Solvent-Dependent Metal Ion–Adenine Recognition. Quantification of the Intramolecular Equilibria between Various Isomers of the Cu^{2+} Complexes Formed in Water–Dioxane Mixtures with the Anions of the Antiviral 9-(2-(Phosphonomethoxy)ethyl)adenine (PMEA), an Adenosine Monophosphate (AMP) Analogue[1]

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The stability constants of the 1:1 complexes formed between Cu^{2+} and 9-(2-(phosphonomethoxy)ethyl)adenine ($PMEA^{2-}$), (phosphonomethoxy)ethane (PME^{2-}), ethylphosphonate (EtP^{2-}), or methylphosphonate (MeP^{2-}) were determined by potentiometric pH titration in water and in water containing 30 or 50% (v/v) 1,4-dioxane (I = 0.1 M, $NaNO_3$; 25 °C). It is shown that the data for MeP^{2-} and EtP^{2-} fit on the same log $K^{Cu}_{Cu(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ straight-line plots previously obtained for simple phosphate monoesters. With these reference lines it could be demonstrated in all solvents that the Cu(PME) complex has a higher stability than expected for a sole phosphonate–Cu^{2+} coordination, and this is attributed to the formation of five-membered chelates involving the ether oxygen present in the $-OCH_2PO_3^{2-}$ residue. For Cu(PMEA) an *additional* stability increase is observed which has to be attributed to a metal ion adenine interaction, giving thus rise to equilibria between *three* different isomers. These equilibria were analyzed for the aqueous solution and the mixtures of water containing 30 or 50% 1,4-dioxane, and it is calculated that 17 (±3), 30 (±4), and 18 (±3)% of Cu(PMEA) exist as an isomer with a sole Cu^{2+}–phosphonate coordination, 34 (±10), 44 (±9), and 28 (±9)% form the mentioned five-membered chelate involving the ether oxygen and the remaining 49 (±10), 26 (±10), and 54 (±9)% are due to an isomer containing also a Cu^{2+}–adenine interaction, $Cu(PMEA)_{cl/Ad}$, respectively. Interestingly the formation degree of $Cu(PMEA)_{cl/Ad}$ passes through a minimum; i.e., small amounts of dioxane inhibit the Cu–adenine interaction—probably with N-3—while larger amounts favor it again. This result parallels a previous observation made with the macrochelate of Cu(5′-AMP) in which the metal ion is bound to the phosphate group and N-7 of the adenine residue. Similar structural alterations have to be expected for other metal ions, e.g., Zn^{2+}. These observations indicate that small alterations of the polarity and water activity in an active-site cavity of an enzyme can considerably alter the structures of substrate complexes.

The dianion of 9-(2-(phosphonomethoxy)ethyl)adenine ($PMEA^{2-}$)[1] may be regarded as an analogue of adenosine 5′-monophosphate (5′-AMP^{2-}) (Figure 1).[3,4] Considering that nucleotides play a key role in many metabolic processes, it is not surprising that artificial nucleotide analogues often display biological activity.[5] Indeed, PMEA and related compounds show antiviral properties against DNA viruses, like herpes viruses, adenoviruses, or poxviruses,[6–8] and also against retroviruses, i.e. human immuno deficiency (HIV) and Moloney murine saroma viruses (MSV);[6,8,9] they also exhibit a cytostatic effect on L-1210 mouse leukemia cells.[6]

As the biological activity of nucleotides generally depends on the presence of metal ions,[10] we recently studied[11] the coordinating properties of $PMEA^{2-}$: it turned out that all the 1:1 complexes formed with Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} (=M^{2+}) are more stable than expected for a sole phosphonate coordination of the metal ion. This increased stability is due to the formation of five-membered chelates which involve the ether oxygen present in the $-OCH_2PO_3^{2-}$ residue of $PMEA^{2-}$ (cf. Figure 1) and which also give rise to equilibrium 1.

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This observation is remarkable because deletion of the ether oxygen or substitution by other groups leads to a loss or at least a considerable reduction of the biological activity.[6,8,12] The

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(1) Abbreviations: Ad, adenine residue; Ado, adenosine; 5′-AMP^{2-}, adenosine 5′-monophosphate; EtP^{2-}, ethylphosphonate; M^{2+}, general divalent metal ion; MeP^{2-}, methylphosphonate; PME^{2-}, dianion of (phosphonomethoxy)-ethane; $PMEA^{2-}$, dianion of 9-(2-(phosphonomethoxy)ethyl)adenine; $R-PO_3^{2-}$, general phosphonate and (in part also) general phosphate monoester ligand; v, volume.

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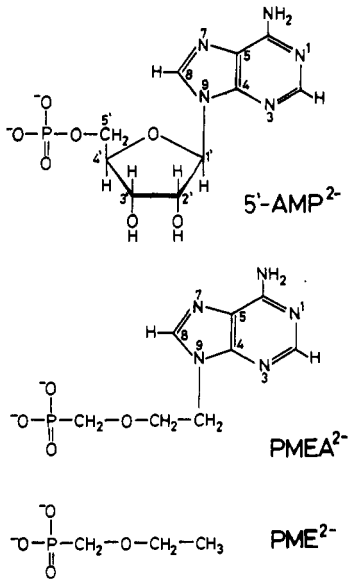

Figure 1. Chemical structure of the dianion of 9-(2-(phosphonomethoxy)-ethyl)adenine ($PMEA^{2-}$) in comparison with the structures of adenosine 5'-monophosphate (5'-AMP^{2-}), which is shown in its dominating anti conformation,[3,4] and the dianion of (phosphonomethoxy)ethane (PME^{2-}).

formation degree of the five-membered chelates varies between about 15 and 30% for the alkaline earth ions and 30 and 50% for the divalent 3d ions and Zn^{2+} or Cd^{2+}. Clearly, the structures of these M(PMEA) species differ significantly from those of the corresponding M(5'-AMP) complexes.[13,14] Moreover, while the adenine moiety affects the stability of the M(5'-AMP) complexes with Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} by forming macrochelates,[13,14] a M^{2+}–adenine interaction occurs to a significant extent only in Cu(PMEA) and Ni(PMEA), where this *third* isomer, aside from the mentioned simple phosphonate complex and the five-membered chelate, is formed to about 50 and 20%, respectively.[11]

To which extent is the formation degree of the various M(PMEA) isomers affected by the polarity of the solvent? Does a reduced solvent polarity favor or inhibit the chelate formations? Answers to questions of this kind are of general interest because our knowledge in this respect is extremely scarce even though it is now well established that the so-called "effective" or "equivalent solution" dielectric constants in proteins[15] or active-site cavities of enzymes[16] are reduced compared to that in bulk water; i.e., the activity of water is decreased[17] due to the presence of aliphatic and aromatic amino acid side chains at the protein–water interface. For example, for the active-site cavities of bovine carbonic anhydrase and carboxypeptidase A these constants are estimated to be 35 and <70, respectively.[16] By employing aqueous solutions that contain 1,4-dioxane, one may expect to simulate to some degree the situation in such cavities; e.g., water containing 30 or 50% (v/v) 1,4-dioxane exhibits dielectric constants of about 53 and 35, respectively.[18]

In aqueous solution all three Cu(PMEA) isomers occur with a significant formation degree;[11] therefore, we selected this system to address the raised questions, in the hope that by employing 30 and 50% (v/v) dioxane–water mixtures as solvents the isomers are still formed to an extent that allows quantification and, hence, possibly also some generalizations. For a quantitative evaluation of the Cu^{2+}/PMEA system it was necessary to study also the Cu^{2+} complexes of the dianion of the PMEA-related (phosphonomethoxy)ethane (PME^{2-}; see Figure 1), as well as of methylphosphonate (MeP^{2-}) and ethylphosphonate (EtP^{2-}).

Experimental Section

Materials. 9-(2-(Phosphonomethoxy)ethyl)adenine, i.e. $H_2(PMEA)^*$, was prepared in the laboratory of A.H.,[19] and (phosphonomethoxy)-ethane, i.e. $H_2(PME)$, by F.G. and co-workers.[20] Both samples were the same as used before.[11,21]

1,4-Dioxane (extra pure) was from Merck AG, Darmstadt, FRG. All the other reagents were the same as used previously.[11]

Measurements. The potentiometric pH titrations and their evaluations were done as described.[11] The calculated acidity constants[11] are so-called practical, mixed or Brønsted constants; for details see ref 22. No "corrections" were applied for the change in solvent from water to the dioxane–water mixtures, though correction factors have been published for such[23,24] and related mixtures.[25] The stability constants are defined, as always, i.e. as concentration constants.

The results for the constants in the tables (vide infra) are the averages of at least 6, usually 8, independent *pairs* of titrations. The stability constants of the binary 1:1 complexes showed no dependence on the excess of Cu^{2+} used; cf. also ref 11.

Results and Discussion

In dealing with adenine derivatives the concentrations used in experiments must be such that self-association[26-28] is negligibly small to guarantee that the properties of the monomeric species are studied. Regarding PMEA it was recently concluded[11] that solutions ≤ 1 mM in PMEA have to be employed; hence, with [PMEA] = 3×10^{-4} M the results certainly refer to monomeric PMEA species. Of course, the other phosphonate derivatives considered in this study do not undergo self-association due to the lack of an aromatic moiety; however, for the sake of uniformity also in these cases the same low concentrations (0.3 mM) were used.

Any kind of additional interactions as indicated for M(PMEA) complexes in equilibrium 1 and the introduction must be reflected in a larger stability[29] than expected for simple phosphonate–metal ion coordination. For this reason the stability of simple phosphonate–Cu^{2+} complexes was studied first with methylphosphonate ($CH_3PO_3^{2-}$; MeP^{2-}) and ethylphosphonate ($CH_3CH_2PO_3^{2-}$; EtP^{2-}) as ligands.

1. Stabilities of Complexes with a Pure Phosphonate–Cu^{2+} Coordination. Phosphonates ($R-PO_3^{2-}$), like $CH_3PO_3^{2-}$ or $CH_3CH_2PO_3^{2-}$, are dibasic and may therefore accept two protons. The first of these is released at a rather low pH, i.e., at least 5 pK_a units below the pK_a of the second proton (eq 2).[11,28] Therefore there is no overlap with eq 2 or the complex formation. Indeed,

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Table I. Negative Logarithms of the Acidity Constants of Monoprotonated Methylphosphonate and Ethylphosphonate (Eq 2) and Logarithms of the Stability Constants of the $Cu(CH_3PO_3)$ and $Cu(CH_3CH_2PO_3)$ Complexes (Eq 3) As Determined by Potentiometric pH Titrations (Exp)[a] in Water and in Water Containing 30 or 50% (v/v) 1,4-Dioxane at 25 °C and I = 0.1 M ($NaNO_3$)[b]

$R-PO_3^{2-}$	% dioxane (v/v)	$pK^H_{H(R-PO_3)}$[d]	$\log K^{Cu}_{Cu(R-PO_3)}$ exp[a]	$\log K^{Cu}_{Cu(R-PO_3)}$ calc[b,c]	$\log \Delta_{R-PO_3}$[e]
$CH_3PO_3^{2-}$	0	7.53 ● 0.01	3.49 ± 0.05	3.47 ± 0.08	0.02 ● 0.09
	30	8.23 ● 0.01	4.47 ± 0.05	4.51 ± 0.03	−0.04 ± 0.06
	50	8.70 ± 0.01	(5.13)[f]	5.16 ± 0.03	(−0.03)[f]
$CH_3CH_2PO_3^{2-}$	0	7.77 ± 0.01	3.61 ± 0.04	3.57 ± 0.08	0.04 ± 0.09
	30	8.47 ± 0.01	(4.61)[f]	4.65 ± 0.03	(−0.04)[f]
	50	8.93 ± 0.01	(5.29)[f]	5.29 ± 0.03	(0.0)[f]

[a] The errors given are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The error limits of the derived data, in the present case for $\log \Delta_{R-PO_3}$, were calculated according to the error propagation after Gauss. The experimental data for the entries in aqueous solution are taken from ref 11. [b] The calculated stability constants for pure Cu^{2+}-phosphonate coordination (calc) are given for comparison; these values are based on straight-line equations[30-32] quantifying the relationship between complex stability and phosphate group basicity (see also Table II and section 2)[c] and the $pK^H_{H(R-PO_3)}$ values of $H(CH_3PO_3)^-$ and $H(CH_3CH_2PO_3)^-$. [c] The parameters of the straight-line equations used for the above calculations are listed in entries 1a, 2, and 3 of Table II. [d] So-called practical constants are listed; see Experimental Section. [e] $\log \Delta_{R-PO_3} = \log K_{exp} - \log K_{calc}$; error limits, 3σ. [f] The values in parentheses are only estimates because the experimental determination of $K^{Cu}_{Cu(R-PO_3)}$ was hampered by the hydrolysis of Cu_{aq}^{2+} (see also section 1).

Table II. Baseline Correlations for Cu^{2+}–Phosphonate or Cu^{2+}–Phosphate Coordination and Phosphonate or Phosphate Group Basicity, for the Solvents Water and Water Containing 30 or 50% (v/v) 1,4-Dioxane (I = 0.1 M, $NaNO_3$; 25 °C),[a] Together with Properties of the Mentioned Solvents.[b,c]

no.[d]	% dioxane (v/v)	mol fraction	ϵ[b]	m	b	R[c]	SD[c]
1a	0	0	78.5	0.453 ± 0.056	0.055 ± 0.340	0.971	0.026
1b	0	0	78.5	0.465 ± 0.025	−0.015 ± 0.164	0.991	0.019
2	30	0.083	52.7	0.559 ± 0.015	−0.089 ± 0.106	0.999	0.01
3	50	0.175	35.2	0.571 ± 0.022	0.190 ± 0.160	0.999	0.01

[a] Straight-line equation: $y = mx + b$, where x represents the pK_a value of any phosphonate or phosphate monoester, $H(R-PO_3)^-$, and y the calculated $\log K^{Cu}_{Cu(R-PO_3)}$ value of the corresponding $Cu(R-PO_3)$ complex; the errors given with the slopes (m) and the y-axis intercepts (b) correspond to one standard deviation (1σ). The listed SD values[c] times 2 or 3 are considered as reasonable error limits for any stability constant calculation in the pK_a range 5–8 for aqueous solution and 6–8.5 for 30% and 6.5–9 for 50% (v/v) 1,4-dioxane–water mixtures.[d] The slopes (m) and intercepts (b) for the straight baseline plots of $\log K^{Cu}_{Cu(R-PO_3)}$ versus $pK^H_{H(R-PO_3)}$ are calculated from equilibrium constants determined earlier[11,30,31] for the following simple phosphonates or phosphate monoesters: (i) aqueous solution (entry 1a) with 4-nitrophenyl phosphate, phenyl phosphate, *n*-butyl phosphate, D-ribose 5′-monophosphate, uridine 5′-monophosphate, and thymidine 5′-monophosphate;[30] (ii) aqueous solution (entry 1b) with the mentioned phosphate monoesters plus methylphosphonate and ethylphosphonate;[11] (iii) for the dioxane–water mixtures (entries 2 and 3) with 4-nitrophenyl phosphate, phenyl phosphate, *n*-butyl phosphate, and D-ribose 5′-monophosphate.[31] [b] The dielectric constants for the dioxane–water mixtures are interpolated from the data given in ref 18. [c] The standard deviations (SD) result from the differences between the experimental and calculated values for the mentioned six (i/entry 1a),[a] eight (ii/entry 1b),[a] and four (iii/entries 2 and 3)[a] ligand systems.[11,30,31] [d] The data for m, b, R, and SD for entry 1a are from Tables V and VI of ref 30, those for entry 1b from Tables V and VI of ref 11, and those for entries 2 and 3 from Tables 2 and 3 of ref 31. [e] Correlation coefficient.

the experimental data of the potentiometric pH titrations of the considered $Cu^{2+}/R-PO_3$ systems are completely described by equilibria 2a and 3a, as long as the evaluation is not carried into the pH range where formation of hydroxo complexes occurs.

$$H(R-PO_3)^- \rightleftharpoons R-PO_3^{2-} + H^+ \qquad (2a)$$

$$K^H_{H(R-PO_3)} = [H^+][R-PO_3^{2-}]/[H(R-PO_3)^-] \qquad (2b)$$

$$Cu^{2+} + R-PO_3^{2-} \rightleftharpoons Cu(R-PO_3) \qquad (3a)$$

$$K^{Cu}_{Cu(R-PO_3)} = [Cu(R-PO_3)]/([Cu^{2+}][R-PO_3^{2-}]) \qquad (3b)$$

The results regarding eqs 2 and 3 for the methylphosphonate and ethylphosphonate systems in water and in water containing 30 or 50% (v/v) 1,4-dioxane are listed in columns 3 and 4 of Table I, respectively.[11,30-32] Some stability constants could only be estimated due to hydrolysis of Cu_{aq}^{2+}; however, despite this handicap, due to the pairwise titration method,[11] the given estimates could still be achieved. Furthermore, the effect of 1,4-dioxane on the acidity of the $H(R-PO_3)^-$ species and on the stability of the $Cu(R-PO_3)$ complexes is in line with previous observations made on analogous phosphate species.[31,33]

2. Correlations between Complex Stability and Ligand Basicity: Construction of Base Lines for $\log K^{Cu}_{Cu(R-PO_3)}$ versus $pK^H_{H(R-PO_3)}$ Plots. Such plots for families of related ligands generally lead to straight lines,[29] and those for phosphate monoester ligands and various metal ions were established several years ago.[30] Recently it was shown for aqueous solutions that on these same straight reference lines also the $\log K^M_{M(R-PO_3)}/pK^H_{H(R-PO_3)}$ data pairs for phosphonates, $R-PO_3^{2-}$, where R represents a noncoordinating residue, are fitting.[11]

The results of Table I allow one to check if this is also true for the 1,4-dioxane–water solvent mixtures. The straight-line equations established previously for the Cu^{2+} complexes with phosphate monoester ligands for water containing 30 or 50% 1,4-dioxane are listed in Table II. With these equations and the acidity constants, $pK^H_{H(R-PO_3)}$, of Table I, the stability constants of the $Cu(CH_3PO_3)$ and $Cu(CH_3CH_2PO_3)$ complexes could be calculated (column 5 of Table I).

The differences between the experimentally determined and the calculated log stability constants for the $Cu(R-PO_3)$ complexes of methylphosphonate and ethylphosphonate are listed in Table I ($\log \Delta_{R-PO_3}$). They are zero within the error limits, thus confirming that the data pairs for these phosphonate complexes fit on the reference lines established previously for the Cu^{2+} complexes of phosphate monoesters. Moreover, these phosphonate results allow now also an extension of the usable pK_a range up to values of 8, 8.5, and 9 for aqueous solution and 30 and 50% dioxane–water mixtures, respectively (see Table II and also Figure 2 in section 4).

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Table III. Negative Logarithms of the Acidity Constants of $H(PME)^-$ (Eq 2) and $H_2(PMEA)^{\pm}$ (Eqs 4 and 5) and Logarithms of the Stability Constants of the Cu(PME) (Eq 3) and Cu(PMEA) Complexes (Eq 7) As Determined by Potentiometric pH Titrations (Exp)[a] in Water and in Water Containing 30 or 50% (v/v) 1,4-Dioxane at 25 °C and I = 0.1 M ($NaNO_3$)[b,c]

$R-PO_3^{2-}$	% dioxane (v/v)	$pK^H_{H_2(R-PO_3)}$[d]	$pK^H_{H(R-PO_3)}$[d]	$\log K^{Cu}_{Cu(R-PO_3)}$ exp[a]	$\log K^{Cu}_{Cu(R-PO_3)}$ calc[b,c]	$\log \Delta_{R-PO_3}$[e]
PME^{2-}	0		7.02 ± 0.01	3.73 ± 0.03	3.25 ± 0.06	0.48 ± 0.07
	30		7.73 ± 0.02	4.62 ± 0.03	4.23 ± 0.03	0.39 ± 0.04
	50		8.17 ± 0.03	5.27 ± 0.06	4.86 ± 0.03	0.41 ± 0.07
$PMEA^{2-}$	0	4.16 ≙ 0.02	6.90 ± 0.01	3.96 ± 0.04[f]	3.19 ± 0.06	0.77 ± 0.07
	30	3.79 ≙ 0.01	7.64 ± 0.01	4.70 ± 0.05[f]	4.18 ± 0.03	0.52 ± 0.06
	50	3.73 ± 0.01	8.05 ± 0.01	5.54 ± 0.07[f]	4.79 ± 0.03	0.75 ± 0.08
5′-AMP^{2-}	0	3.84 ≙ 0.02	6.21 ± 0.01	3.14 ± 0.01	2.87 ± 0.08	0.27 ± 0.08
	30	3.47 ± 0.02	7.00 ± 0.01	3.86 ± 0.02	3.82 ± 0.03	0.04 ± 0.04
	50	3.42 ± 0.02	7.48 ± 0.01	4.73 ± 0.04	4.45 ± 0.02	0.28 ± 0.04

[a] See footnote *a* of Table I. [b] The calculated stability constants for pure Cu^{2+}–phosphonate coordination (calc) are based on straight-line equations quantifying the relationship between complex stability and phosphonate group basicity (Table II)[c] and the $pK^H_{H(R-PO_3)}$ values of $H(PME)^-$ and $H(PMEA)^-$. The corresponding results for the 5′-AMP^{2-} systems are given for comparison; they are taken from ref 34. [c] The parameters of the straight-line equations used for the above calculations are given in entries 1b, 2, and 3 of Table II. [d,e] See footnotes *d* and *e* in Table I. [f] For the additional experimental data regarding Cu^{2+}/PMEA, see Table IV.

3. Stabilities of the Copper Complexes Formed with PME^{2-}, $H(PMEA)^-$, and $PMEA^{2-}$. As PME^{2-} has no more proton binding sites than the simple phosphonates discussed in section 1, the experimental data can again solely be explained by taking into account eqs 2 and 3. The corresponding results are listed in Table III.[34]

For $PMEA^{2-}$ the situation is somewhat more complicated due to the adenine residue which accepts at N-1 a further proton.[4,11,35] The first proton in $H_3(PMEA)^+$ is released from the 2-fold protonated phosphonate group at a low pH as discussed already in section 1 for $H_2(R-PO_3)$ species. The now formed zwitterionic species $H_2(PMEA)^{\pm}$ first releases a proton from the H^+(N-1) site (eq 4) and only then from the still monoprotonated phosphonate residue (eq 5).

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

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

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

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

For an evaluation of the experimental data of the Cu^{2+}/PMEA system aside from eqs 4 and 5 also eqs 6 and 7 must be taken into

$$Cu^{2+} + H(PMEA)^- \rightleftharpoons Cu(H\cdot PMEA)^+ \quad (6a)$$

$$K^{Cu}_{Cu(H\cdot PMEA)} = [Cu(H\cdot PMEA)^+]/([Cu^{2+}][H(PMEA)^-]) \quad (6b)$$

$$Cu^{2+} + PMEA^{2-} \rightleftharpoons Cu(PMEA) \quad (7a)$$

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

account. The acidity constant $K^H_{Cu(H\cdot PMEA)}$ of $Cu(H\cdot PMEA)^+$ (eq 8) is calculated with eq 9. The results regarding eqs 4 and 5 are listed in Table III, and those for eqs 6–9, in Table IV.

$$Cu(H\cdot PMEA)^+ \rightleftharpoons Cu(PMEA) + H^+ \quad (8a)$$

$$K^H_{Cu(H\cdot PMEA)} = [H^+][Cu(PMEA)]/[Cu(H\cdot PMEA)^+] \quad (8b)$$

$$pK^H_{Cu(H\cdot PMEA)} = pK^H_{H(PMEA)} + \log K^{Cu}_{Cu(H\cdot PMEA)} - \log K^{Cu}_{Cu(PMEA)} \quad (9)$$

The solvent influence on the deprotonation of the H^+(N-1) site in $H_2(PMEA)^{\pm}$ and on the $-P(O)_2(OH)^-$ residue in $H(PMEA)^-$ observed now is in accordance with previous observations made at related sites[4,28,36] including the corresponding 5′-AMP species (see also the bottom part of Table III).[34] Comparing the acidity constants of $H_2(PMEA)^{\pm}$, $pK^H_{H_2(PMEA)} \leq 4.16$ (Table III), with those of the $Cu(H\cdot PMEA)^+$ complexes according to eq 8, $pK^H_{Cu(H\cdot PMEA)} \geq 4.42$ (Table IV), demonstrates that Cu^{2+} must be mainly located at the adenine residue and H^+ at the phosphonate group in Cu(H·PMEA) because upon metal ion binding a proton located at a certain site in a ligand can only be acidified but not more strongly bound; this interpretation is also in accordance with the solvent effect[4,28,36] on the values of $pK^H_{Cu(H\cdot PMEA)}$.

The arguments that the metal ions in $M(H\cdot PMEA)^+$ species are bound in an adenosine-type fashion to the nucleic base residue were advanced in detail recently[11] and will not be discussed here. Of course, this kind of evaluation proves only that in $M(H\cdot PMEA)^+$ M^{2+} is predominantly adenine-bound, but it does not allow a conclusion about its distribution between N-1 and N-7 (Figure 1). However, in a recent study about the dichotomy of metal binding in $M(Ado)^{2+}$ complexes it was concluded[35] that Cu^{2+} prefers N-7 in contrast to H^+ which strongly favors N-1.

4. Proof for an Increased Stability of the Cu(PME) and Cu(PMEA) Complexes. The least-squares base lines given in Table II and discussed in section 2 define the relation between phosphonate-complex stability and phosphonate-group basicity. These data are used to construct the plots of $\log K^{Cu}_{Cu(R-PO_3)}$ versus $pK^H_{H(R-PO_3)}$ shown in Figure 2 where also the corresponding data pairs for the Cu^{2+}/PME^{2-} and $Cu^{2+}/PMEA^{2-}$ systems are inserted. The resulting six solid points are considerably above the reference lines in all three solvents, thus proving an increased stability.

A quantitative evaluation of the situation reflected in Figure 2 is possible by calculating with the $pK^H_{H(R-PO_3)}$ values for $H(PME)^-$ and $H(PMEA)^-$ (Table III) and the straight-line equations (Table II) the expected stabilities for the Cu(PME) and Cu(PMEA) complexes having solely a phosphonate–Cu^{2+} coordination. These calculated (calc) stability constants are listed

(34) Liang, G.; Sigel, H. *Inorg. Chem.* **1990**, *29*, 3631–3632.
(35) Sigel, H.; Corfù, N. A.; Ji, L.-n.; Martin, R. B. *Comments Inorg. Chem.* **1992**, *13*, 35–59.
(36) Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1988**, *170*, 617–626.

Table IV. Logarithms of the Stability Constants of the $Cu(H\cdot PMEA)^+$ (Eq 6) and Cu(PMEA) Complexes (Eq 7) As Determined by Potentiometric pH Titrations, Together with the Negative Logarithms of the Acidity Constants (Eqs 8 and 9) of the Corresponding $Cu(H\cdot PMEA)^+$ Complexes for Aqueous Solution and for Water Containing 30 or 50% (v/v) 1,4-Dioxane at 25 °C and I = 0.1 M ($NaNO_3$)[a,b]

% dioxane (v/v)	$\log K^{Cu}_{Cu(H\cdot PMEA)}$	$\log K^{Cu}_{Cu(PMEA)}$	$pK^{H}_{Cu(H\cdot PMEA)}$
0	1.48 ± 0.16	3.96 ± 0.04	4.42 ± 0.17
30	1.78 ± 0.04	4.70 ± 0.05	4.72 ± 0.07
50	2.19 ± 0.05	5.54 ± 0.07	4.70 ± 0.09

[a] See footnote *a* of Table I. [b] The corresponding acidity constants of $H_2(PMEA)^{\pm}$ are listed in Table III.

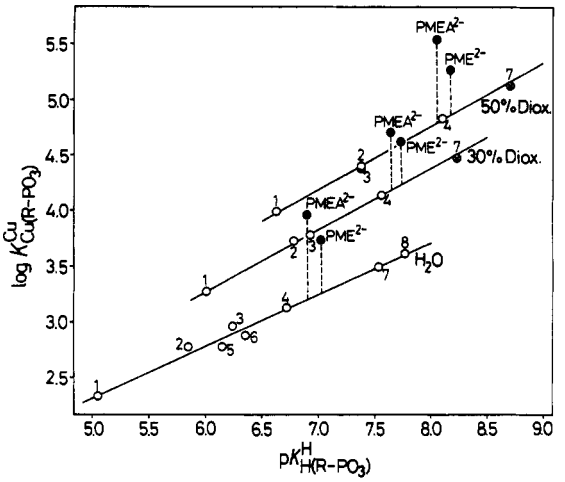

Figure 2. Evidence for an enhanced stability of the Cu(PME) and Cu(PMEA) (●) complexes in mixed dioxane–water solvents based on the relationship between $\log K^{Cu}_{Cu(R-PO_3)}$ and $pK^{H}_{H(R-PO_3)}$ for the Cu^{2+} 1:1 complexes of 4-nitrophenyl phosphate (1), phenyl phosphate (2), D-ribose 5′-monophosphate (3), *n*-butyl phosphate (4), uridine 5′-monophosphate (5), thymidine 5′-monophosphate (6), methylphosphonate (7), and ethylphosphonate (8) in water and in water containing 30 or 50% (v/v) 1,4-dioxane. The least-squares lines are drawn in each case through the data sets shown (○);[11,31] the equations for these reference lines are given in Table II. The data points due to the methylphosphonate system in the mixed solvents (⊛) (see Table I) are shown to prove that simple phosphonates fit within the experimental error limits on the reference lines established with phosphate monoester systems (see also text in section 2). The points due to the Cu^{2+} 1:1 complexes formed with PME^{2-} and $PMEA^{2-}$ (●) in the three mentioned solvents are inserted for comparison (see Table III and section 4). The vertical broken lines emphasize the stability differences to the corresponding reference lines; these differences equal $\log \Delta_{R-PO_3}$ (see eq 10). All the plotted equilibrium constants refer to 25 °C and I = 0.1 M ($NaNO_3$).

in the sixth column of Table III. Their comparison with the measured (exp) stability constants according to eq 10 (regarding

$$\log \Delta_{R-PO_3} = \log K^{Cu}_{Cu(R-PO_3)_{exp}} - \log K^{Cu}_{Cu(R-PO_3)_{calc}} \quad (10a)$$

$$= \log K^{Cu}_{Cu(R-PO_3)} - \log K^{Cu}_{Cu(R-PO_3)_{op}} \quad (10b)$$

eq 10b, see section 5) leads to the stability differences $\log \Delta_{R-PO_3}$ (Table III), which correspond to the vertical broken-line distances seen in Figure 2. Evidently the Cu(PME) and Cu(PMEA) complexes are more stable than expected on the basis of the basicities of their phosphonate groups.

Any kind of chelate formation must be reflected in an increased complex stability;[13,29,37] therefore the positive stability differences $\log \Delta_{R-PO_3}$ prove that to some extent chelates must be formed in all these systems. However, the values due to Cu(PME) are somewhat smaller and evidently rather independent of the solvent

(37) Sigel, H. *Eur. J. Biochem.* **1987**, *165*, 65–72.

Table V. Extent of Chelate Formation According to Equilibrium 1 in the Cu(PME) Species As Quantified by the Dimensionless Equilibrium Constant K_I (Eqs 11 and 14) and the Percentage of $Cu(PME)_{cl}$ (Eq 15) in Aqueous Solution and in Water Containing 30 or 50% (v/v) 1,4-Dioxane at 25 °C and I = 0.1 M ($NaNO_3$)[a]

% dioxane (v/v)	$\log \Delta_{PME}$[a]	K_I	% $Cu(PME)_{cl}$
0	0.48 ± 0.07	2.02 ± 0.47	67 ± 5
30	0.39 ± 0.04	1.45 ● 0.24	59 ± 4
50	0.41 ± 0.07	1.57 ± 0.40	61 ± 6

[a] The values for $\log \Delta_{PME}$ (see eq 10) and their error limits (3σ) are from Table III; see also footnote *a* of Table I. The error limits in all the other columns to the right were calculated according to the error propagation after Gauss by using the errors (with two more digits) listed for $\log \Delta_{PME}$.

while those due to Cu(PMEA) pass through a minimum in 30% dioxane–water.

A corresponding observation has previously been made for the $Cu^{2+}/5'\text{-}AMP^{2-}$ system;[34] these results are also listed in Table III: again a minimum is observed for $\log \Delta_{AMP}$ in 30% dioxane–water mixtures. These different properties exhibited by the Cu(PME) complex on the one hand and the Cu(PMEA) and Cu(5′-AMP) complexes on the other prove that at least partially different binding sites must be involved in the complex-forming processes. These aspects and their quantitative evaluation will be addressed in the following three sections.

5. Structure of the Cu(PME) Species in the Various Solvents. The increased stability of Cu(PME) can only be attributed to the ether oxygen because aside from the phosphonate group PME^{2-} (Figure 1) does not contain another binding site; hence, equilibrium 1 must be operating. The position of this concentration-independent equilibrium between a simple phosphonate-bound species which we designate as the "open" isomer, $Cu(PME)_{op}$, and the five-membered chelate involving the ether oxygen, designated as the "closed" species, $Cu(PME)_{cl}$, is defined by the intramolecular and hence dimensionless equilibrium constant K_I:

$$K_I = [Cu(PME)_{cl}]/[Cu(PME)_{op}] \quad (11)$$

The measured stability for Cu(PME) is defined by eq 3b, yet due to eq 1 it may be rewritten as given in eq 12 and further

$$K^{Cu}_{Cu(PME)} = \frac{[Cu(PME)]}{[Cu^{2+}][PME^{2-}]} = \frac{([Cu(PME)_{op}] + [Cu(PME)_{cl}])}{[Cu^{2+}][PME^{2-}]} \quad (12)$$

$$K^{Cu}_{Cu(PME)} = K^{Cu}_{Cu(PME)_{op}} + K_I K^{Cu}_{Cu(PME)_{op}} = K^{Cu}_{Cu(PME)_{op}}(1 + K_I) \quad (13)$$

$$K_I = \frac{K^{Cu}_{Cu(PME)}}{K^{Cu}_{Cu(PME)_{op}}} - 1 = 10^{\log \Delta} - 1 \quad (14)$$

developed[13,29] to eqs 13 and 14. The stability constant of the open isomer, $K^{Cu}_{Cu(PME)_{op}}$, is not directly accessible by experiments, yet it may be calculated for each solvent with the measured $pK^{H}_{H(PME)}$ value and the equations of the correlation lines given in Table II. In other words, the values for $\log K^{Cu}_{Cu(PME)_{calc}}$ listed in the sixth column of Table III correspond to $\log K^{Cu}_{Cu(PME)_{op}}$ and consequently the stability difference as defined in eq 10b corresponds to the one needed for the calculation of K_I in eq 14; this allows then to calculate (eq 15) the percentage of the closed form, $Cu(PME)_{cl}$, occurring in equilibrium 1.

$$\% Cu(PME)_{cl} = 100K_I/(1 + K_I) \quad (15)$$

The use of eqs 10, 14, and 15 leads to the results summarized in Table V, which prove (i) that the intramolecular equilibrium 1 is operating for Cu(PME) and that both isomers are formed in appreciable amounts and (ii) that the formation degree of $Cu(PME)_{cl}$ is rather independent of the solvent (column 4) despite the fact that the overall stability constant log $K^{Cu}_{Cu(PME)}$ increases by more than 1.5 log units in changing the solvent from water to a 50% dioxane–water mixture (see Table III, column 5).

6. Evaluation of the Increased Stability of the Cu(PMEA) Complex and Conclusions Regarding the Structures of Its Various Isomers in Solution. $PMEA^{2-}$ contains the same $-OCH_2PO_3^{2-}$ residue as PME^{2-}; therefore, equilibrium 1 must also play a role for Cu(PMEA). However, the stability differences, log Δ_{PMEA}, listed in Table III in column 7 are even larger than those of log Δ_{PME}; hence, a further interaction must occur, i.e with a site of the adenine residue as indicated already in section 4.

The only binding sites that an adenine moiety can offer are N-1, N-3, and N-7 (see Figure 1). From space-filling molecular models it is immediately evident that a metal ion bound to the phosphonate group cannot reach N-1; this leaves N-3 and N-7 for a further coordination, and these two cases are considered below.

6.1. Case I Involving N-3. Molecular models reveal that a metal ion chelated to the phosphonate and the ether oxygen (see equilibrium 1) may also interact with N-3 by forming a seven-membered chelate; i.e., no disruption of the ether M^{2+} bond is necessary. Hence, the formation of a macrochelate involving only the phosphonate group and N-3 is highly unlikely and therefore equilibrium scheme 16 results. The purely phosphonate-

$$\begin{array}{l} Cu^{2+} + PMEA^{2-} \overset{K^{Cu}_{Cu(PMEA)op}}{\rightleftharpoons} Cu(PMEA)_{op} \\ \qquad \overset{K_{I/O}}{\rightleftharpoons} Cu(PMEA)_{cl/O} \overset{K_{I/O/N3}}{\rightleftharpoons} Cu(PMEA)_{cl/O/N3} \end{array} \quad (16)$$

coordinated isomer is designated as $Cu(PMEA)_{op}$, the five-membered chelate involving the ether oxygen (eq 1) as $Cu(PMEA)_{cl/O}$, and the 2-fold chelated species involving also N-3 as $Cu(PMEA)_{cl/O/N3}$.

6.2. Case II Involving N-7. The distance to N-7 of a phosphonate-bound M^{2+} in M(PMEA) is rather similar to that, also to N-7, of a phosphate-bound metal ion in a complex with 5′-AMP^{2-} (in the *anti*-conformation; Figure 1). However, N-7 can only be reached from a phosphonate coordinated metal ion in M(PMEA) species if no five-membered chelate with the ether oxygen is formed. Consequently, the open isomer, $Cu(PMEA)_{op}$, may either transform into a macrochelate with N-7 or a five-membered chelate with the ether oxygen, yet both interactions cannot occur at the same time in the same species, giving thus rise to the equilibrium scheme 17. The macrochelated species involving N-7 is designated as $Cu(PMEA)_{cl/N7}$; the other isomers are defined as above in the equilibrium scheme 16.

$$\begin{array}{l} Cu^{2+} + PMEA^{2-} \overset{K^{Cu}_{Cu(PMEA)op}}{\rightleftharpoons} Cu(PMEA)_{op} \overset{K_{I/O}}{\rightleftharpoons} Cu(PMEA)_{cl/O} \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad \underset{K_{I/N7}}{\rightleftharpoons} Cu(PMEA)_{cl/N7} \end{array} \quad (17)$$

6.3. Definition of the Equilibrium Constants. The definition of $K^{Cu}_{Cu(PMEA)_{op}}$ is identical in both schemes 16 and 17.[11] Regarding case I (eq 16) it follows:

$$K_{I/O} = [Cu(PMEA)_{cl/O}]/[Cu(PMEA)_{op}] \quad (18)$$

$$K_{I/O/N3} = [Cu(PMEA)_{cl/O/N3}]/[Cu(PMEA)_{cl/O}] \quad (19)$$

If use is made of the recent derivations,[11] one obtains:

$$K^{Cu}_{Cu(PMEA)} = \frac{[Cu(PMEA)]}{[Cu^{2+}][PMEA^{2-}]}$$

$$= \frac{([Cu(PMEA)_{op}] + [Cu(PMEA)_{cl/O}] + [Cu(PMEA)_{cl/O/N3}])}{[Cu^{2+}][PMEA^{2-}]} \quad (20a)$$

$$= K^{Cu}_{Cu(PMEA)_{op}}(1 + K_{I/O} + K_{I/O}K_{I/O/N3}) \quad (20b)$$

$$K_I = K_{I/tot} = \frac{K^{Cu}_{Cu(PMEA)}}{K^{Cu}_{Cu(PMEA)_{op}}} - 1 = 10^{\log \Delta} - 1 \quad (21a)$$

$$= \frac{[Cu(PMEA)_{cl/tot}]}{[Cu(PMEA)_{op}]} = \frac{([Cu(PMEA)_{cl/O}] + [Cu(PMEA)_{cl/O/N3}])}{[Cu(PMEA)_{op}]} \quad (21b)$$

$$= K_{I/O} + K_{I/O}K_{I/O/N3} = K_{I/O}(1 + K_{I/O/N3}) \quad (21c)$$

Quite analogously[11] for case II (eq 17) it follows, if eq 22 is taken into account:

$$K_{I/N7} = [Cu(PMEA)_{cl/N7}]/[Cu(PMEA)_{op}] \quad (22)$$

$$K^{Cu}_{Cu(PMEA)} = \frac{[Cu(PMEA)]}{[Cu^{2+}][PMEA^{2-}]}$$

$$= \frac{([Cu(PMEA)_{op}] + [Cu(PMEA)_{cl/O}] + [Cu(PMEA)_{cl/N7}])}{[Cu^{2+}][PMEA^{2-}]} \quad (23a)$$

$$= K^{Cu}_{Cu(PMEA)_{op}}(1 + K_{I/O} + K_{I/N7}) \quad (23b)$$

$$K_I = K_{I/tot} = \frac{K^{Cu}_{Cu(PMEA)}}{K^{Cu}_{Cu(PMEA)_{op}}} - 1 = 10^{\log \Delta} - 1 \quad (24a)$$

$$= \frac{[Cu(PMEA)_{cl/tot}]}{[Cu(PMEA)_{op}]} = \frac{([Cu(PMEA)_{cl/O}] + [Cu(PMEA)_{cl/N7}])}{[Cu(PMEA)_{op}]} \quad (24b)$$

$$= K_{I/O} + K_{I/N7} \quad (24c)$$

Clearly, if the second closed isomer, $Cu(PMEA)_{cl/O/N3}$ in scheme 16 and $Cu(PMEA)_{cl/N7}$ in scheme 17, is not formed, eqs 21c and 24c reduce to the two-isomer problem (equilibrium 1) treated in eqs 11–15. Furthermore, it should be noted that the differences between eqs 21c and 24c originate in the different order of the successive equilibria in schemes 16 and 17. This leads to different definitions for the stability constants of some of the species occurring in the intramolecular equilibria (eqs 19 and 22) and, hence, to different dependencies of K_I ($=K_{I/tot}$; see eqs 21c and 24c).

Table VI. Intramolecular Equilibrium Constants According to the Equilibrium Schemes 16 and 17 for the Formation of the Various Possible Isomeric Cu(PMEA) Complexes, Together with the Percentages in Which the Possible Isomers Occur in Aqueous Solution and in Water Containing 30 or 50% (v/v) 1,4-Dioxane at 25 °C and I = 0.1 M ($NaNO_3$)[a]

Case I Involving N-3 (Equilibrium Scheme 16 in Section 6)

no.	% dioxane (v/v)	log Δ_{PMEA}	K_I = $K_{I/tot}$	% $Cu(PMEA)_{cl/tot}$	% $Cu(PMEA)_{op}$	$K_{I/O}$	$K_{I/O/N3}$	% $Cu(PMEA)_{cl/O}$	% $Cu(PMEA)_{cl/O/N3}$
1	0	0.77 ± 0.07	4.89 ± 0.98	83 ± 3	17 ● 3	2.02 ± 0.47	1.42 ± 0.74	34 ± 10[b]	49 ± 10(29)[c]
2	30	0.52 ± 0.06	2.31 ± 0.44	70 ± 4	30 ± 4	1.45 ± 0.23	0.59 ● 0.40	44 ± 9[b]	26 ± 10(19)[c]
3	50	0.75 ● 0.08	4.62 ± 0.99	82 ± 3	18 ± 3	1.57 ± 0.40	1.94 ± 0.97	28 ± 9[b]	54 ± 9(32)[c]

Case II Involving N-7 (Equilibrium Scheme 17 in Section 6)

no.	% dioxane (v/v)	log Δ_{PMEA}	K_I = $K_{I/tot}$	% $Cu(PMEA)_{cl/tot}$	% $Cu(PMEA)_{op}$	$K_{I/O}$	$K_{I/N7}$	% $Cu(PMEA)_{cl/O}$	% $Cu(PMEA)_{cl/N7}$
1	0	0.77 ± 0.07	4.89 ± 0.98	83 ± 3	17 ± 3	2.02 ± 0.47	2.87 ± 1.09	34 ± 10[b]	49 ± 10(20)[c]
2	30	0.52 ± 0.06	2.31 ± 0.44	70 ± 4	30 ± 4	1.45 ± 0.23	0.86 ● 0.51	44 ± 9[b]	26 ● 10(16)[c]
3	50	0.75 ● 0.08	4.62 ● 0.99	82 ± 3	18 ± 3	1.57 ± 0.40	3.05 ± 1.06	28 ± 9[b]	54 ± 9(21)[c]

[a] Regarding log Δ_{PMEA} the same comment as given in footnote *a* of Table V applies. The values for $K_I = K_{I/tot}$ and % $Cu(PMEA)_{cl/tot}$ follow from eqs 21, 24, and (analogously) 15, respectively. The values given in the sixth column for % $Cu(PMEA)_{op}$ result from 100 − % $Cu(PMEA)_{cl/tot}$. The constants $K_{I/O}$ of column 7 are from column 3 of Table V (for the corresponding justification, see text in section 7); with the now known values for K_I and $K_{I/O}$ and eqs 21c and 24c those for $K_{I/O/N3}$ and $K_{I/N7}$ may be calculated, respectively (column 8). [b] These values were calculated via eq 18 with $K_{I/O}$ and % $Cu(PMEA)_{op}$. [c] The values for % $Cu(PMEA)_{cl/O/N3}$ or % $Cu(PMEA)_{cl/N7}$ follow from the difference % $Cu(PMEA)_{cl/tot}$ − % $Cu(PMEA)_{cl/O}$; % $Cu(PMEA)_{cl/O/N3}$ may also be calculated via eq 19 with $K_{I/O/N3}$ and % $Cu(PMEA)_{cl/O}$; analogously % $Cu(PMEA)_{cl/N7}$ follows from eq 22 with $K_{I/N7}$ and % $Cu(PMEA)_{op}$. The results are the same for both calculation methods (aside from possible small differences in the last digit due to differences in rounding) yet the error limits (which are given in parentheses) are understandably larger for the second method.

7. Quantification of the Intramolecular Equilibria Involving the Adenine Residue and the Ether Oxygen in the Cu(PMEA) Species. Values for K_I as defined by eqs 21 and 24 can be calculated with the stability differences log Δ_{PMEA} (eq 10) of Table III; results for case I and for case II are summarized in Table VI. For the calculation of the formation degree of the isomer containing the five-membered chelate with the ether oxygen, i.e. $Cu(PMEA)_{cl/O}$, the justified assumption[11] is made for both cases I and II that $Cu(PME)_{cl}$ (section 5) and $Cu(PMEA)_{cl/O}$ have the same stability. With K_I and $K_{I/O}$ one can now calculate $K_{I/O/N3}$ (case I) and $K_{I/N7}$ (case II) from eqs 21c and 24c, respectively, and thus also the formation degrees of $Cu(PMEA)_{cl/O/N3}$ and $Cu(PMEA)_{cl/N7}$; of course, these latter values result also from the difference between 100 and the sum of the percentages for $Cu(PMEA)_{op}$ and $Cu(PMEA)_{cl/O}$.

It is worth considering in detail schemes 16 (case I) and 17 (case II) together with the quantitative results displayed in Table VI. The dimensionless "overall" equilibrium constant K_I (=$K_{I/tot}$; eqs 21 and 24) follows directly from the experiments and $K_{I/O}$ (eq 18), which quantifies the formation of the five-membered chelate with Cu^{2+} bound to the phosphonate group and the ether oxygen, has of course the same value in both schemes 16 and 17 as it refers to the same $Cu(PMEA)_{cl}$ isomer; therefore, columns 4–7 and 9 contain the same results in the upper and lower parts. Naturally, the values for $K_{I/O/N3}$ (eq 19) and $K_{I/N7}$ (eq 22) (column 8) are different, as they refer to different intramolecular equilibria, yet the formation degrees of the $Cu(PMEA)_{cl/O/N3}$ (upper part) and $Cu(PMEA)_{cl/N7}$ isomers (lower part) are again identical (column 10). This is not surprising as the formation degree of the third isomer follows in both cases (eqs 16 and 17) from the difference 100 minus the sum of the percentages for $Cu(PMEA)_{op}$ and $Cu(PMEA)_{cl/O}$. This means the mathematical treatment proves that the formation degree of this third isomer involving an adenine interaction is exactly of the same size in both cases I and II.

Evidently, at this point the following question arises: Which of the two adenine-bound isomers is actually formed? Or, are even both isomers occurring at the same time? At present these questions cannot unequivocally be answered though careful considerations (cf. ref 11) lead us to suggest that the equilibrium scheme 16 involving the $Cu(PMEA)_{cl/O/N3}$ isomer is the pertinent one. This suggestion may appear as surprising because N-7 of the adenine residue is a very well known binding site for metal ions,[3,13,14,37–40] in contrast to N-3, yet we feel that for steric reasons N-3 is the preferred site in the M(PMEA) complexes. Indeed, the interaction of N-3 of a purine residue with metal ions is becoming more and more apparent in the past few years from X-ray crystal structure[41] and from solution[42,43] studies.

Conclusions

Though the question about the involvement of N-3 versus N-7 in the binding of the adenine residue in Cu(PMEA) cannot unequivocally be answered, it is important to point out (i) that the formation degree of such a species is well quantified (Table VI, columns 8, 10) and (ii) that it is clearly a N site of the adenine residue that is interacting with Cu^{2+}; we therefore designate this isomer now simply as $Cu(PMEA)_{cl/Ad}$. It is most interesting to note that the addition of 1,4-dioxane to an aqueous solution containing Cu(PMEA) initially lowers the formation degree of the $Cu(PMEA)_{cl/Ad}$ isomer, yet upon the addition of more dioxane it increases again.

A similar observation was made for Cu(5′-AMP) for which an isomeric equilibrium exists between a simple phosphate-bound isomer, $Cu(5'\text{-}AMP)_{op}$, and a macrochelated species involving the phosphate group and the N-7 site, designated as $Cu(5'\text{-}AMP)_{cl}$. To illustrate this point further the pertinent previous results[34] are plotted in Figure 3 together with the present ones of Table VI. The formation degree of both $Cu(5\text{-}AMP)_{cl}$ and $Cu(PMEA)_{cl/Ad}$ passes through a minimum upon the addition of dioxane to aqueous solutions of these complex systems.[44]

This fascinating observation means that, independent from the fact that the overall stability constants, log $K^{Cu}_{Cu(R-PO_3)}$, increase continuously in both cases upon the addition of dioxane (Table III), the formation degree of the various isomers is altered

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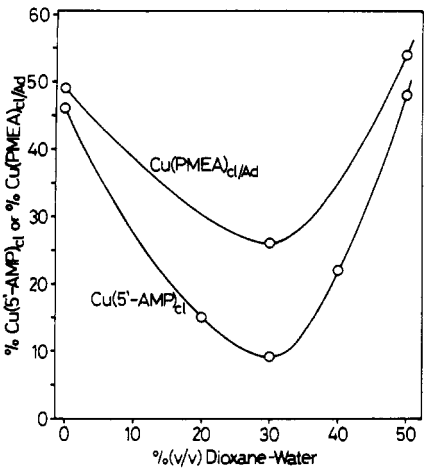

Figure 3. Formation degree of the adenine-chelated species (equilibrium schemes 16 and 17) in the binary Cu^{2+} 1:1 complex systems with $PMEA^{2-}$ (see Table VI) and 5'-AMP^{2-} (from ref 34) as a function of the percentage of 1,4-dioxane added to the aqueous reagent mixtures at 25 °C and I = 0.1 M ($NaNO_3$). $Cu(PMEA)_{cl/Ad}$ represents $Cu(PMEA)_{cl/O/N3}$ and/or $Cu(PMEA)_{cl/N7}$ (see text in Conclusions, while $Cu(5'\text{-}AMP)_{cl}$ represents the macrochelate involving the phosphate group and N-7 of the purine moiety.[13,34]

differently and (yet) unpredictably. Considering that in active-site cavities of metalloproteins, the "solvent" polarity[15,16] and the activity of water[17] are reduced, this is a remarkable result because it demonstrates how nature may alter the structure of a substrate simply by moving it along a protein surface from a more polar into a more apolar region or vice versa.

Similar results as those in Figure 3 are also to be expected for other M^{n+} ions with an affinity for imidazole- or pyridine-type nitrogens. Among the biologically relevant ions Zn^{2+}, which is important, e.g., in nucleic acid metabolism,[10b,e,45,46] has to be placed into this category. It should be emphasized here again that with a decreasing solvent polarity the overall complex stability is increasing despite the changes in the isomeric ratios. This last mentioned category of metal ions is distinctly different from metals like Mg^{2+} or Ca^{2+} that do not undergo a direct adenine interaction;[13,14,37,39,47] however, also for this latter category a decreasing solvent polarity will strongly favor the stability of the $-PO_3^{2-}/M^{2+}$ interaction.[28,33,48]

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(44) The effect of dioxane on $Cu(5'\text{-}AMP)_{cl}$ and $Cu(PMEA)_{cl/Ad}$ is not easy to explain. It seems possible that initially the 1,4-dioxane molecules solvate the hydrophobic adenine residue and inhibit in this way the access of the metal ions to the nitrogen sites. Addition of more dioxane to such a solution will reduce the water activity so much that the aquation (i.e. the solvation by water) of the Cu^{2+} sites is affected, which means that the metal ions are "looking" now for other ligating sites or molecules and consequently now the nitrogens of the adenine residue can overcome their dioxane-solvation barrier and enter again into the metal ion coordination sphere.

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