Stereospecific and Kinetic Control over the Hydrolysis of a Sterically Hindered Platinum Picoline Anticancer Complex

Yu Chen, Zijian Guo, Simon Parsons, and Peter J. Sadler*

Abstract: *cis*-[$PtCl_2(NH_3)$(2-picoline)] (**1**) (AMD473) is a recently reported active anticancer complex. Hydrolysis may be an important step in its intracellular activation and interaction with DNA. In this paper we employed [^1H, ^{15}N] 2D NMR spectroscopy to determine the hydrolysis rates for each chloride ligand of this complex and its 3-picoline analogue **2**. We also report the pK_a values of the aqua and diaqua ligands as well as the X-ray crystal structures of **1** and **2**. For the 3-picoline complex **2** the rate of hydrolysis of the Cl^- *trans* to NH_3 ($k_{1b} = 1.0 \times 10^{-4}\ s^{-1}$, $I =$ 0.1M, 310 K) is similar to that of cisplatin, but slower for the Cl^- *trans* to 3-picoline ($k_{1a} = 4.5 \times 10^{-5}\ s^{-1}$). Both of the first hydrolysis rates for the 2-picoline complex **1** are slower than those of **2**, but in contrast to **2**, the hydrolysis of the Cl^- *trans* to NH_3 (*cis* to 2-picoline) is slower ($k_{1b} = 2.2 \times 10^{-5}\ s^{-1}$) than for the Cl^- *trans* to 2-picoline ($k_{1a} = 3.2 \times 10^{-5}\ s^{-1}$). The crystal structure of **2** revealed that the pyridine ring is tilted by 49° with respect to the Pt square plane, whereas in **1** the ring is almost perpendicular (103°). This introduces steric hindrance by the CH_3 group towards an axial approach to Pt from above, leading to a destabilisation of the expected trigonal-bipyramidal transition state, an effect well-known in substitution reactions of Pt^{II} complexes. The pK_a values for the monoaqua adducts of **1** (6.13 and 6.49) and **2** (5.98 and 6.26 for H_2O *trans* to picoline and NH_3, respectively) and for the diaqua adducts (5.22, 7.16 for **1** and 5.07, 6.94 for **2**) are >0.3 units lower than for cisplatin. The slowness of the hydrolysis, combined with the dominance of (inert) hydroxo species, is expected to contribute to a greatly reduced reactivity of the sterically-hindered 2-picoline complex under intracellular conditions.

Keywords: antitumor agents · bioinorganic chemistry · hydrolysis · platinum · structure elucidation

Introduction

Cisplatin is a widely used anticancer drug; however, there is a need for new agents which do not exhibit cross-resistance and which are less toxic.[1] Most of the active platinum compounds have the general formula *cis*-[$PtAm_2X_2$], where Am is an am(m)ine ligand with at least one NH group and X is a moderately strongly bound anionic leaving group, such as chloride.[2] Recently, there has been interest in pyridine complexes.[3–5] The presence of planar pyridine ligands, as in *cis* or *trans*-[$PtCl_2$(pyridine)$_2$] complexes, can reduce the rates of DNA binding compared to *cis* and *trans*-DDP,[6] and by changing the nature or position of substituents on the pyridine ligands, different binding affinities for DNA can be achieved.[4]

The 2-picoline (2-methylpyridine) complex *cis*-[$PtCl_2(NH_3)$-(2-picoline)] (**1**) (AMD473), a recently reported anticancer complex, has now entered clinical trials.[7] It is reported to possess activity against cisplatin-resistant cell lines, and against a subline of a human ovarian carcinoma xenograph with acquired cisplatin resistance, by injection and oral administration. No chemical studies of the complex have been reported, although it appears to form interstrand DNA cross-links and to bind to plasma proteins much more slowly than cisplatin.[8] For this report we have labelled complex **1** with ^{15}N and compared its hydrolysis behaviour with that of the isomeric 3-picoline derivative **2**, since hydrolysis is likely to be an important initial activation step for this drug. We have also determined the pK_a values for the mono and diaqua complexes, since hydroxo ligands on Pt^{II} are usually inert compared to aqua ligands. The data reveal notable differences between the chemistry of the sterically hindered picoline complex and that of cisplatin.

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Experimental Section

Chemicals and preparation of complexes: 2- and 3-Picoline were purchased from Aldrich. *Cis*-[$PtCl_2(^{15}NH_3)_2$] was prepared according to a reported procedure.[9] Complexes **1** and **2** were prepared by a procedure similar to that described in the literature for natural-abundance, mixed ligand ammine/amine Pt^{II} complexes.[10]

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Complex **1**: ^{1}H NMR: $\delta = 8.87$ (d, $J = 8$ Hz, 1H, H6), 7.81 (m, 1H, H4), 7.49 (d, $J = 8$ Hz, 1H, H3), 7.32 (m, 1H, H5), 3.13 (s, 3H, CH_3); Anal. calcd for $C_6H_{10}Cl_2N^{15}NPt$: C 19.1, H 2.65, N 7.69; found: C 19.28, H 2.97, N 7.57.

Complex **2**: ^{1}H NMR: $\delta = 8.62$ (s, 1H, H2), 8.54 (d, $J = 6$ Hz, 1H, H6), 7.77 (d, $J = 9$ Hz, 1H, H4), 7.37 (m, 1H, H5), 2.35 (s, 3H, CH_3); Anal. found: C 18.82, H 2.70, N 7.31

pH Measurements: These were performed with a Corning 145 pH meter equipped with an Aldrich micro combination electrode calibrated with Aldrich buffer solutions of pH 4, 7 and 10. The values of the pH were adjusted with 1M $HClO_4$ or NaOH as appropriate.

X-ray crystallography: Crystals of complexes **1** ($^{15}NH_3$) and **2** ($^{14}NH_3$) were obtained by the slow evaporation of aqueous solutions containing excess KCl. Data for **1** and **2** were collected on a Stadi-4 diffractometer equipped with an Oxford Cryosystems low-temperature device. Scan modes were both $\omega-\theta$. Complex **2** crystallised as fine delicate needles, which tended to form coaxially aligned clumps and exhibited broad diffraction profiles. For these reasons, $Cu_{K\alpha}$ radiation was used for its greater intensity than $Mo_{K\alpha}$ radiation. The structures were refined by full-matrix least-squares against F^2 (SHELXL 1). H atoms were placed in calculated positions; the CH_3 and NH_3 were modelled as rotating rigid groups. All non-H atoms were refined anisotropically.

Crystal data for the two structures are listed in Table 1, and selected bond lengths and angles are given in Table 2. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-100573. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: (+ 44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Table 1. Crystal structure data for complexes **1** and **2**.

	1	**2**
empirical formula	$C_6H_{10}Cl_2N^{15}NPt$	$C_6H_{10}Cl_2N_2Pt$
M_r	377.1	376.1
colour	yellow	yellow
crystal size (mm)	$0.47 \times 0.39 \times 0.25$	$0.43 \times 0.08 \times 0.08$
crystal shape	block	needle
crystal system	monoclinic	orthorhombic
space group	$P2_1/c$	$Pbca$
a (Å)	9.859(2)	12.287(8)
b (Å)	8.910(2)	7.318(8)
c (Å)	11.197(2)	20.801(14)
β (°)	102.684(15)	90
V (Å^3)	959.6(3)	1871(3)
Z	4	8
λ (Å)	0.71073	1.54178
T (K)	220(2)	220(2)
ρ_{calcd} (g cm^{-3})	2.604	2.420
μ_{calcd} (mm^{-1})	15.119 ($Mo_{K\alpha}$)	30.166 ($Cu_{K\alpha}$)
$F(000)$	688	1240
2θ range (°)	5.9–50	8.5–140
abs. correction ($T_{min/max}$)	Ψ-scans (0.0033/0.0175)	Shelxa (0.0263/0.4036)
refl. collected	3725	4662
unique refl.	1688 ($R_{int} = 0.0358$)	1672 ($R_{int} = 0.0560$)
refl. used	1684	1670
parameters	102	101
$R1$ ($F_0 > 4\sigma(F_0)$)[a]	0.0355	0.0437
$wR2$ (all data)[b]	0.0903	0.1183
$g1$; $g2$[c]	0.0596; 0.0000	0.0746; 0.0000
resid. elec. density (e Å^{-3})	+1.52/−1.16	+1.56/−2.72

[a] $R1 = \Sigma(||F_o| - |F_c||)/\Sigma|F_o|$. [b] $wR2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$. [c] $w = 1/[\sigma^2(F_o^2) + (g1 \times P)^2 + g2 \times P]$; $P = (F_o^2 + 2F_c^2)/3$.

NMR spectroscopy: NMR spectra were recorded on a Bruker DMX500 instrument in 5 mm tubes. All the samples were recorded in 90% H_2O/10% D_2O (0.6 mL) containing 0.1M $NaClO_4$ to maintain a constant ionic strength. The chemical shifts are reported relative to sodium trimethylsilyl[D_4]propionate (through internal dioxane at $\delta = 3.743$) for ^{1}H, and 1M $^{15}NH_4Cl$ in 1.5M HCl for ^{15}N (external). Typical acquisition conditions for ^{1}H spectra were: 45–60° pulses, 2.5 s relaxation delay, 64–256 transients, final digital resolution 0.2 Hz per point. When necessary, the water resonance was suppressed by presaturation, or by means of the WATERGATE pulsed-field-gradient sequence.[11]

Both 1D ^{15}N-edited ^{1}H NMR spectra and 2D [^{1}H, ^{15}N] heteronuclear single-quantum coherence (HSQC) spectra (optimised for $^1J(N,H) = 72$ Hz) were recorded with the use of the sequence of Stonehouse et al.[12] The ^{15}N spins were decoupled by irradiating with the GARP-1 sequence during acquisition.

Data analysis: For the kinetic analysis of NMR data, the appropriate differential equations were integrated numerically, and the rate constants were determined by a nonlinear optimisation procedure by the programme SCIENTIST (Version 2.01, MicroMath). The errors represent one standard deviation. Equilibrium constants were calculated from the equilibrium concentrations of species determined by integration of the 2D spectra.

Titration curves were fitted to the Henderson–Hasselbalch equation using the programme KaleidaGraph (Synergy Software, Reading, PA, USA) on a Macintosh computer.

Table 2. Selected bond lengths (Å) and angles (°) for complexes **1** and **2**.

	1	**2**
Pt–N(1)	2.017(8)	2.008(8)
Pt–N(2)	2.030(8)	2.039(9)
Pt–Cl(1)	2.299(2)	2.296(3)
Pt–Cl(2)	2.322(2)	2.309(3)
N(1)-Pt-N(2)	90.5(3)	90.5(4)
N(1)-Pt-Cl(1)	177.6(2)	177.3(3)
N(2)-Pt-Cl(1)	87.3(2)	86.8(3)
N(1)-Pt-Cl(2)	89.5(2)	90.9(2)
N(2)-Pt-Cl(2)	176.1(3)	178.6(3)
Cl(1)-Pt-Cl(2)	92.70(8)	91.72(10)

Results and Discussion

X-ray crystal structures: We first prepared both natural-abundance and ^{15}N-labeled complexes **1** and **2** and crystallised them for X-ray analysis. Both have a square-planar configuration with angles close to the ideal values of 90° and 180° (Figure 1). In complex **1**, the Pt–Cl(2) bond *trans* to NH_3 is slightly longer (2.322(2) Å) than normal, while the Pt–Cl(1) bond length (2.299(2) Å) is within the normal range. In complex **2** both Pt–Cl bond lengths (2.296(3) Å, 2.309(3) Å) are close to the expected values. The Pt–N(1) bond lengths in both complex **1** (2.017(8) Å) and **2** (2.008(8) Å) are comparable to those of *cis*-$[Pt(py)_2Cl_2]$ (2.01 and 2.04 Å).[13] The most notable feature of the structures is the orientation of the

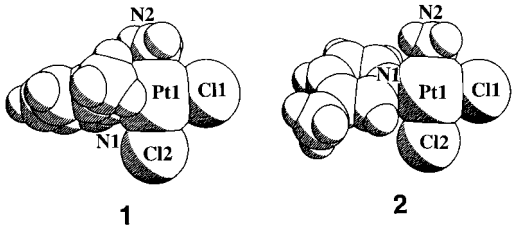


Figure 1. X-ray crystal structures of complexes **1** and **2** illustrating the steric hindrance caused by the 2-methyl group in complex **1**.

picoline ring with respect to the Pt square plane. The 3-picoline ligand is tilted by 48.9°, whereas the 2-picoline ligand is almost perpendicular (102.7°) to the plane, so that the 2-methyl group lies directly over the square plane ($H_3C\cdots Pt$: 3.224 Å). The space-filling model (Figure 1) demonstrates that this introduces steric hindrance to an axial approach to Pt from above. The steric effect leads to a slight twisting of the $[PtN_2Cl_2]$ square plane, with a mean deviation of the atoms from the plane of 0.0406 Å, which is an order of magnitude higher than that for complex **2**.

There are strong intermolecular hydrogen bonds involved in the crystal packing of both complexes (Figure 2). For complex **1,** the three H atoms of the NH_3 ligand are H-bonded to four Cl ligands from two neighbouring molecules, while for complex **2,** H-bonds of similar strength are formed only to three of the four Cl ligands. Such intermolecular H bonds are common in chloro Pt^{II} am(m)ine complexes.[14, 15] A very weak, graphitic type of interaction between the picoline groups of neighbouring molecules of **1** may also be present. In complex **1,** layers containing $Cl\cdots H{-}N$ H-bonding interactions alternate with layers containing picoline groups. In complex **2**, the molecules themselves form layers, with H bonds both within and between layers.

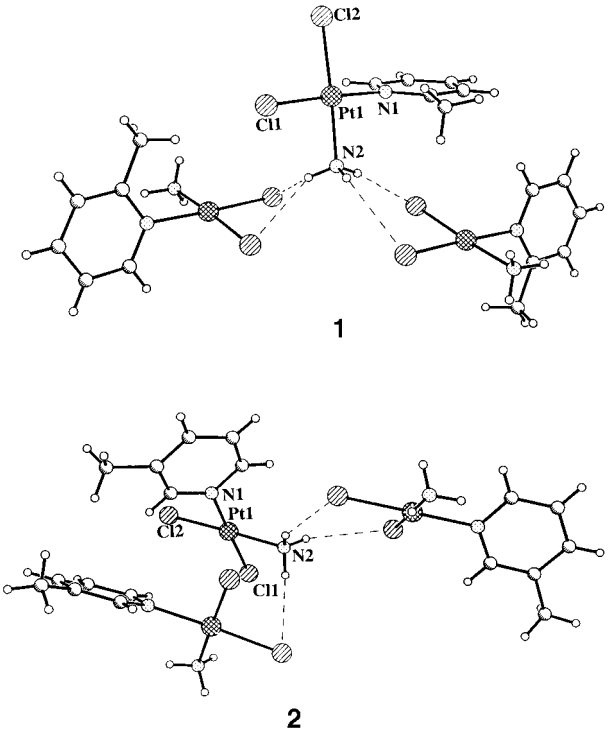
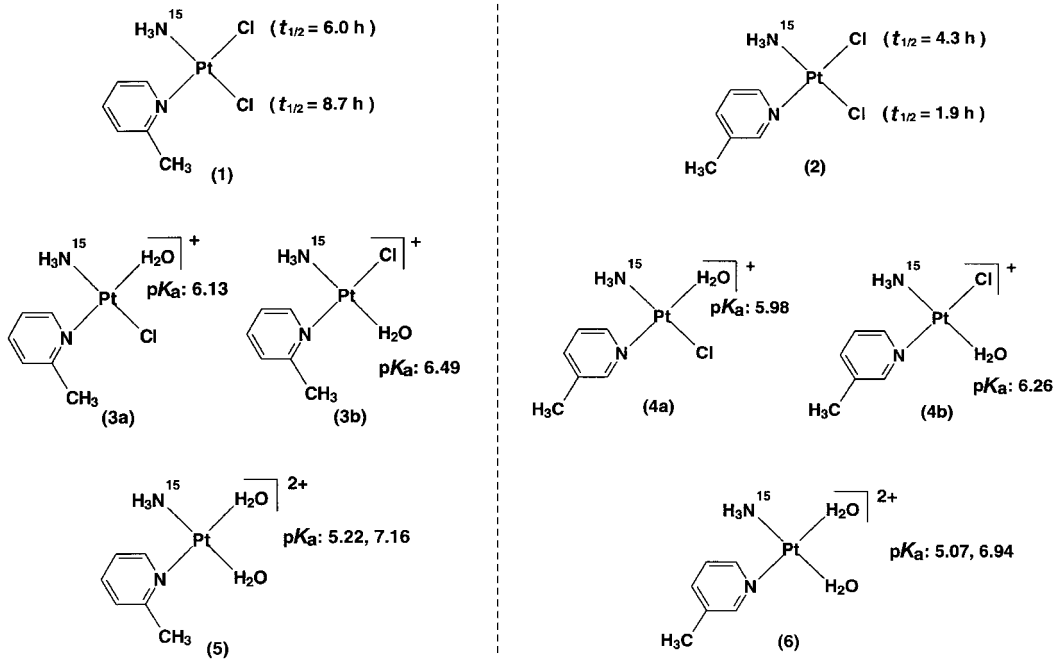


Figure 2. Intermolecular hydrogen bonding in crystals of complexes **1** and **2**; $N{-}H\cdots Cl$ distances for complex **1** range from 2.55 Å to 2.74 Å and for complex **2** from 2.62 Å to 2.81 Å.

Hydrolysis: The [^{1}H, ^{15}N] 2D NMR spectra of $^{15}NH_3$-labelled **1** and **2** in aqueous solutions containing 0.1M $NaClO_4$ were each monitored for a period of over 20 h at 310 K. Initially, a single cross-peak was observed at $\delta = 4.15/-66.52$, which was assigned to the dichloro complex **1**. After 1 h, two additional cross-peaks with similar intensities were detected at $\delta = 4.40/-64.41$ and $4.32/-87.25$. The former peak is consistent with an assignment to complex **3a**, with an ^{15}N shift diagnostic of ^{15}N *trans* to N or Cl,[16] and the latter to **3b** with ^{15}N *trans* to O, Scheme 1. Both peaks increased in intensity for several hours, while the peak for **1** decreased in intensity. After about 2.5 h, a fourth cross-peak appeared at $\delta = 4.41/-82.91$. This was assigned to the diaqua complex **5** (Figure 3A); however, even after 20 h, this accounted for only $<10\,\%$ of the total $^{15}NH_3$–Pt species present.

For the 3-picoline complex **2**, the time-dependence of the [^{1}H, ^{15}N] 2D NMR spectrum was similar to that of complex **1**, except that the cross-peak **c** (Figure 3B), assigned to one of



Scheme 1. Comparison of half-lives for the hydrolysis (310 K) and pK_a values (298 K) of platinum–picoline complexes (0.1M $NaClO_4$).

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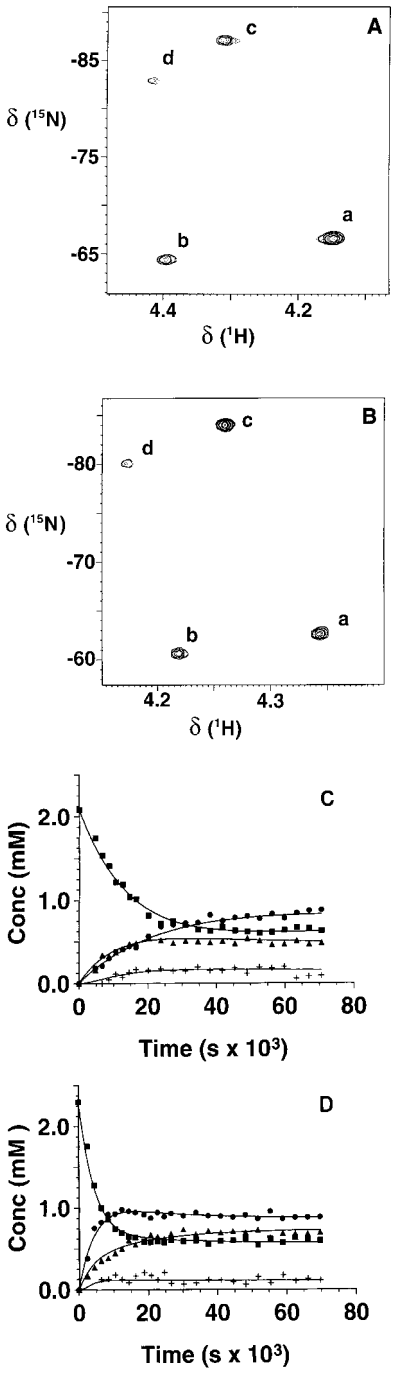


Figure 3. 2D [^{1}H, ^{15}N] HSQC NMR spectra of 3 mM aqueous solutions of: A) *cis*-[$PtCl_2(^{15}NH_3)$(2-picoline)] (**1**) and B) *cis*-[$PtCl_2(^{15}NH_3)$(3-picoline)] (**2**) after 3 h at 310 K. Peak **a** is assigned to the starting complex, peaks **b**,**c** to the two monoaqua complexes (H_2O *cis* to NH_3 and H_2O *trans* to NH_3, respectively), and peak **d** to the diaqua complex. Time dependence of the concentrations of the dichloro and aqua adducts of C) **1** and D) **2**. Labels: **1** and **2** (■), monoaqua complexes **3a** and **4a** (▲), monoaqua complexes **3b** and **4b** (●), diaqua complexes **5** and **6** (+). The curves are the best fits calculated with the rate constants shown in Table 3.

the two monoaqua complexes, was more intense than the other. The ^{15}N chemical shifts for the peaks of the 3-picoline complexes are shifted slightly to lower field with respect to those of the 2-picoline adducts. However, the ^{1}H chemical shifts are very similar. The time-dependence of the concentrations of species detected during hydrolysis of complexes **1** and **2** is shown in Figures 3C and 3D. The assignments of the peaks for the aqua complexes were confirmed by pH titrations (Figure 4), and these allowed the determination of the pK_a value for each monoaqua complex, as well as two pK_a values for each diaqua complex (Scheme 1).

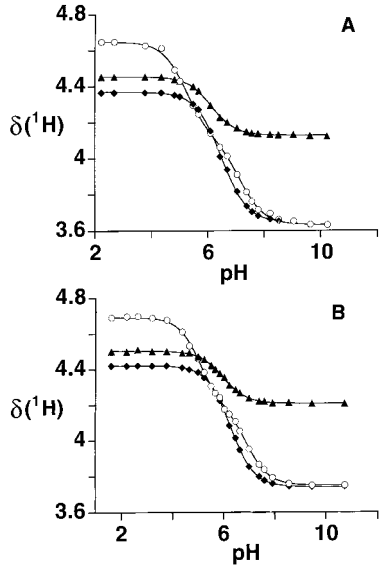
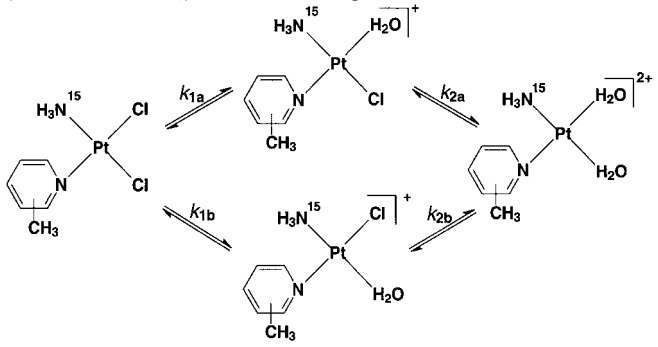


Figure 4. pH-dependence of the ^{1}H NMR chemical shifts of NH_3 in the monoaqua and diaqua complexes: A) complex **1**, and B) complex **2**. The curves represent best fits calculated with the pK_a values listed in Scheme 1. Labels: monoaqua complexes **3a** and **4a** (▲), monoaqua complexes **3b** and **4b** (◆), diaqua complexes **5** and **6** (○).

The NMR data allow the determination of the hydrolysis rates for each individual chloride ligand in the initial complexes **1** and **2**, and in the monoaqua complexes **3a**, **b** and **4a**, **b** (Table 3). It is notable that the hydrolysis rates of the two Cl^- ligands of complex **1** are both slower than those for complex **2** (Table 3). The Cl^- ligand *trans* to NH_3 in complex **1** hydrolyses about four times more slowly than that in the unhindered complex **2**. In complex **2,** the Cl^- ligand *trans* to NH_3 hydrolyses about twice as fast as that *trans* to 3-picoline. This might be expected from the higher *trans* influence of NH_3 (pK_a = 9.29)[17] versus 3-picoline (pK_a = 6.0).[17] However, for complex **1**, the situation is reversed: hydrolysis is faster for the Cl^- ligand *trans* to 2-picoline (pK_a = 6.1).[17] All the first-step hydrolysis rates determined here are slower than that of cisplatin ($t_{1/2}$: 1.75 h at 310 K).[18]

Axial steric interactions have long been known to decrease the rate of substitution reactions of square-planar complexes.[19] For example, the rate of reaction of 2-picoline with $[AuCl_4]^-$ is about 9 times slower than with 3-picoline, but 10 times faster than with 2,6-dimethylpyridine, which blocks both axial sites. In the complexes *cis*-[$Pt(PEt_3)_2$(R)Br], the rate of displacement of Br^- by MeOH decreases dramatically as the steric hindrance by the aryl ligand R, which is *cis* to the leaving group, increases: Ph ≈ *p*-MeC_6H_4 ≫ *o*-MeC_6H_4 > *o*-EtC_6H_4 > 2,4,6-$Me_3C_6H_2$.[20] In an associative mechanism with a trigonal-bipyramidal transition state, the ligands *cis* to the leaving group become axial to the trigonal plane in the 5-coordinate transition state, and interact with the entering and leaving groups at an angle of 90°, so that the steric effect is

Table 3. Rate and equilibrium constants for the hydrolysis of the platinum–picoline complexes **1** (pH = 4.6) and **2** (pH = 4.4) at 310 K (0.1 M $NaClO_4$). Data reported for cisplatin under related conditions (308 K, 0.32 M KNO_3) are listed for comparison.



	Rate constants[a] ($10^{-6}\,s^{-1}$)	Equilibrium constants[b] (10^{-4} M)
1	k_{1a}: 31.9 ± 1.5	K_{1a}: 12.1
2-picoline	k_{1b}: 22.1 ± 1.4	K_{1b}: 21.4
	k_{2a}: 73 ± 14	K_{2a}: 4.8
	k_{2b}: 3.5 ± 2.5	K_{2b}: 2.7
2	k_{1a}: 44.7 ± 1.9	K_{1a}: 23.2
3-picoline	k_{1b}: 103 ± 4	K_{1b}: 28.0
	k_{2a}: 35.0 ± 1.7	K_{2a}: 2.8
	k_{2b}: 78 ± 60	K_{2b}: 2.3
cisplatin[c]	k_1: 75.9	K_1: 43.7

[a] The errors in the rates for the second hydrolysis step are large because the fitting process is relatively insensitive to the rate of the back reaction. Therefore, these constants are not discussed in the text. [b] Constants correspond to kinetic steps indicated, that is K_{1a} to equilibrium between **1** (or **2**) and **3a** (or **4a**), etc. [c] Ref. [18].

more prominent on the ligand in the position *cis* to the bulky ligand.

pK_a values: A change of the methyl group from the 2- to the 3-position only has a small effect on the pK_a values of the aqua ligands, lowering them by about 0.2 units (Scheme 1). The pK_a values for both the monoaqua and diaqua adducts of the 2-picoline and 3-picoline complexes are >0.3 units lower than that of cisplatin. This means that although the sterically hindered 2-picoline complex **1** will exist predominantly (about 70%) as a dichloro adduct under extracellular conditions (0.1 M Cl^-, pH 7.4), under intracellular conditions (4 mM Cl^-, pH 7.4), the hydroxo/chloro and dihydroxo adducts of **1** will predominate (>70%), whereas for cisplatin the dichloro, chloro/aqua and chloro/hydroxo are present in about equal proportions (about 30% each).[21] The slowness of the hydrolysis steps of complex **1** (Table 3) coupled with the dominance of (inert) hydroxo species would both be expected to contribute to its greatly reduced reactivity under intracellular conditions.

Conclusions

The Cl^- ligand *cis* to 2-picoline (*trans* to NH_3) in the complex *cis*-[$PtCl_2(NH_3)$(2-picoline)] (**1**) hydrolyses about 4 times more slowly than that in cisplatin ($t_{1/2}$: 8.7 h at 310 K, compared with 1.8 h for cisplatin), and both Cl^- ligands of **1** hydrolyse more slowly than the 3-picoline analogue **2**. X-ray crystallography has confirmed the steric hindrance introduced by the 2-methyl group of the picoline ligand in **1**. This hindrance has the effect of destabilising the expected trigonal-bipyramidal transition state, an effect well-known in substitution reactions of square-planar Pt^{II} complexes.[19] The pK_a values of the monoaqua and diaqua adducts of both **1** and **2** are >0.3 units lower than those similar cisplatin adducts. This, combined with slower hydrolysis (Table 3), is likely to result in a reduced intracellular activity of complex **1** compared to cisplatin and may contribute to its high activity against cisplatin-resistant cell lines. Our preliminary studies of reactions of complexes **1** and **2** with guanosine 5′-monophosphate (5′-GMP) have established that hydrolysis is the rate-limiting step for both complexes **1** and **2**. The formation of the bis-GMP adduct of complex **1** is about twice as slow as that for complex **2**, which is consistent with the brief report that complex **1** forms DNA cross-links extremely slowly.[8] Further NMR studies should allow detailed insight to be gained into the effect of steric hindrance on the formation of DNA adducts.

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