Ruthenium(III) Coordination to the Exocyclic Nitrogen of 9-Methyladenine and Stabilisation of the Rare Imine Tautomer by Intramolecular Hydrogen Bonding

Aldrik H. Velders,[a] Bart van der Geest,[a] Huub Kooijman,[b] Anthony L. Spek,[b] Jaap G. Haasnoot,[a] and Jan Reedijk*[a]

Keywords: Ruthenium / Bioinorganic chemistry / N ligands / Crystal structure / Hydrogen bonds

The X-ray structure of *trans*-[$RuCl_3(dmtp)_2$(9-MeAde)] shows that this compound is the first crystallographic example of a ruthenium(III) complex with an adenine derivative coordinated as a monodentate ligand through the exocyclic *N*6 nitrogen; two intramolecular hydrogen bonds stabilise the coordination of the adenine, which is present as a neutral ligand in the rare tautomeric imine form with a unique short C6–N6 distance of 1.293(3) Å.

Introduction

In the search for new antitumour-active metal compounds, several chlororuthenium complexes have proven to be promising candidates for clinical studies.[1] In particular, complexes of the type *trans*-$[RuCl_4(L)_2]^-$, with L a heterocyclic ligand like imidazole or indazole, have shown good activity against platinum-resistant tumours.[2,3] The hydrolysis properties[4] of these complexes, and their binding to biologically available ligands present in proteins[5] and nucleic acids,[6] appear to be important factors in the study of a structure-activity relationship. The anionic tetrachlororuthenium(III) complexes are to be considered as prodrugs that hydrolyse rapidly in vivo to form relatively stable neutral *trans*-$[RuCl_3(H_2O)(L)_2]$ complexes.[7] By using the bicyclic nitrogen heterocycle 5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidine, dmtp, we have directly synthesised the first hydrolysis product of such antitumour complexes, *trans*-$[RuCl_3(H_2O)(dmtp)_2]\cdot H_2O$, in which intramolecular hydrogen-bonding interactions of the two dmtp ligands stabilise the coordinated water molecule.[8] This monoaquabis(dmtp)ruthenium(III) complex appears to be a very useful compound for the investigation of the binding properties of this type of ruthenium(III) antitumour complex with biological molecules, such as the heterocyclic nitrogen ligands present in nucleic acids (purines and pyrimidines). In this paper the synthesis and characterisation of the reaction product of *trans*-$[RuCl_3(H_2O)(dmtp)_2]\cdot H_2O$ with the DNA model base 9-methyladenine, 9-MeAde (Figure 1), *trans*-$[RuCl_3(dmtp)_2$(9-MeAde)] (**1**) is presented. The adenine is present in the rare imine tautomer and coordinates to the ruthenium through the exocyclic N6 nitrogen.

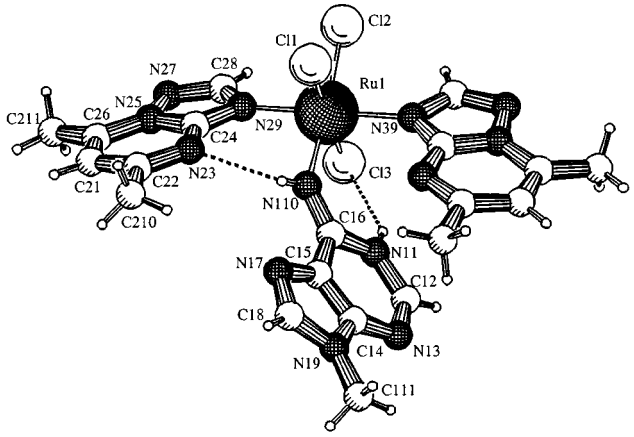
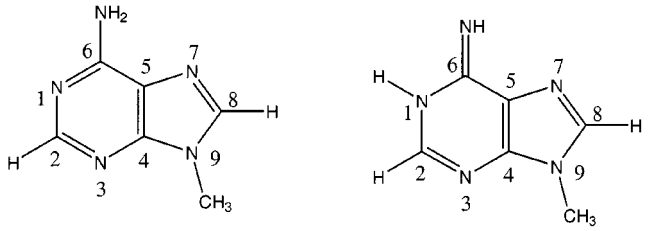


Figure 1. Molecular structure of **1**; intramolecular hydrogen bonding is indicated with dotted lines; the co-crystallised solvent molecule is omitted for clarity; selected metal-ligand bond lengths (Å) and angles (°): Ru(1)−Cl(1) 2.3329(8), Ru(1)−Cl(2) 2.3852(8), Ru(1)−Cl(3), 2.3772(8), Ru(1)−N(29) 2.083(2), Ru(1)−N(39) 2.090(2), Ru(1)−N(110) 2.035(2); Cl(1)−Ru(1)−Cl(3) 178.64(3), N(29)−Ru(1)−N(39) 178.00(8), Cl(2)−Ru(1)−N(110) 175.82(7); donor···acceptor (D···A) distances (Å) and donor−hydrogen···acceptor (D−H···A) angles (°) of the intramolecular hydrogen bonds: N(11)−H···Cl(3) 3.22(9) and 144(3); N(11)−H···N(23) 2.936(3) and 140(3).

Coordination of metal ions to the exocyclic N6 of adenine is difficult, but can occur after deprotonation of the amine.[9] Crystallographic evidence for coordination to the exocyclic nitrogen of deprotonated adenine derivatives has been observed with molybdenum complexes,[10] in a dinuclear platinum complex,[11] and in trinuclear rhodium[12] and ruthenium(II) arene complexes.[13] Crystallographic reports of exocyclic coordination of adenine derivatives in the imine form are even less well-known and have been reported only for a mercury compound,[14] a dimolybdenum complex,[15] and a platinum adduct.[16,17] In DNA, adenine is mainly present in the amine form (Scheme 1) with the N6 and N1 nitrogens acting as hydrogen donor and acceptor atoms, respectively, involved in base pairing with thymine. The imine form of adenine can result in nucleobase mispairing and therefore it is of great biological importance to get a de-

[a] Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University,
P. O. Box 9502, 2300 RA Leiden, The Netherlands
Fax: (internat.) +31-71/527-4671
E-mail: reedijk@chem.leidenuniv.nl
[b] Bijvoet Center for Biomolecular Research, Crystal and Structural Chemistry, Utrecht University,
Padualaan 8, 3584 CH, Utrecht, The Netherlands

  1434−1948/01/0202−0369 $ 17.50+.50/0



Scheme 1. Tautomeric structures of 9-MeAde: the amine form (left) and the imine form (right)

tailed understanding of the factors influencing the formation and properties of such metal complexes.[18−20]

The crystal structure of *trans*-[$RuCl_3(dmtp)_2$(9-MeAde)] (Figure 1) shows a neutral six-coordinated ruthenium(III) complex with three chloride ions in a *mer* configuration, the two dmtp ligands are coordinated through the triazole N9 nitrogens in a mutual *trans* head-to-head orientation. The Ru−N(9) and Ru−Cl bond distances and angles are comparable to those found in related complexes, such as, for example,[8] *trans*-[$RuCl_3(H_2O)(dmtp)_2$]·H_2O and[21] *trans*-[$RuCl_3(H_2O)(admtp)_2$]·H_2O which also display a quasi-octahedral stereochemistry with two *trans*-located triazolopyrimidine ligands. The 9-MeAde is coordinated to the ruthenium through the exocyclic N6 atom, completing the coordination sphere. In the difference density map a hydrogen atom is clearly visible at the N1 site, indicating that the 9-MeAde is neutral and present in the imine form. Also, the short C6−N6 distance of 1.293(3) Å is typical for the imine form of adenine. The average C−N distance for amine groups coordinated to transition metals is 1.39 Å (596 entries in the Cambridge Structural Database, version April 1999).

The N3 site of triazolopyrimidine ligands is known to form intramolecular hydrogen bonds easily with other coordinated ligands like water molecules or amines.[8,21,22] Also, in **1** the N3 atom of one of the two dmtp ligands is involved in an intramolecular hydrogen bond, in this case with the imine proton of the 9-MeAde. The tautomeric imine form of 9-MeAde in **1** is furthermore stabilised by an intramolecular hydrogen bond between the proton on the endocyclic nitrogen N1 and one of the *cis*-coordinated chloride ions. Both hydrogen bonds constrain the 9-MeAde to orient in a *syn* orientation with respect to the N(1) site.

The crystal structure of [$Ru(NH_3)_5$(1-$MeCyt^-$)]$(PF_6)_2$, where 1-$MeCyt^-$ stands for the anion of the pyrimidine 1-methylcytosine,[23] was the first crystallographic report of a DNA base coordinating through the exocyclic nitrogen to a ruthenium(III) complex. More recently, a trinuclear ruthenium-arene complex[13] has been reported in which a deprotonated adenine coordinates contemporaneously through the N1, N7 and N6 nitrogens. The present 9-MeAde complex **1** is the first crystallographic evidence of a purine coordinated to a ruthenium ion though its exocyclic amine as a monodentate ligand in the rare tautomeric imine form. The Ru−N(110) bond length of 2.035(2) Å is significantly shorter than the Ru−N(29) and Ru−N(39) distances [2.083(2) and 2.090(2) Å, respectively] and suggests a considerable π-bonding between the ruthenium and the 9-MeAde ligand.

Ruthenium complexes are known to coordinate preferentially to the N7 nitrogens of oxopurines;[24−27] the N7 is, however, not necessarily the most likely binding site of aminopurines. In fact, in the late seventies it was suggested on the basis of DNA binding studies with single- and double-stranded DNA that ruthenium(III) ammine complexes do not preferentially bind to the N7 of adenine, but possibly coordinate to adenine through the exocyclic nitrogen N6.[28] The absorption spectrum of **1** in the near-UV and visible part of the electromagnetic spectrum shows two main absorption bands at 360 and 500 nm, which have also been observed in several ruthenium(III) ammine complexes with adenine-like ligands.[29,30] The present study unambiguously confirms these absorptions to be characteristic for a ruthenium(III) ion coordinated to the exocyclic, imine group of a nucleobase.

In the solid-state structure of **1**, the two dmtp ligands are not equivalent, but it is likely that in solution all three heterocyclic ligands are to some extent flexible and can rotate around their Ru−N axes. This, in fact, can be concluded from the ^1H NMR spectroscopic data of **1**, in which the two dmtp ligands are indistinguishable. The ^1H NMR spectrum of **1** (Figure 2) shows largely shifted and broadened resonances which is caused by the presence of the paramagnetic ruthenium(III) centre. On the basis of the relative intensities of the peaks, and by comparison with related complexes, the dmtp[8,21,31] and adenine[32,33] proton resonances have been assigned. The dipolar contribution to the isotropic shifts in a ruthenium(III) adenine-κ^{N6} complex are relatively small and the shifts of the H2 and H8 protons can be attributed mainly to the contact contributions;[33] the large shifts of the 9-MeAde ring protons are therefore consistent with a strong π-overlap.

In conclusion, the structure of complex **1** indicates that the use of the triazolopyrimidine dmtp offers the possibility to stabilise, isolate and study the interaction of potential ruthenium antitumour complexes with biological targets like the DNA bases. The intramolecular hydrogen bonds between the dmtp and the 9-MeAde in **1** are not likely to be a prerequisite for N6 coordination, but do play an important role in facilitating the isolation and characterisation of this mode of coordination. The hydrogen-bond interac-

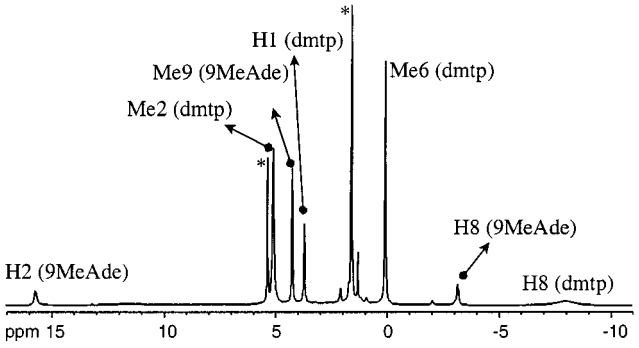


Figure 2. ^1H NMR spectrum of **1** in CD_2Cl_2; the proton numbering is as given in Figure 1; the asterisks indicate residual solvent signals

tion of the N1(H) with a coordinated chloride, on the other hand, is likely to be of influence on the coordination to the adenine N6, also in other (antitumour-active) chlororuthenium complexes. Such a stabilisation of the imine form through (hydrogen) bonding with the N(1) and N(6) of the adenine would inhibit the formation of a normal base pair with thymine; instead, mispairing with guanine or adenine bases might be favoured which eventually can lead to mutations.[18,34,35] The interaction of *trans*-$[RuCl_3(H_2O)(dmtp)_2]\cdot H_2O$ with other nucleobases, as well as the subsequent reactions of the formed adducts, are current subjects of investigation.

Experimental Section

General Remarks: NMR: Data were obtained at 300.13 MHz on a Bruker DPX 300 MHz spectrometer (for CD_2Cl_2 as solvent, δ_H = 5.33 at 295 K). The ^1H NMR signals of **1** could be identified as the paramagnetically shifted and broadened signals with relatively fast relaxation times (on the order of milliseconds). The magnetic moment of **1** was determined on a Johnson Matthey Alfa Product Magnetic Susceptability Balance MK I. Elemental analyses were performed by the Microanalytical Laboratory, University College, Dublin (Ireland). UV/Vis spectra were obtained on a Perkin−Elmer Lambda 900 spectrophotometer. The ligand 5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidine (dmtp) and *trans*-$[RuCl_3(H_2O)(dmtp)_2]\cdot H_2O$ were prepared as described previously.[8] 9-Methyladenine was prepared by methylation of adenine with methyl iodide.[36]

***trans*-[$RuCl_3$(9-MeAde-*N*6)$(dmtp)_2$] (1):** A mixture of *trans*-$[RuCl_3(H_2O)(dmtp)_2]\cdot H_2O$ (0.10 g, 0.2 mmol) and 9-MeAde (0.10 g, 0.7 mmol) was heated in 20 mL ethanol (98%) at 75 °C, without stirring. After 15 minutes, the colour of the initially yellowish suspension turned to dark red and after 30 minutes a red microcrystalline powder precipitated, which was isolated by filtration and washed with ethanol and diethyl ether. Yield, 60 mg (50%). The compound was dissolved in a dichloromethane/ethanol mixture (3:1) and red crystals were obtained by diethyl ether diffusion into this solution. The crystals obtained in this way were not stable in air for long periods of time, most likely because of evaporation of co-crystallised solvent molecules; the X-ray data analyses were done on crystals freshly taken out of the mother liquor. $C_{20}H_{23}Cl_3N_{13}$ (652): calcd. C 36.8, H 3.58, Cl 16.3, N 27.9; found C 35.5, H 3.52, Cl 16.5, N 26.4. − The effective magnetic moment, μ_{eff}, of the complex was determined to be 1.9 Bohr magneton at 20 °C, which is in the range of that expected for a single-electron system like a low-spin ruthenium(III) ion.[37] $-^1H$ NMR ([CD_2Cl_2, at 25 °C): δ = −8.0 [2 H, H8 (dmtp)], −3.1 [1 H, H8 (9-MeAde)], 0.1 [6 H, Me6 (dmtp)], 3.7 [2 H, H1 (dmtp)], 4.2 [3 H, Me9 (9-MeAde)], 5.1 [6 H, Me2 (dmtp)], 15.7 [1 H, H2 (9-MeAde)]. − UV/Vis (CH_2Cl_2): λ = 362 nm (ε = $7.12\cdot10^3$ м$^{-1}$ cm^{-1}), 500 (ε = $2.48\cdot10^3$ м$^{-1}$ cm^{-1}).

X-ray Crystallographic Study: $C_{20}H_{23}Cl_3N_{13}Ru\cdot1/2CH_2Cl_2$, M_r = 695.39, red, plate-shaped crystal (0.02 × 0.20 × 0.40 mm), triclinic, space group *P*1(bar) with *a* = 9.7336(12), *b* = 11.2417(15), *c* = 13.924(2) Å, α = 80.922(7), β = 74.505(8) and γ = 75.410(8)°, *V* = 1414.2(3) Å^3, *Z* = 2, D_x = 1.633 g cm^{-3}, μ(Mo-K_α) = 0.96 mm^{-1}. All data, where relevant, are given with disordered solvent contribution. 15167 Reflections were measured (6389 independent, R_{int} = 0.0461, 1.6° < θ < 27.5°, T = 150 K, Mo-K_α radiation, graphite monochromator, λ = 0.71073 Å) on a Nonius KappaCCD diffractometer with rotating anode; no absorption correction was applied. The structure was solved by automated direct methods (SHELXS-86,[38]). Full-matrix least-squares refinement of 345 parameters on F^2 (SHELXL-97,[38]) resulted in a final *R*1 value of 0.0356 (*wR*2 = 0.0789, GoF = 1.021). H atoms were introduced at calculated positions and included riding on their carrier atoms except for the N−H hydrogens which were located on a difference Fourier map and whose positions were refined. A cavity (1/2,1/2,1/2) with a volume of 206 Å^3 per unit cell was filled with disordered solvent (most likely dichloromethane); the associated electron density was taken into account with the PLATON/SQUEEZE procedure.[39] A total of 42 e per unit cell was found (consistent with the presence of one CH_2Cl_2 molecule) and corrected for. Final residual density was in the range −0.81 to 0.50 e Å^{-3}.

Crystallographic data (excluding structure factors) for the structure(s) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-149915. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Acknowledgments

The authors thank Johnson & Matthey (UK) for their generous loan of $RuCl_3\cdot3H_2O$. This research is sponsored by the Council of Chemical Sciences of the Netherlands Organisation for Scientific Research (NWO), and also support by COST Action D8/0017/97 (Metals in medicine) is kindly acknowledged.

[1] M. J. Clarke, F. Zhu, D. R. Frasca, *Chem. Rev.* **1999**, *99*, 2511.
[2] B. K. Keppler, M. Henn, U. M. Juhl, M. R. Berger, R. Niebl, F. E. Wagner, *Progr. in Clin. Biochem.* **1989**, *10*, 41−69.
[3] B. K. Keppler, *New J. Chem.* **1990**, *14*, 389−403.
[4] O. M. N. Dhubhghaill, W. R. Hagen, B. K. Keppler, K. G. Lipponer, P. J. Sadler, J. Chem. Soc., *Dalton Trans.* **1994**, 3305−3310.
[5] F. Kratz, M. Hartmann, B. K. Keppler, L. Messori, *J. Biol. Chem.* **1994**, *269*, 2581−2588.
[6] M. Hartmann, T. J. Einhauser, B. K. Keppler, *Chem. Commun.* **1996**, 1741−1742.
[7] K.-G. Lipponer, E. Vogel, B. K. Keppler, *Metal-Based Drugs* **1996**, *3*, 243−259.
[8] A. H. Velders, L. Pazderski, F. Ugozzoli, M. Biagini-Cingi, A. M. Manotti-Lanfredi, J. G. Haasnoot, J. Reedijk, *Inorg. Chim. Acta* **1998**, *273*, 259−265.
[9] B. Lippert, *Progr. in Inorg. Chem.* **1989**, *37*, 1−97.
[10] L. Y. Kuo, M. G. Kanatzidis, M. Sabat, A. L. Tipton, T. J. Marks, *J. Am. Chem. Soc.* **1991**, *113*, 9027−9045.
[11] G. Trovo′, G. Bandoli, M. Nicolini, B. Longato, *Inorg. Chim. Acta* **1993**, *211*, 95−99.
[12] D. P. Smith, E. Baralt, B. Morales, M. M. Olmstead, M. F. Maestre, R. H. Fish, *J. Am. Chem. Soc.* **1992**, *114*, 10647−10649.
[13] S. Korn, W. S. Sheldrick, *J. Chem. Soc., Dalton Trans.* **1997**, 2191−2199.
[14] F. Zamora, M. Kunsman, M. Sabat, B. Lippert, *Inorg. Chem.* **1997**, *36*, 1583−1587.
[15] E. F. Day, C. A. Crawford, K. Folting, K. R. Dunbar, G. Christou, *J. Am. Chem. Soc.* **1994**, *116*, 9339−9340.
[16] J. Arpalahti, K. D. Klika, *Eur. J. Inorg. Chem.* **1999**, 1199−1201.
[17] J. Viljanen, K. D. Klika, R. Sillanpaa, J. Arpalahti, *Inorg. Chem.* **1999**, *38*, 4924−4925.
[18] B. Lippert, H. Schoellhorn, U. Thewalt, *Inorg. Chim. Acta* **1992**, *198−200*, 723−732.

[19] J. Sponer, J. E. Sponer, L. Gorb, J. Leszczynski, B. Lippert, *J. Phys. Chem. (A)* **1999**, *103*, 11406−11413.
[20] J. V. Burda, J. Sponer, J. Leszczynski, *J. Biol. Inorg. Chem.* **2000**, *5*, 178−188.
[21] A. H. Velders, F. Ugozzoli, M. Biagini-Cingi, A. M. Manotti-Lanfredi, J. G. Haasnoot, J. Reedijk, *Eur. J. Inorg. Chem.* **1999**, 213−215.
[22] J. M. Salas, M. A. Romero, M. P. Sanchez, M. Quiros, *Coord. Chem. Rev.* **1999**, *195*, 1119−1142.
[23] B. J. Graves, D. J. Hodgson, *J. Am. Chem. Soc.* **1979**, *101*, 5608−5612.
[24] V. M. RodriguezBailey, K. J. LaChanceGalang, P. E. Doan, M. J. Clarke, *Inorg. Chem.* **1997**, *36*, 1873−1883.
[25] K. J. LaChanceGalang, M. Zhao, M. J. Clarke, *Inorg. Chem.* **1996**, *35*, 6021−6026.
[26] M. J. Clarke, H. Taube, *J. Am. Chem. Soc.* **1974**, *96*, 5413−5419.
[27] M. E. Kastner, K. F. Coffey, M. J. Clarke, S. E. Edmonds, K. Eriks, *J. Am. Chem. Soc.* **1981**, *103*, 5747−5752.
[28] M. J. Clarke, M. Buchbinder, A. Kelman, *Inorg. Chim. Acta* **1978**, *27*, L87−L88.
[29] M. J. Clarke, *J. Am. Chem. Soc.* **1978**, *100*, 5068−5075.
[30] M. J. Clarke, *Inorg. Chem.* **1980**, *19*, 1103−1104.
[31] A. H. Velders, *Ph. D. thesis*, Leiden University, **2000**.
[32] M. J. Clarke, *Prog. Clin. Biochem. Med.* **1989**, *10*, 25−39.
[33] V. M. RodriguezBailey, M. J. Clarke, *Inorg. Chem.* **1997**, *36*, 1611−1618.
[34] O. Kennard, W. N. Hunter, *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1254−1277.
[35] M. D. Topal, J. R. Fresco, *Nature* **1976**, *263*, 285−289.
[36] E. G. Talman, W. Bruning, J. Reedijk, A. L. Spek, N. Veldman, *Inorg. Chem.* **1997**, *36*, 854−861.
[37] E. A. Seddon, K. R. Seddon, *The Chemistry of Ruthenium*, Elsevier, Amsterdam, The Netherlands **1984**.
[38] G. M. Sheldrick, SHELXL-97 Program for crystal structure determination. University of Göttingen, Germany, **1997**.
[39] A. L. Spek, PLATON A multi-purpose crystallographic tool. Utrecht University, The Netherlands, 2000. Internet: http://www.cryst.chem.uu.nl/platon/.

Received July 31, 2000
[I00302]