Supporting Information

A Double Diastereoselective Michael Type Addition as an Entry to Conformationally Restricted Tn Antigen Mimics

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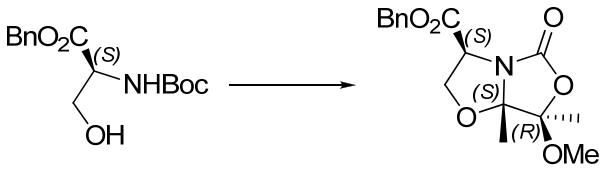
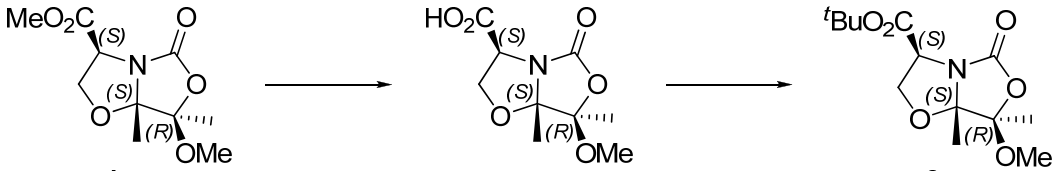
Organic structures

Benzyl (3*S*,7*R*,7a*S*)-7-methoxy-7,7a-dimethyl-5-oxotetrahydro-5*H*-oxazolo[4,3-b]oxazole-3-carboxylate (**3b**)



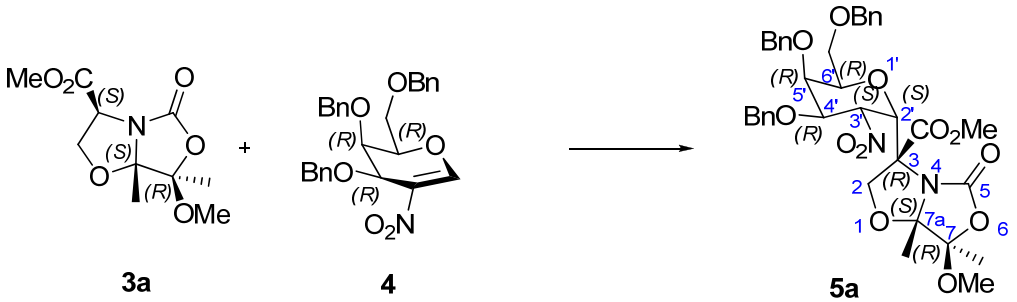
3b

tert-Butyl (3*S*,7*R*,7a*S*)-7-methoxy-7,7a-dimethyl-5-oxotetrahydro-5*H*-oxazolo[4,3-b]oxazole-3-carboxylate (**3c**)



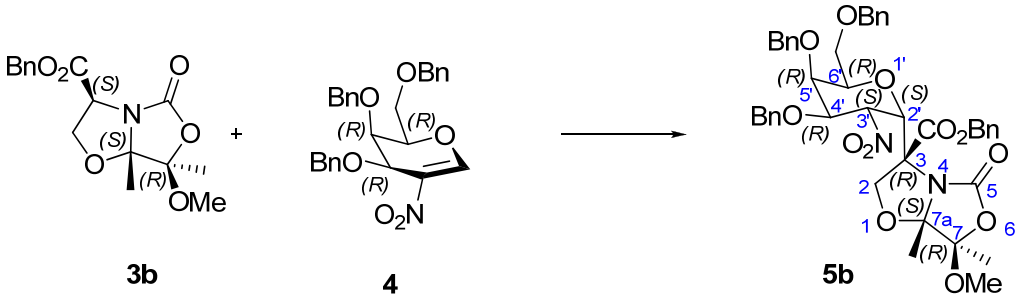
1a **3c**

Methyl (3*R*,7*R*,7a*S*)-3-((2'*S*,3'*S*,4'*R*,5'*R*,6'*R*)-4',5'-bis(benzyloxy)-6'-(benzyloxymethyl)-3'-nitrotetrahydro-2*H*-pyran-2'-yl)-7-methoxy-7,7a-dimethyl-5-oxotetrahydro-2H-oxazolo[3,2-c]oxazole-3-carboxylate (**5a**)



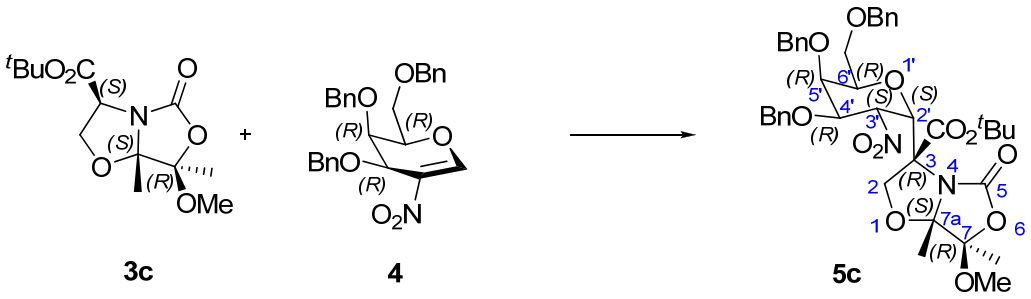
3a **4** **5a**

Benzyl (3*R*,7*R*,7a*S*)-3-((2'*S*,3'*S*,4'*R*,5'*R*,6'*R*)-4',5'-bis(benzyloxy)-6'-(benzyloxymethyl)-3'-nitrotetrahydro-2H-pyran-2'-yl)-7-methoxy-7,7a-dimethyl-5-oxotetrahydro-2H-oxazolo[3,2-c]oxazole-3-carboxylate (**5b**)



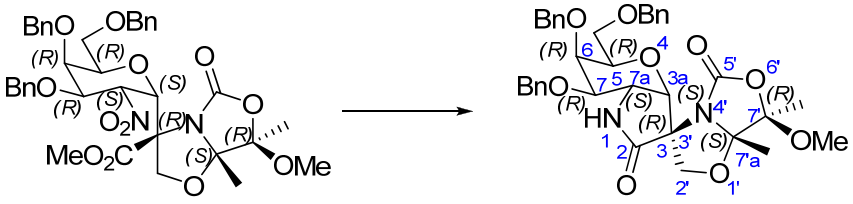
3b **4** **5b**

Tert-butyl (3*R*,7*R*,7a*S*)-3-((2'*S*,3'*S*,4'*R*,5'*R*,6'*R*)-4',5'-bis(benzyloxy)-6'-(benzyloxymethyl)-3'-nitrotetrahydro-2H-pyran-2'-yl)-7-methoxy-7,7a-dimethyl-5-oxotetrahydro-2H-oxazolo[3,2-c]oxazole-3-carboxylate (**5c**)



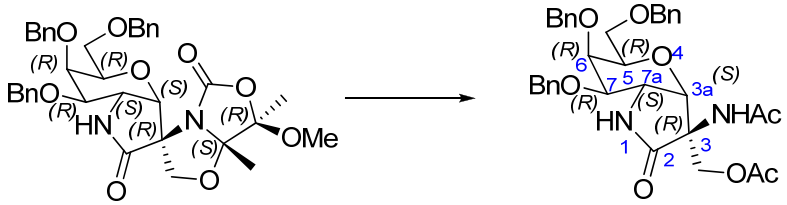
3c **4** **5c**

(3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*,7'*R*,7'a*S*)-6,7-Dibenzyloxy-5-(benzyloxymethyl)-7'-methoxy-7',7'a-dimethyl-spiro[1,3a,5,6,7,7a-hexahydropyran[3,2-b]pyrrol-3,3'-2H-oxazolo[4,3-b]oxazol]-2,5'-dione (**6**)



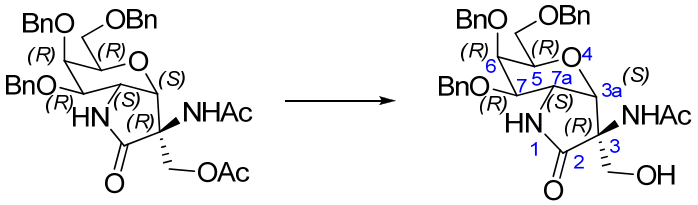
5a **6**

(3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamide-3-acetoxymethyl-6,7-dibenzyloxy-5-(benzyloxymethyl)-2-oxo-1,3a,5,6,7,7a-hexahydropyran[3,2-b]pyrrole (**7**)



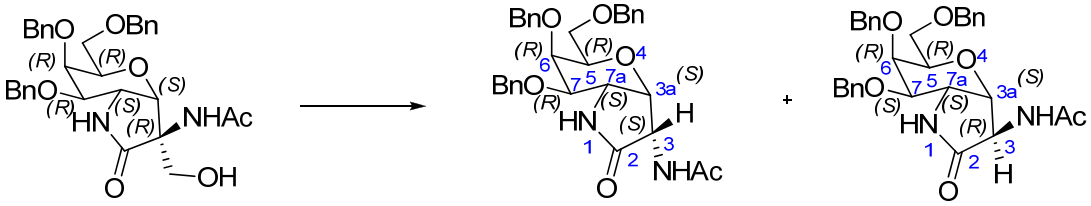
6 **7**

(3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dibenzyloxy-5-(benzyloxymethyl)-3-(hydroxymethyl)-2-oxo-1,3a,5,6,7,7a-hexahydropyrane[3,2-b]pyrrole (**8**)



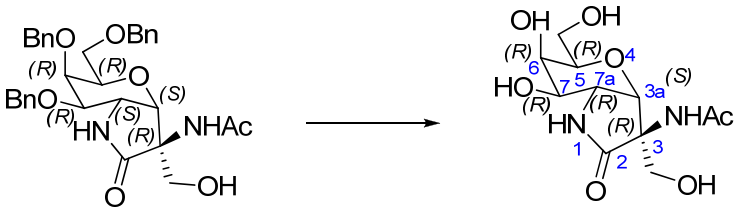
7 **8**

(3*S*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dibenzyloxy-5-(benzyloxymethyl)-2-oxo-1,3a,5,6,7,7a-hexahydropyran[3,2-b]pyrrole (**9a**) and (3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dibenzyloxy-5-(benzyloxymethyl)-2-oxo-1,3a,5,6,7,7a-hexahydropyran[3,2-b]pyrrole (**9b**)



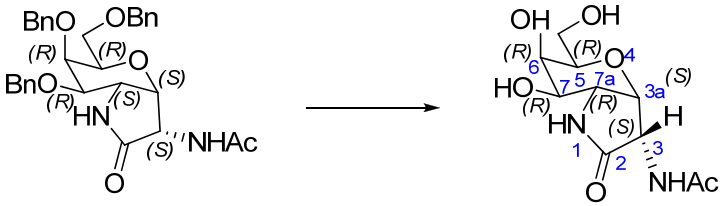
8 **9a** **9b**

(3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dihydroxy-3,5-bis(hydroxymethyl)-2-oxo-1,3a,5,6,7,7a-hexahydropyrano[3,2-b]pyrrole (**2**)



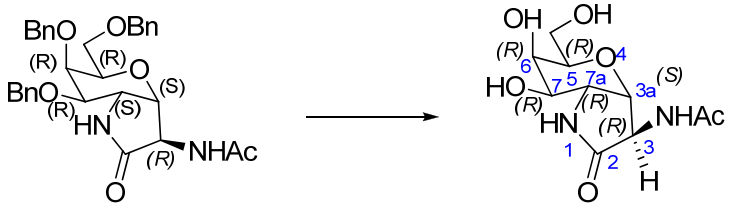
8 **2**

(3*S*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dihydroxy-5-hydroxymethyl-2-oxo-1,3a,5,6,7,7a-hexahydropyrano[3,2-b]pyrrole (**epi-1**)



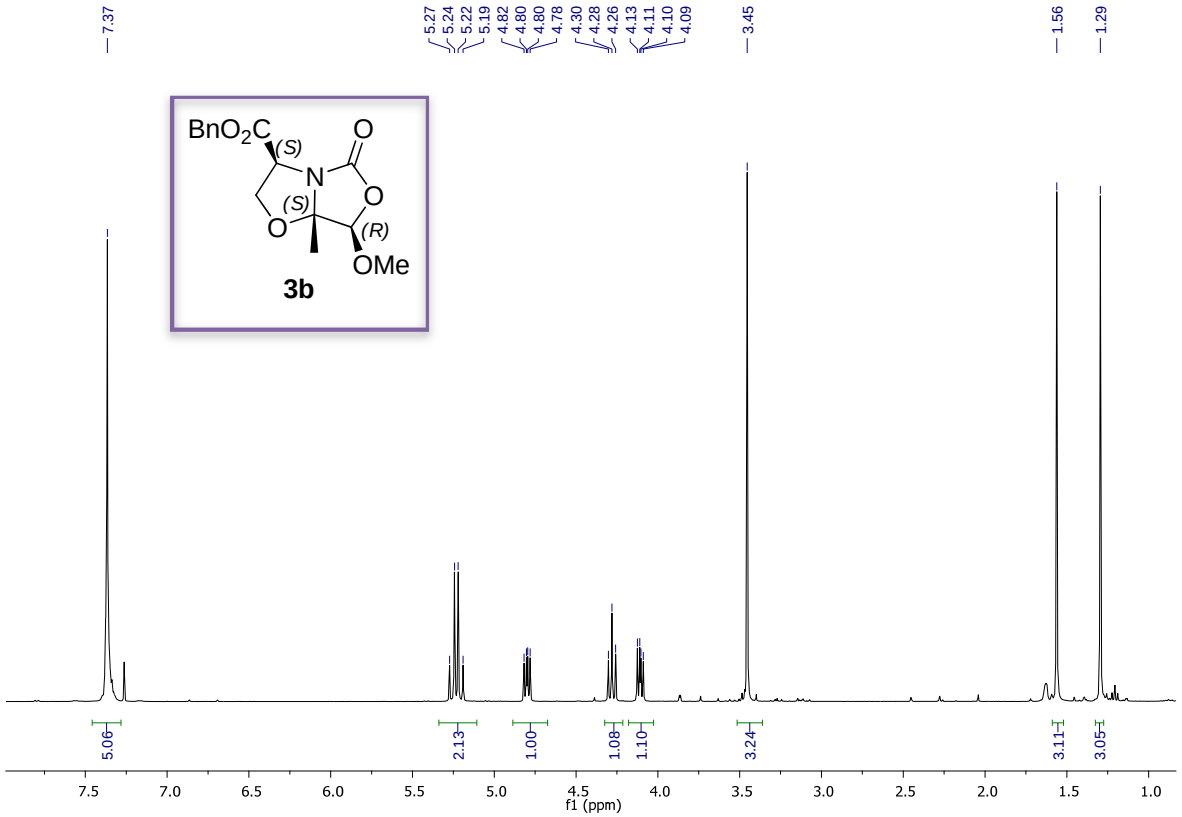
9a **epi-1**

(3R,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dihydroxy-5-hydroxymethyl-2-oxo-1,3a,5,6,7,7a-hexahydropyrano[3,2-b]pyrrole (**1**)

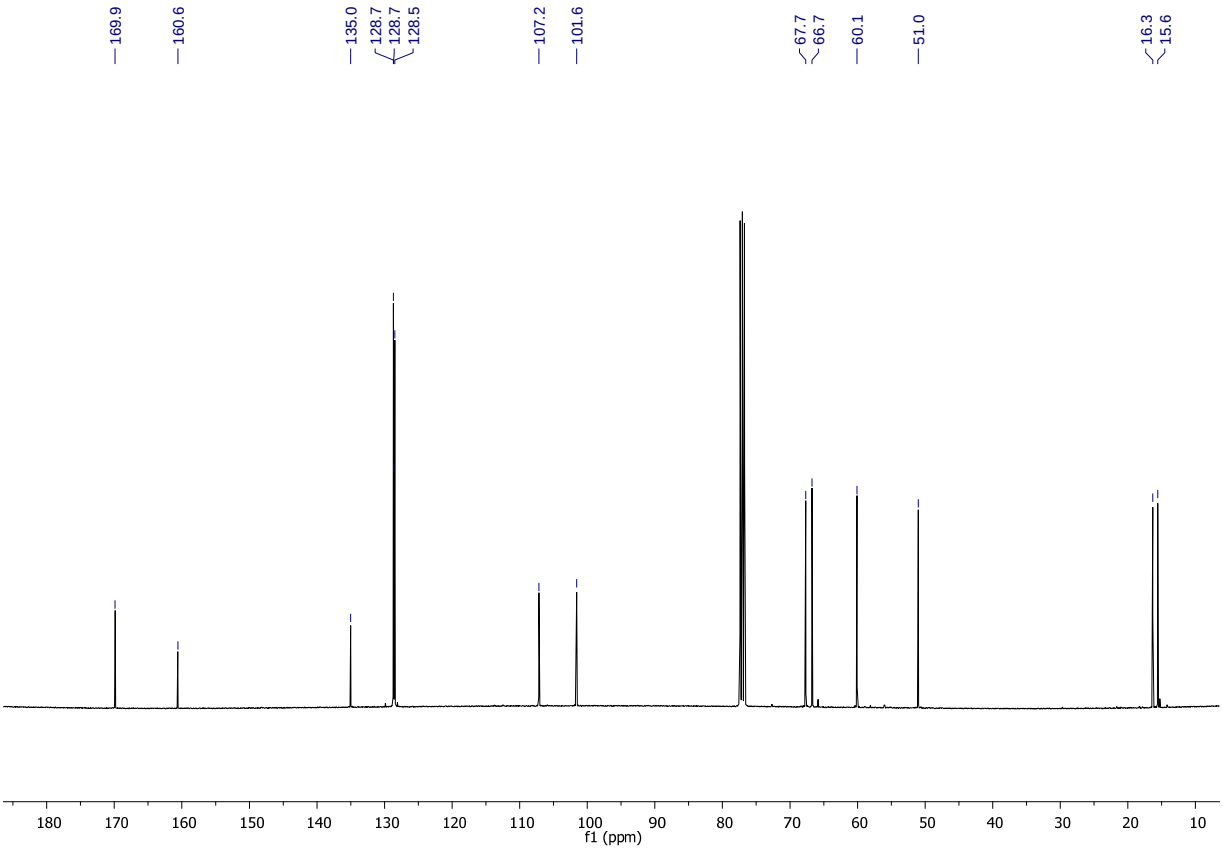


9b **1**

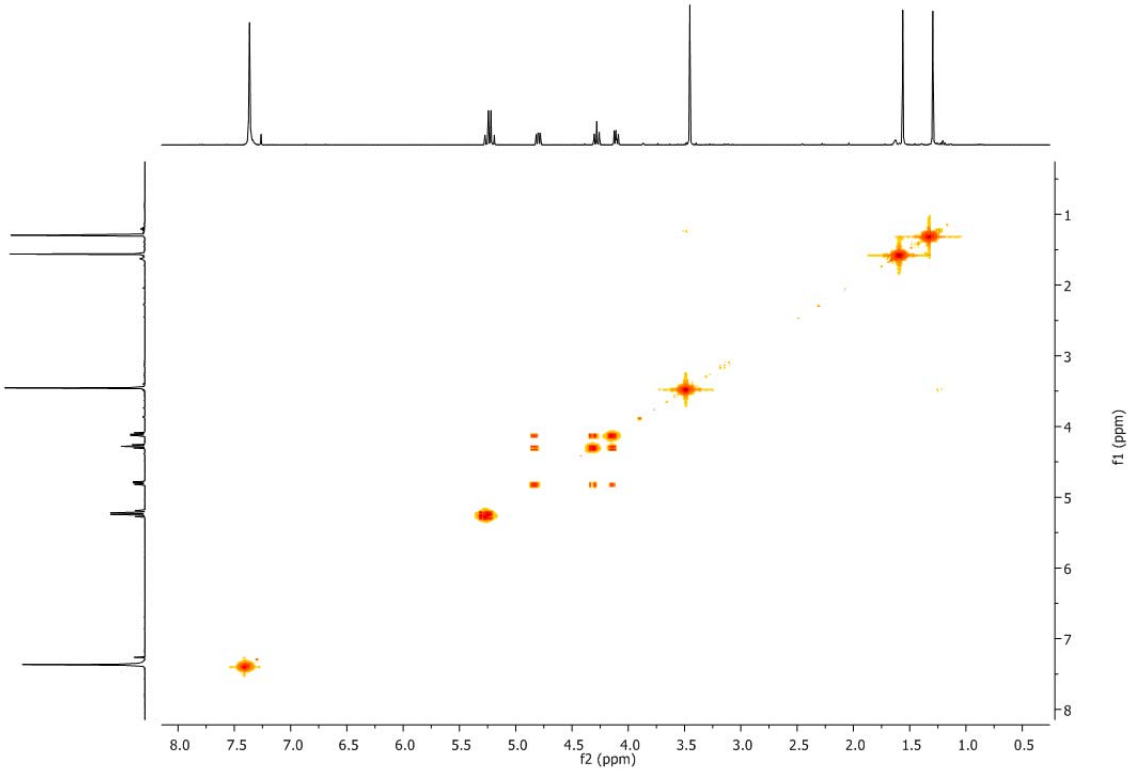
^{1}H NMR 400 MHz in $CDCl_3$



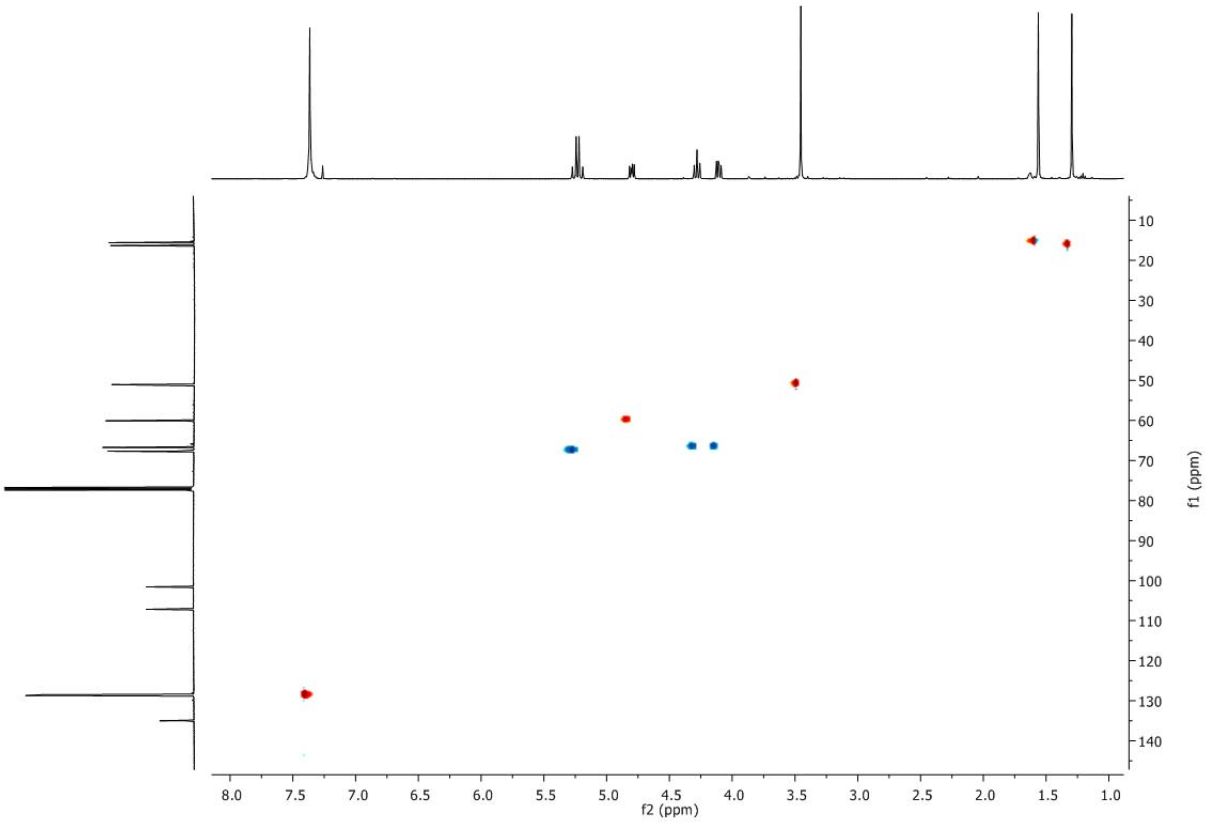
^{13}C NMR 100 MHz in $CDCl_3$



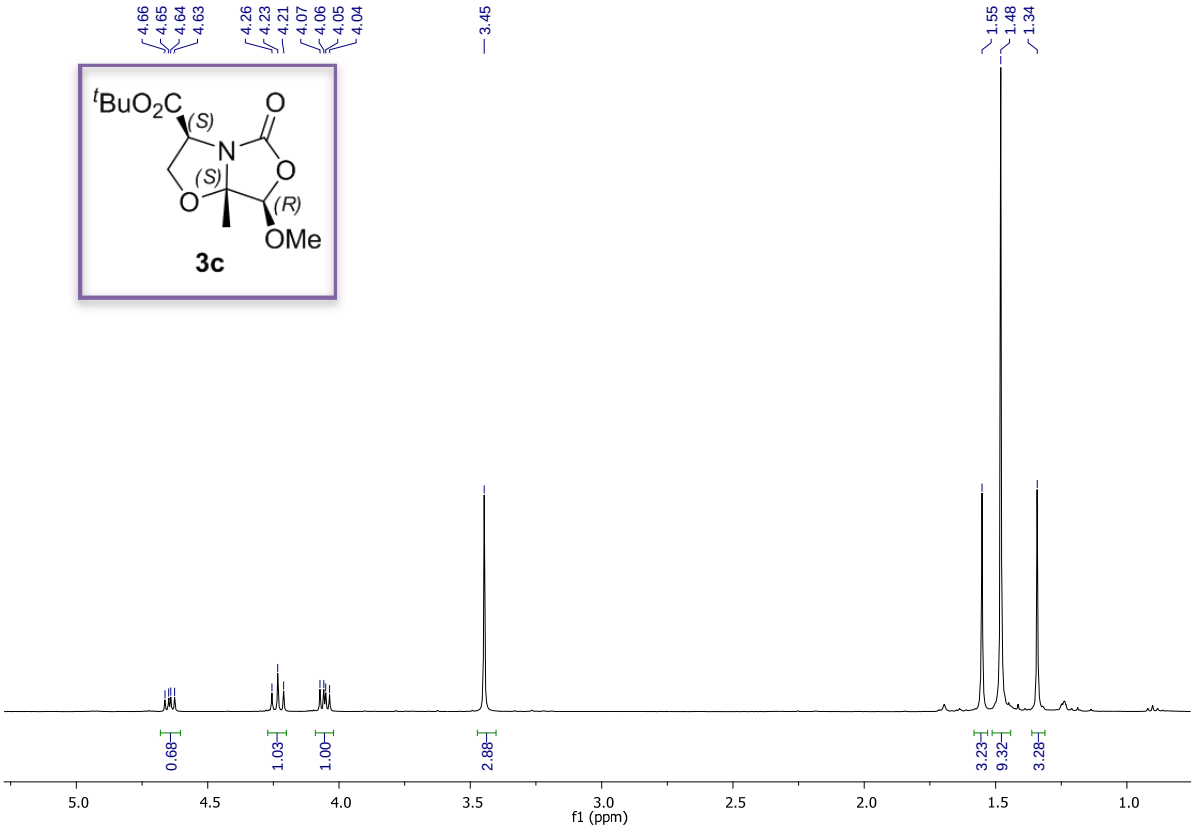
COSY in $CDCl_3$



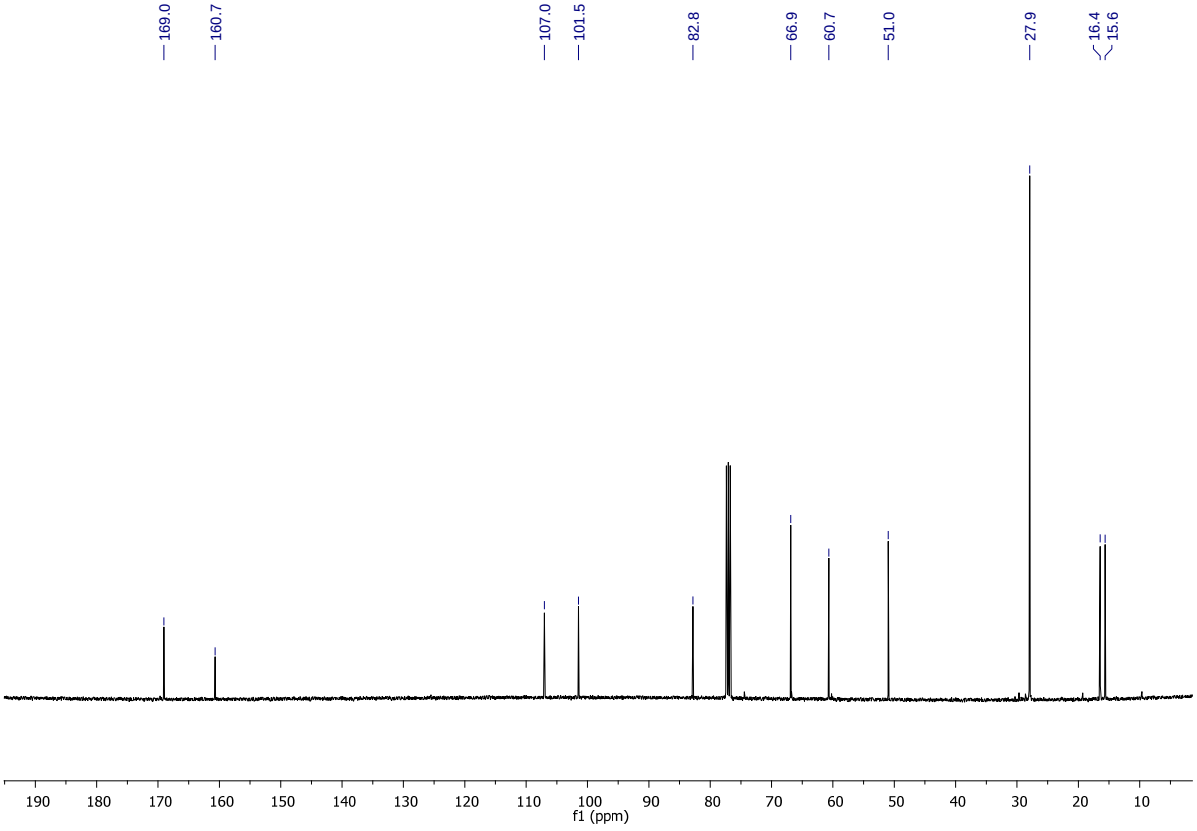
HSQC in $CDCl_3$



^{1}H NMR 400 MHz in $CDCl_3$



^{13}C NMR 100 MHz in $CDCl_3$



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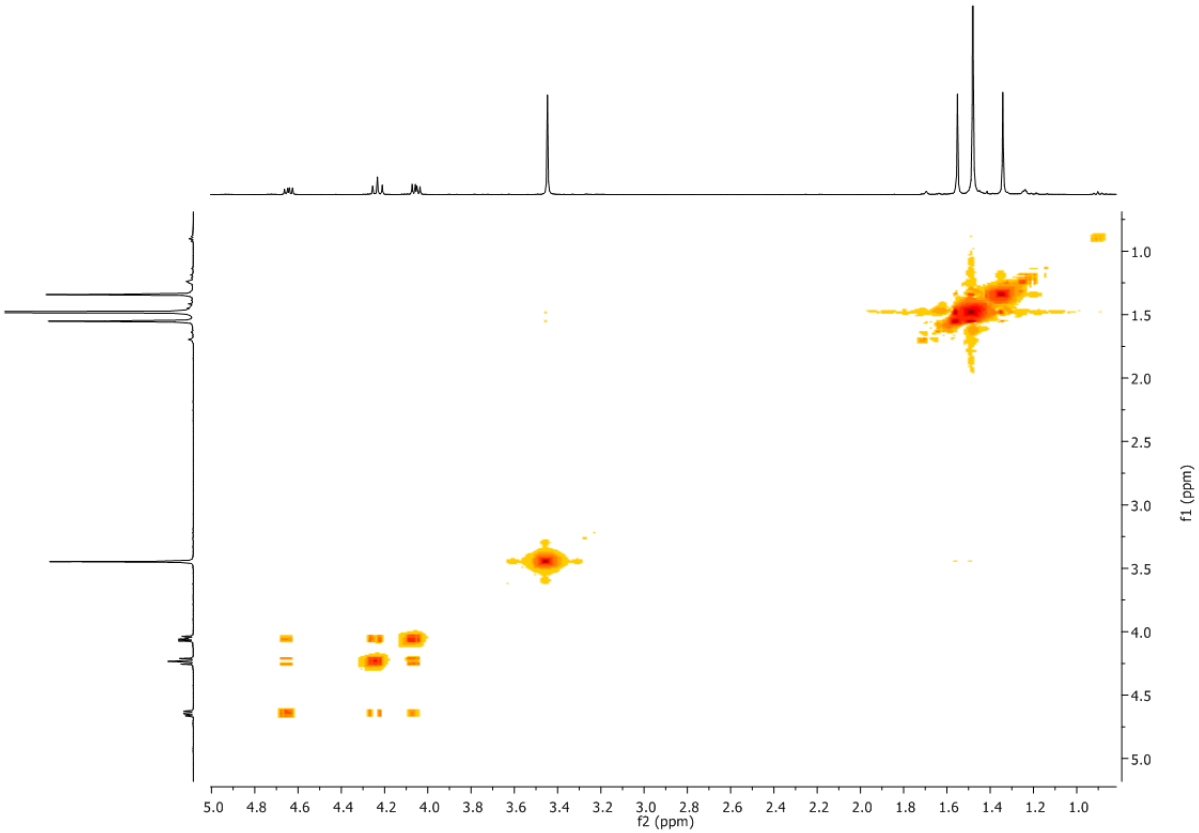

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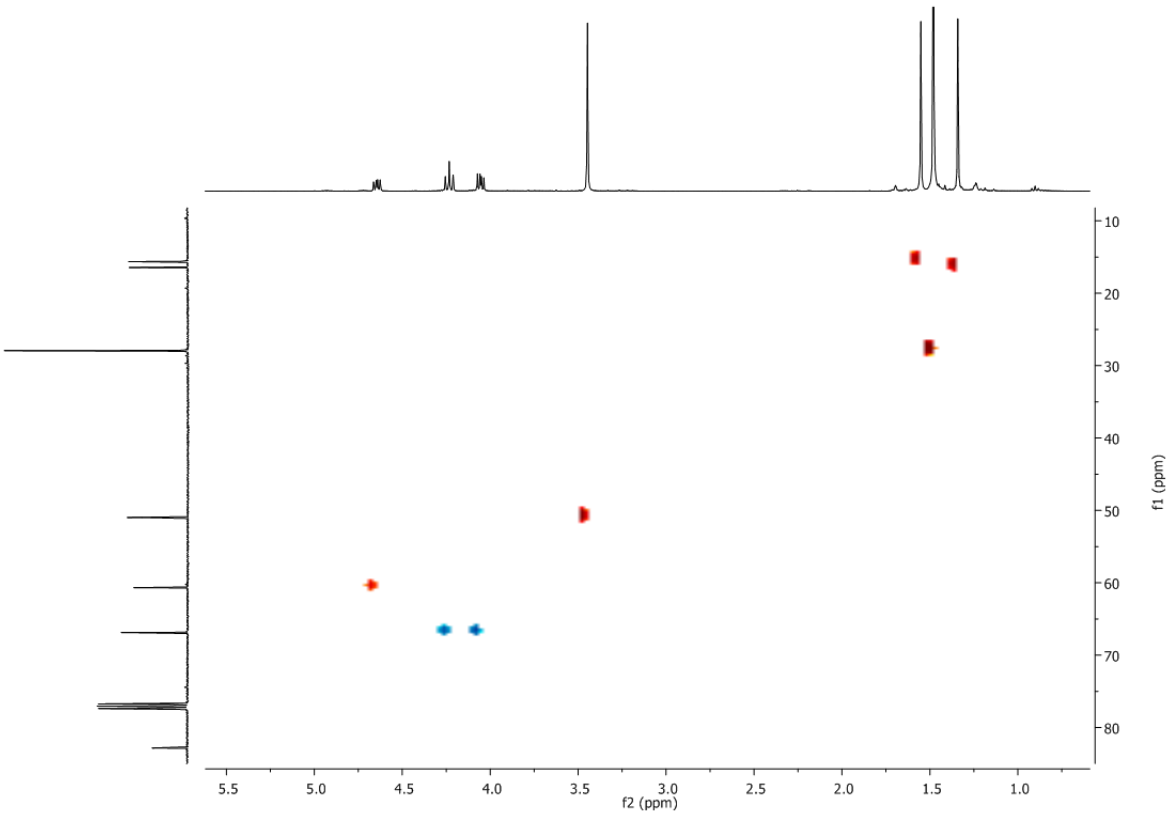
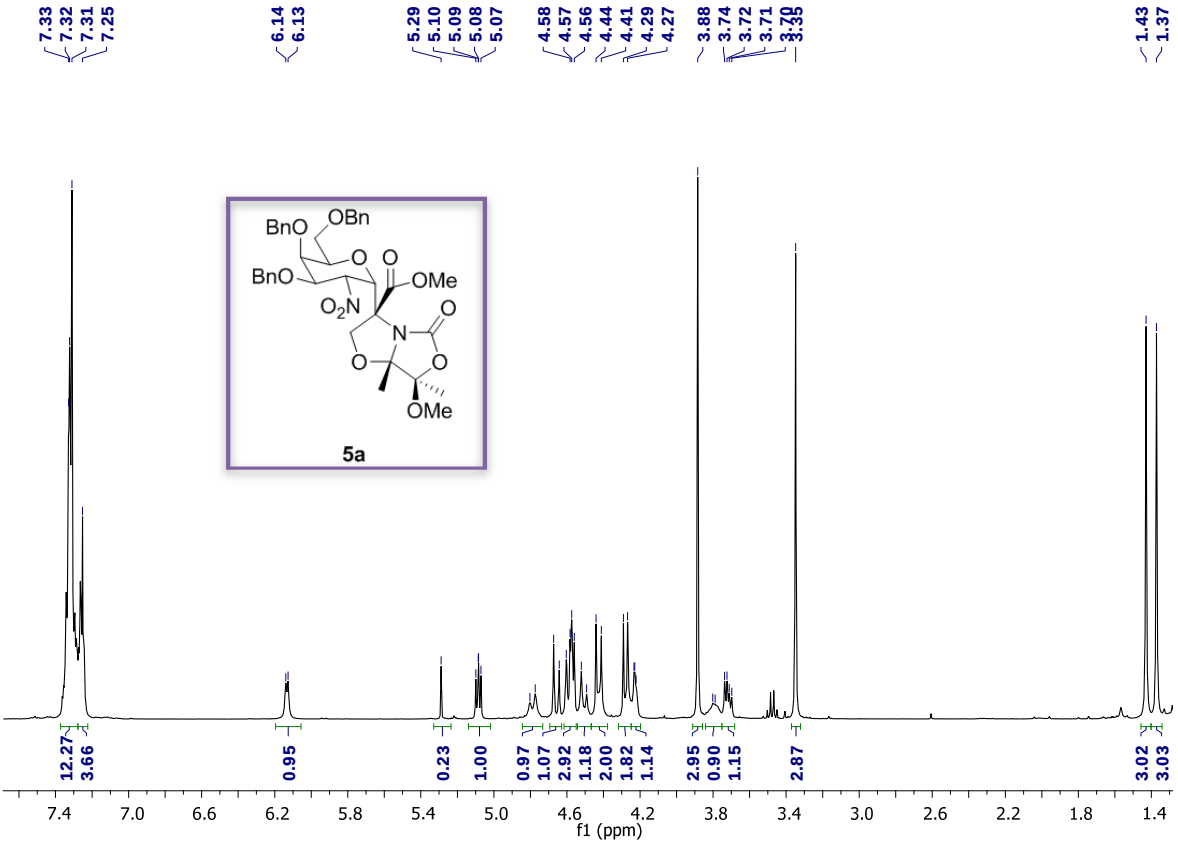


^{1}H NMR 400 MHz in $CDCl_3$



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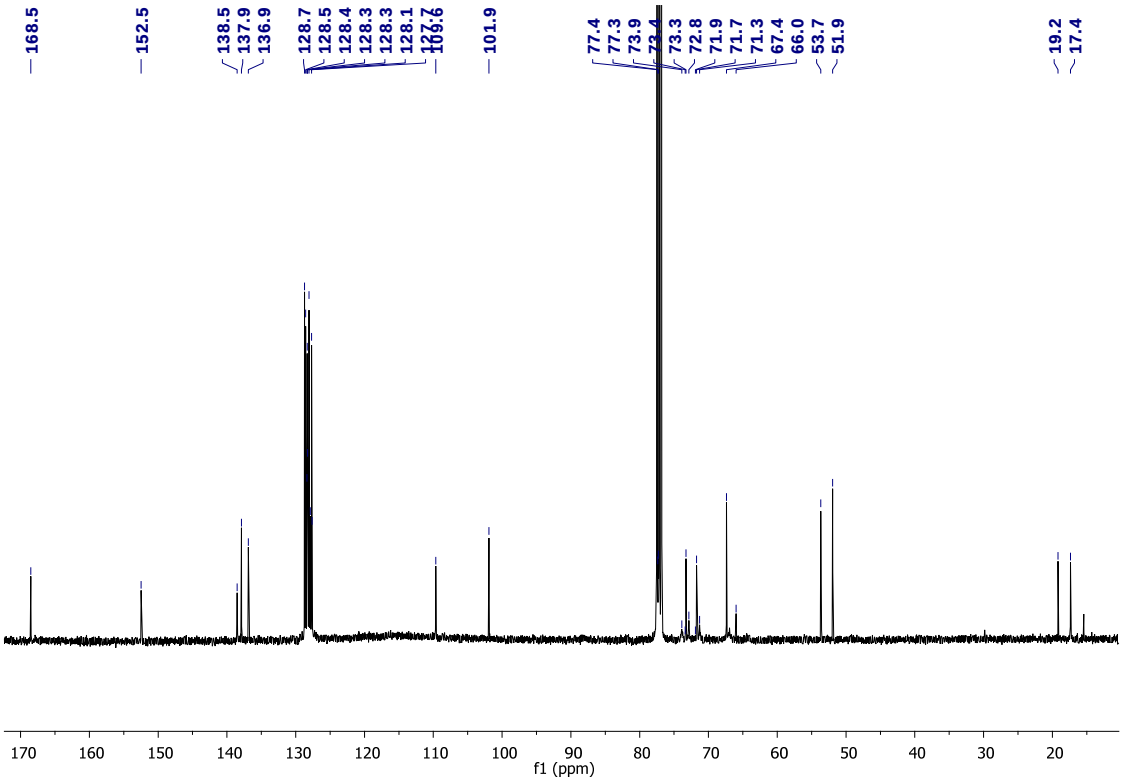
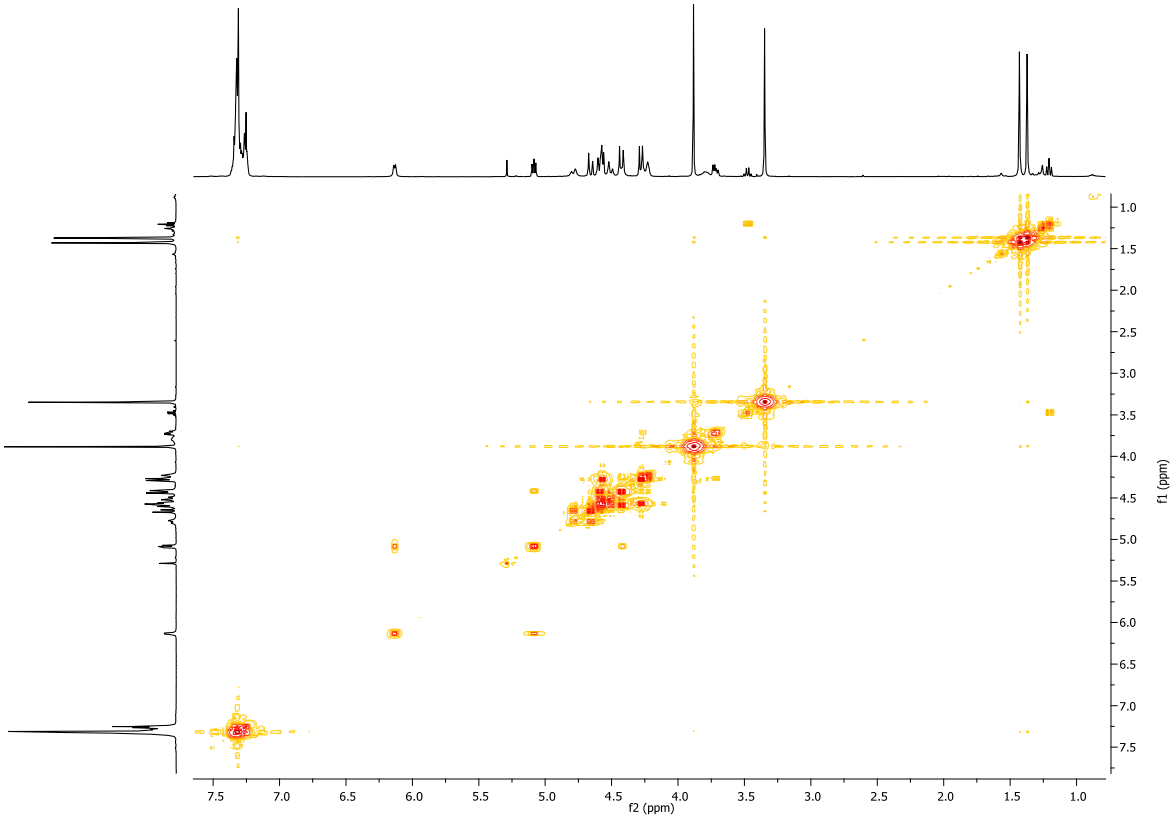


COSY in $CDCl_3$



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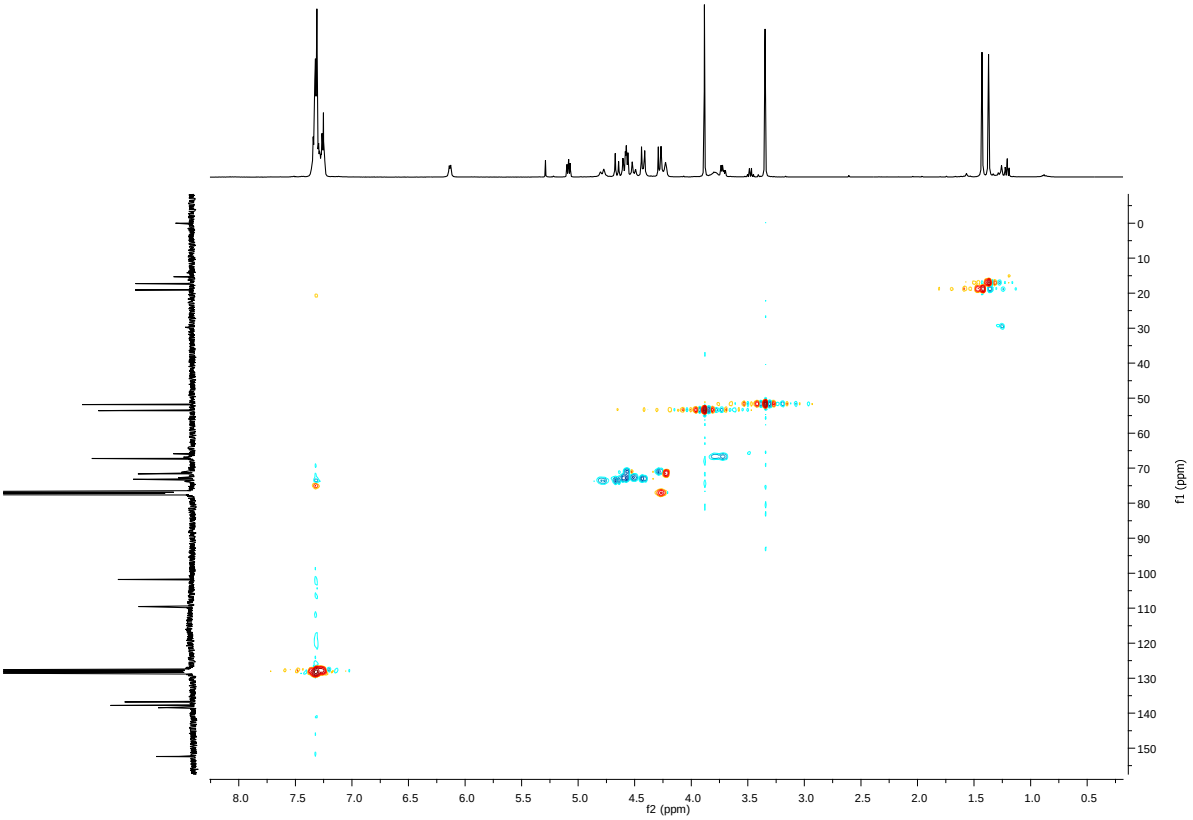
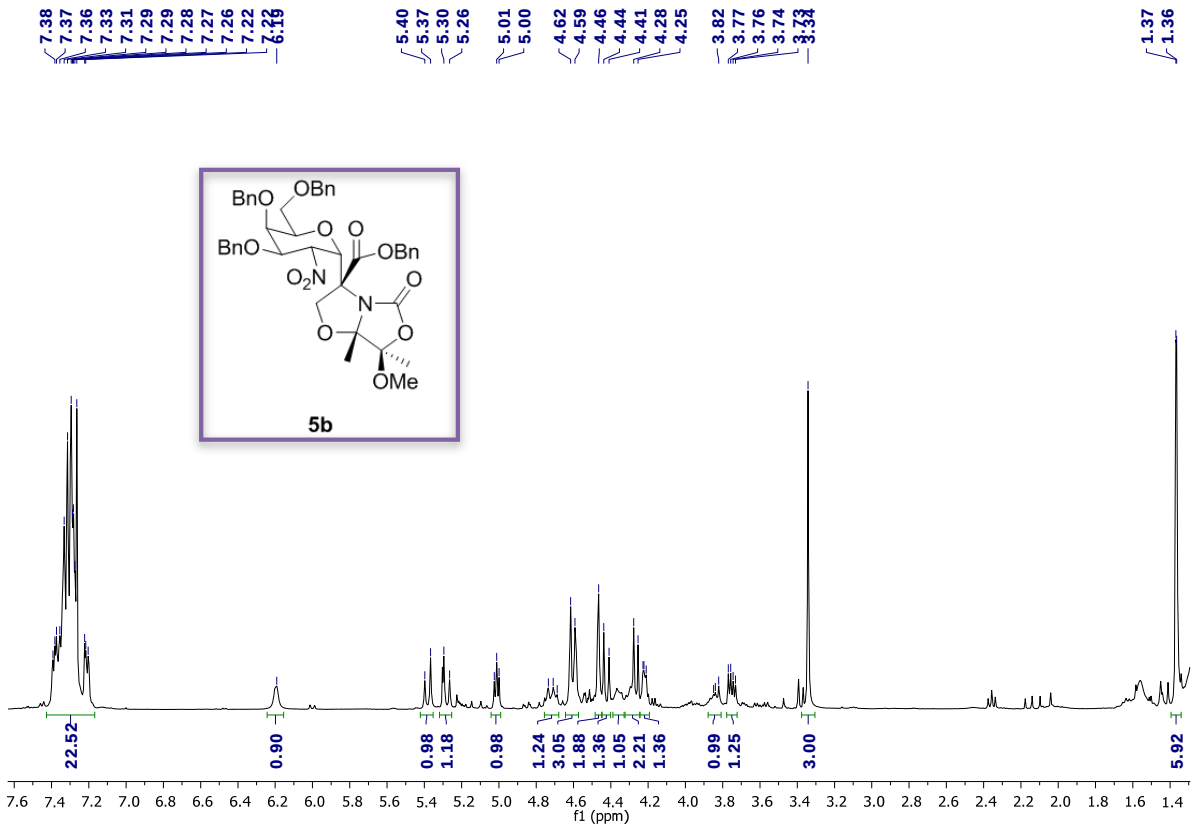
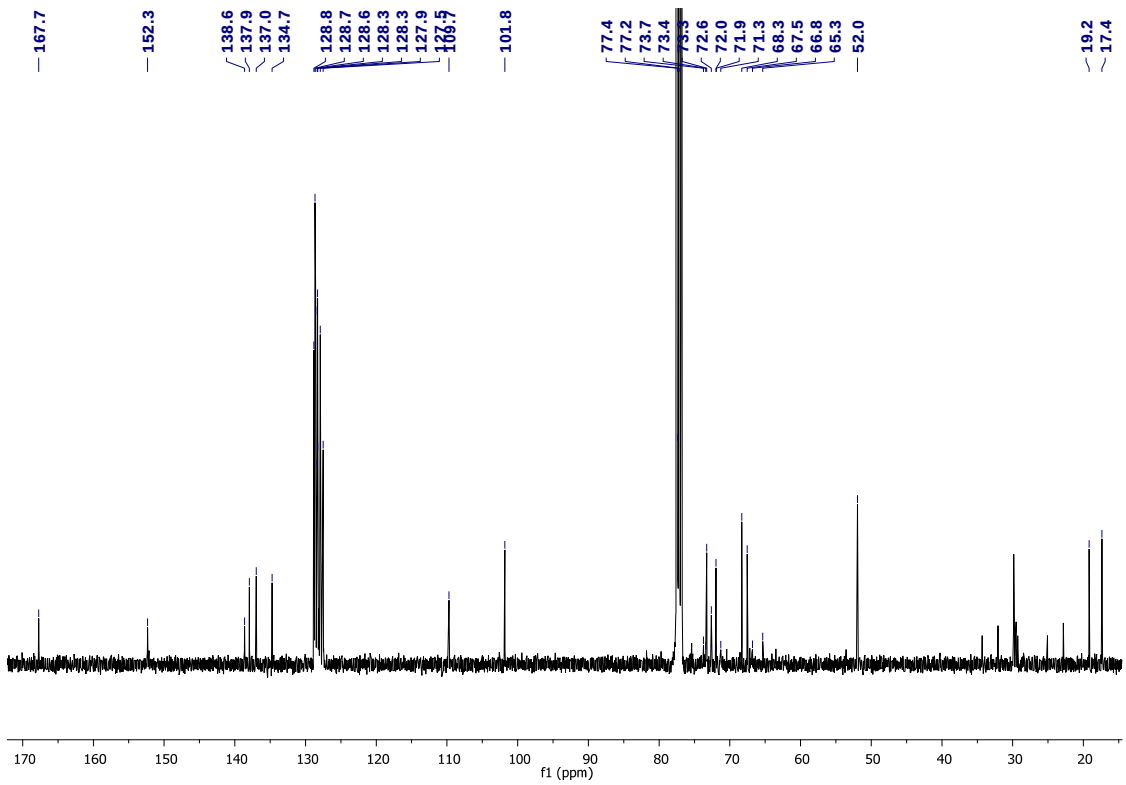


^1H NMR 400 MHz in $CDCl_3$



^{13}C NMR 100 MHz in $CDCl_3$



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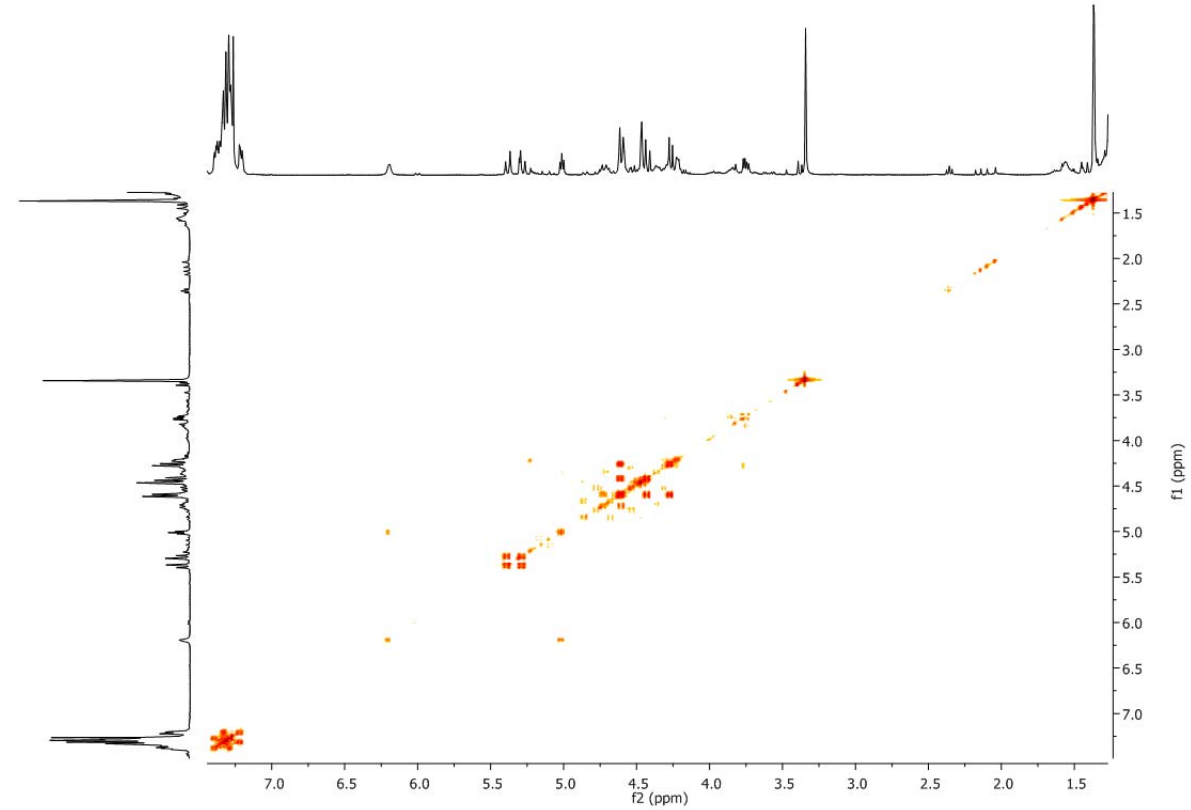

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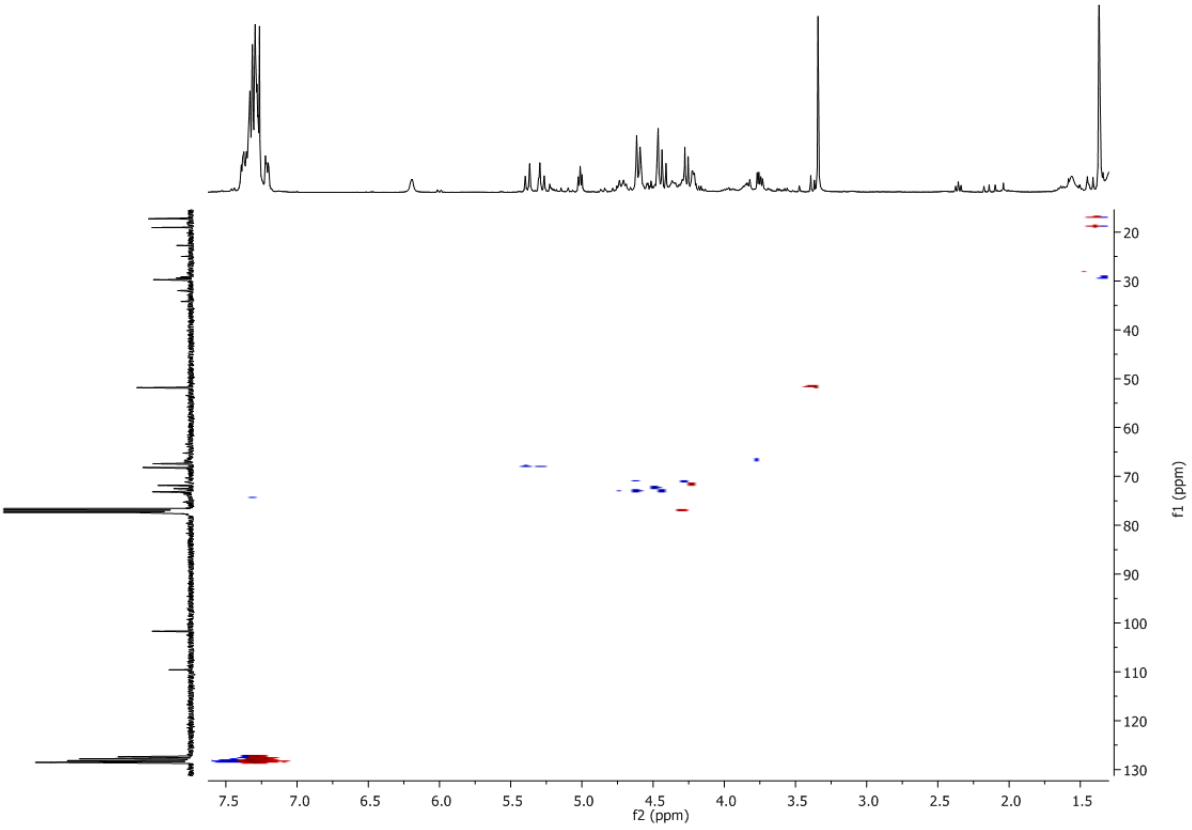
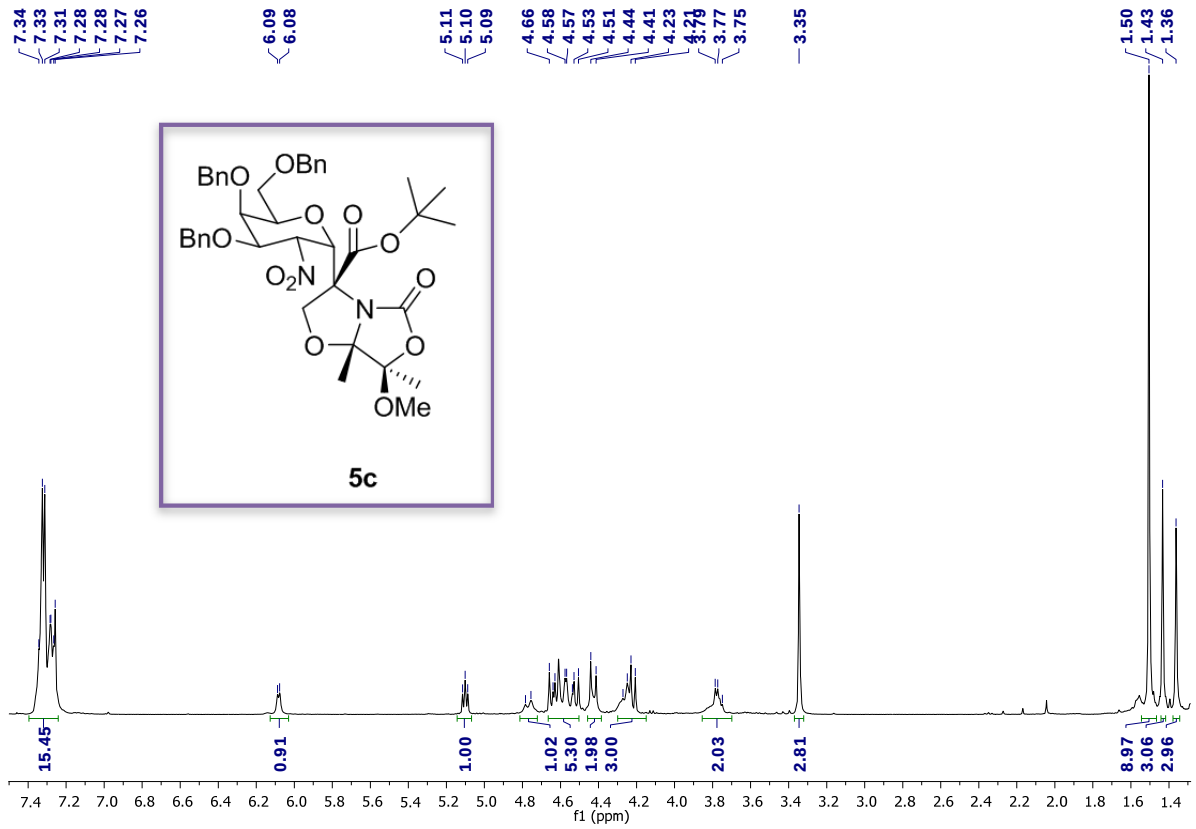
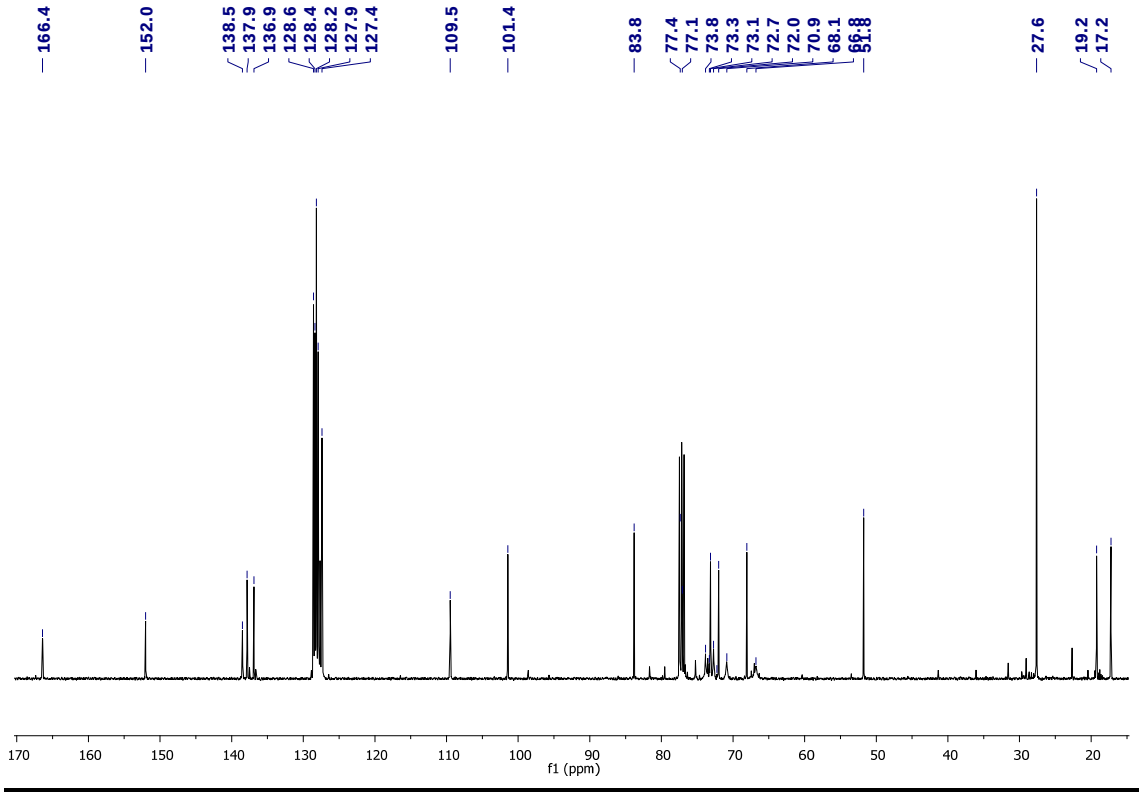


^{1}H NMR 400 MHz in $CDCl_3$



^{13}C NMR 100 MHz in $CDCl_3$



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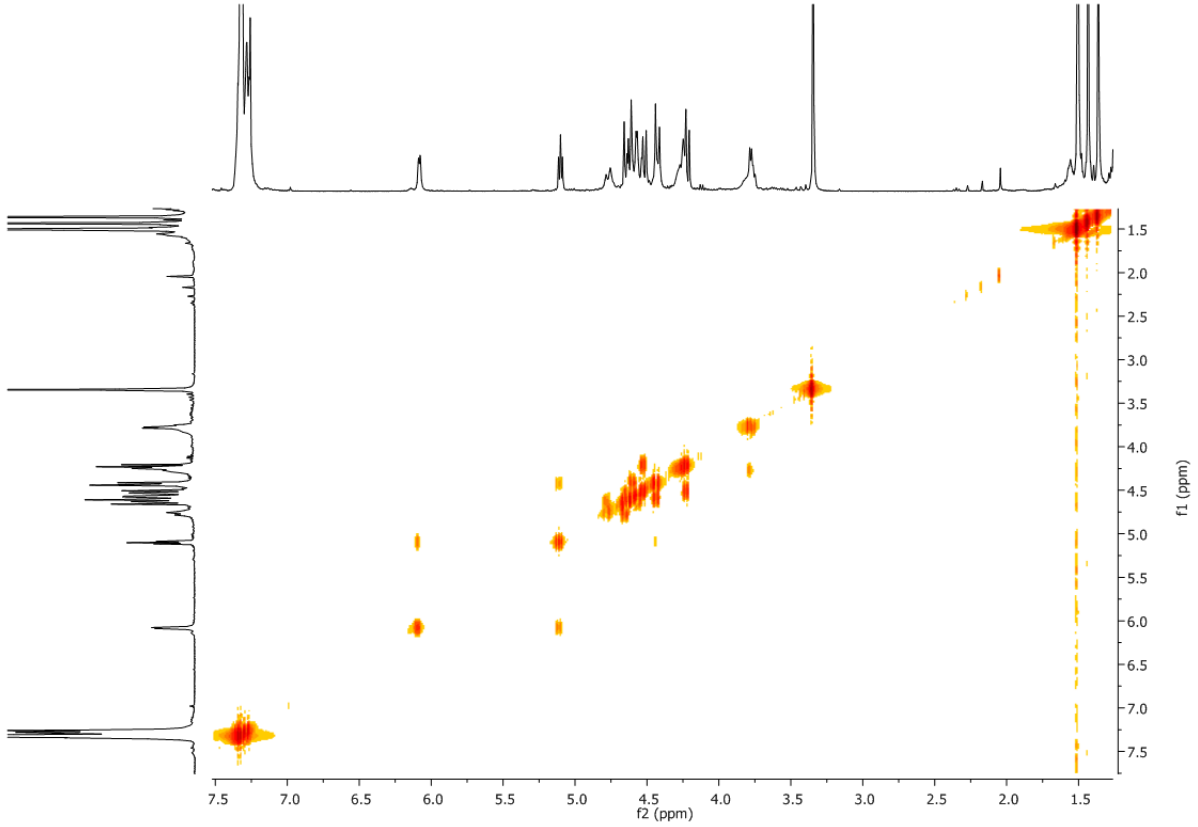

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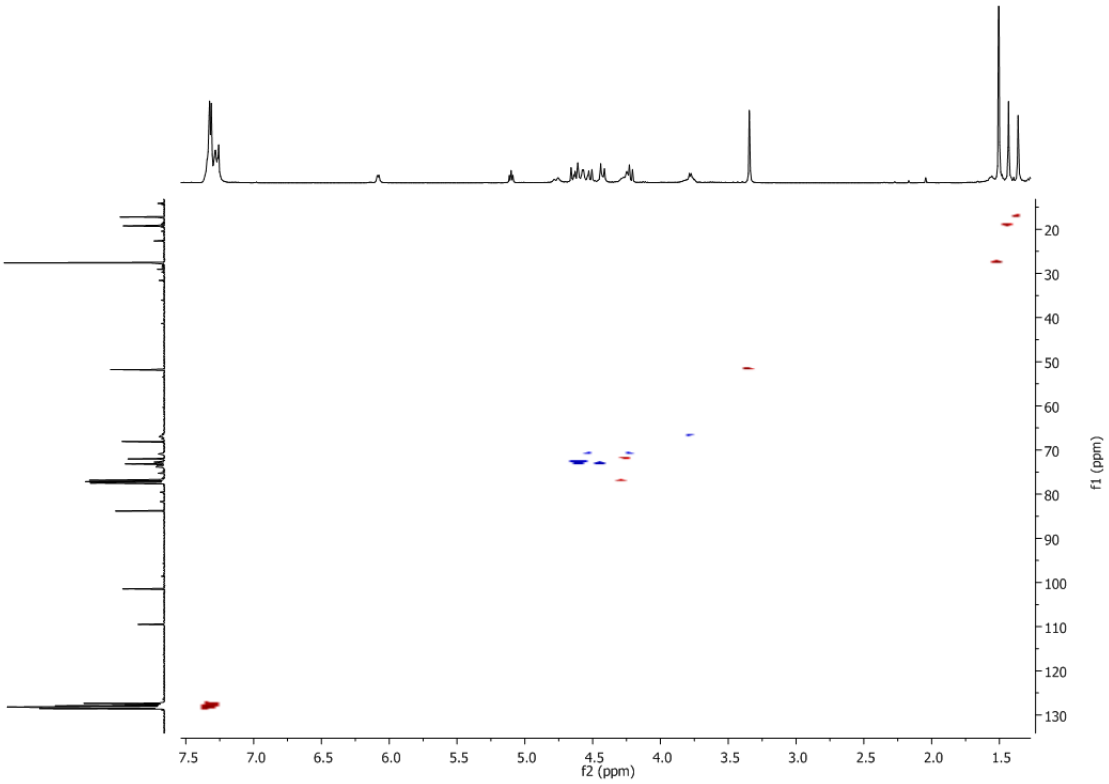

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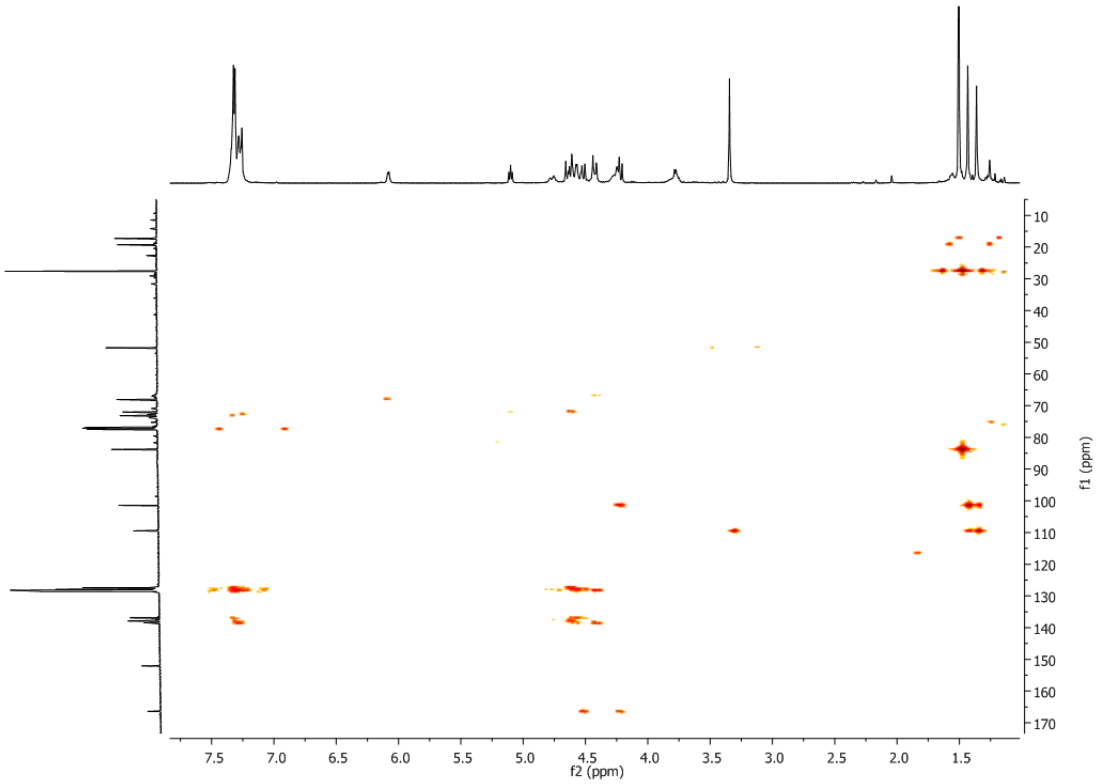
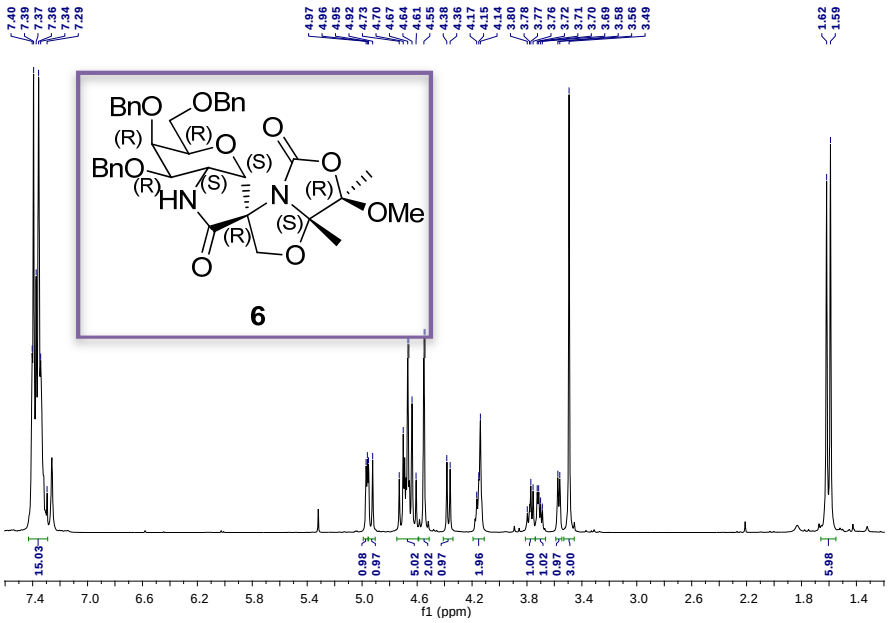


^{1}H NMR 400 MHz in $CDCl_3$



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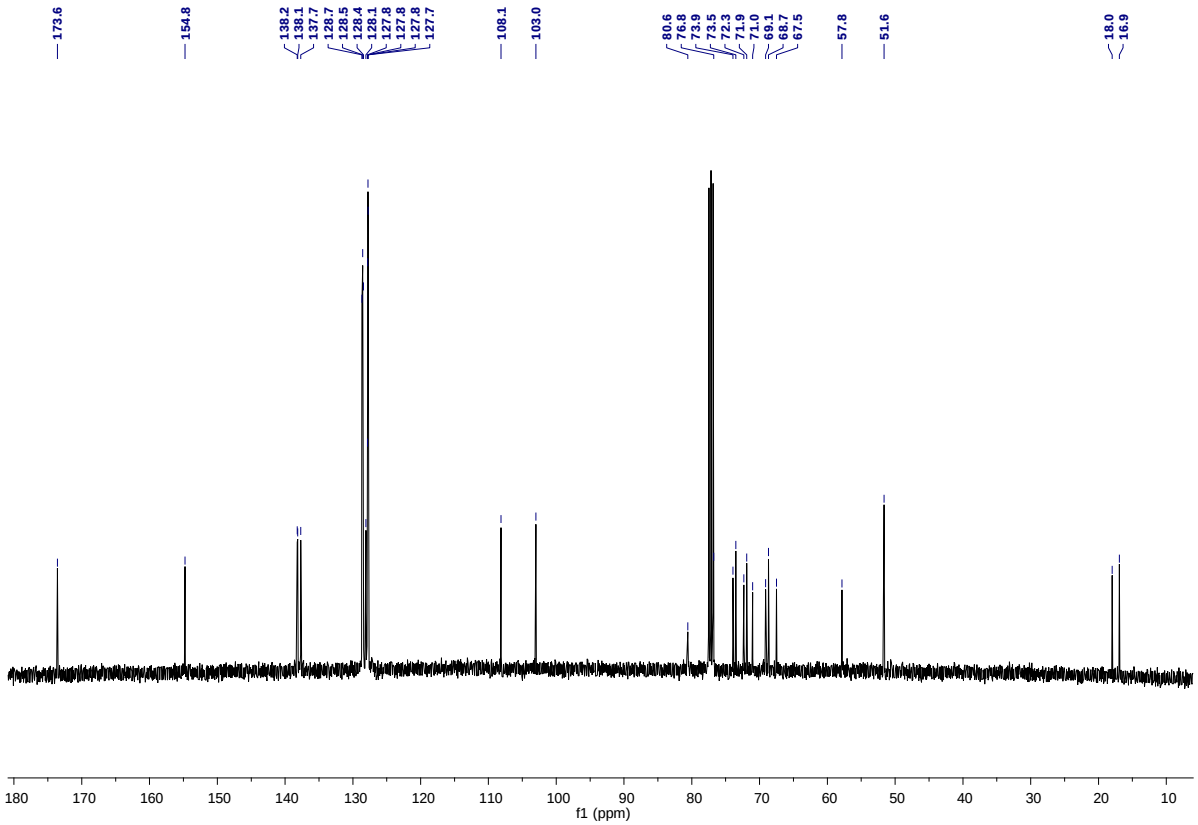

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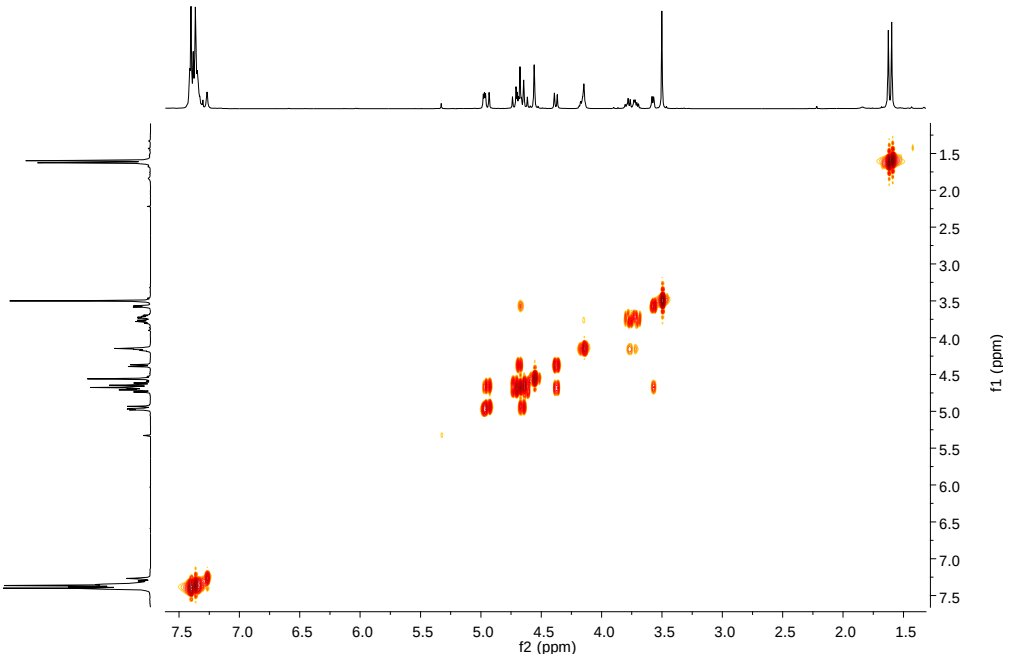

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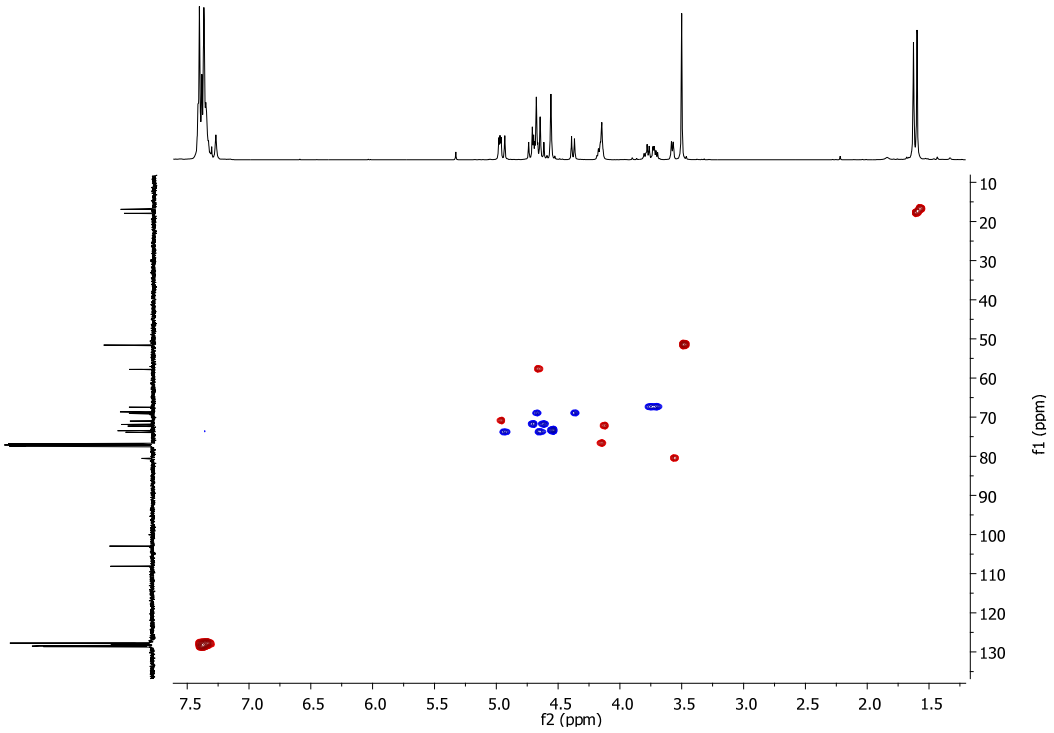

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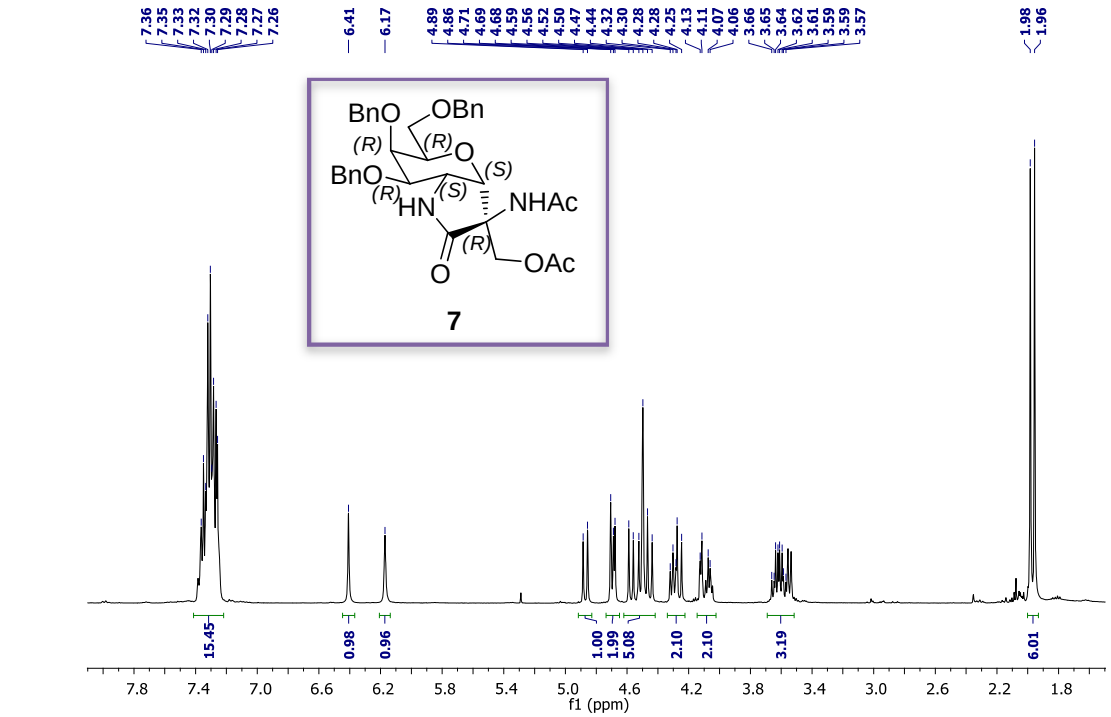

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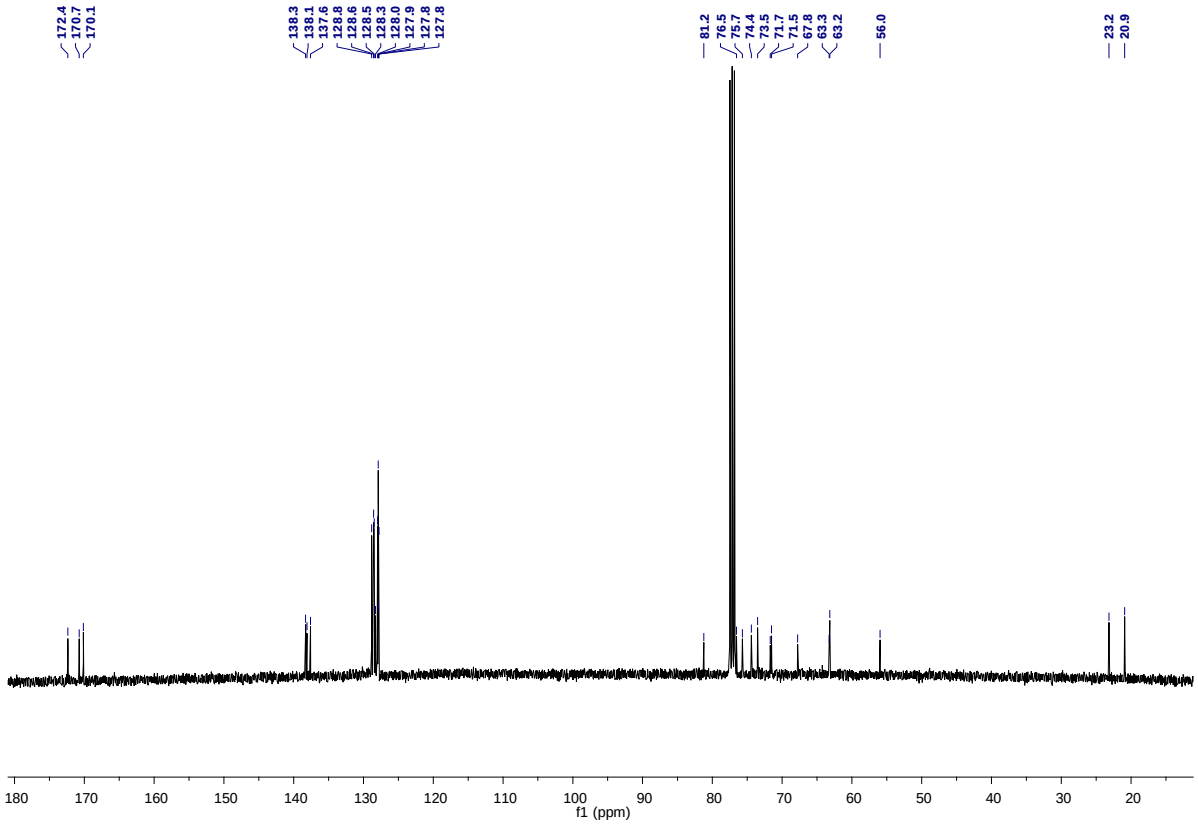
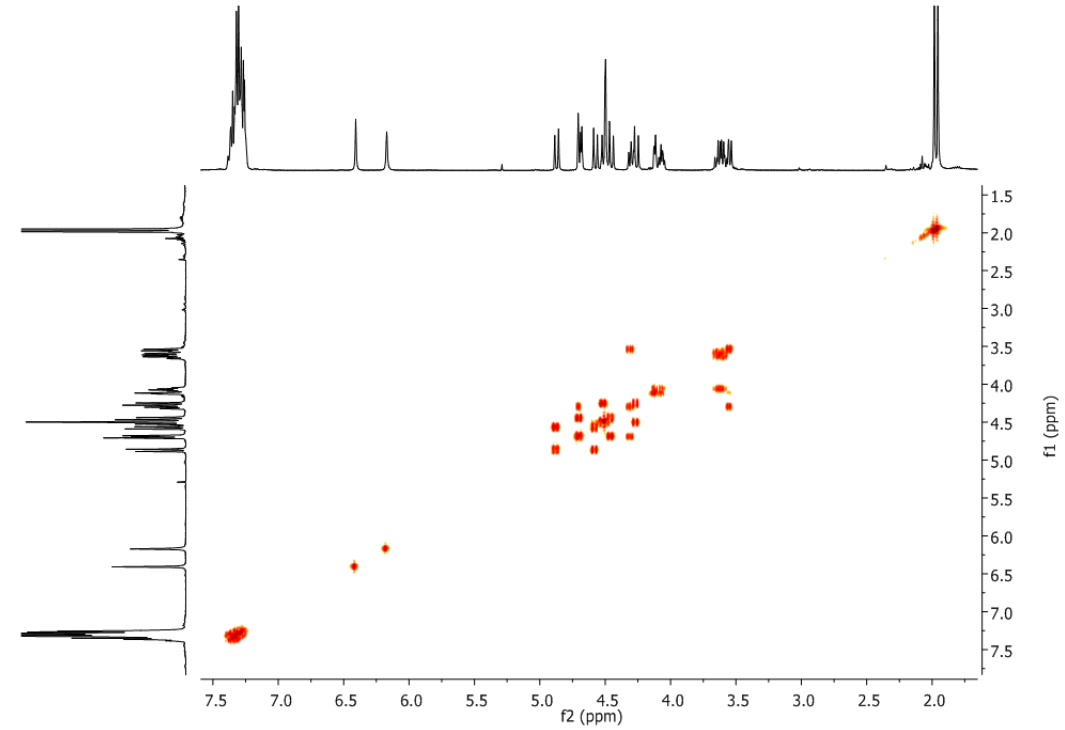
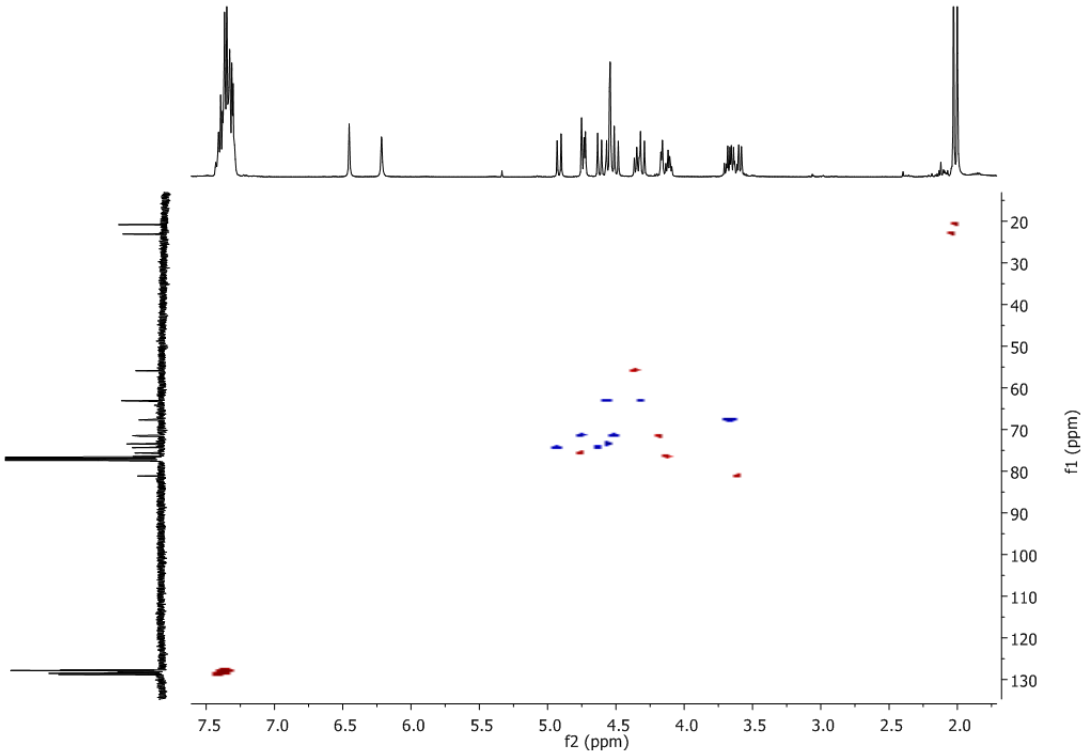


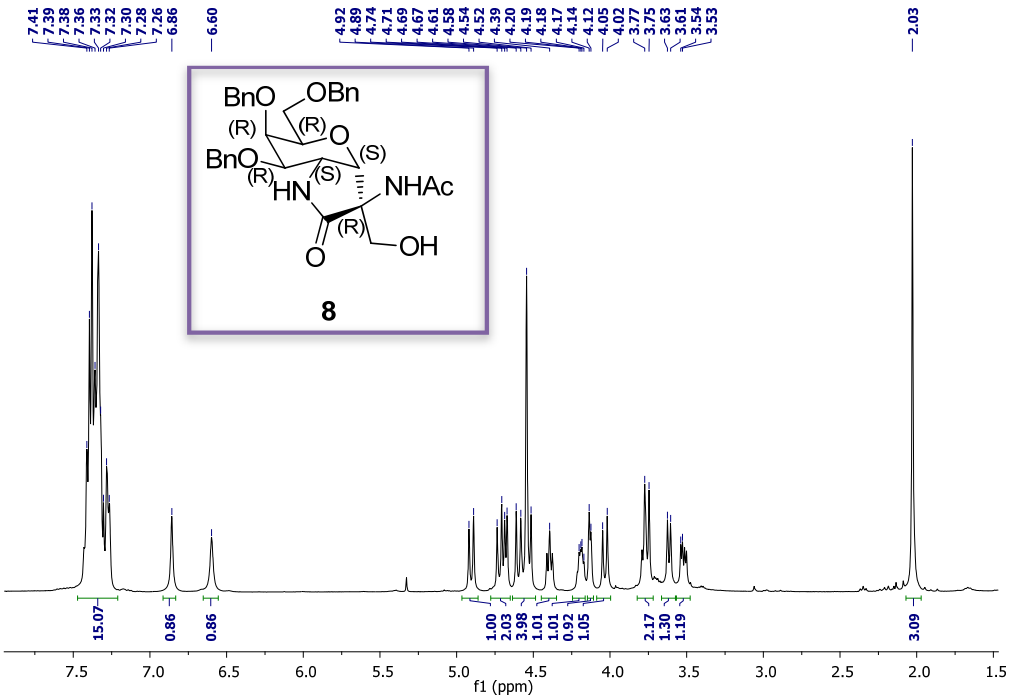
COSY in $CDCl_3$



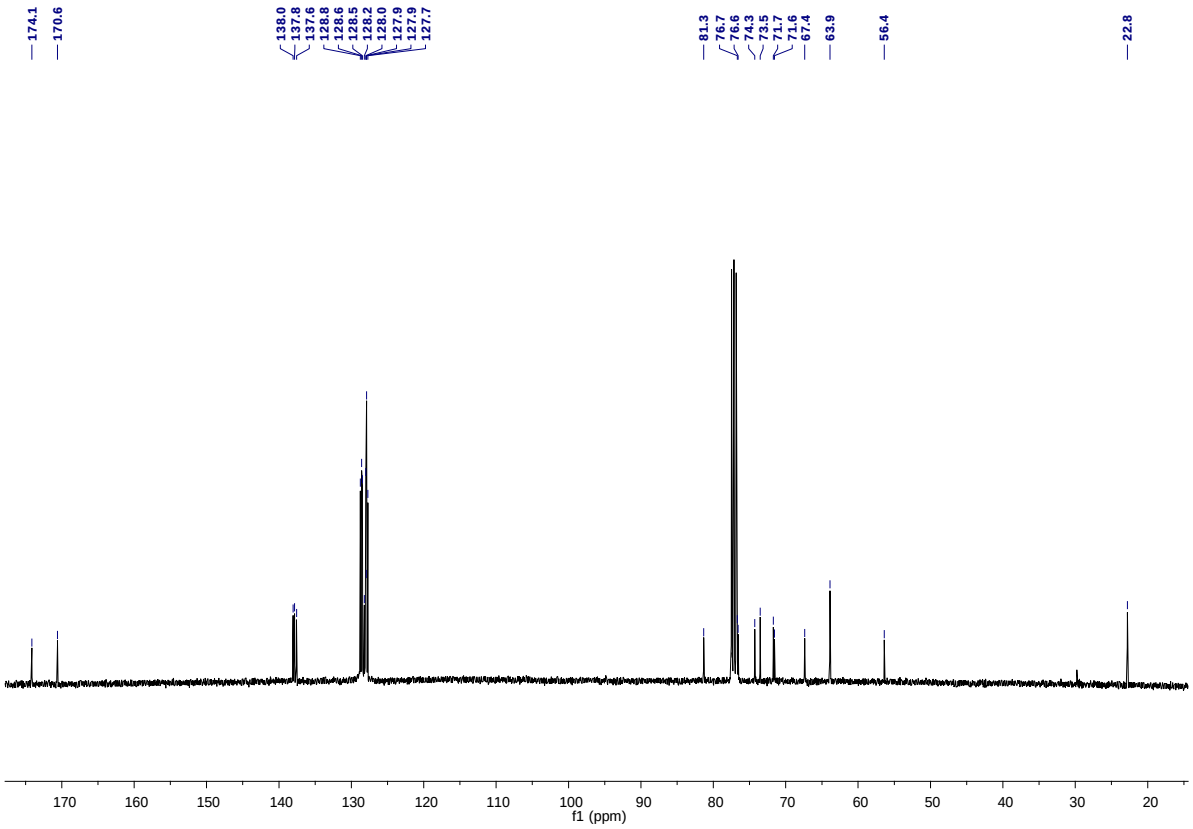
HSQC in $CDCl_3$



^{1}H NMR 400 MHz in $CDCl_3$



^{13}C NMR 400 MHz in $CDCl_3$



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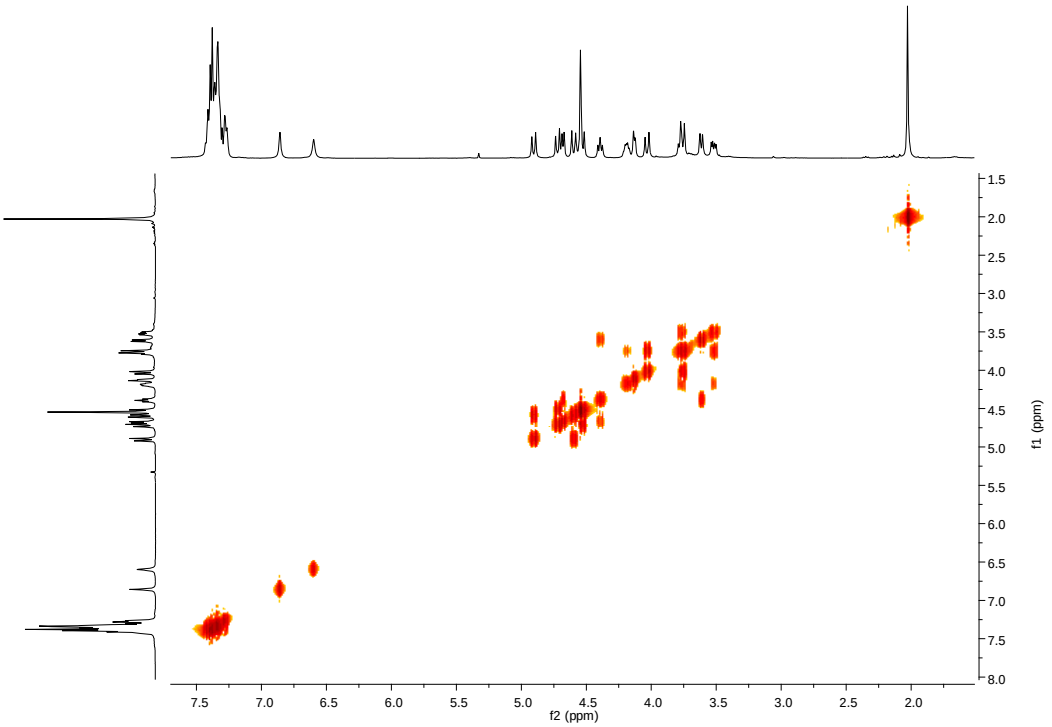

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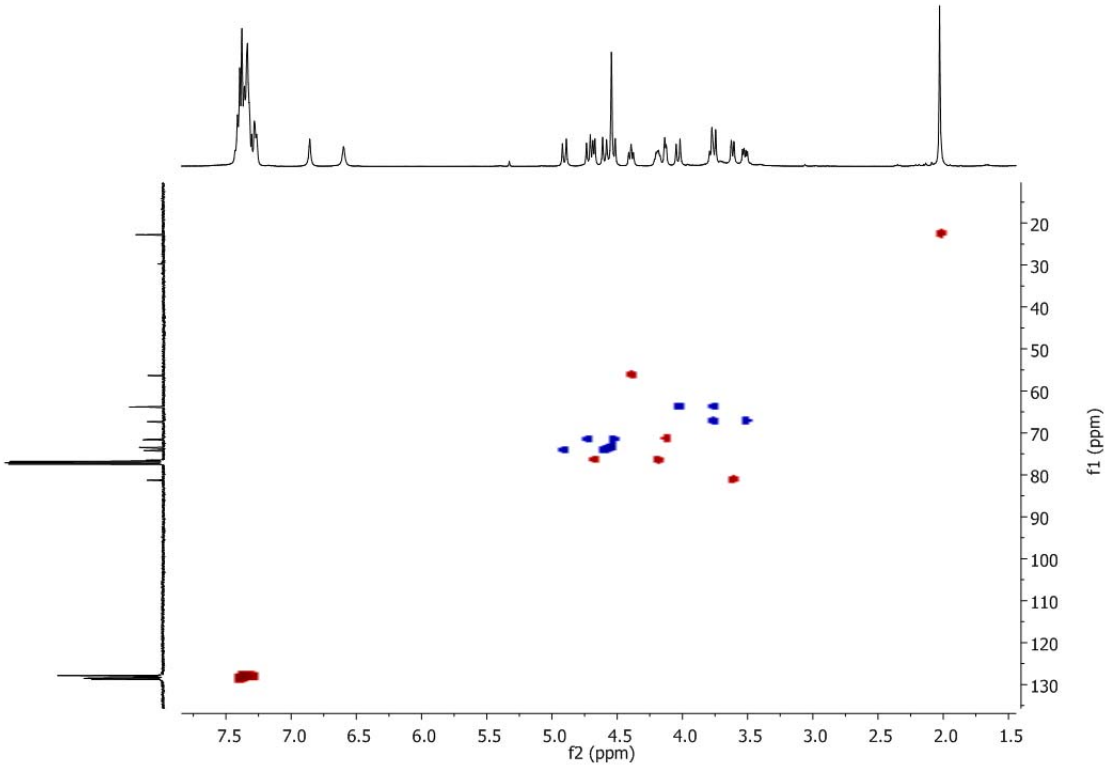
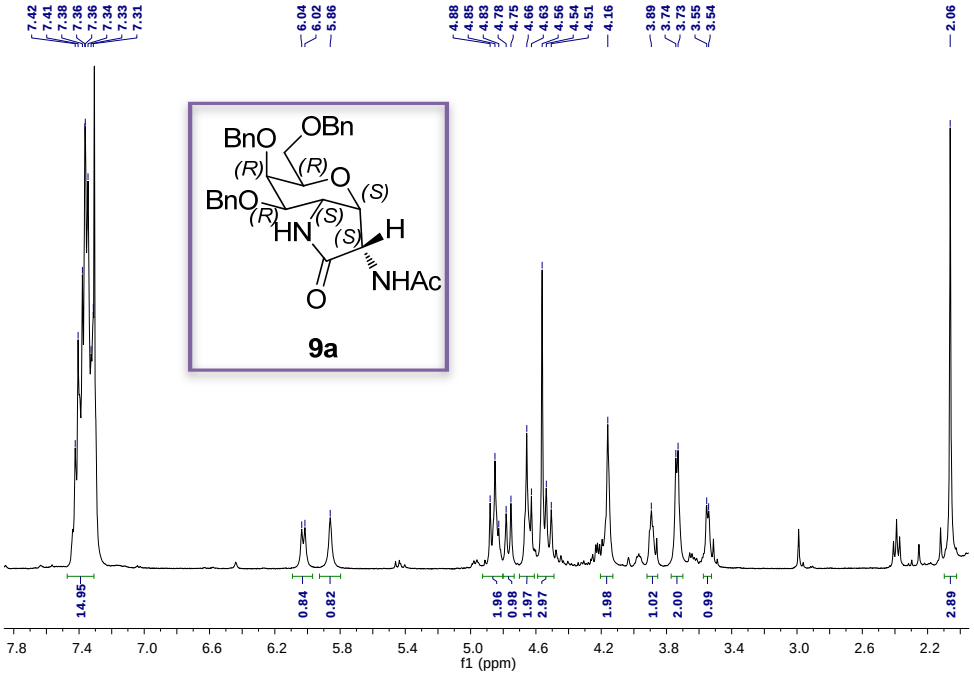
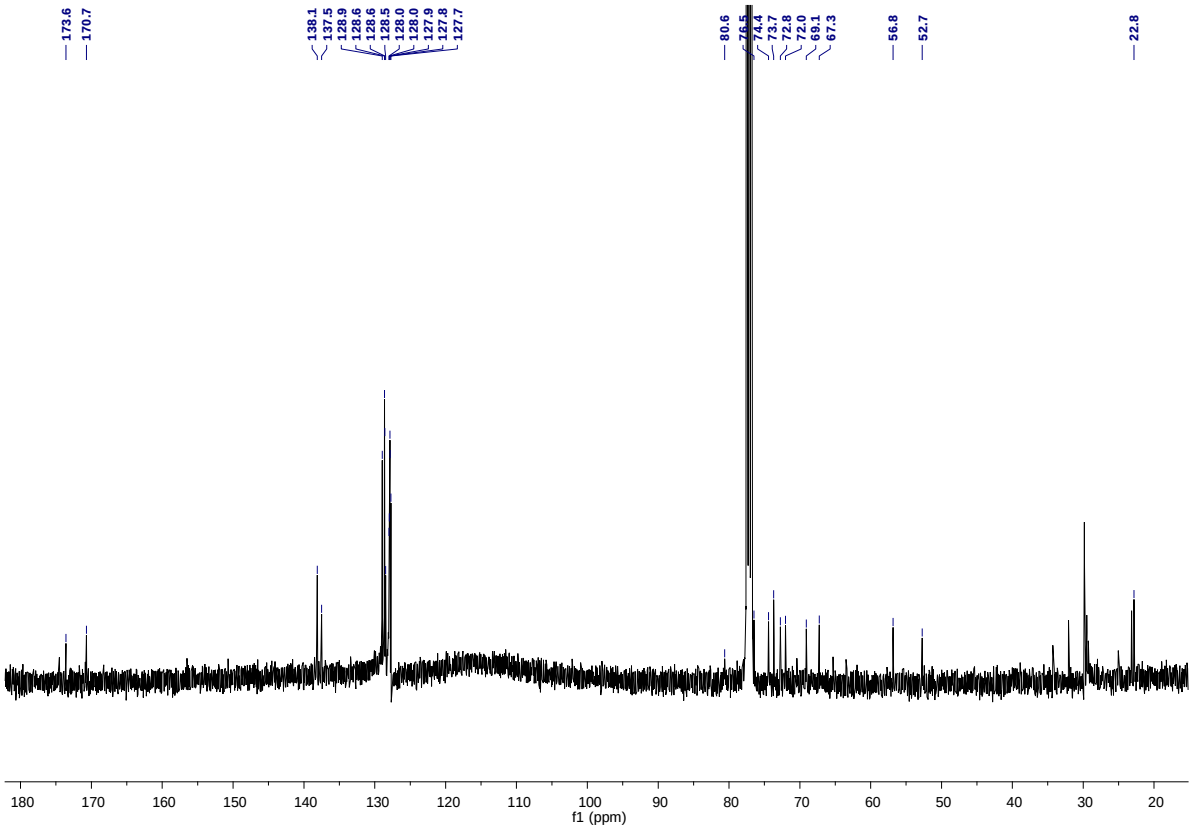


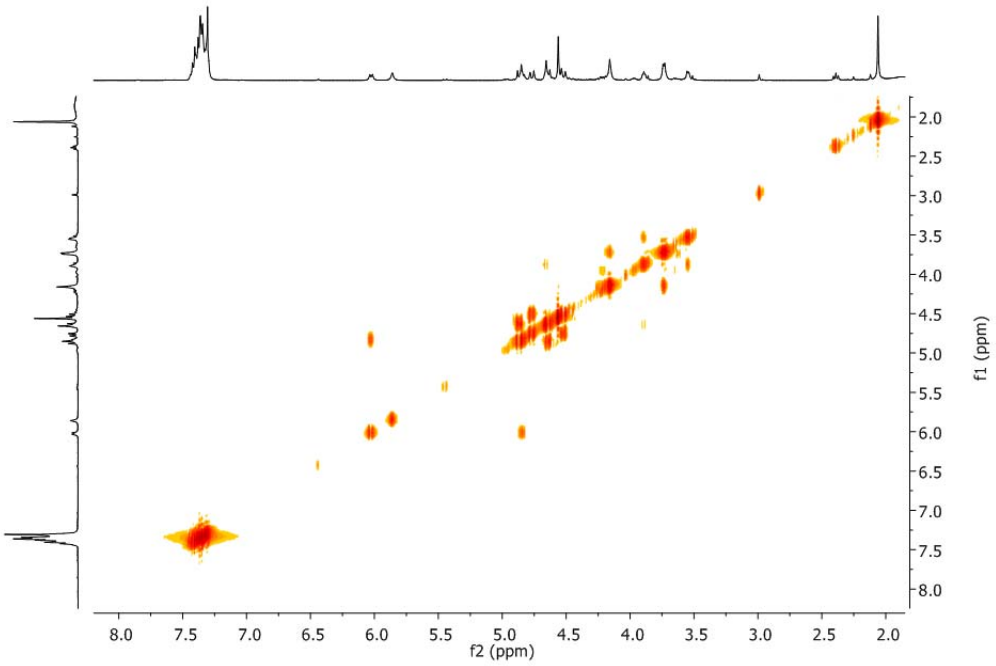
^{1}H NMR 400 MHz in $CDCl_3$



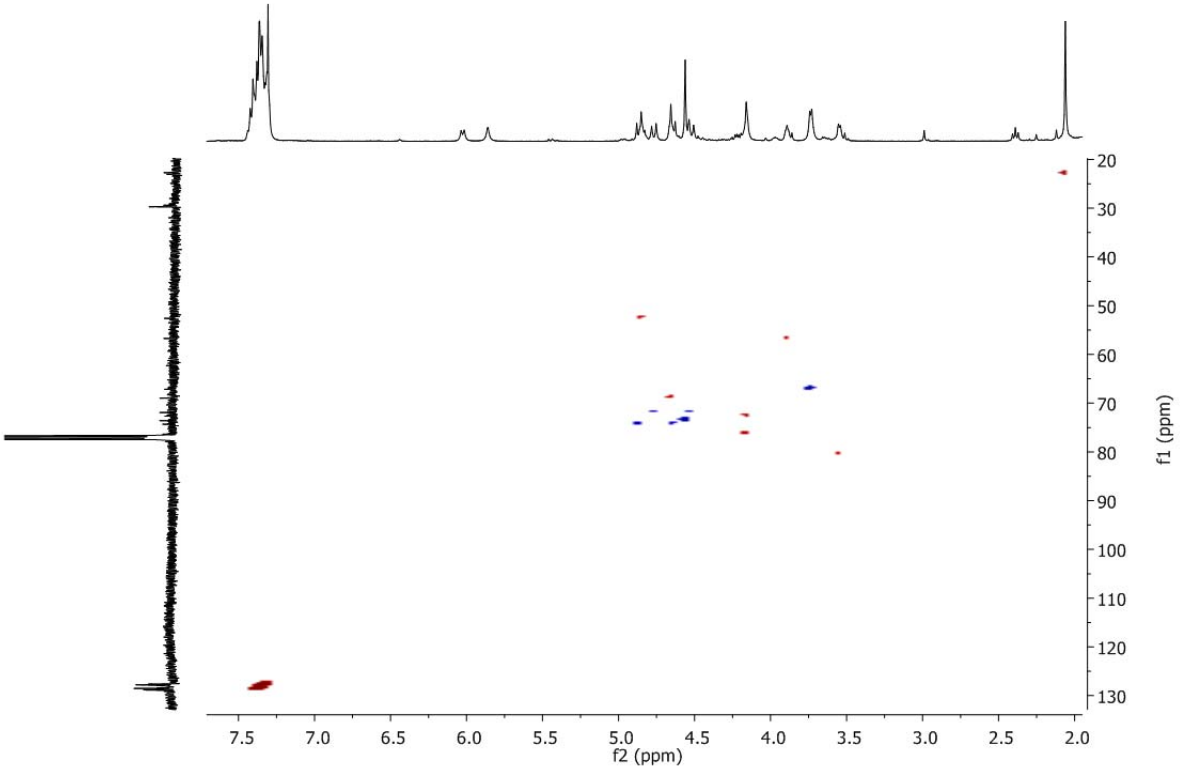
^{13}C NMR 400 MHz in $CDCl_3$



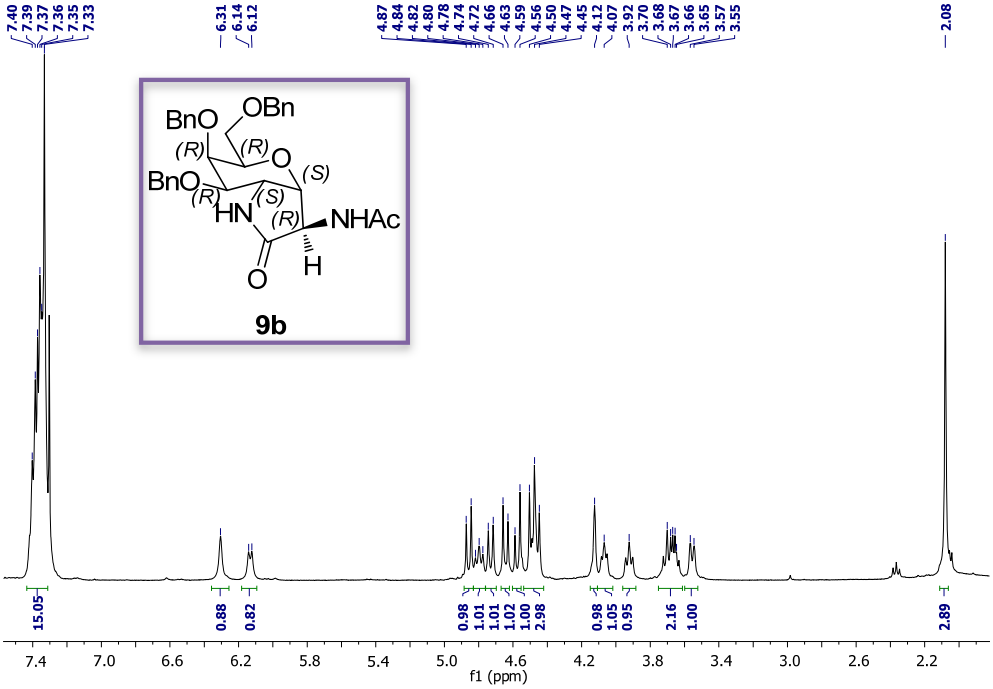
COSY in $CDCl_3$



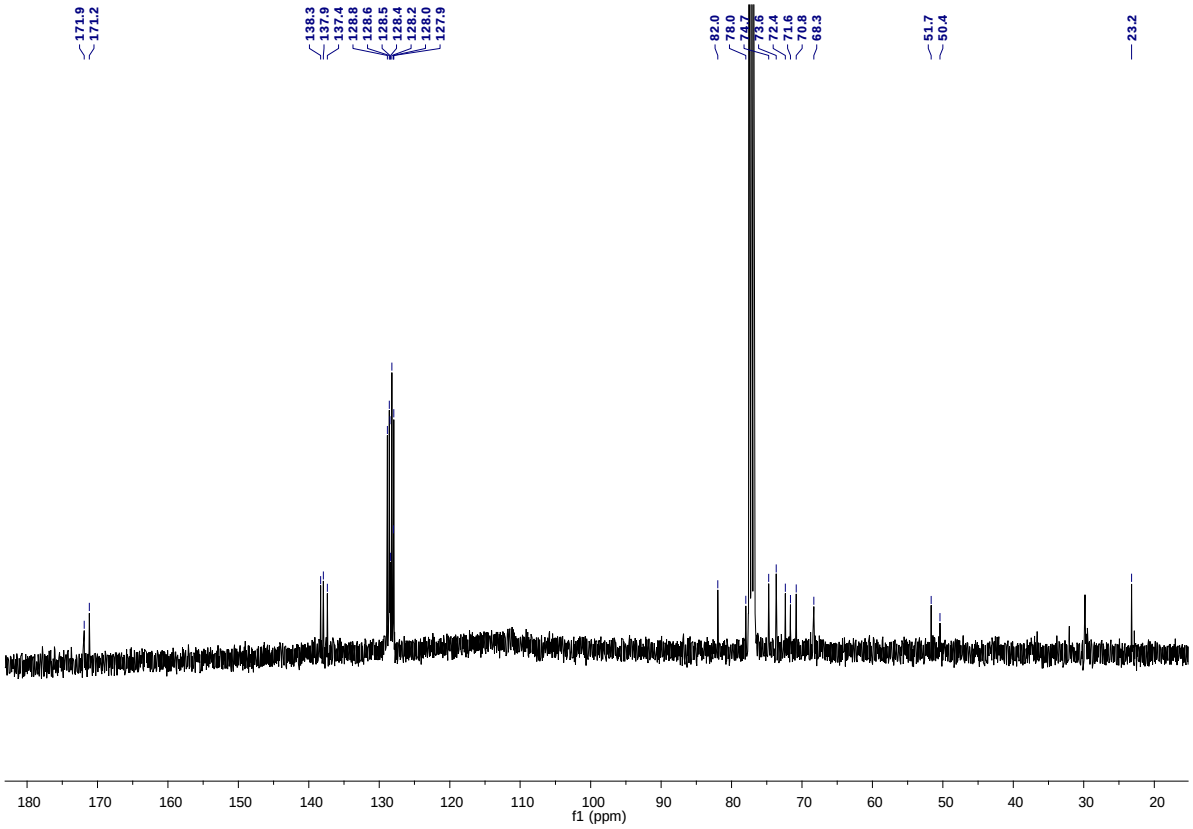
HSQC in $CDCl_3$



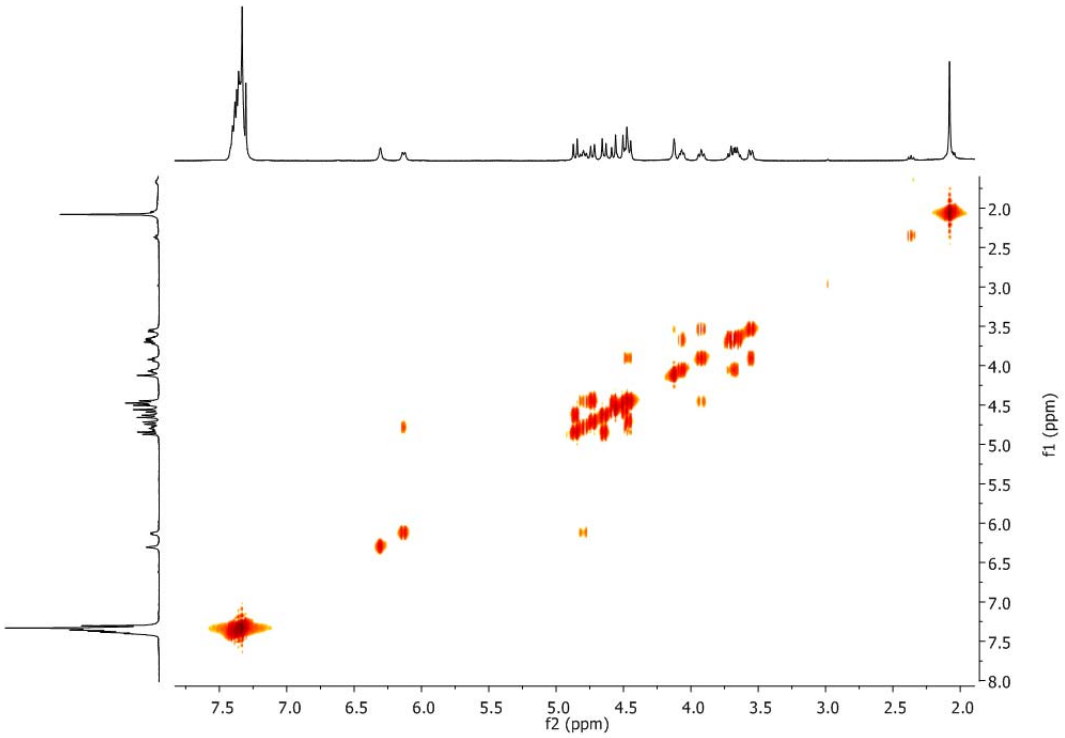
^{1}H NMR 400 MHz in $CDCl_3$



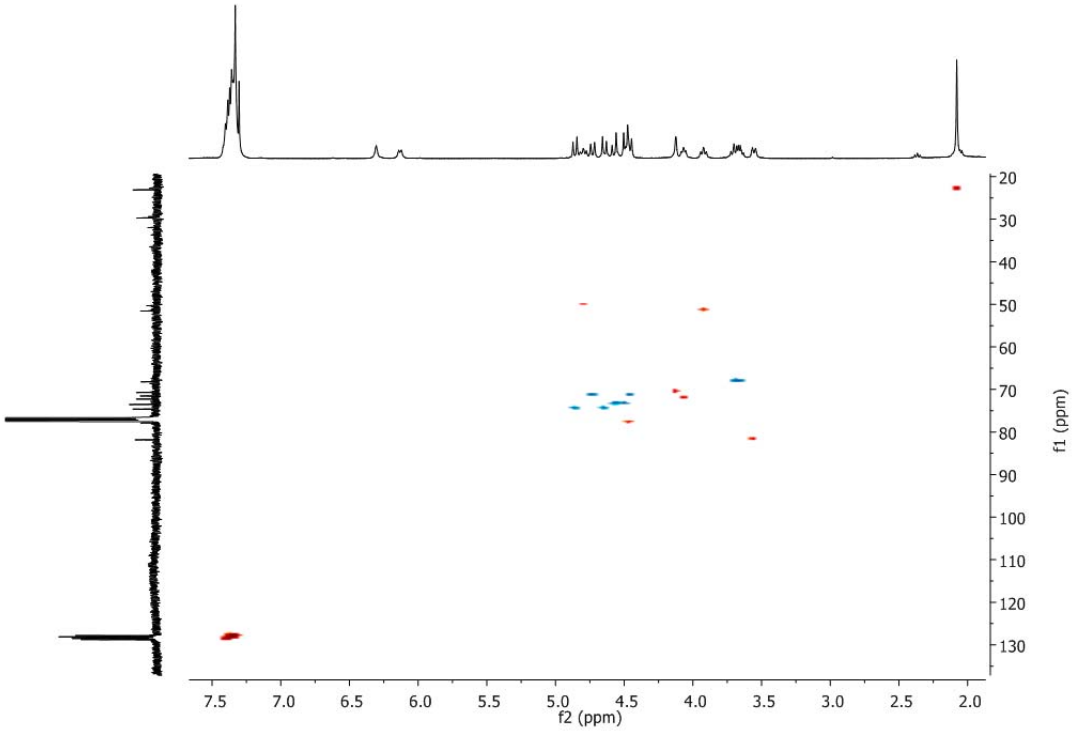
^{13}C NMR 400 MHz in $CDCl_3$



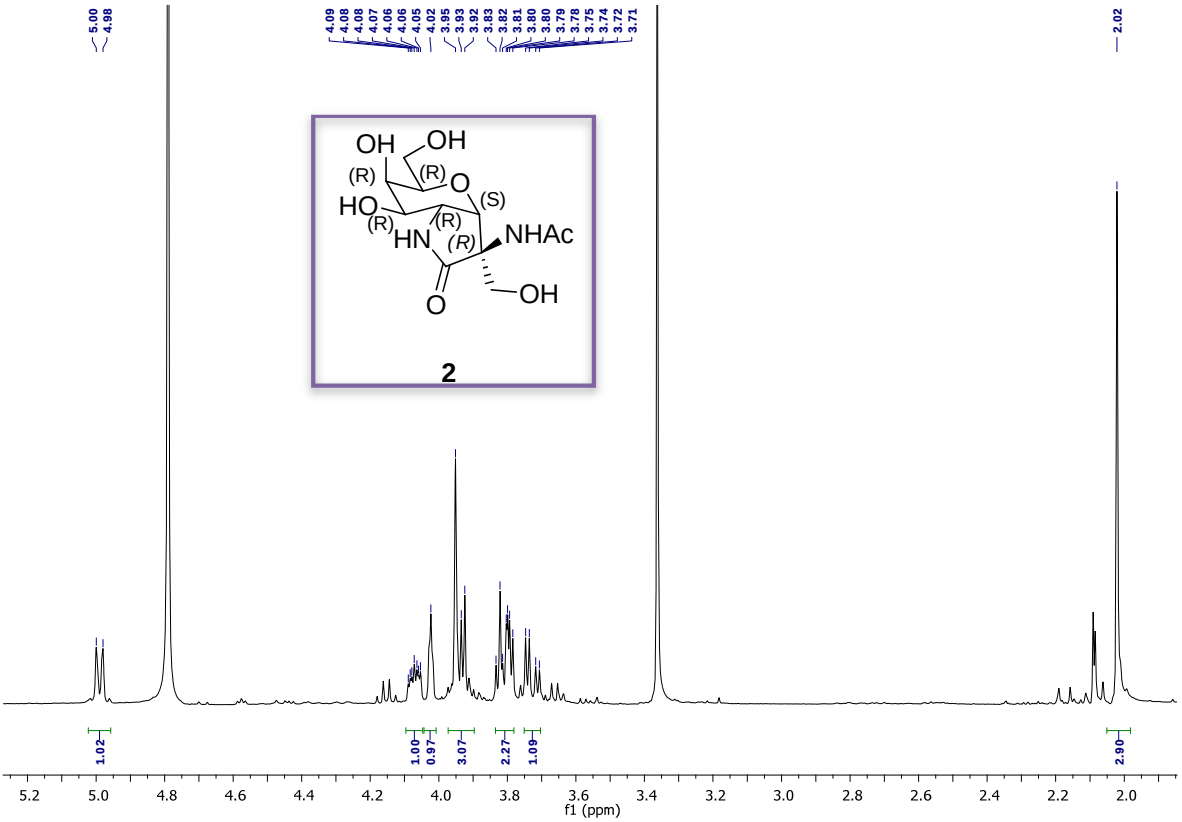
COSY in $CDCl_3$



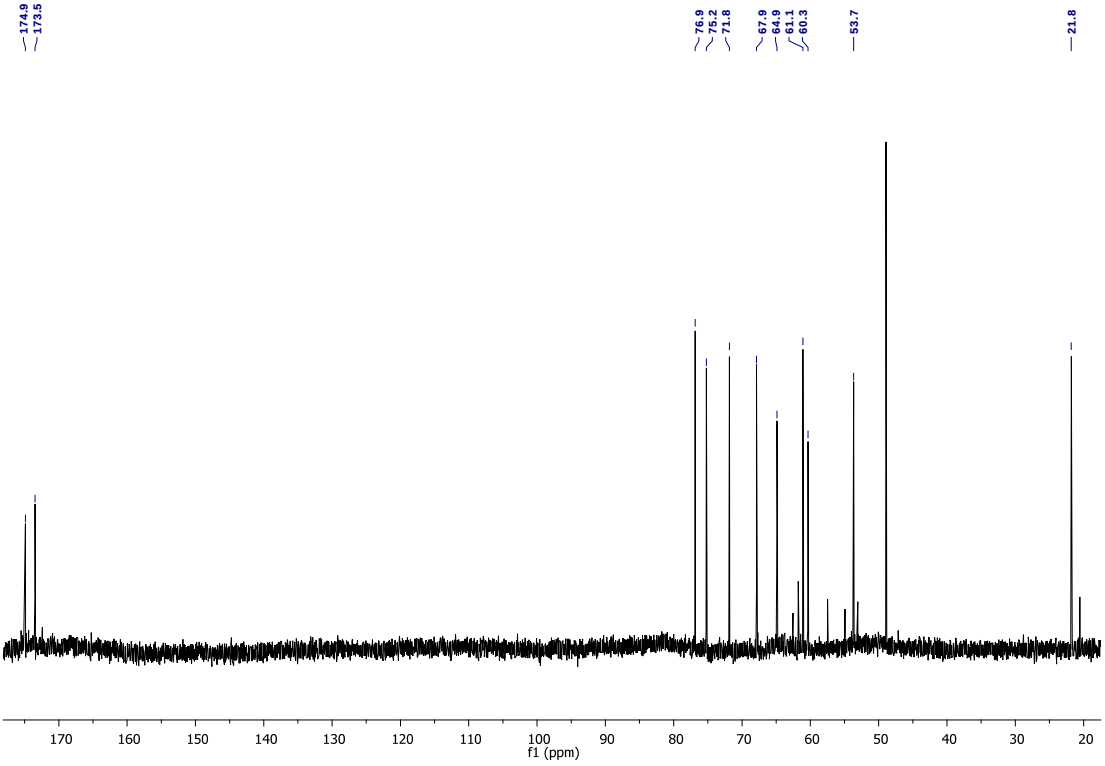
HSQC in $CDCl_3$



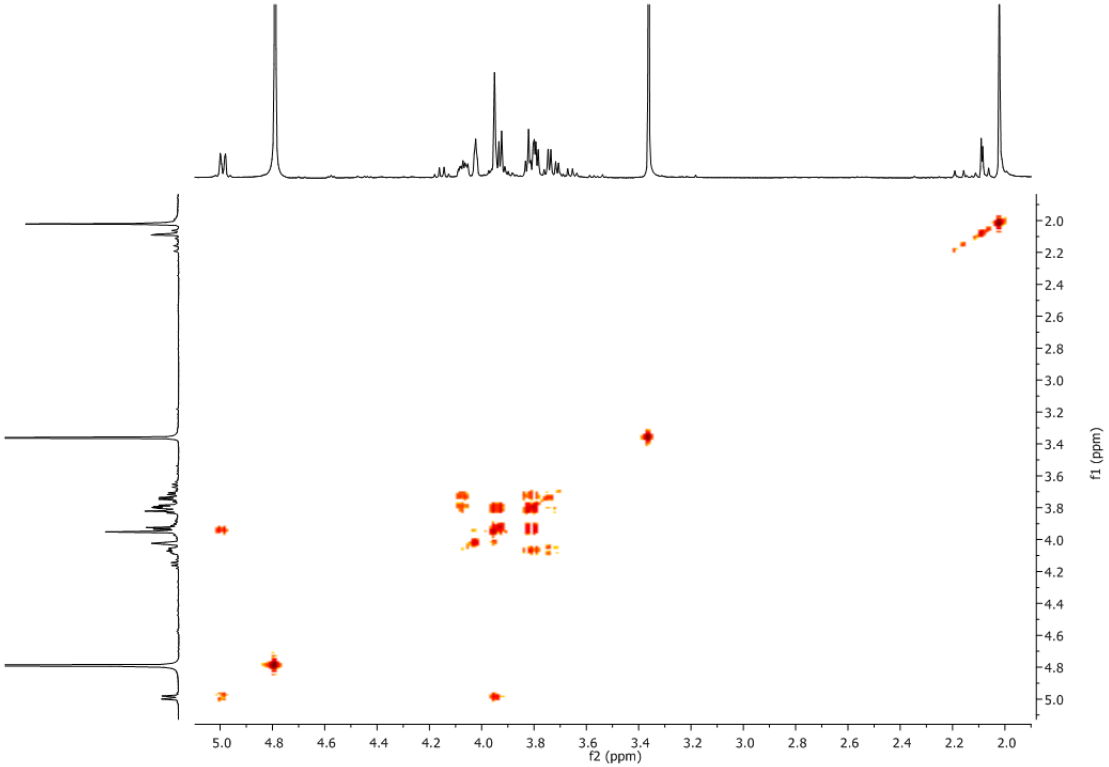
^{1}H NMR 400 MHz in CD_3OD



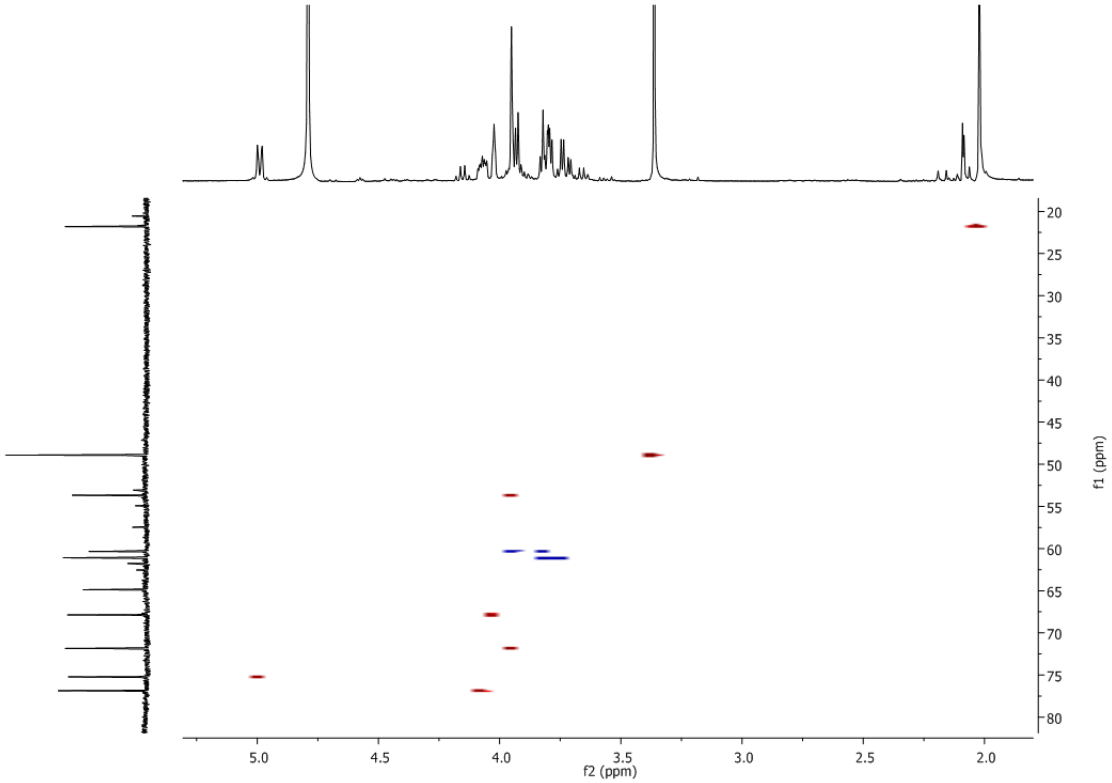
^{13}C NMR 400 MHz in CD_3OD



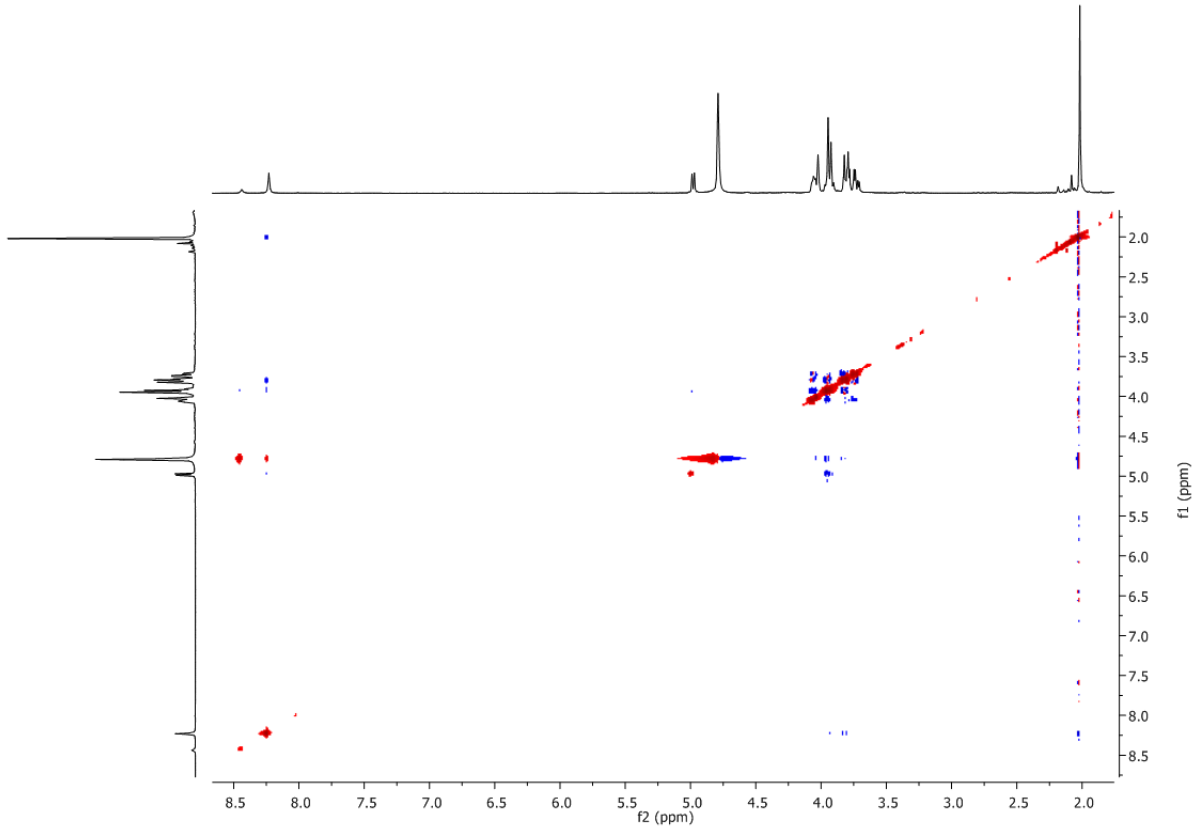
COSY in CD_3OD



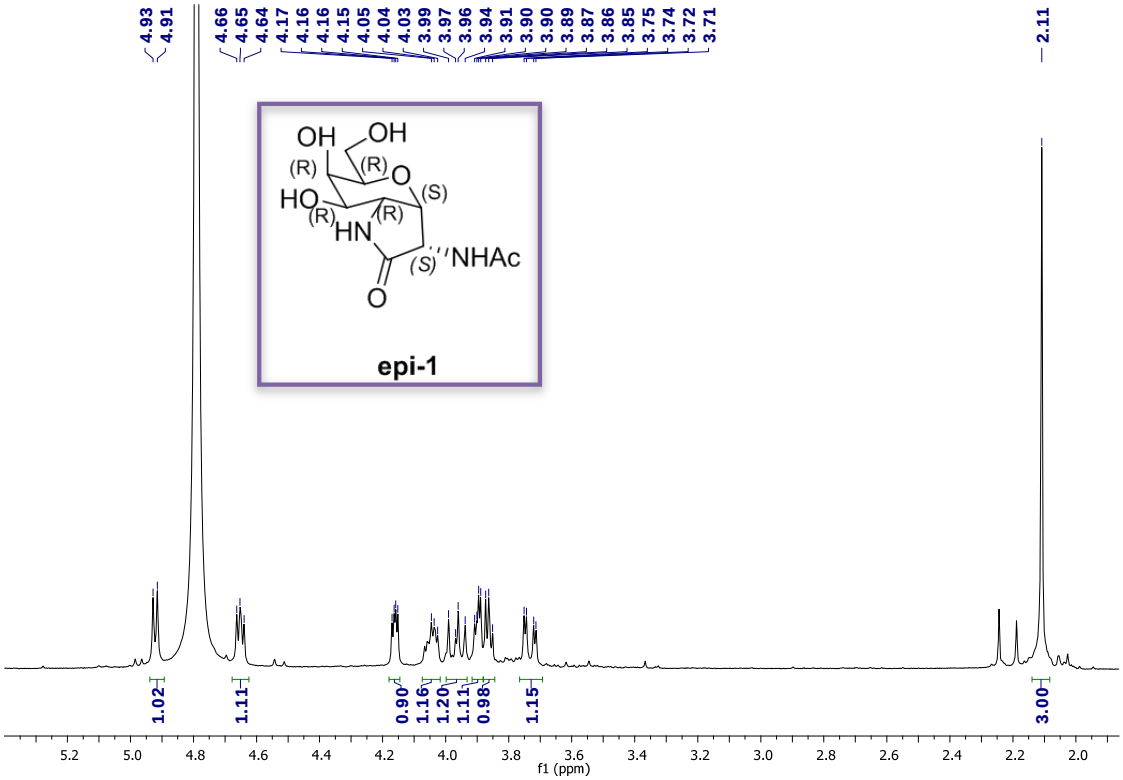
HSQC in CD_3OD



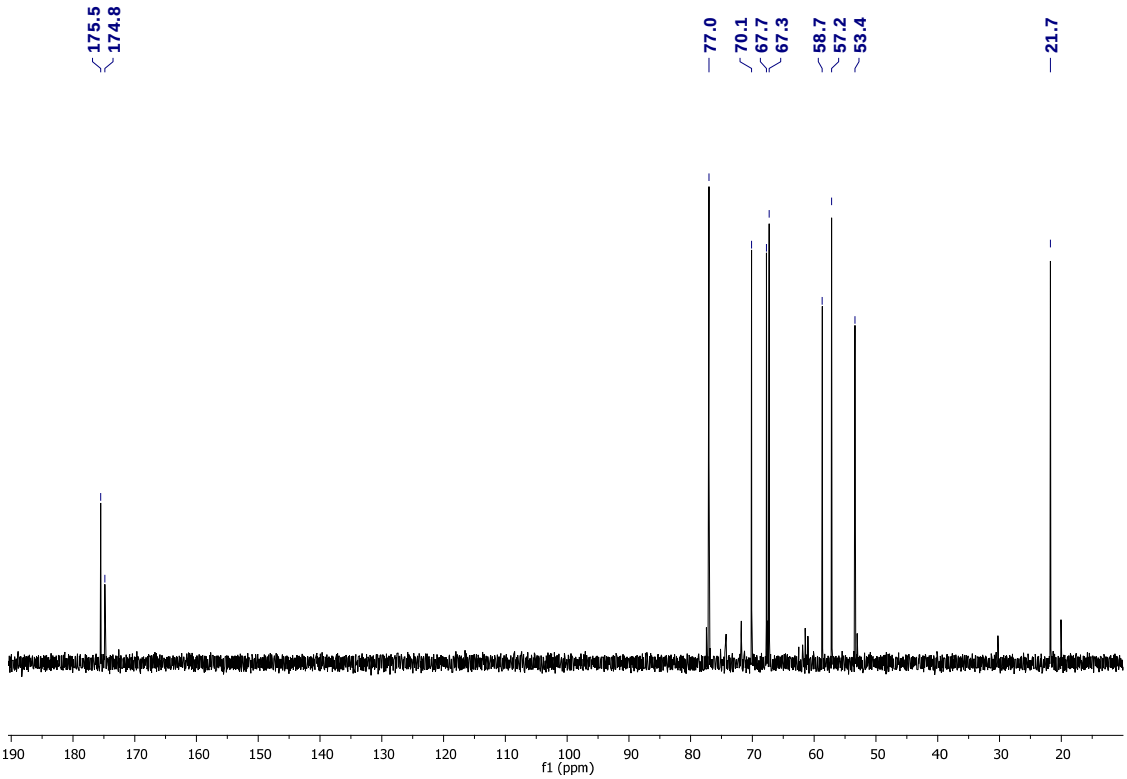
NOESY in H_2O/D_2O (9:1)



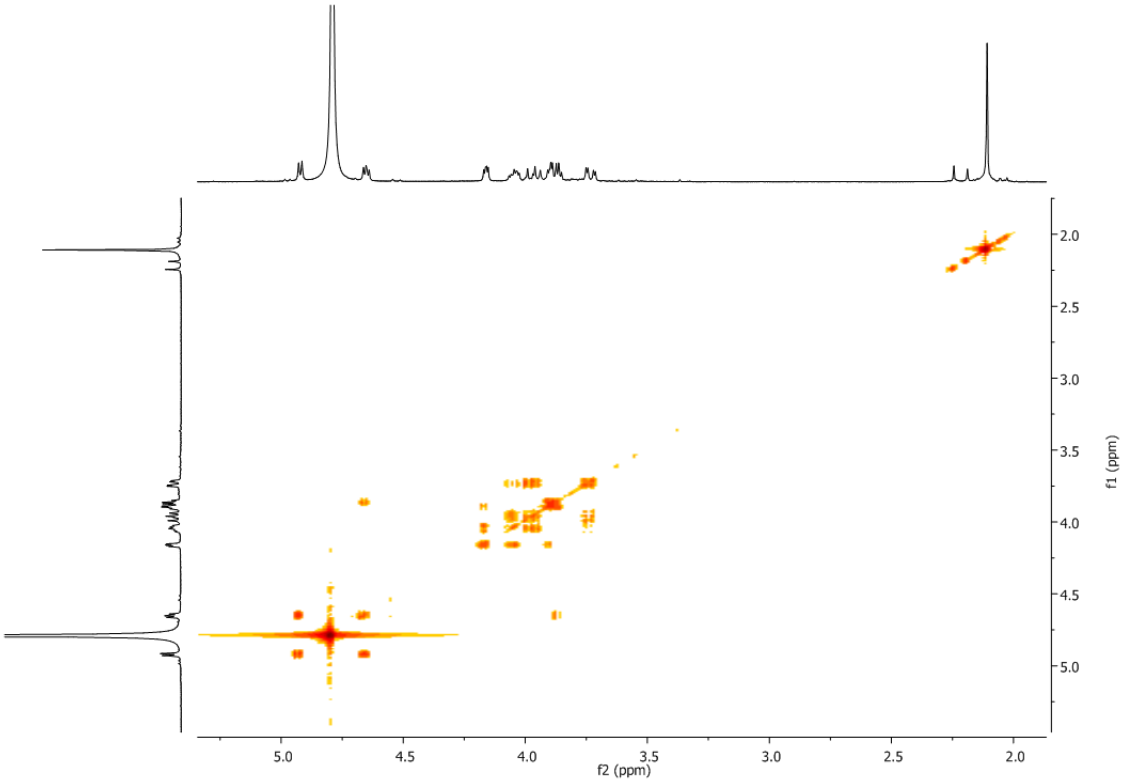
^{1}H NMR 400 MHz in D_2O



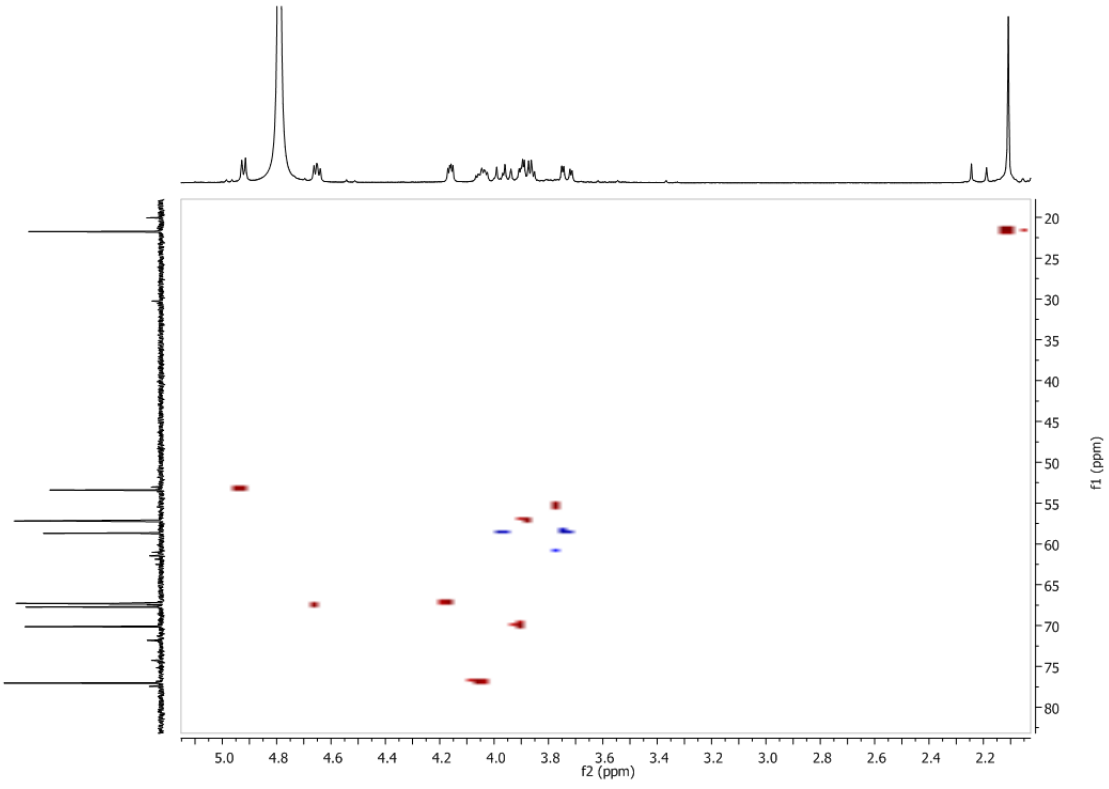
^{13}C NMR 400 MHz in D_2O



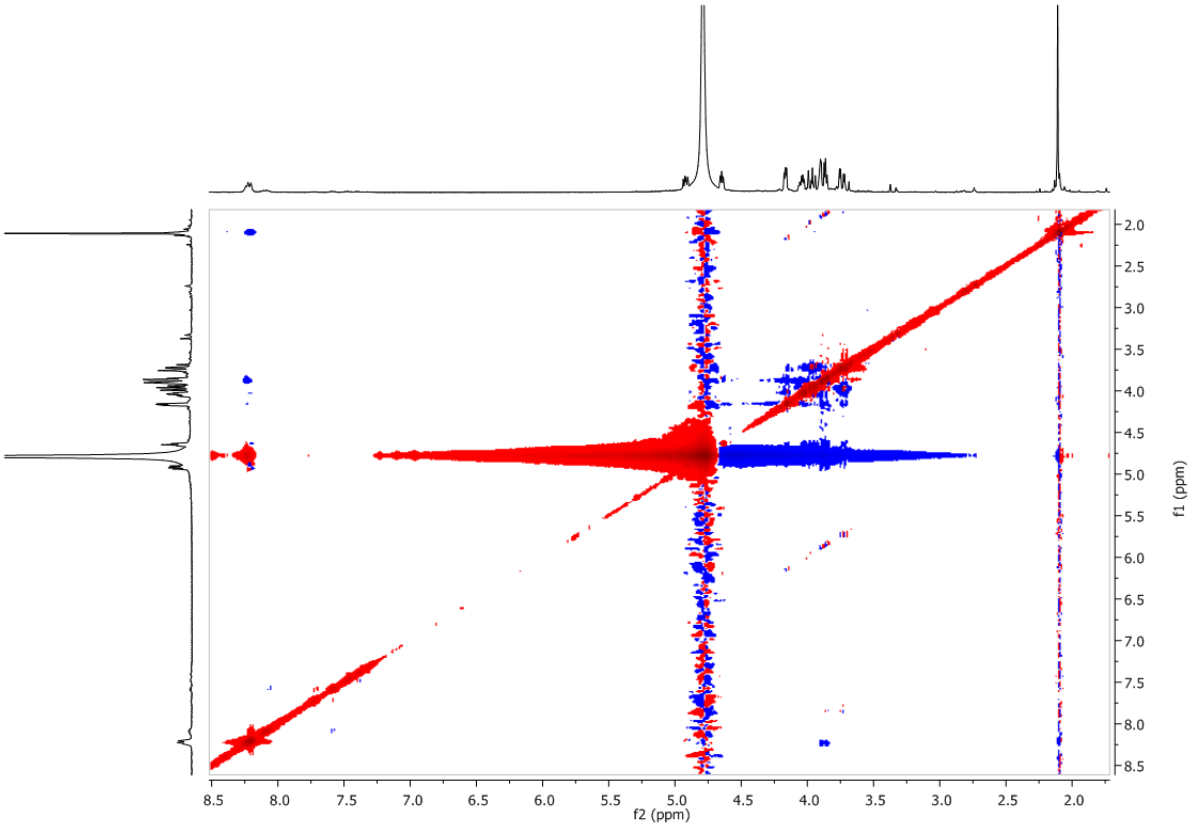
COSY in D_2O


5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)
f1 (ppm)

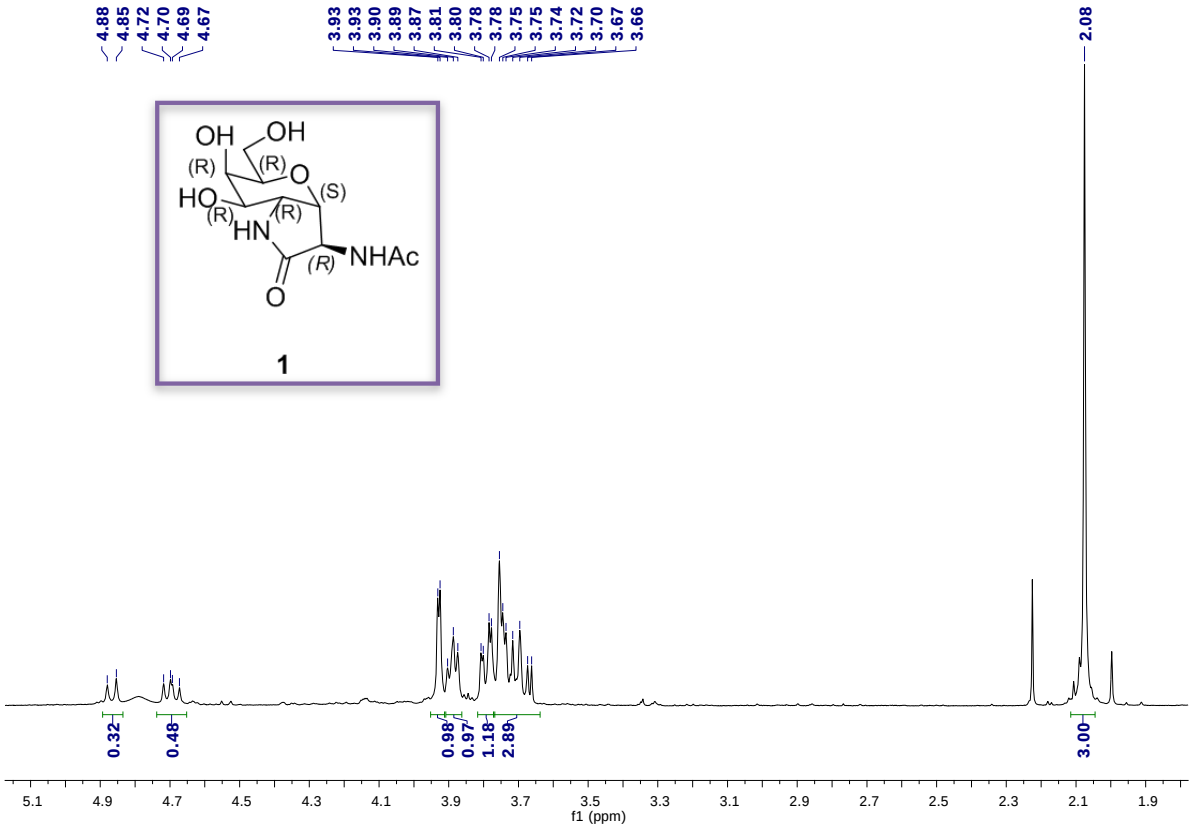
HSQC in D_2O


5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
f2 (ppm)
f1 (ppm)

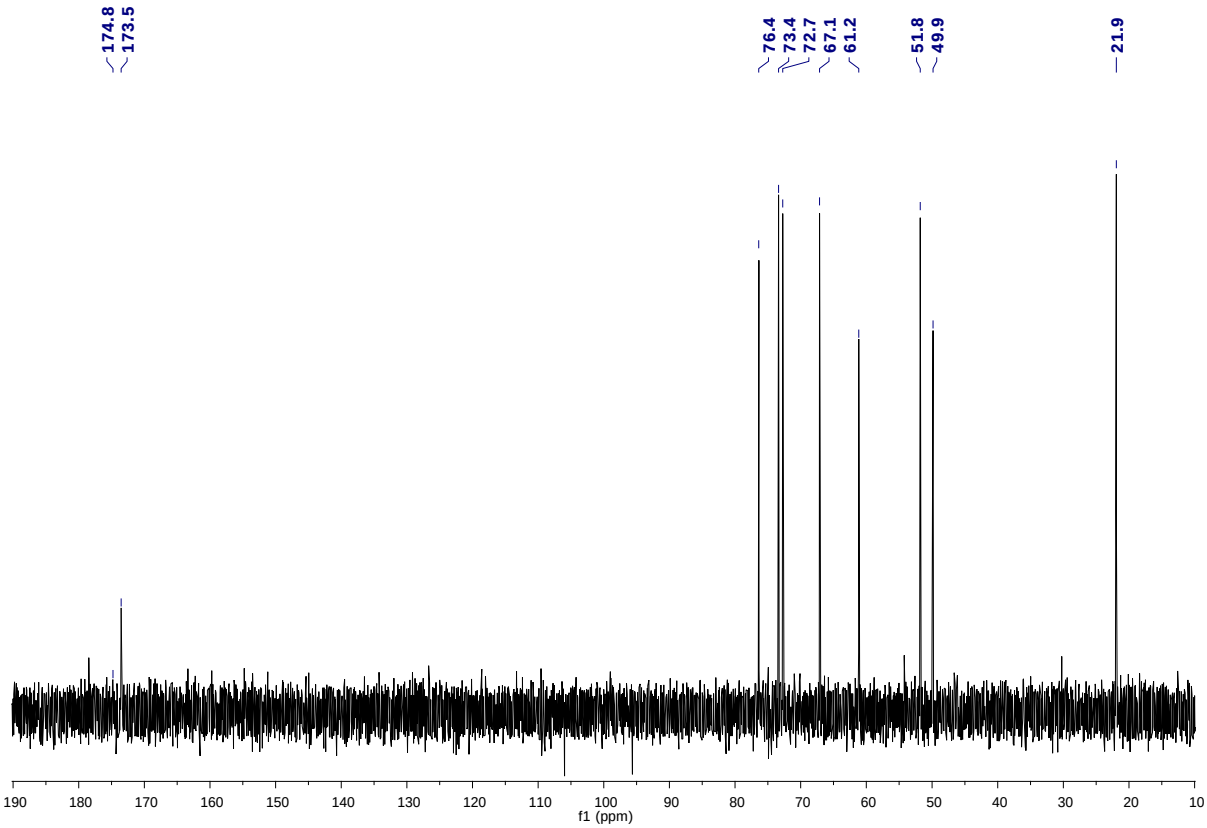
NOESY in D_2O/H_2O (1:9)



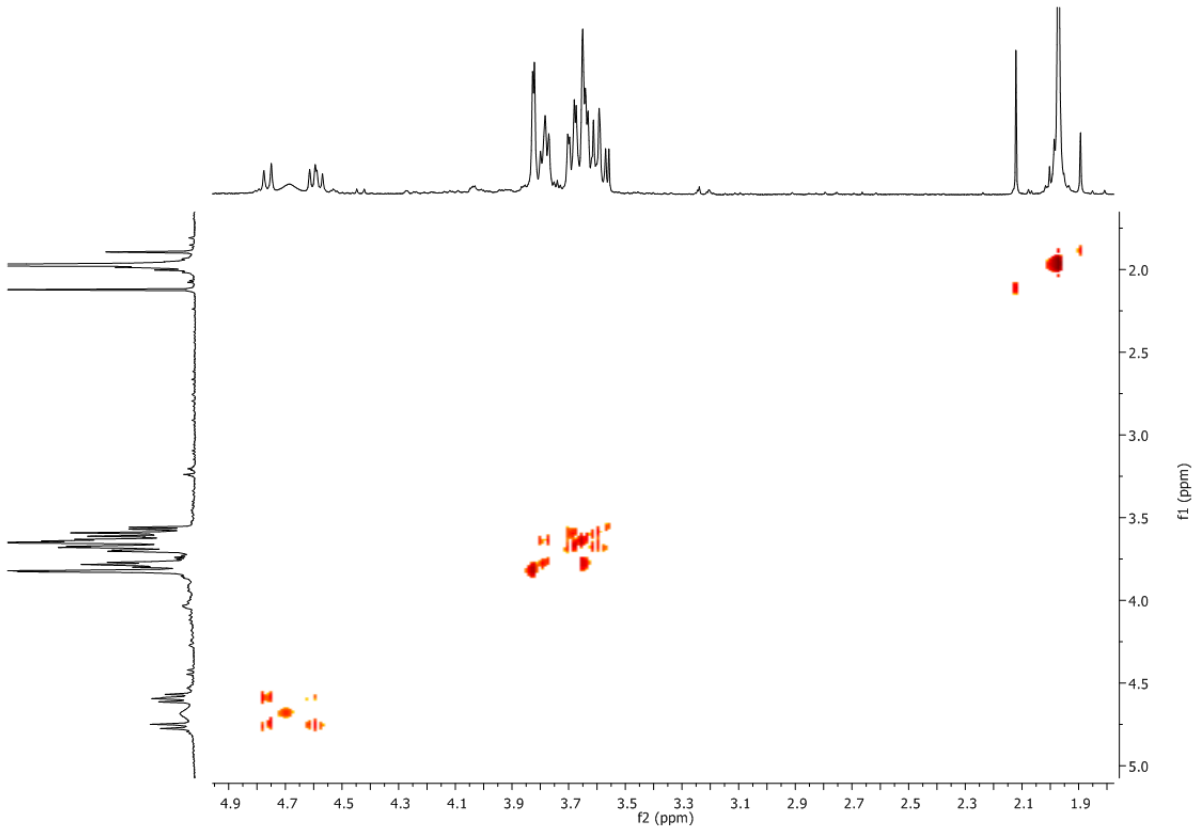
^{1}H NMR in D_2O



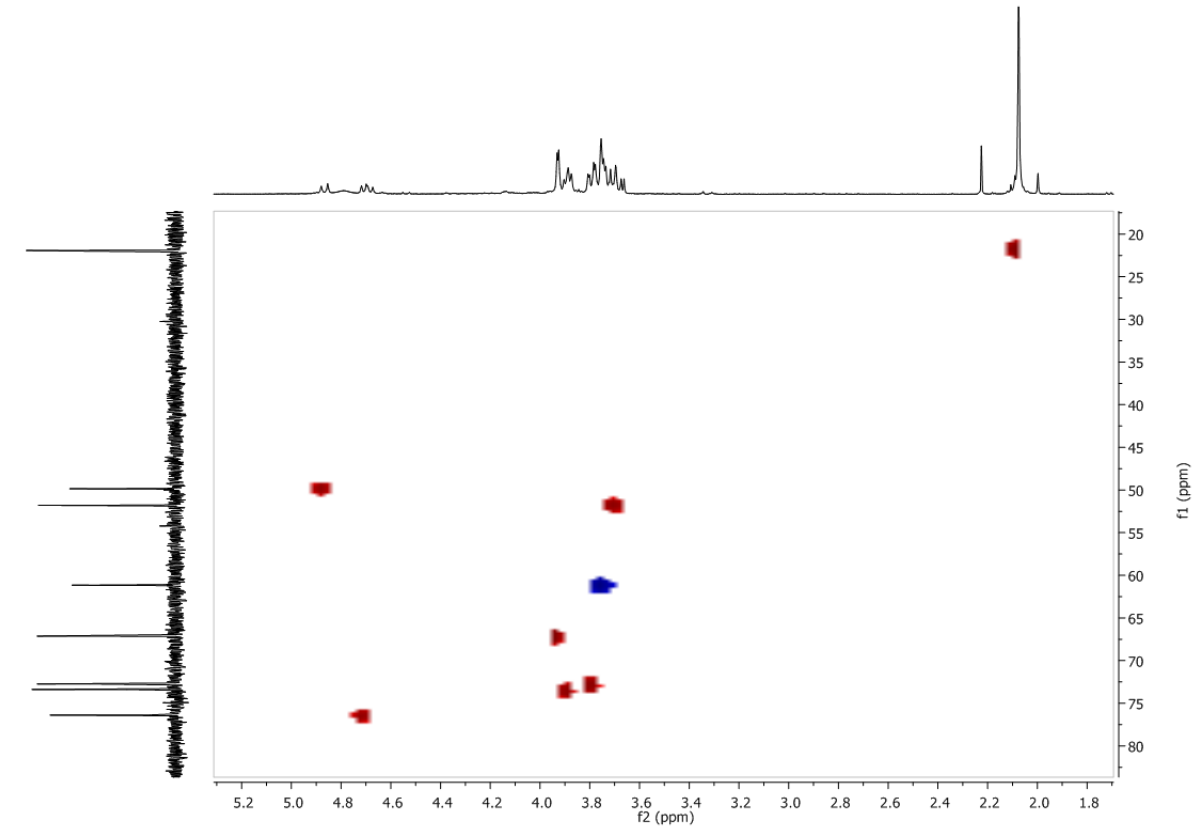
^{13}C NMR in D_2O



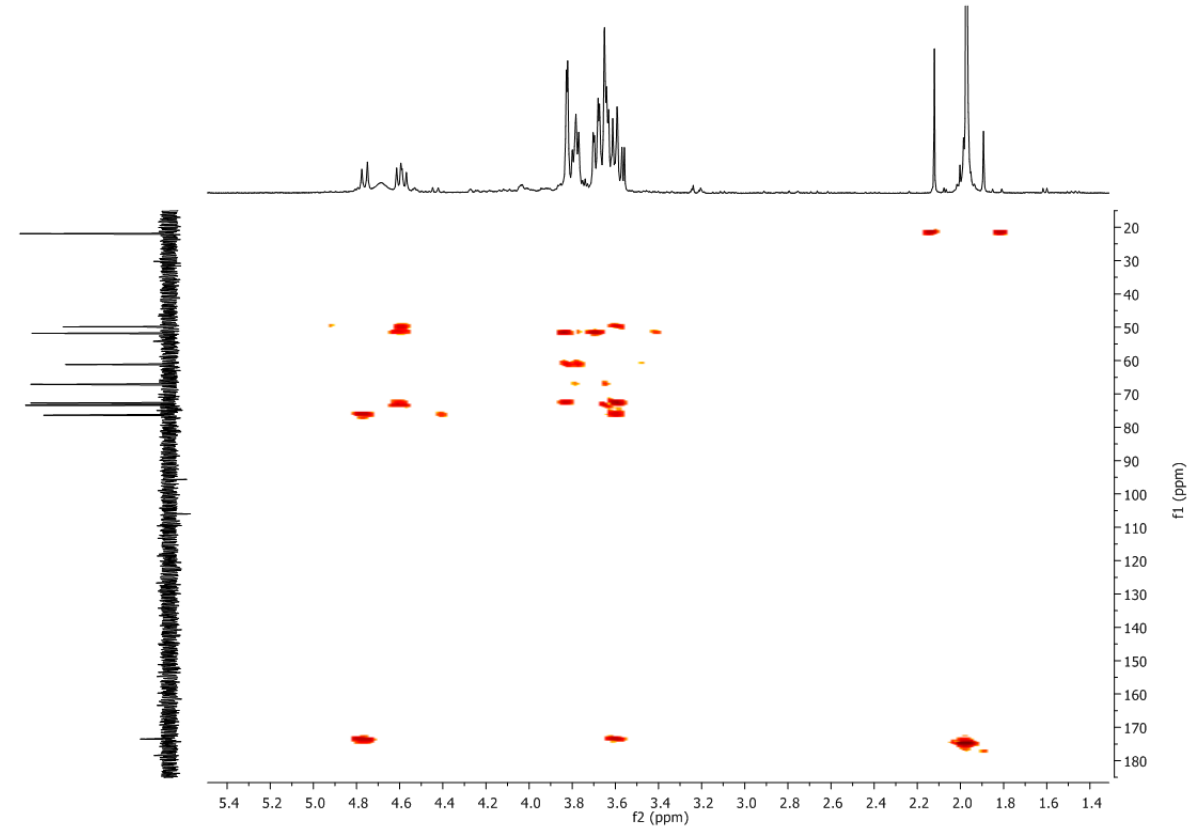
COSY in D_2O



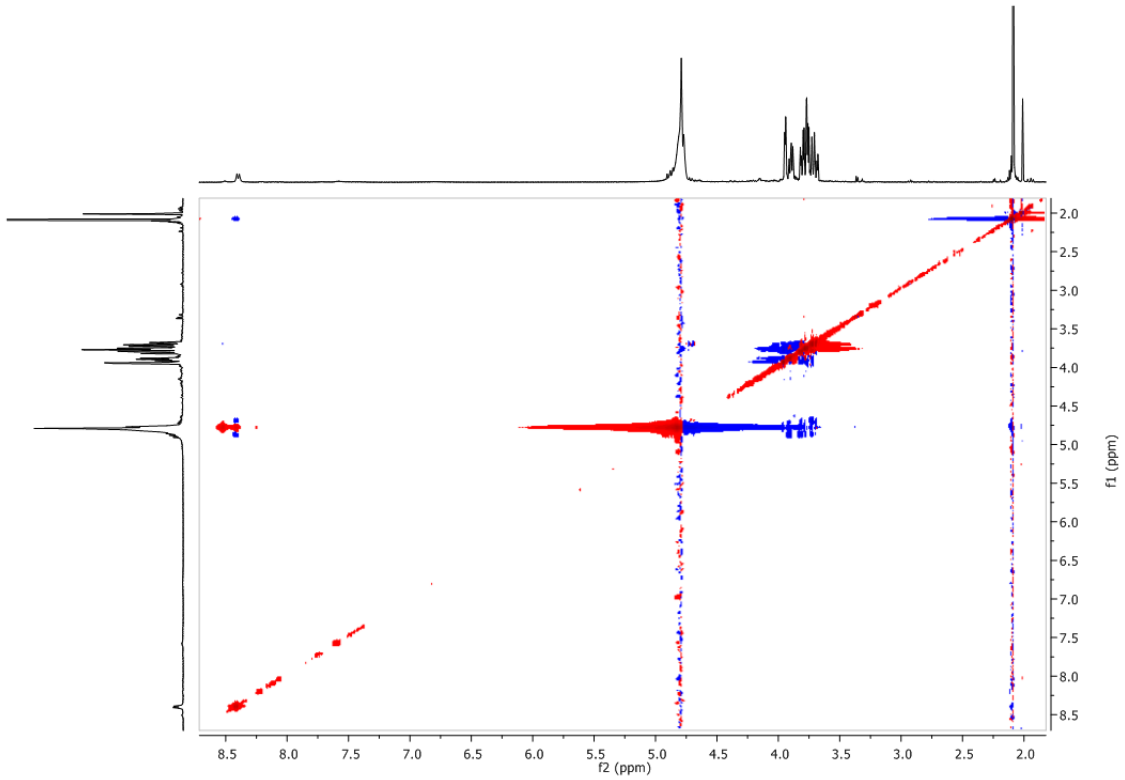
HSQC in D_2O



HMBC in D_2O



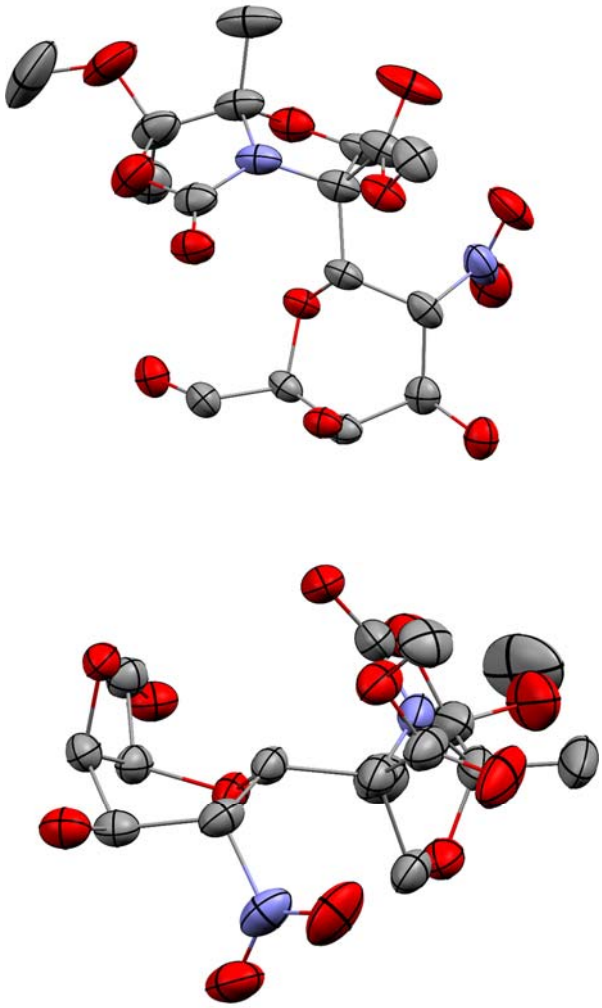
NOESY in D_2O/H_2O (1:9)



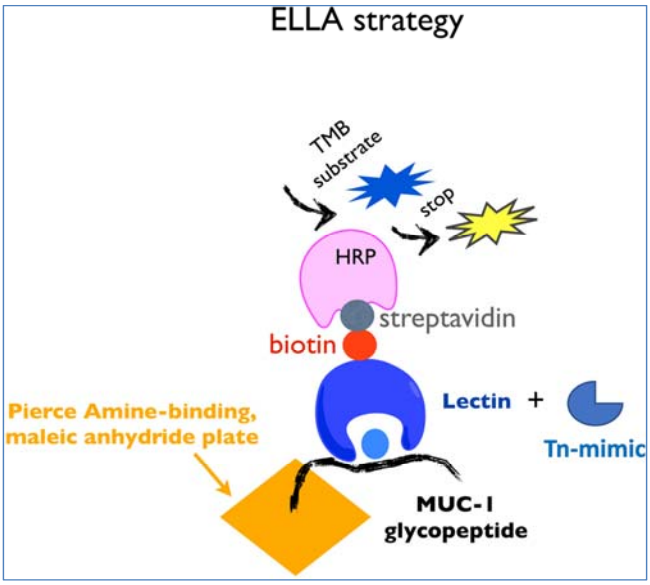
X-ray data of compound 5a

Crystal data for **5a**: $C_{37}H_{42}N_2O_{12}$, M_w = 706.73, colourless prism of 0.50 x 0.22 x 0.20 mm, T= 173 K, Triclinic, space group P 1, Z = 2, a = 10.9237(5) Å, b = 13.2890(6) Å, c = 13.9727(5) Å, α = 63.682°(2) β = 81.338°(2) γ = *81.120°(2),* V = 1788.56(14) Å^3, d_{calc} = 1.312 g cm^{-3}, F(000) = 748, λ = 0.71073 Å (Mo, Ka), μ = 0.098 mm^{-1}, kappa CCD diffractometer, θ range 1.00 - 27.48°, 14718 collected reflections, 928 unique, full-matrix least-squares (SHELXL97), R_1 = 0.0565, wR_2 = 0.1411, (R_1 = 0.0988, wR_2 = 0.1705 all data), goodness of fit = 1.004, residual electron density between 0.255 and -0.231 e Å^{-3}. Hydrogen atoms fitted at theoretical positions.

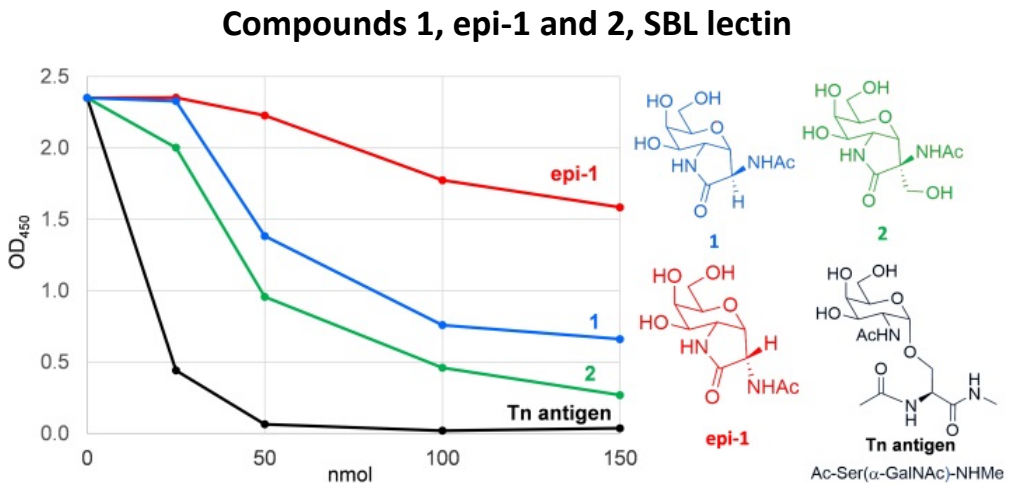
Different views of the ORTEP representation of compound **5a** (Bn groups are omitted for clarity).

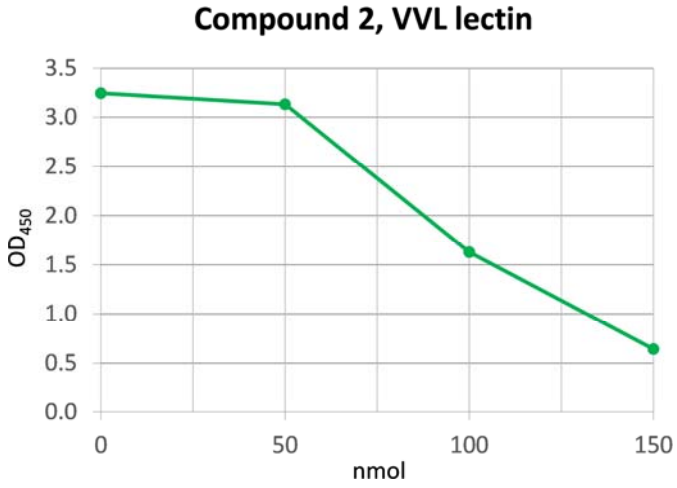


Enzyme-linked lectin assay (ELLA)



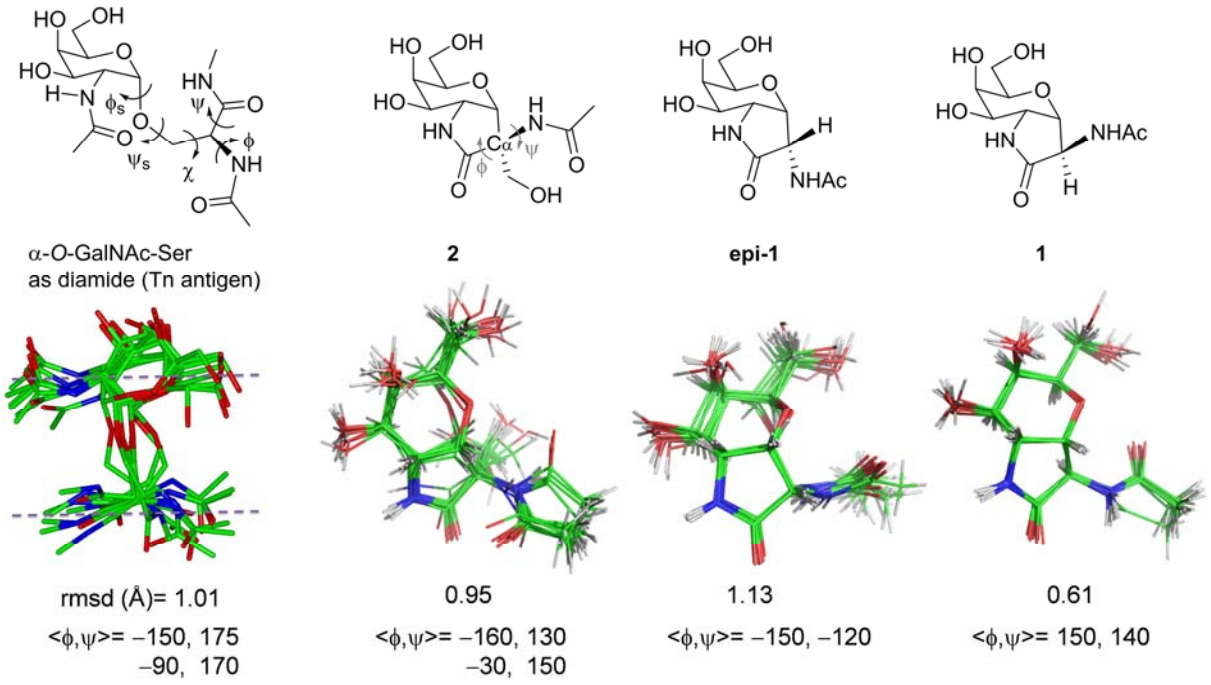
Binding curves obtained for Tn antigen and mimics **1**, **2** and **epi-1** in the competition ELLA assay



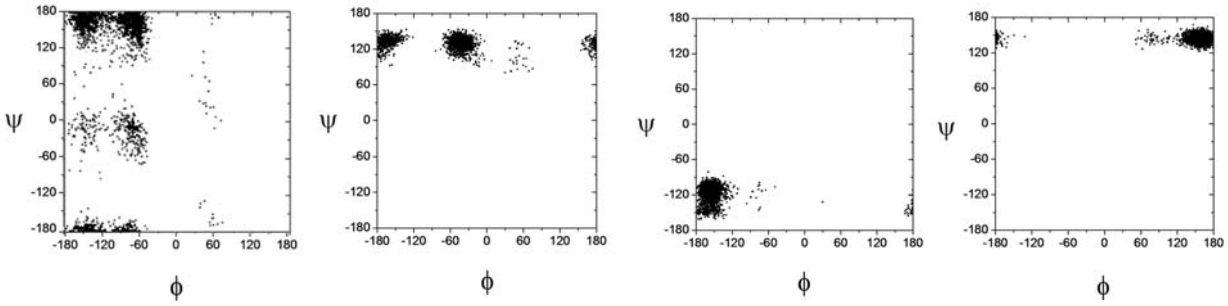


Calculated ensembles for Tn antigen mimics **1, 2** and **epi-1** obtained from the 80 ns MD-tar simulations, showing the RMSDs for heavy atom superimposition.

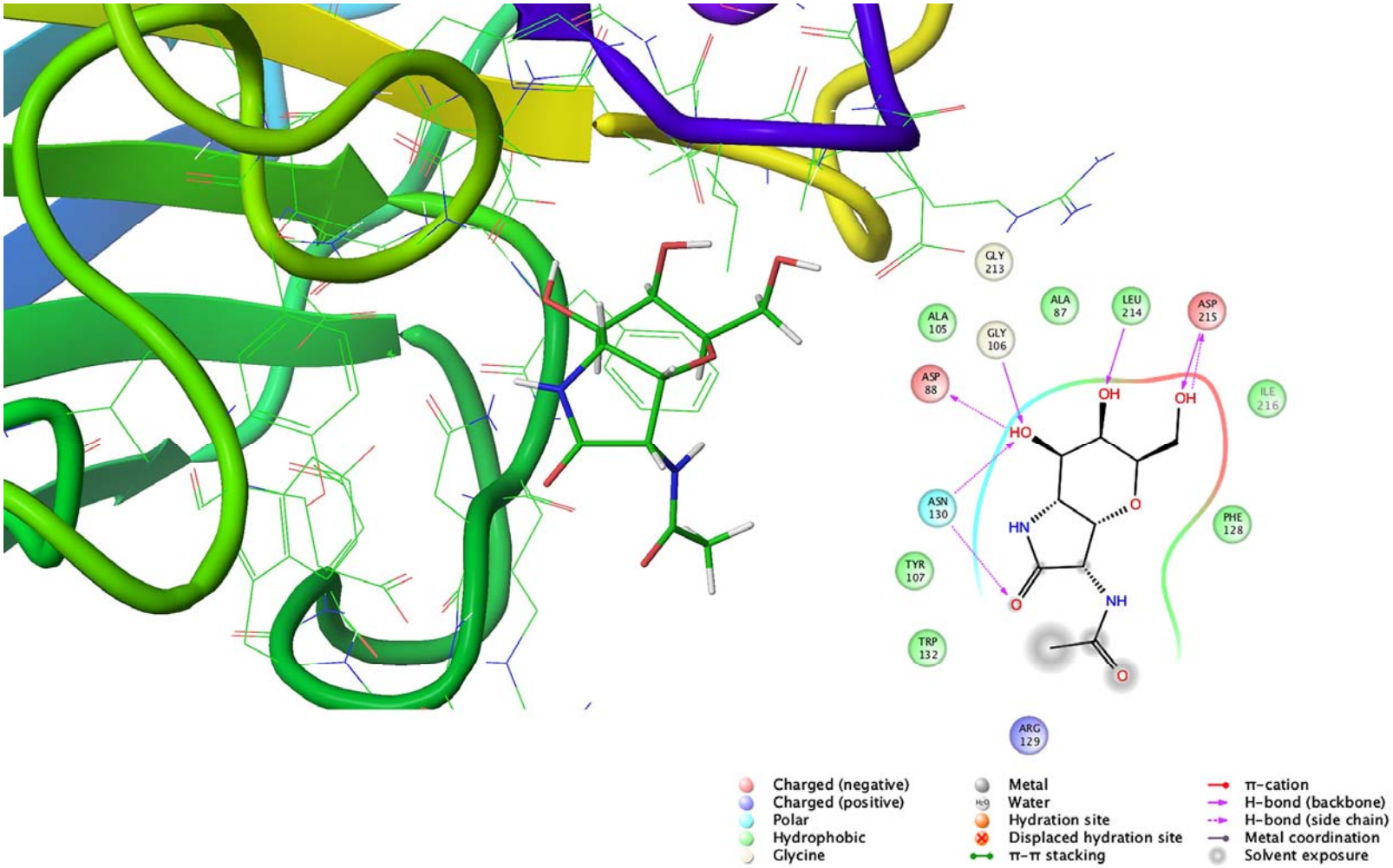
Calculated ensembles



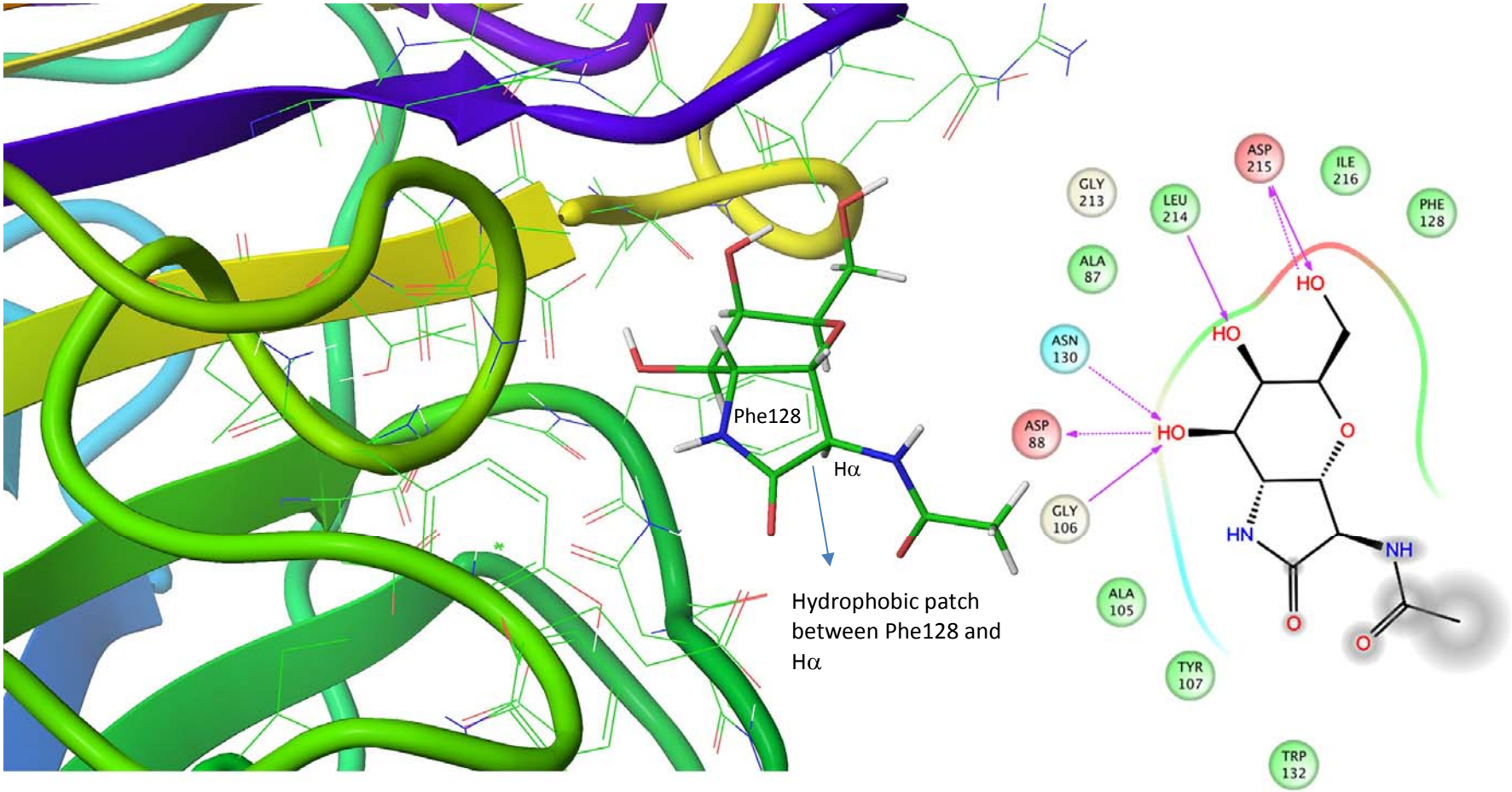
ϕ/ψ distributions obtained from the 80 ns MD-tar simulations



Snapshot taken from the unrestrained 20 ns MD simulation for **SBL:epi-1** complex, together with the ligand interaction diagram obtained with Maestro 9.3.5 software

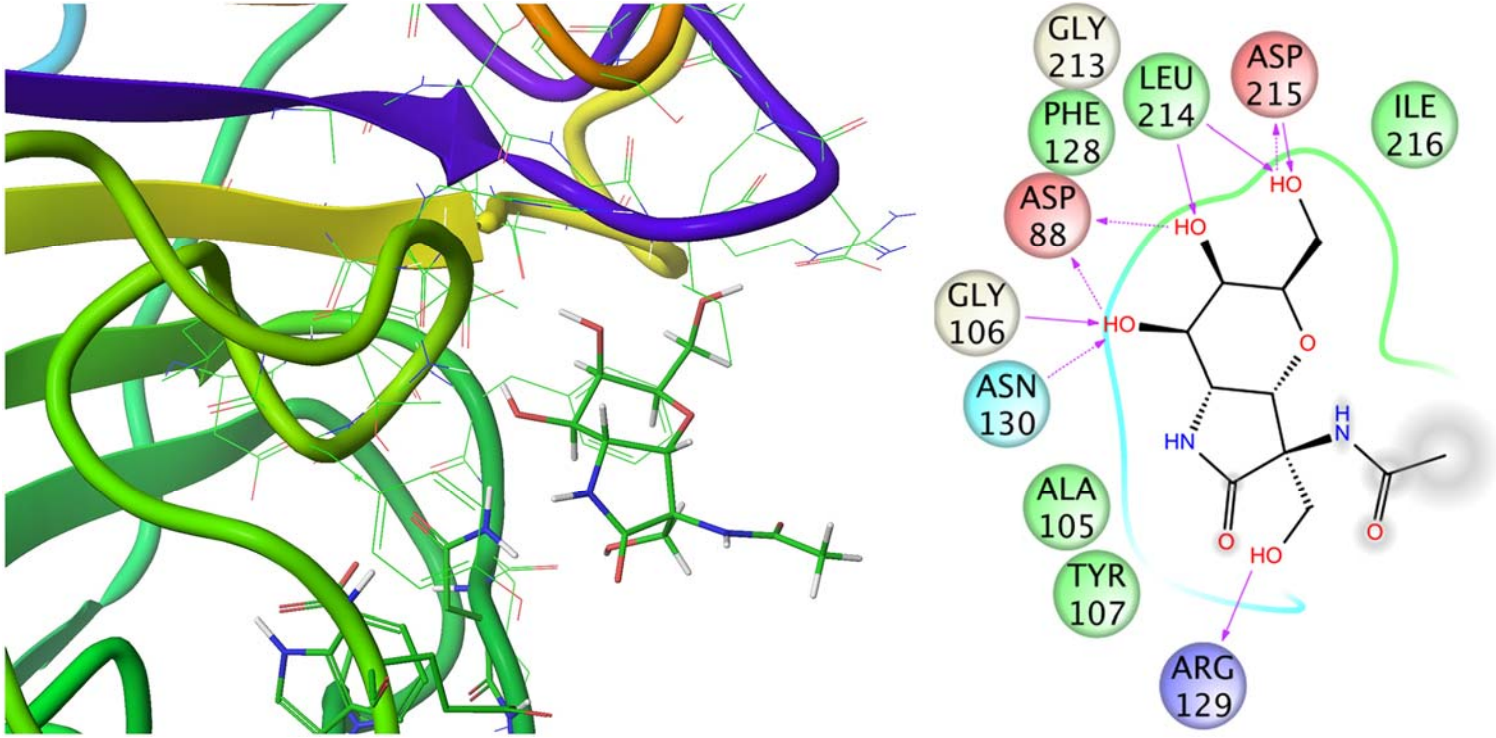


Snapshot taken from the unrestrained 20 ns MD simulation for **SBL:1** complex, together with the ligand interaction diagram obtained with Maestro 9.3.5 software



The difference of affinity to SBL observed between derivatives **1** and **epi-1** could be attributed to the fact that Hα of compound **1** is involved in a hydrophobic patch with the aromatic ring of Phe128 of SBL. This stabilizing interaction is not possible in the case of compound **epi-1**

Snapshot taken from the unrestrained 20 ns MD simulation for **SBL:2** complex, together with the ligand interaction diagram obtained with Maestro 9.3.5 software



The higher affinity of compound **2** may be explained by the extra hydrogen bond present in the bound state for this compound. In fact, the CH_2OH group participates in a high populated hydrogen bond with arginine-129.