Supplementary Material (ESI) for Chemical Communications

Glycosidase Inhibitors as Conformational Transition State Analogues

Ian R. Greig and Ian H. Williams

Description of QM/MM systems studied

All simulations were conducted using the DYNAMO molecular dynamics libraries (Field *et al.*, J. Comput. Chem., **2000**, 21, 1088)

Aqueous model systems

Gas-phase local energy minimum structures for protonated oxazolinium ion **C**, thiazoline **1**, PUGNAc **2** and nagstatin **3c** were determined at the AM1 level of theory. These structures were solvated in a 40 Å x 40 Å x 40 Å periodically repeating cubic box produced using repeated images of a smaller preequilibrated box containing 216 TIP3P water molecules. The AM1 / TIP3P van der Waals parameters of Gao were used throughout,[1] the exception being for the sulfur atom (not parameterized) for which the OPLS values of σ = 3.55 Å and ε = 0.250 kilocalories per mole were used without modification. These starting structures were minimized, heated from 10 K to 300 K over1 ps and equilibrated at 300 K for 100 ps using velocity Verlet dynamics. After equilibration, 600 ps trajectories were determined. Non-bonded interactions were treated with cutoff distances of 15.0 Å, 13.5 Å and 12.0 Å for the pairlist, inner and outer regions respectively.

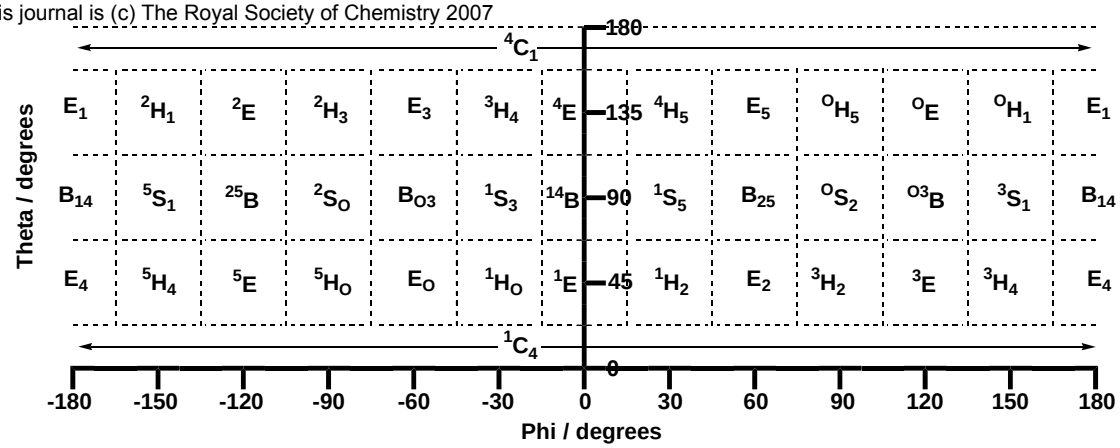
Determination of the potential of the mean force for the reaction of oxazolinium ion **C** took place from its equilibrated structure. A suitably positioned TIP3P water molecule was selected and converted to a QM water molecule and the system was equilibrated for a further 100 ps with the reaction coordinate ($d_1 - d_2$) constrained at a value of 1.3 Å. A series of simulations (each consisting of 20 ps equilibration and 100 ps production) in which the reaction coordinate distance was constrained at 0.05 Å intervals with a force of 3000 kJ mol^{-1} $Å^{-1}$ was conducted and the reaction's free energy profile calculated using the potential of the mean force and weighted histogram analysis approach.

Enzymic system

Structure 2CHN from the Protein Databank was used as the basis for simulations of the *Bacteriodes thetaiotaomicron* GH84 *O*-GlcNAcase (BTOG)–bound thiazoline **1**. Structural and kinetic studies of *Bacteriodes thetaiotaomicron* GH84 *O*-GlcNAcase have shown it to be an excellent mechanistic model for Human *O*-GlcNAcase.[2] Protonation states of amino acid side chains bearing acidic residues at the enzyme's pH optimum were determined on the basis of empirical pH – rate studies[3] and calculations using a variety of theoretical methods. The crystallographically determined structure of *Bacteriodes thetaiotaomicron* GH84 *O*-GlcNAcase consists of four domains with catalytic activity residing in domain 2. Domain 2 (284 amino acid residues treated using the OPLS-AA forcefield) and bound thiazoline (treated quantum mechanically using the AM1 semi-empirical mode) were solvated by 10453 TIP3P water molecules in a 73.7 Å periodically repeating cubic box. Five independent simulations whose starting points were the same minimized structure were conducted using different seeds for the starting velocities. Each system was heated from 10 K to 300 K over 10 ps, equilibrated for a further 20 ps at 300 K. Production trajectories of 130 ps were then produced.

Division of Hexopyranose Conformational Space in regions corresponding to canonical conformations

The regions of conformational spaces corresponding to the 38 canonical hexopyranose conformation are represented diagrammatically below.



For each MD trajectory the fractional occupancies of each region of conformational space were determined (E_1 / B_{14} / E_4 conformations on far left-hand side of the matrices; $^OH_1$ / $^3S_1$ / $^3H_4$ on the far right-hand side of the arrays).

Protonated Hemiacetal

0.0348

0.0000	0.0000	0.0008	0.0212	0.0768	0.0280	0.0080	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0008	0.0808	0.4966	0.1935	0.0544	0.0032	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	0.0012	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

0.0000

Transition State

0.0091

0.0000	0.0000	0.0000	0.0141	0.0705	0.2919	0.3640	0.1849	0.0149	0.0000	0.0000	0.0000
0.0000	0.0000	0.0017	0.0083	0.0008	0.0008	0.0033	0.0191	0.0166	0.0000	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

0.0000

Oxazolinium

0.0004

0.0000	0.0000	0.0000	0.0000	0.0000	0.0012	0.0388	0.2007	0.2843	0.0640	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0028	0.0292	0.0068	0.0084	0.0480	0.2847	0.0308	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

0.0000

Thiazoline

0.0183

0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0291	0.0575	0.0433	0.0100	0.0000	0.0000
0.0000	0.0000	0.0000	0.0008	0.2115	0.5645	0.0075	0.0042	0.0108	0.0375	0.0000	0.0000
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

0.0000

PUGNAc

0.0000

0.0000	0.0000	0.0541	0.0175	0.0000	0.0025	0.1049	0.0008	0.0000	0.0000	0.0000	0.0000
0.0200	0.0000	0.0341	0.0150	0.0541	0.0158	0.0108	0.0000	0.1232	0.0908	0.0050	0.0158
0.0350	0.0000	0.0000	0.0000	0.0283	0.0008	0.0000	0.0000	0.2664	0.1032	0.0000	0.0017

0.0000

Statin

0.015

0.000	0.000	0.002	0.027	0.356	0.485	0.053	0.001	0.000	0.000	0.000	0.000
0.000	0.003	0.025	0.015	0.012	0.008	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.000

BTOG-bound Thiazoline

0.130

0.000	0.000	0.000	0.000	0.001	0.019	0.041	0.044	0.005	0.000	0.000	0.000
0.000	0.000	0.000	0.002	0.541	0.196	0.001	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.000

The similarity indices for pairs of the various ensembles of species studied are given below…

	Protonated hemiacetal **A**	Transition State **B**	Oxazolinium Ion **C**	Thiazoline **1**	PUGNAc **2**	Nagstatin **3c**
Protonated hemiacetal **A**	1.0000	0.0770	0.0098	0.2170	0.1068	0.1630
Transition State **B**		1.0000	0.2418	0.0776	0.2164	0.5984
Oxazolinium Ion **C**			1.0000	0.1850	0.2175	0.0106
Thiazoline **1**				1.0000	0.1137	0.0298
PUGNAc **2**					1.0000	0.0372
Nagstatin **3c**						1.0000

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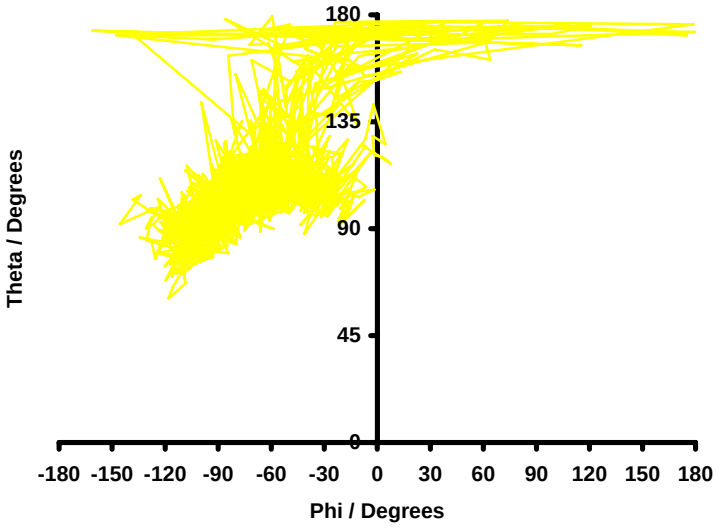
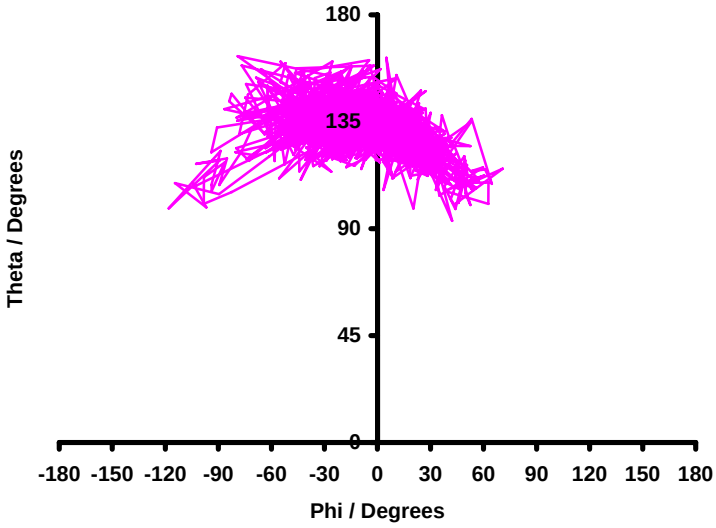
Multiple Dynamic Transformations Between Hexopyranose Ring Conformations in Reaction Pathway Species and Inhibitors

These diagrams show points representing snapshots from the MD trajectories associated with the six ensembles considered. They show multiple conversions between regions of conformational space indicating that, from the perspective of hexopyranose conformational changes, the trajectories are well equilibrated.

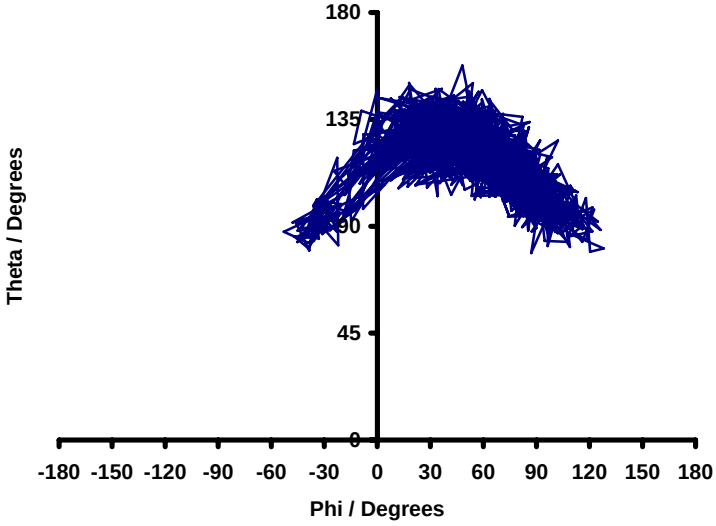
Protonated Hemiacetal



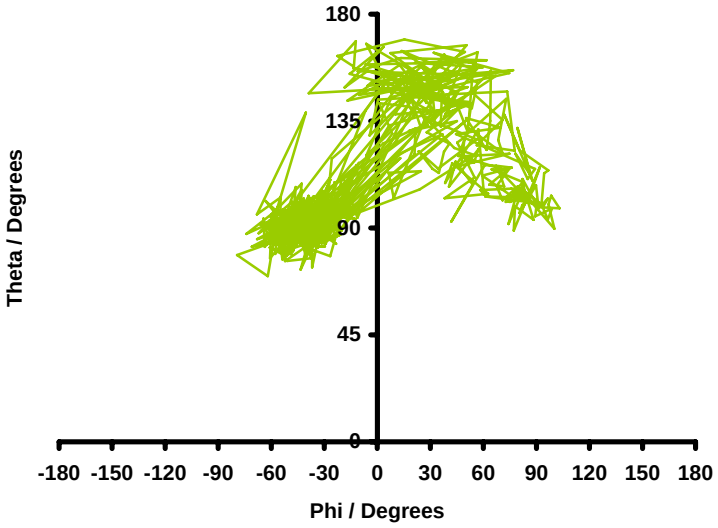
Transition State



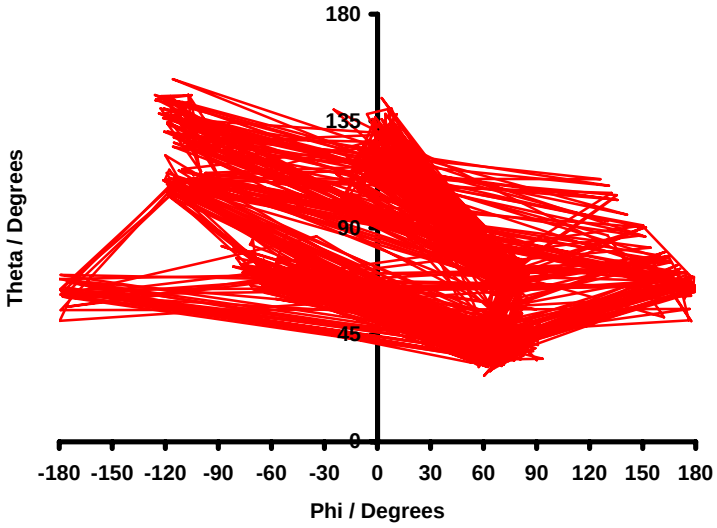
Oxazolinium Ion



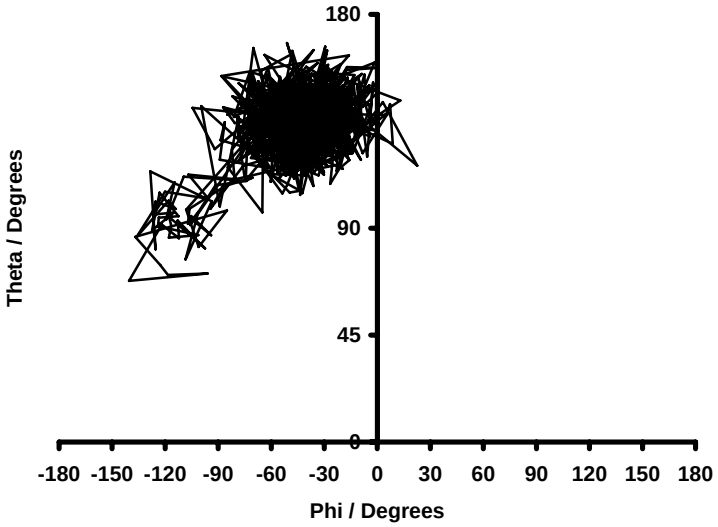
Thiazoline



PUGNAc



Statin



References

[1] J. Gao, X. Xia, *Science* **1992**, 258, 631.

[2] R. J. Dennis, E. J. Taylor, M. S. Macauley, K. A. Stubbs, J. P. Turkenburg, S. J. Hart, G. N. Black, D. J. Vocado, G. J. Davies, *Nature Struct. Mol. Biol.* **2006**, 13, 365;

[3] N. Çetinbaş, M. S. Macauley, K. A. Stubbs, R. Drapala, D. J. Vocadlo, *Biochemistry* **2006**, 45, 3835.