Supporting Information

Ionic-Liquid-based Catch and Release Mass Spectroscopy Tags for Enzyme Monitoring.

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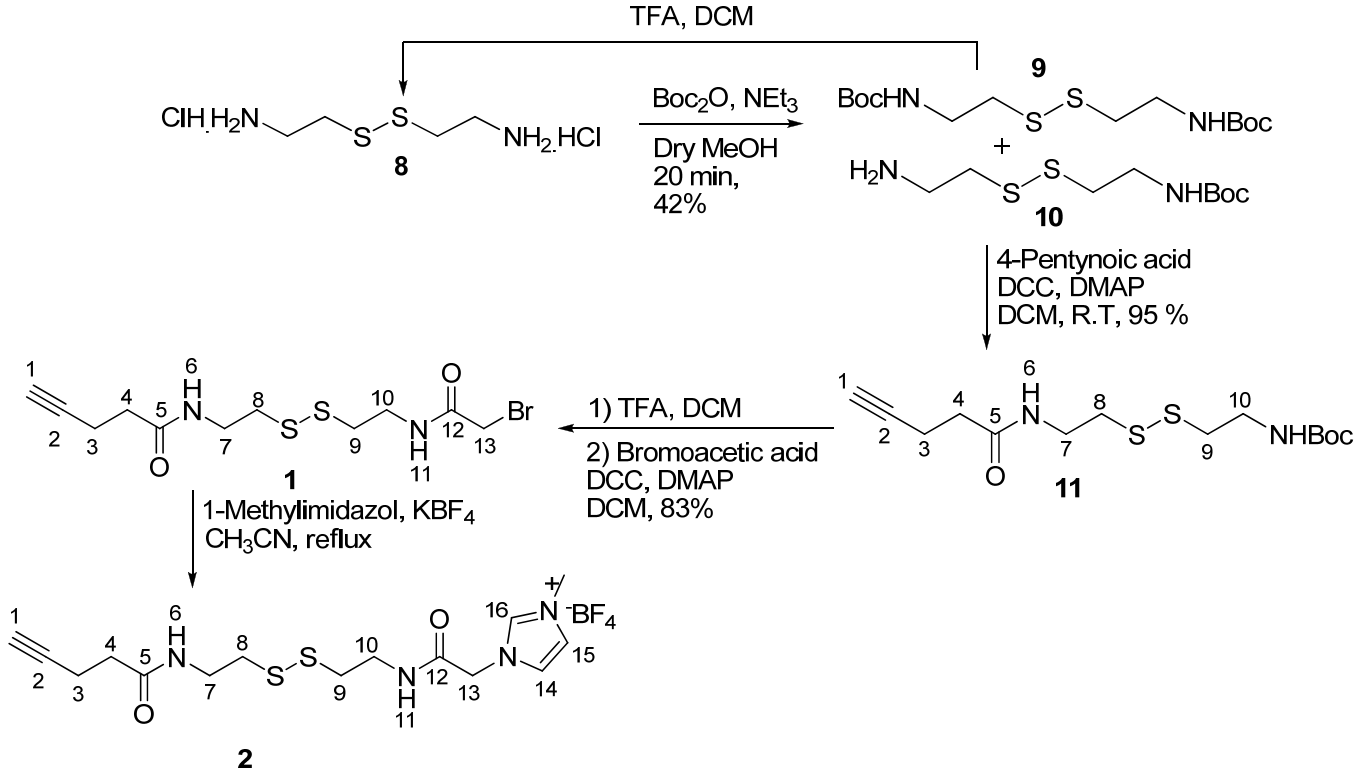
Experimental Procedures

General. Chemicals and β-1,4-GalT (EC 2.4.1.22) were purchased from Aldrich and Fluka and used without further purification. Preactivated molecular sieves kept in an oven at 150 ^{o}C were activated in a standard Microwave (800 W) for 3 minutes (3 x 1 minute) and cooled under vacuum. Dry solvents, where necessary, where obtained by distillation using standard procedures or by passage through a column of anhydrous alumina using equipment from Anhydrous Engineering (University of Bristol) based on the Grubbs' design. Reactions requiring anhydrous conditions were performed under an atmosphere of dry nitrogen; glassware, syringes and needles were either flame dried immediately prior to use or placed in an oven (150 °C) for at least 2 hours and allowed to cool either in a desiccators or under an atmosphere of dry nitrogen; liquid reagents, solutions or solvents were added *via* syringe or cannula through rubber septa; solid reagents were added *via* Schlenk type adapters. Reactions were monitored by TLC on Kieselgel 60 F254 (Merck). Detection was by examination under UV light (254 nm) and by charring with 10% sulfuric acid in ethanol. Flash chromatography was performed using silica gel [Merck, 230−400 mesh (40−63 μm)], the crude material was applied to the column as a solution in CH_2Cl_2 or by pre-adsorption onto silica, as appropriate. Extracts were concentrated under reduced pressure using both a Büchi rotary evaporator (bath temperatures up to 40 °C) at a pressure of either 15 mmHg (diaphragm pump) or 0.1 mmHg (oil pump), as appropriate, and a high vacuum line at room temperature. ^{1}H NMR and ^{13}C NMR spectra were measured in the solvent stated at Varian *INOVA* 400 or 500 instruments, respectively. Chemical shifts quoted in parts per million from $SiMe_4$ and coupling constants (*J*) given in hertz. Multiplicities are abbreviated as: b (broad), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or combinations thereof. Positive ion Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) mass spectra were recorded using an HP-MALDI instrument using gentisic acid matrix. The LC/MS analysis of samples was performed on a Waters LC-MS system ionized by ESI equipped with a Micromass Quattro micro API Mass Spectrometer as a detector and additional Photodiode Array (PDA 2998) and Evaporative Light Scattering (ELSD 2424) detectors. The LC was run on a Luna C18, 4.6 mm x 250 mm analytical column. The capillary and sample cone voltages were 3300 and 35 V. The desolvation and source temperatures were 350^{o}C and 120^{o}C. The cone and desolvation gas flow rates were 50 and 600 liters/hr. Analysis was performed with MassLynx 4.1

Synthesis:



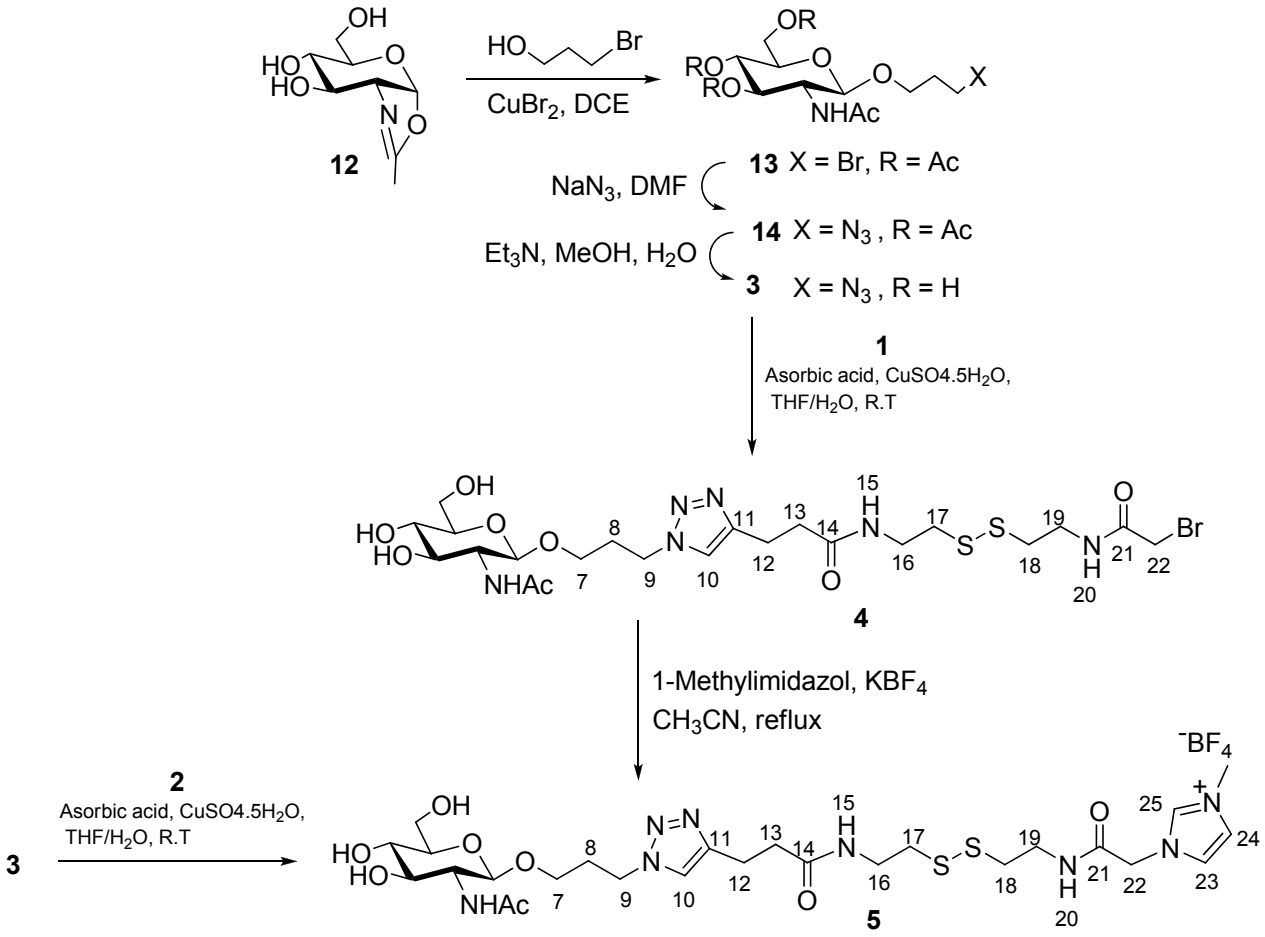
Scheme S1. Synthesis of Linkers **1** (Tag) and **2** (I-Tag)

***N-tert-Butyloxycarbonyl-cystamine* 10.** Prepared following literature procedures from **8**. Spectroscopic data in agreement with literature data. (Jacobsen, K.A.; Fischer, B.; Ji, X. *Bioconjugate Chem.* **1995**, *6*, 255-263.)

***Tert-butyl 2-((2-pent-4-ynamidoethyl)disulfanyl)ethylcarbamate* 11**. To a solution of DCC (1.18 g, 5.7 mmol) in dry DCM (17 mL), were added 4-pentynoic acid (0.56 g, 5.70 mmol) and DMAP (12 mg, 0.095 mmol). A solution of N-*tert*-Butyloxycarbonyl-cystamine **10** (1.2 g, 4.75 mmol) in dry DCM (25 mL) was then added after 10 min and the reaction mixture was left stirring overnight at room temperature. The mixture was then filtered and then washed with H_2O (5 mL x 3) and aqueous HCl (1M). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1:1, hexane:EtOAc), to afford **11** (1.5 g, 95%) as white crystals. mp = 75°C, ^{1}H NMR (400 MHz, $CDCl_3$, ppm) : δ = 6.69 (brs, 1H, H-6), 5.05 (brs, 1H, H-11), 3.59 (q, 2H, J = 6.0 Hz, H-7), 3.44 (q, 2H, J = 6.5 Hz, H-10), 2.84 (t, 2H, $J_{8,7}$ =6.0 Hz, H-8), 2.77 (t, 2H, $J_{9,10}$ = 6.5 Hz, H-9), 2.51 - 2.57 (m, 2H, H-3), 2.44 - 2.47 (m, 2H, H-4), 1.99 (t, 1H, $J_{1,3}$ = 2.38 Hz, H-1), 1.44 (s, 9H, 3x$C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) : δ = 171.4 (C-5), 166.9 (CO *Boc*), 83.0 (C-2), 79.8 ($\underline{C}(CH_3)_3$), 69.2 (C-1) 39.6 (C-10), 38.5 (C-7), 38.1 (C-8), 37.7 (C-9), 35.2 (C-4), 28.4 (3 x $\underline{C}H_3$), 14.8 (C-3). HRMS: (ESI) Calcd for $C_{14}H_{24}N_2O_3S_2Na$ $[M+Na]^+$: 355.1121; found: 355.1117.

***N-(2-((2-(2-bromoacetamido)ethyl)disulfanyl)ethyl)pent-4-ynamide* 1**. To a solution of **11** (458 mg, 1.355 mmol) in DCM (20 ml) was added TFA (10% in DCM, 2 mL) and the resulting mixtured was stirred 1 hour at room temperature. Et_3N (4 mL) was added to neutralize excess of TFA then the reaction mixture was concentrated and co-evaporated with toluene under reduced pressure. The dry residue was redisolved in anhydrous DCM (15 mL) and bromoacetic acid (377 mg, 2.713 mmol, 2 equiv), DMAP (17 mg, 0.139 mmol, 0.1 equiv) and DCC (307 mg, 1.490 mmol, 1.1 equiv) were added. The resulting mixture was stirred for 16 hours at room temperature under nitrogen. The reaction mixture was then filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Tol/EtOAc : 5/5-6/4), to afford **1** (397 mg, 83%) as a pale white solid. ^{1}H NMR (400 MHz, $CDCl_3$ ppm): δ = 7.21 (brs, 1H, H-6), 6.43 (brs, 1H, H-11), 3.91 (s, 2H, H-13), 3.63 (q, 2H, *J* = 6.5 Hz, H-7), 3.61 (q, 2H, *J*=6.5 Hz, H-10), 2.85 (q, *J*=6.60 Hz, 4H, H-8 & H-9), 2.57-2.52 (m, 2H, H-3), 2.45 (td, 2H, *J* = 1.5 , 7.0 Hz, 2H, H-4), 2.02 (t, *J* = 2.69 Hz 1H, H-1); ^{13}C NMR (100 MHz, $CDCl_3$ ppm) : δ = 171.5 (C-12), 166.12 (C-5), 82.9 (C-2), 69.4 (C-1), 39.1 (C-10), 38.4 (C-7), 37.8 (C-8), 37.2 (C-9), 35.2 (C-4), 29.0 (C-13), 14.8 (C-3). HRMS: (CI^+) Calcd for $C_{11}H_{18}{}^{79}BrN_2O_2S_2$, $C_{11}H_{18}{}^{81}BrN_2O_2S_2$ $[M+H]^+$: 352.9993, 354.9973; found: 352.9995, 355.0011.

***N-(2-((2-(1-acetamido-3-methylimidazolium)ethyl)disulfanyl)ethyl)pent-4-ynamide tetrafluoroborate* 2.** To a solution of **1** (20 mg, 0.057 mmol) in acetonitrile (1 mL) were added 1-methylimidazole (34 µL, 0.228 mmol) and KBF_4 (53.9 mg, 0.228 mmol). The reaction mixture was stirred under reflux for 17 hours. After cooling to room temperature, the reaction mixture was filtered and concentrated under reduced pressure, dried under high vacuum and washed with diethyl ether (3x20mL) under sonication. After decantation of the solvent, the oil was dried under vacuum and then purified by re-crystallisation. The residue was dissolved in CH_3CN and toluene was added to give a precipitate, after decantation of the solvent, the residue was dried to afford **2** as a yellow oil (19 mg, 95 % yield). ^{1}H NMR (400 MHz, CD_3CN, ppm) : δ = 8.67 (s, 1H, H-16), 8.06 (brs, 1H, H-6), 7.44 (dd, *J* = 1.5, 2.0 Hz, H-14 or H-15), 7.34 (dd, *J* = 1.5, 2.0 Hz, H-14 or H-15), 7.11 (brs, 1H, H-11), 5.00 (s, 2H, H-13), 3.86 (s, 3H, $NC\underline{H}_3$), 3.52 (q, 2H, *J* = 6.5 Hz, H-7), 3.46 (dt, 2H, *J* = 6.0, 7.0 Hz, H-10), 2.87 (t, *J* = 6.5 Hz, 2H, H-8), 2.81 (dd, 2H, *J* = 6.0, 7.0 Hz, H-9), 2.46-2.42 (m, 2H, H-3), 2.45 (ddd, 1H, *J* = 1.0, 2.5, 6.5 Hz, H-3a), 2.43 (ddd, 1H, *J* = 2.0, 2.5, 6.5 Hz, H-3b), 2.37 (dd, *J* = 1.0, 6.5 Hz, H-4a) 2.35 (dd, 1H, *J* = 2.0, 6.5 Hz, H-4b), 2.18 (t, 1H, *J* = 2.5 Hz, H-1); ^{13}C NMR (100 MHz, CD_3CN, ppm) : δ = 172.6, 166.2 (CO), 138.6 (C-16), 125.1, 124.5 (C-14, C-15), 84.8 (C-2), 70.5 (C-1), 52.5 (C-13), 39.8 (C-7), 39.7 (C-10), 39.2 (C-8), 38.9 (C-9), 37.5 ($N\underline{C}H_3$), 35.9 (C-4), 15.6 (C-3). HRMS: (ESI) Calcd for $C_{15}H_{23}N_4O_2S_2^+$ $[M]^+$ 355.1257; found 355.1261.



Scheme S2. Synthesis of I-Tagged substrate **5**

***3-azidopropyl N-acetyl-2-deoxy-β-D-glucopyranoside* 3.** To a solution of oxazoline **12** (Nakabayashi, S.; Warren, C. D.; Jeanloz, R. W. *Carbohydr. Res.* **1986**, *150*, c7-c10) (500 mg, 1.52 mmol), 3-bromopropan-1-ol (0.69 mL, 7.6 mmol) in dry THF (15 mL) was added anhydrous $CuBr_2$ (340 mg, 1.52 mmol) and left stirring for 2.5 h at 50°C under a nitrogen atmosphere. After cooling the reaction to room temperature, the mixture was filtered through celite and the solvent evaporated. The dried residue was then dissolved in EtOAc (20 mL), washed with H_2O (3 x 20 mL), dried on $MgSO_4$, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluent: EtOAc/Tol: 4/1) to give **13** (444 mg, 65 %). To 300 mg of **13** in DMF (15 mL) were added NaN_3 (414 mg, 6.19 mmol, 10 eq) and the mixture warm to 80°C and left stirring for 18h. The mixture was then cooled to room temperature, filtered through celite and then concentrated under reduced pressure. The dried residue was then purified by flash chromatography (EtOAc:Tol, 5:1) to give **14** as a white solid (258 mg, 94%). To remove the acetyl groups, **14** was dissolved in a mixture of MeOH:Et_3N:H_2O, 8:1:1 (10 mL) and left stirring for 18h at room temperature, co-evaporation of the solvent with Toluene x3 times gave **3** (182 mg, quantitative) without the need for further purification.

1-(3-(2-acetamido-2-deoxy-β-D-glucopyranoside)-propyl)-4-{2-ethyl-[2-(2-((bromo)acetamido)-ethyldisulfanyl)-ethyl]-amido}-[1,2,3]-triazole **4.** To a solution of linker **1** (10 mg, 0.028 mmol) and deprotecting sugar **3** (10.4 mg, 0.034 mmol, 1.2 equiv) in degassed THF (60 µL) was added sodium ascorbate (30 µL of a freshly prepared 0.093 M solution in degassed water, 0.0028 mmol) followed by copper (II) sulphate pentahydrate (30 µL of a 0.047 M solution in degassed water, 0.0014 mmol). The reaction mixture was stirred at room temperature for 20 hours, after which it was concentrated *under* reduced pressure and the residue purified by silica gel column chromatography (DCM:MeOH, 8:2), to afford **4** (16.1 mg, 79%). ^{1}H NMR (400 MHz, MeOD-$CDCl_3$, ppm) : δ = 7.44 (s, 1H, H-10), 4.26-4.23 (m, 2H, H-9), 4.17 (d, 1H, H-1), 3.68 (dd, $J_{6a,5}$ = 2.5 Hz , $J_{6a,6b}$ =12.0 Hz, 1H, H-6a), 3.64 (s, 2H, H-22), 3.63-3.60 (m, 1H, H-7), 3.56 (dd, 1H, $J_{6b,5}$ = 4.5 Hz , $J_{6b,6a}$ =12 Hz, , H-6a), 3.51-3.44 (m, 1H, H-2), 3.36-3.32 (t, 2H, $J_{16,17}$ = 6.5 Hz, H-16), 3.30-3.27 (t, 2H, $J_{19,18}$ = 6.5 Hz, H-19), 3.25-3.23 (m, 1H, H-3), 3.22-3.20 (m, 1H, H-4), 3.20-3.17 (m, 2H, H-7), 3.11-3.06 (m, 1H, H-5), 2.81 (t, 2H, $J_{12,13}$ = 6.5 Hz, H-12), 2.62 (t, 2H, $J_{17,16}$ = 6.5 Hz, H-17), 2.58 (t, 2H, $J_{18,19}$ = 6.5 Hz, H-18), 2.39 (t, 2H, $J_{13,12}$ = 6.5 Hz, H-13), 1.93-1.87 (m, 2H, H-8), 1.84 (s, 3H, C$\underline{H}_3$); ^{13}C NMR (100 MHz, MeOD-$CDCl_3$, ppm) : δ = 170.0-165.9 (CO), 125.6 (C-10), 100.8 (C-1), 75.8 (C-5), 74.6 (C-3), 70.5 (C-4), 68.3 (C-7), 64.8 (C-6), 55.7 (C-2), 46.3 (C-9), 38.6 (C-16), 38.0 (C-19), 37.1 (C-18), 36.7 (C-17), 34.8 (C-13), 29.8 (C-8), 27.7 (C-22), 22.3 ($\underline{C}H_3$), 20.9 (C-12). **HRMS** : (ESI) $[MNa]^+$ calcld for $C_{22}H_{37}BrN_6NaO_8S_2^+$: 679.1195; found : 679.1200.

1-(3-(2-acetamido-2-deoxy-β-D-glucopyranoside)-propyl)-4-{2-ethyl-[2-(1-acetamido-3-methylimidazolium)ethyldisulfanyl)-ethyl]-amido}-[1,2,3]-triazole tetrafluoroborate **5.**

<u>*METHOD A* from **4**</u>

To a solution of **4** (11.4 mg, 0.017 mmol) in acetonitrile (1 mL) and water (0.5 mL) were added 1-methylimidazole (2.7 µL, 0.034 mmol) and KBF_4 (8.7 mg, 0.068 mmol). The reaction mixture was stirred and heated to reflux for 14 hours. After cooling at room temperature, the reaction mixture was filtered and concentrated under reduced pressure, dried under high vacuum and washed with hexane (5 mL) then diethyl ether (3 x 5 mL) with sonication. After decantation, the oil was dried under vacuum to afford **5** as a yellow oil (11.2 mg, 95%).

<u>*METHOD B* directly from **3**</u>

To a solution of **3** (5 mg, 0.016 mmol, 2 eq) and **2** (3.3 mg, 0.008 mmol) in degassed methanol (100 µL) were added an aqueous solution of ascobic acid (0.6 mg in 20 µL of degassed water, 3.2 µmol) and $CuSO_4.5H_2O$ (0.4 mg in 20 µL of degassed water, 1.6 µmol). The mixture was left stirring at room temperture under nitrogen atmosphere overnight. MS showed complete conversion and the solvents were then evaporated under reduce pressure. The residue was purified by reverse phase chromatography C18

(H_2O:MeOH, gradient - 1:0 to 1:1) to give 4.8 mg of expected product **5** (84%). ^{1}H NMR (400 MHz, D_2O, ppm) : δ = 7.73 (s, 1H, H-10), 7.47 (d, $J_{24,23}$ = 2.0 Hz, 1H, H-24), 7.45 (d, $J_{24,23}$ = 2.0 Hz, 1H, H-23), 5.04 (s, 2H, H-22), 4.45 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 4.40 (t, 2H, $J_{9,8}$ = 7.0 Hz, H-9), 3.91 (s, 3H, NC<u>H</u>$_3$), 3.88-3.84 (m, 2H, H-6b, H-7b), 3.74-3.66 (m, 2H, H-6a, H-2), 3.55 (t, 2H, $J_{16,17}$ = 6.5 Hz, H-16), 3.51-3.45 (m, 2H, H-3, H-7a), 3.45-3.41 (m, 4H, H-4, H-5, H-19), 2.99 (t, 2H, $J_{12,13}$ = 7.0 Hz, H-12), 2.82 (t, 2H, $J_{17,16}$ = 6.5 Hz, H-17), 2.73 (t, 2H, $J_{18,19}$ = 6.5 Hz, H-18), 2.59 (t, 2H, $J_{13,12}$ = 7.0 Hz, H-13), 2.14-2.09 (m, 2H, H-8), 2.03 (s, 3H, CH_3 *Ac*); ^{13}C NMR (100 MHz, D_2O ppm) : δ = 167.5 (<u>C</u>O), 165.5, 158.0 (C-14, C-21), 114.5 (C-24), 114.3 (C-23), 100.3 (C-1), 67.2 (C-7), 66.8 (C-5), 64.7 (C-3), 60.9 (C-4), 57.3 (C-7), 51.7 (C-6), 46.6 (1C, C-2), 41.7 (1C, C-22), 37.8 (1C, C-9), 29.4 (1C, C-16), 28.7 (1C, C-19), 27.6 (C-18), 27.3 (C-17), 26.8 (N<u>C</u>H$_3$), 26.2 (C-13), 20.5 (C-8), 13.2 (<u>C</u>H$_3$ *Ac*), 12.0 (C-12); ^{19}F NMR (376 MHz, D_2O) δ (ppm): -150.54. **HRMS :** (ESI) $[M]^+$ calcld for $C_{26}H_{43}N_8O_8S_2^+$ 659.2645 found 659.2639.

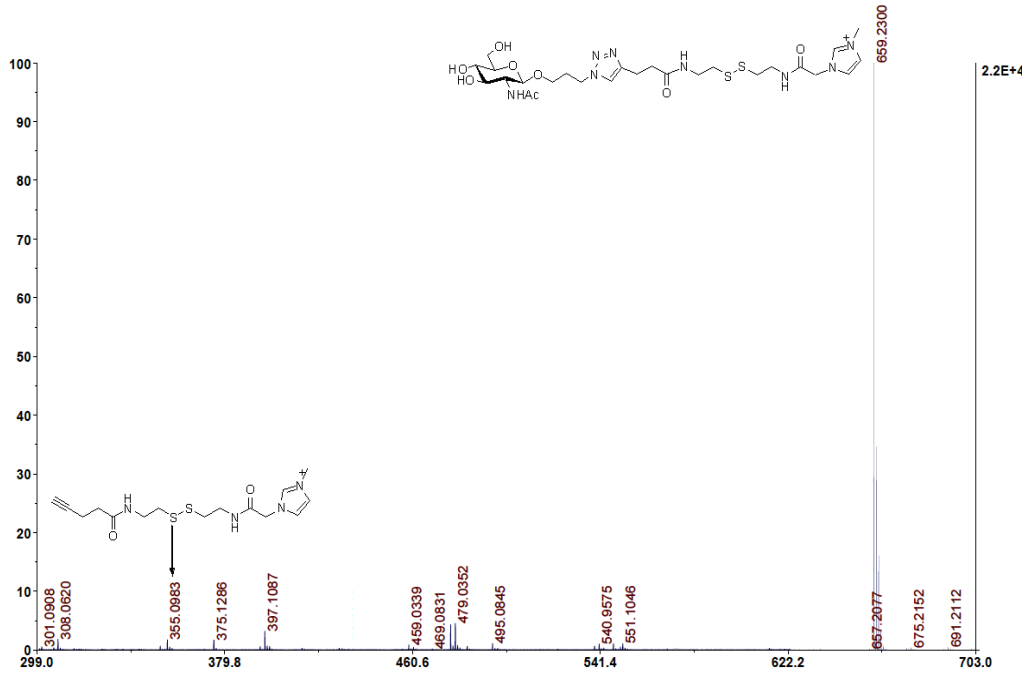
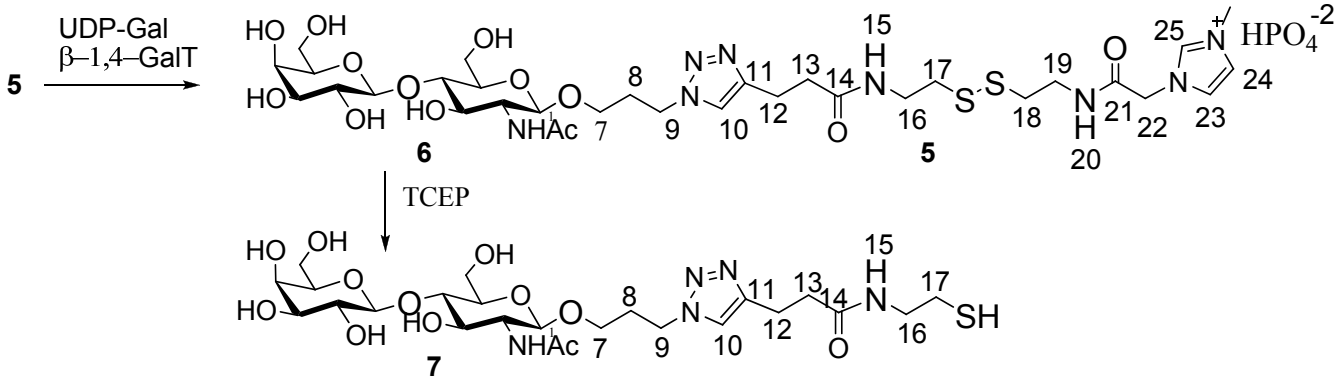


Figure 1S. MALDI-TOF trace of reaction mixture (Method B) showing formation of product **5**.



Scheme S3. Ezymatic reaction of ITagged **5** to yield product **6** and final product release from I-Tag.

Enzymatic assays:

Preparative Enzymatic reaction

Incubations were carried out in 250 μL sodium cacodylate buffer (25 mM, pH 8.2) containing $MnCl_2$ (mM) and bovine serum albumin (1 mg/ml). Acceptor **5** (1.0 mg), UDP-Gal (1.0 mg), alkaline phosphatase (1U), and β-1,4-GalT, EC 2.4.1.22 (100 mU) are incubated at 37 ^{o}C for 72 hr. Every 24 hr., 0.5 mg of UDP-Gal and another 100 mU were added to the incubation mixture to compensate for any hydrolysis of the donor. The reaction was monitored by LC-MS. At the completion of the reaction, the sample was purified on reverse phase C-18 column (Pasteur pipette, 0.7 cm of C18 in height) to afford **6**

1-(3-(2-acetamido-2-deoxy-[4-O-(β-D-galactopyranosy)]-β-D-glucopyranoside)-propyl)-4-{2-ethyl-[2-(1-acetamido-3-methylimidazolium)ethyldisulfanyl)-ethyl]-amido}-[1,2,3]-triazole tetrafluoroborate **6.** (1.2 mg, 96 %). ^{1}H NMR (D_2O, 500 MHz, DOH set at 4.75): δ 7.71 (s, 1H, H-10), 7.47 (d, $J_{24,23}$ = 1.71 Hz, 1H, H-23), 7.45 (d, $J_{24,23}$ = 1.71 Hz, 1H, H-24), 5.89 (s, 2H, H-22), 4.47 (d, 1 H, $J_{1'2'}$ = 8.1 Hz, H-1'), 4.45 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1), 4.40 (ddd, 2H, H-9), 3.91 (s, 3H, N-CH_3), 3.96 (dd, 1H, $J_{6b,6a}$= 12.2 Hz, H-6b), 3.85 (m, 1H, H-7), 3.81 (dd, 1H, $J_{5,6a}$= 5.4 Hz, H-6a), 3.77-3.64 (m, 5H, H-6a', H-6b', H-4', H-5, H-5'), 3.75 (m, 1H, H-3'), 3.68 (m, 1H, H-2'), 3.55 (t, 1H, $J_{2,3}$= 8.6 Hz, H-2), 3.53 (t, 1H, H-3), 3.57-3.48 (m, 1H, H-4), 3.56 (t, $J_{16,17}$ = 6.4 Hz, 2H, H-16a, H-16b), 3.44 (t, $J_{19,18}$ = 6.1 Hz, 2H, H-19a, H-19b), 2.99 (t, $J_{12,13}$ = 6.9 Hz, 2H, H-12a, H-12b), 2.82 (t, $J_{17,16}$ =6.4 Hz, 2H, H-17a, H-17b), 2.73 (t, $J_{18,19}$=6.1 Hz, 2H, H-18a, H-18b), 2.60 (t, $J_{12,13}$ = 6.9 Hz, 2H, H-13a, H-13b), 2.11 (m, 2H, H-8a, H-8b), 2.03 (s, 3 H, C(O)CH_3); **^{13}C NMR** (100 MHz, D_2O) δ (ppm) 170.5-165.9 (3C, 1 x C(O)NH, C14, C-21), 125.9 (C-10), 113.9-114.8 (C-24, C-23), 109.6 (C-1), 101.8 (C-1'), 90.9 (C-22), 79.8, 76.6, 75.0 (C-2), 73.4 (C-2'), 73.3, 71.9 (C-6'), 70.4, 67.2, 60.9 (C-6), 48.5 (C-9), 37.4 (C-17), 37.3 (C-18), 35.7 (C-13), 29.4 (C-8), 28.0 (N-CH_3), 23.3 (C(O)CH_3), 21.5 (C-12). HR-ESI: $C_{32}H_{53}N_8O_{13}S_2^+$ requires $[M]^+$ 821.3168 found: $[M]^+$ 821.3163.

Product Release - ITag Cleavage

To a solution of **6** (0.5 mg) in water (0.5 mL) was added tris(2-carboxyethyl)phosphine hydrochloride (50 mM in H_2O) and the mixture stirred at room temperature for 24h. At the completion of the reaction, the sample was concentrated under reduced pressure and purified by C-18 column chromathography to yield **7** (0.3 mg).

1-(3-(2-acetamido-2-deoxy-[4-O-(β-D-galactopyranosy)]-β-D-glucopyranoside)-propyl)-4-(2-mercaptoethyl) propanamide))-[1,2,3]-triazole **7.** ^{1}H NMR (D_2O, 500 MHz, DOH set at 4.75): δ 7.73 (s, 1H, H-10), 4.47 (d, 1 H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.45 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1), 4.40 (m, 2H, H-9), 3.95 (m, 1H, H-6b), 3.85 (m, 1H, H-7), 3.81 (m, 1H, H-6a), 3.77-3.67 (m, 6H, H-6a', H-6b', H-4', H-5, H-5', H-3'), 3.64 (m, 1H, H-2'), 3.55 (t, 1H, H-2), 3.53 (t, 1H, H-3), 3.57-3.48 (m, 1H, H-4), 3.56 (t, $J_{16,17}$ = 6.0 Hz, 2H, H-16a, H-16b), 2.99 (t, $J_{12,13}$ = 6.9 Hz, 2H, H-12a, H-12b), 2.82 (t, $J_{17,16}$=6.4 Hz, 2H, H-17a, H-17b), 2.73 (m, 2H, H-18a, H-18b), 2.60 (m, 2H, H-13a, H-13b), 2.11 (m, 2H, H-8a, H-8b), 2.02 (s, 3 H, $C(O)CH_3$). HR-ESI: $C_{24}H_{41}N_5O_{12}S_1^+$ requires $[M+Na]^+$ 646.2364 found: $[M+Na]^+$ 646.2359

Calibration and data acquisition

In all the reactions studied, two ions were monitored in order to quantify the progress of the reaction. The ions monitored (ESI) were the acceptor **5** [M^+] and the internal standard **2** [M^+]. The total ion count for each peak was then integrated, and the absolute concentration of each compound was obtained from standard curves after normalising the ionisation efficiencies for each reaction with respect to the internal standard **2**.
A standard curve was constructed for analog **5** at eight different concentrations around those found under the initial reaction conditions needed for initial-rate-method measurements.

For reaction monitoring, acceptor consumption, as judged by their TIC integrated values, were monitored as a function of time. To allow full determination of the kinetic parameters, UDP-Gal was held at a fixed saturating concentration while the ITagged-substrate **5** concentration was varied.

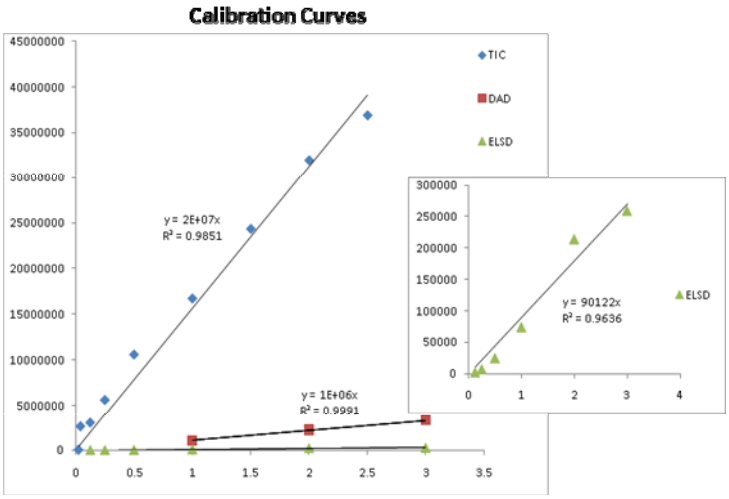


Figure 2S. Concentration curves constructed for compound **5** (60 µL injections) with data collected at eight different concentrations using Total Ion Count (TIC) [♦], Photodiode Array (DAD) [■] and Evaporative Light Scattering (ELSD) [▲] detection. TIC detection was shown to be the more sensitive method of monitoring the reaction at low concentrations.

I-Tag versus non-Tag material - comparative ionization experiment.

5 mM, 1mM and 0.5 mM aqueous solutions of α/β-D-N-Acetylglucosamine (GlcNAc) and of **5** were prepared, respectively and 20 µl aliquots of each solution were directly injected onto the LC-MS and the ionization of the substrates monitor using a 5%–55% MeOH/water gradient over a 15 min period. Traces shown in Figures 3S and 4S. The areas for the different ions were then integrated and area values calculated for each ion. For instance, α/β-D-N-acetylglucosamine shows 2 peaks that correspond to the α and β anomers, 2 ions can be detected corresponding to $M+H^+$ 222 and $M+Na^+$ 244 and the area for each ion was integrated, respectively. For compound **5** (1mM) only M^+ ion is detected. The ionization for **5** was set to 100 %. The data shows that **5** ionizes 17.5 times better than $M+H^+$ of GlcNAc and 13.4 times better than $M+Na^+$ of GlcNAc. When the concentration of sample was reduced, the relative ionization of GlcNAc in comparison to **5** was reduced further.

Table S1.

Substrate	**Ion detected**	**Retention time (min)**	**Relative Ionization (%)**		
			1 mM	**0.05 mM**	**0.025 mM**
α/β-D-N-acetylglucosamine	($M+H^+$) 222	3.06 and 3.20	5.7	3.1	n/a
α/β-D-N-acetylglucosamine	($M+Na^+$) 244	3.06 and 3.20	7.4	5.2	n/a
5	(M^+) 659	7.31	100	100	100
n/a the peaks in the traces were too small to be accurately integrated when only 20 µL were injected					

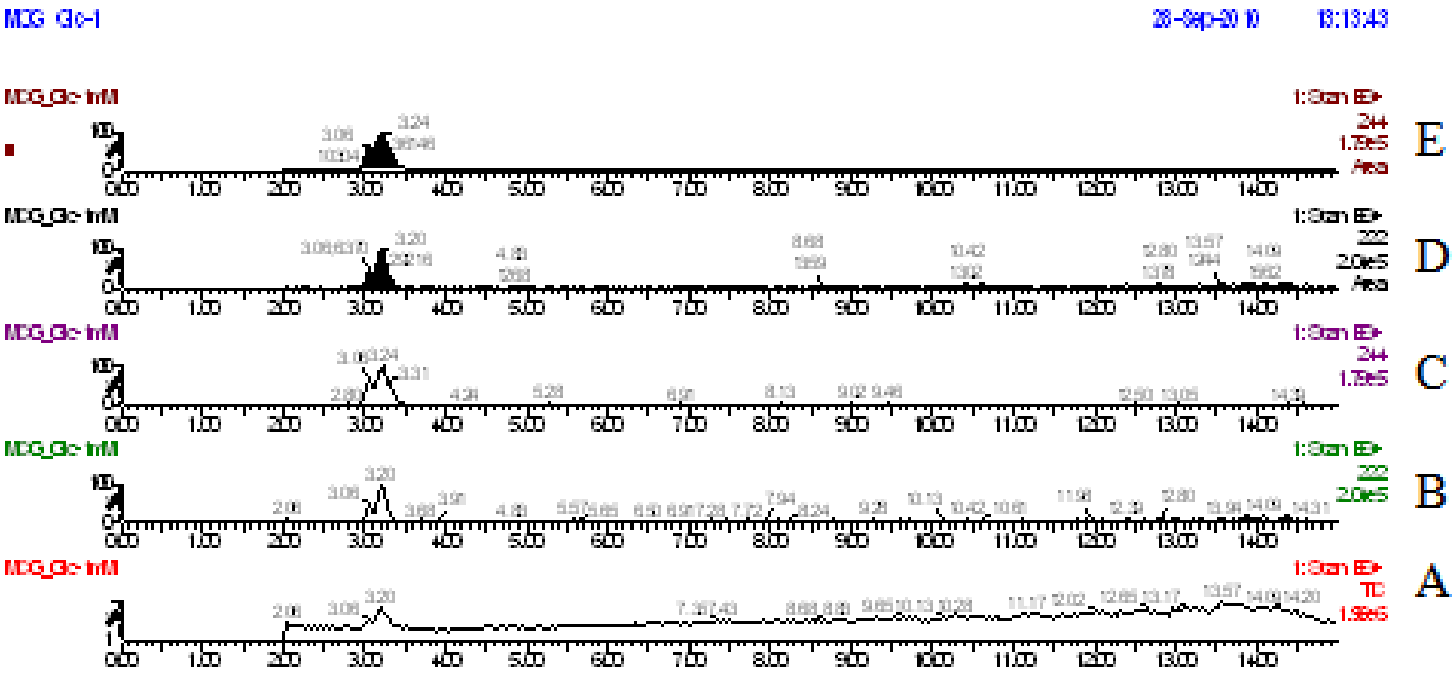


Figure 3S. LC-MS chromathogram of a 20 µL injection of α/β-D-N-Acetylglucosamine (1mM); A) TIC trace showing the corresponding signal for the α and β anomers at 3.06 and 3.20 min; B) TIC trace for GlcNAc [M+H$^+$] 222; C) TIC trace for GlcNAc [M+Na$^+$] 244; D) TIC trace with area integration value of 35586 for [M+H$^+$] 222 and E) TIC trace with an area integration value of 46450 for [M+Na$^+$] 244.

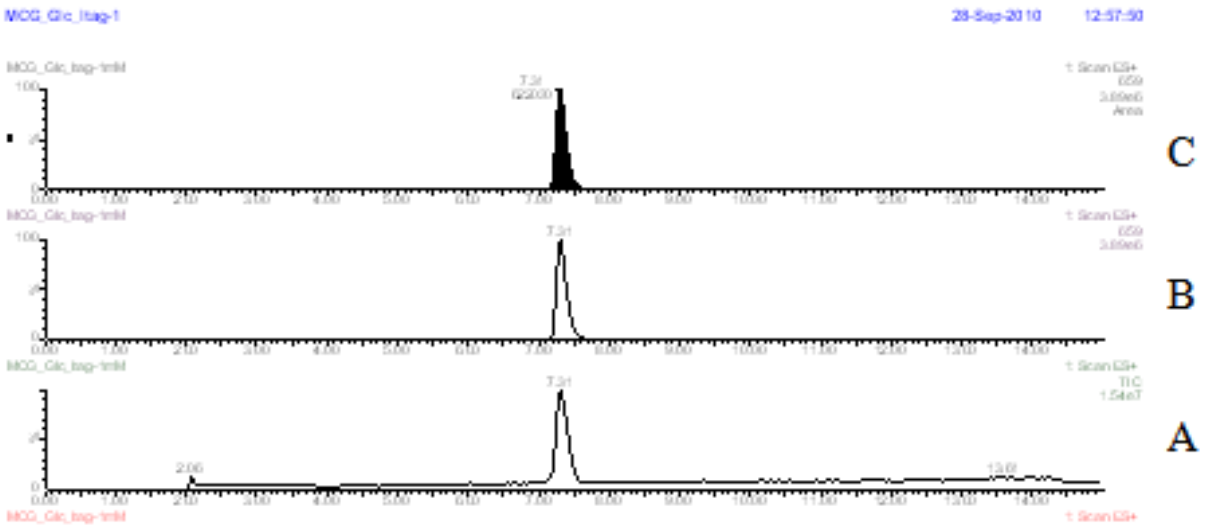
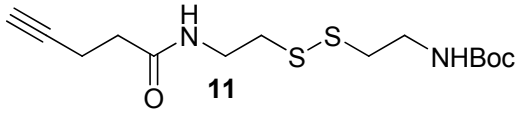


Figure 4S. LC-MS chromathogram of a 20 µL injection of **5** (1mM); A) TIC trace showing the corresponding signal for **5** at 7.31 min; B) TIC trace for GlcNAc [M$^+$] 659; D) TIC trace with an area integration value of 622030 for [M$^+$] 659.

Enzyme kinetics

β-1,4-GalT activity was measured using a LC-MS assay. An aliquot of the reaction after (20 µl) was either directly injected onto the LC-MS (qualitative assay) or after being quenched (quantitative assay) using a 5%–75% acetonitrile/water gradient over a 15 min period. To obtain qualitative activity data, UDP-Gal (1.25 mM), oligosaccharide acceptor **5 (**0.5 mM) and β-1,4-GalT (55 µU) in a 500 µL total solution, were utilized and assayed for eight time points to obtain a linear rate (Figure S2). To obtain kinetic values for N-acetylglucosamine analog **5**, a saturating concentration of UDP-Gal that was held constant at 0.46 mM and enzyme (16.5 µU). The reaction rates were measured at thirteen different concentrations of **5** (0.025 mM to 4 mM). ITagged linker **2** (29 µM) was used as an internal standard to ensure accurate injection volume. The reactions were quenched with the addition of UDP (3.4 mM). All kinetic assays were performed in duplicate. The time of incubation at 37^{o}C was set to 15 min. Kinetic parameters were obtained using Origin 7.0 software (Origin lab).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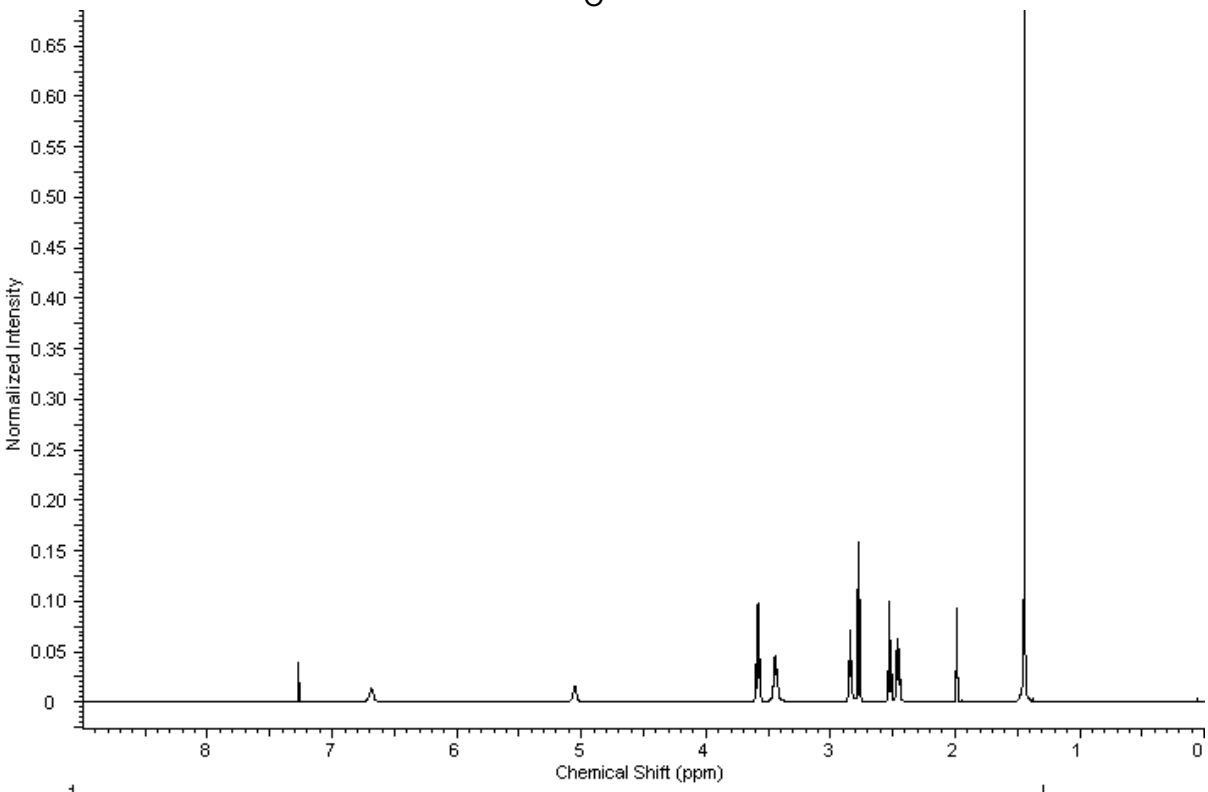
S
S
NHBoc

11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5
4
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2
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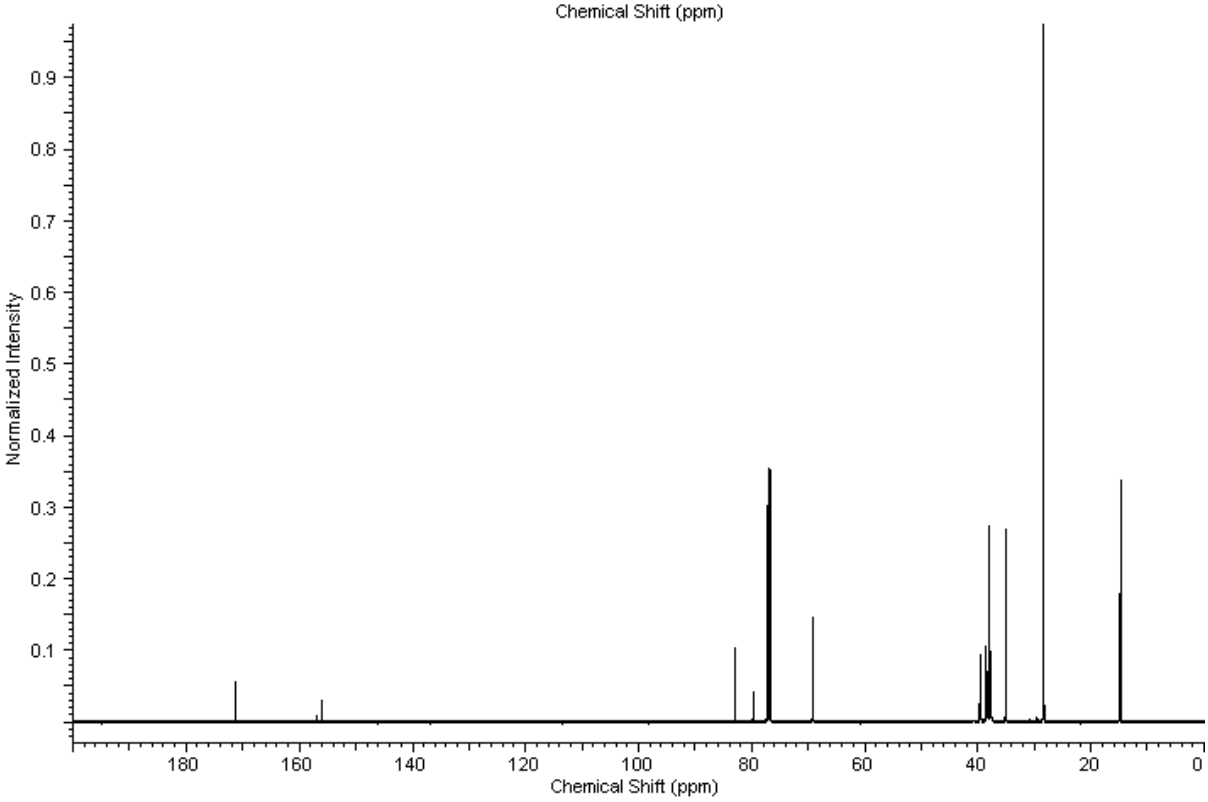
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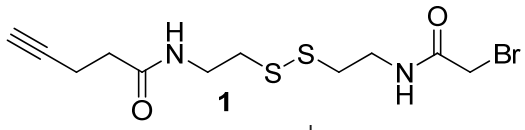
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S
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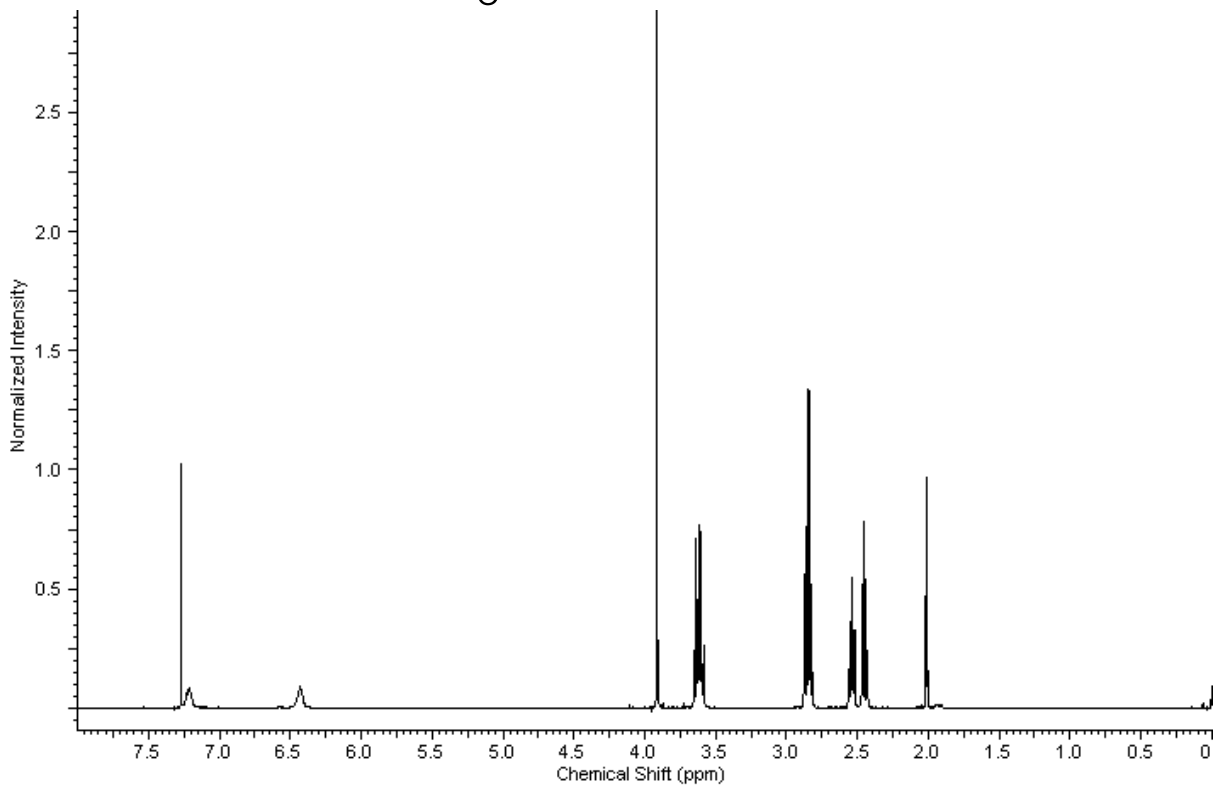
H
O
Br

1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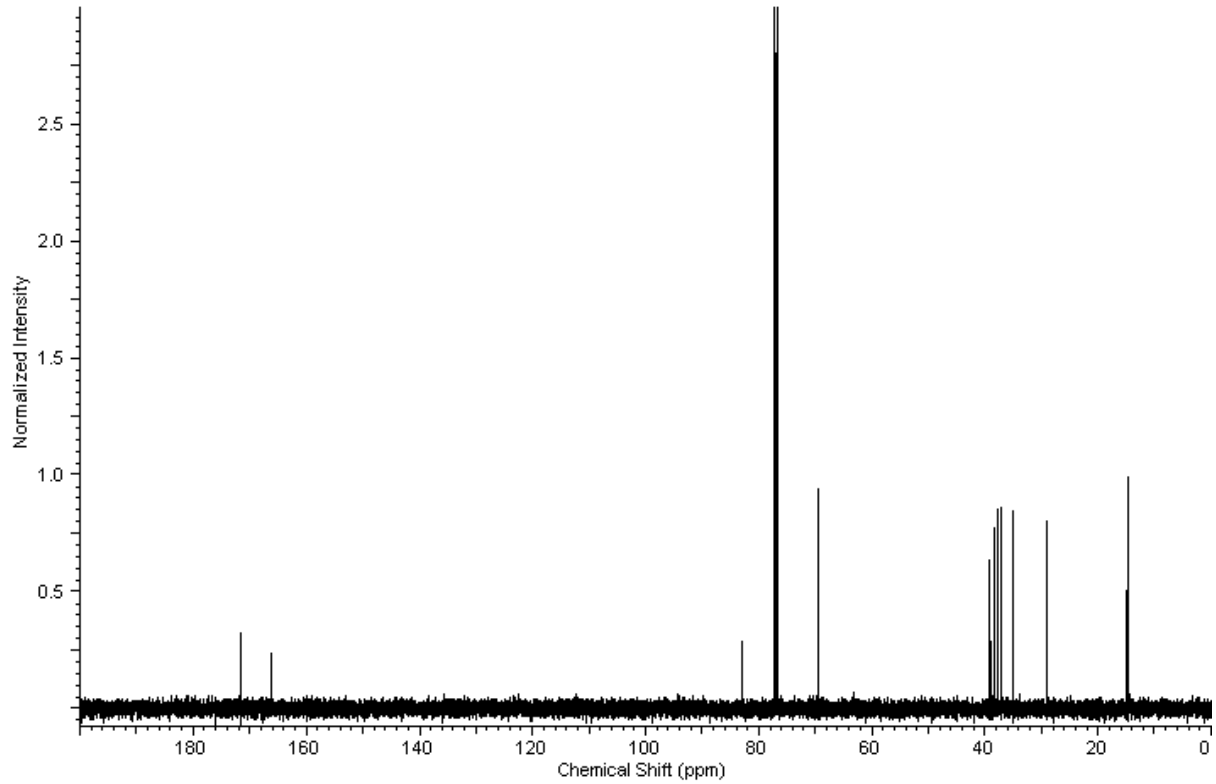
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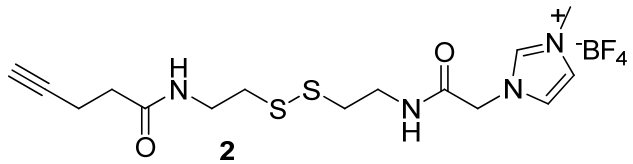
Chemical Shift (ppm)



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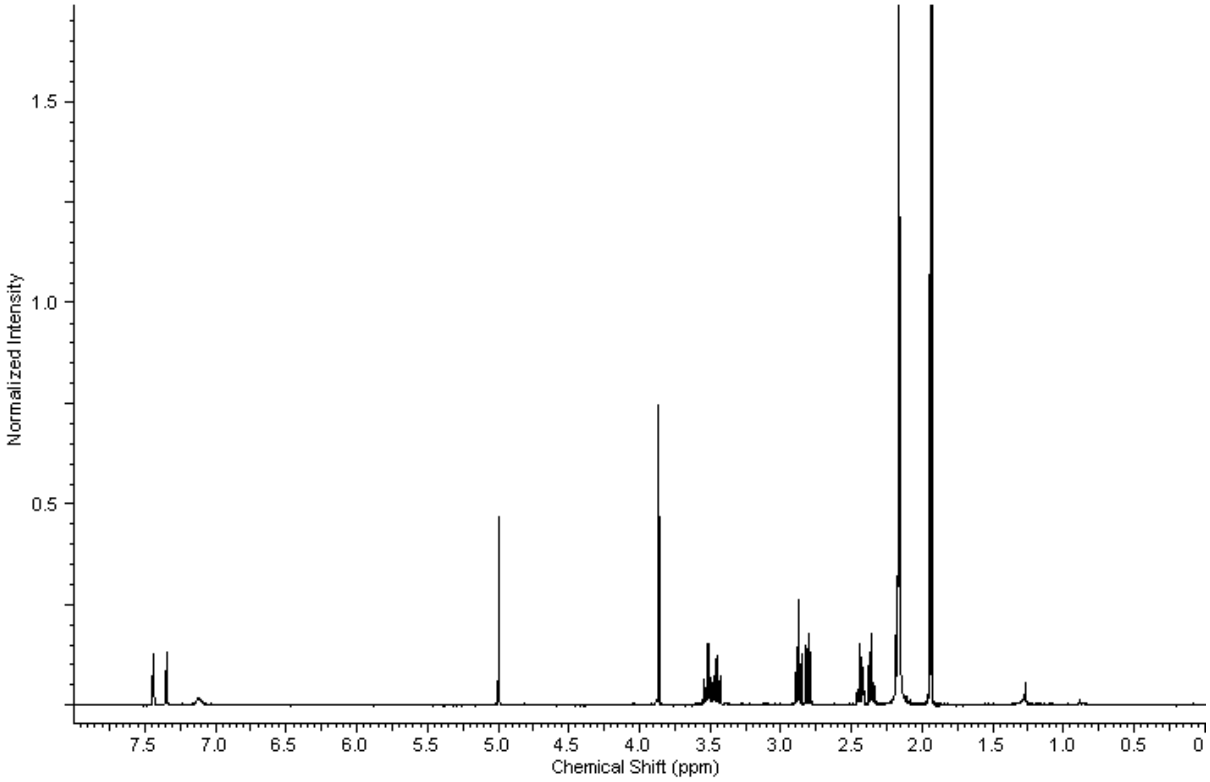
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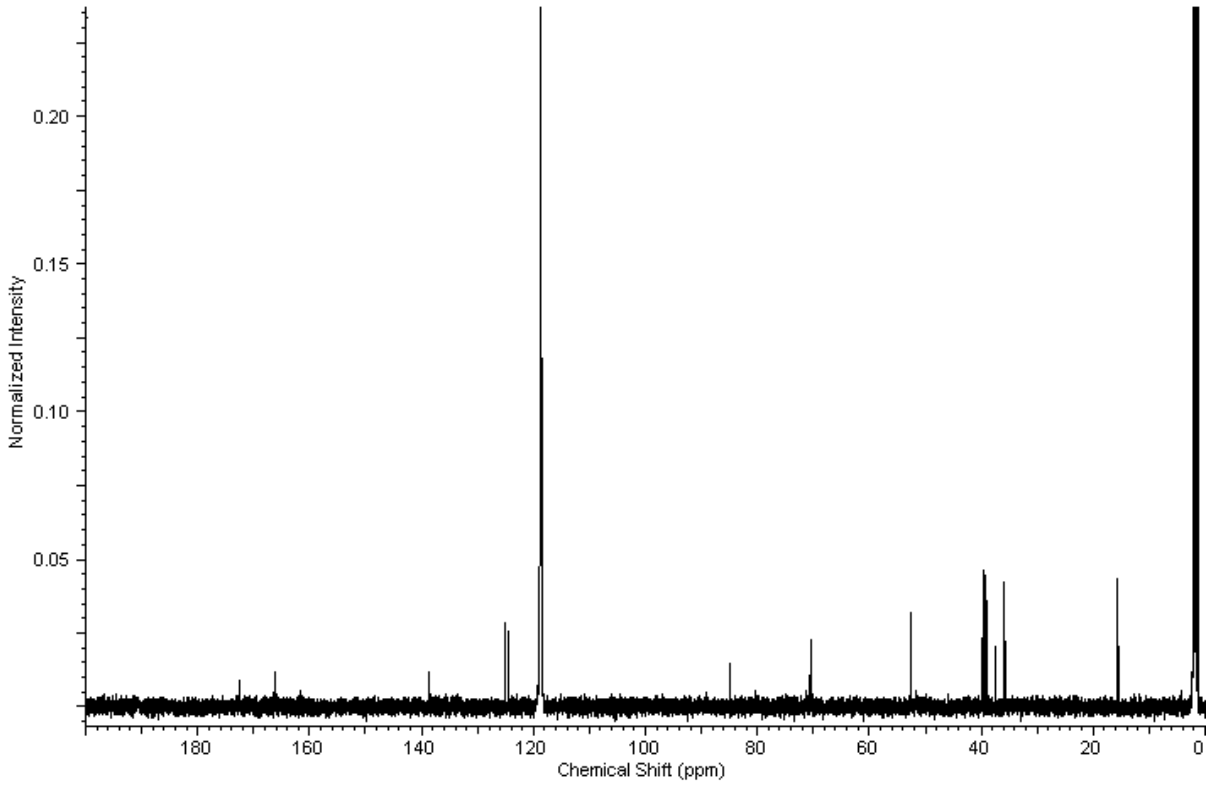
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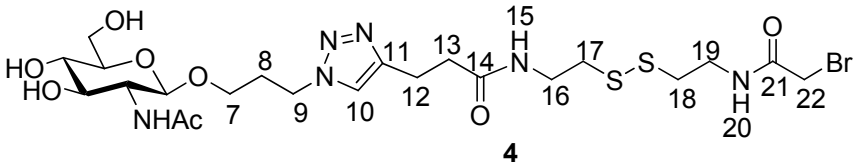
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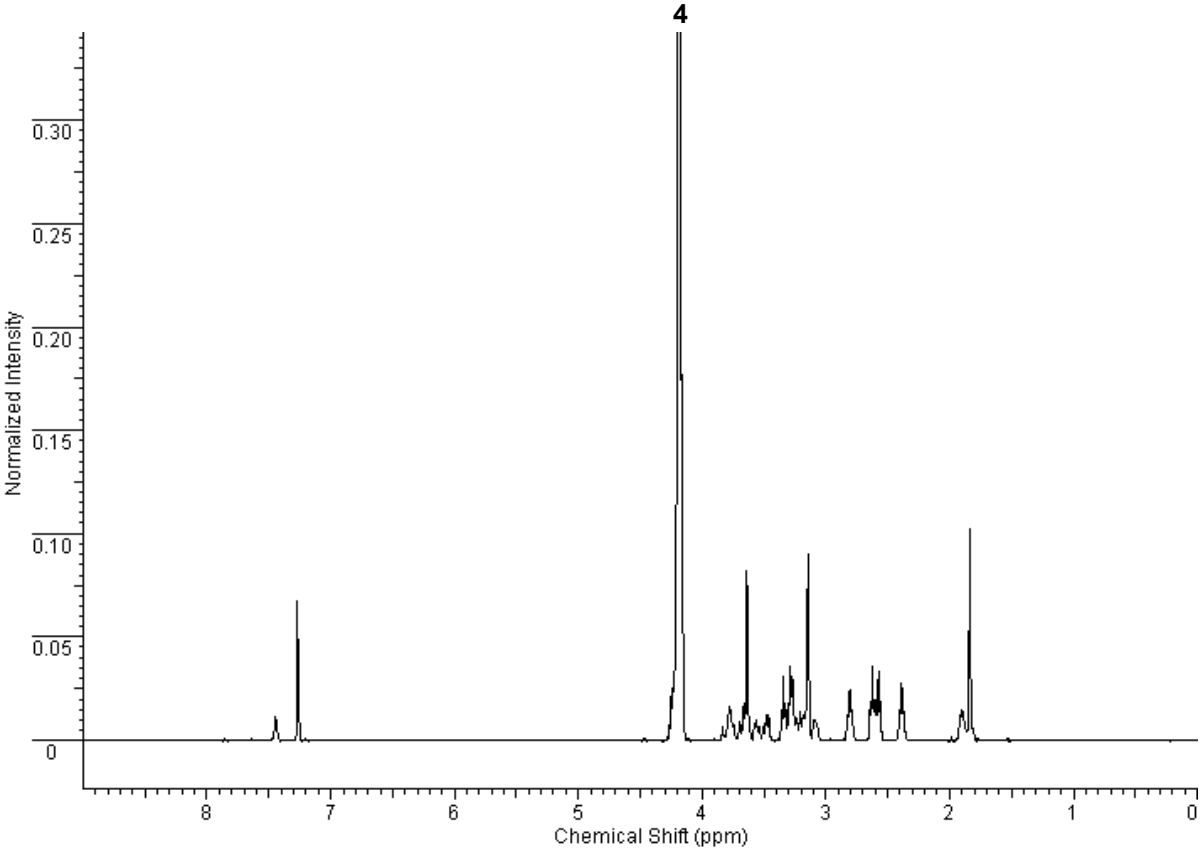
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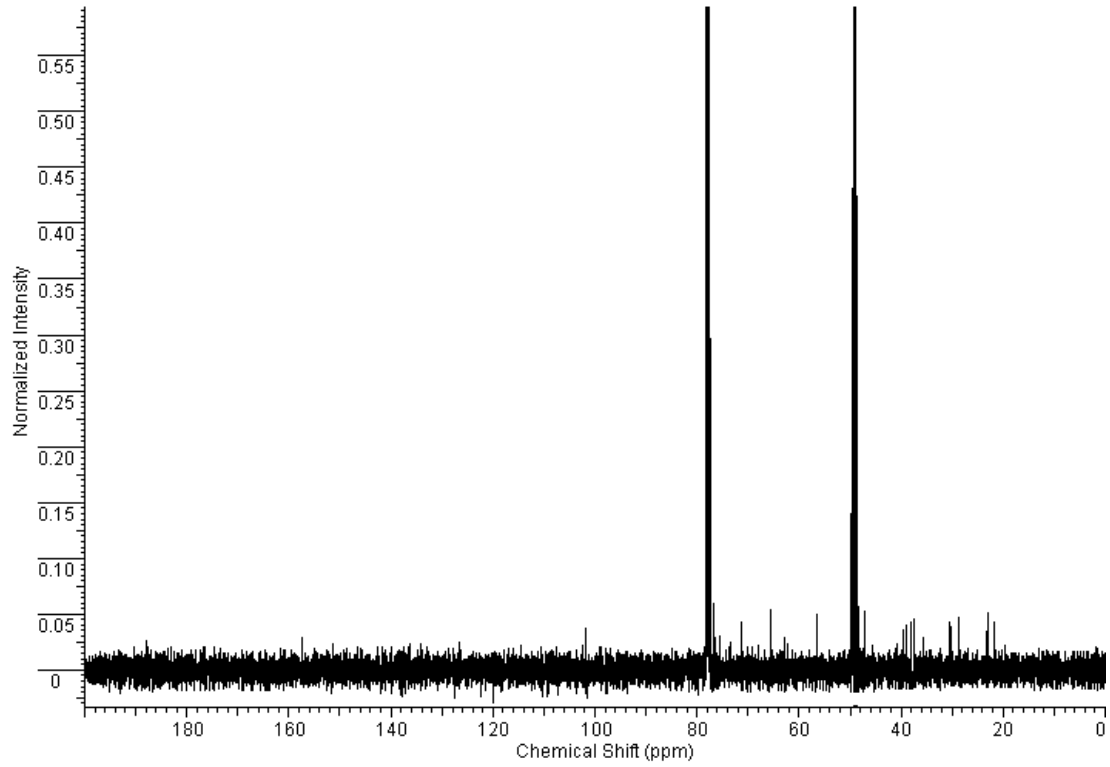
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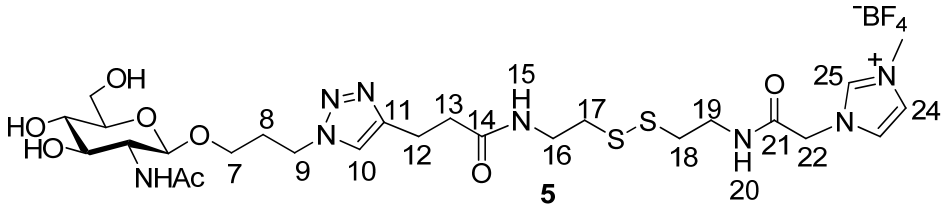
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+
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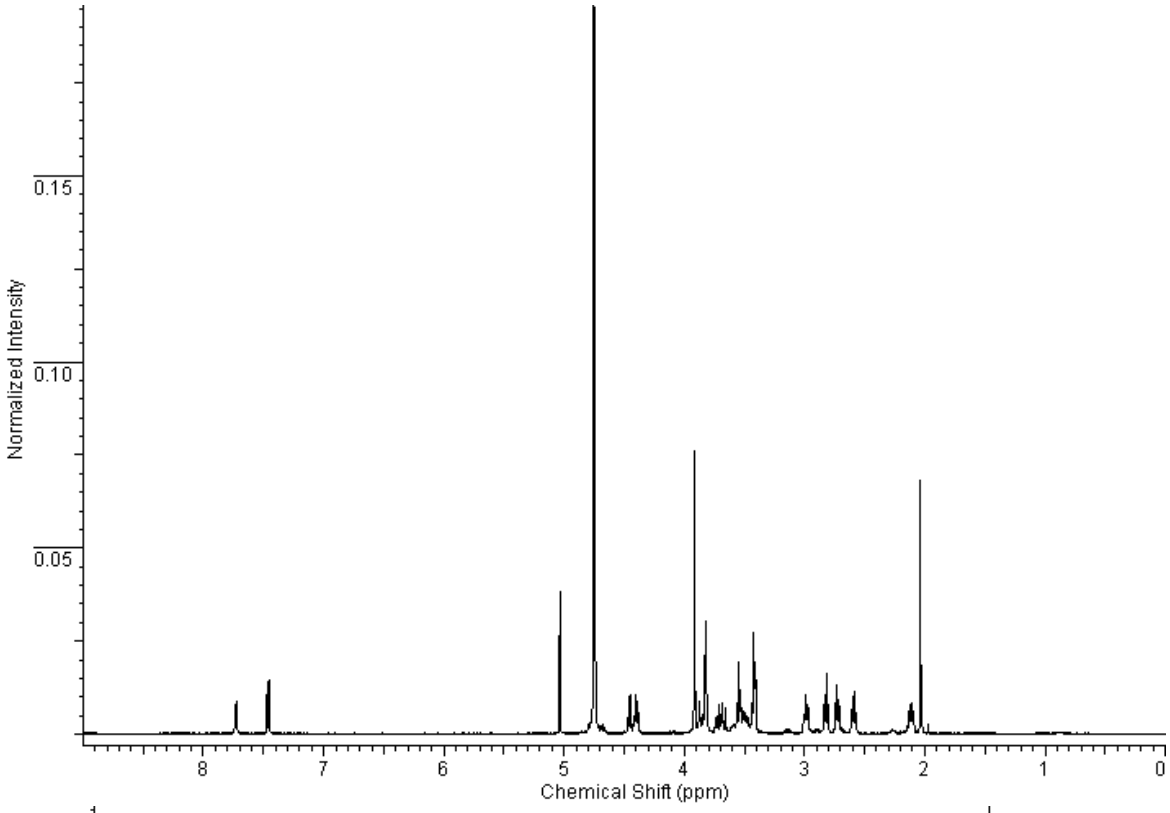
24
$^{-}BF_4$

5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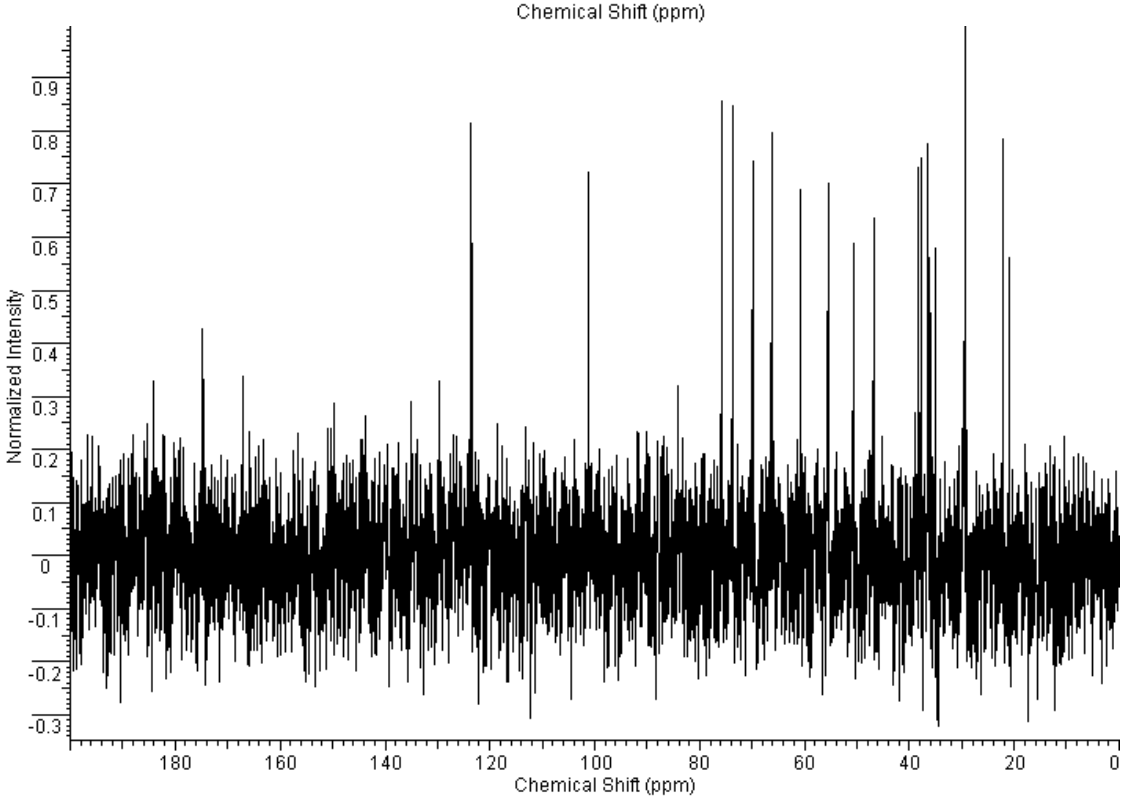
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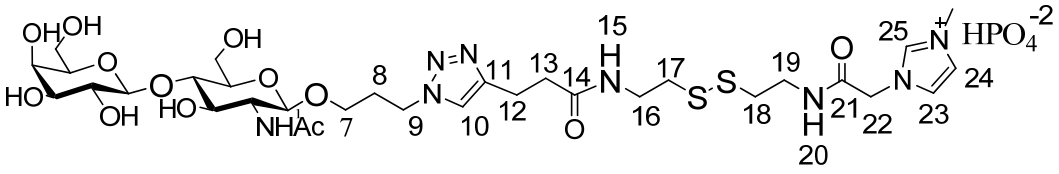
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+
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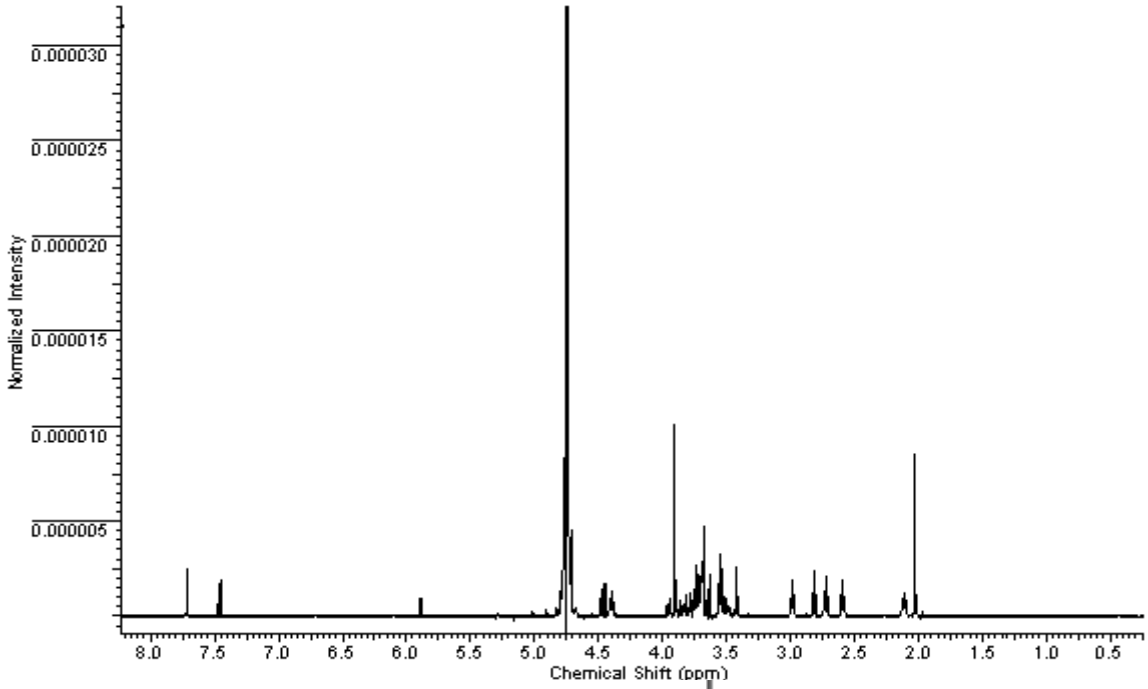
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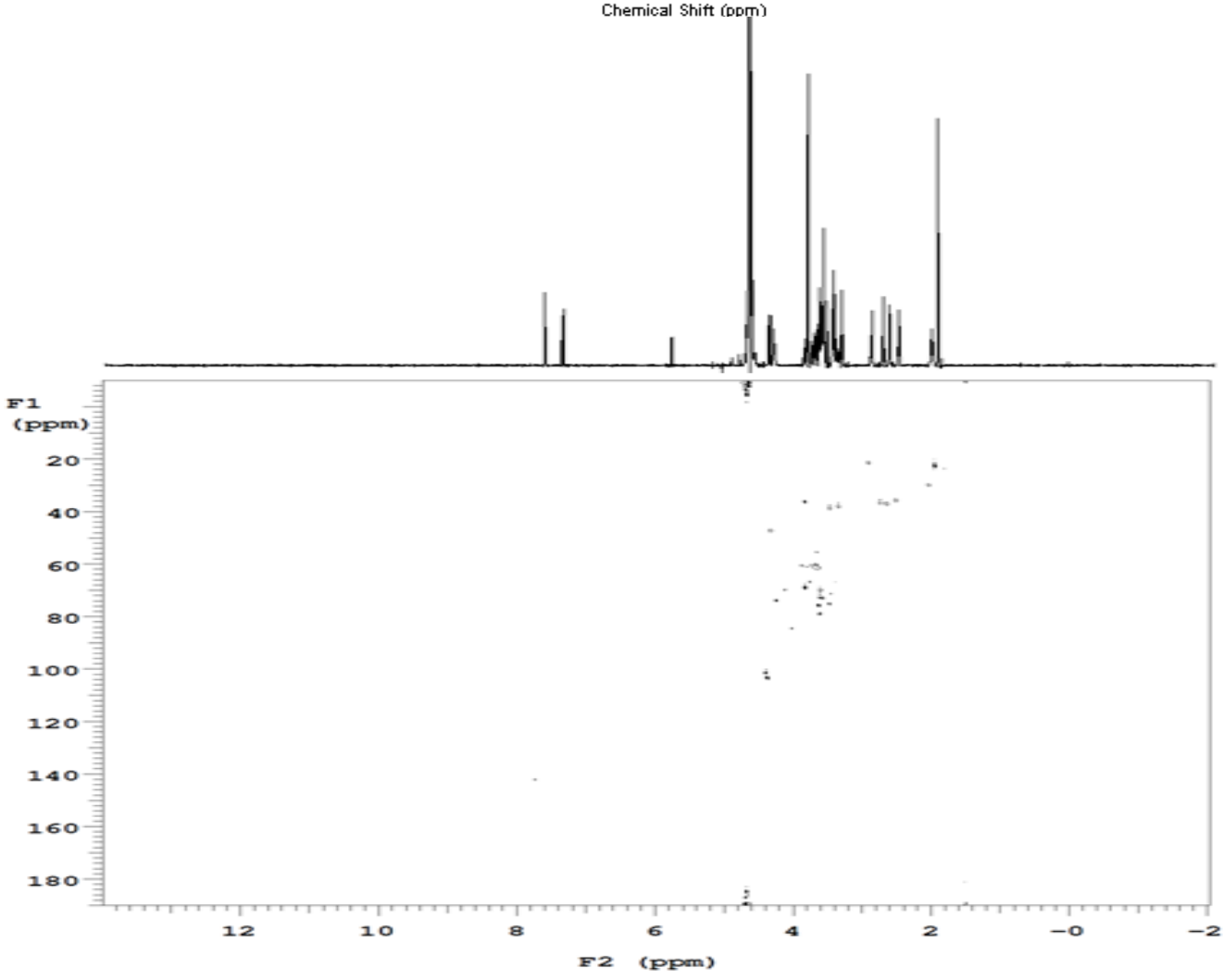
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