Solution Structures of Binary and Ternary Metal Ion Complexes of 9-(5-Phosphonopentyl)adenine (3′-deoxa-PEEA). A Nucleotide Analogue Related to the Antivirally Active 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA)

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The acidity constants of the twofold protonated acyclic 9-(5-phosphonopentyl)adenine, H_2(dPEEA)$^{\pm}$, as well as the stability constants of the M(H;dPEEA)$^+$ and M(dPEEA) complexes with the metal ions 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 by potentiometric pH titrations in aqueous solution at I = 0.1 M ($NaNO_3$) and 25 °C. Application of previously determined straight-line plots of log $K^{M}_{M(R\text{-}PO_3)}$ versus p$K^{H}_{H(R\text{-}PO_3)}$ for simple phosph(on)ate ligands, R-PO_3^{2-}, where R represents a residue which cannot participate in the coordination process, proves that the primary binding site of dPEEA^{2-} is the phosphonate group with all the metal ions studied. However, for the M(dPEEA) systems with Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} a (in part rather small) stability increase is observed which is due to macrochelate formation with the adenine residue, i.e. most likely with N7. The formation degrees of the macrochelates are 17 ± 15%, 28 ± 10%, 46 ± 12%, 42 ± 28%, and 42 ± 9% (3σ), respectively. This means that in this respect dPEEA^{2-} resembles the parent nucleotide adenosine 5′-monophosphate (AMP^{2-}) more than its chain-shortened analogue 9-(4-phosphonobutyl)adenine (dPMEA^{2-}); indeed, in a first approximation macrochelate formation increases for a given metal ion within the series M(dPMEA) < M(dPEEA) < M(AMP). However, the coordinating properties of all three mentioned ligands differ significantly from those of the antivirally active 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA^{2-}) which, due to the presence of an ether oxygen in the aliphatic side chain, has a different coordination chemistry which involves five-membered chelates with the ether oxygen atom. In addition, the stability constants of the mixed ligand complexes formed between Cu(Arm)$^{2+}$, where Arm = 2,2′-bipyridine (Bpy) or 1,10-phenanthroline (Phen), and the H(dPEEA)$^-$ or dPEEA^{2-} species were also measured. Detailed stability constant comparisons reveal that in the monoprotonated ternary Cu(Arm)(H;dPEEA)$^+$ complexes the proton is at the phosphonate group and that stacking between Cu(Arm)$^{2+}$ and H(dPEEA)$^-$ plays a significant role. For the Cu(Arm)(dPEEA) complexes a large increase in complex stability (compared to the stability expected on the basis of the basicity of the phosphonate group) is observed, which is due to intramolecular stack formation between the aromatic ring systems of Phen or Bpy and the purine moiety of dPEEA^{2-}. The formation degree of the stacked isomers is in the order of 65 to 80%. Comparisons of the Cu(Arm)(PA) systems, where PA^{2-} = dPEEA^{2-}, dPMEA^{2-} or AMP^{2-}, reveal that here dPEEA^{2-} resembles its parent AMP^{2-} less closely than dPMEA^{2-} does. The biological implications of these results, including antiviral activities, are shortly discussed.

1. Introduction

Viruses and the diseases they may cause have been studied for more than 100 years. Viruses are classified according to their genetic material into two major groups: RNA and DNA viruses. The human hepatitis A virus (HAV) and polioviruses belong to the first group, whereas the hepatitis B virus (HBV) is classified as a DNA-containing organism.[1]

Within the RNA-virus group special attention has been paid to the *Retroviridae* family to which the human immunodeficiency viruses (HIV-1 and HIV-2) belong.[2] It is well-known today[3] that, at least in theory, the viral replication cycle may be interrupted in many ways. One of the possibilities is to employ acyclic nucleotide analogues[4] and in this category 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA; Figure 1),[5−7] also known as Adefovir,[8] appears to be especially promising. This compound may be con-

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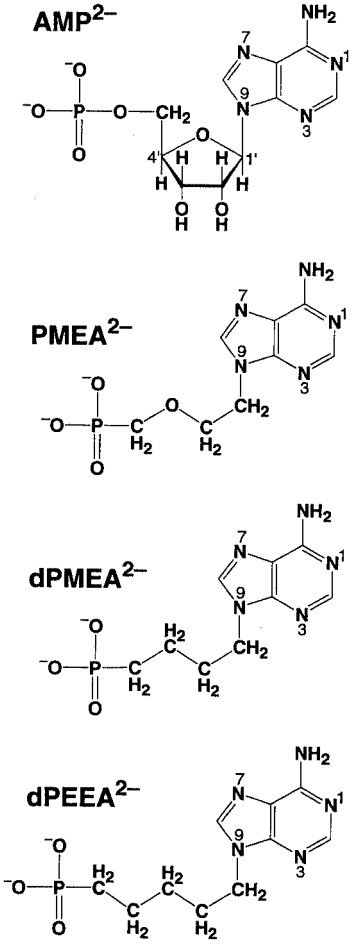


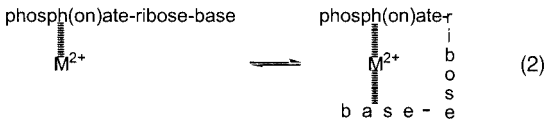
Figure 1. Chemical structures of the dianions of 9-[2-(phosphonomethoxy)ethyl]adenine (= $PMEA^{2-}$ = *Adefovir*), 9-(4-phosphonobutyl)adenine (= $dPMEA^{2-}$ = 3′-deoxa-$PMEA^{2-}$), 9-(5-phosphonopentyl)adenine (= $dPEEA^{2-}$ = 3′-deoxa-$PEEA^{2-}$) and of their parent nucleotide adenosine 5′-monophosphate (AMP^{2-}). The latter is shown in its *anti* conformation.

sidered as an analogue of (2′-deoxy)adenosine 5′-monophosphate [(d)AMP^{2-}; Figure 1]; it is active against HBV and HIV.[8,9] Indeed, its bis(pivaloyloxymethyl)ester (Adefovir dipivoxil) was very recently approved by the US Food and Drug Administration (FDA) for use in hepatitis B therapy.[10]

Since PMEA has a phosphonate group instead of a phosphate unit, it circumvents in blood plasma or in its passage through the cellular membrane the enzyme-catalyzed dephosphorylation that is suffered by nucleotide analogues which contain a monophosphate ester residue.[8,11] Instead, PMEA is diphosphorylated to give the triphosphate-like form PMEApp which is responsible for the therapeutic effect.[8,12] PMEApp is accepted as a substrate by DNA polymerases[13] and after incorporation of the nucleotidyl-like unit into the growing nucleic acid chain leads to its termination due to the lack of a 3′-hydroxyl group. It has been suggested[14,15] that the higher basicity of the phosphonyl group compared to that of a phosphoryl one, together with the presence of the ether-oxygen atom in the aliphatic side chain of $PMEApp^{4-}$, facilitate the correct positioning of the two metal ions needed in the reaction and thus make it initially an even better substrate[16,17] for DNA polymerases than $dATP^{4-}$.[14,15]

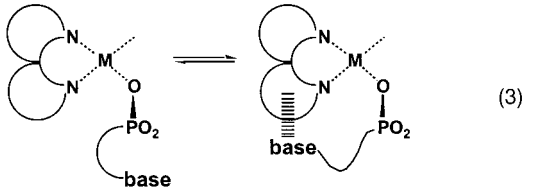
Indeed, PMEA forms with divalent metal ions (M^{2+}), like Mg^{2+}, Mn^{2+} or Zn^{2+}, five-membered chelates involving the ether oxygen as expressed in the intramolecular Equilibrium (1):

Replacement of the ether oxygen by a CH_2 unit gives 9-(4-phosphonobutyl)adenine, also termed 3′-deoxa-PMEA (dPMEA; Figure 1).[18] This compound shows no biological activity,[16] in agreement with the suggestions summarized above, and it also has a coordination pattern[18] different from that of $PMEA^{2-}$,[18–21] yet one which in fact is similar to that of AMP^{2-}.[20,22,23] This means, phosph(on)ate-coordinated metal ions may form macrochelates by interacting with N7 of the adenine residue; this is expressed in the intramolecular Equilibrium (2):[24–26]



Having a broad interest in the metal ion-binding properties of nucleotide analogues,[27–30] we decided to study 9-(5-phosphonopentyl)adenine, which is also known as 3′-deoxa-PEEA (dPEEA; Figure 1), since it is the deoxa analogue of 9-[2-(2-phosphonoethoxy)ethyl]adenine.[31] As expected, dPEEA does not show a useful biological activity but its additional CH_2 group enabled the investigation of the effect that the lengthening of the side chain of dPMEA has on the metal ion-binding properties. Thus, we measured the stability constants of the binary complexes formed between dPEEA and the alkaline earth ions, the divalent ions of the second half of the 3d series as well as Zn^{2+} and Cd^{2+}. We compared the results regarding the position of Equilibrium (2) with those obtained previously for the M(dPMEA) and the M(AMP) complexes.[18,23]

Since weak interactions[32–34] like the aromatic-ring stacking are important for the anchoring process of a substrate in the active site cavity of an enzyme,[35,36] we had previously quantified the extent of intramolecular stacking according to Equilibrium (3)



for the ternary complexes Cu(Arm)(AMP/dPMEA),[37,38] where Arm = 2,2′-bipyridine (Bpy) or 1,10- phenanthroline (Phen). Now the corresponding Cu(Arm)(dPEEA) complexes were studied leading to the interesting result that lengthening of the aliphatic chain by a CH_2 unit diminishes intramolecular stacking [Equilibrium (3)], whereas in the binary M(dPEEA) species macrochelate formation [Equilibrium (2)] is favored.

2. Results and Discussion

2.1 Acidity Constants of $H_2(dPEEA)^{\pm}$

From the structure of $dPEEA^{2-}$ (see Figure 1) it is evident that this species can accept three protons, two at the phosphonate group and one at the N1 site of the adenine residue.[39] Further protonations at an adenine residue are possible at N7 and N3, but these protons are released with $pK_a < 0$;[39−42] similarly, release of the first proton from a twofold protonated phosphonate residue occurs with a pK_a of about 2.[18,43] Hence, in the present study, for which all potentiometric pH titrations were carried out at pH > 4.0, only the following two deprotonation reactions, in which $dPEEA^{2-}$ is abbreviated as PA^{2-}, need to be considered:

$$H_2(PA)^{\pm} \rightleftharpoons H(PA)^{-} + H^{+} \quad (4a)$$

$$K^{H}_{H_2(PA)} = [H(PA)^{-}][H^{+}]/[H_2(PA)^{\pm}] \quad (4b)$$

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

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

Indeed, all the experimental data from the pH titrations in aqueous solution (25 °C; I = 0.1 M, $NaNO_3$) could be excellently fitted by taking into account Equilibria (4) and (5). The acidity constants obtained in the present study for $H_2(dPEEA)^{\pm}$ are given in Table 1 together with some related data.[44−46]

Table 1. Negative logarithms of the acidity constants of $H_2(dPEEA)^{\pm}$ [Equations (4) and (5)] together with the corresponding values of some related systems in aqueous solution at 25 °C and I = 0.1 M ($NaNO_3$)[a][b]

No.	Protonated species	$pK^{H}_{H_2(PA)}$ $(N1)H^{+}$	$pK^{H}_{H(PA)}$ $P(O)_2(OH)^{-}$	Ref.
1	$H(9MeAde)^{+}$	4.10 ± 0.01	−	[41]
2	$CH_3P(O)(OH)_2$	−	7.51 ± 0.01	[43]
3	$CH_3OP(O)(OH)_2$	−	6.36 ± 0.01	[44]
4	$H_2(AMP)^{\pm}$	3.84 ± 0.02	6.21 ± 0.01	[22]
5	$H_2(PMEA)^{\pm}$	4.16 ± 0.02	6.90 ± 0.01	[45]
6	$H_2(dPMEA)^{\pm}$	4.17 ± 0.02	7.69 ± 0.01	[18]
7	$H_2(dPEEA)^{\pm}$	4.17 ± 0.01	7.75 ± 0.01	[c]

[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, mixed or Brønsted constants are listed (see [46] for details). [c] This work.

Comparison of the values listed in Table 1 (entries 1 and 4−7) reveal that the first proton of $H_2(dPEEA)^{\pm}$ is from the $(N1)H^{+}$ site of the adenine residue and the second one from the monoprotonated phosphonate group (see entries 2−7). However, more comparisons are possible; a few are given:

(i) From the comparison of entries 3 and 4 with those of 2 and 5−7 it follows that protonated phosphonate groups are less acidic than the corresponding phosphate groups; this agrees with the lower electronegativity of a C atom compared to an O atom.

(ii) The ether oxygen, due to its electron-withdrawing effect, makes the monoprotonated phosphonate species of $PMEA^{2-}$ more acidic than those of $dPMEA^{2-}$ or $dPEEA^{2-}$ (entries 5−7).

(iii) In fact, the acid-base properties of $H_2(dPMEA)^{\pm}$ and of $H_2(dPEEA)^{\pm}$ are very similar, indicating that the additional CH_2 unit in PEEA has little effect (entries 6, 7).

Finally, it needs to be pointed out that due to the different affinities of the phosph(on)ate groups for protons, the formation degree of the free ligands, PA^{2-}, differs considerably in the physiological pH range around 7.5: Under these conditions, for example, AMP^{2-} is formed to about 95% whereas $dPEEA^{2-}$ occurs only to about 36%.

2.2 Stability Constants of the $M(H;dPEEA)^{+}$ and M(dPEEA) Complexes

The experimental data of the potentiometric pH titrations (see Section 4.3) allow the determination of the stability constants defined by Equilibria (6a) and (7a):

$$M^{2+} + H(PA)^{-} \rightleftharpoons M(H;PA)^{+} \quad (6a)$$

$$K^{M}_{M(H;PA)} = [M(H;PA)^{+}]/([M^{2+}][H(PA)^{-}]) \quad (6b)$$

$$M^{2+} + PA^{2-} \rightleftharpoons M(PA) \quad (7a)$$

$$K^{M}_{M(PA)} = [M(PA)]/([M^{2+}][PA^{2-}]) \quad (7b)$$

Together with Equilibria (4a) and (5a) they are sufficient to obtain excellent fitting of the titration data provided the evaluation is not carried into the pH range where formation of hydroxo species occurs, which was evident from titrations without ligand. Of course, Equilibria (6a) and (7a) are also connected via Equilibrium (8a)

$$M(H;PA)^{+} \rightleftharpoons M(PA) + H^{+} \quad (8a)$$

$$K^{H}_{M(H;PA)} = [M(PA)][H^{+}]/[M(H;PA)^{+}] \quad (8b)$$

and the corresponding acidity constant [Equation (8b)] may be calculated with Equation (9):[47]

$$pK^{H}_{M(H;PA)} = pK^{H}_{H(PA)} + \log K^{M}_{M(H;PA)} - \log K^{M}_{M(PA)} \quad (9)$$

The results are listed in Table 2; the stability constants given for the $M(H;dPEEA)^{+}$ complexes are in part estimates since the formation degree of these species was low (see Section 4.3). The stability constants of the M(dPEEA)

complexes show the usual trends. For the alkaline earth ions the stability of the complexes decreases with increasing ionic radii indicating that metal ion binding at the phosphonate group is (at least) in part inner-sphere. For the divalent 3d metal ions the long-standing experience[48] is confirmed that the stabilities of phosph(on)ate-metal ion complexes often do not strictly follow[18,20,27,28,44,45,49] the Irving−Williams sequence,[50] 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.[19,22,24,25,51]

Table 2. Logarithms of the stability constants of the $M(H;dPEEA)^+$ [Equation (6b)] and M(dPEEA) complexes [Equation (7b)], together with the negative logarithms of the acidity constants of the protonated complexes [Equations (8b) and (9)] in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

M^{2+}	$\log K^{M}_{M(H;dPEEA)}$	$\log K^{M}_{M(dPEEA)}$	$pK^{H}_{M(H;dPEEA)}$
Mg^{2+}	0.4 ± 0.3[b]	1.87 ± 0.05	6.28 ± 0.30
Ca^{2+}	0.2 ± 0.4[b]	1.59 ± 0.06	6.36 ± 0.40
Sr^{2+}	0.1 ± 0.4[b]	1.31 ± 0.07	6.54 ± 0.41
Ba^{2+}	0.0 ± 0.4[b]	1.25 ± 0.06	6.50 ± 0.40
Mn^{2+}	0.69 ± 0.18	2.55 ± 0.03	5.89 ± 0.18
Co^{2+}	0.86 ± 0.21	2.36 ± 0.05	6.25 ± 0.22
Ni^{2+}	1.00 ± 0.15	2.46 ± 0.03	6.29 ± 0.15
Cu^{2+}	1.92 ± 0.12	3.86 ± 0.08	5.81 ± 0.15
Zn^{2+}	1.47 ± 0.22	2.9 ± 0.2[c]	6.32 ± 0.30
Cd^{2+}	1.38 ± 0.15	3.19 ± 0.05	5.94 ± 0.16

[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. [b] The constants listed for these $M(H;dPEEA)^+$ complexes are estimates (see Section 4.3). [c] The experiments with Zn^{2+} were significantly hampered by precipitation; i.e., the pH range accessible for the evaluation of the constants was severely restricted (see Section 4.3).

As far as the $M(H;dPEEA)^+$ complexes are concerned, it is evident that the evaluation of potentiometric pH titration data only allows the determination of their stability constants. 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 binding of a metal ion to a protonated ligand commonly leads to an acidification of the ligand-bound proton.[52,53] Indeed, the acidity constants of the $M(H;dPEEA)^+$ complexes given in column 4 of Table 2 are by about 1.2 to 1.9 log units smaller than $pK^{H}_{H(dPEEA)}$. This shows that the proton in $M(H;dPEEA)^+$ is bound to the phosphonate group, hence, one may tentatively assume that the metal ion is bound preferentially to the nucleobase, since a monoprotonated phosphonate group is only a weak binding site. Indeed, this suggestion agrees with evidence obtained previously for other related $M(H;PA)^+$ species.[20,21,45,54]

Furthermore, the stability constants of the $M(H;dPEEA)^+$ complexes are, within the error limits, identical to the values determined[45] for the corresponding $M(H;PMEA)^+$ and $M(H;dPMEA)^+$ species.[18] Considering that the basicity of the N1 sites in $H(dPEEA)^-$ and $H(dPMEA)^-$ or $H(PMEA)^-$ are also identical (see Table 1; column 3, entries 5−7) and that evidence has been provided for the $M(H;PMEA)^+$ complexes[20,21,45] that the metal ion is mainly located at the nucleobase residue, one may not only conclude that in the $M(H;dPEEA)^+$ complexes the proton is at the phosphonate group, but also that the metal ion is mainly at the adenine residue. The N1 versus N7 dichotomy for metal ion binding to the adenine residue is well-known[55] though there are indications that binding to N7 dominates.[55,56] In any case, the fact that the stabilities of the $M(H;dPEEA)^+$ complexes *follow* the Irving−Williams sequence[50] [in contrast to phosph(on)ate coordinations] also supports[48a] the above conclusion that metal ion binding in the monoprotonated species occurs preferably to a nitrogen atom.

2.3 Evaluation of the Stabilities of the M(dPEEA) Complexes

For the M(dPEEA) complexes the question arises: Does the adenine residue also participate in metal ion binding next to the phosphonate group? Should such an additional interaction with the adenine residue occur, then it has to be reflected in an increased complex stability.[57] 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[45] straight-line correlations which are based on $\log K^{M}_{M(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ plots for simple phosphate monoesters[58] and phosphonates;[45] these ligands are abbreviated as $R\text{-}PO_3^{2-}$, where R represents a non-coordinating residue. The parameters for the corresponding straight-line equations, which are defined by Equation (10),

$$\log K^{M}_{M(R\text{-}PO_3)} = m \cdot pK^{H}_{H(R\text{-}PO_3)} + b \qquad (10)$$

have been tabulated,[19a,25,45,51] i.e., the slopes m and the intercepts b with the y-axis. 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.

The plots of $\log K^{M}_{M(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ according to Equation (10) are shown in Figure 2 for the 1:1 complexes of Ca^{2+}, Co^{2+}, Cd^{2+} and Cu^{2+} as examples, with the data points (empty circles) of the eight simple ligand systems used[45] for the determination of the straight base lines. The four solid circles refer to the corresponding M(dPEEA) complexes; those for the Cd^{2+} and Cu^{2+} species, and possibly also for the Co^{2+} ones, are somewhat above their reference lines, thus proving an increased stability for these complexes, whereas the data point for the Ca^{2+} complex fits within the error limits on the line. Similar properties are observed for the corresponding M(dPMEA) and M(AMP) complexes (crossed circles). In contrast, *all* of the data points[45] of the M(PMEA) complexes (solid squares) are clearly above their reference lines indicating that all these

species are more stable than is expected on the basis of the basicity of the phosphonate group of $PMEA^{2-}$.

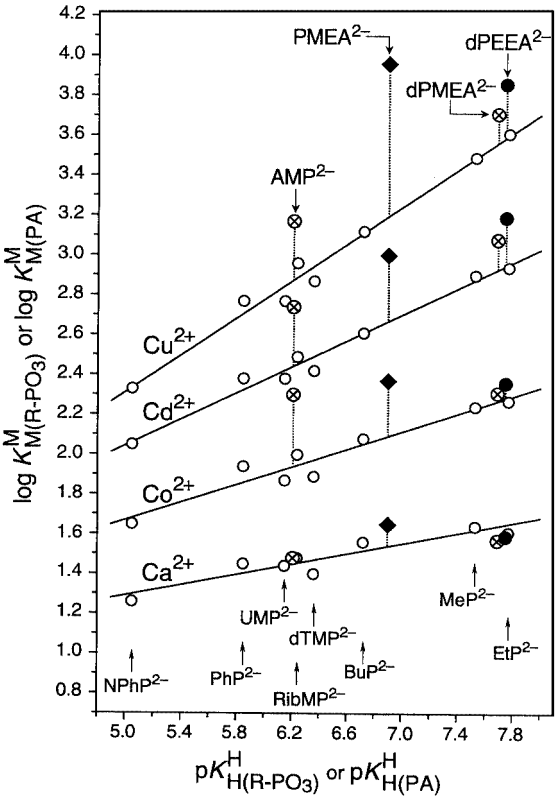


Figure 2. Evidence for an enhanced stability of the Cd(dPEEA) and Cu(dPEEA) and possibly also the Co(dPEEA) complexes, and for the lack of such an enhanced stability of the Ca(dPEEA) species (●), together with the data points for the corresponding metal ion complexes of $dPMEA^{2-}$, AMP^{2-} (⊗) and $PMEA^{2-}$ (◆) based on the relationship between log $K^{M}_{M(R\text{-}PO_3)}$ and $pK^{H}_{H(R\text{-}PO_3)}$ 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 [Equation (10)] are drawn through the corresponding 8 data sets (○) taken from [58] for the phosphate monoesters and from [45] for the phosphonates. The points due to the equilibrium constants for the M^{2+}/dPEEA systems (●) are based on the values listed in Tables 1 and 2; those for the M^{2+}/dPMEA (⊗), M^{2+}/AMP (⊗), and M^{2+}/PMEA systems (◆) are from [18,23] and [45], respectively. The vertical broken lines emphasize the stability differences from the reference lines; they equal log $\Delta_{M/PA}$ as defined in Equation (11). All the plotted equilibrium constants refer to aqueous solutions at 25 °C and I = 0.1 M ($NaNO_3$).

The increased stability of the M(PMEA) complexes has been proven[45] to be largely due to the involvement of the ether oxygen atom in metal ion binding (see Figure 1) which gives rise to the formation of five-membered chelates and thus to the intramolecular Equilibrium (1).[15,19,45] In certain systems, like Ni(PMEA) and Cu(PMEA), a third isomer exists which involves, in addition, N3 of the adenine residue[15,19–21,45] as well as a fourth one involving N7 which, however, occurs only to a minor extent.[18]

Naturally, in the complexes of $dPEEA^{2-}$, $dPMEA^{2-}$ and AMP^{2-}, whose side chains or sugar residue is devoid of an ether oxygen atom (Figure 1), Equilibrium (1) cannot exist and therefore, any possibly observed stability increase has to be attributed to an interaction with the adenine residue. Stability enhancements, like those seen in Figure 2, can be quantified by the differences between the experimentally (exptl) measured stability constants and those calculated (calcd) according to Equation (10); this difference is defined in Equation (11),

$$\log \Delta_{M/PA} = \log K^{M}_{M(PA)_{exptl}} - \log K^{M}_{M(PA)_{calcd}} \quad (11a)$$

$$= \log K^{M}_{M(PA)} - \log K^{M}_{M(PA)_{op}} \quad (11b)$$

where the expressions log $K^{M}_{M(PA)calcd}$ and log $K^{M}_{M(PA)op}$ are synonymous because the calculated value equals the stability constant of the "open" isomer, $M(PA)_{op}$ [see, for example, Equilibria (1) and (2)], in which only a -PO_3^{2-}/M^{2+} interaction occurs. In columns 2–4 of Table 3 the values for the three terms of Equation (11) are listed. The log $\Delta_{M/dPEEA}$ values for Ni(dPEEA), Cu(dPEEA) and Cd(dPEEA) are clearly positive; the same is most likely true for those due to Co(dPEEA) and Zn(dPEEA), especially if one takes into account that the error limits refer to *three times* the standard error. Hence, in all these instances Equilibrium (2) needs to be considered.

Table 3. Stability constant comparisons for the M(dPEEA) complexes between the measured stability constants (exptl; Table 2, column 3) and the calculated stability constants (calcd) based on the basicity of the phosphonate group in $dPEEA^{2-}$ ($pK^{H}_{H(dPEEA)}$ = 7.75; Table 1) and the baseline equations established previously[25,45,51] (see Equation (10) and Figure 2), together with the stability differences log $\Delta_{M/dPEEA}$, as defined by Equation (11). The previously determined stability differences, log $\Delta_{M/dPMEA}$, for M(dPMEA) complexes[18] are given for comparison (aqueous solution; 25 °C; I = 0.1 M, $NaNO_3$)[a]

M^{2+}	log $K^{M}_{M(dPEEA)}$ exptl[b]	log $K^{M}_{M(dPEEA)}$ calcd	log $\Delta_{M/dPEEA}$	log $\Delta_{M/dPMEA}$
Mg^{2+}	1.87 ± 0.05	1.88 ± 0.03	−0.01 ± 0.06	−0.03 ± 0.05
Ca^{2+}	1.59 ± 0.06	1.65 ± 0.05	−0.06 ± 0.08	−0.07 ± 0.07
Sr^{2+}	1.31 ± 0.07	1.37 ± 0.04	−0.06 ± 0.08	−0.06 ± 0.06
Ba^{2+}	1.25 ± 0.06	1.30 ± 0.04	−0.05 ± 0.07	−0.07 ± 0.07
Mn^{2+}	2.55 ± 0.03	2.53 ± 0.05	0.02 ± 0.06	−0.04 ± 0.06
Co^{2+}	2.36 ± 0.05	2.28 ± 0.06	0.08 ± 0.08	0.04 ± 0.08
Ni^{2+}	2.46 ± 0.03	2.32 ± 0.05	0.14 ± 0.06	0.10 ± 0.08
Cu^{2+}	3.86 ± 0.08	3.59 ± 0.06	0.27 ± 0.10	0.16 ± 0.09
Zn^{2+}	2.9 ± 0.2	2.66 ± 0.06	0.24 ± 0.21	0.1 ± 0.2
Cd^{2+}	3.19 ± 0.05	2.95 ± 0.05	0.24 ± 0.07	0.15 ± 0.11

[a] Regarding the error limits (3σ) see footnote [a] of Table 2. [b] These values are from column 3 of Table 2.

A comparison of the log $\Delta_{M/PA}$ values given in columns 4 and 5 of Table 3 for the M(dPEEA) and M(dPMEA) complexes, respectively, reveals that their properties are quite alike with a slightly more enhanced stability of some of the M(dPEEA) species.

2.4 Extent of Macrochelate Formation in M(dPEEA) Systems and Comparison with That Occurring in M(dPMEA) and M(AMP) Species

The position of the concentration-independent Equilibrium (2) between the simple phosphonate-bound species, which we designate as the "open" isomer, $M(dPEEA)_{op}$, and the macrochelated isomer involving the adenine residue, designated as the "closed" species, $M(dPEEA)_{cl}$, is defined by the intramolecular, and hence dimensionless, equilibrium constant K_I given in Equation (12):

$$K_I = [M(dPEEA)_{cl}]/[M(dPEEA)_{op}] \quad (12)$$

Values for K_I can be calculated by known procedures,[22,45,57] i.e., via Equation (13):

$$K_I = 10^{\log \Delta_{M/dPEEA}} - 1 \quad (13)$$

Knowledge of K_I allows then to obtain the percentage of the closed isomer, $M(dPEEA)_{cl}$, according to Equation (14):

$$\% M(dPEEA)_{cl} = 100 \cdot K_I/(1 + K_I) \quad (14)$$

The results are summarized in Table 4 for the M(dPEEA) complexes of the 3d ions including Zn^{2+} and Cd^{2+}. For the complexes of the alkaline earth ions the formation degree of the closed species in Equilibrium (2) is zero within the error limits as follows from the log $\Delta_{M/dPEEA}$ values listed in column 4 of Table 3.

From the results listed in columns 2−4 of Table 4 it follows that at least for Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}, which have the most pronounced affinity for N sites among the ten metal ions studied,[48a] Equilibrium (2) operates and that the adenine residue is involved in metal ion binding. From the three nitrogen atoms available in the purine ring, i.e., N1, N3 and N7 (see Figure 1), N1 cannot be reached by a metal ion already coordinated to the phosphonate group;[19] hence, N3 and N7 remain for macrochelate formation. At this stage one cannot distinguish with absolute certainty between these two possibilities, but we strongly favor an interaction of the phosphonate-coordinated metal ion with N7 because N7 is known to be considerably more basic than N3.[39−42] Furthermore, macrochelate formation in the M(AMP) complexes occurs with certainty via N7;[22,59] hence, it appears that M(dPEEA) and M(dPMEA) resemble in this respect their parent complexes, i.e., M(AMP), more than the M(PMEA) species which contain the antivirally active ligand $PMEA^{2-}$ and involve the ether oxygen atom in metal ion binding (see Section 1).

With the above evidence for macrochelate formation involving N7 in mind, it is interesting to compare the properties of the $dPEEA^{2-}$, $dPMEA^{2-}$ and AMP^{2-} ligands (Figure 1). From the results given in columns 4−6 of Table 4 it follows that the extent of macrochelate formation increases within the series M(dPMEA) < M(dPEEA) < M(AMP). The changes are not dramatic, but the trend within the series seems to be clear for each metal ion. A further interesting observation is that for Cu^{2+}, Zn^{2+} and Cd^{2+} the formation degrees of the $M(dPEEA)_{cl}$ and $M(AMP)_{cl}$ species are quite alike. Hence, it seems that $dPEEA^{2-}$ mimics AMP^{2-} even better than $dPMEA^{2-}$; most likely this is due to the by one CH_2 unit longer side chain of $dPEEA^{2-}$, compared to the one of $dPMEA^{2-}$, and that this allows the phosphonate-bound M^{2+} to reach N7 with less strain.

2.5 Stability of the Mixed Ligand Cu(Arm)(H;dPEEA)$^+$ and Cu(Arm)(dPEEA) Complexes

For the ternary complexes composed of $H(dPEEA)^-$ or $dPEEA^{2-}$, Cu^{2+} and a heteroaromatic amine (Arm), i.e., 2,2′-bipyridine (Bpy) or 1,10-phenanthroline (Phen), the same evaluation procedure holds as described in the first paragraph of Section 2.2 for the binary complexes because complex formation of $Cu(Bpy)^{2+}$ and $Cu(Phen)^{2+}$, due to their high stability,[60] is complete (see also Section 4.3) before the onset of the formation of the mixed ligand complexes occurs. This means that in the case of the ternary systems the experimental data of the potentiometric pH titrations can also be fully described by taking Equilibria (4a)−(7a) into account, but now $M^{2+} = Cu(Arm)^{2+}$. The corresponding stability constants are given in Table 5.

A comparison of the acidity constants of $H_2(dPEEA)^{\pm}$, $pK^H_{H_2(dPEEA)} = 4.17$ and $pK^H_{H(dPEEA)} = 7.75$ (Table 1), with those of the $Cu(Arm)(H;dPEEA)^+$ complexes, i.e.,

Table 4. Increased complex stability, log $\Delta_{M/dPEEA}$ [Equation (11)], and extent of chelate formation according to Equilibrium (2) for some M(dPEEA) complexes, as quantified by the dimensionless equilibrium constant K_I [Equations (12), (13)] and the percentage of $M(dPEEA)_{cl}$ [Equation (14)]. The corresponding percentages for $M(dPMEA)_{cl}$ [18] and $M(AMP)_{cl}$ [23] are given for comparison (aqueous solution; 25 °C; I = 0.1 M, $NaNO_3$)[a]

M^{2+}	log $\Delta_{M/dPEEA}$ [b]	K_I	% $M(dPEEA)_{cl}$	% $M(dPMEA)_{cl}$	% $M(AMP)_{cl}$
Mn^{2+}	0.02 ± 0.06	0.05 ± 0.14	5 ± 13	≤ 5	15 ± 11
Co^{2+}	0.08 ± 0.08	0.20 ± 0.22	17 ± 15	9 ± 17	56 ± 7
Ni^{2+}	0.14 ± 0.06	0.38 ± 0.19	28 ± 10	21 ± 15	75 ± 4
Cu^{2+}	0.27 ± 0.10	0.86 ± 0.43	46 ± 12	31 ± 14	50 ± 7
Zn^{2+}	0.24 ± 0.21[c]	0.74 ± 0.84[c]	42 ± 28	21 ± 37[d]	44 ± 12
Cd^{2+}	0.24 ± 0.07	0.74 ± 0.28	42 ± 9	29 ± 18	50 ± 8

[a] Regarding the error limits (3σ) see footnote [a] of Table 2. [b] From column 4 of Table 3. [c] See footnote [c] in Table 2. [d] The measurements for the Zn(dPMEA) systems were also affected by precipitation.[18]

Table 5. Logarithms of the stability constants of the $M(H;dPEEA)^+$ [Equation (6b)] and M(dPEEA) complexes [Equation (7b)], where $M^{2+} = Cu^{2+}$, $Cu(Bpy)^{2+}$ or $Cu(Phen)^{2+}$, as determined by potentiometric pH titrations in aqueous solution, together with the negative logarithms for the acidity constants of $M(H;dPEEA)^+$ [Equations (8b), (9)] at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

M^{2+}	log $K^{M}_{M(H;dPEEA)}$	log $K^{M}_{M(dPEEA)}$	$pK^{H}_{M(H;dPEEA)}$
Cu^{2+}	1.92 ± 0.12	3.86 ± 0.08	5.81 ± 0.15
$Cu(Bpy)^{2+}$	1.87 ± 0.11	4.07 ± 0.07	5.55 ± 0.13
$Cu(Phen)^{2+}$	2.14 ± 0.12	4.36 ± 0.14	5.53 ± 0.18

See footnote [a] of Table 2.

$pK^{H}_{Cu(Arm)(H;dPEEA)} \approx 5.5$ (Table 5, column 4), reveals that in these complexes, just as in the binary ones, the proton must be located at the phosphonate group, since metal ion coordination must give rise to an acidification.[52,53] In fact, the acidity constants of the $Cu(Arm)(H;dPEEA)^+$ complexes are about 2.2 log units smaller than $pK^{H}_{H(dPEEA)}$, but approximately 1.4 log units larger than $pK^{H}_{H_2(dPEEA)}$.

However, where is the $Cu(Arm)^{2+}$ unit located? Indeed, the $Cu(Arm)(H{\cdot}dPEEA)^+$ species, where the notation $(H{\cdot}dPEEA)^-$ indicates that the proton is at the phosphonate group, may exist in two principally different forms: One, where $Cu(Arm)^{2+}$ is stacked with the purine system of $(H{\cdot}dPEEA)^-$, designated as $[Cu(Arm)/(H{\cdot}dPEEA)]^+_{st}$, and another one, where $Cu(Arm)^{2+}$ is simply coordinated either to the N1/N7 sites of the adenine residue (see Section 2.2), $[(H{\cdot}dPEEA){\cdot}Cu(Arm)]^+_{ade}$, or to the phosphonate group which already carries the proton. However, the formation of the latter species with both the proton and $Cu(Arm)^{2+}$ at the phosphonate group is unlikely, in agreement with the conclusions given in Section 2.2 for the binary $Cu(H;dPEEA)^+$ species, where Cu^{2+} is mainly located at the adenine residue and H^+ at the phosphonate group. Hence, we have to consider the $[(H{\cdot}dPEEA){\cdot}Cu(Arm)]^+_{ade}$ and the $[Cu(Arm)/(H{\cdot}dPEEA)]^+_{st}$ species and take into account the following intramolecular Equilibrium (15):

$$[(H{\cdot}dPEEA){\cdot}Cu(Arm)]^+_{ade} \rightleftharpoons [Cu(Arm)/(H{\cdot}dPEEA)]^+_{st} \qquad (15)$$

An evaluation of the various equilibrium constants, identical to the procedure described recently,[38,61] leads to the conclusion that the stacked species in Equilibrium (15) dominate in the $Cu(Phen)^{2+}$ system with a formation degree of about 70% for the $[Cu(Phen)/(H{\cdot}dPEEA)]^+_{st}$ species. In the case of the $Cu(Bpy)^{2+}$ system the two isomeric species of Equilibrium (15) occur in about *equal* amounts. These results are very similar to those obtained previously[38] for the $Cu(Arm)^{2+}/(H{\cdot}dPMEA)^-$ systems. Furthermore, that stacking is somewhat more pronounced in the system containing Phen is in agreement with the expectation (see also Section 2.7).

2.6 Proof of an Increased Stability of the Mixed Ligand Cu(Arm)(dPEEA) Complexes

One way to quantify the stability of mixed ligand complexes[62,63] is to consider Equilibrium (16a) with its corresponding equilibrium constant [Equation (16b)], which is calculated from Equation (17):

$$Cu(Arm)^{2+} + Cu(dPEEA) \rightleftharpoons Cu(Arm)(dPEEA) + Cu^{2+} \qquad (16a)$$

$$10^{\Delta \log K} = \frac{[Cu(Arm)(dPEEA)][Cu^{2+}]}{[Cu(Arm)^{2+}][Cu(dPEEA)]} \qquad (16b)$$

$$\Delta \log K = \log K^{Cu(Arm)}_{Cu(Arm)(dPEEA)} - \log K^{Cu}_{Cu(dPEEA)} \qquad (17)$$

According to the general rule for complex stabilities, $K_1 > K_2$, Equilibrium (16a) is expected to lie on the left with negative values for $\Delta \log K$ in agreement with statistical considerations, i.e. $\Delta \log K_{Cu/statist} \approx -0.5$.[63] The results for $\Delta \log K_{Cu/Bpy/dPEEA}$ and $\Delta \log K_{Cu/Phen/dPEEA}$ according to Equation (17) are 0.21 ± 0.11 and 0.50 ± 0.16, respectively (see Table 5). These values are clearly larger than the statistically expected value. Hence, it is clear that Equilibrium (16a) is significantly displaced to the right hand side and that these ternary complexes show an increased stability. However, it is difficult to draw quantitative conclusions from these results because the binary Cu(dPEEA) complex itself also shows an increased stability due to macrochelate formation [see Section 2.4 and Equilibrium (2)]. Yet, despite this shortcoming the given results for Equation (17) still prove definitely an increased stability for the Cu(Arm)(dPEEA) complexes.

Another way to evaluate the stability of the ternary Cu^{2+} complexes, independently of that of the corresponding binary complexes, is analogous to the procedure used in Section 2.4 for the binary systems. This means, straight-line correlations for log $K^{Cu(Arm)}_{Cu(Arm)(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ plots, where $R\text{-}PO_3^{2-}$ represents phosphate monoester or phosphonate ligands in which the residue R is unable to interact with $Cu(Arm)^{2+}$, have been defined and they are valid in the pK_a range between 5 and 8.[51][61] These reference lines are seen in Figure 3 where the stability constants log $K^{Cu(Arm)}_{Cu(Arm)(dPEEA)}$ versus the acidity constant $pK^{H}_{H(dPEEA)}$ are also plotted (square symbols) together with the corresponding data[38] of the $Cu(Arm)^{2+}/dPMEA^{2-}$ systems (circles).

The data points in Figure 3 for the two ternary Cu(Arm)(dPEEA) complexes are far above their reference lines proving again the increased complex stability and this must now mean[57] that, aside from the phosphonate-$Cu(Arm)^{2+}$ coordination, further interactions occur. Such an increased stability, which in fact is even more pronounced, is also observed for the ternary Cu(Arm)(dPMEA) systems[38] as one can see in Figure 3. The vertical differences between the mentioned data points and their reference lines can be defined according to Equation (11) (Section 2.3) by keeping in mind that now $M^{2+} = Cu(Arm)^{2+}$. The needed stability of the "open" isomers, $Cu(Arm)(dPEEA)_{op}$, is calculated with $pK^{H}_{H(dPEEA)} = 7.75$ (Table 1, entry 7) and the straight-line parameters given

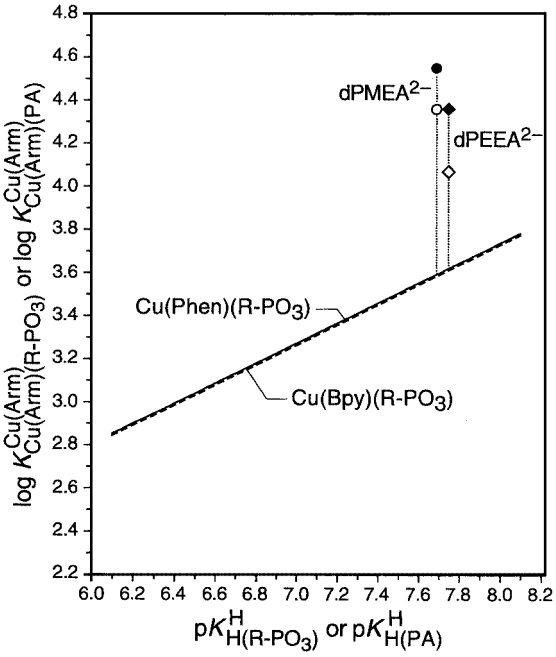


Figure 3. Evidence for an enhanced stability of the ternary Cu(Bpy)(dPEEA) (◇) and Cu(Phen)(dPEEA) (◆) as well as the Cu(Bpy)(dPMEA) (○) and Cu(Phen)(dPMEA) (●) complexes, based on the relationship between log $K^{Cu(Arm)}_{Cu(Arm)(R\text{-}PO_3)}$ or log $K^{Cu(Arm)}_{Cu(Arm)}$ and $pK^{H}_{H(R\text{-}PO_3)}$ or $pK^{H}_{H(PA)}$ in aqueous solution at $I = 0.1$ M ($NaNO_3$) and 25 °C. The plotted data corresponding to the $dPEEA^{2-}$ systems are from Tables 1 and 5 and those corresponding to $dPMEA^{2-}$ are from ref.[38] The two reference lines represent the log K versus pK_a relationship for the ternary Cu(Arm)($R\text{-}PO_3$) complexes [Equation (10)]; it should be emphasized that $R\text{-}PO_3^{2-}$ symbolizes here phosph(on)ate ligands with a group R unable to undergo any kind of hydrophobic, stacking or other type of interaction. The parameters for the straight-line equations are listed in ref.[61]

in refs.[51,61]: log $K^{Cu(Bpy)}_{Cu(Bpy)(dPEEA)op}$ = 3.61 ± 0.07 and log $K^{Cu(Phen)}_{Cu(Phen)(dPEEA)op.}$ = 3.62 ± 0.06. Application of these values together with the corresponding experimental results given in Table 5 (column 3) to Equation (11) leads to the log $\Delta_{Cu/Arm/dPEEA}$ values which quantify in an exact way the enhanced stability of the ternary Cu(Arm)(dPEEA) complexes. These values are listed in the third column of Table 6 (see below).

2.7 Evaluation of the Increased Stability of the Cu(Arm)(dPEEA) Complexes

It is clear[57] that the described enhanced complex stabilities (Table 6, column 3) must originate in additional interactions, i.e., beyond the coordination of $Cu(Arm)^{2+}$ to the phosphonate group. The ligand $dPEEA^{2-}$ offers only two such possibilities: The phosphonate-coordinated $Cu(Arm)^{2+}$ forms (i) a macrochelate with N7 of the adenine residue, as is the case with the binary Cu(dPEEA) complex (Section 2.4), or (ii) an intramolecular stack between the aromatic ring systems of Bpy or Phen and the adenine moiety. This latter type of interaction is known for many examples and can be very significant;[32,35−38,54,63] it is also evident from the results indicated in Section 2.5 for the monoprotonated $Cu(Arm)(H;dPEEA)^{+}$ species.

That the first of these two possible interactions is of no importance was demonstrated in detail recently for the $Cu(Arm)^{2+}$ systems with $dPMEA^{2-}$ [38] as well as for related analogues[36,54,61] and also for AMP^{2-} itself.[37] Hence, the enhanced complex stabilities may only be attributed to intramolecular stack formation; in fact, for Cu(Arm)(AMP) this was also proven to occur in the solid state by crystal structure analyses.[6b,64] Consequently, the Cu(Arm)(dPEEA) complexes occur in the two isomeric forms indicated in Equilibrium (3): In one, $Cu(Arm)^{2+}$ is only coordinated to the phosphonate group and this isomer is designated as $Cu(Arm)(dPEEA)_{op}$ as already indicated in Section 2.6; in the other form, designated as $Cu(Arm)(dPEEA)_{st}$, intramolecular stack formation occurs. A tentative structure of this latter isomer is shown in Figure 4. Consequently, the dimensionless equilibrium constant $K_{I/st}$ due to the intramolecular Equilibrium (3) is defined by Equation (18):

$$K_{I/st} = [Cu(Arm)(dPEEA)_{st}]/[Cu(Arm)(dPEEA)_{op}] \quad (18)$$

Values for $K_{I/st}$ as well as for the percentage of the stacked isomer, $Cu(Arm)(dPEEA)_{st}$, are calculated in analogy to Equations (13) and (14), respectively, and summarized in columns 4 and 5 of Table 6.

The results of Table 6 lead to the following conclusions: (i) Stack formation is always more pronounced in the Cu(Phen)(PA) systems, where PA^{2-} = $dPEEA^{2-}$,

Table 6. Extent of intramolecular stack formation in ternary Cu(Arm)(PA) complexes, where PA^{2-} = $dPEEA^{2-}$, $dPMEA^{2-}$ or AMP^{2-}, as calculated from stability constants determined via potentiometric pH titrations: Given are the stability enhancement log $\Delta_{Cu/Arm/PA}$ [Equation (11)], the intramolecular and dimensionless equilibrium constant $K_{I/st}$ [Equations (13), (18)], and the percentage [Equation (14)] of the stacked $Cu(Arm)(PA)_{st}$ species in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

No.	Cu(Arm)(PA)	log $\Delta_{Cu/Arm/PA}$	$K_{I/st}$	%$Cu(Arm)(PA)_{st}$	Ref.
1a	Cu(Bpy)(dPEEA)	0.46 ± 0.10	1.88 ± 0.66	65 ± 8	[b]
1b	Cu(Phen)(dPEEA)	0.74 ± 0.15	4.50 ± 1.90	82 ± 6	[b]
2a	Cu(Bpy)(dPMEA)	0.78 ± 0.10	5.03 ± 1.39	83 ± 4	[29]
2b	Cu(Phen)(dPMEA)	0.96 ± 0.12	8.12 ± 2.52	89 ± 3	[29]
3a	Cu(Bpy)(AMP)	0.73 ± 0.08	4.37 ± 1.02	81 ± 4	[31]
3b	Cu(Phen)(AMP)	0.99 ± 0.08	8.77 ± 1.81	90 ± 2	[31]

[a] See footnote [a] of Table 2. [b] This work.

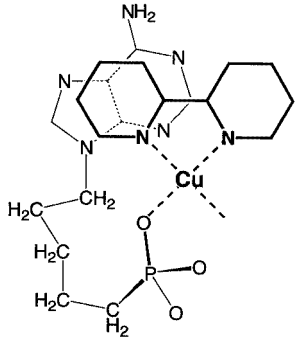


Figure 4. Tentative and simplified structure of a species with an intramolecular stack for Cu(Bpy)(dPEEA) in solution. The orientation of the aromatic rings may vary somewhat from one stacked species to the next; such a stacked complex in solution should not be considered as being rigid.

dPMEA^{2-} or AMP^{2-} (compare entries a with b). This result is understandable, since the overlap of the aromatic ring systems is expected to be more pronounced with the 3-ring system of Phen than with the 2-ring system of Bpy. (ii) The extent of stack formation for a given Arm is within the error limits clearly identical for dPMEA^{2-} and AMP^{2-} (entries 2, 3) and still similar if the Phen/dPEEA^{2-} system (entry 1b) is included in the comparisons (entries 2b, 3b). However, (iii) this is different if the Bpy systems (entries 1a, 2a, 3a) are compared; here the formation degree of Cu(Bpy)(dPEEA)$_{st}$ is clearly lower than the one for Cu(Bpy)(dPMEA) and Cu(Bpy)(AMP). Most likely the pentyl chain is too long to allow an optimal fit with one of the Bpy rings. Indeed, for C_6H_5-$(CH_2)_n$-COO^-/M^{2+}/Phen[63,65] and related systems[66] it has previously been shown that the $(CH_2)_n$ chain may become too long and reach then beyond the rings needed for the intramolecular interaction. In accord with this interpretation are the results for the Cu(Phen)(dPEEA) and Cu(Phen)(dPMEA) systems (entries 1b, 2b); Phen with its 3-ring system is less sensitive than the 2-ring Bpy with regard to the chain length, i.e., to an exchange of the butyl by a pentyl chain. Hence, as far as the stacking interaction in Cu(Arm)(PA) systems is concerned, dPMEA^{2-} mimics AMP^{2-} somewhat better than dPEEA^{2-}.

3. Conclusions

The coordinating properties of dPEEA^{2-} and dPMEA^{2-} do not differ dramatically. However, it is still interesting to note that in binary complexes of divalent 3d metal ions including Zn^{2+} and Cd^{2+}, dPEEA^{2-} resembles the parent nucleotide AMP^{2-} somewhat better than its chain-shortened analogue dPMEA^{2-} (Figure 1): In other words, macrochelate formation between the phosphonate-coordinated metal ion and N7 of the adenine moiety seems to be somewhat more favored in M(dPEEA) than in M(dPMEA) species. In fact, in a first approximation, the extent of macrochelate formation increases for a given M^{2+} within the series M(dPMEA) < M(dPEEA) < M(AMP), though, e.g., for Cu^{2+} and Cd^{2+} the extent is within the error limits identical for the dPEEA^{2-} and AMP^{2-} systems. However, the metal ion-binding properties of all three mentioned ligands differ significantly from those of the antivirally active PMEA^{2-} (see Figure 1); for all its M(PMEA) complexes an ether oxygen-M^{2+} interaction is of relevance which does not occur, of course, with its deoxa analogues or with AMP^{2-}.

In the mixed ligand Cu(Arm)(PA) complexes dPMEA^{2-} resembles its parent AMP^{2-} more closely than dPEEA^{2-}. This means, the extent of intramolecular stack formation is similar in the Cu(Arm)(dPMEA) and Cu(Arm)(AMP) systems and also more pronounced than in Cu(Arm)(dPEEA); this is especially true for Arm = Bpy. It seems that the pentyl chain is somewhat too long and does therefore not favor an ideal fit for the aromatic ring stacks as it appears to be the case with the butyl chain.

It has been suggested[14,15] that diphosphorylated PMEA^{2-}, i.e., PMEApp^{4-}, is initially an excellent substrate for DNA polymerases because of the facilitated formation of the M(P_α)-binding mode which leads then to the also facilitated formation of the reactive M(P_α)-M(P_β,P_γ) mode which is crucial for the transfer of a nucleotidyl unit in the polymerase reaction.[15] This M(P_α) binding is facilitated due to the M^{2+} interaction with the ether oxygen. At present no data are available on the metabolism of dPMEA or dPEEA but if it is assumed that dPMEA and dPEEA are transported to the cell and also diphosphorylated, like PMEA,[12,13] then it would become understandable why these two analogues lack antiviral activity. A facilitated M(P_α) binding (via the ether oxygen) is not possible[14] with dPMEApp^{4-} or dPEEApp^{4-} and thus the transfer of a nucleotidyl unit in the polymerase reaction[15] would also not be facilitated.

4. Experimental Section

4.1 Materials

Twofold protonated 9-(5-phosphonopentyl)adenine, i.e., H_2(dPEEA)$^{\pm}$, was synthesized as described below in Section 4.2 with 1,5-dibromopentane, triethyl phosphite, 1,8-diazabicyclo[1.3.0]undec-1-ene, bromotrimethylsilane and dimethylformamide, which were purchased from Sigma−Aldrich, Prague, Czech Republic.

All aqueous stock solutions for the potentiometric pH titrations were prepared by dissolving the various compounds in deionized, ultrapure (MILLI-Q185 PLUS; from Millipore S.A., 67120 Molsheim, France) CO_2-free water.

2,2′-Bipyridine and 1,10-phenanthroline monohydrate were from Merck AG, Darmstadt, FRG. All the other reagents were the same as used recently.[18]

4.2 Synthesis of 9-(5-Phosphonopentyl)adenine

This compound has been mentioned in the literature[67] in the context of enzyme-catalyzed deamination reactions but its synthesis has not been described and therefore it is given here.

The first step in the synthesis of 9-(5-phosphonopentyl)adenine [= 5-(adenin-9-yl)pentylphosphonic acid] was heating of a mixture of 1,5-dibromopentane (100 g, 0.43 mol) and triethyl phosphite (90 mL, 1.125 equiv.) for 2 days at 110 °C; the resulting ethyl bro-

mide was distilled off. The portion volatile up to a maximum of 100 °C/2 kPa was removed. The residue, i.e. diethyl 5-bromopentylphosphonate, was utilized in the next step without further purification.

The mixture of adenine (20.0 g, 0.148 mol), 1,8-diazabicyclo[1.3.0]undec-1-ene (30 mL) and crude diethyl 5-bromopentylphosphonate (60 mL) in dimethylformamide (200 mL) was heated at 100 °C for 5 h and the solvent evaporated. The residue was extracted with hot chloroform (in total 500 mL) and the solvent evaporated to give by chromatography on a silica gel column (300 mL) in chloroform by extraction with chloroform-methanol mixtures oily diethyl 5-(adenin-9-yl)pentylphosphonate (yield: 11.6 g; 23%). This compound (34 mmol) in acetonitrile (100 mL) was stirred with bromotrimethylsilane (20 mL) till dissolution and left to stand overnight at ambient temperature. The mixture was evaporated in vacuo and the residue codistilled with toluene (2 × 25 mL). Water (150 mL) was added and, after 20 min, the mixture was basified with conc. aqueous ammonia and the solvents evaporated. The residue in minimum water was applied to a column of Dowex 50 × 8 (H^+-form) (250 mL), the column was washed with water to the loss of acidity and UV-absorption of the eluate (monitored at 254 nm). Subsequent elution of the column with diluted (1:10) aqueous ammonia gave a UV-absorbing eluate which was evaporated to dryness in vacuo. This residue in minimum water was basified with ammonia to pH 10 and applied onto a column of Dowex 1 × 2 (acetate form) prewashed with water (200 mL); the column was eluted with water (1 L) and the resin was then stirred batchwise with 1 M formic acid (500 mL), filtered off and washed with boiling water (four 500 mL portions). The combined eluates were evaporated in vacuo, the residue codistilled with water (five 50 mL portions) and crystallized from water. Yield, 5.1 g (52.5%), m.p. 330 °C. $C_{10}H_{16}N_5O_3P$ (285.2): calcd. C 42.11, H 5.65, N 25.55, P 10.86; found C 42.25, H 5.41, N 25.28, P 11.03. ^{1}H NMR (in D_2O, 500 MHz): δ = 8.28 and 8.21 (2 s, 1 H each, H2 and H8 of Ade), 4.27 (t, J = 6.9 Hz, 2 H, H1′), 1.90 (m, 2 H), 1.53 (m, 4 H), 1.36 (m, 2 H, H2′, -3′, -4′, -5′) ppm. ^{13}C NMR (in D_2O, 125.7 MHz): saturated solution − low concentration allowed the detection of proton-bearing carbons only: δ = 154.09 (s, C2), 145.90 (s, C8), 46.92 (s, C1′), 31.63 (s, C2′), 29.98 (d, $J_{C,P}$ = 5.4 Hz, C3′), 35.54 (d, $J_{C,P}$ = 4.4 Hz, C4′), 30.58 (d, $J_{C,P}$ = 121.1 Hz, C5′) ppm.

4.3 Potentiometric pH Titrations. Determination of Equilibrium Constants

The equipment for the titrations,[18] the buffers used for calibration[18] and the evaluation procedures including the computers were the same as before.[43]

The acidity constants $K^H_{H_2(dPEEA)}$ and $K^H_{H(dPEEA)}$ of $H_2(dPEEA)^{\pm}$, where one proton is at the base moiety and the other at the phosphonate group, were determined by titrating 50 mL of aqueous 0.9 m M HNO_3 (25 °C; I = 0.1 M, $NaNO_3$) in the presence and absence of 0.3 mM $dPEEA^{2-}$ under N_2 with 1.6 mL of 0.03 M NaOH. The differences in NaOH consumption between such a pair of titrations were used for the calculations. These conditions correspond to those used recently for the dPMEA system;[18] it needs to be emphasized that at a ligand concentration[22] of 3×10^{-4} M the well-known[32] self-association (via π-stacking) of purines is of no significance. The results for the acidity constants of $H_2(dPEEA)^{\pm}$ are the averages of 19 pairs of independent titrations.

The stability constants $K^M_{M(H;dPEEA)}$ and $K^M_{M(dPEEA)}$ of $M(H;dPEEA)^+$ and M(dPEEA) were determined under the same conditions as the acidity constants, but $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ (25 °C; I = 0.1 M). The conditions correspond to those given recently for the M^{2+}/dPMEA systems,[18] but the M^{2+}/dPEEA ratios were 111:1 (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}), 55.6:1 (Co^{2+}, Ni^{2+}), 53.3:1 (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}), 50:1 (Mn^{2+}), 26.9:1 (Cd^{2+}), 25:1 (Co^{2+}, Ni^{2+}), 13.4:1 (Cd^{2+}), 12.3:1 (Zn^{2+}), 10.6:1 (Cu^{2+}, $Cu(Bpy)^{2+}$, $Cu(Phen)^{2+}$), 5.3:1 (Cu^{2+}, $Cu(Bpy)^{2+}$, $Cu(Phen)^{2+}$) and 5:1 (Zn^{2+}). Since the stability of the $Cu(Arm)^{2+}$ complexes is high,[60] the formation of these species is practically complete under the experimental conditions (titrations of solutions with HNO_3 and HNO_3 + Cu^{2+}/Arm were identical in the lower pH range) and therefore, the evaluation of the titration data of the ternary systems could be done in the way described for the binary ones.[28]

The evaluation of the Zn^{2+}/dPEEA systems was significantly hampered by precipitation, and therefore the pH range accessible for the calculations of the stability constants was severely restricted. In fact, for Zn(dPEEA) only a formation degree of about 2% was reached; hence, the value for Zn(dPEEA) must be considered as an estimate.

However, several of the constants given for the $M(H;dPEEA)^+$ complexes must also be considered as estimates (see Table in Section 2.2), since the formation degree of these species reached only about 3%, in the maximum 18% was reached; for the ternary $Cu(Arm)(H;dPEEA)^+$ complexes a formation degree of nearly 25% was achieved.

The individual results for the stability constants showed no dependence on pH or on the excess of metal ion concentration used. The results are in each case the average of at least five independent pairs of titration curves.

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[7] Abbreviations and definitions: For AMP^{2-}, $dPEEA^{2-}$, $dPMEA^{2-}$ and $PMEA^{2-}$ see Figure 1. Arm, heteroaromatic nitrogen base like Bpy or Phen; Bpy, 2,2′-bipyridine; *I*, ionic

strength of a solution; M^{2+}, general divalent metal ion also including in part $Cu(Arm)^{2+}$; 9MeAde, 9-methyladenine; PA^{2-} = $dPEEA^{2-}$ or any other twofold negatively charged nucleotide or nucleotide analogue; Phen, 1,10-phenanthroline; R-PO_3^{2-}, simple phosphate monoester or phosphonate ligand with R representing a non-interacting residue. Species written without a charge either do not carry one or represent the species in general (i.e. independent of their protonation degree); which of the two possibilities applies is always clear from the context. In formulas like $M(H;dPEEA)^+$, H^+ and $dPEEA^{2-}$ are separated by a semicolon to facilitate reading, yet they appear within the same parenthesis to indicate that the proton is at the ligand without defining its location (see Sections 2.2 and 2.5). The notation $(H{\cdot}dPEEA)^-$ indicates that H^+ is at the phosphonate group (see Section 2.5).

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