trans *Diamine Pt^{II} Anticancer Complex*

Hydrolysis Triggers Oxidation of a *trans* Diamine Platinum(II) Anticancer Complex**

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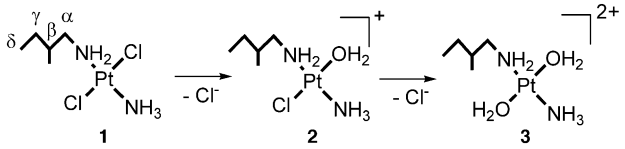
The clinical success of cisplatin (*cis*-diamminedichloroplatinum(II)) as an anticancer drug has prompted the search for more effective platinum anticancer drugs that circumvent some of its limitations, for example, toxicity and resistance.[1–4] It was initially believed, because of the inactivity of transplatin (*trans*-diamminedichloroplatinum(II)), that all *trans*-platinum complexes are ineffective as antitumour agents.[5,6] Recently, several groups have reported that, with appropriate choice of amine, the cytotoxic activity of *trans*-diam(m)inedichloroplatinum(II) complexes can be comparable to, or even greater than, that of cisplatin.[7–11]

An understanding of the aqueous chemistry of diamine Pt^{II} complexes is crucial for establishing their mechanism of action and hence for optimizing drug design. In particular, hydrolysis reactions of chloro Pt^{II} complexes are thought to activate them prior to platination of the target site, DNA, since Pt–OH_2 bonds are more reactive than Pt–Cl bonds.[12] Although both the kinetics and thermodynamics of hydrolysis reactions of cisplatin and related *cis*-diaminodichloro Pt^{II} complexes are well understood, surprisingly little is known about analogous reactions of *trans* complexes.[13–16]

Herein we report studies of the aqueous chemistry of *trans*-[$PtCl_2(NH_3)$(2-Me-butylamine)], **1**, a novel *trans*-platinum complex with cytotoxic activity comparable to that of cisplatin.[17] Unexpectedly, we have found that this complex undergoes facile redox reactions in aqueous solution. This shows that some *trans* complexes can exhibit novel chemistry that may be exploitable in further drug design, and could be relevant to the mechanism of biological activity.

We studied the hydrolysis pathway for **1**, Scheme 1, with ESIMS and ^{1}H 1D and [^{1}H, ^{15}N] 2D NMR spectroscopy.



Scheme 1. Hydrolysis pathway for **1**.

Surprisingly, the ^{1}H 1D NMR spectrum of [$^{15}NH_3$]**1** in water (Figure 1b) was complicated, in particular a range of ^{1}H signals appeared in the $\delta = 5$–6.5 ppm region, which were not seen when NaCl was present to suppress hydrolysis (Figure 1a). Signals at about $\delta = 5$–6.5 ppm (^{1}H) were also observed in the 2D [^{1}H, ^{15}N] spectrum of [^{15}N,^{15}N]**1** in water (see Supporting Information), with associated ^{15}N shifts in the regions −55 to −45 ppm and −25 to −20 ppm.

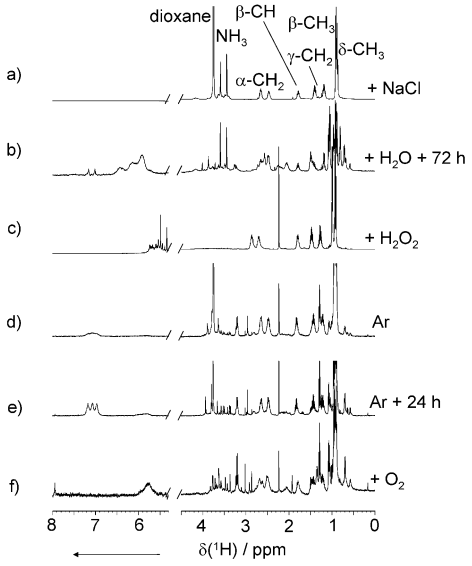


Figure 1. ^{1}H 1D NMR spectra of [$^{15}NH_3$]**1**. a) in 0.1 M NaCl in 90% H_2O/10% D_2O, 310 K; b) after reaction in the dark (72 h at 310 K) in 90% H_2O/10% D_2O, 310 K; c) complex [^{15}N,^{15}N]**1** treated with 25 equiv of H_2O_2; d) complex [$^{15}NH_3$]**1** after reaction with 0.95 equiv of $AgNO_3$ (O_2-free); e) sample from d) after it was allowed to stand for 24 h (O_2-free), and f) sample from e) after the sample had been purged with O_2(g) for 12 h. Signals assignable to Pt^{IV} products (about 5.2–6.5 ppm) appear in spectra b), c) and f). The triplet at about $\delta = 7$ ppm arises from NH_4^+ ions (^{14}N, nuclear spin quantum number $I = 1$), and is observable at low pH values. This signal from NH_4^+ ions disappears after the sample has been purged with O_2(g), as NH_3 is volatile. Water resonances have been omitted for clarity.

To determine whether any of the product signals are assignable to aqua adducts, we investigated aqua complexes prepared by reaction of [^{15}N,^{15}N]**1** with 1.2 mol equivalents of $AgNO_3$ (with subsequent removal of AgCl(s)). Three ^{1}H/^{15}N cross-peaks were observed in both the Pt^{II}–$^{15}NH_2$ and Pt^{II}–$^{15}NH_3$ regions of the spectrum (Figure 2a; ^{1}H/^{15}N chemical shifts (δ/ppm) 4.1 to 4.4/−47 to −44 and 3.5 to 3.8/−66 to −63, respectively). The ^{1}H/^{15}N chemical shifts of these species (Table 1) are consistent with those expected for Pt^{II} species with *trans*-nitrogen atoms,[18] and are assignable to the

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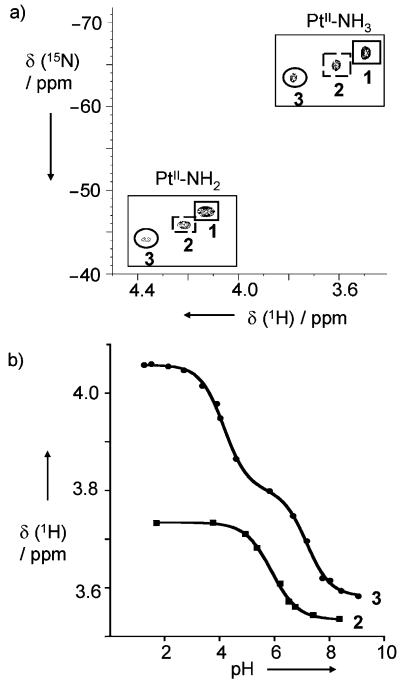

Figure 2. a) [^{1}H, ^{15}N] 2D NMR spectrum of the products formed after treatment of [^{15}N,^{15}N]**1** with $AgNO_3$ (molar ratio 1:1.2) in 0.1 M $NaClO_4$ in 90% H_2O/10% D_2O, 310 K, pH adjusted to 6.2. Assignments for the dichloro (**1**), monoaqua (**2**) and diaqua (**3**) complexes are shown. b) Variation in the Pt-NH_3 ^{1}H NMR chemical shifts of peaks **2** and **3** in the 2D [^{1}H, ^{15}N] NMR spectrum shown in a), assignable to the monoaqua/chloro and diaqua adducts of [^{15}N,^{15}N]**1** in 90% H_2O/10% D_2O, 310 K. The lines are computer-best fits with pK_a values of 5.90 for **2** (monoaqua), and 4.16 and 7.17 for **3** (diaqua).

Table 1: ^{1}H and ^{15}N NMR chemical shifts (ppm) for complexes **1, 2**, and **3** in 90% H_2O/10% D_2O (pH 6.2, 310 K).

Complex	δ ^{1}H,^{15}N [ppm]	
	Pt^{II}-NH_3	Pt^{II}-NH_2
1	3.49, −66.35	4.13, −47.38
2	3.61, −64.89	4.22, −45.85
3	3.78, −63.43	4.37, −44.08

dichloro complex, **1**, the monoaqua complex, **2**, and the diaqua complex, **3**. The assignments of signals for the aqua complexes were confirmed by their pH titration curves. Plots of the variation of the ^{1}H NMR chemical shifts for the Pt–NH_3 signals versus pH were fitted to give pK_a values of 5.90 for **2**, and of 4.16 and 7.17 for **3** (Figure 2b). These values are comparable with those reported for *trans*-$[PtCl(H_2O)(NH_3)_2]^+$ (pK_a = 5.63) and *trans*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$ (pK_{a1} = 4.35, pK_{a2} = 7.40), under similar conditions.[13]

The ^{1}H/^{15}N cross-peaks assignable to monoaqua species were observed in the 2D HSQC spectrum for the hydrolysis products of [^{15}N,^{15}N]**1** formed after 24 h of reaction in 90% H_2O/10% D_2O at 310 K (see Supporting Information). However, in this spectrum, no signals assignable to the diaqua species were observed. It might be expected that hydrolysis of one chloride ligand in a *trans*-$\{PtCl_2\}$ unit might occur at a faster rate than cisplatin on account of the higher *trans* effect of Cl^- compared to NH_3, and that the second step to form *trans*-$\{Pt(H_2O)_2\}^{2+}$ would be very slow. This has indeed been reported to be the case.[14,16,19,20] In fact, it is very difficult to remove the second Cl^- ligand from *trans*-$[PtCl(H_2O)(NH_3)_2]^+$ and requires extensive heating in the presence of Ag^+.[13,19]

Two additional sets of signals were also observed in all the 2D [^{1}H,^{15}N] HSQC spectra recorded for the hydrolysis of [^{15}N,^{15}N]**1** in water (see Supporting Information). Such signals also appeared when aqua adducts were generated by the reaction of [^{15}N,^{15}N]**1** with $AgNO_3$ (molar ratio 1:1.2, in 90% H_2O/10% D_2O, 310 K), Figure 3 (for the complete spectrum

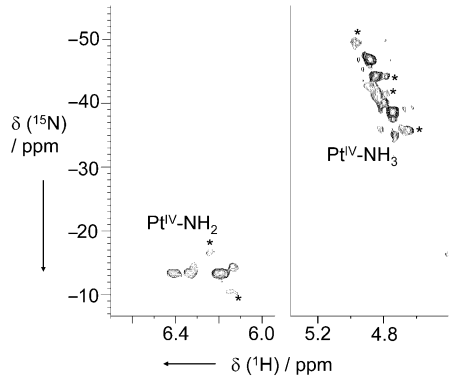

Figure 3. [^{1}H, ^{15}N] 2D NMR spectrum for the Pt^{IV} reaction products formed after reaction of [^{15}N,^{15}N]**1** with $AgNO_3$ (molar ratio 1:1.2) in 0.1 M $NaClO_4$ in 90% H_2O/10% D_2O, 310 K. This spectrum was recorded during the pH titration at pH 1.5. Similar peaks were present at higher pH values, but the Pt–NH_3 signals overlap with the H_2O signals. (* = ^{195}Pt satellites).

see Supporting Information). It can be seen that at least three sets of signals, some with ^{195}Pt satellites, appear in the regions with ^{1}H/^{15}N chemical shifts (δ/ppm) of 5.7 to 6.5/−20 to −10 (Pt^{IV}-NH_2) and 4.6 to 5.2/−50 to −35 (Pt^{IV}-NH_3). The shifts of these cross-peaks are similar to those that we observed for the reaction of [^{15}N,^{15}N]**1** with the strong oxidant H_2O_2, which gave rise to three new sets of Pt–NH_2 and Pt–NH_3 [^{1}H,^{15}N] NMR signals with chemical shifts (δ/ppm) of about 5.6/−24 and 5.3/−40, respectively (see Supporting Information). These signals can be assigned to $^{15}NH_3$–Pt^{IV}-2-Me-(^{15}N)butylamine complexes with additional Cl and OH ligands. Also, the $^1J(^{195}Pt–^{15}N)$ couplings (all about 260 Hz) are consistent with the presence of Pt^{IV} centers.[21]

To investigate whether the presence of air influences the course of the hydrolysis reactions of **1**, aqua adducts were prepared by treating [$^{15}NH_3$]**1** with 0.95 mol equivalents of $AgNO_3$ under argon. In the absence of air, no ^{1}H NMR signals were observed in the δ = 5.2–6.5 ppm region of the 1D ^{1}H (Figure 1d). However, signals in this region appeared after the sample was purged with oxygen overnight (Figure 1f), which suggests that products from the hydrolysis of **1** in water react with oxygen.

Thus, the observed down-field shifts for both ^{1}H and ^{15}N NMR signals upon oxidation of [^{15}N,^{15}N]**1** with H_2O_2 are consistent with the formation of novel Pt^{IV} complexes during the hydrolysis reactions of **1** in air, although they are not only

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trans hydroxy adducts. We can find only one previous report of the spontaneous oxidation of aquated *trans*-diamine Pt^{II} complexes.[13] Appleton et al. reported the detection of NMR peaks corresponding to Pt^{IV} products from reactions of transplatin and silver salts after extensive heating, but did not investigate this reaction further. In the present study, Pt^{IV} peaks appear directly during the hydrolysis of $[^{15}NH_3]$**1**, even in the absence of silver (see Figure 1b). In general, Pt^{II} complexes with diam(m)ine and dihydroxo ligands are more easily oxidised than the dichloro analogues.[22]

Interestingly, ^1H NMR signals for $^{14}NH_4^+$ were observed for $[^{15}NH_3]$**1** in H_2O, under argon (triplet about $\delta = 7.1$ ppm; Figure 1d and 1e). The NH_3 ligand in the complex was labeled with ^{15}N (>95%) in this experiment, and thus the only source of this $^{14}NH_4^+$ was the 2-Me-butylamine ligand (since NO_3^- as a source seems unlikely). This suggests that during the hydrolysis reaction under these conditions the Pt–NH_2 bond of complex **1** is partially cleaved. The mechanism of this cleavage is unclear. Traces of a greyish-black precipitate were observed during the reaction, consistent with formation of Pt^0 (inductively coupled plasma atomic emission spectrometry (ICPAES) analysis after the solid was dissolved in HNO_3/HCl indicated the presence of Pt but not Ag atoms). Ammonia release, therefore, may involve oxidation of the amine.

The formation of Pt^{IV} products was confirmed by ^{195}Pt NMR spectroscopy. Five sets of ^{195}Pt signals were observed in the ^{195}Pt NMR spectrum of **1** that had been treated with $AgNO_3$ (molar ratio **1**/$AgNO_3$ 1:1.2; 0.1 M $NaClO_4$, 90% H_2O/10% D_2O, 310 K), with shifts of −2178, −1867, −1471, −1157, and −50/−78 ppm (Figure 4). The signal at $\delta = -2178$ ppm (a) is assignable to the dichloro complex, **1**, and that at $\delta = -1867$ ppm (b) to the monoaqua complex, **2**. The signals at $\delta = -50$ and −78 ppm (e) are assignable to Pt^{IV} species on the basis of their chemical shifts,[23,24] which confirms that the hydrolysis of **1** results in the formation of both Pt^{II} and Pt^{IV} products. The shift of peak (c), $\delta = -1471$ ppm, is consistent with either an hydroxy-bridged Pt^{II} species or the diaqua complex, **3**, and that of signal (d), $\delta = -1157$ ppm, with an hydroxy-bridged Pt^{II} dimer.[25,26]

ESIMS was used to identify further the products formed from reaction of complex **1** with $AgNO_3$ (0.95 mol equiv) in water. A large number of product signals was observed in the ESIMS spectrum (see Supporting Information), and each set of signals showed a cluster of ions with characteristic isotope splitting patterns for Pt, N and Cl atoms. Fragments observed at *m*/*z* 297, 331, and 349 are assignable to the mononuclear species $[PtC_5N_2H_{15}]^+$, $[PtC_5N_2O_2H_{17}]^+$, and $[PtC_5N_2OClH_{18}]^+$, respectively. With the aid of isotope modeling, the sets of peaks observed at *m*/*z* 667 and 982 can be assigned to the dinuclear species $[Pt_2C_{10}N_4O_2ClH_{35}]^+$ and trinuclear species $[Pt_3C_{15}N_6O_3ClH_{52}]^+$, respectively. This provides further evidence for the formation of di- and trinuclear Pt^{II} hydroxy-bridged species in the hydrolysis reactions of **1**. Since hydroxy-bridged dimers and trimers of **1** appear to be readily formed in aqueous solution, further work on their isolation, DNA binding, and biological testing may be profitable. To our knowledge, no X-ray structures of hydroxy-bridged *trans* diamine complexes are available.

The findings of novel redox reactions of the complex **1** in aqueous solution may aid the design of *trans* diamine anticancer complexes, and the elucidation of structure–activity relationships. Hydrolysis of such *trans* complexes would be expected to be facile inside cells where the Cl^- concentration is lower than outside cells. Oxidation of Pt^{II} to Pt^{IV} ions may be more prevalent in normal cells compared to cancer cells, since cells in solid tumours tend to be hypoxic. If oxidation to Pt^{IV} inactivates the complex, this could provide a useful means of introducing selective toxicity, thus reducing side effects. It may be possible to vary the ease of oxidation by variation of the amine. It will be interesting to determine whether the oxidised products are stable even in the presence of the tripeptide glutathione, which is often present at millimolar concentrations in cells. Further work to investigate reaction pathways accessible to *trans* complexes which are not available to *cis* isomers is warranted.

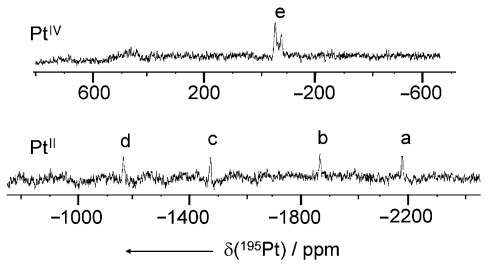

Figure 4. ^{195}Pt-{^1H} NMR spectrum of a solution containing **1** treated with 1.2 mol equivalents of $AgNO_3$ in 90% H_2O/10% D_2O that contains 0.1 M $NaClO_4$ (310 K, about 75 mM). Signals assignable to Pt^{II} complexes **1** and **2** ($\delta = -2178$ ppm (a) and $\delta = -1867$ ppm (b)) and Pt^{IV} species (ca. −50 ppm (e)) are shown. The signal at $\delta = -1157$ ppm (d) may arise from dinuclear Pt^{II} species, and peak **c** at $\delta = -1471$ ppm to an hydroxy-bridged Pt^{II} complex or the diaqua complex **3**.

Experimental Section

$K_2[PtCl_4]$ was provided on loan from Johnson Matthey plc. $^{15}NH_4Cl$, potassium phthalimide-^{15}N, 2-methylbutylamine and *S*-1-bromo-2-methylbutane were purchased from Aldrich. Cisplatin was synthesised by the Dhara method.[27]

The syntheses of *trans*-$[PtCl_2(NH_3)$(*rac*-2-Me-butylamine)], **1**, *trans*-$[PtCl_2(^{15}NH_3)$(*S*-2-Me-(^{15}N)butylamine)], $[^{15}N,^{15}N]$**1**, and *trans*-$[PtCl_2(^{15}NH_3)$(*rac*-2-Me-butylamine)], $[^{15}NH_3]$**1**, were based on previously published procedures,[28,29] and are outlined in the Supporting Information. The monoaqua *trans*-$[PtCl(OH_2)(^{15}NH_3)$(*S*-2-Me-(^{15}N)butylamine)]NO_3 complex, $[^{15}N,^{15}N]$**2**, was prepared in situ by addition of $AgNO_3$ (1.6 mg, 9.4 mmol) to a suspension of $[^{15}N,^{15}N]$**1** (3.7 mg, 9.9 mmol) in 90% H_2O/10% D_2O (0.75 mL). The mixture was allowed to react for 24 h at ambient temperature in the dark, and then centrifuged to remove the white AgCl precipitate. $NaClO_4$ (9.2 mg, 75 mmol) was added to provide a constant ionic strength (0.1 M) for the pH titration. The same procedure was followed to give solutions containing the diaqua complex, *trans*-$[Pt(OH_2)_2(^{15}NH_3)$(*S*-2-Me-(^{15}N)butylamine)]$(NO_3)_2$, $[^{15}N,^{15}N]$**3**, by using 1.2 mol equivalents of $AgNO_3$. In all experiments, it was determined by ICPAES that no residual silver remained in solution.

1D ^1H, 1D ^{195}Pt and 2D [^1H, ^{15}N] HSQC NMR spectra were recorded at 310 K in 5 mm (10 mm for ^{195}Pt) NMR tubes on a Bruker DMX 500 MHz spectrometer (^1H 500.13 MHz, ^{15}N 50.70 MHz, ^{195}Pt 53.76 MHz), with dioxane ($\delta(^1H)$ 3.76 ppm, internal), $^{15}NH_4Cl$ ($\delta(^{15}N)$ 0 ppm, external) and K_2PtCl_4 ($\delta(^{195}Pt)$ −1623 ppm, external)[30] as

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standards. The pH values were determined at 298 K directly in the NMR tube, prior to and after NMR measurements, by using a Corning 145 pH meter equipped with a chloride-free microcombination electrode calibrated with Aldrich standard buffers (pH 4, 7 and 10). The pH was adjusted with dilute solutions of $HClO_4$ and NaOH. The pH titration curves were fitted to the Henderson–Hasselbalch equation by using the Kaleidagraph program (Synergy Software, Reading, PA, USA). ESIMS was performed on a Platform-II mass spectrometer (Micromass, Manchester, UK). ICPAES determination of platinum and silver concentrations were performed with a Thermo Jarrell Ash *IRIS* ICPAES, calibrated with 0–100 ppm standards.

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