Supporting Information for: Efficient and Reproducible Synthesis of an Fmoc-protected Tn Antigen

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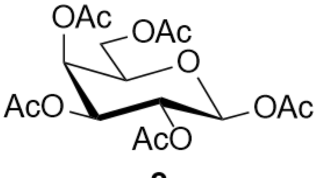
1. General methods and materials for chemical reactions

Solvents were purchased from Caledon Labs (Caledon, Ontario), Sigma-Aldrich (Oakville, Ontario) or VWR Canada (Mississauga, Ontario). Other chemicals were purchased from Sigma-Aldrich, AK Scientific, Oakwood Chemicals, Alfa Aesar or Acros Chemicals and were used without further purification unless otherwise noted. Anhydrous toluene, tetrahydrofuran (THF), diethyl ether and *N*,*N*-dimethylformamide (DMF) were obtained from an Innovative Technology (Newburyport, USA) solvent purification system based on aluminium oxide columns. CH_2Cl_2, pyridine, acetonitrile, *N*,*N*-diisopropylethylamine (DIPEA) and NEt_3 were freshly distilled from CaH_2 prior to use. Purified water was obtained from a Millipore deionization system. All heated reactions were conducted using oil baths on IKA RET Basic stir plates equipped with a P1000 temperature probe. Thin layer chromatography was performed using EMD aluminum-backed silica 60 F254-coated plates and were visualized using either UV-light (254 nm), $KMnO_4$, vanillin, Hanessian's stain, Dragendorff or phosphomolybdic Acid (PMA)'s stain. Preparative TLC was done using glass-backed silica plates (Silicycle) of either 250, 500, 1000 or 2000 μm thickness depending on application. Column chromatography was carried out using standard flash technique with silica (Siliaflash-P60, 230-400 mesh Silicycle) under compressed air pressure. Standard work-up procedure for all reactions undergoing an aqueous wash involved back extraction of every aqueous phase, a drying of the combined organic phases with anhydrous magnesium sulphate, filtration either using vacuum and a sintered-glass frit or through a glass-wool plug using gravity, and concentration under reduced pressure on a rotary evaporator (Buchi or Synthware). ^{1}H NMR spectra were obtained at 300 MHz or 500 MHz, and ^{13}C NMR spectra were obtained at 75 or 125 MHz on Bruker instruments. NMR chemical shifts (δ) are reported in ppm and are calibrated against residual solvent signals of $CHCl_3$ (δ 7.26), DMSO-d5 (δ 2.50), acetone-d5 (δ 2.05), or methanol-d3 (δ 3.31). Spectra were processed using MNOVA 14.1.2 (Mestrelab Research S.L., Santiago de Compostela, Spain). Fourier transform was conducted using a linear phase shift group delay, an exponential-fit 1 Hz apodization, and a zero-filling linear prediction of 65536 points. An automatic phase correction was applied and manually refined to provide a flatter baseline. A baseline correction, employing a Whittaker smoothing function with a 2.60 Hz filter and a smoothing factor of 16384 was employed on the spectrum between −2.00 and 15.00 ppm. Integration was measured using the manual integration tool and referenced to a relevant aliphatic proton. Multiplet analysis was conducted using the algorithm as implemented in the software, confirmed by manual analysis. HRMS were conducted on a Waters XEVO G2-XS TOF instrument with an ASAP probe in CI mode.

2. Specific Experimental Protocols

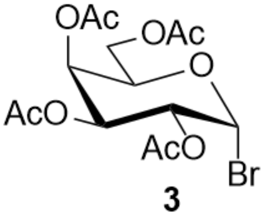
1,2,3,4,6-Penta-*O*-acetyl-β-D-galactopyranose 2



2

D-galactose (50 g, 277.53 mmol) and sodium acetate (68.16 g, 830.92 mmol) were dissolved in acetic anhydride (440 mL) and stirred at reflux for 4 hours. The reaction was cooled to room temperature and the slurry was poured into a large flask with 1.5 kg of crushed ice and stirred until the mixture returned to room temperature. The crystals that had formed were filtered and left to dry overnight. The solid was recrystallized with methanol, filtered, and left to dry again overnight to produce **2** as a white solid with an 83% yield (46.09 g). This reaction was repeated 8 times for this project with scales ranging from 25 to 100 g, and yields between 75-83%. Spectral data is in agreement with published data.[1]

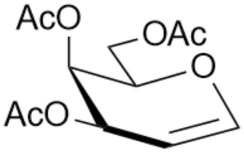
1-Bromo-2,3,4,6-tetra-*O*-acetyl-α- D-galactopyranose 3



3

To a stirring solution of compound **2** (45 g, 115.28 mmol) in dichloromethane (200 mL) at 0 °C, 33% HBr in acetic acid (236 mL) was added dropwise and stirred for approximately 1 hour following the completion of addition. The reaction was then warmed to room temperature and stirred for another 2.5 hours. The solution was washed with $NaHCO_3$ (200 mL x 2), $Na_2S_2O_3$ (200 mL), brine, dried over anhydrous magnesium sulfate, and concentrated *in vacuo.* Cold diethyl ether and hexane (1:1) were used to recrystallize overnight resulting in an 85% yield (40.09 g) of compound **3** as a brown foam. This reaction was repeated 8 times for this project with scales ranging from 20 to 45g, and yields between 81-90%. Spectral data is in agreement with published data.[2]

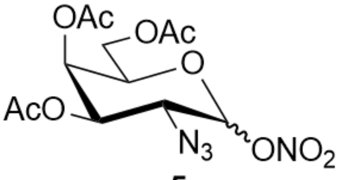
3,4,6-Tri-*O*-acetyl- D-galactal 4



4

Compound **3** (7.40 g, 18.00 mmol) was dissolved in acetonitrile (180 mL) under N_2 and stirred for 5 minutes at 65 °C. Ammonium chloride (7.2 g, 135 mmol) was added followed by a portion wise addition of freshly activated zinc dust (8.88g, 135 mmol) and the reaction was stirred vigorously. The reaction was monitored by TLC (3:1, Hexanes:EtOAc) and changed colour to a golden yellow after 4 hours. The reaction was cooled to room temperature, filtered through celite, and the filtrate was concentrated *in vacuo*. The mixture was purified by silica gel column chromatography (3:1, Hexanes:EtOAc) to yield 4.40 g (90 % yield) of compound **4** (Rf = 0.36) as a golden yellow syrup. This reaction was repeated 8 times for this project with scales ranging from 5.5g to 25g, and yields between 71-94%. Spectral data is in agreement with published data.[1]

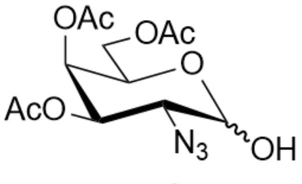
3,4,6-Tri-O-acetyl-2-azido-2-deoxy-d-galactopyranosyl nitrate 5



5

Compound **4** (6.99 g, 25.40 mmol) was dissolved in anhydrous acetonitrile (125 mL), then cooled to -15 °C. CAN (69.6 g, 127.00 mmol) was added to the stirring solution, followed by NaN_3 (4.37 g, 67.31 mmol). The reaction was vigorously stirred at -15 °C for 5 hours under N_2 and was monitored by TLC (1:1; Hexanes: EtOAc). The reaction was then diluted with equal parts diethyl ether and cold water until the mixture changed from bright yellow/orange to clear. The solution was extracted with diethyl ether (3 x 150 ml) and the combined organic was washed with water, brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo.* A mixture of the α and β product were present in the NMR with a combined crude yield of 74%. Additional purification was unnecessary, but when conducted, this reaction was repeated 6 times for this project with scales ranging from 2.3g to 15g, and yields between 42-63% of purified yield (combination of anomers). Due to the overlapping Rf values, these anomers were not separated. Spectral date is in agreement with published data.[3]

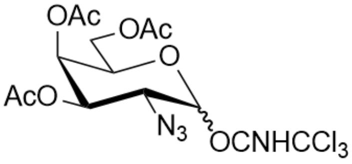
2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-galactopyranose 6



6

To a stirring solution of compound **5** (41.07 g, 109.15 mmol) in acetonitrile (350 mL) at 0 °C was added DIPEA (22.8 mL, 130.98 mmol) and thiophenol (13.4 mL, 130.98 mmol). The reaction was vigorously stirred for 1 hour under N_2 and monitored by TLC (1:1, Hexanes: EtOAc). The solution was concentrated *in vacuo* and the resulting beige waxy solid was triturated with hexanes to remove excess Ph-S-S-Ph. The crude mixture was purified by silica gel column chromatography (1:1, Hexanes: EtOAc) to yield 33 g (85%). of compound **6** (Rf = 0.54; 1:1, Hexanes: EtOAc). Spectral data is in agreement with published data.[4] This reaction was repeated a total of 6 times with scales ranging from 5 g to 41 g with a yield range of 61-85%.

(2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-galactopyranosyl)-2,2,2-trichloroacetimidate 7

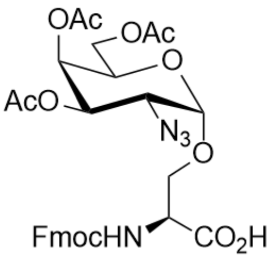


7

To a flame dried round bottom flask charged with a stirring solution of compound **6** (1.30 g, 3.92 mmol) in anhydrous dichloromethane (20 mL) was added Cs_2CO_3 (6.39 g, 19.62 mmol) at 0 °C. Upon vigorous stirring for 5 minutes, freshly distilled trichloroacetonitrile (3.93 mL, 39.24 mmol) was added dropwise and the reaction mixture was allowed to warm to room temperature.

After five minutes, a visible colour change from pale yellow to light brown was observed. The reaction was monitored by TLC immediately after the colour change (1:1 Hexanes: EtOAc) and the mixture was immediately filtered through Celite and washed with dichloromethane after the visible consumption of the starting material. The filtrate was concentrated *in vacuo* and a mixture of the α-trichloroacetimidate and β - trichloroacetimidate **7** were present as seen in the NMR in a 2:5 (α:β) ratio yielding 1.1g (90% yield) with this reaction being repeated a total of 17 times with scales ranging from 100mg to 2g for a crude yield range of 62%-95%. R_f = 0.59 (1:1, Hexanes: EtOAc). Due to the unstable nature of this compound, the α and β anomers were not separated and the subsequent reaction was conducted as soon as all of the solvent was evaporated from the compound. Spectral data is in agreement with published data.[5]

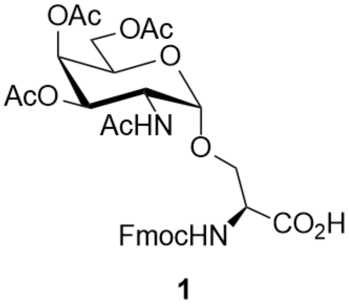
(2*S*)-2-[[(9H-Fluoren-9-ylmethoxy)carbonyl]amino]-3-((2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)oxy)propanoic acid 8



8

To a flame dried round bottom flask with 4 Å molecular sieves, Compound **7** (340 mg, 0.715 mmol) and Fmoc-Ser-OH (351 mg, 1.073 mmol) were dissolved in anhydrous dichloromethane (8.00 ml). $Pd(CH_3CN)_4(BF_4)_2$ (35 mg, 0.0715 mmol) was then added to the solution and the mixture was vigorously stirred at 25 °C. The reaction was monitored by TLC (1:1, hexanes: EtOAc) and stirred overnight. The mixture was then diluted with EtOAc and filtered over Celite. The filtrate was concentrated *in vacuo* and purified by silica gel column chromatography (gradient elution from 3:2 hexanes: EtOAc v/v to 1:1 hexanes: EtOAc v/v) with the reaction being repeated a total of 20 times with scales ranging from 30mg to 400mg to yield 175 mg (32% yield) with a range of yields between 29%-38 % of the α anomer (Rf = 0.35) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 – 7.75 (d, 2H), 7.61 (t, 2H), 7.44 – 7.39 (t, 2H), 7.35 – 7.30 (t, 2H), 5.71 (d, *J* = 8.9 Hz, 1H), 5.56 (d, *J* = 8.5 Hz, 1H), 5.41 – 5.36 (d, 1H), 4.91 (dd, *J* = 10.8, 3.4 Hz, 1H), 4.61 – 4.56 (d, 1H), 4.45 (m, *J* = 7.2 Hz, 2H), 4.25 (t, *J* = 6.9 Hz, 1H), 4.19 (m, *J* = 7.0 Hz, 2H), 4.10 – 4.04 (m, 1H), 4.00 (t, *J* = 6.6 Hz, 1H), 3.95 (d, *J* = 10.7 Hz, 1H), 3.86 (dd, *J* = 10.8, 8.5 Hz, 1H), 2.17 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.6, 169.67, 169.63, 168.9, 156.1, 144.4, 141.3, 127.8, 127.1, 125.1, 120.13, 120.10, 93.8, 72.2, 71.4, 67.43, 67.41, 66.1, 63.0, 61.0, 60.9, 59.6, 56.0, 47.2, 20.70, 20.63, 20.60, HRMS (ESI) *m/z* found 641.2097, calculated 641.2090 for $[M + H]^+$. HRMS (ESI) *m/z* found 641.2097, calculated 641.2090 for $[M + H]^+$.

(2*S*)-2-[[(9H-Fluoren-9-ylmethoxy)carbonyl]amino]-3-((2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)oxy)propanoic acid 1



1

Compound **8** (160 mg, 0.250 mmol) was added to a mixture of THF/Ac_2O/AcOH (3:2:1, v/v/v, 19.2 mL) and treated with zinc and $CuSO_4$ (saturated aq. solution, 800 μl). The reaction was monitored by TLC (1:1, Hexane: EtOAc) and stirred under N_2 for 2 hours at 25 °C. The reaction mixture was diluted with DCM, filtered through Celite, dried with magnesium sulfate, and evaporated *in vacuo* to produce 131 mg of **9** as a white foam (80% yield). This reaction was repeated a total of 7 times with scales ranging from 50mg to 200mg with a yield range between 55%-80% yield Rf = 0.37 (2:1 EtOAc/Hexanes). This product always appeared to retain grease, even with trituration with distilled hexanes or partition between distilled hexanes and distilled acetonitrile (followed by recovery from the acetonitrile phase following evaporation). The residual grease has been marked in the NMR. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.12 (bs, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.61 (t, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.4, 1.3 Hz, 2H), 6.11 (d, *J* = 8.4 Hz, 1H), 5.76 (s, 1H), 5.49 – 5.42 (m, 2H), 4.50 – 4.32 (m, 3H), 4.22 (dd, *J* = 9.5, 5.7 Hz, 3H), 4.12 (d, *J* = 7.2 Hz, 3H), 4.00 (s, 1H), 3.86 (d, *J* = 12.2, 3.2 Hz, 1H), 2.15 (s, 4H), 2.04 (s, 4H), 1.95 (s, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 170.8, 170.1, 168.2, 156.2, 143.8, 143.7, 141.3, 127.8, 127.2, 125.2, 120.1, 92.6, 72.4, 69.5, 69.2, 67.3, 65.6, 64.3, 62.7, 61.3, 60.4 56.0, 47.1, 29.8, 20.73, 20.70, 20.5 657.2296, calculated 657.2291 for [M + H]$^+$.

3. Spectra of novel compounds

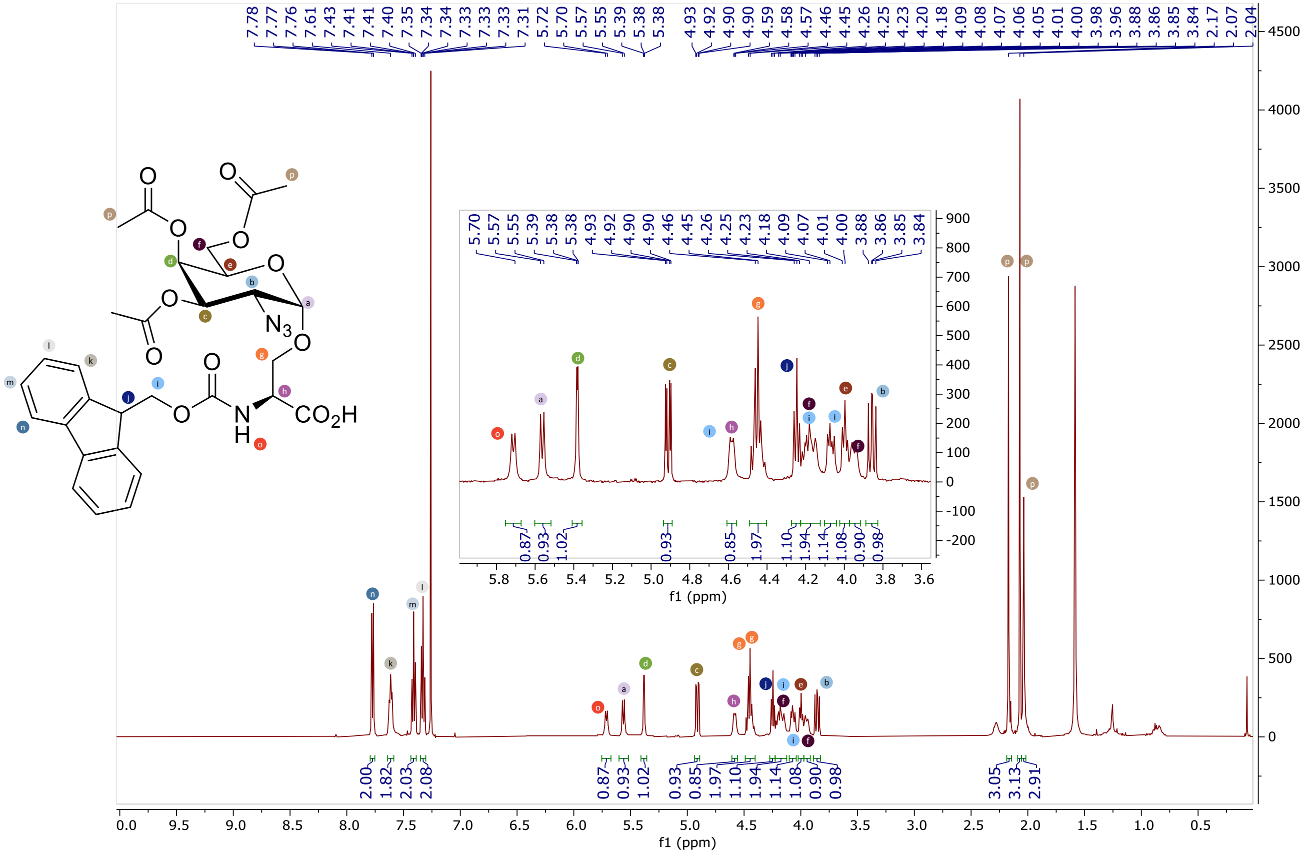


Figure 3.1 ^{1}H NMR (500MHz, CDCl$_3$) of Compound 8

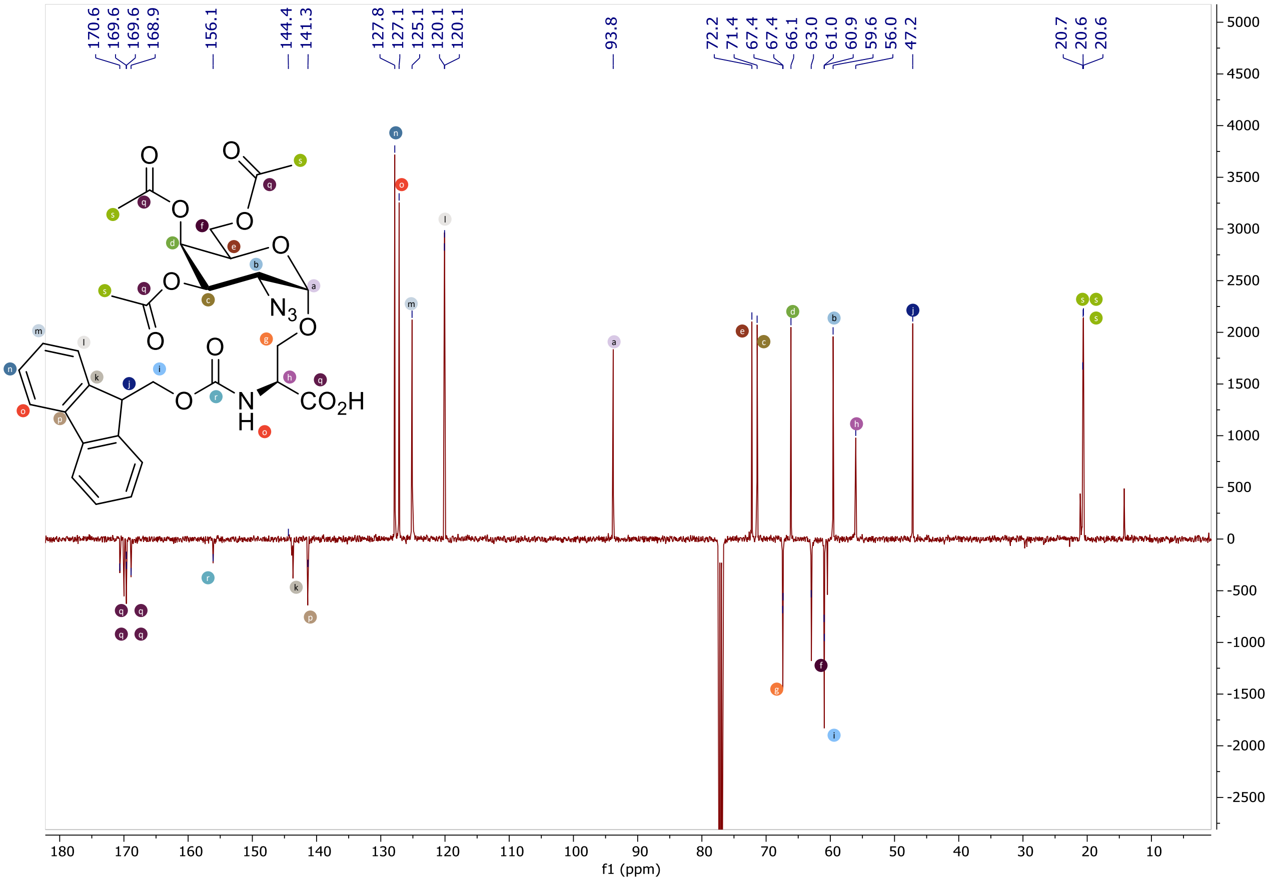


Figure 3.2 DEPT Q (126MHz, $CDCl_3$) of Compound 8

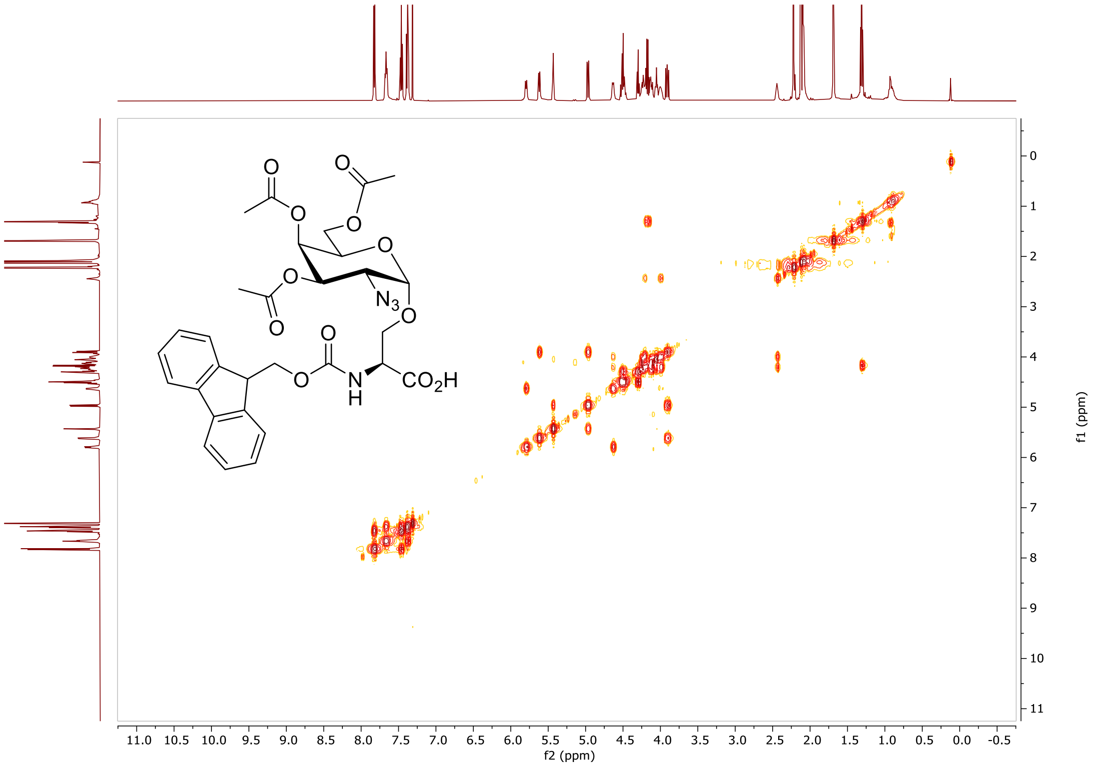


Figure 3.3 2D COSY 45 (500MHz, CDCl₃) of Compound 8

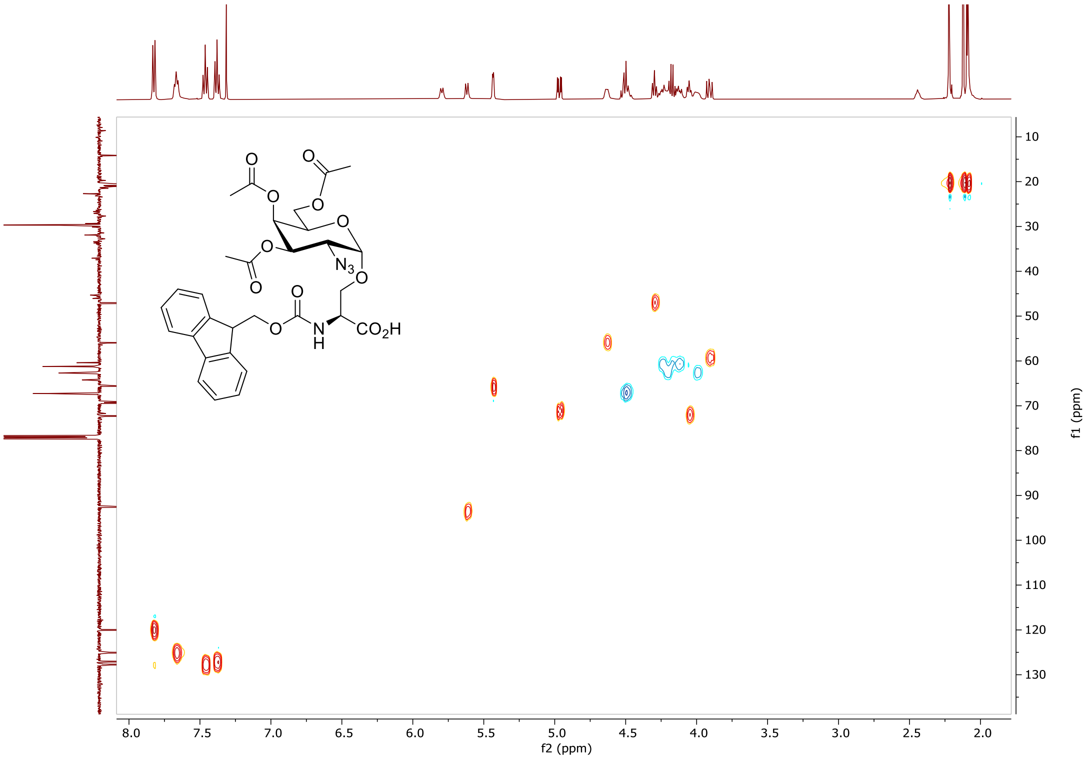


Figure 3.4 ^{1}H-^{13}C ME-HSQC of Compound 8

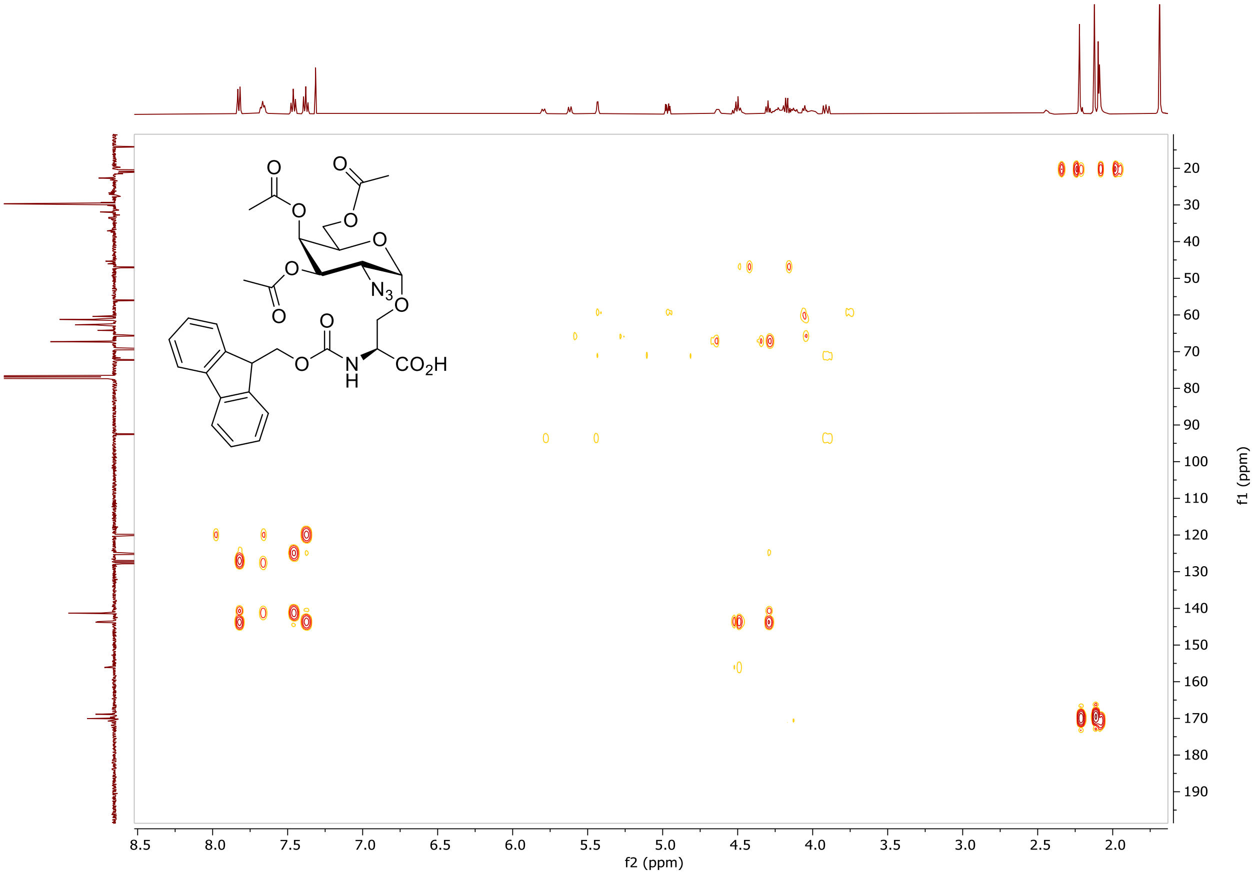


Figure 3.5 ^{1}H-^{13}C HMBC of Compound 8

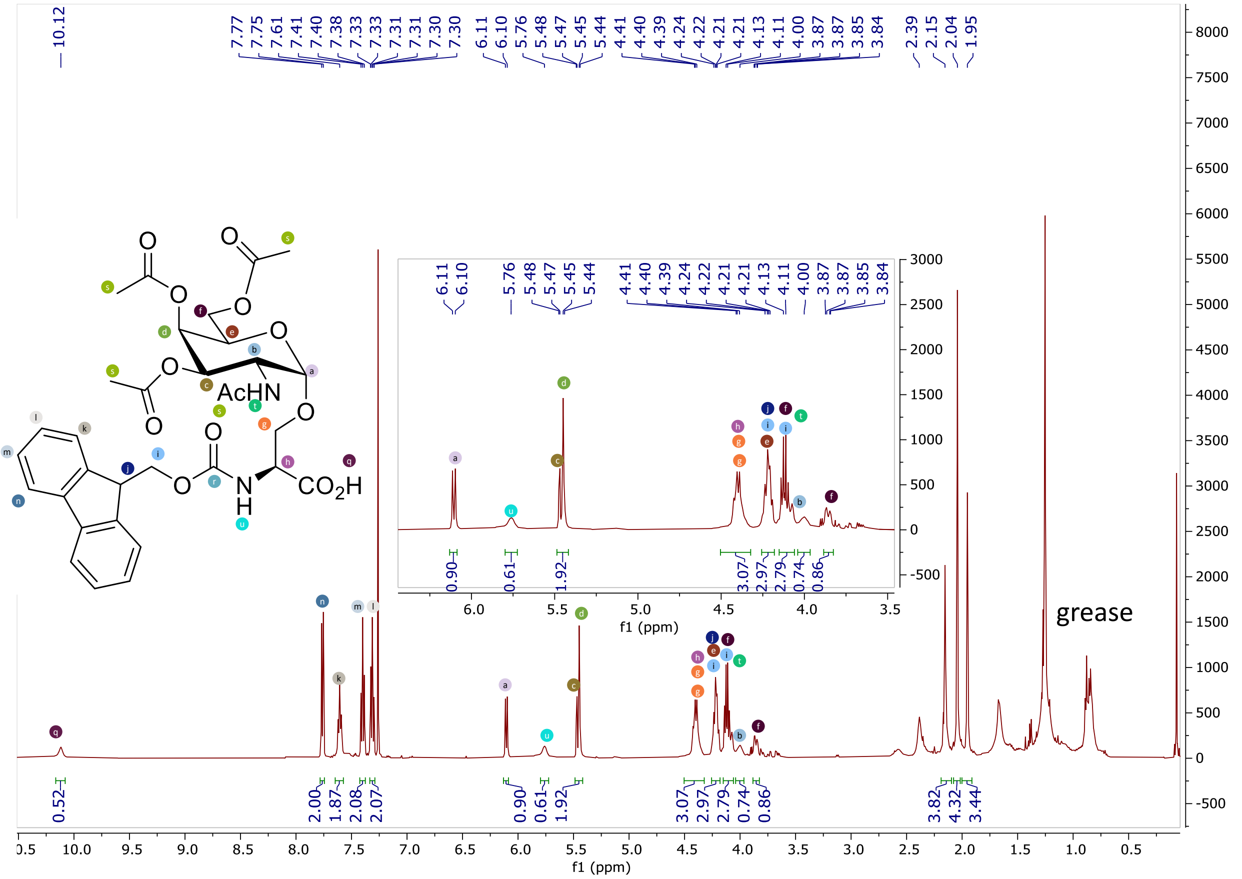


Figure 3.6 ^{1}H NMR (500MHz, $CDCl_3$) of Compound 1

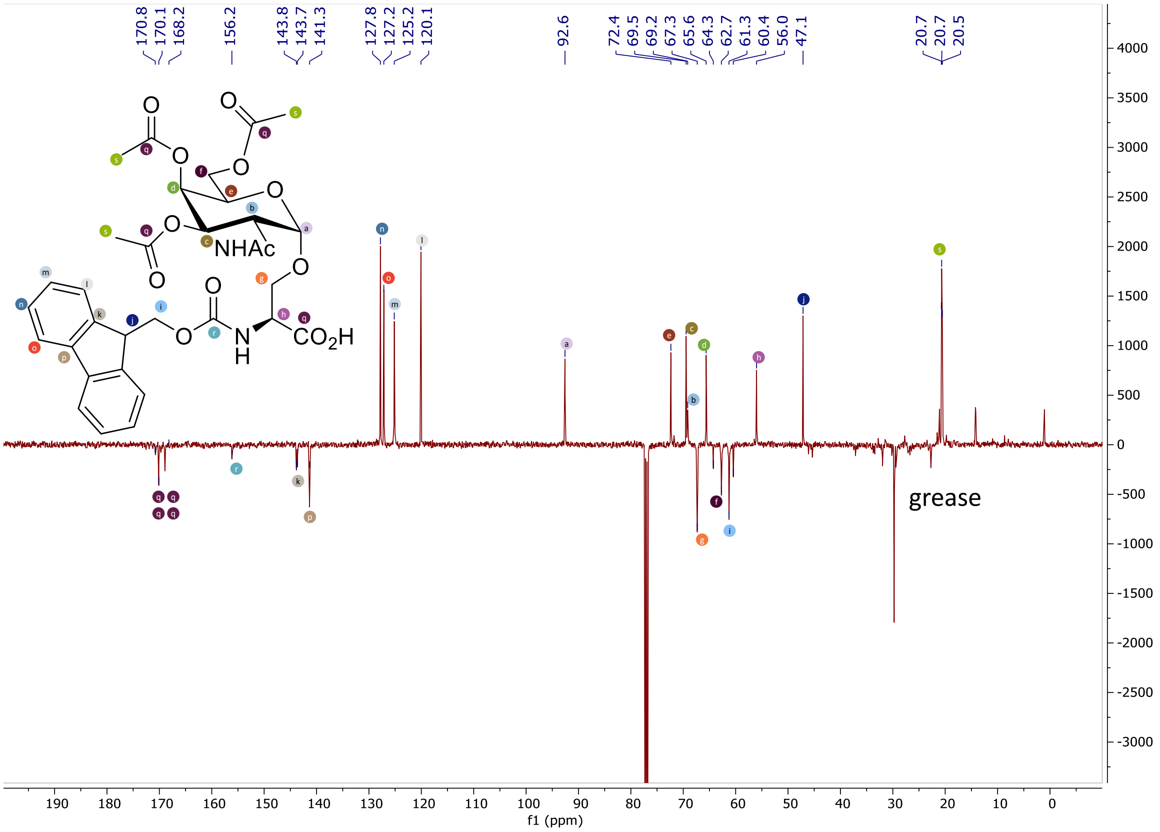


Figure 3.7 DEPT Q (125MHz, $CDCl_3$) of Compound 1

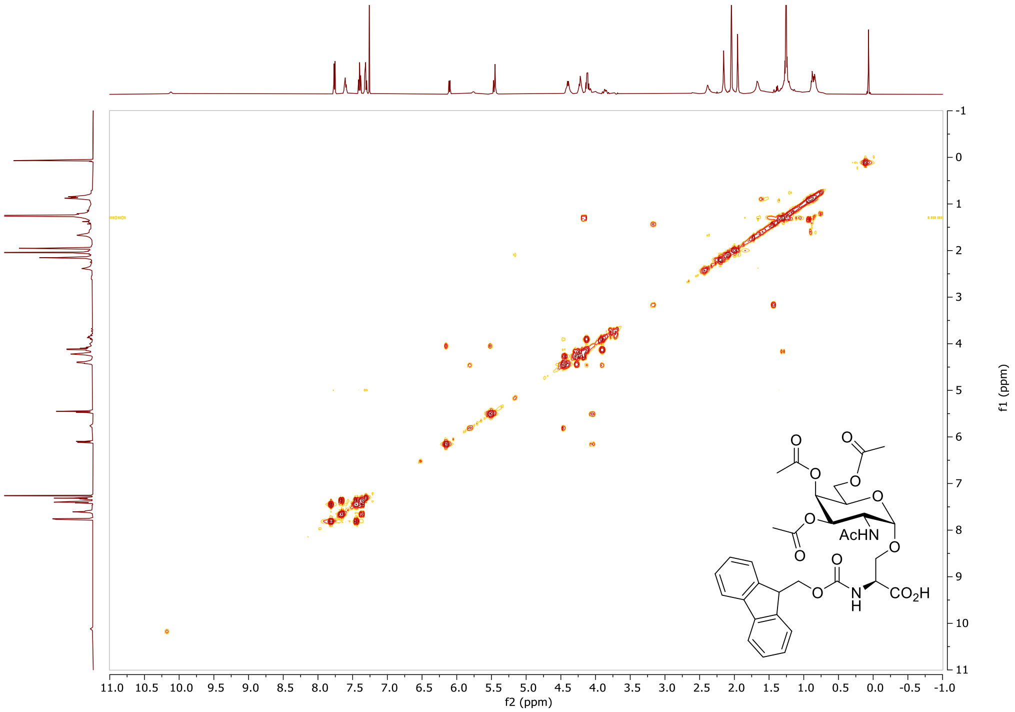


Figure 3.8 2D COSY 45 (500MHz, $CDCl_3$) of Compound 1

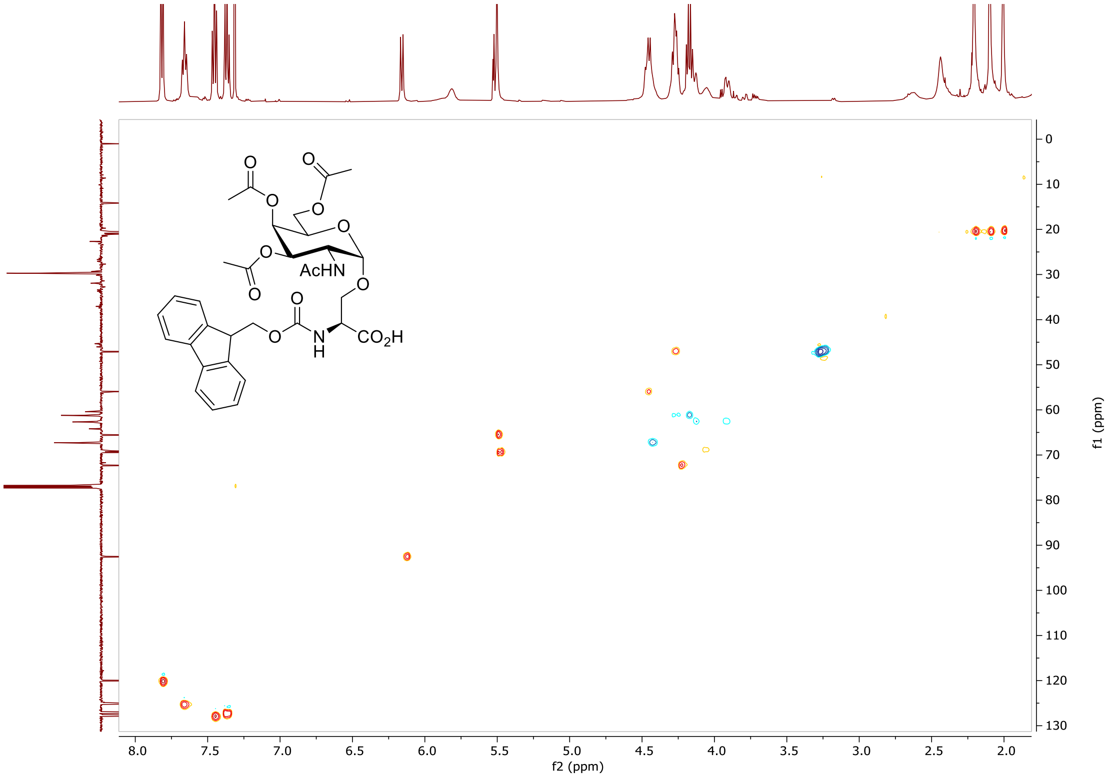


Figure 3.9 ^1H-^{13}C ME-HSQC of Compound 1

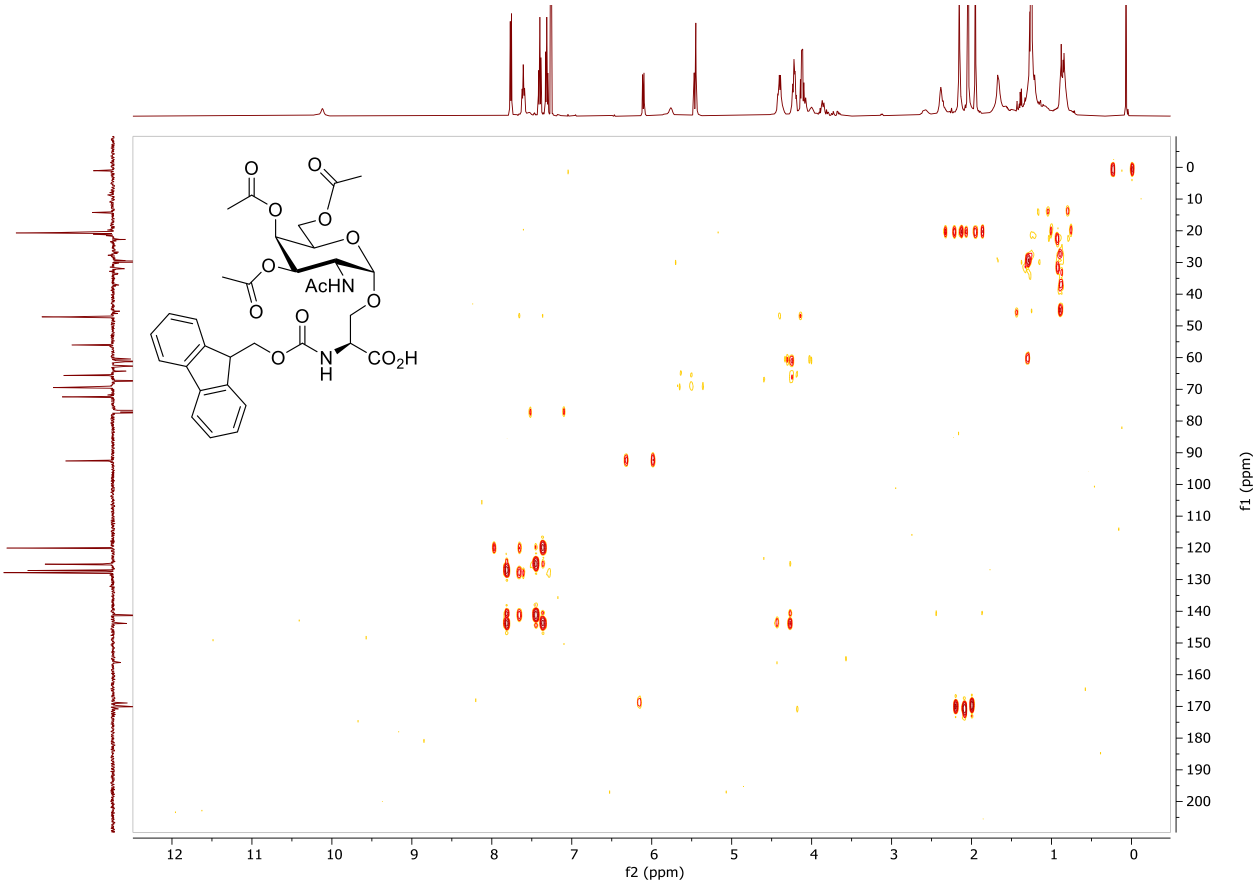


Figure 3.10 ^{1}H-^{13}C HMBC of Compound 1

4. References

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