Supporting information for

VCD spectroscopy reveals that a water molecule determines the conformation of azithromycin in solution

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1. Experimental and computational details

Materials. Azithromycin-dihydrate and chloroform-d_1 were purchased from Sigma Aldrich (Germany) and used without further purification.

IR and VCD spectroscopy. The IR and VCD spectra were recorded on a Bruker Vertex 70/ PMA 50 VCD spectrometer. A solution of azithromycin in CDCl3 at a concentration of 0.185 M was held in a transmission cell with BaF_2 windows with 50 µm path length. Both IR and VCD spectra were recorded at 4 cm^{-1} spectral resolution by accumulating 32 respectively at least ~32000 scans (minimum of 8 hours accumulation time for VCD). Baseline correction of the VCD spectra was done by subtraction of the solvent recorded under identical conditions.

Computational details. Monte-Carlo (MC) based conformational search was done at force field level (MMFF)[1] using Spartan 14.[2] Intermediate geometry optimization on the conformer ensembles from Spartan were done on semi-empirical level using Grimme's extended tight-binding (XTB) toolbox.[3] Removal of duplicates was done based on comparison of distances matrices using own scripts. Final geometry optimizations and frequency calculations were performed at the B3LYP/6-31G(2d,p) level of theory using the Gaussian 09 E.01 software package with tight convergence criteria and ultrafine integration grids.[4] Solvent effects were taken into account implicitly by using the integral equation formalism of the polarizable continuum model (IEFPCM)[5] for chloroform. Vibrational line broadening was simulated by assigning a Lorentzian band shape with half-width at half-height of 8 cm^{-1} to the calculated dipole and rotational strength. The calculated frequencies were scaled by a factor of σ=0.982. The theoretical spectra shown in the main text are simulated according to the Boltzmann weights for the relative zero-point corrected energies ΔE_{ZPC}. Figures are prepared using CylView.[6]

2. Additional spectra

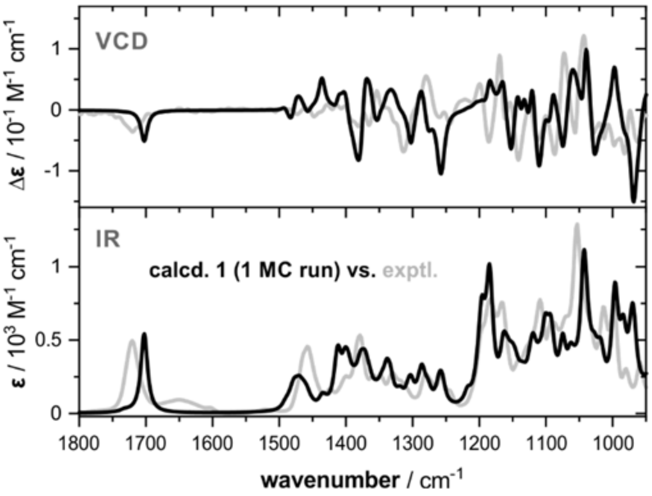

Figure S1. Comparison of experimental and computed spectra of **1** obtained from a single MC search run.

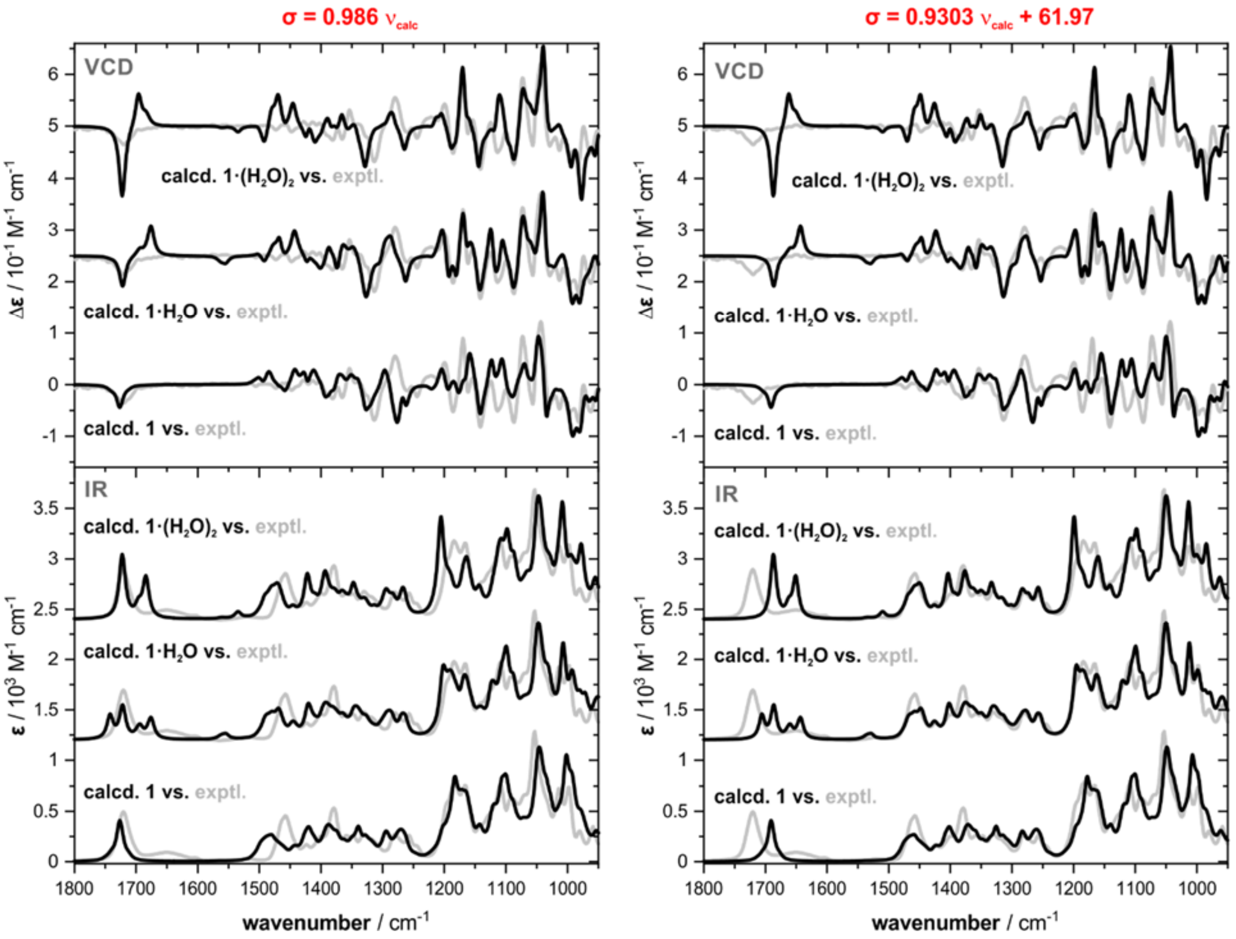

Figure S2. Comparison of experimental and computed spectra of **1**, **1**·H_2O and **1**·$(H_2O)_2$ for different frequency scaling factors σ. The scaling factor used on the right was determined following the approach presented in Reference 7.

3. Conformational analysis

Table S1. Absolute and relative zero-point corrected and Gibbs free energies, E_{ZPC} and G_{298K}, for the lowest energy conformers of **1** which together make up for pop(ΔE)>99% as obtained at the B3LYP/6-31G(2d,p)/ IEFPCM($CDCl_3$) level of DFT.

id	**E_{ZPC} [hartree]**	**G_{298K} [hartree]**	**DE_{ZPC} [kcal/mol]**	**DG_{298K} [kcal/mol]**	**pop(DE) [%]**	**pop(DG) [%]**
azi-*c1*	-2502.620705	-2502.709936	0.00	0.00	46.8	35.8
azi-*c2*	-2502.619714	-2502.709814	0.62	0.08	16.4	31.5
azi-*c3*	-2502.619250	-2502.708755	0.91	0.74	10.0	10.2
azi-*c4*	-2502.619209	-2502.707638	0.94	1.44	9.6	3.1
azi-*c5*	-2502.618371	-2502.707695	1.46	1.41	3.9	3.3
azi-*c6*	-2502.617759	-2502.706680	1.85	2.04	2.1	1.1
azi-*c7*	-2502.617685	-2502.706565	1.90	2.12	1.9	1.0
azi-*c8*	-2502.617509	-2502.707280	2.01	1.67	1.6	2.1
azi-*c9*	-2502.617478	-2502.707588	2.02	1.47	1.5	3.0
azi-*c10*	-2502.617256	-2502.706329	2.16	2.26	1.2	0.8
azi-*c11*	-2502.616720	-2502.706464	2.50	2.18	0.7	0.9
azi-*c12*	-2502.616653	-2502.706455	2.54	2.18	0.6	0.9
azi-*c13*	-2502.616521	-2502.705075	2.63	3.05	0.6	0.2
azi-*c14*	-2502.616518	-2502.707239	2.63	1.69	0.6	2.1
azi-*c15*	-2502.616331	-2502.706442	2.74	2.19	0.5	0.9
azi-*c16*	-2502.615846	-2502.705228	3.05	2.95	0.3	0.2
azi-*c17*	-2502.615776	-2502.706350	3.09	2.25	0.3	0.8
azi-*c18*	-2502.615693	-2502.704714	3.15	3.28	0.2	0.1
azi-*c19*	-2502.615604	-2502.704682	3.20	3.30	0.2	0.1
azi-*c20*	-2502.615453	-2502.705480	3.30	2.80	0.2	0.3
				Σ =	99.1	98.6

Table S2. Absolute and relative zero-point corrected and Gibbs free energies, E_{ZPC} and G_{298K}, for the lowest energy conformers of **1**·H_2O which together make up for pop(ΔE)>99% as obtained at the B3LYP/6-31G(2d,p)/IEFPCM($CDCl_3$) level of DFT

id	**E_{ZPC} [hartree]**	**G_{298K} [hartree]**	**DE_{ZPC} [kcal/mol]**	**DG_{298K} [kcal/mol]**	**pop(DE) [%]**	**pop(DG) [%]**
azi-H_2O-*c1*	-2579.053294	-2579.143764	0.00	0.00	53.4	36.6
azi-H_2O-*c2*	-2579.052657	-2579.143658	0.40	0.07	27.2	32.7
azi-H_2O-*c3*	-2579.051216	-2579.142228	1.30	0.96	5.9	7.2
azi-H_2O-*c4*	-2579.050677	-2579.142051	1.64	1.07	3.3	6.0
azi-H_2O-*c5*	-2579.050455	-2579.141052	1.78	1.70	2.6	2.1
azi-H_2O-*c6*	-2579.049762	-2579.141208	2.22	1.60	1.3	2.4
azi-H_2O-*c7*	-2579.049612	-2579.140889	2.31	1.80	1.1	1.7
azi-H_2O-*c8*	-2579.049544	-2579.140559	2.35	2.01	1.0	1.2
azi-H_2O-*c9*	-2579.049464	-2579.140675	2.40	1.94	0.9	1.4
azi-H_2O-*c10*	-2579.049380	-2579.140080	2.46	2.31	0.8	0.7
azi-H_2O-*c11*	-2579.048942	-2579.140813	2.73	1.85	0.5	1.6
azi-H_2O-*c12*	-2579.048702	-2579.139773	2.88	2.50	0.4	0.5
azi-H_2O-*c13*	-2579.048379	-2579.140137	3.08	2.28	0.3	0.8
azi-H_2O-*c14*	-2579.048104	-2579.139093	3.26	2.93	0.2	0.3
				Σ =	99.1	95.2

Table S3. Absolute and relative zero-point corrected and Gibbs free energies, E_{ZPC} and G_{298K}, for the lowest energy conformers of **1**·$(H_2O)_2$ which together make up for pop(ΔE)>95% as obtained at the B3LYP/6-31G(2d,p)/IEFPCM($CDCl_3$) level of DFT

id	**E_{ZPC} [hartree]**	**G_{298K} [hartree]**	**DE_{ZPC} [kcal/mol]**	**DG_{298K} [kcal/mol]**	**pop(DE) [%]**	**pop(DG) [%]**
azi-$(H_2O)_2$-*c1*	-2655.482072	-2655.574119	0.00	0.00	71.98	59.83
azi-$(H_2O)_2$-*c2*	-2655.47996	-2655.572385	1.33	1.09	7.68	9.53
azi-$(H_2O)_2$-*c3*	-2655.479877	-2655.572316	1.38	1.13	7.03	8.85
azi-$(H_2O)_2$-*c4*	-2655.479133	-2655.571176	1.84	1.85	3.20	2.65
azi-$(H_2O)_2$-*c5*	-2655.478811	-2655.571367	2.05	1.73	2.27	3.24
azi-$(H_2O)_2$-*c6*	-2655.478793	-2655.571856	2.06	1.42	2.23	5.44
azi-$(H_2O)_2$-*c7*	-2655.478372	-2655.571488	2.32	1.65	1.43	3.68
				Σ =	95.82	93.22

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