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Interaction between the DNA Model Base 9-Ethylguanine and a Group of Ruthenium Polypyridyl Complexes: Kinetics and Conformational Temperature Dependence

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The binding capability of three ruthenium polypyridyl compounds of structural formula $[Ru(apy)(tpy)L^{n-}](ClO_4)_{(2-n)}$ [**1a**–**c**; apy = 2,2′-azobis(pyridine), tpy = 2,2′:6′,2″-terpyridine, L = Cl, H_2O, CH_3CN] to a fragment of DNA was studied. The interaction between each of these complexes and the DNA model base 9-ethylguanine (9-EtGua) was followed by means of ^{1}H NMR studies. Density functional theory calculations were carried out to explore the preferential ways of coordination between the ruthenium complexes and guanine. The ruthenium–9-EtGua adduct formed was isolated and fully characterized using different techniques. A variable-temperature ^{1}H NMR experiment was carried out that showed that while the 9-EtGua fragment was rotating fast at high temperature, a loss of symmetry was suffered by the model base adduct as the temperature was lowered, indicating restricted rotation of the guanine residue.

Introduction

Recent studies concerning some ruthenium polypyridyl complexes suggest that such compounds could be an alternative to the use of the classic platinum anticancer drugs.[1] An example of these types of complexes is $Ru(tpy)Cl_3$ (tpy = 2,2′:6′,2″-terpyridine), which shows a remarkable in vitro cytotoxicity and exhibits antitumor activity.[2] α-$[Ru(azpy)_2Cl_2]$ [azpy = 2-phenylazopyridine] was reported to show a very high cytotoxicity, which was found to be even more pronounced than the cytotoxicity shown by cisplatin in most of the applied cell lines.[3,4]

The ultimate target of these kinds of compounds is generally accepted to be DNA.[5] Ruthenium polypyridyl complexes bind to DNA in a variety of covalent and noncovalent modes. One of the most likely ways of interaction between the two molecules appears to be the coordination of the ruthenium center to a DNA base.[6−9]

Various groups have tried to correlate DNA binding of a potential metallodrug to its anticancer activity.[10−20] The models vary from simple model bases, of which the preferred ones are the 9-alkylguanines, to oligonucleotides and larger DNA pieces.

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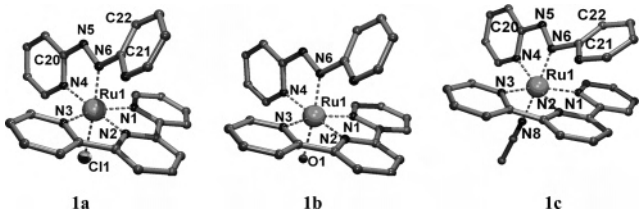

Figure 1. PLATON projections of the cations $[Ru^{II}(apy)(tpy)L]^{n+}$ (**1a**−**c**; L = Cl, H_2O, CH_3CN), with numbering of major atoms. Coordinates were taken from earlier work.[21] Hydrogen atoms and counterions have been omitted for clarity.

NMR spectroscopy can be an important tool that allows the study of whether the metal complex reacts with the model base and, if this reaction occurs, how it develops in time, as well as the structure of the formed products. Further, the experimental conditions can be tuned to resemble physiological conditions as closely as possible.

In the current investigation, a series of complexes with formula $[Ru(apy)(tpy)L^{n-}](ClO_4)_{(2-n)}$ (**1a**−**c**; L = Cl, H_2O, CH_3CN) was selected (see Figure 1).

These complexes are very similar to each other,[21] except for the relative lability of the ligand occupying the sixth coordination position. The labilities of the three chosen ligands should, in principle, be enough to allow coordination of the complex to the model base, albeit their different sizes, shapes, and charges suggest this process could happen following different kinetics in each case. Intercalation of the polypyridyl ligands between DNA base pairs could also be a possible way of interaction of these complexes with DNA.

The reaction between each of the complexes and the model base 9-ethylguanine (9-EtGua) was studied. The 9-EtGua adduct that resulted in all cases (**1d**; see Figure 2) was isolated and completely characterized. Conformational studies were carried out by means of variable-temperature and 2D NMR studies. Structural and electronic properties of the analogous guanine adduct were calculated by density functional theory (DFT) calculations.

Experimental Section

Materials and Reagents. 2,2′-Azobis(pyridine) (apy), Ru(tpy)Cl_3, $[Ru(apy)(tpy)Cl](ClO_4)$, $[Ru(apy)(tpy)(H_2O)](ClO_4)_2 \cdot 2H_2O$, and $[Ru(apy)(tpy)(CH_3CN)](ClO_4)_2$ were synthesized according to the literature methods.[21−23] LiCl, $NaClO_4$ (both Merck), NaClO, $AgNO_3$ (both Acros), tpy (Aldrich), $RuCl_3 \cdot 3H_2O$ (Johnson & Matthey), and 9-EtGua (Sigma) were used as supplied. All other chemicals and solvents were reagent-grade commercial materials and were used as received.

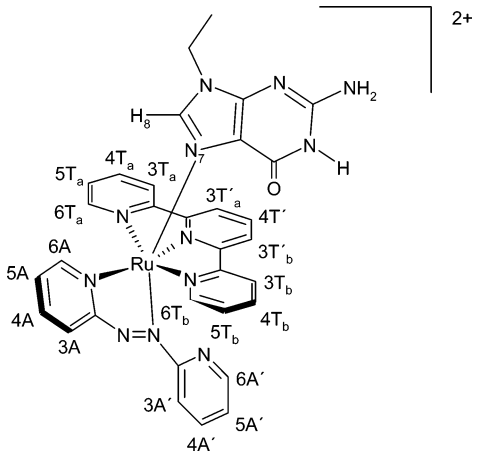

Figure 2. Schematic structure of $[Ru(apy)(tpy)(9\text{-}EtGua)]^{2+}$ (**1d**). A few selected atoms have been labeled, for use in NMR assignments. The subindexes "a" and "b" are only used in the low-temperature spectra. Under low-temperature conditions, the protons in the extreme rings of tpy are not equivalent because of the slow rotation of 9-EtGua on the NMR time scale. As a consequence of this rotation, ring "a" becomes "b" and vice versa.

Physical Measurements. C, H, and N determinations were performed on a Perkin-Elmer 2400 Series II analyzer. Mass spectra were obtained with a Finnigan Aqa mass spectrometer equipped with an electrospray ionization (ESI) source. Fourier transform IR (FTIR) spectra were obtained on a Perkin-Elmer Paragon 1000 FTIR spectrophotometer equipped with a Golden Gate ATR device, using the diffuse-reflectance technique (resolution 4 cm^{-1}). NMR spectra were recorded on a Bruker DPX-300 spectrometer operating at a frequency of 300 MHz, at a temperature of 310 K; on a Bruker Avance-400, at a frequency of 400 MHz, at a temperature of 328 K; and on a Bruker DRX-500 spectrometer operating at a frequency of 500 MHz, at a variable temperature. Chemical shifts were calibrated against tetramethylsilane.

$[Ru(apy)(tpy)(9\text{-}EtGua)]^{2+}$ Titration. The pH titrations were carried out at 310 K in D_2O, by adjustments with DCl and NaOD without the use of any buffer. The pH values were not corrected for the H/D isotope effect. The pH meter was calibrated with Fisher certified buffer solutions of pH 4.00, 7.00, and 10.00.

Synthesis and Characterization of $[Ru(apy)(tpy)(9\text{-}EtGua)](ClO_4)_2$ (1d). $[Ru(apy)(tpy)(H_2O)](ClO_4)_2 \cdot 2H_2O$ (15 mg, 0.019 mmol) and 9-EtGua (4 mg, 0.022 mmol) were vigorously refluxed in 5 mL of absolute EtOH for 24 h. The mixture was left to cool down to room temperature. The product was collected by filtration, washed with a small amount (about 2 mL) of ice-cold water and ether, and dried in vacuo over silica (yield 82%). Anal. Calcd for $C_{32}H_{28}N_{12}O_9Cl_2Ru$: C, 42.9; H, 3.1; N, 18.7. Found: C, 42.7; H, 2.7; N, 18.8. ESI-MS: *m/z* 697.1 ($[Ru(apy)(tpy)(9\text{-}EtGua\text{-}H)]^+$); 348.7 ($[Ru(apy)(tpy)(9\text{-}EtGua)]^{2+}$). ^1H NMR (300 MHz, D_2O, 310 K): δ 9.21 (d, 1H, J = 5.20 Hz), 8.92 (d, 1H, J = 8.22 Hz), 8.48 (t, 1H, J = 8.00 Hz), 8.37 (m, 3H), 8.20 (t, 1H, J = 8.06 Hz), 8.11 (m, 3H), 7.92 (d, 1H, J = 4.99 Hz), 7.64 (m, 3H), 7.41 (dd, 2H, J_1 = 8.70 Hz, J_2 = 14.92 Hz), 7.30 (dd, 1H, J_1 = 4.28 Hz, J_2 = 6.86 Hz), 6.81 (s, 1H), 6.52 (d, 1H, J = 7.98 Hz), 3.83 (dd, 2H, J_1 = 7.21 Hz, J_2 = 14.47 Hz), 1.07 (t, 3H, J = 7.27 Hz).

Computational Details. DFT calculations[24] were performed using the program CPMD[25] with a plane-waves basis set up to an

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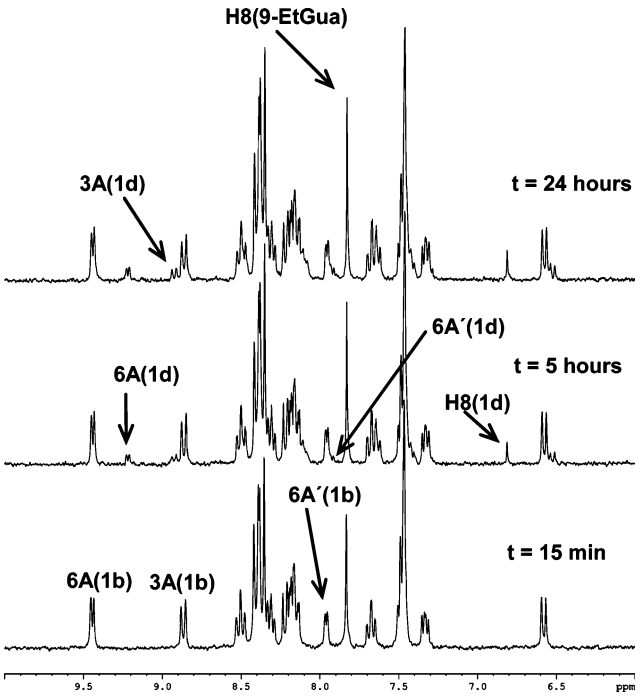

Figure 3. ^{1}H NMR study over 24 h of the reaction between the ruthenium polypyridyl complex **1b** and the DNA model base 9-EtGua in D_2O. Some selected peaks have been labeled with their assignments.

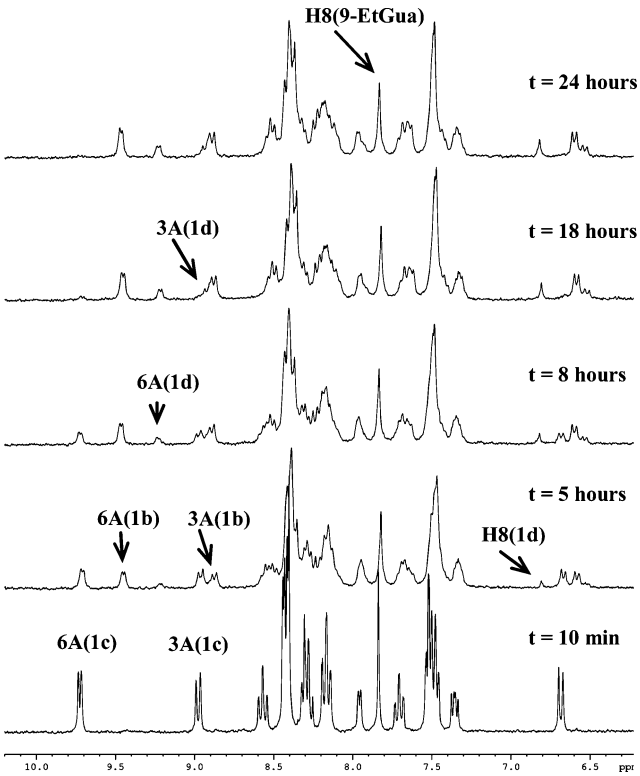

Figure 4. ^{1}H NMR study over 24 h of the reaction between the ruthenium polypyridyl complex **1c** and the DNA model base 9-EtGua in D_2O. Some selected peaks have been labeled with their assignments.

energy cutoff of 70 Ry. Core/valence interactions were described using norm-conserving pseudopotentials of the Martins−Troullier type.[26] Integration of the nonlocal parts of the pseudopotential was obtained via the Kleinman−Bylander scheme[27] for all of the atoms except ruthenium, for which a Gauss−Hermite numerical integration scheme was used. For ruthenium, a semicore pseudopotential was adopted as described in the literature[28] that also incorporates scalar relativistic effects. The gradient-corrected Becke exchange functional and the Perdew correlation functional (BP) were used.[29,30] Isolated system conditions[31] were applied. Calculations were performed in an orthorhombic cubic cell of edges $a = 30$, $b = 29$, and $c = 36$ au. Geometries have been relaxed by iterating geometry optimization runs (based on a conjugate gradient procedure) and molecular dynamics (MD) runs at 0 K up to a gradient of 5.0×10^{-5} au. A fictitious electron mass of 900 au and a time step of 0.1205 fs were used in the MD runs.

Four possible conformers of Ru(apy)(tpy)(Gua), which differ in the orientation of the guanine above the plane of the ligands, were found.

Results and Discussion

^{1}H NMR Studies of the Interaction between Three Ruthenium Polypyridyl Complexes and 9-EtGua. The reaction between the ruthenium polypyridyl complex $[Ru(apy)(tpy)(H_2O)]^{2+}$ and the DNA model base 9-EtGua was studied by ^{1}H NMR (see Figure 3). The conditions of the experiment were chosen to be as close as possible to physiological conditions, using D_2O as a solvent and a temperature of 310 K. The reaction was studied for 24 h, during which the pH was seen to remain neutral.

The signals appearing in this kinetic experiment could be unambiguously assigned by comparison with the ^{1}H NMR spectrum of the isolated model base adduct **1d**, which had been synthesized and characterized by several techniques, vide infra. Although the peaks corresponding to 9-EtGua (CH_3 at 1.07 ppm, CH_2 at 3.83 ppm, and H8 at 6.81 ppm) were found to be shifted with respect to the free base, the peak of choice for the kinetic studies was that corresponding to the proton 6A. This significantly deshielded peak presented a different chemical shift for each of the four complexes (**1a**−**d**), which allowed us to easily distinguish each species in solution as well as to measure the ratio between them.

The model base 9-EtGua was observed to react with the ruthenium complex to give the model base adduct $[Ru(apy)(tpy)(9\text{-EtGua})]^{2+}$. This reaction occurred during the first 5 h when a ruthenium compound−model base ratio of 1:2 was used. No further changes were observed. Despite the 2-fold excess of the model base, only 20% of the ruthenium complex reacted to yield the model base adduct.

The same experiment was carried out starting from the complex $[Ru(apy)(tpy)(CH_3CN)](ClO_4)_2$ (see Figure 4). In this case, the acetonitrile complex was observed to hydrolyze to produce the cation $[Ru(apy)(tpy)(H_2O)]^{2+}$, besides reacting with 9-EtGua as described above. After the 5 h needed by the model base adduct to reach its maximum concentration in the experiment described above, 15% of the ruthenium could be found in the form of the model base adduct in this second case. The 20% obtained in the first experiment was

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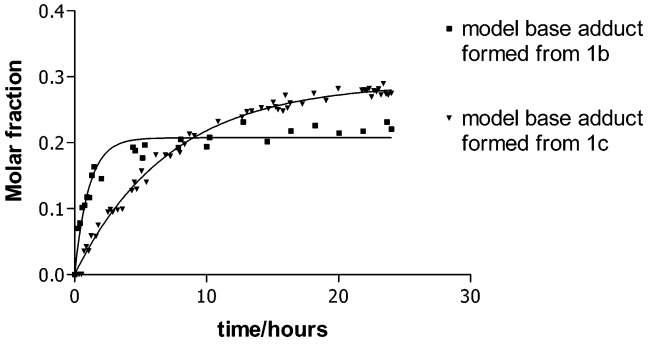

Figure 5. Formation of the model base adduct from two ruthenium complexes (**1b** and **1c**). Molar fraction of $[Ru(apy)(tpy)(9\text{-}EtGua)]^{2+}$ (χ_E) vs time.

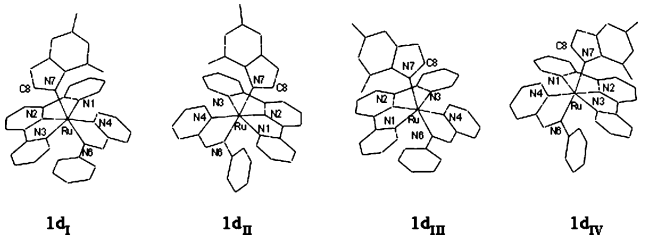

Figure 6. Four models of the $[Ru(apy)(tpy)(Gua)]^{2+}$ adduct obtained by the DFT calculations, with numbering of major atoms as referred to in Table 2.

Table 1. Rate Constants Determined for the Reaction between 9-EtGua and the Ruthenium Polypyridyl Complexes **1b** and **1c**, Respectively

	rate constant k' (h^{-1})	k	half-life of **1d** in solution (h)
1b	0.92 ± 0.08	0.207 ± 0.004	0.8 ± 0.2
1c	0.139 ± 0.004	0.290 ± 0.003	5.0 ± 0.3

obtained in this second experiment after 8 h. The reaction went on until the maximum fraction of the model base adduct was reached. In a total of 18 h from the start of the reaction, 30% of the ruthenium was found to be in the form of $[Ru(apy)(tpy)(9\text{-}EtGua)]^{2+}$.

The reaction between $[Ru(apy)(tpy)Cl]^{+}$ and 9-EtGua proceeded much slower than the other two examples described above. Because of the lower solubility of the ruthenium complex in D_2O, the results obtained in this last case were only regarded in a qualitative way.

The curve of the molar fraction of $[Ru(apy)(tpy)(9\text{-}EtGua)]^{2+}$ (χ_E) vs time (see Figure 5) was fitted with eq 1.

$$\chi_E = k(1 - e^{-k't}) \quad (1)$$

where k is the maximum value of the molar fraction of the ruthenium−model base adduct reached. The values of k and the rate constant k' were calculated, as well as the half-life of the ruthenium−model base adduct (**1d**) in solution (see Table 1).

DFT Calculations. Four different models of the $[Ru(apy)(tpy)(Gua)]^{2+}$ adduct were considered, differing in the orientation of the N1−Ru−N7−C8 torsional angles (see Figure 6). Structures $\mathbf{1d_I}$ and $\mathbf{1d_{II}}$ show an orientation of Gua in such a way that its keto group is wedged between the pyridine ring of apy and the pyridine ring of tpy. This orientation is analogous to that shown in the complex $[RuCl(bpy)_2(9\text{-}EtGua)]^{2+}$, where bpy is 2,2′-bipyridine.[11] In structures $\mathbf{1d_{III}}$ and $\mathbf{1d_{IV}}$, however, the keto group is positioned above the tpy plane. The four models $\mathbf{1d_I}-\mathbf{1d_{IV}}$ were almost isoenergetic, with relative energies of ≤3.8 kcal/mol. The accuracy of these results was validated by relaxing the geometry of $[Ru(apy)(tpy)(H_2O)]^{2+}$ (**1b**) and by comparing it with that of the corresponding X-ray structure. For **1b**, the largest deviation with respect to the X-ray structure[21] occurs for the Ru−OH_2 bond, $\Delta d < 0.1$ Å (4% relative error), while the overall agreement is excellent for all other coordination bonds and angles.

Structural parameters of the most stable isomers of $[Ru(apy)(tpy)(H_2O)]^{2+}$ and $[Ru(apy)(tpy)(Gua)]^{2+}$ are given in Table 2 along with an analysis of the bond ionicity (BI).[32] The four conformational isomers $\mathbf{1d_I}-\mathbf{1d_{IV}}$ present similar coordination geometries with a small difference in the Ru−N7 bond length. The Ru−N7 bond varied by $\Delta d = 0.04$ Å between the most and less thermodynamically stable conformers $\mathbf{1d_I}$ and $\mathbf{1d_{IV}}$. The presence of the keto group of the guanine between the pyridine ring of apy and the pyridine ring of tpy in $\mathbf{1d_I}$ and $\mathbf{1d_{II}}$ or above the tpy plane in $\mathbf{1d_{III}}$ and $\mathbf{1d_{IV}}$ determines also a small rearrangement of the angles.

The binding of the guanine determines a small rearrangement of the apical ligands: the Ru−N7 bond shortens by $\Delta d = 0.04-0.08$ Å ($\Delta BI = 0.06-0.08$) for $\mathbf{1d_I}-\mathbf{1d_{IV}}$, with respect to the Ru−OH_2 bond of **1b**, while the Ru−N6 bond increases by $\Delta d = +0.05-0.04$ Å ($\Delta BI = 0.04$). The coordination geometry corresponds to that of a slightly distorted octahedron that is imposed by the rigidity of the aromatic ring systems of the apy ligand.

The binding of water to the coordinatively unsaturated complex is exothermic by −18.7 kcal/mol, while the binding of the guanine is exothermic by a maximum amount of −47.7 kcal/mol in $\mathbf{1d_I}$ and a minimum of −43.9 kcal/mol in $\mathbf{1d_{IV}}$. The exchange reaction between water and the guanine is exothermic by −29.1 to −25.3 kcal/mol (see Table 2).

Synthesis and Characterization of 1d. pH Titration. Variable-Temperature and 2D NMR studies. The ^{1}H NMR chemical shift values for the model base adduct $[Ru(apy)(tpy)(9\text{-}EtGua)]^{2+}$ (**1d**) in the aromatic region are presented in Table 3.

The coordination of 9-EtGua to ruthenium was proven to occur via the nitrogen N7 by a pH titration experiment of the ^{1}H NMR spectrum of the ruthenium−model base adduct. At low pH, the N7 atom in 9-EtGua is protonated. When the pH is increased, site N7 is deprotonated, causing a shift in the H8 peak toward higher field. The absence of this shift when the experiment was carried out with the ruthenium−model base adduct **1d** was sufficient to prove that the N7 position of 9-EtGua was coordinated to ruthenium.

When a ^{1}H NMR spectrum of the ruthenium−model base adduct was recorded at room temperature, some of the peaks

(32) Based on Boys' orbitals, the bond ionicity BI_{AB} of a bond was calculated as (Alber, F.; Folkers, G.; Carloni, P. *J. Phys. Chem. B* **1999**, *103*, 6121), namely, $BI_{AB} = d_A/d_{AB}$, where d_A is the distance between atom A and the Boys orbital along the AB bond and d_{AB} is the length of the bond between A and B. BI_s help to individualize lone pairs and provide an estimation of the ionicity of chemical bonds.

Table 2. Selected Bond Lengths (Å), Angles (deg), and Bond Ionicities (BIs) of **1b** and **1d$_I$**−**1d$_{IV}$** Compounds[a]

	X-ray of **1b**	calculated structure of **1b**	BI of **1b**	**1d$_I$**	BI of **1d$_I$**	**1d$_{II}$**	BI of **1d$_{II}$**	**1d$_{III}$**	BI of **1d$_{III}$**	**1d$_{IV}$**	BI of **1d$_{IV}$**
Bond											
Ru−O,N7	2.15	2.25	0.82	2.17	0.75	2.19	0.75	2.21	0.74	2.21	0.76
Ru−N1	2.07	2.09	0.73	2.11	0.71	2.09	0.72	2.09	0.73	2.08	0.73
Ru−N2	1.98	1.98	0.71	1.98	0.76	1.98	0.75	1.98	0.70	1.98	0.70
Ru−N3	2.07	2.08	0.73	2.08	0.73	2.09	0.71	2.09	0.73	2.09	0.74
Ru−N4	2.06	2.07	0.74	2.07	0.75	2.08	0.73	2.08	0.74	2.09	0.75
Ru−N6	1.96	1.97	0.68	2.02	0.72	2.01	0.72	2.01	0.72	2.01	0.72
Angles											
N1−Ru−O,N7	87.2	87.0		89.5		85.4		85.9		93.6	
N2−Ru−O,N7	85.9	86.2		88.0		87.5		88.7		89.9	
N3−Ru−O,N7	88.0	88.3		91.0		91.9		96.4		88.9	
N4−Ru−O,N7	95.9	95.3		94.6		96.1		93.6		92.8	
N4−Ru−N6	76.8	77.2		76.2		76.7		76.4		76.2	
N6−Ru−N1	94.3	93.8		88.6		90.7		88.5		88.9	
N6−Ru−N2	101.3	101.5		100.5		100.1		101.6		101.0	
N6−Ru−N3	93.1	93.6		94.7		91.9		93.1		92.9	
N6−Ru−O,N7	172.8	172.2		169.7		172.1		167.2		169.1	
Torsional Angles											
N1−Ru−N7−C8				121.3		133.4		−44.6		−157.6	
relative energies				0.0		0.5		2.5		3.8	
Δ*H* binding wat/gua	−18.7			−47.7		−47.2		−45.2		−43.9	
Δ*H* exchange wat/gua				−29.1		−28.6		−26.6		−25.3	

[a] Relative energies (kcal/mol) of the conformational isomers are given, along with binding energies of water and the guanine and the enthalpy for the reaction of exchange between water and the guanine ligand.

Table 3. Proton Chemical Shift Values (ppm) for the Complexes **1b** and **1d** in the Aromatic Region, Taken in D_2O at 310 K[a]

	proton														
complex	3A	4A	5A	6A	3A′	4A′	5A′	6A′	3T	4T	5T	6T	3T′	4T′	H8
1b	9.01	8.55	8.36	9.46	7.14	7.75	7.34	7.84	8.67	8.19	7.50	7.34	8.67	8.36	
1d	8.92	8.48	8.11	9.21	6.52	7.64	7.30	7.92	8.37	8.11	7.41	7.64	8.37	8.20	6.81

[a] The proton labels are indicated in Figure 2.

appeared broadened. This effect is of great interest in the study of the conformational behavior of the mentioned adduct because these broad resonances suggest hindered rotational behavior of the coordinated 9-EtGua.

Subsequently, a full variable-temperature NMR study was carried out. For this purpose, the solvent was chosen to be MeOH-d_4 because its lower freezing point than that of water allowed a more extensive study. ^{1}H NMR spectra of [Ru(apy)(tpy)(9-EtGua)]$^{2+}$ were recorded in MeOH-d_4 at the following temperatures: 213, 233, 253, 273, 298, 308, and 318 K (see Figure 7). 2D NMR spectra of the compound were recorded at 213 K (see Figure 8) and 328 K (see the Supporting Information). The peaks of the spectra at the highest and lowest temperatures were assigned as indicated in Table 4.

The shifts of the apy protons, as well as that of the proton labeled 4T′ (see Figure 2), remain virtually unaltered by the temperature change. These peaks look sharp in the complete range of temperatures. If the 9-EtGua moiety is disregarded, all of these protons lie on or close to a symmetry plane. The rest of the tpy protons give one set of sharp signals of intensity 2 at 318 K, which split into two sets of sharp signals of intensity 1 at 213 K. At intermediate temperatures, these tpy resonances appear broadened.

If one considers the 9-EtGua moiety to be rotating fast at the NMR time scale at high temperature, its proximity to all tpy protons would be equivalent. This would have the same effect if a symmetry plane were considered, on or close to which the apy protons, as well as the proton labeled 4T′, would lie. The rest of the tpy protons would therefore be equivalent in pairs, and one set of five sharp peaks with intensity 2 would be obtained. As described above, this is what can be seen in the experiment at 318 K (see Figure 7).

Upon a decrease in the temperature, the protons lying on that "symmetry plane" shift slightly, while the rest of the tpy protons broaden first, to finally split into 10 sharp peaks with intensity 1 at 213 K (see Figure 7). This effect is due to the 9-EtGua progressively slowing down its rotational movement, until it has reached a slow rotational movement on the NMR time scale. The molecule has become now asymmetric, and therefore each proton gives a different NMR resonance.

Because the protons of the two extreme pyridine rings of tpy are not equivalent at low temperature, the subindexes "a" and "b" were given to those belonging to each of the rings. In the same way, 3T′a is closer to the "a" ring and 3T′b is closer to the "b" ring.

The NOE H8-3T′a and H8-3Ta cross-couplings (see Figure 8) prove that the 9-EtGua proton H8 is situated between the "a" and the central tpy rings. No NOEs are observed between H8 and 3T′b or 3Tb. Moreover, a strong NOE cross-coupling can be observed between 6A and 6Ta, while the cross-coupling between 6A and 6Tb is much weaker. This difference is due to the presence of the carbonyl group

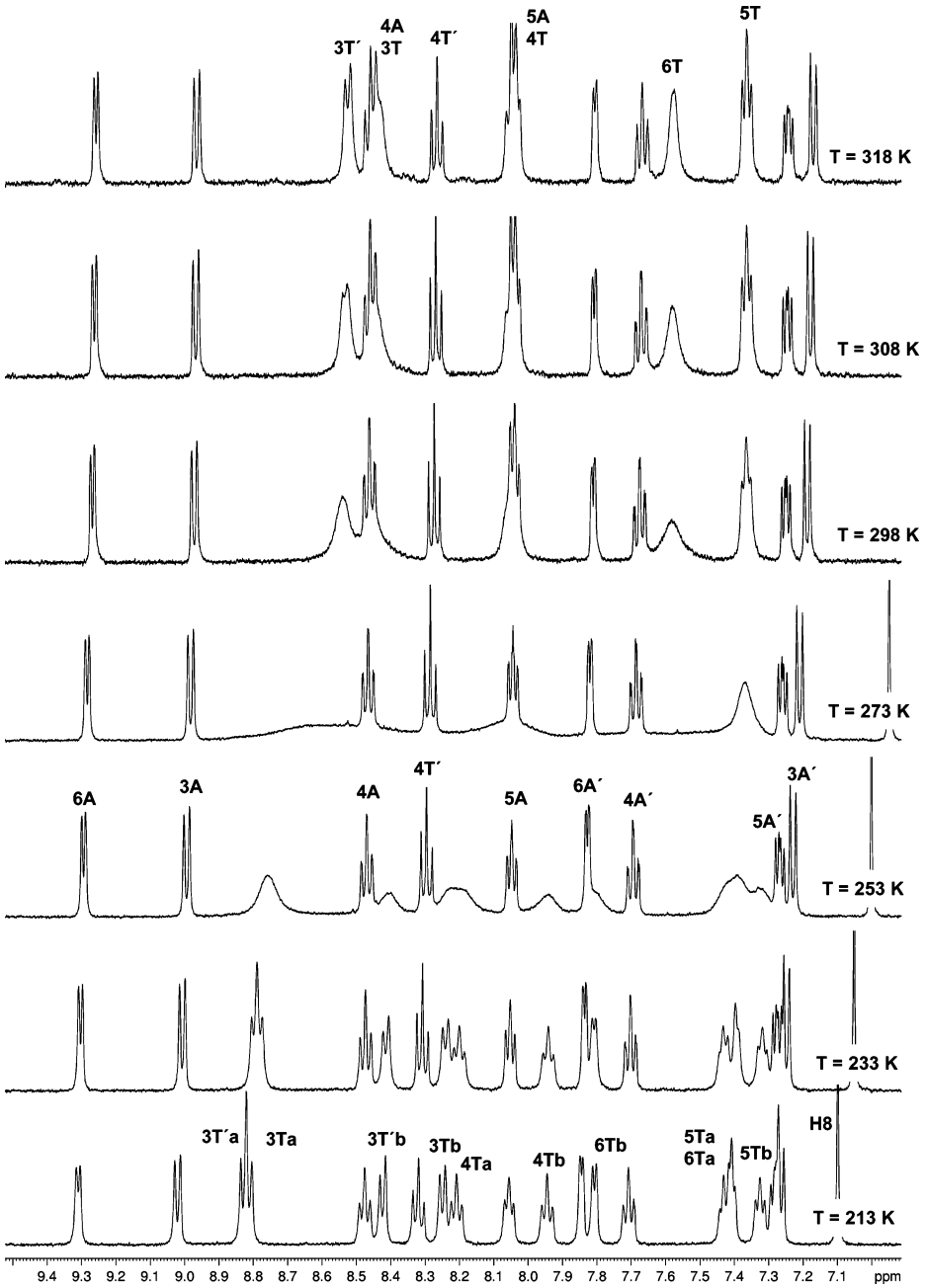

Figure 7. ^{1}H NMR spectra of **1d** in MeOH-d_4 at different temperatures in the range 213−318 K, with labeled peak assignments. The peaks corresponding to H8 were left out at 298, 308, and 318 K for clarity of the figure.

between 6A and 6Tb. The proximity of the carbonyl group to 6Tb could also explain why this proton appears 0.37 ppm downfield with respect to 6Ta. This conformation of a 9-EtGua adduct is analogous to that shown in the crystal structure of the complex $[RuCl(bpy)_2(9\text{-EtGua})]^{2+}$, where bpy is 2,2′-bipyridine.[11]

It can be concluded from the DFT calculations that four conformations of the model base adduct are possible and only two if one neglected the torsion angle of the noncoordinated pyridine ring. This is in agreement with the low-temperature ^{1}H NMR and 2D ^{1}H−^{1}H NMR spectra, which show how only one of these possible conformers is present in a methanolic solution at 213 K, with the carbonyl group being wedged between the tpy and apy ligands (structures $\mathbf{1d_I}$ and $\mathbf{1d_{II}}$ from Figure 6).

Exchange cross-peaks between all of the corresponding tpy resonances can be seen in the ^{1}H−^{1}H NOESY NMR spectrum at 213 K (see Figure 8). This effect suggests that the 9-EtGua moiety is slowly rotating on the NMR time scale around the Ru−N7 bond. The two degenerate positions (structures $\mathbf{1d_I}$ and $\mathbf{1d_{II}}$ from Figure 6) are equivalent in the NMR, in such a way that the "a" ring becomes "b", and vice versa, which explains the absence of H8-3Tb and H8-3T′b cross-couplings.

It has been suggested for analogous compounds[33,34] that the above-mentioned rotation of the 9-EtGua moiety occurs in such a way that the keto group passes over the tpy ligand

(33) Velders, A. H. Ruthenium complexes with heterocyclic nitrogen ligands. Ph.D. Thesis, Leiden University, Leiden, The Netherlands, 2000; pp 156−162.

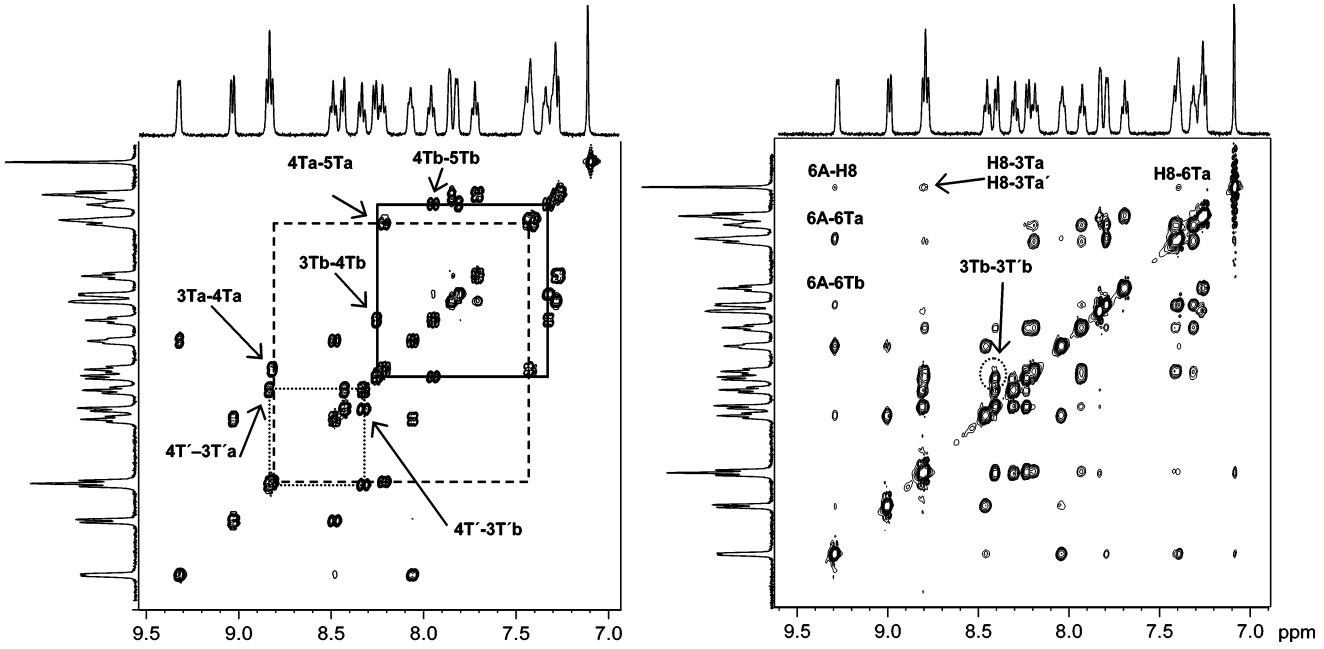

Figure 8. Aromatic region of the ^{1}H−^{1}H COSY (left) and NOESY (right) spectra of **1d** in MeOH-d_4 at 213 K, with some assignments. In the COSY spectrum, the dashed lines indicate the 3Ta−4Ta−5Ta−6Ta COSY cross-peaks. The dotted lines show the 3T′a−4T′−3T′b COSY cross-peaks. The solid lines indicate the 3Tb−4Tb−5Tb−6Tb COSY cross-peaks. Some of these COSY cross-peaks are labeled. In the NOESY spectrum, a few selected cross-peaks are assigned.

Table 4. Proton Chemical Shift Values (ppm) for the Complex **1d** in the Aromatic Region, Taken in MeOH-d_4 at 213 and 328 K[a]

	proton														
T, K	3A	4A	5A	6A	3A′	4A′	5A′	6A′	4T′	H8	3Ta, 3Tb, 3T	4Ta, 4Tb, 4T	5Ta, 5Tb, 5T	6Ta, 6Tb, 6T	3T′a, 3T′b, 3T′
213	9.03	8.48	8.06	9.31	7.27	7.71	7.28	7.85	8.33	7.12	8.83, 8.25	8.21, 7.94	7.43, 7.32	7.43, 7.80	8.85, 8.43
328	8.95	8.46	8.04	9.25	7.16	7.66	7.24	7.80	8.26	6.80	8.43	8.04	7.36	7.58	8.51

[a] The proton labels are indicated in Figure 1.

because a 90° rotation of the model base is hindered by the coordinated pyridine ring of, in the present case, the apy ligand. During this rotation, the molecule passes through two energetic minima, corresponding to the conformers $\mathbf{1d_{III}}$ and $\mathbf{1d_{IV}}$, which lie at higher energies than $\mathbf{1d_I}$ and $\mathbf{1d_{II}}$ (Figure 6). The observation of both H8-6A and H8-6Ta NOE cross-couplings supports this theory.

The guanine derivatives, as well as other smaller model imidazole bases bound to ruthenium polypyridyl complexes, were found to be rotating fast on the NMR time scale, as observed in the cases of the smaller imidazole ligands,[35−37] not rotating at all in the cases in which the model base was stabilized by hydrogen bonds and electrostatic forces,[37,38] and slowly rotating in the intermediate cases.[33,34,36,37] The whole rotation process can be followed by variable-temperature 1D and 2D NMR, as described in this study.

Conclusions

The interaction between a group of ruthenium polypyridyl complexes and a DNA model base was studied. Three very similar complexes differing only in one coordination site, occupied by a leaving group, were chosen for the experiment. The three complexes were proven to bind to 9-EtGua, following different kinetics in each case. Both complexes $[Ru(apy)(tpy)Cl]^+$ and $[Ru(apy)(tpy)(CH_3CN)]^{2+}$ were seen by ^{1}H NMR to hydrolyze to give $[Ru(apy)(tpy)(H_2O)]^{2+}$, besides reacting with 9-EtGua. The reaction from the ruthenium starting complex to the ruthenium−model base adduct is faster in the case of $[Ru(apy)(tpy)(CH_3CN)]^{2+}$ and much slower in the case of the chlorido complex.

The preferential geometry of the ruthenium−model base adduct formed in all cases was inferred from DFT calculations. This 9-EtGua complex shows a very interesting conformational behavior, which has been studied in full detail by means of variable-temperature ^{1}H NMR and 2D COSY and NOESY NMR spectroscopy. At high temperatures, the 9-EtGua moiety is rotating fast at the NMR time scale, while at low temperatures, this model base shows a preferred orientation, with the keto group wedged between the tpy and apy ligands. This behavior is in agreement with the DFT calculations.

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Supporting Information Available: $^1H-^1H$ COSY and NOESY NMR spectra of ruthenium−model base complex [Ru(apy)(tpy)(9-EtGua)]$^{2+}$ in MeOH-d_4 at 328 K. This material is available free of charge via the Internet at http://pubs.acs.org.

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