Formation of Ternary Complexes by Coordination of (Diethylenetriamine)-Platinum(II) to N1 or N7 of the Adenine Moiety of the Antiviral Nucleotide Analogue 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA): Comparison of the Acid–Base and Metal-Ion-Binding Properties of PMEA, (Dien)Pt(PMEA-*N1*), and (Dien)Pt(PMEA-*N7*)**

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Abstract: The synthesis of (Dien)-Pt(PMEA-*N1*), where Dien = diethylenetriamine and $PMEA^{2-}$ = dianion of 9-[2-(phosphonomethoxy)ethyl]adenine, is described. The acidity constants of the threefold protonated H_3[(Dien)-Pt(PMEA-*N1*)]$^{3+}$ complex were determined and in part estimated (UV spectrophotometry and potentiometric pH titration): The release of the proton from the (N7)H^+ site in H_3[(Dien)-Pt(PMEA-*N1*)]$^{3+}$ occurs with a rather low pK_a (= 0.52 ± 0.10). The release of the proton from the -P(O)$_2$(OH)$^-$ group (pK_a = 6.69 ± 0.03) in H[(Dien)-Pt(PMEA-*N1*)]$^+$ is only slightly affected by the N1-coordinated (Dien)Pt^{2+} unit. Comparison with the acidic properties of the H[(Dien)Pt(PMEA-*N7*)]$^+$ species provides evidence that in the (Dien)-Pt(PMEA-*N7*) complex in aqueous solution an intramolecular, outer-sphere macrochelate is formed through hydrogen bonds between the -PO_3^{2-} residue of $PMEA^{2-}$ and a Pt^{II}-coordinated (Dien)-NH_2 group; its formation degree amounts to about 40%. The stability constants of the M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ complexes with M^{2+} = Mg^{2+}, Ca^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} were measured by potentiometric pH titrations in aqueous solution at 25 °C and I = 0.1 M ($NaNO_3$). Application of previously determined straight-line plots of $\log K^{M}_{M(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ for simple phosph(on)ate ligands, R-PO_3^{2-}, where R represents a non-inhibiting residue without an affinity for metal ions, proves that the primary binding site of (Dien)-Pt(PMEA-*N1*) is the phosphonate group with all metal ions studied; in fact, Mg^{2+}, Ca^{2+} and Ni^{2+} coordinate (within the error limits) only to this site. For the Cu[(Dien)Pt(PMEA-*N1*)]$^{2+}$ and Zn[(Dien)Pt(PMEA-*N1*)]$^{2+}$ systems also the formation of five-membered chelates involving the ether oxygen of the $-CH_2-O-CH_2-PO_3^{2-}$ residue could be detected; the formation degrees are about 60% and 30%, respectively. The metal-ion-binding properties of the isomeric (Dien)Pt(PMEA-*N7*) species studied previously differ in so far that the resulting M[(Dien)Pt(PMEA-*N7*)]$^{2+}$ complexes are somewhat less stable, but again Cu^{2+} and Zn^{2+} also form with this ligand comparable amounts of the mentioned five-membered chelates. In contrast, both M[(Dien)Pt(PMEA-*N1*/*N7*)]$^{2+}$ complexes differ from the parent M(PMEA) complexes considerably; in the latter instance the formation of the five-membered chelates is of significance for all divalent metal ions studied. The observation that divalent metal-ion binding to the phosphonate group of (Dien)Pt(PMEA-*N1*) and (Dien)-Pt(PMEA-*N7*) is only moderately inhibited (about 0.2–0.4 log units) by the twofold positively charged (Dien)Pt^{2+} unit at the adenine residue allows the general conclusion, considering that PMEA is a nucleotide analogue, that this is also true for nucleotides and that consequently participation of, for example, two metal ions in an enzymatic process involving nucleotides is not seriously hampered by charge repulsion.

Keywords: acidity constants · DNA · metal-ion complexes · nucleotides · stability constants

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[**] **Abbreviations and definitions**: AMP^{2-}, adenosine 5′-monophosphate; ATP^{4-}, adenosine 5′-triphosphate; av, average; $dAMP^{2-}$, 2′-deoxy-AMP^{2-}; $dATP^{4-}$, 2′-deoxy-ATP^{4-}; $dCMP^{2-}$, 2′-deoxycytidine 5′-monophosphate; $dGMP^{2-}$, 2′-deoxy-GMP^{2-}; dGuo, 2′-deoxyguanosine; Dien, diethylenetriamine = 1,4,7-triazaheptane; En, ethylenediamine = 1,2-diaminoethane; GMP^{2-}, guanosine 5′-monophosphate; IMP^{2-}, inosine 5′-monophosphate; K_a, acidity constant; L, general ligand; M^{2+}, general divalent metal ion; 9MeA, 9-methyladenine; 1MeC, 1-methylcytosine; $PMEA^{2-}$, dianion of 9-[2-(phosphonomethoxy)ethyl]adenine (see Figure 1); $PMEApp^{4-}$, diphosphorylated $PMEA^{2-}$; R-PO_3^{2-}, simple phosphate monoester or phosphonate ligand with R representing a non-coordinating residue (see also legend of Figure 4); TSP, sodium 3-(trimethylsilyl)-1-propanesulfonate; UMP^{2-}, uridine 5′-monophosphate. Species 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 possibilities applies is always clear from the context.

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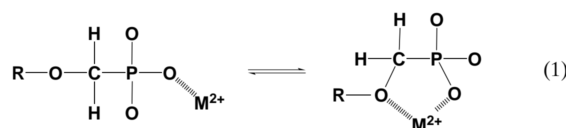
1. Introduction

The dianion of 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$) can be considered as an acyclic-nucleoside phosphonate analogue of (2′-deoxy)adenosine 5′-monophosphate [(d)AMP^{2-}]; it has remarkable antiviral properties,[1] induces apoptosis in human leukemia cell lines[2] and exhibits cytostatic activity in rat and mouse carcinomas and sarcomas.[3] PMEA, also called Adefovir,[1] is phosphorylated in the cell[4] to the diphosphate derivative, $PMEApp^{4-}$, an analogue of (d)ATP^{4-}. In in vitro experiments it was shown that PMEApp inhibits cellular DNA polymerases;[5, 6] it also acts as a substrate/inhibitor for reverse transcriptases,[4a, 6] for example, of the avian myeloblastosis virus,[6] and its incorporation into the growing DNA chain results in chain termination. The oral prodrug of PMEA, that is its bis(pivaloyloxymethyl)ester (also named Adefovir dipivoxil or Preveon),[1] is currently being evaluated in patients infected with human immunodeficiency viruses (HIV-1 and HIV-2) or the hepatitis B virus (HBV).[1, 7]

Recently it was suggested[8, 9] that the reason why $PMEApp^{4-}$ has a greater affinity for several (viral) polymerases than $dATP^{4-}$,[6] is due to the increased basicity[10] of the phosphonyl group (compared with a phosphoryl group) and the possibility of a metal ion (e.g., Mg^{2+}) coordinated to the P_α group of the diphosphophosphonate chain to form a five-membered chelate[11] with the ether oxygen of the acyclic chain (Figure 1). Both properties facilitate the binding of two metal ions to the diphosphophosphonate chain, one being α and the other one being β,γ-bound, thus giving the M(α)–M(β,γ) coordination pattern needed for the enzyme-catalyzed incorporation of the substrate in the growing DNA chain (see [9] and references therein). Indeed, it has been known for years[12] that the presence of the ether oxygen is crucial for an antiviral activity and the existence of the intramolecular Equilibrium (1) for M(PMEA) complexes in solution is well established.[11, 13–15]



(1)

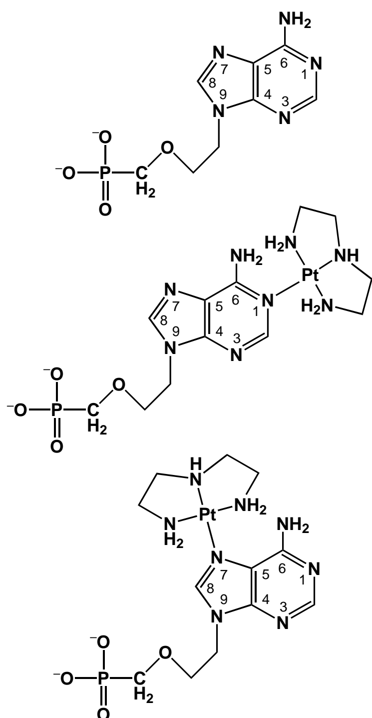


Figure 1. Chemical structure of the dianion of 9-[2-(phosphonomethoxy)-ethyl]adenine ($PMEA^{2-}$; top) and of its corresponding ternary complexes formed by (Dien)Pt^{2+} coordination to N1 and N7 of the adenine moiety giving (Dien)Pt(PMEA-*N1*) (middle) and (Dien)Pt(PMEA-*N7*) (bottom).

The observations summarized above prompted us to combine the antiviral PMEA with platinum(II), also in view of the fact that cisplatin, *cis*-$(NH_3)_2PtCl_2$, is a powerful antitumor drug[16a] and that PMEA exhibits remarkable cytostatic properties.[16b] We selected diethylenetriamine (Dien) as a primary ligand for Pt^{2+} because in the resulting ternary complex with $PMEA^{2-}$ the metal ion has a saturated coordination sphere (Figure 1). The complex initially prepared had the (Dien)Pt^{2+} unit coordinated to N7 of the adenine residue, (Dien)Pt(PMEA-*N7*);[17] its antiviral and cytostatic properties were tested, but no hint for a useful biological activity was observed.[17] Therefore, the subsequently synthesized N1-linkage isomer, (Dien)Pt(PMEA-*N1*) (Figure 1) was not tested.

However, after having studied the acid–base and metal-ion-binding properties of $PMEA^{2-}$ (cf.[9, 11, 15, 18]) and of (Dien)Pt(PMEA-*N7*),[17] we could now also quantify the corresponding properties of the N1 isomer. Consequently, we can report a comparison of the qualities mentioned of $PMEA^{2-}$, (Dien)Pt(PMEA-*N7*), and (Dien)Pt(PMEA-*N1*) (Figure 1) with special attention on the position of Equilibrium (1) in their corresponding metal-ion complexes. In addition, evidence is provided (and quantified) for outer-sphere macrochelate formation in aqueous solution through intramolecular hydrogen bonding between the phosphonate residue and one of the coordinated Dien-NH_2 groups in the (Dien)Pt(PMEA-*N7*) complex. Such a macrochelate does not exist in the (Dien)Pt(PMEA-*N1*) isomer.

2. Results and Discussion

The ternary (Dien)Pt(PMEA-*N1*) complex, the properties of which are investigated in this study (Figure 1), was prepared by mixing aqueous solutions of $[(Dien)Pt(H_2O)](NO_3)_2$ and PMEA, both adjusted to a pH value of about 4.5. Under these conditions the most basic N site of the adenine residue of PMEA, namely N1, is to the largest part deprotonated (see Table 1; entry 5, column 5) and hence, easily accessible for (Dien)Pt^{2+} coordination, thus giving the target compound (Section 4.1). Coordination of (Dien)Pt^{2+} to N1 of the adenine residue of PMEA was proven by $^{1}H,^{1}H$-ROESY and $^{195}Pt,^{1}H$-HMQC NMR spectroscopy (Section 4.2).

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2.1. Acidity constants: Definitions, results, and site attributions: From the structure of (Dien)Pt(PMEA-*N1*) (see Figure 1) it is evident that this species can accept three protons, two at the phosphonate group and one at the N7 site of the adenine residue.[19] A further protonation at N3, after the N1 and N7 sites are blocked, appears in principle possible, however, the basicity of such an N3 site is certainly very low; for example, deprotonation at $(N3)H^+$ in $H_3(adenine)^{3+}$ occurs[20] with $pK_a \simeq -4.2$ and such extreme acidic conditions are not of relevance in the present study. Consequently, the following three deprotonation reactions need to be considered:

$$H_3[(Dien)Pt(PMEA\text{-}N1)]^{3+} \rightleftharpoons H_2[(Dien)Pt(PMEA\text{-}N1)]^{2+} + H^+ \quad (2a)$$

$$K^{H}_{H_3[(Dien)Pt(PMEA\text{-}N1)]} = \frac{[H_2[(Dien)Pt(PMEA\text{-}N1)]^{2+}][H^+]}{[H_3[(Dien)Pt(PMEA\text{-}N1)]^{3+}]} \quad (2b)$$

$$H_2[(Dien)Pt(PMEA\text{-}N1)]^{2+} \rightleftharpoons H[(Dien)Pt(PMEA\text{-}N1)]^{+} + H^+ \quad (3a)$$

$$K^{H}_{H_2[(Dien)Pt(PMEA\text{-}N1)]} = \frac{[H[(Dien)Pt(PMEA\text{-}N1)]^{+}][H^+]}{[H_2[(Dien)Pt(PMEA\text{-}N1)]^{2+}]} \quad (3b)$$

$$H[(Dien)Pt(PMEA\text{-}N1)]^{+} \rightleftharpoons (Dien)Pt(PMEA\text{-}N1) + H^+ \quad (4a)$$

$$K^{H}_{H[(Dien)Pt(PMEA\text{-}N1)]} = \frac{[(Dien)Pt(PMEA\text{-}N1)][H^+]}{[H[(Dien)Pt(PMEA\text{-}N1)]^{+}]} \quad (4b)$$

From previous experience[21] (see also Table 1 below) we expected that Equation (2) occurs at a rather low pH due to the release of the proton from the $(N7)H^+$ site of $H_3[(Dien)Pt(PMEA\text{-}N1)]^{3+}$. If this assumption is correct, it should then be possible to determine the corresponding acidity constant through UV spectrophotometry because protonation/deprotonation reactions at the adenine residue are reflected in absorption changes in the 200–300 nm region.[17] Indeed, Figure 2 shows that the expected spectral changes do occur, which proves that the acid–base reaction takes place at the adenine residue and from Figure 3 it is evident that the

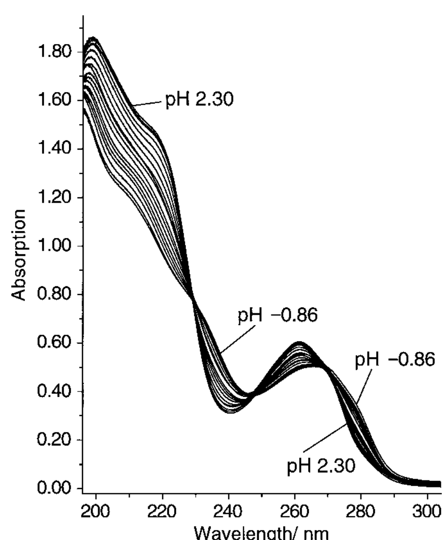

Figure 2. UV absorption spectra measured in 2 cm cells of (Dien)-Pt(PMEA-*N1*) (2.5×10^{-5} M) in aqueous solution in dependence on pH; i.e., the pH values were varied from −0.86, −0.64, −0.37, −0.16, 0.01, 0.16, 0.30, 0.39, 0.50, 0.57, 0.65, 0.75, 0.87, 1.04, 1.18, 1.41, 1.70, 2.05 to 2.30 (25 °C; $I = 0.1$ M, $NaClO_4$, for $[HClO_4] < 0.1$ M; see also Section 4.4).

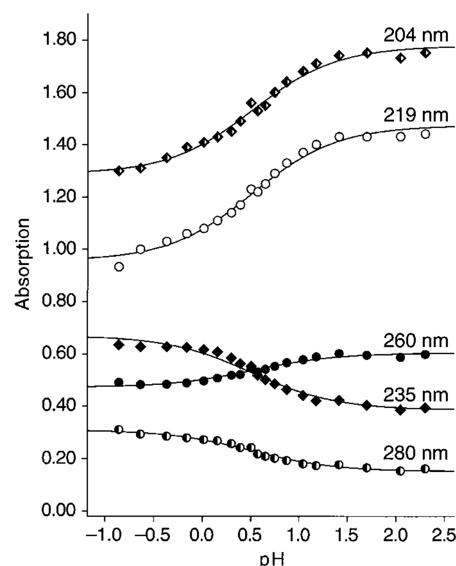

Figure 3. Evaluation of the dependence of the UV absorption of (Dien)-Pt(PMEA-*N1*) at 204, 219, 235, 260 and 280 nm on pH in aqueous solution (see Figure 2) by plotting the absorption A versus pH. The evaluation of this experiment led to the following acidity constants at the mentioned wavelengths: 204 nm, $pK^{H}_{H_3[(Dien)Pt(PMEA\text{-}N1)]} = 0.48 \pm 0.05$; 219 nm, 0.50 ± 0.04; 235 nm, 0.63 ± 0.06; 260 nm, 0.56 ± 0.07; 280 nm, 0.48 ± 0.06 (1σ), which gives the weighted mean $pK^{H}_{H_3[(Dien)Pt(PMEA\text{-}N1)]} = 0.52 \pm 0.08$ (3σ) as the result for this experiment. The solid curves shown are the computer calculated best fits at the mentioned wavelengths through the experimental data points obtained at pH −0.86, −0.64, −0.37, −0.16, 0.01, 0.16, 0.30, 0.39, 0.50, 0.57, 0.65, 0.75, 0.87, 1.04, 1.18, 1.41, 1.70, 2.05 to 2.30 (from left to right) by using the mentioned average result (Section 4.4).

determination of the corresponding pK_a value with a curve-fitting procedure is straight-forward, giving the final result, $pK^{H}_{H_3[(Dien)Pt(PMEA\text{-}N1)]} = 0.52 \pm 0.10$, from two independent series of experiments.

Potentiometric pH titrations in the pH range above 3 only led to the determination of a single acidity constant, that is $pK^{H}_{H[(Dien)Pt(PMEA\text{-}N1)]} = 6.69 \pm 0.03$, which is to be attributed to the release of a proton from the $\text{-}P(O)_2(OH)^-$ group of the $H[(Dien)Pt(PMEA\text{-}N1)]^+$ complex [Eq. (4)]. Indeed, the release of a proton from the twofold protonated phosphonate group, $\text{-}P(O)(OH)_2$, is expected to occur in the pH range 1–2; however, access to this pH range is difficult to achieve with potentiometric pH titrations and if reached, large quantities of a compound are needed. Since these were not available the acidity constant according to Equation (3b) was estimated as described in footnote [g] of Table 1.

The acidity constants obtained in this study for $H_3[(Dien)Pt(PMEA\text{-}N1)]^{3+}$ are listed in Table 1, together with other related data.[22–25] Indeed, comparison of the acidity constants summarized in columns 3, 4 and 6, with the present results confirms the site attributions given above (see also footnote [h] of Table 1).

2.2. Acid–base properties of the adenine residue in the (Dien)Pt(PMEA-*N1*) and (Dien)Pt(PMEA-*N7*) isomers: In free $H_2(PMEA)^{\pm}$ the proton from the adenine moiety is released with $pK^{H}_{H_2(PMEA)} = 4.16$ (Table 1; entry 5, column 5). Coordination of $(Dien)Pt^{2+}$ to either N1 or N7 of PMEA leads to an acidification and the proton is now released with $pK_a =$

Table 1. Negative logarithms of the acidity constants of $H_3[(Dien)Pt(PMEA\text{-}N1)]^{3+}$, $H_3[(Dien)Pt(PMEA\text{-}N7)]^{3+}$ and $H_4(PMEA)^{2+}$, together with the corresponding values of some related systems as determined, unless noted otherwise, by potentiometric pH titrations in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$).[a,b]

No.[c]	Protonated	pK_a for the sites			
	species	$(N7)H^+$	$P(O)(OH)_2$	$(N1)H^+$	$P(O)_2(OH)^-$
1	$H_2(UMP)$		0.7 ± 0.3		6.15 ± 0.01
2	$CH_3OP(O)(OH)_2$		1.1 ± 0.2		6.36 ± 0.01
3	$CH_3P(O)(OH)_2$		2.10 ± 0.03		7.51 ± 0.01
4	$H_2(AnP)$		1.3 ± 0.2		6.49 ± 0.02
5	$H_4(PMEA)^{2+}$	-0.35 ± 0.5[d]	1.22 ± 0.13[d]	4.16 ± 0.02	6.90 ± 0.01
6	$H_3[(Dien)Pt(PMEA\text{-}N7)]^{3+}$		0.78 ± 0.13[f]	1.80 ± 0.10[e]	6.46 ± 0.01
7[h]	$H_3[(Dien)Pt(PMEA\text{-}N1)]^{3+}$	0.52 ± 0.10[e]	1.4 ± 0.2[g]		6.69 ± 0.03
8	$H_2(9MeA)^{2+}$	-0.37 ± 0.06[e]		4.10 ± 0.01	
9	$H[cis\text{-}(NH_3)_2Pt(1MeC)(9MeA\text{-}N7)]^{3+}$			1.93 ± 0.08[d]	
10	$H[cis\text{-}(NH_3)_2Pt(1MeC)(9MeA\text{-}N1)]^{3+}$	0.45 ± 0.09[d]			

[a] The error limits given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] So-called practical or mixed acidity constants are listed (see the second to the last paragraph in Section 4.5). [c] Entry 1 is from ref. [22]; 2 from [23]; 3 and 4 from [24] [$H_2(AnP)$ = acetonylphosphonic acid = $CH_3\text{-}C(O)\text{-}CH_2\text{-}P(O)(OH)_2$]; 5 from [19] (see also [11]); 6 from [17]; 7 this work; 8–10 from [21]. [d] Determined by ^1H NMR shift measurements. Of course, $I > 0.1$ M under the experimental conditions needed for the determination of the values listed in column 3 (see, e.g., Section 4.4). [e] Determined by UV spectrophotometry. [f] Estimated value; for details see footnote [15] in ref. [17]. [g] Estimated value: There is much evidence[24, 25] that the difference $\Delta pK_a = pK^H_{P(O)_2(OH)} - pK^H_{P(O)(OH)_2}$ is constant for a set of related phosphoric/phosphonic acids like those given in entries 1–4, for which $\Delta pK_a = 5.3 \pm 0.2$ (3σ) results. Application of this value to the related acid $H_2[(Dien)Pt(PMEA\text{-}N1)]^{2+}$ (note, the adenine residue is *not* yet protonated) gives $pK^H_{P(O)(OH)_2} = pK^H_{P(O)_2(OH)} - \Delta pK_a = (6.69 \pm 0.03) - (5.3 \pm 0.2) = 1.4 \pm 0.2$. [h] The pK_a values 0.52, 1.4 and 6.69 refer to Equations (2), (3) and (4), respectively.

1.80 from $H_2[(Dien)Pt(PMEA\text{-}N7)]^{2+}$ and $pK_a = 0.52$ from $H_3[(Dien)Pt(PMEA\text{-}N1)]^{3+}$. However, a more detailed comparison by taking the various N sites into account reveals that the situation is more difficult: In $H_2[(Dien)Pt(PMEA\text{-}N7)]^{2+}$ the $(Dien)Pt^{2+}$ unit is at N7 and hence, protonation occurs at N1 and therefore a direct comparison between the acidity constants of this complex and $H_2(PMEA)^{\pm}$ is possible; note, that the charge difference between these species is two in accord with the addition of $(Dien)Pt^{2+}$ to N7. The acidification amounts in this case to $\Delta pK_{a/N1} = pK^H_{H_2(PMEA)} - pK^H_{H_2[(Dien)Pt(PMEA\text{-}N7)]} = (4.16 \pm 0.02) - (1.80 \pm 0.10) = 2.36 \pm 0.10$.

The analogous comparison for the $H_3[(Dien)Pt(PMEA\text{-}N1)]^{3+}$ complex and the $(N7)H^+$ site gives $\Delta pK^*_{a/N7} = pK^H_{H_4(PMEA)} - pK^H_{H_3[(Dien)Pt(PMEA\text{-}N1)]} = (-0.35 \pm 0.5) - (0.52 \pm 0.10) = -0.87 \pm 0.51$ and the negative sign of the result suggests that complex formation of Pt^{2+} at N1 makes N7 more basic. However, this conclusion is misleading[26] since the two species compared, $H_4(PMEA)^{2+}$ and $H_3[(Dien)Pt(PMEA\text{-}N1)]^{3+}$, differ only by a single charge unit, even though the N1-added $(Dien)Pt^{2+}$ carries a charge of two; the reason is that in $H_4(PMEA)^{2+}$ the N1 site is already protonated, increasing the charge and therefore acidifying the $(N7)H^+$ site. For a meaningful comparison the micro acidity constant $pk^{N7\text{-}N1}_{H\cdot N7\text{-}N1//H_3(PMEA)}$ of that $H_3(PMEA)^+$ tautomer is needed in which the N1 site is *free* and the proton resides at N7; with such a value the effect of $(Dien)Pt^{2+}$ coordinated at N1 could truly be evaluated, but it is experimentally not accessible because N1, due to its higher basicity, is always protonated first, that is prior to N7.

Exactly the same difficulty is experienced with the 9-methyladenine (9MeA) systems,[21] the values of which are summarized in entries 8–10 of Table 1. Here one obtains for the analogous comparisons made above $\Delta pK_{a/N1} = (4.10 \pm 0.01) - (1.93 \pm 0.08) = 2.17 \pm 0.08$ and $\Delta pK^*_{a/N7} = (-0.37 \pm 0.06) - (0.45 \pm 0.09) = -0.82 \pm 0.11$; both differences are in perfect accord with those obtained for the PMEA systems. However, in the case of the 9MeA systems it had been possible[21] to estimate the micro acidity constant for the $H(9MeA)^+$ tautomer in which N1 is free and the proton is at N7; that is, $pk^{N7\text{-}N1}_{H\cdot N7\text{-}N1//H(9MeA)} = 2.43 \pm 0.30$. Application of this value gives $\Delta pK_{a/N7} = (2.43 \pm 0.30) - (0.45 \pm 0.09) = 2.0 \pm 0.3$; a result which is identical within the error limits with $\Delta pK_{a/N1} = 2.17 \pm 0.08$. Hence, the acidifying effect of Pt^{2+} coordinated at N1 on the $(N7)H^+$ site equals that of N7-coordinated Pt^{2+} on $(N1)H^+$.

The above conclusion regarding the 9MeA systems is confirmed by another previously reported example of this kind, that is, for the 9-methylhypoxanthine systems.[26] Hence, we can now use these observations and argue that the acidifying effect of $(Dien)Pt^{2+}$ at N1 on the $(N7)H^+$ site of PMEA must be identical to that of $(Dien)Pt^{2+}$ at N7 on the $(N1)H^+$ site. Since the latter value is known for the PMEA systems, namely $\Delta pK_{a/N1} = 2.36 \pm 0.10$ (see above), we can now calculate the micro acidity constant, $pk^{N7\text{-}N1}_{H\cdot N7\text{-}N1//H_3(PMEA)}$, for the $H_3(PMEA)^+$ species in which the proton at the adenine residue is at N7 and *not* at N1; one obtains $pk^{N7\text{-}N1}_{H\cdot N7\text{-}N1//H_3(PMEA)} = pK^H_{H_3[(Dien)Pt(PMEA\text{-}N1)]} + \Delta pK_{a/N1} = (0.52 \pm 0.10) + (2.36 \pm 0.10) = 2.88 \pm 0.14$. Indeed, this micro acidity constant quantifying the acidity of the $(N7)H^+$ site in the $H_3(PMEA)^+$ tautomer compares favorably with the value $pK^H_{H_2(PMEA)} = 4.16 \pm 0.02$ (Table 1), which quantifies the acidity of the N1 site in $H_2(PMEA)^{\pm}$. Evidently, N1 is more basic than N7 by $\Delta pK_a = 1.28 \pm 0.14$;[27, 28] this quantitative result confirms earlier more qualitative conclusions[19, 20, 29, 30] about the basicity order of the nitrogens in the adenine residue.

2.3. Acid–base properties of the $\text{-}P(O)_2(OH)^-$ group in the (Dien)Pt(PMEA-*N1*) and (Dien)Pt(PMEA-*N7*) isomers: Comparison of the acidity constants listed in column 6 of Table 1 shows that the phosphate group is less basic than the phosphonate group (cf. entries 2,3), but that the basicity of the latter one may be reduced by inserting electronegative oxygen atoms in the neighborhood of the phosphonate group, like a carbonyl oxygen in the case of acetonylphosphonate (AnP^{2-}; entry 4) or an ether oxygen as in $PMEA^{2-}$ (entry 5). That the coordination of the twofold positively charged $(Dien)Pt^{2+}$ unit to the adenine residue of $H(PMEA)^-$ also acidifies the $\text{-}P(O)_2(OH)^-$ group (entries 5–7) is expected. However, considering that the distance from the N1 or N7 positions to the phosphonate group is quite alike, if PMEA is stretched out

and the chain orientated away from the adenine residue (i.e., *not* as shown in Figure 1), it is surprising to observe that the effect differs in the two complexes:

$$\Delta pK_{a(N1\text{-}Pt)/PO_3} = (6.90 \pm 0.01) - (6.69 \pm 0.03) = 0.21 \pm 0.03 \quad (5)$$

$$\Delta pK_{a(N7\text{-}Pt)/PO_3} = (6.90 \pm 0.01) - (6.46 \pm 0.01) = 0.44 \pm 0.01 \quad (6)$$

Interestingly, in the related quaternary H[*cis*-$(NH_3)_2$Pt-(dGuo-*N7*)(dGMP-*N7*)]$^+$ complex the -$P(O)_2(OH)^-$ group is also acidified:[31] $\Delta pK_{a(N7\text{-}Pt)/PO_3} = pK^H_{H(dGMP)} - pK^H_{[cis\text{-}(NH_3)_2Pt(dGuo\text{-}N7)(dGMP\text{-}N7)]} = (6.29 \pm 0.01) - (5.85 \pm 0.04) = 0.44 \pm 0.04$. Similar observations have been made for the *average* acidification of the -$P(O)_2(OH)^-$ groups in H_2[*cis*-$(NH_3)_2$Pt(dGMP-*N7*)$_2$] ($\Delta pK_{a/av} = 0.36 \pm 0.04$)[32] and in H_2[(En)Pt(GMP-*N7*)$_2$] ($\Delta pK_{a/av} = 0.38 \pm 0.06$).[33, 34] All these ΔpK_a values are similar to the one given in Equation (6), whereas the average -$P(O)_2(OH)^-$ acidification in H_2[*cis*-$(NH_3)_2$Pt(dCMP-*N3*)$_2$] ($\Delta pK_{a/av} = 0.14 \pm 0.03$)[35] is closer to the result of Equation (5). These differences have previously been explained[32] with outer-sphere macrochelate formation through $Pt(NRH_2)\cdots O_3P$ hydrogen bonds; such a macrochelate formation is not possible for steric reasons in *cis*-$(NH_3)_2$Pt(dCMP-*N3*)$_2^{2-}$, but it is possible in *cis*-$(NH_3)_2$Pt-(dGMP-*N7*)$_2^{2-}$ and its relatives.[32]

Indeed, already more than 15 years ago Martin et al.[36] concluded for the complexes formed between (Dien)Pd^{2+} and AMP^{2-}, IMP^{2-} or GMP^{2-} that in aqueous solution macrochelate formation occurs to some extent between the 5′-phosphate group and coordinated Dien-NH_2 sites. Meanwhile this kind of interaction has been proven[37a] for the (En)Pt(GMP-*N7*)$_2$ complexes by X-ray crystallography in the solid state, and it was also verified by $^1H,^{15}N$-NMR shift and $^1H,^{31}P$-NOE measurements in solution. Analogous results have been presented[37b] for the ([$^{15}N_3$]Dien)Pt(GMP-*N7*) complex, for which it was shown that deprotonation of the monoanionic phosphate group favors the described outer-sphere macrochelate formation.

The summarized results provide the confidence needed to attribute the different acidifications observed for the -$P(O)_2(OH)^-$ groups in the H[(Dien)Pt(PMEA-*N1*)]$^+$ [Eq. (5)] and H[(Dien)Pt(PMEA-*N7*)]$^+$ [Eq. (6)] complexes to outer-sphere macrochelate formation between the phosphonate residue and a Dien-NH_2 group in the latter-mentioned deprotonated complex. Clearly, such a macrochelate can be formed with (Dien)Pt(PMEA-*N7*) as can be seen in Figure 1, where this complex is drawn in such a way as to help to visualize the interaction, but no corresponding hydrogen-bond formation is possible in the (Dien)Pt(PMEA-*N1*) isomer.

To conclude, the results of Equations (5) and (6) can be used for a quantitative evaluation of the formation degree of the macrochelate in (Dien)Pt(PMEA-*N7*) in aqueous solution because the difference $\log \varDelta = (0.44 \pm 0.01) - (0.21 \pm 0.03) = 0.23 \pm 0.03$ is a reflection of this degree of formation. By using described procedures[18, 28, 38] one calculates for the dimension-less equilibrium constant $K_I = 0.70 \pm 0.12$ and for the formation degree of its hydrogen-bonded, outer-sphere macrochelated species 41 ± 4%. This result may be a lower limit because any hydrogen-bond formation occurring with the monoprotonated -$P(O)_2(OH)^-$ group (and this is possible)[37] is not reflected in the calculation. Indeed, for (Dien)Pd(AMP-*N7*) and (Dien)Pd(IMP-*N7*) Martin,[39] using a different approach, concluded recently that about 80% of the complexes are intramolecularly hydrogen-bonded and that the degree of formation of the outer-sphere macrochelate with the monoprotonated phosphate group amounts to about 40%.[39] If these 40% are on top of the present result also an overall value of about 80% is obtained. In any case, it is satisfying to note that the lower limit of 41 ± 4% now obtained is in excellent agreement with the formation degree of 41 ± 5% calculated for the macrochelate in the *cis*-$(NH_3)_2$Pt(dGuo-*N7*)(dGMP-*N7*) complex (see Section 3.4 in ref. [32]) and the 40 ± 7% in the *cis*-$(NH_3)_2$Pt(dGMP-*N7*)$_2^{2-}$ species (see Section 3.2 in ref. [32]); this latter value reflects the *average* situation for each of the *two* possibilities which exist for the macrochelate formation in the *cis*-$(NH_3)_2$Pt(dGMP-*N7*)$_2^{2-}$ complex. These values also prove that there is no significant difference between a phosphate and a phosphonate group as far as the kind of hydrogen bonding indicated is concerned.

2.4. Stabilities of mixed metal-ion complexes formed with (Dien)Pt(PMEA-*N1*): The potentiometric pH titrations carried out in aqueous solution (25°C; $I = 0.1$ M, $NaNO_3$) with (Dien)Pt(PMEA-*N1*) in the presence of Mg^{2+}, Ca^{2+}, Ni^{2+}, Cu^{2+} or Zn^{2+} can be completely accounted for by considering Equilibria (4a) and (7a) provided the evaluation of the

$$M^{2+} + (\mathrm{Dien})\mathrm{Pt}(\mathrm{PMEA}\text{-}N1) \rightleftharpoons \mathrm{M}[(\mathrm{Dien})\mathrm{Pt}(\mathrm{PMEA}\text{-}N1)]^{2+} \quad (7a)$$

$$K^M_{M[(Dien)Pt(PMEA\text{-}N1)]} = \frac{[\mathrm{M}(\mathrm{Dien})\mathrm{Pt}(\mathrm{PMEA}\text{-}N1)]^{2+}]}{[M^{2+}][(\mathrm{Dien})\mathrm{Pt}(\mathrm{PMEA}\text{-}N1)]} \quad (7b)$$

experimental data is not carried into the pH range where hydroxo species are formed; this, however, was evident from the titrations in the absence of ligand (Section 4.5). The measured (exptl) stability constants according to Equation (7b) are listed in the second column of Table 2; none of these constants has been determined before.

Table 2. Comparisons for the M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ complexes between the stability constants [Eq. (7)] determined by potentiometric pH titrations (exptl) and the calculated constants (calcd) based on the basicity of the phosphonate group in (Dien)Pt(PMEA-*N1*) ($pK^H_{H[(Dien)Pt(PMEA\text{-}N1)]} = 6.69 \pm 0.03$, Table 1) and the baseline equations established previously [see Eq. (8) and Figure 4],[11, 28, 38] together with the stability differences $\log \varDelta_{M/(Dien)Pt(PMEA\text{-}N1)}$, as defined by Equation (9) (aqueous solution; 25°C; $I = 0.1$ M, $NaNO_3$).[a]

M^{2+}	$\log K^M_{M[(Dien)Pt(PMEA\text{-}N1)]}$		$\log \varDelta_{M/(Dien)Pt(PMEA\text{-}N1)}$
	exptl	calcd	
Mg^{2+}	1.54 ± 0.05	1.66 ± 0.04	−0.12 ± 0.06
Ca^{2+}	1.29 ± 0.04	1.51 ± 0.05	−0.22 ± 0.06
Ni^{2+}	1.89 ± 0.10	2.06 ± 0.05	−0.17 ± 0.11
Cu^{2+}	3.33 ± 0.10	3.10 ± 0.06	0.23 ± 0.12
Zn^{2+}	2.29 ± 0.07	2.29 ± 0.06	0.00 ± 0.09

[a] For the error limits see footnote [a] of Table 1. The error limits (3σ) of the derived data, in the present case for column 4, were calculated according to the error propagation after Gauss.

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The simple fact that these stability constants can be measured proves that M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ complexes are formed. How can these stability data be evaluated? In earlier studies[11, 22] a linear relationship was established between the logarithms of the stability constants of M(R-PO$_3$) complexes, $\log K^{M}_{M(R-PO_3)}$, and the negative logarithms of the acidity constants of the corresponding monoprotonated H(R-PO$_3$)$^-$ species, $pK^{H}_{H(R-PO_3)}$, for several simple phosphate monoester ligands,[22] including methyl phosphate.[23] The points for the complexes formed with phosphonates such as methanephosphonate (MeP^{2-}) or ethanephosphonate (EtP^{2-}) also fall on the same straight reference line for a given metal ion.[11] The parameters for the corresponding straight-line equations, which are defined by Equation (8), have been tabulated,[11, 18a, 28, 38] that is the slopes

$$\log K^{M}_{M(R-PO_3)} = m \cdot pK^{H}_{H(R-PO_3)} + b \qquad (8)$$

m and the intercepts b with the y axis [Eq. (8)]. Hence, with a known pK_a value for the deprotonation of a -P(O)$_2$(OH)$^-$ group an expected stability constant can be calculated for any phosph(on)ate-metal-ion complex.

Plots of $\log K^{M}_{M(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ according to Equation (8) are shown in Figure 4 for the 1:1 complexes of Ca^{2+}, Ni^{2+} and Cu^{2+}, as examples, with the data points (empty circles) of the eight simple ligand systems used[11] for the determination of the straight baselines. The three solid points refer to the corresponding M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ complexes; those for the Ca^{2+} and Ni^{2+} species are below their reference line indicating a reduced stability due to charge repulsion of (Dien)Pt^{2+} at N1. However, the data point for Cu[(Dien)Pt(PMEA-*N1*)]$^{2+}$ is clearly above its reference line, proving an increased complex stability, which must mean[40] that the phosphonate-coordinated Cu^{2+} is interacting with a further binding site. Since N3 and N7 of the adenine residue (see Figure 1) are close to the (Dien)Pt(N1)$^{2+}$ site, it is not likely that chelates with one of these nitrogens are formed because this would lead to an increase in charge repulsion. Hence, one has to conclude that the ether oxygen allowing the formation of five-membered chelates, as indicated in Equilibrium (1), is the most likely site responsible for the increase in stability. Indeed, this agrees with the properties observed earlier for the M(PMEA) complexes,[9, 11] and in accord herewith it is evident from Figure 4 that all M(PMEA) complexes are more stable than expected from their reference line and also than the M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ species, whereas all of the M[(Dien)Pt(PMEA-*N7*)]$^{2+}$ complexes are even less stable.

2.5. Evaluation of the stabilities of the M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ complexes and comparisons with related species: With Equation (8) and the corresponding parameters[11, 28, 38] stability constants can be calculated (calcd) which reflect the stability of the complexes expected solely on the basis of the basicity of the -PO$_3^{2-}$ group in (Dien)Pt(PMEA-*N1*); these values are listed in column 3 of Table 2. Comparison of the measured and calculated constants according to Equation (9) furnishes the stability differences listed in the final column of

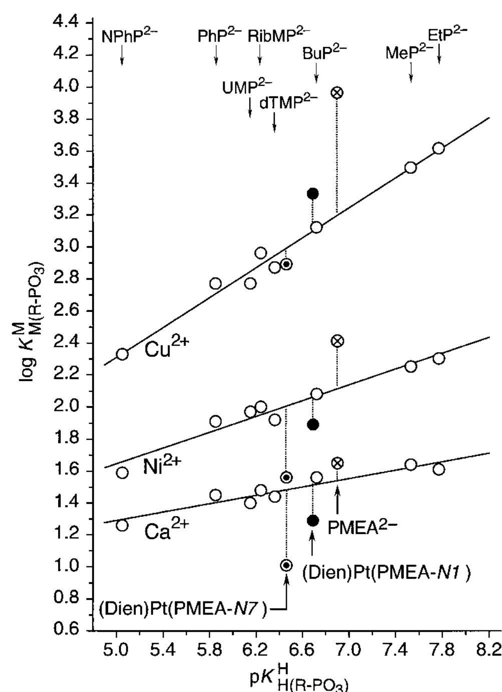

Figure 4. Evidence for a reduced stability of the M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ (●) and M[(Dien)Pt(PMEA-*N7*)]$^{2+}$ (⦿) complexes compared with the enhanced stability of the M(PMEA) complexes (⊗) based on the relationship between $\log K^{M}_{M(R-PO_3)}$ and $pK^{H}_{H(R-PO_3)}$ for M(R-PO$_3$) complexes of some simple phosphate monoester and 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 ethanephosphate (EtP^{2-}) (from left to right). The least squares lines [Eq. (8)][11, 18a, 28, 38] are drawn through the corresponding eight data sets (○) taken from [22] for the phosphate monoesters and from [11] for the phosphonates. The points due to the equilibrium constants for the M^{2+}/(Dien)Pt(PMEA-*N1*) systems (●) are based on the values listed in Tables 1 and 2; those for the M^{2+}/(Dien)Pt(PMEA-*N7*) (⦿) and the M^{2+}/PMEA (⊗) systems are taken from refs. [17] and [11], respectively. The vertical broken lines correspond to the stability differences to the reference lines; they equal $\log \Delta_{M/(Dien)Pt(PMEA-N1)}$ as defined in Equation (9) for the M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ complexes; the analogous definition holds for the other complexes. All the plotted equilibrium constants refer to aqueous solution at 25 °C and $I = 0.1$ M (NaNO$_3$).

Table 2; they correspond to the broken vertical lines seen in Figure 4.

$$\log \Delta_{M/(Dien)Pt(PMEA-N1)} = \log K^{M}_{M[(Dien)Pt(PMEA-N1)]exptl} - \log K^{M}_{M[(Dien)Pt(PMEA-N1)]calcd} \qquad (9)$$

The negative stability differences for the M[(Dien)-Pt(PMEA-*N1*)]$^{2+}$ complexes of Mg^{2+}, Ca^{2+}and Ni^{2+} are identical within their limits of error (Table 2, column 4), giving on average $\log \Delta_{av} = -0.17 \pm 0.06$. It appears that this value reflects the repulsion between M^{2+} coordinated at the -PO$_3^{2-}$ group and the twofold positively charged (Dien)Pt^{2+} located at N1 of PMEA^{2-}. Of course, this (Dien)Pt^{2+} unit also has an effect on the deprotonation of the -P(O)$_2$(OH)$^-$ residue as already discussed in Section 2.3, but as one might expect, this repulsive effect is somewhat larger on the binding of dipositively charged metal ions than on that of the singly charged proton. Therefore, the data points for the three

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mentioned quaternary complexes fall below the reference lines (Table 2, column 4) as seen for the examples (Ca^{2+}/Ni^{2+}) in Figure 4.

However, the Ca^{2+} and Ni^{2+}complexes of (Dien)Pt(PMEA-*N7*) are even less stable than the corresponding M[(Dien)-Pt(PMEA-*N1*)]$^{2+}$ species (Figure 4). In fact, the data points for the M[(Dien)Pt(PMEA-*N7*)]$^{2+}$ complexes of Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+} and Cd^{2+} are on average -0.42 ± 0.04 log units below their reference lines,[17] that is all of these complexes behave identically, only again the Cu^{2+} and Zn^{2+} complexes show an increased stability which is attributable to a contribution from the chelated species in Equilibrium (1).[17]

If one considers only those complexes where the metal ion coordinates solely to the -PO_3^{2-} group, one is faced with the striking observation that for M[(Dien)Pt(PMEA-*N7*)]$^{2+}$ $\log \Delta_{av} = -0.42 \pm 0.04$[17] and for M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ $\log \Delta_{av} = -0.17 \pm 0.06$; that is the (Dien)$Pt^{2+}$ unit at N1 affects metal-ion binding at the phosphonate group by 0.25 ± 0.07 log units less than the same unit at N7 of $PMEA^{2-}$. This difference is difficult to explain because the inhibitory effect for the M[*cis*-$(NH_3)_2$Pt(dGuo-*N7*)(dGMP-*N7*)]$^{2+}$ complexes is with -0.2 log unit[31] also very moderate despite the N7-bound platinum(II). Possibly the access of the metal ion to the phosphonate group is especially hindered by the outer-sphere macrochelate in (Dien)Pt(PMEA-*N7*) (see Section 2.3). In any case, more examples are needed before a conclusive explanation can be offered. At this point it needs to be re-emphasized that the $\log \Delta_{M/PMEA}$ values [defined in analogy to Eq. (9)] are *positive* for all M(PMEA) complexes studied, that is for the metal ions Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}.[9, 11]

The above considerations regarding the $\log \Delta_{av}$ values for the M[(Dien)Pt(PMEA-*N1*/*N7*)]$^{2+}$ complexes tend to suggest that the inhibitory effect of the (Dien)Pt^{2+} unit in a given system should be the same for all metal ions; in fact, this was already confirmed for the M[(Dien)Pt(PMEA-*N7*)]$^{2+}$ complexes.[17] To evaluate the situation for the M[(Dien)-Pt(PMEA-*N1*)]$^{2+}$ complexes we have listed the stability enhancements $\log \Delta_{M/PME\text{-}R}$ in column 2 of Table 3 [defined in analogy to Eq. (9)], which are due to complexes formed with a 2-(phosphonomethoxy)ethyl chain with a non-coordinating residue R of the approximate size of a nucleobase.[41] These $\log \Delta_{M/PME\text{-}R}$ values differ somewhat for the various metal ions as one would expect because the formation degree of the chelate in Equilibrium (1) should depend on the kind of metal ion involved.

Table 3. Stability constant comparison, $\log \Delta_{corr}$ (column 5), for the M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ complexes between the potentiometrically measured stability constants [exptl; Eq. (7)] taken from column 2 in Table 2 and the calculated as well as for the metal-ion–ether oxygen interaction corrected stability constants (calcd/corr) (aqueous solution; 25 °C; $I = 0.1$ M, $NaNO_3$).[a]

M^{2+}	$\log \Delta_{M/PME\text{-}R}$[b]	$\log K^{M}_{M[(Dien)Pt(PMEA\text{-}N1)]}$		$\log \Delta_{corr}$[d]
		exptl	calcd/corr[c]	
Mg^{2+}	0.16 ± 0.04	1.54 ± 0.05	1.82 ± 0.06	-0.28 ± 0.08
Ca^{2+}	0.12 ± 0.05	1.29 ± 0.04	1.63 ± 0.07	-0.34 ± 0.08
Ni^{2+}	0.14 ± 0.07	1.89 ± 0.10	2.20 ± 0.09	-0.31 ± 0.13
Cu^{2+}	0.48 ± 0.07	3.33 ± 0.10	3.58 ± 0.09	-0.25 ± 0.13
Zn^{2+}	0.29 ± 0.07	2.29 ± 0.07	2.58 ± 0.09	-0.29 ± 0.11

[a] For the error limits (3σ) and the error propagation see footnote [a] of Table 2. [b] These differences are defined in analogy to Equation (9); they are taken from Table IV in [41]. [c] The corrected, calculated stability constants were obtained by adding to the calculated values given in column 3 of Table 2 the stability enhancements, $\log \Delta_{M/PME\text{-}R}$ (column 2),[b][41] which result from the M^{2+}–ether oxygen interaction [Eq. (1)] in M(PME-R) complexes. [d] These values correspond to the differences between columns 3 and 4.

If the $\log \Delta_{M/PME\text{-}R}$ values (Table 3, column 2) are added to the calculated values based on Equation (8) (Table 2, column 3) one obtains the "corrected" stability constants listed in column 4 of Table 3. These values reflect the expected stabilities of M(PMEA) complexes in which no nucleobase–metal-ion coordination occurs. Formation of the difference between these values and those determined experimentally (Table 3, column 3) reflects then the charge repulsion of (Dien)Pt^{2+} at N1 on the overall stability of the M(PMEA) complexes involved in Equilibrium (1).

Of course, introduction of a positive charge at the adenine residue of $PMEA^{2-}$ should affect complex formation with all divalent metal ions to the same extent as already indicated above. In fact, all the values given for $\log \Delta_{corr}$ in column 5 of Table 3 are *identical* within their error limits, the arithmetic mean of the five values being $\log \Delta_{corr/av} = -0.29 \pm 0.05$ (3σ). This proves that the stability of all M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ complexes is diminished to the same extent, provided the overall stability of the M(PMEA) complexes occurring in Equilibrium (1) is used as a basis for comparison. It is interesting to note that $\log \Delta_{corr/av} = -0.59 \pm 0.05$ for the M[(Dien)Pt(PMEA-*N7*)]$^{2+}$ series of complexes;[17] hence, we have again a difference in the effects of the (Dien)Pt^{2+} unit between the N1- and N7-coordinated types, which is of the same order $[0.30 \pm 0.07 = (-0.29 \pm 0.05) - (-0.59 \pm 0.05)]$ as the one discussed above (0.25 ± 0.07).

2.6. Comparison of the extent of chelate formation in the two isomeric M[(Dien)Pt(PMEA-*N1*/*N7*)]$^{2+}$ complexes and in the M(PMEA) species: The insights gained in Section 2.5 now allow to further evaluate the results listed in column 4 of Table 2. The value $\log \Delta_{av} = -0.17 \pm 0.06$ (see Section 2.5) obtained from the Mg^{2+}, Ca^{2+} and Ni^{2+} complexes formed with (Dien)Pt(PMEA-*N1*) reflects the decrease in stability expected if only the "open" (op) isomer in Equilibrium (1) is formed. This means that the difference of the differences according to Equation (10) is a reflection of the formation degree of the chelate in Equilibrium (1).

$$\Delta \log \Delta = \log \Delta_{M/(Dien)Pt(PMEA\text{-}N1)} - \log \Delta_{M/(Dien)Pt(PMEA\text{-}N1)/av} \quad (10)$$

The corresponding values for the Cu^{2+} and Zn^{2+} systems are given in entries 2 and 3 of Table 4.

If we term the chelated isomer in Equilibrium (1) as "closed" (cl), the intramolecular equilibrium constant, K_I, for Equilibrium (1) is defined by Equation (11a) which is given as Equation (11b) in a more general form:

$$K_I = \frac{[M[(Dien)Pt(PMEA\text{-}N1)]^{2+}_{cl}]}{[M[(Dien)Pt(PMEA\text{-}N1)]^{2+}_{op}]} \quad (11a)$$

$$= \frac{[M(L)_{cl}]}{[M(L)_{op}]} \quad (11b)$$

The stability enhancement, $\Delta\log \varDelta$ [Eq. (10)], due to chelate formation, is connected with K_I by Equation (12),[11, 28, 38, 40]

$$K_I = 10^{\Delta\log \varDelta} - 1 \quad (12)$$

and the percentage of the closed species follows from Equation (13):

$$\%\ M(L)_{cl} = 100 \cdot K_I/(1+K_I) \quad (13)$$

The corresponding results for the Cu[(Dien)Pt(PMEA-*N1*)]$^{2+}$ and Zn[(Dien)Pt(PMEA-*N1*)]$^{2+}$ complexes are listed in columns 5 and 6 of Table 4 (entries 2, 3); related results are summarized in entries 4–13.[9, 11, 17]

Table 4 allows several interesting comparisons, for example: i) The formation degree of the chelate in Equilibrium (1) is identical within the error limits for the Cu^{2+} complexes of (Dien)Pt(PMEA-*N1*) and (Dien)Pt(PMEA-*N7*) (entries 2, 5). The same is true for the corresponding Zn^{2+} complexes (entries 3, 6). ii) For all other M[(Dien)Pt(PMEA-*N1*/*N7*)]$^{2+}$ complexes studied no indication for chelate formation exists. Points i) and ii) are in accord with other observations[40] and indicate that the affinity of Cu^{2+} and Zn^{2+} toward ether oxygen is especially pronounced; this is also evident from the values listed for log $\varDelta_{M/PME\text{-}R}$ in column 2 of Table 3.[41]

Table 4. Extent of chelate formation according to Equilibrium (1) for the M[(Dien)Pt(PMEA-*N1*)]$^{2+}$, M[(Dien)Pt(PMEA-*N7*)]$^{2+}$ and M(PMEA) complexes as quantified by the dimensionless equilibrium constant K_I [Eqs. (11) and (12)] and the percentage of the chelated isomer $M(L)_{cl}$ [Eq. (13)] (aqueous solution; 25 °C; $I = 0.1$ M, $NaNO_3$).[a]

No.	Ligand (L)	M^{2+}	$\Delta\log \varDelta$[b]	K_I	% $M(L)_{cl}$
1	(Dien)Pt(PMEA-*N1*)	[c]			
2		Cu^{2+}	0.40 ± 0.13	1.51 ± 0.75	60 ± 12
3		Zn^{2+}	0.17 ± 0.11	0.48 ± 0.37	32 ± 17
4	(Dien)Pt(PMEA-*N7*)	[c]			
5		Cu^{2+}	0.32 ± 0.08	1.09 ± 0.38	52 ± 9
6		Zn^{2+}	0.17 ± 0.09	0.48 ± 0.31	32 ± 14
7	$PMEA^{2-}$	[d]			
8		Mg^{2+}	0.16 ± 0.05[e]	0.45 ± 0.17	31 ± 8
9		Ca^{2+}	0.11 ± 0.07[e]	0.29 ± 0.21	22 ± 13
10		Mn^{2+}	0.21 ± 0.08[e]	0.62 ± 0.29	38 ± 11
11		Ni^{2+}	0.30 ± 0.07[e]	(see ref. [9])	50 ± 8[f]
12		Cu^{2+}	0.77 ± 0.07[e]	(see ref. [9])	83 ± 3[f]
13		Zn^{2+}	0.30 ± 0.10[e, g]	1.00 ± 0.46	50 ± 12

[a] For the error limits (3σ) and the error propagation see footnote [a] of Table 2. [b] Defined according (or in analogy) to Equation (10). [c] No evidence was found for chelate formation [Eq. (1)] for the (Dien)Pt(PMEA-*N1*) complexes with Mg^{2+}, Ca^{2+} and Ni^{2+} (this work), and also not for those of (Dien)Pt(PMEA-*N7*) with Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+} and Cd^{2+}.[17] [d] Chelate formation according to Equilibrium (1) also occurs in the $PMEA^{2-}$ complexes of Sr^{2+}, Ba^{2+}, Co^{2+} and Cd^{2+}.[9, 11] [e] These values are defined in refs. [9, 11] as log $\varDelta_{M/PMEA}$. [f] For the Ni(PMEA) and Cu(PMEA) systems the situation is somewhat more complicated[11, 18] because in addition a third isomer exists in which the metal ion is bound to the phosphonate group, the ether oxygen and also to N3 of the adenine moiety (hence, there are two chelate rings in this isomer);[9, 15] from the percentages given above 31 ± 14% and 49 ± 10% are due to this third isomer in the case of Ni(PMEA) and Cu(PMEA), respectively (for further details refs. [9] and [25] should be consulted, but note, the ether oxygen is also involved in this third isomer). [g] Estimated value (see also refs. [9, 11]).

It is further worthwhile to point out that in the absence of the (Dien)Pt^{2+} unit either from N1 or N7, that is if "free" $PMEA^{2-}$ is considered, all ten metal ions studied so far[9, 11] undergo chelate formation with the ether oxygen (Table 4, entries 7–13); even in the case of the Sr^{2+} or Ba^{2+} complexes still a formation degree of about 15% is reached for the chelate.[9, 11]

3. Conclusion

The absolute stability constants of the M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ and M[(Dien)Pt(PMEA-*N7*)]$^{2+}$ complexes for a given metal ion differ somewhat (see, e.g., Figure 4) but the structures of these complexes in solution are quite alike (Table 4). For the complexes with Cu^{2+} and Zn^{2+} Equilibrium (1) is of some relevance, whereas for all the other metal-ion complexes only the open isomer with a sole phosphonate–M^{2+} interaction is of significance. This is rather different for the various parent M(PMEA) complexes for which the chelated species are always of importance even though their formation degree varies between about 15 and 80% (Table 4).[9, 11, 25]

However, the most important result of this study, in a general sense, is the fact that both complexes, (Dien)-Pt(PMEA-*N1*) and (Dien)Pt(PMEA-*N7*), can still act as ligands by binding metal ions through their phosphonate groups. Considering that $PMEA^{2-}$ is a nucleotide analogue, it is evident that this result also applies to purine-nucleoside monophosphates and indeed, for some GMP complexes with an N7-bound Pt^{II} this is also known.[31, 32] Taking together the present and the earlier[17, 31, 32] results, one may conclude that the affinity of the phosph(on)ate group toward divalent metal ions is reduced only by some modest 0.2–0.4 log units by binding of another divalent metal ion to one of the nitrogens of a purine nucleobase. This observation is important because it means that the participation of two (or possibly even more) metal ions in an enzymatic process or in a ribozyme catalysis is not seriously hampered by charge repulsion.

4. Experimental Section

4.1. Synthesis of [(Dien)Pt(PMEA-*N1*)]·HNO_3·0.5 $NaNO_3$·2 H_2O: The materials for the synthesis were the same as used previously[17] for the preparation of (Dien)Pt(PMEA-*N7*).

A solution of [(Dien)Pt(H_2O)]$(NO_3)_2$ (0.413 mmol), obtained from [(Dien)PtI]I[42] and $AgNO_3$ (2 equiv) in water (20 mL) in the dark (24 h at 35 °C) and subsequent cooling to 4 °C followed by filtration of AgI, was brought to pH about 4.5 with 1 M HNO_3. An aqueous solution (10 mL) of PMEA (0.413 mmol) was adjusted to the same pH. The PMEA solution was then slowly added over a 3 h period to the above-mentioned reaction mixture and the resulting solution stirred for 24 h at 35 °C before being brought to dryness (room temperature, N_2 stream). The pale yellow residue was treated twice with methanol (12 h, ambient temperature) and filtered. Isolated yield 41%.

Elemental analysis, potentiometric pH titrations and NMR measurements were consistent with the composition of [(Dien)Pt(PMEA-*N1*)]·HNO_3·0.5 $NaNO_3$·2 H_2O. Anal. calcd (%) for $C_{12}H_{28}N_{9.5}O_{10.5}PPtNa_{0.5}$: C 20.3, H 4.0, N 18.7; found: C 20.3, H 3.8, N 18.3; ^{1}H NMR (D_2O; pD 6.4; TSP as internal standard): δ = 8.60 (s, H2; PMEA), 8.27 (s, H8; PMEA), 4.43 (t, N-CH_2; PMEA), 3.93 (t, O-CH_2; PMEA), 3.56 (d, O-CH_2-PO_3, PMEA).

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4.2. Determination of the structure of (Dien)Pt(PMEA-*N1*) by NMR experiments: Figure 5 shows the ^{1}H,^{1}H-ROESY spectrum of the above compound at pD 6.4. In this spectrum, the cross peak between the H8 proton and the N9-bound methylene moiety is observed at $\delta = 8.27$ and 4.43. Consequently, the other signal in the aromatic region at $\delta = 8.60$ has to correspond to the H2 proton. This assignment agrees with the observations made for the N7-coordinated compound synthesized previously[17] and also with the longitudinal relaxation time T_1 as determined by an inversion recovery experiment, which is about three times larger for the signal of the H2 proton at $\delta = 8.60$ compared with the one of the H8 proton at $\delta = 8.27$. This increased relaxation time is typical for adenine H2 protons.[43] The platinum(II)-facilitated H–D exchange observed[17] for the H8 proton of (Dien)Pt(PMEA-*N7*) is not observed in the ^{1}H NMR spectra of the N1-coordinated compound studied now.

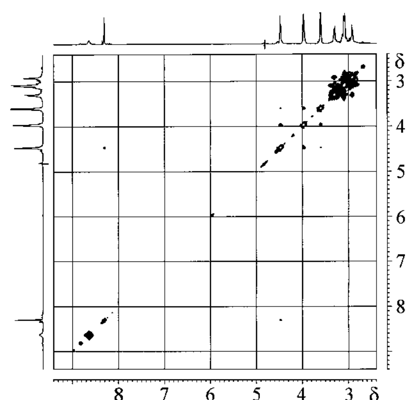

Figure 5. ^{1}H,^{1}H-ROESY spectrum of (Dien)Pt(PMEA-*N1*) in D_2O at pD 6.4 (mixing time 300 ms) with cross-peaks between H8 and N-CH_2.

In the ^{195}Pt,^{1}H-HMQC spectrum, the ^{1}H NMR signal detected at lowest field ($\delta = 8.60$) is assigned to H2 because it couples with the ^{195}Pt NMR signal, thus proving that the platination site is N1. A further indication that the assignment of the platination site is correct, stems from the close similarity of the ^{1}H and ^{195}Pt NMR data obtained now and the data published for the (Dien)Pt(adenosine-*N1*) complex.[44]

A further interesting feature of the ^{195}Pt NMR spectrum is that the signal of the N1-linkage isomer is split ($\delta_1 = -2939$, $\delta_2 = -2923$; relative intensities ca. 2:1). A similar splitting could not be detected unambiguously with the N7-coordinated complex. Such a splitting of ^{195}Pt NMR resonances at ambient temperature was observed before with the (Dien)Pt^{2+} complexes of adenosine (both linkage isomers)[44] and was tentatively attributed to a conformational change of the Dien ligand and/or a restricted rotation about the platinum–nucleoside bond (with Dien in a fixed conformation).

4.3. Materials, equipment and methods: Besides the complex described in Section 4.1, all the other materials needed in the spectrophotometric measurements (Section 4.4) and the potentiometric pH titrations (Section 4.5) were identical with those used before.[17] This is also true for the equipment and the calculation procedures.

The stock solutions of the ligand, i.e., of (Dien)Pt(PMEA-*N1*), were freshly prepared daily by dissolving the compound (Section 4.1) in ultrapure water with one equivalent NaOH and adjusting pH 8.5; the exact concentration was newly determined each time by the evaluation of the corresponding titration pairs described below in Section 4.5.

4.4. Spectrophotometric determination of the first acidity constant of H_3[(Dien)Pt(PMEA-*N1*)]$^{3+}$: The acidity constant, $K^{H}_{H_3[(Dien)Pt(PMEA\text{-}N1)]}$ [Eq. (2)], which refers to the deprotonation of the (N7)H^+ site (Figure 1), was determined by recording UV spectra (sample beam: $HClO_4$, $NaClO_4$ and [(Dien)Pt(PMEA-*N1*)] $= 2.5 \times 10^{-5}$ M or 2.7×10^{-5} M; reference beam: $HClO_4$ and $NaClO_4$) in aqueous solutions at 25 °C in dependence on pH with 2 cm quartz cells. The ionic strength was adjusted to $I = 0.1$ M ($NaClO_4$) in those instances where [$HClO_4$] < 0.1 M; no adjustment was made in solutions with pH ≤ 1, i.e., where [$HClO_4$] $\geqslant 0.1$ M. For further details see ref. [17]; an example of an experimental series is shown in Figure 2 and the corresponding evaluation of the data is exemplified in Figure 3. The final result given in Table 1 is the average of two independent experimental series.

To obtain a well defined absorption of the threefold protonated species, H_3[(Dien)Pt(PMEA-*N1*)]$^{3+}$, it was necessary to record spectra in rather strong acidic solutions. Since the activity coefficients of $HClO_4$ in higher concentrations differ significantly from 1, the procedure described previously[17] was also used now to obtain the interrelation between the concentration of $HClO_4$ and the H^+ activity which provides the pH of the corresponding solution. In the following list the first value refers to the calculated pH and second one given in parentheses to the concentration of $HClO_4$: pH = −0.86 ([$HClO_4$] = 4.84 M), −0.64 (3.63 M), −0.37 (2.42 M), −0.16 (1.69 M), +0.01 (1.21 M), 0.16 (0.91 M), 0.30 (0.67 M), 0.39 (0.54 M), 0.50 (0.42 M), 0.57 (0.36 M), 0.65 (0.30 M), 0.75 (0.24 M), 0.87 (0.18 M), and 1.04 (0.12 M). It may be added that the values measured with the glass electrode were used starting from pH 1.18 ([$HClO_4$] = 0.0605 M) (see also the second to the final paragraph in Section 4.5).

4.5. Determination of equilibrium constants by potentiometric pH titrations: The acidity constant $K^{H}_{H[(Dien)Pt(PMEA\text{-}N1)]}$ [Eq. (4)] of H[(Dien)-Pt(PMEA-*N1*)]$^+$ was determined by titrating 30 mL of an aqueous 0.0008 M HNO_3 in the presence and absence of 0.00041 M (Dien)-Pt(PMEA-*N1*) under N_2 with 1 mL 0.03 M NaOH (25 °C; $I = 0.1$ M, HNO_3). In a second set of experiments the same acidity constant was determined by titrating 30 mL of an aqueous 0.0008 M HNO_3 in the presence and absence of 0.00025 to 0.00029 M (Dien)Pt(PMEA-*N1*) under N_2 with 1.5 mL 0.02 M NaOH (25 °C; $I = 0.1$ M, $NaNO_3$). The acidity constant was calculated as described in ref. [17].

The stability constants $K^{M}_{M[(Dien)Pt(PMEA\text{-}N1)]}$ [Eq. (7)] of the M[(Dien)-Pt(PMEA-*N1*)]$^{2+}$ complexes were determined either under the conditions given in the preceding paragraph for the acidity constant with $NaNO_3$ being partly or fully replaced by $M(NO_3)_2$ ($I = 0.1$ M; 25 °C), or the solutions used for the determination of the acidity constant, $K^{H}_{H[(Dien)Pt(PMEA\text{-}N1)]}$, were used again because only small amounts of (Dien)Pt(PMEA-*N1*) were available; i.e., the solutions were acidified with the equivalent amount of HNO_3 as NaOH had been used in the titration for the acidity constant, and then $M(NO_3)_2$ was added (volume 50 mL; $I = 0.1$ M, $NaNO_3$) and the titration repeated with NaOH (of course, the various dilutions were taken into account in the calculations).

The M^{2+}:ligand ratios employed in the experiments were approximately 88:1 for Mg^{2+}, 81:1 for Ca^{2+}, 58:1 or 41:1 for Ni^{2+}, 65:1 or 46:1 for Zn^{2+} and 13:1 or 8:1 for Cu^{2+}. The stability constants of the M[(Dien)Pt(PMEA-*N1*)]$^{2+}$ complexes were calculated with the "apparent" acidity constants as in ref. [17].[24, 45]

The final result for the acidity constant, $K^{H}_{H[(Dien)Pt(PMEA\text{-}N1)]}$ [Eq. (4)], is the average of eight independent pairs of titrations. It may be added that the direct pH-meter readings were used in the calculations of the acidity constants (see also Section 4.4), i.e., these constants are so-called practical, mixed or Brønsted constants.[46] Their negative logarithms may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values;[46] this conversion term contains both, the junction potential of the glass electrode and the hydrogen ion activity.[46, 47] As the difference in NaOH consumption between pairs of solutions, i.e., with and without ligand (see above),[46] is evaluated, the ionic product of water (K_w) and the conversion term mentioned do not enter into the calculations.

The stability constants, $K^{M}_{M[(Dien)Pt(PMEA\text{-}N1)]}$ [Eq. (7)], of the M[(Dien)-Pt(PMEA-*N1*)]$^{2+}$ complexes are as usual concentration constants. The stability constants calculated individually for the various experiments showed no dependence on the metal-ion concentration. The final results are in each case the averages of at least two different pairs of titrations.

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