Anti-Cancer Pt Drug Adducts with AMP: Novel Direct ^1H and ^{195}Pt NMR Evidence for Slowly Interconverting "Head-to-Tail" Rotamers. Potential Role of Amine Ligand Bulk and NH Groups in Guanine Selectivity and Anti-Cancer Activity

Michael D. Reily and Luigi G. Marzilli*

Contribution from the Department of Chemistry, Emory University, Atlanta, Georgia 30322.
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Abstract: Conclusive evidence is presented that $PtL_2(5'AMP\text{-}N7)_2$ complexes (where L_2 = two monodentate or one bidentate amine ligand(s) and 5'AMP-N7 is 5'AMP coordinated via N7) and the analogous 5'dAMP compounds exhibit detectable restricted rotation about the Pt–N7 bond at ambient temperature even when L_2 *is not* bulky. In particular, ^1H and, in some cases, ^{195}Pt NMR spectra indicate two rotamers as evidenced by the presence of two resolved signals for most of the nuclei. The analogous 5'GMP compounds are known to exhibit such restricted rotation only when L_2 *is* bulky. For *non-bulky* L_2, the rotamer signals for the analogous Cyd and 5MeCyd compounds have not coalesced even at 75 °C; signals for the 5'AMP species have coalesced by 55 °C; and signals for the 5'GMP compounds are known to remain coalesced, even at low T. When ^{195}Pt NMR spectroscopy is used, the preference for the binding of Pt compounds to N7 over N1 of 5'AMP is ca. 10 to 1. Together, these observations suggest appreciable steric effects of the $6NH_2$ group of 5'AMP and even greater steric effects of the exocyclic groups of C derivatives. The high selectivity of Pt compounds for G residues may thus have a steric origin. Several aspects of our spectroscopic studies of the 5'AMP compounds are interesting and revealing. First, the pK_a of the OPO_3H^- of 5'AMP (and 5'dAMP) cis to an NH_2 group was ca. 0.4 unit lower than that cis to a Me_2N group in compounds such as $Pt(N,N\text{-}Me_2en)(5'AMP\text{-}N7)_2$, where $N,N\text{-}Me_2en$ is N,N-dimethylethylenediamine. This result suggests an H bond interaction between the NH_2 of the bidentate ligand and OPO_3^{2-}. Second, the relative ^1H NMR spectral intensities for the two rotamers of $PtL_2(5'AMP\text{-}N7)_2$ were pH dependent. This result can be reasonably explained only if the adenine bases are in a head-to-tail (htt) arrangement. Third, ^{195}Pt NMR signals for the two htt rotamers were resolved for the first time. Fourth, the compounds $Pt(N,N\text{-}Me_2en)X_2$ (X = Cl, Br, I) form two geometric isomers of $Pt(N,N\text{-}Me_2en)(5'\text{-}AMP\text{-}N7)X$ in roughly equal amounts. For X = Cl and Br, one isomer, termed the *reactive* isomer, will add a second 5'AMP to form $Pt(N,N\text{-}Me_2en)(5'AMP\text{-}N7)_2$. Fifth, the isomers of $Pt(N,N\text{-}Me_2en)(5'AMP\text{-}N7)X$ for X = Br and I but not X = Cl exhibit restricted rotation about the Pt–N7 bond. Sixth, the *unreactive* isomer of $Pt(N,N\text{-}Me_2en)(5'AMP\text{-}N7)$ exhibited more rapid rotation about the Pt–N7 bond than the *reactive* isomer. Seventh, the evidence in points four to six, the comparative OPO_3H^- pK_a and H8 T_1 values, and an examination of models all indicate that the *reactive* isomer has the 5'AMP cis to the Me_2N group. The interesting properties of nucleotide compounds revealed by this study are consistent with the latest concepts which have been advanced to explain the anti-cancer effectiveness of Pt-based drugs.

Rosenberg's discovery of the anti-tumor properties of certain platinum complexes with two cis leaving groups such as *cis*-$Pt(NH_3)_2Cl_2$ and $Pt(en)Cl_2$ (en = ethylenediamine)[1] and the subsequent clinical success of the former compound,[2] which has made it now the most widely used anti-cancer drug in the United States,[3] have prompted numerous fundamental studies of the coordination chemistry of Pt compounds to nucleobases.[4-10] The interest in nucleobases arises from the widespread belief that DNA is the molecular target of the drug.[11] The drug appears to attack G (guanine) residues preferentially in DNA,[12,13] and this preference is also manifest in the reactions with monomeric derivatives of nucleic acids. For example, 5'GMP is the most reactive of the four common nucleotide 5'-monophosphates (5'NMP's).[6]

The preference for G is not completely understood,[14] but it is well established that N7 of G is the Pt binding site.[15] The next most reactive Pt target is A (adenine) and then C (cytosine). Unless the amine ligands are bulky, *cis*-$PtL_2(6\text{-oxopurine-N7})_2$ complexes (where L_2 is two unidentate or one bidentate amine ligand, the designation N7 indicates binding mode, and charges are omitted since these depend on pH) exhibit rapid rotation about the Pt–N7 bond on the NMR time scale.[7,9,10,16] When L_2 is bulky,

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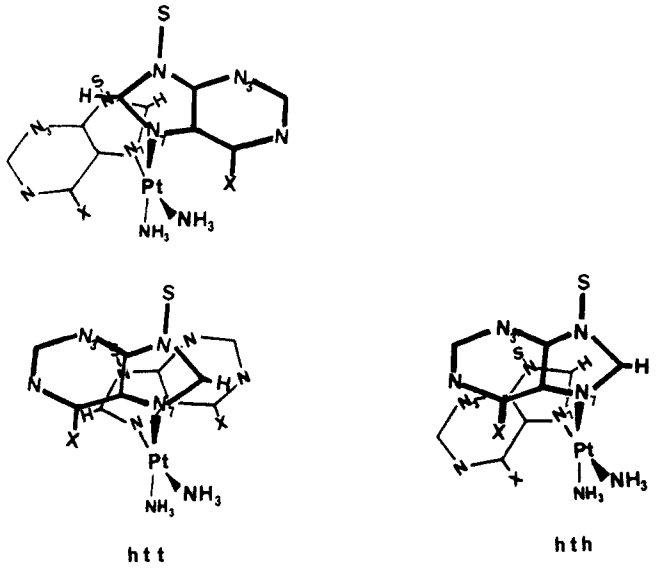
restricted rotation is observed.[7,16,17] Comparisons of the properties of complexes which exhibit restricted rotation to those properties prevalent in Pt anti-cancer compounds led Reedijk and his co-workers to speculate that facile rotation about the Pt–N7 bond may be important in forming the crucial DNA lesion responsible for Pt anti-cancer activity.[16] For the complexes with bulky L_2 groups, restricted rotation is evidenced by the appearance of two H8 signals.[7,16,17] These could be from a "head-to-tail" (htt) pair of diastereomers, each of which have both G H8 groups on opposite sides of the PtN_4 coordination plane or the "head-to-head" (hth) species in which the H8 atoms are on the same side of this plane. For the hth conformation, the two bases are inequivalent. For



htt hth

the htt conformation, there are two rotamers, each with two equivalent bases (C_2 axis), of roughly equal stability. Since both possibilities should lead to equivalent ^{1}H NMR spectra, i.e., two H8 signals of equal size, it is not usually possible to differentiate between all htt and all hth.

The great importance of Pt–G reactions has led to comparatively fewer studies with nucleosides and nucleotides of other nucleobases, especially in recent times when high-field NMR spectrometers have been more routinely applied to such problems. The lower reactivity of these other bases[6] and the presence of two binding sites on A (N1 and N7) have no doubt discouraged further studies with A, C, and T (thymine) nucleotides. A possible corollary of Reedijk's hypothesis[16] is, "Restricted rotation in PtA and PtC adducts inhibits formation of the type of lethal DNA lesion formed by PtG adducts." Since restricted rotation appears to be a steric effect, it is possible that platination of the less sterically hindered G base causes a less significant distortion in the structure of DNA than does platination of A bases. There is mounting evidence that DNA platination by *cis*-PtA_2Cl_2 at the preferred G sites may cause relatively minor DNA distortions.[18] In addition, a new concept has been advanced that such a distortion will permit the DNA lesion caused by *cis*-$Pt(NH_3)_2Cl_2$ to escape repair and thus remain cytotoxic.[19] Indeed, some elegant experiments[19] and calculations[20] support this new idea. In particular, numerous lines of study suggest that the Pt complexes link together two adjacent G residues forming intrastrand cross-links of the type, $Pt(NH_3)_2$(GpG-N7,N7).[12,13,18,19] Calculations suggest that an important structural feature of this type of adduct is a H bond between the amine group and the phosphate group 5′ to the 5′G.[20] Such an NH group would provide little steric hindrance to free rotation about the Pt–N7 bond in simple G nucleoside and nucleotide adducts, and the correlation between drug activity and free rotation might be coincidental.

Consequently, the subject of the conformational and dynamic properties of nucleotide complexes is of considerable fundamental chemical interest. A greater understanding of these phenomena may help to explain the high selectivity of Pt compounds toward G bases. We report an examination of the reactions between Pt anti-cancer agents and several nucleotides and nucleosides derived from A and C. This study has provided a rare opportunity to differentiate between the hth and htt conformations of bis A nucleotide complexes since, in some cases, the relative populations of the species formed in solution is reversibly pH dependent. We also report the first example (for a monomeric species) of the differentiation of such rotamers by ^{195}Pt NMR spectroscopy. This pH effect allows us to probe indirectly the role of NH to phosphate O hydrogen bonding in a manner recently outlined by Martin for relevant Pd complexes.[14] When the AMP ligand is cis to a Me_2N moiety, such as when L_2 = *N,N*-Me_2en, the phosphate group is more basic than when the AMP is cis to an NH_2 group. Along with a new T_1 method, this pK_a dependence allows us to assign ^{1}H NMR signals and differentiate the two geometric isomers of Pt(*N,N*-Me_2en)(5′AMP-N7)X. The two isomers exhibit very different reactivity toward 5′AMP. Thus, when X = Cl or Br, one isomer readily forms, and the other isomer does not form Pt(*N,N*-Me_2en)(5′AMP-N7)$_2$. When X = I, neither isomer forms the bis nucleotide complex. Restricted rotation is observed for both isomers when X = Br and I.

Experimental Section

Instrumentation. ^{1}H NMR spectra were obtained on a Nicolet NB-360 FT spectrometer equipped with a variable temperature unit operating in the Fourier transform mode at 361.08 MHz. Most spectra were recorded with 90% H_2O, 10% D_2O as a solvent and with a Nicolet-supplied version of the 21412 solvent suppression sequence developed by Redfield.[21] The transmitter was set at ca. 10 ppm downfield from the internal standard, TSP. The low-power 450-μs pulse was attenuated to deliver a tip angle of ca. 90°. T_1 measurements were performed in D_2O with a modified inversion–recovery pulse sequence.[22]

^{31}P NMR spectra were recorded on an IBM WP 200 SY spectrometer at 81.01 MHz (90° pulse, 15-s delay). Usually, solutions identical with those used for ^{1}H NMR experiments were employed, but trimethyl phosphate (TMP ~0.01%) was added as an internal standard. ^{195}Pt NMR spectra were recorded on the same instrument operating at 42.93 MHz (Na_2PtCl_6 external reference).

AA spectroscopy was performed on a Perkin-Elmer 306 spectrophotometer equipped with an HG2100 graphite furnace and auto sampler. Typically, 0.001 to 0.03 μg of Pt were used for each measurement. UV spectroscopy was performed on a Perkin-Elmer Lambda 3B.

Materials. Nucleosides and nucleotides were purchased from Aldrich or Sigma and were used as received. *cis*-$Pt(NH_3)_2Cl_2$ was purchased from Aldrich. Pt(en)X_2, Pt(*N,N*-Me_2en)X_2, Pt(*N,N,N′,N′*-Me_4en)X_2, and *cis*-$Pt(MeNH_2)_2X_2$ (X = I, Cl, or Br) were prepared on the basis of the method of Dhara.[23]

Solution Preparation. In a typical experiment, $[Pt(en)(H_2O)_2]^{2+}$ was prepared as a 0.01 M solution by treatment of a vigorously stirred mixture of Pt(en)I_2 (51.5 mg) in a D_2O solution (10 mL) with $AgNO_3$ (33.6 mg) at 35 °C in the dark until AgCl no longer precipitated on addition of NaCl to the filtrate. The AgI was then removed by filtration through Celite, and the clear filtrate (0.25 mL), after pH adjustment, was added immediately to one or two volumes of a solution 0.01 M in nucleoside or nucleotide. After appropriate pH adjustment, such mixtures were either monitored by ^{1}H NMR spectroscopy or allowed to stand for 10 to 20 h. The reported pH values are not corrected for the 10% D_2O content.

In an effort to remove unreacted Pt complex and ligand, we employed a 2 × 20 cm CM-25 Sephadex weak cation exchange column (Na^+ form, H_2O eluent, pH 6, 0.5 mL/min) through which 1.0-mL portions of ca.

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Table I. Selected ^1H and ^{195}Pt NMR Spectral Data for 5'dAMP and 5'AMP and Some Adducts Formed with Pt Compounds[a]

compound	H8	H2	H1' ($^3J_{1'2'}$, Hz)[b]	NH_2	^{195}Pt
5'dAMP	8.47	8.25	6.52 (6.8)	6.89	
Pt(en)(5'dAMP-N7)(H_2O)	9.06	8.33	6.59 (6.0)	7.8[c]	
Pt(en)(5'dAMP-N7)$_2$	9.39	8.20	6.59 (5.8)	7.8[c]	-2685
	9.26	8.19	6.42 (6.0)	7.64	-2690
cis-Pt(NH_3)$_2$(5'dAMP-N7)$_2$	9.32	8.24	6.53 (7.1)	7.0	
	9.20	8.23	6.43 (5.8)		
cis-Pt($MeNH_2$)$_2$-(5'dAMP-N7)$_2$	9.38	8.25	6.52		
	9.23	8.23	6.42 (5.3)		
5'AMP	8.51	8.26	6.14 (5.9)	6.9	
Pt(en)(5'AMP-N7)$_2$	9.49	8.18	6.20 (2.9)	6.7	-2692
	9.37	8.20	6.12 (2.8)		
cis-Pt(NH_3)$_2$(5'AMP-N7)$_2$	9.50	8.20	6.20 (3.3)		
	9.35	8.20	6.08 (3.5)	6.9	-2479
cis-Pt($MeNH_2$)$_2$-(5'AMP-N7)$_2$	9.60	8.29	6.29 (3.4)		
	9.44	8.28	6.17 (4.0)	6.9	-2532
Pt(*N,N*-Me_2en)-(5'AMP-N7)$_2$	9.69	8.32	6.33 (3.5)		
	9.51	8.27	6.28 (2.6)	7.0	-2600
	9.50	8.26	6.22		
	9.47	8.26	6.16 (3.0)		
Pt(*N,N*-Me_2en)(5'AMP-N7)Cl	8.97	8.35	6.22	7.6	
Pt(*N,N*-Me_2en)-(5'AMP-N7)Br	9.12				
	9.09				
	8.95				
	8.93				
Pt(*N,N*-Me_2en)-(5'AMP-N7)I	9.13	8.38			
	9.090	8.33			
	8.94	8.39			
	8.91	8.36			
Pt(tn)(5'AMP-N7)$_2$	9.52	8.10	6.15 (2.88)		
	9.39	8.08	6.06 (~3)		

[a] 90% H_2O, pH 5.5: chemical shifts from internal TSP (±0.005 ppm) or 0.1 M Na_2PtCl_6 (±3 ppm). [b] In the case of deoxynucleotides, $^3J_{1'2''} \cong {}^3J_{1'2'}$ in most cases. [c] Very broad signal. [d] tn = 1,3-propanediamine; Pt(tn)Cl_2 was prepared by known methods.[23]

0.1 M (Pt) solutions of the complexes were passed. In addition to removal of some unidentified Pt species as well as some 5'AMP, this procedure afforded early eluting fractions found to be richer in or, in some cases, containing exclusively the bis-N7,N7 species. Conversely, the later eluting fractions contained much unreacted nucleotide and various other Pt species. From the large number of ^1H NMR signals and from the ^{195}Pt NMR shifts, we believe these fractions contain N1 bound 5'AMP Pt complexes (vide infra).

Results

(I) A Derivatives. (a) ^1H NMR Spectroscopy. (i) Pt(en)-(5'dAMP-N7)$_2$: The 9–5-ppm region of the ^1H NMR spectrum of 5'dAMP (0.02 M) at pH 5.5, 25 °C (90% H_2O/10% D_2O), contains three singlets and a doublet of doublets corresponding to H8, H2, NH_2, and H1' (Table I). Six minutes after the addition of $[Pt(en)(H_2O)_2]^{2+}$ (r = ratio of Pt to nucleotide = 1.0) a single set of new signals was observed (Figure 1, bottom trace). Relative to uncomplexed nucleotide, the H8 signal (assigned by D exchange and T_1 measurements, vide infra) is shifted downfield by 0.59 ppm; the H2 and H1' signals were shifted downfield by ca. 0.10 ppm (Figure 1). We assign these signals to Pt(en)-(5'dAMP-N7)(H_2O). This same chemical shift relationship to uncomplexed nucleotide was observed by Reedijk and his coworkers for *cis*-Pt(NH_3)$_2$(5'GMP-N7)(H_2O).[7,8] Appearing after 15 min were three new sets of signals, all of roughly equal area. Only the two sets with H8 peaks at 9.39 and 9.26 ppm had significant intensity after 3 h of reaction time at 25 °C. These H8 signals contained ca. 80% of the intensity in the 8.4–9.5-ppm region after 30 h. Also, the relative intensity of these two sets was constant throughout the reaction. Both sets have the same chemical shift relationship relative to uncomplexed nucleotide, as was observed for *cis*-Pt(NH_3)$_2$(5'dGMP-N7)$_2$.[7,8] We assign these two sets of signals to the two htt rotamers of Pt(en)-(5'dAMP-N7)$_2$ (Table I). At least seven other base proton signals were observed in the possibly equilibrated mixture and constituted ca. 20% of the intensity in this region. Of these minor components,

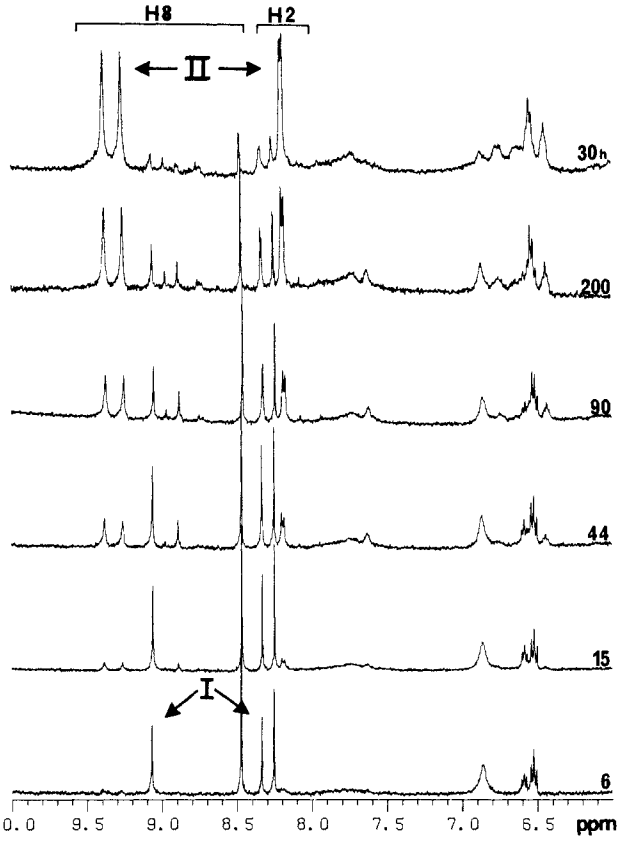

Figure 1. Time dependence of the ^1H NMR spectrum of a 1:1 mixture of 5'dAMP (5 mM) and $[Pt(en)(H_2O)_2]^{2+}$ in 90/10 H_2O/D_2O, pH 5.5. Unless otherwise noted, times indicated are minutes after the mixing of the two solutions as described in the text. Over the course of the reaction the pH changed from 5.5 to 5.6. Signals I and II are assigned to Pt-(en)(5'dAMP-N7)(H_2O) and Pt(en)(5'dAMP-N7)$_2$, respectively. Unlabeled H8 and H2 signals are from 5'dAMP. Note that the Redfield pulse profile is not uniform and signals to higher field are less intense than those to lower field for the same number of nuclei.

one appears to be Pt(en)(5'dAMP-N7)(H_2O) and the others are probably mono-N1 and bis-N1,N7 adducts as well as binuclear species, all known to be formed with A nucleosides and nucleotides under similar conditions.[24,25]

When the reaction was carried out at r = 0.5, under conditions similar to those described above, and followed by ^1H NMR spectroscopy, similar spectral changes and final products were observed. However, there was a relatively larger amount of 5'dAMP remaining at long reaction times.

Reactions similar to those above were carried out for several solutions at various pH values between 3.0 and 7.0. In all cases the major stable soluble species was Pt(en)(5'dAMP-N7)$_2$.

Heating a 3.3 mM solution of Pt(en)(5'dAMP-N7)$_2$ in the presence of 100 mM NaCl at 80 °C, pH 7, for 12 h resulted in complete loss of the signals for the complex. Only the signals for free 5'dAMP were observed.

The effect of increasing concentration of $Eu(NO_3)_3$ on the H8, H1', H2', H4', H5', H5'', and en CH signals of a purified sample of Pt(en)(5'dAMP-N7)$_2$ at pH 5.4 was studied up to a Eu/Pt ratio of 0.11. Above this ratio, precipitation occurred. The upfield lanthanide-induced shifts (LIS) were largest for all four H5' signals and both H8 signals (0.03–0.04 ppm at 0 11 Eu/Pt). Smaller upfield LIS were observed for the signals of the other ribose protons and the LIS for the en CH signal were much smaller (<0.01 ppm at 0.11 Eu/Pt). The ca. 0.005 ppm *downfield* shift of the H2 signals may be indicative of some slight conformational change. The observed LIS are consistent with Eu^{3+} binding to

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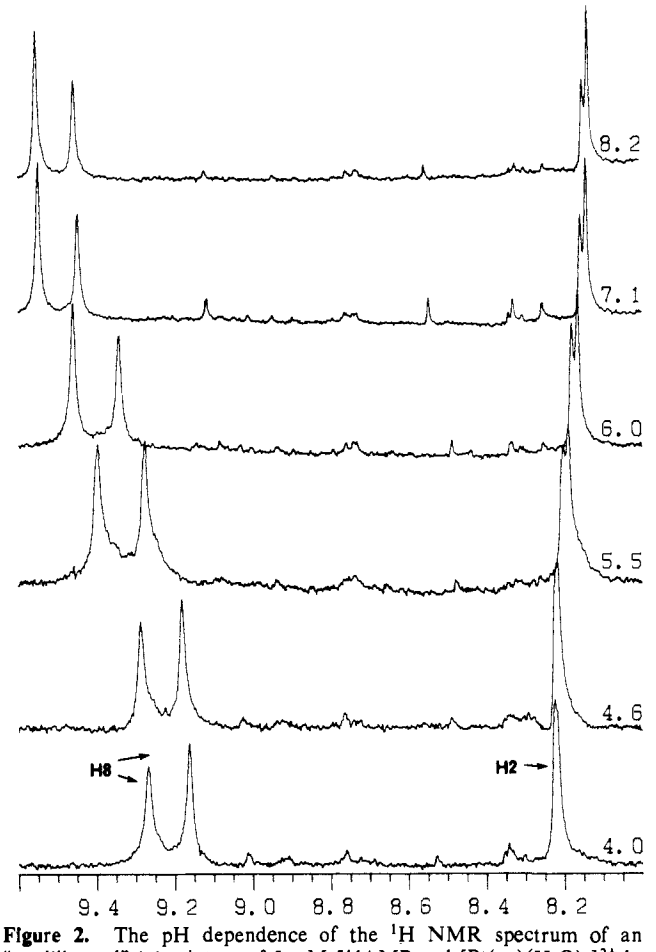


Figure 2. The pH dependence of the ^1H NMR spectrum of an "equilibrated" 1:1 mixture of 5 mM 5'dAMP and $[Pt(en)(H_2O)_2]^{2+}$ in 90/10 H_2O/D_2O. The H8 signals for the two diastereomers of Pt(en)(5'dAMP-N7)$_2$ appear furthest downfield. The corresponding H2 signals are the largest two signals in the 8.0–8.2-ppm region. See note in Figure 1 caption.

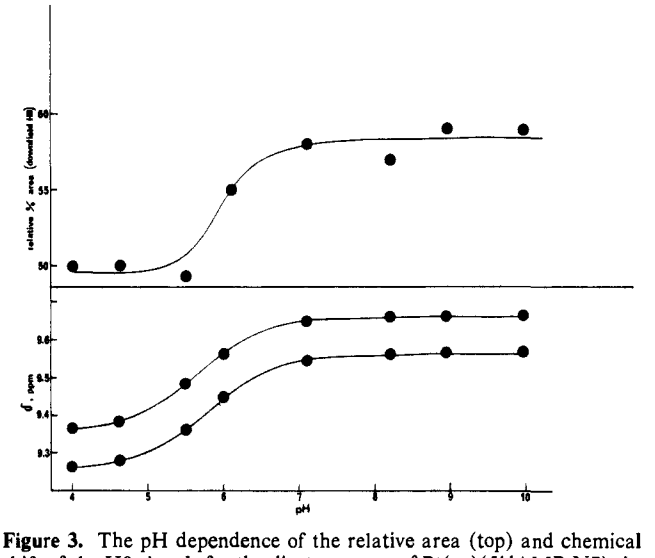


Figure 3. The pH dependence of the relative area (top) and chemical shift of the H8 signals for the diastereomers of Pt(en)(5'dAMP-N7)$_2$ in 90/10 H_2O/D_2O. Top: the fractional areas of the downfield H8 relative to the total area of both H8 signals as determined by integration and deconvolution. Bottom: chemical shifts in ppm from internal TSP. pH values are not corrected for 10% D_2O. See note in Figure 1 caption.

a PO_4^{2-} group in close proximity to H8, such as in the anti-conformer.

The base CH and H1′ region of the ^1H NMR spectrum of Pt(en)(5'dAMP-N7)$_2$ was studied as a function of pH over the range 4–8.2 (Figures 2 and 3). Deprotonation of the OPO_3H^- group, which occurs in this pH range,[14] caused downfield shifts of both H8 signals by 0.32 ppm, with an apparent pK_a of 5.75 ± 0.05 (1.0 unit below that for 5'dAMP). More interestingly, the relative areas of these two H8 signals changed from ca. 50:50 at pH 4.0 to ca. 58:42 at pH >7 (Figures 2 and 3). This finding demonstrates a preference for one conformer over the other in the fully deprotonated form (overall charge 2−).

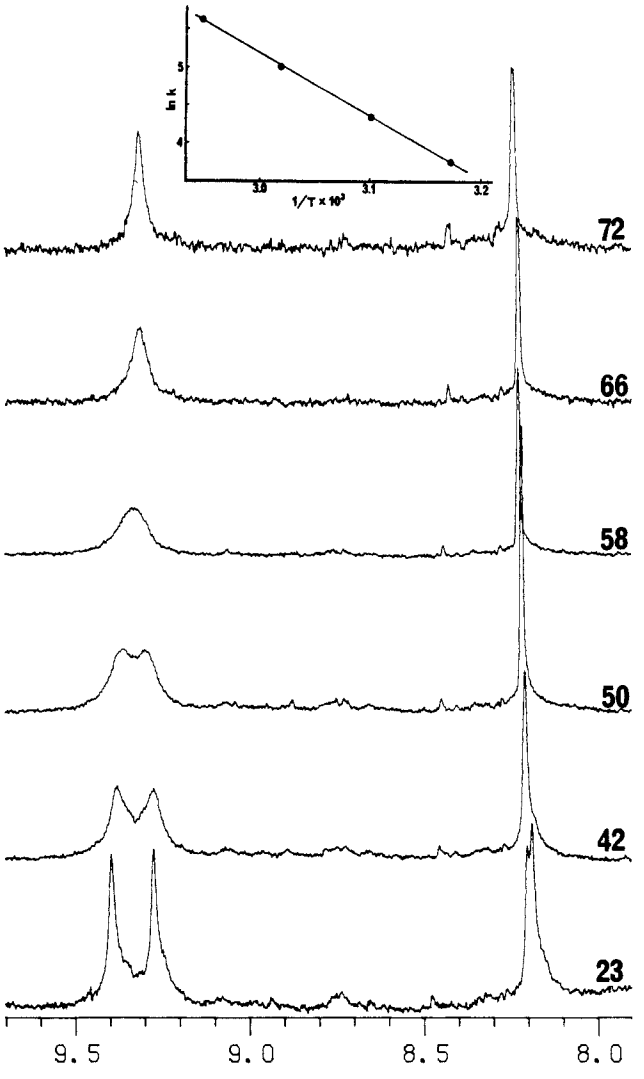


Figure 4. The temperature dependence of the ^1H NMR spectrum of ca. 5 mM Pt(en)(5'dAMP-N7)$_2$, pH 5.5; 90/10 H_2O/D_2O. See Figure 1 for assignments. Insert shows plot of ln k as determined by line shape analysis vs. $1/T$.

Figure 4 shows the effect of temperature on the ^1H NMR spectrum of Pt(en)(5'dAMP-N7)$_2$. The two H8 signals clearly coalesce at ca. 55 °C, after which a further increase in temperature sharpened the merged resonance. Analysis of the data with a Nicolet-supplied two-site exchange line shape simulation program gave an activation energy of 17 ± 1 kcal/mol for rotation in this molecule. A separate analysis of the temperature data by the method of Dimitrov[26] gave identical results.

(ii) ***cis*-Pt(NH$_3$)$_2$(5'AMP-N7)$_2$**: Addition of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ to 2 equiv of 5'AMP under conditions similar to those described above gave analogous results (Table I). The bis-N7,N7 species was purified as described in the Experimental Section.

The effect of pH on the base CH and H1′ region of the ^1H NMR spectrum of *cis*-Pt(NH$_3$)$_2$(5'AMP-N7)$_2$ is shown in Figure 5. In the pH range 3–8.5, both H8 signals shift downfield upon deprotonation of the phosphate groups, with an apparent pK_a of 5.75 ± 0.05, a value identical with that observed for Pt(en)(5'dAMP-N7)$_2$ (vide supra). In the pH range 0.6–3, the H8

(26) Dimitrov, V. S. *Org. Magn. Reson.* **1976**, *8*, 132.

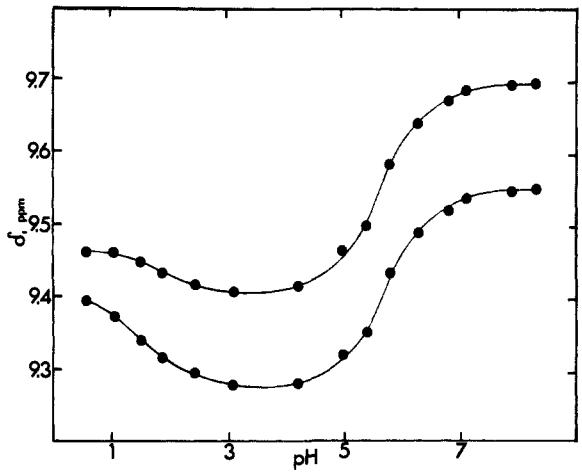


Figure 5. H8 chemical shift vs. pH for *cis*-$Pt(NH_3)_2(5'AMP)_2$ in 90/10 H_2O/D_2O. pH values were measured before and after recording the ^1H NMR spectrum and are not corrected for the 10% D_2O content. Chemical shifts were measured in ppm from internal Me_4N^+ and then corrected to reflect the shift from TSP at pH 7. The pH meter was calibrated at pH 10.0, 7.0, 4.0, and 1.0.

signals shift upfield 0.05–0.1 ppm. The pK_a of N1 for the species *cis*-$Pt(NH_3)_2$(Ado-N7)$_2$ (Ado = adenosine) is reported to be 1.7.[24] The inflection points at ~1.5 in Figure 5 are consistent with N1 protonation, albeit the shape of these curves in this pH range is no doubt influenced somewhat by protonation of the OPO_3H^- groups. AA and UV spectroscopy yielded an extinction coefficient at 260 nm of 2.6×10^4 M^{-1} cm^{-1} per Pt atom. This is in the range expected for a bis AMP species.[24]

(iii) Pt(N,N-Me$_2$en)(5'AMP-N7)$_2$: In reactions similar to those described above but with $[Pt(N,N\text{-}Me_2en)(H_2O)_2]^{2+}$, the bis-N7,N7 species was produced in good yield (ca. 90%) at $r = 0.5$ (Table I). When the complex was formed at pH 5.7, the four resonances for the H8's of Pt(*N,N*-Me$_2$en)(5'AMP-N7)$_2$ appeared as two sets of two signals of equal area with one set larger than the other. The two sets of signals arise from the two htt rotamers; the two equal components of each set arise from the dissymmetry in the chelate ligand. The effect of pH 4.0–8.5) on the four H8 signals of Pt(*N,N*-Me$_2$en)(5'dAMP-N7)$_2$ is presented in the top four curves in Figure 6. Deprotonation of the phosphate group caused rather large downfield shifts (ca. 0.2 ppm) of two of the H8 signals and smaller downfield shifts (ca. 0.1 ppm) of the other two signals. The two signals that shift ca. 0.2 ppm exhibited a pK_a of 5.46 ± 0.02; the other two gave a value of 6.08 ± 0.04 and shifted less than 0.16 ppm. From the difference in intensity of the two peaks that exhibit the largest shift on protonation, it is clear that they are *not* from the same complex. For this reason and those to be presented below, we assign the signals which indicate a higher pK_a (and shift the least on protonation) to H8 of the 5'AMP ligand cis to the Me_2N of the chelate ligand. Four H8 signals are observed even at 75 °C.

(iv) Pt(*N,N*-Me$_2$en)(5'AMP-N7)X (X = Cl, Br, I): When a solution of Pt(*N,N*-Me$_2$en)Cl$_2$ was heated for 48 h at 50 °C in the presence of 1 equiv of 5'AMP (or 5'dAMP) and 100 mM NaCl, with the pH kept between 7.7 and 8.0 with TRIS buffer, the resulting mixture contained ca. 25% Pt(*N,N*-Me$_2$en)-(5'AMP-N7)$_2$ and 25% unchanged 5'AMP. The remaining nucleotide H8 and H2 signals both appeared as singlets at 9.04 and 8.35 ppm (pH 7.5), respectively. The downfield shifts and similar area of these two signals suggested that the species was a new Pt complex with AMP coordinated via N7. We believe this species is "Pt(*N,N*-Me$_2$en)(5'AMP-N7)Cl."

Concentration of this new species was accomplished as follows: The reaction mixture (described above, ~10 mL, 0.05 M in Pt) was added to 6 volumes of cold acetone in a centrifuge tube. The sample was centrifuged and the supernatant removed. The precipitate contained mainly 5'AMP and Pt(*N,N*-Me$_2$en)(5'AMP-N7)$_2$. The supernatant, after concentration, contained largely (>80%) the new species. The pH dependence of the H8 and H2

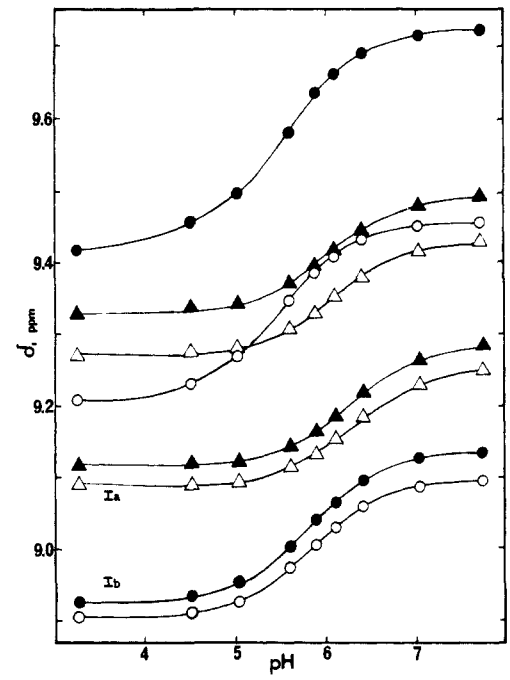


Figure 6. H8 chemical shift vs. pH for Pt(*N,N*-Me$_2$en)(5'AMP-N7)$_2$ and both Pt(*N,N*-Me$_2$en)(5'AMP-N7)Br geometric isomers in 90/10 H_2O/D_2O. The four signals labeled Ia and Ib are assigned to the *reactive* and *unreactive* geometric isomers of Pt(*N,N*-Me$_2$en)(5'AMP-N7)Br, respectively. Chemical shifts were measured from internal dioxane and then adjusted by adding 3.758 ppm to reflect shifts from TSP. Circles indicate 5'AMP ligands trans to the Me_2N group in the bidentate ligand (see text). Open symbols represent one rotamer and closed symbols represent the other rotamer. pH values were measured before and after recording the ^1H NMR spectrum and are not corrected for 10% D_2O content.

shifts for this new species was recorded, and a Henderson–Hasselbach analysis of the data indicated a pK_a of ca. 5.8. A sixfold excess of KI was added to the concentrated solution. The ^1H NMR spectrum of the solution, taken at several times (0.5 to 24 h), revealed the appearance of a pair of new H8 and a pair of new H2 signals of slightly unequal area. There was a concomitant decrease in the original 9.04 (H8) and 8.35 (H2) ppm peaks. The new H8 signals are slightly upfield to that of "Pt(*N,N*-Me$_2$en)(5'AMP-N7)Cl", consistent with a greater shielding effect of I over Cl. The interpretation that the reaction of 5'AMP with Pt(*N,N*-Me$_2$en)Cl$_2$ leads to Pt(*N,N*-Me$_2$en)(5'AMP-N7)$_2$ and "Pt(*N,N*-Me$_2$en)(5'AMP-N7)Cl" requires that we explain (a) the observation of only one chloro species, (b) the reactivity of this species toward I^- but not toward 5'AMP, and (c) the observation of two H8 signals of slightly different area as well as two H2 signals of slightly different area for "Pt(*N,N*-Me$_2$en)(5'AMP-N7)I". (The relative areas of the H8 signals to each other and the H2 signals to each other were similar.)

Since I is more difficult to displace than Cl, we investigated the formation of the iodo adduct directly from Pt(*N,N*-Me$_2$en)I$_2$. Incubation of this diiodo complex with 1 or 2 equiv of 5'AMP for up to 48 h at 50 °C resulted in the formation of the species believed to be "Pt(*N,N*-Me$_2$en)(5'AMP-N7)I" (Table I). In addition, a second set of two H8 signals, nearly equal in intensity to the first, was observed ca. 0.2 ppm downfield from the corresponding signals for "Pt(*N,N*-Me$_2$en)(5'AMP-N7)I". No Pt-(*N,N*-Me$_2$en)(5'AMP-N7)$_2$ was observed after long reaction times ($r = 0.5$). These results are consistent with the formation of *two* Pt(*N,N*-Me$_2$en)(5'AMP-N7)I *geometric* isomers, each of which has two *rotational* isomers. Furthermore, the reaction of Pt-(*N,N*-Me$_2$en)Cl$_2$ with 5'AMP can be understood if (a) one of the *geometric* isomers of Pt(*N,N*-Me$_2$en)(5'AMP-N7)Cl reacts with 5'AMP and the other is *unreactive* and therefore accumulates, (b) both geometric isomers are formed with nearly equal facility, and (c) rotational isomers of the unreactive geometric isomer are undetectable. To differentiate the two geometric isomers of

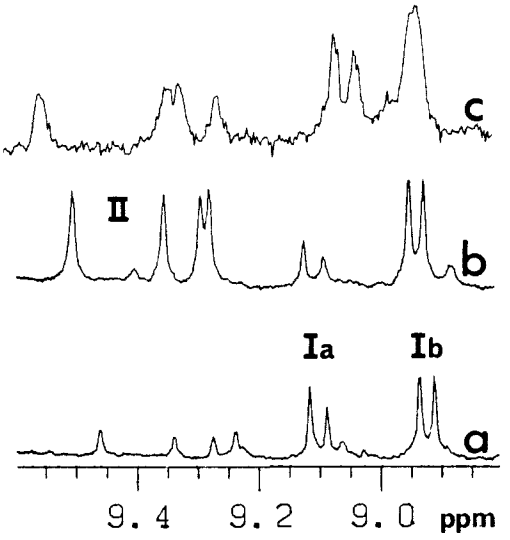


Figure 7. The dependence on r of the ^{1}H NMR spectrum of products formed in the reaction of Pt(N,N-Me_2en)Br_2 with 5'AMP: (a) r = 1.0, 25 °C, (b) r = 0.5, 25 °C, (c) r = 0.5, 75 °C. H8 signals marked Ia and Ib are assigned to the geometric isomers of Pt(*N,N*-Me_2en)(5'AMP-N7)Br with 5'AMP cis (*reactive*) and trans (*unreactive*) to the Me_2N groups, respectively. H8 signals marked II belong to Pt(*N,N*-Me_2en)-(5'AMP-N7)$_2$. Small differences in the shifts of corresponding signals in spectra a and b are attributable to slight differences in final pH values. Reaction conditions were 0.010 M in Pt, pH (initial) 5.6, 45 °C, 24 h elapsed time.

Pt(*N,N*-Me_2en)(5-AMP-N7)X, we designate these as *unreactive* and *reactive*.

To shed further light on these issues, we examined the reaction of Pt(*N,N*-Me_2en)Br_2 with 5'AMP. Pt(*N,N*-Me_2en)(5'AMP-N7)$_2$ was formed, and the relative amount of this species increased with decreasing r (~35% at r = 1). When r = 1, the two sets of signals (with H8's at 9.12, 9.09 and 8.95, 8.93 ppm) attributable to the two Pt(*N,N*-Me_2en)(5'AMP)Br geometric isomers were roughly equal in area and represented ca. 60% of the coordinated 5'AMP (incubation 24 h at 45 °C, pH 5.5, Figure 7, trace a). These signals are almost coincident in shift with those of the iodo analogues, with a slightly smaller spacing between peaks arising from rotational isomers (see Table I). At r = 0.5, ca. 60% of coordinated 5'AMP was in the bis-N7,N7 compound and ca. 25% and 10% were in the *unreactive* and *reactive* isomers, respectively (Figure 7, trace b).

Raising the temperature of a similar sample (Figure 7, trace c) caused broadening and eventual coalescence (at ca. 60 °C) of the two H8 signals centered at 8.94 ppm. The signals assigned to the rotamers of the *reactive* isomer begin to broaden only at 75 °C. Analysis of the pH dependence of the chemical shift of the H8 signals of the two mono-N7(bromo) species yielded pK_a values of 6.36 ± 0.02 and 5.91 ± 0.02 for the *reactive* (downfield) and *unreactive* (upfield) rotamers, respectively (Figure 6).

Molecular models reveal that the H's on the Me groups of *N,N*-Me_2en are in close proximity to H8 of the 5'AMP in one geometric isomer, and the ND_2 group (in D_2O) is close to H8 of 5'AMP in the other isomer. Signals for H8 close to an Me_2N group should have lower T_1 values than those for H8 close to an ND_2 group. The T_1 values for H8 of the *reactive* Pt(*N,N*-Me_2en)(5'AMP-N7)Br isomer are less than those of the *unreactive* isomer and of the H8 signals of Pt(en)(5'AMP-N7)$_2$ (Table II). Further, there is a similar difference in T_1 values for the H8 signals in Pt(*N,N*-Me_2en)(5'AMP-N7)$_2$ (Table II).

All the results agree with the interpretation that, in reactions of 5'AMP with Pt(*N,N*-Me_2en)X_2 (X = Cl, Br, or I), there is little preference for one binding site over the other in initial addition of 5'AMP. The addition of a second 5'AMP proceeds to a detectable extent for only one of the Pt(*N,N*-Me_2en)(5'AMP-N7)X isomers, when X = Cl or Br. When [Pt(*N,N*-Me_2en)$(H_2O)_2]^{2+}$ was used, no buildup of any signals except those belonging to Pt(*N,N*-Me_2en)(5'AMP-N7)$_2$ was observed.

Table II. Selected ^{1}H NMR T_1 Relaxation Data for Pt-5'AMP Adducts[a]

	T_1, s			
	major rotamer		minor rotamer	
compound	H8[b]	H8[c]	H8[b]	H8[c]
Pt(en)(5'AMP-N7)$_2$		1.15		1.10
Pt(*N,N*-Me_2en)(5'AMP-N7)$_2$	0.976	1.21	0.863	1.36
Pt(*N,N*-Me_2en)(5'AMP-N7)Br (*reactive*)	0.974[d]		1.076[d]	
Pt(*N,N*-Me_2en)(5'AMP-N7)Br (*unreactive*)		1.57[d]		1.26[d]

[a] 360-MHz data, pH 3.5, D_2O, 25 °C, concentrations were between 30 and 60 mM in Pt. Spectral conditions are given in the Experimental Section. Estimated error, ±2%. [b] Assigned to 5'AMP cis to the Me_2N group, see text. [c] Assigned to 5'AMP trans to the Me_2N group, see text. [d] Determined at 30 and 60 mM (in Pt) with identical results.

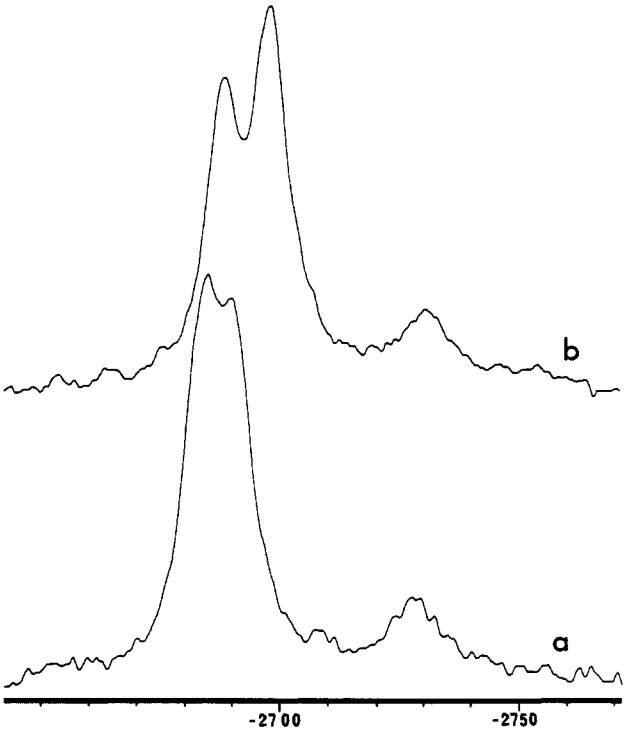


Figure 8. The 42.93-MHz ^{195}Pt NMR spectrum of an "equilibrated" mixture of 5'dAMP (0.2 M) and $[Pt(en)(H_2O)_2]^{2+}$ (0.1 M) in D_2O at pD 5.8 (a) and 7.4 (b). Chemical shifts are referenced to external 0.1 M Na_2PtCl_6. pD was determined by adding 0.4 to the pH meter reading.

(b) ^{31}P NMR Spectroscopy. The ^{31}P NMR spectrum of Pt(en)(5'dAMP-N7)$_2$ (5 mM at pH 5.5) shows two closely spaced signals at −1.50 and −1.65 ppm upfield from internal TMP. At pH 7.1, only one sharp resonance was observed at −1.0 ppm.

(c) ^{195}Pt NMR Spectroscopy. The ^{195}Pt NMR spectral data for several complexes of the type *cis*-PtL_2(5'AMP-N7)$_2$ (and 5'dAMP analogs) are given in Table I. Most of the solutions exhibited single signals for the two bis-N7,N7 rotamers, but in the case of Pt(en)(5'dAMP-N7)$_2$ two signals were observed which we believe are due to the two htt rotamers (Figure 8). At pH 5.5 two closely spaced signals of roughly equal intensity were observed at −2684 and −2690 ppm upfield (from external 0.1 M Na_2PtCl_6). An increase in pH to 7.0 caused a large upfield shift (10–20 ppm) of one of the signals and essentially no shift of the other. In addition, the relative peak areas became unequal at pH 7, as noted above for the H8 signals for this complex. Note the broad peak observed ca. 40 ppm upfield from the main signal. This shift difference is similar to the shift difference observed in N7 and N1 bound *cis*-Pt$(NH_3)_2$(5'AMP)Cl[27] and we attribute

(27) Clore, G. M.; Gronenborn, A. M. *J. Am. Chem. Soc.* **1982**, *104*, 1364.

Table III. Selected ^{1}H NMR Spectral Data for Cyd and 5MeCyd and Some Adducts with Pt(en)[a]

compound	H6	H5	H1′ ($^3J_{1'2'}$, Hz)	$CH_3$5[b]
Cyd	7.85	6.06	5.91 (4.3)	
Pt(en)(Cyd)(H_2O)	7.96	6.15	5.93 (3.9)	
	7.93	*c*	*c* (3.9)	
Pt(en)(Cyd)$_2$	7.94	6.14	5.96 (3.3)	
	7.93	6.13	5.95 (3.3)	
5-MeCyd	7.73		5.96 (3.6)	1.95
Pt(en)(5-MeCyd)(H_2O)	7.79		5.92 (3.6)	2.02[c]
	7.76			
Pt(en)(5-MeCyd)$_2$	7.80		5.95 (3.6)	1.99[c]
	7.77		5.94	

[a] 360-MHz data; PIPES 10 (0.01 M PIPES, 0.1 M $NaNO_3$, 0.001 M EDTA), 99% D_2O, pH 7.0; chemical shifts from internal TSP (±0.005 ppm). [b] In some cases $^4J_{6Me5}$ (~0.6 Hz) was observed. [c] Superimposed resonances.

this broad signal to one or more bis-N7,N1 species. Subsequent purification (as described above) of the analogous 5′AMP compound eliminated this most upfield ^{195} Pt NMR signal, as well as a majority of the minor signals in the ^{1}H NMR spectrum. The ^{195}Pt NMR spectra of fractions which contained large amounts of the materials responsible for the minor ^{1}H NMR signals have a large upfield ^{195}Pt NMR signal. Finally, although the shift range of the ^{195}Pt NMR signal for the various bis-N7N7-Pt species is almost 200 ppm for different amine ligands, a signal is observed ca. 40 ppm upfield from the main signal in all cases prior to purification.

(II) C Derivatives. Complexes formed with Pt(en)Cl_2 (and $[Pt(en)(H_2O)_2]^{2+}$) and 5′dCMP, Cyd, and 5MeCyd (Cyd = cytidine) were studied with ^{1}H NMR spectroscopy; spectral data for these complexes are listed in Tables III and IV. At *r* values ≤2, for the C derivatives studied, two major species were identified by ^{1}H NMR spectroscopy. The relative intensity of the two sets of signals depended on *r*. The predominant set of signals at high *r* is assigned to the mono C species. Conversely, the largest set of signals at low *r* is assigned to the bis species. In the pH range employed in this study (4.0–7.0), N3 is the primary binding site for Pt.[28] The CH protons on the base are far removed from N3 and thus are affected little by Pt complexation; typical shifts of the H5 and H6 protons upon Pt binding are on the order of 0.1 ppm. Also, the strong coupling of the H5 and H6 of Cyd and 5′dCMP ($^3J_{56}$ > 7.6 Hz) complicates the spectra. To overcome this difficulty, we carried out experiments with 5MeCyd, which has a methyl group in place of H5. The following description of the complexes will concentrate largely on the effect of platination on the H6 resonances; other signals will be mentioned where appropriate.

(a) Pt(en)(5′dCMP-N3)(H_2O) and Pt(en)(5′dCMP-N3)$_2$: At *r* values <2 and pH 5.5, two H6 doublets were observed for the mono species along with two doublets corresponding to the bis species (Table IV). At *r* = 2.0, the ratio of mono to bis is ca. 4. In addition, at *r* values >2, two unequal H6 doublets for two as yet unidentified species were observed shifted ca. 0.4–0.45 ppm *upfield* from unreacted 5′dCMP.[29] At *r* = 10, no signals for Pt(en)(5′dCMP-N3)$_2$ or 5′dCMP were observed, and ca. 40% of the 5′dCMP was in the two unidentified species and 60% was in the mono complex.

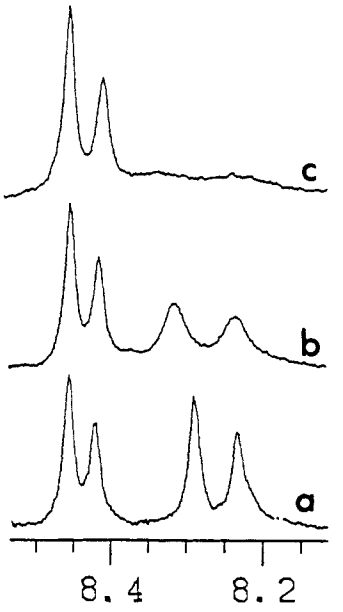


Figure 9. the 7.7–8.7-ppm (amino signal) region of the ^{1}H NMR spectrum of Pt(en)(5′dCMP-N3)$_2$ in 90/10 H_2O/D_2O at (a) pH 4.0, (b) 5.0, and (c) 7.0.

At *r* < 0.5, Pt(en)(5′dCMP-N3)$_2$ was the only species observed with coordinated 5′dCMP. The H6 signals for this coordination compound appear as two doublets, shifted ca. 0.03 ppm downfield from unreacted 5′dCMP (at pH 5.5). The H1′ and H5 signals have shifts very close to those for unreacted 5′dCMP at this pH.

At pH 4.0, four sharp amino proton signals are observed between 8.5 and 8.2 ppm in the ^{1}H NMR spectrum of Pt(en)(5′dCMP-N3)$_2$ (Table IV, Figure 9). Two of these signals (at 8.455 and 8.290 ppm) are of equal intensity and larger than the other two (at 8.420 and 8.233 ppm, also of equal intensity). This difference in area is approximately equal to the difference in the area of the two H6 signals. The two signals are clear evidence for restricted rotation about the C–N bond in each species. At pH 5.5, the line width of the upfield NH singlet roughly doubled and, at pH 7.0, these signals disappeared and the downfield NH signals began to broaden. This pH dependence of the amino proton signals suggests that the protons giving the downfield signal are

Table IV. Selected ^{1}H NMR Spectral Data for 5′dCMP and Some Adducts with Pt(en)[a]

compound	pH	H6	H5	H1′ ($^3J_{1'2'}$, Hz)	NH_2
5′dCMP	4.0	8.080	6.161	6.230	
	5.5	7.958	6.081	6.295 (6.6)	7.2[b]
	7.0	8.040	6.120	6.333 (6.6)	
Pt(en)(5′dCMP-N3)(H_2O)	5.5	7.962	6.095	6.242[b]	7.8[b]
		7.942	6.165		
	7.0	8.040	6.20[b]		
		8.020			
Pt(en)(5′dCMP-N3)$_2$	4.0	7.960	6.062	~6.21	8.455
		7.885	6.050		8.420
					8.290
					8.233
	5.5	7.926	6.070	6.220 (6.5)	8.450
		7.922	6.045	6.610 (6.0)	8.412
					8.310
					8.232
	7.0	7.992	6.082	6.280 (6.6)	8.453
		7.975	6.059	~6.25	8.408

[a] 360-MHz data chemical shifts, in ppm, from internal TSP spectra recorded in 90/10 H_2O/D_2O at ambient *T*. [b] Broad signals.

pointing toward Pt and are possibly H bonded. Then the H's giving the upfield signals would be exposed to solvent and be in more rapid exchange. The relative populations of the diastereomers of Pt(en)(5'dCMP)$_2$ do *not* vary with pH.

(b) Pt(en)(Cyd)$_n$(H$_2$O)$_{2-m}$ Pt(en)(5MeCyd)$_n$(H$_2$O)$_{2-n}$; n = 1,2: The ^{1}H NMR spectrum of Cyd is similar to that of 5'dCMP, with the chemical shift relationship of the H1' and H5 signals reversed (Table IV). 5MeCyd gives one singlet and one doublet in the 8.0–6.0-ppm region (Table IV). The singlet for H6 is slightly broadened by unresolved weak coupling with the adjacent 5-Me group ($^4J \sim 0.6$ Hz). The products formed from Pt(en)Cl$_2$ or [Pt(en)(H$_2$O)$_2$]$^{2+}$ with Cyd and 5MeCyd were similar and had the same dependence on r as the 5'dCMP products. Even when r = 2.0, significant quantities of Pt(en)(Cyd)$_2$ or Pt(en)(5MeCyd)$_2$ were formed. When $r < 0.5$, this bis species is the predominant product.

The ^{1}H NMR spectra for Pt(en)(Cyd)$_2$. Pt(en)(5MeCyd)$_2$, and Pt(en)(5MeCyd)(H$_2$O) were observed as a function of temperature. On an increase in T from 25 to 75 °C, the H6 signals shifted upfield about 0.05 to 0.1 ppm, with no clear sign of coalescence.

Discussion

The strong preference of Pt complexes for G residues,[6] especially in polynucleotides such as DNA,[11-13,20,30-33] is now very well established as an experimental observation. A detailed analysis of comparative equilibrium selectivity suggests an unexplained ~0.7 log unit increase in stability for Pt or Pd binding to N7 of 6-oxopurine nucleosides.[14]

Two possible explanations for this additional stability of G species can be considered. First, the 6-oxo group could form a H bond to the NH group. However, careful work by Martin excludes this explanation for Pd complexes, which are analogous in binding preference to Pt complexes.[14] Second, the 6-amino group of A and the 4-amino and 2-oxo groups of C could sterically interfere with coordination at N1 or N7 of A or N3 of C. The steric interactions may be too small to quantitate, especially for nonlabile Pt(II) compounds.

One property of Pt complexes which appears closely associated with steric effects is the relative ease of rotation about the Pt–N bonds. Thus, although G nucleoside and nucleotide complexes do not exhibit restricted rotation when the other ligands on Pt are small (NH$_3$, en), restricted rotation is observed for bulkier amine ligands such as in *N,N,N',N'*-Me$_4$en complexes.[9,10]

The restricted rotation we have reported for C complexes has been noted previously.[17] However, our work emphasizes that the barrier for rotation about N3 of C derivatives must be rather large (no evidence of rotation even at 75 °C). The difference in intensity of the respective signals for the bis N3,N3 C rotamers is good evidence that the htt arrangement of the cytosine bases observed in the solid also is favored in solution.[34]

Our finding of restricted rotation of some Pt complexes with N7 bound A nucleotides is, to our knowledge, a novel finding. Consistent with past evidence that the restricted rotation in Pt–G compounds can be understood solely as a steric effect, the result provides direct evidence for steric interference by the 6-amino group to rotation of A derivatives.

An alternative explanation for the restricted rotation could be H bonding between the OPO_3^{2-} group and the NH groups of the ligand. Martin's careful study indicates such interactions in Pd compounds lead to increased stability of nucleotide complexes over analogous nucleoside complexes.[14] However, *even* at pH 0.6, where the OPO_3H_2 group is formed, there is still restricted rotation about the Pt–N7 bond of PtL$_2$(5'AMP-N7)$_2$ compounds. Additionally, if such a H-bonding interaction could lead to restricted rotation, then N7 bound GMP Pt complexes would exhibit restricted rotation—and they do not.

It is interesting that even at 75 °C, C derivatives do not undergo rapid rotation about Pt–N3 bonds. Taken together, this evidence would seem supportive of our proposed corollary of the Reedijk hypothesis. However, the initial product of attack of Pt anti-cancer drugs on N7 of A must be a species of the type PtL$_2$(5'-AMP-N7)H$_2$O or PtL$_2$(5'AMP-N7)Cl. In our studies of the *reactive* and *unreactive* isomers of Pt(*N,N*-Me$_2$en)(5'AMP-N7)X as well as of Pt(en)(5'AMP-N7)H$_2$O, we observed restricted rotation only in cases where X = Br or I. Thus, the corollary may not be valid.

Our results, however, are quite consistent with the new idea that a key feature in the PtL$_2$GpG cross-link structure is a H bond between the NH and the phosphate group 5' to the cross-link.[20] The restricted rotation in G adducts of the inactive compounds may be coincidental since all other substituents on the N's of L$_2$ will be bulkier than H. Indeed, our studies of the pK_a's of the AMP compounds reveal clearly that phosphate group···HN H bonding is occurring in these simple nucleotide complexes.

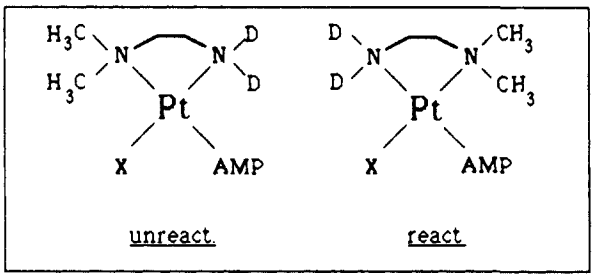
The role of the steric bulk of the exocyclic amino and oxo groups of A and C could well be to discourage intrastrand cross-links and to discourage initial adduct formation. Indeed, the resulting compounds could lead to disruption in the DNA structure, promoting repair of the lesion. In any case, our study provides direct evidence that the 6-NH$_2$ of A derivatives is sterically interfering with binding and that this effect could account for the G selectivity.

The steric size of the 6-NH$_2$ group could also play a role in comparative binding of N7 vs. N1. ^{195}Pt NMR is quite revealing. The N1 bound species occurs 40 ppm to higher field than an N7 bound species.[36] In general, 80% or more of the bis(5'AMP) products are the N7,N7 species. The remainder appears to be the N7,N1 species. We have not tried to examine the N7,N1 products in detail, but we suspect that both htt and hth type species are present and that these are in slow rotation. This interpretation would allow for 8 H8 signals and 8 H2 signals. Thus, 20% of the H8 signal area of the coordinated 5'AMP would be distributed in 8 signals, accounting for the apparent dominance of the bis N7,N7 species as assessed by ^{1}H NMR spectroscopy and the clear identification of the N7,N1 species by ^{195}Pt NMR spectroscopy (the four, 2 htt and 2 hth, ^{195}Pt signals expected should be nearly coincident). In general, the ^{195}Pt NMR signals of rotamers cannot be resolved and we were successful in only one case. However, since the hth species has 2 H8 and one ^{195}Pt signal whereas *each* htt rotamer has an H8 and a ^{195}Pt signal, observation of two ^{195}Pt NMR signals is conclusive evidence for the htt geometry. Although the evidence for the N7,N1 mixture of htt and hth isomers is more circumstantial, the preponderance of N7 to N1 bound nucleotide of ca. 10 to 1 is clear and suggests that the steric effect of the 6NH$_2$ could impede N1 binding relative to N7 binding. The proximity of the 6NH$_2$ group to the N1 site argues in favor of this last statement.

Steric effects also are clearly important in the reaction of Pt(*N,N*-Me$_2$en)X$_2$ with 5'AMP. If we assume an S$_N$2 mechanism with approach of the incoming ligand from above (or below) the coordination plane, then the apparent equal distribution of the geometric isomers of Pt(*N,N*-Me$_2$en)(5'AMP-N7)X suggests little difference in the steric impediment to reaction at either coordination site. However, the ease of addition of the *second* 5'AMP is highly dependent on the geometric isomer.

We assign the geometry below for the Pt(*N,N*-Me$_2$en)-(5'AMP-N7)X species. The evidence for the assignment of the

(28) Marzilli, L. G. *Adv. Inorg. Biochem.* **1981**, *3*, 47.
(29) The spectra are consistent with Pt binding to the exocyclic amino group with deprotonation. Such spectral changes have been observed with 1-methylcytosine, and reaction at the amino group was confirmed by X-ray crystallography (Faggiani, R.; Lippert, B.; Lock, C. J. L.; Speranzini, R. A. *J. Am. Chem. Soc.* **1981**, *103*, 1111).
(30) Tullius, T. D.; Lippard, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 4620.
(31) Marzilli, L. G.; Reily, M. D.; Heyl, B. L.; McMurray, C. T.; Wilson, W. D. *FEBS Lett.* **1984**, *176*, 389.
(32) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; Reedijk, J. *FEBS Lett.* **1984**, *176*, 393.
(33) Cohen, G. L.; Ledner, J. A.; Bauer, W. R.; Ushay, M. H.; Caravana, C.; Lippard, S. J. *J. Am. Chem. Soc.* **1980**, *102*, 2487.
(34) Orbell, J. D.; Marzilli, L. G.; Kistenmacher, T. J. *J. Am. Chem. Soc.* **1981**, *103*, 5126.
(35) The H2 T_1 is roughly twice the H8 T_1 (see Girault et al. ref 15). The T_1 values for the signals assigned to H2 in this study are larger than those assigned to H8.
(36) Marzilli, L. G.; Hayden, Y.; Reily, M. D. *Inorg. Chem.* **1986**, *25*, 974. See also ref 7 and 27.



reactive geometry is as follows: (a) the pK_a of the OPO_3H^- group is 6.36 (0.45 pK_a unit higher than the other geometric isomer), consistent with the 5′AMP being cis to an Me_2N, (b) the short T_1 for the H8 in D_2O, consistent with the H8 being near to a Me_2N and not an ND_2 group,[35] (c) the relatively small shift (<0.17 ppm) of H8 as a function of pH, and (d) the observation of separate rotational isomers even at 75 °C. We are currently examining the products of the reaction of Pt(*N,N,N′,N′*-Me_4en)X_2 with 5′AMP. Regardless of the ultimate identification of the products in this less tractable system, the 5′AMP must be cis to an Me_2N group. The T_1, pK_a, and shift pattern agree with our assignments. Furthermore, the models of the geometric isomers are consistent with greater ease of approach of a second 5′AMP in the *reactive* geometry. In the *reactive* isomer, the chelate Me_2N group should offer greater hindrance to rotation about the Pt–N7 bond than the chelate NH_2 group will offer to such rotation in the *unreactive* isomer. Indeed, at elevated temperature, only the *unreactive* isomer (X = Br) gives evidence of H8 signal coalescence.

In conclusion, restricted rotation of 5′AMP (and 5′dAMP) has been demonstrated with ^1H, ^{195}Pt, and ^{31}P NMR spectroscopy. Our results point to the importance of the $6NH_2$ group steric size and suggest the high selectivity for G could be a consequence of the smaller size of the 6 oxo group. Steric effects of exocyclic groups are probably greater for N1 bound A and for N3 bound C derivatives. Rotation about the Pt–N7 bond for 5′AMP cis to Cl (or H_2O) and NH_2 is facile as in the analogous 5′GMP compounds. However, in bis N7,N7 nucleotide complexes, rotation about Pt–N7 bonds is impeded detectably for 5′AMP but not for 5′GMP. In these simple nucleotide complexes, the lower pK_a of the OPO_3H^- groups on 5′AMP cis to an NH_2 groups points to the importance of H bonding between the NH and OPO_3^{2-} groups. Although relatively simple compounds have been studied, these observations are consistent with emerging new concepts on the nature of the major adducts of Pt anti-cancer drugs with G residues of DNA.[18-20,37] Our results also bear on the less well understood G selectivity and tentatively suggest a steric origin for this selectivity. In any case, these nucleotide complexes exhibit a wealth of intriguing and fundamentally interesting chemistry which deserves study in its own right.[38]

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Registry No. Cyd, 65-46-3; 5-MeCyd, 2140-61-6; 5′-dCMP, 1032-65-1; *cis*-Pt$(NH_3)_2Cl_2$, 15663-27-1; Pt(*N,N*-Me_2en)I_2, 104034-36-8; Pt(*N,N*-Me_2en)Cl_2, 41575-66-0; Pt(*N,N*-Me_2en)Br_2, 52241-19-7; Pt(*N,N,N′,N′*-Me_4en)I_2, 52241-23-3; Pt(*N,N,N′,N′*-Me_4en)Cl_2, 18957-89-6; Pt(*N,N,N′,N′*-Me_4en)Br_2, 52241-22-2; *cis*-Pt$(MeNH_2)_2I_2$, 33634-55-8; *cis*-Pt$(MeNH_2)_2Cl_2$, 15273-32-2; *cis*-Pt$(MeNH_2)_2Br_2$, 15273-31-1; Pt(en)I_2, 23858-10-8; Pt(en)(5′dAMP-N7)$_2$, 104034-37-9; *cis*-Pt(en)$_2$(5′AMP-N7)$_2$, 104051-45-8; Pt(*N,N*-Me_2en)(5′AMP-N7)$_2$, 104051-46-9; Pt(*N,N*-Me_2en)(5′AMP-N7)Cl (isomer 1), 104034-38-0; Pt(*N,N*-Me_2en)(5′AMP-N7)Cl (isomer 2), 104111-05-9; Pt(*N,N*-Me_2en)(5′AMP-N7)Br (isomer 1), 104034-39-1; Pt(*N,N*-Me_2en)(5′AMP-N7)Br (isomer 2), 104111-06-0; Pt(*N,N*-Me_2en)(5′AMP-N7)I (isomer 1), 104034-40-4; Pt(*N,N*-Me_2en)(5′AMP-N7)I (isomer 2), 104111-07-1; Pt(en)(Cyd)(H_2O), 104034-41-5; Pt(en)(Cyd)$_2$, 104034-42-6; Pt(en)(5-MeCyd), 104034-43-7; Pt(en)(5-MeCyd)$_2$, 104051-47-0; Pt(en)(5′dCMP-N_3)(H_2O), 104051-48-1; Pt(en)(5′dCMP-N_3)$_2$, 104034-44-8; ^{125}Pt, 14191-88-9.

(37) Reedijk, J.; Fichtinger-Schepman, A. M. J.; van Oosterom, A. T.; van de Putte, P. *Struct. Bonding*, in press.

(38) **Note Added in Proof**: A species which appears to be identical with the *cis*-Pt$(NH_3)_2$(5′AMP-N7)$_2$ complex studied here was reported while this report was in press (Bose, R. N.; Cornelius, R. D.; Viola, R. E. *J. Am. Chem. Soc.* **1986**, *108*, 4403). However, three alternative species were suggested, all involving a 1:1 stoichiometry for the adducts and direct Pt to phosphate oxygen bonds. We feel that the ^{195}Pt NMR results on the 5′AMP complex (Table I) and all of our results on analogous complexes studied here are inconsistent with all three of the suggested alternative structures.