Articles

Influence of Carrier Ligand NH Hydrogen Bonding to the O6 and Phosphate Group of Guanine Nucleotides in Platinum Complexes with a Single Guanine Ligand

Maria Carlone,[†] Francesco P. Fanizzi,[†] Francesco P. Intini,[†] Nicola Margiotta,[†] Luigi G. Marzilli,[‡] and Giovanni Natile*,[†,§]

Dipartimento Farmaco-Chimico, Università di Bari, via E. Orabona 4, 70125 Bari, Italy, and Department of Chemistry, Emory University, Atlanta, Georgia 30322

Received May 11, 1999

Coordinated *N*,*N*′,*N*″-trimethyldiethylenetriamine (**Me$_3$dien**) has several possible configurations: two have mirror symmetry (*R*,*S* configurations at the terminal nitrogens) and the terminal N−Me's *anti* or *syn* with respect to the central N−Me (*anti*-(*R*,*S*) and *syn*-(*R*,*S*) isomers, respectively), and two are nonsymmetrical (*R*,*R* and *S*,*S* configurations at terminal nitrogens, *rac* denotes a 1:1 mixture of the two isomers). For each configuration, two **Me$_3$dien**Pt**G** atropisomers can be formed (*anti* or *syn* orientation of central N−Me and **G** O6, **G** = guanine derivative), and these can be observed since the terminal N−Me's decrease the rate of **G** rotation about the Pt−N7 bond. In symmetrical *syn*-(*R*,*S*)-**Me$_3$dien**Pt**G** derivatives with **G** = 9-EtG and 3′-GMP, the anti rotamer, which can form O6−NH H-bonds, was slightly favored over the *syn* rotamer but never more than 2:1. This *anti* rotamer is also favored by lower steric repulsion between the terminal N−Me's and **G** O6; thus, the contribution of O6−NH H-bonding to the stability of the anti rotamer could be rather small. With **G** = 5′-GMP, an O6−NH H-bond in the *anti* rotamer and a phosphate−NH H-bond in the *syn* rotamer can form. Only the *syn* rotamer was detected in solution, indicating that NH H-bonds to 5′-phosphate are far more important than to O6, particularly since steric factors favor the *anti* rotamer. Interconversion between rotamers was faster for *syn*-(*R*,*S*)- than for *rac*-**Me$_3$dien** derivatives. This appears to be determined by a smaller steric impediment to **G** rotation of two "quasi equatorial" N−Me's, both on one side of the platinum coordination plane (*syn*-(*R*,*S*) isomer), than one "quasi equatorial" and one "quasi axial" N−Me on either side of the coordination plane (*rac* isomer).

Introduction

Since the discovery of its anticancer activity, many new analogues of *cis*-[$PtCl_2(NH_3)_2$] (cisplatin) have been synthesized and tested for biological activity, and some of these compounds are now well-established anticancer drugs.[1−3]

Carrier amine ligands appear to modulate the anticancer properties of this class of drugs. Activity is usually lost or diminished if the primary or secondary amines on platinum are replaced by tertiary amines.[4] This important dependence of activity on the carrier ligand has led to the hypothesis that O6−NH[5−8] and/or phosphate−NH[8−15] intramolecular hydrogen bonding within the Pt−DNA adduct influences structure and hence activity.

Pt drugs are known to target DNA primarily by forming bifunctional adducts with two adjacent purine bases of the same strand.[9] Therefore, several *cis*-PtA_2**G**$_2$ species, in which A_2 is two unidentate or one bidentate amine ligand and **G** is a unidentate guanine derivative have been studied as model complexes. However, even in the simplest model compound with symmetrical carrier ligands, the **G** bases can be oriented in a head-to-head (HH) or in a head-to-tail (HT) arrangement. There are two possible HT orientations, differing in chirality.

With a nonbulky A_2 carrier ligand, interconversion between *cis*-PtA_2**G**$_2$ atropisomers by rotation around the Pt−N7 bonds is fast on the NMR time scale. Usually a single H8 NMR signal is observed, representing an average signal of all possible guanine orientations.[10−12] Increasing the bulk of the A_2 ligand can slow the Pt−N7 bond rotation so that different atropisomers can be detected by the observation of more than one H8 signal.[6,7,10−15]

[†] Università di Bari.
[‡] Emory University.
[§] E-mail: natile@farmchim.uniba.it.

(1) Bloemink, M. J.; Reedijk, J. In *Metal Ions in Biological Systems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1996; Vol. 32, p 641.
(2) Reedijk, J. *Chem. Commun.* **1996**, 801.
(3) Pasini, A.; Zunino, F. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 615.
(4) Sundquist, W. I.; Lippard, S. J. *Coord. Chem. Rev.* **1990**, *100*, 293.
(5) Sherman, S. E.; Gibson, D.; Wang, A. H. J.; Lippard, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 7368.
(6) Xu, Y.; Natile, G.; Intini, F. P.; Marzilli, L. G. *J. Am. Chem. Soc.* **1990**, *112*, 8177.
(7) Kiser, D.; Intini, F. P.; Xu, Y.; Natile, G.; Marzilli, L. G. *Inorg. Chem.* **1994**, *33*, 4149.
(8) Guo, Z.; Sadler, P. J.; Zang, E. *Chem. Commun.* **1997**, 27.
(9) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. *Biochemistry* **1985**, *24*, 707. Eastman, A. *Biochemistry* **1985**, *24*, 5027.
(10) Cramer, R. E.; Dahlstrom, P. L. *J. Am. Chem. Soc.* **1979**, *101*, 3679.
(11) Cramer, R. E.; Dahlstrom, P. L.; Seu, M. J. T.; Norton, T.; Kashiwagi, M. *Inorg. Chem.* **1980**, *19*, 148.
(12) Cramer, R. E.; Dahlstrom, P. L. *Inorg. Chem.* **1985**, *24*, 3420.
(13) Miller, S. K.; Marzilli, L. G. *Inorg. Chem.* **1985**, *24*, 2421.
(14) Reily, M. D.; Marzilli, L. G. *J. Am. Chem. Soc.* **1986**, *108*, 6785.
(15) Ano, S. O.; Intini, F. P.; Natile, G.; Marzilli, L. G. *J. Am. Chem. Soc.* **1997**, *119*, 8570.

10.1021/ic990517f CCC: $19.00
Published on Web 01/27/2000

In our previous studies, we found that interligand or "second coordination sphere" effects involving both steric and H-bonding interactions influenced the conformation and relative abundance of the rotamers.[16] An important new result of our recent work on *cis*-$PtA_2\mathbf{G}_2$ complexes was the unambiguous indication that the 3′- and the 5′-phosphate group can "reach over" to the NH groups in the six-membered ring of the *cis* guanine and that this interaction will modulate the distribution and conformation when A_2 is bulky.[17]

Therefore we have selected a simpler system (containing a triamine carrier ligand, *N*,*N*′,*N*″-trimethyldiethylenetriamine (**Me$_3$dien**) with two terminal secondary amine groups) to assess H-bond interactions between the NH of carrier ligands and either the O6 or the 5′-phosphate of a nucleotide in a *cis* position, without interference from *cis*-**G** to *cis*-**G** interactions possible in *cis*-$PtA_2\mathbf{G}_2$ intrastrand cross-link models.

In this study, **Me$_3$dien** and [PtX(**Me$_3$dien**)]X (X = I, NO_3) were synthesized and characterized. The nitrogen configurations are kinetically stable in the complexes; therefore the formation of a given isomer is under kinetic control. Under our experimental conditions, only one symmetrical [PtX(**Me$_3$dien**)]X isomer was isolated in the solid state. In solution at neutral pH, however, slow isomerization to a nonsymmetrical isomer and to a second symmetrical isomer takes place. The interaction of $[Pt(D_2O)(\mathbf{Me_3dien})]^{2+}$ with 9-EtG, 3′-GMP, and 5′-GMP was studied by NMR techniques. For comparison, analogous investigations were conducted on the related complexes with *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (**Me$_5$dien**).

Experimental Section

Materials. *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (**Me$_5$dien**) from Aldrich was used as received. *N*,*N*′,*N*″-trimethyldiethylenetriamine (**Me$_3$dien**) was prepared from *N*-methylbis(2-chloroethyl)amine trihydrochloride (Aldrich) as described in ref 18. The complex $[Pt(H_2O)(Me_5dien)](CF_3SO_3)_2$ was prepared from $[PtI(Me_5dien)]_2[Pt_2I_6]$ suspended in water and treated with silver triflate as described in ref 19.

Preparation of [PtI(Me$_3$dien)]I. $K_2[PtCl_4]$ (0.33 g, 0.8 mmol) in water (10 mL) was treated with a solution of KI (1.33 g, 8 mmol) in water (10 mL). The solution was stirred for a few minutes and then treated with a stoichiometric amount of free amine (0.2 g, 0.8 mmol, of **Me$_3$dien**·3HCl in water (10 mL) containing 0.135 g, 2.4 mmol, of KOH). The yellow precipitate which separated after 1 day of stirring at room temperature was collected, washed with water, and dried. Yield of [PtI(**Me$_3$dien**)]I: ∼50%. Anal. Calcd for $C_7H_{19}I_2N_3Pt$: C, 14.1; H, 3.2; N, 7.1. Found: C, 13.8; H, 3.0; N, 7.0.

Preparation of [Pt(NO$_3$)(Me$_3$dien)](NO$_3$). [PtI(**Me$_3$dien**)]I in acetone (0.24 mmol in 100 mL of solvent at 50 °C) was treated with a stoichiometric amount of $AgNO_3$ (82 mg, 0.48 mmol) dissolved in a minimum of water (5 mL). After it was stirred at 50 °C in the dark for 4 h, the mixture was filtered. The filtrate was concentrated to small volume and kept at 4 °C for a few days. The white crystals which formed were collected, washed with acetone, and dried; yield, ∼60%. Anal. Calcd for $C_7H_{19}N_5O_6Pt$: C, 18.1; H, 4.1; N, 15.1. Found: C, 18.2; H, 4.2; N, 15.3.

NMR Spectroscopy. ^{1}H NMR 1D and 2D spectra were obtained with either a Bruker AMWB 300 MHz or a Bruker AVANCE DRX 500 MHz spectrometer. ΔG^* values were determined from line-shape analysis of spectra recorded at different temperatures and using the Eyring equation.

Preparation of Me$_n$dienPtG Complexes (n = 3, 5). To an NMR tube containing the required amount of either [Pt(NO$_3$)(**Me$_3$dien**)](NO$_3$) or $[Pt(H_2O)(Me_5dien)](CF_3SO_3)_2$ (typically 2 mg in 0.5 mL of solvent; for 2D experiments the amount of complex was increased to 8 mg) was added a weighed amount of **G** (ca. 1:1 molar ratio, but in some cases a slight excess of **G** was used). The progress of the reaction at ambient temperature was monitored by ^{1}H NMR spectroscopy. The pH (uncorrected) was maintained at ∼7 and adjusted with DNO_3 as required.

Results and Discussion

Stereochemistry of the Coordinated Me$_5$dien Ligand. The ^{1}H NMR spectrum of $[Pt(H_2O)(\mathbf{Me_5dien})](CF_3SO_3)_2$ in D_2O has three signals for the N−Me's (relative intensities of 3:6:6) and four multiplets (each integrating for two protons) for the protons of the ethylene chains. Two vicinal protons (multiplets at 3.68 and 3.51 ppm) are strongly coupled (ca. 13 Hz); therefore, they are both axial (torsion angle of ca. 180°). The other two vicinal protons (multiplets at 3.04 and 2.96 ppm) are weakly coupled (3 Hz), and therefore they are both equatorial (torsion angle of ca. 60°). Geminal couplings (13 Hz) between the multiplets at 3.68 and 2.96 ppm and between the multiplets at 3.51 and 3.04 ppm indicate that the protons in each pair are in the same methylene group. As expected, the two fused rings of the **Me$_5$dien** ligand have a defined pucker.[20,21]

The multiplet at 3.51 ppm has NOE cross-peaks with the singlets at 3.07 ppm (central N−Me) and 2.77 ppm (two terminal N−Me's), indicating that the corresponding protons are on the same side with respect to the platinum coordination plane. Therefore, the multiplet at 3.51 ppm can be assigned to the axial protons of the methylene groups adjacent to the terminal nitrogens and the singlet at 2.77 ppm to the terminal N−Me's *syn* to the central N−Me. The multiplet at 3.68 ppm has an NOE cross-peak with the singlet at 2.94 ppm (two terminal N−Me's), indicating that the corresponding protons are on the same side of the platinum coordination plane. Therefore the multiplet at 3.68 ppm can be assigned to the axial protons of the methylene groups adjacent to the central nitrogen and the singlet at 2.94 ppm to the terminal N−Me's *anti* to the central N−Me.

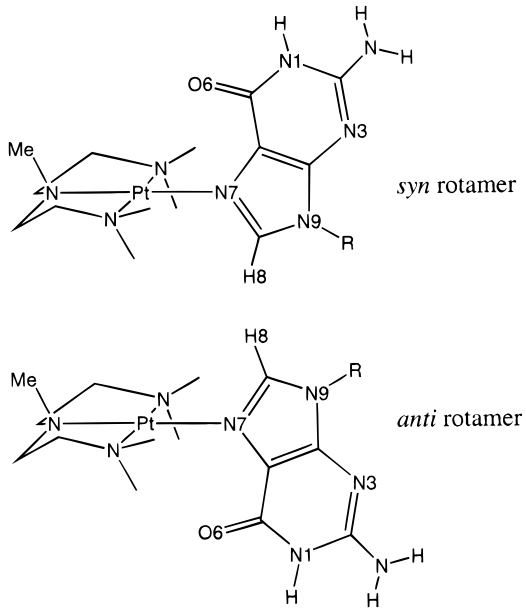
Reaction of $[Pt(H_2O)(Me_5dien)]^{2+}$ with G Derivatives. Reaction of the aqua species with **G** (**G** = 9-EtG, Guo, 5′-dGMP, and 5′-GMP) in water produces **Me$_5$dien**Pt**G** complexes. In all cases two sharp H8 signals were observed, indicating the formation of both possible rotamers; these interconvert slowly on the NMR time scale (Table 1). To classify these rotamers we adopted a stereochemical convention based on the orientation of the O6 of guanine and the central N−Me with respect to the coordination plane.[21,22] Thus, *syn* and *anti* rotamers are defined as the rotamer with O6 of guanine on the same side as and opposite, respectively, the central N−Me with respect to the coordination plane (Chart 1). H8−N−Me NOE cross-peaks

(16) Marzilli, L. G.; Intini, F. P.; Kiser, D.; Wong, H. C.; Ano, S. O.; Marzilli, P. A.; Natile, G. *Inorg. Chem.* **1998**, *37*, 6898. Ano, S. O.; Intini, F. P.; Natile, G.; Marzilli, L. G. *Inorg. Chem.* **1999**, *38*, 2989.
(17) Wong, H. C.; Intini, F. P.; Natile, G.; Marzilli, L. G. *Inorg. Chem.* **1999**, *38*, 1006. Wong, H. C.; Coogan, R.; Intini, F. P.; Natile, G.; Marzilli, L. G. *Inorg. Chem.* **1999**, *38*, 777. Wong, H. C.; Shinozuka, K.; Natile, G.; Marzilli, L. G. *Inorg. Chim. Acta*, in press.
(18) Bencini, A.; Bianchi, A.; Garcia-Espana, E.; Fusi, V.; Micheloni, M.; Paoletti, P.; Ramirez, J. A.; Rodriguez, A.; Valtancoli, B. *J. Chem. Soc., Parkin Trans. 2* **1992**, 1059.
(19) Cini, R.; Intini, F. P.; Maresca, L.; Pacifico, C.; Natile, G. *Eur. J. Inorg. Chem.* **1998**, 1305.
(20) Pitner, T. P.; Martin, R. B. *J. Am. Chem. Soc.* **1971**, *93*, 4400.
(21) Kim, S.-H.; Martin, R. B. *Inorg. Chim. Acta* **1984**, *91*, 11.
(22) Yuriev, E.; Orbell, J. D. *Inorg. Chem.* **1998**, *37*, 6269.

Table 1. ^{1}H NMR Data of H8 of Guanine and N−Me's of the **Dien** Ligand in **Me$_5$dien**Pt**G** and **Me$_3$dien**Pt**G** Complexes

				terminal	
complex	rotamer	H8	central N−Me	*anti*-N−Me	*syn*-N−Me
[Pt(D$_2$O)(**Me$_5$dien**)]$^{2+}$			3.07	2.94	2.77
Me$_5$dien Pt(9-EtG)	*anti*	8.70	3.14	2.75	2.58
	syn	8.61	3.20	2.86	2.48
Me$_5$dien Pt(Guo)	*anti*	8.91	3.14	2.75	2.60
	syn	8.79	3.21	2.86	2.50
Me$_5$dien Pt(5′-dGMP)	*anti*	8.98	3.15	2.74	2.61
	syn	8.94	3.20	2.88	2.49
Me$_5$dien Pt(5′-GMP)	*anti*	9.00	3.16	2.76	2.64
	syn	8.96	3.21	2.89	2.50
[Pt(D$_2$O)(*rac*-**Me$_3$dien**)]$^{2+}$			3.00	2.70	2.53
rac-**Me$_3$dien**Pt(9-EtG)	*anti*	8.38	3.08	2.40	2.23
	syn	8.34	3.11	2.45	2.22
rac-**Me$_3$dien**Pt(3′-GMP)	*anti*	8.65, 8.66	3.10	2.41, 2.43	2.26, 2.27
	syn	8.60, 8.61	3.13	2.48, 2.50	2.24, 2.25
rac-**Me$_3$dien**Pt(5′-GMP)	*anti*	9.04, 9.06	3.10	2.37	2.26, 2.29
	syn	9.00, 9.02	3.10	2.43,2.45	2.22, 2.24
[Pt(D$_2$O)(*syn*-(*R*,*S*)-**Me$_3$dien**)]$^{2+}$			3.06		2.55
syn-(*R*,*S*)-**Me$_3$dien**Pt(9-EtG)	*anti*	8.28	3.14 br		2.25 br
	syn	8.50	3.14 br		2.25 br
syn-(*R*,*S*)-**Me$_3$dien**Pt(3′-GMP)	*anti*	8.58	3.16 br		2.28 br
	syn	8.72	3.16 br		2.28 br
syn-(*R*,*S*)-**Me$_3$dien**Pt(5′-GMP)	*syn*	8.85	3.14		2.21, 2.23
[Pt(D$_2$O)(*anti*-(*R*,*S*)-**Me$_3$dien**)]$^{2+}$			2.94	2.72	
anti-(*R*,*S*)-**Me$_3$dien**Pt(9-EtG)	*anti*, *syn*	8.41	3.03	2.47	

Chart 1. Sketches of Different Rotamers for **Me$_{(3,5)}$dien**Pt**G** Complexes



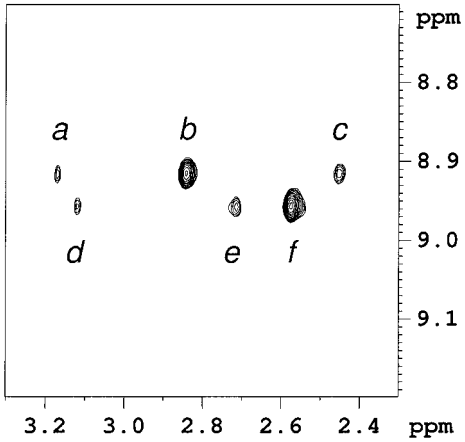


Figure 1. N−Me region of the NOESY/EXY spectrum (5 °C) of **Me$_5$dien**Pt(5′-dGMP). Cross-peaks between H8 of 5′-dGMP and central N−Me and terminal *anti*-N−Me and *syn*-N−Me of **Me$_5$dien** are labeled a, b, and c for the *syn* rotamer and d, e, and f for the *anti* rotamer, respectively.

allowed us to assess the **G** base orientation (Figure 1). Although the two rotamers are formed in a similar amount for 5′-dGMP and 5′-GMP, the *anti*:*syn* ratio is ∼1.6 for the 9-EtG derivative and ∼1.25 for the Guo derivative.

With respect to the aqua species, coordination of the purine base causes the signal of the central N−Me to shift downfield by ∼0.1 ppm; the shift is greater for the *syn* than for the *anti* rotamer. In contrast, the terminal N−Me signals shift upfield; the size of the shift depends upon the *syn* or *anti* configuration of the terminal N−Me's (*syn* N−Me's have "quasi equatorial" character, while *anti* N−Me's have "quasi axial" character) and upon the *syn* or *anti* orientation of the **G**. The "quasi equatorial" *syn* methyl signals undergo an average upfield shift of ∼0.28 and 0.16 ppm for the *syn* and the *anti* rotamer, respectively. For "quasi axial" *anti* N−Me's, the upfield shift is less, 0.19 and 0.07 ppm for the *anti* and *syn* rotamer, respectively.

Finally, for the Guo, 5′-dGMP, and 5′-GMP complexes, the asymmetry of the sugar causes a diastereotopic splitting of terminal methyl signals. The splitting is usually very small (<0.02 ppm) and appears to be greater for "quasi equatorial" *syn* than for "quasi-axial" *anti* methyl signals.

The H8 shift of *anti* rotamers is nearly constant for this series of **G**'s with sugars, suggesting that the 5′-phosphate is away from H8 and does not cause the usual downfield shift of ca. 0.2 ppm. For *syn* rotamers the phosphate effect is small. Compared to "axial" N−Me's, "equatorial" N−Me's appear to obstruct the approach of the phosphate group to H8.

Stereochemistry of the Coordinated Me$_3$dien Ligand. The insolubility of the [PtI(**Me$_3$dien**)]I complex in most organic solvents and in water limited our studies. Therefore [PtI(**Me$_3$dien**)]I was converted into the nitrate complex, which forms the aqua complex in water. ^{1}H NMR spectra, taken at different time intervals, of [Pt(NO$_3$)(**Me$_3$dien**)](NO$_3$) (∼5 mM in D$_2$O at pH 4.3) are shown in Figure 2.

A broad signal at 6.25 ppm was assigned to the terminal

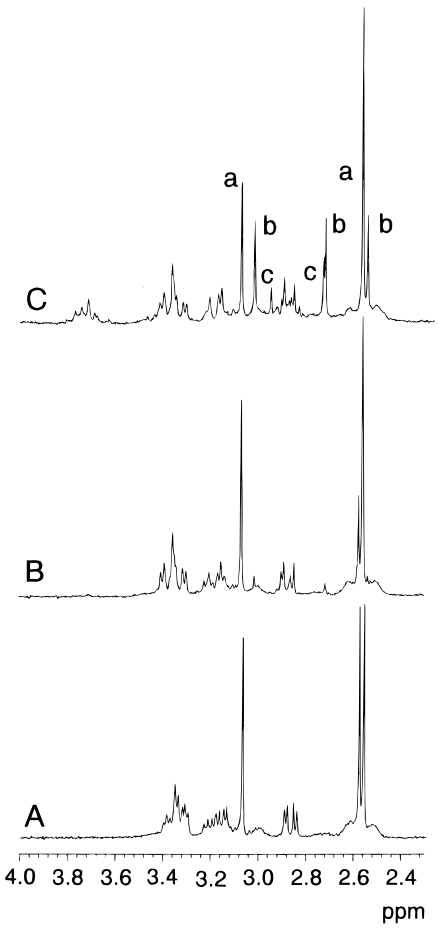
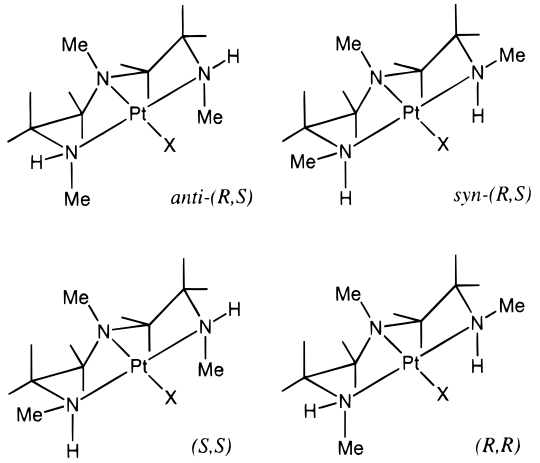


Figure 2. ^{1}H NMR spectra (24 °C) of $[Pt(D_2O)(\mathbf{Me_3dien})]^{2+}$ at different reaction times: A, soon after dissolution; B, after 20 min; C after 60 h. N−Me peaks for *syn*-(*R*,*S*), *rac*, and *anti*-(*R*,*S*) configurations of the **Me$_3$dien** ligand are labeled a, b, and c, respectively.

amine protons, since it decreased with time because of exchange with D_2O. Two methyl resonances are observed at 3.06 and 2.56 ppm (intensity ratio 1:2). The signal at 2.56 ppm, initially a doublet, became a singlet upon NH to ND exchange and is assigned to the terminal N−Me's (a doublet and an overlapping singlet for terminal N−Me's in spectrum B of Figure 2 indicate partial NH to ND exchange). The signal at 3.06 ppm is assigned to the central N−Me. The CH signals of the ethylene bridges fall in the range 2.8−3.4 ppm. A pseudotriplet at 3.32 ppm integrates for four protons and is assigned to the axial ethylene protons, which have a large coupling with the geminal proton and with the axial vicinal proton. Multiplets at 2.85 and 3.15 ppm are assigned to the equatorial protons of the methylene groups.

With time (spectrum C in Figure 2) a new set of three signals of equal intensity appeared in the region of N−Me signals. One signal at 3.0 ppm is close to the signal of the central N−Me of the initial species. The other two signals at 2.54 and 2.71 ppm are only 0.02 ppm upfield and 0.15 ppm downfield with respect to the signal of the terminal N−Me's of the initial species. Significant changes were observed in the region of the methylene signals; in particular, a new multiplet emerged at 3.70 ppm (which had no counterpart in the initial spectrum). The intensity ratio between the new and initial sets of signals was ∼1:2. Finally, a third small set of signals emerged. This set of two singlets (at 2.94 and 2.72 ppm, intensity ratio ca. 1:2) can be tentatively assigned to the central and terminal N−Me signals of a third isomer.

Chart 2. Sketches of Different Configurations for the **Me$_3$dien** Ligand in Platinum Complexes



$[Pt(D_2O)(\mathbf{Me_3dien})]^{2+}$ can form different isomers depending upon the configuration of the **Me$_3$dien** ligand (Chart 2). Two have mirror symmetry (*R*,*S* configurations at the terminal nitrogens) and the terminal N−Me's *anti* or *syn* with respect to the central N−Me (*anti*-(*R*,*S*) and *syn*-(*R*,*S*) isomers, respectively). The two nonsymmetrical isomers have *R*,*R* and *S*,*S* configurations at the terminal nitrogens and constitute a couple of enantiomers (*rac* indicates a 1:1 mixture of *R*,*R* and *S*,*S* enantiomers).

The single resonance for the two terminal methyls observed in the ^{1}H NMR spectrum of a freshly prepared solution of $[Pt(D_2O)(\mathbf{Me_3dien})]^{2+}$ indicates a symmetrical ligand (either *anti*-(*R*,*S*) or *syn*-(*R*,*S*) configuration of the ligand). Although we expected the *anti*-(*R*,*S*) configuration to be preferred on the basis of a smaller sterical strain, X-ray data indicated the formed isomer to have the *syn*-(*R*,*S*) configuration.[23] The new set of three signals of equal intensity in the region of N−Me's (one for the central methyl and two for the terminal methyls) can only be assigned to the aqua species with nonsymmetrical **Me$_3$dien** ligand (*R*,*R* or *S*,*S* configurations at the terminal nitrogens); this compound is formed from the initial symmetrical isomer by inversion of configuration at a terminal nitrogen. The half of the **Me$_3$dien** ligand (ethylene chain and terminal NHMe) where inversion of configuration at nitrogen has occurred is expected to give quite different NMR signals (new singlet at 2.71 ppm for terminal N−Me and a multiplet at 3.70 ppm for methylene protons). Finally, the two very weak signals of roughly 1:2 intensity observed at long reaction time can be assigned to the second symmetrical isomer of the aqua species (*anti*-(*R*,*S*) configuration of the **Me$_3$dien** ligand). It is to be noted that in both Me_3dien and Me_5dien complexes the "quasi axial" terminal N−Me is 0.17 ppm downfield with respect to the "quasi equatorial" N−Me's, which may be a consequence of the magnetic anisotropy of the platinum metal whose shielding effect is greater for "quasi equatorial" than "quasi axial" methyls.

Reaction with 9-EtG. NMR spectra of a freshly prepared solution of $[Pt(NO_3)(\mathbf{Me_3dien})](NO_3)$ in D_2O, pH 7.03, treated with a slight excess of 9-EtG (molar ratio 1:1.4), were recorded before the addition of 9-EtG (A), soon after mixing the reactants (B), and after 20 min (C), 1 h (D), and 36 h (E) (Figure 3).

Spectrum 3B indicates that the reaction is complete in the mixing time of the reagents. The new species, **Me$_3$dien**-Pt(9-EtG), is characterized by two broad 9-EtG H8 signals of

(23) C. Pacifico, F. P. Intini, G. Natile, unpublished results.

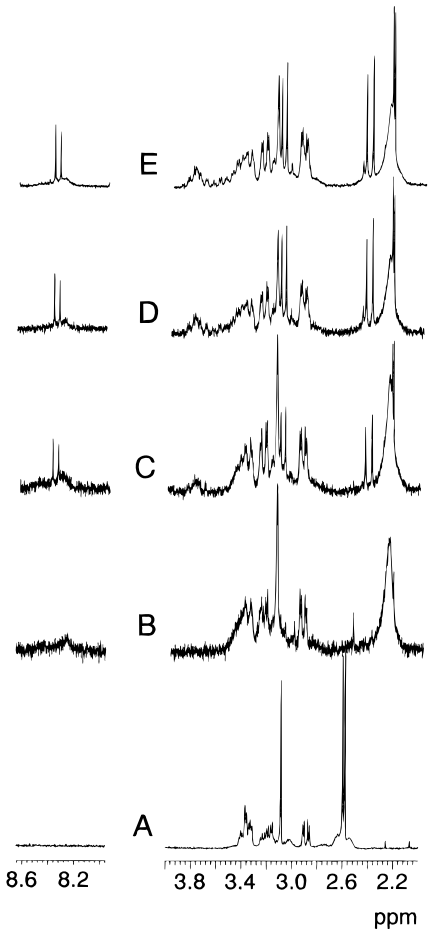


Figure 3. ^{1}H NMR spectra (24 °C) of **Me$_3$dien**Pt(9-EtG) at different reaction times: A, starting [Pt(D$_2$O)(**Me$_3$dien**)]$^{2+}$; B, soon after mixing of the reactants; C, D, and E after 20 min, 1 h, and 36 h, respectively.

different intensity (1:1.8 ratio), a broad signal for the terminal N−Me's of **Me$_3$dien**, and a slightly broad signal for the central N−Me of **Me$_3$dien**.

With time a new set of sharp signals grew in and reached its maximum after 1−2 h. It is characterized by two H8 signals of roughly equal intensity, two central N−Me signals, and four terminal N−Me signals. A third set of very weak resonances is discussed after we assign structures to the first two products.

The first **Me$_3$dien**Pt(9-EtG) complex formed (with two very broad H8 signals separated by ∼0.25 ppm and of relative intensity 1:1.8, one broad terminal N−Me signal, and one rather broad central N−Me signal) most likely is *syn*-(*R*,*S*)-**Me$_3$dien**-Pt(9-EtG) with the **Me$_3$dien** ligand having its original symmetrical configuration. With time, isomerization of the triamine ligand into the *racemic* form (*R*,*R* and *S*,*S* configurations of the terminal nitrogens) leads to the formation of the new set of sharp peaks (two H8 peaks separated by only 0.04 ppm, two central N−Me peaks separated by 0.03 ppm, and four terminal N−Me peaks, two (separated by 0.01 ppm) very close to the terminal Me signal of the initial *syn*-(*R*,*S*) isomer and two (separated by 0.05 ppm) at lower field (0.20 ppm). The spectral features are consistent with the second product being *rac*-**Me$_3$dien**Pt(9-EtG) with two rotamers, as indicated by the two H8, the two central N−Me, and the four terminal N−Me signals (each rotamer having two inequivalent terminal N−Me groups).

The very weak third set of signals consists of one rather broad H8 signal, one central N−Me signal, and one terminal N−Me signal; this set is assigned to the *anti*-(*R*,*S*)-**Me$_3$dien**Pt(9-EtG) isomer. Also for the aqua complex, the isomer with *anti*-(*R*,*S*) configuration of the ligand was much less abundant with respect to those with *syn*-(*R*,*S*) and *rac* configurations. For *anti*-(*R*,*S*)-**Me$_3$dien**Pt(9-EtG) we would expect two signals for each type of proton, one per rotamer in the case of slow rotation, and only one set of signals in the case of fast rotation on the NMR time scale. The observation of only one set of signals indicates that for *anti*-(*R*,*S*)-**Me$_3$dien**Pt(9-EtG) the rotation is fast. The NOESY/EXY spectrum in the region of H8 resonances shown in Figure 4 gives a pictorial view of the different rates of **G** rotation in the three isomers: fast for *anti*-(*R*,*S*) (only one peak, a), moderate for *syn*-(*R*,*S*) (cross-peaks between the signals of the *anti* and *syn* rotamers, b) and very slow for the *rac* isomer (absence of cross-peaks between the two rotamer resonances, c).

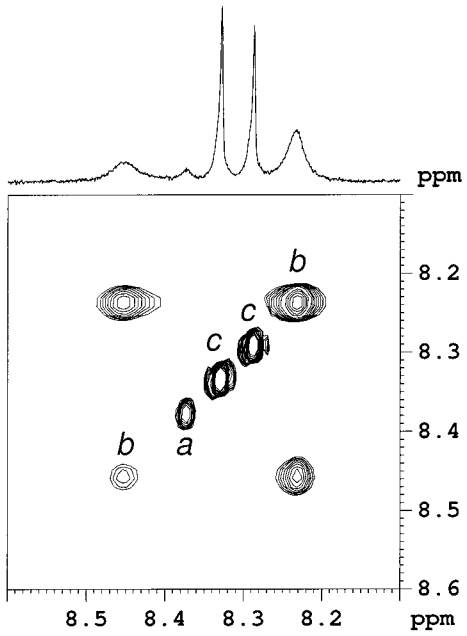


Figure 4. NOESY/EXY spectrum (23 °C) of **Me$_3$dien**Pt(9-EtG) in the region of H8 resonances. Peaks of *anti*-(*R*,*S*), *syn*-(*R*,*S*), and *rac* isomers are labeled a, b, and c, respectively.

The two H8 peaks observed for the two possible rotamers collapse and merge with increasing 9-EtG rotation rate. It is worth noting that the rate of interconversion of the two rotamers is relatively fast in the case of the symmetrical complexes. For *syn*-(*R*,*S*)-**Me$_3$dien**Pt(9-EtG), the ΔG^* of activation could be estimated from line shape analysis of spectra taken at different temperatures (Figure 5) and found to be ca. 14.7 kcal/mol. In contrast, for nonsymmetrical *rac*-**Me$_3$dien**Pt(9-EtG) the ΔG^* was >18.5 kcal/mol. One question to answer is why the *syn*-(*R*,*S*)-**Me$_3$dien**Pt(9-EtG) complex has a large separation between the two H8 peaks and a rather fast rate of rotation about the Pt−N7 bond while the complexes with *rac* configuration of the **Me$_3$dien** ligand have a small separation between the two H8 peaks and a slow rate of rotation about the Pt−N7 bond. The complex with the *syn*-(*R*,*S*) configuration of the **Me$_3$dien** ligand has terminal "quasi equatorial" N−Me's *syn* to the central N−Me. This positioning of the N−Me's renders more pronounced the nonsymmetrical distribution of the steric bulk of the carrier ligand on the two sides of the coordination plane. In contrast, the complexes with *rac* configuration of the **Me$_3$dien** ligand have terminal N−Me's on either side of the coordination plane rendering more symmetrical the distribution of the steric bulk of the carrier ligand on the two sides of the coordination

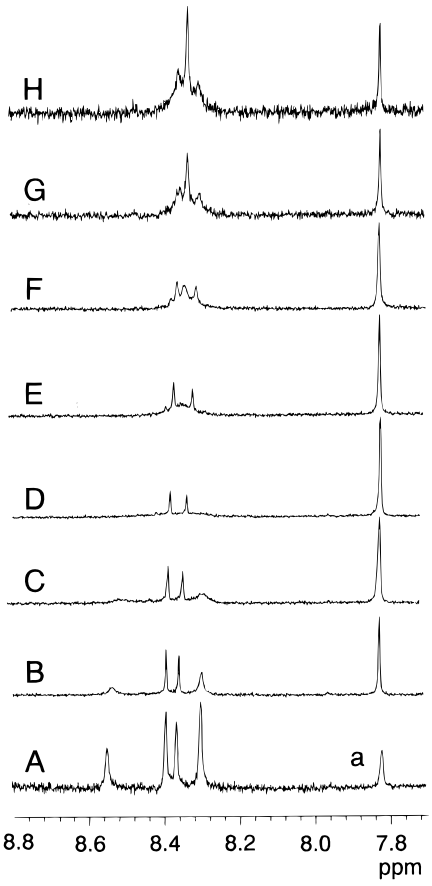


Figure 5. ^{1}H NMR spectra of **Me$_3$dien**Pt(9-EtG) at different temperatures: A, B, C, D, E, F, G, and H = 0, 10, 20, 30, 50, 60, 80, and 90 °C, respectively.

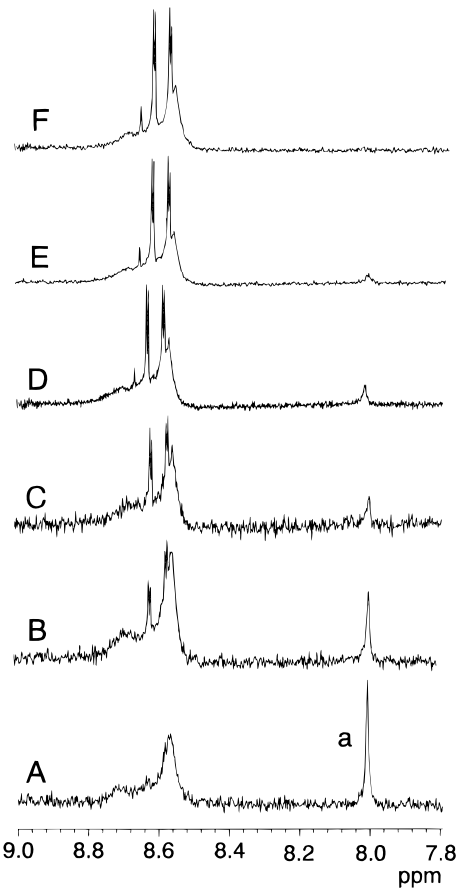


Figure 6. H8 region of ^{1}H NMR spectra of **Me$_3$dien**Pt(3′-GMP) at different reaction times: A, soon after dissolution; B, C, D, E, and F after 50 min, 75 min, 150 min, 8 h, and 30 h, respectively. H8 peak of unreacted 3′-GMP is labeled a.

plane and, as a consequence, the ratio between rotamers approaches 1.

It is less straightforward to explain the faster rate of rotation of EtG about the Pt−N bond in *syn*-(*R*,*S*)-**Me$_3$dien**Pt(9-EtG) with the terminal N−Me's both in "quasi equatorial" positions than in *rac*-**Me$_3$dien**Pt(9-EtG) with only one terminal N−Me in a "quasi equatorial" and the other in a "quasi axial" position. A possible explanation is that in the former case, both terminal N−Me's being on one side of the platinum coordination plane, steric hindrance between the rotating **G** and the N−Me's can be reduced by a tetrahedral distortion of the planar coordination geometry. Such a tetrahedral distortion has been observed in the crystal structure of the nitrate derivative.[23] In contrast, in the case of *rac*-**Me$_3$dien**Pt(9-EtG), the terminal N−Me's being on either side of the platinum coordination plane, a tetrahedral distortion of the planar coordination geometry cannot help in reducing the steric interaction between the terminal N−Me's and the rotating **G**.

Reaction with 3′-GMP. The reaction between [Pt(D$_2$O)(**Me$_3$dien**)]$^{2+}$ and 3′-GMP gave results very similar to those observed in the reaction between [Pt(D$_2$O)(**Me$_3$dien**)]$^{2+}$ and 9-EtG. However, in the case of 3′-GMP, because of the asymmetry of the sugar, the sets of signals arising from (*R*,*R*)- and (*S*,*S*)-**Me$_3$dien**Pt(3′-GMP) isomers are not coincident.

^{1}H NMR spectra of a solution of [Pt(D$_2$O)(**Me$_3$dien**)]$^{2+}$ (pH 7.7) and 3′-GMP (sodium salt, molar ratio 1:1) recorded after 20, 50, 75, 150, and 480 min are reported in Figure 6. The lack of an excess of 3′-GMP slows down the formation reaction, which now overlaps with the reaction of isomerization of the product formed initially. The initial species, *syn*-(*R*,*S*)-**Me$_3$dien**-Pt(3′-GMP), is characterized by two broad signals in the region of H8, a broad signal in the region of terminal N−Me, and a slightly broad signal in the region of central N−Me.

A new set of signals that arises with time is characterized by four sharp H8 resonances, two rather sharp central N−Me signals, and eight sharp terminal N−Me signals. As anticipated, because of the asymmetry of the sugar, the two complexes with *R*,*R* and *S*,*S* configurations at **Me$_3$dien** terminal nitrogens give a noncoincident set of signals; the differences in chemical shifts, however, are very small (<0.03 ppm).

Again the rate of interconversion of the two rotamers was very different for complexes with a symmetrical *syn*-(*R*,*S*)-**Me$_3$dien** and a nonsymmetrical *rac*-**Me$_3$dien** ligand. The ΔG^* of activation was estimated from line shape analysis of NMR spectra taken at different temperatures and found to be ca. 14.7 and >18.3 kcal/mol, respectively, for the two cases.

The ratio between 3′-GMP rotamers was nearly 1:1 for *rac*-**Me$_3$dien**Pt(3′-GMP), while it was ca. 1:2 for *syn*-(*R*,*S*)-**Me$_3$dien**Pt(3′-GMP), as observed for the 9-EtG analogue.

Reaction with 5′-GMP. The reaction of [Pt(D$_2$O)(**Me$_3$dien**)]$^{2+}$ with 5′-GMP (ratio of ca. 1:1.5) in water solution at pH 7.5 was monitored by NMR spectra taken just before mixing of the reactants (A) and after 20 min (B) and 24 h (C), Figure 7. The reaction was almost complete after 20 min (nearly complete disappearance of the signals of the aqua species and appearance of a new set of signals with single rather sharp resonances for H8, central N−Me, and terminal N−Me's). With time a new set with four signals for the H8 protons appeared.

In contrast to the results for the 9-EtG and 3′-GMP complexes, the first reaction product with 5′-GMP had a single sharp

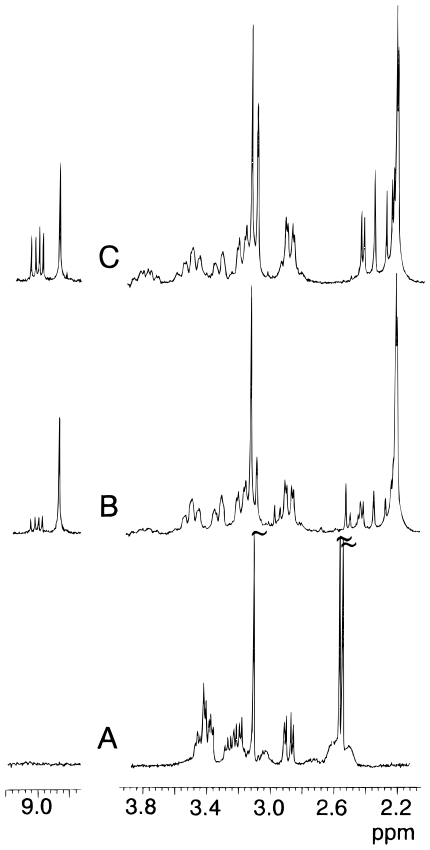


Figure 7. ^{1}H NMR spectra (24 °C) of **Me$_3$dien**Pt(5′-GMP) at different reaction times: A, starting [Pt(D$_2$O)(**Me$_3$dien**)]$^{2+}$; B, soon after mixing of the reactants; and C, after 24 h.

resonance for each type of proton. For example, only one rather sharp signal was observed for H8, instead of the two broad signals observed for the 9-EtG and 3′-GMP derivatives. Beyond any reasonable doubt, the compound formed initially has the **Me$_3$dien** ligand in its original *syn*-(*R,S*) configuration. Either the complex has a fast rate of interconversion between the two possible rotamers or it exists as only one rotamer. It is very unlikely that the interconversion between rotamers is faster in the case of 5′-GMP than in the cases of 9-EtG and 3′-GMP since, in general, 5′-GMP rotates slower than 3′-GMP;[16] therefore, the only reasonable interpretation is that a single rotamer is favored. The symmetrical *syn*-(*R,S*) ligand allows the *syn* rotamer to form NH−5′-phosphate H-bonds (H8 on the same side of the two NH's with respect to the platinum coordination plane); such a H-bonding interaction is not possible in the *anti* rotamer (H8 and NH on opposite sides of the platinum coordination plane). Therefore, only the *syn* rotamer is favored.

The new set of signals growing with time and characterized by having up to four H8 resonances can be assigned easily to the species with the *rac* configuration of the **Me$_3$dien** ligand. Because of the asymmetry of the 5′-GMP sugar, the sets of signals arising from species having the *R,R* and the *S,S* configurations at **Me$_3$dien** terminal nitrogens are not coincident. Moreover, two rotamers are now present (*syn* and *anti*), as observed for the 9-EtG and 3′-GMP cases. It is to be noted that the nonsymmetrical **Me$_3$dien** ligand has NH protons on both sides of the platinum coordination plane; therefore, the rotamers have comparable stability since both rotamers can form H-bonds between the 5′-phosphate and an NH of **Me$_3$dien**.

Chemical Shifts of N−Me and H8 Signals. For **Me$_3$dien**Pt**G** complexes, the magnetic anisotropy of the purine base has a distinct effect on the chemical shift of the N−Me signals of the carrier ligand; this effect resembles that already noted above for **Me$_5$dien**Pt**G** species. The downfield shift of the central N−Me signal is again on the order of 0.1 ppm. The upfield shift of the terminal N−Me signal(s) is on the order of 0.28 ppm for both the "quasi equatorial" *syn* methyls and the "quasi axial" *anti* methyls and for both the *anti* and *syn* rotamers. The small dependence of the chemical shift upon the "axial" or "equatorial" character of the N−Me's and upon the *syn* or *anti* orientation of the G base observed in the case of **Me$_3$dien**Pt**G** complexes as compared to the **Me$_5$dien**Pt**G** case may be a consequence of the greater mobility of the N−Me's and **G** base in the less tight **Me$_3$dien**Pt**G** complex. The 5′-phosphate effect upon the chemical shift of H8 is large for both rotamers of *rac*-**Me$_3$dien**Pt**G** complexes, suggesting that the 5′-phosphate is close to H8. In fact, both rotamers can form phosphate−NH H-bonds.

Conclusions

This work has allowed a direct comparison of H-bond interactions between the NH of carrier ligands and either the O6 or the 5′-phosphate of a nucleotide in a *cis* position, without interference from *cis*-**G** to *cis*-**G** interactions possible in *cis*-PtA_2**G**$_2$ intrastrand cross-link models.

In the case of *syn*-(*R,S*)-**Me$_3$dien**Pt**G** complexes, with both **Me$_3$dien** NH's on one side of the platinum coordination plane and *anti* to the central N−Me, only the *anti* rotamer can form O6−NH H-bonds and no phosphate−NH H-bonds are possible when **G** = 9-EtG and 3′-GMP. In both cases (**G** = 9-EtG and 3′-GMP), the *anti* rotamer was favored, and its H8 signal was upfield of that of the *syn* rotamer ($\Delta\delta \approx 0.25$ ppm), an indication that the G base is more tilted in the *anti* rotamer. This tilting is necessary for an O6−NH H-bond, consistent with such a bond stabilizing the *anti* rotamer. However, it cannot be excluded that the slightly greater abundance of the *anti* rotamer (never exceeding 2:1) reflects instead a destabilization of the *syn* isomer by steric repulsion between O6 and the N−Me's.

In contrast to the apparent small influence on rotamer distribution by an O6−NH H-bond, the 5′-phosphate−NH H-bond can greatly influence rotamer distribution to the extent that only one rotamer was detectable by NMR spectroscopy in the case of *syn*-(*R,S*)-**Me$_3$dien**Pt(5′-GMP). Thus, while we propose that **G** O6−NH H-bonding is weak, **G** 5′-phosphate−NH H-bonding is strong.

Another important result of this investigation is the very different rate of interconversion between rotamers in the cases of the *syn*-(*R,S*) and *rac*-**Me$_3$dien**Pt**G** complexes. In the *syn*-(*R,S*)-**Me$_3$dien**Pt**G** species, both terminal N−Me's of **Me$_3$dien** ligand are *syn* and "quasi equatorial", whereas in *rac*-**Me$_3$dien**Pt**G** one terminal N−Me of **Me$_3$dien** ligand is *syn* and "quasi equatorial" while the other N−Me is *anti* and "quasi axial". Therefore two "quasi equatorial" N−Me's appear to offer a smaller steric impediment to the rotation of the purine base about the Pt−N7 bond than one "quasi equatorial" and one "quasi axial" N−Me. How can this be? A resonable explanation of this apparent dilemma is the possibility, for the *syn*-(*R,S*) isomer, to release the steric hindrance between the rotating G and the "quasi equatorial" methyl by a tetrahedral distortion of the coordination plane (such a distortion has already been observed in the X-ray structure of the nitrate derivative). Such a tetrahedral distortion would not help in the case of the *rac* isomer for which the terminal N−Me's are on either side of the planar coordination plane.

In the case of **Me$_5$dien**Pt**G** complexes, there are no hydrogens on the terminal nitrogens which could participate in H-bond

formation. Moreover there is no way to reduce steric interaction between the rotating G and the four terminal methyls. As a consequence, for all **G**'s both rotamers are formed in comparable yields and the rate of interconversion is always slow on the NMR time scale.

The magnetic anisotropy of the purine base has a characteristic effect upon the chemical shift at the N−Me's of the carrier ligand. The central N−Me signal always undergoes a downfield shift, while the terminal N−Me's undergo an upfield shift. The average value of the shift is smaller for the signals of the central N−Me than for those of the terminal N−Me's. Moreover, particularly in the case of sterically very rigid **Me$_5$dien**Pt**G** complexes, the chemical shift depends also upon the relative disposition of the six-membered-ring of guanine and of the N−Me with respect to the platinum coordination plane (greater when they are on the same side than on opposite sides) and the "quasi axial" or "quasi equatorial" character of terminal N−Me's (greater upfield shift for "quasi equatorial" than for "quasi axial").

Phosphate−NH H-bond formation generally results in a greater downfield shift of the H8 protons. Whenever possible, such an interaction takes place and strongly stabilizes the given molecular structure.

Acknowledgment. This work was supported by NIH Grant GM 29222 (to L.G.M.); MURST (Contribution ex 40%), CNR, and EC (COST Chemistry projects D8/0009/97 and D8/0012/97) (to G.N.).

IC990517F