Supporting Information

Structural insights into heparanase activity using a fluorogenic heparan sulfate disaccharide

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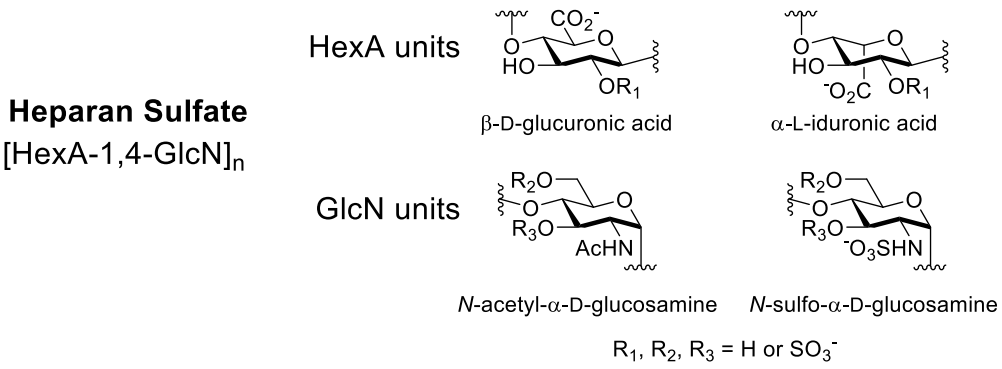


Figure S1 Generic formula for the composition of heparan sulfate, and chemical structures of the individual sugar building blocks.

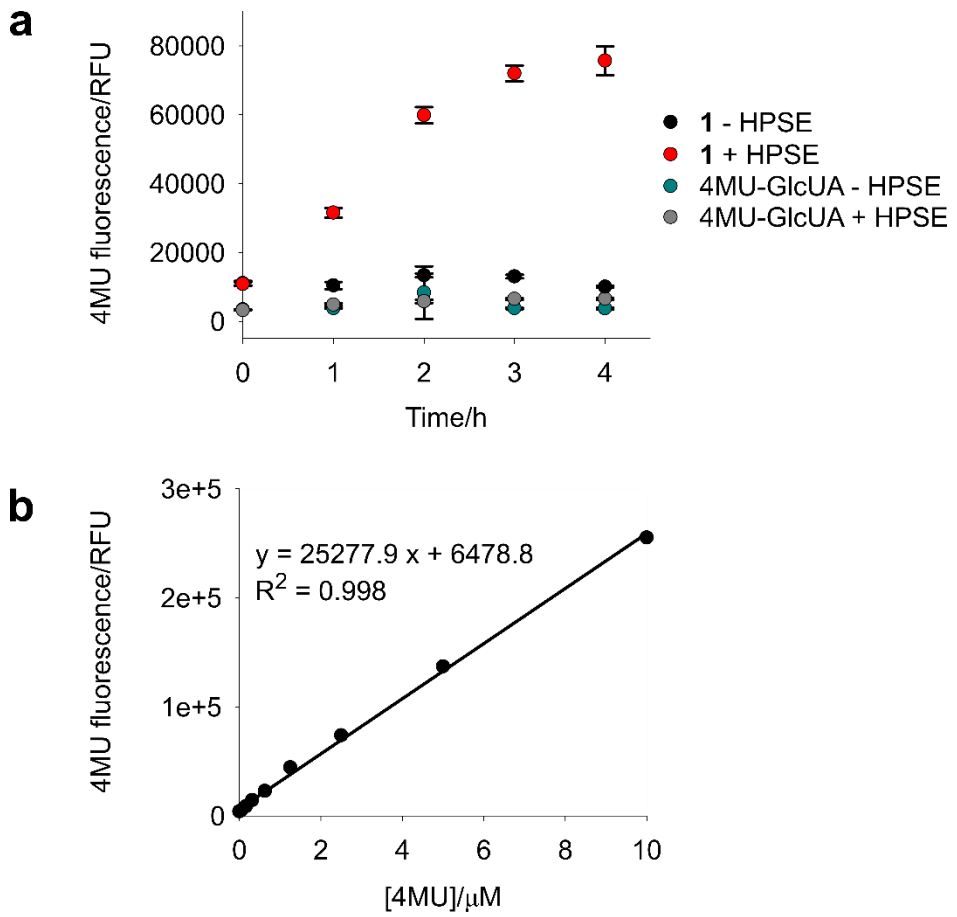
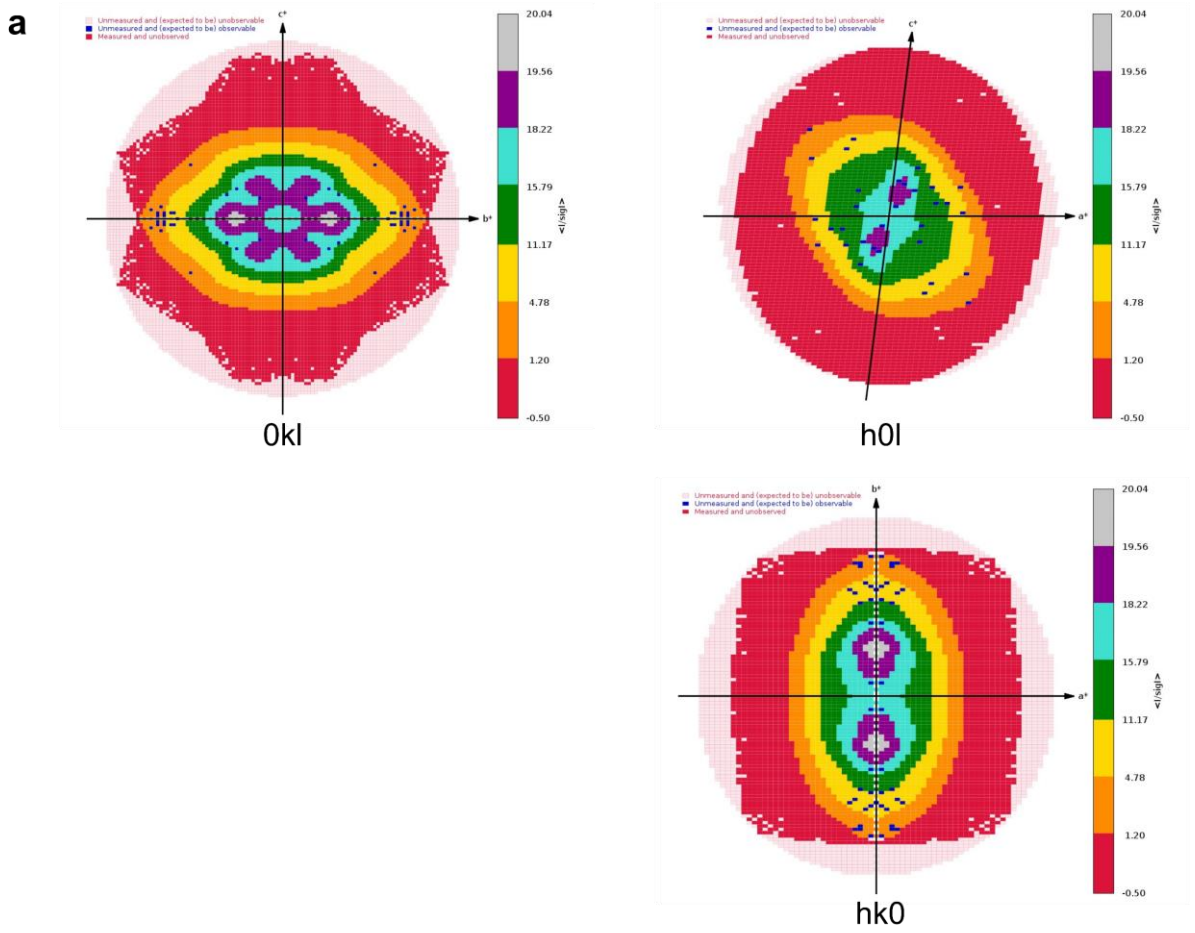


Figure S2 a Time course of 4MU released from **1** or 4MU-GlcA in the presence or absence of HPSE, as measured by 4MU fluorescence. HPSE cleavage of **1** releases 4MU continuously, although some slowdown is observed after 2 h. Essentially no 4MU release is observed using 4MU-GlcA, or for either substrate in the absence of HPSE. **b** Calibration curve of 4MU fluorescence vs 4MU concentration, showing linearity even into the nM concentration range.



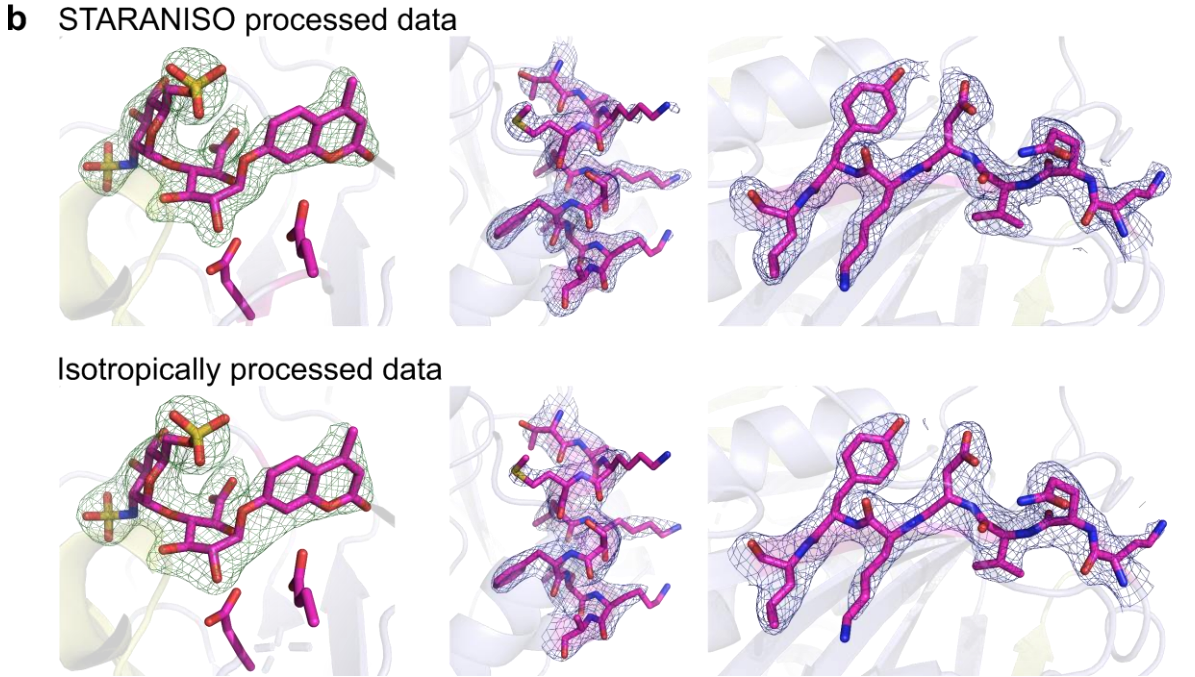
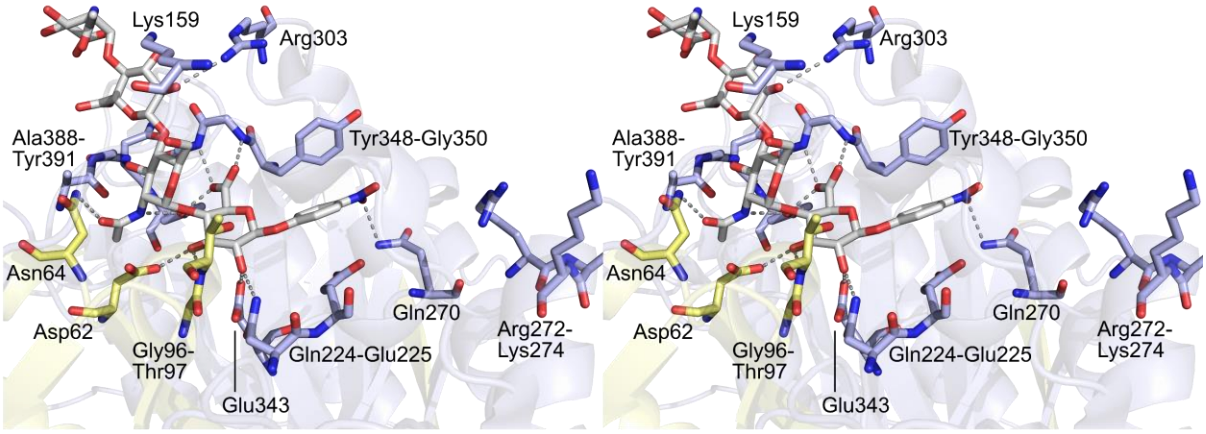
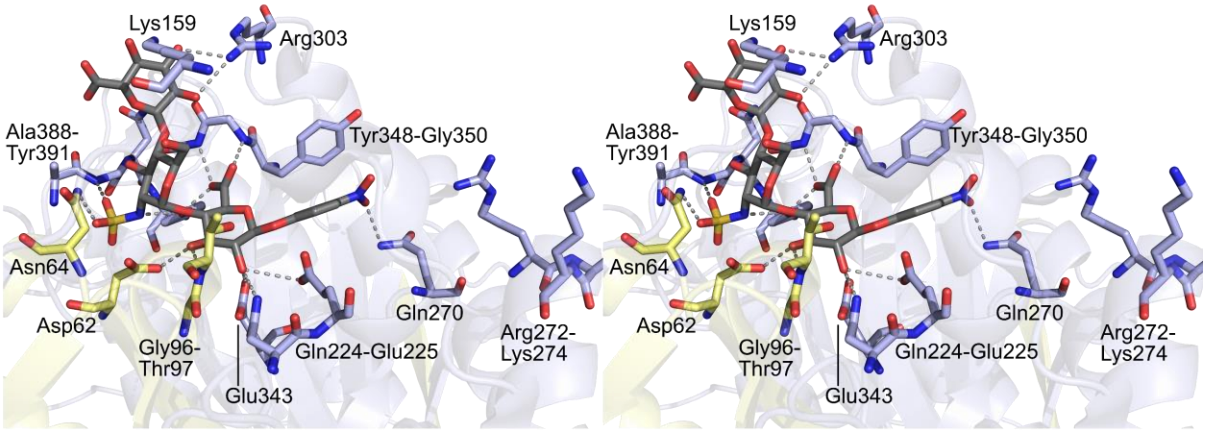


Figure S3 a STARANISO calculated plot of reciprocal lattice points colored according to local mean $I/\sigma(I)$ for the zones (*h*0*l*), (*hk*0), and (0*kl*). A mean $I/\sigma(I) \geq 1.2$ threshold was used as the anisotropic cut-off surface, which defines the resolution limits given in **Table S1**. **b** Comparison of electron densities calculated using STARANISO processed data *vs* isotropically processed data. Electron densities are Refmac σ_A weighted mFo-DFc (green) or 2mFo-DFc (blue) contoured to 3σ (0.17-0.28 electrons/Å^3) or 1σ (0.25 electrons/Å^3) respectively.

5E97 - pNP linked HS (no sulfates) - $^4C_1$ GlcA conformation



5E98 - pNP linked HS (N-sulfated) - $^4C_1$ GlcA conformation



This work - 4MU linked HS (N- and O- sulfated) - $^1S_3$ GlcA conformation

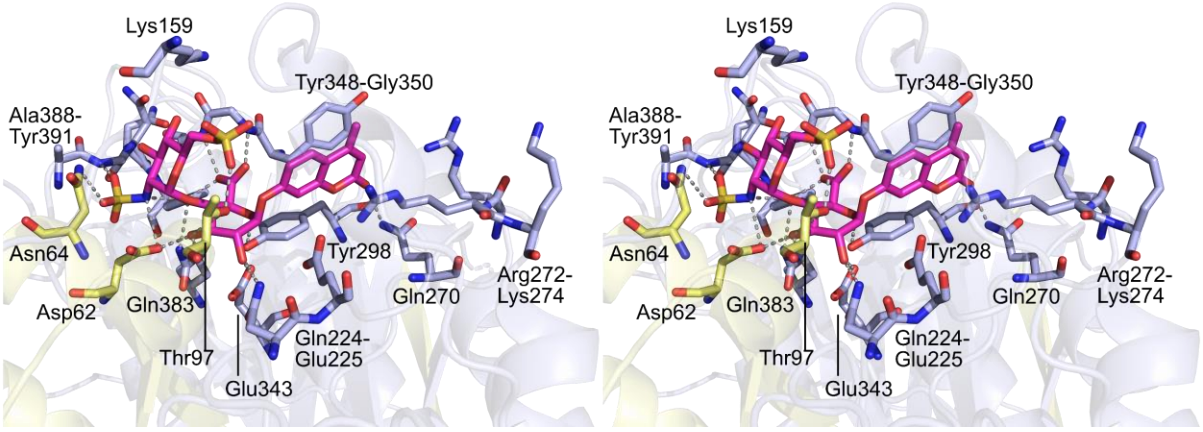


Figure S4 Wall-eyed stereo views of HPSE active site in complex with pNP-linked HS oligosaccharides (PDB accessions 5E97, 5E98), and the 4MU-linked HS disaccharide **1** from this work. HPSE residues are colored blue (50 kDa chain) and yellow (8 kDa chain), and ligands are colored in light gray (5E97), dark gray (5E98) or pink (this work).

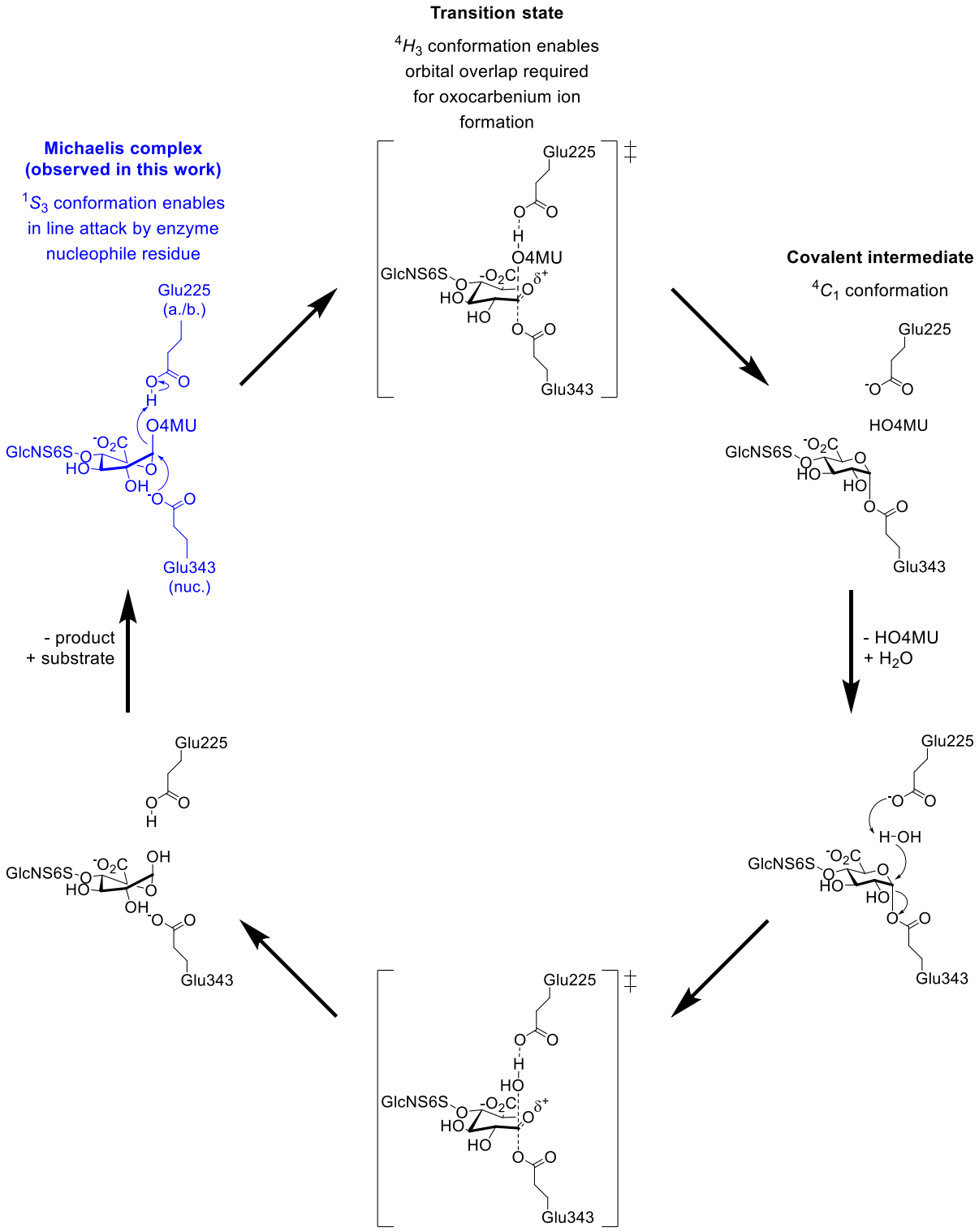


Figure S5 Catalytic cycle for HPSE processing of **1**, illustrating the conformational itinerary undergone by the –1 subsite GlcA. The $^1S_3$ conformation observed for GlcA in the Michaelis complex (blue) facilitates in line attack by the enzyme catalytic nucleophile Glu343.

Table S1

Data collection and refinement statistics

	HPSE-**1**
Data collection	
Space group	P 1 2_1 1
Cell dimensions	
a, *b*, *c* (Å)	44.97, 70.86, 78.46
α, β, γ (°)	90, 97.13, 90
Resolution (Å)	52.40 – 2.07, 1.67, 2.82*
R_{merge}	0.045 (0.667)
R_{pim}	0.025 (0.371)
I / σI	7.8 (1.8)
Completeness – ellipsoidal (%)	91.0 (65.7)
Redundancy	4.1 (4.2)
Refinement	
Resolution (Å)	52.40 – 1.71
No. reflections	24624
R_{work} / R_{free}	0.18/0.24
No. atoms	
Protein	3652
Ligand/ion	87
Water	201
B-factors (Å^2)	
Protein	42.4
Ligand/ion	51.4
Water	39.1
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.59

**Resolution limits of ellipsoid fitted to diffraction cut-off surface defined by eigenvectors*

$$\begin{matrix} 0.758 & 0 & -0.653 \\ 0 & 1 & 0 \\ 0.653 & 0 & 0.758 \end{matrix}$$

Methods

Chemical synthesis

General

Melting points were determined on a DigiMelt MSRS apparatus. Optical rotations were determined on a JASCO P-2000 polarimeter at ambient temperature and are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer at 20 °C. The residual solvent peaks ($CDCl_3$: δH 7.26 and δC 77.16 or CD_3OD: δH 3.31 and δC 49.0) served as internal standards. Coupling constants in Hz were measured from one-dimensional spectra. The analyses of ^{1}H and ^{13}C NMR spectra were assisted by COSY and HSQC experiments. Analytical LRMS and HRMS were performed in a positive or negative ion ESI mode on a Bruker HCT spectrometer and a Bruker micrOTOF$_Q$ spectrometer, respectively. All reagents and solvents were obtained from Sigma-Aldrich, Australia, and were used without further purification, except EtOAc, *n*-hexane, MeOH and DCM which were distilled prior to use. Reactions were monitored by analytical thin layer chromatography (TLC) on silica gel 60 F_{254} plates and visualized by charring with anisaldehyde/H_2SO_4 stain in ethanol. Flash chromatography was performed on silica gel under positive pressure with specified solvent systems.

tert-Butyldiphenylsilyl (3,4-di-*O*-acetyl-2-azido-6-*O*-benzoyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranosiduronate (**8**)

Anhydrous building block **6**[1] (1.02 g, 0.92 mmol) and freshly dried mol sieves (4Å, 1 g) were suspended in anhydrous $CHCl_3$ (45 mL) and stirred (1h). The suspension was cooled to -78 °C and $TiCl_4$ (1.05 mL, 9.58 mmol) was added in three portions. After each addition (at -78 °C) the mixture was brought to 0 °C left stirring each time for 45 min until TLC indicated complete consumption of starting material (R_f = 0.38, Toluene/EtOAc 1:1). The mixture was then poured into a stirred mixture of ice-cold satd $NaHCO_3$ solution and $CHCl_3$ (2:3, v/v, 250 mL). The mixture was separated in a separating funnel and was washed with satd $NaHCO_3$ solution (3 x 30 mL) and the aqueous layer was re-extracted with EtOAc (150 ml). Combined organic layers was washed with brine (50 mL) and dried ($MgSO_4$) and gave after co-evaporation *in vacuo* with toluene crude triol **7** as colourless foam (810 mg). Crude **7** was subjected to rapid silica filtration (EtOAc) and dried under high vacuum before acetylation. Crude **7** in anhydrous pyridine (10 mL) containing a catalytic amount of DMAP was added dropwise to a solution of acetic anhydride/dry pyridine (1:1, v/v, 3.9 mL, 20.5 mmol) at 0 °C and left for 16 h at r.t. The reaction was quenched by dropwise addition of dry MeOH (10 mL) at 0 °C and stirred for 30 min at r.t. After co-evaporation with toluene (3 x 30 mL) crude triacetate **8** was obtained as an off-white foam (686 mg, 77 % yield over 2 steps). The material was sufficiently pure for use in the next step without further purification. An analytical sample was prepared by purification on silica gel (Toluene/EtOAc 5:1) to afford a white foam (R_f = 0.26, Toluene/EtOAc, 3:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06 (dd, 2 H, ArH), 7.83 (dd, 1 H, ArH), 7.10-7.58 (m, 16 H, ArH), 6.10 (dd, 1 H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 10.3 Hz, H3), 5.48 (dd, 1 H, $J_{2',3'}$ 10.7 Hz, $J_{3',4'}$ 9.3 Hz, H3'), 5.38 (d, 1 H, $J_{1,2}$ 2.9 Hz, H1), 5.32 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H1'), 5.19 (dd, 1 H, $J_{4',5'}$ 10.3 Hz, H4'), 4.94 (dd, 1 H, H2), 4.70 (d, 1 H, $J_{4,5}$ 9.8 Hz, H5), 4.54 (dd, A part of ABX, 1 H, $J_{6'a,6'b}$ -12.5 Hz, $J_{5',6'a}$ 2.4 Hz, H-6'a); 4.37 (dd, B part of ABX, 1 H, $J_{5',6'b}$ 3.0 Hz, H6'b), 4.33 (dd, 1 H, H4), 4.03 (ddd, 1 H, H5'), 3.83 (s, 3 H, CO_2Me), 3.36 (dd, 1 H, H-2'), 2.10, 2.04, 1.98 (3 x s, 9 H, 3 x OAc), 1.14 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (C-6), 169.7, 169.6, 168.8 (3 x O*C*(O)Me); 166.3, 165.5 (2 x *C*(O)-Ph), 136.0, 135.9, 134.9, 133.5, 133.4, 132.0, 131.9, 130.3, 130.2, 130.1, 130.0, 129.9, 129.8, 129.0, 128.6, 128.5, 127.9, 127.8, (Ar), 99.1 (C-1'), 91.7 (C-1), 76.6 (C-4), 72.50 (C-2), 71.1 (C-3), 70.6 (C-5), 70.3 (C-3'), 68.6 (C-4'), 68.5 (C-5'), 61.8 (C-6'), 61.0 (C-2'), 53.0 (CO_2CH_3), 27.0 ($C(CH_3)_3$),

20.89, 20.85, 20.78 (3 x OC(O)*C*H$_3$), 19.4 (C(*C*H$_3$)$_3$. LRMS: *m/z* 377.1 [M+Na]$^+$; HRMS: *m/z* calcd for $C_{49}H_{53}N_3O_{16}Si$ [M+Na]$^+$: 990.30929; found: 990.30548.

3,4-Di-*O*-acetyl-2-azido-6-*O*-benzoyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-acetyl-2-*O*-benzoyl-D-glucopyranosiduronate (**9**)

Anhydrous disaccharide **8** (2.45 g, 2.5 mmol) was dissolved in dry THF (10 mL) cooled to -10 °C and a solution of TBAF in THF (1M, 3.3 mL, 3.3 mmol, 1.32 eq) was added dropwise over 5 min and left stirring for 30 min at -10 °C. The mixture was then diluted with $CHCl_3$ (200 mL), washed with satd $NaHCO_3$ solution (5 × 50 mL) and the aqueous layer was re-extracted with $CHCl_3$ (3 × 50 mL). Combined organic layers were washed with brine (50 mL) and dried ($MgSO_4$) and after evaporation *in vacuo* crude **9** was obtained as off white foam. Purification on silica gel (Gradient: Toluene/EtOAc 3:1→EtOAc) gave the hemiacetal **9** as a white foam (1.37 g, 75%), R_f = 0.23 (Toluene/EtOAc, 3:1) as a 6:1 α/β-mixture. α-anomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00 – 8.08 (m, 4 H, ArH), 7.42-7.60 (m, 6 H, ArH), 5.87 (dd, 1 H, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 9.9 Hz, H3), 5.65 (dd, 1 H, $J_{1,2}$ 3.4 Hz, H1), 5.41 (dd, 1 H, $J_{2',3'}$ 10.7 Hz, $J_{3',4'}$ 9.3 Hz, H3'), 5.24 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H1'), 5.17 (dd, 1 H, $J_{4',5'}$ 10.2 Hz, H4'), 4.99 (ddd, 1 H, H2), 4.63 (d, 1 H, $J_{4,5}$ 9.7 Hz, H5), 4.54 (dd, 1 H, $J_{6'a,6'b}$ -12.7 Hz, $J_{5',6'a}$ 2.5 Hz, H6'a), 4.35 (dd, 1 H, $J_{5',6'b}$ 3.1 Hz, H6'b), 4.28 (dd, 1 H, H4), 4.05 (ddd, 1 H, H5'), 3.84 (s, 3 H, CO_2Me), 3.44 (dd, 1 H, H2'), 3.34 (dd, 1 H, $J_{1,1\text{-OH}}$ 4.4 Hz, $J_{2,1\text{-OH}}$ 1.2 Hz, 1-OH), 2.07, 2.03, 2.01 (3 × s, 9 H, 3 × OAc); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.0 (C-6), 169.8, 169.7, 168.9 (3 × O*C*(O)Me); 166.3, 165.9 (2 × C(O)-Ph), 133.8, 133.4, 130.2, 130.1, 129.9, 129.7, 129.0, 128.78, 128.77, 128.6, (Ar), 98.9 (C-1'), 90.7 (C-1), 76.7 (C-4), 71.9 (C-2), 70.5 (C-3', C-3), 70.3 (C-5), 68.63 (C-4'), 68.58 (C-5'), 61.9 (C-6'), 61.1 (C-2'), 53.1 (CO_2*Me*), 20.95, 20.81, 20.76 (3 × OC(O)*Me*).

β-anomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00 – 8.08 (m, 4 H, ArH), 7.42-7.61 (m, 6 H, ArH), 5.59 (dd, 1 H, $J_{2,3}$ 9.7 Hz, $J_{3,4}$ 8.9 Hz, H3), 5.40 (dd, 1 H, $J_{2',3'}$ 10.6 Hz, $J_{3',4'}$ 9.2 Hz, H3'), 5.22 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H1'), 5.17 (dd, 1 H, $J_{4',5'}$ 10.2 Hz, H4'), 5.03 (dd, 1 H, H2), 4.94 (dd, 1 H, $J_{1,2}$ 9.7 Hz, $J_{1,1\text{-OH}}$ 8.7 Hz, H1), 4.54 (dd, 1 H, H6'a), 4.35 (dd, 1 H, H6'b), 4.33 (dd, 1 H, H4), 4.19 (d, 1 H, $J_{4,5}$ 9.7 Hz, H5), 3.98-4.08 (m, 2 H, H-5', 1-OH), 3.85 (s, 3 H, CO_2Me), 3.44 (dd, 1 H, H2'), 2.07, 2.03, 2.00 (3 × s, 9 H, 3 × OAc). Assigned Carbon peaks significantly different to those of α-anomer: ^{13}C NMR (125 MHz, $CDCl_3$): δ 98.90 (C-1'), 96.2 (C-1), 90.7 (C-1'), 74.13 (C-2), 72.8 (C-3), 53.2 (CO_2*Me*). HRMS: *m/z* calcd for $C_{33}H_{35}N_3O_{16}$ [M+Na]$^+$: 752.19151; found: 752.18817.

3,4-Di-*O*-acetyl-2-azido-6-*O*-benzoyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-acetyl-2-*O*-benzoyl-D-glucopyranuronosyl *N*-phenyltrifluoroacetimidate (10)

Dry hemiacetal **9** (5.55g, 7.61 mmol) was dissolved in dry DCM (50 mL) and *N*-phenyltrifluoroacetimidoyl chloride (PTFA-Cl, 3.8 mL, 18.3 mmol, 2.4 eq) was added dropwise at r.t. and stirred for 45 min at r.t. The mixture was cooled to 0 °C and finely powdered K_2CO_3 (10.8 g, 78 mmol) was added and stirring continued for 1h at 0 °C and then 16h at r.t. TLC indicated the reaction was incomplete, so more PTFA-Cl (0.22 mL, 1.32mmol, 0.17 eq) was added at r.t., and more finely powdered K_2CO_3 (1.0 g, 7.2 mmol) was added at 0 °C and stirring continued for 1h at 0 °C and then 2h at r.t. The suspension was diluted with toluene (50 mL), filtered through celite, washed with toluene (5 × 70 mL) and concentrated *in vacuo* at r.t. Purification on silica gel (Gradient: Toluene/EtOAc 7:1→3:1→EtOAc) gave the imidate **10** (5.78g, 78 %) as colourless foam as a 10:1 mixture of anomers. R_f = 0.49 (Toluene/EtOAc, 7:1); major anomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00-8.08 (m, 4H, Ar (Bz)), 7.53-7.67 (m, 2H, Ar (Bz)), 7.42-7.50 (m, 4H, Ar (Bz)), 7.26-7.32 (t, 2 H, m-Ar-N), 7.09-7.14 (t, 1 H, p-Ar-N), 6.77 (d, 2 H, $J_{o,m}$ = 8.0 Hz, o-Ar-N, 6.17 (bs, 1 H, H1), 5.52 (dd, 1 H, H3), 5.33-5.44 (m, 2 H, H3',H2),

5.26 (d, 1 H, $J_{1',2'}$ = 3.7 Hz, H1'), 5.16 (dd, 1 H, $J_{3',4'}$ = 9.4 Hz, $J_{4',5'}$ = 10.2 Hz, H4'), 4.46-4.55 (m, 2 H, H6'a, H4), 4.36 (dd, 1H, $J_{5',6'a}$ = 3.7 Hz, $J_{6'a,6'b}$ = -12.5 Hz, H-6'a), 4.36 (bs, 1 H, H5), 3.83 (s, 3H, CO_2Me), 3.47 (dd, 1 H, $J_{2',3'}$ = 10.7 Hz, H2'), 2.06, 2.03, 2.03 (3 × s, 9 H, 3 × OAc). Major anomer ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9, 169.7, 168.5 (3 × O*C*(O)Me); 167.8 (C-6), 166.2, 165.1 (2 x C(O)-Ph), 143.0 (C=NPh), 133.8, 133.4, 130.1, 129.9, 129.7, 128.9, 128.8, 128.7, 128.6, 124.7, 119.4 (Ar), 115.8 (q, J_{C-F} 286 Hz, (CF_3)), 98.8 (C-1'), 94.3 (C-1), 74.9 (C-5), 74.8 (C-4), 71.8 (C-3), 70.7 (C-2), 70.5 (C-3'), 68.8 (C-5'), 68.6 (C-4'), 62.0 (C-6'), 61.0 (C-2'), 53.2 (O*C*OMe), 20.75, 20.72, 20.72 (3 × O*C*(O)Me).

4'-Methylumbelliferyl (3,4-di-*O*-acetyl-2-azido-6-*O*-benzoyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosiduronate (11)

A mixture of dry donor **10** (983 mg, 1.09 mmol), 7-trimethylsilyl-4-methylumbelliferone[2] (541 mg, 2.18 mmol, 2 eq), and freshly dried mol sieves (AW300, acid washed) was stirred in dry DCE (15 mL) in the dark for 2h at r.t. The suspension was cooled to -25 °C and a stock-solution of $BF_3.OEt_2$ in dry DCE (0.030 mL/mL, 1.35 mL, 0.3 eq) was added dropwise over 10 min, stirred for further 15 min and then at 0 °C for 30 min and 1h at r.t. This sequence was repeated twice by addition of $BF_3.OEt_2$ stock (2nd addition: 0.35 mL, 0.08 eq, 3rd addition 0.467 mL, 0.1 eq) until TLC indicated nearly complete conversion of donor **10**. The mixture was neutralised to pH7 by addition of Et_3N (0.15 mL) at 0 °C. After centrifugation and washing of the residual mol sieves with $CHCl_3$ (6 × 20 mL), the combined organic phases were washed with ice-cold aqueous HCl (0.1 M, 3 × 50 mL), re-extracted with $CHCl_3$ (2 × 30 mL), washed with brine (50 mL), dried ($MgSO_4$) and concentrated to give an off white foam. The crude mixture was acetylated (residual 4-MU and hemiacetal were acetylated, which facilitated purification later; contained around 1.1 mmol hemiacetal and 4-MU) by dissolving in dry pyridine (5 mL) containing a cat. amount of DMAP, followed by dropwise addition of Ac_2O (0.5 mL, 5 eq) at 0 °C and sonicated at r.t. for 90 min. The reaction was quenched by dropwise addition of dry MeOH (15 mL) at 0 °C and stirring was continued at r.t. for 70 min. After co-evaporation with toluene (4 × 25 mL) the crude product was purified on silica gel (Gradient: Toluene/EtOAc 4:1→3:1→1:1→EtOAc, all containing 0.5 % Et_3N) to give the glycoside **11** as an off-white foam (739 mg, 76%), R_f = 0.28 (Toluene/EtOAc, 3:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00-8.08 (m, 4 H, Ar-Bz), 7.55-7.60 (m, 2 H, Ar-Bz), 7.41-7.50 (m, 5 H, Ar-Bz, H5'), 6.95 (d, 1 H, $J_{6',8'}$ = 2.4 Hz, H8'), 6.90 (dd, 1 H, $J_{5',6'}$ = 8.8 Hz, H6'), 6.19 (1 H, $J_{3', Me'}$ = 1.3 Hz, H3'), 5.63 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 8.9 Hz, H3), 5.39-5.47 (m, 3 H, H1,2,3"), 5.29 (d, 1 H, $J_{1",2"}$ = 3.6Hz, H1"), 5.18 (dd, 1 H, $J_{3",4"}$ = 9.4 Hz, $J_{4",5"}$ 10.3 Hz, H4"), 4.55 (dd, A part of ABX, 1 H, $J_{5",6"a,}$ = 2.3 Hz, $J_{6"a,6"b}$ = -12.5 Hz, H6"a), 4.52 (dd, 1 H, H4), 4.36 (dd, B part of ABX, 1 H, $J_{5",6"b}$ 3.3 Hz, H6"b), 4.35 (d, 1H, $J_{4,5}$ = 8.8 Hz, H5), 4.06 (ddd, 1 H, H5"), 3.47 (dd, 1 H, $J_{2",3"}$ = 10.7 Hz, H2"), 3.73(s, 3H, CO_2Me), 2.39 (d, 3 H, Me'), 2.08, 2.04, 2.03 (3 × s, 9 H, 3 × OAc); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.0, 169.7, 169.6 (3 × O*C*(O)Me, 167.6 (C-6), 166.2, 165.3 (2 × C(O)-Ph), 160.9 (C-2'), 159.1 (C-7'), 154.9 (4-MU Fused Ring C = C-8a'), 152.2 (C-4'), 133.8, 133.4, 130.1, 129.9, 129.7, 128.9, 128.8, 128.6 (Ar-Bz), 125.9 (C-5'), 115.8 (Fused ring C = C-4a'), 113.9 (C-6'), 113.4 (C-3'), 104.6 (C-6'), 99.0 (C-1), 98.8 (C-1"), 75.3 (C-4), 74.6 (C-5), 72.8 (C-3), 72.1 (C-2), 70.4 (C-3"), 68.8 (C-5"), 68.6 (C-4"), 61.9 (C-6"), 61.1 (C-2"), 53.2 (CO_2*Me*), 20.79, 20.76, 20.76 (3 × OC(O)*Me*), 18.8 (CH_3'). HRMS: *m/z* calcd for $C_{43}H_{41}N_3O_{18}$ $[M+H]^+$: 888.24634; found: 888.24960.

4'-Methylumbelliferyl (2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl β-D-glucopyranosiduronate (12)

The dried disaccharide **11** (658 mg, 0.741 mmol) was dissolved in dry MeOH (30 mL) and a solution of dry KCN in dry MeOH (24 mg/0.125 mL, 0.37 mmol, 0.5 eq) was added dropwise and stirred for 4h at r.t. More KCN-stock (24 mg/0.125 mL, 0.37 mmol, 0.5 eq), was added dropwise and the reaction was

left stirring o/n at 0 °C. The solution was concentrated in vacuo at 0 °C and the crude product was first purified on silica gel ($CHCl_3$/MeOH/water, 23:5:0.5→MeOH/water, 95:1) to remove KCN, followed by co-evaporation with toluene/MeOH 1:1 at r.t. The crude product was dissolved in isopropanol/water 5:95 and subjected to a second purification on an SPE-cartridge (C-18, 10g, 40 mL) and eluted with isopropanol/water 5:95→15:85→25:75 →35:65→40:60 to give pure deprotected disaccharide **12** as a white solid (278 mg, 68 %); R_f = 0.3 ($CHCl_3$/MeOH/water, 23:5:0.5). ^{1}H NMR (500 MHz, CD_3OD): δ 7.70 (d, 1 H, $J_{5',6'}$ = 9.0 Hz, H5'), 7.07 (dd, 1 H, H6'), 7.02 (d, 1 H, $J_{6',8'}$ = 2.5 Hz, H8'), 6.20 (1 H, $J_{3',\,Me'}$ = 1.3 Hz, H3'), 5.66 (d, 1 H, $J_{1'',2''}$ = 3.6 Hz, H1"), 5.20 (d, 1 H, $J_{1,2}$ = 7.7 Hz H1), 4.31 (d, 1H, $J_{4,5}$ = 9.6 Hz, H5), 3.99 (dd, 1 H, $J_{3,4}$ = 8.9 Hz, H4), 3.85 (dd, 1 H, $J_{2,3}$ = 9.2 Hz, H3), 3.79 (s, 3H, CO_2Me), 3.72-3.78 (m, 3 H, H3", H6"a, H6"b), 3.55 (dd, 1 H, H2), 3.43 (dd, 1 H, $J_{3'',4'',}$ = 8.4 Hz, $J_{4'',5''}$ = 10.0 Hz, H4"), 3.40 (ddd, 1 H, H5"), 3.13 (dd, 1 H, $J_{2'',3''}$ = 10.4 Hz, H2"), 2.45 (d, 3 H, Me'); ^{13}C NMR (125 MHz, CD_3OD): δ 170.4 (GlcA C-6), 163.2 (C-2'), 161.5 (C-7'), 156.0 (4-MU Fused Ring C = C-8a'), 155.4 (4-MU Fused Ring C = C-4'), 127.4 (C-5'), 116.3 (C-4a'), 114.8 (C-6'), 113.1 (C-3'), 105.0 (C-6'), 101.4 (C-1), 99.4 (C-1"), 77.6 (C-3), 77.1 (C-4), 75.5(C-5), 74.6 (C-2), 74.1 (C-5"), 72.3 (C-3"), 71.4 (C-4"), 64.8 (C-2"), 61.8 (C-6"), 53.3 (CO_2Me), 18.6 (CH_3'). HRMS: *m/z* calcd for $C_{23}H_{27}N_3O_{13}$ $[M+Na]^+$: 576.14416; found: 576.14616.

4'-Methylumbelliferyl (2-azido-6-*O*-trichloroethylsulfo-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl β-D-glucopyranosiduronate (13)

To a solution of dry polyol **12** (73 mg, 0.132 mmol) in dry THF (2 mL) was added dropwise a stock-solution of freshly distilled 1,2-dimethyl imidazole in dry THF (c = 38 mg/mL, 1.54 mL, 0.62 mmol, 5 eq) over 10 min at 0 °C. The solution was cooled to -78 °C and 2,2,2-trichloroethoxysulfuryl 2-methyl 3-methylimidazolium triflate[3] (58 mg, 0.132 mmol, 1 eq) was added and stirred for 15 min and then o/n at 0 °C. This exact procedure was repeated twice and after 3 days the reaction was quenched by addition of dry MeOH (2 mL) at 0 °C and 4h at r.t. The mixture was co-evaporated with anhydrous toluene (2 × 20 mL) and the residue was purified on neutral Al_2O_3 (Gradient: $CHCl_3$/MeOH/water, 32:5:0.5→30:5:0.5→23:5:0.5→MeOH/water, 95:1) to give two major fractions A and B, which were each neutralised with strongly acidic ion-exchange resin (Amberlite IR-120, H^+-form) to pH 6, filtered, washed with MeOH (5 × 2 mL) and co-evaporated with anhydrous toluene. The more unpolar fraction A gave pure trichloroethylsulfate **13** (39 mg, 39 %) as white solid (R_f = 0.54, $CHCl_3$/MeOH/water, 23:5:0.5) and the later eluting fraction B gave recovered starting polyol **12** (38mg, 52% recovered), R_f = 0.26, ($CHCl_3$/MeOH/water, 23:5:0.5). ^{1}H NMR (500 MHz, CD_3OD): δ 7.72 (d, 1 H, $J_{5',6'}$ = 8.9 Hz, H-5'), 7.09 (dd, 1 H, H-6'), 7.04 (d, 1 H, $J_{6',8'}$ = 2.4 Hz, H-8'), 6.22 (1 H, $J_{3',\,Me'}$ = 1.2 Hz, H-3'), 5.72 (d, 1 H, $J_{1'',2''}$ = 3.6 Hz, H1"), 5.21 (d, 1 H, $J_{1,2}$ = 7.7 Hz, H1), 4.95 (s, 2 H, CH_2CCl$_3$), 4.54-4.61 (m, 2 H, H-6"a, H-6"b), 4.32 (d, 1 H, $J_{4,5}$ = 9.6 Hz, H5), 3.99 (dd, 1 H, $J_{3,4}$ = 9.0 Hz, H4), 3.86 (dd, 1 H, $J_{2,3}$ = 9.2 Hz H3), 3.80 (s, 3H, CO_2Me), 3.76 (dd, $J_{3'',4''}$ =8.8 Hz, H3"), 3.67 (ddd, 1 H, $J_{4'',5''}$ = 10.0 Hz, H5"), 3.55 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H2), 3.39 (dd, 1 H, H-4"), 3.20 (dd, 1 H, $J_{2'',3''}$ 10.5 Hz, H2"), 2.46 (d, 3 H, Me'). ^{13}C NMR (125 MHz, CD_3OD): δ 170.4 (C-6), 163.2 (C-2'), 161.5 (C-7'), 156.0 (4-MU Fused Ring C = C-8a'), 155.4 (C-4'), 127.4 (C-5'), 116.4 (4-MU Fused Ring C = C-4a'), 114.8 (C-6'), 113.1 (C-3'), 104.9 (C-8'), 101.4 (C-1), 99.5 (C-1"), 94.2 (CCl_3), 80.9 (CH_2CCl_3), 77.6 (C-3), 77.4 (C-4), 75.3 (C-5), 74.6 (C-2), 73.6 (C-6"), 72.3 (C-3"), 71.5 (C-5"), 71.0 (C-4"), 64.5 (C-2"), 53.5 (CO_2Me), 18.6 (CH_3'). HRMS: *m/z* calcd for $C_{25}H_{28}Cl_3N_3O_{16}S$ $[M+H]^+$: 764.03342; found: 764.03517.

4'-Methylumbelliferyl (2-deoxy-6-*O*-sulfo-2-sulfonamido-α-D-glucopyranosyl)-(1→4)-sodium β-D-glucopyranosiduronate, sodium salt (1)

(a) Dry disaccharide **13** (39 mg, 51 μmol) was suspended in MeOH (HPLC-grade, 4.5 mL) and stirred vigorously with NH_4HCO_3 (40 mg, 51 μmol) and freshly activated Zn powder (140 mg, 2.15 mmol) in the dark at r.t. for 48h. The same amounts of reagents were added again and stirring continued for 16 h. ES-MS showed no significant progress after the second addition (77% fully deprotected product and 23% azide intermediate). The suspension was centrifuged, the precipitate was washed under sonication with anhydrous MeOH (8 × 4 mL), centrifuged and the supernatant enriched in more unpolar azide was concentrated *in vacuo* (= MeOH-extract) yielding a white precipitate (11 mg, 44% product and 56% azide by ES-MS). The remaining precipitate was washed with water under sonication and centrifuged (4 × 3 mL) and the supernatant was concentrated *in vacuo* (= aqueous extract) at r.t. to afford crude product as white solid (17 mg). ES-MS analyses of the aqueous extract showed the 91% product and 9% azide. The dried MeOH-extract (11 mg, 44% product and 56% azide) was dissolved in MeOH (HPLC-grade, 1.5 mL), NH_4Cl (15 mg, 1.33 mmol) and freshly activated Zn powder (29 mg, 0.45 mmol) were added and the mixture was sonicated for 1h. ES-MS analyses showed 85% product and still 15% azide. The suspension was diluted with MeOH (50%, v/v, 5 mL), Zn precipitate was centrifuged and washed with 50% MeOH (2 × 3 mL) and water (3 × 3 mL) under sonication, supernatants were combined and concentrated *in vacuo* to afford white solid (22 mg = MeOH-precipitate). (b) Both crude reduction products (MeOH-extract and MeOH precipitate) were combined (~0.043 mmol product, 90 %) dissolved MeOH (HPLC-grade, 1.1 mL) and water (0.24 mL) cooled to 0 °C and an aqueous solution of LiOH (1M, ~0.5 mL) was added dropwise to reach pH 12.5-13.0 and sonicated at 0 °C slowly warming to r.t. After 4.5 h ES-MS-analyses and TLC-analyses (CH_3CN/water, 85:15) indicated the presence of 95% the desired carboxylate. The solution was neutralised at 0 °C by addition of ice-cold aqueous HCl (0.01 M) to pH 8.5, followed by concentration *in vacuo* at r.t. to afford crude carboxylate as a white solid. (c) Dry crude carboxylate (~0.043 mmol) was dissolved in HPLC-grade water (4.3 mL), vigorously stirred followed by addition of Na_2CO_3 (62 mg, 0.58 mmol) and final addition of freshly washed and dried SO_3-pyridine (41 mg, 0.258 mmol, 6 eq) (pH 8.5) and stirring continued o/n. After addition of Na_2CO_3 (51 mg, 480 μmol) and SO_3-pyridine (34 mg, 215 μmol, 5 eq) (pH 8.5) the solution was further stirred for 24h until ES-MS analyses showed the disappearance of the mass of the starting material and showing mainly the mass of final product **1**. The slightly turbid solution was centrifuged, the precipitate was washed with water under sonication (4 × 5 ml) and centrifuged. The combined supernatant was co-evaporated with HPLC-grade water (3 × 7 mL) and dried over P_2O_5 under vacuum to obtain crude sulfated product **1** as an off-white solid. The product was three times purified on a Sephadex G-25 column (water) and pure salt-free **1** was obtained as a white powder (16 mg, 42 % over three steps), R_f = 0.40 (CH_3CN/water, 85:15). ^{1}H NMR (500 MHz, D_2O): δ 7.65 (d, 1 H, $J_{5',6'}$ = 8.9 Hz, H-5'), 7.08 (dd, 1 H, $J_{6',8'}$ 2.5 Hz, H-6'), 7.04 (d, 1 H, H-8'), 6.20 (1 H, $J_{3', Me'}$ = 1.2 Hz, H-3'), 5.71 (d, 1 H, $J_{1'',2''}$ = 3.7 Hz, H1''), 5.27 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H1), 4.38 (dd, A part of ABX, 1 H, $J_{5'',6''a}$ 2.4 Hz, $J_{6''a,6''b}$ = -11.1 Hz, H6''a), 4.20 (dd, B part of ABX, 1 H, $J_{5'',6''b}$ = 2.1 Hz, H6''b), 4.05 (d, 1 H, $J_{4,5}$ = 9.6 Hz, H5), 4.02 (dd, 1 H, H3), 3.89-3.97 (m, 2 H, H4, H5''), 3.73 (dd, 1 H, $J_{2,3}$ =9.4 Hz, H2), 3.65 (dd, 1 H, H3''), 3.61 (dd, 1 H, $J_{3'',4''}$ = $J_{4'',5''}$ = 9.2 Hz, H4''), 3.31 (dd, 1 H, $J_{2'',3''}$ = 10.1 Hz, H2''), 2.40 (d, 3 H, Me'). ^{13}C NMR (125 MHz, D_2O + 2 drops CD_3OD): δ 175.4 (C-6), 156.5 (C-2'), 160.3 (C-7'), 157.2 (4-MU Fused Ring C = C-8a'), 154.7 (C-4'), 127.6 (C-5'), 116.2 (4-MU Fused Ring C = C-4a'), 114.8 (C-6'), 112.2 (C-3'), 104.5 (C-8'), 100.3 (C-1), 98.3 (C-1''), 77.6 (C-5), 77.0 (C-3), 76.9 (C-4), 73.4 (C-2), 72.0 (C-3''), 70.7 (C-4''), 69.9 (C-5''), 67.2 (C-6''), 58.9 (C-2''), 18.9 (CH_3'). HRMS: *m/z* calcd for $C_{22}H_{24}NO_{19}S_2Na_3$ [M-3Na+H]$^{2-}$: 335.52312; found: 335.52377.

HPSE production, crystallization and co-crystal complex formation

Expression, purification and crystallization of recombinant HPSE was performed as previously described[4]. Co-crystal complexes with **1** were generated by soaking unligand HPSE crystals in cryoprotectant solution supplemented with ligand (0.1 M MES pH 5.5, 0.1 M $MgCl_2$, 17% PEG3350, 25% ethylene glycol, 5 mM **1**). Crystals were soaked for 20–40 min, then harvested and directly flash cooled in liquid N_2 for data collection.

Crystal structure solution and refinement

Data for the HPSE-**1** complex were collected at the Diamond Light Source (Harwell, UK). Given the anisotropic nature of the diffraction (**Figure S3**), data were processed using the AutoPROC STARANISO pipeline (Global Phasing Ltd.)[5], which calculates anisotropic resolution limits based on a locally averaged mean $I/\sigma(I)$ cutoff – 1.2 for our data. Detailed crystallographic statistics, including resolution limits along the axes of the STARANISO generated ellipsoid are given in **Table S1**.

The HPSE-**1** complex was solved by directly refining against the unliganded HPSE crystal structure (PDB accession 5E8M) using REFMAC[6]. The resulting solution showed clear difference density for a bound ligand within the enzyme active site. The model was improved by alternating rounds of manual model building and refinement using COOT[7] and REFMAC respectively. Ligand coordinates and dictionaries were generated using JLIGAND[8]. All figures were generated using Pymol.

Initial hydrolysis timecourses

Initial timecourse experiments were carried out in 50 μL volumes of assay buffer (20 mM NaOAc pH 5.0, 150 mM NaCl, 0.1% w/v BSA), containing 500 μM substrate (**1** or 4MU-GlcA (Sigma)) and either 500 nM HPSE or a no enzyme control. Reactions were incubated at 37 °C. At set timepoints, 10 μL reaction volumes were removed and immediately quenched with 10 μL 1 M Na_2CO_3 pH 10.3. Following completion of the timecourse, quenched reaction mixtures were transferred to a black 384 well plate, and 4MU fluorescence measured with a Clariostar microplate reader (BMG Labtech) using the default 4MU fluorophore profile (λ_{Ex}= 360 ± 20 nm; λ_{Em}= 450 ± 30 nm). Data were analysed and plotted using Sigmaplot 13.0 (Systat software).

Michaelis Menten kinetics

Conditions for the Michaelis Menten kinetics were optimized following the timecourse experiment. Reactions were carried out in 10 μL volumes of assay buffer, containing 150 nM HPSE and varying amounts of substrate. Reactions were incubated at 37 °C for 2 h, then immediately quenched with 10 μL 1 M Na_2CO_3 pH 10.3. Quenched reaction mixtures were transferred to a black 384 well plate, and 4MU fluorescence measured with a Clariostar microplate reader using the default 4MU fluorophore profile.

Plots of hydrolysis rate (V) vs substrate concentration ([**1**]) were calculated by subtracting background fluorescence from no enzyme control wells, followed by conversion of 4MU fluorescence to product concentration using a 4MU standard curve (**Figure S2**). Hydrolysis rates were converted to enzyme turnover rates (k_{obs}) using the relationship $V = [E]*k_{obs}$. Michaelis Menten parameters were calculated by fitting plots of k_{obs} vs [**1**] using nonlinear regression to the hyperbolic equation $k_{obs} = k_{cat}*[S]/(K_M+[S])$. Data were analyzed and plotted using Sigmaplot 13.0. Specific activity of HPSE against **1** was calculated using a HPSE molecular weight of 52193.24 $g.mol^{-1}$, and the calculated k_{cat} from Michaelis-Menten kinetics (1.19 h^{-1}; **Figure 3a**)

Heparin inhibition kinetics

Inhibition reactions were carried out in 10 μL volumes of assay buffer, containing 150 nM HPSE, 30 μM **1** and varying amounts of heparin (porcine intenstinal; Sigma H3393). Reactions were incubated at 37 °C for 2 h, then immediately quenched with 10 μL 1 M Na_2CO_3 pH 10.3. 15 μL Quenched reaction mixtures were transferred to a black 384 well plate, and 4MU fluorescence measured with a Clariostar microplate reader using the default 4MU fluorophore profile.

Plots of inhibition vs heparin concentration ([Hep]) were calculated by subtracting background fluorescence from no enzyme control wells, before normalizing hydrolysis rates at each inhibitor concentration (V_I) to the hydrolysis rate in the absence of inhibitor (V_0). IC_{50} Inhibition constants were calculated by fitting plots of V_I/V_0 vs [Hep] using nonlinear regression to the 4 parameter logistic curve function V_I/V_0 = min+((max-min)/(1+([Hep]/IC_{50})^(-Hillslope)). K_I was estimated from IC_{50} using the Cheng-Prusoff approximation $K_I=IC_{50}/(1+[S]/K_M)$[9]. Data were analyzed and plotted using Sigmaplot 13.0.

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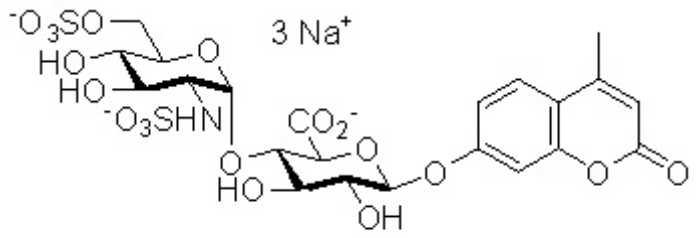

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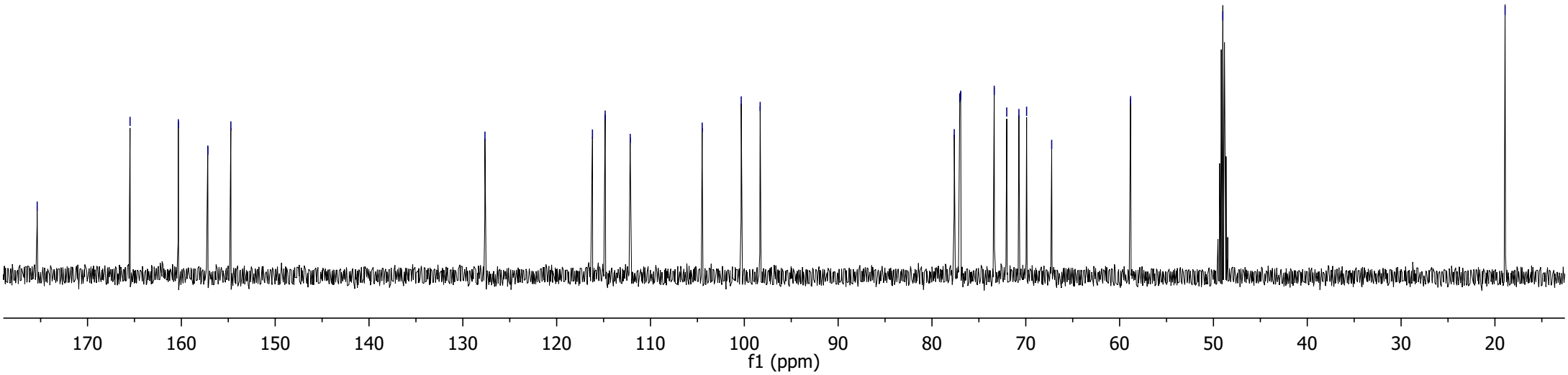

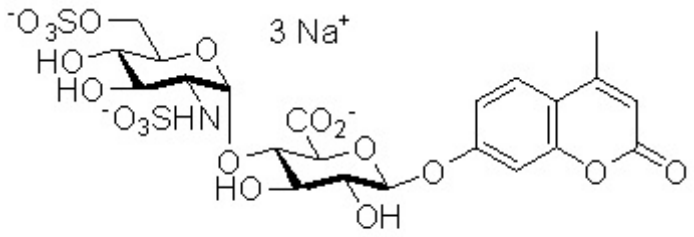
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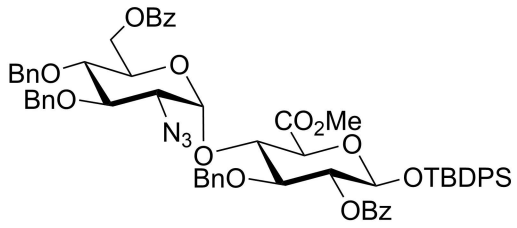
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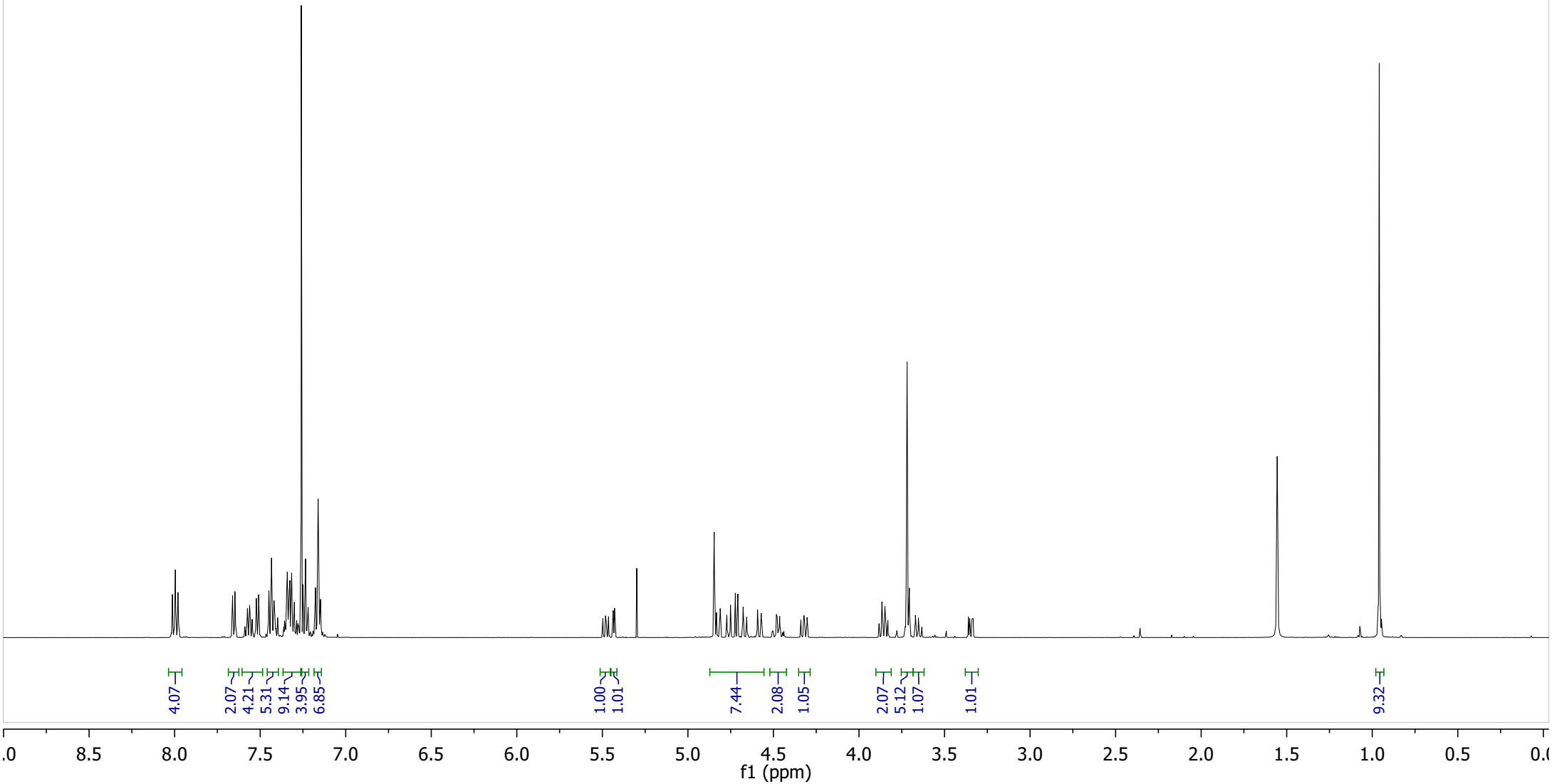
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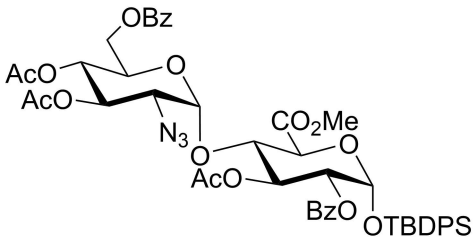
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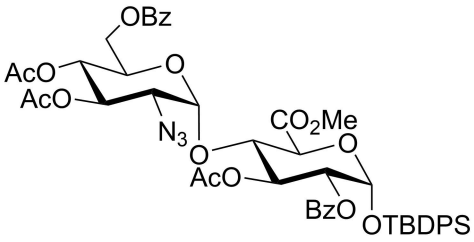
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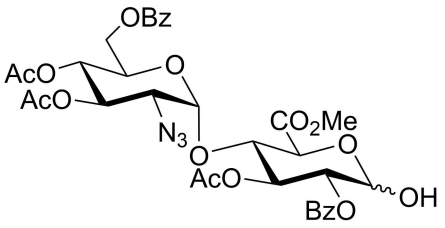
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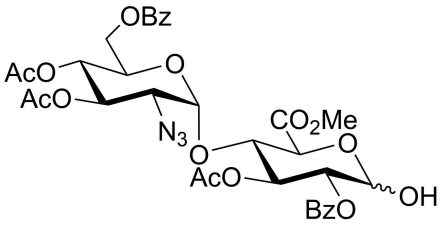
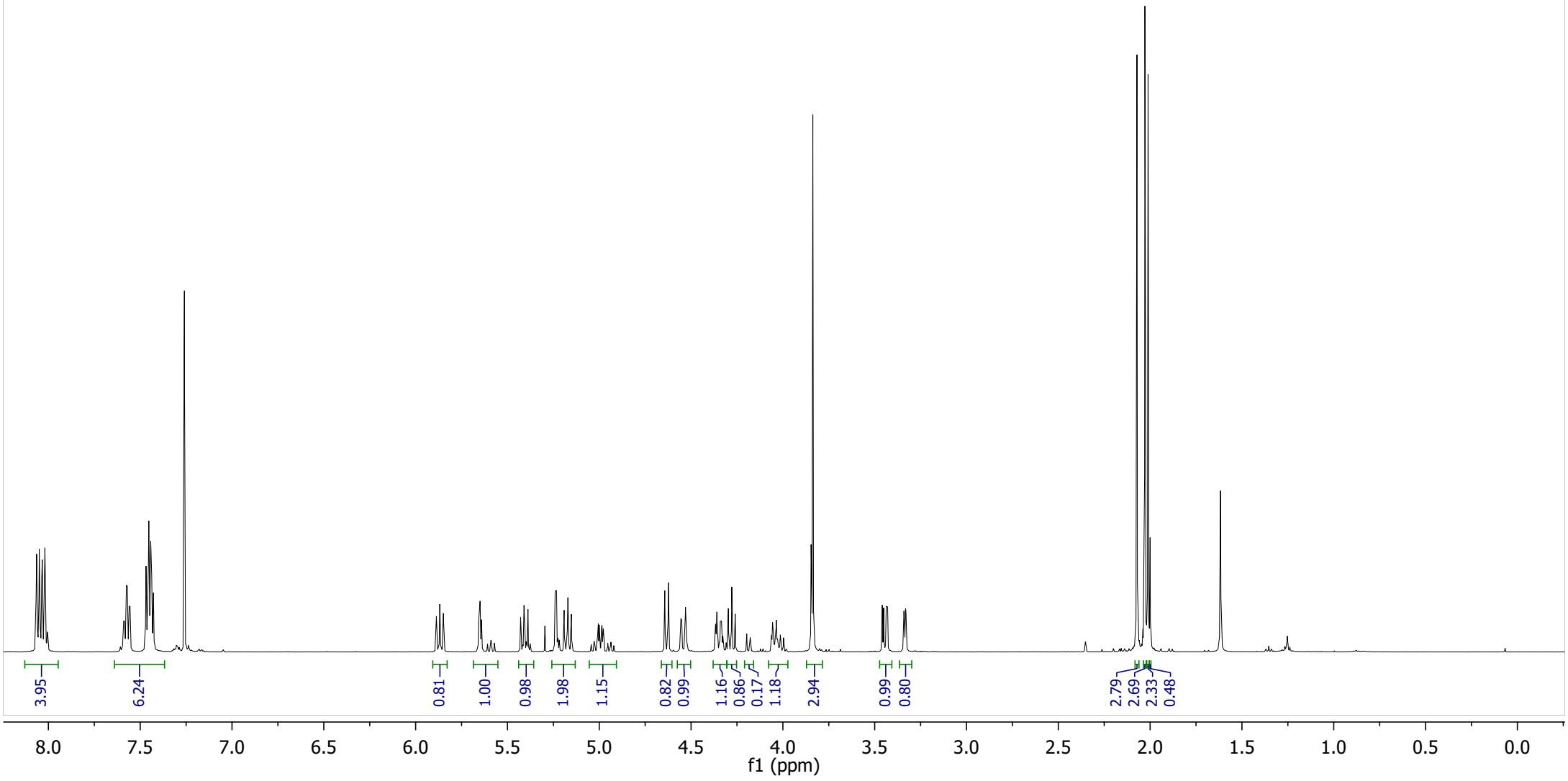
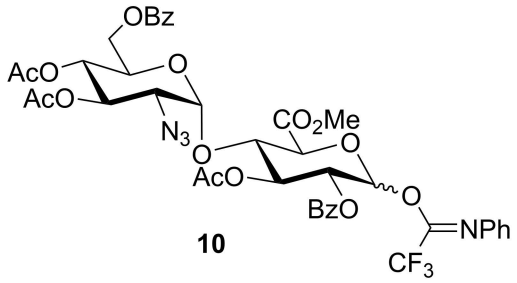
77.16
76.91
76.68
74.13
72.79
71.88
70.50
70.27
68.63
68.58
61.86
61.14
53.24
53.09
20.95
20.81
20.76
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


OBz
AcO
AcO
O
N3
O
CO2Me
O
AcO
BzO
OH

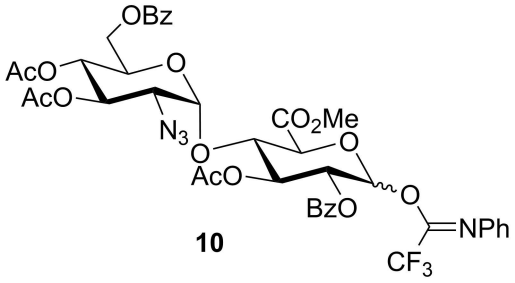
9


3.95
6.24
0.81
1.00
0.98
1.98
1.15
0.82
0.99
1.16
0.86
0.17
1.18
2.94
0.99
0.80
2.79
2.69
2.33
0.48
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
0.5
0.0
f1 (ppm)

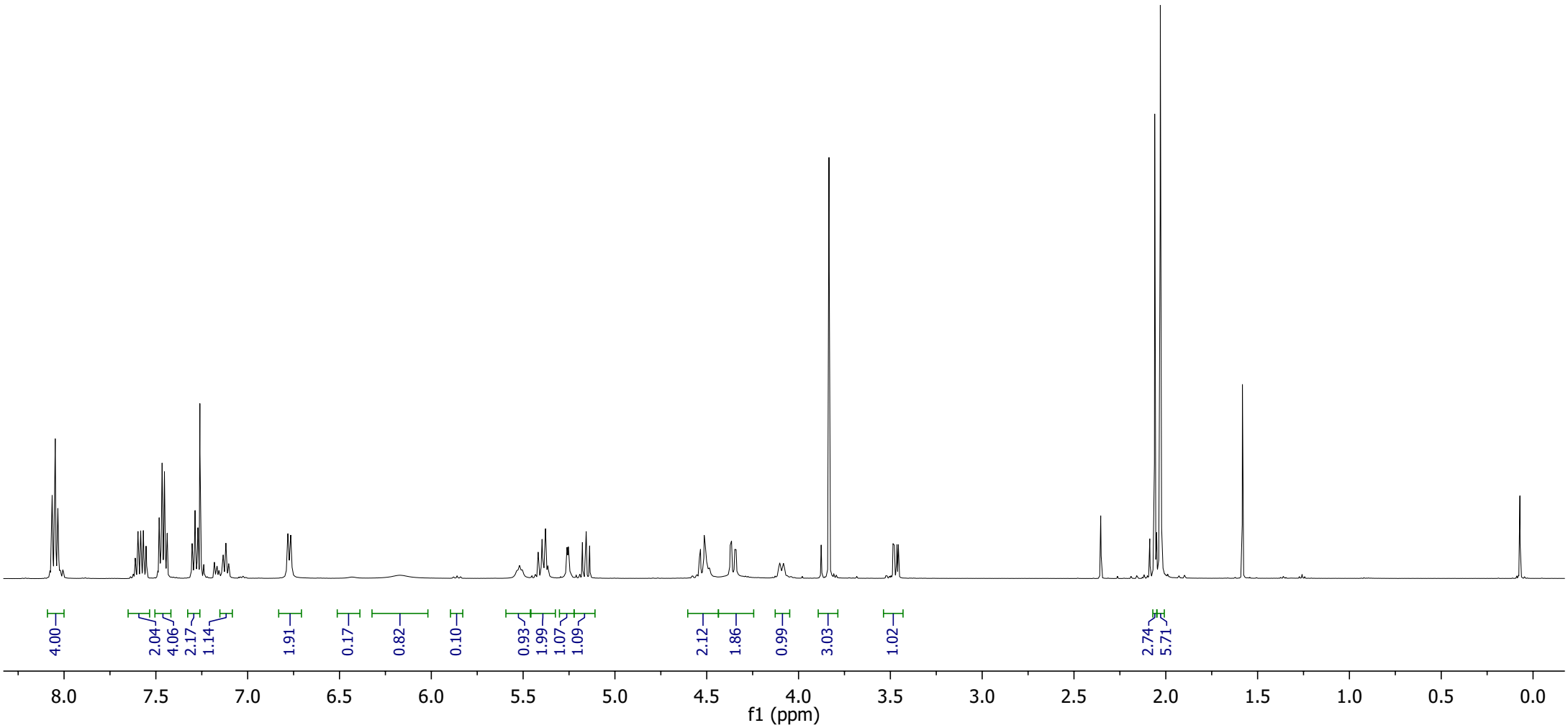


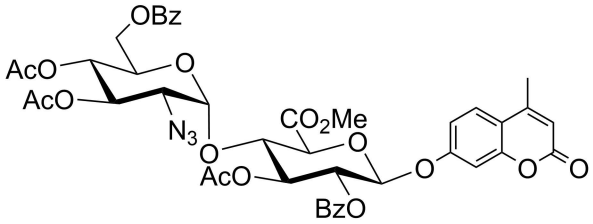
10

169.92
169.68
169.56
167.86
166.24
165.44
165.18
142.98
133.78
133.40
130.15
130.10
129.90
129.70
128.92
128.89
128.85
128.76
128.61
125.43
124.76
119.39
116.95
115.76
99.06
98.86
94.34
74.93
74.78
71.83
70.76
70.55
68.80
68.57
62.01
61.06
53.20
20.78
20.75


OBz
AcO
AcO
O
N3
O
CO2Me
O
AcO
BzO
O
NPh
CF3

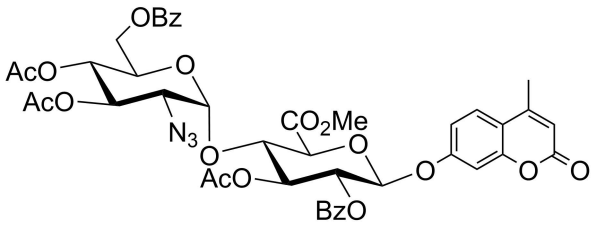
10


4.00
2.04
4.06
2.17
1.14
1.91
0.17
0.82
0.10
0.93
1.99
1.07
1.09
2.12
1.86
0.99
3.03
1.02
2.74
5.71
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
0.5
0.0
f1 (ppm)

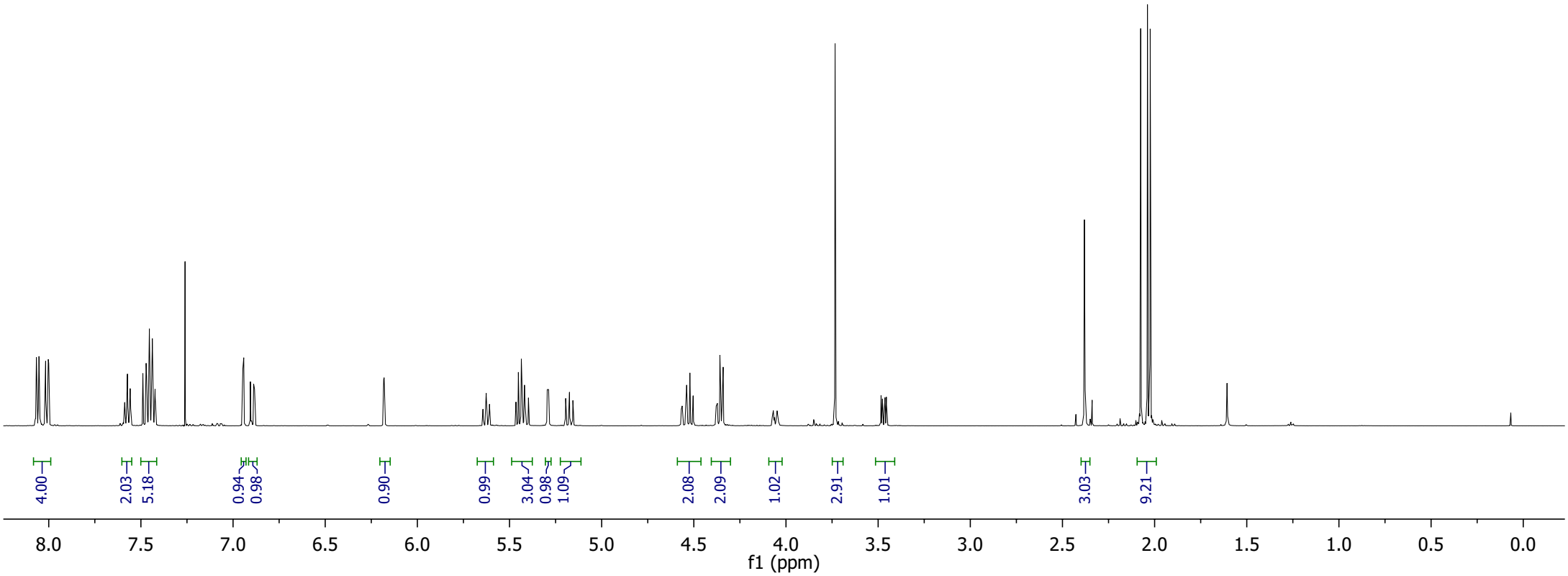

OBz
AcO
AcO
O
N3
O
AcO
CO2Me
O
BzO
O
O
O

11

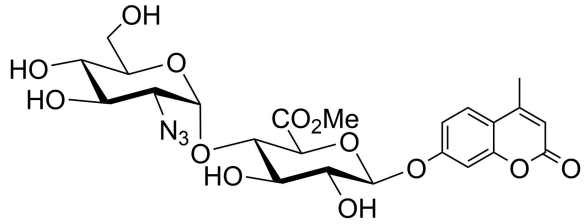
169.96
169.68
169.60
167.63
166.23
165.34
160.91
159.09
154.87
152.22
133.81
133.41
130.06
129.90
129.73
128.86
128.75
128.60
125.86
115.80
113.85
113.43
104.57
98.95
98.83
75.31
74.61
72.78
72.11
70.43
68.79
68.55
61.91
61.10
53.20
20.79
20.76
18.81
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


OBz
AcO
AcO
O
N3
O
CO2Me
O
AcO
BzO
O
O
O

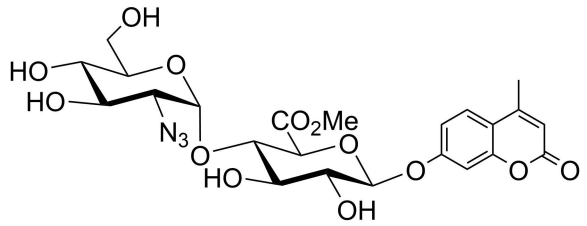
11


4.00
2.03
5.18
0.94
0.98
0.90
0.99
3.04
0.98
1.09
2.08
2.09
1.02
2.91
1.01
3.03
9.21
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
0.5
0.0
f1 (ppm)

170.35
163.22
161.46
155.97
155.39
127.40
116.33
114.79
113.07
104.94
101.36
99.39
77.60
77.14
75.51
74.60
74.07
72.34
71.37
64.79
61.84
53.39
18.63

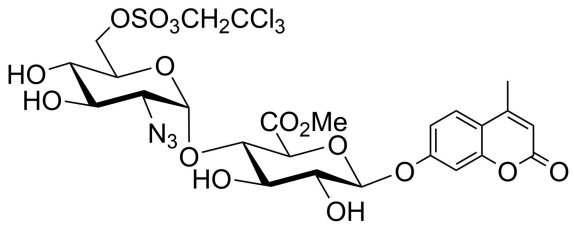


12


OH
HO
HO
O
N3
O
CO2Me
O
HO
OH
O
O
O

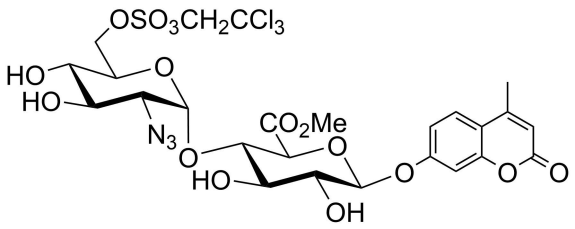
12

1.06
1.98
1.00
1.02
1.00
0.99
1.03
6.17
1.02
1.04
1.05
0.92
1.02
3.27
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)


OSO3CH2CCl3
HO
HO
O
N3
O
CO2Me
O
HO
OH
O
O
O

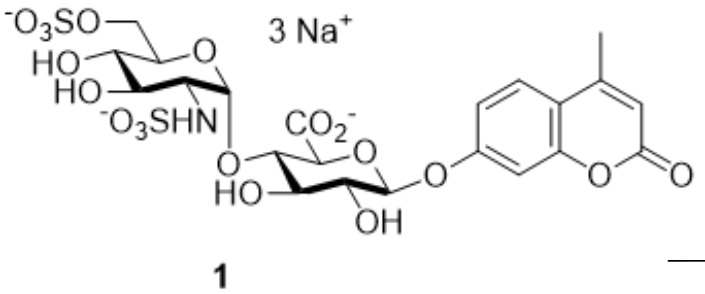
13

170.41
163.24
161.47
156.01
155.41
127.42
116.37
114.84
113.09
104.93
101.41
99.49
94.22
80.87
77.57
77.44
75.35
74.60
73.63
72.33
71.51
70.98
64.53
53.52
18.64
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

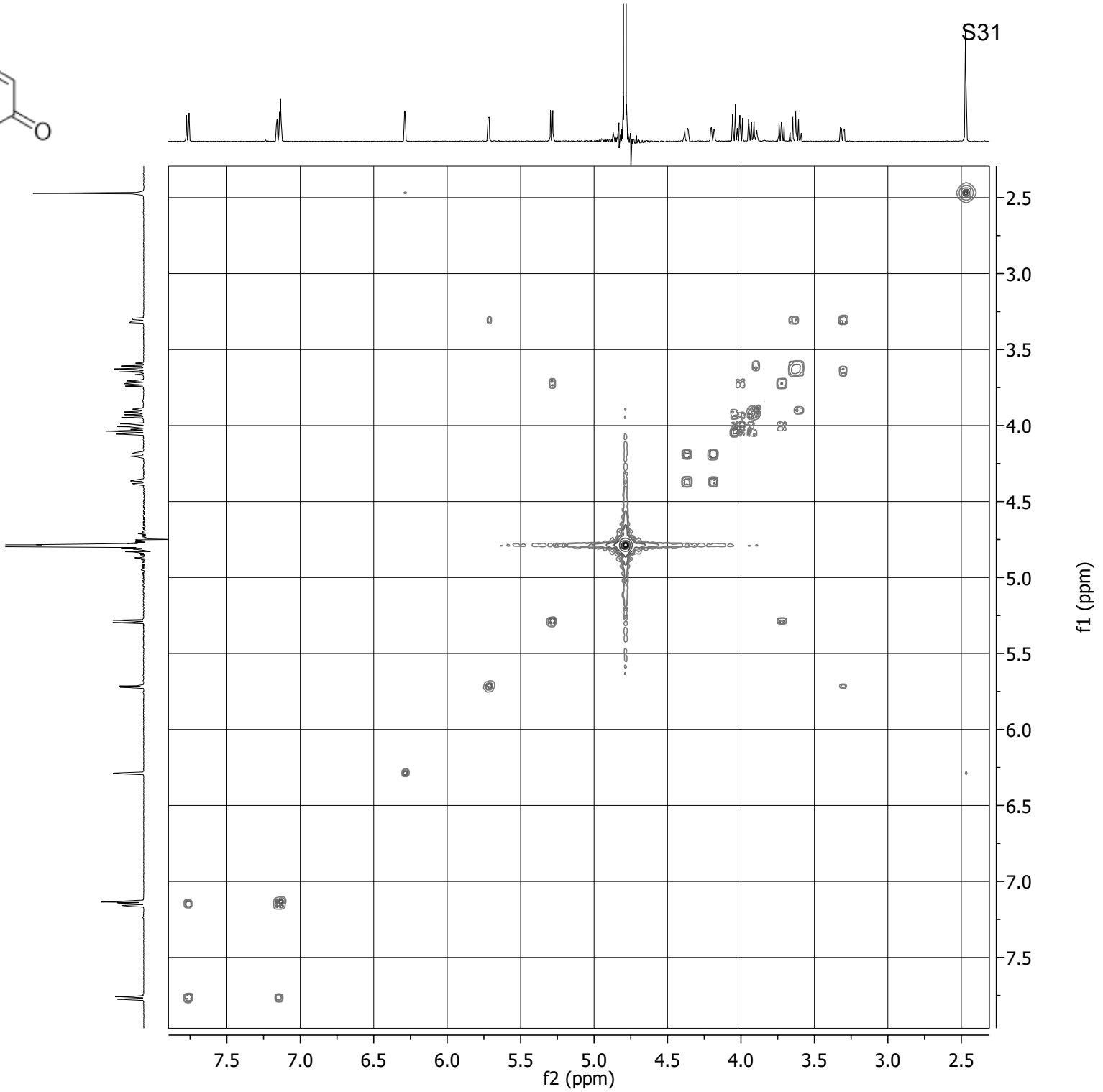

OSO3CH2CCl3
HO
HO
O
N3
O
CO2Me
O
HO
OH
O
O
O

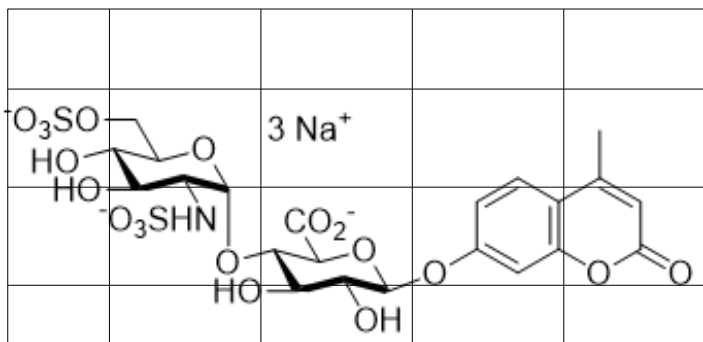
13

1.00
2.00
0.95
0.86
0.98
1.78
1.84
0.93
0.99
1.07
4.10
0.96
1.03
0.93
0.94
3.10
.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0
f1 (ppm)


$^{-}O_3SO$
HO
HO
$^{-}O_3SHN$
3 Na^{+}
CO_2^{-}
O
HO
OH
O
O
O

1

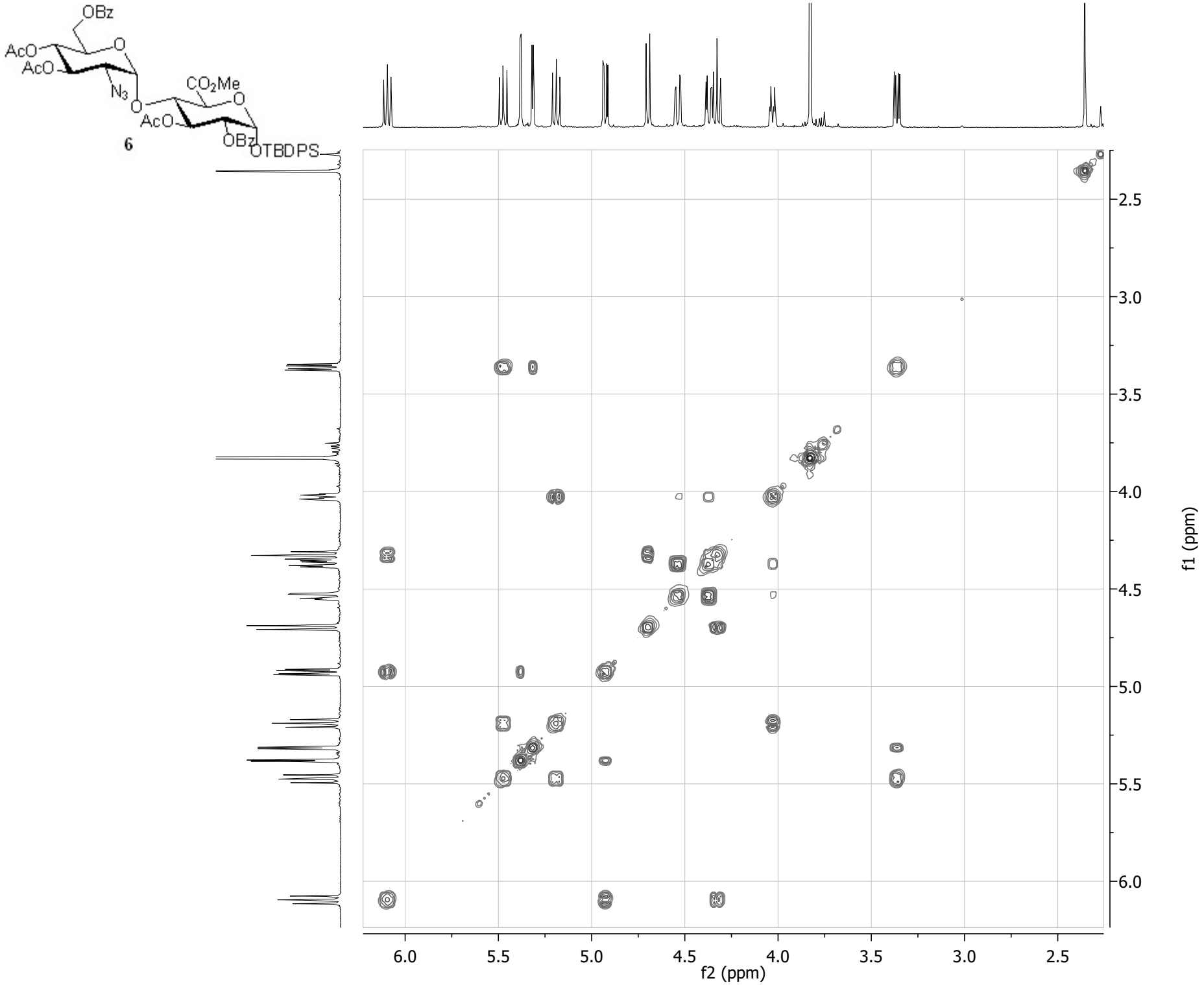

2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f2 (ppm)

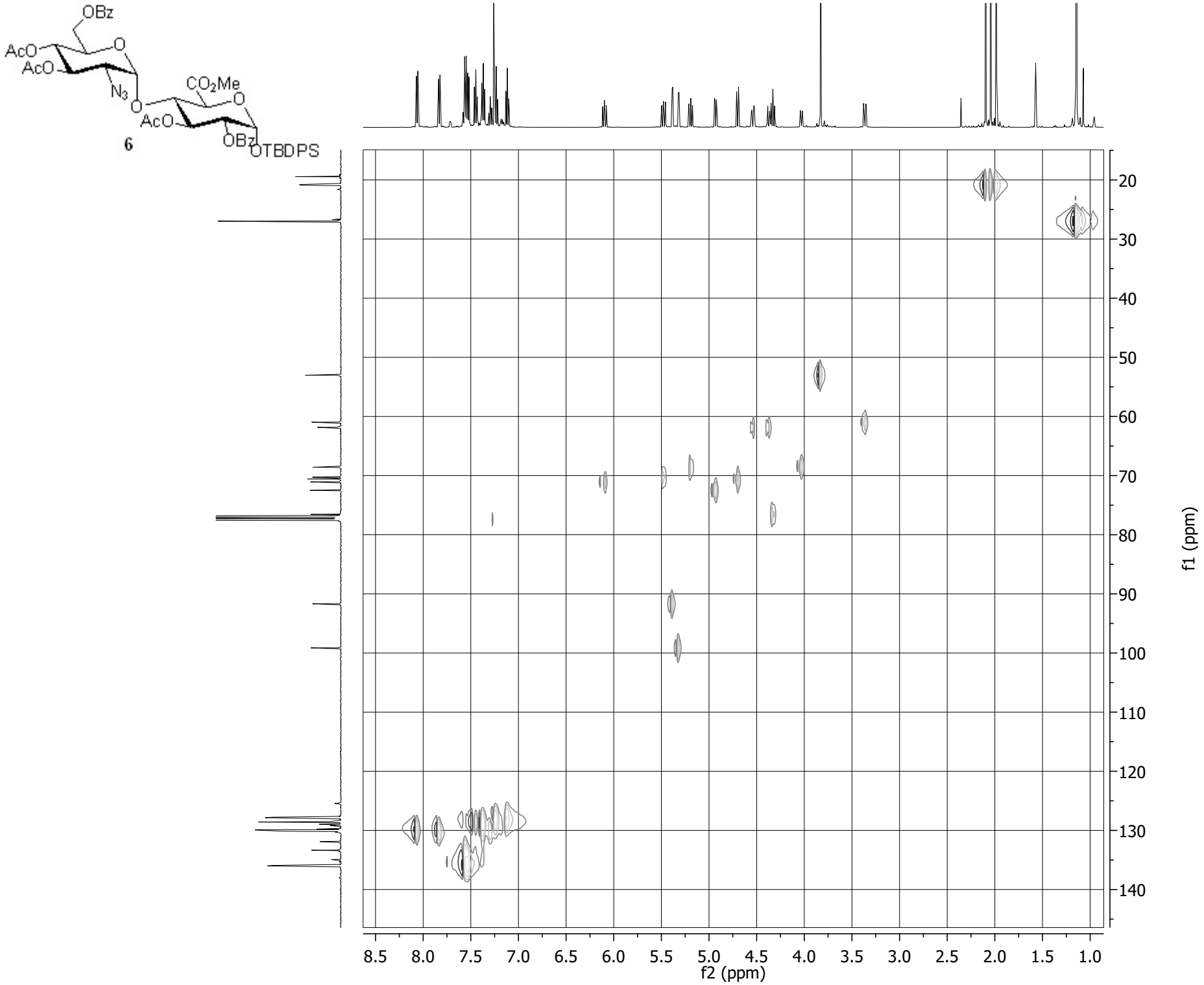


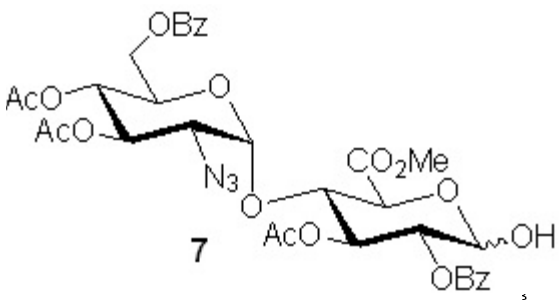
1

f2 (ppm)

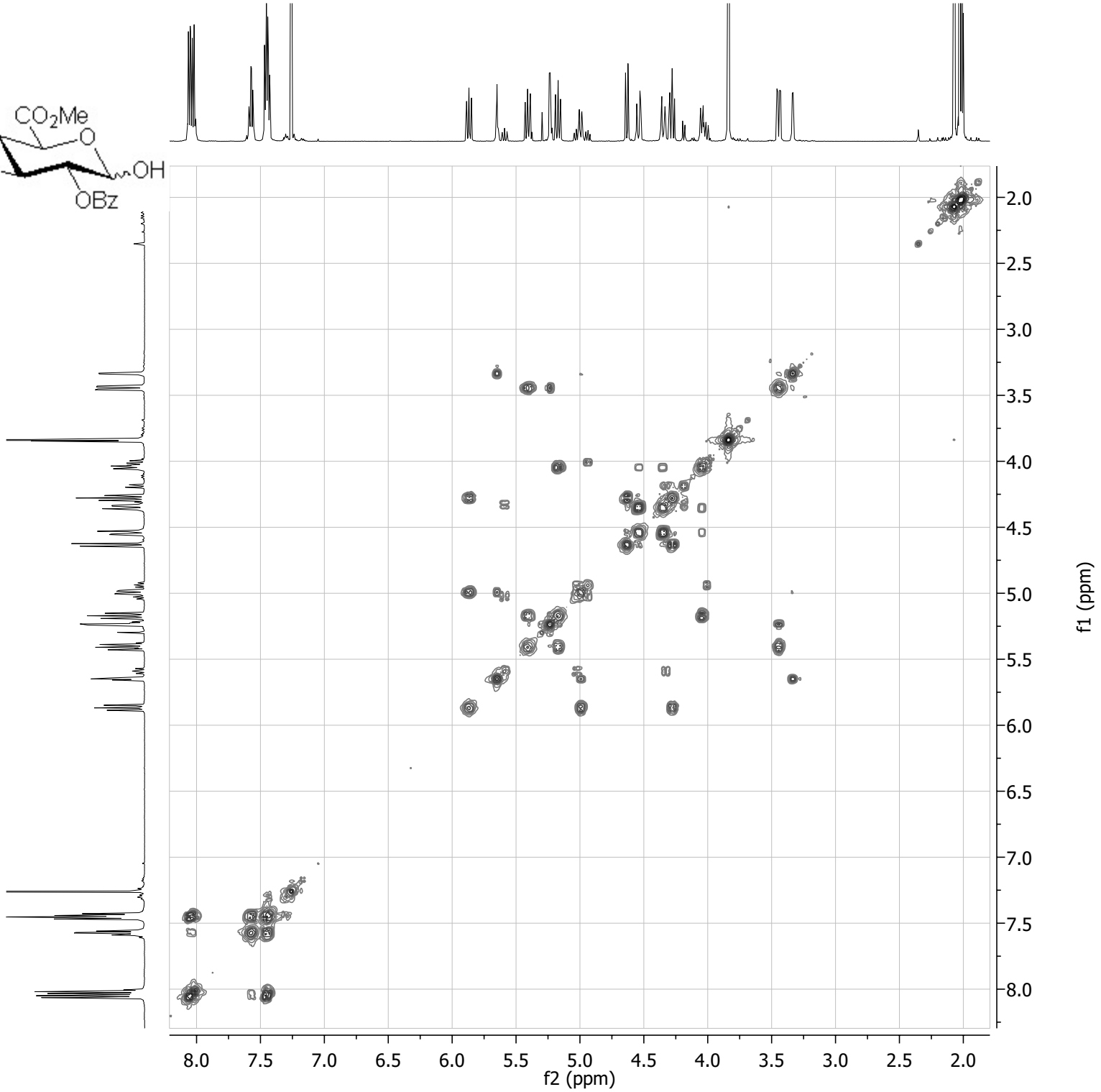
f1 (ppm)

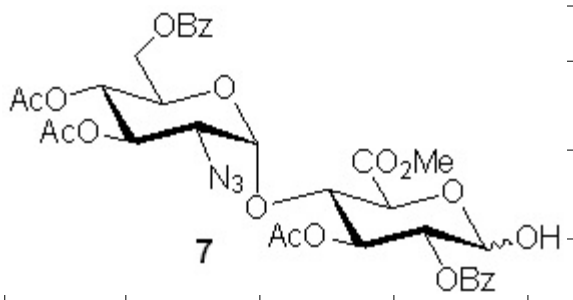

OBz
AcO
AcO
O
N3
O
CO2Me
O
AcO
OBz
OTBDPS
6
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f2 (ppm)
f1 (ppm)


OBz
AcO
AcO
O
N3
O
CO2Me
O
AcO
OBz
OTBDPS
6
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
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)


OBz
AcO
AcO
O
N3
O
CO2Me
O
AcO
OH
OBz

7

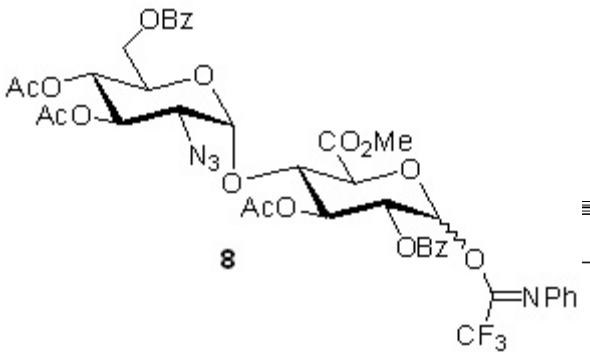

2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)



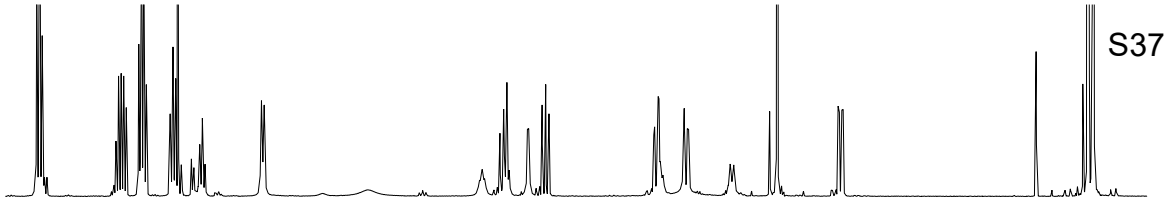
7

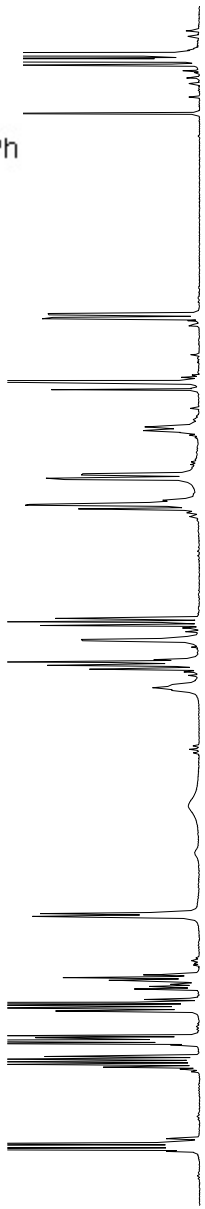
f2 (ppm)

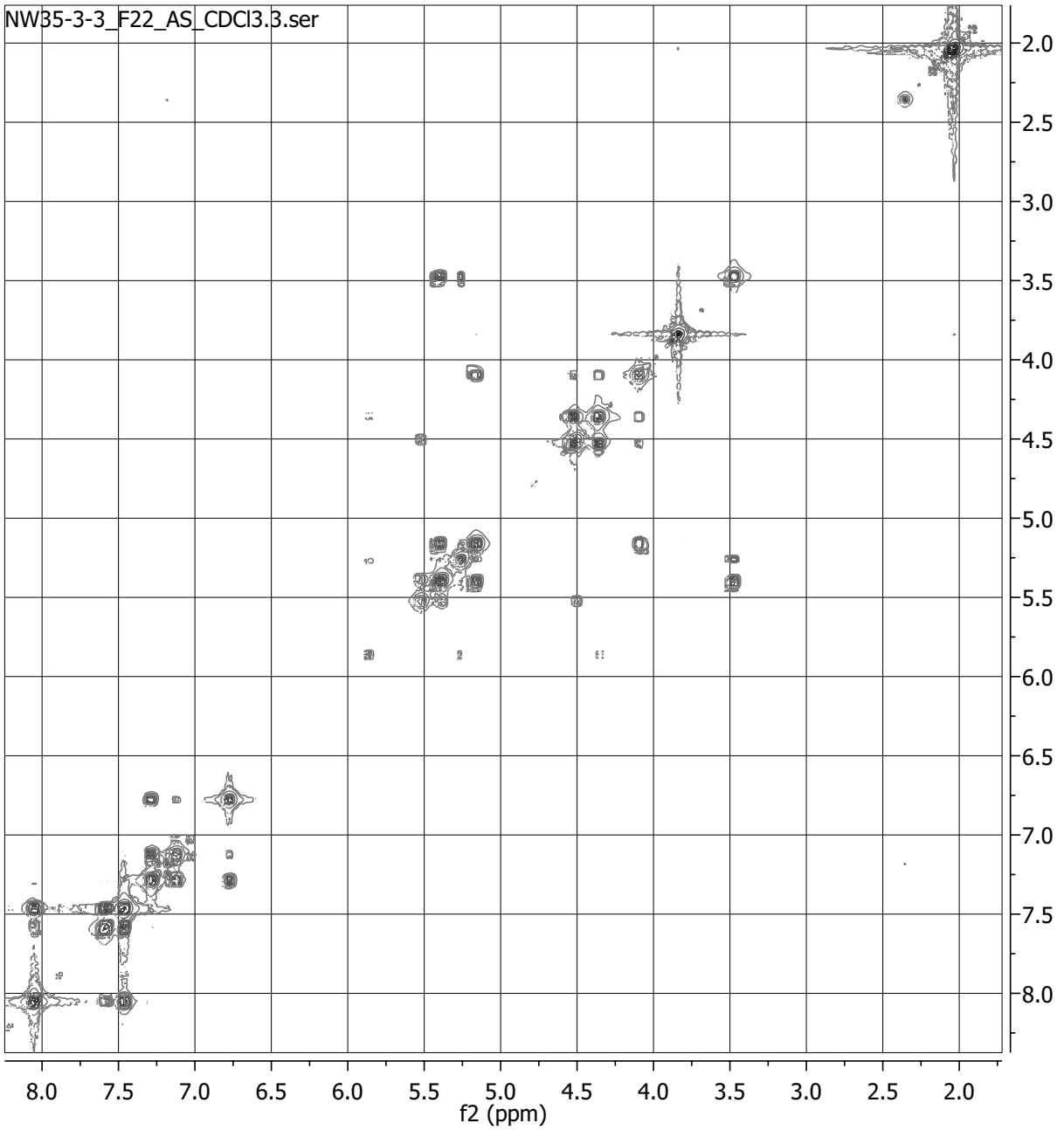
f1 (ppm)

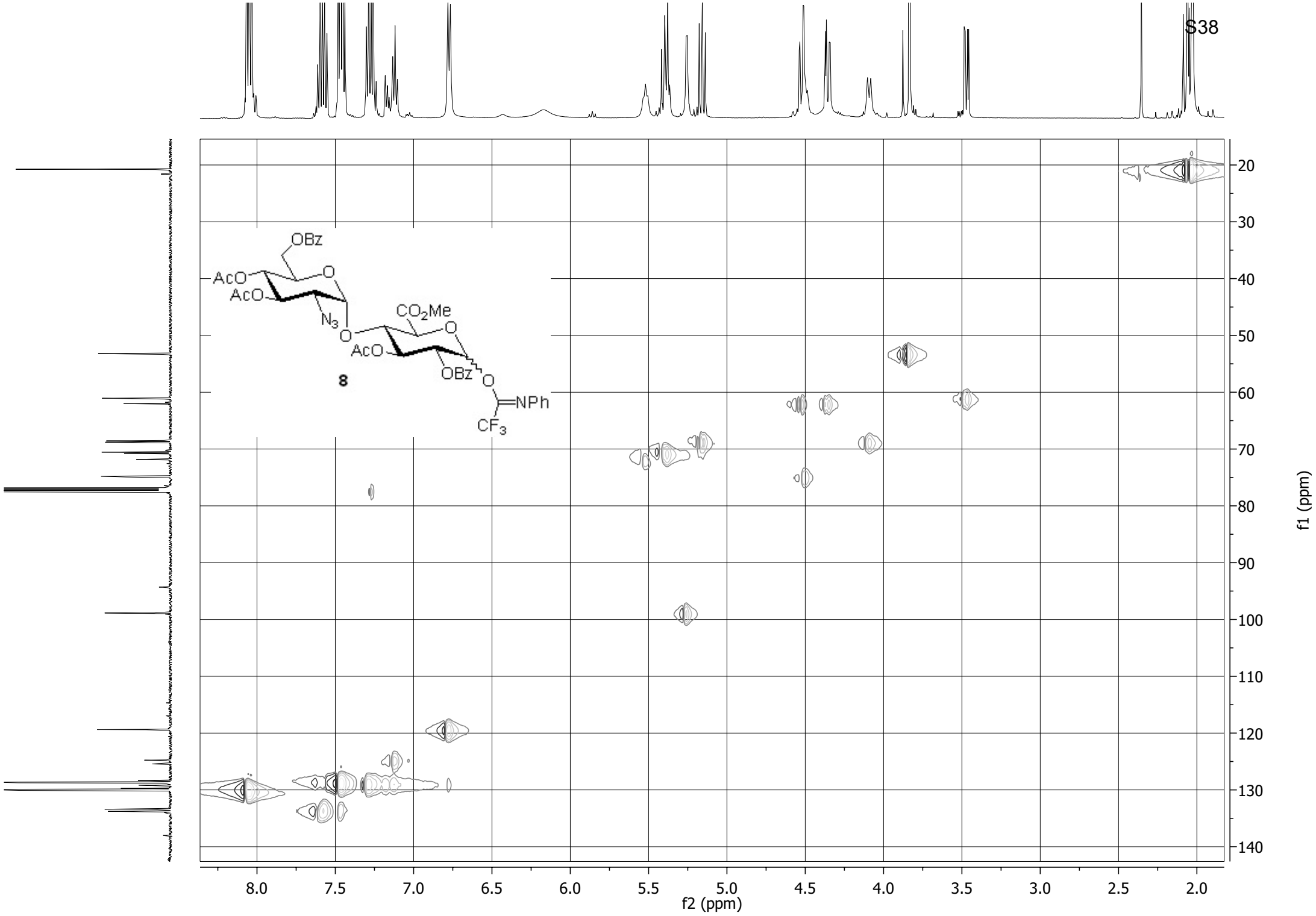

OBz
AcO
AcO
O
N3
O
CO2Me
O
AcO
OBz
O
NPh
CF3

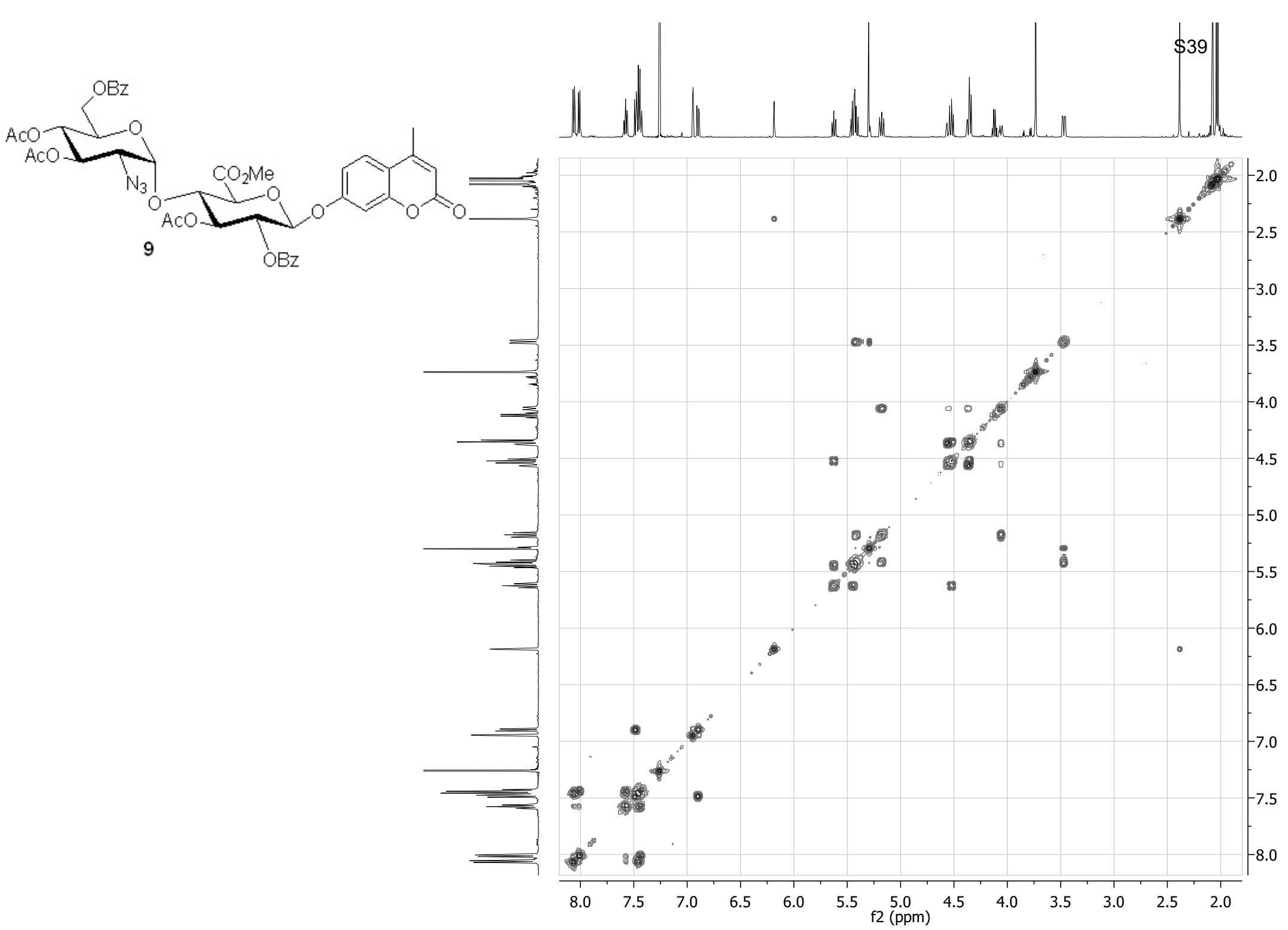
8

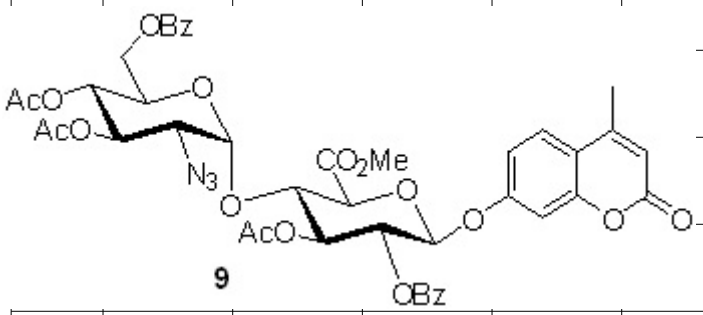





NW35-3-3_F22_AS_CDCl3.3.ser
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)


OBz
AcO
AcO
O
N3
O
CO2Me
O
AcO
OBz
O
NPh
CF3
8
f2 (ppm)
f1 (ppm)

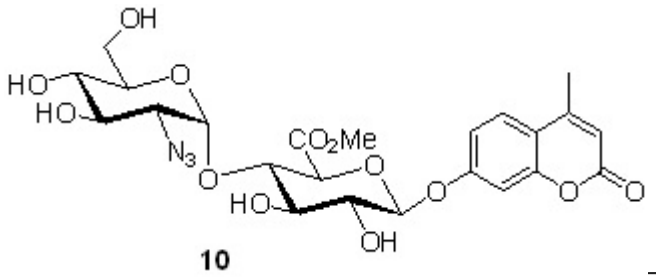

OBz
AcO
AcO
O
N3
CO2Me
O
AcO
O
O
O
O
OBz
9
S39
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)



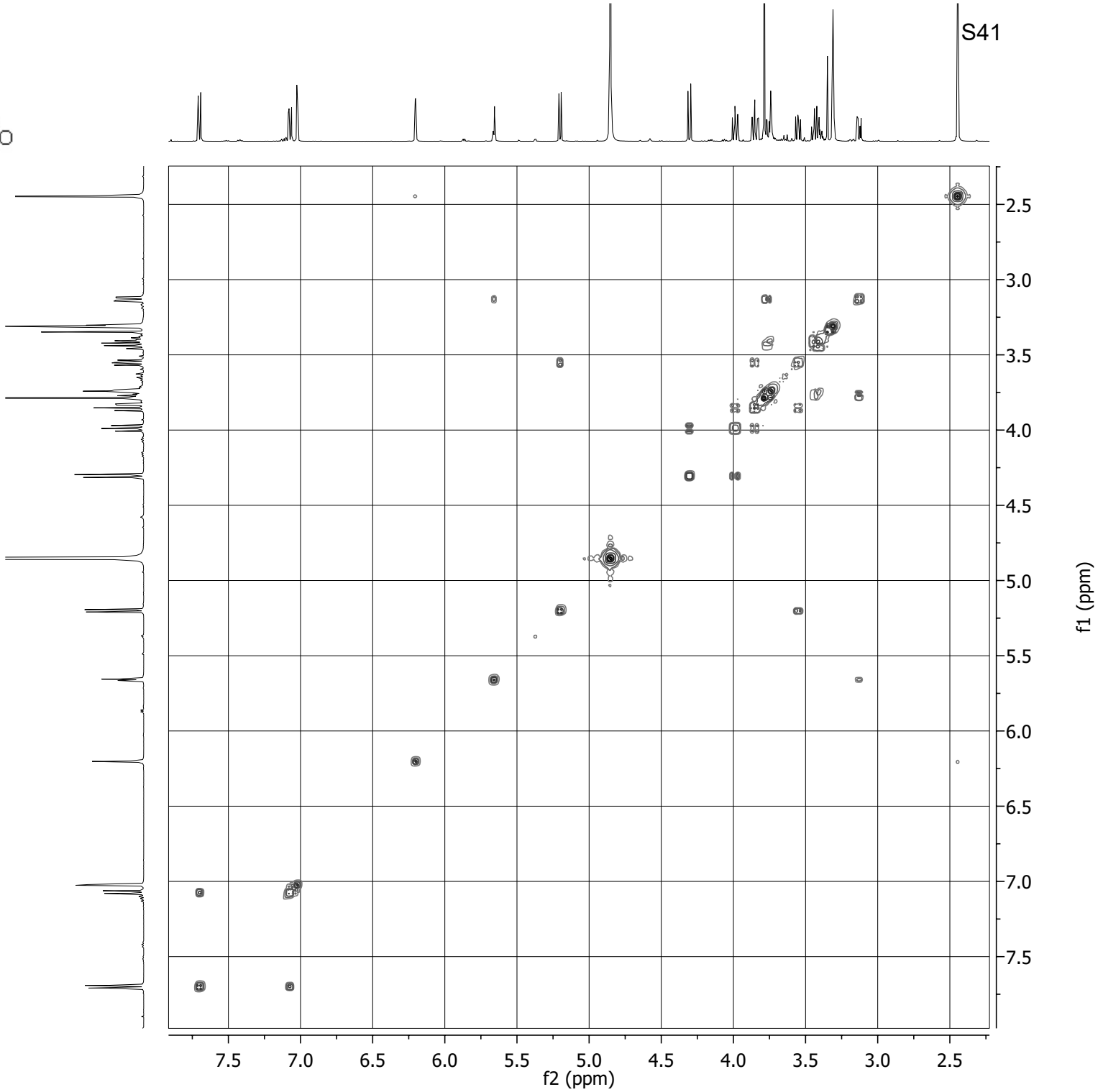
9

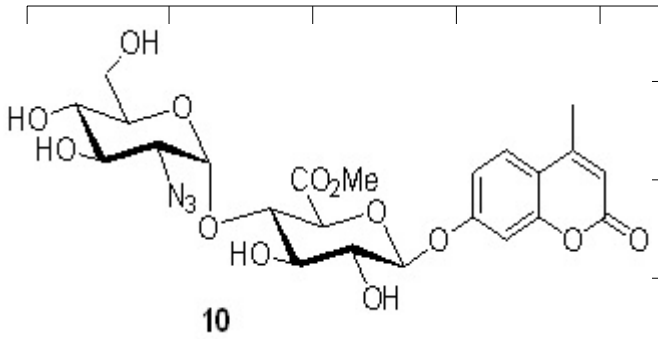
f2 (ppm)

f1 (ppm)


OH
HO
HO
O
N3
O
CO2Me
O
HO
O
O
O
O
OH

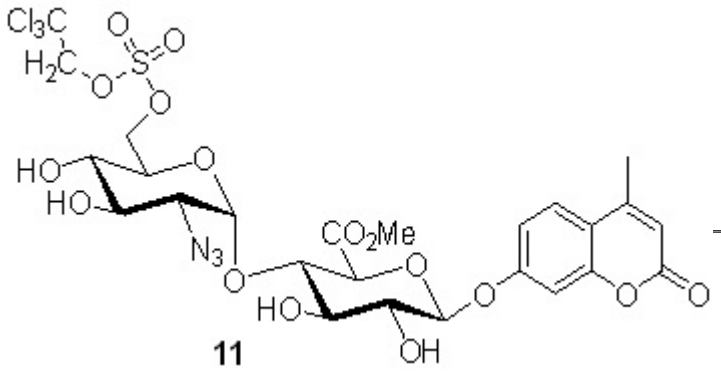
10


2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f2 (ppm)

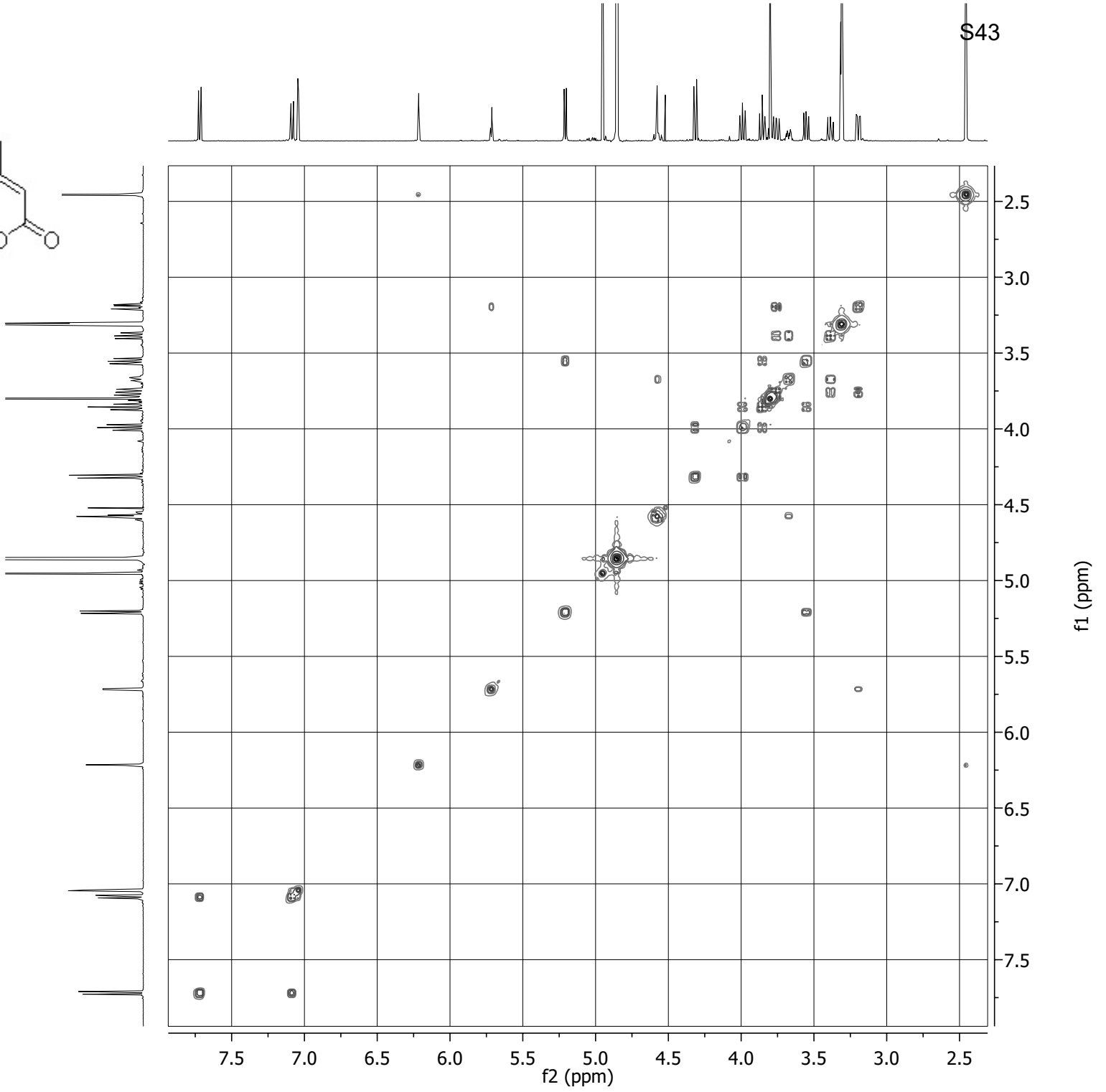

OH
HO
HO
O
N3
O
CO2Me
O
HO
O
OH
O
O
O

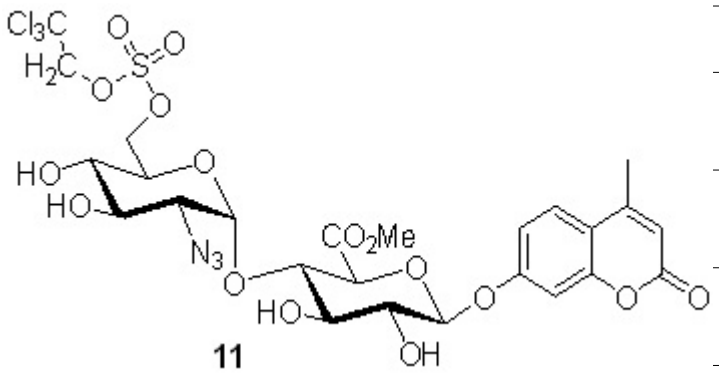
10

7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)


Cl3C
H2C
O
S
HO
HO
N3
CO2Me
HO
OH

11


7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f2 (ppm)
f1 (ppm)


Cl3C
H2C
O
S
HO
HO
N3
CO2Me
HO
OH
O

11

7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)