Quantification of Outer-Sphere Macrochelate Formation in the Ternary *cis*-Diammine−Platinum(II)−Bis-2′-deoxyguanosine 5′-Monophosphate Complex, *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$, and Formation of Quaternary Mixed Metal Ion Species with Magnesium(II), Copper(II), or Zinc(II) in Aqueous Solution

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The acid−base properties of *cis*-$(NH_3)_2Pt(dG)_2^{2-}$, where both dG^{2-} (=2′-deoxyguanosine 5′-monophosphate) are N7-coordinated to the same Pt(II) [the complex is abbreviated as $Pt(dG)_2^{2-}$], are summarized [on the basis of potentiometric pH titration data from B. Song et al. (*Metal-Based Drugs* **1996**, *3*, 131−141)] and a micro acidity constant scheme is developed which allows quantification of the intrinsic acidity of the two $P(O)_2(OH)^-$ groups present in this ternary complex ($I = 0.1$ M, $NaNO_3$; 25 °C). On the basis of comparisons with the corresponding acid−base properties of *cis*-$(NH_3)_2Pt(dCMP{\cdot}H\text{-}N3)_2$ [$(dCMP{\cdot}H)^-$ = phosphate-monoprotonated 2′-deoxycytidine 5′-monophosphate] it is concluded that intramolecular, outer-sphere macrochelates form via $Pt(NH_3)\cdots O_3P$ hydrogen bonds. The formation degree of these macrochelates is quantified; it amounts in aqueous solution in each case (in its lower limit) to about 40% for the various possibilities which exist for the formation of these chelates in the *cis*-$(NH_3)_2Pt(dG)_2$ complexes. The stability constants of the mixed metal ion complexes, $M[Pt(H;dG)(dG)]^+$ and $M[Pt(dG)_2]$, were also determined via potentiometric pH titrations. On the basis of previous measurements with simple phosphate monoesters and phosphonate derivatives, i.e., $R\text{-}PO_3^{2-}$ with R being a noncoordinating residue (Sigel, H.; et al., *Helv. Chim. Acta* **1992**, *75*, 2634−2656), it is shown that the stability of the two mixed metal ion complexes is largely governed by the basicity of the phosphate groups (as quantified via the mentioned microconstants) indicating that the effect of the N7-bound Pt(II) on the phosphate−metal ion binding properties is relatively small. These results suggest that, e.g., a metal ion bound to a nucleobase residue in a nucleotide or in a nucleic acid affects only slightly the metal ion binding capabilities of its phosphate residue or its phosphate backbone.

1. Introduction

Under natural conditions nucleotides and nucleic acids interact with *labile* metal ions,[1−4] whereas therapeutic agents such as the anticancer drug cisplatin, *cis*-$(NH_3)_2PtCl_2$,[5,6] usually form *inert* metal−nucleobase adducts. Thus, it is not surprising that interactions between nucleotides and labile[7,8] as well as inert[9−11] metal ions are in the focus of many efforts. However, so far only little information exists[12,13] about the mutual effects which two different metal ions bound to the same nucleotide exert on each other.

Considering the above situation and also (i) that the significance of metal ions in nucleotide and nucleic acid processes is well documented[2,14] and (ii) that N7 of guanine residues in DNA is generally accepted as being the crucial target of the anticancer drug cisplatin,[5,6] we selected for this study the "units", *cis*-$(NH_3)_2Pt^{2+}$, 2′-deoxyguanosine 5′-monophosphate ($dGMP^{2-}$),

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(1) Abbreviations: AMP^{2-}, adenosine 5′-monophosphate; $dCPM^{2-}$ (or dC^{2-}), 2′-deoxycytidine 5′-monophosphate; $dGMP^{2-}$ (or dG^{2-}), 2′-deoxyguanosine 5′-monophosphate; dGuo, 2′-deoxyguanosine; dien, 1,5-diamino-3-azapentane (diethylenetriamine); en, 1,2-diaminoethane (ethylenediamine); GMP^{2-}, guanosine 5′-monophosphate; *I*, ionic strength; IMP^{2-}, inosine 5′-monophosphate; M^{2+}, divalent metal ion; NMP^{2-} = AMP^{2-}, GMP^{2-}, and IMP^{2-}; $Pt(dG)_2^{2-}$, *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ (see also Figure 1); $R\text{-}PO_3^{2-}$, simple phosphate monoester or phosphonate ligand with R representing a noncoordinating residue (see also caption for Figure 4); tn, 1,3-diaminopropane (trimethylenediamine). 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.

(2) Sigel, H., Sigel, A., Eds. *Interrelations among Metal Ions, Enzymes, and Gene Expression*; Metal Ions in Biological Systems, Vol. 25; M. Dekker, Inc.: New York and Basel, 1989; pp 1−557.

(3) (a) Fraústo da Silva, J. J. R.; Williams, R. J. P. *The Biological Chemistry of the Elements*; Clarendon Press: Oxford, 1991. (b) Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, 1994.

(4) Sträter, N.; Lipscomb, W. N.; Klabunde, T.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2024−2055.

(5) (a) Bloemink, M. J.; Reedijk, J. *Met. Ions Biol. Syst.* **1996**, *32*, 641−685. (b) Reedijk, J. *J. Chem. Soc., Chem. Commun.* **1996**, 801−806.

(6) Whitehead, J. P.; Lippard, S. J. *Met. Ions Biol. Syst.* **1996**, *32*, 687−726.

(7) (a) Sigel, H. *Chem. Soc. Rev.* **1993**, *22*, 255−267. (b) Sigel, H. *ACS Symp. Ser.* **1989**, *402*, 159−204.

(8) Sigel, H.; Song, B. *Met. Ions Biol. Syst.* **1996**, *32*, 135−205.

(9) (a) Lippert, B. *Biometals* **1992**, *5*, 195−208. (b) Lippert, B. *Prog. Inorg. Chem.* **1989**, *37*, 1−97.

(10) (a) Lippert, B. *Met. Ions Biol. Syst.* **1996**, *33*, 105−141. (b) Sabat, M.; Lippert, B. *Met. Ions Biol. Syst.* **1996**, *33*, 143−176.

(11) Sigel, H.; Lippert, B. *Pure Appl. Chem.* **1998**, *70*, 845−854.

(12) Song, B.; Feldmann, G.; Bastian, M.; Lippert, B.; Sigel, H. *Inorg. Chim. Acta* **1995**, *235*, 99−109.

(13) Sigel, H.; Song, B.; Oswald, G.; Lippert, B. *Chem. Eur. J.* **1998**, *4*, 1053−1060.

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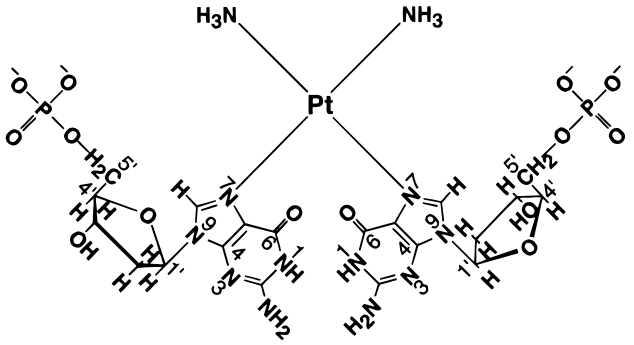


Figure 1. Formal chemical structure of the ternary *cis*-$(NH_3)_2$Pt-$(dGMP)_2^{2-}$ complex. The $dGMP^{2-}$ ligands are depicted in the *anti* conformation, which is usually dominating for purine nucleotides.[17] It may be added that in complexes of the indicated kind the two guanine residues are usually in a head-to-tail[19] configuration (e.g., ref 18) [with a nucleobase−PtN_4 angle close to 50° (e.g., ref 18)].

and the metal ions Mg^{2+}, Cu^{2+}, and Zn^{2+}(=M^{2+}). Since the interaction of *cis*-$(NH_3)_2Pt^{2+}$ with DNA occurs mainly[5,6,15] via intrastrand cross-links formed with adjacent guanine residues, the bis-dGMP complex, *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$, was prepared,[16] in which Pt(II) is N7-coordinated to two neighboring guanine moieties (Figure 1).[17−19]

Similar complexes have been synthesized and studied before by various methods, e.g., *cis*-$(NH_3)_2Pt(GMP)_2^{2-}$,[20,21] *cis*-$(NH_3)_2$-$Pt(IMP)_2^{2-}$,[22] (tn)$Pt(CH_3$-5′-$GMP)_2$,[23] (tn)$Pt(GMP)_2^{2-}$,[24] (tn)-$Pt(dGMP)_2^{2-}$,[24] (en)$Pt(GMP)_2^{2-}$,[18,25,26] and (substituted diamine)-$Pt(GMP)_2^{2-}$.[27] In all cases Pt(II) binding occurs via N7 as confirmed by X-ray analysis in some instances.[18,22,23,25] For the present study the most relevant complex is [(en)Pt(H;GMP-*N7*)$_2$]·$9H_2O$ in which[18] the two guanine bases are in a head-to-tail arrangement,[19] with a dihedral angle of only 36°, which is indicative of substantial intramolecular base stacking; the GMPs are in the *anti* configuration.[18] Figure 1 is drawn accordingly in a simplified manner.

In the (en)Pt(H;$GMP)_2$ complex[18] the phosphate groups are in the vicinity of the H8 atoms and also close to the Pt(II)-bonded amino groups. Indeed, in the solid state[18] "macrochelate rings are formed via intramolecular H-bonding between the monoanionic 5′-phosphate groups and coordinated ethylenediamine NH" sites. This outer-sphere macrochelate formation persists to some extent also in solution with both[18] the $P(O)_2(OH)^-$ and PO_3^{2-} residues, but it is favored, as shown for (dien)Pt(GMP-*N7*),[28] by deprotonation of the monoanionic phosphate group. The first examples of such outer-sphere macrochelates of which we are aware were detected[29] several years ago; i.e., for the complexes of $(dien)Pd^{2+}$ formed with AMP^{2-}, IMP^{2-}, and GMP^{2-} in aqueous solution[29a] and for a complex of *cis*-$(NH_3)_2Pt^{2+}$ with a dinucleotide, d(pGpG), in the solid state.[29b] By including our previous results[12] for *cis*-$(NH_3)_2$-$Pt(dCMP)_2^{2-}$ we are now able to quantify in aqueous solution for the first time the formation degree of such an indicated outer-sphere macrochelate, namely, in the *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ complex.

Are the phosphate groups in the ternary *cis*-$(NH_3)_2Pt$-$(dGMP)_2^{2-}$ complex, despite their partial involvement in hydrogen bonding, also still good acceptor sites for metal ions like Mg^{2+} or Zn^{2+}, i.e., can quarternary mixed metal ion species be formed? And if so, to what extent is the coordination of these metal ions to the dGMP-phosphate groups affected by the nucleobase-bound *cis*-$(NH_3)_2Pt^{2+}$? Indeed, as shown now, M[*cis*-$(NH_3)_2Pt(H;dGMP)(dGMP)]^+$ and M[*cis*-$(NH_3)_2$-$Pt(dGMP)_2$] complexes form in aqueous solution, and they are actually quite stable.

2. Experimental Section

2.1. Materials and Apparatus. The synthesis of Na_2[*cis*-$(NH_3)_2$-$Pt(dGMP)_2]\cdot 11H_2O$ is described in ref 16; the same compound was also used in this study. All other reagents were the same as used recently.[13,16,30]

The equipment used for the potentiometric pH titrations and the desk computer with its frame installations are the same as described previously.[13,30]

2.2. Determination of the Equilibrium Constants. The determination of the acidity constants $K^H_{Pt(H;dG)_2}$, $K^H_{Pt(H;dG)(dG)}$, $K^H_{Pt(dG)_2}$, and $K^H_{Pt(dG)(dG-H)}$ of *cis*-$(NH_3)_2Pt(H;dGMP)_2$, abbreviated as $Pt(H;dG)_2$,[1] was described:[16] 25 mL of aqueous 1.08 mM HNO_3 (25 °C; I = 0.08−0.1 M, $NaNO_3$) were titrated in the presence and absence of 0.4 mM $Pt(dG)_2$ under N_2 with 2 mL of 0.03 M NaOH; the differences in NaOH consumption between two such titrations were used for the calculations.

As only small amounts of $Pt(dG)_2$ were available, to the solutions used for the determination of the acidity constants again HNO_3 was added as well as $M(NO_3)_2$ (total volume: 34 mL), and then the titrations were repeated with NaOH to determine the stability constants of the $M[Pt(H;dG)(dG)]^+$ and $M[Pt(dG)_2]$ complexes. In various instances, after such a titration, to the same solution again HNO_3 was added (total volume: 36 or 38 mL) and the titration with NaOH repeated. Of course, the various dilutions of the solutions were taken into account in the calculations. The $Pt(dG)_2$:M^{2+} ratios were for Mg^{2+} 1:120 and 1:60, for Cu^{2+} 1:4, and for Zn^{2+} 1:33. As a consequence in some experiments I was slightly above 0.1 M; in one experiment with Mg^{2+} (1:120) I reached even a value of 0.16 M.

The stability constants $K^M_{M[Pt(H;dG)(dG)]}$ and $K^M_{M[Pt(dG)_2]}$ for the *cis*-$(NH_3)_2Pt(dG)_2/M^{2+}$ systems were computed for each pair of titrations with a curve-fitting procedure[31] by taking into account the species H^+, $Pt(H;dG)_2$, $Pt(H;dG)(dG)^-$, $Pt(dG)_2^{2-}$, M^{2+}, $M[Pt(H;dG)(dG)]^+$, and

(14) (a) Sigel, A., Sigel, H., Eds. *Interactions of Metal Ions with Nucleotides, Nucleic Acids, and Their Constituents*; Metal Ions in Biological Systems, Vol. 32; M. Dekker, Inc.: New York, Basel, and Hong Kong, 1996; pp 1−814. (b) Sigel, A., Sigel, H., Eds. *Probing of Nucleic Acids by Metal Ion Complexes of Small Molecules*; Metal Ions in Biological Systems, Vol. 33; M. Dekker, Inc.: New York, Basel, and Hong Kong, 1996; pp 1−678.

(15) (a) Takahara, P. M.; Rosenzweig, A. C.; Frederick, C. A.; Lippard, S. J. *Nature* **1995**, *377*, 649−652. (b) Takahara, P. M.; Frederick, C. A.; Lippard, S. J. *J. Am. Chem. Soc.* **1996**, *118*, 12309−12321.

(16) Song, B.; Oswald, G.; Bastian, M.; Sigel, H.; Lippert, B. *Metal-Based Drugs* **1996**, *3*, 131−141.

(17) (a) Martin, R. B.; Mariam, Y. H. *Met. Ions Biol. Syst.* **1979**, *8*, 57−124. (b) Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1987**, *163*, 353−363. (c) Aoki, K. *Met. Ions Biol. Syst.* **1996**, *32*, 91−134.

(18) Barnham, K. J.; Bauer, C. J.; Djuran, M. I.; Mazid, M. A.; Rau, T.; Sadler, P. J. *Inorg. Chem.* **1995**, *34*, 2826−2832.

(19) Head-to-tail means that the H8 atoms of the two guanine residues are on opposite sides of the Pt(II)-coordination plane.[18]

(20) Chu, G. Y. H.; Mansy, S.; Duncan, R. E.; Tobias, R. S. *J. Am. Chem. Soc.* **1978**, *100*, 593−606.

(21) Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Chottard, J.-C. *Biochemistry* **1982**, *21*, 1352−1356.

(22) Kistenmacher, T. J.; Chiang, C. C.; Chalilpoyil, P.; Marzilli, L. G. *J. Am. Chem. Soc.* **1979**, *101*, 1143−1148.

(23) Marzilli, L. G.; Chalilpoyil, P.; Chiang, C. C.; Kistenmacher, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 2480−2482.

(24) Marzilli, L. G.; Chalilpoyil, P. *J. Am. Chem. Soc.* **1980**, *102*, 873−875.

(25) Bau, R.; Gellert, R. W. *Biochimie* **1978**, *60*, 1040.

(26) (a) Berners-Price, S. J.; Frey, U.; Ranford, J. D.; Sadler, P. J. *J. Am. Chem. Soc.* **1993**, *115*, 8649−8659. (b) Berners-Price, S. J.; Ranford, J. D.; Sadler, P. J. *Inorg. Chem.* **1994**, *33*, 5842−5846.

(27) Kiser, D.; Intini, F. P.; Xu, Y.; Natile, G.; Marzilli, L. G. *Inorg. Chem.* **1994**, *33*, 4149−4158.

(28) Guo, Z.; Sadler, P. J.; Zang, E. *J. Chem. Soc., Chem. Commun.* **1997**, 27−28.

(29) (a) Kim, S.-H.; Martin, R. B. *Inorg. Chim. Acta* **1984**, *91*, 11−18. (b) Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *Science* **1985**, *230*, 412−417.

(30) Song, B.; Sigel, H. *Inorg. Chem.* **1998**, *37*, 2066−2069.

M[Pt(dG)$_2$]. The experimental data were evaluated in the pH range between a formation degree of 2% for M[Pt(H;dG)(dG)]$^+$ and the beginning of hydrolysis of M(aq)$^{2+}$ (Cu^{2+}, Zn^{2+}). In the Mg^{2+} system the collection of data was stopped at pH 6.5 to prevent any interference with the deprotonation reactions of the H(N1) sites ($pK^H_{Pt(dG)_2} = 8.73$; vide infra). The formation degree for Mg[Pt(dG)$_2$] and Cu[Pt(dG)$_2$] reached about 40% and 20%, respectively.

The experiments with Zn^{2+} were hampered by a precipitate which appeared soon after Zn[Pt(dG)$_2$] had reached a formation degree of about 15%; this is the main reason why the constants determined for the Zn^{2+} system carry a relatively large error. The calculated stability constants showed no dependence on the excess amount of M^{2+} or the Pt(dG)$_2$ concentration used (the latter varied somewhat due to the procedure described above). There was also no indication that under our experimental conditions M$_2$[Pt(dG)$_2$]$^{2+}$ species formed. The results given in Table 1 (vide infra) are the averages of three titration pairs for the Mg^{2+} complexes and of two pairs each for the Cu^{2+} or Zn^{2+} complexes.

3. Results and Discussion

3.1. Acid−Base Properties of *cis*-(NH$_3$)$_2$Pt(dGMP)$_2^{2-}$ and Its Structure in Solution. Each of the phosphate residues carries a charge of −2 in this ternary complex (see Figure 1), which is often abbreviated below as Pt(dG)$_2^{2-}$, and each may accept two protons; this leads to the 2-fold positively charged species Pt(H$_2$;dG)$_2^{2+}$. The release of the first proton from a P(O)(OH)$_2$ group, which is part of a GMP that also carries a positive charge at N7 (e.g., due to protonation), occurs at very low pH ($pK^H_{H_3(GMP)} = 0.3 \pm 0.2$);[32] hence, one may also conclude for the present case that for both primary protons pK_a < 1. Thus, for the pH range of this study (pH > 3) only the 2-fold-protonated complex, Pt(H;dG)$_2$, is of relevance and the following four deprotonation equilibria need to be considered:

$$Pt(H;dG)_2 \rightleftharpoons Pt(H;dG)(dG)^- + H^+ \quad (1a)$$

$$K^H_{Pt(H;dG)_2} = [Pt(H;dG)(dG)^-][H^+]/[Pt(H;dG)_2] \quad (1b)$$

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

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

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

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

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

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

The protons in equilibria 1 and 2 are released each from a P(O)$_2$(OH)$^-$ residue which follows clearly from the comparison with $pK^H_{H(RibMP)} = 6.24$ of monoprotonated D-ribose 5-monophosphate (RibMP^{2-}).[33] The protons of equilibria 3 and 4 originate from the H(N1) site of the two guanine residues present in Pt(dG)$_2^{2-}$ as is confirmed by $pK^H_{dGuo} = 9.24$ of 2′-deoxyguanosine (dGuo).[11,13] The corresponding acidity constants are

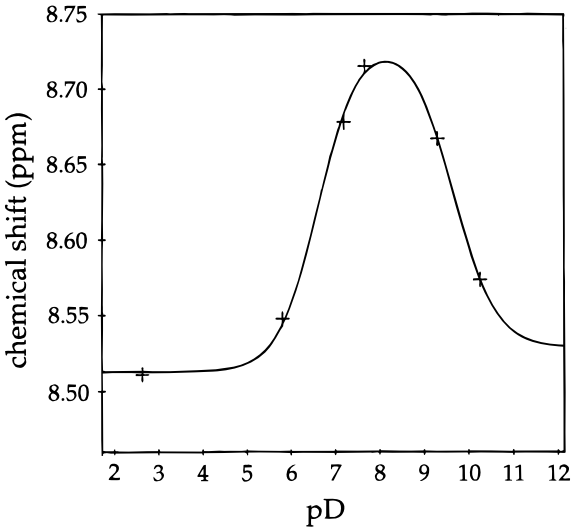


Figure 2. Variation of the chemical shift in dependence on pD for the H8 resonance of 1 mM *cis*-(NH$_3$)$_2$Pt(GMP)$_2$ as measured in D$_2$O at 17 °C. The solid curve is the computer-calculated best fit of the experimental data (taken from ref 21)[35] by using the four stepwise acidity constants given in section 3.1 for *cis*-(NH$_3$)$_2$Pt(D;dGMP)$_2$.

(I = 0.1 M, NaNO$_3$; 25 °C)[16] $pK^H_{Pt(H;dG)_2} = 5.57 \pm 0.03$ (eq 1), $pK^H_{Pt(H;dG)(dG)} = 6.29 \pm 0.02$ (eq 2), $pK^H_{Pt(dG)_2} = 8.73 \pm 0.04$ (eq 3), and $pK^H_{Pt(dG)(dG-H)} = 9.48 \pm 0.04$ (eq 4).

In this context the previously published variation of the chemical shift of H8 with pD for the *cis*-(NH$_3$)$_2$Pt(GMP)$_2$ complex[21] in D$_2$O is further helpful. Transformation of the above given acidity constants by application[34] of eq 5 into the

$$pK_{a/D_2O} = 1.015pK_{a/H_2O} + 0.45 \quad (5)$$

corresponding constants valid for D$_2$O as solvent gives the following values: $pK^D_{Pt(D;dG)_2} = 6.10$ (eq 1), $pK^D_{Pt(D;dG)(dG)} = 6.83$ (eq 2), $pK^D_{Pt(dG)_2} = 9.31$ (eq 3), and $pK^D_{Pt(dG)(dG-H)} = 10.07$ (eq 4). Figure 2 shows the result of the curve-fitting procedure (solid curve)[35] by applying our acidity constants to the previous[21] ^{1}H NMR shift measurements. The excellent fit of the data proves that not only the acidity constants[36] but also the structures of the bis-dGMP and bis-GMP complexes in aqueous solution are very similar; from the so-called[17b,37] "wrong-way shift" in the pD range 6.5 follows that the phosphate groups are in the vicinity of the H8 atoms[21] (see Figure 1). Ionization of the D(N1) sites in the range of pD 9.5 results in an upfield shift (Figure 2) as is common[38] for deprotonation reactions. To conclude, the structures of *cis*-(NH$_3$)$_2$Pt(dGMP)$_2^{2-}$ and *cis*-(NH$_3$)$_2$Pt(GMP)$_2^{2-}$ (see also ref 21) evidently correspond to that described[18] for (en)Pt(GMP)$_2^{2-}$ in the solid state and in solution (see also section 1).

(31) Sigel, H.; Griesser, R.; Prijs, B. *Z. Naturforsch. B* **1973**, *27B*, 353−364.
(32) Sigel, H.; Massoud, S. S.; Corfù, N. A. *J. Am. Chem. Soc.* **1994**, *116*, 2958−2971.
(33) Massoud, S. S.; Sigel, H. *Inorg. Chem.* **1988**, *27*, 1447−1453.
(34) Martin, R. B. *Science* **1963**, *139*, 1198−1203.
(35) The calculation procedure (Newton−Gauss nonlinear least-squares fitting) is based on eq 4 given in ref 17b. The six data points seen in Figure 2 were read out from figure 6 (after its enlargement) in ref 21.
(36) The close similarity for the acidity constants is expected: The acid−base properties of the sites in question differ for H(dGMP)$^-$ and H(GMP)$^-$ only very little; i.e., the pK_a values for the P(O)$_2$(OH)$^-$ and the H(N1) sites are only by Δ pK_a = 0.04 ± 0.02 and 0.07 ± 0.03, respectively, higher for dGMP (25 °C; I = 0.1 M, NaNO$_3$).[30] This small effect is actually offset for the calculations in Figure 2 by the somewhat lower temperature (17 °C) which was used in the experiments[21] for *cis*-(NH$_3$)$_2$Pt(GMP)$_2$.
(37) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32−38.
(38) (a) Fischer, B. E.; Sigel, H. *J. Am. Chem. Soc.* **1980**, *102*, 2998−3008. (b) Blindauer, C. A.; Holý, A.; Dvořáková, H.; Sigel, H. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2353−2363.

3.2. Evidence for Outer-Sphere Macrochelate Formation in *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ Involving $Pt(NH_3)\cdots O_3P$ Hydrogen Bonding. Comparison of the acidity constants given for *cis*-$(NH_3)_2Pt(H;dGMP)_2$ (eqs 1−4) in section 3.1 with those due to $H(dGMP)^-$, i.e., $pK^H_{H(dGMP)} = 6.29 \pm 0.02$ and $pK^H_{dGMP} = 9.56 \pm 0.02$,[16,30] reveals that the *cis*-$(NH_3)_2Pt^{2+}$ unit at N7 (Figure 1) acidifies on average the $P(O)_2(OH)^-$ groups by $\Delta pK_{a/PO_3/av} = (6.29 \pm 0.02) - (1/2)[(5.57 \pm 0.03) + (6.29 \pm 0.02)] = 0.36 \pm 0.04$ and the H(N1) sites by $\Delta pK_{a/N1/av} = (9.56 \pm 0.02) - (1/2)[(8.73 \pm 0.04) + (9.48 \pm 0.04)] = 0.46 \pm 0.06$.

The latter result may be compared with early Raman spectroscopic measurements[20] in D_2O solutions (25 °C; 0.05 M, $NaClO_4$): for GMP^{2-} $pK_{a/D_2O} = 9.8 \pm 0.2$, and for *cis*-$(NH_3)_2Pt(GMP)_2^{2-}$ $pK_{a/D_2O/av} = 9.5 \pm 0.2$.[39] This means that the inductive effect of *cis*-$(NH_3)_2Pt^{2+}$ on the D(N1) deprotonation amounts to $\Delta pK_{a/N1/av} = 0.3 \pm 0.3$, which is close to our result of 0.46 ± 0.06. This confirms again the close relationship between the bis-GMP and bis-dGMP complexes.

It is surprising that the acidifications by the *cis*-$(NH_3)_2Pt^{2+}$ unit at N7 on the H(N1) and the $P(O)_2(OH)^-$ sites are so similar, i.e., $\Delta pK_{a/N1/av} = 0.46 \pm 0.04$ and $\Delta pK_{a/PO_3/av} = 0.36 \pm 0.04$, respectively, 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(II), are part of the same aromatic purine moiety. In fact, $\Delta pK_{a/PO_3/av} = 0.36 \pm 0.04$ seems to be somewhat too large if compared with the average acidification observed[12,16] for *cis*-$(NH_3)_2Pt(H;dCMP)_2$, $\Delta pK_{a/PO_3/av} = 0.14 \pm 0.03$, which clearly appears as more normal. Yet Sadler et al.[26a] also observed $\Delta pK_{a/PO_3/av} = 0.38 \pm 0.06$ for the phosphate[40] pK_a of (en)Pt(H;GMP)$_2$ if compared with free $H(GMP)^-$ ($pK_a = 6.20 \pm 0.04$).[26a] This result is within the error limits identical with our value (0.36 ± 0.04), and thus it confirms excellently the close structural relationship between *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ and (en)Pt$(GMP)_2^{2-}$ already indicated in section 3.1.

The different acidifications in the *cis*-$(NH_3)_2Pt(H;dGMP)_2$ and *cis*-$(NH_3)_2Pt(H;dCMP)_2$ species are indicative of some intramolecular H bonding between the 5′-phosphate groups and the Pt(II)-coordinated NH_3 molecules, giving rise to an intramolecular, though outer-sphere, macrochelate. The formation of such a hydrogen-bonded species should facilitate the release of the proton from the $P(O)_2(OH)^-$ groups because this release should strengthen the hydrogen bond between a Pt(II)-coordinated NH_3 and (now) a PO_3^{2-} group, and exactly this is observed! In other words, the origin for the different acidifications in *cis*-$(NH_3)_2Pt(H;dGMP)_2$ and *cis*-$(NH_3)_2Pt(H;dCMP)_2$, in which the formation of such a macrochelate is not possible for steric reasons, relates to this feature.

For the mentioned (en)Pt$(GMP)_2$ complex it has been shown[18] by X-ray crystallography for the solid state that such outer-sphere macrochelates exist, and it was verified by $^1H\{^{15}N\}$ NMR shift and $^{31}P\{^1H\}$ NOE measurements that their formation occurs to some extent also in solution. Corresponding results have been presented for ([$^{15}N_3$]dien)Pt(GMP-*N7*) (cf. ref 28), (dien)Pd(NMP-*N7*) (cf. ref 29a), and (substituted diamines)Pt(GMP-*N7*) (cf. ref 27) in solution as well as for [(en)Pd(H;GMP-*N7*)$_2$]·$9H_2O$ (cf. ref 18) and [(en)Pd(H;IMP-*N7*)$_2$]·$11H_2O$ (cf. ref 41) in the solid state and in solution. Molecular mechanics calculations[42] also favor hydrogen bonding of the described kind in such macrochelates.

Taken together, these results provide the needed confidence that the above given interpretation regarding the different acidifications observed for *cis*-$(NH_3)_2Pt(H;dGMP)_2$ ($\Delta pK_{a/PO_3/av} = 0.36 \pm 0.04$) and *cis*-$(NH_3)_2Pt(H;dCMP)_2$ ($\Delta pK_{a/PO_3/av} = 0.14 \pm 0.03$) is correct indeed. Hence, our results can be used for a quantitative evaluation of the formation degree of such macrochelates in solution, which is so far missing. This means that the difference $\log \Delta = (0.36 \pm 0.04) - (0.14 \pm 0.03) = 0.22 \pm 0.05$ reflects the formation of the outer-sphere macrochelates in *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$. By using known procedures[32,43] one calculates for the dimensionless intramolecular equilibrium constant $K_I = 0.66 \pm 0.19$ and for the formation degree of the hydrogen-bonded, outer-sphere macrochelated species $40\% \pm 7\%$. These results[44] reflect the *average* situation for each of the *two* possibilities which exist for macrochelate formation in *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ (see Figure 1), because the calculation is based on $\Delta pK_{a/av}$ (see also section 3.4).[45]

3.3. Micro Acidity Constant Scheme for the Phosphate-Monoprotonated *cis*-$(NH_3)_2Pt(H;dGMP)_2$ Complex. Since *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ (Figure 1), if monoprotonated at each phosphate group, is a *symmetrical* diprotonic (or, if the H(N1) sites are included, a tetraprotonic) acid, the statistical expectation for the separation of the acidity constants of the two identical acidic sites, in case they do not affect each other, is $\Delta pK_{a/st} = 0.6$.[12,16] This follows from the symmetry properties: Beginning with Pt$(H;dG)_2$, there are two equivalent ways (Figure 1) for the formation of Pt(H;dG)$(dG)^-$ and also for the protonation of $Pt(dG)_2^{2-}$ to give Pt(H;dG)$(dG)^-$. This means that the formation of the monoprotonated species Pt(H;dG)$(dG)^-$ 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 statistical value is close to the experimental result of $\Delta pK_{a/Pt(H;dG)(dG)} = pK^H_{Pt(H;dG)(dG)} - pK^H_{Pt(H;dG)_2} = (6.29 \pm 0.02) - (5.57 \pm 0.03) = 0.72 \pm 0.04$ (see section 3.1). The difference of only about 0.1 pK unit indicates that the two $P(O)_2(OH)^-$ groups in Pt$(H;dG)_2$ influence each other only slightly and that the distances between these two sites, at least in their protonated form, must be relatively large. This suggestion agrees with the solution structures discussed in sections 3.1 and 3.2.

From the above consideration ($\Delta pK_{a/Pt(H;dG)(dG)} = 0.72$) it is also clear that the buffer regions of Pt$(H;dG)_2$ and Pt(H;dG)$(dG)^-$

(39) In ref 20 only a single value is given, i.e., the two D(N1) deprotonations were considered in an averaged single step.

(40) Only a single value is given[26a] for the two $P(O)_2(OH)^-$ deprotonations in (en)Pt$(H;GMP)_2$.

(41) Rau, T.; van Eldik, R. *Chem. Ber./Recl.* **1997**, *130*, 1551−1555.

(42) (a) Kozelka, J.; Petsko, G. A.; Lippard, S. J.; Quigley, G. J. *J. Am. Chem. Soc.* **1985**, *107*, 4079−4081. (b) Hambley, T. W. *Inorg. Chem.* **1991**, *30*, 937−947.

(43) Sigel, H.; Massoud, S. S.; Tribolet, R. *J. Am. Chem. Soc.* **1988**, *110*, 6857−6865.

(44) One may argue that these results are probably lower limits for the formation degrees of the macrochelates because we consider in our calculations necessarily the different deprotonation properties of *cis*-$(NH_3)_2Pt(H;dGMP)_2$ and *cis*-$(NH_3)_2Pt(H;dCMP)_2$ and attribute these to hydrogen-bond formation. However, the work of Sadler et al.[18] indicates that already in the protonated (en)Pt$(H;GMP)_2$ species some hydrogen-bonded outer-sphere macrochelates form and this has then also to be surmised for *cis*-$(NH_3)_2Pt(H;dGMP)_2$. From this point of view our results represent then actually the difference between phosphate protonated (the concentration of which may be low) and deprotonated forms. To say it differently: We obtain the formation degrees of the outer-sphere macrochelates on top of what is possibly already present in the protonated forms.

(45) One may mention here that from the ΔpK_a values (=0.5−0.7) given in ref 29a for the (dien)Pd(NMP) systems follow formation degrees for the corresponding outer-sphere macrochelates[44] of about 68−80%.[32,43] There is a caveat, however, in that these ΔpK_a values are based on the difference toward the complexes formed with 1,1,4,7,7-pentamethyl-dien and thus there could also be a contribution due to steric inhibition; if so, the given formation degrees would be somewhat too large. However, considering this and especially that these complexes differ significantly from the present one in their composition, one may note that the orders of the formation degrees are still relatively similar.

overlap. Therefore, for a clean quantification of the actual acidity properties of the $P(O)_2(OH)^-$ groups it is necessary to determine the micro acidity constants for the individual sites. In Figure 3 the equilibrium scheme for *cis*-$(NH_3)_2Pt(H;dGMP)_2$ (=$Pt(H;dG)_2$), which is written there as $Pt(dGMP{\cdot}H)_2$ to indicate that the protons are bound at the phosphate groups, is summarized following known routes.[32,46,47] 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 (a, b, and c), but four unknown constants;[46] however, by taking into account the above statistical considerations the present case simplifies because $pK^{H}_{Pt(H;dG)_2} + \log 2 = 5.57 + 0.3 = 5.87 = pk^{Pt(dGMP{\cdot}H)(dGMP)}_{Pt(dGMP{\cdot}H)_2} = pk^{Pt(dGMP)(dGMP{\cdot}H)}_{Pt(dGMP{\cdot}H)_2}$; the analogous reasoning provides $pk^{Pt(dGMP)_2}_{Pt(dGMP{\cdot}H)(dGMP)}$, etc. The corresponding results are given on the arrows in Figure 3.

To make use of these micro acidity constants, which quantify the intrinsic acidities of the $P(O)_2(OH)^-$ groups of the ternary complex (Figure 1), less cumbersome in the following sections, we define

$$pk_{a1/Pt(dG{\cdot}H)_2} = pk^{Pt(dGMP{\cdot}H)(dGMP)}_{Pt(dGMP{\cdot}H)_2} = pk^{Pt(dGMP)(dGMP{\cdot}H)}_{Pt(dGMP{\cdot}H)_2} = 5.87 \pm 0.03 \quad (6)$$

$$pk_{a2/Pt(dG)(dG{\cdot}H)} = pk^{Pt(dGMP)_2}_{Pt(dGMP{\cdot}H)(dGMP)} = pk^{Pt(dGMP)_2}_{Pt(dGMP)(dGMP{\cdot}H)} = 5.99 \pm 0.02 \quad (7)$$

The micro acidity constant, $pk^{Pt(dGMP)(dGMP{\cdot}H)}_{Pt(dGMP{\cdot}H)_2} = 5.87 \pm 0.03$ (eq 6), being identical within the error limits with the macro acidity constant, $pK^{H}_{Pt(dGuo)(dGMP{\cdot}H)} = 5.85 \pm 0.04$, determined[13] for the quaternary *cis*-$(NH_3)_2Pt(dGuo)(dGMP{\cdot}H)^+$ complex confirms the above conclusion that in $Pt(dGMP{\cdot}H)_2$ the two monoprotonated phosphate groups hardly affect each other.

Finally, with a scheme analogous to the one in Figure 3 also the micro acidity constants for the H(N1) sites of *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ can be evaluated;[11,16] however, they are not relevant in the present context and are therefore not discussed.

3.4. Extent of Individual Macrochelate Formation Based on Micro Acidity Constants. Application of the microconstant scheme (Figure 3) and the results summarized in eqs 6 and 7, together with $pK^{H}_{H(dGMP)} = 6.29 \pm 0.01$ of free $H(dGMP)^-$,[16,30] gives the following acidifications for the 2-fold-protonated *cis*-$(NH_3)_2Pt(H;dGMP)_2$ and the monoprotonated *cis*-$(NH_3)_2Pt(dGMP)(H;dGMP)^-$ complexes, respectively:

$$\Delta pk_{a1/Pt(dG{\cdot}H)_2} = (6.29 \pm 0.01) - (5.87 \pm 0.03) = 0.42 \pm 0.03 \quad (8)$$

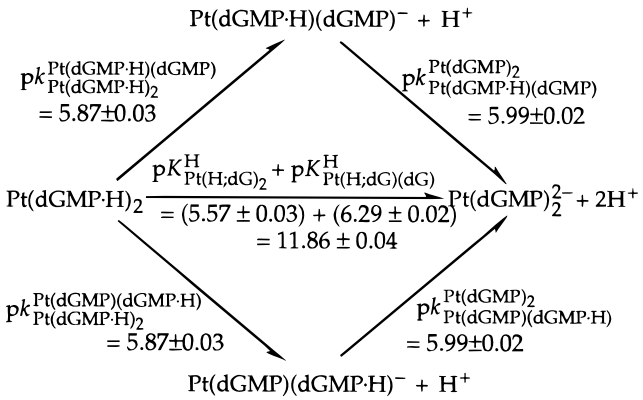
$$\Delta pk_{a2/Pt(dG)(dG{\cdot}H)} = (6.29 \pm 0.01) - (5.99 \pm 0.02) = 0.30 \pm 0.02 \quad (9)$$

For comparison the corresponding micro acidity constants for the *cis*-$(NH_3)_2Pt(H;dCMP)_2$ (eq 10) and *cis*-$(NH_3)_2Pt(dCMP)(H;dCMP)^-$ complexes are needed and taken from our earlier work:[11,16]

$$pk_{a1/Pt(dCMP{\cdot}H)_2} = 6.03 \pm 0.02 \quad (10)$$

$$pk_{a2/Pt(dCMP)(dCMP{\cdot}H)} = 6.17 \pm 0.02 \quad (11)$$

(46) Martin, R. B. *Met. Ions Biol. Syst.* **1979**, *9*, 1−39.
(47) Song, B.; Sigel, R. K. O.; Sigel, H. *Chem. Eur. J.* **1997**, *3*, 29−33.



$$(a)\ K^{H}_{Pt(H;dG)_2} = k^{Pt(dGMP{\cdot}H)(dGMP)}_{Pt(dGMP{\cdot}H)_2} + k^{Pt(dGMP)(dGMP{\cdot}H)}_{Pt(dGMP{\cdot}H)_2}$$

$$(b)\ \frac{1}{K^{H}_{Pt(H;dG)(dG)}} = \frac{1}{k^{Pt(dGMP)_2}_{Pt(dGMP{\cdot}H)(dGMP)}} + \frac{1}{k^{Pt(dGMP)_2}_{Pt(dGMP)(dGMP{\cdot}H)}}$$

$$(c)\ K^{H}_{Pt(H;dG)_2} \cdot K^{H}_{Pt(H;dG)(dG)} = k^{Pt(dGMP{\cdot}H)(dGMP)}_{Pt(dGMP{\cdot}H)_2} \cdot k^{Pt(dGMP)_2}_{Pt(dGMP{\cdot}H)(dGMP)} = k^{Pt(dGMP)(dGMP{\cdot}H)}_{Pt(dGMP{\cdot}H)_2} \cdot k^{Pt(dGMP)_2}_{Pt(dGMP)(dGMP{\cdot}H)}$$

Figure 3. Equilibrium scheme for *cis*-$(NH_3)_2Pt(H;dGMP)_2$, which is written here as $Pt(dGMP{\cdot}H)_2$ 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−c show how the various constants are interlinked with each other.[46] See also the text in section 3.3 and eqs 6 and 7.

The corresponding acidifications follow from the difference formed with $pK^{H}_{H(dCMP)} = 6.24 \pm 0.01$ due to free[12] $H(dCMP)^-$:

$$\Delta pk_{a1/Pt(dC{\cdot}H)_2} = (6.24 \pm 0.01) - (6.03 \pm 0.02) = 0.21 \pm 0.02 \quad (12)$$

$$\Delta pk_{a2/Pt(dC)(dC{\cdot}H)} = (6.24 \pm 0.01) - (6.17 \pm 0.02) = 0.07 \pm 0.02 \quad (13)$$

The differences

$$\log \Delta_1 = (0.42 \pm 0.03) - (0.21 \pm 0.02) = 0.21 \pm 0.04 \quad (14)$$

$$\log \Delta_2 = (0.30 \pm 0.02) - (0.07 \pm 0.02) = 0.23 \pm 0.03 \quad (15)$$

reflect the effect of outer-sphere macrochelate formation on the acidities of *cis*-$(NH_3)_2Pt(H;dGMP)_2$ and *cis*-$(NH_3)_2Pt(dGMP)(H;dGMP)^-$, respectively. Application of described procedures[32,43] gives for the corresponding intramolecular equilibrium constants $K_{I/1} = 0.62 \pm 0.15$ and $K_{I/2} = 0.70 \pm 0.12$, from which the formation degrees of the macrochelates in *cis*-$(NH_3)_2Pt(dGMP)(H;dGMP)^-$ and *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ follow as 38% ± 6% and 41% ± 4%, respectively (the latter reflecting the situation at both sites in *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$; see Figure 1).[44]

As seen in section 3.3 the intrinsic acidity for the release of the first proton from *cis*-$(NH_3)_2Pt(dGMP{\cdot}H)_2$ ($pk_{a1/Pt(dG{\cdot}H)_2}$ =

Table 1. Logarithms of the Stability Constants of the Mixed Metal Ion Complexes, $M[Pt(H;dG)(dG)]^+$ (Eq 16) and $M[Pt(dG)_2]$ (Eq 17), as Determined by Potentiometric pH Titrations in Aqueous Solution, Together with the Negative Logarithms of the Acidity Constants (Eqs 18 and 19) of the Corresponding Monoprotonated $M[Pt(H;dG)(dG)]^+$ Complexes and the Connected Acidifications ΔpK_a or Δpk_a (Eqs 20 and 21) at 25 °C and $I \simeq 0.1$ M ($NaNO_3$)[a-c]

M^{2+}	$\log K^{M}_{M[Pt(H;dG)(dG)]}$ (eq 16)	$\log K^{M}_{M[Pt(dG)_2]}$ (eq 17)	$pK^{H}_{M[Pt(H;dG)(dG)]}$ (eqs 18,19)	$\Delta pK_a(\Delta pk_a)$ (eq 20 (eq 21))	$\log K^{M}_{M[Pt(dGuo)(dGMP)]}$ (ref 13)
Mg^{2+}	1.32 ± 0.09	1.86 ± 0.06	5.75 ± 0.11	0.54 (0.24) ± 0.11	1.21 ± 0.04
Cu^{2+}	2.60 ± 0.15	3.63 ± 0.10	5.26 ± 0.18	1.03 (0.73) ± 0.18	2.60 ± 0.08
Zn^{2+}	1.7 ± 0.2	2.8 ± 0.2	5.2 ± 0.3	1.1 (0.8) ± 0.3	1.81 ± 0.06

[a] The stability constants for the quinternary complexes, $M[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)]^{2+}$ (final column to the right),[13] are given for comparison with the values listed in the second column.[b] [b] The error limits are 3 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 (e.g., column 4) were calculated according to the error propagation after Gauss. The experiments with Zn^{2+} were hampered due to the formation of a precipitate (see section 2.2), and this is the reason for the relatively large error limits. [c] Regarding slight variations in the ionic strength I see section 2.2.

5.87 ± 0.03; eq 6) corresponds to the acidity of *cis*-$(NH_3)_2$Pt(dGuo)(dGMP·H)$^+$ ($pK_a = 5.85 \pm 0.04$),[13] which means that the second phosphate residue in *cis*-$(NH_3)_2Pt(H;dGMP)_2$ has no significant effect on the other site. As the same may be surmised for the bis-dCMP complexes, we may calculate $\Delta pK_{a/Pt(dGuo)(dGMP\cdot H)} = (6.29 \pm 0.01) - (5.85 \pm 0.04) = 0.44 \pm 0.04$ and use then as a reference the corresponding value from the bis-dCMP complex (see eq 12) to obtain the difference $\log \Delta_{Pt(dGuo)(dGMP)} = (0.44 \pm 0.04) - (0.21 \pm 0.02) = 0.23 \pm 0.04$ from which follows[32,43] $K_I = 0.70 \pm 0.16$ and thus 41% ± 5% for the formation degree of the macrochelate in the *cis*-$(NH_3)_2$Pt(dGuo)(dGMP) complex. Clearly, the consistency of these various results is satisfying.

3.5. Stabilities of Mixed Metal Ion Complexes Formed with *cis*-$(NH_3)_2$Pt(dGMP)$_2$ Species. The potentiometric pH titrations carried out in aqueous solution ($I = 0.1$ M, $NaNO_3$; 25 °C) with $Pt(dG)_2$ in the presence of Mg^{2+}, Cu^{2+}, or Zn^{2+} can be completely evaluated by taking into account equilibria 1 and 2 as well as 16 and 17, provided the evaluation of the

$$M^{2+} + Pt(H;dG)(dG)^- \rightleftharpoons M[Pt(H;dG)(dG)]^+ \quad (16a)$$

$$K^{M}_{M[Pt(H;dG)(dG)]} = [M[Pt(H;dG)(dG)]^+]/([M^{2+}][Pt(H;dG)(dG)^-]) \quad (16b)$$

$$M^{2+} + Pt(dG)_2^{2-} \rightleftharpoons M[Pt(dG)_2] \quad (17a)$$

$$K^{M}_{M[Pt(dG)_2]} = [M[Pt(dG)_2]]/([M^{2+}][Pt(dG)_2^{2-}]) \quad (17b)$$

experimental data is not carried into the pH range where hydroxo species form (Cu^{2+}/Zn^{2+}) or is stopped at pH 6.5 (Mg^{2+}) to prevent interference with the deprotonation equilibria 3 and 4 (section 3.1). There was no indication for $M_2[Pt(dG)_2]^{2+}$ complexes, although such species are expected to form if the M^{2+} concentration is high enough and the suitable pH range is reached.

Evidently, equilibria 16 and 17 are connected with each other via equilibrium 18, for which the acidity constant can be calculated with eq 19. The results regarding equilibria 16−18 are listed in Table 1.

$$M[Pt(H;dG)(dG)]^+ \rightleftharpoons M[Pt(dG)_2] + H^+ \quad (18a)$$

$$K^{H}_{M[Pt(H;dG)(dG)]} = [M[Pt(dG)_2]][H^+]/[M[Pt(H;dG)(dG)]^+] \quad (18b)$$

The stabilities of the monoprotonated $M[cis\text{-}(NH_3)_2Pt(H;dG)(dG)]^+$ and the quinternary $M[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)]^{2+}$ complexes for a given metal ion are identical within their error limits (cf. columns 2 and 6 in Table 1). This observation is in

$$pK^{H}_{M[Pt(H;dG)(dG)]} = pK^{H}_{Pt(H;dG)(dG)} + \log K^{M}_{M[Pt(H;dG)(dG)]} - \log K^{M}_{M[Pt(dG)_2]} \quad (19)$$

accord with the identical acid−base properties of the $P(O)_2(OH)^-$ groups in the ternary *cis*-$(NH_3)_2Pt(dGMP\cdot H)_2$ and quaternary *cis*-$(NH_3)_2$Pt(dGuo)(dGMP·H)$^+$ complexes (section 3.3, second to the last paragraph). This means that a Pt(II) N7-coordinated neutral 2′-deoxyguanosine or a singly negatively charged $(dGMP\cdot H)^-$ affects a further, also N7-coordinated dGMP to about the same extent. Of course, the N7-bound dGMPs can interact with additional metal ions only via their phosphate groups. This is different from the situation in M(dGMP) complexes, where a phosphate-coordinated metal ion can in addition interact with N7, giving thus rise to the formation of macrochelates.[30]

That a metal ion coordinated to one phosphate residue in $Pt(dG)_2^{2-}$ may somewhat affect the acid−base properties of the other phosphate group is to be expected. Such an effect may be evaluated via eq 20. Of course, one might also argue that

$$\Delta pK_a = pK^{H}_{Pt(H;dG)(dG)} - pK^{H}_{M[Pt(H;dG)(dG)]} \quad (20a)$$

$$= (6.29 \pm 0.02) - pK^{H}_{M[Pt(H;dG)(dG)]} \quad (20b)$$

one should consider in this comparison the intrinsic acidity, i.e., use the micro acidity constant of the second $P(O)_2(OH)^-$ group of $Pt(H;dG)(dG)^-$ (cf. eq 7 and Figure 2). This then leads to the difference defined in eq 21. Both procedures (see column

$$\Delta pk_a = pk_{a2/Pt(dG)(dG\cdot H)} - pK^{H}_{M[Pt(H;dG)(dG)]} \quad (21a)$$

$$= (5.99 \pm 0.02) - pK^{H}_{M[Pt(H;dG)(dG)]} \quad (21b)$$

5 in Table 1) lead to the conclusion that a metal ion bound to one phosphate group acidifies a proton located at the other. The difference in the extent of the acidification between Mg^{2+} and Cu^{2+} or Zn^{2+} appears to be real and agrees with the analogous observation[12] made for the M^{2+}/*cis*-$(NH_3)_2Pt(dCMP)_2$ systems. Maybe this indicates that Mg^{2+} is partially outer-sphere coordinated[7,32,33] to the phosphate group.

3.6. Comparison of Measured and Expected Stabilities for $M[Pt(H;dG)(dG)]^+$ and $M[Pt(dG)_2]$ Complexes. The stabilities of these two mixed metal ion complexes may be evaluated with the previously established[33,48] linear relationships based on $\log K^{M}_{M(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ plots, where $R\text{-}PO_3^{2-}$ represents simple phosphate monoester or phosphonate ligands in which the residue R is unable to interact with the metal ion

in the complex. The corresponding straight-line equations[48] for the M(R-PO_3) complexes of Mg^{2+}, Cu^{2+}, and Zn^{2+} are

$$\log K^{Mg}_{Mg(R\text{-}PO_3)} = (0.208 \pm 0.015)\, pK^{H}_{H(R\text{-}PO_3)} + (0.272 \pm 0.097) \quad (22)$$

$$\log K^{Cu}_{Cu(R\text{-}PO_3)} = (0.465 \pm 0.025)\, pK^{H}_{H(R\text{-}PO_3)} - (0.015 \pm 0.164) \quad (23)$$

$$\log K^{Zn}_{Zn(R\text{-}PO_3)} = (0.345 \pm 0.026)\, pK^{H}_{H(R\text{-}PO_3)} - (0.017 \pm 0.171) \quad (24)$$

The error limits of log stability constants calculated with given $pK^{H}_{H(R\text{-}PO_3)}$ values and eqs 22, 23, and 24 are ±0.03, ±0.06, and ±0.06 log units (3σ), respectively, in the pK_a range 5−8 (aqueous solution; 25 °C; I = 0.1 M, $NaNO_3$; see Tables 5 and 6 in ref 48 or Table 3 in ref 32).

The plots of log $K^{M}_{M(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ according to eqs 22 and 24 are shown in Figure 4 for the 1:1 complexes of Mg^{2+} and Cu^{2+} with eight simple ligands, which allow phosph(on)ate−M^{2+} coordination only. Also inserted are the stability constants of the monoprotonated M[Pt(H;dG)(dG)]$^+$ (Table 1, column 2) and the deprotonated M[Pt(dG)$_2$] complexes (column 3) in dependence on the corresponding micro acidity constants given in eqs 6 and 7, respectively. Evidently the M[Pt(H;dG)(dG)]$^+$ complexes are somewhat less stable than expected on the basis of the basicity of the phosphate group (i.e., $pk_{a1/Pt(dG\cdot H)_2}$ = 5.87; eq 6), as may be seen from the full circles in Figure 4, which are *below* their reference lines. The vertical distances (broken lines) between these reference lines and the data points (full circles) reflect the stability difference defined in eq 25. The first term on the right is the experimen-

$$\log \Delta_{M[Pt(H;dG)(dG)]} = \log K^{M}_{M[Pt(H;dG)(dG)]} - \log K^{M}_{M[Pt(H;dG)(dG)]_{calcd}} \quad (25)$$

tally measured value (exptl; column 2 in Table 2), and the second one may be calculated (calcd) with $pk_{a1/Pt(dG\cdot H)_2}$ = 5.87 (eq 6) and eqs 22−24; these calculated values are listed in column 3 of Table 2 and the stability differences (eq 25) in column 4. The inhibitory effect of Pt(II) at N7 of dGMP on the metal ion binding property of the phosphate group in Pt(H;dG)(dG)$^-$ with on average approximately −0.2 log unit is not very pronounced. The corresponding inhibition observed[13] for quinternary M[*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)]$^{2+}$ complexes is of the same order.

A similar comparison between measured (exptl) and calculated (calcd) stabilities is also possible for the M[Pt(dG)$_2$] complexes. The expected stabilities may again be calculated by using the straight-line equations 22−24 and the pK_a value (i.e., the microconstant $pk_{a2/Pt(dG)(dG\cdot H)}$ = 5.99; eq 7) of the phosphate residue. This is also illustrated in Figure 4 for the Mg^{2+} and Cu^{2+} complexes; the half-filled data points are significantly above their reference lines. However, this picture is somewhat misleading because after deprotonation of the second phosphate group the symmetrical Pt(dG)$_2^{2-}$ complex (see Figure 1) forms, offering two *identical* and largely independent phosphate groups for binding of a further metal ion. Hence,

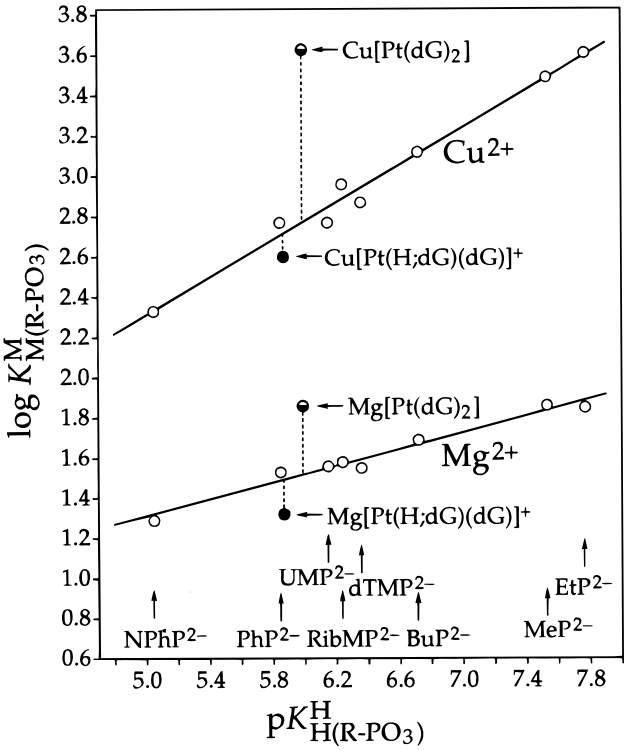


Figure 4. Comparison of the stability of the mixed metal ion M[Pt(H;dG)(dG)]$^+$ (●) and M[Pt(dG)$_2$] complexes (◒) of Mg^{2+} and Cu^{2+} based on the relationship between log $K^{M}_{M(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ for the 1:1 complexes of Mg^{2+} and Cu^{2+} with some simple phosphate monoester or phosphonate ligands (R-PO_3^{2-}): 4-nitrophenyl phosphate (NPhP^{2-}), phenyl phosphate (PhP^{2-}), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5-monophosphate (RibMP^{2-}), thymidine [=1-(2′-deoxy-β-D-ribofuranosyl)thymine] 5′-monophosphate (dTMP^{2-}), *n*-butyl phosphate (BuP^{2-}), methanephosphonate (MeP^{2-}), and ethanephosphonate (EtP^{2-}) (from left to right) (○). The least-squares lines of eqs 22 (Mg^{2+}) and 23 (Cu^{2+}) are drawn through the corresponding eight data sets, which are taken for the phosphate monoesters from ref 33 and for the phosphonates from ref 48. The points due to the equilibrium constants for the mixed metal ion complexes are based on the data given in Table 1 (columns 2 and 3) and the micro acidity constants of eqs 6 and 7. The vertical broken lines emphasize the stability differences to the corresponding reference lines; note: the true stability increase for the M[Pt(dG)$_2$] complexes (◒) is actually 0.3 log unit smaller than indicated by the broken lines (see text in section 3.6). All of the plotted equilibrium constant values refer to aqueous solutions at 25 °C and I = 0.1 M ($NaNO_3$).

the expected complex stability may be calculated, as indicated above, by employing the straight-line equations and the micro acidity constant, but *in addition* 0.3 log unit has to be *added* to the result to obtain the true expected constant (calcd + 0.3), thus taking into account that there are two ways for the formation of this mixed metal ion complex. The stability difference is then defined by eq 26. The corresponding values are listed in columns 5−7 of Table 2.

$$\log \Delta_{M[Pt(dG)_2]} = \log K^{M}_{M[Pt(dG)_2]} - (\log K^{M}_{M[Pt(dG)_2]_{calcd}} + 0.3) \quad (26)$$

From the stability differences given in column 7 of Table 2 it follows that the M[Pt(dG)$_2$] complexes of Cu^{2+} and Zn^{2+} are actually about 0.5 log unit more stable than expected, on the basis of the basicity of the phosphate group. This may be explained either by postulating that a macrochelate is formed involving to a certain extent binding of both phosphate groups to M^{2+} or by postulating that there is a simple charge effect,

(48) Sigel, H.; Chen, D.; Corfù, N. A.; Gregáň, F.; Holý, A.; Strašák, M. *Helv. Chim. Acta* **1992**, *75*, 2634−2656.
(49) Martin, R. B.; Sigel, H. *Comments Inorg. Chem.* **1988**, *6*, 285−314.

Table 2. Stability Constant Comparisons for the Mixed Metal Ion Complexes $M[Pt(H;dG)(dG)]^+$ and $M[Pt(dG)_2]$ between the Measured Stability Constants (Exptl) from Table 1 and the Calculated Stability Constants (Calcd) Based on the Basicity of the Phosphate Groups in $Pt(H;dG)(dG)^-$ or $Pt(dG)_2^{2-}$ (as Defined by the Micro Acidity Constants Given in Eqs 6 and 7) and the Straight Reference-Line Equations Given in Eqs 22−24 (See Also Figure 4) (Aqueous Solution, $I = 0.1$ M, $NaNO_3$; 25 °C)[a]

	$\log K^M_{M[Pt(H;dG)(dG)]}$		$\log \Delta_{M[Pt(H;dG)(dG)]}$	$\log K^M_{M[Pt(dG)_2]}$		$\log \Delta_{M[Pt(dG)_2]}$
M^{2+}	exptl	calcd[b]	(eq 25)	exptl	calcd + 0.3[c]	(eq 26)
Mg^{2+}	1.32 ± 0.09	1.49 ± 0.03	−0.17 ± 0.09	1.86 ± 0.06	1.82 ± 0.03	0.04 ± 0.07
Cu^{2+}	2.60 ± 0.15	2.71 ± 0.06	−0.11 ± 0.16	3.63 ± 0.10	3.07 ± 0.06	0.56 ± 0.12
Zn^{2+}	1.7 ± 0.2	2.01 ± 0.06	−0.3 ± 0.2	2.8 ± 0.2	2.35 ± 0.06	0.45 ± 0.2

[a] Regarding the error limits see footnote *b* of Table 1. [b] Calculated with eqs 22−24 and the microconstant of eq 6. [c] Calculated with eqs 22−24 and the microconstant of eq 7; regarding the addition of 0.3 log unit see text in section 3.6.

i.e., the metal ion bound to one phosphate group is "recognizing" the attracting forces of the other PO_3^{2-} group slightly stronger than the repulsive effect of Pt(II) at N7.

The latter explanation appears to be favored by the related observation (section 3.5) that a Cu^{2+} or Zn^{2+} ion bound to one phosphate group facilitates deprotonation of the other (eq 21 and Table 1, column 5) in the $M[Pt(H;dG)(dG)]^+$ complexes. On the other hand, space-filling molecular models indicate that the two PO_3^{2-} groups of two $dGMP^{2-}$ species, if bound (even in their *anti* conformation) via N7 to *cis*-$(NH_3)_2Pt^{2+}$, may reach a given M^{2+}. This interpretation involving macrochelate formation would also be in accordance with the fact that practically no stability increase (Table 2, column 7) is observed for $Mg[Pt(dG)_2]$ if indeed a partial outer-sphere binding of Mg^{2+} to phosphate groups would occur. Overall it appears that probably both mentioned effects contribute to the observed stability increase of the $Cu[Pt(dG)_2]$ and $Zn[Pt(dG)_2]$ complexes. Assuming that about half of the stability increase of about 0.5 log unit is attributable to macrochelate formation, this would correspond (see refs 8, 32, and 49) to a formation degree of approximately 45% for the macrochelated isomer.

4. Conclusions

One important result of this study is that it provides quantitative information about the extent of outer-sphere macrochelate formation, which occurs via intramolecular $Pt(NH_3)\cdots O_3P$ hydrogen bonding, in the *cis*-$(NH_3)_2Pt(dGMP)(H;dGMP)^-$ and *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ complexes. The formation degree of these macrochelates is considerable; it reaches in aqueous solution approximately 40% in each case and for each individual site (see Figure 1). This result concurs with the observations described previously[18] for the solid state and also (qualitatively) for solutions of the (en)Pt(GMP-*N7*)$_2$ complexes. The persistence of such H bonds in aqueous solution, i.e., against the competition of the H_2O molecules, is remarkable and of general importance with regard to the creation of distinct structures for recognition reactions in biological systems.

The other important result is that the metal ion binding properties of the *cis*-$(NH_3)_2Pt(dGMP)_2^{2-}$ 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. This remarkable observation suggests that, for example, a metal ion bound to a nucleobase residue in a nucleotide and even more so in a nucleic acid, be it single or double stranded, affects only slightly the metal ion binding capabilities of its phosphate residue or its phosphate backbone.

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