Extent of intramolecular π stacks in aqueous solution in mixed-ligand copper(II) complexes formed by heteroaromatic amines and the anticancer and antivirally active 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG).[☆] A comparison with related acyclic nucleotide analogues

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A B S T R A C T

The acyclic nucleoside phosphonate (ANP^{2-}) 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG) is anticancer and antivirally active. The acidity constants of the threefold protonated $H_3(PMEG)^+$ were determined by potentiometric pH titrations (aq. sol.; 25°C; I = 0.1 M, $NaNO_3$). Under the same conditions and by the same method, the stability constants of the binary $Cu(H;PMEG)^+$ and Cu(PMEG) complexes as well as those of the ternary ones containing a heteroaromatic N ligand (Arm), that is, of $Cu(Arm)(H;PMEG)^+$ and Cu(Arm)(PMEG), where Arm = 2,2'-bipyridine (Bpy) or 1,10-phenanthroline (Phen), were measured. The corresponding equilibrium constants, taken from our earlier work for the systems with 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) and 9-[2-(phosphonomethoxy)ethyl]-2,6-diamino-purine (PMEDAP) as well as those for Cu(PME) and Cu(Arm)(PME), where PME^{2-} = (phosphonomethoxy)ethane = (ethoxymethyl)phosphonate, were used for comparisons. These reveal that in the monoprotonated ternary $Cu(Arm)(H;PE)^+$ complexes, the proton and $Cu(Arm)^{2+}$ are at the phosphonate group; the ether oxygen of the $-CH_2-O-CH_2-P(O)_2^-$(OH) residue also participates to some extent in $Cu(Arm)^{2+}$ coordination. Furthermore, the coordinated $Cu(Arm)^{2+}$ forms a bridge with the purine moiety undergoing π-π stacking which is more pronounced with $H\cdot PMEDAP^-$ than with $H\cdot PMEA^-$. Most intense is π stack formation (st) with the guanine residue of $H\cdot PMEG^-$; here the bridged form $Cu(Arm)(H\cdot PMEG)^+_{st}$ occurs next to an open (op), unbridged (binary) stack, formulated as $Cu(Arm)^{2+}/(H\cdot PMEG)^-_{op}$. – The unprotonated and neutral ternary Cu(Arm)(PE) complexes are considerably more stable than the corresponding $Cu(Arm)(R\text{-}PO_3)$ species, where $R\text{-}PO_3^{2-}$ represents a phosph(on)ate ligand with a group R that is unable to participate in any intramolecular interaction. The observed stability enhancements are mainly due to intramolecular stack formation (st) between the aromatic rings of Arm and the purine residue in the Cu(Arm)(PE) complexes and also, to a smaller extent, to the formation of five-membered chelates involving the ether oxygen of the $-CH_2-O-CH_2-PO_3^{2-}$ residue (cl/O) of the PE^{2-} species. The quantitative analysis of the intramolecular equilibria reveals three structurally different Cu(Arm)(PE) isomers; e.g., of Cu(Phen)(PMEG) ca. 1.1% exist as $Cu(Phen)(PMEG)_{op}$, 3.5% as $Cu(Phen)(PMEG)_{cl/O}$, and 95% as $Cu(Phen)(PMEG)_{st}$. Comparison of the various

formation degrees reveals that within a given Cu(Arm)(PE) series the stacking tendency decreases in the order $PMEG^{2-} \geq PMEDAP^{2-} > PMEA^{2-}$. Furthermore, stacking is more pronounced in the acyclic Cu(Arm)(PE) complexes compared with that in the Cu(Arm)(NMP) species, where NMP^{2-} = corresponding parent (2'-deoxy)nucleoside 5'-monophosphate. Here is possibly one of the reasons for the biological activity of the ANPs. One is tempted to speculate that the pronounced stacking tendency of $PMEG^{2-}$, together with a different H-bonding pattern, leads to enhanced binding in the active site of nucleic acid polymerases, thus being responsible for the pronounced anticancer and antiviral activity of PMEG.

Keywords:

Anticancer activity

Antivirals

Aromatic-ring stacking

Isomeric equilibria

Nucleotide analogues

1. Introduction[1]

Since the discovery that the human immunodeficiency virus (HIV) is the causative agent of the acquired immunodeficiency syndrome (AIDS) [3], there have been numerous attempts to find remedies against this retrovirus [4,5]; in fact, against viral diseases in general [4–7]. One of the popular and relatively successful classes of compounds are so-called acyclic nucleoside phosphonates (ANPs) [8]. Among these ANPs, especially two series were and still are in the focus; they differ somewhat in the structure of their "alkyl" chain, that is, R-CH_2-CH_2-O-CH_2-PO_3^{2-} versus R-CH_2-CH(CH_2OH)-O-CH_2-PO_3^{2-} [5].

To the second series belongs (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (HPMPC), which can be considered as an analogue of cytidine 5'-monophosphate (CMP^{2-}) and of its 2'-deoxy derivative ($dCMP^{2-}$) [9]. HPMPC, also known as *Cidofovir*, was approved in 1996 for the treatment of cytomegalovirus retinitis in patients with AIDS [10]. However, new antiviral activities of HPMPC and of related analogues are still being discovered [6,7] and various prodrug forms are studied [11–13] which give rise to higher activities due to an increased bioavailability, e.g., against HIV-1 [12], herpesviruses [11] or orthopoxviruses [11].

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[1] Abbreviations: Those of the methoxyphosphonate ligands are defined in Fig. 1 and its legend. -- AMP^{2-} = adenosine 5'-monophosphate; ANP^{2-} = acyclic nucleoside phosphonate; $ANPpp^{4-}$ = diphosphorylated ANP^{2-}; Arm = heteroaromatic nitrogen base (e.g., Bpy or Phen); ATP^{4-} = adenosine 5'-triphosphate; Bpy = 2,2'-bipyridine; CMP^{2-} = cytidine 5'-monophosphate; $dCMP^{2-}$ = 2'-deoxy-CMP^{2-}; $dGMP^{2-}$ = 2'-deoxy-GMP^{2-}; $dNTP^{4-}$ = 2'-deoxy-NTP^{4-}; GMP^{2-} = guanosine 5'-monophosphate; *I* = ionic strength; K_a = acidity constant; M^{2+} = general divalent metal ion, including in part $Cu(Arm)^{2+}$; NTP^{4-} = nucleoside 5'-triphosphate; Phen = 1,10-phenanthroline; R-PO_3^{2-} = simple phosphate monoester or phosphonate ligand with R being a non-interacting residue. Species denoted without a charge either do not carry one or represent the species in general (i.e., independent of their protonation degree); which of the two possibilities applies, is always clear from the context. In formulae like $Cu(Arm)(H;PMEG)^{+}$, the H^+ ion and $PMEG^{2-}$ are separated by a semicolon to facilitate reading, yet they appear within the same parenthesis to indicate that the proton is at the ligand without defining its location (see Sections 2, 3.2, and 3.3).

An example of the first series mentioned above is 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA; see Fig. 1), also known as *Adefovir*, which was launched in 2002 in the form of its oral prodrug form (*Hepsera* or *Preveon*) [14] for the treatment of patients with chronic hepatitis B virus infections [10]. The prodrug form improves the oral bioavailability and the passage of the drug through the cell membranes [15]. Once inside the cell, the acyclic nucleoside phosphonate (ANP) derivatives are diphosphorylated by kinases leading to their active forms, i.e., $ANPpp^{4-}$ [8,16,17]. These analogues of (2'-deoxy)nucleoside 5'-triphosphates ($(d)NTP^{4-}$) [13] serve as excellent alternative substrates [14,18] for RNA or DNA polymerases [8,13,17], acting as terminators at the 3'-end of the growing nucleic acid chain [10,13] due to the lack of a 3'-OH group. The above also indicates the advantage of the ANPs, which only need to be diphosphorylated in contrast to nucleoside analogues like, e.g., the widely studied [19] *Acyclovir*, guanine(N9)-CH_2-O-CH_2CH_2-OH, where three phosphorylation steps are needed [10].

Closely related to PMEA is 9-[2-(phosphonomethoxy)ethyl]-2,6-diaminopurine (PMEDAP; see Fig. 1), which is about as antivirally active as PMEA [20]. Interestingly, 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG; see Fig. 1) is even more active than PMEA, but it is also more cytotoxic, thus giving a poorer selectivity index [5,21]. However, the cytotoxic effect of PMEG has been evaluated for its application as antitumor agent [22]. In fact, it is active against human cervical cancer cells (*in vitro* and *in vivo*) [23] and non-Hodgkin's lymphoma [24]. Its hexadecyloxypropyl ester also inhibits ocular cell proliferation [25]. Furthermore, it needs to be mentioned that (N6)-substituted 2,6-diaminopurine analogues are metabolized to the corresponding guanine counterparts by N6-methyl-AMP aminohydrolase; thus, these compounds can be considered as prodrugs of PMEG [26]. Taking all this together, it is evident that the molecular properties of PMEG (Fig. 1) [27–31] warrant detailed studies. As a side remark one may mention that derivatives of guanosine and isoguanosine, due to their hydrogen-bonding and π-π stacking properties are used in supramolecular devices [32].

insert Fig. 1 close to here

In all instances the ANP drug is active, as mentioned above, in the diphosphorylated $ANPpp^{4-}$ form, where it constitutes a substrate as an NTP^{4-} analogue for the polymerase. Two metal ions are involved in the mechanism of the polymerase reaction [33]. One of them needs to

be coordinated to the β,γ-phosphate unit and the other to the α-phosph(on)ate group [34–37] to promote the transfer of a nucleotidyl residue [14,18,38]. To achieve this M(α)-M(β,γ) coordination mode, the substrate needs to be correctly positioned in the active-site cavity of the enzyme, and here H-bonding and especially aromatic-ring stacking are expected to be important. In fact, π-π interactions are of relevance in recognition reactions of protein-nucleic acid adducts and they occur, e.g., between an indole residue of a protein-tryptophan and an adenine-RNA moiety in a peptide-RNA complex from a bacteriophage [39,40]. Another example is a zinc finger-RNA adduct with an indole residue intercalated between two guanine moieties [40,41].

Since it is known [42] that substituents at one aromatic ring may directly participate in the stacking interactions with the other, it is of general interest to probe and to compare the stacking properties of the three purine ANPs shown in Fig. 1, and to see what kind of differences, if any, occur. Of course, the substituents at an aromatic-ring system may also contribute to the orientation and stability of the π-stacks, e.g., via CH/π and/or NH/π interactions [43]. As shown over the years, the heteroaromatic N-ligands (Arm), i.e., 2,2'-bipyridine (Bpy) and 1,10-phenanthroline (Phen), have proven very helpful as indicators [44] for evaluating the stacking capabilities of aromatic residues in metal ion complexes [45] of amino acids [46,47] and nucleotides [45,46,48–50]. As bridging link between the two ligands containing aromatic moieties, most often Cu^{2+} was employed [1,2,45,49–51] due to its biological relevance on the one hand [52] and the high stability of the $Cu(Arm)^{2+}$ species [53] on the other. In the present study we have used the same basic 'units' plus the three purine-ANPs seen in Fig. 1.

2. Experimental

Twofold protonated 9-[2-(phosphonomethoxy)ethyl]guanine ($H_2(PMEG)^{\pm}$; Fig. 1) was synthesized according to published procedures [5,54]. All the other reagents were the same as used previously in recent studies [1,2], and the stock solutions were prepared as described [2,55,56]. This also applies to the equipment employed in the potentiometric pH titrations and their evaluations [57], as well as for the experimental procedures in general [55–57], including the determination of the concentration of the NaOH, ligand, and Cu^{2+} stock solutions [58].

The Metrohm instruments were calibrated with buffer solutions and the direct pH meter readings were used in the calculations [56,57] of the acidity constants [55]. These constants of $H_2(PMEG)^{\pm}$ regarding the Equilibria (2a), (3a), and (4a) (*vide infra* in Section 3.1) were measured as described [2] (see also [50]). Because of the direct pH meter reading these constants determined at I = 0.1 M ($NaNO_3$) and 25°C are so-called practical, mixed, or Brønsted constants [59,60]. They may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values [59].

The stability constants $K^{M}_{M(H;PMEG)}$ (Eq. (5)) and $K^{M}_{M(PMEG)}$ (Eq. (6)) (see Section 3.2), where M^{2+} = Cu^{2+}, $Cu(Bpy)^{2+}$ or $Cu(Phen)^{2+}$, of the binary $Cu(H;PMEG)^{+}$ and Cu(PMEG) complexes, as well as of the ternary $Cu(Arm)(H;PMEG)^{+}$ and Cu(Arm)(PMEG) complexes were determined as described recently [2]. The same conditions as used for the determination of the acidity constants were also applied now, but $NaNO_3$ was partly replaced by $Cu(NO_3)_2$/Arm (25°C; I = 0.1 M); for details see Ref. [2]. The individual results for the stability constants showed no dependence on pH or on the excess of metal ion concentrations used. The results are in each case the average of at least six independent pairs of titrations.

Finally, in studies devoted to intramolecular π-π stacks it is important to ascertain that the self-association of the individual reactants is negligible. With $[Cu(Phen)^{2+}]$ = 1.67 to 3.33 mM, as employed here, this goal is achieved because more than 98% of $Cu(Phen)^{2+}$ are present in the monomeric form [2]. For $Cu(Bpy)^{2+}$ this is even more true [61]. Similarly, for AMP^{2-} and related adenine derivatives at concentrations of 0.3 mM about 99.9% are present in their monomeric form [2]. Because the self-stacking tendency of guanines is about half of that of adenines [62] the given 99.9% of monomer are a lower limit for $PMEG^{2-}$. To conclude, the results presented in this study clearly refer to monomeric species.

3. Results and discussion

3.1. Acid-base properties of $H_3(PMEG)^{+}$ and of some related species

The acyclic nucleotide analogue $PMEG^{2-}$ can accept two protons at the phosphonate group

and one more at the N7 site, but it can also release a proton from the (N1)H unit of the guanine residue (Fig. 1), giving thus rise to the following four deprotonation reactions (Eqs. (1)–(4)).

$$H_3(PMEG)^+ \rightleftharpoons H_2(PMEG)^{\pm} + H^+ \quad (1a)$$

$$K^{H}_{H_3(PMEG)} = [H_2(PMEG)^{\pm}][H^+]/[H_3(PMEG)^+] \quad (1b)$$

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

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

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

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

$$PMEG^{2-} \rightleftharpoons (PMEG - H)^{3-} + H^+ \quad (4a)$$

$$K^{H}_{PMEG} = [(PMEG - H)^{3-}][H^+]/[PMEG^{2-}] \quad (4b)$$

insert Table 1 close to here

The corresponding pK_a values of these acidity constants are listed in entry 2 of Table 1 [57,59,63–69]. Comparison with the values of 9-ethylguanine (entry 1) and methylphosphonate (entry 3) or (phosphonomethoxy)ethane (entry 4) reveals that the first proton of $H_3(PMEG)^+$ is released from the twofold protonated phosphonate group, i.e., from $P(O)(OH)_2$, followed by the proton of the $(N7)H^+$ site and the second proton from the $P(O)_2^-(OH)$ group. The release of the final proton according to Equilibrium (4a) from the (N1)H unit of $PMEG^{2-}$ takes place with pK_a = 9.34 (Table 1, footnote "e") and it is thus beyond the pK_a/pH range studied in this work because of the hydrolysis of Cu^{2+}_{aq}. However, it is interesting to note here that the binary stacks of (Phen)(Guo) and $(Phen)(Guo - H)^-$ have, within the error limits, the same stability (K = 42 ± 6 M^{-1} (2σ) [44]) despite the negatively charged $(N1)^-$ in the second adduct.

Entries 4 and 5 of Table 1 referring to (phosphonomethoxy)ethane and 9-methyladenine allow an unequivocal site attribution for the deprotonation reactions of $H_3(PMEA)^+$ and $H_3(PMEDAP)^+$ (see Fig. 1); the first proton being released from $P(O)(OH)_2$ followed by the one from $(N7)H^+$. The third and final proton is released from the $P(O)_2^-(OH)$ group. Both acyclic nucleoside phosphonates are used in the following for comparisons regarding their stacking

tendencies with those in adducts containing $PMEG^{2-}$.

3.2. Stabilities of the Cu(Arm)(H; PMEG)$^+$ and Cu(Arm)(PMEG) complexes

The experimental data of the potentiometric pH titrations of the M^{2+}/PE systems, where M^{2+} = Cu^{2+}, $Cu(Bpy)^{2+}$ or $Cu(Phen)^{2+}$ and PE^{2-} = $PMEG^{2-}$ or one of the ANPs seen in Fig. 1, can be fully described by considering the acidity constants of $H_2(PE)^{\pm}$ (Eqs. (2) and (3)) as well as Equilibria (5a) and (6a):

$$M^{2+} + H(PE)^- \rightleftharpoons M(H;PE)^+ \quad (5a)$$

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

$$M^{2+} + PE^{2-} \rightleftharpoons M(PE) \quad (6a)$$

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

Of course, the evaluation of the experimental data ought not to be carried into the pH range where formation of hydroxo complexes occurs, which is evident from titrations of M^{2+} in the absence of ligand. Since the stabilities of the $Cu(Bpy)^{2+}$ and $Cu(Phen)^{2+}$ complexes are very high [53], their formation is practically complete under the experimental conditions (Ref. [2] and Section 2). Moreover, in formulas like $M(H;PE)^+$ the H^+ and PE^{2-} are separated by a semicolon to facilitate reading, yet they appear within the same parenthesis to indicate that the proton is at the ligand without defining its location.

A further important point is to note that Equilibria (5a) and (6a) are connected via Equilibrium (7a); the corresponding acidity constant (Eq. (7b)) can be calculated with Eq. (8):

$$M(H;PE)^+ \rightleftharpoons M(PE) + H^+ \quad (7a)$$

$$K^{H}_{M(H;PE)} = [M(PE)][H^+]/[M(H;PE)^+] \quad (7b)$$

$$pK^{H}_{M(H;PE)} = pK^{H}_{H(PE)} + \log K^{M}_{M(H;PE)} - \log K^{M}_{M(PE)} \quad (8)$$

The constants for Equilibria (5a), (6a), and (7a) are listed in columns 3, 4, and 5 of Table 2 for the complexes of PMEG, respectively, together with some related data for other PE ligands

[2,57,66,70]. The stability constants for the binary $Cu(H;PMEG)^+$ and Cu(PMEG) complexes will be mainly discussed elsewhere in a different context. Herein we focus on the properties of the ternary complexes and use the data for the binary ones only for comparison, where needed.

insert Table 2 close to here

It may be added that the present evaluation and description of the various PMEG complexes is done in analogy to the recently described situation for several purine- and pyrimidine-nucleotide analogues [1,2], but now, of course, the guanine residue is in the focus.

3.3. Where is the proton located in Cu(Arm)(H;PMEG)$^+$?

Evidently metal ion coordination must give rise to a significant acidification due to charge repulsion between H^+ and Cu^{2+}. A comparison between $pK^{H}_{H_2(PMEG)} = 3.35$ (Table 1, entry 2, column 4) which is due to $(N7)H^+$ deprotonation, and $pK^{H}_{Cu(Arm)(H;PMEG)}$ = ca. 4.9 (Table 2, entries 2b and 2c, column 5) shows that $(N7)H^+$ in $H_2(PMEG)^{\pm}$ is more acidic than $Cu(Arm)(H;PMEG)^+$, consequently the proton cannot be located at N7 but must be at the phosphonate group, which is expressed by writing $Cu(Arm)(H{\cdot}PMEG)^+$ or generally $Cu(Arm)(H{\cdot}PE)^+$. This conclusion agrees with $pK^{H}_{H(PMEG)} = 6.86$ (Table 1, entry 2, column 6), which is due to the monoprotonated phosphonate group, and $pK^{H}_{Cu(Arm)(H;PMEG)}$ = ca. 4.9 for the complexes; this corresponds to an acidification of about 2 pK units at the $P(O)_2^-(OH)$ group.

Of course, the above insight still leaves open the question: Where is $Cu(Arm)^{2+}$ located? There are three possibilities: (i) $Cu(Arm)^{2+}$ is together with H^+ at the phosphonate residue. (ii) It is coordinated at N7 of the purine residue. (iii) $Cu(Arm)^{2+}$ forms a stack with the guanine residue and this is the driving force for the formation of the $Cu(Arm)(H{\cdot}PMEG)^+$ species. Certainly, the actual situation could also be a combination of the three possibilities. In any case, it is evident that the situation is a difficult one. Therefore, we shall first have a look at the stability constants of the complexes assembled in Table 2, and thereafter we continue to evaluate the stabilities and structures of the Cu(Arm)(PMEG) complexes. Maybe these evaluations provide some hints about the situation in the $Cu(Arm)(H{\cdot}PMEG)^+$ species.

3.4. Comparison of the stability constants of the various complexes considered

Three general observations follow from the data in Table 2 and warrant mentioning:

(i) The basicity of the PO_3^{2-} group of $PMEG^{2-}$ and $PMEDAP^{2-}$ is quite similar (Table 1, entries 2 & 7, column 6) as is the stability of their Cu(Arm)(PE) complexes (Table 2, entries 2b/2c and 3b/3c; column 4). This contrasts with the somewhat lower stability of the Cu(Arm)(PMEA) complexes, even though the PO_3^{2-} basicity is very similar again. This leads tentatively to the impression that the shape of the ANP has an influence; indeed, if stack formation should turn out to be important, this would make sense.

(ii) Similarly, the basicities of the $P(O)_2^-(OH)$ group of $H(PMEG)^-$, $H(PMEDAP)^-$, and $H(PMEA)^-$ are quite alike (Table 1; column 3), yet the stabilities of the monoprotonated complexes decrease in the order $Cu(Arm)(H{\cdot}PMEG)^+ > Cu(Arm)(H{\cdot}PMEDAP)^+ > Cu(Arm)(H{\cdot}PMEA)^+$ (Table 2). This order cannot be due to the basicity of the neutral purine residue, because this is lowest in the guanine one (Table 1, columns 4 and 5). Hence, this observation indicates indirectly again that stack formation may be of relevance.

(iii) A comparison of the stability of the binary complexes (Table 2, entries 2a, 3a, and 4a; columns 3 and 4) indicates that $Cu(H{\cdot}PMEG)^+$ and Cu(PMEG) are especially stable. This might be due to pronounced macrochelate formation of the phosphonate-coordinated Cu^{2+} with the N7[(C6)O] site; an interaction which is sterically inhibited in complexes of PMEDAP and PMEA due to $(C6)NH_2$. Indeed, it is known that a nucleobase-C(amino) group is sterically more demanding than a carbonyl substituent [71]; in fact, the latter is also well suited to participate in M^{2+} binding either outersphere via H-bonds or directly by innersphere coordination [72,73].

3.5. Indication for an increased stability of the mixed ligand Cu(Arm)(PMEG) complexes

Already above in point (i) of Section 3.4 we have seen that the ternary Cu(Arm)(PMEG) complexes appear to be especially stable. Indeed, Equilibrium (9a) represents a way to describe the stability of ternary complexes on the basis of their parent binary complexes [74]:

$$Cu(Arm)^{2+} + Cu(PMEG) \rightleftharpoons Cu(Arm)(PMEG) + Cu^{2+} \qquad (9a)$$

$$10^{\Delta \log K_{Cu}} = [Cu(Arm)(PMEG)][Cu^{2+}]/([Cu(Arm)^{2+}][Cu(PMEG)]) \quad (9b)$$

The corresponding equilibrium constant (Eq. (9b)) follows from Eq. (10):

$$\Delta \log K_{Cu} = \log K^{Cu(Arm)}_{Cu(Arm)(PMEG)} - \log K^{Cu}_{Cu(PMEG)} \quad (10)$$

According to the general rule for complex stabilities, i.e., $K_1 > K_2$, Equilibrium (9a) is expected to be on its left side with negative values for $\Delta \log K_{Cu}$. Indeed, statistical considerations predict for $\Delta \log K_{Cu}$ a value of about –0.5 for the coordination of a monodentate ligand to $Cu(Arm)^{2+}$ [75]. However, for simple phosph(on)ates $\Delta \log K_{Cu}$ equals about 0.03 [70,76], in agreement with results obtained for mixed ligand complexes composed of a bidentate heteroaromatic amine and an O-donor site [74,77]. This enhanced stability was attributed to π back-bonding from the metal ion to the aromatic N-ligand, and this in turn favors the polar O-donor binding [77,78].

Comparing the above with the results listed in column 6 of Table 2 shows that $\Delta \log K_{Cu}$ has a positive sign and is also larger than expected for all the ternary complexes, indicating that Equilibrium (9a) is shifted towards its right side. One may add that in the Cu(Arm)(PME) complexes, $\Delta \log K_{Cu}$ being about 0.15 (Table 2, entry 1), the ether oxygen atom (see Fig. 1) participates in metal ion binding in about 75% of the species [70], that is, PME^{2-} acts to a large extent as a bidentate ligand (see Section 3.7 and Ref. [1]). From this follows that in all the other Cu(Arm)(PE) systems, which have a nucleobase residue and a $\Delta \log K_{Cu}$ value larger than about 0.15 log units, the increased stability needs mainly to be attributed to stack formation.

A more detailed comparison of the $\Delta \log K_{Cu}$ values in Table 2 reveals that the $\Delta \log K_{Cu}$ values of 0.30 and 0.59 for the Cu(Bpy)(PMEG) and Cu(Phen)(PMEG) systems, respectively, are relatively small. The reason is that the binary Cu(PMEG) is especially stable (see also point (iii) in Section 3.4); in fact, it is about 1.4 log units more stable than is expected for a simple Cu^{2+} coordination at PO_3^{2-} (calculated with Eq. (11) in Section 3.6, *vide infra*). This then leads to a distorted and misleading view about the stability of the Cu(Arm)(PMEG) complexes.

In other words, though the described results are helpful, the discussed PMEG example reveals also the great shortcoming inherent in the use of Equilibrium (9a) for the quantification of the present type of mixed ligand complexes. Because this quantification rests also on the stability of the binary complexes (Eq. (10)), which themselves may already have an increased

stability, thus affecting the size of $\Delta \log K_{Cu}$, the result will often not be a true reflection of the increased stability of the ternary complex considered.

3.6. Proof of an increased stability for the Cu(Arm)(PMEG) complexes

An alternative way to evaluate the stability of ternary complexes, independently of the properties of the binary ones, rests in the present case for Cu(Arm)(R-PO_3) complexes on the previously established [70,79] straight-line correlations [80] for $\log K^{Cu(Arm)}_{Cu(Arm)(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ plots (Eqs. (12) and (13)). That is, the following evaluation procedure follows closely previous ones [1,2,55]. In these plots R-PO_3^{2-} represents phosphate monoesters or phosphonate ligands, in which the residue R is unable to interact with $Cu(Arm)^{2+}$ [70]. The corresponding straight-line parameters for Cu(R-PO_3) (Eq. (11)) [66,79] are given for comparison:

$$\log K^{Cu}_{Cu(R\text{-}PO_3)} = 0.465 \times pK^{H}_{H(R\text{-}PO_3)} - 0.015 \quad (11)$$

$$\log K^{Cu(Bpy)}_{Cu(Bpy)(R\text{-}PO_3)} = 0.465 \times pK^{H}_{H(R\text{-}PO_3)} + 0.009 \quad (12)$$

$$\log K^{Cu(Phen)}_{Cu(Phen)(R\text{-}PO_3)} = 0.465 \times pK^{H}_{H(R\text{-}PO_3)} + 0.018 \quad (13)$$

The error limits of log stability constants calculated with given $pK^{H}_{H(R\text{-}PO_3)}$ values and Eqs. (11), (12), and (13) are ±0.06, ±0.07, and ±0.06 (3σ), respectively, in the pH range 5–8 [66,70,79]. Relation (11) results from equilibrium constants determined for eight different phosphate monoester/phosphonate ligands [66]. Equations (12) and (13) are based on constants measured for the ternary Cu(Arm)(R-PO_3) complexes, where R-PO_3^{2-} = D-ribose 5-monophosphate, methanephosphonate, and ethanephosphonate [70]. On these $\log K^{Cu(Arm)}_{Cu(Arm)(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ plots, also the data points measured for the corresponding methyl phosphate systems [76] fit, confirming the reliability of the plots.

insert Figure 2 close to here

The reference lines as defined by Eqs. (12) and (13) are seen in Fig. 2 [50,70,79,81], where the stability constants $\log K^{Cu(Arm)}_{Cu(Arm)(PE)}$ (Eq. (6)) versus the acidity constants $pK^{H}_{H(PE)}$ (Eq. (3)) for the PME^{2-}, $PMEG^{2-}$, $PMEDAP^{2-}$, and $PMEA^{2-}$ systems of Fig. 1 are also plotted. The data points for $dGMP^{2-}$ are given for comparison. The data points for all 10 ternary complexes (see

also Table 2) are far above their reference lines, proving an increased stability. This must mean that in the Cu(Arm)(PME) systems the ether oxygen participates in metal ion binding and further that in the Cu(Arm)(PMEG), Cu(Arm)(PMEDAP), and Cu(Arm)(PMEA) systems the nucleobase moiety is responsible for the additionally observed stability enhancements, most likely via aromatic π-π stacking (Fig. 3) (see Section 3.7) as already indicated above.

insert Fig. 3 close to here

The vertical differences seen in Fig. 2 (broken lines) between the data points of the ternary systems and their reference lines, can be defined according to Eq. (14):

$$\log \Delta_{\mathrm{Cu/Arm/PE}} = \log K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)}} - \log K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)op}} \quad (14a)$$

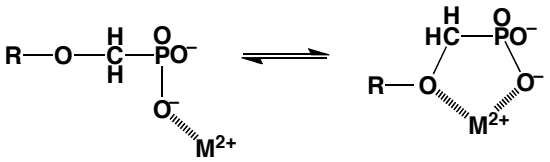
$$= \log K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)exp}} - \log K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)calcd}} \quad (14b)$$

Equations (12) and (13) allow calculating the stability constants for the Cu(Arm)(PE) species in which the metal ion is coordinated solely to the phosphonate group. This species is designated as the 'open' (op) isomer, i.e., $\mathrm{Cu(Arm)(PE)_{op}}$. These calculated (calcd) constants can now be compared with those experimentally (exp) measured (Eq. (6)) leading to Eq. (14). The expressions $\log K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)calcd}}$ (Eq. (14b)) and $\log K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)op}}$ (Eq. (14a)) are synonymous as are $\log K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)}}$ (Eqs. (6, 14a)) and $\log K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)exp}}$ (Eq. (14b)). With Eq. (14) the stability enhancements due to any further interactions, next to the metal ion-phosphonate coordination, can be unequivocally defined. It follows (see also Fig. 2) that the phosphonate group of all the $\mathrm{PE^{2-}}$ species (Fig. 1) is the primary metal ion-binding site. The results of the indicated calculations are summarized in columns 3–5 of Table 3 (see below in Section 3.8).

3.7. Structures and possible isomeric equilibria of the ternary Cu(Arm)(PMEG) complexes

Which additional interactions or binding sites, next to that of the $\mathrm{PO_3^{2-}}$ group, may occur or are possible in metal ion complexes with $\mathrm{PE^{2-}}$ ligands? The most obvious case is the enhanced stability observed for the Cu(Arm)(PME) complexes (Fig. 2) because here in addition only an ether-oxygen interaction can occur. Of course, exactly the same interaction, giving rise to five-membered chelates, may as well take place with $\mathrm{PMEG^{2-}}$ containing a guanine residue (see Fig.

1) as well as with the other PMEs having a purine residue; hence, the following intramolecular Equilibrium (15) between an 'open' (op) and a chelated or 'closed' (cl) isomer, i.e., $Cu(Arm)(PE)_{op}$ and $Cu)Arm)(PE)_{cl/O}$, respectively, needs to be considered:



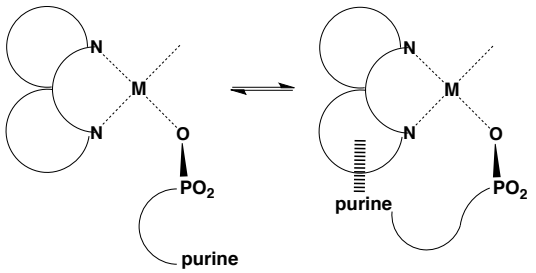
(15)

The position of this equilibrium is defined by the dimensionless constant $K_{I/O}$ (Eq. (16)):

$$K_{I/O} = [Cu(Arm)(PE)_{cl/O}]/[Cu(Arm)(PE)_{op}] \quad (16)$$

The formation of the five-membered chelates involving the ether oxygen occurs certainly within the equatorial part of the coordination sphere of Cu^{2+} because only weak interactions are possible with the apical positions [82], and the stability increase as expressed for Cu(Arm)(PME) by log $\Delta_{Cu/Arm/PME}$ with ca. 0.6 (Eq. (14)) is substantial (Fig. 2). Quite generally, one may add that interactions with ether oxygen atoms or hydroxyl groups are especially favored if via a primary binding site five-membered chelates are formed [64,83].

Since the ternary Cu(Arm)(PMEG) complexes, compared with Cu(Arm)(PME), show further significant stability enhancements (Fig. 2), the guanine moiety (Fig. 1) must also be involved; most likely due to stack formation as indicated already above and as found for the Cu(Arm)(PMEA) [2,70] and Cu(Arm)(PMEDAP) species [2]. Indeed, stack formation between Arm and purine residues is well-known [44]. Hence, Equilibrium (17) is expected to occur.



(17)

The corresponding intramolecular and dimensionless constant $K_{I/st}$ is defined in Eq. (18):

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

$Cu(Arm)(PE)_{op}$ represents the open (op) isomer and $Cu(Arm)(PE)_{st}$ the stacked (st) one.

The question at this point is: Is the observed “extra” stability enhancement (Fig. 2), next to

an intramolecular π-π stacking, possibly in part also due to an interaction of $Cu(Arm)^{2+}$ with an imidazole- or pyridine-type N-atom? At a guanine residue (N1)H is not of relevance as long as it is not deprotonated. Regarding the N3 site one must note that from *o*-aminopyridine and related ligands it is known that M^{2+} binding at the pyridine N is strongly inhibited by a neighboring NH_2 group; for Cu^{2+} the inhibition amounts to about 1.4 log units [84,85]. Furthermore, due to the bulky size of Arm in $Cu(Arm)^{2+}$ a coordination at N3 is further inhibited and therefore not observed in complexes with a 2-aminopurine residue, like in Cu(Arm)(PME2AP), where PME2AP = 9-[2-(phosphonomethoxy)ethyl]-2-aminopurine [55]. Therefore, such an interaction is also not of relevance for Cu(Arm)(PMEG).

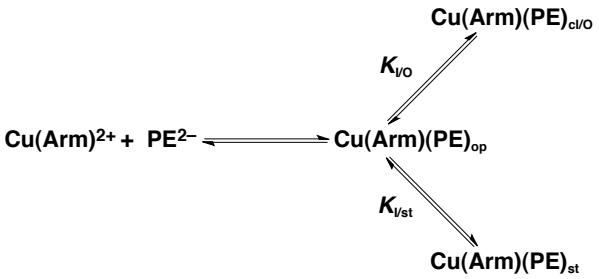
Next, one has to ask: What about the imidazole-type N7? N7 is a well-known binding site for metal ions in complexes of nucleotides and related ligands [37,85–87]. Is this site relevant for the Cu(Arm)(PMEG) species? In the Cu(Arm)(PME2AP) complexes this site is best accessible among all the Cu(Arm)(PE) species because there is no substituent at C6. Ignoring the carbonyl oxygen at C6 in PMEG, because in Cu(Arm)(PMEG) there is no equatorial Cu^{2+} site for its coordination left, the situation in Cu(Arm)(PME2AP) mimics the one in Cu(Arm)(PMEG) well. Furthermore it is well-known that the positive and negative effects of a (C)O unit are small [71,72]. For Cu(Arm)(PME2AP) it was recently concluded [55] by comparing the coordination tendency of Cu^{2+} towards N7 with the inhibition due to the bulky Phen ligand that the N7 site is of no relevance because the indicated difference amounts to –0.17 ± 0.24 log unit. The corresponding evaluation for Cu(Bpy)(PME2AP) gave the difference of –0.33 ± 0.25 log unit [55]. This then means that there is no remarkable affinity of a phosphonate-coordinated $Cu(Arm)^{2+}$ toward N7 of $PME2AP^{2-}$ left [55]. Of course, this result holds for other ternary systems as well, like those containing $PMEG^{2-}$, $PMEA^{2-}$ [2] or $PMEDAP^{2-}$ [2].

From the above reasonings, it follows that next to Equilibrium (15), only Equilibrium (17) with the intramolecular π-π stack formation between the aromatic rings of Bpy or Phen and the purine residue is crucial. About this latter interaction a few more comments are needed: Space-filling molecular models reveal [70] that a purine residue of an ANP^{2-} like $PMEA^{2-}$ or $PMEG^{2-}$, if equatorially chelated to $Cu(Arm)^{2+}$ via the phosphonate group and the ether oxygen, cannot stack well with the aromatic rings of the also equatorially coordinated Arm; a substantial and

strain-free overlap of the aromatic ring systems is possible only if the ether oxygen is not equatorially coordinated to Cu^{2+}. This latter situation is depicted in Fig. 3. Yet, from molecular models [70] it appears that an apical ether-oxygen coordination and simultaneous stack formation might be compatible with each other in the Cu(Arm)(PMEG) species. Hence, various intramolecularly stacked Cu(Arm)(PMEG) complexes are possible, including those with somewhat different orientations of the aromatic rings towards each other. As there is at present no way to distinguish these various isomers and conformers in solution from each other, we treat all the stacked species together and designate them as $Cu(Arm)(PMEG)_{st}$ or generally speaking as $Cu(Arm)(PE)_{st}$. Note, stack formation has not only been proven in the present indirect manner via stability considerations but also directly by spectroscopic methods [44,45,49–51,88,89]. Moreover, intramolecular stack formation between a pyridyl group and a purine ring system occurs in the ternary, dimeric Cu^{2+} complex formed by 2,2'-bipyridine and monoprotonated adenosine 5'-monophosphate as shown for the solid state in a crystal structure study [90].

3.8. Evaluation procedure regarding the intramolecular equilibria involving three isomeric Cu(Arm)(PMEG) species

From the reasonings in the preceding Section 3.7 it follows that Equilibria (15) and (17) are the relevant ones. Hence, these conclusions give rise to the Equilibrium Scheme (19):



(19)

The upper branch reflects Equilibrium (15) and its constant $K_{I/O}$ (Eq. (16)), while the lower branch corresponds to Equilibrium (17) and its dimensionless constant $K_{I/st}$ (Eq. (18)) (see also Fig. 3). Of course, the stability of the "open" isomer, $Cu(Arm)(PE)_{op}$, is defined by Eq. (20):

$$K^{Cu}_{Cu(Arm)(PE)op} = \frac{[Cu(Arm)(PE)]}{[Cu(Arm)^{2+}][PE]} \qquad (20)$$

A situation as described in the Equilibrium Scheme (19) involving three isomeric complexes has previously been analyzed [1,2,64,89,91] and, therefore, below only those equations are given which are needed to understand the interrelations.

Based on Scheme (19) the experimentally accessible equilibrium constant (6b) can be reformulated as in Eq. (21a) and application of Eqs. (16), (18), and (20) leads to Eq. (21b):

$$K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)}} = \frac{[\mathrm{Cu(Arm)(PE)_{op}}] + [\mathrm{Cu(Arm)(PE)_{cl/O}}] + [\mathrm{Cu(Arm)(PE)_{st}}]}{[\mathrm{Cu(Arm)^{2+}}][\mathrm{PE^{2-}}]} \quad (21a)$$

$$= K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)op}} (1 + K_{\mathrm{I/O}} + K_{\mathrm{I/st}}) \quad (21b)$$

From Eq. (14) and Eq. (21) follows Eq. (22), where $\mathrm{Cu(Arm)(PE)_{int/tot}}$ refers to the sum of all the species with an intramolecular (int) interaction:

$$K_{\mathrm{I/tot}} = \frac{K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)}}}{K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)op}}} - 1 \quad (22a)$$

$$= 10^{\log \Delta_{\mathrm{Cu/Arm/PE}}} - 1 \quad (22b)$$

$$= \frac{[\mathrm{Cu(Arm)(PE)_{int/tot}}]}{[\mathrm{Cu(Arm)(PE)_{op}}]} \quad (22c)$$

$$= \frac{[\mathrm{Cu(Arm)(PE)_{cl/O}}] + [\mathrm{Cu(Arm)(PE)_{st}}]}{[\mathrm{Cu(Arm)(PE)_{op}}]} \quad (22d)$$

$$= K_{\mathrm{I/O}} + K_{\mathrm{I/st}} \quad (22e)$$

Evidently, the sum of all dimensionless intramolecular equilibrium constants, $K_{\mathrm{I/tot}}$, can be calculated via Eq. (14) by Eq. (22b). This allows to obtain values for $\mathrm{Cu(Arm)(PE)_{int/tot}}$ and thus also for $\mathrm{Cu(Arm)(PE)_{op}}$. Once $K_{\mathrm{I/tot}}$ is known, the percentage of the total amount of species with an intramolecular interaction, i.e., $\mathrm{Cu(Arm)(PE)_{int/tot}}$, can be calculated according to Eq. (23):

$$\%\ \mathrm{Cu(Arm)(PE)_{int/tot}} = 100 \times K_{\mathrm{I/tot}}/(1 + K_{\mathrm{I/tot}}) \quad (23)$$

Furthermore, in those cases where the stacked species, i.e., $\mathrm{Cu(Arm)(PE)_{st}}$, are not formed, the above equations reduce to the two-isomer problem seen in Equilibrium (15) and $K_{\mathrm{I/tot}}$ equals then $K_{\mathrm{I/O}}$. The results for $K_{\mathrm{I/tot}}$ and the connected data regarding the Cu(Arm)(PE) systems, where $\mathrm{PE^{2-}} = \mathrm{PMEG^{2-}}$, $\mathrm{PMEDAP^{2-}}$ or $\mathrm{PMEA^{2-}}$, as well as the corresponding ones for $K_{\mathrm{I/O}}$ referring to the Cu(Arm)(PME) complexes, are listed in Table 3.

insert Table 3 close to here

The results obtained for the Cu(PME) systems (Table 3, entry 1) show that the formation degree of the 5-membered chelate in Equilibrium (15) is rather high with about 75%. However, for the three other Cu(Arm)(PE) systems which contain a nucleobase residue, it is also clear that $K_{I/tot} >> K_{I/O}$ (cf. the values of entry 1 in column 6 with the other ones in the same column); therefore, it is not surprising that the total amount of species with an intramolecular interaction amounts to about 98% in these instances.

3.9. Formation degrees of the three isomeric Cu(Arm)(PMEG) complexes and of related isomers

A more detailed evaluation regarding Equilibrium Scheme (19) is possible based on the structural identity of the crucial ligand parts of PME^{2-} and the PME derivatives containing a nucleobase residue (Fig. 1). If one makes the justified assumption that $Cu(Arm)(PME)_{cl/O}$ and $Cu(Arm)(PE)_{cl/O}$, where PE^{2-} = $PMEG^{2-}$, $PMEDAP^{2-}$ or $PMEA^{2-}$, have the same stability, the intramolecular equilibrium constant $K_{I/O}$ given in Table 3 (entry 1; column 6) can also be applied here and then, according to Equation (22e), a value for $K_{I/st}$ results. Now the formation degrees of all isomeric species of Equilibrium (19) can be calculated. The results for the three mentioned PE^{2-} ligands are given in Table 4 together with related data for some NMP^{2-} systems [50,92,93].

insert Table 4 close to here

At this point many conclusions may be drawn from the data in Table 4; some are to follow:

(i) All three isomeric complexes of Equilibrium (19) are formed in aqueous solution in appreciable yet rather different amounts in the six Cu(Arm)(ANP) complexes.

(ii) The stacked $Cu(Arm)(ANP)_{st}$ species (Fig. 3) clearly dominate, reaching formation degrees between about 85 and 95%.

(iii) Consequently, the formation degrees of the 5-membered chelates (Eq. (15)) involving the ether oxygen are suppressed to about 10% or less, compared with the approximately 75% present in the Cu(Arm)(PME) systems (Table 4; column 8).

(iv) The $K_{I/st}$ values of the Bpy systems (entries 2b to 4b) are always lower by a factor of ca. 0.5 (within the error limits), compared to those of the Phen systems (entries 2c to 4c). This is

understandable because the overlap of the aromatic ring systems is expected to be more pronounced with the tricyclic Phen than with the bicyclic Bpy.

(v) Interestingly, the ANPs $PMEG^{2-}$ and $PMEDAP^{2-}$ have a very similar shape (Fig. 1) and indeed, the values for % $Cu(Arm)(PE)_{st}$ as well as the corresponding $K_{I/st}$ values are within their error limits the same (see entries 2 and 3 of Table 4). In contrast, $PMEA^{2-}$ (entry 4), which has no substituent at N2, forms the stacked Cu(Arm)(PMEA) isomers with a somewhat lower stability (see the $K_{I/st}$ values) which is in accord with the participation of substituents in stacking interactions [42].

(vi) The entries given in the lower part of Table 4 lead to the observation that stacking in the complexes containing an acyclic nucleotide analogue is more pronounced than in the complexes of the corresponding parent nucleotides; hence, the stabilities are $Cu(Arm)(PMEG)_{st}$ > $Cu(Arm)(dGMP)_{st}$ and $Cu(Arm)(PMEA)_{st}$ > $Cu(Arm)(AMP)_{st}$, as is best seen from the $K_{I/st}$ values in column 7. This result is most likely due to the rigidity of the ribose ring, compared to the acyclic situation in $PMEG^{2-}$ and $PMEA^{2-}$ (Fig. 1) which provides more flexibility.

(vii) Of further interest is the trend-wise observation that stacking with GMP^{2-} (entry 6b), compared to AMP^{2-} (entry 7b), is apparently more pronounced as it is also the case for $PMEG^{2-}$ (entry 2) compared to PMEA (entry 4) (see the above point (v)). As far as the differences between Bpy and Phen in the complexes with the NMP^{2-} ligands are concerned, they fit into the picture indicated above in point (iv).

(viii) Rather surprising is the fact that $dGMP^{2-}$ evidently forms more stable stacking adducts than GMP^{2-} (compare $K_{I/st}$ for entries 5b and 6b). The reason is probably that deletion of the 2'-OH group at the ribose makes the nucleotide somewhat hydrophobic and less well solvated by water; this affects the acid-base properties of the phosphate group slightly, but even more so those of the guanine residue [94], which is in accord with the increased stacking tendency.

To conclude, especially the observations summarized in points (ii), (v), (vi), and (viii) are relevant for the anchoring process of the substrate in the active-site cavity of the nucleic acid polymerase; a correctly adjusted and orientated substrate is crucial for the reaction (see second to the last paragraph in Section 1).

3.10. Isomeric equilibria involving the ternary and monoprotonated Cu(Arm)(H·PMEG)$^{+}$ species

In Section 3.3 we have seen that the proton in the monoprotonated ternary complexes (Table 2, column 3) is located at the phosphonate group. In Section 3.7 it was concluded that the aromatic N sites, i.e., N1 [2], N3, and N7 are not relevant for Cu^{2+} coordination but that stack formation is important. However, still the questions remain: Where is $Cu(Arm)^{2+}$ coordinated and in which interactions is it involved? Does the monoprotonated $P(O)_2^-(OH)$ residue participate in $Cu(Arm)^{2+}$ binding?

In the context of the latter question it was recently concluded [83] that a $P(O)_2^-(OH)$ residue may act as a primary binding site allowing coordination of further sites. The stability constant for this complex formation was estimated based on previous considerations [95] as log $K^{Cu(Arm)}_{Cu(Arm)[P(O)_2(OH)]}$ = 1.0 ± 0.2 and this value was set equal to log $K^{Cu(Arm)}_{Cu(Arm)(H\cdot PE)op}$ [1,2]. Very recent experiments regarding the metal ion-binding properties of an $R(O)_2^-(OH)$ residue have led to new insights and further estimates [96], giving on average log $K^{Cu}_{Cu[ROP(O)_2OR']}$ = 1.35. Since on the one hand heteroaromatic amines like Bpy or Phen may favor the interaction with an O-donor site [74–78] (Section 3.5), yet on the other, the interaction with a monoprotonated phosph(on)ate group may occur [96], at least in part, in an outersphere manner [87,97] with a water molecule between $P(O)_2^-(OH)$ and M^{2+}, we estimate (with a generous error limit) log $K^{Cu}_{Cu[ROP(O)_2OR']}$ = 1.3 ± 0.2 and set this value equal to log $K^{Cu(Arm)}_{Cu(Arm)(H\cdot PE)op}$. With the monoprotonated phosphate group now defined as primary binding site, a scheme analogous to the Equilibrium Scheme (19) can be proposed, i.e., in Scheme (19) PE^{2-} simply needs to be replaced throughout by $H\cdot PE^-$; the same holds for the connected Eqs. (14) to (23). Hence, we are now in the position [1,2] to evaluate the available data for the $Cu(Arm)(H\cdot PMEG)^+$ and its related species, though, as we will see below, there is a caveat with regard to the structure of the stacks.

After having defined the stability of the $Cu(Arm)(H\cdot PMEG)^+_{op}$ complexes, one may calculate, by using the measured stability constants of column 3 in Table 2, the stability enhancements, log $\Delta_{Cu/Arm/H\cdot PMEG}$, as defined in analogy to Eq. (14). The results together with $K^*_{I/tot}$ and % $Cu(Arm)(H\cdot PMEG)^+_{int/tot}$ (calculated in analogy to Eqs. (22b) and (23)) are compiled in Table 5. For comparison the revised results (cf. with [1,2]) obtained for

$Cu(Arm)(H\cdot PMEA)^+$ and Cu(Arm)(H·PMEDAP) are also listed.

insert Table 5 close to here

The results of Table 5 can be further analyzed by using the $K_{I/O}$ values obtained for the Cu(Arm)(PME) complexes (see Section 3.7 plus Tables 3 and 4), because the corresponding stability enhancements due to the interaction with the ether O-atom (Equilibrium (15)) are expected [83] to be independent from the metal ion affinity of the primary binding site. The remaining calculations were carried out analogously to those for the Cu(Arm)(PE) systems given in Table 4. The formation degrees of the various isomers (analogous to Scheme (19)) of the monoprotonated ternary $Cu(Arm)(H\cdot PMEG)^+$ complexes (but see the caveat below) are collected in Table 6, together with those for the $Cu(Arm)(H\cdot PMEDAP)^+$ and $Cu(Arm)(H\cdot PMEA)^+$ ones.

insert Table 6 close to here

Among the conclusions to be drawn from the data in Table 6 are certainly the following ones:

(i) All three isomeric complexes, i.e., $Cu(Arm)(H\cdot PE)^+_{op}$, $Cu(Arm)(H\cdot PE)^+_{cl/O}$, and $Cu(Arm)(H\cdot PE)^+_{st}$, occur in appreciable amounts (Table 6; columns 5, 8, 9), as it is also the case for the uncharged Cu(Arm)(PE) species (Table 4).

(ii) As expected, in all instances it holds: % $Cu(Bpy)(H\cdot PE)^+_{st}$ < % $Cu(Phen)(H\cdot PE)^+_{st}$ (see also the $K^*_{I/st}$ values in column 7).

(iii) For the $Cu(Arm)(H\cdot PMEG)^+$ and the $Cu(Arm)(H\cdot PMEDAP)^+$ systems (entries 2 and 3) the stacked isomers clearly dominate, whereas for the Cu(Arm)(H·PMEA) complexes it appears that the $Cu(Arm)(H\cdot PMEA)^+_{cl/O}$ and $Cu(Arm)(H\cdot PMEA)^+_{st}$ isomers form in more comparable amounts (see in this context footnotes "e" and "f" of Table 6).

(iv) For the Cu(Arm)(PMEG) (Table 4) and $Cu(Arm)(H\cdot PMEG)^+$ (Table 6) systems it appears that the formation degrees of the stacked species are practically identical. However, this conclusion may be compromised by the caveat given below in Section 3.11, whereas for the PMEDAP complexes one may be relatively confident that it holds % $Cu(Arm)(PMEDAP)_{st}$ > % $Cu(Arm)(H\cdot PMEDAP)^+_{st}$ (entries 3 in Tables 4 and 6).

(v) Superficially, the high formation degrees of about 92 to 94% for the

$Cu(Arm)(H{\cdot}PMEG)^{+}_{st}$ isomers appear as most impressive in Table 6. These formation degrees are significantly larger (entry 2) than any other in Table 6 and this is striking, especially if compared with the situation summarized in Table 4 for the neutral Cu(Arm)(PE) systems.

3.11. A caveat regarding the evaluation of the monoprotonated Cu(Arm)(H·PMEG)$^{+}$ systems

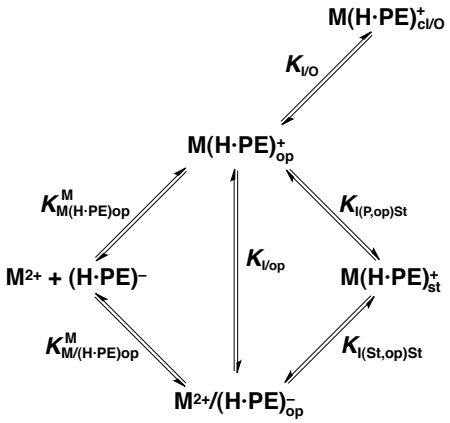
A more careful look at the data regarding the $Cu(Arm)(H{\cdot}PMEG)^{+}$ systems assembled in Table 5 reveals the caveat regarding the conclusion given above in point (v) of Section 3.10: The monoprotonated phosphonate residue of $H(PMEG)^{-}$ is no longer the dominating "primary" binding site because log $K^{Cu(Arm)}_{Cu(Arm)(H\cdot PMEG)op} = 1.3 \pm 0.2$ is smaller than the stability enhancements log $\Delta_{Cu/Arm/H\cdot PMEG}$ which amount to about 1.8 log units (Table 5, entry 2, column 4) indicating the importance of another site. Hence, the data given for $Cu(Arm)(H{\cdot}PMEG)^{+}$ in Table 6 (entry 2) need to be considered with some reservation (see further below).

As far as the $Cu(Arm)(H{\cdot}PMEDAP)^{+}$ systems are concerned, the two values, that is, log $K^{Cu(Arm)}_{Cu(Arm)(H\cdot PMEDAP)op} = 1.3 \pm 0.2$ and log $\Delta_{Cu/Arm/H\cdot PMEDAP} = 1.08 \pm 0.22$ or 1.26 ± 0.22 (Table 5, entry 3, column 4), are close to each other, but the results given in Tables 5 and 6 are still valid in a first approximation. This conclusion is supported by the observation that the percentages calculated previously [2] for $Cu(Arm)(H{\cdot}PMEDAP)^{+}_{st}$, which were based on log $K^{Cu(Arm)}_{Cu(Arm)(H\cdot PMEDAP)op} = 1.0 \pm 0.2$, are within the error limits the same (see Table 6 in [2]) as those calculated now (Table 6, entry 3).

However, what does the observation regarding $Cu(Arm)(H{\cdot}PMEG)^{+}$ as indicated above and also in point (v) of Section 3.10 actually mean? It means that the unbridged aromatic-ring stack, which actually may be considered as a 'binary' stack, symbolized as $Cu(Arm)^{2+}/(H{\cdot}PMEG)^{-}$, contributes to the stability enhancement log $\Delta_{Cu/Arm/H\cdot PMEG}$ = ca. 1.8! Therefore, the percentages listed in Table 6 (entry 2, column 9) refer to the sum of the unbridged plus the Cu^{2+}-bridged stacks (analogous to Fig. 3).

The consequence of the above is that there are two 'open' species, namely $Cu(Arm)(H{\cdot}PMEG)^{+}_{op}$, where Cu^{2+} is at the phosphonate group, and $Cu(Arm)^{2+}/(H{\cdot}PMEG)^{-}_{op}$, that is, the unbridged stack. Each of these two open complexes can lead to the Cu^{2+}-bridged

stack, Cu(Arm)(H·PMEG)$^{+}_{st}$; once via the Cu^{2+}-phosphonate coordinated species, and once via the unbridged (binary-like) stack. In addition, Cu(Arm)(H·PMEG)$^{+}_{op}$ may of course still form the five-membered chelate, Cu(Arm)(H·PMEG)$^{+}_{cl/O}$, in analogy to Eq. (15). These reasonings lead to the Equilibrium Scheme (24), where M^{2+} = Cu(Arm)$^{2+}$ and $(H\cdot PE)^{-}$ = $(H\cdot PMEG)^{-}$:



(24)

The definitions of the two stability constants of the open complexes, $K^{M}_{M(H\cdot PE)op}$ = $K^{Cu(Arm)}_{Cu(Arm)(H\cdot PMEG)op}$ (analogous to Eq. (20)) and $K^{M}_{M/(H\cdot PE)op}$ = $K^{Cu(Arm)}_{Cu(Arm)/(H\cdot PMEG)op}$, follow from the Equilibrium Scheme (24); their dimension is M^{-1}. Similarly, the definitions of all four dimensionless intramolecular equilibrium constants, K_I, also follow from Scheme (24).

One could try to make guesses for the stabilities of the two open complexes and this would then also provide a value for $K_{I/op}$ because Eq. (25) holds:

$$K_{I/op} = \frac{[M^{2+}/(H\cdot PE)^{-}_{op}]}{[M(H\cdot PE)^{+}_{op}]} = K^{M}_{M/(H\cdot PE)op} / K^{M}_{M(H\cdot PE)op} \qquad (25)$$

Furthermore, $K_{I/O}$ could be assumed as being known as was done before (Section 10, Table 6). However, regarding $K_{I(St,op)St}$ and/or $K_{I(P,op)St}$ we were not able to come up with a convincing estimate based on information available to us. Hence, we decided to leave it to the interested readers to make their own guesses regarding the formation degree of the stacked M(H·PE)$^{+}_{st}$ (= Cu(Arm)(H·PMEG)$^{+}_{st}$) species under the indicated conditions. However, that both stacks, Cu(Arm)(H·PMEG)$^{+}_{st}$ and Cu(Arm)$^{2+}$/(H·PMEG)$^{-}_{op}$, form is certain from the results in Table 6 and the above discussion.

4. Concluding remarks

The ANPs seen in Fig. 1 show antiviral activity, PMEA being already in use as a therapeutic agent. The antiviral activities of PMEDAP are similar and those of PMEG are even more pronounced. Most remarkably, PMEG also shows anticancer activities (see Section 1). If one compares the extent of stacking in the Cu(Arm)(ANP) systems, one notes that $PMEA^{2-}$ forms the least stable stacks, whereas those of $PMEG^{2-}$ and $PMEDAP^{2-}$ are of a higher and comparable stability (Table 4, columns 7 and 9); this equality is attributed to shape complementarity (Section 3.9, point (v)), though overall $PMEG^{2-}$ seems actually to be slightly favored in stack formation.

Indeed, the excellent stacking properties of $PMEG^{2-}$ are especially borne out in the monoprotonated $Cu(Arm)(H\cdot PMEG)^{+}$ complexes; here the stability enhancement log $\Delta_{Cu/Arm/HPMEG}$ (analogous to Eq. (14)) is even larger than the metal ion affinity of the "primary" binding site, i.e., of the $P(O)_2^-(OH)$ group (cf. Table 5). This means that the "open" (op) and unbridged $Cu(Arm)^{2+}/(H\cdot PMEG)^{-}_{op}$ stack, which actually may be considered as a "binary" stack, contributes significantly to the observed stability enhancement (Section 3.11). Possibly the remarkable cytotoxic properties of PMEG are a reflection of its intense stacking qualities, which outrun in complexes those of its parent nucleotides dGMP and GMP (Table 4, columns 7 and 9).

Another well-known aspect is that the ether oxygen of the PME chain (Fig. 1) is compulsory for the antiviral activity of the ANPs [98,99]. In accord herewith, 9-(4-phosphonobutyl)adenine (= 3'-deoxy-$PMEA^{2-}$) is devoid of any antiviral activity [98]. It is most likely that the ether oxygen facilitates achieving the two metal ion-containing [33] M(α)-M(β,γ) coordination mode needed for the transfer of a nucleotidyl residue [14,18,38] in the nucleic acid polymerase reaction. This ether-oxygen effect can be modulated via stacking (Section 3.9; point (iii)) and $PMEG^{2-}$ appears here to be especially effective: It reduces in Cu(Arm)(PMEG) the ether-oxygen interacting species to about 5% compared to the ca. 75% occurring in the Cu(Arm)(PME) systems. Though the details are not yet well understood, it is clear that upon mixed ligand complex formation the metal ion-ether oxygen interaction is affected and thus modulated.

Another interesting observation with regard to mechanistic considerations is the larger formation degree of the stacked isomers in the acyclic Cu(Arm)(PMEG) species compared with the parent complex, Cu(Arm)(dGMP) (Table 4). In fact, based on the results assembled in Table 4, several points of relevance for the anchoring process of the substrate in the active-site cavity

of a nucleic acid polymerase, were already discussed in points (ii), (v), (vi), and (viii) of Section 3.9, and shall not be repeated here.

A final point to be emphasized is that the changes in free energy (ΔG^0) connected with intramolecular equilibria like metal ion-ether oxygen or stacking interactions are small, which means that an equilibrium can easily be shifted from one side to another. For example, a stability enhancement of $\log \Delta = 0.1$ (Eq. (14)) corresponds at 25°C only to a change in ΔG^0 of 0.57 kJ mol^{-1}, yet the formation degree of the closed/stacked species increases from zero to ca. 20% (see [2] and for details [79]).

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Table 1

Negative logarithms of the acidity constants of $H_3(PMEG)^+$ [Eqs. (1) to (4)] together with those of some related species as determined by potentiometric pH titrations in aqueous solution at 25°C and I = 0.1 M ($NaNO_3$)[a,b].

No.	Protonated Species	pK_a for the site				Ref.
		$P(O)(OH)_2$	$(N7)H^+$	$(N1)H^+$	$P(O)^-_2(OH)$	
1	$H(9EtG)^+$		3.27 ± 0.03			[63][c]
2	$H_3(PMEG)^+$	1.18 ± 0.05[d]	3.35 ± 0.03		6.86 ± 0.01	–[e]
3	$CH_3P(O)(OH)_2$	2.10 ± 0.03			7.51 ± 0.01	[65]
4	$H_2(PME)$	1.57 ± 0.15[f]			7.02 ± 0.01	[66]
5	$H(9MeA)^+$		(2.96 ± 0.10)[g]	4.10 ± 0.01		[68]
6	$H_3(PMEA)^+$	1.22 ± 0.05[d]		4.16 ± 0.02	6.90 ± 0.01	[66]
7	$H_3(PMEDAP)^+$	1.26 ± 0.05[d]		4.82 ± 0.01	6.94 ± 0.01	[57]

[a] So-called practical, mixed or Brønsted constants are given (see Section 2 and Ref. [59]).

[b] The error limits given are three times the standard error of the mean value (3σ) or the sum of the probable systematic errors, whichever is larger. The error limits of derived data (e.g., entries No. 2, 6, and 7 in column 3) were calculated according to the error propagation after Gauss.

[c] Deprotonation of (N1)H in 9EtG occurs with $pK^H_{9EtG} = 9.57 \pm 0.05$ [63].

[d] Estimate based on $pK^H_{P(O)(OH)_2} = pK^H_{P(O)_2(OH)} - (5.68 \pm 0.05)$; for details see Ref. [64].

[e] This work. Deprotonation of the (N1)H site in $PMEG^{2-}$ occurs with $pK^H_{PMEG} = 9.34 \pm 0.02$ (Eq. (4)).

[f] Estimate; see Ref. [67].

[g] This micro acidity constant reflects the basicity of N7 under conditions where N1 does not carry a proton; i.e., $pk^{N7\text{-}N1}_{H\cdot N7\text{-}N1} = 2.96 \pm 0.10$ holds for the species $^+H\cdot N7(9MeA)N1$. For the macro acidity constant $pK^H_{H_2(9MeA)} = -0.64 \pm 0.06$ holds as measured by UV spectrophotometry [68,69].

Table 2

Logarithms of the stability constants of the ternary Cu(Arm)(H;PMEG)$^+$ [Eq. (5)] and Cu(Arm)(PMEG) [Eq. (6)] complexes, as well as of some related species, as determined by potentiometric pH titrations in aqueous solution, together with the negative logarithms of the acidity constants [Eqs. (7), (8)] of the Cu(Arm)(H;PE)$^+$ complexes (25°C; I = 0.1 M, $NaNO_3$)[a]. The values of the binary complexes (entries (1a), (2a), (3a), and (4a)) as well as the resulting stability differences [Eq. (10)] are given for comparison.

No.	M(PE)	log $K^{M}_{M(H;PE)}$	log $K^{M}_{M(PE)}$	p$K^{H}_{M(H;PE)}$	Δ log K_{Cu}	Ref.
1a	Cu(PME)	–	3.73 ± 0.03	–	–	[66]
1b	Cu(Bpy)(PME)	–	3.86 ± 0.03	–	0.13 ± 0.04	[70]
1c	Cu(Phen)(PME)	–	3.90 ± 0.04	–	0.17 ± 0.05	[70]
2a	Cu(PMEG)	3.26 ± 0.09	4.57 ± 0.08	5.55 ± 0.12	–	–[b]
2b	Cu(Bpy)(PMEG)	3.01 ± 0.08	4.87 ± 0.10	5.00 ± 0.13	0.30 ± 0.13	–[b]
2c	Cu(Phen)(PMEG)	3.15 ± 0.10	5.16 ± 0.13	4.85 ± 0.16	0.59 ± 0.15	–[b]
3a	Cu(PMEDAP)	1.88 ± 0.07	3.94 ± 0.04	4.88 ± 0.08	–	[57]
3b	Cu(Bpy)(PMEDAP)	2.38 ± 0.08	4.90 ± 0.03	4.42 ± 0.09	0.96 ± 0.05	[2]
3c	Cu(Phen)(PMEDAP)	2.56 ± 0.08	5.09 ± 0.04	4.41 ± 0.09	1.15 ± 0.06	[2]
4a	Cu(PMEA)	1.48 ± 0.16	3.96 ± 0.04	4.42 ± 0.17	–	[66]
4b	Cu(Bpy)(PMEA)	1.77 ± 0.11	4.70 ± 0.02	3.97 ± 0.11	0.74 ± 0.04	[70]
4c	Cu(Phen(PMEA)	2.20 ± 0.09	4.97 ± 0.03	4.13 ± 0.10	1.01 ± 0.05	[70]

[a] For the error limits see footnote "b" of Table 1, and for the structure of the ligands see Fig. 1.

[b] This study.

Table 3

Quantification of the stability increase log $\Delta_{Cu/Arm/PE}$ [Eq. (14)] for the Cu(Arm)(PMEG) and related complexes (see Fig. 1) and extent of the total amount of species with an intramolecular interaction [Eqs. (22), (23)] in Cu(Arm)(PE) complexes in aqueous solution (25°C; I = 0.1 M, $NaNO_3$)[a].

No.[a]	Cu(Arm)(PE)	log $K^{Cu(Arm)}_{Cu(Arm)(PE)}$	log $K^{Cu(Arm)}_{Cu(Arm)(PE)op}$	log $\Delta_{Cu/Arm/PE}$	$K_{I/tot}$	% Cu(Arm)(PE)$_{int/tot}$
1b	Cu(Bpy)(PME)	3.86 ± 0.03	3.27 ± 0.07	0.59 ± 0.08	2.89 ± 0.68[b]	74 ± 5[b]
1c	Cu(Phen)(PME)	3.90 ± 0.04	3.28 ± 0.06	0.62 ± 0.07	3.17 ± 0.69[b]	76 ± 4[b]
2b	Cu(Bpy)(PMEG)	4.87 ± 0.10	3.20 ± 0.07	1.67 ± 0.12	45.77 ± 13.15	97.86 ± 0.60
2c	Cu(Phen)(PMEG)	5.16 ± 0.13	3.21 ± 0.06	1.95 ± 0.14	88.13 ± 29.38	98.88± 0.37
3b	Cu(Bpy)(PMEDAP)	4.90 ± 0.03	3.24 ± 0.07	1.66 ± 0.08	44.71 ± 8.02	97.81 ± 0.38
3c	Cu(Phen)(PMEDAP)	5.09 ± 0.04	3.25 ± 0.06	1.84 ± 0.07	68.18 ± 11.49	98.55 ± 0.24
4b	Cu(Bpy)(PMEA)	4.70 ± 0.02	3.22 ± 0.07	1.48 ± 0.07	29.20 ± 4.87	96.69 ± 0.53
4c	Cu(Phen)(PMEA)	4.97 ± 0.03	3.23 ± 0.06	1.74 ± 0.07	53.95 ± 8.86	98.18 ± 0.29

[a] The entry numbers correspond to those of Table 2, where also the references for entries 1, 3, and 4 are given. For the error limits see footnote "b" of Table 1 and for the structures of the ligands see Fig. 1. The values for $K_{I/tot}$ follow from Eqs. (14) and (22b) and those for % Cu(Arm)(PE)$_{int/tot}$ from Eq. (23).

[b] For the Cu(Arm)(PME) systems, $K_{I/tot} = K_{I/O}$ [Eq. (22e)] and % Cu(Arm)(PE)$_{int/tot}$ = % Cu(Arm)(PE)$_{cl/O}$ [see Equilibrium (15) and Eq. (23)].

Table 4

Intramolecular equilibrium constants for the formation of the three differently structured Cu(Arm)(PE) species shown in the Equilibrium Scheme (19) for $PMEG^{2-}$ and several related systems, together with the percentages of these isomers in aqueous solution (25°C; I = 0.1 M, $NaNO_3$)[a,b].

No.[a]	Cu(Arm)(PE)	$K_{I/tot}$	% Cu(Arm)(PE)$_{int/tot}$	% Cu(Arm)(PE)$_{op}$	$K_{I/O}$	$K_{I/st}$	% Cu(Arm)(PE)$_{cl/O}$[c]	% Cu(Arm)(PE)$_{st}$[d]
1b	Cu(Bpy)(PME)	2.89 ± 0.68[b]	74 ± 5[b]	26 ± 5	2.89 ± 0.68	–	74 ± 5	–
1c	Cu(Phen)(PME)	3.17 ± 0.69[b]	76 ± 4[b]	24 ± 4	3.17 ± 0.69	–	76 ± 4	–
2b	Cu(Bpy)(PMEG)	45.77 ± 13.15	97.86 ± 0.60	2.14 ± 0.60	2.89 ± 0.68	42.88 ± 13.17	6 ± 2.5	92 ± 2.5
2c	Cu(Phen)(PMEG)	88.13 ± 29.38	98.88 ± 0.37	1.12 ± 0.37	3.17 ± 0.69	84.96 ± 29.39	3.5 ± 1.5	95 ± 1.5
3b	Cu(Bpy)(PMEDAP)	44.71 ± 8.02	97.81 ± 0.38	2.19 ± 0.38	2.89 ± 0.68	41.82 ± 8.05	6 ± 2	92 ± 2
3c	Cu(Phen)(PMEDAP)	68.18 ± 11.49	98.55 ± 0.24	1.45 ± 0.24	3.17 ± 0.69	65.01 ± 11.51	4.5 ± 1.5	94 ± 1.5
4b	Cu(Bpy)(PMEA)	29.20 ± 4.87	96.69 ± 0.53	3.31 ± 0.53	2.89 ± 0.68	26.31 ± 4.92	10 ± 3	87 ± 3
4c	Cu(Phen)(PMEA)	53.95 ± 8.86	98.18 ± 0.29	1.82 ± 0.29	3.17 ± 0.69	50.78 ± 8.89	6 ± 2	92 ± 2
No.[e]	Cu(Arm)(NMP)					$K_{I/st}$		% Cu(Arm)(NMP)$_{st}$
5b	Cu(Bpy)(dGMP)					13.61 ± 3.56	(8 ± 9)[f]	86 ± 9
5c	Cu(Phen)(dGMP)					19.14 ± 5.11	(6 ± 7)[f]	89 ± 7
6b	Cu(Bpy)(GMP)					5.61 ± 1.25		85 ± 3
7b	Cu(Bpy)(AMP)					4.37 ± 1.02		81 ± 4
7c	Cu(Phen)(AMP)					8.77 ± 1.81		90 ± 2

[a] See footnote "a" of Table 3. Regarding the Refs. for entries 1, 3, and 4 see Table 2.

[b] The values in columns 3 and 4 are from Table 3 (columns 6 and 7). The values in the fifth column for % $Cu(Arm)(PE)_{op}$ follow from 100 – % $Cu(Arm)(PE)_{int/tot}$. The constants for $K_{I/O}$ in the sixth column are from entries 1b and 1c of Table 3 (column 6; for the corresponding justification see text in Sections 3.8 and 3.9); with Eq. (22e) and the known values for $K_{I/tot}$ and $K_{I/O}$ those for $K_{I/st}$ can be calculated (see column 7).

[c] These values were calculated via Eq. (16) with $K_{I/O}$ and % $Cu(Arm)(PE)_{op}$.

[d] The values for % $Cu(Arm)(PE)_{st}$ follow from the difference % $Cu(Arm)(PE)_{int/tot}$ – % $Cu(Arm)(PE)_{cl/O}$ [cf. Eqs. (22c) and (22d)]; % $Cu(Arm)(PE)_{st}$ may also be calculated via Eq. (18) with $K_{I/st}$ and % $Cu(Arm)(PE)_{op}$. The results are the same for both calculation methods, yet the error limits are understandably larger for the second method (data not shown).

[e] The values in entries 5, 6, and 7 are from Refs. [50], [92], and [93], respectively; for details these papers should be consulted.

[f] These percentages are zero within the error limits but they indicate that traces of an isomer involving N7, i.e., $Cu(Arm)(dGMP)_{cl/N7}$, might occur.

Table 5

Quantification of the stability increase log $\Delta_{Cu/Arm/H\cdot PE}$ (analogous to Eq. (14)) for the $Cu(Arm)(H\cdot PMEG)^+$ and related complexes (see Fig. 1) and extent of the total amount of species with an intramolecular interaction (analogous to Eqs. (22) and (23)) in the $Cu(Arm)(H\cdot PE)^+$ complexes in aqueous solution (25°C; I = 0.1 M, $NaNO_3$)[a].

No.[a]	$Cu(Arm)(H\cdot PE)^+$	log $K^{Cu(Arm)}_{Cu(Arm)(H\bullet PE)}$	log $\Delta_{Cu/Arm/H\cdot PE}$[b]	$K^*_{I/tot}$[c]	% $Cu(Arm)(H\cdot PE)_{int/tot}$[c]
2b	$Cu(Bpy)(H\cdot PMEG)^+$	3.01 ± 0.08	1.71 ± 0.22	50.29 ± 25.44	98.05 ± 0.97
2c	$Cu(Phen)(H\cdot PMEG)^+$	3.15 ± 0.10	1.85 ± 0.22	69.79 ± 36.45	98.59 ± 0.73
3b	$Cu(Bpy)(H\cdot PMEDAP)^+$	2.38 ± 0.08	1.08 ± 0.22	11.02 ± 5.96	91.68 ± 4.13
3c	$Cu(Phen)(H\cdot PMEDAP)^+$	2.56 ± 0.08	1.26 ± 0.22	17.20 ± 9.03	94.50 ± 2.73
4b	$Cu(Bpy)(H\cdot PMEA)^+$	1.77 ± 0.11	0.47 ± 0.23	1.95 ± 1.55	66.12 ± 17.81
4c	$Cu(Phen)(H\cdot PMEA)^+$	2.20 ± 0.09	0.90 ± 0.22	6.94 ± 4.01	87.41 ± 6.36

[a] The entry numbers correspond to those of Table 2; the values given above in the third column are from Table 2 (column 3). For the error limits see footnote "b" of Table 1 and for the structures of the ligands see Fig. 1.

[b] Log $\Delta_{Cu/Arm/H\cdot PE}$ is calculated analogously to Eq. (14) by applying log $K^{Cu(Arm)}_{Cu(Arm)(H\cdot PE)op}$ = 1.3 ± 0.2 (see text in Section 3.10).

[c] The values for $K^*_{I/tot}$ follow in analogy to Eqs. (14) and (22b) and those for % $Cu(Arm)(H\cdot PE)_{int/tot}$ from Eq. (23).

Table 6

Intramolecular equilibrium constants for the formation of the three differently structured Cu(Arm)(H·PMEG)$^+$ species and of related complexes. These species are defined in analogy to the Equilibrium Scheme (19), together with the percentages in which these species occur in aqueous solution (25°C; I = 0.1 M, $NaNO_3$)[a,b].

No.[a]	Cu(Arm)(H·PE)$^+$	$K^*_{I/tot}$	% Cu(Arm)(H·PE)$^+_{int/tot}$	% Cu(Arm)(H·PE)$^+_{op}$	$K_{I/O}$	$K^*_{I/st}$	% Cu(Arm)(H·PE)$^+_{cl/O}$ [c]	% Cu(Arm)(H·PE)$^+_{st}$ [d]
2b	Cu(Bpy)(H·PMEG)$^+$	50.29 ± 25.44	98.05 ± 0.97	1.95 ± 0.97	2.89 ± 0.68	47.40 ± 25.45	5.64 ± 3.10	92.4 ± 3.3
2c	Cu(Phen)(H·PMEG)$^+$	69.79 ± 36.45	98.59 ± 0.73	1.41 ± 0.73	3.17 ± 0.69	66.62 ± 36.46	4.47 ± 2.51	94.1 ± 2.6
3b	Cu(Bpy)(H·PMEDAP)$^+$	11.02 ± 5.96	91.68 ± 4.13	8.32 ± 4.13	2.89 ± 0.68	8.13 ± 6.00	24.04 ± 13.21	68 ± 14
3c	Cu(Phen)(H·PMEDAP)$^+$	17.20 ± 9.03	94.50 ± 2.73	5.50 ± 2.73	3.17 ± 0.69	14.03 ± 9.06	17.44 ± 9.45	77 ± 10
4b	Cu(Bpy)(H·PMEA)$^+$	1.95 ± 1.55	66.12 ± 17.81	33.88 ± 17.81	2.89 ± 0.68[e]	–0.94 ± 1.69[e,f]	(97.91 ± 56.39)[e]	<43[f]
4c	Cu(Phen)(H·PMEA)$^+$	6.94 ± 4.01	87.41 ± 6.36	12.59 ± 6.36	3.17 ± 0.69	3.77 ± 4.07	39.91 ± 21.95	48 ± 23

[a] The entry numbers correspond to those of Table 5. For the error limits see footnote "b" of Table 1.

[b] The values in columns 3 and 4 are from Table 5 (columns 5, 6). The values given in the fifth column for % Cu(Arm)(H·PE)$^+_{op}$ follow from 100 – % Cu(Arm)(H·PE)$^+_{int/tot}$. The constants for $K_{I/O}$ in the sixth column are from entries 1b and 1c of Table 3 (column 6) (for the corresponding justification see the text in Sections 3.9 and 3.10); with an equation analogous to Eq. (22e) and the known values for $K^*_{I/tot}$ and $K_{I/O}$ the one for $K^*_{I/st}$ may be calculated (see column 7).

[c] These values were calculated via an equation analogous to Eq. (16) with $K_{I/O}$ and % Cu(Arm)(H·PE)$^+_{op}$.

[d] The above values for % Cu(Arm)(H·PE)$^+_{st}$ follow from the difference % Cu(Arm)(H·PE)$^+_{int/tot}$ – % Cu(Arm)(H·PE)$^+_{cl/O}$ (cf. Eqs. analogous to (22c) and (22d)); % Cu(Arm)(H·PE)$^+_{st}$ may also be calculated via the analogous Eq. (18) with $K^*_{I/st}$ and % Cu(Arm)(H·PE)$^+_{op}$. The results are the

same for both calculation methods, yet the error limits are understandably larger for the second method (data not shown).

[e] This value of $K_{I/O}$ is larger than $K^*_{I/tot}$, which means that the experimental data are not exact enough for a meaningful evaluation regarding $K^*_{I/st}$; in addition, an error in the value for log $\Delta_{Cu/Bpy/H\cdot PMEA}$ (1.3 ± 0.2 was used; see Table 5) has a huge influence. Indeed, in a case like this, small differences play a decisive role and the fact that the value for $K^*_{I/st}$ is *negative*, does not mean that no Cu(Bpy)(H·PMEA)$^+_{st}$ isomer is formed at all. Furthermore, % Cu(Bpy)(H·PMEA)$^+_{cl/O}$ = 97.91 ± 56.39 should be considered as an artefact.[f]

[f] From $K^*_{I/st}$ = –0.94 ± 1.69 follows the upper limit for $K^*_{I/st} \leq 0.75$ and this (in analogy to Eq. (23)) provides for % Cu(Bpy)(H·PMEA)$^+_{st}$ a value of <43 as the upper limit.

Legends for the Figures

Fig. 1. Chemical structures of the dianions of 9-[2-(phosphonomethoxy)ethyl]guanine ($PMEG^{2-}$), 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$), and 9-[2-(phosphonomethoxy)ethyl]-2,6-diaminopurine ($PMEDAP^{2-}$) together with the structure of (phosphonomethoxy)ethane [= PME^{2-} = (ethoxymethyl)phosphonate for R = H]. The four compounds are abbreviated as PE^{2-}. The orientation of $PMEA^{2-}$ (and the same may be assumed for $PMEG^{2-}$) in solution [27] and in the solid state [28] resembles the *anti* conformation of adenosine 5'-monophosphate (AMP^{2-}) [29,30]. The structure of 2'-deoxyguanosine 5'-monophosphate ($dGMP^{2-}$) shown in its dominating *anti* conformation [30,31] at the bottom of the figure, is given for comparison (see text).

Fig. 2. Evidence for an enhanced stability of ternary Cu(Arm)(PE) complexes, where Arm = Bpy (empty symbols) or Phen (full symbols), and PE^{2-} = PME^{2-} (◇,◆), $PMEG^{2-}$(□,■), $PMEDAP^{2-}$ (△,▲), or $PMEA^{2-}$ (○,●), based on the relationship between log $K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(R\text{-}PO_3)}}$ or log $K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(PE)}}$, and $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H(R\text{-}PO_3)}}$ or $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H(PE)}}$ in aqueous solution at I = 0.1 M ($NaNO_3$) and 25ºC. The plotted data are from Tables 1 and *2*. The two reference lines represent the log $K^{\mathrm{Cu(Arm)}}_{\mathrm{Cu(Arm)(R\text{-}PO_3)}}$ versus $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H(R\text{-}PO_3)}}$ relationship for the ternary Cu(Arm)(R-PO_3) complexes (Eqs. (12) and (13)); R-PO_3^{2-} symbolizes phosphonates or phosphate monoesters in which the group R is unable to undergo any kind of hydrophobic, stacking, or other type of interaction, i.e., ligands like D-ribose 5-monophosphate, methanephosphonate or ethanephosphonate [70,79,81]. The broken line holds for Arm = Bpy and the solid line for Arm = Phen. Both straight lines represent the situation for ternary complexes without an intramolecular ligand–ligand interaction. The vertical dotted lines emphasize the stability differences from the reference lines; they equal log $\Delta_{\mathrm{Cu/Arm/PE}}$ as defined in Eq. (14). The data points for Cu(Arm)(dGMP) (☆,★) are given for comparison; the corresponding constants are listed in Ref. [50].

Fig. 3. Tentative and simplified structure of a Cu(Phen)(PMEG) species with an intramolecular stack. The orientation of the aromatic rings may vary among the stacked species; such a stacked complex in solution should not be considered as being rigid.

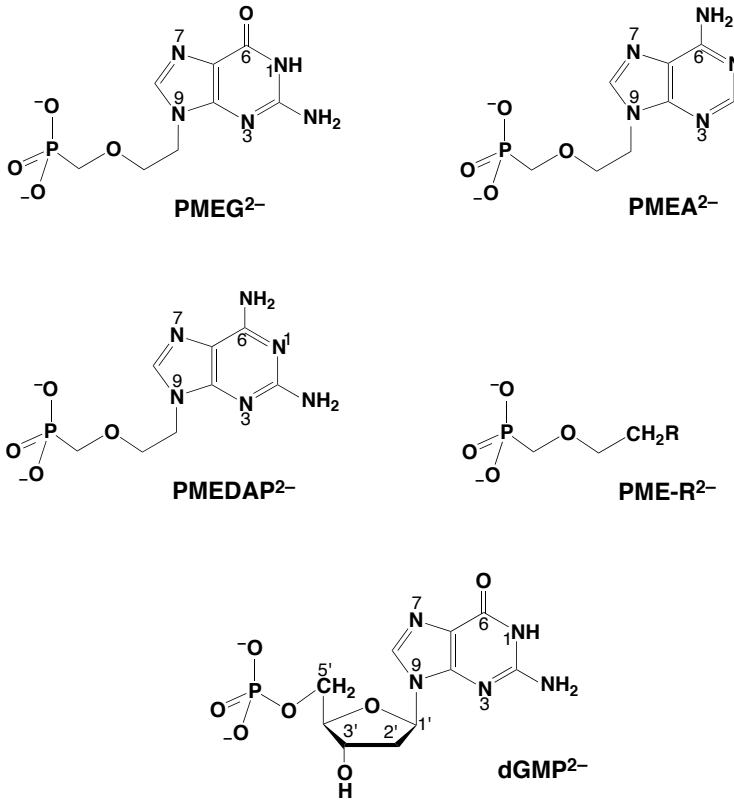


Fig. 1

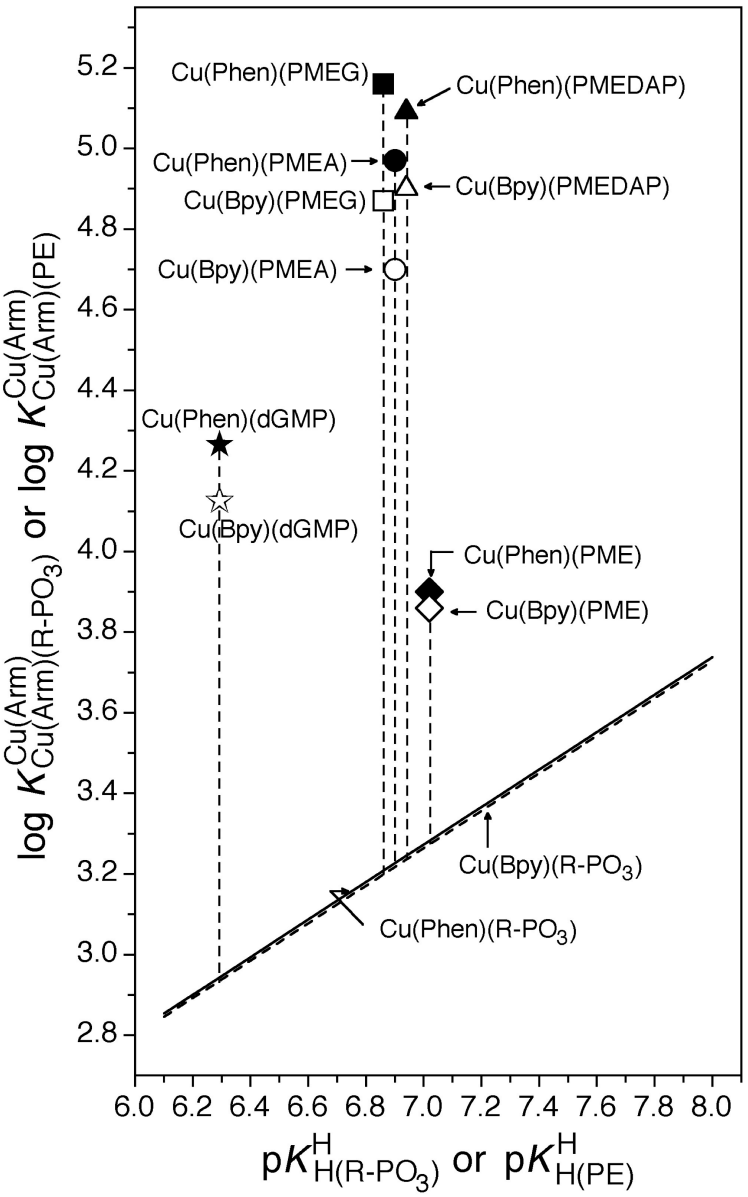

Cu(Phen)(PMEG)
Cu(Phen)(PMEDAP)
Cu(Phen)(PMEA)
Cu(Bpy)(PMEDAP)
Cu(Bpy)(PMEG)
Cu(Bpy)(PMEA)
Cu(Phen)(dGMP)
Cu(Bpy)(dGMP)
Cu(Phen)(PME)
Cu(Bpy)(PME)
Cu(Bpy)(R-PO3)
Cu(Phen)(R-PO3)
log K Cu(Arm) Cu(Arm)(R-PO3) or log K Cu(Arm) Cu(Arm)(PE)
pK H H(R-PO3) or pK H H(PE)
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
6.0 6.2 6.4 6.6 6.8 7.0 7.2 7.4 7.6 7.8 8.0

Fig. 2

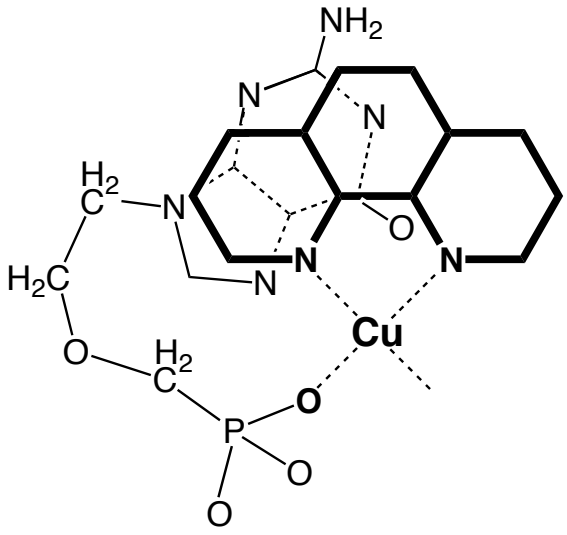
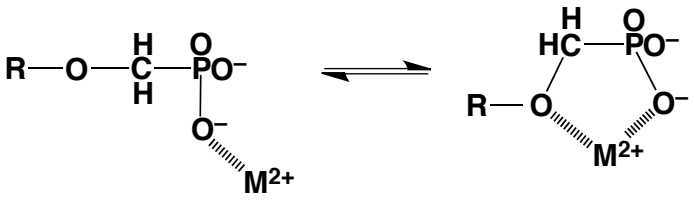
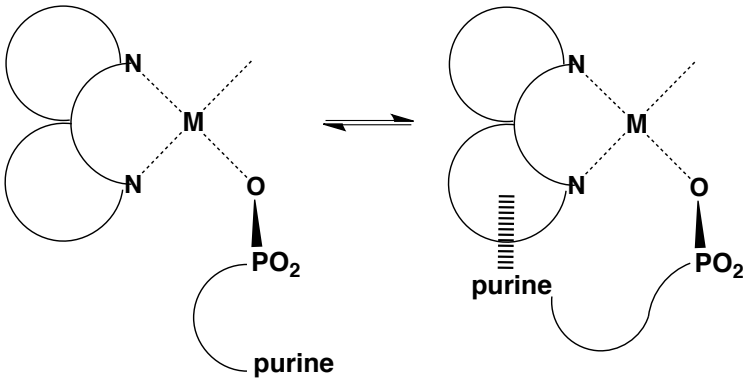


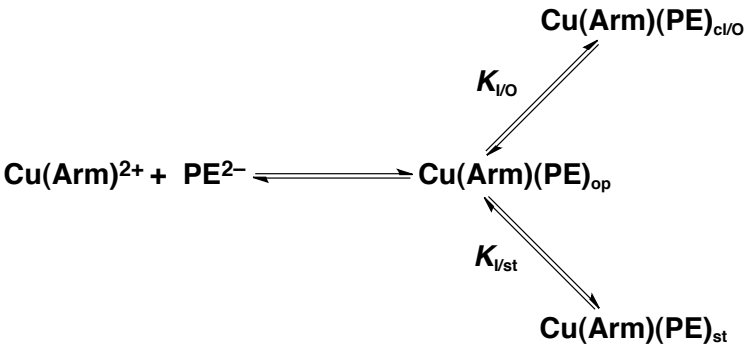
Fig. 3



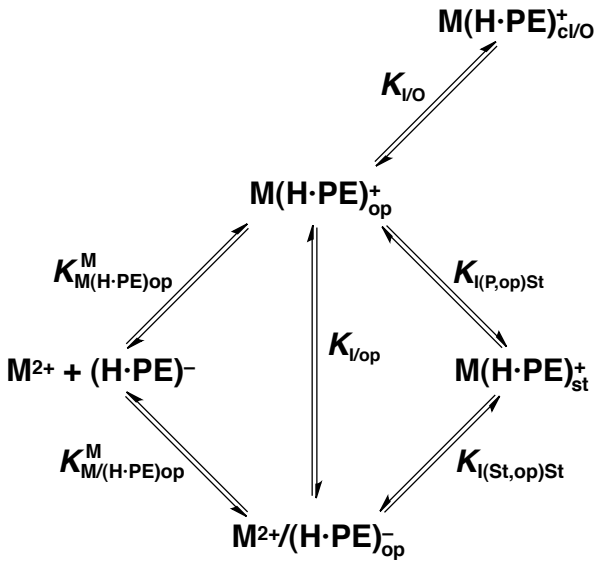
Eq. (15)



Eq. (17)



Eq. (19)


M(H·PE)+cl/O
KI/O
M(H·PE)+op
KMM(H·PE)op
KI(P,op)St
M2+ + (H·PE)−
KI/op
M(H·PE)+st
KMM/(H·PE)op
KI(St,op)St
M2+/(H·PE)−op

Eq. (24)

Synopsis

The guanine residue of 9-[2-(phosphonomethoxy)ethyl]guanine leads to intense intramolecular stacks in the ternary complex formed with Cu(1,10-phenanthroline)$^{2+}$ (aqueous solution). Stacking is more pronounced than with the adenine derivative. The formation degrees of the three isomers, PO_3^{2-} -coordination only, five-membered chelate with the ether-O, and the intramolecular guanine/phenanthroline stack, are determined.

Graphical abstract