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The effects of N7-coordinated *cis*-diammine-platinum(II) on the acid–base properties of guanine derivatives

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Abstract: The effect of the *cis*-$(NH_3)_2Pt^{2+}$ unit, if coordinated to the N7 site of guanine residues, on the acid-base properties of complexes of the type *cis*-$(NH_3)_2Pt(9\text{-EtG})_2^{2+}$, *cis*-$(NH_3)_2Pt(dGuo)(dGMP)$, or *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$, where 9-EtG = 9-ethylguanine, dGuo = 2'-deoxyguanosine, and $dGMP^{2-}$ = 2'-deoxyguanosine 5'-monophosphate, is summarized and various micro acidity constants are derived, which allow to quantify the intrinsic acidities of H(N1) and $-P(O)_2(OH)^-$ sites. In total the acid-base properties of more than 20 different species are considered. The deprotonation of $-P(O)_2(OH)^-$ groups in nucleobase-platinated nucleotide complexes is only slightly facilitated ($\Delta\ pK_a \simeq 0.4$). The acidification of H(N1) sites by N7-bound platinum(II) is more pronounced, e.g., it amounts on average to $\Delta\ pK_a \simeq 0.8$ in *cis*-$(NH_3)_2Pt(dGuo)(dGMP)$. This allows conclusions regarding the situation in intrastrand crosslinks formed between *cis*-$(NH_3)_2Pt^{2+}$ and adjacent guanine residues in DNA.

1. WHY STUDY THE PROPERTIES OF *cis*-DIAMMINE-PLATINUM(II)-GUANINE SPECIES?

Ever since Rosenberg's discovery of *Cisplatin*, *cis*-diamminedichloroplatinum(II), being a powerful antitumor agent [1-3],[a] the interest in the basic chemistry of interactions between metal ions or metal-coordination compounds with nucleobases, nucleotides or nucleic acids, and their possible applications in molecular biology and medicine has increased tremendously (for recent reviews see, e.g. [4,5]). Today, areas of active research [6-8] are, e.g., the application of metal ion compounds as artificial nucleases [9], the probing of DNA [10,11] and RNA [11] structures by metal ion complexes, studies on the gene response to metal ions [12], and the development of novel metal-based compounds for cancer chemo-therapy [13-15], including the 'activation of the *trans*-geometry' of platinum(II) complexes [16].

However, so far little is known about the effect that nucleobase-bound inert metal ions, like platinum(II), exert on the acid-base properties of other nearby sites as well as on the binding properties of such sites toward labile metal ions. With this in mind the authors of this account decided to join forces and to concentrate for the beginning on the corresponding properties of the inert *cis*-$(NH_3)_2Pt^{2+}$ unit. There is now much evidence that *Cisplatin*, *cis*-$(NH_3)_2PtCl_2$, loses in the cell the chloro ligands and exerts then its biological action by preferential binding to the N7 sites of guanine residues of DNA [2,17,18]. In fact, intrastrand crosslinks between adjacent guanine residues represent the majority of adducts [19,20]. The *trans* isomer, i.e. *trans*-$(NH_3)_2PtCl_2$, was found to be inactive [21]. As to the reason of this difference,

a) The reference numbers are given in square brackets and those of equilibria or reactions in parentheses.
Abbreviations: See also Fig. 1 and 2; *cis*-$(NH_3)_2Pt^{2+}$ is often abbreviated as "Pt^{2+}", i.e., e.g. *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ is written as $Pt(dGMP)_2^{2-}$; Dien, 1,4,7-triazaheptane (= bis(2-aminoethyl)amine = diethylenetriamine); En, 1,2-diaminoethane (= ethylenediamine); L, general ligand which is able to accept H^+ and/or M^{n+}; M^{n+}, general metal ion; 1-MeC, 1-methyl-cytosine; pK_a, general (macro) acidity constant; *t*-a_2Pt^{2+}, *trans*-$(CH_3NH_2)_2Pt^{2+}$. 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.

several explanations exist which include differences in binding kinetics (closure of monofunctional adducts), differences in repair of adducts, or simply differences in the "geometrical fit" of the two $Pt(NH_3)_2^{2+}$ isomers with DNA [7].

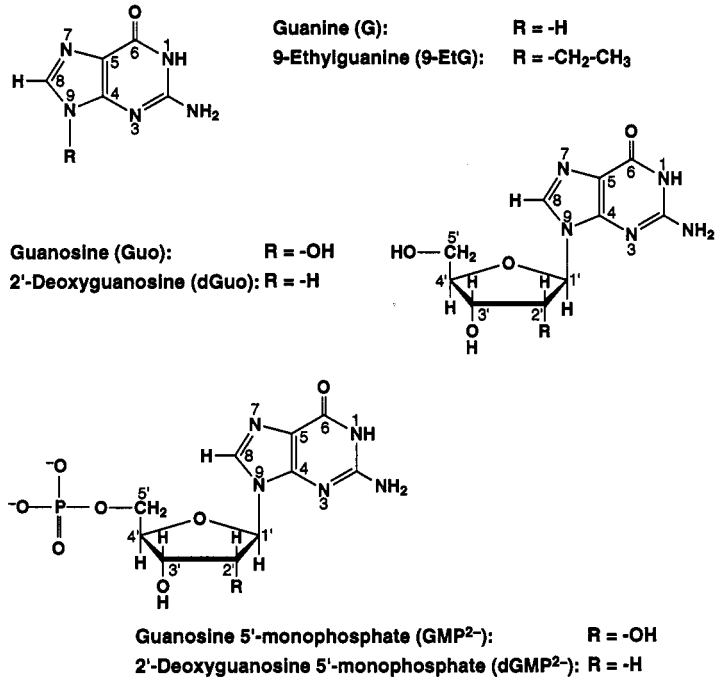


Fig. 1. Guanine derivatives considered in this account. The nucleosides and nucleotides are shown in the dominating *anti* conformation which is usually observed for purines [22-24].

Considering the crucial role of guanine residues in the above mentioned processes, we selected the guanine derivatives shown in Fig. 1 [22-24] as ligands for *cis*-$(NH_3)_2Pt^{2+}$. After the synthesis of various complexes their acid-base properties were studied and compared with those of the free ligands [25-29]. To learn how the still available binding sites, i.e. the phosphate groups, in these higher order complexes, which contain the *cis*-$(NH_3)_2Pt^{2+}$ unit N7-bound to guanine moieties, are affected by the nucleobase-bound Pt(II), we are presently considering also their coordination tendency toward Mg^{2+}, Cu^{2+}, and Zn^{2+} [29-31]. The results available up to now from these studies regarding the acid-base properties are reviewed below. Quite generally, such studies provide insights into the effects which metal ions and protons bound to the same ligand(s) exert on each other.

2. ACID-BASE PROPERTIES OF SEVERAL GUANINE DERIVATIVES

Guanosine (Guo) can accept a proton at N7 and release one from its H(N1) site (see Fig. 1); hence, the following two equilibria together with the expressions defining the acidity constants may be written:

$$H(Guo)^+ \rightleftharpoons Guo + H^+ \qquad K^H_{H(Guo)} = [Guo][H^+]/[H(Guo)^+] \qquad (1a/1b)$$

$$Guo \rightleftharpoons (Guo{-}H)^- + H^+ \qquad K^H_{Guo} = [(Guo{-}H)^-][H^+]/[Guo] \qquad (2a/2b)$$

The acidity constants of the other simple nucleobase derivatives are defined correspondingly. Some results are listed in Table 1 [32-38], where as an abbreviation for the negative logarithm of the acidity constants (see eq. 1,2) the common general term "pK_a" is used; however, the site from which a proton is released is always clearly defined. Deprotonation of the Guo ribose moiety occurs with $pK^H_{(Guo-H)} > 12.0$ [32] and, of course, this is even more true for $(dGuo{-}H)^-$.

The situation for nucleotides is slightly more complicated. For example, GMP^{2-} can accept three protons, i.e., two at its phosphate group and one at the N7 site; this together with the possibility to release a proton from its H(N1) site gives rise to the following four deprotonation reactions:

$$H_3(GMP)^+ \rightleftharpoons H_2GMP^{\pm} + H^+ \qquad (3a)$$

$$K^H_{H_3(GMP)} = [H_2(GMP)^{\pm}][H^+]/[H_3(GMP)^+] \qquad (3b)$$

$$H_2(GMP)^{\pm} \rightleftharpoons H(GMP)^- + H^+ \qquad (4a)$$

$$K^H_{H_2(GMP)} = [H(GMP)^-][H^+]/[H_2(GMP)^{\pm}] \qquad (4b)$$

$$H(GMP)^- \rightleftharpoons GMP^{2-} + H^+ \qquad (5a)$$

$$K^{H}_{H(GMP)} = [GMP^{2-}][H^+]/[H(GMP)^-] \quad (5b)$$

$$GMP^{2-} \rightleftharpoons (GMP\text{–}H)^{3-} + H^+ \quad (6a)$$

$$K^{H}_{dGMP} = [(GMP\text{–}H)^{3-}][H^+]/[GMP^{2-}] \quad (6b)$$

The first proton is released from the phosphoric acid group according to equilibrium 3 at a very low pH, i.e. $pK^{H}_{H_3(GMP)} = 0.3 \pm 0.2$ [32]. The acidity constants for the other three equilibria are listed in Table 2 (*vide infra*) with other related data.

Comparison of the acidity constants for monoprotonated guanine and 9-ethylguanine (see the first two entries in Table 1) shows that replacement of the hydrogen atom at N9 hardly affects the acid-base properties of the H^+(N7) and H(N1) sites. This is in accordance with the similar electronegativities of the H and CH_2CH_3 substituents and it indicates further that the solvation properties of the purine system are hardly altered by this N9 substitution. This is quite different if the hydrogen at N9 is replaced by a ribose residue which gives the above mentioned (eq. 1, 2) nucleoside guanosine (Fig. 1). The entries in the third row of Table 1 demonstrate that now especially the acid-base property of N7 is strongly affected; i.e., the acidity of the H^+(N7) site is increased by about 1.2 pK_a units due to the hydrophilic sugar residue. Comparison with 2'-deoxyguanosine (Table 1, entry 4) reveals that the absence of the 2'-OH group at the ribose moiety enhances the basicity of N7 by $\Delta\ pK_a \simeq 0.2$. The same difference is observed for the pK_a values of $H_2(GMP)^-$ and $H_2(dGMP)^-$ (see entries 3 and 4 in Table 2, *vide infra*). However, the presence of the 5'-phosphate group enhances the basicity of N7 from the nucleoside to the nucleotide on average by $\Delta\ pK_a = 0.38$ (*cf.* entries 3 and 4 of Tables 1 and 2). Its effect on the deprotonation of the H(N1) site is with $\Delta\ pK_a \simeq 0.3$ a bit smaller, but still remarkable. The somewhat higher overall basicity of the 2'-deoxy species, compared with the ribose species, probably results from a somewhat poorer solvation by water of the deoxy species due to the absence of the 2'-OH group. More comparisons between entries 1-4 of Tables 1 and 2 are possible, but are left to the interested reader.

3. ACID-BASE PROPERTIES OF COMPLEXES FORMED WITH PLATINUM(II) OR OTHER DIVALENT METAL IONS AND SUBSTITUTED NUCLEOBASES OR NUCLEOSIDES

N7 coordination of two 9-ethylguanines to *cis*-$(NH_3)_2Pt^{2+}$ leads to the ternary *cis*-$(NH_3)_2Pt(9\text{-EtG})_2^{2+}$ complex, abbreviated as $Pt(9\text{-EtG})_2^{2+}$. As in this complex the N7 sites are occupied by Pt(II), there remain only the N1 sites and their deprotonation is quantified by the following two expressions:

$$Pt(9\text{-EtG})_2^{2+} \rightleftharpoons Pt(9\text{-EtG})(9\text{-EtG–H})^+ + H^+ \quad (7a)$$

$$pK^{H}_{Pt(9\text{-EtG})_2} = [Pt(9\text{-EtG})(9\text{-EtG–H})^+][H^+]/[Pt(9\text{-EtG})_2^{2+}] \quad (7b)$$

$$Pt(9\text{-EtG})(9\text{-EtG–H})^+ \rightleftharpoons Pt(9\text{-EtG–H})_2 + H^+ \quad (8a)$$

$$pK^{H}_{Pt(9\text{-EtG})(9\text{-EtG–H})} = [Pt(9\text{-EtG–H})_2][H^+]/[Pt(9\text{-EtG})(9\text{-EtG–H})^+] \quad (8b)$$

The acidity constants for these two equilibria are given in row 5 of Table 1; the other rows (No. 6-11) contain various related data.

The average acidification of the two H(N1) sites in $Pt(9\text{-EtG})_2^{2+}$ compared with the free ligand may be defined according to equation 9:

$$\Delta\ pK_a = pK^{H}_{9\text{-EtG}} - \tfrac{1}{2}\left(pK^{H}_{Pt(9\text{-EtG})_2} + pK^{H}_{Pt(9\text{-EtG})(9\text{-EtG–H})}\right) \quad (9)$$

$$= (9.57 \pm 0.04) - \tfrac{1}{2}[(8.02 \pm 0.01) + (8.67 \pm 0.01)] = 1.23 \pm 0.04$$

This and corresponding $\Delta\ pK_a$ values are listed in the final column at the right in Table 1. A comparison of entries 5 and 6 of Table 1, under the assumption that coordinated NH_3 or CH_3NH_2 affect Pt^{2+} to about the same extent, indicates in a first approximation that the *cis* and *trans* isomers of $Pt(9\text{-EtG})_2^{2+}$ are acidified by the N7-coordinated Pt(II) about equally. If one of the 9-ethylguanine ligands is replaced by the

TABLE 1. Negative Logrithms of Acidity Constants[a] of Free and M^{n+}-(N7)-Coordinated Guanine Derivatives as Determined by Potentiometric pH Titrations in Aqueous Solution at 25°C and I = 0.1 M ($NaNO_3$) Together with the Extent of Acidification of the H(N1) Site by the N7-Coordinated M^{n+} as Expressed by Δ pK_a (*cf.* eq. 9)

No.[b]	Acid	pK_a for H^+(N7)	pK_a for H(N1)	Δ pK_a
1	$H(Guanine)^+$	3.3[c]	9.4[c]	
2	$H(9\text{-EtG})^+$	3.27 ± 0.04	9.57 ± 0.04	
3	$H(Guo)^+$	2.11 ± 0.04	9.22 ± 0.01	
4	$H(dGuo)^+$	2.30 ± 0.04	9.24 ± 0.03	
5	*cis*-$(NH_3)_2Pt(9\text{-EtG})_2^{2+}$		8.02±0.01/8.67±0.01 (8.34 ± 0.02)	1.23 ± 0.04[d]
6	*t*-$a_2Pt(9\text{-EtG})_2^{2+}$		8.01±0.01/8.81±0.01 (8.41 ± 0.01)	1.16 ± 0.04[d]
7	*t*-$a_2Pt(1\text{-MeC})(9\text{-EtG})^{2+}$		8.12 ± 0.01	1.45 ± 0.04
8	$Ni(9\text{-EtG})^{2+}$		7.8 ± 0.3	1.8 ± 0.3
9	$Cu(Guo)^{2+}$		7.05 ± 0.55[e]	2.2 ± 0.6
10	$(NH_3)_5Ru^{II}(Guo)^{2+}$		8.7 ± 0.2[f]	0.5 ± 0.2
11	$(NH_3)_2Ru^{III}(Guo)^{3+}$		7.36 ± 0.05[f]	1.86 ± 0.05

[a] The error limits are *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The error limits of the derived data (in the above case for Δ pK_a) were calculated according to the error propagation after Gauss. [b] Entries 2 and 5 are from ref. [25]; 3 from ref. [32]; 4 from ref. [29]; 6, 7, and 8 from ref. [27]. [c] These values were measured "near 25°C and 0.1 M ionic strength" (ref. [23]). Similar values are $pK^H_{H(G)}$ = 3.3 (25°C, I undefined, ref. [33]; temperature and I undefined, ref. [34]) and pK^H_G = 9.42 (25°C, I = 0.1 M, ref. [35]). [d] The average of the acidification on both H(N1) sites is considered (see eq. 9). [e] This value is calculated (see ref. [36]) from the data given in ref. [37]; 20°C, I = 1 M ($NaNO_3$). [f] From ref. [38]; 25°C, I = 0.1 M (LiCl).

acid-base inactive 1-methylcytosine to give *t*-$a_2Pt(1\text{-MeC})(9\text{-EtG})^{2+}$ (entry 7), the remaining H(N1) site in 9-EtG is by Δ p$K_a \simeq$ 0.3 more strongly acidified (*cf.* with entry 6). Entries 8 and 9 show that Ni^{2+} and Cu^{2+} have a more pronounced polarizing power than Pt^{2+}: Δ p$K_a \simeq$ 2. On the other hand, the $(NH_3)_5Ru^{2+}$, if bound to N7 of guanosine, acidifies N1 by only Δ p$K_a \simeq$ 0.5 (entry 10). Oxidation of Ru(II) to Ru(III) enhances the polarizing power of the metal ion significantly and leads to Δ p$K_a \simeq$ 1.9 (entry 11).

As the distances between the N7 and N1 sites in the examples given in entries 7-11 of Table 1 are identical, the different extents of acidification, the effects of which are transmitted through the σ and π frameworks, have to be due to the properties of the metal ions [39]. As their charge in examples 7-10 is identical, this is probably, aside from the differences in the ionic radii, a reflection of their differing π back-bonding capabilities. In any case, the acidifying effect, e.g. of Δ pK_a = 1.23, in *cis*-$(NH_3)_2Pt(9\text{-EtG})_2^{2+}$ (Table 1, entry 5), means that the H(N1) site is transformed into an even better H donor suitable for hydrogen bonding than is the case in the uncomplexed guanine residue. Indeed, the excellent properties of N7-platinated 9-EtG to form adducts with nucleobases via hydrogen bonding were already proven [25].

4. ACID-BASE PROPERTIES OF COMPLEXES CONTAINING *cis*-$(NH_3)_2Pt^{2+}$ AND NUCLEOTIDES

Coordination of two $H(dGMP)^-$ to *cis*-$(NH_3)_2Pt^{2+}$ via N7 leads to *cis*-$(NH_3)_2Pt(dGMP{\cdot}H)_2$, which is abbreviated as $Pt(dGMP{\cdot}H)_2$ and where "GMP·H" indicates that the proton is at the phosphate group. This species can lose two protons from the two $-P(O)_2(OH)^-$ groups and another two from the H(N1) sites. In the species *cis*-$(NH_3)_2Pt(dCMP{\cdot}H)_2$ (see Fig. 2), which is used for comparisons, of course, only the protons from the $-P(O)_2(OH)^-$ groups can be ionized, as there remain no acid-base sites in the cytidine moieties which are coordinated via N3 to *cis*-$(NH_3)_2Pt^{2+}$. As an example, the various deprotonation reactions for *cis*-$(NH_3)_2Pt(dGMP{\cdot}H)_2$ are defined below:

$$Pt(dGMP{\cdot}H)_2 \rightleftharpoons Pt(dGMP{\cdot}H)(dGMP)^- + H^+ \quad (10a)$$

$$K^H_{Pt(dGMP{\cdot}H)_2} = [Pt(dGMP{\cdot}H)(dGMP)^-][H^+]/[Pt(dGMP{\cdot}H)_2] \quad (10b)$$

$$Pt(dGMP{\cdot}H)(dGMP)^- \rightleftharpoons Pt(dGMP)_2^{2-} + H^+ \quad (11a)$$

$$K^H_{Pt(dGMP{\cdot}H)(dGMP)} = [Pt(dGMP)_2^{2-}][H^+]/[Pt(dGMP{\cdot}H)(dGMP)^-] \quad (11b)$$

$$Pt(dGMP)_2^{2-} \rightleftharpoons Pt(dGMP)(dGMP{-}H)^{3-} + H^+ \quad (12a)$$

$$K^H_{Pt(dGMP)_2} = [Pt(dGMP)(dGMP{-}H)^{3-}][H^+]/[Pt(dGMP)_2^{2-}] \quad (12b)$$

$$Pt(dGMP)(dGMP{-}H)^{3-} \rightleftharpoons Pt(dGMP{-}H)_2^{4-} + H^+ \quad (13a)$$

$$K^H_{Pt(dGMP)(dGMP-H)} = [Pt(dGMP{-}H)_2^{4-}][H^+]/[Pt(dGMP)(dGMP{-}H)^{3-}] \quad (13b)$$

In principle, each of the two $-P(O)_2(OH)^-$ groups in $Pt(dGMP{\cdot}H)_2$ could accept an additional proton, but the release of the first proton from a $-P(O)(OH)_2$ group, which is part of a GMP that carries a positive charge at N7 (e.g., due to protonation), occurs at very low pH ($pK^H_{H3(GMP)} = 0.3 \pm 0.2$ [32]) and is therefore not considered in the present context. Hence, the protons in equilibria 10 and 11 are released from the $-P(O)_2(OH)^-$ groups and those of equilibria 12 and 13 originate from the H(N1) sites of the two guanine moieties [31]. The various acidity constants of the complexes containing nucleotides (see Fig. 2) are summarized in Table 2 together with the data for the free ligands [40] and an additional related example, $(Dien)Pd(GMP{\cdot}H)^+$ [41].

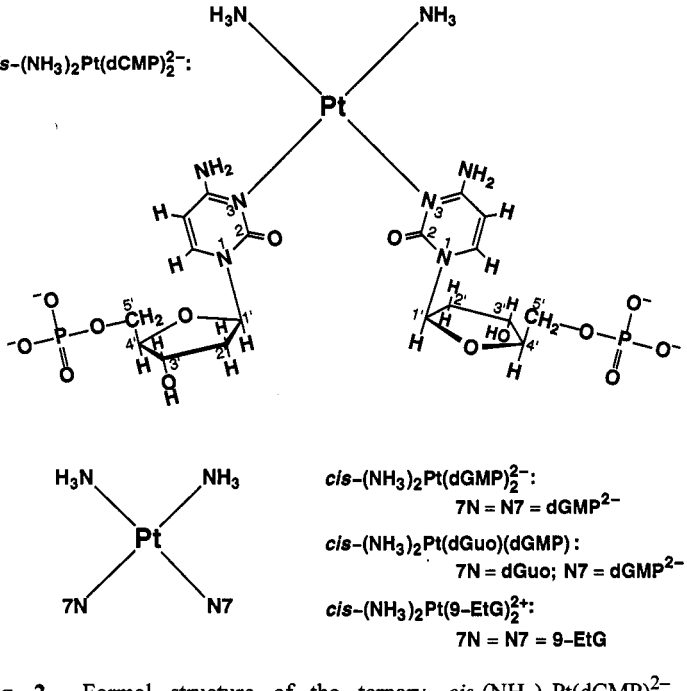


Fig. 2. Formal structure of the ternary *cis*-$(NH_3)_2Pt(dCMP)_2^{2-}$ complex, as well as of analogous, higher order complexes involving N7-coordinated guanine derivatives (see Fig. 1). The $dCMP^{2-}$ ligands are depicted in their *anti* conformation which is usually dominating; for guanosine and its derivatives also the *anti* conformation is favored [22,23].

From the results in Table 2 it is immediately evident that deprotonation of the $-P(O)_2(OH)^-$ residues occurs in all species relatively close to pH 6, whereas the proton from the H(N1) site of the guanine moieties is released in the pH range of about 9. The average acidifying effect of the *cis*-$(NH_3)_2Pt^{2+}$ unit coordinated at N7 on the H(N1) sites in *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ is $\Delta\ pK_{a/av} = 0.46 \pm 0.06$ (Table 2, entry 7). The same effect in *cis*-$(NH_3)_2Pt(dGMP{\cdot}H)_2$ on the two $-P(O)_2OH)^-$ groups is of the same order, i.e. $\Delta\ pK_{a/av} = 0.36 \pm 0.04$. This observation is somewhat surprising because in the latter case only a through-space effect can operate whereas in the other case both sites, i.e. H(N1) and the N7-coordinated Pt^{2+}, are part of the same aromatic purine moiety. It appears that the value $\Delta\ pK_{a/av} = 0.36 \pm 0.04$ is somewhat too large if compared with the results $\Delta\ pK_a = 0.44 \pm 0.04$ and $\Delta\ pK_a = 0.39 \pm 0.05$ due to the complexes *cis*-$(NH_3)_2Pt(dGuo)(dGMP{\cdot}H)^+$ and $(Dien)Pd(GMP{\cdot}H)^+$, respectively (entries 8,9), because in these latter two examples only a single $-P(O)_2(OH)^-$ group is acidified. Consequently, the average acidification, $\Delta\ pK_{a/av} = 0.14 \pm 0.03$, observed for *cis*-$(NH_3)_2Pt(dCMP{\cdot}H)_2$ appears as more normal.

The somewhat different acidifications in the $Pt(dGMP{\cdot}H)_2$ and $Pt(dCMP{\cdot}H)_2$ species are possibly due to some intramolecular H-bonding between the 5'-phosphate groups and coordinated NH_3, giving rise to an intramolecular, though outer-sphere macrochelate; such a species has been observed by X-ray crystallography for $[Pt(En)(GMP\text{-}N7)_2]{\cdot}9H_2O$ [42] and there is also evidence [42] that it occurs to some extent in

TABLE 2. 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 H^+(N7) and H(N1) Sites of Free Guanine Nucleotides and Their N7-Coordinated Pt^{2+} Complexes (see Fig. 2), as well as of Some Related Ligands and Complexes. The Acidifying Properties of Pt^{2+} and Pd^{2+} Are Expressed via $\Delta\ pK_a$, i.e., in Analogy to Equation 9

No.[b]	Acid	pK_a for H^+(N7)	pK_a for $-P(O)_2(OH)^-$	pK_a for H(N1)	$\Delta\ pK_a$ for $-P(O)_2(OH)^-$	$\Delta\ pK_a$ for H(N1)
1	$H_2(CMP)^{\pm}$	4.33±0.04[c]	6.19±0.02			
2	$H_2(dCMP)^{\pm}$	4.46±0.01[c]	6.24±0.01			
3	$H_2(GMP)^{\pm}$	2.48±0.04	6.25±0.02	9.49±0.02		
4	$H_2(dGMP)^{\pm}$	2.69±0.03	6.29±0.01	9.56±0.02		
5	$H(dGuo)^+$	2.30±0.04		9.24±0.03		
6[d]	*cis*-$(NH_3)_2Pt(dCMP{\cdot}H)_2$		5.73±0.02/6.47±0.02 (6.10±0.03)		0.14±0.03[e]	
7	*cis*-$(NH_3)_2Pt(dGMP{\cdot}H)_2$		5.57±0.03/6.29±0.02 (5.93±0.04)	8.73±0.04/9.48±0.04 (9.10±0.06)	0.36±0.04[e]	0.46±0.06[e]
8	*cis*-$(NH_3)_2Pt(dGuo)(dGMP{\cdot}H)^+$		5.85±0.04	8.20±0.03/9.05±0.10 (8.62±0.10)	0.44±0.04	0.78±0.11[e,f]
9	$(Dien)Pd(GMP{\cdot}H)^+$				0.39±0.05	1.06±0.05

[a] See footnote *a* of Table 1. [b] Entry 1 is from ref. [40]; 2 and 6 from ref. [30]; 3 from ref. [32]; 4 from ref. [28,29]; 5 and 8 from ref. [29]; 7 from ref. [28,31]. The values of entry 9 were calculated from the acidity constants given in ref. [41], which were determined by ^{1}H-NMR shift experiments in D_2O at 34°C and $I \simeq 0.5$ M (KNO_3). [c] This value refers to the deprotonation of the H^+(N3) site of the cytidine moiety. [d] *Cis*-$(NH_3)_2Pt^{2+}$ is N3-coordinated to dCMP (see Fig. 2). [e] The average of the acidification on both sites, i.e. the two $-P(O)_2(OH)^-$ or the two H(N1) sites, present in these complexes is considered. [f] $\Delta\ pK_a = \frac{1}{2}\ (pK^H_{Guo} + pK^H_{dGMP}) - \frac{1}{2}\ (pK^H_{Pt(dGuo)(dGMP)} + pK^H_{[Pt(dGuo)(dGMP)-H]}) = \frac{1}{2}\ [(9.24\pm0.03) + (9.56\pm0.02)] - \frac{1}{2}\ [(8.20\pm0.03) + (9.05\pm0.10)] = 0.78\pm0.11$.

solution as well. In fact, the formation of such a hydrogen-bonded outer-sphere species should facilitate the release of the proton from the $-P(O)_2(OH)^-$ group, as this release should strengthen the hydrogen bond between the Pt(II)-coordinated NH_3 and the $-PO_3^{2-}$ group. Consequently, one is tempted to attribute the difference $\log \Delta = (0.36 \pm 0.04) - (0.14 \pm 0.03) = 0.22 \pm 0.05$ to the formation of such macrochelates; by using known procedures [32,43] one calculates from this difference a formation degree of approximately 40% for the hydrogen-bonded, outer-sphere macrochelated species.

The already discussed average acidification, $\Delta\ pK_{a/av} = 1.23 \pm 0.04$, for *cis*-$(NH_3)_2Pt(9\text{-}EtG)_2^{2+}$ (Table 1, entry 5) is quite significant; that the one for *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ is considerably lower, i.e. $\Delta\ pK_{a/av} = 0.46 \pm 0.06$ (Table 2, entry 7), is probably the result of the counterbalance in the charge by the two $-PO_3^{2-}$ residues in the latter species. This interpretation agrees with the acidifications observed for the *cis*-$(NH_3)_2Pt(dGuo)(dGMP)$ and (Dien)Pd(GMP) complexes, which carry only a single $-PO_3^{2-}$ group. Additional comparisons between entries 5-11 and 6-9 of Tables 1 and 2, respectively, are possible, but left to the reader.

One further aspect warrants discussion: Figure 2 shows that *cis*-$(NH_3)_2Pt(dCMP)_2^{2-}$ is a 'symmetrical' species; the same applies to *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$, *cis*-$(NH_3)_2Pt(9\text{-}EtG)_2^{2+}$, and *trans*-$(CH_3NH_2)_2Pt(9\text{-}EtG)_2^{2+}$. The statistical expectation for the separation of the acidity constants of two identical acidic sites in the same molecule, which do *not* affect each other, is $\Delta\ pK_{a/st} = 0.6$ [28,30]. This follows from the symmetry properties of, e.g., *cis*-$(NH_3)_2Pt(dCMP{\cdot}H)_2$, i.e., there are two equivalent ways for the formation of $Pt(dCMP{\cdot}H)(dCMP)^-$ and also for the protonation of $Pt(dCMP)_2^{2-}$ (see Fig. 2) to give $Pt(dCMP{\cdot}H)(dCMP)^-$. This means, the formation of this monoprotonated species is two times favored by a factor of 2, which gives overall a factor of 4, i.e. $\Delta\ pK_{a/st} = 0.6$. This value has to be compared with the following experimental results which are taken from Tables 1 and 2:

$$\Delta\ pK_{a/Pt,dCMP\cdot H} = pK^H_{Pt(dCMP\cdot H)(dCMP)} - pK^H_{Pt(dCMP\cdot H)_2} = (6.47\pm0.02) - (5.73\pm0.03) = 0.74\pm0.03 \quad (14)$$

$$\Delta\ pK_{a/Pt,dGMP\cdot H} = pK^H_{Pt(dGMP\cdot H)(dGMP)} - pK^H_{Pt(dGMP\cdot H)_2} = (6.29\pm0.02) - (5.57\pm0.03) = 0.72\pm0.04 \quad (15)$$

$$\Delta\ pK_{a/Pt,dGMP} = pK^H_{Pt(dGMP)(dGMP-H)} - pK^H_{Pt(dGMP)_2} = (9.48\pm0.04) - (8.73\pm0.04) = 0.75\pm0.06 \quad (16)$$

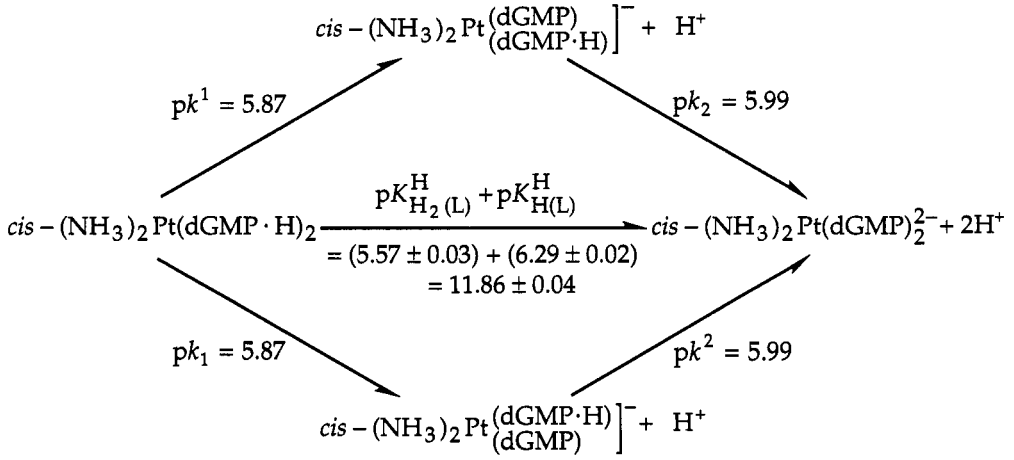
$$\Delta\, pK_{a/Pt,9\text{-}EtG} = pK^{H}_{Pt(9\text{-}EtG)(9\text{-}EtG\text{-}H)} - pK^{H}_{Pt(9\text{-}EtG)_2} = (8.67\pm0.01) - (8.02\pm0.01) = 0.65\pm0.01 \quad (17)$$

$$\Delta\, pK_{a/t\text{-}a_2Pt,9\text{-}EtG} = pK^{H}_{ta_2Pt(9\text{-}EtG)(9\text{-}EtG\text{-}H)} - pK^{H}_{ta_2Pt(9\text{-}EtG)_2} = (8.81\pm0.01) - (8.01\pm0.01) = 0.80\pm0.01 \quad (18)$$

For the first four examples (eq. 14-17) the $\Delta\, pK_a$ values are close to the statistical expectation; they are on average only about 0.1 pK unit larger than $\Delta\, pK_{a/st} = 0.6$. In other words, the mutual influence that the two corresponding acidic sites in these complexes exert on each other is quite small, which indicates that they react independently and consequently the distances between these sites (at least in the protonated forms) must be relatively large. Indeed, for the $-P(O)_2(OH)^-$ groups (eq. 14,15) this is not surprising, but for the H(N1) sites (eq. 16,17) it is certainly somewhat surprising because the two guanine moieties, which lose a proton from their H(N1) site, are linked via their N7 site to the same platinum(II). Of interest is the comparison between the properties of *cis*-$(NH_3)_2Pt(9\text{-}EtG)^{2+}$ (eq. 17) and *trans*-$(CH_3NH_2)_2Pt(9\text{-}EtG)_2^{2+}$ (eq. 18); though not very large, it is clear that a mutual effect between the two acidic sites in the *trans* complex exists.

5. MICRO ACIDITY CONSTANTS FOR SOME DI- (AND TETRA-) PROTONIC PLATINUM(II) COMPLEXES

From the results summarized in equations 14-18 it is clear that the buffer regions of all the Pt(II) species considered are strongly overlapping. Therefore, for a clean quantification of the actual acidity properties of the $-P(O)_2(OH)^-$ groups and of the H(N1) sites as well, it is necessary to determine the micro acidity constants valid for the individual sites. Following known routes [43-45], we have summarized in Fig. 3, as an example, the equilibrium scheme for *cis*-$(NH_3)_2Pt(dGMP{\cdot}H)_2$ defining the micro acidity constants (k) and giving their interrelation with the macro acidity constants (K). There are three independent equations (a), (b), and (c), but four unkown constants [44]; however, by taking into account the above statistical considerations the matter becomes simple for the symmetric acids (*cf.* Fig. 2) because, e.g., $pK^{H}_{Pt(dGMP{\cdot}H)_2} + \log 2 = 5.57 + 0.3 = 5.87 = pk^1 = pk_1$; the analogous reasoning provides $pk_2 = pk^2 = pK^{H}_{Pt(dGMP{\cdot}H)(dGMP)} - \log 2 = 6.29 - 0.3 = 5.99$, etc.. The corresponding results are given on the arrows in Figure 3.



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

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

Fig. 3. Equilibrium scheme for *cis*-$(NH_3)_2Pt(dGMP{\cdot}H)_2$ (which is written in the given way 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 (a), (b), and (c) show how the various constants are interlinked with each other [44]. See also the text in Section 5 and the results summarized in Table 3 which include also other related systems.

The micro acidity constants $pk^1 = pk_1$ and $pk_2 = pk^2$, for the five symmetrical platinum(II) complexes, $Pt(dCMP{\cdot}H)_2$, $Pt(dGMP{\cdot}H)_2$, $Pt(dGMP)_2^{2-}$, $Pt(9\text{-}EtG)_2^{2+}$, and $t\text{-}a_2Pt(9\text{-}EtG)_2^{2+}$, are given in rows 1-3 and 5, 6 of Table 3. Entry 4 refers to the unsymmetrical species Pt(dGuo)(dGMP) which will be discussed further below. In columns 3 and 4 of Table 3 the micro acidity constants are given, whereas columns 5 and 6 provide the differences between the pK_a values of the free ligands (Tables 1 and 2) and the values for pk^1 and pk_2. Thus, these values quantify the acidifying effect of Pt^{2+} on the individual sites. Finally, the average between the values given for $\Delta\, pk_1$ and $\Delta\, pk_2$ (columns 5, 6) results in $\Delta\, pk_{a/av}$ (final column to the right) and these values are identical, of course, with the values listed under $\Delta\, pK_a$ in Tables 1 and 2, if the same systems are considered (*cf.* also eq. 9).

Of the many comparisons possible we give just two: (i) The micro acidity constants pk^1 for the *cis*-$(NH_3)_2Pt(9\text{-}EtG)_2^{2+}$ and *trans*-$(CH_3NH_2)_2Pt(9\text{-}EtG)_2^{2+}$ complexes (entries 5, 6 of Table 3) are identical within their error limits, which means, there is no difference between the *cis* and the *trans* complex as far as the deprotonation of the first H(N1) site is concerned, whereas the values for pk_2 differ; i.e., in the *trans* complex the two 9-ethylguanines feel each other somewhat while this is practically not the case in the *cis* complex. Consequently, for the latter species pk^1 and pk_2 are nearly equal while for the *trans* complex these values differ. – (ii) Comparison of the micro acidity constant $pk^1 = 5.87 \pm 0.03$ (entry 2) for the $Pt(dGMP{\cdot}H)_2$ complex with the macro acidity constant $pK^H_{Pt(dGuo)(dGMP{\cdot}H)} = 5.84 \pm 0.04$ (Table 2, row 8) of the $Pt(dGuo)(dGMP{\cdot}H)^+$ complex reveals that the two values are identical within their error limits. This observation confirms the conclusion already made above that the two monoprotonated phosphate groups in the *cis*-$(NH_3)_2Pt(dGMP{\cdot}H)_2$ complex are so far apart that they hardly affect each other.

The pK_a values for the release of the two protons from the two H(N1) sites present in *cis*-$(NH_3)_2Pt(dGuo)(dGMP)$ are separated from each other only by 0.85 ± 0.10 pK unit (difference between the two values listed in Table 2, entry 8, column 5), which means that the two corresponding buffer regions are overlapping. Consequently, for this complex also a micro acidity constant evaluation is necessary. This is more complicated because pk^1 no longer equals pk_1, etc., but the micro acidity constant for the release of the final H(N1) proton from Pt(dGuo–H)(dGMP)$^-$ could be estimated ($pk_2 = 8.86$ [29]). With this value and equations (a), (b), and (c) given in Fig. 3 the other micro acidity constants can be calculated; these results are given in entry 4 of Table 3. Of course, application of the micro acidity constants pk^1 and pk_1 allows to calculate [29] the ratio R between the two isomeric monodeprotonated quarternary complexes:

$$R = \frac{[cis\text{-}(NH_3)_2Pt(dGuo-H)(dGMP)^-]}{[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP-H^-)]} = \frac{10^{-8.39}}{10^{-8.65}} = 10^{0.26} \simeq \frac{2}{1} \simeq \frac{67}{33} \qquad (19)$$

TABLE 3. Micro Acidity Constants for *cis*-$(NH_3)_2Pt(L)_2$ Species (defined in analogy to Fig. 3) and Extent of the Acidification (Δ pk; see text in Section 5) by Nucleobase-Coordinated Pt(II) on $-P(O)_2(OH)^-$ Groups and H(N1) Sites; Pt(dGuo)(dGMP) (entry 4) and $t\text{-}a_2Pt(9\text{-}EtG)_2^{2+}$ (entry 6) are Considered for Comparisons (aqueous solutions at 25°C; $I = 0.1$ M, $NaNO_3$)[a]

No.	$Pt(L)_2$	$pk^1 = pk_1$	$pk_2 = pk^2$	$\Delta\, pk_1$	$\Delta\, pk_2$	$\Delta\, pk_{a/av}$
1	$Pt(dCMP{\cdot}H)_2$	6.03±0.02	6.17±0.02	0.21±0.02	0.07±0.02	0.14±0.03
2	$Pt(dGMP{\cdot}H)_2$	5.87±0.03	5.99±0.02	0.42±0.03	0.30±0.02	0.36±0.04
3	$Pt(dGMP)_2^{2-}$	9.03±0.04	9.18±0.04	0.53±0.04	0.38±0.04	0.46±0.06
4	Pt(dGuo)(dGMP)	8.39/8.65[b,c]	8.86/8.60[b,d]	0.85/0.91	0.70/0.64	0.78±0.11
5	$Pt(9\text{-}EtG)_2^{2+}$	8.32±0.01	8.37±0.01	1.25±0.04	1.20±0.04	1.23±0.06
6	$t\text{-}a_2Pt(9\text{-}EtG)_2^{2+}$	8.31±0.01	8.51±0.01	1.26±0.04	1.06±0.04	1.16±0.06

[a] Regarding the error limits see footnote *a* of Table 1. Rows 1 and 2 give micro acidity constants for the deprotonation of $-P(O)_2(OH)^-$ groups and rows 3-6 for H(N-1) sites. [b] This is an unsymmetrical acid and therefore pk^1 and pk_1 are not equal; this holds also for pk_2 and pk^2. [c] The first value refers to the release of the first proton from the H(N1) 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. [29]. [d] The first value refers to the release of the second proton from the remaining H(N1) site in *cis*-$(NH_3)_2Pt(dGuo–H)(dGMP)^-$ and the second value correspondingly to the release of H^+ from *cis*-$(NH_3)_2Pt(dGuo)(dGMP–H)^-$; *cf.* ref. [29].

Evidently, *cis*-$(NH_3)_2$Pt(dGuo–H)(dGMP)$^-$ dominates with about 67% whereas the other (N1)-monodeprotonated species occurs with about 33%. Certainly, this result is an estimate [29], but still it proves that both tautomeric forms of [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)–H]$^-$ occur simultaneously in appreciable amounts (see also Section 6 below).

6. CONCLUSIONS

Among the most remarkable results summarized in this account is the observation that a proton at a phosphate group, e.g. in the ternary *cis*-$(NH_3)_2$Pt(dGMP·H)$_2$ or the quarternary *cis*-$(NH_3)_2$Pt(dGuo)(dGMP·H)$^+$ complexes (Fig. 2), is only slightly acidified by the N7-coordinated *cis*-$(NH_3)_2Pt^{2+}$ unit ($\Delta\ pK_a \simeq 0.4$; Table 2). This contrasts with the more significant acidification (on average $\Delta\ pK_a \simeq 0.8$) of the same platinum unit on the H(N1) sites of the last mentioned phosphate-deprotonated complex. As the overall charge of *cis*-$(NH_3)_2$Pt(dGuo)(dGMP) equals that of a DNA-intrastrand cross-link unit formed with *cis*-$(NH_3)_2Pt^{2+}$ and two adjacent guanine residues, the summarized results are also meaningful for the effects of *cis*-$(NH_3)_2Pt^{2+}$ if bound to DNA. This means, based on the mentioned acidification one has to expect that a low, but significant portion (approximately 6% [29]) of the guanine residues which carry a platinum(II) at N7 are deprotonated at N1 under physiological conditions. In any case, the results indicate that the acidification of the H(N1) sites by platinum(II) transfers these sites into better H donors, which means that these sites become even more suitable for hydrogen bonding than is the case already for the uncomplexed nucleobases.

A further important observation regarding DNA is that the formation of complexes of the type M[*cis*-$(NH_3)_2$Pt(dGMP·H)(dGMP)]$^+$ [31] or M[*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)]$^{2+}$ [29] is only slightly inhibited; i.e., the affinity of the -PO_3^{2-} group for divalent metal ions (Mg^{2+}, Cu^{2+}, Zn^{2+}) [46,47] is only little affected by the platinum(II) coordinated at N7 of the same nucleotide unit [29,31]. Consequently, one may expect that a nucleobase-bound platinum(II) in DNA affects the metal ion-binding properties of the phosphate backbone only little. This conclusion is important regarding the binding of metal ions like K^+ or Mg^{2+}. Quite generally one should point out that the metal ion binding properties of, e.g., the *cis*-$(NH_3)_2$Pt(dGMP)$_2^{2-}$ complex, as well as of its monoprotonated form, are largely governed by the basicities of the phosphate groups and that the repulsive effect of *cis*-$(NH_3)_2Pt^{2+}$, coordinated at N7, is not very pronounced, allowing thus the formation of mixed metal ion complexes [31]. This result is remarkable not only with regard to nucleic acids, as indicated above, but also with regard to nucleotides and the possible structures of their complexes in enzyme reactions, which often involve two metal ions [48,49]. Finally, it may be mentioned in this context that we recently succeeded in binding the (Dien)Pt^{2+} unit to N3 of an adenine residue [50] and this allowed to study the effect of this unit on the acid-base properties of the corresponding N1 and N7 sites.

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