Supporting Information

Can Simulations and Modeling Decipher NMR Data for Conformational Equilibria? Arginine-Vasopressin

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Computational details

Long-scale molecular dynamics simulation

Table S1 Parameters for the unrestrained long-scale molecular-dynamics simulation of Arg[8]-vasopressin

Force field	ff99SB[1] (Amber 10, Amber 14 CUDA)[2]
Initial conformation	open (PDB ID: 1YF4);[3] neutralized with 2 Cl^- (ions08.lib, frcmod.ionsjc_tip4pew[4, 5])
Solvation	explicit, TIP4PEw,[6] truncated octahedral box
Temperature and Pressure	T= 300K, p= 1 bar (Berendsen coupling,[7] 1.0 ps external heath bath)
Minimization	8,945 steps: 500 steps steepest-descent followed by conjugate-gradient method
Molecular dynamics	2 fs time steps, SHAKE algorithm,[8] 8.0 Å non-bonded cut-off, Particle Mesh Ewald method,[9] periodic boundary conditions
Simulation time	23,000 ns

Representative conformations of Arg[8]-vasopressin

Analysis of the conformational space of 11 µs MD simulation revealed 4 main ring conformations for AVP: *open, saddle, clinched open, twisted saddle*. A detailed description of the conformations has been published previously.[10]

The most populated overall state of each cluster (ring-state type, main ring conformation) was chosen as representative for further DFT and NMR calculations; *saddle* and *clinched open* each of with *extended* and *folded* tail. The mean torsions of the representatives are given in Table S2.

Table S2 Mean backbone torsions and standard deviations (±) of representative conformationas of Arg[8]-vasopressin

		saddle$_{ext}$		*saddle$_{fold}$*		*cl.open$_{ext}$*		*cl.open$_{fold}$*		*tw.saddle*		*open*	
		state3[a]		**state6**		**state12**		**state14**		**state19**		**state27**	
Tyr[2]	phi	-80.9	±21.4	-78.5	±18.5	-101.2	±28.8	-86.8	±25.4	-84.6	±29.0	-112.0	±37.9
	psi	144.2	±12.8	143.2	±11.6	-16.4	±23.3	-22.9	±21.1	162.1	±13.1	134.6	±18.8
Phe[3]	phi	-63.0	±9.5	-62.7	±9.1	-99.9	±32.8	-106.6	±26.0	-52.0	±16.8	54.9	±12.0
	psi	-20.7	±13.6	-22.5	±12.5	157.4	±13.5	154.5	±16.0	127.3	±15.1	4.4	±31.3
Gln[4]	phi	-86.5	±17.4	-89.2	±16.3	-66.4	±18.0	-69.4	±11.3	55.0	±8.4	-135.9	±23.8
	psi	-7.6	±17.8	-4.7	±16.5	-18.2	±25.7	-21.8	±18.7	12.4	±21.1	151.6	±19.2
Asn[5]	phi	-113.3	±21.2	-113.8	±21.0	-112.1	±27.8	-109.9	±30.4	-106.1	±29.4	-75.4	±19.8
	psi	-27.4	±21.8	-26.4	±23.3	74.5	±62.7	92.8	±57.7	-8.7	±47.3	124.7	±31.9
Cys[6]	phi	-126.3	±20.0	-126.6	±21.0	-111.1	±34.9	-117.3	±38.9	-120.8	±28.3	-128.8	±30.4
	psi	132.7	±34.5	139.8	±23.1	145.9	±19.2	145.6	±20.1	143.8	±24.2	149.0	±22.7
Pro[7]	phi	-67.8	±11.3	-64.6	±11.1	-66.3	±11.1	-64.8	±11.5	-67.7	±11.2	-67.5	±11.5
	psi	-63.0	±11.3	-62.7	±11.3	-99.9	±32.8	-106.6	±26.0	-52.0	±16.8	53.3	±12.0
Arg[8]	phi	-20.8	±13.8	150.3	±12.5	150.3	±16.0	150.3	±16.0	127.3	±15.1	4.4	±31.3
	psi	-86.5	±17.4	-89.2	±16.3	-69.4	±18.0	-69.4	±11.3	55.0	±8.4	-135.9	±23.8

[a]PDB files of the representative states are available under these designations from the SI (ESM4) of our previous publication (http://dx.doi.org/10.1007/s00894-014-2485-0); Abbreviations: clinched (cl.); twisted (tw.); ext (extended); fold (folded)

After extending the MD-simulation to 23 µs, the conformational space was again clustered using DASH[11]. The resulting representatives were assigned to the former representatives already defined for the first 11 µs of the MD simulation via circular similarity of backbone torsions in order to ensure consistency. From the 23 µs MD simulation the representatives of the five most populated ring state types were chosen in order to calculate their free energies and populations. The first four clusters were the already identified main conformations from the 11 µs MD; the additional conformation (*variants*) was seen for the first time after 11 µs. V*ariants* is a *clinched open* variant; a rotamer of the peptide-bond between residue Gln[4] and Asn[5] of the *clinched open* conformation (Figure S1). *Variants* was added to the selection, although low populated in the simulation, because it occurred only once and at the end of the 23 µs simulation with no following interconversion. Thus, it could not be ruled out that this cluster might be another main cluster of AVP. However, the thermodynamic calculations (metadynamics) showed that the conformation is the least stable (*cf.* main text) and it was not considered further for DFT-NMR-calculations.

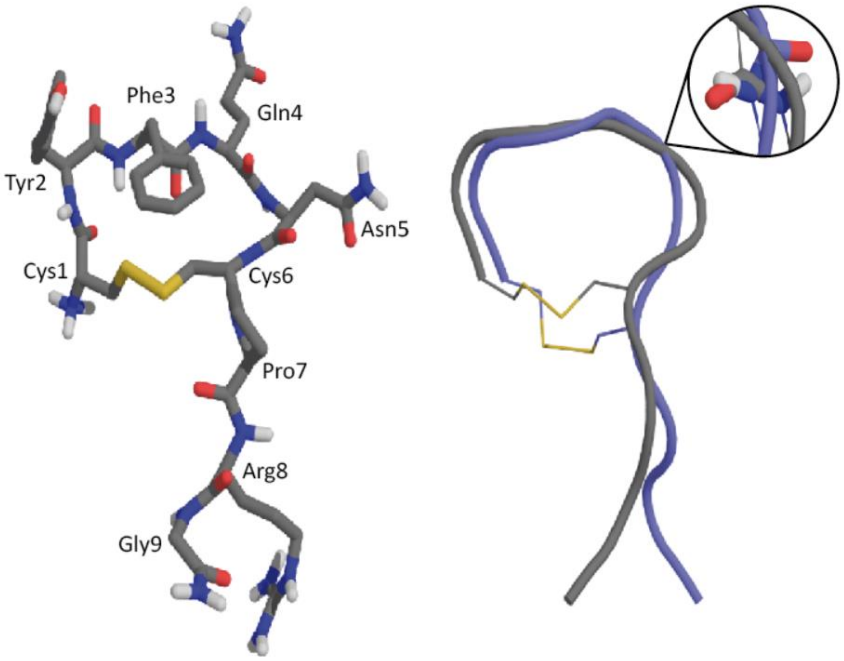

Figure S1 Representative for the ring-state type *variants* (grey). Left: stick depiction of *variants*. Right: cartoon depiction of *variants and* ring alignment with the representative for *clinched open* (blue).
Variants is a 4,5 peptide-bond rotamer of *clinched open* as illustrated in the zoom. The conformation *variants* occurred for the first time at the end of 23 µs MD simulation of Arg[8]-vasopressin and is expected to be populated insignificantly in aqueous solution according to thermodynamic calculations.

Cartesian coordinates of the B3LYP/6-31G(d) optimized geometries are given below as Gaussian Archive Entries.

Metadynamics simulations

We used a combination of metadynamics,[12, 13] in its well-tempered variant (WT),[14] the multiple-walker technique[15] and the path collective variable (PCV)[16, 17] to determine the free-energy differences between AVP conformations (identified from the 23 μs trajectory) in water. Four main ring conformers identified previously[10] and one new found in the extended 23 μs MD simulation were used as starting geometries for the metadynamics simulations. The PCV used was a numerical assignment to the most similar AVP conformer based on the RMSD of the backbone atoms of the ring residues. An analysis of the unbiased 23 μs MD trajectory using this PCV showed that 90% of the frames can be uniquely assigned to one of the five ring conformers. We were therefore able to use this single PCV for the metadynamics simulation.

The simulation boxes and topologies used for the AMBER MD simulations were converted to GROMACS[18] format. The simulation configuration used the same water model, temperature and thermodynamic ensemble as the reference unbiased simulation. Particle mesh Ewald (PME) was used to treat electrostatic interactions, using a cut-off distance of 1.0 nm and each of the five models were equilibrated for 20 ns. Gaussian hills with an initial height of 0.6 kcal mol^{-1} applied every ps were used. The Gaussian functions were rescaled in the WT scheme using a bias factor of 10. The metadynamics simulations were performed using GROMACS with the PLUMED plug-in.[19]

Calculation of chemical shifts and correlation with experimental values

Representative structures were taken from the 23 µs MD simulation and optimized using the B3LYP hybrid density functional,[20] the 6-31G(d) basis set[21] and the default polarizable continuum model (PCM) for water solvent using Gaussian09.[22] These optimized structures were then used to calculate ^{1}H and ^{13}C chemical shifts using the GIAO formalism,[23] again with PCM-water. The chemical shifts were obtained from the calculated magnetic shielding using the correlation formulae given in the main text. These formulae were obtained by linear regression of a training set of experimental chemical shifts[24] and the corresponding shielding values at level B3LYP/6-31G* with PCM-water (Figure S2).

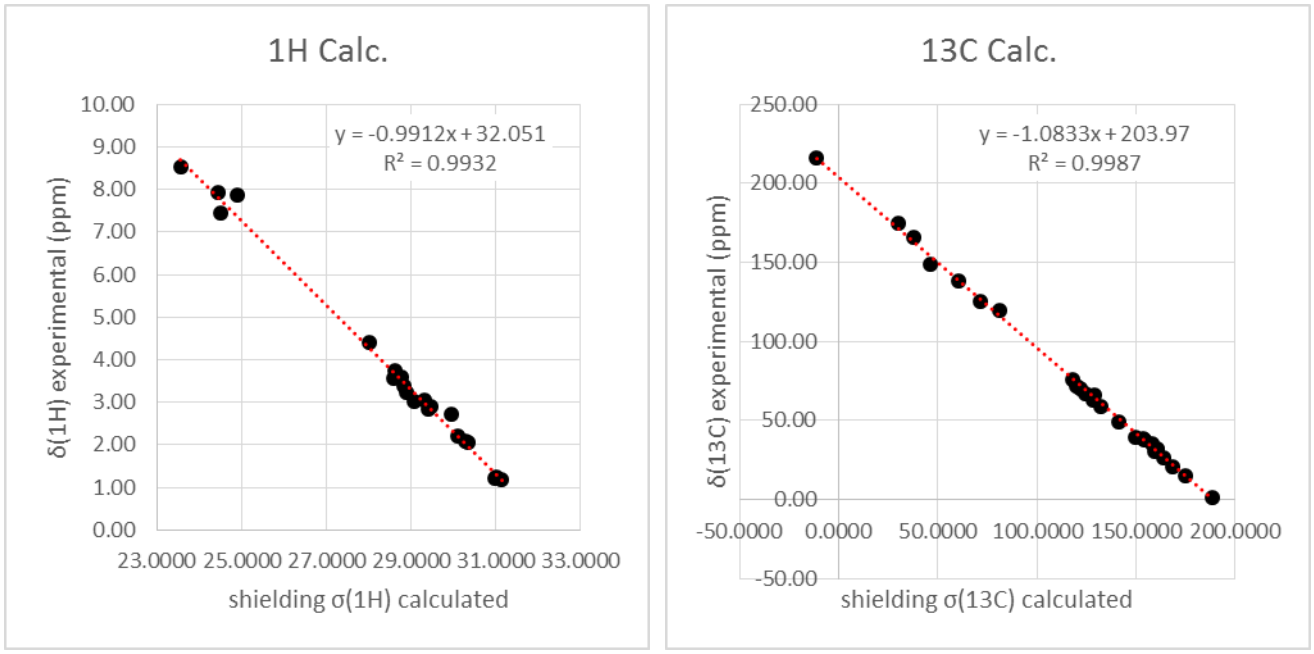

Figure S2 Linear regression of magnetic shielding at level B3LYP/6-31G(d) with PCM water and a training set of experimental chemical shifts (ppm) for ^{1}H and ^{13}C

Table S3-4 show the calculated ^{13}C and ^{1}H chemical shifts (δ ppm) for the individual conformations and the equilibrium mixtures (Eq. 1-4) calculated from the metadynamics free-energy differences, both assuming a single (*extended*) tail conformation and using the *extended*:*folded* equilibrium determined from the 23 µs MD simulation for each representative conformation.

$$\delta_{saddle_{eq.}} = 0.7314 \times \delta_{saddle_{ext}} + 0.2686 \times \delta_{saddle_{fold}} \quad (1)$$

$$\delta_{cl.open_{eq.}} = 0.6263 \times \delta_{cl.open_{ext}} + 0.3737 \times \delta_{cl.open_{fold}} \delta_{open} \quad (2)$$

$$\delta_{equilibrium_{ext}} = 0.6865 \times \delta_{saddle_{ext}} + 0.2951 \times \delta_{cl.open_{ext}} + 0.0043 \times \delta_{tw.saddle} + 0.0141 \times \delta_{open} \quad (3)$$

$$\delta_{equilibrium_{eq.}} = 0.6865 \times (0.7314 \times \delta_{saddle_{ext}} + 0.2686 \times \delta_{saddle_{fold}}) + 0.2951 \times (0.6263 \times \delta_{cl.open_{ext}} + 0.3737 \times \delta_{cl.open_{fold}}) + 0.0043 \times \delta_{tw.saddle} + 0.0141 \times \delta_{open} \quad (4)$$

Table S3 Calculated ^{13}C chemical shifts (B3LYP/6-31G(d), PCM water) of Arg^{8}-vasopressin

		Calculated ^{13}C chemical shifts (ppm)									
		Individual conformations								**Metadynamics equilibrium**	
			Saddle			*Clinched open*		*Twisted saddle*	*Open*	Equilibrium	**Equilibrium**
res	atom	*ext*	*fold*	*equil*[a]	*ext*	*fold*	*equil*[b]	*ext*	*ext*	*ext*[c]	***equilibrium***[d]
Cys^{1}	C$^{\alpha}$	57.90	57.27	57.73	56.81	58.08	57.28	57.23	58.08	57.58	57.60
Cys^{1}	C$^{\beta}$	43.53	43.38	43.49	49.46	49.98	49.66	46.52	45.04	45.32	45.34
Tyr^{2}	C$^{\alpha}$	63.12	63.82	63.30	65.73	65.80	65.76	58.02	63.64	63.87	64.01
Tyr^{2}	C$^{\beta}$	37.72	39.58	38.22	40.42	40.46	40.44	39.97	42.43	38.59	38.94
Tyr^{2}	C$^{\delta1}$	133.17	131.80	132.80	134.21	134.62	134.37	135.89	134.23	133.51	133.30
Tyr^{2}	C$^{\delta2}$	131.08	133.13	131.63	133.02	132.52	132.83	133.95	133.37	131.70	132.02
Tyr^{2}	C$^{\varepsilon1}$	118.14	117.08	117.85	115.46	114.56	115.13	115.79	115.83	117.31	117.01
Tyr^{2}	C$^{\varepsilon2}$	118.09	116.37	117.63	115.90	115.61	115.79	117.39	116.41	117.42	117.07
Phe^{3}	C$^{\alpha}$	62.93	62.34	62.77	54.22	56.81	55.19	62.29	62.98	60.36	60.54
Phe^{3}	C$^{\beta}$	37.98	38.02	37.99	41.37	44.82	42.66	40.51	39.87	39.02	39.40
Phe^{3}	C$^{\delta1}$	131.31	131.04	131.23	134.45	133.75	134.19	131.16	130.93	132.23	132.10
Phe^{3}	C$^{\delta2}$	131.63	131.33	131.55	133.42	131.86	132.84	133.24	134.32	132.20	131.98
Phe^{3}	C$^{\varepsilon1}$	131.22	131.40	131.27	130.95	131.08	131.00	131.95	130.27	131.13	131.18
Phe^{3}	C$^{\varepsilon2}$	131.67	131.34	131.58	130.01	131.21	130.46	131.21	129.58	131.15	131.22
Phe^{3}	C$^{\zeta}$	129.93	129.83	129.90	129.57	128.92	129.32	128.48	128.24	129.79	129.70
Gln^{4}	C$^{\alpha}$	57.93	56.76	57.61	61.15	58.78	60.26	61.77	55.39	58.86	58.38
Gln^{4}	C$^{\beta}$	30.23	27.62	29.53	28.74	27.92	28.44	25.60	40.73	29.92	29.35
Gln^{4}	C$^{\gamma}$	34.65	29.18	33.18	29.41	29.45	29.42	33.47	33.86	33.09	32.08
Asn^{5}	C$^{\alpha}$	58.15	56.35	57.67	53.20	52.10	52.79	53.70	53.33	56.61	56.15
Asn^{5}	C$^{\beta}$	41.48	43.06	41.90	36.42	35.29	36.00	42.79	40.79	39.98	40.15
Cys^{6}	C$^{\alpha}$	55.48	54.06	55.10	53.99	55.27	54.47	55.96	52.63	55.00	54.88
Cys^{6}	C$^{\beta}$	45.80	49.45	46.78	50.39	48.02	49.50	46.15	52.71	47.25	47.67
Pro^{7}	C$^{\alpha}$	66.11	66.08	66.10	66.59	66.73	66.64	65.76	66.07	66.25	66.26
Pro^{7}	C$^{\beta}$	31.87	32.15	31.95	31.93	32.19	32.03	31.61	31.67	31.88	31.96
Pro^{7}	C$^{\gamma}$	26.37	27.70	26.73	27.51	27.54	27.52	26.92	26.81	26.72	26.97
Pro^{7}	C$^{\delta}$	51.28	51.17	51.25	50.72	51.23	50.91	49.90	50.72	51.10	51.13
Arg^{8}	C$^{\alpha}$	56.93	58.60	57.38	56.10	58.71	57.08	56.21	55.59	56.66	57.26
Arg^{8}	C$^{\beta}$	36.06	32.65	35.14	36.20	33.57	35.22	36.46	35.94	36.10	35.18
Arg^{8}	C$^{\gamma}$	30.23	26.10	29.12	28.70	27.17	28.13	27.37	28.20	29.74	28.81
Arg^{8}	C$^{\delta}$	44.58	44.90	44.66	46.36	45.84	46.16	44.45	46.34	45.13	45.13
Gly^{9}	C$^{\alpha}$	42.47	44.61	43.04	42.59	46.31	43.98	42.80	42.54	42.51	43.31

[a]Eq. 1; [b]Eq. 2; [c]Eq. 3; [d]Eq. 4; Abbreviations: extended (ext); folded (fold); equilibrium (equil)

Table S4 Calculated ^{1}H chemical shifts (B3LYP/6-31G(d), PCM water) of Arg^{8}-vasopressin

		Calculated ^{1}H chemical shifts (ppm)									
		Individual conformations								**Metadynamics equilibrium**	
			Saddle			*Clinched open*		*Twisted saddle*	*Open*	Equilibrium	**Equilibrium**
res	atom	*ext*	*fold*	*equil*[a]	*ext*	*fold*	*equil*[b]	*ext*	*ext*	*extendedt*[c]	***equilibrium***[d]
Cys^{1}	H$^{\alpha}$	3.87	3.91	3.88	3.63	4.26	3.86	4.40	4.10	3.80	3.88
Cys^{1}	H$^{\beta a}$	3.39	3.69	3.47	3.37	2.65	3.10	2.84	3.10	3.38	3.35
Cys^{1}	H$^{\beta b}$	3.05	2.74	2.97	3.32	4.20	3.65	3.49	2.85	3.13	3.17
Tyr^{2}	H$^{\alpha}$	4.52	4.26	4.45	3.95	3.81	3.90	4.87	4.40	4.35	4.29
Tyr^{2}	H$^{\beta a}$	3.14	3.20	3.16	3.11	2.87	3.02	3.12	3.12	3.13	3.12
Tyr^{2}	H$^{\beta b}$	3.99	2.75	3.66	2.95	2.64	2.84	2.75	2.98	3.66	3.40
Tyr^{2}	H$^{\delta 1}$	7.37	7.24	7.33	7.33	5.73	6.73	7.48	7.18	7.36	7.15
Tyr^{2}	H$^{\delta 2}$	7.25	6.95	7.17	7.25	7.09	7.19	7.54	7.37	7.25	7.18
Tyr^{2}	H$^{\varepsilon 1}$	7.10	6.41	6.91	6.72	6.32	6.57	6.74	6.71	6.98	6.81
Tyr^{2}	H$^{\varepsilon 2}$	6.52	6.42	6.49	6.56	6.49	6.54	6.94	6.73	6.54	6.51
Phe^{3}	H$^{\alpha}$	4.64	5.02	4.75	4.48	4.71	4.57	4.03	3.52	4.58	4.67
Phe^{3}	H$^{\beta a}$	3.68	3.71	3.69	3.12	2.62	2.94	2.72	2.47	3.49	3.44
Phe^{3}	H$^{\beta b}$	2.73	2.74	2.73	3.19	3.20	3.19	2.63	3.90	2.88	2.88
Phe^{3}	H$^{\delta 1}$	7.08	7.10	7.09	7.15	7.41	7.25	7.49	7.10	7.11	7.14
Phe^{3}	H$^{\delta 2}$	7.07	7.17	7.09	7.57	7.56	7.57	7.26	7.61	7.23	7.24
Phe^{3}	H$^{\varepsilon 1}$	7.37	7.42	7.38	7.66	7.56	7.63	7.56	7.34	7.46	7.45
Phe^{3}	H$^{\varepsilon 2}$	7.43	7.47	7.44	7.52	7.60	7.55	7.51	7.30	7.46	7.47
Phe^{3}	H$^{\zeta}$	7.39	7.40	7.39	7.48	7.64	7.54	7.44	7.24	7.41	7.43
Gln^{4}	H$^{\alpha}$	4.45	4.79	4.54	4.12	4.27	4.17	3.28	4.26	4.34	4.42
Gln^{4}	H$^{\beta a}$	2.05	1.38	1.87	2.60	1.55	2.21	1.76	1.25	2.20	1.96
Gln^{4}	H$^{\beta b}$	2.26	2.52	2.33	1.84	2.40	2.05	2.37	2.37	2.14	2.25
Gln^{4}	H$^{\gamma a}$	2.00	1.81	1.95	2.12	2.31	2.19	1.51	2.19	2.03	2.02
Gln^{4}	H$^{\gamma b}$	2.25	2.20	2.23	2.23	2.38	2.29	0.50	2.66	2.24	2.25
Asn^{5}	H$^{\alpha}$	4.73	4.93	4.78	4.71	4.61	4.68	4.97	5.10	4.73	4.76
Asn^{5}	H$^{\beta a}$	2.75	2.36	2.64	2.50	2.32	2.44	2.71	2.63	2.67	2.58
Asn^{5}	H$^{\beta b}$	2.63	2.84	2.68	3.31	2.84	3.14	2.39	2.50	2.83	2.81
Cys^{6}	H$^{\alpha}$	5.09	5.05	5.08	5.03	4.72	4.91	4.66	4.43	5.06	5.02
Cys^{6}	H$^{\beta a}$	2.78	2.48	2.70	3.18	2.96	3.10	3.23	2.86	2.90	2.82
Cys^{6}	H$^{\beta b}$	2.97	3.19	3.03	3.41	3.41	3.41	3.72	3.34	3.11	3.15
Pro^{7}	H$^{\alpha}$	4.16	4.07	4.14	4.20	4.00	4.12	4.19	4.16	4.17	4.13
Pro^{7}	H$^{\beta a}$	1.95	2.12	1.99	2.05	2.16	2.09	1.93	2.03	1.98	2.02
Pro^{7}	H$^{\beta b}$	2.23	2.37	2.27	2.37	2.36	2.37	2.23	2.34	2.27	2.30
Pro^{7}	H$^{\gamma a}$	2.26	2.47	2.32	2.39	2.50	2.43	2.25	2.17	2.30	2.35
Pro^{7}	H$^{\gamma b}$	2.03	2.09	2.05	2.11	2.12	2.11	1.99	2.01	2.06	2.07
Pro^{7}	H$^{\delta a}$	3.95	3.80	3.91	3.90	4.10	3.97	3.66	3.68	3.93	3.92
Pro^{7}	H$^{\delta b}$	3.79	3.69	3.76	3.74	4.14	3.89	3.54	3.60	3.77	3.80
Arg^{8}	H$^{\alpha}$	4.31	3.86	4.19	4.33	3.80	4.13	4.41	4.32	4.32	4.18
Arg^{8}	H$^{\beta a}$	1.88	2.38	2.02	1.46	2.29	1.77	1.68	1.39	1.75	1.93
Arg^{8}	H$^{\beta b}$	2.51	1.71	2.29	2.21	1.98	2.13	2.05	2.20	2.41	2.24
Arg^{8}	H$^{\gamma a}$	1.86	2.00	1.90	1.80	1.97	1.86	1.98	1.84	1.84	1.89
Arg^{8}	H$^{\gamma b}$	2.19	2.28	2.22	2.06	2.23	2.12	2.19	1.83	2.15	2.18
Arg^{8}	H$^{\delta a}$	3.38	3.17	3.32	3.40	3.55	3.45	3.16	3.28	3.38	3.36

Arg^8	$H^{\delta b}$	3.27	3.09	3.22	3.33	3.18	3.27	3.32	3.40	3.29	3.24
Gly^9	$H^{\alpha a}$	4.37	4.57	4.43	4.43	3.28	4.00	4.38	4.39	4.39	4.30
Gly^9	$H^{\alpha b}$	3.26	4.34	3.55	3.29	3.81	3.48	3.27	3.24	3.27	3.53
Tyr^2	H^N	6.03	5.52	5.90	5.21	5.48	5.31	8.92	6.62	5.81	5.75
Phe^3	H^N	4.93	5.17	4.99	5.40	6.28	5.73	4.90	5.90	5.08	5.22
Gln^4	H^N	4.97	4.62	4.88	5.26	5.30	5.28	5.59	6.03	5.08	5.02
Gln^4	$H^{\varepsilon 21}$	4.52	4.45	4.50	4.39	4.41	4.39	4.25	4.44	4.48	4.47
Gln^4	$H^{\varepsilon 22}$	5.18	4.82	5.08	4.92	4.81	4.88	4.48	6.34	5.12	5.04
Asn^5	H^N	6.49	6.97	6.62	6.39	5.58	6.09	6.40	5.53	6.44	6.44
Asn^5	$H^{\delta 21}$	4.54	4.40	4.50	4.50	4.40	4.47	4.86	5.03	4.54	4.50
Asn^5	$H^{\delta 22}$	5.20	5.04	5.16	4.98	4.86	4.93	5.17	5.39	5.14	5.10
Cys^6	H^N	6.98	7.32	7.07	5.98	6.36	6.12	6.85	6.12	6.67	6.78
Arg^8	H^N	5.75	6.01	5.82	5.69	5.92	5.78	5.80	5.62	5.73	5.81
Arg^8	H^{ε}	3.83	4.07	3.90	4.62	4.00	4.39	4.39	4.67	4.08	4.05
Gly^9	H^N	4.88	6.60	5.35	4.99	7.12	5.79	4.97	4.95	4.92	5.47
$(Gly^9)NH_2$	H^{N1}	4.76	7.23	5.42	4.77	6.67	5.48	4.76	4.78	4.76	5.43
$(Gly^9)NH_2$	H^{N2}	4.43	4.88	4.55	4.43	4.29	4.38	4.41	4.43	4.43	4.50

[a]Eq. 1; [b]Eq. 2; [c]Eq. 3; [d]Eq. 4; Abbreviations: extended (ext); folded (fold); equilibrium (equil)

Calculation of interatomic distances and correlation with experimental values

To calculate interatomic distances, the longest sections of the 23 µs MD trajectory of Arg^8-vasopressin that were occupied entirely by a distinct ring state were chosen (278 ns *saddle*$_{ext}$; 212 ns *saddle*$_{fold}$, 136 ns *clinched open*$_{ext}$; 67 ns *clinched open*$_{fold}$, 191 ns *twisted saddle* and 220 ns *open*). The individual distance-trajectories corresponding to the experimental NOE distances were extracted from each representative MD-section. The equilibrium distances are calculated as the 1/6 power means of the distances within each state, weighted according to the distribution given by the metadynamics simulations (Eq. 5-8).

$$r_{equilibrium_{ext}} = (0.6865 \times r_{saddle_{ext}}^{1/6} + 0.2951 \times r_{cl.open_{ext}}^{1/6} + 0.0043 \times r_{tw.saddle}^{1/6} + 0.0141 \times r_{open}^{1/6})^6 \quad (5)$$

$$r_{equilibrium_{eq.}} = \left[0.6865 \times (0.7314 \times r_{saddle_{ext}}^{1/6} + 0.2686 \times r_{saddle_{fold}}^{1/6}) + 0.2951 \times (0.6263 \times r_{cl.open_{ext}}^{1/6} + 0.3737 \times r_{cl.open_{fold}}^{1/6}) + 0.0043 \times r_{tw.saddle}^{1/6} + 0.0141 \times r_{open}^{1/6}\right]^6 \quad (6)$$

The distances for the main states (*saddle* and *clinched open*) were refined by taking the relative populations of *extended* and *folded* tail conformations (Eq. 5-6) into consideration.

$$r_{saddle_{eq.}} = (0.7314 \times r_{saddle_{ext}}^{1/6} + 0.2686 \times r_{saddle_{fold}}^{1/6})^6 \quad (7)$$

$$r_{cl.open_{eq.}} = (0.6263 \times r_{cl.open_{ext}}^{1/6} + 0.3737 \times r_{cl.open_{fold}}^{1/6})^6 \quad (8)$$

The results of the statistical analysis of the correlation between calculated and experimental distances are given in the main text. Figure S3 shows the plot of calculated vs. experimental interatomic distances at pH 6.0 and pH 4.7 and calculated distances are listed in Table S5. Mean unsigned errors (MUE) and root mean square deviations (RMSD) are the same order of magnitude as the experimental error limits (pH 6.0 ±0.5 Å, pH 4.7 ±0.7 Å) for all individual conformations and equilibrium distances calculated from metadynamics. The results are discussed in the main text. At pH 6.0, the number of experimental distances is decreased due to proton exchange. As long as only a small number of experimental distances are available and if the experimental error limits are relatively higher than the differences between representative conformations, the linear regression remains insignificant.

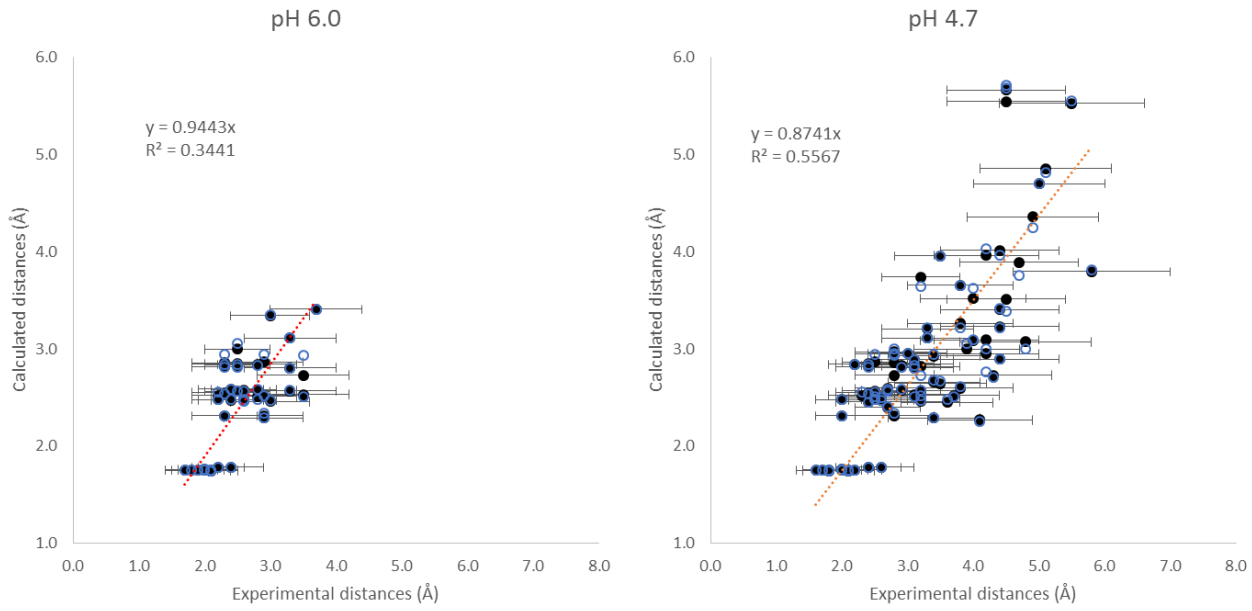

Figure S3 Linear regression of calculated equilibrium and experimental NOE distances at pH 6.0 (left) and pH 4.7 (right). Open blue circles indicate the equilibrium conformations with extended tail. The arrow bars show the error limits of the experimental NOE constraints.

Table S5 Calculated (r^6 weighted) interatomic distances of conformations of Arg^8-vasopressin in solution (TIP4PEw water)

				Interatomic distances (Å)					
				Individual conformations				**Metadynamics equilibrium**	
res	atom	res	atom	*Saddle*[a] *equilibrium*	*Clinched open*[b] *equilibrium*	*Twisted saddle extended*	*Open extended*	Equilibrium[c] *extended*	**Equilibrium[d] *equilibrium***
Cys^1	H^{α}	Cys^1	$H^{\beta a}$	2.49	2.66	2.61	2.98	2.53	2.55
Cys^1	H^{α}	Cys^1	$H^{\beta b}$	2.94	2.69	2.55	2.51	2.94	2.85
Cys^1	$H^{\beta a}$	Cys^1	$H^{\beta b}$	1.76	1.75	1.75	1.75	1.76	1.76
Cys^1	H^{α}	Tyr^2	H	2.26	2.28	2.33	2.30	2.26	2.27
Cys^1	H^{α}	Phe^3	H	6.30	4.00	6.39	6.16	5.55	5.53
Cys^1	$H^{\beta a}$	Cys^6	H	5.44	5.88	4.76	4.30	5.68	5.55
Cys^1	$H^{\beta a}$	Cys^6	H^{α}	5.94	5.09	4.95	5.22	5.71	5.66
Tyr^2	H	Tyr^2	H^{α}	2.88	2.93	2.89	2.93	2.89	2.89
Tyr^2	H^{α}	Tyr^2	$H^{\beta a}$	2.53	2.50	2.49	2.59	2.56	2.52
Tyr^2	H^{α}	Tyr^2	$H^{\beta b}$	2.90	2.64	3.01	2.78	2.81	2.82
Tyr^2	H^{α}	Tyr^2	H^{δ}*($H^{\delta 1}$)	4.03	3.83	4.25	3.46	4.03	3.96
Tyr^2	$H^{\beta a}$	Tyr^2	$H^{\beta b}$	1.75	1.74	1.75	1.75	1.75	1.75
Tyr^2	$H^{\beta a}$	Tyr^2	H^{δ}*($H^{\delta 1}$)	3.24	3.22	3.57	2.91	3.22	3.23
Tyr^2	$H^{\beta b}$	Tyr^2	H^{δ}*($H^{\delta 1}$)	2.66	2.88	2.41	3.17	2.70	2.73
Tyr^2	$H^{\delta 1}$	Tyr^2	H^{ε}*($H^{\varepsilon 1}$)	2.48	2.47	2.48	2.49	2.48	2.48
Tyr^2	H	Phe^3	H	4.63	2.30	4.57	4.36	3.81	3.80
Tyr^2	H^{α}	Phe^3	H	2.28	3.38	2.38	2.20	2.57	2.57
Tyr^2	$H^{\beta a}$	Phe^3	H	3.97	4.12	3.90	3.94	3.96	4.01
Phe^3	H	Phe^3	H^{α}	2.77	2.91	2.78	2.23	2.80	2.80
Phe^3	H	Phe^3	$H^{\beta a}$	3.18	2.89	2.49	3.30	3.00	3.09
Phe^3	H	Phe^3	$H^{\beta b}$	2.47	2.88	2.48	3.78	2.62	2.60
Phe^3	H^{α}	Phe^3	$H^{\beta a}$	2.40	2.57	2.45	2.77	2.47	2.46
Phe^3	H^{α}	Phe^3	$H^{\beta b}$	2.60	2.78	2.98	2.66	2.68	2.66
Phe^3	H^{α}	Phe^3	H^{δ}*($H^{\delta 1}$)	4.04	3.59	3.18	3.41	3.75	3.89
Phe^3	$H^{\beta a}$	Phe^3	$H^{\beta b}$	1.74	1.75	1.75	1.75	1.74	1.74
Phe^3	$H^{\beta a}$	Phe^3	H^{δ}*($H^{\delta 1}$)	2.95	3.01	2.88	3.06	3.00	2.97
Phe^3	$H^{\beta b}$	Phe^3	H^{δ}*($H^{\delta 1}$)	3.10	3.05	3.28	3.00	3.10	3.09
Phe^3	$H^{\delta 1}$	Phe^3	H^{ε}*($H^{\varepsilon 1}$)	2.47	2.47	2.47	2.48	2.47	2.47
Phe^3	$H^{\varepsilon 1}$	Phe^3	H^{ζ}	2.48	2.48	2.48	2.48	2.48	2.48
Phe^3	H^{α}	Gln^4	H	3.49	2.38	2.15	3.06	3.11	3.11
Phe^3	$H^{\beta a}$	Gln^4	H	4.11	3.61	4.13	4.17	3.96	3.96
Phe^3	$H^{\beta b}$	Gln^4	H	3.86	3.44	4.19	4.12	3.65	3.74
Gln^4	H	Gln^4	H^{α}	2.92	2.82	2.23	2.94	2.88	2.89
Gln^4	H	Gln^4	$H^{\beta a}$	2.58	2.76	3.49	3.11	2.67	2.64
Gln^4	H	Gln^4	$H^{\beta b}$	3.39	2.97	3.94	3.27	3.22	3.26
Gln^4	H	Gln^4	$H^{\gamma *}$($H^{\gamma a}$)	3.49	3.21	3.37	3.75	3.41	3.40
Gln^4	H^{α}	Gln^4	$H^{\beta a}$	2.89	2.70	2.95	2.68	2.81	2.83
Gln^4	H^{α}	Gln^4	$H^{\beta b}$	2.55	2.58	2.52	2.67	2.57	2.56
Gln^4	H^{α}	Gln^4	$H^{\gamma *}$($H^{\gamma a}$)	2.90	3.24	2.68	3.12	3.05	3.00
Gln^4	$H^{\beta a}$	Gln^4	$H^{\beta b}$	1.76	1.75	1.76	1.76	1.76	1.76
Gln^4	$H^{\beta a}$	Gln^4	$H^{\gamma *}$($H^{\gamma a}$)	2.84	2.86	2.92	2.94	2.84	2.85
Gln^4	$H^{\varepsilon 1}$	Gln^4	$H^{\varepsilon 2}$	1.75	1.75	1.75	1.75	1.75	1.75
Gln^4	H^{α}	Cys^6	H	4.62	4.85	4.02	5.80	4.70	4.70

				Interatomic distances (Å)					
				Individual conformations				Metadynamics equilibrium	
res	atom	res	atom	*Saddle*[a] *equilibrium*	*Clinched open*[b] *equilibrium*	*Twisted saddle extended*	*Open extended*	Equilibrium[c] *extended*	**Equilibrium[d]** ***equilibrium***
Asn^{5}	H	Asn^{5}	H^{α}	2.96	2.94	2.94	2.86	2.95	2.95
Asn^{5}	H	Asn^{5}	H^{β}*($H^{\beta a}$)	2.86	3.10	2.81	2.63	2.94	2.93
Asn^{5}	H^{α}	Asn^{5}	H^{β}*($H^{\beta a}$)	2.54	2.49	2.50	2.49	2.52	2.52
Asn^{5}	$H^{\delta 1}$	Asn^{5}	$H^{\delta 2}$	1.75	1.75	1.75	1.75	1.75	1.75
Asn^{5}	H	Cys^{6}	H	2.17	3.49	2.49	4.30	2.51	2.53
Asn^{5}	H^{α}	Cys^{6}	H	3.53	2.58	3.32	2.26	3.22	3.21
Cys^{6}	H	Cys^{6}	H^{α}	2.97	2.92	2.94	2.93	2.95	2.95
Cys^{6}	H	Cys^{6}	$H^{\beta a}$	2.83	2.80	3.19	2.70	2.73	2.82
Cys^{6}	H	Cys^{6}	$H^{\beta b}$	3.68	3.17	3.25	3.65	3.62	3.52
Cys^{6}	H^{α}	Cys^{6}	$H^{\beta a}$	2.93	2.72	2.61	2.96	2.94	2.86
Cys^{6}	H^{α}	Cys^{6}	$H^{\beta b}$	2.44	2.70	2.57	2.54	2.48	2.51
Cys^{6}	$H^{\beta a}$	Cys^{6}	$H^{\beta b}$	1.75	1.75	1.75	1.75	1.75	1.75
Cys^{6}	H	Pro^{7}	$H^{\delta a}$	4.78	5.03	5.02	4.96	4.81	4.86
Cys^{6}	H	Pro^{7}	$H^{\delta b}$	4.20	4.74	4.72	4.62	4.25	4.36
Cys^{6}	H^{α}	Pro^{7}	$H^{\delta a}$	2.50	2.58	2.66	2.63	2.51	2.52
Cys^{6}	H^{α}	Pro^{7}	$H^{\delta b}$	2.49	2.36	2.34	2.36	2.48	2.45
Pro^{7}	H^{α}	Pro^{7}	$H^{\beta a}$	2.84	2.84	2.84	2.84	2.84	2.84
Pro^{7}	H^{α}	Pro^{7}	$H^{\beta b}$	2.31	2.31	2.31	2.31	2.31	2.31
Pro^{7}	H^{α}	Pro^{7}	$H^{\gamma *}$	3.66	3.65	3.65	3.65	3.65	3.65
Pro^{7}	$H^{\beta a}$	Pro^{7}	$H^{\beta b}$	1.78	1.78	1.78	1.79	1.78	1.78
Pro^{7}	$H^{\beta a}$	Pro^{7}	$H^{\gamma *}$	2.45	2.46	2.46	2.46	2.45	2.45
Pro^{7}	$H^{\beta b}$	Pro^{7}	$H^{\gamma *}$	2.50	2.49	2.49	2.49	2.50	2.50
Pro^{7}	H^{γ}*	Pro^{7}	H^{δ}*	2.29	2.29	2.29	2.29	2.29	2.29
Pro^{7}	$H^{\delta b}$	Pro^{7}	$H^{\delta b}$	1.78	1.78	1.79	1.78	1.78	1.78
Pro^{7}	H^{α}	Arg^{8}	H	2.33	2.26	2.38	2.36	2.34	2.31
Arg^{8}	H	Arg^{8}	H^{α}	2.75	2.68	2.93	2.94	2.93	2.73
Arg^{8}	H	Arg^{8}	$H^{\beta a}$	2.94	2.98	2.77	2.75	2.77	2.95
Arg^{8}	H	Arg^{8}	$H^{\beta b}$	3.50	3.55	3.34	3.34	3.39	3.51
Arg^{8}	H^{α}	Arg^{8}	$H^{\beta a}$	2.84	2.86	2.81	2.81	2.83	2.84
Arg^{8}	H^{α}	Arg^{8}	$H^{\beta b}$	2.57	2.57	2.60	2.59	2.59	2.57
Arg^{8}	$H^{\beta a}$	Arg^{8}	$H^{\beta b}$	1.75	1.75	1.76	1.76	1.75	1.75
Arg^{8}	$H^{\beta a}$	Arg^{8}	H^{γ}*	2.57	2.56	2.57	2.58	2.56	2.57
Arg^{8}	$H^{\beta b}$	Arg^{8}	H^{γ}*	2.60	2.59	2.60	2.61	2.59	2.60
Arg^{8}	H^{γ}*	Arg^{8}	H^{δ}*	2.40	2.40	2.40	2.39	2.40	2.40
Gly^{9}	H	Gly^{9}	$H^{\alpha 1,2}$	2.49	2.50	2.50	2.50	2.50	2.50
Gly^{9}	$H^{\alpha 1,2}$	Gly^{9}	$H^{N1,2}$	3.07	3.09	3.00	3.00	3.00	3.07
Gly^{9}	H^{N1}	Gly^{9}	H^{N2}	1.75	1.75	1.75	1.75	1.75	1.75

[a]Eq. 7; [b]Eq. 8; [c]Eq. 5; [d]Eq. 6

Experimental details

Sample preparation for NMR

Arg^8-vasopressin was obtained from Bachem (UK) Ltd as the trifluoroacetate salt of the chemically synthesized peptide, having a purity (by HPLC) of >96%. Mass spectrometry of the synthesized material gave a molecular mass of 1084.55 Da, in close agreement to the calculated molecular mass of 1086.26 Da for the reduced form of the peptide.

Samples of 5.0 mg dry weight were dissolved in 320 µl of 90% H_2O/ 10% D_2O to give a peptide concentration of 14.4 mM. The pH of the sample was measured to be 4.7 and NMR spectra were recorded without adjustment. In addition the sample was dried by lyophilization, then redissolved in 320 µl of 20 mM potassium phosphate buffer (pH 6.5) in 90% H_2O/ 10% D_2O and NMR spectra were recorded at a pH measured as 6.0. NMR spectra of Arg^8-vasopressin in D_2O at both pH 4.7 and pH 6.0 were recorded at least 2h after redissolving the extensively dried samples in 99.9% D_2O (Sigma Aldrich).

NMR experiments

NMR spectroscopy was performed on a Varian Inova 600 MHz spectrometer, equipped with 5-channels, a 5 mm triple resonance ($^1H/^{13}C/^{15}N$) coldprobe and actively shielded pulse field z-axis gradients.

Proton resonance assignments were achieved using a combination of 2D ^1H-^1H total chemical shift correlation spectroscopy (TOCSY),[25] and ^1H-^1H nuclear Overhauser effect spectroscopy (NOESY) NMR spectra.[26] Spectra were acquired as 2048 complex points, with 32 transients for each of 512 increments and a spectral width of 10.0 ppm in both dimensions. Mixing times of 60 and 75 ms for the TOCSY experiment and 200 and 300 ms for the NOESY experiment were used. Water suppression was achieved through use of the watergate 3919 sequence.[27]

Resonance assignments for carbon and nitrogen at natural abundance were obtained through the use of gradient heteronuclear single quantum coherence (gHSQC) experiments. A standard ^{13}C-^1H gHSQC NMR spectrum,[28, 29] was acquired as 1024 complex points in t2 (observe ^1H dimension) and 280 increments in t1 (indirect ^{13}C dimension) using 32 transients over spectral widths of 6000.60 Hz (10.0 ppm) and 21114.68 Hz (140.0 ppm) respectively. The transmitter offsets were initially set to the water position in the ^1H and to 70 ppm in the ^{13}C dimension, but other combinations of offset and sweep width were later used to focus on the aliphatic and aromatic regions. A 2D sensitivity enhanced ^{15}N-^1H gHSQC NMR spectrum[28, 30, 31] was acquired as 2048 complex points in t2 (observe ^1H dimension) and 128 increments in t1 (indirect ^{15}N dimension) using 32 transients over spectral widths of 6000.60 Hz (10.0 ppm) and 2431.06 Hz (40.0 ppm) respectively. The transmitter offsets were set to the water position in the ^1H and to 120 ppm in the ^{15}N dimension. States−TPPI quadrature detection was employed in the ^{15}N-dimension.[32]

Spectral processing and format conversion was performed using NMRPipe[33] and visualized with NMRView[34]. Arg^8-vasopressin spectra were assigned using Analysis v2.0.7 from the CcpNMR software suite.[35] Proton and ^{13}C chemical shifts were referenced to 3-trimethyl silyl propane sulfonic acid (DSS) and ^{15}N chemical shifts were referenced to an external liquid ammonia. The ^{1}H, ^{13}C and ^{15}N chemical shifts of the major populated *trans*-Pro^7 isomer of Arg^8-vasopressin in H_2O/pH 4.7, D_2O/pH 4.7, H_2O/pH 6.0, D_2O/pH 6.0 are given in Table S6-9. The volumes of assigned peaks were determined using the box sum method in Analysis with an r^{-6} distance calibration against the fixed distance between the Tyr^2 H^{δ} and H^{ϵ} atoms. A 20% change (the default) in the calculated target distance was taken. These experimentally derived distances are listed in Table S10.

Experimental ^{1}H, ^{13}C and ^{15}N chemical shifts

Table S6 Experimental NMR chemical shifts (δ ppm) of Arg^{8}-vasopressin in H_2O at pH 6.0/ 298 K

Residue	H^N	N	H^α	C^α	Others
Cys^{1}	-	-	3.97	56.18	3.27:$H^{\beta a}$; 3.12:$H^{\beta b}$ 44.23:C^{β}
Tyr^{2}	8.57$^{?}$	123.79$^{?}$	4.64	58.02$^{?}$	2.80$H^{\beta a}$; 2.96:$H^{\beta b}$; 7.05:H^{δ}*; 6.83:H^{ε}* 39.10:C^{β}; 133.34:C^{δ}*; 118.46:C^{ε}*
Phe^{3}	8.04	122.55$^{?}$	4.54	58.37	3.01:$H^{\beta a}$; 3.31:$H^{\beta b}$; 7.23:H^{δ}*; 7.40:H^{ε}*; 7.37:H^{ζ} 39.42:C^{β}; 131.99:C^{δ}*; 131.81:C^{ε}*; 130.17:C^{ζ}
Gln^{4}	8.32	120.80	4.12	57.82	2.05:$H^{\beta a}$; 2.12:$H^{\beta b}$; 2.29:H^{γ}*; 6.89:$H^{\varepsilon a}$; 7.53:$H^{\varepsilon b}$ 28.66:C^{β}; 33.99:C^{γ}; 114.24:N^{ε}
Asn^{5}	8.30	118.20	4.77	-	2.86:H^{β}*; 6.92:$H^{\delta a}$; 7.63:$H^{\delta b}$ 38.74:C^{β}; 114.53:N^{δ}
Cys^{6}	8.15	122.39	4.89	-	3.18:$H^{\beta a}$; 2.93:$H^{\beta b}$ 41.69:C^{β}
trans-Pro^{7}	-	-	4.45	63.52	1.93:$H^{\beta a}$; 2.31:$H^{\beta b}$; 2.06:H^{γ}*; 3.73:$H^{\delta a}$; 3.83:$H^{\delta b}$ 32.16:C^{β}; 27.60:C^{γ}; 50.79:C^{δ}
Arg^{8}	8.63	123.97	4.32	56.45	1.80:$H^{\beta a}$; 1.90:$H^{\beta b}$; 1.67:H^{γ}*; 3.22:H^{δ}*; 7.22:H^{ε} 30.76:C^{β}; 27.28:C^{γ}; 43.46:C^{δ}; 86.76:N^{ε}
Gly^{9}	8.45	113.01	3.93*	45.06	
NH_2^{10}	-	-	-	-	7.09:H^{N1}; 7.48:H^{N2}; 109.16:N

$^{?}$tentative assignment; *degenerate atoms

Table S7 Experimental NMR chemical shifts (δ ppm) of Arg^{8}-vasopressin in D_2O at pH 6.0/ 298 K

Residue	H^N	N	H^α	C^α	Others
Cys^{1}	-	-	3.98	56.01	3.28:$H^{\beta a}$; 3.12:$H^{\beta b}$ 44.20:C^{β}
Tyr^{2}	-	-	4.64	58.02	2.81:$H^{\beta a}$; 2.97:$H^{\beta b}$; 7.06:H^{δ}*; 6.83:H^{ε}* 39.11:C^{β}; 133.35:C^{δ}*; 118.38:C^{ε}*
Phe^{3}	8.05	-	4.54	58.33	3.01:$H^{\beta a}$; 3.31:$H^{\beta b}$; 7.23:H^{δ}*; 7.40:H^{ε}*; 7.37:H^{ζ} 39.37:C^{β}; 131.99:C^{δ}*; 131.81:C^{ε}*; 130.18 C^{ζ}
Gln^{4}	-	-	4.12	57.75	2.05:$H^{\beta a}$; 2.13:$H^{\beta b}$; 2.29:H^{γ}*; 6.89:$H^{\varepsilon a}$; 7.53:$H^{\varepsilon b}$ 28.60:C^{β}; 33.93:C^{γ}
Asn^{5}	8.31	-	4.78	53.08	2.86:H^{β}*; 6.92:$H^{\delta a}$; 7.63:$H^{\delta b}$ 38.66:C^{β}
Cys^{6}	8.15	-	4.90	54.27	3.19:$H^{\beta a}$; 2.93:$H^{\beta b}$ 41.67:C^{β}
trans-Pro^{7}	-	-	4.46	63.51	1.94:$H^{\beta a}$; 2.32:$H^{\beta b}$; 2.07:H^{γ}*; 3.74:$H^{\delta a}$; 3.84:$H^{\delta b}$ 32.16:C^{β}; 27.61:C^{γ}; 50.80:C^{δ}
Arg^{8}	8.62	-	4.32	56.36	1.81:$H^{\beta a}$; 1.91:$H^{\beta b}$; 1.68:H^{γ}*; 3.23:H^{δ}* 30.72:C^{β}; 27.28:C^{γ}; 43.34:C^{δ}
Gly^{9}	8.46	-	3.92*	44.93	
NH_2^{10}	-	-	-	-	-

*degenerate atoms

Table S8 Experimental NMR chemical shifts (δ ppm) of Arg^8-vasopressin in H_2O at pH 4.7/ 298 K

Residue	H^N	N	H^α	C^α	Others
Cys^1	-	-	4.29	55.39	3.46:$H^{\beta a}$; 3.25:$H^{\beta b}$ 42.87:C^β
Tyr^2	8.90	125.26	4.67	-	2.85:$H^{\beta a}$; 2.95:$H^{\beta b}$; 7.07:H^δ*; 6.84:H^ε* 39.25:C^β; 133.36:C^δ*; 118.49:C^ε*
Phe^3	8.15	123.04	4.48	58.63	3.01:$H^{\beta a}$; 3.30:$H^{\beta b}$; 7.22:H^δ*; 7.40:H^ε*; 7.38:H^ζ 39.31:C^β; 131.96:C^δ*; 131.81:C^ε*; 130.17:C^ζ
Gln^4	8.32	121.11	4.12	57.93	2.05:$H^{\beta a}$; 2.13:$H^{\beta b}$; 2.30:H^γ*; 6.90:$H^{\varepsilon a}$; 7.53:$H^{\varepsilon b}$ 28.64:C^β; 33.96:C^γ; 114.18:N^ε
Asn^5	8.33	118.14	4.80	-	2.88:H^β*; 6.93:$H^{\delta a}$; 7.63:$H^{\delta b}$ 38.83:C^β; 114.55:N^δ
Cys^6	8.14	122.09	4.92	-	3.21:$H^{\beta a}$; 2.94:$H^{\beta b}$ 41.28:C^β
trans-Pro^7	-	-	4.46	63.48	1.94:$H^{\beta a}$; 2.32:$H^{\beta b}$; 2.07:H^γ*; 3.75:$H^{\delta a}$; 3.85:$H^{\delta b}$ 32.18:C^β; 27.60:C^γ; 50.80:C^δ
Arg^8	8.65	124.06	4.32	56.50	1.81:$H^{\beta a}$; 1.91:$H^{\beta b}$; 1.69:$H^{\gamma a}$; 1.68:$H^{\gamma b}$; 3.23:H^δ*; 7.21:H^ε 30.75:C^β; 27.28:C^γ; 43.46:C^δ; 86.76:N^ε
Gly^9	8.44	112.97	3.94*	45.04	
NH_2^{10}	-	-	-	-	7.09:H^{N1}; 7.48:H^{N2}; 109.17:N

[?]tentative assignment; *degenerate atoms

Table S9 Experimental NMR chemical shifts (δ ppm) of Arg^8-vasopressin in D_2O at pH 4.7/ 298 K

Residue	H^N	N	H^α	C^α	Others
Cys^1	-	-	4.29	55.22	3.46:$H^{\beta a}$; 3.25:$H^{\beta b}$ 42.83:C^β
Tyr^2	-	-	4.66	58.12	2.85:$H^{\beta a}$; 2.96:$H^{\beta b}$; 7.07:H^δ*; 6.84:H^ε* 39.27:C^β; 133.37:C^δ*; 118.42:C^ε*
Phe^3	-	-	4.47	58.59	3.02:$H^{\beta a}$; 3.30:$H^{\beta b}$; 7.21:H^δ*; 7.40:H^ε*; 7.37:H^ζ 39.28:C^β; 131.96:C^δ*; 131.81:C^ε*; 130.18:C^ζ
Gln^4	8.33	-	4.12	57.85	2.05:$H^{\beta a}$; 2.13:$H^{\beta b}$; 2.30:H^γ*; 6.90:$H^{\varepsilon a}$; 7.54:$H^{\varepsilon b}$ 28.58:C^β; 33.89:C^γ
Asn^5	8.30	-	4.81	53.04	2.89:H^β*; 6.94:$H^{\delta a}$; 7.63:$H^{\delta b}$ 38.75:C^β
Cys^6	-	-	4.92	53.97	3.21:$H^{\beta a}$; 2.93:$H^{\beta b}$ 41.26:C^β
trans-Pro^7	-	-	4.46	63.49	1.94:$H^{\beta a}$; 2.33:$H^{\beta b}$; 2.07:H^γ*; 3.75:$H^{\delta a}$; 3.85:$H^{\delta b}$ 32.18:C^β; 27.61:C^γ; 50.81:C^δ
Arg^8	8.65	-	4.31	56.41	1.82:$H^{\beta a}$; 1.91:$H^{\beta b}$; 1.68:H^γ*; 3.23:H^δ* 30.71:C^β; 27.28:C^γ; 43.34:C^δ
Gly^9	8.45	-	3.93*	44.91	
NH_2^{10}	-	-	-	-	7.48:H^N*

*degenerate atoms

Experimental NOE distances

Table S10 Experimental NOE distances of Arg^8-vasopressin

				NOE distances (Å)					
				pH 4.7			pH 6.0		
res	atom	res	atom	Constraint	Error limits		Constraint	Error limits	
				r_{exp}	+	-	r_{exp}	+	-
Cys^1	H$^{\alpha}$	Cys^1	H$^{\beta a}$	2.4	0.5	0.5	2.3	0.5	0.5
Cys^1	H$^{\alpha}$	Cys^1	H$^{\beta b}$	2.8	0.6	0.6	2.3	0.5	0.5
Cys^1	H$^{\beta a}$	Cys^1	H$^{\beta b}$	2.0	0.3	0.4	2.0	0.2	0.4
Cys^1	H$^{\alpha}$	Tyr^2	H	4.1	0.8	0.8	-	-	-
Cys^1	H$^{\alpha}$	Phe^3	H	5.5	1.1	1.1	-	-	-
Cys^1	H$^{\beta a}$	Cys^6	H	4.5	0.9	0.9	-	-	-
Cys^1	H$^{\beta a}$	Cys^6	H$^{\alpha}$	4.5	0.9	0.9	-	-	-
Tyr^2	H	Tyr^2	H$^{\alpha}$	4.4	0.9	0.9	-	-	-
Tyr^2	H$^{\alpha}$	Tyr^2	H$^{\beta a,b}$	2.3	0.5	0.5	2.2	0.4	0.4
Tyr^2	H$^{\alpha}$	Tyr^2	H$^{\beta b,a}$	2.4	0.5	0.5	2.3	0.5	0.5
Tyr^2	H$^{\alpha}$	Tyr^2	H$^{\delta}$*	4.2	0.8	0.8	-	-	-
Tyr^2	H$^{\beta a}$	Tyr^2	H$^{\beta b}$	1.8	0	0.4	2.1	0.3	0.4
Tyr^2	H$^{\beta a}$	Tyr^2	H$^{\delta}$*	4.4	0.9	0.9	-	-	-
Tyr^2	H$^{\beta b}$	Tyr^2	H$^{\delta}$*	4.3	0.9	0.9	-	-	-
Tyr^2	H$^{\delta 1}$	Tyr^2	H$^{\varepsilon}$*	2.5	0.5	0.5	2.6	0.5	0.5
Tyr^2	H	Phe^3	H	5.8	1.2	1.2	-	-	-
Tyr^2	H$^{\alpha}$	Phe^3	H	3.2	0.6	0.6	3.3	0.7	0.7
Tyr^2	H$^{\beta a}$	Phe^3	H	4.4	0.9	0.9	-	-	-
Phe^3	H	Phe^3	H$^{\alpha}$	3.1	0.6	0.6	3.3	0.7	0.7
Phe^3	H	Phe^3	H$^{\beta a}$	4.2	0.8	0.8	-	-	-
Phe^3	H	Phe^3	H$^{\beta b}$	3.8	0.8	0.8	-	-	-
Phe^3	H$^{\alpha}$	Phe^3	H$^{\beta a}$	3.2	0.6	0.6	3.0	0.6	0.6
Phe^3	H$^{\alpha}$	Phe^3	H$^{\beta b}$	3.4	0.7	0.7	-	-	-
Phe^3	H$^{\alpha}$	Phe^3	H$^{\delta}$*	4.7	0.9	0.9	-	-	-
Phe^3	H$^{\beta a}$	Phe^3	H$^{\beta b}$	2.1	0.4	0.4	2.1	0.4	0.4
Phe^3	H$^{\beta a}$	Phe^3	H$^{\delta}$*	2.8	0.6	0.6	-	-	-
Phe^3	H$^{\beta b}$	Phe^3	H$^{\delta}$*	4.0	0.8	0.8	-	-	-
Phe^3	H$^{\delta 1}$	Phe^3	H$^{\varepsilon}$*	2.6	0.5	0.5	2.6	0.5	0.5
Phe^3	H$^{\varepsilon 1}$	Phe^3	H$^{\zeta}$	2.0	0.3	0.4	2.2	0.4	0.4
Phe^3	H	Gln^4	H	-	-	-	3.7	0.7	0.7
Phe^3	H$^{\alpha}$	Gln^4	H	3.3	0.7	0.7	3.3	0.7	0.7
Phe^3	H$^{\beta a}$	Gln^4	H	3.5	0.7	0.7	-	-	-
Phe^3	H$^{\beta b}$	Gln^4	H	3.2	0.6	0.6	-	-	-
Gln^4	H	Gln^4	H$^{\alpha}$	3.1	0.6	0.6	-	-	-
Gln^4	H	Gln^4	H$^{\beta a}$	3.5	0.7	0.7	-	-	-
Gln^4	H	Gln^4	H$^{\beta b}$	3.8	0.8	0.8	-	-	-
Gln^4	H	Gln^4	H$^{\gamma *}$(H$^{\gamma a}$)	4.4	0.9	0.9	-	-	-
Gln^4	H$^{\alpha}$	Gln^4	H$^{\beta a}$	2.9	0.6	0.6	2.5	0.5	0.5
Gln^4	H$^{\alpha}$	Gln^4	H$^{\beta b}$	2.7	0.5	0.5	2.5	0.5	0.5
Gln^4	H$^{\alpha}$	Gln^4	H$^{\gamma *}$(H$^{\gamma a}$)	3.9	0.8	0.8	2.5	0.5	0.5
Gln^4	H$^{\beta a}$	Gln^4	H$^{\beta b}$	2.1	0.3	0.4	-	-	-

NOE distances (Å)									
				pH 4.7			pH 6.0		
res	atom	res	atom	Constraint	Error limits		Constraint	Error limits	
				r_{exp}	+	-	r_{exp}	+	-
Gln^4	$H^{\beta a}$	Gln^4	$H^{\gamma *}(H^{\gamma a})$	2.4	0.5	0.5	2.5	0.5	0.5
Gln^4	$H^{\varepsilon 1}$	Gln^4	$H^{\varepsilon 2}$	1.8	0.0	0.4	1.8	0.1	0.4
Gln^4	H^{α}	Cys^6	H	5.0	1.0	1.0	-	-	-
Gln^4	H^{α}	Asn^5	H	-	-	-	3.0	0.6	0.6
Asn^5	H	Asn^5	H^{α}	3.0	0.6	0.6	-	-	-
Asn^5	H	Asn^5	$H^{\beta}*$	3.4	0.7	0.7	-	-	-
Asn^5	H^{α}	Asn^5	$H^{\beta}*$	3.2	0.6	0.6	2.9	0.6	0.6
Asn^5	$H^{\delta 1}$	Asn^5	$H^{\delta 2}$	1.7	0.1	0.3	1.7	0	0.3
Asn^5	H	Cys^6	H	3.1	0.6	0.6	3.5	0.7	0.7
Asn^5	H^{α}	Cys^6	H	3.3	0.7	0.7	-	-	-
Cys^6	H	Cys^6	H^{α}	2.8	0.6	0.6	-	-	-
Cys^6	H	Cys^6	$H^{\beta a}$	3.2	0.6	0.6	-	-	-
Cys^6	H	Cys^6	$H^{\beta b}$	4.0	0.8	0.8	-	-	-
Cys^6	H^{α}	Cys^6	$H^{\beta a}$	2.5	0.5	0.5	2.9	0.6	0.6
Cys^6	H^{α}	Cys^6	$H^{\beta b}$	2.5	0.5	0.5	2.8	0.6	0.6
Cys^6	$H^{\beta a}$	Cys^6	$H^{\beta b}$	2.0	0.3	0.4	2.0	0.3	0.4
Cys^6	H	Pro^7	$H^{\delta a}$	5.1	1.0	1.0	-	-	-
Cys^6	H	Pro^7	$H^{\delta b}$	4.9	1.0	1.0	-	-	-
Cys^6	H^{α}	Pro^7	$H^{\delta a}$	3.7	0.7	0.7	-	-	-
Cys^6	H^{α}	Pro^7	$H^{\delta b}$	3.6	0.7	0.7	-	-	-
Pro^7	H^{α}	Pro^7	$H^{\beta a}$	2.2	0.4	0.4	2.3	0.5	0.5
Pro^7	H^{α}	Pro^7	$H^{\beta b}$	2.0	0.2	0.4	2.3	0.5	0.5
Pro^7	H^{α}	Pro^7	$H^{\gamma *}$	3.8	0.8	0.8	-	-	-
Pro^7	$H^{\beta a}$	Pro^7	$H^{\beta b}$	2.6	0.5	0.5	2.4	0.5	0.5
Pro^7	$H^{\beta a}$	Pro^7	$H^{\gamma *}$	2.4	0.5	0.5	2.4	0.5	0.5
Pro^7	$H^{\beta b}$	Pro^7	$H^{\gamma *}$	2.6	0.5	0.5	2.6	0.5	0.5
Pro^7	$H^{\gamma}*$	Pro^7	$H^{\delta}*$	3.4	0.7	0.7	2.9	0.6	0.6
Pro^7	$H^{\delta b}$	Pro^7	$H^{\delta b}$	2.4	0.5	0.5	2.2	0.4	0.4
Pro^7	H^{α}	Arg^8	H	2.8	0.6	0.6	2.9	0.6	0.6
Arg^8	H	Arg^8	H^{α}	2.8	0.6	0.6	3.5	0.7	0.7
Arg^8	H	Arg^8	$H^{\beta a}$	4.2	0.8	0.8	-	-	-
Arg^8	H	Arg^8	$H^{\beta b}$	4.5	0.9	0.9	-	-	-
Arg^8	H^{α}	Arg^8	$H^{\beta a}$	3.1	0.6	0.6	2.8	0.6	0.6
Arg^8	H^{α}	Arg^8	$H^{\beta b}$	2.9	0.6	0.6	2.8	0.6	0.6
Arg^8	$H^{\beta a}$	Arg^8	$H^{\beta b}$	2.2	0.4	0.4	2.1	0.4	0.4
Arg^8	$H^{\beta a}$	Arg^8	$H^{\gamma}*$	2.5	0.5	0.5	2.4	0.5	0.5
Arg^8	$H^{\beta b}$	Arg^8	$H^{\gamma}*$	2.7	0.5	0.5	2.6	0.5	0.5
Arg^8	$H^{\gamma}*$	Arg^8	$H^{\delta}*$	2.7	0.5	0.5	-	-	-
Gly^9	H	Gly^9	$H^{\alpha 1,2}$	2.5	0.5	0.5	-	-	-
Gly^9	$H^{\alpha 1,2}$	Gly^9	$H^{N1,2}$	4.8	1.0	1.0	-	-	-
Gly^9	H^{N1}	Gly^9	H^{N2}	1.6	0.1	0.3	1.9	0.1	0.4

Gaussian archive entries for the B3LYP/6-31G(d)-optimized geometries.

Saddle, folded:


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Saddle, extended:

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2S2)]\\@

Clinched open, folded:

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with folded tail conformation\\2,1\N,-4.6682334799,8.0993140401,13.31
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RevD.01\State=1-A\HF=-4313.5754649\RMSD=7.546e-09\RMSF=2.142e-06\Dipol
e=-9.3182369,-3.6441124,-8.9227906\Quadrupole=207.4827637,-101.2244415
,-106.2583222,-49.7858048,259.7104929,20.5083893\PG=C01 [X(C46H67N15O1
2S2)]\\@

Clinched open, extended:


```
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 l_open\\2,1\N,-9.3435783672,-12.4949768434,2.5153303585\H,-9.694391987
 4,-13.1779261134,1.8211119347\H,-10.137759484,-11.9119649439,2.7978609
 187\H,-8.9856452812,-12.9745120635,3.3534778048\C,-8.2632699003,-11.69
 26504466,1.8506910731\H,-8.2705617264,-10.696151436,2.294555961\C,-6.8
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 3,-12.7753040285,3.7803917189\C,-8.6556394425,-11.663084404,0.35477259
 34\O,-9.442834375,-12.5063183906,-0.0764313241\N,-8.0492226972,-10.737
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 .4329860119,-9.0948366248,-6.2103359879\H,-6.7420075617,-9.3422812609,
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 996,-18.1234921974,0.9647070557\N,-5.3589543873,-17.7054604215,-0.5044
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 .3614104429,0.265520001\H,-4.7787534082,-19.1234226302,0.8827481359\C,
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 10559253,-19.8796248561,0.1032133477\O,-2.3989200201,-20.0518117631,1.
 3192942622\N,-1.3177563658,-20.4363532038,-0.6409733303\H,-0.640170377
 3,-21.0383353717,-0.1929320713\H,-1.2527900089,-20.3061849217,-1.64044
 23222\C,-3.6030480579,-17.3276931838,1.1823896445\O,-2.6058377048,-16.
 7036880636,0.8193450618\N,-4.2088472346,-17.141917444,2.3792195914\H,-
 4.9799334821,-17.7382954429,2.6494156477\C,-3.6788976679,-16.273853645
 2,3.4161793005\H,-2.6079239531,-16.1601954914,3.2251400695\C,-4.275234
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 464315\H,-4.1274428566,-14.4481764025,2.3615235454\S,-6.0877466545,-14
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03\H,-8.5036501134,-18.0746748481,9.1931667268\C,-7.9319421489,-17.547
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39,-18.6157205937,6.9276914232\N,-9.2954638628,-17.8743669169,5.071257
6459\H,-10.1336120417,-17.3079585699,5.0534173659\C,-8.6935905986,-18.
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6.7924842302,-18.5158197539,4.5476857932\H,-7.1183854593,-18.845852347
7,2.9094047276\N,-9.2884682641,-17.7594583712,2.7525739524\H,-10.26939
78288,-17.5226164764,2.7150135435\H,-8.7469676304,-17.8072159832,1.889
1350585\C,-6.2798588702,-18.1163065079,10.6428512316\O,-5.6651680774,-
19.1686044428,10.8211063758\N,-6.900297617,-17.4276340844,11.634309282
1\H,-7.4758691547,-16.6374292213,11.3734456094\C,-7.1414388091,-18.018
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,12.9738280657\O,-9.1410270253,-19.0047519619,11.9642805376\N,-8.83855
29665,-19.2022184436,14.2103740872\H,-8.2616531748,-19.0482034484,15.0
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Twisted saddle, extended:

1\1\FAU-CCC-CCDH173\FOpt\RB3LYP\6-31G(d)\C46H67N15O12S2(2+)\CLARK\23-A
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,7.0336633398,-9.9968301557\H,5.7786842663,6.5201520252,-8.4846886719\
H,7.1046279972,7.4980372998,-8.6285305788\C,5.2619704319,8.5217194046,
-8.9562854103\H,4.8465765776,8.5965721412,-7.9506218898\C,6.1083113637
,9.7577187113,-9.30753096\H,6.8292900672,9.9488660217,-8.5091476943\H,
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682,7.734436115,-9.8679485066\H,1.0757375305,6.9018978986,-9.315233947
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18238894\H,-3.7674038676,7.7259129528,-11.5405023952\C,-2.535242593,6.
1693008044,-12.3912652802\O,-3.4884893434,5.6243728648,-13.2019124264\
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08262,11.2260429449,-13.5293904958\C,8.4543571899,19.3225538005,-12.07
95253168\O,7.716251286,20.299737373,-11.9447981197\N,9.6621926574,19.3
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587063,20.4173150476,-14.9363976986\O,8.7593972366,19.5528482329,-15.2
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,22.0941619843,-15.440432973\H,9.724240805,21.4127513519,-16.717917836
7\\Version=EM64L-G09RevC.01\State=1-A\HF=-4313.5804546\RMSD=3.550e-09\
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16244,29.149813,-23.9381885,41.9635208,27.3753322,-55.7098266\PG=C01 [
X(C46H67N15O12S2)]\\@

Open, extended:

1\1\FAU-CCC-CCDH173\FOpt\RB3LYP\6-31G(d)\C46H67N15O12S2(2+)\CLARK\24-A
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\\2,1\N,0.4475246287,-3.1779434367,-3.5701330464\H,-0.5341706603,-2.88
25080235,-3.5394377666\H,0.630961857,-3.882061537,-2.8241126635\H,0.60
74771251,-3.6323651944,-4.4749533667\C,1.4175750482,-2.0519324222,-3.3
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