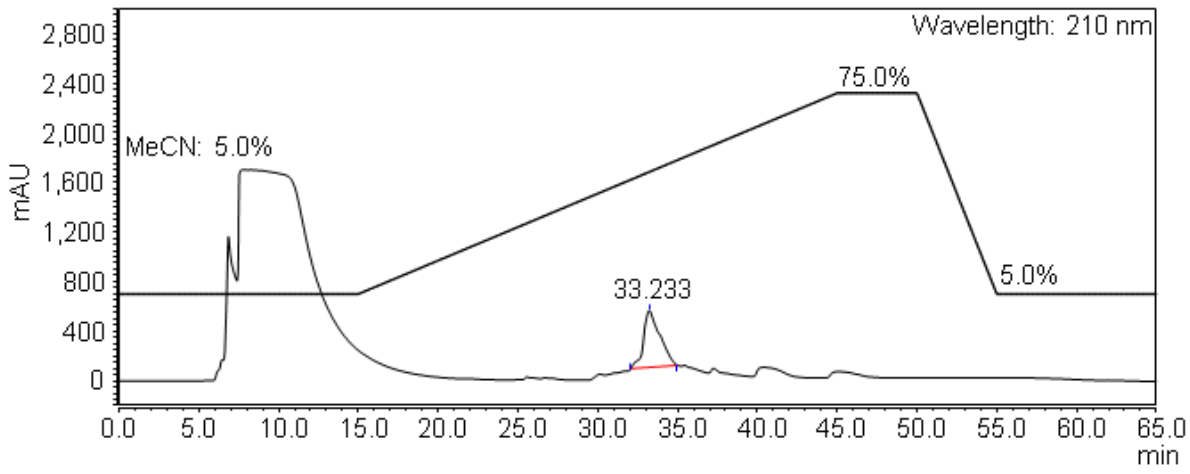
HPLC trace of 20a



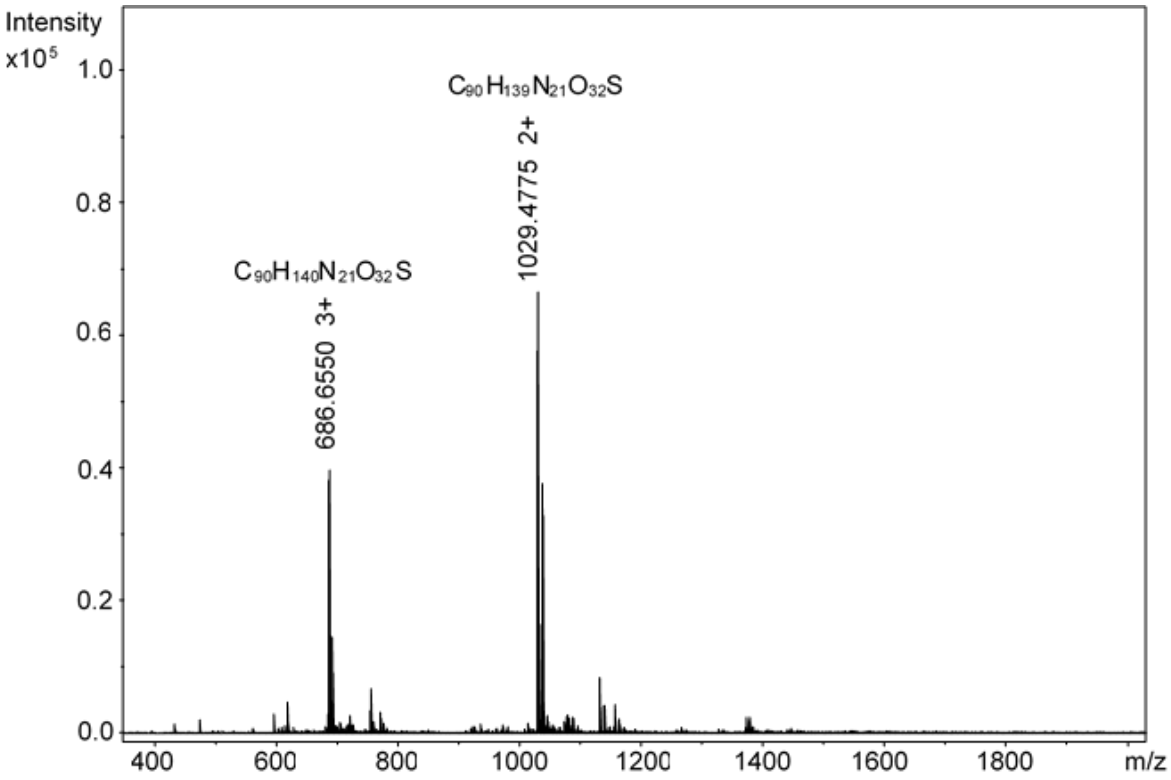


20b

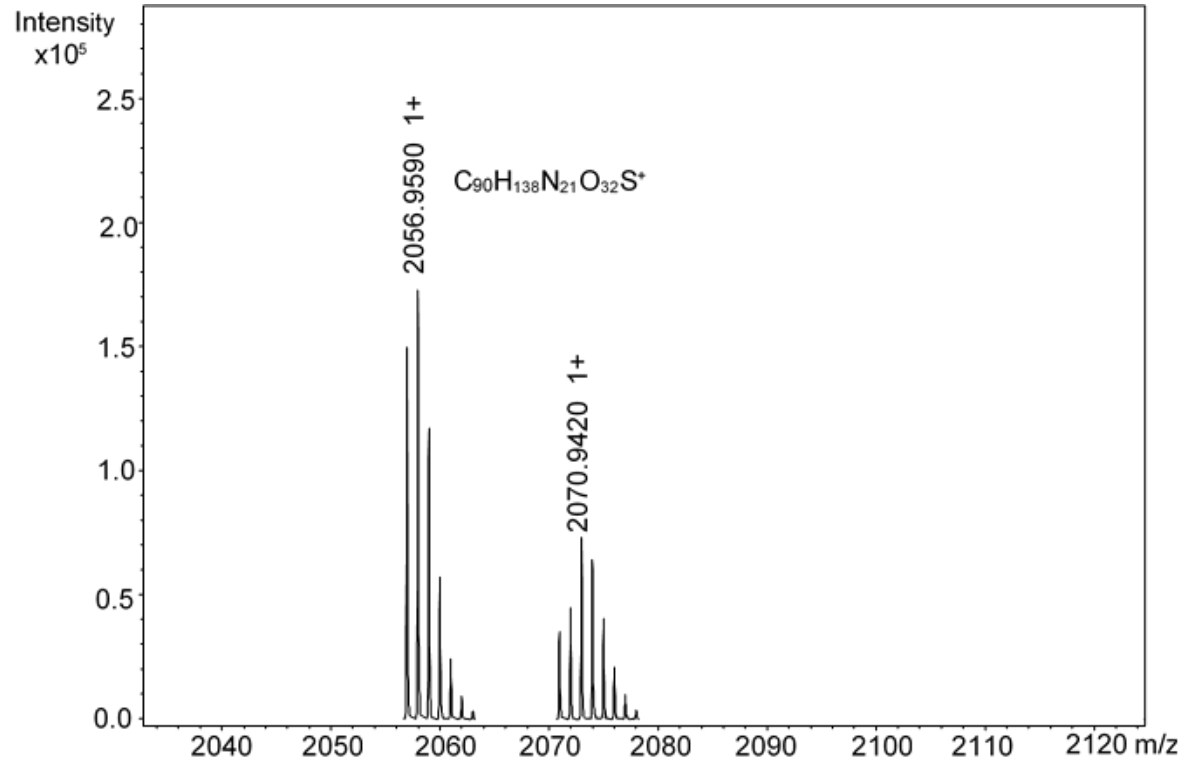
HPLC trace of 20b:

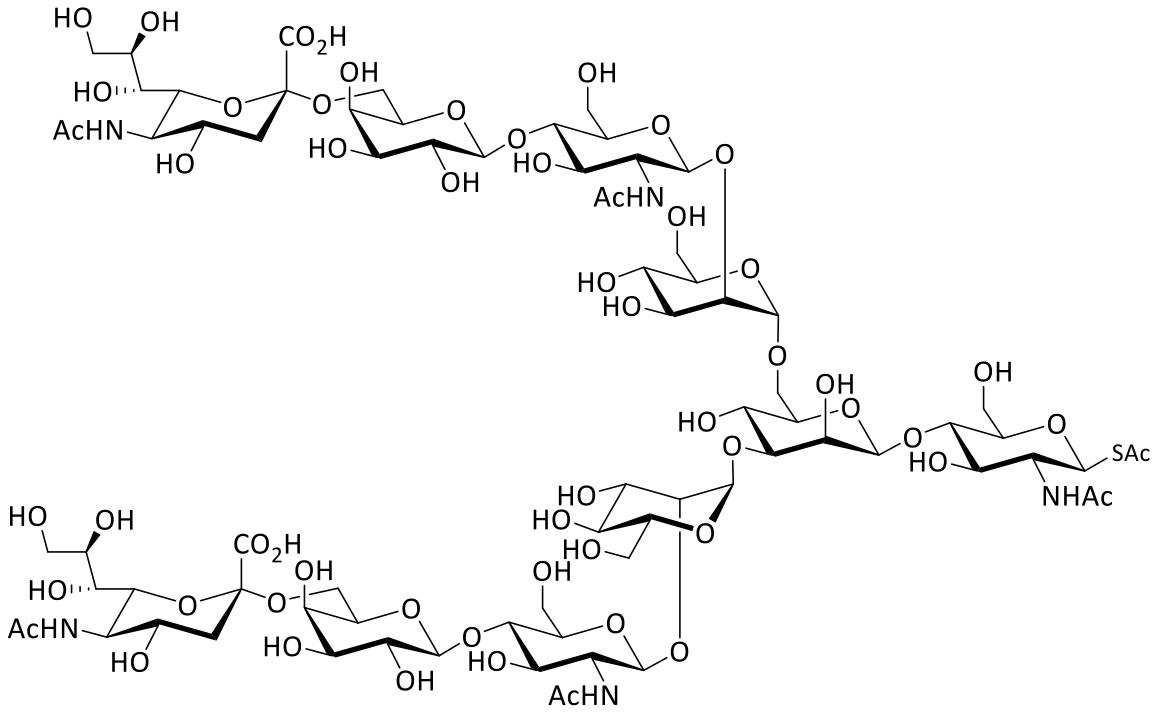


Mass spectrum of 20b:

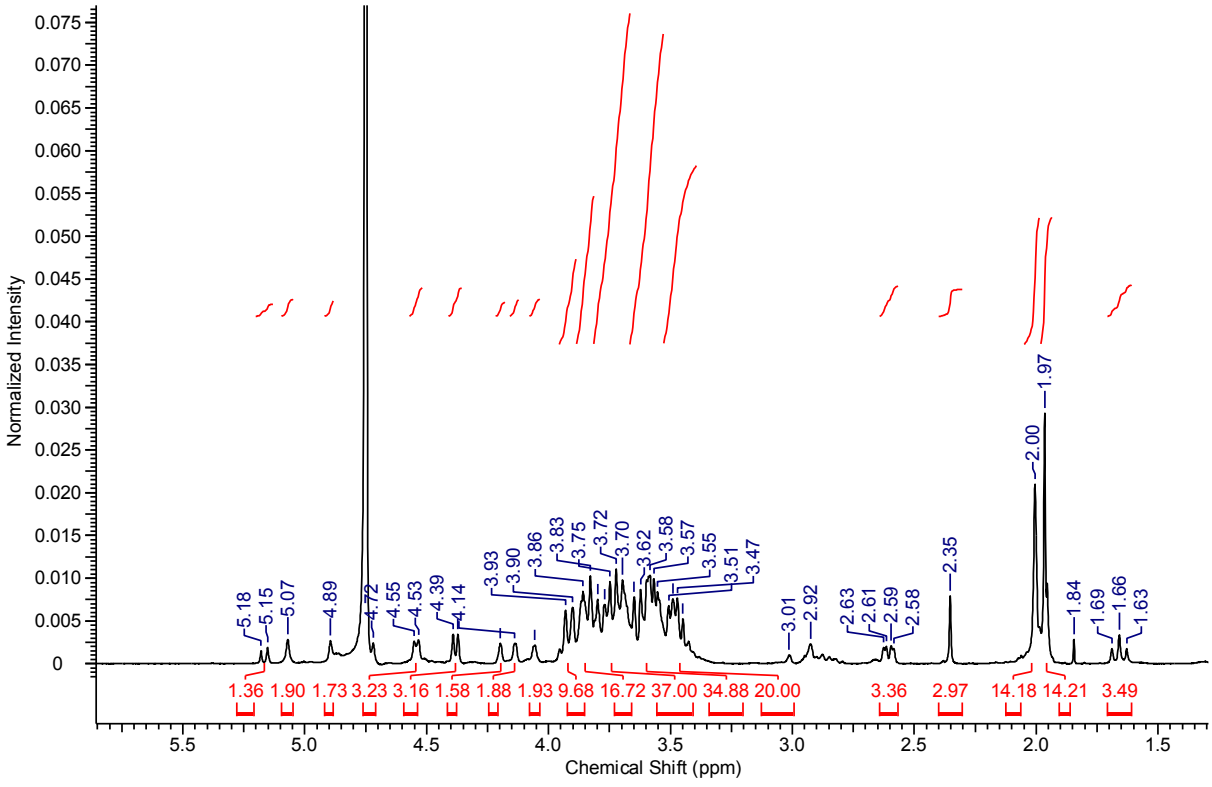


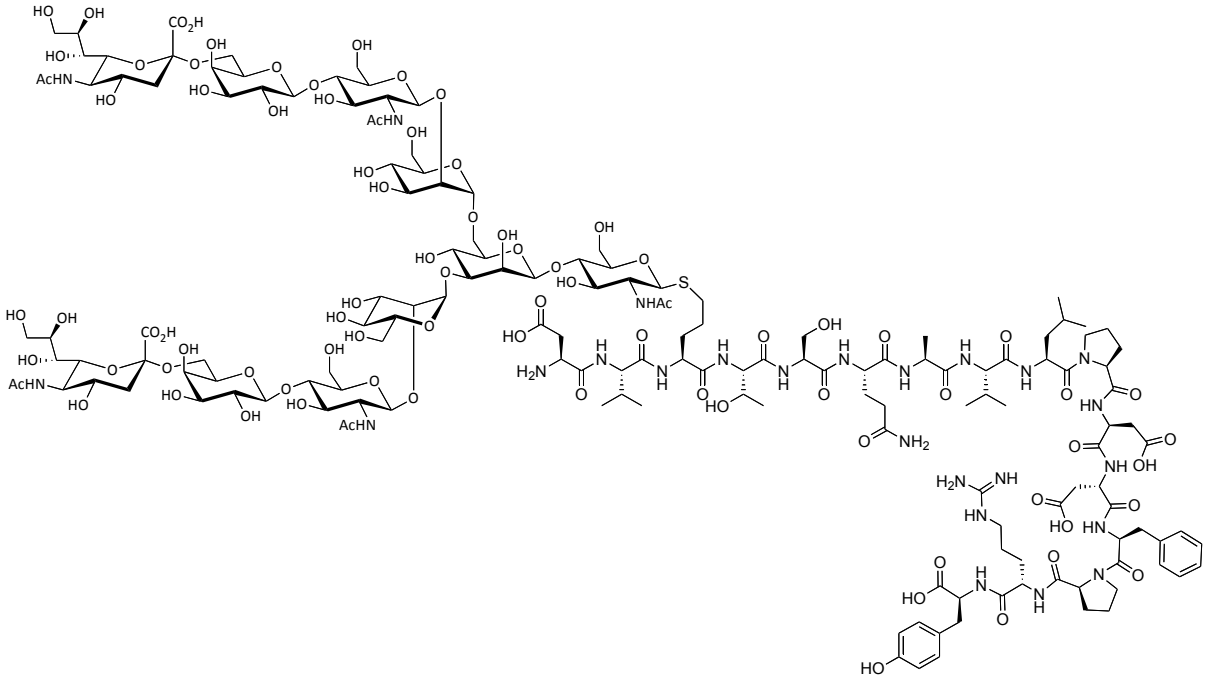
Deconvoluted mass spectrum of 20b:





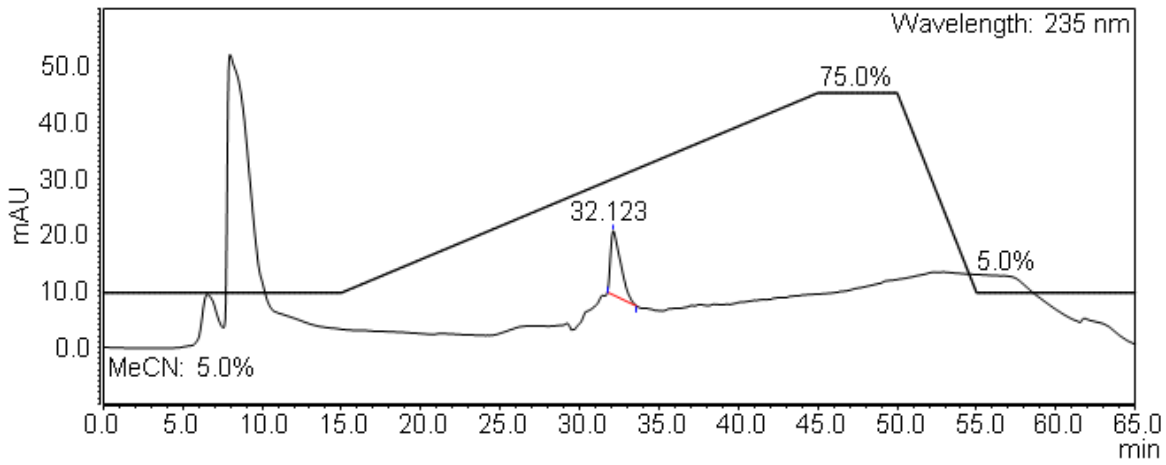
Sialoglycan thioacetate 22a



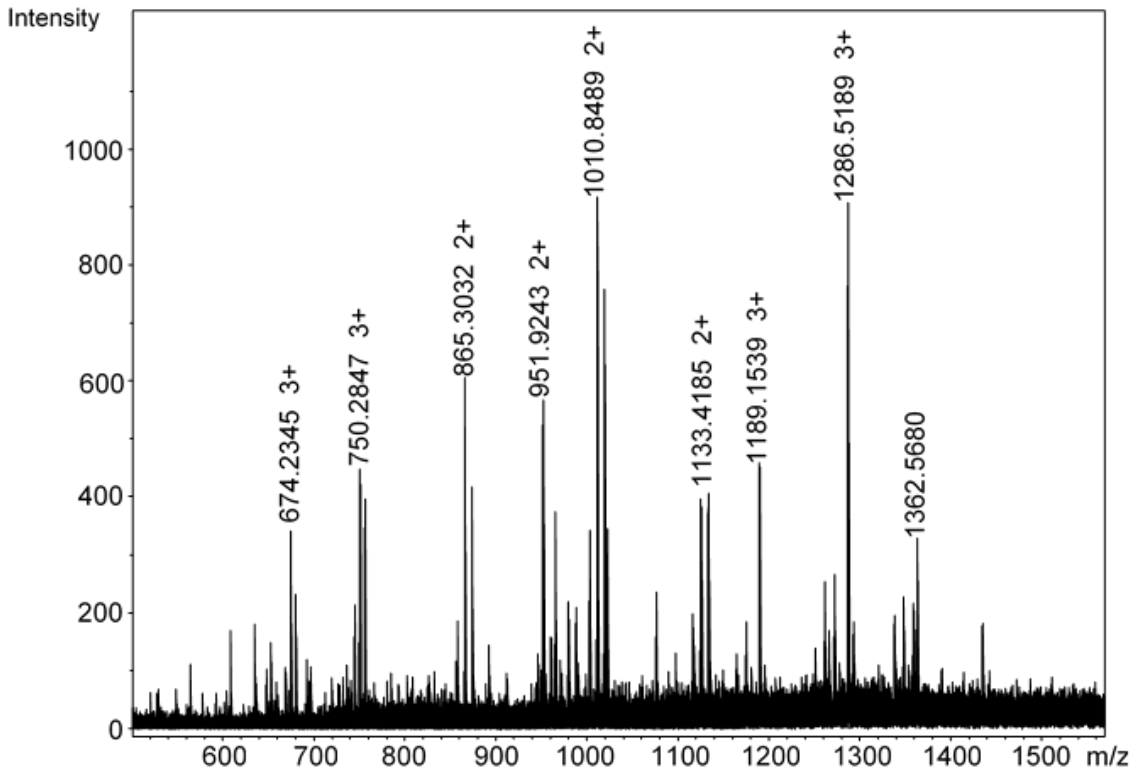


Sialoglycan neoglycopeptide 23

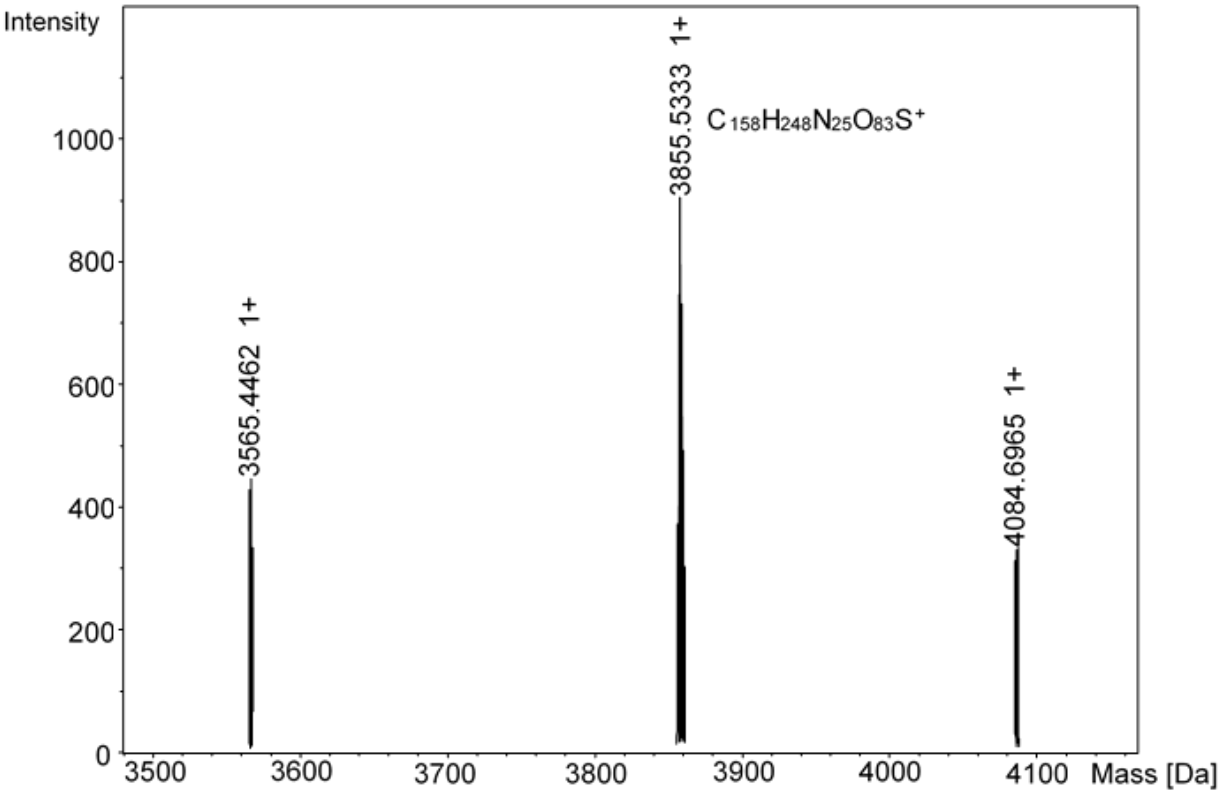
HPLC trace of 23:



Mass spectrum of 23



Deconvoluted mass spectrum of 23:



References

1. P. W. R. Harris, S. H. Yang and M. A. Brimble, *Tetrahedron Lett.,* 2011, **52**, 6024-6026.

2. Z. Amso, R. Kowalczyk, M. Watson, Y-E. Park, K. E. Callon, D. S. Musson, J. Cornish, M. A. Brimble, *Org. Biomol. Chem.*, 2016, **14**, 9225-9238.

3. Chalker, J. M. M., *Angew. Chem., Int. Ed. 51* (8), 1835-1839.

4. Lin, Y. A.; Chalker, J. M.; Davis, B. G., *J. Am. Chem. Soc.* **2010,** *132* (47), 16805-16811.

5. Gamblin, D. P.; Garnier, P.; van Kasteren, S.; Oldham, N. J.; Fairbanks, A. J.; Davis, B. G., *Angew. Chem., Int. Ed.* **2004,** *43* (7), 828-833

6. Rakotomanomana, N.; Lacombe, J.-M.; Pavia, A. A., *Carbohydr. Res.* **1990,** *197* (0), 318-323.

7. Watt, G. M.; Lund, J.; Levens, M.; Kolli, V. S. K.; Jefferis, R.; Boons, G.-J., *Chemistry & Biology* **2003,** *10* (9), 807-814.

8. Ito, H.; Kamachi, T.; Yashima, E., *Chem. Commun.* **2012,** *48* (45), 5650-5652.

9. Black, W. A. P.; Colquhoun, J. A.; Dewar, E. T., *Carbohydr. Res.* **1967,** *5* (3), 362-365.

10. Murakami, H.; Minami, T.; Ozawa, F., *J. Org. Chem.* **2004,** *69* (13), 4482-4486.

11. Boal, A. K.; Guryanov, I.; Moretto, A.; Crisma, M.; Lanni, E. L.; Toniolo, C.; Grubbs, R. H.; O'Leary, D. J., *J. Am. Chem. Soc.* **2007,** *129* (22), 6986-6987.