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# Platinum Complexes with NH Groups on the Carrier Ligand and with Only One Guanine or Hypoxanthine Derivative. Informative Models for Assessing Relative Nucleobase and Nucleotide Hydrogen-Bond Interactions with Amine Ligands in Solution

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Complexes of the type *syn*-(*R*,*S*)-$Me_3$dienPt**L** ($Me_3$dien = *N*,*N*′,*N*′′-trimethyldiethylenetriamine; **L** = guanine or hypoxanthine derivative) have two rotamers, a feature useful for assessing hydrogen-bond interactions between a $Me_3$dien NH group and either the O6 or the phosphate group of the coordinated **L**. The two rotamers are defined as *endo* and *exo* for the rotamer with the six-membered ring of the purine on the same side and on the opposite side, respectively, of the coordination plane as the N–Me's. For **L** = 5′-GMP and 5′-IMP the *endo* rotamer is the exclusive form (at neutral and basic pH) or is present at 90% and more (low pH where 5′-phosphate group is protonated). A 5′-phosphate group can be positioned to form a direct H-bond with a $Me_3$dien NH group only in the *endo* form; such an H-bond explains this high *endo* preference. Such a direct phosphate–NH H-bond is not possible for other complexes used in this study because either **L** has no phosphate group (9-EtG, Guo) or the phosphate is at the 3′-position (3′-GMP and 3′-IMP), too far for H-bonding. Nevertheless, a preference for the *endo* rotamer was observed for these **L** also. This result is opposite to that expected both from potential steric repulsion of the **L** O6 with the N–Me groups and also from the lack of a potential favorable H-bond interaction between **L** O6 and a $Me_3$dien NH. For the 9-EtG adduct, the temperature dependence of the *endo*/*exo* equilibrium and the activation parameters for *endo*/*exo* interconversion suggest that the preference for the *endo* rotamer arises from the hydration of the $Me_3$dien NH groups; such hydration is favorable in the *endo* rotamer. At basic pH, N1H deprotonation increases the H-bond capacity of O6, and the *exo* rotamer increases in stability, becoming the dominant rotamer for the 9-EtG and Guo adducts. For **L** = 3′-GMP and 3′-IMP, stabilization of the *endo* form upon phosphate deprotonation at neutral pH was observed. This result is attributed to an H-bonding network involving water, the 3′-phosphate, and the $Me_3$dien NH groups.

## Introduction

Cisplatin (*cis*-$PtCl_2(NH_3)_2$) displays exceptional anticancer activity when employed in the treatment of testicular, ovarian, cervical, head and neck, esophageal, and non-small-cell lung cancers.[1] Continued research into the mechanism of action of cisplatin is being carried out to understand why it is so extraordinarily effective, especially against testicular cancer, and to allow the rational design of new derivatives that will overcome the toxic side effects and the resistance problems encountered with cisplatin. Classical structure−activity relationships of platinum anticancer drugs have led, over the past decades, to the synthesis and screening of many cisplatin analogues of the formula *cis*-$PtX_2A_2$, but progress in identifying superior carrier ligands has been rather limited (X = anion of intermediate binding strength; $A_2$ = two unidentate or one bidentate amine ligand).[2]

Although transplatin (*trans*-$PtCl_2(NH_3)_2$) is inactive, some trans-platinum complexes of the types *trans*-$PtCl_2L_2$ and *trans*-$PtCl_2(NH_3)L$ (L = planar N-donor ligand) are active against cisplatin-resistant tumors.[3−5] Polynuclear platinum compounds, such as BBR3464 ([(*trans*-$PtCl(NH_3)_2)_2(\mu$-*trans*-

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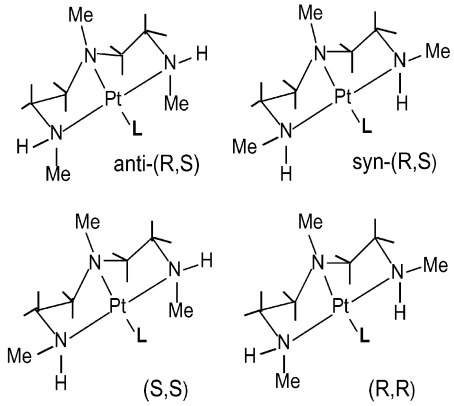
$Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2)](NO_3)_4$), have also been investigated and are currently in clinical trials.[6,7] The former complexes cannot give the 1,2-intrastrand cross-links formed by cisplatin, while the latter compounds, unlike mononuclear species, can produce long-range lesions.

The major DNA adduct formed by cisplatin, the intrastrand cross-link between adjacent **G**'s, is mimicked by *cis*-$PtA_2\mathbf{G}_2$ models, where **G** is a guanine derivative. Models with bulky $A_2$ ligands,[8−13] designed to reduce the rate of rotation around the Pt−**G** bonds, have been extensively investigated in our laboratories and have contributed to the elucidation of some features of the cisplatin adducts.

Recent results from studies on oligomers led to the hypothesis that the very small size of the NH group, not its hydrogen-bonding ability, is responsible for the good activity exhibited by Pt compounds with amine carrier ligands bearing multiple NH protons.[9,14] In contrast, for amines lacking NH groups, bulky substituents projecting out of the coordination plane could clash with neighboring nucleobases.[15]

Traditionally, $^1$H NMR spectroscopy has been used to investigate the role of phosphate/amine-NH interactions, not only in complexes with two **G**'s but also in complexes with one **G**, for which there is no interference from dipole−dipole interactions of *cis*-**G**'s. The chemical shift behaviors of Pt−NH and **G** H8 NMR signals suggested the existence of such H-bonding interactions in Pt(en)(5′-GMP)$_2$[16] and in Pt(dien)-(5′-GMP) (en = 1,2-diaminoethane; dien = diethylenetriamine);[17,18] in the latter complex the O6/NH interaction, promoted by deprotonation of N1H of **G**, has also been monitored spectroscopically. Crystallographic studies of Pt-(dien) complexes with substituted 6-oxopurines[19] and with the trinucleotide d(ApGpA)[20] showed inter- and intracomplex H-bridges between O6 and NH, the former being stronger than the latter.

**Chart 1.** Configurations of the Amine Ligand in $Me_3$dienPt**L** Complexes




In this work, 1D and 2D NMR investigations of several $Me_3$dienPt**L** complexes (**L** = 5′-GMP, 5′-IMP, Me-5′-GMP, 3′-GMP, 3′-IMP, Guo, and 9-EtG) are reported. The coordinated $Me_3$dien ligand (*N*,*N*′,*N*″-trimethyldiethylenetriamine) can adopt two symmetrical and two unsymmetrical configurations (Chart 1).[21,22] Moreover, because of the unsymmetrical relationship of the carrier ligand with respect to the coordination plane, and because the **L** base is lopsided and normally lies with its plane perpendicular to this plane, two rotamers are possible. By convention, the rotamers with **L** O6 on the same and on the opposite side of the Pt coordination plane as the central N-Me are designated as *endo* and *exo*, respectively (Chart 2). The rate of interconversion is high enough to ensure fast equilibration between rotamers but slow enough to allow detection of separate sets of NMR signals for the two conformers.

A previous study examined the interconversion between rotamers as a function of the carrier ligand configurations.[21] In the present study we focused our attention upon the symmetrical *syn*-(*R*,*S*)-$Me_3$dienPt**L** species. This configuration of $Me_3$dien places the three N−Me groups on one side of the platinum coordination plane and the two NH's on the opposite side of the coordination plane (Chart 1). The investigation has been extended to include guanine and hypoxanthine bases with different substituents in the 9-position and to different pH values to assess H-bond interactions between the NH's of the carrier ligand and either the O6 or the phosphate of the coordinated **L**. The assignment of the *endo* and *exo* conformations to the two rotamers, which in the previous work was only tentative and not always correct,[21] has now been based entirely on experimental evidence.

Evidence is mounting that H-bonding interactions clearly identified in solids[23,24] may not persist in solution.[25] For

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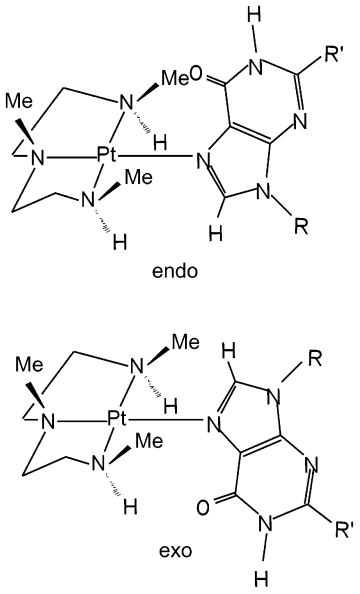
**Chart 2.** Rotamers of *syn*-(*R*,*S*)-$Me_3$dienPtL Complexes[a]




[a] R is ethyl for 9-EtG, a sugar residue for Guo, and a sugar−phosphate for nucleotides; R′ = H (hypoxanthine) or $NH_2$ (guanine).

example, *cis*-Pt$(NH_3)_2$(5′-GMP)$_2$ forms NH to 5′-phosphate interactions in the solid but second-sphere interactions in solution,[25] in which the 5′-phosphate of each 5′-GMP interacts with the N1H of the *cis*-5′-GMP. Thus, H-bonds and conformers found in the solid may be much less important in solution. H-bonds in solution cannot be assessed so directly as in the solid, and therefore, *syn*-(*R*,*S*)-$Me_3$-dienPt**L** adducts comprise an informative series to assess H-bonding. Specifically, for example, in *syn*-(*R*,*S*)-$Me_3$-dienPt(5′-GMP) with 5′-GMP in the normal anti conformation, the $Me_3$dien NH groups can H-bond to either the **L** O6 (in one rotamer) or the 5′-phosphate (in the other rotamer).

## Experimental Section

**Materials.** $Me_3$dien and [Pt$(NO_3)$($Me_3$dien)]$(NO_3)$ were prepared as previously described.[21] Guanosine 5′-monophosphate (5′-GMP), inosine 5′-monophosphate (5′-IMP), guanosine 3′-monophosphate (3′-GMP), inosine 3′-monophosphate (3′-IMP), guanosine (Guo), and 9-ethylguanine (9-EtG) were used as received (Sigma). The phosphate methyl ester derivative of guanosine 5′-monophosphate (Me-5′-GMP) was prepared as described previously.[26]

**Preparation of Adducts.** Solutions of adducts (5 mM) were prepared by treatment of [Pt$(NO_3)$($Me_3$dien)]$(NO_3)$, in $D_2O$ (0.6 mL) at pH* ∼3−4 or ∼6 (asterisk denotes that no correction for deuterium effect was introduced), with a stoichiometric amount of **L** (**L** = guanine or hypoxanthine derivative). Reactions were monitored by $^1$H NMR spectroscopy until the disappearance of free **L** was indicated or until a constant intensity ratio between free and complexed **L** was attained. Standard $DNO_3$ and NaOD solutions (in $D_2O$) were used for adjusting the pH* of each sample directly in the NMR tubes when necessary. 1D NMR studies were performed at 23 °C and 2−5 °C (the low temperature permitting observation of sharper H8 NMR signals). NOESY experiments were performed at −5 °C (solvent $D_2O/CD_3OD$, 80:20 v/v) and −20 °C (solvent $D_2O/CD_3OD$, 65:35 v/v).

**Table 1.** H8 and H2 NMR Chemical Shifts (ppm) and *endo*:*exo* Rotamer Ratios for *syn*-(*R*,*S*)-$Me_3$dienPt**L** Complexes in $D_2O$

| | | | H8 (H2) | | |
|---|---|---|---|---|---|
| L | *T* (°C) | pH* | *endo* | *exo* | *endo*:*exo* ratio |
| 5′-GMP | 2 | 3.0 | 8.63 | 8.73 | 9.0 |
| | | 6.5 | 8.84 | 8.73 | >60 |
| | | 10.2 | 8.70 | *a* | *a* |
| 5′-IMP | 2 | 3.3 | 9.00 (8.30) | 9.12 (8.31) | 23 |
| | | 5.6 | 9.13 (8.29) | 9.12 (8.29) | >44 |
| | | 10.2 | 9.02 (8.17) | *a* | *a* |
| Me-5′-GMP | 2 | 2.5 | 8.57 | 8.67 | 7.3 |
| | | 6.5 | 8.57 | 8.67 | 7.3 |
| | | 9.9 | 8.44 | 8.55 | 3.5 |
| 3′-GMP | 5 | 3.0 | 8.57 | 8.76 | 2.5 |
| | | 7.0 | 8.60 | 8.74 | 4.0 |
| | | 9.9 | 8.40 | 8.58 | 1.2 |
| 3′-IMP | 5 | 3.4 | 8.99 (8.33) | 9.17 (8.33) | 2.8 |
| | | 7.0 | 8.98 (8.30) | 9.10 (8.30) | 3.5 |
| | | 10.3 | 8.74 (8.20) | 8.93 (8.22) | 1.7 |
| Guo | 5 | 3.1 | 8.54 | 8.74 | 2.3 |
| | | 7.3 | 8.52 | 8.70 | 2.8 |
| | | 10.3 | 8.31 | 8.54 | 0.8 |
| 9-EtG | 5 | 7.2 | 8.27 | 8.50 | 2.0 |
| | | 10.2 | 8.08 | 8.33 | 0.6 |

[a] Only the *endo* rotamer present at high pH*.

**Table 2.** N−Me Chemical Shifts (ppm) for *syn*-(*R*,*S*)-$Me_3$dienPt**L** Complexes in $D_2O$ at pH* ∼7

| L | *T* (°C) | rotamer | central N−Me | terminal N−Me's |
|---|---|---|---|---|
| 5′-GMP | 2 | *endo* | 3.13 | 2.19, 2.21 |
| | | *exo* | 3.09 | 2.28[b] |
| 5′-IMP[a] | 2 | *endo* | 3.15 | 2.18, 2.19 |
| | | *exo* | 3.14 | 2.30[b] |
| Me-5′-GMP | 2 | *endo* | 3.28 | 2.35, 2.36 |
| | | *exo* | 3.24 | 2.46[b] |
| 3′-GMP | 5 | *endo* | 3.15 | 2.23, 2.24 |
| | | *exo* | 3.11 | 2.29, 2.33 |
| 3′-IMP | 5 | *endo* | 3.18 | 2.20, 2.22 |
| | | *exo* | 3.14 | 2.28, 2.33 |
| Guo | 5 | *endo* | 3.15 | 2.23[b] |
| | | *exo* | 3.11 | 2.29[b] |
| 9-EtG | 5 | *endo* | 3.15 | 2.21[b] |
| | | *exo* | 3.11 | 2.29[b] |

[a] pH* = 5.6. [b] Broad.

**1D NMR Spectroscopy.** All NMR spectra were collected on a Varian Unity 600 spectrometer (spectral width, 6 kHz). The residual HOD peak was used as reference. In a typical experiment, a selective presaturation pulse (10 dB) was applied for 1 s to the residual HOD resonance. The FID was accumulated for 64 transients in blocks containing 16k data points. Before Fourier transformation, the FID's were baseline corrected for DC offset before an exponential multiplication apodization function with a 0.2 Hz line broadening was applied. Selected $^1$H NMR chemical shifts of the purine base and the $Me_3$dien ligand are reported in Tables 1 and 2.

**2D NMR Spectroscopy.** 2D NMR Spectra (512 × 2048 matrixes with a spectral window of 6−7 kHz in each dimension) were recorded on a Varian Unity 600 spectrometer. For the NOESY experiments, a 500 ms mixing time was used. A 1 s presaturation pulse was typically used to saturate the HOD resonance. Spectra were processed by using Felix 97.0 (MSI) on a Silicon Graphics INDY R4400 workstation. Typical processing involved zero-filling

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the $t_1$ dimension to 2048 points, exponential multiplication (0.4 Hz line broadening) in $t_2$, and applying a sine bell function shifted 90° over all points in $t_1$.

**Line-Shape Analysis.** In one case (*syn*-(*R,S*)-$Me_3$dienPt(9-EtG)) the rate constants for interconversion between *endo* and *exo* rotamers at different temperatures were calculated from line-shape analyses of $^1$H NMR spectra collected on a Bruker AVANCE 300 MHz spectrometer. Simulation of the spectra was performed in the temperature range 268−343 K using DNMR6. The enthalpy and the entropy of activation ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) were evaluated by using the Eyring equation. To avoid ligand isomerization, a pH* value of 1 was used.

## Results

***syn*-(*R,S*)-$Me_3$dienPt(5′-GMP).** Previous studies,[21] carried out at neutral pH and 24 °C, reported the detection of only one rotamer for *syn*-(*R,S*)-$Me_3$dienPt(5′-GMP). This rotamer was assigned an *endo* conformation on the basis of the assumption that this rotamer was stabilized by an H-bond interaction between the 5′-phosphate and an NH of $Me_3$dien (H-bonding is possible when both groups are on the same side of the platinum coordination plane). However, at lower temperature and pH* (5 °C and pH* 3) a small amount of the second rotamer can also be detected. In the NOESY spectrum (5 °C and pH* 3), EXSY cross-peaks of moderate size between the H8, NH, and terminal N−Me resonances of the two rotamers (Figure 1) indicate a moderate rate of exchange between the two rotamers.

Upon increase of the pH* from ∼3 to ∼7.5, the H8 resonance of the *endo* rotamer shifted downfield by 0.26 ppm; the H8 resonance of the *exo* rotamer did not appear to shift until its complete disappearance at neutral pH. The H8 signal of the *endo* rotamer shifted upfield by 0.18 ppm from pH* 7.5 to pH* 10. In the pH* range of 3−6.5 (both rotamers present in equilibrium), the amine NH signals of the *endo* rotamer shifted downfield (by 0.31 and 0.37 ppm), whereas those of the *exo* rotamer did not shift (Figure 2). In this as in all other adducts reported in this study, NH to ND exchange was observed. Typically, complete exchange required weeks at pH* ∼3 but only minutes at pH* ∼7. These observations are consistent with the literature showing the exchange is catalyzed by hydroxide ion.[27]

The downfield shift of the NH resonances and the increase in abundance coincident with the phosphate deprotonation are clear indications that the major rotamer has the *endo* conformation, which allows H-bond formation between the 5′-phosphate group and amine NH's.

***syn*-(*R,S*)-$Me_3$dienPt(5′-IMP).** The NMR spectrum of this 5′-IMP adduct has twice as many aromatic signals as that of the 5′-GMP analogue. The behavior of this adduct was very similar to that just described for the 5′-GMP complex (two rotamers observable only at low temperature and low pH*, with the *endo* rotamer much more abundant than the *exo* rotamer). In titration experiments performed at 2 °C, the abundance of the *endo* rotamer increased from 96 to 100% as the pH* was raised from ∼3 to ∼6. In the range of pH* 3−7 the H8 and H2 resonances of the major rotamer shifted downfield by 0.22 and 0.03 ppm, respectively, while no shift was observed for the H8 and H2 signals of the minor rotamer (visible up to pH* 6). From pH* ∼7 to pH* ∼10 the aromatic signals of the major rotamer (now the exclusive form) shifted upfield ($\Delta\delta$ = 0.11 and 0.12 ppm for H8 and H2,

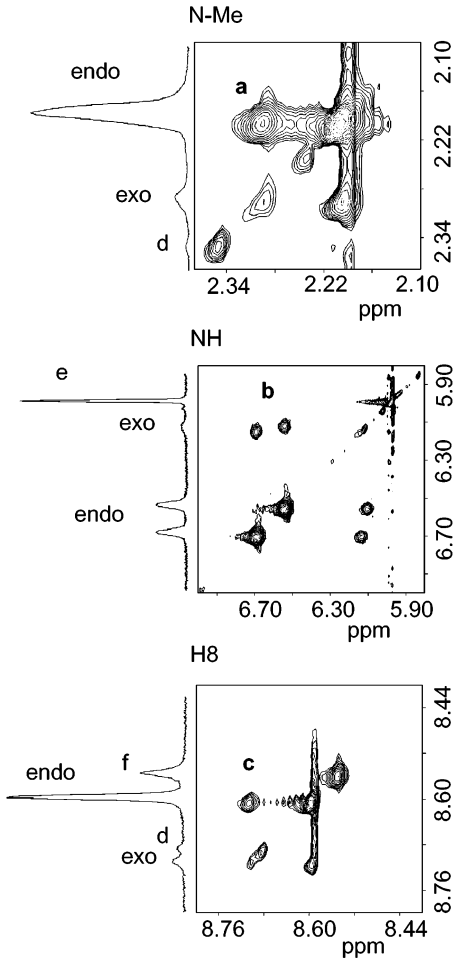


**Figure 1.** Terminal N−Me, NH, and H8 regions of the NOESY spectrum, recorded at 5 °C and pH* 3, for $Me_3$dienPt(5′-GMP) in $D_2O$. EXSY cross-peaks between *endo* and *exo* rotamers are labeled a−c for terminal N-Me, NH, and H8 protons, respectively: d = signals assignable to (*R,R*)- and (*S,S*)-$Me_3$dienPt(5′-GMP); e = H1′ proton signal, overlapped for all GMP species; f = free H8 5′-GMP signal.

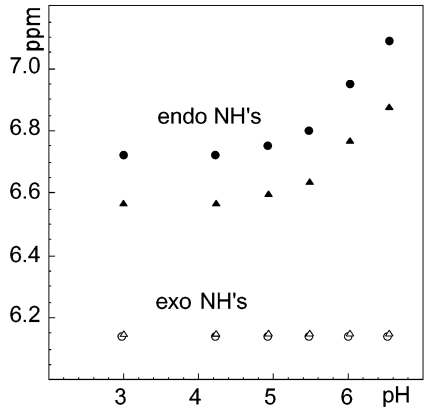


**Figure 2.** Dependence of NH resonances upon pH* for *syn*-(*R,S*)-$Me_3$dienPt(5′-GMP) at 2 °C.

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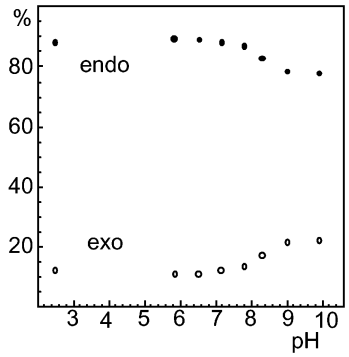


**Figure 3.** Distribution of the *endo* and *exo* rotamers as a function of pH* for *syn*-(*R*,*S*)-$Me_3$dienPt(Me-5′-GMP) at 2 °C.

respectively). By analogy with the 5′-GMP adduct, the major rotamer of the 5′-IMP adduct has the *endo* conformation.

***syn*-(*R*,*S*)-$Me_3$dienPt(Me-5′-GMP).** As for the 5′-GMP adduct, the NMR spectrum of the phosphate methyl ester derivative, *syn*-(*R*,*S*)-$Me_3$dienPt(Me-5′-GMP), also has only one H8 signal at 23 °C. However, at 2 °C two signals were observed at pH* ∼3 in the intensity ratio of ∼88:12, indicating the presence of both rotamers in equilibrium. The interconversion between rotamers is shown by the presence of H8, NH, and terminal N−Me EXSY cross-peaks in the NOESY spectrum. In the titration experiment performed at 2 °C, the H8 signals did not shift as the pH* was increased from ∼3 to ∼7.5, while from pH* ∼7.5 to ∼10 both H8 signals shifted upfield by 0.15 ppm concomitant with N1H deprotonation. The amine NH signals did not shift over the pH range in which they were detectable (pH* ∼3 to pH* ∼7).

The rotamer distribution as a function of pH* is shown in Figure 3. As the N1H was deprotonated, the concentration of the *endo* rotamer decreased slightly while that of the *exo* rotamer increased slightly. This is not the case for the 5′-GMP and 5′-IMP adducts, for which the percentage of the major rotamer increased from acidic to neutral pH and remained the exclusive form at basic pH. The different behavior of the Me-5′-GMP adduct is attributed to the impossibility for this nucleotide to achieve a dinegative charge on the phosphate (see Discussion).

***syn*-(*R*,*S*)-$Me_3$dienPt(3′-GMP).** In a preliminary study of this adduct,[21] the two broad H8 signals observed at 24 °C and pH* 7.7 were explained by the presence of two interconverting rotamers; however, because of the lack of experimental data, the assignment of a conformation to each rotamer was only tentative and, in fact, not correct. In a 1D spectrum taken at 5 °C and pH* 3, we found that the two H8 signals were relatively sharp and that the major (8.57 ppm) to minor (8.76 ppm) ratio is ∼2.5:1. The presence of strong EXSY cross-peaks in a 2D NOESY spectrum at −5 °C indicates that the rate of exchange between rotamers is still significant. However, at lower temperature, −20 °C, a NOESY spectrum has almost no EXSY cross-peaks.[28] At −20 °C the orientation of the **L** base in each rotamer could be determined from this NOESY spectrum (Figure 4). Both the more intense, more upfield H8 signal and the less intense, more downfield H8 signal have cross-peaks with the corresponding signals assigned to terminal N−Me groups (within each rotamer the two terminal N−Me's are not equivalent because of the asymmetry of the ribophosphate N9-substituent; however, in the case of the major rotamer the separation between the two N−Me signals is very small, Figure 4). Because each one of the two NOE cross-peaks of the minor species is almost as big as the global cross-peak of the major form, we conclude that the less abundant rotamer has the *exo* conformation, which is characterized by having H8 and N-Me's on the same side of the coordination plane and therefore close to each other (Chart 2). Accordingly, the more favored rotamer must have the *endo* conformation (H8 and the N−Me's on opposite sides of the platinum coordination plane).

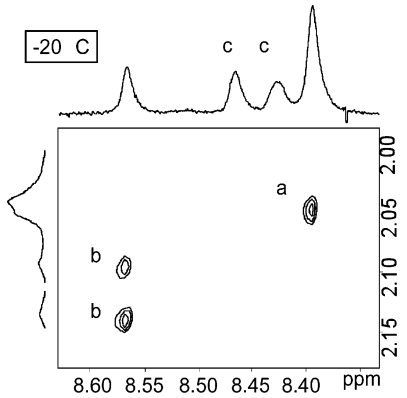


**Figure 4.** Terminal N−Me and H8 region of the NOESY spectrum, recorded at −20 °C and pH* 3, for $Me_3$dienPt(3′-GMP) (solvent $D_2O/CD_3$-OD, 65:35 v/v). N−Me···H8 NOE cross-peaks are labeled a and b for *endo* and *exo* rotamers, respectively. Signals labeled c belong to the species with (*R*,*R*) and (*S*,*S*) configurations of the $Me_3$dien ligand.

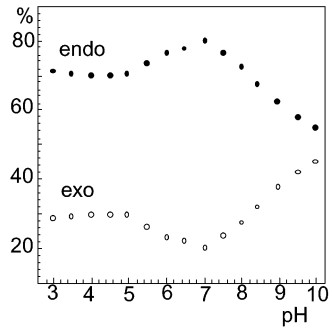


**Figure 5.** Distribution of the *endo* and *exo* rotamers as a function of pH* for *syn*-(*R*,*S*)-$Me_3$dienPt(3′-GMP) at 5 °C.

The pH* dependence of the rotamer distribution was studied at 5 °C (Figure 5, Table 1). When the pH* was raised from 3.0 to ∼7.5 (a range over which phosphate deprotonation takes place), the *endo*:*exo* ratio increased from ∼2.5 to ∼4. Only a very small downfield shift of the H8 signal of the *endo* rotamer was observed in this pH range. From pH* ∼7.5 to pH* ∼10, the *endo*:*exo* ratio drastically decreased from ∼4 to ∼1.2, and both H8 signals underwent

(28) Although the NOESY spectra indicate slower exchange between rotamers at −20 °C than at −5 °C, the resonance signals are nevertheless broader at −20 °C than at −5 °C. It is likely that dynamic processes other than interconversion between rotamers, such as slower changes in sugar pucker and *syn*/*anti*-3′-GMP conformation, cause the observed line broadening.

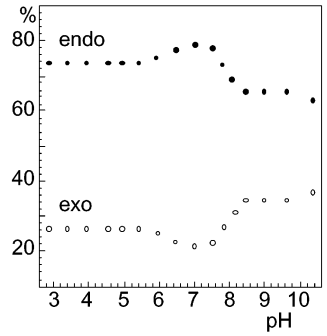


**Figure 6.** Distribution of the *endo* and *exo* rotamers as a function of pH* for *syn*-(*R*,*S*)-Me$_3$dienPt(3′-IMP) at 5 °C.

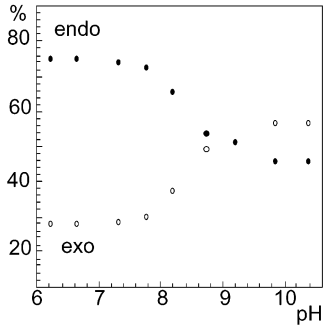


**Figure 7.** Distribution of the *endo* and *exo* rotamers as a function of pH* for *syn*-(*R*,*S*)-Me$_3$dienPt(Guo) at 5 °C.

an upfield shift that is typical for N1 deprotonation ($\Delta\delta$ of ~0.2 ppm).[26,29−33]

***syn*-(*R*,*S*)-Me$_3$dienPt(3′-IMP).** The usually more upfield aromatic signals assigned to H2 were related to their H8 counterparts on the basis of signal intensities. The behavior of this 3′-IMP adduct was practically identical to that of the analogous 3′-GMP complex. Experiments performed at 5 °C indicated that from pH* ~3 to pH* ~7.5 the *endo*:*exo* ratio increased from ~2.8 to ~3.5, while from pH* 7.5 to pH* 10 the *endo*:*exo* ratio decreased from ~3.5 to ~1.7 (Table 1, Figure 6). All four aromatic signals remained nearly unchanged over the range of pH* 3−7.5 but shifted upfield as the pH* was raised from 7.5 to 10 ($\Delta\delta$ of ~0.25 ppm for H8 and ~0.12 ppm for H2).

***syn*-(*R*,*S*)-Me$_3$dienPt(Guo).** The H8 signals observed for this guanosine adduct have a pattern similar to that described above for the 3′-GMP adduct, and the assignment was performed by analogy (Table 1). In the titration experiment performed at 5 °C, the *endo*:*exo* ratio decreased from ~2.8 to ~0.8 as the pH* was increased from ~6 to ~10 (Figure 7). For the same pH* range the H8 signals shifted upfield by 0.23 and 0.20 ppm for the *endo* and *exo* rotamer, respectively.

***syn*-(*R*,*S*)-Me$_3$dienPt(9-EtG).** The *syn*-(*R*,*S*)-Me$_3$dienPt-(9-EtG) complex was also investigated. At 5 °C and pH* of ~7, the H8 signals of this compound were clearly broader than those observed for the 3′-GMP adduct at the same temperature and pH* values, indicating that the rate of interconversion between the two rotamers is slightly faster for the 9-EtG adduct. By analogy with the 3′-GMP case, the more intense and more upfield H8 signal was assigned to the *endo* rotamer (8.28 ppm) while the less intense and more downfield signal was assigned to the *exo* rotamer (8.52 ppm). The *endo*:*exo* ratio decreased from ~2.1 at pH* ~6 to ~0.6 at pH* ~10, as deprotonation of N1H took place (Figure 8). Over the same pH* range, both the *endo* and *exo* H8 signals shifted upfield, as a consequence of N1H deprotonation ($\Delta\delta$ of 0.19 and 0.17 ppm, respectively).

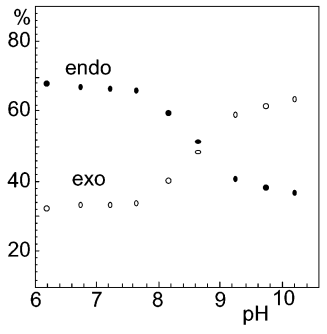


**Figure 8.** Distribution of the *endo* and *exo* rotamers as a function of pH* for *syn*-(*R*,*S*)-Me$_3$dienPt(9-EtG) at 5 °C.

**Rate Constants and Activation Parameters for Interconversion between Rotamers in *syn*-(*R*,*S*)-Me$_3$dienPt(9-EtG).** For the 9-EtG complex the rate constants for interconversion were evaluated by spectral simulation between 268 and 343 K (Figure 9). The enthalpy and entropy of activation ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, respectively) were estimated from plots of ln($k$/$T$) vs 1/$T$, which were linear within experimental error (Figure 10). With respect to the transition state, the *endo* rotamer is lower in enthalpy (by $-65 \pm 1$ kJ mol$^{-1}$) and similar in entropy (by $-2 \pm 2$ J K$^{-1}$ mol$^{-1}$). In contrast, the *exo* rotamer is lower in enthalpy by $-56 \pm 1$ kJ mol$^{-1}$ but higher in entropy by $+28 \pm 2$ J K$^{-1}$ mol$^{-1}$. From the activation parameters, it is possible to calculate the difference in enthalpy ($\Delta H$) and entropy ($\Delta S$) between *endo* and *exo* rotamers. Compared to the *exo* rotamer, the *endo* rotamer is lower in both enthalpy and entropy (by $9 \pm 1$ kJ mol$^{-1}$ and $30 \pm 2$ J K$^{-1}$ mol$^{-1}$, respectively). Evaluation of a plot of ln([*exo*]/[*endo*]) vs 1/$T$ gave $\Delta H$ and $\Delta S$ values similar to values obtained from the kinetic study.

## Discussion

**5′-Phosphate/NH Interactions.** In a previous study[21] of the complex *syn*-(*R*,*S*)-Me$_3$dienPt(5′-GMP), at neutral pH* and 24 °C, evidence for only one rotamer in solution was found. The apparently unique behavior of the 5′-GMP adduct was explained by the assumption that the tendency of the 5′-phosphate to form an H-bond with the NH of the carrier ligand renders the *endo* conformation, which places the 5′-phosphate and the NH's on the same side of the platinum coordination plane, by far the more favored of the two possible rotamers. The present study shows that, at lower pH* and temperature, it is possible to detect a small amount

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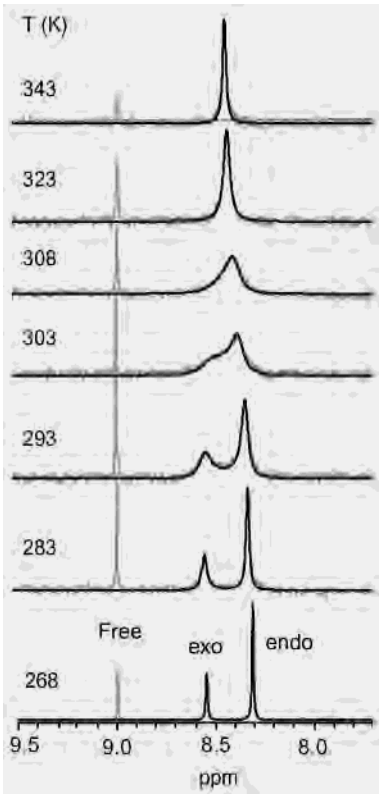


**Figure 9.** Experimental (pH* 1, gray line) and simulated (dark line) NMR spectra in the H8 resonance region for $Me_3$dienPt(9-EtG). The temperature of the sample is indicated at the left of each spectrum.

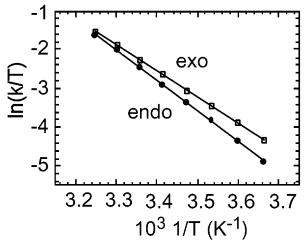


**Figure 10.** ln($k/T$) as a function of $1/T$ for *endo* and *exo* rotamers of *syn*-(*R*,*S*)-$Me_3$dienPt(9-EtG) at pH* 1.

of the second rotamer also. At pH* 3 and 2 °C, the ratio between major and minor rotamers was 9:1 for the 5′-GMP adduct and 7.3:1 for the Me-5′-GMP adduct investigated for the first time in this work.

When the pH* was raised from 3 to 6.5 at 2 °C, where the 5′-phosphate group of 5′-GMP is deprotonated, the major rotamer became the exclusive species in solution. In contrast, no change in rotamer distribution was observed for the Me-5′-GMP adduct for the same pH* change (major:minor ratio of 7.3 at pH* 6.5), in accord with the absence of phosphate deprotonation for Me-5′-GMP (Table 1).

N1H deprotonation at basic pH* did not change the rotamer distribution in the case of 5′-GMP (the major rotamer remains the only detectable form), whereas for Me-5′-GMP the concentration of the major rotamer decreased (major:minor ratio of 3.5 at pH* 10). In this respect, the *syn*-(*R*,*S*)-$Me_3$dienPt(Me-5′-GMP) complex follows the same trend as all the other *syn*-(*R*,*S*)-$Me_3$dienPt**L** complexes lacking a 5′-phosphate group.

The H-bond interaction between a 5′-phosphate and an NH of the carrier ligand (possible only for the *endo* rotamer) fully explains the changes in rotamer populations, as a function of pH*, observed for the 5′-GMP and Me-5′-GMP adducts. In the former complex, the *endo* rotamer increased with pH*, becoming the exclusive species at neutrality, because of further deprotonation of the 5′-phosphate around pH* 6. In contrast, for Me-5′-GMP, where further deprotonation of the phosphate group is not possible, the relative abundance of the two rotamers remained constant between pH* ∼3 and pH* ∼7.

Moreover, when the pH* was raised from 7 to 10, no change in rotamer population was observed for 5′-GMP, whereas for Me-5′-GMP the *exo* rotamer (O6 and amine NH on the same side of the coordination plane) increased slightly in abundance. Stabilization of the *exo* form as the pH* was raised to 10 is in accord with an increase of the H-bond capacity of O6 following N1H deprotonation.[30,31,33] For these adducts, the resulting O6/amine NH interaction can compete with phosphate/amine-NH H-bond interaction only in the case of Me-5′-GMP, which cannot form a dinegative 5′-phosphate group.

The above conclusion is further supported by the carrier ligand NH chemical shifts. For both the 5′-GMP and the Me-5′-GMP adducts, the spectrum of the *endo* rotamer exhibited well-resolved signals for the two NH protons of $Me_3$dien ($\Delta\delta$ of 0.16 and 0.10 ppm for 5′-GMP and Me-5′-GMP, respectively). In contrast, the NH resonances for the *exo* rotamer were almost unresolved. Only the NH resonances of the *endo* form of the 5′-GMP adduct shifted downfield (by 0.31 and 0.37 ppm, respectively) as the pH* was increased from ∼2.5 to ∼6.5 (at higher pH* a fast exchange between NH and $D_2O$ occurred even at 2 °C).[16] These results can be used to support the presence of 5′-phosphate−NH H-bonds. The marked nonequivalence of the two NH's observed in the major rotamer probably stems from the preferential H-bond interaction of the 5′-phosphate with one of the two NH groups.[34] In contrast, in the minor rotamer the 5′-phosphate cannot interact with the amine NH's (being on opposite sides of the platinum coordination plane) and the splitting of the NH resonances is negligibly small. The strong dependence upon pH of the chemical shift of the NH signals only for the major rotamer of the 5′-GMP adduct and not for the analogous species with Me-5′-GMP is in accord with deprotonation of the phosphate group at pH* ∼6 being possible only for the former complex. A downfield shift of the NH signals of the amine carrier ligand upon deprotonation of the 5′-phosphate of a *cis*-nucleotide has been reported.[16,33]

**Phosphate Group Effect on H8 Chemical Shift.** For **L** = 5′-GMP and 5′-IMP, it was possible to observe the so-called "wrong-way chemical shift".[2,29,33] When the pH* was raised from ∼3 to ∼7, the H8 resonance of the *endo* rotamer shifted significantly downfield ($\Delta\delta$ = 0.25 ppm for 5′-GMP and 0.23 ppm for 5′-IMP). Such a shift is simultaneous with

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deprotonation of the 5′-phosphate group, which takes place at pH* ∼6. The 5′-phosphate group close to the H8 proton can explain the observed downfield shift; however, such a situation occurs only in the *endo* rotamer, in which the H8 and the NH's of the amine are on the same side of the platinum coordination plane. Therefore, this observation clearly demonstrates that in the adduct the downfield shift requires the 5′-phosphate group to be brought into the vicinity of the H8 proton by an extra interaction such as H-bond formation with a NH proton.[33]

The "wrong-way chemical shift" was not observed in the adduct with Me-5′-GMP, which cannot undergo deprotonation of the phosphate as the pH* is raised from 3 to 7.

**O6/NH and 3′-Phosphate/NH Interactions.** Compounds lacking a 5′-phosphate (**L** = 3′-GMP, 3′-IMP, Guo, and 9-EtG) favor the *endo* rotamer at acidic and neutral pH*. Besides having the O6 on the more crowded side of the coordination plane, this rotamer also cannot form O6−NH H-bonds. As the pH* was increased from 7 to 10, the *endo*:*exo* ratio invariably decreased. The stabilization of the *exo* rotamer as the pH* was raised to 10 is in accordance with an increase of the H-bond capacity of O6 following N1H deprotonation.[20,30,31,33]

At pH* ∼10 the exo rotamer becomes dominant for **L** = Guo and 9-EtG adducts (*endo*:*exo* ratio of 0.8 and 0.6, respectively). However, for the adducts with **L** = 3′-GMP and 3′-IMP, the *endo* rotamer still remains the major form, although it is less abundant than at acidic and neutral pH (*endo*:*exo* ratios of 1.2 and 1.7, respectively). In addition, for the complexes of the 3′-nucleotides, titration experiments (Figures 5 and 6) have revealed that the *endo* rotamer was further stabilized as the pH* was raised from 3 to 6 in conjunction with the deprotonation of the 3′-phosphate group. Because in the *endo* rotamer the 3′-phosphate and the amine NH's are on the same side of the coordination plane, it is worth discussing if a direct H-bond interaction between the 3′-phosphate and an amine NH is possible. Models reveal that the distance between a phosphate oxygen and the NH is too long, regardless of how one rotates the phosphate group.[35] This point has been made frequently in the past for bis adducts.[9,15,31,33] However, second-sphere interactions between the two *cis*-3′-GMP's in bis adducts are complicated and obscure other interactions. Although a phosphate group located in the 3′ position is much less suited for interaction with NH groups of a carrier ligand than a phosphate in the 5′-position, it is conceivable that an intervening water molecule can facilitate an H-bond network, thus accounting for this stabilization.

**Factors Disfavoring O6/NH Interactions.** The preference at acidic and neutral pH for the *endo* rotamer (O6 on the same side of the N−Me groups with respect to the coordination plane) also for purine derivatives lacking a 5′-phosphate was unexpected because this conformer is characterized both by unfavorable interligand steric interactions and by the absence of any H-bond interaction between O6 and an NH of the *cis*-amine. A deeper insight into the factors affecting the stability of different conformers was gained by the kinetic and thermodynamic investigation performed on the *syn*-(*R*,*S*)-$Me_3$dienPt(9-EtG) complex. The *endo* rotamer had lower values of $\Delta H$ and $\Delta S$ with respect to the *exo* rotamer, by 9 kJ $mol^{-1}$ and 30 J $K^{-1}$ $mol^{-1}$, respectively. It is conceivable that, in the *exo* rotamer, as a consequence of an O6/NH interaction, there is a release of water molecules of solvation from the amine protons with consequent increase in enthalpy and entropy. However, the stabilizing effect of the greater $\Delta S$ does not compensate for the destabilizing effect of the greater $\Delta H$ (upon desolvation of the amine protons), and the *exo* rotamer becomes the less abundant form. A parallel investigation dealing with *cis*-$PtA_2\mathbf{G}_2$ complexes ($A_2$ = bidentate diamine ligand with one Me and one H on the nitrogen donors) also showed that the rotamer with O6 on the same side of the NH with respect to the coordination plane was characterized by higher enthalpy and entropy, and NH desolvation phenomena were invoked as a possible explanation.[36]

The role played by solvation/desolvation of the amine protons can also be clearly understood from the trend in activation parameters. The transition state for the interconversion between the two rotamers is rather high in $\Delta H$ with respect to both the *endo* and *exo* ground states, as expected from a large increase in steric interaction between the $Me_3$-dien ligand and the guanine base when the latter traverses through the platinum coordination plane. However, with respect to the transition state, the *endo* rotamer (in which there is no competition between O6 of guanine and solvent molecules for interaction with amine protons) has significantly lower enthalpy than the *exo* rotamer. The transition state, while having entropy comparable to that of the *endo* ground state ($\Delta S^{\ddagger}$ of only 2 J $K^{-1}$ $mol^{-1}$), has considerably lower entropy with respect to the *exo* ground state ($\Delta S^{\ddagger}$ = −28 J $K^{-1}$ $mol^{-1}$). This lower entropy of the transition state must clearly have a molecular basis, which we believe is very likely to be the solvation of the amine protons, as the weak O6/NH interaction no longer impedes access of water to these NH groups.

## Conclusions

The mono adducts with a specially designed carrier ligand studied here simultaneously reinforce, clarify, and expand upon observations and conclusions we have reached from the study of bis adducts.[8−15,33] The bis adducts are more faithful models of DNA cross-links. However, interactions between the two *cis*-**L** ligands in bis adducts complicate the interpretation of **L** to carrier ligand interactions. The absence of a second **L** in these mono adducts allowed us to gain a better and more precise understanding of the relative importance of carrier ligand H-bonding interactions.

In the absence of any complication arising from the presence of a phosphate group, an H-bond interaction between the O6 of a coordinated purine and the NH of the

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carrier ligand in a mono adduct contributes little to the stability of the rotamer in which such an interaction can take place (*exo* rotamer). This result agrees with our previous findings with bis adducts. The temperature dependence of both the equilibrium and the interconversion rate between *endo* and *exo* rotamers in a mono adduct provides important results helping to explain the weakness of the O6/ amine-NH interactions. When this interaction is established, the release of water molecules of solvation from the amine protons causes an increase in entropy, which does not fully compensate for the concomitant increase in enthalpy. As a consequence, the *endo* rotamer in which the NH's are left completely free to interact with solvent molecules is the more stable rotamer (*endo*:*exo* ratio of ~2.2 for **L** = 9-EtG).

A strict analogy is observed between derivatives of hypoxanthine and guanine adducts. However, at acidic pH, the adducts with hypoxanthine derivatives (3′-IMP and 5′-IMP) appear to have a slightly lower preference for the *exo* rotamer than the corresponding compounds with guanine derivatives (3′-GMP and 5′-GMP). Such behavior would be in accord with a slightly weaker ability for hypoxanthine adducts to participate in an O6/NH interaction as compared to the guanine adducts. The lower electron density on O6 (and hence lower H-bond accepting ability) arises from the absence of the electron-donating 2-$NH_2$ group in hypoxanthine derivatives.

At pH* above 8.5, N1H deprotonation increases the H-bond acceptor capacity of O6, rendering the O6/amine-NH interaction more competitive. For Guo and 9-EtG adducts, this interaction is responsible for inverting the order of stability, and the *endo:exo* ratio, which was ~2.2 at acidic pH*, becomes ~0.7 at pH* 10.

For *syn*-(*R*,*S*)-$Me_3$dienPt**L** complexes with **L** = 5′-GMP and 5′-IMP, the evidence clearly indicates the occurrence of 5′-phosphate/amine NH interactions. There is a striking preference for the *endo* form even at very acidic pH, where only a very small amount of the *exo* form is present. In addition, downfield shifts of amine NH signals and the "wrong-way chemical shift" of H8 are all consistent with the occurrence of such H-bonding. Models and X-ray structures confirm that the 5′-phosphate group is structurally capable of forming such a direct H-bond.

For the 3′-GMP and 3′-IMP adducts, the *endo* rotamer is preferred (*endo*:*exo* ratio of 1.2 and 1.7, respectively). However, this preference is higher than for the 9-EtG adduct, thus providing evidence that a weak interaction can take place between the 3′-phosphate and the amine *cis*-NH groups when both types of groups are on the same side of the platinum coordination plane. This interaction is undoubtedly indirect and most likely involves a hydrogen-bond water network. No evidence for this type of weak interaction was found in studies of the more complicated bis adducts.

Finally, data for the Me-5′-GMP adduct along with the data for other adducts lead to the following order of decreasing carrier ligand NH H-bond interactions: 5′-$PO_4^{2-}$ > 5′-$PO_4H^-$ > 5′-$MePO_4^-$ > **L**$^-$O6 > 3′-$PO_4^{2-}$ > 3′-$PO_4H^-$ ~ **L** O6.

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