Kinetics and Mechanism of the Complexation of *trans*-Diamminedichloroplatinum(II) with the Purine Nucleoside Inosine in Aqueous Solution

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Kinetics of the complexation of *trans*-$[PtCl_2(NH_3)_2]$ (**1**), and its hydrolysis products *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ (**2**) and *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (**3**), with the purine nucleoside inosine (L) has been studied by HPLC in aqueous solution at 318.2 K (pH = 2.8–3.4, I = 0.1 M). The relative ability of **1**–**3** to bind inosine is about 1:200:10 as given by the second-order rate constants $k_3 = (6.5 \pm 0.4) \times 10^{-3}$ M^{-1} s^{-1} (**1**), $k_4 = 1.4 \pm 0.1$ M^{-1} s^{-1} (**2**), and $k_5 = (6.6 \pm 0.6) \times 10^{-2}$ M^{-1} s^{-1} (**3**). An excess of ligand gives stepwise formation of the 1:2 complex. When [L] < 0.02 M, hydrolysis of the first chloro ligand ($k_1 = (1.05 \pm 0.03) \times 10^{-3}$ s^{-1}) is the rate-limiting step in the binding of inosine to **1** as well as to the 1:1 complex (**4**), in which the fourth ligand is Cl^- ($k_6 = (9.4 \pm 0.7) \times 10^{-5}$ s^{-1}). In higher ligand concentration direct substitution of Cl^- becomes significant both in **1** and **4**; the second-order rate constant for the latter is $k_7 = (8 \pm 1) \times 10^{-4}$ M^{-1} s^{-1}. Under acidic conditions aquation of **2** is very slow ($k_2 = (4 \pm 2) \times 10^{-6}$ s^{-1}) and strongly reversible. In basic solution (pH > 11), instead, hydrolysis of **2** is irreversible ($k_{2,OH} = (2 \pm 0.2) \times 10^{-5}$ s^{-1}). Competition of inosine and Cl^- for **2** and **3** was employed to study the Cl^- anation of aquated Pt(II) species. The second-order rate constants are $k_{-1} = 2.2 \pm 0.4$ M^{-1} s^{-1} for **2** and $k_{-2} = 0.20 \pm 0.02$ M^{-1} s^{-1} for **3**. Thus, the equilibrium constants for the stepwise hydrolysis of **1** are $K_1 = (4.8 \pm 1.2) \times 10^{-4}$ M and $K_2 = (1.8 \pm 0.8) \times 10^{-5}$ M. The rate constant for the chloride anation of the 1:1 complex (**5**) bearing H_2O as the fourth ligand is $k_{-6} = 0.62 \pm 0.12$ M^{-1} s^{-1}, which gives the equilibrium constant $K_6 = (1.5 \pm 0.5) \times 10^{-4}$ M for the reaction between **4** and **5**. The second-order rate constant for the conversion of **5** into the 1:2 complex (**6**) is $k_8 = 0.35 \pm 0.03$ M^{-1} s^{-1}. Comparison of the kinetic data obtained to those of the cis isomer reveals considerable differences in the hydrolysis reactions and in the formation of monofunctional adducts. In particular, the properties of the diaqua species differ markedly. By contrast, the second complexation step appears to be kinetically similar in both configurations.

Introduction

Coordination of Pt(II) diammines to nucleic acids and their constituents has received considerable interest in recent years.[1] It is well documented that only the cis isomer shows anticarcinogenic activity, whereas the corresponding trans isomer is far less active at equimolar doses.[2,3] The reason for this is not completely understood, however, because when bound bifunctionally to DNA, the trans isomer inhibits replication to the same extent than the cis isomer.[4] It has been suggested, for example, that the inactivity of *trans*-$[PtCl_2(NH_3)_2]$ may result from the selective removal of Pt bound to DNA[4a] or from the formation of different Pt–DNA adducts for stereochemical reasons.[5] Since Pt(II) is a typical inert metal ion, the differential activity of these isomers may also result from kinetic factors.[1c] Unfortunately, comparison of the kinetic parameters is difficult because most of the studies deal with the cis isomer only, whereas quantitative data for the trans isomer are rather limited.

In the case of aquated Pt(II) species the diaqua derivative of the cis isomer has been reported to form a monodentate adduct with DNA about 10 times faster than the monoaqua–monochloro species of either isomer.[6] Early findings with the dichloro species indicated that the rate of reaction of both isomers with DNA is mainly governed by their rate of hydrolysis.[7] A recent study has shown that the rate of the initial binding of both dichloro compounds to DNA is almost the same and corresponds to the rate of hydrolysis of these species.[3] In contrary, another Pt–DNA study suggests the order trans > cis for the hydrolysis rate,[8] in agreement with findings without DNA[9] and the expected order of the trans effect Cl > NH_3.[10] In fact, there seems to be quite a controversy concerning the hydrolysis rate of the trans isomer, since also the order cis > trans has been reported in aqueous solution.[11]

In acidic medium the hydrolysis products react much faster with nucleobases than the dichloro species in the case of the cis isomer.[12] Instead, very little information is available on the relative reactivity of the corresponding trans compounds.[1c] This

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(1) (a) Lippert, B. In *Progress in Inorganic Chemistry*; Lippard, S. J., Ed.; Wiley: New York, 1989; Vol. 37, pp 1–97. (b) Sundquist, W. I.; Lippard, S. J. *Coord. Chem. Rev.* **1990**, *100*, 293–322. (c) Bruhn, S. L.; Toney, J. H.; Lippard, S. J. In *Progress in Inorganic Chemistry*; Lippard, S. J., Ed.; Wiley: New York, 1990; Vol. 38, pp 477–516.
(2) Pinto, A. L.; Lippard, S. J. *Biochim. Biophys. Acta* **1985**, *780*, 167–180.
(3) Bancroft, D. P.; Lepre, C. A.; Lippard, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 6860–6871.
(4) (a) Ciccarelli, R. B.; Solomon, M. J.; Varshavsky, A.; Lippard, S. J. *Biochemistry* **1985**, *24*, 7533–7540. (b) Heiger-Bernays, W. J.; Essigmann, J. M.; Lippard, S. J. *Biochemistry* **1990**, *29*, 8461–8466.
(5) (a) van der Veer, J. L.; Ligtvoet, G. J.; van den Elst, H.; Reedijk, J. *J. Am. Chem. Soc.* **1986**, *108*, 3860–3862. (b) Gibson, D.; Lippard, S. J. *Inorg. Chem.* **1987**, *26*, 2275–2279. (c) Lepre, C. A.; Chassot, L.; Costello, C. E.; Lippard, S. J. *Biochemistry* **1990**, *29*, 811–823.
(6) Schaller, W.; Reisner, H.; Holler, E. *Biochemistry* **1987**, *26*, 943–950.
(7) Johnson, N. P.; Hoeschele, J. D.; Rahn, R. O. *Chem.-Biol. Interact.* **1980**, *30*, 151–169.
(8) Pinto, A. L; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4616–4619.
(9) (a) Tucker, M. A.; Colvin, C. B.; Martin, D. S. *Inorg. Chem.* **1964**, *3*, 1373–1383. (b) Panasuyk, V. D.; Malashok, N. F. *Russ. J. Inorg. Chem.* **1986**, *13*, 1405–1408.
(10) Basolo, F.; Pearson, R. G. *Mechanisms of Inorganic Reactions*; Wiley: New York, 1967; Chapter 5.
(11) Miller, S. E.; Gerard, K. J.; House, D. A. *Inorg. Chim. Acta* **1991**, *190*, 135–144.
(12) Arpalahti, J.; Mikola, M.; Mauristo, S. *Inorg. Chem.* **1993**, *32*, 3327–3332.

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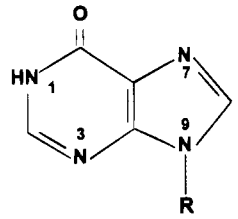
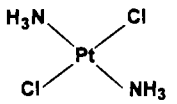
Chart 1



Inosine (L)



trans-[$PtCl_2(NH_3)_2$]

may be attributed to experimental difficulties in preparing the aqua derivatives of *trans*-[$PtCl_2(NH_3)_2$], as outlined recently by Appleton et al.[13] For example, preparation of the diaqua derivative from the dichloro species with Ag^+ requires prolonged treatment at elevated temperatures in the case of the trans isomer, while considerably milder conditions are sufficient for the cis compound. In addition, the first hydrolysis step appears to be strongly reversible,[3,13] which makes the following of specific complexation reactions difficult.

In this paper we report a detailed kinetic and mechanistic analysis of the complexation of *trans*-[$PtCl_2(NH_3)_2$] (**1**), and its hydrolysis products *trans*-[$PtCl(NH_3)_2(H_2O)]^+$ (**2**) and *trans*-[$Pt(NH_3)_2(H_2O)_2]^{2+}$ (**3**), with the model nucleobase inosine in slightly acidic aqueous solution at 318.2 K. The main purpose of the work was to study quantitatively the coordination properties of the different Pt(II) compounds giving special emphasis to the reactions of **2** and **3**. Very recently, we have shown that the substitution inert OH group facilitates the isolation of *trans*-[$PtCl(OH)(NH_3)_2]\cdot H_2O$,[14] which can be used as a convenient source for **2**. In order to make direct comparisons with the complexation abilities of the corresponding cis derivatives measured earlier,[12] inosine (Chart 1) was chosen for the model nucleobase.

Experimental Section

Materials and Solutions. The nucleobase derivatives were commercial products from Sigma and they were used as received. DMF (E. Merck AG) was dried over 5 Å molecular sieves. *trans*-[$PtCl_2(NH_3)_2$] was prepared and its purity checked as reported earlier.[15] The procedure for the preparation of *trans*-[$PtCl(OH)(NH_3)_2]\cdot H_2O$ is given elsewhere.[14] The corresponding dihydroxo derivative was obtained by hydrolyzing the dichloro compound under basic conditions.[6,11] A mixture of 150 mg of **1** (0.5 mmol) and 1.1 equiv of NaOH in 40 mL of water was heated on a water bath until **1** dissolved (about 10 min), after which 1.1 equiv of NaOH was added to the pale yellow solution, and the whole was stirred under nitrogen at 55−65 °C for 24 h in the dark. Two equivalents of $AgClO_4$ was added to the clear, pale yellow solution while warm, and the resulting gray precipitate was removed by filtration. The filtrate was concentrated on a rotary evaporator until yellow crystals appeared. The crystals were redissolved by gentle warming, and any insoluble material was removed by centrifugation. The dihydroxo compound crystallized as a dihydrate (pale yellow needles) upon cooling the supernatant in an ice bath. The crystals were filtered off, were washed first with 500 μL of ice-cold water and then with 500 μL of ice-cold ethanol, and were briefly air dried. An additional crop of crystals was obtained upon concentration of the mother liquid. Total yield: 90 mg (60%). The geometry of the compound was ascertained as previously described.[15] The crude product contains small amounts of the monoaqua−monochloro species (less than 2%), as evident from the HPLC analysis after inosine derivatization (see text). Recrystallization from a minimum amount of 5×10^{-3} M NaOH solution gave analytically pure product.[16] The dihydroxo compound dissolves very readily in water, acid, and in alkali.

Stock solutions of **3** (0.05 M) for kinetic purposes were prepared by dissolving the dihydroxo compound in 0.2 M HNO_3, and they were stable at least for 1 month. Stock solutions of **2** (0.01 M) prepared by dissolving the desired amount of *trans*-[$PtCl(OH)(NH_3)_2]\cdot H_2O$ in water were more stable than those prepared in 0.05 M HNO_3. The latter gave yellow crystals after 48 h when stored in a refrigerator.[17] For control purposes **2**[3] and **3**[13] were prepared also by literature methods. Stock solutions of the 1:1 complexes, *trans*-[$PtCl(NH_3)_2$(Ino-N7)]$^+$ and *trans*-[$Pt(NH_3)_2$(Ino-N7)$(H_2O)]^{2+}$, were obtained by LC-fractionation of the mixtures of inosine (0.01 mmol) in excess of **2** (0.05 mmol) or **3** (0.1 mmol), respectively.[18]

Kinetic Measurements. Kinetics of the complexation of inosine with *trans*-[$PtCl_2(NH_3)_2$] and its hydrolysis products in unbuffered aqueous solution (pH = 2.8−3.4) at 318.2 K was studied by HPLC as previously described.[12] The pH of the reaction mixtures adjusted with HNO_3 or NaOH remained practically constant (within 0.1 unit) in each measurement. Signal height or peak area was used as the measure of the concentration. The reactions were started by adding a known amount of the desired platinum compound to a prethermostated reaction mixture.[19] In the case of *trans*-[$PtCl_2(NH_3)_2$], about 8 mg of the compound was rapidly dissolved in water by sonication at about 310 K to give a 1 mM solution. The reaction was started immediately after the dissolution, which took less than 1 min. For control purposes, **1** was dissolved in dry DMF to give a 0.02 M solution and the desired amount (<50 μL) of fresh solution was added to the reaction mixture. Samples withdrawn from the reaction mixture at suitable time intervals were chromatographed either directly or indirectly after the addition of suitable reagents. When the compound **4** was monitored by using **1** or **2** as starting materials, the samples were diluted with 1 M NaCl (1:1) in order to prevent aquation of **4**. In the case of analyzing **5**, the samples were made alkaline (pH > 11) at 273 K to convert the reactive aqua ligands into inert hydroxo groups.[20] To improve chromatographic resolution the latter were acidified with 0.05 M HNO_3 just before injection into the chromatograph.

The hydrolysis reaction of **2**, and the chloride anation of **3**, were followed indirectly by converting unreacted Pt(II) species into more easily HPLC-detectable inosine complexes. In the former case, samples from the reaction mixture were diluted with 0.1 M inosine solution (1:1) and the mixture was kept at 318.2 K for 65 s before injection into HPLC.[21] During this treatment unreacted **2** is converted completely into **4**. In the latter case, aliquots from the reaction mixture containing **3** and varying amounts of Cl^- ($[Cl^-]_T > 20\ [Pt]_T$) were treated with 0.1 M inosine solution (1:1) for 30 min at 318.2 K, during which unreacted **3** was converted into **6**. Generally the samples were stored in an ice bath less than 1 h before injection into the chromatograph.

Peak heights/areas of the 1:1 complexes were transformed into the concentration by employing a suitable pyrimidine derivative (1-methyl- or 1,3-dimethyluracil) as an internal standard both in the reaction mixtures and in the calibration samples. In the case of **5** the calibration sample was prepared from a known amount of inosine in excess of **3**. Addition of a large excess of Cl^- to this mixture converted **5** into **4**. The employment of known amounts of **3** or isolated **4** as a starting material in excess of inosine gave calibration samples for **6**.

Results and Discussion

Scheme 1 depicts the assumed reaction pathway for the complexation of inosine (L) with *trans*-[$PtCl_2(NH_3)_2$] and its hydrolysis products in slightly acidic aqueous solution. Under

(13) Appleton, T. G.; Bailey, A. J.; Barnham, K. J.; Hall, J. R. *Inorg. Chem.* **1992**, *31*, 3077−3082.
(14) Arpalahti, J.; Sillanpää, R.; Mikola, M. *J. Chem. Soc., Dalton Trans.* **1994**, 1499−1500.
(15) Arpalahti, J.; Lippert, B. *Inorg. Chim. Acta* **1987**, *138*, 171−173.
(16) Satisfactory elemental analysis were obtained for N and H. The crystal structure determination of $H_{12}N_2O_4Pt$ is in progress.
(17) The crystals were identified as *trans*-[$PtCl_2(NH_3)_2$] by X-ray analysis; see: Milburn, G. H. W.; Truter, M. R. *J. Chem. Soc. A* **1966**, 1609−1616.
(18) Arpalahti, J.; Lehikoinen, P. *Inorg. Chem.* **1990**, *29*, 2564−2567.
(19) The ionic strength was adjusted to 0.1 M with $NaClO_4$.
(20) Arpalahti, J. *Inorg. Chem.* **1990**, *29*, 4598−4602.
(21) Hydrolysis reactions under basic conditions were stopped with 0.1 M inosine in 0.02 M HNO_3.

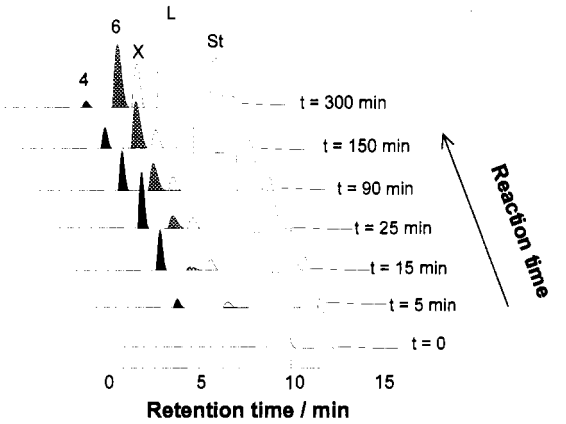
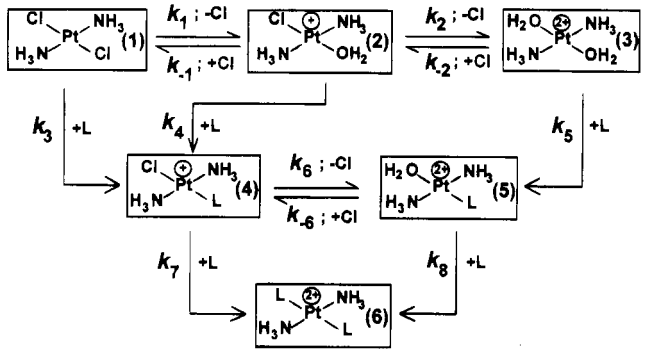


Figure 1. HPLC analysis of a mixture containing **1** (0.1 mM) and L (40 mM) at selected time intervals tracing compounds **4** and **6**. St denotes 1,3-dimethyluracil (0.15 mM), and × is an unknown decomposition product of inosine. A Serva $RP^{-1}8$ column (endcapped) using 6% MeOH in 0.05 M $NaClO_4$ (pH ≈ 4) as an eluent and flow rate 0.8 mL/min was used.

Scheme 1



these conditions Pt(II) is expected to bind inosine through the N7 position in all cases, whereas coordination to 1-methyl- or 1,3-dimethyluracil employed as internal standards is unlikely. The latter has no available nitrogen atom for Pt(II), and in 1-methyluracil the single available coordination site (N3) is protonated at pH 3, which efficiently prevents platination at this site.[22] According to chromatographic analysis the concentration of the internal standard remained constant in each kinetic run. It is further assumed that deprotonation of the aqua ligand(s) in **2**, **3**, and **5** does not markedly interfere in the complex formation with inosine at about pH 3. The reported pK_a values are 5.63 for **2** and 4.35 and 7.40 for **3**.[13] In the case of **5**, the data obtained for various Pt(II) species containing three nitrogen donors, viz., 6.24 for $[Pt(dien)(H_2O)]^{2+}$ [18] and 5.78 for *cis*-$[Pt(NH_3)_2(Ino\text{-}N7)(H_2O)]^{2+}$,[20] suggest a pK_a value above 5.5 for this compound.

Complexation of *trans*-$[PtCl_2(NH_3)_2]$. In a high excess of inosine the conversion rate of **2** into **4**, and **5** into **6**, is expected to be far greater that the hydrolysis rate of **1** and **4**, respectively, analogously to that observed recently for the corresponding cis isomer.[12] In other words, steady-state approximation can be applied to both **2** and **5**. The fact that LC analysis showed no trace of **5** during the complex formation (even without added Cl^-) supports these assumptions. A typical HPLC tracing of **4** and **6** is depicted in Figure 1. According to Scheme 1, the formation and disappearance of **4** obeys the rate law for two consecutive reactions, in which both steps consist of two parallel reactions.[23] Thus, the time-dependent concentration of **4** may

(22) pK_a 9.3 for uridine: Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32–38.
(23) Steps **1** → **2** and **4** → **5** are of first-order reactions, while steps **1** → **4** and **4** → **6** refer to pseudo-first-order reactions.

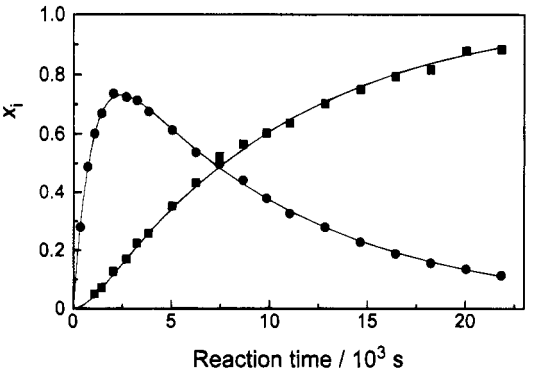


Figure 2. Time-dependent mole fractions of **4** (●) and **6** (■) from the total amount of Pt(II) in the reaction of **1** (2×10^{-4} M) with excess of L (0.011 M). Both lines represent computer simulations.

be expressed by eq 1, where $k_{f,obs}$ stands for the sum constant

$$[ML]_t = [MCl_2]_T \frac{k_{f,obs}}{k_{d,obs} - k_{f,obs}} (e^{-k_{f,obs}t} - e^{-k_{d,obs}t}) \quad (1)$$

$$\frac{[ML]_t}{[ML]_{max}} = \frac{e^{-k_{f,obs}t} - e^{-k_{d,obs}t}}{e^{-k_{f,obs}t_{max}} - e^{-k_{d,obs}t_{max}}} \quad (2)$$

$k_1 + k_3[L]_T$, and $k_{d,obs}$ for $k_6 + k_7[L]_T$. $[L]_T$ is the total ligand concentration, while $[MCl_2]_T$ denotes the total concentration of **1**. Here, and in subsequent equations, charges are omitted for clarity. Due to the poor aqueous solubility of the compound **1** the term $[MCl_2]_T$ in eq 1 may be inaccurate. To avoid this problem the rate constants were also computed by eq 2, where $[ML]_t$ denotes the concentration of **4** at the moment t and $[ML]_{max}$ denotes the maximum concentration of **4** at t_{max} during the kinetic run. In a typical kinetic run **4** reaches the maximum mole fraction of about 0.75 from the total amount of platinum in less than 45 min (Figure 2), while its conversion into **6** is considerably slower; i.e. the transformation of **1** into **4** is much faster than overall step **4** → **6**. This indicates that the rate constant $k_{d,obs}$ may be obtained independently from the disappearance of **4** by using eq 3, where the term $\ln [ML]_0$ denotes

$$\ln [ML]_t = -k_{d,obs}t + \ln [ML]_0 \quad (3)$$

the concentration of **4** after 2 h, i.e., approximately after 5 half-lives of the first step. Strictly linear plots of $\ln [ML]_t$ vs t gave the $k_{d,obs}$ values listed in Table 1. A least-squares fit was employed to compute the rate constant $k_{f,obs}$ by eqs 1 and 2, also using the $k_{d,obs}$ values obtained from the disappearance of **4**.

Inspection of the data collected in Table 1 reveals that the different computational methods give compatible values for both $k_{f,obs}$ and $k_{d,obs}$. Plotting of $k_{f,obs}$ vs $[L]_T$ gives $k_1 = (1.05 \pm 0.03) \times 10^{-3}$ s^{-1} and $k_3 = (6.5 \pm 0.4) \times 10^{-3}$ M^{-1} s^{-1} as the intercept and slope, respectively (Figure S1A, supplementary material). Similar treatment for $k_{d,obs}$ yields $k_6 = (9.5 \pm 0.2) \times 10^{-5}$ s^{-1} and $k_7 = (7.7 \pm 0.3) \times 10^{-4}$ M^{-1} s^{-1} (Figure S1B). Even when differences in experimental conditions are taken into account, the k_1 value obtained in this study appears to be higher than most of the data presented in literature, viz. 9.8×10^{-5} s^{-1} ($T = 293.2$ K, $I = 0.3$),[9a] 9.6×10^{-5} s^{-1} ($T = 310.2$ K, pH = 6.5),[3] 6.62×10^{-5} s^{-1} ($T = 298.2$ K, $I = 0.01$),[9b] and 1.90×10^{-5} s^{-1} ($T = 298.2$ K, $I = 0.1$).[11] However, for the following reasons we believe our k_1 value is reliable. Control experiments employing freshly dissolved **1** in DMF gave values very similar to those obtained for $k_{f,obs}$ and $k_{d,obs}$ using aqueous solution of **1** (Table 1), which rule out possible inaccuracies due to the poor aqueous solubility of **1**. The reliability of our

Table 1. Observed Rate Constants, k_i, for the Hydrolysis and Complexation of **1** and **4** in the Presence of Varying Amounts of Inosine (L) and Cl^- at 318.2 K[a]

$[L]_T/10^{-2}$ M	$[Cl^-]_T/10^{-2}$ M	$k_{f,obs}$[b]$/10^{-3}$ s^{-1}	$k_{d,obs}$[c]$/10^{-4}$ s^{-1}
1.11		1.06 (1.06)	1.05 (1.03)
2.21		1.24 (1.28)	1.13 (1.09)
2.25		1.18 (1.19)	1.08 (1.07)
2.26		0.97[d]	1.07[d]
4.03		1.38	1.27
4.15		1.31 (1.04)	1.26 (1.46)
5.97		1.44 (1.46)	1.43 (1.41)
7.52		1.56 (1.80)	1.56 (1.39)
8.92		1.63 (1.71)	1.65 (1.59)
10.5		1.71 (1.67)	1.71 (1.75)
4.03	1	0.88	0.97
4.02	2	0.76	0.82
4.02	4	0.65	0.66
4.05	6	0.61	0.57
4.03	8	0.50	0.52
4.03	12	0.44[e]	0.47[e]

[a] $I = 0.1$ M, $[Pt]_T = 2 \times 10^{-4}$ M. [b] Calculated by eq 2 using the $k_{d,obs}$ values measured independently. In general, fittings of both rate constants gave compatible results. The data in parentheses refer to eq 1. [c] Obtained by eq 3 from the disappearance of **4**. [d] **1** dissolved in DMF. [e] $I = 0.12$ M.

data is further supported by the nice correlation between experimental and computed values for $[ML]_t$ and $[ML_2]_t$ (Figure 2).[24] Moreover, the observations that $k_4[L]_T$ is at least 15 times greater than $k_{f,obs}$ or $k_{d,obs}$ and $k_8[L]_T > 30k_{d,obs}$ are in line with the mechanistic assumptions made above. In addition, the fact that the k_6 value given above agrees very well with that found independently (vide infra) supports the reliability of k_1 and k_3. The rate constant for the hydrolysis of **1** is about five times greater than that for the cis isomer, whereas the direct substitution of the chloro ligand with the incoming nucleoside occurs about three times faster in the trans compound (Table 6). Both of these findings are in line with the trans order $Cl^- > NH_3$. A similar difference has been reported also earlier for the hydrolysis[9a] and solvolysis[25] of these species. However, it is worth noting that aquation of **1** is strongly reversible, as will be shown below. This may seriously affect the magnitude of k_1 if not taken into consideration or if the experimental conditions are poorly chosen. In our case, high ligand excess ($[L]_T > 55\ [Pt]_T$) effectively prevents the Cl^- anation.

Complexation of the Aquation Products of *trans*-$[PtCl_2(NH_3)_2]$. The formation of the 1:1 complexes **4** and **5** was studied independently by employing isolated **2** or **3** as a starting material. According to HPLC analysis, excess of **2** converts inosine to a single product **4** with a retention time of 6.3 min,[26] while **3** prepared from the crude product of the dihydroxo species gave two signals under identical elution conditions, the major one at 5.2 min, which is assigned to **5**, and the minor one at 6.3 min. The latter signal disappeared upon recrystallization of the dihydroxo compound. This procedure appeared to be very sensitive in detecting impurities of **2** in **3**, because of the trans effect $Cl^- \gg H_2O$. The magnitude of the relevant rate constants ($k_4 \approx 20k_5$; Table 6) indicates that even the presence of 5% impurity of **2** in **3** gives rise to almost equal amounts of **4** and **5** when $[Pt]_T \gg [L]_T$. For comparison, the reaction of inosine with an excess of **3** prepared[13] by treating **1** with $AgNO_3$ at 363 K gave two products (at 5.2 and 6.3 min) in almost equal amounts.

(24) Conventional rate equation of two consecutive reactions were used to compute $[ML_2]$ values.
(25) Sundquist, W. I.; Ahmed, K. J.; Hollis, L. S.; Lippard, S. J. *Inorg. Chem.* **1987**, *26*, 1524−1528.
(26) Diphenyl column (250 × 4 mm, Serva) using 0.05 M $NaClO_4$ (pH ≈ 4) as an eluent, with flow rate 0.8 mL/min.

Table 2. Observed Rate Constants, $k_{i,obs}$, for the Complexation of **2** with Inosine at 318.2 K[a]

$[L]_T/10^{-4}$ M	$[Pt]/10^{-4}$ M	$k_{4,obs}$[b]$/10^{-3}$ s^{-1}	$(k_6 + k_7[L]_T)_{obs}$[c]$/10^{-5}$ s^{-1}
0.1	1.84	0.26	
0.5	4.61	0.68	
0.5	6.90	0.98	
0.5	9.21	1.36	
0.5	13.8	1.98	
20.0	1.0	2.78[d]	8.85 (9.56)[e]
40.0	1.0	5.33[d]	9.57 (9.72)
57.0	1.0	7.81[d]	9.65 (9.86)
80.0	1.0	10.5[d]	9.83 (10.0)

[a] $I = 0.1$ M. [b] Obtained from the disappearance of the free ligand in Pt(II) excess. [c] Obtained from the disappearance of **4** in ligand excess. [d] Calculated by eq 2 from the time-dependent concentration of **4** using independently determined values for $(k_6 + k_7[L]_T)_{obs}$. [e] Data in parentheses refer to calculated values ($k_6 = 9.4 \times 10^{-5}$ s^{-1} and $k_7 = 8.0 \times 10^{-4}$ M^{-1} s^{-1}; Table 6).

Table 3. Observed Rate Constants, $k_{i,obs}$, for the Complexation of **3** and **5** with Inosine at 318.2 K[a]

$[L]_T/10^{-4}$ M	$[Pt]/10^{-4}$ M	$k_{5,obs}$[b]$/10^{-4}$ s^{-1}	$k_{8,obs}$[c]$/10^{-4}$ s^{-1}
0.2	5.4	0.43	
0.2	10.8	0.85	
0.2	27.0	1.94	
0.2	47.2	3.28	
5.0			1.71 (1.92)
10.0			3.34 (3.52)
15.0			5.39 (5.37)
20.0			6.95 (7.53)
61.1	1.9	3.96[d] [3.67][e]	[24.6]
118.0	1.9	7.86[d] [7.52]	[45.0]
148.0	1.9	9.58[d] [8.96]	[58.7]

[a] $I = 0.1$ M. [b] Obtained from the disappearance of the free ligand in Pt(II) excess. [c] Obtained from the disappearance of **5** employed as a starting material. The data in parentheses are calculated from the formation of **6**; ligand excess at least 10-fold. [d] Obtained by eq 2 from the time-dependent concentration of **5** using the values $(0.35[L]_T)$ s^{-1} for $k_{8,obs}$. [e] The data in brackets refer to fitting of both rate constants by eq 2.

The observed pseudo-first-order rate constants, $k_{i,obs}$ listed in Tables 2 and 3, were calculated from the disappearance of the free ligand by eq 3, where L stands for ML and $k_{i,obs}$ for $k_{d,obs}$. In both cases plots of $k_{i,obs}$ vs $[Pt]_T$ were linear over the the whole concentration range employed (Figure S2, supplementary material). The data in Table 2 give $k_4 = 1.44 \pm 0.03$ M^{-1} s^{-1}, while those in Table 3 yield $k_5 = 0.069 \pm 0.002$ M^{-1} s^{-1}. The small deviation of k_5 at low $[Pt]_T$ values may result from the formation of 1:2 complex, although the Pt excess employed was at least 20-fold.

The complexation ability of **2** and **3** was studied also in ligand excess to ascertain the purity of the aquated Pt(II) species. By using **2** as a starting material, the time-dependent concentration profile of **4** was rather similar to that found above by using **1** as a starting compound, but almost all of Pt(II) transformed into **4** before any appreciable formation of **6** indicating that $k_4 \gg k_6 + k_7[L]_T$. The observed rate constants listed in Table 2 were computed by eq 2,[27] and they gave the mean value of $k_4 = 1.35 \pm 0.04$ M^{-1} s^{-1}. This agrees very well with that found in excess Pt(II) supporting the reliability of the data. In each case also the values found for $k_6 + k_7[L]_T$ are in excellent agreement with those calculated using independently measured values for k_6 and k_7. For comparison, rate constants of 0.95 M^{-1} s^{-1} ($T = 298.2$ K, $I = 5$ mM, pH = 5−6)[7] and 4.7 M^{-1} s^{-1} ($T = 310.2$ K, $I = 10$ mM, pH = 5.5)[6] have been reported for the reaction of **2** with DNA.

(27) In eq 2 $k_{4,obs}$ stands for $k_{f,obs}$, and $(k_6 + k_7[L]_T)_{obs}$ for $k_{d,obs}$.

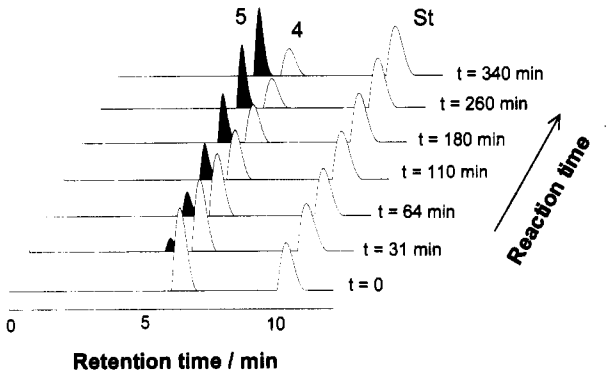


Figure 3. HPLC trace of the transformation of isolated **4** (0.02 mM, hatched signal) into **5** (dark signal). St denotes 1-methyluracil (0.025 mM). A Serva diphenyl column using 0.05 M $NaClO_4$ (pH ≈ 4) as an eluent and flow rate 0.8 mL/min was used.

In the case of **3**, instead, both **5** and **6** began to form without delay after mixing of the reactants and the maximum concentration of **5** formed represented only about 10% of the total amount of platinum employed. This indicates that $k_8 > k_5$; i.e. **6** is formed faster from **5** than **5** from **3**, which is in agreement with the expected trans order Ino-*N7* > H_2O. The rate constants $k_{5,obs}$ and $k_{8,obs}$ listed in Table 3 were computed by eq 2 from the time-dependent concentration of **5**.[28] However, the unfavorable ratio of steps **3** → **5** and **5** → **6** makes the accurate quantification of **5** rather difficult. Hence, the rate constant k_8 was determined independently from the conversion of isolated **5** into **6** in ligand excess. The observed rate data are included in Table 3. In each case the values found by first-order rate equation for $k_{8,obs}$ from the disappearance of **5** and from the formation of **6** were compatible. The mean value of $k_8 = 0.35 \pm 0.03$ M^{-1} s^{-1} was used to calculate $k_{8,obs}$ for the overall reaction **3** → **6**, which gave $k_5 = 0.064 \pm 0.004$ M^{-1} s^{-1} as the mean. This agrees well with that found above in excess Pt. Further support to data is given by the reasonable agreement between the calculated and fitted values for $k_{8,obs}$.

Interconversion of 1:1 Pt–Inosine Complexes. As seen in Figure 3, HPLC offers a convenient method for the simultaneous analysis of both 1:1 complexes **4** and **5**. In aqueous solution hydrolysis or aquation of the compound **4** obeys a first-order rate law, while the reverse reaction is of second-order; i.e., anation of the compound **5** depends also on the concentration of Cl^- ions. By employment of isolated **4** as a starting material, the rate law for the formation of **5** may be expressed by eq 4, which yields eq 5 upon integration.[29] The

$$\frac{d[ML(H_2O)]_t}{dt} = k_6[MLC]_t - k_{-6}[ML(H_2O)]_t^2 \quad (4)$$

$$[ML(H_2O)]_t = [MCl]_T \frac{2k_6(1 - e^{ts})}{k_6(1 - e^{ts}) - s(1 + e^{ts})} \quad (5)$$

$$s = \sqrt{k_6^2 + 4k_6k_{-6}[MCl]_T}$$

employment of a least-squares fit to eq 5 gave $k_6 = (9.3 \pm 0.2) \times 10^{-5}$ s^{-1} and $k_{-6} = 0.68 \pm 0.06$ M^{-1} s^{-1}.[30] On the other hand, also the concentration of **4** can be used to calculate both rate constants by taking into account that $[MLCl]_t = [MLCl]_T - [ML(H_2O)]_t$, which gave the values $(9.1 \pm 0.2) \times 10^{-5}$ s^{-1} and 0.65 ± 0.02 M^{-1} s^{-1} for k_6 and k_{-6}, respectively.

The large value obtained for k_{-6} indicates that the anation reaction is very sensitive to the concentration of free Cl^- ions. The semipreparative HPLC method employed for the isolation of **4** is assumed to give a compound essentially free from unbound Cl^-. However, the measurement of free Cl^- ions is difficult because these are slowly released from **4** even when stored in an ice bath. Therefore the equilibrium between **4** and **5** was studied also in Cl^- excess by using isolated **5** as a starting material ($[Cl^-]_T = 0.5-1.0$ mM; $[Cl^-]_T:[Pt]_T > 25$). Under these conditions the time-dependent concentration of **5** may be expressed by eq 6. Least-squares fit to the kinetic data gave k_6

$$[ML(H_2O)]_t = [ML(H_2O)]_T \frac{k_6 + k_{-6}[Cl]_T(e^{-(k_6 + k_{-6}[Cl]_T)t})}{k_6 + k_{-6}[Cl]_T} \quad (6)$$

$= (9.6 \pm 0.5) \times 10^{-5}$ s^{-1} and $k_{-6} = 0.53 \pm 0.03$ M^{-1} s^{-1},[31] which are in reasonable agreement with the values reported above. The data obtained for the hydrolysis of **4** nicely correlate with the rate constants of $(5.5-6.8) \times 10^{-5}$ s^{-1} reported for the closure of the monofunctional adducts of **1** on both single- and double-stranded DNA ($T = 310.2$ K, $I = 3$ mM).[3] This supports the assumption that hydrolysis of Cl^- is the rate-limiting step in the formation of bifunctional adduct from the monofunctional adduct bearing Cl^- as the fourth ligand.

Chloride Anation of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$. The stepwise conversion of **1** into **4** and then **6** in a 0.04 M inosine solution in the presence of varying amounts of Cl^- ($[Cl^-]_T$, $[L]_T > 50[Pt]_T$) was employed to study the chloride anation of **2**. Using the steady-state approximation for **2** and **5** the rate constants $k^{Cl}_{f,obs}$ and $k^{Cl}_{d,obs}$ for the formation and disappearance of **4** may be calculated by eq 2,[32] analogously to that given above for the complexation of **1**. The rate data obtained are included in Table 1. As evident from Scheme 1, the observed rate constants, $k^{Cl}_{f,obs}$ and $k^{Cl}_{d,obs}$, may be expressed under these conditions by eqs 7 and 8, respectively. Consequently, a linear

$$\frac{1}{k^{Cl}_{f,obs} - k_3[L]_T} = \frac{k_{-1}}{k_1k_4}\frac{[Cl]_T}{[L]_T} + \frac{1}{k_1} \quad (7)$$

$$\frac{1}{k^{Cl}_{d,obs} - k_7[L]_T} = \frac{k_{-6}}{k_6k_8}\frac{[Cl]_T}{[L]_T} + \frac{1}{k_6} \quad (8)$$

correlation is expected between $1/(k^{Cl}_{f,obs} - k_3[L]_T)$ and $[Cl^-]_T/[L]_T$ with an intercept of $1/k_1$ and a slope of k_{-1}/k_1k_4 (Figure 4A), which gives $k_{-1} = 2.2 \pm 0.4$ M^{-1} s^{-1} using the k_1 and k_4 values listed in Table 6. The intercept yields $k_1 = (9.4 \pm 1.4) \times 10^{-4}$ s^{-1}, which agrees within the limits of error with the value found above. Similarly, the plot of $1/(k^{Cl}_{d,obs} - k_7[L]_T)$ vs $[Cl^-]_T/[L]_T$ gives $k_6 = (9.1 \pm 0.5) \times 10^{-5}$ s^{-1} and $k_{-6} = 0.56 \pm 0.04$ M^{-1} s^{-1} (Figure 4B), which agree very well with those found above from the interconversion of 1:1 complexes. Together with the consistency of the k_1 values this strongly supports the reliability of the method and the k_{-1} value reported. Other estimates for k_{-1} are 0.49 M^{-1} s^{-1} ($T = 303.2$ K, $I = 0.1$ M)[9b] and 0.0305 M^{-1} s^{-1} ($T = 298.2$ K, $I = 0.1$ M).[11]

The knowledge of the rate constants for the aquation and anation reactions gives the equilibrium constants for the

(28) In this case $k_{5,obs}$ stands for $k_{f,obs}$, and $k_{8,obs}$ for $k_{d,obs}$.
(29) For the integration of eq 4, see: Benson, S. W. *The Foundations of Chemical Kinetics*; McGraw-Hill: New York, 1960; pp 29–31. Initial hydrolysis of **4** was taken into account by extrapolating the total concentration of **4** to represent the "real" value, viz. $[MLCl]_T = [MLCl]_t + [ML(H_2O)]_t$.
(30) Mean values of two independent measurements.
(31) Mean values of two independent measurements using also the concentration of **4**.
(32) In eq 2 $k^{Cl}_{f,obs}$ stands for $k_{f,obs}$, and $k^{Cl}_{d,obs}$ for $k_{d,obs}$.

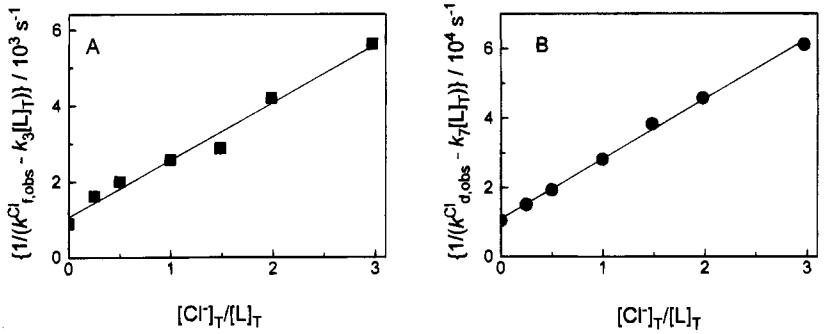


Figure 4. Plots of (A) $1/(k^{Cl}_{f,obs} - k_3[L]_T)$ and (B) $1/(k^{Cl}_{d,obs} - k_7[L]_T)$ vs $[Cl^-]_T/[L]_T$ showing the effect of $[Cl^-]_T$ on the rate constants for the formation ($k^{Cl}_{f,obs}$) and disappearance ($k^{Cl}_{d,obs}$) of **4** when $[L]_T = 0.04$ M. The slope of the plot (A) gives k_{-1} for the Cl^- anation of **2** upon multiplication with k_1k_4 (see eq 7), while that of B yields k_{-6} for the Cl^- anation of **5** by multiplying with the term k_6k_8 (eq 8); see Scheme 1.

Table 4. Observed Rate Constants, $k_{-2,obs}$, for the Cl^- Anation of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ at 318.2 K[a]

$[Cl^-]_T/10^{-4}$ M	$k_{-2,obs}/10^{-4}\ s^{-1}$
3.0	0.67
5.0	1.07
7.5	1.59
10.0	2.03
20.0	4.12

[a] $I = 0.1$ M; $[Pt]_T = 2 \times 10^{-5}$ M.

hydrolysis of **1** and **4** as the ratio of k_i/k_{-i};[33] viz., $K_1 = (4.8 \pm 1.2) \times 10^{-4}$ M and $K_6 = (1.5 \pm 0.5) \times 10^{-4}$ M. Despite the inconsistency of the individual rate constants the K_1 value obtained agrees fairly well with the literature data, viz., 2.39×10^{-4} M,[9b] 3.2×10^{-4} M,[9a] and 6.22×10^{-4} M.[11] For comparison, $K_1 = (3.2 \pm 0.15) \times 10^{-3}$ M and $K_6 = (2.8 \pm 0.4) \times 10^{-4}$ M have been reported under identical conditions for the corresponding compounds of cis configuration.[12] Thus, hydrolysis of first chloro ligand is thermodynamically more unfavorable in *trans*- than in *cis*-$[PtCl_2(NH_3)_2]$, whereas the rate constant for the hydrolysis is greater for the trans compound. By contrast, in the case of 1:1 inosine complexes the configuration of the compounds plays only a minor role in the hydrolysis of the chloro ligand both kinetically and thermodynamically.

Chloride Anation of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. There are several problems associated with experimental determination of k_{-2}, as outlined recently by Appleton et al.[13] However, the rate data given above indicate that a reasonable fast conversion of **3** into **6** occurs at high [L] values, while the conversion of **4** into **6** is much slower. Thus, treatment of known mixtures of **3** and Cl^- with an excess of inosine for 30 min converts practically all of unreacted **3** into **6** provided that $k_8[L]_T > 20\ k_{-6}[Cl^-]_T$. During this treatment less than 20% from **4** formed by steps **3** → **2** → **4** is converted into **6**. Consequently, the diminution of the signal for **6** can be used to calculate the rate constant $k_{-2,obs}$ by eq 3, where ML_2 stands for ML and $k_{-2,obs}$ for $k_{d,obs}$. The plots ln $[ML_2]$ vs t were strictly linear for 2 half-lives after which an upfield curvature appeared most probably due the side reaction mentioned above. The rate constants obtained at different $[Cl^-]_T$ values (Table 4) give $k_{-2} = 0.20 \pm 0.02\ M^{-1}\ s^{-1}$.

The affinity of **3** for Cl^- appears to be much higher than that obtained for other Pt(II) species containing two aqua ligands in trans positions; e.g., $0.058\ M^{-1}\ s^{-1}$ for $[Pt(H_2O)_4]^{2+}$ and $2.4 \times 10^{-4}\ M^{-1}\ s^{-1}$ for *trans*-$[PtCl_2(H_2O)_2]$.[34] This behavior can be attributed to the cis effect of the ligands $NH_3 > H_2O > Cl^-$, since the influence of charge is reported to be negligible.[9a] These results are in agreement with the proposal that in the case of very weakly trans directing systems (e.g. H_2O) the cis effect may be substantial.[10]

Aquation of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$. Due to the weak trans effect of the H_2O group aquation of **2** is expected to be very slow, which makes this reaction difficult to study. In particular, the employment of **1** as a starting material is inappropriate under acidic conditions, because the first aquation of step of **1** is highly unfavorable regarding to the formation of **2**. Therefore, aquation of **2** is generally not observed.[6,7] Very recently, we have reported the procedure for facile isolation of *trans*-$[PtCl(OH)(NH_3)_2]\cdot H_2O$.[14] This compound is an excellent starting material to study this reaction, because it generates **2** *in situ* when dissolved in acidic medium. Further experimental and mechanistic problems arise, however, from the tendency of the liberated Cl^- ions to bind **2** rather than **3**, particularly in the early stage of aquation, as evident from magnitude of the rate constants k_{-1} and k_{-2}. In addition, the step **2** → **1** is strongly reversible, which makes the total aquation of **2** exceedingly complicated, and the kinetic analysis difficult. However, using the theory of stepwise equilibrium, one can determine the equilibrium constant K_2 for the aquation of **2** by eq 9, where K_1 is the equilibrium constant for the first aquation

$$K_2 = \frac{\{[MCl(H_2O)]_T - [MCl(H_2O)]\}^2\{K_1 + [MCl(H_2O)]\}K_1}{[MCl(H_2O)]\{K_1 + 2[MCl(H_2O)]\}^2} \quad (9)$$

step of **1** and the terms $[MCl(H_2O)]_T$ and $[MCl(H_2O)]$ represent the total and equilibrium concentrations of **2**, respectively (for the derivation of eq 9, see the supplementary material). The measurement of the concentration of **2** by HPLC was facilitated by its carefully controlled precolumn derivatization with inosine. In 0.05 M inosine solution (pH ≈ 3) **2** is almost completely converted into **4** in 65 s (about 6 half-lives), during which the side reactions via steps **1** → (**2**) → **4** and **4** → **6** do not significantly affect the amount of **4**. According to the HPLC tracing of **4**, the system reaches the equilibrium state after about 72 h (Figure 5). Four independent measurements (Table 5) gave the value of $(1.8 \pm 0.8) \times 10^{-5}\ M^{-1}$ for the equilibrium constant K_2 when $K_1 = (4.8 \pm 1.2) \times 10^{-4}\ M^{-1}$. Multiplication of K_2 with the rate constant k_{-2} gives $k_2 = (4 \pm 2) \times 10^{-6}\ s^{-1}$, which agrees reasonable well with the initial rate constant found for the disappearance of derivatized **2**, viz. $(5 \pm 1) \times 10^{-6}\ s^{-1}$. When compared to **1**, acidic hydrolysis of **2** is much slower and also thermodynamically more unfavorable, which reflects the trans effect $Cl^- \gg H_2O$. Under basic conditions (pH > 11), instead, hydrolysis of **2** is irreversible with the rate constant $k_{2,OH} = (2.0 \pm 0.1) \times 10^{-5}\ s^{-1}$, in agreement with the trans effect $OH^- > H_2O$.

(33) The rate constants are given in Table 6.
(34) The reported values of 6.65×10^{-3} and 2.92×10^{-5} at 298.2 K, respectively, corrected to 318.2 K, see: Gröning, Ö.; Elding, L. I. *Inorg. Chem.* **1989**, *28*, 3366–3372.

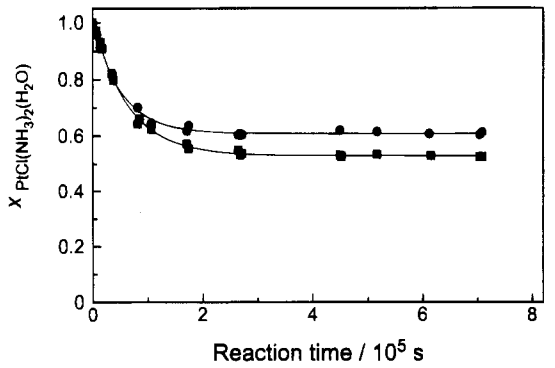


Figure 5. Time-dependent mole fraction of unhydrolyzed **2** from the total amount of Pt(II) based on HPLC tracing of its 1:1 precolumn derivative with inosine. Reaction mixtures were prepared by dissolving *trans*-$[PtCl(OH)(NH_3)_2]\cdot H_2O$ in 0.1 M $NaClO_4$ (pH ≈ 3) to give a 4×10^{-5} M (■) and 8×10^{-5} M (●) solution.

Table 5. Equilibrium Constants K_2 for the Reaction **2** ↔ **3** in Aqueous Solution at 318.2 K[a]

$b/10^{-5}$ M^{-1}	$c/10^{-5}$ M^{-1}	K_2[d]$/10^{-5}$ M^{-1} s^{-1}
4.0	2.1	1.5
8.0	4.9	1.5
10.0	6.1	1.8
20.5	13.0	2.3

[a] $I = 0.1$ M. [b] Initial concentration of **2**. [c] Equilibrium constant of **2** (after about 9 days). [d] Calculated by eq 9.

Table 6. Summary of the Rate Constants for the Stepwise Complexation of *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ with Inosine at 318.2 K[a]

	$k_i/10^{-4}$ s^{-1}		$k_j/10^{-3}$ M^{-1} s^{-1}	
reacn step[b]	*cis*[c]	*trans*[d]	*cis*[c]	*trans*[d]
1 → **2**	1.9 ± 0.2	10.5 ± 0.3		
1 → **4**			1.9 ± 0.4	6.5 ± 0.4
2 → **1**			60 ± 15	2200 ± 400
2 → **3**	2.3 ± 0.3	0.04 ± 0.02		
2 → **4**			140 ± 10	1400 ± 100
3 → **2**			980 ± 140	200 ± 20
3 → **5**			670 ± 30	66 ± 6
4 → **5**	2.1 ± 0.1	0.94 ± 0.07		
4 → **6**			0.8 ± 0.1	0.8 ± 0.1
5 → **4**			750 ± 70	620 ± 120
5 → **6**			310 ± 30	350 ± 30

[a] In 0.1 M $NaClO_4$. [b] See Scheme 1 for details. [c] Data from ref 12. [d] This work.

Comparison with *cis*-$[PtCl_2(NH_3)_2]$. The rate data obtained are summarized in Table 6. Comparison with the corresponding reactions of the cis isomer reveals considerable differences, but also substantial similarities, which nicely demonstrates the importance of the trans effect in the reactivity of Pt(II).

Acidic hydrolysis of the dichloro species is faster for the trans isomer, whereas the reverse is true for the monochloro–monoaqua species. In addition, these reactions are more strongly reversible in the case of the trans isomer; the pK_1's are 2.5 and 3.3 and the pK_2's are 3.6 and 4.7 for the cis and trans isomer, respectively. The relative ability of *cis*-$[PtCl_2(NH_3)_2]$ and its hydrolysis products to bind inosine is 1:70:350, as noted previously.[12] In the case of trans isomer the second-order rate constants k_3, k_4, and k_5 give the ratio 3:740:35 when normalized to the rate constant of 1.9×10^{-3} M^{-1} s^{-1} found for the reaction of *cis*-$[PtCl_2(NH_3)_2]$ with inosine under similar conditions. Together with the hydrolysis data these findings imply that kinetic factors may play significant role in the biological inactivity of the trans isomer.

Due to its higher reactivity the dichloro species of the trans compound is more easily deactivated because of the strongly reversible nature of the first hydrolysis step, in particular. In plasma the high Cl^- concentration (0.103 M) almost completely prevents the hydrolysis of the trans isomer. Since the OH group is inert toward substitution relative to the H_2O group,[18,20] the reactivities of the first hydrolysis products decrease with increasing pH. The effect is more profound in the case of the trans isomer ($pK_a = 5.63$)[13] than in the cis compound ($pK_a = 6.41$).[35] Thus, at cellular pH = 7.4 both monochloro–monoaqua species seem to bind nucleobases at comparable rates but the affinity for Cl^- remains higher for the trans isomer.

The most striking features between these isomers are, however, the differences in the formation and reactivity of the diaqua species and the similarities in the formation of bis(nucleobase) adduct. The rate data obtained indicate that the formation of the diaqua derivative is much more difficult in trans than in cis geometry, even in neutral solution. In addition, the diaqua species of the trans isomer is far less reactive than that of the cis isomer, at least in acidic medium as evident from the relevant rate constants. On the other hand, the second complexation step appears to kinetically similar in both configurations. Evidently, the trans effect for $NH_3 \approx$ that for Ino-*N*7. The only significant difference (though small) can be seen in the hydrolysis rate of the Cl^- group in the 1:1 complex, in agreement with earlier observations found from Pt–DNA interactions.[3] Thus, from a kinetic point of view it is tempting to postulate that the biological differences of *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ are, at least partly, due to the markedly different properties of the diaqua species of these isomers, although only the first hydrolysis products are usually considered as active intermediates in these cases. It is worth noting that the rate constant of the cis diaqua species decreases by a factor of 10 upon deprotonation of one H_2O group.[20] In trans geometry similar behavior should *increase* the rate constant of the monoaqua–monohydroxo species because of the trans effect $OH^- > H_2O$. Preliminary experiments reveal, however, that this is not the case. Instead, the rate constant decreases in a similar manner as found for the cis isomer.

Concluding Remarks. The relative ability of **1**–**3** to bind inosine is about 1:200:10 as given by the ratio of $k_3:k_4:k_5$. Expectedly, coordinated water molecule is a better leaving group than the chloro ligand. All rate parameters obtained are in line with the trans order $Cl^- > NH_3 \approx$ Ino-*N*7 $> H_2O$. Excess of ligand gives stepwise formation of 1:2 complex. When [L] < 0.02 M, hydrolysis of first chloro ligand is the rate-limiting step in the binding of inosine to **1** as well as to the 1:1 complex (**4**), in which the fourth ligand is Cl^-. In higher ligand concentration direct substitution of Cl^- becomes significant in both **1** and **4**. Under acidic conditions both hydrolysis steps of **1** are thermodynamically unfavorable ($K_1 = 4.8 \pm 1.2) \times 10^{-4}$ M^{-1} and $K_2 = (1.8 \pm 0.8) \times 10^{-5}$ M^{-1}). In basic solution (pH > 11), instead, hydrolysis of **2** is irreversible. Comparison of the kinetic data obtained to those of the cis isomer reveals considerable differences in the hydrolysis reactions and in the formation of monofunctional adducts. In particular, the properties of the diaqua species differ markedly. By contrast, the second complexation step appears to be kinetically similar in both configurations.

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Supplementary Material Available: Text and equations giving the derivation of eq 9 and figures showing the plots of $k_{f,obs}$ vs $[L]_T$ and $k_{d,obs}$ vs $[L]_T$ (Figure S1A,B) and plots of $k_{4,obs}$ vs $[Pt]_T$ and $k_{5,obs}$ vs $[Pt]_T$ (Figure S2) (2 pages). Ordering information is given on any current masthead page.

(35) Berners-Price, S. J.; Frenkiel, T. A.; Frey, U.; Ranford, J. D.; Sadler, P. J. *J. Chem. Soc., Chem. Commun.* **1992**, 789–791.