Complex Formation of the Antiviral 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA) and of Its N1, N3, and N7 Deaza Derivatives with Copper(II) in Aqueous Solution**

Claudia A. Blindauer, Abdul H. Emwas, Antonín Holý, Hana Dvořáková, Einar Sletten, and Helmut Sigel*

Abstract: The stability constants of the 1:1 complexes formed between Cu^{2+} and the anions of the N1, N3, and N7 deaza derivatives of 9-[2-(phosphonomethoxy)-ethyl]adenine (PA^{2-}), $Cu(H;PA)^{+}$ and Cu(PA), were determined by potentiometric pH titration in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$) and compared with previous results for 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$) and (phosphonomethoxy)ethane (PME^{2-}). A microconstant scheme reveals that in $Cu(H;PA)^{+}$ Cu^{2+} is coordinated to the nucleobase, H^{+} being at the phosphonate group, in about 90% of the $Cu(H;PMEA)^{+}$ and $Cu(H;1\text{-deaza-PMEA})^{+}$ species, but only in about 37% and 12% of the corresponding complexes with $H(3\text{-deaza-PMEA})^{-}$ and $H(7\text{-deaza-PMEA})^{-}$, respectively. Straight-line plots of $\log K^{Cu}_{Cu(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ for simple phosph(on)ate ligands show that all the Cu(PA) complexes, including those with $PMEA^{2-}$ and PME^{2-}, are more stable than expected simply from the basicity of the $-PO_3^{2-}$ group; to some extent five-membered chelates ($Cu(PA)_{cl/O}$) involving the ether oxygen of the $-CH_2-O-CH_2-PO_3^{2-}$ chain are formed, and in all complexes an *additional* nucleobase–metal-ion interaction occurs. Based on ^1H NMR line-broadening measurements and structural considerations it is concluded that in Cu(3-deaza-PMEA) the interaction occurs with N7 whereas in Cu(7-deaza-PMEA), Cu(1-deaza-PMEA), and Cu(PMEA) it occurs with N3. The proof of a metal ion–N3 interaction is important (and also of relevance regarding DNA) because so far this interaction has received little attention. In all Cu(PA) systems three major isomeric species are in equilibrium; for example, 17(±3)% of Cu(PMEA) exists as an isomer with a sole Cu^{2+}–phosphonate coordination, 34(±10)% as $Cu(PMEA)_{cl/O}$, and in 49(±10)% the Cu^{2+} is bound to the phosphonate group, the ether O, and N3. In contrast, 54(±8)% of Cu(5′-AMP) occurs as an isomer with sole Cu^{2+}–phosphate coordination and 46(±8)% as a macrochelate involving N7 too.

Keywords
adenine · isomerizations · NMR spectroscopy · nucleotides · stability constants

1. Introduction

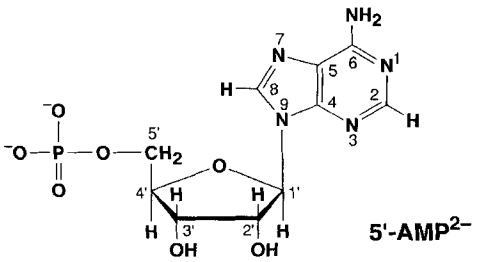
Aside from their importance as components of DNA and RNA, nucleotides and their derivatives play a key role in a wide range of biologically significant reactions.[1] Consequently, a variety of nucleoside and nucleotide analogues have been designed as potential therapeutic agents.[2] One of these substances, the dianion of 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$), can be regarded[3] as an acyclic analogue of adenosine 5′-monophosphate ($5'\text{-}AMP^{2-}$) (Scheme 1).[4]

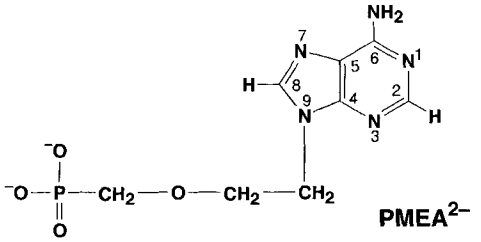
PMEA is active against various viruses, including human immunodeficiency viruses (HIV-1 and HIV-2).[5] After its twofold phosphorylation by cellular nucleotide kinases,[6] the resulting triphosphate analogue inhibits the viral DNA polymerase or

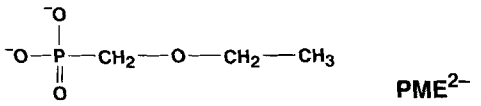
[*] Prof. Dr. H. Sigel, Dipl.-Chem. C. A. Blindauer
Institut für Anorganische Chemie, Universität Basel
Spitalstrasse 51, CH-4056 Basel (Switzerland)
Fax: Int. code +(61)267-1017
e-mail: sigel@ubaclu.unibas.ch
Prof. Dr. E. Sletten, Cand. Scient. A. H. Emwas
Department of Chemistry, University of Bergen, N-5007 Bergen (Norway)
Fax: Int. code +(5)589-490
e-mail: einar.sletten@kj.uib.no
Dr. A. Holý, Dr. H. Dvořáková
Institute of Organic Chemistry and Biochemistry, Academy of Sciences,
CZ-16610 Prague (Czech Republic)
Fax: Int. code +(2)243-10090
e-mail: uochb@uochb.cas.cz

[**] **Abbreviations and definitions:** $2'\text{-}AMP^{2-}$, adenosine 2′-monophosphate; $3'\text{-}AMP^{2-}$, adenosine 3′-monophosphate; ATP^{4-}, adenosine 5′-triphosphate; M^{2+}, divalent metal ion; $PA^{2-} = PME^{2-}$, $PMEA^{2-}$, and its twofold negatively charged deaza derivatives; $R-PO_3^{2-}$, simple phosphate monoester or phosphonate ligand with R representing a noncoordinating residue (see also Figure 1); $TuMP^{2-}$, tubercidin 5′-monophosphate (=7-deaza-5′-AMP^{2-}). Although the IUPAC nomenclature for the deazaadenine compounds is 3H-imidazo[4,5b]pyridine-7-amine (1-deazaadenine), imidazo[4.5c]pyridine-4-amine (3-deazaadenine), and pyrrolo[2,3d]pyrimidine-4-amine (7-deazaadenine), the trivial names and the numbering system for purines are retained in the present study to facilitate the comparison with the parent compound, $PMEA^{2-}$, and other adenine derivatives. For example, 1-deaza-PMEA is thus named 9-[2-(phosphonomethoxy)ethyl]-1-deazaadenine. In mathematical expressions and tables, 1-, 3-, and 7-deaza-PMEA are written as 1d-, 3d-, and 7d-PMEA. In the text the expression "PMEAs" encompasses PMEA as well as its three deaza derivatives. 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.

0947-6539/97/0309-1526 $ 17.50+.50/0



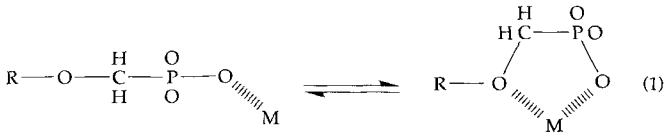




Scheme 1. Structures of the dianion of 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$), adenosine 5'-monophosphate (5'-AMP^{2-}), and (phosphonomethoxy)-ethane (PME^{2-} = ethoxymethanephosphonate). 5'-AMP^{2-} is shown in its dominating *anti* conformation (ref. [4]).

reverse transcriptase activity by terminating the growing nucleic acid chain.[7] The fact that most relevant enzymes, like DNA and RNA polymerases, kinases, and ATP synthases, are metal-ion- (often Zn^{2+})-dependent[1c, 8] and that they use nucleotides as substrates only in the form of (mostly Mg^{2+}) complexes[8, 9] has led us to study the metal-ion-binding properties of $PMEA^{2-}$ and of its base-deficient analogue (phosphonomethoxy)ethane (PME) for the alkaline earth and several divalent 3d metal ions, including Zn^{2+} and Cd^{2+}.[10–12]

In both ligands, it is principally the phosphonate group that determines the stability of the complexes.[10–12] In addition, a five-membered chelate involving the ether oxygen of the (phosphonomethoxy)ethyl chain is formed according to Equilibrium (1). The stability of most of the M(PMEA) complexes is



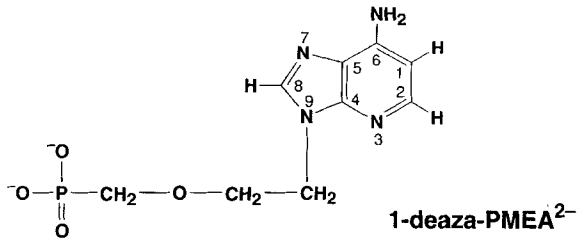
determined by the formation of these two isomeric species, as follows from a comparison of their stability contants with those of the M(PME) complexes. This observation is relevant because the ether oxygen atom is essential for the physiological effects of PMEA.[13]

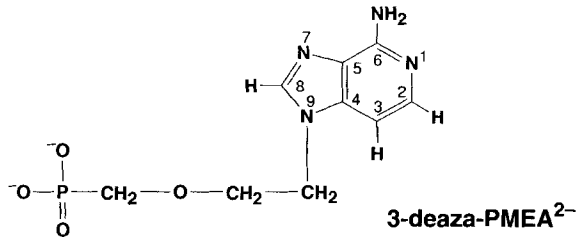
For Cu^{2+} and Ni^{2+} the situation is somewhat different. Cu(PMEA) in particular shows a further significant stability enhancement compared with Cu(PME), which must be ascribed to an interaction with the nucleobase.[14] Considering space-filling molecular models and the fact that N1 cannot be reached by a metal ion already coordinated to the phosphonate group, one can conclude[10–12] that two additional isomeric complexes involving the adenine ring are possible:

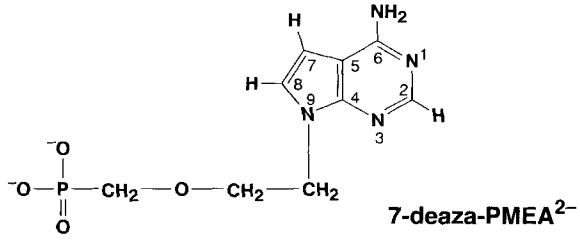
1) One with the five-membered chelate [Equilibrium (1)] still intact, but with a further seven-membered chelate involving N3, and
2) one with Cu^{2+} coordinated to the phosphonate group and N7, thus forming a macrochelate.

Until now, it could not be determined with certainty[10, 12] whether the isomer involving N3 or the one with N7 dominates, or even if both isomers occur simultaneously in solution. Steps to resolve this ambiguity are presented in this study. Since the Zn^{2+}/PMEA system, which would be the most relevant[1c] from a biological point of view, cannot be studied in detail due to the formation of a precipitate,[10] we concentrated our efforts on the complexes formed with Cu^{2+}, an ion which is itself also of biological relevance.[15]

We measured the stabilities of the Cu^{2+} complexes of the 1-, 3-, and 7-deaza analogues of PMEA by potentiometric pH titrations and compared them with those of Cu(PME) and Cu(PMEA) in order to draw conclusions about the metal-ion-coordination patterns within the Cu(PMEA) species; these are further substantiated by ^1H NMR line-broadening studies applying the







paramagnetic properties of Cu^{2+}. Evidently the Cu(PMEA) isomer involving N3 is the crucial one. This adds further evidence regarding the importance of this so far rather neglected site[16] for metal-ion binding at the adenine residue. The N3 position is clearly well suited for metal ion coordination if favorable conditions are provided.

  0947-6539/97/0309-1527 $ 17.50+.50/0

2. Results and Discussion

2.1. Definition of the Equilibrium Constants and Stability of the $Cu(H;PA)^+$ and Cu(PA) Complexes: The experimental conditions for the potentiometric pH titrations in water at 25 °C and $I = 0.1$ M ($NaNO_3$) were again carefully selected[10] such that the results certainly apply for the monomeric species derived from $PMEA^{2-}$ and its deaza derivatives; when considered together in the following, these are abbreviated as PA^{2-}. In the pH range of the present study the PAs can be protonated at both the nucleobase and the phosphonate group, $-PO_3^{2-}$, which is the most basic site.[10, 17] The resulting $H(PA)^-$ species may then be protonated at the adenine residue to form $H_2(PA)^{\pm}$. This protonation occurs for $H(PMEA)^-$, $H(3\text{-deaza-PMEA})^-$, and $H(7\text{-deaza-PMEA})^-$ at N1 and for $H(1\text{-deaza-PMEA})^-$ at N3.[17] The corresponding deprotonation steps are defined in Equations (2)–(3) below. Their acidity constants and those for

$$H_2(PA)^{\pm} \rightleftharpoons H(PA)^- + H^+ \quad (2a)$$

$$K^H_{H_2(PA)} = [H(PA)^-][H^+]/[H_2(PA)^{\pm}] \quad (2b)$$

$$H(PA)^- \rightleftharpoons PA^{2-} + H^+ \quad (3a)$$

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

$K^H_{H_4(PA)}$ and $K^H_{H_3(PA)}$, as well as the corresponding sites where the protons are located, have recently been determined by ^1H NMR shift measurements; the deprotonation reactions of $H_2(PA)^{\pm}$ were also measured by potentiometric pH titrations.[17] These latter results are summarized in columns 2 and 3 of Table 1, together with some related data.[4b, 18]

The experimental data for the potentiometric pH titrations of the Cu^{2+}/PA systems can be described completely by Equilibria (2a)–(5a), if the evaluation is not carried into the pH range where hydroxo complexes form. The acidity constant of Equilibrium (6a) may be calculated with Equation (7). The results are listed in Table 1. The analysis of pH titrations

$$Cu(H;PA)^+ \rightleftharpoons Cu(PA) + H^+ \quad (6a)$$

$$K^H_{Cu(H;PA)} = [Cu(PA)][H^+]/[Cu(H;PA)^+] \quad (6b)$$

$$pK^H_{Cu(H;PA)} = pK^H_{H(PA)} + \log K^{Cu}_{Cu(H;PA)} - \log K^{Cu}_{Cu(PA)} \quad (7)$$

yields only the amount and distribution of species of a net charge type, such as $Cu(H;PA)^+$, and further information is required to locate the binding sites of the proton and the metal ion (see Section 2.2). Similarly, the stability constants of the Cu(PA) complexes also warrant a more detailed analysis considering Equilibrium (1) and the possibility of additional nucleobase–metal-ion interactions (see Sections 2.4–2.6).

2.2. Structural Considerations for the Monoprotonated $Cu(H;PA)^+$ Complexes: The acidity constants $K^H_{Cu(H;PA)}$ of the $Cu(H;PA)^+$ complexes and $K^H_{H_2(PA)}$ of the $H_2(PA)^{\pm}$ species differ only by 1 log unit or less (columns 6 and 2 in Table 1). A proton located at a certain site in a ligand must be acidified upon metal-ion binding to another site in the same ligand; that is, if $pK^H_{M(H;PA)} \gg pK^H_{H_2(PA)}$ the proton would have to be bound at the phosphonate group. However, such an unequivocal situation does not occur in Table 1, and in any case the metal ion could be bound at the nucleobase, but to some extent also at the $-PO_2(OH)^-$ group.

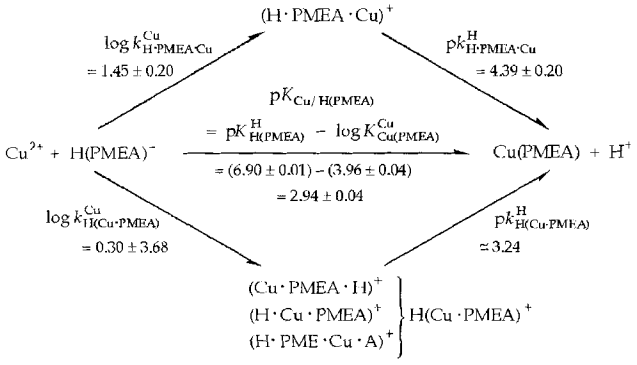
The microconstant scheme (Scheme 2), in which we use PMEA as an example, and which was developed in analogy to similar problems discussed previously,[19] enabled us to identify the main binding site of Cu^{2+} in $Cu(H;PA)^+$. In $(H\cdot PMEA\cdot Cu)^+$ the metal ion is coordinated either to N1 or N7 of the adenine residue[20] and the proton is located at the phosphonate group. In $H(Cu\cdot PMEA)^+$ the Cu^{2+} ion is bound at the phosphonate group and the proton either at N1[21] or also at the phosphonate group.[22] The equilibrium scheme shows the reaction between Cu^{2+} and $H(PMEA)^-$ leading to Cu(PMEA) and H^+, proceeding via $(H\cdot PMEA\cdot Cu)^+$ (upper part) and/or $H(Cu\cdot PMEA)^+$ (lower part). There are three independent equations [(8a,b,c) in Scheme 2], but four unknown microconstants; hence, one of the four needs to be determined or estimated.

Table 1. Negative logarithms of the acidity constants of $H_2(PA)^+$ [Eqs. (2), (3)] and logarithms of the stability constants of the $Cu(H;PA)^+$ [Eq. (4)] and Cu(PA) complexes [Eq. (5)], together with the negative logarithms of the acidity constants for the corresponding $Cu(H;PA)^+$ species [Eqs. (6), (7)], as determined by potentiometric pH titrations in water at 25 °C and $I = 0.1$ M ($NaNO_3$) [a]. The data for systems with adenosine (Ado) or tubercidin (Tu = 7-deazaadenosine) are given for comparison.

PA^{2-}/Ado/Tu	$pK^H_{H_2(PA)}$ [b]	$pK^H_{H(PA)}$ [b]	$\log K^{Cu}_{Cu(H;PA)}$	$\log K^{Cu}_{Cu(PA)}$	$pK^H_{Cu(H;PA)}$
Ado	3.61 ± 0.03 [c]			0.80 ± 0.12 [d]	
Tu	5.29 ± 0.02 [e]			1.06 ± 0.08 [e]	
PME^{2-}		7.02 ± 0.01 [f]		3.73 ± 0.03 [f]	
$PMEA^{2-}$	4.16 ± 0.02 [f]	6.90 ± 0.01 [f]	1.48 ± 0.16 [f]	3.96 ± 0.04 [f]	4.42 ± 0.17 [f]
1d-$PMEA^{2-}$	5.49 ± 0.02 [b]	7.03 ± 0.02	2.13 ± 0.10	4.66 ± 0.08	4.50 ± 0.13
3d-$PMEA^{2-}$	6.61 ± 0.02	7.83 ± 0.01	3.01 ± 0.09	4.60 ± 0.08	6.24 ± 0.12
7d-$PMEA^{2-}$	5.62 ± 0.02	7.00 ± 0.01	2.52 ± 0.17	3.83 ± 0.08	5.69 ± 0.19

[a] The errors given are *three 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 $pK^H_{Cu(H;PA)}$ [Eq. (7)], were calculated by means of Gauss error propagation. [b] The values given for $pK^H_{H_2(PA)}$ refer to the deprotonation of the $H^+(N1)$ site, except for $H_2(1\text{-deaza-PMEA})^{\pm}$ where the proton is liberated from the $H^+(N3)$ site (cf. ref. [17]); the values for $pK^H_{H(PA)}$ refer to the deprotonation of the $-P(O)_2(OH)^-$ residue. The values for the deaza derivatives are from ref. [17]. [c] This value refers to $pK^H_{H(Ado)}$ [4b]. [d] Average of the constants determined in four different laboratories; taken from the final column in Table IV of ref. [18a]. [e] $I = 0.5$ M ($NaNO_3$), 25 °C; from ref. [18b]. The pK_a value is for $pK^H_{H(Tu)}$. [f] From ref. [10].

0947-6539/97/0309-1528 $ 17.50+.50/0 *Chem. Eur. J.* **1997**, *3*, No. 9



$$K^{Cu}_{Cu(H;PMEA)} = k^{Cu}_{H\cdot PMEA\cdot Cu} + k^{Cu}_{H(Cu\cdot PMEA)} \quad (8a)$$

$$\frac{1}{K^{H}_{Cu(H;PMEA)}} = \frac{1}{k^{H}_{H\cdot PMEA\cdot Cu}} + \frac{1}{k^{H}_{H(Cu\cdot PMEA)}} \quad (8b)$$

$$K^{Cu}_{Cu(PMEA)} \cdot K^{H}_{H(PMEA)} = k^{Cu}_{H\cdot PMEA\cdot Cu} \cdot k^{H}_{H\cdot PMEA\cdot Cu} = k^{Cu}_{H(Cu\cdot PMEA)} \cdot k^{H}_{H(Cu\cdot PMEA)} \quad (8c)$$

Scheme 2. The interrelation between the monoprotonated Cu(H;PMEA)$^+$ species is shown, where the metal ion may either be coordinated at the adenine residue (upper part of the scheme), i.e. (H·PMEA·Cu)$^+$, or at the phosphonate group (lower part of the scheme), i.e. H(Cu·PMEA)$^+$ (see [22]), and the other species also in equilibrium with these two complexes. The scheme defines microconstants (k) and gives their interrelations with the macroconstants (K) [Eqs. (3), (5)]; the arrows indicate the directions for which the constants are defined. The macroconstants are from Table 1; the microconstants were derived by applying the Equations (8a), (8b), and (8c), together with the assumptions described in the text in Section 2.2 and ref. [23], to give $\log k^{Cu}_{H\cdot PMEA\cdot Cu} = 1.45 \pm 0.20$ (see also Table 2). The error limits of the various constants were calculated by Gauss error propagation; they correspond to three times the standard error. Regarding the large error of $\log k^{Cu}_{H(Cu\cdot PMEA)}$ (arrow at the left in the lower path) see ref. [24].

A value for $\log k^{Cu}_{H\cdot PMEA\cdot Cu}$ may be estimated[23] based on the stability constant of the Cu(adenosine)$^{2+}$ complex: $\log K^{Cu}_{Cu(Ado)} = 0.80 \pm 0.12$ (Table 1). This value needs to be corrected[23] for 1) the different basicities of N1 in H(PMEA)$^-$ and adenosine, and 2) the charge effect which the $-PO_2(OH)^-$ group exerts on Cu^{2+} at the N1 site; this then gives $\log k^{Cu}_{H\cdot PMEA\cdot Cu} = 1.45 \pm 0.20$.[23] This value with its (estimated)[23] error limit is given on the arrow in the upper left of Scheme 2. The other three microconstants can now be calculated; the results are given on the various arrows in the scheme.[24]

An analogous analysis for Cu(H;PA)$^+$ of the deaza derivatives of PMEA[25] gives the constants collected in Table 2, where the values from Scheme 2 for Cu(H;PMEA)$^+$ are again listed to facilitate comparisons and to help identify the various microconstants; for example, PMEA in Scheme 2 has to be replaced by 1-deaza-PMEA, etc. With the microconstants summarized in columns 3 and 7 of Table 2 an estimate of the ratio R [Eq. (9)] of the species (H·PA·Cu)$^+$ to H(Cu·PA)$^+$ (see also Scheme 2), which carry the metal ion at the nucleobase residue or the phosphonate group, respectively, can be made (column 8). Now the

$$R = \frac{[(H\cdot PA\cdot Cu)^+]}{[H(Cu\cdot PA)^+]} = \frac{k^{Cu}_{H\cdot PA\cdot Cu}}{k^{Cu}_{H(Cu\cdot PA)}} \quad (9)$$

degrees of formation of the (H·PA·Cu)$^+$ species in which Cu^{2+} is coordinated to the nucleobases, that is, their percentages, can be calculated (Table 2, column 9).

Evidently in more than 90% of the Cu(H;PMEA)$^+$ species the metal ion is bound to the adenine residue; this agrees excellently with the previous conclusion[10] based on somewhat different arguments. No conclusion can be drawn about the distribution of Cu^{2+} between the N1 and N7 site in the (H·PMEA·Cu)$^+$ species, but previous evaluations for 9-methyladenine[20b] and adenosine[18a, 20b] indicate that about 75% are N7 and 25% N1 bound. Since the result of the calculation in Scheme 2 indicates that the deprotonation reaction proceeds (mainly) along the upper path and since the estimation for the stability of the (H·PMEA·Cu)$^+$ species is based on the stability of the Cu(Ado)$^{2+}$ complex, there is no hint that chelate formation plays a role in the stability of the protonated Cu(H;PMEA)$^+$ complexes.

The observation that the degree of formation of the (H·PA·Cu)$^+$ species decreases in the series 1-deaza-PMEA > 3-deaza-PMEA > 7-deaza-PMEA (Table 2, column 9) is most interesting. This decreasing affinity of the nucleobase residues toward Cu^{2+} is also reflected in the properties of the unprotonated Cu(PA) complexes, especially of Cu(7-deaza-PMEA), as will be discussed below.

2.3. Evaluation of the Stability of the Cu(PA) Complexes: The stability of the Cu(PA) complexes may be evaluated by means of the straight-line correlation for a $\log K^{Cu}_{Cu(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ plot [Eq. (9)], where $R-PO_3^{2-}$ represents phosphate monoester or phosphonate ligands in which the residue R is unable to

Table 2. Results of the analysis regarding the microconstants for the reaction of Cu^{2+} with H(PA)$^-$ to give Cu(PA) and H^+ via the isomers of Cu(H;PA)$^+$. All constants listed below are defined analogously to the constants given on the various arrows in Scheme 2 (25 °C; $I = 0.1$ M, $NaNO_3$) [a]. Also given are the ratios $R = [(H\cdot PA\cdot Cu)^+]/[H(Cu\cdot PA)^+]$ [Eq. (9)] and the percentages of the (H·PA·Cu)$^+$ species in which Cu^{2+} is coordinated to the nucleobase moiety.

H(PA)$^-$	ΔpK_a [b]	$\log k^{Cu}_{H\cdot PA\cdot Cu}$ [c]	$pK_{Cu/H(PA)}$ [d]	$pk^{H}_{H\cdot PA\cdot Cu}$ [e]	$\log K^{Cu}_{Cu(H;PA)}$ [f]	$\log k^{Cu}_{H(Cu\cdot PA)}$ [g]	R [h]	% (H·PA·Cu)$^+$ [i]
H(PMEA)$^-$	0.55 ± 0.04	1.45 ± 0.20	2.94 ± 0.04	4.39 ± 0.20	1.48 ± 0.16	0.30 ± 3.68	≈14	≈93
H(1d-PMEA)$^-$	1.88 ± 0.04	2.06 ± 0.20	2.37 ± 0.08	4.43 ± 0.22	2.13 ± 0.10	1.30 ± 1.33	≈5.8	≈85
H(3d-PMEA)$^-$	3.00 ± 0.04	2.58 ± 0.20	3.23 ± 0.08	5.81 ± 0.22	3.01 ± 0.09	2.81 ± 0.19	0.59 ± 0.37	37(49;18)
H(7d-PMEA)$^-$	0.33 ± 0.03	1.61 ± 0.17	3.17 ± 0.08	4.78 ± 0.19	2.52 ± 0.17	2.46 ± 0.20	0.14 ± 0.08	12(18;6)

[a] See footnote [a] of Table 1 and the comments in Section 2.2; regarding some of the error limits see [24]. [b] $\Delta pK_a = pK^{H}_{H_2(PA)} - pK^{H}_{H(Ado)}$ (or $-pK^{H}_{H(Tu)}$) [25]; the corresponding values are listed in column 2 of Table 1. [c] See the left-hand arrow of the upper path in Scheme 2; $\log k^{Cu}_{H\cdot PA\cdot Cu} = \log K^{Cu}_{Cu(Ado)} + \Delta pK_a m + (0.40 \pm 0.15)$, where $m = 0.46$; see text in Section 2.2 and ref. [23]; for 7-deaza-PMEA the estimates are based on tubercidin data [25]. [d] Calculated according to the definition given on the horizontal arrow in Scheme 2 with the macroconstants listed in columns 3 and 5 of Table 1. [e] See the right-hand arrow on the upper path in Scheme 2; with the other two constants known, the values for this microconstant now follow from the properties of cyclic systems. [f] From column 4 of Table 1. [g] See the left-hand arrow in the lower part of Scheme 2. The constants were calculated by Equation (8a) of Scheme 2 with the values given above in columns 3 and 6 (see also ref. [24]). [h] Calculated according to Equation (9). [i] Percentage (H·PA·Cu)$^+ = 100 \times R/(1 + R)$. The values given in parentheses represent the upper (first value) and lower limits (second value) of the nucleobase-bound species. The values for $pk^{H}_{H(Cu\cdot PA)}$ (see arrow at the right in the lower part of Scheme 2) are not listed but they can easily be calculated in analogy to footnote [e].

interact with the metal ion.[10, 26] The error limits of log stability constants calculated with given $pK^{H}_{H(R-PO_3)}$ values and Equation (10) are ± 0.06 log units (3σ) in the pK_a range 5–8 (see Tables 5, 6 in ref. [10] or Table 3 in ref. [27]).

$$\log K^{Cu}_{Cu(R-PO_3)} = (0.465 \pm 0.025) \times pK^{H}_{H(R-PO_3)} - (0.015 \pm 0.164) \quad (10)$$

The plot of $\log K^{Cu}_{Cu(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ according to Equation (10) is shown in Figure 1 for the 1:1 complexes of Cu^{2+}

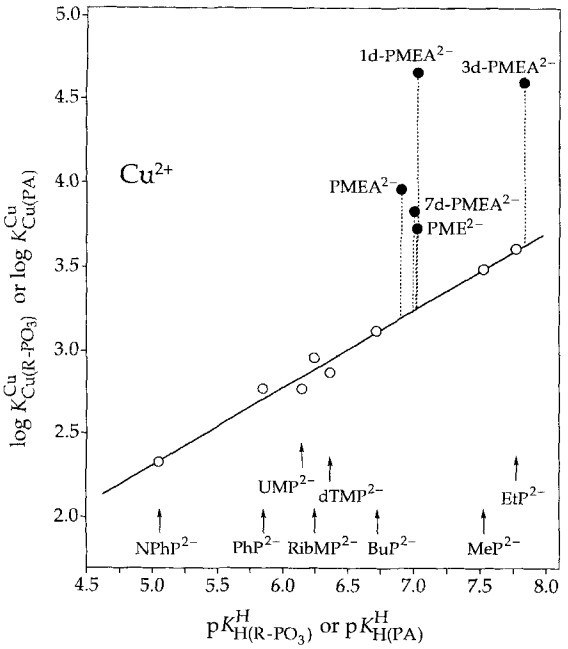


Figure 1. Evidence for enhanced stability of the Cu^{2+} 1:1 complexes of the PA^{2-} ligands (●), based on the relationship between $\log K^{Cu}_{Cu(R-PO_3)}$ and $pK^{H}_{H(R-PO_3)}$ for the 1:1 complexes of Cu^{2+} with some simple phosphate monoester or phosphonate ligands ($R-PO_3^{2-}$): 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate (PhP^{2-}), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5-monophosphate ($RibMP^{2-}$), thymidine [= 1-(2-deoxy-β-D-ribofuranosyl)thymine] 5′-monophosphate ($dTMP^{2-}$), *n*-butyl phosphate (BuP^{2-}), methanephosphonate (MeP^{2-}), and ethanephosphonate (EtP^{2-}) (○). The least-squares line of Equation (11) is drawn through the corresponding 8 data sets taken from ref. [26] for the phosphate monoesters and from ref. [10] for the phosphonates. The points due to the equilibrium constants for the Cu^{2+}/PA^{2-} systems (●) are based on the macro acidity constants listed in Table 1. The vertical broken lines emphasize the stability difference from the reference line; they equal $\log \Delta_{Cu/PA}$ as defined in Equation (10) for the PME^{2-} and $PMEA^{2-}$ systems; for the deaza derivatives this is only approximately true (Section 2.3). All the constants refer to aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$).

with eight simple ligands allowing only phosph(on)ate–Cu^{2+} coordination. The five solid points, which refer to the Cu^{2+} complexes of PME^{2-}, $PMEA^{2-}$, 1-deaza-$PMEA^{2-}$, 3-deaza-$PMEA^{2-}$, and 7-deaza-$PMEA^{2-}$ are considerably above the reference line, thus proving an increased stability for these complexes. A quantitative evaluation is possible by calculating with the straight-line Equation (10) the expected (calcd) stabilities, provided the pK_a values for the deprotonation of the $-PO_2(OH)^-$ group of the PAs are known.

For PME this is clearly $pK^{H}_{H(PME)} = 7.02$ (Table 1) because the phosphonate group is the only basic site, and for PMEA it is $pK^{H}_{H(PMEA)} = 6.90$, as this acidity constant is more than 2.7 log units away from the other pK_a value (Table 1); that is, there is practically no overlap between the buffer regions of $H_2(PMEA)^{\pm}$ and $H(PMEA)^-$. For the deaza derivatives this is different; here the two pK_a values are separated only by about 1.2–1.5 log units, hence, $H(PA)^-$ exists in the two isomeric forms $(PA\cdot H)^-$ and $(H\cdot PA)^-$, in which the proton is nucleobase- or phosphonate-bound, respectively. In the previous analysis[17] it was concluded that the pK_a value for $(H\cdot PA)^-$ is well represented by the microconstant $pk^{H}_{H\cdot PA} = pK^{H}_{H(PMEA)} = 6.90 \pm 0.02$ for 1-deaza- and 7-deaza-PMEA, for which a detailed microconstant scheme was developed. The pK_a value of H(3-deaza-PMEA)$^+$ is about 0.8 log units higher than that of the other PAs (Table 1, column 3), which was attributed, in agreement with ^1H NMR shift experiments,[17] to the location of the $-PO_2(OH)^-$ group in a "hydrophobic" environment close to H2 inhibiting the release of the proton. Of course, once the proton is replaced by Cu^{2+} the steric orientation of the whole residue as well as its solvation will change and the $-PO_3^{2-}/Cu^{2+}$ group will be exposed to the aqueous solvent just as with all the other PAs. Consequently, the basicity of $-PO_3^{2-}$ in 3-deaza-$PMEA^{2-}$, as far as its effect on metal-ion binding is concerned, is also best described by the micro acidity constant $pk^{H}_{H\cdot(3d-)PMEA} = 6.90$. Hence, taking $pk^{H}_{H\cdot PA} = 6.90$ for all *three* deaza derivatives, the stability constants for the Cu(PA) complexes having only a phosphonate–Cu^{2+} coordination can be calculated with Equation (9) (Table 3, column 3).

Table 3. Stability constant comparisons between the measured stability constants (exp) from Table 1 and the calculated stability constants (calcd) based on the basicity of the phosphonate residues [a] and on the reference-line Equation (10) for the Cu(PA) complexes of PME^{2-}, $PMEA^{2-}$, and its deaza derivatives. The values for $\Delta\log\Delta_{Cu/PA}$ [Eq. (12)] reflect the further stability increase due to a nucleobase–Cu^{2+} interaction ($I = 0.1$ M, $NaNO_3$; 25 °C) [b].

PA^{2-}	$\log K^{Cu}_{Cu(PA)}$ [Eq. (5)] exp [c]	calcd	$\log\Delta_{Cu/PA}$ [Eq. (11)]	$\Delta\log\Delta_{Cu/PA}$ [Eq. (12)]
PME^{2-}	3.73 ± 0.03	3.25 ± 0.06	0.48 ± 0.07	
$PMEA^{2-}$	3.96 ± 0.04	3.19 ± 0.06	0.77 ± 0.07	0.29 ± 0.10
1d-$PMEA^{2-}$	4.66 ± 0.08	3.19 ± 0.06	1.47 ± 0.10	0.99 ± 0.12
3d-$PMEA^{2-}$	4.60 ± 0.08	3.19 ± 0.06	1.41 ± 0.10	0.93 ± 0.12
7d-$PMEA^{2-}$	3.83 ± 0.08	3.19 ± 0.06	0.64 ± 0.10	0.16 ± 0.12

[a] The employed acidity constants are $pK^{H}_{H(PME)} = 7.02$ and $pK^{H}_{H(PMEA)} = 6.90$ (Table 1), and for the deaza derivatives $pk^{H}_{H\cdot PA} = pK^{H}_{H(PMEA)} = 6.90$ (see text in Section 2.3). [b] See footnote [a] of Table 1. [c] From column 5 of Table 1.

Comparison of these calculated (calcd) stability constants for the Cu(PA) complexes with the measured (exp) ones by means of Equation (11a) yields the stability differences listed in column 4 of Table 3 [regarding Eq. (11b) see Section 2.6]. Evi-

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

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

dently all Cu(PA) complexes are more stable than expected on the basis of the basicity of the $-PO_3^{2-}$ group, thus confirming the conclusions from Figure 1.

2.4. Structural Considerations for the Cu(PA) Complexes in Solution and Formulation of the Isomeric Equilibria: The positive stability differences mentioned (Table 3, column 4) prove that chelates must be formed to some extent with $PMEA^{2-}$, its deaza

0947-6539/97/0309-1530 $ 17.50+.50/0 *Chem. Eur. J.* **1997**, *3*, No. 9

derivatives, and PME^{2-}.[14] The latter is crucial because PME^{2-} can form only the five-membered chelate seen in Equilibrium (1). Thus, the stability difference $\log\Delta_{Cu/PME} = 0.48 \pm 0.07$ is solely due to the formation of this chelate, and since the (phosphonomethoxy)ethyl chain is identical with that in the four other PAs, any stability increase larger than that observed for Cu(PME) must be attributed to an *additional* nucleobase–Cu^{2+} interaction in the complexes of $PMEA^{2-}$ and its deaza derivatives. This additional stability enhancement is quantified by Equation (12), and the results are listed in column 5 of Table 3.

$$\Delta\log\Delta_{Cu/PA} = \log\Delta_{Cu/PA} - \log\Delta_{Cu/PME} \quad (12)$$

The facts already summarized under points (1) and (2) in the introduction lead to the Equilibria (13) and (14). In both

$$Cu^{2+} + PA^{2-} \overset{K^{Cu}_{Cu(PA)_{op}}}{\rightleftharpoons} Cu(PA)_{op} \overset{K_{l/O}}{\rightleftharpoons} Cu(PA)_{cl/O} \quad (13)$$
$$Cu(PA)_{op} \overset{K_{l/N7}}{\rightleftharpoons} Cu(PA)_{cl/N7}$$

$$Cu^{2+} + PA^{2-} \overset{K^{Cu}_{Cu(PA)_{op}}}{\rightleftharpoons} Cu(PA)_{op} \overset{K_{l/O}}{\rightleftharpoons} Cu(PA)_{cl/O} \quad (14)$$
$$Cu(PA)_{cl/O} \overset{K_{l/O/N3}}{\rightleftharpoons} Cu(PA)_{cl/O/N3}$$

schemes first an "open" complex forms between Cu^{2+} and the phosphonate group of PA^{2-}, $Cu(PA)_{op}$, also seen in Equilibrium (1), which is followed by the five-membered chelate with the ether oxygen, $Cu(PA)_{cl/O}$, yet, in Equation (13) a macrochelate of the phosphonate-coordinated Cu^{2+} with N7 also occurs, designated as $Cu(PA)_{cl/N7}$, whereas in Equation (14) Cu^{2+}, already involved in the five-membered chelate, forms a further – now seven-membered—chelate with N3, $Cu(PA)_{cl/O/N3}$.

The $\Delta\log\Delta_{Cu/PA}$ values [Eq. (11), column 5 of Table 3] point to the following conclusions: In Cu(3-deaza-PMEA) the additional stability increase must be due to a (N7)–Cu^{2+} interaction because N1 is sterically not accessible and N3 is absent. Correspondingly, in Cu(7-deaza-PMEA) the relatively small extra stability has to be attributed to a (N3)–Cu^{2+} interaction. These observations prove that both Equilibria (13) and (14) can operate, but which is more important for the Cu^{2+} complexes of $PMEA^{2-}$ and 1-deaza-$PMEA^{2-}$? In Cu(1-deaza-PMEA) the largest *extra* stability increase due to a nucleobase–metal-ion interaction occurs, and we attribute it largely to an interaction with N3 rather than with N7, recalling that in H_2(1-deaza-PMEA)$^{\pm}$ the nucleobase is protonated at N3,[17] and that this N3 is the most basic one in all the PMEAs studied. This conclusion and the favorable steric arrangement[10] suggest that for Cu(PMEA) too Equation (14) is the more important and that a (N3)–Cu^{2+} interaction is mainly responsible for its additional stability increase.

2.5. Conclusions from ^{1}H NMR Line-Broadening Experiments Regarding the Nitrogen–Cu^{2+} Binding Sites for the Various PMEAs and AMPs: To confirm the above conclusions and to obtain information by an independent method about the various Cu^{2+}-binding sites at the nucleobases, ^{1}H NMR line-broadening experiments were carried out. Paramagnetic relaxation, which leads to line broadening, arises in NMR spectroscopy when an unpaired electron spin interacts with a nuclear spin. A large magnetogyric ratio of the electron compared with that of the proton makes the dipolar coupling to the electron spin a very effective means of relaxation for the nuclear spin.[28] Scalar interactions between the electron and nuclear spins have similar effects. In the simplest possible case a ligand molecule exchanges between a paramagnetic environment (e.g. bound to Cu^{2+}, $S = {}^1/_2$) and a "free" state, with the ligand present in solution in vast excess over the paramagnetic center (e.g., amount 10^2–10^4 times greater). The effect is observed as a dramatic decrease in spin–lattice relaxation time (T_1) and spin–spin relaxation time (T_2). The latter effect is related to the paramagnetically induced line broadening ($\Delta = 1/\pi T_2$), which is measured at half height of the resonance signals (error ca. $\pm 10\%$).

Generally, geometric information obtained from line-broadening measurements is expected to be less reliable since T_2 usually is dominated by scalar interactions (also called contact contributions).[29] However, in several cases assignments of binding sites of paramagnetic ions based on T_2 measurements have proved to be in qualitative agreement with those obtained from T_1 data.[30] In the present case the scalar (through-bond) effect on T_2 is expected to be relatively similar for both H2 and H8 for binding at N3 or N7, respectively (Scheme 1). Thus the residual dipolar (through-space) relaxation component of T_2 should enable us to distinguish between the two binding sites.[31]

At the top of Figure 2 the paramagnetically induced line-broadening versus metal ion/ligand ratios for the H2 and H8 protons of PMEA are plotted. The effect on H2 is dominant, indicating that N3 rather than N7 is the binding site in a chelate involving the purine base. However, monodentate binding at N1 could also produce similar line broadening at H2. In order to eliminate this possibility the titration was repeated with the 1-deaza derivative, which lacks the N1 binding site. The results shown in Figure 2 (second from top) are indeed quite convincing.

A macrochelate where Cu^{2+} binds to the phosph(on)ate group *and* N7 is sterically possible [Section 2.4, Eq. (13)]. To check if such a model is also energetically favorable 3-deaza-PMEA was titrated. The line-broadening data quite clearly show no paramagnetic effects on H8 (Figure 2; second from bottom) but a dramatic broadening of H2. Since N1 cannot be reached by Cu^{2+} coordinated to the phosphonate group, the effect on H2 must arise from monodentate N1 coordination. This result is, however, less surprising than it may appear at first sight: 3-deaza-PMEA is the most basic derivative among all the deaza compounds (Table 1) and this disfavors macrochelate formation with N7 for two reasons: i) At pD $\approx$ 8.8 only about 70% of the species are present as 3-deaza-$PMEA^{2-}$, while the remaining 30% are protonated at the phosphonate group[32] and thus not available for Cu^{2+}–phosphonate binding. This contrasts with all the other PMEAs, for which about 95% exist as PA^{2-} at pD 8.8. ii) The N1 site of 3-deaza-PMEA is 2.45 and 1.0 log units *more* basic than the corresponding site in PMEA and 7-deaza-PMEA, respectively (cf. $pK^{H}_{H2(PA)}$ in Table 1), which greatly favors monodentate N1 binding of Cu^{2+}. Hence, 3-deaza-PMEA is rather special.

  0947-6539/97/0309-1531 $ 17.50+.50/0

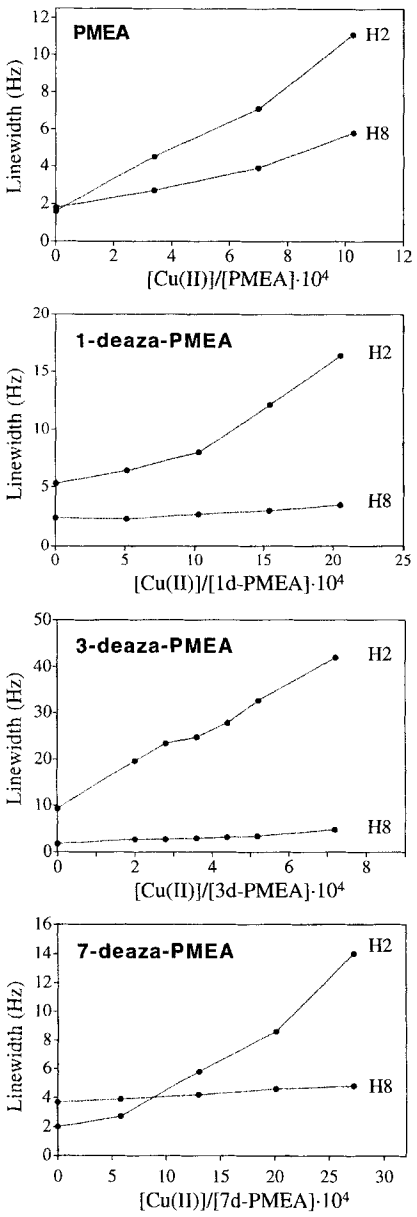


Figure 2. The effect of increasing amounts of Cu^{2+} on the ^1H NMR linewidths of H2 and H8 of $PMEA^{2-}$ and of its deaza derivatives. The ^1H NMR line-broadening experiments were carried out at 25 °C in D_2O at pD ≈ 8.8 with [PA] ≈ 3.0 mM.

Finally, 7-deaza-PMEA was titrated with Cu^{2+} to check whether monodentate coordination at N7 induced significant line broadening of the H8 signal. The results (Figure 2, bottom) reveal H2 broadening, and when compared with the 1-deaza (and 3-deaza) results they clearly suggest that the major species for Cu(7-deaza-PMEA) is a chelate involving N3 and the phosphonate group. Furthermore, if monodentate N7 coordination was of importance for the $Cu^{2+}/PMEA^{2-}$ system this should result in a significant difference in line broadening of H8 in PMEA compared with that of the 7-deaza analogue. There does indeed appear to be some difference, but not a large one; hence, the above conclusion that Equilibrium (14) is the dominant pathway for line broadening in the Cu(PMEA) system is confirmed.

The observed effects of the metal-ion–ligand interactions are the result of rapid ligand exchange in the coordination sphere of the metal ion on the NMR timescale. Thus, as already indicated above, the observed line broadening is induced both by monodentate metal-ion binding *and* chelate formation. Therefore, it should be revealing to study adenosine 5′-monophosphate ($5'\text{-}AMP^{2-}$) too, because Cu(5′-AMP) shows an increased complex stability (see also Table 4) that is *not* observed for Cu(TuMP), where $TuMP^{2-}$ = 7-deaza-$5'\text{-}AMP^{2-}$;[33] this earlier result proves that macrochelate formation in M(5′-AMP) complexes occurs with N7. Indeed, titrations of $5'\text{-}AMP^{2-}$ with Cu^{2+} monitored by NMR show that N7 rather than N1 or N3 is the preferred coordination site (Figure 3, upper part) indicating also for this system that the phosphate group has a "guiding" effect, that is, that the phosphate-coordinated Cu^{2+} forms a macrochelate via N7 within the NMR timescale, thus confirming rather old results[34] and corroborating the interpretation given for Cu(PMEA) (Figure 2, top).

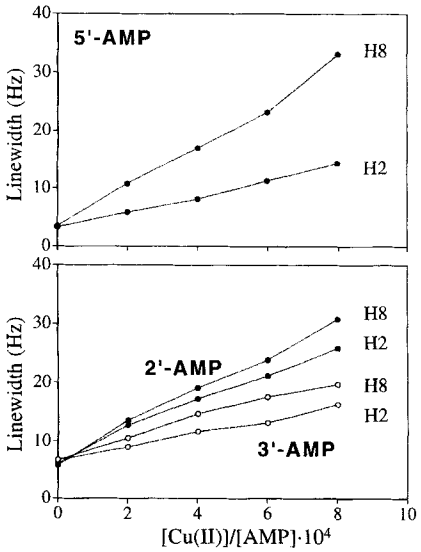


Figure 3. Effect of increasing amounts of Cu^{2+} on the ^1H NMR linewidths of H2 and H8 of $5'\text{-}AMP^{2-}$, $2'\text{-}AMP^{2-}$ (●), and $3'\text{-}AMP^{2-}$ (○). [AMP^{2-}] ≈ 3.0 mM; otherwise as given in the legend of Figure 2.

To complete the picture, we also titrated $2'\text{-}AMP^{2-}$ and $3'\text{-}AMP^{2-}$ with Cu^{2+} (Figure 3, bottom). All three AMPs are fully deprotonated at pD 8.8, and as the Cu^{2+}/AMP^{2-} ratios are identical in the three experiments, the extent of the line broadening can be directly compared. Figure 3 shows that the line-broadening effect of Cu^{2+} on the H8 signal of $3'\text{-}AMP^{2-}$ is less pronounced than on those of 2′- and $5'\text{-}AMP^{2-}$. Furthermore, the observed broadening of the H8 signal of $2'\text{-}AMP^{2-}$ indicates monodentate binding at N7 because Cu^{2+} coordinated to the 2′-phosphate group cannot reach N7. Most important, however, is the fact that the broadening of the signal of H2 in $2'\text{-}AMP^{2-}$ is *greater* than that in $5'\text{-}AMP^{2-}$, which indicates that chelate formation of the 2′-phosphate-bound Cu^{2+} occurs with N3. This conclusion fits with previous results based on potentiometric pH titrations that revealed a higher complex stability for Cu(2′-AMP) than expected on the basis of the basicity of the 2′-phosphate group alone.[35]

The present results for the $Cu^{2+}/3'\text{-}AMP^{2-}$ system (Figure 3, lower part) also agree with these earlier results;[35] here the

0947-6539/97/0309-1532 $ 17.50+.50/0 *Chem. Eur. J.* **1997**, *3*, No. 9

line-broadening effect of Cu^{2+} is the smallest among all three AMPs (see also ref. [34]), and it is slightly larger for the H8 signal than for that of H2, an observation which agrees with the fact that N7 of an adenine residue is more basic than N3.[36] Cu^{2+} binding to these two nitrogens occurs in a monodentate fashion, because Cu^{2+} coordinated at the 3′-phosphate cannot reach N7 and can hardly reach N3, unless 3′-AMP^{2-} adopts the less-favored *syn* conformation.[35] In agreement with this, a possible stability increase of Cu(3′-AMP) is just at the limit of significance.[35]

These results for the Cu^{2+}/AMP^{2-} systems are reassuring regarding those obtained for the PMEAs (Figure 2): The relative effects on H2 compared with H8 prove that the preferred binding region in $PMEA^{2-}$ is the pyrimidine rather than the imidazole part; this differs distinctly from the situation in 5′-AMP^{2-} (Figure 3). Finally, the potentiometric data plotted in Figure 1 and the results summarized in Table 3 (column 4) clearly show that the stabilities of all four Cu(PA) complexes are larger than expected; they decrease in the order 1-deaza-$PMEA^{2-} \gtrsim$ 3-deaza-$PMEA^{2-} >$ $PMEA^{2-} >$ 7-deaza-$PMEA^{2-}$. Also the NMR data indicate that 7-deaza-PMEA forms the least stable complex.

2.6. Formation Degree of the Various Isomeric Cu^{2+} Complexes Formed with PA^{2-} of the PMEAs in Aqueous Solution: As seen in Section 2.4, the Cu^{2+} complexes of $PMEA^{2-}$ and its three deaza derivatives are more stable than expectations based on the basicity of their $-PO_3^{2-}$ groups suggest [Eq. (11)] and more importantly, they are also more stable than the Cu(PME) complex (see Table 3, column 5, [Eq. (12)], which encompasses the isomers seen in Equilibrium (1); this proves that the adenine residues must also participate in complex formation.

There are two unequivocal cases: For structural reasons Cu^{2+} can undergo the mentioned adenine interaction in 3-deaza-$PMEA^{2-}$ and 7-deaza-$PMEA^{2-}$ complexes only through N7 or N3, that is, by Equilibria (13) and (14), respectively. Furthermore, from the line-broadening studies it follows that the Cu^{2+} interaction occurs with $PMEA^{2-}$ and 1-deaza-$PMEA^{2-}$ overwhelmingly at N3, so there are three major isomers and Equilibrium (14) is (mainly) operating for these two PMEAs too. Thus, the degree of formation of the various possible isomers has to be evaluated for $PMEA^{2-}$, 1-deaza-$PMEA^{2-}$, and 7-deaza-$PMEA^{2-}$ based on Equilibrium (14) and for 3-deaza-$PMEA^{2-}$ on Equilibrium (13).

In Equilibrium (14) the experimentally accessible overall stability constant as defined in Equation (5) may be rewritten as given in Equations (15b), (15c), and (15d).[37] The definitions

$$K^{Cu}_{Cu(PA)} = \frac{[Cu(PA)]}{[Cu^{2+}][PA^{2-}]} \quad (15a)$$

$$= \frac{([Cu(PA)_{op}] + [Cu(PA)_{cl/O}] + [Cu(PA)_{cl/O/N3}])}{[Cu^{2+}][PA^{2-}]} \quad (15b)$$

$$= K^{Cu}_{Cu(PA)_{op}} + K_{I/O} \cdot K^{Cu}_{Cu(PA)_{op}} + K_{I/O} \cdot K_{I/O/N3} \cdot K^{Cu}_{Cu(PA)_{op}} \quad (15c)$$

$$= K^{Cu}_{Cu(PA)_{op}} (1 + K_{I/O} + K_{I/O} \cdot K_{I/O/N3}) \quad (15d)$$

involved in these expressions [see also Eq. (14)] are laid out in Equations (16)–(18). The stability increase $\log \Delta_{Cu/PA}$, defined

$$K^{Cu}_{Cu(PA)_{op}} = \frac{[Cu(PA)_{op}]}{[Cu^{2+}][PA^{2-}]} \quad (16)$$

$$K_{I/O} = \frac{[Cu(PA)_{cl/O}]}{[Cu(PA)_{op}]} \quad (17)$$

$$K_{I/O/N3} = \frac{[Cu(PA)_{cl/O/N3}]}{[Cu(PA)_{cl/O}]} \quad (18)$$

in Equation (11) and below partially abbreviated as $\log\Delta$, is connected with the overall intramolecular equilibrium constant K_I, often written as $K_{I/tot}$, by Equation (19). For Equilibrium (13)

$$K_I = K_{I/tot} = \frac{K^{Cu}_{Cu(PA)}}{K^{Cu}_{Cu(PA)_{op}}} - 1 = 10^{\log\Delta} - 1 \quad (19a)$$

$$= \frac{[Cu(PA)_{cl/tot}]}{[Cu(PA)_{op}]} = \frac{([Cu(PA)_{cl/O}] + [Cu(PA)_{cl/O/N3}])}{[Cu(PA)_{op}]} \quad (19b)$$

$$= K_{I/O} + K_{I/O} \cdot K_{I/O/N3} = K_{I/O}(1 + K_{I/O/N3}) \quad (19c)$$

Equations (20a)–(20d) apply. Aside from the definitions in

$$K^{Cu}_{Cu(PA)} = \frac{[Cu(PA)]}{[Cu^{2+}][PA^{2-}]} \quad (20a)$$

$$= \frac{([Cu(PA)_{op}] + [Cu(PA)_{cl/O}] + [Cu(PA)_{cl/N7}])}{[Cu^{2+}][PA^{2-}]} \quad (20b)$$

$$= K^{Cu}_{Cu(PA)_{op}} + K_{I/O} \cdot K^{Cu}_{Cu(PA)_{op}} + K_{I/N7} \cdot K^{Cu}_{Cu(PA)_{op}} \quad (20c)$$

$$= K^{Cu}_{Cu(PA)_{op}} (1 + K_{I/O} + K_{I/N7}) \quad (20d)$$

Equations (16) and (17), Equation (21) is used in the above

$$K_{I/N7} = \frac{[Cu(PA)_{cl/N7}]}{[Cu(PA)_{op}]} \quad (21)$$

expressions. The connection between $K_I = K_{I/tot}$ and the experimentally accessible values for $\log\Delta_{Cu/PA}$ ($= \log\Delta$) is given by Equation (22).

$$K_I = K_{I/tot} = \frac{K^{Cu}_{Cu(PA)}}{K^{Cu}_{Cu(PA)_{op}}} - 1 = 10^{\log\Delta} - 1 \quad (22a)$$

$$= \frac{[Cu(PA)_{cl/tot}]}{[Cu(PA)_{op}]} = \frac{([Cu(PA)_{cl/O}] + [Cu(PA)_{cl/N7}])}{[Cu(PA)_{op}]} \quad (22b)$$

$$= K_{I/O} + K_{I/N7} \quad (22c)$$

Of course, if the isomers $Cu(PA)_{cl/O/N3}$ [Eq. (14)] or $Cu(PA)_{cl/N7}$ [Eq. (13)] are not formed, $K_{I/O/N3}$ [Eq. (18)] or $K_{I/N7}$ [Eq. (21)] become zero and the above Equations (19c) or (22c) reduce to $K_I = K_{I/tot} = K_{I/O}$, that is, to the two-isomer problem appropriate for the treatment of Cu(PME). In this case only the species $Cu(PA)_{op}$ and $Cu(PA)_{cl/O}$ exist, and the situation corresponds to Equilibrium (1). Hence, $K_{I/O}$ for Cu(PME) can be calculated based on the results obtained for $\log\Delta_{Cu/PME}$.

If three isomers are formed [Schemes (13) and (14)], K_I ($= K_{I/tot}$) can be calculated according to Equations (19a) or (22a) from the $\log\Delta_{Cu/PA}$ values in column 4 of Table 3. Hence,

Table 4. Intramolecular equilibrium constants for the formation of the various isomeric Cu(PA) complexes as defined in the Equilibria (13) and (14), together with the percentages in which the isomers occur in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$). The values for the Cu^{2+}/5′-AMP system [27] are given for comparison [a].

Equilibria (14) involving N3 of the adenine residue

No.	PA^{2-}	$\log\Delta_{Cu/PA}$ [Eq. (11)]	$K_1 = K_{1/tot}$ [Eq. (19a)]	% $Cu(PA)_{cl/tot}$ [Eq. (19b)]	% $Cu(PA)_{op}$ [Eq. (19b)]	$K_{1/O}$ [Eq. (17)]	$K_{1/O/N3}$ [Eqs. (18), (19c)]	% $Cu(PA)_{cl/O}$ [Eqs. (1), (14)] [b]	% $Cu(PA)_{cl/O/N3}$ [c] [Eq. (14)]
1	PME^{2-}	0.48±0.07			33±5	2.02±0.47		67±5	
2	$PMEA^{2-}$	0.77±0.07	4.89±0.98	83±3	17±3	2.02±0.47	1.42±0.74	34±10	49±10
3	1d-$PMEA^{2}$	1.47±0.10	28.51±6.80	96.6±0.8	3.4±0.8	2.02±0.47	13.1±4.7	6.8±2.2	90±2
4	7d-$PMEA^{2}$	0.64±0.10	3.37±1.01	77±5	23±5	2.02±0.47	0.67±0.63	46 ±15	31±16

Equilibria (13) involving N7

No.	PA^{2-}	$\log\Delta_{Cu/PA}$ [Eq. (11)]	$K_1 = K_{1/tot}$ [Eq. (22a)]	% $Cu(PA)_{cl/tot}$ [Eq. (22b)]	% $Cu(PA)_{op}$ [Eq. (22b)]	$K_{1/O}$ [Eq. (17)]	$K_{1/N7}$ [Eqs. (21), (22c)]	% $Cu(PA)_{cl/O}$ [Eqs. (1), (13)] [b]	% $Cu(PA)_{cl\,N7}$ [c] [Eq. (13)]
5	3d-$PMEA^{2-}$	1.41±0.10	24.70±5.92	96.1±0.9	3.9±0.9	2.02±0.47	22.7±5.9	7.9±2.6	88±3
6	5′-AMP^{2-} [d]	0.27±0.06			54±8		0.86±0.26		46±8

[a] See footnote [a] of Table 1. The values in the third column are from column 4 of Table 3. The values given in the sixth column for percentage $Cu(PA)_{op}$ follow from 100 − % $Cu(PA)_{cl/tot}$. The constants $K_{1/O}$ result from the Cu^{2+}/PME^{2-} system and are taken from ref. [10] (see text in Section 2.6); with the now known values for K_1 and $K_{1/O}$ and Equations (19c) and (22c) those for $K_{1/O/N3}$ and $K_{1/N7}$ may be calculated (column 8). [b] These results were calculated with Equation (17) with $K_{1/O}$ and percentage $Cu(PA)_{op}$. [c] The values for percentage $Cu(PA)_{cl/O/N3}$ and $Cu(PA)_{cl/N7}$ follow from the difference in percentages $Cu(PA)_{cl/tot}$ − $Cu(PA)_{cl/O}$; for further details see Tables 4 or 11 in refs. [12] or [10], respectively. [d] See also ref. [38].

the concentration fraction of $Cu(PA)_{op}$ becomes known [Eqs. (19b) or (22b)]. Assuming that the stability of the $Cu(PME)_{cl/O}$ isomer represents the stability of the $Cu(PA)_{cl/O}$ isomers of the PMEAs well, as they all contain the structurally identical (phosphonomethoxy)ethyl chain, then $K_{1/O/N3}$ or $K_{1/N7}$ can also be calculated with Equations (19c) or (22c), respectively, and hence, the degree of formation of all isomers can be known. The results are summarized in Table 4, together with the values for the Cu^{2+}/5′-AMP^{2-} system.[27, 38]

The most obvious conclusion from Table 4 is that aside from the -PO_3^{2-} group all three additional binding sites of PMEA or its deaza derivatives, that is, the ether O as well as N3 and N7 of the adenine residue, participate in complex formation: a) the formation degree of $Cu(PME)_{cl/O}$ is considerable, about 67% [Eq. (1) and Table 4, column 9]; b) of the 77% due to Cu(7-deaza-PMEA)$_{cl/tot}$ (no. 4 in Table 4) about 31% occurs with a N3 interaction involving a seven- and also a five-membered chelate, $Cu(7\text{-deaza-PMEA})_{cl/O/N3}$, next to 46% of the $Cu(7\text{-deaza-PMEA})_{cl/O}$ isomer [Eq. (14)]; and c) of the approximately 96% of the $Cu(3\text{-deaza-PMEA})_{cl/tot}$ about 88% (no. 5 in Table 4) is present as the N7 macrochelate, $Cu(3\text{-deaza-PMEA})_{cl/N7}$ [Eq. (13)], and about 8% remains as $Cu(3\text{-deaza-PMEA})_{cl/O}$. Furthermore, 3-deaza-$PMEA^{2-}$ is better suited for the formation of macrochelates than is 5′-AMP^{2-}, which forms only about 46% of the $Cu(5'\text{-AMP})_{cl/N7}$ isomer (Table 4, no. 6). This is because the 3-deazaadenine residue is more basic overall than the adenine residue (Table 1) and, owing to its "aliphatic" chain, the ligand itself is more flexible than 5′-AMP^{2-} with its relatively rigid ribose moiety.

The Cu^{2+} complexes of 1-deaza-$PMEA^{2-}$ and $PMEA^{2-}$, which prefer N3 interactions over N7 (Sections 2.4 and 2.5), form considerable amounts of $Cu(PA)_{cl/O/N3}$ (Table 4, Nos. 2 and 3): $Cu(1\text{-deaza-PMEA})_{cl/O/N3}$ is favored (about 90% formed) much more than $Cu(PMEA)_{cl/O/N3}$ (about 49% formed). Since N3 is the most basic nucleobase site in 1-deaza-PMEA (Table 1), this result is not surprising. What is surprising, rather, is the fact that if a metal ion is correctly positioned at suitable "primary" binding sites then N3 binds metal ions with a relatively pronounced affinity, despite its low basicity within the adenine ring (N1 > N7 > N3; cf. ref. [36]).

Comparison of all the results in Table 4 shows that the Cu^{2+}–nucleobase interaction is least pronounced in the Cu^{2+}/7-deaza-$PMEA^{2-}$ system; this result concurs with those described for the protonated complexes (Section 2.2; Table 2) and also with the NMR line-broadening observations of Section 2.5.

Finally, it needs to be emphasized that this analysis proves that the main three isomers of all PMEAs occur simultaneously in appreciable amounts in equilibrium with each other. For example, Cu(PMEA) itself consists of about 17% $Cu(PMEA)_{op}$, 34% $Cu(PMEA)_{cl/O}$, and 49% $Cu(PMEA)_{cl/O/N3}$.

3. Conclusions

The analyses presented in this study, which involve the three deaza isomers of PMEA, the determination of the stability constants of the corresponding Cu^{2+} complexes by potentiometric pH titrations, and ^1H NMR line-broadening studies of the various ligands in the presence of Cu^{2+}, prove that three main isomers of the Cu(PMEA) complex exist and that in one of them the adenine residue is bound to Cu^{2+} via N3! To the best of our knowledge this is the first time that a three-isomer problem has been solved in all its facets, including the binding sites and degrees of formation of the various isomeric complexes that occur in solution in equilibrium with each other.

The finding that metal-ion binding occurs at N3 is of general interest because so far not much is known (see refs. [16,35]) about the metal-ion-binding properties, especially in solution, of this purine nitrogen site. The present results demonstrate that despite its low basicity[36] N3 can bind metal ions well *provided* these are orientated or positioned by suitable "primary" coordination. For this reason Cu^{2+} forms macrochelates involving N7 with 5′-AMP^{2-} and 3-deaza-$PMEA^{2-}$, whereas in its complexes of 2′-AMP^{2-},[35] 7-deaza-$PMEA^{2-}$, 1-deaza-$PMEA^{2-}$, and $PMEA^{2-}$ it interacts with N3!

This observation regarding N3 is not only of importance for nucleotides but possibly even more so for the metal-ion-binding properties of DNA, where N3 is exposed to the solvent in the minor groove.[39] The results described here for Cu^{2+} also hold for other metal ions with a tendency to coordination with N

0947-6539/97/0309-1534 $ 17.50+.50/0 *Chem. Eur. J.* **1997**, *3*, No. 9

sites. Examples are Zn^{2+}, which is crucial for the activity of many enzymes,[40a] but also Cd^{2+}, which is generally toxic to biological systems.[40b]

The properties of $PMEA^{2-}$ and 5′-AMP^{2-} that depend only on the qualities of the adenine moiety, such as hydrogen bonding or stacking interactions, are expected to be very similar or even identical. Furthermore, it follows from the formation of monoprotonated $Cu(H;PMEA)^{+}$ species (Section 2.2) in which the metal ion is mainly located at the adenine residue and the proton at the phosphonate group (Table 2, first row) that the adenine residue of PMEA may form adenosine-type complexes.

As the lengths of the D-ribose 5-monophosphate residue and of the (phosphonomethoxy)ethyl residue are also very similar,[3a] a metal ion coordinated at the phosphate group of 5′-AMP^{2-} or at the phosphonate group of $PMEA^{2-}$ is placed at about the same distance from the adenine moiety; in other words, the "open" isomers of the corresponding complexes are structurally quite alike. However, there are also crucial differences: 5′-AMP^{2-} coordinates alkaline earth ions only through the phosphate group, whereas divalent 3d ions and Zn^{2+} or Cd^{2+} also form macrochelates additionally involving N7 of the adenine moiety.[27, 38] In contrast, $PMEA^{2-}$ interacts with all metal ions studied so far[10, 12] not only through the phosphonate group, but to a remarkable extent also through the neighboring ether oxygen, forming five-membered chelates as illustrated in Equilibrium (1). Furthermore, as has now been shown, the interaction with the adenine residue occurs through N3! Hence, the structures of the complexes formed with 5′-AMP^{2-} and $PMEA^{2-}$, having only a phosphate- or phosphonate–metal ion interaction, are similar, while those isomers that comprise chelates have very different structures in solution. At this point, it may be recalled that the ether O atom of PMEA is not only important for its *metal-ion-binding properties* but that it is also essential for the *biological activity*, for example, its antiviral action.[13]

Regarding isomeric equilibria, that is to say, equilibria between different structural forms of complexes with the same composition, it is important to note that the conversion of an "open" form into a chelated form with a formation degree of about 20% is connected only with a stability difference of $\log\Delta = 0.1$ [Eq. (10)]. In other words, ΔG° changes by only 0.6 kJ mol^{-1}.[14, 41] Since isomeric equilibration is fast, nature has here a tool to achieve high selectivity without employing high energy barriers[42] because formation of 20% of a given isomer in equilibrium is more than enough to serve in an enzyme reaction as substrate or inhibitor.

4. Experimental Section

4.1. Materials: The free acids of 9-[2-(phosphonomethoxy)ethyl]adenine, 9-[2-(phosphonomethoxy)ethyl]-1-deazaadenine, 9-[2-(phosphonomethoxy)-ethyl]-3-deazaadenine and 9-[2-(phosphonomethoxy)ethyl)]-7-deazaadenine were synthesized according to published procedures.[43] The aqueous stock solutions of the PMEA ligands were freshly prepared daily just before the titrations by dissolving the substance in distilled, CO_2-free water and adding 2 equiv of NaOH.

The sodium salt of adenosine 5′-monophosphate and adenosine 2′-monophosphoric acid and adenosine 3′-monophosphoric acid were purchased from Sigma (St. Louis, MO, USA). The disodium salt of 1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (Na_2EDTA), potassium hydrogenphthalate, HNO_3, NaOH (Titrisol), and the nitrate salts of Na^{+} and Cu^{2+} (all *pro analysi*) were from Merck (Darmstadt, FRG).The buffer solutions (pH 4.64, 7.00, 9.00 based on the NBS scale; now NIST) for calibration were from Metrohm (Herisau, Switzerland).

4.2. Potentiometric pH Titrations: The exact concentration of the Cu^{2+} stock solutions was determined by potentiometric pH titration via the EDTA complex. The NaOH solutions used for the titrations were standardized with potassium hydrogenphthalate. The exact concentration of the ligand solutions was newly determined in each experiment by the evaluation of the titration pairs described below. The potentiometric pH titrations were all carried out with a Metrohm E536 potentiograph equipped with an E535 dosimat and a Metrohm 6.0202100 (NB) combined macro glass electrode. The set was calibrated with the above-mentioned Metrohm buffer solutions. The direct pH readings were used in the calculations of the acidity constants,[17] so these are so-called practical, mixed or Brønsted constants. Their negative logarithms given for aqueous solutions at $I = 0.1$ M ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values.[44]

The acidity constants $K^{H}_{H_2(PA)}$ and $K^{H}_{H(PA)}$ of the twofold protonated 1-, 3-, and 7-deaza-PMEAs were determined as described recently[17] by titration of an aqueous solution of HNO_3 (30 mL, 1.35 mM, $I = 0.1$ M, $NaNO_3$) under N_2 at 25 °C in the presence and absence of the PMEA derivative (0.4 mM, all present in the stock solution as PA^{2-}) with NaOH (1.5 mL, 0.03 M). The difference in NaOH consumption between such a pair of corresponding solutions was evaluated.

The conditions for the determination of the stability constants $K^{Cu}_{Cu(H;PA)}$ and $K^{Cu}_{Cu(PA)}$ were the same as given above, but some of the $NaNO_3$ was now replaced by $Cu(NO_3)_2$ ($I = 0.1$ M; 25 °C). The Cu^{2+}:ligand ratios were 1:1 and 5.6:1; with 1-deaza-PMEA the ratio 2.8:1 was also applied. The constants were calculated with an IBM-compatible desktop computer (with an 80-486 processor), connected to an Epson Stylus printer and a Hewlett-Packard 7475A plotter; a curve-fitting procedure was used taking into account the species H^{+}, $H_2(PA)^{\pm}$, $H(PA)^{-}$, PA^{2-}, Cu^{2+}, $Cu(H;PA)^{+}$, and Cu(PA). The experimental data were taken every 0.1 pH unit starting from about 3% complex formation until the beginning of the hydrolysis of $Cu^{2+}_{(aq)}$, which was evident from the titrations without ligand. The results are the averages of at least seven independent pairs of titration curves.

4.3. ^{1}H NMR Line-Broadening Measurements: The ^{1}H NMR experiments were performed at 400.13 MHz on a Bruker Avance DMX400 spectrometer. Samples of PMEA and its deaza derivatives were dissolved in D_2O at concentrations around 3.0 mM. In these dilute solutions it is expected that excessive stacking of the purine bases is avoided.[41] The pH was adjusted to a pH-meter reading of 8.4, corresponding to a pD of 8.8. A stock solution of 0.22 mM $Cu(NO_3)_2$ in D_2O was used in the titration of the solutions containing the PMEAs. Aliquots of 2 μL were added directly into the NMR tube and thoroughly mixed before the ^{1}H NMR spectra were recorded. The tetramethylammonium (TMA^{+}) ion was added as a chemical shift standard and the ^{1}H NMR signals of the various protons were assigned as previously.[17] Where appropriate, the measured linewidths include coupling, such as between H1 and H2 in 1-deaza-PMEA. The spectra were recorded at 25 °C using a standard one-pulse sequence with presaturation of the residual HDO water signal. Exponential multiplication of the FIDs was performed before the Fourier transform to improve the signal-to-noise ratio.

Acknowledgements: The competent technical assistance of Rita Baumbusch in the preparation of the manuscript is gratefully acknowledged. This study was supported by the Swiss National Science Foundation (HS), the Norwegian Research Council (ES), the EU-HCM programme, Contract ER-BCHRXCT920016 (ES and, via the Swiss Government, HS) and within the COST D1 programme by the Swiss Federal Office for Education and Science (HS), and the Governments of Norway (ES) and the Czech Republic (AH).

Received: February 4, 1997 [F603]

[1] a) *Probing of Nucleic Acids by Metal Ion Complexes of Small Molecules* (Eds.: A. Sigel, H. Sigel), in *Met. Ions Biol. Syst.*, *Vol. 33*, M. Dekker, New York, Basel, Hong Kong, **1996**, pp. 1–678; b) *Interactions of Metal Ions with Nucleotides, Nucleic Acids, and Their Constituents* (Eds.: A. Sigel, H. Sigel), in *ibid.*, *Vol. 32*, **1996**, pp. 1–814; c) *Interrelations among Metal Ions, Enzymes, and Gene Expression* (Eds.: A. Sigel, H. Sigel), in *ibid.*, *Vol. 25*, **1989**, pp. 1–557.

[2] a) *Nucleotide Analogues as Antiviral Agents* (Ed.: J. C. Martin), in *ACS Symp. Ser.*, *Vol. 401*, American Chemical Society, Washington, DC, **1989**; b) P. Alexander, A. Holý, *Collect. Czech. Chem. Commun.* **1994**, *59*, 2127–2165.

[3] a) C. H. Schwalbe, W. Thomson, S. Freeman, *J. Chem. Soc. Perkin Trans. 1* **1991**, 1348–1349. b) Of course, PMEA is actually more closely related to 2′-deoxy-5′-AMP or even 2′,3′-dideoxy-5′-AMP, but for these less information is available and therefore 5′-AMP is used for comparisons. With regard to the metal-ion-binding properties this does not matter, because the ribose moiety does not commonly participate in the binding of the biologically significant metal ions [3c]. c) H. Sigel, *Chem. Soc. Reviews* **1993**, *22*, 255–267.

[4] Regarding the *anti*/*syn* conformations see: a) R. B. Martin, Y. H. Mariam, *Met. Ions Biol. Syst.* **1979**, *8*, 57–124; b) R. Tribolet, H. Sigel, *Eur. J. Biochem.* **1987**, *163*, 353–363.

[5] a) R. Pauwels, J. Balzarini, D. Schols, M. Baba, J. Desmyter, I. Rosenberg, A. Holý, E. De Clercq, *Antimicrob. Agents Chemother.* **1988**, *32*, 1025–1030; b) E. De Clercq, A. Holý, I. Rosenberg, *ibid.* **1989**, *33*, 185–191.

[6] a) A. Merta, I. Votruba, J. Jindřich, A. Holý, T. Cihlář, I. Rosenberg, M. Otmar, T. Y. Herve, *Biochem. Pharmacol.* **1992**, *44*, 2067–2077; b) S. A. Foster, J. Černý, Y.-c. Cheng, *J. Biol. Chem.* **1991**, *266*, 238–244; c) J. Balzarini, E. De Clercq, *ibid.* **1991**, *266*, 8686–8689.

[7] a) J. Balzarini, Z. Hao, P. Herdewijn, D. G. Johns, E. De Clercq, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 1499–1503; b) J. Neyts, E. De Clercq, *Biochem. Pharmacol.* **1994**, *47*, 39–41.

[8] A. S. Mildvan, *Magnesium* **1987**, *6*, 28–33.

[9] L. Stryer, *Biochemistry*, W. H. Freeman, New York, **1988**; L. Stryer, *Biochemie*, Spektrum, Heidelberg, Berlin, New York, **1991**, p. 431.

[10] H. Sigel, D. Chen, N. A. Corfù, F. Gregáň, A. Holý, M. Strašák, *Helv. Chim. Acta* **1992**, *75*, 2634–2656.

[11] a) D. Chen, F. Gregáň, A. Holý, H. Sigel, *Inorg. Chem.* **1993**, *32*, 5377–5384; b) D. Chen, M. Bastian, F. Gregáň, A. Holý, H. Sigel, *J. Chem. Soc. Dalton Trans.* **1993**, 1537–1546; c) M. Bastian, D. Chen, F. Gregáň, G. Liang, H. Sigel, *Z. Naturforsch.* **1993**, *48b*, 1279–1287; d) B. Song, A. Holý, H. Sigel, *Gazz. Chim. Ital.* **1994**, *124*, 387–392.

[12] a) H. Sigel, *Coord. Chem. Rev.* **1995**, *144*, 287–319; b) H. Sigel, *J. Indian Chem. Soc.* (P. Ray Award Lecture) **1997**, *74*, 261–271.

[13] a) A. Holý, E. De Clercq, I. Votruba, *ACS Symp. Ser.* **1989**, *401*, 51–71; b) A. Holý, I. Votruba, A. Merta, J. Černý, J. Veselý, J. Vlach, K. Šedivá, I. Rosenberg, M. Otmar, H. Hřebabecký, M. Trávniček, V. Vonka, R. Snoeck, E. De Clercq, *Antiviral Res.* **1990**, *13*, 295–312; c) A. Holý in *Advances in Antiviral Drug Design, Vol. 1* (Ed.: E. De Clercq), JAI Press, Greenwich, Connecticut, USA, **1993**, pp. 179–231; d) for sulfur, see: D. Villemin, F. Thibault-Starzyk, *Synth. Commun.* **1993**, *23*, 1053–1059.

[14] R. B. Martin, H. Sigel, *Comments Inorg. Chem.* **1988**, *6*, 285–314.

[15] a) W. Kaim, J. Rall, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 43–60; b) *Metalloenzymes Involving Amino Acid Residue and Related Radicals* (Eds.: H. Sigel, A. Sigel), in *Met. Ions Biol. Syst., Vol. 30*, M. Dekker, New York, Basel, Hong Kong, **1994**, pp. 1–494; c) G. Berthon, *Agents Actions* **1993**, *39*, 210–217; d) *Electron Transfer Reactions in Metalloproteins* (Eds.: H. Sigel, A. Sigel), in *Met. Ions Biol. Syst., Vol. 27*, M. Dekker, New York, Basel, Hong Kong, **1991**, pp. 1–537.

[16] C. Meiser, B. Song, E. Freisinger, M. Peilert, H. Sigel, B. Lippert, *Chem. Eur. J.* **1997**, *3*, 388–398.

[17] C. A. Blindauer, A. Holý, H. Dvořáková, H. Sigel, *J. Chem. Soc. Perkin Trans. 2* **1997**, in press.

[18] a) H. Sigel, N. A. Corfù, L.-n. Ji, R. B. Martin, *Comments Inorg. Chem.* **1992**, *13*, 35–59; b) L.-n. Ji, N. A. Corfù, H. Sigel, *J. Chem. Soc. Dalton Trans.* **1991**, 1367–1375.

[19] a) B. Song, D. Chen, M. Bastian, R. B. Martin, H. Sigel, *Helv. Chim. Acta* **1994**, *77*, 1738–1756; b) R. K. O. Sigel, B. Song, H. Sigel, *J. Am. Chem. Soc.* **1997**, *119*, 744–755.

[20] The repeatedly discussed N1 versus N7 dichotomy will not be considered here: a) ref. [18a]; b) R. B. Martin, *Met. Ions Biol. Syst.* **1996**, *32*, 61–89; c) a Cu^{2+}/N3 interaction could also be postulated but this could only occur within a chelate (see Sections 2.4, 2.6) and would lead here to the $(H\cdot PME\cdot Cu\cdot A)^+$ isomer considered in ref. [22].

[21] If a PA species contains the N1 atom, this is always the most basic site in the corresponding nucleobase [17] (see also footnote [b] in Table 1).

[22] $H(Cu\cdot PMEA)^+$ encompasses the following isomers: 1) $(H\cdot Cu\cdot PMEA)^+$; Cu^{2+} and H^+ are both bound to the phosphonate group and the metal ion does not form any chelates involving the nucleobase; 2) $(H\cdot PME\cdot Cu\cdot A)^+$; Cu^{2+} and H^+ are both bound to the phosphonate group but Cu^{2+} forms chelates with N3 and/or N7 [note: Should any chelate formation with the $-PO_2(OH)^-$ group occur in the $(H\cdot PMEA\cdot Cu)^+$ isomer (see also [20c]) this would then also give $(H\cdot PME\cdot Cu\cdot A)^+$]; 3) $(Cu\cdot PMEA\cdot H)^+$; the proton is at the nucleobase [21] and Cu^{2+} at the phosphonate group. Since chelate formation of phosphonate-coordinated Cu^{2+} with a *protonated* nucleobase is highly unlikely, such an additional isomer is not included. Analogous considerations can also be made for the other $H(Cu\cdot PA)^+$ systems.

[23] a) This estimate is made in the following way: The stability constant of $Cu(adenosine)^{2+}$, $\log K^{Cu}_{Cu(Ado)} = 0.80\pm0.12$ (Table 1), is corrected for the different basicities of the N1 site in $H(PMEA)^-$ and adenosine [i.e., $\Delta pK_a = pK^H_{H_2(PMEA)} - pK^H_{H(Ado)} = (4.16\pm0.02) - (3.61\pm0.03) = 0.55\pm0.04$ (Table 1)] by applying the average of the slopes ($m = 0.46$) of the regression lines for $\log K$ versus pK_a plots [18a, 23b] for N1- and N7-type ligands. This gives the "corrected" value $(0.80\pm0.12) + (0.25\pm0.04) = 1.05\pm0.13$, which needs to be further corrected for the charge effect that the $-PO_2(OH)^-$ group exerts on Cu^{2+} at the N1 site [the effect of the same group on H^+(N1) is taken care of by ΔpK_a], corresponding to 0.40 ± 0.15 log units, as is known from various other cases where the distances between the positive and negative charges are of a comparable size [23c]. Hence, $\log k^{Cu}_{H\cdot PMEA\cdot Cu} = (1.05\pm0.13) + (0.40\pm0.15) = 1.45\pm0.20$; b) the average of the corresponding slopes given in ref. [20b] is very similar, i.e., $m = 0.47$; c) M. Bastian, H. Sigel, *J. Coord. Chem.* **1991**, *23*, 137–154.

[24] The exceedingly large error of the value on the arrow at the left in the lower part of Scheme 2 (and in column 7 of Table 2) results from the fact that according to Equation (7a) of Scheme 2, $k^{Cu}_{H(Cu\cdot PMEA)} = K^{Cu}_{Cu(H;PMEA)} - k^{Cu}_{H\cdot PMEA\cdot Cu} = 10^{(1.48\pm0.16)} - 10^{(1.45\pm0.20)}$; here a difference is calculated between two large and very similar values with overlapping error limits. In other words, the result 0.30 ± 3.68 is "meaningless" (except that it indicates a *small* value) because the reaction overwhelmingly follows the upper path in Scheme 2 (see also columns 3, 6, and 7 in Table 2). The fact that the value for $k^{Cu}_{H(Cu\cdot PMEA)}$ must be *small* (despite its large error limit) permits the estimation of values for R and the percentage of $(H\cdot PA\cdot Cu)^+$ as given in columns 8 and 9, respectively, of Table 2.

[25] a) One may argue that for the deaza derivatives of PMEA the estimation of a value for $\log k^{Cu}_{H\cdot PA\cdot Cu}$ by means of $\log K^{Cu}_{Cu(A\text{-}de)}$ is no longer appropriate. However, for 3-deaza-PMEA, which also has the N1 and N7 sites, this is clearly no problem; hence, the analysis was made as in ref. [23]. In the case of 7-deaza-PMEA, which has only the N1 site left, the estimation was made by using the values for tubercidin (=7-deazaadenosine; see Table 1) in the analysis (carried out in analogy to [23]). Finally, for 1-deaza-PMEA the procedure used for PMEA [23] was again employed because in a first approximation the coordinating properties of the N1/N7 combination in the adenine residue are considered as being similar to those of the N3/N7 combination in the 1-deazaadenine residue. This leads to $\log k^{Cu}_{H\cdot(1d-)PMEA\cdot Cu} = 2.06\pm0.20$ (Table 2, column 3) for $[H\cdot(1d\text{-})PMEA\cdot Cu]^+$, which may directly be compared with $\log K^{Cu}_{Cu(Xao-H)} = 2.58\pm0.03$ [25b] for $Cu(Xao-H)^+$ of xanthosinate, $(Xao-H)^-$, because the acidity constants $pK^H_{H(1d-PMEA)} = 5.49\pm0.02$ (Table 2) and $pK^H_{Xao} = 5.47\pm0.03$ [25b] are identical and because xanthosine is apparently deprotonated at the H(N3) site [20b]. That the stability of $Cu(Xao-H)^+$ is somewhat larger than that of $[H\cdot(1d\text{-})PMEA\cdot Cu]^+$ is not surprising because in the first case the negative charge is on the nucleobase, whereas in the second case it is on the $-PO_2(OH)^-$ residue; furthermore, xanthosine possesses two carbonyl groups which might also participate in metal ion binding to some extent [25b]. Hence, this comparison shows that $\log k^{Cu}_{H\cdot(1d-)PMEA\cdot Cu} = 2.06\pm0.20$ is of the correct order. Moreover, even if this value were as low as 1.83, 50% of Cu^{2+} would still be bound to the nucleobase in $Cu(H;1d\text{-}PMEA)^+$, i.e. $R = 1$ [Eq. (9) and Table 2]. Should it be as large as 2.35 then the species $[H\cdot(1d\text{-})PMEA\cdot Cu]^+$ would occur at 96% [$R = 21.4$; Eq. (9)]; b) Y. Kinjo, R. Tribolet, N. A. Corfù, H. Sigel, *Inorg. Chem.* **1989**, *28*, 1480–1489.

[26] S. S. Massoud, H. Sigel, *Inorg. Chem.* **1988**, *27*, 1447–1453.

[27] H. Sigel, S. S. Massoud, N. A. Corfù, *J. Am. Chem. Soc.* **1994**, *116*, 2958–2971.

[28] a) G. Navon, G. Valensin, *Met. Ions Biol. Syst.* **1987**, *21*, 1–45; b) E. Sletten, N. Å. Frøystein, *ibid.* **1996**, *32*, 397–418.

[29] W. G. Espersen, R. B. Martin, *J. Am. Chem. Soc.* **1976**, *98*, 40–44.

[30] L. G. Marzilli, *Prog. Inorg. Chem.* **1977**, *23*, 255–378.

[31] N. Å. Frøystein, E. Sletten, *Inorg. Chim. Acta* **1987**, *138*, 49–53.

[32] a) The given percentages were calculated with $pK^D_{H(3d-PMEA)} = 8.40$, which was obtained from the equation $pK_{a/D_2O} = pK_{a/H_2O} \times 1.015 + 0.45$ [32b] from the value given in Table 1 for $pK^H_{H(3d-PMEA)}$; b) R. B. Martin, *Science* **1963**, *139*, 1198–1203.

[33] H. Sigel, S. S. Massoud, R. Tribolet, *J. Am. Chem. Soc.* **1988**, *110*, 6857–6865.

[34] H. Sigel, *Experientia* **1966**, *22*, 497–499.

[35] S. S. Massoud, H. Sigel, *Eur. J. Biochem.* **1989**, *179*, 451–458.

[36] a) Adenine may accept three protons in total [20b]: the first at N1 ($pK_a = 4.2$ [20b]), a second one [17] at N7 ($pK_a \approx -0.4$ [36b]) and a final one at N3 ($pK_a \approx -4.2$ [36b]) in very strong acids; b) R. L. Benoit, M. Fréchette, *Can. J. Chem.* **1984**, *62*, 995–1000.

[37] Detailed derivations of the various equations are given in refs. [10] and [12]; here they are summarized only to the extent needed to make the presented results understandable.

[38] H. Sigel, B. Song, *Met. Ions Biol. Syst.* **1996**, *32*, 135–205.

[39] K. Aoki, *Met. Ions Biol Syst.* **1996**, *32*, 91–134; see p. 121.

[40] a) *Zinc and Its Role in Biology and Nutrition* (Ed.: H. Sigel), in *Met. Ions Biol. Syst., Vol. 15*, M. Dekker, New York, Basel, **1983**, pp. 1–493; b) *Handbook on Metals in Clinical and Analytical Chemistry* (Eds.: H. G. Seiler, A. Sigel, H. Sigel), M. Dekker, New York, Basel, Hong Kong, **1994**, pp. 283–297 (Cd) and 667–674 (Zn).

[41] H. Sigel, *ACS Symp. Series* **1989**, *402*, 159–204.

[42] H. Sigel, *Pure Appl. Chem.* **1989**, *61*, 923–932.

[43] a) H. Dvořáková, A. Holý, *Collect. Czech. Chem. Commun.* **1993**, *58*, 1419–1429; b) A. Holý, I. Rosenberg, H. Dvořáková, *ibid.* **1989**, *54*, 2190–2210; c) A. Holý, I. Rosenberg, *ibid.* **1987**, *52*, 2801–2809.

[44] H. Sigel, A. D. Zuberbühler, O. Yamauchi, *Anal. Chim. Acta* **1991**, *255*, 63–72.

0947-6539/97/0309-1536 $ 17.50+.50/0