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# [$^1$H,$^{15}$N] NMR studies of the aquation of *cis*-diamine platinum(II) complexes

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This contribution is dedicated to Professor Bernhard Lippert.

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ABSTRACT

Two $^{15}$N-labelled *cis*-Pt(II) diamine complexes with dimethylamine ($^{15}$N-dma) and isopropylamine ($^{15}$N-ipa) ligands have been prepared and characterised. [$^1$H,$^{15}$N] HSQC NMR spectroscopy is used to obtain the rate and equilibrium constants for the aquation of *cis*-[$PtCl_2$($^{15}$N-dma)$_2$] at 298 K in 0.1 M $NaClO_4$ and to determine the p$K_a$ values of *cis*-[$PtCl(H_2O)$($^{15}$N-dma)$_2$]$^+$ (6.37) and *cis*-[$Pt(H_2O)_2$($^{15}$N-dma)$_2$]$^{2+}$ (p$K_{a1}$ = 5.17, p$K_{a2}$ = 6.47). The rate constants for the first and second aquation steps ($k_1 = (2.12 \pm 0.01) \times 10^{-5}$ s$^{-1}$, $k_2 = (8.7 \pm 0.7) \times 10^{-6}$ s$^{-1}$) and anation steps ($k_{-1} = (6.7 \pm 0.8) \times 10^{-3}$ M$^{-1}$ s$^{-1}$, $k_{-2} = 0.043 \pm 0.004$ M$^{-1}$ s$^{-1}$) are very similar to those reported for cisplatin under similar conditions, and a minor difference is that slow formation of the hydroxo-bridged dimer is observed. Aquation studies of *cis*-[$PtCl_2$($^{15}$N-ipa)$_2$] were precluded by the close proximity of the NH proton signal to the $^1H_2O$ resonance.



## 1. Introduction

An understanding of the aqueous chemistry of diamine Pt(II) anticancer complexes is crucial for establishing their mechanism of action since hydrolysis reactions of chloro Pt(II) complexes are thought to activate them prior to platination of the target site (DNA) and aqua ligands are more labile to substitution than hydroxo ligands [1]. In the continued search for novel platinum anticancer drugs able to circumvent some of the limitations of cisplatin, for example toxicity and resistance, platinum complexes in the *trans* geometry are of current interest [2]. Since the *trans* isomer of cisplatin is inactive, it was initially believed that all *trans*-platinum complexes are ineffective as antitumour agents, but it is now recognised that substitution of $NH_3$ in *trans*-[$PtCl_2$(L)(L′)] can give cytotoxicity in the micromolar range, as well as activity in cisplatin resistant cell lines. Included amongst the classes of active *trans*-platinum(II) complexes so far discovered are those with aliphatic amines as spectator ligands [3]. A comparison of the aquation solution chemistry of related *cis*- and *trans*-Pt(II) amine complexes is an important first step in understanding their different mechanisms of biological activity.

[$^1$H,$^{15}$N] HSQC NMR spectroscopy has been used extensively to study the hydrolysis of cisplatin and a range of other $^{15}$N-labelled platinum am(m)ine complexes [4]. In this report we have used this technique to explore the aquation chemistry of *cis*-[$PtCl_2$($^{15}$N-amine)$_2$], for the $^{15}$N-labelled amines dimethylamine (dma) and isopropylamine (ipa). The results highlight the influence on the aquation chemistry of classical Pt(II) anticancer complexes in the *cis* geometry of substituting the $NH_3$ ligands by aliphatic amines. The aquation chemistry of a series of related *trans*-[$PtCl_2$($^{15}$N-amine)($^{15}$N-amine′)] complexes, incorporating $^{15}$N-labelled aliphatic amine ligands, will be reported elsewhere [5].

## 2. Experimental

### 2.1. Preparation of compounds

Starting materials $K_2PtCl_4$, methylamine $^{15}$N hydrochloride, phthalimide $^{15}$N potassium salt, hydrazinium hydroxide and 2-bromopropane were purchased from Prolabo, Chambers Hispania, S. L. and Sigma–Aldrich. All solvents chloroform, methanol, tetrahydrofuran (THF), ethyl ether and dimethylformamide (DMF) were purchased from Sigma–Aldrich and purified by standard methods prior to use [6].

#### 2.1.1. $^{15}$N-isopropylamine

$^{15}$N-isopropylamine was synthesised *via* the Gabriel synthesis [7].

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*2.1.2. Synthesis of cis-[PtCl$_2$($^{15}$N-dma)$_2$] and cis-[PtCl$_2$($^{15}$N-ipa)$_2$]*

Full synthetic procedures will be reported elsewhere [5]. Briefly, $^{15}$N-labelled amine ($^{15}$N-dma or $^{15}$N-ipa) was added to a $K_2PtCl_4$ suspension in a THF/$H_2O$ mixture, using a ratio of 4:1 amine:Pt. After stirring for 14 h in the dark at room temperature, the yellow precipitate was collected, washed with water and chloroform and air dried to give:

*cis*-[PtCl$_2$($^{15}$N-dma)$_2$] (56%). Elemental analysis, *Anal.* Calc. for $C_4H_{14}N_2Cl_2Pt$: C, 13.48; H, 3.96; N, 7.86. Found: C, 13.66; H, 3.93; N, 7.90%. $\delta_H$ (300.13 MHz; DMSO-$d_6$; $Me_4Si$) 2.47 (6H, d, $CH_3$), 5.64 (1H, d of sept, $J_{HH}$ 5.5, $J_{HN}$ 73.8, NH); $\delta_{NH}$ (50.68 MHz; 500.13 MHz; DMSO-$d_6$; $Me_4Si$) −29.53, 5.65 ($J_{NPt}$ 368.4, $J_{HPt}$ 69.8); $\delta_{CH}$ (125.76 MHz; 500.13 MHz; DMSO-$d_6$; $Me_4Si$) 43, 2.6.

*cis*-[PtCl$_2$($^{15}$N-ipa)$_2$] (15%). Elemental analysis, *Anal.* Calc. for $C_6H_{18}N_2Cl_2Pt$: C, 18.75; H, 4.72; N, 7.29. Found: C, 19.21; H, 4.74; N, 7.30%. $\delta_H$ (300.13 MHz; DMSO-$d_6$; $Me_4Si$) 1.08 (6H, d, $J_{HH}$ 6.3, $CH_3$), 2.97 (1H, sept, $J_{HH}$ 6.45, CH), 4.63 (2H, dd, $J_{HH}$ 6.41, $J_{HN}$ 71.3, $NH_2$); $\delta_{NH}$ (50.68 MHz; 500.13 MHz; DMSO-$d_6$; $Me_4Si$) −2.89, 4.63 ($J_{NPt}$ 338.5).

### 2.2. NMR spectroscopy

For characterisation of the $^{15}$N-labelled complexes NMR spectra were recorded on either Bruker AMX-300 ($^1$H 300.13 MHz) or DRX-500 ($^1$H 500.13 MHz, $^{15}$N 50.68 MHz, $^{13}$C 125.76 MHz) spectrometers. $^1$H and $^{13}$C chemical shifts are reported relative to tetramethylsilane ($Me_4Si$) or to residual signals of deuterated solvents and $^{15}$N NMR spectra are referenced to liquid ammonia [8].

For the aquation studies $^1$H 1D and 2D [$^1$H,$^{15}$N] HSQC NMR spectra were recorded at 298 K on a Bruker Avance 600 MHz spectrometer ($^1$H 600.1 MHz, $^{15}$N 60.8 MHz) fitted with a pulsed field gradient module and 5 mm triple resonance probehead. 2D [$^1$H,$^{15}$N] HSQC NMR spectra (optimised for $^1J$ ($^{15}$N,$^1$H = 72 Hz)) were recorded using the standard Bruker phase sensitive HSQC pulse sequence. $^1$H chemical shifts were referenced to internal 1,4-dioxane (3.76 ppm), $^{15}$N shifts were calibrated externally against $^{15}NH_4Cl$ (1.0 M in 1.0 M HCl in 10% $D_2O$/90% $H_2O$).

### 2.3. pH measurements

pH values were determined using a Shindengen pH Boy-P2 (su19A) meter. To avoid leaching of chloride into the bulk sample, aliquots of 5 μl of the solution were placed on the electrode. The meter was calibrated using pH buffers at pH 6.9 and 4.0. Adjustments in pH were made using 0.1 M and 0.01 M $HClO_4$ or 0.1 M and 0.01 M NaOH.

### 2.4. p$K_a$ determination

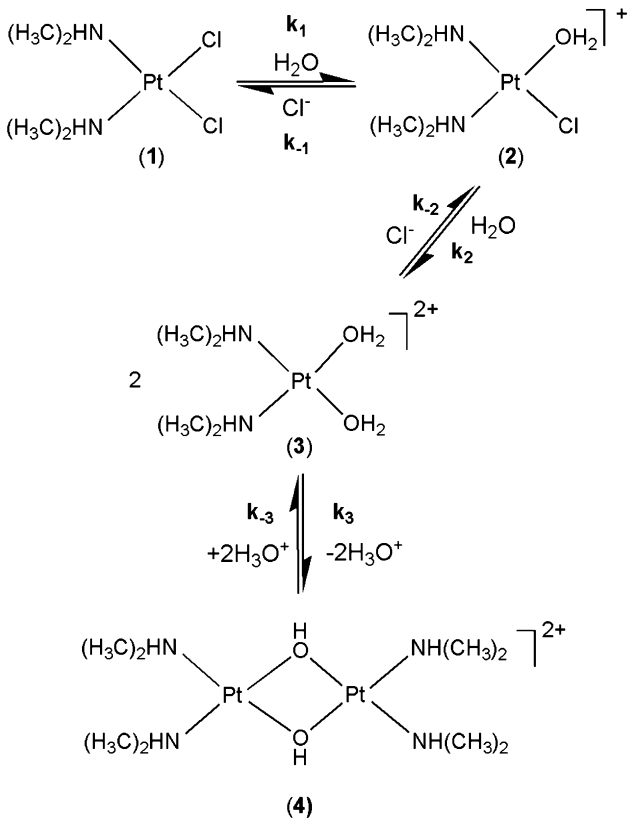
In order to obtain a solution of predominantly monoaqua and diaqua species the following preparative method was performed. $AgNO_3$ (0.68 mg, 4.00 mmol) was dissolved in $H_2O$ (1.0 ml) and 1.8 equiv. were added to 1 ml of a solution of *cis*-[PtCl$_2$($^{15}$N-dma)$_2$] (0.75 mg, 2.11 mmol) in 0.113 M $NaClO_4$ in 10% $D_2O$/90% $H_2O$. 1,4-dioxane (10 μl) was added and the solution ([Pt] = 2.09 mM) was incubated for 24 h at 298 K and then centrifuged to remove the AgCl precipitate. 1D $^1$H and 2D [$^1$H,$^{15}$N] HSQC NMR spectra were recorded in the pH range *ca.* 3–9.5.

Kaleidagraph (Synergy Software, Reading, PA) was used to analyse the pH titration data using the following equations:

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

$$\delta = (\delta_{AB} + \delta_{AA}[H^+]/K_{a1} + \delta_{BB}K_{a2}/[H^+])/(1 + [H^+]/K_{a1} + K_{a2}/[H^+]) \quad (2)$$

where $K_a$ is the acid dissociation constant for the monoaqua species (**2**), $K_{a1}$ and $K_{a2}$ those for the diaqua species (**3**) and $\delta_A$, $\delta_B$, $\delta_{AA}$, $\delta_{AB}$ and $\delta_{BB}$ are the limiting $^1$H or $^{15}$N chemical shifts of




**Scheme 1.**

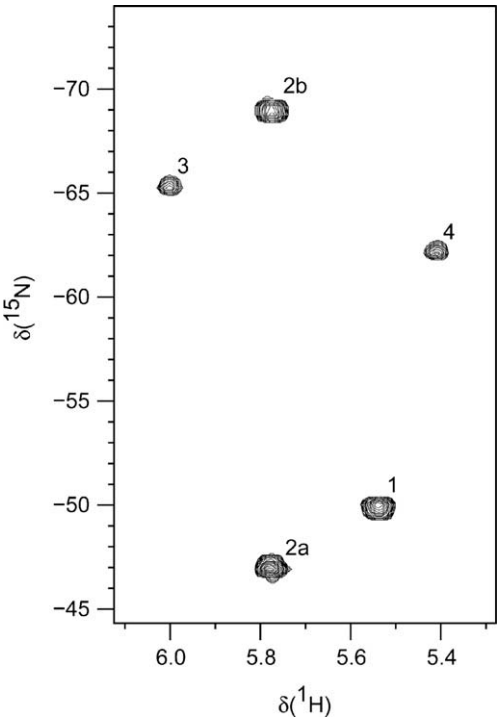



**Fig. 1.** 2D [$^1$H,$^{15}$N] HSQC NMR spectrum of *cis*-[PtCl$_2$($^{15}$N-dma)$_2$] at 298 K in 0.1 M $NaClO_4$ (10% $D_2O$/90% $H_2O$) after 69 h at pH 5.4. The peak assignments are shown in Table 1. Note that the $^1$H and $^{15}$N shifts of **2** and **3** shift with pH (Fig. 2).