# Discovery of a New Drug-Like Series of OGT Inhibitors by Virtual Screening.

Elena M. Loi[1,2], Tihomir Tomašič[1], Cyril Balsollier[2,1], Kevin van Eekelen[2], Matjaž Weiss[1], Martina Gobec[1], Matthew G. Alteen[3], David J. Vocadlo[3], Roland J. Pieters[2], Marko Anderluh[1]

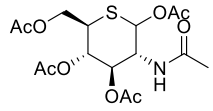
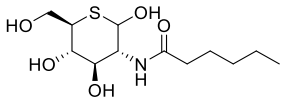
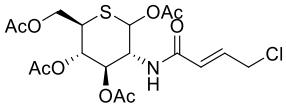
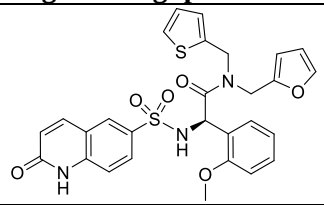
[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Ljubljana, 1000, Slovenia*

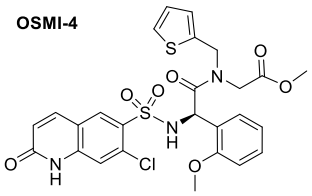
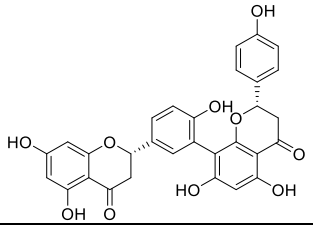
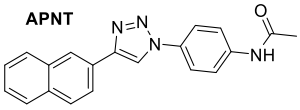
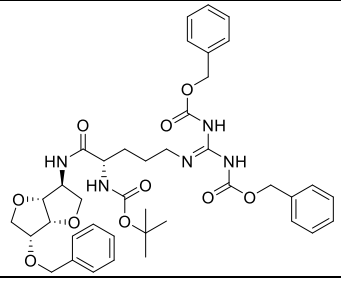
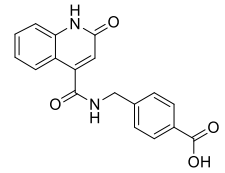
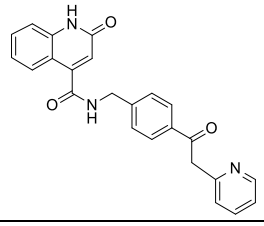
[2]*Department of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, 3584 CG Utrecht, The Netherlands*

[3]*Department of Chemistry, Simon Fraser University, Burnaby, BC, V5A 1S6, Canada*

# Supplementary information

**Table S1**. List of most potent OGT inhibitors reported to date and their properties.

| Name | Structure | Inhibitory parameter | Issue | Reference |
|---|---|---|---|---|
| **Substrate analogues** | | | | |
| **$Ac_4$-5S-GlcNAc** |  | $EC_{50}$ = 5 μM | Cell permeable<br>Off-target effects<br>Poor aqueous solubility | Dorfmueller, H. C. et al. Amino Acids 40, 781–792 (**2011**). |
| **5S-GlcNHex** |  | Similar to $Ac_4$-5S-GlcNAc | Cell permeable<br>Off-target effects | Liu, T. W. et al. Angew. Chemie - Int. Ed. 57, 7644–7648 (**2018**). |
| **$Ac_4$-ES1** |  | Similar to $Ac_4$-5S-GlcNAc | Cell permeable<br>Covalent inhibitor<br>Selective | Worth, M. et al. Chem. Commun. 55, 13291–13294 (**2019**). |
| **High-throughput and virtual screening identified inhibitors** | | | | |
| **OSMI-1** |  | $IC_{50}$ = 2.7 μM | Cell permeable<br>Off-target effects | Ortiz-Meoz, R. F. et al. ACS Chem. Biol. 10, 1392–1397 (**2015**). |

| | | | | |
|---|---|---|---|---|
| **OSMI-2**<br>**OSMI-3**<br>**OSMI-4** |  | OSMI-2 (acid form) $K_D$ = 140 nM<br>OSMI-3 (acid form) $K_D$ = 5 nM<br>OSMI-4 (acid form) $K_D$ = 8 nM | Very potent<br>Cell permeable<br>Specific | Martin, S. E. S. et al. J. Am. Chem. Soc. 140, 13542–13545 (**2018**). |
| **L01** |  | $IC_{50}$ = 21.8 μM | Cell permeable<br>Natural product<br>Off-target effects | Liu, Y. et al. Sci. Rep. 7, 12334 (**2017**) |
| **APNT and APBT** |  | APNT:<br>$IC_{50}$ = 66.7 μM<br>APBT<br>$IC_{50}$ = 139 μM | Cell permeable<br>Non-competive<br>Poor aqueous solubility<br>Low potency | Wang, Y.- et al. J. Med. Chem. 60, 263–272 (**2017**). |
| **LQMed 330** |  | $IC_{50}$ = 11.7 μM | Not selective<br>Unknown solubility and permeability | Albuquerque, S. O. et al. Eur. J. Pharm. Sci. 154, 105510 (**2020**) |
| **OGT inhibitors from our group** | | | | |
| **F20** |  | $IC_{50}$ = 116.0 μM | Cell permeable<br>Low potency | Zhang, H. et al. MedChemComm 9, 883–887 (**2018**). |
| **6b** |  | $IC_{50}$ = 144.5 μM | Low potency | Weiss, M. et al. Front. Chem. 9, 205 (**2021**). |

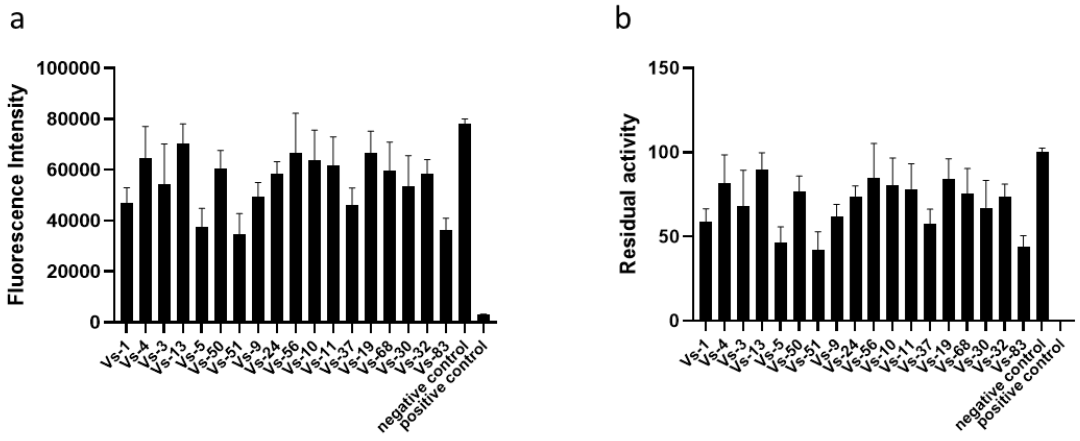
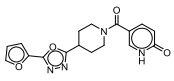
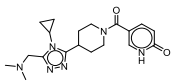
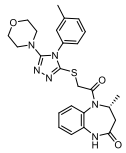
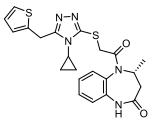
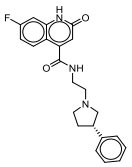
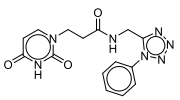
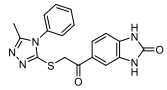
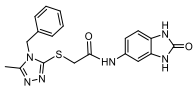
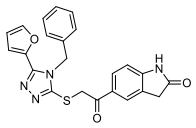
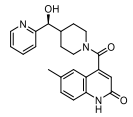
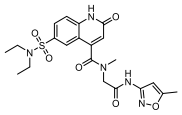
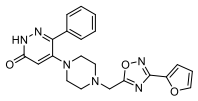
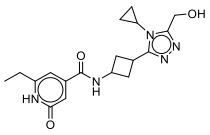
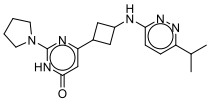
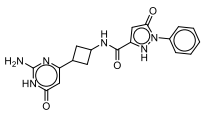
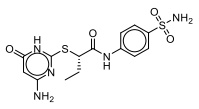
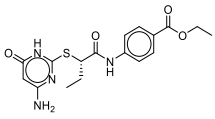
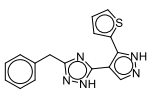



**Figure S1**. Results of the screening with OGT fluorescent activity assay. The compounds were screened at a fixed concentration of 100 μM in two independent experiments. The results are expressed as fluorescence intensity values (a) or normalized residual activity values (b), with respect to the negative (DMSO) and positive (OSMI-4) controls.

**Table S2.** Details of the commercially available compounds screened in this study.

| Name | Structure | Vendor | ID | Purity declared by vendor |
|---|---|---|---|---|
| **Vs-1** |  | Life Chemicals | F5596-0429 | ≥90% |
| **Vs-4** |  | ChemBridge | 87364924 | ≥85% |
| **Vs-3** |  | Enamine | Z92480524 | ≥90% |
| **Vs-13** |  | Enamine | Z90105807 | ≥90% |
| **Vs-24** |  | ChemBridge | 39842207 | ≥85% |
| **Vs-30** |  | ChemBridge | 72669148 | ≥85% |

| | | | | |
|---|---|---|---|---|
| **Vs-5** |  | Enamine | Z24159125 | ≥90% |
| **Vs-50** |  | Enamine | Z24541217 | ≥90% |
| **Vs-51** |  | Enamine | Z65683626 | ≥90% |
| **Vs-9** |  | ChemBridge | 87867766 | ≥85% |
| **Vs-56** |  | Enamine | Z220335192 | ≥90% |
| **Vs-32** |  | ChemBridge | 82571109 | ≥85% |
| **Vs-10** |  | ChemBridge | 41869522 | ≥85% |
| **Vs-11** |  | ChemBridge | 46452758 | ≥85% |
| **Vs-37** |  | ChemBridge | 53010325 | ≥85% |
| **Vs-19** |  | ChemBridge | 7555184 | ≥90% |
| **Vs-68** |  | ChemBridge | 7915441 | ≥90% |
| **Vs-83** |  | ChemBridge | 40085129 | ≥85% |

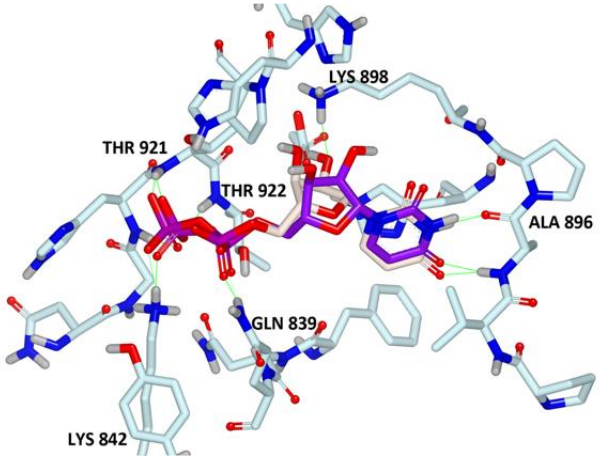



**Figure S2.** Validation of the docking experiment: overlay of the co-crystallized (beige) and re-docked (purple) ligand UDP. PDB entry: 4N39. RMSD value: 1.45.

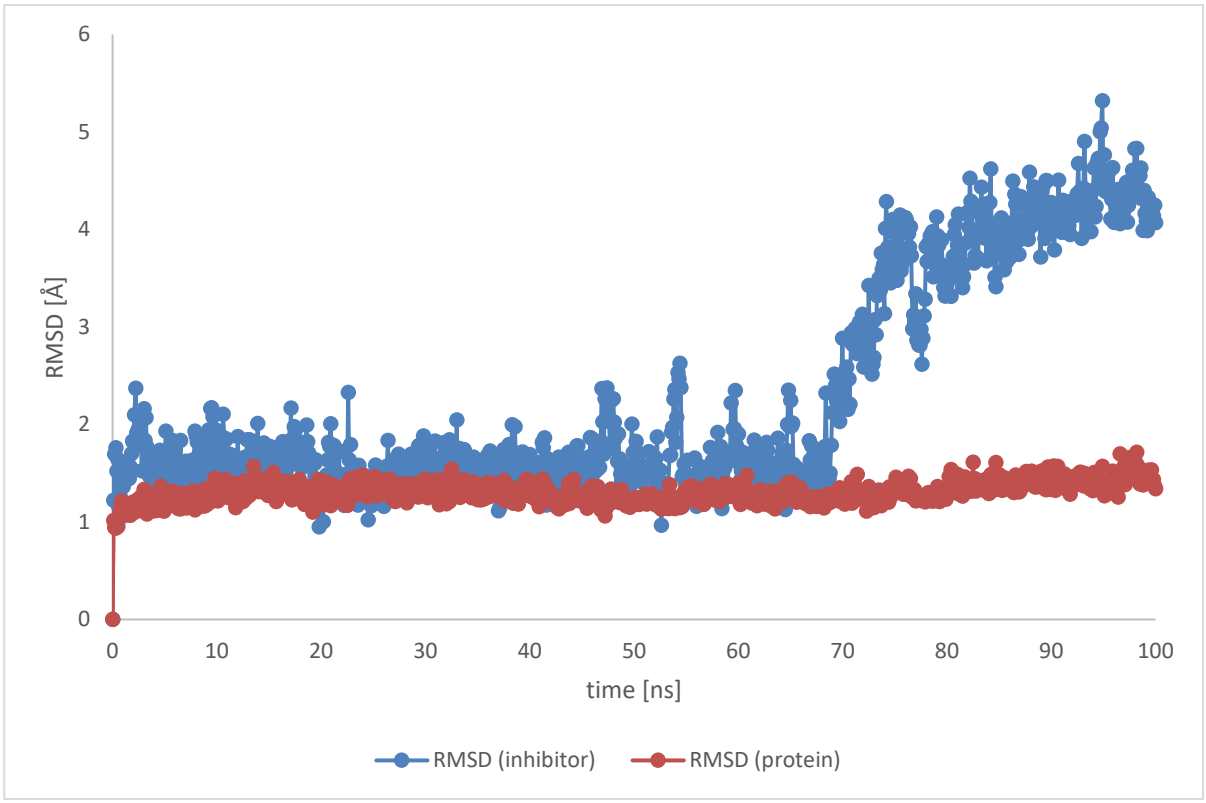



**Figure S3.** Protein (in red) and ligand (in blue) RMSD values during the 100 ns molecular dynamics simulation.

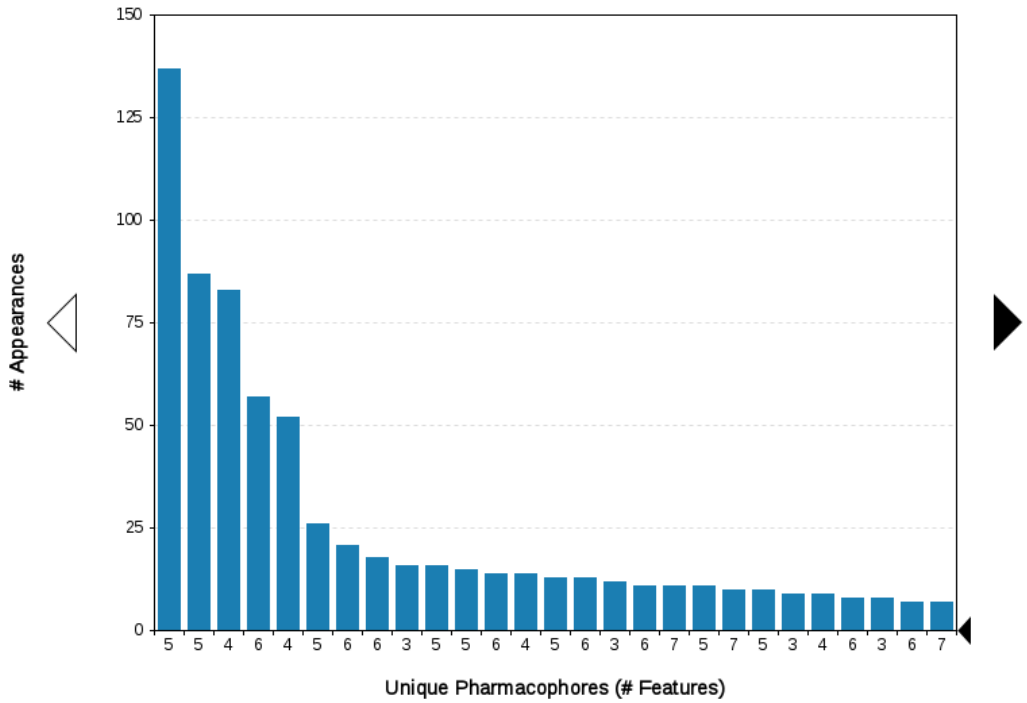
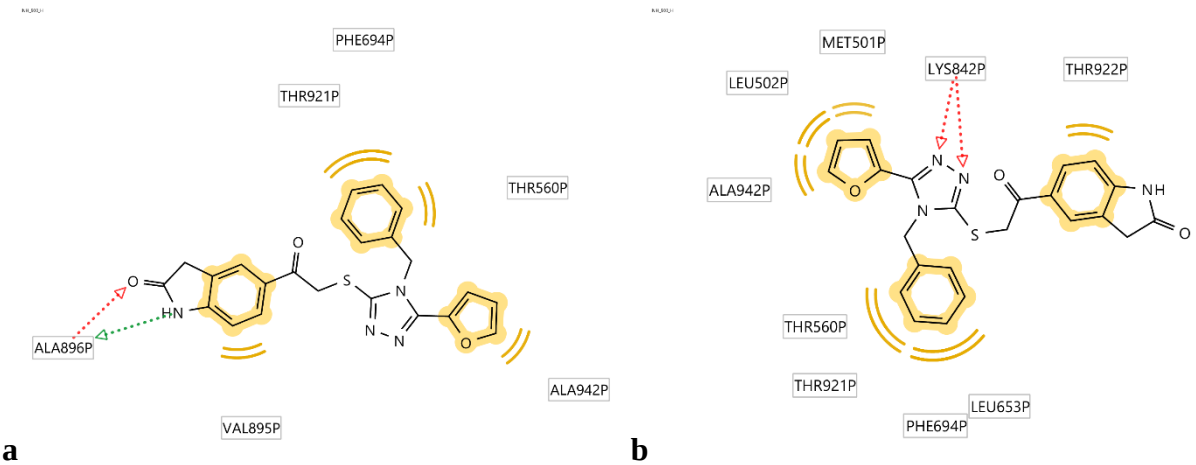
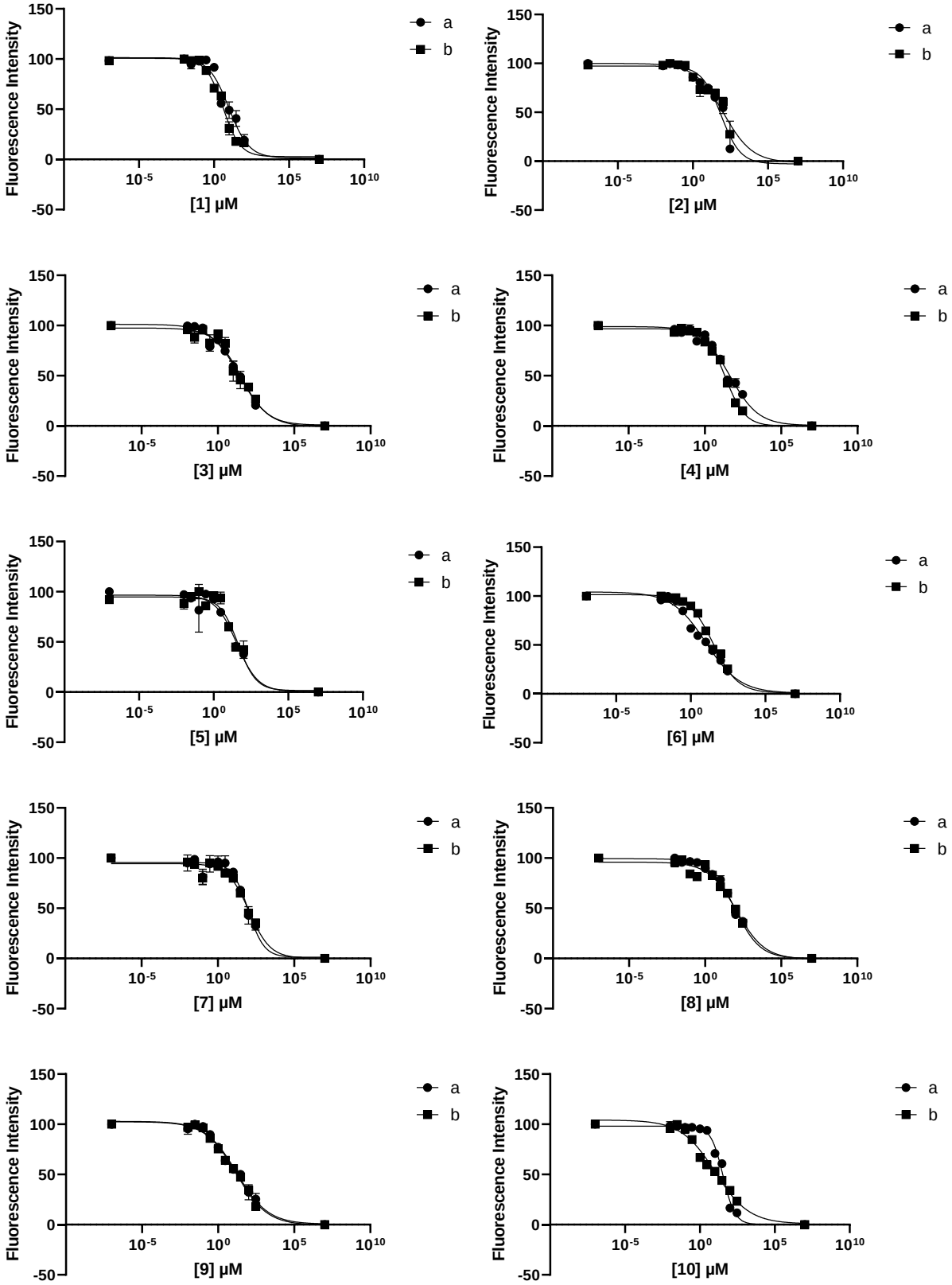



**Figure S4.** Plot of the most frequent unique structure-based pharmacophore models derived from the molecular dynamics simulations of the OGT in complex with Vs-51. The numbers below the bars indicate the numbers of interaction features observed during molecular dynamics simulation for the pharmacophore models.




**a** **b**

**Figure S5.** Schematic representation of the interactions between OGT and Vs-51 observed in **a**) the first and **b)** the second most frequently occurring structure-based pharmacophore model.


Fluorescence Intensity
150
100
50
0
-50
10-5
100
105
1010
[1] μM
[2] μM
[3] μM
[4] μM
[5] μM
[6] μM
[7] μM
[8] μM
[9] μM
[10] μM
a
b

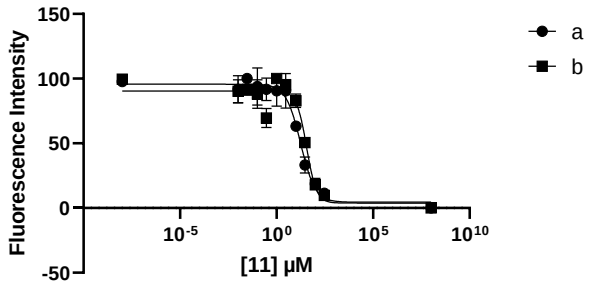



**Figure S6.** $IC_{50}$ curves of compounds **1**-**11** measured in two independent experiments. Normalized fluorescence intensity values are expressed as a percentage of the DMSO control.

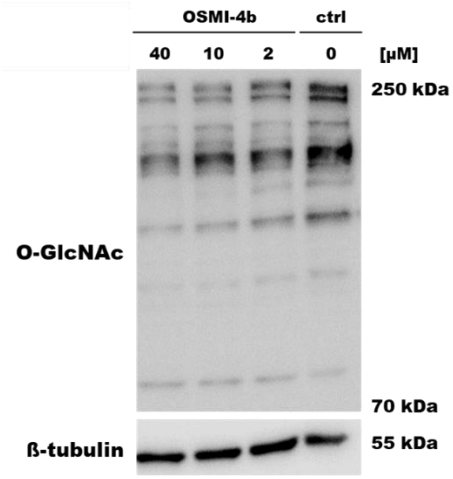
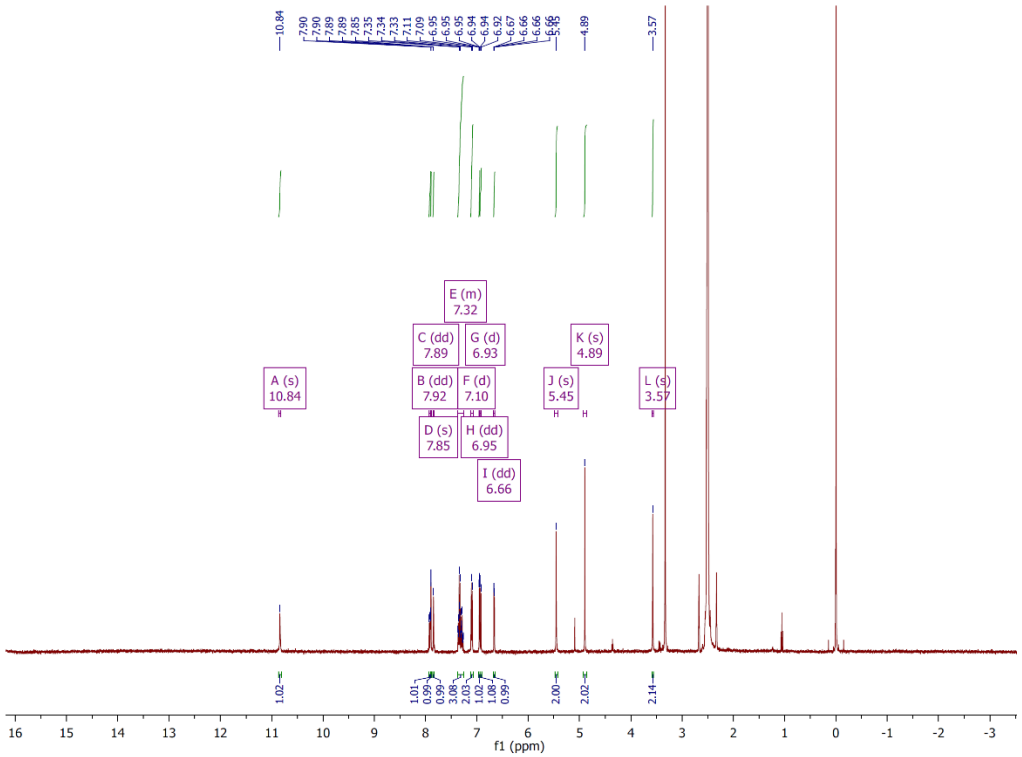
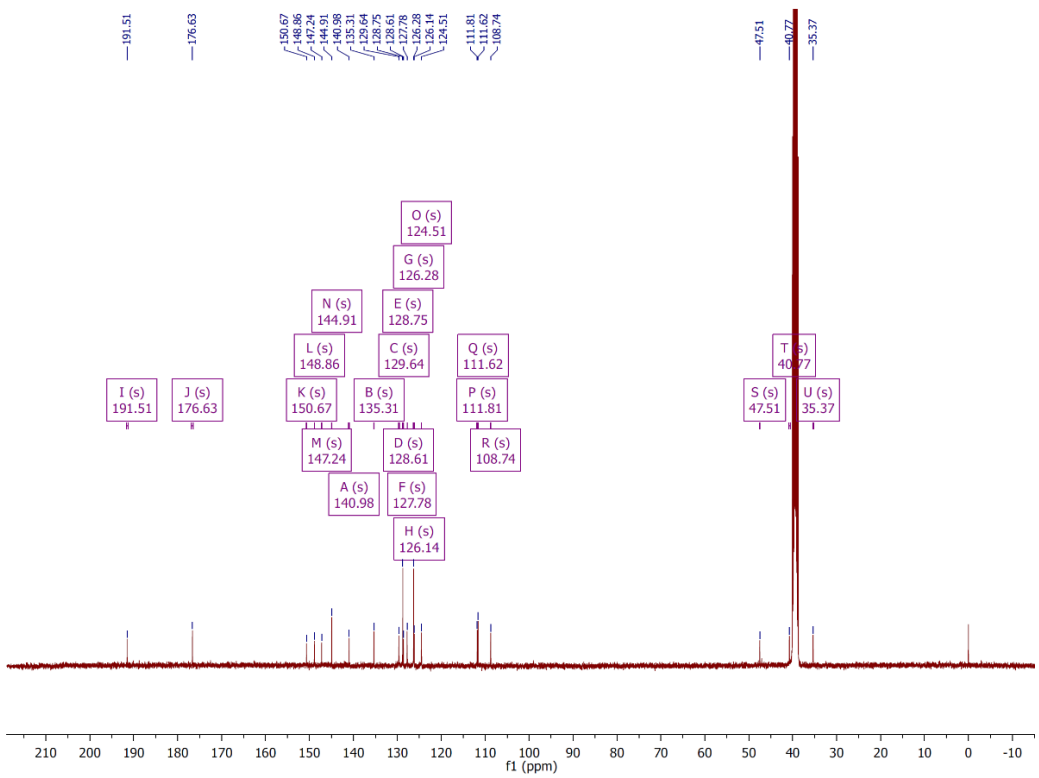



**Figure S7.** Representative picture of western blot analysis of O-GlcNAc levels after treating AMO1 cells with OSMI-4b for 4 hours (two independent experiments).

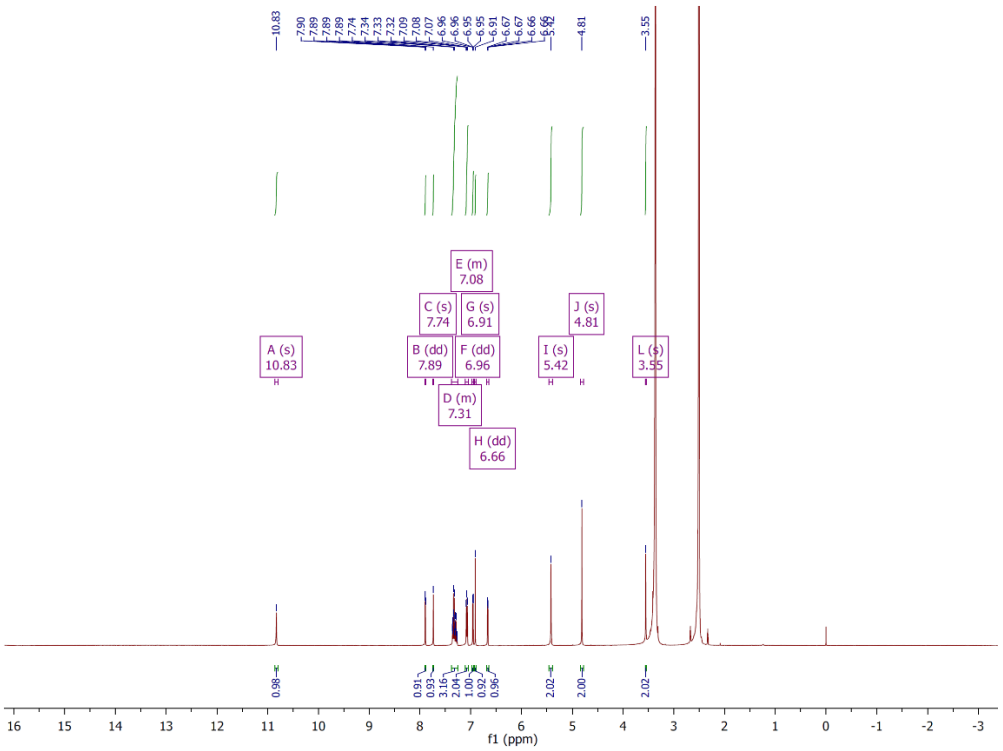
Compound **1** $^{1}$H NMR



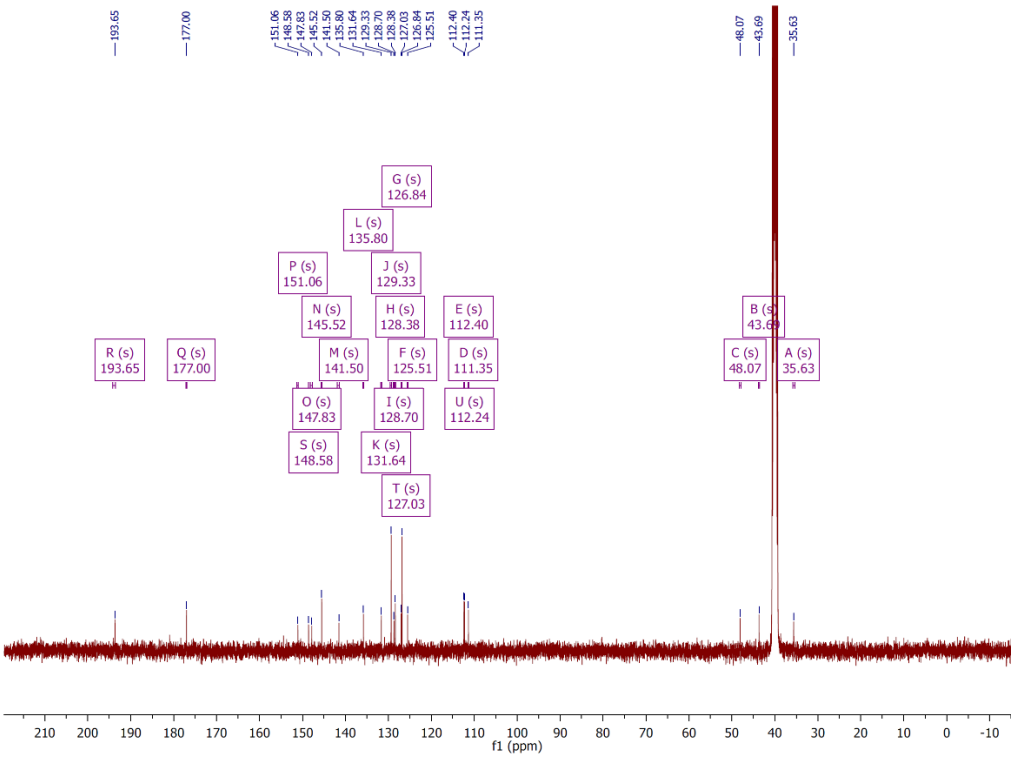

Compound **1** $^{13}$C NMR



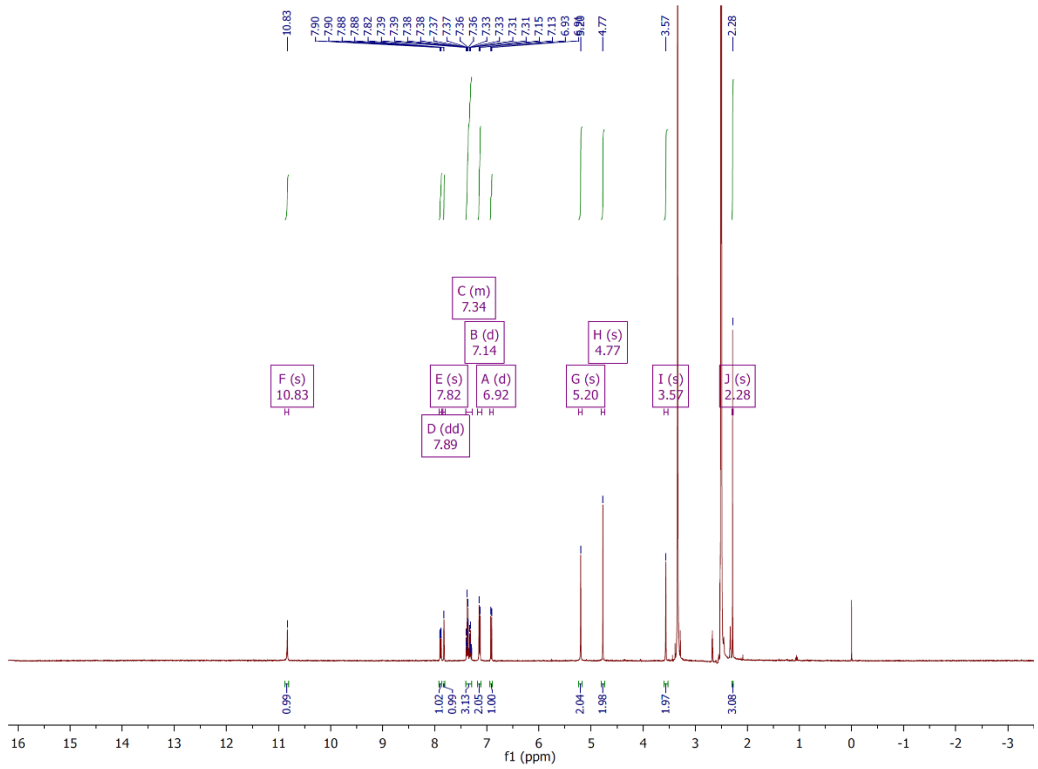
Compound **2** $^{1}$H NMR



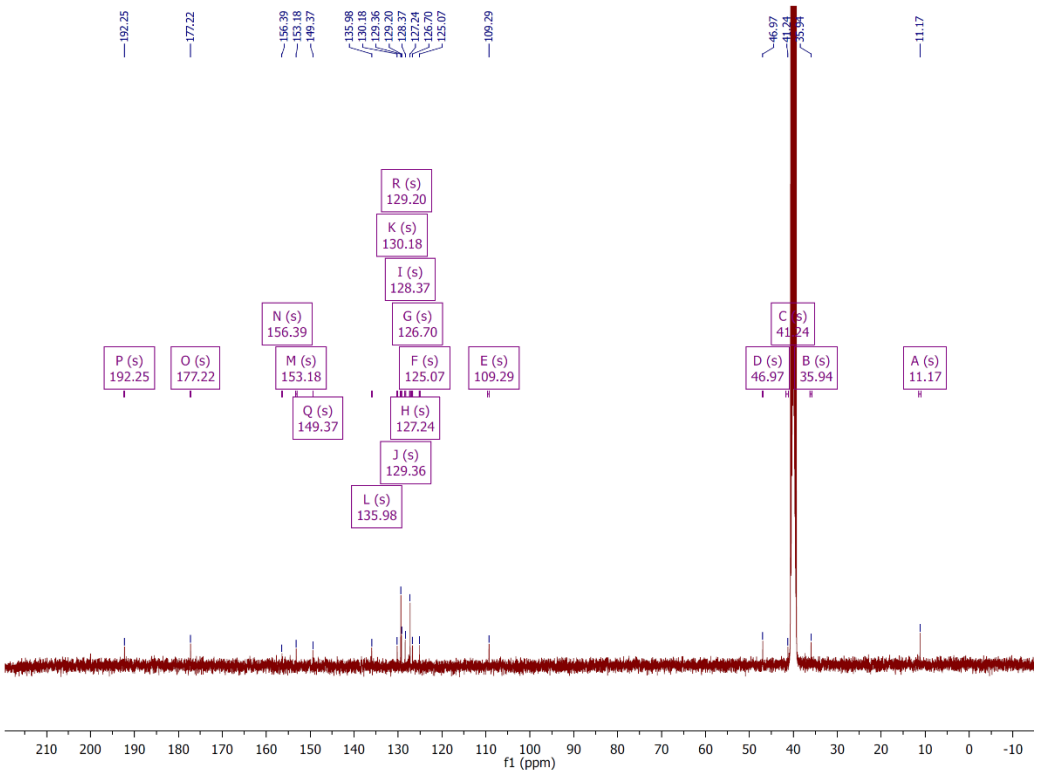

Compound **2** $^{13}$C NMR



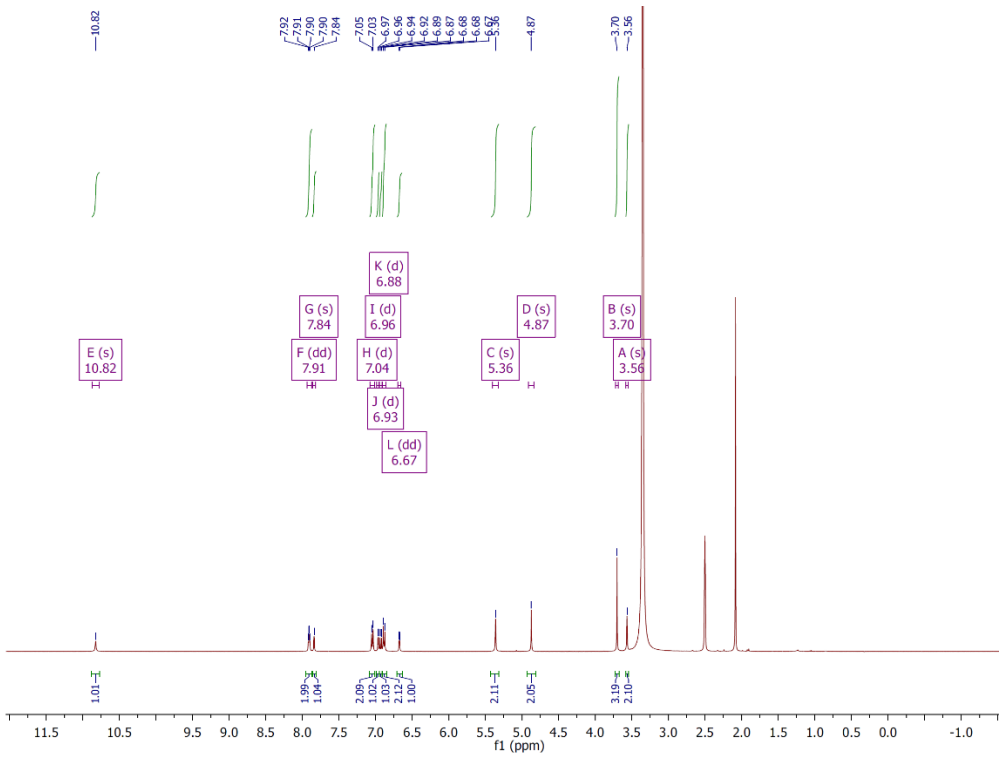
Compound **3** $^{1}$H NMR



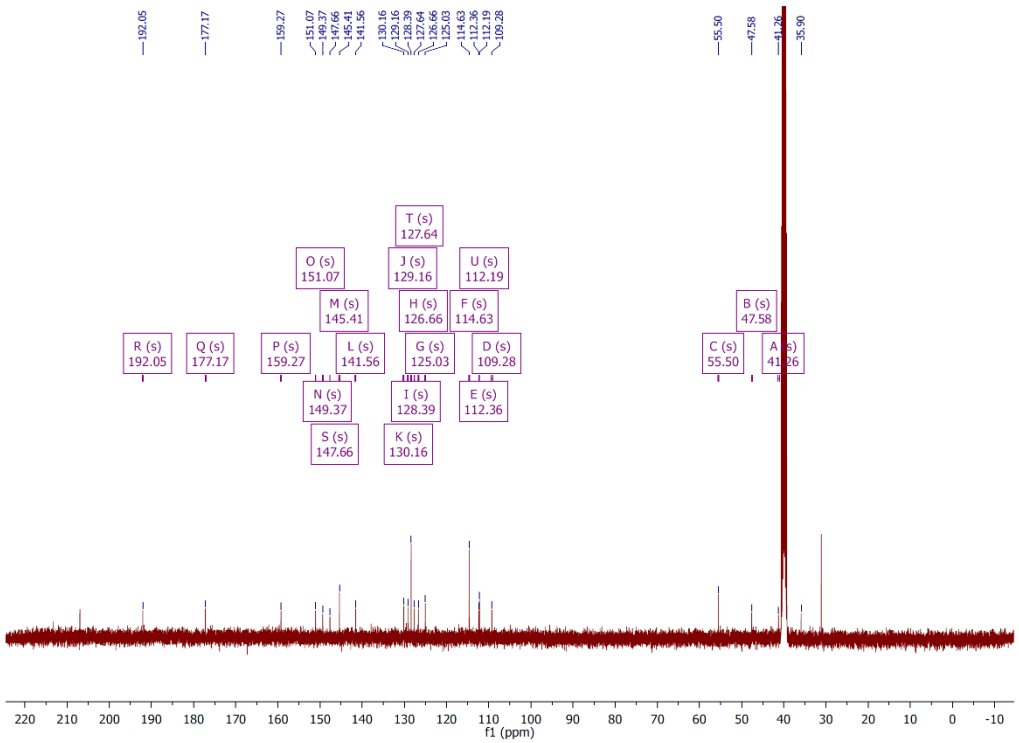

Compound **3** $^{13}$C NMR



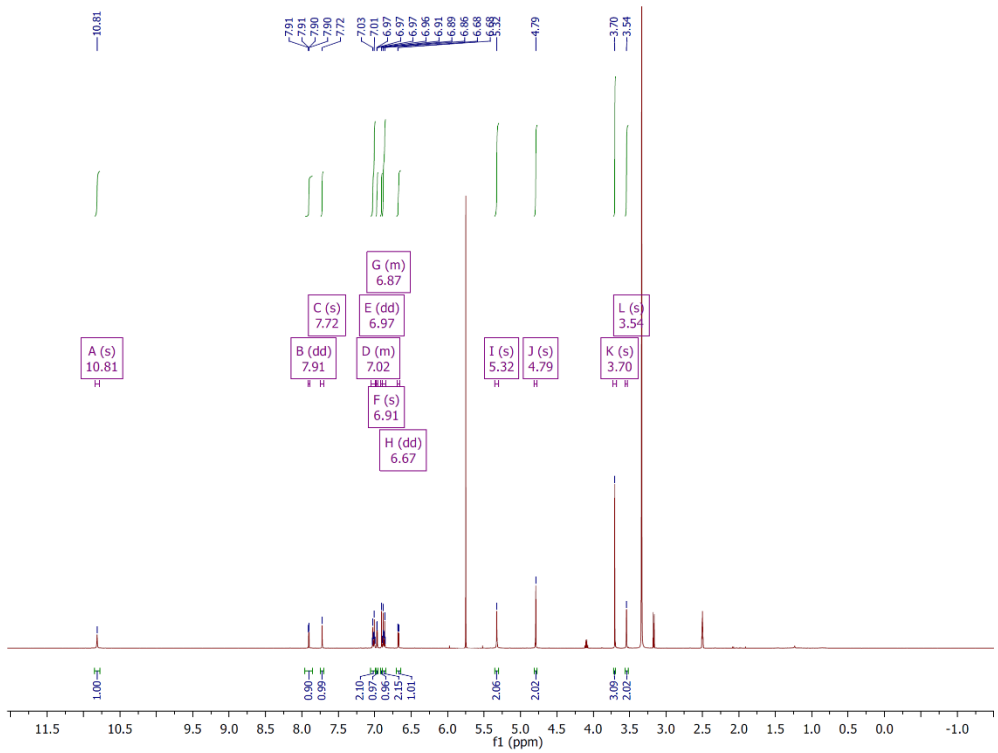
Compound **4** $^{1}$H NMR



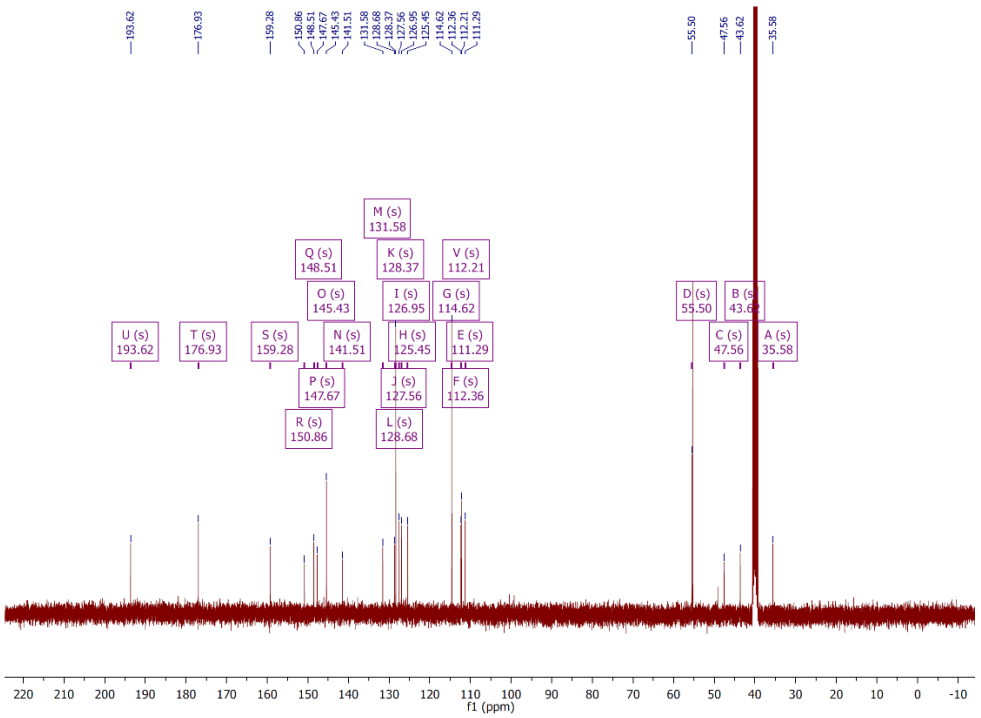

Compound **4** $^{13}$C NMR



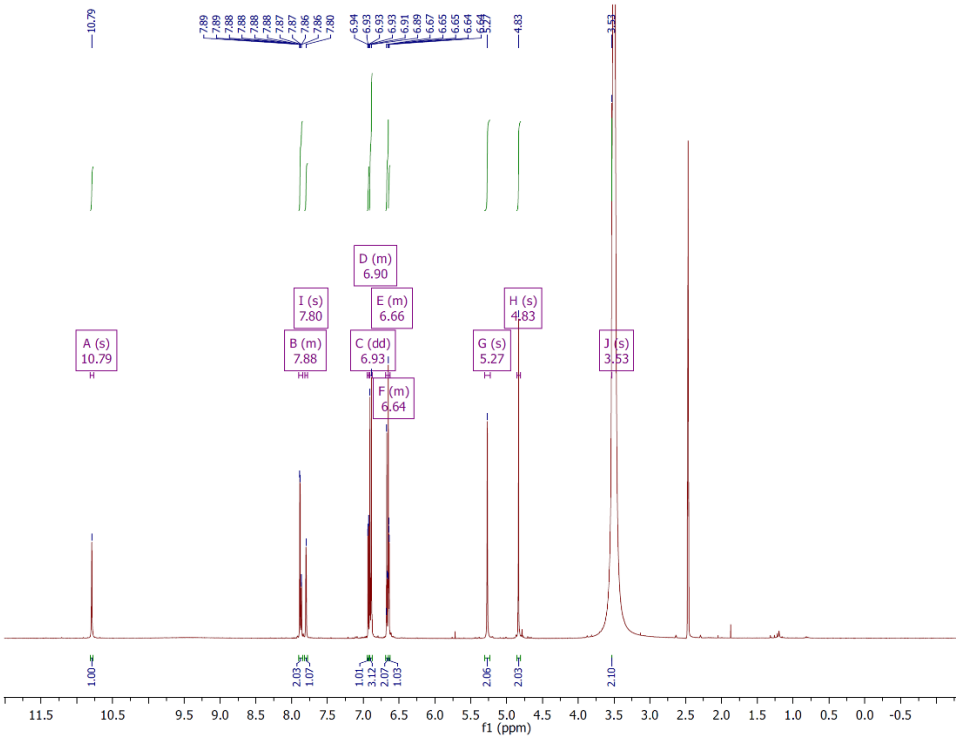
Compound **5** $^{1}$H NMR



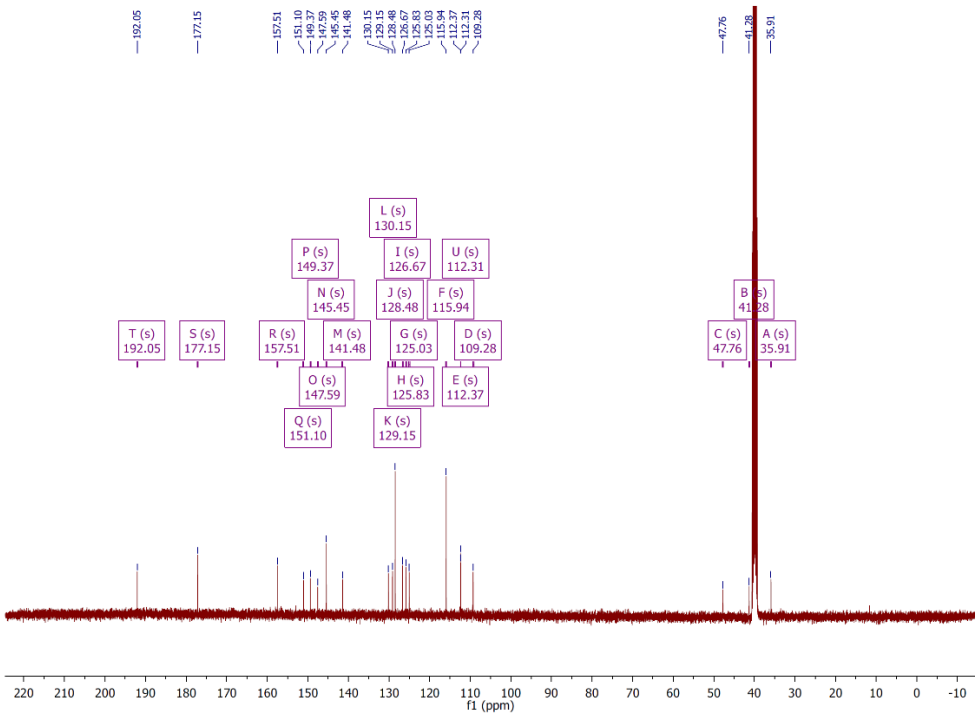

Compound **5** $^{13}$C NMR



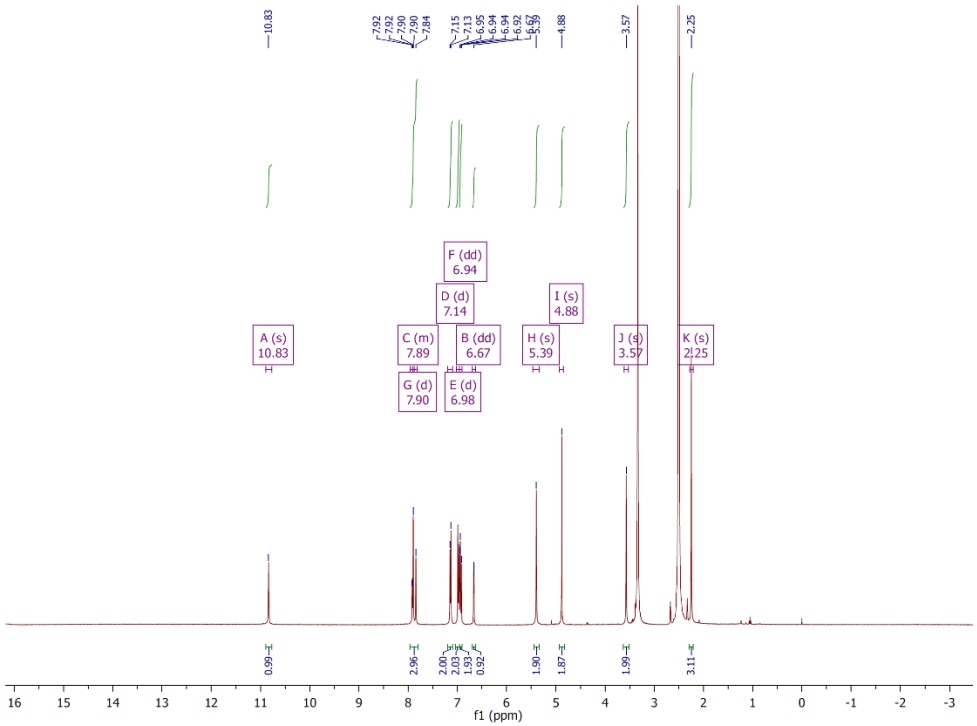
Compound **6** $^{1}$H NMR



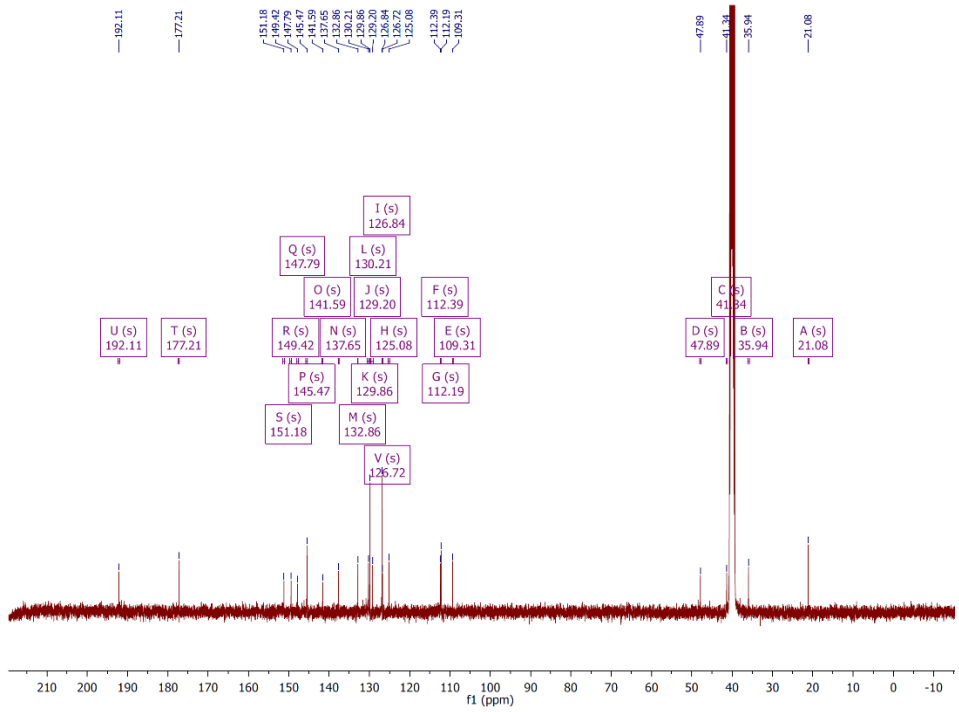

Compound **6** $^{13}$C NMR



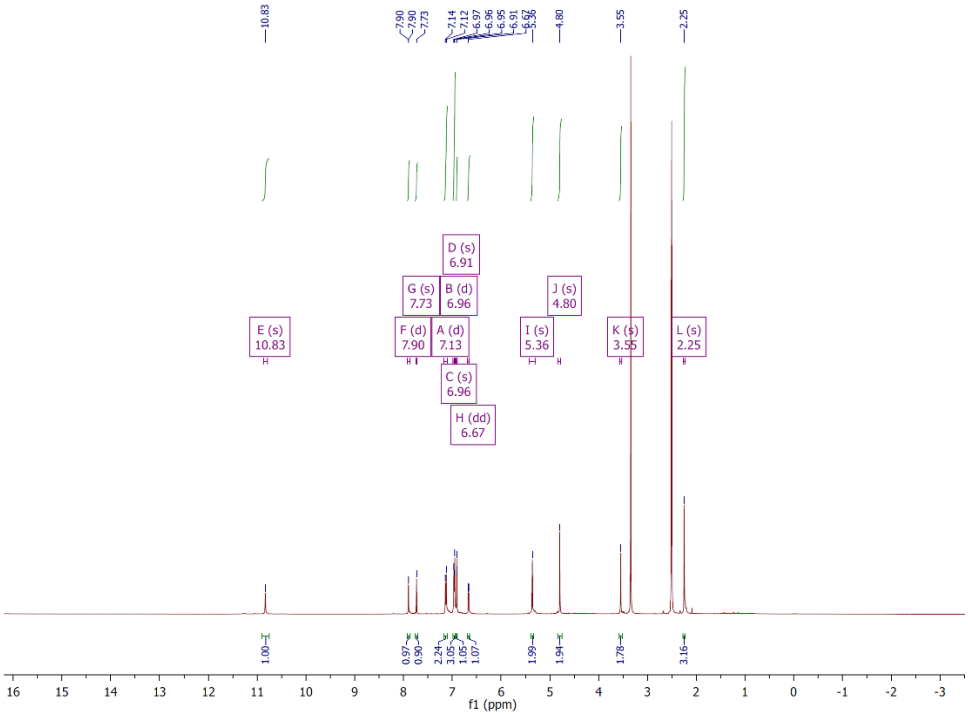
Compound **7** $^{1}$H NMR



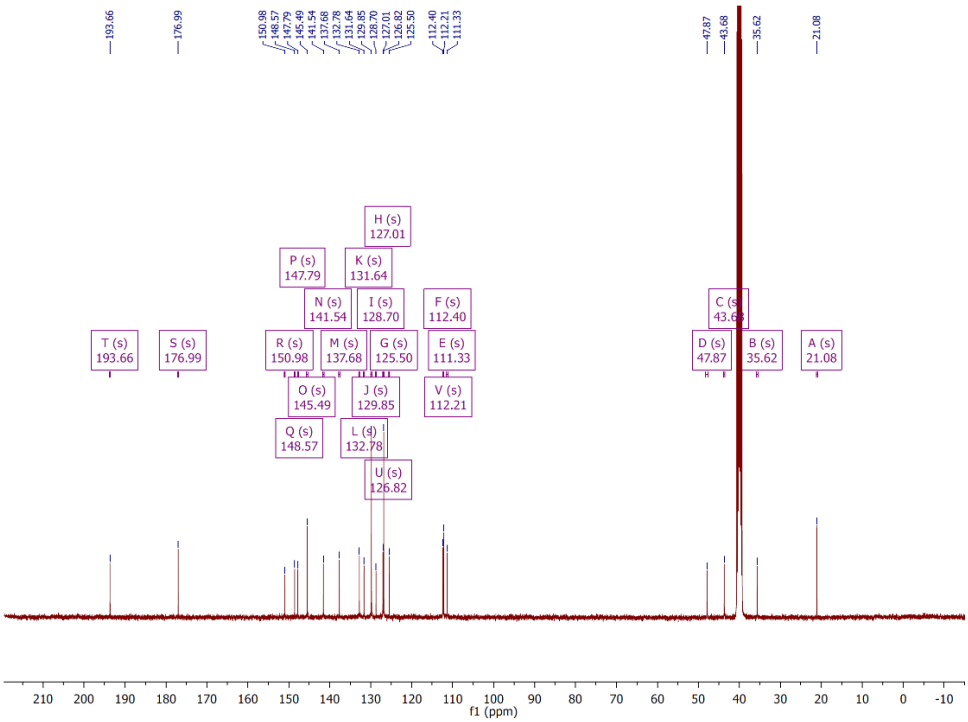

Compound **7** $^{13}$C NMR



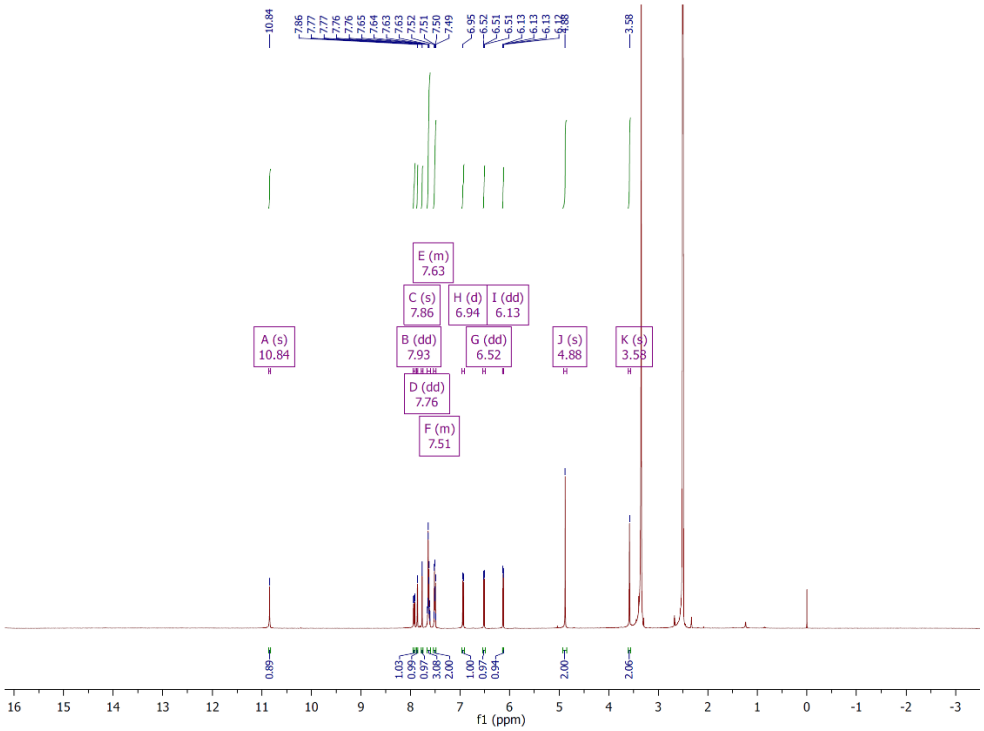
Compound **8** $^{1}$H NMR



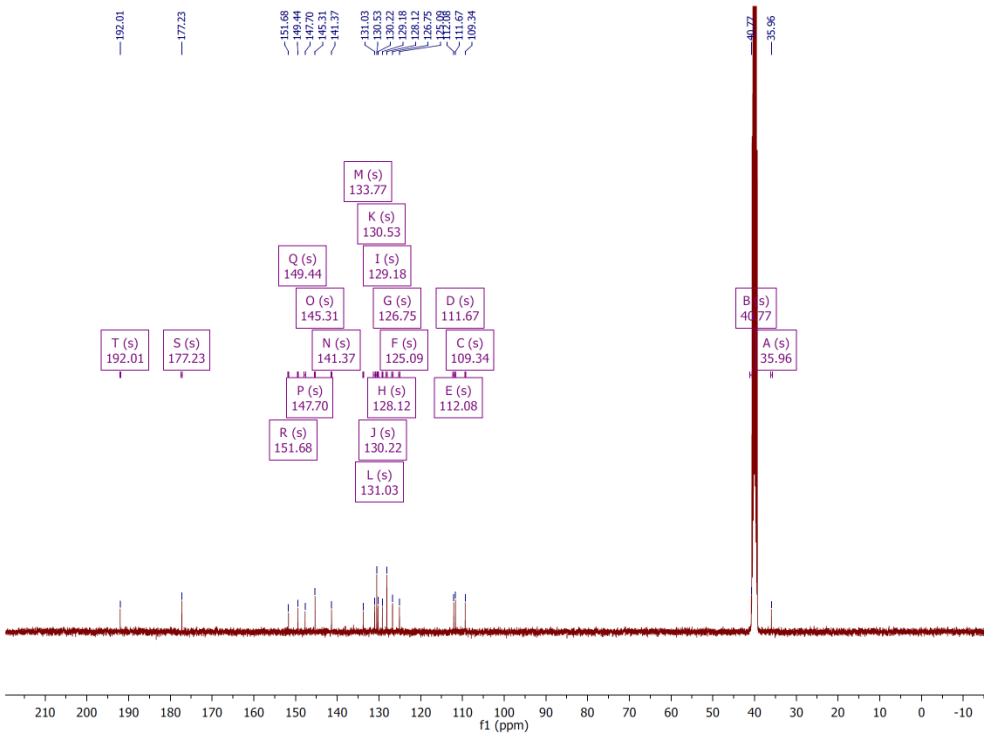

Compound **8** $^{13}$C NMR



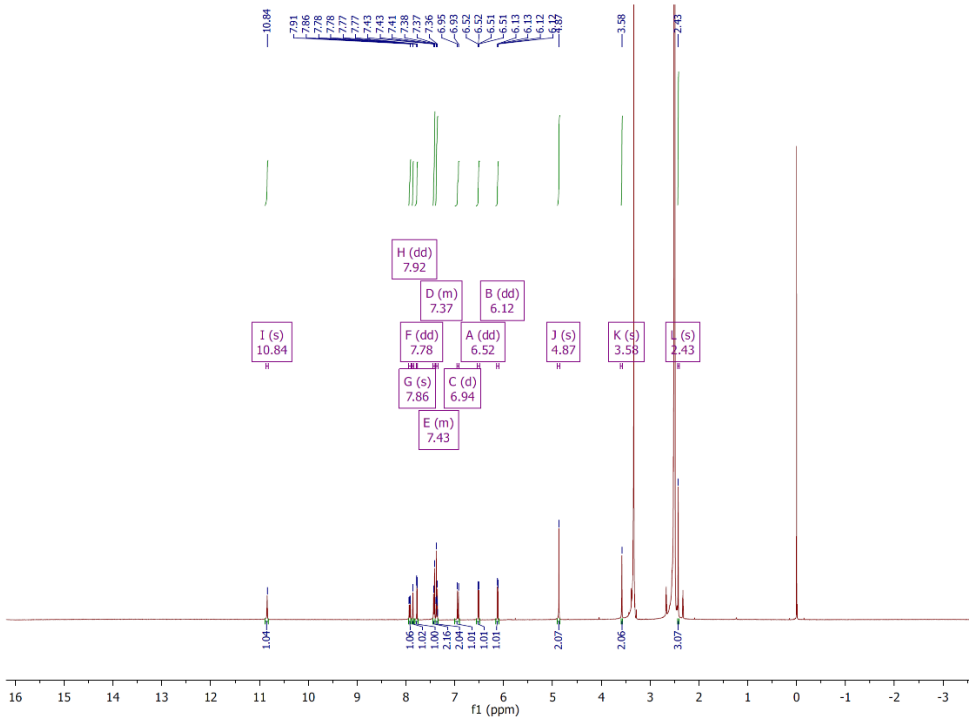
Compound **9** $^{1}$H NMR



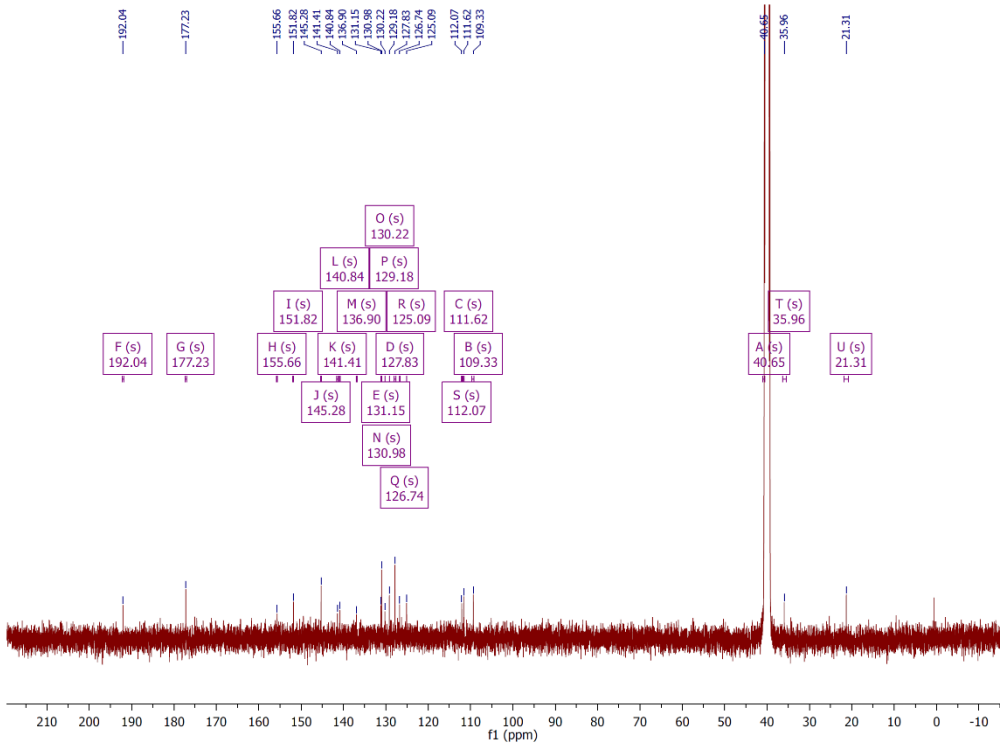

Compound **9** $^{13}$C NMR



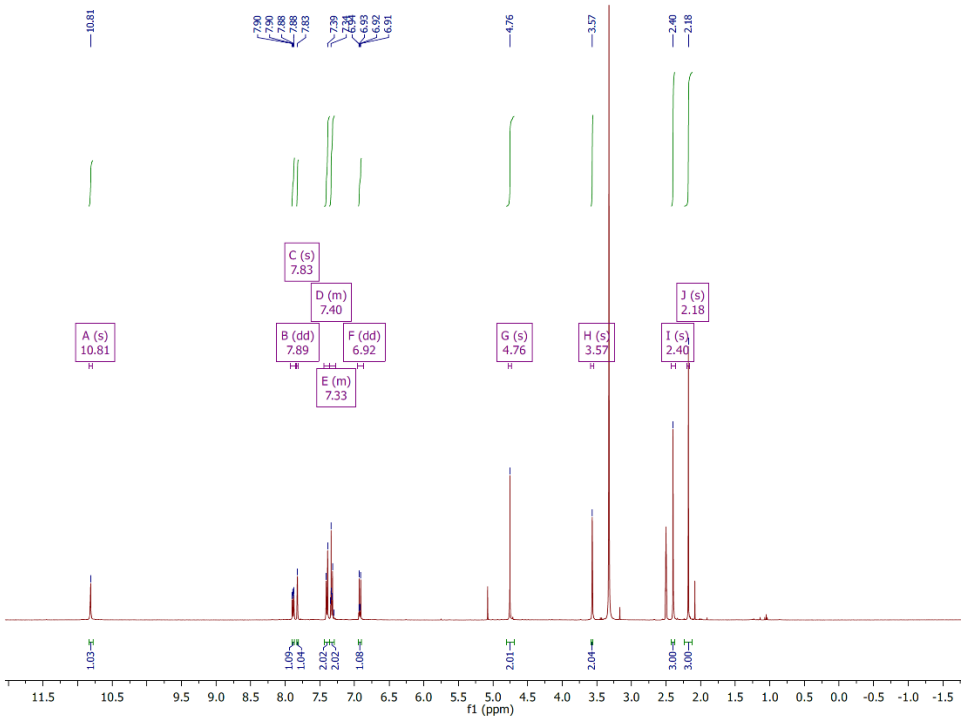
Compound **10** $^{1}$H NMR



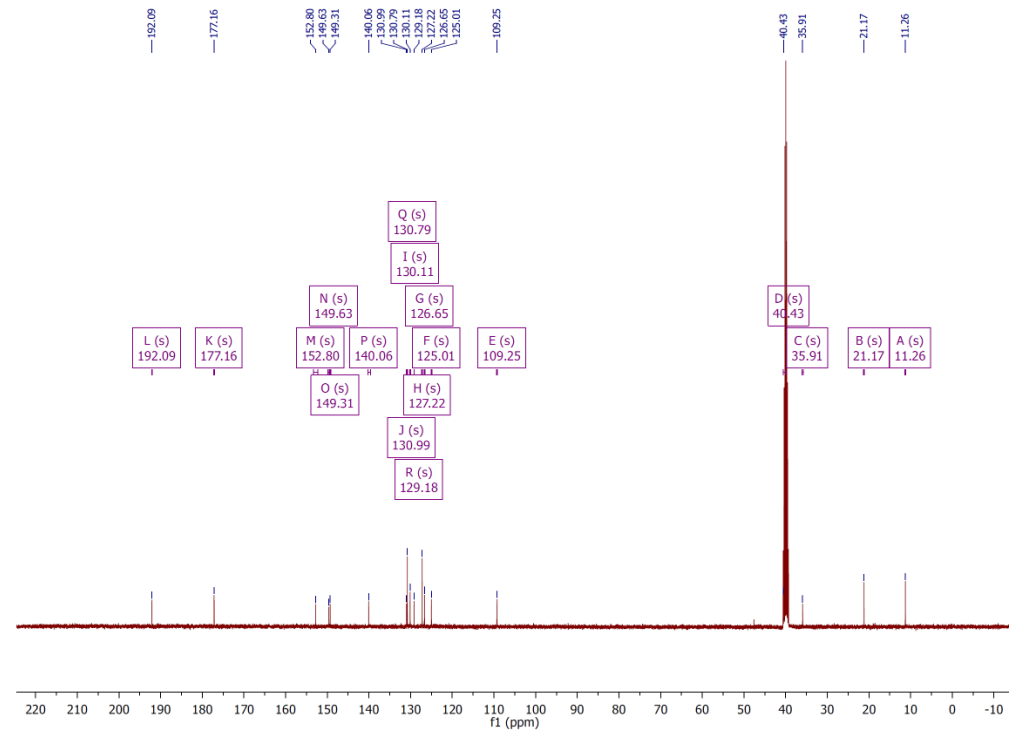

Compound **10** $^{13}$C NMR



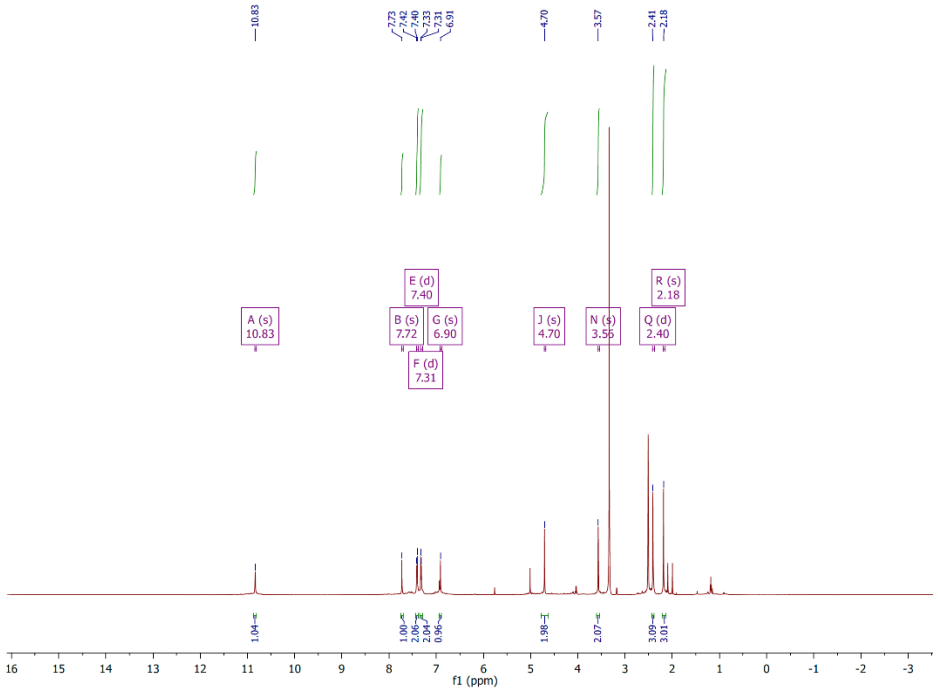
Compound **11** $^{1}$H NMR



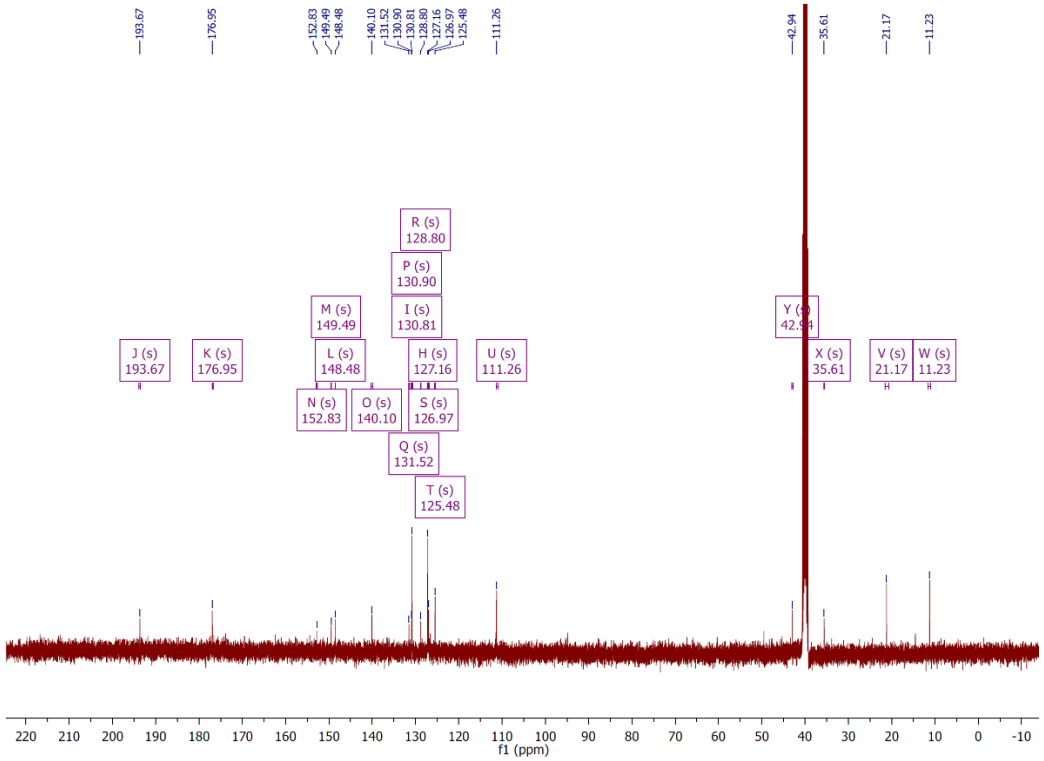

Compound **11** $^{13}$C NMR



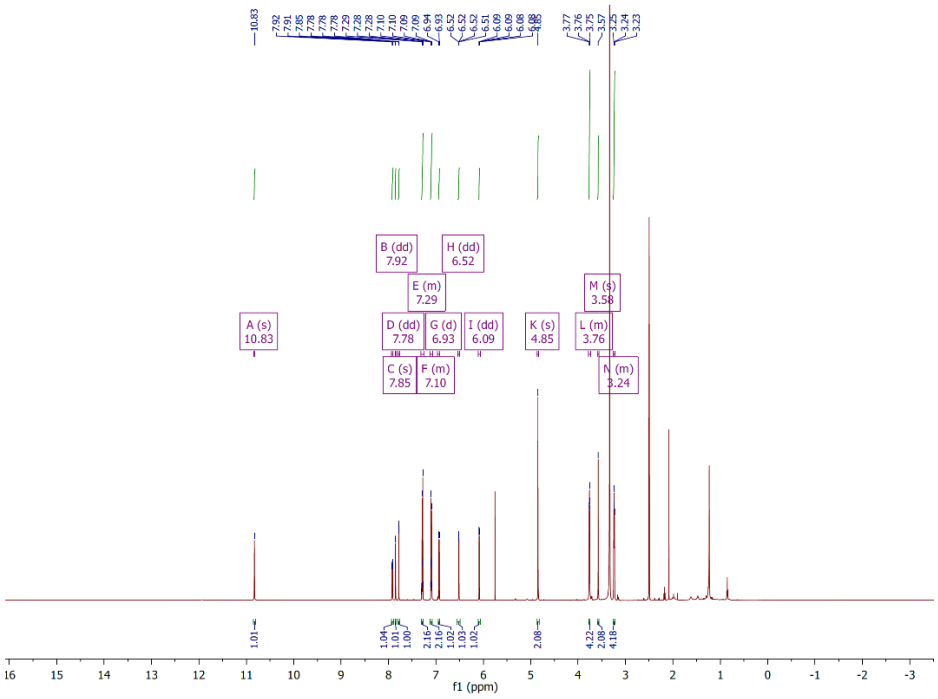
Compound **12** $^{1}$H NMR




Compound **12** $^{13}$C NMR



Compound **13** $^{1}$H NMR




Compound **13** $^{13}$C NMR