from one oxygen to another, there will be an almost instantaneous electric charge redistribution. Not only a proton or deuteron but also a negative charge undergoes quasi-rotation, and the resulting pulse of electron current through the ion may generate an electromagnetic pulse capable of relaxing the nuclear spin. We call this mechanism quasi-spin rotation (QSR).

QSR may be analyzed by using concepts from FT NMR.[29] An electromagnetic pulse rotates the spin magnetization vector through an angle α;

$$\alpha = \gamma_p H_{x,y} \tau_w \tag{16}$$

where $H_{x,y}$ is the effective pulse amplitude (a field with x and y axis components) and τ_w is the pulse width. Our pulses have amplitude H and occur at random orientations to the external field. Since H_z components are ineffective

$$H_{x,y}^2 = H_x^2 + H_y^2 = (2/3)H^2 \tag{17}$$

The fractional change in z-axis magnetization induced by a weak pulse is given by (for small α)

$$\Delta M_z/M_z = 1 - \cos\alpha \simeq (1/2)\alpha^2 \tag{18}$$

Equation 18, applied to FT NMR, describes a change in bulk magnetization involving an entire spin ensemble. In our mechanism, each pulse interacts with only one spin, and eq 18 describes the probability that a single QSR pulse relaxes that spin. The QSR relaxation rate from eq 16, 17, and 18 is

$$R_{QSR} = (\Delta M_z/M_z)/\Delta t = (1/3)\gamma_p^2 H^2 \tau_w^2 \tau_r^{-1} \tag{19}$$

where τ_r may be identified as the quasi-rotation jump time of eq 15.

The following order of magnitude calculation indicates that eq 19 can account for the observed rate. Assume that when a proton quasi-rotates, the pulse generates a field equivalent to one Bohr magneton, β, at a distance of 1 Å from the ^{31}P nucleus. Then $H^2 = \beta^2/(1\ \text{Å})^6 \simeq 10^8\ G^2$. Let the pulse width be the time required for a proton to jump between the H-bond double minima, or approximately the oscillation period of a proton in a hydrogen bond, $\tau_w = 10^{-14}$ s. If $\tau_r = 10^{-11}$ s, then eq 19 gives $R_{QSR} \simeq 3 \times 10^{-2}\ s^{-1}$, in agreement with our data for R_{RES}.

An interesting relationship is obtained by multiplying eq 19 and 15:

$$R_{CSA}R_{QSR} = (2/45)\omega_p^2(\Delta\sigma)^2\gamma_p^2H^2\tau_w^2 \tag{20}$$

The temperature-dependent term, τ_r, is canceled. We have tested eq 20 with our data, using $R_{RES} = R_{QSR}$. Unfortunately, our data are not ideally suited for this purpose. At high temperatures, where R_{RES} is accurately known, R_{CSA} is quite uncertain; at low temperatures the reverse is true. Nevertheless, the following averages result from using data over the most reliable range (10–50 °C). At pH 10.3 and 145.75 MHz, $R_{CSA}R_{RES} \times 10^4 = 3.5 \pm 0.5$ in D_2O and 3.3 ± 0.7 in 20% D_2O, and the activation energies in both solvents were zero within experimental error (0.1 ± 0.6 and 0.3 ± 1.0 kcal/mol). At pH 2 the average combined rates ($\times 10^4$) were 5.5 ± 1.2 in D_2O and 7.0 ± 1.4 in 20% D_2O, with identical activation energies (-0.8 ± 1.0 kcal/mol) in both solvents.

The results above confirm our expectation that temperature effects are small or absent in eq 20. There is also no significant solvent isotope effect, even though it is quite important in both R_{CSA} and R_{QSR} alone. Solvent isotope effects on E^*_{RES} and E^*_{CSA} enter through τ_r which includes a major contribution due to tunneling in 20% D_2O but not in 100% D_2O. Equation 20 will be insensitive to isotope effects if the pulse angle α depends only on the quantity of charge shifted during a pulse; i.e., if the isotope effects on H^2 and τ_w^2 are opposite and cancel. This leaves $\Delta\sigma$ as the only real variable in eq 20. We interpret pH effects on ($R_{CSA}R_{RES}$) to show that $\Delta\sigma$ varies as the phosphate ion is protonated. $\Delta\sigma$ is greater for $H_2PO_4^-$ (at pH 2) than for HPO_4^{2-} (at pH 10.3).

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Interaction of *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ with Ribose Dinucleoside Monophosphates[1]

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Abstract: The reactions of five dinucleoside monophosphates (IpI, GpG, ApA, GpC, and ApC) with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$, with both reactants ca. 4×10^{-4} M in water (pH 5.5), have been studied. Sephadex chromatography and LC, ^1H NMR, and CD analyses have been used to characterize the complexes formed. The three homodinucleotides have a geometry leading to N(7)–N(7) chelation of the metal. IpI and GpG give a single N(7)–N(7) chelated complex while ApA also gives other products due to competitive N(1) binding to the metal. GpC and ApC lead to mixtures of several complexes and in both cases cytosine appears to have more affinity for the platinum than do guanine and adenine. In the case of GpC one of the complexes appears as an N(7)G–N(3)C platinum chelate, the CD of which supports a left-handed helical arrangement of the bases. As far as the perturbation of the DNA structure upon binding of the *cis*-$(NH_3)_2Pt^{II}$ moiety is concerned, these results bring further support to the hypothesis of intrastrand cross-linking of adjacent guanines occurring after a local denaturation or premelting of the DNA. They also suggest that cross-linking of adjacent guanine and cytosine could occur for a left-handed sequence of the polynucleotide.

The mechanism of action of the antitumor *cis*-dichlorodiammineplatinum(II) complex[3] is still the subject of active investigations.[4,5] There is much evidence which suggests that DNA is the primary target of the platinum(II) drug.[6-9] Several studies

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with DNA show that platinum binding is related to the % (G + C) content and indicate preferential binding to guanine[10-15] followed by adenine.[11,16] In vivo the active form of the *cis*-dichloro complex could be the species of the aquated complex *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ corresponding to the pH of the medium.[16-19] On the nucleoside level, the diaquo complex reacts only with Guo, Ado, and Cyd.[18,19] The corresponding apparent formation constants for the 1:1 complexes have almost the same value (log K = 3.7, 3.6, and 3.5, respectively).[19] Kinetic studies reveal a higher reaction rate for Guo than for Ado and Cyd.[20] The order of nucleophilicity toward the diaquo or the dichloro complex has been recently reported to be GMP > AMP ≫ CMP.[16] However, it has been shown, in the case of CpG reacting with $Pt(en)Cl_2$, that platinum is bound to cytosine first and then to guanine.[21] Spectroscopic studies, including ^1H NMR,[22-26] UV,[18] and Raman,[25,26] of several platinum complexes of nucleosides and nucleotides show that the main binding sites on the bases are N(7) and N(1) for adenine, N(7) for guanine, and N(3) for cytosine. Crystal structures have been reported for *cis*-bis-Guo,[27,28] *cis*-bis(5'-IMP),[29,30] 9-methyladenine[31] -platinum(II) complexes, and a *cis*-bis(3'-CMP) complex,[32] which, respectively, display N(7) or N(3) but no bidendate coordination.

Three binding modes have been considered in order to explain the strong perturbation of the DNA structure by the platinum bifunctional complexes. First, the occurrence of interstrand cross-linking has been demonstrated and measured in vitro and in vivo. However, such cross-links do not appear to be the most important cytotoxic event.[8,33,34] Second, intrastrand cross-linking is supported by the X-ray structures of bisnucleoside and -nucleotide cis complexes,[27,30] by the spectral evidence for the favored formation of such bis complexes in solution with Ino[25] and 5'-GMP,[26] and by the inhibition of 9-aminoacridine intercalation in DNA after binding of the *cis*-$(NH_3)_2Pt^{II}$ moiety.[35] Third, a cis-bidentate platinum fixation to the N(7) and O(6) sites of a guanine[12,13,26,29,36,37] could lead to the dissociation of G-C base pair hydrogen bonds, this local denaturation allowing further cross-linking initially unlikely with native DNA.[38]

The present study is concerned with the binding of the *cis*-$(NH_3)_2Pt^{II}$ moiety to several dinucleoside monophosphates. It had been initially proposed, from CD results, that this reaction with ApA and ApC formed interbase links within the dinucleotides. Conformational considerations and pH data first suggested the two 6-NH_2 groups of ApA and adenine N(1) and cytosine-4-NH_2 of ApC as the chelating sites.[39] However, the Ado amino groups being poor ligands of heavy metals, it has been later postulated that the cross-linking should involve the N(7) and/or N(1) positions of two adjacent stacked bases.[25] We have recently shown that IpI reacts with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ to give a single product which is the N(7)-N(7) cross-linked dinucleotide complex $[Pt(NH_3)_2(IpI)]^+$.[40] We now report the results of our study of the reaction between *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and three homodinucleotides with purine bases, IpI, GpG, and ApA, and two heterodinucleotides with a purine and a pyrimidine base, GpC and ApC.

Experimental Section

GpG and ApC ammonium salts and IpI, ApA, and GpC free acids were from Sigma Ltd. ApA ammonium salt was from Boehringer. The purity of the dinucleotides has been checked by LC reverse phase analysis on a Waters Associates C18 micro-Bondapak column.

cis-$[Pt(NH_3)_2Cl_2]$ was prepared according to ref 41. The solution of *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ was freshly prepared from the dichloro complex as in ref 19 ($C = 5 \times 10^{-2}$ M).

The stoichiometric reactions (1 Pt per dinucleotide) were run at ca. 10^{-4} M concentrations (determined by UV absorption), with ca. 3 mg of dinucleotide per experiment, in doubly distilled water. The solution of the diaquo complex was added with a Hamilton syringe. The pH values of the solutions were checked at the beginning of the reaction and found to be in the range 5-5.5. In these conditions the diaquo and monoaquo monohydroxy species are predominant ($pK_1 = 5.51$ and $pK_2 = 7.37$ for the diaquo complex) and there is no significant dimerization of the latter.[18] The solutions were stoppered and kept in the dark at 37 °C for 70-90 h (137 h when *cis*-$[Pt(NH_3)_2Cl_2]$ was used). The crude reaction mixture was treated with a saturated KCl solution, in order to eliminate any aquo-Pt species, and lyophilized. The stability of the KCl-treated solutions has been checked by UV spectroscopy; no change in the absorption was noticeable after 2 weeks (up to 6 weeks in the case of ApA).

(1) Abbreviations used: in oligonucleotides A, C, G, and I represent adenosine, cytidine, guanosine, and inosine, respectively, and p to the left of a nucleoside symbol indicates a 5'-phosphate and to the right it indicates a 3'-phosphate. The dinucleoside monophosphates IpI, GpG, ApA, GpC, and ApC will be occasionally referred to as dinucleotides. Ado = adenosine; Cyd = cytidine; Guo = guanosine; Ino = inosine; 5'-GMP = 5'-guanosine monophosphate; 5'-IMP = 5'-inosine monophosphate, etc. LC = high-pressure liquid chromatography; NMR = nuclear magnetic resonance; s = singlet; d = doublet; CD = circular dichroism; en = ethylenediamine; dien = diethylenetriamine.

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The G-25 and G-10 Sephadex chromatography was monitored at 254 nm with an LKB analyzer. The G-25 Sephadex chromatography was performed with a 100 × 1.6 cm column, equilibrated and eluted with a 5×10^{-2} M KCl solution. The dead volume of the column was 76 mL and 8.4-mL fractions were collected. Those corresponding to the peak of the dinucleotide complex(es) were joined and lyophilized and the product was submitted to G-10 Sephadex chromatography (fractionation range 0–700) with a 60 × 2 cm column eluted with doubly distilled water. The desalted fractions were lyophilized, and the product was dissolved in D_2O and lyophilized twice in this solvent for ^1H NMR analysis. In each case the ^1H NMR spectrum of the Sephadex-isolated product has been compared to that of the KCl-treated crude reaction mixture after lyophilization in D_2O.

LC analyses were performed on a Waters Associates liquid chromatograph, with 254-nm detection, on a Waters C18 micro-Bondapak (reverse phase) column, using an aqueous $CH_3CO_2NH_4$ solution (10^{-2} M) as eluant A and $CH_3CO_2NH_4$ (10^{-2} M) in H_2O/CH_3OH (1:1) as eluant B at pH 7.3. The preparative separations were performed with the same column and eluant conditions except in the case of the GpC reaction (pH 6).

^1H NMR spectra have been recorded either on a Bruker WH 270 spectrometer (270 MHz, BNC 28 computer) or a Cameca TSN 250 (250 MHz, Nicolet 1180 computer) using standard Fourier transform techniques. The sweep width was 4000 (WH 270) or 3000 Hz (TSN 250) and the acquisition time ~1 s (8K data points). The D_2O used was 99.95% deuterated. In the case of spectra recorded in D_2O, the residual HDO signal was saturated by decoupling at the HDO resonance frequency in order to improve the dynamic range of the experiment. The concentrations of the samples were 5×10^{-3} to $\sim 10^{-3}$ M. For the low-concentration ones, block averaging techniques were used and the resolution of the input ADC was fixed at 12 bits. In the case of spectra recorded in H_2O, a special procedure was used on the Cameca TSN 250 spectrometer. Excitation of the sample was performed using one 333-μs pulse immediately followed by another 333-μs pulse with inverted phase. A 30-dB attenuator was inserted at the output of the rf amplifier. It can be shown that the power spectrum of this excitation sequence displays a large zero area at 3000 Hz of the carrier. Adjusting the carrier frequency at 3000 Hz from the H_2O resonance led to an attenuation by a factor of ~1000 of the H_2O signal, with low distortion of the spectrum in the 10–6-ppm region. This technique is based upon published experiments[42,43] and more details will be given elsewhere. Probe temperature was ca. 17 °C. Chemical shifts are referred to DSS (4,4-dimethyl-4-silapentanesulfonic acid sodium salt) used as internal reference. The pH of the ^1H NMR samples was monitored with an Ingold 405-M_3 (150 mm) microelectrode directly in the NMR tube, using a Metrohm E 532 digital pH meter. The pD values of the D_2O solutions were calculated according to pD = pH (meter reading) + 0.40.[44] The pD variations were obtained by addition of small aliquots of D_2O solutions of DCl or NaOD.

The CD measurements have been performed on a Jobin Yvon Mark III dichrograph, in the 350–220-nm region, at room temperature. The dinucleotides were ca. 10^{-4} M in 0.05 M NaCl solutions. The molar ellipticities $[\theta]$ are given per nucleotide residue; the molar extinction coefficients necessary to calculate the $[\theta]$ values were determined from the ratio of the optical densities of the dinucleotide solutions before and after incubation with the Pt(II) complex assuming that no change in concentration occurs during the incubation. When the reaction product is a mixture, only a mean extinction coefficient is obtained by this procedure; this mean value has been used for the different fractions of the mixture which have been separated by LC.

Results

Reaction of IpI with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$. The stoichiometric reaction of the diaquo complex with IpI (free acid), 4.2×10^{-4} M in water (pH 5.5) at 37 °C for 90 h, gives a solution with a red-shifted λ_{max} at 253 nm compared to 248 nm for the IpI initial solution and a ratio of optical densities (IpI + Pt):IpI = 0.89. After KCl treatment, Sephadex chromatography and LC analysis show that there is no unreacted IpI and only one monomeric product (>95%).

The ^1H NMR spectrum of free IpI, 5×10^{-3} M in D_2O at pD 5.5, gives the following signals (δ ppm, DSS): H(8)-3′, s, 8.35; H(8)-5′, s, 8.43; H(2)-3′ and 5′, two very close resonances at 8.16; H(1′)-3′, d, 5.82 and -5′, d, 5.92 ($J \sim 3$ Hz) assigned according to published data.[45] Figure 1 presents four ^1H NMR spectra of the isolated product, later mentioned IpI[Pt], at different pDs. At pD 5.5 the respective integrations of the signals at 9.06, 8.70, 8.26, 6.26, and 6.04 ($J \sim 6.5$ Hz) ppm are in the ratio 1:1:2:1:1. The two low-field signals can be completely removed at pD 7.5 by deuterium exchange with D_2O (24 h at 37 °C; no exchange is observed for IpI in the same conditions). Therefore they can be assigned to two H(8) protons in α position to coordinated N(7).[22,25] The signals at 8.28 and 8.23 ppm are attributed to the H(2) protons[22,25] and those at 6.26 and 6.04 ppm to the two riboses H(1′). Acidification of the pD 11.5 solution regenerates the corresponding H(2) and H(1′) signals. The chemical-shift difference between the 9.06- and 8.70-ppm signals shows that the two H(8) protons (initially at 8.35 and 8.43 ppm) experience different environments in the complex. Their resonances should be shifted downfield by platinum coordination, a shift reported to be of the order of 0.60 ppm.[25] Therefore one of them is also shifted upfield probably in part by the ring current diamagnetic anisotropy of the other purine ring. Such an upfield shift of 0.13 ppm has already been invoked to explain the H(8) chemical shift difference between mono- and *cis*-bis-Ino complexes.[25] For the

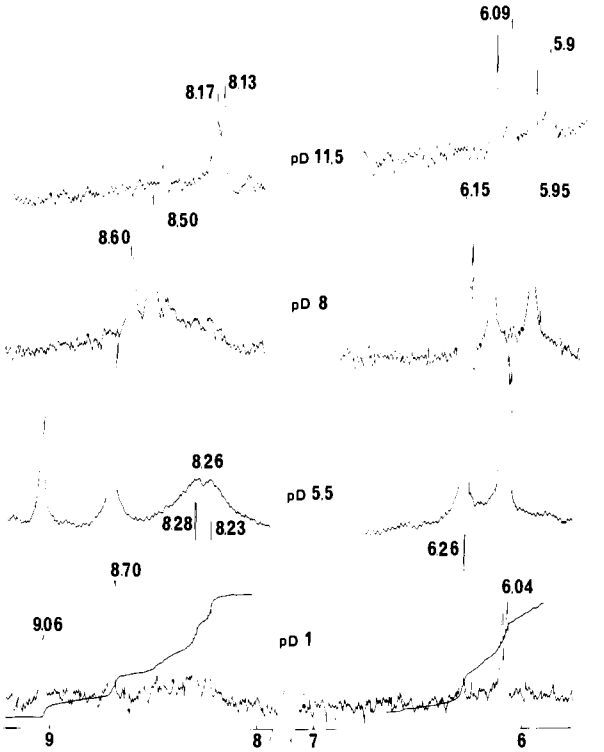

Figure 1. ^1H NMR spectra (270 MHz, D_2O, DSS, 17 °C) of the IpI[Pt] complex at various pDs.

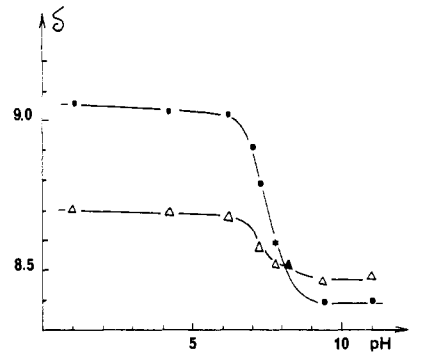

Figure 2. Variation of the chemical shifts of the H(8) protons vs. pH for a 10^{-2} M solution of the IpI[Pt] complex.

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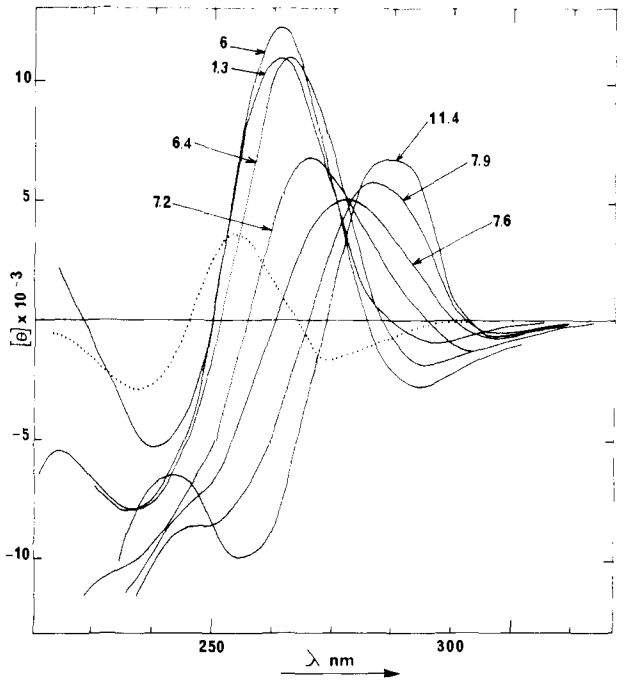

Figure 3. CD spectra of the IpI[Pt] complex 1.3×10^{-4} M, NaCl 0.1 M, 25 °C, at different pHs: (—) between 1.3 and 11.4; (···) IpI 1.2×10^{-4} M, NaCl 0.1 M, pH 4.7.

two complexes *cis*-$[Pt(NH_3)_2(Ino)_2]Cl_2$ and $[Pt(en)(Ino)_2]Cl_2$ respective overall downfield shifts of 0.81 and 0.50 ppm vs. Ino have been reported.[22] The H(2) 8.28- and 8.23-ppm signals (initially at 8.16 ppm) have experienced the expected small downfield shift due to N(7) coordination (0.03–0.1 ppm).[22,25]

To get a better understanding of the influence of the pH, we have studied the evolution of the ^1H NMR spectrum of IpI[Pt] in H_2O from pH 1 to 10.9. The effect of pH on the chemical shifts of the two H(8) protons is depicted in Figure 2. The assignment of the signals above pH 8.1 is tentative and based on the apparent best profiles of the curves of δ vs. pH. The pK_a of the apparent acid involved is in the vicinity of 7.5 (H_2O). However, the curves do not represent a single process, and several equilibria including deprotonation of one and of the other inosine could be involved.[46] In this pH region the phosphate deprotonation ($pK_a \sim 1$) should not interfere.

The CD spectra of the IpI[Pt] complex at different pHs are presented in Figure 3. The spectrum at pH 6 shows a positive maximum red shifted from 253 to 262 nm and a three times larger intensity when compared to that of the free dinucleotide at pH 4.7. Between pH 6.4 and 8, the CD spectra present a pseudo-isoelliptic point at 272 nm. We have checked the influence of possible intermolecular interactions and found that there is no change of $[\theta]$ at 262 nm for the IpI[Pt] complex from 9×10^{-4} to 3×10^{-5} M concentrations in NaCl (0.05 M) at pH 5.20.

Reaction of GpG with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$. The stoichiometric reaction of the diaquo complex with GpG (ammonium salt), 4.2×10^{-4} and 3×10^{-4} M in water (pH 5.5) at 37 °C, for 96 and 72 h gives a solution with a red-shifted λ_{max} at 261 nm compared to 253 nm for the GpG initial solution and a ratio of optical densities (GpG + Pt):GpG = 0.87. The chromatographic analyses show that there is no unreacted GpG and that the crude product contains more than 95% of a single monomeric species, GpG[Pt].

The ^1H NMR spectrum of free GpG, 5×10^{-3} M in D_2O at pH 5.6, gives the following signals (δ ppm, DSS): H(8), two s, 7.97 and 7.90; H(1′), two d, 5.85 (J = 5 Hz) and 5.76 (J = 3 Hz).[47] The ^1H NMR spectrum of the GpG[Pt] complex at pD 5.5 exhibits two singlets at 8.54 and 8.32 ppm, one singlet at 6.06 ppm, and one doublet at 5.90 ppm (J = 7 Hz). Their respective integrations are in the ratio 1:1:1:1. The two low-field signals can be completely removed by deuterium exchange with D_2O at pD 11.3 (18 h at 37 °C). Therefore they can be assigned to two H(8) protons in α position to N(7) coordinated to platinum.[26] The two other signals are attributed to the ribose H(1′) protons. The assignment of the H(8) signals (initially at 7.97 and 7.90 ppm) is in agreement with the 0.4–0.7-ppm overall downfield shifts reported for several 5′-GMP mono or bis complexes.[22,24,26] It is noteworthy that the H(8) exchange in D_2O requires a higher pH than in the case of the IpI[Pt] complex. It has already been reported that this exchange is considerably slower for the bis-(5′-GMP) than for the bis-Ino complex.[26] The influence of pH on the ^1H NMR spectrum of GpG[Pt] has been studied in D_2O because of the slow exchange of the H(8) protons. It is very similar to that observed for IpI[Pt]; however, the apparent monotitration profile is not as clear-cut. The pK_a of the apparent acid involved is of the order of 8.5 (D_2O).

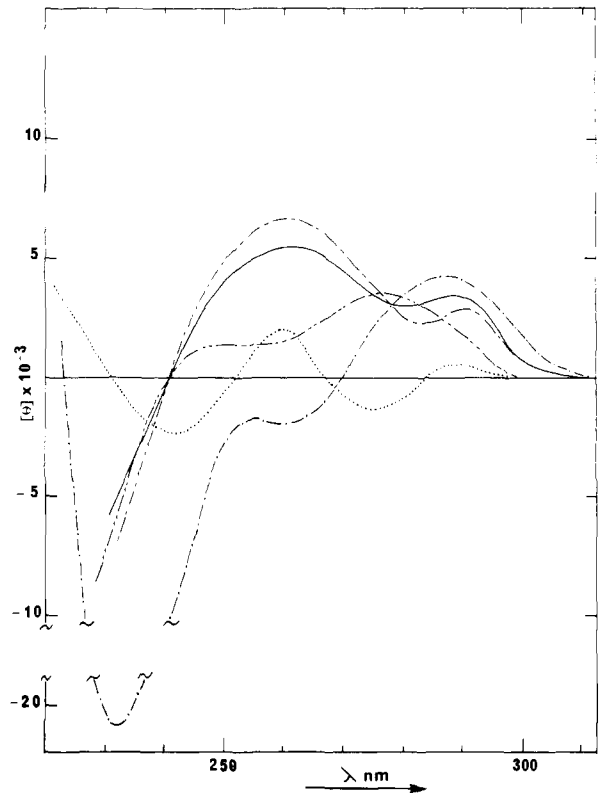

Figure 4. CD spectra of the GpG[Pt] complex 10^{-4} M, NaCl 0.05 M, 25 °C, at different pHs: (—) 7.1; (- - -) 2.5; (-···-) 8.1; (-·-) 10.9; (···) GpG 10^{-4} M, NaCl 0.05 M, pH 7.1.

The CD spectra of the GpG[Pt] complex and of free GpG at pH 7.1 (Figure 4) exhibit important differences. They both present a positive band at 290 nm which is enhanced in the complex. But the latter shows another large positive band at 262 nm instead of the bisignated curve, with a minimum at 275 nm, present in free GpG. The evolution of the CD spectrum of GpG[Pt] with pH presents some analogy with that observed for IpI[Pt] (Figure 3) with two types of curves at acidic and basic pH.

Reaction of ApA with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$. The stoichiometric reaction of the diaquo complex with ApA (free acid and ammonium salt), 3.8×10^{-4} and 5×10^{-4} M in water (pH 5.5) at 37 °C, for 70 and 90 h gives a solution with a red-shifted λ_{max} at 264 nm compared to 258 nm for the ApA initial solution, and a ratio of optical densities (ApA + Pt):ApA = 0.83. Sephadex chromatography shows that there is no unreacted ApA and that the monomeric product gives a rather broad, unsymmetrical peak (preceded and followed by two small ones, each ~5% of the mixture, which have not been further investigated). The reaction run with *cis*-$[Pt(NH_3)_2Cl_2]$, 5×10^{-4} M in water (pH 5.5) at 37 °C, for 137 h gives a similar G-25 chromatogram but unreacted ApA is still present. LC analysis of the monomeric product reveals

(46) N. Ogasawara and Y. Inoue, *J. Am. Chem. Soc.*, **98**, 7048 (1976).

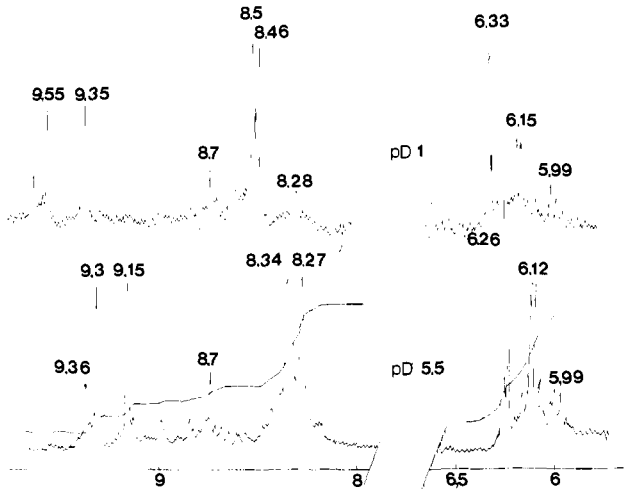

Figure 5. ^1H NMR spectra (270 MHz, D_2O, DSS, 17 °C) of the mixture of complexes (at pD 5.5 and 1) from the stoichiometric reaction between ApA and *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$.

a complex mixture with two major peaks (~70% of the mixture and in a ca. 75:25 ratio). The product from the main peak has been isolated by preparative LC.

The ^1H NMR spectrum of free ApA, 5.7×10^{-3} M in D_2O at pD 6 (20 °C), gives the following signals (δ ppm, DSS): H(8)-3′ and -5′, 8.22; H(2)-3′, s, 7.98; H(2)-5′, s, 8.11; H(1′), two d, 5.84 ($J \sim 3$ Hz) and 5.96 ($J \sim 3.5$ Hz), assigned according to published data.[47-49] The spectra of the mixture at pD 5.5 and 1 are presented in Figure 5. At pD 5.5 the signals at 9.36, 9.30, 9.15, and 8.70 ppm suggest the presence of protons in α position to nitrogen atoms coordinated to platinum.[23,24] The three lower field ones can be easily removed by exchange with D_2O, at basic pH, and even slowly and at similar rates at pD 5.5 (~50% after 38 h at 37 °C). Therefore they can be assigned to H(8) protons α to coordinated N(7).[23,48] The ^1H NMR spectrum of the isolated main complex, at pD 7, exhibits four resonances at 9.26, 9.20, 8.34, and 8.27 ppm in 1:1:1:1 ratio which can be, respectively, assigned to two H(8) and two H(2) protons of an ApA[Pt] complex. The signals of the H(8) protons (initially at 8.22 ppm) have experienced rather large downfield shifts due to platinum coordination. The preferred N(7) binding of [Pt(dien)Cl]Cl to the 6-methylaminopurine riboside gives an H(8) downfield shift of 0.60 ppm[23] and a 1.01-ppm shift has been reported for the H(8) of platinated Ado, coordinated in both N(7) and N(1) positions.[23] The 8.34- and 8.27-ppm H(2) signals (initially at 8.11 and 7.98 ppm) are in agreement with the downfield shifts of 0.35–0.10 ppm reported for the H(2) resonances of N(7)-coordinated Ado and 5′-AMP.[23,24] Among the other resonances of the mixture, the signal at 8.70 ppm, which cannot be removed by proton exchange, can be assigned to an H(2) proton α to a coordinated N(1), in agreement with the downfield shifts of 0.95–0.57 ppm reported in the case of N(1) + N(7) coordinated Ado and 5′-AMP.[23,24]

The CD spectra of free ApA, the mixture of complexes, and the isolated ApA[Pt] complex are represented in Figure 6. The spectrum of the reaction mixture is in good agreement with that previously reported.[39] The spectrum of the ApA[Pt] complex at pH 7 shows a maximum and a minimum which are both red shifted, compared to those of the bisignated curve of ApA, respectively, from 272 to 281 and 252 to 260 nm. The amplitude between the peak and trough of the signal is ca. 50% that of free ApA.

Reaction of GpC with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$. The stoichiometric reaction of the diaquo complex with GpC (free acid), 6.2×10^{-4} and 2.2×10^{-4} M in water (pH 5.5) at 37 °C,

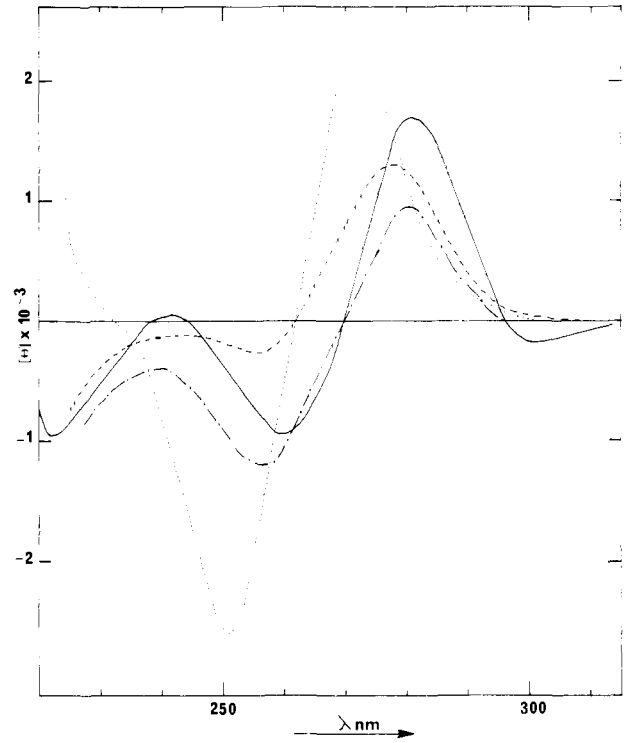

Figure 6. CD spectra of the ApA[Pt] complex from preparative LC, 2×10^{-5} M, (—) pH 7, (----) pH 0.9; of the reaction mixture 5×10^{-4} M, NaCl 0.05 M, (-·-) pH 6.2; ApA 9×10^{-5} M, NaCl 0.1 M, (···) pH 6.8.

for 90 and 96 h gives a solution with a red-shifted λ_{max} at 264 nm compared to 257 nm for the GpC initial solution, and a ratio of optical densities (GpC + Pt):GpC = 1.07. Sephadex chromatography shows that there is no unreacted GpC and that the monomeric product gives an unresolved broad peak (preceded by a small one, less than 5% of the mixture, which has not been further investigated). Preparative LC has led to the isolation of three fractions (a–c). Fraction b contains only one complex while fractions a and c, when kept for 8 days after separation, at a 10^{-3} M concentration, both give the same equilibrium mixture of two constituents (~55:45). Fraction c also contains a minor component. If the reaction is run with an excess of platinum, a new peak appears on the LC chromatogram with a longer retention time.

The ^1H NMR spectrum of free GpC, 5×10^{-3} M in D_2O, at pD 5.5 gives the signals (δ ppm, DSS) G:H(8), s, 8.06; C:H(6), d, 8.03 ($J = 8$ Hz) and H(5), d, 5.98 ($J = 8$ Hz); riboses H(1′), two d, 5.89 ($J = 4$ Hz) and 5.84 ($J \sim 3$ Hz), assigned according to published data.[47] The ^1H NMR spectra of the equilibrated fractions a and c, at pD 6 (Figure 7), confirm that they contain the same mixture of two complexes. The minor component of fraction c exhibits the resonance at 8.54 ppm which is the only one of the reaction mixture to be removed by deuterium exchange at pD 11.3 (18 h at 37 °C) and which can therefore be assigned to an H(8) α to the coordinated N(7) of a guanine.[24,26] The crude mixture before Sephadex desalting exhibits three distinct doublets for the 7.6-ppm group of resonances, at 7.60, 7.69, and 7.72 ppm ($J = 8$ Hz). We tentatively assign them to three H(6) protons (initially at 8.03 ppm) of N(3)-coordinated cytosines assuming that, as in the case of CpG,[21] and despite platinum binding, they would experience an overall upfield shift. In fact the complexation of Cyd and of 5′-CMP with [Pt(dien)Cl]Cl gives downfield shifts of the H(6) and H(5) protons, respectively, of 0.11–0.28 and 0.12–0.26 ppm,[23,24] but in the case of the stoichiometric reaction of CpG with $Pt(en)Cl_2$ (10^{-2} M at 57 °C) an upfield shift of 0.11 ppm has been observed for the H(6) of one of the CpG complexes.[21] To these H(6) signals of coordinated cytosines would correspond the two downfield-shifted H(5) doublets (initially at 5.98 ppm) at 6.50 (b) and 6.16 ppm (a and c), and others (for

(47) P. O. Ts'o, N. S. Kondo, M. P. Schweizer, and D. P. Hollis, *Biochemistry*, **8**, 997 (1969).
(48) S. I. Chan and J. H. Nelson, *J. Am. Chem. Soc.*, **91**, 168 (1969).
(49) C. H. Lee, F. S. Ezra, N. S. Kondo, R. H. Sarma, and S. S. Danyluk, *Biochemistry*, **15**, 3627 (1976).

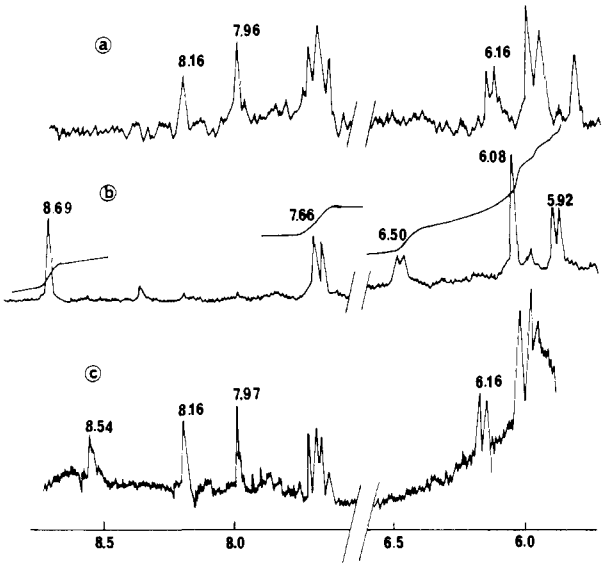

Figure 7. ^{1}H NMR spectra (250 MHz, D_2O, DSS, 17 °C) of the three preparative LC fractions (a–c at pD 6) of the mixture of complexes resulting from the stoichiometric reaction between GpC and *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$.

a and c) could overlap with deshielded H(1′) signals. The 8.16- and 7.96-ppm singlets of fractions a and c should correspond to H(8) protons of unbound guanines. Complex b appears also with an N(3)-coordinated cytosine. Its 8.69-ppm singlet, which is far downfield, could correspond to the H(8) of an N(7)-coordinated guanine; however, no or slight deuterium exchange occurred with D_2O at pD 11.3 (18 h at 37 °C). An N(1)G coordination is unlikely in this case.[26] The fact that the complexes of fractions a and c do not involve guanine coordination is also supported by the appearance of an important 8.31-ppm singlet characteristic of the H(8) of an N(7)-coordinated guanine (exchanged with D_2O at pD 11.3), when the reaction is run with an excess of platinum.

The CD spectra of GpC and of fractions a, b, and c are presented in Figure 8. The spectra of fractions a and c are similar, in agreement with the LC and ^{1}H NMR data (the third component of fraction c does not have a large contribution to the CD spectrum). The spectrum of fraction b is different from the others with three positive bands at 304, 258, and 220 nm. We have checked the influence of the concentration in this case, in NaCl, 0.05 M at pH 6. The amplitudes of the bisignated signal between λ_+ 258 nm and λ_- 280 nm show an overall 40% decrease when the concentration varies from 8×10^{-4} down to 8×10^{-6} M, revealing a significant contribution of intermolecular interactions. There is no change of the CD spectrum of fraction a between pH 1.8 and 6 and, at higher pH, one observes a blue shift of the minimum and a decrease of the intensity of the signal. For fraction b, increasing the pH from 6 to 11.1 completely suppresses the couplet (with an isoelliptic point at 268 nm).

The reaction of ApC with the diaquo complex, in similar conditions to those of GpC, gives a mixture of three main complexes in comparable proportions, and no preparative separation has been attempted yet (microfilm supplement).

Discussion

Before discussing the results we find it worth mentioning that for all the characterized complexes and especially IpI[Pt] and GpG[Pt] no coupling of the H(8) protons with the ^{195}Pt isotope is observed in the ^{1}H NMR spectra recorded at 250 and 270 MHz. Such a coupling is seen for platinum complexes of nucleosides and nucleotides studied at 60 MHz.[22,24] An absence of coupling has already been noted for bis(Ino), bis(5′-IMP), and bis(5′-GMP) complexes and can be seen for *cis*-$[Pt(NH_3)_2(Guo)_2]^{2+}$ studied at 100 MHz.[25,26,50] We have evidence showing that this decoupling is due to a dominant chemical shift anisotropy relaxation process for ^{195}Pt, which is greater the higher the molecular weight of the complexes studied and the higher the magnetic field used.[51]

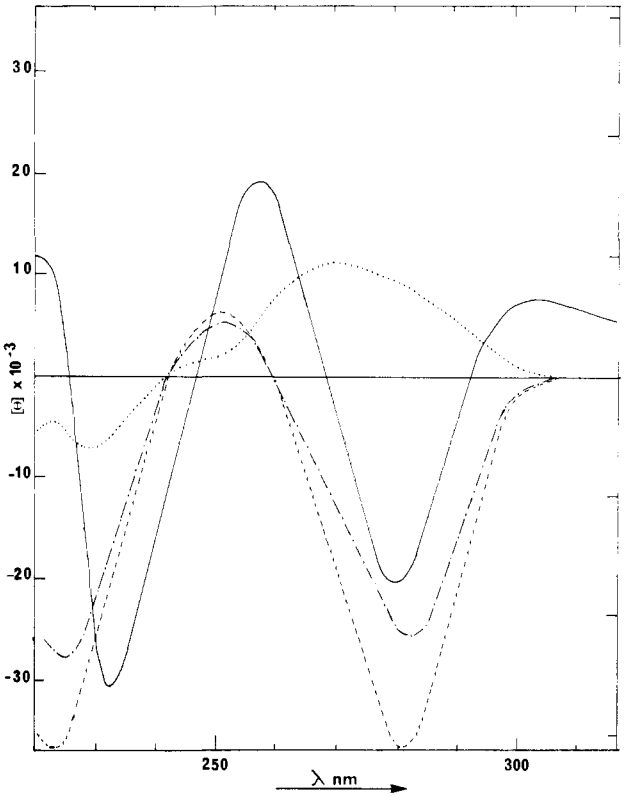

Figure 8. CD spectra of GpC and of the a, b, and c LC fractions from the mixture of complexes resulting from the stoichiometric reaction between GpC and *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$: (···) GpC 1.5×10^{-4} M, NaCl 0.05 M, pH 7.4; (----) fraction a, 1.2×10^{-4} M, NaCl 0.05 M, pH 5.9; (—) fraction b, 0.9×10^{-4} M, NaCl 0.05 M, pH 1.2–6; (-·-) fraction c, 0.9×10^{-4} M, NaCl 0.05 M, pH 5.6.

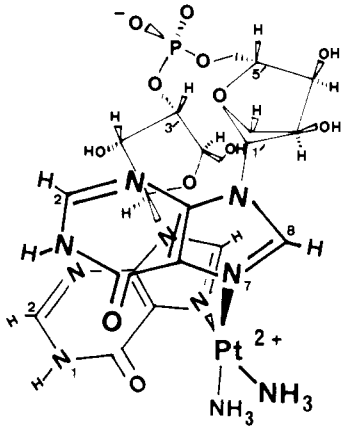

Figure 9. Schematic representation of the IpI[Pt] complex in the stacked anti,anti conformation at pH <6: $[Pt(NH_3)_2(IpI)]^+$.

Reaction of IpI and GpG with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$. In each case the stoichiometric reaction gives a single monomeric complex and ^{1}H NMR indicates that the two N(7) are coordinated to platinum within an N(7)–N(7) chelate of the metal. Considering that in IpI the two bases are preferentially oriented in the anti,anti conformation with a right-handed screw turn of the

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(51) J. Y. Lallemand, J. Soulié, and J. C. Chottard, *J. Chem. Soc., Chem. Commun.*, 436 (1980). In the case of the *cis*-$[Pt(NH_3)_2(Guo)_2](NO_3)_2$ complex the following evolution of the ^{195}Pt–H(8)G coupling has been observed: at 90 MHz, 60 °C, sharp satellites, 27 °C, beginning of coalescence, and 4 °C, coalescence of the satellites; at 250 MHz, 17 °C, no visible coupling, 90 °C, appearance of the coupling with a pattern similar to that observed at 90 MHz and 4 °C.

stack,[45] it seems reasonable to assume that the chelated IpI[Pt] complex presents a head-to-head arrangement of the purines as schematically represented in Figure 9. This is in agreement with the CD measurements, which show no sign inversion between the IpI and the IpI[Pt] complex spectra. Examination of the CPK model suggests that relatively stacked conformations can be adopted by the head-to-head purines of the IpI[Pt] complex, which could lead to the observed upfield shift of an H(8) NMR signal. It is known from X-ray structures of *cis*-bis(5′-IMP) complexes that the dihedral angle between the purines, in a head-to-tail arrangement, is of the order of 41°,[29,30] a small value compared to the 85.6° observed in the case of the *cis*-bis(3′-CMP) complex.[32] The CD spectrum of the IpI[Pt] complex, in the 250-nm region, is of the bisignated type, as for IpI, but with a larger amplitude. If one uses the amplitude between the peak and trough of the CD curve as a criterion of base–base interaction,[52] it follows that this interaction is larger in the complex than in the free dinucleotide. We must note that the classical geometrical relationship between the two interacting bases of a dinucleotide[53] is not relevant in the case of the chelated complex; nonetheless, the relative orientation of the transition moments must be such that they are strongly coupled. The conformation or conformational equilibrium of IpI[Pt] at pH 6 ($[Pt(NH_3)_2(IpI)]^+$) is kept in acidic medium, as evidenced by a nearly unaltered CD spectrum down to pH 1.3. Different CD signals are observed between pH 8.5 and 11.5 and the latter could be characteristic of the doubly deprotonated form. From the evolution of the CD spectra as a function of pH (Figure 3) it looks as if two major species are in equilibrium. Such a simplified interpretation of the data agrees with the apparent monotitration profile of the curves of H(8) chemical shifts vs. pH. Assuming that the doubly deprotonated IpI[Pt] complex is unstacked, one can write the following equilibria:

$$\begin{array}{ccc} IpI[Pt]_s & & IpI^-[Pt]_s \\ \uparrow\downarrow & \overset{-H^+}{\rightleftharpoons} & \uparrow\downarrow \\ IpI[Pt]_u & & IpI^-[Pt]_u \end{array} \rightleftharpoons IpI^{2-}[Pt]_u$$

The ^1H NMR and CD data appear to reflect only one overall equilibrium between two stacked and unstacked forms, suggesting that among the possible deprotonated species an unstacked form could be predominating.

The case of the GpG[Pt] complex is very similar to that of IpI[Pt]. In both cases the curves of the H(8) chemical shifts vs. pH reflect the influence of N(1)H dissociation on the environment of the H(8) protons. The apparent pK_as, 7.5 (H_2O) for IpI[Pt] and 8.5 (D_2O) for GpG[Pt], are to be compared, respectively, to 8.8 (H_2O) for Ino[54] and 9.8 (D_2O) for 5′-GMP.[26,54] Such a decrease of the N(1)H pK_a is noteworthy since only 0.3 and 0.6 log unit decreases have been determined in the case of the bis-(5′-GMP) complexes formed, respectively, from the cis and trans diammine diaquo complexes.[26] N(7)-Platination alone probably does not account for this large decrease and some proximity effect of the two head-to-head purines (Figure 9) could be involved.

The CD spectrum of the GpG[Pt] complex at neutral pH bears almost no resemblance with the spectra described for solutions of GpG plus Zn^{2+} and Cu^{2+}.[55] However, the authors give no indication either on the species present in the solution or on their evolution with time. It is noteworthy that the changes of the CD signal of GpG upon its chelation into the GpG[Pt] complex lead to a significant increase of ellipticity at 275 nm, where free GpG exhibits a minimum (Figure 4). Therefore the formation of intrastrand cross-links between adjacent guanines might account, at least partially, for the characteristic enhancement of ellipticity, at ca. 275 nm, reported for the binding of the *cis*-$(NH_3)_2Pt^{II}$ moiety to DNA at low Pt/DNA(P) ratios.[14,56,57]

Reaction of ApA with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$. This stoichiometric reaction gives two main products, the major one being an N(7)–N(7) chelated ApA[Pt] complex, analogous to IpI- and GpG[Pt]. It is noteworthy that for these three chelated complexes, at pD 5.5, one H(1′) signal appears as a doublet and the other as a singlet. For the free dinucleoside monophosphates both signals exhibit a $J_{1'2'}$ coupling and this coupling is also observed for the bis-Ino,[25] bis-Guo,[50] and bis(5′-GMP)[26] complexes. It is known that the ribose coupling constants are highly sensitive to stacking interactions.[45,49,52] The CD bisignated curve of ApA[Pt], with half the intensity of that of ApA (Figure 6), indicates a significant interaction between the chelated purines. For ApA, calculations predict an 80% intensity decrease of the signal when the bases are set perpendicular instead of parallel to one another.[58] Acidic pH lowers the intensity of the signal but this effect is very small compared to that observed with pure ApA, where the signal collapses entirely, indicating a complete unstacking of the bases. The spectrum of the reaction mixture is only slightly sensitive to temperature, showing a 15% decrease from 13 to 54 °C to be compared with a 60% decrease for ApA in the same temperature interval.[59] This agrees with the coexistence in the mixture of a chelated species with stacked bases and of monocoordinated species with variable interactions between the purines.

ApA, like the other homodinucleotides IpI and GpG, has a geometry favoring N(7)–N(7) chelation of platinum, but N(1) competitive binding of the metal is probably the main reason for the formation of a mixture of complexes.

Reaction of GpC with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$. This reaction gives a mixture of three main components, two of which appear to be in slow equilibrium. The three of them have an N(3) coordinated cytosine. The ^1H NMR spectra of fractions a and c exhibit similar H(5)–H(6)C and H(1′) signal patterns and two identical H(8)G resonances. This suggests that the two slowly equilibrating components are two isomers of the cytosine-monocoordinated dinucleotide complex $[Pt(NH_3)_2(GpC)Cl]^+$. Considering GpC in the stacked anti,anti conformation (or syn,anti), these isomers could correspond to two different positions of the chloride ligand either "inside the stack" (in close proximity to the guanine) or "outside the stack".[60]

For the single complex of fraction b, the cytosine H(5) and H(6) proton assignments do not agree with an anti,anti-type conformation of the dinucleotide (with a right-handed screw turn of the stack) which would imply a shielding effect of the guanine ring current and a deshielding effect of the 5′-phosphate affecting, respectively, the H(5) and H(6) protons.[47] This is supported by the CD data, which suggest a strong interaction between the purine and pyrimidine bases due to conservative excitonic coupling, mostly of intramolecular origin, and show that the sign of the couplet (λ_+ 258, λ_- 280 nm) (Figure 7) is inverted when compared to those of the IpI[Pt] and ApA[Pt] complexes. This may reveal a different handedness of the distorted helical arrangement of the bases.[61] A left-handed helical arrangement has been very recently characterized for a d(CpG) trimer.[62] CPK models show that N-

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(57) As noted by a referee a marked enhancement of the CD spectrum of 5′-GMP has been reported for the complex $[Pt^{II}(trimethylenediamine)(5'\text{-}GMP)_2]^{2-}$ (during submission of this paper): L.-G. Marzilli and P. Chalilpoyil, *J. Am. Chem. Soc.*, **102**, 873 (1980).
(58) W. C. Johnson, M. S. Itzkowitz, and I. Tinoco, *Biopolymers*, **11**, 225 (1972).
(59) J. W. Pettegrew, D. W. Miles, and H. Eyring, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 1785 (1977).
(60) There is no free rotation of Pt-bound ortho-disubstituted pyridines around the Pt–N bond: M. Orchin and P. J. Schmidt, *Inorg. Chim. Acta Rev.*, **2**, 123 (1968).
(61) C. A. Bush and J. Brahms in "Physicochemical Properties of Nucleic Acids", Vol. 2, Jules Duchesnes, Ed., Academic Press, New York, 1973, Chapter 12.
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(7)G-N(3)C platinum chelation seems very unfavorable for GpC in the anti,anti conformation and that in this highly constrained structure one would expect the H(8)G to be upfield shifted by the ring current of the cytosine, which is not the case. On the contrary, N(7)G-N(3)C chelation is easily achieved with the guanine ring in the syn conformation, leading to a left-handed GpC[Pt] chelate. Such a chelate fits the ^{1}H NMR data (Figure 7b); however, the H(8) of the guanine appears to be only very slowly exchangeable with D_2O at pD 11.3. It is noteworthy that no or slight deuterium exchange of a guanine H(8) at pD 12 has also been reported in the case of *trans*-$[Pt(NH_3)_2(5'\text{-GMP})_2]^{2-}$, from Raman and ^{1}H NMR data.[26] If one compares these results to the exchange that we did observe for GpG[Pt], one can note that the CPK model of the left-handed GpC[Pt] complex shows that the H(8)G is in close proximity with the O(2) of the cytosine, a situation comparable to that of the two H(8)G of the *trans*-bis(5'-GMP) complex which are close to the O(6) of the other guanine, due to the "head-to-tail" arrangement of the bases. Such a proximity might contribute to the slowing down of the H(8)G deuterium exchange.

The analysis of the mixture from the ApC reaction reveals preferential binding of cytosine over adenine with competitive N(1) and N(7) binding for the latter. Owing to the complexity of the ^{1}H NMR spectrum of the mixture and to the nonconservative character of its CD signal, no conclusion can be drawn concerning the presence of a chelated platinum complex without separation of the components.

Conclusion

Among the five studied dinucleoside monophosphates, IpI, GpG, ApA, GpC, and ApC, reacting with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$, the first three homodinucleotides have a geometry leading to N(7)-N(7) chelation of the metal. IpI and GpG give a single N(7)-N(7) chelated complex while ApA also gives other products due to competitive N(1) binding to the metal. GpC and ApC lead to mixtures of several complexes and in both cases cytosine appears to have more affinity for the platinum of the diaquo complex than do guanine and adenine.

The CD data in relation with the ^{1}H NMR data, particularly in the cases of the $[Pt(NH_3)_2(IpI)]^+$ and $[Pt(NH_3)_2(ApA)]^+$ complexes, show that a significant interaction exists between the two purines which are in a mutual orientation different from parallelism.

In the case of GpC one of the complexes appears as an N-(7)G-N(3)C platinum chelate, the CD of which suggests a left-handed helical arrangement of the bases.

As far as the perturbation of the DNA structure upon binding of the *cis*-$(NH_3)_2Pt^{II}$ moiety is concerned,[4,38,63] our results show that an efficient cross-linking of two adjacent guanines could be favored and contribute to the characteristic enhancement of CD ellipticity, at ca. 275 nm, observed at low Pt/DNA(P) ratios, bringing further support to the hypothesis of intrastrand cross-linking.[29,64] Because of the stereochemical constraints inherent in this type of interaction, such a chelation of two adjacent guanines could only occur after a local denaturation of DNA. This could be the result of a primary interaction of one base with the *cis*-diamminediaquoplatinum(II) complex,[12,13,29,36,63] or of a preexisting localized premelted region of kinked DNA.[65,66] Moreover, it seems that an N(7)G-N(3)C cross-linking of adjacent guanine and cytosine could occur for a left-handed segment of the polynucleotide.[62]

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Supplementary Material Available: ^{1}H NMR of GpG[Pt] (Figure 10); LC chromatograms of the ApA, GpC (including the separated a-c fractions), and ApC reaction mixtures (Figure 11); CD spectra of GpC[Pt] (fraction b) from pH 1.2 to 11.1 (Figure 12); Sephadex analysis, ^{1}H NMR (Figure 13), and CD data of the ApC mixture (6 pages). Ordering information is given on any current masthead page.

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Intermolecular Association of 1,N^6-Ethenoadenosine in Aqueous Solution. Vapor Pressure Osmometric and Heat of Dilution Studies

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Abstract: Self-association of 1,N^6-ethanoadenosine (εAdo) in aqueous solution was studied by vapor pressure osmometry (VPO) and heat of dilution measurements. The association process was found to be accounted by an isodesmic (indefinite) noncooperative self-association model and a simple monomer-dimer reaction cannot account for the data based on VPO experiments. The intermolecular stacking equilibrium quotient was determined to be 18.7 ± 0.8 M^{-1}. Thus, the self-complexing affinity is increased on going from adenosine (Ado) to εAdo. The enthalpy of self-association was also determined from a solution calorimetric dilution study; $\Delta H^\circ = -35.6 \pm 1.0$ kJ/mol.

εAdo[2] was prepared by Barrio et al.[3] in 1972, although the first derivative having the imidazo[2,1-*i*]purine skeletal structure was reported by Kochetkov et al.[4] a year earlier. The presence of a fused imidazole ring alters the properties of the adenine ring

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