Cisplatin−DNA Cross-link Models with an Unusual Type of Chirality-Neutral Chelate Amine Carrier Ligand, *N,N′*-Dimethylpiperazine (Me_2ppz): Me_2ppzPt(guanosine monophosphate)$_2$ Adducts That Exhibit Novel Properties

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Most simple *cis*-$PtA_2\mathbf{G}_2$ complexes that model the G−G cross-link DNA lesions caused by the clinically used anticancer drug *cis*-$PtCl_2(NH_3)_2$ undergo large fluxional motions at a rapid rate (A_2 = two amines or a diamine; **G** = guanine derivative). The carrier amine ligands in active compounds have NH groups, but the fundamental role of the NH groups has been obscured by the dynamic motion. To assess carrier ligand effects, we examine retro models, *cis*-$PtA_2\mathbf{G}_2$ complexes, in which dynamic motion has been reduced by the incorporation of steric bulk into the carrier ligands. In this study we introduce a new approach employing the chirality-neutral chelate (**CNC**) ligand, **Me_2ppz** (*N,N′*-dimethylpiperazine). Because they lie in the Pt coordination plane, the methyl groups of **Me_2ppz** do not clash with the O6 of the base of **G** ligands in the ground state, but such clashes sterically hinder dynamic motion. NMR spectroscopy provided conclusive evidence that **Me_2ppz**Pt$(GMP)_2$ complexes (GMP = 5′- and 3′-GMP) exist as a slowly interconverting mixture of two dominant head-to-tail (HT) conformers and a head-to-head (HH) conformer. Since the absence of carrier ligand chirality precluded using NMR methods to determine the absolute conformation of the two HT conformers, we used our recently developed CD pH jump method to establish chirality. The most abundant HT **Me_2ppz**Pt$(5′\text{-GMP})_2$ form had Λ chirality. Previously this chirality was shown to be favored by phosphate-cis **G** N1H hydrogen-bonding interligand interactions; such interactions also favor the HT conformers over the HH conformer. For typical carrier ligands, **G** O6 and phosphate interactions with the carrier ligand NH groups also favor the HT forms. These latter interactions are absent in **Me_2ppz**Pt$(GMP)_2$ complexes, but the HT forms are still dominant. Nevertheless, we do find the first evidence for an HH form of a simple *cis*-$PtA_2\mathbf{G}_2$ model with A_2 lacking any NH groups. In previous studies, the absence of the HH conformer in *cis*-$PtA_2\mathbf{G}_2$ complexes lacking carrier NH groups may be due to the presence of out-of-plane carrier ligand bulk. Such bulk forces both **G** O6−**G** O6 and **G** O6−carrier ligand clashes, thereby disfavoring the HH form. The major DNA cross-link adduct has the HH conformation. Thus, for anticancer activity, the small bulk of the NH group may be more important than the H-bonding interaction.

Introduction

Cisplatin (*cis*-$PtCl_2(NH_3)_2$) is one of the most widely used drugs in the treatment of several cancers.[1,2] Despite the success of cisplatin, problems regarding resistance and side effects have encouraged the development of new platinum drugs that might show activity against a wider range of malignancies with fewer side effects. Undertaking such a task requires knowledge of the biological targets of platinum drugs, the interactions that ensue, and the reasons that certain structural features of Pt compounds make some successful as antitumor agents and others unsuccessful.

It is widely accepted that DNA is the primary target responsible for the anticancer activity of cisplatin. DNA replication is inhibited in the presence of cisplatin,[2] and more recent studies suggest that transcription is also affected.[3] X-ray structural[1,2,4] and NMR data[2,4,5] have identified N7 of the guanine base as the primary DNA binding site. The cross-links formed by cisplatin (and its analogues) have been investigated and identified from X-ray structures,[2,4] NMR spectroscopy,[2,4] and enzyme digestion studies.[1,4,6] However, the fluxional nature of the adduct has hampered elucidation of the structures both in solution and in the solid state.[7,8] The primary cisplatin−DNA adduct has been determined to be a 1,2-intrastrand cross-link between adjacent guanines (d(GpG)).[6,9,10] Consequently, this cross-link is most often used as a model for investigations

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regarding the effect of cisplatin on DNA. Although it is not certain that the 1,2-intrastrand cross-link is responsible for cisplatin's anticancer activity, the isomer, *trans*-$PtCl_2(NH_3)_2$, which is unable to form this cross-link due to geometrical constraints, is inactive. It should be noted, however, that other types of trans Pt compounds are active.[11−13]

Although exceptions exist, the following structural features appear to be important for *cis*-type platinum drugs with the general formula *cis*-$PtX_2(amine)_2$ to exhibit antitumor effects: (1) anionic leaving groups of intermediate lability (e.g., Cl^-); (2) amine ligands with at least one hydrogen.[2,14] This last requirement has led to speculation regarding the importance of hydrogen-bonding interactions between the carrier ligand NH groups and oxygen atoms in the guanine and phosphate groups in or near the DNA lesion.[15] The extent and relative ease of rotation about the Pt−G N7 bond are important because rotation about this bond is critical for monoadducts to form cross-links between adjacent purines (guanine or adenine) in DNA.[16,17] Both the leaving and amine ligands can influence monoadduct rotation. Thus, carrier ligand NH hydrogen-bonding interactions may influence both adduct formation and conformation.

Induced distortions in DNA feature prominently in hypotheses to explain cisplatin's mechanism of action. It is hypothesized that this distortion is not efficiently recognized by repair enzymes in tumor cells and thereby inhibits the replication process (i.e., the distorted DNA is an unsuitable template), eventually leading to cell death.[2,18] In distorted cisplatin intrastrand cross-linked DNA adducts, the dominant conformer appears to have the guanines in a head-to-head (HH) orientation (Figure 1). Conformers with the head-to-tail (HT) orientation are thought to be favored in *cis*-$PtA_2\mathbf{G}_2$ adducts (A_2 = two monodentate or one bidentate amine ligand; bold letter **G** = guanine derivative *not* linked by a phosphodiester group).

Statistics favor the HH conformer, which should be twice as abundant as either HT conformer in most *cis*-$PtA_2\mathbf{G}_2$ adducts (in common adducts the *cis*-PtA_2 moiety has C_2 symmetry). However, when both N's of the carrier ligand have at least two H's, no evidence has been found for the HH conformer of *cis*-$PtA_2\mathbf{G}_2$ adducts in solution, even though such species have been found in the solid state.[19,20] Furthermore, no HH form has been reported when the carrier ligand has no NH groups. In some intermediate cases, usually with one NH on each N, HH conformers have been observed, but at equilibrium the HT forms have always dominated.[21]

Assessment of the solution conformation of *cis*-$PtA_2\mathbf{G}_2$ adducts with the less bulky ligands is complicated by dynamic motion that equilibrates the rotamers rapidly on the NMR time scale. Bulk has been shown to be necessary to slow the process. However, in a recent trapping experiment, we were unable to find any evidence for HH forms in solution and observed only HT forms with nonbulky carrier ligands.[22]

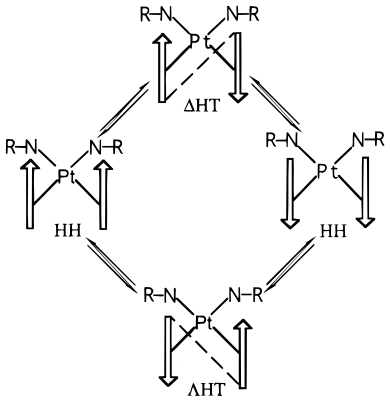

Figure 1. Schematic representation of the interconversion between HT and HH atropisomers of *cis*-$PtA_2\mathbf{G}_2$ (A_2 = **Me$_2$ppz**) complexes viewed with the **G** coordination sites forward and piperazine ligands to the rear (mostly omitted for clarity). Arrows represent the **G** bases with H8 at the head. The two possible HT rotamers are differentiated by an imaginary line (dotted line in this figure) drawn between identical points (generally the O6 atom) of each of the **G** ligands. The resulting line (viewed from the **G** side of the coordination plane) has either a positive slope (ΔHT) or a negative slope (ΛHT). R represents CH_3 groups when A_2 = **Me$_2$ppz**.

The evidence for this dichotomy that linked adducts favor the HH form and unlinked adducts favor the HT form has accumulated through extensive studies spanning a quarter of a century.[7] However, our most recent investigations have begun to challenge the validity of the dichotomy.[23] Thus, the implicit assumptions in drug design that only HH forms are relevant to activity and that the carrier ligand has to interact with such an HH form must now be viewed with caution. For example, one leading hypothesis to explain activity involves the bonding of a damage recognition protein to DNA adducts.[3,24] This binding helps to avoid repair of DNA damage, allowing the adduct to persist long enough to cause cell death. The results suggest that the structural changes induced by the cisplatin 1,2-intrastrand cross-links are responsible for this specific recognition.[24] We hypothesize that binding by the damage recognition protein will be weaker when the cross-link adopts an HT form.[23] Such lesions would not be well protected by the protein and would be better substrates for repair. Thus, we suspect that enhanced repair may be the reason for the lack of success in the search for clinically useful drugs with a carrier ligand very different from $(NH_3)_2$. On the basis of this hypothesis, we believe that understanding those factors which favor HH over HT forms is important for designing new anticancer drugs.

In this study, models with the carrier ligand, *N*,*N*′-dimethylpiperazine (**Me$_2$ppz**, Figure 2), of the type **Me$_2$ppz**Pt$\mathbf{G}_2$ were investigated with the aid of NMR and CD spectroscopy for **G** = guanosine 5′-monophosphate (5′-GMP) and guanosine 3′-monophosphate (3′-GMP).

Experimental Section

Materials. 5′-GMP and 3′-GMP were received from Sigma. $PtCl_2$(**Me$_2$ppz**) was prepared by a modification[25] of the original method of Mann and Watson.[26]

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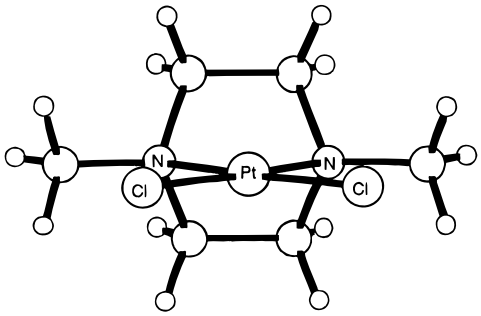

Figure 2. Ball and stick model of the cisplatin analogue, dichloro(*N*,*N*′-dimethylpiperazine)platinum(II) ($PtCl_2$(**Me$_2$ppz**)).

^{1}H NMR Spectroscopy. All samples (~2.5−5 mM in Pt) used for NMR experiments were prepared in D_2O by treating the chloro complex with $AgNO_3$ (1.7 equiv) in the dark for 1 day. After AgCl was removed by filtration, the guanine derivative (~2 equiv) was added. (Often the **G**:Pt ratios were slightly higher than 2:1 to ensure complete reaction of Pt.) All NMR studies were performed on either a GE QE 300 MHz or GE Omega 600 MHz instrument. A presaturation pulse was employed to suppress the HOD peak, and the residual HOD peak was used as reference. At least 128 scans were collected in each experiment. Saturation transfer experiments were conducted using a presaturation pulse of 3 s and pulse delay of 1 s. In addition to irradiation of the peaks of interest, the appropriate symmetrical positions were irradiated in order to eliminate any power spillage effects in these experiments. Deuterated nitric acid and sodium hydroxide solutions were used in pH studies.

Circular Dichroism (CD) Spectroscopy. All samples used for CD experiments were prepared from respective NMR samples and diluted to ~0.07 mM **G** with deionized water. Ten acquisitions from 400 to 200 nm were collected on a JASCO J-600 CD spectropolarimeter for each sample at a scan speed of 50 nm/min. In pH jump experiments, only one acquisition at a scan speed of 200 nm/min was collected at 10 °C.

Results

General Features of *cis*-Pt$A_2$$G_2$ Adducts. In simple *cis*-Pt$A_2$$G_2$ complexes, rotation about the Pt−**G** N7 bond interconverts HH and HT atropisomers in solution (Figure 1). Steric effects introduced by the A_2 ligand can lower the rate of rotation. Generally, when A_2 is not bulky (e.g., the ammonia groups in cisplatin), rotation about the Pt−**G** N7 bond is fast; when A_2 is bulky, rotation is restricted by **G** O6 clashes with A_2, allowing observation of the NMR signals of individual HT and HH conformers.[16,17] The **G** H8 ^{1}H NMR signal is usually the best signal type for detecting and identifying such conformers.

The asymmetry of the **G** ribose residue and the local symmetry of the *cis*-PtA_2 moiety influence the number of NMR signals that can be observed for each *cis*-Pt$A_2$$G_2$ conformer. We limit this discussion to *cis*-Pt$A_2$$G_2$ cases in which the **G** bears an asymmetric ribose residue and the symmetry of the *cis*-PtA_2 moiety is C_2 or higher. Because the **G** bears a chiral ribose moiety, the HT conformers are not enantiomers; thus, the ΔHT and ΛHT rotamers can be distinguished by NMR spectroscopy. The two **G**'s in each HT conformer are equivalent and are related by a C_2 axis; the H8's are magnetically equivalent in each, giving only one H8 signal for each HT rotamer. The H8 signals from the two HT rotamers are well separated because the base tilting with respect to the coordination plane is different, leading to different contributions of the anisotropy to the shifts. These factors also influence the CD signal. The transition exhibiting the CD signal is centered in the **G** bases of the *cis*-PtG_2 chromophore. Because of the short time scale of electronic transitions, a CD spectrum is the weighted sum of the CD signal of all conformers. Many conformational features, such as N/S sugar pucker or the syn/anti relationship of a sugar to the base in a nucleotide, can influence the CD spectrum. However, the rate of change of such features is too fast to allow assessment by NMR methods. Consequently, the NMR signals of each conformer we detect actually reflect an average of "subconformers." The *cis*-PtG_2 moieties in the ΔHT and ΛHT conformers are nearly enantiomeric; thus, the CD signals are expected to have nearly opposite shapes but the intensities are not expected to be the same since the conformers are not enantiomers. Also the average base tilting in the *cis*-PtG_2 chromophores is not the same for the ΔHT and ΛHT forms[21,27−30] and the sugar can have different average relationships to the **G** base within the **G** residue; thus, care must be taken in interpreting all such CD spectra. Even in the hypothetical case of a 50:50 mixture of ΔHT and ΛHT forms and the complete absence of the HH form, a CD signal should be observed since the contributions of the CD signals of each HT form do not cancel. In addition, as the pH is changed, the relative position of the sugar to the base can change; at high pH, the electronic transition in the chromophore changes as the base N1H is deprotonated. Thus, even if the relative abundance of conformers with distinct NMR signals is insensitive to pH, the CD spectrum can change with pH.

In the case under consideration, only one HH atropisomer is possible. However, the asymmetry imposed on the complex by the sugar residue renders the two **G**'s in the HH rotamer nonequivalent. The two H8 signals expected for the HH conformer should be of equal intensity since they arise from the same single species. Thus, it is possible to observe up to four H8 signals (one for each HT atropisomer and two from the HH conformer). The HH form has a CD signal but it is generally weak and difficult to detect.[21] The weakness of the CD signal probably arises from the inherent symmetry of the base chromophore arrangement since it is possible for both **G** bases to orient so that the two bases have a mirror relationship.

General Observations for Me$_2$ppzPt(GMP)$_2$ Complexes. ^{1}H NMR spectra provided evidence for one HH and two HT conformers for the **Me$_2$ppz**Pt(5′-GMP)$_2$ and **Me$_2$ppz**Pt(3′-GMP)$_2$ complexes. HT forms dominated over the HH atropisomer. Variable-temperature and saturation transfer experiments utilizing the H8 signals confirmed that interconversion between the atropisomers via rotation about the Pt−**G** N7 bonds is relatively slow.

G = 5′-GMP, ^{1}H NMR Spectra. Four H8 peaks were observed in the ^{1}H NMR spectrum of **Me$_2$ppz**Pt(5′-GMP)$_2$. The two dominant H8 signals are unequal in intensity and are assigned to the two HT forms (HTa and HTb). The two most downfield peaks (HHd1 and HHd2 for the more and less downfield peaks, respectively) are small but of equal size; these are thus assigned to the HH conformer. The reaction was carried out at pH ~4 in order to ensure binding only to N7, and platinum coordination at N7 was confirmed because no shift of the H8 signals was observed when the pH was lowered from 3.3 to

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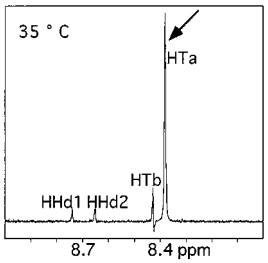

Figure 3. Difference NMR spectrum from the saturation transfer experiment with **Me$_2$ppz**Pt(5′-GMP)$_2$, pH 3.0, 35 °C. The arrow signifies an irradiated peak.

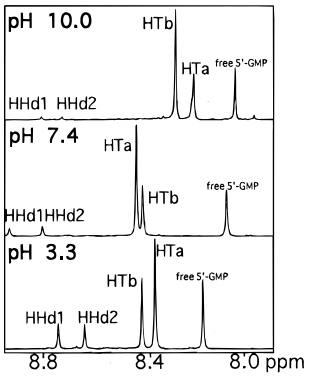

Figure 4. pH Dependence of NMR spectra of **Me$_2$ppz**Pt(5′-GMP)$_2$, room temperature.

1.3. A variable-temperature study (pH 7.6), conducted in order to assess the energy barrier of **Me$_2$ppz**Pt(5′-GMP)$_2$ atropisomerization, revealed that by 80 °C all four peaks (HHd1, HHd2, HTa, HTb) appear to broaden to a similar extent (Supporting Information), but coalescence was not achieved. Transfer of magnetization was observed between the four H8 signals; transfer of magnetization to the HTb, HHd1 and HHd2 signals was observed when HTa was irradiated at 35 °C (Figure 3).

Relative atropisomer distribution and H8 chemical shifts were found to be pH dependent (Figure 4). Upon increase of the pH from 3.3 to 7.4, the percentages for the HTa, HTb, and HH atropisomers changed from 50%, 26%, and 24% to 60%, 28%, and 12%, respectively. By pH 10.0, the percentages of the HTa, HTb and HH atropisomers were 29%, 70%, and 1%, respectively. From pH 3.3 to 7.4, all four H8 signals shifted downfield (HHd1 and HHd2 by ∼0.2 ppm, HTa by 0.12 ppm, and HTb by 0.05 ppm), as expected from the "wrong-way" shift upon 5′-phosphate group deprotonation.[31,32] From pH 7.4 to 10.0, the H8 signals all shifted upfield (HHd1 and HHd2 by ∼0.10 ppm, HTa by 0.23 ppm, and HTb by 0.13 ppm). Upfield shifts of the H8 signal are often observed upon **G** N1H deprotonation.[31,33]

CD Spectra. A positive feature at ∼285 nm and negative feature at ∼250 nm were observed for **Me$_2$ppz**Pt(5′-GMP)$_2$ at pH 7.5 and below (Figure 5). The signal intensity was stronger at pH 7.5 ($\Delta\epsilon$ values of ∼4 and ∼−4 at 285 and 250 nm, respectively) than at pH 3.2 ($\Delta\epsilon$ values of ∼2 and ∼−1.5 at 285 and 250 nm, respectively). This CD signal shape is characteristic of the ΛHT *cis*-PtA$_2$**G**$_2$ form.[27,29,34,35] The signal shape inverted at pH 10.3, with a negative band at ∼285 nm. A pH jump experiment was conducted to eliminate the possibility that the observed changes in the CD spectra were simply reflective of a pH-dependent change in the electronic transitions and not due to a redistribution of the HT atropisomers (Figure 6). The pH of the sample was raised to 9.0; the sample was left for >1 h and then cooled to 10 °C. The pH was then dropped to 7.1, and the CD spectrum was immediately recorded after 30 s and monitored continuously thereafter for up to an hour. Between 30 s and 10 min, the intensity of the characteristic ΛHT signal shape was found to increase substantially; no increase was observed after this time. These CD results are consistent with the pH-dependent HT atropisomer redistribution observed by ^{1}H NMR (at pH 8.9, integration of H8 signals revealed 51% and 48% of the HTa and HTb forms, respectively; at pH 7.4, the percentages were 60% and 28%, respectively). Therefore the CD signal correlates well with the major HT form.

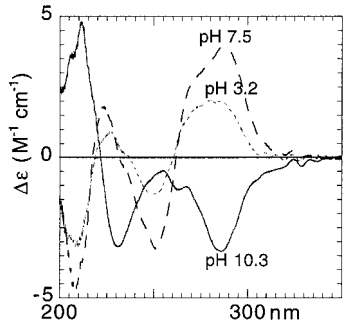

Figure 5. CD spectra of **Me$_2$ppz**Pt(5′-GMP)$_2$, collected at room temperature.

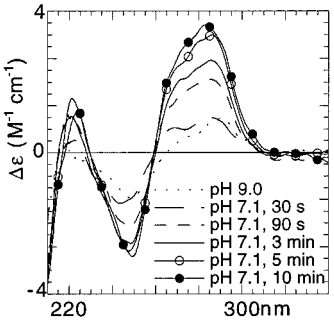

Figure 6. CD spectra of **Me$_2$ppz**Pt(5′-GMP)$_2$ pH-jump experiment, collected at 10 °C.

Table 1. Atropisomer Percentage and **G** H8 ^{1}H NMR Chemical Shifts as a Function of pH for **Me$_2$ppz**Pt(GMP)$_2$ Adducts

complex	pH	HTa % (ppm)	HTb % (ppm)	HH % (ppm)
Me$_2$ppz Pt(5′-GMP)$_2$	3.3	50 (8.37)	26 (8.42)	24 (8.63,8.73)
	7.4	60 (8.49)	28 (8.47)	12 (8.83, 8.96)
	10.0	29 (8.26)	70 (8.34)	1 (8.76, 8.83)
Me$_2$ppz Pt(3′-GMP)$_2$	3.3	44 (8.40)	48 (8.34)	8 (8.58, 8.63)
	7.5	49 (8.50)	47 (8.35)	4 (8.59, 8.64)
	10.4	38 (8.13)	62 (8.19)	<1 (8.48, 8.54)

G = 3′-GMP, ^{1}H NMR Spectra. Four H8 ^{1}H NMR signals observed in the spectrum of **Me$_2$ppz**Pt(3′-GMP)$_2$ were assigned to the two HT atropisomers and the HH conformer. Similar HT atropisomer distributions were observed at pH 3.3 and 7.5 (Table 1). At pH 10.4, HTb was clearly favored. The HH form became less favored as the pH was raised. Changes in H8 chemical shifts were relatively small when the pH was raised from 3.3 to 7.5. All H8 signals shifted upfield when the pH was raised to 10.4; the H8 signals for the HH rotamer each shifted upfield by ∼0.10

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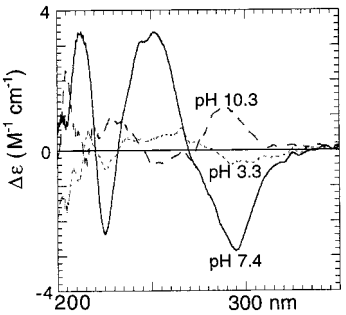

Figure 7. CD spectra of **Me$_2$ppz**Pt(3′-GMP)$_2$, collected at room temperature.

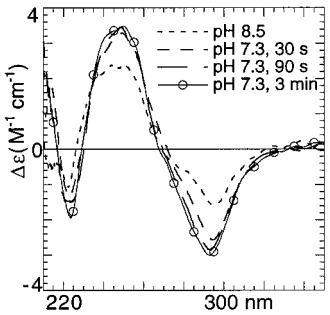

Figure 8. CD spectra of **Me$_2$ppz**Pt(3′-GMP)$_2$ pH-jump experiment, collected at 10 °C.

ppm, and HTa and HTb shifted upfield by 0.37 and 0.16 ppm, respectively.

CD Spectra. A negative band at ∼290 nm and a positive band at ∼255 nm were observed for **Me$_2$ppz**Pt(3′-GMP)$_2$ at pH 3.3 and 7.4 (Figure 7). This CD signal shape is characteristic of a ΔHT *cis*-PtA$_2$**G**$_2$ rotamer.[28,29,34,35] The signal intensity is stronger at pH 7.4 ($\Delta\epsilon$ values of ∼−3 and ∼3 at ∼290 nm and ∼255 nm, respectively) than at pH 3.3 ($\Delta\epsilon$ values $< \pm 1$ were observed at similar wavelengths). At pH 10.3, the CD signal shape inverts (positive band at ∼290 nm and negative band at ∼255 nm), and the signal intensity at this pH is relatively weak. A pH-jump experiment performed at 10 °C (Figure 8) revealed a slight increase in the signal intensity (shape characteristic of the ΔHT form) over a short time interval after the pH was lowered from 8.5 to 7.3, thereby suggesting that the CD signal reflects an increase in the ΔHT conformer population over this pH adjustment. This finding correlates well with the HT redistribution observed by ^{1}H NMR spectroscopy.

Discussion

Me$_2$ppz represents a new type of ligand in a cisplatin analogue which we have termed a chirality-neutral chelate (**CNC**) ligand. The two most distinctive features of this **CNC** ligand, compared to previously studied cisplatin analogues, are the lack of instantaneous dissymmetry and the absence of equatorial or axial NH groups. Such NH groups have been postulated to play an important role in the anticancer activity of cisplatin and many of its analogues.[2] At any point in time, the *cis*-PtA$_2$**G**$_2$ moiety generally lacks a plane of symmetry. For the *cis*-Pt(NH$_3$)$_2$ moiety itself, the NH's are positioned to interact with the nucleic acid target. These interactions will simultaneously break the symmetry and influence the position of the nucleic acid bases.

One tool for elucidating the hydrogen bonding between the amine NH groups and **G** moieties has been the assessment of the spectral features and the relative stabilities of atropisomers in *cis*-PtA$_2$**G**$_2$ systems.[27−30,34] For example, in our solution studies of less dynamic *cis*-PtA$_2$**G**$_2$ type complexes, we have been able to explain the atropisomer distribution by identifying new interligand interactions.[28,30] We have used two types of specially designed amines (Figure 9), chirality-controlling chelates (**CCC**)[29,30] and a hybrid ligand.[27,28] In addition to the widely invoked amine−phosphate group and amine−**G** O6 hydrogen-bonding interligand interactions, we established that cis **G**−cis **G** interactions (dipole(**G**)−dipole(**G**), **G** NH−phosphate group) and **G**−diamine steric interactions also modulate the stability of the conformers formed.[27−30,34] In addition, we have made the first steps in extending this information on "second-sphere communication" to the dynamic analogues of the type *cis*-Pt(NH$_3$)$_2$**G**$_2$.[35]

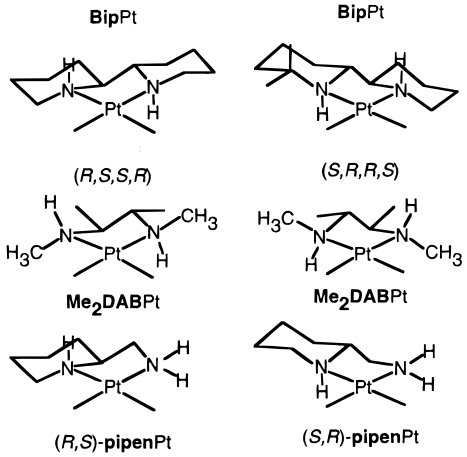

Figure 9. Sketches of **Me$_2$DAB**Pt (**Me$_2$DAB** = *N*,*N*′-dimethyl-2,3-diaminobutane) and **Bip**Pt (**Bip** = bipiperidine) **CCC** ligands with *S*,*R*,*R*,*S* and *R*,*S*,*S*,*R* configurations and the hybrid **pipen**Pt (**pipen** = 2-(aminomethyl)piperidine) moiety with stereochemistry noted at the N and C asymmetric centers, respectively.

These studies with less dynamic analogues have been very revealing, but the conformational diversity of the nucleotide ligands had previously obscured these interligand interactions. Solid-state studies have also not been very revealing, in part because the dynamic nature of the adducts has impeded crystallization but also because of the absence of solvent effects in the crystalline state. Therefore, it seemed wise to investigate systems which would reveal the interactions. Since the **Me$_2$ppz** ligand has no amine hydrogens, amine−phosphate group or amine−**G** O6 hydrogen bonding cannot influence atropisomer preference in **Me$_2$ppz**Pt**G**$_2$ complexes. Thus, the objective of this study was to exploit the opportunity this **CNC** ligand offers for gaining new insight concerning cis **G** interactions and steric effects.

Although the finding that the HH form was the least stable atropisomer for the **Me$_2$ppz**Pt(GMP)$_2$ adducts is a common observation in our work with (**CCC**)Pt**G**$_2$ complexes,[29,30,36] the H8 shifts for HHd1 and HHd2 H8 signals do not conform to the normal pattern (Table 1). (Characteristically, the two H8 signals from HH rotamers exhibit a chemical shift difference of ∼1 ppm and are positioned on either side of the two HT signals.[29,30,36]) The unusual shift pattern might suggest that the equivalent intensity of the HHd1 and HHd2 H8 signals may not indicate that the minor form is an HH form. However, there is no doubt that the minor species is an HH rotamer with both GMP's bound via N7. We can exclude the only reasonable interpretation, namely, that the HHd1/HHd2 signals arise from

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a species with one GMP bound via N1 and one GMP bound via N7. Platinum coordination at N7 only was confirmed because no change in shift of the HHd1/HHd2 signals was observed when the pH was lowered from 3.3 to 1.3. Furthermore, the broadening of the four H8 signals with increasing temperature (Supporting Information) and the exchange of magnetization observed in saturation transfer experiments (Figure 3) for **Me$_2$ppz**Pt(5′-GMP)$_2$ demonstrate interconversion of the forms responsible for the observed H8 signals. These findings and the similar spectra for the **Me$_2$ppz**Pt(3′-GMP)$_2$ complex are completely consistent with assignment of the four H8 peaks to the HT and HH rotamers. Finally, the slow rotation evident from the NMR spectra of the **Me$_2$ppz**Pt(GMP)$_2$ complexes is consistent with the high rotational energy barrier (∼19 kcal/mol) calculated by molecular mechanics for a model with an N7-bound guanine, namely the [**Me$_2$ppz**Pt(9-ethylguanine)$_2$]$^{2+}$ complex.[37] (This calculated value is higher than the calculated ∼13−15 kcal/mol rotational energy barrier threshold correlating with restricted rotation.[34,38]) We shall consider below the reasons for the very downfield position of *both* HH H8 signals.

Factors Influencing Distribution. Our work indicates that the dipole(**G**)−dipole(**G**) interaction is the principal factor favoring the HT conformation in *cis*-PtA$_2$**G**$_2$ complexes both in solution and in the solid state.[29] However, the absolute conformation (ΔHT or ΛHT) of the dominant HT form depends on a variety of factors. Normally, we determine the conformation by assessing NOE cross-peaks to a chiral carrier ligand with known absolute configuration.[29] The high symmetry of the **Me$_2$ppz** ligand precludes the use of NMR methods to establish the absolute conformation. Therefore, we utilized the enhanced CD signals such as those observed previously for *cis*-PtA$_2$**G**$_2$ complexes.[39] We have shown that two different CD signal shapes are characteristic of the ΛHT and ΔHT atropisomers.[27−29,34] In the case of **Me$_2$ppz**Pt(5′-GMP)$_2$, one HT form (HTa) clearly dominates over the other HT conformer (HTb) at approximately neutral pH (Figure 4). Thus, CD spectroscopy was used as an aid in identifying the conformation of the preferred HT atropisomer (HTa) of **Me$_2$ppz**Pt(5′-GMP)$_2$. The shape of the signal at pH 7.5 was shown to be dictated by the HTa atropisomer in a pH jump experiment (Figure 6). This shape (Figure 5) is characteristic of the ΛHT conformation, thereby indicating that HTa is the ΛHT form.

Our previous analysis of experimental data for our complexes with bulky chiral diamine ligands established that, below pH 7.5, 5′-GMP and 3′-GMP complexes are biased toward the ΛHT and ΔHT conformations, respectively.[28,34] This bias also appears evident for complexes with nonchiral amines, including *cis*-Pt(NH$_3$)$_2$**G**$_2$ complexes.[35] Examination of models, as well as aspects of the experimental data, indicates that these biases arise from second-sphere communication, i.e., the interaction of the phosphate group of one **G** with N1H of the cis **G**.

Another factor influencing which HT conformer is preferred is the interaction of the 5′ phosphate group with the carrier ligand NH groups when **G** = 5′-GMP. For all **G** ligands, **G** O6 hydrogen bonds to the carrier ligand NH groups are possible, but our recent results indicate that these hydrogen bonds are not of great importance.[29,30] Since the **Me$_2$ppz** ligand has no NH groups, **Me$_2$ppz**Pt**G**$_2$ adducts do not contain NH−**G** O6 or NH−phosphate hydrogen bonds involving the carrier ligand. The **Me$_2$ppz** methyl groups could have unfavorable steric interactions with the **G** O6 and to a lesser extent with the 5′-phosphate group of 5′-GMP, but models suggest that such interactions are weak. Therefore, the only favorable interactions possible are dipole(**G**)−dipole(**G**) and phosphate−cis **G** NH interactions between the two **G** moieties.

The pH dependence of the **Me$_2$ppz**Pt(5′-GMP)$_2$ atropisomer distribution (Figure 4) was studied in order to assess the influence of the phosphate−cis **G** N1H interactions, which are known to be strongest at pH ∼7.[34] HTa is the major **Me$_2$ppz**Pt(5′-GMP)$_2$ HT form at pH ∼3 and becomes slightly more preferred as the pH is raised to ∼7. At higher pH values, the ratio between the two HT forms is ∼1. By pH 9.5, HTb has now become the dominant HT conformer (Table 1). (Careful analysis was required since the chemical shifts of the two HT signals are pH sensitive, and they overlap at pH 6.4 and again at pH 9.3, Supporting Information).

CD spectra at low and high pH support the importance of phosphate−cis **G** N1H hydrogen bonding in HT forms. Like the CD signal at pH 7.5, the signal at pH 3.2 exhibits the shape characteristic of the ΛHT rotamer, but the signal is of weaker intensity, reflecting the reduced dominance of the ΛHT form (Figure 5). Inversion of the signal shape as the pH was raised to pH 10.3 supports the NMR data showing that the ΔHT conformer is now preferred. Past work has shown that the ΔHT conformer is favored at high pH for **G** = 5′-GMP.[27,30,34] Thus the CD and NMR data are consistent and indicate HTa = ΛHT and HTb = ΔHT.

The NMR spectra of **Me$_2$ppz**Pt(3′-GMP)$_2$ revealed a ratio between HT conformers of ∼1 at pH ∼3 and ∼7. However, as the pH was raised to ∼10, a dominant form (HTb) emerged (Table 1). The shape of the CD signal of **Me$_2$ppz**Pt(3′-GMP)$_2$ at pH ∼7 resembles that characteristic of the ΔHT conformer (Figure 7); this is the form shown previously to have the most favored phosphate−cis **G** N1H hydrogen-bonding potential in the 3′-GMP complexes. The signal at pH ∼3 exhibits a shape similar to that observed at pH ∼7, but its intensity is significantly lower. At pH ∼10, the signal has inverted and thus resembles that characteristic of the ΛHT, although it is likewise of low intensity. Thus, the CD spectra for **Me$_2$ppz**Pt(3′-GMP)$_2$ suggest that the relative HT rotamer distribution behaves as expected from phosphate−cis **G** N1H hydrogen bonding. However, it is not clear why the HT distribution of **Me$_2$ppz**Pt(3′-GMP)$_2$ conformers (HT ratio is ∼1 from pH ∼3 to pH ∼7) observed in the NMR spectra does not correlate so well with the proposed phosphate−cis **G** N1H hydrogen-bonding hypothesis at low and neutral pH. Nevertheless, the emergence of the dominant HTb form at high pH is consistent with the loss of favored phosphate−cis **G** N1H stabilizing forces in the less abundant HTa form at high pH. From these CD observations and the pH jump experiment (Figure 8), HTa = ΔHT and HTb = ΛHT for **Me$_2$ppz**Pt(3′-GMP)$_2$.

Recently, studies with **CCC** ligands (**Me$_2$DAB** and **Bip**, Figure 9) have indicated that dipole(**G**)−dipole(**G**) interactions favoring the HT forms over the HH form probably depend on the degree of tilt of the **G** bases.[29,30] In the (**CCC**)Pt(GMP)$_2$ complexes, the preferred HT form had the more downfield-shifted H8 signal than the minor HT form, indicating a less tilted arrangement of the **G** bases in the major HT form. (Different orientations/tilt arrangements of the **G** bases will produce different ring-current effects. The shift of the H8 signal can be useful in evaluating such effects. More specifically, when bases are tilted such that the H8 atoms are positioned in closer

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proximity to the five- or six-membered rings of the adjacent base, the **G** bases exert a more significant ring-current effect on one another, and a less downfield-shifted H8 signal is expected.[40] Thus, when both **G** bases in an HT form are less tilted, the H8 signal is more downfield.) In the case of **Me$_2$ppz**Pt(5′-GMP)$_2$ at pH ∼7, the major HT form (HTa) has the downfield H8 signal but the separation of HT H8 signals is small (0.02 ppm, Table 1). A separation of only ∼0.05 ppm was observed between H8 signals of the HT forms in **Me$_2$ppz**Pt-(5′-GMP)$_2$ at pH 3.3 (Table 1). The analogous value for **Me$_2$DAB**Pt(5′-GMP)$_2$ is ∼0.3 ppm at pH ∼3.[29] Thus the difference in the degree of tilt between the two observed HT forms of **Me$_2$ppz**Pt(5′-GMP)$_2$ may be minimal in comparison to that in other *cis*-PtA$_2$(GMP)$_2$ complexes. Moreover, potential hydrogen bonds between the NH's of the A$_2$ ligand and the **G** moieties in (**CCC**)Pt**G**$_2$ complexes have been proposed to favor base tilting.[29,30] However, although the favored HT atropisomer cannot form such H-bonds, the bases are tilted in order to avoid bulk associated with the N-alkyl groups of the **CCC** carrier ligand. This bulk projects somewhat out of the plane. Thus, the cause for the tilting appears to be different for the two HT conformers of a given (**CCC**)Pt**G**$_2$ adduct, e.g. (*R*,*S*,*S*,*R*)-**Bip**Pt-(5′-GMP)$_2$.[30] Since neither H-bonding nor steric interactions are important for the **G** O6 groups in these **Me$_2$ppz**Pt**G**$_2$ complexes, the **G** bases may be less likely to assume different degrees of tilt with respect to the coordination plane in the two HT forms.

As mentioned above, the small separation between the H8 shifts of the HH form in these **Me$_2$ppz**Pt**G**$_2$ complexes is unusual. The more common ∼1 ppm shift difference between the H8 signals previously observed for the HH conformer in *cis*-PtA$_2$**G**$_2$ complexes has been explained by the relative canting of the **G**'s.[29] One base (which can form an H-bond) is canted toward the second base, placing the H8 of the first base into the shielding cone of the second base (which is not canted). The small separation of the unusually downfield H8 signals for the HH form observed in this study suggests that both **G**'s are relatively uncanted. Such an arrangement may be most sterically favored. Moreover, the absence of NH's in the **Me$_2$ppz** ligand eliminates hydrogen-bonding interactions as potential factors that may encourage the base(s) to cant.

For both the 5′-GMP and 3′-GMP complexes, the percentage of the HH conformer decreased as the pH was raised (Table 1). This decrease may be due to an increase in unfavorable electrostatic interactions between the phosphate groups as they deprotonate at higher pH and between the bases as N1H deprotonates. Another interesting observation is that a larger percentage of the HH form was observed for the 5′-GMP complex than for the 3′-GMP complex (Table 1). A relatively high percentage of the HH rotamer has also been reported for 5′-GMP complexes with **Bip** and **Me$_2$DAB** compared to other **G**'s.[29,30] Hydrogen bonding between the NH's of **Me$_2$DAB** and phosphate group/O6 of the 5′-GMP has been suggested as helping to stabilize the HH form; however, such hydrogen bonding cannot be offered as an explanation for the stabilization of the HH rotamer in **Me$_2$ppz**Pt(5′-GMP)$_2$ complex since the **Me$_2$ppz** ligand has no NH groups. Perhaps the flexibility of the 5′-phosphate group renders it capable of some stabilizing interactions with the cis **G** (e.g., **G** phosphate group−cis **G** 2′-OH, 3′-OH, or 2-NH$_2$ hydrogen bonds). The position of the phosphate group also appears to play an important role in stabilizing the different HT forms since the percentages of the two different HT forms are more or less equal in the **Me$_2$ppz**Pt-(3′-GMP)$_2$ complex at low pH, whereas HTa dominates over HTb by a factor of ∼2 in **Me$_2$ppz**Pt(5′-GMP)$_2$.

Conclusion

The results for the **Me$_2$ppz**Pt(GMP)$_2$ complexes investigated can be summarized as follows: the **G** bases are in slow rotation, the two dominant conformers are HT forms, and the HH rotamer is present in solution. This is the first report of an HH form for a *cis*-PtA$_2$**G**$_2$ complex when A$_2$ lacks an NH group. Furthermore, the H8 signals of this HH form do not conform to typical previously reported shift patterns for HH forms; both signals are relatively downfield and closely spaced, suggesting that the **G** bases orient in a similar, less tilted manner. We hypothesize that the in-plane position of the N−Me groups of **Me$_2$ppz** allows ample space for the HH form to exist without significant clashes between the **G** O6 groups. Other carrier ligands lacking NH groups have significant out-of-plane bulk and generally form inactive compounds. Thus, the small bulk of the NH group may be more important than the H-bonding interaction for anticancer activity. Given the finding that carrier ligands favoring HH forms in cross-links are associated with anticancer activity, **Me$_2$ppz** platinum complexes should be tested for activity.

One HT form clearly dominates over the other at low pH when **G** = 5′-GMP. In comparison, an approximately equal distribution between the two HT forms is observed when **G** = 3′-GMP. Moreover, the largest amount of the HH form is observed when **G** = 5′-GMP. Thus, the position of the phosphate group appears to influence significantly the observed atropisomer distributions in **Me$_2$ppz**Pt(GMP)$_2$ complexes. A similar finding for (**CCC**)Pt(GMP)$_2$ complexes was interpreted similarly, but the results were clouded by hydrogen-bonding interactions with the carrier ligand.[29,30]

In previous studies of *cis*-PtA$_2$(GMP)$_2$ complexes, the ΛHT and ΔHT forms were preferred in 5′- and 3′-GMP complexes, respectively. CD and NMR spectroscopy indicate that the ΛHT atropisomer predominates in solution near physiological pH in the **Me$_2$ppz**Pt(5′-GMP)$_2$ complex, in agreement with results for other *cis*-PtA$_2$(5′-GMP)$_2$ complexes.[29,35] However, both HT forms are nearly equally stable for **Me$_2$ppz**Pt(3′-GMP)$_2$. At this time the reasons for these differences are not apparent, and additional studies are in progress in order to understand the unique features of **Me$_2$ppz**Pt**G**$_2$ complexes.[37]

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Supporting Information Available: Figures of the temperature and pH dependence of the H8 ^{1}H NMR signals of **Me$_2$ppz**Pt(5′-GMP)$_2$. This material is available free of charge via the Internet at http://pubs.acs.org.

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