Kinetics and Mechanism of the Complexation of *trans*-Diamminediaquaplatinum(II) with the 6-Oxopurine Nucleosides Inosine and 1-Methylinosine in Aqueous Solution as a Function of the pH

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Kinetics of complexation of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with the model nucleobases inosine and 1-methylinosine has been studied in aqueous solution at 298.2 K in the pH range 2.8−9.6 (I = 0.1 M) by using HPLC as an analytical tool. The complexation of aquated *trans*-$Pt^{II}(NH_3)_2$ with the nucleobases employed can be quantitatively explained by replacement of the aqua ligand with the nucleobase, while the hydroxo group bound to Pt(II) is inert to substitution reaction relative to the coordinated water molecule. The stepwise acidity constants of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ obtained from kinetic measurements were in excellent agreement with those found potentiometrically ($pK_{a1} = 4.48 \pm 0.02$, $pK_{a2} = 7.20 \pm 0.05$). The reactivity of the Pt(II) dication is 7−8 times higher than that of the monocation despite the moderate trans effect $OH^- > H_2O$. In excess of the nucleobase, stepwise formation of 1:1 and 1:2 complexes is observed. 1-Methylinosine forms only N7-bound species, as does inosine when pH < 5.5. Although deprotonation of inosine N1H offers an additional binding site for Pt(II), the ability of the N7 site to accommodate Pt(II) is increased by the loss of the N1H proton, as well. Consequently, the N7 binding mode predominates in the formation of 1:1 complexes throughout the pH range studied. In the case of 1-methylinosine the second complexation step is mechanistically straightforward. With inosine, instead, proton transfer formally from N1H to the deprotonated OH group bound to Pt(II) gives substitution labile aqua ligand, the reactivity of which is comparable to that of the dicationic species. Even at high pH the N7 site is preferred over the N1 site also in 1:2 complexes. At pH 9.5, the inosine N1/N7 binding ratio is about 0.6 in 1:1 complexes, whereas the approximate percentage of different 1:2 complexes formed is as follows (Pt(II) binding sites in parentheses): (N7,N7) 44%, (N1,N7) 44%, and (N1,N1) 12%. In *trans*-$[Pt(NH_3)_2(InoH\text{-}N7)(H_2O)]^{2+}$ the inosine N1H proton is acidified by 1.7 log units, whereas in *trans*-$[Pt(OH)(NH_3)_2(InoH\text{-}N7)]^{+}$ the influence of the Pt(II) unit on the N1H acidity is 1.4 log units.

Introduction

In the complexation of isomeric $[PtCl_2(NH_3)_2]$ with nucleic acids and their constituents the labile chloro ligand(s) are substituted by the nitrogen atoms of the base moieties.[1,2] In aqueous solution a complicated reaction pathway is expected, because the stepwise aquation of the dichloro species and the Cl^- anation of the aqua forms efficiently compete with the formation of Pt(II)−nucleobase complexes.[3,4] Thus, evaluation of the significance of various binding modes may become difficult if the dichloro compounds are employed as starting materials. By contrast, the situation is more straightforward with the diaqua species. Much attention has been paid on the complexation of the diaqua form of the cis isomer, in particular, because of the biological activity of *cis*-$[PtCl_2(NH_3)_2]$. At present, kinetics for the reactions of aquated *cis*-Pt(II) diammine with nucleosides,[3,5,6] mononucleotides,[7] short oligonucleotides,[8] and DNA[9] are relatively well characterized. By contrast, data for the inactive trans isomer are scanty.[4] This may be attributed to experimental difficulties in preparing the diaqua derivative of *trans*-$[PtCl_2(NH_3)_2]$.[10] However, we have recently shown that substitution inert OH^- group facilitates the isolation of *trans*-$[Pt(OH)_2(NH_3)_2]\cdot 2H_2O$,[4,11] which is a convenient precursor for the diaqua species by generating *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ in acidic medium.

Despite intensive studies the reason for the different biological activity of isomeric $[PtCl_2(NH_3)_2]$ is not completely understood, because when bound bifunctionally to DNA the trans isomer inhibits replication to the same extent as the cis isomer.[12] It has been suggested that the inactivity of *trans*-$[PtCl_2(NH_3)_2]$ may result from stereochemical reasons[13] or from different

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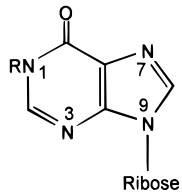
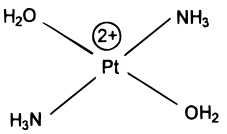
Chart 1



Inosine (InoH, R = H)
1-Methylinosine (MeIno, R = CH_3)



trans-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ ion

biological processing of Pt−DNA adducts formed with these isomers,[1c] for example. It is of considerable interest that certain *trans*-Pt(II) diamines other than the diammine compound do exhibit antitumor activity.[2b] Thus, the differential activity of *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ may result from kinetic factors as well, as has often been proposed but also disputed.[1c,2b,9b] From the chemical point of view, *trans*-$[PtCl_2(NH_3)_2]$ and *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ react faster with nucleobases than the corresponding cis derivatives, whereas with isomeric $[Pt(NH_3)_2(H_2O)_2]^{2+}$ the reverse is true under acidic conditions.[3,4] These findings are in line with the trans effect $Cl^- > NH_3 > H_2O$.[14] Under neutral conditions, however, the complexation abilities of the diaqua species may be quite different because of the deprotonation of the aqua ligand(s), which decreases the reactivity of the cis isomer[5] but may increase that of the trans isomer due to the trans effect $OH^- > H_2O$.[4] For these reasons, and to extend our knowledge on Pt(II)−nucleobase interactions, we now report a detailed kinetic study of the complexation of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with the model nucleobases inosine and its 1-methyl derivative in aqueous solution at 298.2 K in the pH range 2.8−9.6 by using HPLC as an analytical tool. The choice of the ligands allows direct comparisons with our previous results with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$.[5]

Experimental Section

Materials and Solutions. The nucleobase derivatives were purchased from Sigma, 4-morpholineethanesulfonic acid and -propanesulfonic acid were obtained from Aldrich, triethanolamine and acetic acid were purchased from Merck, and 2-ethylpyridine was obtained from Koch-Light Laboratories; they all were used as received.[15] Solutions of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (1 mM) were prepared by dissolving the desired amount of *trans*-$[Pt(OH)_2(NH_3)_2]\cdot 2H_2O$[4,11] in 0.01 M $HClO_4$. A stock solution of *trans*-$[Pt(OH)(NH_3)_2$(Ino-N7)] (1 mM) was obtained by dissolving a known amount of *trans*-$[PtCl(NH_3)_2$(InoH-N7)]NO_3[16] in aqueous alkali (pH > 10) and stirring the mixture for 7 days at about 20 °C. The completeness of the aquation was verified by HPLC.[4] Both solutions were stable for several weeks when stored in a refrigerator. The buffer solutions employed were prepared by adding strong alkali or acid to the aqueous solution of the buffer agent.

Kinetic Measurements. HPLC was employed to study the complexation of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with inosine and its 1-methyl derivative in aqueous solution at 298.2 K in the pH range 2.8−9.6. High excess of the ligand ($[L]_T > 20[Pt]_T$) provided pseudo-first-order conditions for the complex formation, while the total Pt(II) concentration was kept below 0.2 mM to avoid the influence of possible sidereactions (e.g. the formation of OH-bridged Pt(II) species)[10] on the complexation. The following buffers were used to maintain the desired pH value (the pH range employed in parentheses): HAc (3.8−5.0), 2-ethylpyridine (5.0−6.0), MES (5.0−6.2), MOPS (6.2−7.2), and TEA (7.2−8.5). Above pH 8.4 self-buffering of the ligand was employed in the case of inosine complexation. The measurements in Pt(II) excess were carried out in unbuffered solution, and the desired pH value was accomplished by adding strong alkali to the reaction mixture. The sulfonic acid derivatives MES and MOPS were chosen for buffer agents to facilitate chromatographic analysis, particularly in the case of inosine, since they do not significantly absorb UV light at 260 nm. In addition, these zwitterions show only a weak affinity for hard metal ions,[17,18] and there is no obvious reason why they should favor Pt(II). Of the softer donors in the sulfonic acid derivatives, the S atom is coordinatively saturated and the N atom of the morpholine ring is sterically hindered.

The employment of acetic acid as a buffer agent is ambiguous, because acetate ion can coordinate to Pt(II).[19] However, this side reaction can be hampered in a significant manner by using a high ligand to buffer ratio (20:1), and a pH range where the protonated form predominates. The reactions were started by adding the desired Pt(II) species into the prethermostated reaction mixture, and the complex formation was traced by HPLC as previously described[20] by employing an end-capped RP-18 column (5 μm, E. Merck AG) and water−methanol mixtures (0.05 M $NaClO_4$, pH 3) as eluents. Peak areas were used as the measure of the concentration in all cases.

The time-dependent concentration of different 1:1 complexes was employed to calculate pseudo-first-order rate constants for their formation and disappearance by eq 1. Here $[M]_0$ is the initial

$$[ML]_t = [M]_0 \frac{k'^{\,i}_{f,obs}}{k'_{d,obs} - k'_{f,obs}}(e^{-k'_{f,obs}t} - e^{-k'_{d,obs}t}) \quad (1)$$

$$\frac{[ML]_t}{[ML]_{max}} = \frac{e^{-k'_{f,obs}t} - e^{-k'_{d,obs}t}}{e^{-k'_{f,obs}t_{max}} - e^{-k'_{d,obs}t_{max}}} \quad (2)$$

concentration of Pt(II) and $[ML]_t$ is the concentration of the 1:1 complex at the moment t. The terms $k'_{f,obs}$ and $k'_{d,obs}$ stand for the rate constant for the formation of all 1:1 or 1:2 species, respectively, while the term $k'^{\,i}_{f,obs}$ represents the rate constant for the formation of the given 1:1 complex. The rate constants $k'_{f,obs}$ and $k'_{d,obs}$ were also computed by eq 2, where $[ML]_{max}$ denotes the concentration of the 1:1 complex at the moment t_{max}. With inosine, the disappearance of isolated N7-bound 1:1 complex gave the sum rate constant, $k'_{a,obs}$, for the 1:2 complexes by eq 3, where $[ML]_0$ is initial concentration of the complex.[21] In all

$$\ln[ML]_t = -k'_{a,obs}t + \ln[ML]_0 \quad (3)$$

cases peak areas were transformed into the concentration by employing 1-methyluracil or 1,2-dimethyluracil as an internal standard and by taking into account that N7 coordination predominates with inosine when pH > 4. For calibration purposes a known amount of the ligand was transformed into the N7-bound complex in Pt excess ($[Pt]_T > 50[L]_T$) under acidic conditions.

Potentiometric Measurements. The protonation constants of *trans*-$[Pt(OH)_2(NH_3)_2]$ were determined potentiometrically at 298.2 K in 0.1 M $NaClO_4$ under nitrogen by titrating with a standard 0.1 M $HClO_4$ solution using a Metrohm combined glass electrode. The pH meter was calibrated with Metrohm standard buffer solutions (pH 4.00 and 7.00).

Results and Discussion

1-Methylinosine. Throughout the pH range studied HPLC tracing of the reactions of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with excess

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(15) Abbreviations: acetic acid (HAc), inosine (InoH), 1-methylinosine (MeIno), 4-morpholineethanesulfonic acid (MES) and -propanesulfonic acid (MOPS), triethanolamine (TEA).
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Table 1. Observed Second-Order Rate Constants, $k_{i,obs}/10^{-4}$ M^{-1} s^{-1}, for the Formation of N7-Bound 1:1 and 1:2 Complexes between Aquated *trans*-$Pt^{II}(NH_3)_2$ and 1-Methylinosine in Aqueuos Solution at 298.2 K[a]

pH	$k_{f,obs}$	$k_{d,obs}$	pH	$k_{f,obs}$	$k_{d,obs}$
2.92	105	810	5.50	20	320
3.00	114[b]		5.64	20	230
3.21	98	830	5.87	17	170
3.35	105[b]		6.03	16	130
3.40	98	820	6.36	12	70
3.70	109[b]		6.76	9.7	44
3.98	98[b]		7.03	7.1	16
4.05	95[b]		7.12	6.5	15
4.17	74	720	7.40	5.0	8.0
4.46	62	680	7.63	4.2	4.1
4.48	66[b,c]		7.87	3.0	2.2
4.67	53	630	8.01	2.0	1.7
4.80	48[b,c]		8.12	1.6	1.5
5.03	32	520	8.22	1.2	1.2
5.14	27	470			

[a] $I = 0.1$ M. [b] In Pt(II) excess. [c] Refer to the inital value.

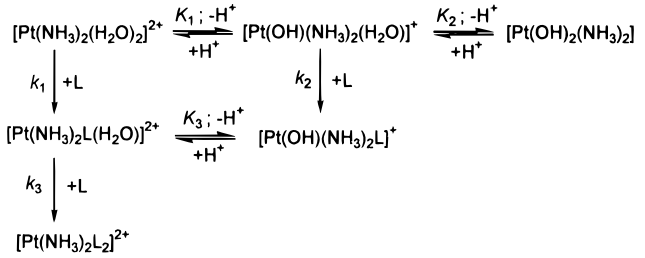
of 1-methylinosine revealed the formation of two products. The one with the shorter retention time is dominant only in the very beginning of the reactions and is assigned to a 1:1 complex, whereas the second product is clearly an end product, i.e. a 1:2 complex. The fact that a high excess of Pt(II) gave the former as a single product supports these assignments. In both products Pt(II) is assumed to bind to the N7 site of the nucleobase.[5] The relative amounts of these products formed during the reactions suggest that the second complexation step is much faster than the first, particularly at low pH. At higher pH both steps become slower, but the effect is more notable for the formation of bis-adduct. The observed second-order rate constants are listed in Table 1. The similarity of the values found for $k_{1,obs}$ in ligand and Pt(II) excess supports the validity of the data. Near pH 4.5 the plots of ln[L] vs t for measurements in Pt(II) excess showed upfield curvature, however, which suggests a decrease in effective Pt(II) concentration. Most probably this refers to the formation of OH-bridged species,[10] although the total concentration of Pt(II) was only about 10 mM. Nevertheless, the initial rate constants obtained in these cases are in fair agreement with the data found in ligand excess.

As seen in Table 1 the rate constants for both steps decrease with increasing pH. Since 1-methylinosine acts as neutral ligand under these conditions (pK_a 1.39),[22] the decrease in $k_{i,obs}$ must be attributed to the deprotonation of the aqua ligand(s) of Pt(II) which gives substitution inert OH group(s).[5,20] Accordingly, the complexation pathway in excess of the ligand (L) may be depicted by Scheme 1. The observed second-order rate constant $k_{f,obs}$ for the formation of 1:1 complex may be expressed by eq 4. Here K_1 and K_2 denote the acidity constants of *trans*-

$$k_{f,obs} = \frac{k_1[H^+]^2 + k_2K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (4)$$

$[Pt(NH_3)_2(H_2O)_2]^{2+}$and *trans*-$[Pt(OH)(NH_3)_2(H_2O)]^+$ ions, respectively. Least-squares fit to the kinetic data gave $k_1 = (1.05 \pm 0.01) \times 10^{-2}$ M^{-1} s^{-1}, $k_2 = (1.3 \pm 0.1) \times 10^{-3}$ M^{-1} s^{-1}, $K_1 = 10^{-(4.49\pm0.02)}$ M and $K_2 = 10^{-(7.2\pm0.1)}$ M.[23] The values found for the acidity constants from kinetic data are in excellent agreement with those obtained by potentiometric titrations,[24]

Scheme 1



viz. $K_1 = 10^{-(4.48\pm0.02)}$ M and $K_2 = 10^{-(7.20\pm0.05)}$ M which supports the validity of the data. In the literature, pK_a values of 4.32 and 7.38 have been reported for *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and *trans*-$[Pt(OH)(NH_3)_2(H_2O)]^+$ at 293.2 K, respectively.[25] The order $k_1 > k_2$ found for the rate constant is rather unexpected because of the trans effect $OH^- > H_2O$.[4] This suggests that the statistical factor and the decrease in charge of Pt(II) species when one reactive group (H_2O) changes to an inert one (OH^-) are more dominant than this moderate trans effect (*vide infra*).

Like above, the observed decrease of $k_{d,obs}$ with increasing pH refers to deprotonation of the aqua ligand in the 1:1 complex. According to Scheme 1, the term $k_{d,obs}$ may be expressed by eq 5, where K_3 stand for the acidity constant of the aqua ligand in

$$k_{d,obs} = k_3\frac{[H^+]}{[H^+] + K_3} \quad (5)$$

trans-$[Pt(NH_3)_2(MeIno\text{-}N7)(H_2O)]^+$. The values obtained by the least-squares fit were $k_3 = (8.09 \pm 0.07) \times 10^{-2}$ M^{-1} s^{-1} and $K_3 = 10^{-(5.27\pm0.02)}$ M. Comparison of the former to the rate constant for the formation 1:1 complex shows that substitution of one of the aqua ligands in *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with a nitrogen-bound nucleobase renders the remaining one more labile, in agreement with the trans effect N > O.[14] Quite interestingly, the aqua ligand in *trans*-$[Pt(NH_3)_2(MeIno\text{-}N7)(H_2O)]^+$ is about 0.5 log unit more acidic than that in the cis isomer, which will be discussed later in this paper.

Inosine. HPLC traces of the reaction of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with inosine at selected pH values are shown in Figure 1. Below pH 5.5 only two products **1a** and **2a** were detected. On the basis of their time-dependent formation, the former is assigned to a 1:1 and the latter to a 1:2 complex. Most probably both **1a** and **2a** have Pt(II) coordinated to the N7 site of inosine.[5] Above pH 5.5 three additional products appeared. The one with shortest retention time (**1b**) is assigned to N1-platinated 1:1 complex. Compound **2b** denotes a 1:2 complex, in which one ligand binds Pt(II) through the N7 site and the other through the deprotonated N1 site. The findings that in an excess of inosine **1a** gave only **2a** when the pH < 5.5, whereas above this pH both **2a** and **2b** were detected, and that the amount of **2b** formed increased with increasing pH strongly support these assignments. Product **2c** was formed in detectable amounts only at pH > 7.8. Evidently this compound is a 1:2 complex, in which Pt(II) binds both ligands through the N1 site, since it was not formed when **1a** was used as a starting material. The simplified reaction pathway of aquated *trans*-$Pt^{II}(NH_3)_2$ $[M(aq)_2]$ in an excess of inosine (L) may thus be expressed by Scheme 2, in which charges and protolysis reactions are omitted for clarity. Although the overall complexation pattern is very complicated, the resolution of **1a** and **1b** facilitated an independent study of the different steps.

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(23) Least-squares fitting using log $k_{f,obs}$ values and eq 4 in logarithmic form gave $k_1 = (1.04 \pm 0.05) \times 10^{-2}$ M^{-1} s^{-1}, $k_2 = (1.2 \pm 0.1) \times 10^{-3}$ M^{-1} s^{-1}, $K_1 = 10^{-(4.52\pm0.07)}$ M and $K_2 = 10^{-(7.3\pm0.1)}$ M.
(24) Mean values of three independent measurements.
(25) Jensen, K. A. *Z. Anorg. Allgem. Chem.* **1939**, *242*, 87−91. See also ref 10.

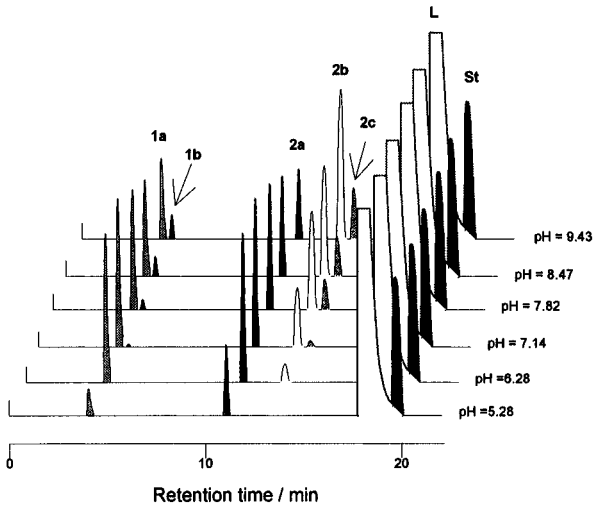
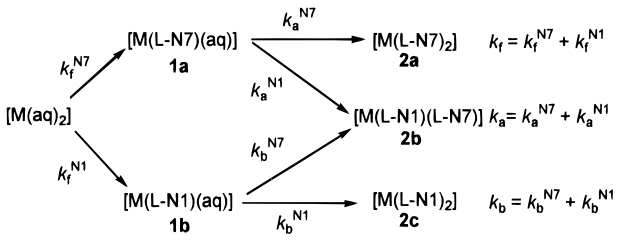


Figure 1. HPLC traces of the mixtures of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (0.2 mM) and inosine (L, 0.06−0.1 M) at selected pH values using water−methanol mixtures (0.05 M $NaClO_4$, pH 3) as eluents, linear gradient from 100:0 to 91:9 in 6 min, initial delay 4 min, flow rate 1 mL/min. The chromatograms were recorded when **1a** showed maximum area. St denotes 1,3-dimethyluracil. Notation of signals as Pt(II) binding sites: (**1a**) N7, (**1b**) N1, (**2a**) N7,N7, (**2b**) N1,N7, (**2c**) N1,N1.

Scheme 2



1:1 Complexes. Table 2 records the observed second-order rate constants for the complexation of aquated *trans*-$Pt^{II}(NH_3)_2$ with inosine. The data found at pH 7.01 (TEA buffer) and at pH 7.14 (MOPS buffer) are in good agreement, which shows that the sulfonic acid derivatives are useful and convenient buffers in the presence of Pt(II). Similarly, those found at pH 4.77 (HAc) and at pH 5.28 (MES) are in accordance with the general trend of the rate constants, suggesting that acetate ion does not significantly compete with inosine when proper experimental conditions are fulfilled. Further support for the use of acetic acid buffer could be seen in the binding of 1-methylinosine to the complex **1a** (*vide infra*), where plots ln[ML] vs *t* were strictly linear over 3 half-lives and gave $k_{m,obs} = 0.063 \pm 0.001\ M^{-1}\ s^{-1}$ at pH 5.00 (HAc) and $k_{m,obs} = 0.060 \pm 0.001\ M^{-1}\ s^{-1}$ at pH 5.12 (MES).

As seen in Tables 1 and 2, the observed rate constants for the formation of 1:1 complexes, $k_{f,obs}$, are practically identical for inosine and its 1-methyl derivative at low pH (when N7 coordination predominates). However, above pH 6 deprotonation of inosine N1H results in higher values also for $k_{f,obs}{}^{N7}$. By taking the protolysis reactions of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and inosine into account the detailed reaction pathway for the formation of **1a** may be depicted by Scheme 3, while the observed rate constant, $k_{f,obs}{}^{N7}$, may be expressed by eq 6. Here

$$k_{f,obs}{}^{N7} = \frac{k_1[H^+]^3 + k_2K_L[H^+]^2 + k_3K_1[H^+]^2 + k_4K_1K_L[H^+]}{([H^+]^2 + K_1[H^+] + K_1K_2)([H^+] + K_L)} \quad (6)$$

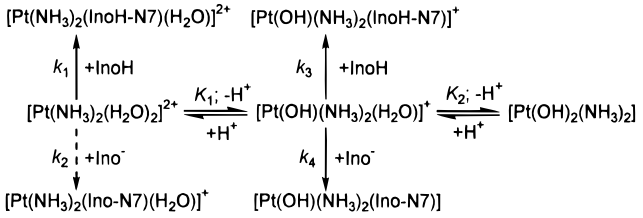
K_L denotes the acidity constant of inosine ($K_L = 10^{-8.8}$ M).[26]

Table 2. Observed Second-Order Rate Constants, $k_{i,obs}/10^{-4}\ M^{-1}\ s^{-1}$, for the Formation of Different 1:1 and 1:2 Complexes between Aquated *trans*-$Pt^{II}(NH_3)_2$ and Inosine in Aqueuos Solution at 298.2 K[a]

pH	$k_{f,obs}{}^{N7}$	$k_{f,obs}{}^{N1}$	$k_{a,obs}{}^{N7}$	$k_{a,obs}{}^{N1}$	$k_{b,obs}{}^{N7}$ [b]	$k_{b,obs}{}^{N1}$ [c]
2.77	112		828			
3.36[d]			828			
4.13	82.0		829			
4.77	52.0		751			
5.28	25.1		490			
5.53	23.9		336			
5.62[d]			332			
5.66	20.0		262			
5.98	17.5		141			
6.04[d]			146			
6.22	16.0		90			
6.36[d]			87	2.7		
6.38	14.6		76	2.8		
6.86	11.5	0.1	25.6	2.6		
7.01[e]	10.0	0.5	19.3	2.2		
7.14[f]	8.6	0.5	16.0	2.0	67	3.0
7.61[d]			7.46	1.64		
7.82	3.9	0.85	6.25	1.8	17	3.2
8.21[d]			4.20	1.48		
8.44[d]			3.47	1.35		
8.47	1.6	0.78	2.75	1.0	5.5	2.4
8.82	0.95	0.55	1.82	0.67	3.7	1.7
9.03[d]			1.73	0.79		
9.10	0.53	0.28	1.2	0.49	2.1	1.3
9.43	0.27	0.18	0.63	0.26	1.2	0.6
9.63[d]			0.29	0.14		

[a] $I = 0.1$ M. Data obtained by eq 1. [b] Obtained by eq 10 from the formation of **2b**, see text. [c] Calculated as $k_{b,obs} - k_{b,obs}{}^{N7}$, see Scheme 2. [d] Obtained by eq 3 from the disappearance of isolated **1a**. [e] In TEA buffer. [f] In MOPS buffer.

Scheme 3

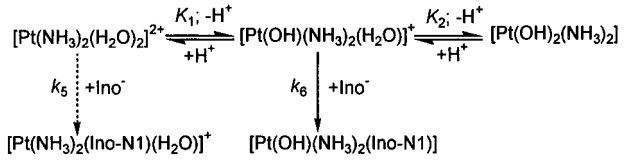


Least-squares fit to the data gave $k_1 = (1.14 \pm 0.03) \times 10^{-2}\ M^{-1}\ s^{-1}$, $k_3 = (1.6 \pm 0.1) \times 10^{-3}\ M^{-1}\ s^{-1}$, $k_4 = (5.8 \pm 0.2) \times 10^{-3}\ M^{-1}\ s^{-1}$.[27] However, no reliable value could be obtained for k_2. In fact, neglecting this term in eq 6 did not significantly change the value of the remaining parameters or their errors, which indicates that the reaction between *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and anionic inosine contributes only little on the overall reaction. This is reasonable considering the difference of about 4 orders of magnitude in their pK_a values. A similar observation was made also in the case of the cis isomer.[5] The order $k_1 > k_4 > k_3$, which is in line with findings for 1-methylinosine shows that factors decreasing the reactivity of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ upon deprotonation of one of the aqua ligands override the increased nucleophility of the anionic ligand.

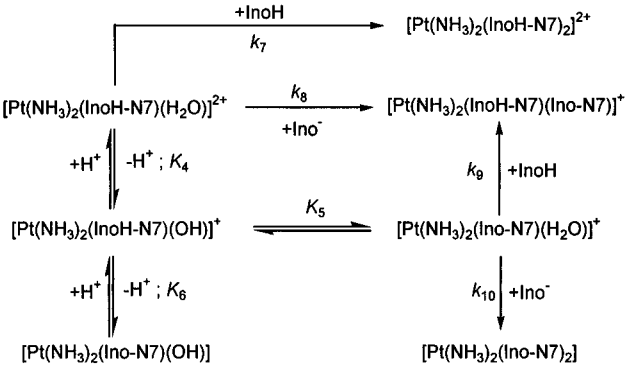
Attempts to convert the peak areas of **1b** into the concentration were unsuccessful, because of the small amount of **1b** formed in Pt(II) excess even at high pH. Hence, the data listed for $k_{f,obs}{}^{N1}$ in Table 2 were obtained indirectly by subtracting

(26) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32−38.
(27) The data quoted refer to fitting using logarithmic values for $k_{f,obs}{}^{N7}$ and eq 6 in logarithmic form. Normal scale fit gave $k_1 = (1.14 \pm 0.01) \times 10^{-2}\ M^{-1}\ s^{-1}$, $k_3 = (1.56 \pm 0.05) \times 10^{-3}\ M^{-1}\ s^{-1}$, $k_4 = (6 \pm 3) \times 10^{-3}\ M^{-1}\ s^{-1}$.

Scheme 4



Scheme 5



$k_{f,obs}{}^{N7}$ from $k_{f,obs}$ using values obtained by eq 1. The reactions proposed for the formation of **1b** are shown in Scheme 4. Least-squres fit to the data for $k_{f,obs}{}^{N1}$ gave $k_6 = (4.4 \pm 0.2) \times 10^{-3}$ M^{-1} s^{-1} by eq 7. Like above, no reliable value could be

$$k_{f,obs}{}^{N1} = \frac{(k_5[H^+]^2 + k_6K_1[H^+])K_L}{([H^+]^2 + K_1[H^+] + K_1K_2)([H^+] + K_L)} \quad (7)$$

obtained for the parameter k_5. The values found for k_4 and k_6 indicate that *trans*-$[Pt(OH)(NH_3)_2(H_2O)]^+$ may slightly favor the N7 over the N1 site in anionic inosine, which parallels the behavior of the corresponding cis derivative,[5] though the latter more clearly favors the N7 site.

Formation of 1:2 Complexes. Table 2 lists also the observed rate constants for the formation of different 1:2 complexes **2a**, **2b**, and **2c**. Complex formation via **1a** (Scheme 2) gave the rate constants $k_{a,obs}{}^{N7}$ and $k_{a,obs}{}^{N1}$ from the disappearance of **1a** by using the amount of **2a** formed in each case. As seen in Table 2, the employment of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ or isolated **1a** as starting materials gave compatible values for $k_{a,obs}{}^{N7}$ and $k_{a,obs}{}^{N1}$. The reaction pathway proposed for the formation of **2a** is depicted in Scheme 5. The observed second-order rate constant, $k_{a,obs}{}^{N7}$, may thus be expressed by eq 8. However,

$$k_{a,obs}{}^{N7} = \frac{k_7[H^+]^3 + k_9K_4K_5[H^+]^2 + k_8K_L[H^+]^2 + k_{10}K_4K_5K_L[H^+]}{([H^+]^2 + K_4[H^+] + K_4K_6 + K_4K_6[H^+])(K_L + [H^+])} \quad (8)$$

the large number of unknown parameters (seven) makes a direct least-squares fitting to eq 8 rather speculative. To improve the reliability of the numerical treatment, and to check the validity of the proposed reaction pathway, the equilibrium constants K_4, K_5, and K_6 were calculated from the data obtained for the binding of 1-methylinosine to **1a**. The reaction pathway for this mixed-ligand 1:2 complex corresponds to that of **2a** in Scheme 5 when protolysis of the ligand is ignored. Least-squares fit to the kinetic data for this system gave $k_{m1} = 0.090 \pm 0.002$ M^{-1} s^{-1}, $k_{m2} = 0.05 \pm 0.03$ M^{-1} s^{-1}, $K_4 = 10^{-(5.4\pm0.1)}$ M, $K_5 = 0.02 \pm 0.01$, and $K_6 = 10^{-(7.4\pm0.1)}$ M by eq 8, in which $k_{m,obs}$, k_{m1}, and k_{m2} stand for $k_{a,obs}{}^{N7}$, k_7, and k_9, respectively, and the term $K_L = 0$. The value found for k_{m1} is in agreement

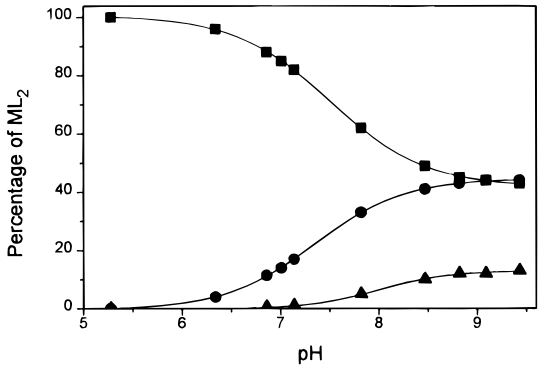


Figure 2. Distribution of different 1:2 complexes formed in the reaction of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with excess of inosine as a function of the pH. Notation: **2a** (□), **2b** (●), and **2c** (▲).

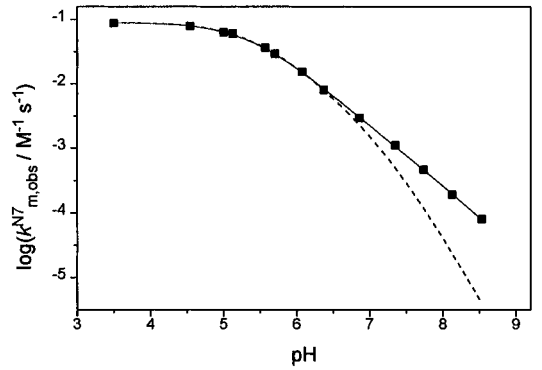


Figure 3. Observed rate constant, log $k_{m,obs}$, for the binding of 1-methylinosine to the complex **1a** as function of the pH. Both lines refer to computed values by eq 8 including the term K_5 in Scheme 5 (solid line), or ignoring the term K_5 (dashed line). See text for further details.

with the corresponding rate parameter given above for 1-methylinosine. Figure 3 depicts the plot of log $k_{m,obs}$ vs pH for the reaction of **1a** with 1-methylinosine, in which the solid line represents computer simulation. If the equilibrium described by K_5 in Scheme 5 is ignored, the fitted values for k_{m1} and K_4 are not markedly changed, but the term K_6 becomes negative and the standard deviation of the fit increases. Further support to the proposed mechanism is seen in Figure 3, where the dashed line represents the calculated pH dependence of $k_{m,obs}$ using the values given above for k_{m1}, K_4, and K_6 but omitting reaction steps described by the terms K_5 and k_9 from the overall reaction. Above pH 7 the observed values for $k_{m,obs}$ are significantly higher than those calculated using the simplified reaction pathway. Thus, the formation of **2a** is mechanistically analogous to that proposed earlier for the corresponding cis isomer.[5]

Deprotonation of the aqua ligand in *trans*-$[Pt(NH_3)_2(InoH\text{-}N7)(H_2O)]^{2+}$ followed by proton transfer formally from N1H to the OH group bound to Pt(II) represents, in fact, deprotonation of N1H in this compound. The acidity constant for N1H can be obtained as $K_4K_5 = 10^{-7.10}$ M from the properties of a cyclic system. Thus, in *trans*-$[Pt(NH_3)_2(InoH\text{-}N7)(H_2O)]^{2+}$ the inosine N1H proton is acidified by 1.7 log units, which agrees with findings reported earlier for similar systems.[20,28] In *trans*-$[Pt(OH)(NH_3)_2(InoH\text{-}N7)]^+$ the influence of the Pt(II) unit on the N1H acidity is about 1.4 log units, which is in line with the charge effects on the Pt(II) moieties. For comparison, in *trans*-

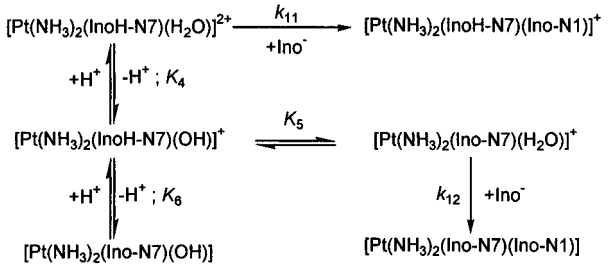
(28) For example, see: (a) Raudaschl-Sieber, G.; Schöllhorn, H.; Thewalt, U.; Lippert, B. *J. Am. Chem. Soc.* **1985**, *107*, 3591−3595. (b) Miller, S. K.; Marzilli, L. G. *Inorg. Chem.* **1985**, *24*, 2421−2425. (c) van der Veer, J. L.; van den Elst, H.; Reedijk, J. *Inorg. Chem.* **1987**, *26*, 1536−1540.

Table 3. Rate and Equilibrium Constants for Protolysis and Complexation of Aquated *cis*- and *trans*-$Pt^{II}(NH_3)_2$ with the Model Nucleobases Inosine and 1-Methylinosine at 298.2 K[a]

reaction	cis[b]	trans
pK_a		
$[M(H_2O)_2]^{2+} \leftrightarrow [M(OH)(H_2O)]^+$	5.64	4.48
$[M(OH)(H_2O)]^+ \leftrightarrow [M(OH)_2]$	7.40	7.20
$[M(L\text{-}N7)(H_2O)]^{2+} \leftrightarrow [M(OH)(L\text{-}N7)]^+$ [c]	5.79	5.27
$[M(LH\text{-}N7)(H_2O)]^{2+} \leftrightarrow [M(OH)(LH\text{-}N7)]^+$ [d]	5.78	5.4
$[M(OH)(LH\text{-}N7)]^+ \leftrightarrow [M(OH)(L\text{-}N7)]$[d]	7.57	7.4
$[M(OH)(LH\text{-}N7)]^+ \leftrightarrow [M(L\text{-}N7)(H_2O)]^+$ [d]	1.2[e]	1.7[e]
$[M(L\text{-}N1)(H_2O)]^+ \leftrightarrow [M(OH)(L\text{-}N1)]$[d]		6.4
Rate Constants $k_i/10^{-3}$ M^{-1} s^{-1} for 1:1 Complexes		
$[M(H_2O)_2]^{2+} \rightarrow [M(L\text{-}N7)(H_2O)]^{2+}$ [c]	114	10.5
$[M(OH)(H_2O)]^+ \rightarrow [M(OH)(L\text{-}N7)]^+$ [c]	8.3	1.3
$[M(H_2O)_2]^{2+} \rightarrow [M(LH\text{-}N7)(H_2O)]^{2+}$ [d]	120	11.4
$[M(OH)(H_2O)]^+ \rightarrow [M(OH)(LH\text{-}N7)]^+$ [d]	10−13	1.6
$[M(OH)(H_2O)]^+ \rightarrow [M(OH)(L\text{-}N7)]$[d]	63	5.8
$[M(OH)(H_2O)]^+ \rightarrow [M(OH)(L\text{-}N1)]$[d]	40	4.4
Rate Constants $k_j/10^{-3}$ M^{-1} s^{-1} for 1:2 Complexes		
$[M(L\text{-}N7)(H_2O)]^{2+} \rightarrow [M(L\text{-}N7)_2]^{2+}$ [c]	81	81
$[M(LH\text{-}N7)(H_2O)]^{2+} \rightarrow [M(LH\text{-}N7)_2]^{2+}$ [d]	80	82
$[M(L\text{-}N7)(H_2O)]^+ \rightarrow [M(L\text{-}N7)(LH\text{-}N7)]^+$ [d]	30	60
$[M(L\text{-}N7)(H_2O)]^+ \rightarrow [M(L\text{-}N7)_2]$[d]	300	470
$[M(LH\text{-}N7)(H_2O)]^{2+} \rightarrow [M(LH\text{-}N7)(L\text{-}N1)]^+$ [d]	1000	800
$[M(L\text{-}N7)(H_2O)]^+ \rightarrow [M(L\text{-}N7)(L\text{-}N1)]$[d]	200	210
$[M(L\text{-}N1)(H_2O)]^+ \rightarrow [M(L\text{-}N1)(LH\text{-}N7)]$[d]		45
$[M(L\text{-}N1)(H_2O)]^+ \rightarrow [M(L\text{-}N1)(L\text{-}N7)]$[d]		120
$[M(L\text{-}N1)(H_2O)]^+ \rightarrow [M(L\text{-}N1)_2]$[d]		100

[a] $I = 0.1$ M. In the subsequent reactions M denotes the $Pt^{II}(NH_3)_2$ entity. [b] Data from ref 5. [c] L = 1-methylinosine. [d] L = inosine. [e] -log *K*.

Scheme 6

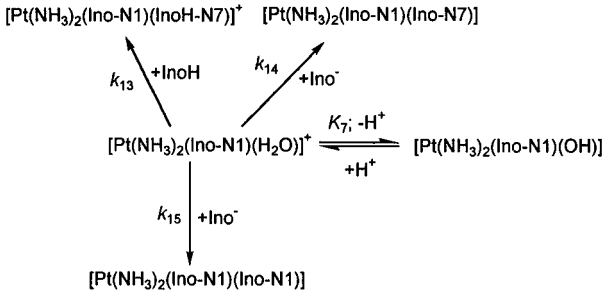


$[PtCl(NH_3)_2(InoH\text{-}N7)]^+$ the acidity difference is about 1.3 log units when compared to the free nucleoside,[16] while a difference of 0.67 log units has been given for the acidity constants of the N1H sites of the two 9-ethylguanines (egua) in *cis*-$[Pt(NH_3)_2(egua)_2]^{2+}$.[29]

The employment of the values found for the equilibrium constants gave $k_7 = 0.082 \pm 0.004$ M^{-1} s^{-1}, $k_9 = 0.06 \pm 0.01$ M^{-1} s^{-1}, and $k_{10} = 0.47 \pm 0.03$ M^{-1} s^{-1} by least-squares fitting to eq 8. No reliable value could be observed for k_8, which is in line with the pK_a difference of about 3 log units of these species. Comparison with the corresponding reactions in cis geometry reveals no significant differences (Table 3). In both cases the rate parameters for anionic inosine are higher than those of the neutral form.

Figure 2 depicts the distribution of different end products in the reaction of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with excess of inosine in the pH range 5−9. The percentage of each species was obtained from HPLC data assuming approximately equal molar extinction coefficients for **2a** and **2b** at 260 nm, consistent with findings from reactions of isolated **1a** with inosine. Below pH 6 the predominant end product is **2a**, whereas at pH 9.5 the amounts of **2a** and **2b** are almost equal. Even at high pH, **2c** is only a minor product contributing less than 15% from the total amount of 1:2 complexes. As shown in Scheme 2, **2b** is formed parallel from **1a** and **1b**. The detailed reaction pathway via **1a** is depicted in Scheme 6. Thus, the rate constant $k_{a,obs}{}^{N1}$ may be expressed by eq 9, which gave $k_{11} = 0.80 \pm 0.03$ M^{-1} s^{-1} and $k_{12} = 0.21 \pm 0.02$ M^{-1} s^{-1} by least-squares fit.

Scheme 7



$$k_{a,obs}{}^{N1} = \frac{(k_{11}[H^+]^2 + k_{12}K_4K_5[H^+])K_L}{([H^+]^2 + [H^+]K_4 + K_4K_6 + K_4K_5[H^+])(K_L + [H^+])} \quad (9)$$

The proposed reactions for the formation of **2b** and **2c** via **1b** are shown in Scheme 7. In the pH range 5−9 protonation of the nonplatinated N7 site of **1b** may be neclected, since its pK_a is expected to be near 2.5.[20] The rate constants $k_{b,obs}{}^{N1}$ for **2c** and $k_{b,obs}{}^{N7}$ for **2b** were obtained indirectly from their sum constant, $k_{b,obs}$. The latter was computed from the time-dependent formation and disappearance of **1b** by eq 2 using the $k_{f,obs}{}^{N1}$ given above.[30] According to Scheme 2, rate equation of parallel consecutive two-step reaction, in which both of the second steps consist of two parallel reactions may be applied to the overall formation of **2b** (eq 10). In this system other

$$[\mathbf{2b}] = [A]_0\left(\frac{k_{f,obs}^{N7}k_{a,obs}^{N1}}{k_{f,obs}(k_{a,obs}-k_{f,obs})}(1-e^{-k_{f,obs}t}) - \frac{k_{f,obs}^{N7}k_{a,obs}^{N1}}{k_{a,obs}(k_{a,obs}-k_{f,obs})}(1-e^{-k_{a,obs}t})\right) + [A]_0\left(\frac{k_{f,obs}^{N1}k_{b,obs}^{N7}}{k_{f,obs}(k_{b,obs}-k_{f,obs})}(1-e^{-k_{f,obs}t}) - \frac{k_{f,obs}^{N1}k_{b,obs}^{N7}}{k_{b,obs}(k_{b,obs}-k_{f,obs})}(1-e^{-k_{b,obs}t})\right) \quad (10)$$

rate constants (seven) are known which leaves $k_{b,obs}{}^{N7}$ as the single parameter to be fitted in each run. The corresponding rate constant $k_{b,obs}{}^{N1}$ was obtained by subtracting $k_{b,obs}{}^{N7}$ from $k_{b,obs}$ (Table 2). Due to the minor contribution of the N1 binding in the first complexation step only data above pH 7.1 were employed to calculate the rate constants for **2b** and **2c**. In all cases the error in $k_{b,obs}{}^{N7}$ values found by least-squares fit was less than 5%, and the concentrations of **2b** obtained by HPLC were in good agreement with fitted curve (Figure 4).

As shown in Scheme 7, the formation of **2c** and **2b** via **1b** may expressed by eqs 11 and 12, respectively. Due to the limited amount of data the acidity constant K_7 was computed from the formation of **2c**. The least-squares fit to the data listed in Table 2 for $k_{b,obs}{}^{N1}$ and $k_{b,obs}{}^{N7}$ gave $k_{15} = 0.10 \pm 0.02$ M^{-1} s^{-1} and $K_7 = 10^{-(6.4\pm0.1)}$ M by eq 11, and $k_{13} = 0.045 \pm 0.001$

(29) Schröder, G.; Lippert, B.; Sabat, M.; Lock, C. J. L.; Faggiani, R.; Song, B.; Sigel, H. *J. Chem. Soc., Dalton Trans.* **1995**, 3767−3775.

(30) In eq 2 $k_{b,obs}$ stands for $k_{d,obs}$. Peak areas of **1b**, rather than concentrations, were used in computations.

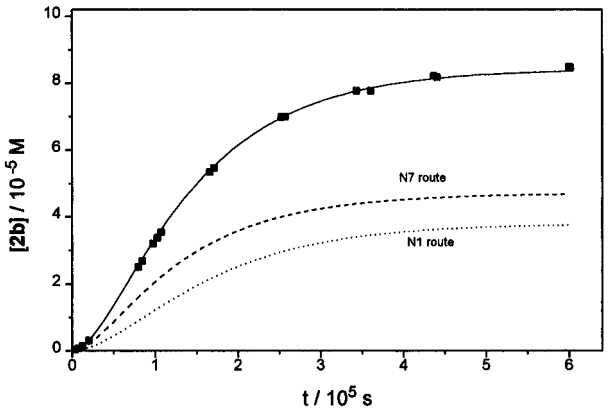


Figure 4. Time-dependent concentration of **2b** in the reaction of *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (0.2 mM) with inosine (0.06 M) at pH 8.82 (298.2 K). Solid line computed by eq 10. The dashed line refers to the formation of **2b** via **1a** (N7), and the dotted line, via **1b** (N1).

$$k_{b,obs}^{N1} = \frac{k_{15}K_L[H^+]}{([H^+] + K_7)([H^+] + K_L)} \quad (11)$$

$$k_{b,obs}^{N7} = \frac{[H^+](k_{13}[H^+] + k_{14}K_L)}{([H^+] + K_7)([H^+] + K_L)} \quad (12)$$

$M^{-1}\ s^{-1}$ and $k_{14} = 0.12 \pm 0.01\ M^{-1}\ s^{-1}$ by eq 12, respectively. Thus, the affinity of **1b** to bind anionic inosine through the N1 and N7 sites seems to be almost equal, as seen in values for k_{15} and k_{14}.

Comparison with the Cis Isomer. Mechanistically the complexation of aquated *cis*- and *trans*-$Pt^{II}(NH_3)_2$ with the model nucleobases inosine and 1-methylinosine are analogous. However, the rate and equilibrium constants for protolysis and complexation steps of various species formed in these reactions (Table 3) show considerable differencies, but also substantial similarities, which cannot be completely explained by the trans effects of the relevant ligands.

Comparison of the kinetic data for isomeric $[Pt(NH_3)_2(H_2O)_2]^{2+}$ reveals that $k_1(\text{cis}) \approx 10k_1(\text{trans})$, in agreement with the trans effect N > O, whereas for $[Pt(OH)(NH_3)_2(H_2O)]^+$ the difference is $k_2(\text{cis}) \approx 6k_2(\text{trans})$. With both isomers $k_2 < k_1$, which can be attributed to the decrease in charge of Pt(II) species due to the deprotonation of one of the H_2O ligands, and to the statistical factor when one labile group (H_2O) changes to an inert one (OH^-). For example, in the complexation of inosine with aquated *cis*-Pt(II) derivatives at 318.2 K a 5-fold decrease in rate constants has been observed when one H_2O ligand is substituted with Cl^-.[3] The stronger diminution of the rate constants for *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ upon deprotonation of one of the aqua ligands as compared to the trans isomer may be rationalized as follows. With *cis*-$[Pt(OH)(NH_3)_2(H_2O)]^+$, strong hydrogen bonding between H_2O and OH^- groups is feasible, which renders both groups more alike, i.e. both are something between H_2O and OH^-. Since OH^- is substitution inert relative to the coordinated water molecule,[5,20] the hydrogen-bonded H_2O group becomes a poorer leaving group in cis compound. For geometric reasons this is not possible with the trans isomer. However, in this case the trans effect $OH^- > H_2O$ makes the remaining aqua ligand more labile.[4] Thus, the difference in k_2 becomes smaller than that in k_1 in these isomers.

The ability of *cis*- and *trans*-$[Pt(NH_3)_2(\text{MeIno-N7})(H_2O)]^+$ ions to bind the second nucleobase is practically equal despite the weak trans effect NH_3 > N(nucleobase), as seen in the aquation rate of isomeric $[PtCl(NH_3)_2(\text{InoH-N7})]^+$, for example.[4] This suggests an extra stabilization of the aqua ligand in cis configuration, probably due to hydrogen bonding to the C(6)O group of the nucleobase,[6a] rather than differences in steric hindrances of these square planar complexes on the incoming ligand. The observation that the pK_a of the aqua ligand of *cis*-$[Pt(NH_3)_2(\text{MeIno-N7})(H_2O)]^+$ is about 0.5 unit higher than that of the trans isomer nicely correlates with H-bond formation in cis geometry. H-bonding of one of the hydrogens of the aqua group to C(6)O increases the electron density of the oxygen atom of the aqua ligand and makes the remaining hydrogen atom less acidic. To some extent, the higher pK_a of the cis compound may also be attributed to the trans effect NH_3 > N(nucleobase).[14] The data in Table 3 reveal that the isomeric inosine N7 complexes behave in a similar manner when inosine bears a proton at N1. By contrast, anionic inosine has a different effect on isomeric complexes, i.e. $k_j(\text{trans}) > k_j(\text{cis})$. Qualitatively this is in line with the reasoning given above because in the cis compound deprotonation of inosine N1H should strengthen the proposed H-bonding between the aqua ligand and C(6)O, which renders the aqua ligand less labile. In the trans isomer, instead, labilization of the aqua is expected due to the anionic ligand in the trans position.

The binding modes of both isomers appear to very similar. With 1-methylinosine and with neutral inosine they form only N7-bound 1:1 and 1:2 complexes. In the case of anionic inosine both isomers seem to slightly prefer the N7 site over the N1 site in 1:1 complexes and also in 1:2 complexes.

Conclusions. The complexation of aquated *trans*-$Pt^{II}(NH_3)_2$ with the model nucleobases employed can be quantitatively explained by replacement of the aqua ligand with the nucleobase, while the hydroxo group bound to Pt(II) is inert to substitution reaction relative to the coordinated water molecule. The reactivity of the Pt(II) dication is 7−8 times higher than that of the monocation despite the moderate trans effect $OH^- > H_2O$. In excess of the nucleobase stepwise formation of 1:1 and 1:2 complexes is observed. 1-Methylinosine forms only N7-bound species, as does also inosine when pH < 5.5. Although deprotonation of inosine N1H offers an additional binding site for Pt(II), the ability of the N7 site to accommodate Pt(II) is increased by the loss of the N1H proton, as well. Consequently, the N7 binding mode predominates in the formation of 1:1 complexes throughout the pH range studied. In the case of 1-methylinosine the second complexation step is mechanistically straightforward. With inosine, instead, proton transfer formally from N1H to deprotonated OH group bound to Pt(II) gives substitution labile aqua ligand, the reactivity of which is comparable to that of the dicationic species. Even at high pH the N7 site is preferred over the N1 site in 1:2 complexes. At pH 9.5, for example, the inosine N1/N7 binding ratio is about 0.6 in 1:1 complexes, whereas the approximate percentage of different 1:2 complexes formed is as follows (Pt(II) binding sites in parentheses): (N7,N7) 44%, (N1,N7) 44%, and (N1,N1) 12%. In *trans*-$[Pt(NH_3)_2(\text{InoH-N7})(H_2O)]^{2+}$ the inosine N1H proton is acidified by 1.7 log units, whereas in *trans*-$[Pt(OH)(NH_3)_2(\text{InoH-N7})]^+$ the influence of Pt(II) on the N1H acidity is 1.4 log units.

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