

Supporting Data for:**NMR and EPR structural delineation of copper (II) complexes formed by kanamycin A in water.****Nicola D'Amelio¹, Elena Gaggelli¹, Nicola Gaggelli¹, Elena Molteni¹, Maria Camilla Baratto¹, Gianni Valensin^{1*}, Małgorzata Jeżowska-Bojczuk² and Wojciech Szczepanik²**¹*Department of Chemistry and the NMR Center, University of Siena, Via A.Moro 2, Siena 53100, Italy*²*Faculty of Chemistry, University of Wrocław, F.Joliot-Curie 14, Wrocław 50-383, Poland*

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Experimental Supplementary Informations on Molecular Dynamics Calculations.

In this procedure, the potential energy is a function of the difference between the distance constraints provided by the user and corresponding distances found in a given conformer. No other potential energy terms are present except van der Waals repulsions. At the beginning of the calculation an arbitrary number of different conformers is generated by randomly varying torsional dihedral angles. Then, the potential energy is minimised by a simulated annealing procedure in the torsion angle space in which the system is brought to high temperature to allow all possible high energy starting conformations and subsequently cooled down in order to stabilize it in the potential energy minima, which better satisfy the imposed constraints.

In particular we performed the calculation with the program DYANA using 10000 steps and 300 random relative starting positions of kanamycin and Cu(II). Since the program does not support amino-sugars, the library was modified by treating kanamycin as a residue linked to Cu(II) through a long chain of linkers without van der Waals radius. These linkers can freely rotate around their bonds and enable to sample all possible relative positions of the ligand with respect to copper before the minimization step. The kanamycin "residue" was built allowing completely free rotation around the bonds connecting the rings and the bonds linking the substituents. In addition each ring was allowed to assume all possible chair or boat conformations (2 chairs and 6 boats) yielding a total of 512 conformers. Each conformer was then allowed to have 50 different metal - ligand relative positions before the minimization routine.

The structures of the complexes thus generated only rely on experimental data and no assumption is made on the conformation assumed by the ligand before co-ordination.

Table S1. ^1H chemical shifts (ppm) of KanA 0.5 mM in D_2O at pH 5.5, 7.4 and 12.0 (values uncorrected for the isotopic effect), $T=298$ K. Chemical shifts were referenced to internal TSP.

Proton	pH 5.5	pH 7.4	pH 12.0
H_1'	5.592	5.563	5.342
H_2'	3.684	3.652	3.621
H_3'	3.794	3.774	3.728
H_4'	3.394	3.385	3.340
H_5'	4.027	4.030	3.782
H_6'	3.456	3.434	3.007
	3.194	3.176	2.782
H_1	3.530	3.282	2.921
H_2 (eq)	2.493	2.224	1.981
H_2 (ax)	1.896	1.560	1.242
H_3	3.530	3.181	2.908
H_4	3.757	3.592	3.341
H_5	3.919	3.809	3.680
H_6	3.874	3.547	3.273
H_1''	5.153	5.118	5.060
H_2''	3.954	3.812	3.519
H_3''	3.510	3.343	3.026
H_4''	3.719	3.608	3.354
H_5''	3.956	3.958	3.943
H_6''	3.853	3.825	3.796
	3.800	3.809	

Table S2. Non selective ($R^{\text{n sel}}$, s^{-1}), single-selective (R^{sel} , s^{-1}) and double-selective ($R^{\text{b sel}}$, s^{-1}) relaxation rates of H₂ protons of KanA 0.5 mM in D₂O at pH 5.5, 7.4 and 12.0 (values uncorrected for the isotopic effect), T = 298 K. Values for the rotational correlation time, τ_R , were obtained from the geminal ($r = 0.177$ nm) cross-relaxation rates (σ (s^{-1}) = $R^{\text{b sel}} - R^{\text{sel}}$)

Proton	R^{sel}	$R^{\text{b sel}}$	σ	τ_R (ps)
pH 5.5				
H2 (eq)	2.48	2.75	0.27	217
H2 (ax)	2.62	2.78	0.16	249
pH 7.4				
H2 (eq)	2.24	2.52	0.28	215
H2 (ax)	2.21	2.53	0.32	203
pH 12.0				
H2 (eq)	2.09	2.38	0.29	212
H2 (ax)	2.07	2.23	0.16	249

Table S3. EPR magnetic parameters of Cu(II)-KanA complexes at pH 7.4 and 12.0.

	pH=7.4	pH=12.0
g_{\parallel}	2.220	2.231
G_{\perp}	2.057	2.052
A_{\parallel}^a	196.4	194.7
A_{\perp}^a	21.4	17.2
$n^{\circ} N^b$	2	2
$A_{\text{Niso}}^{a,c}$	12.4	12.2

^a Coupling constants are given in cm^{-1} and multiplied by a factor of 10^4

^b Number of equatorial nitrogen donor atoms

^c Isotropic superhyperfine coupling constant

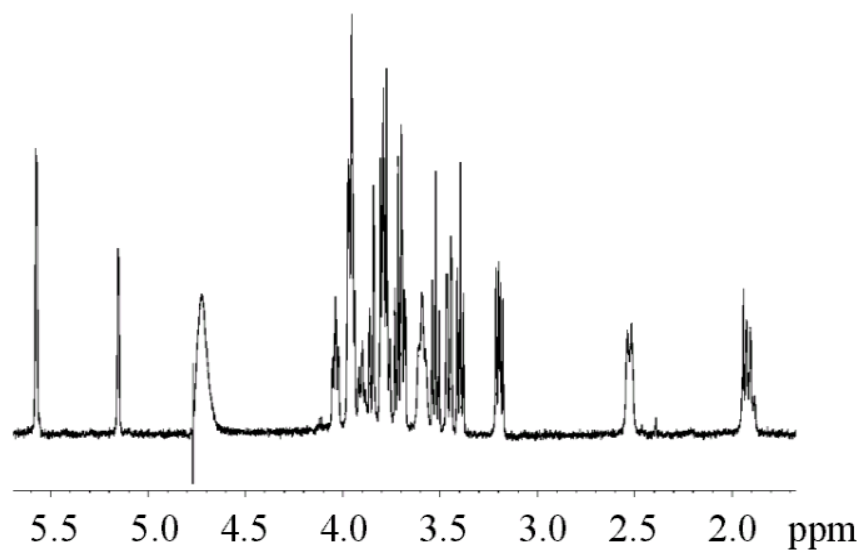


Figure S1. ¹H-NMR spectrum of KanA 0.5 mM in D₂O at 298 K and pH 5.5 (value uncorrected for the isotopic effect) recorded at 600 MHz.
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