Binding of 9-Methylhypoxanthine and 9-Ethylguanine to [*cis*-Ru(2,2′-bipyridine)$_2$]$^{2+}$. NMR and X-ray Structure of *cis*-Chlorobis(2,2′-bipyridine)(9-ethylguanine-κN^7)ruthenium(II) Chloride

Paul M. van Vliet, Jaap G. Haasnoot, and Jan Reedijk*

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Received August 5, 1993•

The synthesis and characterization of [*cis*-Ru(bpy)$_2$(9mhyp-κN^7)L]$^{+/2+}$ and [*cis*-Ru(bpy)$_2$(9egua-κN^7)L]$^{+/2+}$ (bpy = 2,2′-bipyridine, 9mhyp = 9-methylhypoxanthine, 9egua = 9-ethylguanine), where L = Cl$^-$ or H_2O, is reported. Both ions are isolated as chlorides and hexafluorophosphates and show similar proton NMR patterns for the *cis*-configurated bpy ligands. The aqua complexes (L = H_2O) have been isolated for both Ru(nucleobase-κN^7) complexes as hexafluorophosphate salts. [*cis*-Ru(bpy)$_2$(9egua-κN^7)Cl]Cl crystallizes in the triclinic space group $P\bar{1}$ with a = 11.895(4) Å, b = 12.407(2) Å, c = 13.329(5) Å, α = 91.5(2)°, β = 90.8(3)°, γ = 111.3(2)°, and Z = 1 for the formula $C_{54}H_{46}Cl_4N_{18}O_5Ru_2$. The structure was solved by direct methods and refined by Fourier and full-matrix least-squares techniques, resulting in R_w = 0.0751 for 7346 independent significant reflections. The guanine moiety is positioned with the H(8) proton directed toward the coordinating chlorine and the keto group between the pyridyl groups of the bpy ligands. The distances from the oxygen atom of the keto group to the centroids of the bpy pyridyl rings are 3.95(3) and 3.60(3) Å, respectively. An additioned stabilizing interaction between the lone pairs located on the keto group and the π systems of the pyridyl groups is deduced from comparison of the proton NMR data of the crystals and the same complex formed in solution. A statistical survey in the Cambridge Structural Database (CSD) has shown that the presented X-ray structure is the first example of such an interaction for a coordination compound. The 6-ketopurine orientation is important for studies on the covalent binding of [*cis*-Ru(diimine)$_2$]$^{2+}$ compounds to the N(7) site of guanine in B-DNA.

Introduction

Quite a few metal complexes show *in vitro* tumor-inhibiting effects.[1] It is likely that metal–DNA interactions are involved in these effects.[2-4] For amine complexes of Pt(II) and Ru(II) containing reactive chloro or aqua ligands, the pyrimidine N(7) sites of DNA are likely targets, because they are readily accessible in the major DNA groove.[3] The antitumor properties of [*cis*-Pt(NH$_3$)$_2$Cl$_2$] (cisplatin) have been intensely studied both in the chemical and in the medical area of science, and major progress has been achieved. From this work, it is well known that when cisplatin is incubated with DNA, intrastrand Pt(guanine-κN^7, guanine-κN^7) chelates are formed in statistically larger amounts as would be expected. These observations have led to a great deal of understanding of the antitumor properties of the drug cisplatin.[4]

A few Ru compounds have also been shown to exhibit antitumor effects. The most promising examples are [*trans*-Ru(dmso)$_4$Cl$_2$] (dmso = dimethyl sulfoxide) and [H$_2$im][*trans*-Ru(Him)$_2$Cl$_4$] (Him = imidazole), the latter being already tested in preclinical trials.[5] For both Ru complexes, the binding to DNA has been studied, and in the case of [*trans*-Ru(dmso)$_4$Cl$_2$] also DNA model compounds were used. [H$_2$im][*trans*-Ru(Him)$_2$Cl$_4$] inhibits *Escherichia coli* DNA polymerase I after preincubation with DNA.[6] [*trans*-Ru(dmso)$_4$Cl$_2$] binds *in vitro* to plasmid DNA, causing inhibition of restriction enzymes which normally cut the DNA at guanine-rich sites.[7] When [*trans*-Ru(dmso)$_4$Cl$_2$] is reacted with 2′-deoxyguanosine or d(GpG), a *cis*-configurated N(7) bis adduct[8] and a Ru(2′-deoxyguanylyl-κN^7-(3′→5′)-2′-deoxyguanosine-κN^7) chelate[9] are formed, respectively. This complex is therefore, at least for single-stranded DNA, just like cisplatin, capable of binding two guanine ligands at the N(7) sites in a *cis* configuration.

Covalent binding of rigid Ru complexes to DNA in general is not yet very well developed, and therefore it was considered interesting to study the binding of Ru compounds to relatively simple guanine derivatives in aqueous solutions at neutral pH. Ru(II) compounds of the form [Ru(diimine)$_2$Cl$_2$] are quite stable and were found suitable for proton NMR studies.[10] The Λ isomer of [*cis*-Ru(phen)$_2$Cl$_2$] (phen = 1,10-phenanthroline) has been shown to have an enantioselective binding toward B-DNA.[11] As a matter of fact, covalent binding of [*cis*-Ru(bpy)$_2$(OH$_2$)$_2$]$^{2+}$ (bpy = 2,2′-bipyridine) to DNA has been reported as an undesired side effect of probing DNA with a derivative of [Ru(bpy)$_3$]$^{2+}$.[12] The bpy diaqua complex was, among other compounds of the forms [L$_4$Ru(OH$_2$)$_2$]$^{2+}$ and [L$_5$RuOH$_2$]$^{2+}$, recently used in enantioselectivity studies with B-DNA.[13] Several studies have shown that bpy induces only minimal intercalative binding with DNA.[14] Therefore [*cis*-Ru(bpy)$_2$Cl$_2$] was expected to be a useful

* Author to whom correspondence should be addressed.
• Abstract published in *Advance ACS Abstracts*, March 15, 1994.

(1) Keppler, B. K. *New J. Chem.* **1990**, *14*, 389.
(2) Lippert, B. *Prog. Inorg. Chem.* **1989**, *37*, 1.
(3) Clarke, M. J. *Prog. Clin. Biochem. Med.* **1989**, *10*, 25.
(4) (a) Reedijk, J. *Inorg. Chim. Acta* **1992**, *198–200*, 873 and references therein. (b) Sundquist, W. I.; Lippard, S. J. *Coord. Chem. Rev.* **1990**, *100*, 293.
(5) (a) Alessio, E.; Mestroni, G.; Nardin, G.; Attia, W. A.; Calligaris, M.; Sava, G.; Zorzet, S. *Inorg. Chem.* **1988**, *27*, 4099. (b) Keppler, B. K.; Henn, M.; Juhl, U.M.; Berger, M. R.; Niebl R.; Wagner, F. E. *Prog. Clin. Biochem. Med.* **1989**, *10*, 41.
(6) Holler, E.; Schaller, W.; Keppler, B. K. *Arzneim-Forsch./Drug Res.* **1991**, *41*(2), 1065.
(7) Loseto, F.; Alessio, E.; Mestroni, G.; Lacidogna, G.; Nassi, A.; Giordano, D.; Coluccia, M. *Anticancer Res.* **1991**, *11*, 1549.
(8) Cauci, S.; Viglino, P.; Esposito, G.; Quadrifoglio, F. *J. Inorg. Biochem.* **1991**, *43*, 739.
(9) Esposito, G.; Cauci, S.; Foglari, F.; Alessio, A.; Scocchi, M.; Quadrofoglio, F.; Viglino, P. *Biochemistry* **1992**, *31*, 7094.
(10) Seddon, E. A.; Seddon, K. R. *The Chemistry of Ruthenium*, Elsevier, Amsterdam, 1984.
(11) (a) Barton, J. K.; Lolis, E. *J. Am. Chem. Soc.* **1985**, *107*, 708. (b) Danishefsky, A. Ph.D. Thesis, Columbia University, 1987.
(12) Telser, J.; Cruickshank, K. A.; Schanze, K. S.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 7221.
(13) (a) Grover, N.; Gupta, N.; Thorp, H. H. *J. Am. Chem. Soc.* **1992**, *114*, 3390. (b) Gupta, N.; Grover, N.; Neyhart, G. A., Singh, P.; Thorp, H. H *Inorg. Chem.* **1993**, *32*, 310.
(14) Morgan, R. J.; Chatterjee, S.; Baker, A. D.; Strekas, T. C. *Inorg. Chem.* **1991**, *30*, 2687 and references therein.

0020-1669/94/1333-1934$04.50/0 © 1994 American Chemical Society

Table 1. Proton Chemical Shifts of (**2**) [*cis*-Ru(bpy)$_2$(9egua-κN^7)OH$_2$](PF$_6$)$_2$ (**2**) and [*cis*-Ru(bpy)$_2$(9mhyp-κN^7)OH$_2$](PF$_6$) (**4**) in $(CD_3)_2CO$

	bpy a[a]								bpy b[a]								base				
compd	H(3)	H(4)	H(5)	H(6)	H(3′)	H(4′)	H(5′)	H(6′)	H(3)	H(4)	H(5)	H(6)	H(3′)	H(4′)	H(5′)	H(6′)	H(8)	NH_2	NH	CH_2	CH_3
2	8.76	8.28	7.88	9.46	8.67	8.02	7.38	7.83	8.80	8.30	7.76	9.05	8.62	7.96	7.33	7.98	7.16	7.25	6.70	3.91	1.16
4	8.75	8.27	7.89	9.46	8.66	8.03	7.35	7.82	8.80	8.27	7.72	9.01	8.63	8.00	7.39	7.98	7.56		7.14		3.69

[a] The bpy ligands **a** and **b** are defined according Figure 4.

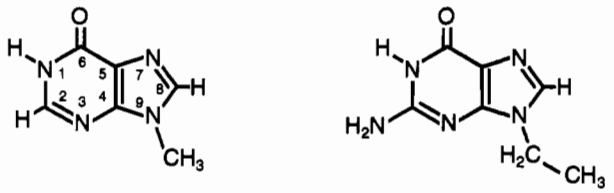

Figure 1. Schematic representation of 9-methylhypoxanthine (9mhyp) (left) and 9-ethylguanine (9egua) (right).

model compound for studies of covalent binding to DNA. The present paper describes the binding of [*cis*-Ru(bpy)$_2$Cl$_2$] in aqueous solutions at pH 7 to the nucleobases 9-methylhypoxanthine (9mhyp) and 9-ethylguanine (9egua) (Figure 1), including a crystal structure of [*cis*-Ru(bpy)$_2$(9egua-κN^7)Cl]Cl.

Experimental Section

Synthesis and Characterization. Materials. 9-Ethylguanine (Sigma) and 2,2′-bipyridine (Janssen Chimica) were obtained commercially and used without further purification. [*cis*-Ru(bpy)$_2$Cl$_2$][15] and [Ru(bpy)$_2$CO$_3$][16] were synthesized according to published methods. 9-Methylhypoxanthine was prepared by methylation of adenine[17] (Sigma) and subsequent oxidation.[18] Water was obtained from a Millipore filtration system. All prepared compounds gave satisfactory elementary analyses.

[*cis*-Ru(bpy)$_2$(9egua-κN^7)Cl]Cl (1). (a) A 2-mg sample of [*cis*-Ru(bpy)$_2$Cl$_2$] (4 μmol) in 1 mL of D_2O was heated at 100 °C in an Eppendorf cup for 10 min, and after cooling, 1.5 mg of 9-ethylguanine (8 μmol) was added. This mixture was kept at 37 °C for 24 h and used for ^{1}H NMR measurements without further purification.

(b) A 50-mg quantity of [*cis*-Ru(bpy)$_2$Cl$_2$] was refluxed for 16 h with the appropriate amount 9-ethylguanine in 50 mL (30:70 v:v) of aqueous ethanol. The solvent was evaporated *in vacuo*, and the residue was taken up in water. The product was purified on a CM Sephadex C-25 column using a NaCl gradient. One fraction was collected, and the water was removed *in vacuo*. The residue was taken up in absolute ethanol, the NaCl was filtered off, and the product was precipitated by adding diethyl ether, isolated, and dried *in vacuo*. Reaction ratios (Ru:base) of 1:1 to 1:10 and different solvents (methanol, diethylene glycol, dimethylformamide) were tried, but in all cases only the 1:1 product was obtained.

[*cis*-Ru(bpy)$_2$(9egua-κN^7)OH$_2$](PF$_6$)$_2$ (2). (a) A 50-mg sample of [*cis*-Ru(bpy)$_2$Cl$_2$] was refluxed for 16 h with the appropriate amount of 9-ethylguanine in 50 mL (30:70, v:v) of aqueous ethanol. The reaction mixture was concentrated, a concentrated solution of NH_4PF_6 in water was added, and the precipitate was collected after a period of standing at 4 °C. The product was purified on an aluminum oxide-90 column with ethanol as eluent. The solvent was removed *in vacuo*, and the complex was recrystallized from an acetone–water mixture.

(b) [*cis*-Ru(bpy)$_2$(OH$_2$)$_2$]$^{2+}$ was prepared by dissolving 10 mg (21 μmol) of [Ru(bpy)$_2$CO$_3$] in a pH 4 solution of *p*-toluenesulfonic acid in (30:70, v:v) aqueous ethanol, and the solution was kept in the dark to prevent isomerization to the *trans* isomer. The solution was then adjusted with NaOH to pH 7, and 7.5 mg (42 μmol) of 9-ethylguanine was added. The reaction mixture was refluxed for 16 h. A concentrated solution of NH_4PF_6 in water was added, and the volume was reduced *in vacuo* until a precipitate could be collected. The product was purified on a short aluminum oxide-90 column with ethanol as eluent. The solvent was removed *in vacuo*, and the complex was recrystallized from an acetone–water mixture. In order to investigate the formation of a disubstituted product, a reaction ratio of Ru:base = 1:10 was also tried; however, only the 1:1 product was obtained.

[*cis*-Ru(bpy)$_2$(9mhyp-κN^7)Cl]Cl (3). Compound **3** was synthesized analogously to **1**, by following procedures a and b.

[*cis*-Ru(bpy)$_2$(9mhyp-κN^7)OH$_2$](PF$_6$)$_2$ (4). Compound **4** was synthesized analogously to **2**, by following the procedures a and b.

Methods. Instrumentation. ^{1}H nuclear magnetic resonance spectra were recorded on a Bruker WM 300 spectrometer at 300 MHz in D_2O or $(CD_3)_2CO$. Assignments were made on the basis of homonuclear COSY experiments. The chemical shifts (δ) (Table 1) are reported in parts per million (ppm) downfield from $(CH_3)_4Si$ in cases of $(CD_3)_2CO$ solutions. $[(CH_3)_4N](NO_3)$, set at 3.18 ppm, was used as reference for D_2O solutions. Before the spectra were recorded, the products **1** and **3** were dissolved in D_2O and lyophilized. Where necessary, the HDO signal was reduced by selective saturation. The pH titrations were carried out in D_2O by adjustments with DCl and NaOD without the use of a buffer. The pH values were not corrected for the H/D isotope effect.

^{13}C spectra were recorded at 50.1 MHz on a JEOL 200 spectrometer at 50.1 MHz and at 75.4 MHz on a Bruker WM-300 spectrometer. In both cases, the samples were used as concentrated as possible and were filtered once before the measurements.

IR spectra were recorded on a Perkin-Elmer 580B spectrophotometer as CsI pellets and Nujoll mulls. UV–vis spectra were recorded with a Perkin-Elmer 330 spectrophotometer.

Elemental analyses were carried out by the Chemical Services Unit of University College Dublin except for the Cl analysis, which was performed at our own laboratory. Cl was analyzed potentiometrically, after using the method of Schöninger for sample destruction.

The chloride abstraction/addition experiments performed on **1** were characterized by ^{1}H NMR and were carried out under an argon atmosphere in subdued light. Three samples of 4 mg of **1** in 0.5 mL of D_2O were kept at room temperature for 4 days. One sample was used as a blank, and 1.5-equiv quantities of $AgNO_3$ (per Ru) were added to each of the remaining two samples. The AgCl precipitate was removed by centrifugation. To increase the Cl^- concentration, a 5-fold excess of LiCl was added to one of the remaining samples.

Single-Crystal X-ray Analysis. **1** was crystallized from a (1:1) mixture of benzyl alcohol and formic acid in an attempt to obtain the ester by slow reaction. As a result of slow evaporation of formic acid, crystals appeared after a few days. A needle shaped, ruby colored prism with approximate dimensions 0.2 × 0.1 × 0.07 mm^3 was mounted on a CAD-4 diffractometer, and diffraction data were collected using graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). A total of 14 243 reflections were collected, of which 13 740 independent reflections were measured by a $\omega/2\theta$ scan with 2θ range 2–33°; h = −18 → 18, k = −19 → 19, and l = 0 → 20. The value for R_{int} proved to be 0.0419. Transmission coefficients ranged from 90.66 to 108.24. Data were corrected for Lorentz and polarization effects. Absorption correction was applied by the DIFABS program[19] (μ = 6.0 cm^{-1}, on the basis of the localized structure). Atomic scattering factors were taken from the literature.[20] The structure was solved by direct methods, leading to the metal center, chlorine atoms, and major parts of the bpy and guanine rings. The rest of the atoms were located in the Fourier difference maps except for the amino hydrogen atoms. A disordered water molecule was found at a center of symmetry and refined with an occupation factor of 0.5 and an isotropic temperature factor. All located hydrogen atoms were placed at 1.00 Å from their carrier atoms with isotropic temperature factors which were refined as groups. The density of various crystals measured by flotation at different times, varied between 1.4 and 1.5 g·cm^{-3}, indicating evaporation of crystal solvent during storage. ^{1}H NMR measured in $(CD_3)_2SO$ of dissolved crystals showed the presence of small amounts of benzyl alcohol and formic acid. A search for and analysis of voids in the structure (grid 0.2 Å, probe radius 1.2 Å) resulted in a potential solvent area of 498 Å^3, i.e. 27.2% of the unit cell. Attempts to describe the solvent molecules by disorder models were unsuccessful.

(15) Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**, *17*, 3334.
(16) Johnson, E. C.; Sullivan, B. P.; Salmon, D. J.; Adeyemi, S. A.; Meyer, T. J. *Inorg. Chem.* **1978**, *17*, 2211.
(17) Krüger, M. *Z. Physiol. Chem.* **1893**, *18*, 423.
(18) Elion, G. B. *J. Org. Chem.* **1962**, *27*, 2478.
(19) Walker, N.; Stuart, D. *Acta Crystallogr.* **1983**, *A39*, 158.
(20) *International Tables for X-ray Crystallography*; Kynoch Press: Birmingham, U.K., 1974; Vol. 4.

Table 2. Crystallographic Data for [*cis*Ru(bpy)$_2$(9egua-κN^7)Cl]Cl·1.5H$_2$O (**5**)

empirical formula	$C_{54}H_{46}N_{18}O_5Cl_2Ru_2$
fw	1371.03
space group	$P\bar{1}$
a, b, c (Å)	11.895(4), 12.407(2), 13.329(5)
α, β, γ (deg)	91.5(2), 90.8(3), 111.3(2)
V (Å^3)	1832(3)
Z	1
ρ_{obs} (g·cm^{-3})	1.4–1.5
ρ_{calc} (g·cm^{-3})	1.243
T (K)	294
$\lambda_{Mo\ K\alpha}$ (Å)	0.710 73
μ (cm^{-1})	6.0
R	0.0733[a]
R_w	0.0751[b]

[a] $R = \sum(|F_o| - |F_c|)/\sum|F_o|$. [b] $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$; $w = 1/[\sigma^2(F_o) + 0.027974F_o^2]$.

Solvent correction by the BYPASS program[21] failed. Weighted full-matrix refinement in space group $P\bar{1}$ (378 variables; anisotropic thermal parameters for most non-hydrogen atoms) converged to $R = 0.0733$ and $R_w = 0.0751$ for 7346 significant independent reflections [$I > 2.5\sigma(I)$]. Refinement in space group $P1$ led to significantly higher R and R_w values. Calculations were performed with SHELXS-86 [22] (structure determination), SHELX-76 [23] (refinement), and the EUCLID package[24] (molecular geometry and illustrations) running on a μVAX-3400 computer. Table 2 lists all relevant crystallographic data.

Crystallographic Statistical Analysis. The January 1993 release of the Cambridge Structural Database (CSD)[25] was searched using QUEST for entries containing a pyridyl and a keto group present within the same residue or present in different residues. Structures with both fragments present within the same chemical fragment were omitted. The double-bond character of the keto group was defined by a bond length range of 1.12–1.25 Å. The entries containing a distance smaller than 4.00 Å between the oxygen of the keto group and the centroid of the pyridyl ring, were selected using the GSTAT89 program.

Results and Discussion

^{1}H NMR Measurements. When 9mhyp or 9egua is incubated with [*cis*-Ru(bpy)$_2$Cl$_2$] at neutral pH at 37 °C, only the N(7) mono adduct is formed. N(7) coordination for each guanine derivative is clearly shown by plotting the chemical shift of the H(8) proton as a function of pH (Figure 2). For both ligands the N(7) (de)protonation is absent. Coordination through N(7) of guanine is preferred by most metal ions at neutral pH[2-4,26] and for 9egua this was shown previously only for Ru(II) in [(η^6-C$_6$H$_6$)Ru(9egua-κN^7)Cl$_2$].[27]

The 1:1 binding of the base to the *cis*-Ru(bpy)$_2$ moiety, leads to an unsymmetrical complex, and therefore a splitting of the bpy pattern in the proton NMR spectrum (Table 1) is expected. The H(6) protons are near different ligands, and therefore these differ significantly in chemical shift values. Monofunctional binding has been also confirmed from integration of the alkyl resonances of 9mhyp and 9egua with respect to bpy resonances in the proton NMR spectra. One could expect (for steric reasons) a relatively high energy barrier for the second step, forming a bis adduct of *cis* octahedral Ru(II) and purine systems. Therefore various reaction ratios at elevated reaction temperatures were tried, but in all cases only the 1:1 products were obtained.

Table 3. Atomic Fractional Coordinates and Equivalent Isotropic Thermal Parameters for Non-Hydrogen atoms in [*cis*-[Ru(bpy)$_2$(9egua-κN^7)Cl]Cl·1.5H$_2$O (**5**) (Standard Deviations in Parentheses)

atom	x/a	y/b	z/c	U_{eq}[a]/U_{iso}[b] (Å^2)
Ru(1)	0.18811(3)	0.37088(4)	0.20682(3)	0.0350(1)
Cl(1)	0.1842(1)	0.5480(1)	0.1343(1)	0.0484(4)
Cl(2)	0.1392(2)	0.2652(2)	0.7970(1)	0.0720(7)
Ow(1)	0.8295(5)	0.4886(6)	0.1050(4)	0.074(2)
Ow(2)	1/2	0	1/2	0.26(1)[b]
O(6)	0.2015(4)	0.3035(5)	0.4791(3)	0.060(2)
N(1)	0.0445(4)	0.3018(4)	0.5791(3)	0.044(1)
N(2)	–0.0969(6)	0.2996(6)	0.6956(4)	0.061(2)
N(3)	–0.1210(4)	0.3524(4)	0.5317(3)	0.045(1)
N(7)	0.0544(4)	0.3755(4)	0.3112(3)	0.038(1)
N(9)	–0.1132(4)	0.4011(5)	0.3575(4)	0.044(1)
N(1b)	0.3380(4)	0.4755(4)	0.2853(3)	0.043(1)
N(1′b)	0.3209(4)	0.3702(5)	0.1143(4)	0.046(1)
N(1a)	0.0466(4)	0.2574(5)	0.1226(3)	0.046(1)
N(1′a)	0.1890(4)	0.2183(5)	0.2543(4)	0.046(1)
C(2)	–0.0582(5)	0.3193(5)	0.5993(4)	0.041(2)
C(4)	–0.0695(5)	0.3631(5)	0.4412(4)	0.038(1)
C(5)	0.0331(4)	0.3494(5)	0.4114(4)	0.037(1)
C(6)	0.1023(5)	0.3165(5)	0.4860(4)	0.042(2)
C(8)	–0.0345(5)	0.4057(5)	0.2816(4)	0.043(1)
C(10)	–0.2251(5)	0.4226(6)	0.3501(5)	0.050(2)
C(11)	–0.3295(7)	0.3190(8)	0.319(1)	0.090(4)
C(2b)	0.4464(5)	0.4880(6)	0.2442(5)	0.050(2)
C(2′b)	0.4360(5)	0.4249(7)	0.1492(5)	0.053(2)
C(2a)	0.0234(6)	0.1424(6)	0.1397(5)	0.054(2)
C(2′a)	0.1081(6)	0.1240(5)	0.2121(6)	0.054(2)
C(3b)	0.5557(6)	0.5530(7)	0.2838(6)	0.067(3)
C(3′b)	0.5355(7)	0.422(1)	0.0988(8)	0.096(4)
C(3a)	–0.065(1)	0.0552(8)	0.0853(8)	0.095(4)
C(3′a)	0.1084(9)	0.0100(7)	0.2296(8)	0.084(3)
C(4b)	0.5547(7)	0.6193(8)	0.3811(8)	0.086(3)
C(4′b)	0.5119(8)	0.349(1)	0.0044(7)	0.092(4)
C(4a)	–0.1379(9)	0.084(1)	0.0220(8)	0.095(4)
C(4′a)	0.200(1)	0.0007(9)	0.2934(9)	0.103(5)
C(5b)	0.442(7)	0.6083(8)	0.4216(6)	0.068(3)
C(5′b)	0.3950(8)	0.2980(9)	–0.0329(6)	0.080(4)
C(5a)	–0.1217(7)	0.202(1)	0.0079(6)	0.082(3)
C(5′a)	0.2870(9)	0.1052(8)	0.3395(8)	0.087(4)
C(6b)	0.3415(6)	0.5379(6)	0.3710(5)	0.054(2)
C(6′b)	0.3016(7)	0.3075(7)	0.0215(5)	0.062(2)
C(6a)	–0.0238(6)	0.2862(6)	0.0575(5)	0.053(2)
C(6′a)	0.2784(6)	0.2074(6)	0.3131(5)	0.056(2)

[a] $U_{eq} = 1/3(U_{11} + U_{22} + U_{33})$. [b] Occupation factor 0.5.

When [*cis*-Ru(bpy)$_2$Cl$_2$] is dissolved in aqueous media, the [*cis*-Ru(bpy)$_2$(OH$_2$)Cl]$^+$ and [*cis*-Ru(bpy)$_2$(OH$_2$)$_2$]$^{2+}$ species are formed.[10] On the other hand, the [*cis*-Ru(bpy)$_2$(base-κN^7)Cl]$^+$ cation shows a remarkable stability toward hydrolysis in D$_2$O at 37 °C. This stability is visualized in Figure 3. The H(6a) proton of bpy near the chloro ligand shows a downfield shift when the coordinated chlorine atom is abstracted by silver ions, in agreement with the formation of the aqua complex. The peak at 9.35 ppm again disappears when the chloride concentration is raised, showing that this process is reversible. The chloride concentration could therefore have an influence on the second binding step involving 9mhyp or 9egua and the [*cis*-Ru(bpy)$_2$(base-κN^7)X] cation (X = H$_2$O, Cl$^-$). [*cis*-Ru(bpy)$_2$(OH$_2$)$_2$]$^{2+}$ without chloride ions in the reaction mixture can be obtained from [Ru(bpy)$_2$CO$_3$] dissolved in an acidic solution. However reaction of [*cis*-Ru(bpy)$_2$(OH$_2$)$_2$]$^{2+}$ with an excess 9mhyp or 9egua gave still only the mono aqua adducts mentioned above.

Chloride is only present as an impurity when the Ru–base complexes are isolated with noncoordinating counterions like hexafluorophosphate. In those cases, a water molecule is present as a ligand. Though the IR and UV–vis evidence for coordinated water was not immediately clear, successive recrystallizations of the crude products showed the same feature as shown in Figure 3, in favor of the most downfield H(6a) resonance. The resonance

(21) Van der Sluis, P.; Spek, A. L. *Acta Crystallogr.* **1990**, *A46*, 194.
(22) Sheldrick, G. M. SHELXS-86: Program for crystal structure determination. University of Göttingen, Germany, 1986.
(23) Sheldrick, G. M. SHELX-76: Program for crystal structure determination. University of Cambridge, U.K., 1976.
(24) (a) Spek, A. L. The EUCLID package. In *Computational Crystallography*; Sayre, D., Ed.; Clarendon Press: Oxford, U.K., 1982; p 528. (b) Spek, A. L. *Acta Crystallogr.* **1990**, *A46*, C34.
(25) CAOS/CAMM Center, Faculty of Science, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands.
(26) Miller, S. K.; Van der Veer, D. G.; Marzilli, L. G. *J. Am. Chem. Soc.*, **1985**, *107*, 1048.
(27) Sheldrick, W. S.; Heeb, S. *Inorg. Chim. Acta* **1990**, *168*, 93.

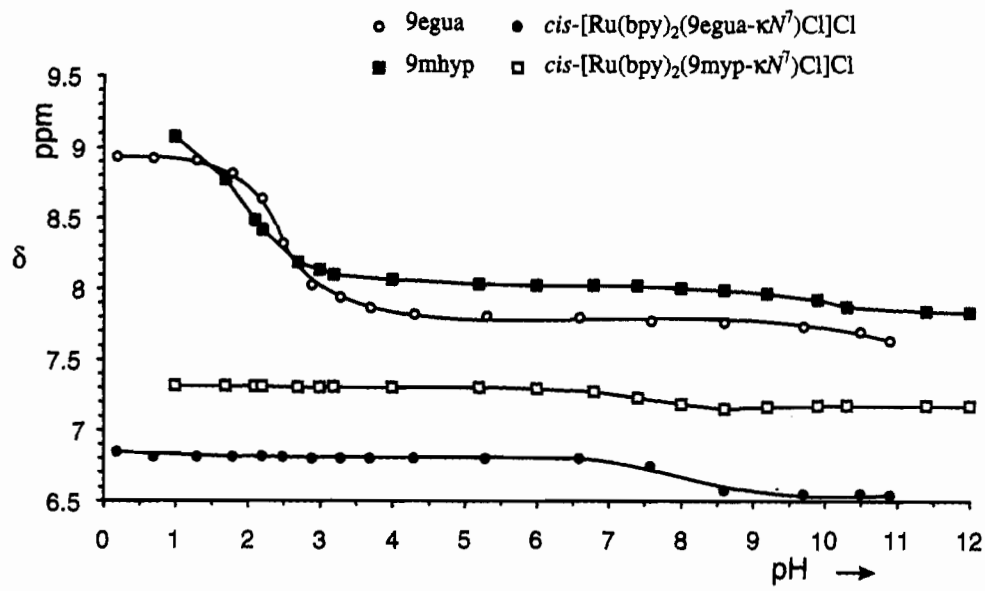

Figure 2. Plot of the chemical shift vs. pH of the H8 of 9egua, [*cis*-Ru(bpy)$_2$(9egua-κN^7)Cl]Cl (**1**), 9mhyp, and [*cis*-Ru(bpy)$_2$(9mhyp-κN^7)Cl]Cl (**3**).

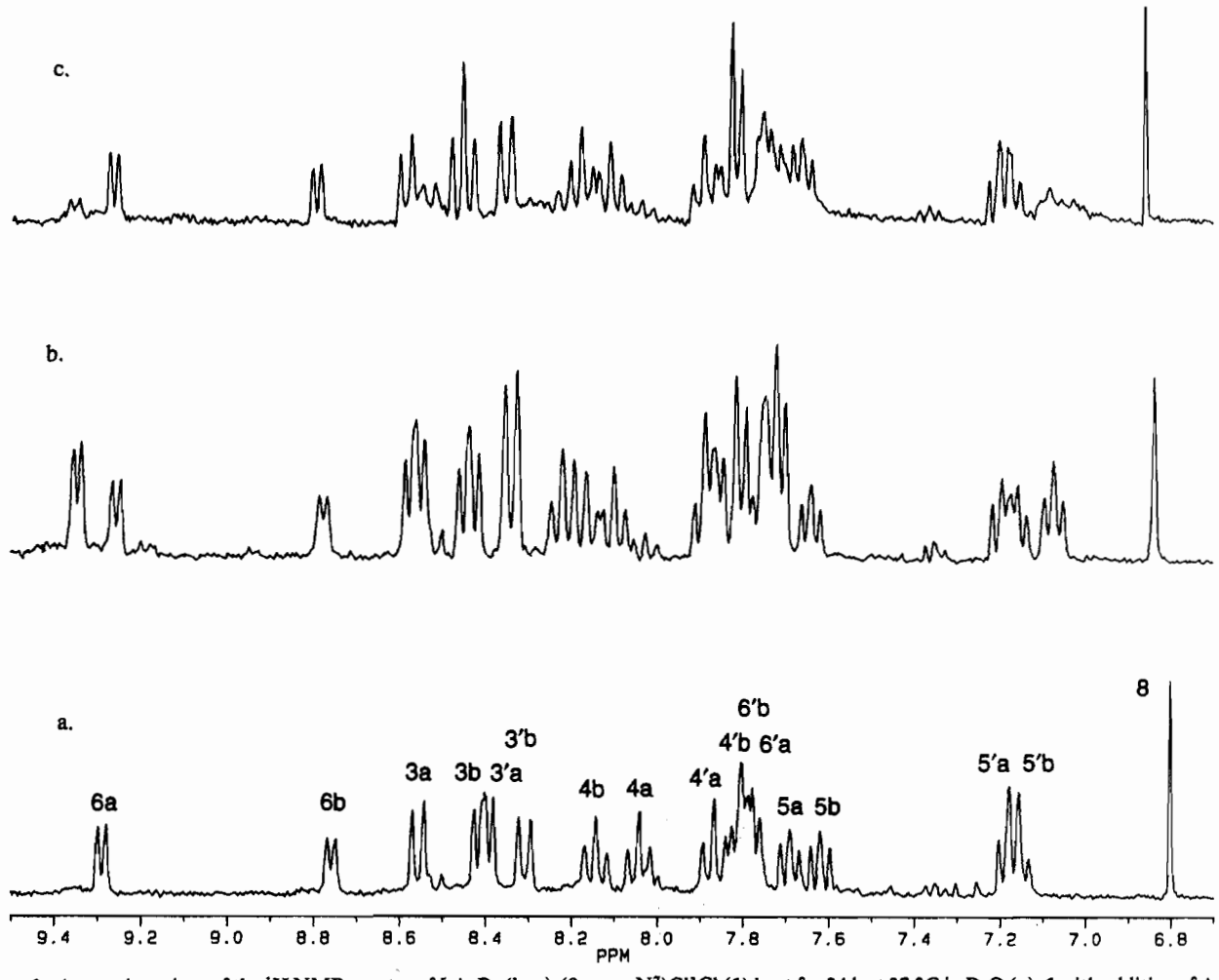

Figure 3. Aromatic regions of the ^{1}H NMR spectra of [*cis*-Ru(bpy)$_2$(9egua-κN^7)Cl]Cl (**1**) kept for 24 h at 37 °C in D_2O (a), **1** with addition of $AgNO_3$ (b), and **1** with addition of $AgNO_3$ and subsequent addition of an excess LiCl (c).

of bound water could not be observed at room temperature, indicating fast proton exchange on the NMR time scale with bulk water.

The H(8) resonance of coordinated 9mhyp and 9egua shows a large upfield shift, indicating a stronger influence of the bpy π clouds than is caused by N(7) coordination (Figure 2). The bpy patterns of **2** and **4** show a striking resemblance (Table 1). This implies that 9mhyp is a good model compound for guanine; it can be easily prepared and has an extra non-exchangeable H(2) proton compared to guanine, which is likely to give extra structural information in D_2O studies.

Description of the Structure of [*cis*-Ru(bpy)$_2$(9egua-κN^7)Cl]Cl·1.5H$_2$O (5). The compound crystallizes in the triclinic space group $P\bar{1}$, so the Λ and Δ isomers are both present in the unit cell. Metal complexes containing nucleobases are usually difficult to crystallize. Various solvent mixtures were tried, but the obser-

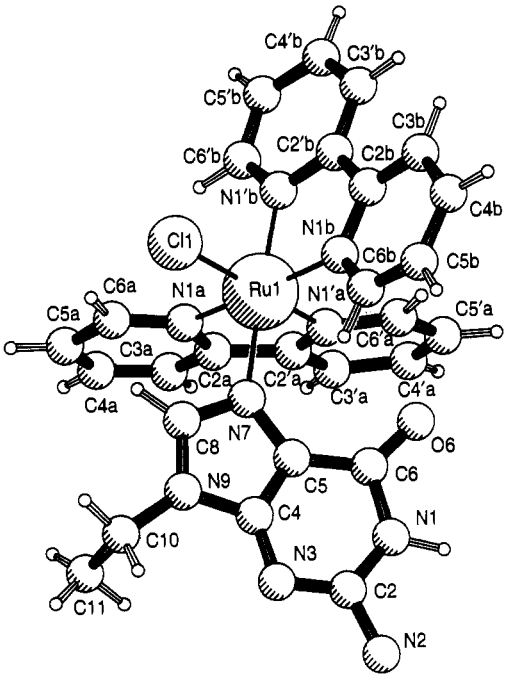

Figure 4. Perspective view and atomic numbering of the cationic unit in the title compound. Only the Λ isomer is shown.

vation that the compound did crystallize as needles from an acetic acid–ethanol mixture when sufficient amounts of ethyl acetate were formed resulted in the idea to try an analogous procedure with the aromatic ester to be formed from formic acid and benzyl alcohol. However, due to evaporation of formic acid, crystals appeared before formation of significant amounts of the ester.

During the refinement of the structure, only diffuse solvent electrons varied in density, indicating evaporation of crystal solvent. The BYPASS program has been designed for cases where description of solvent disorder fails,[21] but its application to this problem proved not to be possible due to relatively large potential solvent areas present in the unit cell. Therefore the refinement had to be stopped at a stage where the *R* value had reached a minimum, without description of solvent disorder.

A projection of the cationic unit of the Λ isomer is shown in Figure 4. The atomic labeling system is the same as the one used in the description of the proton NMR. Relevant intramolecular distances and angles are given in Tables 4 and 5. The bond lengths around Ru and bite angles of the bpy ligands are within the ranges that might be expected from related compounds.[27,28] The structure is stabilized by staggered stacking of the pyridyl moieties of symmetry related units, with a closest contact for C(4'b)···C(2'b) of 3.40(2) Å. The coordinated chloride ion is involved in weak hydrogen bonding with Cl(1)···Ow(1) = 3.205(6) Å; the noncoordinating chlorine atom forms a hydrogen bond with N(1) having a N(1)···Cl(2) distance of 3.197(6) Å and the angle N(1)–H(1)···Cl(2) measures 156.1(7)°.

The most interesting feature of the structure presented here is the conformation of the 9egua ligand, which is of great interest for studies on covalent binding of [*cis*-Ru(diimine)]$^{2+}$ species to N(7) of guanine in B-DNA.[11,13] The planar purine moiety is situated with the keto group between the pyridyl rings of the bpy ligands. A space-filling model of **5** visualizes a barrier for rotation about the Ru–N(7) bond (see Figure 5), indicating that H(8) protons of rotamers would differ significantly in chemical shift. Dissolved crystals of **5** have proton NMR resonances identical to those of **2**, which means that rotamers are not formed at 37 °C. This is a strong indication that the 6-ketopurine conformation found in **5** is also the preferred one in solution. The distances of

(28) Eggleston, D. S.; Goldsby, K. A.; Hodgson, D. J.; Meyer, T. J. *Inorg. Chem.* **1985**, *24*, 4573.

Table 4. Intramolecular Distances (Å) in [*cis*-Ru(bpy)$_2$(9egua-κ*N*^{7})Cl]Cl·1.5H$_2$O (**5**) (Standard Deviations in Parentheses)

Ru(1)–Cl(1)	2.439(2)	N(1'a)–C(2'a)	1.321(9)
Ru(1)–N(7)	2.143(5)	N(1'a)–C(6'a)	1.360(9)
Ru(1)–N(1b)	2.038(5)	C(4)–C(5)	1.355(8)
Ru(1)–N(1'b)	2.020(5)	C(5)–C(6)	1.441(8)
Ru(1)–N(1a)	2.054(5)	C(10)–C(11)	1.47(1)
Ru(1)–N(1'a)	2.016(6)	C(2b)–C(2'b)	1.45(1)
O(6)–C(6)	1.252(8)	C(2b)–C(3b)	1.35(1)
N(1)–C(2)	1.346(8)	C(2'b)–C(3'b)	1.37(1)
N(1)–C(6)	1.411(7)	C(2a)–C(2'a)	1.47(1)
N(2)–C(2)	1.368(8)	C(2a)–C(3a)	1.38(1)
N(3)–C(2)	1.327(7)	C(2'a)–C(3'a)	1.44(1)
N(3)–C(4)	1.348(7)	C(3b)–C(4b)	1.52(1)
N(7)–C(5)	1.388(7)	C(3'b)–C(4'b)	1.50(2)
N(7)–C(8)	1.303(8)	C(3a)–C(4a)	1.35(2)
N(9)–C(4)	1.388(8)	C(3'a)–C(4'a)	1.41(2)
N(9)–C(8)	1.376(8)	C(4b)–C(5b)	1.39(1)
N(9)–C(10)	1.452(8)	C(4'b)–C(5'b)	1.38(2)
N(1b)–C(2b)	1.366(8)	C(4a)–C(5a)	1.42(2)
N(1b)–C(6b)	1.355(8)	C(4'a)–C(5'a)	1.45(2)
N(1'b)–C(2'b)	1.357(9)	C(5b)–C(6b)	1.37(1)
N(1'b)–C(6'b)	1.414(9)	C(5'b)–C(6'b)	1.37(1)
N(1a)–C(2a)	1.377(9)	C(5a)–C(6a)	1.39(1)
N(1a)–C(6a)	1.340(9)	C(5'a)–C(6'a)	1.36(1)

Table 5. Bond Angles (deg) and Selected Torsion Angles (deg) in [*cis*-Ru(bpy)$_2$(9egua-κ*N*^{7})Cl]Cl·1.5H$_2$O (**5**) (Standard Deviations in Parentheses)

Cl(1)–Ru(1)–N(7)	89.7(3)	Ru(1)–N(1a)–C(6a)	125.9(6)
Cl(1)–Ru(1)–N(1b)	85.9(3)	C(2a)–N(1a)–C(6a)	119.5(6)
Cl(1)–Ru(1)–N(1'b)	90.8(3)	Ru(1)–N(1'a)–C(2'a)	116.6(5)
Cl(1)–Ru(1)–N(1a)	97.0(3)	Ru(1)–N(1'a)–C(6'a)	123.8(5)
Cl(1)–Ru(1)–N(1'a)	174.9(3)	C(2'a)–N(1'a)–C(6'a)	118.6(7)
N(7)–Ru(1)–N(1b)	98.6(3)	N(1)–C(2)–N(2)	115.8(6)
N(7)–Ru(1)–N(1'b)	176.9(4)	N(1)–C(2)–N(3)	123.7(6)
N(7)–Ru(1)–N(1a)	85.9(3)	N(2)–C(2)–N(3)	120.5(7)
N(7)–Ru(1)–N(1'a)	93.4(4)	N(3)–C(4)–N(9)	123.4(6)
N(1b)–Ru(1)–N(1'b)	78.4(4)	N(3)–C(4)–C(5)	131.2(6)
N(1b)–Ru(1)–N(1a)	174.7(4)	N(9)–C(4)–C(5)	105.3(6)
N(1b)–Ru(1)–N(1'a)	97.6(4)	N(7)–C(5)–C(4)	111.1(6)
N(1'b)–Ru(1)–N(1a)	97.1(4)	N(7)–C(5)–C(6)	131.4(6)
N(1'b)–Ru(1)–N(1'a)	86.3(4)	C(4)–C(5)–C(6)	117.4(6)
N(1a)–Ru(1)–N(1'a)	79.3(4)	O(6)–C(6)–N(1)	119.7(6)
C(2)–N(1)–C(6)	126.4(6)	O(6)–C(6)–C(5)	129.9(6)
C(2)–N(3)–C(4)	110.9(6)	N(1)–C(6)–C(5)	110.4(6)
Ru(1)–N(7)–C(5)	135.1(5)	N(7)–C(8)–N(9)	112.1(6)
Ru(1)–N(7)–C(8)	119.9(5)	N(9)–C(10)–C(11)	113.1(7)
C(5)–N(7)–C(8)	105.0(5)	N(1b)–C(2b)–C(2'b)	113.9(6)
C(4)–N(9)–C(8)	106.4(6)	N(1b)–C(2b)–C(3b)	125.6(7)
C(4)–N(9)–C(10)	126.3(6)	C(2'b)–C(2b)–C(3b)	120.5(7)
C(8)–N(9)–C(10)	127.1(6)	N(1'b)–C(2'b)–C(2b)	114.4(6)
Ru(1)–N(1b)–C(2b)	116.2(5)	N(1'b)–C(22)–C(3'b)	123.7(8)
Ru(1)–N(1b)–C(6b)	127.1(5)	C(2b)–C(2'b)–C(3'b)	122.0(8)
C(2b)–N(1b)–C(6b)	116.7(6)	N(1a)–C(2a)–C(2'a)	113.5(6)
Ru(1)–N(1'b)–C(2'b)	116.7(5)	N(1a)–C(2a)–C(3a)	121.5(8)
Ru(1)–N(1'b)–C(6'b)	124.4(6)	C(2'a)–C(2a)–C(3a)	124.7(8)
C(2'b)–N(1'b)–C(6'b)	118.6(7)	N(1'a)–C(2'a)–C(2a)	116.0(6)
Ru(1)–N(1a)–C(2a)	114.5(5)	N(1'a)–C(2'a)–C(3'a)	122.3(8)
C(2a)–C(24)–C(3'a)	121.5(7)	C(4b)–C(5b)–C(6b)	117.8(8)
C(2b)–C(3b)–C(4b)	115.6(8)	C(4'b)–C(5'b)–C(6'b)	119.4(9)
C(2'b)–C(3'b)–C(4'b)	116.6(9)	C(4a)–C(5a)–C(6a)	117.5(9)
C(2a)–C(3a)–C(4a)	118.5(9)	C(4'a)–C(5'a)–C(6'a)	117(1)
C(2'a)–C(3'a)–C(4'a)	118.0(9)	N(1b)–C(6b)–C(5b)	125.6(8)
C(3b)–C(4b)–C(5b)	118.6(8)	N(1'b)–C(6'b)–C(5'b)	122.2(8)
C(3'b)–C(4'b)–C(5'b)	119.3(9)	N(1a)–C(6a)–C(5a)	121.3(8)
C(3a)–C(4a)–C(5a)	121.1(10)	N(1'a)–C(6'a)–C(5'a)	125.3(8)
C(3'a)–C(4'a)–C(5'a)	118.7(10)		

Cl(1)–Ru(1)–N(7)–C(8)	39.9(5)
N(1a)–Ru(1)–N(7)–C(8)	−57.1(5)

O(6) to the centroid of the bpy 'a and b pyridyl rings are 3.95(3) and 3.60(3) Å, respectively, and therefore it is likely that an interaction of the lone pairs of guanine carbonyl groups with the pyridyl π systems is present. Comparable keto–phenyl interactions are found in proteins, and *ab initio* calculations of the interaction of the formamide oxygen with the benzene π system have shown that such a geometry has a stabilizing enthalpic

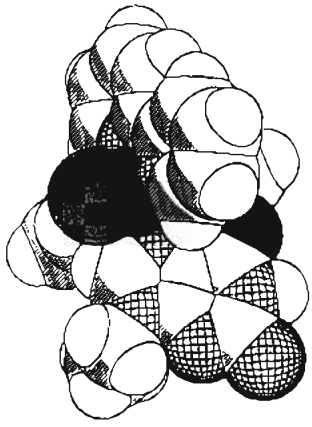

Figure 5. Space-filling model of the Δ isomer of the title compound clearly indicating a potential energy barrier for rotation around the Ru–N(7) bond. The perspective is equivalent to that of Figure 4.

effect.[29] Prolonged ^{13}C NMR measurements of **2**, however, did not reveal the chemical shift of the keto carbon, most likely due to concentration problems. It appeared to be of interest whether a keto–pyridyl interaction is common in inorganic structures. Therefore an extensive CSD search was carried out, but only organic structures applied to the distance limit of 4.0 Å for the oxygen to the centroid of the pyridyl ring. Therefore it can be concluded that the presented X-ray structure is the first example of this interaction in a coordination compound.

Further studies of the [*cis*-Ru(bpy)$_2$Cl$_2$] complexes with other guanosine derivatives are in progress.[30]

(29) Thomas, K. A.; Smith, G. M.; Thomas, T. B.; Feldman, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 4843.

Concluding Remarks

[*cis*-Ru^{II}(bpy)$_2$L$_2$]$^{n+}$ (L = Cl$^-$, H$_2$O) compounds react with the alkylated 6-ketopurines 9-methylhypoxanthine and 9-ethylguanine in a 1:1 ratio. The sixth ligand can be either a Cl$^-$ or a H$_2$O ligand. The crystal structure of [*cis*-Ru(bpy)$_2$(9egua-κN^7)Cl]Cl·1.5H$_2$O and its ^{1}H NMR data show that the purine is positioned with the keto group between the pyridyl rings of the *cis* configurated bpy ligands. The preferred purine conformation is unique for a coordination complex and could be of great importance for the binding of Ru–polypyridyl complexes to DNA.

Acknowledgment. The authors thank Mr. F. B. Hulsbergen[31] for chloride analysis, Mr. S. Gorter[31] for diffraction data collection, and Dr. R. A. G. de Graaff,[31] Dr. A. L. Spek,[32] Dr. J. A. Kanters,[32] and Mr. A. Schouten[32] for useful discussions on the X-ray determination. This research was sponsored by the Netherlands Organization for Chemical Research (SON), with financial aid from the Netherlands Organization for the Advancement of Research (NWO). Johnson Matthey Ltd. (Reading, U.K.) is gratefully acknowledged for a generous loan of RuCl$_3$·3H$_2$O.

Supplementary Material Available: Tables of crystal data and details of the structure determination, atomic coordinates for the hydrogen atoms, thermal parameters of all non-hydrogen atoms, and all bond distances and angles and a partial crystal packing plot of the cationic units of [*cis*-Ru(bpy)$_2$(9egua-κN^7)Cl]Cl·1.5H$_2$O (11 pages). Ordering information is given on any current masthead page.

(30) Van Vliet, P. M.; Toekimin, S. M. S.; Haasnoot, J. G.; Reedijk, J. *J. Inorg. Biochem.* **1991**, *43*, 448.
(31) Leiden University, The Netherlands.
(32) Laboratorium voor Kristal- en Structuurchemie, Utrecht University, The Netherlands.