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Acid–Base and Metal-Ion-Binding Properties of 9-[2-(2-Phosphonoethoxy)ethyl]adenine (PEEA), a Relative of the Antiviral Nucleotide Analogue 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA). An Exercise on the Quantification of Isomeric Complex Equilibria in Solution

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The acidity constants of 3-fold protonated 9-[2-(2-phosphonoethoxy)ethyl]adenine, $H_3(PEEA)^+$, and of 2-fold protonated (2-phosphonoethoxy)ethane, $H_2(PEE)$, and the stability constants of the $M(H;PEEA)^+$, M(PEEA), and M(PEE) complexes with M^{2+} = Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} have been determined (potentiometric pH titrations; aqueous solution; 25 °C; I = 0.1 M, $NaNO_3$). It is concluded that in the $M(H;PEEA)^+$ species, the proton is at the phosphonate group and the metal ion at the adenine residue. The 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 residue that does not affect metal-ion binding, proves that the M(PEEA) complexes of Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} as well as the M(PEE) complexes of Co^{2+}, Cu^{2+}, and Zn^{2+} have larger stabilities than is expected for a sole phosphonate coordination of M^{2+}. For the M^{2+} complexes without an enhanced stability (e.g., Mg^{2+} or Mn^{2+}), it is concluded that M^{2+} binds in a monodentate fashion to the phosphonate group of the two ligands. Combination of all of the results allows the following conclusions: (i) The increased stability of the Co(PEE), Cu(PEE), Zn(PEE), and Co(PEEA) complexes is due to the formation of six-membered chelates involving the ether-oxygen atom of the aliphatic residue ($-CH_2-O-CH_2CH_2-PO_3^{2-}$) of the ligands with formation degrees of about 15–30%. (ii) Cd(PEEA) forms a macrochelate with N7 of the adenine residue (formation degree about 30%); Ni(PEEA) has similar properties. (iii) With Zn(PEEA), both mentioned types of chelates are observed, that is, $Zn(PEEA)_{cl/O}$ and $Zn(PEEA)_{cl/N7}$, with formation degrees of about 13 and 41%, respectively; the remaining 46% is due to the "open" isomer $Zn(PEEA)_{op}$ in which the metal ion binds only to the PO_3^{2-} group. (iv) Most remarkable is Cu(PEEA) because a fourth isomer, $Cu(PEEA)_{cl/O/N3}$, is formed that contains a six-membered ring involving the ether oxygen next to the phosphonate group and also a seven-membered ring involving N3 of the adenine residue with a very significant formation degree of about 50%. Hence, $PEEA^{2-}$ is a truly ambivalent ligand, its properties being strongly dependent on the kind of metal ion involved. Comparisons with M^{2+} complexes formed by the dianions of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) and related ligands reveal that five-membered chelates involving an ether-oxygen atom are considerably more stable than the corresponding six-membered ones. This observation offers an explanation of why PMEA is a nucleotide analogue with excellent antiviral properties and PEEA is not.

1. Introduction

The use of nucleotide analogues as therapeutic agents has a long tradition,[1,2] and all building blocks of a nucleotide have been altered and varied over the years (see, for example, ref 3 and the refs therein). Within the large group of acyclic

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nucleotide analogues (see refs 4 and 5 and the refs therein),[‖] 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA)[6] turned out to be an especially successful compound; it is active against a wide range of viruses,[5] including herpes viruses, poxviruses,[7] hepadnaviruses, and retroviruses, and it also has antineoplastic and immunomodulatory activities.[5] In 2002, PMEA, now known as *Adefovir*,[5] was approved by the U.S. Food and Drug Administration (FDA)[8] in its oral prodrug form, that is, its bis(pivaloyloxymethyl)ester form (Adefovir dipivoxil),[5] for the treatment of hepatitis B patients who suffer from the infection of a DNA virus. For the same treatment, the same compound, but under the name *Hepsera*, was also approved in early 2003 for "community marketing" by the European Agency for the Evaluation of Medicinal Products (EMEA).[5,9]

The dianion of PMEA, which can be considered as an analogue of (2′-deoxy)adenosine 5′-monophosphate [(d)AMP^{2-}] (see Figure 1),[10−14] or maybe even more precisely, as an analogue of 2′,3′-bisdeoxyadenosine 5′-monophosphate, is converted in the cells[5] to its diphosphorylated form, PMEApp^{4-}, which is an analogue of (2′-deoxy)adenosine 5′-triphosphate [(d)ATP^{4-}]. This triphosphate analogue is initially recognized by nucleic acid polymerases as a substrate and incorporated in the growing nucleic acid chain, which is then terminated because of the lack of a 3′-hydroxy group. In the polymerase reaction, one of the two metal ions[15] needs to be coordinated to the β,γ-phosphate units and the other to the α-phosphate group[16] to promote the transfer of a nucleotidyl residue.[17] The observation[18,19] that PMEApp^{4-} is initially a better substrate than the parent ATP^{4-} was

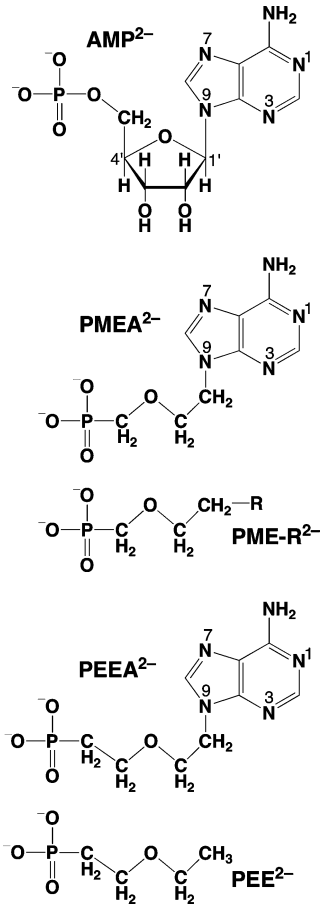
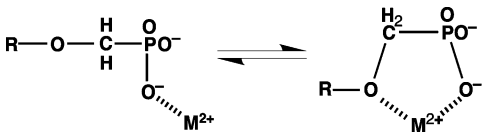


Figure 1. Chemical structures of adenosine 5′-monophosphate (AMP^{2-}) and the dianions of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA^{2-} = Adefovir)[5] and 9-[2-(2-phosphonoethoxy)ethyl]adenine (PEEA^{2-}), together with the structures of PME−R^{2-}, i.e., a derivative of (phosphonomethoxy)-ethane (= PME^{2-} = ethoxymethanephosphonate) with a noninteracting residue R,[10] and PEE^{2-} {= dianion of (2-phosphonoethoxy)ethane = [2-(2-ethoxy)ethyl]phosphonate}. PME−R^{2-} and PEE^{2-} represent the metal-ion-coordinating properties of the ether-phosphonate chains occurring in PMEA^{2-} and PEEA^{2-}, respectively. A further ligand to be considered in this study is 9-(5-phosphonopentyl)adenine,[11] which is abbreviated as dPEEA^{2-} (= 3′-deoxa-PEEA^{2-}) to indicate that its structure corresponds to that of PEEA^{2-} except that the ether-O atom is replaced by a CH_2 group. It should also be noted that AMP^{2-} is shown in its dominating anti conformation[12,13] and that the orientation of PMEA^{2-} in solution[14] resembles this anti conformation.

rationalized by the suggestion[20,21] that the ether-oxygen atom present in PMEA facilitates the M^{2+}/α-phosph(on)ate coordination and, thus, the transfer of a nucleotidyl residue by the formation of a five-membered chelate ring, as is expressed in a simplified manner in equilibrium 1:



(1)

In fact, it is well-known that this ether oxygen is crucial for the biological activity of PMEA and that its replacement

‖ For Abbreviations and Definitions, see end of paper.

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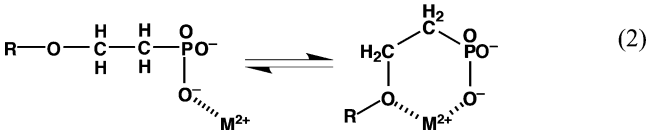
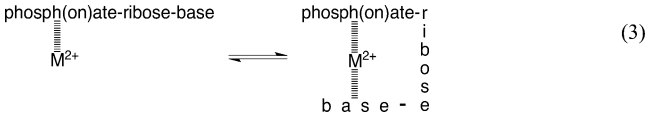
by an S atom[22] or a CH_2 unit[18] results in inactive compounds. Similarly, the position of this O atom within the aliphatic chain is crucial; already, the insertion of one additional CH_2 unit deprives the resulting analogue, that is, 9-[2-(2-phosphonoethoxy)ethyl]adenine (PEEA; see Figure 1), of any useful antiviral activity.[19,23] For this reason, we decided to study the coordination chemistry of $PEEA^{2-}$ in detail and to compare it with that of[21,24,25] $PMEA^{2-}$. Considering that equilibrium 1, with the formation of a five-membered chelate, is apparently crucial for the biological activity of PMEA, we initially focused our attention especially on the position of equilibrium 2, in which a six-membered chelate is formed:



(2)

In other words, the question was: are five-membered rings, involving an ether-oxygen atom, more stable than six-membered ones?

To be able to quantify the effect of the adenine residue on the stability of the M(PEEA) complexes, where M^{2+} = Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}, we included in the study (2-phosphonoethoxy)ethane (PEE; see Figure 1), which represents well the alkyl ether−phosphonate chain of $PEEA^{2-}$ and, of course, also allows the formation of six-membered chelates, as indicated in equilibrium 2. Such a separate evaluation of the role of the alkyl ether−phosphonate chain and, therefore, indirectly, also of the adenine residue in M(PEEA) complexes is necessary because the formation of macrochelates, according to equilibrium 3,



(3)

by an intramolecular interaction of a phosphate-bound metal ion with N7 of the adenine residue is well-known to occur in certain M(AMP) complexes.[26,27] Analogous macrochelates are also observed in M^{2+} complexes formed with 9-(5-phosphonopentyl)adenine,[11] which is abbreviated in its dianionic form as $dPEEA^{2-}$ (= 3′-deoxa-$PEEA^{2-}$) to indicate that its structure differs from that of $PEEA^{2-}$ only by the replacement of the ether-O atom by a CH_2 unit (Figure 1).

In fact, the results show that metal ions such as Mg^{2+} coordinate only to the phosphonate group of $PEEA^{2-}$ and even metal ions such as Zn^{2+} reach only a low formation degree of the six-membered chelate in equilibrium 2. Therefore, these findings are in agreement with the indicated low antiviral activity of this nucleotide analogue because, under these circumstances, no facilitated M^{2+}/α-phosph(on)ate binding is expected to occur with $PEEApp^{4-}$, in contrast to the situation with $PMEApp^{4-}$.[21] However, because of the interactions of some metal ions with the nitrogen atoms of the adenine residue, further isomeric complexes are formed; for example, four isomeric species could be quantified for Cu(PEEA), making $PEEA^{2-}$ a fascinating molecule because its coordination chemistry practically differs from metal ion to metal ion.

2. Experimental Section

2.1. Materials and Equipment. The disodium salt of [2-(2-ethoxy)ethyl]phosphonate (PEE) was prepared as described recently,[28] and PEEA was synthesized as given in ref 23 (see also ref 5). The aqueous stock solutions of the ligands were freshly prepared daily by dissolving the substances in deionized ultrapure CO_2-free water and by adding the necessary equivalents of NaOH to give a pH of about 8.5.

All of the other reagents were the same as those used in recent studies.[29−31] This also applies for the equipment employed in the potentiometric pH titrations (see also below) and their evaluations,[31] as well as for the experimental procedures regarding the determination of the concentration of the NaOH, ligand, and metal-ion-stock solutions.[29,32]

2.2. Potentiometric pH Titrations. The pH titrations for the determination of the equilibrium constants in aqueous solution were recorded with a Metrohm E536 potentiograph connected to a Metrohm E665 dosimat and a Metrohm 6.0222.100 combined macro glass electrode. The pH calibration was done with the buffer solutions (pH 4.00, 7.00, 9.00 based on the NBS scale; now NIST) obtained from Metrohm AG.

The direct pH meter readings were used in the calculations of the acidity constants; that is, these constants determined at $I = 0.1$ M ($NaNO_3$) and 25 °C are so-called practical, mixed, or Brønsted constants.[33] They may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values;[33] this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[33,34] It should be emphasized that the ionic product of water (K_w) and the mentioned conversion term do not enter into our calculation procedures because we always evaluate the differences in NaOH consumption between a pair of solutions, that is, with and without the ligand. The stability constants determined are, as usual, concentration constants.

All equilibrium constants were calculated by curve-fitting procedures using a Newton-Gauss nonlinear least-squares program in the way and with the equipment described in a recent study.[31]

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2.3. Determination of Equilibrium Constants Involving PEE. The acidity constants $K^{H}_{H_2(PEE)}$ (eq 7) and $K^{H}_{H(PEE)}$ (eq 8) of $H_2(PEE)$ and $H(PEE)^-$, respectively, where the protons are at the phosphonate group, were determined by titrating 25 mL of aqueous 0.02 M HNO_3 (25 °C; $I = 0.1$M, $NaNO_3$) in the presence and absence of 3.4 mM ligand (PEE^{2-}) under N_2 with 2.5 mL of 0.2 M NaOH. The differences in NaOH consumption between the pair of titrations were used for the calculations. The pH range from 2 to 7.6 was evaluated, and this already initially corresponds to a neutralization degree of 66% for the equilibrium $H_2(PEE)/H(PEE)^-$; at pH 7.6, a neutralization degree of 77% is reached for the $H(PEE)^-/PEE^{2-}$ system, meaning that only about 23% of the $H(PEE)^-$ species remains untitrated. The final result for $K^{H}_{H_2(PEE)}$ is the average of six pairs of independent titrations.

The acidity constant $K^{H}_{H(PEE)}$ of $H(PEE)^-$ (eq 8) was also determined by titrating 50 mL of aqueous 0.42 mM HNO_3 (25 °C; $I = 0.1$M, $NaNO_3$) in the presence and absence of 0.40 mM ligand (PEE^{2-}) under N_2 with 0.75 mL of 0.03 M NaOH. The differences in NaOH consumption between the pair of titrations were evaluated in the pH range 5.9−8.3, which corresponds approximately to pK_a ±1.2. In other words, the initial formation degree of $H(PEE)^-$ at pH 5.9 amounts to about 94%, and at pH 8.3, about 6% is left, meaning that now approximately 94% is present as PEE^{2-}. The final result for p$K^{H}_{H(PEE)}$ is the average of, in total, 46 pairs of independent titrations including the ones mentioned above; it also confirms the previous result.[28]

The stability constants $K^{M}_{M(PEE)}$ of the M(PEE) complexes (eq 13) were determined under the same conditions used for the acidity constant of $H(PEE)^-$ but $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ (25 °C; $I = 0.1$ M). The M^{2+}:ligand ratios employed were close to 83:1 (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}), 67:1 (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}), 42:1 (Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}), and 21:1 (Co^{2+}, Zn^{2+}, Cd^{2+}). The ratios for Cu^{2+}: PEE were 8.4:1 and 4.2:1 as given in ref 28.

The stability constants were calculated as described[3,29] by collecting the experimental data for the M^{2+}/PEE systems every 0.1 pH unit in the pH range up to 90% neutralization of $H(PEE)^-$ or until the beginning of the hydrolysis of $M(aq)^{2+}$; the latter was clear from the titrations without the ligand.

The individual results for the stability constants showed no dependence on pH or on the excess of metal-ion concentration used. The results are always the averages of at least five (usually six) independent pairs of titrations.

2.4. Determination of Equilibrium Constants Involving PEEA. The acidity constants $K^{H}_{H_3(PEEA)}$ (eq 4), $K^{H}_{H_2(PEEA)}$ (eq 5), and $K^{H}_{H(PEEA)}$ (eq 6) of $H_3(PEEA)^+$, $H_2(PEEA)^{\pm}$, and $H(PEEA)^-$, respectively, where one proton is at the adenine residue and the other two protons are at the phosphonate group, were determined by titrating 25 mL of aqueous 0.032 M HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 3.9 mM ligand ($PEEA^{2-}$) under N_2 with 2.7 mL of 0.3 M NaOH. Again, the differences in NaOH consumption between the pair of titrations were used for the calculations. The pH range evaluated was from 2.0 to 6.6. At pH 2.0, only about 23.5% of the $H_3(PEEA)^+$ species is left, approximately 76% of the ligand is already present as $H_2(PEEA)^{\pm}$ in addition to traces of $H(PEE)^-$. At pH 6.6, only about 0.3% of $H_2(PEEA)^{\pm}$ remains, but approximately 77% of the ligand is now present as $H(PEEA)^-$ and 22.7% as $PEEA^{2-}$. The final result for $K^{H}_{H_3(PEEA)}$ is the average of five pairs of independent titrations.

The acidity constants $K^{H}_{H_2(PEEA)}$ (eq 5) and $K^{H}_{H(PEEA)}$ (eq 6) of $H_2(PEEA)^{\pm}$ and $H(PEEA)^-$, respectively, were also determined by titrating 50 mL of aqueous 0.90 mM HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 0.30 mM ligand ($PEEA^{2-}$) under N_2 with 1.7 mL of 0.03 M NaOH. The differences in NaOH consumption between the pair of titrations were evaluated in the pH range 3.6−8.5. In this case, the initial formation degree of $H_2(PEEA)^{\pm}$ at pH 3.6 amounts to about 77%, and at pH 8.5, 4% of the ligand exists as $H(PEEA)^-$ and 96% as $PEEA^{2-}$. The final results for these two constants are the averages of, in total, 47 pairs of independent titrations, including the ones from above, and they are in accord with previous values.[28]

The stability constants $K^{M}_{M(H;PEEA)}$ (eq 12) and $K^{M}_{M(PEEA)}$ (eq 13) of the $M(H;PEEA)^+$ and M(PEEA) complexes, respectively, were determined under the same conditions used for the acidity constants of $H_2(PEEA)^{\pm}$ and $H(PEEA)^-$, but now, $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ (25 °C; $I = 0.1$ M). The M^{2+}/ligand ratios employed were close to 111:1 (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, and Ni^{2+}), 89:1 (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Ni^{2+}), 56:1 (Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}), and 28:1 (Co^{2+}, Zn^{2+}, and Cd^{2+}). The ratios for Cu^{2+}/PEEA were about 11:1 and 5.5:1, as given in ref 28.

The stability constants were calculated[35] considering the species H^+, $H_2(PEEA)^{\pm}$, $H(PEEA)^-$, $PEEA^{2-}$, M^{2+}, $M(H;PEEA)^+$, and M(PEEA) by using the experimental data at every 0.1 pH unit in the pH range up to a 90% neutralization of $H(PEEA)^+$ or until the beginning of the hydrolysis of $M(aq)^{2+}$; the latter was clear from the titrations without the ligand. However, several of the constants given for the $M(H;PEEA)^+$ complexes, especially those for the alkaline earth ion complexes, must be considered as estimates[32] (see Table 2, below) because the formation degree of these species reached only about 3%; the maximum was reached for $Mg(H;PEEA)^+$ at 7%. In all of the other instances, the maximum formation degree of $M(H;PEEA)^+$ varied between about 5 and 30%.

Finally, it needs to be emphasized that the results for the stability constants showed no dependence on the excess of metal-ion concentration employed in the various experiments. The final results are always the averages of at least five (usually six) independent pairs of titrations for each system.

2.5. Determination of the Acidity Constant of $H_3(dPEEA)^+$. For reasons of comparison, we also determined the acidity constant $K^{H}_{H_3(dPEEA)}$ (analogous to eq 4) of $H_3(dPEEA)^+$, where the proton is at the phosphonate group, together with the acidity constants for the $H_2(dPEEA)^{\pm}$ and $H(dPEEA)^-$ species by titrating 25 mL of aqueous 0.032 M HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 3.9 mM ligand ($dPEEA^{2-}$) under N_2 with 2.7 mL of 0.3 M NaOH. The differences in NaOH consumption between the pair of titrations were used for the calculations in the pH range 2.0−6.6. At pH 2.0, about 53% of the $H_3(dPEEA)^+$ species is left; approximately 46.5% of the ligand is already present as $H_2(dPEEA)^{\pm}$ in addition to small amounts of $H(dPEEA)^-$. At pH 6.6, only about 0.3% of $H_2(dPEEA)^{\pm}$ remains, but approximately 93% of the ligand is now present as $H(dPEEA)^-$ and 6.7% as $dPEEA^{2-}$. The final result for $K^{H}_{H_3(dPEEA)}$ is the average of six pairs of independent titrations; the pK_a values measured now for the $H_2(dPEEA)^{\pm}$ and $H(dPEEA)^-$ species agreed with the published ones.[11]

3. Results and Discussion

It is well-known that nucleobases and their derivatives can undergo self-association via π stacking.[12,36] Therefore, the experimental conditions for the determination of the acidity constants of $H_2(PEEA)^{\pm}$ and of the stability constants of the

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Table 1. Negative Logarithms of the Acidity Constants of $H_3(PEEA)^+$ (Eqs 4−6) Together with Those of Some Related Species as Determined by Potentiometric pH Titrations in Aqueous Solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[a,b]

		pK_a for the site				
number	acid	$P(O)(OH)_2$	(N1)H	$P(O)_2^-(OH)$	Δ p$K_{a/P(OH)/P(OH)_2}$	ref
1	$H(9MeAde)^+$		4.10 ± 0.01			38
2	$H_3(PEEA)^+$	1.49 ± 0.08	4.15 ± 0.01	7.12 ± 0.01	5.63 ± 0.08	*c*
3	$H_2(PEE)$	1.71 ± 0.10		7.07 ± 0.01	5.36 ± 0.10	*c*
4	$H_3(PMEA)^+$	1.22 ± 0.13[d]	4.16 ± 0.02	6.90 ± 0.01	5.68 ± 0.13	24
5	$H_2(PME)$	1.57 ± 0.15[e]		7.02 ± 0.01	5.45 ± 0.15	24
6	$H_3(dPEEA)^+$	2.06 ± 0.08[c]	4.17 ± 0.01	7.75 ± 0.01	5.69 ± 0.08	11
7	$H_3(dPMEA)^+$	1.98 ± 0.13	4.17 ± 0.02	7.69 ± 0.01	5.71 ± 0.13	39
8	$CH_3P(O)(OH)_2$	2.10 ± 0.03		7.51 ± 0.01	5.41 ± 0.03	40
9	$H_2(PMCh)^+$	1.11 ± 0.12[f]		6.57 ± 0.01	5.46 ± 0.12	29
10	$H_2(PMEDAPy)^+$	1.14 ± 0.15		6.62 ± 0.01	5.48 ± 0.15	30
11	$CH_3OP(O)(OH)_2$	1.1 ± 0.2		6.36 ± 0.01	5.26 ± 0.2	41
12	$H_2(UMP)$	0.7 ± 0.3		6.15 ± 0.01	5.45 ± 0.3	42
13	$H_2(FMN)$	0.7 ± 0.5		6.18 ± 0.01	5.48 ± 0.5	43
14	$H_3(AMP)^+$	0.4 ± 0.2[g]	3.84 ± 0.02	6.21 ± 0.01	5.81 ± 0.2	27, 44

[a] So-called practical, mixed, or Brønsted constants are given (see Section 2.2 and ref 33). [b] The error limits given are 3 times the standard error of the mean value (3σ) or the sum of the probable systematic errors, which ever is larger. The error limits of the derived data, in the present case, for column 6, were calculated according to the error propagation after Gauss. [c] Measured in this study. [d] Determined by ^{1}H NMR shift experiments; from ref 14. [e] Estimate; from ref 30. [f] From ref 30. [g] Determined by ^{1}H NMR shift experiments; from ref 12.

$M(H;PEEA)^+$ and M(PEEA) complexes (see below) by potentiometric pH titrations (25 °C; $I = 0.1$ M, $NaNO_3$) were selected such that the results refer to monomeric species. With ligand concentrations of 0.3 mM, this is ascertained as has been shown previously for PMEA.[24]

3.1. Acidity Constants of $H_3(PEEA)^+$ and of Related Acids. $PEEA^{2-}$ (see Figure 1) can accept three protons, two at the phosphonate group and one at the N1 site of the adenine residue.[14] Further protonations are possible at N7 and N3, but these protons are released with pK_a < 0;[37,38] therefore, they are not considered in this study. At pH > 0, the strongest acid that exists in aqueous solution based on $PEEA^{2-}$ is $H_3(PEEA)^+$. Hence, the following three deprotonation reactions need to be considered:

$$H_3(PEEA)^+ \rightleftharpoons H_2(PEEA)^{\pm} + H^+ \quad (4a)$$

$$K^H_{H_3(PEEA)} = [H_2(PEEA)^{\pm}][H^+]/[H_3(PEEA)^+] \quad (4b)$$

$$H_2(PEEA)^{\pm} \rightleftharpoons H(PEEA)^- + H^+ \quad (5a)$$

$$K^H_{H_2(PEEA)} = [H(PEEA)^-][H^+]/[H_2(PEEA)^{\pm}] \quad (5b)$$

$$H(PEEA)^- \rightleftharpoons PEEA^{2-} + H^+ \quad (6a)$$

$$K^H_{H(PEEA)} = [PEEA^{2-}][H^+]/[H(PEEA)^-] \quad (6b)$$

In the case of the adenine-free PEE^{2-} (Figure 1), only the phosphonate group can be protonated, giving the uncharged species $H_2(PEE)$; the corresponding reactions are

$$H_2(PEE) \rightleftharpoons H(PEE)^- + H^+ \quad (7a)$$

$$K^H_{H_2(PEE)} = [H(PEE)^-][H^+]/[H_2(PEE)] \quad (7b)$$

$$H(PEE)^- \rightleftharpoons PEE^{2-} + H^+ \quad (8a)$$

$$K^H_{H(PEE)} = [PEE^{2-}][H^+]/[H(PEE)^-] \quad (8b)$$

The acidity constants measured in this study for $H_3(PEEA)^+$ and $H_2(PEE)$ are listed in Table 1 together with some related data.[38−44] From entries 1−3 of Table 1, it follows that the protons of $H_3(PEEA)^+$ are released from the acidic sites in the order $P(O)(OH)_2$ > $(N1)H^+$ > $P(O)_2^-(OH)$. This order is further confirmed by the other entries in Table 1.

The data in Table 1 offer comparisons for many conclusions; some are given as follows:

(i) Entries 8 and 11 show that phosphonate groups are more basic than phosphate groups.

(ii) Entries 4 and 8 reveal that the ether oxygen in the alkyl chain of PMEA makes the protonated phosphonate group more acidic.

(iii) If the distance of the ether-O atom from the phosphonate group increases (cf. entries 2 and 3 with 4 and 5), the acidification by the O atom decreases.

(iv) The observation that the replacement of the O atom by a CH_2 unit (cf. entries 2 and 4 with 6 and 7) leads to an increased basicity of the phosphonate group agrees with points ii and iii.

(v) All adenine-nucleotide analogues (entries 2, 4, 6, and 7) show the same N1 basicity, which is, by about 0.3 pK units, more pronounced than that of the parent AMP (entry 14).

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(vi) Interestingly, the difference,

$$\Delta pK_{a/P(OH)/P(OH)_2} = pK_{a/P(O)_2(OH)} - pK_{a/P(O)(OH)_2} \quad (9)$$

is independent of the absolute size of the pK_a values of the phosphonic acids; it is, within the error limits, identical for entries 3, 5, 8, 9, and 10 and amounts, on average, to 5.43 ± 0.06 (3σ).

(vii) Of course, the positively charged $(N1)H^+$ site of the adenine residue is expected to facilitate the release of the proton from the $P(O)(OH)_2$ group, and indeed, the difference according to eq 9 is larger and for entries 2, 4, 6, and 7, on average, amounts to 5.68 ± 0.05. Hence, the acidification of the $(N1)H^+$ site on the $P(O)(OH)_2$ group can be quantified by eq 10

$$\Delta pK_a^* = (5.68 \pm 0.05) - (5.43 \pm 0.06) = 0.25 \pm 0.08 \quad (10)$$

and it is in the expected order.[45]

Finally, one may note that the $\Delta pK_{a/P(OH)/P(OH)_2}$ values for entries 11−14 of Table 1, which refer to phosphate monoesters, are, within their error limits, identical to the differences given above for the phosphonates. This observation allows one further important generalization: if $K^H_{P(O)_2(OH)}$ for a monoprotonated phosphonate derivative or a phosphate monoester is known, $K^H_{P(O)(OH)_2}$ can be estimated according to eq 11:

$$pK^H_{P(O)(OH)_2} = pK^H_{P(O)_2(OH)} - (5.43 \pm 0.06) \quad (11)$$

Because many acidity constants for $P(O)_2^-(OH)$ groups exist in the literature,[46−48] this insight promises to be useful in many instances.

3.2. Stability Constants of the $M(H;PEEA)^+$, M(PEEA), and M(PEE) Complexes. The experimental data of the potentiometric pH titrations (see Sections 2.3 and 2.4) allow the determination of the stability constants according to equilibrium 12a for the $M(H;PEEA)^+$ complexes:

$$M^{2+} + H(PEEA)^- \rightleftharpoons M(H;PEEA)^+ \quad (12a)$$

$$K^M_{M(H;PEEA)} = [M(H;PEEA)^+]/([M^{2+}][H(PEEA)^-]) \quad (12b)$$

The formation of the neutral species M(PE), where PE^{2-} = PEE^{2-} or $PEEA^{2-}$, is defined by equilibrium 13a:

$$M^{2+} + PE^{2-} \rightleftharpoons M(PE) \quad (13a)$$

$$K^M_{M(PE)} = [M(PE)]/([M^{2+}][PE^{2-}]) \quad (13b)$$

Equilibria 8a and 13a, in the case of PEE, and equilibria 5a, 6a, 12a, and 13a, in the case of PEEA, are sufficient to obtain an excellent fitting of the titration data provided that the evaluation is not carried into the pH range where the formation of hydroxo species occurs; this was evident from titrations without the ligand.

Of course, equilibria 12a and 13a, in the case of PEEA, are also connected via equilibrium 14a:

$$M(H;PEEA)^+ \rightleftharpoons M(PEEA) + H^+ \quad (14a)$$

$$K^H_{M(H;PEEA)} = [M(PEEA)][H^+]/[M(H;PEEA)^+] \quad (14b)$$

and the corresponding acidity constants (eq 14b) may be calculated with eq 15:

$$pK^H_{M(H;PEEA)} = pK^H_{H(PEEA)} + \log K^M_{M(H;PEEA)} - \log K^M_{M(PEEA)} \quad (15)$$

The results are listed in Table 2; the stability constants given for the $M(H;PEEA)^+$ complexes are, in part, estimates because the formation degree of these species was low (see Section 2.4). The stability constants of the M(PEE) and M(PEEA) species show the usual trends. For the alkaline earth ions, complex stability decreases with increasing ionic radii, indicating that M^{2+} binding at the phosphonate group is (at least), in part, innersphere. For the divalent 3d metal ions, the long-standing experience[49] is confirmed that the stabilities of phosph(on)ate−metal-ion complexes often do not strictly follow (e.g., refs 10, 27, 41, and 50−52) the

Table 2. Logarithms of the Stability Constants of the $M(H;PEEA)^+$ (Eq 12) and M(PE) Complexes (Eq 13), Where PE^{2-} = $PEEA^{2-}$ or PEE^{2-}, Together with the Negative Logarithms of the Acidity Constants of the Protonated $M(H;PEEA)^+$ Species (Eqs 14 and 15) as Determined by Potentiometric pH Titrations in Aqueous Solution at 25 °C and I = 0.1 M ($NaNO_3$)[a]

PE^{2-}	M^{2+}	$\log K^M_{M(H;PEEA)}$	$\log K^M_{M(PE)}$	$pK^H_{M(H;PEEA)}$
$PEEA^{2-}$	Mg^{2+}	0.4 ± 0.3[b]	1.74 ± 0.06	5.78 ± 0.31
	Ca^{2+}	0.2 ± 0.4[b]	1.52 ± 0.04	5.80 ± 0.40
	Sr^{2+}	0.1 ± 0.4[b]	1.27 ± 0.05	5.95 ± 0.40
	Ba^{2+}	0.0 ± 0.4[b]	1.20 ± 0.06	5.92 ± 0.40
	Mn^{2+}	0.76 ± 0.22	2.41 ± 0.05	5.47 ± 0.23
	Co^{2+}	0.83 ± 0.15	2.21 ± 0.04	5.74 ± 0.16
	Ni^{2+}	1.20 ± 0.17	2.41 ± 0.06	5.91 ± 0.18
	Cu^{2+}	2.09 ± 0.17	3.98 ± 0.11	5.23 ± 0.20
	Zn^{2+}	1.23 ± 0.14	2.78 ± 0.05	5.57 ± 0.15
	Cd^{2+}	1.28 ± 0.13	2.89 ± 0.05	5.51 ± 0.14
PEE^{2-}	Mg^{2+}		1.73 ± 0.03	
	Ca^{2+}		1.51 ± 0.04	
	Sr^{2+}		1.26 ± 0.07	
	Ba^{2+}		1.24 ± 0.08	
	Mn^{2+}		2.36 ± 0.02	
	Co^{2+}		2.24 ± 0.04	
	Ni^{2+}		2.18 ± 0.06	
	Cu^{2+}		3.44 ± 0.03	
	Zn^{2+}		2.53 ± 0.03	
	Cd^{2+}		2.73 ± 0.04	

[a] For the error limits, see footnote *b* of Table 1. [b] The constants listed for these $M(H;PEEA)^+$ complexes are estimates (see Section 2.4 and refs 11 and 32). The constants given for the Cu^{2+} systems are identical to those in ref 28.

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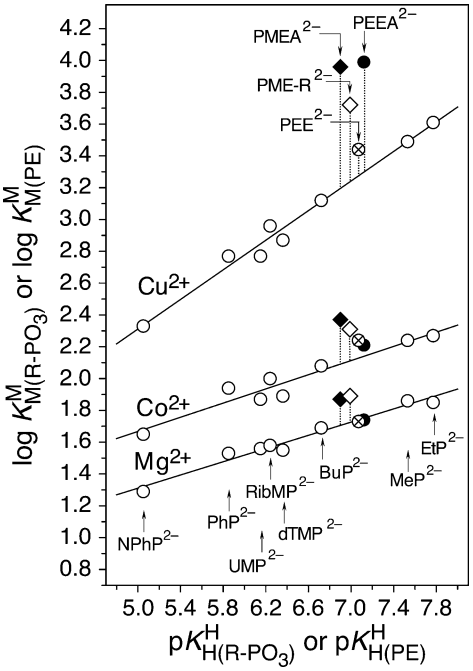

Figure 2. Evidence for enhanced stabilities of some M(PEEA) (●) and M(PEE) (⊗) complexes in comparison with those of the corresponding M(PMEA) (◆) and M(PME-R) (◇) species, based on the relationship between log $K^{M}_{M(R-PO_3)}$ and $pK^{H}_{H(R-PO_3)}$ for M(R−PO_3) complexes of some simple phosphate 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 ethanephosphonate (EtP^{2-}) (from left to right). The least-squares lines (eq 16) are drawn through the corresponding eight data sets (○) taken from ref 42 for the phosphate monoesters and from ref 24 for the phosphonates. The points due to the equilibrium constants for the M^{2+}/PEEA (●) and M^{2+}/PEE systems (⊗) are based on the values listed in Tables 1 and 2; those for the M^{2+}/PMEA systems (◆) are from ref 24, and those for the M^{2+}/PME-R systems (◇) are based on $pK^{H}_{H(PME-R)} = 6.99$ [average of the pK_a values for $H(PME)^-$ and $H(PMEC)^-$] and the stability enhancements listed in ref 10 (see also Table 4, column 5). The vertical broken lines emphasize the stability differences to the reference lines; they equal log $\Delta_{M/PE}$, as defined in eq 17 for the M(PEEA) and M(PEE) complexes. All of the plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$).

Irving−Williams sequence,[53] an observation in agreement with the fact that, in ligands of this kind, the phosph(on)ate group is always the main stability-determining binding site[25,26,50−52] in M(PE)-type complexes (see Section 3.4 and Figure 2).

3.3. Some Comments on the Structure of the M(H;PEEA)$^+$ Complexes. The evaluation of potentiometric pH titration data only allows the determination of the stability constants of the M(H;PEEA)$^+$ complexes. Further information is required to detect the binding sites of the proton and the metal ion. At first, one may ask where the proton is located because the binding of M^{2+} to a protonated ligand commonly leads to an acidification of the ligand-bound proton.[54,55] Indeed, the acidity constants of the M(H;PEEA)$^+$ complexes given in column 5 of Table 2 are, by about 1.2−1.9 log units, smaller than $pK^{H}_{H(PEEA)}$ but about 1.1−1.8 log units larger than $pK^{H}_{H_2(PEEA)}$. This shows that the proton in M(H;PEEA)$^+$ is bound to the phosphonate group; hence, one may tentatively assume that the metal ion is bound preferentially to the nucleobase because a monoprotonated phosphonate group is only a weak binding site.[56] Indeed, this suggestion agrees with evidence obtained previously for other related M(H;PEEA)$^+$-type species.[24,57,58]

Furthermore, the stability constants of the M(H;PEEA)$^+$ complexes are, within the error limits, identical to the values determined for the corresponding[24] M(H;PMEA)$^+$ and M(H;dPMEA)$^+$ species.[39] Considering that the basicity of the N1 sites in $H(PEEA)^-$ and $H(PMEA)^-$ or $H(dPMEA)^-$ are also identical (see Table 1, column 4, entries 2, 4, and 7) and that evidence has been provided for the M(H;PMEA)$^+$ complexes[24,57] that M^{2+} is mainly located at the nucleobase residue, one may not only conclude that in the M(H;PEEA)$^+$ complexes the proton is at the phosphonate group, but also that M^{2+} is mainly at the adenine residue. The N1 versus N7 dichotomy for metal-ion binding to the adenine residue is well-known,[59] though there are indications that binding to N7 dominates.[59,60] In any case, the fact that the stabilities of the M(H;PEEA)$^+$ species *follow* the Irving−Williams sequence[53] [in contrast to phosph(on)ate coordinations] also supports[49] the above conclusion that metal-ion binding in the monoprotonated species occurs preferably to a nitrogen atom.

3.4. Evaluation of the Stabilities of the M(PEEA) and M(PEE) Complexes. $PEEA^{2-}$ offers four potential binding sites for the coordination of metal ions: the 2-fold negatively charged phosphonate group, the ether oxygen of the $-CH_2CH_2-O-CH_2CH_2-PO_3^{2-}$ chain (Figure 1), and the adenine residue with its N7 and N3 sites; N1 is not accessible for a phosphonate-bound metal ion.[24,25a] Of course, PEE^{2-} offers the phosphonate group and the ether oxygen. The phosphonate group is clearly the primary binding site for all metal ions considered in this study, and therefore, any participation in M^{2+} binding of one (or more) of the other potential sites has to be reflected in a relative stability increase.[61] Hence, it is necessary to define the stability of a pure PO_3^{2-}/M^{2+} interaction. This can be done by applying the previously defined[25a,51,52] straight-line correlations, which are based on log $K^{M}_{M(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ plots for simple phosphate monoesters[42] and phosphonates;[24] these ligands are abbreviated as R−PO_3^{2-}, where R represents a noncoordinating residue. The parameters for these straight-line equations, that is, the slopes *m* and the intercepts *b* with

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the *y* axis, which are defined by eq 16,

$$\log K^{M}_{M(R-PO_3)} = m \cdot pK^{H}_{H(R-PO_3)} + b \qquad (16)$$

were tabulated.[24,25a,51,52] Hence, with a known pK_a for the deprotonation of a $P(O)_2(OH)^-$ group, an expected stability constant can be calculated for any phosph(on)ate−M^{2+} complex.

Plots of log $K^{M}_{M(R-PO_3)}$ versus p$K^{H}_{H(R-PO_3)}$ according to eq 16 are shown in Figure 2 for the 1:1 complexes of Mg^{2+}, Co^{2+}, and Cu^{2+}, as examples, with the data points (empty circles) of the eight simple ligand systems[24,42] used for the determination of the straight reference lines.[24] The data point due to Cu(PEE) is above its reference line, indicating that the ether-oxygen atom participates in Cu^{2+} binding and that equilibrium 2 is of relevance, but more remarkable is the much more enhanced stability of the Cu(PEEA) complex, which means that in addition to the ether-oxygen atom, the adenine residue must also be involved in M^{2+} binding. The data points for Co(PEE) and Co(PEEA) are just barely, but to an almost equal extent, above their reference line, indicating that the interaction with the ether oxygen is weak and that the adenine residue does not participate in M^{2+} coordination. The data points of the Mg^{2+} complexes with PEE^{2-} and $PEEA^{2-}$ fit on the reference line, as seen in Figure 2, demonstrating that only a PO_3^{2-}/M^{2+} binding is of relevance. These observations contrast with those for the M(PME-R) complexes where *all* of the data points are above their reference lines, meaning that Mg^{2+}, Co^{2+}, and Cu^{2+} also clearly interact with the ether-oxygen atom and that equilibrium 1 is of importance in these instances;[21] furthermore, comparison of these data with those for the corresponding M(PMEA) species reveals, in addition, that the adenine residue is of no relevance for Mg^{2+} binding,[20,21] of possibly small relevance for Co^{2+},[20] and of significant relevance for Cu^{2+}.[21,39]

Stability enhancements such as those seen in Figure 2 can be quantified by the differences between the experimentally (exptl) measured stability constants and those calculated (calcd) according to eq 16; this difference is defined in eq 17, where PE^{2-} = PEE^{2-} and $PEEA^{2-}$:

$$\log \Delta_{M/PE} = \log K^{M}_{M(PE)_{exptl}} - \log K^{M}_{M(PE)_{calcd}} \qquad (17a)$$

$$= \log K^{M}_{M(PE)} - \log K^{M}_{M(PE)_{op}} (= \log \Delta) \qquad (17b)$$

Clearly, the expressions log $K^{M}_{M(PE)_{calcd}}$ and log $K^{M}_{M(PE)_{op}}$ are synonymous because the calculated value equals the stability constant of the "open" isomer, $M(PE)_{op}$ (see, for example, equilibria 1−3), in which only a PO_3^{2-}/M^{2+} interaction occurs.

The values for the three terms of eq 17 are listed in columns 4, 5, and 6 of Table 3. The log $\Delta_{M/PE}$ values for the Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Mn^{2+} complexes of PEE^{2-} (entries 1b−5b) and $PEEA^{2-}$ (entries 1a−5a) are evidently zero within the error limits. This means that there is no stability enhancement, and consequently, the "open" isomer in equilibrium 2 dominates the situation. A comparison of the log $\Delta_{M/PE}$ values of entries 7a−10a with 7b−10b reveals that the stability enhancements of the M(PEEA) complexes involving Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} are more pronounced, and this reveals that the adenine residue is also of relevance (see Sections 3.6−3.8).

Table 3. Stability Constant Comparisons for the M(PE) Complexes, Where PE^{2-} = $PEEA^{2-}$ or PEE^{2-}, According to Eq 17, That Is, Between the Experimentally Measured (exptl) and the Calculated (calcd) Log Stability Constants, the Latter Being Based on the Reference-Line Equations (eq 16)[24,25a,51,52] and the p$K^{H}_{H(PE)}$ Values (Table 1) of the Monoprotonated $H(PE)^-$ Species (Aqueous Solution; 25 °C; I = 0.1 M, $NaNO_3$)[a]

			log $K^{M}_{M(PE)}$		
number	PE^{2-}	M^{2+}	exptl[b]	calcd	log $\Delta_{M/PE}$
1a	$PEEA^{2-}$	Mg^{2+}	1.74 ± 0.06	1.75 ± 0.03	−0.01 ± 0.07
2a		Ca^{2+}	1.52 ± 0.04	1.57 ± 0.05	−0.05 ± 0.06
3a		Sr^{2+}	1.27 ± 0.05	1.32 ± 0.04	−0.05 ± 0.06
4a		Ba^{2+}	1.20 ± 0.06	1.24 ± 0.04	−0.04 ± 0.07
5a		Mn^{2+}	2.41 ± 0.05	2.38 ± 0.05	0.03 ± 0.07
6a		Co^{2+}	2.21 ± 0.04	2.14 ± 0.06	0.07 ± 0.07
7a		Ni^{2+}	2.41 ± 0.06	2.17 ± 0.05	0.24 ± 0.08
8a		Cu^{2+}	3.98 ± 0.11	3.30 ± 0.06	0.68 ± 0.13
9a		Zn^{2+}	2.78 ± 0.05	2.44 ± 0.06	0.34 ± 0.08
10a		Cd^{2+}	2.89 ± 0.05	2.74 ± 0.05	0.15 ± 0.07
1b	PEE^{2-}	Mg^{2+}	1.73 ± 0.03	1.74 ± 0.03	−0.01 ± 0.04
2b		Ca^{2+}	1.51 ± 0.04	1.56 ± 0.05	−0.05 ± 0.06
3b		Sr^{2+}	1.26 ± 0.07	1.31 ± 0.04	−0.05 ± 0.08
4b		Ba^{2+}	1.24 ± 0.08	1.24 ± 0.04	0.00 ± 0.09
5b		Mn^{2+}	2.36 ± 0.02	2.37 ± 0.05	−0.01 ± 0.05
6b		Co^{2+}	2.24 ± 0.04	2.13 ± 0.06	0.11 ± 0.07
7b		Ni^{2+}	2.18 ± 0.06	2.15 ± 0.05	0.03 ± 0.08
8b		Cu^{2+}	3.44 ± 0.03	3.27 ± 0.06	0.17 ± 0.07
9b		Zn^{2+}	2.53 ± 0.03	2.42 ± 0.06	0.11 ± 0.07
10b		Cd^{2+}	2.73 ± 0.04	2.73 ± 0.05	0.00 ± 0.06

[a] For the error limits, see footnote *b* of Table 1. [b] From column 4 of Table 2.

3.5. Comparison of the Extent of Ether-Oxygen Binding in M(PEE) and M(PME-R) Complexes. The Two-Isomer Problem. With the stability enhancements seen in Table 3 for several of the M(PEE) complexes at hand, we shall evaluate these systems first because, here, the interpretation is unequivocal as an increased stability can only be attributed to the formation of a six-membered chelate, as seen in equilibrium 2. If one designates the "open" isomer as $M(PEE)_{op}$ and the chelated or "closed" species as $M(PEE)_{cl/O}$, the dimensionless equilibrium constant $K_{I/O}$ for the concentration-independent equilibrium 2 is defined by eq 18:

$$K_{I/O} = [M(PEE)_{cl/O}]/[M(PEE)_{op}] \qquad (18)$$

The measured stability constants due to equilibrium 13a are defined by eq 13b, where [M(PEE)] represents the sum of the concentrations of *all* of the M(PEE) isomers present. Consequently, expressions 13a and 13b may be rewritten as given in equilibrium 19 and eq 20:

$$M^{2+} + PEE^{2-} \rightleftharpoons M(PEE)_{op} \rightleftharpoons M(PEE)_{cl/O} \qquad (19)$$

$$K^{M}_{M(PEE)} = \frac{[M(PEE)_{op}] + [M(PEE)_{cl/O}]}{[M^{2+}][PEE^{2-}]} \qquad (20a)$$

$$= \frac{[M(PEE)_{op}]}{[M^{2+}][PEE^{2-}]} + \frac{[M(PEE)_{cl/O}]}{[M^{2+}][PEE^{2-}]} \qquad (20b)$$

Table 4. Extent of Chelate Formation According to Equilibrium 2 as Expressed by the Dimensionless Equilibrium Constants $K_{I/O}$ (Eqs 18 and 23) and the Percentages of $M(PEE)_{cl/O}$ (Eq 24) Based on the log $\Delta_{M/PEE}$ Values Listed in Column 6 (Entries 1b−10b) of Table 3 for the M(PEE) Complexes. The Corresponding Information Is Also Provided for the M(PME−R) Complexes Regarding Equilibrium 1 (Aqueous Solution; 25 °C; $I = 0.1$ M, $NaNO_3$)[a]

	M(PEE) complexes			M(PME−R) complexes		
M^{2+}	log $\Delta_{M/PEE}$	$K_{I/O}$	% $M(PEE)_{cl/O}$	log $\Delta_{M/PME-R}$[b]	$K_{I/O}$	% $M(PME-R)_{cl/O}$
Mg^{2+}	−0.01 ± 0.04	∼0	≤7	0.16 ± 0.04	0.45 ± 0.13	31 ± 6
Ca^{2+}	−0.05 ± 0.06	∼0	≤2	0.12 ± 0.05	0.32 ± 0.15	24 ± 9
Sr^{2+}	−0.05 ± 0.08	∼0	≤7	0.09 ± 0.05	0.23 ± 0.14	19 ± 9
Ba^{2+}	0.00 ± 0.09	∼0	≤19	0.11 ± 0.05	0.29 ± 0.15	22 ± 9
Mn^{2+}	−0.01 ± 0.05	∼0	≤9	0.19 ± 0.06	0.55 ± 0.21	35 ± 9
Co^{2+}	0.11 ± 0.07	0.29 ± 0.21	22 ± 13	0.20 ± 0.06	0.58 ± 0.22	37 ± 9
Ni^{2+}	0.03 ± 0.08	0.07 ± 0.20	7 ± 17 (≤22)	0.14 ± 0.07	0.38 ± 0.22	28 ± 12
Cu^{2+}	0.17 ± 0.07	0.48 ± 0.24	32 ± 11	0.48 ± 0.07	2.02 ± 0.49	67 ± 5
Zn^{2+}	0.11 ± 0.07	0.29 ± 0.21	22 ± 13	0.29 ± 0.07	0.95 ± 0.31	49 ± 8
Cd^{2+}	0.00 ± 0.06	∼0	≤13	0.30 ± 0.05	1.00 ± 0.23	50 ± 6

[a] For the error limits, see footnote *b* of Table 1. [b] The values in this column are from Table IV in ref 10.

Considering that the stability of the "open" isomer is defined by eq 21,

$$K^{M}_{M(PEE)_{op}} = [M(PEE)_{op}]/([M^{2+}][PEE^{2-}]) \qquad (21)$$

from eqs 17, 18, 20, and 21 follow[44,61] eqs 22 and 23:

$$K^{M}_{M(PEE)} = K^{M}_{M(PEE)_{op}} + K^{M}_{M(PEE)_{cl/O}} \qquad (22a)$$

$$= K^{M}_{M(PEE)_{op}}(1 + K_{I/O}) \qquad (22b)$$

$$K_{I/O} = \frac{K^{M}_{M(PEE)}}{K^{M}_{M(PEE)_{op}}} - 1 \qquad (23a)$$

$$= 10^{\log \Delta} - 1 \qquad (23b)$$

Of course, once $K_{I/O}$ is known, the percentage of the "closed" or chelated isomer occurring in equilibrium 2 follows from eq 24:

$$\%\ M(PEE)_{cl/O} = 100 \cdot K_{I/O}/(1 + K_{I/O}) \qquad (24)$$

Application of this procedure[44,61] yields the results listed in columns 2, 3, and 4 of Table 4 for the M(PEE) species. However, as outlined in the Introduction, one of the main questions of this study was: are five- or six-membered chelates more stable? In other words, for a given M^{2+}, is equilibrium 1 or equilibrium 2 more displaced to the right side? To address this question, we used the available[10] log $\Delta_{M/PME-R}$ values, which refer to M(PME-R) complexes (see Figure 1), and carried out the analogous calculations (eqs 18−24) for the M(PEE) species. The corresponding results are summarized at the right-hand side of Table 4.

On the basis of the data in Table 4, one may draw, among others, the following conclusions:

(i) The formation degrees of the chelates of the M(PEE) complexes vary in the narrow range between about 0 and 30%; in fact, chelates are only formed with Co^{2+}, Cu^{2+}, and Zn^{2+}. For Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Ni^{2+}, and Cd^{2+}, the log $\Delta_{M/PEE}$ values and, correspondingly, the $K_{I/O}$ values are zero, within the error limits, meaning that equilibrium 2 is far on its left side and chelates occur only in trace amounts, if at all.

Table 5. Comparisons of the Stability Enhancements (Eq 17) Observed for the M(PEEA) and M(PEE) Complexes According to Eq 25 (Aqueous Solution; 25 °C and $I = 0.1$ M, $NaNO_3$)[a]

M^{2+}	log $\Delta_{M/PEEA}$[b]	log $\Delta_{M/PEE}$[b]	Δlog $\Delta_{M/PEEA/PEE}$
Mg^{2+}	−0.01 ± 0.07	−0.01 ± 0.04	0.00 ± 0.08
Ca^{2+}	−0.05 ± 0.06	−0.05 ± 0.06	0.00 ± 0.08
Sr^{2+}	−0.05 ± 0.06	−0.05 ± 0.08	0.00 ± 0.10
Ba^{2+}	−0.04 ± 0.07	0.00 ± 0.09	−0.04 ± 0.11
Mn^{2+}	0.03 ± 0.07	−0.01 ± 0.05	0.04 ± 0.09
Co^{2+}	0.07 ± 0.07	0.11 ± 0.07	−0.04 ± 0.10
Ni^{2+}	0.24 ± 0.08	0.03 ± 0.08	0.21 ± 0.11
Cu^{2+}	0.68 ± 0.13	0.17 ± 0.07	0.51 ± 0.15
Zn^{2+}	0.34 ± 0.08	0.11 ± 0.07	0.23 ± 0.11
Cd^{2+}	0.15 ± 0.07	0.00 ± 0.06	0.15 ± 0.09

[a] For the error limits, see footnote *b* of Table 1. [b] From column 6 of Table 3.

(ii) For the M(PME-R) species, the situation is different; *all* of the mentioned metal ions form chelates to some extent with formation degrees between about 20 and 65%!

(iii) Most importantly, a comparison of the listed data for the M(PEE) and M(PME-R) species shows that five-membered chelates (equilibrium 1) involving M^{2+}−phosphonate binding and an ether-oxygen atom interaction are significantly more stable than the corresponding six-membered chelates (equilibrium 2). This observation explains[21] why PMEA has excellent antiviral properties and PEEA does not (see Introduction).

3.6. Extent of Chelate Formation in M(PEEA) Complexes. In Some Instances, the Adenine Residue Participates in Metal-Ion Binding! Already, in the last paragraph of Section 3.4, it was concluded that the stability enhancement for several of the M(PEEA) complexes is more pronounced than that of the corresponding M(PEE) complexes. To place this conclusion on quantitative grounds, the log $\Delta_{M/PE}$ values of eq 17 are compared according to eq 25:

$$\Delta\log \Delta_{M/PEEA/PEE} = \log \Delta_{M/PEEA} - \log \Delta_{M/PEE} \qquad (25)$$

Indeed, the results of Table 5 prove that the M(PEEA) complexes of Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} experience a more pronounced stability enhancement than the corresponding M(PEE) species, and this is evidence that, in these instances, the adenine residue also participates in M^{2+} binding. Consequently, chelate formation of the phosphonate-bound M^{2+} with the ether-oxygen atom and/or the adenine residue

Table 6. Extent of Total Chelate Formation Involving the Ether Oxygen and/or the Adenine Residue as Expressed by the Dimensionless Equilibrium Constants $K_{I/tot}$ (Analogous to Eqs 18 and 23) and the Percentages of $M(PEEA)_{cl/tot}$ (Analogous to Eq 24) Based on the log $\Delta_{M/PEEA}$ Values Listed in Column 6 (entries 1a−10a) of Table 3 for the M(PEEA) Complexes (Aqueous Solution; 25 °C; $I = 0.1$ M, $NaNO_3$)[a]

M^{2+}	log $\Delta_{M/PEEA}$	$K_{I/tot}$	$\%M(PEEA)_{cl/tot}$
Mg^{2+}	-0.01 ± 0.07	~0	≤13[b]
Ca^{2+}	-0.05 ± 0.06	~0	≤2[b]
Sr^{2+}	-0.05 ± 0.06	~0	≤2[b]
Ba^{2+}	-0.04 ± 0.07	~0	≤7[b]
Mn^{2+}	0.03 ± 0.07	~0	≤21[b]
Co^{2+}	0.07 ± 0.07	0.17 ± 0.19	15 ± 14[b]
Ni^{2+}	0.24 ± 0.08	0.74 ± 0.32	42 ± 11
Cu^{2+}	0.68 ± 0.13	3.79 ± 1.43	79 ± 6
Zn^{2+}	0.34 ± 0.08	1.19 ± 0.40	54 ± 8
Cd^{2+}	0.15 ± 0.07	0.41 ± 0.23	29 ± 11

[a] For the error limits, see footnote *b* of Table 1. [b] A comparison with the corresponding results in Table 4 shows that, in these instances, no chelate formation occurs and if it occurs (Co^{2+}), it involves the ether oxygen (see also the text in Section 3.6), i.e., $\%M(PEEA)_{cl/tot} = \%M(PEEA)_{cl/O}$.

occurs, and this means, further, that the log $\Delta_{M/PEEA}$ values listed in Table 3 (column 6) encompass both interactions.

Therefore, with the above conclusion in mind, we calculated for the M(PEEA) systems the *total* (tot) amount of chelated species formed because these data in comparison with those of the M(PEE) complexes in Table 4 should provide new insights regarding the ether-oxygen and/or adenine-residue participation in M^{2+} binding. For the calculation procedure, eqs 19−24 can be applied if M(PEE) is replaced by M(PEEA), $K_{I/O}$ by $K_{I/tot}$, and $M(PEE)_{cl/O}$ by $M(PEEA)_{cl/tot}$. The results of these calculations are listed in Table 6.

A comparison of the results given in Table 4 (at the left) for the M(PEE) complexes with those in Table 6 for the M(PEEA) systems reveals the following facts:

(i) Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Mn^{2+} do not form chelates with PEE^{2-} and $PEEA^{2-}$, or only do so in trace amounts, meaning that equilibrium 2 is on its left side.

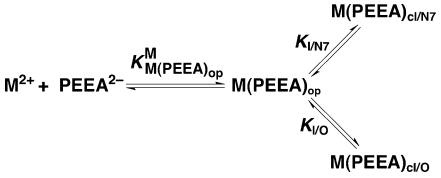
(ii) In Co(PEE) and Co(PEEA), the extent of the ether-oxygen interaction is identical, within the error limits; the formation degree of the chelate in equilibrium 2 amounts to about 18% in both systems.

(iii) For Cd(PEE), no stability enhancement is observed (Table 4), and thus, no ether-oxygen interaction occurs. Consequently, the complete stability enhancement observed for Cd(PEEA) is to be attributed to macrochelate formation, according to equilibrium 3, of the phosphonate-coordinated metal ion by interacting with N7 of the adenine residue. This corresponds to the type of macrochelation observed in M(AMP) complexes.[27,44] It may be added that N1 cannot be reached by a phosph(on)ate-bound metal ion,[25,57a] that N3 only participates if a further directing site is actively involved (see Section 3.8),[39,57a] and, most importantly,[37,38,62] that N7 is considerably more basic than N3. This site attribution to N7 agrees with that reached[11] for the M(dPEEA) species (Figure 1); indeed, the extent of macrochelate formation for Cd(dPEEA) and Cd(PEEA) is, within the error limits, the same, with values of $42 \pm 9\%$ and $29 \pm 11\%$, respectively, and this is exactly what one would expect if the ether oxygen is not involved.

(iv) The same arguments as those given in point iii also hold for Ni(PEEA); again, macrochelate formation with N7 reaches about the same formation degree in Ni(dPEEA) and in Ni(PEEA), that is, $28 \pm 10\%$ (cf. ref 11) and $42 \pm 11\%$ (Table 6), respectively. A Ni^{2+}/ether-O interaction occurs in Ni(PEEA) only in trace amounts (Table 4), if at all (see Section 3.7).

(v) However, the comparison between the data for the M(PEE) and M(PEEA) complexes of Cu^{2+} and Zn^{2+} reveals that both types of interactions are important, that is, the one with the ether oxygen as well as the one with the adenine residue.

3.7. Isomeric Equilibria in Zn(PEEA) and Related Systems. A Three-Isomer Problem. From a comparison of the log $\Delta_{Zn/PE}$ values for Zn(PEE) and Zn(PEEA) in Table 5, and as also concluded in point v of the preceding section, it is clear that, for Zn(PEEA), both the ether-O (equilibrium 2) and the N7 isomers (equilibrium 3; see also point iii of Section 3.6) play a role; we designate these chelated species generally as $M(PEEA)_{cl/O}$ and $M(PEEA)_{cl/N7}$, respectively. Indeed, if one makes the assumption that in a first approximation, the log Δ values are additive, one obtains, with log $\Delta_{Zn/PEE} = 0.11$ (ether-oxygen interaction; Table 4) and log $\Delta_{Zn/dPEEA} = 0.24$ (N7 interaction),[11] a sum of 0.35 log units, which is in accord with log $\Delta_{Zn/PEEA} = 0.34 \pm 0.08$ (Table 6). The same reasoning for Cu(PEEA) gives a sum of 0.44 log units [= 0.17 (ether O) + 0.27 (N7)[11]], which is considerably smaller than log $\Delta_{Cu/PEEA} = 0.68 \pm 0.13$; this indicates that the situation for the Cu(PEEA) system may be more complicated and that further isomers occur (see Section 3.8). However, for the present, we shall concentrate on equilibrium scheme 26



(26)

and evaluate the situation for Zn(PEEA). The corresponding data for Cu(PEEA) and Ni(PEEA) are calculated for further comparisons.

On the basis of equilibrium scheme 26, the experimentally accessible overall stability constant, as defined in eq 13b, may be rewritten as given in eqs 27b, 27c, and 27d:[24,25a]

$$K^{M}_{M(PEEA)} = \frac{[M(PEEA)]}{[M^{2+}][PEEA^{2-}]} \quad (27a)$$

$$= \frac{[M(PEEA)_{op}] + [M(PEEA)_{cl/O}] + [M(PEEA)_{cl/N7}]}{[M^{2+}][PEEA^{2-}]} \quad (27b)$$

$$= K^{M}_{M(PEEA)_{op}} + K_{I/O}K^{M}_{M(PEEA)_{op}} + K_{I/N7}K^{M}_{M(PEEA)_{op}} \quad (27c)$$

$$= K^{M}_{M(PEEA)_{op}}(1 + K_{I/O} + K_{I/N7}) \quad (27d)$$

(62) Sigel, H. *Pure Appl. Chem.* **2004**, *76*, 1869−1886.

Table 7. Intramolecular Equilibrium Constants (Eqs 18, 28, and 29) for the Formation of the Isomeric Species $M(PEEA)_{op}$, $M(PEEA)_{cl/O}$, and $M(PEEA)_{cl/N7}$ (see Equilibrium Scheme 26), Together with the Percentages of Which the Isomers Occur in Aqueous Solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

M^{2+}	$K_{I/tot}$ (eq 29a)	% $M(PEEA)_{cl/tot}$ (eqs 24, 29b)	% $M(PEEA)_{op}$ (eq 29b)	$K_{I/O}$ (eq 18)	$K_{I/N7}$ (eqs 28, 29c)	% $M(PEEA)_{cl/O}$ (eq 18)[b]	% $M(PEEA)_{cl/N7}$ (eq 28)[b]
Ni^{2+}	0.74 ± 0.32	42 ± 11	58 ± 11	0.07 ± 0.20	0.67 ± 0.38	4 ± 12	38 ± 16
Cu^{2+}	3.79 ± 1.43	79 ± 6	21 ± 6	0.48 ± 0.24	3.31 ± 1.45[c]	10 ± 6	69 ± 8[c]
Zn^{2+}	1.19 ± 0.40	54 ± 8	46 ± 8	0.29 ± 0.21	0.90 ± 0.45	13 ± 10	41 ± 13

[a] For the error limits, see footnote *b* of Table 1. The values listed in columns 2 and 3 are from columns 3 and 4 of Table 6, respectively. The values given in the fourth column for % $M(PEEA)_{op}$ follow from 100 − % $M(PEEA)_{cl/tot}$. The constants $K_{I/O}$ of column 5 are from column 3 of Table 4 (see text in Section 3.7); with the values now known for $K_{I/tot}$ and $K_{I/O}$ and eq 29c, those for $K_{I/N7}$ may be calculated (column 6). [b] Calculated in analogy to eq 18 with $K_{I/O}$ and % $M(PEEA)_{op}$. The values for % $M(PEEA)_{cl/N7}$ follow from the difference expression % $M(PEEA)_{cl/tot}$ − % $M(PEEA)_{cl/O}$; they may also be calculated in analogy to eq 28 with $K_{I/N7}$ and % $M(PEEA)_{op}$. The results are the same for both calculation methods, but the error limits are understandably larger for the second method. [c] These values are too large; they also contain a contribution from a Cu^{2+}/N3 interaction, see Section 3.8 and Table 8.

The necessary definitions are analogous to eqs 18 and 21; in addition, only eq 28 is needed:

$$K_{I/N7} = [M(PEEA)_{cl/N7}]/[M(PEEA)_{op}] \tag{28}$$

The connection between $K_{I/tot}$ (see Table 6) and the experimentally accessible values for $\log \Delta_{M/PEEA}$ ($= \log \Delta$) is given by eq 29:

$$K_{I/tot} = \frac{K^{M}_{M(PEEA)}}{K^{M}_{M(PEEA)_{op}}} - 1 = 10^{\log \Delta} - 1 \tag{29a}$$

$$= \frac{[M(PEEA)_{cl/tot}]}{[M(PEEA)_{op}]} = \frac{[M(PEEA)_{cl/O}] + [M(PEEA)_{cl/N7}]}{[M(PEEA)_{op}]} \tag{29b}$$

$$= K_{I/O} + K_{I/N7} \tag{29c}$$

Of course, if the isomer $M(PEEA)_{cl/N7}$ (eq 26) is not formed, $K_{I/N7}$ (eq 28) becomes zero and the above eq 29c reduces to $K_{I/tot} = K_{I/O}$, that is, to the two-isomer problem appropriate for the treatment of the M(PEE) complexes (Section 3.5). In this case, only the species $M(PEEA)_{op}$ and $M(PEEA)_{cl/O}$ exist, and the situation corresponds to equilibrium 2.

If three isomers are formed according to equilibrium scheme 26, $K_{I/tot}$ can be calculated (see also Section 3.6 and Table 6) according to eq 29a from the $\log \Delta_{M/PEEA}$ values in column 2 of Table 6. Hence, the concentration fraction of $M(PEEA)_{op}$ becomes known (eq 29b). Assuming that the stability, $K_{I/O}$, of the $M(PEE)_{cl/O}$ isomers (Table 4, left part) represents the stability of the $M(PEEA)_{cl/O}$ isomers well because both of them contain the structurally identical (phosphonoethoxy)ethyl chain, $K_{I/N7}$ can also be calculated with eq 29c, and hence, the formation degrees of all of the isomers become known (Table 7).

The most obvious conclusions from Table 7 are as follows:

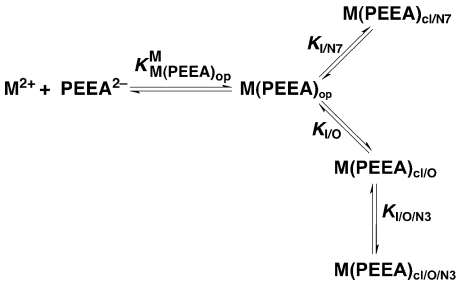
(i) In the Zn(PEEA) system, all three isomers indicated in Scheme 26 occur in appreciable amounts; aside from the open isomer (ca 46%), $Zn(PEEA)_{cl/O}$ reaches a formation degree of about 13% and $Zn(PEEA)_{cl/N7}$ one of about 41%. This latter value is remarkable because it is, within the error limits, identical to the one calculated[11] for Zn(dPEEA) (42%).

(ii) The results for the Ni(PEEA) system confirm the conclusion in Section 3.6 that $Ni(PEEA)_{cl/O}$ occurs, if at all, only in trace amounts. The formation degree of $38 \pm 16\%$ for $Ni(PEEA)_{cl/N7}$ is, within the error limits, identical to the result given in Table 6 ($42 \pm 11\%$), which was obtained by considering only $Ni(PEEA)_{op}$ and $Ni(PEEA)_{cl/N7}$; it also agrees with the value[11] for $Ni(dPEEA)_{cl/N7}$ ($28 \pm 10\%$).

(iii) The suspicion expressed already in the first paragraph of this section regarding Cu(PEEA) is confirmed; all three isomers, according to equilibrium scheme 26, occur, but the formation degree of $69 \pm 8\%$ for $Cu(PEEA)_{cl/N7}$ is considerably larger than the $46 \pm 12\%$ calculated previously[11] for $Cu(dPEEA)_{cl/N7}$. Considering that the error limits used amount to 3σ, it is evident that the indicated difference is real.

3.8. The Cu(PEEA) System—A Four-Isomer Problem. Because there is no convincing explanation why macrochelate formation with N7 of the adenine residue should be considerably more pronounced in Cu(PEEA) than in its deoxa analogue Cu(dPEEA) (see Figure 1 and its legend), one has to postulate the occurrence of a fourth isomer and that this is responsible for the additional stability enhancement. Indeed, for the Cu(PMEA) system, it has been shown[57a] that an isomer, which involves the ether oxygen and, thus, contains a five-membered chelate ring (equilibrium 1) together with a seven-membered one involving N3 of the adenine residue, is the *majority* species.[39,63] Because the formation degree of the six-membered chelate $Cu(PEE)_{cl/O}$ at about 32% is remarkably high, it is most likely that the analogous species $Cu(PEEA)_{cl/O}$ undergoes a further interaction with N3, thus, giving rise to an isomer that contains the combination of six- and seven-membered chelate rings and that we designate as $Cu(PEEA)_{cl/O/N3}$. In this context, it is important to emphasize that, for steric reasons, a macrochelate involving *only* N3 cannot be formed by $PEEA^{2-}$ and Cu^{2+}. If one tries to form such a species with molecular models, one automatically forces the ether oxygen into the coordination sphere of the metal ion, thus, giving rise to $Cu(PEEA)_{cl/O/N3}$. This isomer together with those considered in equilibrium scheme 26 leads then to equilibrium scheme 30, which is written in a general way by using M^{2+}:

(63) Gómez-Coca, R. B.; Holý, A.; Vilaplana, R. A.; González-Vílchez, F.; Sigel, H. *Bioinorg. Chem. Appl.* **2004**, *2*, 331−352.



(30)

The four equilibrium constants seen in equilibrium scheme 30 are defined by the already mentioned eqs 18 (in analogy), 21, and 28 together with the also necessary eq 31:

$$K_{I/O/N3} = [M(PEEA)_{cl/O/N3}]/[M(PEEA)_{cl/O}] \quad (31)$$

With these definitions, the measured overall stability constant (eq 13b) can again be redefined, as given in eqs 32b−32d:

$$K^{M}_{M(PEEA)} = \frac{[M(PEEA)]}{[M^{2+}][PEEA^{2-}]} \quad (32a)$$

$$= \frac{[M(PEEA)_{op}] + [M(PEEA)_{cl/N7}] + [M(PEEA)_{cl/O}] + [M(PEEA)_{cl/O/N3}]}{[M^{2+}][PEEA^{2-}]} \quad (32b)$$

$$= K^{M}_{M(PEEA)_{op}} + K_{I/N7}K^{M}_{M(PEEA)_{op}} + K_{I/O}K^{M}_{M(PEEA)_{op}} + K_{I/O/N3}K_{I/O}K^{M}_{M(PEEA)_{op}} \quad (32c)$$

$$= K^{M}_{M(PEEA)_{op}}(1 + K_{I/N7} + K_{I/O} + K_{I/O}K_{I/O/N3}) \quad (32d)$$

The connection between the overall intramolecular equilibrium constant $K_{I/tot}$ (see Section 3.6 and Table 6) and the also accessible stability enhancement $\log \Delta$ (eq 17) is given by eqs 33a−33e:

$$K_{I/tot} = \frac{K^{M}_{M(PEEA)}}{K^{M}_{M(PEEA)_{op}}} - 1 = 10^{\log \Delta} - 1 \quad (33a)$$

$$= \frac{[M(PEEA)_{cl/tot}]}{[M(PEEA)_{op}]} \quad (33b)$$

$$= \frac{([M(PEEA)_{cl/N7}] + [M(PEEA)_{cl/O}] + [M(PEEA)_{cl/O/N3}])}{[M(PEEA)_{op}]} \quad (33c)$$

$$= K_{I/N7} + K_{I/O} + K_{I/O/N3}K_{I/O} \quad (33d)$$

$$= K_{I/N7} + K_{I/O}(1 + K_{I/O/N3}) \quad (33e)$$

The value for $K_{I/tot}$, as calculated in Section 3.6 (see Table 6), is listed again in column 3 of Table 8 (entry 1). The relation between $K_{I/tot}$ and the other three intramolecular equilibrium constants follows from eqs 33b and 33c. Based on the reasonable assumption[20] (see also Section 3.7) that the stability of the $M(PEEA)_{cl/O}$ isomer is well-represented by that of the six-membered $M(PEE)_{cl/O}$ species (Figure 1) and the stability of the $M(PEEA)_{cl/N7}$ isomer by that of the $M(dPEEA)_{cl/N7}$ macrochelate, values for $K_{I/O}$, which defines the position of equilibrium 2, and $K_{I/N7}$, which refers to equilibrium 3, are also known (see Section 3.7 and ref 11). Hence, values for the only unknown constant in eq 33e, $K_{I/O/N3}$ (eq 31), can be obtained, and consequently, the formation degrees for all four isomers appearing in equilibrium scheme 30 can be calculated. The corresponding results are summarized in Table 8 for the Cu(PEEA) system together with those obtained earlier[39,63] for the Cu(PMEA) system; as far as the error limits are concerned, it needs to be emphasized that *3 times* the standard errors (3σ) are given.

From the results in Table 8, several interesting conclusions are evident:

(i) The formation degrees of the isomers involving N3, that is, $Cu(PEEA)_{cl/O/N3}$ and $Cu(PMEA)_{cl/O/N3}$, are identical within the error limits. Considering that N3 must have the same basicity in $PEEA^{2-}$ and $PMEA^{2-}$, this is not surprising; however, it is surprising that once a "guiding" binding site is close by, the N3 site also becomes an "active" binding site for metal ions.[11,57a,63]

(ii) The minority species in the Cu(PEEA) system is $Cu(PEEA)_{cl/O}$, with a formation degree of only about 10%. This confirms the conclusion reached in Section 3.5 (Table 4) that five-membered chelate rings involving an ether-oxygen atom are more stable than six-membered ones. Indeed, $Cu(PMEA)_{cl/O}$ reaches, with about 34%, a much higher formation degree.

(iii) Considering that $Cu(PMEA)_{cl/N7}$, with a formation degree of about 8%, is the minority species in the corresponding system and that $Cu(PEEA)_{cl/N7}$ occurs with a formation degree of about 18%, this observation may be taken as support of the earlier conclusion[11] that $dPEEA^{2-}$ is more suitable for an N7 interaction, because of its somewhat larger chain, than its analogue $dPMEA^{2-}$. In other words, in as far as macrochelate formation with N7 is concerned, $dPEEA^{2-}$ resembles AMP^{2-} more closely than $dPMEA^{2-}$.

4. Conclusions

With regard to the structure−function relationship for nucleotide analogues and their antiviral activity, as already indicated in the Introduction, one may conclude the following: If one assumes that PMEA and PEEA are transported to the cell and diphosphorylated, as it is known for PMEA,[5,64,65] then it becomes understandable why PMEA shows an antiviral activity and PEEA does not.[19,23] PMEA allows a facilitated $M(P_\alpha)$ binding (via the ether oxygen; equilibrium 1) and, thus, also an enhanced formation of the $M(P_\alpha)-M(P_\beta,P_\gamma)$-binding mode, which is crucial for the transfer of a nucleotidyl unit in the polymerase reaction.[20,21] In contrast, PEEA does not give rise to such a facilitated $M(P_\alpha)$ binding with biologically relevant metal ions, like Mg^{2+}, Mn^{2+}, or Zn^{2+}, because the six-membered chelate ring involving the ether-oxygen atom (equilibrium 2) is considerably less stable than the corresponding five-membered ring (Section 3.5; Table 4), and therefore, no promotion of the $M(P_\alpha)-M(P_\beta,P_\gamma)$-binding mode results. In other words, for the biological activity of an acyclic-nucleoside phosphonate

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Table 8. Intramolecular Equilibrium Constants (K_I) for the Formation of the Various Cu(PA) Isomers, Where PA^{2-} = $PEEA^{2-}$ and $PMEA^{2-}$ (see Figure 1), as Defined in the Equilibrium Scheme 30, Together with the Percentages in Which the Four Isomers Occur in Aqueous Solution at 25 °C and I = 0.1 M ($NaNO_3$)[a]

PA^{2-}	log $\Delta_{Cu/PA}$	$K_{I/tot}$	% $Cu(PA)_{cl/tot}$	% $Cu(PA)_{op}$	$K_{I/O}$
$PEEA^{2-}$	0.68 ± 0.13[b]	3.79 ± 1.43[b]	79 ± 6[b]	21 ± 6[b]	0.48 ± 0.24[c]
$PMEA^{2-}$	0.77 ± 0.07	4.89 ± 0.95	83 ± 3	17 ± 3	2.02 ± 0.49
PA^{2-}	$K_{I/N7}$	$K_{I/O/N3}$	% $Cu(PA)_{cl/O}$	% $Cu(PA)_{cl/N7}$	% $Cu(PA)_{cl/O/N3}$
$PEEA^{2-}$	0.86 ± 0.43[d]	5.10 ± 4.35[e]	10 ± 6[f]	18 ± 10[g]	51 ± 13[h]
$PMEA^{2-}$	0.45 ± 0.30	1.20 ± 0.73	34 ± 10	7.7 ± 5.3	41 ± 12

[a] For the error limits (3σ), see footnote *b* of Table 1. The values for the Cu(PMEA) system are from ref 39 (see also ref 63). The origins of the various values for the Cu(PEEA) system are given below in footnotes *b*−*h*. [b] The values for log $\Delta_{Cu/PEEA}$, $K_{I/tot}$, and % $Cu(PEEA)_{cl/tot}$ are from Table 6. The value for % $Cu(PEEA)_{op}$ follows from 100 − % $Cu(PEEA)_{cl/tot}$. [c] From column 3 in Table 4; see also the text in Section 3.8. [d] From Table 4 (column 3) in ref 11; this value refers to the Cu(dPEEA) system; see also the text in Section 3.8. [e] This value follows from eq 33e because all of the other intramolecular equilibrium constants are now known. [f] Calculated with $K_{I/O}$ and % $Cu(PEEA)_{op}$ by application of the equation analogous to eq 18. [g] Calculated with $K_{I/N7}$ and % $Cu(PEEA)_{op}$ by application of eq 28. [h] This value follows from the difference expression % $Cu(PEEA)_{cl/tot}$ − % $Cu(PEEA)_{cl/O}$ − % $Cu(PEEA)_{cl/N7}$; it could also be calculated with $K_{I/O/N3}$ and % $Cu(PEEA)_{cl/O}$ by application of eq 31.

derivative, the ether oxygen is compulsory (see Introduction), but it must also be correctly positioned to become effective. It is further worthwhile to note that another related analogue, 9-[3-(phosphonomethoxy)propyl]adenine (PMPA), Ade−CH_2−CH_2−CH_2−O−CH_2−PO_3^{2-}, is biologically inactive (see page 56 of ref 18) despite its possibility to form five-membered chelates. Hence, other factors are important as well, and we suspect that the inactivity of $PMPA^{2-}$ is most likely due to an inappropriate orientation and a distance between the adenine residue and the PO_3^{2-} group that is not ideal; this distance as well as the orientation is very similar for $PMEA^{2-}$ and AMP^{2-} (Figure 1); that is, the phosph(on)-ate group is close to H8 of the adenine residue with both compounds.[14]

However, aside from the biological insights that this study on the metal-ion-binding properties of $PEEA^{2-}$ has provided, this ligand is fascinating in its own rights from a coordination chemical point of view because of its ambivalent metal-ion-binding properties: (i) Several metal ions such as Mg^{2+} or Mn^{2+} coordinate to $PEEA^{2+}$ only in a monodentate fashion to the phosphonate group. (ii) This contrasts with the properties of Co^{2+}, which acts to a certain degree in a bidentate manner, forming six-membered chelates involving, in addition to the PO_3^{2-} group, the ether-O atom (equilibrium 2). (iii) Other metal ions such as Cd^{2+} form a macrochelate (equilibrium 3) involving N7 of the adenine residue, and (iv) Zn^{2+} combines both properties by giving rise to the formation of all three mentioned isomers, that is, $Zn(PEEA)_{op}$, $Zn(PEEA)_{cl/O}$, and $Zn(PEEA)_{cl/N7}$. (v) Most impressive are the properties of Cu^{2+} that give rise to the formation of four different isomers, the three mentioned under point iv plus a fourth one, namely, $Cu(PEEA)_{cl/O/N3}$. The remarkable fact here is that N3 becomes an "active" binding site once a suitable and "guiding" further interaction is available close by (in the present case, the ether-O atom). Hence, the M^{2+}/$PEEA^{2-}$ systems provide an instructive exercise on how to deal with isomeric complex equilibria.

The observation regarding N3 is of relevance for nucleic acids. For example, in the major groove of DNA, N7 is exposed for metal-ion binding, and in the minor grove, it is N3.[13] That the more basic[38,62] N7 site is well-suited for metal-ion binding is by now general knowledge,[16c,26,50,51] whereas this property of N3 has only more recently been recognized.[55,57a,66]

Finally, with regard to the isomeric equilibria described in this study, that is, of equilibria between different structural forms of complexes with the same composition, it is important to note that the conversion of an "open" form into a chelated species with a formation degree of about 20% is connected only with a stability difference of log Δ = 0.1 (eq 17). In other words, $\Delta G°$ changes by 0.6 kJ mol^{-1} only.[52,61] Because isomeric equilibration with biologically relevant metal ions is usually fast, nature has here a tool to achieve high selectivity without employing high energy barriers[67] because a formation of 20% of a given isomer in equilibrium is more than enough to serve in an enzyme reaction as a substrate or inhibitor.

Abbreviations and Definitions. Abbreviations, see also Figure 1, and definitions relevant to this paper are (d)ATP^{4-}, (2′-deoxy)adenosine 5′-triphosphate; dPMEA, 9-(4-phosphonobutyl)adenine = 3′-deoxa-$PMEA^{2-}$ (see Figure 1); FMN^{2-}, flavin mononucleotide = riboflavin 5′-phosphate; I, ionic strength; K_a, general acidity constant; M^{2+}, general divalent metal ion; 9MeAde, 9-methyladenine; PA^{2-} = $PEEA^{2-}$ or $PMEA^{2-}$ (see Figure 1 and Section 3.8); PE^{2-} = $PEEA^{2-}$ and PEE^{2-} [also sometimes includes other nucleotide analogues (Sections 3.1-3.7)]; PMCh, *O*-phosphonatomethylcholine; $PMEC^{2-}$, dianion of 9-[2-(phosphonomethoxy)ethyl]cytosine; $PMEDAPy^{-}$, anion of 1-[2-(phosphonomethoxy)ethyl]-2,4-diaminopyrimidine = quaternary 1-[2-(phosphonomethoxy)ethyl] derivative of 2,4-diaminopyrimidine; R−PO_3^{2-}, simple phosphate monoester or phosphonate ligand with R representing a noninteracting residue; UMP^{2-}, uridine 5′-monophosphate. Species written without a charge either do not carry one or represent the species in general (i.e., independent of their protonation degree); which one of the two possibilities applies is always clear from the context. In

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formulas such as $M(H;PEEA)^{+}$, the H^{+} ion and $PEEA^{2-}$ are separated by a semicolon to facilitate reading, yet they appear within the same set of parentheses to indicate that the proton is at the ligand without defining its location.

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