Stereospecific Hydrogen-Bonding in Mononucleotide Adducts of Platinum Anticancer Complexes in Aqueous Solution

Susan J. Berners-Price,[+] Urban Frey,[‡] John D. Ranford,[‡] and Peter J. Sadler*[,‡]

Contribution from the School of Science, Griffith University, Nathan, Queensland 4111, Australia, and the Department of Chemistry, Birkbeck College, University of London, Gordon House and Christopher Ingold Laboratories, 29 Gordon Square, London WC1H 0PP, U.K.

Received February 12, 1993

Abstract: The ammine and amine N–H ^{1}H NMR resonances of Pt(II) complexes containing 5′-phosphate derivatives of nucleobases undergo significant low-field shifts in aqueous solution consistent with stereospecific H-bonding between Pt–NH protons and *cis* nucleotide 5′-phosphate groups. The effects are strongest for NH protons held rigidly in a chelate ring (ethylenediamine, en, complexes), when the phosphate is fully deprotonated, and when rotation about Pt–N7 (head-to-tail isomerism) is slow, as in $[Pt(en)(5'\text{-AMP-}N7)_2]^{2+}$. The temperature dependences of Pt–NH ^{1}H NMR resonances for these complexes are low ($<|-5.6 \times 10^{-3}|$ ppm K^{-1}) but not simply diagnostic of H-bonding. The nucleobase complexes investigated were *cis*-$[Pt(X)(Y)(^{15}NH_3)(^{15}NH_2CH_3)]^{2+}$, $[Pt(^{15}N\text{-en})(X)(Y)]^{2+}$, and $[Pt(N,N\text{-}Me_2\text{-en})(X)(Y)]^{2+}$ where X = Y = 5′-GMP, 5′-dGMP, 3′-GMP, G, 5′-AMP, or 3′-AMP or Y = Cl or H_2O. Data for the complexes *trans*-$[Pt(X)(Y)(NH_3)_2]^{n+}$, X, Y = 5′-GMP, Cl, H_2O are reported for comparison with the *cis* analogues, and the shifts are rationalized in terms of *cis* and *trans* influences.

Introduction

A wide range of Pt(II) and Pt(IV) complexes containing ammines and primary or secondary amines exhibit anticancer activity[1] and the N–H bonds in these complexes are thought to play a key role in their mechanism of action.[2] The primary target site for platinum anticancer drugs is DNA[3] and the critical lesions may involve intrastrand cross-links between two guanine bases (GpG, *N7*,*N7* chelate), or an adenine and a guanine (ApG *N7*,*N7* chelate).[4]

There are many reports in the literature that H-bonding stabilizes platinum ammine and amine complexes containing nucleobases. Such interactions may strongly influence the kinetics of reactions and contribute to the preference of Pt for particular bases and particular cross-links. Hydrogen bonds between N–H protons of coordinated amines and C6O and 5′-phosphates of nucleobases in the solid state have been described.[5] For example, in the crystal structures of[6] $[Pt(en)G_2]Cl_{1.5}I_{0.5}\cdot 2H_2O$ and $Na_2[Pt(en)(5'\text{-GMP})_2]\cdot 3H_2O$ there is intermolecular H-bonding between the C6O carbonyl and the en NH_2 of a neighboring molecule.[7,8] In the crystal structure[9] of *cis*-$[Pt(NH_3)_2\{d(pGpG)\text{-}N7(1),N7(2)\}]^{2+}$ there appears to be only weak interligand ammine–C6O H-bonding but stronger intramolecular H-bonding with the terminal 5′-phosphate. Weak ammine–C6O bonds have also been detected for *cis*-$[Pt(NH_3)_2\{d(CpGpG)\text{-}N7(2),N7(3)\}]^{2+}$, but phosphate–ammine contacts are absent.[10] In the recent crystal structure of $[Pt(dien)\{(ApGpA)\text{-}N7(2)\}]^{2+}$,[11] there are both types of weak H-bonds, and these appear to determine the dihedral angle between guanine and the PtN_4 coordination plane. However, the molecular structure and conformation of $[Pt(dien)\{(ApGpA)\text{-}N7(2)\}]^{2+}$ are different in the solid state and in solution. This was attributed to crystal packing effects. Thus H-bonding interactions of N–H protons have been clearly demonstrated in the solid state, but their existence in solution remains to be established.

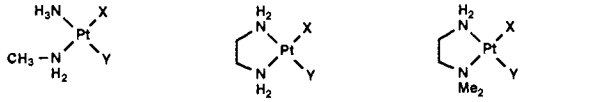
In solution, ^{1}H NMR studies of H-bonding in ammine and amine platinum nucleotide complexes have been made only at low pH: Xu et al.[12] inferred from H8–NH ^{1}H NMR NOE data for $[Pt(N,N\text{-dimethyl-2,3-diaminobutane})X_2]^{2+}$ (X = 5′-GMP or 5′-dGMP) obtained at pH 3 (^{14}NH peaks), that NH–C6O H-bonding was predominant. However, there is indirect evidence for NH–phosphate H-bonding from other reported work. The downfield shifts of ^{31}P NMR resonances of phosphate groups 5′ to GpG units have been interpreted in terms of H-bonding with an ammine or amine ligand,[13–15] and the pK_a's of the 5′-phosphate groups of 5′-AMP and 5′-dAMP have been reported to be significantly lowered when these nucleotides are coordinated *cis* to an NH_2 group in complexes such as $[Pt(en)(5'\text{-AMP-}N7)_2]^{2+}$.[16] The existence of ammine–phosphate H-bonding in a d(GpG)-platinated decanucleotide duplex has been inferred from analysis of CH NMR shifts, coupling constants, and sugar ring puckers.[17] Theoretical calculations too have suggested that such H-bonds

* To whom correspondence should be addressed.
+ Griffith University.
‡ University of London.

(1) Farrell, N. *Transition Metal Complexes as Drugs and Chemotherapeutic Agents*; Kluwer Academic: Dordrecht, 1989.
(2) (a) Reedijk, J. *J. Inorg. Biochem.* **1991**, *43*, 80. (b) Reedijk, J. *Inorg. Chim. Acta* **1992**, *198*–200, 873–881.
(3) Pinto, A. L.; Lippard, S. J. *Biochim. Biophys. Acta* **1985**, *780*, 167–180.
(4) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153–1181.
(5) Lippert, B. *Prog. Inorg. Chem.* **1989**, *37*, 1–97.
(6) Abbreviations: 3′-AMP, adenosine 3′-monophosphate; 5′-AMP, adenosine 5′-monophosphate; dien, diethylenetriamine; DMF, dimethylformamide; en, 1,2-diaminoethane (ethylenediamine); G, guanosine; 3′-GMP, guanosine 3′-monophosphate; 5′-GMP, guanosine 5′-monophosphate; 5′-dGMP, 2′-deoxyguanosine 5′-monophosphate; h/h, head-to-head; h/t, head-to-tail (rotamers of purine complexes about Pt–N7 bond, H8's of two coordinated purines on same side or opposite sides of PtN_4 plane, respectively); HMQC, heteronuclear multiple quantum coherence; TSP, sodium trimethylsilyl[*2,2,3,3-d*$_4$]propionate. The charges on nucleobases are ignored in formulas.
(7) Gellert, R. W.; Bau, R. *J. Am. Chem. Soc.* **1975**, *97*, 7379–7380.
(8) Gellert, R. W. Ph.D. Thesis, University of California, 1980. Bau, R.; Gellert, R. W. Unpublished.
(9) Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 7368–7381.
(10) Admiraal, G.; van der Veer, J. L.; de Graaff, A. G.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1987**, *109*, 592–594.
(11) Admiraal, G.; Alink, M.; Altona, C.; Dijt, F. J.; van Garderen, J.; de Graaff, R. A. G.; Reedijk, J. *J. Am. Chem. Soc.* **1992**, *114*, 930–938.
(12) Xu, Y.; Natile, G.; Intini, F. P.; Marzilli, L. G. *J. Am. Chem. Soc.* **1990**, *112*, 8177–8179.
(13) Den Hartog, J. H. J.; Altona, C.; van der Marel, G. A.; Reedijk, J. *Eur. J. Biochem.* **1985**, *147*, 371–379.
(14) Spellmeyer Fouts, C.; Marzilli, L. G.; Byrd, R. A.; Summers, M. F.; Zon, G.; Shinozuka, K. *Inorg. Chem.* **1988**, *27*, 366–376.
(15) Bloemink, M. J.; Heetebrij, R. J.; Inagaki, K.; Kidani, Y.; Reedijk, J. *Inorg. Chem.* **1992**, *31*, 4656–4661.
(16) Reily, M. D.; Marzilli, L. G. *J. Am. Chem. Soc.* **1986**, *108*, 6785–6793.

0002-7863/93/1515-8649$04.00/0

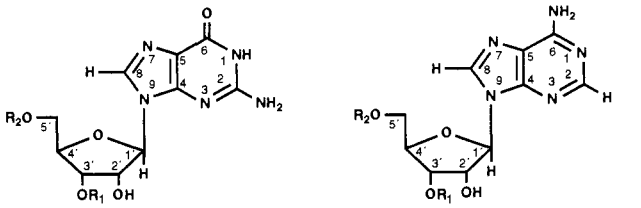
Chart I



1 : X,Y = Cl 8 : X,Y = Cl 13 : X,Y = 5′-GMP

2 : X,Y = 5′-GMP 9 : X,Y = 5′-GMP 16 : X,Y = 5′-AMP

3 : X,Y = 5′-AMP 14 : X,Y = 5′-AMP



Guanosine R_1 = H, R_2 = H Adenosine R_1 = H, R_2 = H

3′-GMP $R_1 = PO_3H_2$, R_2 = H 3′-AMP $R_1 = PO_3H_2$, R_2 = H

5′-GMP R_1 = H, $R_2 = PO_3H_2$ 5′-AMP R_1 = H, $R_2 = PO_3H_2$

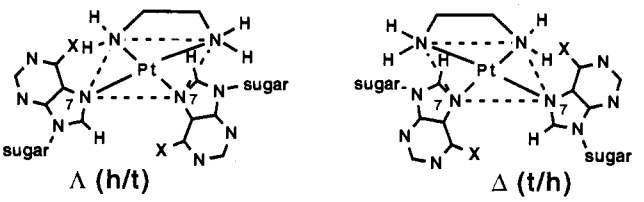
(either direct or via an intervening water molecule) contribute to the stability of the adducts.[18-21]

The Pt–NH ^{1}H NMR resonances of platinum anticancer complexes cannot be readily detected in D_2O solutions because the half-life for H–D exchange is only a few minutes at ambient temperatures,[22,23] and in H_2O solutions, the intense H_2O peak causes severe dynamic range problems. In addition, N–H resonances are often broadened by the quadrupolar effects of ^{14}N (I = 1, 99.6% natural abundance). Resonances for NH protons of lipophilic platinum amines in nonaqueous media have been reported,[24] and $^1J(^{14}N-^1H)$ couplings have been observed,[5] but the latter were reported to be unaffected by nucleobase coordination to platinum.

Recently, we applied ^{15}N-edited ^{1}H NMR spectroscopy and 2D [^{1}H, ^{15}N] heteronuclear multiple quantum coherence (HMQC) NMR spectroscopy to the direct detection of ammine and amine N–H resonances of ^{15}N-labeled platinum complexes in aqueous solution.[25,26] Effective water suppression was achieved, and resonances from complexes in the 0.2–10 mM concentration range were readily observable within a few minutes. Two-dimensional correlations of ^{1}H and ^{15}N NMR chemical shifts provided assignments of ^{1}H NMR resonances.

In this work we have investigated whether Pt–NH ^{1}H NMR shifts provide direct evidence for H-bonding interactions in nucleobase complexes in aqueous solutions. We have studied both ammine and amine (methylamine and ethylenediamine) Pt(II) complexes with G and A purine bases, with various sites of phosphorylation (3′ or 5′, Chart I), and also the effects of pH and temperature.

Chart II



Λ (h/t) Δ (t/h)

There are three stereoisomers of square-planar complexes having two *cis* ligands with C_s local symmetry.[27] The head-to-tail enantiomers (for example as illustrated in Chart II, H8's of coordinated purines on the opposite side of the PtN_4 plane, X is C=O for G, NH_2 for A) are expected to be greatly favored over the head-to-head isomer.[27-29] When the purine carries a chiral ribose, the h/t enantiomers become diastereomers, and, in principle, can be distinguished by NMR.[29] By comparing G and A derivatives it is possible to examine the effects of rotation of the purine about Pt–N7 on H-bonding. Fast rotation has been established previously for mononucleobase G complexes unless there is steric hinderance from bulky groups on the coordinated amine.[30-32] The slower rotation in AMP complexes has been attributed to steric hinderance provided by the 6-amino group.[16]

Our studies provide direct evidence for a special role for mononucleotide 5′-phosphate groups in H-bonding with Pt–N–H protons in aqueous solution.

Materials and Methods

Materials. Nucleobases, KCl, KI, NEt_4Cl, and $^{15}NH_4Cl$ were purchased from Aldrich or Sigma. $K_2[PtCl_4]$ and *cis*-$[PtCl_2(NH_3)_2]$ were obtained from Johnson Matthey. $^{15}NH_2CH_3$·HCl was purchased from MSD Isotopes. *trans*-$[PtCl_2(^{15}NH_3)_2]$ was the gift of Dr. T. G. Appleton (University of Queensland, Australia).

Preparation of Complexes. ***cis*-$[PtCl_2(^{15}NH_3)_2]$ (6)** and **$[Pt(^{15}N$-en)$Cl_2]$ (8)** were synthesized as described previously[33] and recrystallized from aqueous potassium chloride or dilute HCl. The diaqua complex $[Pt(^{15}N\text{-en})(H_2O)_2]^{2+}$ was prepared by the addition of slightly less than 2 mol equiv of $AgNO_3$ (33.1 mg, 195 μmol) to a solution of the dichloro complex (32.8 mg, 100 μmol) in DMF-d_7 (300 μL), incubation at 310 K for ca. 24 h, removal of the AgCl precipitate by centrifugation, and dilution with 95% H_2O/5% D_2O (7.7 mL). The final Pt concentration was 12 mM, and the solution had a pH of 3.1 and contained 3% DMF. The $^1H\{^{15}N\}$ spectrum of this solution showed the presence of ca. 7% unreacted $[Pt(^{15}N\text{-en})Cl_2]$. Stock solutions of *cis*- and *trans*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$ were similarly prepared from their respective dichloro complexes.

***cis*-$[PtCl_2(NH_3)(NH_2CH_3)]$ (1).** ^{15}N-Labeled and unlabeled **1** were prepared from labeled or unlabeled *cis*-$[PtCl_2(NH_3)_2]$, respectively, according to a reported method.[34] Elemental Anal. found (calculated): Cl, 22.61 (22.58); C, 4.15 (3.82); H, 2.58 (2.57); N, 8.67 (8.92). The purity was also checked by ^{1}H NMR, deposited Figure D1. Solubility in H_2O at 298 K was ca. 5 mM.

(17) Herman, F.; Kozelka, J.; Stoven, V.; Guittet, E.; Girault, J.-P.; Huynh-Dinh, T.; Igolen, J.; Lallemand, J.-Y.; Chottard, J.-C. *Eur. J. Biochem.* **1990**, *194*, 119–133.

(18) Kozelka, J.; Petsko, G. A.; Lippard, S. J.; Quigley, G. J. *J. Am. Chem. Soc.* **1985**, *107*, 4079–4081.

(19) Kozelka, J.; Petsko, G. A.; Quigley, G. J.; Lippard, S. J. *Inorg. Chem.* **1988**, *25*, 1075–1077.

(20) Hambley, T. W. *Inorg. Chem.* **1991**, *30*, 937–942.

(21) Kozelka, J.; Chottard, J.-C. *Biophys. Chem.* **1990**, *35*, 165–178.

(22) Koubek, E.; House, D. A. *Inorg. Chim. Acta* **1992**, *191*, 103–107.

(23) A curious exception appears to be *cis*-$\{Pt(G)(X)(NH_2Me)_2\}$, X = Cl or G, for which slow N–H exchange has been detected via NH–CH coupling, but no N–H resonance was reported: Miller, S. K.; Marzilli, L. G. *Inorg. Chem.* **1985**, *24*, 2421–2425.

(24) Cherchi. V.; Faraglia, G.; Sindellari, L. In *Lectures in Bioinorganic Chemistry*; Nicolini, M., Sindellari, L., Eds.; Cortina Int.: Verona, 1991; p 103.

(25) Berners-Price, S. J.; Frenkiel, T. A.; Ranford, J. D.; Sadler, P. J. *J. Chem. Soc., Dalton Trans.* **1992**, 2137–2139.

(26) Berners-Price, S. J.; Frenkiel, T. A.; Frey, U.; Ranford, J. D.; Sadler, P. J. *J. Chem. Soc., Chem. Commun.* **1992**, 789–791.

(27) Cramer, R. E.; Dahlstrom, P. L. *Inorg. Chem.* **1985**, *24*, 3420–3424.

(28) Marcelis, A. T. M.; Korte, H. J.; Krebs, B.; Reedijk, J. *Inorg. Chem.* **1982**, *21*, 4059–63.

(29) There are only a few examples of h/h rotamers in crystalline complexes (ref 5), and the h/h form of [Pt(*N*,*N*-dimethyl-2,3-diaminobutane)(5-GMP)$_2$]$^{2+}$ has been detected as a minor isomer in solution (ref 12). For the Pt(^{15}N-en) complexes, the h/t enantiomers should give rise to only two nonequivalent NH resonances. If rotation is fast on the NMR time scale, these become a single resonance. For the two h/t diastereomers a total of four NH resonances should be observed, becoming two peaks in the fast exchange situation, although the additional nonequivalence introduced by the chiral ribose might be small. The Δ and Λ assignments in Chart II are those of ref 27.(30) Cramer, R. E.; Dahlstrom, P. L. *J. Am. Chem. Soc.* **1979**, *101*, 3679–3681.

(31) Marcelis, A. T. M.; van der Veer, J. L.; Zoetslook, J. C. M.; Reedijk, J. *Inorg. Chim. Acta* **1983**, *78*, 195–203.

(32) Inagaki, K.; Dijt, F. J.; Lempers, E. L. M.; Reedijk, J. *Inorg. Chem.* **1988**, *27*, 382–387.

(33) Kerrison, S. J. S.; Sadler, P. J. *J. Chem. Soc., Chem. Commun.* **1977**, 861–864.

(34) Abrams, M. J.; Giandomenico, C. M.; Vollano, J. F.; Schwartz, D. A. *Inorg. Chim. Acta* **1987**, *131*, 3–4.

***cis*-[Pt(X)$_2$(^{15}NH$_3$)(^{15}NH$_2$CH$_3$)]$^{2+}$, X = 5′-GMP (2), 5′-AMP (3).** A typical sample preparation was as follows. *cis*-[PtCl$_2$(^{15}NH$_3$)(^{15}NH$_2$CH$_3$)] (0.9 mg, 2.8 μmol) and Na$_2$5′-GMP·3H$_2$O (3.3 mg, 7.2 μmol) were weighed directly into an NMR tube and 0.5 mL of 0.1 M phosphate buffer, pH 7.0 in 95% H$_2$O/5% D$_2$O, was added to give a final concentration of 6 mM Pt and 14 mM 5′-GMP. The sample was ultrasonicated for ca. 30 s, then shielded from light by Al foil, and incubated for 2 h at 343 K in a water bath. The pH of the sample was adjusted using HNO$_3$ and KOH solutions.

[Pt(*N,N*-Me$_2$-en)Cl$_2$]. The procedure of Alink et al.[35] was followed. *N,N*-Me$_2$-en (44 mg, 0.50 mmol) in 0.1 M HCl (0.50 mL) was added dropwise with stirring to a hot solution of K$_2$PtCl$_4$ (0.21 g, 0.50 mmol) in H$_2$O (2 mL). The color changed from deep red to orange and a precipitate formed after 5 min. After stirring for 10 min the mixture was refrigerated overnight, and the yellow crystalline solid was filtered, washed with H$_2$O (2 × 1 mL), and dried *in vacuo*: yield 0.105 g, 59%.

[Pt(*N,N*-Me$_2$-en)(5′-GMP)$_2$]$^{2+}$ (13). [Pt(*N,N*-Me$_2$-en)Cl$_2$] (35.6 mg, 0.10 mmol) and AgNO$_3$ (33.9 mg, 0.20 mmol) were reacted overnight at 310 K in DMF-d_7 (300 μL), and the AgCl precipitate was removed by centrifugation. A 150-μL aliquot of the resultant solution was added to Na$_2$5′-GMP·3H$_2$O (46 mg, 0.10 mmol) in 95% H$_2$O/5% D$_2$O (0.40 mL) in a 5-mm NMR tube. A white precipitate formed initially but redissolved on heating at 333 K for 10 min. The final solution contained 91 mM Pt and 182 mM 5′-GMP, and the pH was adjusted to 6.2 with 1 M HNO$_3$ before acquiring the ^{1}H NMR spectrum.

[Pt(*N,N*-Me$_2$-en)(5′-AMP)$_2$]$^{2+}$ (16). This was prepared in a similar way by *in situ* reaction of [Pt(*N,N*-Me$_2$-en)(H$_2$O)$_2$]$^{2+}$ and 5′-AMP. The pH of the solution (91 mM in Pt and 182 mM in 5′-AMP) was raised from 4.8 to 6.2 before acquiring the ^{1}H NMR spectrum.

***trans*-[Pt(5′-GMP)$_2$(^{15}NH$_3$)$_2$]$^{2+}$ (5).** An aliquot (0.1 mL) of a stock solution of Na$_2$5′-GMP·3H$_2$O (99.7 mM) was added to 0.4 mL of a 9.9 mM solution of *trans*-[Pt(H$_2$O)$_2$(^{15}NH$_3$)$_2$]$^{2+}$ in 95% H$_2$O/5% D$_2$O to give a solution 7.9 mM in Pt and 19.9 mM in 5′-GMP. The pH was adjusted to 4.39, and this increased to 5.16 over a period of 2 days at 310 K. NMR spectra were recorded after readjusting the pH to 4.3 and 6.5.

[Pt(^{15}N-en)(H$_2$O)(5′-GMP)]$^{2+}$ (26). An aliquot (0.1 mL) of a stock solution of 5′-GMP (37.7 mM, pH adjusted to 6.41 with 0.5 M HNO$_3$) was added to 0.4 mL of a 10.8 mM stock solution of the diaqua complex, to give a solution 8.6 mM in Pt and 7.5 mM in 5′-GMP, pH 4.82 which decreased to 4.34 over a period of 6 h.

[Pt(^{15}N-en)X$_2$]$^{2+}$, X = G (12), 3′-GMP (11), 5′-GMP (9), 5′-dGMP (10), 3′-AMP (15), 5′-AMP (14). These complexes were all prepared directly in NMR tubes, and the solutions usually contained a slight excess of nucleobase relative to Pt. The preparation of the X = 5′-GMP complex was typical. An aliquot (0.10 mL) of a stock solution of Na$_2$5′-GMP·3H$_2$O (110 mM) was added to 0.4 mL of the 12.5 mM stock solution of the diaqua complex to give a solution 10 mM in Pt and 22 mM in 5′-GMP. The solution was heated at 323 K for 15 min and left at ambient temperature for 5 h. The final pH was 7.08. For the solution containing 3′-GMP, a white precipitate formed on addition of the nucleotide to the diaqua complex, but this dissolved on heating to 343 K for 1 min. The sample was incubated at 310 K for 12 h, and the final pH was 3.45. The 5′-AMP and 3′-AMP samples were incubated for 12 h at 310 K, and the final pH values were 3.13 and 3.60, respectively. The pH values of all solutions were adjusted as required with 0.2 M HNO$_3$ or NaOH.

In order to compare ^{1}H NMR spectra under identical conditions, the guanine series of complexes was prepared (directly in NMR tubes) in 20 mM phosphate buffer (pH 6.5, 95% H$_2$O/5% D$_2$O). An initial ultrasonication for ca. 30 s was used to aid dissolution of [Pt(^{15}N-en)Cl$_2$] (10 mM) in a solution containing a slight excess of the base. The solutions were heated at 343 K for 10 min and then incubated overnight at 310 K. A 1:2 solution of [Pt(^{15}N-en)Cl$_2$] (2 mM) and 5′-GMP was also studied (i.e., in the absence of phosphate buffer) for comparison. After incubation at 310 K for 15 h the pH was 6.12.

NMR Spectroscopy. 500.13 MHz ^{1}H{^{15}N} NMR spectra were recorded on a Bruker AM-500 spectrometer fitted with a BSV-7 transmitter, a BFX-5 X nucleus decoupler, and a 5 mm inverse probehead. Sample spinning was not used. All samples were prepared in 95% H$_2$O/5% D$_2$O, and the water signal was preirradiated (usually for 1.5 s) by means of a DANTE sequence.[36] One-dimensional ^{15}N-edited ^{1}H spectra were recorded using a spin-echo difference sequence,[37] optimized for ^1J(N,H) = 73 Hz ($\tau = 1/2J$). For some samples, improved H$_2$O suppression was obtained with $\tau = n/2J$, $n = 3, 5, 7$. Typically 16 transients were acquired.

Two-dimensional [^{1}H, ^{15}N] HMQC spectra were recorded as previously described[26] using the standard sequence,[38] modified to include a pair of purge pulses for improved suppression of signals from protons not bound to ^{15}N, as proposed by Otting and Wüthrich.[39] The sequence was optimised for ^1J(N,H) = 73 Hz ($1/2J$ = 6.8 ms), and the lengths of the purge pulses were adjusted for each sample in the range 0–3 ms to maximize solvent suppression, with the two pulses of different duration to avoid refocussing effects. All 2D spectra were acquired using the TPPI method[40] to give absorption mode line shapes with sign discrimination in F_1. During the acquisition time of the 2D experiments, the ^{15}N spins were decoupled by irradiating with the GARP-1 sequence[41] at a field strength of 1.7 kHz. 2D spectra were processed using sine-bell weightings in both dimensions.

For each sample a standard protocol was adopted: a 1D spectrum with water suppression by presaturation was acquired followed by an ^{15}N-edited spectrum and an edited and ^{15}N-decoupled 1D spectrum. Usually a 12–24 min 2D [^{1}H, ^{15}N] HMQC spectrum was also then acquired. Some 500-MHz ^{1}H and 50.7-MHz ^{15}N NMR spectra were obtained on a JEOL GSX 500, and 270-MHz ^{1}H NMR spectra on a JEOL GSX270.

^{31}P NMR spectra were obtained on Bruker WM200 (81 MHz) or Bruker AM 400 (162 MHz) spectrometers at 298 K. The samples were the same as those used for ^{1}H NMR. Typical pulsing conditions: 60–70° pulses, 8 K data points, 5-s relaxation delay, a final resolution of 1 Hz, 100–500 transients.

^{1}H NMR spectra were referenced to TSP (usually added to samples just before NMR spectra were taken as microliter aliquots of a 10 mg/mL solution) or dioxan (3.767 ppm relative to TSP), ^{15}N spectra (at 50.67 MHz) to 1.5 M NH$_4$Cl in 1 M HCl (external), and ^{31}P to 85% H$_3$PO$_4$ (external).

Measurements of pH. These were made at ca. 298 K directly in the NMR tube, before and after recording spectra, using a Corning 240 meter equipped with an Aldrich micro combination electrode calibrated with Aldrich buffer solutions at pH 4, 7, and 10.

Results

Our preliminary NMR work[25] on 5′-GMP adducts of cisplatin suggested that it was possible to detect H-bonding from the chemical shift behavior of the Pt–NH$_3$ protons. However, this was complicated by the direct overlap of NH$_3$ resonances with the intense water peak. A similar downfield shift is observed on formation of *trans*-[Pt(5′-GMP-*N7*)$_2$(^{15}NH$_3$)$_2$]$^{2+}$ (**5**) from *trans*-[PtCl$_2$(^{15}NH$_3$)$_2$] (**4**): 0.66 ppm at pH 6.5 (cf. 0.59 for cisplatin at pH 7.9) and 0.55 ppm at pH 4.3 (c.f. 0.39 ppm for cisplatin, pH 4.4). These data together with those for aqua complexes and analogous *cis* species reported previously[25,26] are collected in Table I. This includes a reassignment of resonances for the NH$_3$ ligands in *cis*-[PtCl(5′-GMP-*N7*)(^{15}NH$_3$)$_2$]$^+$ (**22**, see Discussion). In the present work we sought to avoid the problems caused by overlap of NH$_3$ resonances and the intense H$_2$O peak by the use of primary amines which have N–H shifts to lower field of water. First we studied mixed-ligand monoammine–monomethylamine complexes and then chelated 1,2-diaminoethane complexes. Our strategy involved establishing that H-bonding is indeed being observed by studying pH and temperature dependences of NH peaks. The role of 5′-phosphates was investigated by comparing 5′- and 3′-nucleotide monophosphates with nucleosides of guanine and adenine.

***cis*-[PtCl$_2$(NH$_3$)(NH$_2$CH$_3$)] (1).** The ^{1}H NMR spectrum of unlabeled **1** in DMF-d_7 consists of three resonances (deposited Figure D1): a triplet for the CH$_3$ protons with ^{195}Pt satellites and two broad resonances for the NH$_2$ and NH$_3$ protons. In contrast, ^{15}N labeled-**1** gives rise to sharp multiplets with resolved fine structure for both the NH$_3$ and NH$_2$ protons due to the different

(35) Alink, M.; Nakahara, H.; Hirano, T.; Inagaki, K.; Nakanishi, M.; Kidani, Y.; Reedijk, J. *Inorg. Chem.* **1991**, *30*, 1236–1241.
(36) Morris, G. A.; Freeman, R. *J. Magn. Reson.* **1978**, *29*, 433–462.
(37) Freeman, R.; Mareci, T. H.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 341–345.
(38) Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301–315.
(39) Otting, G.; Wüthrich, K. *J. Magn. Reson.* **1988**, *76*, 569–574.
(40) Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967–974.
(41) Shaka, J.; Barker, P. B.; Freeman, R. *J. Magn. Reson.* **1985**, *64*, 547–552.

Table I. ^1H and ^{15}N NMR Chemical Shifts (δ) and Coupling Constants (Hz) for *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ and Their Aqua and 5′-GMP Adducts at 300 K (Unless Otherwise Stated)[h]

complex		pH	δH8	δH1′	J(Pt,H)	δ^1H ($\delta^{15}N$) obsd	δ^1H ($\delta^{15}N$) calc[a]
trans-$[PtCl_2(^{15}NH_3)_2]$	**4**	*b*			53	3.60 (–66.6)	
trans-$[Pt(H_2O)_2(^{15}NH_3)_2]^{2+}$	**17**	4.3			49	4.03 (–63.7)	4.10 (–62.6)
trans-$[PtCl(H_2O)(^{15}NH_3)_2]^{+}$ [c]	**18**	4.3				3.87 (–65.4)	3.85 (–64.6)
trans-$[Pt(5'\text{-GMP-}N7)_2(^{15}NH_3)_2]^{2+}$	**5**	4.3	8.77	6.02		4.15	4.06 (–56.8)
		6.5	8.91	6.03		4.26 (–59.2)	
trans-$[Pt(H_2O)(5'\text{-GMP-}N7)(^{15}NH_3)_2]^{2+}$ [c]	**19**	4.6	8.66	6.02		4.12 (–61.4)	4.08 (–59.7)
cis-$[PtCl_2(^{15}NH_3)_2]$	**6**	*b*			63	4.08 (–68.8)	
cis-$[Pt(H_2O)_2(^{15}NH_3)_2]^{2+}$	**20**	low[g]				4.51 (–87.2)	4.50 (–88.0)
cis-$[PtCl(H_2O)(^{15}NH_3)_2]^{+}$	**21**	low[g]				4.33 (–66.8) (*trans* Cl)	
						4.25 (–90.0) (*trans* O)	
cis-$[PtCl(5'\text{-GMP-}N7)(^{15}NH_3)_2]^{+}$	**22**	6.5[d]	8.61	5.99		4.38 (–66.4) (*trans* Cl)	4.31 (–63.9)
						4.09 (–69.1) (*trans* N)	4.12 (–68.6)
cis-$[Pt(5'\text{-GMP-}N7)_2(^{15}NH_3)_2]^{2+}$	**7**	6.5[d]	8.63 (8.14)[e]	5.90 (5.92)[e]			
		7.9[d,f]				4.67 (–66.6)	
		4.4[d]				4.47 (–67.5)	4.33 (–64.5)
cis-$[Pt(H_2O)(5'\text{-GMP-}N7)(^{15}NH_3)_2]^{2+}$	**23**	4.1				4.48 (–85.1) (*trans* O)	
						4.37 (–66.6) (*trans* N)	

[a] Calculated values based on the following influences on chemical shifts (*trans* T, *cis* C, in ppm) compared to Z = Cl:

ligand (Z)	$T(\delta)_Z$		$C(\delta)_Z$	
	^{15}N (lit. value)	^1H	^{15}N (lit. value)	^1H
H_2O	–21.2 (–18.8)	+0.17	+2.0 (+2.2)	+0.25
5′-GMP	+0.2	+0.04	+4.9	+0.23

Literature values are those of Appleton et al. (ref 49). The values for 5′-GMP complexes should be independent of H-bonding (note 50). [b] 154 mM NaCl. [c] Tentative assignment. [d] 310 K. [e] Free 5′-GMP. [f] 0.5 M NH_4^+. [g] Limiting low pH chemical shifts from titration curves (ref 26). [h] Data for the *cis* complexes from refs 25 and 26.

Table II. ^1H and ^{15}N Chemical Shifts (δ) and Coupling Constants (J, Hz) for ^{15}N-Labeled *cis*-$[PtX_2(NH_3)(NH_2CH_3)]$ Complexes at Various Temperatures in DMF-d_7 or 95% H_2O/5% D_2O

complex, X (solvent)	T (K)	NH_2 δ^1H[a]	NH_2 $\delta^{15}N$	NH_3 δ^1H[a]	NH_3 $\delta^{15}N$[b]	CH_3 δ^1H	H8 δ^1H
1, Cl (DMF)	298	4.84(68.8)		4.24(62.7)		2.459[c]	
1, Cl	275	4.82(66)	–62.5	4.20(61)	–66.3	2.440	
(0.14 M NaCl)	298	(4.7)[d]		4.14(64)	–65.8(319)	2.441[c]	
	333	4.63(65)		4.05(64)		2.448	
2, 5′-GMP	275	nd		nd		2.371	8.669/8.767
(0.10 M P_i, pH 7)	298	5.22	–61.7	4.61	–64.4	2.356	8.593/8.672
	333	5.09		*d*		2.357	8.535/8.609
3, 5′-AMP	275	5.61/5.94	–58.1	(4.53/4.59)[e]	(–61.6/–54.7)[e]	2.334/2.365	9.365/9.503/9.560/9.702
(0.1 M P_i, pH 7)		5.69/5.84	–58.0				
	298	5.53/5.86	–57.5	*d*		2.334/2.364	9.383/9.516/9.560/9.695
		5.58/5.77	–57.5				
	333	5.47/5.79		(4.88/4.91)[e]		2.340/2.368	9.414/9.541/9.571/9.699
		5.49/5.71					

[a] ^2J (^1H, ^{195}Pt) in parentheses; nd not detected. [b] ^1J (^{15}N, ^{195}Pt) in parentheses. [c] ^3J ($^1H(CH_3)$, $^1H(NH_2)$) = 6.2, ^2J (^1H, ^{195}Pt) = 44 in H_2O, ^3J ($^1H(CH_3)$, $^1H(NH_2)$) = 6.3, ^2J (^1H, ^{195}Pt) = 48 in DMF-d_7. [d] Beneath H_2O. [e] Uncertain assignments.

^{15}N–^1H, ^{195}Pt–^1H, and ^1H–^1H couplings (deposited Figure D1 and Table II). ^1H, ^1H-^{15}N-Edited and [^1H, ^{15}N] HMQC NMR spectra of ^{15}N-**1** in 0.14 M Cl^- (to suppress hydrolysis) were recorded at different temperatures, and the chemical shifts and coupling constants are listed in Table II.

Reaction of 1 with 5′-GMP. After incubation of **1** with 5′-GMP (1:2.6 mol ratio) in 0.1 M phosphate buffer pH 7.0 for 10 min at 343 K, the ^1H NMR spectrum showed three major peaks in the H8 region, assignable to free 5′-GMP (8.196 ppm) and *cis*-$[Pt(5'\text{-GMP-}N7)_2(^{15}NH_3)(^{15}NH_2CH_3)]^{2+}$, **2**. Two minor peaks were also visible at 8.643 and 8.725 ppm assignable to the two isomers of the monoadduct *cis*-$[PtCl(5'\text{-GMP})(^{15}NH_3)(^{15}NH_2CH_3)]^{+}$. After additional incubation for 2 h, the latter resonances were no longer visible, and resonances for species **2** were observed as the sole product. Lowering the temperature to 275 K broadened all the resonances of complex **2**. The line width of the H8 signal at 8.767 ppm was ca. twice as large as that of the peak at 8.669 ppm, and the NH_2 and NH_3 ^1H NMR resonances were apparently broadened beyond detection. However when the temperature of the sample was raised to 333 K, the $^1H\{^{15}N\}$ resonance for the NH_2 protons was a well-resolved quartet although that for the NH_3 protons was not resolvable from the water peak. ^1H and ^{15}N NMR data for **2** are listed in Table II.

The reaction of **1** with 5′-GMP in a 1:1 mol ratio also gave **2** as the major product together with hydrolyzed **1**. The monocomplex was observed as a minor intermediate. The ready formation of bis GMP complexes at 1:1 cisplatin/GMP ratios, and even when cisplatin is present in excess, has been noted previously.[42]

Reaction of 1 with 5′-AMP. The ^1H NMR spectrum (298 K) of a solution containing ^{15}N-**1** and 5′-AMP in a 1:2.3 mol ratio after incubation in 0.1 M phosphate buffer for 10 min at 343 K showed five major peaks in the H8 region, one assignable to free 5′-AMP (8.589 ppm) and four of equal intensities assignable to *cis*-$[Pt(5'\text{-AMP-}N7)_2(^{15}NH_3)(^{15}NH_2CH_3)]^{2+}$, **3**. Two minor signals were also observed at 9.122 and 9.245 ppm with respective intensities of ca. 2:1, probably due to the monocomplex *cis*-$[PtCl(5'\text{-AMP-}N7)(^{15}NH_3)(^{15}NH_2CH_3)]^{+}$. After further incubation

(42) Marcelis, A. T. M.; van Kralingen, C. G.; Reedijk, J. *J. Inorg. Biochem.* **1980**, *13*, 213–222.

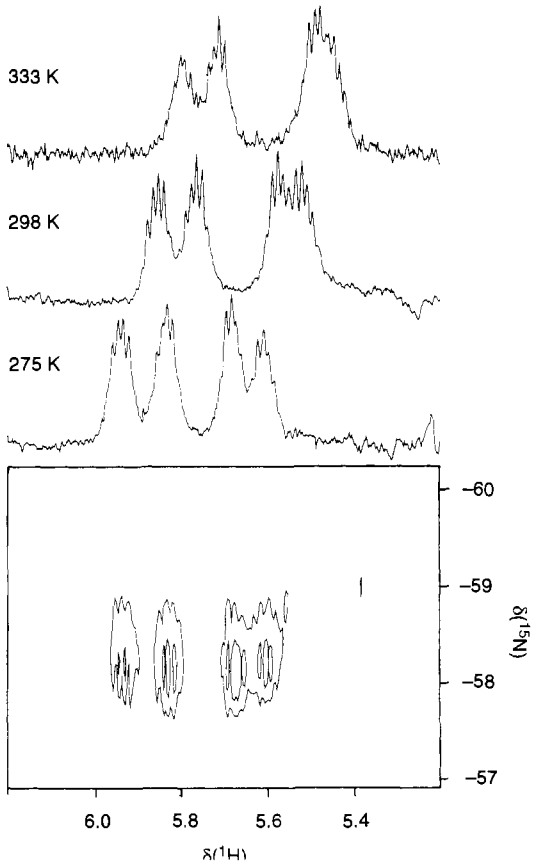


Figure 1. 500 MHz ^{15}N-edited ^{1}H{^{15}N} spectra at different temperatures and the corresponding [^{1}H, ^{15}N] 2D spectrum (50.67 MHz ^{15}N) at 275 K of a solution containing 5.4 mM *cis*-[$PtCl_2(^{15}NH_3)(^{15}NH_2CH_3)$] (**1**), 12.4 mM 5′-AMP in 100 mM phosphate buffer (95% H_2O/5% D_2O) pH 7.0 after incubation for 2 h at 343 K. Only the NH_2 region (assignable to the bis-5′-AMP complex **3**) is shown.

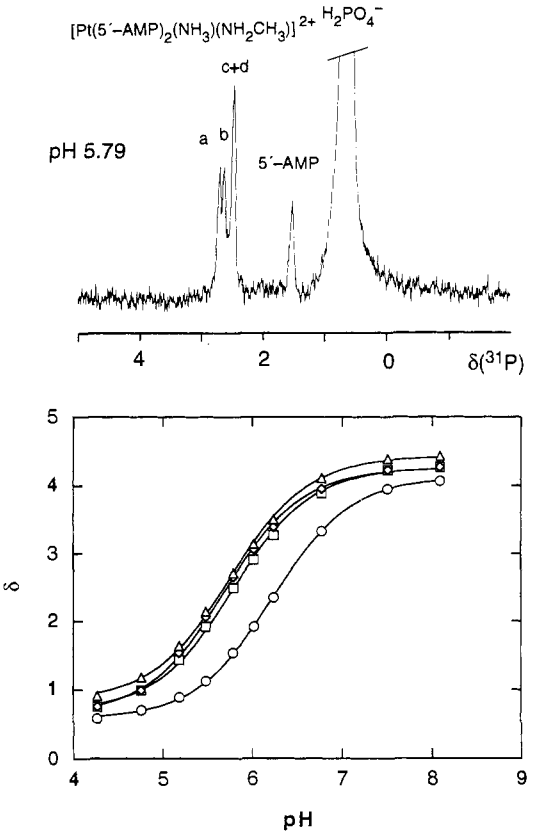


Figure 2. Top: ^{31}P spectrum (162 MHz) of a solution containing 5.4 mM **1**, 12.4 mM 5′-AMP, and 100 mM phosphate (95% H_2O/5% D_2O) pH 5.79 after incubation for 2 h at 343 K. The resonances are assignable to rotamers of the bis-5′-AMP complex **3**. Bottom: plots of ^{31}P NMR chemical shifts vs pH for peaks a (Δ),b (♦), and c + d (□) for complex **3** and free 5′-AMP (○). The solid line are best fits calculated with the pK_a values listed in Table III.

for 2 h at 343 K, the only product observed was the bis adduct **3**. The 2D [^{1}H, ^{15}N] HMQC spectrum consisted of four sets of multiplets for the NH_2 protons with different ^{1}H chemical shifts but the same ^{15}N shifts, Table II. Lowering the temperature to 275 K increased the dispersion of the ^{1}H (and ^{15}N) resonances slightly, and the appearance of the multiplets was consistent with them being doublets of quartets (geminal NH_2 and vicinal CH_3 couplings), although a detailed analysis was not attempted. At higher temperatures the dispersion decreased, Figure 1. It is notable that both the ammine and amine N–H resonances of **1** experience shifts to lower field (0.8–1.2 ppm) on formation of the 5′-AMP complex **3**. These shifts are about twice as large as those observed on formation of the 5′-GMP adduct **2** (ca. 0.5 ppm).

pK_a Values for Complexes 2 and 3. These were determined from the pH dependence of the ^{31}P chemical shifts. One ^{31}P peak was observed for the bis-5′-GMP complex **2**, but three resonances were observed for bis-5′-AMP complex **3** with intensity ratios of 1:1:2 (Figure 2, interpretable as four resonances of equal intensity with two overlapping, indicative of slow head–to–tail rotation of coordinated 5′-AMP). The fits to the titration data are shown in Figure 2, and the pK_a values[43] and ^{31}P chemical shifts are listed in Table III.

Adducts of [Pt(^{15}N-en)Cl_2] with 5′-GMP, 5′-dGMP, 3′-GMP, and G. The ^{15}N-edited ^{1}H{^{15}N} spectrum of [Pt(^{15}N-en)(5′-GMP-*N7*)$_2$]$^{2+}$ **9** at 310 K, pH 6.2 consisted of two broadened peaks ($\Delta\nu_{1/2}$ 30 Hz) of approximately equal intensities (Figure 3A), assignable to the en-$^{15}NH_2$ protons. On lowering the temperature, both peaks shifted to lower field, but the low field resonance shifted to a greater extent so that the two peaks became further apart, Figure 3B. Increasing the pH of the solution had a similar effect to lowering the temperature (Figure 3): at pH 7.7 (310 K) the spectrum of the en-NH_2 protons was similar to that at pH 6.2 and 283 K. Two broad resonances were observed at 6.15 and 5.91 ppm, with the latter having a larger area than the former (ca. 1:1.3). When the pH was lowered, both peaks shifted to high field, but the shift of the low field peak was greater so that the peaks became increasingly closer together, and at pH 4.3

Table III. ^{1}H and ^{31}P NMR Chemical Shifts and pK_a's for *cis*-[$Pt(X)_2(Am)_2$] Complexes and 5′-GMP and 5′-AMP for Comparison

				δ[a]		
$(X)_2$	$(Am)_2$	complex	^{1}H/^{31}P	acid	base	pK_a[b]
5′-GMP	$(NH_3)(CH_3NH_2)$	**2**	P	0.76	4.14	5.95
5′-GMP	(en)	**9**	P	0.94	4.42	5.82
5′-GMP	(en)	**9**	NHa	5.75	6.15	(5.82)
		9	NHb	5.75	5.91	(5.82)
5′-AMP	$(NH_3)(CH_3NH_2)$	**3**	Pa	0.84	4.43	5.75
		3	Pb	0.64	4.25	5.69
		3	Pc,d	0.69	4.26	5.79
5′-AMP	(en)	**14**	H8a	9.404	9.669	5.63
		14	H8b	9.281	9.579	5.72
		14	NHa	6.40	7.22	5.76
		14	NHb	6.29	7.32	5.73
		14	NHc	6.14	5.84	5.78
		14	NHd	6.03	5.78	5.86
5′-GMP			P	0.56	4.13	6.20
5′-AMP			P	0.58	4.12	6.23

[a] Limiting chemical shifts from computer fits to the data. [b] Errors ±0.04; brackets indicate that pK_a from ^{31}P data was used to derive limiting shifts.

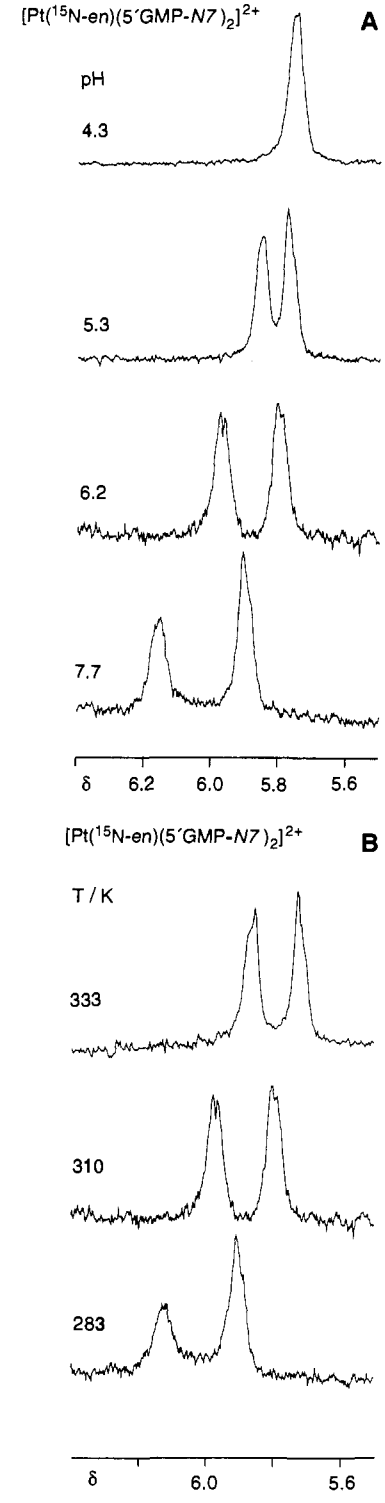


Figure 3. ^{15}N-Edited ^{1}H{^{15}N} NMR spectra of a 2 mM solution of [Pt(^{15}N-en)(5′-GMP-*N7*)$_2$]$^{2+}$ **9** (A) at various pH values (310 K) and (B) at various temperatures (pH 6.2).

only a single resonance was observed (δ 5.75). The presence of 20 mM phosphate did not significantly affect the chemical shifts of **9**.

Two NH peaks were observed also in the ^{15}N-edited ^{1}H-{^{15}N} spectrum of [Pt(^{15}N-en)(5′-dGMP-*N7*)$_2$]$^{2+}$ (**10**) at pH 6.2 (Table IV). On the other hand, only a single NH resonance was observed for both [Pt(^{15}N-en)(3′-GMP-*N7*)$_2$]$^{2+}$ (**11**) (δ 5.72) and [Pt(^{15}N-en)(G-*N7*)$_2$]$^{2+}$ (**12**) (δ 5.67) under similar conditions (Figure D2), with chemical shifts similar to that of the 5′-GMP complex **9** at low pH. A pK_a value of 5.82 was determined for the 5′-phosphate groups in **9** by ^{31}P NMR (Figure 4). Deprotonation

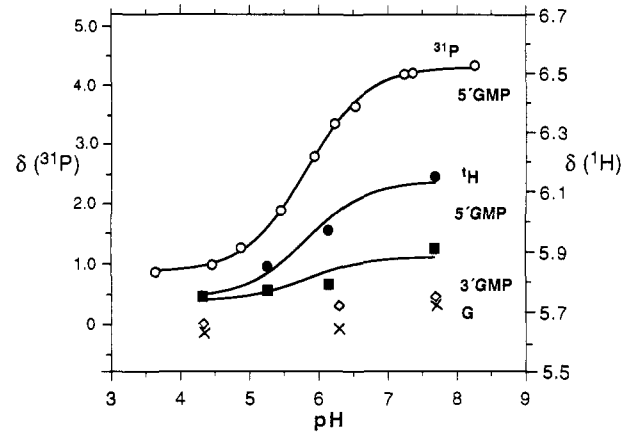


Figure 4. Dependence of the ^{31}P NMR resonance of [Pt(^{15}N-en)(5′-GMP)$_2$]$^{2+}$ (**9**) on pH (○), and the ^{1}H NMR NH resonances of 2 mM solutions of [Pt(^{15}N-en)X$_2$], X = 5′-GMP (●, ■) (**9**), 3′-GMP (◇) (**11**), and G (×) (**12**). The curves are those appropriate for a pK_a value of 5.82, the value derived from fitting the points of the ^{31}P titration.

of the 5′-phosphate groups is accompanied by downfield shifts of the two NH signals by ca. 0.40 and 0.16 ppm. A reasonable fit for the pH dependence of the NH ^{1}H NMR chemical shifts was obtained using the pK_a value derived from the ^{31}P data (5.82, Figure 4 and Table III). In contrast, the pH dependences of NH resonances for the 3′-GMP and G complexes **11** and **12** were much less marked, the resonances being only slightly deshielded with increasing pH (Figure 4). A small splitting of the NH_2 peak for **11** was observed below 300 K (deposited Figure D3).

In order to investigate the possible influence of intermolecular effects on Pt–NH ^{1}H NMR shifts, we studied the concentration dependence of the spectrum of complex **9** [Pt(^{15}N-en)(5′-GMP-*N7*)$_2$]$^{2+}$ at pH 7.1. The chemical shift of the low-field resonance decreased by only 0.05 ppm with a 4-fold dilution of a 10 mM solution, and there was no significant change in the chemical shift of the high field NH resonance.

The 2D [^{1}H, ^{15}N] spectrum of **9** showed that the two NH ^{1}H resonances are associated with the same ^{15}N chemical shift (−30.2 ppm), indicating that they arise from nonequivalent protons on the same N atom (*vide infra*).

[Pt(^{15}N-en)(H_2O)(5′-GMP-*N7*)]$^{2+}$ (26). Further evidence for the nonequivalence of the two Pt-NH_2 protons on the same coordinated N atom in 5′-GMP complexes was provided by the 2D [^{1}H, ^{15}N] spectrum of **26** (Figure 5), generated *in situ* by addition of 1 mol equiv of 5′-GMP to an aqueous solution of [Pt(^{15}N-en)(H_2O)$_2$]$^{2+}$. The ^{1}H–^{15}N two-dimensional cross-peak at 5.64/−31.5 ppm is assignable to the NH_2 group *trans* to 5′-GMP-*N7*, whereas the pair of peaks at 5.76, 5.70/−45.5 ppm are unambiguously assignable, on account of the ^{15}N shift, to the two NH_2 protons *trans* to O. This result shows that en-NH_2 protons on the *same* N atom, that is *cis* to 5′-GMP-*N7*, are nonequivalent.

[Pt(*N*,*N*-Me_2-en)(5′-GMP-*N7*)$_2$]$^{2+}$ (13). This complex was studied because now only one coordinated N atom has attached protons, and the restricted rotation about Pt–N7 for the 5′-GMP ligand cis to NMe_2[35] might lead to stronger H-bonding for the other 5′-GMP ligand. A sample of **13** was prepared by addition of 2 mol equiv of 5′-GMP to a 100 mM solution of [Pt(*N*,*N*-Me_2-en)(H_2O)$_2$]$^{2+}$. The NH proton resonances from this sample were observable within a few hours even with ^{15}N at *natural abundance* using ^{15}N-edited ^{1}H NMR spectroscopy. The normal ^{1}H spectrum of the sample at pH 6.2 was consistent with formation of **13** as the major product. However, a significant amount of free 5′-GMP was also present, together with two additional sets of minor peaks assignable to protons of coordinated 5′-GMP in a monoadduct **28**, Table IV. The ^{15}N-edited ^{1}H spectrum of the sample (deposited Figure D4), consisted of two clearly resolved resonances in the NH_2 region assignable to **13**, δ 6.34 and 5.77,

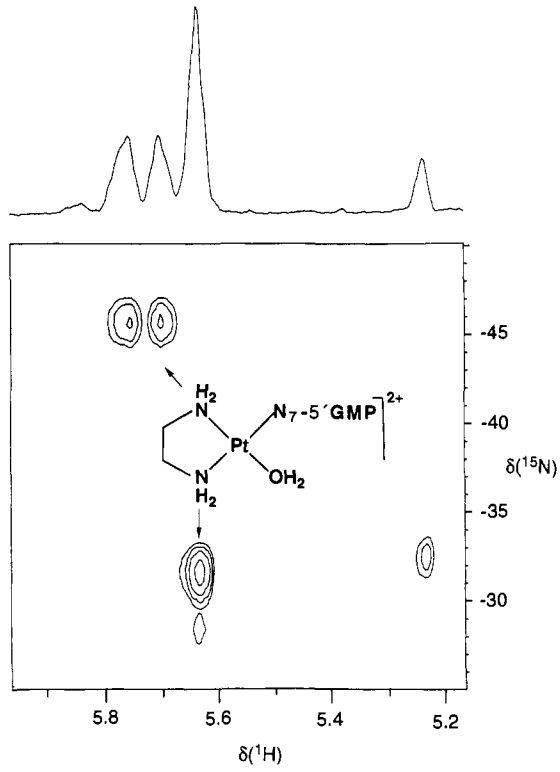


Figure 5. [^{1}H, ^{15}N] 2D NMR spectrum of an 8.6 mM solution of [Pt(^{15}N-en)(H_2O)(5′-GMP-*N7*)]$^{2+}$ (**26**), pH 4.8, 300 K. The nonequivalence of the two protons on the coordinated nitrogen *cis* to 5′-GMP can be seen. The minor cross-peak at 5.24/−32.2 ppm is assigned to residual [Pt(^{15}N-en)Cl_2] (**8**) present in the stock solution of [Pt(^{15}N-en)(H_2O)$_2$]$^{2+}$.

with the latter being slightly more intense. The decrease in intensity of the more deshielded resonance is similar to that found for **9**, although this peak is much more strongly deshielded in **13** (by ca. 0.4 ppm, Table IV). Minor NH_2 peaks were also resolved at 6.42 and 5.91 ppm (Table IV).

Adducts of [Pt(^{15}N-en)Cl_2] with 5′-AMP and 3′-AMP. [Pt(^{15}N-en)(5′-AMP-*N7*)$_2$]$^{2+}$ (**14**) was prepared by reaction of [Pt(^{15}N-en)(H_2O)$_2$]$^{2+}$ (**24**) (10 mM) with 2.2 mol equiv of 5′-AMP. The ^{1}H NMR spectrum was similar to that reported by Reily and Marzilli[16] for the two head-to-tail isomers of **14** with two sets of signals resolved for each of the H8, H2, and H1′ protons (Table V). The pH dependences of the chemical shifts of the two H8 signals are shown in deposited Figure D5, from which an average pK_a value of 5.72 was derived, Table III. The relative intensity of the two H8 signals changed from ca. 1:1.2 at pH 4.4 to 1.2:1 at pH 7.8, showing that the preference for one rotamer over the other is pH dependent, as noted previously.[16]

The ^{15}N-edited ^{1}H{^{15}N} spectrum of **14**, [Pt(^{15}N-en)(5′-AMP-*N7*)$_2$]$^{2+}$, consists of four signals assignable to en-NH_2 protons. The chemical shifts of these resonances are highly dependent on pH (Figure 6). At pH 1.0, the four peaks are relatively close together, and as the pH increases they separate into two pairs, one set becoming increasingly deshielded whilst the other becomes increasingly shielded so that at pH 7.8 the two pairs are separated by 1.45 ppm. Two-dimensional [^{1}H, ^{15}N] spectra recorded at pH 4.3 and 7.8 enabled the NH resonances from the two different rotamers to be distinguished on the basis of very slight differences in ^{15}N chemical shift (Table V). The effect of pH on each of the NH signals is shown in Figure 6B. The four curves give rise to an average pK_a value of 5.78 ± 0.05, a value close to that derived from H8 shift data, showing that the chemical shifts of the four NH resonances depend on the protonation state of the 5′-phosphate groups. It is evident from Figure 6B that the chemical shifts of the Pt–NH_2 protons in one rotamer are more strongly dependent on pH than those of the other rotamer.

When the phosphate groups are fully deprotonated, the most downfield-shifted Pt–NH proton resonances for the two rotamers of the bis-5′-AMP complex **14** are 2.05 and 1.94 ppm to low field of [Pt(^{15}N-en)Cl_2], a much greater deshielding than was observed for the analogous 5′-GMP complex **9** (0.91 ppm). Figure 7 shows the effect of temperature on the four Pt–NH ^{1}H NMR peaks of the sample of **14** at pH 7.8. All four resonances exhibit linear temperature dependences, shifting to low field with decreasing temperature (Table VI). Three of the resonances have similar temperature dependences, but the fourth has a smaller dependence. This results in overlap of the two low field peaks at high temperature. The absence of coalescence as the peaks overlap, confirms that each of the two rotamers contributes one Pt–NH proton resonance to the low field pair and one to the high field pair.

We prepared [Pt(^{15}N-en)(3′-AMP-*N7*)$_2$]$^{2+}$ (**15**) by the analogous reaction of [Pt(^{15}N-en)(H_2O)$_2$]$^{2+}$ (10 mM) with 2.2 mol equiv of 3′-AMP. The ^{1}H spectrum of the equilibrated mixture (at pH 7.0, 300 K) contained intense resonances attributable to unreacted 3′-AMP and a major set of peaks assignable to **15** (Table V). In contrast to the 5′-AMP complex, only one set of signals was observed (although broadened) when the phosphate group is in the 3′-position. At least 12 other 3′-AMP resonances were present in the ^{1}H NMR spectrum between 9.4 and 8.7 ppm and constituted ca. 30% of the intensity in this region. We did not attempt to identify these minor components. The ^{15}N-edited ^{1}H spectrum contained a broad signal ($\Delta\nu_{1/2}$ 50 Hz) at 5.94 ppm assignable to the NH_2 protons of **15**, with an associated ^{15}N cross-peak at −27.7 ppm in the 2D [^{1}H, ^{15}N] spectrum.

[Pt(*N*,*N*-Me$_2$-en)(5′-AMP)$_2$]$^{2+}$. Complex **16** was prepared by addition of 2 mol equiv of 5′-AMP to a 100 mM solution of [Pt(*N*,*N*-Me$_2$-en)(H_2O)$_2$]$^{2+}$ (**27**). The ^{1}H spectrum showed that a large amount of free 5′-AMP remained, but there were also two pairs of H8 peaks (δ 9.78, 9.59 and 9.61, 9.51, Table V) with a relative intensity ratio of 1:1.1. The chemical shifts are in agreement with those reported by Reily and Marzilli[16] for the two h/t rotamers of **16** at pH 6.1. No other peaks were present that could be assigned to 5′-AMP adducts. The ^{15}N-edited ^{1}H NMR spectrum of **16** (with ^{15}N in natural abundance, 20 500 transients) showed two major broad resonances (δ 7.2 and 6.3) which we assign to the two nonequivalent NH protons in the two rotamers. Separate resonances for the two rotamers were not resolved. In comparison, the four NH peaks of the en complex **14** had chemical shifts of 7.00, 6.95, 5.94, and 5.87 ppm at this pH and temperature. Three minor signals were also observed in the ^{15}N-edited ^{1}H spectrum at 5.52, 5.73, and 5.89 ppm.

Temperature Dependences. The Pt–NH resonances of most of the complexes studied here exhibited linear temperature dependences, shifting to low field with decreasing temperature, Table VI. Many of the observed temperature coefficients of the nucleobase complexes are very similar to those of the parent dichloro complexes (ca. -3.3×10^{-3} ppm K^{-1}), but low values for some of the NH resonances in AMP complexes (ca. -1 to -2×10^{-3} ppm K^{-1}) are notable. For the 3′-GMP complex **11** only a single Pt–NH ^{1}H NMR resonance was observed at high temperatures, but below 300 K two peaks were resolved, and these became increasingly further apart with decreasing temperature (deposited Figure D3, Table VI).

Discussion

The vast majority of active platinum anticancer complexes contain ammine ligands or amines with at least one hydrogen on the coordinated nitrogen (primary or secondary amines),[1] and it is widely believed that Pt–NH H-bonding plays a critical role in their mechanism of action.[2] From X-ray crystal structures,[5,7,9-11] it is clear that N–H hydrogens can form H-bonds to 5′-phosphate groups and C6 carbonyl groups in purine nucleotide adducts and that both intra- and intermolecular H-bonding can occur.

Table IV. Chemical Shifts for Guanine and Aqua Adducts of [Pt(^{15}N-en)Cl_2] and [Pt(*N,N*-Me_2-en)Cl_2] at 300 K (Unless Otherwise Stated)

complex		pH	δH8	δH1′	δen-CH_2	δN^1H (δ^{15}NH)
[Pt(^{15}N-en)Cl_2]	**8**	7.7			2.69	5.27
		6.2				5.27
		4.8				5.24 (–32.2)
		6.5[g]			2.62	5.28 (–31.3)
[Pt(^{15}N-en)(H_2O)$_2$]$^{2+}$	**24**	3.11			2.54	5.76
[Pt(^{15}N-en)Cl(H_2O)]$^+$	**25**	3.11				5.57, 5.49
		a				5.52 (–29.5), 5.46 (–50.4)
[Pt(^{15}N-en)(H_2O)(5′-GMP-*N7*)]$^{2+}$	**26**	4.8	8.79	6.05	2.70	5.64 (–31.5) (*trans* N)
						5.76, 5.70 (–45.5) (*trans* O)
[Pt(^{15}N-en)(5′-GMP-*N7*)$_2$]$^{2+}$	**9**	1.4[b]	8.92	5.92	2.84	5.68
		6.1[b]	8.64	5.93	2.84	5.79, 5.96
		6.7[g]	8.70	5.93	2.85	5.86, 6.11 (–30.2)
[Pt(^{15}N-en)(5′-dGMP-*N7*)$_2$]$^{2+}$	**10**	6.2[g]	8.60	6.31	2.87	5.81, 5.97[c] (–30.5)
[Pt(^{15}N-en)(3′-GMP-*N7*)$_2$]$^{2+}$	**11**	4.3	8.42	5.95	2.86	5.69
		6.2[g]	8.44	5.93	2.86	5.72 (–31.5)
		7.7	8.43	5.92	2.86	5.74
[Pt(^{15}N-en)(G-*N7*)$_2$]$^{2+}$	**12**	4.3	8.33	5.89	2.86	5.66
		6.2[g]	8.33	5.92	2.86	5.67 (–30.7)
		7.7	8.21	5.87	2.87	5.72
[Pt(*N,N*-Me_2-en)(H_2O)$_2$]$^{2+}$	**27**	3.0			2.77 m[d]	
[Pt(*N,N*-Me_2-en)(5′-GMP-*N7*)$_2$]$^{2+}$	**13**	6.2	8.70, 8.53	5.80, 5.90	*e*	5.77, 6.34
[Pt(*N,N*-Me_2-en)(X)(5′-GMP-*N7*)][f]	**28**		8.80, 8.56	5.95, 5.87		5.91, 6.42

[a] Hydrolysis of [Pt(^{15}N-en)Cl_2]. [b] 310 K. [c] Plus underlying broad peaks 5.3–5.9 ppm. [d] NMe δ 2.84. [e] Not analyzed. [f] Minor product, tentative assignment, possibly X = H_2O or Cl, or minor rotamer of **13**. [g] 20 mM phosphate buffer.

Table V. Chemical Shifts for 3′-AMP and 5′-AMP and Their Adducts of [Pt(^{15}N-en)]$^{2+}$ and [Pt(*N,N*-Me_2-en)]$^{2+}$ at 300 K

complex		pH	δH8	δH2	δH1′	δen-CH_2	δN^1H (δ^{15}NH)
[Pt(^{15}N-en)(5′-AMP-*N7*)$_2$]$^{2+}$	**14**	4.25	9.29	8.23	6.11	2.95	6.44, 6.03 (–27.1)
			9.42	8.21	6.21		6.34, 6.14 (–26.9)
(5′-AMP)		4.25	8.54	8.30	6.15		
[Pt(^{15}N-en)(5′-AMP-*N7*)$_2$]$^{2+}$	**14**	7.75	9.58	8.18	6.15	2.95	7.21, 5.78 (–26.55)
			9.67	8.16	6.23		7.32, 5.85 (–26.47)
(5′-AMP)		7.75	8.64	8.27	6.15		
[Pt(*N,N*-Me_2-en)(5′-AMP-*N7*)$_2$]$^{2+}$	**16**	6.16	9.78/9.59	8.20–8.31[a]	6.07–6.26[a]	2.87–3.22[b]	7.16, 6.32[c]
			9.61/9.51				
[Pt(^{15}N-en)(3′-AMP-*N7*)$_2$]$^{2+}$	**15**	4.3	9.09 br[d]	8.31	6.13	2.94, 2.91	5.92[e]
		7.0	9.11 br[d]	8.31	6.14	2.95, 2.91	5.93[e] (–27.7)
(3′-AMP)		7.0	8.37	8.24	6.11		

[a] Partially overlapped by peaks of free 5′-AMP. [b] CH_2 and NMe. [c] Additional minor peaks also resolved at 5.52, 5.73, and 5.89 ppm. [d] Plus at least 12 minor peaks 8.74–9.4 ppm. [e] Plus several additional broad NH ^{1}H resonances in the region 5.3–6.3 ppm (^{15}N shifts not resolved).

However, the conditions under which such H-bonds are favored in solution are not clear, nor how great a contribution they make to stabilizing nucleotide adducts. It has been proposed that Pt–NH H-bonding plays a role in stabilizing intermediates formed during the course of reactions of cisplatin with nucleotides. For example 5′-GMP and particularly 5′-dGMP react with *cis*-[Pt(H_2O)$_2$(NH_3)$_2$]$^{2+}$ more rapidly than 3′-GMP, G, or dG.[44] Such interactions may provide a kinetic driving force toward GpG cross-links in oligonucleotides.[45]

In this work we have probed H-bonding in mononucleotide adducts of cisplatin and related complexes in aqueous solution using ^{1}H NMR spectroscopy. The problems of detecting N–H protons in H_2O solutions can be overcome via the use of multipulse techniques and inverse detection technology.[25,26,37–39] With these methods protons bonded to ^{15}N can be selectively detected. Although this can be done with natural abundance ^{15}N, it takes several hours of instrument time and requires high concentrations of complexes (>ca. 40 mM in a 5-mm tube). By enriching complexes with >95% ^{15}N, it is possible to work at low millimolar concentrations, and even approach micromolar concentrations of physiological relevance. Also, with enriched complexes, it is feasible to carry out 2D [^{1}H,^{15}N] HMQC experiments and correlate ^{1}H and ^{15}N shifts. This usually provides assignments of ^{1}H resonances, since the chemical shifts of ^{15}N ligands bound to Pt(II) cover a wide range and are diagnostic of the *trans* ligand.[46] It is essential to work in H_2O solutions since N–H protons of coordinated ammines and amines usually exchange with deuterons of D_2O within a few minutes. In general, acid or base catalysis of N–H exchange in H_2O solutions of platinum complexes does not appear to be a problem, and resonances can be observed over a wide pH range (e.g., 2–9).

Initially we investigated 5′-GMP adducts of cisplatin.[25] The NH_3 ^{1}H resonance of *cis*-[Pt(5′-GMP-*N7*)$_2$(NH_3)$_2$]$^{2+}$ (**7**) is substantially shifted downfield (by 0.59 ppm at pH 7.9) when compared to *cis*-[$PtCl_2$(NH_3)$_2$] (**6**), and this reduces to 0.39 ppm when the pH of **7** is lowered to 4.4. Similar results were obtained for the *trans* complex **5** in comparison with **4** (Table I). These data are consistent with H-bonding between Pt–NH_3 and the phosphate group of a *cis*-5′-GMP, the strongest interaction being with the deprotonated phosphate. For example, the N–H protons involved in H-bonding in peptides and proteins are deshielded and often display low-temperature coefficients due to restricted accessibility to solvent protons,[47,48] *vide infra*. It was not feasible to determine the temperature coefficient of the NH_3 resonance for **7** since the peak is located directly beneath the H_2O peak. To

(43) Our pK_a values for 5′-GMP and 5′-AMP (-OPO_3^{2-}/-OPO_2(OH)$^-$, 6.20 and 6.23, respectively) are close to the literature values (6.1 and 6.19, I = 0.1, 298 K: *Critical Stability Constants*; Martell, A. E., Smith, R. M., Eds.; Plenum Press: New York, Vol. 2, 1975; pp 280, 289).

(44) Green, M.; Garner, M.; Orton, D. H. *Trans. Met. Chem.* **1992**, *17*, 164–176.

(45) Laoui, A.; Kozelka, J.; Chottard, J.-C. *Inorg. Chem.* **1988**, *27*, 2751–2753.

(46) (a) Pregosin, P. S. *Annu. Rep. NMR Spectrosc.* **1986**, *17*, 287–349. (b) Pregosin, P. S. *Coord. Chem. Rev.* **1982**, *44*, 247–291.

(47) Urry, D. W.; Ohnishi, T.; Long, M. M.; Mitchell, L. W. *Int. J. Pep. Prot. Res.* **1975**, *7*, 367–378.

(48) Feeney, J. In *Drug Action at the Molecular Level*; Roberts, G. C. K., Ed.; MacMillan Press: London, 1977; pp 55–76.

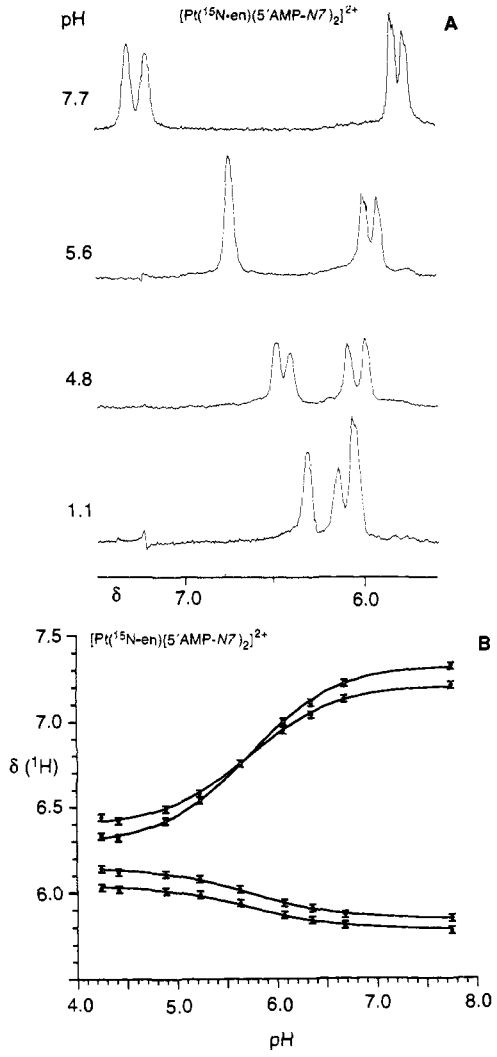


Figure 6. (A) Dependence of the ^{15}N-edited $^{1}H\{^{15}N\}$ NMR resonances of $[Pt(^{15}N\text{-en})(5'\text{-AMP})_2]^{2+}$ (**14**, 10 mM, 300 K) on pH. (B) Fits to the titration curves for the NH resonances using the pK_a values listed in Table III.

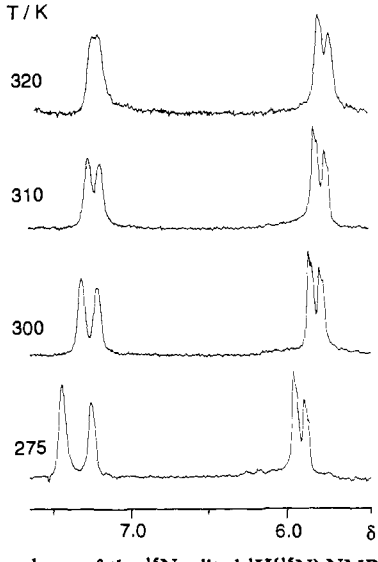


Figure 7. Dependence of the ^{15}N-edited $^{1}H\{^{15}N\}$ NMR resonances of $[Pt(^{15}N\text{-en})(5'\text{-AMP})_2]^{2+}$ (**14**) (10 mM, pH 7.8) on temperature.

avoid this problem we turned our attention to primary amine complexes.

Amine Complexes. First we studied the mixed ligand complex *cis*-$[Pt(5'\text{-GMP})_2(^{15}NH_3)(^{15}NH_2CH_3)]^{2+}$ (**2**) to verify that the N–H peaks would be further shifted to low field and clear of the H_2O signal, and subsequently we investigated ethylenediamine (en) complexes in which the NH_2 protons are held more rigidly in space in a chelate ring and therefore potentially able to form stronger H-bonds.

Table VI. Temperature Dependence of NH Shifts in $[P(X)_2(Am)_2]$ Complexes

				$-10^3\ \delta/T$ (ppm K^{-1})	
$(Am)_2$	$(X)_2$	complex	pH	NH_3	NH_2 (a,b,c,d)
$(NH_3)(CH_3NH_2)$	Cl	**1**	7	2.6	3.3
$(NH_3)(CH_3NH_2)$	5'-GMP	**2**	7		3.7
$(NH_3)(CH_3NH_2)$	5'-AMP	**3**	7		2.6, 2.2, 3.4, 2.5
(en)	Cl	**8**	6.2–7.8		3.2/3.3[a]
(en)	5'-GMP	**9**	6.2		5.5, 3.6
(en)	3'-GMP	**11**	6.2		5.4 (av),[b] 3.7
(en)	G	**12**	6.2		3.1
(en)	5'-AMP	**14**	7.8		4.3, 1.1, 4.1, 4.1

[a] Complex present as an impurity in solutions of **9** and **14**, respectively. [b] Average value: 8.0 < 290 K, 3.7 > 290 K.

For complex **2**, *cis*-$[Pt(5'\text{-GMP-}N7)_2(^{15}NH_3)(^{15}NH_2CH_3)]^{2+}$, the NH_3 peak is again very close to that for H_2O and is very difficult to study, whereas the NH_2 peak of the methylamine ligand is to lower field of water. In comparison with the dichloro complex **1**, the NH_2 resonance of **2** is shifted to low field by ca. 0.5 ppm, again consistent with H-bonding. The NH_2 protons remained magnetically equivalent. Head-to-tail isomerism, involving rotation about the Pt–N7 bond, would be expected to be rapid on the NMR time scale in this complex since there are no bulky groups on the coordinated N's.[30,31] The broadest H8 resonance for **2** at low temperature may be assignable to the 5'-GMP *cis* to CH_3NH_2, the bulkiest amine ligand. The temperature coefficient for the NH_2 proton resonance of this complex (-3.7×10^{-3} ppm K^{-1}) does not provide evidence for any difference in H-bonding compared to the parent dichloro complex **1**, Table VI.

The observation of four H8 resonances and four NH_2 peaks of equal intensities for the 5'-AMP adduct of the methylamine–ammine mixed ligand complex **3** suggests that head-to-tail (h/t) isomerism of coordinated AMP (Chart II) is slow on the NMR time scale and that the h/t and t/h conformations have similar energies. The ^{31}P NMR spectrum can also be interpreted as consisting of four peaks, two of which have the same shift (c,d in Figure 2). Slow h/t isomerism in 5'-AMP (as opposed to 5'-GMP) Pt(II) amine complexes has been reported previously.[16] The lowering of the pK_a of the 5'-phosphate from 6.23 in 5'-AMP itself to 5.7 in **3** (Table III) is consistent with the stabilization of the deprotonated phosphate by H-bonding. A similar lowering of pK_a has been reported[16] for complex **14** (by 1.0 unit, but source of pK_a for free 5'-AMP not stated), *vide infra*. Hydrogen bonding appears to restrict solvent access to three of the four nonequivalent NH_2 protons to a small extent as shown by the lowering of their temperature coefficients, Table VI.

The importance of the 5'-phosphate group in H-bonding was clearly demonstrated by our studies of ethylenediamine (en) complexes. The NH_2 ^{1}H NMR resonance of $[Pt(^{15}N\text{-en})Cl_2]$ (**8** δ 5.27) occurs to low field of the H_2O resonance allowing strongly deshielded resonances in Pt–^{15}Nen complexes to be readily observed using ^{15}N-edited ^{1}H NMR methods, without the possibility of overlap with the intense H_2O resonance, as was the case for *cis*-$[Pt(5'\text{-GMP-}N7)_2(NH_3)_2]^{2+}$ (**7**). The substantial low-field shifts for the NH resonances of 5'-GMP and 5'-dGMP complexes are consistent with the presence of H-bonding. Moreover, the parallel pH dependences of the ^{31}P 5'-phosphate and the NH_2 ^{1}H NMR resonances of **9** (Figure 4) provides strong evidence for the close association between these two groups via intramolecular H-bonding. The lack of large low-field NH shifts and lack of a marked pH dependence for the 3'-GMP and G complexes shows that 5'-phosphates play a special role in such

H-bonding processes. For the 3′-GMP complex **11**, two NH resonances were resolved below 300 K, and these had markedly different temperature coefficients (Table VI). A possible explanation for the nonequivalence of the NH protons in this case is that intermolecular H-bonding to the 3′-phosphate group contributes to the shielding at low temperatures.

In chelated en complexes, the NH_2 protons are more rigidly held and consequently H-bonding is stronger (low-field NH shifts ca. twice as large as those for NH_3 complexes). The largest low field shifts were observed for 5′-AMP complexes. It is reasonable to assume that the slower heat-to-tail rotation of coordinated AMP compared to GMP leads to stronger H-bonding and a shift in the equilibrium toward H-bonded compared to non-H-bonded complexes. Significant changes in rotamer populations were seen on deprotonation of the 5′-phosphate of the 5′-AMP complex **14** (as noted previously from H8 studies[16]), and this may result from an increase in the population of H-bonded species at high pH. In contrast to the 5′-AMP complex, only one set of NH peaks was observed for the 3′-AMP–N7 complex **15**. They were broadened suggesting that there is less restricted rotation about the Pt–N7 bond when the phosphate is in the 3′-compared to 5′-position bringing the two h/t rotamers into fast (or intermediate) exchange on the NMR time scale. As for GMP, the low field shifts of Pt–NH protons of 3′-AMP complexes are smaller than those for 5′-AMP complexes consistent with the presence of strong H-bonding only when a 5′-phosphate group is present.

The data for [Pt(^{15}N-en)(H_2O)(5′-GMP-*N7*)]$^{2+}$ (**26**) and [Pt(*N*,*N*-Me_2-en)(5′-GMP-*N7*)$_2$]$^{2+}$ (**13**) support the view that the two NH resonances observed for [Pt(en)(5′-GMP-*N7*)$_2$]$^{2+}$ (**9**) arise from nonequivalent hydrogens on the same coordinated N of en, the marked nonequivalence being attributable to stereospecific H-bonding with the 5′-phosphate. The apparent difference in the areas of the two Pt–NH ^{1}H NMR peaks for **9** (Figure 3, same ^{15}N shifts) is probably due to T_2 relaxation effects.

Temperature coefficients for N–H ^{1}H NMR resonances have been most thoroughly studied for peptides, and low values are usually ascribed to H-bonding interactions which protect the N–H protons from solvent. In freely solvent-accessible environments, coefficients of up to ca. $|-9 \times 10^{-3}|$ ppm K^{-1} are commonly observed,[47,48] and completely shielded H-bonded protons have coefficients of $\langle|-5 \times 10^{-3}|$ ppm K^{-1}. Thus most of the temperature coefficients determined in this work are low suggesting a high degree of shielding from solvent even for the parent dichloro complexes **1** and **8**. However, it should be noted that the rules for peptides have been established empirically, and the origins of the temperature dependences are not well understood. Therefore it cannot be assumed that the magnitudes of the effects in platinum ammine and amine complexes will be the same, and empirical rules will have to be established for this series too.

Unfortunately few X-ray structures of ammine or amine Pt(II) mononucleotide complexes are available. The crystal structures of [Pt(en)G_2]$Cl_{1.5}I_{0.5}$·$2H_2O$ and Na_2[Pt(en)(5′-GMP)$_2$]·$3H_2O$ (h/t isomers) show only intermolecular H-bonding between N–H protons and C6O.[7,8] Na_2[Pt(en)(5′-GMP)$_2$]·$3H_2O$ was crystallized at pH 7 (in the presence of tBuOH) and has deprotonated phosphate groups. It therefore appears that crystal packing effects determine the structure in the solid state and disrupt the intramolecular NH–5′-phosphate H-bonds which we detect in solution. The NOE data of Xu et al.[12] on [Pt(*N*,*N*-dimethyl-2,3-diaminobutane)X_2]$^{2+}$ (X = 5′-GMP or 5′-dGMP) are consistent with our finding that NH-5′-phosphate H-bonding is weak at low pH when the phosphate group is protonated. These workers suggested that NH–C6O H-bonding predominates for this complex at pH 3.

Ammine Complexes. If H-bonding to the phosphate of 5′-GMP in the *cis* position to NH_3 plays a major role in the low-field shift of ^{1}H NMR resonances, then the shifts we reported previously[25] for *cis*-[PtCl(5′-GMP-*N7*)(NH_3)$_2$]$^+$ (**22**) are difficult to understand, since the NH_3 ligand *trans* to 5′-GMP is more strongly deshielded than that *cis* to 5′-GMP, although intramolecular H-bonding to the deprotonated 5′-phosphate would be expected to occur more readily for a *cis* NH_3. The complex was detected as an intermediate in the reaction of **6** with 2 mol equiv of 5′-GMP (pH 6.5), and assignments of ^{1}H peaks for the ammine ligands *trans* (δ 4.38) and *cis* (δ 4.09) to 5′-GMP were based on a comparison with those for *cis*-[Pt(H_2O)(5′-GMP-*N7*)(NH_3)$_2$]$^{2+}$ (**23**) (pH 4.1). The ^{15}N chemical shifts allowed unambiguous assignments of NH_3 ligands *trans* to H_2O and GMP, respectively. With the aid of the new data reported in the present paper it is possible to reassign the NH_3 peaks for **22**.

We have now analyzed NH_3 ^{1}H NMR shifts following the approach of Appleton and co-workers,[49] who have previously investigated the influence of different neutral or anionic ligands (Z) on the ^{15}N chemical shifts (δ_N) of ammine ligands *trans* or *cis* to Z in *cis*-[Pt(NH_3)$_3$Z]$^{n+}$ complexes. In general, *cis* influences on δ_N are smaller than *trans* influences but are still significant. We have compared ^{15}N and ^{1}H shifts of NH_3 resonances for *cis*-[$PtCl_2$(NH_3)$_2$] (**6**), *cis*-[PtCl(H_2O)(NH_3)$_2$]$^+$ (**21**), and *cis*-[Pt(H_2O)(5′-GMP-*N7*)(NH_3)$_2$]$^{2+}$ (**23**) and calculated[50] approximate *trans* (T) and *cis* (C) influences of H_2O and 5′-GMP (pH 4.3) relative to Z = Cl in *cis*-[PtZ_2(NH_3)$_2$] using the relationships

$$T(\delta)H_2O = \delta(NH_3)_{trans}\ (\mathbf{21}) - \delta(NH_3)\ (\mathbf{6})$$

$$C(\delta)H_2O = \delta(NH_3)_{cis}\ (\mathbf{21}) - \delta(NH_3)\ (\mathbf{6})$$

and

$$T(\delta)5'\text{-GMP} = \delta(NH_3)_{trans}\ (\mathbf{23}) - \delta(NH_3)\ (\mathbf{21})$$

$$C(\delta)5'\text{-GMP} = \delta(NH_3)_{cis}\ (\mathbf{23}) - \delta(NH_3)\ (\mathbf{21})$$

The derived values (footnote to Table I) were used to calculate the ^{15}N and ^{1}H chemical shifts for the series of diammineplatinum(II) complexes using the expressions

$$\delta(NH_3\ trans\ \text{to Y in } cis\text{-}[PtZY(NH_3)_2]^{m+} = \delta(\mathbf{6}) + T(\delta)Y + C(\delta)Z$$

and

$$\delta(NH_3\ \text{in } trans\text{-}[PtZY(NH_3)_2]^{m+}) = \delta(\mathbf{4}) + C(\delta)Y + C(\delta)Z$$

Overall there is good correspondence between the observed and calculated values, Table I. As might be expected for ^{1}H NMR shifts, in contrast to ^{15}N which is directly bonded to Pt(II), *cis* influences are larger than *trans* influences. Based on the calculated values, our previous assignment for the ^{1}H NMR NH_3 resonances of *cis*-[PtCl(5′-GMP-*N7*)(NH_3)$_2$]$^+$ (**22**) should be reversed, so that the cross peaks at δ 4.38/–66.4 and 4.09/–69.1 are reassigned to NH_3 ligands trans to Cl and 5′-GMP, respectively. Now the ammine ligand *cis* to 5′-GMP experiences the greater deshielding, consistent with a role in H-bonding.

There is also a good correspondence between the experimental and calculated ^{1}H shifts of NH_3 ligands in the *trans* complexes, *trans*-[Pt(5′-GMP-*N7*)$_2$(NH_3)$_2$]$^{2+}$ (**5**) and *trans*-[Pt(H_2O)(5′-GMP-*N7*)(NH_3)$_2$]$^{2+}$ (**19**). For the former complex, the downfield shift compared to *trans*-[$PtCl_2$(NH_3)$_2$] increased from 0.55 ppm at pH 4.3 to 0.66 at pH 6.5, again consistent with stronger H-bonding interactions involving a deprotonated 5′-phosphate group.

Conclusions

Direct observation of Pt–NH ^{1}H NMR resonances of ammine and amine ligands in Pt(II) complexes containing derivatives of

(49) Appleton, T. G.; Hall, J. R.; Ralph, S. F. *Inorg. Chem.* **1985**, *24*, 4685–4693.

guanine and adenine in aqueous solution provides evidence for the formation of H-bonds with the 5′-phosphate groups of nucleotides coordinated in the *cis* position. The interaction results in substantial low-field shifts of NH ^{1}H NMR resonances. These shifts are largest, and H-bonding is probably strongest, when the NH protons are constrained in chelate rings (ethylenediamine), when purine head-to-tail rotation is slow (5′-AMP), and when the phosphate group is fully deprotonated ($-OPO_3^{2-}$). The H-bonds are stereospecific: one of the *cis* NH_2 protons in, e.g., $[P(en)(5'\text{-GMP})_2]^{2+}$ or $[Pt(en)(5'\text{-AMP})_2]^{2+}$ is more deshielded than the other. The temperature dependences of the Pt–NH ^{1}H NMR shifts are low (-1 to -5×10^{-3} ppm K^{-1}), but do not, as yet, provide a simple diagnostic test for H-bonding. Hydrogen bonding lowers the pK_a's of nucleotide 5′-phosphates (by 0.5 units in the case of $[Pt(en)(5'\text{-GMP})_2]^{2+}$), and results in deshielding of their ^{31}P resonances.

Our data are consistent with previous reports of Pt–NH H-bonding to 5′-phosphate groups in crystals of diammino Pt(II) polynucleotide complexes[5,9,11] and with the predictions of theoretical calculations.[18-21] Few crystal structures of Pt(II) ammine or amine mononucleotide complexes are available with which to compare our solution data, and in those structures which have been determined[7,8] only intermolecular C6O–NH H-bonds have been observed. Although it is clear that interactions of NH protons with 5′-phosphate groups occur in solution, H-bonding to C6O groups could also contribute to the low-field NH shifts.

Chemical shift ranges for Pt–NH protons in Pt(II) ammine and amine complexes are beginning to emerge (ca. 3.6–4.7 for NH_3 protons, and 4.6–7.3 ppm for NH_2 protons). The use of amines rather than ammonia as ligands in these studies is advantageous since H-bonded NH_3 protons are often shifted downfield beneath the intense H_2O peak, and their detection becomes difficult (requiring the use of relaxation agents for example). The sensitivity of ^{15}N-edited NMR spectroscopy with an inverse probe is high enough to allow detection of Pt–NH resonances from samples of the more soluble platinum complexes with ^{15}N in natural abundance. The NH ^{1}H NMR chemical shifts of Pt(II) ammine complexes can be rationalized in terms of *cis* and *trans* influences, as has been done previously for ^{15}N NMR shifts.[49] However, in contrast to ^{15}N shifts, ^{1}H shifts are dominated by *cis* influences rather than *trans* influences.

It will now be of interest to determine whether 5′-phosphates also play critical roles in determining the behavior of NH resonances of adducts of platinum anticancer complexes with polynucleotides. The nucleobases in GpG cross-links are usually constrained in a head-to-head fashion,[4] and, on the basis of the present work, this situation should give rise to large low-field NH ^{1}H NMR shifts of H-bonded ammine or amine protons. We shall report the results of such experiments elsewhere.[51]

Acknowledgment. We thank the UK Medical Research Council, the Australian National Health and Medical Research Council (R. Douglas Wright Award to S.J.B.P.), The Royal Society, The Swiss National Science Foundation, the Wolfson Foundation, the Science and Engineering Research Council, and University of London Intercollegiate Research Service for their support for this work. We are grateful to D. T. A. Frenkiel (National Institute for Medical Research) for advice and assistance with the inverse probe experiments and for comments on the script. We thank Professor J. Reedijk (University of Leiden) and Dr. J. Feeney (National Institute for Medical Research) for helpful discussions and Professor R. Bau (University of Southern California) for providing the X-ray coordinates for $[Pt(en)G_2]Cl_{1.5}I_{0.5}\cdot 2H_2O$ and unpublished crystallographic data on $Na_2[Pt(en)(5'\text{-GMP})_2]\cdot 3H_2O$.

Supplementary Material Available: ^{1}H NMR spectra of *cis*-$[PtCl_2(NH_3)(NH_2CH_3)]$ (**1**) in DMF-d_7: (A) unlabeled **1** (500 MHz) and (B) ^{15}N-labeled **1** (270 MHz). The natural abundance ^{15}N satellites on the NH_3 peak (^1J = 73 Hz) for the unlabeled complex can just be discerned in (A). Some of the ^{195}Pt satellites are indicated; they are broadened via chemical shift anisotropy relaxation (Figure D1). ^{15}N-Edited ^{1}H{^{15}N} NMR spectra of a 10 mM solutions of $[Pt(^{15}N\text{-en})X_2]^{2+}$ (X = 5′-GMP **9**, 3′-GMP **11**, and G **12**) in 20 mM phosphate buffer pH 6.2, 300 K (Figure D2). Temperature dependence of the ^{15}N-edited ^{1}H NMR spectrum of a 10 mM solution of $[Pt(^{15}N\text{-en})(3'\text{-GMP-}N7)_2]^{2+}$ (**11**), pH 6.2, showing the nonequivalence of the NH_2 protons of en at low temperature (Figure D3). ^{15}N-Edited ^{1}H NMR spectrum of a 100 mM solution of $[Pt(N,N\text{-Me}_2\text{-en})(5'\text{-GMP})_2]^{2+}$ (**13**, natural abundance ^{15}N), pH 6.2, 300 K, showing resonances which can be assigned to nonequivalent NH_2 protons of coordinated *N*,*N*-Me_2-en. The spectrum (Figure D4) is the result of 20 000 transients (15 h). Fits to the titration curves for the H8 resonances of $[Pt(^{15}N\text{-en})(5'\text{-AMP})_2]^{2+}$ (**14**) using the pK_a values listed in Table III (Figure D5) (5 pages). Ordering information is given on any current masthead page.

(50) In these calculations the ^{15}N and ^{1}H chemical shifts of *cis*-$[Pt(H_2O)(5'\text{-GMP-}N7)(NH_3)_2]^{2+}$ are at pH 4.1. This is below the pK_a of the 5′-phosphate of GMP, and H-bonding should make little contribution to them.

(51) Berners-Price, S. J.; Ranford, J. D.; Sadler, P. J. *Manuscript* in preparation.