Equilibrium and Kinetic Studies of the Aquation of the Dinuclear Platinum Complex $[\{trans\text{-}PtCl(NH_3)_2\}_2(\mu\text{-}NH_2(CH_2)_6NH_2)]^{2+}$: p$K_a$ Determinations of Aqua Ligands via [^{1}H,^{15}N] NMR Spectroscopy

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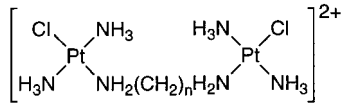
By the use of [^{1}H,^{15}N] heteronuclear single quantum coherence (HSQC) 2D NMR spectroscopy and electrochemical methods we have determined the hydrolysis profile of the bifunctional dinuclear platinum complex $[\{trans\text{-}PtCl(^{15}NH_3)_2\}_2(\mu\text{-}^{15}NH_2(CH_2)_6{}^{15}NH_2)]^{2+}$ (1,1/t,t ($n = 6$), ^{15}N-**1**), the prototype of a novel class of potential antitumor complexes. Reported are estimates for the rate and equilibrium constants for the first and second aquation steps, together with the acid dissociation constant ($pK_{a1} \approx pK_{a2} \approx pK_{a3}$). The equilibrium constants determined by NMR at 25 and 37 °C ($I = 0.1$ M) were similar, $pK_1 \approx pK_2 = 3.9 \pm 0.2$, and from a chloride release experiment at 37 °C the values were found to be $pK_1 = 4.11 \pm 0.05$ and $pK_2 = 4.2 \pm 0.5$. The forward and reverse rate constants for aquation determined from this chloride release experiment were $k_1 = (8.5 \pm 0.3) \times 10^{-5}$ s^{-1} and $k_{-1} = 0.91 \pm 0.06$ M^{-1} s^{-1}, where the model assumed that all the liberated chloride came from **1**. When the second aquation step was also taken into account, the rate constants were $k_1 = (7.9 \pm 0.2) \times 10^{-5}$ s^{-1}, $k_{-1} = 1.18 \pm 0.06$ M^{-1} s^{-1}, $k_2 = (10.6 \pm 3.0) \times 10^{-4}$ s^{-1}, $k_{-2} = 1.5 \pm 0.6$ M^{-1} s^{-1}. The rate constants compare favorably with other complexes with the $[PtCl(am(m)ine)_3]^+$ moiety and indicate that the equilibrium of all these species favors the chloro form. A pK_a value of 5.62 was determined for the diaquated species $[\{trans\text{-}Pt(^{15}NH_3)_2(H_2O)\}_2(\mu\text{-}^{15}NH_2(CH_2)_6{}^{15}NH_2)]^{4+}$ (**3**) using [^{1}H,^{15}N] HSQC NMR spectroscopy. The speciation profile of **1** and its hydrolysis products under physiological conditions is explored.

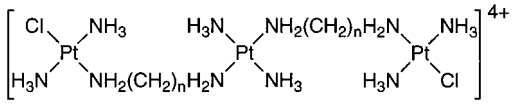
Introduction

The compound $[\{trans\text{-}PtCl(NH_3)_2\}_2(\mu\text{-}NH_2(CH_2)_6NH_2)]^{2+}$, (1,1/t,t ($n = 6$), **1**), is the prototypical compound of a new class of multinuclear platinum am(m)ine complexes that exhibit novel antitumor and DNA-binding properties.[1,2] This new class includes the phase I clinical candidate $[\{trans\text{-}PtCl(NH_3)_2\}_2\{\mu\text{-}trans\text{-}Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2\}]^{4+}$, (1,0,1/t,t,t ($n = 6$), or BBR3464).[3,4]



1,1/t,t (n = 6)

1



1,0,1/t,t,t (n = 6)

BBR3464

The flexibility, charge, and hydrogen-bonding capability of these compounds are thought to be related to their improved cytotoxic properties relative to cisplatin and its derivatives.[5] Dinuclear platinum complexes react with DNA faster than cisplatin[6,7] and produce a different DNA adduct profile, highlighted by long-range *interstrand* cross-links.[7] Because these interstrand cross-links appear to contribute to the increased antitumor activity of these compounds, it is important to understand how these adducts form.

[^{1}H,^{15}N] HSQC NMR spectroscopy has recently been shown to be a powerful method for examining kinetics of DNA platination reactions by cisplatin,[8−11] and we are currently using this technique to examine the kinetics and mechanism of formation of both inter- and intrastrand cross-links by di- and trinuclear platinum am(m)ine complexes. The technique requires the use of ^{15}N-labeled compounds and allows observation of

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(1) Farrell, N. *Comments Inorg. Chem.* **1995**, *16*, 373−389.
(2) Farrell, N. In *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Chaires, J. B., Eds.; JAI Press: New Haven, CT, 1996; Vol. 2; pp 187−216.
(3) Farrell, N.; Spinelli, S. In *Uses of Inorganic Chemistry in Medicine*; Farrell, N., Ed.; Royal Society of Chemistry: Cambridge, U.K., 1999; pp 124−134.
(4) Brabec, V.; Kašpárková, J.; Vrána, O.; Nováková, O.; Cox, J. W.; Qu, Y.; Farrell, N. *Biochemistry* **1999**, *38*, 6781−6790.
(5) Farrell, N.; Qu, Y.; Bierbach, U.; Valsecchi, M.; Menta, E. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Wiley VCH: New York, 1999; pp 479−496.
(6) Farrell, N. P.; de Almeida, S. G.; Skov, K. A. *J. Am. Chem. Soc.* **1988**, *110*, 5018−5019.
(7) Zaludová, R.; Zakovská, A.; Kašpárková, J.; Balcarová, Z.; Kleinwächter, V.; Vrána, O.; Farrell, N.; Brabec, V. *Eur. J. Biochem.* **1997**, *246*, 508−517.

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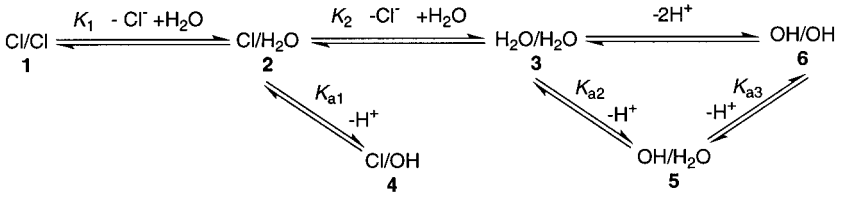
Scheme 1



all platinated species at low (micromolar) concentrations. The spectra are simplified because only ^{1}H and ^{15}N resonances derived from platinum am(m)ine species are seen and the ^{15}N shifts are sensitive to the nature of the trans ligand.[12,13] A kinetic study of the stepwise formation of a 1,4 interstrand GG DNA cross-link by ^{15}N-**1** will be reported elsewhere.[14]

Since aquation is usually the rate-determining step in the binding of Pt(II) ammine complexes to DNA and aqua ligands are more labile to substitution than hydroxo ligands, it is important to understand the speciation profile of the di- and trinuclear complexes in aqueous solution. A generalized reaction profile for dinuclear platinum a(m)mine complexes such as **1** is shown in Scheme 1.

[^{1}H,^{15}N] NMR spectroscopy has been used previously to study the hydrolysis of cisplatin,[15] [PtCl(dien)]$^+$,[16] and *cis*- and *trans*-[PtCl$_2$(NH$_3$)(cyclohexylamine)].[17] The NMR technique is particularly valuable for the reliable determination of the pK_a values of coordinated aqua ligands, because hydrolysis products can be measured directly without interference from other species (e.g., hydroxo-bridged oligomers) which can complicate the interpretation of potentiometric titration curves.

In this work we have used a combination of [^{1}H,^{15}N] HSQC NMR spectroscopy and chloride concentration determinations, using a chloride sensitive electrode, to study the hydrolysis profile of **1**. Reported are estimates for the rate and equilibrium constants for the first and second aquation steps, together with the acid dissociation constant (pK_{a1} $\approx$ pK_{a2} $\approx$ pK_{a3}).

Experimental Section

Preparation of [{*trans*-PtCl(NH$_3$)$_2$}$_2$(μ-NH$_2$(CH$_2$)$_6$NH$_2$)](NO$_3$)$_2$ (^{15}N-1). The ^{15}N (100% enriched) *trans*-[PtCl$_2$(^{15}NH$_3$)$_2$] was prepared from the literature preparation of *trans*-[PtCl$_2$(NH$_3$)$_2$] with the use of ^{15}NH$_4$Cl.[18] ^{15}N-Labeled 1,6-hexanediamine was prepared from 1,6-dibromohexane by the standard method using commercially available ^{15}N (100% enriched) potassium phthalimide.[19] AgNO$_3$ (1.10 g, 6.48 mmol) was added to a solution of *trans*-[PtCl$_2$(^{15}NH$_3$)$_2$] (2.0 g, 6.7 mmol) in 220 mL of DMF. The solution was worked up similarly to the literature procedure previously described.[20] Yield: 1.13 g of [{*trans*-PtCl(^{15}NH$_3$)$_2$}$_2$$\mu$-(^{15}NH$_2$(CH$_2$)$_6^{15}NH_2$)](NO$_3$)$_2$ (42% based on *trans*-DDP). ^{1}H NMR (D$_2$O): δ (ppm) 1.31, 1.62, 2.62. Anal. Calcd for C$_6$H$_{28}$N$_8$Cl$_2$O$_6$Pt$_2$: C, 9.29; H, 3.64; N, 15.22; Cl, 9.14. Found: C, 9.22; H, 3.38; N, 14.99; Cl, 9.00.

NMR Spectroscopy. The NMR spectra were recorded on a Varian UNITY-INOVA-600 MHz spectrometer (^{1}H, 599.92 MHz; ^{15}N, 60.79 MHz). The ^{1}H NMR chemical shifts are internally referenced to 1,4-dioxane (δ 3.767) and the ^{15}N chemical shifts externally referenced to ^{15}NH$_4$Cl (1.0 M in 1.0 M HCl in 5% D$_2$O in H$_2$O). The two-dimensional [^{1}H,^{15}N] heteronuclear single-quantum coherence (HSQC) NMR spectra (decoupled by irradiation with the GARP-1 sequence during the acquisition) were recorded using the sequence of Stonehouse et al.[21] and processed as described previously.[11] Concentrations of species were determined by integration of the two-dimensional cross peaks using the Varian VNMR software.

pH Measurements. The pH values of the NMR samples were measured using a Shindengen pH Boy-P2 (su19A) pH meter and calibrated against pH buffers at pH 6.9 and 4.0. Aliquots of 5 μL of the solution were placed on the electrode, and the pH was recorded (the aliquots were not returned to the sample). Adjustments in pH were made using 0.04, 0.2, and 1.0 M HClO$_4$, or 0.04, 0.2, and 1.0 M NaOH.

Hydrolysis Experiments: (a) [^{1}H,^{15}N] NMR. ^{15}N-**1** (nitrate salt) (2.11 mg, 2.73 μmol) was dissolved in 500 μL of a solution of 0.1 M NaClO$_4$ in 95%H$_2$O/5%D$_2$O to give a final concentration of 5.45 mM. The sample was incubated for 8 days at 25 °C, during which time the pH decreased from 5.5 to 4.8. The [^{1}H,^{15}N] NMR spectrum was recorded at 25 °C, then at 37 °C, after the sample had been incubated for 15 h at this temperature.

(b) Cl$^-$ Release Study. Fifty milliliter solutions of 2 mM 1,1/t,t (n = 6) **1** (nitrate salt) were prepared in deionized water. The release of chloride was followed at 37 °C in a thermostated cell. The Cl$^-$ ion concentration was monitored over time using a Fisher Scientific Chloride ion selective electrode and a Fisher Accumet model 925 pH/ion meter. Data were averaged over 3 runs.

The concentrations of **1**, **2**, and **3** were calculated from the amount of chloride found in the solution in two ways. The first was based on the assumption that all the liberated chloride came from **1** to form **2** and thus **3** is absent from the solution. The second method assumed that the two Pt environments behaved independently, and so aquation of the chloro ligand from **2** was equally as probable as aquation of the first chloro ligand from **1**. Thus the concentrations of **1**, **2**, and **3** were calculated so that the ratio **1**:**2** is equal to **2**:**3** at each time point. The data were then fitted to appropriate reversible aquation models using the program SCIENTIST (MicroMath, version 2). Copies of the models together with the primary input and calculated data are provided as Supporting Information.

pK_a Determination of [{*trans*-Pt(OH$_2$)(^{15}NH$_3$)$_2$}$_2$(μ-^{15}NH$_2$(CH$_2$)$_6$-^{15}NH$_2$)]$^{2+}$ (3). ^{15}N-**1** (nitrate salt) (5.03 mg, 6.49 μmol) and AgNO$_3$ (1.61 mg, 1.5 equiv) were suspended in DMF-d_7 (170 μL) and incubated overnight at 37 °C. The solution was centrifuged, and a 102 μL aliquot of the supernatant was diluted with a solution of NaClO$_4$ (100 mM, 1898 μL). A drop (ca. 5 μL) of 1,4-dioxane in D$_2$O (5%) was added. Examination of the [^{1}H,^{15}N] NMR spectrum showed only one peak derived from overlapped signals for **2** and **3**. Since it was therefore not possible to determine the pK_a's of the individual aquated species by titration of this solution, additional AgNO$_3$ (0.90 mg) was added to

(8) Barnham, K. J.; Berners-Price, S. J.; Frenkiel, T. A.; Sadler, P. J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1874−1877.
(9) Berners-Price, S. J.; Barnham, K. J.; Frey, U.; Sadler, P. J. *Chem.—Eur. J.* **1996**, *2*, 1283−1291.
(10) Reeder, F.; Guo, Z.; Murdoch, P. D.; Corazza, A.; Hambley, T. W.; Berners-Price, S. J.; Chottard, J. C.; Sadler, P. J. *Eur. J. Biochem.* **1997**, *249*, 370−382.
(11) Davies, M. S.; Berners-Price, S. J.; Hambley, T. W. *J. Am. Chem. Soc.* **1998**, *120*, 11380−11390.
(12) Appleton, T. G.; Hall, J. R.; Ralph, S. F. *Inorg. Chem.* **1985**, *24*, 4685−4693.
(13) Berners-Price, S. J.; Sadler, P. J. *Coord. Chem. Rev.* **1996**, *151*, 1−40.
(14) Berners-Price, S. J.; Cox, J. W.; Davies, M. S.; Qu, Y.; Farrell, N. Manuscript in preparation.
(15) Berners-Price, S. J.; Frenkiel, T. A.; Frey, U.; Ranford, J. D.; Sadler, P. J. *J. Chem. Soc., Chem. Commun.* **1992**, 789−791.
(16) Guo, Z.; Chen, Y.; Zang, E.; Sadler, P. J. *J. Chem. Soc., Dalton Trans.* **1997**, 4107−4111.
(17) Barton, S. J.; Barnham, K. J.; Habtemariam, A.; Sue, R. E.; Sadler, P. J. *Inorg. Chim. Acta* **1998**, *273*, 8−13.
(18) Kaufmann, G. B.; Cowan, D. O. *Inorg. Synth.* **1963**, *7*, 239−245.
(19) Sheehan, J. C.; Bolhofer, W. A. *J. Am. Chem. Soc.* **1950**, *72*, 2786−2788.
(20) Qu, Y.; Farrell, N. *Inorg. Chem.* **1992**, *31*, 930−932.
(21) Stonehouse, J.; Shaw, G. L.; Keeler, J.; Laue, E. D. *J. Magn. Reson., Ser. A.* **1994**, *107*, 174−184.

ensure complete formation of **3**. The solution was sonicated and incubated overnight at 37 °C. The solution was then centrifuged and the supernatant decanted and filtered through a 0.45 μm filter to remove the precipitated AgCl. The measured pH was 5.02. [^{1}H,^{15}N] NMR spectra were recorded for this solution titrated over the pH range 2.0−9.2.

The pH titration data were analyzed using the equation

$$\delta = (\delta_A[H^+] + \delta_B K_a)/([H^+] + K_a) \quad (1)$$

where K_a is the acid dissociation constant for one Pt−OH_2 group of the diaqua complex **3** and δ_A and δ_B are the chemical shifts of the diaqua (**3**) and dihydroxo (**6**) complexes, respectively. For fitting, the program KaleidaGraph (Synergy Software, Reading, PA) was used.

Results

Fully ^{15}N-labeled [{*trans*-PtCl($^{15}NH_3$)$_2$}$_2$(μ-$^{15}NH_2$(CH$_2$)$_6$-$^{15}NH_2$)]$^{2+}$ (**1**) was prepared by the synthetic method described previously using ^{15}N-labeled starting materials.[20] The [^{1}H,^{15}N] NMR spectrum of ^{15}N-**1** (in DMF-d_7) showed two major ^{1}H/^{15}N cross peaks, one at δ 4.24/−65.5 in the region expected[13] for Pt−NH_3 trans N and the other, at δ 5.41/−46.5, consistent with Pt−NH_2 trans to Cl.[22] As anticipated, the four Pt−NH_3 groups and the two Pt−NH_2 groups in **1** are magnetically equivalent, but the relative volumes of the NH_3:NH_2 cross peaks (2.2:1) were lower than the expected ratio of 3:1. This is likely to be a consequence of differing rates of exchange with solvent and/or relaxation effects. HPLC purification of ^{15}N-**1** indicated that the purity was >95% and the presence of impurities was evident in the [^{1}H,^{15}N] NMR spectrum, with six minor ^{1}H/^{15}N cross peaks in the Pt−NH_3 region (δ 4.54/−67.6, 4.76/−64.1, 4.78/−63.9, 4.86/−62.8, 4.60/−70.1, and 4.65/−66.3) and one minor cross peak in the Pt−NH_2 region (δ 5.33/−44.5).[23]

Aquation of 1. A 5.45 mM aqueous solution of ^{15}N-**1** in 0.1 M $NaClO_4$ was monitored by [^{1}H,^{15}N] NMR. The spectrum recorded after 8 days is shown in Figure 1. In addition to the peaks for **1** (3.85/−64.5, 4.99/−46.8), peaks assignable to the aquachloro complex **2** were visible at 4.08/−62.2 and 5.08/−64.3 in the $^{15}NH_3$ and $^{15}NH_2$ regions, respectively (Figure 1). These peaks correspond to the {$PtON_3$} group of **2**, and, as the peaks for the {$PtClN_3$} group are not visible, they must be coincident with those of **1**. The shift to high field of the Pt−NH_2 ^{15}N resonance by 14 ppm is consistent with replacement of the trans chloro ligand by the oxygen donor of H_2O.[13] For the Pt−NH_3 groups the ^{15}N shift is not diagnostic of the nature of the cis ligand, but significant shifts in both ^{1}H and ^{15}N dimensions allow peaks for **1** and **2** to be distinguished. The ^{1}H and ^{15}N shifts of **2** are consistent with the measured pH (4.9) of the equilibrium solution (see Figure 3). The assignment was confirmed when the peaks at 4.08/−62.2 and 5.08/−64.3 both disappeared after the addition of 200 mM chloride to a similar equilibrium solution and incubation for 26 h at 25 °C.

The [^{1}H,^{15}N] NMR spectrum cannot distinguish between the {$PtON_3$} group of the aquachloro complex **2** and the diaqua complex **3** (Scheme 1). However, given that the two positively charged {PtN_3} groups are separated by a six carbon chain linker, it is reasonable to assume that they act independently of one another and the equilibrium constant for the second aquation step (K_2) will be similar to that of the first (K_1). Measurement of Pt−NH_3 ^{1}H/^{15}N peak volumes in the spectrum of the 5.45 mM solution of ^{15}N-**1** in 0.1 M $NaClO_4$ at equilibrium (8 days, 25 °C) gave a ratio of 91.8:8.2 for the two peaks at 3.85/−64.5 and 4.08/−62.2. (Figure 1). The major peak is derived from the total concentration of **1** plus 50% of **2**, and the minor peak to the total concentration of **3** plus 50% of **2**. By taking into account this overlap we can estimate a ratio for **1**:**2**:**3** of 0.855:0.127:0.019. From these data were estimated equilibrium constants of 3.9 ± 0.2 for the first (pK_1) and second (pK_2) aquation steps (at 25 °C). Comparison of the intensities of the Pt−NH_2 peaks at 4.99/−46.8 and 5.08/−64.3 gave a lower relative concentration of the aquated species, and this discrepancy is reflected in the relatively large error in the K values. When the sample was incubated for a further 15 h at 37 °C, the [^{1}H,^{15}N] NMR spectrum (at 37 °C) showed no significant difference in the ratio of the Pt−NH_3 (or Pt−NH_2) **1**:**2/3** peaks, indicating that, within the experimental error, the equilibrium constants K_1 and K_2 are comparable at 25 and 37 °C.

There was no apparent difference in the [^{1}H,^{15}N] NMR recorded within 10 min of dissolution and that recorded after 8 days, indicating that equilibrium is achieved very rapidly. However, the small extent of aquation means that small increases in the concentration of the minor aquated species could not be measured accurately by comparing peak volumes of **1** and **2**. It was therefore not feasible to use this technique to measure the rate of aquation of **1**, and instead we followed the release of chloride from a solution of **1** in H_2O at 37 °C with a chloride selective electrode. The data were treated in two ways which either ignored or included the second aquation step in the

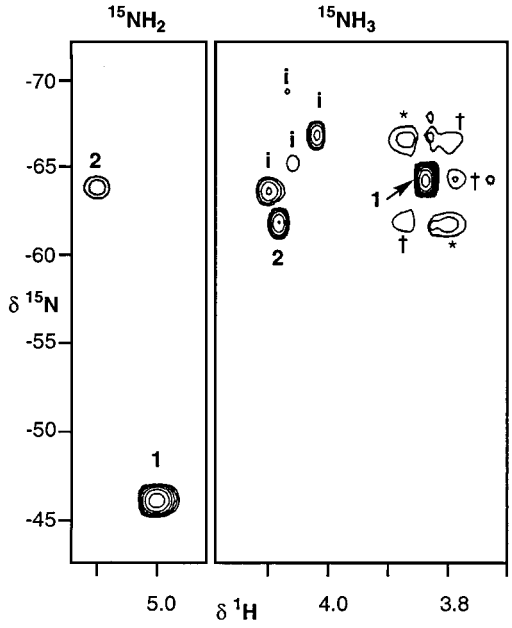


Figure 1. [^{1}H,^{15}N] HSQC spectrum of a 5.4 mM solution of fully ^{15}N-labeled **1** in 0.1 M $NaClO_4$ (in 5%D_2O/95%H_2O) after 8 days at 25 °C. The resonances are assigned to the Pt−NH_3 and Pt−NH_2 protons in **1** and the aquachloro species **2**. (See Scheme 1.) The peaks for **2** are superimposed with those of the diaqua species **3,** but this latter species represents <1% of the total species at equilibrium. The peaks for **1** include a contribution from the nonaquated {PtN_3Cl} group of **2** (i.e., 50% of the total concentration of **2**), and this overlap is taken into account in the calculation of the equilibrium constants shown in Table 2. Peaks labeled "*" are ^{195}Pt satellites and "†" are artifacts, as observed previously with use of the pulse sequence.[9,11] Peaks labeled "i" are due to Pt−$^{15}NH_3$ impurities in the sample of ^{15}N-**1** (see text).

(22) Qu, Y.; Valsecchi, M.; del Greco, L.; Spinelli, S.; Farrell, N. *Magn. Reson. Chem.* **1993**, *31*, 920−924.

(23) The peaks corresponding to impurities did not shift or change in intensity throughout all the experiments. Although these impurity species are unassigned, their presence in the sample of ^{15}N-**1** did not compromise the studies on the hydrolysis equilibria and pK_a measurements because the chemical shift and/or peak volumes of the hydrolysis products **2** and **3** could be measured without interference from these minor species over the entire pH range (2−9). The impurities will not be discussed further.

Table 1. ^{1}H and ^{15}N Chemical Shifts for **1**, the Aquated Species **3**, and **4** and pK_a Values for **3**

	δ ^{1}H[b]		δ ^{15}N[c]		pK_a	
complex[a]	NH_2	NH_3	NH_2	NH_3	^{1}H	^{15}N
1 (Cl/Cl)	4.99	3.85	−46.8	−64.5		
3 (H_2O/H_2O)	5.13[d]	4.11[d]	−65.4 ± 0.2[d]	−62.1 ± 0.2[d]	5.62 ± 0.03 (NH_3) 5.62 ± 0.02 (NH_2)	5.56 ± 0.04 (NH_3) 5.60 ± 0.04 (NH_2)
4 (OH/OH)	4.48[d]	3.83[d]	−57.0 ± 0.2[d]	−63.3 ± 0.2[d]		

[a] 0.1 M $NaClO_4$ in 95%H_2O/5%D_2O, 25 °C. [b] Referenced to dioxane at 3.767 ppm. [c] Referenced to $^{15}NH_4Cl$ (external). [d] Values obtained by fitting eq 1.

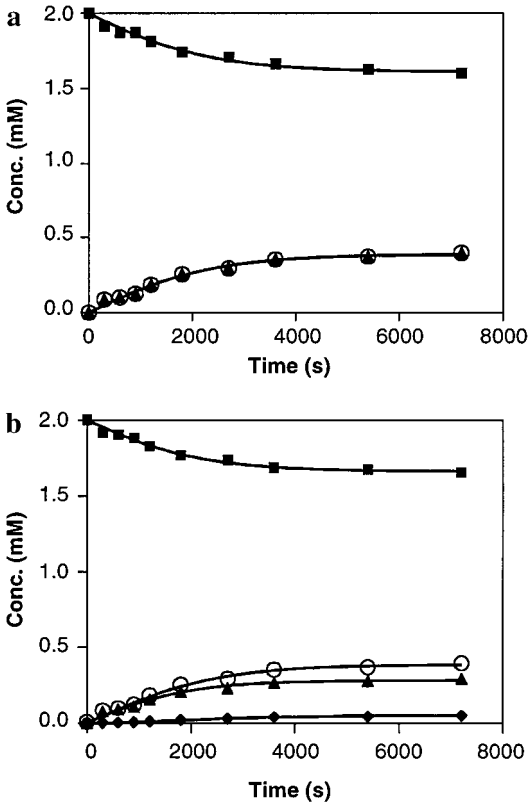


Figure 2. Time dependence of species present during the aquation of **1** at 37 °C as determined from the concentration of free chloride (○) using a chloride sensitive electrode. In panel a, concentrations of **1** (■) and **2** (▲) were calculated assuming that only one bound chloro group is liberated, and in panel b, concentrations of **1** (■), **2** (▲), and **3** (◆) were calculated assuming that aquation of both Pt sites is equally probable, and so the same ratio of **3**:**2** and **2**:**1** exists at each time point. The curves are computer best fits for the rate constants shown in Table 2.

calculation of the aquated species present, based on the concentration of liberated chloride (Figure 2). The kinetic fits to these sets of data afforded the values for the forward (k_1, k_2) and reverse (k_{-1}, k_{-2}) rate constants shown in Table 2. From the data shown in Figure 2a we calculated an equilibrium constant (pK_1) of 4.03 ± 0.04 at 37 °C, and when the second aquation step was included (Figure 2b), the values for pK_1 and pK_2 were 4.17 ± 0.03 and 4.2 ± 0.3, respectively.The agreement in the pK values obtained by the two (electrochemical and NMR) methods is reasonable, given the different conditions, the assumptions made in the calculations, and the possible errors in the NMR method.[15]

pK_a Determinations of the Diaqua Adduct 3. The concentration of **2** in the aqueous solution of ^{15}N-**1** at equilibrium was too low to use this sample to measure the acid dissociation constant of the monoaqua chloro complex (K_{a1}). However, assuming that the two {PtN_3} groups act independently, the pK_a values for the coordinated water molecule in **2** should be similar to that of the diaqua species **3** and also the aquahydroxo species **5** (K_{a2} and K_{a3}, Scheme 1). This assumption is reasonable given that for **3** the H_2O molecules are coordinated to two positively charged {PtN_3} groups and the distance between them is approximately 16.1 Å. This situation is very different from the much studied dicarboxylate systems where two distinct pK_a values are still found for chain lengths greater than seven carbon atoms. Recent computational calculations indicate that the pK_a shifts will be of the order of 0.2 pH units when the carboxylate groups are separated by a distance of 16 Å.[24] In the case of dicarboxylates it is the electrostatic repulsion between two negative charges in the dianion which destabilizes this molecule relative to the species where only one end is deprotonated. This manifests itself in an increase in pK_a (less facile dissociation) for the second deprotonation step.[24] On the other hand, both ends of the dinuclear platinum complexes carry a positive charge and the same charge difference (+1) occurs for the two deprotonation steps of the diaqua species **3**. By analogy with the dicarboxylate situation, the inherent electrostatic repulsion of the platinum centers (a combination of charge and {Pt−(NH_3)$_2$}−{Pt(NH_3)$_2$} repulsive interactions[25]) should not be dramatically increased upon production of an aqua species. It is unlikely that the difference in pK_a due to electrostatic factors will be measurable within the limits of the error and the two platinum centers may be considered independent to a first approximation.

A sample of the aquated form **3** (in 0.1 M $NaClO_4$) was prepared from **1** by removal of Cl^- by treatment with Ag^+. The [^{1}H,^{15}N] NMR spectrum of the solution at pH 5.0 contained two major cross-peaks at 5.02/−63.7 and 4.05/−62.3 assignable to the Pt−NH_2 and Pt−NH_3 groups of **3**. This solution was titrated over the pH range 2−9.2, and the pK_a(s) of the aqua ligand(s) of **3** were determined by [^{1}H,^{15}N] NMR by monitoring the pH dependences of the ^{1}H and ^{15}N shifts of both the Pt−$^{15}NH_3$ and Pt−$^{15}NH_2$ groups (Figure 3). The shifts of the Pt−$^{15}NH_2$ group (trans to O) are more sensitive to pH than those of the Pt−$^{15}NH_3$ groups (Table 1). The ^{1}H chemical shift changes of ∼0.6 ppm when deprotonation occurs trans to the observed am(m)ine group and ∼0.3 ppm when the am(m)ine is cis to the deprotonation are similar to those observed previously for cisplatin monoaqua and diaqua systems[15] and for the two monoaqua isomers of *cis*-[$PtCl_2$(NH_3)(cyclohexylamine)].[17] At pH values below 5.5 the ^{1}H shift of the $^{15}NH_2$ group of **3** (δ 4.80 to 5.12) occurs to high frequency of the ^1H_2O resonance whereas above pH 6 it lies to lower frequency (δ 4.67 to 4.48). In the range pH 5.6−5.9 the $^{15}NH_2$ resonance of **3** is not observable in the [^{1}H,^{15}N] NMR spectrum, due to its proximity to the ^1H_2O resonance. All four pH titration curves were fitted to eq 1 to give the pK_a values listed in Table 1. The data derived from the ^{1}H shifts are more precise due to the poorer resolution in the ^{15}N dimension (δ ca. ±0.1 ppm).

(24) Rajasekaran, E.; Jayaram, B.; Honig, B. *J. Am. Chem. Soc.* **1994**, *116*, 8238−8240.
(25) Qu, Y.; Farrell, N. *J. Inorg. Biochem.* **1990**, *40*, 255−264.

Table 2. Rate and Equilibrium Constants for the Hydrolysis of **1** (Scheme 1) in Comparison to Cisplatin, $[PtCl(dien)]^+$, and $[PtCl(NH_3)_3]^+$

compound	T (°C)	k_1 (s^{-1})	k_{-1} (M^{-1} s^{-1})	pK_1	ref
1,1/t,t ($n = 6$) (**1**)	37	$(8.5 \pm 0.3) \times 10^{-5}$	0.91 ± 0.06	4.03[a]	this work
				3.9[b]	
	25			3.9[b]	this work
cisplatin	37[c]			2.72	15
	25[d]	5.18×10^{-5}	7.68×10^{-3}	2.17	29
$[PtCl(dien)]^+$	20[e]	$(6.50 \pm 0.13) \times 10^{-5}$	0.537 ± 0.005	3.92	26
$[PtCl(NH_3)_3]^+$	20[e]	$(1.11 \pm 0.03) \times 10^{-5}$	0.137 ± 0.002	4.09	26

[a] Based on k_1 and k_{-1} values obtained from the chloride release experiment (2 mM solution of **1** in H_2O, 37 °C) with values derived from the single aquation model (Figure 2a), $K_1 = (9.3 \pm 0.9) \times 10^{-5}$ M. For the model where the second aquation step occurs (Figure 2b), the rate constants are $k_1 = (7.9 \pm 0.2) \times 10^{-5}$ s^{-1}, $k_{-1} = 1.18 \pm 0.06$ M^{-1} s^{-1}, $k_2 = (10.6 \pm 3.0) \times 10^{-4}$ s^{-1}, $k_{-2} = 1.5 \pm 0.6$ M^{-1} s^{-1}. [b] Based on the ratio of **1**:**2**/**3** in the [^{1}H,^{15}N] NMR spectrum of a 5.4 mM solution of **1** in 0.1 M $NaClO_4$ (in 5%D_2O/95%H_2O) at equilibrium (Figure 1). [c] 95%H_2O/5%D_2O. [d] $I = 0.1$ M. [e] Unbuffered aqueous solution; $4.2 < pH < 5$.

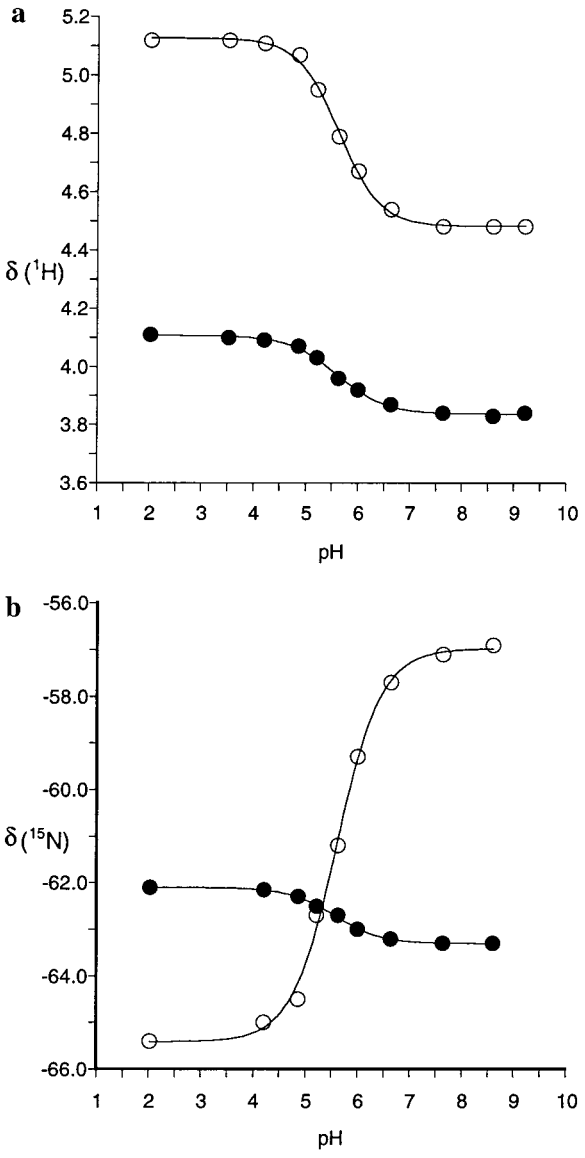


Figure 3. Plots of ^{1}H (a) and ^{15}N (b) chemical shifts vs pH for Pt−NH_3 (●) and Pt−NH_2 (○) groups in the diaqua complex **3**. The resulting δ and pK_a values are listed in Table 1. Proton exchange between acidic and basic forms is fast on the (^{1}H and ^{15}N) NMR time scale, and average peaks are seen.

Identical values (pK_a 5.62) are obtained from the fits of the ^{1}H shifts of the $^{15}NH_2$ and $^{15}NH_3$ groups.

Discussion

^{15}N-Labeling of **1** and use of [^{1}H,^{15}N] NMR spectroscopy has allowed us to study the speciation profile for this dinuclear platinum complex in aqueous solution. The [^{1}H,^{15}N] NMR method has the advantage that the concentrations of aquated species can be measured directly at low (micromolar) concentrations, even when other products are present in the solution, and so avoids some of the problems associated with other methods.[15] The possible hydrolysis reactions of **1** are shown in Scheme 1.

When the dichloro complex is dissolved in water, one of the bound chloro ligands dissociates to give the monoaqua monochloro complex **2** until an equilibrium (K_1) is established between **1**, **2**, and the liberated chloride. It is reasonable to assume that the two $PtClN_3$ groups will act independently of one another, which means that the equilibrium constant for the second aquation step (K_2) will be similar to that of the first. Therefore, the reaction does not proceed further to produce significant amounts of the diaqua complex **3**, as the relatively small value for K_2 effectively prevents further chloride ion release. Depending on the pH of the solution, the bound water molecules may deprotonate to give the chlorohydroxo species **4** and negligible amounts of the aquahydroxo (**5**) and dihydroxo (**6**) species, with the species distribution determined by the pK_a's.

These [^{1}H,^{15}N] NMR studies indicate that the equilibrium between **1** and **2** is reached very rapidly and the equilibrium concentrations lie largely on the side of the dichloro complex. For a 5.4 mM aqueous solution of the nitrate salt of **1**, the coordinated chloride is only aquated to 15% at 25 °C. The equilibrium constant (Table 2) is comparable to those reported recently[26] for $[PtCl(dien)]^+$ and $[PtCl(NH_3)_3]^+$, which are also $PtClN_3$ systems. Both of these complexes are aquated in aqueous solution with rate constants in the 10^{-5} s^{-1} range (20 °C), but the extent of aquation is limited by the relatively high chloride anation rate constants (10^{-1} M^{-1} s^{-1}). Early studies by Gray and co-workers[27,28] commented on the inertness of $[PtCl(dien)]^+$ where no net hydrolysis was detectable by conductometry,[28] and in a recent [^{1}H,^{15}N] NMR study Guo et al.[16] were unable to detect any aquated species in 5 mM aqueous solutions of $[PtCl(dien)]^+$. Kozelka[26] recently proposed that the small extent of aquation, rather than its low rate constant, explains why the aquation could not be detected in these previous studies. Chloride release studies measured for (unlabeled) **1** in water showed that the equilibrium between **1** and **2** is reached within ~2.5 h at 37 °C (Figure 2). The calculated aquation rate constant (k_1) (Table 2) (10^{-5} s^{-1} range) is magnitude similar to rate constants for cisplatin,[29] $[PtCl(NH_3)_3]^+$,[26] and $[PtCl(dien)]^+$.[26] The reverse rate constant (k_{-1}) (which corresponds to the

(26) Marti, N.; Hoa, J. H. B.; Kozelka, J. *Inorg. Chem. Commun.* **1998**, *1*, 439−442.
(27) Basolo, F.; Gray, H. B.; Pearson, R. G. *J. Am. Chem. Soc.* **1960**, *82*, 4200−4203.
(28) Gray, H. B. *J. Am. Chem. Soc.* **1962**, *84*, 1548−1552.
(29) Hindmarsh, K.; House, D. A.; Turnbull, M. M. *Inorg. Chim. Acta* **1997**, *257*, 11−18.

chloride anation of **2**) is of a magnitude similar to those of [PtCl(NH_3)$_3$]$^+$ and [PtCl(dien)]$^+$,[26] and so in this case also it can be concluded that the extent of aquation is limited by the relatively fast anation, particularly when compared to cisplatin.

[^{1}H,^{15}N] NMR allowed also the rapid determination of the pK_a value of the aqua ligands in the diaqua complex **3**. Only one set of peaks was observed throughout the titration range, showing, as expected, that there is fast exchange between protonated and deprotonated species on the (^{1}H and ^{15}N) NMR time scale. There is only one point of inflection in the titration curves, and the good fits of eq 1 to the experimental data substantiate our assumption that the two PtN_3O groups in the dinuclear complex act independently of one another and deprotonation of one H_2O ligand does not influence the other. It is notable, however, that, if the two pK_a's are close to one another, it will be difficult to discern more than one inflection. To a first approximation the measured pK_a value of 5.62 will correspond to all three dissociation constants (K_{a1}, K_{a2}, and K_{a3}) defined in Scheme 1. This value is lower (0.8 log units) than for *cis*-[PtCl(H_2O)(NH_3)$_2$]$^+$ (pK_a = 6.41)[15] and lower (0.4 log units) than for [Pt(NH_3)$_3$(OH_2)]$^{2+}$ (pK_a = 6.0,[30] 6.37[31]). It is also lower than the reported values for [Pt(dien)(H_2O)]$^{2+}$ (6.53,[32] 6.0,[16] 6.24,[33] 5.87,[34] 6.13[35]). The low pK_a value means that complex **2**, the major hydrolysis product of **1**, will be largely in the less reactive hydroxo form (**4**) at physiological pH. On the basis of the calculated equilibrium and dissociation constants listed in Tables 1 and 2 we calculate that at physiological pH (7.2) and an intracellular Cl^- concentration of 22.7 mM (the recent value quoted by Jennerwein and Andrews[36] for A2008 human ovarian cancer cells), [{*trans*-PtCl($^{15}NH_3$)$_2$}$_2$(μ-$^{15}NH_2(CH_2)_6{}^{15}NH_2$)]$^{2+}$ (**1**) will be 99.6% in the dichloro form. Of the trace amounts of hydrolyzed species (0.4% of the total) the OH/Cl form dominates (**4**(96.3%):**2**(2.5%):**6**(1:1%)**5**(0.03%):**3**(0.02%)).

In conclusion, this study shows that when dissolved in aqueous solution, aquation of **1** occurs rapidly and equilibrium is achieved more rapidly ($t_{1/2}$ = 23 min) than for cisplatin ($t_{1/2}$ = 165 min),[29] under similar conditions (37 °C). However, aquation occurs only to a limited extent with the position of the equilibrium favoring the dichloro species. The aquation and anation rate constants are similar to those of other complexes which share the $PtClN_3$ coordination sphere. The smaller extent of hydrolysis and the lower pK_a value (ca. 5.62) of the monoaqua chloro species compared to cisplatin (6.41) will have a major influence on differences in the speciation and reactivity of these platinum antitumor complexes under biological conditions. The relatively small extent of hydrolysis and the evidence of high DNA affinity (rapid DNA binding) suggest that formation of aqua species may not be a necessary step in DNA adduct formation for this class of compounds.[7] Furthermore, the differences in solution chemistry of polynuclear complexes (as represented by **1**) and cisplatin are not sufficient to explain the differences in antitumor activity, and other factors such as DNA interactions may be more important. Besides the cationic nature of **1**, maintenance of the integrity of the complex may make for more efficient DNA targeting in the biological milieu. These hydrolysis studies support the opinion that polynuclear complexes represent a new and unique direction in the development of platinum antitumor agents.

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Supporting Information Available: SCIENTIST models used to determine the rate constants given in Table 2 for the aquation of **1** based on one aquation only and two aquation steps, together with the primary input and calculated data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(30) Gonnet, F.; Reeder, F.; Kozelka, J.; Chottard, J.-C. *Inorg. Chem.* **1996**, *35*, 1653−1658.
(31) Appleton, T. G.; Hall, J. R.; Ralph, S. F.; Thompson, C. S. M. *Inorg. Chem.* **1989**, *28*, 1989−1993.
(32) Lim, M. C.; Martin, R. B. *J. Inorg. Nucl. Chem.* **1976**, *38*, 1911−1914.
(33) Arpalahti, J.; Lehikoinen, P. *Inorg. Chem.* **1990**, *29*, 2564−2567.
(34) Erickson, L. E.; Erickson, H. L.; Meyer, T. H. *Inorg. Chem.* **1987**, *26*, 997−999.
(35) Alcock, R. M.; Hartley, F. R.; Rogers, D. E. *J. Chem. Soc., Dalton Trans.* **1973**, 1070−1073.
(36) Jennerwein, M.; Andrews, P. A. *Drug Metab. Dispos.* **1995**, *23*, 178−184.