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[^{1}H, ^{15}N] Heteronuclear Single Quantum Coherence NMR Study of the Mechanism of Aquation of Platinum(IV) Ammine Complexes

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The aquation and hydrolysis of a series of platinum(IV) complexes of the general form *cis*, *trans*, *cis*-[$PtCl_2(X)_2(^{15}NH_3)_2$] (X = Cl^-, $O_2CCH_3^-$, OH^-) have been followed by [^{1}H,^{15}N] Heteronuclear Single Quantum Coherence NMR spectroscopy. Negligible aquation (<5%) is observed for the complexes where X = $O_2CCH_3^-$ or OH^- over 3−4 weeks. Aquation of *cis*-[$PtCl_4(^{15}NH_3)_2$] (**1**) is observed, and the rate of aquation increases with increasing pH and upon the addition of 0.01 mol equiv of the platinum(II) complex *cis*-[$PtCl_2(^{15}NH_3)_2$] (cisplatin). The first aquated species formed from *cis*-[$PtCl_4(NH_3)_2$] has one of the axial chloro groups (relative to the equatorial NH_3 ligands) replaced by an aqua/hydroxo ligand. The second observed substitution occurs in an equatorial position. Peaks that are consistent with five of the eight possible aquation species were observed in the NMR spectra.

Introduction

The aquation and substitution reactions of platinum(IV) complexes have been investigated extensively and were significant in the development of the understanding of the mechanism of ligand substitution at octahedral metal centers in the 1960s.[1−21] These studies almost exclusively focused on *trans*-[PtN_4X_2] or [PtN_5X] (N = N-donor ligand, X = halide or pseudohalide) complexes using spectrophotometry, conductivity, changes in pH, or via exchange of radiolabeled Cl^- to monitor reaction progress and as a result, incorporated only one or two substitution steps.[4]

The first aquation/hydrolysis studies on *cis*- and *trans*-[$PtCl_4(NH_3)_2$] complexes were carried out by Werner and coworkers[22,23] and Jørgensen[24] in the late 1800s using conductivity measurements that afforded inconsistent results. Grinberg and Korableva,[6] using the same techniques, found that the rate of aquation of the *trans*-isomer was 3-fold greater than found for the *cis*-isomer, and that the inconsistent kinetic data of the earlier studies[22−24] resulted from exposure to light and also from the presence of residual platinum(II) "impurities" that act as catalysts. Other workers have verified both effects on Pt(IV) substitution reactions.[7,8,13,19,20]

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The body of evidence from early workers suggests that ligand exchange on octahedral platinum(IV) complexes occurs via an S_N1-like (dissociative) substitution[2] but is also facilitated by Pt(II) catalysts. Basolo and co-workers followed chloro ligand exchange between platinum(II) and platinum(IV) complexes and proposed a two-electron inner-sphere transfer mechanism in which a chloro ligand bridging the two Pt centers was responsible.[3,4] An induction period of 1−2 h was observed prior to ligand substitution, which was ascribed to in situ platinum(IV) reduction to platinum(II) which then catalyzed platinum(IV) ligand exchange.[5,12] Additional experimental support for the $Pt^{IV}-Cl-Pt^{II}$ transition state was provided by Cox et al.[25] who observed incorporation of ^{195}Pt into the Pt(IV) species from labeled ^{195}Pt(II) complexes.

There is renewed interest in platinum(IV) complexes stemming from their potential as chemotherapeutic agents,[26−28] particularly in light of the recent U.S. FDA consideration of satraplatin (JM216),[29] and accordingly the rates and stereochemical preferences of their aquation behavior is of importance. Indeed, it has been shown that the aquation of separate clinical preparations of the Pt(IV) drug tetraplatin proceeded at differing rates, and this was suggested to affect Pt(IV) drug pharmacokinetics.[30] In the current study the aquation/hydrolysis of three Pt(IV) complexes that incorporate a *cis*-$\{PtCl_2(NH_3)_2\}$ equatorial core, differing only in the nature of the axial ligands (Cl^-, OH^-, or $O_2CCH_3^-$), is explored. *cis*-$[PtCl_4(NH_3)_2]$ (**1**) is of particular relevance in this context because it was shown in the seminal work by Rosenberg to interfere with cell division in *Escherichia coli*[31,32] which is the observation that ultimately resulted in the development of cisplatin as a chemotherapeutic agent.

We have chosen to investigate this group of complexes because they encompass a range of reduction potentials,[33−35] and it has been shown previously that the reactions and speciation of Pt(IV) complexes in cancer cells and simulated biological environments correlate with these reduction potentials.[26,36−39] The application of [^{1}H,^{15}N] Heteronuclear Single Quantum Coherence (HSQC) 2D NMR spectroscopy to follow the reaction pathways of ^{15}N-labeled platinum am(m)ine complexes is powerful because all platinated species are detected simultaneously, and the ^{15}N chemical shift is sensitive to the nature of the ligand *trans* to the ^{15}N-am(m)ine group, permitting identification of individual species and quantification of the peak volumes over the course of a reaction.[40] [^{1}H,^{15}N] NMR methods have been previously applied by us and others to study the aquation reactions of cisplatin[41−43] and other mononuclear[44−47] and polynuclear[48−50] Pt(II) am(m)ine anticancer complexes. The hydrolytic behavior of *cis*-$[PtCl_2(NH_3)(c\text{-}C_6H_{11}NH_2)]$,[51] a major metabolite in samples from patients treated with the Pt(IV) anticancer complex satraplatin, has been investigated by [^{1}H,^{15}N] NMR, and Nemirovski et al. have used [^{1}H,^{13}C] HSQC 2D NMR to follow the loss of isotopically labeled acetate axial ligands from a Pt(IV) complex to infer reduction to Pt(II),[52] but this is the first such study to investigate the aquation reactions of Pt(IV) ammine complexes.

Experimental Section

Synthesis of ^{15}N-Labeled Cisplatin and Platinum(IV) Analogues *cis*-$[PtCl_2(^{15}NH_3)_2]$.[53,54] $K_2[PtCl_4]$ (0.70 g, 1.7×10^{-3} mol) was dissolved in water (10 mL), and a saturated solution of KI (1.0 g, 6.0×10^{-3} mol, ∼1 mL) was added. This mixture was stirred at room temperature for 15 min, affording a black solution. $^{15}NH_4Cl$ (0.20 g, 3.7×10^{-3} mol) in 1 M KOH solution (2.5 mL) was added dropwise with stirring, and the mixture was then heated to 40 °C for 30 min. The bright yellow *cis*-$[PtI_2(^{15}NH_3)_2]$ that precipitated was collected, washed with water, ethanol, and ether and dried in a desiccator, yielding 0.74 g, 1.5×10^{-3} mol, 91%.

Dry *cis*-$[PtI_2(^{15}NH_3)_2]$ (0.50 g, 1.0×10^{-3} mol) was dissolved in an aqueous solution of $AgNO_3$ (0.356 g, 2.10×10^{-3} mol, ∼2.5 mL) in slight excess. The reaction vessel was protected from light

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and stirred at 37 °C for 1 h. The AgI precipitate was filtered off through a syringe and microfilter, after centrifuging the reaction mixture. A few drops of 1 M HCl were added to the filtrate until no further AgCl precipitation was apparent. The solution was again filtered, and excess KCl (0.70 g, 9.0×10^{-3} mol) was added to the filtrate, which was stirred at 37 °C precipitating *cis*-[$PtCl_2(^{15}NH_3)_2$] (cisplatin) as a yellow powder. This was recrystallized from aqueous sodium chloride solution, washed with cold water, ethanol, and ether and dried in a desiccator, yielding 0.27 g, 8.9×10^{-4} mol, 87%. IR (KBr, cm^{-1}) 3280 s, 3209 s, 1639 w, 1535 w, 1313 s, 1293 m, 802 s.

***cis*, *trans*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (3).** [55,56] Dry *cis*-[$PtCl_2(^{15}NH_3)_2$] (0.20 g, 6.6×10^{-4} mol) was suspended in water (5 mL) and a 10-fold excess of H_2O_2 (3% w/v, 7.0 mL, 6.0×10^{-3} mol, **Caution!**) was added. The mixture was stirred for 1 h at 50 °C, affording a pale yellow powder. *cis*, *trans*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (**3**) was recrystallized from hot water, collected and washed with cold water, ethanol, and ether, and dried in a desiccator, yielding 0.109 g, 3.27×10^{-4} mol, 49%. IR (KBr, cm^{-1}) 3516 vs, 3393 vw, br, 3258 s, 3176 m, 2900−3100 br, 2727 s, 2671 vw, 2513 vw, 2450 w, 2278 w, 2278 w, 2113 vw, 1876 vw, 1819 w, 1606 w, 1589 m, 1551 w, 1361 m, 1171 vw, 1040 vs, 966 m, 906 m, 820 vw, 557 vs, 532 m, 454 s.

***cis*-[$PtCl_4(^{15}NH_3)_2$] (1).** *cis*, *trans*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (**3**) was synthesized as described above from *cis*-[$PtCl_2(^{15}NH_3)_2$] (0.20 g, 6.6×10^{-4} mol), and the isolated product *cis*, *trans*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (**3**) was suspended in 10 M HCl solution (5 mL) and stirred for 1 h at 50 °C. Heat was then removed and the mixture stirred overnight to give a pale yellow solid, *cis*-[$PtCl_4(^{15}NH_3)_2$] (**1**). This was recrystallized from HCl (4 M), collected and washed with cold water, ethanol, and ether, and dried in a desiccator, yielding 0.115 g, 3.08×10^{-4} mol, 47%. IR (KBr, cm^{-1}) 3264 s, 3177 vs, 3076 m, 2661 w, 2616 m, 2187 w, 2154 w, 1678 m br, 1563 s, 1552 s, 1454 w, 1324 s, 1309 s, 864 m, 834 w, 521 w.

***cis*, *trans*, *cis*-[$PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2$] (10).** [33] *cis*, *trans*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (**3**) (0.019 g, 5.2×10^{-4} mol) was suspended in CH_2Cl_2 (25 mL). A large excess of acetic anhydride (5 mL, 0.05 mol) was added and a drying tube sealed over the reaction vessel. The mixture was stirred for 3 weeks, with addition of further acetic anhydride (1 mL, 0.01 mol) every 48 h. The buff colored *cis*, *trans*, *cis*-[$PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2$] (**10**) was collected, recrystallized from water and washed with cold water, ethanol, and ether, and dried in a desiccator yielding 0.1490 g, 3.55×10^{-4} mol, 54%. IR (KBr, cm^{-1}) 3265 s, 3202 s, 3082 s, 1654 vs, 1618 s, 1590 m, 1564 m, 1429 w, 1402 w, 1363 s, 1299 vs, 1272 vs, 1048 w, 1023 w, 942 m, 864 w, 701 s, 532 w, 510 w.

***cis*-[$Pt(OH)_4(^{15}NH_3)_2$] (9).** [57,58] ^{15}N-labeled cisplatin (0.05 g, 1.66×10^{-4} mol) was stirred with 1.90 equiv of $AgNO_3$ (0.052 g, 3.15×10^{-4} mol) in water (0.5 mL) overnight protected from light. The solution was centrifuged and the precipitated AgCl was filtered off. To the filtrate, containing the complex *cis*-[$Pt(OH_2)_2(^{15}NH_3)_2]^{2+}$, was added H_2O_2 (30% v/v, ∼2 mL) and the mixture stirred at 45 °C for 8 h. The solution was then warmed to ∼80 °C for 2 h to decompose excess peroxide. The solution was concentrated by rotary evaporation, acetone was added, and the complex that precipitated out was collected at the pump.

NMR Studies. Aquation of *cis*-[$PtCl_4(^{15}NH_3)_2$] (1). *cis*-[$PtCl_4(^{15}NH_3)_2$] (**1**) (1.45×10^{-3} g, 3.89×10^{-6} mol) was dissolved in 100 mM $NaClO_4$ solution (1.300 mL) (***Caution!** perchlorates are explosive*.) by sonicating at 298 K for 5 min in the dark, giving a 2.99 mM solution. A 650 μL aliquot was transferred to an NMR tube and D_2O (40 μL) was added to give a concentration of *cis*-[$PtCl_4(^{15}NH_3)_2$] of 2.82 mM. The aquation reaction was studied at three different initial pH values (5.7, 4.3, and 3.6). The pH of the $NaClO_4$ solution was adjusted to 4.3 and 3.6 by addition of 0.1 M $HClO_4$ prior to the addition of the Pt reagents, and the pH values quoted are those of the solutions following the addition of Pt.

Aquation of *cis*, *trans*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (3). *cis*, *trans*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (**3**) ($1.30 \times 10^{-3}$ g, 3.87×10^{-6} mol) was dissolved in 1.300 mL of 100 mM $NaClO_4$ in H_2O. A 650 μL aliquot was transferred to an NMR tube and D_2O (40 μL) was added to give a concentration of *cis*, *trans*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (**3**) of 3.65 mM. The initial pH of the solution was 4.3.

Aquation of *cis*, *trans*, *cis*-[$PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2$] (10). *cis*, *trans*, *cis*-[$PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2$] (**10**) ($1.60 \times 10^{-3}$ g, 3.81×10^{-6} mol) was dissolved in 1.300 mL of 100 mM $NaClO_4$ in H_2O. A 650 μL aliquot was transferred to an NMR tube and D_2O (40 μL) was added to give a concentration of *cis*, *trans*, *cis*-[$PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2$] (**10**) of 3.59 mM. The initial pH of the solution was 4.3.

The samples were either immediately placed in the NMR spectrometer at 25 °C or wrapped in aluminum foil and placed in a thermostatted water bath at 25 °C between data collection times.

For the Pt(II) catalyzed reactions, 0.01 mol equiv of *cis*-[$PtCl_2(^{15}NH_3)_2$] were added to the platinum(IV) solution. This was carried out by dissolving ^{15}N-cisplatin (∼1.50×10^{-3} g) in 5% D_2O/95% H_2O (1 mL) by sonicating at 298 K for 2 min, giving a ∼5 mM stock solution, of which 4 μL was added to the Pt(IV) reaction mixture. The addition of this small volume has a negligible effect on the Pt(IV) concentration.

Data Collection. $^1H\{^{15}N\}$ NMR spectra were recorded at 400.13 MHz on a Varian UNITY 400 MHz spectrometer fitted with a pulsed field gradient module and a 5 mm triple-resonance probehead. The ^1H spectra were acquired with water suppression using the WATERGATE sequence.[59] Both the 1D ^{15}N-edited ^1H NMR spectra and 2D [^1H,^{15}N] HSQC NMR spectra (optimized for $^1J(^{15}N-^1H) = 72$ Hz) were recorded using the sequence of Stonehouse et al.[60] The ^{15}N signals were decoupled by irradiating with the GARP-1 sequence at a field strength of 1 kHz during the acquisition time. Samples were not spun during the acquisition of data. All samples (including buffers, acids, etc.) were prepared in 95% H_2O/5% D_2O (for a deuterium lock but with minimal loss of signal as a result of deuterium exchange). Spectral acquisition parameters and data analysis were as previously described.[42,61,62]

Results

Fully ^{15}N-labeled *cis*-[$PtCl_4(^{15}NH_3)_2$] (**1**), *cis*, *trans*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (**3**), *cis*-[$Pt(OH)_4(^{15}NH_3)_2$] (**9**), and *cis*, *trans*, *cis*-[$PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2$] (**10**) were prepared according to published methods using ^{15}N-labeled starting materials.[33,53−56] The ^1H and ^{15}N NMR chemical shifts of these peaks are given in Table 1. The conversion of *cis*, *trans*,

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Table 1. ^{1}H and ^{15}N Chemical Shifts of Platinum(IV) Complexes in 0.1 M $NaClO_4$ in 5% D_2O/95% H_2O[a]

complex	^{1}H	^{15}N	*trans*-donor[b]
cis-$[PtCl_4(^{15}NH_3)_2]$ (**1**)	6.04	−35.0	Cl
fac-$[PtCl_3(OH)(^{15}NH_3)_2]$ (**2**)	5.84	−34.3	Cl
cis, *trans*, *cis*-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (**3**)	5.74	−34.2	Cl
mer-$[PtCl_3(OH)(^{15}NH_3)_2]$ (**4a**)	5.88	−32.9	Cl
mer-$[PtCl_3(OH)(^{15}NH_3)_2]$ (**4b**)	5.60	−50.9	O
cis,cis,cis-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (**5a**)	5.81	−32.0	Cl
cis,cis,cis-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (**5b**)	5.80	−53.4	O
trans,cis,cis-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (**7**)	5.82	−51.5	O
fac-$[PtCl(OH)_3(^{15}NH_3)_2]$ (**8**)	5.75	−51.6	O
cis-$[Pt(OH)_4(^{15}NH_3)_2]$ (**9**)	5.31	−47.8	O
cis, *trans*, *cis*-$[PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2]$ (**10**)	6.46	−41.0	Cl

[a] $^{1}H/^{15}N$ chemical shifts are quoted for the first appearance of a peak in the solution with a starting pH of 4.3. Values for **3** and **9** are derived from the pH titration curves (Supporting Information, Figure S1) for the authentic samples at this pH. The chemical shifts are sensitive to pH and change during the course of the reactions. [b] The ligand donor atom lying *trans* to the $^{15}NH_3$ group.

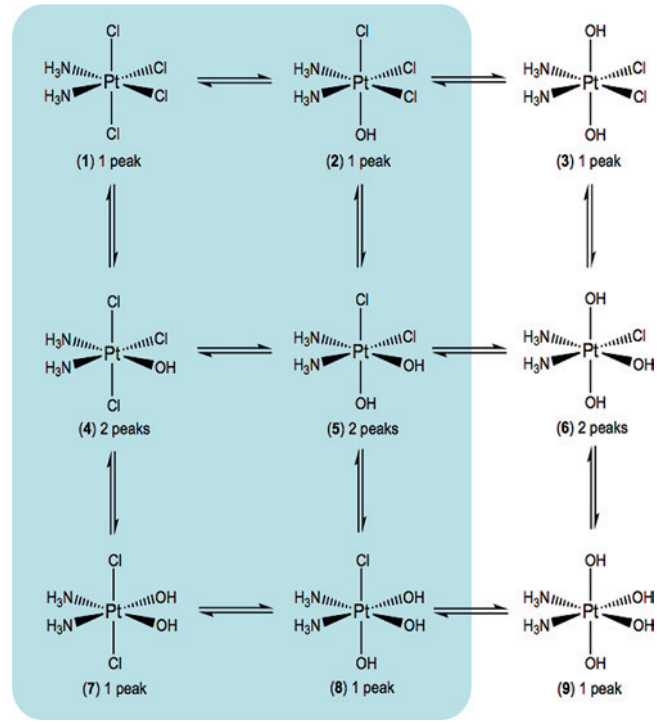
cis-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ to *cis*, *trans*, *cis*-$[PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2]$ is well understood[63−65] and there was no spectral evidence to suggest replacement of the two equatorial Cl ligands by acetato ligands.

The $^{1}H/^{15}N$ peaks of complexes give rise to ^{195}Pt satellites. The high intensity of the ^{195}Pt satellites is a consequence of the octahedral geometry of Pt(IV) complexes and the relatively low magnetic field (400 MHz) at which the spectra were recorded, reducing line broadening observed previously for square planar Pt(II) complexes on 600 MHz instruments because of chemical shift anisotropy relaxation effects.[40] The $^{1}H/^{15}N$ shifts of the Pt(IV) complexes are observed approximately 2 ppm downfield in the ^{1}H dimension and ∼30 ppm upfield in the ^{15}N dimension relative to their Pt(II) counterparts.

Aquation/Hydrolysis Studies. The aquation and hydrolysis reactions of the three ^{15}N-labeled Pt(IV) complexes in 0.1 M $NaClO_4$ (5% D_2O/95% H_2O) were monitored by $[^{1}H,^{15}N]$ HSQC NMR spectroscopy. The reactions were unbuffered because previous experiments with Pt(II) showed that both phosphate and acetate bind to platinum.[42,50] Sodium perchlorate was selected as supporting electrolyte because the perchlorate anion does not efficiently bind to platinum(II).[66] In initial experiments we found that when the solution pH was greater than 5.7 the $[^{1}H,^{15}N]$ signals were lost as a result of rapid ^{1}H exchange between coordinated NH_3 ligands and the solvent. Consequently all reactions were carried out with initial solution pH values below 5.7.

Aquation of *cis*-$[PtCl_4(^{15}NH_3)_2]$ (1). The aquation of *cis*-$[PtCl_4(NH_3)_2]$ (**1**) was studied at three different initial pH values (5.7, 4.3, and 3.6). Duplicate experiments in which 1% *cis*-$[PtCl_2(NH_3)_2]$ was added as catalyst were also carried out. The eight possible species arising from aquation/hydrolysis of *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) are shown in Scheme 1 and were considered when assigning the peaks observed

Scheme 1. Possible Species Arising from Aquation/Hydrolysis of *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) and the Expected Number of $[^{1}H,^{15}N]$ Peaks Afforded by Each Complex[a]



[a] Detected species arising from aquation and hydrolysis of **1** are highlighted.

in the $[^{1}H,^{15}N]$ 2D HSQC NMR spectra. Species ranging from monoaqua/hydroxo through to tetraaqua/hydroxo are possible as the NH_3 ligands are not labile under the experimental conditions. Further, both axial and equatorial aquation is possible, giving rise to a range of geometric isomers. The peaks were assigned on the basis of their order of appearance in the spectra and whether a partner peak could be identified. Of the eight possible aquated species, five have pairs of chemically equivalent NH_3 ligands, as does the "parent" *cis*-$[PtCl_4(NH_3)_2]$ (**1**), and are expected to give rise to single peaks in the $[^{1}H,^{15}N]$ 2D HSQC NMR spectra. Three aquation products (**4**, **5**, and **6**) have inequivalent $^{15}NH_3$ ligands resulting in pairs of peaks in the spectra (Scheme 1).

The pH of the reaction mixtures decreased over time to between 2.7 and 3.2 in all the reactions studied, consistent with the hydrolysis of aqua to hydroxo ligands following coordination to the hard platinum(IV) metal center. Many of the peaks exhibited pH dependent shifts, but their identities were verified by analysis of consecutive spectra. All aquated ligands in Scheme 1 are consequently shown in their hydroxo form. However, at pH values below 3.5 significant proportions of the aqua form may be present.

Figure 1 shows selected $^{1}H/^{15}N$ HSQC spectra collected for the Pt(II) catalyzed aquation of *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) in 0.1 M $NaClO_4$ (95% H_2O/5% D_2O) with an initial pH of 4.3. The intensity of the $^{1}H/^{15}N$ peak due to *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) diminishes throughout the course of the reaction, but its chemical shift is invariant at $\delta(^{1}H/^{15}N) = 6.04/-35.0$. The first new peak to appear in all reactions is

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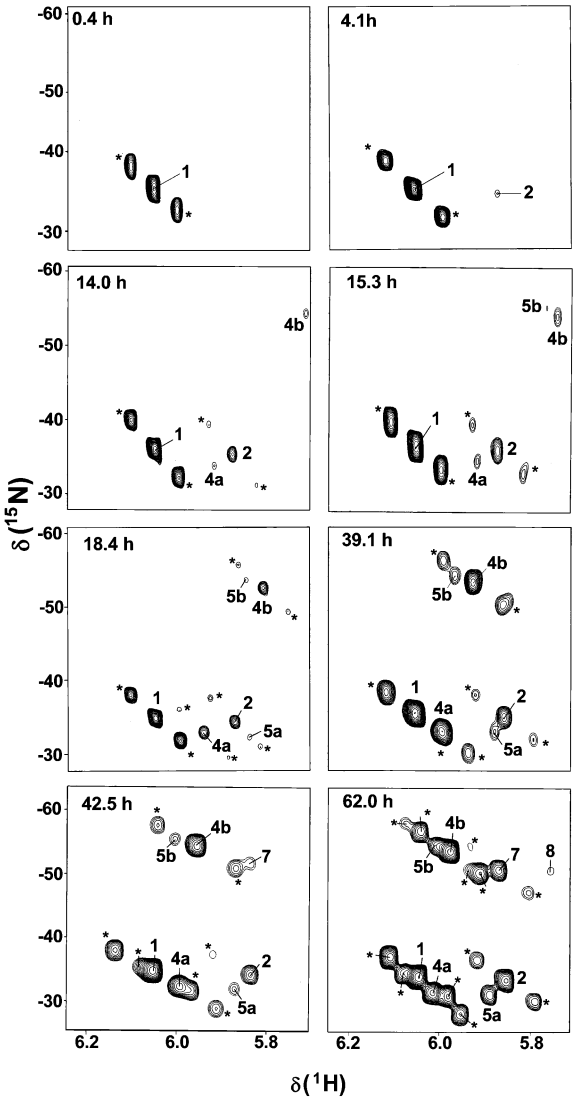


Figure 1. [^{1}H,^{15}N] HSQC NMR spectra for the aquation of *cis*-[$PtCl_4(NH_3)_2$] (**1**) with the addition of 1% *cis*-[$PtCl_2(NH_3)_2$] in 0.1 M $NaClO_4$ (initial pH = 4.3) at 298 K. (Key: * = ^{195}Pt satellite, species numbers are defined in Table 1).

at δ(^{1}H/^{15}N) = 5.84/−34.3 which shifts slightly to 5.86/−34.2 over the course of the reaction. No partner peak was found indicating two equivalent NH_3 ligands, and the ^{15}N shift of this peak denotes a *trans* Cl^- ligand. This indicates substitution of one of the axial Cl^- ligands giving [$PtCl_3(OH)(^{15}NH_3)_2$] (**2**) in which the three chloro ligands lie in a facial arrangement.

A pair of peaks labeled **4a** and **4b** (initially observed at δ ^{1}H/^{15}N = 5.88/−32.9 and 5.60/−50.9, respectively) arise simultaneously (after ∼3 h) and maintain similar volumes. The ^{15}N shift of peak **4b** is ∼20 ppm more shielded than **4a**, consistent with aquation of one of the equatorial positions of *cis*-[$PtCl_4(NH_3)_2$] (**1**) forming the complex [$PtCl_3(OH)$-$(^{15}NH_3)_2$] (**4**) with the chloro ligands in a meridional arrangement. The ^{1}H/^{15}N shifts of both peaks of this species are, as expected for an aquated complex, sensitive to changes in pH, with **4a** moving to δ ^{1}H/^{15}N = 6.02/−32.0 and **4b** to 5.98/−54.6 as the pH dropped to 2.7 over the course of the reaction.

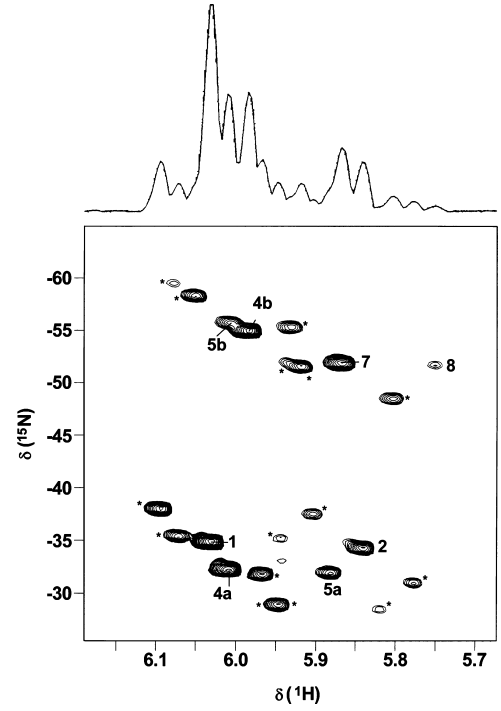


Figure 2. [^{1}H,^{15}N] HSQC NMR spectrum of the reaction mixture following aquation of *cis*-[$PtCl_4(^{15}NH_3)_2$] in the presence of 0.01 molar equivalents of *cis*-[$PtCl_2(^{15}NH_3)_2$] in 100 mM $NaClO_4$ (initial pH = 5.7) after 90 h at 298 K. (Key: * ^{195}Pt satellite, labels as defined in Table 1).

The paired peaks **5a** and **5b** (initially observed at δ ^{1}H/^{15}N = 5.81/−32.0 and 5.80/−53.4) are the next to appear in all the reactions studied and are assigned to the complex *cis*, *cis*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (**5**). Both of these peaks exhibit pH dependences, with the ^{1}H/^{15}N peak representing NH_3 *trans* to OH_2/OH (**5b**) moving 0.2 ppm in the ^{1}H dimension to 6.03/−55.2. The next peak to appear is at δ ^{1}H/^{15}N at 5.82/−51.5. No partner for this peak has been identified, and its ^{15}N shift is indicative of a complex in which both NH_3 ligands are *trans* to O donor ligands. This indicates that the peak could represent species labeled **7**, **8**, or **9** in Scheme 1. It is assigned here as *trans*, *cis*, *cis*-[$PtCl_2(OH)_2(^{15}NH_3)_2$] (**7**) on the basis of the reaction sequence. Further, comparison of the ^{1}H/^{15}N shifts of this species with those of pre-prepared **9** under similar pH conditions show these are not the same species (Supporting Information, Figure S2). The peak **7** does not exhibit a strong pH dependence, moving to 5.86/−51.8 in the final spectrum (60 h). This can be explained on the basis of its late appearance in the reaction by which time the pH of the reaction had already significantly dropped and stabilized.

A peak appears at δ ^{1}H/^{15}N = 5.75/−51.6 in the final spectrum in Figure 1 obtained after 62 h of reaction. No partner peak has been identified and it is thus assigned to *fac*-[$PtCl(OH)_3(^{15}NH_3)_2$] (**8**) on the basis of its order of appearance and its position indicating OH_2/OH groups *trans* to the NH_3 ligands. This peak is also seen in Figure 2 which shows the [^{1}H,^{15}N] HSQC NMR spectrum of the analogous reaction that was initiated at pH 5.7 after 90 h at 298 K. The ^{1}H, ^{15}N shifts of this peak at pH 2.7 are inconsistent with the shifts observed for pre-prepared **9** (see below), further supporting its assignment as **8**.

Analysis of the sequence of appearance of species in the spectra suggests that the reaction follows the pathway outlined in Scheme 1. In most spectra there is no evidence for the formation of *cis*, *trans*, *cis*-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (**3**) from the aquation of **1**, under the conditions used. This is supported by comparison of $^1H/^{15}N$ chemical shifts of **3** as a function of pH (Supporting Information, Figure S1), which were independently determined from pH titration of the final reaction mixture of the aquation of separately prepared **3** (Supporting Information, Figure S3, see below). However, a weak ^1H, ^{15}N peak at about 5.94/−32.7 seen in Figure 2 has shifts that are similar to those expected for **3** at the observed pH of 2.7 (Supporting Information, Figure S1) and therefore we cannot rule out the presence of a trace amount. There is no evidence, on the basis of the $^1H/^{15}N$ chemical shifts of separately prepared *cis*-$[Pt(OH)_4(NH_3)_2]$ (**9**) at various pH values (Supporting Information, Figure S1), for the formation of this ultimate product, where all chloro ligands have been replaced.

Rates of Reaction. The complexity of the aquation and hydrolysis of *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) precludes a complete kinetic analysis. Scheme 1 shows that for the six species observed in the aquation of *cis*-$[PtCl_4(NH_3)_2]$ there are seven reactions possible. A further complication arises when the impact of the Pt(II) catalyst on the rates is considered. It has been previously found that the rate of reaction for Pt(IV) complexes is first order with respect to [Pt(IV)], [Pt(II)], and the incoming ligand.[9]

The time dependence of the species formed in the aquation of *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) with an initial pH of 4.3 in the presence of cisplatin is shown in Figure 3. The relative contribution of (**1**) decreases over time until after 62 h it accounts for ∼35% of the total Pt(IV). *fac*-$[PtCl_3(OH)(^{15}NH_3)_2]$ (**2**) is the first aquated species to be detected and is apparent after ∼1.6 h of reaction. The concentration of **2** only very slowly increases such that after 62 h it accounts for 10% of the total Pt. The second new species to be observed, *mer*-$[PtCl_3(OH)(^{15}NH_3)_2]$ (**4**), is first observed after ∼3 h of reaction and increases in concentration so that at 62 h it accounts for 41% of the platinum. *cis,cis,cis*-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (**5**) is not detected until 15 h into the reaction; its abundance increases so that at 62 h it accounts for 8% of the total Pt. *trans,cis,cis*-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (**7**) is first observed some 40 h into the reaction, while *fac*-$[PtCl(OH)_3(^{15}NH_3)_2]$ (**8**) is only observed in the final spectrum collected at 62 h.

The rate at which aquation/hydrolysis of *cis*-$[PtCl_4(NH_3)_2]$ (**1**) takes place is higher when the solution is less acidic. This is shown by comparing the 24 h spectra of the three reactions that began with pH's of 5.7, 4.3, and 3.6. For the reaction initially at pH 5.7, after 24 h 86% of **1** remained, while for the solution with an initial pH of 4.3, **1** represented 98.4% of the total, and for that with an initial pH of 3.6, 98.7% of **1** remained. By 50 h, the reaction mixture initially at pH 5.7 contained 43% **1** and 44% **4**, while the other two reactions still had in excess of 96% **1**. A similar pH dependence was found for the cisplatin catalyzed reactions. In those reactions in which 1% *cis*-$[PtCl_2(^{15}NH_3)_2]$ was added, $[^1H,^{15}N]$ HSQC NMR spectra were also acquired in the region where Pt(II) signals are observed. In no spectrum was cisplatin or one of its aquated species observed, but this is not unexpected given that the concentration of Pt(II) added is approximately at the level of detection by this technique,[40] and no reductants were present in the reaction solution.

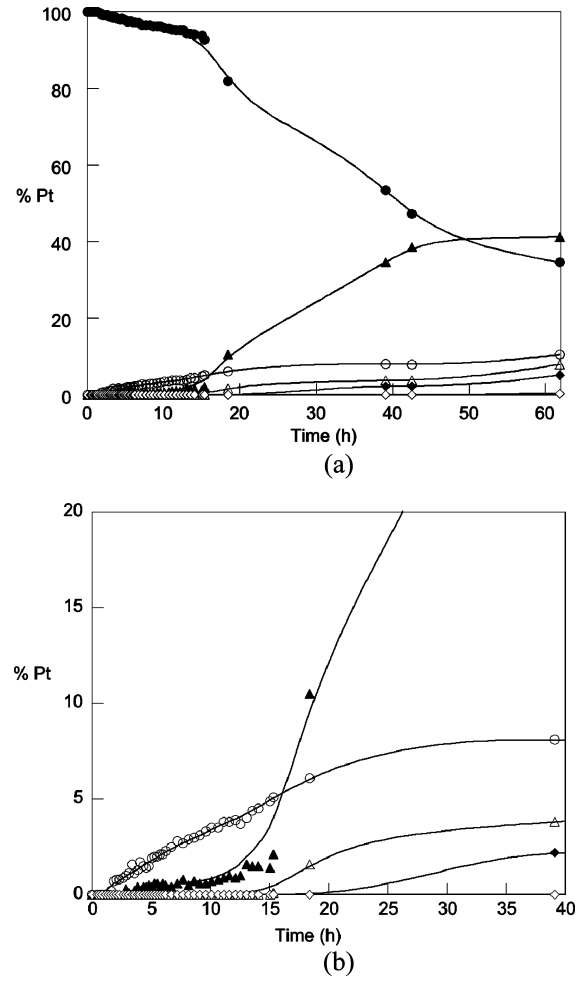


Figure 3. Time dependence of species in the aquation of *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) in 100 mM $NaClO_4$ with an initial pH of 4.3 with 1% *cis*-$[PtCl_2(^{15}NH_3)_2]$ catalyst over (a) 60 h and (b) expansion showing minor species. (Key: ● = **1**, ○ = **2**, ▲ = **4**, △= **5**, ◆ = **7**, ◇ = **8**).

A substantial increase in the rate of aquation of *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) is seen on the addition of 1% cisplatin to the reaction mixture. Figure 4 shows the time dependence of species formed from *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) at an initial pH of 3.6 both in the absence and presence of 1% cisplatin catalyst added. At this low pH the rate of reaction is the slowest of those studied and the effect of Pt(II) on the rate is most pronounced. Where no cisplatin is added to the reaction mixture, after 62 h **1** accounts for 90% of platinum species. At this time **2** accounts for 3.6% and **4** for 6.2% of the total. Under the same conditions with 1% cisplatin added, **1** accounts for 45% of the total platinum, and **2** (7.1%), **4** (35.6%), **5** (6.2%), and **7** (5.8%) are also present. Taking only the first substitution into account this amounts to a 5.5 fold increase in the rate of the reaction.

The analogous reactions that began at pH 5.7 show that the cisplatin catalyzed reaction is approaching equilibrium after 24 h, where **1** contributes 33.5% of the total platinum.

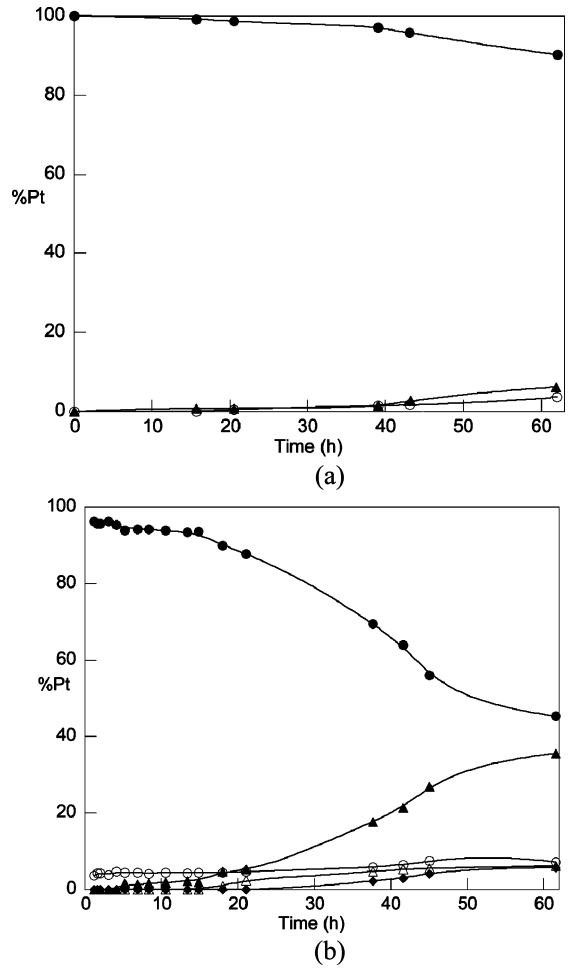
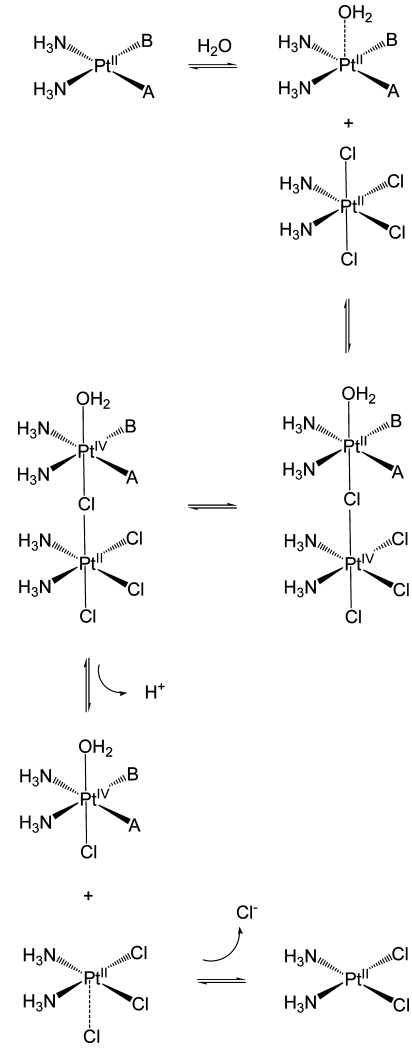


Figure 4. Time dependence of species in the aquation of *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) in 100 mM $NaClO_4$ with an initial pH of 3.6 with (a) no added *cis*-$[PtCl_2(^{15}NH_3)_2]$ and (b) 1% *cis*-$[PtCl_2(^{15}NH_3)_2]$ catalyst added to the reaction mixture. (Key: ● = **1**, ○ = **2**, ▲ = **4**, △= **5**, ◆ = **7**).

This value decreases nominally, so that after 90 h some 31.5% of the reaction mixture remains as **1**. The relative contributions of other species do not significantly change over the same time period. The uncatalyzed reaction maintained 86% **1** after 24 h reaction time, but the reaction had approached equilibrium by ∼70 h reaction time with **1**, ∼33%, **2**, 5%, **4**, 50%, **5**, 5%, and **7**, 8.5%. Over the course of the reaction, the pH fell to approximately 2.7. At this pH, it is likely that the solution is sufficiently acidic that a significant fraction of the hydroxo ligands are protonated and can be displaced by chloro ligands.

Aquation of *cis*-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (3) and *cis*, *trans*, *cis*-$[PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2]$ (10). Negligible aquation was observed for *cis*, *trans*, *cis*-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (**3**) in 0.1 M $NaClO_4$ over a 25 day period, showing only ∼4% aquation in the presence of cisplatin as catalyst (Supporting Information, Figure S3) and none in the absence of added cisplatin. The additional $^1H/^{15}N$ peak is at δ $^1H,^{15}N$ = 5.16/−48.8 which represents an NH_3 ligand *trans* to a O-donor and from **3** this would logically be *mer*-$[PtCl(OH)_3(^{15}NH_3)_2]$ (**6**). However no partner peak is observed in the *trans* Cl region of the spectrum although, given the low abundance of the species, it is possible that it remains undetected, particularly if it lies close to the peak for **3** or its ^{195}Pt satellites. *cis*-$[Pt(OH)_4(^{15}NH_3)_2]$ (**9**) is discounted on the basis of its chemical shifts (Supporting Information, Figures S1 and S2). *cis*, *trans*, *cis*-$[PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2]$ (**10**) in 0.1 M $NaClO_4$ underwent negligible aquation over a 25 day period, the spectrum being essentially unchanged and the starting material accounting for in excess of 98% of the total Pt (Supporting Information, Figure S4).

Scheme 2. Mechanism for Ligand Substitution on Platinum(IV) Complexes Catalyzed by Platinum(II), As Proposed by Basolo et al.[2,4] (A = Cl^- or H_2O, B = Cl^- or H_2O)



Discussion

Basolo and co-workers proposed that platinum(IV) aquation and substitution proceeds via an inner-sphere electron transfer mechanism involving platinum(II).[3,4] It is possible to determine whether all aquation products are equally likely based on the proposed mechanism. An inner-sphere mechanism consists of two processes, precursor complex formation followed by electron transfer, as shown in Scheme 2.

The bridging ligand depicted in the intermediate in Scheme 2 is a chloro ligand. Mason[9] reported the order of reactivity of the bridge was $I^- \gg Br^- > SCN^- > Cl^- \gg OH^-$, with the rate with Cl^- some 1000 fold greater than with OH^-. On this basis, **3** and **6**, both having axial aqua/hydroxo ligands, and *cis*, *trans*, *cis*-$[PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2]$ (**10**)

with axial acetato ligands are expected to undergo extremely slow aquation, and this was indeed observed for both **3**, which showed ~4% aquation over a period of 25 days, and **10** where it was less than 2%. Likewise, the lack of detectable **3** and **6** during the aquation of **1** can be understood in terms of OH^- ligands being poor electron bridges; the Pt(IV) complexes require at least one axial chloro ligand for the inner sphere mechanism to occur, and as the axial chloro electron bridge is handed from the Pt(IV) center to the Pt(II) complex during this process, the aquation products all retain an axial chloro ligand. *mer*-$[PtCl_3(OH)(^{15}NH_3)_2]$ (**4**) and *trans*, *cis*, *cis*-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (**7**) which both have the "*reactive trans dihalo axis*" are not expected to attain high relative concentration because they should undergo more rapid reaction than complexes with one or two hydroxo ligands in the axial sites.

Equatorially aquated species (**4** and **7**) most likely arise from aquation of cisplatin and then oxidation via the inner sphere mechanism and are therefore more prevalent than might otherwise be expected. Substitution rates on Pt(II) are considerably greater than on Pt(IV) with the aquation equilibrium of cisplatin attained over a period of less than one day,[41,42] with *cis*-$[PtCl(OH_2)(NH_3)_2]^+$ the dominant species in that equilibrium. The apparent "incubation period" prior to the appearance of **4** is probably associated with the time required for aquation in the Pt(II) state.

It is notable that *cis*, *trans*, *cis*-$[PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2]$ (**10**) did not undergo observable aquation over a period of 3 weeks because the plasma of patients treated with the closely related complex, Satraplatin, *cis*, *trans*, *cis*-$[PtCl_2(O_2CCH_3)_2(NH_3)$(cyclohexylamine)] is reported to yield two or three aquated biotransformation products (JM383, *cis*, *trans*, *cis*-$[Pt(OH)_2(O_2CCH_3)_2(NH_3)$(cyclohexylamine)], and JM518 and JM559, the two isomers of *cis*, *trans*, *cis*-$[PtCl(OH)(O_2CCH_3)_2(NH_3)$(cyclohexylamine)]).[67−70] Indeed, in some cases JM383 is reported to be a major metabolite.[71] The in vivo aquation of Satraplatin is probably not occurring via platinum(II) catalyzed ligand exchange because this necessarily leads to loss of one or both of the axial acetate ligands, and the concentrations of platinum(II) and platinum(IV) in biological environments are likely to be too low to allow such mechanisms to operate. Thus, in the absence of Pt(II) catalysis, aquation and hydrolysis of the chloro ligands will have occurred via an S_N1 dissociative mechanism with loss of the chloro ligand in the rate-determining step.

Conclusions

Analysis of consecutive [^{1}H,^{15}N] HSQC NMR spectra of *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**) show substitution of chloro ligands by aqua/hydroxo ligands in both axial and equatorial positions relative to the non-labile NH_3 ligands. Substitution is seen to occur first in the axial position with subsequent substitution occurring in equatorial positions. Of the eight possible aquation products only five are observed. The addition of a 1% "catalytic amount" of *cis*-$[PtCl_2(^{15}NH_3)_2]$ to the reaction mixture greatly enhances the rate of substitution, particularly early in the reaction profile. Aquation of both *cis*, *trans*, *cis*-$[PtCl_2(OH)_2(^{15}NH_3)_2]$ (**3**) and *cis*, *trans*, *cis*-$[PtCl_2(O_2CCH_3)_2(^{15}NH_3)_2]$ (**10**) is considerably slower than observed for *cis*-$[PtCl_4(^{15}NH_3)_2]$ (**1**), and the addition of *cis*-$[PtCl_2(^{15}NH_3)_2]$ does not dramatically increase the rate of aquation for these complexes, consistent with the requirement of an axial chloro ligand for catalyzed hydrolysis of Pt(IV) ammine complexes.

Acknowledgment. This work was supported by grants from the Australian Research Council.

Supporting Information Available: Plots of the ^{1}H/^{15}N chemical shifts of **3** and **9** as a function of pH, [^{1}H,^{15}N] HSQC NMR spectra of 25 day samples of both **3** and **10** in 100 mM $NaClO_4$, and **9** in 100 mM $NaClO_4$ at a pH of 3.5 are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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