Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information for

Synthesis of *uronic*-Noeurostegine – a potent bacterial β-glucuronidase inhibitor[#]

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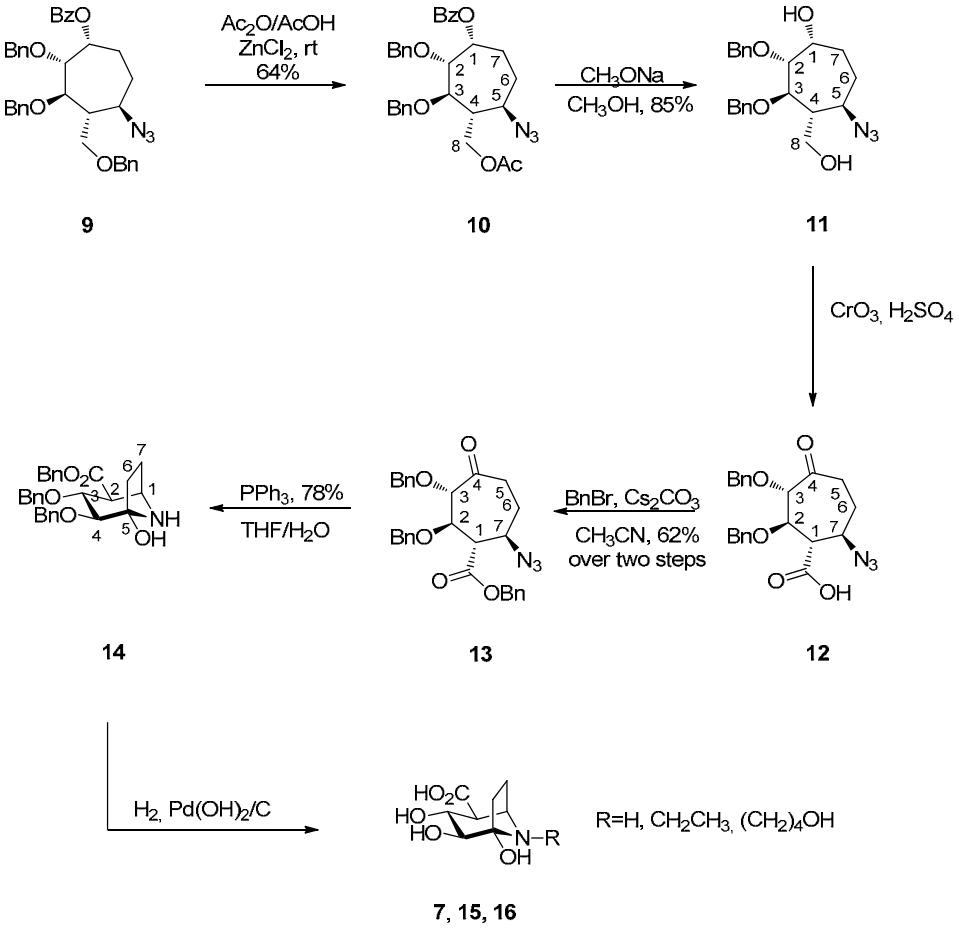
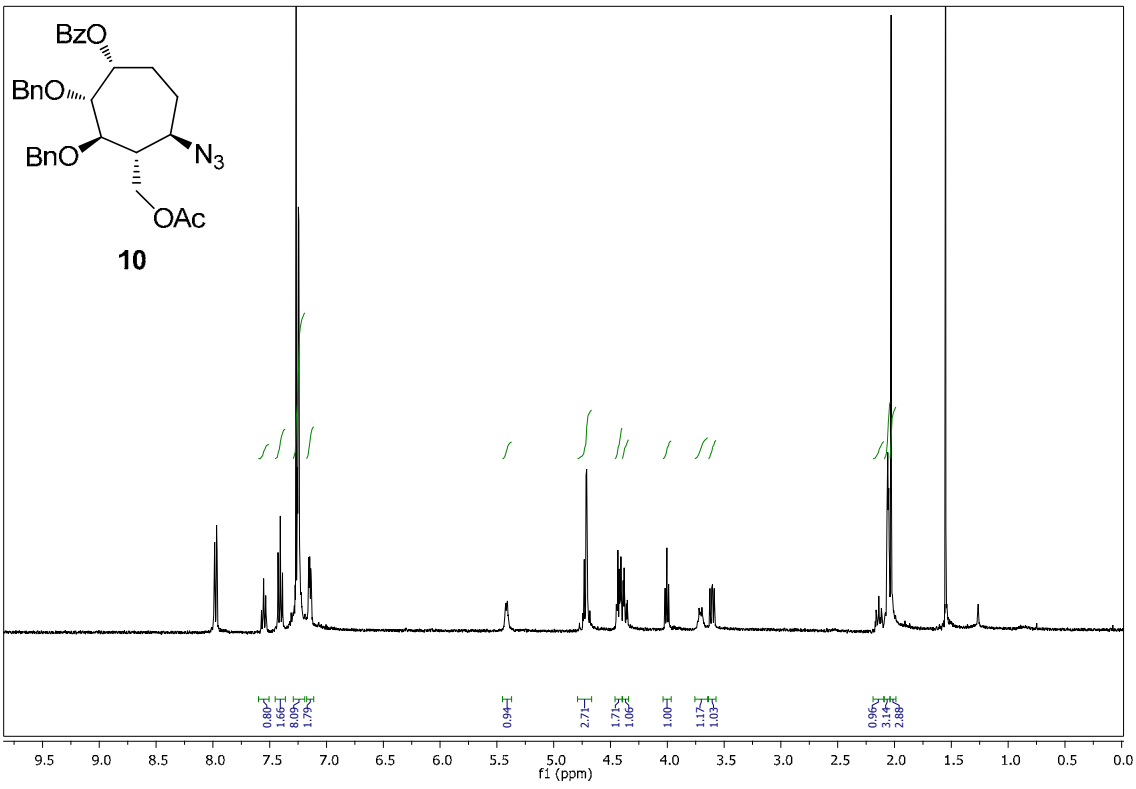
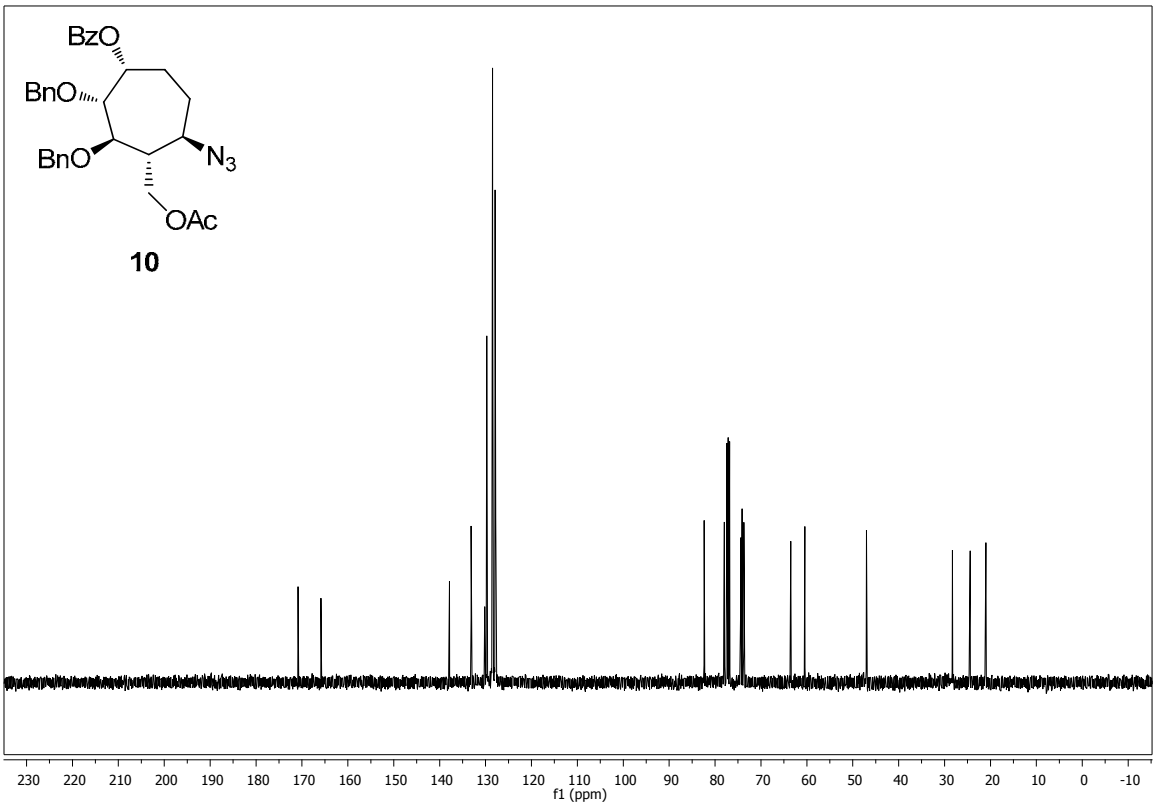
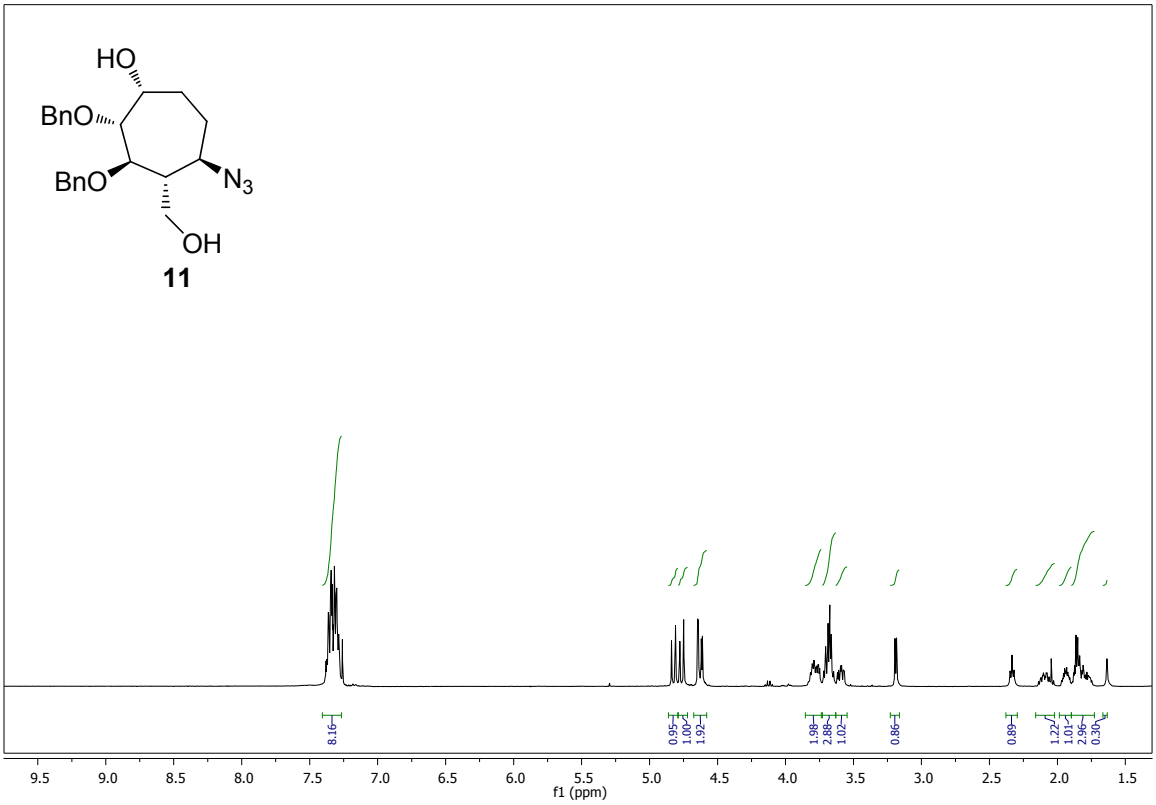
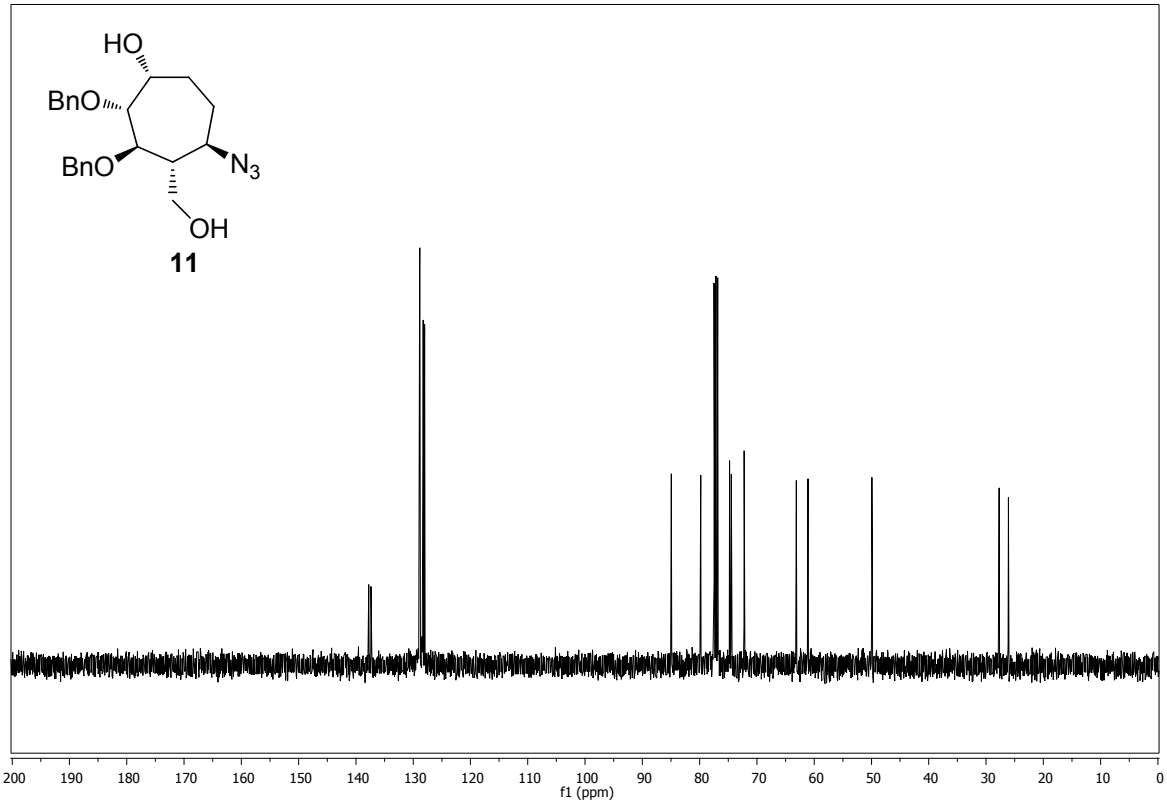


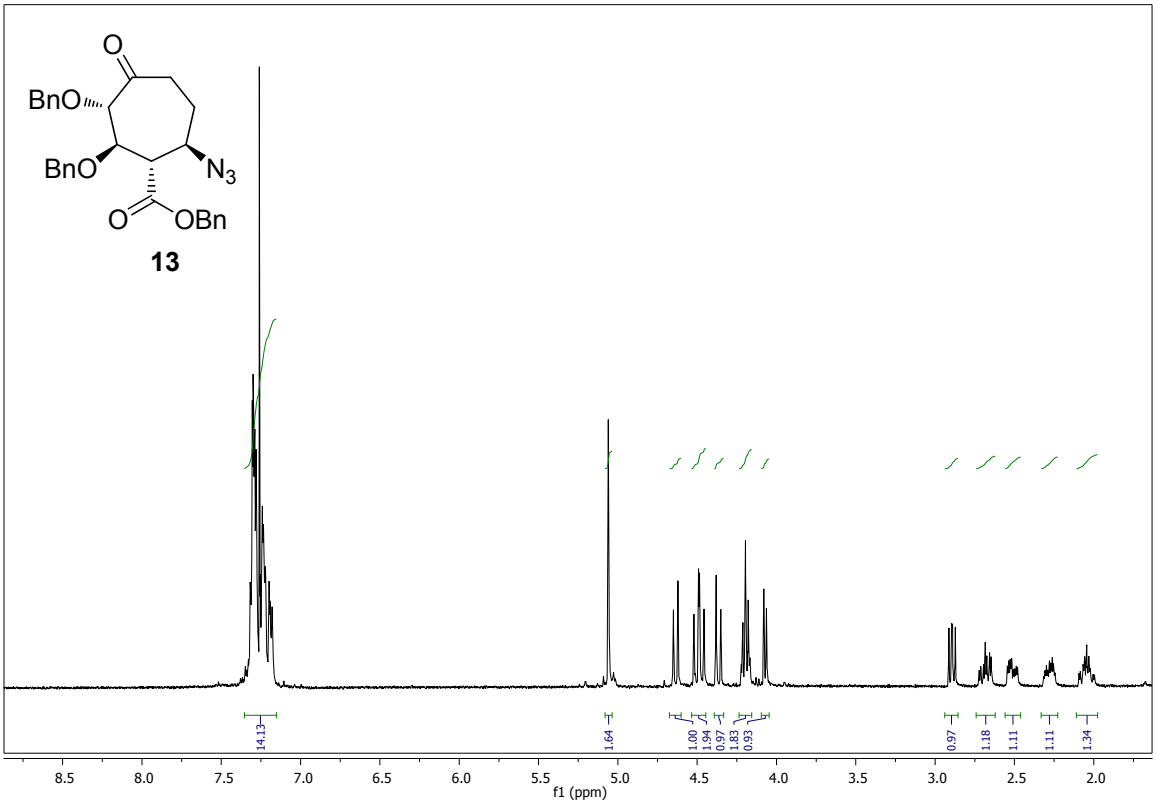
NMR Spectra

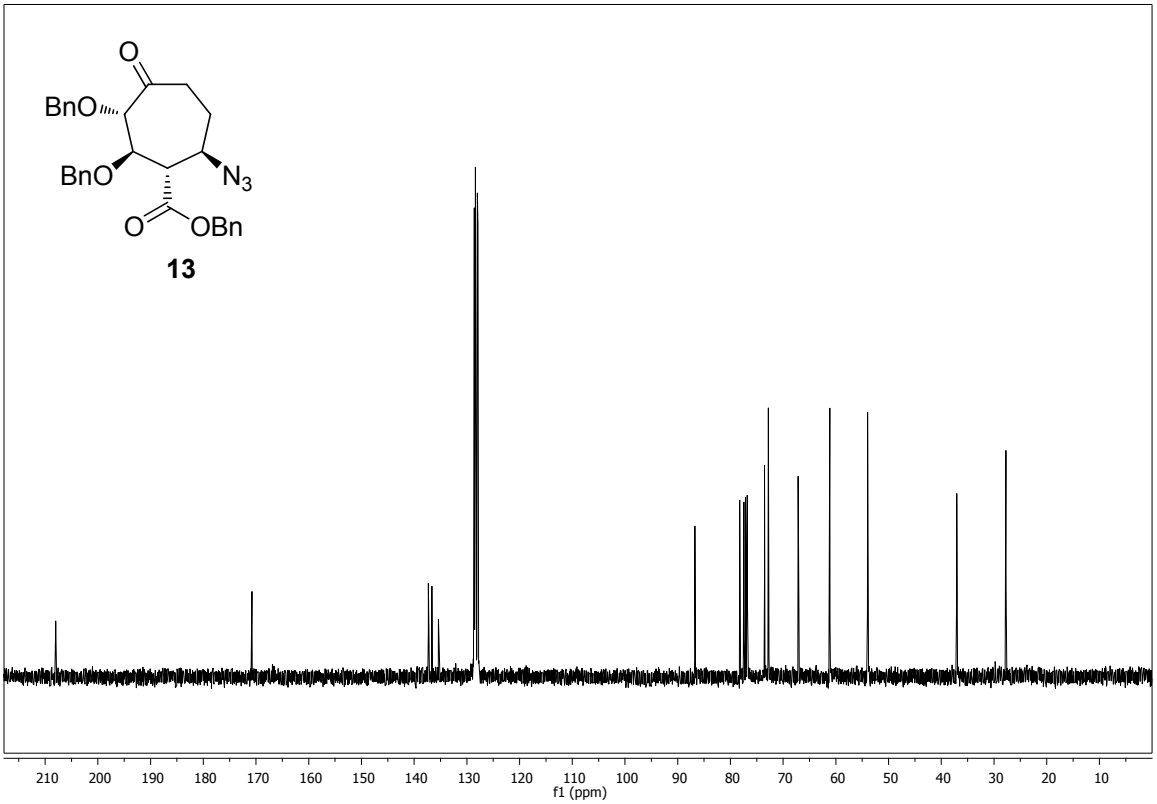


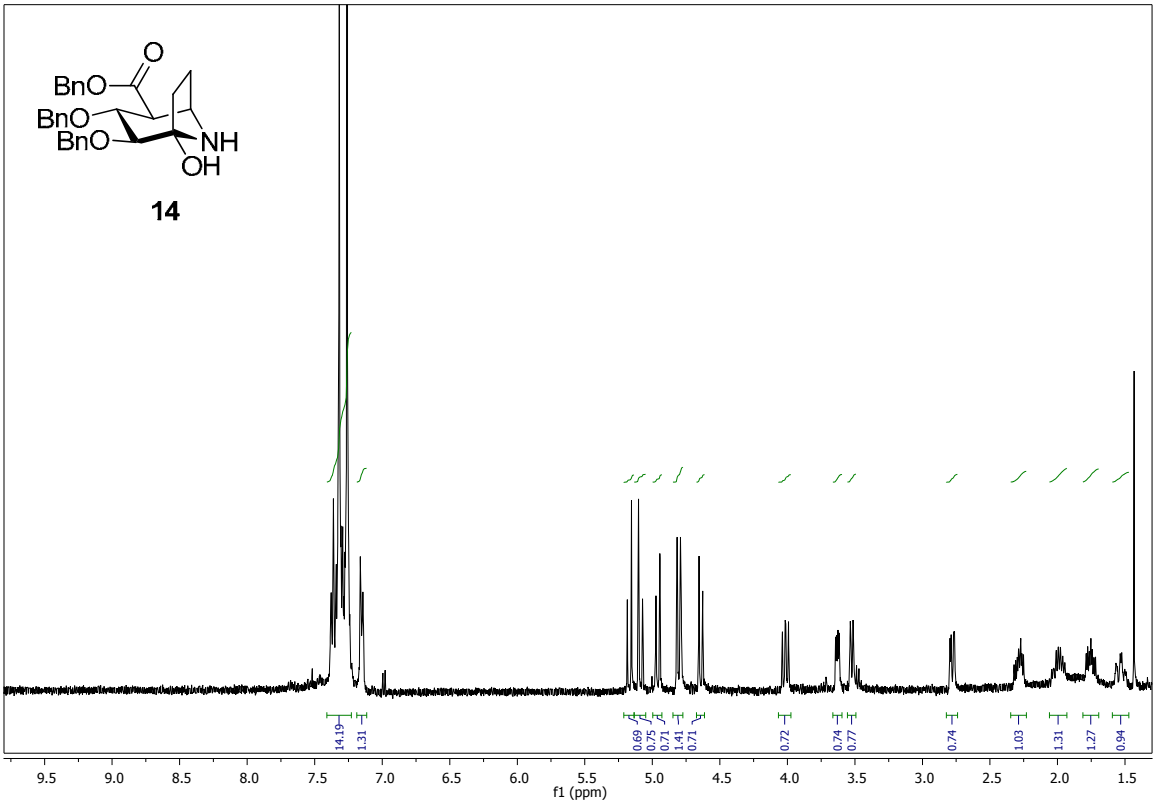


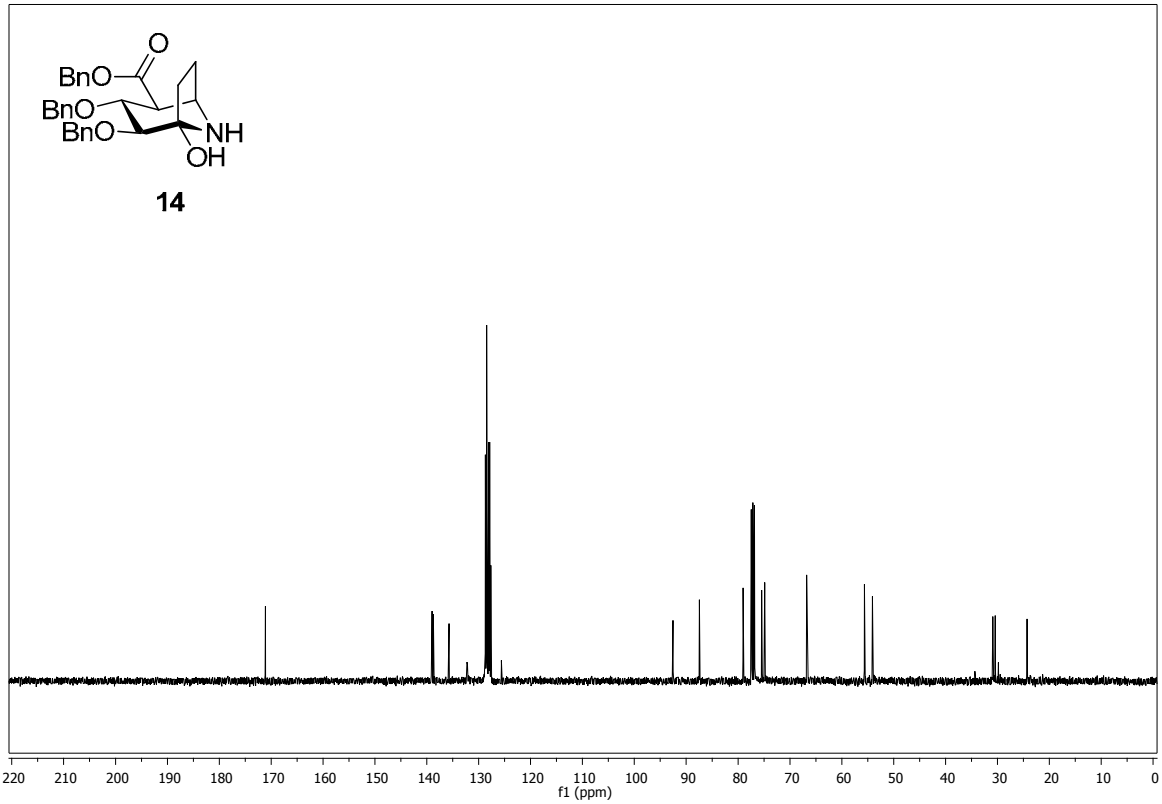


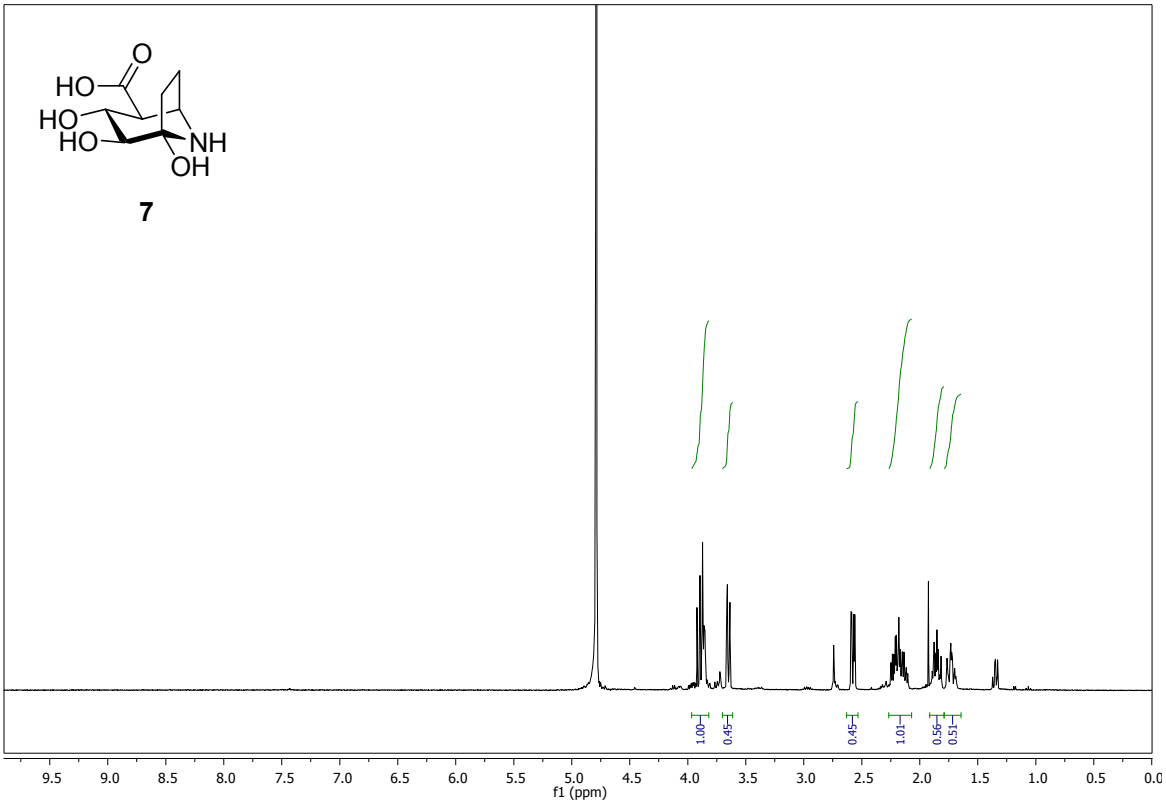












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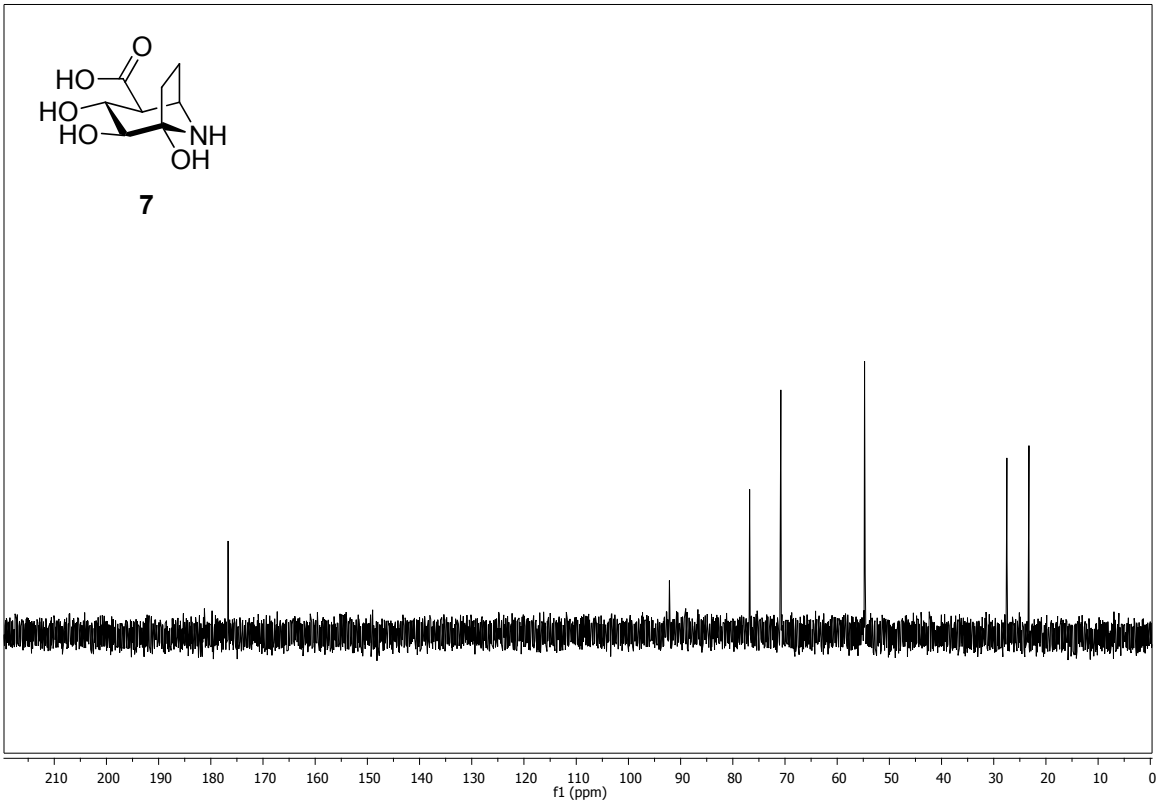

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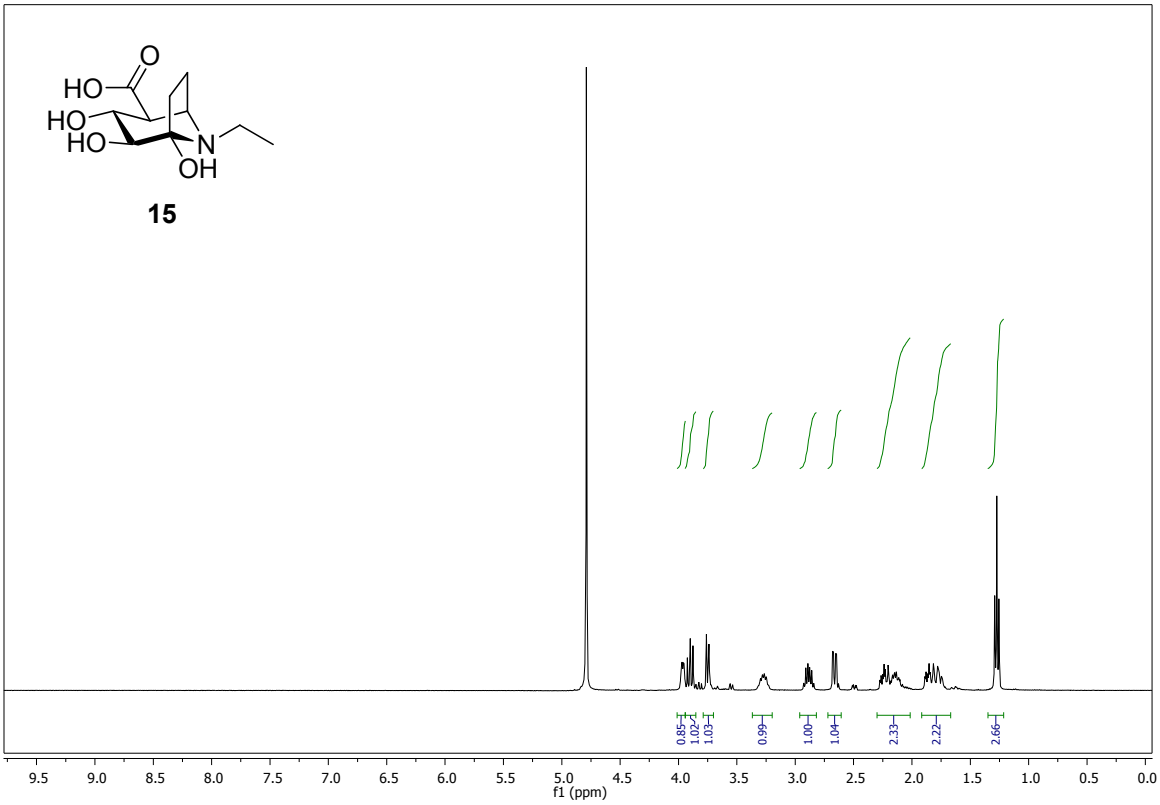
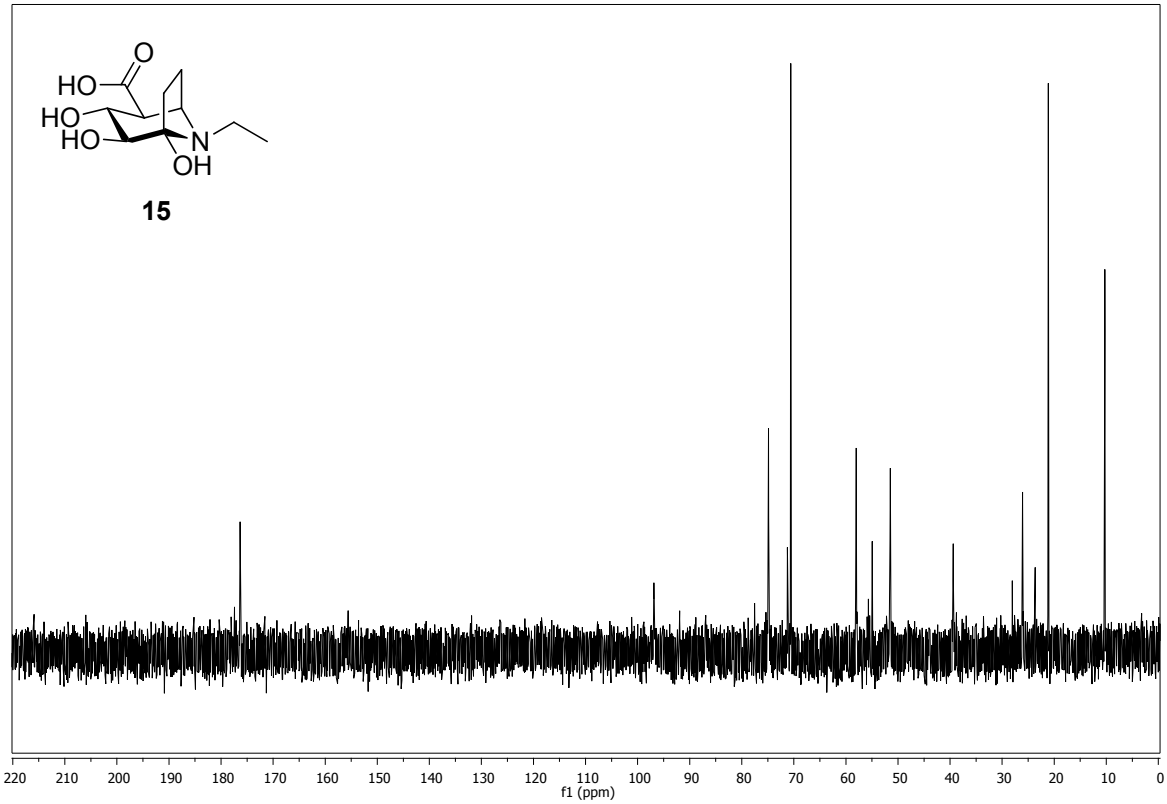
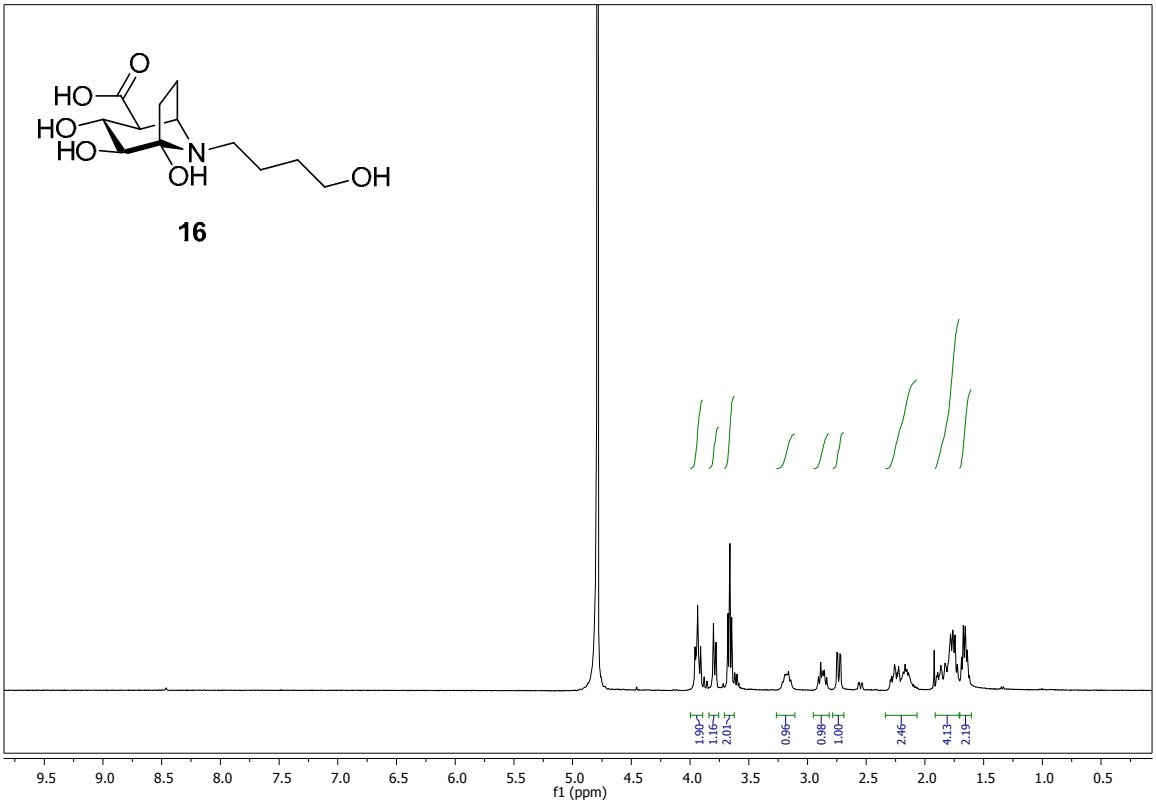
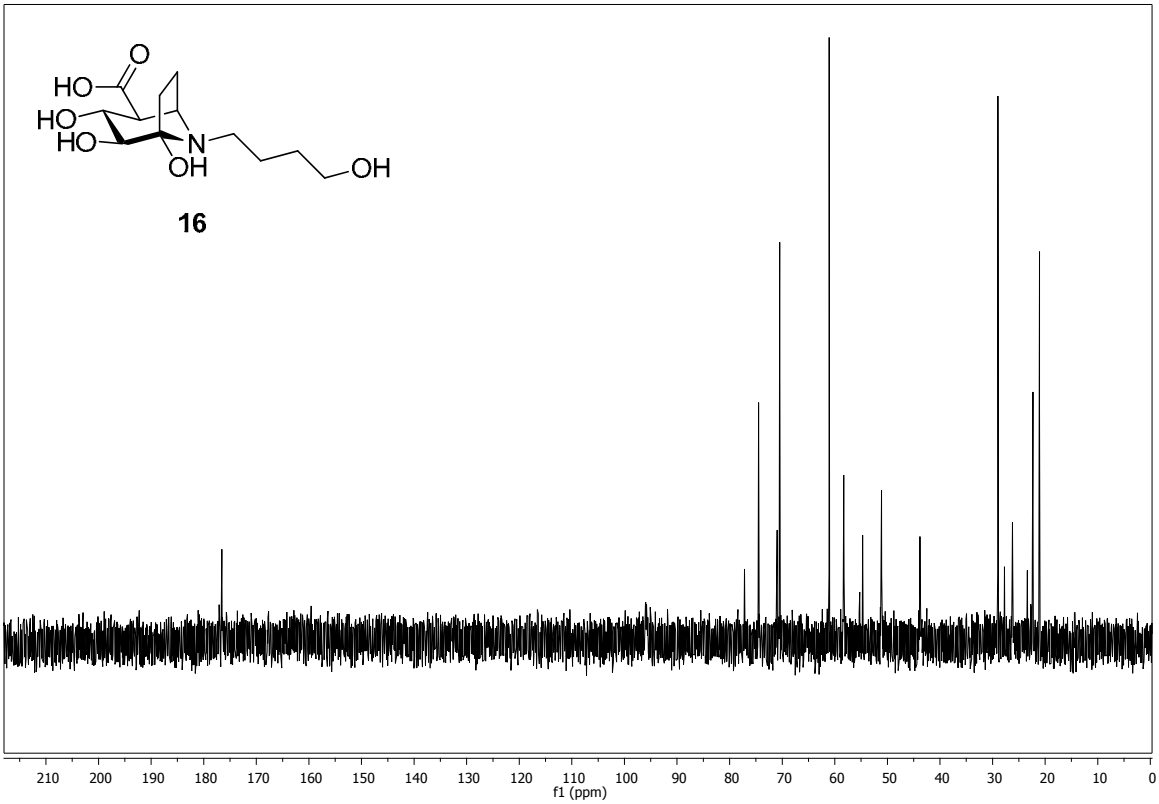


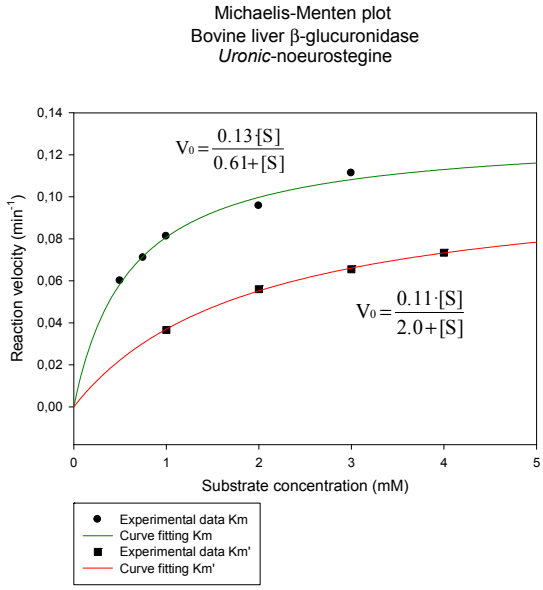


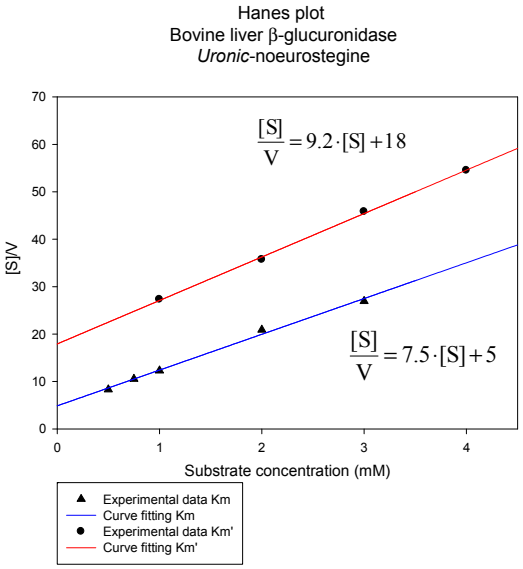




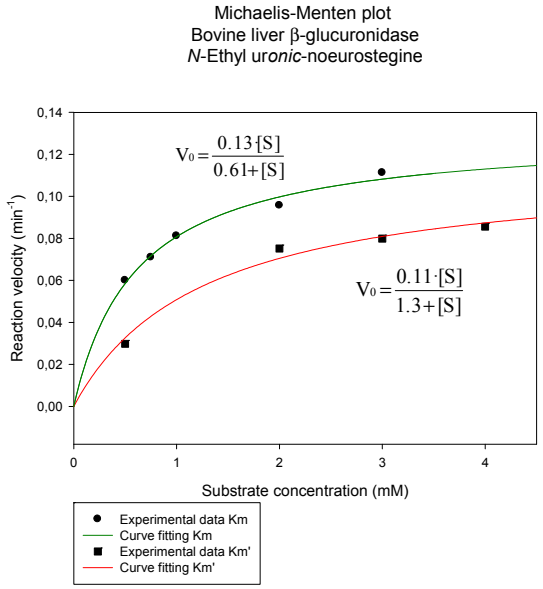
Michaelis-Menten and Hanes plots

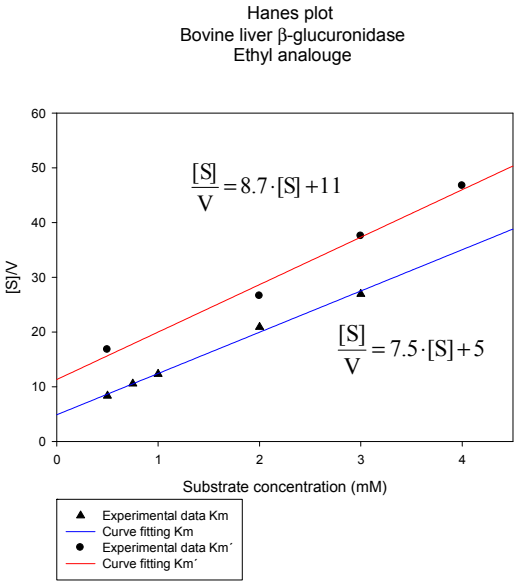
Michaelis-Menten and Hanes plots for inhibition of bovine liver β-glucuronidase and *E. coli* β-glucuronidase by *uronic*-noeurostegine and two analogues thereof.



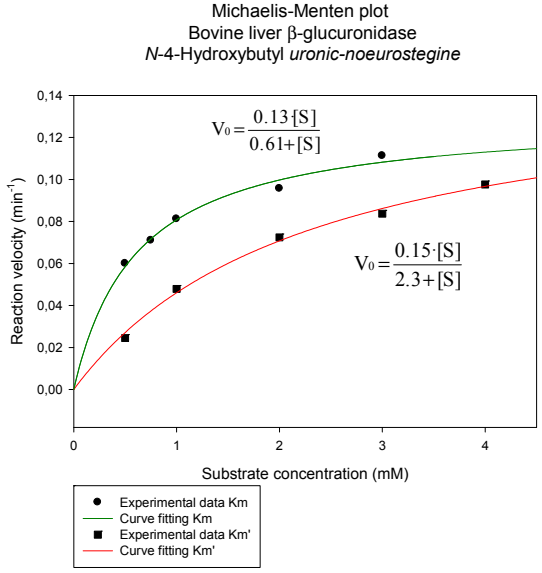


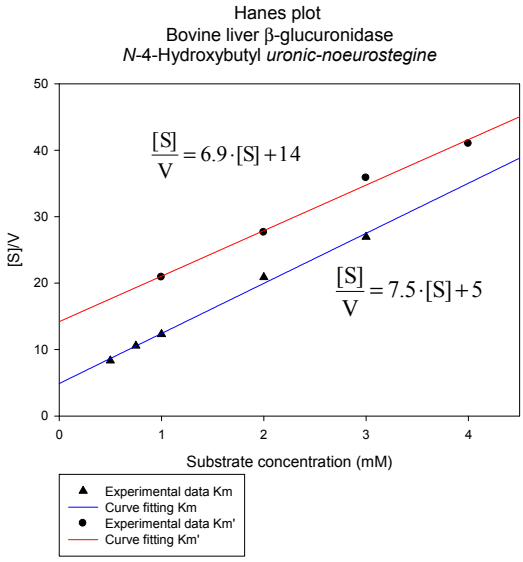
[I] = 5 μM, K_i = 2.3 μM.



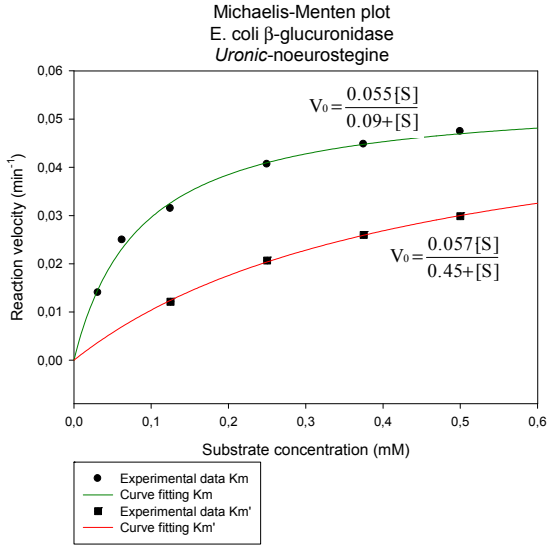


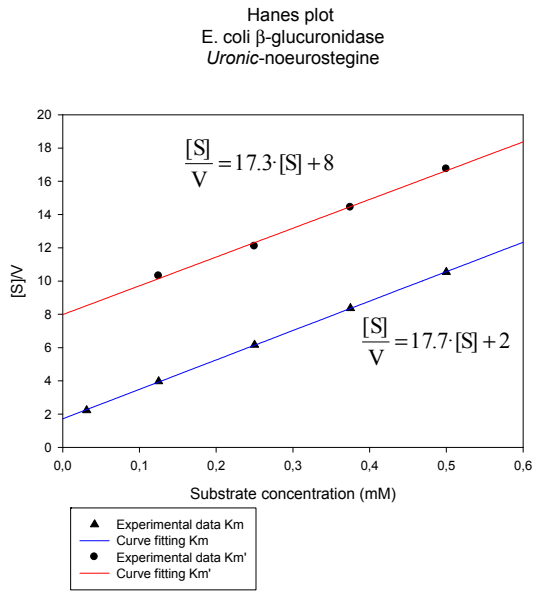
[I] = 10 μM, K_i = 9.5 μM.



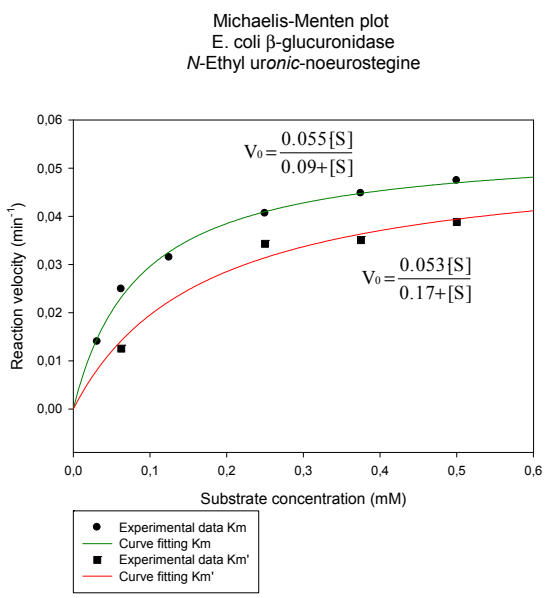


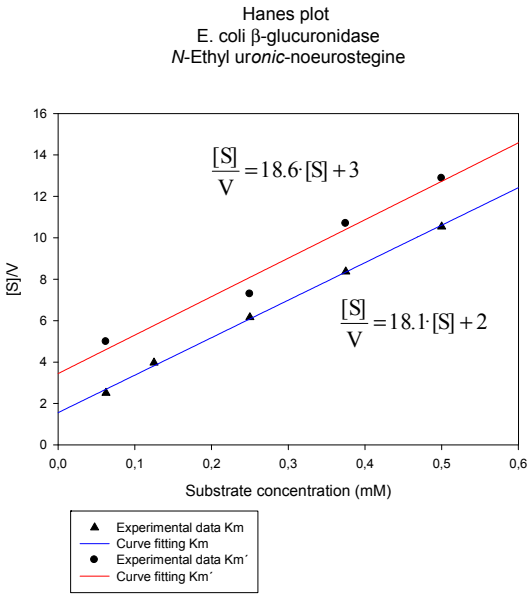
[I] = 10 μM, K_i = 3.6 μM.



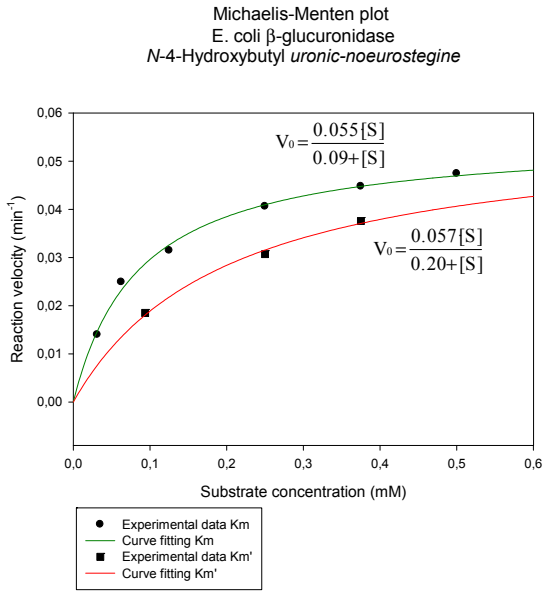


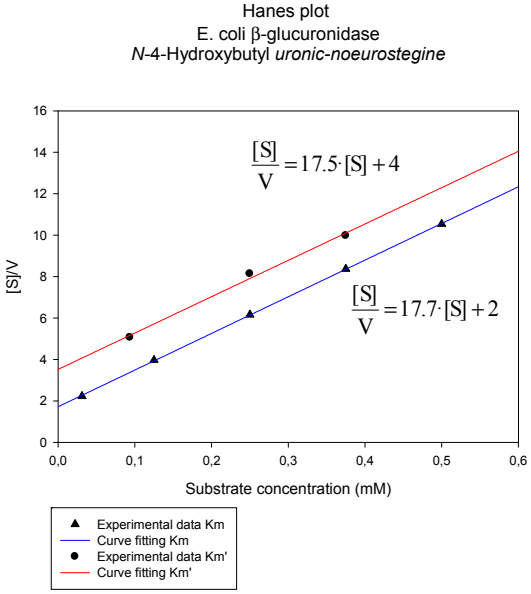
[I] = 0.25 μM, K_i = 60 nM.





[I] = 1 μM, K_i = 1.0 μM.





[I] = 1 μM, K_i = 0.74 μM.

Computational methods:

Ligand modelling: Compound **7** was constructed in Maestro version 9.2.[1] The protonation state of the nitrogen was determined by Epik[2] to be 8.38 yielding a zwitter ionic structure. Both compound **7** and the co-crystallised ligand were minimised in MacroModel[3] utilizing the MMFFs force field[4] in implicit water by conjugated gradient method. Conformational search was also performed in

Marcromodel[3] using the MCMM method, utilizing the MMFFs force field and implicit solvent. The global energy conformations were used as input structures for Glide docking.

Protein Preparation: The protein structure (PDB:3K4D) was prepared for Glide docking utilizing the Protein Preparation wizard in Schrödinger Suite 2011.[5] The catalytic Glu413 was selected to be protonated.

Glide Docking:[6] The co-crystallized ligand was used to binding site definition. The extra precision scoring function was applied in all dockings.[7] Two poses were obtained of compound **7** with GlideScores of -12.03 and -11.30 kcal/mol, respectively. The docking of the co-crystallized ligand yielded one pose with a GlideScore of -12.26 kcal/mol and an all atom RMSD of 0.20 Å.

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[1] Maestro, version 9.2, Schrödinger, LLC, New York, NY, 2011.
[2] a) Epik, version 2.2, Schrödinger, LLC, New York, NY, 2011. b) J. C. Shelley, A. Cholleti, L. L. Frye, J. R. Greenwood, M. R. Timlin and M. Uchiyama, *J. Comput. Aided Mol. Des.* 2007**, 21**, 681–691.
[3] MacroModel, version 9.9, Schrödinger, LLC, New York, NY, 2011
[4] a) T. A. Halgren, *J. Comput. Chem.* 1996*,* **17**, 490-519, b) T. A. Halgren, *J. Comput. Chem.* 1999, **20**, 730-748
[5] Schrödinger Suite 2011 Protein Preparation Wizard; Epik version 2.2, Schrödinger, LLC, New York, NY, 2011; Impact version 5.7, Schrödinger, LLC, New York, NY, 2011; Prime version 3.0, Schrödinger, LLC, New York, NY, 2011.
[6] a) Glide, version 5.7, Schrödinger, LLC, New York, NY, 2011, b) R. A. Friesner, J. L. Banks, R. B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. P. Repasky, E. H. Knoll, D. E. Shaw, M. Shelley, J. K. Perry, P. Francis and P. S. Shenkin, *J. Med. Chem.*, 2004, **47**, 1739–1749.
[7] R. A. Friesner, R. B. Murphy, M. P. Repasky, L. L. Frye, J. R. Greenwood, T. A. Halgren, P. C. Sanschagrin and D. T. Mainz, *J. Med. Chem.*, 2006, **49**, 6177–6196.