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Coordination of 9-Ethylguanine to the Mixed-Ligand Compound α-[Ru(azpy)(bpy)Cl_2] (azpy = 2-Phenylazopyridine and bpy = 2,2′-Bipyridine). An Unprecedented Ligand Positional Shift, Correlated to the Cytotoxicity of This Type of [RuL_2Cl_2] (with L = azpy or bpy) Complex

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The striking difference in cytotoxic activity between the inactive *cis*-[Ru(bpy)$_2$Cl$_2$] and the recently reported highly cytotoxic α-[Ru(azpy)$_2$Cl$_2$] (α indicating the isomer in which the coordinating Cl atoms, pyridine nitrogens, and azo nitrogens are in mutual *cis*, *trans*, *cis* orientation) encouraged the synthesis of the mixed-ligand compound *cis*-[Ru(azpy)(bpy)Cl$_2$]. The synthesis and characterization of the only occurring isomer, i.e., α-[Ru(azpy)(bpy)Cl$_2$], **1** (α denoting the isomer in which the Cl ligands are *cis* related to each other and the pyridine ring of azpy is *trans* to the pyridine ring of bpy), are described. The solid-state structure of **1** has been determined by X-ray structure analysis. The IC_{50} values obtained for several human tumor cell lines have indicated that compound **1** shows mostly a low to moderate cytotoxicity. The binding of the DNA model base 9-ethylguanine (9-EtGua) to the hydrolyzed species of **1** has been studied and compared to DNA model base binding studies of *cis*-[Ru(bpy)$_2$Cl$_2$] and α-[Ru(azpy)$_2$Cl$_2$]. The completely hydrolyzed species of **1**, i.e., α-[Ru(azpy)(bpy)(H$_2$O)$_2$]$^{2+}$, has been reacted with 9-EtGua in water at room temperature for 24 h. This resulted in the monofunctional binding of only one 9-EtGua, coordinated via the N7 atom. The product has been isolated as α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)](PF$_6$)$_2$, **2**, and characterized by 2D NOESY NMR spectroscopy. The NOE data show that the 9-EtGua coordinates (under these conditions) at the position *trans* to the azo nitrogen atom. Surprisingly, time-dependent ^{1}H NMR data of the 9-EtGua adduct **2** in acetone-d_6 show an unprecedented positional shift of the 9-EtGua from the position *trans* to the azo nitrogen to the position *trans* to the bpy nitrogen atom, resulting in the adduct α′-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)](PF$_6$)$_2$ (α′ indicating 9-EtGua is *trans* to the bpy nitrogen). This positional isomerization of 9-EtGua is correlated to the cytotoxicity of **1** in comparison to both the cytotoxicity and 9-EtGua coordination of *cis*-[Ru(bpy)$_2$Cl$_2$], α-[Ru(azpy)$_2$Cl$_2$], and β-[Ru(azpy)$_2$Cl$_2$]. This positional isomerization process is unprecedented in model base metal chemistry and could be of considerable biological significance.

Introduction

After the successful development of *cis*-[$PtCl_2(NH_3)_2$], cisplatin,[1] as a medicine against cancer, several ruthenium compounds have more recently been under investigation for their antitumor activity.[2,3] Within various groups of ruthenium anticancer complexes structure−activity relationships have been explored by designing several derivatives and by studying the interaction with biological targets, e.g., DNA.[2,3]

The cytotoxic activity of the dichlorobis(2-phenylazopyridine)ruthenium(II) complexes against a series of human

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(1) Wong, E.; Giandomenico, C. M. *Chem. Rev.* **1999**, *99*, 2451.
(2) Reedijk, J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3611.
(3) Clarke, M. J. *Coord. Chem. Rev.* **2002**, *232*, 69.

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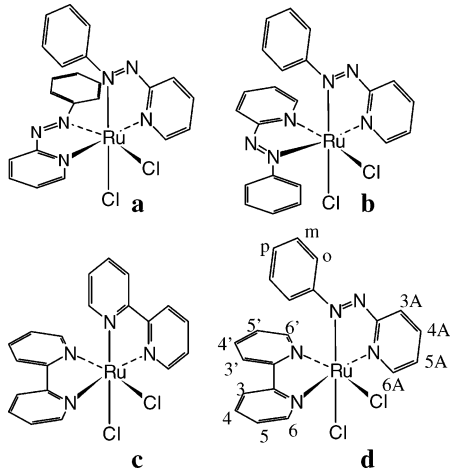

Figure 1. Schematic structures of α-[Ru(azpy)$_2$Cl$_2$] (a), *β*-[Ru(azpy)$_2$Cl$_2$] (b), *cis*-[Ru(bpy)$_2$Cl$_2$] (c), and α-[Ru(azpy)(bpy)Cl$_2$] (d), with the numbering used for NMR assignments.

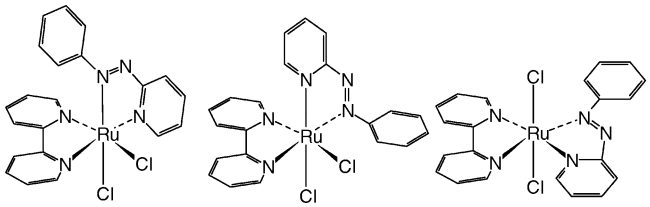

Figure 2. Theoretically possible isomers of [Ru(azpy)(bpy)Cl$_2$]: α-*cis*-[Ru(azpy)(bpy)Cl$_2$] (left), *β*-*cis*-[Ru(azpy)(bpy)Cl$_2$] (middle), and *trans*-[Ru(azpy)(bpy)Cl$_2$] (right).

tumor cell lines has been reported.[4] More data about the cytotoxic activity of the [RuL$_2$Cl$_2$] (L = 2-phenylazopyridine, 4-methyl-2-phenylazopyridine, and *o*-tolylazopyridine) complexes have recently been published.[5] The different isomeric complexes show distinct cytotoxicities.[4,5] In particular, the α isomers (α indicating the configuration in which the Cl ligands are in a mutual *cis* position, the pyridine rings are *trans* positioned, and the coordinating azo nitrogen atoms are in a *cis* orientation) of the [Ru(azpy)$_2$Cl$_2$] complexes and also the methylated derivatives show the biologically important features of a high stability (no isomerization) and a very high cytostatic activity.[4,5] In contrast, the structurally related *cis*-[Ru(bpy)$_2$Cl$_2$] (Figure 1) is inactive.[6] The striking difference in activity between the highly cytotoxic α-[Ru(azpy)$_2$Cl$_2$] and the inactive *cis*-[Ru(bpy)$_2$Cl$_2$] encouraged the investigation of the mixed-ligand compound [Ru(azpy)(bpy)Cl$_2$] (Figure 1) for its cytotoxicity. In theory three isomers (Figure 2) would be expected for [Ru(azpy)(bpy)Cl$_2$], two isomers with the Cl ligands *cis* and one isomer with the Cl ligands in a *trans* position. However, in the literature only the two *cis* isomers are mentioned, albeit poorly and erroneously characterized (vide infra).[7] A careful and detailed study described below observes only one isomer, which is fully characterized, and the molecular structure is reported.

It is generally accepted that DNA is a major target for platinum and ruthenium anticancer compounds. DNA model-base binding studies with α-[Ru(azpy)$_2$Cl$_2$] and *cis*-[Ru(bpy)$_2$Cl$_2$] have been performed with the use of several DNA model bases. The model base 9-ethylguanine (9-EtGua) coordinates monofunctionally to both the α-[Ru(azpy)$_2$] and *cis*-[Ru(bpy)$_2$] moieties.[8,9] Interestingly, the 9-EtGua model base coordinated to the α-[Ru(azpy)$_2$] moiety can have two orientations, whereas 9-EtGua coordinated to the *cis*-[Ru(bpy)$_2$] moiety is fixed in one orientation.[9] Combination of these 9-EtGua studies with detailed orientational studies using 1-methylbenzimidazole[10−13] have indicated that rather small differences in orientational freedom of the DNA model bases might explain the observed differences in cytotoxicity of α-[Ru(azpy)$_2$Cl$_2$] and *cis*-[Ru(bpy)$_2$Cl$_2$]. To confirm and further investigate this statement, DNA model base studies have also been performed with the hybrid species α-[Ru(azpy)(bpy)Cl$_2$], which will be presented in this Article.

Experimental Section

Materials. [Ru(bpy)Cl$_3$]$_n$·*x*H$_2$O was prepared as described in the literature.[14] This precursor complex used in the synthesis of the mixed-ligand compound α-[Ru(azpy)(bpy)Cl$_2$] was referred to as the Ru(IV) species [Ru(bpy)Cl$_4$] in early literature.[14] However, a later review[15] reported the identity of the material as being the polynuclear species [Ru(bpy)Cl$_3$]$_n$·*x*H$_2$O, although experimental evidence for this hypothesis was not given.[15] Elemental analyses of the precursor complex used for the synthesis of **1** suggested the material to be [Ru(bpy)Cl$_3$(H$_2$O)$_{0.75}$(RuCl$_3$)$_{0.15}$]$_n$ (data not shown). The model base 9-ethylguanine (Sigma) was used without purification. For column purification neutral aluminum oxide (ICN AluminaN Akt. 1) was used.

α-[Ru(azpy)(bpy)Cl$_2$], 1. This compound was prepared by a modification of the literature procedure for the synthesis of *cis*-[Ru(azpy)(bpy)Cl$_2$].[7] A green solution of [Ru(bpy)Cl$_3$]$_n$·*x*H$_2$O (1.00 g) in 75 mL of dimethylformamide was mixed with azpy (0.65 g, 3.55 mmol) and refluxed for 50 min. The reaction mixture was filtered, and the filtrate was evaporated to dryness. The solid residue was dissolved in 50 mL of chloroform, filtered, and concentrated to approximately 10 mL by rotary evaporation. This mixture was separated over a neutral alumina column, with chloroform as eluent. A blue fraction eluted first, followed by the violet product fraction. Between these two, a minor fraction containing both blue and violet species was collected. All three fractions were concentrated and

(4) Velders, A. H.; Kooijman, H.; Spek, A. L.; Haasnoot, J. G.; de Vos, D.; Reedijk, J. *Inorg. Chem.* **2000**, *39*, 2966.
(5) Hotze, A. C. G.; Caspers, S. E.; de Vos, D.; Kooijman, H.; Spek, A. L.; Flamigni, A.; Bacac, M.; Sava, G.; Haasnoot, J. G.; Reedijk, J. *J. Biol. Inorg. Chem.* **2004**, *9*, 354.
(6) Nováková, O.; Kaspárková, J.; Vrána, O.; van Vliet, P. M.; Reedijk, J.; Brabec, V. *Biochemistry* **1995**, *34*, 12369.
(7) Popov, A. M.; Egorova, M. B.; Dmitrieva, R. I.; Gindin, V. A.; Drobachenko, A. V.; Knorunzhii, V. V. *Problemy Sovrenennoi Khimii Koordinatsionnykh Soedineii*; Izd. Leningrad GOS Univ Leningrad: Leningrad, 1987.
(8) Van Vliet, P. M.; Haasnoot, J. G.; Reedijk, J. *Inorg. Chem.* **1994**, *33*, 1934.
(9) Hotze, A. C. G.; Velders, A. H.; Ugozzoli, F.; Biagini-Cingi, M.; Manotti-Lanfredi, A. M.; Haasnoot, J. G.; Reedijk, J. *Inorg. Chem.* **2000**, *39*, 3838.
(10) Velders, A. H. Ph.D. Thesis, Leiden University, 2000.
(11) Velders, A. H.; Hotze, A. C. G.; Haasnoot, J. G.; Reedijk, J. *Inorg. Chem.* **1999**, *38*, 2762.
(12) Velders, A. H.; Hotze, A. C. G.; Van Albada, G.; Haasnoot, J. G.; Reedijk, J. R. *Inorg. Chem.* **2000**, *39*, 4073.
(13) Velders, A. H.; Hotze, A. C. G.; Reedijk, J. *Chem. Eur. J.* **2004**, submitted for publication.
(14) Krause, R. A. *Inorg. Chim. Acta* **1977**, *22*, 209.
(15) Krause, R. A. *Struct. Bonding* **1987**, *67*, 1.

Table 1. Crystallographic Data for **1**

empirical formula	$C_{21}H_{17}N_5Cl_2Ru(H_2O)_{0.5}$	V, Å^3	4112.2(8)
fw	520.38	D_{calcd}, g cm^{-3}	1.681
cryst syst	monoclinic	μ_{calcd}, cm^{-1}	1.043 (Mo Kα, graphite monochromator)
space group	C2/*c* (No. 15)	T, K	150
Z	8	R1[a]	0.0296 [for 4699$F_o > 4\sigma(F_o)$]
a, *b*, *c*, Å	36.056(4), 7.9181(10), 16.039(2)	wR2[b]	0.0657
β, deg	116.099(10)	GOF	1.020

[a] $R1 = \sum||F_o| - |F_c||/\sum|F_o|$. [b] $wR2 = [\sum[w(F_o^2 - F_c^2)^2/\sum[w(F_o^2)^2]]^{1/2}$.

recrystallized by slow addition of diethyl ether. The blue fraction yielded 80 mg of crystalline α-[Ru(azpy)$_2$Cl$_2$], while the violet fraction yielded 0.51 g of pure α-[Ru(azpy)(bpy)Cl$_2$]. The minor blue-purple fraction yielded after slow crystallization pure α-[Ru(azpy)(bpy)Cl$_2$]. Recrystallization from chloroform−ether resulted in crystals suitable for X-ray diffraction studies. Yield: 0.60 g (45%). Anal. Calcd for $RuC_{21}H_{17}N_5Cl_2 \cdot H_2O$: C 47.65, H 3.62, N 13.23. Found: C 47.34, H 3.72, N 13.53. ^{1}H NMR (300 MHz, chloroform-*d*): δ 9.75 (d, 6A), 9.61 (d, 6), 8.47 (d, 3A), 7.96 (m, 3′ + 4A), 7.87 (d, 3), 7.76 (m, 4′ + 5A), 7.70 (t, 4), 7.36 (t, 5), 7.15 (t, *p* + 5′), 7.13 (t, *m* + 6′), 6.88 (d, *o*).

α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)](PF$_6$)$_2$, 2. α-[Ru(azpy)(bpy)Cl$_2$] (50.0 mg, 9.76×10^{-5} mol) was refluxed for 1.5 h in 25 mL of water containing 34.6 mg (2.04×10^{-4} mol) of AgNO$_3$. A 27.0 mg (1.51×10^{-4} mol) sample of 9-EtGua was added to the filtered solution, and the mixture was stirred for 1 day at room temperature. The solution was then filtered to remove unreacted 9-EtGua. A concentrated aqueous solution of 0.5 g of NH_4PF_6 was then added. A red precipitate was collected by filtration and washed with cold water. The product was dried in vacuo over P_4O_{10}. Yield: 0.050 g (55%). ^{1}H NMR (600 MHz, acetone-d_6, 293 K): δ 11.20 (s, NH1), 9.06 (d, 6), 8.96 (d, 6A), 8.87 (d, 3A), 8.45 (t, 3′ + 4A), 8.37 (d, 3), 8.15 (m, 4′ + 4), 7.98 (t, 5A), 7.76 (t, 5), 7.69 (s, H$_2$O), 7.47 (t, 5′), 7.38 (d + s, 6′ + 8), 7.30 (t, *p*), 7.15 (t, *m*), 6.87 (d, *o*), 6.75 (s, NH$_2$), 3.93 (q, CH$_2$), 1.17 (t, CH$_3$).

ESI MS: *m*/*z* 638.2, {[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)]$^{2+}$ − H$^+$}; 619.1, [Ru(azpy)(bpy)(9-EtGua-H)]$^+$; 309.6, [Ru(azpy)(bpy)(9-EtGua)]$^{2+}$.

Methods and Instrumentation. NMR experiments were performed on Bruker 300 DPX and 600 DMX spectrometers. Spectra were recorded in CDCl$_3$ and acetone-d_6 unless otherwise denoted and calibrated on the residual solvent peaks. 2D ^{1}H−^{1}H NOESY experiments were performed with a mixing time of 0.8 s. Elemental analyses (C, H, and N) were carried out on a Perkin-Elmer 2400 CHNS analyzer. Mass spectra were performed on a Finnigan MAT 900 instrument equipped with an electrospray interface (ESI).

Crystal Structure Determination of 1. A crystal suitable for X-ray structure determination was glued to the top of a glass capillary and transferred into the cold nitrogen stream on a Nonius Kappa CCD diffractometer on a rotating anode. The crystal data and details on the data collection are presented in Table 1. The unit-cell parameters were checked for the presence of a higher lattice symmetry.[16] The structure was solved by automated direct methods using SHELXS86[17] and refined on F^2 using full-matrix least-squares techniques (SHELXL-97).[18] The water hydrogen atoms were located on a difference Fourier map, and their coordinates were included as parameters on the refinement. All other hydrogen atoms were included in the refinement on calculated positions riding on their carrier atoms. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were included in the refinement with a fixed isotropic displacement parameter related to the value of the equivalent isotropic displacement parameter of their carrier atoms. The intensities of 25939 reflections were measured ($1.6° < \theta < 27.46°$, $-45 < h < +46$, $-10 < k < +10$, $-20 < l < +20$, φ and ω area detector scans, with a crystal to detector distance of 40 mm, 2.9 h X-ray exposure time), 4699 of which were unique ($R_{int} = 0.0508$, $R_\sigma = 0.0471$), using a black crystal of approximate dimensions $0.10 \times 0.15 \times 0.30$ mm. No absorption correction was applied. A total of 270 parameters were refined. The final residual density was in the range $-0.60 < \Delta\rho < +0.57$ e Å^{-3}. Neutral atom scattering factors and anomalous dispersion corrections were taken from the *International Tables for Crystallography*.[19] Geometrical calculations and illustrations were performed with Platon.[20]

Results and Discussion

General Information. To synthesize the mixed-ligand complex α-[Ru(azpy)(bpy)Cl$_2$], a modified procedure has been applied based upon a method used in the literature.[7] The crude product obtained after reaction contained a mixture of α-, *β*-, and *γ*-[Ru(azpy)$_2$Cl$_2$] (30%), the mixed-ligand compound **1** (60%), and *cis*-[Ru(bpy)$_2$Cl$_2$] (10%), as determined by NMR spectroscopy. To obtain the compound α-[Ru(azpy)(bpy)Cl$_2$] pure, the mixture was separated over a neutral alumina column. The synthesis of α-[Ru(azpy)(bpy)Cl$_2$] (α indicating the pyridine ring of the azpy ligand is *trans* to the pyridine ring of the bpy ligand) was originally reported by Popov et al.[7] They also reported that a small amount (1:20) of *β*-[Ru(azpy)(bpy)Cl$_2$] (*β* indicating the Cl ligands are in *cis* positions to each other and the azo nitrogen is *trans* to the pyridine ring of the bpy ligand) was generated as a side product (Figure 2).[7] Unfortunately, they only report some TLC data and IR data, but no NMR data. Under the experimental conditions of Popov et al.[7] and other experimental conditions, i.e., low-boiling-point solvents, variable reaction times, and relatively low temperatures, no other isomers could be obtained, suggesting that Popov et al.[7] have incorrectly characterized their products. It is not yet clear why the α isomer is thermodynamically favored.

To investigate the interaction of **1** with DNA model bases, it was decided to start from α-[Ru(azpy)(bpy)(H$_2$O)$_2$]$^{2+}$, which is expected to react faster with nitrogen bases than the corresponding parent compound. For this reason **1** was first reacted with AgNO$_3$ before the model base 9-EtGua was added. The reaction of α-[Ru(azpy)(bpy)(H$_2$O)$_2$]$^{2+}$ with

(16) Spek, A. L. *J. Appl. Crystallogr.* **1988**, *21*, 578.
(17) Sheldrick, G. M. *Shelxs86, Program for crystal structure determination*; University of Göttingen: Göttingen, Germany, 1986.
(18) Sheldrick, G. M. *Shelxl-97-2, Program for crystal structure refinement*; University of Göttingen: Göttingen, Germany, 1997.
(19) Wilson, A. J. C., Ed. *International Tables for Crystallography*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1992; Vol. C.
(20) Spek, A. L. *PLATON—A multi-purpose crystallographic tool*; Utrecht University: Utrecht, The Netherlands, 2003.

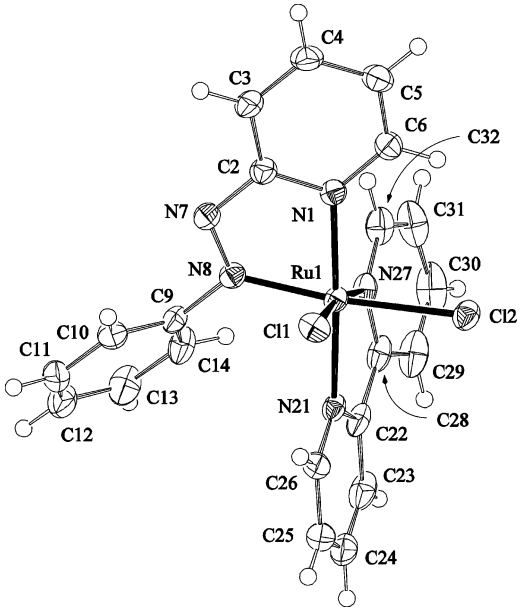

Figure 3. Atomic displacement ellipsoid plot[21] of **1** drawn at the 50% probability level.

9-EtGua was carried out at room temperature, as under these conditions only the kinetically favored adduct with the 9-EtGua *trans* to the Nazo (Nazo = azo nitrogen) atom (α-[Ru(azpy)(bpy)(9-EtGua)(H_2O)]$^{2+}$) was formed. Nevertheless, the reaction was also performed at higher temperatures (40 °C and under reflux), resulting in mixtures of α- and α′-[Ru(azpy)(bpy)(9-EtGua)(H_2O)]$^{2+}$ (α′ indicating 9-EtGua is *trans* to the bpy nitrogen). The isomerization reaction of α- to α′-[Ru(azpy)(bpy)(9-EtGua)(H_2O)]$^{2+}$ in acetone-d_6 was found to be almost complete after about 3 weeks at room temperature (in fact, approximately only 20% of α is still present at this time). After this three week period, the ratio of α to α′ did not change in time anymore. The isomerization also took place in H_2O, albeit much slower, so it seems likely that especially acetone facilitates this isomerization process.

Single-Crystal Structure Determination of 1. The molecular structure of **1** (Figure 3) shows the azpy ligand and bpy ligand coordinated in such a way that the pyridine ring of the azpy ligand is *trans* positioned relative to the pyridine ring of the bpy ligand, defined as the α configuration. Crystallographic data are summarized in Table 1. Selected bond distances and angles are listed in Table 2. The Ru−Npy (Npy = pyridine nitrogen) and Ru−Nazo (Nazo = coordinating azo nitrogen) distances of the azpy ligand are comparable to those in the related compound[21] α-[Ru(azpy)$_2$-Cl_2]. The N═N distance is 1.313(3) Å. The angles N8−Ru−N1 and N21−Ru−N27 are 77.26(7)° and 78.79(7)°, respectively, revealing considerable distortion of the octahedron. The angle Cl1−Ru−Cl2 is 88.72(2)°, which is slightly smaller than the corresponding angle[21] in α-[Ru(azpy)$_2$$Cl_2$] (89.44°). The crystal structure contains one water molecule located on a crystallographic 2-fold rotation axis connecting two symmetry-related molecules of **1** by donating a H-bond from OH to one (*trans* to Nazo nitrogen atom) of the coordinated chloride ions (distance O···Cl = 3.408(3) Å).

(21) Seal, A.; Ray, S. *Acta Crystallogr.* **1984**, *C40*, 932.

Table 2. Selected Bond Distances (Å) and Angles (deg) of **1**

Bond Distances (Å)			
Ru(1)−N(1)	2.0279(17)	Ru(1)−Cl(2)	2.4179(7)
Ru(1)−N(8)	1.937(2)	N(8)−N(7)	1.313(3)
Ru(1)−N(21)	2.0639(17)	N(8)−C(9)	1.439(3)
Ru(1)−N(27)	2.0507(18)	N(7)−C(2)	1.381(3)
Ru(1)−Cl(1)	2.3978(7)		

Bond Angles (deg)			
Cl(1)−Ru(1)−Cl(2)	88.72(2)	Cl(2)−Ru(1)−N(27)	85.08(6)
Cl(1)−Ru(1)−N(1)	86.02(5)	N(1)−Ru(1)−N(8)	77.26(7)
Cl(1)−Ru(1)−N(8)	87.55(6)	N(1)−Ru(1)−N(21)	177.44(8)
Cl(1)−Ru(1)−N(21)	94.62(5)	N(1)−Ru(1)−N(27)	100.83(7)
Cl(1)−Ru(1)−N(27)	171.14(6)	N(8)−Ru(1)−N(21)	100.27(1)
Cl(2)−Ru(1)−N(1)	95.27(6)	N(8)−Ru(1)−N(27)	99.39(8)
Cl(2)−Ru(1)−N(8)	171.86(5)	N(21)−Ru(1)−N(27)	78.79(7)
Cl(2)−Ru(1)−N(21)	87.23(6)		

NMR Characterization of 1. The ^{1}H NMR spectrum of **1** in $CDCl_3$ shows 15 proton resonances, which indicate the formation of the mixed-ligand complex **1**. The resonances have been assigned using 2D COSY and NOESY NMR spectroscopy. Two characteristics of the azpy and bpy ligands are important to take into account first. (1) The resonances corresponding to the H6 atoms of both azpy and bpy ligands have a smaller *J* coupling than the resonances corresponding to the H3 atoms. (2) The bpy ligand shows a characteristic H3−H3′ NOE, which allows immediate assignment of all resonances of the bpy ligand.

Two proton resonances (with small *J* coupling) are observed at 9.75 and 9.61 ppm, i.e., at relatively low field, indicating a considerable deshielding effect of nearby Cl ligands. X-ray data (vide supra) show two H6 atoms, H6A and H6, close to the Cl ligands (H6A−Cl_1 = 4.165 Å, H6A−Cl_2 = 2.890 Å, H6−Cl_1 = 2.687 Å, and H6−Cl_2 = 4.021 Å). Using the H3−H3′ NOE of the bpy ligand, the signal at 9.61 ppm is assigned to the H6 atom of the bpy ligand. The resonance corresponding to H6′ of the bpy ligand appears at high field. As a consequence the signal at 9.75 ppm is assigned as the H6A resonance. The 2D NOESY spectrum (Figure 4) shows strong cross-peaks between the H6 and *ortho* protons and between the H6A and H6′ resonances. These two cross-peaks confirm the α configuration. The upfield position of the H6′ resonance further establishes the α configuration, as this hydrogen atom is within the shielding cone of the azpy pyridine ring. These NOESY NMR data are in correspondence with X-ray data; i.e., the distances H6A−H6′ and H6−H*o* are, respectively, 3.044 and 4.990 Å (the latter one is the average value of H6 to the two *ortho* hydrogens as in solution fast rotation of the phenyl ring occurs).

NMR Structural Characterization of the Product of the Reaction of α-[Ru(azpy)(bpy)(H_2O)$_2$]$^{2+}$ with the DNA Model Base 9-Ethylguanine. As the α-[Ru(azpy)(bpy)] moiety is asymmetric, two different coordination sites are present in α-[Ru(azpy)(bpy)(H_2O)$_2$]$^{2+}$. Using different conditions, i.e., other reaction temperatures and an excess of 9-EtGua, did not result in coordination of two 9-EtGua model bases. For this reason two different monofunctional adducts with one coordinated 9-EtGua would be expected upon reaction of α-[Ru(azpy)(bpy)(H_2O)$_2$]$^{2+}$ with 9-EtGua: one with the 9-EtGua positioned *trans* to the azo nitrogen of the

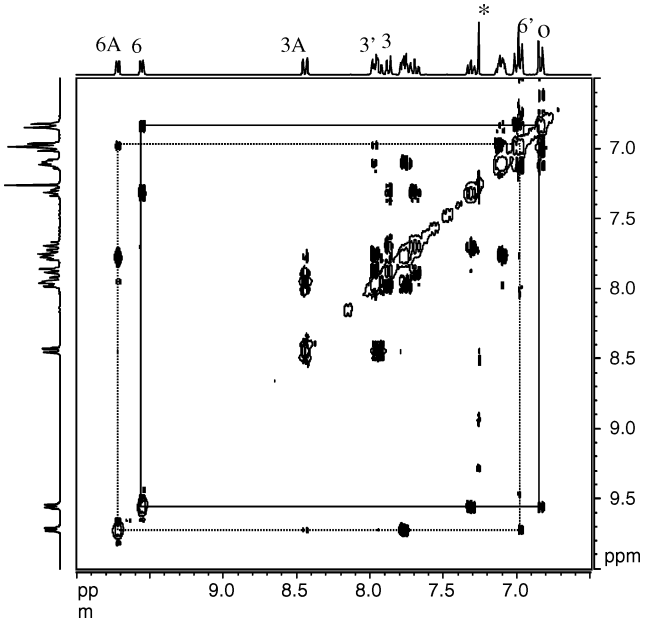

Figure 4. 2D ^{1}H−^{1}H NOESY spectrum (300 MHz) of **1** in $CDCl_3$ (asterisk denotes $CHCl_3$) showing the NOEs, which prove the α configuration, the solid line indicating the H6−*o* NOE and the dashed line indicating the H6A−H6′ NOE.

Table 3. Selected Proton Chemical Shift Values (ppm) for α-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$ (α) and α′-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$ (α′) in Acetone-d_6 at 293 K and 600 MHz

	6 6A	5 5A	4 4A	3 3A	6′ *o*	5′ *m*	4′ *p*	3′	NH(1)	H8	NH_2	H_2O
α	9.06	7.76	8.13	8.37	7.38	7.47	8.16	8.45	11.20	7.38	6.75	7.69
	8.96	7.98	8.45	8.87	6.87	7.15	7.30					
α′	8.76	7.61	8.15	8.48	7.28	7.48	8.19	8.53	11.14	7.19	6.79	8.84
	9.13	8.08	8.44	8.88	6.98	7.13	7.30					

azpy ligand and one with the model base positioned *trans* to the bpy nitrogen. Nevertheless, the 1:1 reaction of 9-EtGua with α-[Ru(azpy)(bpy)(H$_2$O)$_2$]$^{2+}$ for 24 h at room temperature results in the formation of only one adduct. This adduct is isolated as α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)](PF$_6$)$_2$ with 9-EtGua *trans* to the azo nitrogen atom (Nazo), as concluded from ^{1}H NMR and 2D NOESY data (vide infra). The 9-EtGua signals are clearly present in the ^{1}H NMR spectrum (600 MHz, 293 K) in pure acetone-d_6 (Table 3, Figure 5), i.e., the NH_2 group at 6.75 ppm, the H8 at 7.38 ppm, the NH(1) at 11.20 ppm (not shown), and the CH_2 and CH_3 at 3.93 and 1.17 ppm (not shown), respectively.

Only one set of 9-EtGua resonances arises with integration values corresponding to a 1:1 adduct. The 9-EtGua ligand coordinates to ruthenium via its N7 atom, which is easily concluded on the basis of the fact that the H8 resonance shows NOE cross-peaks to the ligand backbone (vide infra). The H6−H*o* and H6A−H6′ NOE cross-peaks, together with the H6′ resonance at high field, agree with the retained configuration of the azpy and bpy ligands with respect to **1** (Figure 6). 2D NOESY NMR has also been used to determine the position and orientation of the 9-EtGua model base. The 2D NOESY spectrum (Figure 6) has been recorded at 263 K (600 MHz), as at this temperature the H8 and H6′ resonances do not overlap. The 2D NOESY spectrum shows the H6−H_2O, H8−H6A, and H8−H6′ cross-peaks. The cross-peak between the H6 and H_2O resonances shows the vicinity of the H6 atom to the water ligand, directly proving that the water ligand is *cis* to the azo nitrogen and that the 9-EtGua is coordinated *trans* to the azo nitrogen. The H8−H6A and H8−H6′ (the latter one is difficult to see due to the closeness to the diagonal) NOE cross-peaks indicate the orientation of 9-EtGua with the H8 atom wedged between the azpy and bpy pyridine rings. In this orientation the keto group is directed toward the aqua ligand. A hydrogen bond between the aqua ligand and the keto group of 9-EtGua is likely to stabilize this conformation, as has also been reported for the related α- and β-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$ complexes.[9,10] The H6A resonance is now located upfield of the H6 resonance, which is the opposite of the pattern in the parent compound **1** (although it should be noted that the ^{1}H NMR spectra of **1** and α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)]$^{2+}$ are recorded in different solvents). The H6A atom in the 9-EtGua adduct is now no longer deshielded by the *trans*-azo Cl ligand like in **1**, but shielded by the five-membered ring of the 9-EtGua model base. The absence of the NH_2 resonance in the spectrum of α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)](PF$_6$)$_2$ at 263 K is an interesting feature. Variable-temperature ^{1}H NMR measurements of **2** reveal (data not shown) that the NH_2 resonance broadens extensively upon cooling and seems to resharpen again at 203 K. The same phenomenon is observed in variable-temperature ^{1}H NMR measurements of the isomerization adduct α′. The origin for this behavior is not clear, but might be due to the formation of intermolecular hydrogen bonds at low temperature.

In Situ Isomerization of α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)]$^{2+}$ (9-EtGua *trans* to the Nazo Atom) to α′-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)]$^{2+}$ (9-EtGua *trans* to the Bpy Nitrogen). An in situ and spontaneous isomerization of the complex takes place when it is allowed to stand in acetone-d_6. After approximately three weeks α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)](PF$_6$)$_2$ has been almost completely converted into another compound according to NMR data (Figure 5, Table 3). The ^{1}H NMR spectra show that all resonances of α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)](PF$_6$)$_2$ have been extensively diminished in intensity and are replaced by new resonances. The resonances corresponding to H6, H6A, H*o*, H_2O, and H8 of α′-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)](PF$_6$)$_2$ are very obviously shifted relative to those of the original α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)](PF$_6$)$_2$. The ^{1}H and 2D NMR data clearly prove that the α configuration of the [Ru(azpy)(bpy)] moiety is retained and that 9-EtGua is still coordinated to the ruthenium via its N7 atom. All signals have been assigned using 2D COSY and NOESY NMR spectroscopy.

The 2D NOESY spectrum (600 MHz) of the new adduct is recorded at 263 K as at this temperature the signals show less overlap. The spectrum displays cross-peaks other than those of the original adduct, H8−H*o*, H8−H6, and H6A−H_2O couplings (Figure 7). Especially the NOE cross-peak between the H6A and H_2O resonances (Figure 7) proves that the 9-EtGua model base has been shifted from the position *trans* to the azo nitrogen to the position *trans* to the bpy

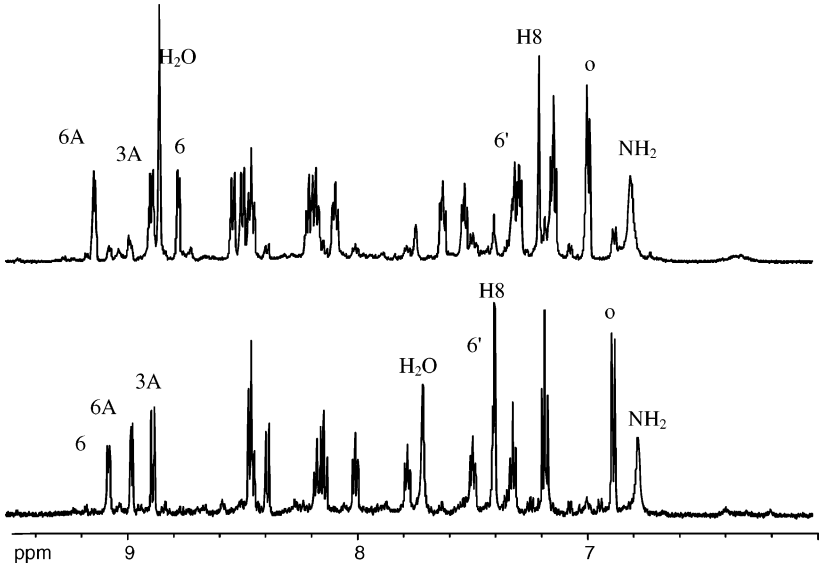

Figure 5. Aromatic region of the ^{1}H NMR spectra (600 MHz) of α-[Ru(azpy)(bpy)(9-EtGua)(H_2O)]$^{2+}$ (lower spectrum) and the α′-[Ru(azpy)(bpy)(9-EtGua)(H_2O)]$^{2+}$ appearing after three weeks (upper spectrum) in acetone-d_6 at 293 K. The most important resonances are indicated.

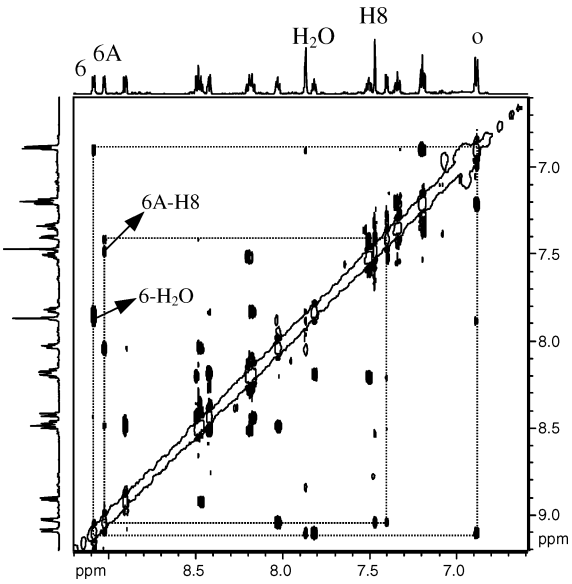

Figure 6. 2D ^{1}H−^{1}H NOESY spectrum (600 MHz) and some assignments (proton numbering as in Figures 1 and 8) of the aromatic region of α-[Ru(azpy)(bpy)(9-EtGua)(H_2O)](PF_6)$_2$ in acetone-d_6 at 263 K. The H6A−H8 and H6−H_2O NOEs are indicated; the H8−H6′ is difficult to see in this picture due to the closeness to the diagonal. The dotted lines indicate the H6−H*o* NOE and H6A−H6′ NOE of the α-[Ru(azpy)(bpy)] backbone.

nitrogen (Figure 8), resulting in α′-[Ru(azpy)(bpy)(9-EtGua)(H_2O)]$^{2+}$ (α′ indicating 9-EtGua is *trans* to the bpy nitrogen atom). The H8−H*o* cross-peak indicates that the H8 atom of 9-EtGua is in the vicinity of the *ortho* atoms, and together with the H8−H6 NOE, it suggests that the H8 is wedged between the phenyl ring and the pyridine ring. In this position again a hydrogen bond is possible between the keto group and the coordinated water. An upfield shift is noted of the H6 resonance in α′-[Ru(azpy)(bpy)(9-EtGua)(H_2O)]$^{2+}$ related to the chemical shift of the H6 resonance in **1** (although it should be noted that the ^{1}H NMR spectra of **1** and α′-[Ru(azpy)(bpy)(9-EtGua)(H_2O)]$^{2+}$ are recorded in different solvents). In comparison to that of the parent α-[Ru(azpy)(bpy)Cl_2] complex, the H6 atom in the *cis* adduct is now shielded due to the five-membered ring of the 9-EtGua model base and no longer deshielded by the chloride *cis* to the azo nitrogen. The H_2O signal shows a relatively large downfield shift in α′-[Ru(azpy)(bpy)(9-EtGua)(H_2O)]$^{2+}$ relative to the α adduct (Figure 5). The origin for this downfield shift of the H_2O resonance might be that the hydrogen bonding between the keto group and the coordinated water ligand in the α′ isomer is significantly stronger than in the α isomer. The reason for this phenomenon is not clear, yet.

In addition to the NMR characterization of this isomerization product, also a mass spectrum was taken of the NMR sample of **2**, after it stood in acetone-d_6 for three weeks. The mass spectrum from this aged sample displays the same peaks as a fresh sample, which proves that the [Ru(azpy)(bpy)(9-EtGua)(H_2O)]$^{2+}$ moiety is still present.

Mechanism of Isomerization. Although the isomerization mechanism has not been investigated in full detail, a few comments can be made. The stronger π-accepting properties of the azo nitrogen related to the bpy nitrogen[22] will lead to the kinetic favoring of substitutions at the position *trans* to the azo nitrogen. On the other hand, the kinetic *trans* effect in octahedrally coordinated complexes is not as pronounced as in square planar complexes. It is assumed that the stronger hydrogen bonding in the α′ isomer thermodynamically drives the isomerization. This hypothesis is further confirmed by experimental model base studies (data not shown) with the model base 1-methylimidazole (1-MeIm). The compound α-[Ru(azpy)(bpy)(1-MeIm)(H_2O)]$^{2+}$ also shows in situ isomerization in acetone-d_6, but this results in an equilibrium between the α′ and α isomers. This equilibrium probably stems from the absence of a thermodynamically favored isomer, as no keto group is present in 1-MeIm which could be involved in hydrogen bonding (data not shown).

Isomerization Process and Cytotoxicity. Compound **1** appears to be an important and interesting model com-

(22) Juris, A.; Balzani, V.; Barigelletti, F.; Campagna, S.; Belser, P.; von Zelewsky, A. *Coord. Chem. Rev.* **1988**, *84*, 85.

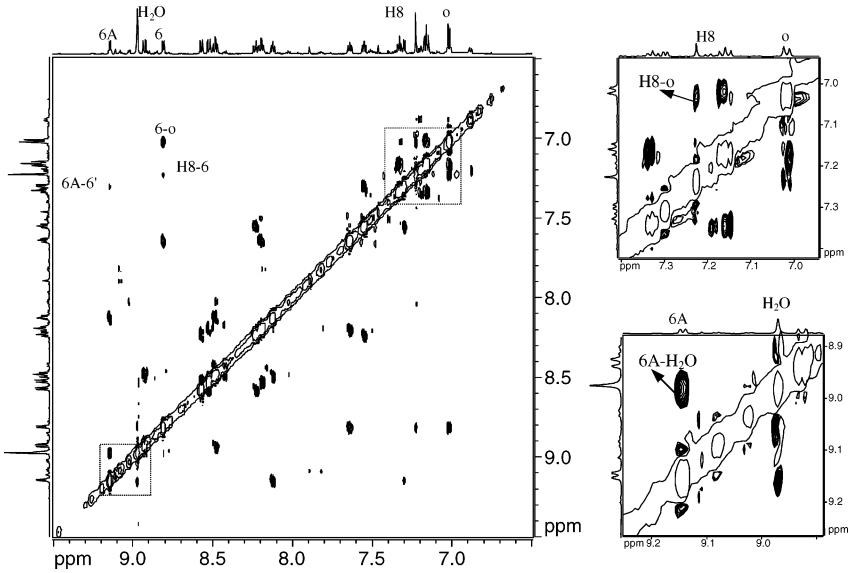

Figure 7. 2D $^1H-^1H$ NOESY spectrum (600 MHz) and some assignments (proton numbering as in Figures 1 and 8) of the aromatic region of α′-[Ru(azpy)(bpy)(9-EtGua)(H_2O)](PF_6)$_2$ in acetone-d_6 at 263 K. This measurement has been performed using an old sample of **2** (three weeks in acetone-d_6). In the left panel the NOEs H6−H*o* and H6A−H6′ are indicated to prove the α configuration. The NOEs H6−H8, H6A−H_2O (enlargement), and H8−H*o* (enlargement) prove the position and orientation of 9-EtGua in the α′ adduct.

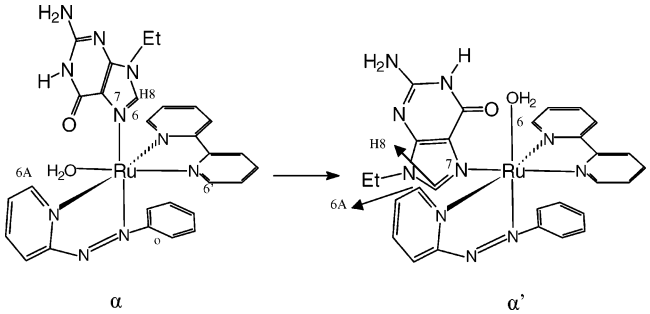

Figure 8. Schematic representation of the α (left) and α′ (right) isomers of [Ru(azpy)(bpy)(9EtGua)(H_2O)]$^{2+}$ (and numbering used for the NMR assignment on the right-hand side also indicated by arrows).

pound for the understanding of the cytotoxicity of the [Ru(azpy)$_2$Cl$_2$] compounds. In fact, IC_{50} values of **1** determined in several human tumor cell lines[23] (A498, EVSA-T, H226, IGROV, M19, MCF-7, and WIDR) show that **1** displays mostly a low to moderate cytotoxicity (IC_{50} = 2500−20000 ng/mL) (Supporting Information Table S1). It is interesting to compare the cytotoxicity of **1** with those of the highly cytotoxic[4,5] α-[Ru(azpy)$_2$Cl$_2$], moderately cytotoxic[4,5] β-[Ru(azpy)$_2$Cl$_2$], and inactive *cis*-[Ru(bpy)$_2$Cl$_2$] (see Supporting Information Table S1) to find some structure−activity relationships for this kind of complex. The low cytotoxicity of **1** (in most cell lines) would have been difficult to explain beforehand and might be caused by a different accessibility to DNA coordination or by interaction with other biological targets. Different electronic properties of the azpy versus bpy ligand might also be responsible for the different cytotoxicities of the highly cytotoxic [Ru(azpy)$_2$Cl$_2$] compounds relative to the inactive *cis*-[Ru(bpy)$_2$Cl$_2$] and in most cell lines lowly cytotoxic **1**.

To explain the difference in cytotoxicity of this kind of complex, initially DNA model base binding studies have been used, as it is generally thought that DNA might be the target of antitumor-active ruthenium complexes.[3] DNA model base studies with α-[Ru(azpy)$_2$Cl$_2$] and *cis*-[Ru(bpy)$_2$Cl$_2$] have shown that both compounds bind only one 9-EtGua model base coordinated via the N7 atom,[8,9] in accordance to the results presented above.

The observed positional shift of 9-EtGua in **2** is unprecedented in metal nucleobase chemistry. Often another kind of isomerization is observed, i.e., the shift of the metal from one coordination site of the model base to the other. Especially in platinum adenine chemistry several examples of linkage isomerization are known.[24,25] Also in ruthenium chemistry this linkage isomerization has been described for pentaammine(hypoxanthine)ruthenium complexes, and again in this case it is the coordination site of the model base which varies.[26] Competition between the metal ion and a proton will occur with kinetically labile species; a metal bound at low pH via the N7 atom of 9-EtGua will cross over to the N1 site if the pH is raised.[27] Changes in pH[24−27] or changes in oxidation state[28,29] are generally involved in such linkage isomerization processes. Furthermore, linkage isomerization processes of *cis*- and *trans*-[$PtCl_2(NH_3)_2$]-like species in oligonucleotides are driven by strained initial structures.[30−32]

(23) The cytotoxicity of the ruthenium(II) complexes was tested in vitro applying the human tumor cell lines EVSA-T (breast cancer), WIDR (colon cancer), IGROV (ovarian cancer), M19 (melanoma), A498 (renal cancer), and H226 (non-small-cell lung cancer) using the SRB test to determine the cell viability.

(24) Arpalahti, J.; Klika, K. D. *Eur. J. Inorg. Chem.* **1999**, 1199.
(25) Arpalahti, J.; Klika, K. D.; Molander, S. *Eur. J. Inorg. Chem.* **2000**, 1007.
(26) Clarke, M. J. *Inorg. Chem.* **1977**, *16*, 738.
(27) Lippert, B. *Coord. Chem. Rev.* **2000**, *200−202*, 487.
(28) Lippert, B.; Schollhorn, H.; Thewalt, U. *J. Am. Chem. Soc.* **1986**, *108*, 6616.
(29) Pichierri, F.; Holthenrich, D.; Zangrando, E.; Lippert, B.; Randaccio, L. *J. Biol. Inorg. Chem.* **1996**, *1*, 439.
(30) Yang, D.; van Boom, S. S. G. E.; Reedijk, J.; Van Boom, J. H.; Wang, A. H. J. *Biochemistry* **1995**, *34*, 12912.
(31) Dalbies, R.; Payet, D.; Leng, M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8147.
(32) Comess, K. M.; Costello, C. E.; Lippard, S. J. *Biochemistry* **1990**, *29*, 2102.

Very remarkably, in the case of the isomerization of **2**, it was proven that the model base remains coordinated at the N7 atom of 9-EtGua, but moves to another coordination site of the metal complex. In the 9-EtGua studies performed with the symmetric compounds α-[Ru(azpy)$_2$Cl$_2$] and *cis*-[Ru(bpy)$_2$Cl$_2$], however, it was impossible to determine whether the 9-EtGua can switch positions between the two coordination sites, as both sites are equivalent.

More useful for comparison are the two isomeric complexes[10] *β*- and *β*′-[Ru(azpy)$_2$(9-EtGua)X]$^{2+}$ (*β* indicates the isomer with the 9-EtGua *trans* to the Nazo atom, *β*′ the isomer with the 9-EtGua *trans* to the bpy nitrogen). The backbone *β*-[Ru(azpy)$_2$] results in the same sort of coordination sites as the α-[Ru(azpy)(bpy)] backbone, i.e., one site *trans* to the azo nitrogen and one site *trans* to the bpy nitrogen (Figure 1).

A mixture of the two adducts *β*- and *β*′-[Ru(azpy)$_2$(9-EtGua)X]$^{2+}$ is isolated as described in earlier studies[10] under experimental conditions slightly different from those of the now reported α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)]$^{2+}$ (i.e., *β*-[Ru(azpy)$_2$(NO$_3$)$_2$] in reaction with 9-EtGua in water at 40 °C, for 4 days). However, if the reaction between *β*-[Ru(azpy)$_2$(NO$_3$)$_2$] and 9-EtGua is performed at room temperature and the reaction time is only 1 day, only the *β* isomer is obtained and can be isolated as *β*-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$, in which the model base is also coordinated *trans* to the azo nitrogen (data not shown). In acetone-d_6 this isomer is slowly converted into the *β*′ isomer, in which the 9-EtGua model base is coordinated *trans* to the bpy nitrogen atom (data not shown). This isomerization of *β*-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$ into *β*′-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$ takes place at a slower rate than in the case of **2**; even after 2 months in acetone-d_6 at room temperature the conversion is not complete (*β*′:*β* = 1.6:1) and becomes an equilibrium.

In earlier studies[9,11,12] the difference in flexibility of potential DNA adducts has been used to explain the distinction in cytotoxic activity of the highly active α-[Ru(azpy)$_2$Cl$_2$] and inactive *cis*-[Ru(bpy)$_2$Cl$_2$]. Now, with the above-mentioned positional shift of 9-EtGua in mind, it becomes attractive to correlate such isomerization processes of DNA (model base) adducts with the presence or absence of cytotoxic activity. The 9-EtGua adducts of the moderately cytotoxic *β*-[Ru(azpy)$_2$] moiety and lowly cytotoxic α-*cis*-[Ru(azpy)(bpy)] moiety show the isomerization of the kinetically favored *trans*-Nazo adduct into the thermodynamically stable *trans*-Nbpy adduct. Concerning the 9-EtGua adduct of the highly cytotoxic α-[Ru(azpy)$_2$] moiety (in which the 9-EtGua is also coordinated *trans* to the Nazo nitrogen atom), this positional isomerization process is not detectable. The 9-EtGua might switch between the two coordination sites in α-[Ru(azpy)$_2$(H$_2$O)$_2$]$^{2+}$, but this would result in the same adduct due to the C_2 axis. Consequently, there is only the kinetically favored *trans*-Nazo adduct and no possibility to isomerize to a thermodynamically favored adduct. So it might be hypothesized that the stable *trans*-Nazo 9-EtGua adduct is a prerequisite for a biologically relevant DNA adduct, and in this way the activity of α-[Ru(azpy)$_2$Cl$_2$] is explained. Consequently, it is interesting to correlate the incomplete isomerization of *β*-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$ into *β*′-[Ru(azpy)$_2$(9-EtGua)(H$_2$O)](PF$_6$)$_2$ to the cytotoxicity of the parent compound *β*-[Ru(azpy)$_2$Cl$_2$]. The fact that *β*-[Ru(azpy)$_2$Cl$_2$] is a factor of 10 less cytotoxic than the analogous α isomer, but still shows considerable activity, might be explained by the fact that only part of the "active *trans*-Nazo 9-EtGua adduct" is converted into the inactive *trans*-Nbpy adduct. Thus, it might be speculated that the cytotoxicity of this type of complex is related to the stability of DNA adducts *trans* to the Nazo nitrogen of the azpy ligand, although more DNA studies are needed to prove this statement. Moreover, the time scale of conversion of one adduct into the other has now only been determined in acetone-d_6. Therefore, it is impossible to state if and how fast these processes might occur in biological fluids and whether these processes occur on the time scale of, for example, IC$_{50}$ determinations.

Conclusions

The X-ray structure and NMR data of the compound α-[Ru(azpy)(bpy)Cl$_2$] presented above finally and unambiguously identify the earlier reported compound as the α isomer, and the formation of other isomers is excluded. The compound α-[Ru(azpy)(bpy)Cl$_2$] shows a low to moderate cytotoxicity in several cell lines, but is an interesting compound in the series of structurally related complexes, i.e., α-[Ru(azpy)$_2$Cl$_2$], *β*-[Ru(azpy)$_2$Cl$_2$], and *cis*-[Ru(bpy)$_2$Cl$_2$], with respect to the cytotoxic activity and DNA model base binding. The spontaneous isomerization in α-[Ru(azpy)(bpy)(9-EtGua)(H$_2$O)](PF$_6$)$_2$ of the 9-EtGua model base from the position *trans* to the Nazo atom to the position *cis* to the Nazo atom is remarkable especially in correlation to low to moderate cytotoxicity and with respect to the behavior of the 9-EtGua adducts of the related complexes α-[Ru(azpy)$_2$Cl$_2$] and *β*-[Ru(azpy)$_2$Cl$_2$]. In particular, it is hypothesized that the kinetically favored and stable adduct with 9-EtGua *trans* to the Nazo atom is responsible for the cytotoxicity of α-[Ru(azpy)$_2$Cl$_2$]. The fact that the *trans*-Nazo 9-EtGua adduct is partly converted into the "*trans*-Nbpy adduct" in the case of the moderately cytotoxic complex *β*-[Ru(azpy)$_2$Cl$_2$] and lowly cytotoxic α-*cis*-[Ru(azpy)(bpy)Cl$_2$] might explain the decreased cytotoxicity.

These positional isomerization processes involving a nucleobase, which switches to another coordination site of the metal, could thus be of considerable biological significance in that the thermodynamically most stable adduct is different from the kinetically favored one. This isomerization might therefore have consequences for the coordination of this kind of complex to DNA and subsequently, with DNA as the target, might indeed influence the cytotoxicity and antitumor activity.

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COST Action D20 allowing regular research exchanges to partner laboratories inside EU countries is gratefully acknowledged. Dr. Dick de Vos (PCN, Haarlem) is kindly acknowledged for determination of the IC_{50} values.

Supporting Information Available: Table S1, comparison of the IC_{50} values (μM) of a series of structurally related ruthenium(II) complexes, i.e., α-[Ru(azpy)(bpy)Cl$_2$], *cis*-[Ru(bpy)$_2$Cl$_2$], β-[Ru(azpy)$_2$Cl$_2$],[4,5] and α-[Ru(azpy)$_2$Cl$_2$],[4,5] against a series of human tumor cell lines (PDF) and X-ray crystallographic files for **1** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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