Unusual Coordination of the Rare Neutral Imine Tautomer of 9-Methyladenine Chelating in the *N*6,*N*7-Mode to Ruthenium(II) Complexes

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The coordination of the DNA model base 9-methyladenine (9-MeAde) to both complexes *cis*-[Ru(bpy)$_2$Cl$_2$] and α-[Ru(azpy)$_2$(NO$_3$)$_2$] is reported. Structural characterisation using 2D NMR techniques and variable-temperature NMR studies between 25 and –55 °C show that in the compounds α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$ (**1**) and *cis*-[Ru(bpy)$_2$(9-MeAde)](PF$_6$)$_2$ (**2**), 9-MeAde is coordinated to the ruthenium ion in a chelating mode via its N7 and exocyclic N6 atoms. The NMR spectroscopic data unambiguously prove the occurrence of the rare imine tautomer of 9-MeAde in these complexes, which is clearly stabilised by the bidentate coordination of 9-MeAde in both complexes. Variable-temperature NMR spectra and 2D COSY data show that the H2 resonance of 9-MeAde in **1** and **2** appears as a doublet at low temperatures and shows a COSY cross peak to the NH1 resonance of 9-MeAde, which confirms the protonation at the N1 site. This protonated N1 site, together with an observed pK_a of ca. 6.5 of compound **1** is in agreement with the presence of the imine tautomeric form of 9-MeAde. As the binding mode of 9-MeAde to both *cis*-[Ru(bpy)$_2$] and α-[Ru(azpy)$_2$] moieties is the same, this binding mode does not explain the earlier observed difference in cytotoxicity between *cis*-[Ru(bpy)$_2$Cl$_2$] and α-[Ru(azpy)$_2$Cl$_2$].

Introduction

Currently, several ruthenium complexes are under investigation for their antitumour properties, and amongst them are polypyridyl−ruthenium complexes.[1−3] The in vitro cytotoxicity of three isomeric bis(2-phenylazopyridine)ruthenium(II) complexes, [Ru(azpy)$_2$Cl$_2$], has recently been reported.[4] The so-called α isomer, α-[Ru(azpy)$_2$Cl$_2$] (α corresponding with the isomer in which the coordinating pairs Cl, N(py), and N(azo) are *cis*, *trans*, and *cis*, respectively), shows remarkably high cytotoxicity against a series of tumour-cell lines.[4] This high cytotoxicity is in contrast to the low cytotoxicity of its isomeric complexes[4] β-[Ru(azpy)$_2$Cl$_2$] and γ-[Ru(azpy)$_2$Cl$_2$] and the structurally related complex[5] *cis*-[Ru(bpy)$_2$Cl$_2$] (where bpy is 2,2′-bipyridine). Therefore, in the search for a structure-activity relationship of antitumour-active ruthenium complexes, the investigation of complexes of the type *cis*-[Ru(LL)$_2$Cl$_2$] (with LL being a heterocyclic bidentate ligand like azpy or bpy) is very useful. Since it is generally accepted that DNA might be the target for antitumour-active ruthenium complexes,[1−3] as in the case for antitumour-active platinum complexes,[6−8] the interaction of the [Ru(azpy)$_2$Cl$_2$] complexes with DNA model bases has been studied.[3,9] The α-[Ru(azpy)$_2$Cl$_2$] compound is poorly soluble in aqueous solutions, and therefore the analogous water-soluble nitrate complex, α-[Ru(azpy)$_2$(NO$_3$)$_2$], has been synthesized and used for DNA model base binding studies.[9]

The reaction of the DNA model base 9-ethylguanine (9-EtGua) to α-[Ru(azpy)$_2$(NO$_3$)$_2$] and the structurally similar, but noncytotoxic *cis*-[Ru(bpy)$_2$Cl$_2$], results in monofunctional binding of 9-EtGua in both cases.[9,10] The 9-EtGua binding studies and rotational aspects of smaller model bases, like 1-methylbenzimidazole, show that the α-[Ru(azpy)$_2$] moiety[3] is more versatile in its coordination to heterocycles than the *cis*-[Ru(bpy)$_2$] moiety.[11,12] To obtain a better understanding of all factors influencing the biological properties of α-[Ru(azpy)$_2$Cl$_2$] and *cis*-[Ru(bpy)$_2$Cl$_2$] (Figure 1), the binding of other DNA model bases and oligonucleotides is under investigation. The interaction with the DNA model base 9-methyladenine is presented in this paper. Of all DNA nucleobases, the adenine base exhibits the most flexible binding-site behaviour with metal ions.[13] In 9-substituted adenine derivatives the ring nitrogen atoms N1 and N7 are the predominant binding sites.[13] In certain cases metal ions may also bind to the exocyclic amino group after deprotonation of the exocyclic N6 nitrogen atom.[14−18]

Bidentate coordination of adenine derivatives is shown in dinuclear complexes in which the adenine derivative acts as

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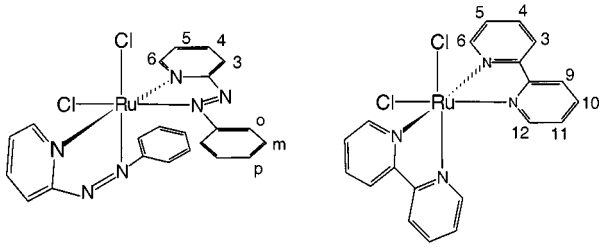

Figure 1. Comparison of the schematic structures of the Λ enantiomers of α-[Ru(azpy)$_2$Cl$_2$] (left) and *cis*-[Ru(bpy)$_2$Cl$_2$] (right)

a bridging ligand between the two metal sites. This bridging coordination occurs via the N6 and N7 atoms of the 9-ethyladenine (9-EtAde), like in the dinuclear Mo structure[19] [Mo$_2$(O$_2$CCHF$_2$)$_2$(9-EtAde)$_2$(MeCN)$_2$](BF$_4$)$_2$·2MeCN and a related dinuclear Rh structure.[20,21] In the tetrameric carbonylrhodium(I) complex [(CO)$_2$Rh(μ-3-MeAde$_{-H}$)]$_4$ the bridging *N*6,*N*7 coordination of deprotonated 3-methyladenine (3-MeAde) is also shown.[22] In a dirhenium carboxylate complex the bridging coordination of adenine occurs via its N1 and N6 atoms.[23] Bidentate coordination in which the N6 and N7 atoms of adenine (Ade) derivatives are coordinated to the same metal site have been reported for the tetrameric (cymene)Ru^{II} complex[24] [{Ru(Ade$_{-H}$)-(η^6-*p*-cymene)}$_4$](CF$_3$SO$_3$)$_4$, the trinuclear complex [{Ru(9-EtAde$_{-H}$)(η^6-*p*-cymene)}$_3$](CF$_3$SO$_3$)$_3$, and in related complexes[25] with adenosine bases. Also, in the case of other metal complexes like Mo, Ir, and Rh, an *N*6,*N*7 coordination of adenine derivatives to one metal site has been observed.[26−28]

In DNA, adenine is mainly present in its amine tautomeric form (Figure 2) in which form it can take part in Watson−Crick base pairing with thymine. It is estimated[29]

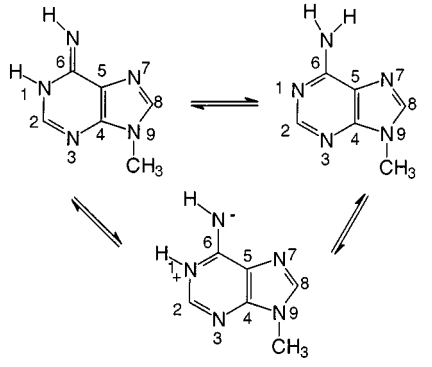

Figure 2. Tautomeric structures of 9-MeAde: the imine form (left) the amine form (right) and the zwitterionic form (bottom)

that the ratio of the amine to the imine tautomer (Figure2) in neutral adenine is approximately 10^4 to 10^5. This tautomeric equilibrium might be influenced by metal coordination.[29−31] To the best of our knowledge, only a few examples of metallated adenine derivatives are known in which it is explicitly proven by NMR spectroscopy and/or a crystal structure that the adenine derivative is in the neutral imine form.[14,17−21] The imine form of adenine in DNA might result in nucleobase mispairing, which eventually results in mutations if not repaired.[29,30] Regarding the potential antitumour activity of ruthenium complexes, it is of biological relevance to obtain a better insight in their binding to adenine derivatives. In this paper the binding of 9-methyladenine (9-MeAde) to α-[Ru(azpy)$_2$(NO$_3$)$_2$] and *cis*-[Ru(bpy)$_2$Cl$_2$] is presented. NMR spectroscopic data show that in these adducts, 9-MeAde is coordinated in its rare imine form, likely stabilised by the *N*6,*N*7 coordination.

Results and Discussion

Synthesis and Characterisation

On dissolving α-[Ru(azpy)$_2$(NO$_3$)$_2$] in H$_2$O, hydrolysis of the nitrate ions occurs, resulting in α-[Ru(azpy)$_2$(H$_2$O)$_2$]$^{2+}$,[3,9] which consecutively coordinates to 9-MeAde. Also upon reaction of *cis*-[Ru(bpy)$_2$Cl$_2$] and 9-MeAde it is the aqua complex, *cis*-[Ru(bpy)$_2$(H$_2$O)$_2$]$^{2+}$, that coordinates to 9-MeAde. The reaction of α-[Ru-(azpy)$_2$(NO$_3$)$_2$] with 9-methyladenine (9-MeAde) was carried out at 40 °C because we have observed[9] that isomerisation from the α to the β isomer in aqueous solution does not occur at or below 40 °C. Both in α-[Ru(azpy)$_2$(NO$_3$)$_2$] and in *cis*-[Ru(bpy)$_2$Cl$_2$], two coordination sites, which are identical due to the presence of a C_2 axis in the complexes, are in theory available for coordination to DNA model bases after hydrolysis of the anions. However, NMR spectroscopy clearly proves the coordination of only one 9-MeAde ligand to the α-[Ru(azpy)$_2$] and *cis*-[Ru(bpy)$_2$] moieties. Also, when the reaction of α-[Ru(azpy)$_2$(NO$_3$)$_2$] or *cis*-[Ru(bpy)$_2$Cl$_2$] with 9-MeAde was repeated in a ratio 1:2, no bifunctional adducts of the type α-[Ru(azpy)$_2$(9-MeAde)$_2$](PF$_6$)$_2$ or *cis*-[Ru(bpy)$_2$(9-MeAde)$_2$](PF$_6$)$_2$ were obtained. The starting materials α-[Ru(azpy)$_2$(NO$_3$)$_2$] and *cis*-[Ru(bpy)$_2$Cl$_2$] were racemic mixtures containing equal amounts of Λ and Δ isomers, but as 9-MeAde is not chiral, no diastereoisomers were formed and consequently only one set of signals was observed. Nucleosides do have a chiral centre and on reaction of α-[Ru(azpy)$_2$(NO$_3$)$_2$] with guanosine[9] and adenosine[32] two different sets of signals were observed for the diastereoisomeric complexes. The binding pattern of adenine derivatives to metal complexes

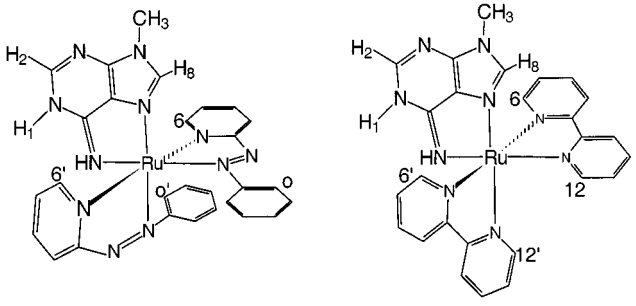

Figure 3. *N*6,*N*7-Bidentate coordination of 9-MeAde in the Λ enantiomers of α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$ (left) and *cis*-[Ru(bpy)$_2$(9-MeAde)](PF$_6$)$_2$ (right) (with proton numbering used for the NMR assignments)

is known to be very complex (vide supra), and the bidentate coordination of 9-MeAde to the α-[Ru(azpy)$_2$] and *cis*-[Ru(bpy)$_2$] moieties was not for certain beforehand. Moreover, NMR characterisation of bidentate coordination and confirmation of the imine tautomer of adenine derivatives in metal complexes is not very clear from literature, and especially the assignment of adenine resonances is sometimes confusing.[20,21] Therefore, in the presented work, a detailed NMR study was performed in order to investigate the binding mode of 9-MeAde in the bifunctional ruthenium(II) complexes.

Mass spectrometry data of α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$ show two molecule-ion peaks; apparently a part of the sample is protonated and a part of the sample is deprotonated, i.e. *m*/*z* = 616 {α-[Ru(azpy)$_2$(9-MeAde)]$^{2+}$ − H$^+$}$^+$ and *m*/*z* = 308 {α-[Ru(azpy)$_2$(9-MeAde)]$^{2+}$}. The same observation was made in *cis*-[Ru(bpy)$_2$(9-MeAde)](PF$_6$)$_2$, i.e. two signals appear in the mass spectra, belonging to *cis*-[Ru(bpy)$_2$(9-MeAde)]$^{2+}$ and {*cis*-[Ru(bpy)$_2$(9-MeAde)]$^{2+}$ − H$^+$}$^+$. The elemental analysis of *cis*-[Ru(bpy)$_2$(9-MeAde)](PF$_6$)$_2$ shows the presence of co-crystallised water and acetone. The pseudo-crystalline material did not allow single-crystal X-ray analysis.

NMR Structural Characterisation of α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$

The ^{1}H NMR spectrum of the reaction product of α-[Ru(azpy)$_2$(NO$_3$)$_2$] with 9-methyladenine (9-MeAde), i.e. α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$ (**1**), in [D$_6$]acetone at 25 °C (see Figure 4, Table 1) shows two sets of azpy signals, which are the first indication of the formation of a 1:1 adduct. The α-[Ru(azpy)$_2$] backbone is C_2-symmetric, so both coordination sites are equivalent. The coordination of a DNA model base to one of the two coordination sites results in a compound that no longer has a C_2 axis, resulting in non-equivalent azpy ligands. The integration of the signals of 9-MeAde relative to the azpy signals confirms the monofunctional coordination of 9-MeAde. The assignment of all signals was made from 2D NOESY and COSY NMR spectroscopic data at 25 °C and variable-temperature ^{1}H NMR measurements (vide infra). Characteristic signals of coordinated 9-MeAde are H8 at δ = 8.51, H2 at δ = 8.18, NH1 as a broad signal at δ = 12, and the NH6 signal overlapping with the *meta* signals at δ ≈ 7.4. These NMR spectroscopic data do not answer the question whether 9-MeAde is present in its neutral imine form or protonated amine form, since four resonances of 9-MeAde would be expected in both cases. This question will be addressed in the next section.

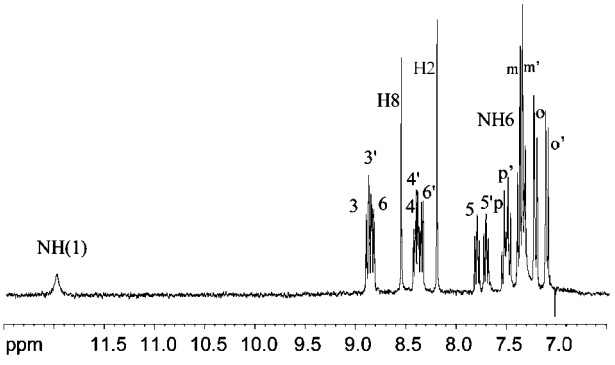

Figure 4. Low-field region of the ^{1}H NMR spectrum of α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$ in [D$_6$]acetone at 25 °C

No isomerisation of the α-[Ru(azpy)$_2$] moiety had taken place, since the interligand NOE cross-peak between the pyridine H6 protons and the phenyl ring *ortho* signals, H6-*o*′ and H6′-*o*, indicate presence of the α isomer.[3,9] The H8 resonance of the coordinated 9-MeAde was determined by its intraligand NOE cross peak with the CH$_3$ signal. The H8 signal of the coordinated 9-MeAde has an NOE cross peak to the *ortho* signal of one of the azpy ligands. This confirmed the *N*7 coordination of 9-MeAde and indicated the orientation of 9-MeAde in the complex with the H8 situated above the aza bond of the azpy ligand. In comparison to the resonance of H6, the signal of H6′ is shifted upfield by almost 0.5 ppm. This is easily explained by the orientation of 9-MeAde, which causes the H6′ of the azpy ligand to be shielded by the five-membered ring of 9-MeAde.

The experimental conditions used for the reaction of α-[Ru(azpy)$_2$(NO$_3$)$_2$] and 9-MeAde were the same as for the analogous reaction with 9-EtGua previously reported,[9] resulting in α-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$ with a coordinated water molecule in the final adduct. Therefore, a coordinated water molecule was to be expected in the complex with 9-MeAde; however, the ^{1}H NMR spectrum shows no resonance to be assigned to this coordinated water ligand. This observation, in fact, is the first indication of a bidentate coordination of 9-MeAde to the α-[Ru(azpy)$_2$] moiety. Even though in theory, the absence of the proton

Table 1. Proton chemical shift values [ppm] for 9-methyladenine (9-MeAde) and α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$ (**1**) in D$_2$O (a) and [D$_6$]acetone (b) at 25 °C

	6/6′	3/3′	4/4′	5/5′	*p*/*p*′	*m*/*m*′	*o*/*o*′	H8	H2	NH(1)	NH(6)
9-MeAde (a)								8.20	7.93		
9-MeAde (b)								7.81	8.20		7.2
1 (a)	8.51	8.75	8.26/	7.59	7.51	7.33	7.06	8.07	8.02		
pH < 5	7.95	8.75	8.26	7.59	7.51	7.33	7.22				
1 (b)	8.83	8.91	8.38	7.80	7.50	7.39	7.24	8.53	8.20	11.9	7.4
	8.35	8.91	8.41	7.71	7.48	7.38	7.12				

resonance of coordinated water could also be caused by rapid exchange with residual water present in [D_6]acetone. To exclude the possibility of water exchange by traces of H_2O in [D_6]acetone (98%), [D_6]acetone (100%) was used, and again no proton resonance of a coordinated water molecule could be assigned.

The ^{1}H NMR spectrum of **1** in D_2O at 25 °C and pH ≈ 5, shows that the H8 resonance (δ = 8.10) is shifted downfield in comparison to the free ligand (δ = 7.93), whereas the H2 resonance is shifted upfield (from δ = 8.20 for the free ligand to δ = 8.03 in the complex). In [D_6]acetone the H8 (δ = 8.52) resonance of 9-MeAde in the complex is shifted significantly downfield corresponding to the free base (δ = 7.81) (Table 1). This downfield shift is most probably due to the deshielding effect of the phenyl ring of the azpy ligand. The phenyl ring rotates fast on the NMR time scale around the CN axis, but is likely to spend most of the time in an orientation with the phenyl ring not in-plane with the chelate, but more perpendicular to it. This would imply the H(*o*) atoms to be on average close to the H8, which is confirmed by the NOE cross peak between these two signals.

Variable-Temperature NMR Experiments of α-[Ru(azpy)$_2$-(9-MeAde)](PF$_6$)$_2$

The above-mentioned NMR spectroscopic data of **1** at room temperature show four aromatic 9-MeAde signals that suggest the 9-MeAde to be present in the imine tautomer. Therefore, the possible imine tautomer and coordination mode of 9-MeAde in the complex was further investigated by using variable-temperature NMR, as it is known that low-temperature measurements can shift signals apart and slow down rotational or exchange properties. In fact, upon lowering the temperature of **1** in [D_6]acetone from

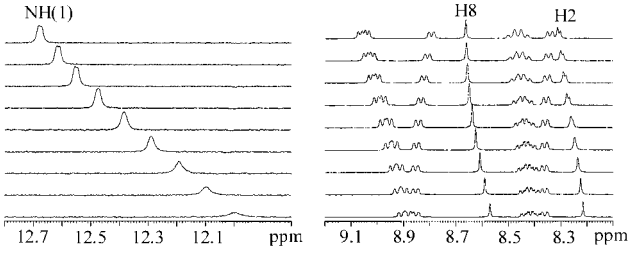

Figure 5. Variable-temperature ^{1}H NMR spectra of parts of the aromatic region of α-[Ru(azpy)$_2$(9MeAde)](PF$_6$)$_2$ in [D_6]acetone from 25 °C (bottom spectrum) to −55 °C (top spectrum); the shifting and sharpening of the NH1 signal (left) and the doubling of the H2 signal (right), prove the protonated N1 site

room temperature (25 °C) to −55 °C (Figure 5), the low-field broad signal (δ = 11.9) sharpened and shifted downfield (δ = 12.7). More importantly, the H2 signal of 9-MeAde also shifted downfield and became a doublet at −25 °C (see Figure 5). At temperatures below −55 °C, the H2 signal overlaps with the H6′ signal (not shown in Figure 5). 2D COSY experiments at −50 °C provide a cross peak between the singlet at δ = 12.8 and the doublet at δ = 8.3 (see Figure 6). This cross peak unambiguously confirms the signal at δ = 12.8 to be the NH1 (which means that this site is protonated) and the signal at δ = 8.3 to be the H2 proton. Although the NH1 resonance is not as clearly split into a doublet as the H2 resonance, probably due to line-broadening as the result of exchange with bulk water present in [D_6]acetone, the ^3J coupling of NH1 (which is hardly seen) is the same as the H2 resonance, i.e. 2.9 Hz. The exchange of the NH1 with bulk water is confirmed by the EXSY cross peak between residual water present in [D_6]acetone and the NH1 signal. In the 2D ROESY spectrum a weaker cross peak between the bulk water resonance and H2 was also observed, which is attributed to an exchange-COSY signal. The fact that the ^3J splitting of the H2 resonance is only observed at low temperatures is due to slower exchange of the NH1 proton with bulk water.

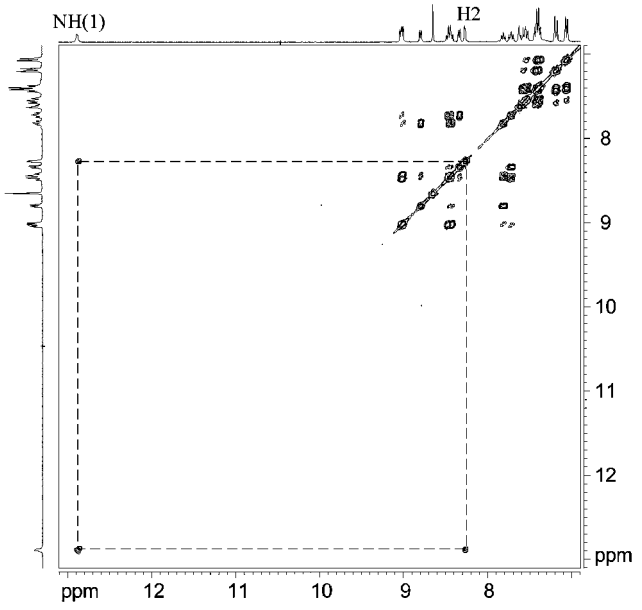

Figure 6. Low-field region of the COSY spectrum of α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$ in [D_6]acetone at −50 °C; the cross peak between the 9-MeAde NH(1) and H2 resonances is indicated with a dashed line

As the adduct **1** was isolated at pH ≈ 5, the protonation of N1 is not likely when 9-MeAde is in its amine form as the pK_a of an *N*7-metallated 9-MeAde is thought to be < 4, as in the case of Pt complexes.[33,34] Thus, in compound **1** the N1 protonation would only be possible if the 9-MeAde was present in its imine form. However, 9-MeAde being present in the zwitterionic form (Figure 2) could not a priori be excluded, as in that case the N1 and N6 atoms were also both protonated. For example, in the case of a (cymene)Ru^{II} complex [{Ru(Ade$_{-H}$)(η^6-*p*-cymene)}$_4$]-(CF$_3$SO$_3$)$_4$, Ade is probably present in the zwitterionic form, as shown by the relatively long N6−C6 distance in the crystal structure.[24] The combination of NMR spectroscopic and crystal structural data of the complex[19] [Mo$_2$(O$_2$CCHF$_2$)$_2$(9-EtAde)$_2$(MeCN)$_2$](BF$_4$)$_2$·2MeCN is in agreement with the results presented here. This crystal structure shows that the 9-EtAde molecules are bridging

ligands coordinated via the N7 and N6 nitrogen atoms and, importantly, are present in the neutral imine tautomer. The ^{1}H NMR spectrum of this Mo structure at −40 °C shows the NH1 signal at low field and the H2 resonance of 9-EtAde as a doublet, and so these resonances have a similar appearance as the 9-MeAde resonances of the present sample **1** at −55 °C. This confirms the presence of the imine form of 9-MeAde in sample **1**. In DNA, adenine in the imine form is very exceptional, but metal coordination might influence the equilibrium.[29−31] The imine form of 9-MeAde in **1** must apparently be stabilised by the bidentate coordination via the N7 and exocyclic N6 atoms. The presence of 9-MeAde coordinated in a bidentate fashion in **1** was also confirmed by the absence of an NMR signal of a coordinated water (vide supra), and by the pH experiments showing only one protonation step (vide infra).

As at low temperatures, 9-MeAde resonances no longer overlap with azpy resonances. 2D NOESY experiments were also performed at −50 °C. For example, at −50 °C the NH6 singlet was clearly seen at δ = 7.6, whereas at room temperature this signal showed overlap with the *meta* signals at δ ≈ 7.4. 2D NOESY and ROESY NMR spectroscopic data (600 MHz, Figure 7) at −50 °C show NOE signals between the H8 and H_o resonances, and between the NH6 and H6 resonances, confirming the orientation of 9-MeAde caused by the chelating mode of 9-MeAde via its N7 and N6 nitrogen atoms, i.e. the H8 positioned above the aza bond and the NH6 close to the H6 of the azpy ligand. In the NOE level of the 2D ROESY spectrum NH1 shows an NOE cross peak to NH6, which confirms the imine form. The NH1−NH6 distance (2.25 Å) of 9-EtAde, which is also present in the neutral imine form in the structure $[Rh_2(DTolF)_2(9\text{-}EtAde)_2(CH_3CN)][BF_4]_2$ (DTolF = *N*,*N*′-*para*-ditolylformamidinate), shows that an NOE cross-peak between NH1 and NH6 in compound **1** is likely.[20,21] However, the assignment[20] of the NH1 and NH6 signals of $[Rh_2(DTolF)_2(9\text{-}EtAde)_2(CH_3CN)][BF_4]_2$ is confusing as it was done by selective decoupling. By this method it is impossible to discriminate between both signals. On the other hand, it is also stated[21] that the NH1 and H2 resonances of $[Rh_2(DTolF)_2(9\text{-}EtAde)_2(CH_3CN)][BF_4]_2$ were determined by selective decoupling. In this presented work we have shown that 2D COSY NMR is a powerful technique for assigning the NH1 and H2 resonances of 9-MeAde in the imine form, and by using 2D NOESY NMR, the coupling between the NH1 and NH6 resonances proves the assignment of the NH6 resonance of 9-MeAde.

Protonation and Deprotonation of α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$

A pH titration of **1** in the pH range from 1 to 14, followed by ^{1}H NMR spectroscopy, shows only one protonation step, indicating a pK_a of 6.5 for NH1 (see Supporting Information, S1). The fact that only one protonation step was observed confirms the bidentate coordination of 9-MeAde. In the case of α-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$, two protonation steps were observed;[9] one for N1 of 9-EtGua and one for coordinated

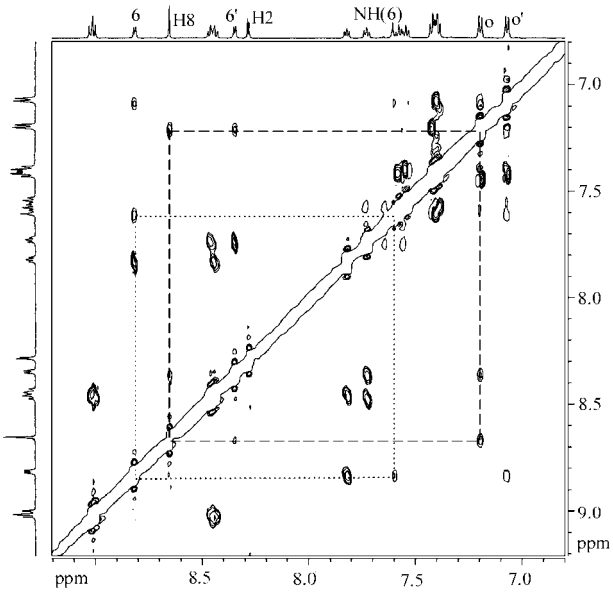

Figure 7. Aromatic region of the NOESY spectrum of α-[Ru(azpy)$_2$(9-MeAde)](PF$_6$)$_2$ in [D$_6$]acetone at −50 °C; the cross peaks between the 9-MeAde H8 and H_o resonances and the H6 and NH(6) signals are indicated with dashed and dotted lines, respectively

H_2O. The acidity of the free imine tautomer of 9-MeAde is estimated to have a pK_a for N1 of about 12.[15] In the case of adenine in the Hg complex [Hg(9-MeAde-*N*6)(1,3-dimeU-*C*5]NO_3·H_2O, in which 9-MeAde is present in its imine form and coordinated only via its exocyclic N6 nitrogen atom, the pK_a for deprotonation of NH1 is 4.5.[15] Therefore, in **1** the effect of ruthenium(II) is an increase in the acidity by about 5.5 log units in comparison to the acidity of the free imine tautomer of 9-MeAde; this shift seems to be reasonable in comparison to the Hg compound.[15]

The deprotonation behaviour of 9-MeAde of **1** in [D$_6$]-acetone was more complicated than in water. Depending on the pH at which the product of the reaction between α-[Ru(azpy)$_2$(NO$_3$)$_2$] and 9-MeAde was isolated, a mixture of **1** and the deprotonated form, α-[Ru(azpy)$_2$(9-MeAde$_{-H}$)](PF$_6$) (**1$_{-H}$**) could be obtained. In the ^{1}H NMR spectrum of either pure **1** or a mixture of **1** and **1$_{-H}$**, one set of signals was observed at room temperature. In the fully protonated sample, lowering the temperature of the sample only resulted in a shift of the signals and appearance of the NH1 and H2 resonances as doublets, as mentioned above and shown in Figure 5. However, in the case of a sample in which both **1** and **1$_{-H}$** were present, lowering the temperature resulted in ^{1}H NMR spectra showing signal broadening (from ca. 5 °C to −45 °C) and on further cooling, re-sharpening in two sets of signals at −75 °C (in a **1**/**1$_{-H}$** ratio of 1.5:1). This signal broadening and re-sharpening is likely due to **1** and **1$_{-H}$** being in fast exchange at the NMR time-scale at room temperature, intermediate exchange at temperatures from ca. 5 °C to −45 °C, and at slow exchange at −75 °C, resulting in the appearance of signals of both **1** and **1$_{-H}$**. After addition of some HNO_3

(1 M) to this sample of **1** and $\mathbf{1_{-H}}$, ^{1}H NMR spectroscopic data at −75 °C showed that the signals of $\mathbf{1_{-H}}$ were converted into one set of resonances (those of **1** only). Similarly, the addition of an excess of triethylamine in situ to **1** in [D_6]acetone at −75 °C resulted in one set of signals of α-[Ru(azpy)$_2$(9-MeAde$_{-H}$)](PF_6). In particular, the disappearance of the NH1 signal and the appearance of H2 as a singlet resonance instead of a doublet after addition of excess triethylamine in situ, indicated deprotonation of the NH1 site of 9-MeAde in the imine form. The significant upfield shift of the NH6 signal to δ = 5.7 can be seen as a characteristic after deprotonation of the NH1 site.

NMR Structural Characterisation of *cis*-[Ru(bpy)$_2$(9-MeAde)](PF_6)$_2$

The downfield region of the ^{1}H NMR spectrum of *cis*-[Ru(bpy)$_2$(9-MeAde)](PF_6)$_2$ (**2**) at 25 °C in [D_6]acetone (Table 2) shows two sets of bpy multiplet signals and the characteristic four singlet signals (H8, H2, NH1, and NH6) of the coordinated 9-MeAde ligand. The fact that the two bpy ligands were non-equivalent upon coordination of 9-MeAde, and the integration of the signals of 9-MeAde relative to the bpy signals, confirmed the presence of just one molecule of 9-MeAde. With the use of 2D NOESY and COSY experiments the bpy and 9-MeAde signals were assigned. The H8 resonance of 9-MeAde was assigned using the intraligand NOE coupling between the CH_3 and H8 signals. Interestingly, the H8 signal appears at higher field than the H2 resonance in contrast to the signals observed in the analogous ^{1}H NMR spectrum of **1** in which the H8 and H2 resonances appear in opposite order. The H8 signal at δ = 8.00 is shifted downfield with respect to the H8 of free 9-MeAde. The H2 resonance at δ = 8.11 is shifted upfield (δ = 0.3) relative to free 9-MeAde. Similar to the previously discussed NMR spectroscopic data of **1**, the absence of a coordinated water signal in the ^{1}H NMR spectrum of **2** suggests the bidentate coordination of 9-MeAde to the *cis*-[Ru(bpy)$_2$] moiety.

Variable-temperature ^{1}H NMR spectra show, similar to **1**, downfield shifting and sharpening of the NH1 signal of 9-MeAde and downfield shifting of the H2, H8, and NH6 signals of 9-MeAde upon decreasing the temperature. Remarkable again, is the splitting of the H2 signal into a doublet, most clearly seen at −50 °C (Figure 8). The NH1 and H2 signals were confirmed by a COSY cross peak between these two signals (see Supporting Information, Figure S2). A 2D ROESY NMR spectrum (600 MHz) (see Supporting Information, Figure S3) at −50 °C shows an NOE cross peak between NH1 and NH6 and together with the COSY cross peak NH1−H2, this confirms the imine tautomer of 9-MeAde. The H6 signal of bpy shows an NOE cross peak to NH6 and the H8 resonance shows an NOE cross peak to the H6′ signal. These NOE data are in agreement with the orientation of 9-MeAde caused by the bidentate coordination via its N7 and N6 nitrogen atoms. In summary, the 2D NMR spectroscopic data and variable-temperature ^{1}H NMR measurements indicate that in **2** 9-MeAde is in the neutral imine form and this tautomeric form is most likely stabilised by the chelating coordination via its N7 and N6 atoms. In this coordination mode the large difference in chemical shift of the H6 and H6′ resonances is explained because the H6′ atom is in the shielding cone of 9-MeAde.

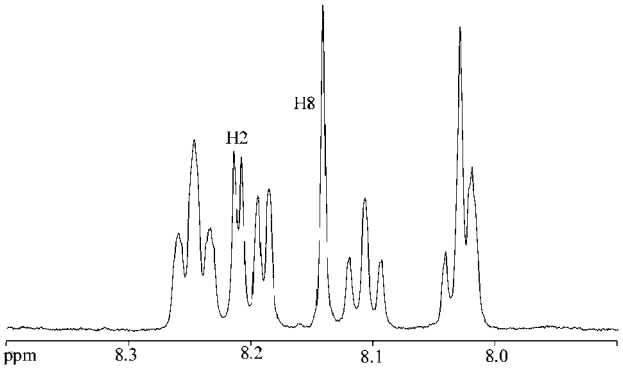

Figure 8. Enlargement of the aromatic region of the ^{1}H NMR spectrum in [D_6]acetone at −50 °C of *cis*-[Ru(bpy)$_2$(9-MeAde)](PF_6)$_2$ showing the 9-MeAde H2 signal as a doublet

Analogous to the results of **1**, after addition of triethylamine in situ at −50 °C, the ^{1}H NMR spectra of **2** show the disappearance of the NH1 signal, the appearance of the H2 signal, no longer as a doublet, and the NH6 resonance dramatically shifted upfield to δ = 5.3. These results are consistent with the formation of *cis*-[Ru(bpy)$_2$(9-MeAde$_{-H}$)](PF_6), in which 9-MeAde is still present in the imine form, now deprotonated at N1. Unfortunately, due to the poor solubility of **2** in water, the pK_a of this compound could not be determined.

Conclusions

The strong differences in biological activity between the structurally similar complexes *cis*-[Ru(bpy)$_2$Cl$_2$] and α-[Ru(azpy)$_2$Cl$_2$] encouraged us to investigate the binding of DNA model bases to the hydrolysis products of both com-

Table 2. Proton chemical shift values [ppm] for *cis*-[Ru(bpy)$_2$(9-MeAde)](PF_6)$_2$ (**2**) in [D_6]acetone at 25 °C

	6/6′	5/5′	4/4′	3/3′	12/12′	11/11′	10/10′	9/9′	H8	H2	NH(1)	NH(6)
2[a]	9.32 8.59	8.00 7.63	8.22/ 8.22	8.77 8.77	8.11 8.00	7.77 7.75	8.07 8.00	8.70 8.65	8.00	8.11	11.7	7.01

[a] The proton chemical shift values of **2** in D_2O could not be determined due to poor solubility of **2** in D_2O.

plexes. In this paper the coordination of 9-MeAde to both *cis*-$[Ru(bpy)_2(H_2O)_2]^{2+}$ and α-$[Ru(azpy)_2(H_2O)_2]^{2+}$ is presented. NMR spectroscopic data prove that, although the ruthenium ions in these two complexes have two available coordination sites, only one 9-MeAde coordinates to the metal ion. NMR structural characterisation using 2D NMR techniques and variable-temperature ^1H NMR spectra show that in both compounds α-$[Ru(azpy)_2$(9-MeAde)$](PF_6)_2$ (**1**) and in *cis*-$[Ru(bpy)_2$(9-MeAde)$](PF_6)_2$ (**2**), 9-MeAde is present in its rare neutral imine tautomeric form, apparently stabilised by the chelating coordination via its N7 and exocyclic N6 atoms. As the coordination mode of 9-MeAde is the same in both the azpy complex **1**, as in the bpy complex **2**, the difference in cytotoxicity of the parent chloride complexes cannot be explained by differences in the coordination of 9-MeAde around the ruthenium centre. At present, further investigation is focussed on the steric properties of the *cis*-$[Ru(LL)_2Cl_2]$ complexes (with LL being a heterocyclic bidentate ligand), to investigate the influence of steric hindrance of the LL ligand toward the free coordination sites in order to obtain a structure-activity relationship of antitumour-active ruthenium complexes. Nevertheless, the interesting imine tautomer of 9-MeAde, stabilised by the bidentate coordination to the ruthenium ion reported here, represents a structural model for any metal ion of interest with a nucleobase where coordination influences the tautomeric equilibrium. There might be biological consequences of the occurrence of the imine form of 9-MeAde upon coordination of 9-MeAde to ruthenium(II). It has already been questioned whether Pt coordination does shift the tautomer equilibrium towards the imine form, but Lippert et al. reported[35] that for the compound $[Cl_3Pt(9MeA)]^-$ the shift in favour for the imine tautomer is too small to explain the observed preferential Ademine−Thymine (AT) transversion. It is generally accepted that the mutation pathway of the AT transversion involves a pairing between one of the two adenines in the rare imine tautomer and the other adenine in the normal amine tautomer in a *syn* conformation.[35] Speculatively, the obvious shift towards the imine tautomer of 9-MeAde presented in this study might result in AT transversions when these ruthenium compounds coordinate to DNA.

In conclusion, a very interesting bidentate coordination of 9-MeAde in the complexes α-$[Ru(azpy)_2$(9-MeAde)$](PF_6)_2$ and *cis*-$[Ru(bpy)_2$(9-MeAde)$](PF_6)_2$ is observed in which 9-MeAde is present in its neutral imine tautomer.

Experimental Section

General Remarks: NMR experiments were performed at 300.13 MHz with a Bruker 300 DPX spectrometer and at 600.13 MHz with a Bruker 600 DPX spectrometer. Spectra were recorded in $[D_6]$acetone, calibrated on the residual solvent peak (δ_H = 2.06) using standard Bruker pulse programs. NOESY and ROESY spectra were recorded with mixing times of 1.0 and 0.5 s, respectively. The pH-dependent experiments were performed in D_2O and the pH was adjusted by HNO_3 (0.1 M) and NaOH (0.1 M). The direct pH-meter readings were used to estimate the pK_a values (no correction for the pD value was performed). Elemental analyses were carried out by the Chemical Services Unit of the University College, Dublin. Mass spectra were obtained by the chemical services of the Gorlaeus Laboratories with a Finnigan MAT 900 instrument equipped with an electrospray interface (ESI). Hydrated $RuCl_3$ was used as received from Johnson and Matthey, Inc. 2-(Phenylazopyridine) and α-$[Ru(azpy)_2Cl_2]$ were synthesised according to published methods.[36−38] α-$[Ru(azpy)_2(NO_3)_2]$ was prepared as described previously.[9] 9-Methyladenine was prepared by methylation of adenine with methyl iodide.[39] *cis*-$[Ru(bpy)_2Cl_2]$ $2H_2O$ was prepared according to a literature procedure.[40]

α-$[Ru(azpy)_2$(9-MeAde)$](PF_6)_2$ (1): 9-Methyladenine (0.027 g, 0.18 mmol) was added to solution of α-$[Ru(azpy)_2(NO_3)_2]$ (0.10 g, 0.17 mmol) in 32 mL of water. The purple reaction mixture was stirred at 40 °C for 7 d. After filtration, a concentrated aqueous solution of NH_4PF_6 was added and the precipitate was collected by filtration. The purple solid was purified by dissolution in acetone and addition of diethyl ether, and this purification step was repeated several times. Yield 0.12 g (77%). ESI MS: *m*/*z* = 616 (92) $\{\alpha\text{-}[Ru(azpy)_2(9\text{-}MeAde)]^{2+} - H^+\}^+$, 308 (100) $\{\alpha\text{-}[Ru(azpy)_2(9\text{-}MeAde)]^{2+}\}$. $C_{28}H_{25}F_{12}N_{11}P_2Ru$ (906.58): calcd. C 37.1, H 2.78, N 17.0; found C 37.1, H 2.76, N 16.1.

***cis*-$[Ru(bpy)_2$(9-MeAde)$](PF_6)_2$ (2):** A mixture of *cis*-$[Ru(bpy)_2Cl_2]$ (0.100 g, 0.19 mmol) and 9-MeAde (0.035 g, 0.23 mmol) in 70 mL of water and 30 mL of ethanol was heated under reflux for 16 h. The reaction mixture was concentrated by rotary evaporation and filtered at room temperature before a concentrated aqueous solution of NH_4PF_6 was added. The resulting orange precipitate was isolated by filtration and recrystallised from acetone/water. The orange crystalline material was washed with diethyl ether. Yield: 0.08 g (68%). ESI MS: *m*/*z* = 562 (68) $\{cis\text{-}[Ru(bpy)_2(9\text{-}MeAde)]^{2+} - H^+\}^+$,281 (100) $\{cis\text{-}[Ru(bpy)_2(9\text{-}MeAde)]^{2+}\}$. $C_{26}H_{23}F_{12}N_9P_2Ru{\cdot}C_3H_6O{\cdot}2.5H_2O$ (852.53 + 58 + 45): calcd. C 36.8, H 2.77, N 13.3; found C 36.8, H 3.05, N 13.3.

Note added in proof (November 9, 2001): The proposed structural details of **1** and **2** as discussed in this paper are confirmed by the recently elucidated crystal structure of α-$[Ru(azpy)_2$(3-MeAde)$](PF_6)$, which will be reported soon.

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