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Supporting Information

Chemical Synthesis Elucidates the Immunological Importance of a Pyruvate Modification in the Capsular Polysaccharide of *Streptococcus pneumoniae* Serotype 4

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Supporting Information

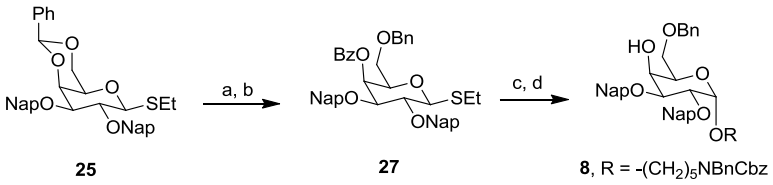
1. General information:

Commercial grade solvents were used unless stated otherwise. Dry solvents were obtained from a Waters Dry Solvent System. Solvents for chromatography were distilled prior to use. Sensitive reactions were carried out in heat-dried glassware and under an argon atmosphere. Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates precoated with a 0.25 mm thickness of silica gel. Spots were visualized by staining with vanillin solution (6% (w/v) vanillin and 10% (v/v) sulfuric acid in 95% EtOH) or Hanessian's stain (5% (w/v) ammonium molybdate, 1% (w/v) cerium(II) sulfate and 10% (v/v) sulfuric acid in water). Silica column chromatography was performed on Fluka Kieselgel 60 (230-400 mesh).

^{1}H, ^{13}C and two-dimensional NMR spectra were measured with a Varian 400-MR , 600-MR and Bruker Avance 700 spectrometer at 296 K. Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective residual solvent peaks ($CDCl_3$: δ 7.27 in ^{1}H and 77.23 in ^{13}C NMR; CD_3OD: δ 3.31 in ^{1}H and 49.15 in ^{13}C NMR; D_2O: δ 4.80 in ^{1}H NMR; Acetone-d_6: δ 2.05 in ^{1}H and 29.92 in ^{13}C NMR).The following abbreviations are used to indicate peak multiplicities: *s* singlet; *d* doublet; *dd* doublet of doublets; *t* triplet; *dt* doublet of triplets; *q* quartet; *m* multiplet. Coupling constants (*J*) are reported in Hertz (Hz). Optical rotation (OR) measurements were carried out with a Schmidt & Haensch UniPol L1000 polarimeter at λ = 589 nm and a concentration (c) expressed in g/100 mL in the solvent noted in parentheses. High resolution mass spectrometry (HRMS) was performed at the Free University Berlin, Mass Spectrometry Core Facility, with an Agilent 6210 ESI-TOF mass spectrometer. Infrared (IR) spectra were measured with a Perkin Elmer 100 FTIR spectrometer.

2. Experimental Procedures

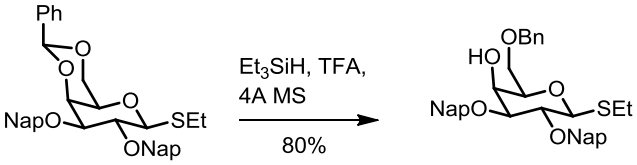
2.1. Synthesis of Galactose building block 8:



25 **27** **8**, R = -$(CH_2)_5$NBnCbz

SI-Scheme 1. Synthesis of Gal acceptor **8**. Reaction conditions: a) Et_3SiH, TFA, CH_2Cl_2, 4Å MS, 80%; b) BzCl, pyridine, 75%; c) $HO(CH_2)_5NBnCbz$, NIS, TfOH, CH_2Cl_2/ether (1:3), 63% (α) and 23% (β) (α:β = 3:1); d) NaOMe, MeOH, reflux, 90%.

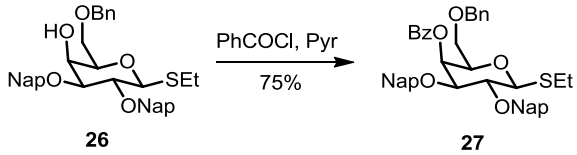
2.2. Synthesis of Galactose building block 26:



25 **26**

Compound **25**[1] (5.5 g, 9.2 mmol) was stirred in DCM (90 mL) with activated 4Å MS (5.0 g) for 10 min before cooling to 0 ^oC. Added triethylsilane (11.86 mL, 74.2 mmol) followed by TFA (4.29 mL, 55.7 mmol) dropwise and stirred the reaction mixture at room temperature for 4 h before quenching with water. Extracted the aqueous layer with CH_2Cl_2, and washed the organic layer with sat. aq. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated to obtain oil. Purification by flash column chromatography using toluene and acetone as eluent (0 to 7.5%) afforded the compound **26** as colorless oil (4.4 g, 80%). $[\alpha]_D^{20}$ = + 29.7^o (c = 1.10, $CHCl_3$); IR ν_{max} (film) 3570, 2858, 1362, 1081, 818, 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 – 7.74 (m, 8H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.52 – 7.41 (m, 5H), 7.40 – 7.29 (m, 4H), 5.09 (d, *J* = 9.7 Hz, 1H), 5.01 – 4.77 (m, 3H), 4.60 (d, *J* = 1.3 Hz, 2H), 4.49 (dd, *J* = 9.7, 1.6 Hz, 1H), 4.17 (s, 1H), 3.88 – 3.70 (m, 3H), 3.69 – 3.55 (m, 2H), 2.91 – 2.66 (m, 2H), 1.35 (td, *J* = 7.4, 1.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 135.9, 135.3, 133.4, 133.3, 133.2 (2C), 128.6, 128.5, 128.2, 128.1 (2C), 127.9 (2C), 127.8 (2C), 127.1, 126.8, 126.5, 126.3, 126.2, 126.1, 126.0, 125.9, 85.3, 82.4, 78.2, 77.1, 76.0, 73.9, 72.3, 69.5, 67.1, 25.0, 15.3; HRMS (ESI): Calcd for $C_{37}H_{38}O_5S$ $[M+Na]^+$ 617.2338, found: 617.2342.

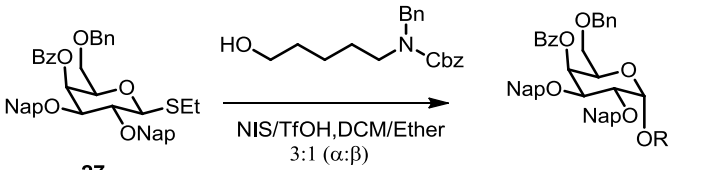
2.3. Synthesis of Galactose building block 27:



26 **27**

To a 0 ^{o}C cooled solution of **26** (4.3 g, 7.23 mmol) in pyridine (30 mL) was added benzoyl chloride (2.52 mL, 21.7 mmol) and stirred at room temperature for 13 h. Diluted the reaction mixture with water and extracted the aqueous layer with ether. Washed the organic layer with water, 1.0 M HCl, brine, dried over Na_2SO_4, filtered and concentrated to obtain oil. Purification by flash column chromatography using hexanes and ethyl acetate as eluent (0 to 10%) afforded the compound **27** as oil (3.8 g, 75%). $[\alpha]_D^{20}$ = + 79.9º (c = 1.60, $CHCl_3$); IR ν_{max} (film) 2862, 1721, 1272, 1095, 815, 701 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 – 8.07 (m, 2H), 7.84 – 7.71 (m, 6H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.65 – 7.55 (m, 2H), 7.54 – 7.34 (m, 9H), 7.32 – 7.28 (m, 2H), 7.27 – 7.20 (m, 2H), 5.97 (dd, *J* = 3.0, 0.9 Hz, 1H), 5.04 (t, *J* = 10.9 Hz, 2H), 4.96 (d, *J* = 10.6 Hz, 1H), 4.76 (d, *J* = 11.7 Hz, 1H), 4.59 (d, *J* = 9.2 Hz, 1H), 4.53 (d, *J* = 11.7 Hz, 1H), 4.46 (d, *J* = 11.8 Hz, 1H), 3.91 – 3.86 (m, 1H), 3.85 – 3.73 (m, 2H), 3.67 (dd, *J* = 9.5, 5.9 Hz, 1H), 3.60 (dd, *J* = 9.5, 7.0 Hz, 1H), 2.83 (qq, *J* = 12.6, 7.4 Hz, 2H), 1.37 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 137.7, 135.8, 135.4, 133.8, 133.4 (2C), 133.3, 133.2, 133.1, 130.3, 130.2, 130.0, 128.6 (2C), 128.5, 128.2, 128.1 (2C), 127.9, 127.8 (2C), 127.8, 127.2, 127.0, 126.6, 126.3, 126.1, 125.9, 85.6, 81.3, 78.0, 76.3, 76.1, 73.9, 71.9, 68.5, 67.8, 25.2, 15.3; HRMS (ESI): Calcd for $C_{44}H_{42}O_6S$ $[M+Na]^+$ 721.2600, found: 721.2600.

2.4. Synthesis of Galactose linker building block 28:



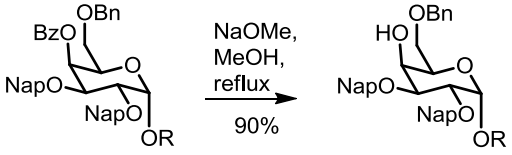
27 **28, R = $(CH_2)_5$NBnCbz**

Stirred a solution of microwave activated 4 Å acid washed molecular sieves (AWMS) (3.2 g), compound **27** (2 g, 2.86 mmol) and C5 aminopentyl linker (1.21 g, 3.72 mmol) in a mixture of ether and DCM (3:1; 36 mL : 12 mL) at room temperature for 15 min. Cooled the reaction mixture to 0 ^{o}C and added NIS (0.78 g, 3.15 mmol) followed by TfOH (0.25 mL, 0.28 mmol) and stirred for 30 min. Diluted the reaction mixture with aq. sat. $Na_2S_2O_3$. Extracted the

aqueous layer with ether, and dried the organic layer over Na_2SO_4, filtered and concentrated to obtain oil. Purification by flash column chromatography using hexanes and ethyl acetate as eluent (0 to 25%) afforded α-anomer **28** (1.75 g, 63%), and β-anomer (0.63 g, 23%) as oils, indicating a modest selectivity of ~ 3:1 (α :β)

NMR analysis: Because of the C5 aminopentyl linker the anomeric protons in the ^{1}H nmr were submerged or the peaks were broadened and so, the confirmation of the linkage could not be established easily. From ^{13}C for α-anomer, the anomeric carbon was at 98.4 ppm thereby indicating an α- linkage and the $J_{C,H}$ was 167.7 Hz. For β-anomer, the ^{13}C value was 104.1 ppm for the anomeric indicating a β-anomer and the $J_{C,H}$ coupling for β-anomer was 158.8 Hz indicating a β-anomer. **α-anomer**: $[\alpha]_D^{20}$ = + 95.8º (c = 0.74, $CHCl_3$); IR ν_{max} (film) 2928, 1720, 1698, 1270, 1103, 1057, 740 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.84 – 7.70 (m, 6H), 7.64 (dd, *J* = 12.7, 5.0 Hz, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.51 – 7.12 (m, 23H), 5.92 (s, 1H), 5.18 (bs, 2H), 5.03 (d, *J* = 11.4 Hz, 1H), 4.98 (d, *J* = 12.3 Hz, 1H), 4.90 (bs, 1H), 4.84 (d, *J* = 12.2 Hz, 1H), 4.77 (d, *J* = 11.4 Hz, 1H), 4.45 (m, 4H), 4.17 (bs, 2H), 3.99 (dd, *J* = 10.0, 3.6 Hz, 1H), 3.65 (bs, 1H), 3.54 (d, *J* = 6.3 Hz, 2H), 3.44 (bm, 1H), 3.22 (bm, 2H), 1.58 (bm, 4H), 1.31 (bm, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 166.0, 138.1, 137.9, 136.1, 135.9, 133.4, 133.3, 133.1, 133.0, 130.2, 130.0, 128.7, 128.6, 128.5, 128.5, 128.2, 128.1, 128.0 (3C), 127.8 (3C), 127.7, 127.4, 127.0, 126.7, 126.2, 126.1, 126.0, 125.9, 125.8, 98.0, 76.7, 75.2, 73.7, 73.5, 72.1, 69.0, 68.9, 68.4, 68.2, 67.3, 60.5, 50.6, 50.4, 47.3 (2C), 29.3, 28.1, 27.7, 23.6, 21.2, 14.4; HRMS (ESI): Calcd for $C_{62}H_{61}NO_9$ $[M+Na]^+$ 986.4244, found: 986.4144.

2.5. Synthesis of Galactose linker building block 8:

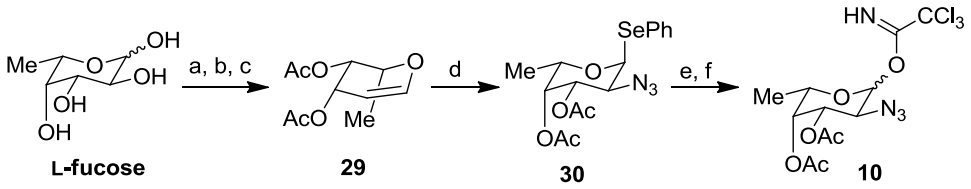


28, R = $(CH_2)_5$NBnCbz **8, R = $(CH_2)_5$NBnCbz**

To a solution of compound **28** (1.5 g, 1.55 mmol) in a mixture of MeOH and THF (2 : 1; 10 mL : 5 mL) at room temperature was added a 0.5 M solution of NaOMe in methanol (0.78 mL, 0.39 mmol) and the reaction mixture heated to 50 ^{o}C for 30 h. The reaction was neutralized with Amberlite 120 H^+ resin, filtered and concentrated. Purification by flash column chromatography using hexanes and ethyl acetate as eluent (0 to 40%) afforded the compound **8** as oil (1.2 g, 90%). $[\alpha]_D^{20}$ = + 61.8º (c = 2.90, $CHCl_3$); IR ν_{max} (film) 3462, 2920,

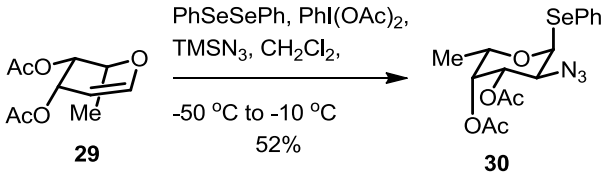
1693, 1226, 1088, 1043, 731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 – 7.67 (m, 8H), 7.57 – 7.41 (m, 6H), 7.39 – 7.06 (m, 15H), 5.19 (s, 2H), 4.99 (d, *J* = 11.8 Hz, 2H), 4.91 (d, *J* = 11.7 Hz, 1H), 4.84 (bd, *J* = 11.4 Hz, 2H), 4.59 (d, *J* = 12.0 Hz, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 4.49 (bd, *J* = 12.7 Hz, 2H), 4.15 (s, 1H), 3.96 (d, *J* = 12.3 Hz, 2H), 3.75 (dd, *J* = 10.0, 5.4 Hz, 1H), 3.68 (dd, *J* = 9.9, 6.3 Hz, 1H), 3.63 (d, *J* = 8.3 Hz, 1H), 3.39 (bs, 1H), 3.23 (bm, 2H), 2.69 (s, 1H), 1.75 – 1.44 (bm, 4H), 1.32 (bm, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 136.1, 135.9, 133.4 (2C), 133.2, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0 (2C), 127.8 (3C), 127.4, 126.8, 126.6, 126.3, 126.2, 126.1 (2C), 125.9, 97.5, 77.8, 76.2, 73.7, 73.5, 73.0, 69.8, 68.6, 68.3, 68.2, 67.3, 29.3, 23.6; HRMS (ESI): Calcd for $C_{55}H_{57}NO_8$ $[M+Na]^+$ 882.3982, found: 882.3918.

2.6. Synthesis of Fucose building block 10:[2]



SI-Scheme 2. Synthesis of FucNAc donor **8**. Reaction conditions: a) Ac_2O, pyridine, quant; b) HBr, AcOH, Ac_2O, 0 ^oC to RT; c) NMI, Zn, EtOAc, 80 ^oC, 84% over two steps; d) Ph_2Se_2, $PhI(OAc)_2$, $TMSN_3$, CH_2Cl_2, -30 ^oC to -10 ^oC, 52%; e) NIS, THF/H_2O, 91%; f) CCl_3CN, K_2CO_3, 98%.

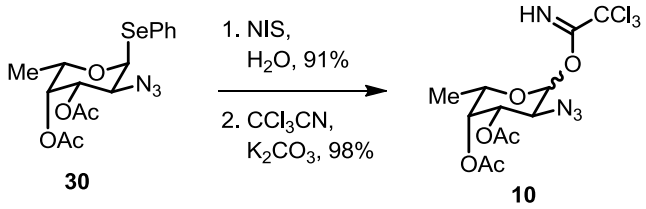
2.7. Synthesis of Fucose building block 30:



To a solution of fucal **29**[2] (14.5 g, 67.7 mmol) and diphenyl diselenide (21.1 g, 67.7 mmol) in CH_2Cl_2 (220 mL) at -50 ^oC was added bisacetate iodobenzene (21.8 g, 67.7 mmol) followed by trimethylsilyl azide (17.9 mL, 135 mmol). The reaction mixture was warmed to -10 ^oC over a period of 1.5 h by which time no starting material was observed by TLC. The solvent was removed under vacuum to obtain the crude as reddish brown oil. Purification by flash column chromatography using cyclohexane and ethyl acetate as eluent (0 to 30%) afforded the compound **30** as oil (14.5 g, 52%). $[\alpha]_D^{20}$ = - 217.1° (c = 1.48, $CHCl_3$); IR ν_{max} (film) 2939, 2109, 1742, 1368, 1219, 1083, 1018, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.63 –

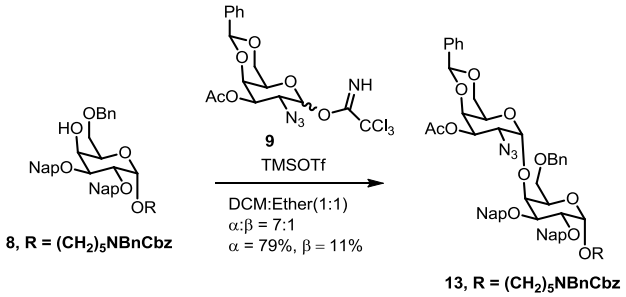
7.53 (m, 2H), 7.35 – 7.23 (m, 3H), 5.96 (d, *J* = 5.4 Hz, 1H), 5.33 (dd, *J* = 3.3, 1.3 Hz, 1H), 5.14 (dd, *J* = 10.9, 3.2 Hz, 1H), 4.51 (q, *J* = 6.5, 0.7 Hz, 1H), 4.24 (dd, *J* = 10.9, 5.4 Hz, 1H), 2.18 (s, 3H), 2.07 (s, 3H), 1.10 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.8, 134.8, 129.3, 129.1, 128.3, 128.2, 128.1, 84.5, 71.7, 70.2, 67.6, 58.9, 20.8, 20.7, 15.9; HRMS (ESI): Calcd for $C_{16}H_{19}N_3O_5Se$ $[M+Na]^+$ 436.0388, found: 436.0400.

2.8. Synthesis of Fucose building block 10:



A solution of azidoselenide **30** (5.0 g, 12.1 mmol) in a mixture of THF, water and acetone (1:1: 0.5; 28 mL: 28 mL: 14 mL) was cooled to 0 ^{o}C. *N*-iodosuccinimide (5.4 g, 24.2 mmol) was added and the reaction mixture stirred at room temperature for 30 min. The reaction was diluted with ethyl acetate and the organic layer washed with sat. aq. $Na_2S_2O_3$ and brine respectively. Dried the organic layer over Na_2SO_4, filtered and concentrated to obtain the oil. Purification by flash column chromatography using hexanes and ethyl acetate as eluent (0 to 60%) afforded the compound as a 1:1 mixture of anomers (3.0 g, 91%). Dissolved the lactol (0.75 g, 2.7 mmol) in dichloroethane, and added trichloroacetonitrile (1.37 mL, 13.7 mmol) at room temperature followed by K_2CO_3 (1.02 g, 7.2 mmol) and stirred for 4 h. Filtered the reaction over celite and washed the celite with dichloromethane and removed the solvents under vacuum to obtain the compound **10** as a mixture of anomers (1.12 g, 98%, α : β = 1 : 5.5). The NMR was clean and matched the one reported in literature and hence taken to the next step without further purification.[3] $[\alpha]_D^{20}$ = - 19.2^{o} (c = 1.64, $CHCl_3$); IR ν_{max} (film) 2993, 2114, 1751, 1729, 1679, 1235, 1216, 1070, 1031, 840, 793 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) reported only β-anomer : δ 8.77 (s, 1H), 5.68 (d, *J* = 8.5 Hz, 1H), 5.25 (dd, *J* = 3.4, 0.9 Hz, 1H), 4.91 (dd, *J* = 10.8, 3.4 Hz, 1H), 3.97 – 3.83 (m, 2H), 2.21 (s, 3H), 2.08 (s, 3H), 1.24 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 169.9, 161.1, 97.0, 71.8, 70.5, 69.4, 60.5, 20.8 (2C), 16.2; HRMS (ESI): Calcd for $C_{12}H_{15}Cl_3N_4O_6$ $[M+Na]^+$ 438.9955, found: 438.9940.

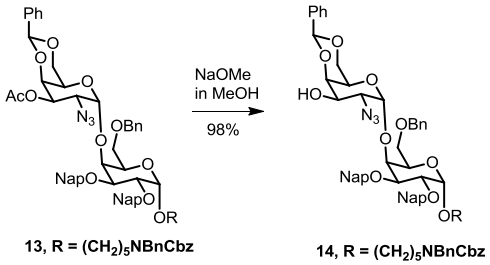
2.9. Synthesis of disaccharide 13:



To a solution of donor **8** (1.75 g, 2.03 mmol) and acceptor **9** (1.26 g, 2.65 mmol) in a mixture of ether and CH_2Cl_2 (1:1; 11.6 mL : 11.6 mL) at 0 ^oC was added TMSOTf (0.037 mL, 0.20 mmol) and the reaction mixture stirred at 0 ^oC for 15 min. Quenched the reaction by adding a drop of Et_3N and removed the solvents under vacuum. Purification by flash column chromatography using hexanes and ethyl acetate as eluent (0 to 20%) afforded α-anomer **13**, (1.89 g, 79%) as oil and β-anomer, (0.26 g, 11%) as oil. The selectivity for the glycosylation ranged from 10:1 to 7:1 (α:β)

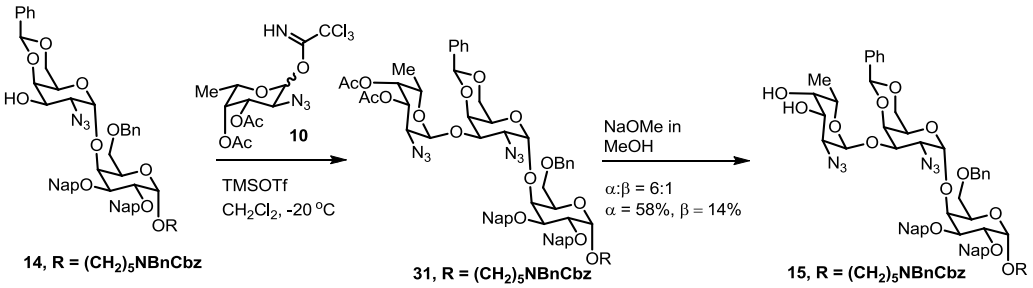
NMR analysis: ^1H nmr analysis of α-anomer showed only one anomeric proton that was distinct with J = 3.5 Hz. The other anomeric proton was embedded within the napthyl methylene protons. For the β-anomer the coupling constant was J = 8.1 Hz. ^{13}C indicated a value of 99.5 and 97.1 ppm for α-anomer and 101.8 and 97.7 ppm for β-anomer. The $J_{C,H}$ coupling for fraction-1 was 170.4 Hz and 166.2 Hz indicating two α-anomeric linkages and was 168.6 Hz and 164.3 Hz for fraction-2 indicating one α- and one β-anomeric linkages. **α-anomer**: $[\alpha]_D^{20}$ = + 129.0^o (c = 1.25, $CHCl_3$); IR ν_{max} (film) 2925, 2109, 1744, 1694, 1225, 1089, 1042, 748 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.87 – 7.70 (m, 7H), 7.58 (d, J = 8.6 Hz, 1H), 7.53 – 7.04 (m, 25H), 5.24 (dd, J = 11.1, 3.3 Hz, 1H), 5.19 (s, 1H), 5.17 (s, 2H), 5.08 (d, J = 3.5 Hz, 1H), 4.97 (d, J = 11.9 Hz, 1H), 4.94 – 4.81 (m, 4H), 4.54 (s, 2H), 4.47 (d, J = 10.0 Hz, 2H), 4.30 (d, J = 2.7 Hz, 1H), 4.14 (d, J = 2.9 Hz, 1H), 4.07 (s, 1H), 4.02 (dd, J = 10.3, 3.6 Hz, 1H), 3.98 – 3.86 (m, 4H), 3.64 – 3.46 (m, 3H), 3.35 (bs, 1H), 3.20 (bm, 2H), 3.02 (d, J = 11.7 Hz, 1H), 2.16 (s, 3H), 1.56 (bm, 4H), 1.38 – 1.06 (bm, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 138.1, 137.8, 137.7, 136.1, 133.4 (2C), 133.2, 133.0, 129.1, 128.7, 128.6 (2C), 128.3 (2C), 128.2 (2C), 128.1, 128.0 (2C), 127.9, 127.8, 127.4, 127.2, 126.6, 126.3 (2C), 126.2, 126.0 (2C), 125.9, 125.5, 100.5, 99.0, 97.3, 77.2, 76.5, 74.6, 73.7, 73.4, 73.3, 73.1, 70.4, 69.0, 68.8, 68.3, 67.2, 62.3, 58.0, 29.3, 23.6, 21.2; HRMS (ESI): Calcd for $C_{70}H_{72}N_4O_{13}$ $[M+Na]^+$ 1199.4994, found: 1199.4902.

2.10. Synthesis of disaccharide 14:



To a solution of the compound **13** (1.85 g, 1.57 mmol) in a mixture of methanol and THF (2 : 1; 16 mL : 8 mL) was added a 0.5 M solution of NaOMe in MeOH (0.31 mL, 0.15 mmol) and stirred for 12 h at room temperature. The reaction was neutralized using Amberlite 120 H^+ resin, filtered, and concentrated. Purification by flash column chromatography using hexanes and ethyl acetate as eluent (0 to 20%) afforded the compound **14** (1.74 g, 98%) as white foam. $[\alpha]_D^{20}$ = + 88.9º (c = 1.15, $CHCl_3$); IR ν_{max} (film) 3474, 2925, 2111, 1740, 1694, 1234, 1088, 1041, 748 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.68 (m, 8H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.55 – 7.43 (m, 5H), 7.42 – 7.07 (m, 20H), 5.18 (bs, 3H), 5.01 (d, *J* = 3.4 Hz, 1H), 4.98 (s, 1H), 4.94 (s, 1H), 4.92 – 4.78 (m, 3H), 4.54 (s, 2H), 4.48 (bd, *J* = 9.2 Hz, 2H), 4.28 (s, 1H), 4.03 – 3.83 (m, 6H), 3.66 – 3.52 (m, 4H), 3.50 (dd, *J* = 10.5, 3.4 Hz, 1H), 3.47 – 3.32 (bm, 1H), 3.30 – 3.13 (m, 2H), 3.07 (d, *J* = 11.6 Hz, 1H), 1.79 – 1.43 (bm, 4H), 1.41 – 1.10 (bm, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 137.7, 137.6, 136.1, 135.9, 133.4, 133.3, 133.2, 133.0, 129.4, 128.7, 128.6 (2C), 128.4, 128.3, 128.2 (2C), 128.1 (2C), 128.0, 127.9 (2C), 127.3, 126.7, 126.4 (2C), 126.3, 126.2, 126.1, 125.9, 125.6, 101.0, 99.2, 97.0, 77.2, 75.5, 75.4, 74.5, 73.7, 73.0, 72.8, 69.1, 69.0, 68.3, 67.7, 67.3, 62.6, 61.4, 29.3, 23.6; HRMS (ESI): Calcd for $C_{68}H_{70}N_4O_{12}$ $[M+Na]^+$ 1157.4888, found: 1157.4923.

2.11. Synthesis of trisaccharide 15:

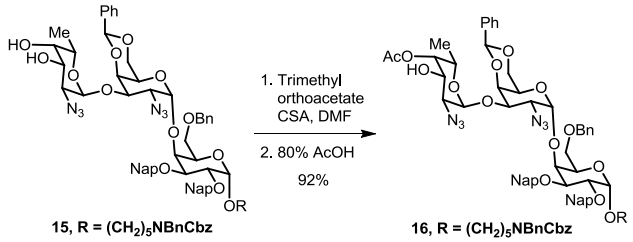


To a solution of donor compound **10** (0.5 g, 1.2 mmol) and acceptor **14** (1.05 g, 0.92 mmol) in DCM (10 mL) at -20 ^oC was added TMSOTf (0.017 mL, 0.092 mmol) and the reation mixture warmed to 0 ^oC over 30 min. Quenched the reaction by addition of two drops of Et_3N, and evaporated. Purification by flash column chromatography using toluene and acetone as eluent

(0 to 25%) afforded the compound **31** as a mixture of anomers that could not be separated easily at this step. To a solution of trisaccharide **31** in a mixture of methanol and THF (2 : 1; 7 mL : 3.5 mL) was added 0.5 M solution of NaOMe in MeOH (0.185 mL, 0.092 mmol) and stirred at room temperature for 12 h. The reaction was neutralized with Amberlite 120 H^+ resin, filtered, and concentrated. Purification by flash column chromatography using hexanes and ethyl acetate as eluent (0 to 25%) afforded α-anomer **14** (0.70 g, 58%) and β-anomer (0.12 g, 14%) as white foams.

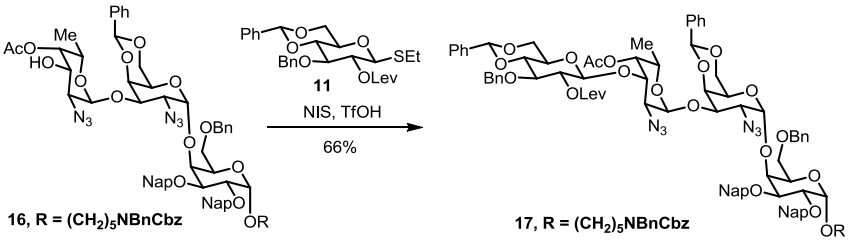
NMR analysis: ^{1}H nmr of α-anomer contained three α-anomeric protons with chemical shift and coupling constant of 5.09 ppm (J = 3.5 Hz), 4.99 ppm, and 4.83 ppm (J = 3.6 Hz). ^{13}C had values of 100.5, 99.2, and 97.0 ppm. The $J_{C,H}$ coupling was 174.5, 168.4, and 167.0 Hz indicating three α-anomeric linkages. ^{1}H nmr of β-anomer contained two α-anomeric protons based on chemical shift and coupling constant of 5.00 ppm (J = 3.5 Hz), and 4.91 ppm and he β-anomeric proton at 4.06 ppm (J = 7.1 Hz). ^{13}C had a value of 99.1 (2C) and 97.0 ppm The $J_{C,H}$ coupling was 168.6, and 167.1 Hz indicating two α-anomeric linkages, the β linkage overlapped with one of the α and hence could not be calculated. **α-anomer**: $[\alpha]_D^{20}$ = + 71.6º (c = 1.06, $CHCl_3$); IR ν_{max} (film) 3488, 2923, 2111, 1740, 1694, 1234, 1088, 1040, 747 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.87 – 7.78 (m, 5H), 7.78 – 7.71 (m, 2H), 7.57 (d, J = 8.2 Hz, 1H), 7.52 – 7.42 (m, 5H), 7.41 – 7.08 (m, 20H), 5.21 (s, 1H), 5.18 (bs, 2H), 5.09 (d, J = 3.5 Hz, 1H), 5.00 (bs, 1H), 4.95 (bs, 2H), 4.91 (bs, 2H), 4.83 (d, J = 3.7 Hz, 1H), 4.60 – 4.50 (m, 2H), 4.47 (bs, 2H), 4.34 (s, 1H), 4.17 (q, J = 7.4 Hz, 1H), 4.22 – 4.11 (m, 1H), 4.10 – 3.85 (m, 6H), 3.83 (dd, J = 10.8, 3.2 Hz, 1H), 3.72 (dd, J = 10.8, 3.5 Hz, 1H), 3.70 – 3.50 (m, 5H), 3.41 (bs, 1H), 3.21 (bm, 2H), 3.09 (d, J = 11.7 Hz, 1H), 2.47 (s, 1H, OH), 2.23 (s, 1H, OH), 1.59 (bm, 4H), 1.28 (bm, 2H), 1.14 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 137.8, 137.7, 136.1, 136.0, 133.4 (2C), 133.2, 133.1, 129.2, 128.7 (2C), 128.7, 128.6, 128.4, 128.2, 128.1, 128.0 (2C), 127.9, 127.1, 126.4 (2C), 126.3, 126.1 (2C), 125.9, 125.6, 100.9, 100.5, 99.3, 97.0, 77.5, 77.4 (2C), 77.2, 77.0, 76.9, 75.7, 75.5, 74.1, 73.7, 73.1, 72.6, 71.7, 69.2, 69.0, 68.8, 68.3, 67.4, 67.3, 66.7, 62.4, 61.1, 59.0, 29.3, 23.6, 16.5; HRMS (ESI): Calcd for $C_{74}H_{79}N_7O_{15}$ $[M+Na]^+$ 1328.5532, found: 1328.5551.

2.12. Synthesis of trisaccharide 16:



To a solution of the trisaccharide compound **15** (0.65 g, 0.40 mmol) in DMF (2.4 mL) at room temperature was added trimethyl orthoacetate (0.38 mL, 2.99 mmol) and *p*-TSA (0.014 g, 0.075 mmol) and the reaction mixture stirred for 30 min. Triethylamine (4 drops) was added and the solvent removed under vacuum using toluene as an azeotrop. To the crude was added 80% acetic acid (4.66 mL) and the reaction mixture stirred for 1 h at room temperature. The solvent was removed under vacuum, azeotroped with toluene to obtain oil. Purification by flash column chromatography using hexanes and ethyl acetate as eluent (10 to 50%) afforded the compound **16** as white foam (0.62 g, 92%). $[\alpha]_D^{20}$ = + 66.5º (c = 1.10, $CHCl_3$); IR ν_{max} (film) 2925, 2112, 1746, 1697, 1233, 1089, 1042, 746 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.89 – 7.79 (m, 5H), 7.76 (dd, *J* = 6.7, 2.6 Hz, 2H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.54 – 7.43 (m, 5H), 7.42 – 7.21 (m, 19H), 7.17 (s, 1H), 5.20 (bs, 3H), 5.10 (d, *J* = 3.5 Hz, 1H), 5.08 (dd, *J* = 3.4, 1.2 Hz, 1H), 5.01 (bs, 1H), 4.98 – 4.89 (m, 4H), 4.83 (d, *J* = 3.7 Hz, 1H), 4.61 – 4.51 (m, 2H), 4.48 (d, *J* = 6.7 Hz, 2H), 4.35 (s, 1H), 4.26 (q, *J* = 6.4 Hz, 1H), 4.19 (dd, *J* = 10.7, 3.5 Hz, 1H), 4.05 (d, *J* = 3.0 Hz, 1H), 4.04 – 3.93 (m, 4H), 3.89 (t, *J* = 8.8 Hz, 1H), 3.83 (dd, *J* = 10.8, 3.2 Hz, 1H), 3.72 (dd, *J* = 10.8, 3.4 Hz, 1H), 3.67 (d, *J* = 11.7 Hz, 1H), 3.64 – 3.50 (m, 3H), 3.42 (bs, 1H), 3.22 (bm, 2H), 3.10 (d, *J* = 11.7 Hz, 1H), 2.22 (s, 3H), 1.57 (bm, 4H), 1.40 – 1.16 (bm, 2H), 1.00 (d, *J* = 6.6 Hz, 3H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 138.1, 137.7, 136.0 (2C), 133.4 (2C), 133.2, 133.1, 129.3, 128.7, 128.6 (2C), 128.4 (2C), 128.2, 128.1, 128.0 (3C), 127.9, 127.4, 127.1, 126.4, 126.3, 126.1 (2C), 125.9, 125.6, 101.0, 100.5, 99.2, 97.0, 77.4, 77.1, 75.7, 75.5, 74.0, 73.7, 73.1, 72.5, 69.2, 68.9, 68.3, 67.6, 67.3 (2C), 65.8, 62.3, 61.0, 59.0, 29.3, 23.6, 21.0, 16.5.; HRMS (ESI): Calcd for $C_{76}H_{81}N_7O_{16}$ $[M+Na]^+$ 1370.5637, found: 1370.5479.

2.13. Synthesis of tetrasaccharide 17:



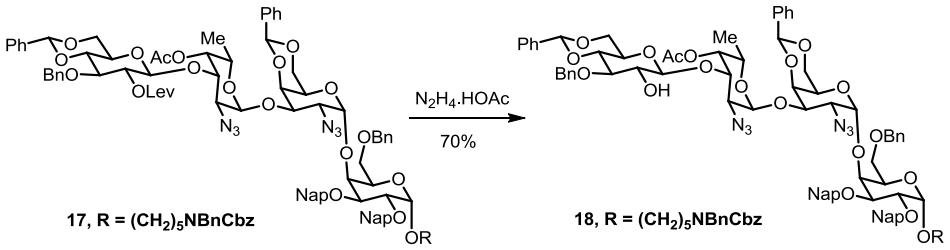
Stirred a solution of acceptor **16** (0.25 g, 0.18 mmol), donor **11** (0.14 g, 0.27 mmol) (dried under vaccum overnight) and activated 4 Å MS (0.39 g) in DCM (2 mL) for 1 h at room temperature. After cooling to -30 ^{o}C added NIS (0.063 g, 0.27 mmol) followed by TfOH (8.2 µL, 0.093 mmol) and stirred the reaction mixture for 1h. Quenched the reaction using 0.05 mL of Et_3N. Diluted the RM with DCM and washed the organic layer with sat. aq. $Na_2S_2O_3$, water, brine, dried over Na_2SO_4, filtered and concentrated to obtain yellow oil. Purified the crude by flash chromatography using hexanes and ethyl acetate as eluent (10% to 50%) to obtain the compound **17** as white foam (0.22, 66%).

NMR analysis: ^{1}H nmr indicated three α- and one β-anomeric protons based on chemical shift and coupling constant at 5.02 ppm (J = 3.5 Hz), 4.98 ppm, and 4.70 ppm (J = 3.6 Hz) for α and 4.57 ppm (J = 7.6 Hz) for β. ^{13}C indicated a value of 100.8, 99.1 and 97.0 ppm for α and 100.0 for β. The $J_{C,H}$ coupling was 173.9, 172.0, and 170.6 Hz for three α-anomeric and 164.9 Hz for the β-anomeric linkages.

$[\alpha]_D^{20}$ = + 36.6º (c = 0.90, $CHCl_3$); IR ν_{max} (film) 2925, 2111, 1746, 1696, 1233, 1089, 1042, 748 cm^{-1}; ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.02 – 7.73 (m, 8H), 7.64 (d, J = 8.1 Hz, 1H), 7.60 (dd, J = 8.5, 1.6 Hz, 1H), 7.55 – 7.20 (m, 34H), 5.71 (s, 1H), 5.36 (s, 1H), 5.25 (d, J = 2.6 Hz, 1H), 5.15 (m, 3H), 5.06 (s, 1H), 5.02 – 4.86 (m, 6H), 4.83 (d, J = 6.7 Hz, 1H), 4.80 (d, J = 2.6 Hz, 1H), 4.72 (d, J = 11.9 Hz, 1H), 4.60 (s, 2H), 4.51 (s, 2H), 4.39 (s, 1H), 4.34 (dd, J = 10.9, 3.3 Hz, 1H), 4.30 – 4.20 (m, 3H), 4.17 (s, 1H), 4.09 – 3.91 (m, 5H), 3.86 – 3.70 (m, 4H), 3.71 – 3.59 (m, 3H), 3.57 (dd, J = 10.9, 3.7 Hz, 1H), 3.54 – 3.38 (m, 2H), 3.33 (dd, J = 12.4, 1.4 Hz, 1H), 3.24 (bs, 2H), 2.78 – 2.64 (m, 2H), 2.64 – 2.48 (m, 2H), 2.13 (s, 3H), 2.12 (s, 3H), 1.57 (bs, 4H), 1.37 (bs, 2H), 0.98 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 172.0, 170.9, 139.7 (2C), 139.6, 139.4, 139.0, 137.7, 134.4, 134.3, 134.0, 133.9, 129.8, 129.7, 129.6, 129.3 (2C), 129.2, 129.1, 129.0, 128.9 (2C), 128.8 (2C), 128.7 (2C), 128.6 (2C), 128.5, 128.3, 128.0, 127.5, 127.3, 127.2 (2C), 127.1, 127.0, 126.8, 126.7, 126.5, 126.2, 101.8, 101.6, 101.4, 100.1, 98.0, 82.1, 80.2, 78.3, 78.3, 76.4, 76.3, 75.2, 74.6, 74.2, 74.1, 73.9, 73.1, 72.6, 71.1, 70.0, 69.6, 69.3, 68.6, 67.5, 66.8, 66.7, 63.4, 60.3, 59.7, 38.2, 30.0, 29.9, 28.7,

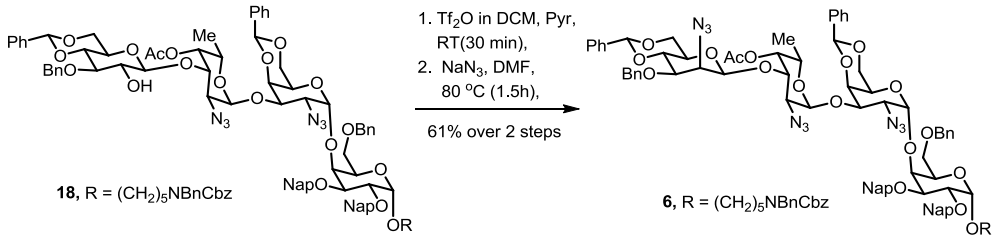
24.2, 20.8, 16.8; HRMS (ESI): Calcd for $C_{101}H_{107}N_7O_{23}$ $[M+Na]^+$ 1808.7316, found: 1808.7196.

2.14. Synthesis of tetrasaccharide 18:



Compound **17** (0.18 g, 0.10 mmol) was dissolved in a mixture of toluene, ethanol and DCM (2 : 1 : 0.5; 3.6 mL : 1.8 mL : 0.9 mL). Hydrazine acetate (0.046 g, 0.50 mmol) was then added. After 30 min at room temperature diluted the reaction with water and extracted the aqueous with ether. Washed the organic layer with brine, dried over Na_2SO_4, filtered, and concentrated to obtain oil Purified the crude by flash chromatography using hexanes and ethyl acetate as eluent, (0 to 30%) to obtain the compound **18** as oil (0.12 g, 70%). $[\alpha]_D^{20}$ = + 42.0º (c = 1.00, $CHCl_3$); IR ν_{max} (film) 3494, 2869, 2111, 1730, 1695, 1234, 1088, 1041, 746 cm^{-1}; ^1H NMR (400 MHz, Acetone-d_6) δ 8.04 – 7.73 (m, 8H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.60 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.56 – 7.07 (m, 34H), 5.66 (s, 1H), 5.38 (s, 1H), 5.34 (d, *J* = 2.5 Hz, 1H), 5.16 (d, *J* = 3.1 Hz, 3H), 5.06 (s, 1H), 5.02 – 4.88 (m, 5H), 4.86 (q, *J* = 12.0 Hz, 2H), 4.62 (s, 1H), 4.61 (s, 2H), 4.51 (s, 2H), 4.41 (s, 1H), 4.38 (dd, *J* = 10.9, 3.3 Hz, 1H), 4.33 (q, *J* = 6.1 Hz, 1H), 4.28 (d, *J* = 2.7 Hz, 1H), 4.24 – 4.17 (m, 2H), 4.14 (d, *J* = 4.0 Hz, 1H), 4.10 – 3.93 (m, 5H), 3.83 – 3.71 (m, 2H), 3.70 – 3.55 (m, 6H), 3.49 – 3.38 (m, 3H), 3.34 (dd, *J* = 12.4, 1.5 Hz, 1H), 3.24 (bs, 2H), 2.17 (s, 3H), 1.57 (bs, 4H), 1.42 – 1.24 (bs, 2H), 1.03 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 171.1, 139.5, 138.8, 138.7, 138.5, 138.3, 136.8 (2C), 133.5, 133.4, 133.1, 133.0, 128.7, 128.6, 128.4 (2C), 128.3, 128.0 (2C), 127.9 (3C), 127.8, 127.7 (2C), 127.6, 127.5, 127.1, 126.6, 126.4, 126.3, 126.2 (2C), 126.1, 125.9, 125.8, 125.6, 101.5, 101.0, 100.7, 100.5, 99.2, 97.1, 81.3, 81.1, 77.4, 77.2, 75.6, 75.4, 74.7, 74.6, 74.2, 74.0, 73.5, 73.0, 72.3, 71.7, 70.2, 69.1, 68.7, 68.5, 67.7, 66.6, 66.2, 65.7, 62.6, 59.2, 59.02, 29.1, 23.3, 20.1, 15.9; HRMS (ESI): Calcd for $C_{96}H_{101}N_7O_{21}$ $[M+Na]^+$ 1710.6948, found: 1710.6809.

2.15. Synthesis of tetrasaccharide 6:



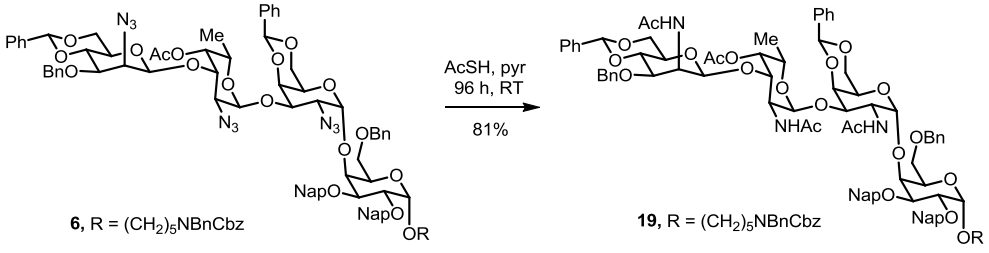
To a solution of compound **18** (0.05 g, 0.03 mmol) in DCM (0.6 mL) was added pyridine (0.06 mL, 0.74 mmol) followed by a 1.0 M solution of triflic anhydride in DCM (0.089 mL, 0.089 mmol) and stirred the reaction mixture at room temperature for 30 min. Quenched the reaction with sat. aq. $NaHCO_3$ and extracted the aqueous with DCM, washed with brine, dried over Na_2SO_4, filtered, and concentrated to obtain oil which was dried on high vacuum for 2 h. The crude was then taken in DMF (0.6 mL), added NaN_3 (0.0057 g, 0.089 mmol) and heated the reaction to 80 ^oC for 1.5 h. After cooling to room temperature, diluted the reaction with sat. aq. NH_4Cl and extracted the aqueous layer with ethyl acetate. Washed the organic layer with brine, dried over Na_2SO_4, filtered, and concentrated to obtain oil. Purified the crude by flash chromatography using hexanes and ethyl acetate as eluent (0 to 40%) to obtain the compound **6** as white foam (0.031 g, 61%).

NMR analysis: ^1H nmr showed a coupling constant of J= 1.1 Hz for the β-mannoside linkage which before inversion was 7.2 Hz. The $J_{C,H}$ coupling was 180.0, 176.0, 172.0 and 160.0 Hz

$[\alpha]_D^{20}$ = + 21.0^o (c = 1.50, $CHCl_3$); IR ν_{max} (film) 2916, 2860, 2112, 1737, 1697, 1234, 1088, 1045, 746 cm^{-1}; ^1H NMR (400 MHz, Acetone-d_6) δ 8.01 – 7.76 (m, 8H), 7.64 (d, *J* = 8.6 Hz, 1H), 7.60 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.55 – 7.17 (m, 34H), 5.68 (s, 1H), 5.36 (s, 1H), 5.30 (d, *J* = 3.3 Hz, 1H), 5.15 (d, *J* = 3.4 Hz, 3H), 5.07 (s, 1H), 5.02 (d, *J* = 1.3 Hz, 1H), 5.01 – 4.90 (m, 5H), 4.82 (d, *J* = 12.2 Hz, 1H), 4.74 (d, *J* = 12.2 Hz, 1H), 4.60 (s, 2H), 4.51 (s, 2H), 4.40 (s, 1H), 4.37 (dd, *J* = 10.9, 3.3 Hz, 1H), 4.35 – 4.23 (m, 2H), 4.17 (s, 1H), 4.14 (dd, *J* = 10.4, 4.9 Hz, 1H), 4.09 – 3.94 (m, 6H), 3.93 – 3.83 (m, 2H), 3.79 (t, *J* = 10.3 Hz, 1H), 3.74 (dd, *J* = 10.9, 3.4 Hz, 1H), 3.63 (m, 3H), 3.55 (dd, *J* = 10.9, 3.7 Hz, 1H), 3.51 – 3.36 (m, 2H), 3.33 (d, *J* = 11.0 Hz, 1H), 3.24 (bs, 2H), 2.16 (s, 3H), 1.57 (bs, 4H), 1.36 (bs, 2H), 1.04 (d, *J* = 6.5 Hz, 3H).; ^{13}C NMR (100 MHz, Acetone-d_6) δ 171.5, 139.7, 139.6 (2C), 139.4, 139.1, 137.7 (2C), 134.4, 134.3, 134.0, 133.9, 129.7, 129.6, 129.3 (2C), 129.2, 129.1, 128.9 (3C), 128.8, 128.7, 128.6 (2C), 128.5, 128.4, 128.0, 127.5, 127.3, 127.2, 127.1, 127.0, 126.8, 126.7, 126.5, 102.2, 101.5 (2C), 100.2, 98.7, 98.0, 79.2, 78.3, 78.2, 77.7, 76.5, 76.3, 75.1, 74.3, 73.9, 73.2, 73.1,

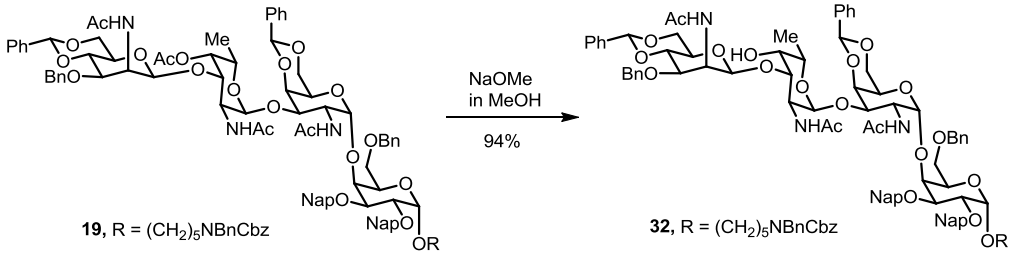
72.6, 70.8, 69.9, 69.6, 69.1, 68.6, 68.0, 67.5, 66.2, 64.7, 63.4, 60.1, 59.7, 30.0, 24.2, 20.9, 16.7; HRMS (ESI): Calcd for $C_{96}H_{100}N_{10}O_{20}$ $[M+Na]^+$ 1735.7013, found: 1735.6995.

2.16. Synthesis of tetrasaccharide 19:



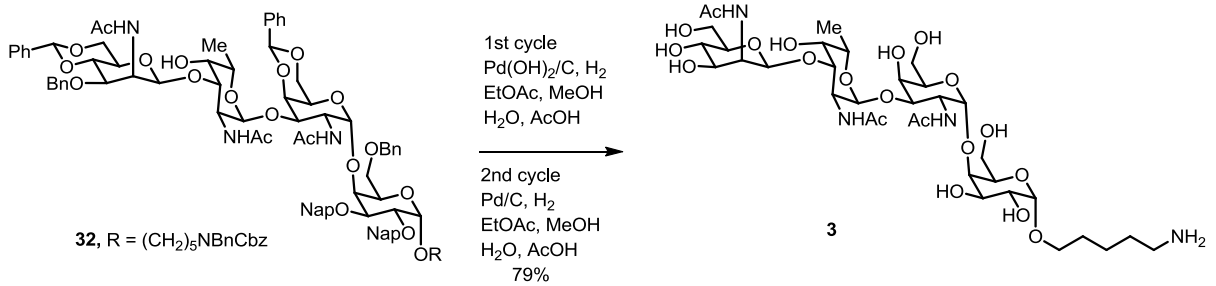
To a solution of compound **6** (0.03 g, 0.018 mmol) in pyridine (0.5 mL) was added thioacetic acid (0.15 mL, 2.18 mmol) and the reaction stirred at room temperature for 96 h. (Since the reaction was not able to be monitored by TLC, LC-MS was taken after 48 h and it showed the presence of diacetamide. Added further 0.07 mL of thioacetic acid and continued stirring for additional 48h (LC-MS showed no starting material or mono- or diacetamide). The solvent were removed under vacuo and the crude azeotroped twice with toluene. Purified the crude by flash chromatography using DCM, acetone and MeOH as eluent (5% each of MeOH and acetone upto 50 %) to obtain the compound **19** as foam (0.025 g, 81%). $[\alpha]_D^{20}$ = + 28.0º (c = 1.38, $CHCl_3$); IR ν_{max} (film) 3434, 2934, 2860, 1738, 1674, 1234, 1093, 1045, 749 cm^{-1}; ^1H NMR (400 MHz, Acetone-d_6) δ 7.89 (m, 8H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.65 – 7.54 (m, 2H), 7.55 – 7.14 (m, 34H), 6.47 (d, *J* = 9.8 Hz, 1H), 6.24 (d, *J* = 9.1 Hz, 1H), 5.55 (s, 1H), 5.44 (s, 1H), 5.24 (d, *J* = 2.5 Hz, 1H), 5.14 (s, 2H), 5.10 (d, *J* = 3.6 Hz, 1H), 5.06 – 4.94 (m, 5H), 4.90 (d, *J* = 12.7 Hz, 1H), 4.81 (d, *J* = 1.7 Hz, 1H), 4.77 (d, *J* = 11.9 Hz, 1H), 4.74 – 4.66 (m, 1H), 4.57 (d, *J* = 11.7 Hz, 1H), 4.53 – 4.45 (m, 5H), 4.41 (s, 1H), 4.39 – 4.28 (m, 3H), 4.28 – 4.22 (m, 1H), 4.20 (dd, *J* = 10.0, 4.7 Hz, 1H), 4.11 (dd, *J* = 10.3, 3.4 Hz, 1H), 4.08 – 3.97 (m, 4H), 3.84 (t, *J* = 9.6 Hz, 1H), 3.79 – 3.52 (m, 6H), 3.40 (m, 3H), 3.22 (s, 2H), 2.13 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.94 (s, 3H), 1.56 (bs, 4H), 1.41 – 1.30 (m, 2H), 1.14 (d, *J* = 6.5 Hz, 3H).; ^{13}C NMR (100 MHz, Acetone-d_6) δ 171.6, 171.0, 170.4 (2C), 139.8 (2C), 139.5, 139.3, 139.2, 137.8, 137.6, 134.3 (2C), 134.0, 133.9, 129.5, 129.3, 129.2, 129.2, 128.9, 128.8 (4C), 128.7, 128.6 (4C), 128.5 (2C), 128.0 (2C), 127.2, 127.1, 127.0, 126.9, 126.8, 126.6, 126.4 (2C), 102.2, 101.9, 101.4, 100.0, 98.4, 98.2, 79.1, 78.6, 77.3, 76.9, 76.7 (2C), 73.8, 73.7, 73.0 (2C), 71.3, 70.5, 70.2, 69.8, 69.2, 68.7 (2C), 68.0, 67.4, 65.8, 63.3, 50.8, 49.6, 49.4, 30.0, 24.2, 23.8, 23.7, 23.2, 20.8, 16.9; HRMS (ESI): Calcd for $C_{102}H_{112}N_4O_{23}$ $[M+Na]^+$ 1783.7615, found: 1783.7609.

2.17. Synthesis of tetrasaccharide 32:



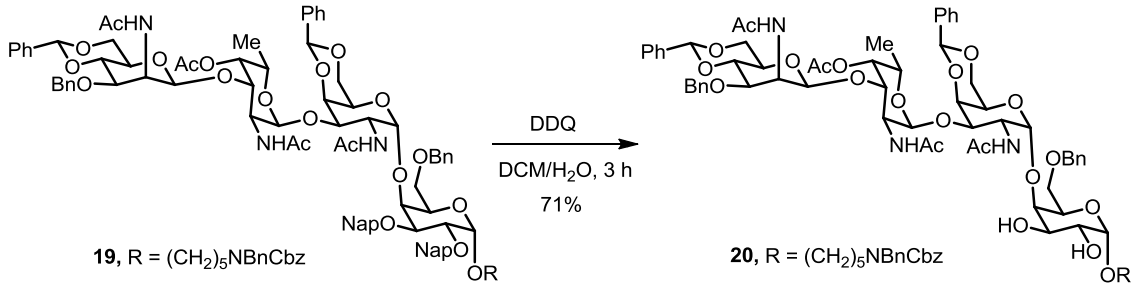
To a solution of compound **19** (0.021 g, 0.012 mmol) in MeOH (0.45 mL) was added a solution of 0.5 M NaOMe in MeOH (5.96 µL, 2.98 µmol) and the reaction stirred for 2.5 h at room temperature. Diluted the reaction with MeOH, neutralized with Amberlite 120 H^+ resin, filtered, and concentrated to obtain the compound **32** as foam (0.0193 g, 94%). $[\alpha]_D^{20}$ = + 49.1º (c = 1.90, $CHCl_3$); IR ν_{max} (film) 3354, 2929, 2860, 1667, 1372, 1096, 1045, 751 cm^{-1}; ^1H NMR (400 MHz, Acetone-d_6) δ 8.02 – 7.78 (m, 8H), 7.73 (d, *J* = 7.9 Hz, 1H), 7.63 – 7.53 (m, 2H), 7.53 – 7.17 (m, 34H), 7.10 (d, *J* = 9.5 Hz, 1H), 6.46 (d, *J* = 9.1 Hz, 1H), 5.56 (s, 1H), 5.42 (s, 1H), 5.14 (s, 2H), 5.10 (d, *J* = 3.6 Hz, 1H), 5.05 – 4.81 (m, 9H), 4.78 – 4.68 (m, 1H), 4.61 – 4.45 (m, 6H), 4.45 – 4.25 (m, 4H), 4.20 (dd, *J* = 10.1, 4.8 Hz, 1H), 4.17 (d, *J* = 6.5 Hz, 1H), 4.13 (dd, *J* = 10.3, 3.6 Hz, 1H), 4.09 – 3.97 (m, 3H), 3.94 (d, *J* = 9.9 Hz, 1H), 3.91 – 3.82 (m, 2H), 3.75 (d, *J* = 10.1 Hz, 1H), 3.73 – 3.52 (m, 5H), 3.50 – 3.29 (m, 4H), 3.21 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.55 (bs, 4H), 1.39 – 1.30 (m, 2H), 1.26 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 172.4, 170.5, 170.4, 139.9, 139.8, 139.5, 139.3, 139.1, 137.8, 137.6, 134.3 (2C), 134.0, 133.8, 130.3, 130.0, 129.6, 129.5, 129.3, 129.2 (3C), 129.0, 128.9, 128.8 (2C), 128.7 (2C), 128.6 (3C), 128.5, 128.4 (2C), 128.1, 128.0, 127.1 (3C), 127.0, 126.9, 126.8, 126.6, 126.4, 102.2, 102.0, 101.3, 100.0, 99.4 (2C), 79.0, 78.6, 78.1, 77.0, 76.8, 76.7, 74.6, 73.7, 73.1, 73.0, 71.4, 70.2, 69.9, 69.6, 69.2, 68.7, 68.6, 68.0, 67.4, 67.3, 63.3, 51.8, 49.5, 48.7, 24.2, 23.6 (2C), 23.2, 17.2; HRMS (ESI): Calcd for $C_{100}H_{110}N_4O_{22}$ $[M+Na]^+$ 1741.7509, found: 1741.7503.

2.18. Synthesis of tetrasaccharide 3:



To compound **32** (0.019 g, 0.011 mmol) was added MeOH, water, ethyl acetate and acetic acid (3:1:1:0.1-0.3 mL: 0.1 mL:0.1 mL:10 µL). Purged the reaction mixture before and after addition of $Pd(OH)_2$ (20%) (0.036 g, 0.052 mmol) with argon for 2-3 min. The reaction mixture was purged with hydrogen and stirred for 24 h under an atmosphere of hydrogen. Filtered off palladium over celite, washed with methanol and 1:1 mixture of MeOH and water, concentrated the filtrate to obtain oil. (LCMS at this point showed no SM but onlypartially debenzylated compounds). Resubjected the crude for a second cycle with the same solvent combination, Pd/C (0.036 g, 10% Pd; 50% water) stirred for 20 h. Filtration as above, followed by LCMS showed only compound. Washed the crude with hexanes and decanted followed by acetone and decantation to obtain the compound **3** as foam (0.0075 g, 79%). $[\alpha]_D^{20}$ = + 33.3º (c = 0.56, H_2O)[4]; IR ν_{max} (film) 3354, 2929, 2860, 1667, 1372, 1096, 1045, 751 cm^{-1}; ^1H NMR (400 MHz, d_2o) δ 5.10 (d, *J* = 2.3 Hz, 1H), 5.05 (d, *J* = 2.5 Hz, 1H), 4.94 (s, 1H), 4.90 (d, *J* = 3.8 Hz, 1H), 4.53-4.48 (m, 2H), 4.45 (dd, *J* = 11.2, 3.7 Hz, 1H), 4.24 (s, 2H), 4.18-4.07 (m, 4H), 4.07 – 3.92 (m, 5H), 3.93 – 3.82 (m, 2H), 3.82 – 3.68 (m, 5H), 3.67 – 3.52 (m, 2H), 3.46-3.39 (m, 1H), 3.06 (t, *J* = 8.0 Hz, 2H) 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 1.79-1.66 (m, 4H), 1.56-1.46 (m, 2H), 1.30 (d, *J* = 6.5 Hz, 3H).; ^{13}C NMR (100 MHz, d_2o) δ 175.4, 173.6 (2C), 98.7, 98.3, 98.2, 95.2, 77.2, 76.3, 73.2, 72.6, 71.7, 71.5, 70.3, 69.0, 68.1 (2C), 67.9, 67.6, 66.8 (2C), 60.4, 60.3, 60.2, 53.3, 49.0, 47.6, 39.3, 27.9, 26.4, 22.3, 22.1, 21.9, 21.8, 15.4; HRMS (ESI): Calcd for $C_{35}H_{62}N_4O_{20}$ $[M+Na]^+$ 881.3855, found: 881.3790.

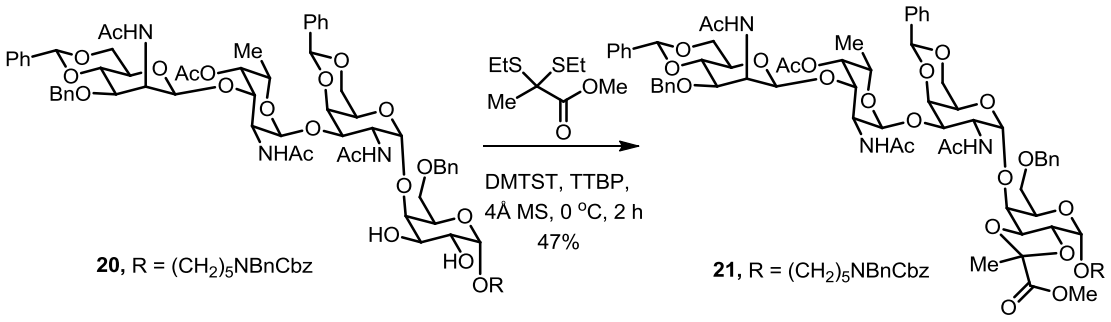
2.19. Synthesis of tetrasaccharide 20:



To a solution of compound **19** (0.035 g, 0.02 mmol) in a mixture of DCM (1.08 mL) and water (0.06 mL) was added DDQ (0.014 g, 0.06 mmol) at room temperature and stirred for 3 h. Diluted the reaction mixture with water and DCM. Washed the organic layer with sat aq. $Na_2S_2O_3$, and sat. aq. $NaHCO_3$ solution, dried over Na_2SO_4, filtered and concentrated to obtain the crude as oil. Purified the crude by flash chromatography [hexanes and ethyl acetate as eluent (0 to 50%)] to obtain the compound **20** as foam (0.021g, 71%). $[\alpha]_D^{20}$ = + 12.2º (c = 0.50, $CHCl_3$); IR ν_{max} (film) 3384, 2936, 1740, 1661, 1373, 1091, 1026, 698 cm^{-1}; ^1H NMR

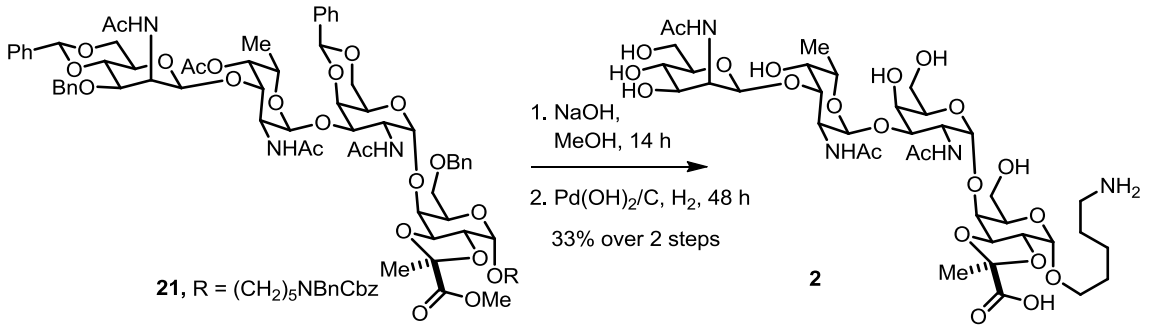
(600 MHz, Acetone-d_6) δ 7.52 (d, *J* = 7.1 Hz, 2H), 7.48 (d, *J* = 6.5 Hz, 2H), 7.45 – 7.08 (m, 26H), 5.65 (s, 1H), 5.56 (s, 1H), 5.27 (d, *J* = 1.5 Hz, 1H), 5.17 (s, 2H), 5.14 – 5.07 (m, 1H), 5.05 (d, *J* = 1.7 Hz, 1H), 4.92 – 4.74 (m, 3H), 4.73 – 4.67 (m, 1H), 4.62 (d, *J* = 5.8 Hz, 1H), 4.54 – 4.49 (m, 5H), 4.51 (d, *J* = 14.2 Hz, 3H), 4.45 (d, *J* = 3.4 Hz, 1H), 4.40 (d, *J* = 6.3 Hz, 1H), 4.28 – 4.17 (m, 2H), 4.17 – 4.10 (m, 3H), 4.10 – 4.02 (m, 2H), 4.01 (bs, 1H), 3.85 (t, *J* = 9.6 Hz, 2H), 3.81 – 3.49 (m, 7H), 3.46 – 3.33 (m, 2H), 3.25 (bs, 2H), 2.11 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.65 – 1.46 (m, 4H), 1.43 – 1.23 (m, 2H), 1.17 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (151 MHz, acetone-d_6) δ 171.6, 170.9, 170.5 (2C), 139.9 (2C), 139.5, 139.4, 139.2, 129.6, 129.3, 129.2, 128.9 (2C), 128.8, 128.7, 128.6, 128.5, 128.4, 128.1, 127.2 (2C), 102.3, 101.9, 101.6, 100.1, 99.5, 98.2, 79.2, 77.3 (2C), 76.8, 76.4, 73.8, 73.7, 73.6, 71.4, 71.3, 71.2, 71.0, 70.5, 70.4, 70.0, 69.2, 69.0, 68.7, 68.0, 67.5, 65.8, 63.3, 50.8, 49.6, 49.4, 32.7, 30.4, 30.3, 30.1, 30.0, 29.9, 29.7, 29.6, 29.5, 24.2, 23.8, 23.7, 23.2, 20.8, 16.9; HRMS (ESI): Calcd for $C_{80}H_{96}N_4O_{23}$ $[M+Na]^+$ 1503.6363, found: 1503.6434.

2.20. Synthesis of pyruvated tetrasaccharide 21:



To a solution of compound **20** (0.010 g, 6.75 µmol) in DCM (0.5 mL) was added methyl 2,2-bis (ethylthio) propanoate[5] (0.0084 g, 0.04 mmol), and 2,4,6-tri-tert-butylpyridine (0.023 g, 0.094 mmol), followed by 4Å MS and the reaction mixture stirred at room temperature for 10 min. Cooled the reaction mixture to 0 ^{o}C and added a solution of DMTST (0.0092 g, 0.04 mmol) in DCM (0.2 mL) over a period of 2 h at 0 ^{o}C. Quenched the reaction mixture with 0.1 mL of Et_3N, filtered and removed the solvents under vacuum. Purified the crude by flash chromatography (DCM, Acetone and MeOH as eluent, 50%) to obtain the compound **21** as mixture of *R* and *S* pyruvates (0.005 g, 47%). HRMS (ESI): Calcd for $C_{84}H_{101}N_4O_{25}$ $[M+H]^+$ 1565.6755, found: 1565.6733. For ^{1}H and ^{13}C, see attached spectra for details (S55-S56).

2.21. Synthesis of pyruvated tetrasaccharide 2:



To compound **21** in MeOH (0.5 mL) was added aq. NaOH (0.085 µL 3.75 M) and the reaction mixture stirred for 14 h at room temperature. Removed the solvent under vacuum, diluted with water and cooled to 0 ^{o}C. Neutralized the reaction mixture with three drops of acetic acid at 0 ^{o}C. Extracted the aqueous with EtOAc, dried the organic over Na_2SO_4, filtered and concentrated to obtain the crude compound, which by ^{1}H NMR showed no more the acetate and methyl ester peaks. Taken to the next step without further purification.

A solution of the crude compound in a mixture of MeOH, EtOAc and water (3:2:1; 0.3 mL: 0.2 mL: 0.1 mL) was purged with argon for 3 min followed by the addition of $Pd(OH)_2/C$ (10%). Purged the reaction mixture with H_2 and stirred the reaction mixture for 48 h under H_2 atmosphere. Filtered the reaction mixture through a PTFE filter and washed the filtrate with a mixture of 1:1 MeOH: water. Removed the solvents under vacuum to obtain the crude compound. Purified the crude by HPLC to obtain 4 fractions. Fraction 4 was the pure compound **2** (1 mg, 33%). The other fractions were depyruvated tetrasaccharide **3** and a mixture of the unwanted isomer and an unknown compound. ^{1}H NMR (600 MHz, D_2O) δ 5.43 (d, *J* = 3.1 Hz, 1H), 5.05 (d, *J* = 2.8 Hz, 1H), 4.91 (d, *J* = 3.6 Hz, 2H), 4.48 (d, *J* = 3.9 Hz, 1H), 4.42 (s, 1H), 4.37 (dd, *J* = 11.1, 3.9 Hz, 1H), 4.29 (dd, *J* = 7.3, 4.6 Hz, 1H), 4.25 – 4.15 (m, 2H), 4.15 – 4.00 (m, 5H), 3.95 (dd, *J* = 12.0, 1.8 Hz, 1H), 3.93 – 3.90 (m, 1H), 3.88 (dd, *J* = 11.2, 3.0 Hz, 1H), 3.87 – 3.78 (m, 3H), 3.77 – 3.63 (m, 6H), 3.54 (t, *J* = 9.8 Hz, 1H), 3.40 (ddd, *J* = 9.7, 5.5, 2.1 Hz, 1H), 3.03 (t, *J* = 7.6 Hz, 2H), 2.06 (s, 6H), 2.05 (s, 4H), 1.83 – 1.65 (m, 4H), 1.58 (s, 3H), 1.29 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (176 MHz, D_2O) δ 175.9, 175.5, 173.7, 173.6, 108.0, 98.7, 98.3, 96.6, 95.1, 76.3, 74.5, 73.4, 73.0, 72.8, 72.6, 72.5, 71.8, 70.7, 68.3, 68.1, 67.6, 66.9, 61.5, 60.4, 60.2, 53.3, 49.0, 47.6, 39.3, 28.0, 26.4, 22.7, 22.4, 22.2, 21.9 (2C), 15.5; HRMS (ESI): Calcd for $C_{38}H_{64}N_4O_{22}$ $[M+Na]^+$ 951.3910, found: 951.3928.

2.22. Deletion Sequences

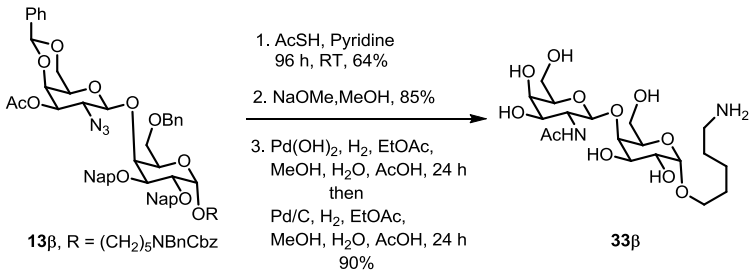
2.22.1. General procedures for the preparation of deletion sequences:

A) Deacylation: To a solution of compound in MeOH (0.06 mL) was added a solution of 0.5 M NaOMe in MeOH (0.013 mL, 6.37 μmol) and the reaction mixture stirred for 2 h at room temperature. Diluted the reaction mixture with MeOH, neutralized with Amberlite 120 H^+ resin, filtered and concentrated.

B) Azide to N-acetamide: To a solution of compound in pyridine (0.6 mL) was added thioacetic acid (0.091 mL, 1.27 mmol) and the reaction mixture stirred at room temperature for 18 h. Removed the solvents under vacuo and azeotroped the reaction mixture twice with toluene to obtain the crude as yellow oil. Purified the crude by flash chromatography using DCM and EtOAc as eluent (0 to 20%).

C) Global Deprotection: Dissolved the compound in a mixture of MeOH, water, ethyl acetate and acetic acid (3:1:1:0.1-0.3 mL: 0.1 mL:0.1 mL:10 µL). Purged the reaction mixture before and after addition of $Pd(OH)_2$ (20%) with argon for 2-3 min. The reaction mixture was then purged with hydrogen and stirred for 24 h under an atmosphere of hydrogen. Filtered off palladium over celite, washed with methanol and 1:1 mixture of MeOH and water, and concentrated the filtrate. Resubjected the crude for a second cycle with the same solvent combination, Pd/C (10% Pd; 50% water) stirred for 20 h. Filtered as above and washed the crude with hexanes and acetone and decanted to obtain the compound as foam after drying.

2.22.2. Disaccharide β-deletion sequence:

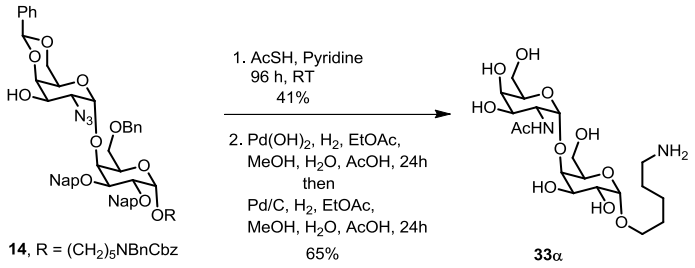


13β, R = $(CH_2)_5$NBnCbz

33β

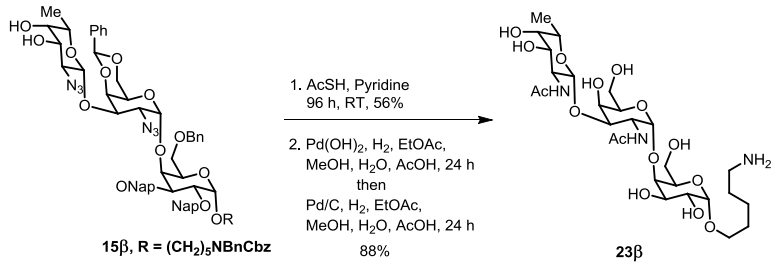
Following general procedures B, A and C the β-disaccharide deletion sequence **33β** was obtained. ^{1}H NMR (400 MHz, D_2O) δ 4.94 (d, *J* = 3.8 Hz, 1H), 4.64 (d, *J* = 8.3 Hz, 1H), 4.15 (s, 1H), 3.98-3.89 (m, 4H), 3.87 – 3.65 (m, 8H), 3.62 – 3.47 (m, 1H), 3.03 (t, *J* = 7.4 Hz, 2H), 2.08 (s, 3H), 1.78-1.63 (m, 4H), 1.53-1.42 (m, 2H).; ^{13}C NMR (100 MHz, D_2O) δ 174.9, 102.6, 98.2, 76.9, 74.7, 70.8, 70.1, 69.3, 68.4, 67.8, 67.7, 61.0, 60.6, 52.5, 39.2, 27.9, 26.3 (2C), 22.2; HRMS (ESI): Calcd for $C_{19}H_{36}N_2O_{11}$ $[M+H]^+$ 469.2397, found: 469.2383.

2.22.3. Disaccharide α-deletion sequence:



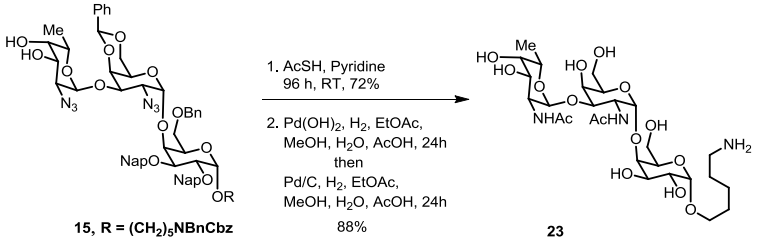
Following general procedures B, and C the β-disaccharide deletion sequence **33α** was obtained ^{1}H NMR (400 MHz, D_2O) δ 4.83 (d, *J* = 2.9 Hz, 1H), 4.76 (d, *J* = 3.3 Hz, 1H), 4.22 (t, *J* = 6.3 Hz, 1H), 4.03 (dd, *J* = 11.2, 3.7 Hz, 1H), 3.94 – 3.66 (m, 5H), 3.66 – 3.48 (m, 5H), 3.51 – 3.32 (m, 2H), 2.85 (t, *J* = 7.5 Hz, 2H), 1.92 (s, 3H), 1.63 – 1.46 (m, 4H), 1.38 – 1.20 (m, 2H).; ^{13}C NMR (100 MHz, D_2O) δ 174.3, 98.2, 98.1, 77.8, 71.6, 70.7, 69.0, 68.2, 68.1, 67.8, 67.0, 60.5, 60.4, 50.1, 39.3, 27.9, 26.4, 22.3, 21.8; HRMS (ESI): Calcd for $C_{19}H_{36}N_2O_{11}$ $[M+H]^+$ 469.2397, found: 469.2383.

2.22.4. Trisaccharide β-deletion sequence:



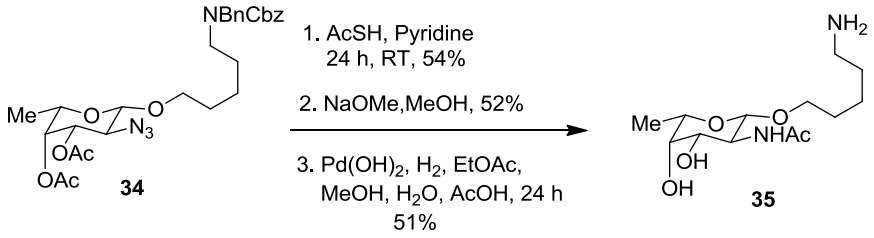
Following general procedures B, and C the β-trisaccharide deletion sequence **23β** was obtained ^{1}H NMR (400 MHz, D_2O) δ 4.90 (d, *J* = 3.8 Hz, 1H), 4.88 (d, *J* = 4.0 Hz, 1H), 4.48 (d, *J* = 7.4 Hz, 1H), 4.23 (t, *J* = 6.4 Hz, 1H), 4.13 (dd, *J* = 11.2, 3.7 Hz, 1H), 4.06 – 3.87 (m, 4H), 3.80 (ddd, *J* = 14.2, 10.6, 3.1 Hz, 2H), 3.73 – 3.55 (m, 9H), 3.53 – 3.38 (m, 1H), 2.93 – 2.89 (m, 2H), 1.98 (s, 3H), 1.95 (s, 3H), 1.68 – 1.49 (m, 4H), 1.48 – 1.28 (m, 2H), 1.21 (d, *J* = 6.4 Hz, 3H).; ^{13}C NMR (100 MHz, D_2O) δ 174.9, 174.3, 100.6, 98.4, 98.2, 78.2, 75.7, 71.6, 71.0, 70.8, 70.6, 70.4, 69.0, 68.3, 67.9, 66.3, 60.5, 60.2, 52.5, 48.9, 39.3, 28.0, 26.4, 22.3, 22.2, 22.1, 15.6; HRMS (ESI): Calcd for $C_{27}H_{49}N_3O_{15}$ $[M+Na]^+$ 678.3061, found: 678.3003.

2.22.5. Trisaccharide α-deletion sequence:



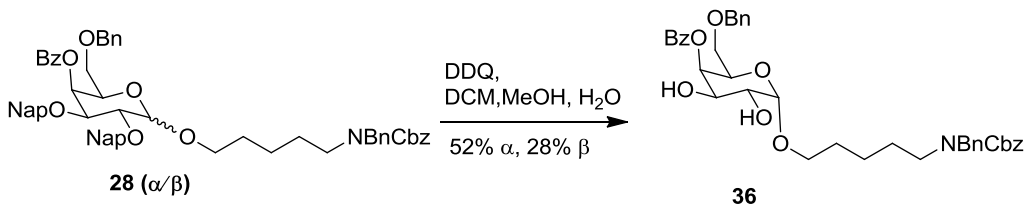
Following general procedures B, and C the α-trisaccharide deletion sequence **23** was obtained ^{1}H NMR (400 MHz, D_2O) δ 4.94 (d, *J* = 3.8 Hz, 1H), 4.90 (d, *J* = 2.2 Hz, 1H), 4.75 (d, *J* = 3.8 Hz, 1H), 4.36 (t, *J* = 5.9 Hz, 1H), 4.30 (dd, *J* = 11.1, 3.7 Hz, 1H), 4.06 – 4.00 (m, 2H), 3.96 (d, *J* = 8.2 Hz, 2H), 3.93 – 3.78 (m, 5H), 3.74 (d, *J* = 2.5 Hz, 1H), 3.71 – 3.52 (m, 5H), 3.51 – 3.38 (m, 1H), 2.91 (t, *J* = 7.5 Hz, 2H), 1.94 (s, 6H), 1.69 – 1.49 (m, 4H), 1.46 – 1.25 (m, 2H), 1.14 (d, *J* = 6.5 Hz, 3H).; ^{13}C NMR (100 MHz, D_2O) δ 174.1, 173.6, 98.5, 98.2 (2C), 77.2, 72.9, 71.5, 70.9, 70.4, 69.0, 68.2, 68.1, 67.9, 67.5, 67.1, 60.4, 60.2, 49.4, 49.1, 39.3, 27.9, 26.4, 22.3, 22.1, 21.9, 15.3; HRMS (ESI): Calcd for $C_{27}H_{49}N_3O_{15}$ $[M+Na]^+$ 678.3061, found: 678.3068.

2.22.6. Synthesis of FucNAc deletion sequence:



Following general procedures B, A, and C the FucNAc deletion sequence **35** was obtained ^{1}H NMR (400 MHz, CD_3OD) δ 4.20 (d, *J* = 8.4 Hz, 1H), 3.85 – 3.67 (m, 2H), 3.56 – 3.42 (m, 3H), 3.43 – 3.31 (m, 1H), 3.21 (dt, *J* = 3.1, 1.4 Hz, 1H), 2.80 (t, *J* = 7.5 Hz, 2H), 1.88 (s, 3H), 1.65 – 1.44 (m, 4H), 1.44 – 1.26 (m, 2H), 1.17 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.2, 103.2, 73.4 (2C), 72.0, 70.2, 54.0, 50.0, 40.7, 29.9, 28.3, 23.2, 17.0; HRMS (ESI): Calcd for $C_{13}H_{26}N_2O_5$ $[M+Na]^+$ 313.1739, found: 313.1728.

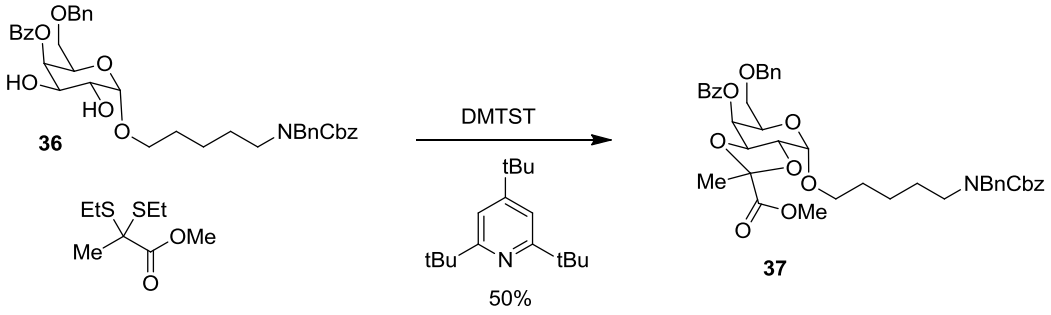
2.22.7. Synthesis of Gal-(2,3)-pyruvate compound 36:



To a mixture (α/β) of compound **28** (0.3 g, 0.31 mmol) in a mixture of DCM (20 mL) and MeOH (5 mL) was added DDQ (0.177 g, 0.77 mmol) at room temperature. After 1 h, 0.1 mL of water was added and the reaction mixture stirred at room temperature for 3 h. Added further 140 mg of DDQ and stirred the reaction mixture for 12 h, by which time there was no

more starting material by TLC. Diluted the reaction mixture with DCM (5 mL) and washed the organic layer with sat. aq. $NaHCO_3$ solution, dried over Na_2SO_4, filtered and concentrated to obtain the crude as oil. Purified the crude by flash chromatography [hexanes and ethyl acetate as eluent (0 to 50%)] to obtain two fractions. Fr-1 was pure α-anomer compound **36** (0.110 g, 52%) and fr-2 was the β-anomer (0.060 g, 28%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.08 – 8.01 (m, 2H), 7.71 – 7.58 (m, 1H), 7.57 – 7.46 (m, 2H), 7.46 – 7.06 (m, 15H), 5.66 (s, 1H), 5.18 (s, 2H), 4.92 (s, 1H), 4.60 – 4.32 (m, 4H), 4.25 (s, 1H), 4.16 – 3.96 (m, 2H), 3.96 – 3.82 (m, 1H), 3.83 – 3.63 (m, 1H), 3.63 – 3.35 (m, 4H), 3.26 (s, 2H), 1.58 (s, 4H), 1.37 (s, 2H).; ^{13}C NMR (101 MHz, Acetone-d_6) δ 166.4, 139.5, 133.8, 131.5, 130.5, 129.4, 129.3, 129.0, 128.7, 128.3, 128.2, 128.0, 100.2, 73.8, 73.0, 70.8, 70.1, 70.0, 69.5, 68.8, 67.4, 24.2; HRMS (ESI): Calcd for $C_{40}H_{45}NO_9$ $[M+Na]^+$ 706.2992, found: 706.2996.

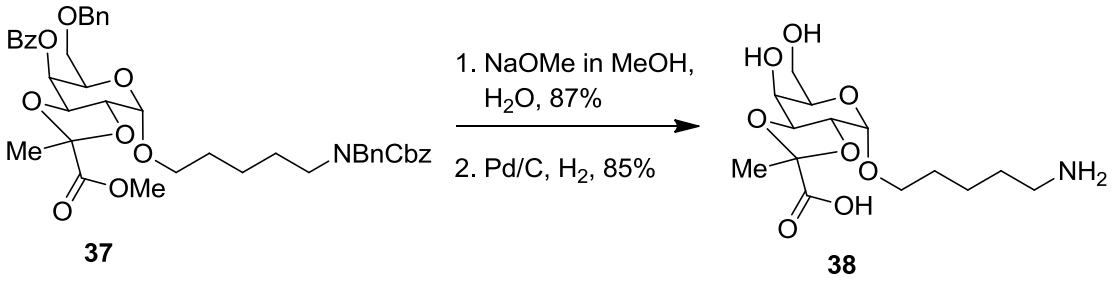
2.22.8. Synthesis of Gal-(2,3)-pyruvate compound 37:



To a solution of compound **36** (0.032 g, 0.047 mmol) in DCM (2 mL) was added methyl 2,2-bis(ethylthio)-propanoate (0.02 g, 0.094 mmol), 2,4,6-tri-tert-butylpyridine (0.093 g, 0.37 mmol), followed by 4Å MS (0.1 g) and the reaction mixture stirred at room temperature for 20 min. Cooled the reaction mixture to -30 ^{o}C and added a solution of DMTST (0.042 g, 0.18 mmol) in DCM (1 mL) and warmed the reaction mixture to -10 ^{o}C followed by 0 ^{o}C over 1 h. Quenched the reaction mixture with 0.1 mL of triethylamine and removed the solvents under vacuum. Purified the crude by flash chromatography [hexanes and ethyl acetate as eluent (0 to 50%)] to obtain two fractions. Fr-1 was the pure compound **37** (0.018 g, 50%, mixture of *R* and *S* isomer) and fr-2 was the recovered starting material (0.007 g). ^{1}H NMR (400 MHz, Acetone-d_6, mixture of *R* and *S* isomers) δ 8.18 – 7.91 (m, 2H), 7.75 – 7.59 (m, 1H), 7.59 – 7.46 (m, 2H), 7.47 – 6.92 (m, 14H), 6.02 – 5.67 (m, 1H), 5.33 (s, 1H), 5.17 (s, 1H), 4.61 – 4.48 (m, 2H), 4.49 – 4.32 (m, 3H), 4.32 – 3.98 (m, 2H), 3.84 – 3.44 (m, 2H), 3.36 – 3.16 (m, 4H), 1.57 (bs, 4H), 1.48 (s, 3H), 1.37 (s, 2H), 1.45 – 1.15 (m, 2H).; ^{13}C NMR (101 MHz, Acetone-d_6) δ 170.1, 169.9, 165.8, 165.7, 139.5, 139.35, 134.2, 134.1, 130.9, 130.4 (2C), 129.6, 129.4, 129.3, 129.0 (2C), 128.7, 128.6, 128.3, 128.2, 128.0, 106.5, 106.4, 98.3, 98.2,

75.5, 75.2, 73.8, 73.8, 73.5, 70.3, 69.9, 69.8, 69.2, 69.1, 69.0, 67.4, 52.6, 52.2, 24.1, 22.9, 22.9.; HRMS (ESI): Calcd for $C_{44}H_{49}NO_{11}$ $[M+Na]^+$ 790.3203, found: 790.3207.

2.22.9. Synthesis of Gal-(2,3)-pyruvate compound 38:



To compound **37** (0.015 g, 0.020 mmol) in MeOH (2.4 mL) was added a solution of NaOMe in MeOH (0.059 mL, 0.029 mmol, 0.5 M) and the reaction mixture stirred for 14 h. Added 0.25 mL of water and stirred for additional 1.5 h. Removed the solvent under vacuum, diluted the reaction mixture with water, cooled to 0 ^oC and neutralized the reaction mixture with three drops of acetic acid. Extracted the aqueous with ethyl acetate and dried the organic over Na_2SO_4, filtered and concentrated to obtain the crude compound (0.011 g, 87%), which by ^1H NMR showed no more the benzoate and methyl ester peaks. The crude compound (0.005 g, 7.7 µmol) was dissolved in a mixture of solvent (MeOH:water:EtOAc – 0.6 mL:0.15 mL:0.15 mL) and was purged with argon for 3 min followed by the addition of 10% Pd/C (0.0016 g). Purged again the reaction mixture with argon and then with hydrogen and stirred for 12 h under hydrogen atmosphere. Filtered the reaction mixture through a PTFE filter and washed the filtrate with a mixture of 1:1 MeOH: water. Removed the solvents under vacuum to obtain the compound **38** (0.0022 g, 85%), which was pure by ^1H NMR. ^1H NMR (400 MHz, D_2O, mixture of *R* and *S* isomers) δ 5.35 (s, 1H), 5.30 (s, 0.5H), 4.42 (s, 0.5H), 4.37 (s, 1H), 4.09 – 3.99 (m, 1.5H), 3.98 – 3.89 (m, 2H), 3.89 – 3.72 (m, 6H), 3.73 – 3.54 (m, 2H), 3.00 (q, *J* = 7.4 Hz, 3H), 1.81 – 1.59 (m, 7H), 1.56 (s, 3H), 1.54 (s, 1.5H), 1.52 – 1.38 (m, 3H).; ^{13}C NMR (101 MHz, D_2O) δ 176.6, 176.5, 107.7 (2C), 96.8, 96.4, 74.9, 74.2, 71.9 (2C), 71.9, 71.8, 70.9, 68.2, 67.9, 67.3, 67.1, 61.0, 39.3, 39.2, 27.9, 27.6, 26.4, 26.3, 22.7, 22.5, 22.4, 22.2.; HRMS (ESI): Calcd for $C_{14}H_{25}NO_8$ $[M+Na]^+$ 358.1478, found: 358.1489.

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1. S. D. Khaja, V. Kumar, M. Ahmad, J. Xue, K. L. Matta, *Tetrahedron Lett.* **2010**, *51*, 4411-4414.
2. a) Y. V. Mironov, A. A. Sherman, N. E. Nifantiev, *Tetrahedron Lett.* **2004**, *45*, 9107-9110; b) R. E. J. N. Litjens, R. den Heeten, M. S. M. Timmer, H. S.

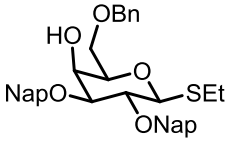
Overkleeft, G. A. van der Marel, *Chem. Eur. J.* **2005**, *11*, 1010-1016; c) E. Bedini, D. Esposito, M. Parrilli, *Synlett* **2006**, *2006*, 825-830; d) A.-B. Alhassan, D. C. McCutcheon, M. Zeller, P. Norris, *J. Carbohydr. Chem.* **2012**, *31*, 371-383.

3. K. Sarkar, I. Mukherjee, N. Roy, *J. Carbohydrate. Chem*. **2003**, *22*, 95-107.

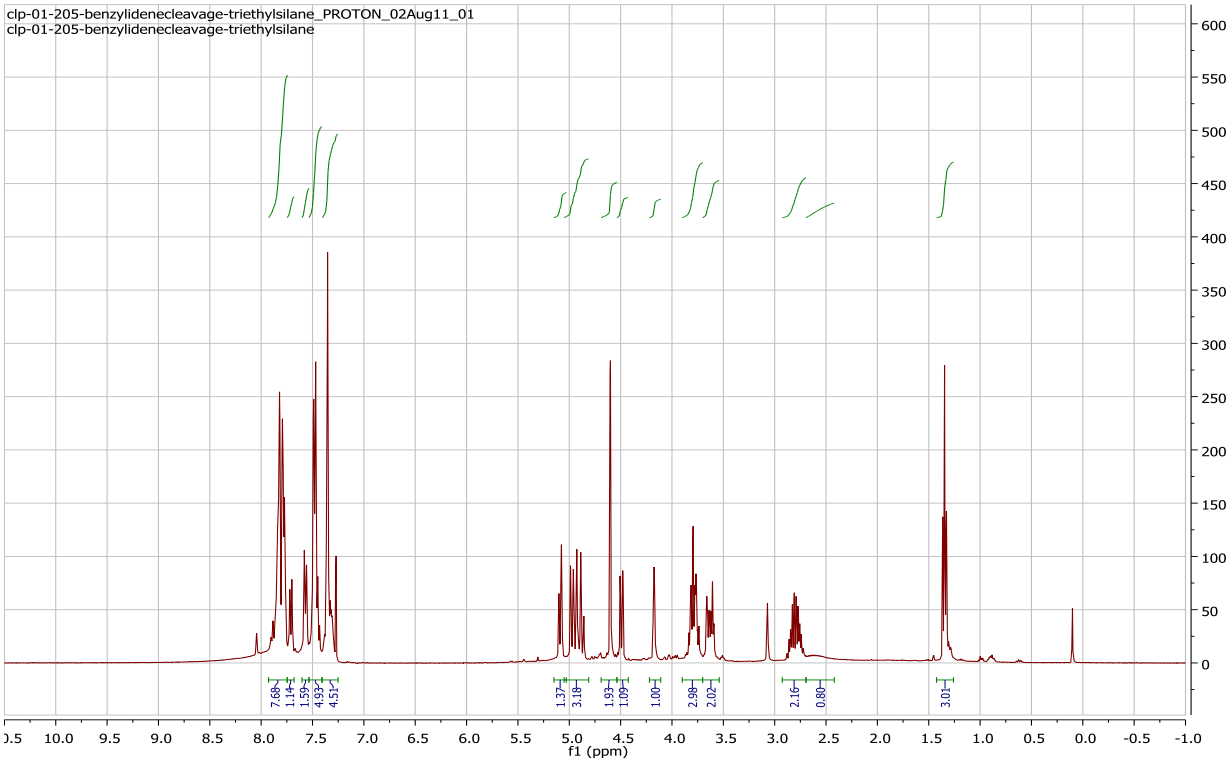
4. P.-E. Jansson, B. Lindberg, U. Lindquist, *Carbohydr. Res.* **1981**, *95*, 73-80.

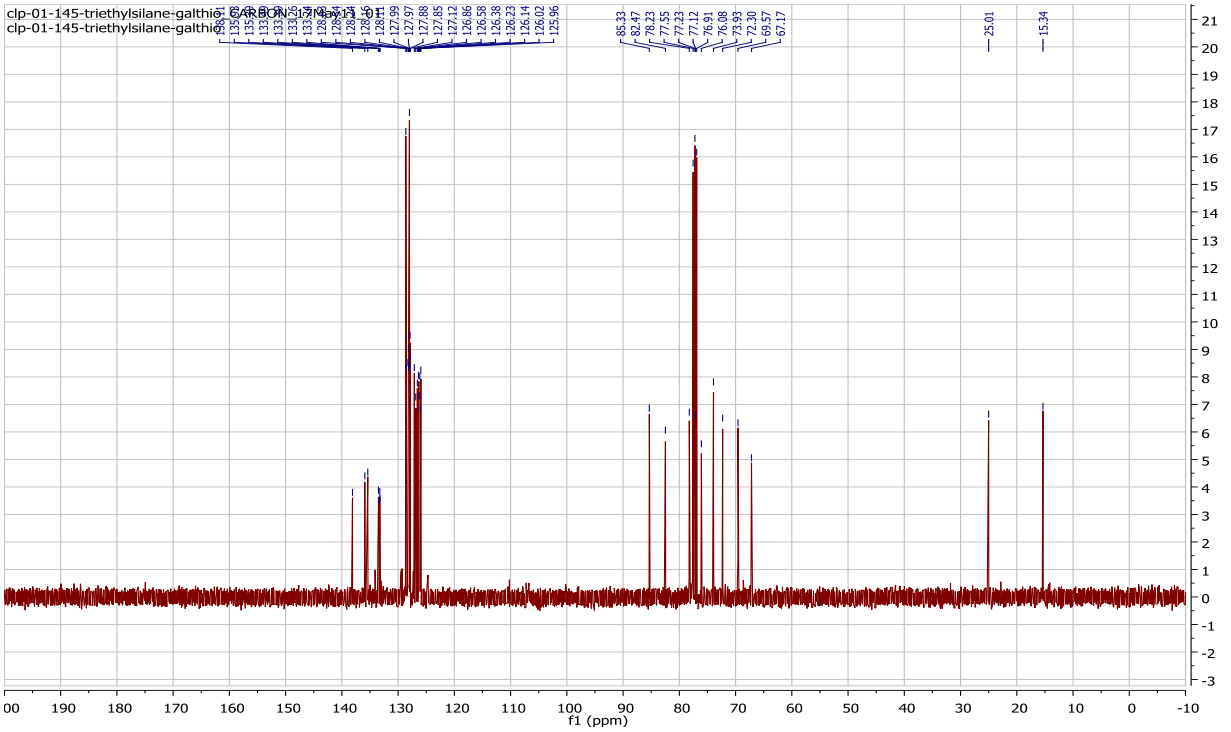
5. I. Bajza, J. Kerékgyártó, J. Hajkó, L. Szilágyi, A. Lipták, *Carbohydr. Res.* **1994**, *253*, 111-120.

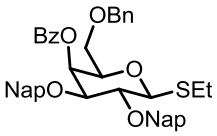
6. NMR spectra of compounds:



26



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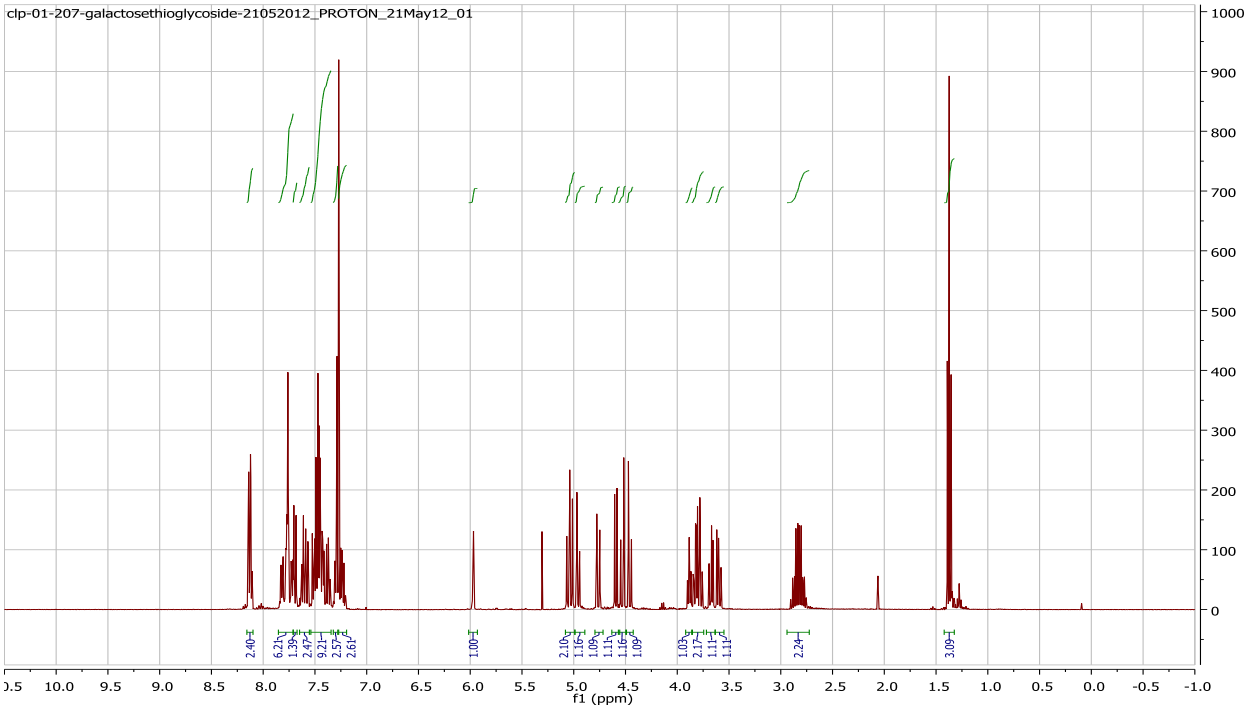
BzO
O
NapO
SEt
ONap

27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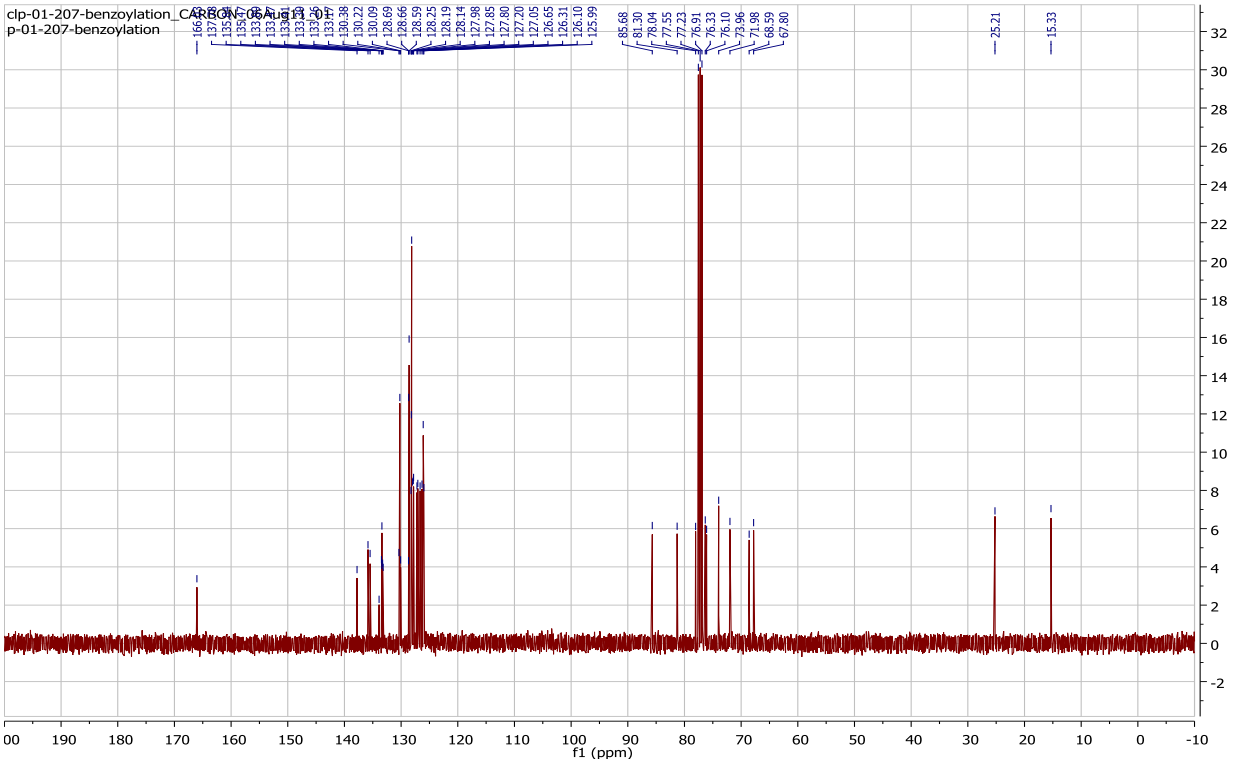
f1 (ppm)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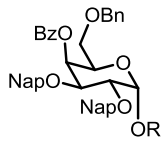
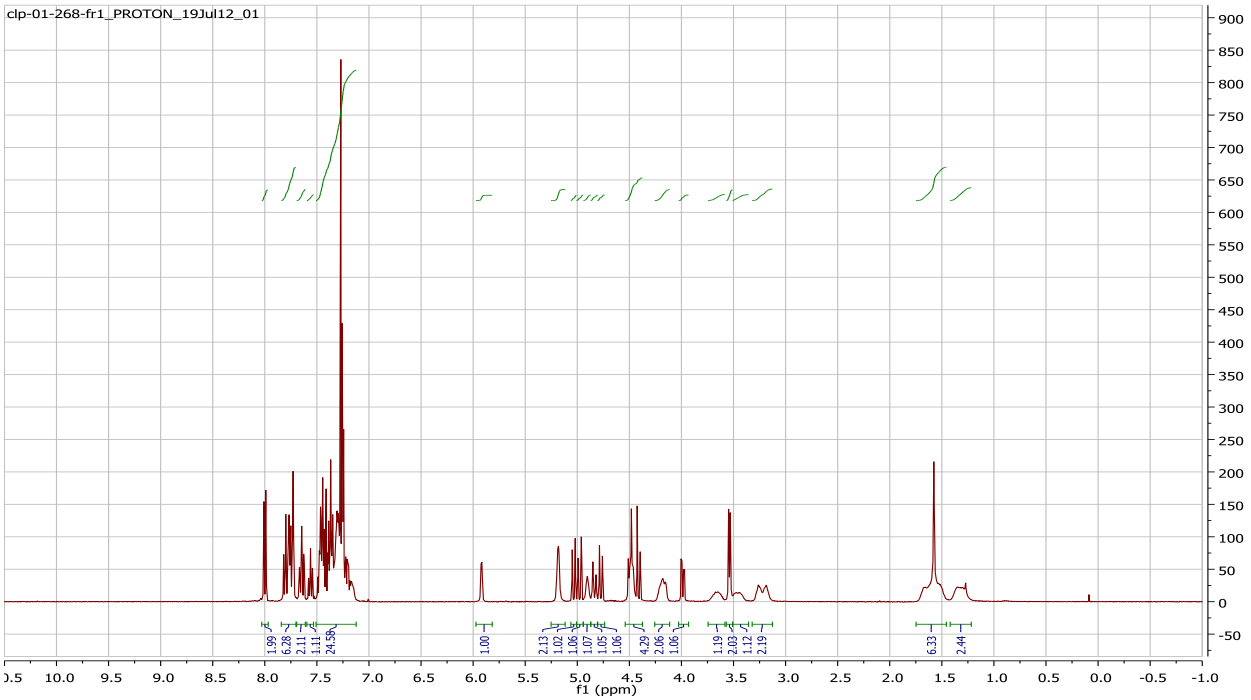
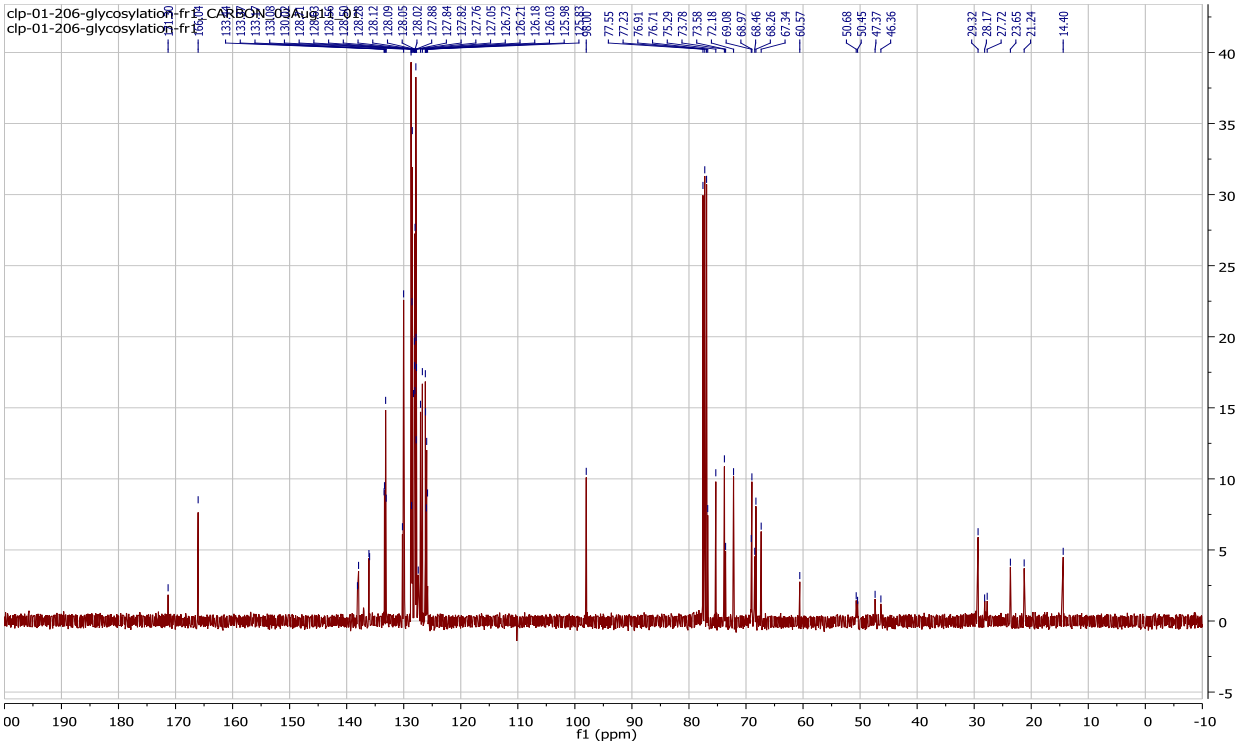
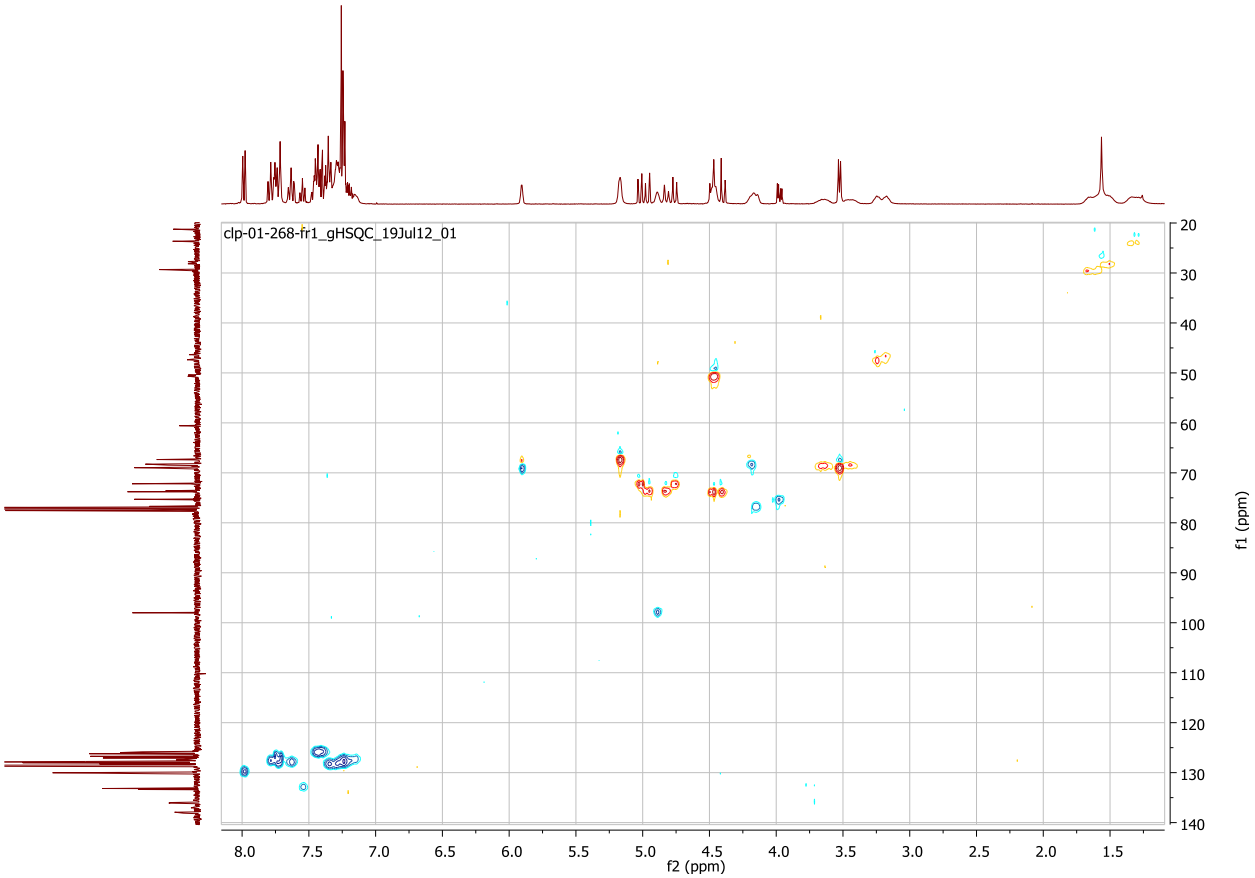
p-01-207-benzoylation
f1 (ppm)



28, R = $(CH_2)_5NBnCbz$



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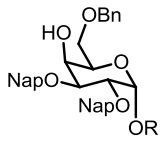
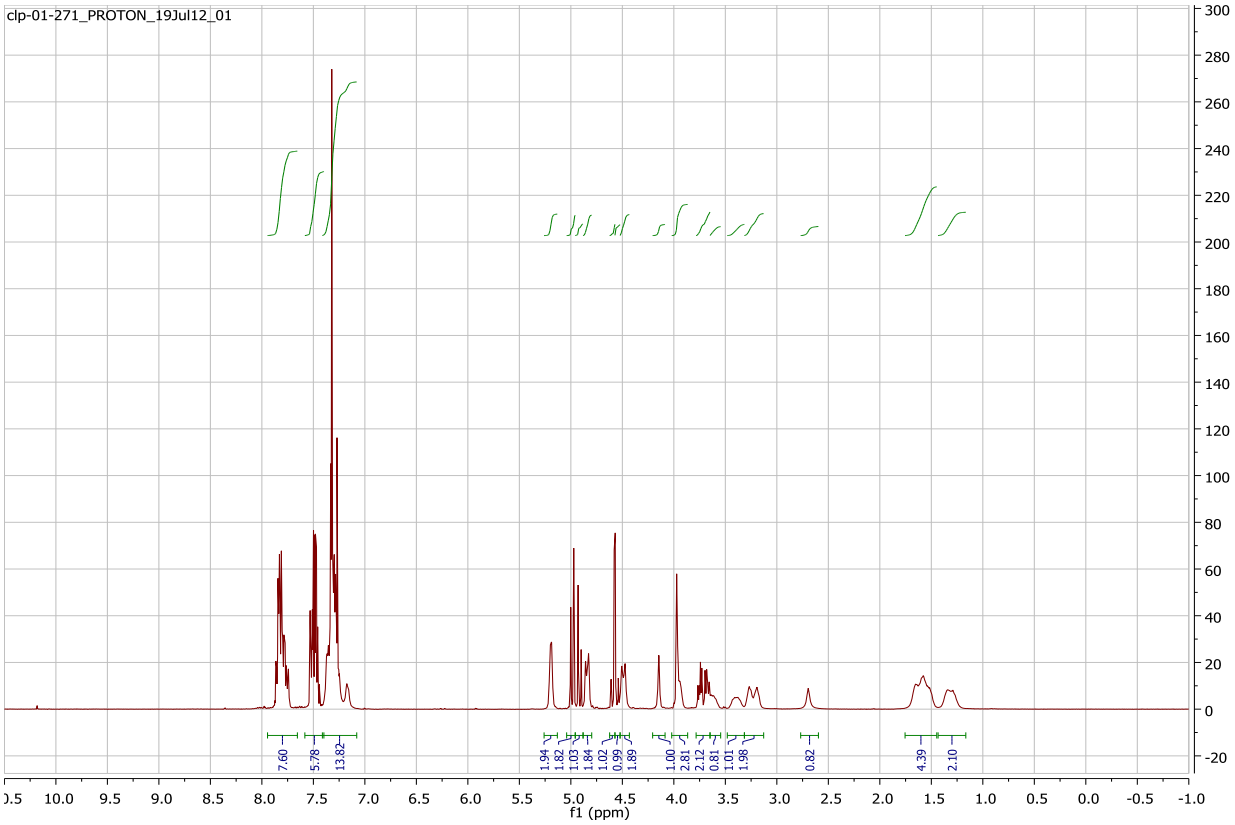
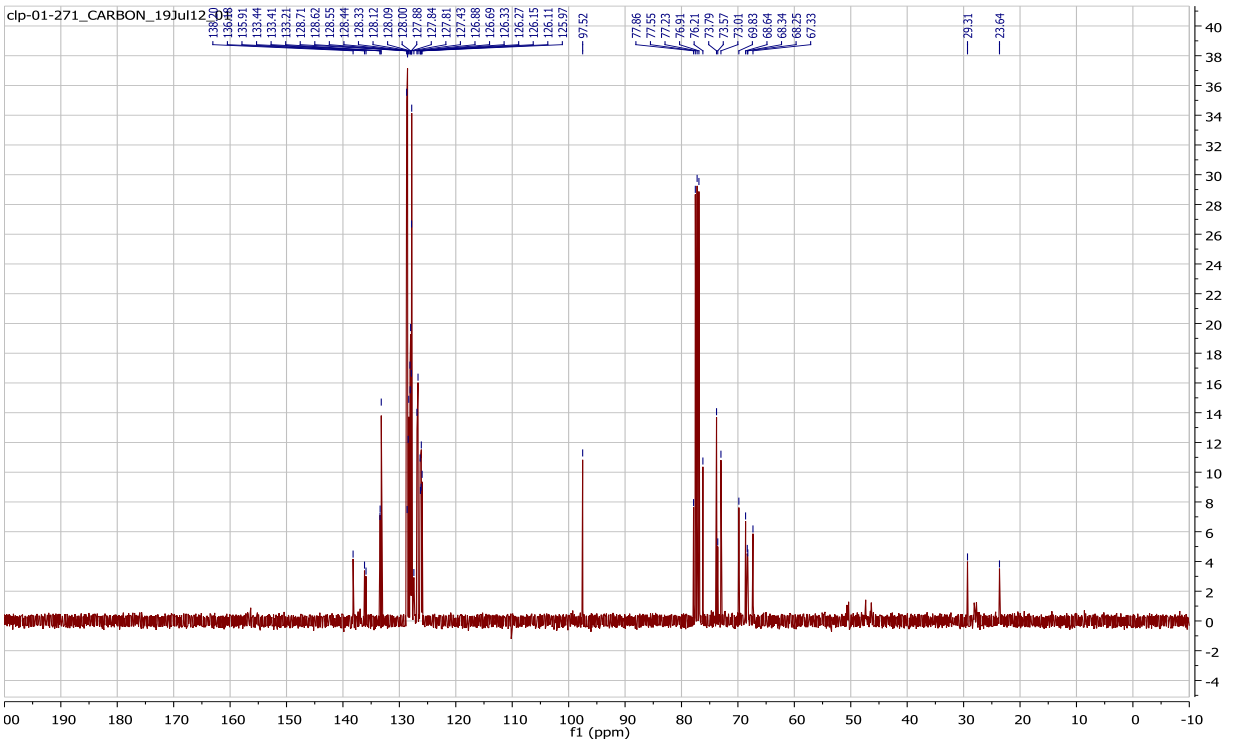
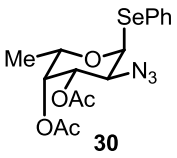
f1 (ppm)



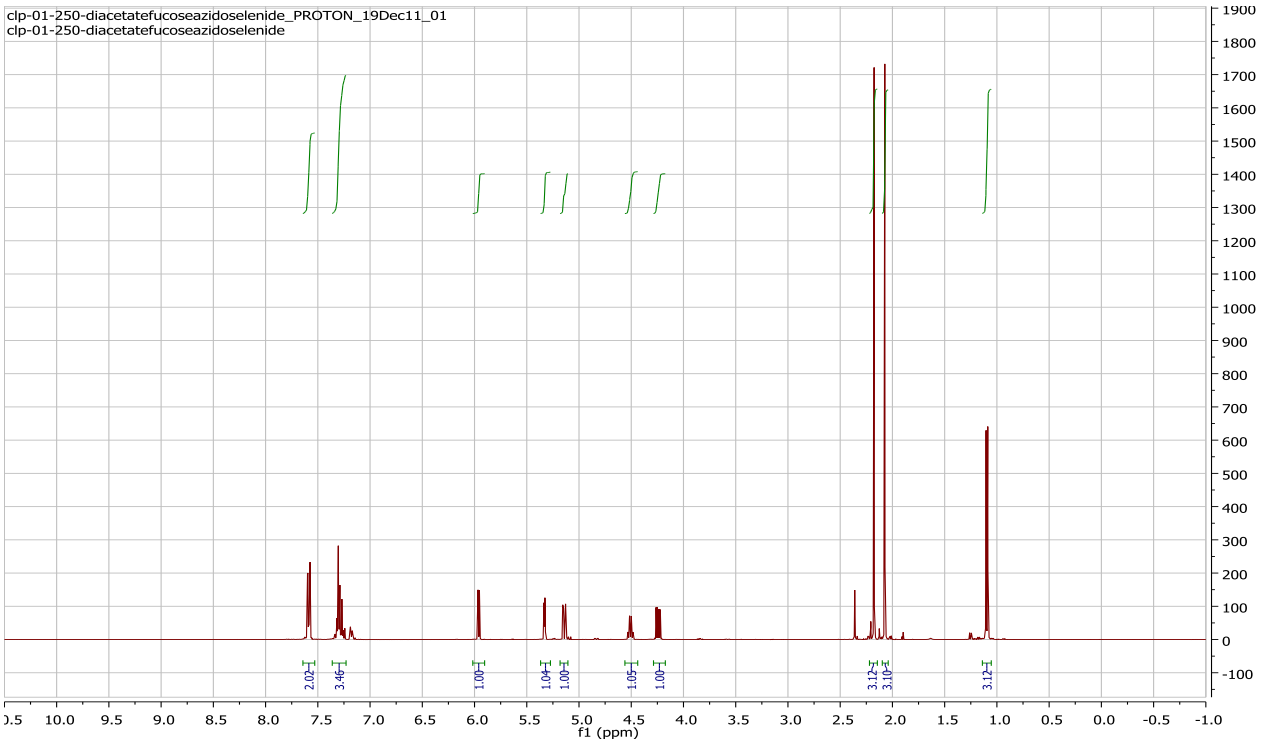
8, R = $(CH_2)_5NBnCbz$






SePh
Me
O
N3
OAc
OAc

30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clp-01-250-diacetatefucoseazidoselenide
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1.00
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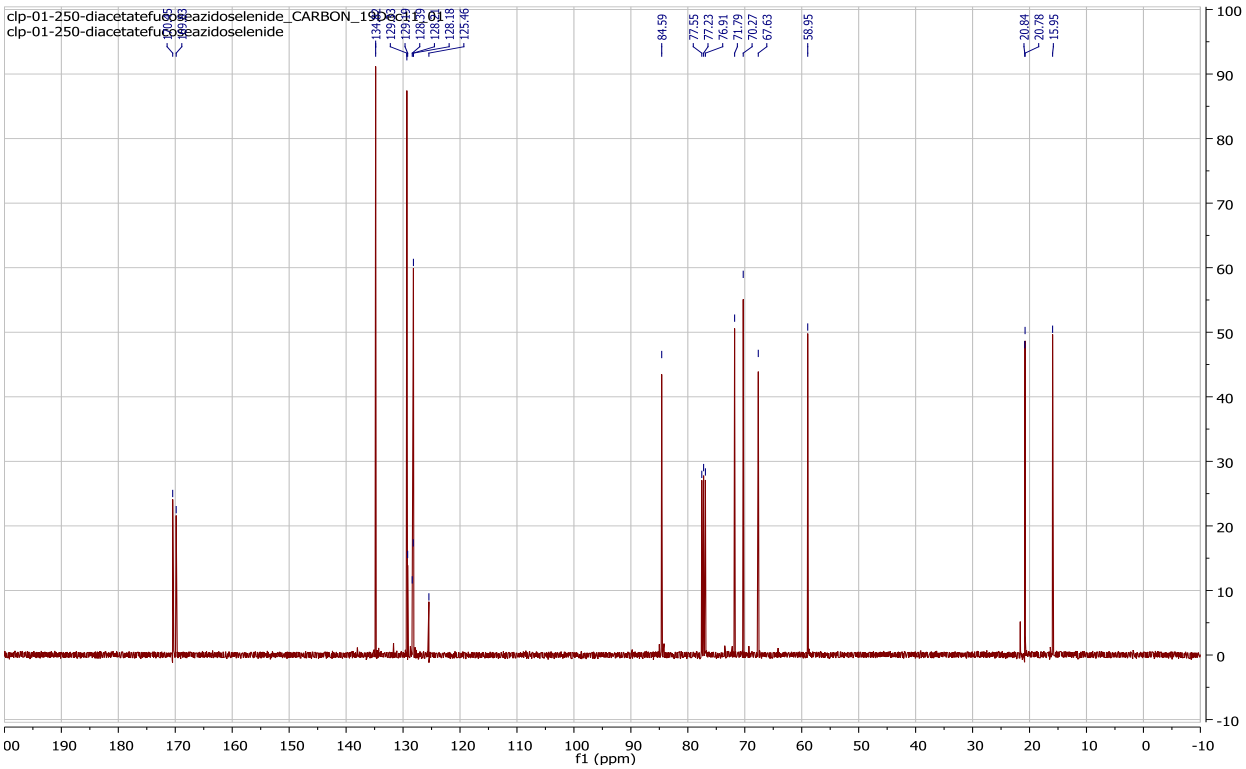
1.00
3.12
3.10
3.12
f1 (ppm)


clp-01-250-diacetatefucoseazidoselenide_CARBON_19Dec11_01
clp-01-250-diacetatefucoseazidoselenide
169.95
169.83
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129.93
129.49
128.39
128.21
128.18
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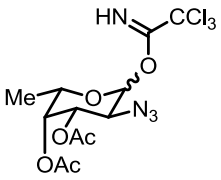
58.95
20.84
20.78
15.95
f1 (ppm)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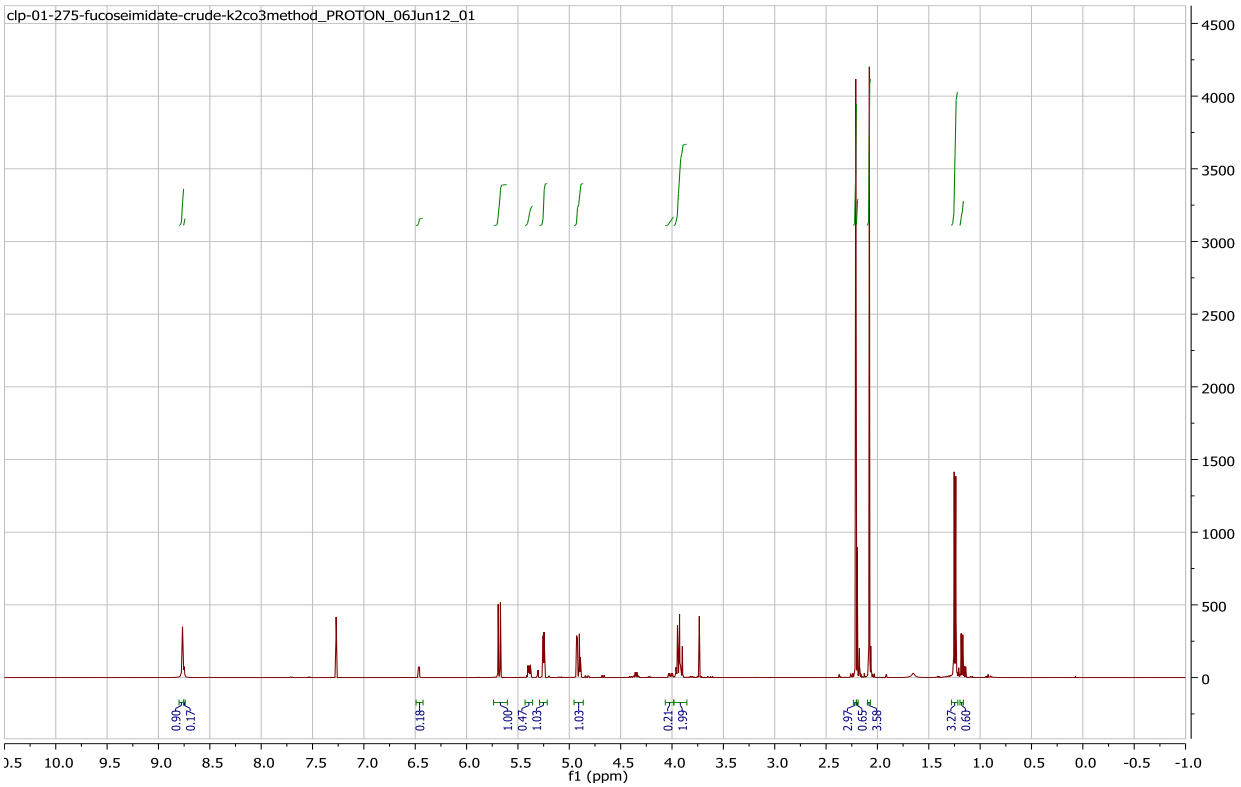
CCl3
O
Me
O
N3
OAc
OAc

10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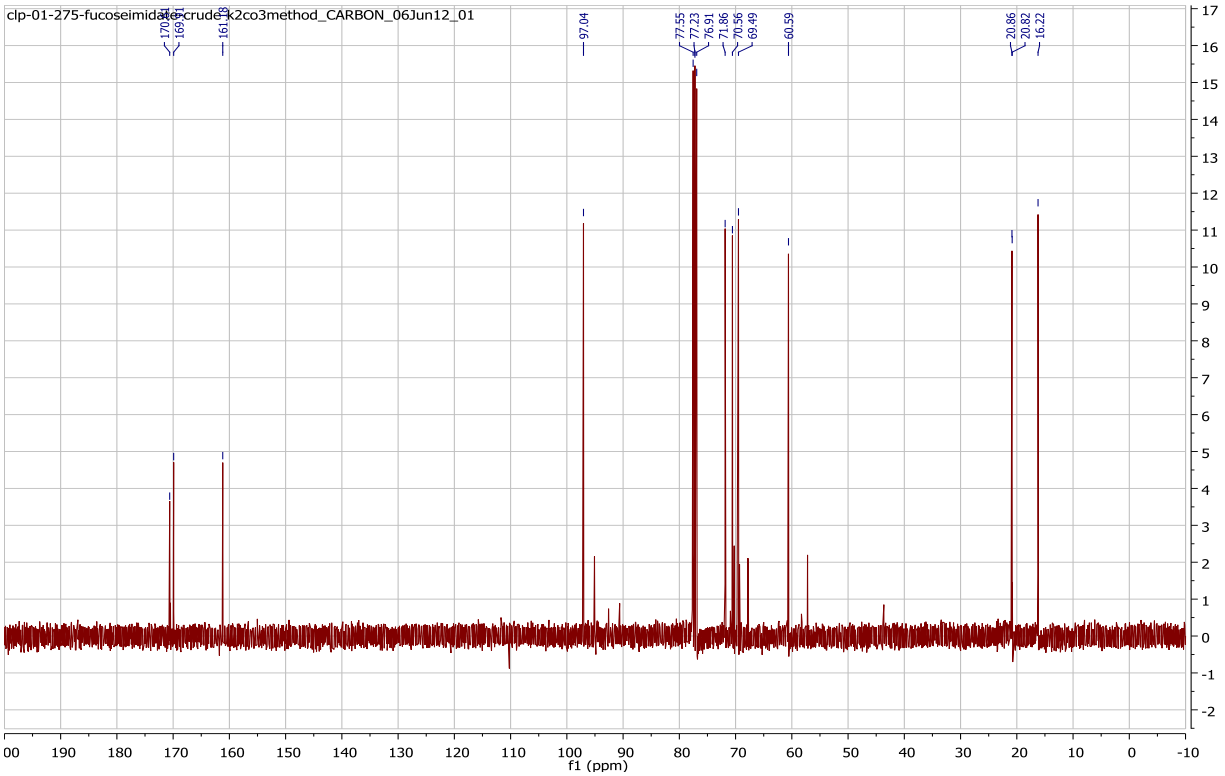
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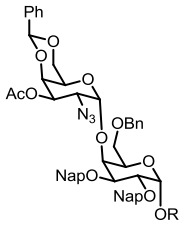
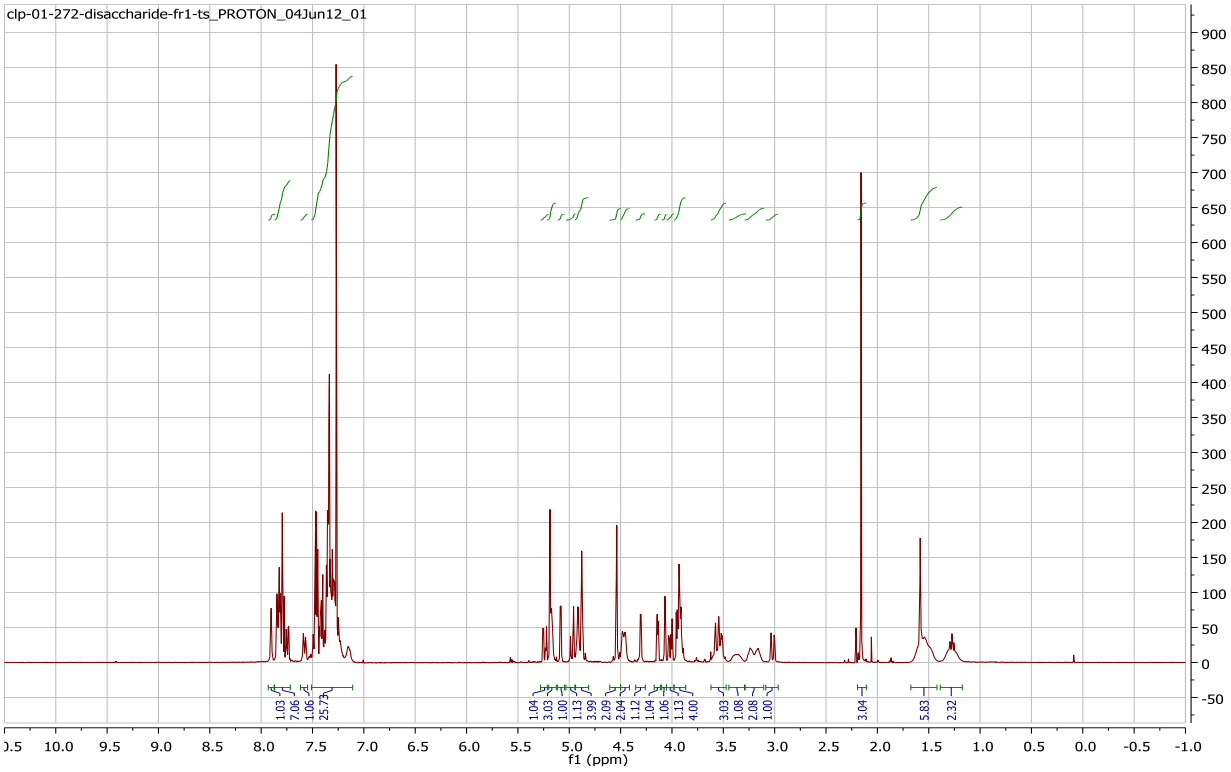
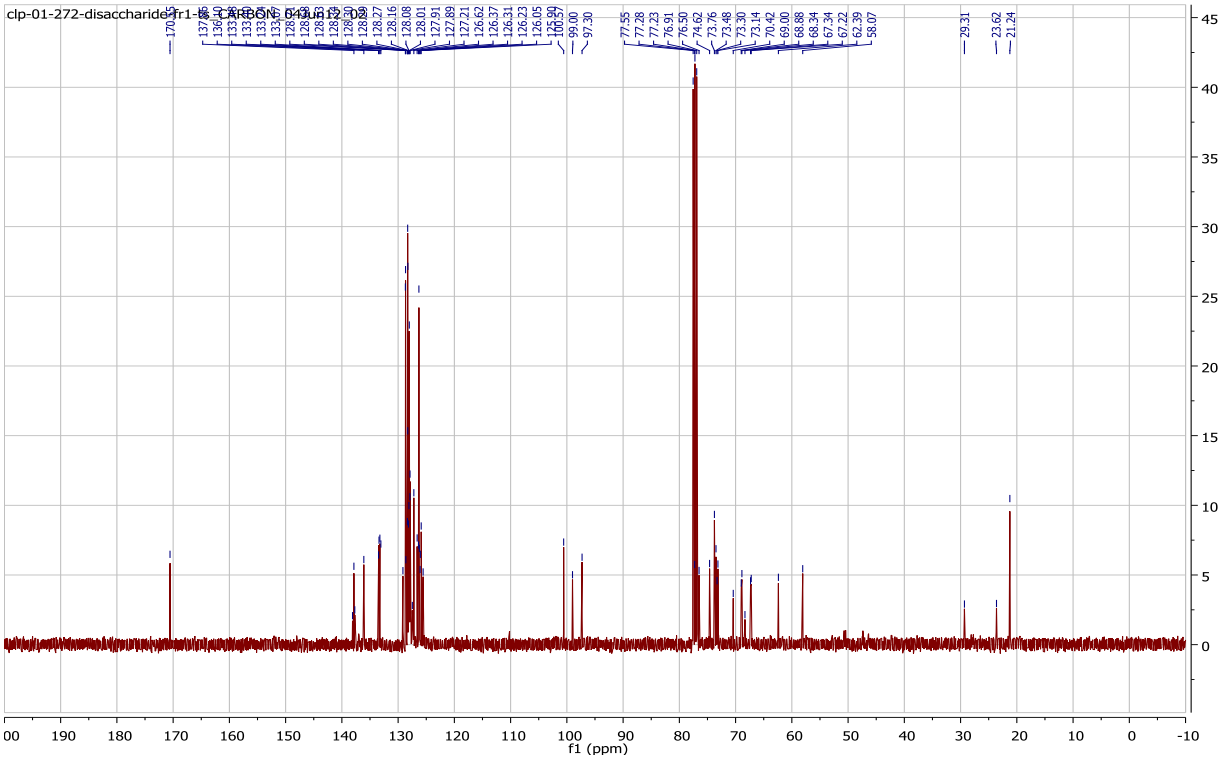
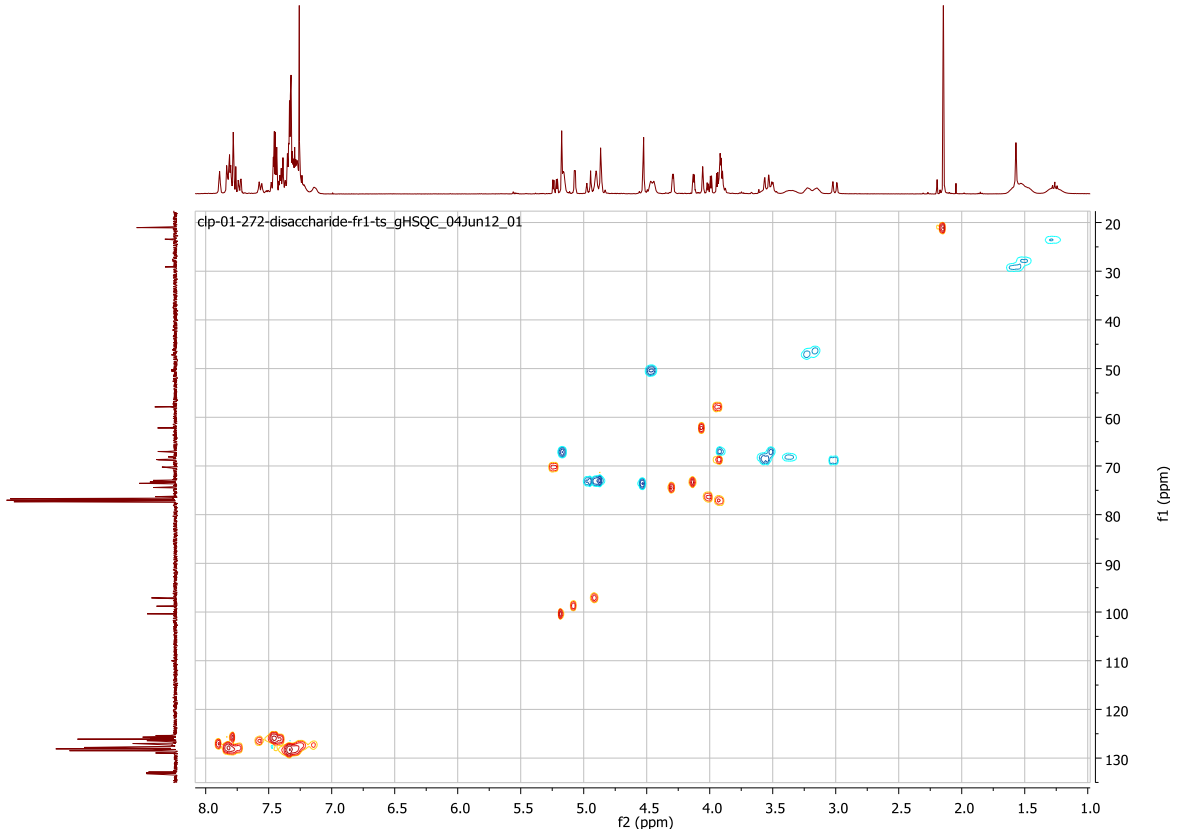
60.59
20.86
20.82
16.22
f1 (ppm)



13, R = $(CH_2)_5NBnCbz$



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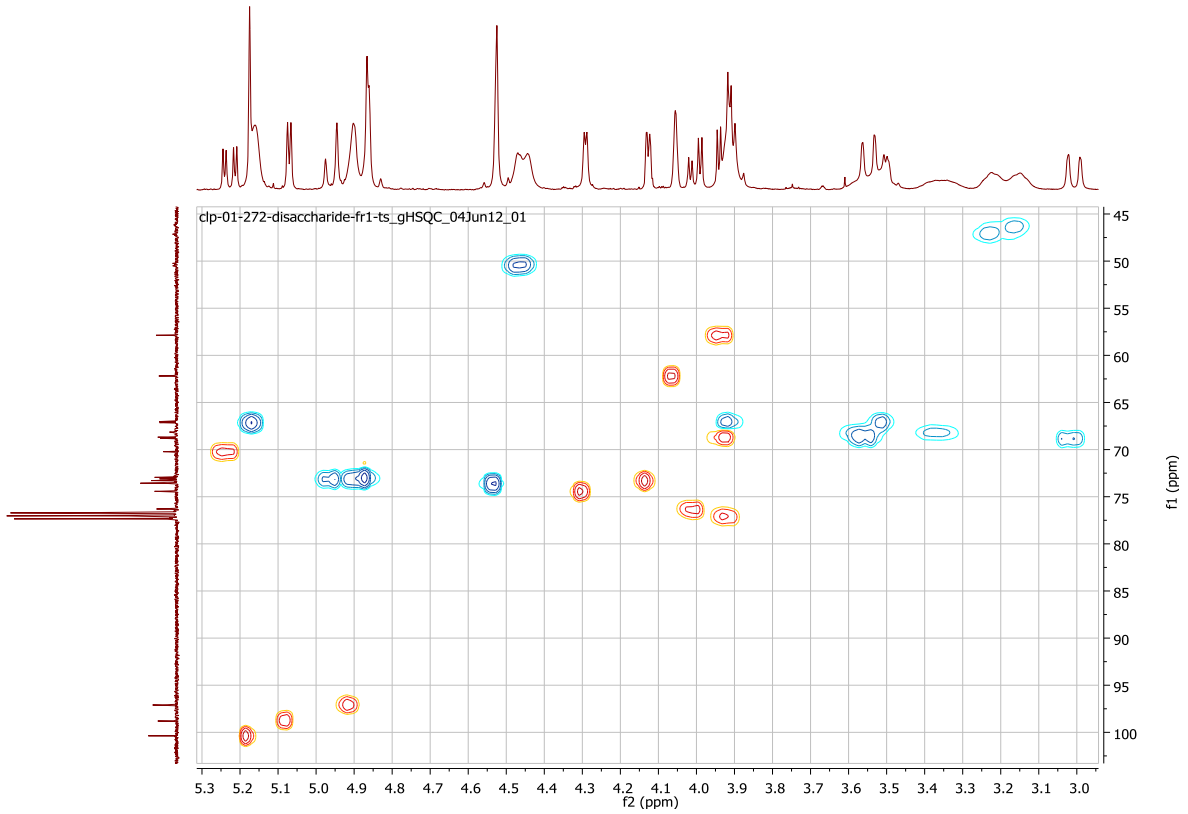
f1 (ppm)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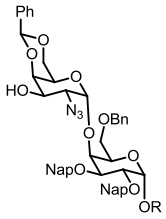
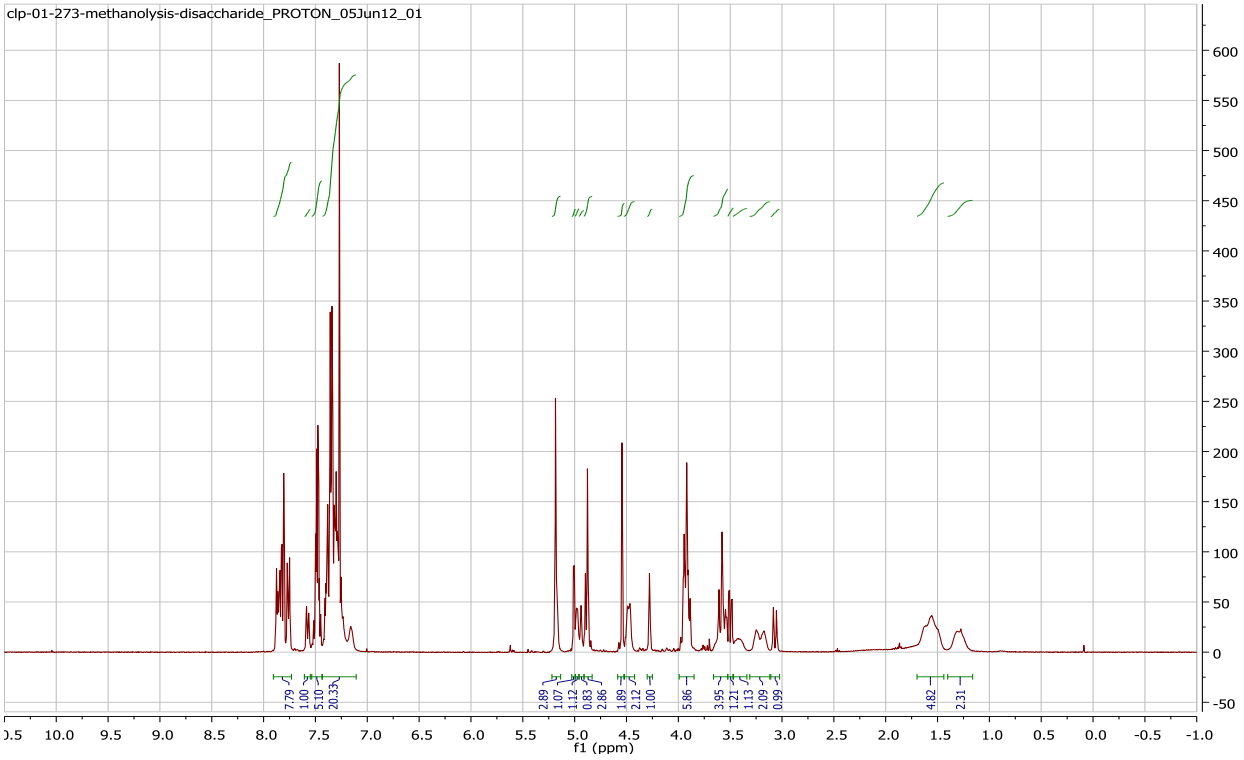
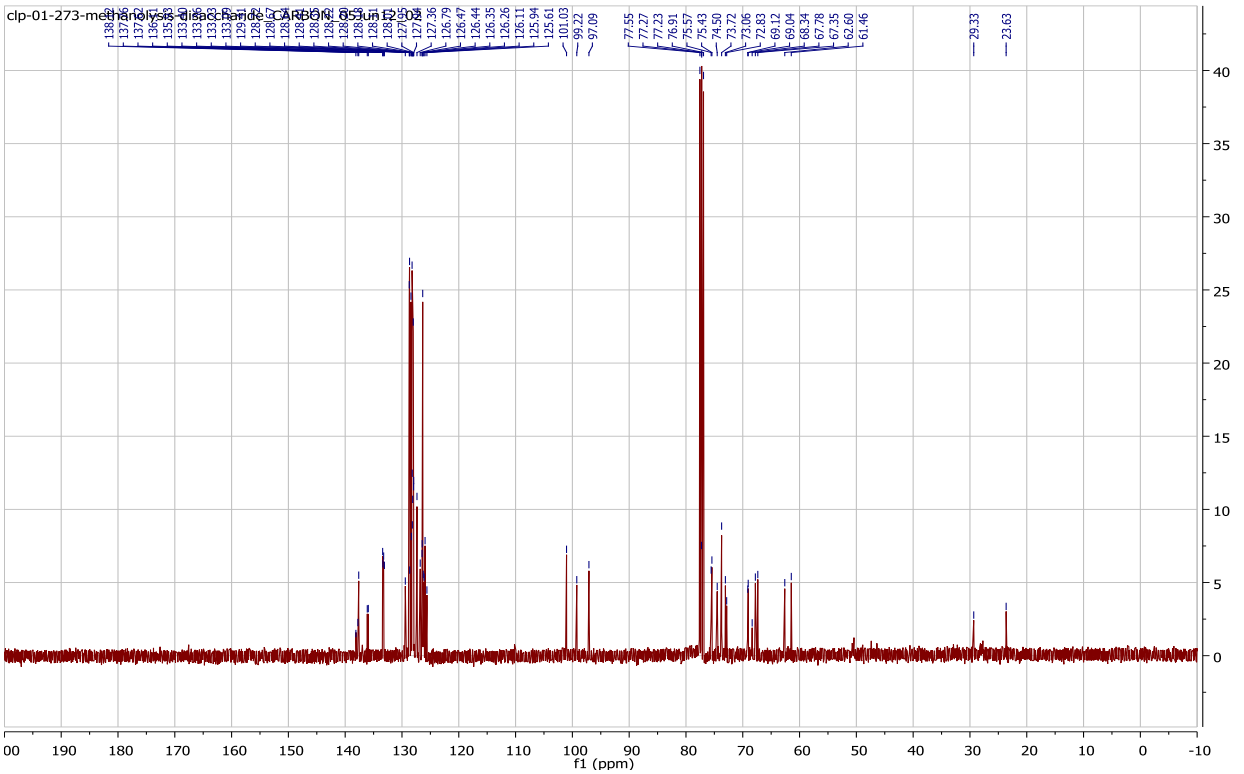
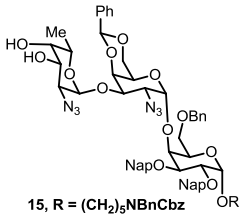
f2 (ppm)
f1 (ppm)



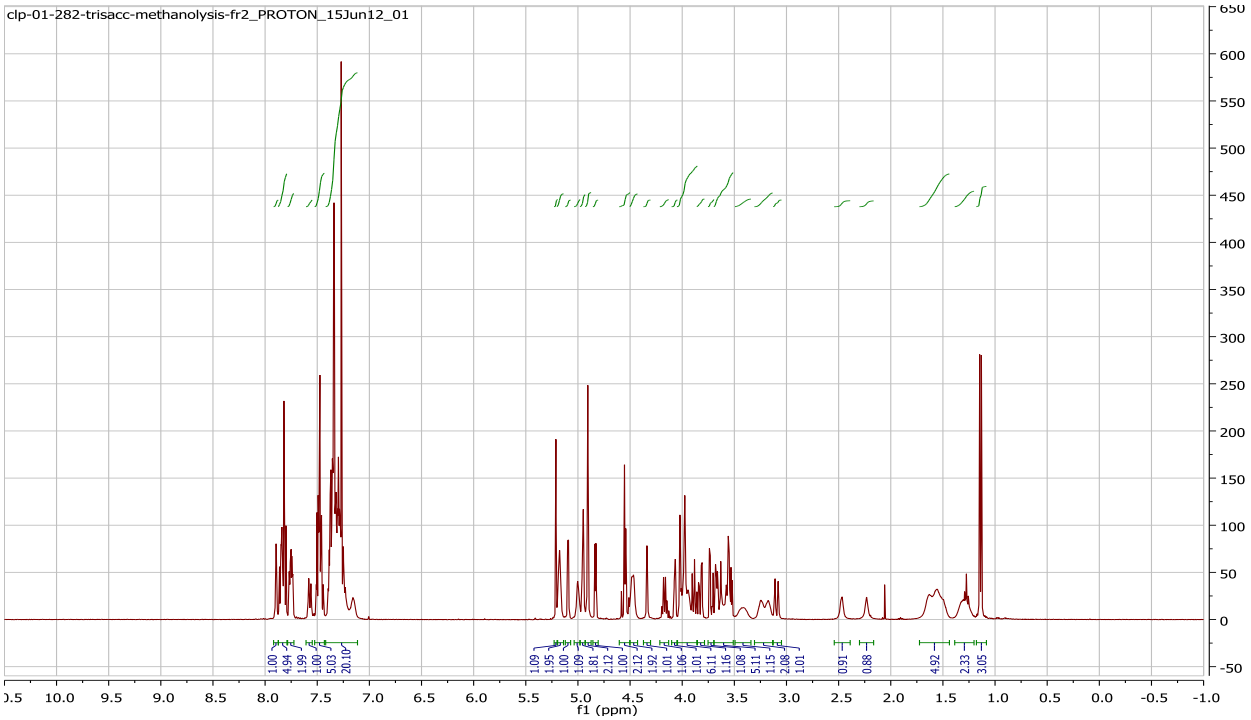
14, R = $(CH_2)_5NBnCbz$

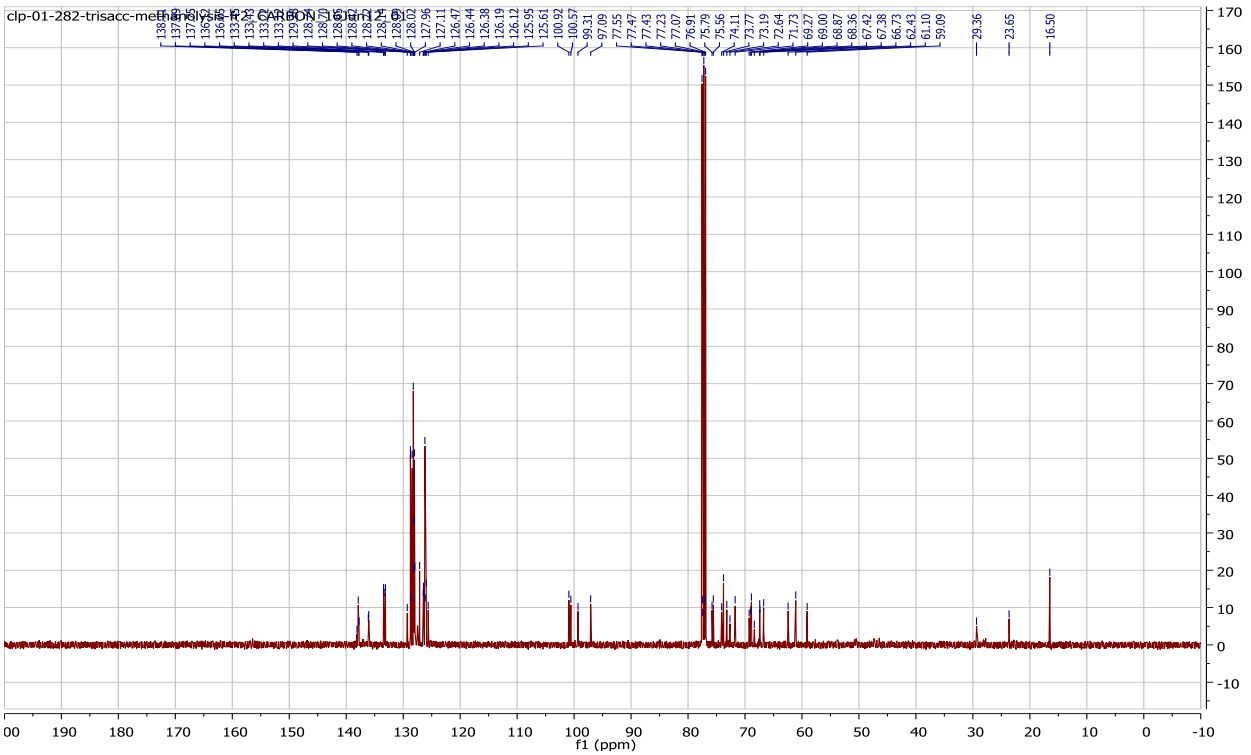


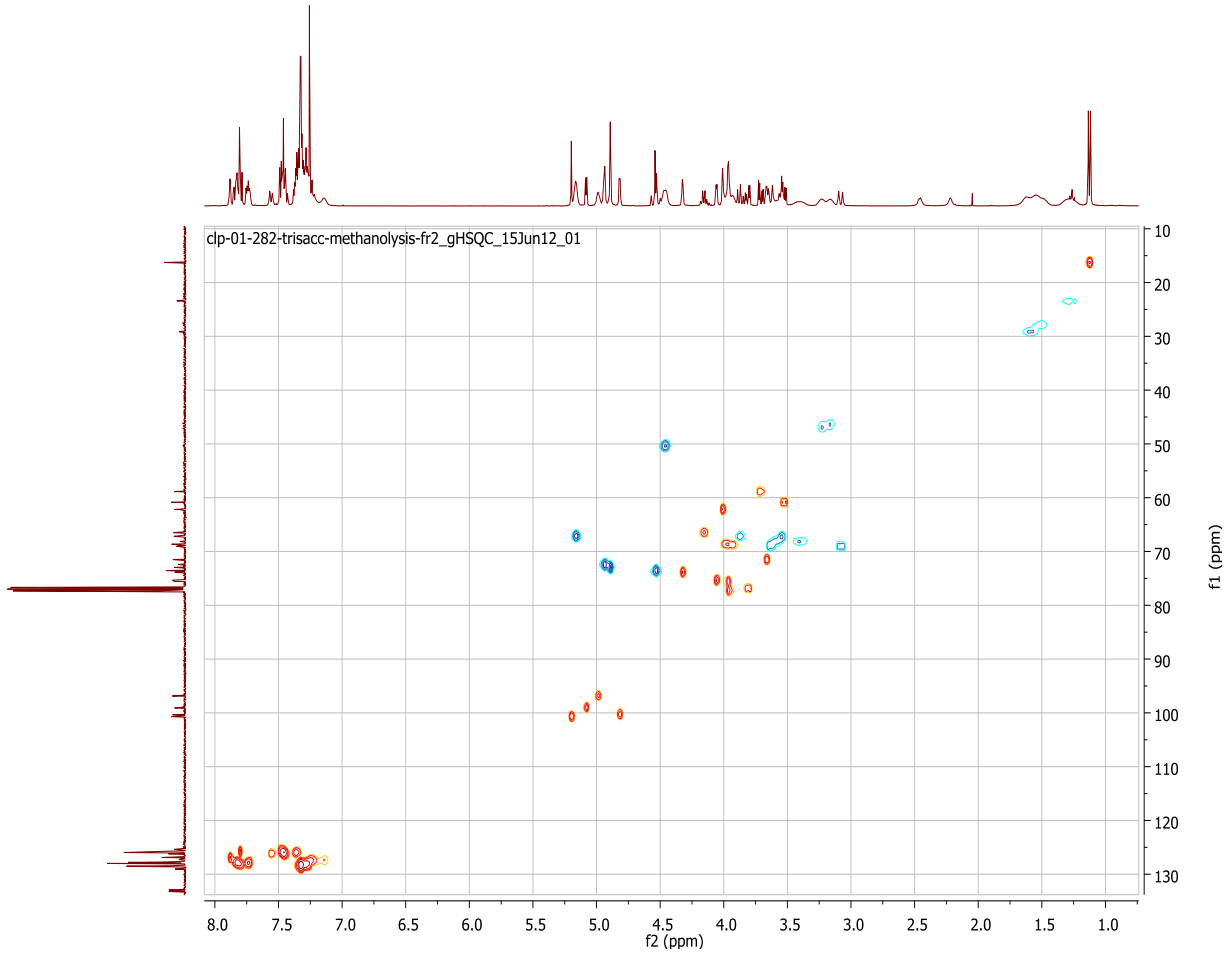


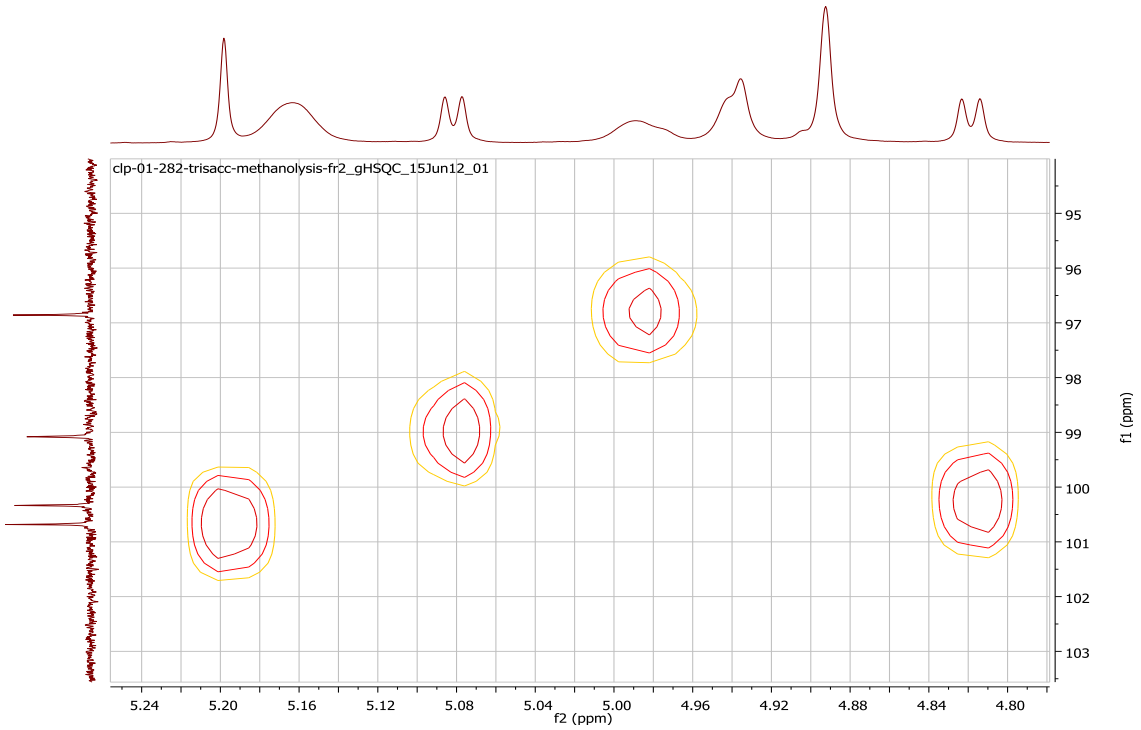


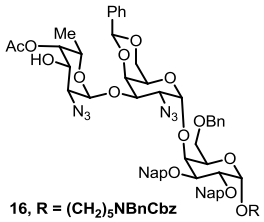
15, R = $(CH_2)_5NBnCbz$



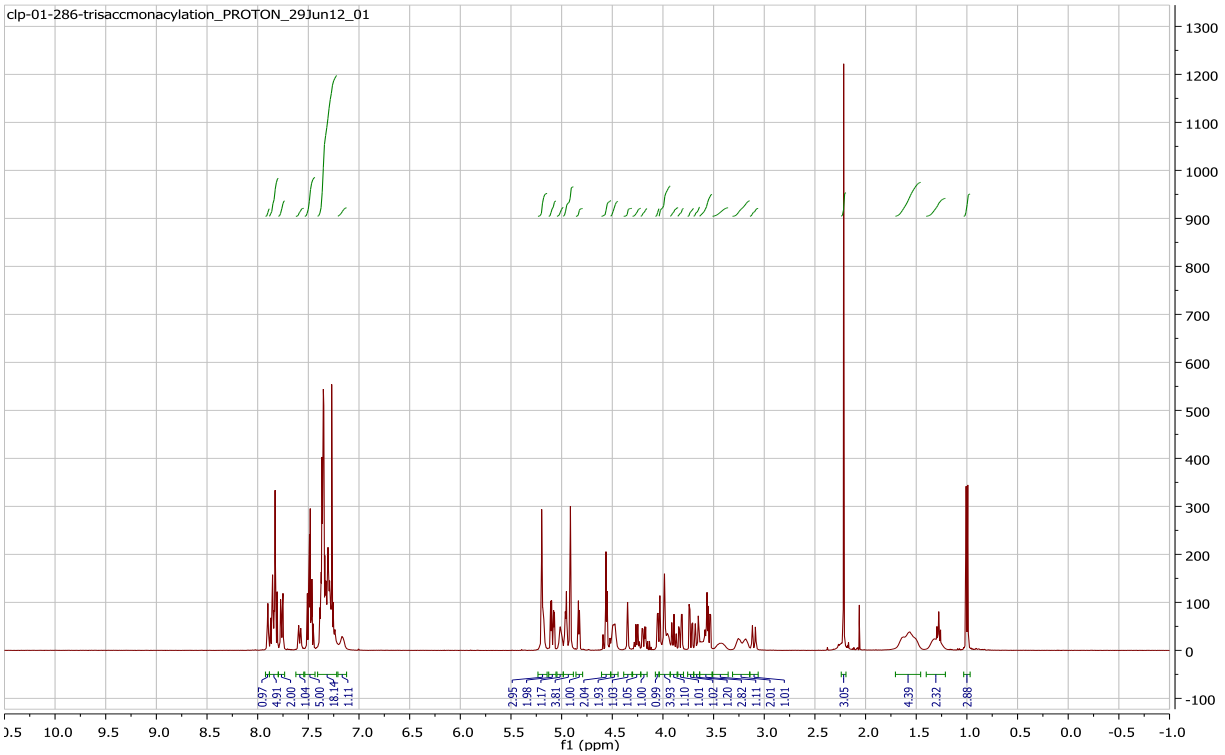


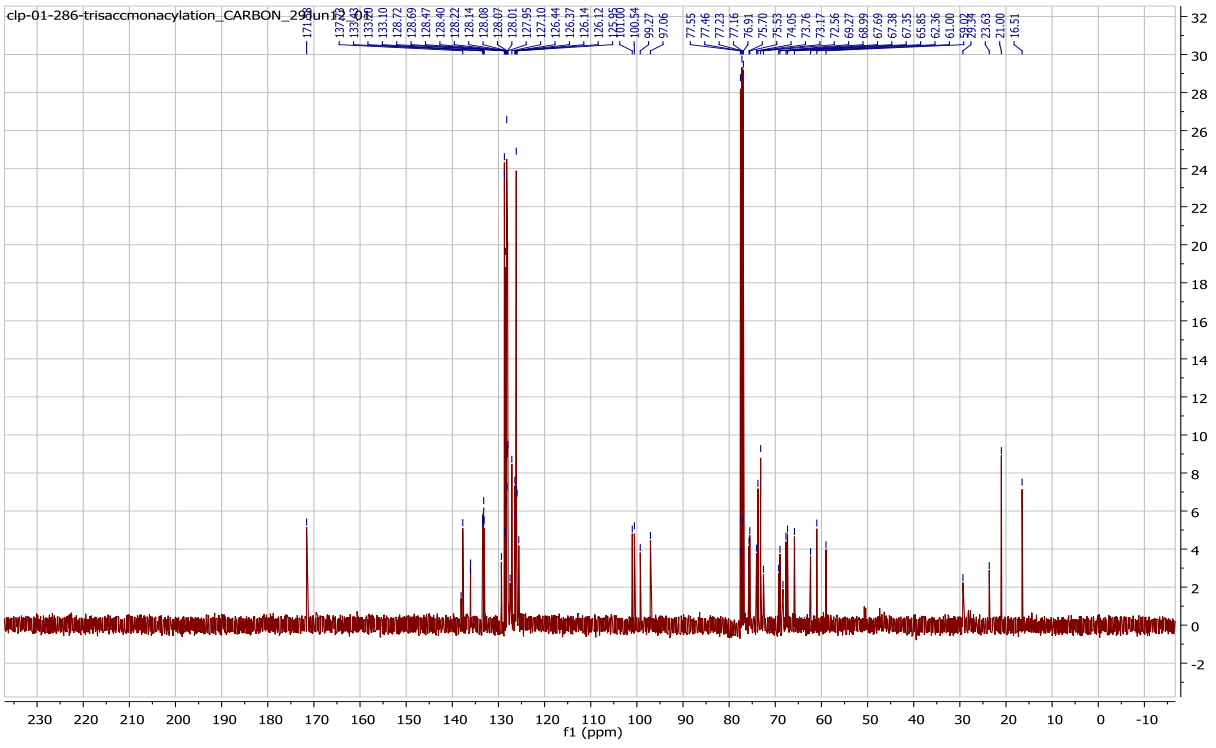

clp-01-282-trisacc-methanolysis-fr2_gHSQC_15Jun12_01
f2 (ppm)
f1 (ppm)

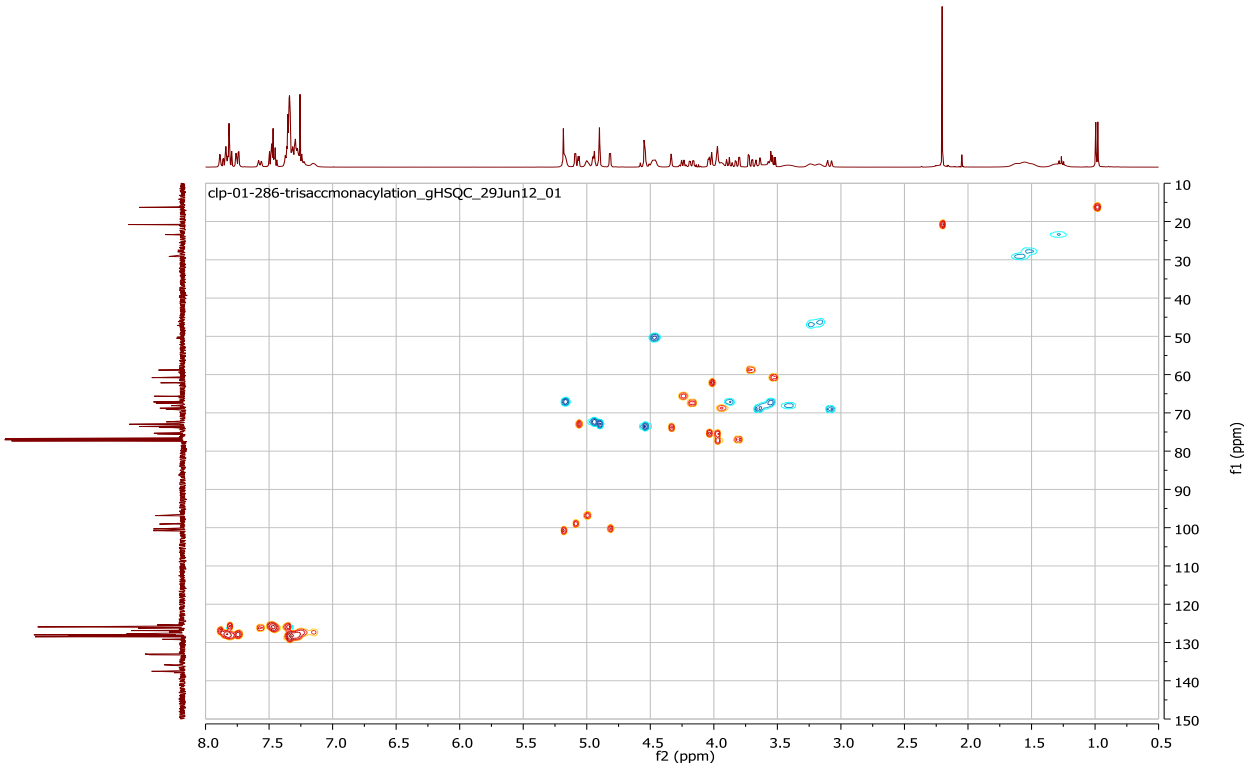

clp-01-282-trisacc-methanolysis-fr2_gHSQC_15Jun12_01
f2 (ppm)
f1 (ppm)

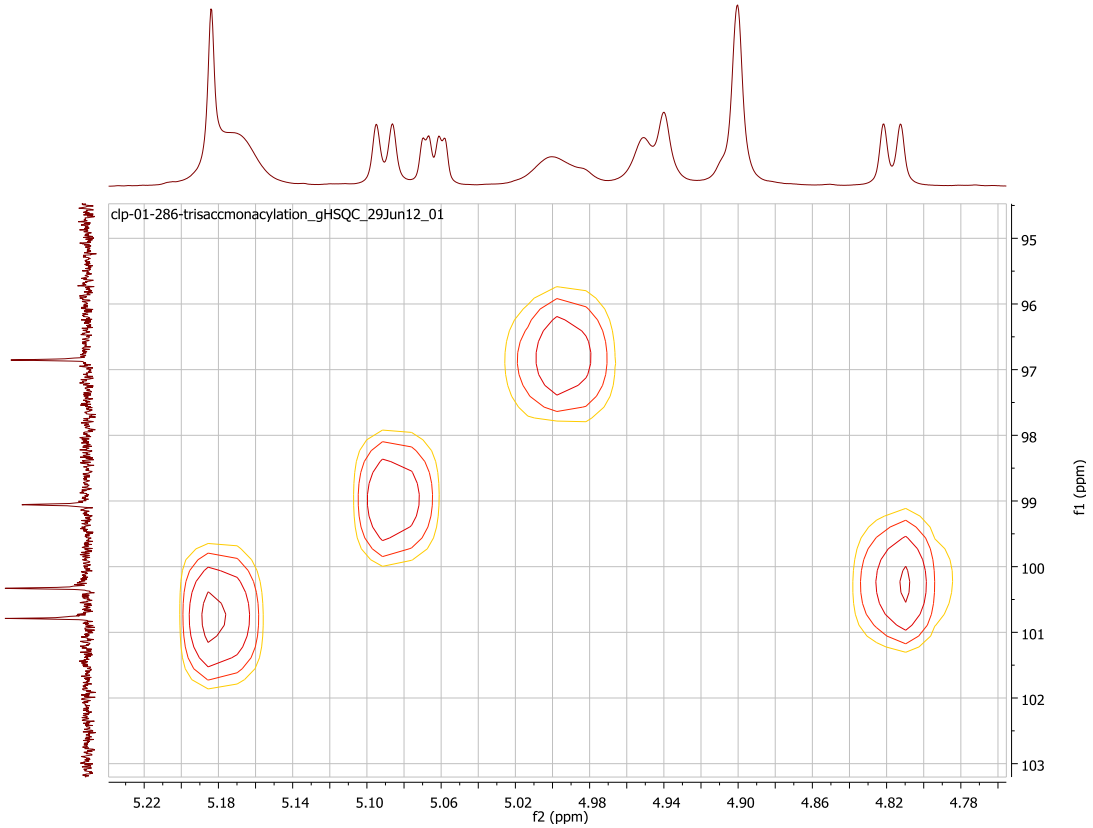


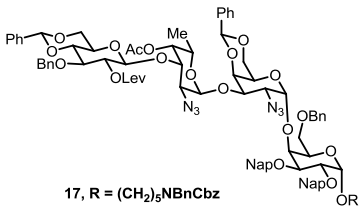
16, R = $(CH_2)_5NBnCbz$



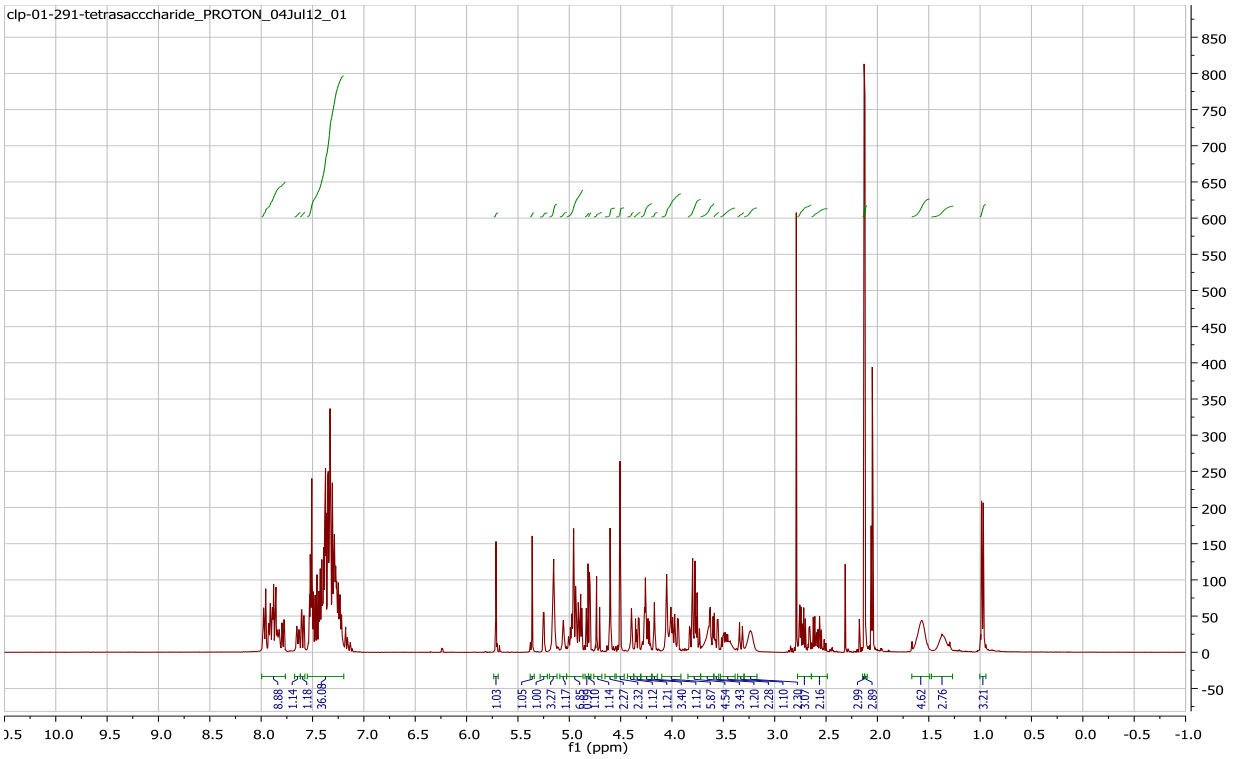


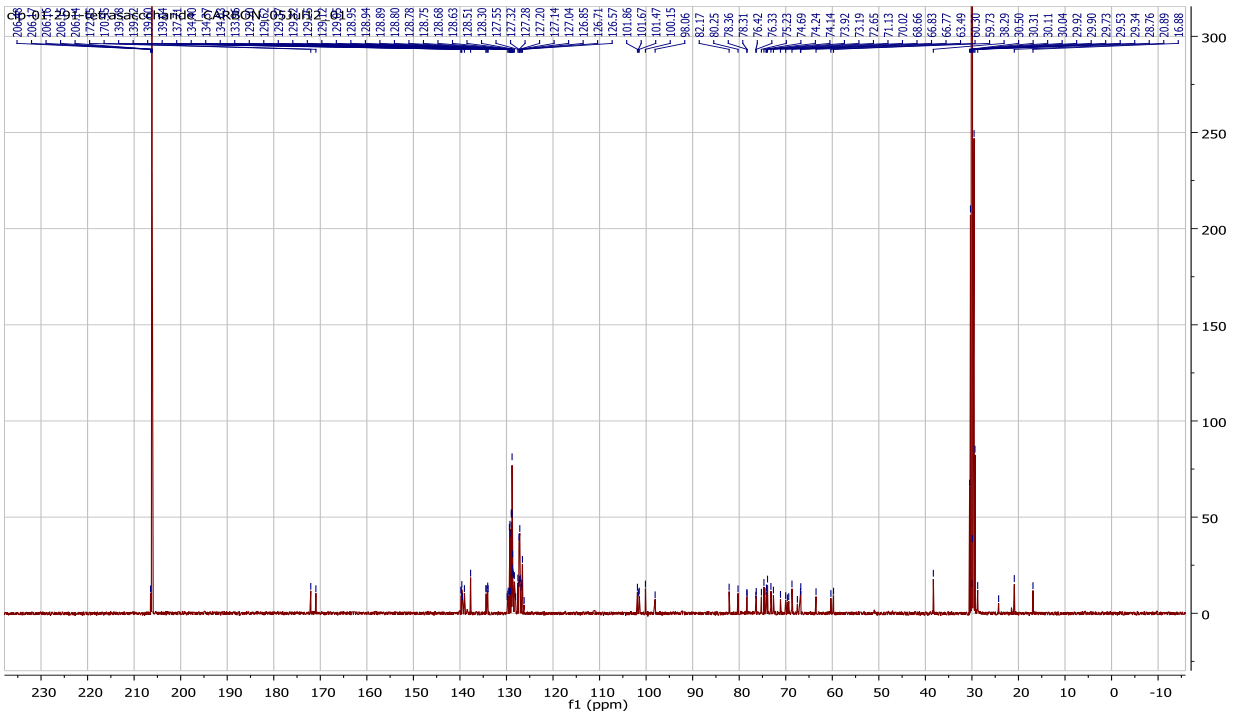

clp-01-286-trisaccmonacylation_gHSQC_29Jun12_01
f2 (ppm)
f1 (ppm)

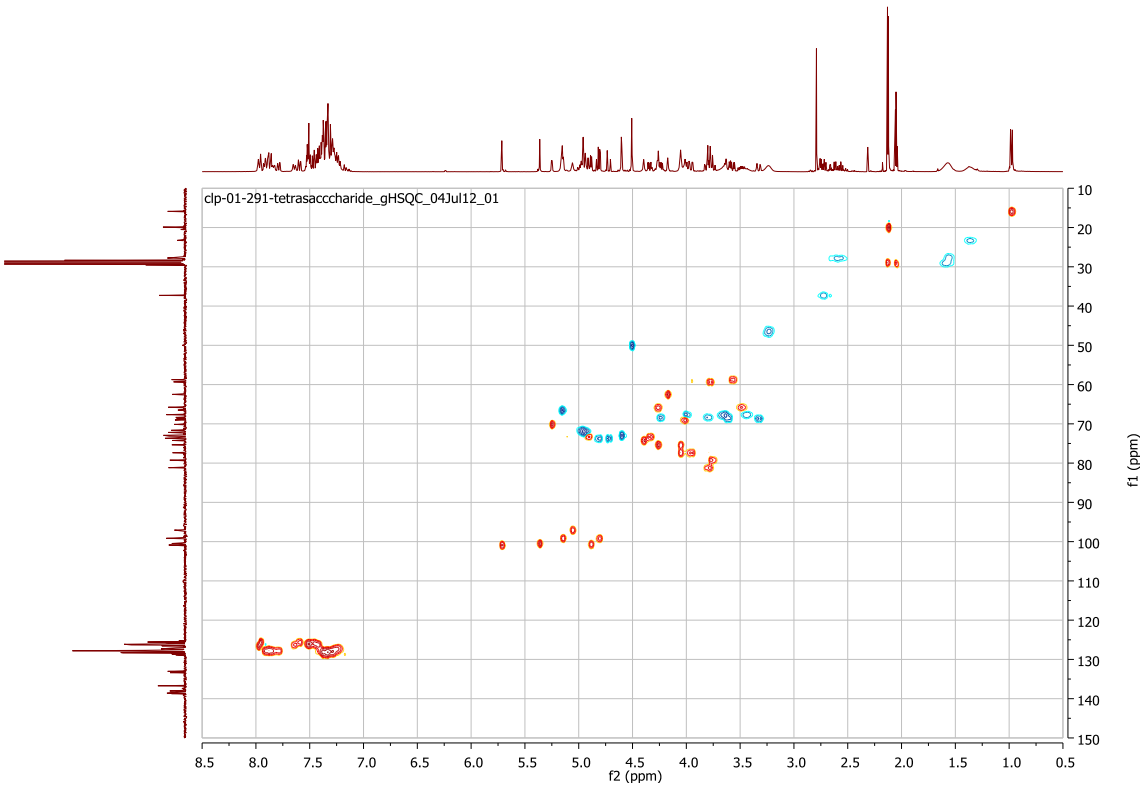

clp-01-286-trisaccmonacylation_gHSQC_29Jun12_01
f2 (ppm)
f1 (ppm)

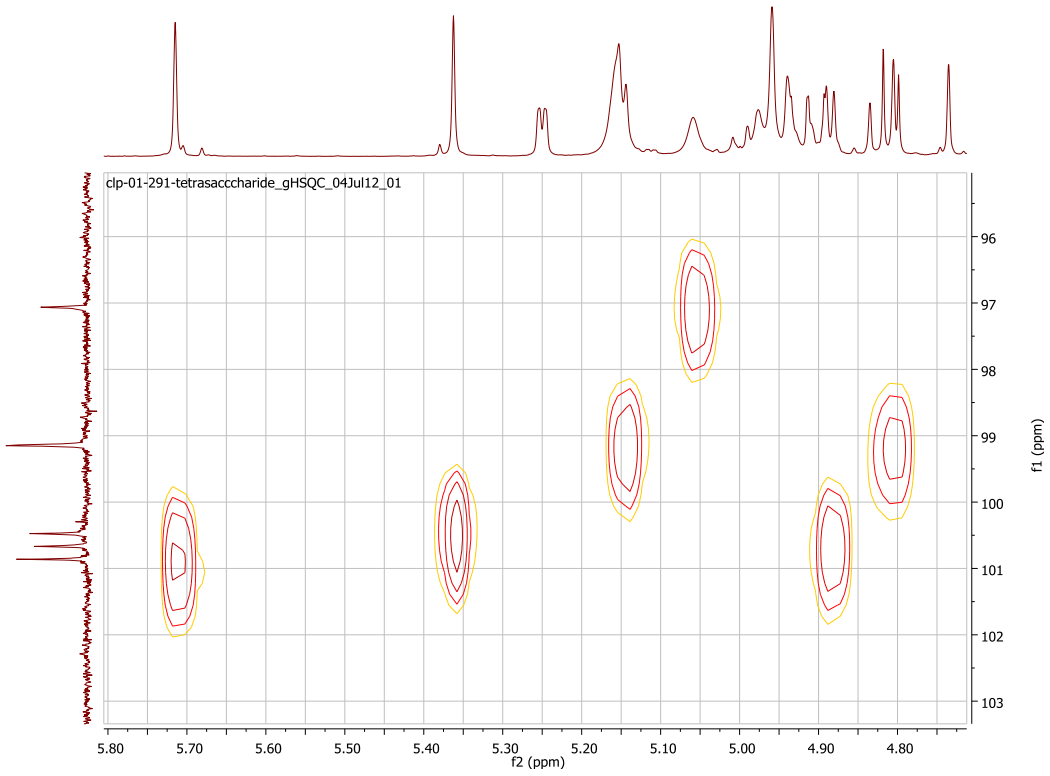


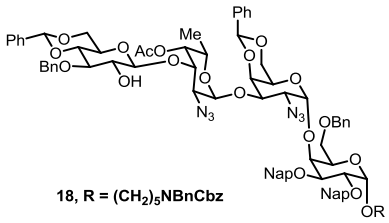
17, R = $(CH_2)_5NBnCbz$



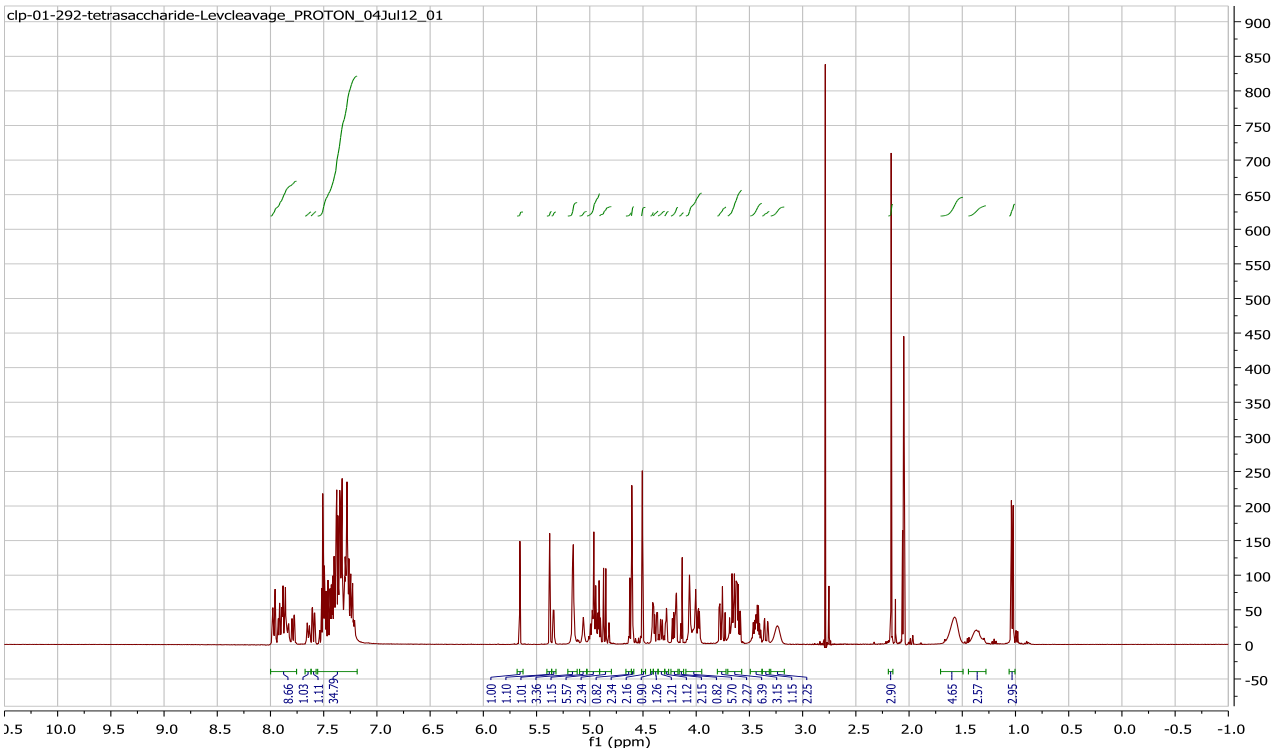


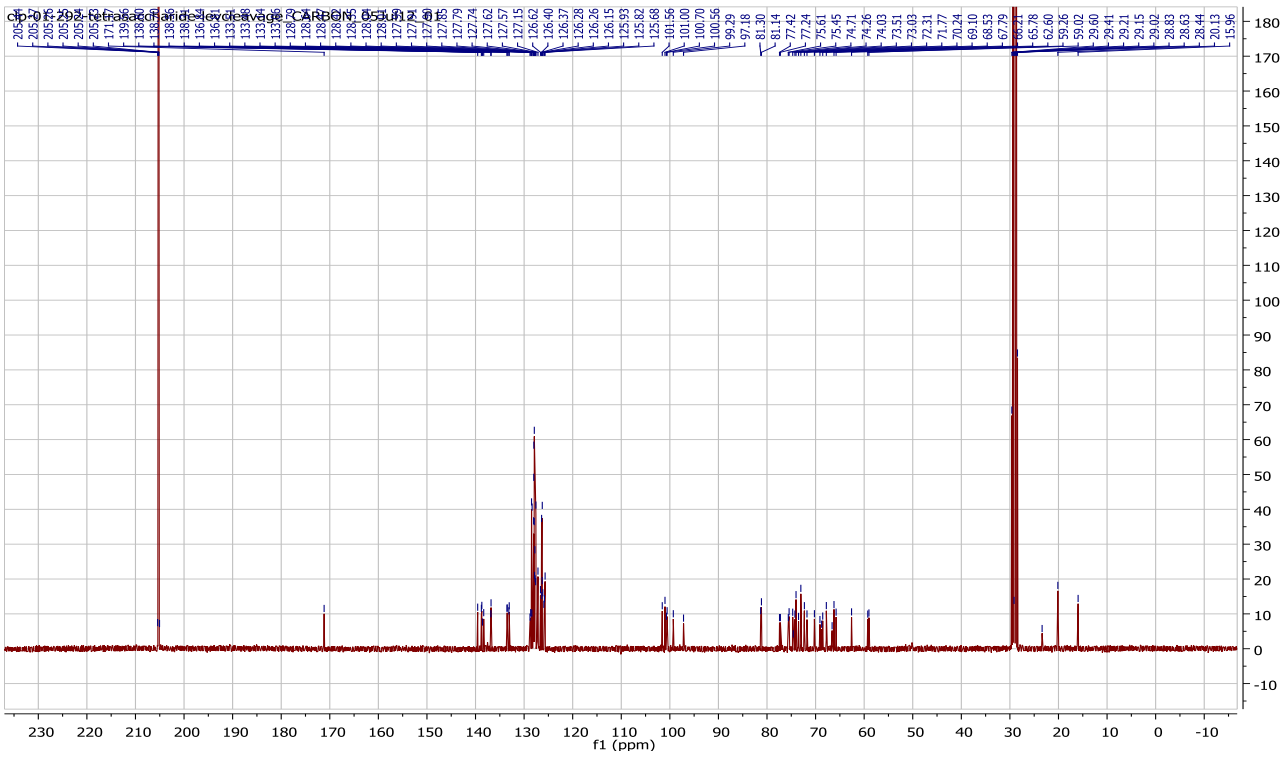

clp-01-291-tetrasacccharide_gHSQC_04Jul12_01
f2 (ppm)
f1 (ppm)

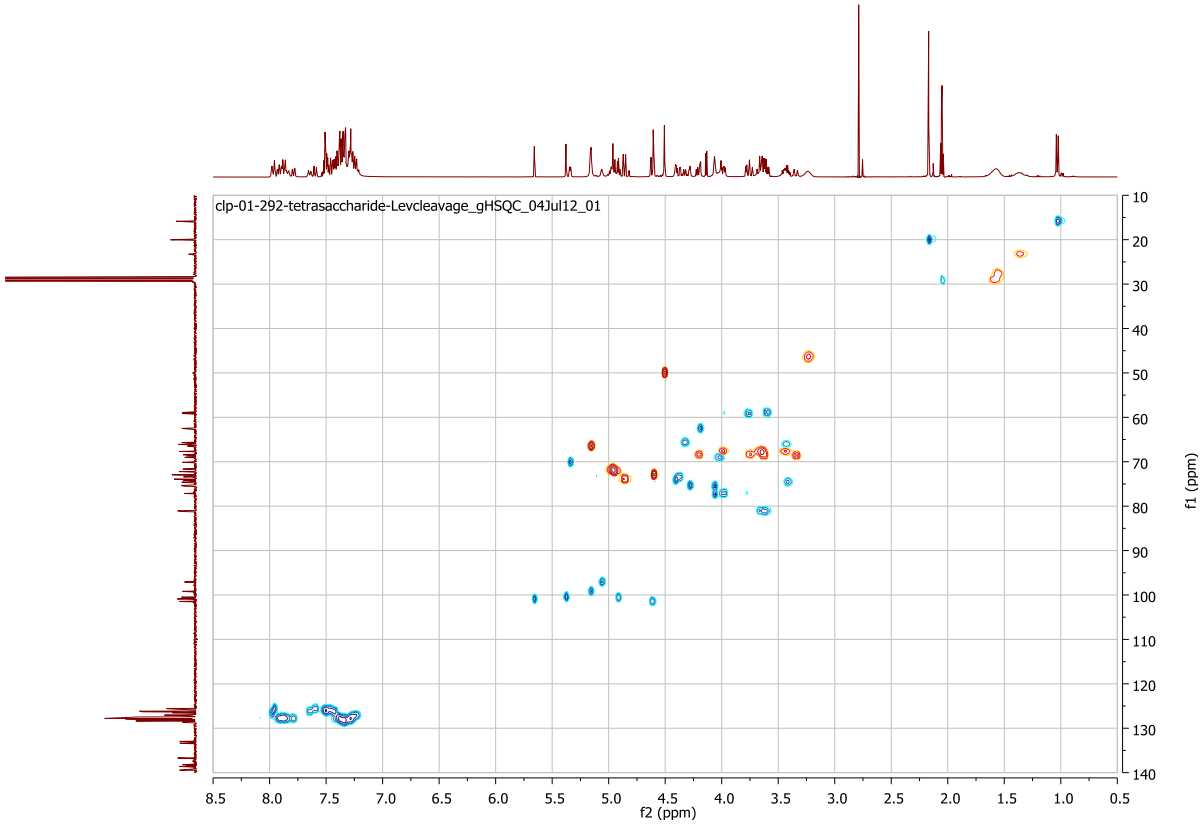

clp-01-291-tetrasacccharide_gHSQC_04Jul12_01
f2 (ppm)
f1 (ppm)

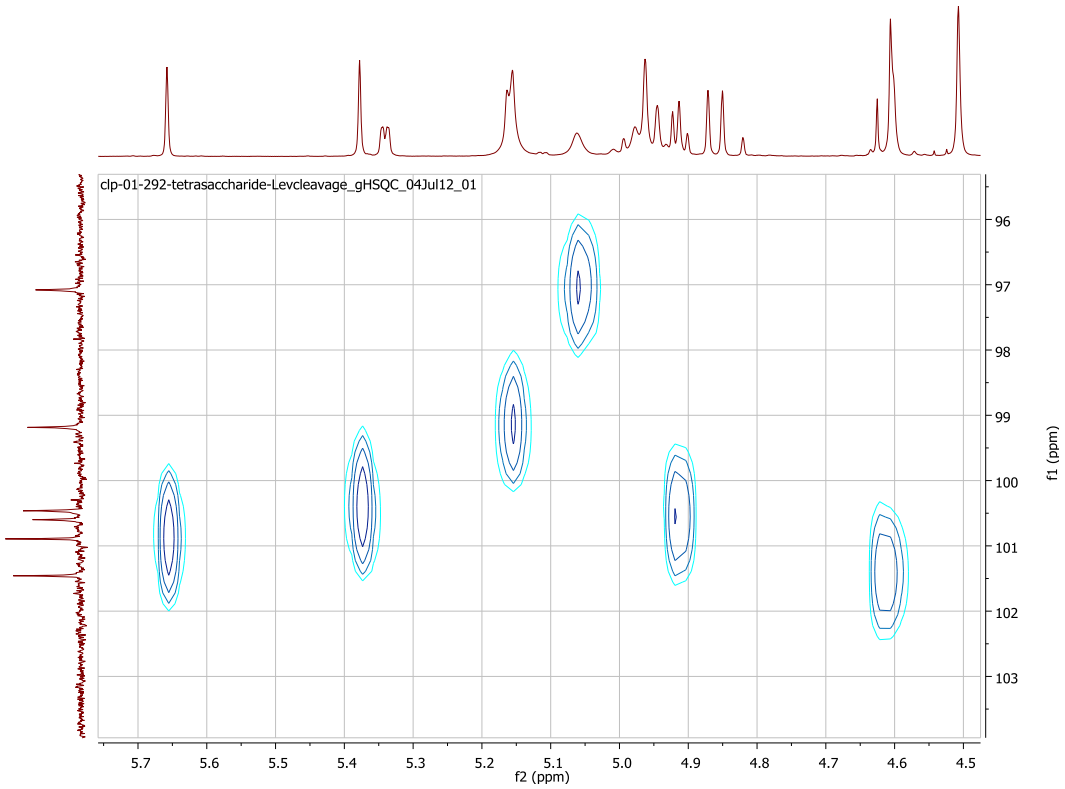


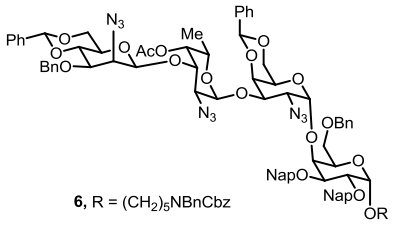
18, R = $(CH_2)_5NBnCbz$



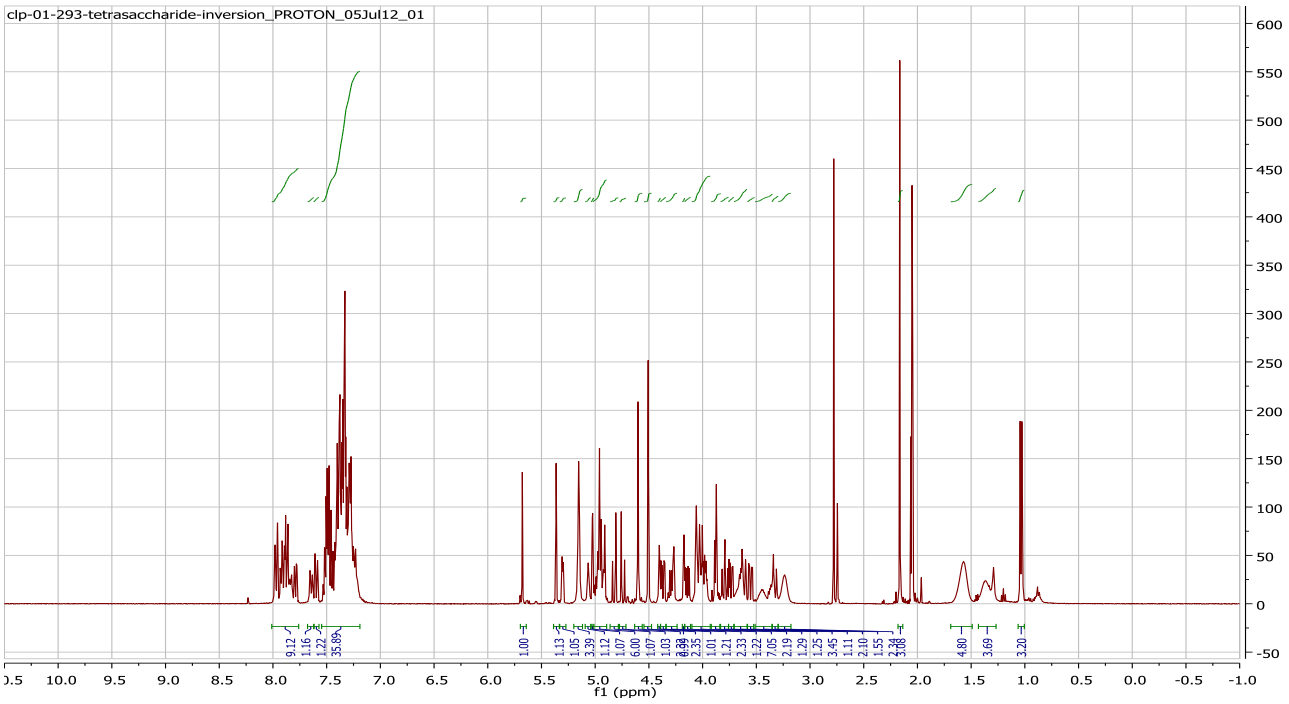


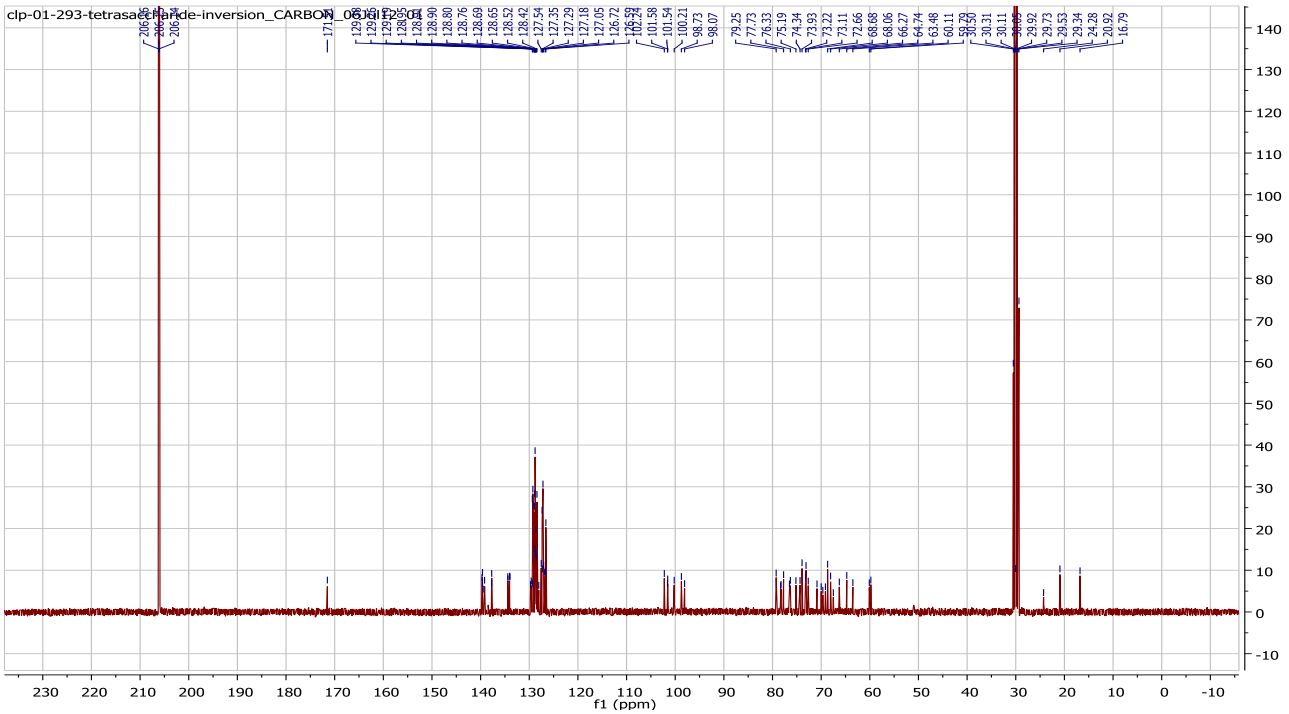

clp-01-292-tetrasaccharide-Levcleavage_gHSQC_04Jul12_01
f2 (ppm)
f1 (ppm)

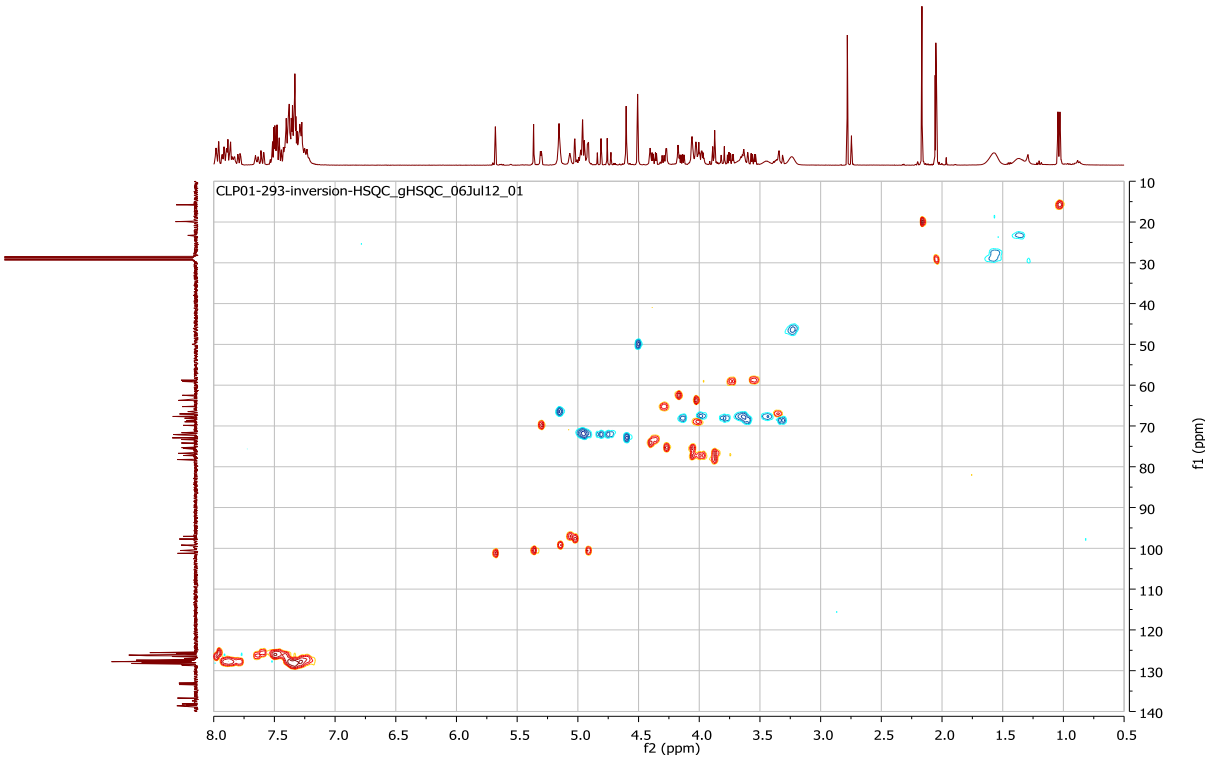

clp-01-292-tetrasaccharide-Levcleavage_gHSQC_04Jul12_01
f2 (ppm)
f1 (ppm)

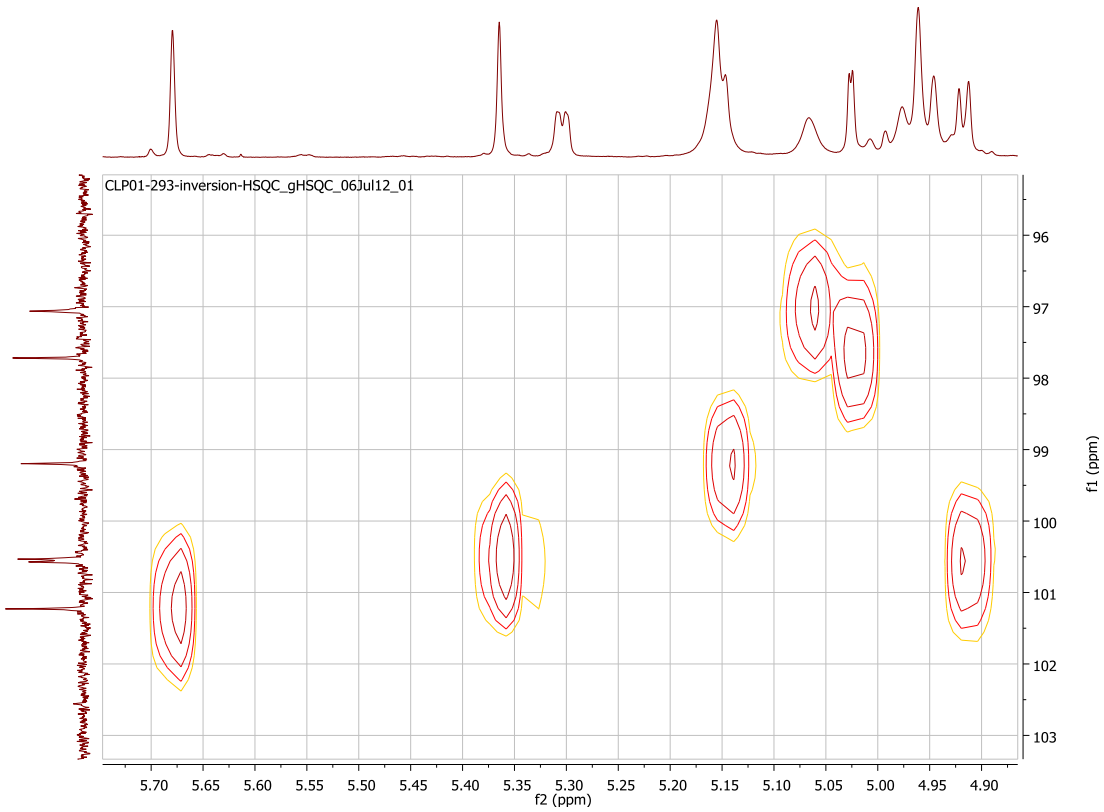


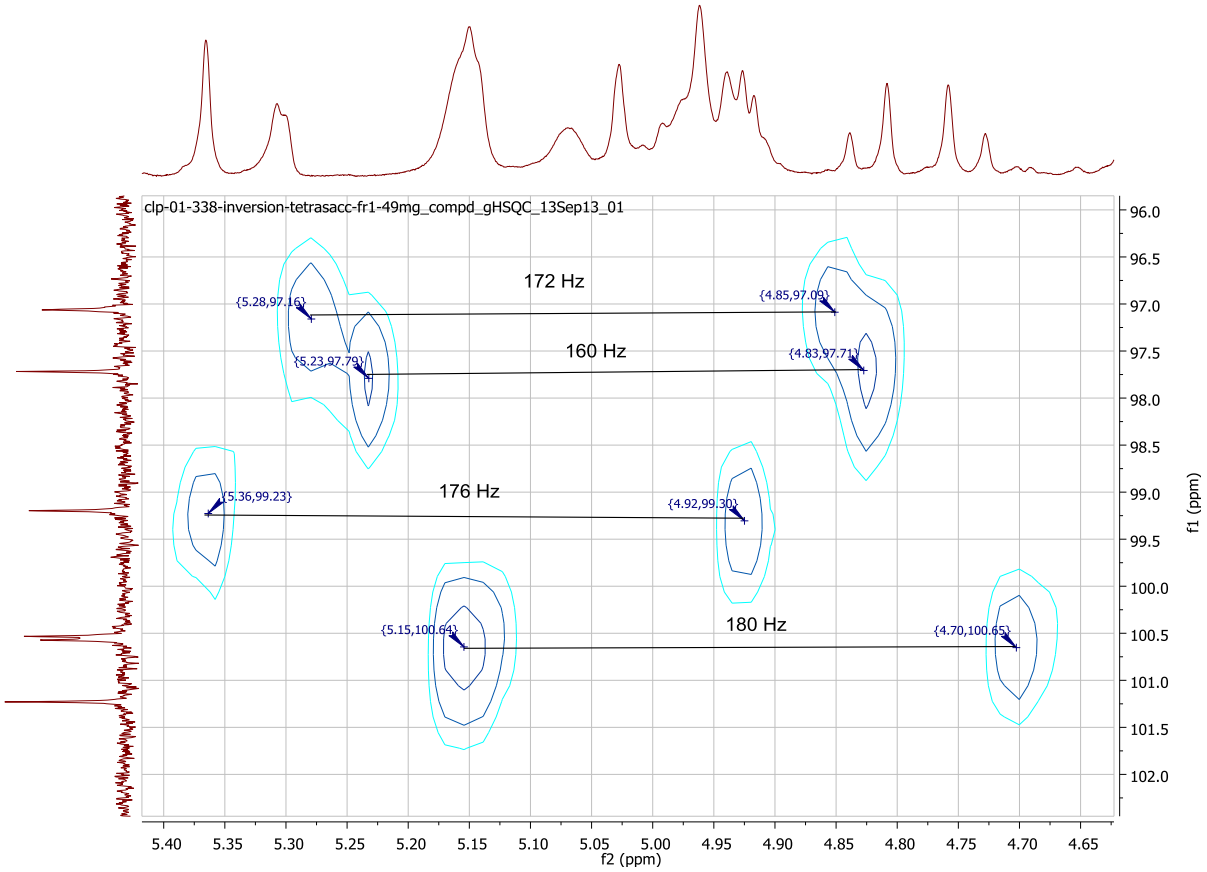
6, R = $(CH_2)_5NBnCbz$

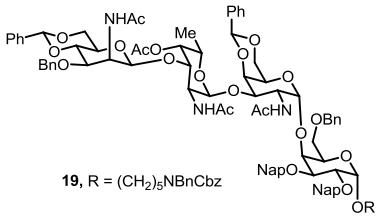




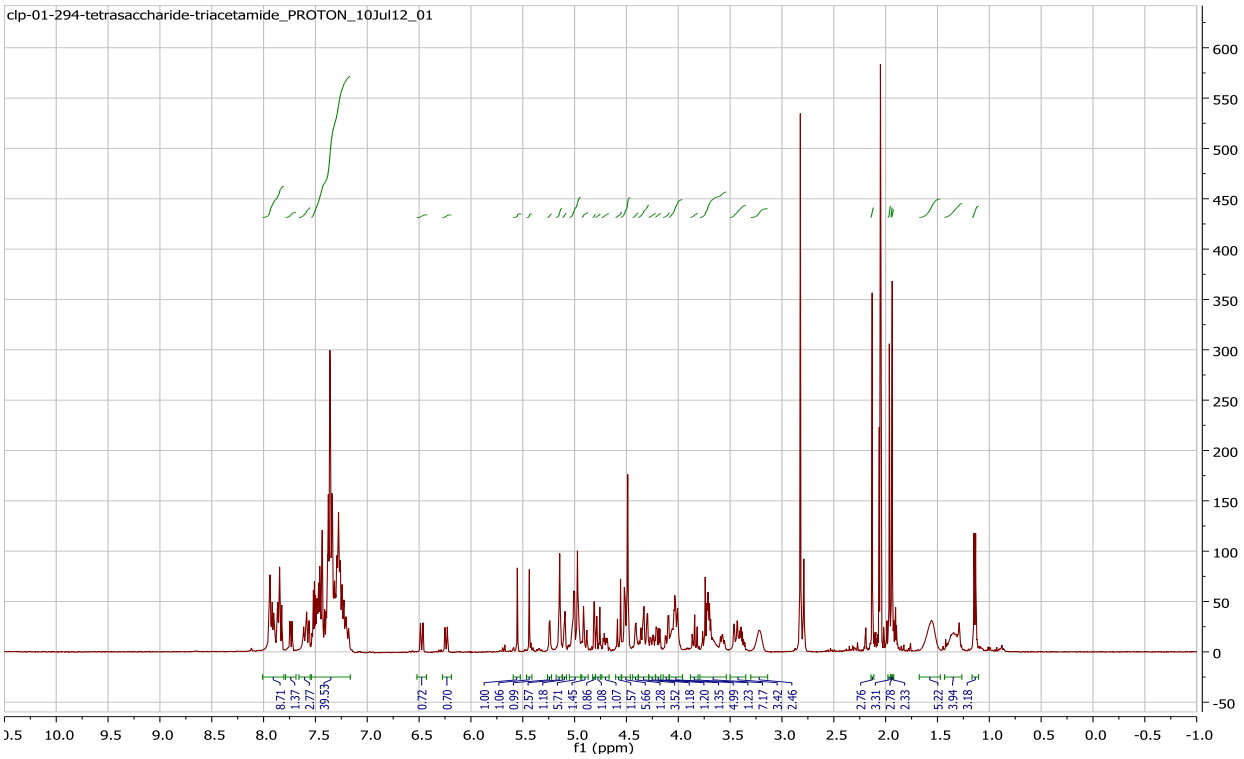

CLP01-293-inversion-HSQC_gHSQC_06Jul12_01
f2 (ppm)
f1 (ppm)

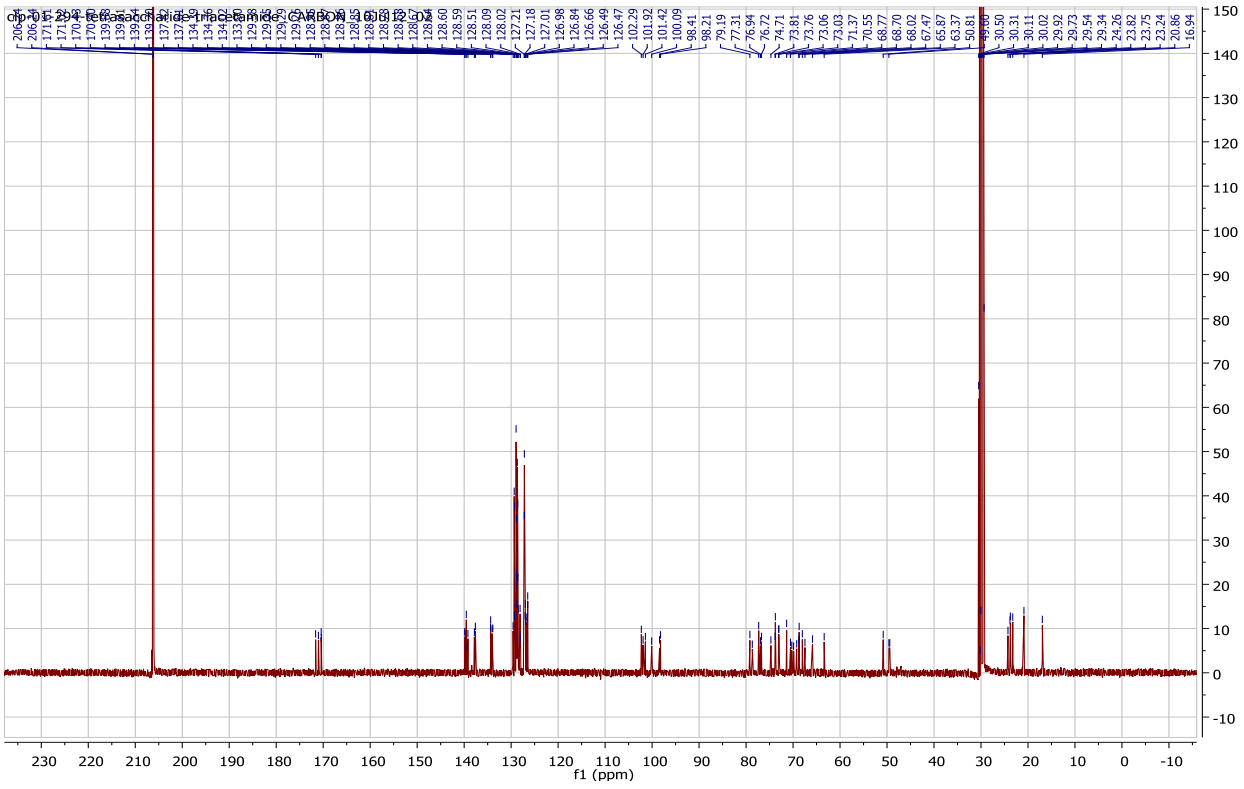

CLP01-293-inversion-HSQC_gHSQC_06Jul12_01
f2 (ppm)
f1 (ppm)

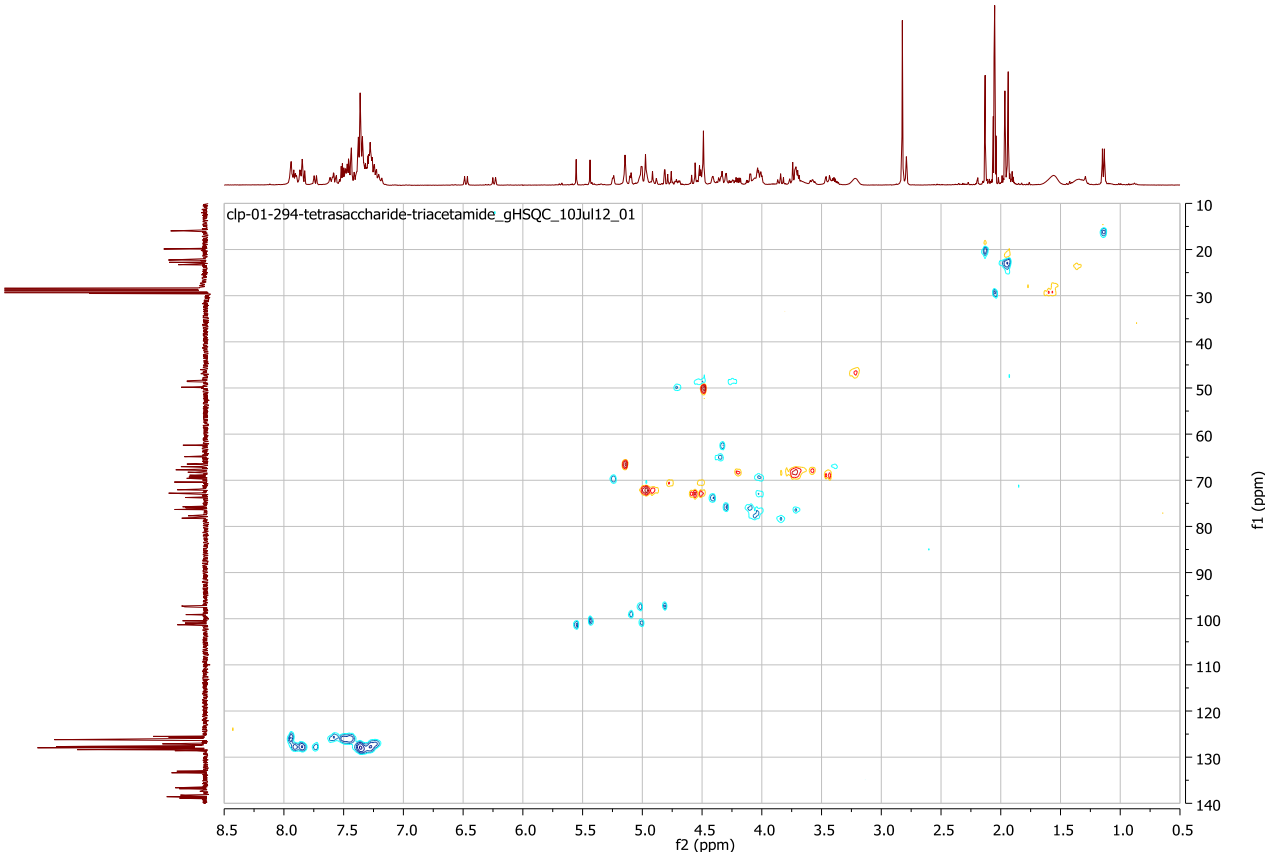

clp-01-338-inversion-tetrasacc-fr1-49mg_compd_gHSQC_13Sep13_01
172 Hz
160 Hz
176 Hz
180 Hz
{5.28,97.16}
{5.23,97.79}
{4.85,97.09}
{4.83,97.71}
{5.36,99.23}
{4.92,99.30}
{5.15,100.64}
{4.70,100.65}
f2 (ppm)
f1 (ppm)

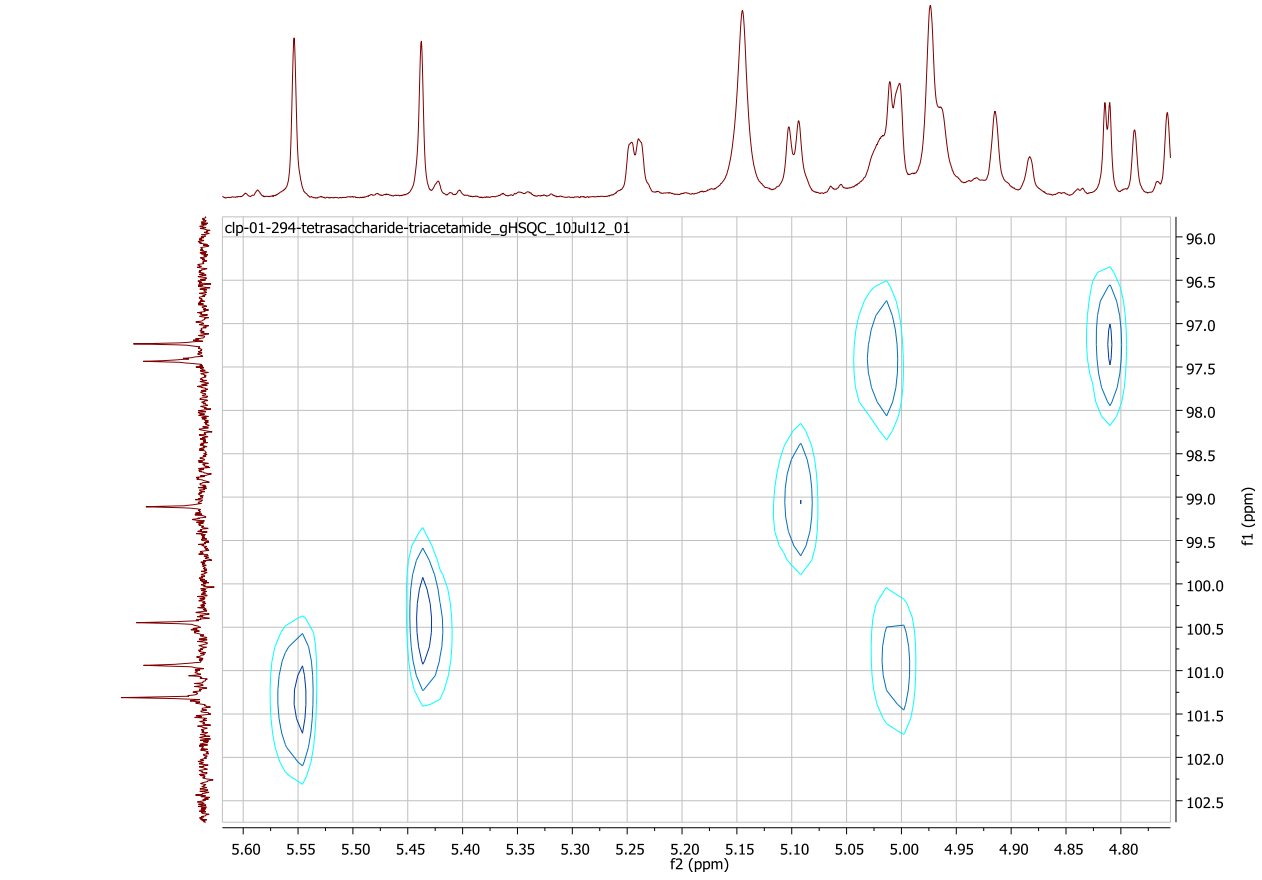


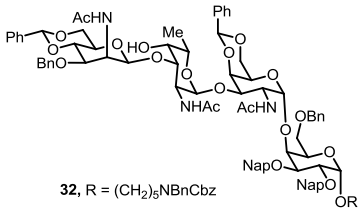
19, R = $(CH_2)_5NBnCbz$



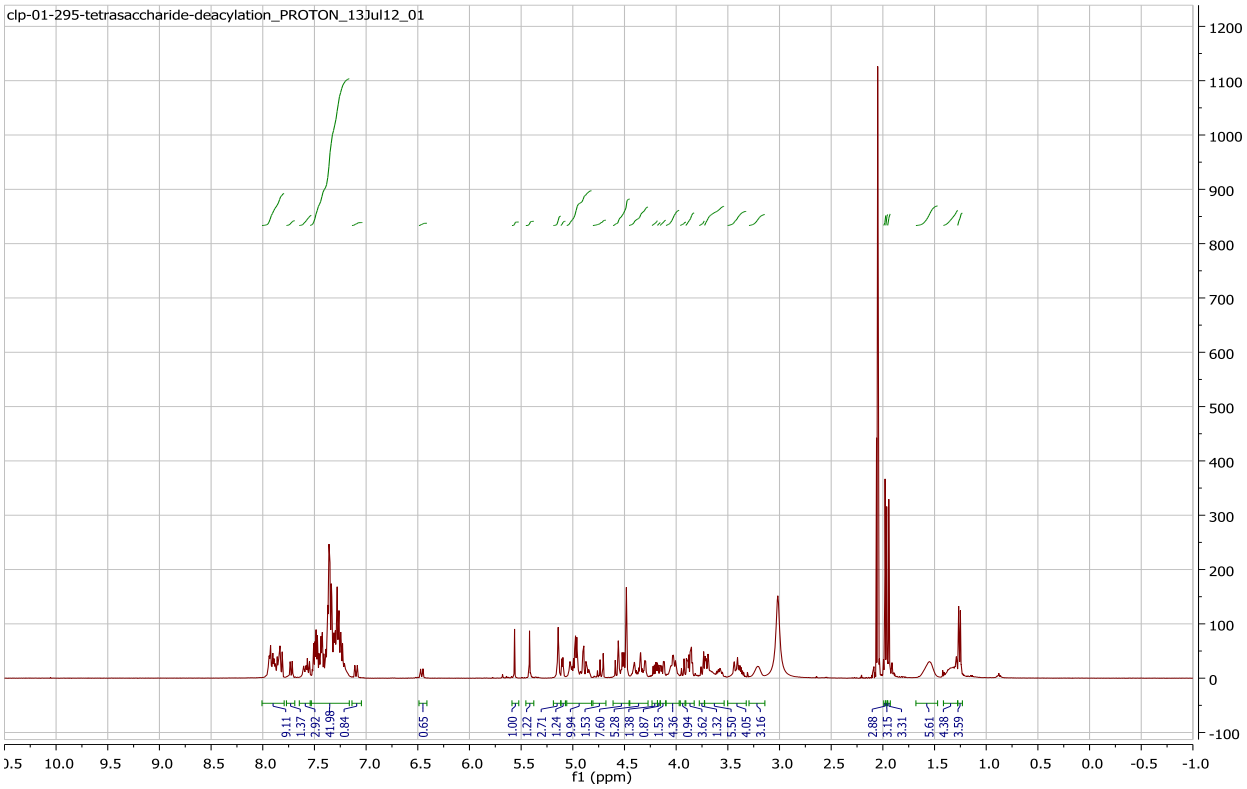


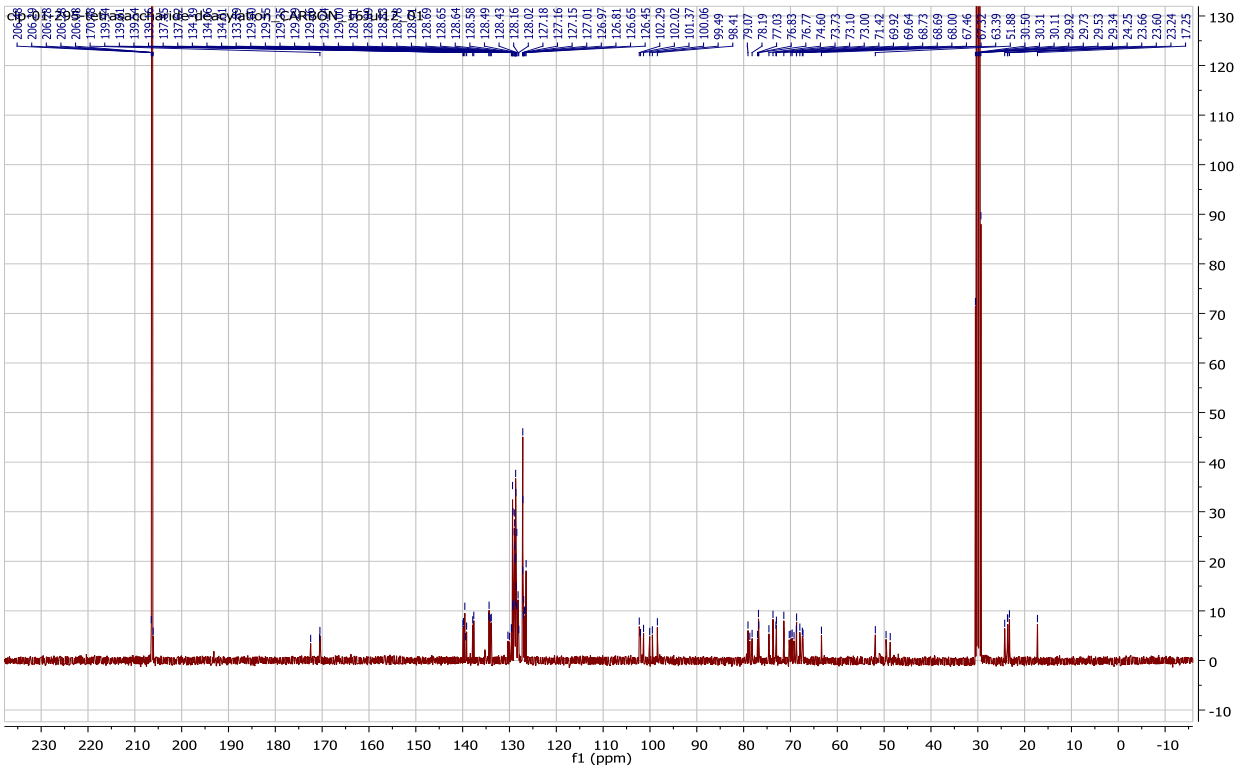

clp-01-294-tetrasaccharide-triacetamide_gHSQC_10Jul12_01
f2 (ppm)
f1 (ppm)

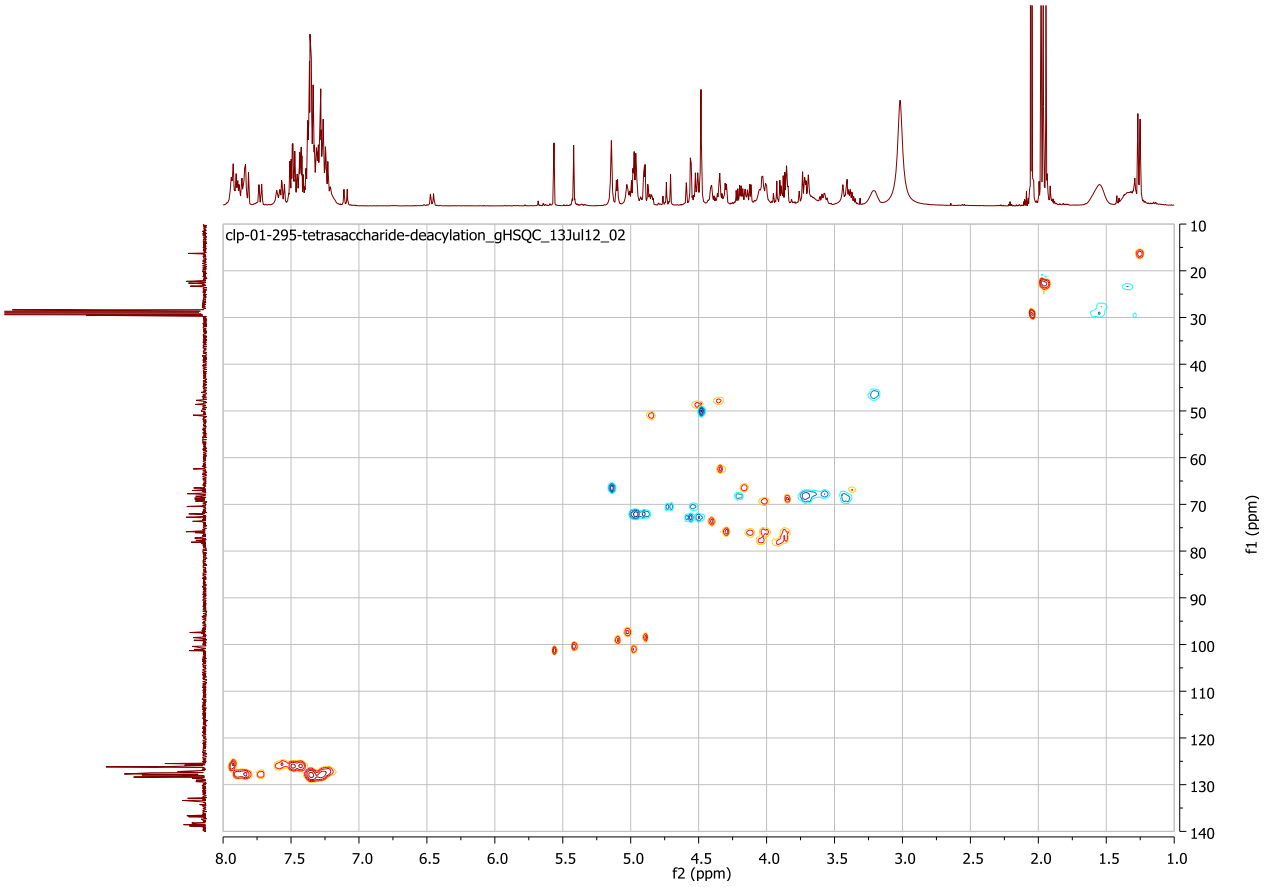

clp-01-294-tetrasaccharide-triacetamide_gHSQC_10Jul12_01
f2 (ppm)
f1 (ppm)

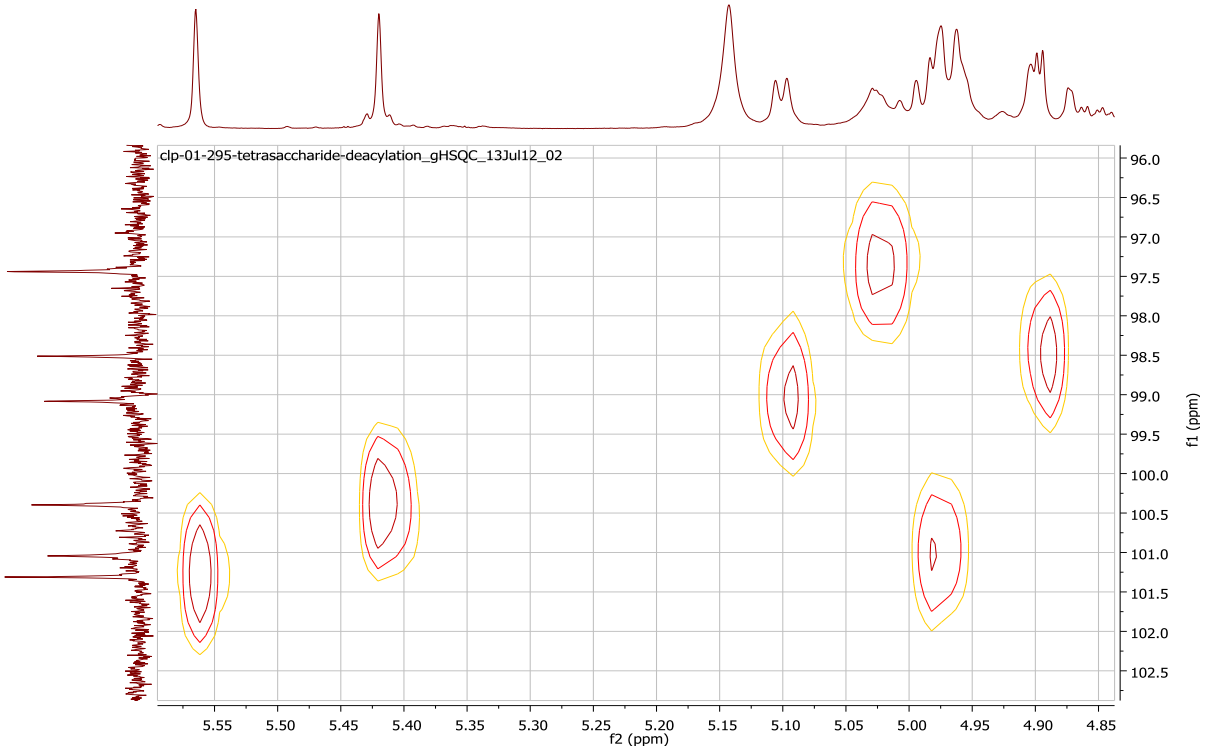


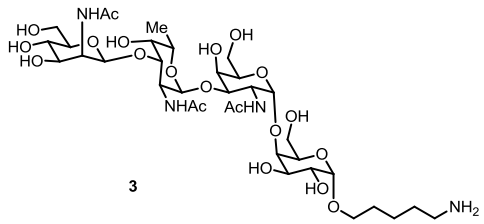
32, R = $(CH_2)_5NBnCbz$



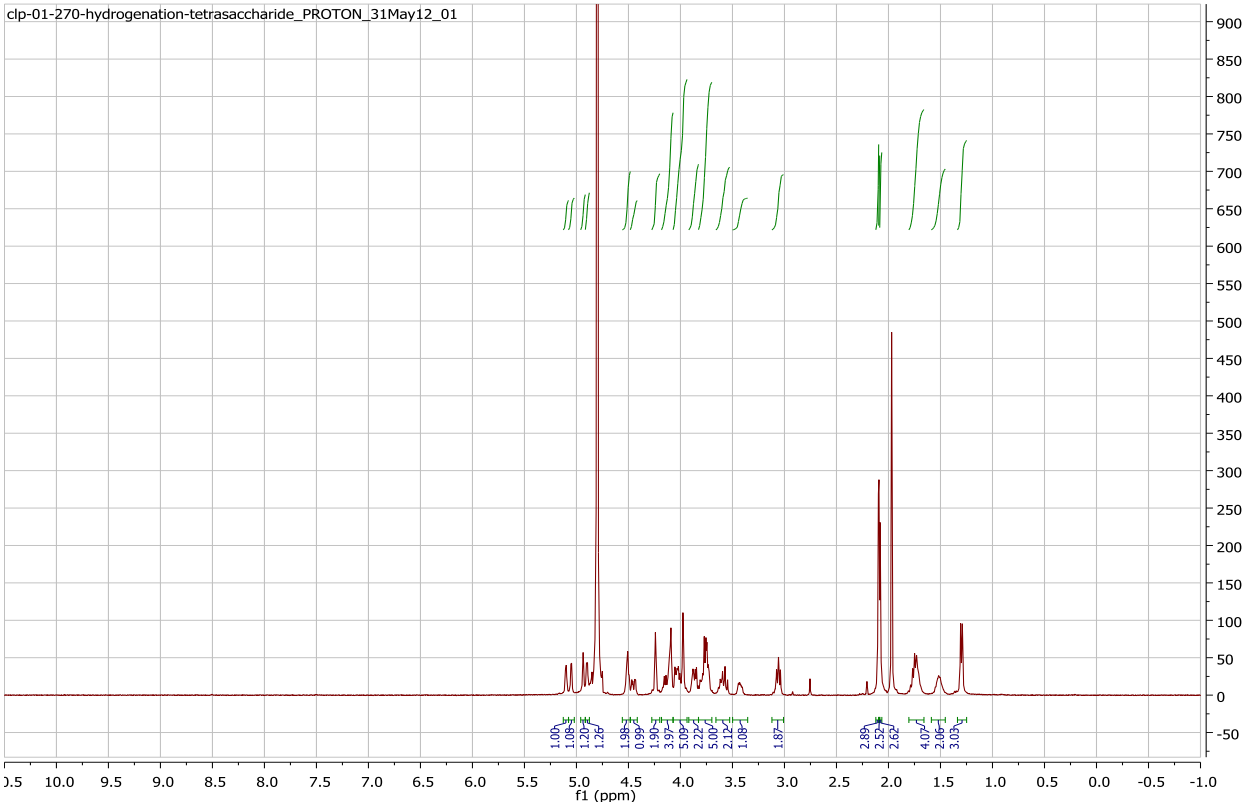


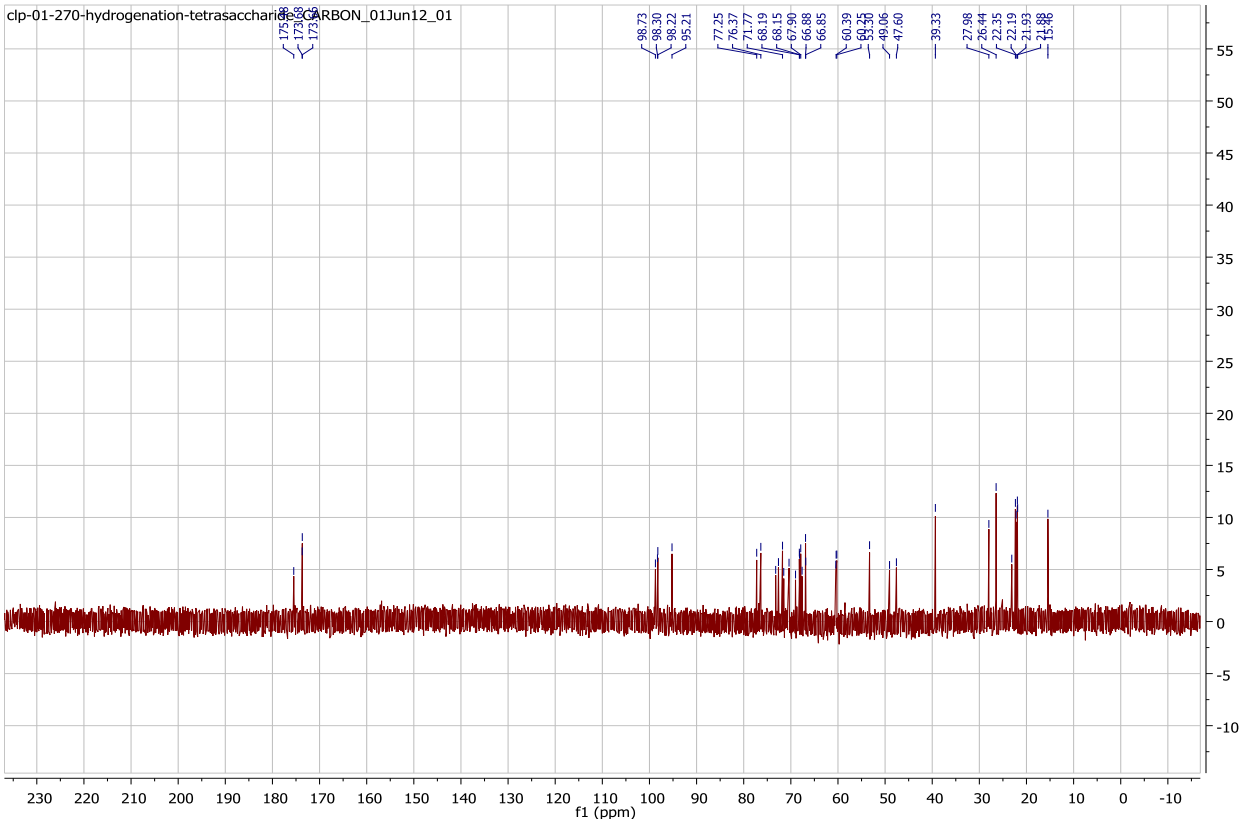

clp-01-295-tetrasaccharide-deacylation_gHSQC_13Jul12_02
f2 (ppm)
f1 (ppm)

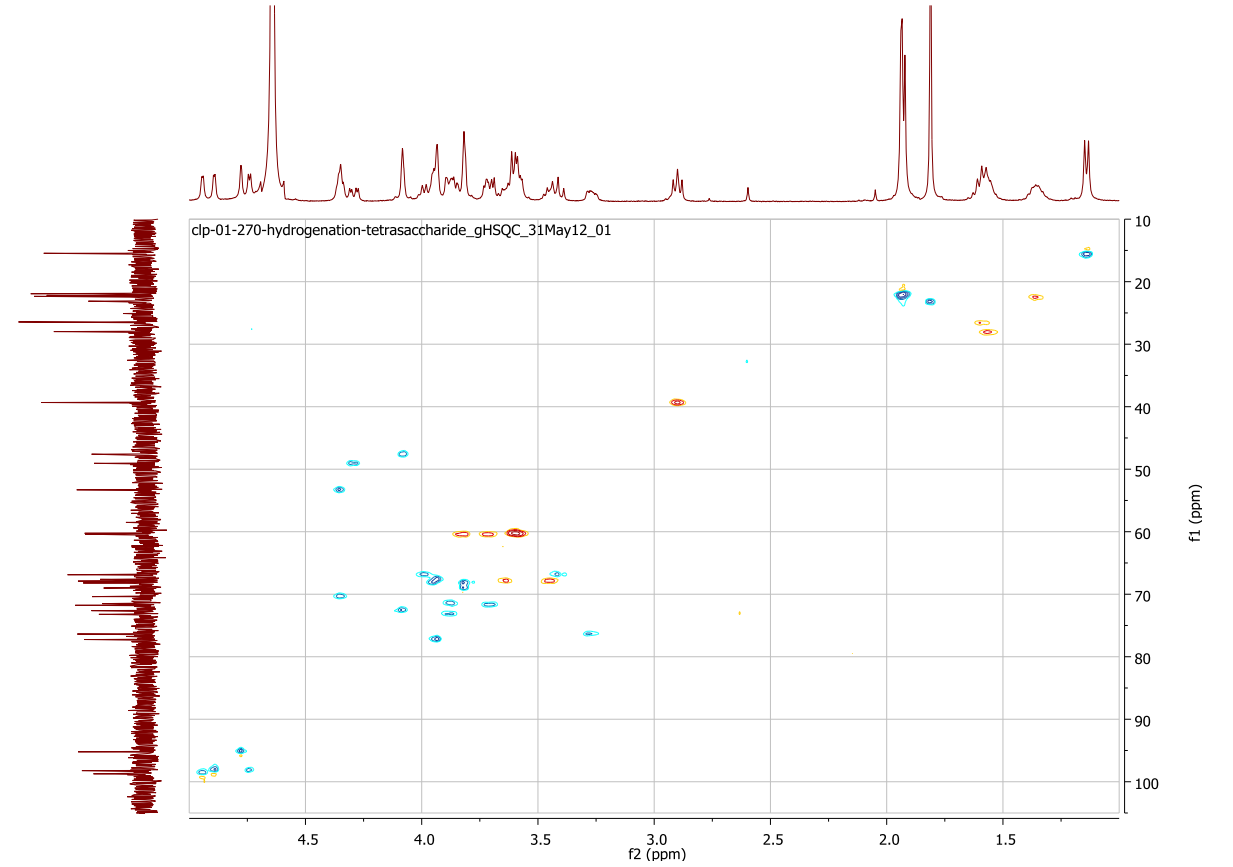

clp-01-295-tetrasaccharide-deacylation_gHSQC_13Jul12_02
f2 (ppm)
f1 (ppm)

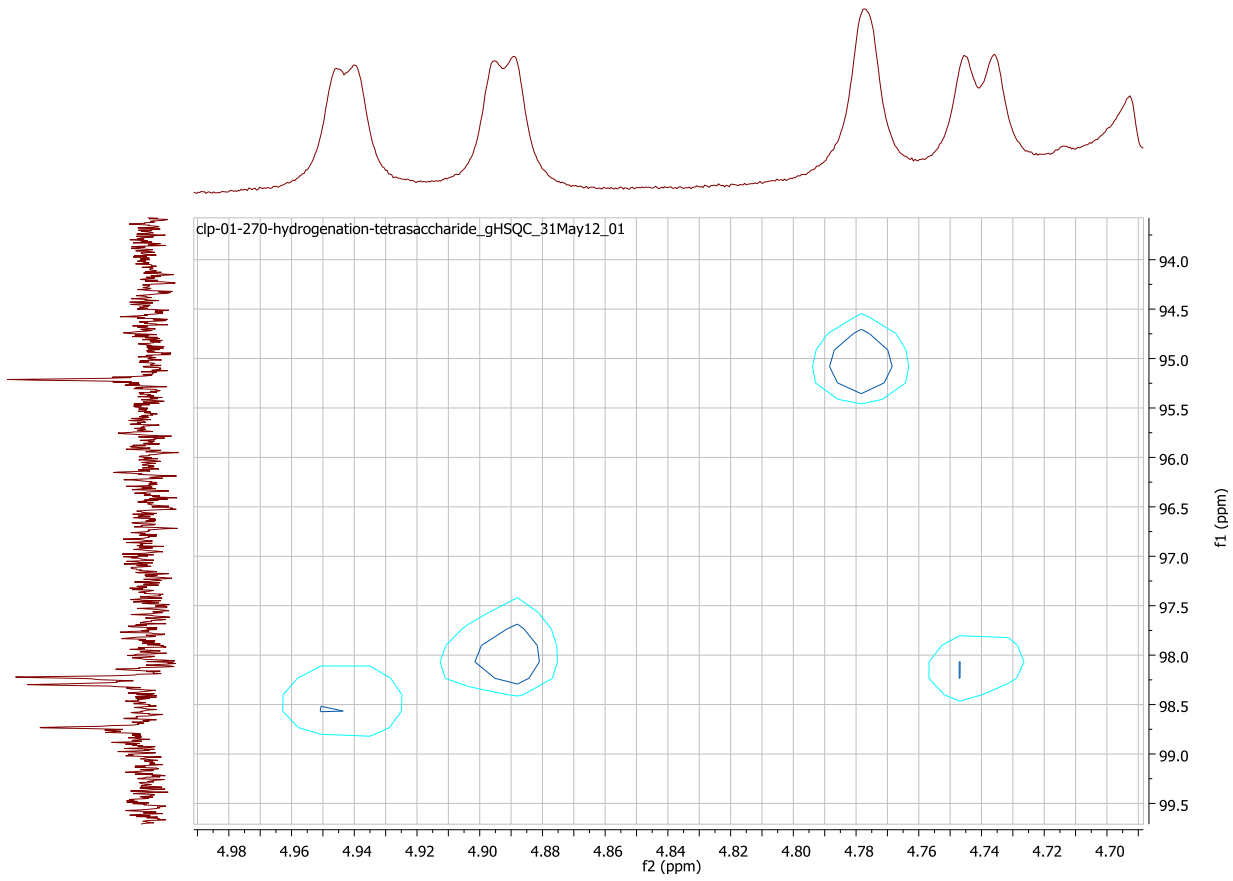

NHAc
HO
HO
HO
O
HO
Me
O
O
NHAc
O
AcHN
HO
OH
O
O
OH
O
HO
HO
O
NH2

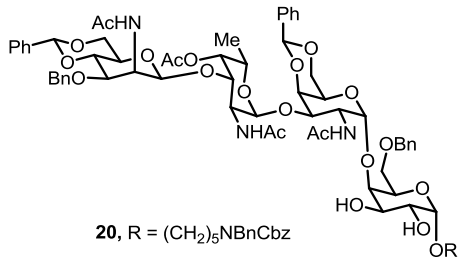
3


clp-01-270-hydrogenation-tetrasaccharide_PROTON_31May12_01
f1 (ppm)

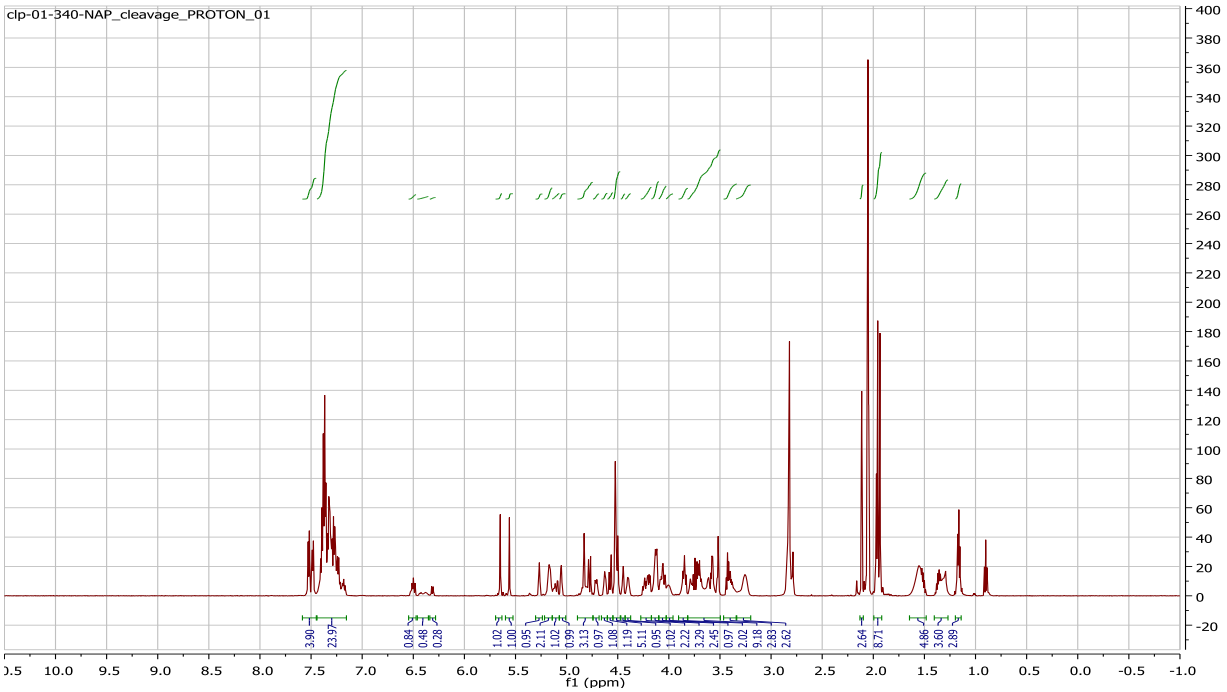

clp-01-270-hydrogenation-tetrasaccharide_CARBON_01Jun12_01
175.88
173.68
173.49
98.73
98.30
98.22
95.21
77.25
76.37
71.77
68.19
68.15
67.90
66.88
66.85
60.39
60.25
53.30
49.06
47.60
39.33
27.98
26.44
22.35
22.19
21.93
21.88
15.46
f1 (ppm)

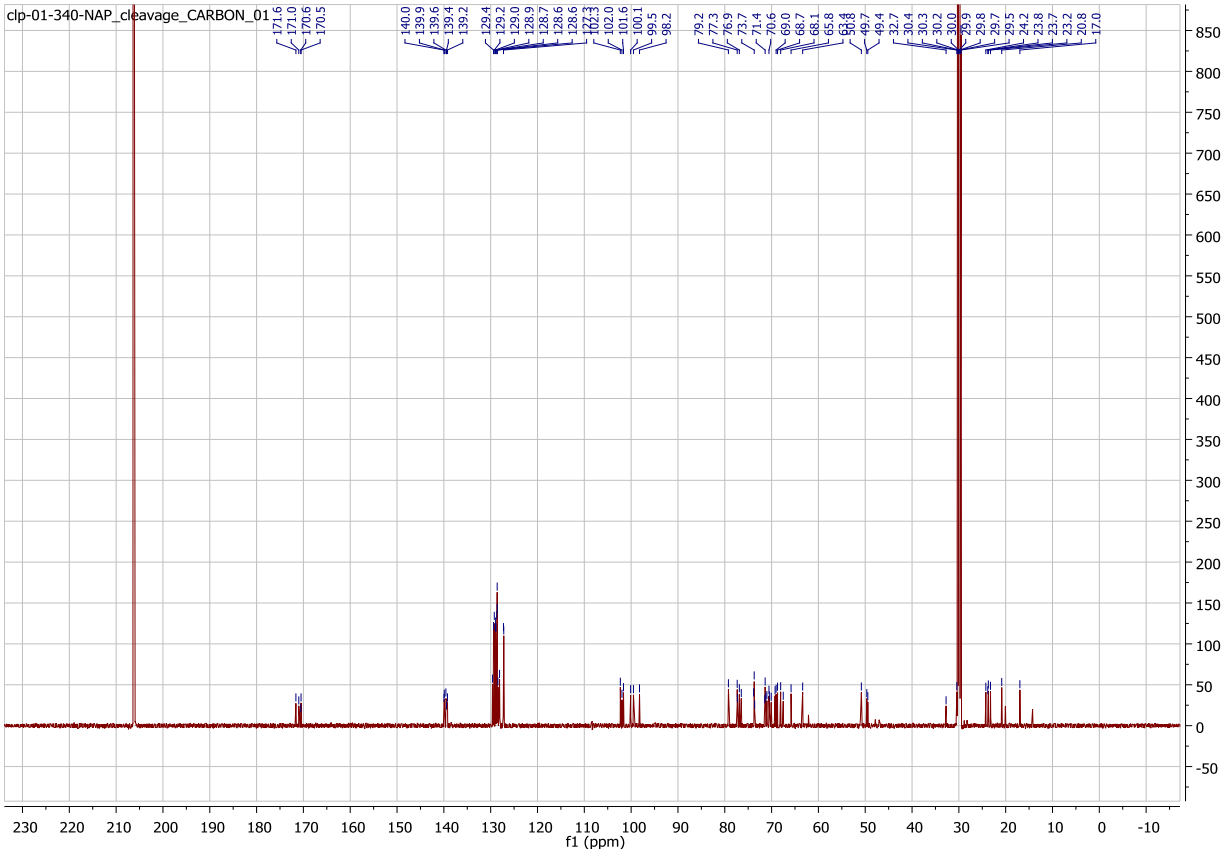

clp-01-270-hydrogenation-tetrasaccharide_gHSQC_31May12_01
f2 (ppm)
f1 (ppm)

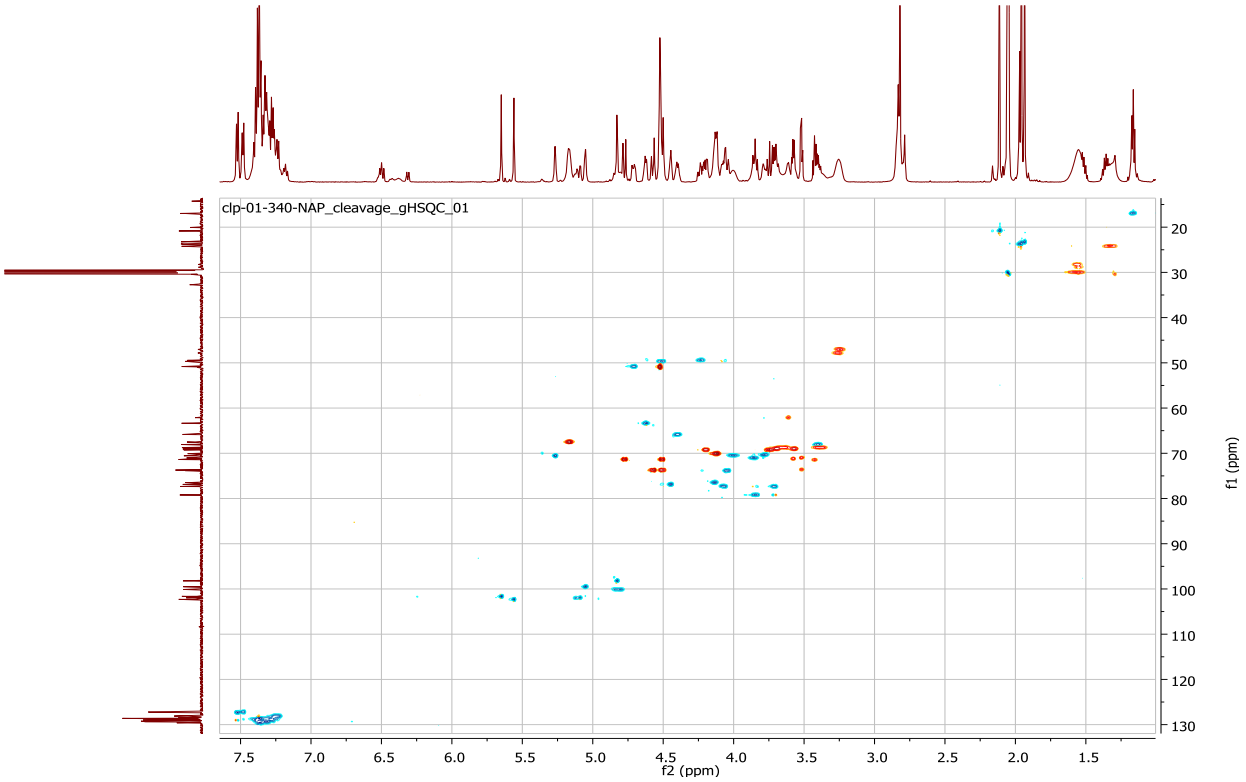

clp-01-270-hydrogenation-tetrasaccharide_gHSQC_31May12_01
f2 (ppm)
f1 (ppm)

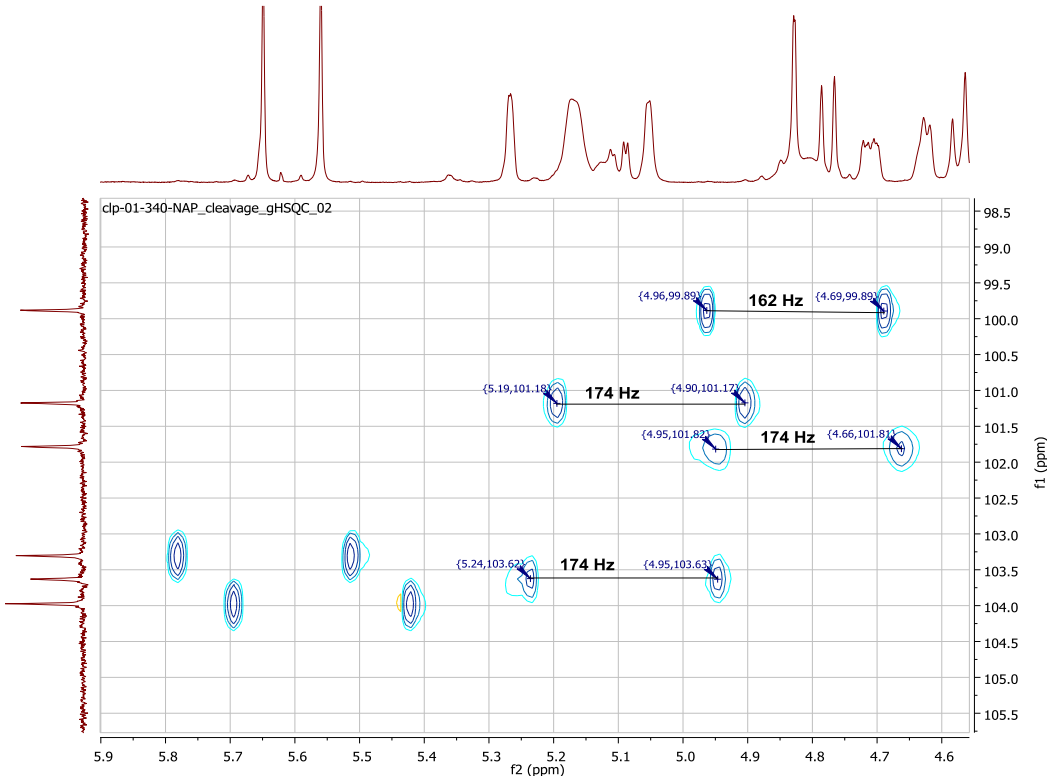


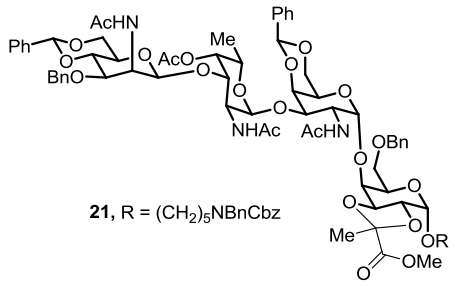
20, R = $(CH_2)_5NBnCbz$



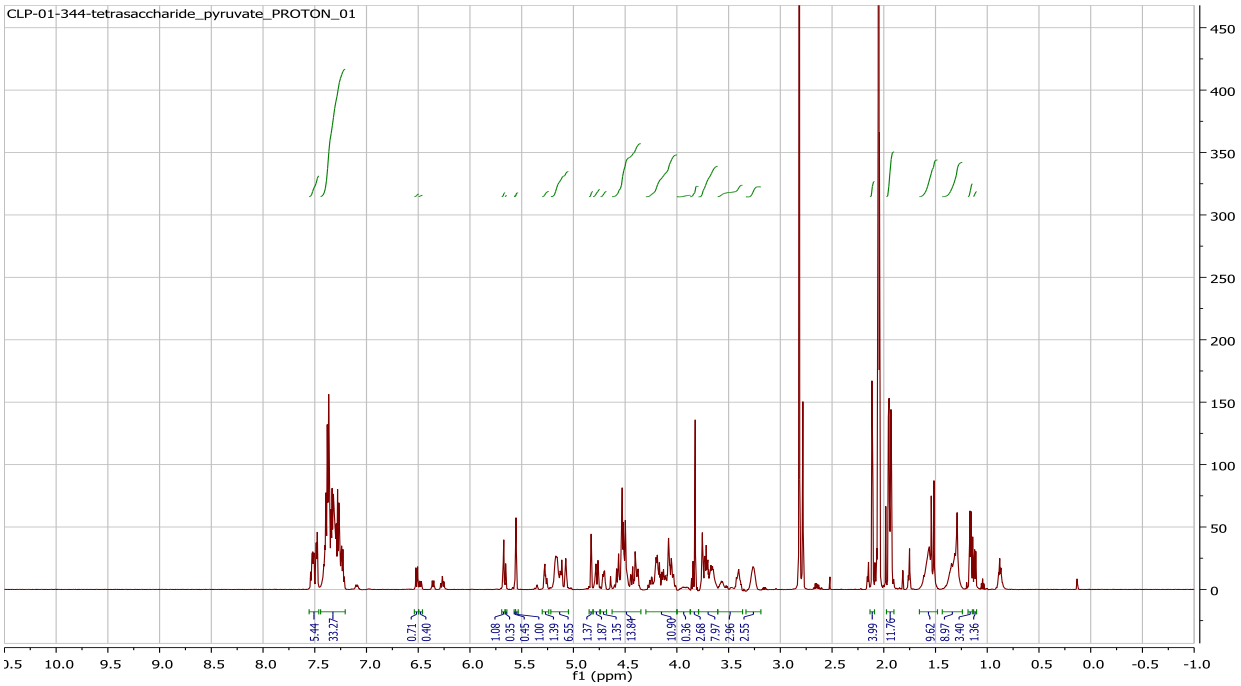


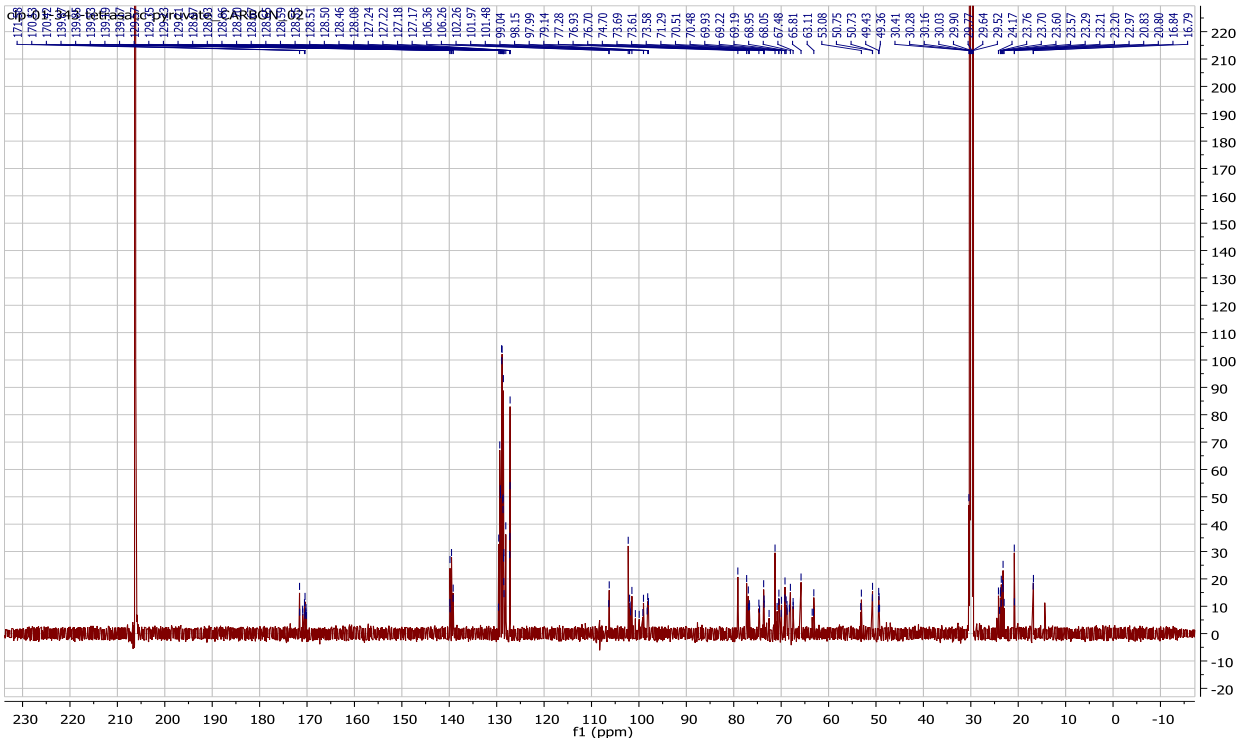

clp-01-340-NAP_cleavage_gHSQC_01
f2 (ppm)
f1 (ppm)

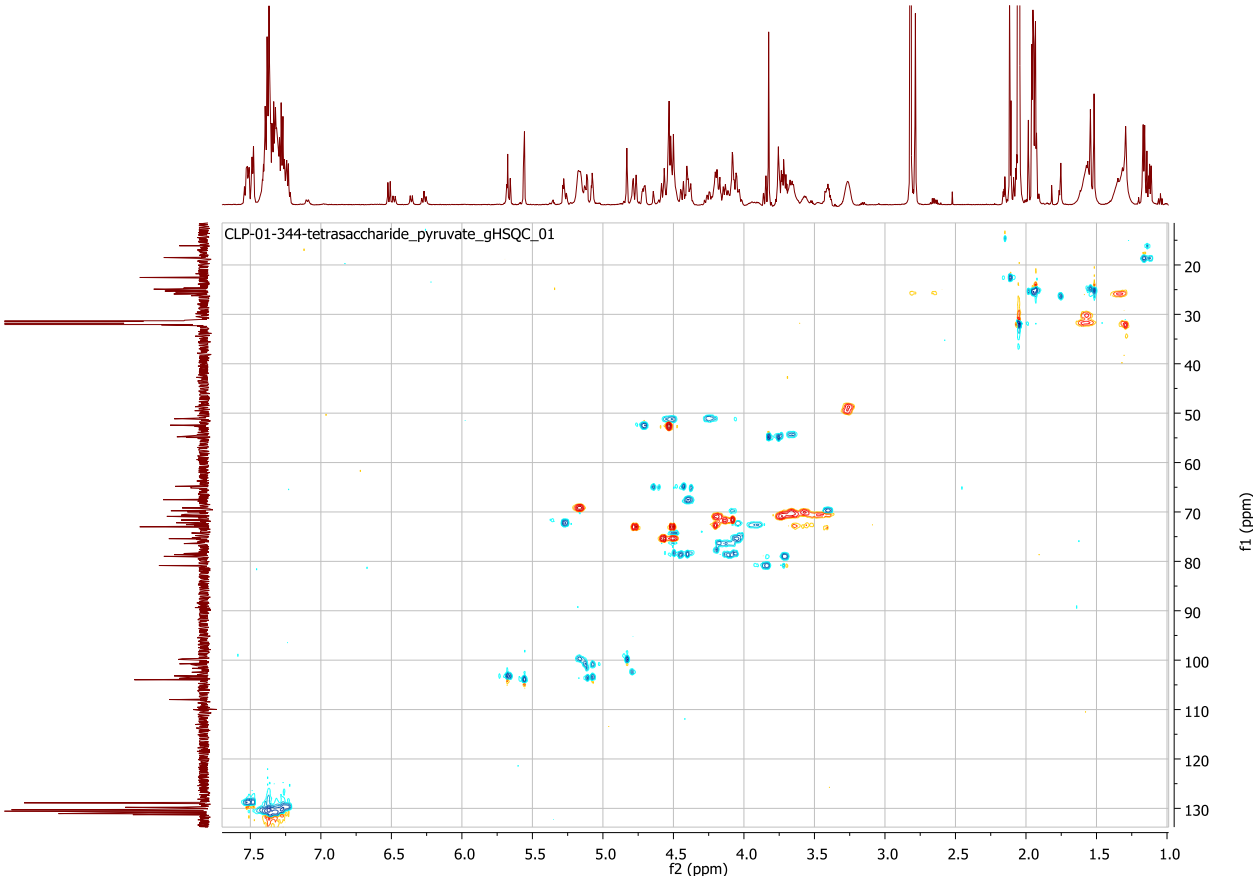

clp-01-340-NAP_cleavage_gHSQC_02
{4.96,99.89}
162 Hz
{4.69,99.89}
{5.19,101.18}
174 Hz
{4.90,101.17}
{4.95,101.82}
174 Hz
{4.66,101.81}
{5.24,103.62}
174 Hz
{4.95,103.63}
f2 (ppm)
f1 (ppm)

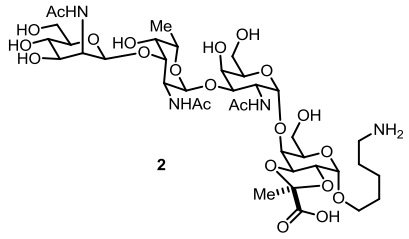


21, R = $(CH_2)_5NBnCbz$

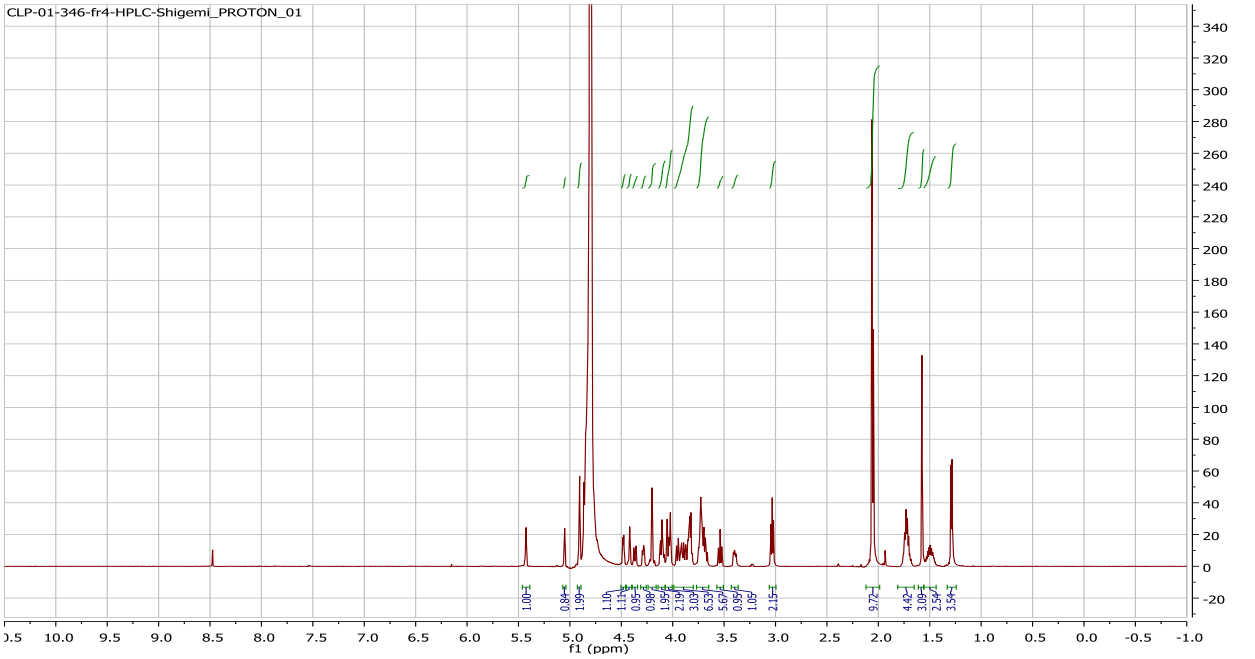


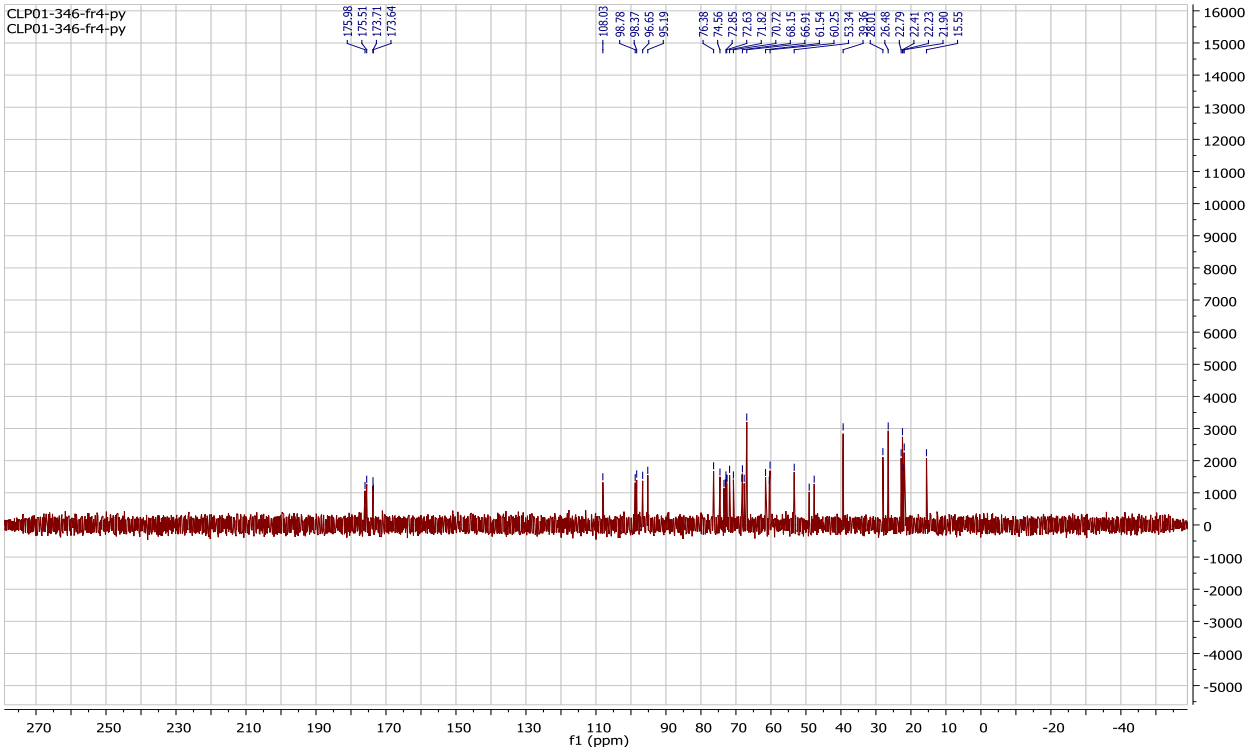



CLP-01-344-tetrasaccharide_pyruvate_gHSQC_01
f2 (ppm)
f1 (ppm)

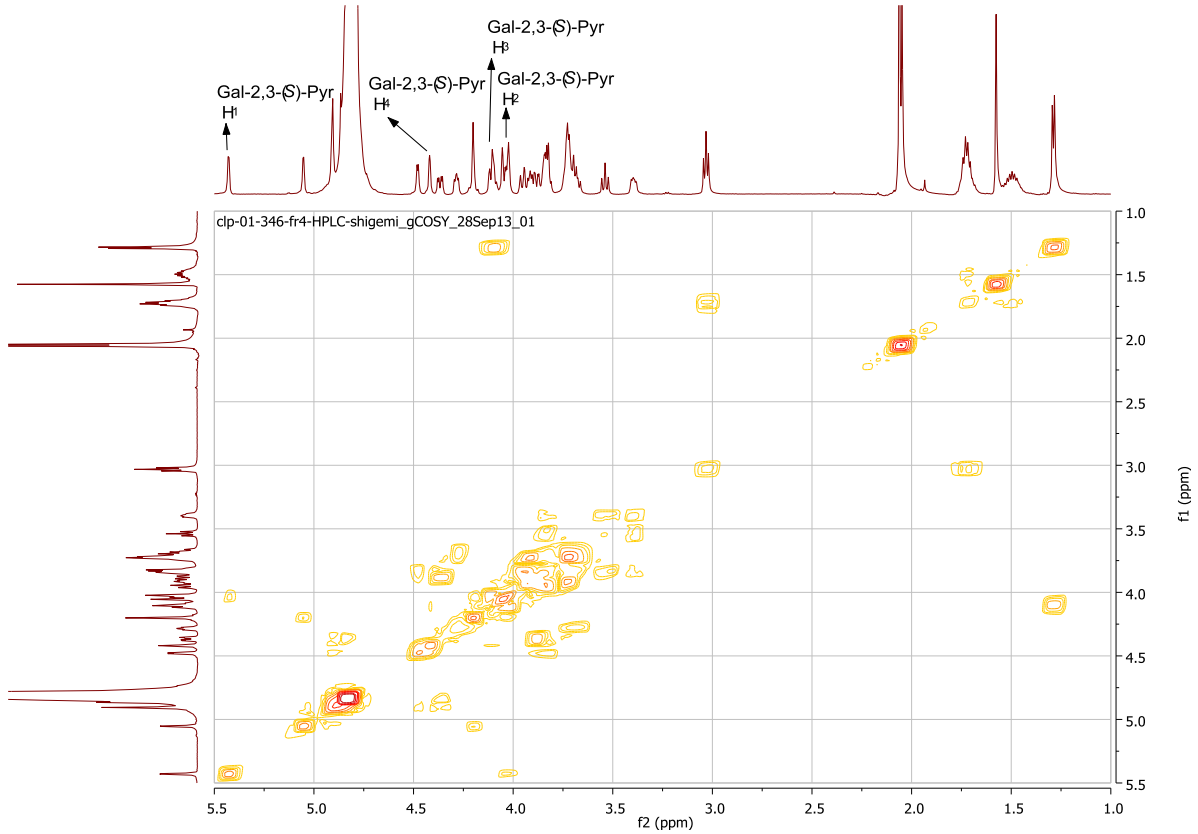

AcHN
HO
HO
HO
O
HO
Me
O
O
NHAc
O
HO
OH
O
O
AcHN
O
OH
NH2
O
O
Me
O
O
OH
O

2

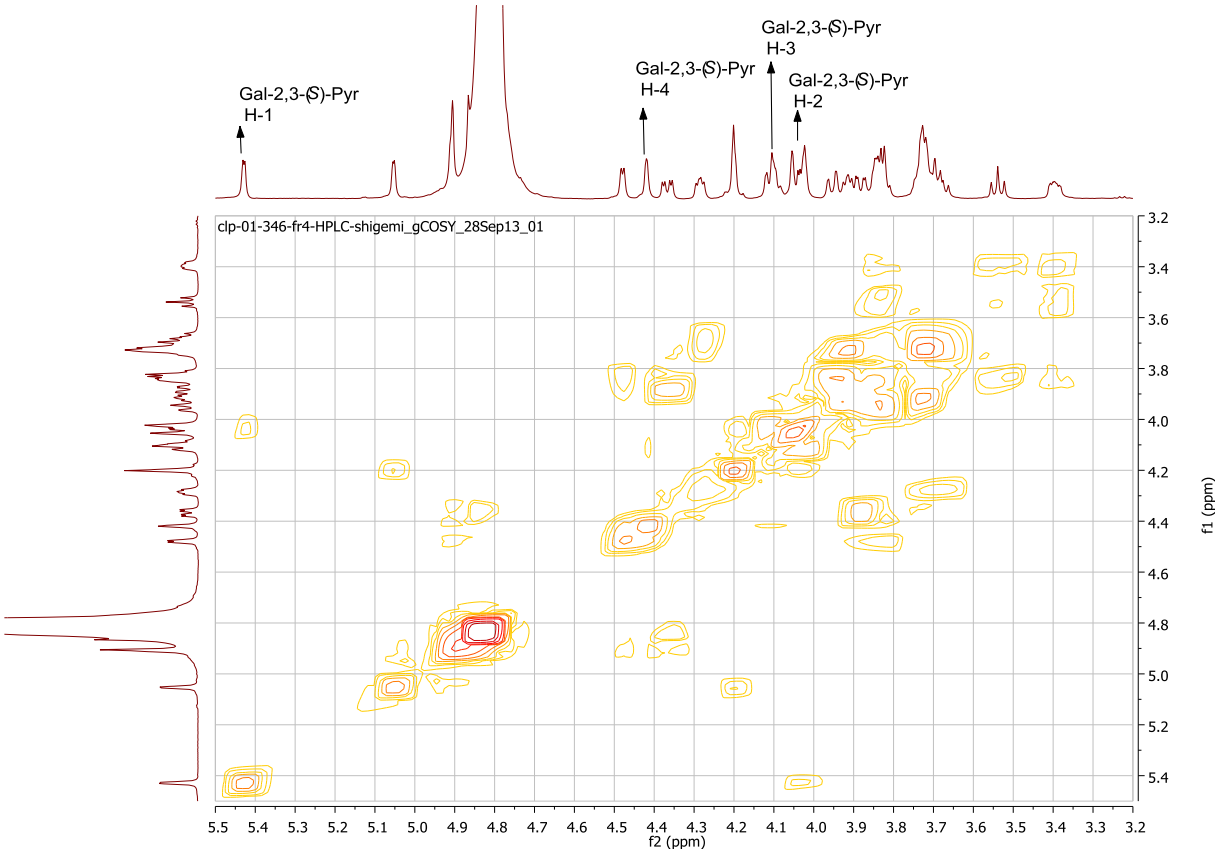

CLP-01-346-fr4-HPLC-Shigemi_PROTON_01
f1 (ppm)


CLP01-346-fr4-py
CLP01-346-fr4-py
f1 (ppm)

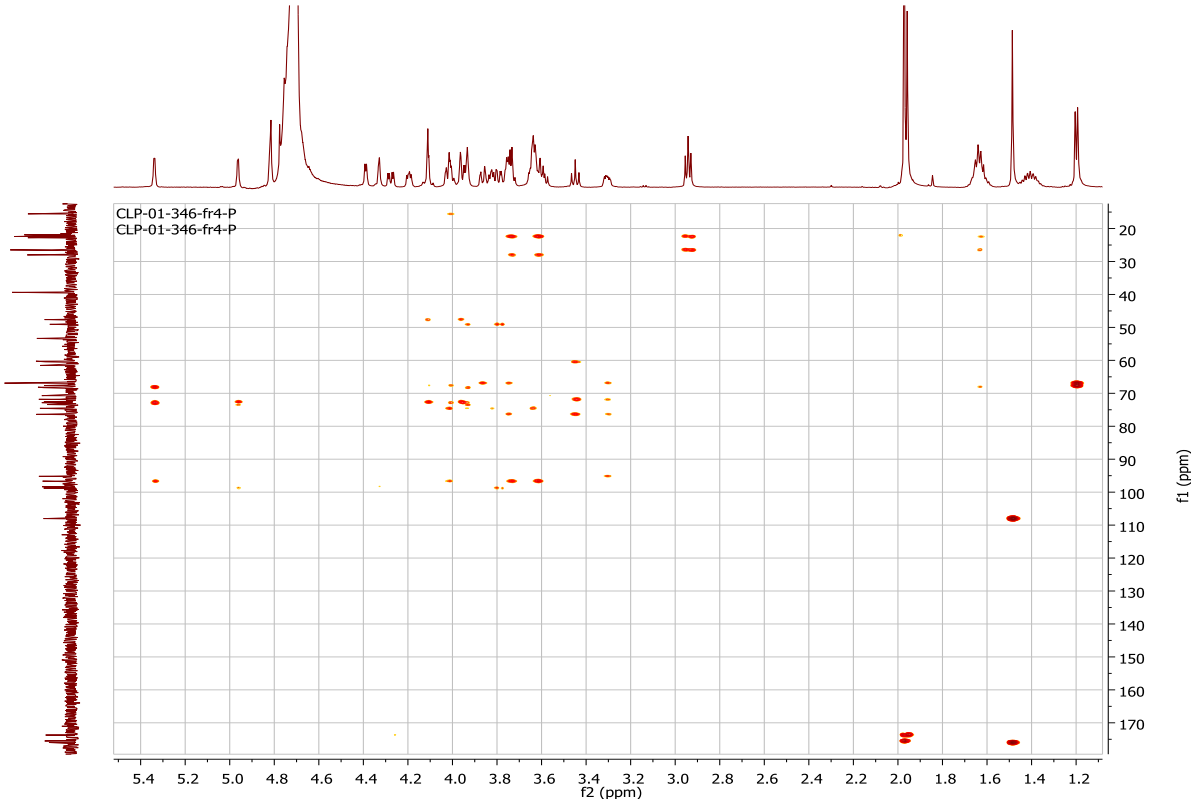
COSY- Compound **2**



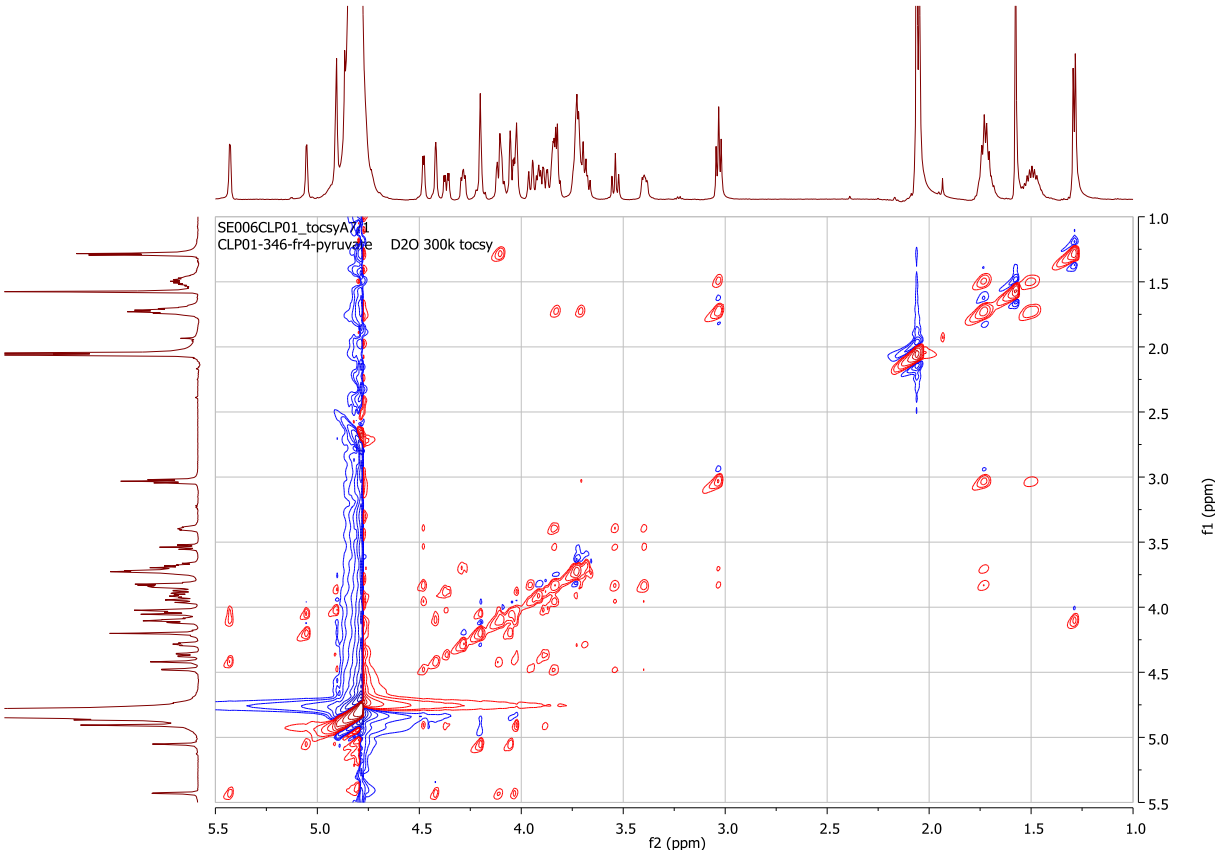
COSY- Compound **2**-Expanded



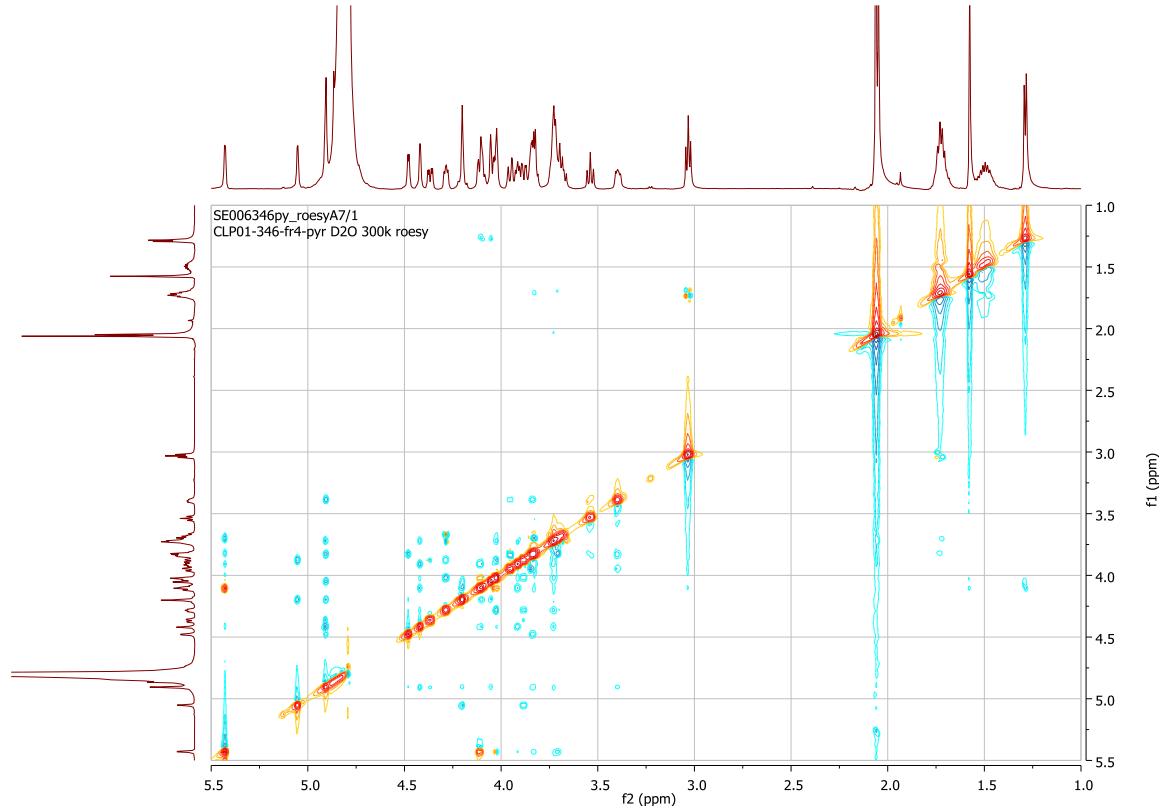
HMBC- Compound **2**



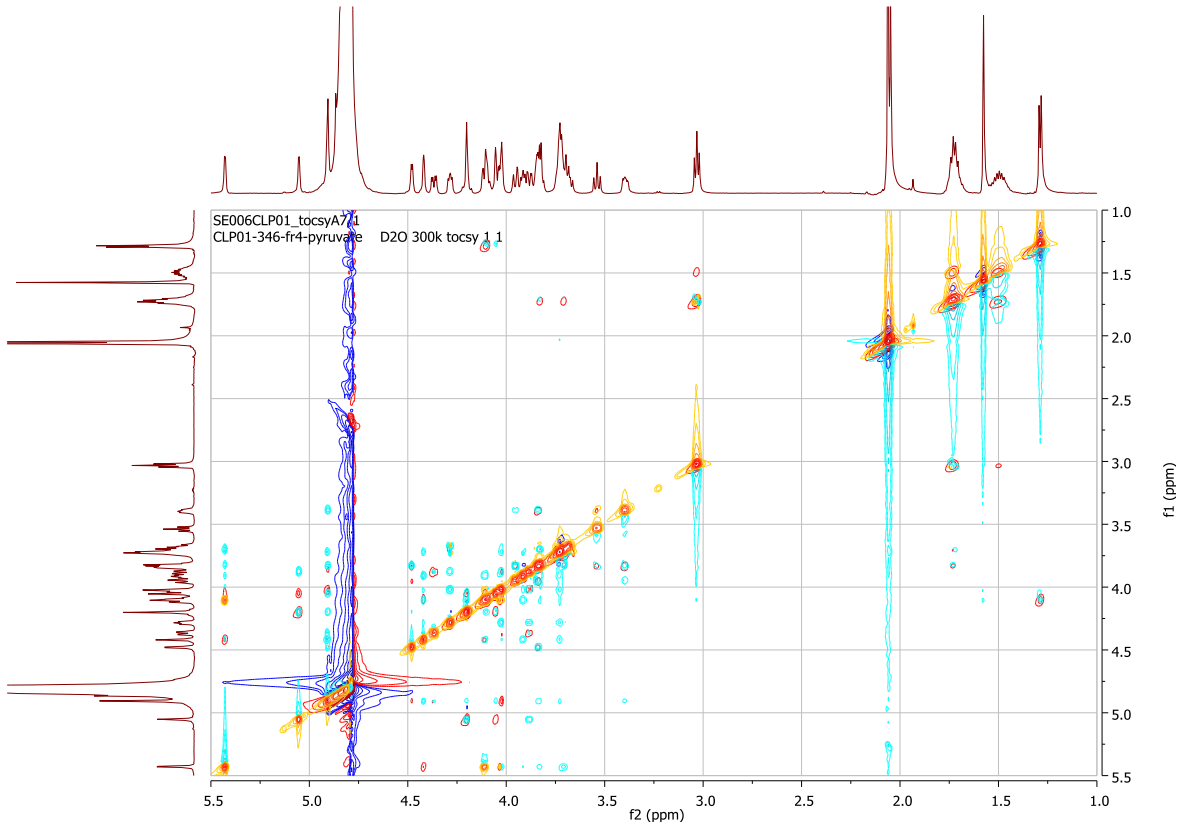
TOCSY- Compound **2**



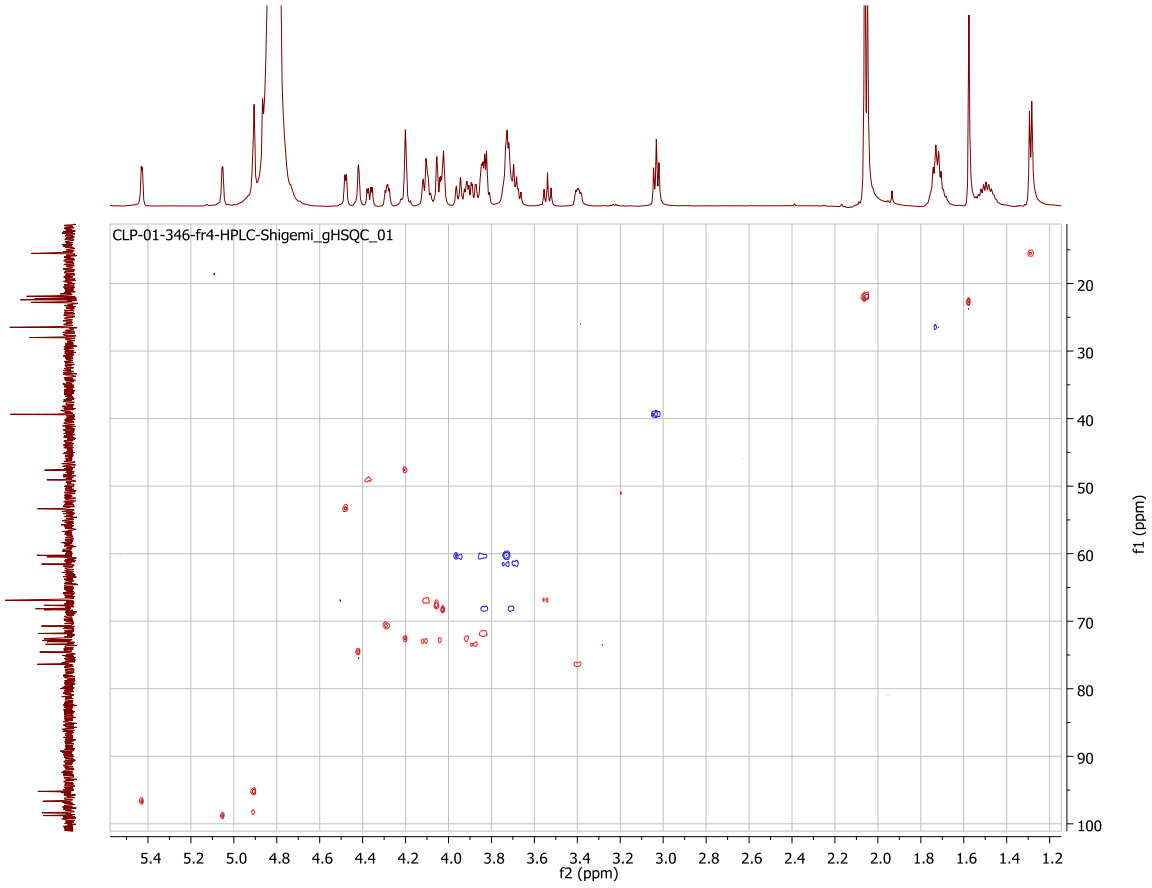
ROESY- Compound **2**



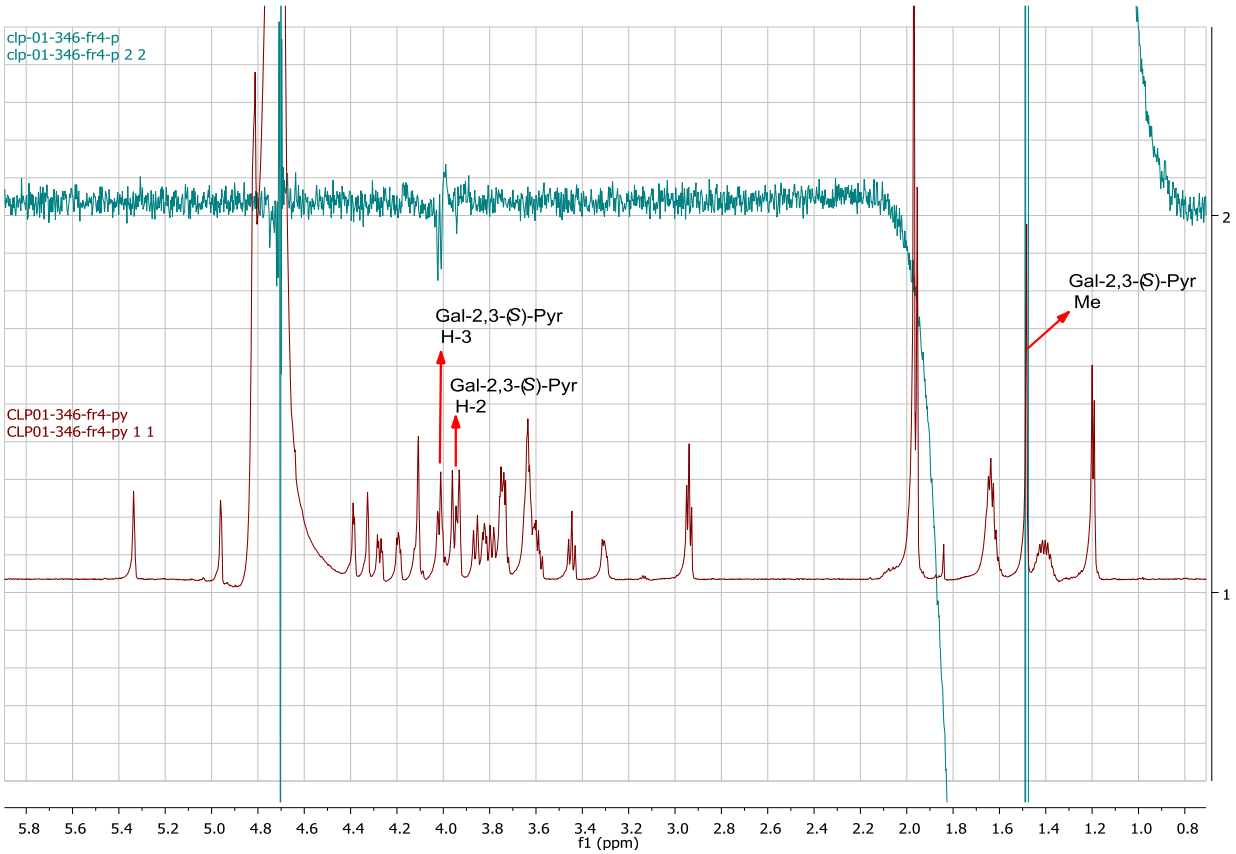
TOCSY, ROESY overlay- Compound **2**

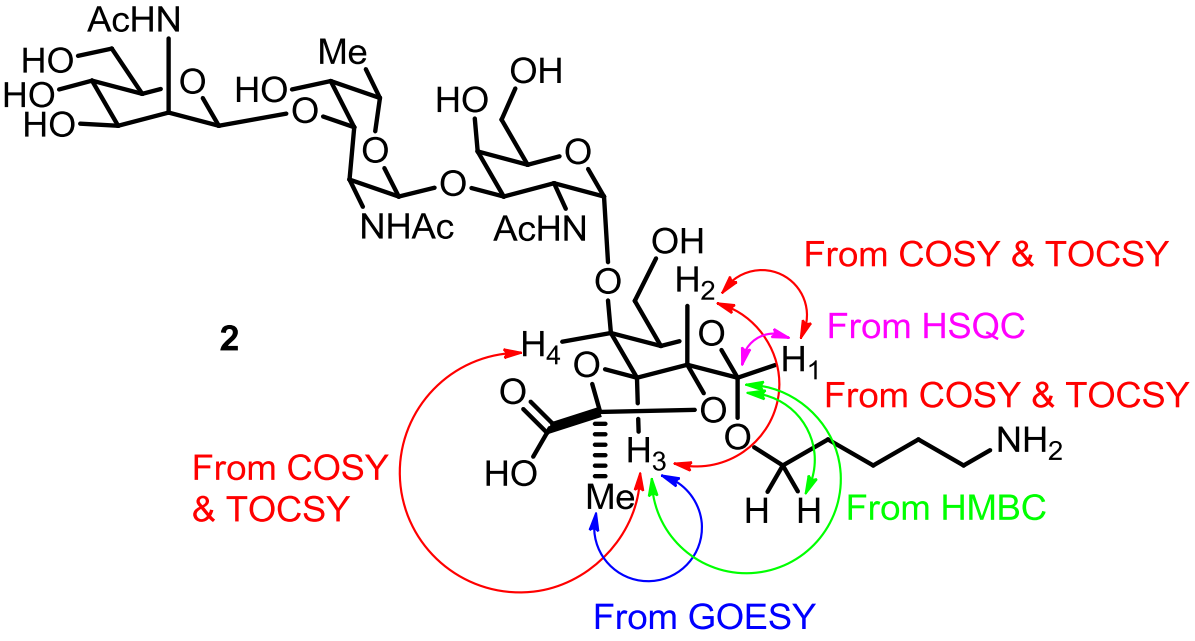


HSQC- Compound **2**

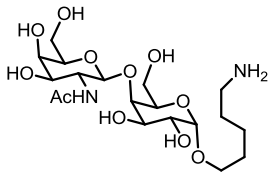


GOESY- Compound **2**

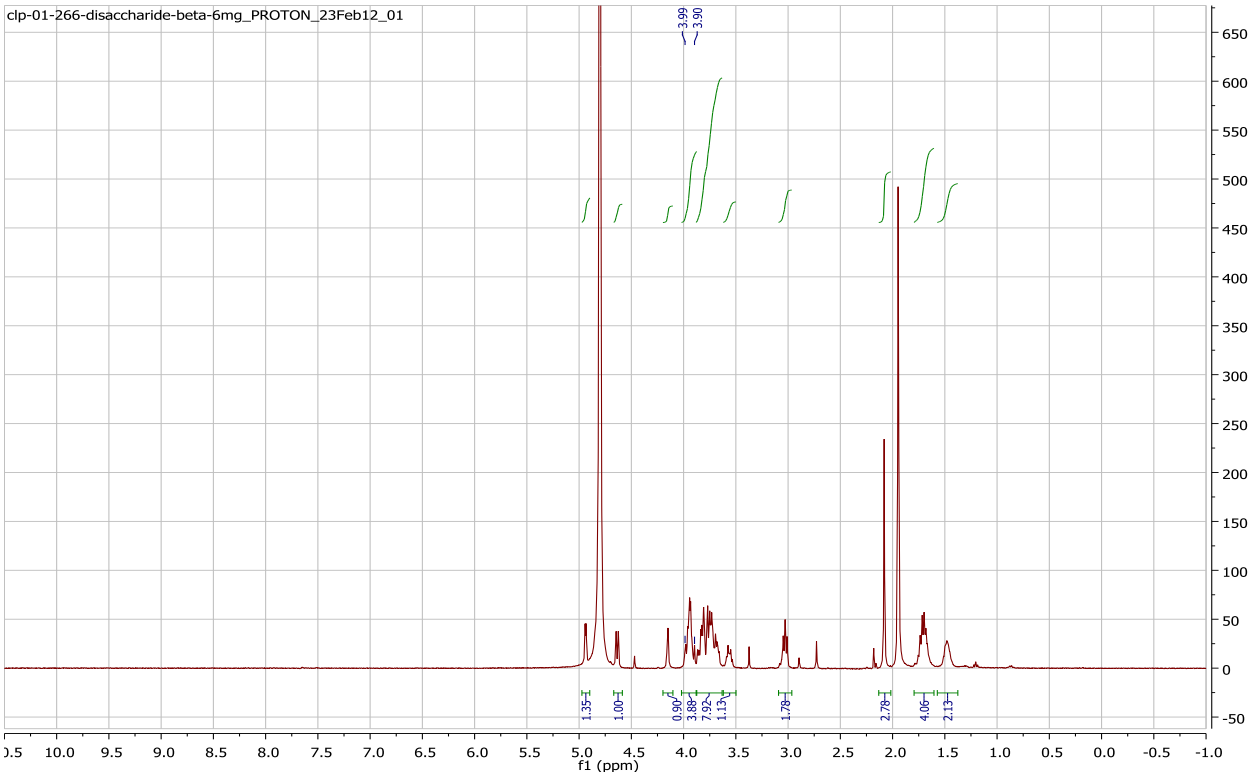


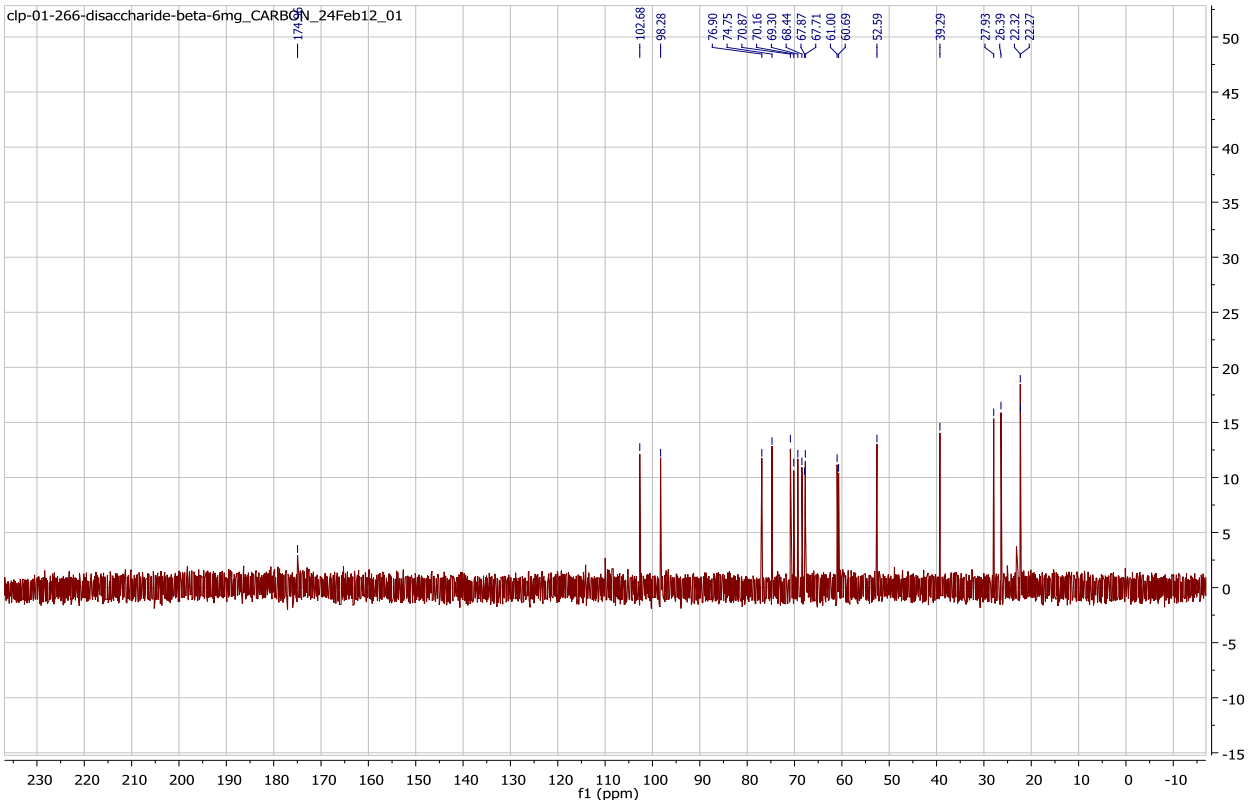

AcHN
HO
HO
HO
O
HO
Me
O
O
HO
OH
O
O
NHAc
AcHN
OH
O
H2
From COSY & TOCSY
From HSQC
2
H4
O
O
H1
From COSY & TOCSY
O
O
O
NH2
From COSY
& TOCSY
HO
H3
Me
H H
From HMBC
From GOESY

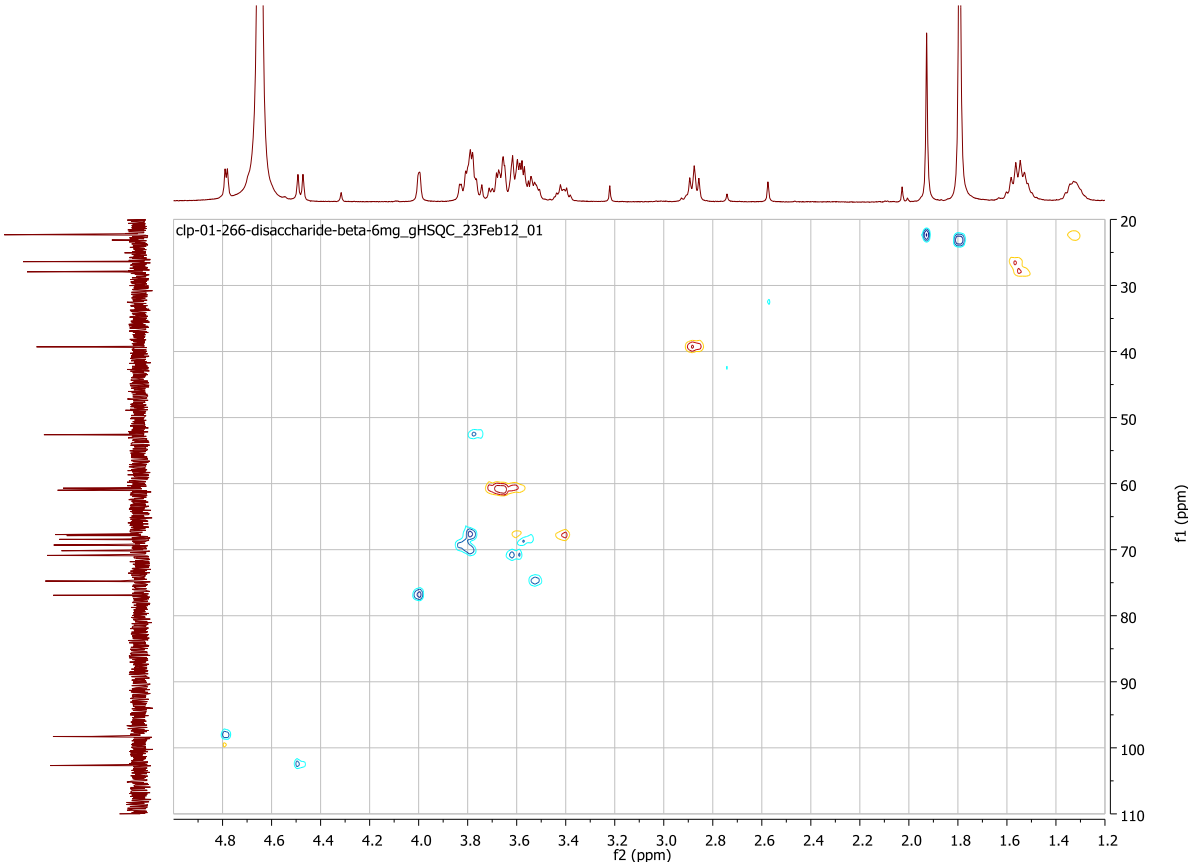
Gal-2, 3-(*S*)-Pyr

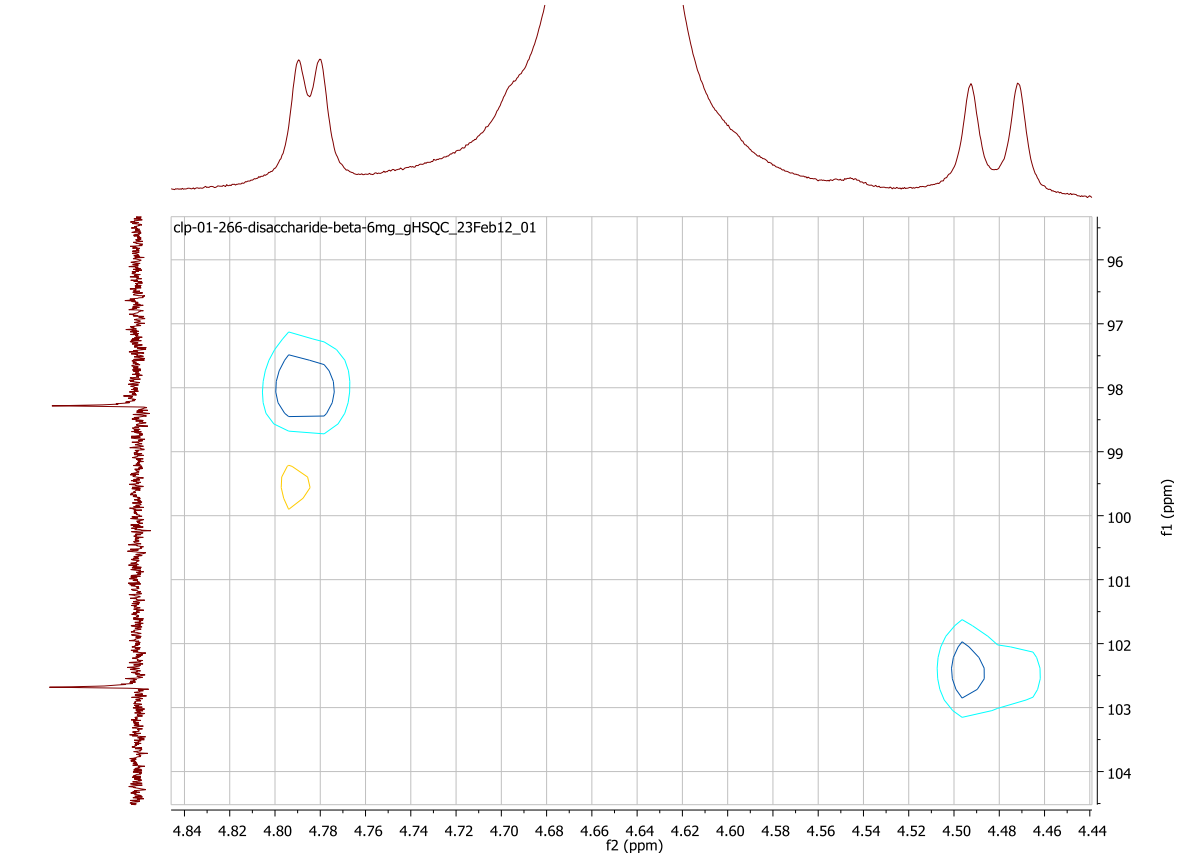

OH
HO
O
OH
HO
O
NH2
AcHN
O
HO
HO
O

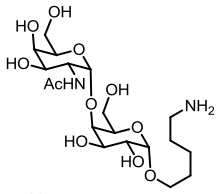
33β


clp-01-266-disaccharide-beta-6mg_PROTON_23Feb12_01
3.99
3.90
1.35
1.00
0.90
3.88
7.92
1.13
1.78
2.78
4.06
2.13
f1 (ppm)

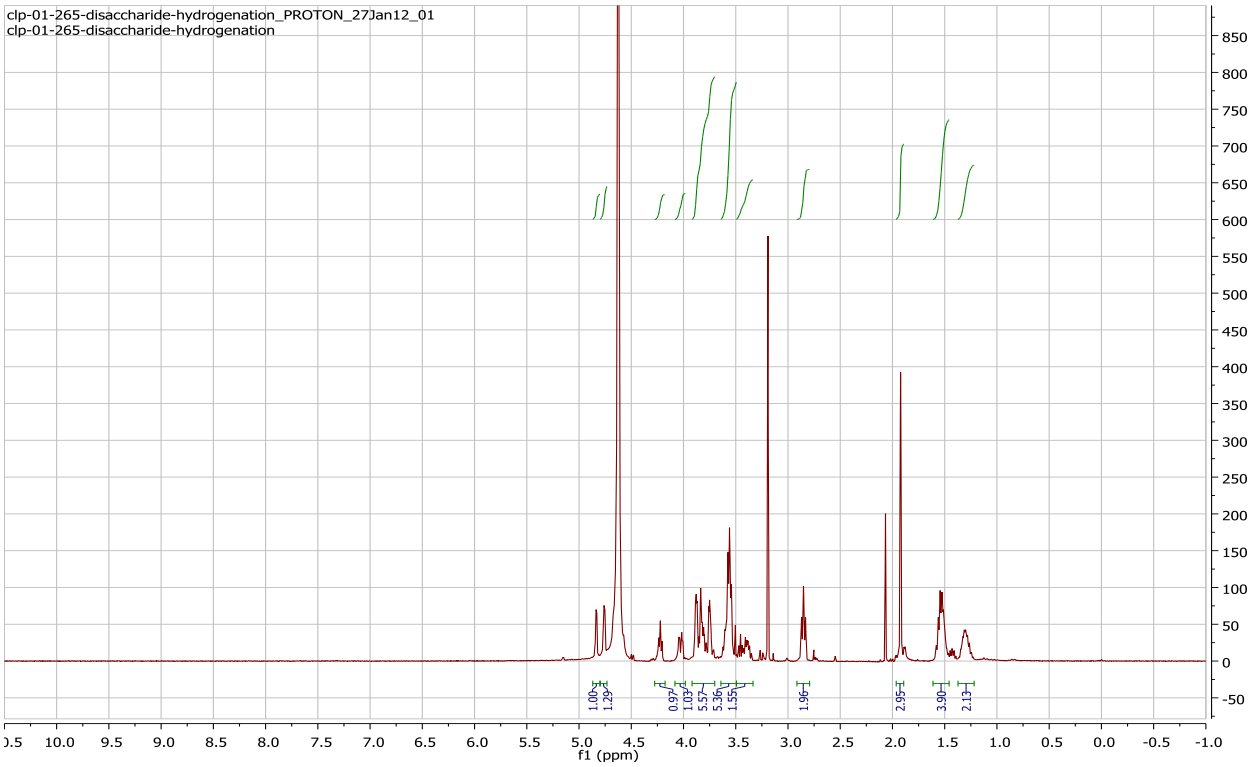

clp-01-266-disaccharide-beta-6mg_CARBON_24Feb12_01
174.58
102.68
98.28
76.90
74.75
70.87
70.16
69.30
68.44
67.87
67.71
61.00
60.69
52.59
39.29
27.93
26.39
22.32
22.27
f1 (ppm)

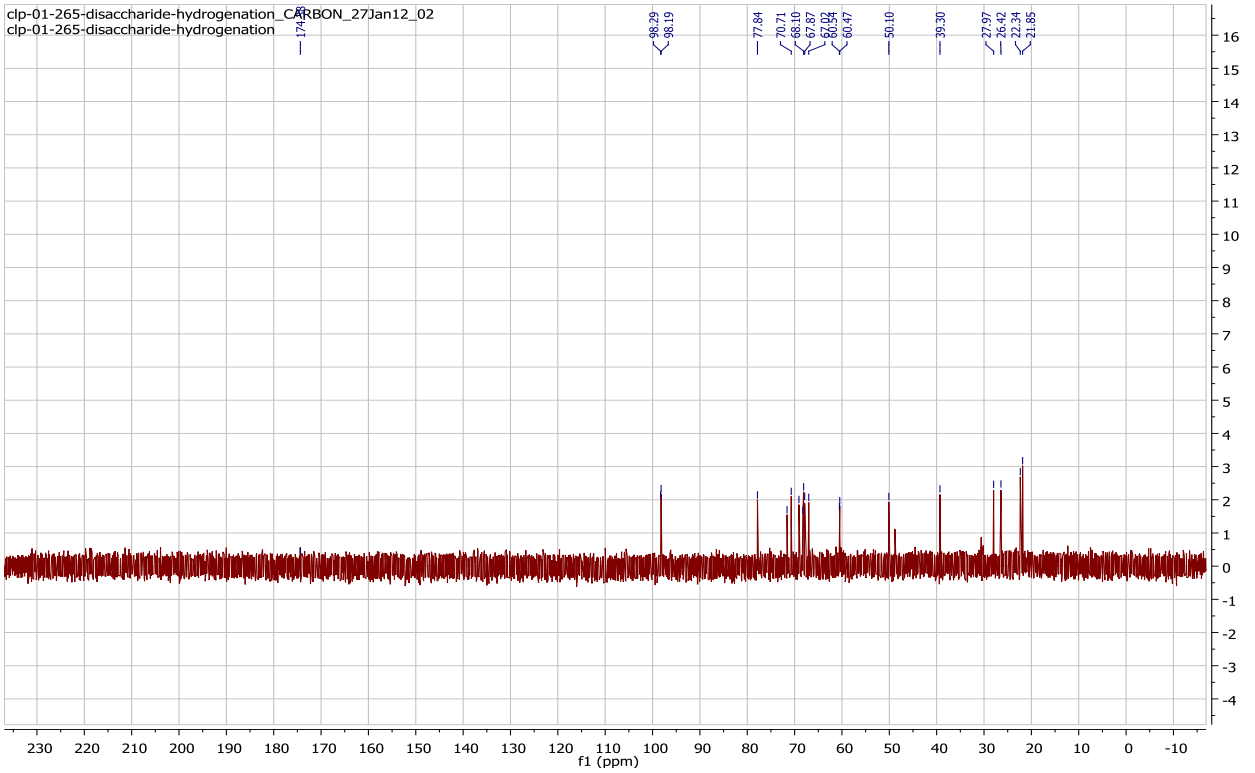

clp-01-266-disaccharide-beta-6mg_gHSQC_23Feb12_01
f2 (ppm)
f1 (ppm)

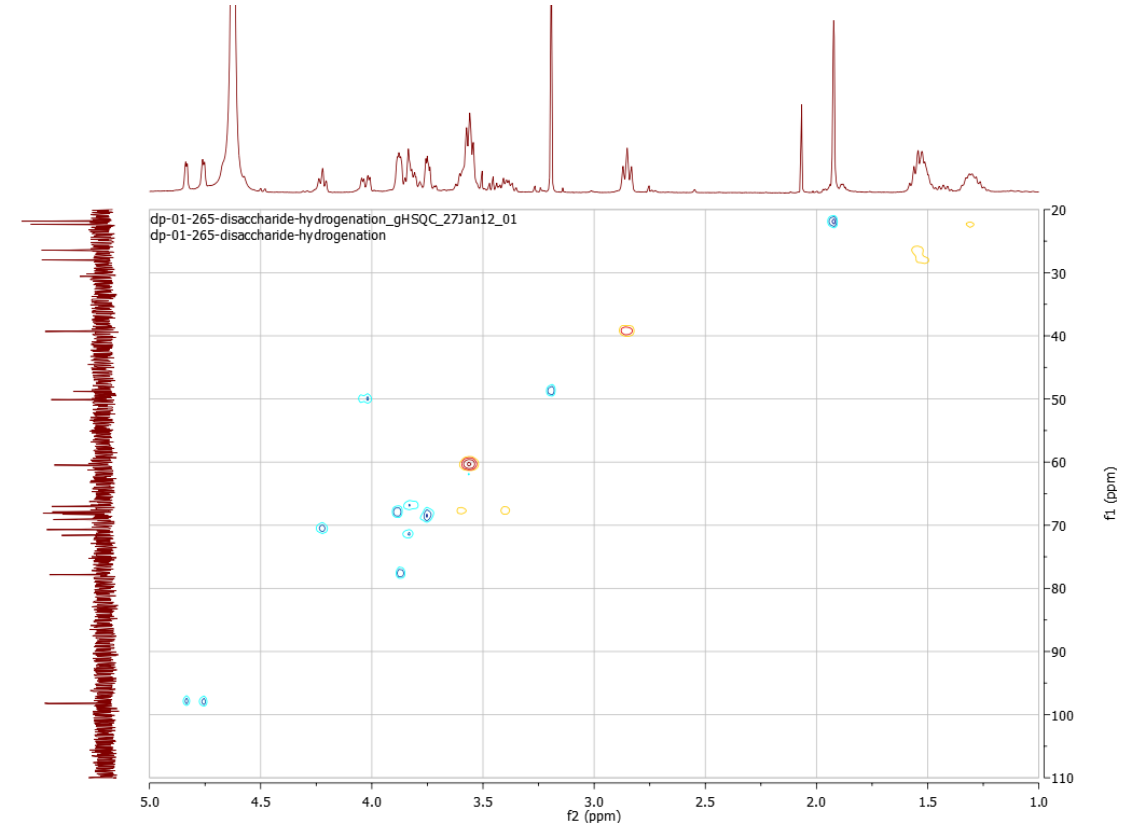

clp-01-266-disaccharide-beta-6mg_gHSQC_23Feb12_01
f2 (ppm)
f1 (ppm)

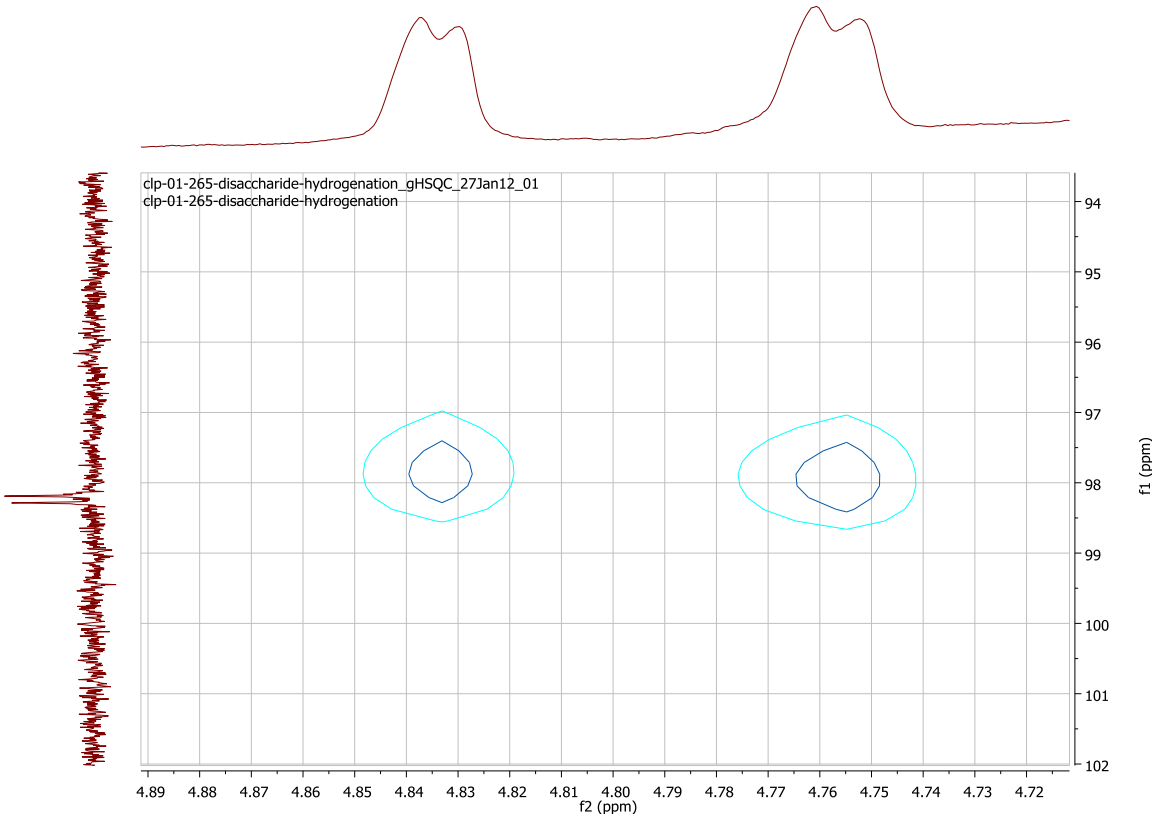

OH
HO
HO
O
AcHN
OH
O
NH2
O
HO
HO
O

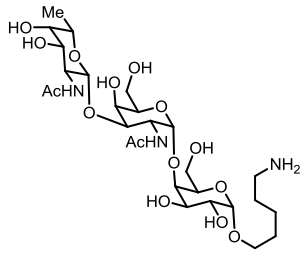
33α


clp-01-265-disaccharide-hydrogenation_PROTON_27Jan12_01
clp-01-265-disaccharide-hydrogenation
1.00
1.29
0.97
1.03
5.57
5.36
1.55
1.96
2.95
3.90
2.13
f1 (ppm)

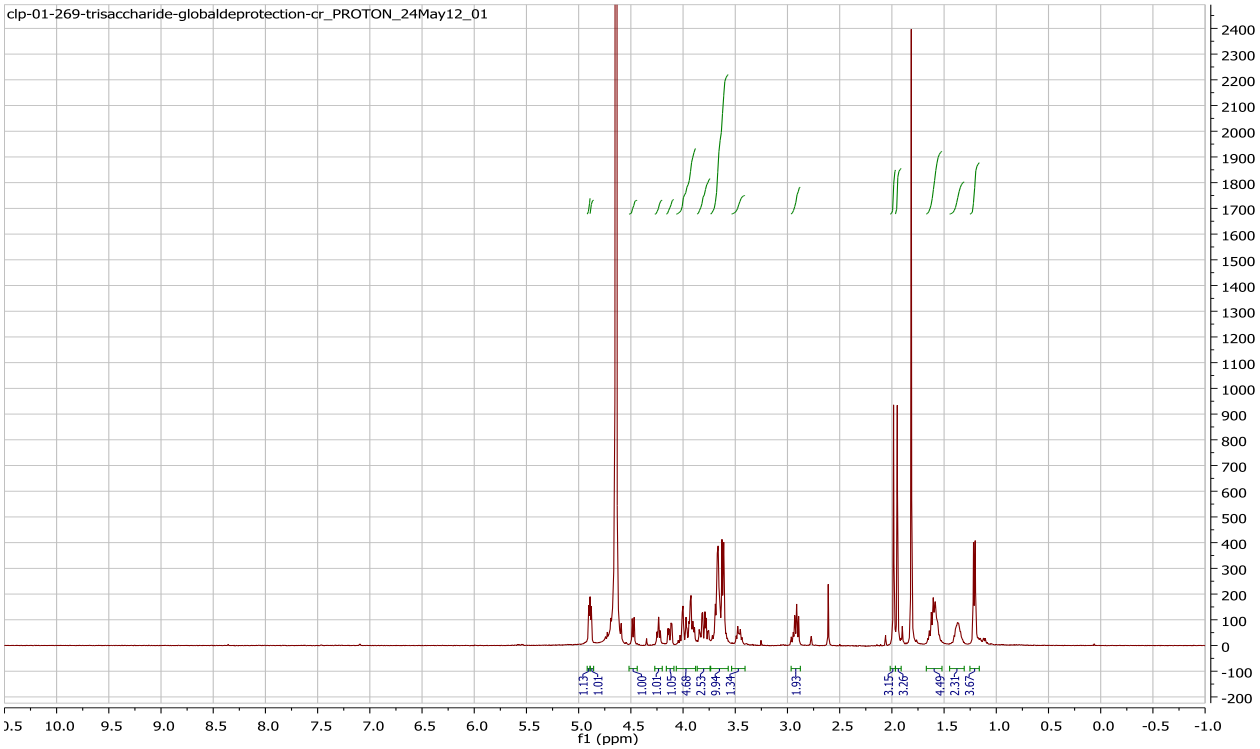

clp-01-265-disaccharide-hydrogenation_CARBON_27Jan12_02
clp-01-265-disaccharide-hydrogenation
174.47
98.29
98.19
77.84
70.71
68.10
67.87
60.54
60.47
50.10
39.30
27.97
26.42
22.34
21.85
f1 (ppm)

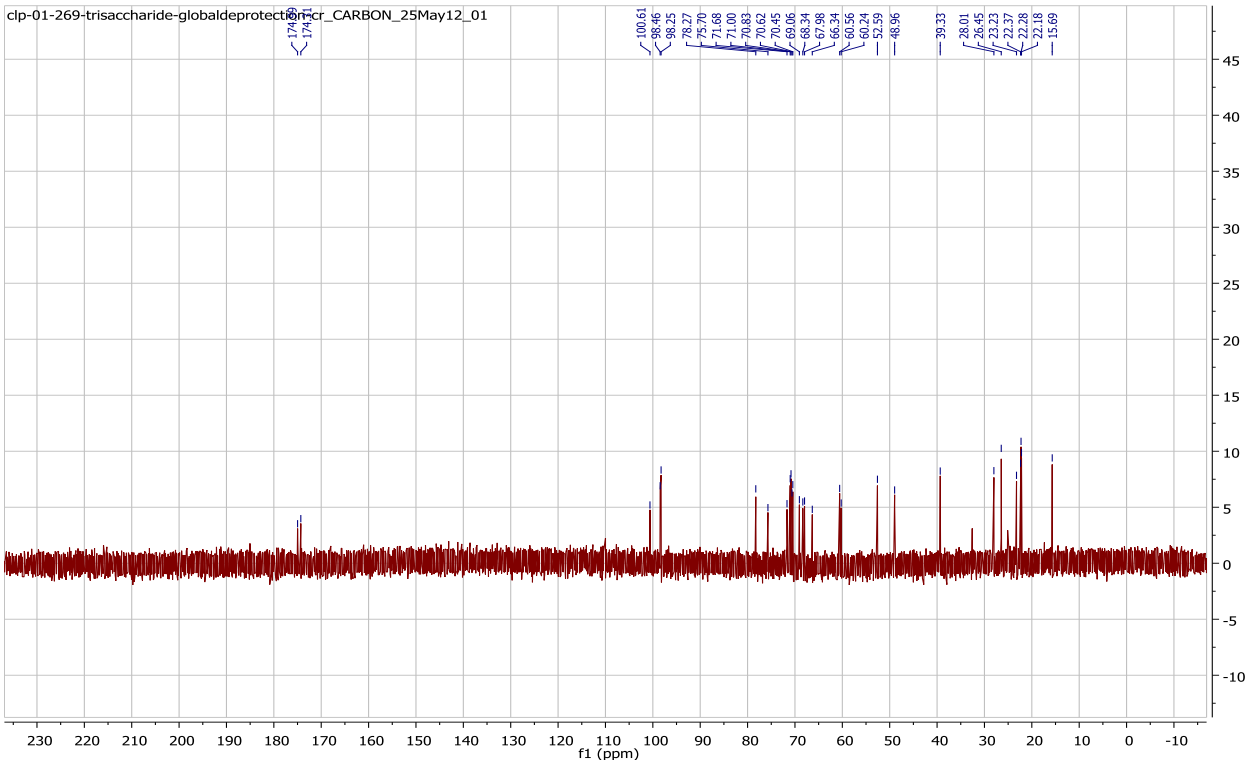

dp-01-265-disaccharide-hydrogenation_gHSQC_27Jan12_01
dp-01-265-disaccharide-hydrogenation
f2 (ppm)
f1 (ppm)

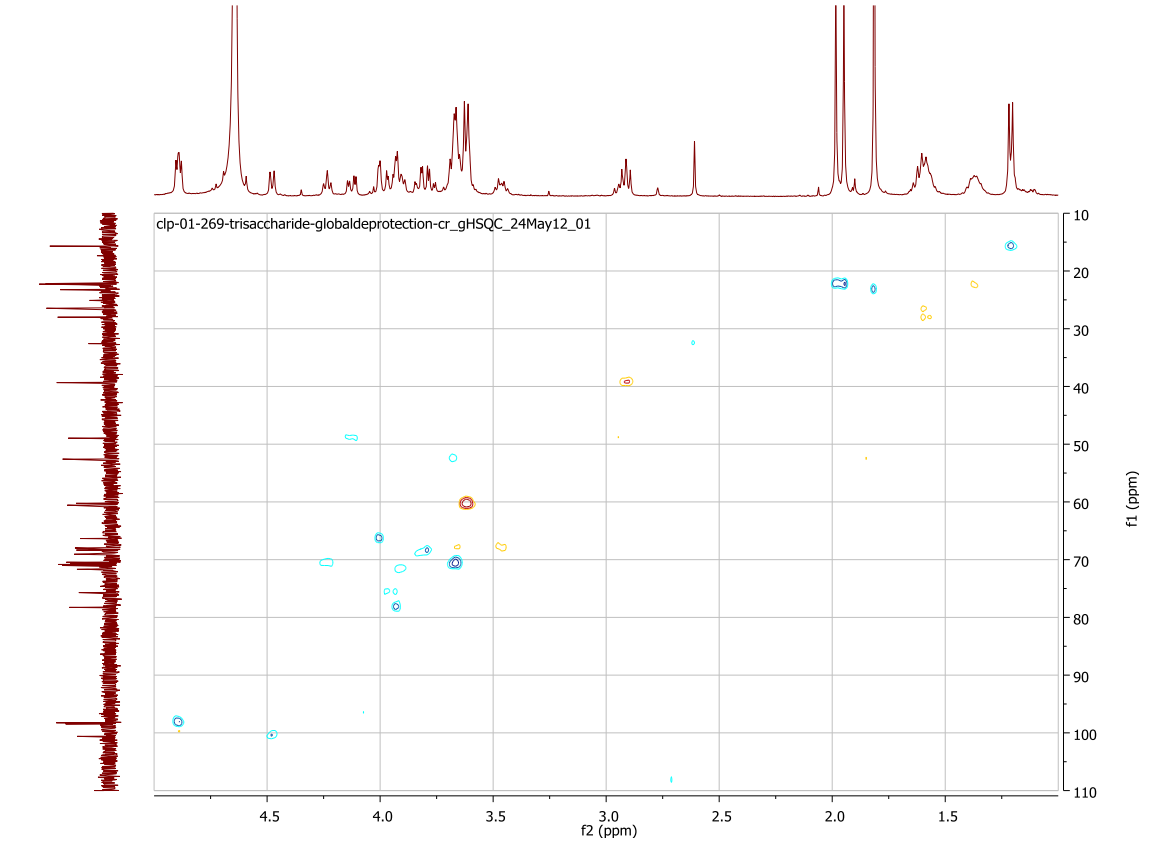

clp-01-265-disaccharide-hydrogenation_gHSQC_27Jan12_01
clp-01-265-disaccharide-hydrogenation
f2 (ppm)
f1 (ppm)

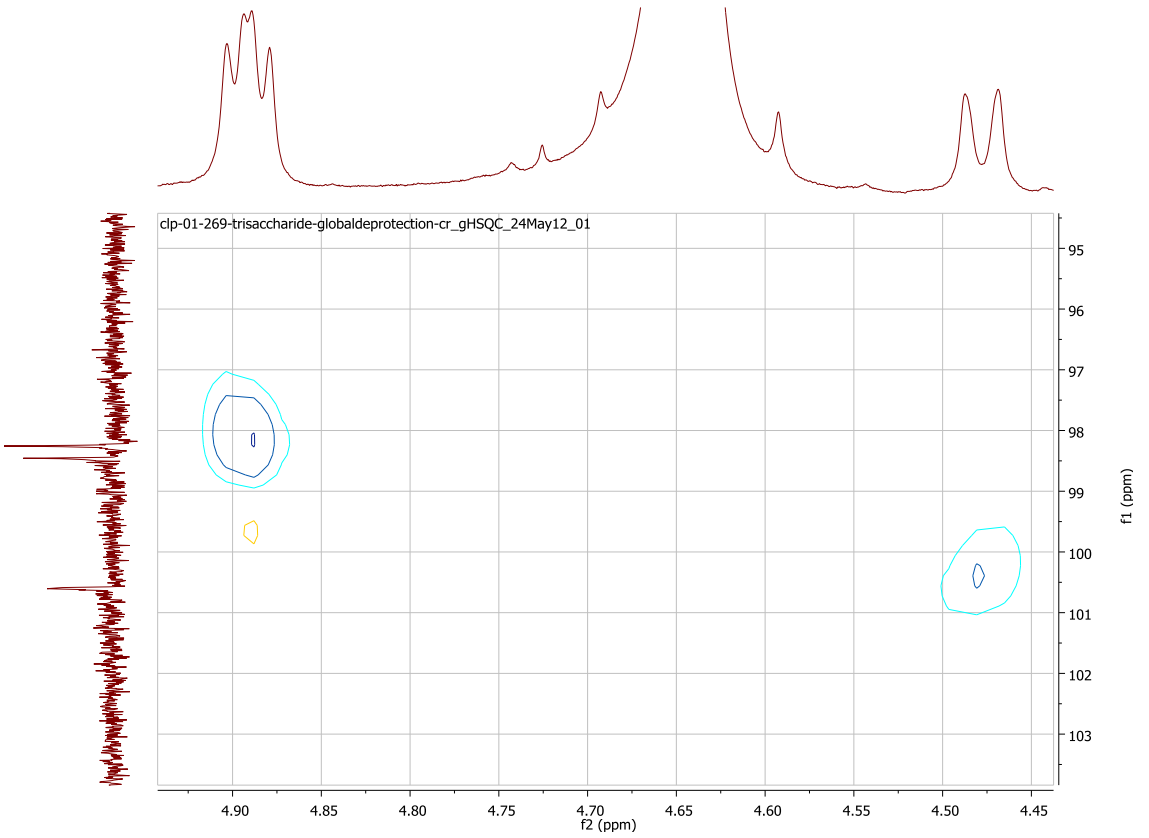


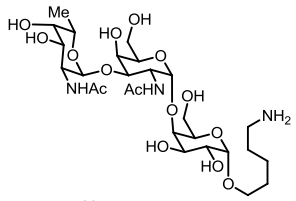
23β, R = $(CH_2)_5NH_2$



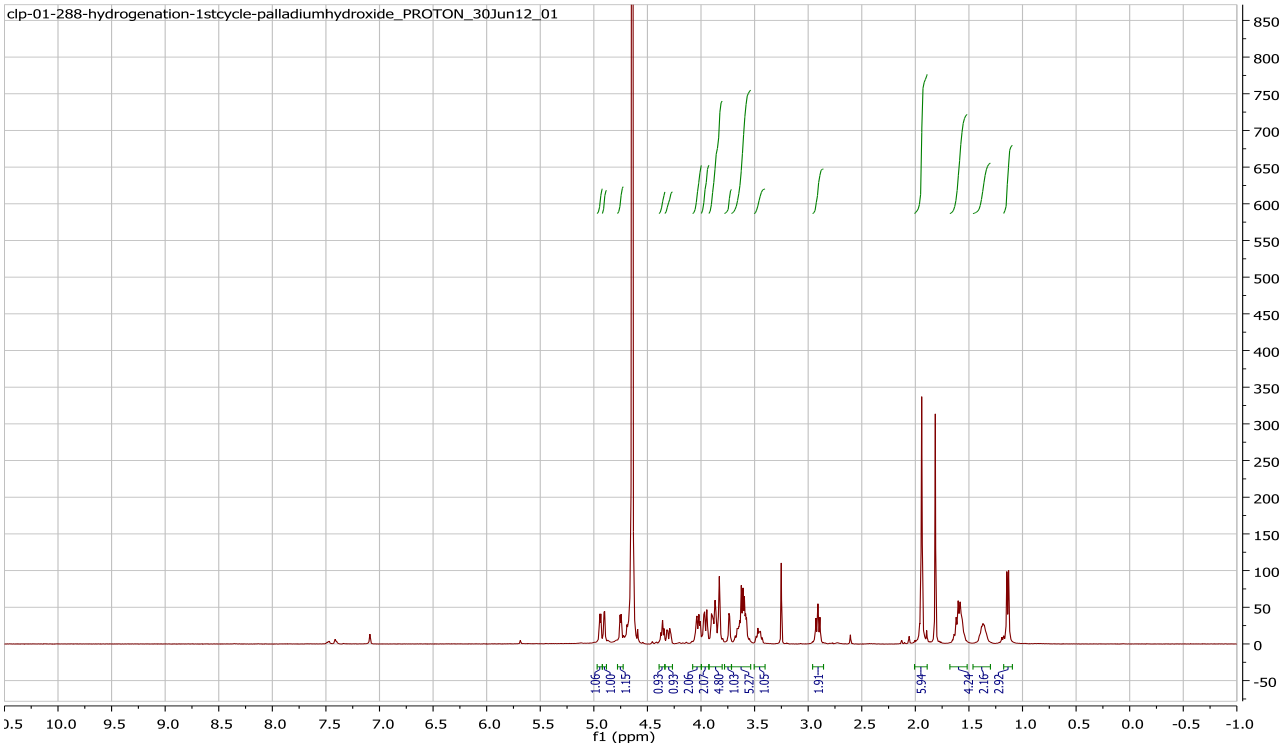


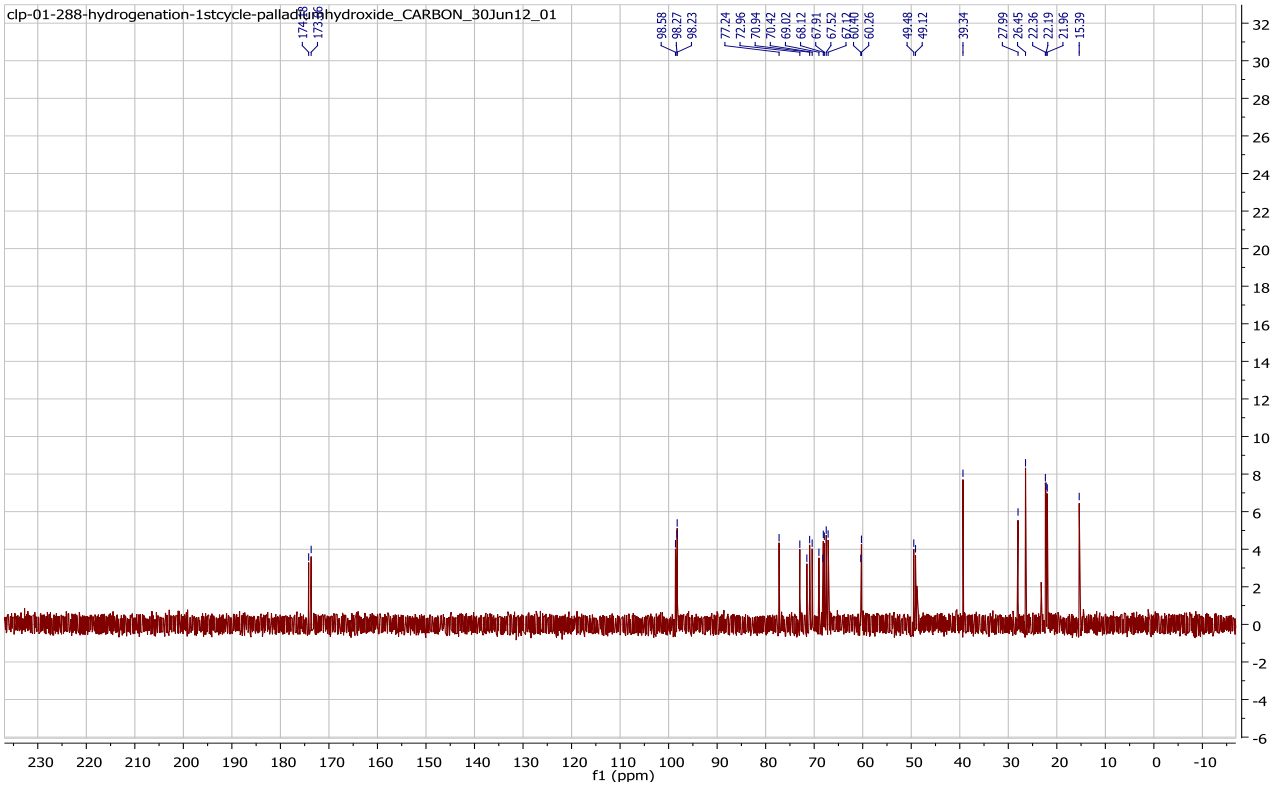

clp-01-269-trisaccharide-globaldeprotection-cr_gHSQC_24May12_01
f2 (ppm)
f1 (ppm)

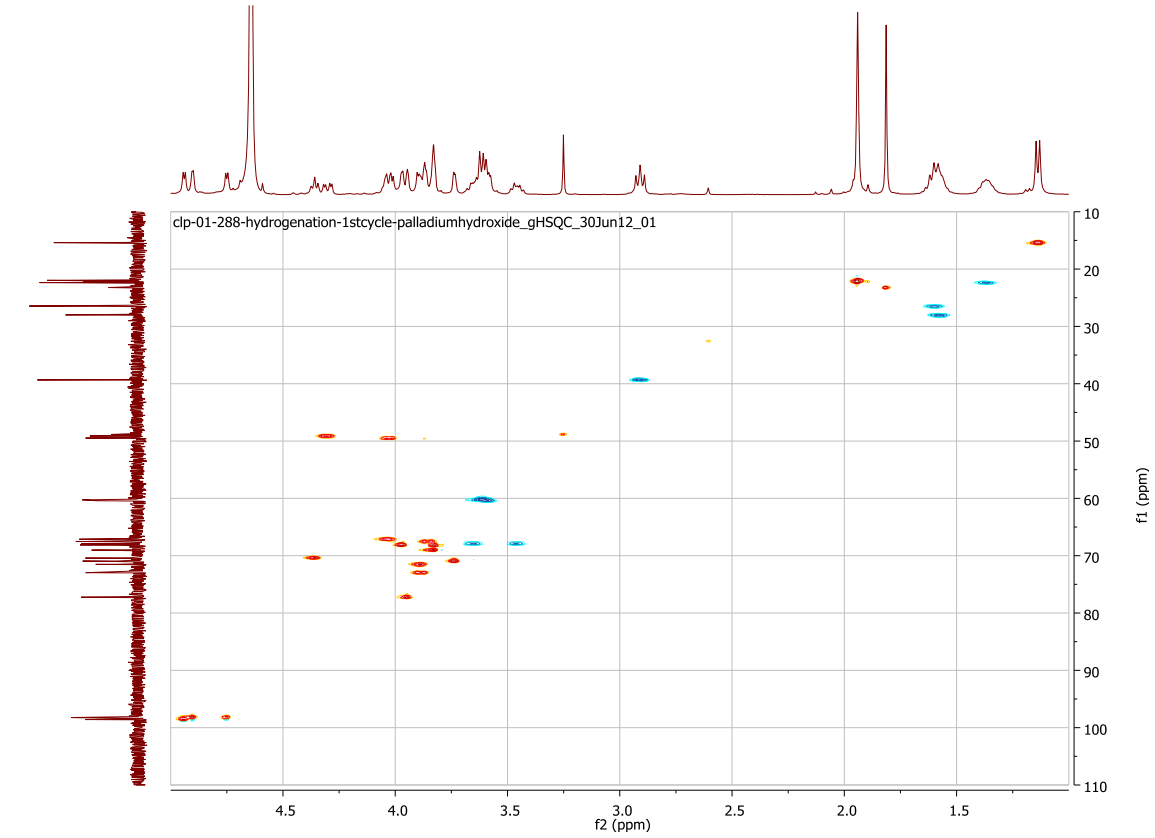

clp-01-269-trisaccharide-globaldeprotection-cr_gHSQC_24May12_01
f2 (ppm)
f1 (ppm)

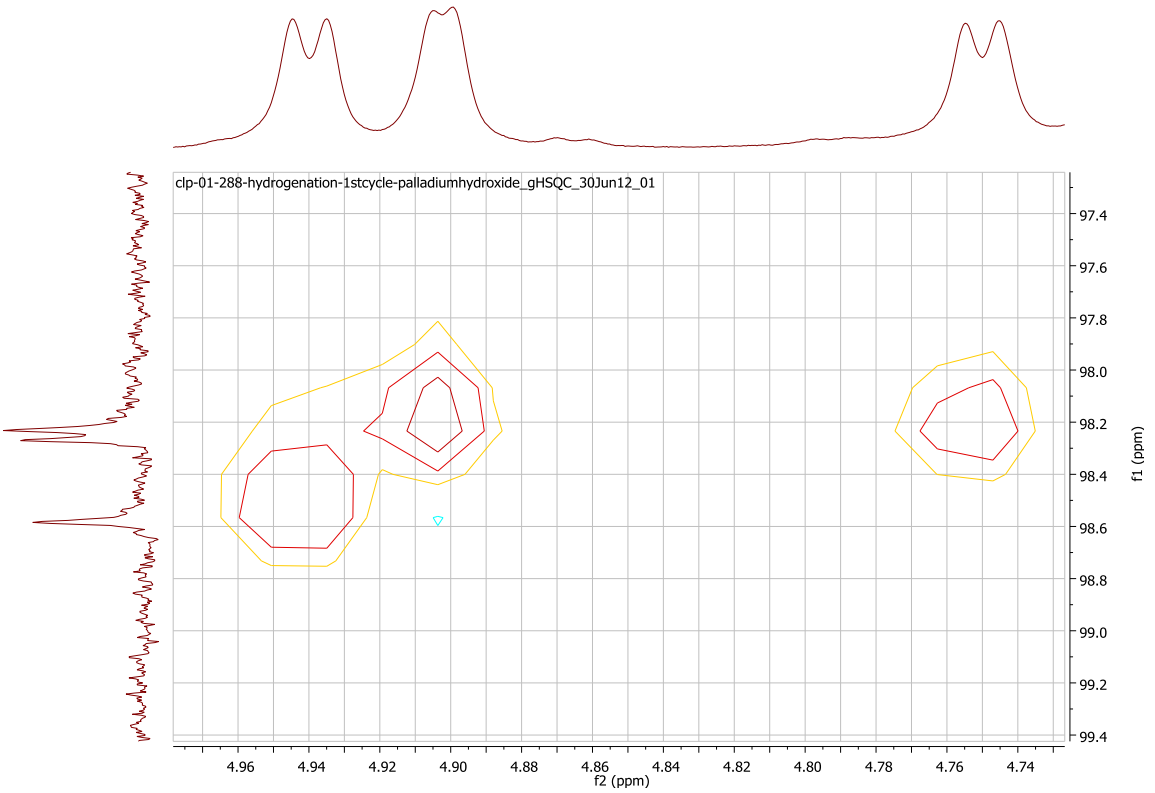

Me
HO
HO
O
NHAc
O
HO
OH
O
AcHN
OH
O
HO
O
HO
O
NH2

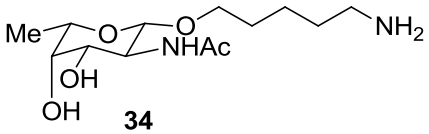
23α


clp-01-288-hydrogenation-1stcycle-palladiumhydroxide_PROTON_30Jun12_01
f1 (ppm)

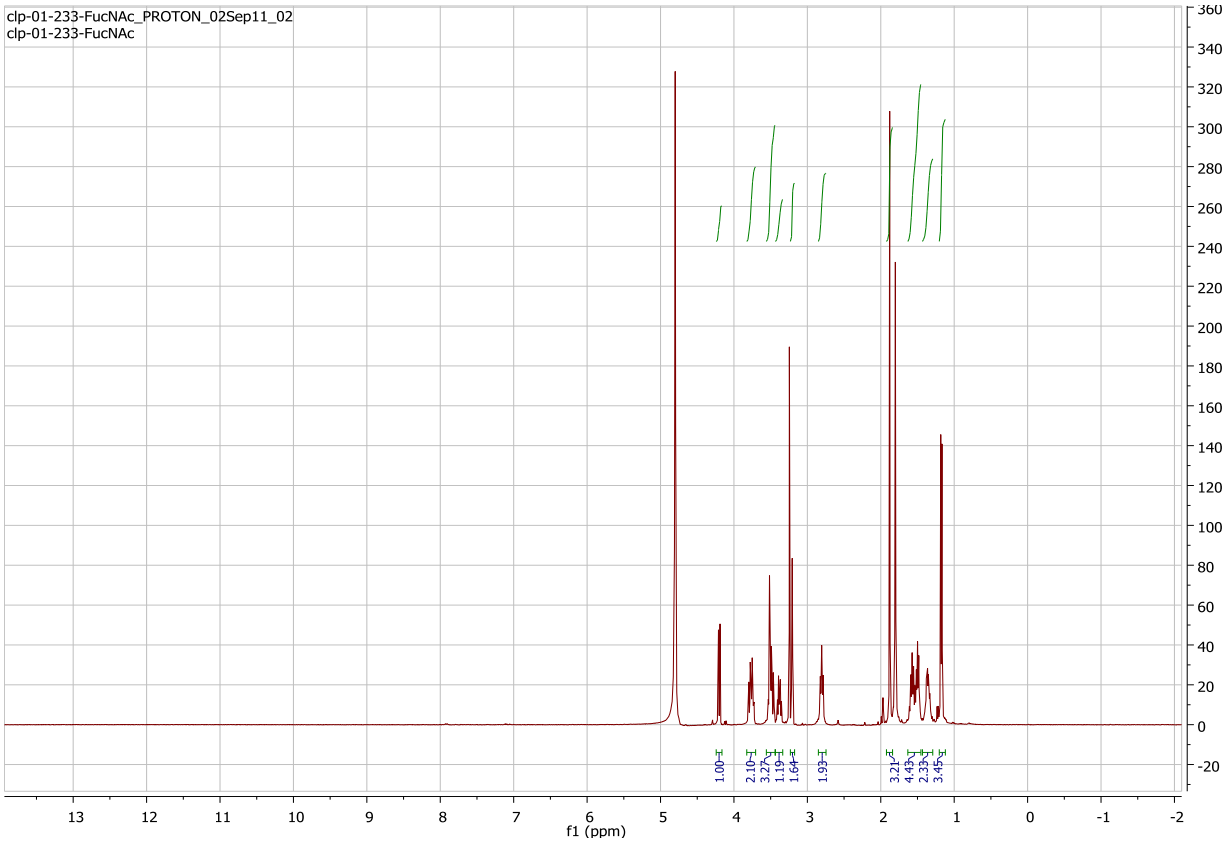

clp-01-288-hydrogenation-1stcycle-palladiumhydroxide_CARBON_30Jun12_01
98.58
98.27
98.23
77.24
72.96
70.94
70.42
69.02
68.12
67.91
67.52
67.12
60.40
60.26
49.48
49.12
39.34
27.99
26.45
22.36
22.19
21.96
15.39
f1 (ppm)

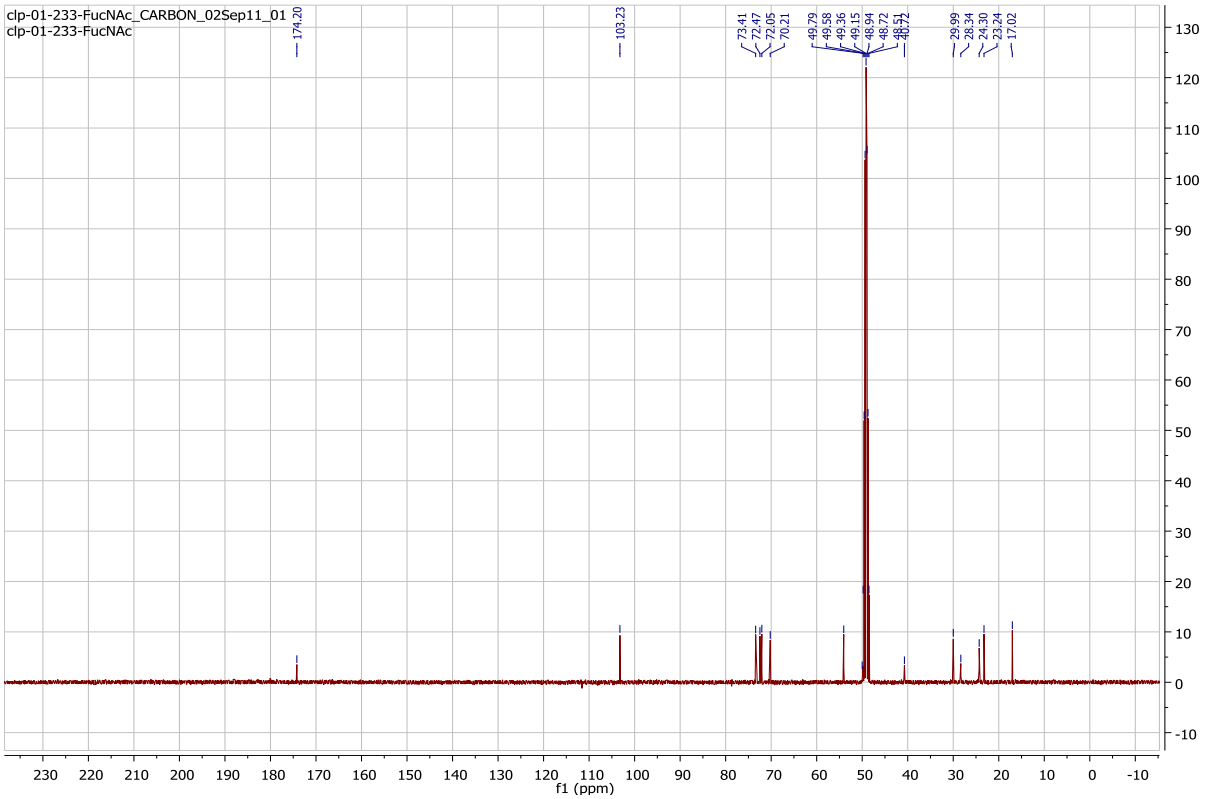

clp-01-288-hydrogenation-1stcycle-palladiumhydroxide_gHSQC_30Jun12_01
f2 (ppm)
f1 (ppm)

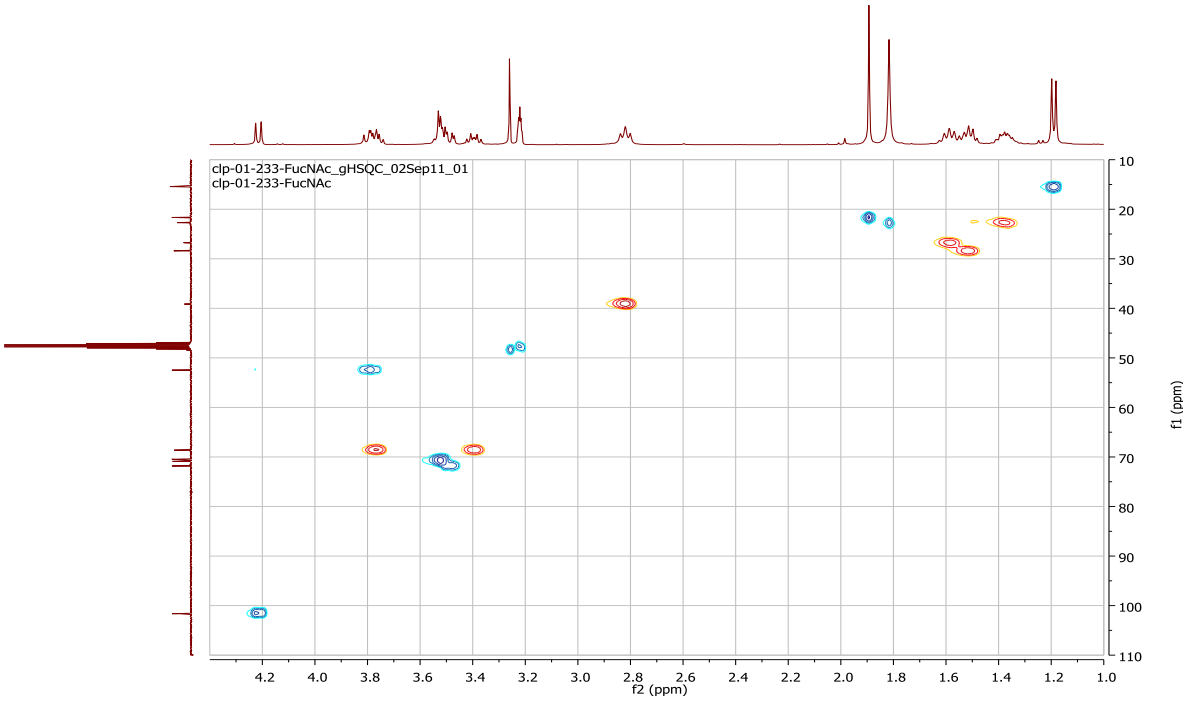

clp-01-288-hydrogenation-1stcycle-palladiumhydroxide_gHSQC_30Jun12_01
f2 (ppm)
f1 (ppm)

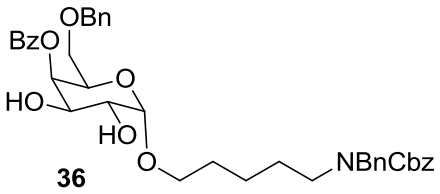

Me
O
O
NHAc
NH2
OH
OH

34

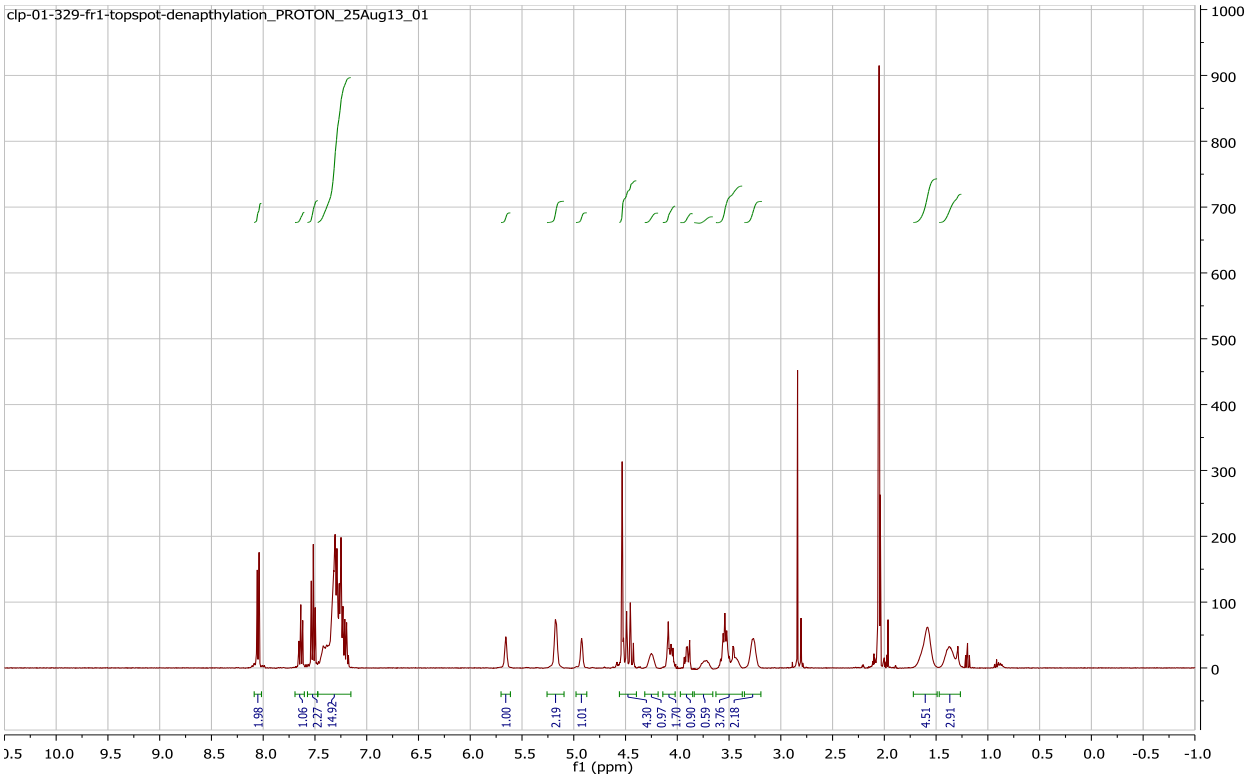

clp-01-233-FucNAc_PROTON_02Sep11_02
clp-01-233-FucNAc
1.00
2.10
3.27
1.19
1.64
1.93
3.21
4.45
2.35
3.45
f1 (ppm)

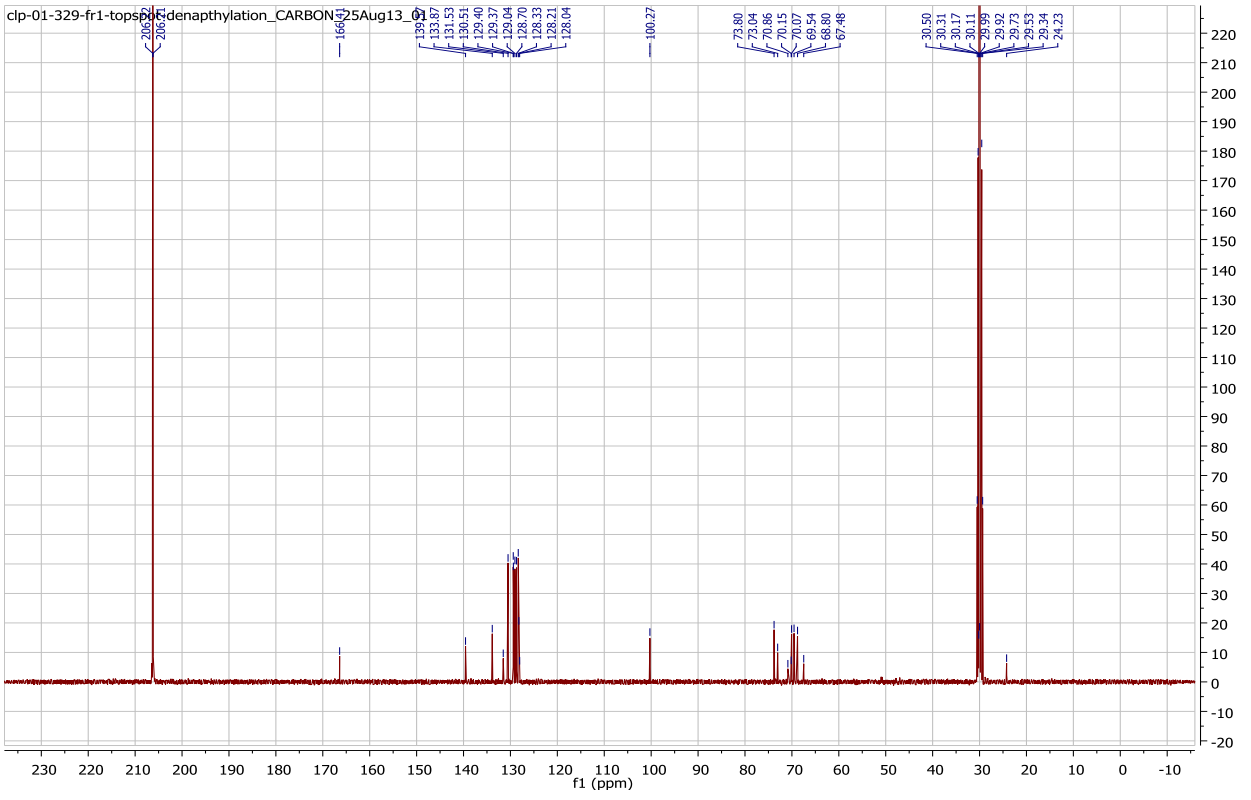

clp-01-233-FucNAc_CARBON_02Sep11_01
clp-01-233-FucNAc
174.20
103.23
73.41
72.47
72.05
70.21
49.79
49.58
49.36
49.15
48.94
48.72
48.51
48.29
29.99
28.34
24.30
23.24
17.02
f1 (ppm)

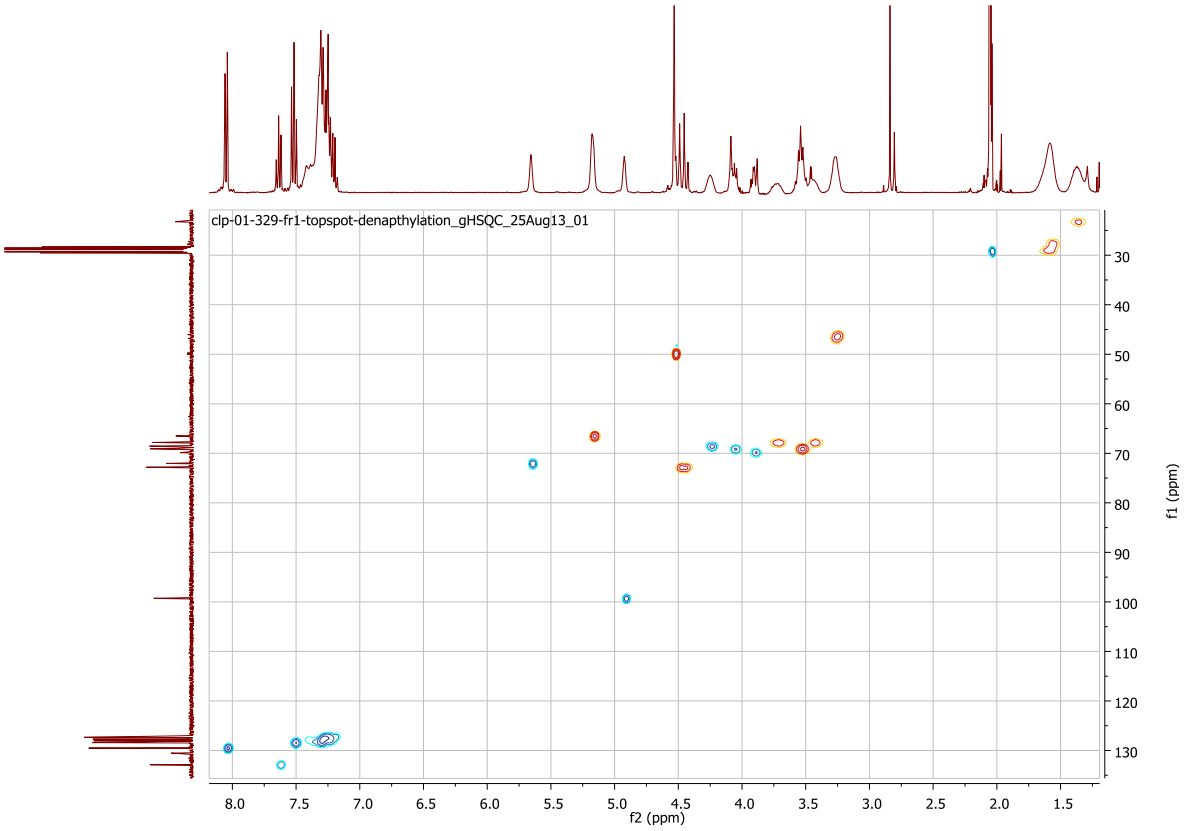

clp-01-233-FucNAc_gHSQC_02Sep11_01
clp-01-233-FucNAc
f2 (ppm)
f1 (ppm)

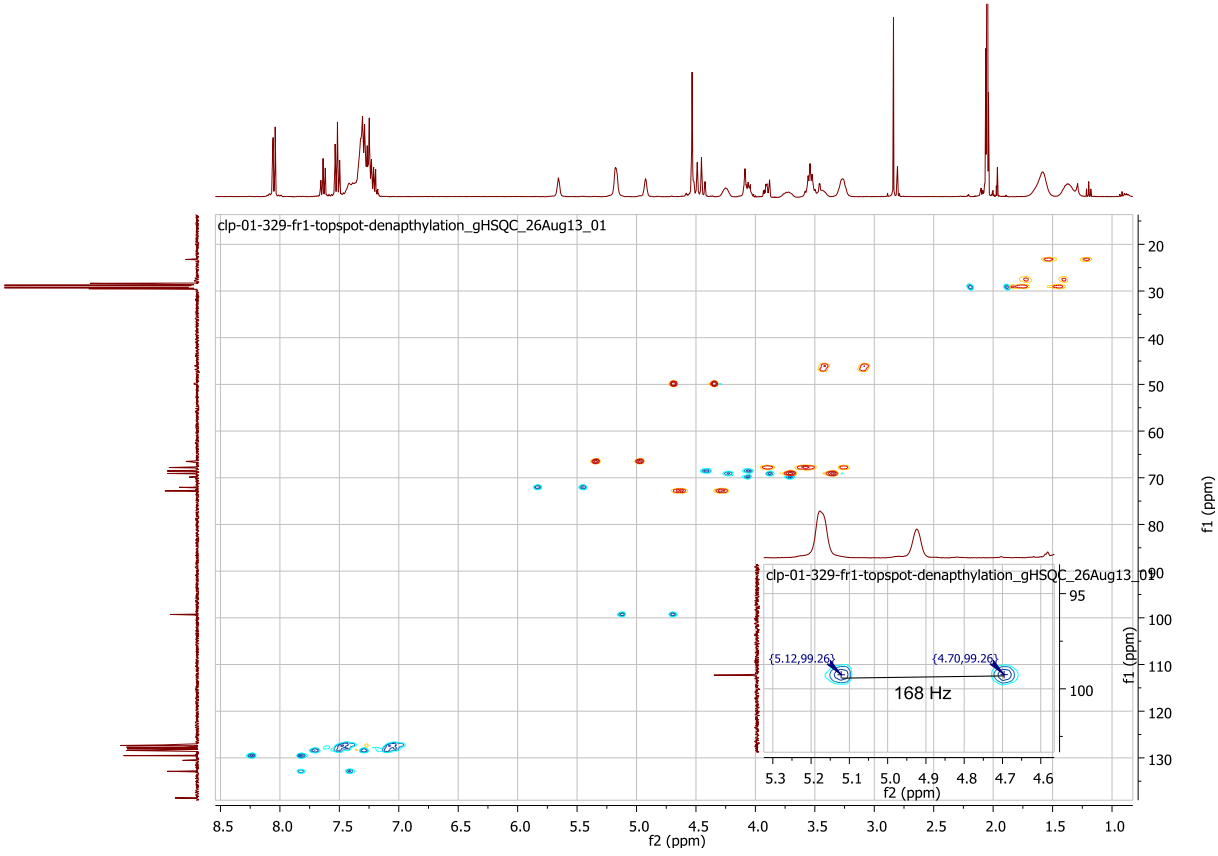

OBn
BzO
O
HO
HO
O
NBnCbz

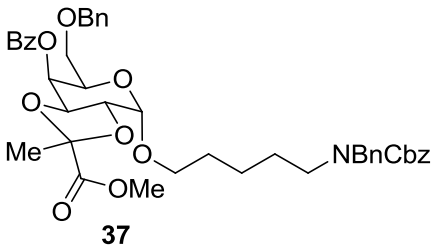
36


clp-01-329-fr1-topspot-denapthylation_PROTON_25Aug13_01
f1 (ppm)

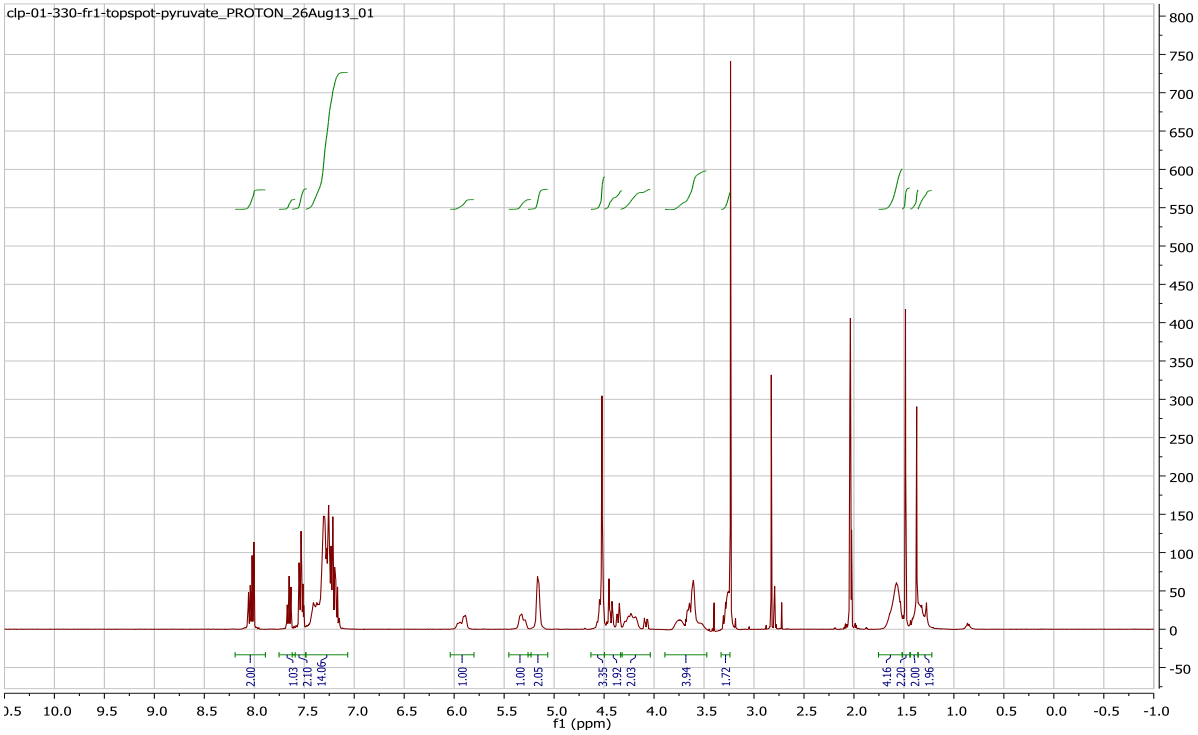

clp-01-329-fr1-topspot-denapthylation_CARBON_25Aug13_01
f1 (ppm)


clp-01-329-fr1-topspot-denapthylation_gHSQC_25Aug13_01
f2 (ppm)
f1 (ppm)


clp-01-329-fr1-topspot-denapthylation_gHSQC_26Aug13_01
clp-01-329-fr1-topspot-denapthylation_gHSQC_26Aug13_02
{5.12,99.26}
{4.70,99.26}
168 Hz
f2 (ppm)
f1 (ppm)


OBn
BzO
O
O
O
Me
O
NBnCbz
OMe
O

37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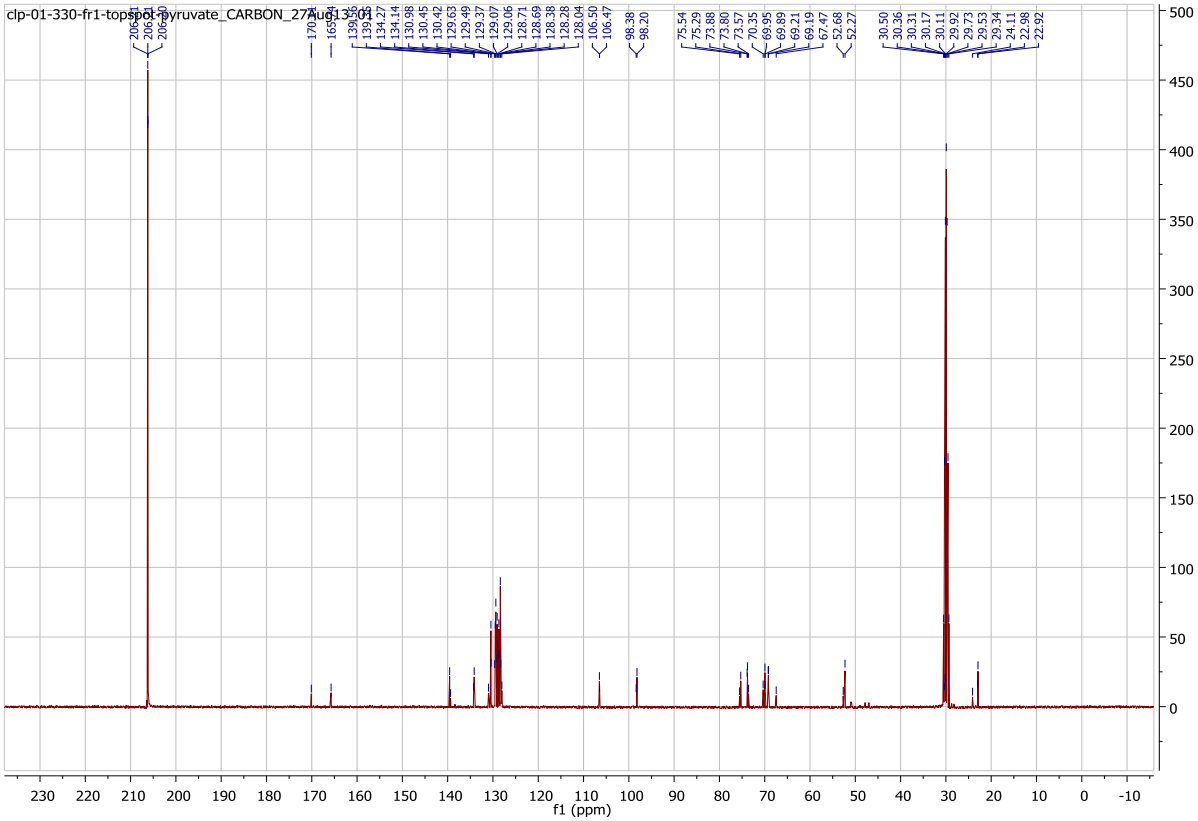
f1 (ppm)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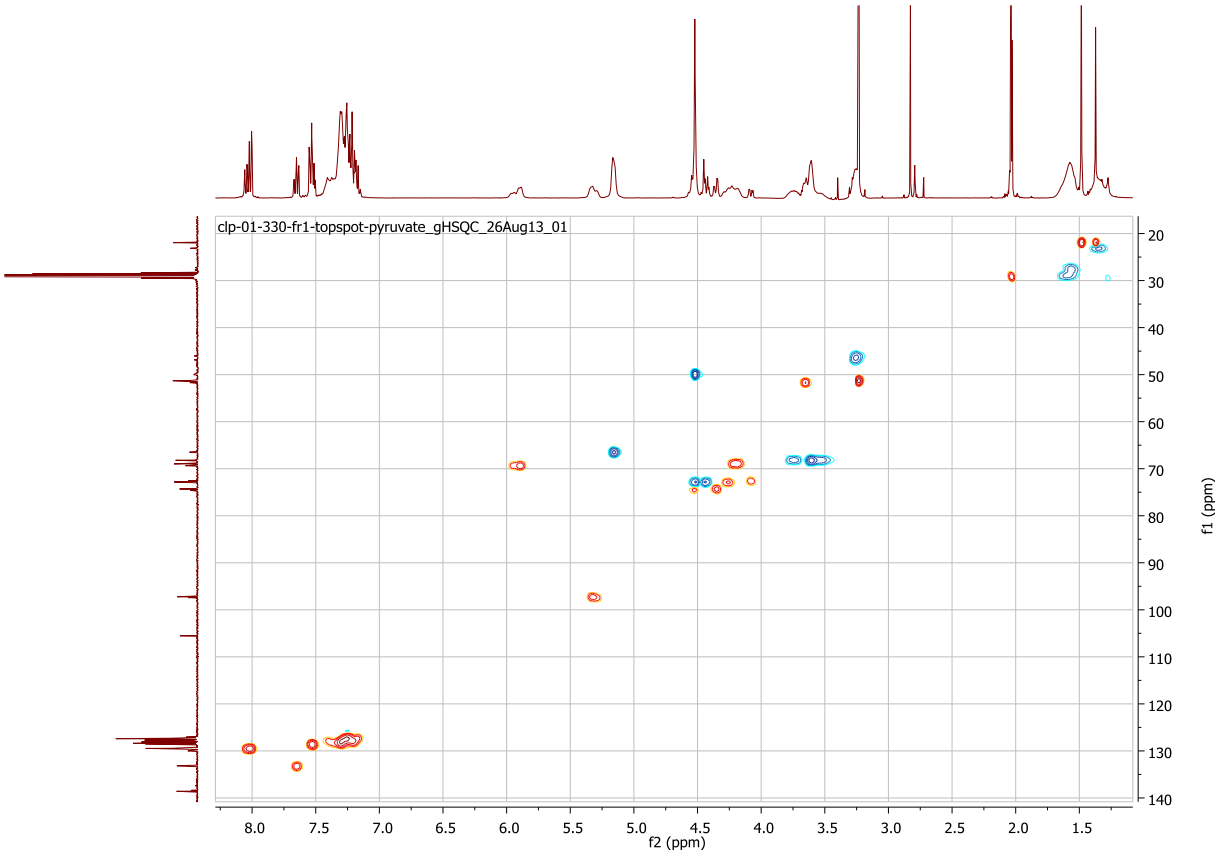
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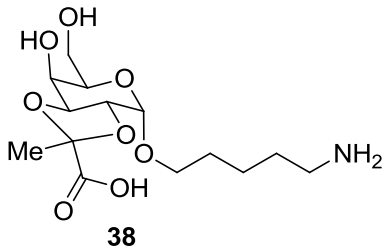
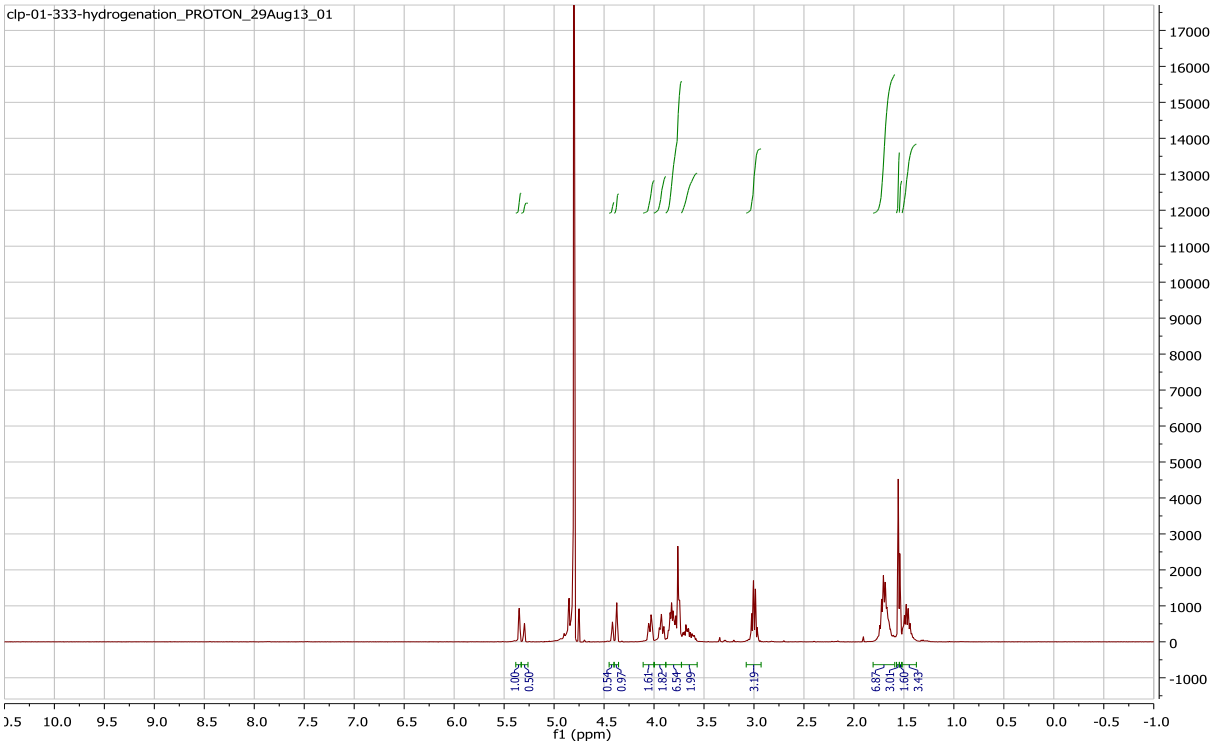
f1 (ppm)


OH
HO
O
O
O
Me
O
NH2
OH
O

38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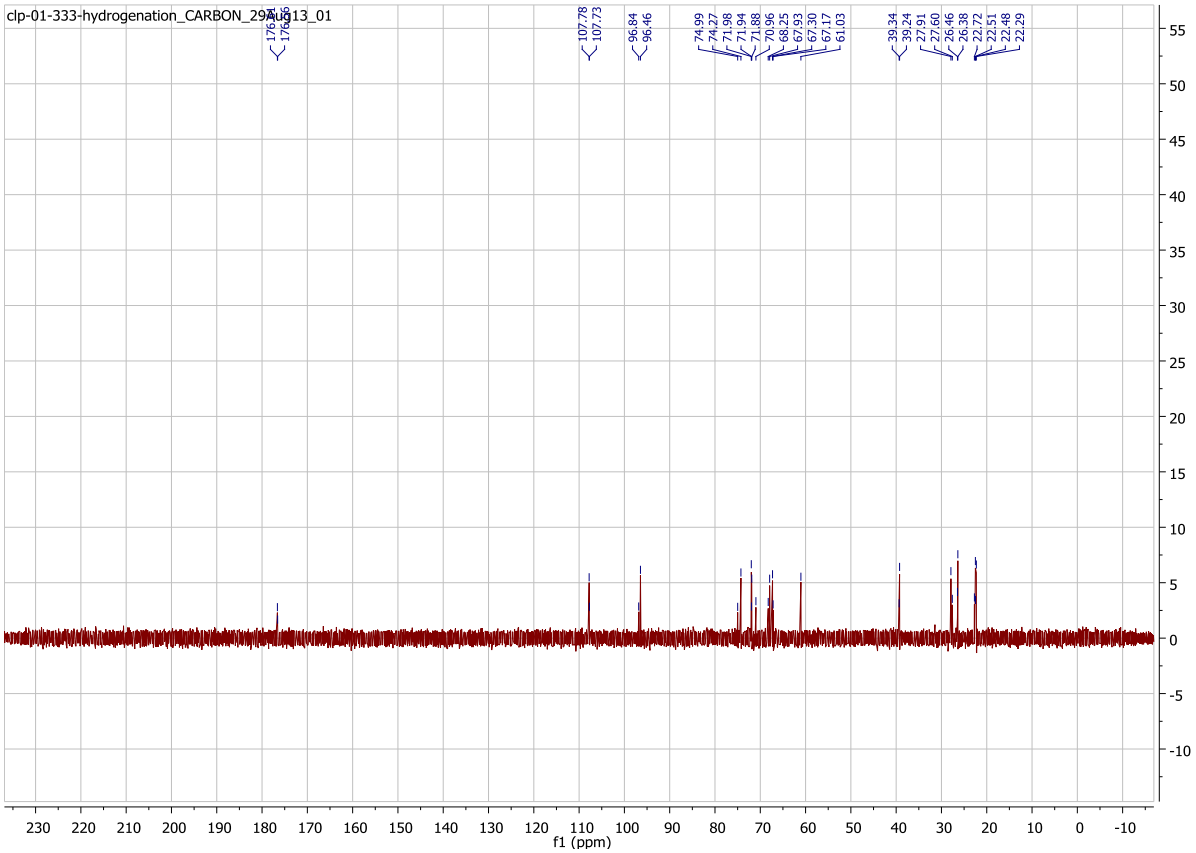
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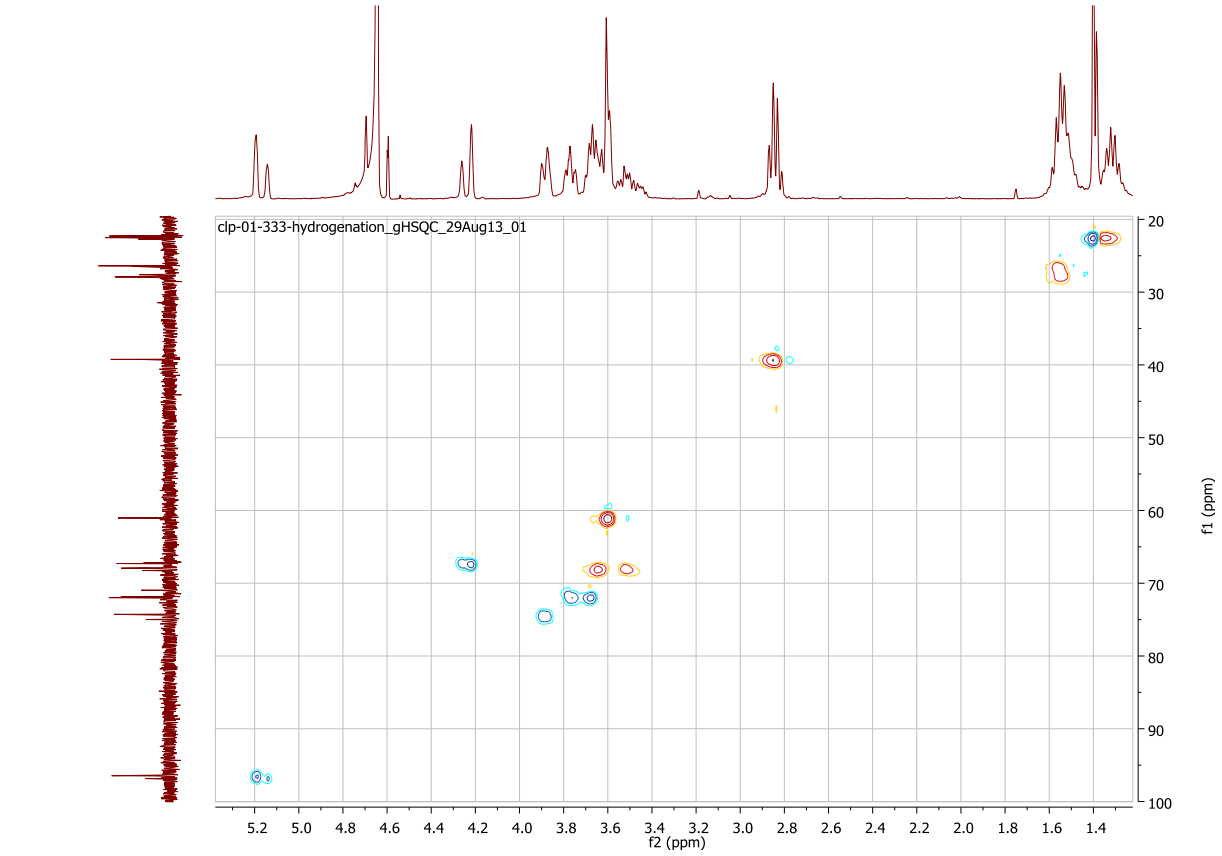
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f2 (ppm)
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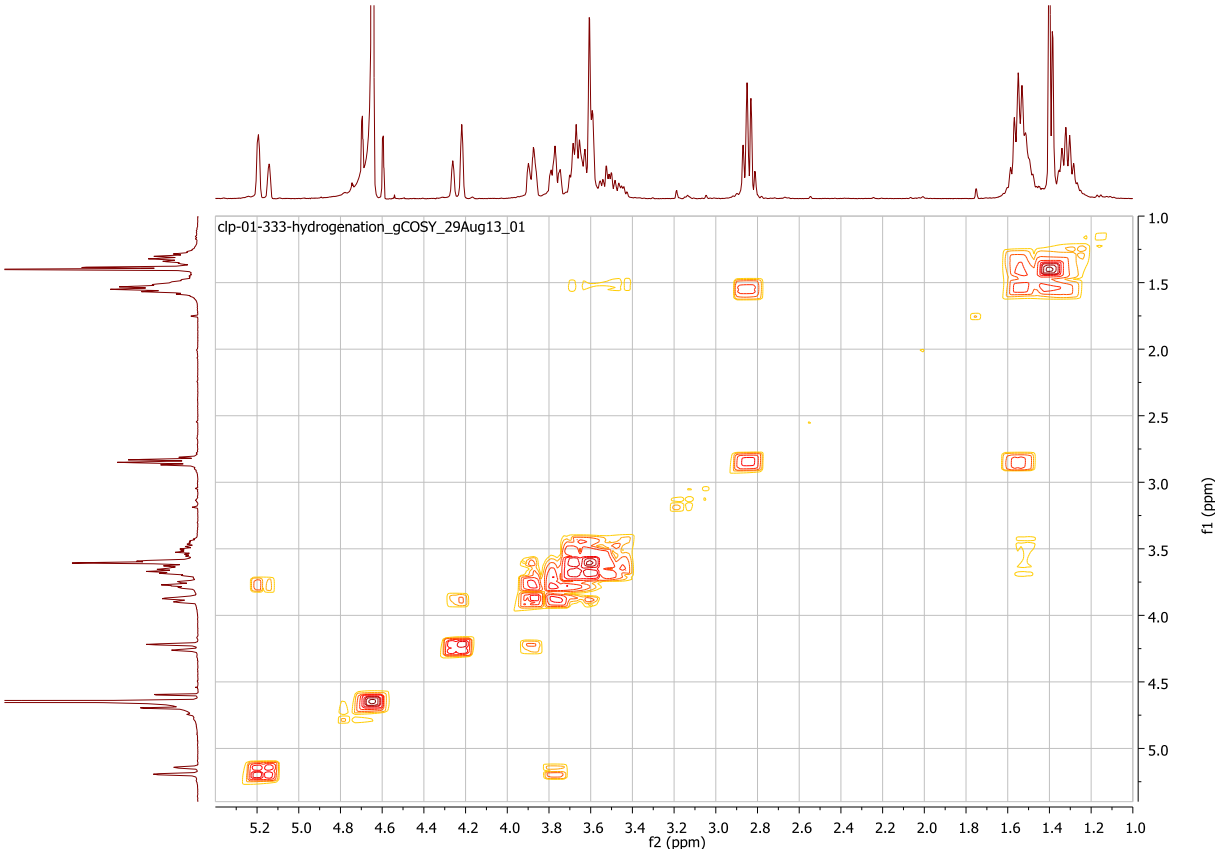
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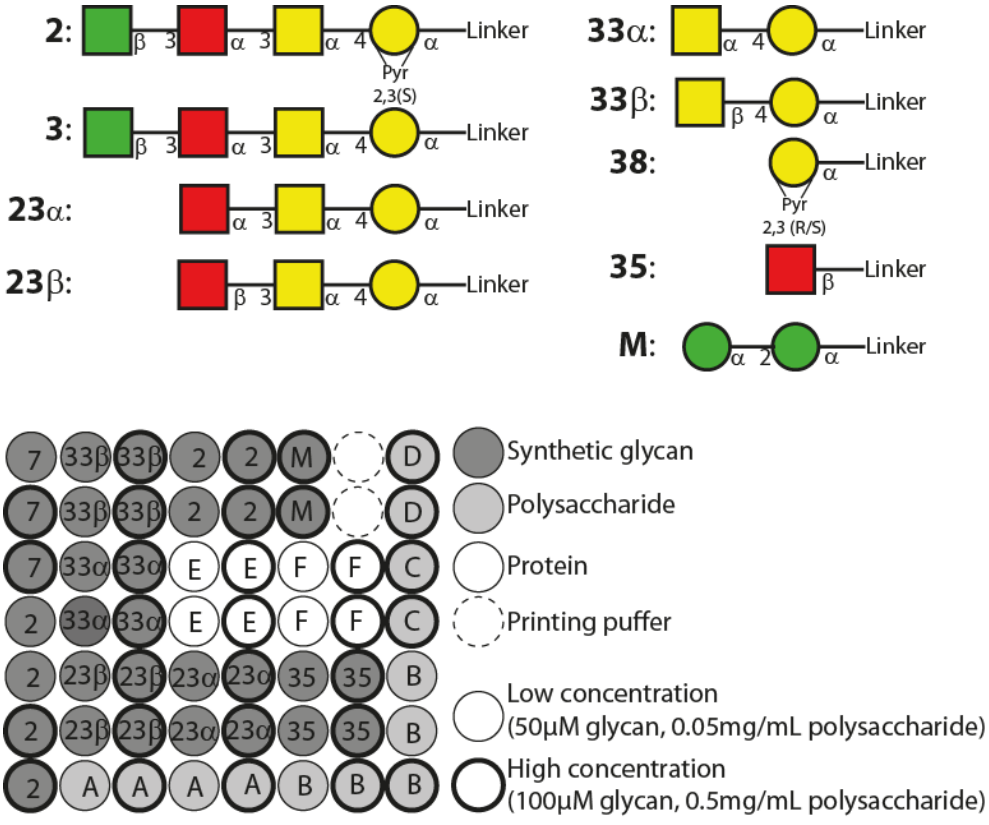
f2 (ppm)
f1 (ppm)

7. Biological experiments

a. Preparation of glycan microarrays

Substances were spotted on NHS activated glass slides (CodeLink; Surmodics) in 50 mM sodium phosphate buffer pH 8.5 at the concentration given in supplementary figure 1 using a Scienion S3 microarray printer equipped with a type 4 coated nozzle. Approximately 0.4 nL of each substance was printed per spot. Humidity was set to 65% during the entire print run. Afterwards, the slides were incubated overnight at room temperature in a humidity saturated chamber. Quenching was performed for one hour at room temperature in 100 mM ethanolamine; 50mM sodium phosphate pH 9. Slides were dried by centrifugation for five minutes at 300 g in CombiSlide (Eppendorf) slide holder and stored dried at 4 °C until use.



Polysaccharides
A: *S. pneumoniae* cell wall polysaccharide
B: *S. pneumoniae* type 4 CPS
C: *S. pneumoniae* type 3 CPS
D: *S. pneumoniae* type 2 CPS

Proteins
E: Carrier protein CRM_{197}
F: BSA-Dimannose conjugate

Supplementary figure 1: Printing pattern of glycan arrays. The dimannoside with aminolinker (M) was used as printing control (kind gift of Dr. Hidenori Tanaka).

b. Glycan array incubation

Directly before use, glycan arrays were blocked by incubation with 1% BSA in phosphate buffered saline (PBS) for 30 min at room temperature. Slides were washed three times for 5 min with PBS and dried by centrifugation. To allow multiwell incubation, a 64 well gasket (FlexWell 64 grid, Grace BioLabs) was attached to the slide. Dilutions of SP4 rabbit typing serum (SSI Diagnostica) were prepared in 1% BSA-PBS also containing the binding inhibitor (SP4 CPS or *S. pneumoniae* CWPS, 5 µg/mL). Samples were incubated for 20 min at room temperature, applied to the slide, and incubated for 1 h at room temperature. Slides were washed three times for 5 min with PBS containing 0.1% Tween-20 (PBS-T). Secondary antibody solution (FITC labeled goat anti-rabbit IgG; Abcam; diluted 1 in 200 in 1% BSA-PBS) was applied to the wells and the slides were incubated for 30 min at room temperature in the dark. Afterwards, slides were washed twice with PBS-T. The multiwell gasket was removed, the slide washed once with PBS, rinsed shortly with water and dried by centrifugation. Intensities were read out with a GenePix 4300A microarray reader (Molecular Devices) and spots were analyzed using GenePix Pro 7 (Molecular Devices) using the mean fluorescence intensity of every spot subtracted by the local background. Spot diameter was kept identical for all substances. Mean of four spots from duplicates was calculated, and error bars represent standard deviation.