Contribution from the Department of Chemistry,
Emory University, Atlanta, Georgia 30322

# Interaction of Platinum Antitumor Agents with Guanine Nucleosides and Nucleotides. $^{195}$Pt and $^{1}$H NMR Spectroscopic Characterization of Compound III

SANDRA K. MILLER and LUIGI G. MARZILLI*

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Three species (I, II, and III) are formed between guanosine (Guo) and *cis*-$[Pt(NH_3)_2Cl_2]$ at a ratio of 1:10 with excess $Cl^-$ that are analogous to previously described products formed under similar conditions from guanosine 5′-monophosphate (GMP) instead of Guo. Species I and II are identified as *cis*-$[Pt(NH_3)_2Cl(Guo)]^+$ and *cis*-$[Pt(NH_3)_2(Guo)_2]^{2+}$, respectively, by $^{195}$Pt and $^{1}$H NMR spectroscopy, p$K_a$ (~8.5), and charge determination (1+ and 2+, respectively). The p$K_a$ (~1.5) and charge of III (1+) from Guo are consistent with either an N7, O6 chelate deprotonated at N1 or a dimer in which two $Pt(NH_3)_2Cl$ units are linked at N7 and N1 of an N1-deprotonated bridging $Guo^-$. However, III has two $^{195}$Pt NMR signals consistent only with the latter structure. Furthermore, analogous compounds are formed by Guo treated with *cis*-$[Pt(NH_2CH_3)_2Cl_2]$. Four $CH_3$ $^{1}$H NMR signals are observed for III, consistent with the bridged structure but not with the N7, O6 chelate structure. Results obtained with the phosphate methyl ester of GMP and 1-methylguanosine treated with *cis*-$[Pt(NH_3)_2Cl_2]$ and Guo treated with $Pt(en)Cl_2$ also supported these assignments for the composition and structure of I, II, and III. On reinvestigation of the $^{195}$Pt NMR spectra of solutions containing I, II, and III derived from GMP and *cis*-$[Pt(NH_3)_2Cl_2]$, we obtained results that led to the same composition and structural assignments for GMP as for Guo although III for the GMP product was less tractable than the related III formed with Guo. The similarity in results for Guo and GMP clearly rules out an N7, O6 chelate structure for III formed with GMP.

## Introduction

Early observations indicated that the broad-spectrum antitumor agent *cis*-$[Pt(NH_3)_2Cl_2]$ preferentially attacks G (guanine residues) in DNA.[1-5] This finding prompted numerous investigations that have more definitively confirmed, with a variety of experimental methods, that G is attacked preferentially[6-10] and that, in the major DNA adducts, both Cl ligands are eventually replaced either with two G residues or with one G and one A (adenine).[10] Consequently, numerous investigations into the coordination chemistry of the $PtA_2$ unit [where $A_2$ = $(NH_3)_2$ or en (ethylenediamine)] with G nucleotides and nucleosides (GMP = guanosine 5′-monophosphate and Guo = guanosine, respectively) have been reported.[11-33]

The principal conclusions of these studies were as follows: (a) N7 of G is the favored coordination site. (b) Rotation about the Pt–N7 bond is rapid unless $A_2$ is replaced by a bulkier ligand.[16,31-32] (c) The 6-oxo group does not bind directly to Pt. Reedijk's laboratory has extended the analysis to a consideration of the effect of platination on the sugar conformation.[14] The weight of evidence for N7 coordination included X-ray crystallography of isolated nucleosides[15,16] and nucleotides.[17,18] General agreement has recently been reached concerning points a, b, and c except for a paper by Clore and Gronenborn[19] (*CG*), which calls all these conclusions into question.

These authors (*CG*) reported observing three species (I, II, and III) on treatment of GMP with *cis*-$[Pt(NH_3)_2Cl_2]$ in the presence of 0.5 M KCl and excess Pt complex over GMP (10:1). Species III, formed at long reaction times, was assigned a structure in which N7 and O6 were coordinated as a chelate to one Pt center. Species I and II were both considered to be rotational isomers of *cis*-$[Pt(NH_3)_2Cl(GMP)]^-$.

Key points in the structural assignments were (i) the high ratio of Pt to GMP, which should preclude compounds such as *cis*-$[Pt(NH_3)_2(GMP)_2]^{2-}$, (ii) the presence of excess Cl, which would have favored Cl coordination over monodentate GMP coordination, (iii) the apparent rotational interconversion of I and II at high temperature, and (iv) the occurrence of only one $^{195}$Pt NMR signal for III.

Since the study contradicted prevailing opinion, we decided to reinvestigate the (*CG*) experiments and to extend these to other Pt complexes and to other G derivatives. Independently, Reedijk and co-workers (*DCHMR*)[20] also examined the system. The emphasis of the two reinvestigations was different, however, with *DCHMR* focusing on I and II with GMP and our laboratory focusing on III with GMP and other G derivatives. We also examined the $^{195}$Pt NMR spectral properties. Our studies are quite complementary, and where overlap occurred, we are in close agreement with *DCHMR* as to the nature of these three species. In fact, *DCHMR* concluded that the $^{195}$Pt NMR spectroscopy in the *CG* study had been incorrectly performed and predicted

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**Table I.** $^1$H NMR Spectral Data for Several G Derivatives Including *cis*-$[Pt(NH_3)_2Cl_2]$ Adducts[a]

| | H8 | | | | H1′ | | | |
|---|---|---|---|---|---|---|---|---|
| species | GMP | MeGMP | Guo | 1-MeGuo | GMP | MeGMP | Guo | 1-MeGuo |
| I | 8.58 | 8.48 | 8.49 | 8.48 | 6.00 | 6.00 | 5.98 | 5.98 |
| II | 8.62 | 8.45 | 8.39 | 8.37 | 5.92 | 5.91 | 5.90 | 5.88 |
| III | 8.41[b] | 8.34[b] | 8.34 | | 5.95 | 5.96 | 5.93 | |
| G | 8.17 | 8.08 | 8.01 | 7.98 | 5.94 | 5.95 | 5.92 | 5.90 |

[a] In ppm; summarized from supplementary Tables SI–SIV. [b] Two resonances; see supplementary material.

spectral features that we have, in fact, observed.

## Experimental Section

**Materials.** The following were obtained from Sigma: guanosine, 1-methylguanosine (1-MeGuo), and guanosine 5′-monophosphate. The following were obtained from Aldrich: *cis*-$[Pt(NH_3)_2Cl_2]$, $D_2O$ (99.8% atom % D), and the sodium salt of 3-(trimethylsilyl)propionic acid (TSP) (99+%).

The compounds $[Pt(en)Cl_2]$ and *cis*-$[Pt(NH_2CH_3)_2Cl_2]$ were prepared by known methods[33] and were kindly provided by Barbara L. Heyl and Christina S. Fouts, respectively. The K(MeGMP) (potassium salt of phosphate methyl ester of GMP) was prepared according to ref 34.

**Instrumentation.** The $^1$H NMR spectra were collected on a Nicolet 360NB spectrometer (360 MHz) and the $^{195}$Pt NMR spectra of an IBM WP-200SY spectrometer (42.93 MHz).

The pH of the solutions was determined on an Orion Model 70/A digital analyzer using an Ingold pH electrode.

**NMR Studies.** The $^1$H NMR spectra were referenced to TSP and collected at 23 °C with 5-mm tubes. The HOD peak in the $^1$H NMR spectra (8K or 16K) was suppressed by selective decoupling, and the number of transients varied from 40 to 300 depending on the concentration of the sample and the percent of H8 deuterium exchange. The reaction solutions were in $D_2O$, 0.05 M in phosphate buffer at pH 6.5 (no correction for the isotope effect on the glass electrode). The pH was adjusted with 1 M KOH and 1 M $HNO_3$. The platinum complex to base ratio and the chloride concentration were varied depending on the compound desired. Specific conditions were as follows: I, 3 mM G, 3 mM Pt, 0.5 M KCl; II, 6 mM G, 3 mM Pt, 3–0.5 M KCl; III, 3 mM G, 30 mM Pt, 0.05 or 0.5 M KCl. Details of the individual experiments are contained in the supplementary material. The reaction solutions were heated at 80 °C, and the time course of the reactions was followed by $^1$H NMR.

The $^{195}$Pt NMR spectra were obtained with 10-mm tubes and a 2-mL sample size. A sweep width of 50 000 Hz was used with an observation frequency (O1) of −5000 Hz, an acquisition time of 0.16 s, and a relaxation delay of 0.3 μs. The peaks were referenced to 0.1 M $K_2[PtCl_6]$, which was located with O1 = 90 000 Hz. A spectral reference value of 92 403.56 Hz was used without repeating the reference each time, since it decomposes with time. A left shift was applied 8–16 times to remove rolls in the base line, and a line broadening of 50 Hz was applied prior to transformation. Solutions for the $^{195}$Pt NMR studies were similar to those used in the $^1$H NMR studies but higher reactant concentrations were used as follows: I and II, 20 mM G, 20 mM Pt, 0.5 M KCl, 1 h, 80 °C; II, 75 mM G, 25 mM Pt, no KCl, 2 h, 80 °C; III, 15 mM G, 60 mM Pt, 0.5 M KCl, 8 h, 80 °C. In all cases $^1$H NMR spectra were also recorded.

**pH Titrations.** The solutions used to assess the $^1$H NMR shift dependence of species I, II, and Guo were obtained by heating a solution containing Guo (15 mM), *cis*-$[Pt(NH_3)_2Cl_2]$ (15 mM), 0.05 M phosphate buffer at pH 6.5, and 0.5 M KCl for 1 h at 80 °C. For species III, a similar solution, but with *cis*-$[Pt(NH_3)_2Cl_2]$ (60 mM), was heated for 8 h. The solutions at 25 °C were adjusted to the desired pH with KOH and $HNO_3$. Several samples were used, and the solvent was either $D_2O$ or 90% $H_2O$/10% $D_2O$. For the $D_2O$ solutions, 0.4 was added to the pH meter reading.[22] To account for the pH dependence of TSP shift, 0.015 ppm was added to all shifts of solutions below pH 4. For 10% $D_2O$ solutions a Nicolet-supplied version of the Redfield 21412 pulse sequence with irradiation at ~10 ppm, a pulse width of 480 μs, and a sweep width of 4200 Hz was used. The low-power transmitter was attenuated so that the pulse corresponded to a 90° tip angle. Spectra were measured with 200 transients stored in 16K memory. Spectral parameters for $D_2O$ solutions were given above.

**Column Chromatography.** The charges on species I, II, and III were determined by using ion-exchange Sephadex chromatography. A 1-mL solution containing I, II, and III for each G base was prepared with 15 μmol of G ligand and 60 μmol of Pt complex. The solutions were concentrated by rotary evaporation before application to the column. A solution containing Guo complexes was passed through a cation-exchange column (column 1 × 4 cm, CM-Sephadex C-25, Sigma) and eluted with 0.2 M NaCl. A solution containing GMP complexes was passed through an anion-exchange column (column 1 × 8 cm, DEAE-Sephadex C-25, Sigma) and eluted with deionized water. A solution containing MeGMP complexes was passed through both a cation- and an anion-exchange column (columns 1 × 8 cm) and eluted with deionized water. The effluent fractions containing G derivatives were identified by UV absorbance after spotting on a silica gel plate treated with a fluorescent indicator (254-nm phosphor, Analtech).

## Results

**NMR Spectroscopy Results for *cis*-$[Pt(NH_3)_2Cl_2]$. (1) GMP. (a) H8 Resonances.** To a first approximation, all three studies agree experimentally on the formation of three species from the resonances in this part of the spectrum. However, our results and those of *DCHMR* do *not* reveal a constant ratio between I and II and *do* reveal two closely spaced signals for H8 of III. Our chemical shift results are summarized in Table I, and the summary of several experiments appears in the supplementary material.

**(b) H1′ Resonances.** There is a major difference between this study and that of *CG*.[14] The complex *cis*-$[Pt(NH_3)_2(GMP)_2]^{2-}$ has a H1′ (doublet) slightly *upfield* from the resonance for GMP (pH 6.5). In the *CG* paper it was stated that all three Pt complexes had resonances ca. 0.08 ppm downfield from GMP. This downfield shift for both I and II was a major point used by *CG* to exclude both I and II, which have H8 resonances close to that reported for *cis*-$[Pt(NH_3)_2(GMP)_2]^{2-}$,[11] from being this latter complex. In this study and the *DCHMR* study, the H1′ signal for II is upfield from that of free GMP, and we agree with the *DCHMR* conclusion that II is in fact *cis*-$[Pt(NH_3)_2(GMP)_2]^{2-}$, which has an H1′ shift upfield from that in GMP.[11]

**(c) $^{195}$Pt Resonances.** One major point made by *CG* was that the $^{195}$Pt resonance of *cis*-$[Pt(NH_3)_2(GMP)_2]^{2-}$ could not be observed and therefore II could not be this compound. We have had no difficulty in observing the expected resonance for *cis*-$[Pt(NH_3)_2(GMP)_2]^{2-}$ at −2455 ppm[19] when II was present under the *CG* conditions. The $^{195}$Pt NMR spectrum for a solution with I, II, and III in the *CG* report, as pointed out by *DCHMR*, does not extend to −2455 ppm, where the signal for II would be found. Extensive attempts to observe the $^{195}$Pt NMR spectrum for III under conditions where I and II are absent were not very successful (see below). Nevertheless, a signal at −2345 ppm could be observed when III was present. This peak was never found unless there was a signal at −2295 ppm, where the signal for I is observed.

**(d) Conclusion.** In this study, two key spectroscopic criteria (H1′ $^1$H NMR resonances and $^{195}$Pt NMR resonances) clearly identify II as *cis*-$[Pt(NH_3)_2(GMP)_2]^{2-}$, in agreement with *DCHMR* but in conflict with *CG*. The signal at −2295 ppm in the $^{195}$Pt NMR spectrum of a solution containing I, II, and III is likely to be a combination of one signal from I and one from III.

**(2) MeGMP.** Essentially the same results were observed for this nucleotide as described above for GMP. The principal consequence of methylation is a ~0.1-ppm upfield shift of the H8 signal for all species except II in comparison to the analogous GMP shifts. For II, the upfield shift of the H8 resonance is slightly greater, ~0.15 ppm. This shift effect of the phosphate group of ~0.1–0.2 ppm was also found by *DCHMR* on protonation of the phosphate group of II (GMP). Since there are two more $PO_4$ groups in II compared to I, the greater effect on II can be understood. One consequence of the effect is that, whereas for GMP,

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**Table II.** $^{195}$Pt NMR Spectral Data for *cis*-$PtA_2$ Complexes with G Derivatives[a]

| | $A_2 = (NH_3)_2$ | | | | $A_2$ = en |
|---|---|---|---|---|---|
| species | GMP | MeGMP | Guo | 1-MeGuo | Guo |
| I | −2295 | | −2308 | −2306 | −2548 |
| III | −2295 | | −2306 | | −2548 |
| | −2345 | | −2345 | | −2571 |
| II | −2455 | −2445 | −2445 | −2439 | −2662 |

[a] In ppm, referenced to $PtCl_6^{2-}$; summarized from Tables SI–SIV.

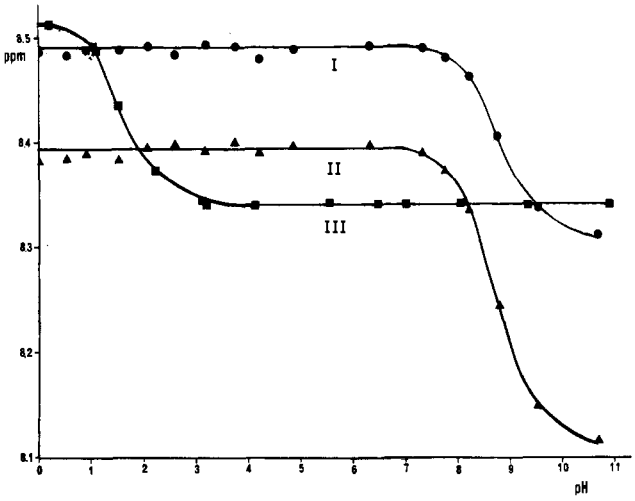


**Figure 1.** Graph of H8 chemical shift (ppm) vs. pH for products I, II, and III from the reaction of Guo with *cis*-$[Pt(NH_3)_2Cl_2]$.

the order of H8 shifts (downfield to upfield) is II, I, III, free ligand, for other species in this study, the order if I, II, III (where applicable), free ligand. In contrast, for all species the H1′ order (downfield to upfield) is I, III (where applicable), free ligand, II. The $^{195}$Pt NMR shifts for I and II with MeGMP are similar to those for the analogous GMP complexes (Table II). Again, III probably has two signals at −2319 ppm and at −2382 ppm.

**(3) Guo. (a) H8 Resonances.** The H8 resonances of I, II, and III are also observed for Guo excluding any significant role for the $PO_4$ group in metal binding. The shifts observed for I and II are very similar to those found with MeGMP. Thus, a clear relationship to the GMP Pt species is established. Two H8 signals could not be resolved for III, however.

**(b) H1′ Resonances.** As mentioned above, the identical pattern was observed for Guo as for the G nucleotides.

**(c) pH Titration.** The shift dependence of H8 for I, II, and III as a function of pH (0–11) was determined (Figure 1). Species III had a constant H8 chemical shift of 8.34 ppm from pH 3 to 11. There was a downfield shift of 0.2 ppm between pH 3 and 0 with $pK_a$ = 1.5. Species I and II had constant H8 chemical shifts of 8.49 and 8.40 ppm, respectively, between pH 0 and 6.5. Above pH 6.5 the H8 signals for I and II shifted upfield and reached final values above pH 10 of ca. 8.3 and ca. 8.1 ppm for I and II, respectively.

**(d) $^{195}$Pt Resonances.** In a solution where I and II were essentially eliminated, two clear peaks could be observed for III (Figure 2a). The downfield peak is close to that for I (Figure 2b) whereas the upfield peak is ca. 40 ppm upfield.

**(4) 1-MeGuo.** No species analogous to III was observed with this nucleoside. Otherwise, the H8, H1′, and $^{195}$Pt resonances for I and II were very similar to the corresponding resonances for Guo (Tables I and II).

**$^1$H NMR Studies with Guo and Other Pt Complexes. *cis*-$[Pt(NH_2CH_3)_2Cl_2]$.** We hoped that the methyl signals of this complex would provide information regarding the composition of species III. Species corresponding to I, II, and III could be easily identified by the $^1$H NMR chemical shifts for H8 and H1′ as listed in Table III.

The methyl region (~2.5 ppm) contained signals of unreacted *cis*-$[Pt(NH_2CH_3)_2Cl_2]$ and some small amount of decomposition products complicating the interpretation of this region. The unreacted *cis*-$[Pt(NH_2CH_3)_2Cl_2]$ and its decomposition products could be almost completely separated from the desired complexes by cation-exchange chromatography as described above for Guo. Fractions containing G bases were lyophilized, dissolved in the $D_2O$ phosphate buffer, and examined by $^1$H NMR spectroscopy.

A solution containing only species I and II was prepared and passed through the cation-exchange column. Species I eluted first. The $^1$H NMR spectrum of a $D_2O$ solution (pH 6 prepared as above) containing I has two triplets at 2.5 ($J$ = 6.0 Hz) and 2.9 ppm ($J$ = 6.5 Hz). The $^1$H NMR spectrum of a similar solution containing II has one triplet at 2.4 ppm ($J$ = 5.8 Hz). At acid pH the exchange of the NH to ND is slow,[35] and coupling between NH and $CH_3$ accounts for the triplets. The pH of the two solutions was raised to 8 and the $^1$H NMR spectra contained sharp singlets at 2.35 ppm for II and at 2.29 and 2.55 ppm for I. The proton coupling for species I was regenerated as follows. The sample was lyophilized and redissolved in $H_2O$. The solution was heated in warm water for 15 min to facilitate exchange. The pH of the solution was lowered to 5.2, and the solution was lyophilized. The residue was redissolved in $D_2O$, and the $^1$H NMR spectrum again exhibited $CH_3$ coupling.

A solution containing I, II, and III was prepared and passed through the cation-exchange column. Species I and III eluted

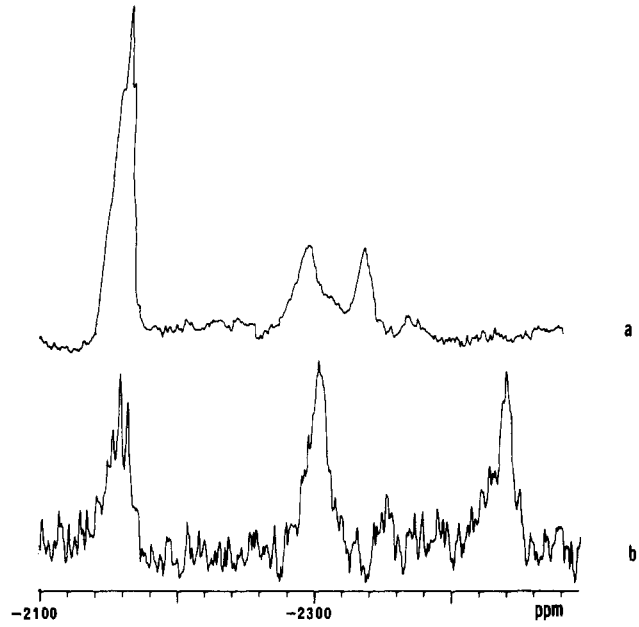


**Figure 2.** $^{195}$Pt NMR spectra of (a) III, prepared by heating a solution of Guo (15 mM) + *cis*-$[Pt(NH_3)_2Cl_2]$ (60 mM) in 0.5 M KCl in pH 6.5 phosphate buffer for 8 h at 80 °C, (b) I and II, prepared from Guo (20 mM) + *cis*-$[Pt(NH_3)_2Cl_2]$ (20 mM) as in part a but with only 2 h of heat.

**Table III.** $^1$H NMR Spectral Data[a] for *cis*-$PtA_2$ Adducts of Guanosine

| | | | | N–$CH_3$[b] | |
|---|---|---|---|---|---|
| species | $A_2$ | H8 | H1′ | trans N | trans Cl |
| I | $(NH_3)_2$ | 8.49 | 5.98 | | |
| | en | 8.48 | 6.00 | | |
| | $(NH_2CH_3)_2$ | 8.51 | 5.98 | 2.29 | 2.51 |
| II | $(NH_3)_2$ | 8.39 | 5.90 | | |
| | en | 8.43 | 5.89 | | |
| | $(NH_2CH_3)_2$ | 8.43 | 5.90 | 2.35 | |
| III | $(NH_3)_2$ | 8.34 | 5.93 | | |
| | en | 8.34 | 5.95 | | |
| | $(NH_2CH_3)_2$ | 8.36 | 5.94 | 2.26, 2.37 | 2.54 |

[a] Shifts in ppm. [b] For unreacted *cis*-$[Pt(NH_2CH_3)_2Cl_2]$, 2.47 ppm.

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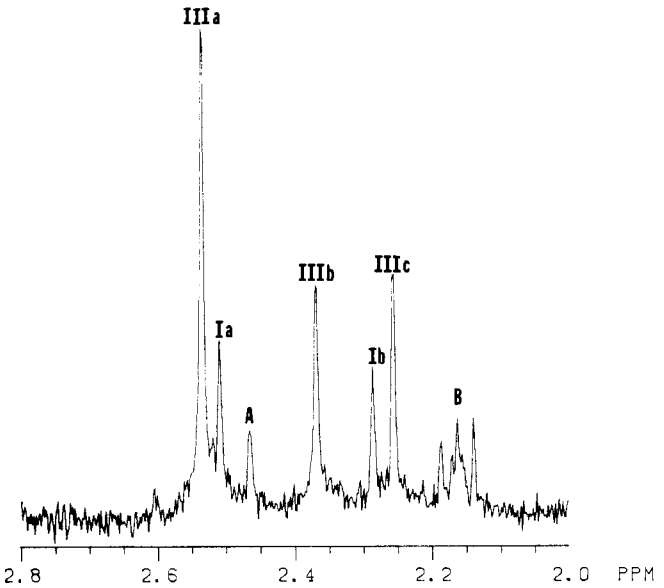


**Figure 3.** Methyl region of the $^1H$ NMR spectrum of a pH 7 $D_2O$ solution containing complexes I and III from the reaction of Guo with *cis*-$[Pt(NH_2CH_3)_2Cl_2]$. The products were purified by cation-exchange chromatography, and the NH groups are exchanged to ND. The $CH_3$ resonance assignments are as follows: IIIa, both ligands trans to Cl in III, IIIb, and IIIc, the ligands trans to the bridging $Guo^-$; Ia, ligands trans to Cl and Ib, trans to N in I; A, unreacted *cis*-$[Pt(NH_2CH_3)_2Cl_2]$; B, a resonance for TSP.

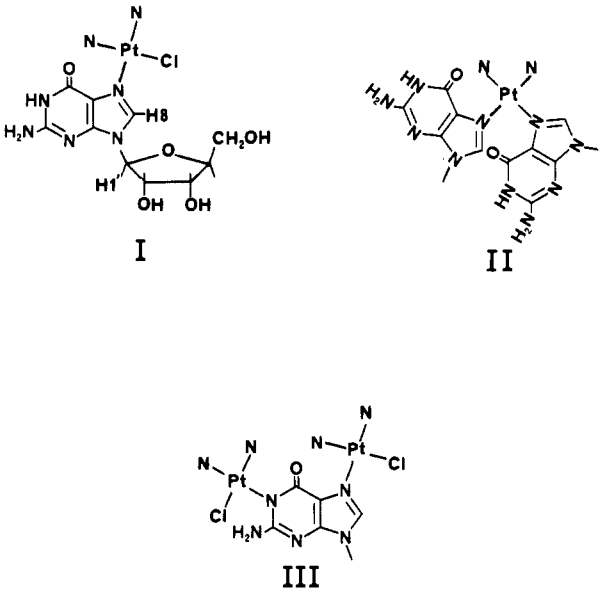


**Figure 4.** Partial structures for species I, II, and III. For I, the complete structure of Guo is illustrated.

together, followed by II. The $^1H$ NMR spectrum of the solution containing I and III at pH 8 has three signals corresponding to III at 2.26, 2.37, and 2.54 ppm (Figure 3). The two upfield signals are of equal size, and the downfield signal was twice the intensity of the upfield signals. The chemical shifts for species I, II, and III are summarized in Table III.

**[Pt(en)Cl$_2$].** Analogous reactions of Guo and $[Pt(en)Cl_2]$ produced species corresponding to I, II, and III. The $^1H$ and $^{195}Pt$ NMR spectra of these species are similar to those obtained for Guo complexes of other Pt adducts (Table III), although the en displaces all the $^{195}Pt$ NMR shifts.

## Discussion

**The Nature of I and II for GMP.** $^{195}Pt$ NMR signals are very sensitive to the nature of the coordinated ligands; signals for coordination environments *cis*-$[Pt(NH_3)_2Cl_2]$, *cis*-$[Pt(NH_3)_2ClL]$, and *cis*-$[Pt(NH_3)_2L_2]$, where L is a purine N donor, are expected at −2160, ∼−2325, and ∼−2450 ppm, respectively.[19,36] The observation of the $^{195}Pt$ NMR signal of II at −2455 ppm, along with the $^1H$ NMR results presented in Table I and in the *DCHMR* study, confirms that II is *cis*-$[Pt(NH_3)_2(GMP)_2]^{2-}$ and not a rotamer of I. There is agreement in all three studies that I is *cis*-$[Pt(NH_3)_2Cl(GMP)]^-$. It should be noted that the suggestion by *CG* that the two rotamers of I would have unequal populations is based on the false premise that each rotamer would interact differently with the adjacent Cl and $NH_3$ groups. The rotamers differ primarily in the orientation of the purine with respect to the coordination plane. In either rotamer, the 6-oxo group could interact with the adjacent $NH_3$ group and avoid the vicinity of the Cl ligand. The B/M dihedral angle[34] can be similar for each rotamer.

**The Nature of III for GMP.** We were primarily interested in assessing the structure of III. In Figure 4, we present the proposed structures for I, II, and III with emphasis on the coordination environment. *DCHMR* suggested that III for GMP might be a mixture of products with structures closely related to structure III in Figure 4. We found that III formed from GMP was particularly difficult to study, and we will return to consider it later after a discussion of III with Guo.

**The Nature of III for Guo.** We found III for Guo produced by reaction with *cis*-$[Pt(NH_3)_2Cl_2]$ to be much more tractable than III for GMP. The H8 resonance for III with Guo was apparently a single resonance. Rather forcing conditions are needed to produce III, and relatively poorly defined $^{195}Pt$ NMR spectra were obtained for III with GMP, despite many attempts to obtain a suitable $^{195}Pt$ NMR spectrum. In contrast, the spectrum of III for Guo gave signals of approximately the correct intensity relative to that for unreacted *cis*-$[Pt(NH_3)_2Cl_2]$. Two resonances are found, and the positions of these resonances at −2306 and −2345 ppm are clearly in the region expected for a *cis*-$[Pt(NH_3)_2ClL]$ coordination environment. Additionally, the higher field resonance is ca. 40 ppm upfield, and this difference is that expected between an N7 donor and an N1 donor.[20] Contrary to the suggestion by *CG*, this compound cannot be an N7, O6 chelate since then only one $^{195}Pt$ NMR signal should be observed. In the *CG* study with GMP, the $^{195}Pt$ NMR spectrum presented was obtained on a solution with I present. Since the resonance for I and the downfield resonance for III probably coincide, *CG* believed only one $^{195}Pt$ NMR signal could be attributed to III. We have nearly completely eliminated I with GMP by using long reaction times in some of our experiments, but the $^{195}Pt$ NMR signals for III were also gone. Only the signal for *cis*-$[Pt(NH_3)_2Cl_2]$ was observed. For Guo, this problem could be avoided.

The pH titration for III (Figure 1) is consistent with a dimer bridged by N1 and N7. In particular, there is no titratable proton at high pH corresponding to N1H deprotonation such as the $pK_a$ value of ca. 8.5 found here for I and II for Guo. This value is typical for N7-platinated G derivatives.[20] However, at low pH, a protonation site is observed for III with a $pK_a$ value of 1.5. This value is expected on the basis of reported values for related N1- and N7-bridged Pt complexes of 9-alkyl-6-oxopurines.[22,37] The replacement of a proton at N1 by Pt increases the basicity at N3 because Pt is less electron accepting than a proton. *DCHMR* also observed a downfield shift for H8 at low pH for III with GMP, but interpretation of this effect is complicated by protonation of the phosphate group.

Further evidence for such a bridged species is found in our observation that, of the G derivatives studied, only 1-MeGuo did not form III. However it readily formed I and II. The similar shifts of the $^{195}Pt$ and $^1H$ NMR resonances for I and II for Guo and 1-MeGuo confirm that these are essentially identical species,

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**Table IV.** Charges of Species I, II, and III

| | | | III | | | |
|---|---|---|---|---|---|---|
| species | I | II | Cl, Cl | Cl, $H_2O$ | $H_2O$, $H_2O$ | N7, O6[a] |
| GMP | 1− | 2− | 1− | 0 | 1+ | 1− |
| MeGMP | 0 | 0 | 0 | 1+ | 2+ | 0 |
| Guo | 1+ | 2+ | 1+ | 2+ | 3+ | 1+ |

[a] Deprotonated at N1.

except for the $CH_3$ group. Therefore, there is no ready explanation for failure of 1-MeGuo to form III unless III has a deprotonated N1.

Treatment of Guo with [Pt(en)$Cl_2$] also produced I, II, and III. The similar $^1$H NMR shifts and the presence of two similar-intensity $^{195}$Pt NMR signals for III strongly suggest that, in III, the cis arrangement of the amine ligands is preserved also for products derived from *cis*-[Pt($NH_3$)$_2Cl_2$].

To further characterize III, we decided to examine the products formed from *cis*-[Pt($NH_2CH_3$)$_2Cl_2$] and Guo. Again, on the basis of $^1$H NMR spectra for coordinated Guo, I, II, and III must be very similar to the products formed by *cis*-[Pt($NH_3$)$_2Cl_2$]. The signals for the $CH_3$ resonances are informative. Signals for $CH_3NH_2$ ligands trans to Cl appear at lower field than signals for $CH_3NH_2$ ligands trans to N donors. This pattern is clear from the shifts of *cis*-[Pt($NH_2CH_3$)$_2Cl_2$], I, and II in Table III. For structure III, two $CH_3$ resonances should be downfield and two upfield, as observed (Table III). The two downfield resonances are coincident.

Thus, on the basis of $^{195}$Pt NMR and $CH_3$ $^1$H NMR shifts, it is likely that III contains Cl in the coordination sphere as illustrated in Figure 4. To further confirm this composition, we examined the relationship of the charges of I, II, and III derived from *cis*-[Pt($NH_3$)$_2Cl_2$] and Guo, GMP, and MeGMP. The bridging guanine carries one negative charge. The charges expected for I, II, and III are summarized in Table IV. Passage of a solution of I, II, and III for Guo through a cation-exchange column led to elution of I and III together, followed by II—in agreement with the proposed charges. Similarly, I, II, and III for MeGMP are all uncharged and elute similarly in either cation or anion columns.

The only reasonable alternative ligands to Cl would be $H_2O$, OH, Guo, or amine. However, no inflection point was observed for III between pH 3 and 11. Thus monodentate $H_2O$ and OH are excluded. Alternatively OH could act as a bridging ligand—but this composition leads to a positively charged species for III with MeGMP. In addition, an N1- and N7-bridged species also bridged by OH would not be sterically feasible. Finally, neither an amine ligand nor another Guo ligand is likely to complete the coordination sphere since these possibilities are greatly outside the range of $^{195}$Pt NMR shifts observed and would have led to the observation of additional $^1$H NMR resonances.

**Conclusion.** This study has provided strong evidence for the nature of III for Guo starting with *cis*-[Pt($NH_2CH_3$)$_2Cl_2$], [Pt(en)$Cl_2$], and *cis*-[Pt($NH_3$)$_2Cl_2$]. The natures of III formed by the latter complex and GMP most probably are similar. Subsequent reactions may occur for III formed by GMP. Perhaps paramagnetic impurities formed at long reaction times may interfere with $^{195}$Pt NMR observations. However, the upfield $^{195}$Pt NMR signal for solutions containing I and III confirms that an N1-bound species is formed at least during early stages of the reaction.

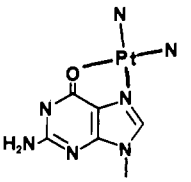

**Figure 5.** Hypothetical structure for an N7, O6 chelate with N1 deprotonated.

Since the charge on III is clearly 1+ for the Guo/*cis*-[Pt($NH_3$)$_2Cl_2$] reaction, N1H must be deprotonated. The proposed structure of III in Figure 4 would also explain the pH titration behavior of III in this study and that of *DCHMR* and the failure of III to form with 1-MeGuo. Nevertheless, titration behavior alone does not clearly eliminate an N7, O6 chelate deprotonated at N1 for species III (Figure 5). However, such an explanation for the titration behavior cannot be easily rationalized since the p$K_a$ for N1H would need to be lowered by ~8.0 p$K_a$ units to ~1.5. Such N1H deprotonation increases the metal binding ability of O6.[38-40] From Table IV, it is also clear that the charge on III and an N7, O6 chelate deprotonated at N1 would be the same for III with Guo, GMP, and MeGMP. If III were such a chelate, only one $^{195}$Pt NMR resonance should be found and, for A = $NH_2CH_3$, only two $CH_3$ signals should be found. An N7, O6 chelate is clearly unable to explain the two $^{195}$Pt and the four $CH_3$ $^1$H NMR signals, and therefore, this study clearly rules out this structure for III.

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**Registry No.** I, 82758-00-7; II, 62661-45-4; III, 96825-28-4; *cis*-[Pt($NH_3$)$_2Cl_2$], 15663-27-1; *cis*-[Pt($NH_2CH_3$)$_2Cl_2$], 15273-32-2; [Pt(en)$Cl_2$], 14096-51-6.

**Supplementary Material Available:** Complete tables containing $^1$H and $^{195}$Pt NMR chemical shifts from individual experiments for Guo, GMP, MeGMP, and 1-MeGuo Pt adducts (4 pages). Ordering information is given on any current masthead page.

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