Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

1,2-*cis* Alkyl glycosides: Straightforward glycosylation from unprotected 1-thioglycosyl donors

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Contents

1. Flow-injection-analysis–ESI-TOF-MS (FIA–ESI-TOF-MS) instrumentation

An AB Sciex QSTAR Elite triple quadrupole mass spectrometer (Concord, Ontario, Canada) was outfitted with a flow-injection analysis (FIA) device to provide rapid and effective injection of multiple samples for ESI-TOF-MS analysis. A picture of the FIA equipment is shown in Figure S1.

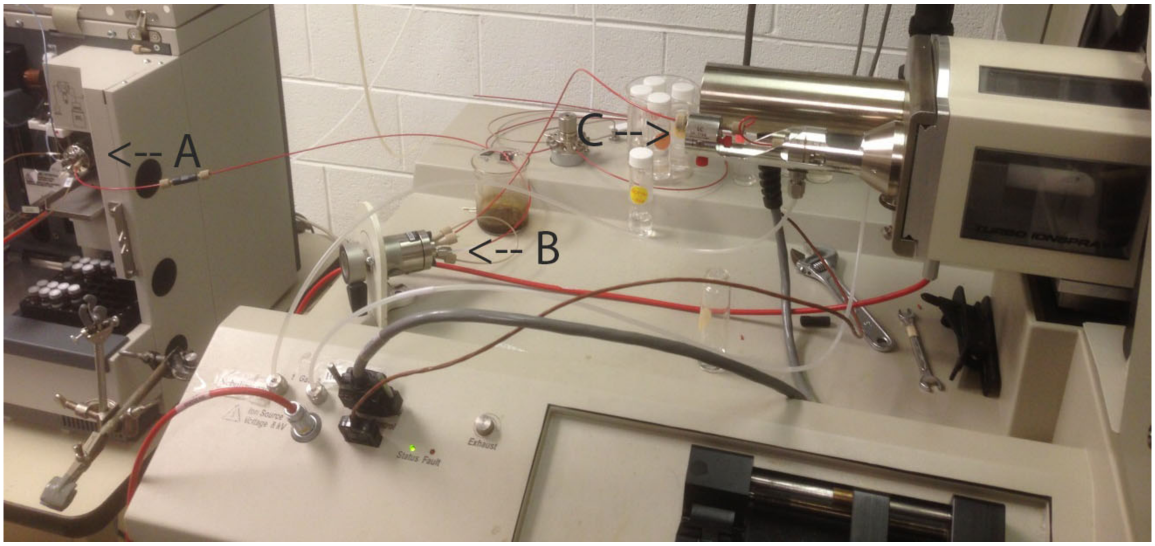


Figure S1 Flow-injection device outfitted on QSTAR Elite TOF-MS instrument. **A**: LC Pump [Dionex Ultimate fully integrated micro-, capillary- and nano-HPLC system (Sunnyvale, CA, USA)] **B**: 6-Port manual injection valve (Rheodyne, Rohnert Park, CA, USA), and **C**: Injection port for AB Sciex QSTAR Elite time-of-flight mass spectrometer (Concord, Ontario, Canada) with an ESI ion source.

FIA–ESI-TOF-MS conditions

- FIA flow
  - Solvent: MeOH
  - Flow rate: 120 μL/min
- Manual injection
  - Sample concentration: 10 μg/mL; 25 μL diluted to 1.00 mL with MeOH for injection
  - Loop volume: 2.5 μL
  - Syringe size: 250 μL
  - Injections at 2-min intervals; 10 times each

2. High-throughput screening/reaction optimization by flow-injection analysis–ESI-TOF-MS (FIA–ESI-TOF-MS)

Before the screening for glycosylation conditions, we carefully examined whether the ionizing properties of deuterated and nondeuterated glycoside isomers are identical. To establish a quantitative calibration curve, deuterium-labeled glycoside solution [10 μg/mL, propargyl 2,3,4,6-tetra-*O*-(acetyl-d_3)-α-

D-galactopyranoside (**4**)] and non-labeled glycoside solution [propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4a**)] were prepared separately (see Experimental Section, main text), mixed in five concentration ratios, and diluted to the same volume by MeOH to afford five standards (Table S1). A six-port manual injection valve was installed between the LC pump and the ESI-QTOF instrument. MeOH was chosen as the FIA solvent. Each sample was injected into the six-port manual injection valve with a 2.5-μL sample loop and analyzed by ESI-QTOF-MS for 10 replicates. Non-labeled glycoside ($[M_H+Na]^+$ ion) and labeled ($[M_D+Na]^+$ ion) were observed at *m/z* 409 and *m/z* 421, respectively (Figure S2). Integration of peak areas under extracted peak profiles at *m/z* 409 and *m/z* 421 were performed, and peak area ratios were calculated for each entry. Experimental results indicated that the ionizing properties of the deuterated and nondeuterated glycoside isomers are almost identical, which enables rapid quantification of the nondeuterated glycoside when deuterated glycoside is added as an internal standard (Figure S3).

Table S1 Comparison of $\frac{[C_H]}{[C_D]}$ [a] and $\frac{PA_H}{PA_D}$ [b]

entry	$\frac{[C_H]}{[C_D]}$ [a]	$\frac{[PA_H]}{[PA_D]}$ [b]	Standard deviation
1	0.2	0.2061	0.0095
2	0.4	0.3955	0.0094
3	1	0.9946	0.0158
4	2.5	2.5619	0.0354
5	5	5.2201	0.4221

[a] Concentration ratios of non-labeled standards/labeled standards. [b] Peak area ratios using isotope-diluted FIA–ESI-TOF-MS.

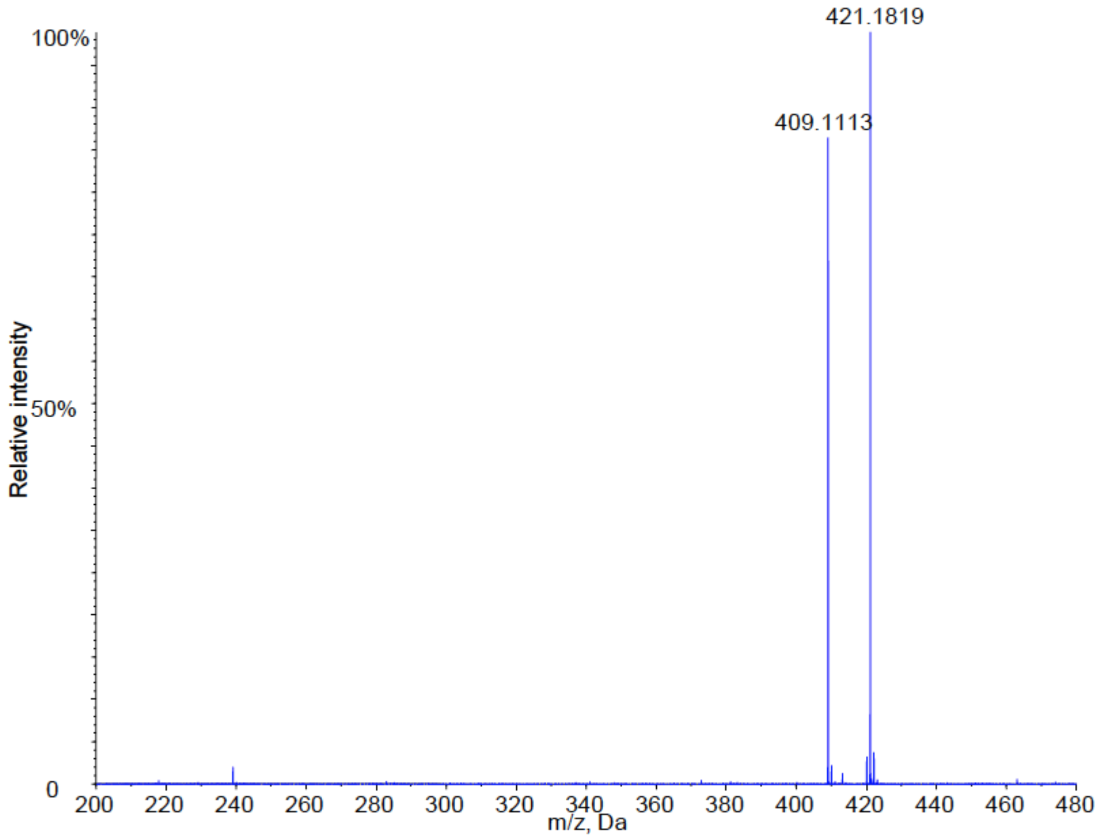


Figure S2 ESI-TOF-MS of product **4a** ([M_H+Na)$^+$] ion at *m/z* 409) and internal standard (deuterated) **4** ([M_D+Na)$^+$] ion at *m/z* 421)

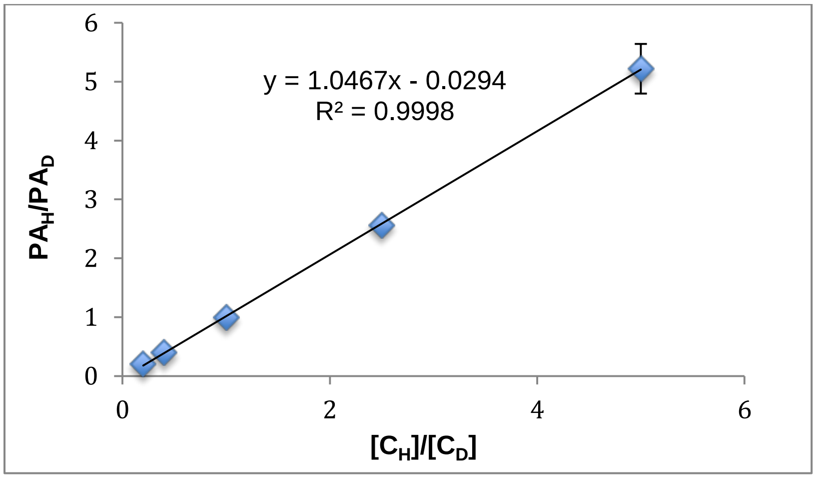


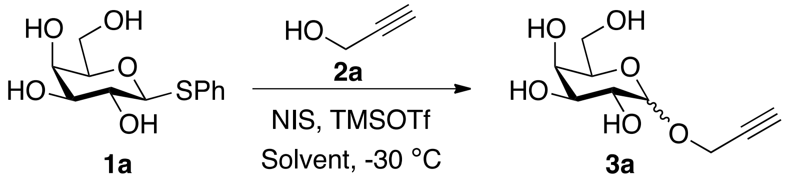
Figure S3 Relationship between concentration ratios of standards ($\frac{[C_H]}{[C_D]}$) and peak area ratios ($\frac{PA_H}{PA_D}$).

For a general analytical method, the internal standard was dissolved in CH_2Cl_2 to afford a 10 μg/mL solution. The crude product (non-labeled glycoside) was also dissolved in CH_2Cl_2 to get an estimated 10 μg/mL solution. Samples (25 μL) of both labeled and non-labeled solutions were mixed and diluted with MeOH to get a 1 mL of solution. The solution was injected into the six-port injection valve, and the injection was repeated 10 times at 2-min intervals. The peaks of labeled ($[M_D+Na]^+$ ion at *m/z* 421) and non-labeled ($[M_H+Na]^+$ ion at *m/z* 409) glycoside were observed (Figure S2). After analyzing the ratio of the two peak areas, the concentration of the non-labeled glycoside solution was obtained.

3. Optimization of reaction studies: Effects of added solvents.

Several solvents known to favor α-glycosylation, including DMF, CH_2Cl_2, Et_2O and THF, were added to the reaction mixture to explore whether they can increase the α/β ratios. The data listed below in Table S2 indicate no enhancement in the productions of the α anomer.

Table S2 Effects of added solvents on yield and α/β ratios.



entry	Solvent added	Equiv	yield	α:β
1	DMF	6.0	36	10:1
2	CH_2Cl_2	6.0	65	3:1
3	Et_2O	6.0	55	5:1
4	THF	6.0	18	5:1
5	DMF	1.2	53	10:1
6	CH_2Cl_2	1.2	66	10:1
7	Et_2O	1.2	58	7:1
8	THF	1.2	54	8:1
9	DMF	0.2	76	10:1
10	CH_2Cl_2	0.2	74	9:1
11	Et_2O	0.2	67	9:1
12	THF	0.2	74	3:1

4. ^{1}H NMR, ^{13}C NMR, COSY NMR and HSQC NMR spectra

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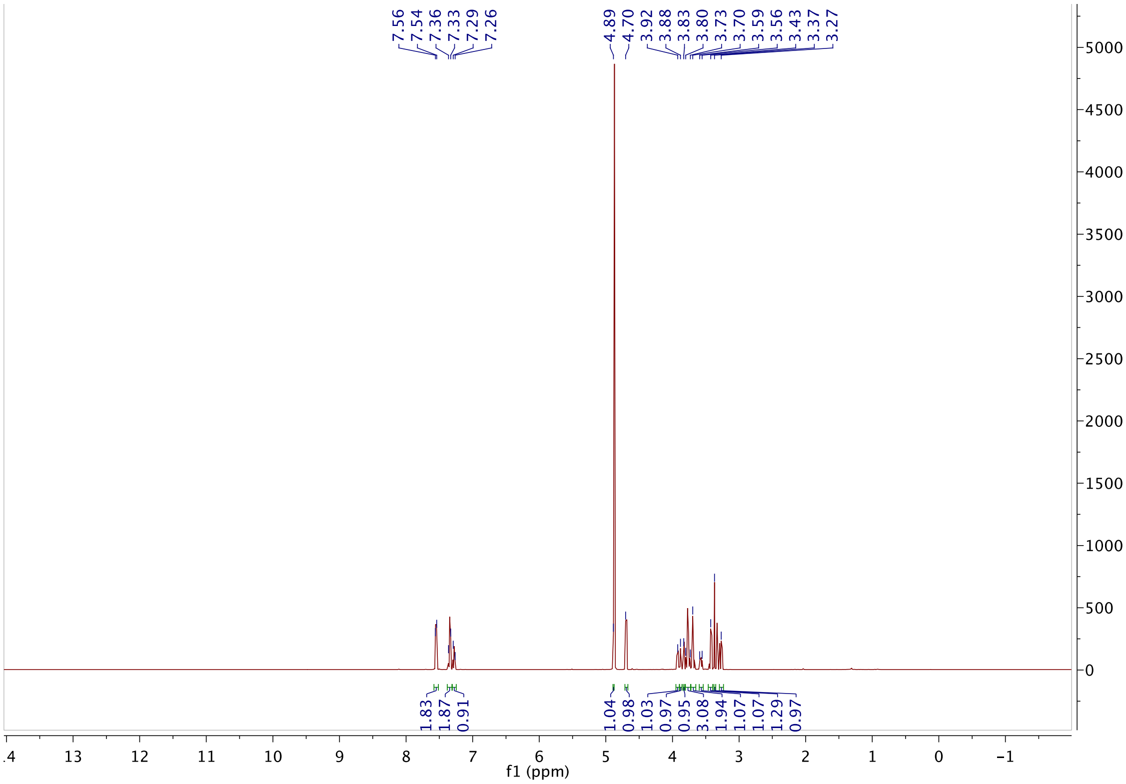
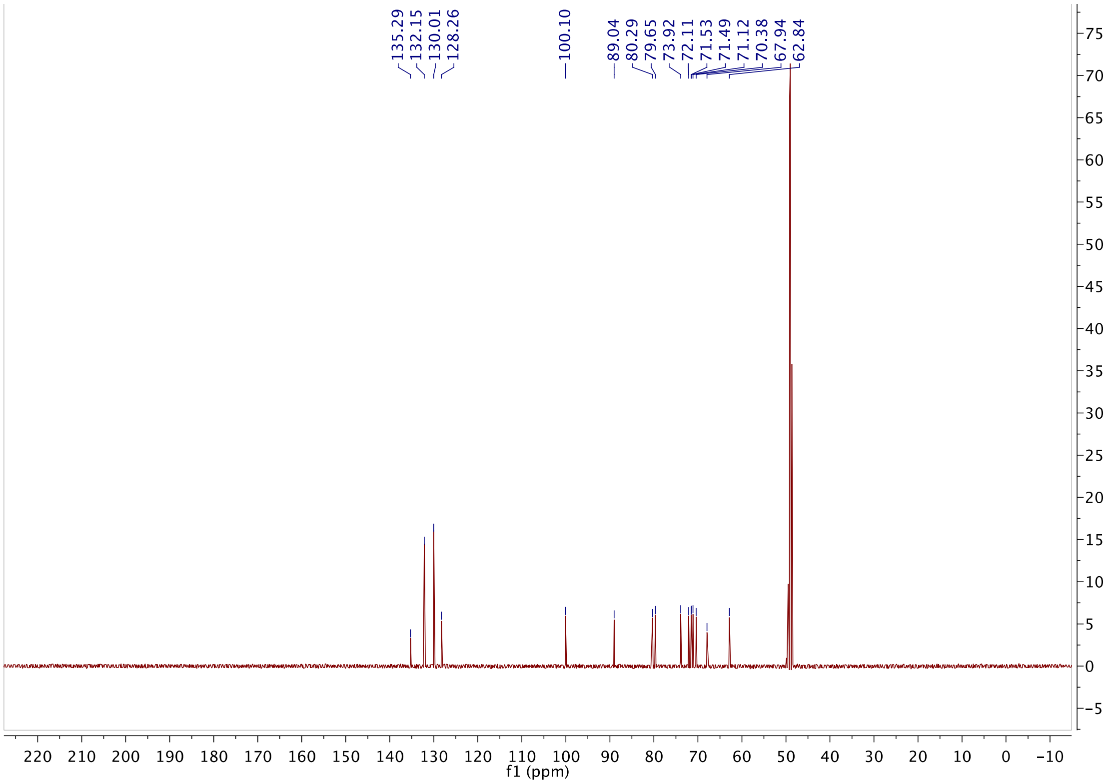
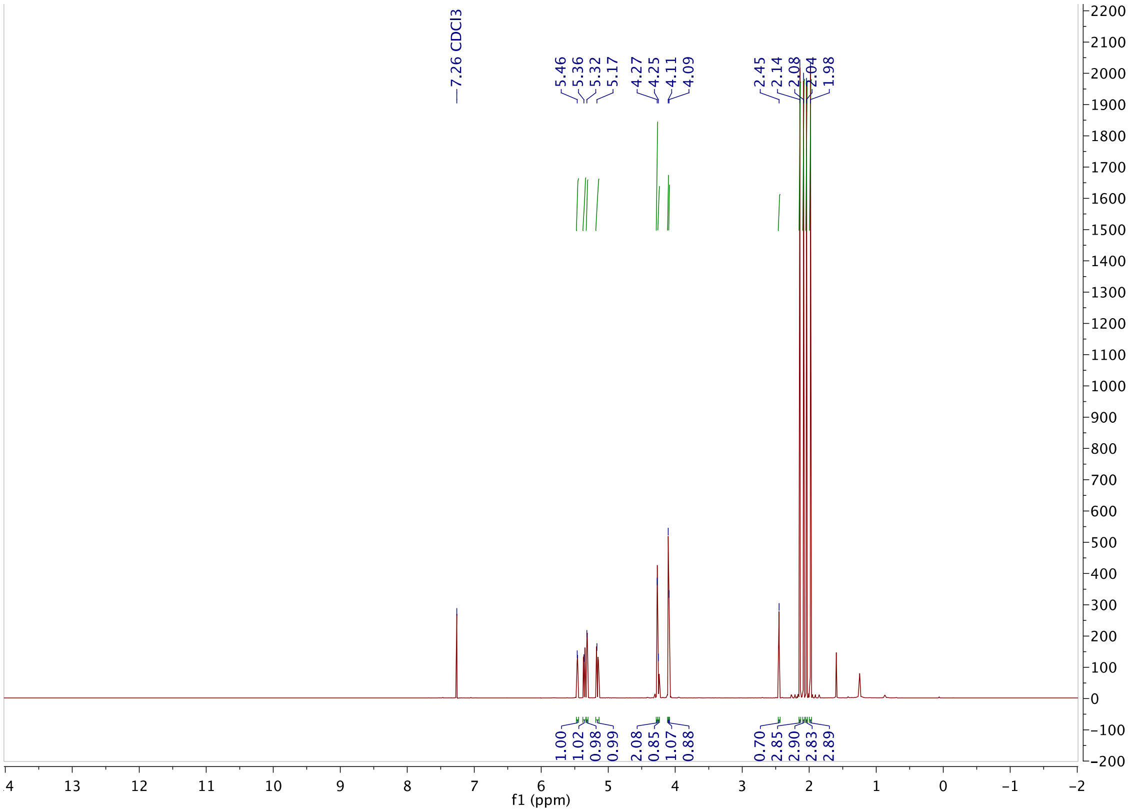


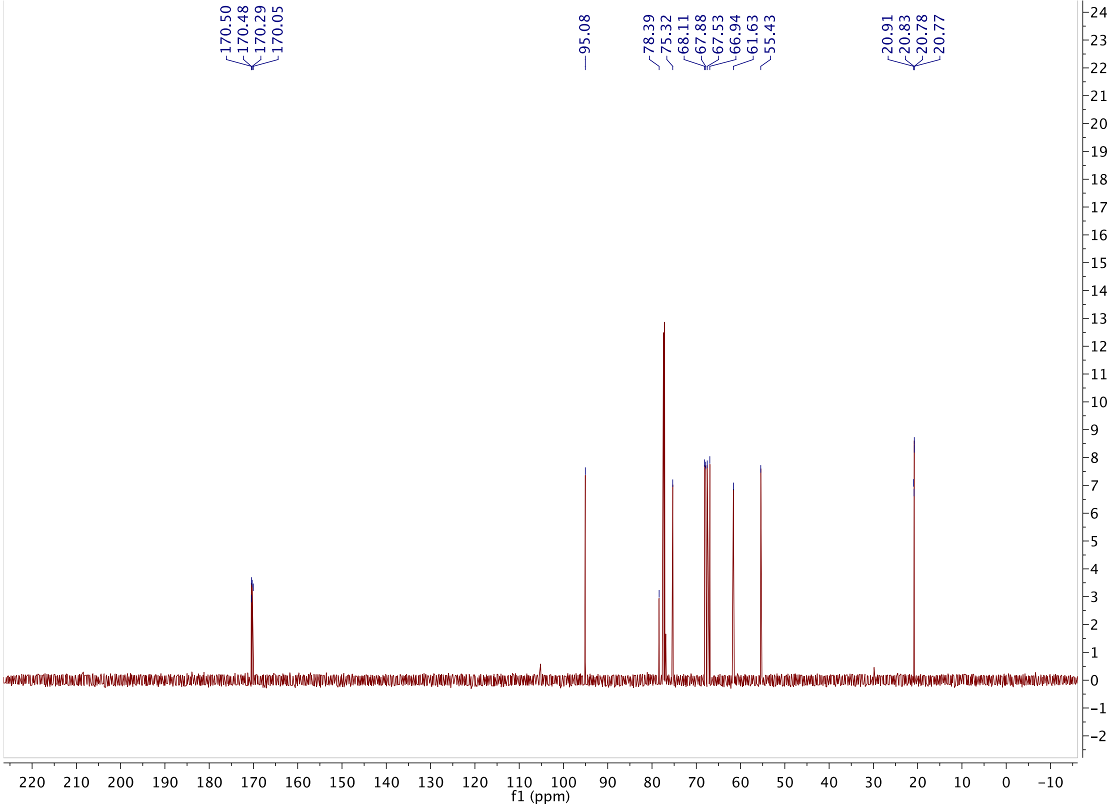
^{1}H NMR (500 MHz, $CDCl_3$: Phenyl *α*-D-galactopyranosyl-(1→6)-1-thio-*β*-D-glucopyranoside (**1dβ**)



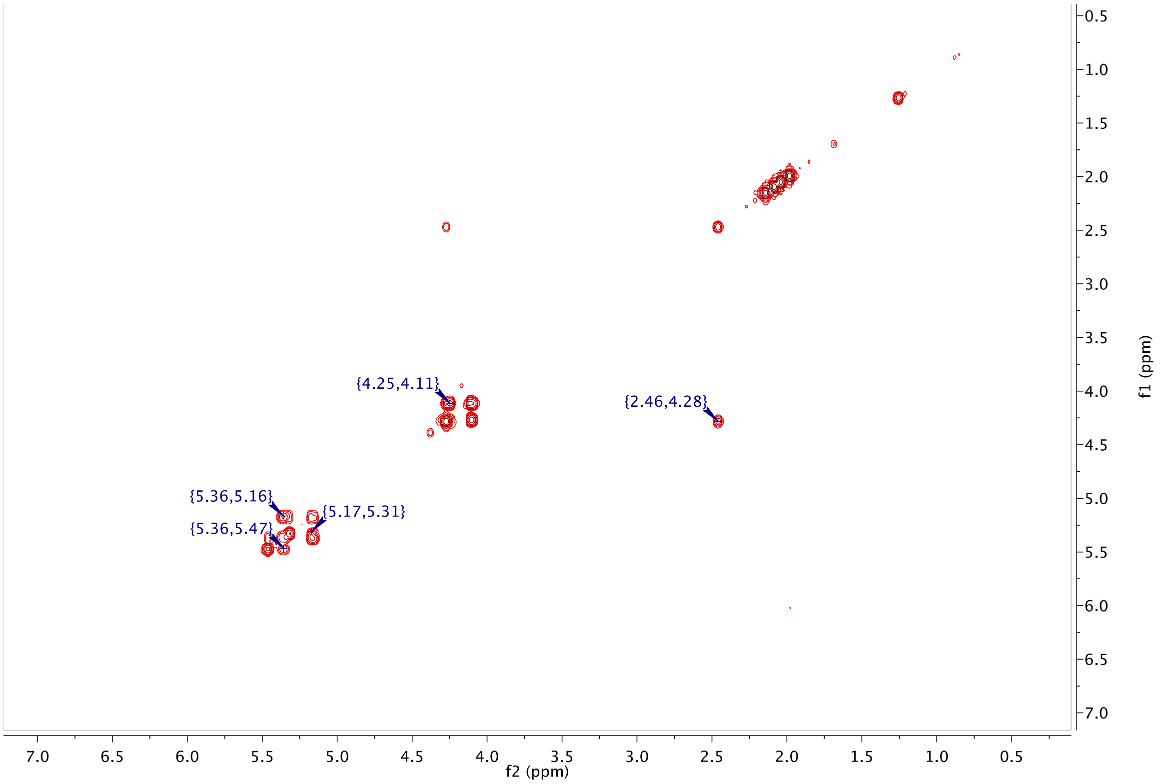
^{13}C NMR (125 MHz, $CDCl_3$):Phenyl *α*-D-galactopyranosyl-(1→6)-1-thio-*β*-D-glucopyranoside(**1dβ**)



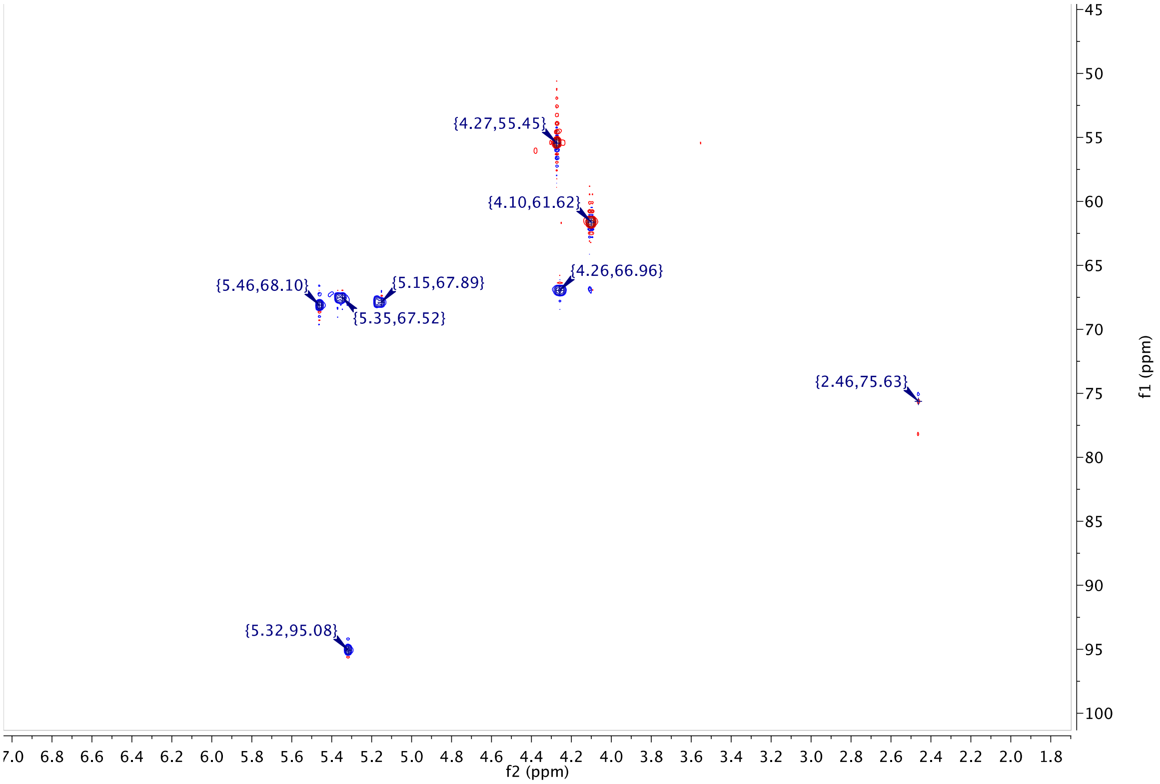
^{1}**H NMR** (500 MHz, $CDCl_3$): Propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4a**)



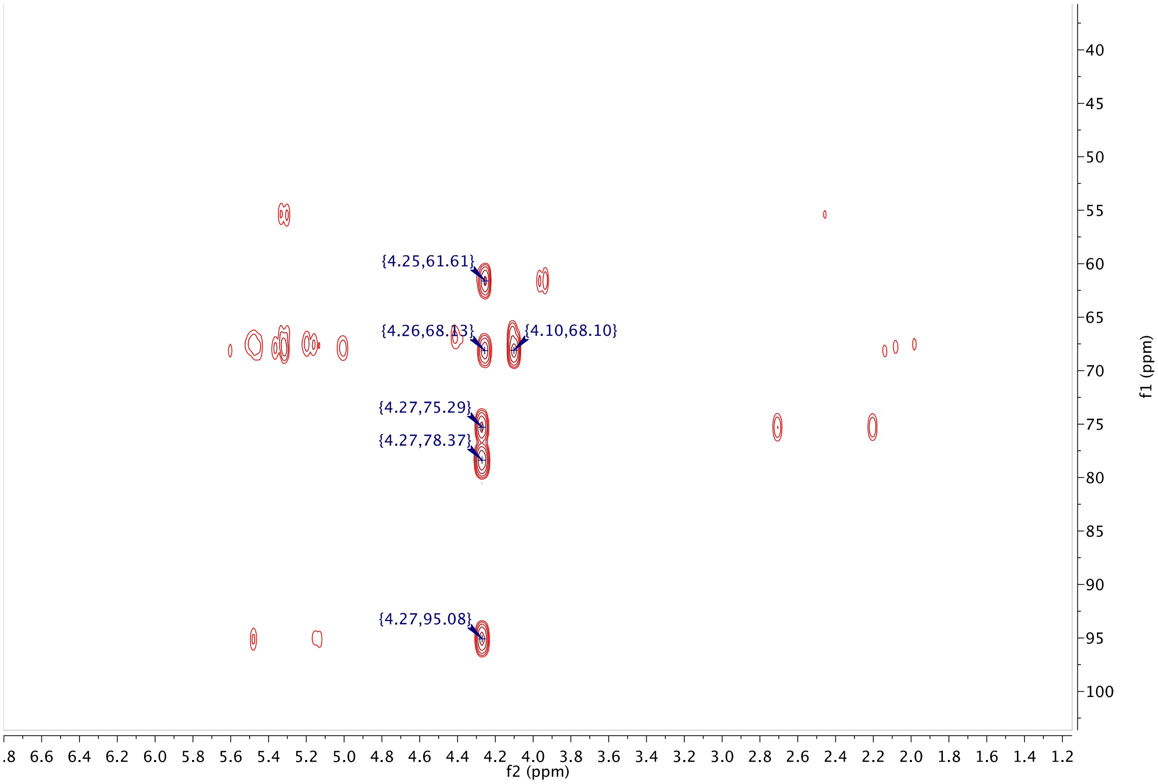
^{13}**C NMR** (125 MHz, $CDCl_3$): Propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4a**)



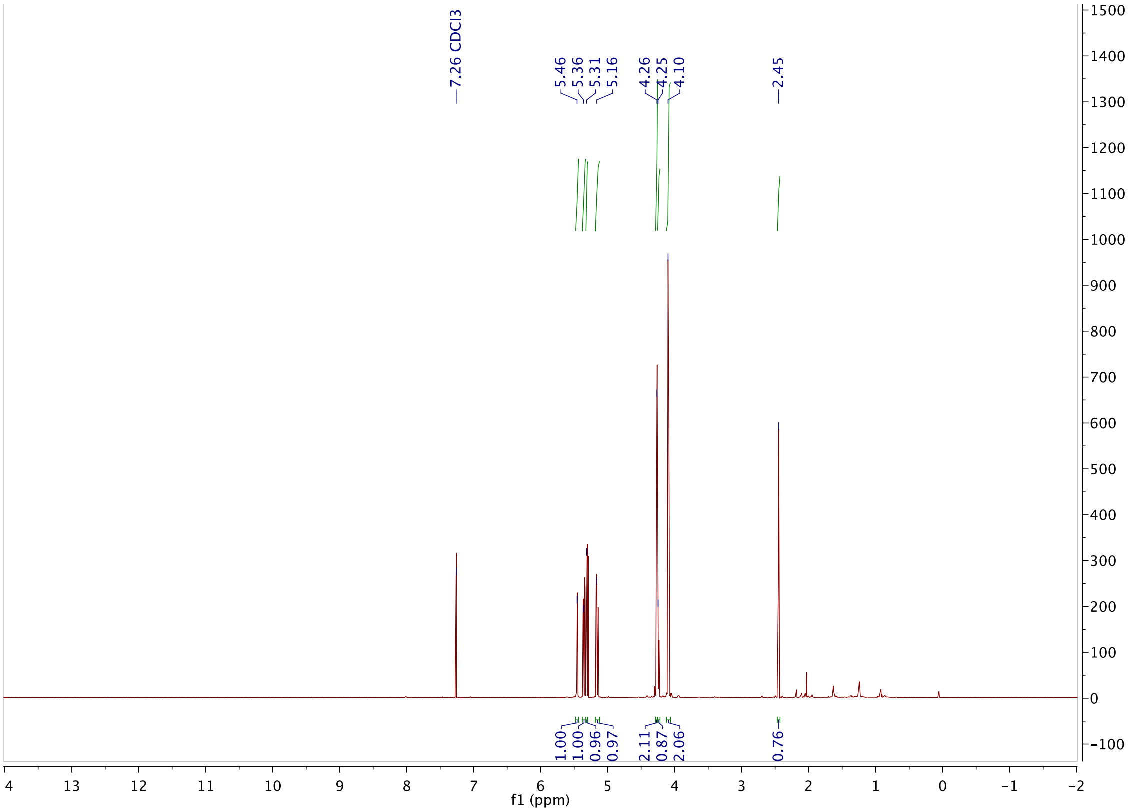
gCOSY NMR: Propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4a**)



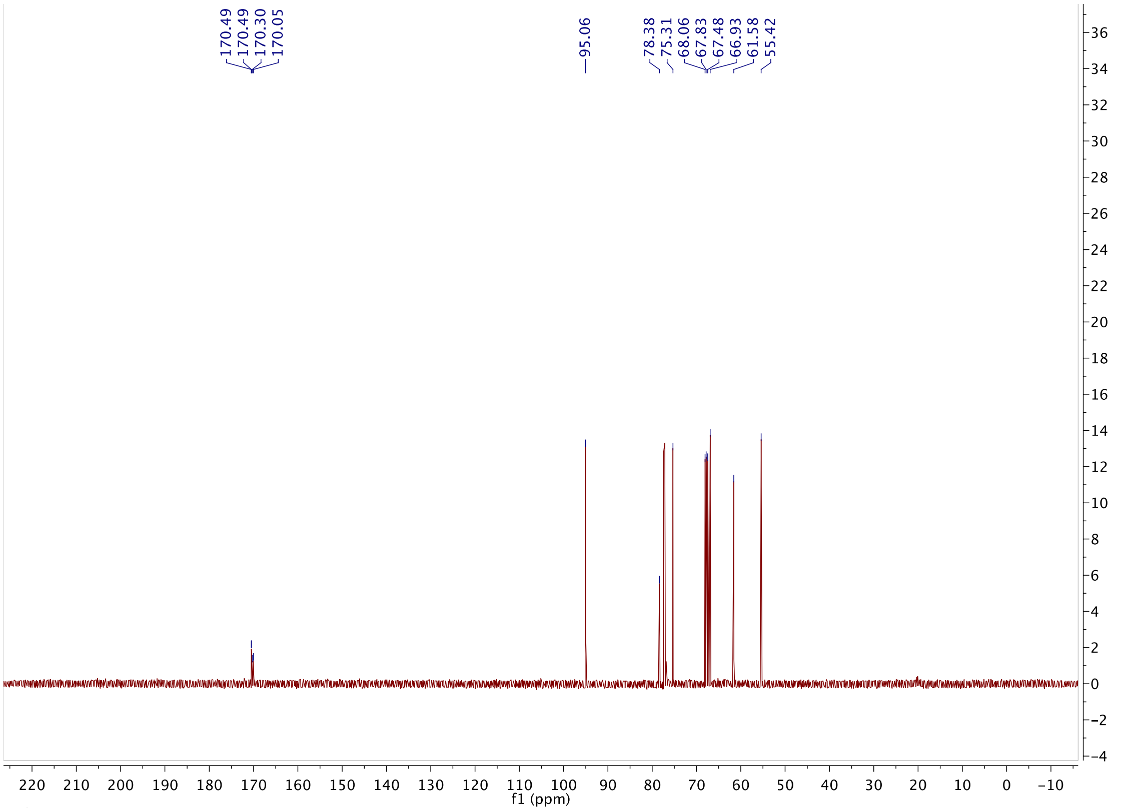
gHSQC NMR: Propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4a**)



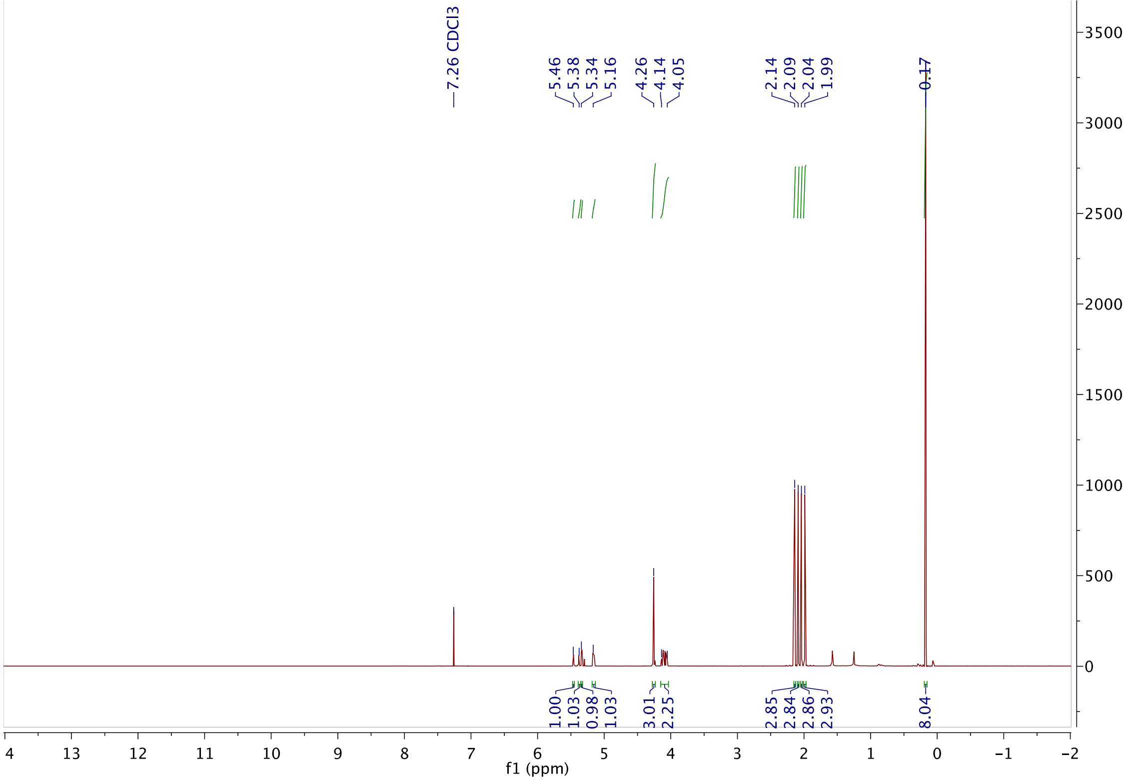
gHMBC NMR: Propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4a**)



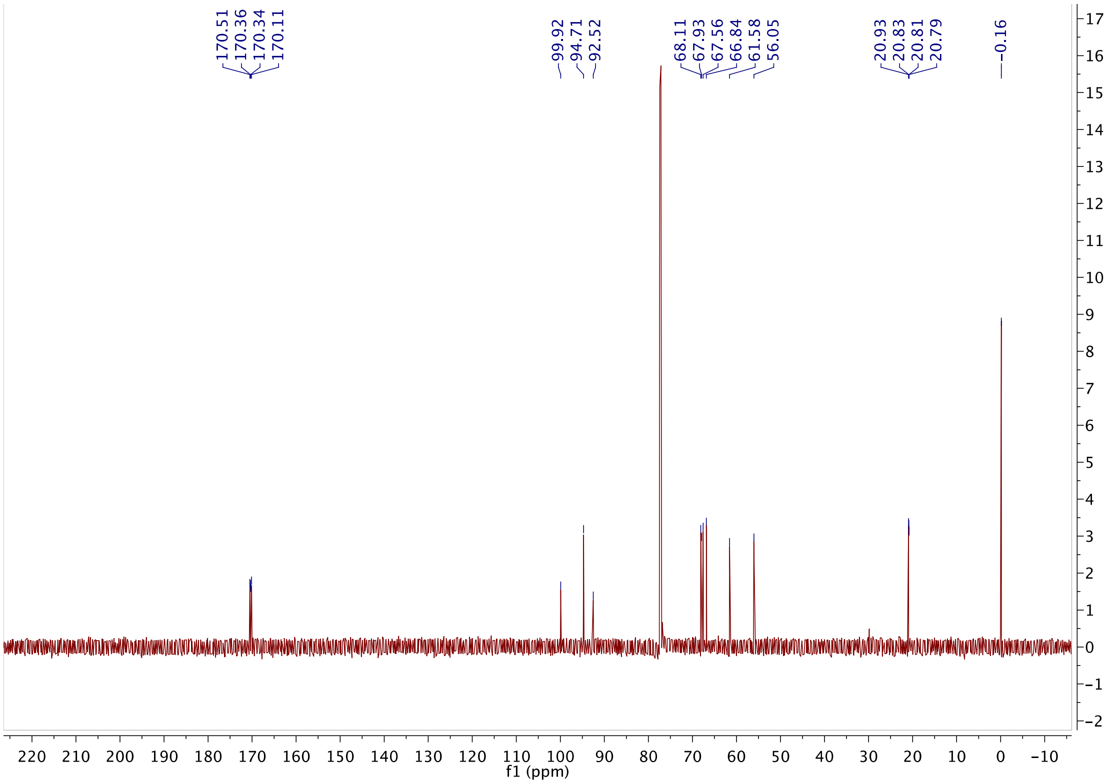
^{1}H NMR (500 MHz, CDCl$_3$): Propargyl 2,3,4,6-tetra-*O*-(acetyl-*d$_3$*)-α-D-galactopyranoside (**4**)



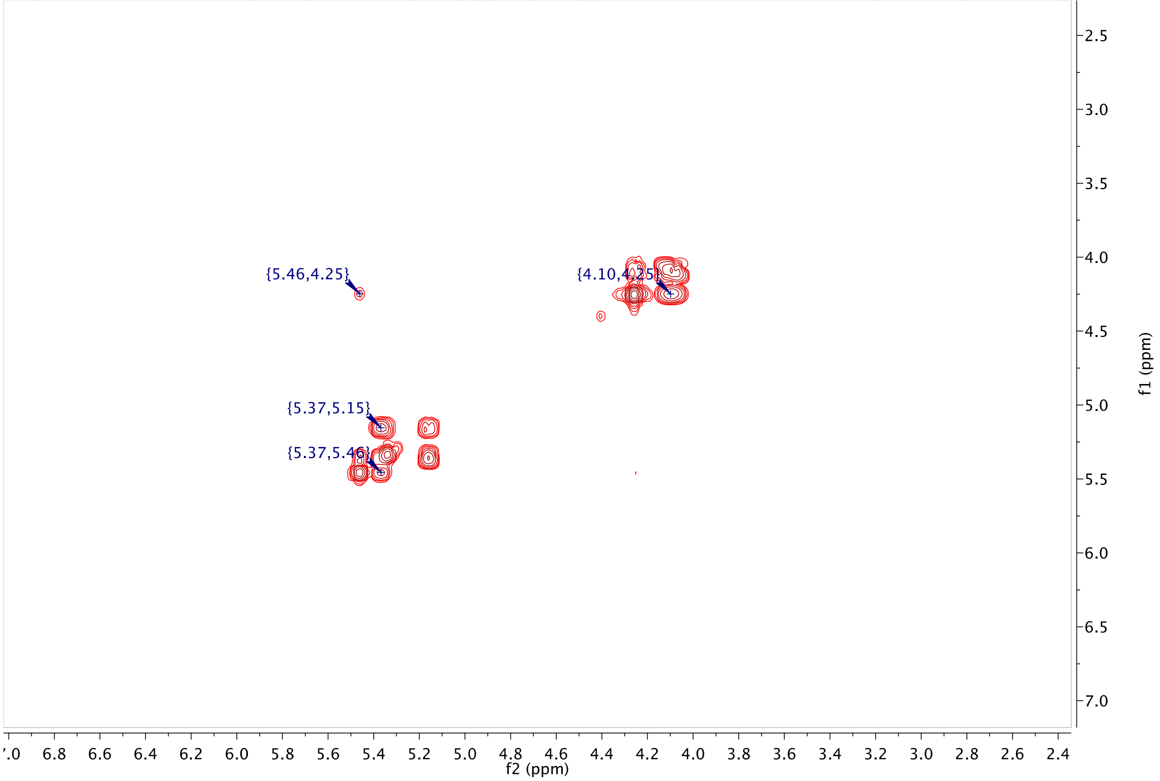
^{13}C NMR (125 MHz, CDCl$_3$): Propargyl 2,3,4,6-tetra-*O*-(acetyl-*d*$_3$)-α-D-galactopyranoside (**4**)



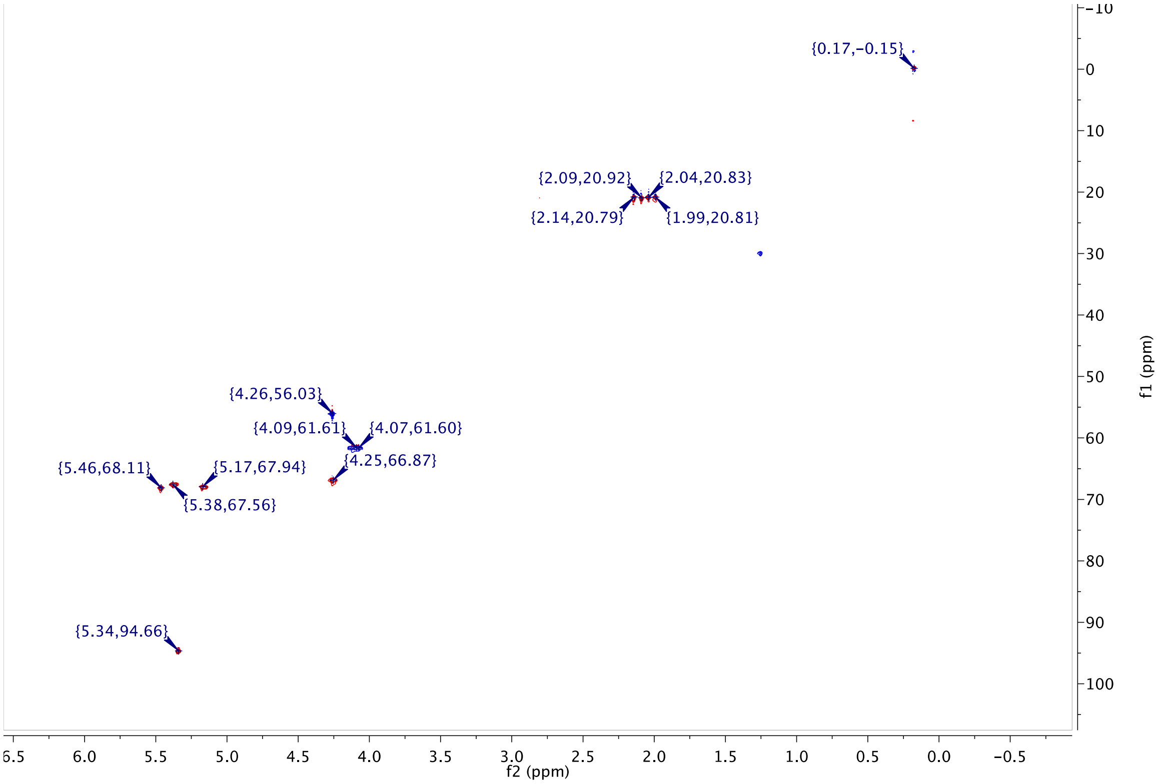
^{1}H NMR (500 MHz, CDCl$_3$): 3-(Trimethylsilyl)propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4b**)



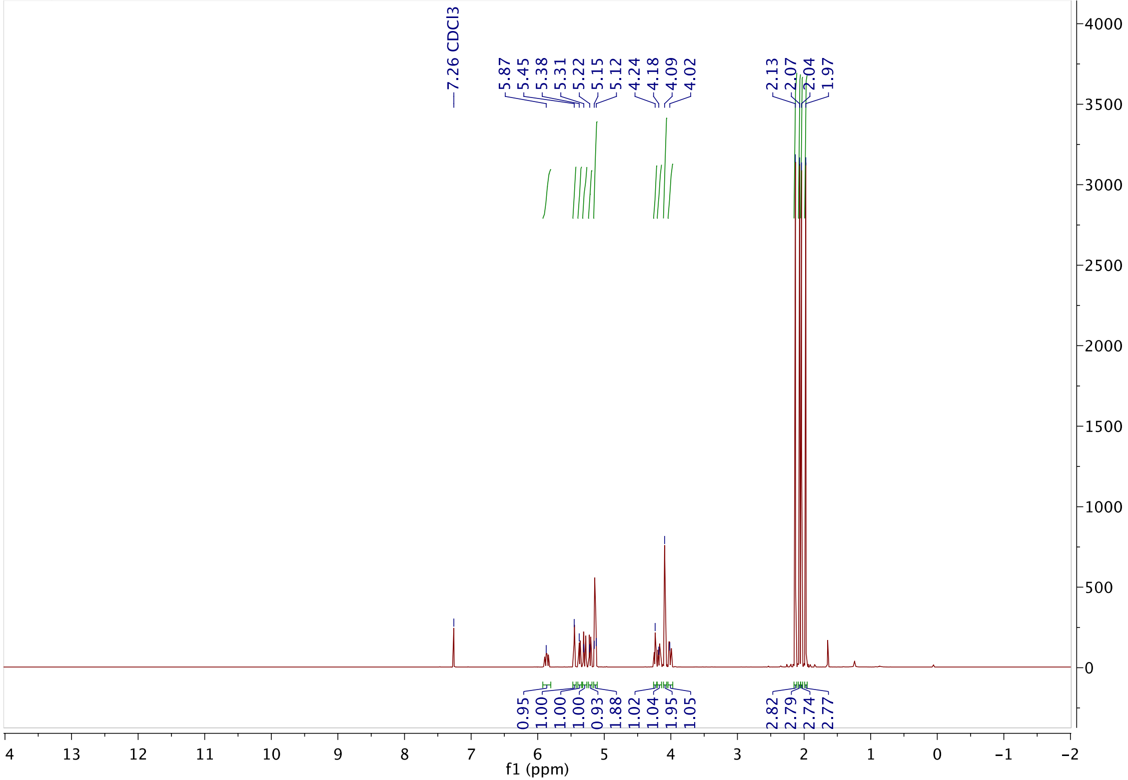
^{13}C NMR (125 MHz, $CDCl_3$): 3-(Trimethylsilyl)propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4b**)



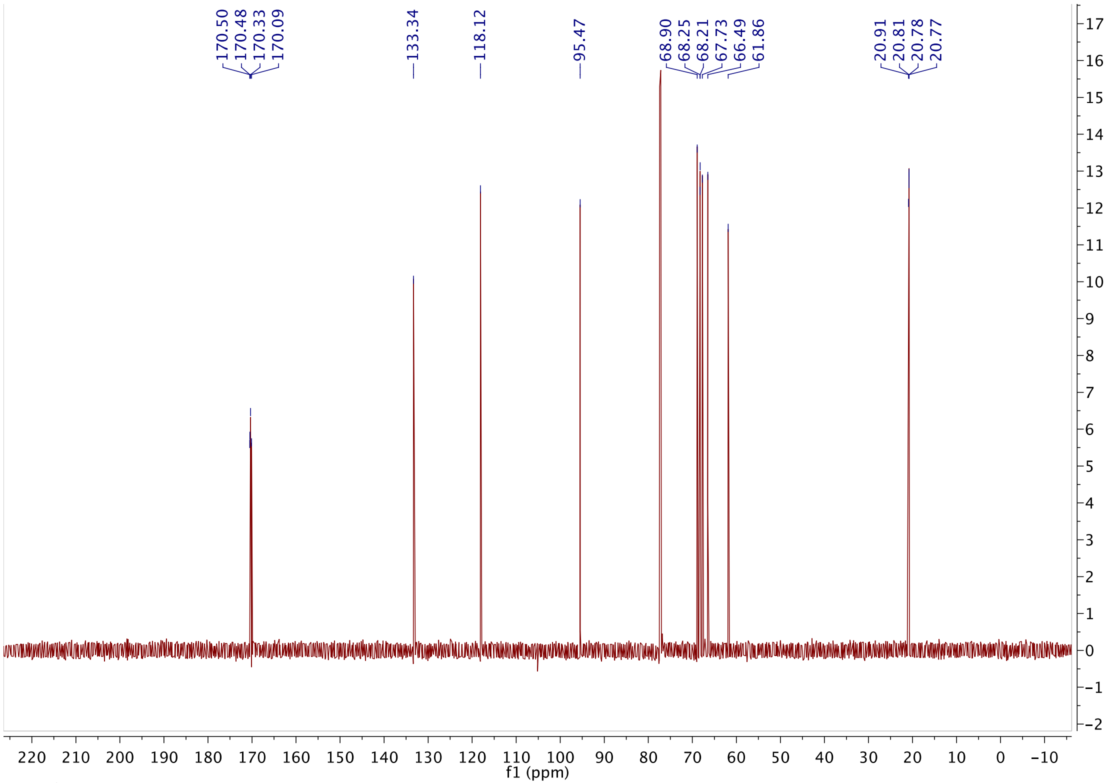
gCOSY NMR: 3-(Trimethylsilyl)propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4b**)



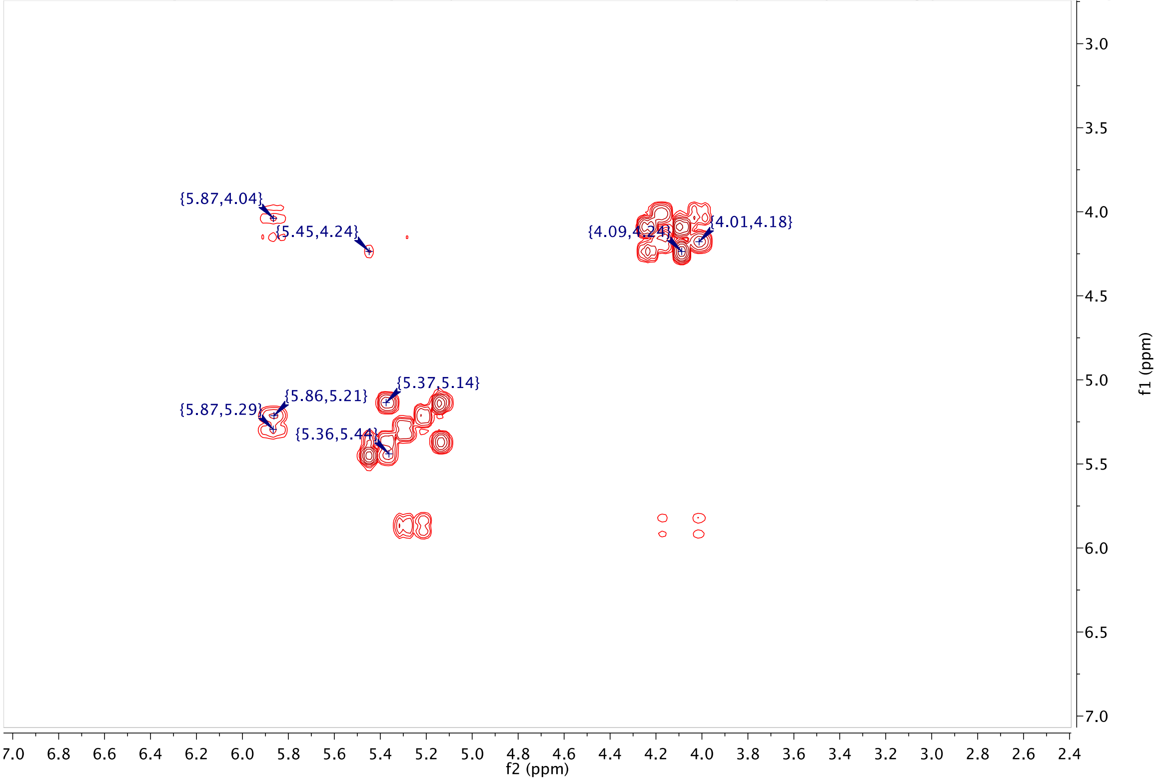
gHSQC NMR: 3-(Trimethylsilyl)propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4b**)



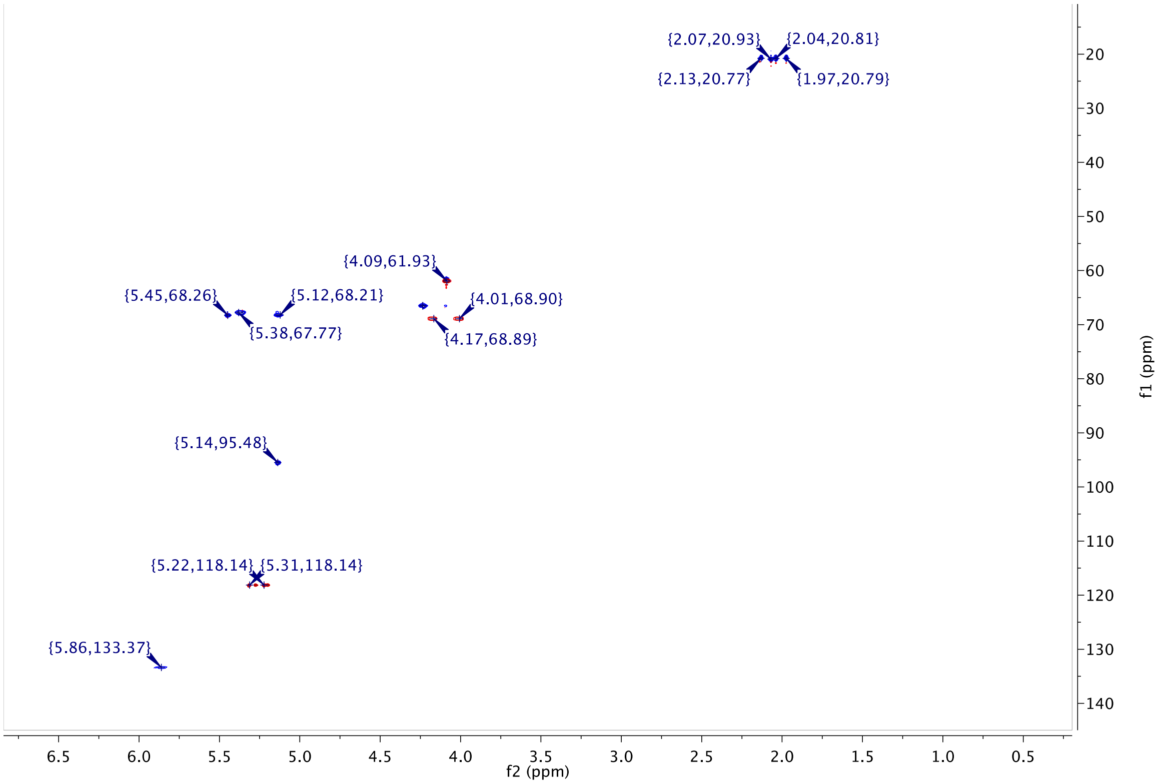
^{1}H NMR (500 MHz, $CDCl_3$): Allyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4c**)



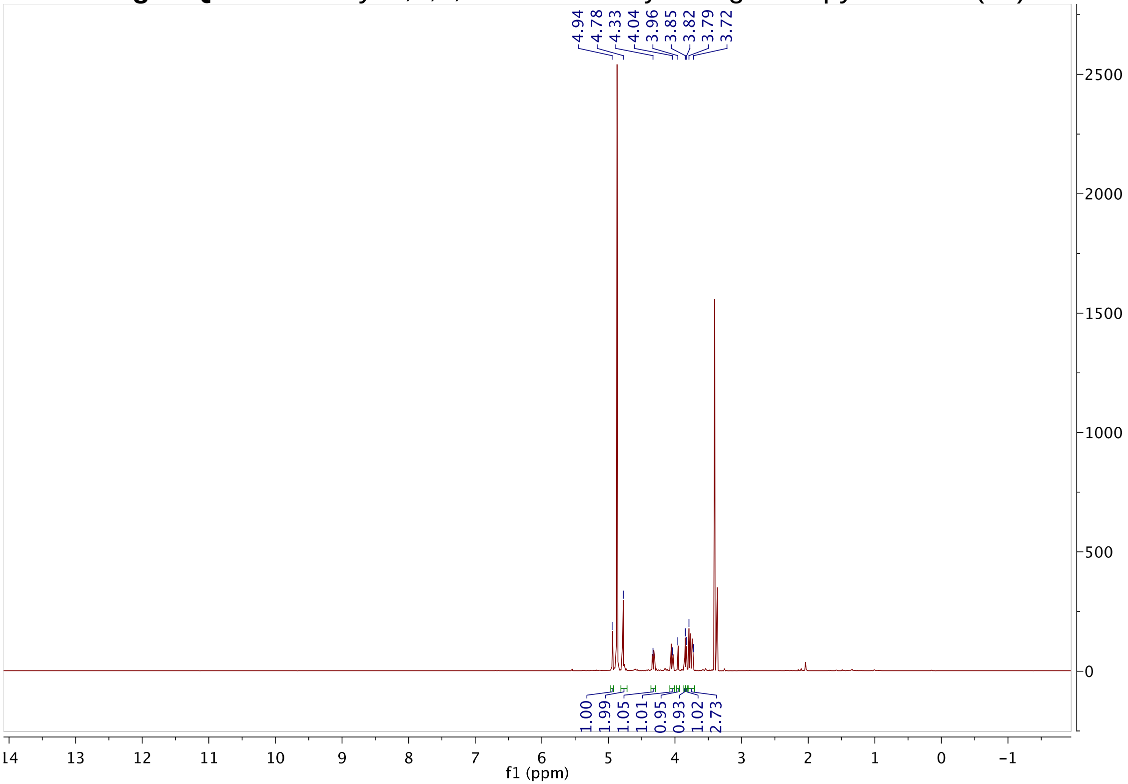
^{13}**C NMR** (125 MHz, CDCl$_3$): Allyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4c**)



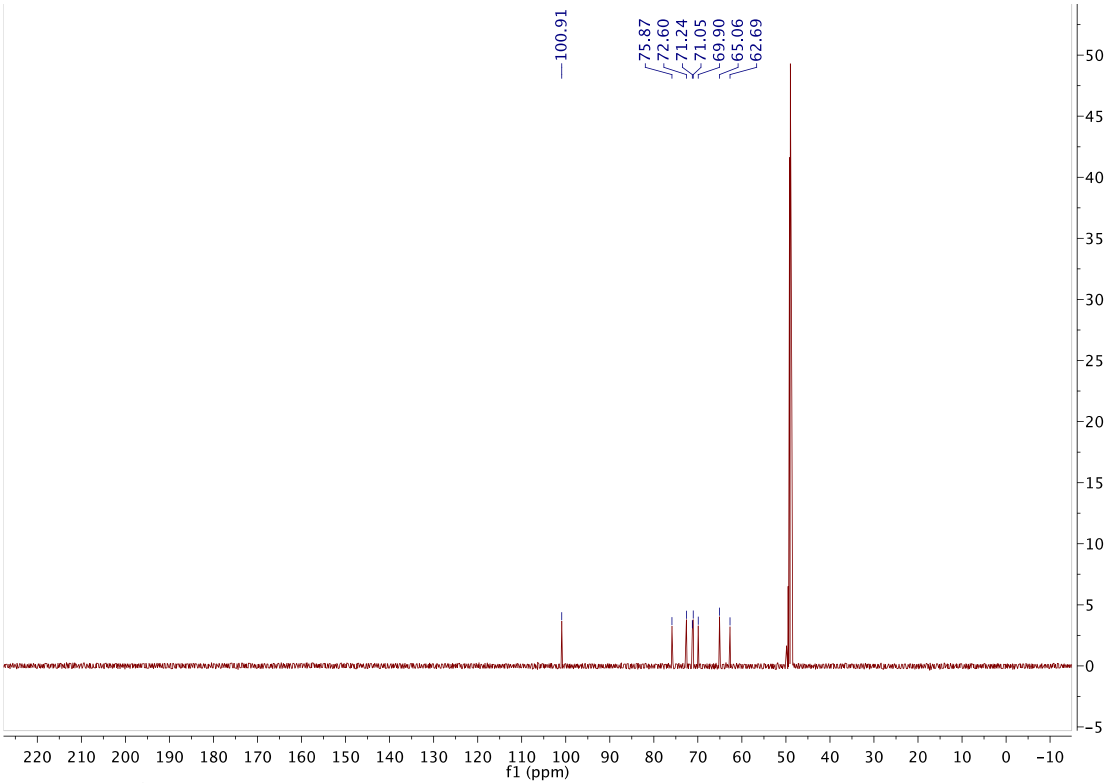
gCOSY NMR: Allyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4c**)



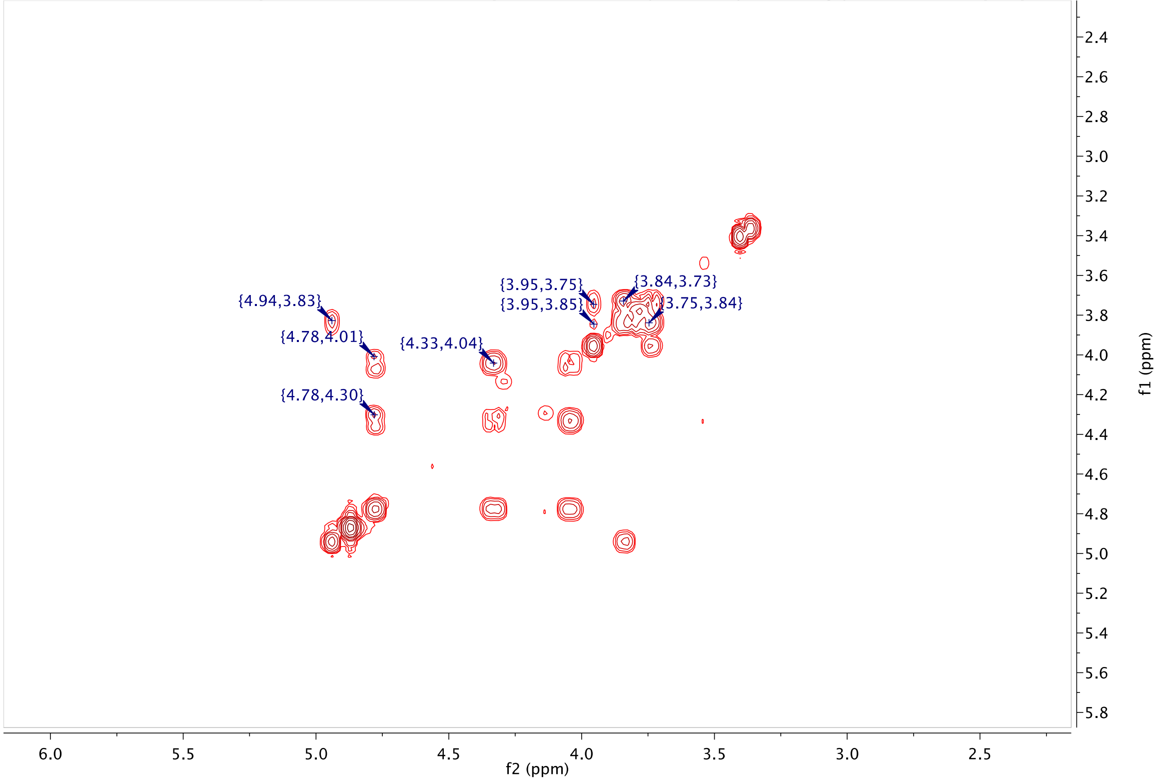
gHSQC NMR: Allyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4c**)



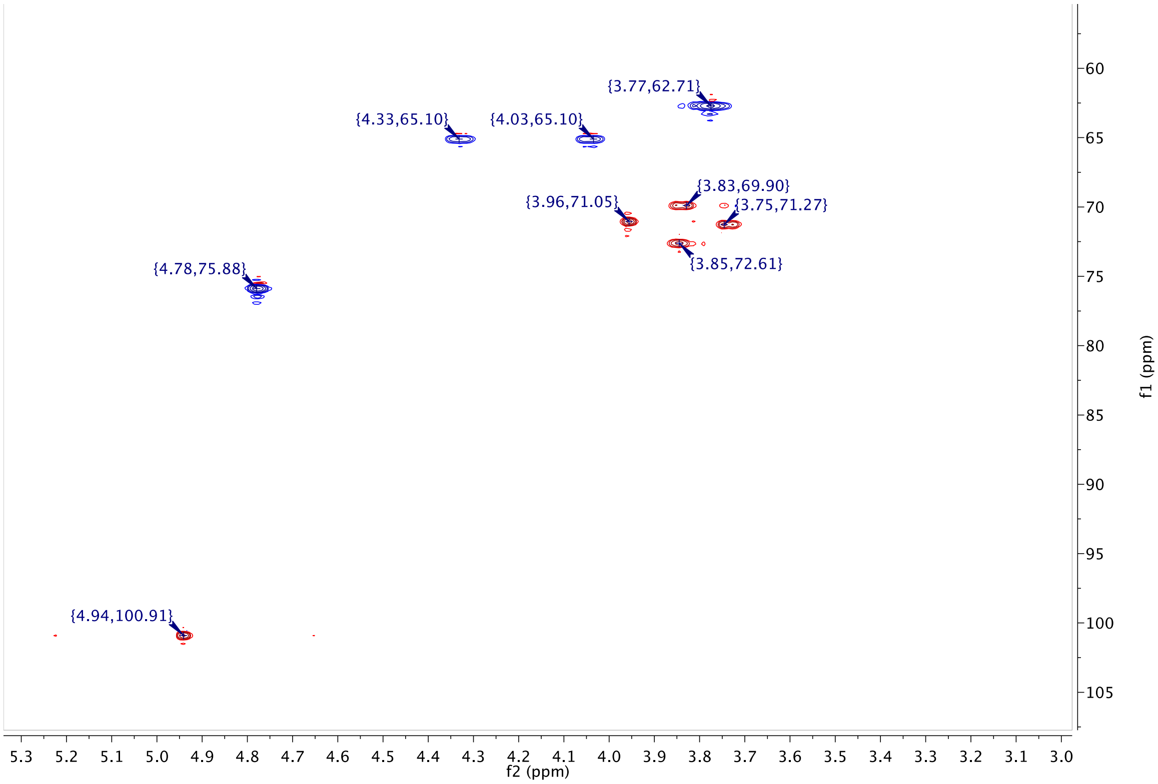
^{1}H NMR (500 MHz, CD_3OD): 2-Nitroethyl α-D-galactopyranoside (**4d**)



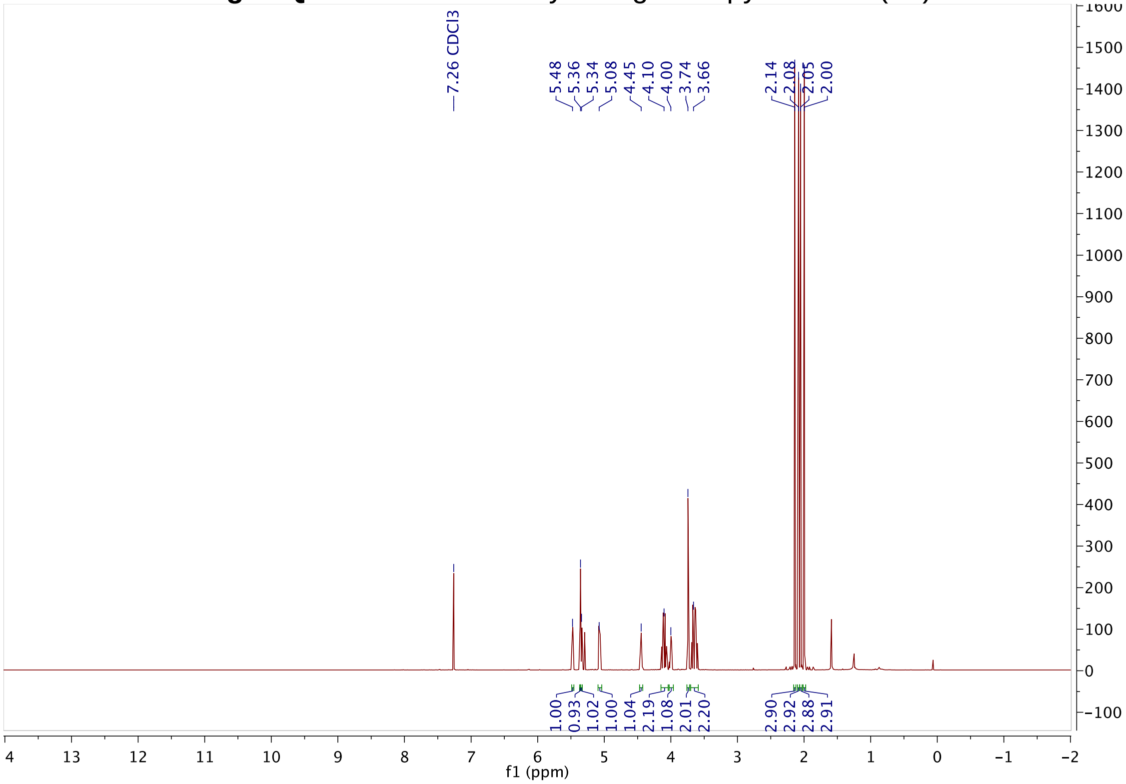
^{13}**C NMR** (125 MHz, CD_3OD): 2-Nitroethyl α-D-galactopyranoside (**4d**)



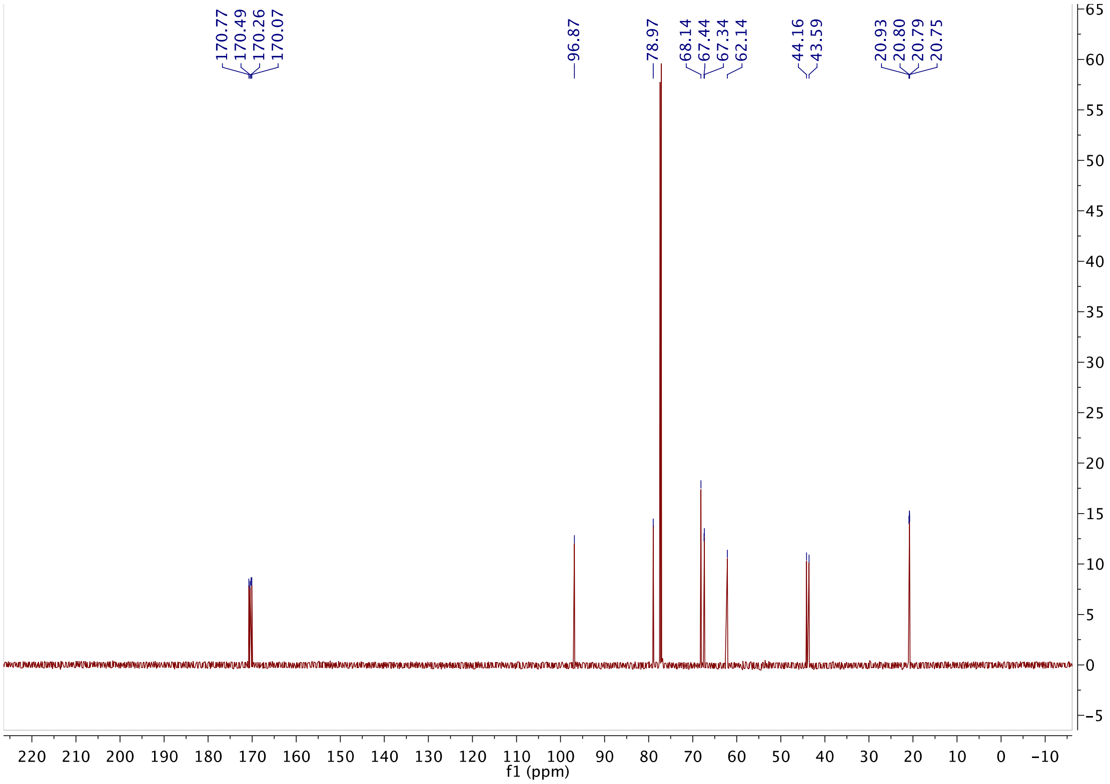
gCOSY NMR: 2-Nitroethyl α-D-galactopyranoside (**4d**)



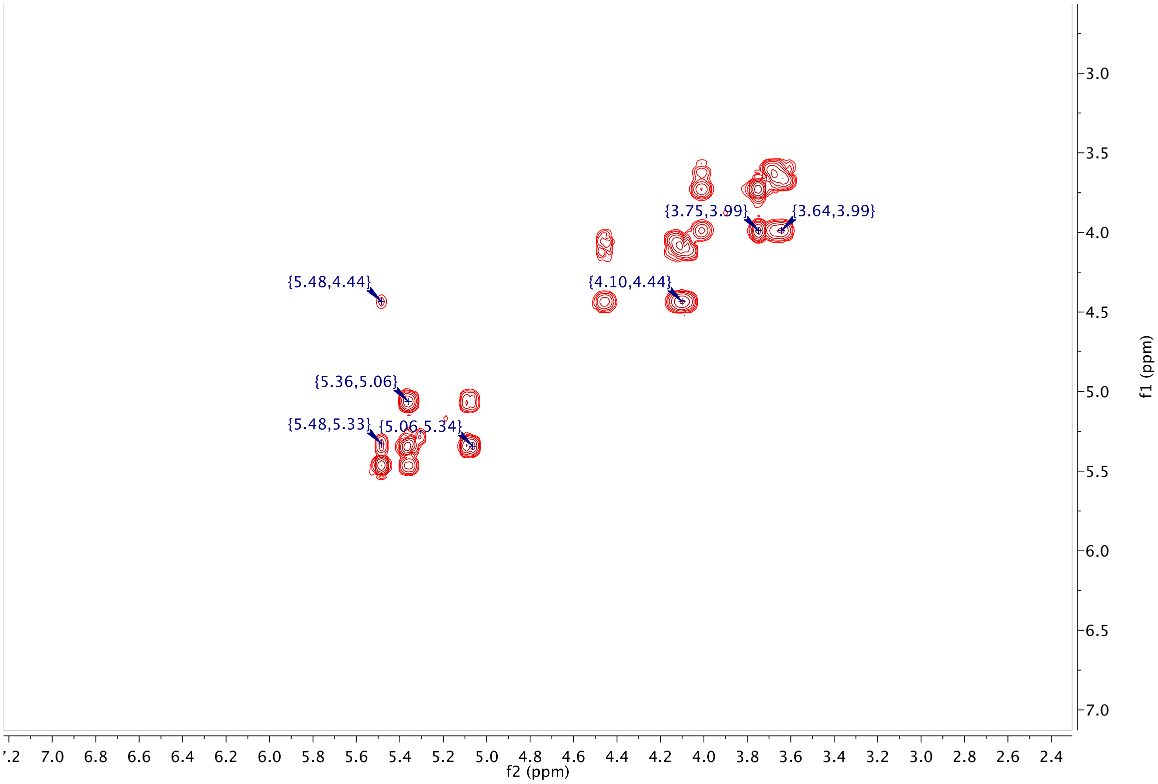
gHSQC NMR: 2-Nitroethyl α-D-galactopyranoside (**4d**)



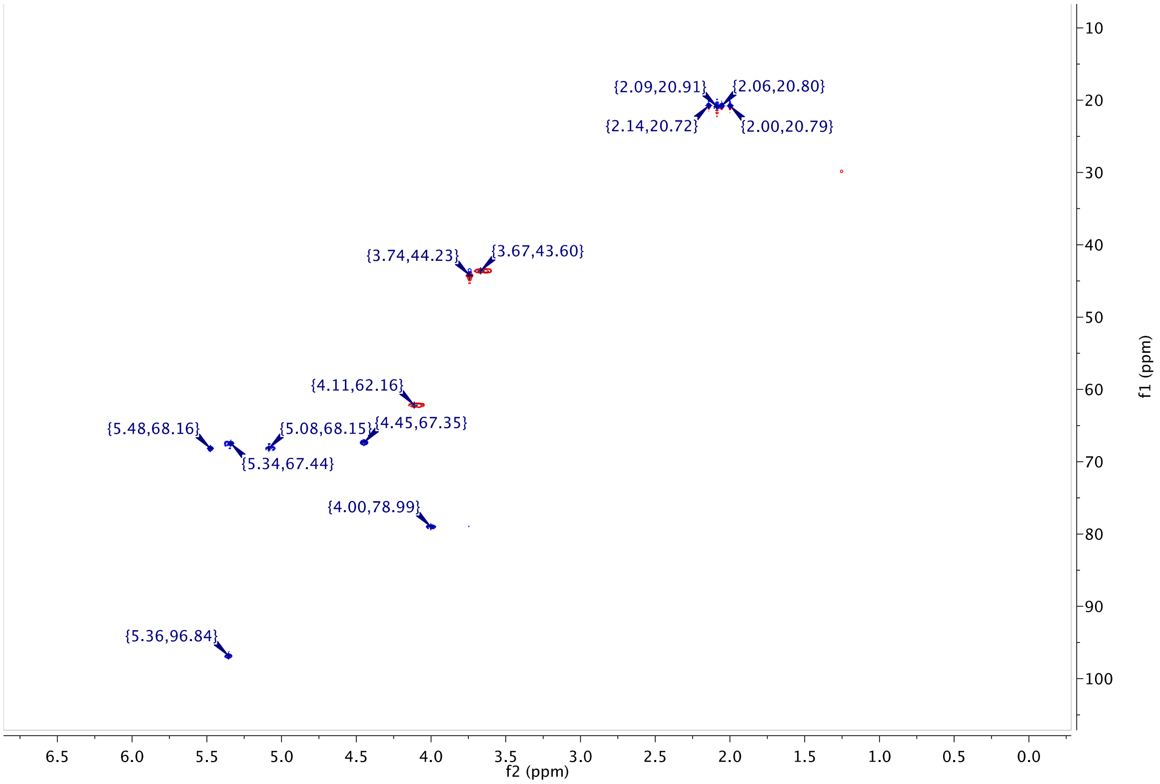
^{1}H NMR (500 MHz, $CDCl_3$): 1,3-Dichloro-2-propyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4e**)



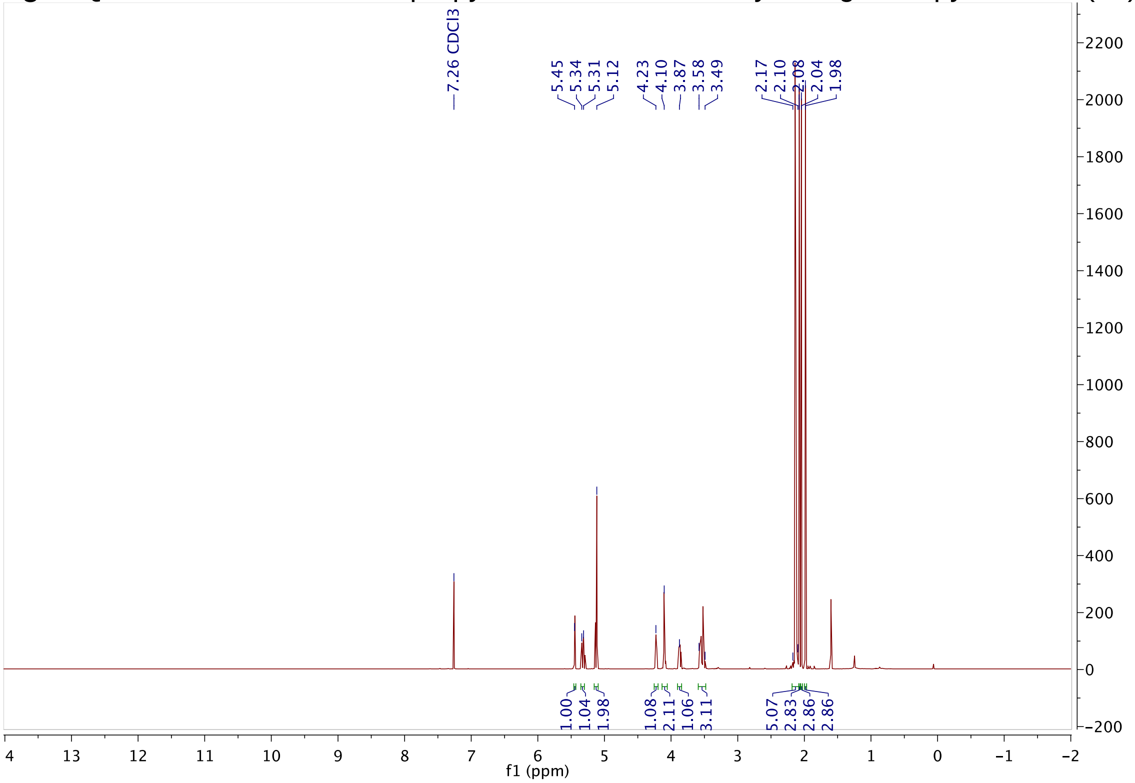
^{13}C NMR (125 MHz, CDCl$_3$): 1,3-Dichloro-2-propyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4e**)



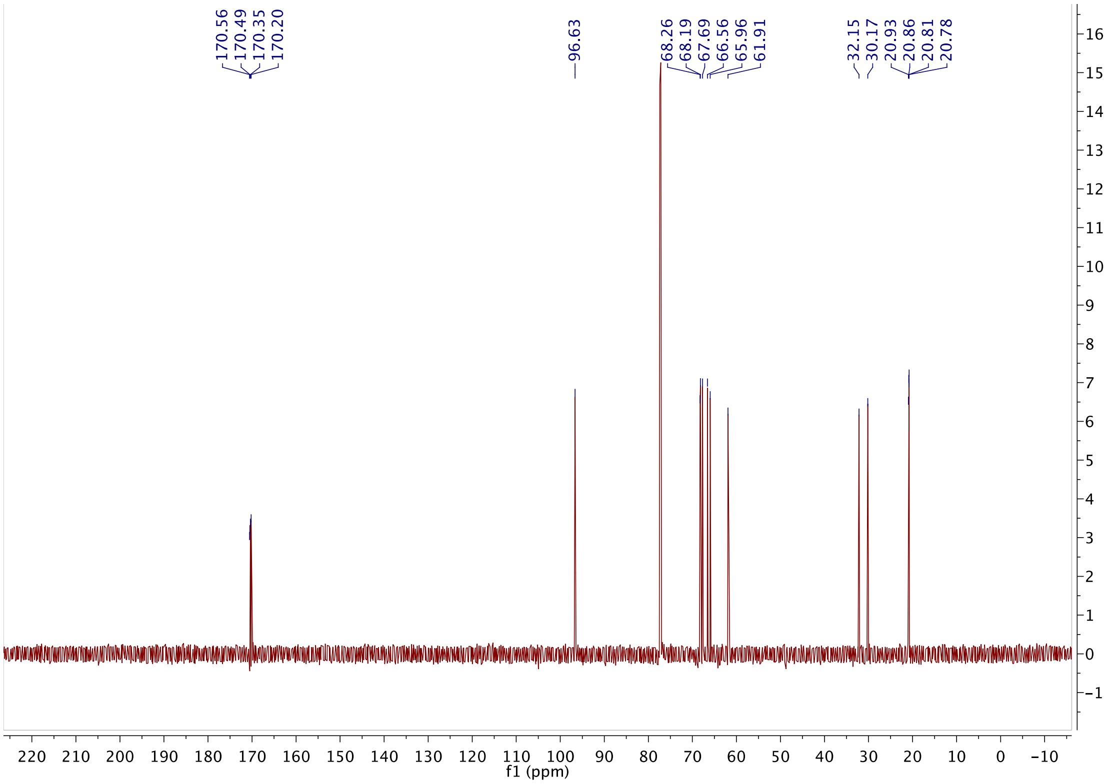
gCOSY NMR: 1,3-Dichloro-2-propyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4e**)



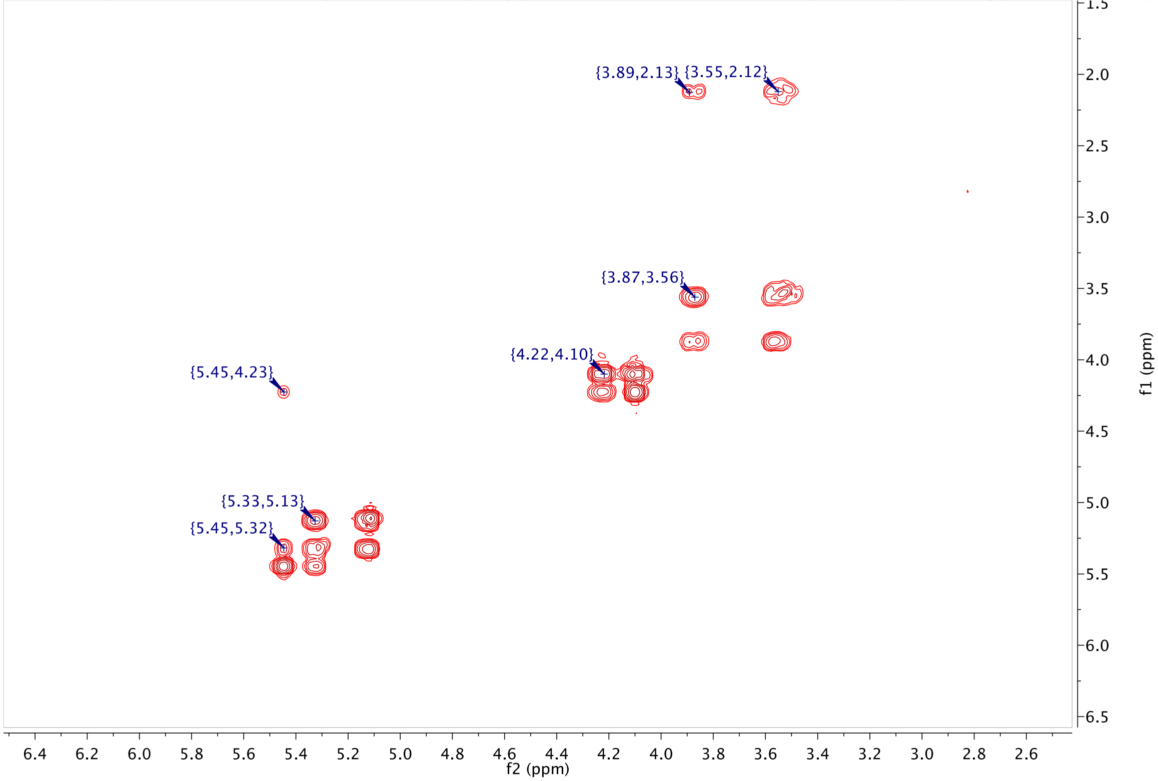
gHSQC NMR: 1,3-Dichloro-2-propyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4e**)



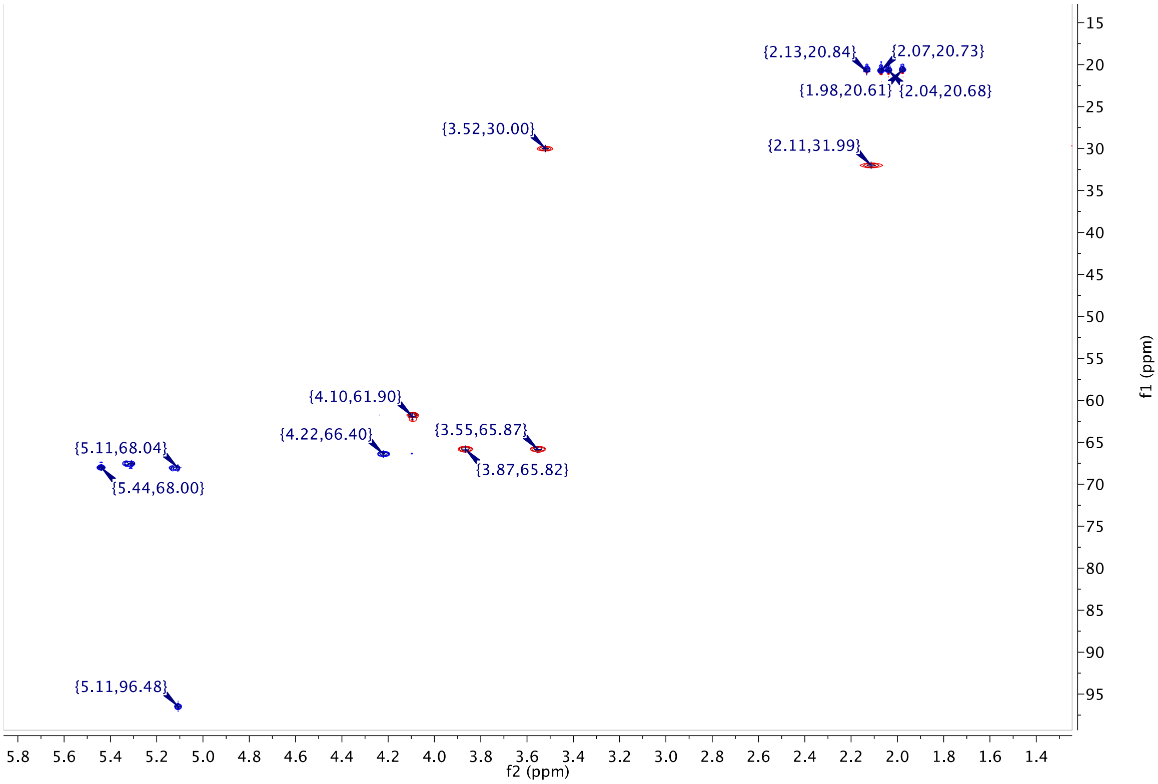
^{1}H NMR (500 MHz, $CDCl_3$): 3-Bromopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4f**)



^{13}**C NMR** (125 MHz, CDCl$_3$): 3-Bromopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4f**)



gCOSY NMR: 3-Bromopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4f**)



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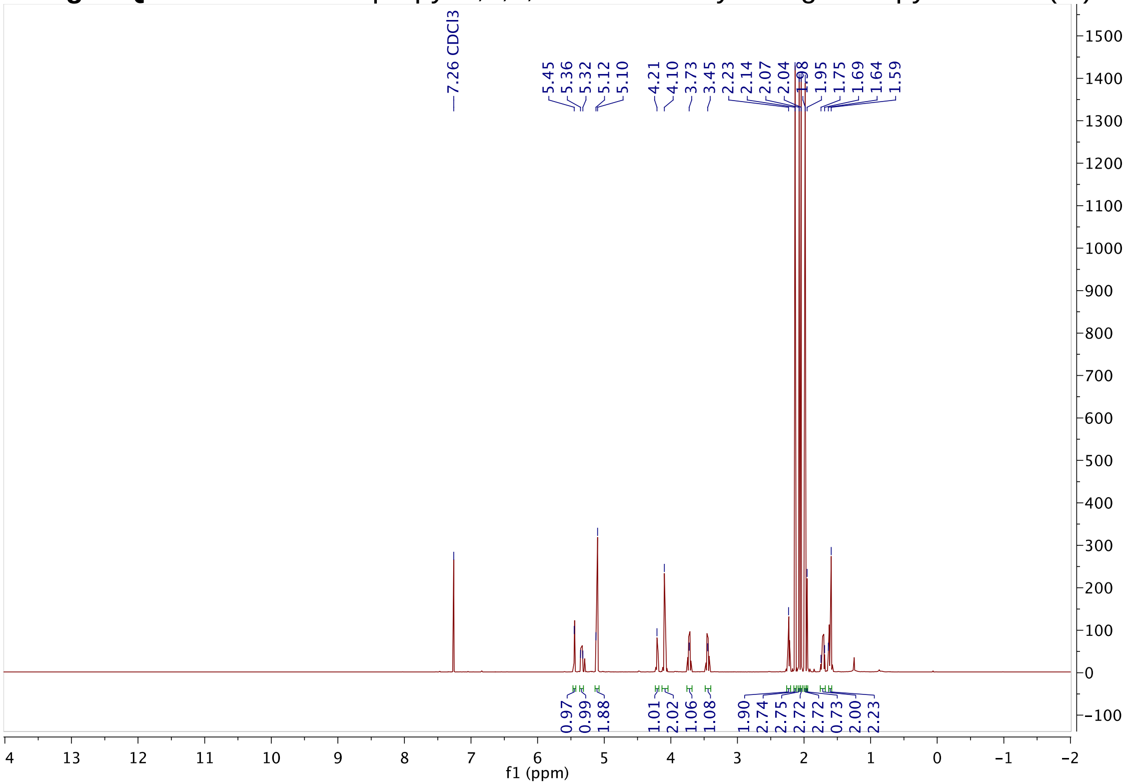
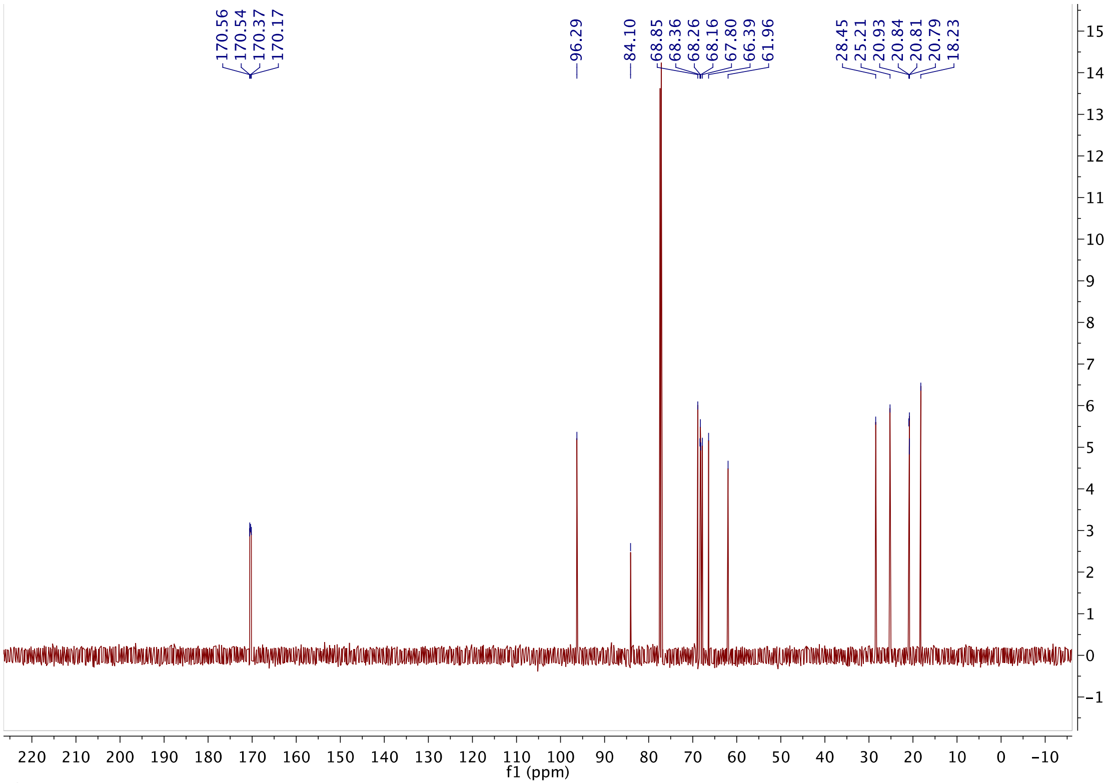
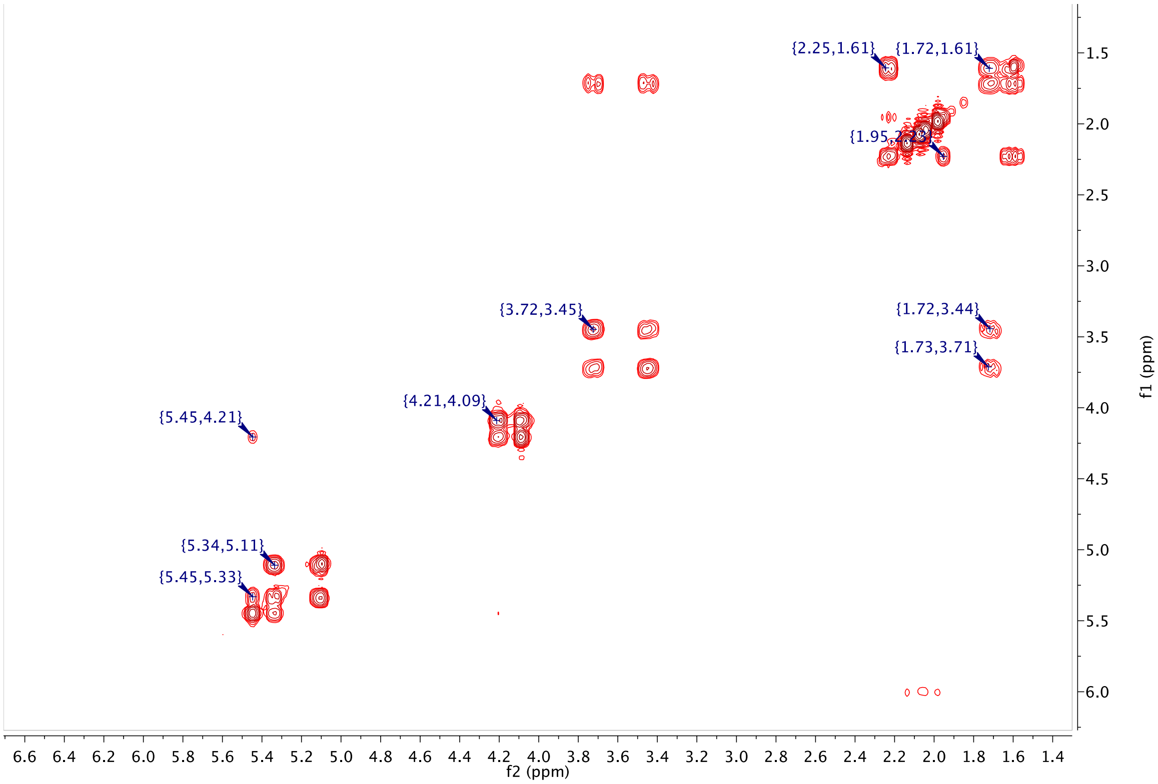


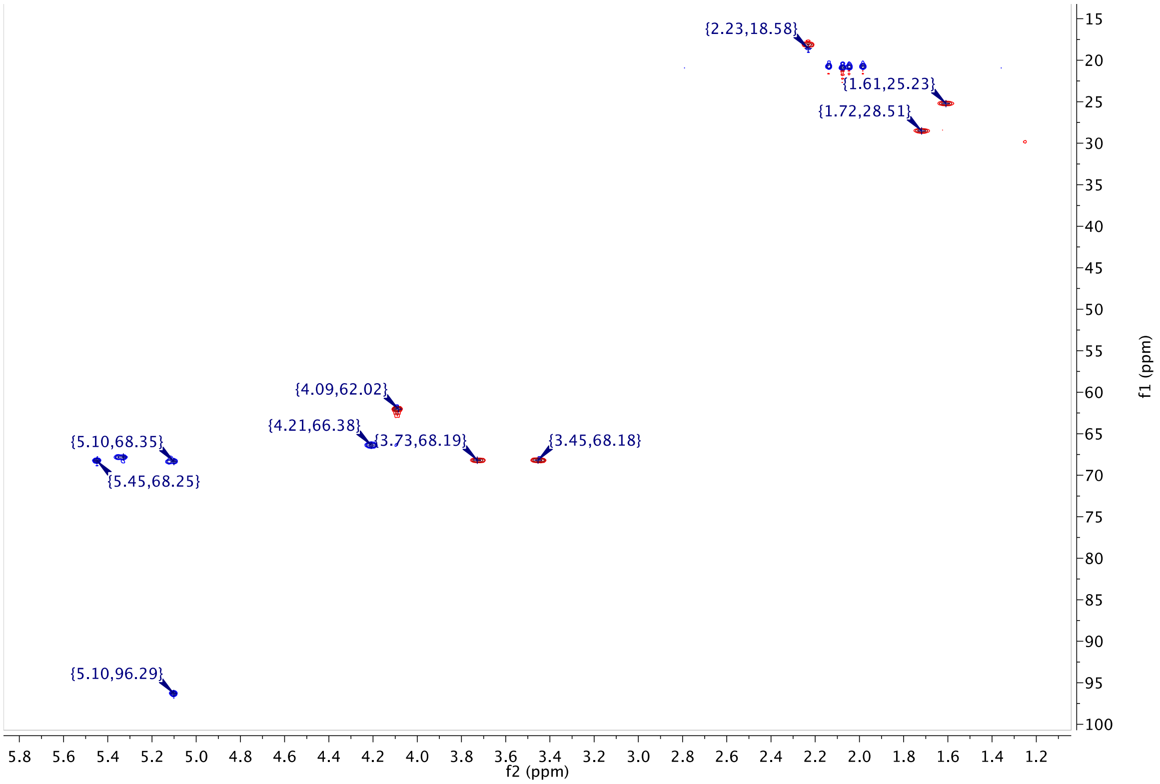
^{1}H NMR (500 MHz, CDCl$_3$): 5-Hexynyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4g**)



^{13}**C NMR** (125 MHz, $CDCl_3$): 5-Hexynyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4g**)



gCOSY NMR: 5-Hexynyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4g**)



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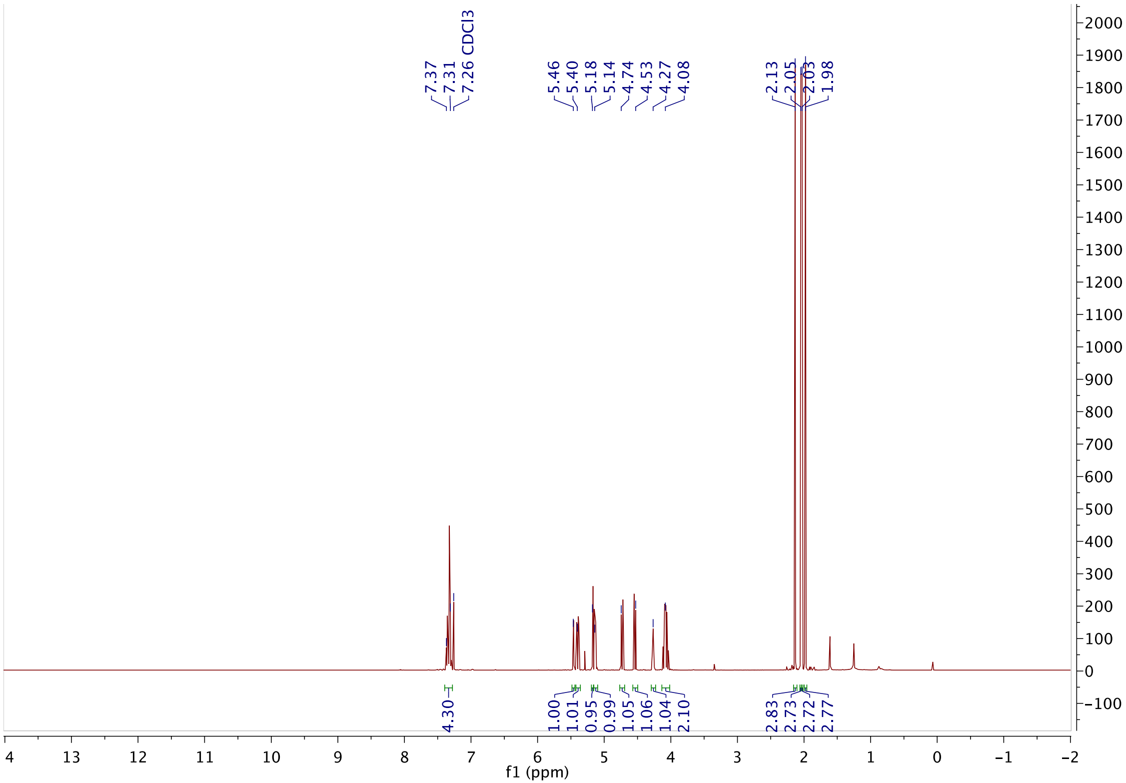
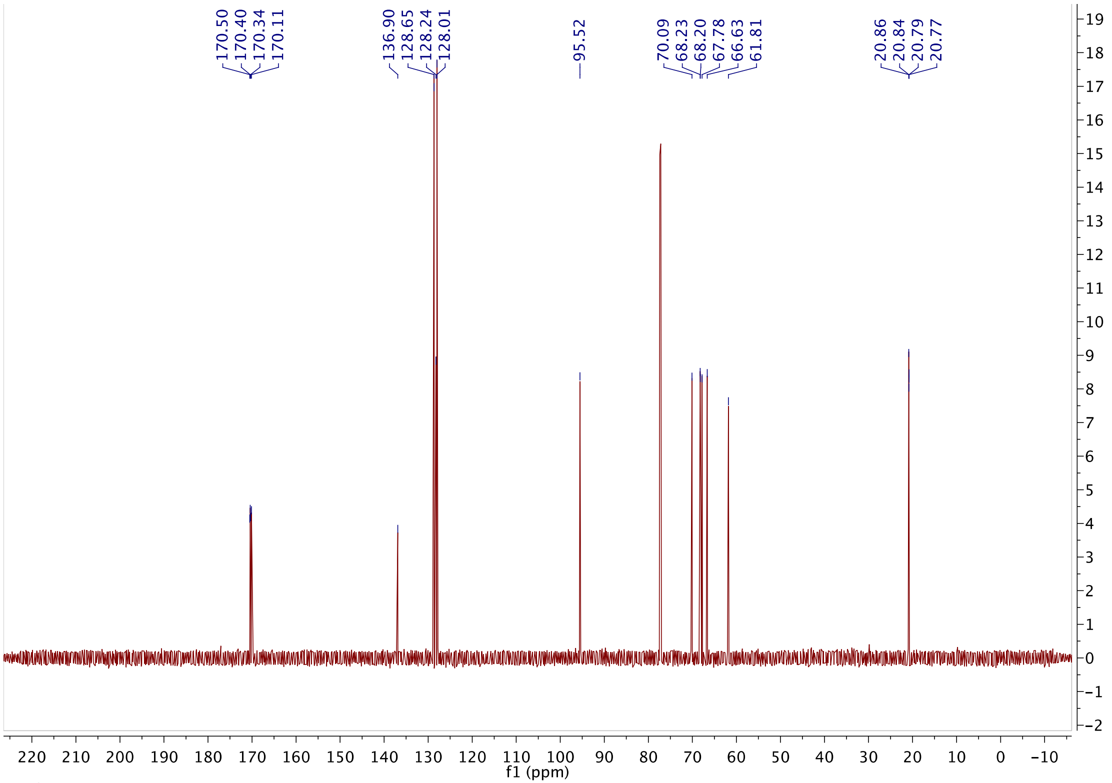
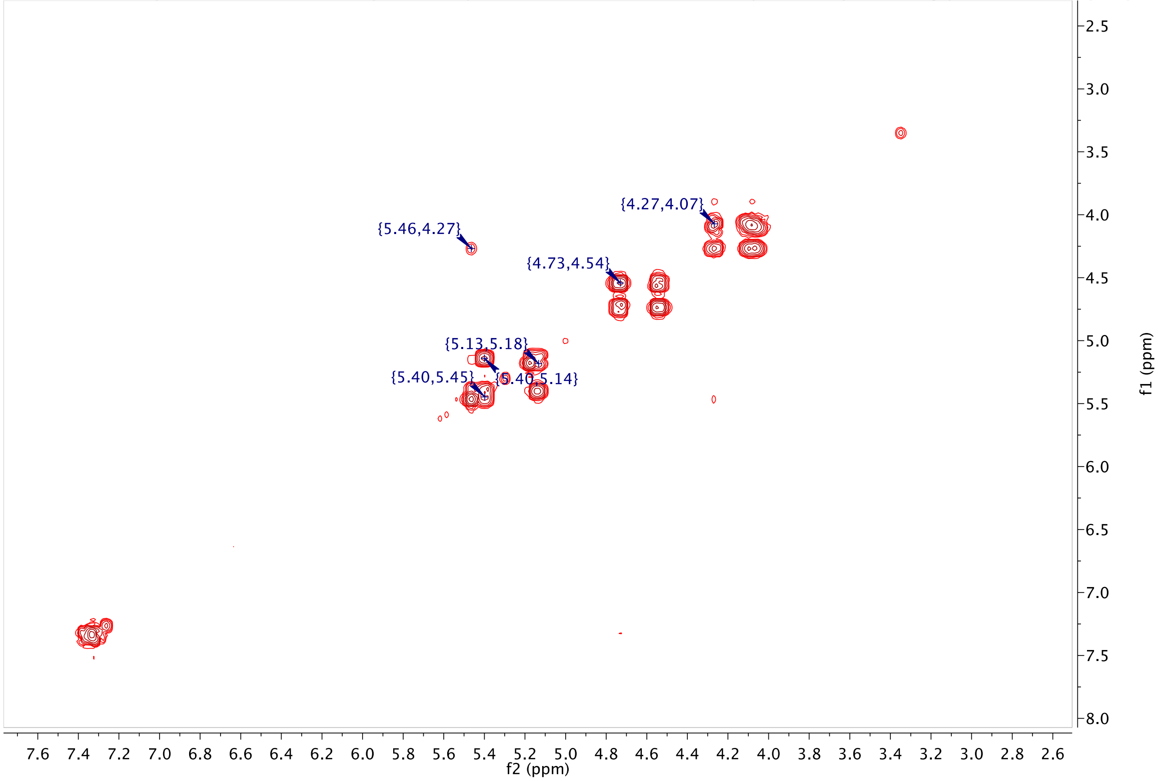


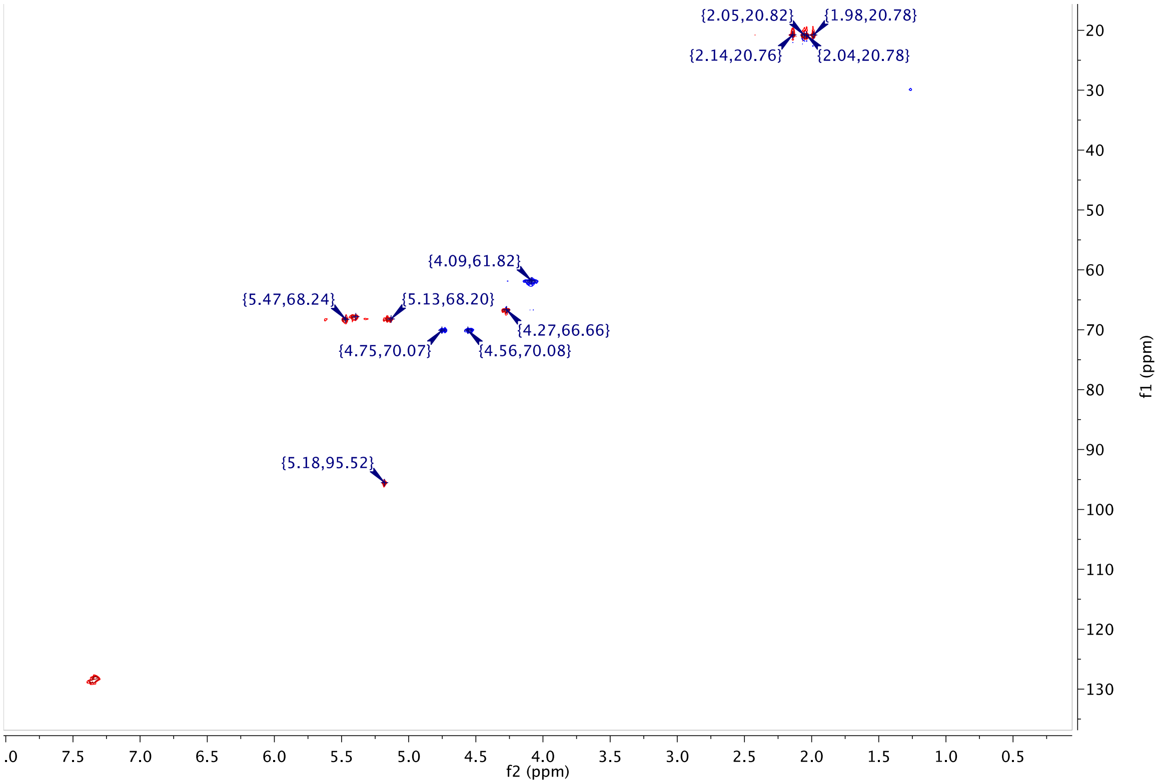
^{1}H NMR (500 MHz, $CDCl_3$): Benzyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4h**)



^{13}C NMR (125 MHz, $CDCl_3$): Benzyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4h**)



gCOSY NMR: Benzyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4h**)



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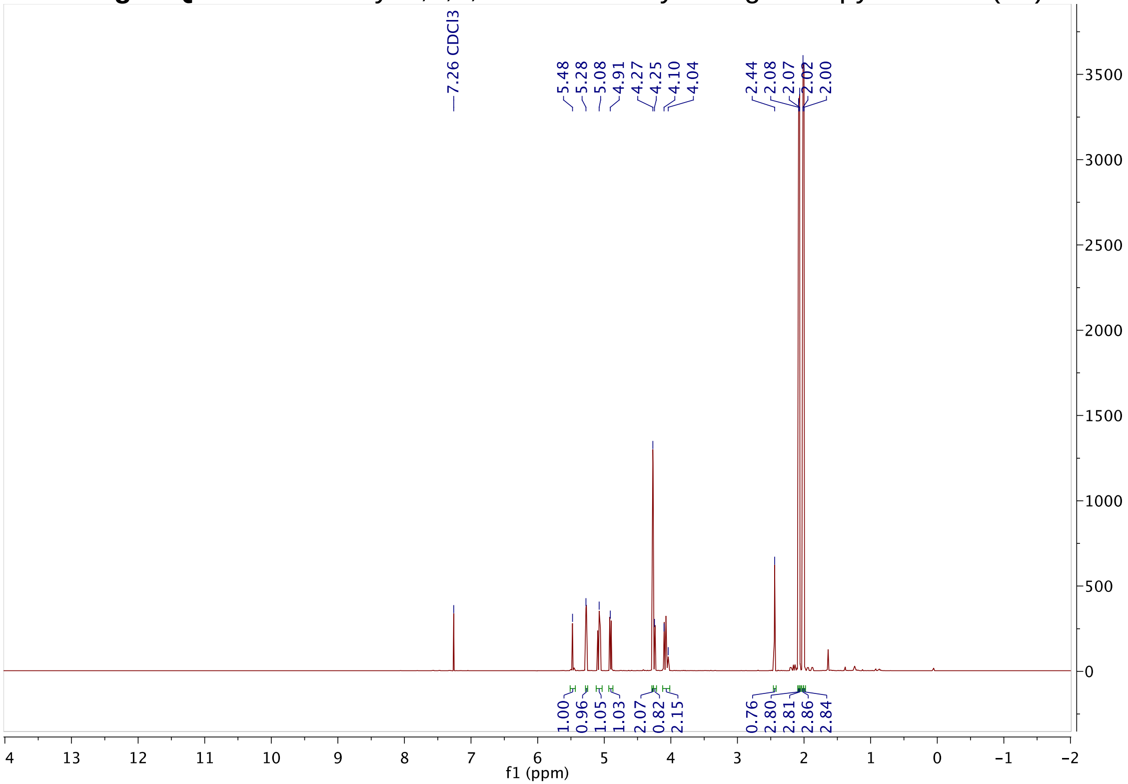
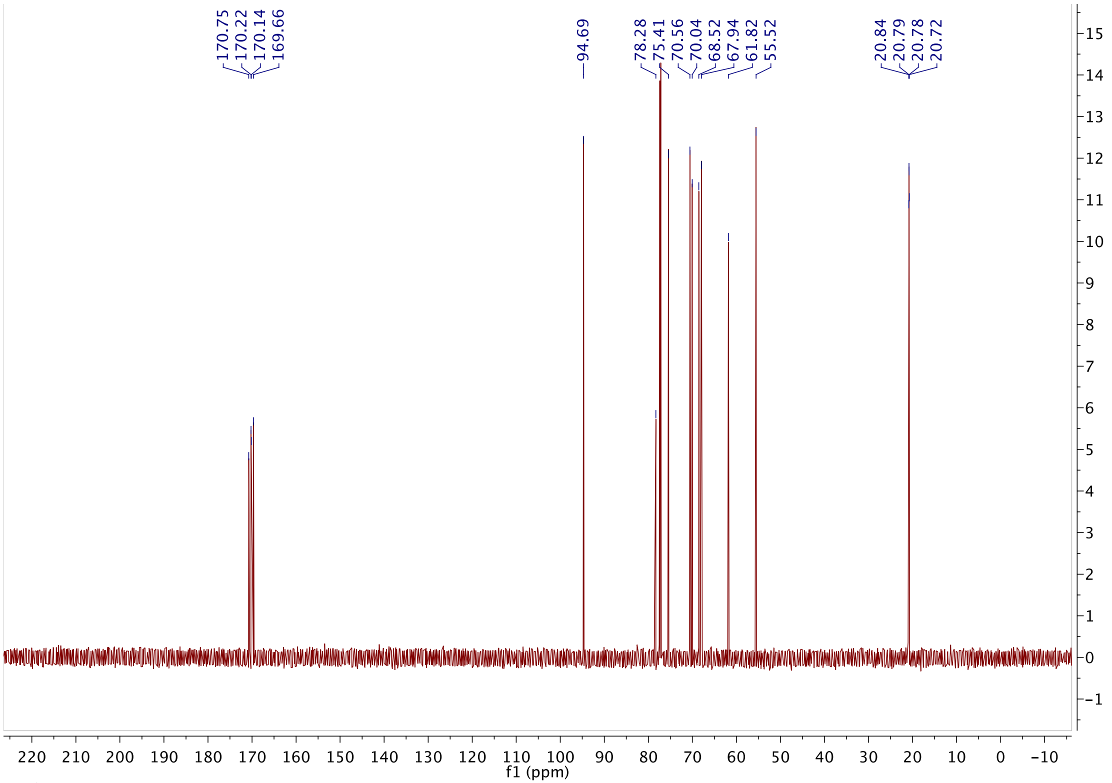
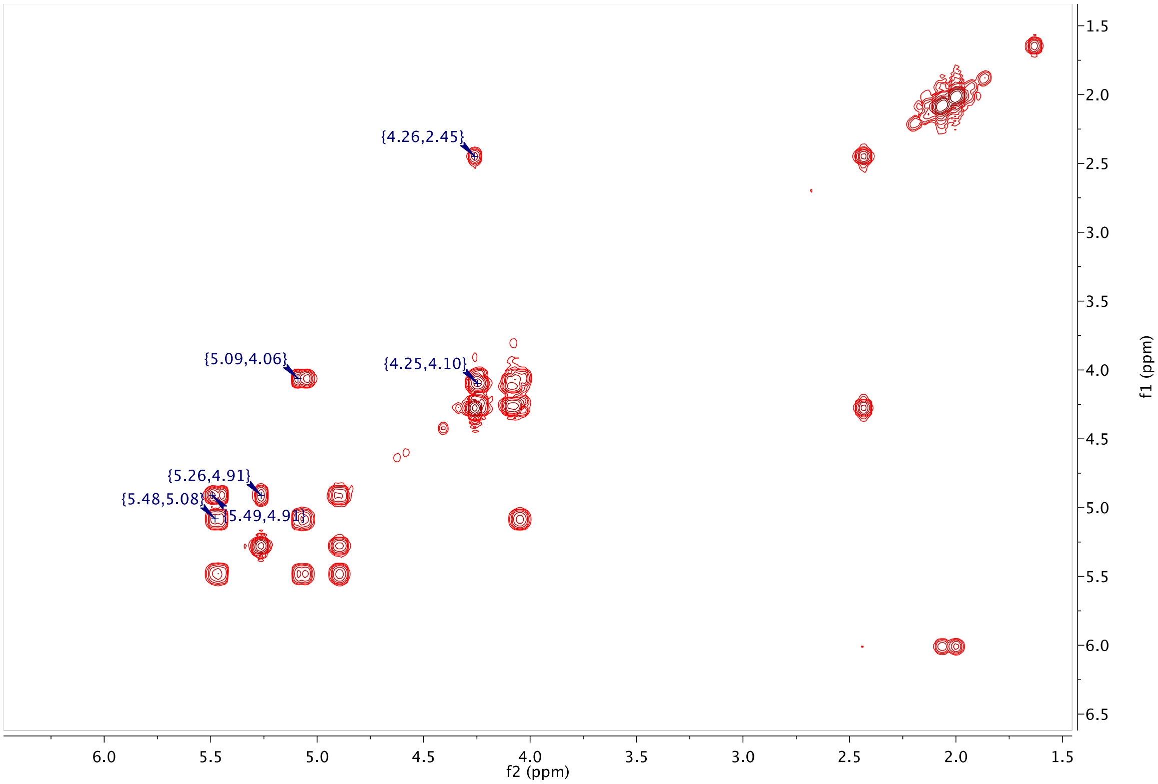


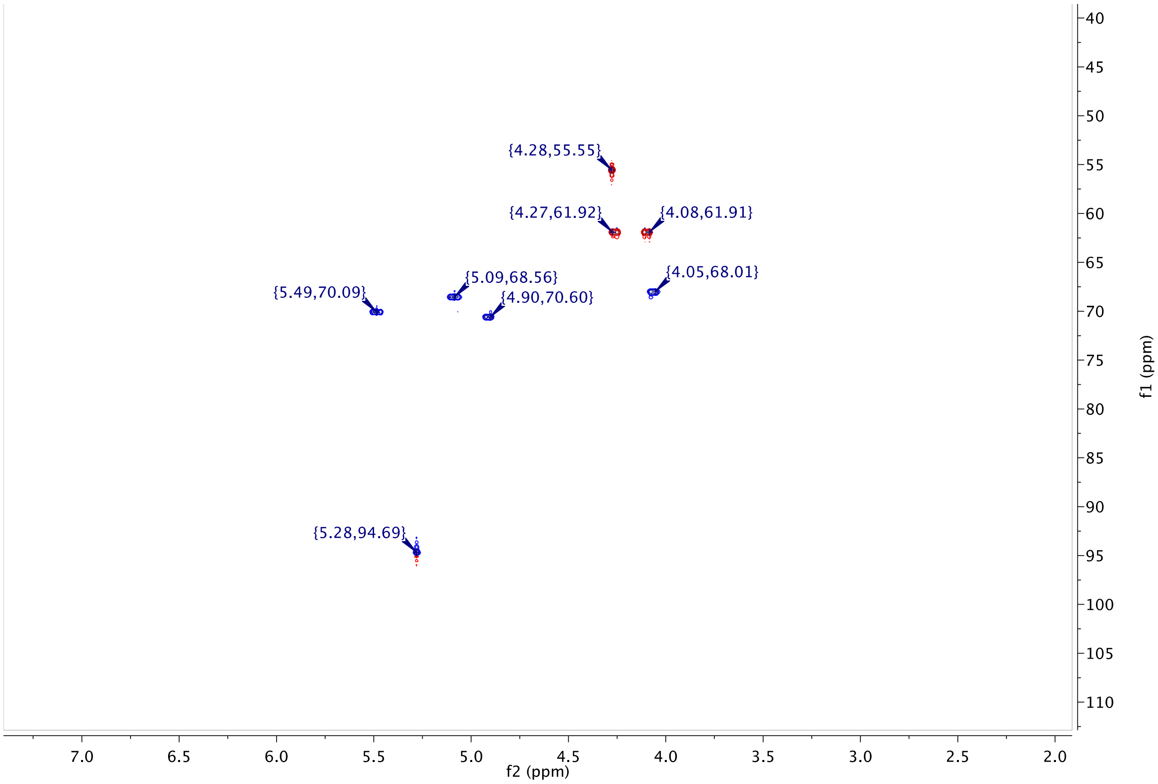
^{1}**H NMR** (500 MHz, $CDCl_3$): Propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**4i**)



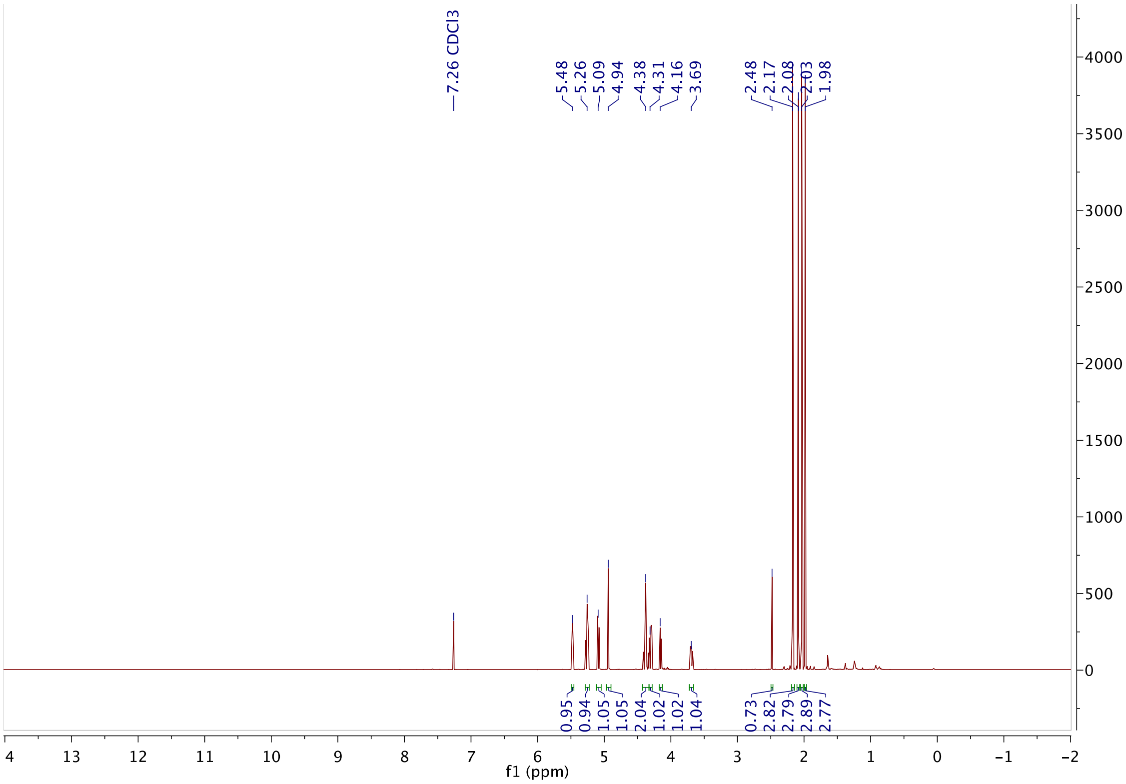
^{13}**C NMR** (125 MHz, $CDCl_3$): Propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**4i**)



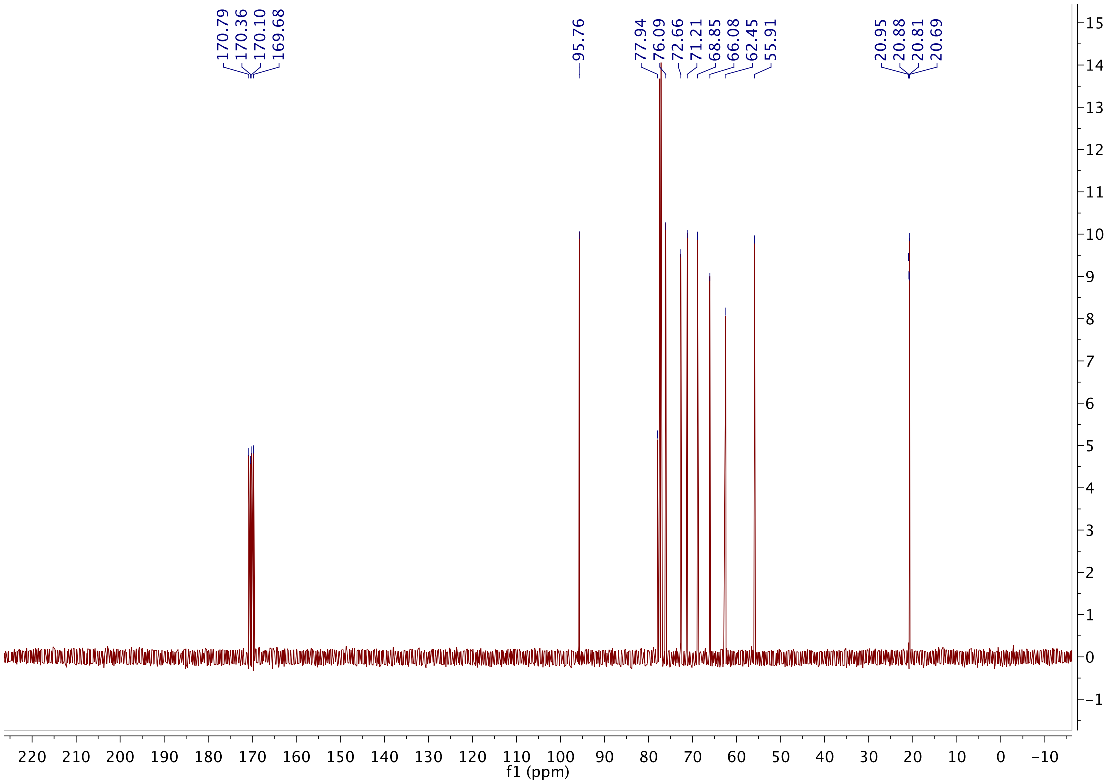
gCOSY NMR: Propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**4i**)



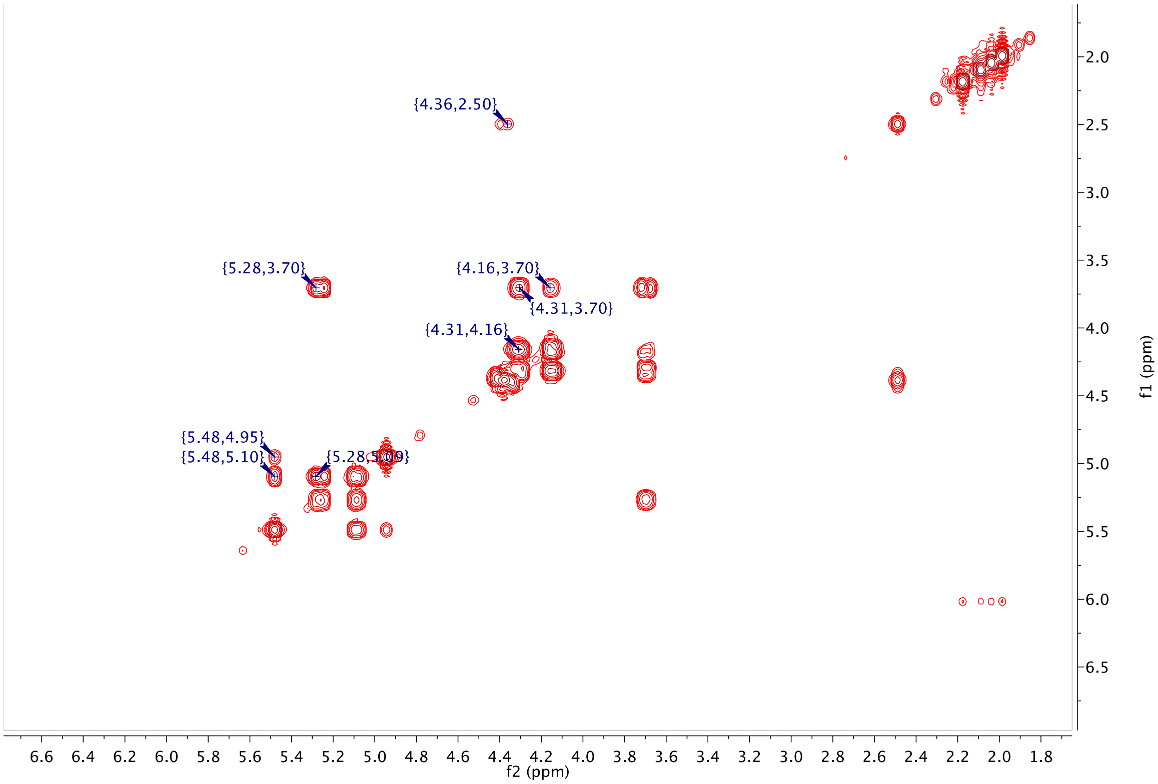
gHSQC NMR: Propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**4i**)



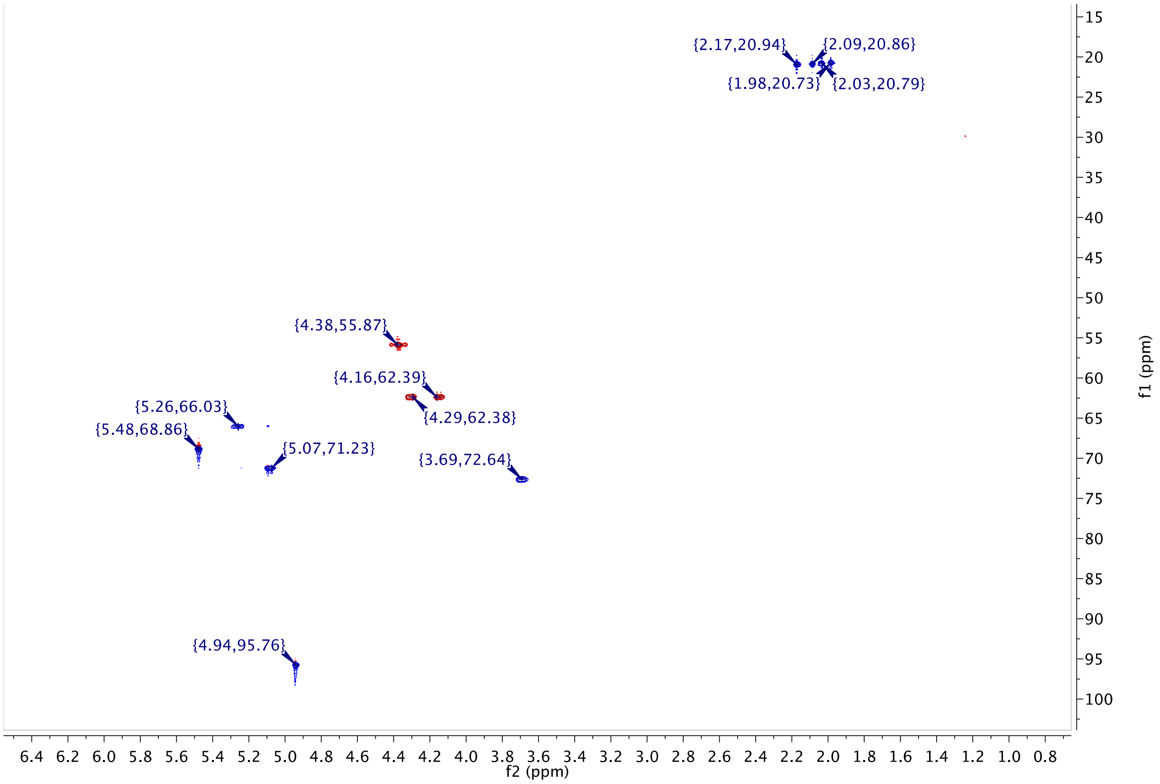
^{1}H NMR (500 MHz, $CDCl_3$): Propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside (**4j**)



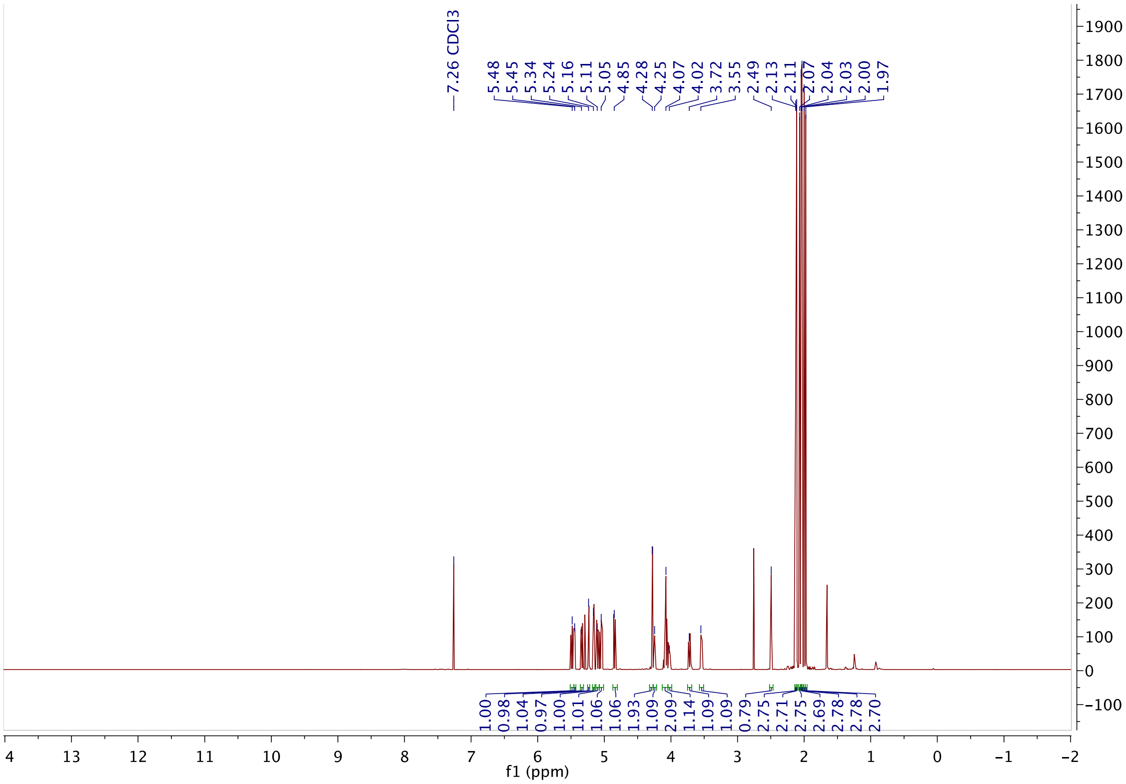
^{13}**C NMR** (125 MHz, $CDCl_3$): Propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside (**4j**)



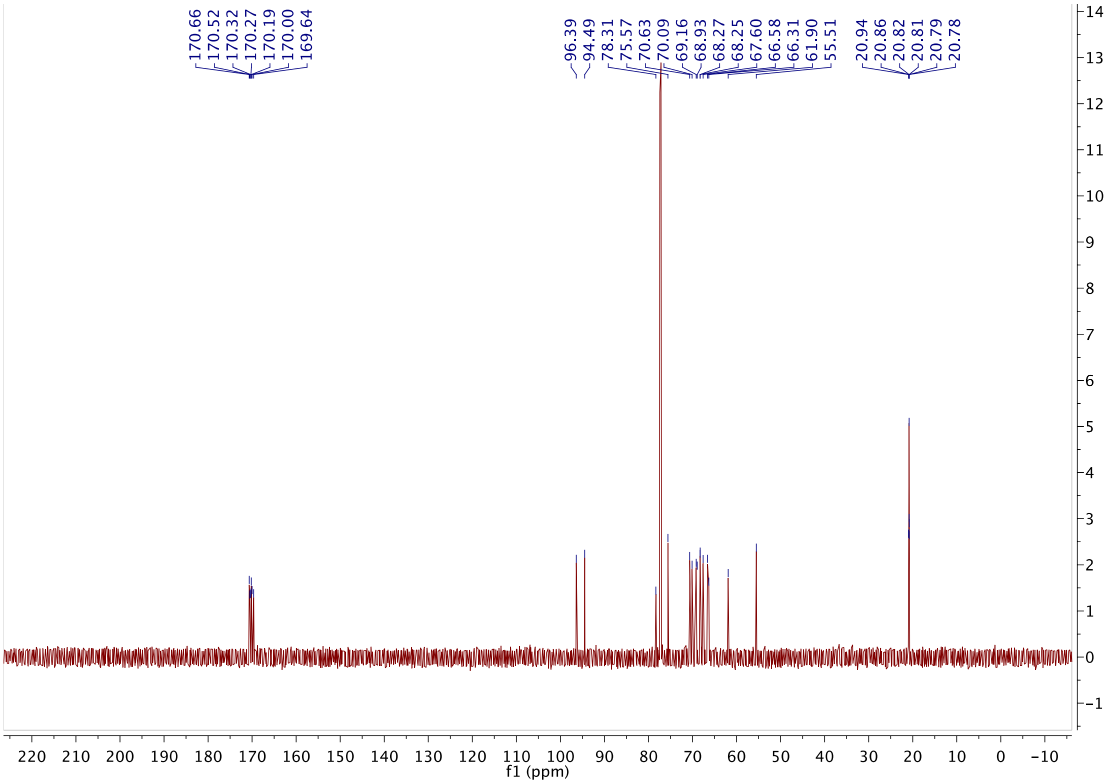
gCOSY NMR: Propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside (**4j**)



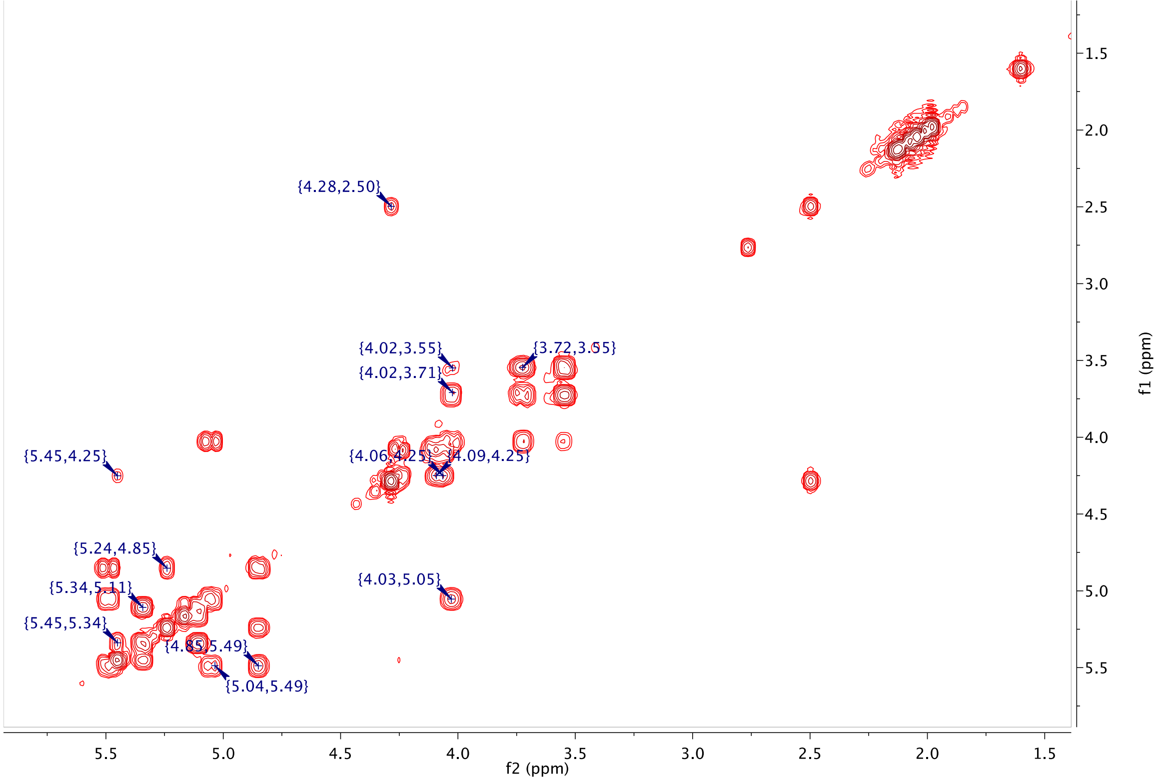
gHSQC NMR: Propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside (**4j**)



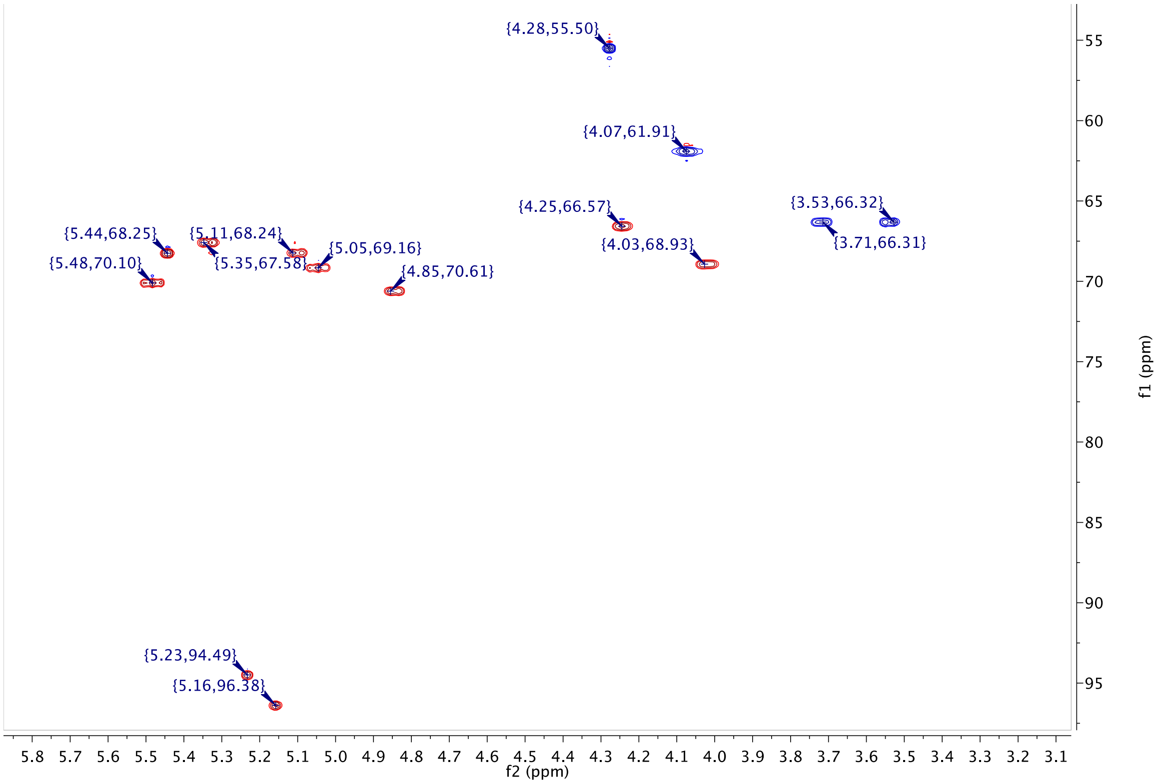
^{1}H NMR (500 MHz, $CDCl_3$): Propargyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside **(4k)**



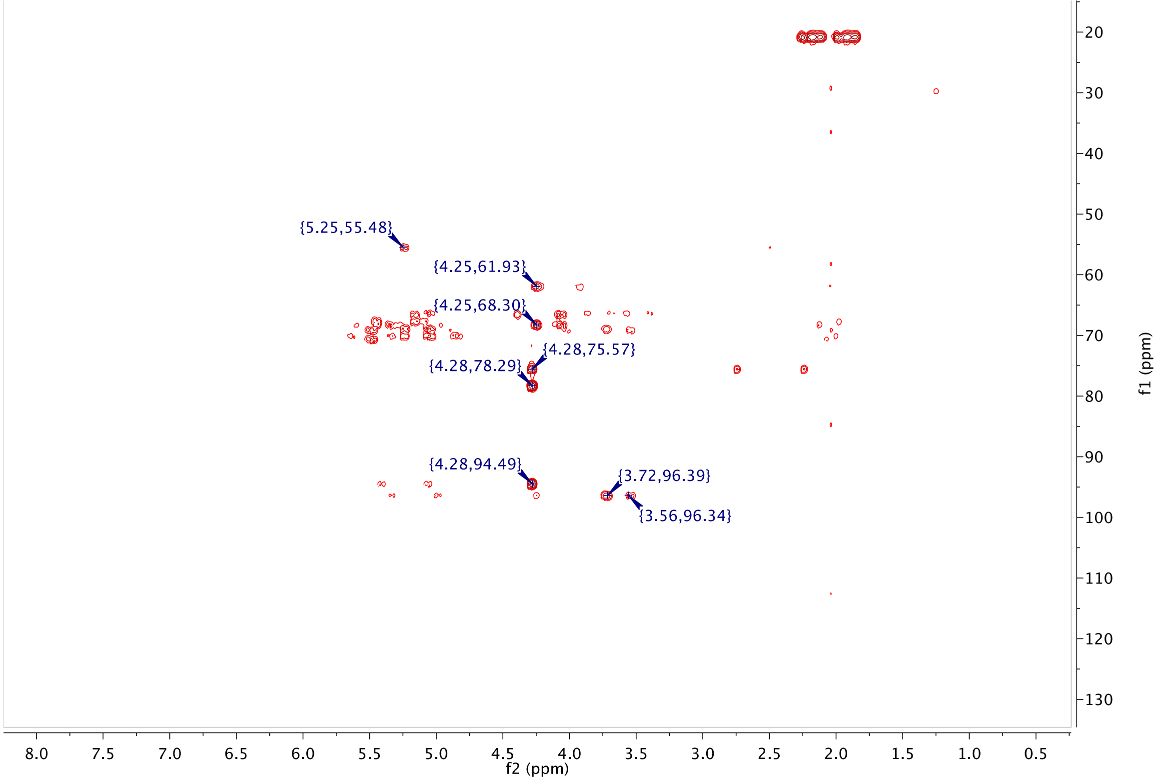
^{13}C NMR (125 MHz, $CDCl_3$): Propargyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside **(4k)**



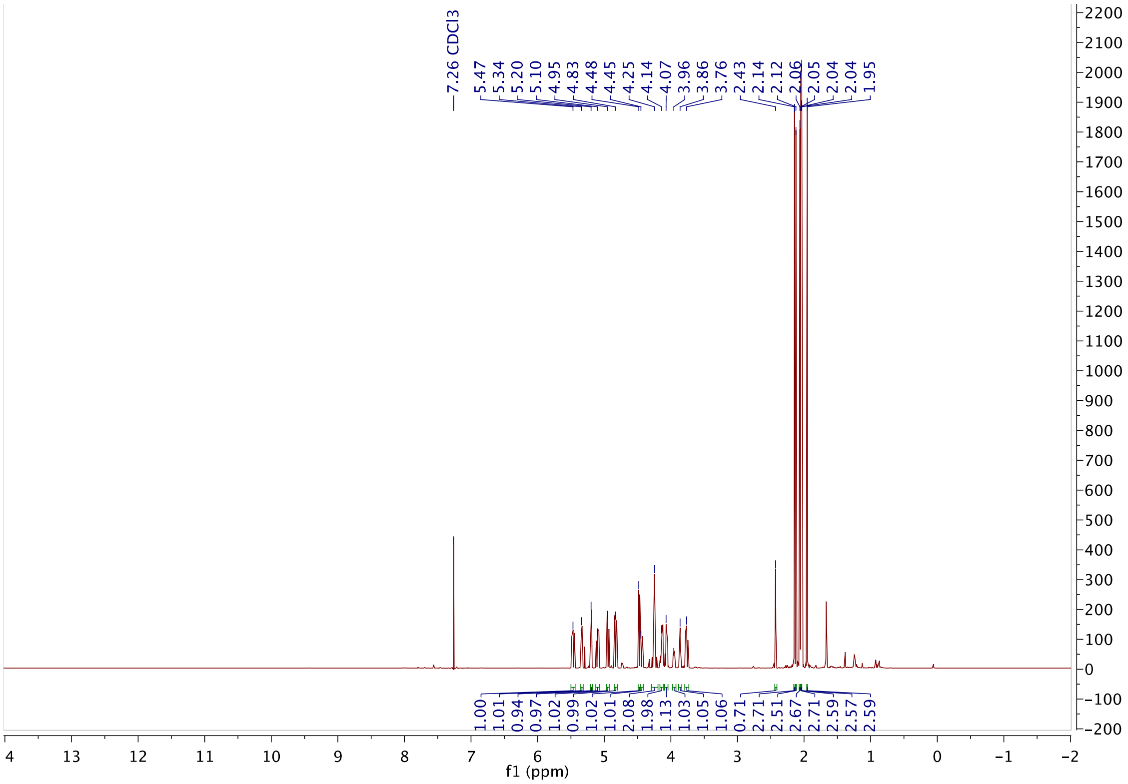
gCOSY NMR: Propargyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside **(4k)**



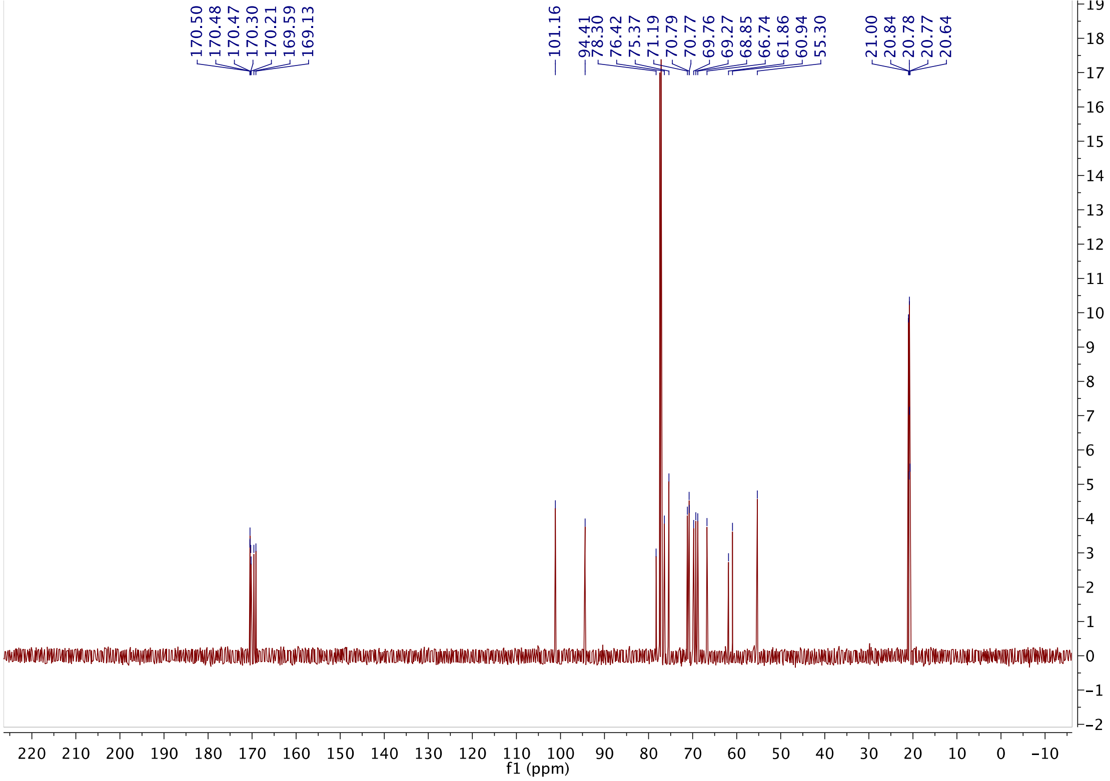
gHSQC NMR: Propargyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside **(4k)**



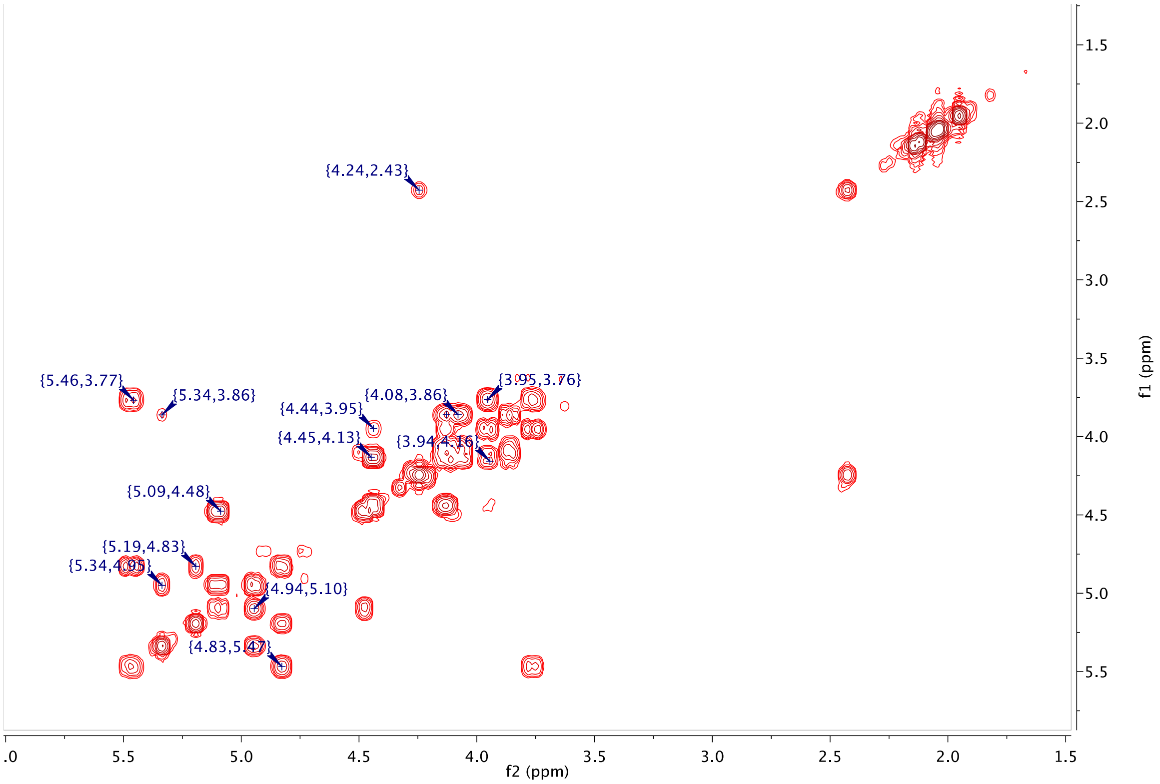
gHMBC NMR: Propargyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside **(4k)**



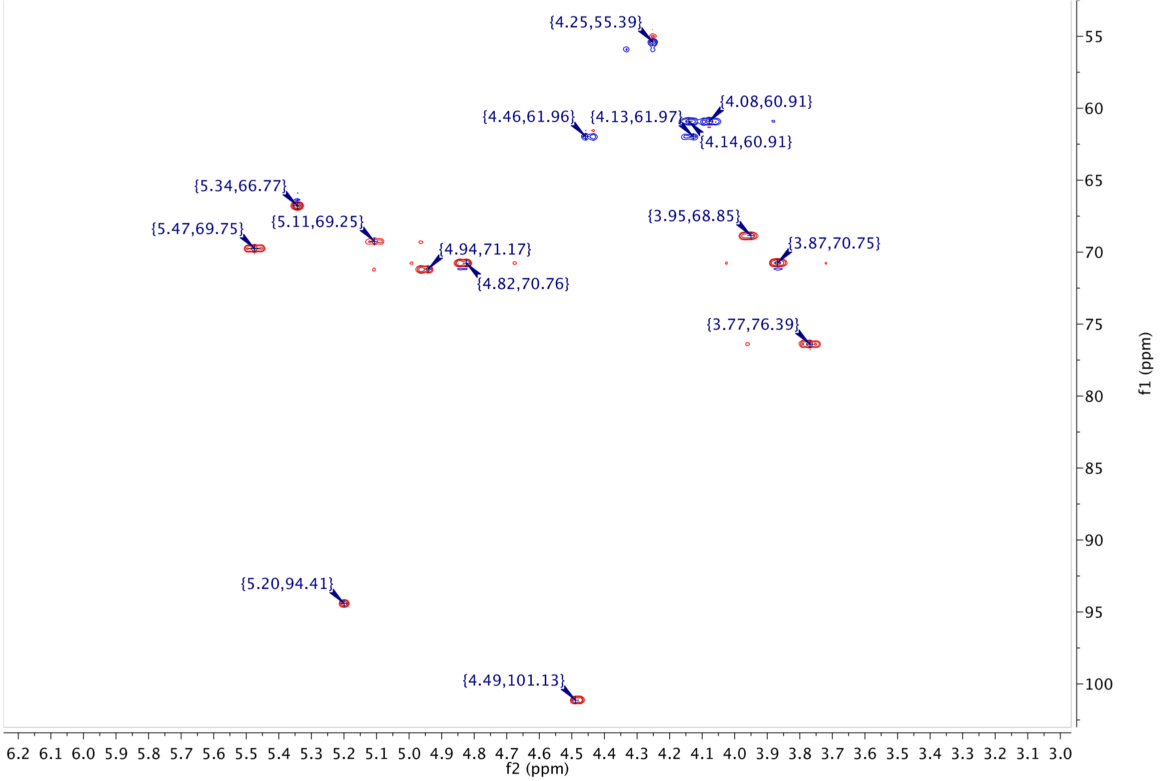
^{1}H NMR (500 MHz, $CDCl_3$): Propargyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside **(4l)**



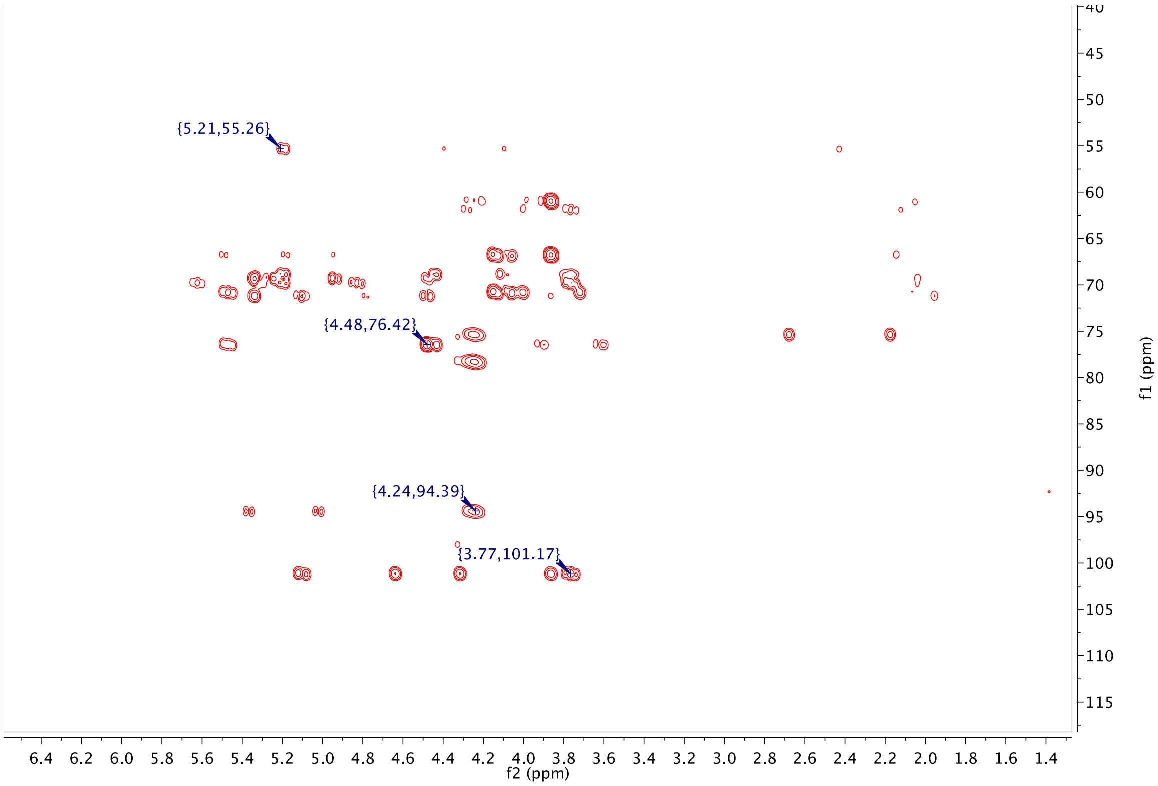
^{13}C NMR (125 MHz, $CDCl_3$): Propargyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside **(4l)**



gCOSY NMR: Propargyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside **(4l)**



gHSQC NMR: Propargyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside **(4l)**



gHMBC NMR: Propargyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside **(4l)**