marked variations in this property. In compounds such as $(Cp^{4i})_2Ae$, which are readily soluble in aromatics and even alkanes, tight ion pairing between metal and ligand can endow the complexes with a type of "pseudo-covalency" that suppresses ligand exchange and rearrangement.

The effects of encapsulating ligands on the properties of alkaline-earth metallocenes demonstrate some of the control that is available over the reactions of highly ionic compounds. We expect elaboration of this principle to broaden even further the range of chemistry accessible to organometallics of the heavy s-block elements.

Acknowledgement is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. Funds for the X-ray diffraction facility at Vanderbilt University were provided through NSF Grant CHE-8908065. We thank Dr. Paul N. Swepston of Molecular Structure Corp. for obtaining the crystal structure of $(Cp^{4i})_2Ca$ and Professors S. C. Blackstock and C. M. Lukehart for helpful discussions.

Supplementary Material Available: Tables of atomic fractional coordinates, bond distances and angles involving non-hydrogen atoms, dihedral angles between Cp and *i*-Pr groups in $[(C_3H_7)_4C_5H]_2Ca$ and $[(C_3H_7)_4C_5H]_2Ba$, and anisotropic thermal parameters (25 pages); listings of observed and calculated structure factor amplitudes (71 pages). Ordering information is given on any current masthead page.

Interaction of Bis(platinum) Complexes with the Mononucleotide 5′-Guanosine Monophosphate. Effect of Diamine Linker and the Nature of the Bis(platinum) Complex on Product Formation

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Abstract: The reactions of bis(platinum) complexes containing bidentate or monodentate coordination spheres with the mononucleotide guanosine 5′-monophosphate (GMP) have been studied in solution by ^1H and ^{195}Pt NMR spectroscopy. The structures of the complexes are $[\{cis\text{-}PtCl_2(NH_3)\}_2(diamine)]$ (bidentate coordination spheres, 2,2/c,c) and $[\{trans\text{-}PtCl(NH_3)_2\}_2(diamine)]Cl_2$ (monodentate coordination spheres, 1,1/t,t) where diamine = 1,4-butanediamine ($NH_2(CH_2)_4NH_2$, BN) or 2,5-dimethyl-2,5-hexanediamine ($NH_2C(CH_3)_2(CH_2)_2C(CH_3)_2NH_2$, TMET). The 2,2/c,c complexes were studied as the aqua species $[\{cis\text{-}Pt(H_2O)_2(NH_3)\}_2(diamine)]^{4+}$ by reaction of the iodide with 4 equiv of $AgNO_3$ in water. In the case of the monodentate bis(platinum) complexes, the stepwise substitution of two GMP groups is easily seen, giving $[\{trans\text{-}Pt(GMP)(NH_3)_2\}_2(diamine)]$. The rate of reaction at 30 °C as measured by $t_{1/2}$ from the spectra was faster with TMET (3.5 h) than with BN (4.5 h). For bis(platinum) complexes with bidentate coordination spheres, the final product is $[\{cis\text{-}Pt(GMP)_2(NH_3)\}_2(diamine)]^{4-}$ but the mode of formation is dependent on the diamine. Whereas no intermediate species were observed with the linear $NH_2(CH_2)_4NH_2$, the sterically more demanding $NH_2C(CH_3)_2(CH_2)_2C(CH_3)_2NH_2$ gave in the initial stages of the reaction the species with only one GMP bound to each Pt $[\{Pt(GMP)(H_2O)(NH_3)\}_2(diamine)]$. All GMP ligands are N7 bound. The reactions are discussed in relation to the DNA binding and antitumor activity of the complexes.

The mechanism of action of the antitumor activity of bis(platinum) complexes containing two platinum–amine units linked by a variable-length diamine chain is of considerable interest. We have now reported the synthesis and properties of complexes containing bidentate coordination spheres $[\{cis\text{-}PtCl_2(NH_3)\}_2(diamine)]$ and monodentate coordination spheres $[\{trans\text{-}PtCl(NH_3)_2\}_2(diamine)]Cl_2$.[1,2] The antitumor activity of both sets of complexes is characterized by activity in both murine and human tumor cell lines with natural and acquired resistance to cisplatin.[2-4] It is particularly noteworthy that the presence of a cisplatin group is not necessary for antitumor activity, especially in cisplatin-resistant cells. DNA-binding studies have shown that an important interaction, inaccessible to cisplatin, is the formation of interstrand cross-links through binding of the two platinum centers to opposite DNA strands.[2,5] Our present studies include the description of this DNA-binding mode and its importance in the mechanism of cytotoxicity of these complexes.

The mechanism by which bis(platinum) complexes overcome cisplatin resistance is important, because it is possible that unique modes of the DNA binding of bis(platinum) complexes inaccessible to monomeric species are responsible for overcoming the resistance, either through enhanced cytotoxicity or because the bis(platinum)-induced DNA lesions are inherently more difficult to repair. Thus, there may exist a molecular basis to enhancement of non-cross-resistance, and the exploration of this aspect may eventually lead to discovery of platinum complexes with a spectrum of clinical anticancer activity different from cisplatin and its "second-generation" analogues.

The modes of DNA binding of bis(platinum) complexes with bidentate coordination spheres (2,2/c,c) are a complex array including interstrand cross-links (by each Pt binding to opposite strands) and intrastrand cross-links (by cisplatin-like binding to two adjacent bases on a single strand). Bis(platinum) complexes with monodentate coordination spheres, on the other hand, can

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Table 1. Principal NMR Spectral Parameters for the Reaction Products of 5′-Guanosine Monophosphate (GMP) with Bis(platinum) and Related Complexes[a]

complex	δ (¹H), ppm: H8	δ (¹H), ppm: H1′ ($J_{1'-2'}$, Hz)	δ (¹H), ppm: diamine	δ (¹⁹⁵Pt), ppm
GMP	8.20	5.95 (6.0)		-
[{*trans*-Pt(GMP)$(NH_3)_2$}$_2$BN] (I)	8.87	6.02 (4.4)	2.86 (m) 2.25 (m)	−2568
[{*trans*-Pt(GMP)$(NH_3)_2$}$_2$TMET] (II)	8.90	6.04 (4.2)	2.2 (m) 1.4	−2573
[{*cis*-Pt$(GMP)_2(NH_3)$}$_2$BN]$^{4-}$ (III)	8.62 8.60	5.88 (3.1) 5.86 (2.5)	2.59 (m) 1.92 (m)	−2473
[{*cis*-Pt$(GMP)_2(NH_3)$}$_2$TMET]$^{4-}$ (IV)	8.58[b] 8.53	5.97 (4.9) 5.89 (3.7)	1.75 (m) 1.38	−2482
[Pt(GMP)dien][c]	8.86	6.0 (3.9)	-	
cis-[Pt(H_2O)(GMP)$(NH_3)_2$][c]	8.89	6.02 (1.7)	-	
cis-[PtCl(GMP)$(NH_3)_2$]$^-$[d]	8.58	6.0	-	−2295
cis-[Pt$(GMP)_2(NH_3)_2$]$^{2-}$[d]	8.62	5.92	-	−2455

[a] As per Experimental Section. Integration as expected. Singlets except where noted. Counter anions omitted for clarity BN = $H_2N(CH_2)_4NH_2$. TMET = $H_2NC(CH_3)_2(CH_2)_2C(CH_3)_2NH_2$. In bis(platinum) complexes, structure of starting material is given in parentheses. For the products of complexes III and IV, ^{31}P NMR spectra give one peak at −0.57 and −0.66 ppm from trimethyl phosphate as reference. [b] Broad peaks that sharpen upon increase of temperature. [c] See refs 11 and 18 for details. dien = diethylenetriamine. [d] See ref 10 for details.

only form interstrand cross-links and a possible intrastrand cross-link by binding of each Pt atom to bases on the same strand. This latter mode would be equivalent but structurally quite different to a cisplatin adduct. To examine how these complexes bind to DNA and the factors affecting the formation of the different adducts possible, we are investigating the reactions of the various bis(platinum) species with mono- and oligonucleotides. In principle, a major source of sequence specificity and DNA affinity in bis(platinum) complexes is alteration of the diamine backbone. In this paper, we present the results of reactions of a simple mononucleotide, 5′-guanosine monophosphate (GMP), with bis(platinum) complexes 2,2/c,c and 1,1/t,t containing a straight-chain diamine, 1,4-butanediamine (BN), vs a sterically hindered diamine, 2,5-dimethyl-2,5-hexanediamine, a 1,4-butanediamine substituted by methyl groups in all C1 and C4 positions (TMET). Studies on the behavior of the hydrolysis products of these complexes have shown differences due to both the nature of the diamine and the coordination sphere,[6] and we wish to examine how these features affect reaction with other nucleophiles, especially purines and pyrimidines involved in DNA binding.

Experimental Section

Guanosine 5′-monophosphate disodium salt (GMP) was from Sigma Ltd. and was used without further purification. NMR spectra were run on a Bruker 250-MHz spectrometer at 30 °C. ^{195}Pt NMR spectra were obtained typically with a spectrum width of 25 or 62.5 kHz, 5000–10000 scans, and a relaxation delay of 0.18–0.5 s between 15-μs pulses and referenced to a Na_2PtCl_6 solution in D_2O as the external reference. [{*trans*-PtCl$(NH_3)_2$}$_2$$NH_2(CH_2)_4NH_2$]$Cl_2$ (I) and [{*trans*-PtCl$(NH_3)_2$}$_2$$NH_2C(CH_3)_2(CH_2)_2C(CH_3)_2NH_2$]$Cl_2$ (II) (1,1/t,t complexes) were prepared according to literature procedures.[2,6] Both complexes were allowed to react with 2 equiv of GMP in D_2O, and the reactions were followed by ^{1}H and ^{195}Pt NMR spectroscopy (C = 0.02 M). The pD (uncorrected) of the solutions varied from 6.8 to 7.1 (complex I) and from 6.8 to 7.0 (complex II) during the reaction. The solutions of [{*cis*-Pt$(D_2O)_2(NH_3)$}$_2$$NH_2(CH_2)_4NH_2$]$^{4+}$ (III) and [{*cis*-Pt$(D_2O)_2$-(NH_3)}$_2$$NH_2C(CH_3)_2(CH_2)_2C(CH_3)_2NH_2$]$^{4+}$ (IV) (2,2/c,c complexes) were freshly prepared from the corresponding iodide complexes[6] (C = 0.03 M). The solutions were immediately allowed to react with GMP (1:4 stoichiometry), and the reactions were followed by ^{1}H and ^{195}Pt NMR spectroscopy. The pD of the reaction mixtures was also monitored during the reaction, giving final values of 6.8 (complex III) and 7.1 (complex IV).

The chemical shift dependence on pD (uncorrected) of the final products from the reactions of complexes I and II with GMP were measured both before and after each spectrum was recorded, and the pD was changed by the addition of small amounts of NaOD or DCl in D_2O.

Results and Discussion

The complexes studied are shown in Figure 1. The abbreviations used are 1,1/t,t for the monodentate complexes I and II,

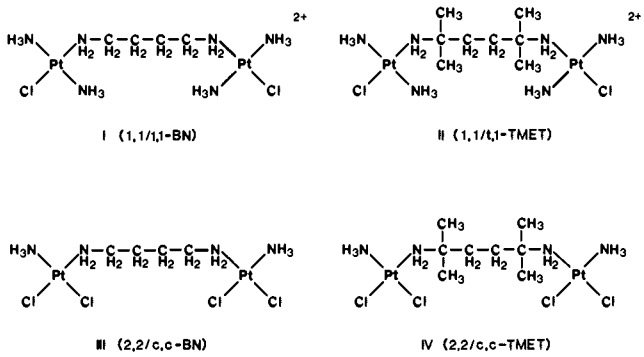
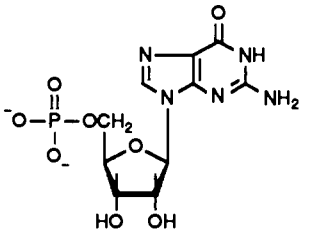


Figure 1. Structures of bis(platinum) complexes containing monodentate and bidentate coordination spheres.

which contain one unique chloride trans to the diamine bridge, and 2,2/c,c for the bidentate III and IV, which contain two cis chlorides on each platinum atom. The complexes were synthesized by the published routes.[2,6] The 1,1/t,t complexes were studied as the chloride complexes because of their suitable water solubility. The 2,2 complexes were studied as their aqua complexes by treatment of the intermediate iodo complex [{*cis*-Pt$I_2(NH_3)$}$_2$-(diamine)] with a stoichiometric amount of $AgNO_3$, as previously reported.[6] The reactions were studied by ^{1}H and ^{195}Pt NMR spectroscopy, and the parameters for the final products are given in Table I. The reactions were performed in unbuffered solution because use of phosphate buffer is inadvisable due to reactivity of phosphate with Pt complexes.[7-9] Use of non-phosphate-containing buffer such as PIPES gave immediate precipitates, especially with the aqua complexes, and their use was therefore not feasible. In all cases, the pH of the unbuffered reaction mixtures varied between 6.7 and 7.1 during the reaction, a very close approximation to physiological conditions. GMP was used as the disodium salt of the dianion



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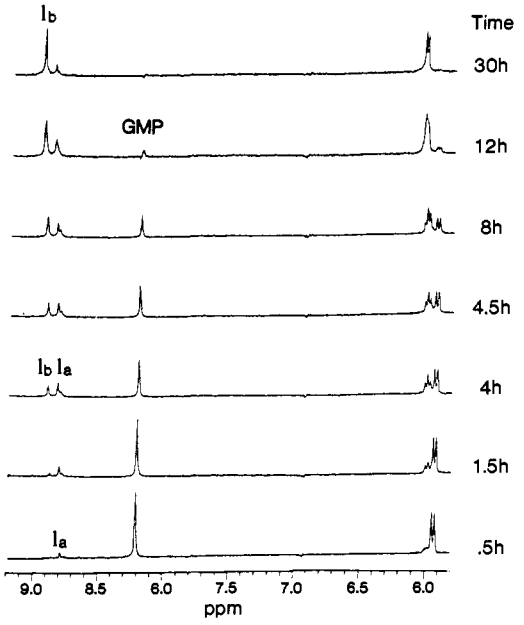

Figure 2. Reaction of [{*trans*-$PtCl(NH_3)_2$}$_2$($NH_2(CH_2)_4NH_2$)]Cl_2 (I) with GMP followed by ^1H NMR spectroscopy showing the changes in the H8 and H1′ chemical shifts. Conditions as per Experimental Section.

Reactions of 1,1/t,t Complexes with GMP. The combined ^1H and ^{195}Pt NMR spectra show the stepwise substitution of chloride by GMP with formation of the asymmetric intermediate [{*trans*-$PtCl(NH_3)_2$}–(diamine)–{*trans*-$Pt(GMP)(NH_3)_2$}]$^+$ and conversion to the final product [{*trans*-$Pt(GMP)(NH_3)_2$}$_2$(diamine)]. Note that the designation of total charges on these complexes follows the formulation of Miller and Marzilli,[10] allowing a formal 2– charge for each GMP. In both cases, the H8 peaks of bound GMP are shifted slightly downfield during the course of the reaction. These minor changes (<0.1 ppm) in the chemical shifts are probably due to the altered concentrations of each species as the reaction proceeds and the change in pH during the reaction.

Complex I, [{*trans*-$PtCl(NH_3)_2$}$_2$($H_2N(CH_2)_4NH_2$)]Cl_2. The ^1H NMR spectra of the reaction of I with GMP is shown in Figure 2. As the reaction proceeds with GMP binding to platinum, a signal ($H8_a$) first appears at 8.80 ppm, and later on another signal ($H8_b$) arises at 8.87 ppm. As the reaction proceeds, the signal of free GMP at 8.20 ppm decreases in intensity, and both new signals at first increase. The approximate half-life for disappearance of GMP, calculated when integration of free GMP is equal to integration of all Pt–GMP peaks, is 4.5 h. After the half-life of the reaction, the peak $H8_b$ still grows, but peak $H8_a$ mostly disappears. At intermediate times, a slight shoulder appears on the $H8_a$ peak. The final pH of the reaction in unbuffered solution was 7.1 from an initial value of 6.8, and the chemical shift values were 8.87 ppm for $H8_a$ and 8.95 ppm for $H8_b$.

The chemical shift of the sugar H1′ protons of the GMP product is shifted downfield from free GMP. During the reaction, the two sets of signals corresponding to the two species that give rise to the $H8_a$ and $H8_b$ signals are not clearly separated at the field strength employed, resulting in an apparent triplet. The final product shows only one pair of H1′ signals centered at 6.02 ppm (J = 4.4 Hz). Examination of Figure 2 shows that a small signal due to $H8_a$ still persists even after 30 h. Excess GMP added to the reaction mixture after 30 h results in complete disappearance of $H8_a$.

The ^{195}Pt chemical shift of complex I is at −2437 ppm.[6] When GMP is added, signal I_b emerges at −2568 ppm within 30 min, Figure 3. While the peak of complex I decreases in intensity, the signal designated as I_b grows in and no other peaks are observed. The chemical shifts are consistent with substitution of

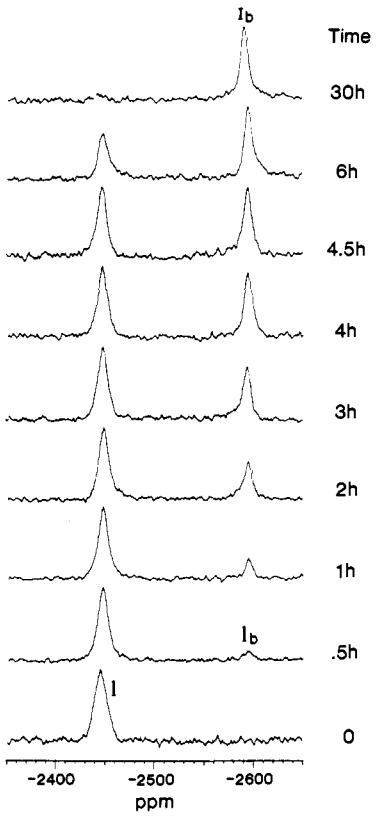

Figure 3. Reaction of [{*trans*-$PtCl(NH_3)_2$}$_2$($NH_2(Ch_2)_4NH_2$)]Cl_2 (I) with GMP followed by ^{195}Pt NMR spectroscopy. Conditions as per Experimental Section.

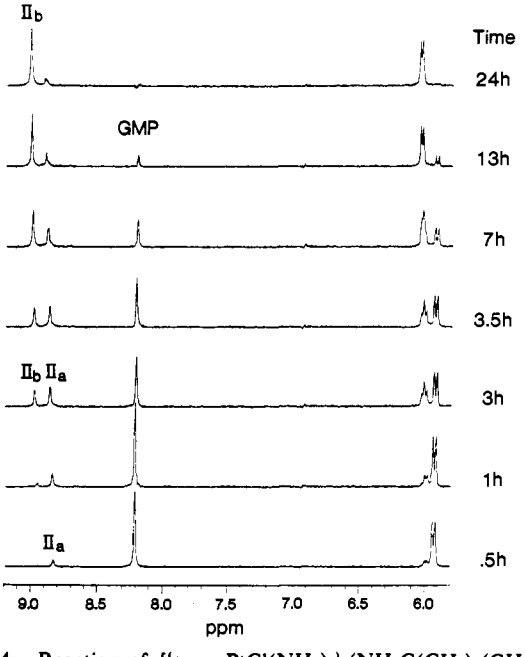

Figure 4. Reaction of [{*trans*-$PtCl(NH_3)_2$}$_2$($NH_2C(CH_3)_2(CH_2)_2C(CH_3)_2NH_2$)]$Cl_2$ (II) with GMP followed by ^1H NMR spectroscopy showing the changes in the H8 and H1′ chemical shifts. Conditions as per Experimental Section.

Cl by GMP[10] and formation of [{*trans*-$Pt(GMP)(NH_3)_2$}$_2$$NH_2(CH_2)_4NH_2$]. The approximate half-life of the reaction is $4^1/_2$ h, calculated when Pt signals are of equal intensity, and is consistent with the value found from the ^1H NMR spectra.

Complex II, [{*trans*-$PtCl(NH_3)_2$}$_2$($H_2NC(CH_3)_2(CH_2)C(CH_3)_2NH_2$)]$Cl_2$. The reaction of complex II with GMP, Figure 4, is similar to that of I. In the ^1H NMR spectrum, two new signals appear at 8.83 and 8.95 ppm at the beginning of the

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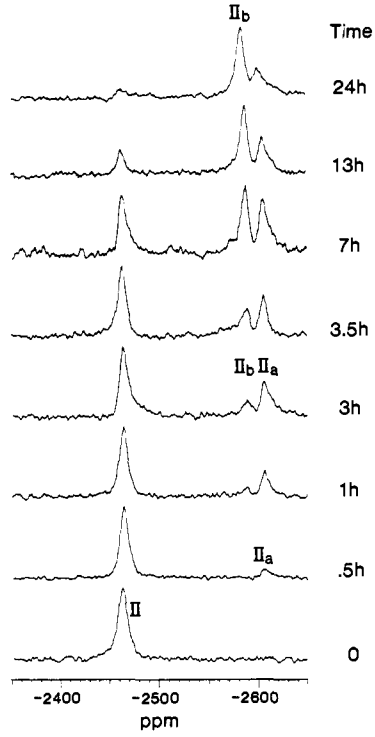


Figure 5. Reaction of [{*trans*-$PtCl(NH_3)_2$}$_2$($NH_2C(CH_3)_2(CH_2)_2C(CH_3)_2NH_2$)]$Cl_2$ (II) with GMP followed by ^{195}Pt NMR spectroscopy. Conditions as per Experimental Section.

reaction. The final pH of the reaction mixture was 7.0, and after 24 h the major peak appears at 9.01 ppm, with only a minor resonance at 8.90 ppm. The $t_{1/2}$ for disappearance of free GMP is 3.5 h and is thus faster than for complex I, and excess GMP drives the reaction to completion. The behavior of the H1′ protons is similar to that of I, the final spectrum showing the expected doublet centered at 6.04 ppm (J = 4.15 Hz).

Examination of the CH_3 region of the spectrum also shows the presence of three peaks during the reaction—corresponding to starting material and the two products. The final spectrum shows one signal at 1.39 ppm.

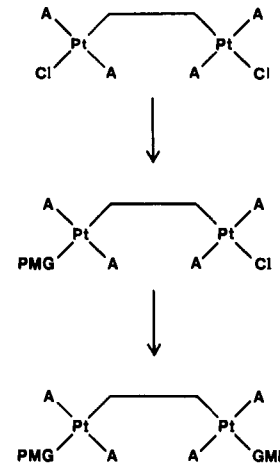
The ^{195}Pt chemical shift of complex II is at −2454 ppm. When GMP is added, signal II_a appears at −2595 ppm, and another signal II_b appears at −2580 ppm after 90 min, Figure 5. While the initial signal due to complex II is diminishing, both new peaks increase in intensity. As the reaction proceeds, both the signals of complex II and signal II_a decrease and only II_b increases. After 24 h, signal II_a still persists. The final chemical shift values are −2590 ppm (II_a) and −2573 ppm (II_b). The pattern of appearance and disappearance of these peaks follows very closely that found from the ^{1}H NMR study, with an approximate half-life for disappearance of GMP of $3^1/_2$ h.

Analysis of Spectra. The reaction of GMP with the monomer *cis*-$[PtCl_2(NH_3)_2]$ and its aqua derivative has been rigorously studied,[10,11] and our analysis follows closely the conclusions from this system. The most likely explanation of the appearance of two H8 peaks during the reaction is that we are observing the stepwise formation of the final product, Scheme I (charges omitted for clarity).

There are three different species—the original complex, the asymmetric intermediate [{*trans*-$PtCl(NH_3)_2$}-(diamine)-{*trans*-$Pt(GMP)(NH_3)_2$}]$^+$ (corresponding to I_a and II_a), and the final product [{*trans*-$Pt(GMP)(NH_3)_2$}$_2$(diamine)] (I_b and II_b).

In principle, restricted rotation of the GMP molecules around the backbone could give rise to two peaks in the ^{1}H NMR spectrum. Restricted rotation with guanosine or GMP is only observed however with bulky substituents on the amine nitrogens.[12,13] In symmetrical complexes such as I_b and II_b, the two coordination units act independently of each other and we would not expect to see the different conformers at room temperature. Further, the behavior is not that expected of conformational preference because the ratio of the two peaks is not constant. The binding of both GMP molecules is through N7, according to the pD behavior of the H8 resonances (see below). The possibility that different H8 chemical shifts are due to N7 and/or N1 binding is ruled out by the value of the shift and the absence of any peaks attributable to Pt–N1 species in the ^{195}Pt NMR spectrum.

Scheme I



The persistence of a small amount of the intermediates I_a and II_a is consistent with studies of the Cl^- dependence of *cis*-$[PtCl_2(NH_3)_2]$ with GMP.[11,14] In our case, the starting materials contained chloride as counter anion and during the reaction more Cl^- is produced upon displacement by the incoming nucleotide. This will tend to slow the reaction down; the fact that excess GMP drives the reaction to completion confirms the interpretation.

The ^{195}Pt chemical shifts of the products are consistent with a PtN_4 coordination sphere.[25] In the case of II, the observation of two peaks with PtN_4 coordination may be attributed to the slightly different environment of [{$Pt(GMP)(NH_3)_2$}($NH_2(C(CH_3)_2(CH_2)_2C(CH_3)_2NH_2)$R], where R = $PtCl(NH_3)_2$ (II_a) or $Pt(GMP)(NH_3)_2$ (II_b). These two groups will exert slightly different electronic effects that, together with the effect of the methyl groups, could allow for separation in this case. If this is correct, then there should also be two peaks observable in the $[PtN_3Cl]$ region, but only a possible shoulder is apparent. Quadrupolar relaxation results in the broad line width for ^{195}Pt NMR peaks when the donor atoms are ^{14}N.[15] The width at half-height of the peaks presented in Figures 2 and 4 are of the order of 600 Hz. The field strength employed in the present study may not be sufficient to resolve all possible species. Likewise, we do not observe any intermediates in the reaction of I.

A principal result of platination of mono- and oligonucleotides is to switch the sugar pucker from S-type (C2′-endo) to N-type (C3′-endo).[16] The value of $J_{H1'-H2'}$ is sensitive to this change.[17] The lower values of $J_{H1'-H2'}$ observed for the products I_b and II_b in comparison to free GMP indicate that the sugar conformation is altered in the direction of the N conformer. The chemical shift of the H1′ protons is also shifted slightly downfield, in agreement with the results obtained on complexes with only one GMP bound per Pt [Pt(GMP)(dien)][18] and *cis*-$[PtCl(GMP)(NH_3)_2]$.[10,11]

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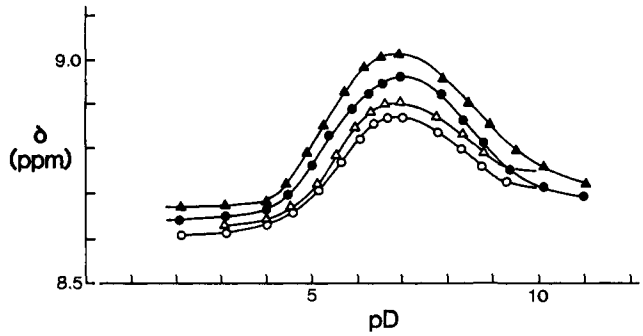

Figure 6. Variation in chemical shifts for the H8 protons versus pD for the products of complexes I and II with GMP: ○, I_a; ●, I_b; △, II_a; ▲, II_b. The species refer to those in Figures 2 and 4.

pD Dependence of Reaction Products of 1,1/t,t Complexes I and II with GMP. The chemical shift of the H8 proton of free GMP is affected by variation of pH. Platination of N7 removes this sensitivity. The pD dependence of the products of reactions of I and II with GMP are depicted in Figure 6. The titration curves for both complexes and all four H8 signals follow the same pattern, and all protons exhibit similar chemical shifts, indicating that they experience similar environments. The shape of the curve is also very similar to that of *cis*-$[Pt(NH_3)_2(GMP)_2]^{2-}$, furnishing further evidence that the coordination of GMP occurs through N7.[11] The chemical shift does not change significantly between pD 2.0 and 4.0, the region of N7 protonation (pK_a of N7 of free GMP = 2.4).[19,20] The major variations are downfield shifts occurring between pD 4.0 and 7.0 followed by upfield shifts from pD 7.0 to 10.0. These are the regions of phosphate deprotonation and N1 deprotonation, respectively.[11,14] The chemical shift changes to lower field from pD 4.0 to 7.0 are 0.3 ppm for $H8_{Ib}$, 0.33 ppm for $H8_{IIb}$, 0.23 ppm $H8_{Ia}$, and 0.25 ppm for $H8_{IIa}$.

Reactions of 2,2/c,c Complexes with GMP. Complexes containing bidentate coordination spheres [{*cis*-$PtCl_2(NH_3)$}$_2$(diamine)] are only sparingly soluble in water. As with cisplatin, their reactions with nucleotides are best studied by using the aqua species. In this case, however, the ^{1}H NMR spectra of the reaction mixtures using 4 equiv of GMP are very complicated and the intermediates corresponding to stepwise displacement are extremely difficult to distinguish. Indeed, one of the interesting features of the chemistry of symmetrical bis(platinum) complexes (i.e., containing equivalent coordination spheres) is their mechanism of substitution with small ligands. At least initially, the two platinum atoms are independent and have equal probability of reaction. Upon reaction of the first Pt and substitution of the first ligand, a combination of possible intermediate species arises, which may even give rise to more than one product. This aspect has been exemplified previously in the reaction of chloride ion with bis(platinum) tetraamines [{*cis*-$Pt(NH_3)_2(H_2N(CH_2)_nNH_2)$}$_2$]$Cl_4$.[1]

The parameters for the final products are given in Table I. The final pH of the solutions were 7.0 (complex III) and 7.1 (complex IV) and did not vary greatly from the starting samples. At this pH, the aqua species may form a variety of hydroxo-bridged complexes,[6] but this did not affect the expected outcome of the reactions. The complexity of the ^{1}H NMR spectra precludes detailed analysis. Selected ^{195}Pt NMR spectra, along with the ^{1}H NMR spectra of the final products, are shown in Figure 7. The combined spectra show that, for complex III, only the final product corresponding to [{*cis*-$Pt(GMP)_2(NH_3)$}$_2$(diamine)]$^{4-}$ is observed, whereas for complex IV with the sterically hindered diamine, an intermediate corresponding to one GMP bound to each Pt, [{$Pt(GMP)(H_2O)(NH_3)$}$_2$(diamine)], is clearly seen. We will discuss the ^{195}Pt NMR results for each of these compounds.

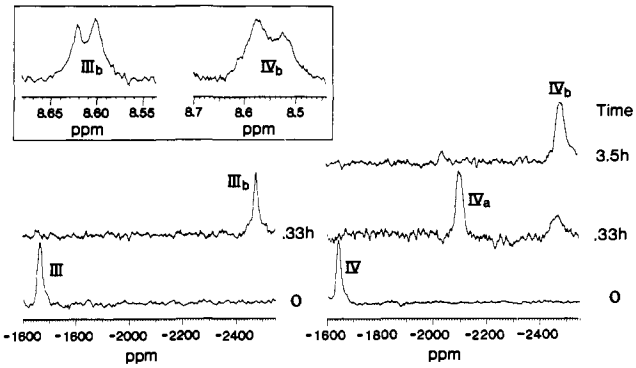

Figure 7. Reactions of [{*cis*-$Pt(H_2O)_2(NH_3)$}$_2$($NH_2(CH_2)_4NH_2$)]$^{4+}$ (complex III) and [{*cis*-$Pt(H_2O)_2(NH_3)$}$_2$($NH_2C(CH_3)_2(CH_2)_2C(CH_3)_2NH_2$)]$^{4+}$ (complex IV) with GMP followed by ^{195}Pt NMR spectroscopy. Conditions as per Experimental Section. The inset shows the ^{1}H NMR spectra of the final products in each case corresponding to [{*cis*-$Pt(GMP)_2(NH_3)$}$_2$(diamine)] taken upon completion of the reaction at 30 °C.

Complex III, [{*cis*-$PtCl_2(NH_3)$}$_2$($H_2N(CH_2)_4NH_2$)]. The initial peak of the tetraaqua species at −1672 ppm rapidly disappears, and one new peak arises at −2473 ppm in the ^{195}Pt NMR spectrum, Figure 7. The corresponding value for *cis*-$[Pt(NH_3)_2(GMP)_2]^{2-}$ is −2455 ppm.[10] No other peaks are observed at any time during the reaction, which is complete within 20 min. While the purpose of this present study was not to examine detailed kinetic effects, the reaction does appear faster than for *cis*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$.[21,22] Comparison under strictly identical conditions is required, however, before further conclusions can be drawn.

The final spectrum of complex III shows two H8 resonances at 8.62 and 8.60 ppm corresponding to the inequivalent GMP ligands trans to NH_3 and trans to diamine NH_2 in the product [{*cis*-$Pt(GMP)_2(NH_3)$}$_2$(diamine)]. The H1′ region also shows a broad set of two doublets centered at 5.85 ppm. The H1′ protons are now shifted upfield as found for *cis*-$[Pt(GMP)_2(NH_3)_2]^{2-}$.[10,11]

Complex IV, [{*cis*-$PtCl_2(NH_3)$}$_2$($H_2NC(CH_3)_2(CH_2)_2C(CH_3)_2NH_2$)]. In contrast to III, when complex IV reacts with GMP, Figure 7, the initial peak of the starting material at −1660 ppm disappears rapidly within 20 min with the concomitant appearance of two new peaks at −2101 ppm (IV_a) and −2476 ppm (IV_b). In the initial stages of the reaction, the principal species is IV_a. After approximately 1 h, peak IV_a begins to decrease in intensity with a continued increase in intensity of IV_b. Minor peaks are also observed as the reaction proceeds further, possibly due to intermediates viii and ix (see Scheme II). The final product IV_b after 3.5 h gives the major signal at −2482 ppm.

The ^{1}H NMR spectra, although complicated, also confirm the trend of this reaction. In the early stages of the reaction, a set of peaks centered at 8.9 ppm appears. These gradually decrease in intensity while giving rise to a broad set centered at 8.55 ppm. The final spectrum (corresponding to IV_b of the ^{195}Pt NMR spectrum) taken at 3.5 h also shows the expected doublet, but in this case the peaks are broadened. Raising the temperature to 70 °C gives two sharp peaks at 8.58 and 8.53 ppm, indicating that there is some restricted rotation around the Pt–N7 bonds. This is consistent with previous observations when two GMP molecules are cis to a sterically hindered amine.[12,13] An interesting observation is that, at 70 °C, the intensities of the peaks are not equal; the upfield peak decreases in intensity. Heating in D_2O results in loss of the H8 peak through deuterium exchange.[23] The different environments of the two GMP molecules—one trans NH_3 and the other relatively more sterically crowded trans to the $H_2NC(CH_3)_2$ moiety of the bridging diamine—may result in selective deuteration. The H1′ protons give rise to a complicated

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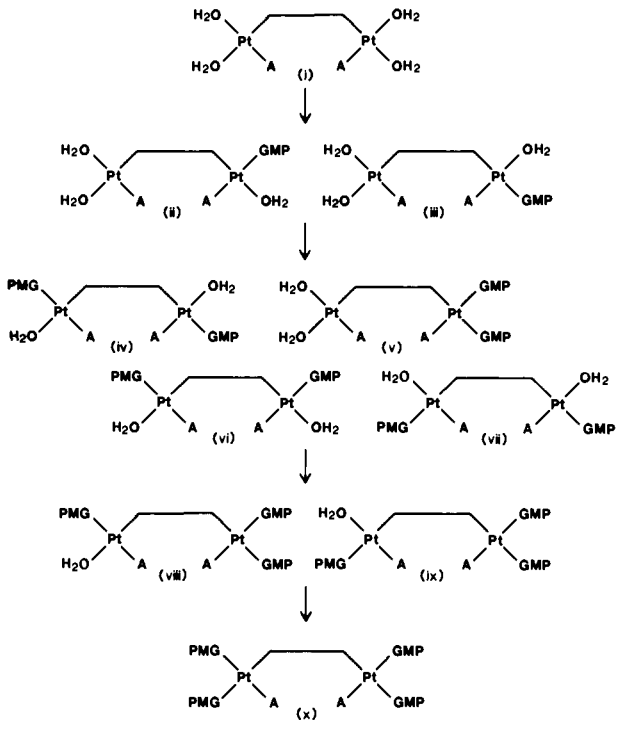
Scheme II



pattern at 5.9 ppm but upfield from free GMP.

The H8 chemical shifts in *cis*-$[Pt(H_2O)(GMP)(NH_3)_2]$ and [Pt(GMP)(dien)] are also approximately 0.2 ppm downfield from that of *cis*-$[Pt(GMP)_2(NH_3)_2]^{2-}$.[11,18] The first set of peaks that arise in the ^1H NMR spectrum of complex IV and GMP also show the approximate chemical shift of II_b, with only one GMP bound per platinum (see above and Table I). Thus, the values of the chemical shifts of both H8 and H1′ in the final reaction products of III and IV correspond to full substitution and formation of $[\{cis\text{-}Pt(GMP)_2(NH_3)\}_2(diamine)]^{4-}$.

Analysis of Spectra. In the present case, the overall reaction may be represented as in Scheme II (charges are omitted for clarity).

The number of isomers is obtained by considering that the first GMP can enter trans to NH_3 or trans to the diamine NH_2 (ii and iii). The second GMP can enter also on the unreacted Pt trans to NH_3 or trans to the diamine NH_2 (iv, vi, and vii) or on the same Pt as the first GMP (v). Reaction of the third molecule of GMP gives rise to the trisubstituted intermediates viii and ix. These considerations are important for understanding the details of bis(platinum)–DNA interactions (see also below). There exists a total of 10 species in solution if we consider only N7-bound GMP, independent of multiple forms arising from restricted rotation. Chelate formation by binding of both N7 and a phosphate oxygen of GMP has been observed, under conditions of excess Pt complex, in the study of 6-oxopurine mononucleotides with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$.[24] The stoichiometric reactions we carry out do not favor such chelate formation. In agreement with this argument, no peaks indicative of chelate formation were observed when the reactions of III and IV were monitored by ^{31}P NMR spectroscopy (see footnote *a*, Table I). Instead the final products gave peaks at −0.57 ppm (complex III) and −0.66 ppm (complex IV) from trimethyl phosphate as reference. For complex IV, the ^{31}P NMR spectra taken after 0.33 h showed two peaks at −0.18 and −0.66 ppm. No downfield peak indicative of chelate formation[24] was observed at any time. The discussion assumes here only the possibility of GMP binding through unique N7 sites.

The systematic ^{195}Pt NMR chemical shift differences are as expected for the displacement of H_2O by a N donor.[25] Thus, no intermediates are seen in the reaction of complex III, and the only species observed is the final product, species x of Scheme II, $[\{cis\text{-}Pt(GMP)_2(NH_3)\}_2(BN)]^{4-}$. In the case of complex IV, the intermediate species IV_a observed at early time points corresponds to species iv, vi, or vii where only one GMP is bound to each Pt in $[\{Pt(GMP)(H_2O)(NH_3)\}_2(diamine)]$. The ^1H NMR spectra confirm this interpretation. Thus, the presence of the Me groups on the diamine backbone slows down the attack of the second GMP on the monosubstituted $[\{Pt(GMP)(H_2O)(NH_3)\}_2(diamine)]$. This is in contrast to the 1,1/t,t case discussed above where complex II actually reacted at a slightly faster rate than complex I. Molecular models show that the approach of the second GMP is sterically hindered by the presence of both the CH_3 groups on the amine-bonded carbon and by the exocyclic C6=O group of the bound mononucleotide.

Relationship to DNA Binding and Antitumor Activity. Comparison of the DNA binding of monodentate and bidentate bis(platinum) complexes shows some intriguing differences.[2] Thus, for the 2,2/c,c bidentate complexes, interstrand cross-linking *decreases* with increasing r_b (or total Pt bound), whereas it *increases* with increasing r_b for the monodentate species.[2] Further, although the general features of the biological activity are similar, the trends are not the same. Particularly, there is a difference in activity across a panel of ovarian tumor cell lines between 1,1 and 2,2 complexes.[26] A principal goal of this work is to see if we can systematically design complexes with an altered spectrum of tumor specificity based on altered modes of DNA binding. The work summarized in this paper gives us some insights into the DNA binding of the two sets of complexes. In the case of reaction with GMP, which we may consider as a model for the initial reaction with DNA, we have seen that the nature of the diamine causes some difference between reactions of the 1,1/t,t (monodentate coordination) and 2,2/c,c (bidentate coordination) bis(platinum) complexes.

The stepwise displacement of the chlorides of bis(platinum) complexes I and II with GMP may be represented as

$$ClPt\text{–}PtCl \rightarrow (GMP)Pt\text{–}PtCl \rightarrow (GMP)Pt\text{–}Pt(GMP)$$

(see also Scheme I). The initial approach of a bis(platinum) complex to DNA must obviously be monodentate binding, presumably to a guanine N7 site. In the case of the 1,1/t,t (both Pt units monodentate) complexes, the next step may involve a base on the opposite strand (interstrand cross-link formation) or the same strand (bis(Pt) intrastrand cross-link formation). The present studies cannot distinguish between the likelihood of these possibilities. The kinetics of the second step will, in principle, be affected by steric effects in the diamine backbone, even though the affinity of each individual platinum atom for a DNA base (or indeed any nucleophile) is identical. Assuming equivalent reactivity in the formation of the first platinum–purine bond, sequence specificity may arise from at least two factors. Upon formation of the first purine bond, there is still the possibility for rotation of the (DNA-bound) platinum coordination sphere around the platinum–purine bond and the steric effects of the backbone may be more important in dicating how and where the second platinum–purine (or pyrimidine) bond, and thus the interstrand cross-link, forms.

In the stepwise formation of a fully purinated 2,2/c,c complex, the first two steps of Scheme II are the most important. The formation of the first platinum–purine (or pyrimidine) bond sets up a competition between intrastrand cross-link formation through further reaction of the initially bound platinum and interstrand cross-link formation by complexation of the second "free" platinum atom, Figure 8. The observation of intermediate species in the reaction of complex IV implies that steric effects of the backbone can favor bis(Pt) interstrand cross-linking over the intrastrand cross-linking of one individual platinum atom. The reactive bonds (aqua or chloro) on each platinum atom may then react further

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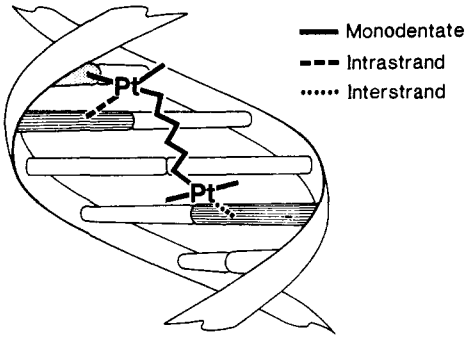

Figure 8. Possible pathways of reaction of 2,2/c,c bis(platinum) complexes with DNA leading to an interstrand (Pt,Pt) or intrastrand cross-link (one Pt only).

to give the limiting tetrafunctional structure. If the second reaction is closing of the intrastrand cross-link, the intermediate species formed now contains one platinum bound in a bidentate manner to a relatively rigid 17-membered dinucleotide chelate.[27] This situation fixes the first platinum coordination sphere, as there is no rotation possible around the platinum-purine bonds. Only rotation around the C-C bonds of the diamine backbone can place the second platinum atom in a favorable position for bonding and thus interstrand cross-link formation.

These considerations may help to explain the relative DNA binding and antitumor activity of the two structurally different sets of bis(platinum) complexes.[2] In 2,2/c,c species, cisplatin-like activity will ensue when intrastrand adducts are formed. The relative degree of other novel adducts ("non-cisplatin") may dictate how different the activity will be in comparison to cisplatin. This difference is thus marked for the 1,1/t,t complex although the presence of a 2+ charged species may not be advantageous in a pharmacological sense because of the expected reduced cellular uptake. We note further that the formation of the interstrand cross-link will also be affected by geometry; the 1,1/t,t complex is only one of three possible isomers for bis(platinum) complexes with monodentate coordination spheres. Current synthetic efforts are aimed at developing routes to the other possible isomers.

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Design of Discriminating Hosts for Noble Metal Ions with Double Functions of Thia and Amide Donors in Macrocyclic Structures

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Abstract: A novel tetradentate, dithia diamide **9** (6,6-dimethyl-5,7-dioxo-1,11-dithia-4,8-diazacyclotetradecane, "dioxo[14]aneN_2S_2") and a pentadentate, trithia diamide macrocyclic ligand **10** (12,12-dimethyl-11,13-dioxo-1,4,7-trithia-10,14-diazacyclohexadecane, "dioxo[16]aneN_2S_3") have been synthesized and their ligand properties examined. They smoothly encapsulate only divalent noble metal ions Pt^{II} (to **17** and **19**, respectively) and Pd^{II} (to **18** and **20**, respectively) but not other typical transition-metal ions, Cu^{II}, Ni^{II}, or Co^{II}. Moreover, **9** and **10** can effectively remove Pt^{II} from *cis*-$[Pt^{II}(NH_3)_2Cl_2]$ to yield Pt^{II}-in complexes **17** and **19**, respectively. Pt^{II} complex **19** possesses a four-coordinated, square-planar geometry with $(N^-)_2S_2$ donors (N^- denotes a deprotonated amide anion), where the central S(4) atom is not coordinated, as shown by the X-ray crystal structure resolved by the heavy-atom method with 2543 unique reflections with $|F_o| > 4\sigma(F_o)$. Final R and R_w were 0.040 and 0.060, respectively: monoclinic, space group $P2_1/c$ with a = 11.753 (6) Å, b = 9.574 (3) Å, and c = 19.183 (9) Å, β = 126.78 (3)°, and V = 1729 (1) Å^3; ρ_c = 2.096 g cm^{-3} for Z = 4, and formula weight 545.62. The cyclic voltammetry of **19** in dimethyl formamide (DMF) displays a 2e$^-$ oxidation at +0.81 V vs SCE ($Pt^{II} \rightarrow Pt^{IV}$) and a 2e$^-$ reduction at +0.32 V ($Pt^{IV} \rightarrow Pt^{II}$), implying that the Pt^{II} state is stabilized by the square-planar $(N^-)_2S_2$ coordination and that the electrochemically oxidized Pt^{IV} state requires additional axial S(4) and DMF donors for stabilization. The two amide anions in Pt^{II}-in complexes **17** and **19** are reversibly protonated to Pt^{II}-out complexes **27** and **28**. Treatment of **28** with an equimolar amount of **10** yields 2:1 macrocycle-Pt^{II} complex **29**. In **9** and **10** discriminating functions are endowed by the combination of the characteristic S donors and amide groups in the macrocyclic skeleton to concertedly work only on Pt^{II} and Pd^{II} ions.

Introduction

We have already introduced the amide-containing macrocyclic polyamines **1**[1-6] and **2**[7-9] as novel ligands having hybrid features of oligopeptides (e.g. triglycine **3**) and polyamines **4** and **5**, respectively. As with triglycine complexes **6**,[10] these macrocyclic ligands interact with several divalent transition-metal ions such

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