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Crystallographic and NMR evidence of the unusual N6,N7-didentate chelation of 3-methyladenine coordinated to the cytotoxic α-dichlorobis(2-phenylazopyridine)ruthenium(II) complex †

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Adenine bases have recently been found to coordinate to the cytotoxic alpha isomer of dichlorobis(2-phenylazopyridine)ruthenium(II) complexes in the rare imine form, stabilised by N6,N7-didentate coordination, which is now proven by X-ray structure determination of the compound α-[Ru(azpy)$_2$(3-MeAde$_{-H}$)]PF$_6$ (azpy = 2-phenyl-azopyridine, 3-MeAde$_{-H}$ = deprotonated 3-methyladenine).

Ruthenium complexes have attracted much attention in the search for new anticancer agents.[1] The so-called α-isomer of the dichlorobis(2-phenylazopyridine)ruthenium(II) complexes,[2] α-[Ru(azpy)$_2$Cl$_2$] (α corresponds to the isomer in which the coordination pairs Cl, N(py), and N(azo) are *cis*, *trans* and *cis*, respectively) shows remarkably high cytotoxicity against a series of human tumour cell lines.[3] It is generally accepted that DNA might be the ultimate target for antitumour-active ruthenium complexes,[1] as in the case of antitumour-active platinum complexes.[4] Therefore, the interaction of the cytotoxic α-[Ru(azpy)$_2$Cl$_2$] with DNA-model bases has been studied. With 9-ethylguanine (9-EtGua) the monofunctional adduct α-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$ is formed, fully characterised by NMR spectroscopy.[5] The coordination of adenine bases to the α-[Ru(azpy)$_2$] moiety results in didentate coordination *via* the N6 and N7 atoms as concluded from NMR data[6] of α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$, and shown in the X-ray structure of α-[Ru(azpy)$_2$(3-MeAde$_{-H}$)](PF$_6$)$_2$ reported here. The single-crystal structure determination of α-[Ru(azpy)$_2$-(3-MeAde$_{-H}$)]PF$_6$, **1**, ‡ is the first crystallographic evidence of a mononuclear ruthenium(II) complex in which an adenine model base is present in the imine form and stabilised by chelating coordination *via* both its N7 and N6 atoms. There are some differences between 9-MeAde and 3-MeAde like the increased ligating power of 3-alkylated 6-aminopurines over the 9-alkylated 6-aminopurines[7] and tautomeric structures[8] (Fig. 1). Nevertheless, the coordination mode of 9-MeAde and 3-MeAde to metal ions is very similar. A few crystal structures of cobalt[9] and platinum[7] complexes with N(3) alkylated 6-aminopurine derivatives with N7 coordination are known. Another example is a rhodium complex with a bridging 3-MeAde ligand (*via* the N7 and N6 sites).[10]

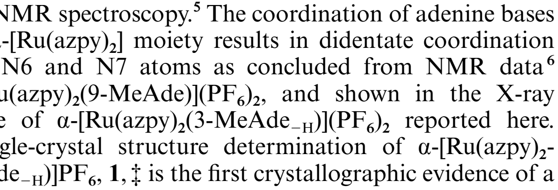

Fig. 1 Tautomeric structures (and numbering used for NMR assignments) of 3-MeAde: the amine form (left) and the imine form (right).

The molecular structure of **1** (Fig. 2) shows the 3-MeAde base coordinated in a didentate fashion *via* its N6 and N7 atoms. In fact the 3-MeAde ligand is found to be disordered over two positions, related by a two-fold rotation around the axis bisecting the angle N8–Ru–N28 (see Fig. S1, ESI). Since bond distances and valence angles of both components are linked, only geometric parameters for the major component are listed. The Ru–N6 distance of 2.215(10) Å and Ru–N7 distance of 2.033(11) Å are comparable to those in the polynuclear ruthenium structures [{Ru(ade)(η^6-*p*-cymene)}$_3$]]$^{3+}$-(ade = adenine), [{Ru(9-EtAde$_{-H}$(η^6-*p*-cymene)}$_3$]$^{3+}$ (9-EtAde$_{-H}$ = deprotonated 9-ethyladenine) and [{Ru(5-AMP)(η^6-*p*-MeC$_6$-H$_4$Pr)}$_3$]·7.5H$_2$O (5′-AMP = adenosine 5′-monophosphate) in which the adenine derivatives also coordinate in a N6,N7-didentate fashion.[11] In contrast to **1**, these complexes are polynuclear: the adenine derivatives function as bridging ligands *via* the N1 or N9 sites.

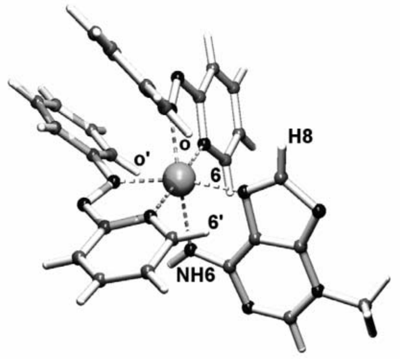

Fig. 2 Molecular structure of the Δ-enantiomer of α-[Ru(azpy)$_2$-(3-MeAde$_{-H}$)](PF$_6$), **1**.

The 3-MeAde ligand in **1** shows the short C6–N6 distance of 1.276(16) Å typical for the imine form of adenine. The average C–N distance for coordinating and non-coordinating imines is 1.297 Å (Cambridge Structural Database,[12] February 2002). It has been estimated from NDDO energy differences that the rare imine form of 3-MeAde is somewhat more likely than for 9-MeAde and adenine (ΔE_{pot} = 57.9 and 195 for the imine form of respectively 3-MeAde and 9-MeAde relative to ΔE_{pot} = 0 for the most stable tautomer *i.e.* the amine form).[8] The amine–imine tautomeric equilibrium might be influenced by metal

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† Electronic supplementary information (ESI) available: plots of the 3-MeAde ligand in **1** and of the intermolecular hydrogen bonding of the NH(6) group of one 3-MeAde moiety towards N9 of another 3-MeAde ligand. See http://www.rsc.org/suppdata/dt/b2/b204665m/

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coordination[13] and in the case of **1** the imine form is probably stabilised by didentate coordination. In the ruthenium cymene complexes the adenine bases are apparently present in the imine form (C6–N6 = 1.309 Å and 1.306 Å respectively).[11a] Instead of didentate coordination also stabilisation of the imine form by hydrogen bonding might occur, like the imine form of 9-MeAde which is only coordinated *via* its N6 atom in the compound[14] *trans*-[RuCl$_3$(9-MeAde-*N6*)(dmtp)$_2$] (dmtp = 5,7-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidine). The molecular structure of **1** shows only one PF$_6$ counter ion which also confirms that the 3-MeAde base is deprotonated (see also Notes and references). Deprotonation of 3-MeAde in the imine form likely occurs at the N9 site (*vide infra*) leaving the N6 site protonated. However, the hydrogen atom on the exocyclic N6 site was difficult to locate during the structure determination due to disorder of the 3-MeAde ligand. On the other hand, the N6–N9 distance between two symmetry related molecules (3.221(16) Å) indicate the presence of an hydrogen atom between these two nitrogens (see Fig. S2, ESI). In addition, the NMR data (*vide infra*) clearly show the NH(6) resonance and although these NMR data are obtained in solution this hydrogen position was consecutively included in the X-ray diffraction analysis.

The ^{1}H NMR spectrum of **1** in acetone-d_6 (293 K) shows the purine resonances H2 at 7.99 ppm (8.19), H8 at 7.55 (7.80) and NH(6) at 6.42 (7.22) ppm (the resonances of the free model base are indicated in brackets). Characterisation of **1** by 2D NOESY NMR is interesting also in comparison with NMR data of the related compound[6] α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$. In **1** the N3 site is methylated and this CH$_3$ resonance shows an intraligand NOE peak with the H2 resonance. The H2 signal shows no further NOE interactions. The NOE couplings H8-*o* and H8-6′ are in agreement with the didentate coordination of 3-MeAde as observed in the X-ray structure and confirm the NMR-data[6] of α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$. The strong NH6–H6 coupling (and weak NH6-*o*′) is also important as these observations prove the protonation of the N6 site and the NH6–H6 coupling is in agreement with the short distance observed in the X-ray structure (3.0339 Å). The NMR data show that in **1** the N6 site is protonated and the N9 site deprotonated and the latter probably causes the relatively high field H8 resonance in comparison to the analogous but neutral 9-MeAde compound.[6] In fact, protonation of **1** during NMR analysis (by adding an excess of 1 M HNO$_3$ to the sample of **1** in acetone-d_6) shifts the H8 resonance from 7.55 to 8.33 ppm, which is a chemical shift value comparable to the H8 resonance of the analogous 9-MeAde complex. This relatively low field position of the H8 resonance (8.33 ppm) is explained by the deshielding effect of the phenyl ring (of the azpy ligand with the N(py) and N(azo) *fac* with N7 of 3-MeAde).[6]

In conclusion, to the best of our knowledge, this is the first crystallographic report of a mononuclear ruthenium(II) complex in which an adenine model base is present in the imine form, stabilised by the chelating coordination mode *via* its N6 and N7 atoms. The data presented here unambiguously confirm the conclusions based on NMR data. This kind of DNA adduct might be a clue in developing a structure–activity relationship for cytotoxic ruthenium complexes.

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Notes and references

‡ The compound α-[Ru(azpy)$_2$(3-MeAde$_{-H}$)]PF$_6$, **1**, has been synthesized (3-MeAde obtained from Aldrich) analogously to the synthesis of the related 9-MeAde compound.[6] The compound has been purified on a neutral alumina column (eluent: acetone–methanol, 25 : 1, fraction 1) which causes deprotonation of 3-MeAde. Single crystals of **1** suitable for X-ray crystallographic studies were grown after addition of an excess of diethyl ether to fraction 1 obtained from the column. Analyses: RuC$_{28}$H$_{24}$N$_{11}$PF$_6$·0.7H$_2$O Found (calc.): C, 43.3 (43.5); H 3.45 (3.31); 19.8 (19.9)%. ^{1}H NMR of **1** (acetone-d_6, 20 °C, 600.13 MHz, azpy ligand with N(py) and N(azo) *fac* with N7 of purine is denoted without primes; the other azpy ligand with primes): δ 3.79 (s, CH$_3$), 6.42 (s, NH(6)), 7.06 (d, *o*′), 7.13 (d, *o*), 7.34 (t, *m*′), 7.38 (t, *m*), 7.49 (t, *p*′), 7.52 (t, *p*), 7.55 (s, H8), 7.63 (t, 5′), 7.70 (t, 5), 7.76 (d, 6′), 7.99 (s, H2), 8.32 (t, 4), 8.36 (t, 4′), 8.54 (d, 6), 8.90 (d, 3), 8.93 (d, 3′).

Crystal data for **1**: C$_{28}$H$_{24}$N$_{11}$RuPF$_6$, M_r = 760.62, black crystal (0.01 × 0.05 × 0.10 mm), triclinic, space group $P\bar{1}$ (no. 2), a = 7.6447(12), b = 13.318(3), c = 16.527(4) Å, α = 81.295(10), β = 76.875(10), γ = 83.945(10)°, V = 1615.4(6) Å^3, Z = 2, D_c = 1.5638(6) g cm^{-3}, F(000) = 764, μ(Mo-Kα) = 0.608 mm^{-1}. 46051 reflections measured, 5245 independent, R_{int} = 0.322, R_σ = 0.253, 1.0 < θ < 24.50°, T = 100 K, Mo-Kα radiation, graphite monochromator, λ = 0.71073 Å, Nonius KappaCCD diffractometer on rotating anode, no absorption correction. The structure was solved by automated Patterson methods.[15] The 3-MeAde ligand is disordered over two positions (refined ratio 0.754(7) : 0.246). Equality restraints were applied to corresponding bond lengths and angles of the two disorder components. Hydrogens were placed at calculated positions riding on their carrier atoms. Based on NMR-data (see discussion), a hydrogen was included on the imine moiety of the 3-MeAde ligand. Its position was derived from that of a neighbouring H-bond acceptor. Ordered non-hydrogen atoms were refined with anisotropic displacement parameters; disordered atoms were refined with isotropic displacement parameters, hydrogen atoms with fixed isotropic displacement parameters were related to the displacement parameters of their carrier atoms. Disordered solvent in solvent accessible voids was accounted for using the PLATON/SQUEEZE algorithm. Refinement[16] on F^2 of 405 parameters converged at a final $wR2$ value of 0.212, $R1$ = 0.092 (for 2582 reflections with $F_o > 4\sigma(F_o)$), S = 1.02, $-0.93 < \Delta\rho < 0.72$ e Å^{-3}. CCDC reference number 184379. See http://www.rsc.org/suppdata/dt/b2/b204665m/ for crystallographic data in CIF or other electronic format.

1 M. J. Clarke, F. Zhu and D. R. Frasca, *Chem. Rev.*, 1999, **99**, 2511.
2 R. A. Krause and K. Krause, *Inorg. Chem.*, 1980, **19**, 2600.
3 A. H. Velders, H. Kooijman, A. L. Spek, J. G. Haasnoot, D. de Vos and J. Reedijk, *Inorg. Chem.*, 2000, **39**, 2966.
4 B. Lippert, *Cisplatin. Chemistry and Biochemistry of a Leading Anticancer Drug*, Verlag Helvetica Chimica Acta and Wiley-VCH, Weinheim, 1999.
5 A. C. G. Hotze, A. H. Velders, F. Ugozzoli, M. Biagini-Cingi, A. M. Manotti-Lanfredi, J. G. Haasnoot and J. Reedijk, *Inorg. Chem.*, 2000, **39**, 3838.
6 A. C. G. Hotze, M. E. T. Broekhuisen, K. v. d. Schilden, A. H. Velders, J. G. Haasnoot and J. Reedijk, *Eur. J. Inorg. Chem.*, 2002, 369.
7 J. D. Orbell, D. Solorzano, L. G. Marzilli and T. J. Kistenmacher, *Inorg. Chem.*, 1982, **21**, 2630.
8 C. Bartzsch, C. Weiss and H. J. Hofmann, *J. Prakt. Chem.*, 1984, **326**, 407.
9 (*a*) T. Sorrell, L. A. Epps, T. J. Kistenmacher and L. G. Marzilli, *J. Am. Chem. Soc.*, 1978, **100**, 5756; (*b*) P. J. Toscano, C. C. Chiang, T. J. Kistenmacher and L. G. Marzilli, *Inorg. Chem.*, 1981, **20**, 1513.
10 W. S. Sheldrick and B. Gunther, *J. Organomet. Chem.*, 1991, **402**, 265.
11 (*a*) S. Korn and W. S. Sheldrick, *Inorg. Chim. Acta*, 1997, **254**, 85; (*b*) S. Korn and W. S. Sheldrick, *J. Chem. Soc., Dalton Trans.*, 1997, 2191.
12 F. H. Allen and O. Kennard, *Chem. Des. Autom. News*, 1993, **8**, 31.
13 B. Lippert, H. Schollhorn and U. Thewalt, *Inorg. Chim. Acta*, 1992, **200**, 723.
14 A. H. Velders, B. van der Geest, H. Kooijman, A. L. Spek, J. G. Haasnoot and J. Reedijk, *Eur. J. Inorg. Chem.*, 2001, 369.
15 P. T. Beurskens, G. Admiraal, G. Beurskens, W. P. Bosman, S. Garcia-Granda, R. O. Gould, J. M. M. Smits and C. Smylkalla, The DIRDIF99 program system, Technical report of the Crystallography Laboratory, University of Nijmegen, The Netherlands, 1999.
16 G. M. Sheldrick, SHELXL-97 Program for crystal structure refinement, University of Göttingen, Germany, 1997.