Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION

Direct aqueous synthesis of cyanomethyl thioglycosides from reducing sugars; ready access to reagents for protein glycosylation.

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Table S1 Extent of protein modification as a percentage of total lysozyme

Sugar	0	1	2	3	4	5	6	Average (mean)	Conversion
Glc	-	-	-	-	26	48	26	5.0	100%
Gal	-	-	-	1	28	46	25	5.0	100%
Man	9	33	38	18	2	-	-	1.7	91%
Lac	3	9	27	35	16	9	-	2.8	97%
Maltotriose	4	26	43	21	7	<1	-	2.0	96%

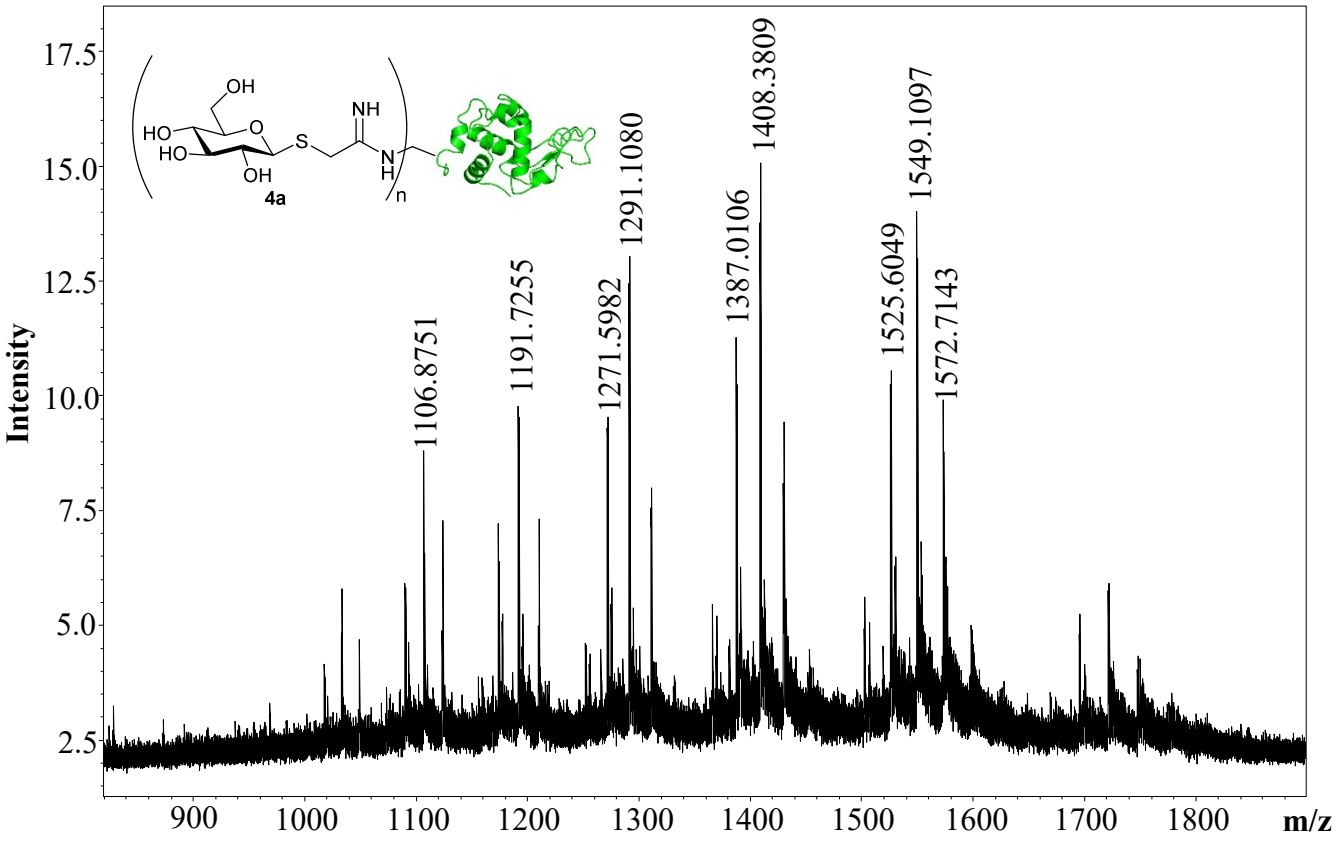


Fig. S1 HRMS of lysozyme modified with glucose reagent **3a**

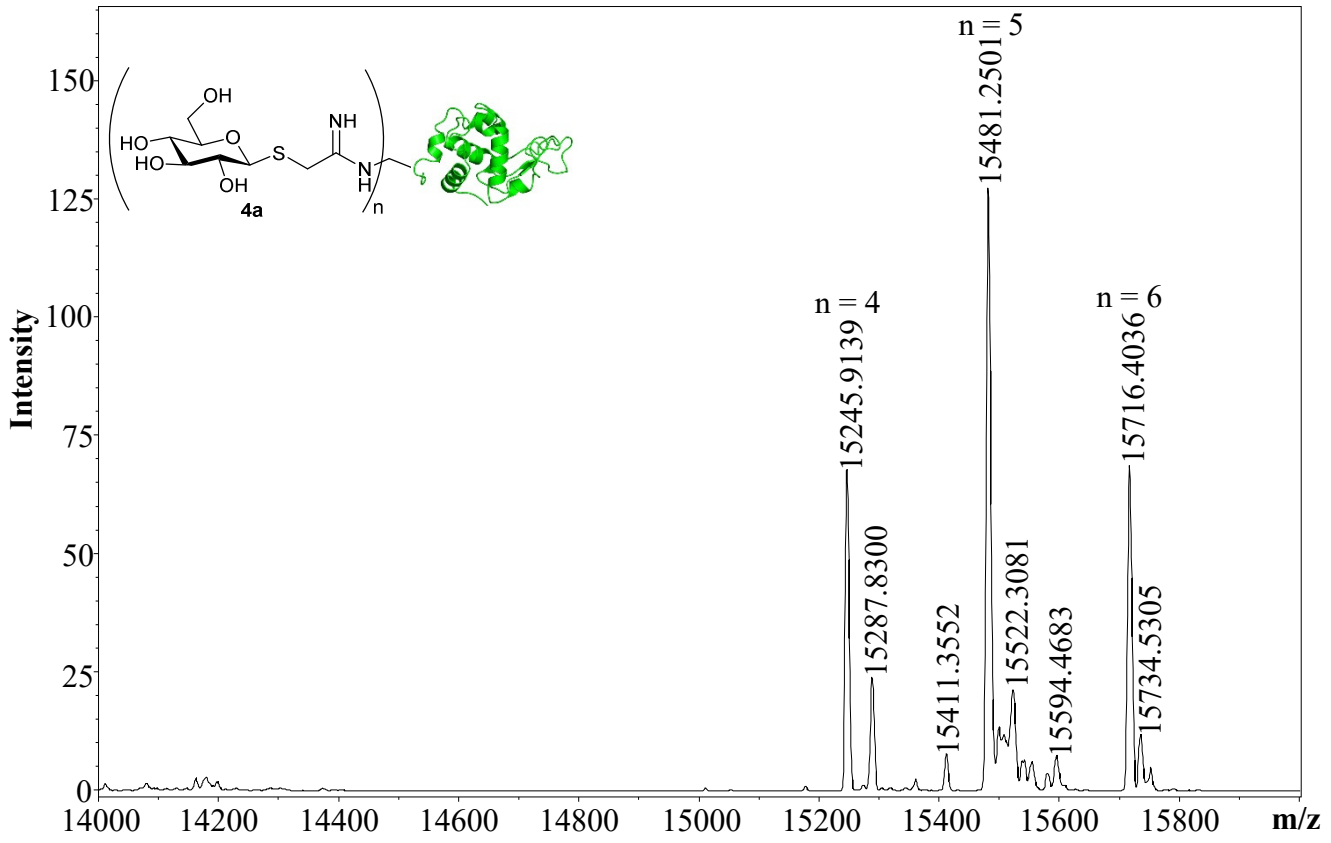


Fig. S2 Deconvoluted HRMS of lysozyme modified with glucose reagent **3a**

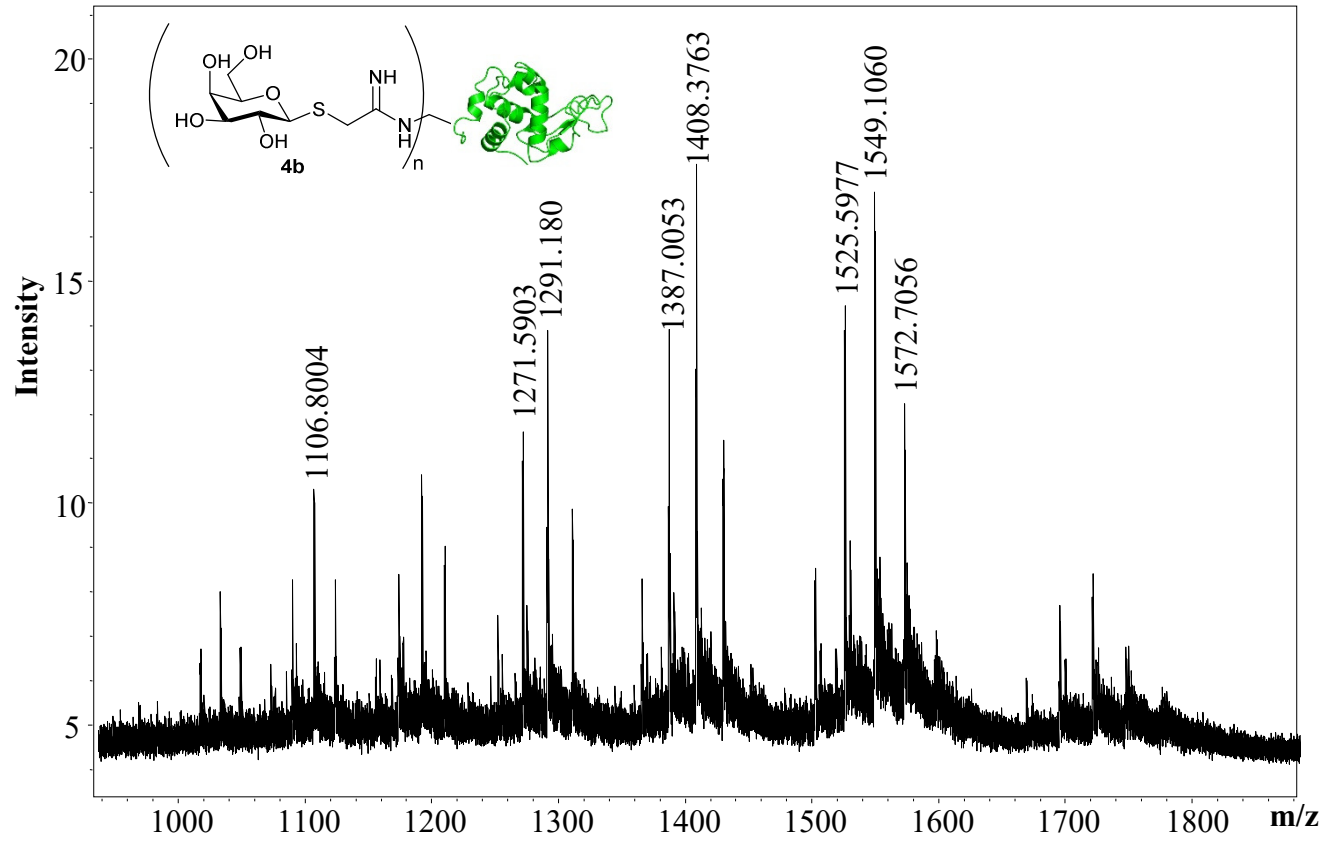


Fig. S3 HRMS of lysozyme modified with galactose reagent **3b**

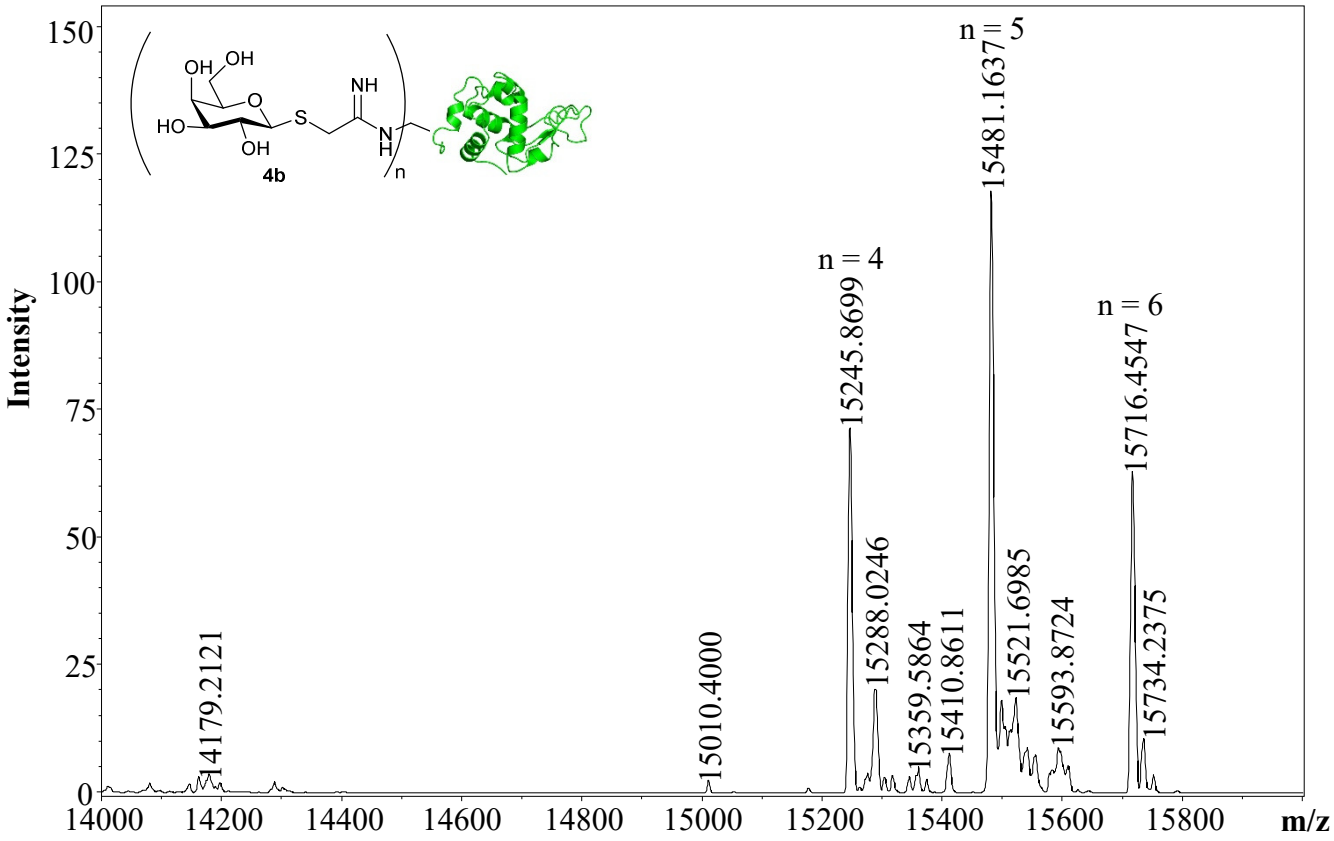


Fig. S4 Deconvoluted HRMS of lysozyme modified with galactose reagent **3b**

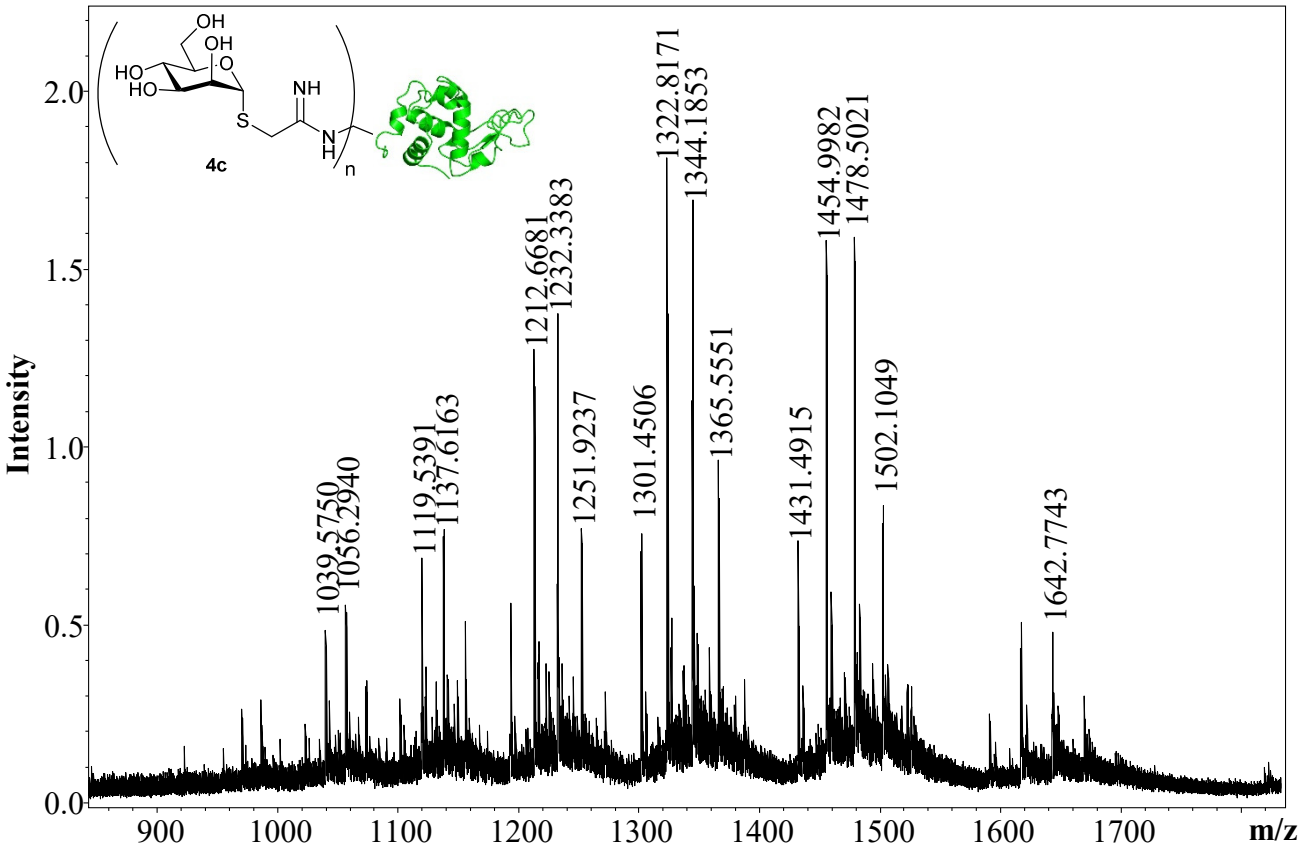


Fig. S5 HRMS of lysozyme modified with mannose reagent **3c**

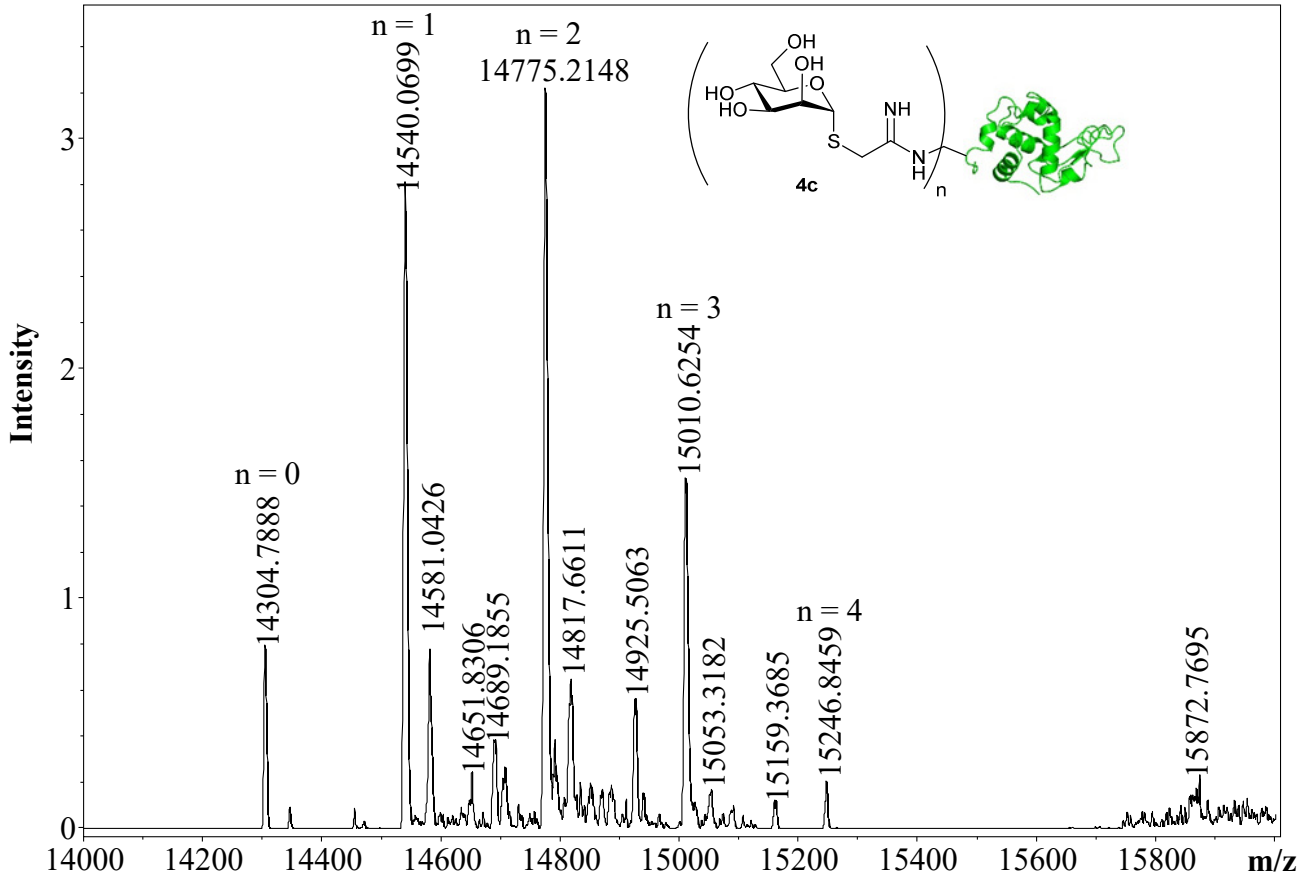


Fig. S6 Deconvoluted HRMS of lysozyme modified with mannose reagent **3c**

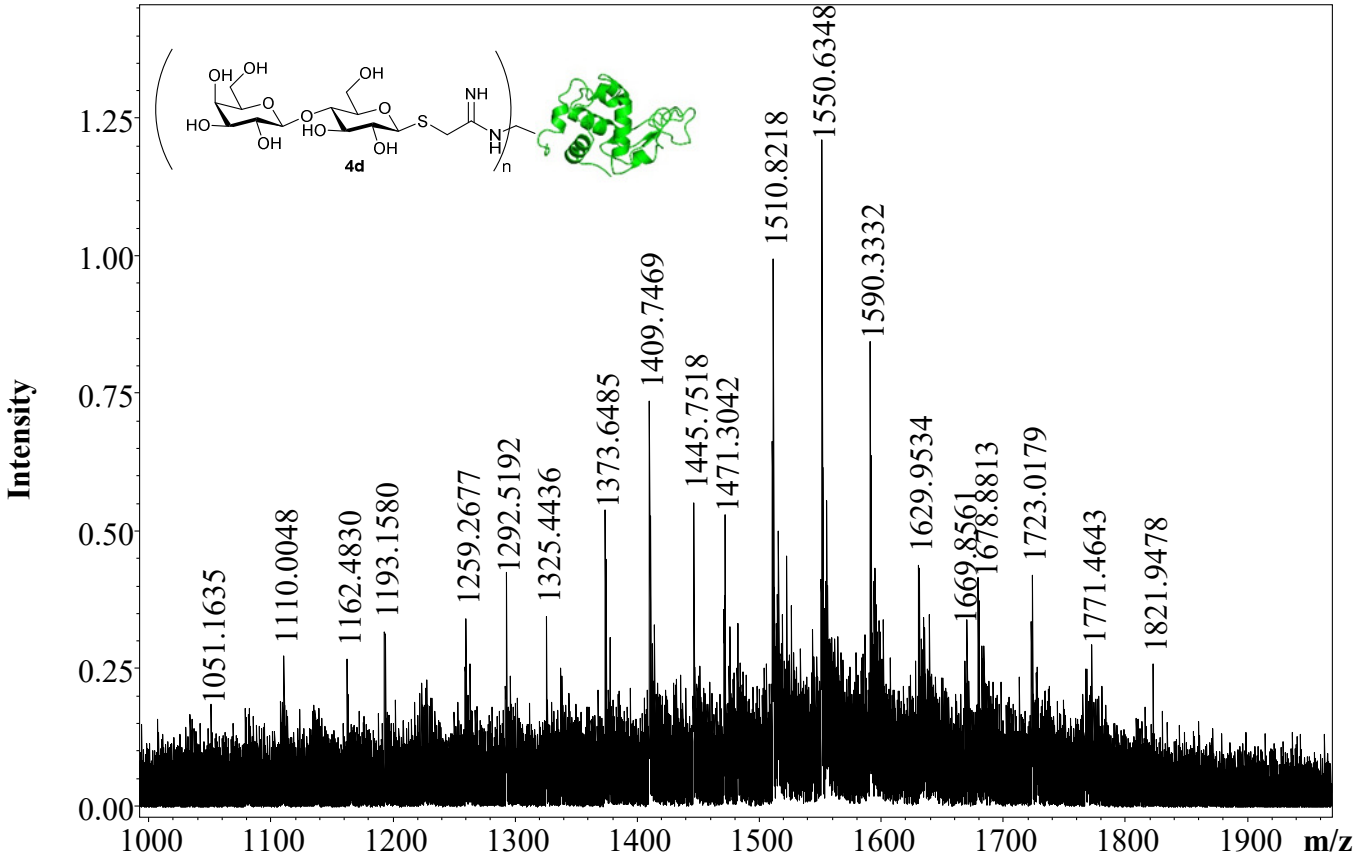


Fig. S7 HRMS of lysozyme modified with lactose reagent **3d**

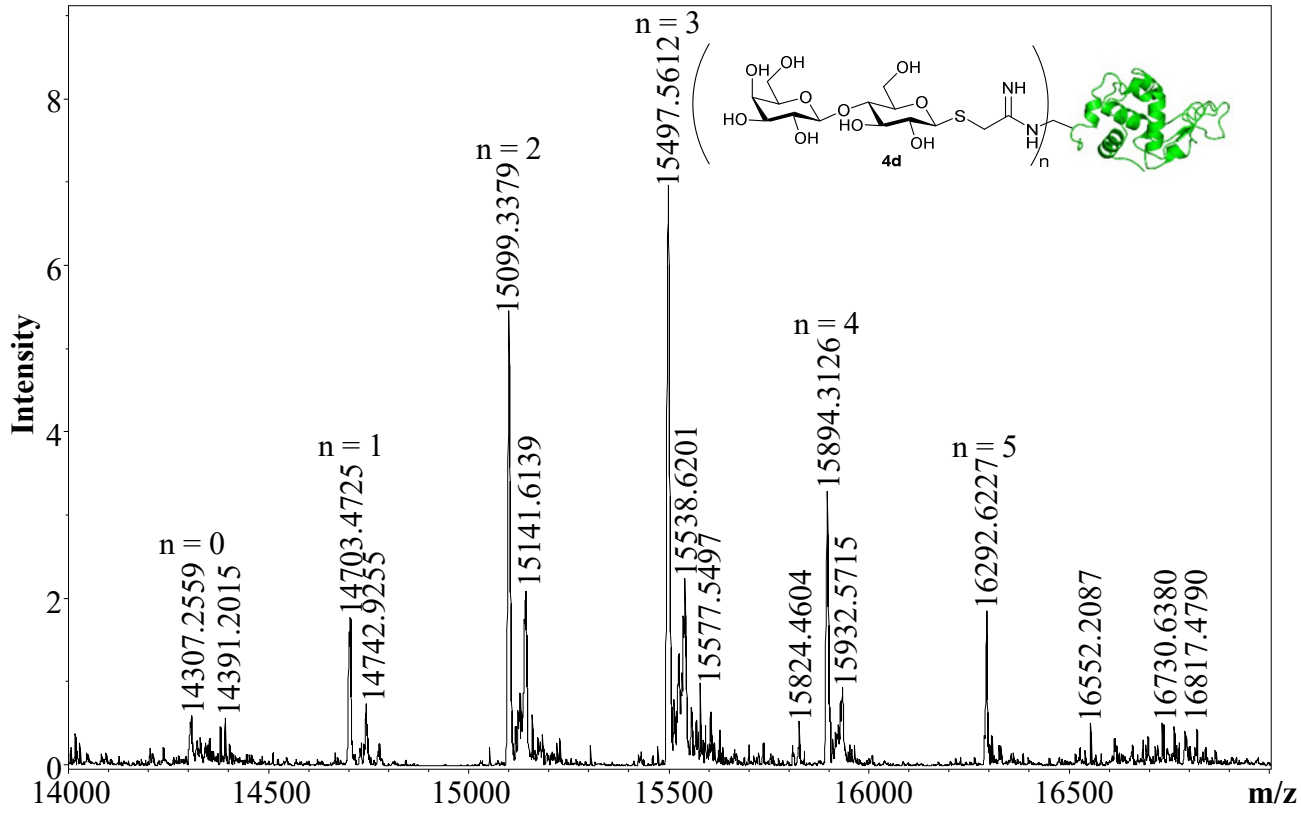


Fig. S8 Deconvoluted HRMS of lysozyme modified with lactose reagent **3d**

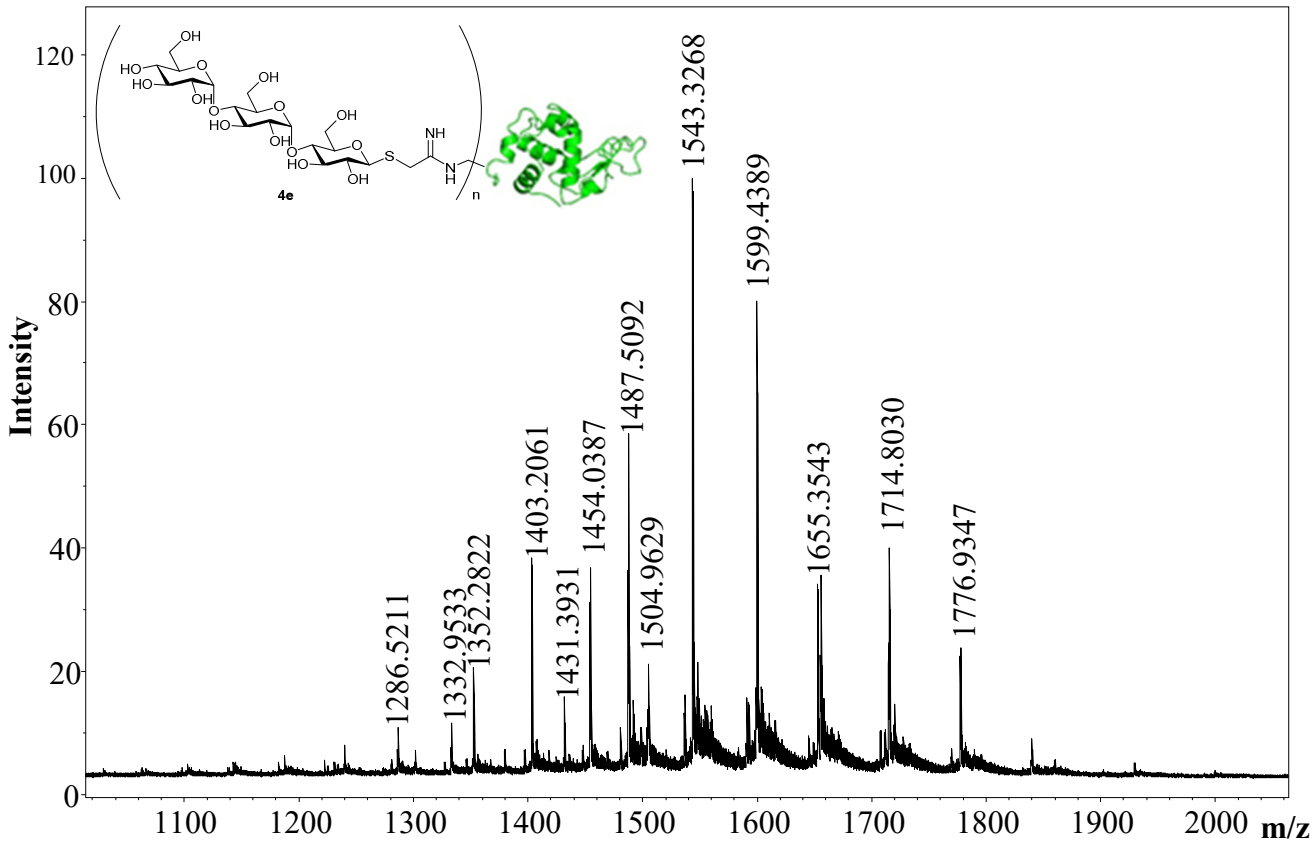


Fig. S9 HRMS of lysozyme modified with maltotriose reagent **3e**

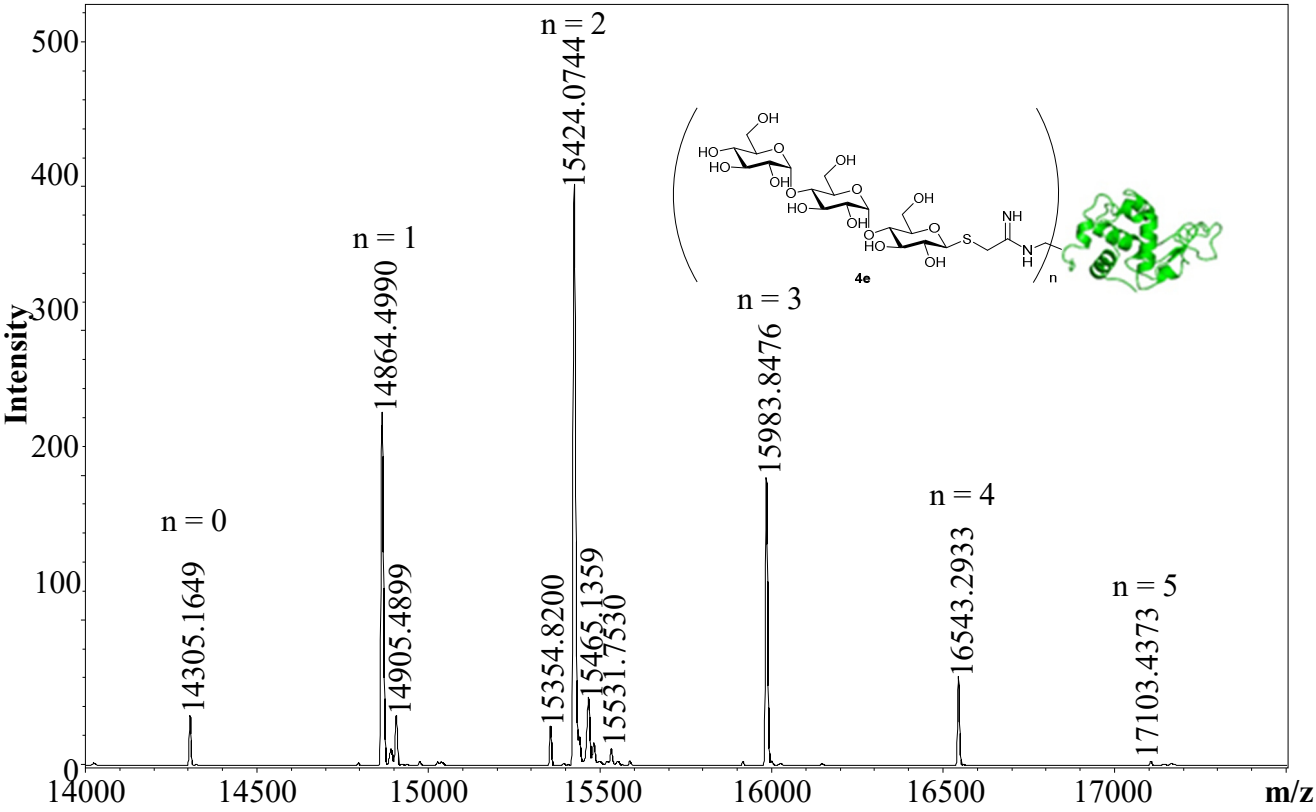


Fig. S10 Deconvoluted HRMS of lysozyme modified with maltotriose reagent **3e**

General Experimental

Reagents were used as supplied without further purification unless otherwise stated. Lysozyme from hens' egg white was purchased from Sigma-Aldrich and used as received. HPLC–grade solvents were used for reactions. Petroleum ether (Petrol) refers to the fraction of light petroleum ether boiling in the range 40-60 °C. Solvent was removed under reduced pressure using a Buchi^{TM} rotary evaporator. Thin Layer Chromatography (t.l.c.) was carried out on Merck Silica Gel 60F254 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, 13C) spectra were recorded on either an Agilent 400–MR instrument operating for 1H NMR at 400 MHz, and at 100 MHz for 13C NMR, or an Agilent 600–MR instrument operating for 1H NMR at 600 MHz, and at 150 MHz for 13C 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, and HMBC. High resolution mass spectra were recorded by Dr. Marie Squire and Dr. Amelia Albrecht 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 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 Bruker FTIR spectrometer with Alpha's Platinum ATR single reflection diamond as neat samples.

Protein Mass Spectrometry Analysis

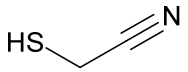
High resolution accurate mass samples were analysed on a maXis 3G UHR-Qq-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) coupled to a Dionex Ultimate 3000 LC system (ThermoFisher) .

5 uL sample was injected. ESI-L Low Concentration Tuning Mix (Agilent Technologies) injected after each sample as a calibrant. Processed using Compass software (Bruker Daltonik GmbH, Bremen, Germany)

LC MS analysis used the following column: Agilent ZORBAX, SB-C18, 5µ, 2.1 x 150 mm; A flow rate of 200 µL/min and the following solvent profile of acetonitrile (Scharlau, Acetonitrile, Multisolvent®, HPLC grade) with water (purified using a MilliQ deionising

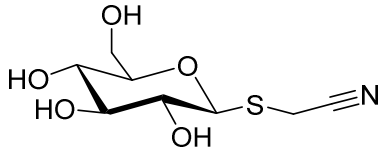
system) with 0.5% formic acid was ultilised: a hold on 100% water for five minutes, followed by a gradient from 0% - 80% CH_3CN/H_2O (0.5% formic acid) over 10 minutes, an isocratic hold on 80% CH_3CN for two minutes and then a restoration to 0% CH_3CN over two minutes. The method was finished with an isocratic hold on 0% CH_3CN over five minutes.
The ESI source was set at 4 kV, 200°C, 1 bar nebuilisation gas and 8 L/min dry gas.

Mercaptoacetonitrile[1]



Chloroacetonitrile (6.3 mL, 0.1 mol) and thioacetic acid (7.05 mL, 0.1 mol) were dissolved in CH_2Cl_2 (70 mL) and cooled to -30 °C under an atmosphere of N_2. Triethylamine (16.7 mL, 0.12 mol) was added dropwise over 0.5 h. The reaction was stirred at -30 °C for another 1 h, and then gradually warmed to RT. Water (5 mL) was added, and the organic layer was washed with acetic acid (10%, 2 x 15 mL) followed by water (2 x 15 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The liquid produced was dissolved in MeOH (100 mL) and Amberlyst® 15 (H^+ form, 3 g) was added. The reaction was heated to reflux under an atmosphere of N_2 and stirred for 20 h. The reaction was filtered, Amberlyst® 15 (H^+ form, 0.3 g) was added, and the mixture was concentrated *in vacuo* at RT to afford mercaptoacetonitrile **45** (5.96 g, 82%) as a pale yellow liquid; υ_{max} (neat) 2247 cm^{-1} (C≡N); δ_H (400 MHz, $CDCl_3$)[1] 2.37 (1H, t, *J* 8.2 Hz, SH), 3.29 (2H, d, *J* 8.2 Hz, CH_2); δ_C (100.5 MHz, $CDCl_3$) 9.6 (t, $\underline{C}H_2$), 118.2 (s, $\underline{C}N$).

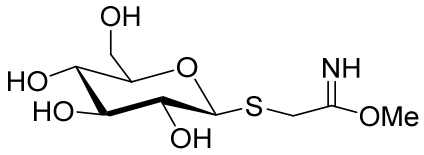
Cyanomethyl 1-thio-β-D-glucopyranoside 2a



D-Glucose (200 mg, 1.11 mmol) was dissolved in water (4 mL). Triethylamine (2.5 mL, 34 mmol) was added, the reaction was cooled to 0 °C, and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride (0.92 g, 5.5 mmol) was dissolved in mercaptoacetonitrile

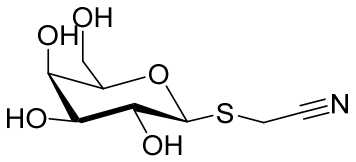
(1.3 g, 17.8 mmol) and the resulting solution was added dropwise to the aqueous D-glucose solution over 2 min. The reaction was stirred at 0 °C for 0.5 h, and was then diluted with water (5 mL) and washed with CH_2Cl_2 (5 x 10 mL). The aqueous layer was filtered through a column of Amberlite® IR-120 (Na^+ form, produced by treating the H^+ form with 1 M aqueous NaOH solution and then washing with water until pH 7), lyophilised, pre-adsorbed onto silica, and purified by flash column chromatography ($CHCl_3$:MeOH, 5:1) to afford cyanomethyl 1-thio-β-D-glucopyranoside **2a** (232 mg, 89%) as a pale yellow oil; $[\alpha]_D^{20}$ -67 (*c*, 1.0 in MeOH) [lit. $[\alpha]_D^{22}$ -60.2 (*c*, 5.05 in water)][2] ; υ_{max} (neat) 2248 cm^{-1} (C≡N); δ_H (400 MHz, D_2O) 3.37 - 3.47 (2H, m, H-2 & H-3), 3.49 - 3.55 (2H, m, H-4 & H-5), 3.68, 3.79 (2H, ABq, *J* 17.6 Hz, C<u>H</u>$_2$CN) 3.72 (1H, dd, $J_{5,6}$ 5.5 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 3.91 (1H, dd, $J_{5,6'}$ 1.8 Hz, $J_{6,6'}$ 12.3 Hz, H-6'), 4.72 (1H, d, $J_{1,2}$ 9.8 Hz, H-1); δ_C (100.5 MHz, D_2O) 14.4 (t, <u>C</u>H$_2$CN), 60.7 (t, C-6), 69.2 (d, C-2), 71.8 (d, C-3), 77.0, 80.0 (2 x d, C-4 & C-5), 84.4 (d, C-1), 118.6 (s, CH$_2$<u>C</u>N); HRMS (ESI-TOF): calcd. for $C_8H_{13}NO_5SNa^+$: 258.0407. Found: 258.0414 (MNa^+).

2-Imino-2-methoxyethyl 1-thio-β-D-glucopyranoside 3a[2]



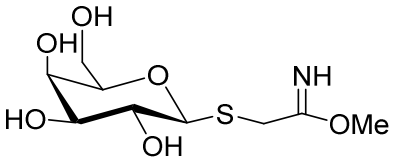
Sodium metal (8 mg, 0.36 mmol) was dissolved in MeOH (3.6 mL). Cyanomethyl 1-thio-β-D-glucopyranoside **2a** (83.6 mg, 0.36 mmol) was added and the reaction was stirred for 64 h. The reaction mixture was then concentrated *in vacuo* and analysed by ^1H NMR to reveal the formation of 2-imino-2-methoxyethyl 1-thio-β-D-glucopyranoside **47** (35% conversion). The crude material was subsequently used without purification for conjugation experiments; υ_{max} (neat) 1654 cm^{-1} (C=N); δ_H (400 MHz, CD_3OD) 4.32 Hz (1H, d, *J* 10.2 Hz, H-1); HRMS (ESI-TOF): calcd. for $C_9H_{18}NO_6S^+$: 268.0849. Found: 268.0860 (MH^+).

Cyanomethyl 1-thio-β-D-galactopyranoside 2b



D-Galactose (200 mg, 1.11 mmol) was dissolved in water (4 mL). Triethylamine (2.4 mL, 33 mmol) was added, the reaction was cooled to 0 °C, and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride (1.12 g, 6.7 mmol) was dissolved in mercaptoacetonitrile (1.6 g, 22 mmol) and the resulting solution was added dropwise to the aqueous D-galactose solution over 2 min. The reaction was stirred at 0 °C for 0.5 h, and was then diluted with water (5 mL) and washed with CH_2Cl_2 (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), lyophilised, pre-adsorbed onto silica, and purified by flash column chromatography ($CHCl_3$:MeOH, 5:1) to afford cyanomethyl 1-thio-β-D-galactopyranoside **2b** (106 mg, 41%) as a pale yellow oil; $[\alpha]_D^{20}$ +13 (*c*, 0.1 in MeOH) [lit. $[\alpha]_D^{20}$ -51.5 (*c*, 5.05 in water)][2]; υ_{max} (neat) 2252 cm^{-1} (C≡N); δ_H (400 MHz, D_2O) 3.62 - 3.80 (5H, m, H-2, H-4, H-5, H-6 & H-6'), 3.69, 3.81 (2H, ABq, *J* 17.6 Hz, CH_2) 3.99 (1H, d, $J_{2,3}$ 3.1 Hz, H-4), 4.66 (1H, d, $J_{1,2}$ 9.4 Hz, H-1); δ_C (100.5 MHz, D_2O) 14.5 (t, $\underline{C}H_2CN$), 61.0 (t, H-6), 68.6 (d, C-4), 69.2 (d, C-2), 73.8, 79.1 (2 x d, C-3 & C-5), 84.9 (d, C-1), 118.6 (s, $CH_2\underline{C}N$); HRMS (ESI-TOF): calcd. for $C_8H_{13}NO_5SNa^+$: 258.0407. Found: 258.0413 (MNa^+).

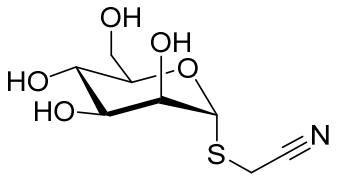
2-Imino-2-methoxyethyl 1-thio-β-D-galactopyranoside 3b[2]



Sodium metal (5 mg, 0.22 mmol) was dissolved in MeOH (2.5 mL). Cyanomethyl 1-thio-β-D-galactopyranoside **2b** (52 mg, 0.22 mmol) was added and the reaction was stirred for 64 h. The reaction mixture was then concentrated *in vacuo* and analysed by ^1H NMR to reveal the formation of 2-imino-2-methoxyethyl 1-thio-β-D-galactopyranoside **3b** (50% conversion).

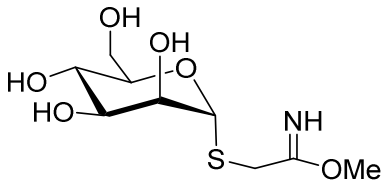
The crude material was subsequently used without purification for the conjugation experiments; υ_{max} (neat) 1651 cm^{-1} (C=N); δ_H (400 MHz, CD_3OD)[3] 4.27 (1H, d, $J_{1,2}$ 9.4 Hz, H-1); HRMS (ESI-TOF): calcd. for $C_9H_{18}NO_6S^+$: 268.0849. Found: 268.0853 (MH^+).

Cyanomethyl 1-thio-α-D-mannopyranoside 2c



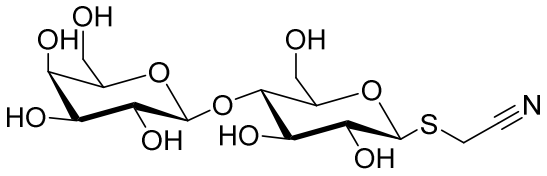
D-Mannose (100 mg, 0.56 mmol) was dissolved in water (2 mL). Triethylamine (1.2 mL, 11.1 mmol) was added, the reaction was cooled to 0 °C, and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride (0.56 g, 3.3 mmol) was dissolved in mercaptoacetonitrile (0.8 g, 11.1 mmol) and the resulting solution was added dropwise to the aqueous D-mannose solution over 2 min. The reaction was stirred at 0 °C for 0.5 h, and was then diluted with water (2 mL) and washed with CH_2Cl_2 (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), lyophilised, pre-adsorbed onto silica, and purified by flash column chromatography ($CHCl_3$:MeOH, 5:1) to afford cyanomethyl 1-thio-α-D-mannopyranoside **2c** (103 mg, 78%) as a yellow oil; $[\alpha]_D^{20}$ +80 (*c*, 1.0 in MeOH); υ_{max} (neat) 3327 cm^{-1} (OH), 2255 cm^{-1} (CN); δ_H (400 MHz, D_2O) 3.60, 3.65 (2H, ABq, *J* 17.6 Hz, $SC\underline{H}_2CN$), 3.71 - 3.76 (2H, m, H-3 & H-4), 3.80 (1H, dd, $J_{5,6}$ 5.5 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 3.88 (1H, dd, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.1 Hz, H-6'), 3.91 - 3.97 (1H, m, H-5), 4.08 (1H, d, $J_{2,3}$ 1.2 Hz, H-2), 5.47 (1H, s, H-1); δ_C (100.5 MHz, D_2O) 15.3 (t, $S\underline{C}H_2CN$), 60.4 (t, C-6), 66.7 (d, C-3), 70.8 (d, C-2), 71.0 (d, C-4), 73.5 (d, C-5), 84.8 (d, C-1), 118.2 (s, $SCH_2\underline{C}N$); HRMS (ESI-TOF): calcd. for $C_8H_{13}NO_5SNa^+$: 258.0407. Found: 258.0401 (MNa^+).

2-Imino-2-methoxyethyl 1-thio-α-D-mannopyranoside 3c[2]



Sodium metal (13.7 mg, 0.60 mmol) was dissolved in MeOH (6 mL). Cyanomethyl 1-thio-α-D-mannopyranoside **2c** (140 mg, 0.60 mmol) was added and the reaction was stirred for 64 h. The reaction mixture was then concentrated *in vacuo* and analysed by ^{1}H NMR to reveal the formation of 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside **3c** (22% conversion). The crude material was subsequently used without purification for conjugation experiments; υ_{max} (neat) 3311 cm^{-1} (OH), 1649 cm^{-1} (C=NH); δ_H (400 MHz, CD_3OD) 5.34 (1H, s, H-1); HRMS (ESI-TOF): calcd. for $C_9H_{18}NO_6S^+$: 268.0849. Found: 268.0848 (MH^+).

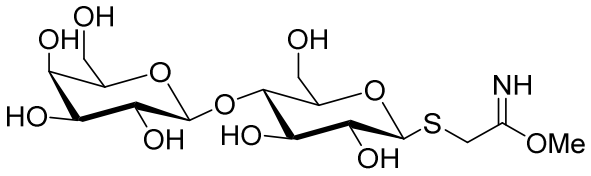
Cyanomethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 2d



β-D-Galactopyranosyl-(1→4)-D-glucopyranose (103 mg, 0.3 mmol) was dissolved in water (2 mL). Triethylamine (0.84 mL, 6 mmol) was added, the reaction was cooled to 0 °C, and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride (0.303 g, 1.8 mmol) was dissolved in mercaptoacetonitrile (0.439 g, 6 mmol) and the resulting solution was added dropwise to the aqueous β-D-galactopyranosyl-(1→4)-D-glucopyranose solution over 2 min. The reaction was stirred at 0 °C for 0.5 h, and was then diluted with water (2 mL) and washed with CH_2Cl_2 (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), lyophilised, pre-adsorbed onto silica, and purified by flash column chromatography ($CHCl_3$:MeOH, 5:1) to afford cyanomethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside **2d** (77 mg, 65%) as a yellow oil; $[\alpha]_D^{20}$ -25 (*c*, 0.1 in MeOH); υ_{max} (neat) 3298 cm^{-1} (OH), 2251 cm^{-1} (CN); δ_H (400 MHz, CD_3OD) 3.37 (1H, t, *J* 9.8 Hz, H-2$_A$),

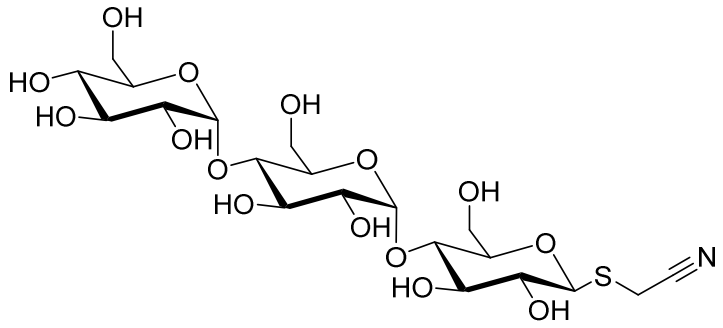
3.42 - 3.65 (6H, m, H-3_A, H-4_A, H-5_A, H-2_B, H-3_B & H-5_B), 3.66 - 3.88 (6H, m, C$\underline{H}_2$, H-6_A, H-4_B, H-6_B & H-6'$_B$), 3.94 (1H, dd, $J_{5,6}$ 2.0 Hz, $J_{6,6'}$ 12.1 Hz, H-6'$_A$), 4.37 (1H, d, $J_{1,2}$ 7.4 Hz, H-1_B), 4.60 (1H, d, $J_{1,2}$ 9.4 Hz, H-1_A); δ_C (100.5 MHz, CD_3OD) 13.1 (t, $\underline{C}H_2CN$), 60.5 (t, C-6_A), 61.1 (t, C-6_B), 68.9 (d, C-4_B), 71.1, 72.6, 73.3, 75.6, 76.4, 78.8, 79.4 (7 x d, C-2_A, C-3_A, C-4_A, C-5_A, C-2_B, C-3_B & C-5_B), 83.9 (d, C-1_A), 103.6 (d, C-1_B), 117.3 (s, $CH_2\underline{C}N$); HRMS (ESI-TOF): calcd. for $C_{14}H_{24}NO_{10}S^+$: 398.1115. Found: 398.1109 (MH^+).

2-Imino-2-methoxyethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 3d[2]



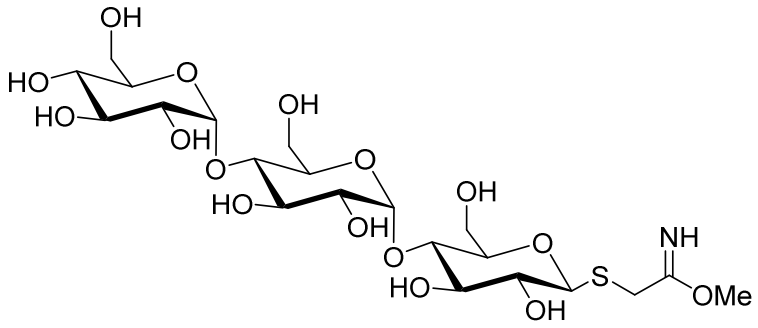
Sodium metal (7.6 mg, 0.33 mmol) was dissolved in MeOH (3.3 mL). Cyanomethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside **2d** (130 mg, 0.33 mmol) was added and the reaction was stirred for 64 h. The reaction mixture was then concentrated *in vacuo* and analysed by ^{1}H NMR to reveal the formation of 2-imino-2-methoxyethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside **3d** (85% conversion). The crude material was subsequently used without purification for conjugation experiments; υ_{max} (neat) 3279 cm^{-1} (OH), 1652 cm^{-1} (C=NH); δ_H (400 MHz, CD_3OD) 4.36 (1H, d, *J* 7.4 Hz, H-1_B), 4.45 (1H, d, *J* 7.4 Hz, H-1_A); HRMS (ESI-TOF): calcd. for $C_{15}H_{28}NO_{11}S^+$: 430.1378. Found: 430.1392 (MH^+).

Cyanomethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 2e



α-D-Glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-β-D-glucopyranose (101 mg, 0.2 mmol) was dissolved in D_2O (1.3 mL). Triethylamine (0.56 mL, 4 mmol) was added, the reaction was cooled to 0 °C, and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride (0.202 g, 1.2 mmol) was dissolved in mercaptoacetonitrile (0.292 g, 4 mmol) and the resulting solution was added dropwise to the aqueous α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-β-D-glucopyranose solution over 2 min. The reaction was stirred at 0 °C for 0.5 h, and was then diluted with water (2 mL) and washed with CH_2Cl_2 (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), lyophilised, pre-adsorbed onto silica, and purified by flash column chromatography ($CHCl_3$:MeOH, 5:1) to afford cyanomethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside **2e** (101 mg, 90%) as a yellow oil; $[\alpha]_D^{20}$ +54 (*c*, 1.0 in MeOH); υ_{max} (neat) 3279 cm^{-1} (OH), 2248 cm^{-1} (CN); δ_H (600 MHz, CD_3OD) 3.26 (1H, t, *J* 9.2 Hz), 3.33 (1H, t, *J* 9.6 Hz), 3.42 - 3.47 (2H, m), 3.48 - 3.53 (2H, m), 3.57 (1H, t, *J* 9.2 Hz), 3.61 (1H, t, *J* 9.4 Hz), 3.63 - 3.70 (3H, m), 3.73 - 3.84 (7H, m), 3.84 - 3.88 (2H, m), 3.90 (1H, dd, *J* 12.4 Hz, *J* 2.0 Hz), 4.59 (1H, d, $J_{1,2}$ 9.6 Hz, H-1_A), 5.15 (1H, d, $J_{1,2}$ 3.7 Hz, H-1_B), 5.19 (1H, d, $J_{1,2}$ 3.9 Hz, H-1_C); δ_C (151 MHz, CD_3OD) 13.0 (t, S<u>C</u>H_2CN), 60.7, 60.9, 61.3 (3 x t, 3 x C-6), 70.1, 71.9, 72.4, 72.5, 72.8, 73.4, 73.5, 73.6, 77.8, 79.3, 79.4, 79.9, 83.9 (d, C-1_A), 101.2 (d, C-1_C), 101.5 (d, C-1_B), 117.2 (SCH_2<u>C</u>N); HRMS (ESI-TOF): calcd. for $C_{20}H_{33}NO_{15}SNa^+$: 582.14633. Found: 582.1461 (MNa^+).

2-Imino-2-methoxyethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 3e



Sodium metal (4 mg, 0.17 mmol) was dissolved in MeOH (1.7 mL). cyanomethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside **2e** (97 mg, 0.17 mmol) was added and the reaction was stirred for 64 h. The reaction mixture was then concentrated *in vacuo* and analysed by ^{1}H NMR to reveal the formation of 2-imino-2-methoxyethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside **3e** (60% conversion). The crude material was subsequently used without purification for conjugation experiments; υ_{max} (neat) 3289 cm^{-1} (OH), 1652 cm^{-1} (C=NH); δ_H (400 MHz, D_2O) 4.69 (1H, d, $J_{1,2}$ 10.6 Hz, H-1_A), 5.29 - 5.35 (2H, m, H-1_B & H-1_C); HRMS (ESI-TOF): calcd. for $C_{21}H_{38}NO_{16}S^+$: 592.1906. Found: 592.1907 (MH^+).

Conjugation of 2-imino-2-methoxyethyl 1-thio-β-D-glucopyranoside 3a to lysozyme

Lysozyme (1 mg, 0.070 μmol) was dissolved in sodium borate buffer (0.2 mL, 0.2 M, pH 8.5). A portion of the 2-imino-2-methoxyethyl 1-thio-β-D-glucopyranoside **3a** crude reaction mixture (50 μL, approximately 1.8 μmol of **3a**) was added to the lysozyme solution and the reaction was stirred. After 5 h the reaction was dialysed against water (2 x 500 mL), and filtered; HRMS (ESI-TOF): 15246, 54% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)_4$: 15247), 15481, 100% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)_5$: 15482), 15715, 54% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)_6$: 15717).

Conjugation of 2-imino-2-methoxyethyl 1-thio-β-D-galactopyranoside 3b to lysozyme

Lysozyme (1 mg, 0.070 μmol) was dissolved in sodium borate buffer (0.2 mL, 0.2 M, pH 8.5). A portion of the 2-imino-2-methoxyethyl 1-thio-β-D-galactopyranoside **3b** crude reaction mixture (50 μL, approximately 2.2 μmol of **3b**) was added to the lysozyme solution

and the reaction was stirred. After 5 h the reaction was dialysed against water (2 x 500 mL), and filtered; HRMS (ESI-TOF): 15010, 2% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)_3$: 15012), 15246, 61% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)_4$: 15247), 15481, 100% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)_5$: 15482), 15717, 54% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)_6$: 15717).

Conjugation of 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside 3c to lysozyme

Lysozyme (1 mg, 0.070 μmol) was dissolved in sodium borate buffer (0.2 mL, 0.2 M, pH 8.5). A portion of the 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside **3c** crude reaction mixture (50 μL, approximately 1.1 μmol of **3c**) was added to the lysozyme solution and the reaction was stirred. After 5 h the reaction was dialysed against water (2 x 500 mL), and filtered; HRMS (ESI-TOF): 14305, 25% (calcd. for lysozyme: 14307), 14540, 87% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)$: 14542), 14775, 100% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)_2$: 14777), 15011, 47% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)_3$: 15012), 15247, 6% (calcd. for lysozyme-$(C(NH)CH_2SC_6H_{11}O_5)_4$: 15247).

Conjugation of 2-imino-2-methoxyethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 3d to lysozyme

Lysozyme (1 mg, 0.070 μmol) was dissolved in sodium borate buffer (0.2 mL, 0.2 M, pH 8.5). A portion of the 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside **3d** crude reaction mixture (50 μL, approximately 1.7 μmol of **3d**) was added to the lysozyme solution and the reaction was stirred. After 5 h the reaction was dialysed against water (2 x 500 mL), and filtered; HRMS (ESI-TOF): 14307, 9% (calcd. for lysozyme: 14307), 14703, 25% (calcd. for lysozyme-$(C(NH)CH_2SC_{12}H_{21}O_{10})$: 14704), 15099, 78% (calcd. for lysozyme-$(C(NH)CH_2SC_{12}H_{21}O_{10})_2$: 15101), 15497, 100% (calcd. for lysozyme-$(C(NH)CH_2SC_{12}H_{21}O_{10})_3$: 15498), 15894, 47% (calcd. for lysozyme-$(C(NH)CH_2SC_{12}H_{21}O_{10})_4$: 15895), 16293, 27% (calcd. for lysozyme-$(C(NH)CH_2SC_{12}H_{21}O_{10})_5$: 16292).

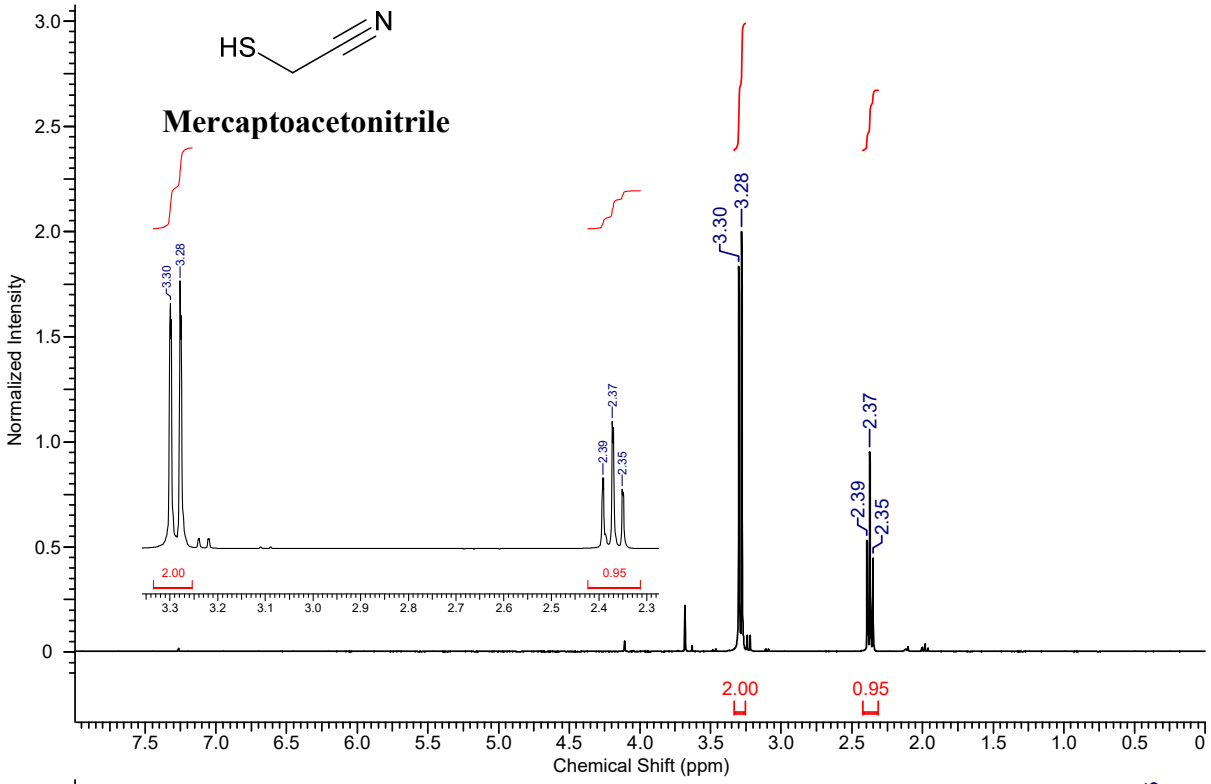
Conjugation of 2-imino-2-methoxyethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 3e to lysozyme

Lysozyme (1 mg, 0.070 μmol) was dissolved in sodium borate buffer (0.2 mL, 0.2 M, pH 8.5). A portion of crude 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside **3e** (3 mg, approximately 3 μmol of **3e**) was added to the lysozyme solution and the reaction was stirred.

After 5 h the reaction was dialysed against water (2 x 500 mL), and filtered; HRMS (ESI-TOF): 14305, 9% (calcd. for lysozyme: 14307), 14864, 61% (calcd. for lysozyme-($C(NH)CH_2SC_{18}H_{31}O_{15}$): 14866), 150424, 100% (calcd. for lysozyme-($C(NH)CH_2SC_{18}H_{31}O_{15}$)$_2$: 15425), 15983, 50% (calcd. for lysozyme-($C(NH)CH_2SC_{18}H_{31}O_{15}$)$_3$: 15984), 16543, 15% (calcd. for lysozyme-($C(NH)CH_2SC_{18}H_{31}O_{15}$)$_4$: 16543), 17103, 1% (calcd. for lysozyme-($C(NH)CH_2SC_{18}H_{31}O_{15}$)$_5$: 17102).

References

1. Gaumont, A. C.; Wazneh, L.; Denis, J. M., *Tetrahedron* **1991,** *47* (27), 4927-4940.
2. Lee, Y. C.; Stowell, C. P.; Krantz, M. J., *Biochemistry* **1976,** *15* (18), 3956-3963.
3. Pearce, O. M. T.; Fisher, K. D.; Humphries, J.; Seymour, L. W.; Smith, A.; Davis, B. G., *Angew. Chem., Int. Ed.* **2005,** *44* (7), 1057-1061.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2.00
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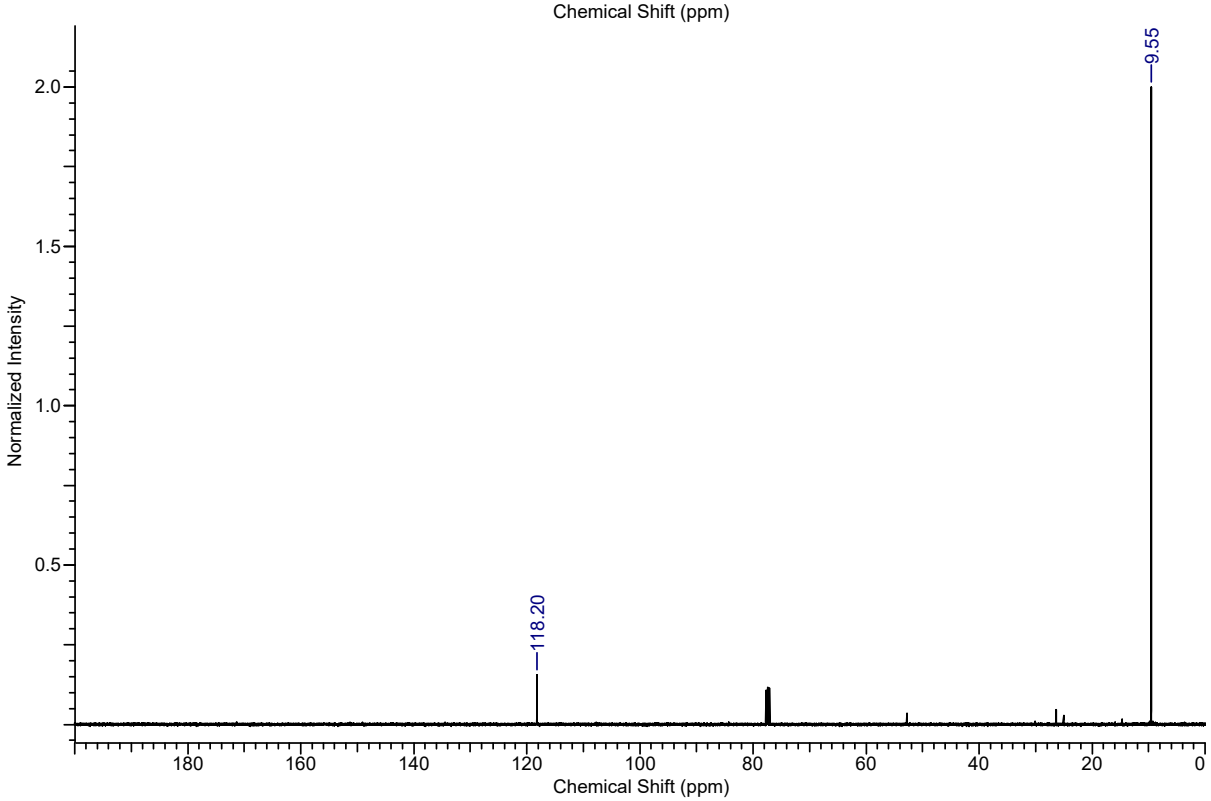
Normalized Intensity
Chemical Shift (ppm)
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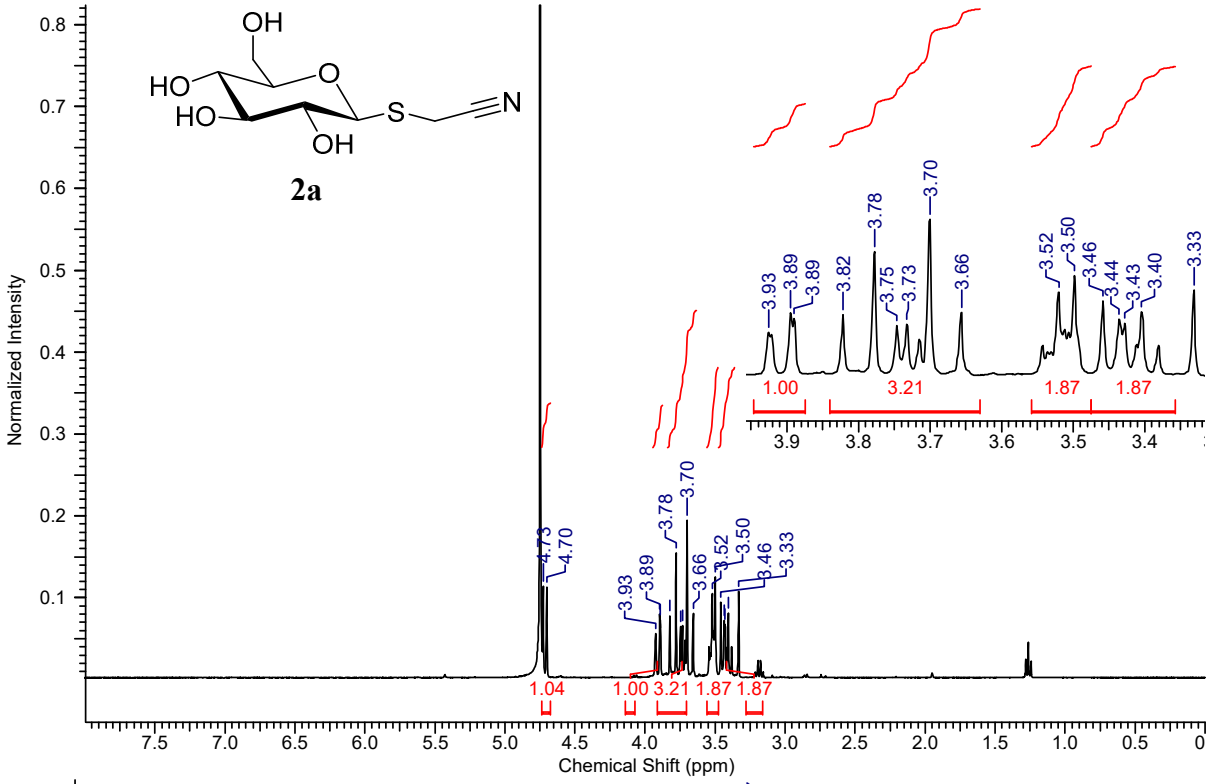
118.20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HO
O
HO
S
N
OH
2a
Normalized Intensity
Chemical Shift (ppm)
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3.78
3.70
3.66
3.52
3.50
3.46
3.33
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3.21
1.87
1.87
3.89
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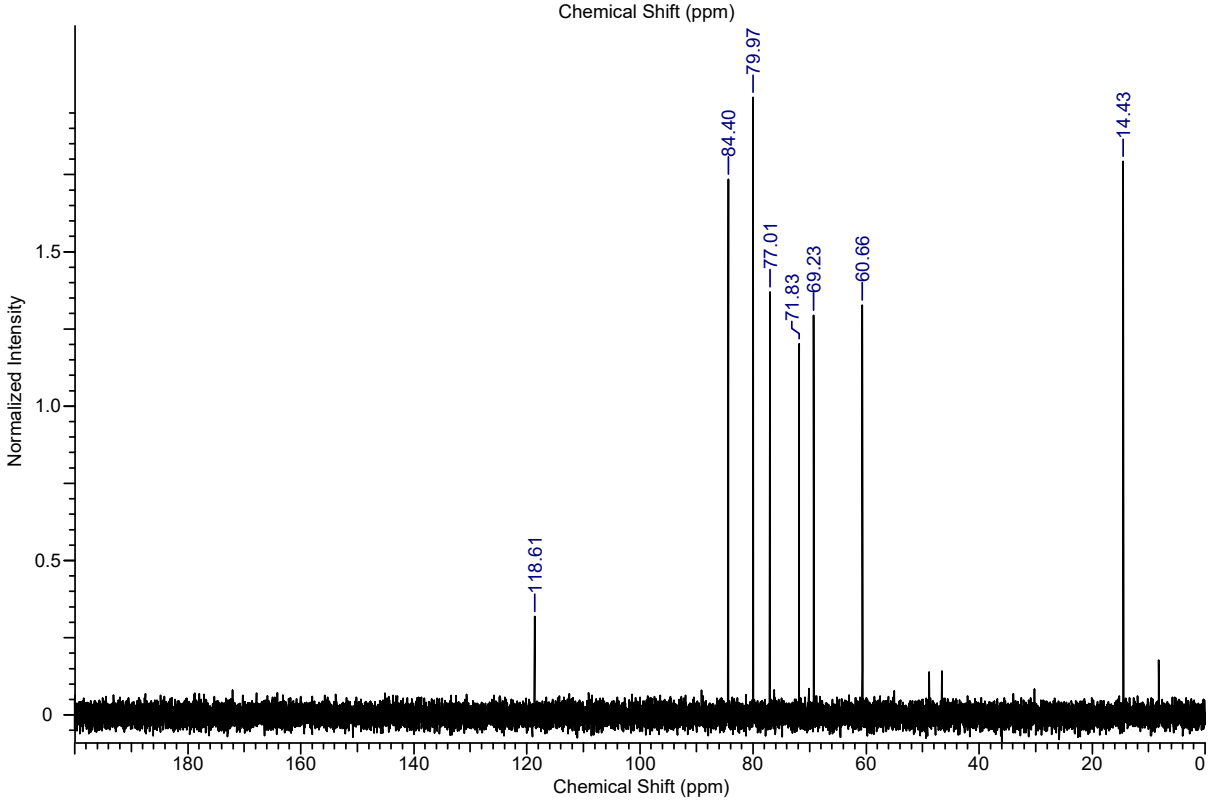
3.75
3.73
3.44
3.43
3.40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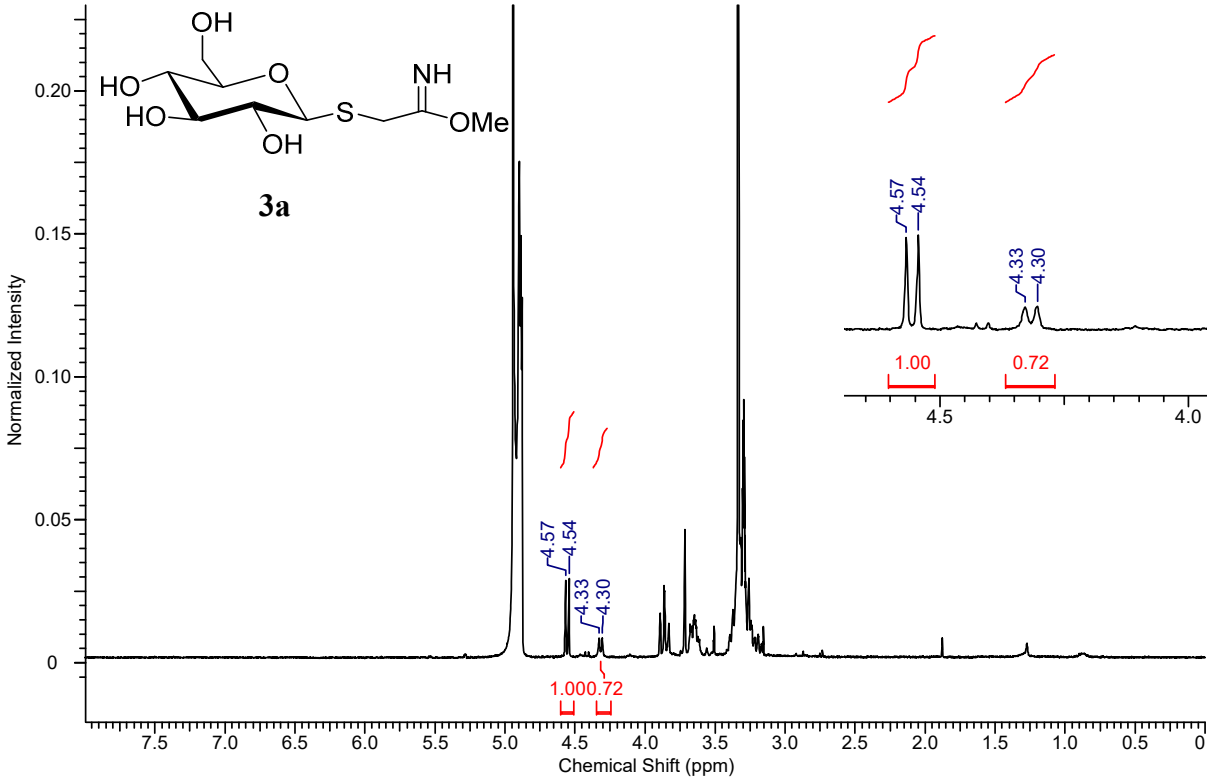
60.66
14.43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HO
O
NH
HO
S
OMe
OH
3a
Normalized Intensity
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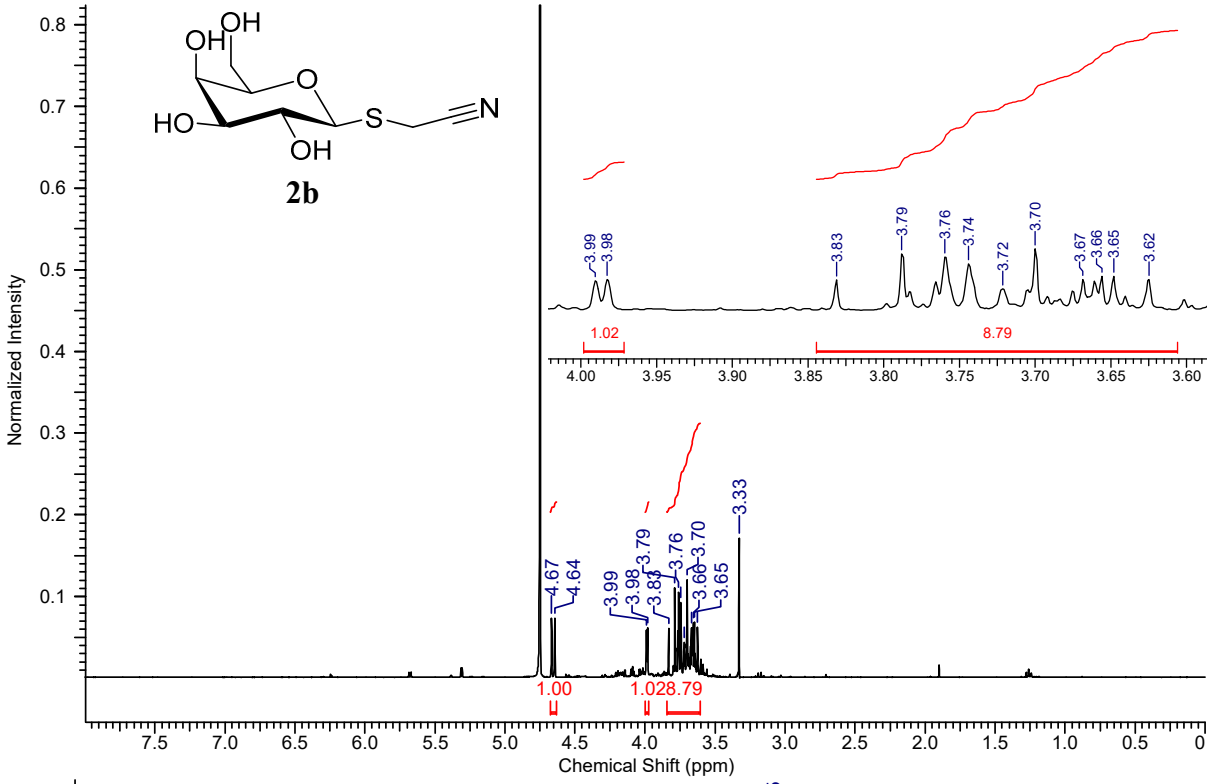
0.10
0.05
0
4.57
4.54
4.33
4.30
1.00
0.72
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)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OH
O
HO
S
N
OH
2b
Normalized Intensity
Chemical Shift (ppm)
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3.65
3.62
3.33
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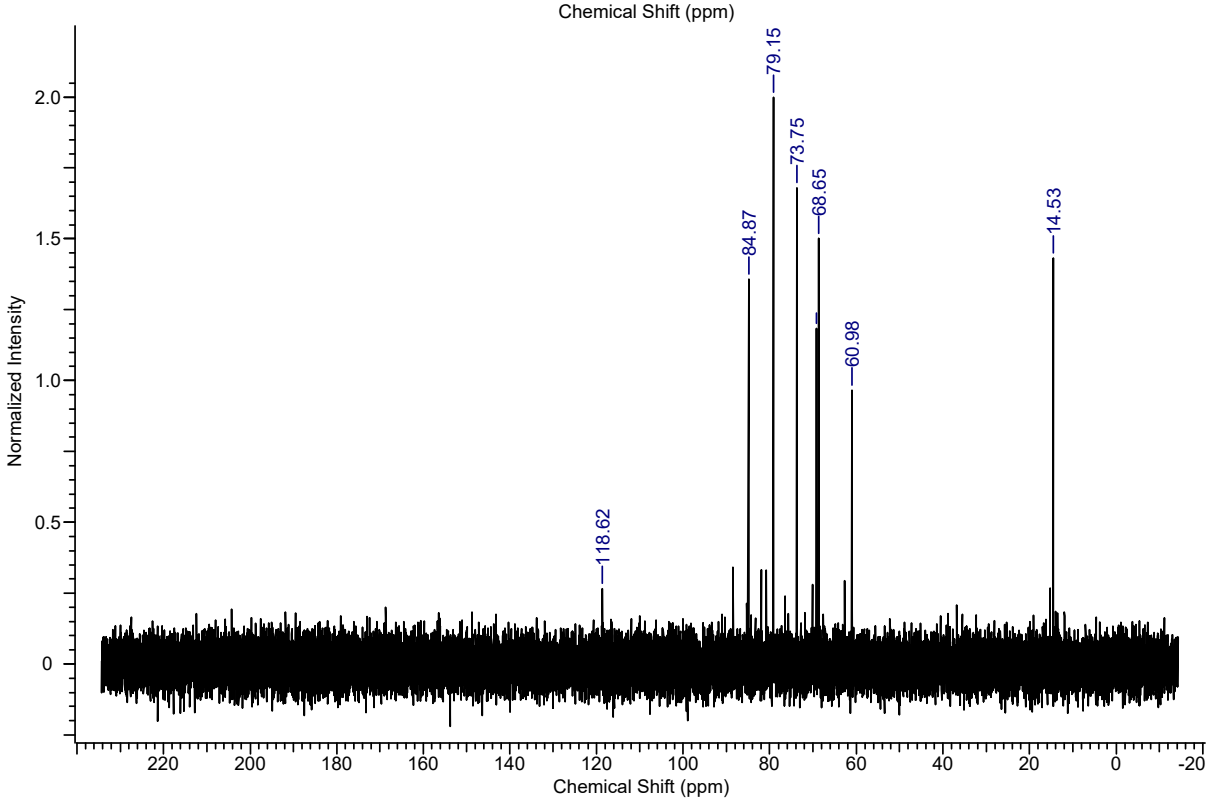
1.02
8.79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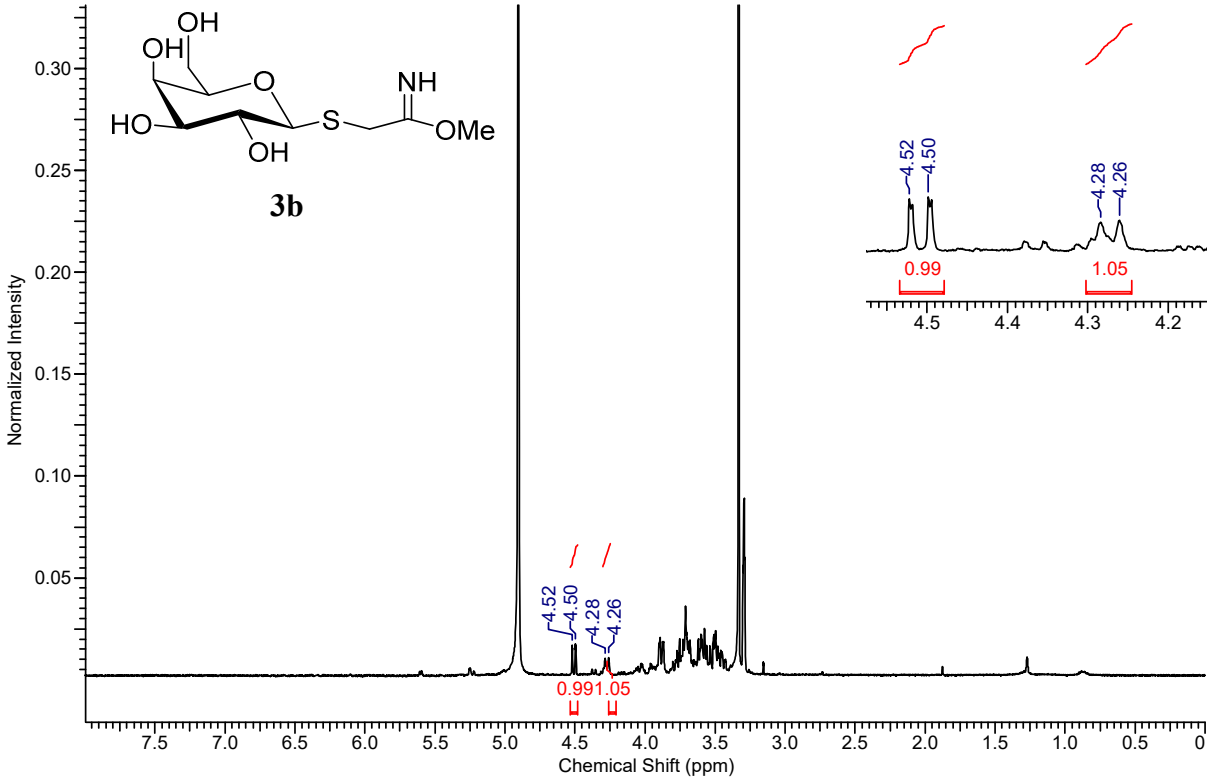
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OH
O
NH
HO
S
OMe
OH
3b
Normalized Intensity
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0.05
4.52
4.50
4.28
4.26
0.99
1.05
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
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1.5
1.0
0.5
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Chemical Shift (ppm)
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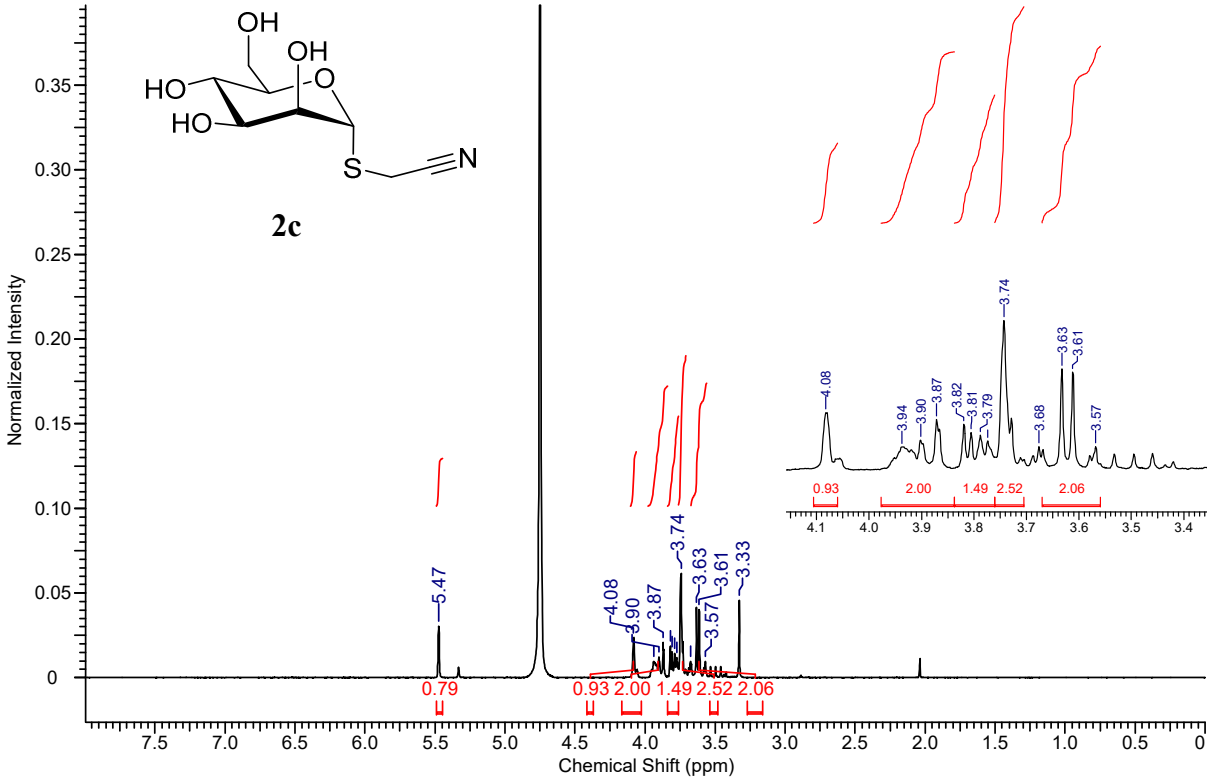
4.4
4.3
4.2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OH
HO
HO
O
S
N
2c
Normalized Intensity
Chemical Shift (ppm)
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3.61
3.57
3.33
0.79
0.93
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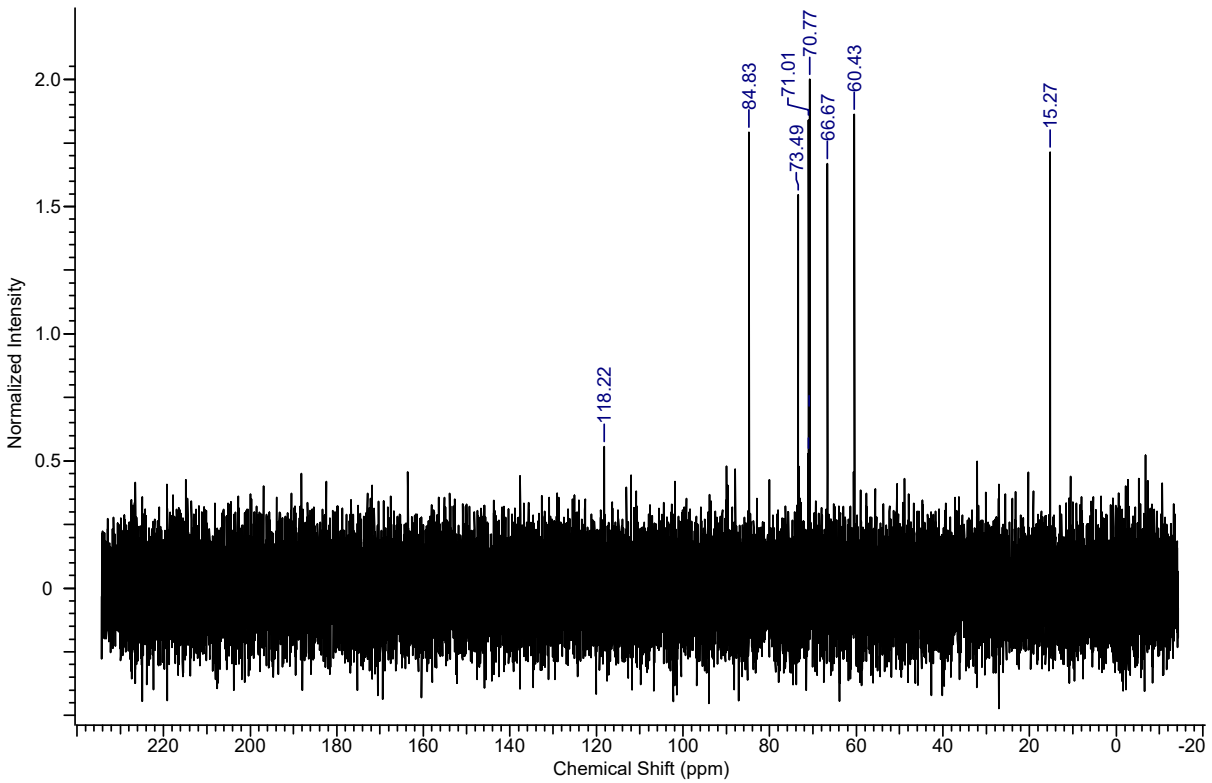
1.49
2.52
2.06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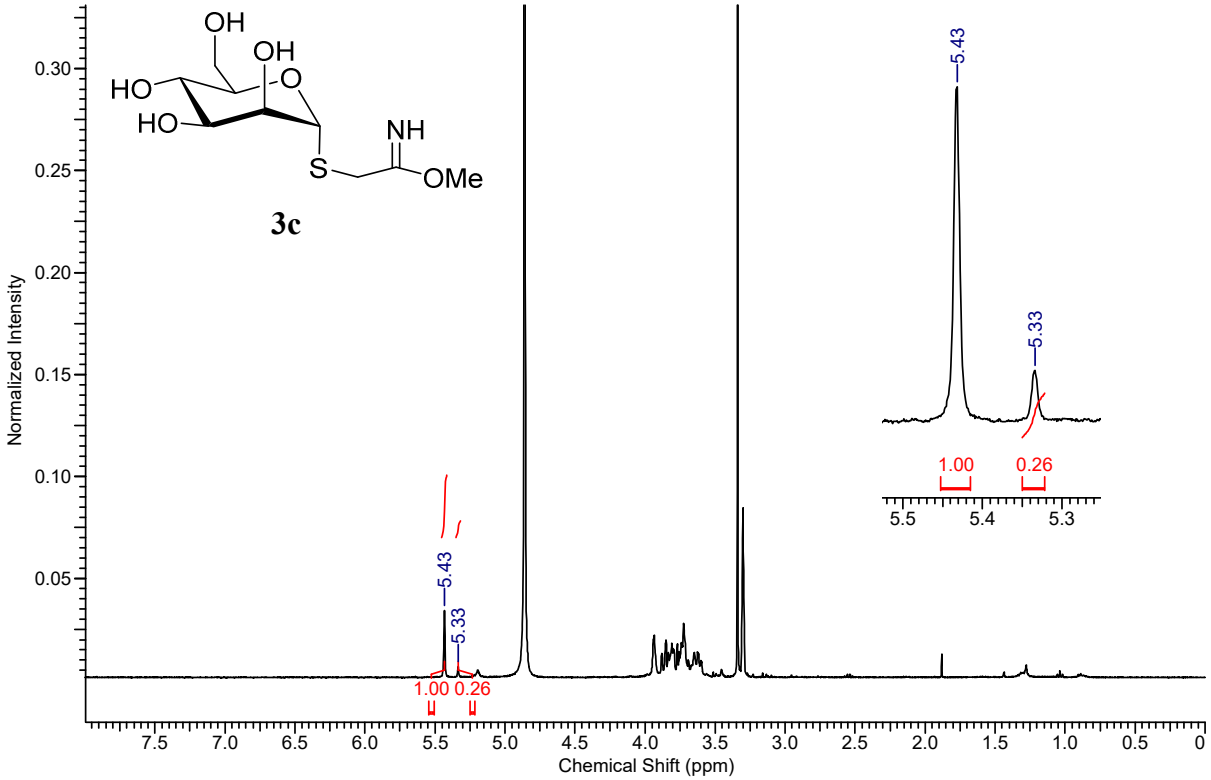
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OH
HO
HO
O
NH
S
OMe
3c
Normalized Intensity
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0.15
0.10
0.05
5.43
5.33
1.00
0.26
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
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1.0
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Chemical Shift (ppm)
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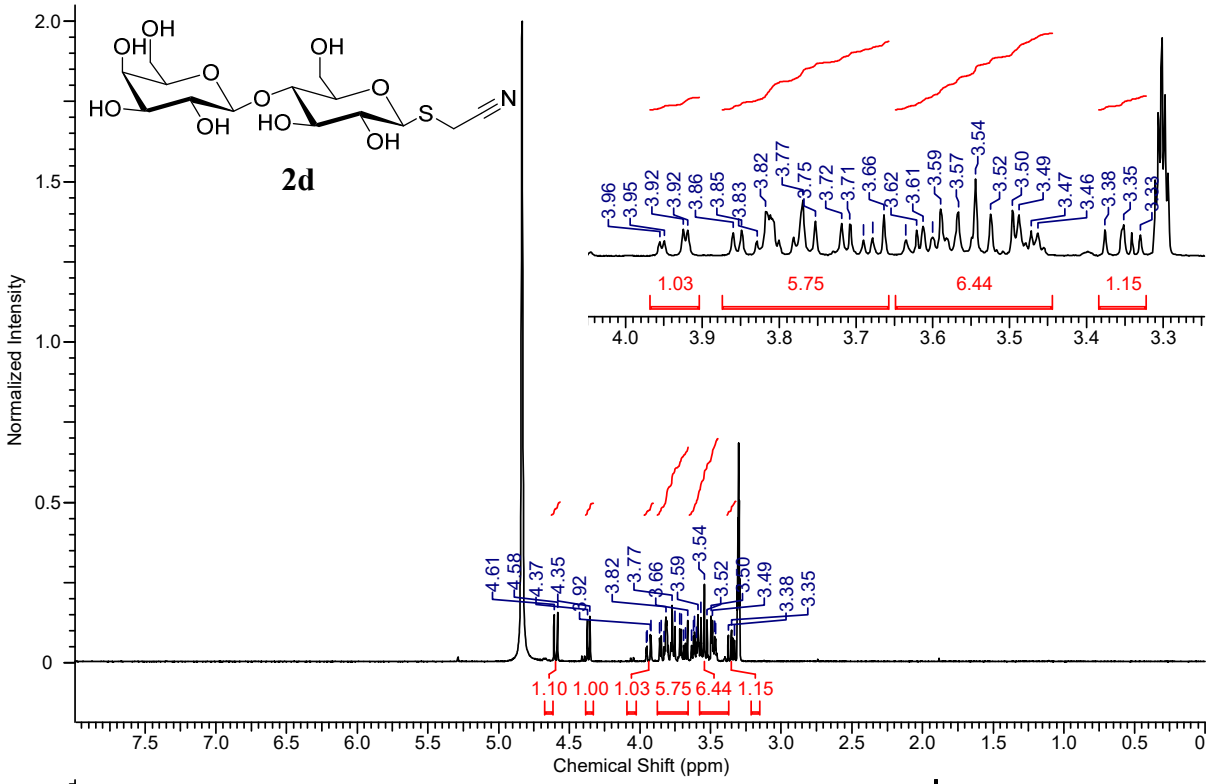
5.5
5.4
5.3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HO
O
OH
S
N
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3.54
3.52
3.50
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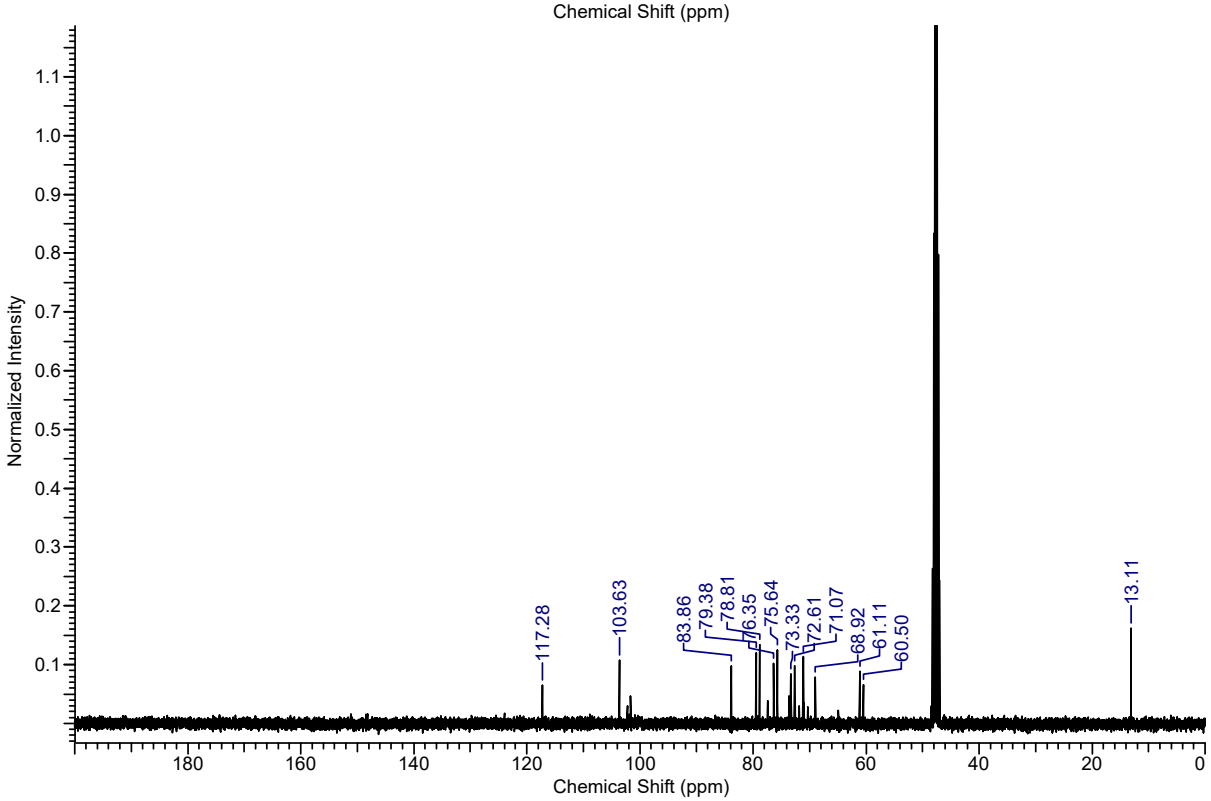
Normalized Intensity
Chemical Shift (ppm)
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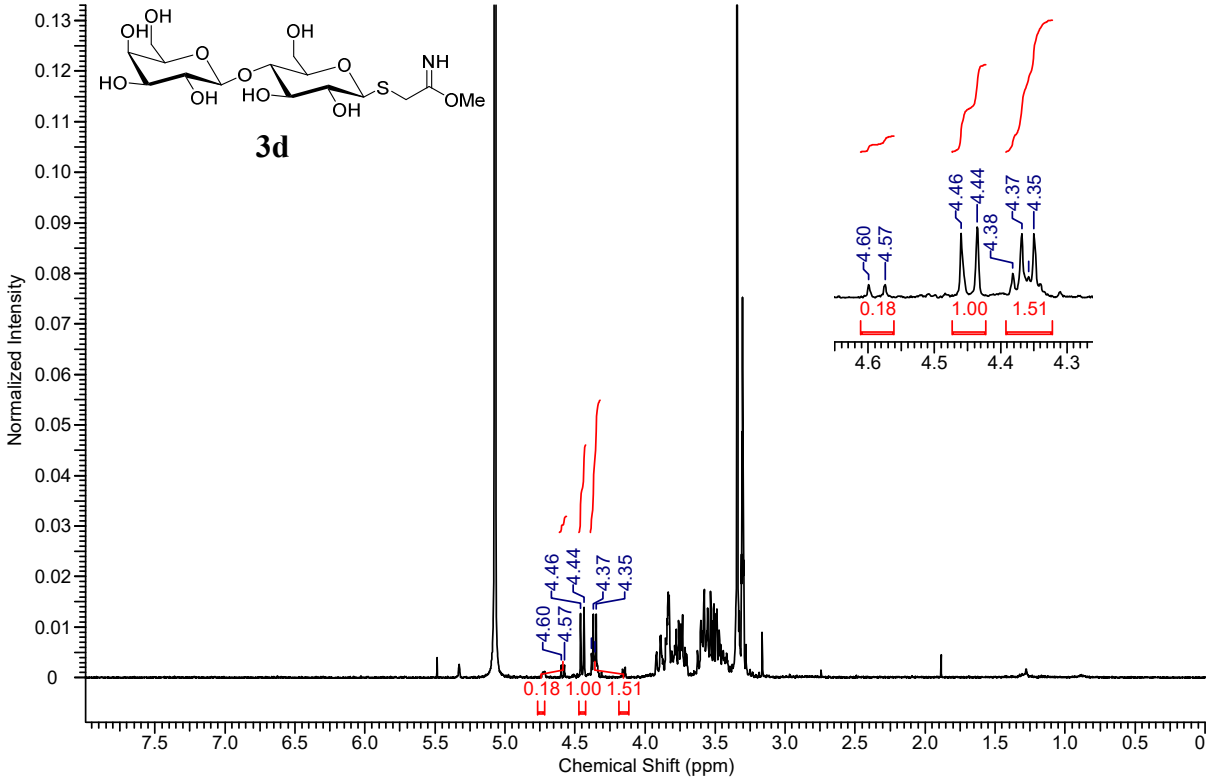
61.11
60.50
13.11


3d
Normalized Intensity
Chemical Shift (ppm)
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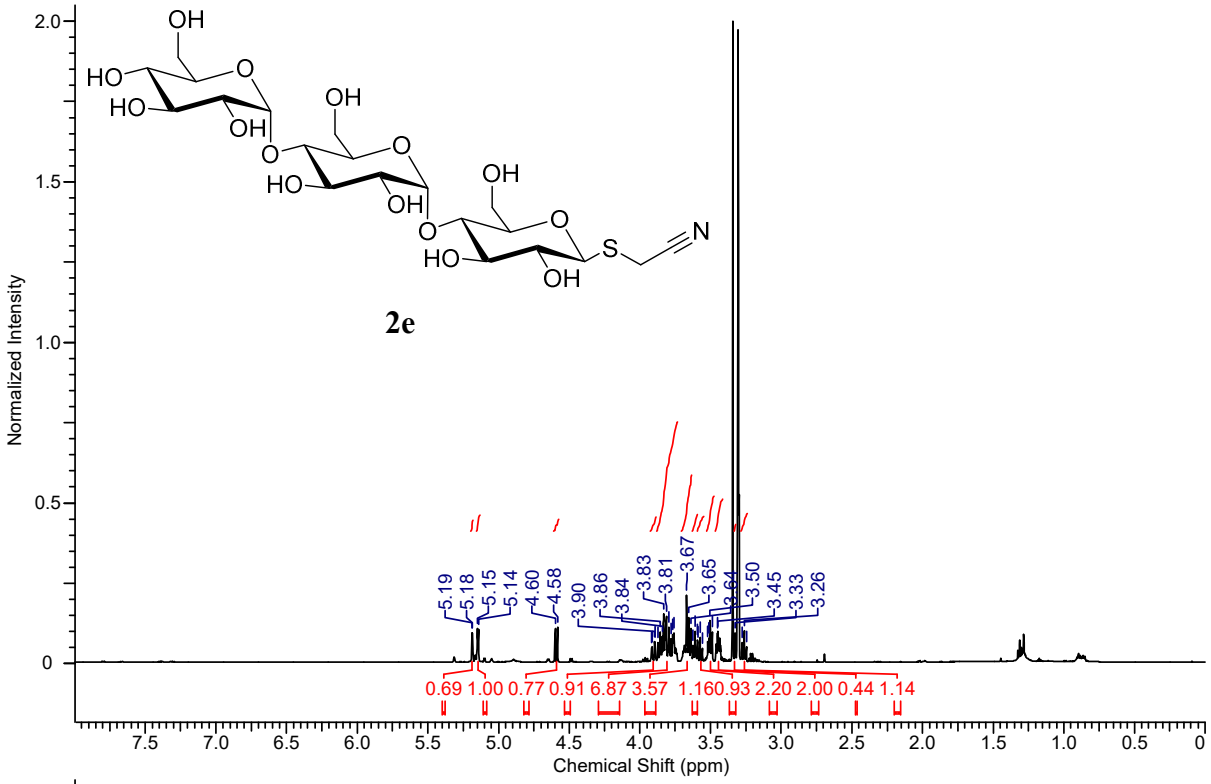
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OH
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O
HO
OH
OH
O
O
HO
S
N
OH
2e
Normalized Intensity
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1.0
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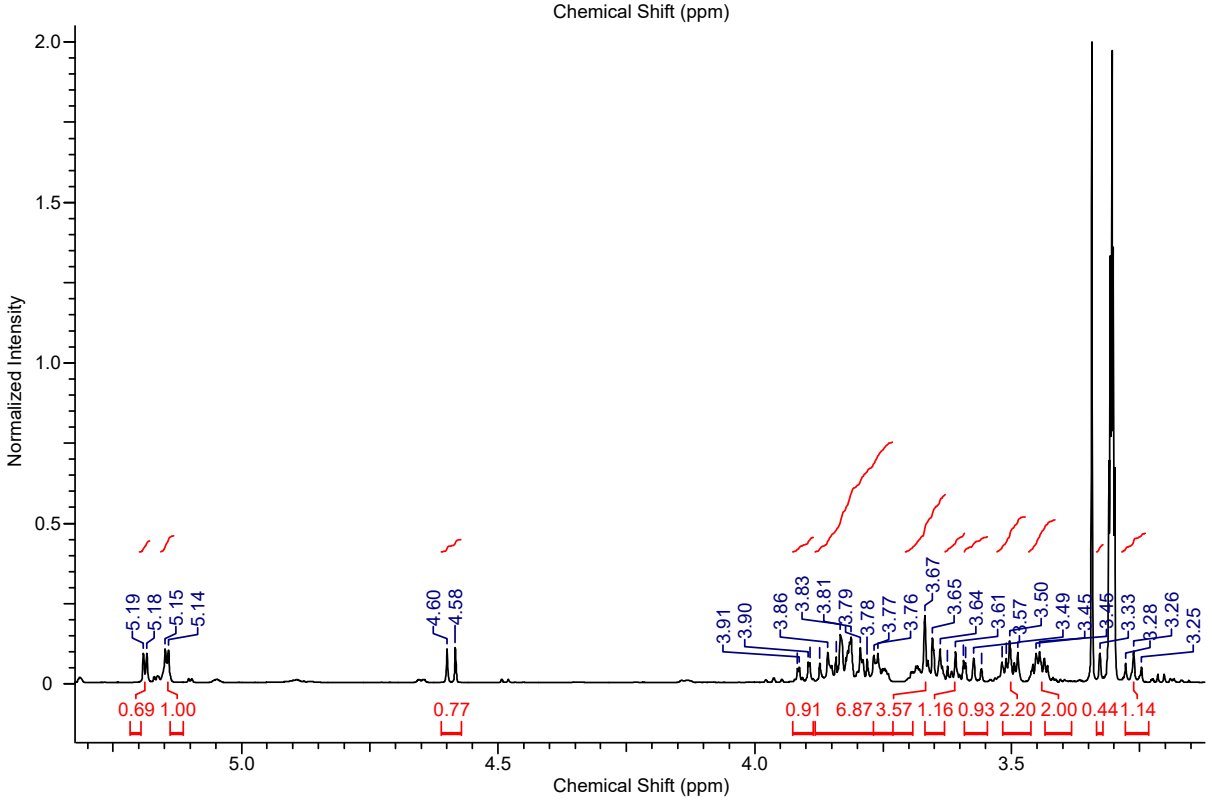
0
5.19
5.18
5.15
5.14
4.60
4.58
3.90
3.86
3.84
3.83
3.81
3.67
3.65
3.64
3.50
3.45
3.33
3.26
0.69 1.00 0.77 0.91 6.87 3.57 1.16 0.93 2.20 2.00 0.44 1.14
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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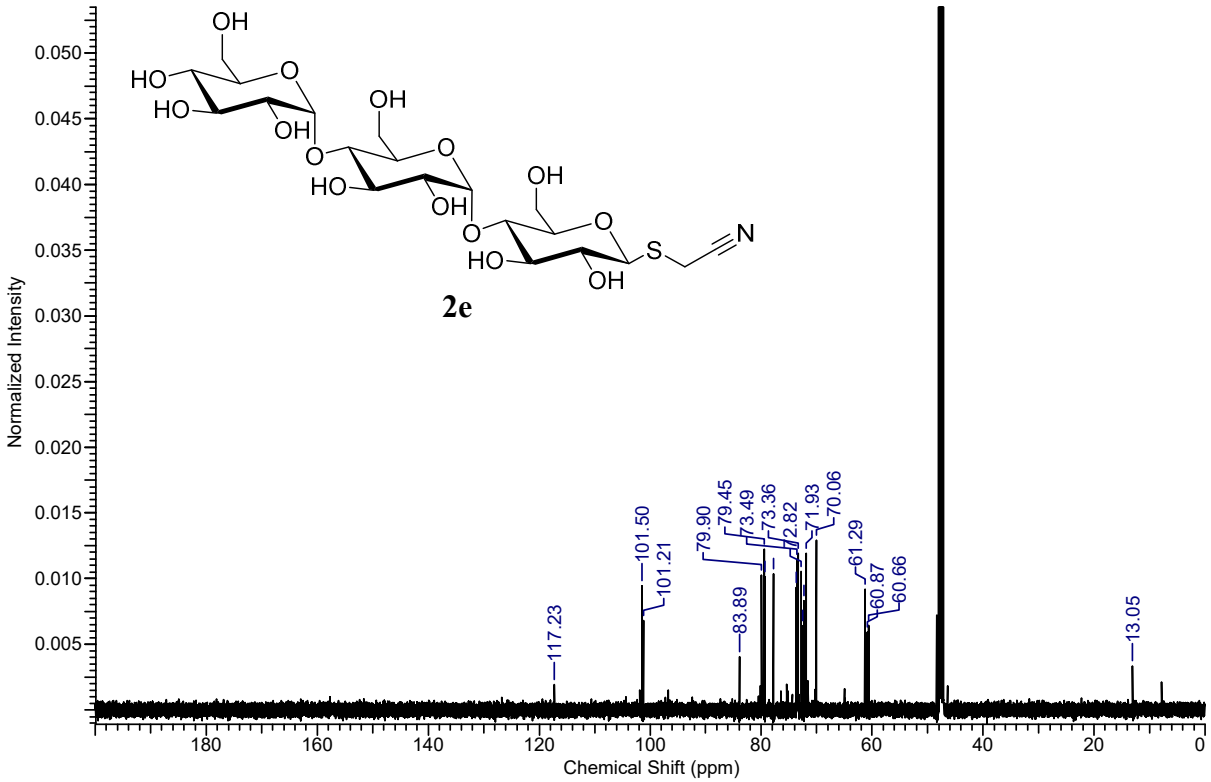
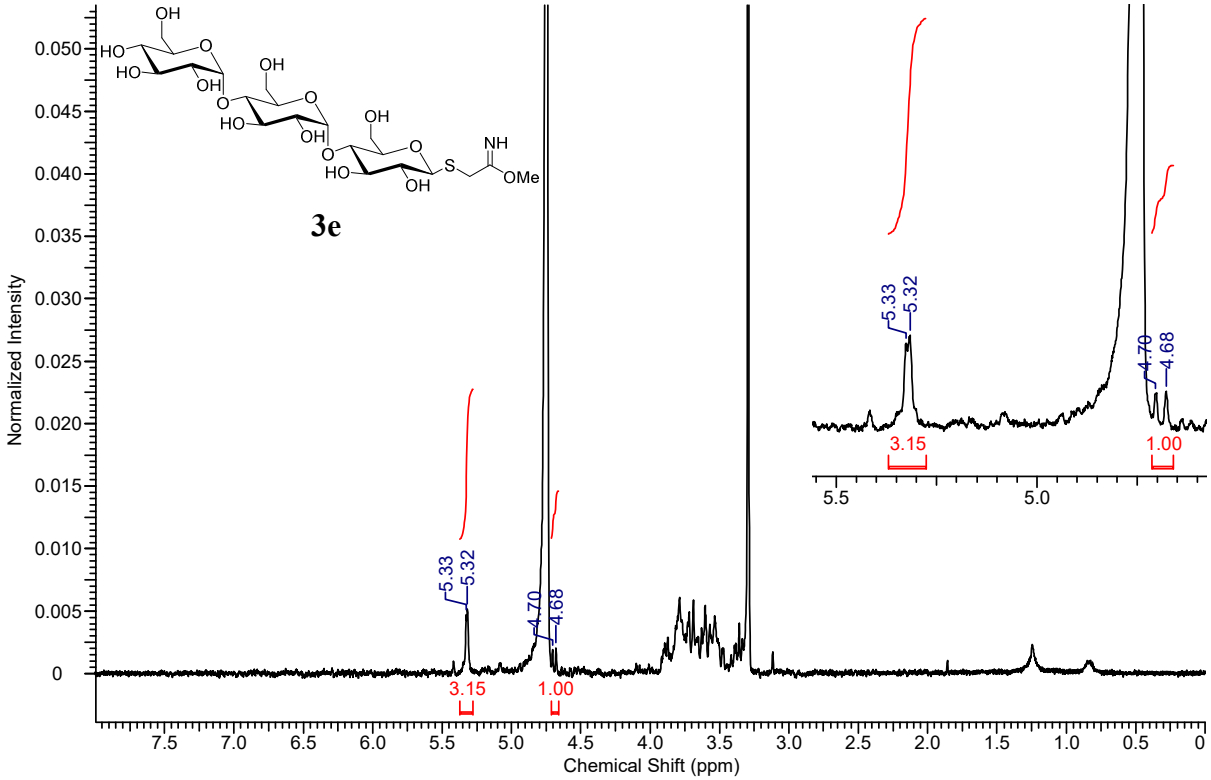
3.61
3.57
3.50
3.49
3.45
3.46
3.33
3.28
3.26
3.25
0.69 1.00
0.77
0.91 6.87 3.57 1.16 0.93 2.20 2.00 0.44 1.14
5.0 4.5 4.0 3.5
Chemical Shift (ppm)


OH
HO
HO
O
OH
OH
O
HO
OH
O
OH
O
HO
S
N
OH
Normalized Intensity
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
117.23
101.50
101.21
83.89
79.90
79.45
73.49
73.36
72.82
71.93
70.06
61.29
60.87
60.66
13.05
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

2e


3e
Normalized Intensity
Chemical Shift (ppm)
5.33
5.32
4.70
4.68
3.15
1.00