Effects of (N7)-Coordinated Nickel(II), Copper(II), or Platinum(II) on the Acid–Base Properties of Guanine Derivatives and Other Related Purines[+]

Bin Song,[a] Jing Zhao,[a] Rolf Griesser,[a] Cordula Meiser,[b] Helmut Sigel,*[a] and Bernhard Lippert*[b]

This study is dedicated to Professor Dr. R. Bruce Martin, University of Virginia, Charlottesville (USA), on the occasion of his 70th birthday, with the very best wishes of the authors for all his future endeavors and with deep appreciation for friendship and unselfish advice provided over many years to H.S. and B.L.

Abstract: The effect of Ni^{2+}, Cu^{2+}, and *cis*-a_2Pt^{2+} or *trans*-a_2Pt^{2+} (where a = NH_3 or CH_3NH_2), if coordinated to the N7 site of guanine residues, on the acid–base properties of complexes containing guanine derivatives as ligands is considered. The various acidity constants were determined by potentiometric pH titrations. Over 60 acidity constants are listed; about half of these are new. In many instances micro acidity constants have been derived that allow a quantification of the intrinsic acid–base properties of a certain site, which are otherwise blurred by the pK_a values of overlapping buffer regions. This material allows many comparisons; among these is the observation that the acidifying properties of (N7)-coordinated divalent metal ions on the corresponding (N1)H sites in a guanine derivative decrease in the following series: Cu^{2+} ($\Delta pK_a = 2.2 \pm 0.3$) > Ni^{2+} (1.7 ± 0.15) > Pt^{2+} (1.4 ± 0.1) ~ Pd^{2+} (1.4). The data also indicate that the effects are similar for guanine and hypoxanthine residues, but that they are more pronounced for adenine derivatives because in the latter case a (N7)-bound M^{2+} affects a (N1)H^+ site; hence, a further charge effect is operative here. The available material does not yet allow certain prediction of the more subtle differences occurring between the *cis* and *trans* isomers of Pt^{2+} complexes, but replacement of, for example, NH_3 in the coordination sphere of Pt^{2+} with CH_3NH_2 has an effect. Of course, as one might expect, the effect of (N7)-bound Pt^{2+} in guanine nucleotide complexes is smaller on the more remote phosphate groups than it is on the closer (N1)H sites. By evaluation (by means of micro acidity constants) of data available for hypoxanthine derivatives it is also shown that $(N1)^-$-bound Pt^{2+} has an acidifying effect on the $(N7)H^+$ site comparable to that of (N7)-coordinated Pt^{2+} on the (N1)H site.

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[a] Prof. Dr. H. Sigel, Dr. B. Song, J. Zhao, Dr. R. Griesser
Institut für Anorganische Chemie, Universität Basel
Spitalstr. 51, CH-4056 Basel (Switzerland)
Fax: (+41) 61-267-1017
E-mail: sigel@ubaclu.unibas.ch

[b] Prof. Dr. B. Lippert, Dr. Cordula Meiser
Fachbereich Chemie, Universität Dortmund
Otto-Hahn-Strasse 6, D-44227 Dortmund (Germany)
Fax: (+49) 231-755-3797
E-mail: lippert@pop.uni-dortmund.de

[+] **Abbreviations and definitions:** See also Figure 1 and its legend; a, ammonia or methylamine; CMP^{2-}, cytidine 5′-monophosphate; $dCMP^{2-}$, 2′-deoxycytidine 5′-monophosphate; dien, diethylenetriamine = 3-azapentane-1,5-diamine; edta, 1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; G, guanine derivative (see also Figure 1); GP^{2-}, GMP^{2-} (guanosine 5′-monophosphate) and/or $dGMP^{2-}$ (2′-deoxyguanosine 5′-monophosphate); L, general ligand; M^{2+}, divalent metal ion; 1-MeC, 1-methylcytosine. Species which are given in the text without a charge either do not carry one or represent the species in general (i.e., independent from their protonation degree); which of the two versions applies is always clear from the context. A formula like $(9\text{-EtG} - H)^-$ means that the ligand has lost a proton; in the present case it is to be read as 9-ethylguanine (9-EtG) *minus* H^+. In formulas like [(dien)-Pd(H;Ado)]$^{3+}$ H and Ado are separated by a semicolon to facilitate reading, yet they appear within the same parenthesis to indicate that the proton is located at the ligand.

1. Introduction

Under natural conditions nucleotides and nucleic acids interact with *labile* metal ions[1–3] whereas therapeutic agents such as the anticancer drug cisplatin, *cis*-$[(NH_3)_2PtCl_2]$,[4–6] usually form *inert* metal–nucleobase adducts.[7] There is now much evidence that cisplatin loses the chloro ligands in the cell and then exerts its biological action by preferential binding of the *cis*-$(NH_3)_2Pt^{2+}$ unit to the N7 sites of guanine residues of DNA.[5, 6] In contrast, the *trans* isomer, *trans*-$[(NH_3)_2PtCl_2]$, is found to be inactive.[8, 9]

As far as labile metal ions are concerned, it may be emphasized that for nucleotide systems involved in transphosphorylations there is now much evidence that in simple[10] as well as in enzymatic reactions[3] two (or even more) metal ions are involved. There is further evidence that in reactive intermediates a metal ion–N7 interaction occurs not only in the metal ion-promoted hydrolysis[10, 11] of ATP but also in enzymatic reactions as proposed recently for Zn^{2+};[12] similarly, adenosine N7 nitrogens are important in a Mg^{2+}-dependent ribozyme.[13]

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Considering the above situation it is surprising to find that there are only a few studies[14–23] that deal with the effects exerted by metal ions bound to a certain site of a nucleobase derivative on other nearby sites. There is hardly a study which does this in a systematic way. To our knowledge, no experimental investigation exists that compares the properties of analogous complexes formed with different metal ions. We now report the effects that the (N1)H sites in particular experience upon N7 coordination of Ni^{2+}, Cu^{2+}, or Pt^{2+} to the guanine derivatives shown in Figure 1.[24–26] We also include a

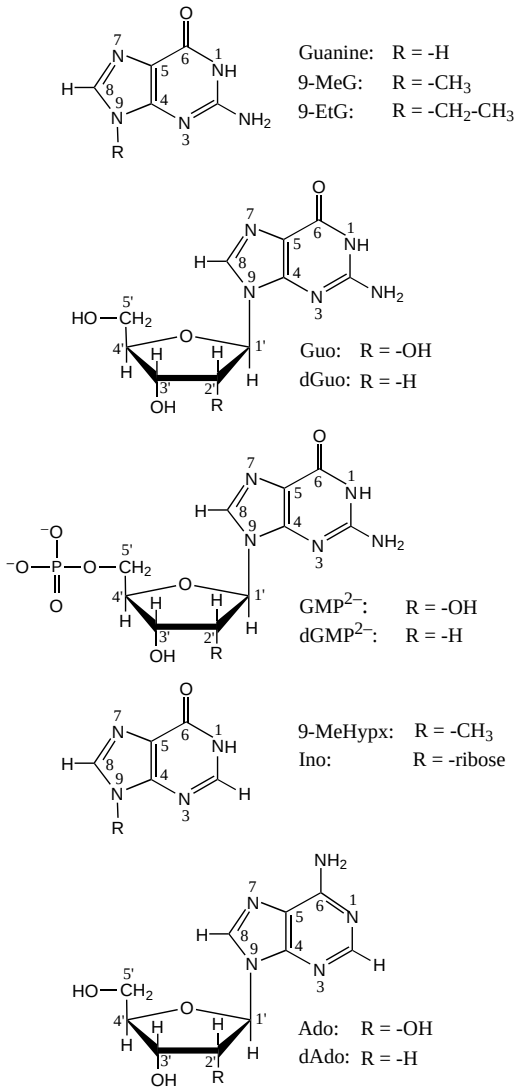

Figure 1. Guanine derivatives considered in this study: 9-MeG, 9-methylguanine; 9-EtG, 9-ethylguanine; Guo, guanosine; dGuo, 2′-deoxyguanosine; GMP^{2-}, guanosine 5′-monophosphate; $dGMP^{2-}$, 2′-deoxyguanosine 5′-monophosphate. The nucleosides and nucleotides are shown in the dominating *anti* conformation which is usually observed for purines.[24b–26] 9-Methylhypoxanthine (9-MeHypx), inosine (Ino), adenosine (Ado), and 2′-deoxyadenosine (dAdo), are shown for comparison (see Sections 2.1, 2.2 and 2.4 as well as Tables 1, 2 and 3).

few data on Pt^{2+} complexes containing GMP or dGMP as well as on some other related purine ligands. These results allow interesting comparisons, including those between *cis* and *trans* isomers of Pt^{2+} units.

2. Results and Discussion

2.1. Comparison of the acid–base properties of some simple guanine derivatives and acidification of the (N1)H site via (N7)-coordinated Ni^{2+} or Cu^{2+}: The acid–base properties of guanine and of various derivatives (G) were determined by potentiometric pH titrations. All these derivatives can accept a proton at N7 and release one from the (N1)H site. Consequently, the two deprotonation reactions (1) and (2), in which G represents the neutral guanine derivative and $(G-H)^-$ the corresponding species deprotonated at N1, need to be considered.

$$H(G)^+ \rightleftharpoons G + H^+ \quad (1a)$$

$$K^H_{H(G)} = [G][H^+]/[H(G)^+] \quad (1b)$$

$$G \rightleftharpoons (G-H)^- + H^+ \quad (2a)$$

$$K^H_G = [(G-H)^-][H^+]/[G] \quad (2b)$$

The neutral guanine derivatives G interact via N7 with divalent metal ions (M^{2+}) to give the $M(G)^{2+}$ complexes, and the (N1)-deprotonated ligands react to yield the $M(G-H)^+$ species. Only these two kinds of complexes form, since the experiments involving metal ions were carried out at a large M^{2+}:G ratio (see Sections 4.4.1–4.4.3). Consequently, the experimental data of the potentiometric pH titrations could be fully explained by taking into account Equations (1) and (2) as well as the following two complex-forming equilibria (3) and (4), as long as the evaluation of the data was not carried into the pH range where hydroxo-complex formation occurs.

$$M^{2+} + G \rightleftharpoons M(G)^{2+} \quad (3a)$$

$$K^M_{M(G)} = [M(G)^{2+}]/([M^{2+}][G]) \quad (3b)$$

$$M^{2+} + (G-H)^- \rightleftharpoons M(G-H)^+ \quad (4a)$$

$$K^M_{M(G-H)} = [M(G-H)^+]/([M^{2+}][(G-H)^-]) \quad (4b)$$

Of course, the complex $M(G)^{2+}$ formed according to Equation (3) may lose a proton from its H(N1) site to give $M(G-H)^+$ according to Equilibrium (5a). The corresponding acidity constant, $K^H_{M(G)}$, may be calculated with Equation (6).[22]

$$M(G)^{2+} \rightleftharpoons M(G-H)^+ + H^+ \quad (5a)$$

$$K^H_{M(G)} = [M(G-H)^+][H^+]/[M(G)^{2+}] \quad (5b)$$

$$pK^H_{M(G)} = pK^H_G + \log K^M_{M(G)} - \log K^M_{M(G-H)} \quad (6)$$

The results determined for the various systems are summarized in Table 1 together with some related values regarding 9-methylhypoxanthine (9-MeHypx) and inosine (Ino) (see Figure 1).[27–31] The acidity constants given for the simple ligands agree well with the data available in the literature.[24, 32] This is also true for entries 8 and 9 of Table 1, in other words the early data given by Fiskin and Beer[31] for $H(Guo)^+$ agree with those determined recently.[30]

Comparison of entries 4 and 5 in Table 1 for the deprotonation of the $(N7)H^+$ site shows that replacement of a hydrogen

Table 1. Negative logarithms of the acidity constants [Eqs. (1), (2)][a] of some monoprotonated guanine derivatives (G) and logarithms of the stability constants of the corresponding $M(G)^{2+}$ and $M(G-H)^{+}$ complexes [Eqs. (3), (4)] as determined by potentiometric pH titrations in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[b] together with some derived, related data [Eqs. (5) – (7)].

	$H(G)^{+}$	$pK^{H}_{H(G)}$ $(N7)H^{+}$ [Eq. (1)]	pK^{H}_{G} (N1)H [Eq. (2)]	M^{2+}	$\log K^{M}_{M(G)}$ [Eq. (3)]	$\log K^{M}_{M(G-H)}$ [Eq. (4)]	$pK^{H}_{M(G)}$ [Eqs. (5),(6)]	$\Delta pK'_a$ [Eq. (7)]
1	H(9-MeHypx)$^{+}$	1.87 ± 0.01	9.21 ± 0.01					
2	H(9-MeG)$^{+}$	3.11 ± 0.06	9.56 ± 0.02	Ni^{2+}	1.81 ± 0.06	3.46 ± 0.07	7.91 ± 0.09	1.65 ± 0.09
3	H(9-MeG)$^{+}$	3.11 ± 0.06	9.56 ± 0.02	Cu^{2+}	2.37 ± 0.09	4.2 ± 0.3	7.7 ± 0.3	1.9 ± 0.3
4	H(guanine)$^{+}$	3.29 ± 0.03	9.36 ± 0.01					
5	H(9-EtG)$^{+}$	3.27 ± 0.03[c]	9.57 ± 0.05[c]	Ni^{2+}	1.76 ± 0.10	3.48 ± 0.13	7.85 ± 0.17[d]	1.72 ± 0.18
6	H(9-EtG)$^{+}$	3.27 ± 0.03[c]	9.57 ± 0.05[c]	Cu^{2+}	2.42 ± 0.09	4.7 ± 0.4	7.3 ± 0.4	2.3 ± 0.4
7[e]	H(Ino)$^{+}$	1.06 ± 0.06	8.76 ± 0.03					
8[e]	H(Guo)$^{+}$	2.11 ± 0.04	9.22 ± 0.01					
9[f]	H(Guo)$^{+}$	2.20 ± 0.15	9.24 ± 0.03	Cu^{2+}	2.15 ± 0.04	4.34 ± 0.55	7.05 ± 0.55	2.2 ± 0.6
10	H(dGuo)$^{+}$	2.30 ± 0.04[g]	9.24 ± 0.03[g]	Cu^{2+}	2.12 ± 0.14	4.7 ± 0.4	6.7 ± 0.4	2.5 ± 0.4
11	H(dGuo)$^{+}$	2.30 ± 0.04[g]	9.24 ± 0.03[g]	Ni^{2+}	1.53 ± 0.09	3.20 ± 0.18	7.57 ± 0.20	1.67 ± 0.20

[a] So-called practical (or mixed) acidity constants[27] are listed; see Section 4.3. [b] The error limits are *three times* the standard error of the mean value (3σ) or the sum of the probable systematic errors, whichever is larger. The error limits of the derived data, in the present case for $pK^{H}_{M(G)}$ [Eq. (6)] and $\Delta pK'_a$ [Eq. (7)], were calculated according to the error propagation after Gauss. [c] These results confirm previously published data[28] (see also ref. [29]). [d] This result is in close agreement with a previous one.[29b] [e] From ref. [30]. [f] Taken and partially calculated from the data given in ref. [31]. [g] The previous result[19] is confirmed.

atom at N9 by an ethyl group (see Figure 1) has no effect, whereas the corresponding substitution with a methyl group (entry 2) leads to a slight acidification ($\Delta pK_a = 0.18 \pm 0.07$). As far as the deprotonation of the H(N1) site is concerned, 9-methylguanine and 9-ethylguanine behave identically within the error limits, whereas the same site in guanine is more acidic by $\Delta pK_a = 0.2 \pm 0.05$. In contrast to the relatively minor effects which alkyl residues exert upon substitution of (C9)H, the corresponding substitution by a ribose or a 2′-deoxyribose residue leads to a significant acidification of a proton at N7, that is, to $\Delta pK_a = 1.18 \pm 0.05$ and 0.99 ± 0.04 for guanosine (entry 8) and 2′-deoxyguanosine (entry 10), respectively, compared with guanine (entry 4).

Of interest is also the replacement of the $(C2)NH_2$ group by a hydrogen atom, that is, the change from a guanine derivative to a hypoxanthine one: both the $(N7)H^{+}$ and the (N1)H sites become more acidic, though the latter site to a lesser extent. A comparison of the results obtained for 9-methylguanine and 9-methylhypoxanthine (entries 2 and 1 of Table 1) reveals that deletion of the amino group at C2 makes the $(N7)H^{+}$ site more acidic by $\Delta pK_a = 1.24 \pm 0.06$, whereas the effect on (N1)H, with $\Delta pK_a = 0.35 \pm 0.02$, is less pronounced; this is somewhat astonishing, because the considered substitution at C2 is just next to N1 (see Figure 1). Very similar values result for the corresponding nucleosides (entries 7 and 8): $\Delta pK_{a/N7} = 1.05 \pm 0.07$ and $\Delta pK_{a/N1} = 0.46 \pm 0.03$.

The acidification of the (N1)H sites of the various guanine derivatives listed in Table 1 caused by Ni^{2+} or Cu^{2+} coordinated to N7 is quite pronounced,[33] as the results in the last column on the right in Table 1 demonstrate. These correspond to the differences defined in Equation (7). Despite the large

$$\Delta pK'_a = pK^{H}_{G} - pK^{H}_{M(G)} \quad (7)$$

error limits, especially for the Cu^{2+} systems (see Section 4.4.1), it is clear that the effect of the two metal ions differs somewhat; the acidification exerted by Ni^{2+} (entries 2, 5 and 11) amounts on average to $\Delta pK'_a = 1.7 \pm 0.15$ and that by Cu^{2+} to $\Delta pK'_a = 2.2 \pm 0.3$, yet for a given metal ion the extent of the acidification does not significantly differ between the various guanine derivatives.

The different effect of the two metal ions is interesting, and, as we shall see in the next section, the acidifying properties of Pt^{2+} are even smaller; they amount tentatively to $\Delta pK'_a \approx 1.45$ if the data for entry 1 and 6 of Table 2 are compared; the other data given in Table 2 cannot be directly compared with the situation described above because in these instances two 9-ethylguanines are coordinated to a Pt^{2+} unit (see Section 2.4 for details). The slightly smaller acidification by Pt^{2+} than by Ni^{2+} agrees with the somewhat larger ionic radius of the former. Values for Zn^{2+} systems could not be obtained because of the formation of hydroxo complexes, however, it appears safe to assume that the acidifying effect of N7-bound Zn^{2+} on the (N1)H site is close to the results given above for Ni^{2+}.

2.2. Effect of (N7)-coordinated isomeric Pt^{2+} complexes on the deprotonation of the (N1)H site of 9-ethylguanine and of some related ligands: In this section we consider especially Pt^{2+} complexes which contain two 9-ethylguanine ligands, coordinated by N7, in their coordination sphere. Evidently such $[a_2Pt(9\text{-}EtG)_2]^{2+}$ species, where "a" represents ammonia or methylamine, can release in total two protons from each of the two (N1)H sites. Consequently, the following two deprotonation equilibria, Equations (8) and (9), need to be

$$Pt(G)_2^{2+} \rightleftharpoons Pt(G)(G-H)^{+} + H^{+} \quad (8a)$$

$$pK^{H}_{Pt(G)_2} = [Pt(G)(G-H)^{+}][H^{+}]/[Pt(G)_2^{2+}] \quad (8b)$$

$$Pt(G)(G-H)^{+} \rightleftharpoons Pt(G-H)_2 + H^{+} \quad (9a)$$

$$pK^{H}_{Pt(G)(G-H)} = [Pt(G-H)_2][H^{+}]/[Pt(G)(G-H)^{+}] \quad (9b)$$

considered; G again represents any uncharged guanine derivative and Pt^{2+} either *cis*- or *trans*-a_2Pt^{2+} units. These acidity constants are listed in Table 2, together with a few related data.[34, 35]

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Table 2. Negative logarithms of the acidity constants[a] for the (N1)H site of free and a_2Pt^{2+}-(N7)-coordinated 9-ethylguanine [Eqs. (2), (8), and (9)] as determined by potentiometric pH titrations in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$) together with the corresponding constants for some related systems (entries 12–18; No. 17 and 18 refer to 34 °C and $I = 0.5$ M, KNO_3). The extent of the acidification of the (N1)H site by the (N7)-coordinated a_2Pt^{2+} is expressed by $\Delta pK'_a$ [Eq. (7)] or ΔpK^*_a [Eq. (10)].

	Acid	$pK^H_{Pt-OH_2}$ $Pt-OH_2$[b]	pK_a (N1)H [Eqs. (2), (5), (8)]	$pK^H_{Pt(G)(G-H)}$ (N1)H [Eq.(9)]	$\Delta pK'_a$ or ΔpK^*_a [Eqs.(7), (10)]
1[c]	9-EtG		9.57 ± 0.05		
2	*cis*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$		8.01 ± 0.03[d]	8.66 ± 0.01[d]	1.24 ± 0.06
3[e]	*trans*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$		7.90 ± 0.02	8.54 ± 0.05	1.35 ± 0.07
4	*cis*-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$		7.92 ± 0.02	8.58 ± 0.02	1.32 ± 0.06
5	*trans*-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$		7.99 ± 0.03[f]	8.77 ± 0.05[f]	1.19 ± 0.08
6	*trans*-$[(CH_3NH_2)_2Pt(1\text{-MeC})(9\text{-EtG})]^{2+}$		8.12 ± 0.01[g]		1.45 ± 0.05
7[h]	dGuo		9.24 ± 0.03		
8[i]	*cis*-$[(NH_3)_2Pt(dGuo)(H_2O)]^{2+}$	4.91 ± 0.15	8.28 ± 0.06		0.96 ± 0.07
9[i]	*trans*-$[(NH_3)_2Pt(dGuo)(H_2O)]^{2+}$	5.60 ± 0.17	8.42 ± 0.04		0.82 ± 0.05
10[i]	*cis*-$[(NH_3)_2Pt(dGuo)(Cl)]^{+}$		7.84 ± 0.05		1.40 ± 0.06
11[i]	*trans*-$[(NH_3)_2Pt(dGuo)(Cl)]^{+}$		8.24 ± 0.03		1.00 ± 0.04
12[j]	$H(Ado)^+$		3.61 ± 0.03		
13[i]	*cis*-$[(NH_3)_2Pt(H;dAdo)(H_2O)]^{3+}$	5.28 ± 0.16	1.7 ± 0.1		1.9 ± 0.1
14[i]	*trans*-$[(NH_3)_2Pt(H;dAdo)(H_2O)]^{3+}$	4.80 ± 0.08	1.7 ± 0.3		1.9 ± 0.3
15[i]	*cis*-$[(NH_3)_2Pt(H;dAdo)(Cl)]^{2+}$		1.7 ± 0.1		1.9 ± 0.1
16[i]	*trans*-$[(NH_3)_2Pt(H;dAdo)(Cl)]^{2+}$		1.7 ± 0.2		1.9 ± 0.2
17[k]	$H(Ado)^+$		3.78 ± 0.03		
18[k]	$[(dien)Pd(H;Ado)]^{3+}$		1.92 ± 0.1		1.9 ± 0.1

[a] See footnotes [a] and [b] of Table 1. [b] These values hold for the release of H^+ from a H_2O molecule coordinated to a_2Pt^{2+}. [c] From entry 5 of Table 1. [d] These results are in close agreement with previously published data[28] (see also ref. [29]). [e] Because of solubility problems in the presence of NO_3^- for this system the ionic strength was adjusted with Cl^- (Na^+) ($I = 0.1$ M). [f] These results agree well with previous ones.[29b] [g] This value is also given in ref. [29b]. [h] From entry 10 of Table 1. [i] We are grateful to Dr. J. Kozelka for providing us with Table S1, that is, the Supplementary Material of ref. [17]. The mentioned table lists acidity constants determined in D_2O (24 °C; I close to 0.1 M since [complex] = 0.01–0.04 M) by ^1H NMR shift measurements (δ_{H8}) in dependence on pH*, that is, the pH-meter reading. We corrected the listed values by taking into account that pD = pH-meter reading + 0.40[34] (see also ref. [25]); these results, now valid for D_2O solutions, were transformed to constants valid for H_2O as solvent by applying the equation $pK_{a/H_2O} = (pK_{a/D_2O} - 0.45)/1.015$[35] and these final results are given above in entries 8–11 and 13–16. [j] From ref. [25]. This value is used for the calculations regarding $\Delta pK'_a$ [Eq. (7); column 6], that is, it is assumed that the pK_a values for $H(Ado)^+$ and $H(dAdo)^+$ are very similar. This assumption is supported by a comparison of the results given in entries 8 and 10 of Table 1 for the deprotonation of the (N1)H site in Guo and dGuo, respectively. [k] Based on results listed in Table III of ref. [15a] (34 °C; $I = 0.5$ M, KNO_3). These values were also determined by ^1H NMR shift measurements in D_2O in dependence on pH*; these data were transformed as described in [i] to constants valid for H_2O as solvent, and these final results are given above in entries 17 and 18.

Comparison of entries 2 and 3 of Table 2 shows that (N7)-bound *cis*- and *trans*-$(NH_3)_2Pt^{2+}$ have a slightly different effect on (N1)H in 9-ethylguanine: In the *trans* isomer the acidification of the first (N1)H site is larger by $\Delta pK_a = 0.11 \pm 0.04$, and this also applies to the release of the second proton from the other (N1)H site in $[(NH_3)_2Pt(9\text{-EtG})(9\text{-EtG} - H)]^+$, where $\Delta pK_a = 0.12 \pm 0.05$. Hence, the average acidification of the (N7)-coordinated $(NH_3)_2Pt^{2+}$ as defined by Equation (10) is slightly more pronounced for the *trans* isomer (with $\Delta pK^*_a = 1.35 \pm 0.07$) than for the *cis* complex (with $\Delta pK^*_a = 1.24 \pm 0.06$; Table 2, last column on the right).

$$\Delta pK^*_a = pK^H_G - \tfrac{1}{2}(pK^H_{Pt(G)_2} + pK^H_{Pt(G)(G-H)}) \quad (10)$$

Considering the above results one notes with surprise that replacement of the Pt^{2+}-coordinated ammonia (NH_3) by methylamine (CH_3NH_2; entries 4, 5) reverses the situation. This means that the *cis* isomer is now on average more acidic, with $\Delta pK^*_a = 0.13 \pm 0.10$, than the *trans* compound. Tentatively one may suggest that this is due to the higher basicity of methylamine ($pK^H_{H(CH_3NH_2)} = 10.64 \pm 0.01$; 25 °C; $I = 0.1$ M[36]) compared with that of ammonia ($pK^H_{H_4N} = 9.38 \pm 0.01$; 25 °C; $I = 0.1$ M, $NaNO_3$[37]).

That the electron donating/withdrawing properties of the ligands coordinated to Pt^{2+} affect its acidifying properties is confirmed by the results summarized in entries 7–16 of Table 2. For $[(NH_3)_2Pt(dGuo)(OH)]^+$ (cf. ref. [38]) too the *cis* isomer is slightly more acidic, by $\Delta pK_a = 0.14 \pm 0.08$ (entries 8, 9). This difference becomes quite pronounced if OH^- is replaced by Cl^- to give the $[(NH_3)_2Pt(dGuo)(Cl)]^+$ species, where again the *cis* isomer is more acidic, but this time by $\Delta pK_a = 0.40 \pm 0.07$. This large effect in the latter example may possibly be attributed to the π-electron-accepting properties of Cl^- due to its empty 3d orbitals.

As far as the acidification of the $(N1)H^+$ site of $H(dAdo)^+$ by the different isomers of N7-bound $[(NH_3)_2Pt(H_2O)]^{2+}$ or $[(NH_3)_2Pt(Cl)]^+$ units is concerned, no conclusion regarding a *cis*/*trans* effect can be made based on the available pK_a values (entries 13–16 of Table 2) because these are identical within their error limits (1.7 ± 0.3). However, that the acidification as such in these instances with $\Delta pK'_a = 1.9$ is larger than in all the other examples is certain and also in perfect agreement with the effect observed for Pd^{2+} (entries 17, 18) in $[(dien)Pd(H;Ado\text{-}N1)]^{3+}$. Indeed, this result is understandable because of the more significant charge repulsion due to the positively charged $(N1)H^+$ site present in these examples.

However, to make the indicated *cis/trans* ambiguity even more difficult to understand, one has to note that the isomers of $[(NH_3)_2Pt(dGuo)(H_2O)]^{2+}$ and $[(NH_3)_2Pt(dAdo)(H_2O)]^{2+}$ (cf. ref. [39]) behave differently with regard to the acidification of the Pt^{2+}-coordinated H_2O molecule: In the first example (entries 8, 9; column 3) the *cis* isomer is by $\Delta pK_a = 0.69 \pm 0.23$ more acidic than the *trans* isomer, whereas for $[(NH_3)_2Pt(dAdo)(H_2O)]^{2+}$ (entries 13, 14) the situation is reversed; now the *cis* isomer is with $\Delta pK_a = 0.48 \pm 0.18$ *less*

acidic. Hence, at present it appears that more experimental data are needed before the varying extents of acidifications in *cis/trans* isomers of Pt^{2+}-nucleobase complexes can be predicted, yet the considerations given below in Section 2.3 should also be noted.

Despite the difficulties indicated above, one further comparison is possible: In entries 2–6 of Table 2 the N_4 donor set in the coordination sphere of Pt^{2+} is kept constant; two aliphatic and two aromatic nitrogen atoms are bound to Pt^{2+}. The average acidification of the various isomeric *cis*- and *trans*-$Pt(9\text{-EtG})_2^{2+}$ complexes listed in entries 2–5 in Table 2 amounts to $\Delta pK_a^* = 1.28 \pm 0.10$; this value is approximately 0.2 log units smaller than the one observed for the *trans*-$[(CH_3NH_2)_2Pt(1\text{-MeC})(9\text{-EtG})]^{2+}$ complex, where 1-MeC = 1-methylcytosine. The possibly more valid comparison between *trans*-$[(CH_3NH_2)_2Pt(1\text{-MeC})(9\text{-EtG})]^{2+}$ (entry 6) and *trans*-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$ (entry 5) amounts to $\Delta\Delta pK_a = (1.45 \pm 0.05) - (1.19 \pm 0.08) = 0.26 \pm 0.09$. In any case, the acidification of (N7)-coordinated a_2Pt^{2+} is somewhat more pronounced if only a single (N1)H site is available for acidification. This result is understandable, since the second proton is released from a species which now carries an overall charge of only +1.

2.3. Comparison of the properties of guanine nucleobases and water as ligands in *cis* and *trans* Pt^{2+} complexes: The difficulty described in Section 2.2 in rationalizing the effects of Pt^{2+} binding to N7 of guanine bases on the acidity of the (N1)H proton contrasts with that of the diaqua and mixed aquachloro species of *cis*- and *trans*-$(NH_3)_2Pt^{2+}$. The properties of the latter can be qualitatively explained by applying the concept of the *trans* influence.[40] Thus, the pK_a values for the aqua ligands (in the case of the diaqua species they refer to pK_{a1}) of *trans*-$[(NH_3)_2Pt(H_2O)_2]^{2+}$ (4.35),[41] *trans*-$[(NH_3)_2Pt(Cl)(H_2O)]^{+}$ (5.63),[41] *cis*-$[(NH_3)_2Pt(H_2O)_2]^{2+}$ (5.93,[42] 5.37,[43] 5.24[44]), and *cis*-$[(NH_3)_2Pt(Cl)(H_2O)]^{+}$ (6.85,[42] 6.41[43]) follow the expectation for the sequence of the *trans* influence, which is $NH_3 > Cl^- > OH_2$, despite differences in charge. In other words, the acidity of an aqua ligand is, to a first approximation, dependent on its binding strength to Pt^{2+}, which in turn is influenced by the nature of the donor atom *trans* to the aqua ligand. Considering the spread of pK_a values in these systems (2.5 log units) the given interpretation is rather straightforward.

In contrast, the variation in the pK_a values of the mono(dGuo) complexes with a +1 charge (see entries 10 and 11 in Table 2) is much smaller (0.40 log units) and this is even more so for the bis(9-ethylguanine) complexes of a +2 charge ($\leq$0.1 log units; entries 2–5 in Table 2). Clearly, the sequence of the pK_a values for the mono(dGuo) complexes (*cis*-$[(NH_3)_2Pt(dGuo)(Cl)]^{+}$, 7.84; *trans*-$[(NH_3)_2Pt(dGuo)(Cl)]^{+}$, 8.24; *cis*-$[(NH_3)_2Pt(dGuo)(OH)]^{+}$, 8.28; *trans*-$[(NH_3)_2Pt(dGuo)(OH)]^{+}$, 8.42; see entries 8–11) does not follow the *trans* influence; for example, if the *trans* influence were to be the determining factor, the (N1)H site of *trans*-$[(NH_3)_2Pt(dGuo)(OH)]^{+}$, with an O atom *trans* to N7 of dGuo, should be more acidic than *cis*-$[(NH_3)_2Pt(dGuo)(Cl)]^{+}$, with its N7 atom of dGuo *trans* to NH_3, yet it is just the other way around.

We have previously shown that the concept of the *trans* influence is suitable to explain in a qualitative way the magnitude of ^3J coupling values between ^{195}Pt and $^1H8$ in the ^1H NMR spectra of Pt^{2+} complexes of 9-EtG-*N7*.[45] This is because a Karplus-type dependence is not expected to operate in these compounds since the Pt-N7-C8-H8 fragments are coplanar. Moreover, any effect due to varying angles between the Pt^{2+} coordination plane and the nucleobase plane should be minimal, considering that large dihedral angles of 70–90° are observed in virtually all guanine complexes of *cis*- and *trans*-$(NH_3)_2Pt^{2+}$. Consequently, it has been proposed[45] that the value of ^3J reflects a measure of the strength of the bond between Pt^{2+} and N7 of the guanine nucleobase. Thus, for 9-EtG ligands this value is largest for OH_2 *trans* to 9-EtG (32.2 Hz), and smallest for NH_3 *trans* to 9-EtG (22.0–23.9 Hz), with Cl^- *trans* to 9-EtG displaying an intermediate value (28.8 Hz) (solvent: D_2O in all cases). Unfortunately not enough high-resolution X-ray data for Pt^{2+}–N7 bond lengths are available to substantiate this conclusion.

The fact that the pK_a values for the (N1)H acidity and the ^3J coupling values for $^{195}Pt - {}^1H8$ do not correlate[46] suggests that the strength of the Pt^{2+}–N7 bond to guanine is not the (sole) determinant of the acidity of the (N1)H site. In principle, π back-bonding effects from Pt^{2+} to the nucleobase could also play a role; hence the dihedral angle between the guanine plane and the Pt^{2+} coordination plane could likewise be important. However, as indicated above, we do not consider these ($80 \pm 10°$) variations as being crucial. It thus appears that more subtle effects, such as differences in the stabilization of the guaninate anion by solvent (water) molecules, could become important, for example through H bonding, which could involve (probably several) water molecules as well as the other ligands at Pt^{2+}. Theoretical calculations might help to better understand the described phenomena.

2.4. Comparison of the effect of (N7)- or (N1)-coordinated (dien)Pt^{2+} and of related M^{2+} units on the deprotonation of (N1)H or (N7)H^+ sites of hypoxanthine derivatives: Up to now only the effect of (N7)-coordinated Pt^{2+} on the N1 site of purine derivatives was considered. How does (N1)-bound Pt^{2+} affect the properties of the N7 site? To this end we evaluated previously published ^1H NMR shift measurements[16] in D_2O in dependence on pH* (i.e., the pH-meter reading) for Pt^{2+} complexes of 9-methylhypoxanthine (see Figure 1). We enlarged the published figures[16] and read from these the evidently carefully obtained experimental data, transformed pH* to pD[34] and applied our previously described[25] nonlinear least-squares fitting procedure. Part A of Figure 2 proves that the following comparisons are reliable: it shows that the ^1H NMR shifts measured previously[16] in dependence on pD for 9-methylhypoxanthine can be fitted excellently with the acidity constants that we have now measured by potentiometric pH titrations (entry 1 of Table 1) if transformed[35] to pK_a values valid for D_2O as solvent. The other three parts of Figure 2 present the curve fits carried out by us through the data for Pt^{2+} complexes,[16] which led to pK_a values valid for D_2O as solvent. The results obtained were then transformed[34] to results for water (H_2O) as solvent, and these acidity constants are assembled in Table 3 (entries 2, 3

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Table 3. Negative logarithms of the acidity constants[a] for the (N1)H and $(N7)H^+$ sites of free and Pt^{2+}-coordinated 9-methylhypoxanthine or inosine [analogous to Eqs. (1), (2), (8), (9)] as determined by various methods in aqueous solution under somewhat varying conditions.[b–f] The extent of the acidification of the (N1)H site by (N7)-coordinated Pt^{2+} and of the $(N7)H^+$ site by (N1)-coordinated Pt^{2+} is expressed by $\Delta pK'_a$ [analogous to Eq. (7)].

	Acid	pK_a $(N7)H^+$	pK_a (N1)H	$\Delta pK'_a$
1[b]	H(9-MeHypx)$^+$	1.87 ± 0.01	9.21 ± 0.01	
2[c]	(dien)Pt(9-MeHypx-*N7*)$^{2+}$		7.67 ± 0.08	1.54 ± 0.08
3[c]	(dien)Pt(H;9-MeHypx-*N1*)$^{2+}$	3.02 ± 0.25		-1.15 ± 0.25
4[c]	*cis*-$(NH_3)_2$Pt(H;9-MeHypx-*N1*)(9-MeHypx-*N7*)$^{2+}$	2.80 ± 0.09	7.85 ± 0.15	$-0.93 \pm 0.09/1.36 \pm 0.15$
5[d]	H(Ino)$^+$	1.06 ± 0.06	8.76 ± 0.03	
6[e]	(dien)Pt(Ino-*N7*)$^{2+}$		7.24 ± 0.10	1.52 ± 0.10
7[e]	(dien)Pt(H;Ino-*N1*)$^{2+}$	2.30 ± 0.10		-1.24 ± 0.12
8[f]	H(Ino)$^+$		8.88 ± 0.03	
9[f]	(dien)Pd(Ino-*N7*)$^{2+}$		7.44 ± 0.03	1.44 ± 0.04

[a] See footnotes [a] and [b] of Table 1. [b] $I = 0.1$ M, $NaNO_3$; 25 °C. Values from entry 1 in Table 1. With these acidity constants obtained from potentiometric pH titrations it is possible to fit the chemical shift data in dependence on pD as given in Figures 3 and 5 of ref. [16]; the present fit is shown in part A of Figure 2. [c] $I \approx 0.01 - 0.1$ M, ambient temperature. These results were obtained from the data published in Figures 3 and 5 of ref. [16] as described in the text of Section 2.4 and the legend for Figure 2. [d] $I = 0.1$ M, $NaNO_3$; 25 °C. From entry 7 in Table 1. [e] $I = 0.1$ M, $NaClO_4$; 25 °C. From ref. [47]; error limits estimated. [f] $I = 0.5$ M, KNO_3; 34 °C. The values given in ref. [15a] were transformed to water (H_2O) as solvent as described in footnote [i] of Table 2.

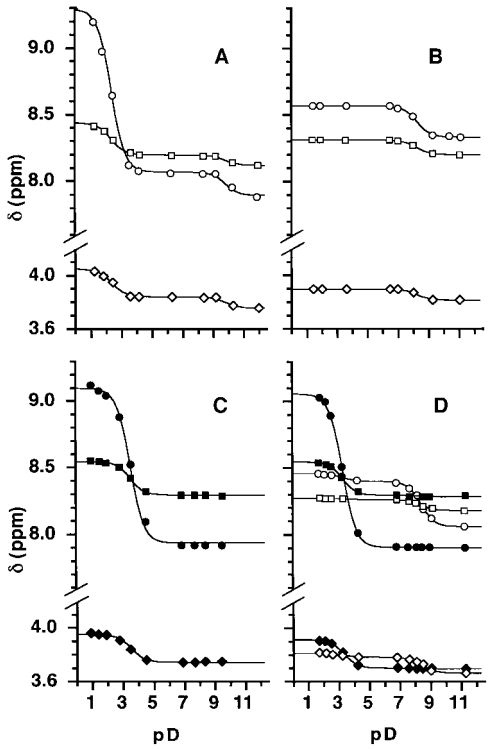


Figure 2. Variation of the chemical shift in dependence on pD for 9-methylhypoxanthine (A) and several of its Pt^{II} complexes (B, C, and D). The solid curves in A show the excellent fit of the experimental data pairs taken from ref. [16] (ppm vs. $pD = pH^* + 0.40$)[34] with $pK^D_{(9\text{-MeHypx})} = 2.35$ and $pK^D_{9\text{-MeHypx}} = 9.80$; these values follow[35] from $pK^H_{(9\text{-MeHypx})} = 1.87$ and $pK^H_{9\text{-MeHypx}} = 9.21$ (Table 1, entry 1) as obtained now from potentiometric pH titrations. For the [(dien)Pt(9-MeHypx-*N7*)]$^{2+}$ (B), [(dien)Pt(9-MeHypx-*N1*)]$^+$ (C), and *cis*-[$(NH_3)_2$Pt(9-MeHypx-*N1*)(9-MeHypx-*N7*)]$^+$ (D) systems and their H2 (□,■), H8 (○,●), and (N9)-CH_3 (◇,◆) protons (open symbols: free and (N7)-coordinated 9-MeHypx; closed symbols: (N1)-coordinated 9-MeHypx) an independent fitting procedure[25] was carried out and in each case the weighted mean of the three resulting values was calculated; with these acidity constants (i.e., $pK_{a/D_2O} = 8.24$ for B, $pK_{a/D_2O} = 3.52$ for C, and $pK_{a/N7/D_2O} = 3.29$ and $pK_{a/N1/D_2O} = 8.42$ for D) the solid curves seen in parts B, C, and D have been computed. These acidity constants (B–D) valid for D_2O as solvent were transformed[35] to H_2O as solvent; these results are given in entries 2, 3, and 4 of Table 3, respectively (see also text in Section 2.4).

and 4), together with some related data for [(dien)-Pt(inosine)]$^{2+}$ complexes, which originate from kinetic experiments[47] (entries 6, 7), as well as an example[15a] for $(dien)Pd^{2+}$ in which the acidity constants were also determined by ^1H NMR shift measurements (entry 9).

Entry 2 of Table 3 shows that $(dien)Pt^{2+}$, if coordinated to N7 of 9-methylhypoxanthine, leads to an acidification of the (N1)H site by $\Delta pK'_a = 1.54 \pm 0.08$; this result is close to that observed for *trans*-[$(CH_3NH_2)_2$Pt(1-MeC)(9-EtG)]$^{2+}$ with $\Delta pK'_a = 1.45 \pm 0.05$ (Table 2, entry 6) and indicates thus that the trends described in this section for hypoxanthine derivatives apply also to guanines. Furthermore, entries 5 and 6 of Table 3 show that the acidification on inosine in [(dien)Pt-(Ino-*N7*)]$^{2+}$ is practically identical ($\Delta pK'_a = 1.52 \pm 0.10$) to that seen in entry 2. The fact that Pd^{2+} behaves very similarly to Pt^{2+} follows from entries 8 and 9 where $\Delta pK'_a = 1.44 \pm 0.04$ is given for the [(dien)Pd(Ino-*N7*)]$^{2+}$ complex; indeed, the acidifications listed in entries 6 and 9 for the two comparable species are identical within their error limits.

A most interesting result is obtained if $(dien)Pt^{2+}$ coordinates through N1 to 9-methylhypoxanthine. From entries 1 and 3 in Table 3, it follows that in [(dien)Pt(9-MeHypx-*N1*)]$^+$ the N7 site is *more* basic than in the free ligand; this is why $\Delta pK'_a$ carries a negative sign, $\Delta pK'_a = -1.15 \pm 0.25$. The same result, within the error limits ($\Delta pK'_a = -1.24 \pm 0.12$), is obtained for [(dien)Pt(H;Ino-*N1*)]$^{2+}$ (entry 7). The most fascinating case in this context is certainly the coordination of the *cis*-$(NH_3)_2Pt^{2+}$ unit to N1 of one 9-MeHypx and to N7 of a second one giving the *cis*-[$(NH_3)_2$Pt(H;9-MeHypx-*N1*)(9-MeHypx-*N7*)]$^{2+}$ complex (entry 4), in which the (N7)-coordinated 9-methylhypoxanthine is acidified at its (N1)H site whereas in the (N1)-coordinated case the N7 site becomes again *more* basic than the free ligand.

Though there is one previous quantitative example[47] as well as a few qualitative ones,[48, 49] this is the first time that the apparent basicity-enhancing effect of (N1)-coordinated Pt^{2+} on the N7 site is described in a quantitative manner in several examples (entries 3, 4, and 7), making the result unequivocal. From the qualitative examples mentioned it becomes in

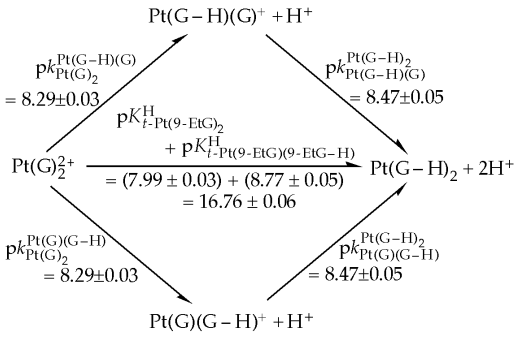
addition clearer that the effect described for hypoxanthine derivatives also operates in guanines[49] and adenines.[48]

What is the reason for the apparent basicity enhancement of the N7 site upon Pt^{2+} coordination to N1 of a purine-type residue as seen in entries 3, 4, and 7 of Table 3? One is tempted to conclude that Pt^{2+} replaces a proton at the (N1)H site and that H^+ is always more polarizing than a metal ion, which is in principle correct. However, substitution at the N1 site also means that the $(N1)Pt^+$ site carries an overall charge of only one whereas that of the $(N7)Pt^{2+}$ site is two (entries 2, 6, 9). Hence, if a valid comparison between the effect of Pt^{2+} at N7 versus that at N1 is attempted, one has to consider equally charged ligands and complexes!

If we use the neutral inosine ligand, Ino, as an example, Pt^{2+} coordination at N7 gives Pt(Ino-*N7*)$^{2+}$ (Table 3, entry 6). To obtain a complex of the same overall charge upon Pt^{2+} coordination at $(N1)^-$ one has to consider the tautomer $H(Ino-H)^{\pm}$; this means a zwitterionic inosine ligand with the proton at N7 and a negative charge at N1, which gives the complex $[Pt(H;Ino-H/\mathit{N1})]^{2+}$. Clearly, the tautomeric $H(Ino-H)^{\pm} \rightleftharpoons$ Ino equilibrium is far over to its right side,[15b] but fortunately the intrinsic or micro acidity constant of $(N7)H^+$ in $H(Ino-H)^{\pm}$ has still been estimated,[50] $pk^{Ino-H}_{H(Ino-H)} = 3.3$. This value describes the acidity of the $(N7)H^+$ proton in the zwitterion $H(Ino-H)^{\pm}$. Comparison of this intrinsic $(N7)H^+$ acidity constant with $pK_a = 2.30$ of the $[(dien)Pt(H;Ino-H)]^{2+}$ complex in Table 3 (entry 7) gives $\Delta pk'_a = 1.0$; in other words, if the intrinsic acidity of the $(N7)H^+$ site as described by the micro acidity constant is considered, the problem described above for the macro acidity constants no longer exists, but the system behaves normally and Pt^{2+} coordination at $(N1)^-$ in $H(Ino-H)^{\pm}$ gives rise to an acidification of the $(N7)H^+$ site in the order observed for the (N7)-coordinated Pt^{2+} complexes (see Table 3). Estimation of the micro acidity constant for H(9-MeHypx$-$H)$^{\pm}$ gives[51] $pk^{(9\text{-MeHypx}-H)}_{H(9\text{-MeHypx}-H)} = 4.1$ and so one obtains for the system of entry 3 $\Delta pk'_a = 4.1 - 3.02 = 1.1$ in perfect agreement with the preceding example.[52]

2.5. Micro acidity constants for the $[a_2Pt(9\text{-}EtG)_2]^{2+}$ complexes: The *cis*- and *trans*-$[a_2Pt(9\text{-}EtG)_2]^{2+}$ complexes (entries 2–5 of Table 2) are evidently *symmetrical* diprotonic acids regarding the release of the proton from the (N1)H sites and this fact also warrants consideration. The statistical expectation for the separation of the acidity constants of two identical acidic sites, which do not affect each other, is $\Delta pK_{a/st} = 0.6$.[53] This follows from the symmetry properties: Beginning with $[a_2Pt(9\text{-}EtG)_2]^{2+}$ there are two equivalent ways for $Pt(G-H)(G)^+$ to form, and also for the protonation of $Pt(G-H)_2$ to give $Pt(G-H)(G)^+$. This means the formation of the monoprotonated species $Pt(G-H)(G)^+$ is twice favored by a factor of 2, which gives a factor of 4 overall, so $\Delta pK_{a/st} = 0.6$.

Comparison of the above statistical value with the differences $pK^H_{Pt(G-H)(G)} - pK^H_{Pt(G)_2}$ of entries 2–5 in Table 2 shows that these differences vary between 0.64 ± 0.05 (entry 3)—which is within its error limits identical with the statistical value—and 0.78 ± 0.06 (entry 5); this indicates that the two acidic sites in these $[a_2Pt(9\text{-}EtG)_2]^{2+}$ complexes behave rather independently. However, from these considerations it is also clear that the buffer regions of the two species, $Pt(G)_2^{2+}$ and $Pt(G-H)(G)^+$, are strongly overlapping [Eqs. (8) and (9)]. Therefore, for a clear quantification of the actual acidity properties of the (N1)H sites in the $[a_2Pt(9\text{-}EtG)_2]^{2+}$ complexes it is necessary to consider the micro acidity constants for the individual sites. Following known routes,[31, 54, 55] in Figure 3 the equilibrium scheme for *trans*-$[(CH_3NH_2)_2$-Pt(9-EtG)$_2]^{2+}$, which is written there as *t*-Pt(9-EtG)$_2^{2+}$ or even simply as $Pt(G)_2^{2+}$, is summarized. The definition of the micro acidity constants (k) and their interrelation with the macro acidity constants (K) is evident from the scheme. There are three independent equations, (11a), (11b), and (11c), but four unknown constants;[54] however, by taking into account the above statistical considerations the present case (Table 2, entry 5) simplifies because $pK^H_{t\text{-}Pt(9\text{-}EtG)_2} + \log 2 = 7.99 + 0.3 = 8.29 = pk^{Pt(G-H)(G)}_{Pt(G)_2} = pk^{Pt(G)(G-H)}_{Pt(G)_2}$; the analogous reasoning provides $pk^{Pt(G-H)_2}_{Pt(G-H)(G)}$, and so on. The corresponding results are given on the arrows in Figure 3.



$$K^H_{t\text{-}Pt(9\text{-}EtG)_2} = k^{Pt(G-H)(G)}_{Pt(G)_2} + k^{Pt(G)(G-H)}_{Pt(G)_2} \quad (11a)$$

$$\frac{1}{K^H_{t\text{-}Pt(9\text{-}EtG)(9\text{-}EtG-H)}} = \frac{1}{k^{Pt(G-H)_2}_{Pt(G-H)(G)}} + \frac{1}{k^{Pt(G-H)_2}_{Pt(G)(G-H)}} \quad (11b)$$

$$K^H_{t\text{-}Pt(9\text{-}EtG)_2} \cdot K^H_{t\text{-}Pt(9\text{-}EtG)(9\text{-}EtG-H)} = k^{Pt(G-H)(G)}_{Pt(G)_2} \cdot k^{Pt(G-H)_2}_{Pt(G-H)(G)} = k^{Pt(G)(G-H)}_{Pt(G)_2} \cdot k^{Pt(G-H)_2}_{Pt(G)(G-H)} \quad (11c)$$

Figure 3. Equilibrium scheme for *trans*-$[(CH_3NH_2)_2Pt(9\text{-}EtG)_2]^{2+}$, which is written here as *t*-Pt(9-EtG)$_2^{2+}$ or $Pt(G)_2^{2+}$, defining the micro acidity constants (k) and showing their interrelation with the macro acidity constants (K). The arrows indicate the directions for which the acidity constants are defined. Equations (11a), (11b), and (11c) show how the various constants are interlinked with each other.[54] See also the text in Section 2.4 and the results summarized in Table 4, which also include other related systems.

With a scheme analogous to the one in Figure 3 the micro acidity constants for the (N1)H sites of the other $[a_2Pt(9\text{-}EtG)_2]^{2+}$ complexes given in entries 2–4 of Table 2 may also be evaluated. The corresponding results are summarized in Table 4, where the values for *trans*-$[(CH_3NH_2)_2Pt(EtG)_2]^{2+}$ as taken from Figure 3 are repeated too. Use of and comparisons with these latter values should facilitate the site attributions regarding the other complexes.

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Table 4. Negative logarithms of the micro acidity constants for $a_2Pt(9\text{-EtG})_2^{2+}$ complexes (defined in analogy to Figure 3) and extent of the acidification (Δpk; see text in Section 2.5), by (N7)-coordinated a_2Pt^{2+} on the corresponding (N1)H sites. The microconstants given in Figure 3 for *trans*-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$ are also listed below in entry 5 for reasons of comparisons (aqueous solutions at 25 °C; $I = 0.1$ M, $NaNO_3$)

[a]	$a_2Pt(9\text{-EtG})_2^{2+}$	$pk^{Pt(G-H)(G)}_{Pt(G)_2} = pk^{Pt(G)(G-H)}_{Pt(G)_2}$	$pk^{Pt(G-H)_2}_{Pt(G-H)(G)} = pk^{Pt(G-H)_2}_{Pt(G)(G-H)}$	$\Delta pk_{a/1}$[b]	$\Delta pk_{a/2}$[b]	$\Delta pk_{a/av}$[c]
1	9-EtG[b]					
2	*cis*-$[(NH_3)_2Pt(G)_2]^{2+}$	8.31 ± 0.03	8.36 ± 0.01	1.26 ± 0.06	1.21 ± 0.05	1.24 ± 0.06
3	*trans*-$[(NH_3)_2Pt(G)_2]^{2+}$	8.20 ± 0.02	8.24 ± 0.05	1.37 ± 0.05	1.33 ± 0.07	1.35 ± 0.07
4	*cis*-$[(CH_3NH_2)_2Pt(G)_2]^{2+}$	8.22 ± 0.02	8.28 ± 0.02	1.35 ± 0.05	1.29 ± 0.05	1.32 ± 0.06
5	*trans*-$[(CH_3NH_2)_2Pt(G)_2]^{2+}$	8.29 ± 0.03	8.47 ± 0.05	1.28 ± 0.06	1.10 ± 0.07	1.19 ± 0.08

[a] The entry numbers correspond to those used in Table 2. See also footnote [b] of Table 1. [b] The acidity constant of the free ligand, $pK^H_{9\text{-EtG}} = 9.57 \pm 0.05$, is used for the various comparisons, i. e., $\Delta pk_{a/1} = pK^H_{9\text{-EtG}} - pk^{Pt(G-H)(G)}_{Pt(G)_2}$, and $\Delta pk_{a/2} = pK^H_{9\text{-EtG}} - pk^{Pt(G-H)_2}_{Pt(G-H)(G)}$. [c] $\Delta pk_{a/av} = 1/2(\Delta pk_{a/1} + \Delta pk_{a/2})$; these results correspond to those given in Table 2 for ΔpK^*_a[Eq. (10)].

In columns 3 and 4 of Table 4 the micro acidity constants are given, whereas columns 5 and 6 provide the differences, $\Delta pk_{a/1}$ and $\Delta pk_{a/2}$, between the $pK^H_{9\text{-EtG}}$ value [Eq. (2)] of free 9-ethylguanine and the two micro acidity constants of the $[a_2Pt(9\text{-EtG})_2]^{2+}$ complexes. Hence, these latter values quantify the acidifying effect of a_2Pt^{2+} on the individual (N1)H sites. Finally, the average of the $\Delta pk_{a/1}$ and $\Delta pk_{a/2}$ values results in $\Delta pk_{a/av}$ (final column to the right) and these values are identical, of course, with the values listed under ΔpK^*_a in Table 2.

Of the many comparisons possible in Table 4 we will consider just two: 1) The values of $\Delta pk_{a/1}$ in column 5 are nearly identical within their error limits; in fact, these limits overlap with the average of the four values [$= 1.32\,(\pm 0.08)$ (3σ)]; this indicates that the acidifying effect of a_2Pt^{2+} on the release of the first proton from one of the two (N1)H sites is very similar. Furthermore, this averaged value of $\Delta pK_a = 1.32 \pm 0.08$, and the acidification observed for *trans*-$[(CH_3NH_2)_2Pt(1\text{-MeC})(9\text{-EtG})]^{2+}$, $\Delta pK'_a = 1.45 \pm 0.05$ (Table 2, entry 6), which has only a single (N1)H site, are also rather similar; hence, one may wish to compare this result ($\Delta pK_a \approx 1.4 \pm 0.1$) with those given in Section 2.1 for Ni^{2+} and Cu^{2+} complexes. 2) In accord with the preceding point, we observe that the *cis*/*trans* effects become manifest especially in the deprotonation of the second (N1)H site as is evident from the values given for $\Delta pk_{a/2}$ in column 6 of Table 4 (see also Section 2.2).

2.6. Acid–base properties of GMP or dGMP complexes with (N7)-bound *cis*- or *trans*-$(NH_3)_2Pt^{2+}$ units: If we abbreviate GMP^{2-} and $dGMP^{2-}$ (see Figure 1) as GP^{2-} and focus for the moment on those a_2Pt^{2+} complexes which have two GP^{2-} ligands coordinated by N7 in their coordination sphere, we may concentrate on the properties of the $[a_2Pt(GP)_2]^{2-}$ species. Each of the phosphate residues carries a charge of minus two (cf., e.g., Figure 4) and each may accept two protons; this leads to the twofold positively charged species $[a_2Pt(GP \cdot H_2)_2]^{2+}$.[56] The release of the first proton from a $-P(O)(OH)_2$ group, which is part of a GP that also carries a positive charge at N7, occurs at a very low pH, that is, $pK_a < 1$ for both primary protons.[20] Hence, for physiological conditions only the twofold protonated complex $[a_2Pt(GP \cdot H)_2]$ is of relevance and, hence, the four deprotonation equilibria Equations (12) – (15) need to be considered (for simplicity the amine "a" is omitted).[56]

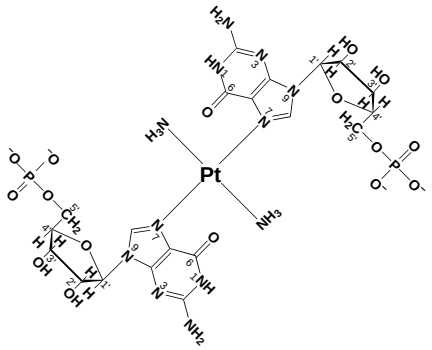

Figure 4. Formal structure of the *trans*-$[(NH_3)_2Pt(GMP)_2]^{2-}$ complex.

$$Pt(GP \cdot H)_2 \rightleftharpoons Pt(GP \cdot H)(GP)^- + H^+ \quad (12a)$$

$$K^H_{Pt(GP \cdot H)_2} = [Pt(GP \cdot H)(GP)^-][H^+]/[Pt(GP \cdot H)_2] \quad (12b)$$

$$Pt(GP \cdot H)(GP)^- \rightleftharpoons Pt(GP)_2^{2-} + H^+ \quad (13a)$$

$$K^H_{Pt(GP \cdot H)(GP)} = [Pt(GP)_2^{2-}][H^+]/[Pt(GP \cdot H)(GP)^-] \quad (13b)$$

$$Pt(GP)_2^{2-} \rightleftharpoons Pt(GP)(GP - H)^{3-} + H^+ \quad (14a)$$

$$K^H_{Pt(GP)_2} = [Pt(GP)(GP - H)^{3-}][H^+]/[Pt(GP)_2^{2-}] \quad (14b)$$

$$Pt(GP)(GP - H)^{3-} \rightleftharpoons Pt(GP - H)_2^{4-} + H^+ \quad (15a)$$

$$K^H_{Pt(GP)(GP-H)} = [Pt(GP - H)_2^{4-}][H^+]/[Pt(GP)(GP - H)^{3-}] \quad (15b)$$

The available results for $[a_2Pt(GP \cdot H)_2]$ complexes, determined by potentiometric pH titrations, are summarized in Table 5, together with some related data. Most of the acidity constants given are taken from our earlier work,[18–20, 53] but because of the values that have now been determined for *trans*-$[(NH_3)_2Pt(GMP \cdot H)_2]$ several new insights are gained.

The first two protons are released from the monoprotonated phosphate residues in $Pt(GP \cdot H)_2$ according to Equilibria (12) and (13), as follows clearly from the comparison of the pK_a values due to $H(dGMP)^-$ (entry 2), $H(GMP)^-$ (entry 5), and $H(dCMP)^-$, as well as the corresponding *cis*-$[(NH_3)_2Pt(dCMP \cdot H)_2]$ complex (entries 7, 8). The third and fourth proton of the $Pt(GP)_2^{2-}$ complexes (entries 3, 6) are released according to Equilibria (14) and (15) from the (N1)H sites as follows from a comparison with the data given in entry 1 of Table 5 for 2′-deoxyguanosine (dGuo).

Table 5 allows many comparisons; a few follow here. Replacement of the (C2′)OH group by a hydrogen atom

Table 5. Negative logarithms of the acidity constants[a] as determined by potentiometric pH titrations in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$) for the deprotonation of monoprotonated phosphate groups, $-P(O)_2(OH)^-$, and for the (N7)H^+ and (N1)H sites of free GPs and their (N7)-coordinated Pt^{2+} complexes (an example is given in Figure 4) [Eqs. (12) – (15)], as well as of some related ligands and complexes. The acidifying properties of the $(NH_3)_2Pt^{2+}$ unit are expressed by ΔpK_a^*, that is, in analogy to Equation (10).[b]

	Acid	pK_a for (N7)H^+	pK_a for $-P(O)_2(OH)^-$	pK_a for (N1)H	ΔpK_a^* for $-P(O)_2(OH)^-$	ΔpK_a^* for (N1)H
1[c]	H(dGuo)$^+$	2.30 ± 0.04		9.24 ± 0.03		
2[d]	H_2(dGMP)$^\pm$	2.69 ± 0.03	6.29 ± 0.01	9.56 ± 0.02		
3[e]	*cis*-[$(NH_3)_2$Pt(dGMP·H)$_2$]		$5.57 \pm 0.03/6.29 \pm 0.02$	$8.73 \pm 0.04/9.48 \pm 0.04$	0.36 ± 0.04	0.46 ± 0.06
4[f]	*cis*-[$(NH_3)_2$Pt(dGuo)(dGMP·H)]$^+$		5.85 ± 0.04	$8.20 \pm 0.03/9.05 \pm 0.10$	0.44 ± 0.04	0.78 ± 0.11
5[g]	H_2(GMP)$^\pm$	2.48 ± 0.04	6.25 ± 0.02	9.49 ± 0.02		
6	*trans*-[$(NH_3)_2$Pt(GMP·H)$_2$]		$5.60 \pm 0.02/6.64 \pm 0.02$	$8.30 \pm 0.03/8.98 \pm 0.02$	0.13 ± 0.03	0.85 ± 0.04
7[h]	H_2(dCMP)$^\pm$	4.46 ± 0.01[i]	6.24 ± 0.01			
8[h]	*cis*-[$(NH_3)_2$Pt(dCMP·H)$_2$]		$5.73 \pm 0.02/6.47 \pm 0.02$		0.14 ± 0.03	

[a] See footnotes [a] and [b] of Table 1. [b] This means, the average of the acidification on both sites, that is, the two $-P(O)_2(OH)^-$ or the two (N1)H sites present in these complexes are considered. [c] From entry 10 of Table 1. [d] From refs. [18, 57]. [e] From refs. [18, 20, 29a]. [f] From ref. [19]. [g] From ref. [30]. [h] From ref. [53]. [i] Deprotonation occurs here from the (N3)H^+ site of the cytosine ring.

(see Figure 1) makes the N7 site more basic as follows from the pK_a values due to H_2(GMP)$^\pm$ (entry 5) and H_2(dGMP)$^\pm$ (entry 2); this is confirmed by the results obtained for Guo and dGuo (see entries 8 and 10 of Table 1). However, in the present context it is important to note that the effect of the replacement of the OH group by a H atom at C2′ is insignificant for the properties of the phosphate group and quite small for those of the (N1)H site. Hence, the acid – base properties of the *cis*-[$(NH_3)_2$Pt(dGMP·H)$_2$] and *trans*-[$(NH_3)_2$Pt(GMP·H)$_2$] complexes can directly be compared (entries 3 and 6). It is interesting to see that the release of the first proton from one of the two $-P(O)_2(OH)^-$ groups occurs within the error limits in both complexes with the same pK_a, whereas the deprotonation of the second monoprotonated phosphate group is considerably retarded in the *trans* isomer. A consequence of this is that in the *cis* complex the acidity between the two $-P(O)_2(OH)^-$ groups differs only by $\Delta pK_a = 0.72 \pm 0.04$, whereas in the *trans* isomer $\Delta pK_a = 1.04 \pm 0.03$.

The above observation made at the phosphate groups contrasts strongly with the situation at the (N1)H sites (entries 3 and 6). Here the ΔpK_a values for the *cis* (0.75 ± 0.06) and *trans* isomer (0.68 ± 0.04) are identical within the error limits. However, the overall acidification is much more pronounced in the *trans*-[$(NH_3)_2$Pt(GMP)$_2$]$^{2-}$ complex, where $\Delta pK_a^* = 0.85 \pm 0.04$ compared with $\Delta pK_a^* = 0.46 \pm 0.06$ for the *cis* complex. Hence, this observation corresponds to that for the *cis*- and *trans*-[$(NH_3)_2$Pt(9-EtG)]$^{2+}$ isomers, where the effect in the *trans* species was also somewhat more pronounced. The strong acidification of the (N1)H sites in *trans*-[$(NH_3)_2$Pt(GMP)$_2$]$^{2-}$ is surprising because it exceeds or at least corresponds to the effect in the neutral *cis*-$(NH_3)_2$Pt(dGuo)(dGMP) species for which the average acidification amounts to 0.78 ± 0.11 (entry 4 in Table 5). Finally, the average acidification of the monoprotonated phosphate groups by the (N7)-bound Pt^{2+} in *trans*-[$(NH_3)_2$Pt(GMP·H)$_2$] is rather small, at $\Delta pK_a^* = 0.13 \pm 0.03$, but this observation will be discussed below in connection with the micro acidity constants.

2.7. Micro acidity constants for nucleotide-containing [$(NH_3)_2$Pt(L)$_2$] complexes: The complexes in entries 3, 6 (Figure 4), and 8 of Table 5 are symmetrical and, if present in their protonated state, diacidic with regard to the $-P(O)_2(OH)^-$ groups and the (N1)H sites. As the separation of the pK_a values for a given kind of site is small, i.e. between 0.68 ± 0.04, a value close to the statistical expectation of 0.6 (see Section 2.5), and 1.04 ± 0.03 for the $-P(O)_2(OH)^-$ groups and the (N1)H sites (see entry 6 of Table 5), the various buffer regions are evidently overlapping. Therefore, only a micro acidity constant analysis will allow a quantification of the intrinsic acid – base properties of a given site. The details of such an analysis (in analogy to Figure 3; see Section 2.5) are shown in Figure 5 for the deprotonation of the monoprotonated phosphate groups in *trans*-[$(NH_3)_2$Pt(GMP·H)$_2$]. These results, as well as those for several other nucleotide complexes formed by $(NH_3)_2Pt^{2+}$, are summarized in Table 6.

The most surprising result from Table 6 is probably the observation that the acidification for the release of the proton from the second $-P(O)_2(OH)^-$ group in *trans*-[$(NH_3)_2$Pt(GMP)(GMP·H)]$^-$ has a *negative* sign, $\Delta pk_{a/2} = -0.09 \pm 0.03$ (see entry 2). In other words, the release of this proton is slightly inhibited compared to the situation in free H(GMP)$^-$; this indicates that the effect of the (N7)-coordinated Pt^{2+} is somewhat overcompensated by the other already deprotonated $-PO_3^{2-}$ group (cf. also Figure 4). In the *cis*-[$(NH_3)_2$Pt(dGMP)(dGMP·H)]$^-$ complex, this is different; here $\Delta pk_{a/2} = 0.30 \pm 0.02$ (entry 3). On the other hand it should be noted that the intrinsic acidity for the release of the first proton from a $-P(O)_2(OH)^-$ group in the three complexes given in entries 2 – 4 of Table 6 (see column 3) is identical within the error limits; pk^1 is between 5.85 ± 0.04 and 5.90 ± 0.02. Hence, there is *no* difference between the *cis* and *trans* isomer and the value is also not affected by the presence of a second $-P(O)_2(OH)^-$ group.

The stronger acidification of the $-P(O)_2(OH)^-$ group ($\Delta pk_{a/1} = 0.42 \pm 0.03$) in *cis*-[$(NH_3)_2$Pt(dGMP·H)$_2$], compared with the one for *cis*-[$(NH_3)_2$Pt(dCMP·H)$_2$] ($\Delta pk_{a/1} = 0.21 \pm 0.02$), has been used[19] to calculate the degree of formation of the outer-sphere macrochelate involving a $Pt(NH_3) \cdots O_3P$ hydrogen bond which amounts to $38 \pm 6\%$ in the *cis*-[$(NH_3)_2$Pt(dGMP)(dGMP·H)] species; similarly, for *cis*-[$(NH_3)_2$Pt(dGMP)$_2$]$^{2-}$ $41 \pm 4\%$ are obtained[19] for each of the two sites in this *cis* complex.[58] Such outer-sphere macrochelate formation has been shown before by NMR

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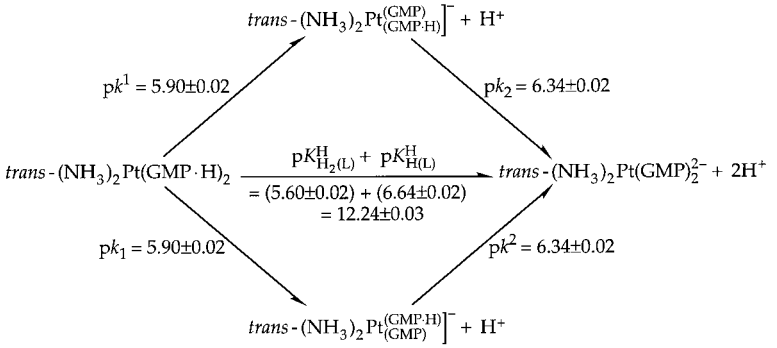

$$K^{H}_{H_2(L)} = k^1 + k_1 \qquad (16a)$$

$$\frac{1}{K^{H}_{H(L)}} = \frac{1}{k_2} + \frac{1}{k^2} \qquad (16b)$$

$$K^{H}_{H_2(L)} \cdot K^{H}_{H(L)} = k^1 \cdot k_2 = k_1 \cdot k^2 \qquad (16c)$$

Figure 5. Equilibrium scheme for *trans*-[$(NH_3)_2Pt(GMP\cdot H)_2$] (which is written thus to indicate that the protons are bound at the phosphate group) defining the micro acidity constants (k) and showing their interrelation with the macro acidity constants (K). The arrows indicate the directions for which the acidity constants are defined. Equations (16a), (16b), and (16c) show how the various constants are interlinked with each other.[54] See also the text in Section 2.7 and the results summarized in Table 6 which include also other related systems.

measurements to occur in aqueous solution[59, 60] as well as in the solid state[59, 61] as indicated by X-ray crystal analysis. The present result, $\Delta pk_{a/1} = 0.35 \pm 0.03$ (entry 2; column 5 of Table 6), suggests that such an outer-sphere macrochelate also forms with the *trans*-[$(NH_3)_2Pt(GMP)(GMP\cdot H)]^-$ species to approximately $28 \pm 6\,\%$.[62] The formation of a second outer-sphere macrochelate in *trans*-[$(NH_3)_2Pt(GMP)_2]^{2-}$ appears from the present results ($\Delta pk_{a/2} = -0.09 \pm 0.03$ as discussed above) as rather unlikely, but for a final answer the acidifying properties of Pt^{2+} in *trans*-[$(NH_3)_2Pt(CMP\cdot H)_2$] would have to be known.

Finally, the first two entries in Table 6 demonstrate that the consideration of $\Delta pk_{a/av}$ ($= \Delta pK^*_a$) alone can lead to misinterpretations. The rather significant acidification as expressed by $\Delta pk_{a/1}$ for *trans*-[$(NH_3)_2Pt(GMP\cdot H)_2$] is overcompensated by $\Delta pk_{a/2}$, and this is the reason for the relatively small value observed for the averaged (overall) acidification, $\Delta pk_{a/av} = 0.13 \pm 0.03$. This example proves the necessity to consider micro constants and thus the intrinsic acid–base properties of a site.

As far as the deprotonation of (N1)H sites is concerned, the *trans*-[$(NH_3)_2Pt(GMP)_2]^{2-}$ complex is evidently more closely related in its properties to *cis*-[$(NH_3)_2Pt(dGuo)$-$(dGMP)$] than to *cis*-[$(NH_3)_2$-$Pt(dGMP)_2]^{2-}$ (entries 5–7). This shows again (see also Section 2.2) that the subtle differences that occur between *cis* and *trans* isomers are difficult to predict at this stage of our knowledge.

3. Conclusions

The results summarized in this study show that the acidifying effect of (N7)-coordinated divalent metal ions on the deprotonation of (N1)H sites in guanine derivatives decreases in the series $Cu^{2+} > Ni^{2+} > Pt^{2+} \approx Pd^{2+}$, the ΔpK_a values being in the order of about 2.2 ± 0.3 (Section 2.1) $> 1.7 \pm 0.15$ (Section 2.1) $> 1.4 \pm 0.1$ (Sections 2.2 and 2.4) ≈ 1.4 (Section 2.4), respectively. This series reflects the decreasing charge density of the divalent metal ions, which depends mainly on the radii and the coordination numbers. In this context it would be interesting to have the analogous information for Zn^{2+} too, because nucleic acid polymerases and many related enzymes depend on the presence of this metal ion.[1–3, 12] Unfortunately, data for Zn^{2+} complexes cannot easily be obtained owing to hydrolysis reactions. They are, however, expected to be similar to those observed for the Ni^{2+} species.

The available material also indicates that the effects of (N7)-coordinated M^{2+} on (N1)H sites are similar for guanine and hypoxanthine residues, whereas for adenine residues the

Table 6. Negative logarithms of the micro acidity constants for nucleotide-containing $(NH_3)_2Pt(L)_2$ species (defined in analogy to Figure 5) and extent of the acidification (Δpk; see text in Section 2.7) by (N7)-coordinated $(NH_3)_2Pt^{2+}$ on the corresponding $-P(O)_2(OH)^-$ groups and (N1)H sites. The microconstants given in Figure 5 for *trans*-[$(NH_3)_2Pt(GMP\cdot H)_2$] are also listed below in entry 2 to facilitate comparisons (aqueous solutions at 25 °C; $I = 0.1$ M, $NaNO_3$).[a]

	$(NH_3)_2Pt(L)_2$	$pk^1 = pk_1$	$pk_2 = pk^2$	$\Delta pk_{a/1}$[b]	$\Delta pk_{a/2}$[b]	$\Delta pk_{a/av}$[c]
1	*cis*-[$(NH_3)_2Pt(dCMP\cdot H)_2$]	6.03 ± 0.02	6.17 ± 0.02	0.21 ± 0.02	0.07 ± 0.02	0.14 ± 0.03
2	*trans*-[$(NH_3)_2Pt(GMP\cdot H)_2$]	5.90 ± 0.02	6.34 ± 0.02	0.35 ± 0.03	-0.09 ± 0.03	0.13 ± 0.03
3	*cis*-[$(NH_3)_2Pt(dGMP\cdot H)_2$]	5.87 ± 0.03	5.99 ± 0.02	0.42 ± 0.03	0.30 ± 0.02	0.36 ± 0.04
4[d]	*cis*-[$(NH_3)_2Pt(dGuo)(dGMP\cdot H)]^+$	5.85 ± 0.04		0.44 ± 0.04		0.44 ± 0.04
5	*cis*-[$(NH_3)_2Pt(dGMP)_2]^{2-}$	9.03 ± 0.04	9.18 ± 0.04	0.53 ± 0.04	0.38 ± 0.04	0.46 ± 0.06
6[e]	*cis*-[$(NH_3)_2Pt(dGuo)(dGMP)$]	8.39/8.65[f]	8.86/8.60[g]	0.85/0.91	0.70/0.64	0.78 ± 0.11
7	*trans*-[$(NH_3)_2Pt(GMP)_2]^{2-}$	8.60 ± 0.03	8.68 ± 0.02	0.89 ± 0.04	0.81 ± 0.03	0.85 ± 0.04

[a] See footnote [b] of Table 1. Entries 1–4 give micro acidity constants for the deprotonation of $-P(O)_2(OH)^-$ groups and entries 5–7 for (N1)H sites. [b] The acidity constants given in Table 5 for the uncoordinated ligands are used in the various comparisons, that is, $\Delta pk_{a/1} = pK_{a/P(O)_2(OH)}$ (or $pK_{a/(N1)H}$) $- pk^1$ and $\Delta pk_{a/2} = pK_{a/P(O)_2(OH)}$ (or $pK_{a/(N1)H}$) $- pk_2$. [c] $\Delta pk_{a/av} = 1/2(\Delta pk_{a/1} + \Delta pk_{a/2})$; these results correspond to those given in columns 6 and 7 of Table 5 under ΔpK^*_a [Eq. (10)]. [d] The complex *cis*-$(NH_3)_2Pt(dGuo)(dGMP\cdot H)^+$ has only a single $-P(O)_2(OH)^-$ group and therefore the measured acidity constant[19] equals the micro acidity constant. [e] This is an unsymmetrical acid and therefore pk^1 and pk_1 are not equal; this holds also for pk_2 and pk^2. [f] The first value refers to the release of the first proton from the (N1)H site of the (N7)-coordinated dGuo in *cis*-$(NH_3)_2Pt(dGuo)(dGMP)$ and the second value to the same reaction of the also (N7)-bound $dGMP^{2-}$; for details see ref. [19]. [g] The first value refers to the release of the second proton from the remaining (N1)H site in *cis*-$(NH_3)_2Pt(dGuo-H)(dGMP)^-$ and the second value correspondingly to the release of H^+ from *cis*-$(NH_3)_2Pt(dGuo)(dGMP-H)^-$; see ref. [19].

acidification appears to be more pronounced; this is understandable because in the latter case a (N7)-bound M^{2+} affects a (N1)H^+ site; hence, a further charge effect is operating.

The much more subtle differences observed between *cis* and *trans* isomers of Pt^{2+} complexes are at this stage difficult to explain. From the available results it is clear that all four ligand atoms bound to Pt^{2+} have an effect on the acidifying properties of this metal ion and as a consequence of this, replacement of NH_3 in *cis*-$(NH_3)_2Pt^{2+}$, for example, by CH_3NH_2 alters the acidifying effect of (N7)-coordinated Pt^{2+} somewhat. Of course, as one might expect, the effect of (N7)-bound Pt^{2+} in guanine nucleotide complexes is smaller on the more remote phosphate groups than it is on the closer (N1)H sites. The effect of (N7)-coordinated metal ions on the hydrogen bonding properties of nucleobases, especially regarding DNA, has been discussed recently[19] in a different context and is not repeated here.

4. Experimental Section

4.1. Synthesis of the platinum(II) complexes: The following compounds were prepared as previously: *cis*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$,[63] *trans*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$,[64] *trans*-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$,[65] and *trans*-$[(CH_3NH_2)_2Pt(1\text{-MeC})(9\text{-EtG})]^{2+}$.[66]

cis-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$ was prepared in analogy to its NH_3 species from *cis*-$[(CH_3NH_2)_2PtCl_2]$,[67] $AgNO_3$, and 9-EtG. The product was passed over Sephadex G10 and the fraction with the desired compound was identified by ^1H NMR (D_2O, pD 6.7, TSP): $\delta = 8.27$ (s, H8); 4.18 (q, CH_2 of 9-EtG); 1.45 (t, CH_3 of 9-EtG) and ^{195}Pt NMR spectroscopy (D_2O, pD 6.7): $\delta = -2512$ (relative to $[PtCl_6]^{2-}$).

trans-$[(NH_3)_2Pt(GMP)_2]\cdot 5H_2O$ was prepared from *trans*-$[(NH_3)_2PtCl_2]$ (75 mg, 0.25 mmol) and Na_2GMP (407 mg, 1 mmol) which were stirred in water (45 mL) with the pH adjusted to 6 (HNO_3) for 12 h at 60 °C and an additional 5 h at 60 °C with pH adjusted to 2.1. The greyish precipitate that formed was filtered and redissolved in 15 mL H_2O at 70 °C, and the solution allowed to evaporate. 102 mg (39 %) of the compound was obtained this way. ^1H NMR (D_2O, pD 4.3): $\delta = 8.78$ (s, H8); 6.04 (d, H1′); 4.54, 4.39, 4.17 (m, sugar); $C_{20}H_{42}N_{12}O_{21}PtP_2$ (1043.8): calcd C 23.0, H 4.1, N 16.1; found C 22.7, H 3.9, N 16.3.

4.2. Materials for the titration experiments: Aside from the complexes described in Section 4.1, the free ligands 9-methylguanine, 9-ethylguanine, and 9-methylhypoxanthine were needed, and these were purchased from Chemogen, Konstanz (Germany). Guanine, 2′-deoxyguanosine, and GMP (disodium salt) were from Sigma Chemical Company, St. Louis (MO, USA). The nitrate salts of Na^+, Ni^{2+}, and Cu^{2+}, potassium hydrogen phthalate, the disodium salt of edta, HNO_3, and NaOH (Titrisol) (all pro analysi) were from Merck AG, Darmstadt (Germany).

The aqueous stock solutions of the mentioned ligands and of the various platinum(II) complexes (Section 4.1) were freshly prepared daily and the exact concentration was newly determined each time (see below); in the case of the complexes, the pH of the stock solutions was adjusted with NaOH to about 7.5 prior to the determination of their concentration. All solutions were prepared with deionized, ultrapure (MILLI-Q185 PLUS, from Millipore S.A., 67120 Molsheim, France), and CO_2-free water. The ligand concentration of solutions used for the potentiometric pH titrations was always below 1 mM, which means that self-association is certainly negligible for these guanine derivatives.[30]

The titer of the NaOH used for the titrations was established with potassium hydrogen phthalate. The exact concentrations of the $M(NO_3)_2$ stock solutions were determined by potentiometric pH titration via their $M(edta)^{2-}$ complexes by measuring the proton equivalents liberated from $H(edta)^{3-}$ upon complex formation.

4.3. Potentiometric pH titrations: The pH titrations were carried out with a Metrohm E536 potentiograph equipped with an E665 dosimat and a 6.0202 100(NB) combined macro glass electrode. The buffer solutions (pH 4.64, 7.00, 9.00, based on the NIST scale; for details see ref. [27]) used for calibration were also from Metrohm AG, Herisau (Switzerland). The direct pH-meter readings were used to calculate the acidity constants; i.e., these constants are so-called practical, mixed, or Brønsted constants.[27] Their negative logarithms given for aqueous solutions at $I = 0.1$ M ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values;[27] this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[27, 68] No conversion term is necessary for the stability constants of the metal ion complexes; these are as usual concentration constants.

The ionic product of water (K_w) and the mentioned conversion term do not enter into the calculations because we evaluate the *differences* in NaOH consumption between solutions with and without ligand[27, 69] (see also below), a procedure which also furnishes the concentration of the ligands directly.

All constants were calculated with an IBM-compatible desk computer with a Pentium processor (connected to an Epson Stylus 1000 printer and a Hewlett–Packard 7475A plotter) by a curve-fit procedure using a Newton–Gauss nonlinear least-squares program.

4.4. Determination of the equilibrium constants

4.4.1. 9-Methylguanine; acidity and complex stability constants: The acidity constants $K^H_{H(9\text{-MeG})}$ and $K^H_{9\text{-MeG}}$ of H(9-MeG)$^+$ and 9-MeG [Eqs. (1) and (2)] were determined under two somewhat different sets of conditions, i.e., by titrating 25 mL of aqueous 6.0 mM HNO_3 ($I = 0.1$ M, $NaNO_3$; 25 °C) in the presence and absence of 0.88 mM 9-MeG under N_2 with 3 mL 0.06 M NaOH or by titrating 25 mL of aqueous 3.0 mM HNO_3 ($I = 0.1$ M, $NaNO_3$; 25 °C) in the presence and absence of 0.43 mM 9-MeG under N_2 with 3 mL 0.03 M NaOH. From the first set of experiments, the constants were calculated by employing the difference in NaOH consumption between the two mentioned titrations within the pH range 2.4–10.6 corresponding to about 16 % neutralization for the equilibrium H(9-MeG)$^+$/9-MeG and about 92 % for 9-MeG/(9-MeG – H)$^-$, and within the pH range 2.7–10.7 corresponding to about 28 % neutralization for the equilibrium H(9-MeG)$^+$/9-MeG, and about 93 % for 9-MeGH/(9-MeG – H)$^-$ in the second set of experiments. The final result is the average of 16 independent pairs of titrations.

The stability constants $K^M_{M(9\text{-MeG})}$ [Eq. (3)] and $K^M_{M(9\text{-MeG–H})}$ [Eq. (4)] for the M(9-MeG)$^{2+}$ and M(9-MeG – H)$^+$ complexes, respectively, for Ni^{2+} and Cu^{2+} were determined under the last mentioned conditions used for the acidity constants, but $NaNO_3$ was partly replaced by $Ni(NO_3)_2$ or $Cu(NO_3)_2$ ($I = 0.1$ M; 25 °C). The ratios of M^{2+}:9-MeG were for Ni^{2+} 18:1 and 36:1, for Cu^{2+} 7:1, 8.5:1, and 11:1. The two stability constants were computed with a curve-fitting procedure by taking into account the species H^+, H(9-MeG)$^+$, 9-MeG, (9-MeG – H)$^-$, M^{2+}, M(9-MeG)$^{2+}$, and M(9-MeG – H)$^+$ (see also ref. [70]). Throughout, the data were collected (every 0.1 pH unit) from the lowest accessible pH to the beginning of the hydrolysis of M(aq)$^{2+}$; the latter was evident from the titrations without 9-MeG. The individual results showed no dependence on the excess of M^{2+} used in the experiments. The final results are the averages of 8 and 6 independent pairs of titrations for the Ni^{2+} and Cu^{2+} complexes, respectively. The determination of the stability of the Cu(9-MeG – H)$^+$ complex was significantly hampered by the beginning of the hydrolysis of Cu(aq)$^{2+}$; therefore, here and also in other instances (Sections 4.4.2 and 4.4.3) the error limit of the corresponding stability constant is rather large.

4.4.2. 9-Ethylguanine; acidity and complex stability constants: The acidity constant $K^H_{9\text{-EtG}}$ [Eq. (2)] of 9-EtG was determined in three different sets of titrations. In the first one 25 mL of aqueous 0.12 mM HNO_3 ($I = 0.1$ M, $NaNO_3$; 25 °C) were titrated in the presence and absence of 0.7 mM 9-EtG under N_2 with 1 mL of 0.03 M NaOH. The two other sets involved titration of 25 mL of aqueous 5.0 mM HNO_3 in the presence and absence of 1.0 mM 9-EtG under N_2 with 3 mL of 0.06 M NaOH or titration of 25 mL of aqueous 3.0 mM HNO_3 in the presence and absence of 0.43 mM 9-EtG under N_2 with 3 mL of 0.03 M NaOH ($I = 0.1$ M, $NaNO_3$; 25 °C). In the two latter sets of titrations the acidity constant $K^H_{H(9\text{-EtG})}$ [Eq. (1)] for H(9-EtG)$^+$ was determined in addition. The experimental data were evaluated within the pH range 7.9–10.4 corresponding to about 2 % to 87 % neutralization for the equilibrium 9-EtG/(9-EtG – H)$^-$ in case of the first titration set. The constants in the two other experimental sets were calculated within the pH range corresponding to about 15 % (pH 2.5) neutralization for the equilibrium H(9-EtG)$^+$/9-EtG and about 94 % (pH 10.8) neutralization for 9-EtG/(9-EtG – H)$^-$ or within the pH range 2.6–10.5 which corre-

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sponds to about 18% neutralization for the equilibrium $H(9\text{-EtG})^+$/9-EtG and about 89% for 9-EtG/$(9\text{-EtG}-H)^-$. The final results are the averages of 12 and 16 independent pairs of titrations for $pK^H_{H(9\text{-EtG})}$ and $pK^H_{9\text{-EtG}}$, respectively.

The stability constants $K^M_{M(9\text{-EtG})}$ and $K^M_{M(9\text{-EtG}-H)}$ for the $M(9\text{-EtG})^{2+}$ and $M(9\text{-EtG}-H)^+$ complexes of Cu^{2+} and Ni^{2+} [Eqs. (3) and (4)] were determined under the conditions of the last-mentioned set of titrations used for the acidity constants but $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ ($I = 0.1$M; 25 °C). The M^{2+} to ligand ratios were 39:1 and 77:1 for Ni^{2+}, and 7.5:1 and 15:1 for Cu^{2+}. The calculations were done analogously to those described in Section 4.4.1. The final results are the averages of four independent pairs of titrations in the case of both metal ions.

4.4.3. 2′-Deoxyguanosine; acidity and complex stability constants: The constants $K^H_{H(dGuo)}$ [Eq. (1)] and K^H_{dGuo} [Eq. (2)] of $H(dGuo)^+$ were determined by titrating 25 mL of aqueous 6.0 mM HNO_3 ($I = 0.1$M, $NaNO_3$; 25 °C) in the presence and absence of 0.93 mM dGuo under N_2 with 3 mL 0.06M NaOH. The pH range 2.5–10.4 used for the calculations by employing the difference in NaOH consumption between the two mentioned titrations, corresponded to about 61% (pH 2.5) neutralization for the equilibrium H·dGuo/dGuo and 94% (pH 10.4) for dGuo/$(dGuo-H)^-$. The final results for the two acidity constants are the averages of 16 independent titration pairs.

The stability constants $K^M_{M(dGuo)}$ and $K^M_{M(dGuo-H)}$ for the $M(dGuo)^{2+}$ and $M(dGuo-H)^+$ complexes [Eqs. (3) and (4)] of Cu^{2+} and Ni^{2+} were determined under the conditions given for the acidity constants but $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ ($I = 0.1$M; 25 °C). The metal to ligand ratios used in the experiments were 36:1 and 18:1 for $Ni^{2+}$and 11:1, 9:1 and 5.6:1 for Cu^{2+}. The two stability constants were computed by taking into account in analogy the species indicated in Section 4.4.1. The final results are the averages of 3 and 7 independent pairs of titrations for the stability constants of the complexes formed with Ni^{2+} and Cu^{2+}, respectively.

4.4.4. Guanine; acidity constants: The acidity constants $K^H_{H(Guanine)}$ and $K^H_{Guanine}$ for guanine [Eqs. (1) and (2)] were determined by titrating 50 mL of aqueous 3.0 mM HNO_3 ($I = 0.1$M, $NaNO_3$; 25 °C) in the presence and absence of 0.4 mM guanine under N_2 with 3 mL 0.06M NaOH. The calculations were processed in the pH range 3.0–10.4, which corresponds approximately to a neutralization degree of 34% for the equilibrium $H(guanine)^+$/guanine and to about 92% for guanine/$(guanine-H)^-$. It should be mentioned that because of solubility problems guanine was dissolved in a basic solution which was then acidified before the titrations. The results for the two acidity constants are the averages of 7 independent pairs of titrations.

4.4.5. 9-Methylhypoxanthine; acidity constants: The acidity constants $K^H_{H(9\text{-MeHypx})}$ and $K^H_{9\text{-MeHypx}}$ [Eqs. (1) and (2)] were determined by titrating 25 mL of aqueous 6.0 mM HNO_3 ($I = 0.1$M, $NaNO_3$; 25 °C) in the presence and absence of 1.0 mM 9-MeHypx under N_2 with 3 mL 0.06M NaOH. The pH range used for the calculations corresponds to a neutralization degree of about 73% (pH 2.3) for the equilibrium $H(9\text{-MeHypx})^+$/9-MeHypx and to one of about 96% (pH 10.6) for 9-MeHypx/$(9\text{-MeHypx}-H)^-$. The final results are the averages of 8 pairs of titrations.

4.4.6. cis-[$(NH_3)_2$Pt(9-EtG)$_2$]$^{2+}$; acidity constants: For *cis*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$ the acidity constants $K^H_{Pt(9\text{-EtG})_2}$ and $K^H_{Pt(9\text{-EtG})(9\text{-EtG}-H)}$ [Eqs. (8) and (9)] were determined by titrating 25 mL of aqueous 0.12 mM HNO_3 ($I = 0.1$M, $NaNO_3$; 25 °C) in the presence and absence of 0.35 mM *cis*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$ or 50 mL of aqueous 0.06 mM HNO_3 ($I = 0.1$M, $NaNO_3$; 25 °C) in the presence and absence of 0.27 mM *cis*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$ under N_2 with 1 mL 0.03M NaOH. The constants were calculated within a pH range corresponding to a neutralization degree of about 4% (pH 6.6) for the equilibrium *cis*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$/*cis*-$[(NH_3)_2Pt(9\text{-EtG})(9\text{-EtG}-H)]^+$ and of about 98% (pH 10.4) for *cis*-$[(NH_3)_2Pt(9\text{-EtG})(9\text{-EtG}-H)]^+$/*cis*-$[(NH_3)_2Pt(9\text{-EtG}-H)_2]$. The final results are the averages of seven independent pairs of titrations.

4.4.7. trans-[$(NH_3)_2$Pt(9-EtG)$_2$]$^{2+}$; acidity constants: Because of the low solubility of the nitrate salt of this complex in aqueous solution, HCl and NaCl had to be used instead of HNO_3 and $NaNO_3$, and also in much lower concentrations than usual. The acidity constants $K^H_{Pt(9\text{-EtG})_2}$ and $K^H_{Pt(9\text{-EtG})(9\text{-EtG}-H)}$ of *trans*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$ [Eqs. (8) and (9)] were determined by titrating 75 mL of aqueous 0.04 mM HCl ($I = 0.1$M, NaCl; 25 °C) in the presence and absence of 0.035 mM *trans*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$ under N_2 with 1 mL 0.01M NaOH. The constants were calculated within the pH range corresponding to about 2% (pH 6.2) neutralization for the equilibrium *trans*-$[(NH_3)_2Pt(9\text{-EtG})_2]^{2+}$/*trans*-$[(NH_3)_2Pt(9\text{-EtG})(9\text{-EtG}-H)]^+$ and about 92% (pH 9.6) neutralization for *trans*-$[(NH_3)_2Pt(9\text{-EtG})(9\text{-EtG}-H)]^+$/*trans*-$[(NH_3)_2Pt(9\text{-EtG}-H)_2]$. The final results are the averages of ten independent pairs of titrations.

4.4.8. cis-[$(CH_3NH_2)_2$Pt(9-EtG)$_2$]$^{2+}$; acidity constants: The corresponding acidity constants as defined by Equations (8) and (9) were determined by titrating 25 mL of aqueous 0.12 mM HNO_3 ($I = 0.1$M, $NaNO_3$; 25 °C) in the presence and absence of 0.40 mM *cis*-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$ or 50 mL 0.06 mM HNO_3 ($I = 0.1$M, $NaNO_3$; 25 °C) in the presence and absence of 0.09 mM *cis*-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$ under N_2 with 1 mL 0.03M NaOH. The pH range used in the calculations corresponded to an approximate neutralization degree of 1% (pH 6.1) for the equilibrium *cis*-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$/*cis*-$[(CH_3NH_2)_2Pt(9\text{-EtG})(9\text{-EtG}-H)]^+$ and to one of about 98% (pH 10.3) for *cis*-$[(CH_3NH_2)_2Pt(9\text{-EtG})(9\text{-EtG}-H)]^+$/*cis*-$[(CH_3NH_2)_2Pt(9\text{-EtG}-H)_2]$. The corresponding results are the averages of seven independent pairs of titrations.

4.4.9. trans-[$(CH_3NH_2)_2$Pt(9-EtG)$_2$]$^{2+}$; acidity constants: The compound *trans*-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$ is not easily dissolved in aqueous solution. Therefore three different stock solutions were prepared: The first one contained 0.0398 g substance in 75 mL water (ca. $6.7 \cdot 10^{-4}$M) and the second one 0.02052 g substance in 82.5 mL water (ca. $3.1 \cdot 10^{-4}$M). For the third stock solution only 0.01129 g substance was dissolved in 82.5 mL water, corresponding to a concentration of about $1.7 \cdot 10^{-4}$M. From the first stock solution 25 mL of a titration solution which was 0.402 mM in the complex and 0.12 mM in HNO_3 were prepared. From the second and third stock solutions 50 mL of titration solutions were made which were 0.248 and 0.136 mM in the complex, respectively, and each was also 0.06 mM in HNO_3; the ionic strength was always adjusted to 0.1M with $NaNO_3$ (25 °C). These solutions as well as the corresponding ones without complex were titrated under N_2 with 1 mL 0.03M NaOH. The pH range used for the calculations corresponded to about 1% (pH 6.0) neutralization for the equilibrium *trans*-$[(CH_3NH_2)_2Pt(9\text{-EtG})_2]^{2+}$/*trans*-$[(CH_3NH_2)_2Pt(9\text{-EtG})(9\text{-EtG}-H)]^+$ and 93% (pH 9.9) for *trans*-$[(CH_3NH_2)_2Pt(9\text{-EtG})(9\text{-EtG}-H)]^+$/*trans*-$[(CH_3NH_2)_2Pt(9\text{-EtG}-H)_2]$. The results for the two constants according to Eqs. (8) and (9) are the averages of nine independent pairs of titrations.

4.4.10. trans-[$(CH_3NH_2)_2$Pt(1-MeC)(9-EtG)]$^{2+}$; acidity constant: The acidity constant according to Equation (8) of this complex was determined by titrating 25 mL of aqueous 0.12 mM HNO_3 ($I = 0.1$M, $NaNO_3$; 25 °C) in the presence and absence of 0.35 mM *trans*-$[(CH_3NH_2)_2Pt(1\text{-MeC})(9\text{-EtG})]^{2+}$ under N_2 with 1 mL 0.03M NaOH. The constant was calculated in the pH range 6.4–9.8, which corresponds to a neutralization degree of about 2% to 98% for the equilibrium *trans*-$[(CH_3NH_2)_2Pt(1\text{-MeC})(9\text{-EtG})]^{2+}$/ *trans*-$[(CH_3NH_2)_2Pt(1\text{-MeC})(9\text{-EtG}-H)]^+$. The result is the average of five titration pairs.

4.4.11. trans-[$(NH_3)_2$Pt(GMP)$_2$]$^{2-}$; acidity constants: The constants $K^H_{Pt(GP\cdot H)_2}$, $K^H_{Pt(GP\cdot H)(GP)}$, $K^H_{Pt(GP)_2}$, and $K^H_{Pt(GP)(GP-H)}$ for *trans*-$[(NH_3)_2Pt(GMP)_2]^{2-}$ as defined in Equations (12)–(15) were determined by titrating 25 mL of aqueous 0.8 mM HNO_3 ($I = 0.1$M, $NaNO_3$; 25 °C) in the presence and absence of 0.2 to 0.3 mM complex under N_2 with 2 mL 0.02M NaOH. The constants were computed by employing the difference in NaOH consumption between the two mentioned titrations in the pH range (pH 3.9) corresponding to about 2% neutralization for the equilibrium $Pt(GP\cdot H)_2$/$Pt(GP\cdot H)(GP)^-$ and about 87% (pH 9.8) or 93% (pH 10.1) for the one of $Pt(GP)(GP-H)^{3-}$/$Pt(GP-H)_2^{4-}$. The final results given for the four acidity constants are the averages from eight independent pairs of titrations.

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[51] The difference $pK^{H}_{H(9\text{-MeHypx})} - pK^{H}_{H(Ino)} = (1.87 \pm 0.01) - (1.06 \pm 0.06) = 0.8$ (see Table 1, entries 1 and 7) represents the effect of the exchange of a methyl group versus a ribose residue at N9 on the basicity of N7. Consequently, $pk^{(9\text{-MeHypx}-H)}_{H(9\text{-MeHypx}-H)} = pk^{(Ino-H)}_{H(Ino-H)} + 0.8 = 3.3 + 0.8 = 4.1$.

[52] We expect that the same reasonings based on micro acidity constants hold also for guanine and adenine derivatives and their Pt^{II} complexes, and therefore we will next attempt to study such examples and to obtain also the corresponding micro acidity constants of the ligands.

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