Electronic Supplementary Material (ESI) for Medical Chemistry Communications

Intramolecular Hydrogen Bonding to Improve Membrane Permeability and Absorption in Beyond Rule of Five Chemical Space

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Electronic Supplementary Information

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Conformational Searching in the Gas Phase to Determine the Lowest Energy Conformation

Lowest energy conformers were calculated in Macromodel (Schrodinger Inc.) using the OPLS 2005 force field and the systematic dynamic sampling approach in the gas phase. Conformers were generated using 12 steps for each rotatable bond. Torsional sampling was set to intermediate, and mirror image conformations were retained. Only conformers within an energy window of 42 $kJmol^{-1}$ were considered. Structures with an RMSD of atom positions of less than 1Å were regarded as duplicates and eliminated from the analysis. Only the lowest energy conformer was considered for analysis of internal hydrogen bonding. We only use the lowest energy conformer for the analysis, since the energy difference between the lowest energy conformation and the second lowest energy conformation is 7.73 kcal/mol for cyclosporine, 4.80 kcal/mol for atazanavir, and 2.93 kcal/mol for aliskiren. Therefore, we propose that analysis of only the lowest energy conformer is sufficient for most small molecules which form internal hydrogen bonds. Computation time for a full conformational analysis for a typical drug-like molecule with molecular weight of 450-500 Dalton was less than 10 minutes. The computations can be readily parallelised which enables the analysis of library-size data sets of for example 20,000 compounds on 40 processors in 3-4 days, depending on the complexity of the molecular structures. The process also scales linearly with the number of processors, i.e. twice the number of processors halves the computation time required.

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Variable Temperature NMR Experiments to Determine Temperature coefficients for Exchangeable Protons

In the past variable temperature NMR experiments have been used to detect if exchangeable protons are involved in intramolecular hydrogen bonds or are not solvent exposed, their uses and limitations have been detailed, for proteins, by Williamson and Baxter.[1] For our studies, and after performing a series of calibration experiments, we established the following limits in temperature coefficient (TC) in lipophilic solvents, such as deuterated chloroform and deuterated toluene:

- For TCs less negative than -3 ppb K^{-1} there is a strong intramolecular hydrogen bond,

- TC between -5.0 and -3.0 ppb K^{-1} is considered a weak intramolecular hydrogen bonds,

- TC between -5.0 and -8.0 ppb K^{-1} there are no intramolecular hydrogen bonds present.

- For TC more negative than -8.0 ppb K^{-1} the environment in which the measured proton sits is subject to large conformational changes when temperature is changed, and therefore no intramolecular hydrogen bonds are present.

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Cyclosporine A (CsA)

Cyclosporine A was obtained from commercial sources. We followed the NMR assignment of CsA performed by Kessler,[2] and measured the TC of the NH protons in deuterated chloroform. NMR derived TC data on CsA (Table SI-1) suggests the formation of three strong IHBs, and one weak IHB in NH87, while the changes in the chemical shifts of the OH proton with temperature are caused by the changes in orientation of the adjacent alkyl side chain (Figure SI-1). The differences in the temperature coefficients reported by Kessler[2] and coworkers for NH 87 could be due to the lower concentration at which we conducted our experiments, 0.9 mM, versus 40 mM.

Table SI-1 Chemicals shifts and TCs in CDCl3 of the exchangeable protons of CsA.

Proton number	Chemical shift at 298K (ppm)	TC (ppb K^{-1})	R^2
NH 89	7.916	-2.8	0.998
NH 87	7.636	-5.0	0.994
NH 86	7.428	-1.4	0.996
NH 88	7.122	-0.9	0.995
OH 90	3.833	-9.2	0.999

The nOes we measured are in agreement with the nOe structures described by Kessler[2] (Figure SI-2). The lowest energy conformation calculated in the gas phase (Figure 5) clearly predicts well for the number of intramolecular hydrogen bonds observed by NMR but is not in good agreement with the nOes measured, suggesting the overall predicted conformation is different to the solution conformation. This is perhaps not a surprise given the size and flexibility of this large molecule.

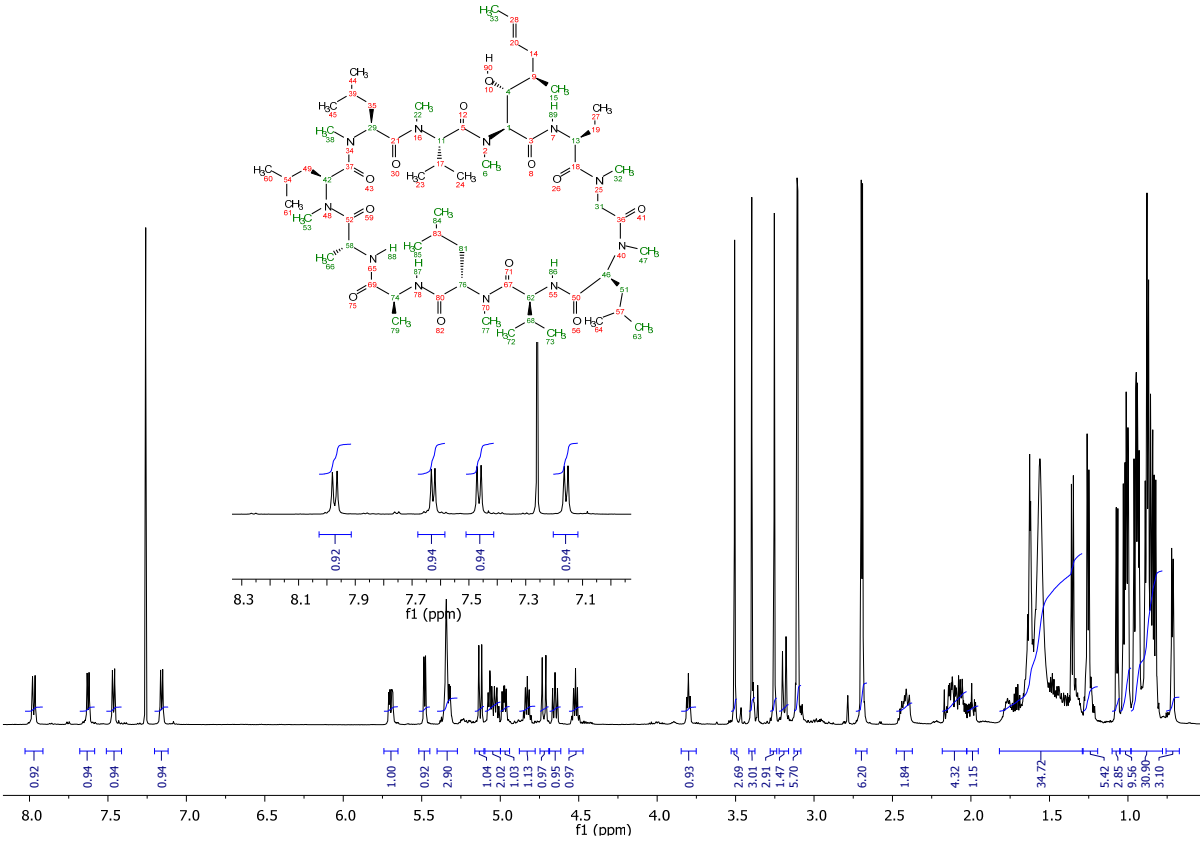

Figure SI-1. ^{1}H NMR spectrum of CsA in CDCl3, in the expanded region of the spectra are all four NH protons.

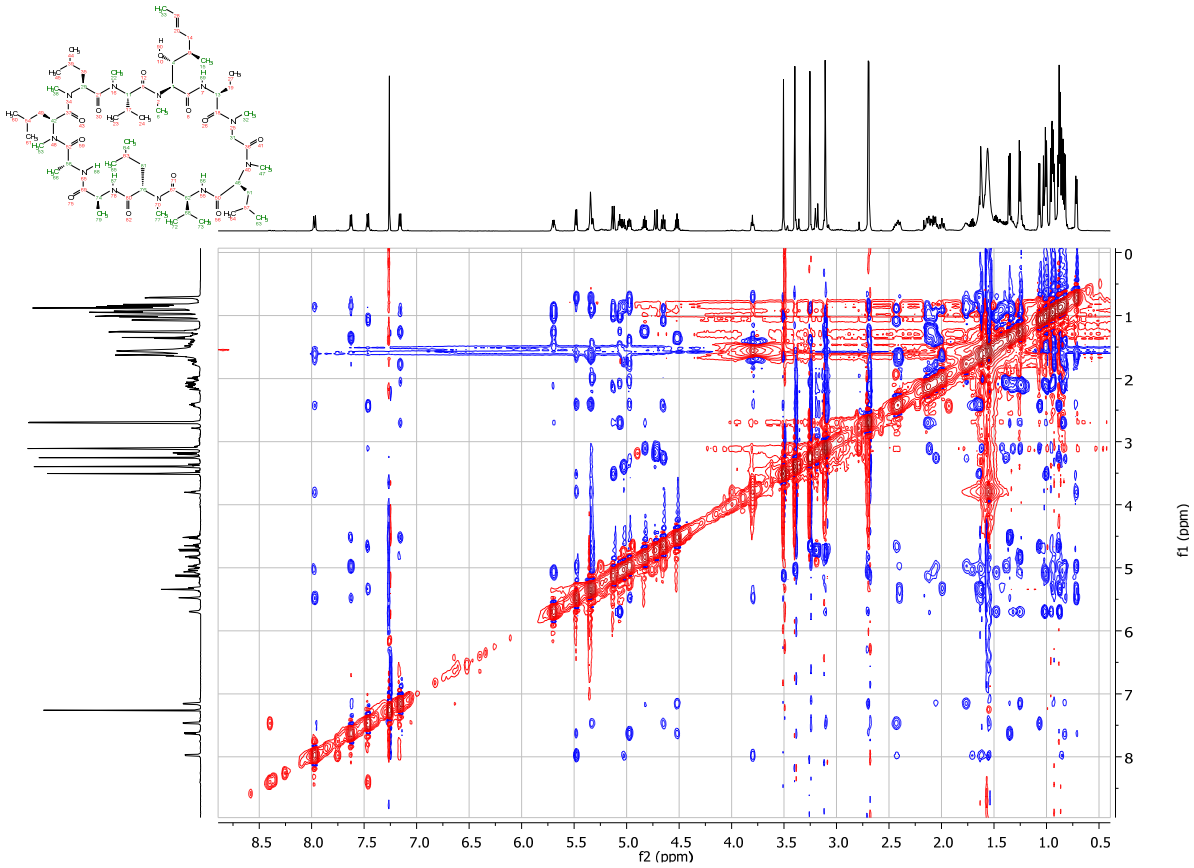

Figure SI-2. 2D NOESY spectrum of CsA in CDCl3 solution.

Atazanavir

Atazanavir was prepared according to the synthesis of Xu.[3] NMR TC experiments for atazanavir were carried out in deuterated toluene due to the greater solubility and low dielectric constant of 2.3 at 296K. TC data suggests the formation of two strong and two weak intramolecular hydrogen bonds (Table SI-2) (Figure SI-3).

Table SI-2 Chemicals shifts and TCs in d8-toluene of the exchangeable protons of atazanavir.

Proton number	Chemical shift at 298K (ppm)	TC (ppb K^{-1})	R^2
NH4	5.241	-2.6	0.980
NH 11	6.609	-4.1	1.0
NH 18	5.561	-0.7	0.116
NH 22	5.388	-3.5	0.996

Detailed analysis of the NOESY data (Figure SI-4) in deuterated toluene helped us to map the correlations and compare that to the lowest energy conformation calculated in the gas phase (Figure 6). The data suggests some interactions are in agreement with the model while others are less well predicted. The nOe observed from the t-butyl group labelled as 28-30, to the biphenyl group together with the temperature coefficients for NH-18 and NH-11, -0.7 and -4.1 ppb K^{-1}, respectively, are in good agreement with the lowest energy conformation. The lack of linearity for the temperature coefficient of NH-18 could be due to a combination of factors, we hypothesised that a change in the conformation could be responsible for this. The interactions of the two other NH protons, NH-4 being involved in an IHB and NH-22 involved in a weak interaction, was not predicted in the gas phase model. Unfortunately the –OH proton was not observed, as it is potentially involved in rapid exchange with the trace amount of water present in the sample.

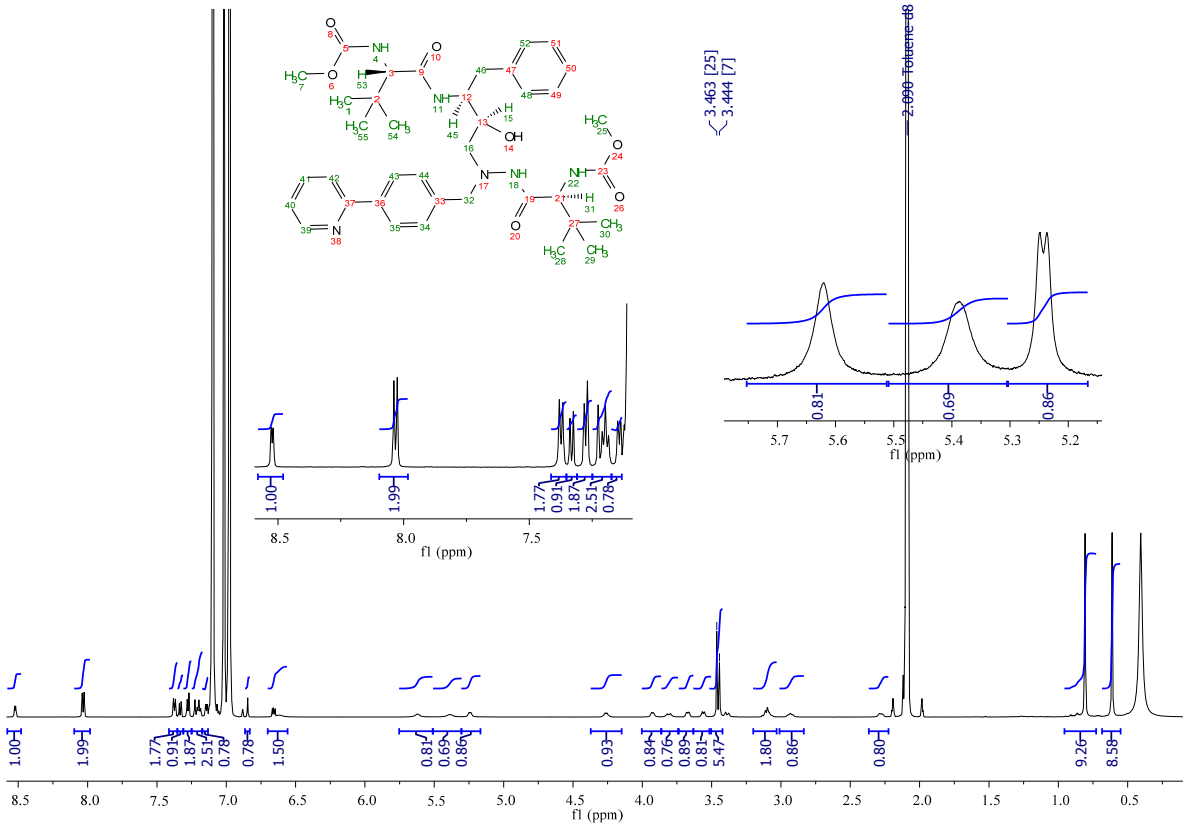

Figure SI-3. ^{1}H NMR spectrum of atazanavir in d8-toluene, in the expanded region of the spectra are the aromatic and three of the NH protons.

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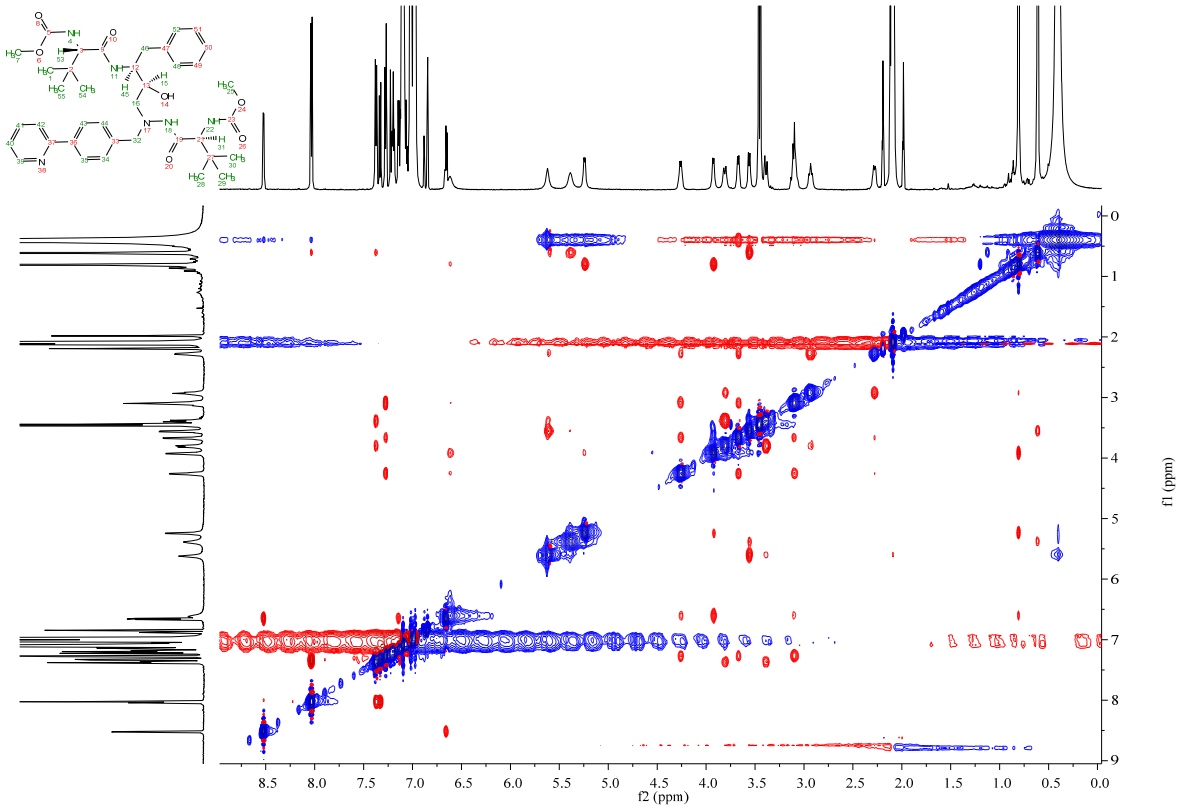
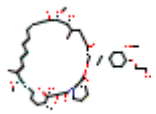
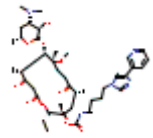
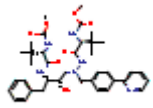
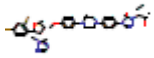
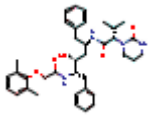
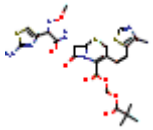
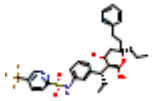
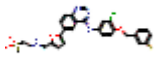
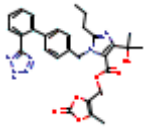


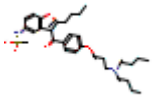
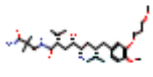
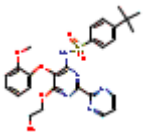
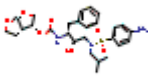
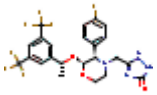
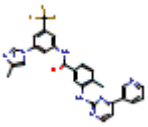
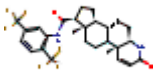
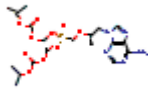
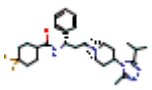
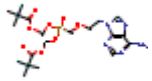
Figure SI-4. 2D NOESY spectrum of atazanavir in d8-toluene solution.

Aliskiren

Aliskiren was purchased from commercial sources but due to the very low solubility of the compound in solvents such as deuterated chloroform or toluene we were not able to produce data of sufficient quality for TC or nOe analysis.

Table SI-3 Drug compounds with MW >500 Da that were approved by the FDA (2000-2010).

Structure	Generic Name	MW (Da)	clogP	TPSA (Å^2)	NO CNT (Revised NO CNT)	NHOH CNT (Revised NHOH CNT)	nROT	IHB
	Everolimus	958	7.1	205	15 (12)	3 (0)	9	3
	Telithromycin	812	3.7	172	15 (14)	1 (0)	11	1
	Atazanavir	705	5.9	171	13 (10)	5 (2)	16	3
	Posaconazole	701	4.1	116	12 (11)	1 (0)	12	1
	Lopinavir	629	6.1	120	9 (6)	4 (1)	15	3
	Cefditoren Pivoxil	621	2.7	175	13 (12)	2 (1)	11	1
	Tipranavir	603	7.7	106	7 (7)	2 (2)	11	0
	Lapatinib	581	6.0	106	8 (7)	2 (1)	11	1
	Olmesartan Medoxomil	559	2.7	162	12 (11)	2 (1)	11	1

Structure	Generic Name	MW (Da)	clogP	TPSA (Å^2)	NO CNT (Revised NO CNT)	NHOH CNT (Revised NHOH CNT)	nROT	IHB
	Dronedarone	557	8.6	89	7 (6)	1 (0)	18	1
	Aliskiren	552	3.5	146	9 (7)	4 (2)	19	2
	Bosentan	552	4.2	146	11 (9)	2 (0)	10	2
	Darunavir	548	2.9	140	10 (8)	3 (1)	12	2
	Aprepitant	534	4.8	83	7 (7)	2 (2)	6	0
	Nilotinib	530	5.8	98	8 (8)	2 (2)	6	0
	Dutasteride	529	4.9	58	4 (4)	2 (0)	2	0
	Tenofovir Disoproxil	519	0.8	185	15 (15)	1 (1)	17	0
	Maraviroc	514	3.3	63	6 (5)	1 (0)	8	1
	Adefovir Dipivoxil	501	1.4	167	13 (13)	1 (1)	13	0

Supplementary references

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2 H. Kessler, M. Kock, T. Wein and M. Gehrke, *Helv. Chim. Acta*, 1990, **73**, 1818.

3 Z. Xu, J. Singh, M.D. Schwinden, B. Zheng, T.P. Kissick, B. Patel, M.J. Humora, F. Quiroz, L. Dong, D.-M. Hsieh, J.E. Heikes, M. Pudipeddi, M.D. Lindrud, S.K. Srivastava, D.R. Kronenthal and R.H. Mueller, *Org. Proc. Res. Dev.* 2002, **6**, 323.