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[^{1}H,^{15}N] Nuclear magnetic resonance studies of [Pt(dien)Cl]$^+$ (dien = diethylenetriamine): hydrolysis and reactions with nucleotides‡

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The anticancer drug cisplatin is activated *via* hydrolysis before binding to DNA. Although [Pt(dien)Cl]$^+$ **1** (where dien is diethylenetriamine) has been extensively used as a model for the first step in the binding of cisplatin to DNA and other biological targets, the hydrolysis behaviour of this complex is not well understood. In this work we have used a two-dimensional [^{1}H,^{15}N] NMR approach, and have established correlations between δ(^{15}N) and ^1J(^{195}Pt–^{15}N) for Pt–NH_2 and Pt–NH groups which are useful for assignment of resonances and identification of *trans* ligands, as has been previously recognised for Pt–NH_3 complexes. Although it has previously been suggested that the rate of reaction of complex **1** with guanosine 5′-monophosphate is determined by the rate of hydrolysis, no NMR peaks for an aqua intermediate were detectable, and no hydrolysis of **1** alone was observed after 7 d at 298 K. This raises new questions about the mechanisms of such reactions.

The therapeutic effects of cisplatin are believed to depend largely on the formation of bifunctional guanylyl(3′-5′)guanosine (GpG) intrastrand cross-links on DNA.[1] The drug, which exists in neutral form in circulating plasma, hydrolyses after passing through the cell membrane into cytoplasm to give the aqua complex $[Pt(NH_3)_2Cl(H_2O)]^+$ and to a lesser extent $[Pt(NH_3)_2(H_2O)_2]^{2+}$.[2] The aqua complexes are believed to be the active species in DNA binding.

Recently it has been shown that certain monofunctional adducts formed between *cis*-$[PtCl_2(NH_3)_2]$ or *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and GG sequences of duplex DNA can be very long-lived.[3,4] Although the biological functions of these monofunctional adducts are not known, they are the precursors of the bifunctional adducts, and could also be targets for the recognition by other cellular components. In the case of *trans* iminoether Pt^{II} complexes, monofunctional adducts may be responsible for the antitumour activity.[5]

The monofunctional complex [Pt([^{15}N]dien)Cl]Cl (dien = diethylenetriamine) **1** has been used extensively as a model for the first step in binding of platinum antitumour compounds to DNA,[6] although the compound itself is inactive as an antitumour agent. Previous kinetic analyses of reactions of **1** with nucleotides[7–9] are consistent with hydrolysis as the initial step, although there has been no direct detection of the aqua complex during the reaction. However, while studying reactions of pyridine with [Pt(dien)X]X (X = *e.g.* Cl, Br or I), Gray and co-workers[10,11] commented on the inertness of **1** in water. They stated that 'before adding pyridine, the conductances of the solution of [Pt(dien)X]X corresponded to one to one electrolytes and did not change with time. Thus no hydrolysis occurred'. Martin and Bahn[12] studied the substitution of Br in [Pt(dien)Br]$^+$ by chloride, using ion exchange and radioisotope tagging, and concluded that aquation contributed partially to the replacement, and a hydrolysis rate constant of 1.3×10^{-4} s^{-1} at 298 K was evaluated for [Pt(dien)Br]$^+$.

Our previous work has illustrated the value of two-dimensional [^{1}H,^{15}N] NMR spectroscopy in following the detailed courses of reactions of cisplatin with nucleotides, and in particular has allowed the detection of aqua intermediates.[4,13] We have shown recently in preliminary studies that [Pt([^{15}N]dien)Cl]Cl can be prepared and used to monitor H-bonding interactions within nucleotide adducts.[14] Therefore we have re-examined the kinetics reactions of **1** with nucleotides in an attempt to detect aqua intermediates. The diagnostic value of Pt–^{15}N couplings and ^{15}N chemical shifts for Pt–NH_2 and Pt–NH groups is also discussed.

Experimental

Materials

Nitrogen-15 labelled diethylenetriamine was synthesised according to the reported procedure,[15] and the complex [Pt([^{15}N]dien)Cl]Cl was prepared according to the literature method for the unlabelled complex.[16] Stock solutions of [Pt([^{15}N]dien)Cl]NO_3 and [Pt([^{15}N]dien)(H_2O)]$(NO_3)_2$ were prepared by reacting [Pt([^{15}N]dien)Cl]Cl with 1 and 2 mol equivalents of $AgNO_3$, respectively, for 24 h in the dark. Complexes [Pt([^{15}N]dien)Br]Br and [Pt([^{15}N]dien)I]I were prepared *in situ* by reacting **1** with an excess of KBr or KI, respectively. The sodium salts of guanosine 5′-monophosphate (5′-GMP) and guanosine 3′-monophosphate (3′-GMP) were obtained from Sigma.

NMR spectroscopy

The NMR spectra were recorded on the following instruments: Varian Unity-plus 500 (^{1}H, 500 MHz; ^{15}N 50.7 MHz), Varian Unity and Inova 600 (^{1}H, 600 MHz; ^{15}N, 60.8 MHz). The ^{1}H NMR chemical shifts are internally referenced to 1,4-dioxane (δ 3.74) and the ^{15}N chemical shifts externally referenced to 1 M [^{15}N]H_4Cl in 1.5 M HCl. For ^{1}H one-dimensional NMR spectra, water suppression was achieved by using the WATERGATE sequence.[17] The one-dimensional ^{15}N-edited ^{1}H and two-dimensional [^{1}H,^{15}N] heteronuclear single-quantum coherence (HSQC) NMR spectra (decoupled by irradiation with the GARP-1 sequence during acquisition) were recorded using the sequences of Stonehouse *et al.*[18] All the spectra were recorded at 298 K and all samples were shielded from the light to avoid possible photochemical reactions. Concentrations of species were determined by integration of the two-dimensional NMR cross-peaks using VNMR software.

pH Measurements

The pH values of the NMR samples were adjusted with 1 M $HClO_4$ or NaOH and determined using a Corning 240 pH meter equipped with an Aldrich microcombination electrode, calibrated with buffer solutions at pH 4, 7 and 10.

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Table 1 Proton and ^{15}N chemical shifts of $\{Pt([^{15}N]dien)\}^{2+}$ complexes (298 K)

		NH		NH_2		$^1J(^{195}Pt–^{15}N)$/Hz	
Compound	pH	$\delta(^1H)$	$\delta(^{15}N)$ (*trans* to)	$\delta(^1H)$	$\delta(^{15}N)$ (*trans* to N)	NH	NH_2
$[Pt([^{15}N]dien)Cl]Cl$	5.40	6.65	8.1 (Cl)	5.23	−33.8	392	326
				5.02	−33.8		326
$[Pt([^{15}N]dien)(H_2O)]^{2+}$	4.25	6.90	−8.5 (O)	5.32	−31.8	425	334
				5.52	−31.8		321
$[Pt([^{15}N]dien)(OH)]^{+}$	8.65	7.15	−1.7 (O)	5.13	−34.9	348	303
				4.93	−34.9		
$[Pt([^{15}N]dien)Br]Br$	6.00	6.65	15.1 (Br)	5.21	−34.6	389	315
				5.01	−34.6		315
$[Pt([^{15}N]dien)I]I$	6.35	6.63	25.7 (I)	5.17	−36.4	370	298
				4.98	−36.4		301
$[Pt([^{15}N]dien)(L\text{-Met-}S)]^{+}$ [a]	≈6.0	7.08	26.0 (S)	5.68	−31.8	318	323
				5.83	−31.8		323
$[Pt([^{15}N]dien)(5'\text{-GMP-}N^7)]^{2+}$	6.45	6.76	5.9 (N)	5.90	−29.2	*b*	*b*
				5.68	−29.2		
				5.51	−29.2		
				5.40	−29.2		
$[Pt([^{15}N]dien)(3'\text{-GMP-}N^7)]^{2+}$	6.74	6.72	6.0 (N)	5.48	−30.3	*b*	*b*
				5.32	−30.3		

[a] L-Met-*S* = L-methionine. [b] Satellites too broad to observe.

NMR sample preparation

All the samples were in 90% H_2O–10% D_2O (0.7 ml), and contained 0.1 M $NaClO_4$ to maintain a constant ionic strength. The concentrations of platinum complexes in samples for hydrolysis and pH titration studies were in the range of 1 to 5 mM. The concentration of $[Pt([^{15}N]dien)Cl]Cl$ **1** for kinetic studies was 1 mM, and the ratio of Pt : 5′-GMP was varied from 1 : 0.5 to 1 : 10. After mixing the solutions of the Pt complex and the nucleotide, the pH value was adjusted to 6.4 to 6.9 when necessary. The use of buffers was avoided because of the possibility of interaction with Pt. Reactions of **1** (3 mM) with 3′-GMP (1 : 1), KBr and KI (excess) were carried out at pH 6 to 7, and samples were incubated for 1–5 h prior to recording the spectra.

Calculations of rate constants

For the second-order kinetic analysis of NMR data, the appropriate differential equations were integrated numerically, and the rate constants were determined by a non-linear optimization procedure using the program SCIENTIST (version 2.01, MicroMath Inc.). First-order constants were calculated using the program KALEIDAGRAPH[19] on a Macintosh computer.

Results

First we characterized complex **1** and the related bromo and iodo derivatives.

$[^1H,^{15}N]$ NMR spectroscopy of $[Pt([^{15}N]dien)X]X$ (X = Cl, Br or I)

The two-dimensional NMR spectrum of $[Pt([^{15}N]dien)Cl]Cl$ **1** showed three cross-peaks, two with ^{15}N chemical shifts of δ −33.8, in the region expected for Pt–NH_2 *trans* N and therefore assignable to the non-equivalent protons on the two *trans* Pt–NH_2 groups of **1**. The third cross-peak, with an ^{15}N chemical shift of δ 8.1, can be assigned to the Pt–NH *trans* to Cl^-. The two cross-peaks for the Pt–NH_2 groups of $[Pt([^{15}N]dien)Br]Br$ and $[Pt([^{15}N]dien)I]I$ have very similar ^{15}N chemical shifts to those of **1**, Table 1, whereas the ^{15}N chemical shifts of the cross-peak assignable to Pt–NH show a progressive downfield shift on changing from Cl (δ 8.1) to Br (δ 15.1) to I (δ 25.7) (Table 1). The $^{195}Pt–^{15}N$ coupling constants for these complexes were measurable from the satellites observed in the two-dimensional spectra and are listed in Table 1. To allow a discussion of the influence of *trans* ligands on ^{15}N chemical shifts and coupling constants in conjunction with the data for O ligands described below, data for $[Pt([^{15}N]dien)(L\text{-Met-}S)]^+$ were also obtained (Table 1).

Hydrolysis of $[Pt([^{15}N]dien)Cl]^+$ 1

A 5 mM aqueous solution of **1** (pH 5.4) was monitored by ^1H and two-dimensional $[^1H,^{15}N]$ HSQC NMR spectroscopy for 7 d at 298 K. Apart from the peaks assigned to the starting complex, no new cross-peaks appeared in the spectra. Similarly the spectrum of a solution of $[Pt([^{15}N]dien)Cl]NO_3$ was also unchanged after 24 h at 298 K. Considering the detection limit of this technique, it was concluded that the rate of hydrolysis of $[Pt([^{15}N]dien)Cl]^+$ is $\ll 10^{-8}$ s^{-1}.

It was expected, by analogy with Pt–NH_3 and Pt–NH_2 complexes,[13] that the Pt–NH ^{15}N resonance of hydrolysed species would be shifted to high field by *ca.* 20 ppm relative to **1**. To confirm this, the aqua complex was prepared and characterized by NMR spectroscopy.

Characterisation of $[Pt([^{15}N]dien)(H_2O)]^{2+}$ 2

The two-dimensional $[^1H,^{15}N]$ HSQC NMR spectrum of **2** at pH 4.25, prepared by removal of Cl^- by treatment with Ag^+, contains a major cross-peak at δ 6.90/−8.5 (Fig. 1), assignable to an NH group *trans* to oxygen. The other two major cross-peaks in the spectrum can be assigned to the *trans* NH_2 groups of **2**. A weak cross-peak at δ 7.15/−1.7, in the ^{15}N chemical shift region of NH *trans* to an oxygen, was unassigned.

This solution was titrated over the pH range 1 to 11 to determine the pK_a of the aqua ligand. The pH dependences of the ^1H NMR shifts are shown in Fig. 2. The computer best fits gave rise to a pK_a of 6.0 ± 0.2. This value is comparable to that reported previously for this complex and for $[Pt(NH_3)_3(H_2O)]^{2+}$ using other methods (Table 2).[3a,20–23] It was notable that $^1J(^{195}Pt–^{15}N)$ values for the hydroxo complex $[Pt([^{15}N]dien)(OH)]^+$ were *ca.* 80 Hz smaller for Pt–NH and *ca.* 30 Hz smaller for Pt–NH_2.

Reactions of $[Pt([^{15}N]dien)Cl]Cl$ 1 with nucleobases

In order to investigate the pathways of reaction of complex **1** with nucleotides, we studied the kinetics of binding of **1** to

Table 2 pK_a Values for some platinum mono-aqua complexes (298 K, I = 0.1 M)

Compound	pK_a	Ref.
$[Pt(dien)(H_2O)]^{2+}$	6.13	20
	5.87	22
	6.24	23
	6.0	This work
$[Pt(NH_3)_3(H_2O)]^{2+}$	6.37	21
	6.0	3

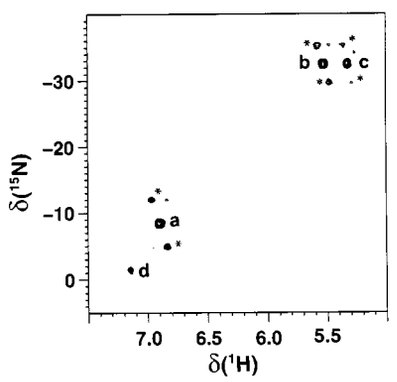

Fig. 1 Two-dimensional [^{1}H,^{15}N] HSQC NMR spectrum of [Pt([^{15}N]-dien)$(H_2O)]^{2+}$, pH 4.25, 298 K. Peak **a** is assigned to the NH group, and peaks **b** and **c** to the two NH_2 groups. Peak **d** has not been assigned. * Indicates ^{195}Pt satellites

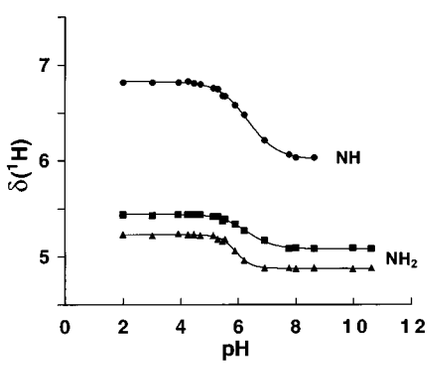

Fig. 2 Plots of ^{1}H NMR chemical shifts of Pt–NH_2 and Pt–NH of $[Pt([^{15}N]dien)(H_2O)]^{2+}$ *vs.* pH. The solid lines are best fits corresponding to a pK_a value of 6.0 ± 0.2. The Pt–NH cross-peak was not observed at pH values above 9, presumably due to an increase in exchange rate at high pH

5′-GMP, and also studied an equilibrium solution of the 3′-GMP adduct to allow comparison of NH shifts and assessment of the role of the 5′-phosphate group in H-bonding.[14]

The reaction of **1** (1 mM) with 5′-GMP proceeded rapidly, and at a molar ratio of 1:0.5 was essentially complete within 4 h at 298 K. Only peaks assignable to **1** and the adduct $[Pt([^{15}N]dien)(5'\text{-GMP-}N^7)]^{2+}$ (charge on 5′-GMP ignored) were seen. Plots showing the time dependence of the formation of the product are shown in Fig. 3. Attempts to fit these curves together with those for the disappearance of **1** using second-order kinetics were unsuccessful, giving k_2 values which varied from 0.06 M^{-1} s^{-1} at 1:10 to 0.2 M^{-1} s^{-1} at 1:0.5 molar ratio. When the 1:4 and 1:10 reactions were treated as pseudo-first-order reactions, k_1 values of 1.3 and 1.5 × 10^{-4} s^{-1}, respectively, were obtained.

The two-dimensional [^{1}H, ^{15}N] NMR spectrum of the adduct of **1** with 3′-GMP is shown in Fig. 4. The cross-peaks observed at δ 5.48/−30.3 and 5.32/−30.3 are assignable to the Pt–NH_2 groups of $[Pt([^{15}N]dien)(3'\text{-GMP-}N^7)]^{2+}$, and that at δ 6.72/6.0 to Pt–NH. It is notable that, in contrast to the 5′-GMP adduct, only two cross-peaks are observed for Pt–NH_2.

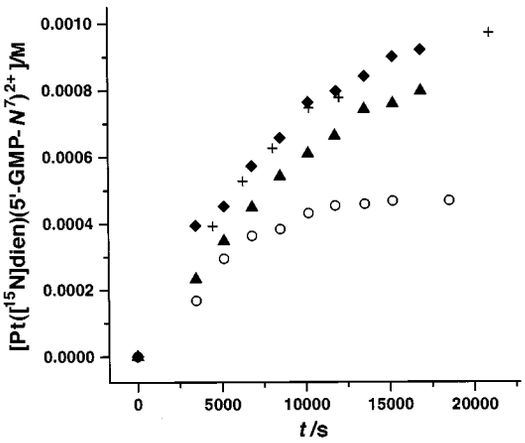

Fig. 3 Variation of the concentrations of $[Pt([^{15}N]dien)(5'\text{-GMP-}N^7)]^{2+}$ with time during reactions of **1** with 5′-GMP at molar ratios of 1:0.5 (○), 1:1 (▲), 1:4 (+) and 1:10 (◆)

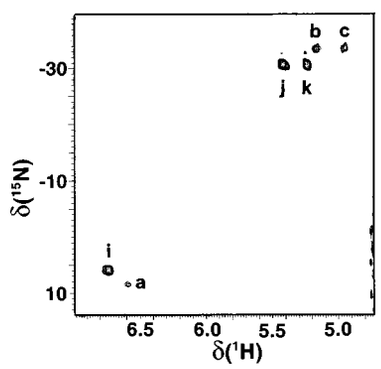

Fig. 4 Two-dimensional [^{1}H,^{15}N] NMR spectrum of a *ca.* 1:1 solution of [Pt([^{15}N]dien)Cl]Cl **1** (3 mM) and 3′-GMP, pH 6.74, 298 K, after 5 h incubation. Peak **i** is assigned to Pt–NH and peaks **j** and **k** to Pt–NH_2 of $[Pt([^{15}N]dien)(3'\text{-GMP-}N^7)]^{2+}$. Peaks **a**, **b** and **c** are due to unreacted **1**

Discussion

Nitrogen-15 labelling of dien [15] allows the study of the kinetics of reactions of complex **1**, a species which is of much interest in the platinum anticancer field in relation to the formation of monofunctional adducts,[5] using two-dimensional [^{1}H,^{15}N] NMR spectroscopy. This method has previously been shown to be valuable for the detection and characterisation of reaction intermediates and adducts.[4,24]

Analysis of the kinetic data for reactions of **1** with 5′-GMP showed that the reaction is not a simple second-order process. The k_1 values obtained from the pseudo-first-order treatment (*ca.* 1.4 × 10^{-4} s^{-1}, 0.1 M $NaClO_4$, *ca.* pH 6.6, 298 K) are comparable to those reported previously by Slavin *et al.*[9] (k_1 10^{-4} s^{-1}, 0.5 M $NaClO_4$, pH 6.8, 313 K) by UV spectroscopy, and Djuran *et al.*[7] (k_1 6.2 × 10^{-5} s^{-1}, 0.1 M phosphate, pH 6, 295 K) by ^{1}H NMR spectroscopy (GMP H[8] resonance). Slavin *et al.*[9] used pseudo-first-order kinetics to establish a two-term rate law (first-order and second-order pathways), whereas Djuran *et al.*[7] found that the second-order pathway was negligible even at low Pt:GMP ratios (1:1 and 1:2). Both groups attributed the first-order pathway to hydrolysis of **1**. However, in the present work, no peaks for an aqua/hydroxo intermediate were observed by two-dimensional [^{1}H,^{15}N] NMR during the course of the reaction. Since it was possible that the aqua species was present at too low a concentration to detect, hydrolysis of **1** was studied independently. This showed that the rate of hydrolysis of either **1** or its nitrate salt is much slower than the rate of the reactions with 5′-GMP. In fact, Gray and co-workers [10,11] noted the inertness of **1** towards hydrolysis some 30 years ago, and there appear to be no other reports of hydrolysis studies of **1**.

Therefore either the rate-limiting step in reactions of **1** with

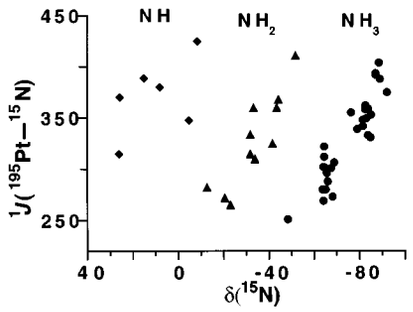

Fig. 5 Plot of $^1J(^{195}Pt–^{15}N)$ *versus* $\delta(^{15}N)$ of Pt–NH_3, Pt–NH_2 and Pt–NH, showing a similar dependence on the *trans* ligand. Data are taken from Table 1, and from the literature[21,28–30]

5′-GMP does not involve hydrolysis, or the hydrolysis is catalysed by the presence of the nucleotide. Mechanisms involving dien ring-opening can be considered but we observed no peaks for such intermediates and ring-opening seems unlikely at pH values as high as 6.8. Arpalahti and co-workers[25] have proposed a mechanism for the substitution of guanosine in [Pt(dien)(Guo)] (Guo = guanosine) by thiourea which involves a five-co-ordinate intermediate containing a ring-opened dien ligand. However, their reactions were carried out at pH 3. It is likely that complex **1** and 5′-GMP form an initial recognition complex. Outer-sphere interactions are thought to be important in the recognition of DNA by cisplatin.[26] Such a recognition complex could be a five-co-ordinate intermediate and could facilitate hydrolysis or phosphate substitution. However there is no NMR evidence for this. The second-order rate constants for the reactions of $[Pt(dien)(H_2O)]^{2+}$ with inosine and 1-methylinosine have been shown[23] to decrease dramatically with increase of pH due to the formation of the inert hydroxo complex $[Pt(dien)(OH)]^+$.

In order to confirm the chemical shifts, the aqua complex was prepared by treatment of **1** with Ag^+, and further characterized by a pH titration. The pK_a value (6.0) determined from the variation of the ^1H NMR NH and NH_2 chemical shifts is similar to values reported previously for this complex and the related triammine complex, Table 2. It was notable that the $^1J(^{195}Pt–^{15}N)$ values for the Pt–NH and Pt–NH_2 groups of $[Pt(dien)(OH)]^+$ are significantly smaller than those of $[Pt([^{15}N]dien)(H_2O)]^{2+}$ **2**. Similarly, Appleton *et al.*[27] have observed that $^1J(^{195}Pt–^{15}N)$ for Pt–NH_3 *trans* to O in $[Pt([^{15}N]H_3)_3(OH)]^+$ is *ca.* 90 Hz smaller than that for the protonated form $[Pt([^{15}N]H_3)_3(H_2O)]^{2+}$.

As can be seen in Fig. 5, measurements of both $^1J(^{195}Pt–^{15}N)$ and the ^{15}N chemical shift are valuable for distinguishing between Pt–NH, Pt–NH_2 and Pt–NH_3 resonances and for assignment of the *trans* ligand. In general, ligands with high *trans* influences give rise to smaller coupling constants (L-Met-*S* < I < Br < Cl < H_2O) and cause a low-field shift of the ^{15}N resonance. The dominant contribution to one-bond coupling constants between ^{195}Pt and ^{15}N is usually interpreted in terms of the Fermi contact interaction involving Pt 6s and N 2s orbitals.[31] The usefulness of $^1J(^{195}Pt–^{15}N)$ values is limited by the difficulty in determining them for larger molecules especially at high observation frequencies on account of the dominance of relaxation *via* chemical shift anisotropy.[13] For example, the ^{195}Pt satellites for the GMP complexes studied here were too broad to observe.

We have previously suggested[14] that both the 5′-phosphate and C^6 carbonyl groups of $[Pt([^{15}N]dien)(5′\text{-GMP-}N^7)]^{2+}$ are involved in the stabilization of this adduct. As can be seen from Fig. 4 and Table 1, two of the ^1H NMR peaks for Pt–NH_2 of $[Pt([^{15}N]dien)(5′\text{-GMP-}N^7)]^{2+}$ are shifted to low field compared to similar peaks of the 3′-GMP adduct, consistent with the presence of Pt–$NH_2 \cdots$ 5′-phosphate H-bonding in the former. This effect has also been observed for Pt–NH_3 complexes,[24] and such interactions are thought to be important for adducts of Pt drugs with DNA.[32]

Conclusion

We have shown that reactions of $[Pt([^{15}N]dien)Cl]^+$ with nucleotides can readily be monitored by two-dimensional $[^1H,^{15}N]$ NMR spectroscopy, and that Pt–NH shifts and $^1J(^{195}Pt–^{15}N)$ coupling constants are diagnostic of the *trans* ligand, as are those of Pt–NH_2 and Pt–NH_3. Kinetic analyses of reactions **1** with 5′-GMP reported here, and those reported previously,[7,9] appear to be consistent with hydrolysis as the rate-limiting step but no evidence for this was found in the NMR spectra. Moreover the rate of hydrolysis of **1** itself was found to be negligible, confirming the previous report of its inertness in water.[10] The details of the mechanism of reaction of **1** with GMP are therefore unclear, and this has often been the case in reported kinetic studies of other reactions of **1**. Proton NMR shifts of the Pt–NH_2 protons suggest that H-bonding interactions with the 5′-phosphate group are involved in the stabilization of $[Pt([^{15}N]dien)(5′\text{-GMP-}N^7)]^{2+}$. We are now using these NMR methods for investigating DNA adducts of complex **1**.

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References

1 S. E. Sherman and S. J. Lippard, *Chem. Rev.*, 1987, **87**, 1153.
2 M. C. Lim and R. B. Martin, *J. Inorg. Nucl. Chem.*, 1976, **38**, 1911.
3 (*a*) F. Gonnet, F. Reeder, J. Kozelka and J.-C. Chottard, *Inorg. Chem.*, 1996, **35**, 1653; (*b*) F. Reeder, F. Gonnet, J. Kozelka and J.-C. Chottard, *Chem. Eur. J.*, 1996, **2**, 1068.
4 K. J. Barnham, S. J. Berners-Price, T. A. Frenkiel, U. Frey and P. J. Sadler, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1874; S. J. Berners-Price, K. J. Barnham, U. Frey and P. J. Sadler, *Chem. Eur. J.*, 1996, **2**, 1283.
5 V. Brabec, O. Vrána, O. Nováková, V. Kleinwächter, F. P. Intini, M. Coluccia and G. Natile, *Nucleic Acids Res.*, 1996, **24**, 336.
6 See, for example, C. J. van Garderen, L. P. A. van Houte, H. van den Elst, J. H. van Boom and J. Reedijk, *J. Am. Chem. Soc.*, 1989, **111**, 4123; G. Admiraal, M. Alink, C. Altona, F. J. Dijt, C. J. van Garderen, R. A. G. de Graaff and J. Reedijk, *J. Am. Chem. Soc.*, 1992, **114**, 930.
7 M. I. Djuran, E. L. M. Lempers and J. Reedijk, *Inorg. Chem.*, 1991, **30**, 2648.
8 R. N. Bose, S. Moghaddas, E. L. Weaver and E. H. Cox, *Inorg. Chem.*, 1995, **34**, 5878.
9 L. L. Slavin, E. H. Cox and R. N. Bose, *Bioconjugate Chem.*, 1994, **5**, 316.
10 F. Basolo, H. B. Gray and R. G. Pearson, *J. Am. Chem. Soc.*, 1960, **82**, 4200.
11 H. B. Gray, *J. Am. Chem. Soc.*, 1962, **84**, 1548.
12 D. S. Martin and E. L. Bahn, *Inorg. Chem.*, 1967, **6**, 1653.
13 S. J. Berners-Price and P. J. Sadler, *Coord. Chem. Rev.*, 1996, **151**, 1.
14 Z. Guo, P. J. Sadler and E. Zang, *Chem. Commun.*, 1997, 27.
15 E. Zang and P. J. Sadler, *Synthesis*, 1997, 410.
16 G. Annibale, M. Brandolisio and B. Pitteri, *Polyhedron*, 1995, **14**, 451.
17 M. Piotto, V. Saudek and V. Sklenar, *J. Biomol. NMR*, 1992, **2**, 661.
18 J. Stonehouse, G. L. Shaw, J. Keeler and E. D. Laue, *J. Magn. Reson., Ser. A*, 1994, **107**, 174.
19 KALEIDAGRAPH, Synergy Software, Reading, PA, 1994.
20 R. M. Alcock, F. R. Hartley and D. E. Rogers, *J. Chem. Soc., Dalton Trans.*, 1973, 1070.
21 T. G. Appleton, J. R. Hall, S. F. Ralph and C. S. M. Thompson, *Inorg. Chem.*, 1989, **28**, 1989.

22 L. E. Erickson, H. L. Erickson and T. Y. Meyer, *Inorg. Chem*., 1987, **26**, 997.
23 J. Arpalahti and P. Lehikoinen, *Inorg. Chem*., 1990, **29**, 2564.
24 See, for example, S. J. Berners-Price, U. Frey, J. D. Ranford and P. J. Sadler, *J. Am. Chem. Soc*., 1993, **115**, 8649.
25 M. Mikola, J. Vihanto and J. Arpalahti, *J. Chem. Soc*., *Chem. Commun*., 1995, 1759.
26 S. K. C. Elmroth and S. J. Lippard, *Inorg. Chem*., 1995, **34**, 5234.
27 T. G. Appleton, J. R. Hall and S. F. Ralph, *Inorg. Chem*., 1985, **24**, 4685.
28 S. J. S. Kerrison and P. J. Sadler, *J. Chem. Soc*., *Chem. Commun*., 1977, 861.
29 S. J. S. Kerrison, Ph.D. Thesis, University of London, 1981.
30 I. M. Ismail, Ph.D. Thesis, University of London, 1982.
31 P. S. Pregosin, H. Omura and L. M. Venanzi, *J. Am. Chem. Soc*., 1973, **95**, 2047.
32 J. Reedijk, *Inorg. Chim. Acta*, 1992, **198–200**, 873.

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