Electronic Supplementary Information

A click-flipped enzyme substrate boosts the performance of the diagnostic screening for hunter syndrome

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Supporting Information

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1) Supporting Figures

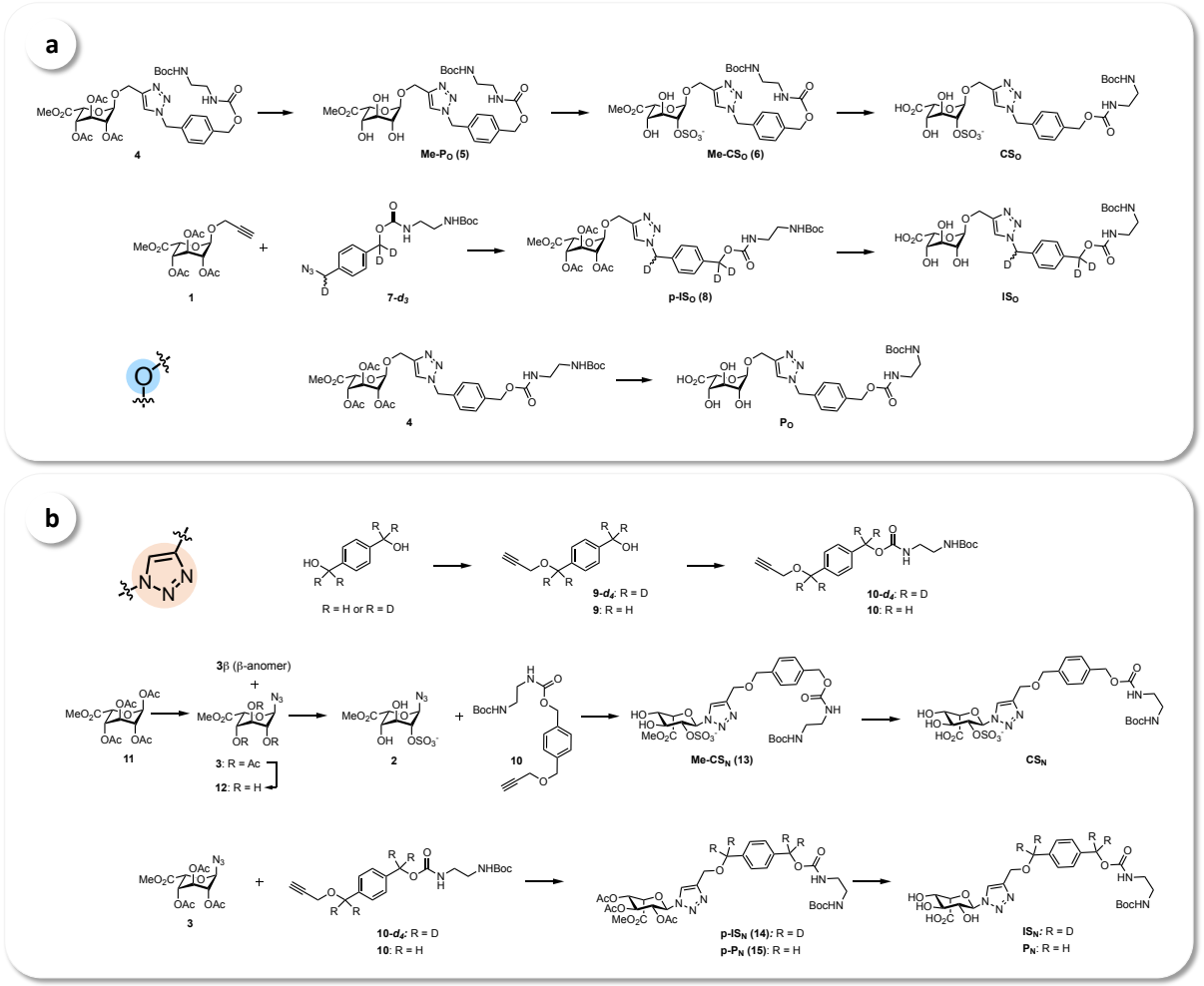
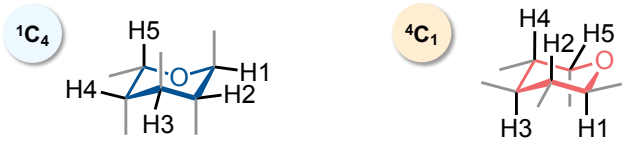


Figure S1. Synthesis of click substrates, products, and internal standards. (a) *O*-linked compounds (**CS$_O$**, **P$_O$**, **IS$_O$**), (b) triazole-linked compounds (**CS$_N$**, **P$_N$**, **IS$_N$**).



compound		H1	H2	H3	H4	H5
CS_O	$^1C_4$ chem. shift (ppm)	5.09	4.14	4.03	3.94	4.38
	multiplicity ($^3J_{HH}$)	s	m	m	m	d (2.3 Hz)
cpd 2	$^1C_4$ chem. shift (ppm)	5.78	4.23	4.38	4.57	4.99
	multiplicity ($^3J_{HH}$)	s	m	m	m	d (2.0 Hz)
	click flip					
Me-CS_N	$^4C_1$ chem. shift (ppm)	6.38	4.21	4.04	4.61	5.00
	multiplicity ($^3J_{HH}$)	d (9.4 Hz)	t (8.9 Hz)	t (8.9 Hz)	dd (8.9, 6.7 Hz)	d (6.7 Hz)
CS_N	$^4C_1$ chem. shift (ppm)	6.22	4.75	3.92	4.05	4.54
	multiplicity ($^3J_{HH}$)	d (7.9 Hz)	t (7.9 Hz)	t (8.4 Hz)	dd (8.4, 5.5 Hz)	d (5.4 Hz)
P_N	$^4C_1$ chem. shift (ppm)	6.06	3.95	3.62	3.80	4.39
	multiplicity ($^3J_{HH}$)	d (9.1 Hz)	t (9.1 Hz)	t (9.4 Hz)	dd (9.5, 6.1 Hz)	d (6.1 Hz)

Figure S2. Overview of ^{1}H NMR signals (600 MHz, chemical shift and vicinal coupling constant, $^3J_{HH}$) of **CS_O** and the iduronyl azide in $^1C_4$ conformation, as well as click-flipped triazole-linked compounds **Me-CS_N**, **CS_N**, and **P_N** in $^4C_1$ conformation. The $^3J_{HH}$ values indicate the predominant conformation (see Haasnoot *et al.*[1]): $^3J_{HH}$ of ≲2 Hz for $^1C_4$, and $^3J_{HH}$ of ~8-9 Hz (H1-H2, H2-H3, H3-H4) as well as ~5-6 Hz (H4-H5) for $^4C_1$. $^3J_{HH}$ obtained for **P_O** (4-7 Hz; as previously reported[2]) indicate $^1C_4/^2S_0$ conformation.[1] [* measured at 400 MHz]

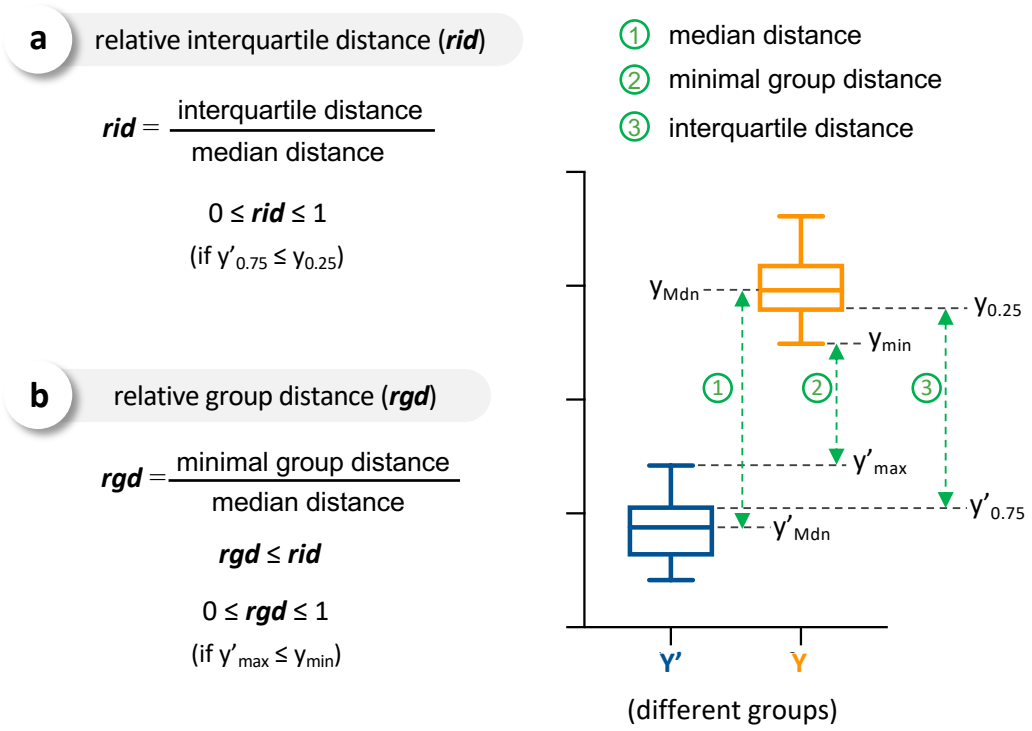


Figure S3. The relative interquartile distance (*rid*) and the relative group distance (*rgd*) are reliable statistical measures to compare different substrates and assays for the discrimination of affected from non-affected patient samples (Y: random newborns, Y': affected patients, Mdn: median, $y_{0.25}$ = first quartile of Y, $y'_{0.75}$ = third quartile of Y').

2) General Methods

All reagents, unless otherwise noted, were purchased from commercial sources without further purification. Solvents used for flash column chromatography were purchased from Donau Chemie AG (Austria) and distilled. Unless otherwise noted, reactions were carried out in dry solvents. Anhydrous acetonitrile (Sigma Aldrich, Germany) and anhydrous DMF (ACROS, Belgium) were commercially obtained and stored under argon. Dichloromethane (DCM), methanol, THF or diethyl ether were dried using PURESOLV- columns (Inert Corporation, USA) to obtain anhydrous solvents. Organic layers obtained after extractive workup were dried using anhydrous Na_2SO_4 or $MgSO_4$ (Sigma Aldrich, Germany). Reactions were carried out under an atmosphere of argon in air-dried glassware and magnetic stirring. Sensitive liquids were transferred via syringe. Thin layer chromatography was performed using TLC plates on aluminum support (Merck, silica gel 60, fluorescent indicator 254). Column chromatography was performed using a BUCHI Sepacore Flash System (2 x BUCHI Pump Module C-605, BUCHI Pump Manager C-615, BUCHI UV Photometer C-635, and BUCHI Fraction Collector C-660) or a Reveleris® X2 Flash Chromatography/Prep Purification System (BUCHI, Switzerland). Stationary phases used were either silica gel 60 (40-63 µm) as obtained from Merck (Germany) or one of the following RP-columns: Kinetex® 5 µm C18 100 Å, AXIA Packed LC Column 100 x 30.0 mm; Luna® 10 µm C18 100 Å, 250 x 21.2 mm; Luna® 10 µm C18 100 Å, 250 x 10 mm from Phenomenex (USA), or SNAP Ultra C18 cartridges from Biotage® (Sweden). Solvents used for preparative HPLC were purchased from VWR.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200, Bruker Avance UltraShield 400 or Bruker Ascend 600 spectrometer at 20 °C. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane and calibrated using solvent residual peaks. Data is shown as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, bs = broad signal), coupling constants (J, Hz) and integration.

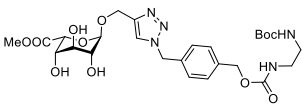
HPLC analysis for reaction monitoring were performed on a 1200 series system (Agilent Technologies, USA) using a Kinetex® (5 µm C18 100 Å, 50 x 4.6 mm, Phenomenex, USA) column and water/acetonitrile gradient elution. Peak detection was enabled via DAD (Agilent Technologies) as well as a Bruker HCT Esquire Ion Trap MS.

HRMS measurements were performed on a Thermo Ultimate 3000 system with a CTC PAL autosampler using flow injection with isocratic water/acetonitrile elution coupled with a Thermo Q Exactive Focus.

In general, no unexpected or unusually high safety hazards were encountered.

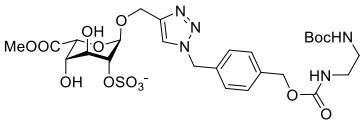
3) Synthesis

Me-P_O (5)



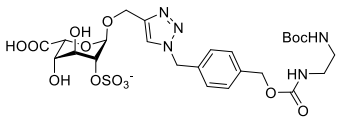
Compound **4**[2] (150 mg, 0.21 mmol, 1 eq) was dissolved in MeOH (40 mL) and the solution was purged with argon. After addition of molecular sieves (400 mg, 3Å) the mixture was stirred overnight. The reaction was filtrated and the solvent was evaporated. The residue was purified by preparative HPLC (C18, 5-95% MeCN in H_2O, gradient elution) to afford **5** (83 mg, 67%) as a white solid; ^{1}H NMR (600 MHz, CD_3OD) δ 7.98 (s, 1H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 5.58 (s, 1H), 5.06 (s, 1H), 4.98 (d, *J* = 2.9 Hz, 1H), 4.80 (d, *J* = 12.6 Hz, 1H), 4.71 (d, *J* = 2.9 Hz, 1H), 4.67 (d, *J* = 12.5 Hz, 1H), 3.90–3.84 (m, 1H), 3.80 (t, *J* = 4.7 Hz, 1H), 3.75 (s, 2H), 3.51- 3.48 (m, 1H), 3.35 (s, 2H), 3.19–3.15 (m, 2H), 3.14–3.09 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 172.1 (s, 1C), 158.8 (s, 1C), 158.6 (s, 1C), 145.6 (s, 1C), 139.0 (s, 1C), 136.4 (s, 1C), 129.3 (d, 2C), 129.3 (d, 2C), 125.4 (d, 1C), 102.1 (d, 1C), 80.1 (s, 1C), 71.6 (d, 1C), 71.5 (d, 1C), 71.0 (d, 1C), 70.6 (d, 1C), 66.9 (t, 1C), 62.1 (t, 1C), 54.6 (t, 1C), 52.6 (q, 1C), 41.8 (t, 1C), 41.2 (t, 1C), 28.7 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 596.2562 for $C_{26}H_{38}N_5O_{11}^+$, found 596.2557.

Me-CS_O (6)



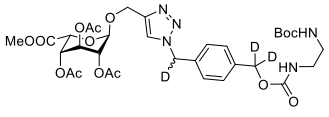
Me-P_O (65 mg, 0.11 mmol, 1 eq) was dissolved in MeOH (10 mL) and dibutyltin(IV) oxide (41 mg, 0.16 mmol, 1,5 eq) was added. The mixture was heated to reflux until dibutyltin(IV)oxide completely dissolved. The reaction was allowed to cool to 30 °C and then concentrated. The residue was co-evaporated with toluene (10 mL), redissolved in DMF (10 mL) and sulfur trioxide trimethylamine complex (23 mg, 0.16 mmol, 1,5 eq) was added. The reaction mixture was stirred overnight at 55 °C, allowed to cool to room temperature and then quenched by addition of MeOH (10 mL). The mixture was concentrated and the residue was extracted with water (2 mL). The aqueous extract was purified by preparative HPLC (C18, 5-30% MeCN in H_2O, gradient elution) affording a 4:1 mixture of the 2-*O*-sulfated **Me-CS_O** (32 mg, 43%) and its 4-*O*-sulfated isomer (8 mg, 11%) as white solids; **Me-CS_O** (**6**, 2-*O*-Sulfate): ^{1}H NMR (600 MHz, CD_3OD) δ 8.01 (s, 1H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 5.58 (s, 2H), 5.24 (s, 1H), 5.06 (s, 2H), 4.82–4.66 (m, 3H), 4.26–4.22 (m, 1H), 4.08 – 4.03 (m, 1H), 3.87 (bs, 1H), 3.77 (s, 3H), 3.20–3.10 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 172.2 (s, 1C), 158.9 (s, 1C), 158.6 (s, 1C), 145.6 (s, 1C), 138.8 (s, 1C), 136.4 (s, 1C), 128.3 (d, 2C), 128.3 (d, 2C), 125.6 (d, 1C), 100.2 (d, 1C), 80.1 (s, 1C), 74.7 (d, 1C), 70.4 (d, 1C), 69.8 (d, 1C), 68.8 (d, 1C), 66.9 (t, 1C), 62.3 (t, 1C), 54.6 (t, 1C), 52.7 (q, 1C), 41.8 (t, 1C), 41.2 (t, 1C), 28.7 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 676.2131 for $C_{26}H_{38}N_5O_{14}S^+$, found 676.2125; 4-*O*-Sulfate (isomer): ^{1}H NMR (600 MHz, CD_3OD) δ 7.98 (d, 1H), 7.37 (d, *J* = 8.0 Hz, 5H), 7.31 (d, *J* = 8.0 Hz, 4H), 5.58 (s, 2H), 5.06 (s, 2H), 4.97 (d, *J*= 3.6 Hz, 1H), 4.84 (d, *J* = 3.3 Hz, 1H), 4.82–4.65 (m, 2H), 4.53–4.50 (m, 1H), 4.14 (t, *J* = 5.0 Hz, 1H), 3.75 (s, 3H), 3.50- 3.45 (m, 1 H), 3.20–3.10 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 171.1 (s, 1C), 158.9 (s, 1C), 158.6 (s, 1C), 145.7 (s, 1C), 138.8 (s, 1C), 136.4 (s, 1C), 128.3 (d, 2C), 128.3 (d, 2C), 125.5 (d, 1C), 102.1 (d, 1C), 80.1 (s, 1C), 76.6 (d, 1C), 71.6 (d, 1C), 70.4 (d, 1C), 69.6 (d, 1C), 66.9 (t, 1C), 62.4 (t, 1C), 54.6 (t, 1C), 52.8 (q, 1C), 41.8 (t, 1C), 41.2 (t, 1C), 28.8 (q, 3C).

CS_O



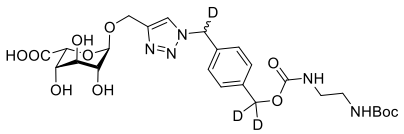
To a solution of **6** (20 mg, 0.03 mmol, 1eq, 4:1 mixture of 2-*O*-sulfate and 4-*O*-sulfate) in THF (5.4 mL) and water (0.47 mL) cooled to 0 °C was added aqueous NaOH (1.33 mL, 0.1 M). After 4 h the reaction was quenched by addition of aqueous HCl (0.1 M) until pH 7. The reaction was concentrated and the residue was purified by preparative HPLC (C18, 5-30% MeCN in H_2O, gradient elution) affording **CS_O** (14.4 mg) and its 4-*O*-sulfated isomer (3.6 mg) as white solids (92%); **CS_O** (2-*O*-Sulfate): ^{1}H NMR (600 MHz, D_2O) δ 8.23 (s, 1H), 7.40- 7.26 (m, 4H), 6.29 (d, *J* = 8.51 Hz, 1H), 5.58 (d, *J* = 15.7 Hz, 1H), 5.55 (d, *J* = 15.1 Hz, 1H), 5.09 (s, 1H), 5.04 (s, 2H), 4.79-4.68 (m, 2H), 4.38 (d, *J* = 2.3 Hz, 1H), 4.15–4.13 (m, 1H), 4.03 (td, *J* = 3.0, 1.0 Hz, 1H), 3.94 (bt, *J* = 2.8 Hz, 1H), 3.22–3.06 (m, 4H), 1.29 (s, 9H); ^{13}C NMR (150 MHz, D_2O) δ 175.9 (s, 1C), 158.3 (s, 1C), 158.2 (s, 1C), 143.8 (s, 1C), 136.9 (s, 1C), 134.6 (s, 1C), 128.3 (d, 2C), 128.1 (d, 2C), 125.4 (d, 1C), 97.9 (d, 1C), 80.8 (s, 1C), 73.8 (d, 1C), 68.8 (d, 1C), 68.7 (d, 1C), 67.9 (d, 1C), 66.2 (t, 1C), 60.3 (t, 1C), 53.5 (t, 1C), 40.2 (t, 1C), 39. 6 (t, 1C), 27.5 (q, 3C); 4-*O*-Sulfate (isomer): ^{1}H NMR (600 MHz, D_2O) δ 8.23 (s, 1H), 7.40–7.26 (m, 4H), 6.29 (d, *J* = 8.5 Hz, 1H), 5.58 (d, *J* = 15.7 Hz, 1H), 5.55 (d, *J* =15.1 Hz, 1H), 5.04 (s, 2H), 4.93 (d, J= 3.24 Hz, 1H), 4.79–4.68 (m, 2H), 4.52 (bt, *J* = 3.7 Hz, 1H), 4.48 (d, *J* = 2.6 Hz, 1H), 4.22 (bt, *J* = 4.68 Hz, 1H), 3.60–3.53 (m, 1H), 3.22–3.06 (m, 4H), 1.29 (s, 9H); ^{13}C NMR (150 MHz, D_2O) δ 175.4 (s, 1C), 158.3 (s, 1C), 158.2 (s, 1C), 143.8 (s, 1C), 136.7 (s, 1C), 134.8 (s, 1C), 128.6 (d, 2C), 127.9 (d, 2C), 125.3 (d, 1C), 99.9 (d, 1C), 80.2 (s, 1C), 76.5 (d, 1C), 69.2 (d, 1C), 68.6 (d, 1C), 68.6 (d, 1C), 66.6 (t, 1C), 60.5 (t, 1C), 53.5 (t, 1C), 40.8 (t, 1C), 40.6 (t, 1C), 27.4 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 662.1974 for $C_{25}H_{36}N_5O_{14}S^+$, found 662.1967.

p-IS_O (8)



Compound **1**[2] (70 mg, 0.19 mmol, 1 eq) and click marker **7-d_3**[2] (73 mg, 0.21 mmol, 1.1 eq) were dissolved in THF (10.5 ml) and Et_3N (23 mg, 0.23 mmol, 1.2 eq) was added, followed by addition of CuI (3.6 mg, 0.019 mmol, 0.1 eq) under an argon atmosphere. The reaction mixture was stirred overnight, filtrated and concentrated. The residue was purified by column chromatography (90 g SiO_2, 20-100% EtOAc in hexanes, gradient elution) to afford **8** (125 mg, 92%) as a white solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.28- 7.24 (m, 2H), 5.52 (s, 1H), 5.49 (d, *J* = 26.7 Hz, 1H) 5.24 (bs, 1H), 5.11–5.08 (m, 2H), 5.03–5.00 (m, 1H), 4.89–4.83 (m, 3H), 4.78–4.74 (m, 1H), 4.70 (d, *J* = 12.6 Hz, 1H), 3.77 (s, 3H), 3.31–3.23 (m, 4H), 2.05 (s, 3H), 2.04 (s, 13H), 1.42 (s, 16H), 1.94 (s, 3H), 1.42 (s, 19H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.6 (s, 1C), 169.5 (s, 1C), 169.2 (s, 1C), 168.4 (s, 1C), 156.7 (s, 1C), 156.6 (s, 1C), 144.4 (s, 1C), 137.5 (s, 1C), 134.3 (s, 1C), 128.9 (d, 2C), 128.5 (d, 2C), 122.9 (d, 1C), 97.5 (d, 1C), 79.8 (s, 1C), 67.2 (d, 1C), 66.7 (d, 2C), 66.6 (d, 1C), 62.0 (t, 1C), 53.8 (t, $^1J_{CD}$ = 21 Hz, 1C), 52.8 (q, 1C), 41.8 (t, 1C), 40.7 (t, 1C), 28.5 (q, 3C), 20.9 (q, 1C), 20.8 (q, 1C), 20.7 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 725.3068 for $C_{32}H_{41}D_3N_5O_{14}^+$, found 725.3057.

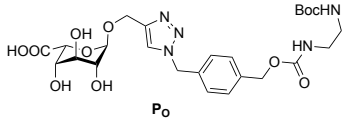
IS_O



A solution of **8** (65 mg, 0.089 mmol, 1 eq) in THF (25 mL) and water (3.85 mL) was cooled to 0 °C and aqueous NaOH (4.48 mL, 0.45 mmol, 5 eq, 0.1M) was added. After 4h the reaction was quenched by addition of aqueous HCl (0.1 M) until pH 7. The reaction was concentrated and the residue was purified by preparative HPLC (C18, MeCN in H_2O, 5-30%, gradient elution) to give **IS_O** (32 mg, 62%) as a white solid; ^{1}H NMR (600 MHz, CD_3OD) δ 7.94 (s, 1H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.21 (d, *J* = 8.1 Hz, 2H), 5.46 (s, 1H), 4.71 (d, *J* = 5.3 Hz, 1H), 4.66 (d, *J* = 12.7 Hz, 1H), 4.21 (d, *J* = 4.1 Hz, 1H), 3.65 (dd, *J* = 6.8, 4.4 Hz, 1H), 3.50 (t, *J* = 6.7 Hz, 1H), 3.22 (dd, *J* = 6.5,

5.3 Hz, 1H), 3.10–3.04 (m, 2H), 3.03–2.99 (m, 2H), 1.31 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 177.0 (s, 1C), 158.9 (s, 1C), 158.6 (s, 1C), 145.9 (s, 1C), 138.8 (s, 1C), 136.4 (s, 1C), 129.4 (d, 2C), 129.3 (d, 2C), 125.6 (d, 1C), 101.4 (d, 1C), 80.1 (s, 1C), 73.9 (d, 1C), 72.8 (d, 1C), 72.4 (d, 1C), 71.9 (d, 1C), 62.2 (t, 1C), 54.3 (t, $^{1}J_{CD}$ = 21 Hz, 1C), 41.8 (t, 1C), 41.2 (t, 1C), 28.8 (t, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 585.2594 for $C_{25}H_{33}D_3N_5O_{11}^+$, found 585.2583.

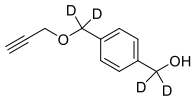
P_O



P_O

We have previously described the synthesis of **P_O** starting from compound **4**.[1] 20 mg of remaining **P_O** were re-purified by preparative HPLC (C18, 5-30 % MeCN in H_2O, gradient elution) immediately before any analytical measurements.

[4-[(Propyn-1-yloxy)methyl-*d*$_2$-]phenyl]-*d*$_2$-methanol (9-*d*$_4$)

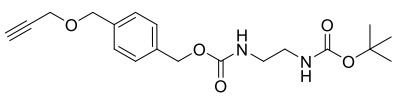


To a suspension of NaH (422 mg, 10.54 mmol, 1.5 eq) in THF (40 mL) was slowly added a solution of 1,4-(benzene)dimethane-*d*$_4$-ol[3] (1 g, 7.03 mmol, 1 eq) in THF (20 mL). After stirring for 1 h, tetrabutylammonium iodide (260 mg, 0.7 mmol, 0.1 eq) and propargyl bromide (80% in toluene, 1.18 mL, 10.55 mmol, 1.5 eq) were added. The mixture was stirred overnight at 60 °C and then quenched by addition of saturated sodium bicarbonate solution. The reaction was extracted with diethyl ether, dried with sodium sulfate and concentrated. Purification via column chromatography (12 g SiO_2, 0-47% EtOAc in hexanes, gradient elution) gave **9-*d*$_4$** (370 mg, 29%) as a yellowish oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.33 (m, 4H), 4.17–4.14 (m, 2H), 2.47 (t, *J* = 2.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.7 (s, 1C), 136.7 (s, 1C), 128.5 (d, 2C), 127.3 (d, 2C), 79.8 (s, 1C), 74.8 (d, 1C), 57.1 (t, 1C); HR-ESI-ORBITRAP $[M+Na]^+$ m/z calcd. 203.0981 for $C_{11}H_8D_4O_2Na^+$, found 203.0979.

General procedure A – Carbamate coupling

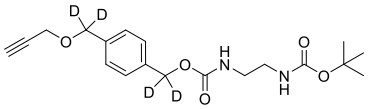
To a solution of alcohol (1 eq) in THF (1.76 mL/mmol) cooled to 0°C was added sodium carbonate (1 eq) and triphosgene (0.5 eq). The reaction was stirred overnight, filtrated and concentrated under reduced pressure at room temperature. The residue was dissolved in THF (0.35 mL/mmol) and added to a solution of amine (1.1 eq) in THF (1.41 mL/mmol) and aqueous NaOH (1 N, 1.76 mL/mmol). The reaction was stirred overnight and then extracted with dichloromethane. The combined organic layer was concentrated and the residue was purified by column chromatography (90 g SiO_2, 10-30% EtOAc in hexanes, gradient elution).

***N*-click marker (10)**



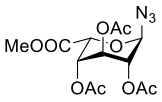
Following general procedure A, compound **9**[4] (0.33 g, 1.89 mmol, 1 eq) was reacted with N-Boc-diaminoethane (0.33 g, 2.08 mmol, 1.1 eq) to afford **5** (251 mg, 48%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 4H), 5.14 (bs, 1H), 5.09 (s, 2H), 4.81 (bs, 1H), 4.61 (s, 2H), 4.17 (d, *J* = 2.4 Hz, 2H), 3.32–3.24 (m, 4H), 2.47 (t, *J* = 2.4 Hz, 1H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7 (s, 1C), 156.4 (s, 1C), 137.3 (s, 1C), 136.2 (s, 1C), 128.3 (d, 4C), 79.7 (s, 1C), 79.6 (s, 1C), 74,7 (d, 1C), 71.2 (t, 1C), 66.5 (t, 1C), 57.1 (t, 1C), 41.6 (t, 1C), 40.6 (t, 1C), 28.5 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 363.1914 for $C_{19}H_{26}N_2O_5^+$, found 363.1911.

Deuterated *N*-click marker (10-*d*$_4$)



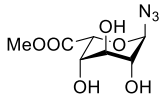
Following general procedure A, **9-*d*$_4$** (83 mg, 0.46 mmol, 1 eq) was reacted with N-Boc-diaminoethane (1.1 eq) to afford **5-*d*$_4$** (57 mg, 34%) as a white solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35 (s, 4H), 5.12 (bs, 1H), 4.81 (bs, 1H), 4.16 (d, *J* = 2.3 Hz, 2H), 3.35–3.20 (m, 4H), 2.47 (t, *J* = 2.3 Hz, 1H), 1.43 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.8 (s, 1C), 156.6 (s, 1C), 137.3 (s, 1C), 136.2 (s, 1C), 128.4 (d, 4C), 79.8 (s, 1C), 79.7 (s, 1C), 74.8 (d, 1C), 57.1 (t, 1C), 41.7 (t, 1C), 40.7 (t, 1C), 28.5 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 367.2166 for $C_{19}H_{23}D_4N_2O_5^+$, found 367.2161.

Azido- 2,3,4-triacetyl-α-L-idopyranuronic acid, methyl ester (3)



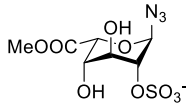
1,2,3,4-Tetraacetyl-α-L-idopyranuronic acid, methyl ester (**11**)[5] (1 g, 2.66 mmol, 1 eq) was dissolved in DCM (27.5 mL) and TMS-N_3 (0.337 g, 2.92 mmol, 1.1 eq) was added at room temperature under argon. $SnCl_4$ (1 M in DCM, 2.66 mL, 2.66 mmol, 1 eq) was added and the mixture was stirred for 2 h. The reaction was quenched by addition of saturated sodium bicarbonate solution and extracted with DCM. The organic layer was dried with Na_2SO_4 and the solvent was evaporated. Purification by column chromatography (90 g SiO_2, 20-50% EtOAc in hexanes, gradient elution) afforded pure **3** (800 mg, 83%) as a colorless oil and the β-anomer **3β** (150 mg) as a by-product.
3: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.56 (d, *J* = 3.4 Hz, 1H), 5.15 (t, *J* = 3.1 Hz, 1H), 5.12 (t, *J* = 4.2 Hz, 1H), 4.93 (d, *J* = 3.2 Hz, 1H), 4.74 (t, *J* = 4.0 Hz, 1H), 3.80 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.5 (s, 1C), 169.4 (s, 1C), 169.1 (s, 1C), 168.1 (s, 1C), 86.7 (d, 1C), 68.6 (d, 1C), 67.2 (d, 1C), 67.1 (d, 1C), 66.8 (d, 1C), 52.9 (q, 1C), 20.9 (q, 1C), 20.8 (q, 1C), 20.7 (q, 1C); HR-ESI-ORBITRAP $[M+Na]^+$ m/z calcd. 382.0857 for $C_{13}H_{78}N_3O_9Na^+$, found 382.0855;
3β (β–anomer): ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.19 (t, J= 3.1 Hz, 1H), 5.11-5.09 (m, 1H), 5.02 (d, *J* = 1.8 Hz, 1H), 4.925-4.91 (m, 1H), 4.62 (d, *J* =2.1 Hz, 1H), 3.79 (s, 3H), 2.143 (s, 3H), 2.140 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.4 (s, 1C), 169.3 (s, 1C), 168.0 (s, 1C), 166.9 (s, 1C), 85.2 (d, 1C), 73.8 (d, 1C), 66.6 (d, 1C), 66.0 (d, 1C), 65.9 (d, 1C), 52.9 (q, 1C), 20.8 (q, 1C), 20.8 (q, 1C), 20.7 (q, 1C).

1-Azido-α-L-idopyranuronic acid methylester (12)



3 (120 mg, 0.33 mmol, 1 eq) was dissolved in anhydrous methanol (50 mL) and 3Å molecular sieves (1 g) were added at room temperature. After 2 days the reaction was filtrated and the solvent was removed. Purification by reversed phase column chromatography (30 g C18, 10-30% MeCN in H_2O, gradient elution) gave **12** (60 mg, 78%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD) δ 5.36 (d, *J* = 5.1 Hz, 1H), 4.72 (d, *J* = 4.0 Hz, 1H), 3.88–3.82 (m, 2H), 3.79 (s, 3H), 3.37 (dt, J_1 = 5.2 Hz, J_2 = 1.0 Hz, 1H) ^{13}C NMR (100 MHz, CD_3OD) δ 170.5 (s, 1C), 89.4 (d, 1C), 72.2 (d, 1C), 70.6 (d, 1C), 70.6 (d, 1C), 69.8 (d, 1C), 51.2 (q, 1C); HR-ESI-ORBITRAP $[M+Na]^+$ m/z calcd. 256.0540 for $C_7H_{11}N_3O_6Na^+$, found 256.0539.

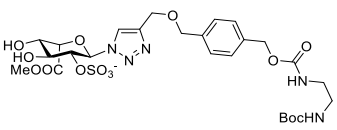
1-Azido-α-L-idopyranuronic acid methyl ester, 2-*O*-sulfate (2)



12 (44 mg, 0.19 mmol, 1 eq) was dissolved in anhydrous MeOH (10 mL) and dibutyltin(IV) oxide (70 mg, 0.28 mmol, 1.5 eq) was added. The reaction mixture was refluxed for 40 min until dibutyltin oxide completely dissolved. The reaction mixture was cooled to room temperature and concentrated. The residue was co-evaporated with toluene (2x) and then dissolved in anhydrous DMF (5 mL). Sulfur trioxide–trimethylamine complex (65 mg, 0.47 mmol, 2.5 eq) was added and the reaction mixture was heated at 55 °C for 24 h. The reaction mixture cooled to room temperature and quenched by addition of MeOH. The mixture was

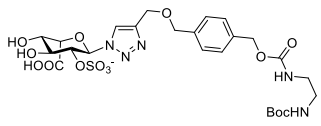
concentrated under vacuum and the residue was purified by preparative HPLC (C18, 5-20% MeCN in H_2O, gradient elution) to afford the trimethylammonium salt of **2** (17.8 mg, 30%) as a colorless solid.[1]H NMR (400 MHz, CD_3OD) δ: 5.78 (bs, 1H), 4.99 (d, *J* = 2.0 Hz, 1H), 4.57 (m, 1H), 4.38 (m, 1H), 4.23 (m, 1H), 3.80 (s, 3H) 2.97 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 169.3 (s, 1C), 88.0 (d, 1C), 73.0 (d, 1C), 72.5 (d, 1C), 67.2 (d, 1C), 66.5 (d, 1C), 51.5 (q, 1C); HR-ESI-ORBITRAP $[M]^-$ m/z calcd. 312.0132 for $C_7H_{10}N_3O_9S^-$, found 312.0146.

Me-CS$_N$ (13)



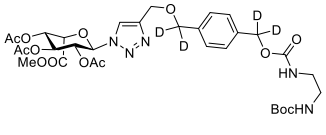
CuI (2 mg, 0.01 mmol, 0.1 eq) was added to a solution of **2** (30 mg, 0.10 mmol, 1 eq), **10** (40 mg, 0.11mmol, 1.1 eq) and Et_3N (0.15 µL, 0.12 mmol, 1.2 eq) in DMF (3 mL). The reaction mixture was stirred overnight, filtrated and concentrated. The residue was purified by preparative HPLC (C18, 5-30% MeCN in H_2O, gradient elution) to obtain **13** (5.4 mg, 80%) as a white solid; ^{1}H NMR (600 MHz, CD_3OD) δ 8.25 (s, 1H), 7.35 (s, 4H), 6.38 (d, *J* = 9.4 Hz, 1H), 5.07 (s, 2H), 5.00 (d, *J* = 6.7 Hz, 1H), 4.65 (s, 2H), 4.61 (dd, *J* = 8.9, 6.7 Hz, 1H), 4.60 (s, 2H), 4.21 (t, *J* = 8.9 Hz, 1H), 4.04 (t, *J* = 8.9 Hz, 1H), 3.81 (s, 3H), 3.20–3.16 (m, 2H), 3.16–3.11 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 171.1 (s, 1C), 159.0 (s, 1C), 158.6 (s, 1C), 146.2 (s, 1C), 139.1 (s, 1C), 137.9 (s, 1C), 129.1 (d, 2C), 128.9 (d, 2C), 124.1 (d, 1C), 87.0 (d, 1C), 80.1 (s, 1C), 76.5 (d, 1C), 75.0 (d, 1C), 73.7 (d, 1C), 73.0 (t, 1C), 72.5 (d, 1C), 67.2 (t, 1C), 64.0 (t, 1C), 52.8 (q, 1C), 41.8 (t, 1C), 41.2 (t, 1C), 28.7 (q, 3C); HR-ESI-ORBITRAP $[M]^-$ m/z calcd. 674.1985 for $C_7H_{10}N_3O_9S^-$, found 674.1968.

CS$_N$



To a solution of **13** (18 mg, 0.027 mmol, 1eq) in THF (5.4 mL) and water (0.47 ml) cooled to 0 °C was added aqueous NaOH (0.1 M, 1.33 mL, 0.133 mmol, 5 eq). The reaction mixture was stirred for 4 h and then quenched by addition of aqueous HCl (0.1 M) to adjust the pH to 7. The reaction was concentrated and the residue was purified by preparative HPLC (C18, 5-30% MeCN in H_2O, gradient elution) to afford **CS$_N$** (14 mg, 80%) as a white solid; ^{1}H NMR (600 MHz, D_2O) δ 8.23 (s, 1H), 7.38 (s, 4H), 6.22 (d, *J* = 7.9 Hz, 1H), 5.08 (s, 2H), 4.75 (t, *J* = 7.9 Hz, 1H), 4.71 (s, 2H), 4.57 (s, 2H), 4.54 (d, *J* = 5.4 Hz, 1H), 4.05 (dd, *J* = 8.4, 5.5 Hz, 1H), 3.92 (t, *J* = 8.4 Hz, 1H), 3.20–3.11 (m, 4H), 1.35 (s, 9H); ^{13}C NMR (150 MHz, D_2O) δ 174.9 (s, 1C), 158.4 (s, 1C), 158.2 (s, 1C), 143.7 (s, 1C), 136.8 (s, 1C), 136.5 (s, 1C), 128.9 (d, 2C), 127.8 (d, 2C), 124.9 (d, 1C), 83.9 (d, 1C), 80.9 (s, 1C), 78.1 (d, 1C), 73.8 (d, 1C), 72.0 (d, 1C), 71.4 (t, 1C), 69.8 (d, 1C), 66.5 (t, 1C), 62.0 (t, 1C), 40.2 (t, 1C), 39.7 (t, 1C), 27.6 (q, 3C); HR-ESI-ORBITRAP M^- m/z calcd. 660.1896 for $C_{25}H_{34}N_5O_{14}S^-$, found 660.1821.

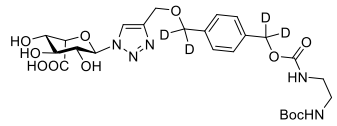
p-IS$_N$ (14)



3 (50 mg, 0.14 mmol, 1 eq) and **10-*d*$_4$** (56 mg, 0.15 mmol, 1.1 eq) were dissolved in THF (7 mL) and Et_3N (23 µL, 0.17 mmol, 1.2 eq) was added. CuI (3 mg, 14 µmol, 0.1 eq) was added and the reaction mixture was stirred overnight. The mixture was filtrated and concentrated. The residue was purified by column chromatography (20 g SiO_2, 20-95% EtOAc in hexanes, gradient elution) to afford **14** (90 mg, 89%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.33 (s, 4H), 6.70–6.64 (m, 1H), 5.63–5.56 (m, 2H), 5.29–5.23 (m, 1H), 5.16 (bs, 1H), 4.88 (d, *J* = 5.8 Hz, 1H), 4.84 (bs, 1H), 4.66 (s, 2H), 3.83 (s, 3H), 3.34–3.20 (m, 4H), 2.08 (s, 3H), 2.04 (s, 3H), 1.94 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6 (s, 1C), 169.5 (s, 1C), 169.1 (s, 1C), 168.4 (s, 1C), 156.8 (s, 1C), 156.5 (s, 1C), 146.0 (s, 1C), 137.7 (s, 1C), 136.1 (s, 1C), 128.4 (d, 2C), 128.3 (d, 2C), 121.6 (d, 1C), 83.3 (d, 1C), 79.7 (s,

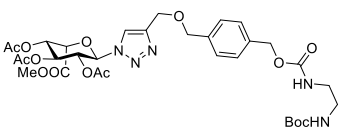
1C), 71.3 (d, 1C), 69.2 (d, 1C), 68.6 (d, 1C), 68.3 (d, 1C), 63.5 (t, 1C), 52.9 (q, 1C), 41.7 (t, 1C), 40.8 (t, 1C), 28.5 (q, 3C), 20.7 (q, 1C), 20.6 (q, 1C), 20.4 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 726.3130 for $C_{32}H_{40}D_4N_5O_{14}^+$, found 726.3123.

IS_N



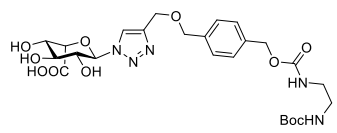
To a solution of **14** (70 mg, 0.11 mmol, 1 eq) in THF (21 mL) and water (1.49 mL) cooled to 0 °C was added aqueous NaOH (0.1 M, 5.5 mL, 0.55 mmol, 5 eq). The reaction mixture was stirred for 4 h and then quenched by addition of aqueous HCl (0.1 M) until pH 7. The reaction was concentrated and the residue was purified by preparative HPLC (C18, 5-30% MeCN in H_2O, gradient elution) to afford **IS_N** (20 mg, 31%) as a white solid; ^{1}H NMR (600 MHz, CD_3OD) δ 8.19 (s, 1H), 7.35 (s, 1H), 6.04 (d, *J* = 9.2 Hz, 1H), 4.65 (s, 2H), 4.38 (d, *J* = 6.2 Hz, 1H), 3.94 (t, *J* = 9.1 Hz, 1H), 3.79 (dd, *J* = 9.5, 6.2 Hz, 1H), 3.60 (t, *J* = 9.3 Hz, 1H), 3.21–3.15 (m, 3H), 3.15- 3.10 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.1 (s, 1C), 159.0 (s, 1C), 158.6 (s, 1C), 145.7 (s, 1C), 139.0 (s, 1C), 137.8 (s, 1C), 129.1 (d, 2C), 128.9 (d, 2C), 125.0 (d, 1C), 87.0 (d, 1C), 80.1 (s, 1C), 76.2 (d, 1C), 75.3 (d, 1C), 74.2 (d, 1C), 72.5 (d, 1C), 63.8 (t, 1C), 41.8 (t, 1C), 41.2 (t, 1C), 28.7 (q, 3C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 586.2657 for $C_{25}H_{32}D_4N_5O_{11}^+$, found 586.2655.

p-P_N (15)



3 (0.3 g, 0.84 mmol, 1 eq) and **10** (0.33 g, 0.92 mmol, 1.1 eq) were dissolved in THF (18 mL) and Et_3N (0.14 mL, 1 mmol, 1.2 eq) was added. CuI (16 mg, 0.084 mmol, 0.1 eq) was added and the reaction mixture was stirred overnight. The mixture was filtrated and concentrated, and the residue was purified by column chromatography (90 g SiO_2, 20-100% EtOAc in hexanes) to afford **15** (474 mg, 79%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.33 (s, 4H), 6.69-6.64 (m, 1H), 5.62-5.56 (m, 2H), 5.30-5.23 (m, 1H), 5.16 (bs, 1H), 5.08 (s, 2H), 4.88 (d, *J* = 5.9 Hz, 2H), 4.85 (bs, 1H), 4.67 (s, 2H), 4.56 (s, 2H), 3.83 (s, 3H), 3.33–3.18 (m, 4H), 2.08 (s, 3H), 2.04 (s, 3H), 1.94 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6 (s, 1C), 169.5 (s, 1C), 169.1 (s, 1C), 168.4 (s, 1C), 156.8 (s, 1C), 156.6 (s, 1C), 146.0 (s, 1C), 137.8 (s, 1C), 136.2 (s, 1C), 128.4 (d, 2C), 128.2 (d, 2C), 121.6 (d, 1C), 83.3 (d, 1C), 79.8 (s, 1C), 72.2 (t, 1C), 71.3 (d, 1C), 69.2 (d, 1C), 68.6 (d, 1C), 68.3 (d, 1C), 66.6 (t, 1C), 63.6 (t, 1C), 53.0 (q, 1C), 41.7 (t, 1C), 40.8 (t, 1C), 28.5 (q, 3C), 20.7 (q, 1C), 20.6 (q, 1C), 20.4 (q, 1C); HR-ESI-ORBITRAP $[M+H]^+$ m/z calcd. 722.2879 for $C_{32}H_{44}N_5O_{14}^+$, found 722.2875.

P_N



To a solution of **15** (50 mg, 0.069 mmol, 1 eq) in THF (21 mL) and water (3.54 mL) cooled to 0 °C was added aqueous NaOH (0.1 M, 3.46 mL, 0.346 mmol, 5 eq). After stirring for 4 h the reaction mixture was quenched by addition of aqueous HCl (0.1 M) until pH 7. The reaction was concentrated and the residue was purified by preparative HPLC (C18, 5-30% MeCN in H_2O, gradient elution) to afford **P_N** (39 mg, 97%) as a white solid; ^{1}H NMR (600 MHz, CD_3OD) δ 8.20 (s, 1H), 7.34 (s, 4H), 6.06 (d, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 4.65 (s, 2H), 4.57 (s, 2H), 4.39 (d, *J* = 6.1 Hz, 1H), 3.95 (t, *J* = 9.1 Hz, 1H), 3.80 (dd, *J* = 9.5, 6.1 Hz, 1H), 3.62 (t, *J* = 9.2 Hz, 1H), 3.20–3.155 (m, 2H), 3.16–3.10 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.1 (s, 1C), 158.9 (s, 1C), 158.6 (s, 1C), 145.6 (s, 1C), 139.0 (s, 1C), 137.8 (s, 1C), 129.1 (d, 2C), 128.9 (d, 2C), 125.0 (d, 1C), 86.9 (d, 1C), 80.1 (s, 1C), 76.1 (d, 1C), 75.2 (d, 1C), 74.2 (d, 1C), 72.9 (t, 1C), 72.4 (d, 1C), 67.2 (t, 1C), 63.9 (t, 1C), 41.8 (t, 1C), 41.2 (t, 1C), 28.7 (q, 3C); HR-ESI-ORBITRAP $[M-H]^-$ m/z calcd. 580.2249 for $C_{25}H_{34}N_5O_{11}^-$, found 580.2259.

4) Analytical Methods, Enzyme and DBS Assays

Materials and reagents

Acetonitrile (LC-MS Grade), methanol (Ultra LC-MS Grade) and water (Ultra LC-MS Grade) were obtained from Carl Roth GmbH (Austria). Acetone (MilliporeSigma HPLC) and isopropyl alcohol (MilliporeSigma HPLC) were purchased from VWR (USA). Recombinant human iduronate-2-sulfatase (I2S) enzyme was purchased from R&D Systems® (Catalog Number: 2449-SU). All other chemicals were obtained from Sigma Aldrich (Germany). DBS quality control cards were kindly provided by the CDC (Center for Disease Control and Prevention, USA). Anonymized DBS samples of newborns were provided by the Medical University of Vienna and the Vienna General Hospital, and anonymized DBS samples of affected patients were kindly provided by the Villa Metabolica of the University Medical Center of the Johannes Gutenberg University Mainz (Germany). The institutional ethics committee approved the study (EK 478/2009, EK 1687/2014, and approved extensions).

Equipment: MT-plate shaker TiMix 5 control equipped with incubation hood TH15 (Edmund Bühler GmbH, Germany), Centrifuge 5810R from Eppendorf (Germany), DBS Puncher® Instrument from Perkin Elmer (USA), balance (PCB 200-2) from Kern&Sohn GmbH (Germany), pH-meter (seven compact) from Mettler Toledo (USA).

I2S enzyme kinetics and inhibition

Assay buffer

Sodium acetate trihydrate (3.4 g) was dissolved in 200 mL of water. To the magnetically stirred solution were added 600 µL of acetic acid followed by addition of lead (II) acetate trihydrate (813 mg) in small portions. The mixture was stirred for 20 minutes until a clear homogenous solution was obtained. The pH was adjusted to 5.00 by dropwise addition of 0.1 M sodium hydroxide solution. Finally, water was added to reach a total volume of 250 mL. The buffer solution was homogenized and used for further applications.[6]

I2S stock solution

The I2S sample obtained from R&D Systems® (Catalog Number: 2449-SU) was diluted in freshly prepared assay buffer and vortexed for 1 minute to reach a concentration of 20 µg/mL.

Enzyme kinetics at various substrate concentrations

Substrates (**CS_O**, **CS_N**) were dissolved in assay buffer resulting in 4 mg/mL stock solutions (S-stock). The corresponding internal standards (**P_O** in case of **CS_O**, **P_N** in case of **CS_N**) were dissolved in assay buffer resulting in 20 µg/mL stock solutions (IS-stock). The following table shows the used mixing procedure to prepare eight individual assay cocktails (assay CT) in 1000 µL portions of both assay sets, which were vortexed for 1 minute before use.

	blank	blank-IS	0.3 mM	0.6 mM	1.2 mM	1.8 mM	2.4 mM	3.0 mM
IS-stock (µL)	0	500	500	500	500	500	500	500
S-stock (µL)	0	0	50	100	200	300	400	500
buffer (µL)	1000	500	450	400	300	200	100	0

Duplicates of each standard solution (10 µL) were placed in a 96 well plates. Then 10 µL of the I2S stock solution in assay buffer was added to each well. The plates were sealed using adhesive aluminum foil and incubated at 37 °C with shaking (250 rpm) for 1 h, 4 h, and 24 h respectively. The final assay conditions were: 5 ng/µL internal standards, 10 ng/µL I2S and substrate concentrations from 150 µM to 1.5 mM (for both $\mathbf{CS_O}$ and $\mathbf{CS_N}$).

The assay was stopped by adding 60 µL of acetonitrile to each well. The plate was covered with aluminum foil and subjected to orbital shaking (350 rpm) at room temperature for 2 min. The plate was then centrifuged for 1 min at 3200 rpm. Finally, 25 µL of each well were transferred into a new plate and diluted with 125 µL water prior to LC-MS/MS analysis.

Product inhibition ('crossed assay')

Products of the intended enzymatic desulfation ($\mathbf{P_O}$, $\mathbf{P_N}$), which were also used for MS tuning prior to analysis, were dissolved in methanol to reach a concentration of 1 mg/mL (P-stock). Assay cocktails were prepared by mixing equal amounts of described stock solutions (see above). The following table shows the used mixing procedure to prepare six individual assay cocktails. First, a solution of the product to be added (either $\mathbf{P_O}$ or $\mathbf{P_N}$) was placed into Eppendorf tubes and concentrated by means of a heated nitrogen flow (40 °C) for 15 minutes. Then 110 µL of the 'opposite' assay cocktail ($\mathbf{CS_N}$ + $\mathbf{IS_N}$ in case of $\mathbf{P_O}$, $\mathbf{CS_O}$ + $\mathbf{IS_O}$ in case of $\mathbf{P_N}$) was added. The resulting mixtures were vortexed for 3 minutes before further use.

	blank	0 µM	125 µM	250 µM	500 µM
assay cocktail (µL)	0	110	110	110	110
P-stock (µL)	0	0	8	16	32
assay buffer (µL)	110	0	0	0	0

To perform the actual enzyme assay, triplicates of each final cocktail solution (10 µL) were placed in a 96 well plate. Then 10 µL of the I2S stock solution was added to each well. The plates were sealed by means of adhesive aluminum foil and incubated at 37 °C with shaking (250 rpm) for 1 h and 4 h, respectively. The final 'crossed assay' conditions were: 5 ng/µL internal standard ($\mathbf{IS_O}$ in case of $\mathbf{CS_O}$, $\mathbf{IS_N}$ in case of $\mathbf{CS_N}$), 10 ng/µL I2S, 1.5 mM substrate concentration ($\mathbf{CS_O}$ or $\mathbf{CS_N}$) and concentrations of the opposed product ($\mathbf{P_N}$ in case of $\mathbf{CS_O}$, $\mathbf{P_O}$ in case of $\mathbf{CS_N}$, respectively) of the enzymatic degradation between 0 and 1mM.

The assay was stopped by adding 60 µL of acetonitrile to each well. The plate was covered with aluminum foil and subjected to orbital shaking (350 rpm) at room temperature for 2 min. The plate was centrifuged for 1 min at 3200 rpm, and 25 µL of each well were transferred into a new plate and diluted with 125 µL water prior to LC-MS/MS analysis.

Dried blood spot assays

Anonymized DBS samples of newborns were provided by the Medical University of Vienna and the Vienna General Hospital, and anonymized DBS samples of affected patients were kindly provided by the Villa Metabolica of the University Medical Center of the Johannes Gutenberg University Mainz (Germany) with written consent of the patients. The institutional ethics committee of the Medical University of Vienna approved the study (EK 478/2009, EK 1687/2014, and approved extensions) in accordance to the WMA (World Medical Association) declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (2013).

Preparation of assay cocktail

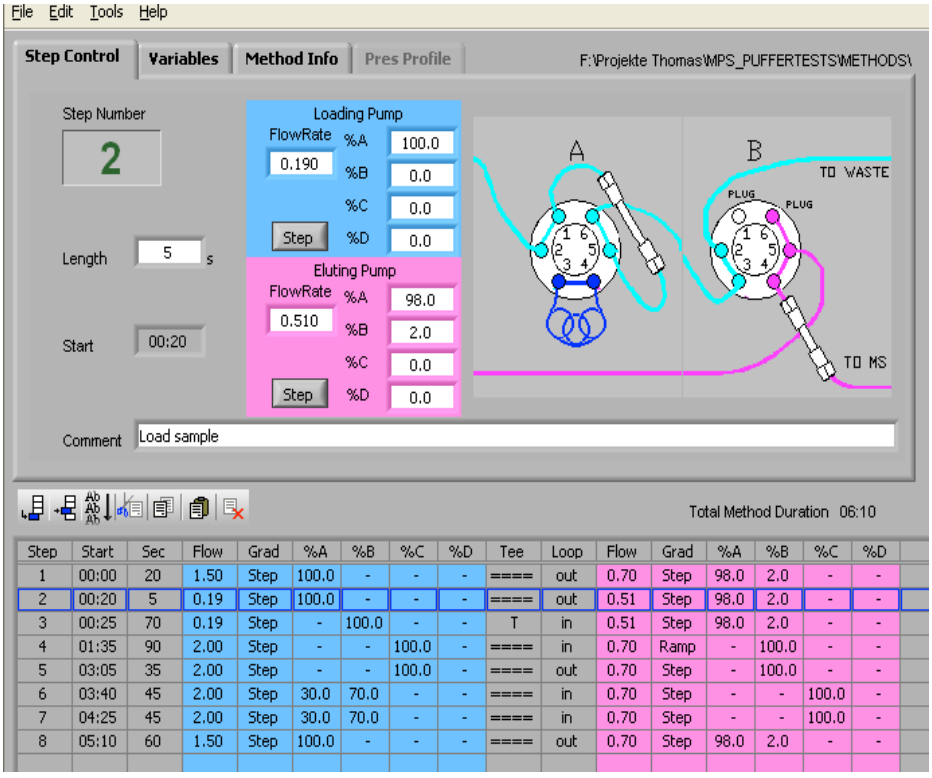
A 2 mg/mL stock solution of the substrate (**CS_O** or **CS_N**) in assay buffer and a 20 µg/mL stock solution of the corresponding internal standard (**P_O** or **P_N**, respectively) in assay buffer were freshly prepared and mixed in a 1:1 ratio to obtain an assay cocktail containing 1 mg/mL substrate and 10 µg/mL internal standard.

Sample preparation

DBS cards were punched as 3.2 mm spots into a 96 well plate. 30 µL of assay cocktail (containing **CS_O/IS_O** or **CS_N/IS_N**, respectively) were added, the plate was covered with adhesive aluminum foil and incubated for 22 h at 37°C and at 500 rpm orbital shaking. The plate was then centrifuged for 3 minutes at 3000 rpm and the assays were quenched by addition of 100 µL MeCN to each well. The plate was covered with aluminum foil and subjected to orbital shaking (350 rpm) at room temperature for 2 min. The plate was centrifuged for 15 min at 3200 rpm and subsequently cooled to 4 °C for 10 min to complete precipitation. Volumes of 50 µL of the supernatants were transferred into a new plate and diluted with 100 µL water prior to LC-MS/MS analysis. All spots were incubated and measured in duplicates.

Liquid chromatography and mass spectrometry

Chromatography was performed on a TLX2 system with Accela pumps and a PAL RTC autosampler (Switzerland). Samples (1 µL) were analyzed using a Waters ACQUITY CSH column (C18, 130 Å, 1.7 µm, 2.1 x 50 mm) and a Turboflow HTC column (Thermo Cyclom P, 0.5 x 50 mm) maintained at 40 °C. Solvent A contained 0.1% formic acid in water. Solvent B contained 0.1% formic acid in MeCN/MeOH = 1:1 (v/v). A mixture of MeCN/2-propanol/acetone = 45:45:10 (v/v) was used as solvent C. The autosampler was run at room temperature. The total flow rate during separation was 0.7 mL/min with an injection volume of 1 µL, using a turboflow® online sample pre-treatment according to the following gradient conditions:



Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B	%C	%D
1	00:00	20	1.50	Step	100.0	-	-	-	====	out	0.70	Step	98.0	2.0	-	-
2	00:20	5	0.19	Step	100.0	-	-	-	====	out	0.51	Step	98.0	2.0	-	-
3	00:25	70	0.19	Step	-	100.0	-	-	T	in	0.51	Step	98.0	2.0	-	-
4	01:35	90	2.00	Step	-	-	100.0	-	====	in	0.70	Ramp	-	100.0	-	-
5	03:05	35	2.00	Step	-	-	100.0	-	====	out	0.70	Step	-	100.0	-	-
6	03:40	45	2.00	Step	30.0	70.0	-	-	====	in	0.70	Step	-	-	100.0	-
7	04:25	45	2.00	Step	30.0	70.0	-	-	====	in	0.70	Step	-	-	100.0	-
8	05:10	60	1.50	Step	100.0	-	-	-	====	out	0.70	Step	98.0	2.0	-	-

Mass spectrometric detection was performed on a Thermo Scientific TSQ Quantum Ultra (USA) operated in positive ion electrospray ionization (ESI) mode. The source parameters of the mass spectrometer were optimized and used as follows: ion spray voltage = 4500 V; vaporizer temperature = 289°C; shealth gas pressure = 35 psi; ion sweep gas pressure = 2.0 psi, aux gas pressure = 35 psi; capillary temperature = 289 °C, skimmer offset = 0; argon was used as collision gas; nitrogen was used as nebulizer, auxiliary and curtain gas. Data was processed using LCquan™ 2.7 (Thermo Scientific, USA) and analyzed using Prism 8 (GraphPad, USA). Table S1 shows the settings for multireaction monitoring. Table S2 shows the results of the analysis of clinical samples.

Table S1. Multireaction Monitoring

	Precursor (m/z)	Product 1 (m/z)	Product 2 (m/z)	Dwell time (ms)
P-O	582.196	482.125	482.155	100
IS-O	585.321	485.285	485.315	100
P-N	582.300	482.285	482.315	100
IS-N	586.300	486.285	486.315	100

Table S2. Results of DBS assays using I2S sets **CS_O/P_O** and **CS_N/P_N**; shown as "mean values (SD)" (µM/h)

I2S	CS_O/P_O	CS_N/P_N
CDC QCL[a]	0.02 (0.02)	0.27 (0.04)
CDC QCM[a]	0.15 (0.02)	1.26 (0.09)
CDC QCH[a]	0.18 (0.04)	1.96 (0.16)
Affected patients (n=5)[a]	0.01 (0.01)	0.10 (0.07)
Random patients (n=22)[a]	1.83 (1.03)	4.65 (1.45)

[a] blank-corrected

5) NMR Analysis of Iduronyl Triazoles at Different pH

Materials and reagents

Sodium deuteroxide solution (40 wt.% in D_2O, 99.5 atom% D) was purchased from Sigma Aldrich (Germany). Acetic acid-d_4 (CD_3COOD, 99.5 atom% D) and deuterium oxide (D_2O, 99.9 atom% D) were obtained from Cambridge Isotope Laboratories, inc. (USA).

Experimental procedure

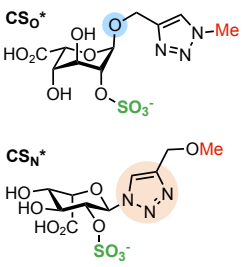
200 µL of acetic acid-d_4 were diluted in 1800 µL D_2O to obtain a 1.75 mM stock solution of CD_3COOD. 92.6 µL of sodium deuteroxide solution were diluted in 900 µL deuterium oxide to obtain a 1 M NaOD stock solution. Solutions of defined pH* values (A, B, C, D and neutral) were prepared according to the Table shown below. 1 mg of substance to be analyzed was dissolved in 1 mL D_2O and then 100 µL of this solution were mixed with 500 µL of the required deuterated buffer solution. ^1H NMR measurements (Bruker Ascend 600 spectrometer) were performed at 20 °C immediately after homogenization. pH* (for a comparison and the relation of pH, pH* and pD see Athur Krężel *et al.*[7]) of all solutions was measured before adding the sample solution and after NMR measurement. NMR analysis did not show any shift of the conformations of **CS_N** and **P_N** from $^4C_1$ to $^1C_4$. We exclusively detected these compounds in the $^4C_1$ conformation (>99%) over the measured pH range.

	NaOD (µL)	CD_3COOD (µL)	D_2O (µL)	pH* pre-NMR	pH* post-NMR
A	0	25	975	2.84	3.22
B	25	100	875	4.19	4.22
C	25	39	936	4.96	5.00
D	25	25	950	5.99	5.87
neutral	0	0	1000	7.01	7.18

6) Docking Studies

Ligand preparation

We used OpenEye OMEGA[8] (version 2017.Jun) to generate conformers of the compounds using the energy-minimised models **CS_O*** and **CS_N*** as starting structures. The maximum number of conformers for both compounds was set to 500. After generating the conformers, we filtered them to keep only those in which the sugar adopted chair ($^1C_4$ and $^4C_1$) conformations. We proceeded to cluster the remaining conformers hierarchically, using the RMSD of all heavy atoms as a distance metric, with the number of clusters set to 20.



For each compound, we generated two ensembles of 20 conformers, one per sugar conformation, using representative models selected from the clusters identified in the previous paragraph.

Protein Preparation

We used the X-RAY structure of I2S determined by Demydchuk *et al.*[9] (PDBID 5FQL). All the post translational modifications were removed, as were the solvent molecules and ions except for the calcium ion and one water molecule adjacent to the binding site. The water molecule in question

facilitates hydrogen bonding between the ligand and residues Y348 and K479. In addition to that, all the side chains atoms of the sulfooxy-serine past the beta-carbon were removed, and the residue identifier changed to DDZ so that HADDOCK (see below) could rebuild the missing atom with parameters that would enable the formation of the covalent bond.

Docking

The guru interface of the HADDOCK2.2 webserver[10,11] was used to perform the docking. HADDOCK can automatically rebuild the side chains of the standard AA residues in addition to some modified residues for which topology and parameters are supported. One of those is dihydroxy-alanine (PDB ligand ID DDZ), for which we made the following modifications:

1. Removal of one of the two beta-carbon oxygens.
2. Scaling down the non-bonded interaction parameters of the CB atom by a factor of 10 to allow for short distances with the ligand sulfate oxygens (the regular non-bonded parameters would not allow for bond distances to be reached).

HADDOCK makes use of distance restraints to drive the docking towards desirable solutions. In this case, we defined restraints with two goals in mind. The first, to place the compound sulfate group within bond distance to the beta-carbon of the dihydroxy-alanine, and the second, to maintain the geometry of the binding site components throughout the docking run.

To dock the ligand into the binding pocket, we defined two ambiguous restraints: The first between the three sulfate oxygens of the compounds and the beta-carbon of the dihydroxy-alanine, using the carbon-oxygen bond distance as our target distance, and the second between the three sulfate oxygens and the calcium ion present in the binding site, using the reference structure distance as target distances.

Further, to keep the calcium ion in its position throughout the run we defined distance restraints between it and its coordinating atoms (NE2 of H335, OD1 of D45 and D46, and OD2 of D334), once again using the reference structure distances.

Finally, the water molecule in the binding site was restrained to protein atoms in its proximity (NZ of K479 and K347, and the hydroxyl oxygen of Y348). We also defined a set of restraints that would maintain the distance of the compound sulfate oxygens within hydrogen bond limits of this water molecule.

The default parameters of HADDOCK were adapted for small molecule docking as followed. The weight of the van der Waals energy term for the initial rigid-body docking stage (it0) scoring was increased to 1 and that of the electrostatic energy term for the final water refinement (itw) scoring was decreased to 0.1. Further, since we are using an ensemble of models as starting point for the docking, the sampling was increased to 2000/400/400 for it0, it1 and itw, respectively, along with the number of structures to analyse, which was also set to 400. The clustering method was set to RMSD with a cut-off of 2 Å. In addition to the default final models, models containing the water molecules were saved to disk. All distance restraints were active for the entire run (the were entered to the server in the unambig category). All other parameters were left to their default settings.

Results

Table S3. HADDOCK scores [a.u.] of the truncated substrates **CS_O*** and **CS_N*** in the conformations $^1C_4$ and $^4C_1$.

Substrate	$^1C_4$	$^4C_1$
CS_O*	–54.7 ± 1.7	–64.9 ± 1.5[a]
CS_N*	–58.7 ± 1.7[a]	–64.8 ± 2.3

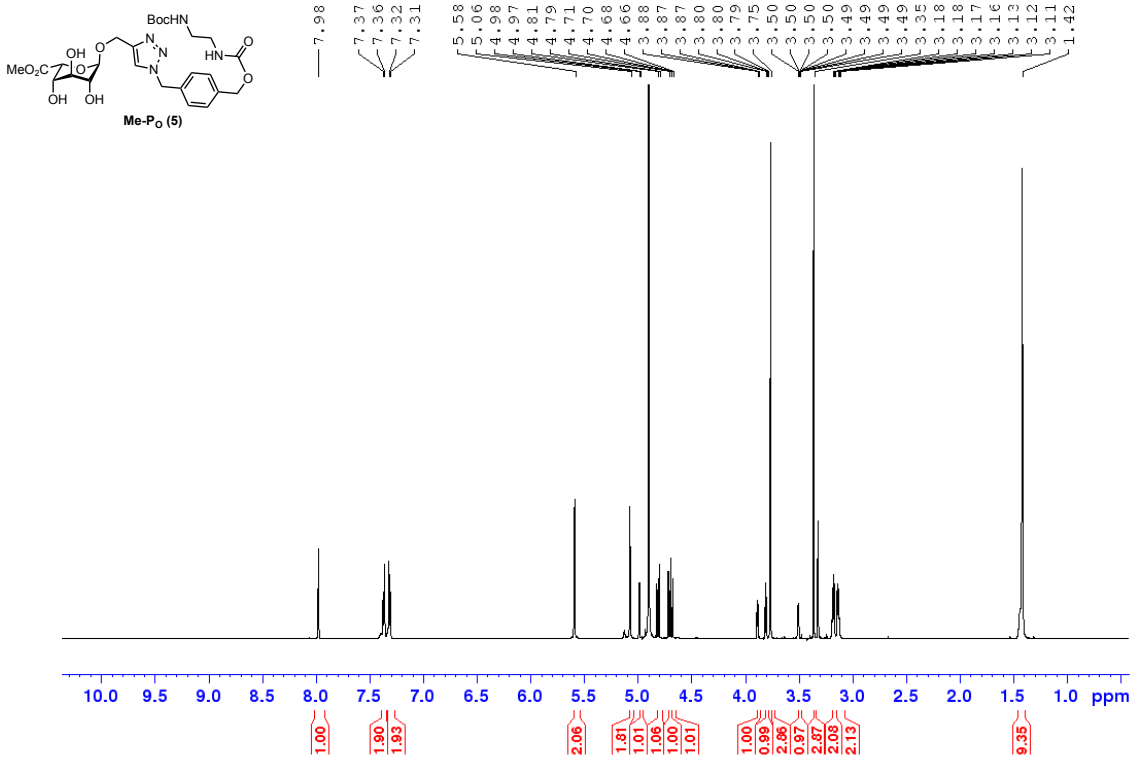
[a] These entries correspond to substrates in conformations that were not observed experimentally.

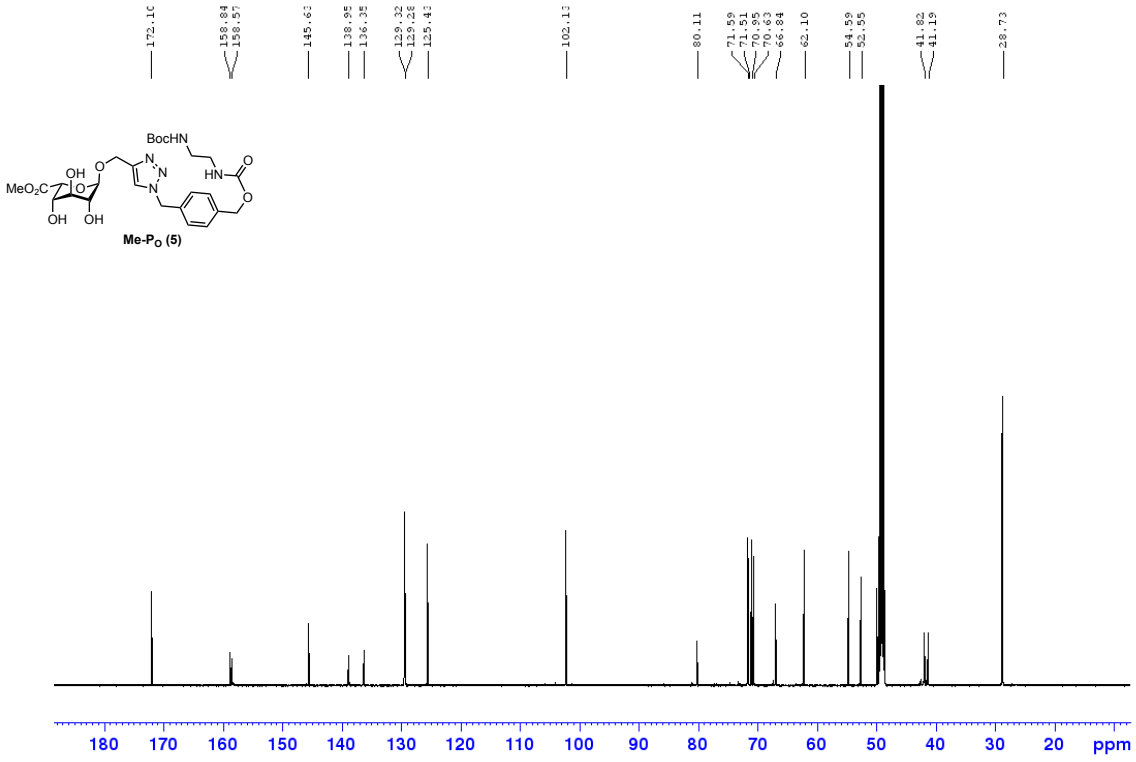
Table S4. Detailed results from HADDOCK simulations.

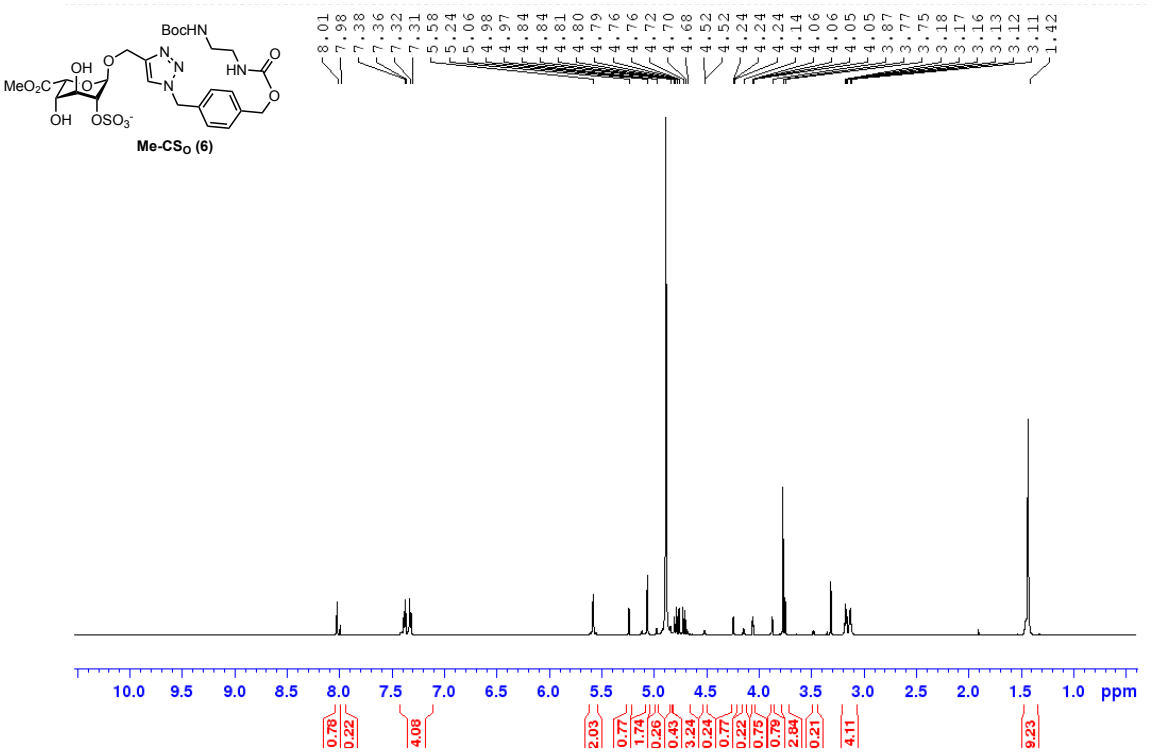
Substrate		**CS_O***		**CS_N***	
conformation		$^1C_4$	$^4C_1$	$^1C_4$	$^4C_1$
total	no. of structures clustered	395	390	394	387
	no. of clusters	6	3	5	4
	% structures clustered	98.8	97.5	98.5	96.8
top cluster no.		1	1	1	3
HADDOCK score [a.u.]		**-54.7 ± 1.7**	**-64.9 ± 1.5**	**-58.7 ± 1.7**	**-64.8 ± 2.3**
cluster size		164	270	298	99
RMSD[a] [Å]		0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.0	0.3 ± 0.2
energy	van der Waals	5.1 ± 1.1	-1.5 ± 3.0	3.8 ± 1.9	1.5 ± 1.4
	electrostatic	-365.3 ± 23.4	-397.9 ± 2.8	-375.9 ± 17.5	-429.2 ± 27.6
	desolvation	-5.7 ± 4.1	-7.2 ± 3.0	-6.7 ± 2.0	-7.6 ± 1.3
	restraint violation	1.2 ± 0.4	1.2 ± 0.2	1.3 ± 0.7	1.3 ± 0.3
buried surface area [Å^2]		509.8 ± 23.2	524.3 ± 16.7	535.9 ± 32.5	527.8 ± 5.3

[a] from the best model within each cluster

7) NMR Spectra



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OH
OSO3-
Me-CSO (6)
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7.31
5.58
5.24
5.06
4.98
4.97
4.84
4.84
4.81
4.80
4.79
4.76
4.76
4.72
4.70
4.68
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3.11
1.42
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0.76
0.22
4.06
2.03
0.77
1.74
0.26
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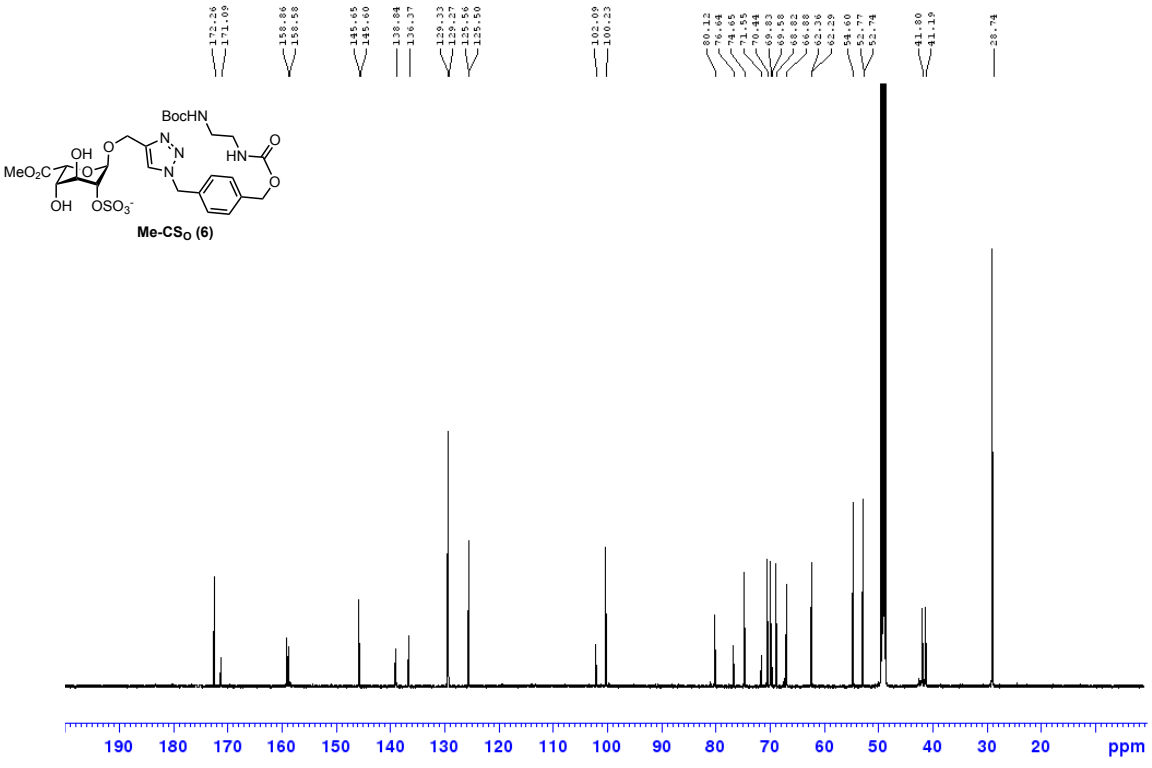
0.21
4.11
9.23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71.55
70.44
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66.88
62.36
62.29
54.60
52.77
52.74
41.80
41.19
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OH
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Me-CSO (6)
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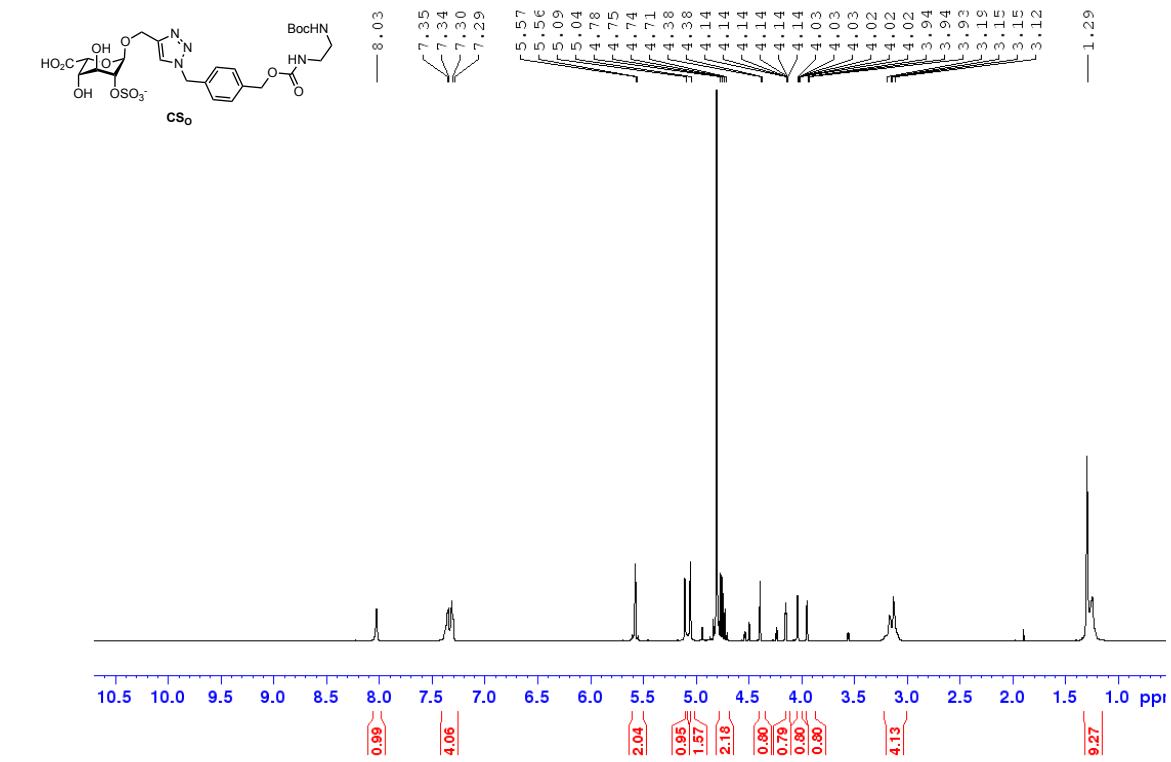
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3.19
3.15
3.12
1.29
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
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2.04
0.95
1.57
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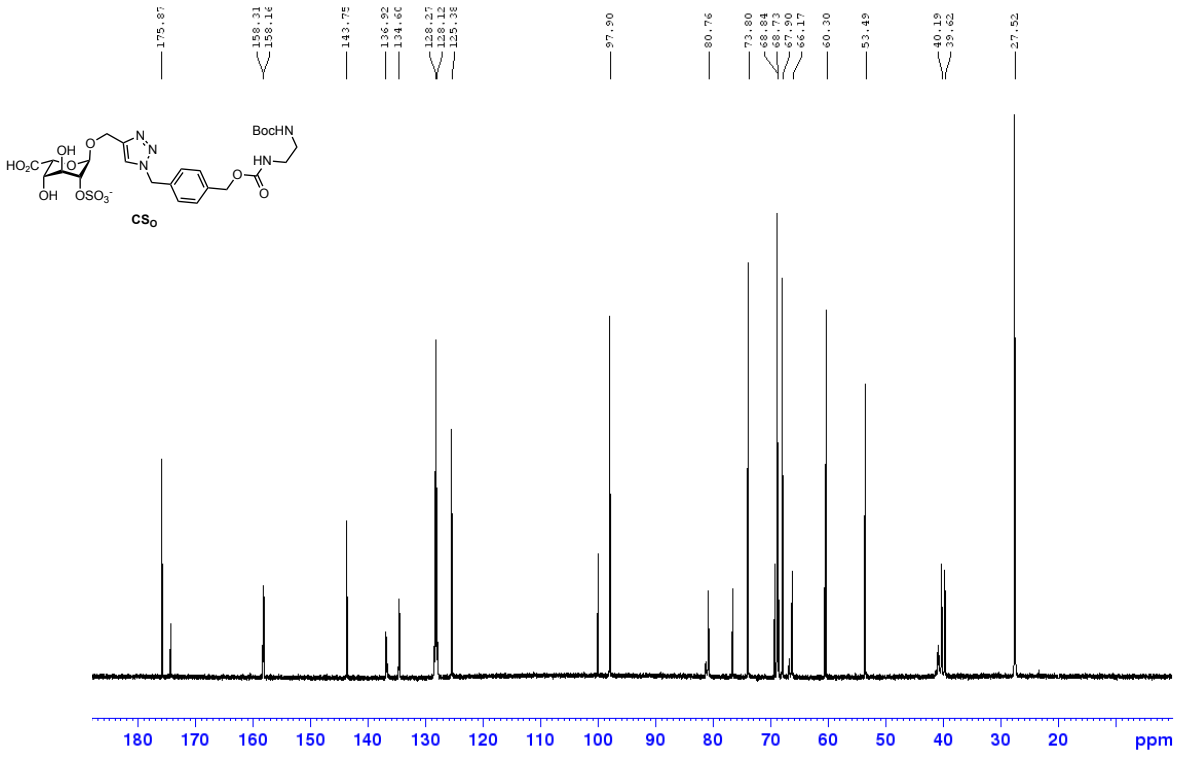
0.80
0.79
0.80
0.80
4.13
9.27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68.73
67.90
66.17
60.30
53.49
40.19
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BocHN
HN
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HO2C
OH
OSO3-
CSO
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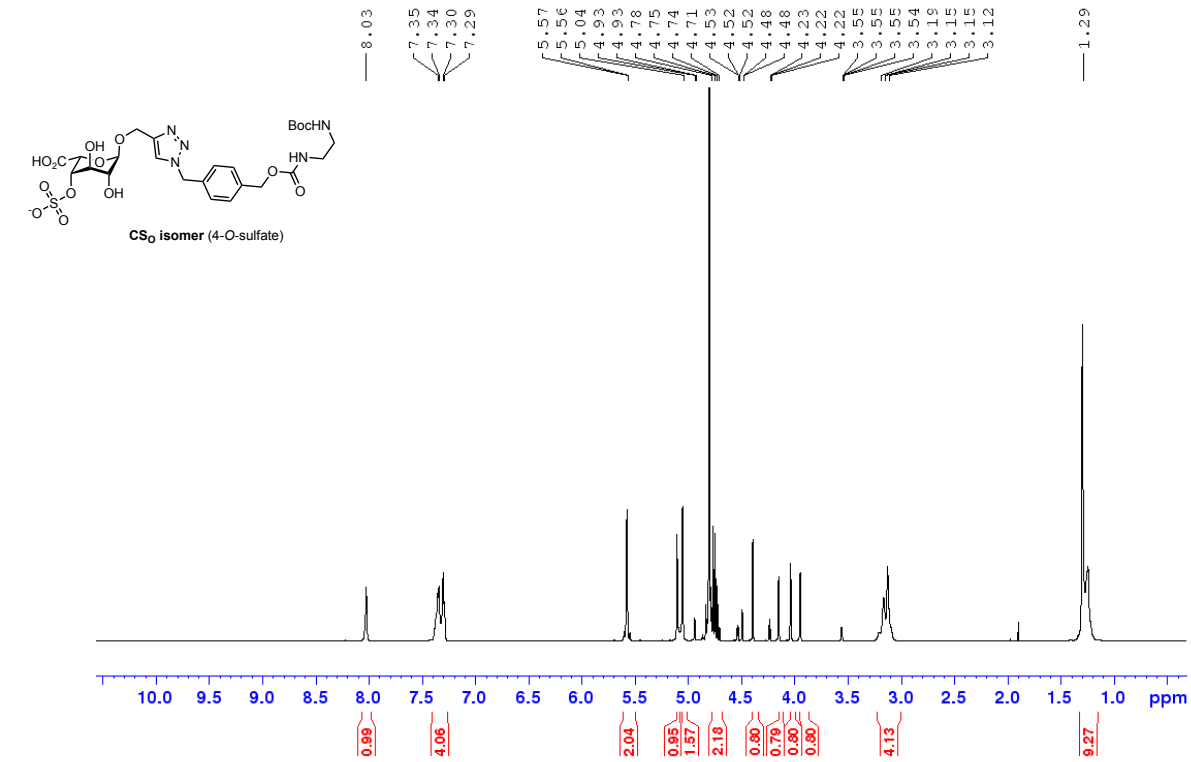
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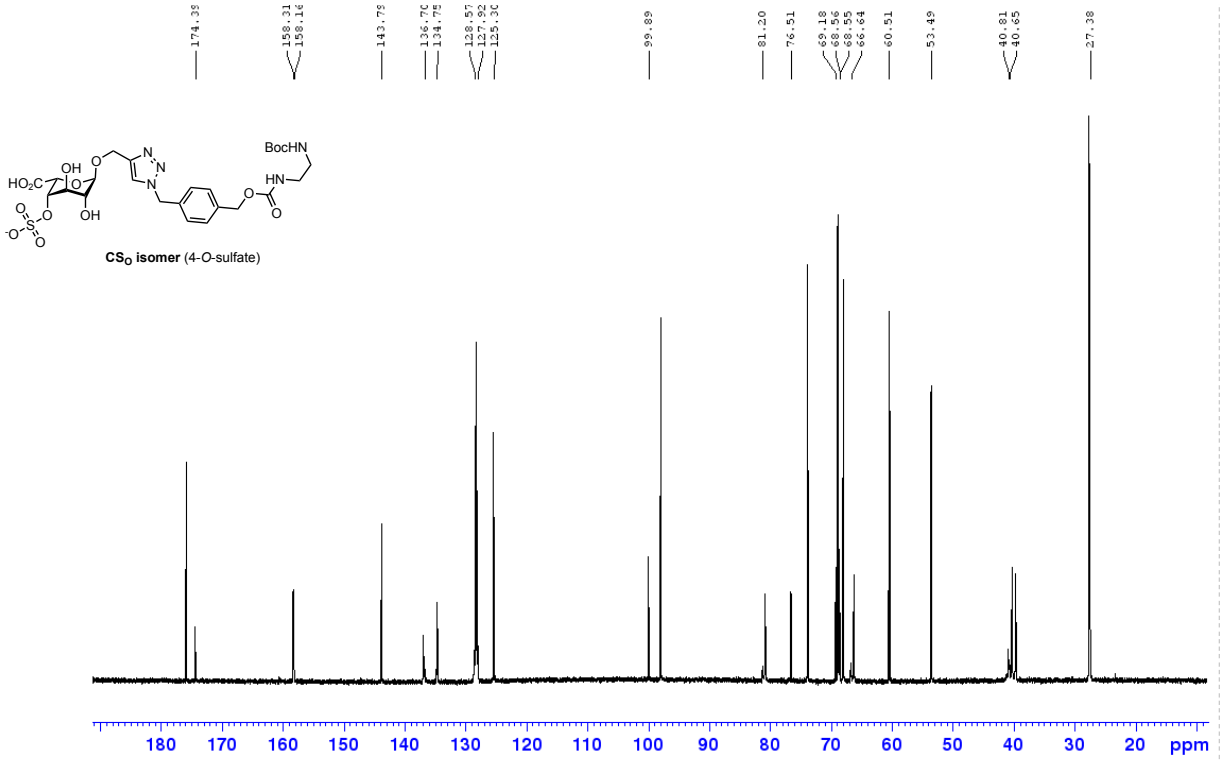
4.48
4.23
4.22
4.22
3.55
3.55
3.55
3.54
3.19
3.15
3.15
3.12
1.29
BocHN
HN
O
HO2C
OH
OH
O
O
O
S
O
-O
CSO isomer (4-O-sulfate)
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.99
4.06
2.04
0.95
1.57
2.18
0.80
0.79
0.80
0.80
4.13
9.27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134.75
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68.55
66.64
60.51
53.49
40.81
40.65
27.38
BocHN
HN
O
HO2C
OH
OH
O
O
O
S
O
-O
CSO isomer (4-O-sulfate)
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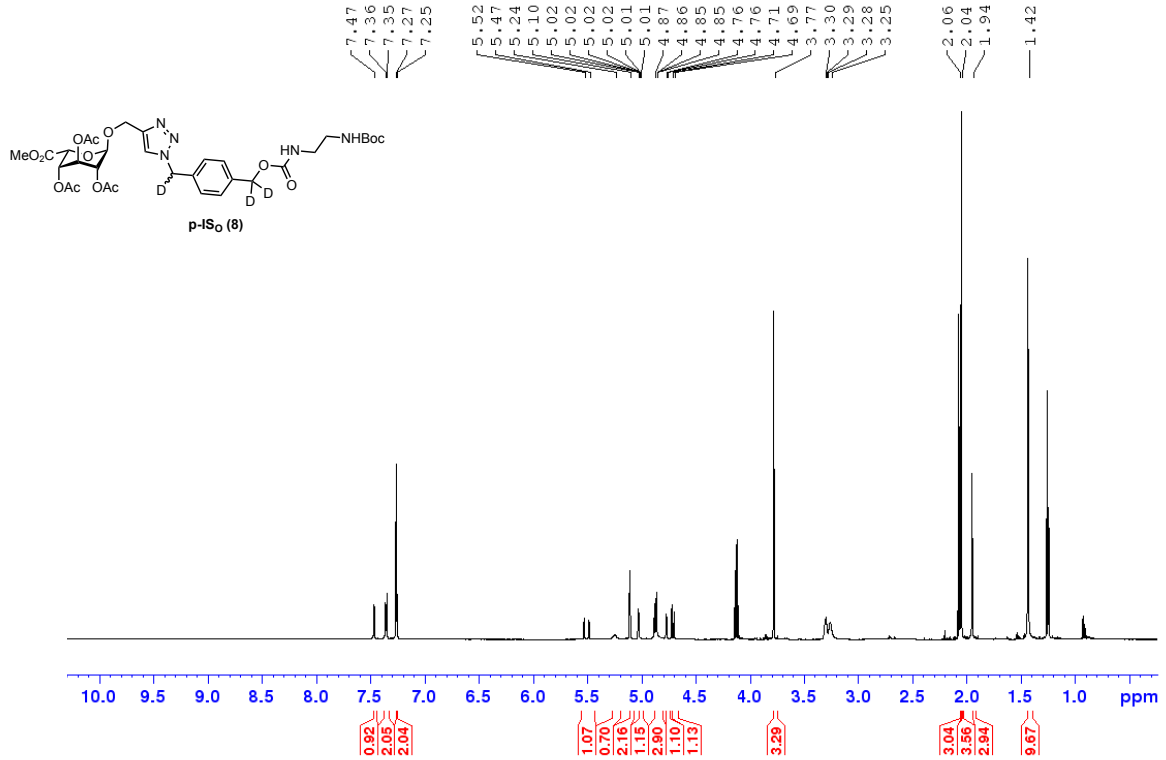
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3.25
2.06
2.04
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1.42
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OAc
O
N
N
N
D
HN
NHBoc
O
D
D
O
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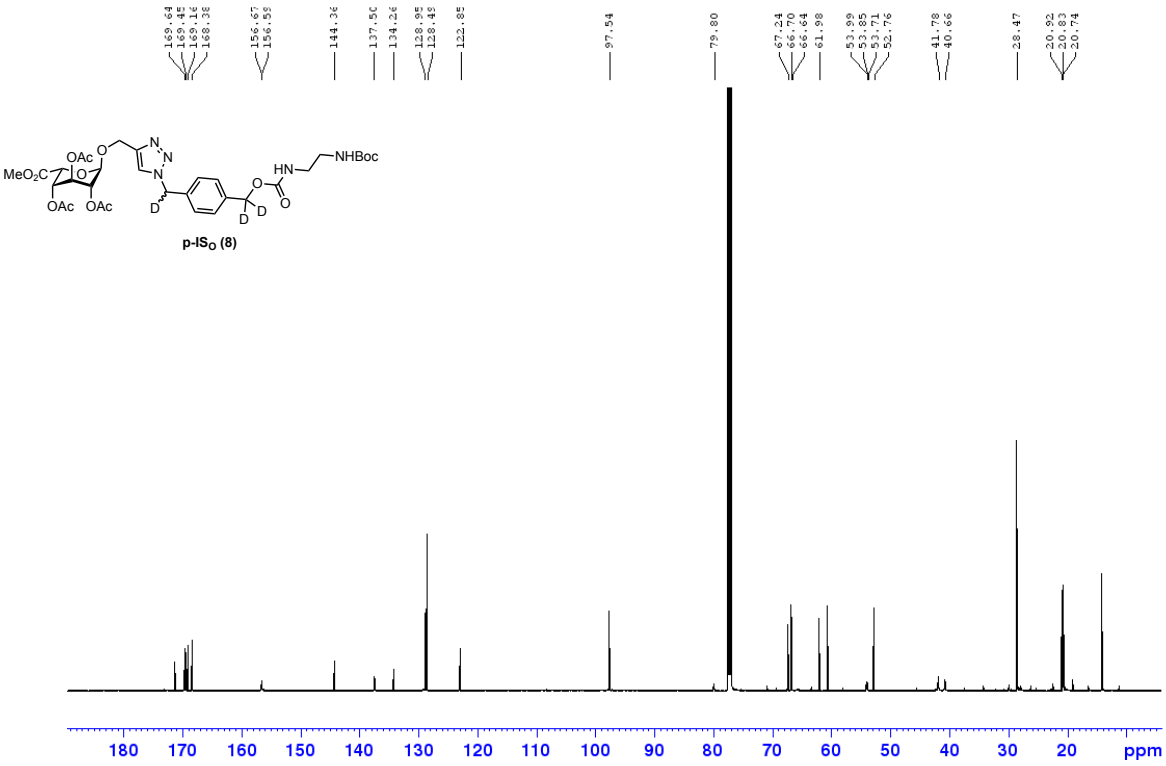
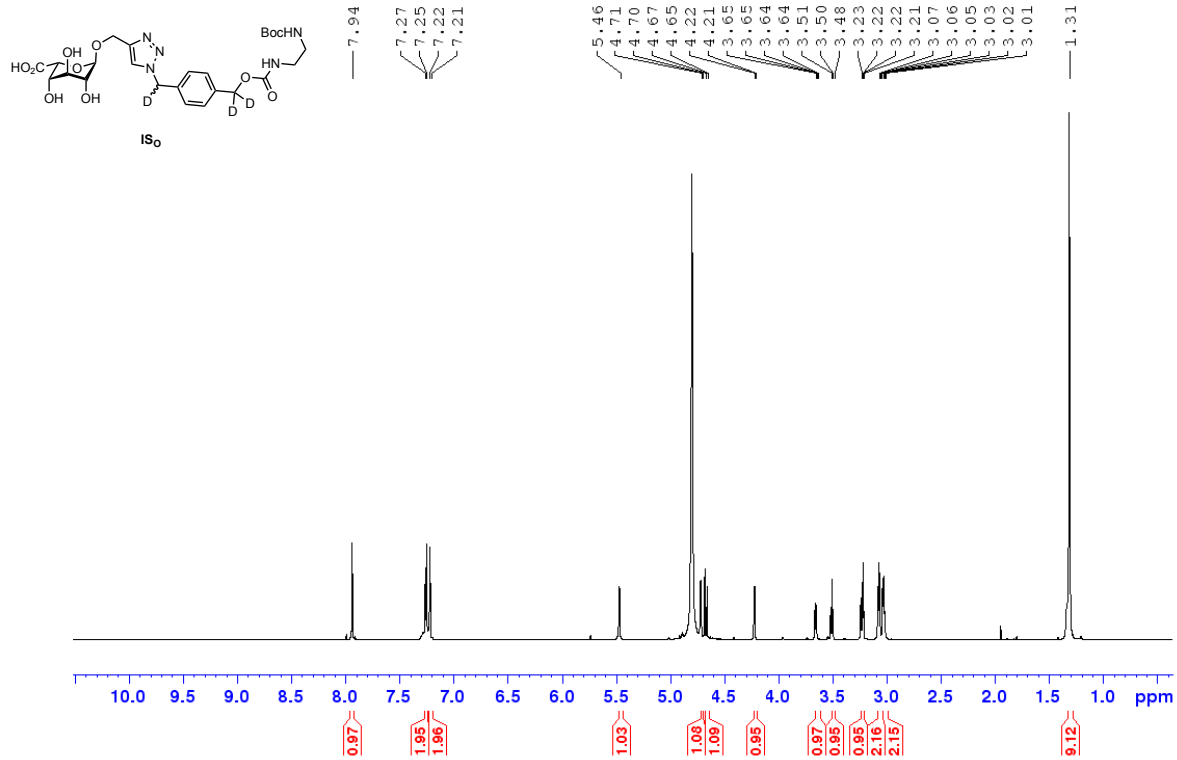
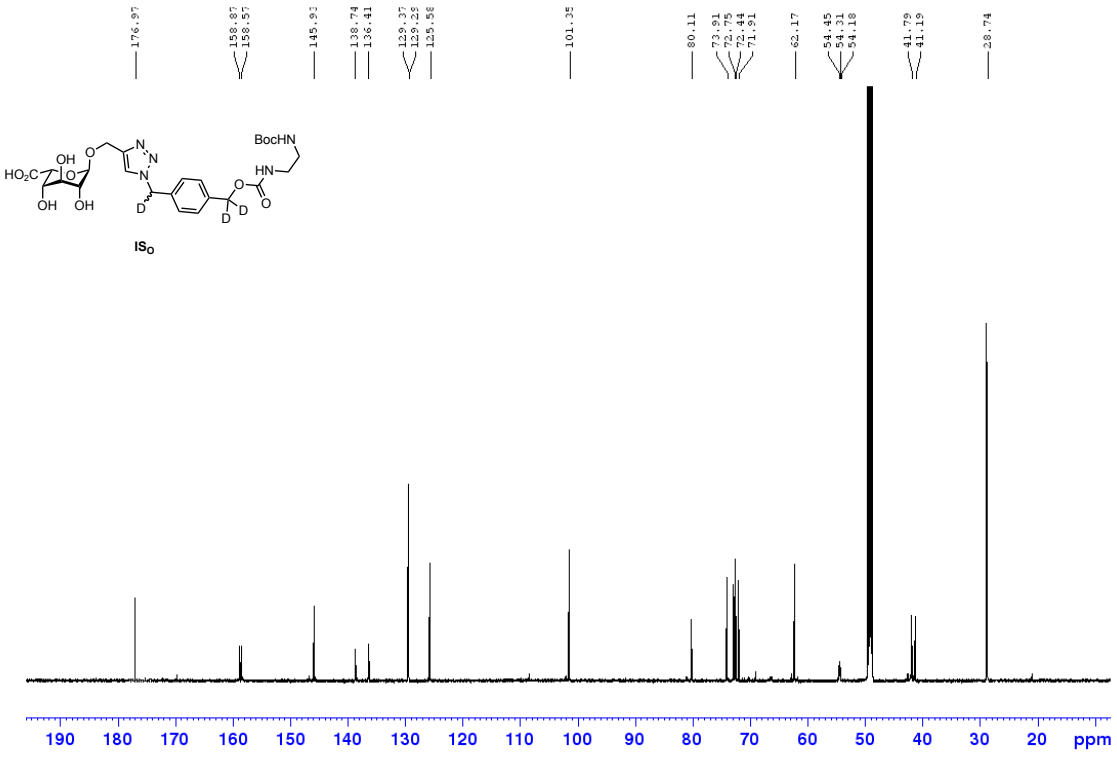
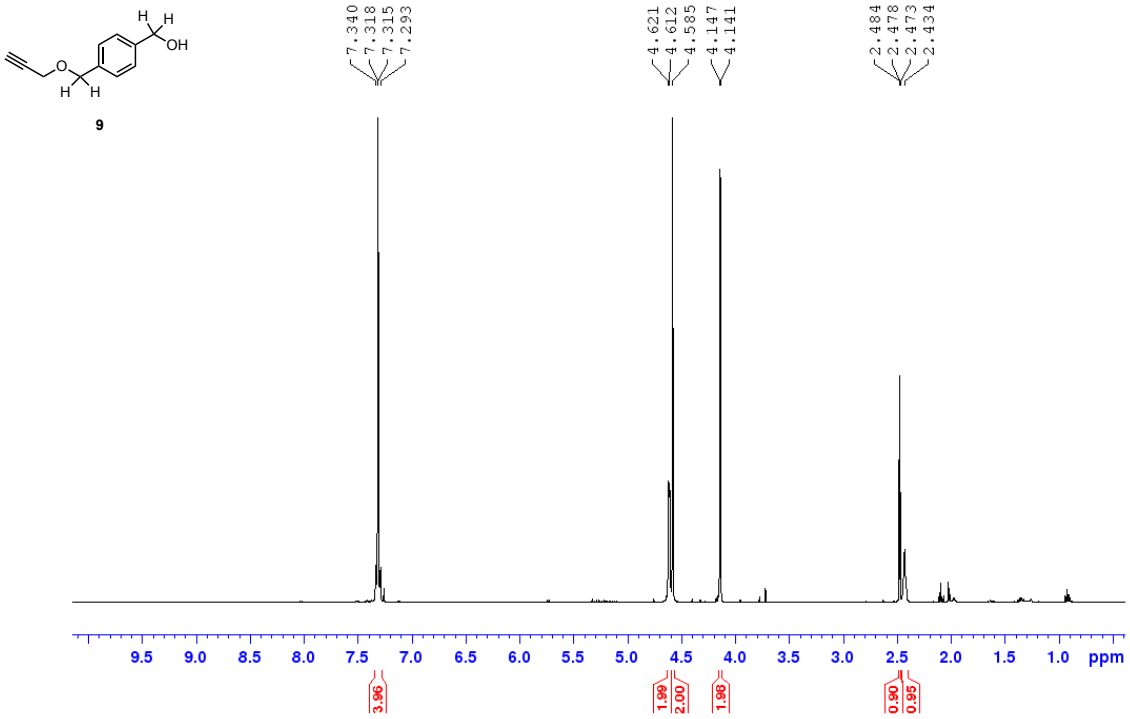
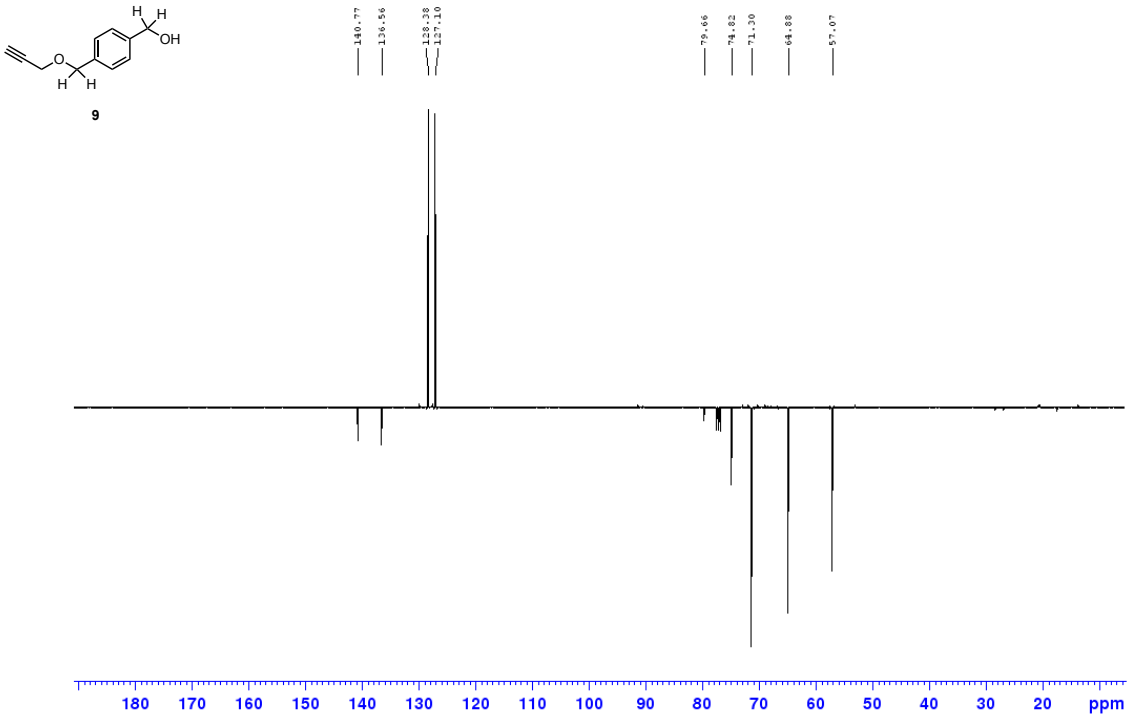
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.92
2.05
2.04
1.07
0.70
2.16
1.15
2.90
1.10
1.13
3.29
3.04
3.56
2.94
9.67

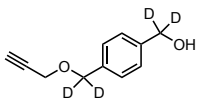

169.64
169.45
169.16
168.38
156.67
156.59
144.36
137.50
134.26
128.95
128.49
122.85
97.54
79.80
67.24
66.70
66.64
61.98
53.99
53.85
53.71
52.76
41.78
40.66
28.47
20.92
20.83
20.74
OAc
MeO2C
OAc
OAc
O
N
N
N
D
HN
NHBoc
O
D
D
O
p-IS$_O$ (8)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


BocHN
HN
HO2C
OH
OH
OH
D
D
D
O
IS0
7.94
7.27
7.25
7.22
7.21
5.46
4.71
4.70
4.67
4.65
4.22
4.21
3.65
3.65
3.64
3.64
3.51
3.50
3.48
3.23
3.22
3.22
3.21
3.07
3.06
3.05
3.03
3.02
3.01
1.31
10.0 9.5 9.0 8.5 8.0 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 ppm
0.97
1.95
1.96
1.03
1.08
1.09
0.95
0.97
0.95
0.95
2.16
2.15
9.12


176.97
158.87
158.57
145.90
138.74
136.41
129.37
129.26
125.58
101.35
80.11
73.91
72.75
72.44
71.91
62.17
54.45
54.31
54.18
41.79
41.19
28.74
BocHN
HN
HO2C
OH
OH
OH
D
D
D
O
IS0
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

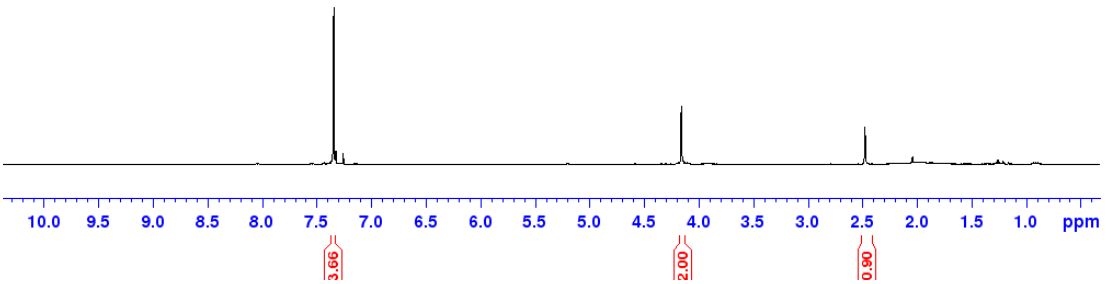

9
7.340
7.318
7.315
7.293
4.621
4.612
4.585
4.147
4.141
2.484
2.478
2.473
2.434
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
3.96
1.99
2.00
1.98
0.90
0.95

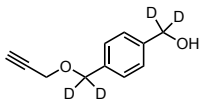

9
140.77
136.56
128.38
127.10
79.66
74.82
71.30
64.88
57.07
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


D
D
OH
O
D
D

9-d_4

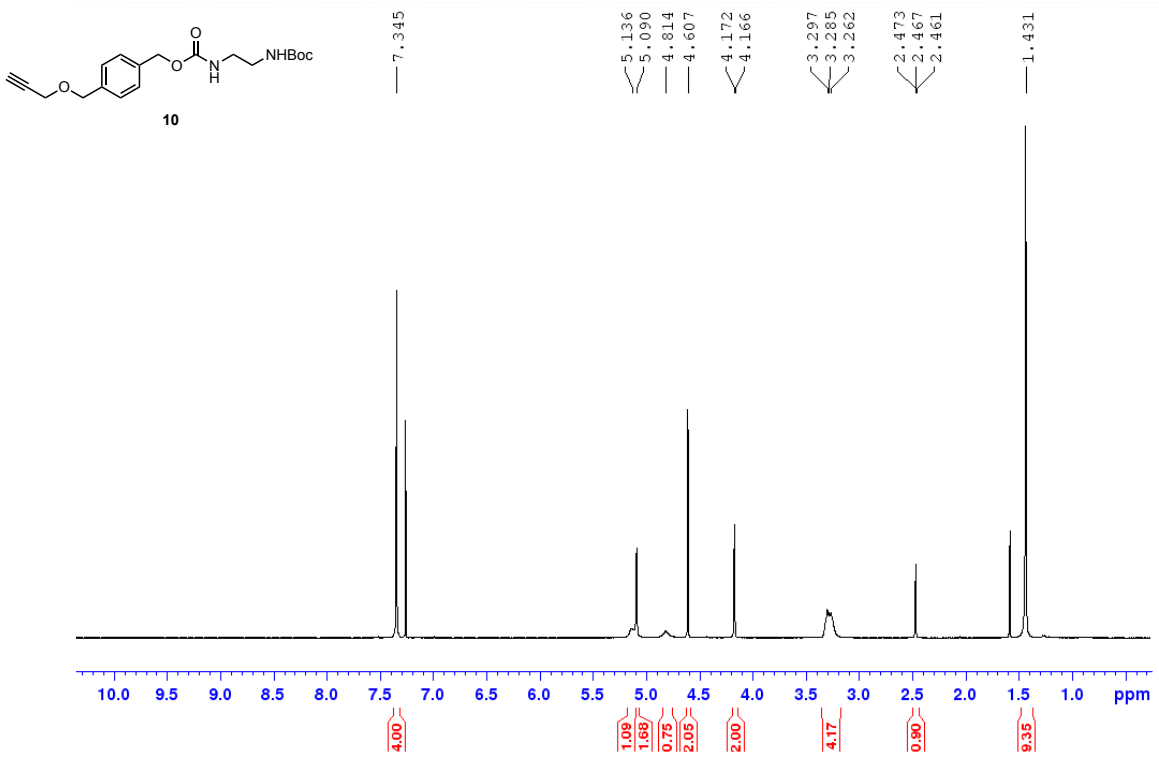

7.350
7.345
4.160
4.157
4.154
4.152
2.477
2.471
2.465

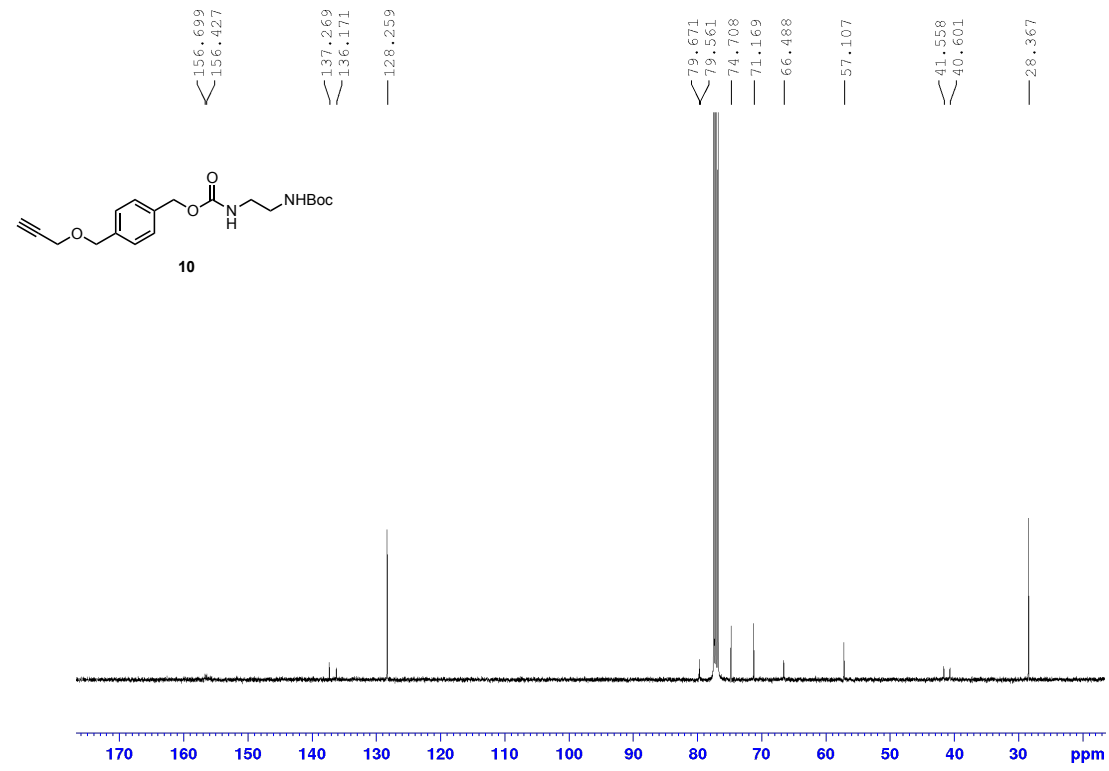

10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
3.66
2.00
0.90

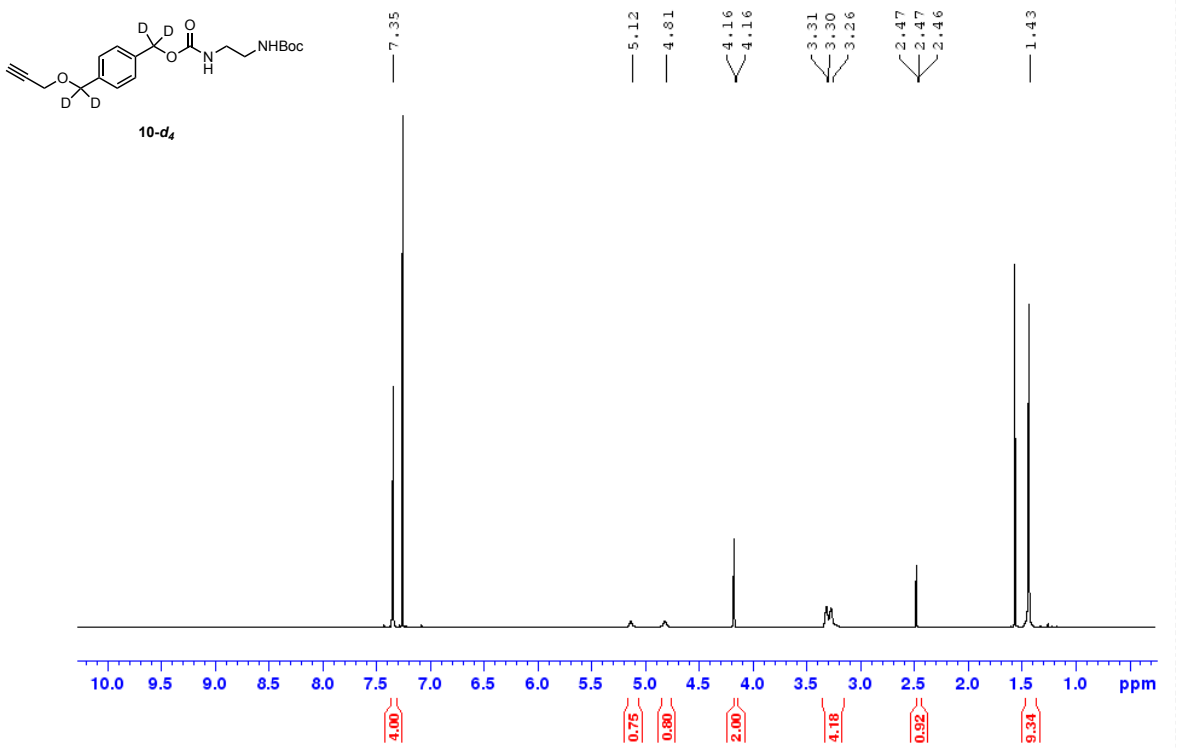

D
D
OH
O
D
D

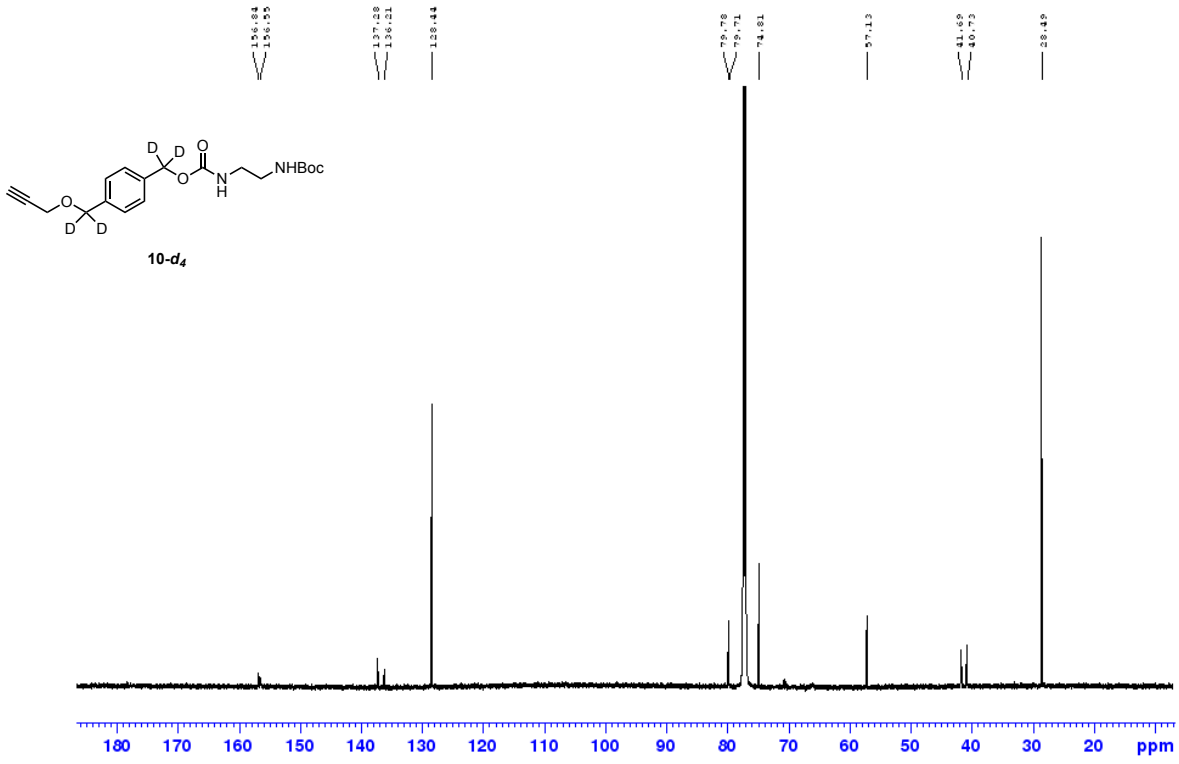
9-d_4

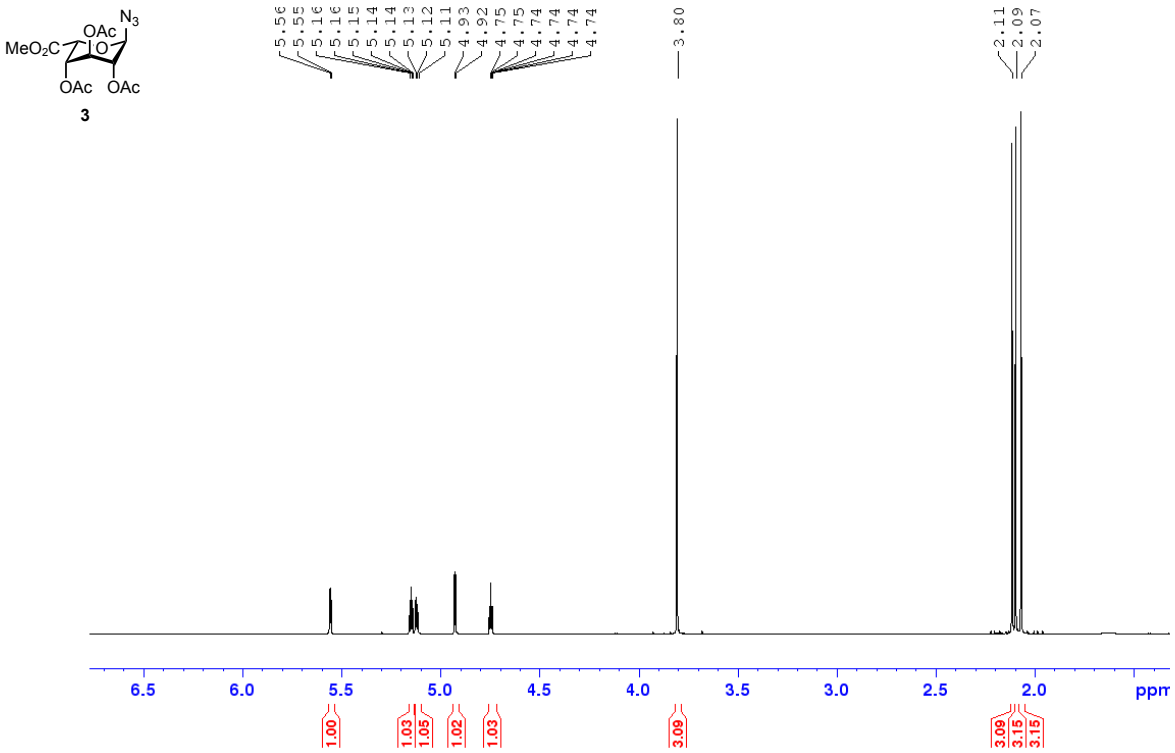
140.68
136.69
128.53
127.25
79.74
74.78
57.07
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

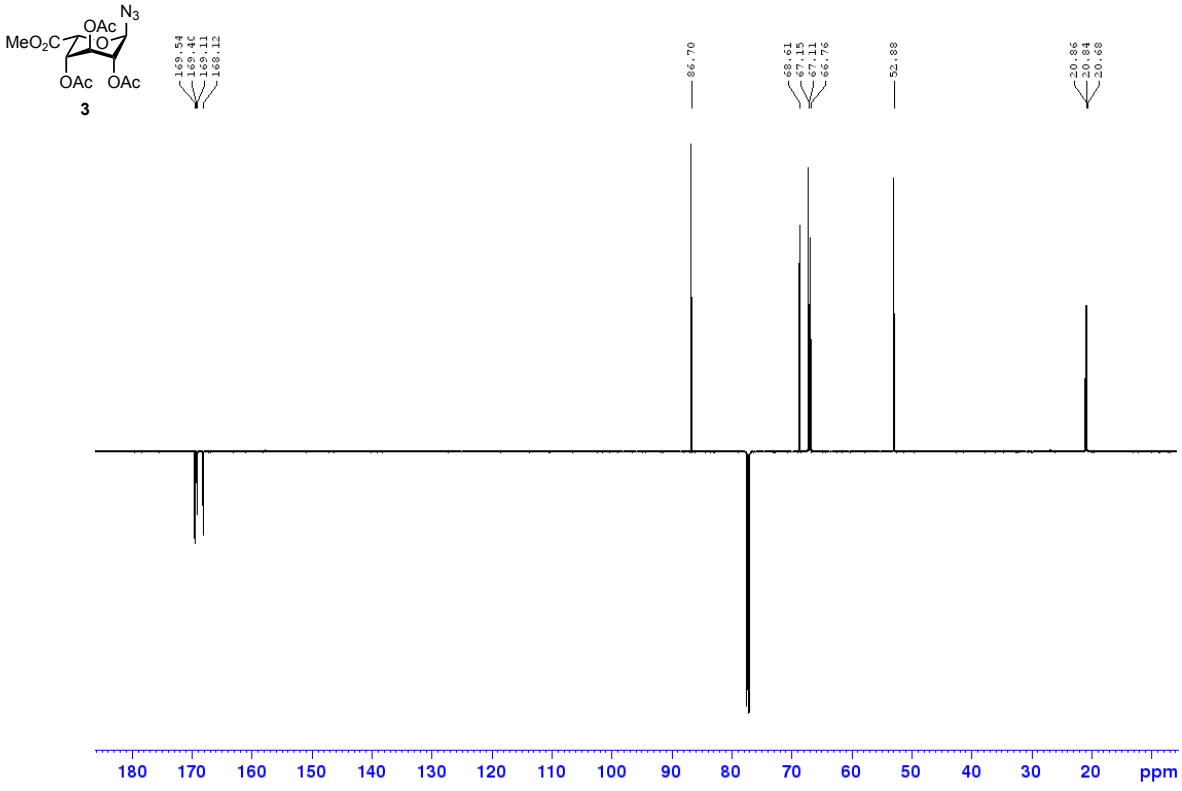

NHBoc
10
7.345
5.136
5.090
4.814
4.607
4.172
4.166
3.297
3.285
3.262
2.473
2.467
2.461
1.431
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
4.00
1.09
1.68
0.75
2.05
2.00
4.17
0.90
9.35

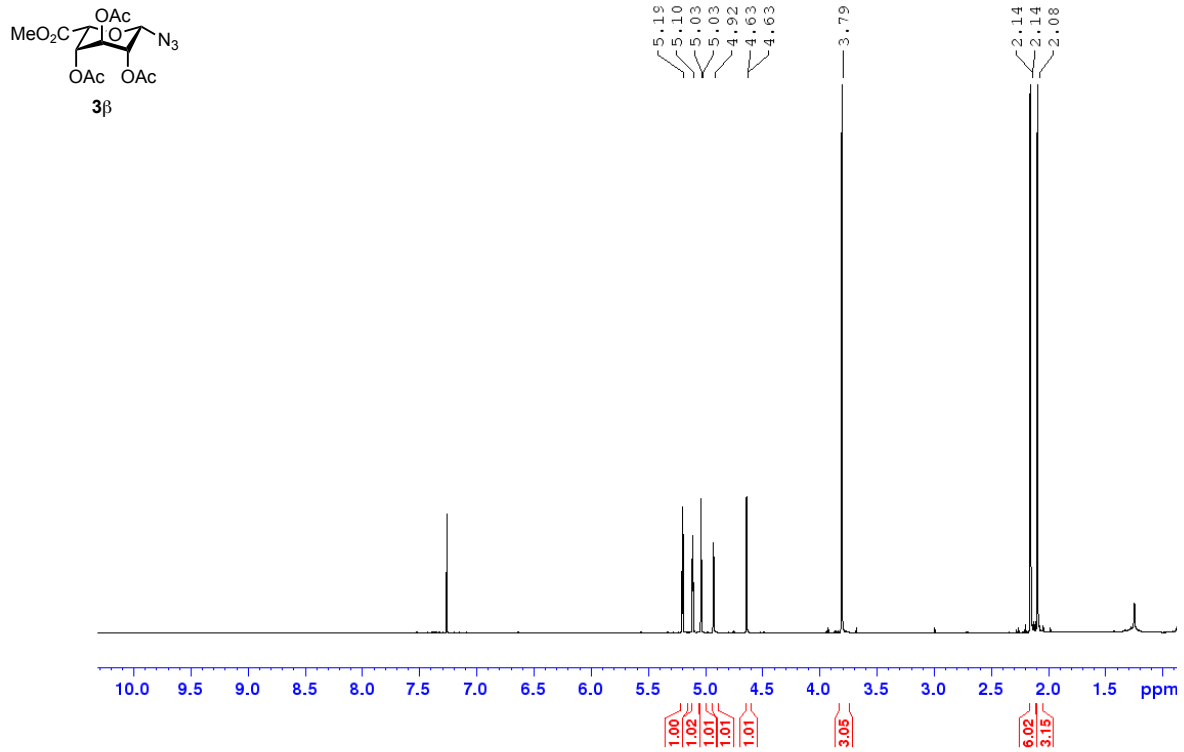

NHBoc
10
156.699
156.427
137.269
136.171
128.259
79.671
79.561
74.708
71.169
66.488
57.107
41.558
40.601
28.367
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 ppm

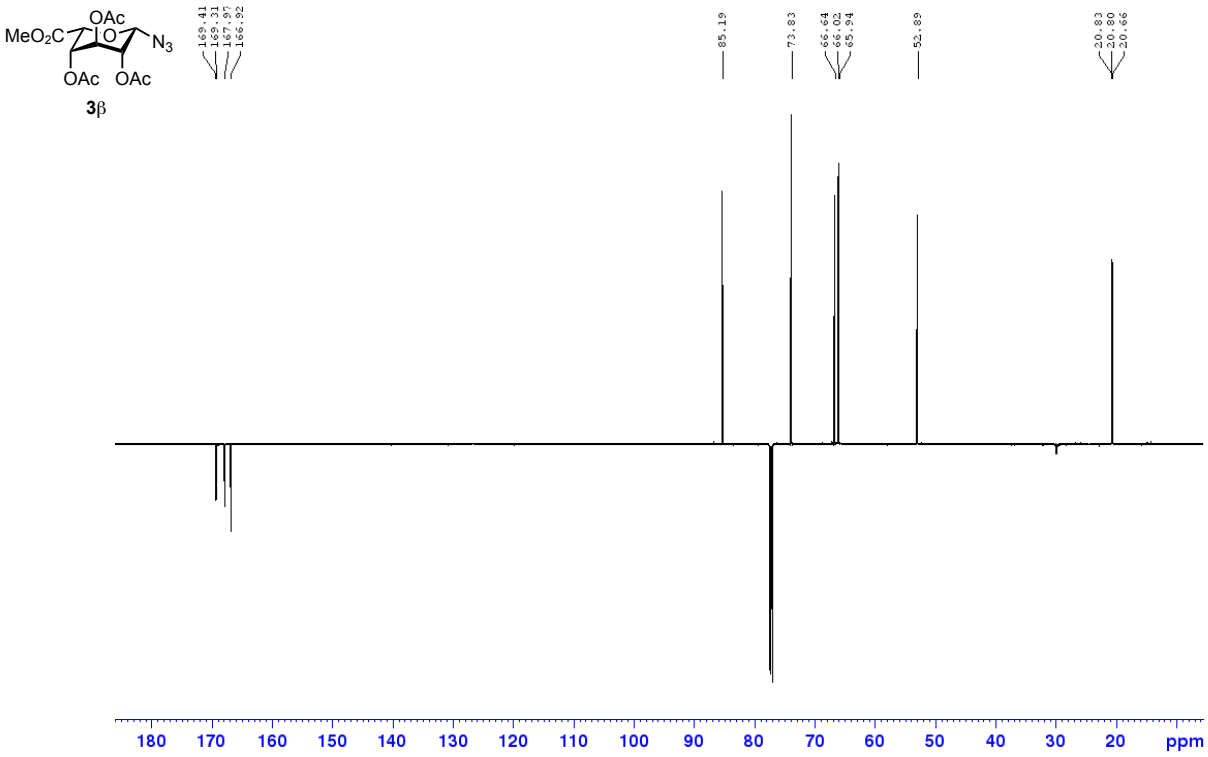

NHBoc
10-d_4
7.35
5.12
4.81
4.16
4.16
3.31
3.30
3.26
2.47
2.47
2.46
1.43
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
4.00
0.75
0.80
2.00
4.18
0.92
9.34

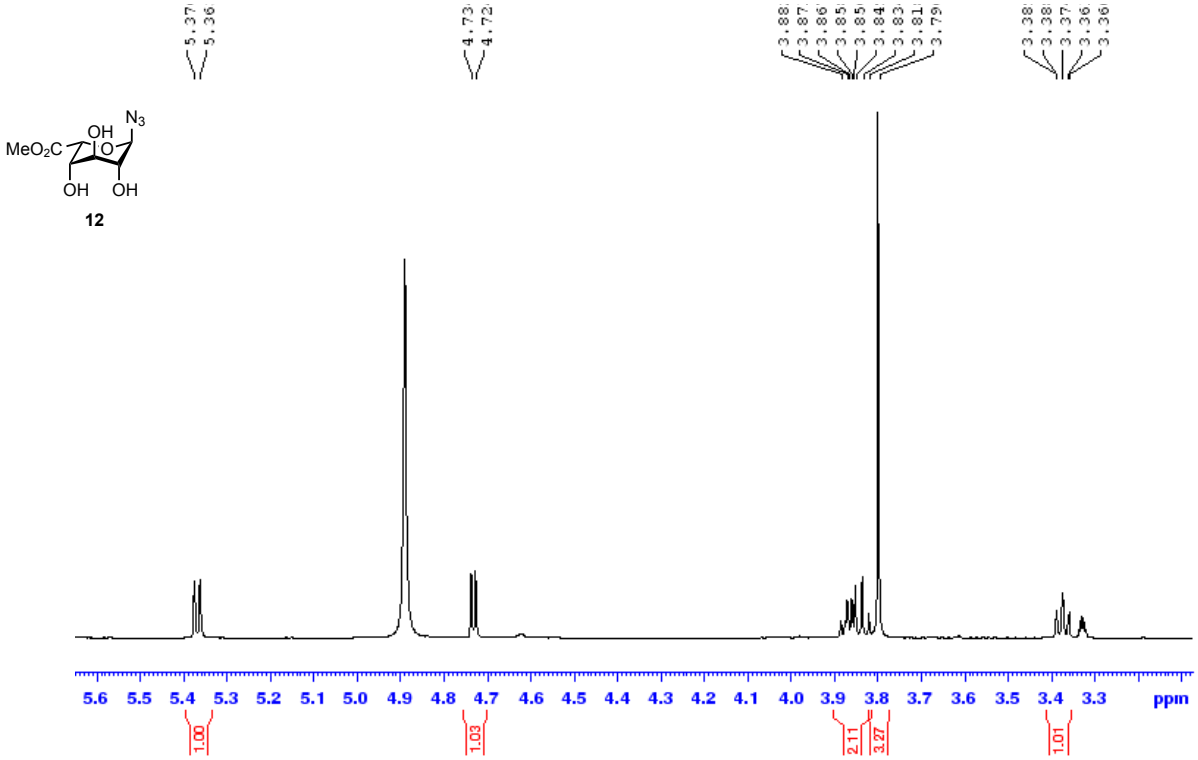

NHBoc
10-d_4
156.84
156.55
137.28
136.21
128.44
79.78
79.71
74.81
57.13
41.69
40.73
28.49
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

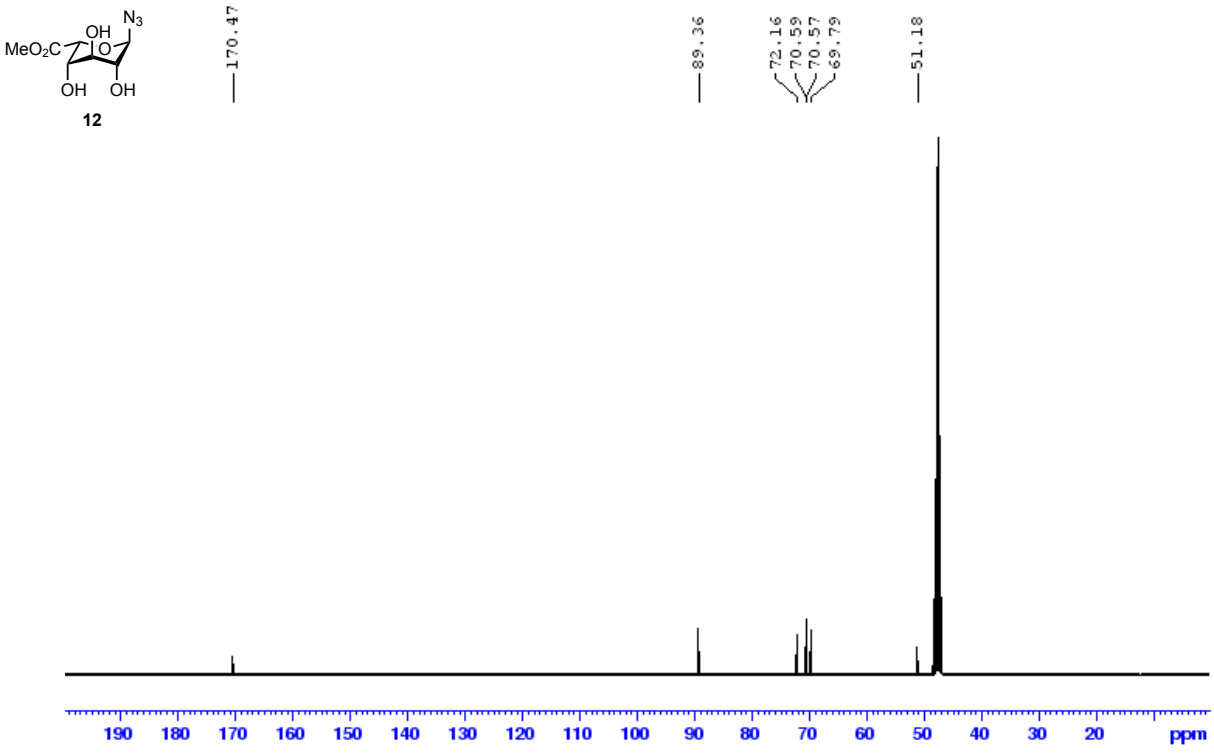

N3
OAc
MeO2C
O
OAc
OAc
3
5.56
5.55
5.16
5.15
5.14
5.13
5.12
5.11
4.93
4.92
4.75
4.75
4.74
4.74
4.74
4.74
3.80
2.11
2.09
2.07
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.00
1.03
1.05
1.02
1.03
3.09
3.09
3.15
3.15

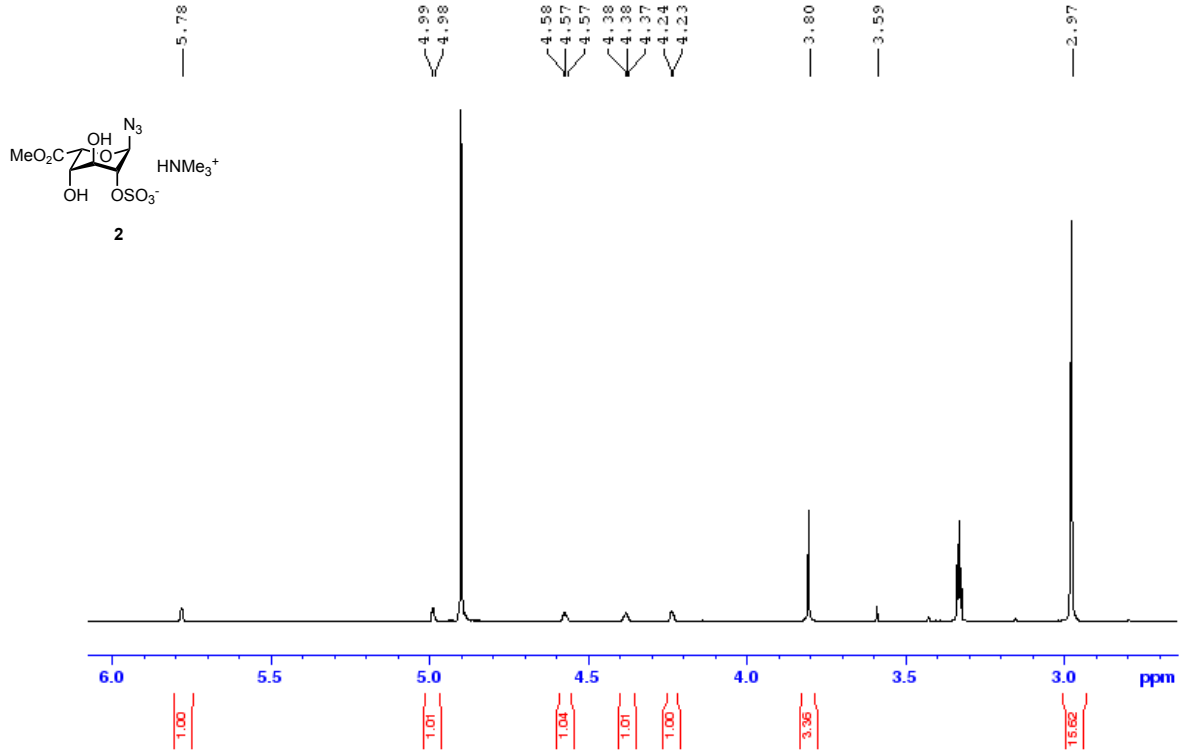

N3
OAc
MeO2C
O
OAc
OAc
3
169.54
169.45
169.11
168.12
86.70
68.61
67.15
67.11
66.76
52.88
20.86
20.84
20.68
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

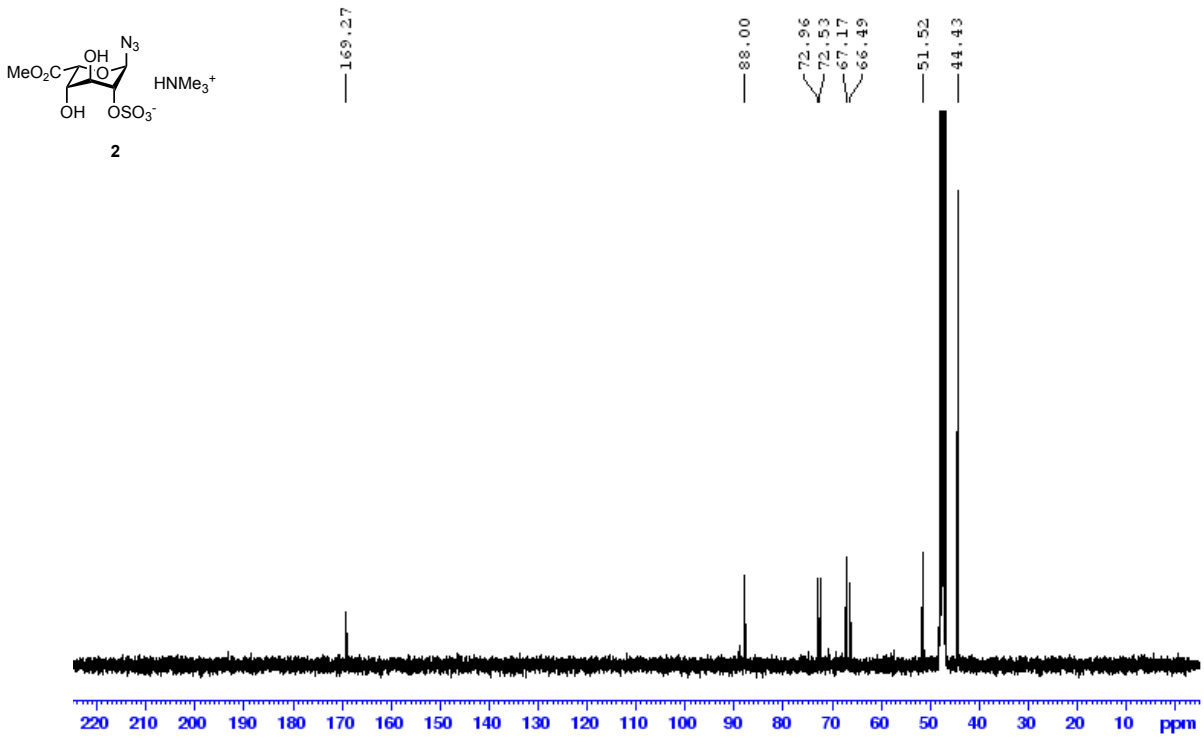

OAc
MeO2C
O
N3
OAc OAc
3β
5.19
5.10
5.03
4.92
4.63
4.63
3.79
2.14
2.14
2.08
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm
1.00
1.02
1.01
1.01
1.01
3.05
6.02
3.15

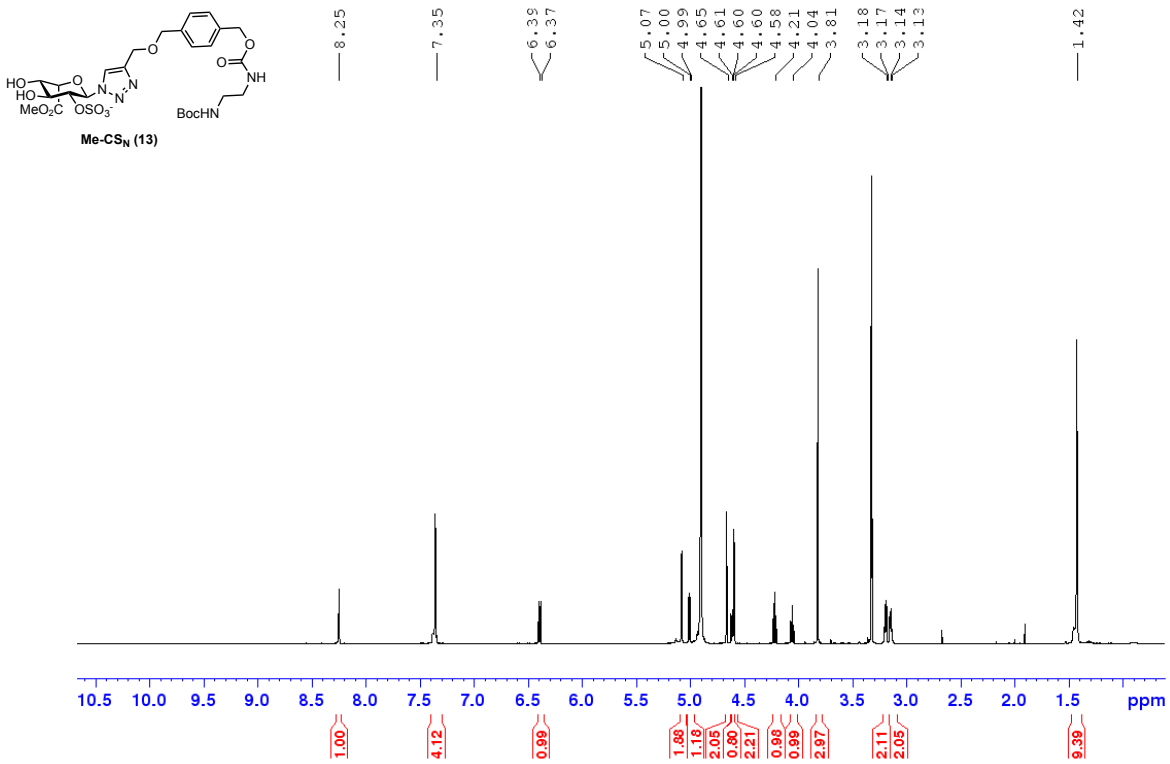

OAc
MeO2C
O
N3
OAc OAc
3β
169.41
169.31
167.97
166.92
85.19
73.83
66.64
66.02
65.94
52.89
20.83
20.80
20.66
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

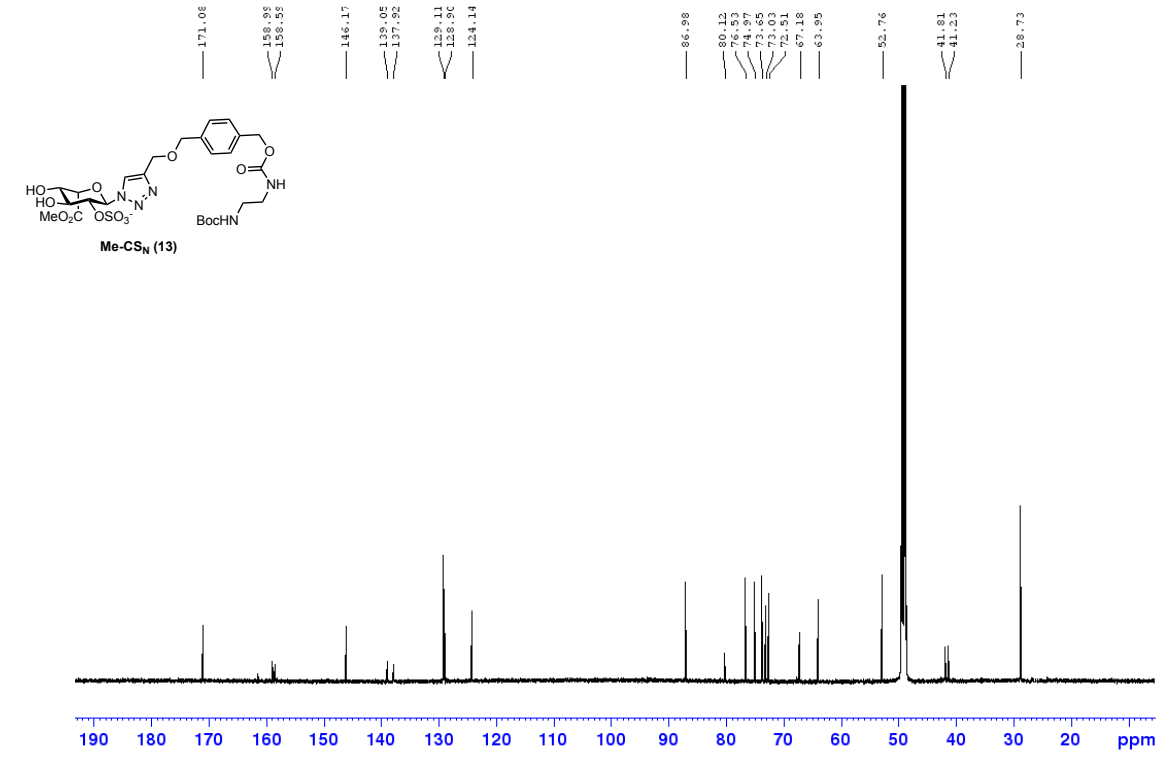

5.37
5.36
4.73
4.72
3.88
3.87
3.86
3.85
3.85
3.84
3.83
3.81
3.79
3.38
3.38
3.37
3.36
3.36
N3
OH
MeO2C
O
OH
OH
12
5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3
ppm
1.00
1.03
2.11
3.27
1.01

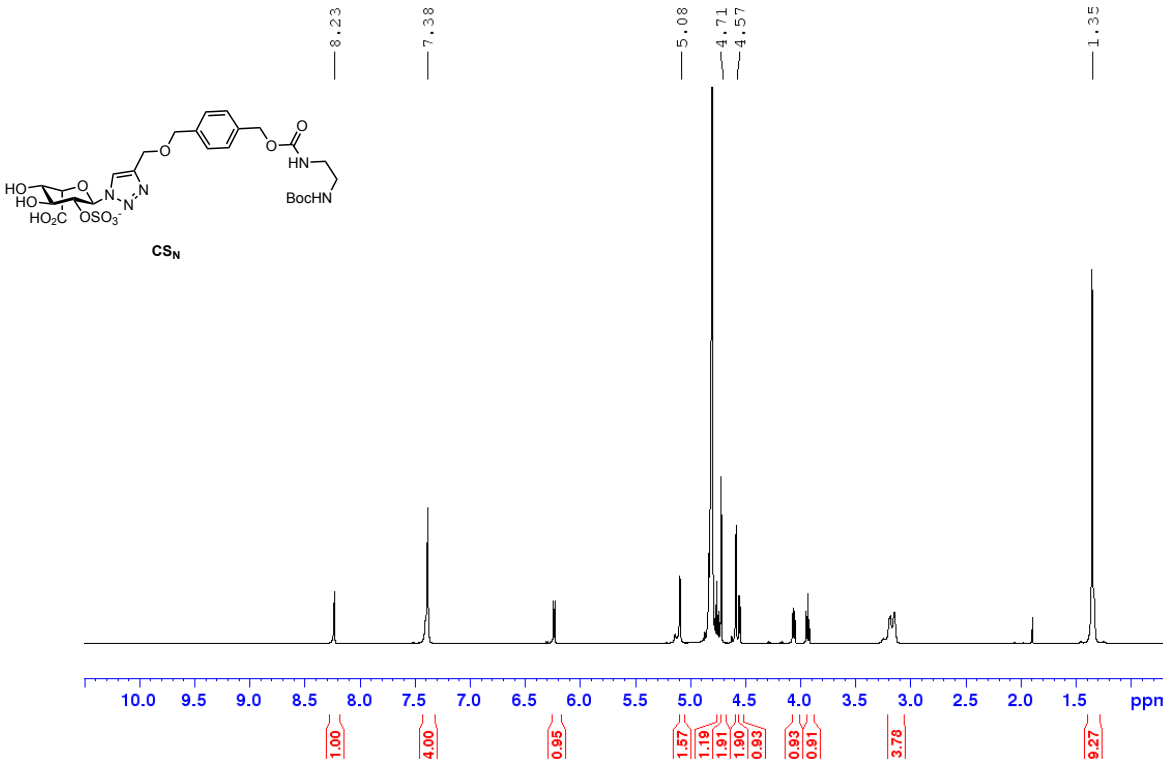

N3
OH
MeO2C
O
OH
OH
12
170.47
89.36
72.16
70.59
70.57
69.79
51.18
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
ppm

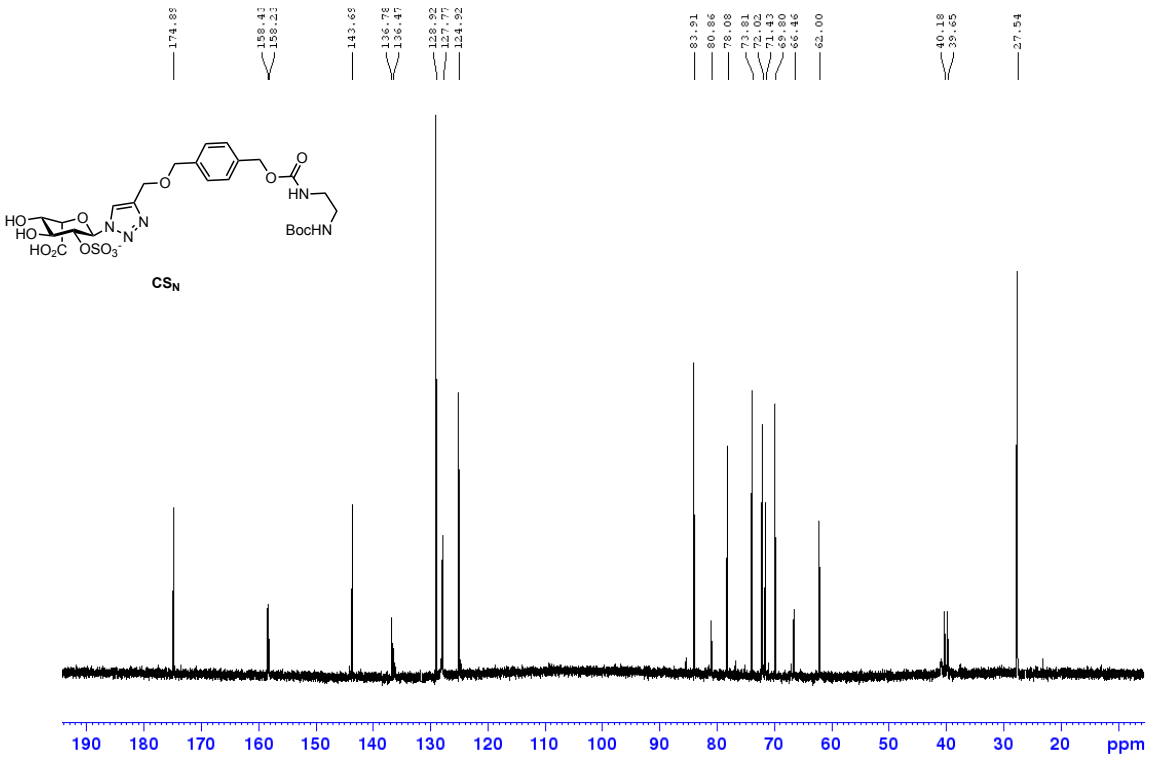

5.78
4.99
4.98
4.58
4.57
4.57
4.38
4.38
4.37
4.24
4.23
3.80
3.59
2.97
N3
OH
MeO2C
O
HNMe3+
OH
OSO3-
2
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm
1.00
1.01
1.04
1.01
1.00
3.96
15.62

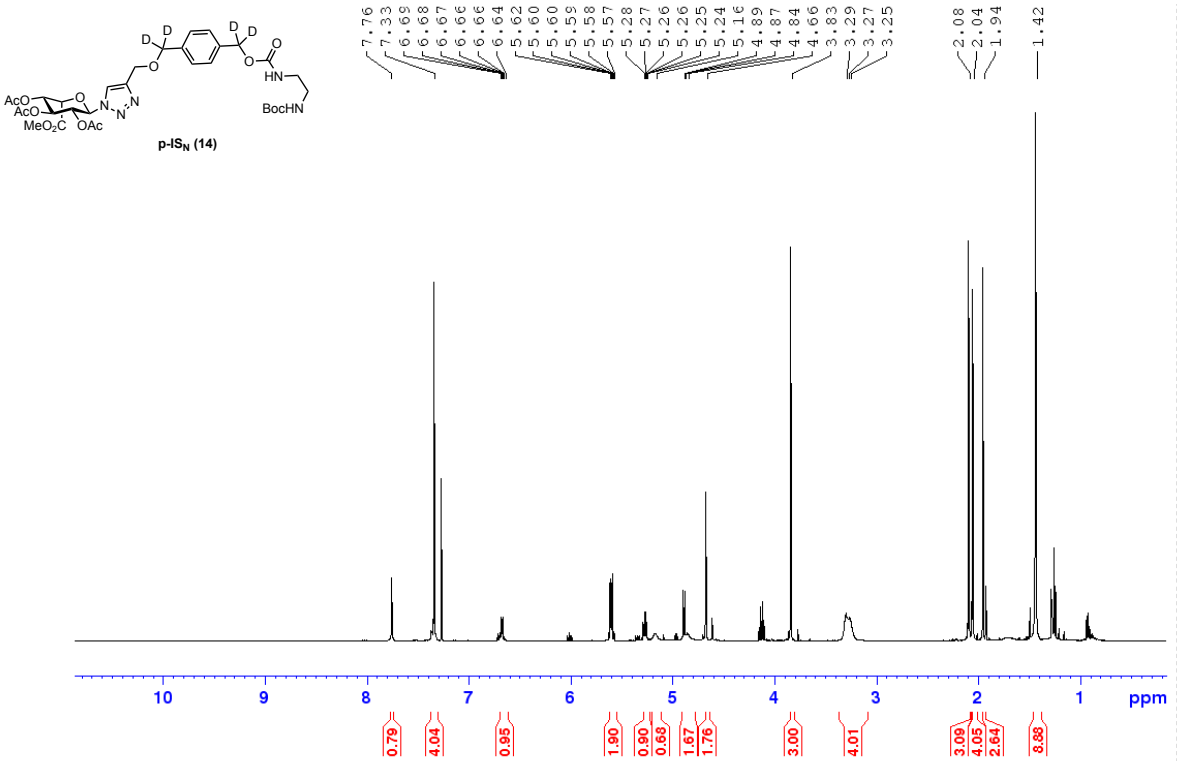

169.27
88.00
72.96
72.53
67.17
66.49
51.52
44.43
N3
OH
MeO2C
O
HNMe3+
OH
OSO3-
2
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

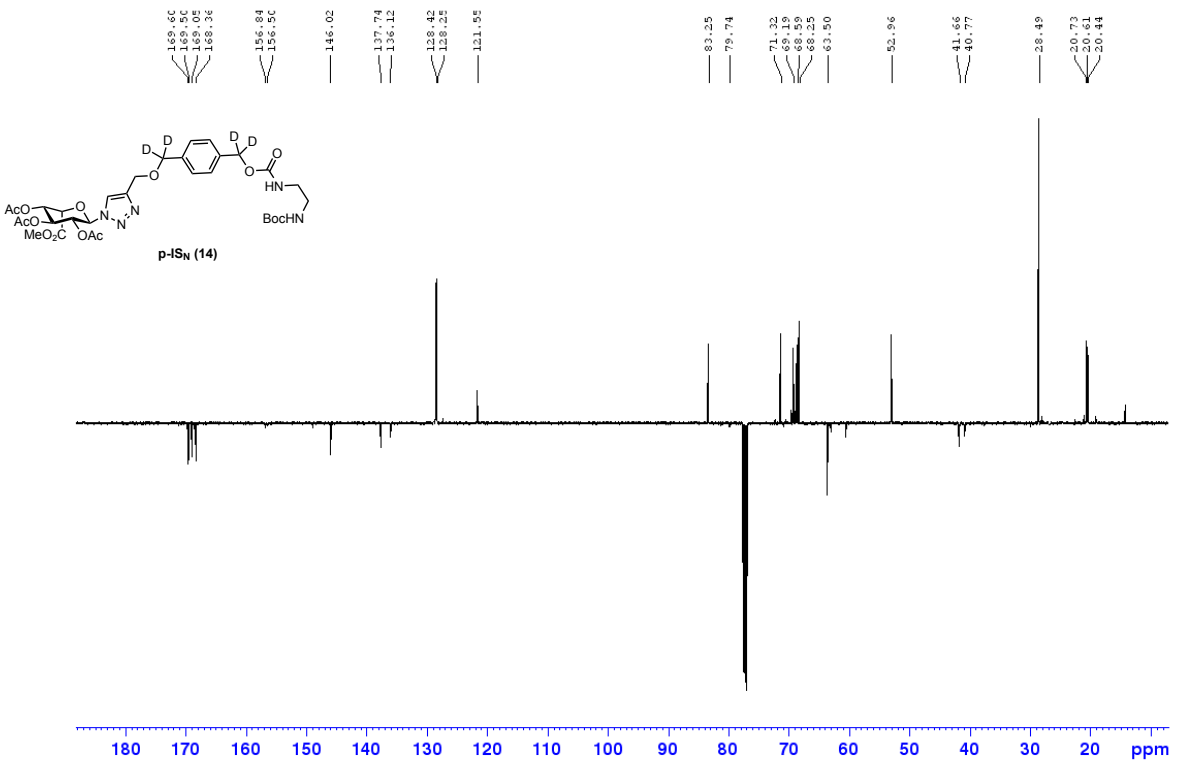

Me-CS$_N$ (13)
8.25
7.35
6.39
6.37
5.07
5.00
4.99
4.65
4.61
4.60
4.60
4.58
4.21
4.04
3.81
3.18
3.17
3.14
3.13
1.42
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm
1.00
4.12
0.99
1.88
1.18
2.05
0.80
2.21
0.98
0.99
2.97
2.11
2.05
9.39

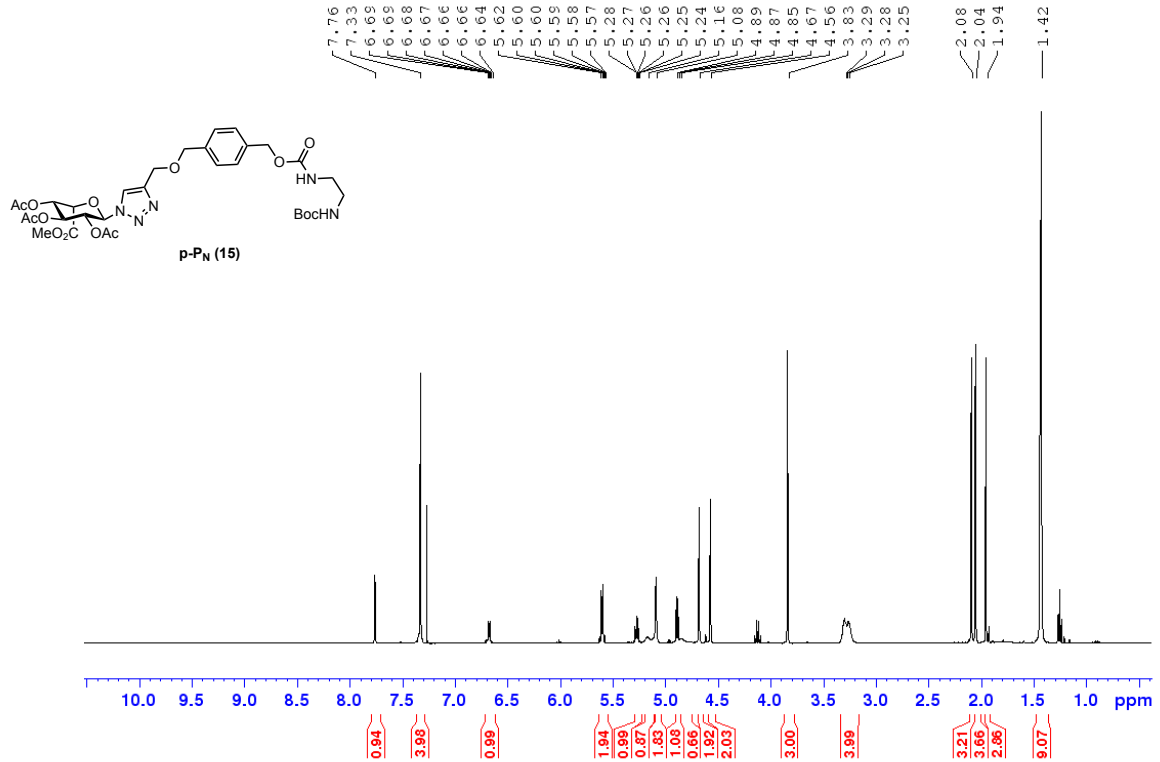

Me-CS$_N$ (13)
171.08
158.99
158.55
146.17
139.05
137.92
129.11
128.90
124.14
86.98
80.12
76.53
74.97
73.65
73.03
72.51
67.18
63.95
52.76
41.81
41.23
28.73
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

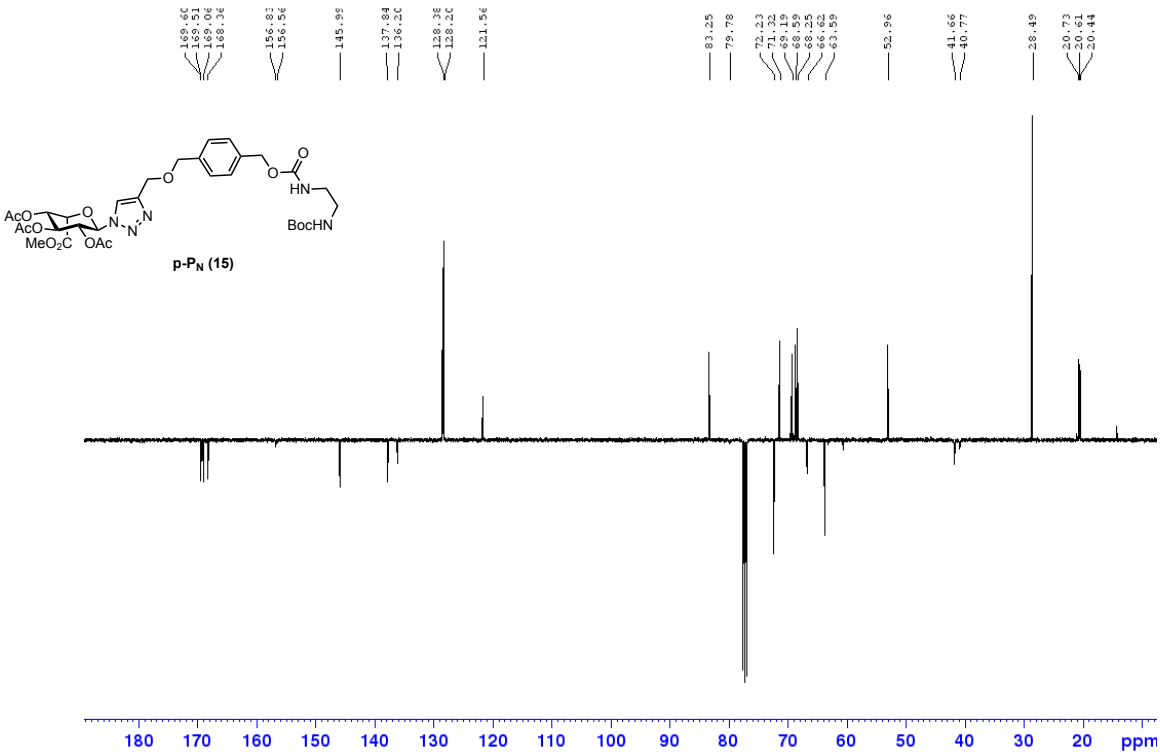

8.23
7.38
5.08
4.71
4.57
1.35
HO
HO
HO2C
OSO3-
O
N
N
N
O
O
HN
BocHN
CSN
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm
1.00
4.00
0.95
1.57
1.19
1.91
1.90
0.93
0.93
0.91
3.78
9.27

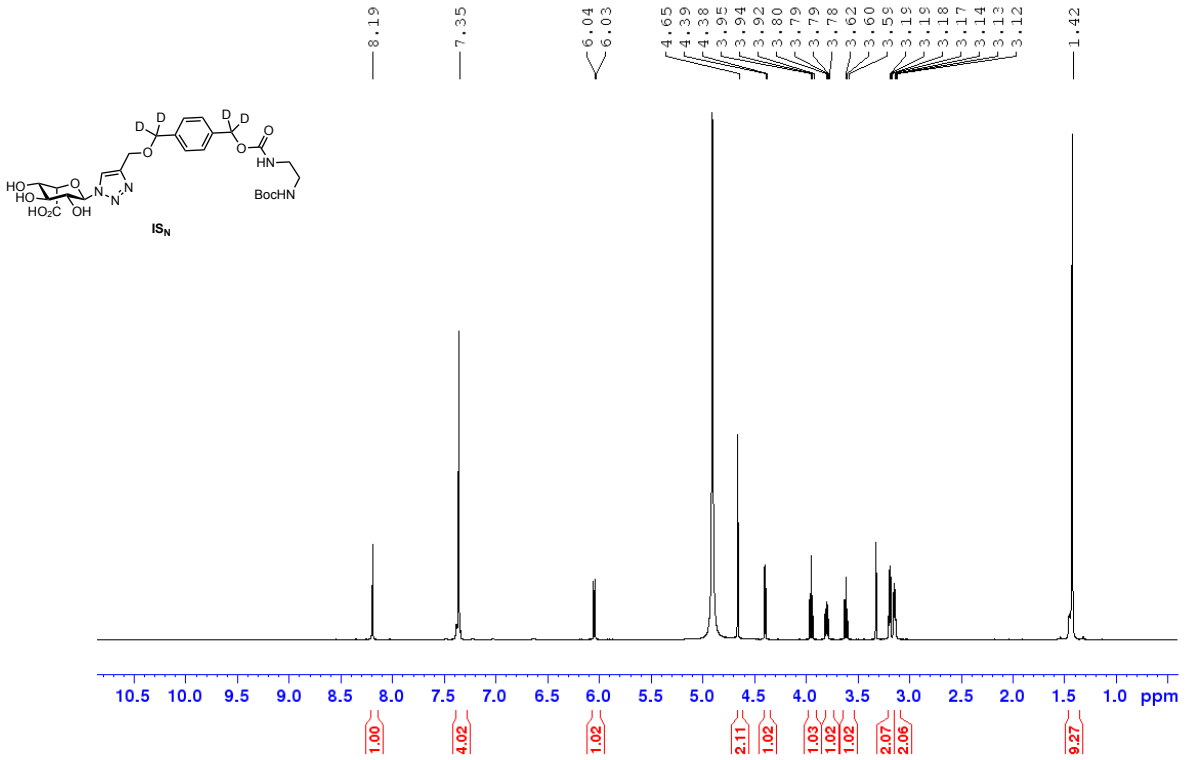

174.88
158.41
158.23
143.69
136.78
136.47
128.92
127.77
124.92
83.91
80.86
78.08
73.81
72.02
71.43
69.00
66.46
62.00
40.18
39.65
27.54
HO
HO
HO2C
OSO3-
O
N
N
N
O
O
HN
BocHN
CSN
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

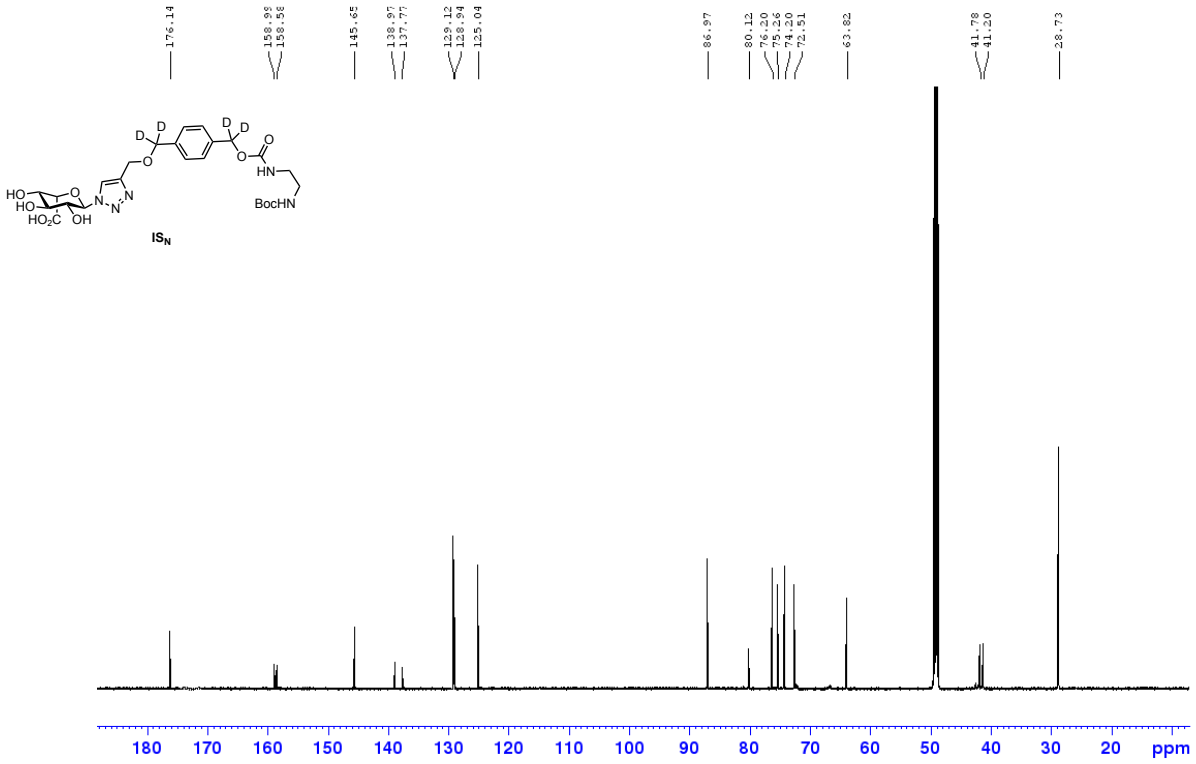

p-IS$_N$ (14)
7.76
7.33
6.69
6.68
6.67
6.66
6.66
6.64
5.62
5.60
5.60
5.59
5.58
5.57
5.28
5.27
5.26
5.26
5.25
5.24
5.16
4.89
4.87
4.84
4.66
3.83
3.29
3.27
3.25
2.08
2.04
1.94
1.42
10
9
8
7
6
5
4
3
2
1
ppm
0.79
4.04
0.95
1.90
0.90
0.68
1.67
1.76
3.00
4.01
3.09
4.05
2.64
8.88

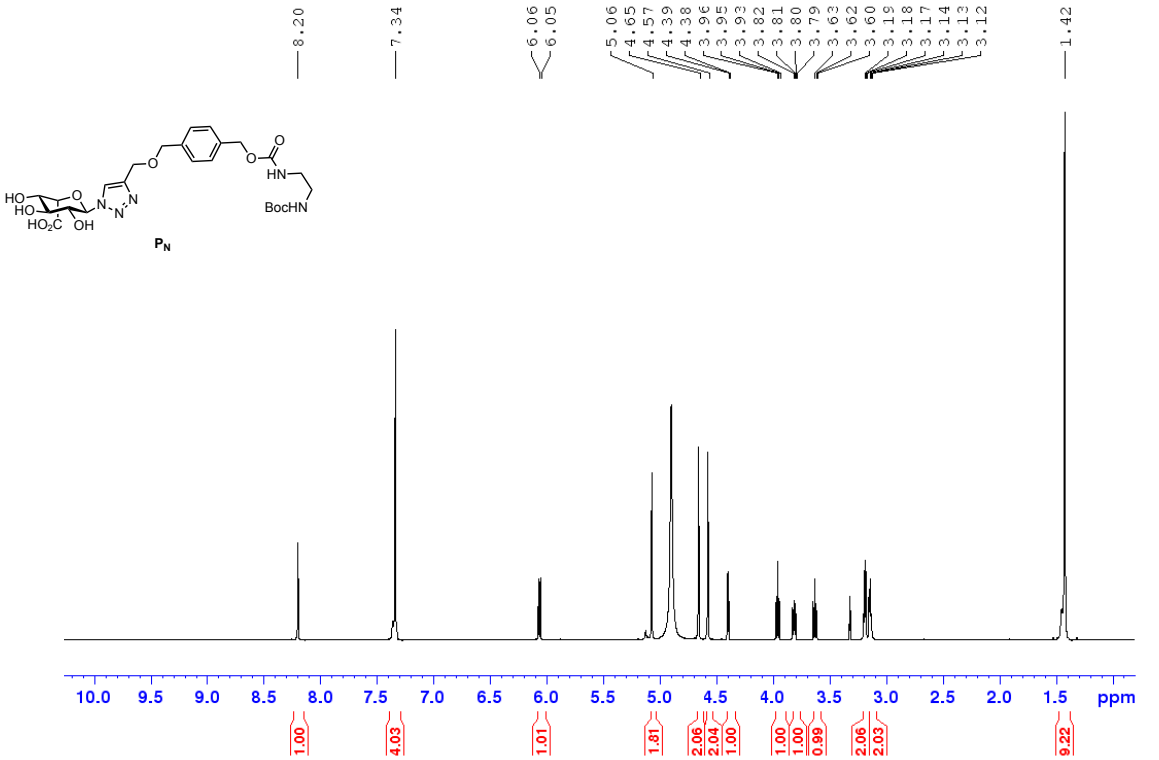

p-IS$_N$ (14)
169.66
169.56
169.05
168.36
156.84
156.56
146.02
137.74
136.12
128.42
128.25
121.55
83.25
79.74
71.32
69.19
68.59
68.25
63.50
52.96
41.66
40.77
28.49
20.73
20.61
20.44
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

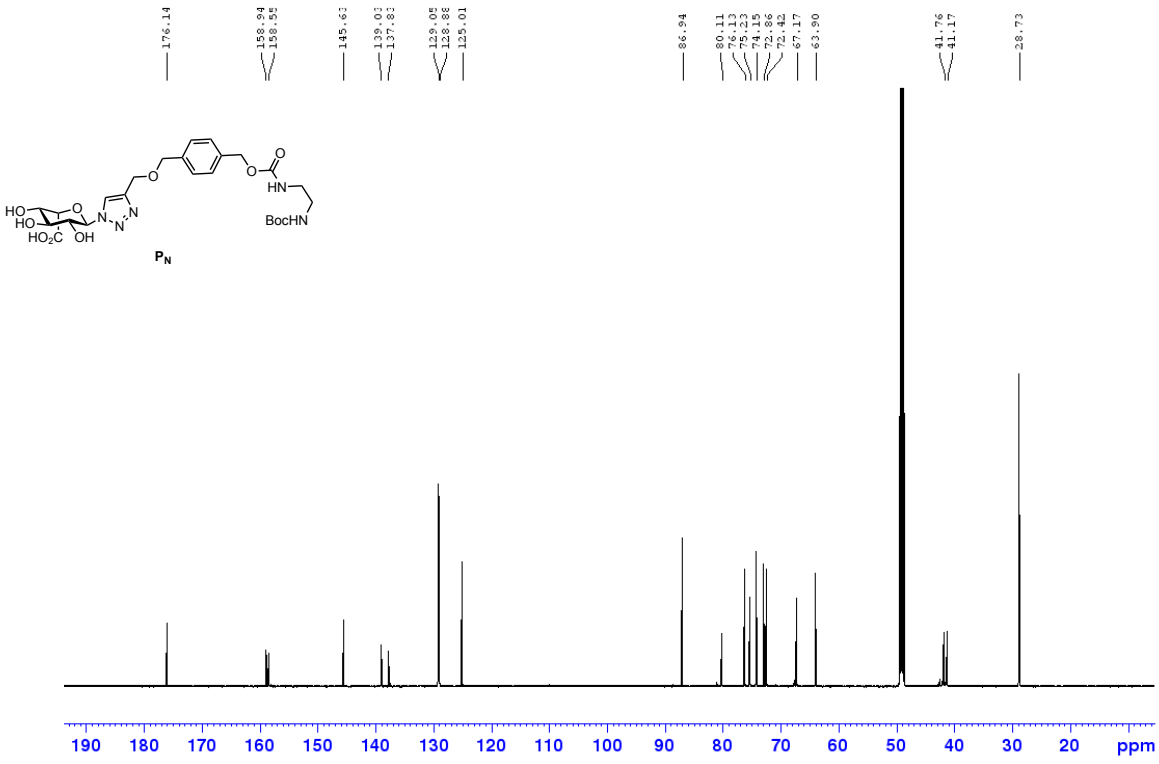

7.76
7.33
6.69
6.69
6.68
6.67
6.66
6.66
6.64
5.62
5.60
5.60
5.59
5.58
5.57
5.28
5.27
5.26
5.26
5.25
5.24
5.16
5.08
4.89
4.87
4.85
4.67
4.56
3.83
3.29
3.28
3.25
2.08
2.04
1.94
1.42
AcO
AcO
MeO2C
OAc
O
N
N
N
O
O
HN
BocHN
p-PN (15)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.94
3.98
0.99
1.94
0.99
0.87
1.83
1.08
0.66
1.92
2.03
3.00
3.99
3.21
3.66
2.86
9.07


169.60
169.51
169.06
168.36
156.83
156.56
145.95
137.84
136.20
128.38
128.20
121.56
83.25
79.78
72.23
71.32
69.19
68.59
68.25
66.62
63.59
52.96
41.66
40.77
28.49
20.73
20.61
20.44
AcO
AcO
MeO2C
OAc
O
N
N
N
O
O
HN
BocHN
p-PN (15)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


8.19
7.35
6.04
6.03
4.65
4.39
4.38
3.95
3.94
3.92
3.80
3.79
3.79
3.78
3.62
3.60
3.59
3.19
3.19
3.18
3.17
3.14
3.13
3.12
1.42
10.5 10.0 9.5 9.0 8.5 8.0 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 ppm
1.00
4.02
1.02
2.11
1.02
1.03
1.02
1.02
2.07
2.06
9.27


176.14
158.99
158.58
145.65
138.97
137.77
129.12
128.94
125.04
86.97
80.12
76.20
75.26
74.20
72.51
63.82
41.78
41.20
28.73
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


8.20
7.34
6.06
6.05
5.06
4.65
4.57
4.39
4.38
3.96
3.95
3.93
3.82
3.81
3.80
3.79
3.63
3.62
3.60
3.19
3.18
3.17
3.14
3.13
3.12
1.42
HO
HO
HO2C
OH
O
N
N
N
O
O
HN
BocHN
PN
10.0
9.5
9.0
8.5
8.0
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
ppm
1.00
4.03
1.01
1.81
2.06
2.04
1.00
1.00
1.00
0.99
2.06
2.03
9.22


176.14
158.94
158.55
145.63
139.03
137.83
129.05
128.88
125.01
86.94
80.11
76.13
75.13
74.15
72.86
72.42
67.17
63.90
41.76
41.17
28.73
HO
HO
HO2C
OH
O
N
N
N
O
O
HN
BocHN
PN
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

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