Supporting Information (SI) for

Conformational sampling of macrocyclic drugs in different environments – Can we find the relevant conformations?

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1. ^{1}H NMR assignments

Table S5. ^{1}H NMR assignment (ppm) of roxithromycin in $CDCl_3$ and D_2O

Table S1. Number of conformations and comparison of the calculated minimum energy conformer (MEC) to crystal structures

Compounds	Crystal structure	Number of conformations						RMSD of MEC to crystal structure[#] (Å)					
		Vacuum			GB-Solvation			Vacuum			GB-Solvation		
		MC	MOE	OME	MC	MOE	OME	MC	MOE	OME	MC	MOE	OME
Erythromycin	1YI2	1022	148	168	1254	1374	364	1.51	4.90	2.57	0.87	1.05	2.12
	2J0D							2.34	4.19	2.96	2.05	2.24	2.77
	3FRQ							1.65	4.26	2.42	1.46	1.73	2.36
	QIFKEX							1.56	4.83	2.78	1.05	1.27	2.28
	NAVTAF							1.21	4.78	2.47	0.38	0.70	1.85
	LAPDEN							1.97	4.45	3.13	1.55	1.71	2.75
Clarithromycin	NAVSUY01	63	335	1079	1321	1390	1677	3.02	3.53	3.17	3.02	2.77	3.26
	CIWJIC							3.14	2.42	2.91	1.35	3.11	2.27
	WANNUU							3.34	2.39	3.04	1.25	3.18	2.45
Azithromycin	1YHQ	7462	90	160	1248	1631	250	1.38	1.50	2.11	2.24	2.33	2.74
	GEGJAD							1.46	1.62	3.79	1.95	2.62	1.50
Roxithromycin	1JZZ	1193	69	479	3440	2111	1220	4.45	2.21	3.92	3.67	2.49	2.19
	FUXYOM							3.43	4.45	2.77	3.77	4.16	4.12
	KAHWAT							3.85	2.56	3.01	3.95	2.32	2.67
Telithromycin	1YIJ	288	277	838	1934	4800	4062	1.40	2.86	2.95	1.19	1.96	3.56
	1P9X							5.85	5.17	6.23	5.29	6.06	5.75
	4V7S							3.86	4.17	5.00	5.03	4.29	4.95
	4V7Z							3.75	4.10	4.87	4.90	4.15	4.83
	4WF9							1.84	2.48	2.14	2.13	2.32	2.65
Danoprevir	3SU1	239	556	3836	1749	4247	5108	2.33	3.28	3.07	1.72	3.29	3.29
Grazoprevir	3SUG	164	1032	2815	1933	3045	5060	2.66	3.49	3.50	3.32	3.03	2.79
Vaniprevor	3SU4	69	939	2128	2908	3327	4104	2.50	2.83	3.64	2.13	3.10	3.70
Asunaprevir	4WH6	101	1236	3248	1240	1908	4968	3.95	3.98	4.90	3.95	4.64	4.81
	MIYWOI							2.90	3.61	4.03	2.12	4.39	3.72
Telaprevir	3SV6	182	651	3298	1296	4221	4721	3.09	3.53	3.41	3.44	2.88	2.53
	LERJID							4.31	4.27	5.32	3.91	4.62	5.19
	3SV7							3.90	3.93	4.80	3.85	4.62	4.72

[#] RMSD of minimum energy conformer (MEC) compared to the crystal structure on the same row, color code according to the RMSD (Green: < 2.0 Å, Yellow: 2.0-4.0 Å and Red: > 4.0 Å)

Table S2. Comparison of conformers most similar to crystal structures to the corresponding crystal structure by structural similarity (by RMSD) and to the predicted minimum energy conformations (by energy)

Compounds	Crystal structure	RMSD to crystal structure[#] (Å)						Potential energy gap[*] (kcal/mol)					
		Vacuum			GB-Solvation			Vacuum			GB-Solvation		
		MC	MOE	OME	MC	MOE	OME	MC	MOE	OME	MC	MOE	OME
Erythromycin	1YI2	1.41	1.14	1.06	0.51	0.84	1.6	6.36	17.69	2.62	12.67	4.15	17.65
	2J0D	2.16	1.82	1.81	1.38	1.07	1.85	6.36	23.93	14.64	26.07	21.03	21.17
	3FRQ	1.00	1.45	1.15	0.74	0.63	1.61	25.00	21.18	14.11	6.73	6.67	21.11
	QIFKEX	1.41	1.17	1.05	0.57	0.81	1.63	6.36	21.23	2.62	8.66	6.98	15.95
	NAVTAF	1.20	1.32	1.06	0.26	0.7	1.35	1.66	17.69	2.62	2.32	0.01	2.41
	LAPDEN	1.59	1.08	1.61	1.04	0.45	1.82	6.36	23.93	2.62	12.67	6.98	17.65
Clarithromycin	NAVSUY01	2.85	2.02	2.14	2.65	1.94	2.00	20.01	18.69	23.34	19.95	23.58	22.31
	CIWJIC	2.94	1.23	0.86	0.73	0.72	0.82	15.86	23.68	7.97	2.40	12.88	5.98
	WANNUU	3.10	1.05	0.83	0.79	0.69	0.80	15.86	23.68	9.33	5.90	12.88	7.99
Azithromycin	1YHQ	0.49	1.16	1.07	0.50	0.86	1.24	5.50	22.92	12.83	2.13	4.26	11.48
	GEGJAD	1.03	1.23	1.22	0.82	0.77	1.33	3.42	11.14	13.83	3.24	7.07	11.58
Roxithromycin	1JZZ	4.45	2.10	1.51	2.10	1.04	1.23	17.63	16.3	20.82	22.81	7.64	13.35
	FUXYOM	2.46	2.10	2.38	2.04	2.58	2.18	24.06	22.74	22.38	24.84	23.17	21.56
	KAHWAT	3.00	1.58	1.05	2.68	0.88	1.14	7.44	21.42	7.84	19.33	2.31	11.09
Telithromycin	1YIJ	0.98	1.69	1.09	0.69	0.98	1.17	16.19	23.89	22.46	10.30	10.06	13.8
	1P9X	2.25	3.46	2.66	2.12	2.00	1.84	19.19	22.81	24.87	9.40	21.73	20.43
	4V7S	1.46	2.51	1.64	0.92	1.05	1.12	11.74	22.82	21.63	10.16	13.34	20.01
	4V7Z	1.54	2.52	1.58	1.10	1.14	1.17	11.74	22.82	21.63	13.44	13.24	20.01
	4WF9	1.63	2.00	1.51	1.28	1.54	1.47	10.48	18.40	15.51	12.79	21.86	4.15
Danoprevir	3SU1	1.60	2.21	1.34	0.92	0.82	1.04	21.21	17.44	13.32	10.87	2.16	13.33
Grazoprevir	3SUG	1.57	1.59	1.27	0.89	1.03	1.22	5.91	20.94	17.78	7.26	1.45	18.91
Vaniprevor	3SU4	2.27	1.34	1.10	0.84	0.95	0.86	1.29	22.98	20.83	4.61	10.22	8.12
Asunaprevir	4WH6	2.00	2.10	1.52	1.44	1.02	1.23	12.57	22.65	20.75	4.16	3.11	15.66
	MIYWOI	1.74	1.86	1.08	0.95	0.96	1.14	10.22	23.52	21.58	4.47	3.21	10.83
Telaprevir	3SV6	3.51	1.55	1.50	1.03	0.84	1.15	14.59	22.22	14.13	15.20	6.15	14.89
	LERJID	3.54	1.48	1.03	0.76	0.92	1.15	14.59	22.22	14.13	3.87	4.31	17.71
	3SV7	3.38	1.41	1.44	0.95	0.93	1.11	14.59	23.19	17.74	10.16	6.72	14.08

Energy difference between the "conformer most similar to the specific crystal structure" (by RMSD) and minimum energy conformer predicted for each compound by the three methods in vacuum and water, color coded according to the energy (Green: < 5.0 kcal/mol., Yellow: 5-10 kcal/mol., and Red: > 10.0 kcal/mol.). [#] RMSD of the "conformer most similar to the crystal structure" compared to the crystal structures on the same row, color coded according to the RMSD (Green: < 2.0 Å, Yellow: 2.0-4.0 Å and Red: > 4.0 Å).

Table S3. Comparison of the macrocyclic core in the minimum energy conformers (MEC) obtained by MC, MOE and OMEGA to the cores of the corresponding crystal structures (by RMSD).[#]

Macrocyclic drugs	Crystal Structures	Apolar			Polar		
		MC	MOE	OME	MC	MOE	OME
Erythromycin	1YI2	1.37	1.45	1.48	0.44	0.45	1.00
	2J0D	1.20	1.55	1.51	0.63	0.73	1.12
	QIFKEX	1.33	1.56	1.50	0.69	0.78	0.87
	NAVTAF	1.19	1.44	1.31	0.48	0.61	1.04
	LAPDEN	1.61	1.86	1.39	1.17	1.23	1.18
Azithromycin	1YHQ	0.52	1.09	1.30	0.85	1.16	1.44
	GEGJAD	0.46	1.15	1.48	1.01	0.94	1.56
Clarithromycin	NAVSUY	1.50	1.63	1.18	1.17	2.04	1.40
	CIWJIC	1.47	1.52	1.17	1.09	1.83	1.34
	WANNU	1.59	1.45	1.41	0.69	1.86	1.40
Roxithromycin	1JZZ	1.16	0.38	1.45	1.14	0.93	0.78
	FUXYOM	1.45	1.77	1.56	1.56	1.93	1.71
	KAHWAT	1.18	1.17	1.75	1.16	1.46	1.16
Telithromycin	1YIJ	1.08	1.41	1.26	1.47	1.43	1.53
	1P9X	1.71	1.34	1.50	1.36	2.04	1.51
	4V7S	1.06	1.42	1.33	1.52	1.42	1.57
	4V7Z	0.83	1.08	1.26	1.37	1.50	1.46
	4WF9	1.24	1.44	1.37	1.44	1.63	1.46
Danoprevir	3SU1	1.31	1.12	1.43	1.11	1.15	1.67
Grazoprevir	3SUG	0.33	0.35	0.79	0.952	1.44	1.19
Vaniprevir	3SU4	0.78	1.22	1.42	0.78	0.56	0.54
Average RMSD (Å) =		1.16	1.30	1.37	1.05	1.29	1.28

[#] RMSDs $\leq$1.0 Å have been color coded in green, those in the range of 1.0-1.25 in yellow.

Table S4: Comparison of minimum energy conformation (MEC) obtained by MC, MOE and OMEGA to the conformations determined by NMR spectroscopy for roxithromycin in chloroform and water [#]

Conformations in chloroform

NMR conformations #	MC	MOE	OMEGA
Conf 1	3.67	2.09	3.22
Conf 2	3.35	2.42	3.34
Conf 3	3.34	2.46	3.77

Conformations in water

NMR conformation #	MC	MOE	OMEGA
Conf 2	2.77	2.51	2.19
Conf 4	2.64	3.29	3.47
Conf 5	2.96	3.43	3.29
Conf 6	4.17	1.91	2.14
Conf 7	2.43	3.08	2.98
Conf 8	3.86	1.03	1.86

[#]MECs obtained in apolar environment (ε=1) were compared to conformations obtained in chloroform, and MECs obtained in polar environment (ε=80) to those in water.

Figure S1. Overlays of the different conformations found in the crystalline state of each HCV NS3/4A protease inhibitor. Overlays were generated by alignment of the heavy atoms and the color used for the PDB and CSD codes match those of the carbon atoms in the corresponding structures.

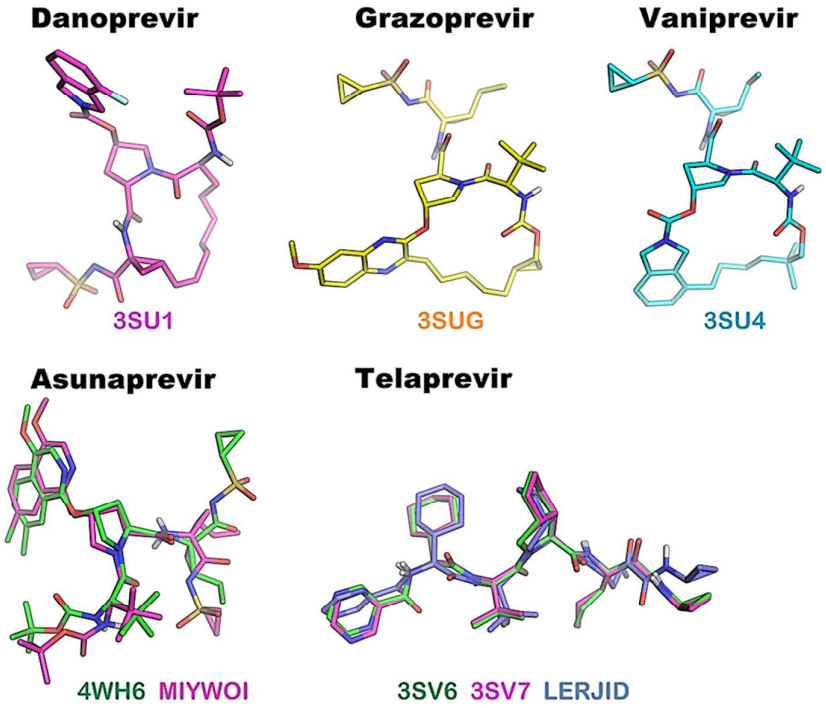


Figure S2. Radius of gyration (Rgyr) calculated for the erythronolides and HCV NS3/4A protease inhibitors. For each compound Rgyr has been calculated for the conformation(s) adopted in the crystal structures and for the conformational ensembles generated by MC (green), MOE (pink) and OMEGA (yellow) in apolar and polar environments. Rgyr was calculated using the MOE Software, and a fixed scale has been used to facilitate comparisons between compounds. Box plots show minimum and maximum values as whiskers, the boxes span the 25th-75th percentile range.

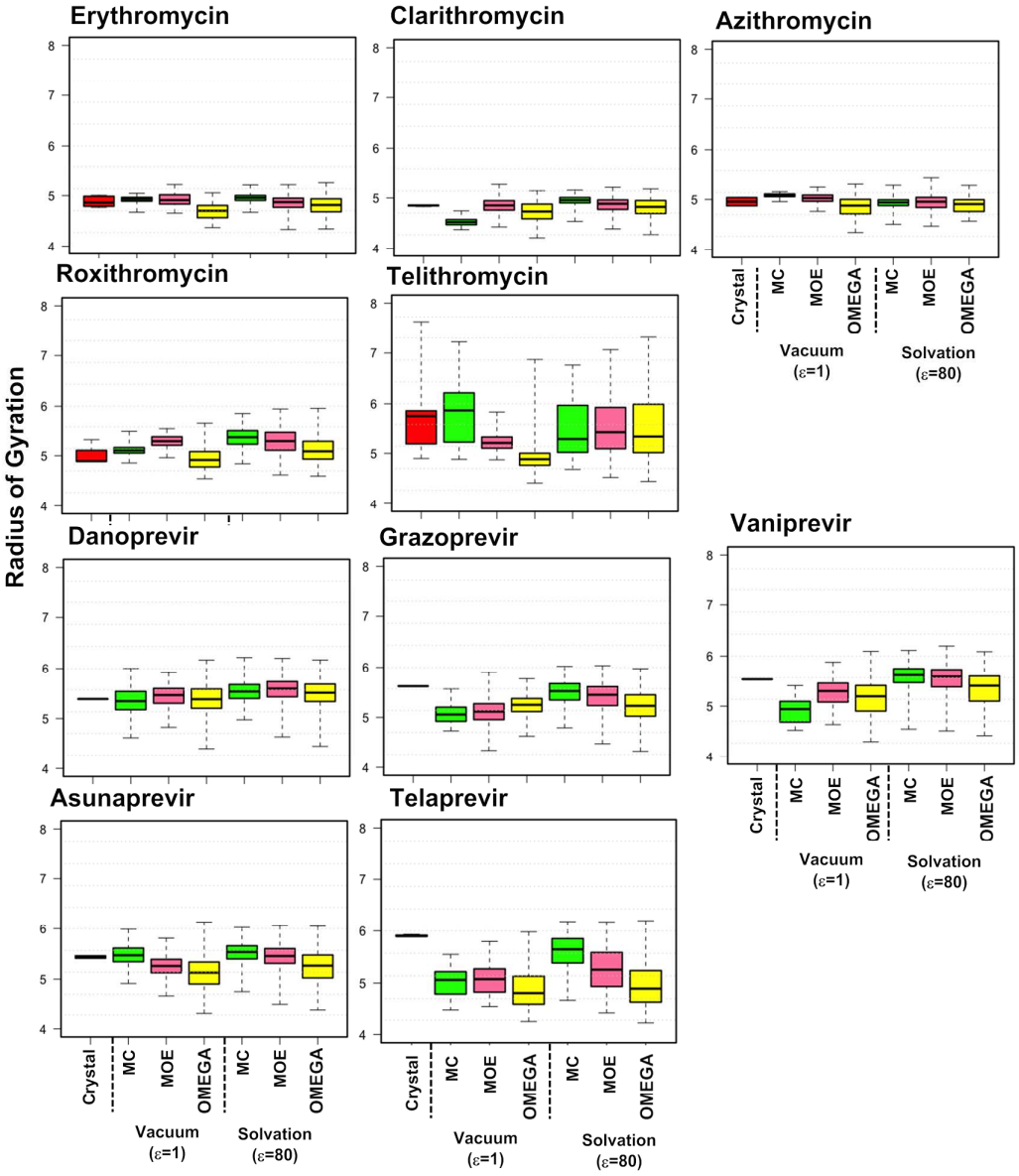


Figure S3. Polar surface area (PSA) calculated for the erythronolides and HCV NS3/4A protease inhibitors. For each compound PSA has been calculated for the conformation(s) adopted in the crystal structures and for the conformational ensembles generated by MC (green), MOE (pink) and OMEGA (yellow) in apolar and polar environments. PSA was calculated based on the surface area of the molecule that arises from oxygen and nitrogen atoms, plus their attached hydrogen atoms, using the Schrödinger software. A fixed scale has been used to facilitate comparisons between the compounds. Box plots show minimum and maximum values as whiskers, the boxes span the 25th-75th percentile range.

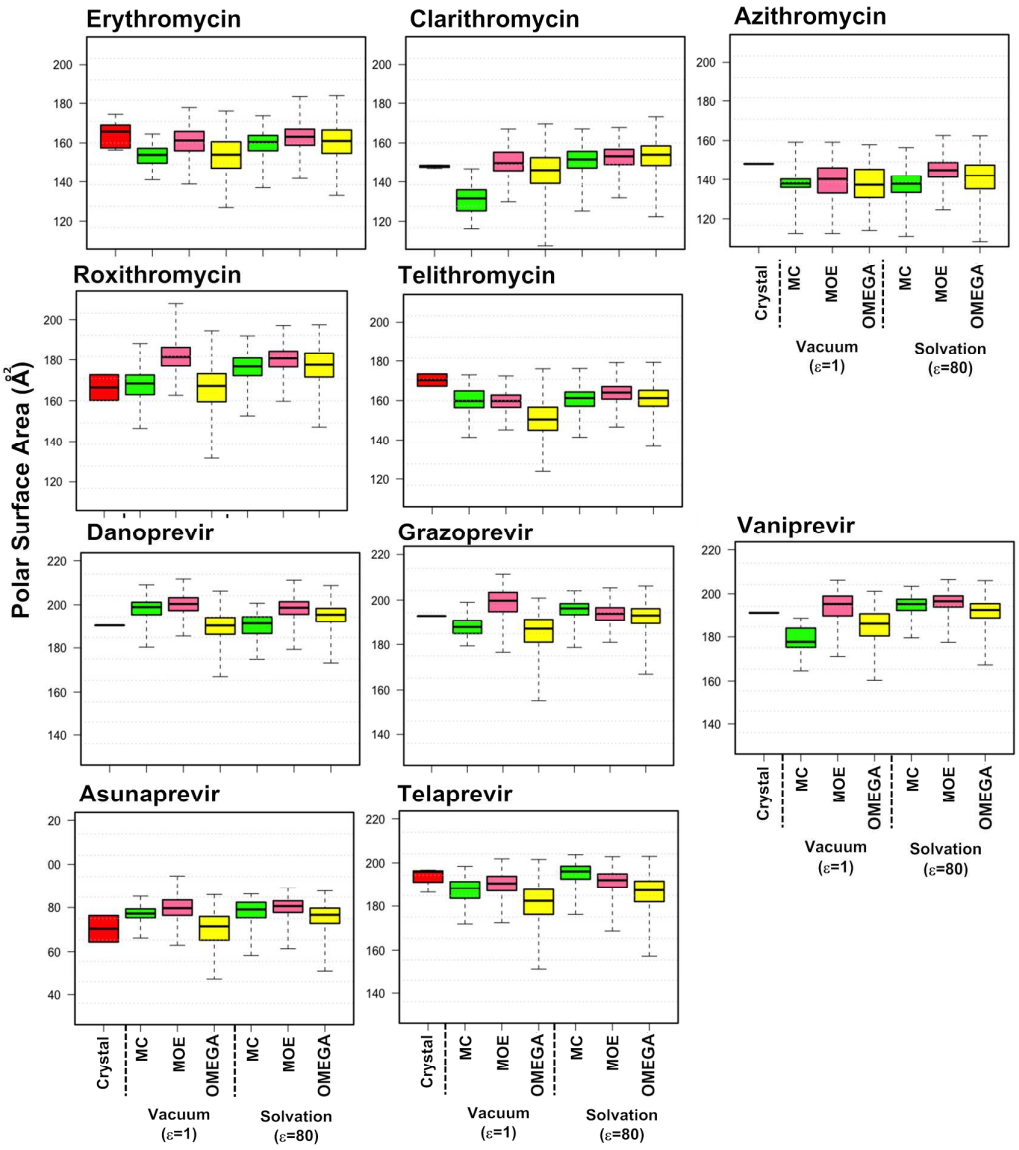


Figure S4. The number of IMHBs in the crystal structures and in the conformational ensembles generated by MC (green), MOE (pink) and OMEGA (yellow) in apolar and polar environments for the HCV inhibitors. Box plots show minimum and maximum values as whiskers; the 25th, 50th and 75th percentiles as boxes; and MECs as red stars.

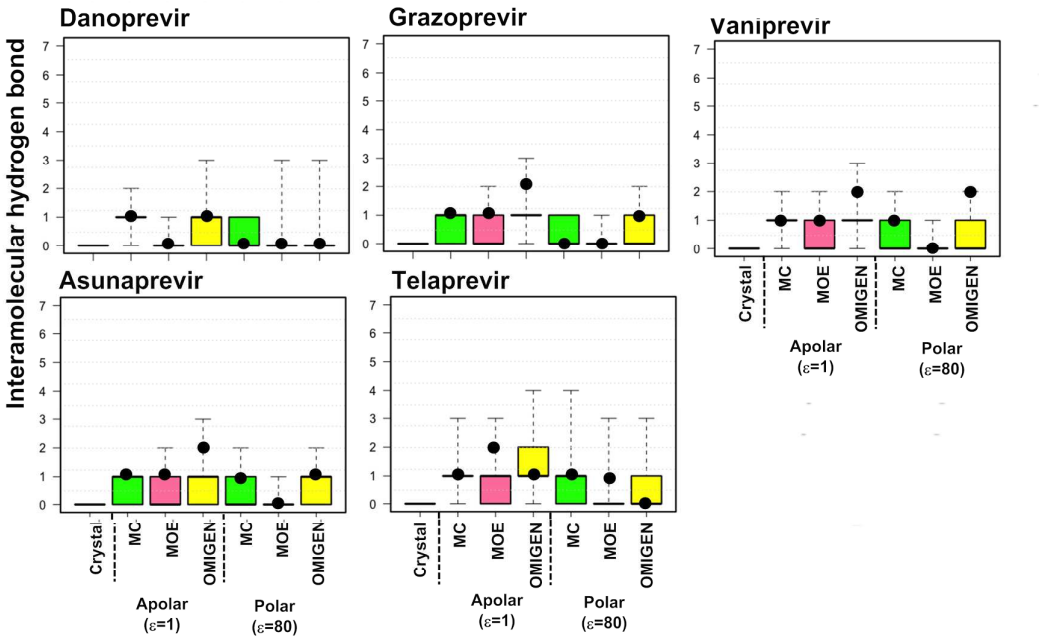
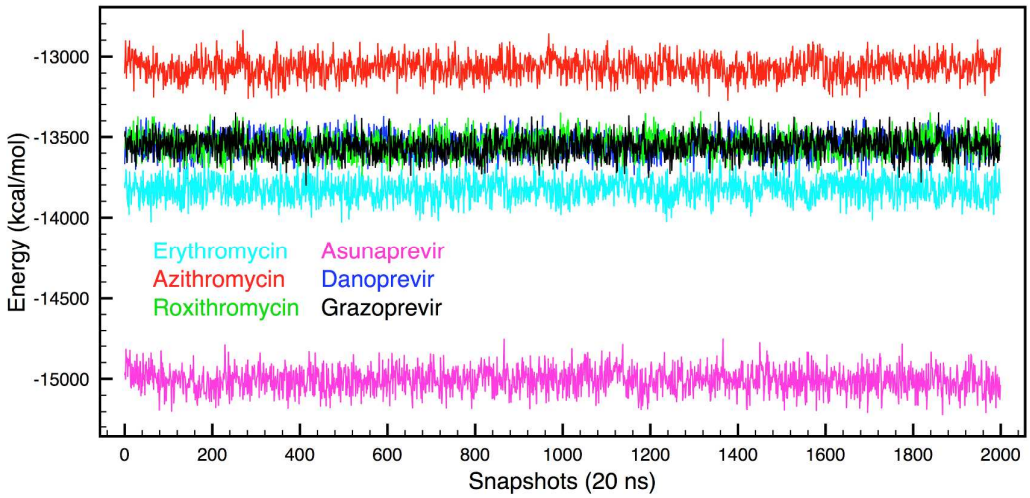


Figure S5. Comparison of potential energies in chloroform (A) and water (B) as a measure of system stability.

A. MD trajectories from chloroform



B: *MD trajectories from water*

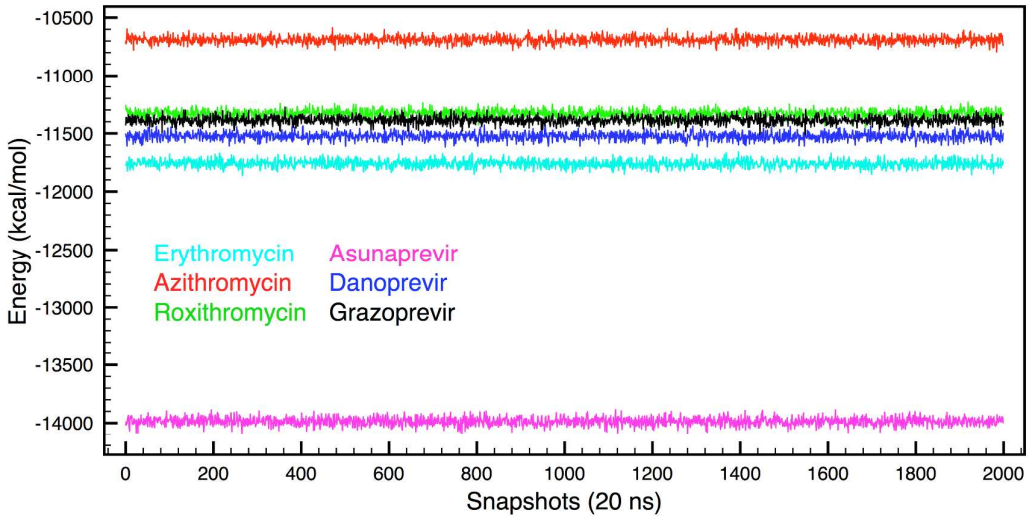
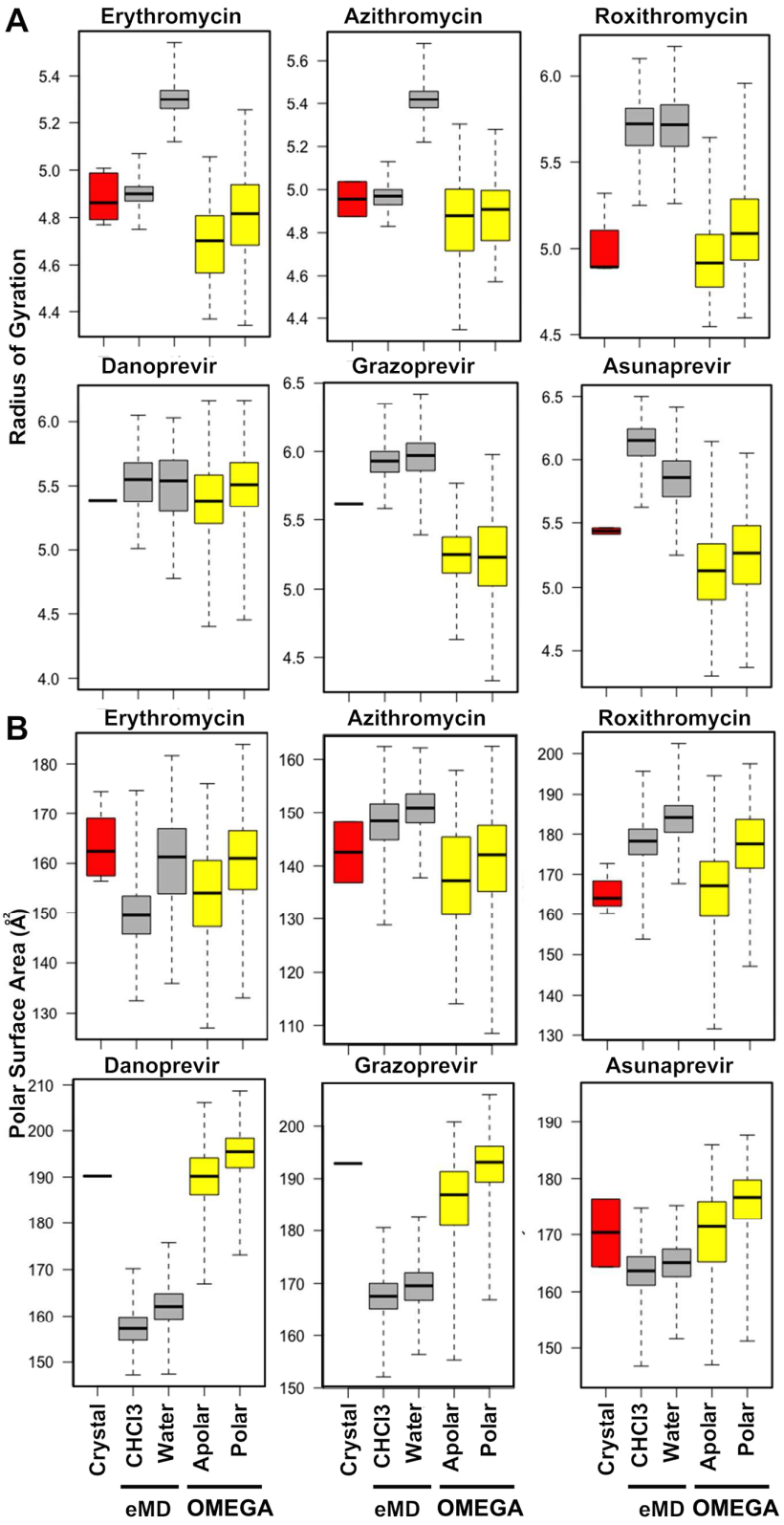
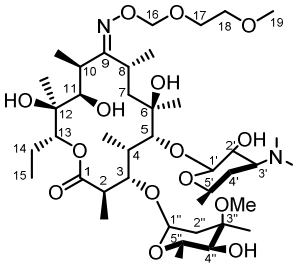


Figure S6. Comparison of molecular property space of conformations from eMD with ensembles from OMEGA. **A**: Radius of Gyration; **B**: Polar Surface Area



NMR-analysis of Roxithromycin



1. ^{1}H NMR assignments

Proton assignments were derived from TOCSY, NOESY, COSY, and HSQC NMR spectra recorded at 25 °C on a 900 MHz BRUKER Avance III HD NMR spectrometer equipped with a TCI cryoprobe.

Table S5. ^{1}H NMR assignment (ppm) of roxithromycin in $CDCl_3$ and D_2O

	Macrocycle			**Sugars**	
	$CDCl_3$	D_2O		$CDCl_3$	D_2O
1	-	-	1'	4.43	4.6
2	2.9	3.06	2'	3.49	3.53
2-Me	1.18	1.23	2'-OH	3.28	-
3	3.98	3.84	3'	2.49	3.48
4	2.03	2.02	3'-NMe_2	-	2.86
4-Me	1.09	1.07	4'	1.70, 1.27	2.12, 1.57
5	3.54	3.53	5'	3.5	3.89
6	-	-	5'-Me	1.23	1.31
6-Me	1.49	1.44	1"	4.84	4.95
7	2.35, 1.58	1.67, 4.56	2"	2.36, 1.56	2.53, 1.68
8	3.75	3.74	3"-Me	1.24	1.25
8-Me	1.03	1.14	3"-OMe	3.31	3.32
9	-	-	4"	3.02	3.23
10	2.67	2.94	4"-OH	2.21	
10-Me	1.19	1.19	5"	4	4.13
11	3.82	3.68	5"-Me	1.28	1.31
11-OH	4.31	-			
12	-	-			
12-Me	1.14	1.23			
12-OH	3.14	-			
13	5.1	5.14			
14	1.92, 1.47	1.87, 1.55			
15	0.85	0.85			
16	5.19, 5.17	5.22, 5.19			
17	3.80, 3.72	3.84			
18	3.57, 3.56	3.64			
19	3.42	3.4			

2. Interproton distances

NOE build-ups were recorded without solvent suppression with mixing times of 100, 200, 300, 400, 500, 600, and 700 ms. The relaxation delay was set to 2.5 s, and 16 scans were recorded with 16384 data points in the direct dimension and 512 data points in the indirect dimension. Distances were calculated using geminal methylene protons (1.78 Å) as reference. The NOE peak intensities were calculated according to ([cross peak1 × cross peak2]/[diagonal peak1 × diagonal peak2])$^{0.5}$. At least 4 mixing times giving a linear ($R^2 > 0.97$) initial build-up rate (σ_{ij}) were used. The interproton distances (r_{ij}) were calculated according to the equation $r_{ij}=r_{ref}(\sigma_{ref}/\sigma_{ij})^{(1/6)}$.

Table S6. Interproton distances (Å) for roxithromycin derived from NOE build-up measurements in $CDCl_3$

No.	Proton A	Proton B	σ	R^2	Distance rAB (Å)
1	13	11	0.0000091	0.99	2.31
2	3	5	0.0000250	0.99	1.95
3	11	4	0.0000013	0.99	3.19
4	2	4	0.0000110	0.99	2.24
5	3	4	0.0000130	0.99	2.18
6	11	10	0.0000147	0.99	2.13
7	10	7B	0.0000049	0.99	2.61
8	8	6Me	0.0000357	0.98	1.84
9	2	4Me	0.0000314	0.99	1.88
10	10	8Me	0.0000335	0.99	1.86
11	10	12Me	0.0000475	0.99	1.75
12	5	4Me	0.0000021	0.98	2.94
13	5	6Me	0.0000257	0.98	1.94
14	1"	2"A	0.0000068	0.99	2.43
15	1"	2Me	0.0000435	0.99	1.78
16	1"	3	0.0000310	0.99	1.88
17	1'	5'	0.0000272	0.98	1.92
18	1'	3'	0.0000464	0.99	1.76
19	4"	5"Me	0.0000235	0.99	1.97
20	1'	4Me	0.0000138	0.99	2.15
21	5	5"	0.0000071	0.99	2.41
22	11	12Me	0.0000075	0.97	2.44
ref.	2"A	2"B	0.0000433	0.99	1.78
ref.	17A	17B	0.0000380	0.76	1.76

Table S7. Interproton distances (Å) for roxithromycin derived from NOE build-up measurements in D_2O

No.	Proton A	Proton B	σ	R^2	Distance rAB (Å)
1	13	11	0.0000193	0.99	2.93
2	11	4	0.0000623	0.99	2.41
3	2	4	0.0000314	0.99	2.70
4	10	4	0.0000035	0.98	3.90
5	3	5	0.0000235	0.99	2.84
6	5	4	0.0000110	0.99	3.22
7	3	2	0.0000084	0.99	3.37
8	3	4	0.0000167	0.99	3.00
9	11	10	0.0000426	0.99	2.57
10	13	12Me	0.0000036	0.98	3.89
11	3	2Me	0.0000060	0.99	3.56
12	10	8Me	0.0000073	0.99	3.45
13	10	12Me	0.0000243	0.99	2.82
14	2	4Me	0.0000131	0.98	3.13
15	5	6Me	0.0000228	0.98	2.85
16	3	6Me	0.0000038	0.98	3.85
17	3	4Me	0.0000018	0.99	4.35
18	10	8Me	0.0000364	0.98	2.64
19	4	6Me	0.0000029	0.98	4.02
20	1"	2	0.0000018	0.98	4.35
21	1'	4Me	0.0000185	0.99	2.95
22	1'	5"	0.0000660	0.99	2.39
23	1'	3"Me	0.0000080	0.99	3.40
24	1'	6Me	0.0000028	0.98	4.04
ref.	2"A	2"B	0.0003853	0.99	1.78
ref.	4'A	4'B	0.0004024	0.98	1.77

3. Monte Carlo molecular mechanics (MCMM) conformational search

The conformational searches were performed using the Monte Carlo algorithm with intermediate torsion sampling, 50 000 Monte Carlo steps, and a RMSD cut2off set to 2.0 Å, followed by molecular mechanics energy minimization with the software Macromodel (v.9.1) as implemented in the Schrödinger package. The energy minimization was performed using the Polak2Ribiere type conjugate gradient (PRCG) with maximum iteration steps set to 5000. All conformations within 42 kJ/mol from the global minimum were saved. The results of the four independent searches performed using OPLS27006 or Amber* as force field, and with $CHCl_3$ or H_2O as solvation model, are given below. The ensembles from the conformational searches in one solvent were combined, and elimination of redundant conformations by comparisons of the heavy atom coordinates applying the RMSD cutoff 121.5 Å was performed, according to Table S8. These ensembles were combined and redundant conformations were eliminated again (RMSD cutoff = 1.5 Å), giving the ensemble used for NAMFIS analysis (66 conformers).

Table S8. Results of the MCMM conformational analysis

		Number of conformations		
		Total[a]	Within 12.6 kJ/mol[b]	Following redundant conformer elimination[c]
$CHCl_3$	OPLS	177	13	62
	Amber*	197	25	
H_2O	OPLS	172	14	38
	Amber*	127	14	

[a]Total number of unique conformations found. The global minimum was found for all investigated compounds at least 30 times. [b]Conformations found within 12.6 kJ/mol (3.0 kcal/mol) of the global minimum. [c]Conformations obtained after redundant conformation elimination with the RMSD cutoff 1.5 Å ($CHCl_3$) and 1.Å (H_2O) for heavy atoms. These ensembles were again combined and reduced by redundant conformer elimination (RMSD cutoff = 1.5 Å) giving the ensemble used as input in the NAMFIS analysis (66 conformers).

4. NAMFIS analysis

Solution ensembles were determined by fitting the experimentally measured distances to those back2calculated for computationally predicted conformations following previously described protocols. $C/_2$ signals were treated according to the equation $d=(((d_1^{-6})+(d_2^{-6}))/2)^{-1/6}$, and methyl signals according to $d=(((d_1^{-6})+(d_2^{-6})+(d_3^{-6}))/3)^{-1/6}$. The NAMFIS ensemble analyses were validated using standard methods, that is through evaluation of the reliability of the conformational restraints by the addition of 10% random noise to the experimental data, by the random removal of individual restraints, and by comparison of the experimentally observed and back2calculated distances. Since the orientations of flexible parts of molecules are not as well predicted by the conformational searches as the more rigid parts, only macrocycle interactions were included in the initial NAMFIS analyses, corresponding to distance 1213 for $CDCl_3$ and distance 1219 for D_2O (Table S6 and S7).

Table S9. Experimentally determined and back-calculated (NAMFIS) interproton distances (Å).

$CDCl_3$		D_2O	
Exp.	Calc.	Exp.	Calc.
2.31	2.80	2.93	2.90
1.95	2.57	2.41	2.82
3.19	3.67	2.70	2.44
2.24	2.63	3.90	4.00
2.18	2.53	2.84	2.32
2.13	2.68	3.22	2.91
2.61	2.94	3.37	2.90
1.84	2.59	3.00	2.62
1.88	2.57	2.57	2.68
1.86	2.70	3.89	2.96
1.75	2.72	3.56	3.26
2.94	3.14	3.45	3.55
1.94	2.66	2.82	2.74
		3.13	2.70
		2.85	2.81
		3.85	3.93
		4.35	3.90
		2.64	2.97
		4.02	2.58
RMS=0.61		RMS=0.49	

Table S10. Conformational populations derived by NAMFIS analysis of roxithromycin in $CDCl_3$ and D_2O solutions

$CDCl_3$		D_2O	
Conf. No.	%[a]	Conf. No.	%[a]
1	17	2	14
2	12	4	59
3	71	5	7
		6	6
		7	4
		8	8

[a]Population of the indicated conformer in solution, as deduced by NAMFIS analysis, all other molar fractions are 1% or less. The conformations are shown below.

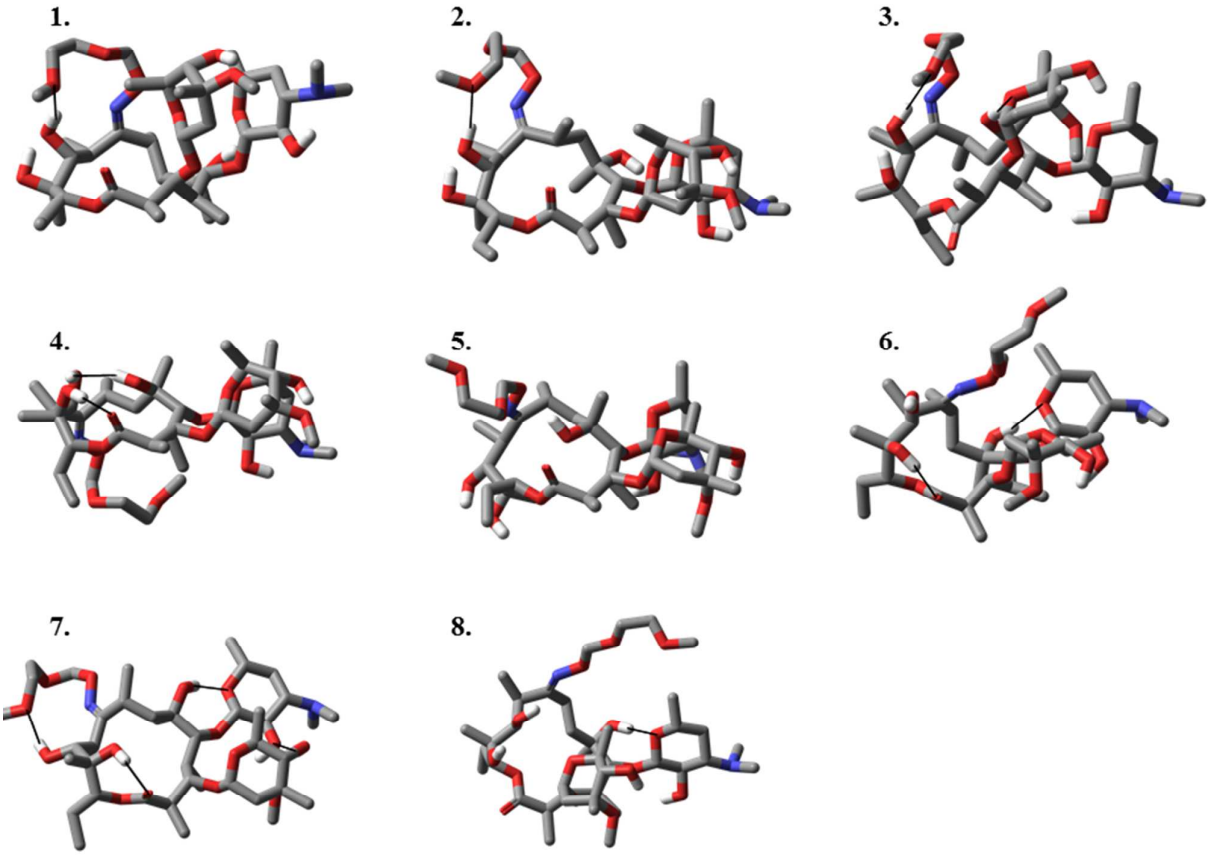


Figure S7. Roxithromycin conformations in solution, as selected by NAMFIS analysis. Population percentages are given in Table S10. Hydrogen bonds are indicated by black lines. Non-polar hydrogens are omitted for clarity