Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Polyhedron 22 (2003) 1067–1076
POLYHEDRON
www.elsevier.com/locate/poly

Stability and structure of binary and ternary metal ion complexes in aqueous solution of the quaternary 1-[2-(phosphonomethoxy)ethyl] derivative of 2,4-diaminopyrimidine ($PMEDAPy^-$). Properties of an acyclic nucleotide analogue

Alfonso Fernández-Botello [a,b], Antonín Holý [c], Virtudes Moreno [b], Helmut Sigel [a,*]

[a] *Department of Chemistry, Inorganic Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland*
[b] *Inorganic Chemistry Department, Faculty of Chemistry, University of Barcelona, E-08028 Barcelona, Spain*
[c] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences, CZ-16610 Prague, Czech Republic*

Received 6 November 2002; accepted 17 January 2003

Abstract

The acidity constants of the twofold protonated quaternary 1-[2-(phosphonomethoxy)ethyl] derivative of 2,4-diaminopyrimidine, $H_2(PMEDAPy)^+$, were determined by potentiometric pH titrations in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$) and compared with the corresponding constants of also twofold protonated *O*-phosphonatomethylcholine, $H_2(PMCh)^+$, an analogue of phosphocholine; the corresponding acidity constants are quite similar. By the same experimental method and under the same conditions the stability constants of the $M(PMEDAPy)^+$ complexes with the metal ions $M^{2+} = Mg^{2+}$, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} or Cd^{2+} and also of the mixed ligand complexes, $Cu(Arm)(PMEDAPy)^+$, where Arm = Bpy (2,2′-bipyridine) or Phen (1,10-phenanthroline), have also been determined. Application of the previously determined straight-line plots of $\log K^{M}_{M(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ for simple phosph(on)ate ligands, $R-PO_3^{2-}$, where R represents a residue that does not affect complex formation, proves that the positively charged pyrimidinium residue somewhat inhibits, due to charge repulsion, complex formation with the $-PO_3^{2-}$ group. In fact, a comparison with previous results obtained for $R-CH_2CH_2-O-CH_2-PO_3^{2-}$ (=PME–R^{2-}) ligands (R is non-interacting) with the data obtained now for the binary complexes of $PMEDAPy^-$ shows that for all 10 divalent metal ions studied the inhibition amounts to $\Delta\log \Delta_{M/PMEDAPy} = 0.42 \pm 0.04$. This 'constant' repulsive effect is evidence that in the $M(PMEDAPy)^+$ complexes the ether oxygen does not participate in complex formation; this is different in the M(PME–R) complexes, where five-membered chelates form in equilibrium, the extent being dependent on the kind of metal ion involved. The results for the mixed ligand $Cu(Arm)(PMEDAPy)^+$ complexes show a significant increase in complex stability of about 0.6 log unit (compared to the stability expected on the basis of the basicity of the phosphonate group), which is due to intramolecular stack formation between the aromatic ring systems of Phen or Bpy and the pyrimidinium moiety of $PMEDAPy^-$. The formation degree of the stacked isomer in the $Cu(Arm)(PMEDAPy)^+$ systems is on the order of 70%, though it is somewhat more pronounced with Phen than with Bpy. Comparisons with the Cu(Arm)(N) systems, where N = cytidine 5′-monophosphate (CMP^{2-}) or 1-[2-(phosphonomethoxy)ethyl]cytosine ($PMEC^{2-}$), reveal that the stacking properties of all three pyrimidine derivatives are similar. An explanation is offered why $PMEDAPy^-$ shows no biological activity compared to several other closely related acyclic nucleotide analogues, which have antiviral properties.

Keywords: Acidity constants; Charge repulsion; Phosphonate complexes; Pyrimidine nucleotide analogue; Stability constants; Stacking interactions; Ternary complexes

1. Introduction

Acyclic nucleoside phosphonates are promising antiviral agents [1,2]. Like with their parent nucleotides [3],

* Corresponding author. Tel.: +41-61-267-1007; fax: +41-61-267-1017.
E-mail address: helmut.sigel@unibas.ch (H. Sigel).

0277-5387/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0277-5387(03)00093-7

two series of compounds are derived, either from the pyrimidines [1,2] or purines [1]. An example for the latter kind is 9-[2-(phosphonomethoxy)ethyl]-2,6-diaminopurine (PMEDAP) which is a powerful antiviral drug active both against DNA viruses [4] and retroviruses [5]. An example of an active compound derived from the pyrimidine series is (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (Cidofovir; HPMPC) which is employed against cytomegaloviruses [6].

Considering the structures of the two mentioned compounds, an effort was undertaken to combine their two main structural features, i.e., the pyrimidine part of HPMPC and the two amino functions of PMEDAP. Consequently, the quaternary 1-[2-(phosphonomethoxy)ethyl] derivative of 2,4-diaminopyrimidine was synthesized [7]. The resulting compound is {[2-(2,4-diaminopyrimidin-io)ethoxy]methyl}phosphonate [7], sometimes also (not quite correctly) named as 1-[2-(phosphonomethoxy)ethyl]-2,4-diaminopyrimidine from where its abbreviation PMEDAPy originates (see Fig. 1).

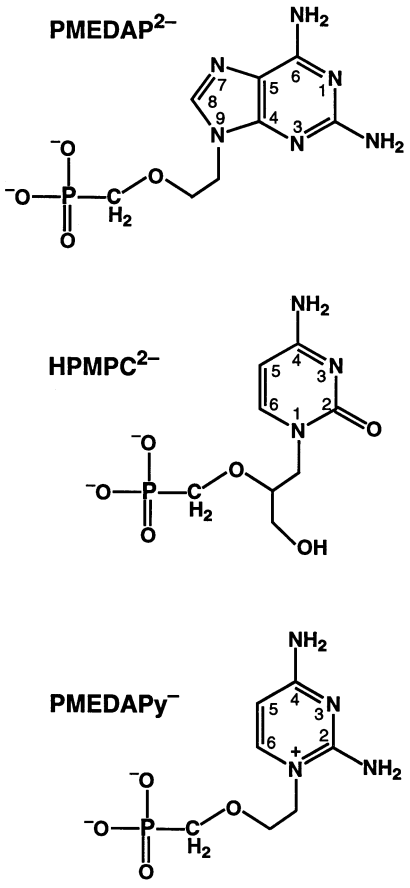
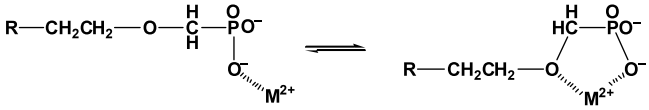


Fig. 1. Chemical structures of the anions of 9-[(2-phosphonomethoxy)ethyl)]-2,6-diaminopurine ($PMEDAP^{2-}$), 1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine ($HPMPC^{2-}$) and 1-[2-(phosphonomethoxy)ethyl]-2,4-diaminopyrimidine ($PMEDAPy^{-}$; see also the second paragraph of Section 1).

Biological studies of PMEDAPy showed that this compound does not exhibit any antiviral activity against DNA viruses or retroviruses nor any cytostatic activity [7]. Why is this compound inactive in contrast to the related PMEDAP and HPMPC? An answer to this question would clearly facilitate the search for other antivirally active nucleotide analogues.

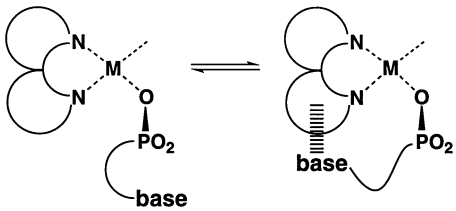
It is known [8,9] that the acyclic nucleoside phosphonates of the mentioned kind are biologically active in the form of their diphosphorylated derivatives serving as substrates for DNA polymerases, and that here metal ions are playing a crucial role [10,11]. Therefore, we have now studied in the course of our investigations [12–16] on antivirally active nucleotide analogues the metal ion-binding properties of $PMEDAPy^{-}$. It had previously been suggested [17,18] that metal ion binding to the ether oxygen facilitates the formation of the reactive species in the active site cavity of polymerases and therefore we studied the position [19,20] of the intramolecular equilibrium (1)



(1)

to see if the positively charged pyrimidinium residue has an effect on its position.

The correct anchoring of a substrate in the active-site cavity of an enzyme is a further crucial point for biological activity. Since this anchoring process usually occurs via hydrogen bonding [21] or stacking interactions [22], we have also investigated the stacking properties [12,23–25] of the quaternary 2,4-diaminopyrimidinium residue by using equilibrium (2) as a model.



(2)

This means, $PMEDAPy^{-}$ was linked via a Cu^{2+} ion to 1,10-phenanthroline (Phen) or 2,2′-bipyridine (Bpy) and then the position of the intramolecular equilibrium (2) was determined.

It is shown now that the positively charged pyrimidinium residue strongly inhibits the metal ion–ether oxygen interaction in $M(PMEDAPy)^{+}$ complexes whereas the stacking properties of the quaternary 2,4-diaminopyrimidinium residue in the Cu(Arm)-$(PMEDAPy)^{+}$ complexes, where Arm = Bpy or Phen,

are very close to those of uncharged pyrimidine derivatives.

2. Experimental

2.1. Materials and measurements

Monoprotonated {[2-(2,4-diaminopyrimidin-io)ethoxy]methyl}phosphonate, $H(PMEDAPy)^{\pm}$, was synthesized as described [7]. The aqueous stock solution of the ligand was freshly prepared daily by dissolving the substance in deionized, ultrapure (MILLI-Q185 PLUS; from Millipore S.A., 67120 Molsheim, France) CO_2-free water and adding 1 equivalent of NaOH; its exact concentration was determined as described [26]. All the other reagents, including monoprotonated *O*-phosphonatomethylcholine, $H(PMCh)^{\pm}$, and the buffers employed for pH calibration, were the same as used recently [26].

The equipment for the potentiometric pH titrations [26] and the computers used for their evaluation [15] are the same as recently, except that for several experiments a Metrohm 716 DMS-Titrino (Metrohm AG, Herisau, Switzerland) connected with an IBM-compatible desk computer with a Pentium processor (software Titnet 2.4 from Metrohm) and a Hewlett-Packard Desk Jet 1600C Color Smart printer was used. A Metrohm 6.0222.100 combined macro glass electrode was employed. The data obtained with the different equipment agreed within the error limits.

The pH calibration of the instruments was done with buffers [26] and the direct pH meter readings were used in the calculations [15] of the acidity constants; i.e., these constants determined at $I = 0.1$ M ($NaNO_3$) and 25 °C are so-called practical, mixed or Brønsted constants [27]. They may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values [27]; this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity [27,28]. It should be emphasized that the ionic product of water (K_w) and the mentioned conversion term do not enter into our calculation procedures because we always evaluate the differences in NaOH consumption between a pair of solutions, i.e., with and without ligand (see Section 2.2). The stability constants determined are, as usual, concentration constants.

2.2. Determination of equilibrium constants

The acidity constants $K^{H}_{H_2(PMEDAPy)}$ (Eqs. (3a) and (3b)) and $K^{H}_{H(PMEDAPy)}$ (Eqs. (4a) and (4b)) of $H_2(PMEDAPy)^{+}$ and $H(PMEDAPy)^{\pm}$, respectively, where the protons are at the phosphonate group, were determined by titrating 25 ml of aqueous 0.02 M HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 3.4 mM ligand ($PMEDAPy^{-}$) under N_2 with 2.5 ml of 0.2 M NaOH. The differences in NaOH consumption between such a pair of titrations were used for the calculations. The pH range evaluated was 1.9–7.1 which corresponds initially already to a neutralization degree of about 85% for the $H_2(PMEDAPy)^{+}$/$H(PMEDAPy)^{\pm}$ system (Eqs. (3a) and (3b)), i.e., only 15% of $H_2(PMEDAPy)^{+}$ are left for titration. At pH 7.1 a neutralization degree of 75% is reached for the $H(PMEDAPy)^{\pm}$/$PMEDAPy^{-}$ system meaning that only about 25% of $H(PMEDAPy)^{\pm}$ remain untitrated. The final result for $K^{H}_{H_2(PMEDAPy)}$ is the average of 6 pairs of independent titrations.

An analogous set of six titration pairs was carried out with *O*-phosphonatomethylcholine ($PMCh^{-}$; $(CH_3)_3N^{+}–CH_2CH_2–O–CH_2–PO_3^{2-}$) to determine for reasons of comparison also in this case the acidity constant $K^{H}_{H_2(PMCh)}$ for the release of the first proton from the $–P(O)(OH)_2$ residue of $H_2(PMCh)^{+}$. From the same experiments (see above) also a value for $K^{H}_{H(PMCh)}$ was obtained which was within the error limits identical with the one previously measured [26].

The acidity constant $K^{H}_{H(PMEDAPy)}$ of $H(PMEDAPy)^{\pm}$ (Eqs. (4a) and (4b)) was also determined by titrating 50 ml of aqueous 0.4 mM HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 0.3 mM ligand ($PMEDAPy^{-}$) under N_2 with 0.8 ml of 0.03 M NaOH. The differences in NaOH consumption between such a pair of titrations were evaluated in the pH range 4.9–8.3 which corresponds approximately to $pK_a \pm 1.7$, i.e. to 6.6 ± 1.7 (see Table 1 in Section 3.1). In other words, the initial formation degree of $H(PMEDAPy)^{\pm}$ amounts to about 98% and at pH 8.3 about 2% are left, meaning that now 98% of $PMEDAPy^{-}$ are present. The final result is the average of in total 48 pairs of independent titrations including the ones from above.

The stability constants $K^{M}_{M(PMEDAPy)}$ of the $M(PMEDAPy)^{+}$ complexes (Eqs. (5a) and (5b)) were determined under the same conditions as used in the second set of experiments for the acidity constant of $H(PMEDAPy)^{\pm}$ but now $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ (25 °C; $I = 0.1$ M). The M^{2+}:ligand ratios employed now were identical to those used in a recent study [26]. The evaluation of the experimental data was done by a curve fitting procedure using a Newton–Gauss non-linear least-squares program which was applied in the described way.

For all M^{2+}/PMEDAPy systems it holds that the results showed no dependence on the excess of M^{2+} used in the experiments. The final results given for the stability constant $K^{M}_{M(PMEDAPy)}$ of the various complexes (see Section 3.2) are always the averages of at least five (usually six) independent pairs of titrations.

Table 1
Negative logarithms of the acidity constants (Eqs. (3a), (3b), (4a) and (4b)) for aqueous solutions of $H_2(PMEDAPy)^+$ and $H(PMEDAPy)^\pm$ as well as of some related species at 25 °C and $I = 0.1$ M ($NaNO_3$) as determined by potentiometric pH titrations

No.	$H_2(R–PO_3)$[a]	$pK^H_{H_2(R–PO_3)}$	$pK^H_{H(R-PO_3)}$	ΔpK_a[b]	Ref.
1	$H_2(PMEDAPy)^+$	1.14 ± 0.15	6.62 ± 0.01	5.48 ± 0.15	–[c]
2	$H_2(PMCh)^+$	1.11 ± 0.12[c]	6.57 ± 0.01[d]	5.46 ± 0.12	–
3	$CH_3P(O)(OH)_2$	2.10 ± 0.03	7.51 ± 0.01	5.41 ± 0.03	[29]
4	$CH_3OP(O)(OH)_2$	1.10 ± 0.20	6.36 ± 0.01	5.26 ± 0.20	[30]
5	$H_2(PME)$	1.57 ± 0.15[e]	7.02 ± 0.01		[19]

The errors given are three times the standard error of the mean value or the sum of the probable sytematic errors, whichever is larger. So-called practical (or mixed) acidity constants [27] are listed; see Section 2.1.

[a] See the list of abbreviations (Appendix A).

[b] $\Delta pK_a = pK^H_{H(R-PO_3)} - pK^H_{H_2(R–PO_3)}$.

[c] This work.

[d] From Ref. [26].

[e] Estimated value based on the results of entries 1–4; no measured value is available.

3. Results and discussion

3.1. Acid-base properties of $H_2(PMEDAPy)^+$

For the quaternary 2,4-diaminopyrimidinium residue we could not detect any proton affinity down to a pH of 1. Therefore, the proton-accepting properties of $PMEDAPy^-$ are defined by the $–PO_3^{2-}$ group which may accept two protons. Hence, if protonated, for the release of these protons from $H_2(PMEDAPy)^+$ the following two equilibria need to be considered:

$$H_2(PMEDAPy)^+ \rightleftharpoons H(PMEDAPy)^\pm + H^+ \quad (3a)$$

$$K^H_{H_2(PMEDAPy)} = [H(PMEDAPy)^\pm][H^+]/[H_2(PMEDAPy)^+] \quad (3b)$$

$$H(PMEDAPy)^\pm \rightleftharpoons PMEDAPy^- + H^+ \quad (4a)$$

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

The acidity constants for these two equilibria were determined by potentiometric pH titrations in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$). The results are listed in Table 1 together with the corresponding acidity constants of several closely related species [19,26,29,30].

If one considers the structure of $PMEDAPy^-$ in Fig. 1, it is evident that the structure of *O*-phosphonatomethylcholine ($PMCh^-$; $(CH_3)_3N^+–CH_2CH_2–O–CH_2–PO_3^{2-}$) is closely related to it. Indeed, comparison of entries 1 and 2 in Table 1 shows that the acidity constants for the release of the first protons from the $–P(O)(OH)_2$ group are identical within the error limits for both, $H_2(PMEDAPy)^+$ and $H_2(PMCh)^+$. This confirms that the nucleobase residue does not participate in the acid–base reactions. This conclusion is further substantiated by entries 3 and 4 of Table 1 and the ΔpK_a values which result from the differences between the various acidity constants and which are listed in column 5, since all these ΔpK_a values are identical within the error limits. This observation is remarkable since it proves that the release of both protons from the $–P(O)(OH)_2$ group is affected to the same extent by the positively charged residue.

A further comparison is most interesting, namely the one between entries 1 and 5, i.e., for the monoprotonated $H(PME)^-$ [$CH_3CH_2–O–CH_2–P(O)_2(OH)^-$] and $H(PMEDAPy)^\pm$ species. This comparison shows the effect of the positively charged pyrimidinium group ($DAPy^+$) on the deprotonation of the relatively distant $–P(O)_2(OH)^-$ group in $H(PMEDAPy)^\pm$, and it results in $\Delta pK_{a/DAPy/H(PMEDAPy)} = pK^H_{H(PME)} - pK^H_{H(PMEDAPy)} = (7.02 \pm 0.01) - (6.62 \pm 0.01) = 0.40 \pm 0.01$. Indeed, this value is very similar to that obtained for the corresponding comparison between entries 2 and 5 involving the $(CH_3)_3N^+$ residue, i.e., $\Delta pK_{a/(CH_3)_3N/H(PMCh)} = (7.02 \pm 0.01) - (6.57 \pm 0.01) = 0.45 \pm 0.01$.

3.2. Stability constants of $M(PMEDAPy)^+$ complexes

All of the experiments with PMEDAPy and the alkaline earth ions, several divalent 3d ions as well as Zn^{2+} or Cd^{2+}, including also $Cu(Bpy)^{2+}$ and $Cu(Phen)^{2+}$ ($= M^{2+}$), may be completely described by considering equilibria (4a), (4b), (5a) and (5b) (see Section 2.2), as long as the evaluation is not carried into the pH range where hydroxo complexes form.

$$M^{2+} + PMEDAPy^- \rightleftharpoons M(PMEDAPy)^+ \quad (5a)$$

$$K^M_{M(PMEDAPy)} = [M(PMEDAPy)^+]/([M^{2+}][PMEDAPy^-]) \quad (5b)$$

The stability constants determined in this study are listed in column 3 of Table 2 together with related data [31]. These constants (entries 1–10) show the usual trends, that is, that complex stability of the alkaline earth ions decreases with increasing ionic radii. For the divalent 3d metal ions the long standing experience

Table 2
Logarithms of the stability constants of $M(PMEDAPy)^{+}$ complexes (Eqs. (5a) and (5b)) as determined by potentiometric pH titrations (column 3) in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$), together with the corresponding calculated stability constants for an unaffected metal ion-phosphonate coordination in $M(R–PO_3)$ complexes (see text in Section 3.2) and the resulting stability differences log $\Delta_{M/PMEDAPy}$ (Eq. (6)). The corresponding stability enhancements (Eq. (7)) due to a M^{2+}–ether oxygen interaction in the M(PME–R) complexes are also given (column 6; from [31]) together with the overall inhibitory effect of the positively charged pyrimidinium group in the $M(PMEDAPy)^{+}$ complexes as defined by Eq. (8) (see column 7)

No.	M^{2+}	log $K^{M}_{M(PMEDAPy)}$	log $K^{M}_{M(R-PO_3)}$	log $\Delta_{M/PMEDAPy}$	log $\Delta_{M/PME-R}$	Δ log Δ_{M}
1	Mg^{2+}	1.43 ± 0.05	1.65 ± 0.03	−0.22 ± 0.06	0.16 ± 0.04	0.38 ± 0.07
2	Ca^{2+}	1.20 ± 0.04	1.50 ± 0.05	−0.30 ± 0.06	0.12 ± 0.05	0.42 ± 0.08
3	Sr^{2+}	0.94 ± 0.07	1.27 ± 0.04	−0.33 ± 0.08	0.09 ± 0.05	0.42 ± 0.09
4	Ba^{2+}	0.82 ± 0.11	1.20 ± 0.04	−0.38 ± 0.12	0.11 ± 0.05	0.49 ± 0.13
5	Mn^{2+}	2.06 ± 0.03	2.26 ± 0.05	−0.20 ± 0.06	0.19 ± 0.06	0.39 ± 0.08
6	Co^{2+}	1.86 ± 0.05	2.03 ± 0.06	−0.17 ± 0.08	0.20 ± 0.06	0.37 ± 0.10
7	Ni^{2+}	1.75 ± 0.05	2.04 ± 0.05	−0.29 ± 0.07	0.14 ± 0.07	0.41 ± 0.10
8	Cu^{2+}	3.06 ± 0.03	3.06 ± 0.06	0.00 ± 0.07	0.48 ± 0.07	0.48 ± 0.10
9	Zn^{2+}	2.11 ± 0.04	2.27 ± 0.06	−0.16 ± 0.07	0.29 ± 0.07	0.45 ± 0.10
10	Cd^{2+}	2.45 ± 0.04	2.58 ± 0.05	−0.13 ± 0.06	0.30 ± 0.05	0.43 ± 0.08
11	$Cu(Bpy)^{2+}$	3.60 ± 0.04	3.09 ± 0.07	+0.51 ± 0.08		
12	$Cu(Phen)^{2+}$	3.73 ± 0.04	3.10 ± 0.06	+0.63 ± 0.07		

The error limits given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The error limits (3σ) of the derived data (in columns 5-7) were calculated according to the error propagation after Gauss.

[19,20,32,33] is confirmed that the stabilities of phosph(on)ate-metal ion complexes often do not strictly follow [34] the Irving–Williams sequence [35].

What is the effect of the relatively distant positively charged pyrimidinium group on the stability of the $M(PMEDAPy)^{+}$ complexes? This question can be addressed by making use of the recently constructed [19] (see also [32,33]) log $K^{M}_{M(R-PO_3)}$ versus p$K^{H}_{H(R-PO_3)}$ plots for $M(R–PO_3)$ complexes, where $R–PO_3^{2-}$ are simple phosph(on)ate ligands, that is, their residue R does not affect complex formation (see Fig. 2). Plots of this type result as expected [36] in straight lines; the parameters of these (least-squares) reference lines are summarized in Table 5 of [19] for the binary complexes and in Table 5 of [23] for the ternary (mixed ligand) complexes (see also Table 1 in Ref. [32]). This previous achievement allows now the calculation of the stability constant of a pure phosph(on)ate coordination with the acidity constant of any monoprotonated phosph(on)ate group.

With these reference line equations and p$K^{H}_{H(PMEDAPy)}$ of $H(PMEDAPy)^{\pm}$ (Table 1, entry 1), the logarithms of the stability constants, log $K^{M}_{M(R-PO_3)}$, for the corresponding $M(R–PO_3)$ complexes were calculated (Table 2, column 4). The stability of these complexes reflects the basicity of the $–PO_3^{2-}$ group, that is, of a hypothetical '$PMEDAPy^{2-}$' in which no additional effect of the positively charged pyrimidinium ring exists on metal ion binding at the phosphonate group. Now the differences between the measured and these calculated stability constants can be formed according to Eq. (6) (see column 5 of Table 2):

$$\log \Delta_{M/PMEDAPy} = \log K^{M}_{M(PMEDAPy)} - \log K^{M}_{M(R-PO_3)} \quad (6)$$

Nearly all of these stability differences are clearly negative (entries 1–10); hence, the positively charged pyrimidinium residue reduces the stability of these complexes.

The described result probably becomes even more apparent from the two examples of log $K^{M}_{M(R-PO_3)}$ versus p$K^{H}_{H(R-PO_3)}$ plots in Fig. 2 [19,37]. The data pairs for the Mg^{2+} and Zn^{2+} complexes with $PMEDAPy^{-}$ are clearly below the reference lines; the distance from a given data point to its reference line (full line) corresponds to log $\Delta_{M/PMEDAPy}$ as defined in Eq. (6).

3.3. A more detailed appraisal of the effect of the positively charged pyrimidinium residue

From Fig. 2 and especially from the data in column 5 of Table 2 (entries 1–10), it is evident that the log $\Delta_{M/PMEDAPy}$ values vary depending on the kind of metal ion considered. As the affinity of the various metal ions toward ether oxygen differs [36], this effect also needs to be taken into account. Indeed, analogous to Eq. (6) a stability enhancement may be defined for M(PME–R) complexes which reflects the extent of the participation of the ether oxygen in equilibrium (1) (for details see [18–20,31,32]). This stability enhancement is expressed by Eq. (7),

$$\log \Delta_{M/PME-R} = \log K^{M}_{M(PME-R)} - \log K^{M}_{M(PME-R)_{op}} \quad (7)$$

where $K^{M}_{M(PME-R)_{op}}$ represents the stability constant of the open (op) isomer seen at the left side in equilibrium (1). Values for log $\Delta_{M/PME-R}$ were established previously [31] and they are listed in column 6 of Table 2. All of these values are positive and from the two examples (Mg^{2+}, Zn^{2+}) shown in Fig. 2, it is evident

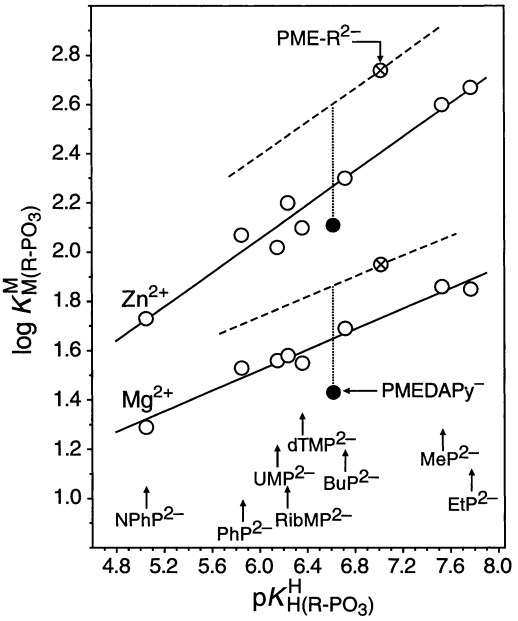


Fig. 2. Evidence for a reduced stability of the $Mg(PMEDAPy)^+$ and $Zn(PMEDAPy)^+$ complexes (●), based on the relationship between $\log K^{M}_{M(R-PO_3)}$ and $pK^{H}_{H(R-PO_3)}$ for the 1:1 complexes of Mg^{2+} and Zn^{2+} with some simple phosphate monoester or phosph(on)ate ligands ($R-PO_3^{2-}$) (○): 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate (PhP^{2-}), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5-monophosphate ($RibMP^{2-}$), thymidine [= 1-(2′-deoxy-β-D-ribofuranosyl)thymine] 5′-monophosphate ($dTMP^{2-}$), *n*-butyl phosphate (BuP^{2-}), methanephosphonate (MeP^{2-}), and ethanephosphonate (EtP^{2-}) (from left to right). The least-squares lines are drawn through the corresponding eight data sets (○) taken from Ref. [37] for the phosphate monoesters and from Ref. [19] for the phosphonates. The parameters for the slopes *m* and the intercepts *b* of the straight lines are tabulated in Refs. [19] or [32]. The points due to the equilibrium constants for the M^{2+}/PME–R systems (⊗) are based on the values measured for PME and 1-[2-(phosphonomethoxy)ethyl]cytosine (for details see Ref. [31]). These points quantify the extent of the participation of the ether oxygen in the $-CH_2CH_2-O-CH_2-PO_3^{2-}$ chain in complex formation, and they define the broken lines parallel to the main (solid) reference lines. The values for the PMEDAPy systems (●) are from Tables 1 and 2. The vertical dotted lines emphasize the reduction in complex stability due to charge repulsion between the positively charged pyrimidinium residue and M^{2+} in the $M(PMEDAPy)^+$ complexes; the length of these dotted lines equals $\Delta \log \Delta_M$ as defined in Eq. (8) (Table 2, column 7). All the plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$).

that they are significantly above their reference lines. It may be added that these values allow to calculate [19,20,31–33,36] the formation degree of the closed (cl) isomer in equilibrium (1); it varies for the M(PME–R) complexes [31] between about 19 and 67% for $Sr(PME-R)_{cl}$ and $Cu(PME-R)_{cl}$, respectively; a further representative example is $Zn(PME-R)_{cl}$ with about 49%.

The given $\log \Delta_{M/PME-R}$ values (Table 2, column 6), if added to the calculated stability of the $M(R-PO_3)$ complexes (column 4), reflect the expected stability of the $M(PMEDAPy)^+$ complexes (by considering the basicity of the $-PO_3^{2-}$ group of $PMEDAPy^-$) if the pyrimidinium ring would be 'innocent'. However, this is clearly not the case as we have already seen (Table 2, column 5; Section 3.2), in fact, the repulsive influence of the pyrimidinium ring on the stability of the $M(PMEDAPy)^+$ complexes can now obviously be quantified by Eq. (8) (cf. also Fig. 2):

$$\Delta\log \Delta_M = -\log \Delta_{M/PMEDAPy} + \log \Delta_{M/PME-R} \quad (8)$$

The corresponding $\Delta\log \Delta_M$ values are listed in column 7 of Table 2 and these values correspond to the vertical dotted distances seen in Fig. 2. It is revealing to observe that all these $\Delta\log \Delta_M$ values are identical within their error limits. The arithmetic (and the weighted) mean of the 10 values gives $\Delta\log \Delta_{M/av} = 0.42 \pm 0.04$ (3σ), and again, all other values overlap within the error limits with this result. In other words, the effect of the positively charged pyrimidinium ring on the stability of the $M(PMEDAPy)^+$ complexes is for all metal ions studied identical and this must mean that all of them exist only in a phosphonate-coordinated form, equilibrium (1) being of no significance, that is, being on its left side. Considering that an M^{2+}–ether oxygen interaction would bring the pyrimidinium ring very close to the metal ion giving rise to an increased repulsion, this observation is easily rationalized. The mentioned average inhibiting effect of the positively charged pyrimidinium ring on M^{2+} binding (the altered basicity of the $-PO_3^{2-}$ group already taken into account) corresponds to that of the same positively charged unit on the release of H^+ from the $-P(O)_2(OH)^-$ group, i.e., $\Delta pK_{a/DAPy/H(PMEDAPy)} = 0.40 \pm 0.01$ (see the last paragraph in Section 3.1).

In addition, it is interesting to note that the $(CH_3)_3N^+$ group of *O*-phosphonatomethylcholine ($PMCh^-$), i.e., $(CH_3)_3N^+-CH_2CH_2-O-CH_2-PO_3^{2-}$, has within the error limits the same stability-inhibiting effect in $M(PMCh)^+$ complexes [26], i.e., $\Delta\log \Delta_{M/av/M(PMCh)} = 0.46 \pm 0.05$. In these latter type complexes the one with Cu^{2+} is apparently a little bit more destabilized than those of the other metal ions [26], whereas in the present case $Cu(PMEDAPy)^+$ shows the same properties as all the other $M(PMEDAPy)^+$ complexes studied.

3.4. Stability of the mixed ligand $Cu(Arm)(PMEDAPy)^+$ complexes

For the ternary complexes composed of $PMEDAPy^-$, Cu^{2+} and a heteroaromatic amine (Arm), i.e., 2,2′-bipyridine (Bpy) or 1,10-phenanthroline (Phen), the same evaluation procedure holds as indicated in the first paragraph of Section 3.2 because complex formation of $Cu(Bpy)^{2+}$ and $Cu(Phen)^{2+}$, due to their high stability [38], is complete (for details see [26]) before the onset of the formation of the mixed ligand complexes. This means, also for the ternary

complexes the situation described by equilibria (5a) and (5b) applies, but now $M^{2+} = Cu(Arm)^{2+}$. The corresponding determined stability constants are given in entries 11 and 12 of Table 2 (column 3).

One way to quantify the stability of mixed ligand complexes [39,40] is to consider Eq. (9a); the corresponding constant (Eq. (9b)) is calculated with Eq. (10):

$$Cu(Arm)^{2+} + Cu(PMEDAPy)^{+} \rightleftharpoons Cu(Arm)(PMEDAPy)^{+} + Cu^{2+} \quad (9a)$$

$$10^{\Delta\log K} = \frac{[Cu(Arm)(PMEDAPy)^{+}][Cu^{2+}]}{[Cu(Arm)^{2+}][Cu(PMEDAPy)^{+}]} \quad (9b)$$

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

In case that a further identification of $\Delta\log K$ for a certain equilibrium is needed, this will be given by additional subscripts, like $\Delta\log K_{Cu/Arm/PMEDAPy}$.

According to the general rule for complex stabilities that $K_1 > K_2$, Eq. (9a) is expected to lie on the left with negative values for $\Delta\log K$ in agreement with statistical considerations, i.e., $\Delta\log K_{Cu/statist} \simeq -0.5$ [40]. The values for the corresponding Bpy and Phen systems according to Eq. (10) are (constants, from Table 2, entries 9,11,12):

$$\Delta\log K_{Cu/Bpy/PMEDAPy} = (3.60 \pm 0.04) - (3.06 \pm 0.03) \quad (11a)$$

$$= 0.54 \pm 0.05 \quad (11b)$$

$$\Delta\log K_{Cu/Phen/PMEDAPy} = (3.73 \pm 0.04) - (3.06 \pm 0.03) \quad (12a)$$

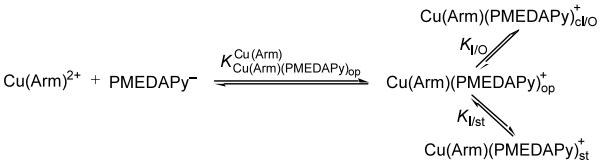
$$= 0.67 \pm 0.05 \quad (12b)$$

These values are clearly larger than the statistically expected value; furthermore, since $\Delta\log K_{Cu/Arm/PMEDAPy} \simeq 0.6$ corresponds to $10^{\Delta\log K} = 4$, the position of Eq. (9a) is significantly shifted to the right hand side. Consequently, these ternary complexes show an increased stability!

This increased stability of the ternary Cu^{2+} complexes may also be quantified independently of the stability of the binary $Cu(PMEDAPy)^{+}$ complex. This means, by using the previously established [23,32] straight-line correlations for $\log K^{Cu(Arm)}_{Cu(Arm)(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ plots, where $R-PO_3^{2-}$ represents phosphate monoester or phosphonate ligands in which the residue R is unable to interact with $Cu(Arm)^{2+}$. The stability of these $Cu(Arm)(R-PO_3)$ complexes corresponds to the stability of the open species, $Cu(Arm)(PMEDAPy)^{+}_{op}$, seen at the left in equilibrium (2), and these calculated values are given in the fourth column of entries 11 and 12 of Table 2. Application of these data to Eq. (6) confirms (see Table 2, column 5) the above conclusion that the ternary complexes $Cu(Bpy)(PMEDAPy)^{+}$ and $Cu(Phen)(PMEDAPy)^{+}$ are more stable indeed than expected. Clearly, such an increased stability proves [36] that a further interaction must take place.

3.5. Evaluation of the increased stability of the $Cu(Arm)(PMEDAPy)^{+}$ complexes. Conclusions regarding their structure in solution

In principle, considering the structure of PMEDAPy (Fig. 1), one could think of two reasons leading to an increased stability: (i) An interaction of the metal ion with the ether oxygen (cl/O) or (ii) aromatic-ring stacking (st) between the aromatic rings of the coordinated ligands. This is expressed in equilibrium scheme (13):



(13)

However, since there is no evidence for a Cu^{2+}–ether oxygen interaction in the binary $Cu(PMEDAPy)^{+}$ complex, which behaves like all the other binary complexes studied (see Section 3.3, Table 2, column 7), there is no reason to assume that this is different in the ternary complexes since the distance to the positive charge, which is responsible for the inhibition of the M^{2+}–ether interaction, is the same in the binary and in the ternary complexes. This positive charge at N1 of the quaternary pyrimidinium ring is of course also the reason why there is no indication for any metal ion interaction with N3. Hence, only the lower part of equilibrium scheme (13) is of relevance and accordingly the necessary constants can be defined as given in Eqs. (14) and (15):

$$K^{Cu(Arm)}_{Cu(Arm)(PMEDAPy)_{op}} = \frac{[Cu[(Arm)(PMEDAPy)^{+}_{op}]}{[Cu(Arm)^{2+}][PMEDAPy^{-}]} \quad (14)$$

$$K_{I/st} = \frac{[Cu(Arm)(PMEDAPy)^{+}_{st}]}{[Cu(Arm)(PMEDAPy)^{+}_{op}]} \quad (15)$$

As shown previously [20,22,23], Eqs. (5b), (14) and (15) can be combined to allow the calculation of values for the dimensionless intramolecular equilibrium constant $K_{I/st}$:

$$K_{I/st} = \frac{K^{Cu(Arm)}_{Cu(Arm)(PMEDAPy)}}{K^{Cu(Arm)}_{Cu(Arm)(PMEDAPy)_{op}}} - 1 \quad (16a)$$

$$= 10^{\log \Delta} - 1 \quad (16b)$$

The expression $\log \Delta$ which appears in Eq. (16b) is defined analogously to Eq. (6), i.e., as $\log \Delta_{Cu(Arm)/PMEDAPy}$. Of course, knowledge of $K_{I/st}$ allows calculation of the percentage of the stacked isomer occurring in the lower part of equilibrium scheme (13) as well as in equilibrium (2):

$$\%Cu(Arm)(PMEDAPy)^{+}_{st} = 100K_{I/st}/(1 + K_{I/st}) \quad (17)$$

Table 3
Extent of intramolecular stack formation in ternary Cu(Arm)(N) complexes, where N = PMEDAPy$^-$, PMEC^{2-} or CMP^{2-}, as calculated from stability constants determined via potentiometric pH titrations: given is the stability enhancement log $\Delta_{Cu(Arm)/N}$ (Eq. (6)), the intramolecular and dimensionless equilibrium constant K_I (Eqs. (15), (16a) and (16b)), and the percentage of the stacked Cu(Arm)(N)$_{st}$ species in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$)

No.	Cu(Arm)(N)	log $\Delta_{Cu(Arm)/N}$	$K_{I/st}$	%Cu(Arm)(N)$_{st}$	Ref.
1a	Cu(Bpy)(PMEDAPy)$^+$	0.51 ± 0.08	2.24 ± 0.60	69 ± 6	–[a]
b	Cu(Phen)(PMEDAPy)$^+$	0.63 ± 0.07	3.27 ± 0.69	77 ± 4	–[a]
2a	Cu(Bpy)(PMEC)	–[b]	–[b]	63 ± 8	[42]
b	Cu(Phen)(PMEC)	–[b]	–[b]	67 ± 4	[42]
3a	Cu(Bpy)(CMP)	0.31 ± 0.08	1.04 ± 0.38	51 ± 9	[41]
b	Cu(Phen)(CMP)	0.42 ± 0.07	1.63 ± 0.44	62 ± 6	[41]

See footnote 'a' of Table 2.
[a] This work.
[b] In these cases a M^{2+}–ether oxygen interaction occurs also in one of the isomers of the ternary complexes. For an example of such a system see Table 7 in Ref. [23].

Application of Eqs. (6), (16a), (16b) and (17) leads to the results summarized in Table 3 where also some related data [41,42] are given.

The results in Table 3 confirm in several ways that the arguments initially presented are correct and that intramolecular stack formation according to equilibrium (2) is the reason for the observed enhanced stability of the ternary complexes: (i) The formation degree of the stacked isomer involving Phen is somewhat larger than the one with Bpy (Table 3, entry 1); considering the extent of the possible overlap of the aromatic rings, this is most reasonable and also in accord with related earlier observations [22,41,43]. Furthermore, (ii) the cytosine moiety is structure-wise relatively similar to the quaternary 2,4-diaminopyrimidinium residue, and indeed, the formation degree of stacking is also rather similar; it amounts for Cu(Bpy)(PMEC) and Cu(Phen)(PMEC), where PMEC, 1-[2-(phosphonomethoxy)ethyl]cytosine, to 63 ± 8 and 67 ± 7%, respectively (Table 3, entry 2). Similarly, for the complexes with the parent nucleotide cytidine 5′-monophosphate (CMP^{2-}) the percentages of stacking are 51 ± 9% and 62 ± 6% for Cu(Bpy)(CMP) and Cu(Phen)(CMP), respectively (Table 3, entry 3). That the values for the present systems appear to be slightly larger may be due to the positive charge at the pyrimidine ring which possibly promotes charge transfer interactions within the stacks somewhat.

A tentative and simplified structure of the stacked isomer occurring in equilibrium (1) and the equilibrium scheme (13) is shown in Fig. 3. Certainly, this intramolecular stacking interaction could in addition be proven via spectrophotometric (charge transfer) measurements [44] as previously carried out for related mixed ligand nucleotide systems [12,22,45,46]. We considered such an attempt as superfluous in the present case, especially as stack formation between Pt(Phen)(ethylenediamine)$^{2+}$ and CMP is also known [22] and crystal structure

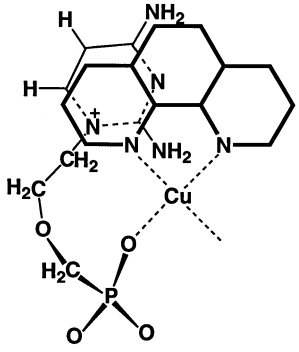


Fig. 3. Tentative and simplified structure of a Cu(Phen)(PMEDAPy)$^+$ species with an intramolecular stack. The orientation of the aromatic rings may vary among the stacked species; such a complex in solution should not be considered as being rigid.

studies of related Cu(Arm)(nucleotide) complexes exist [47].

Finally, the described results allow a further interesting comparison: In Section 3.4 in the context of Eqs. (11a), (11b), (12a) and (12b) we have seen that the values for $\Delta\log K_{Cu/Arm/PMEDAPy}$ are significantly positive and in the order of about 0.6. However, the corresponding values for simple but related systems like those with ethanephosphonate (EtP^{2-}) are $\Delta\log K_{Cu/Bpy/EtP} = 0.01 \pm 0.02$ and $\Delta\log K_{Cu/Phen/EtP} = 0.02 \pm 0.02$ [23]; moreover, if other simple O-donor ligands like acetate [40] or methyl phosphate [48] are employed, similar results are obtained, i.e., values for $\Delta\log K_{Cu/Arm/O\text{-}donor}$ are always close to zero. Hence, the results of Eqs. (11b) and (12b) reflect practically completely the stability contribution of the intramolecular stack formation. Indeed, the values of $\Delta\log K_{Cu/Arm/PMEDAPy}$ are within the error limits identical with the corresponding log $\Delta_{Cu(Arm)/PMEDAPy}$ values given in Table 3 (column 3, entry 1). This demonstrates the internal consistency of the results presented and it also confirms the above conclusion that the observed increased complex stability

of the ternary species is solely due to stack formation and that an ether oxygen–metal ion interaction is of no significance.

4. Conclusions

Anchoring of a substrate in the active-site cavity of an enzyme is certainly an important condition for biological activity. The present results show that the quaternary 2,4-diamino-pyrimidinium moiety is able to undergo stacking interactions and considering its structure this is most likely also the case as far as hydrogen bonding is concerned. Hence, it seems to us that the observed biological inactivity of PMEDAPy must have other reasons.

It was previously concluded [17,18] that the metal ion–ether oxygen interaction facilitates the correct positioning of the metal ions along the triphosph(on)ate chain and that this is crucial for the biological activity, i.e., the insertion of this kind of artificial acyclic nucleotide analogue, like $PMEApp^{4-}$, into the growing nucleic acid chain as catalyzed by metal ion-dependent DNA polymerases. Of course, due to the lack of a 3′-hydroxy group, after insertion chain termination occurs. We suggest now that $PMEDAPy^{-}$ has no antiviral effects, i.e., is biologically inactive, despite the presence of the essential ether oxygen because it is unable to coordinate to a metal ion due to the nearby positive charge at N1 of the pyrimidine ring.

Acknowledgements

The competent technical assistance of Mrs Rita Baumbusch and Mrs Astrid Sigel in the preparation of this manuscript is gratefully acknowledged. This study was supported by the Swiss National Science Foundation (H.S.) and within the COST D20.002 programme by the Swiss Federal Office for Education & Science (H.S.) and the Ministry of Education of the Czech Republic (A.H.), as well as by the University of Barcelona (V.M.). The study is also part of the research project No. 4055905 of the Institute of Organic Chemistry and Biochemistry in Prague, Czech Republic (A.H.).

Appendix A: Abbreviations and definitions

Arm	heteroaromatic nitrogen base, e.g. Bpy or Phen
Bpy	2,2′-bipyridine
CMP^{2-}	cytidine 5′-monophosphate
$HPMPC^{2-}$	see Fig. 1
I	ionic strength
M^{2+}	any divalent metal ion (in some instances also $Cu(Bpy)^{2+}$ and $Cu(Phen)^{2+}$ are represented by this abbreviation)
Phen	1,10-phenanthroline
$PMCh^{-}$	*O*-phosphonatomethylcholine
PME^{2-}	dianion of (phosphonatomethoxy)ethane (= ethoxymethane phosphonate)
$PMEA^{2-}$	dianion of 9-[2-(phosphonomethoxy)ethyl]adenine
$PMEApp^{4-}$	diphosphorylated $PMEA^{2-}$
$PMEC^{2-}$	dianion of 1-[2-(phosphonomethoxy)ethyl]cytosine
$PMEDAP^{2-}$	see Fig. 1
$PMEDAPy^{-}$	see Fig. 1
$PME–R^{2-}$	derivative of PME^{2-} with a residue R that does not affect complex formation
$R–PO_3^{2-}$	simple phosph(on)ate ligand with a residue R that does not affect complex formation (see also legend to Fig. 2) (in a few instances also other compounds with a $–PO_3^{2-}$ group, e.g. $PMEDAPy^{-}$, are represented by this abbreviation)

Species that are given in the text without a charge either do not carry one or represent the species in general (i.e., independent of their degree of protonation); which of the two versions applies is always clear from the context.

References

[1] A. Holý, J. Günter, H. Dvořáková, M. Masojídková, G. Andrei, R. Snoeck, J. Balzarini, E. De Clercq, J. Med. Chem. 42 (1999) 2064.
[2] A. Holý, I. Votruba, M. Masojídková, G. Andrei, R. Snoeck, L. Naesens, E. De Clercq, J. Balzarini, J. Med. Chem. 45 (2002) 1918.
[3] H. Sigel, Chem. Soc. Rev. 22 (1993) 255.
[4] E. De Clercq, T. Sakuma, M. Baba, R. Pauwels, J. Balzarini, I. Rosenberg, A. Holý, Antiviral Res. 8 (1987) 261.
[5] (a) J. Balzarini, C.F. Perno, D. Schols, E. De Clercq, Biochem. Biophys. Res. Commun. 178 (1991) 329;
(b) L. Naesens, J. Balzarini, I. Rosenberg, A. Holý, E. De Clercq, Eur. J. Clin. Microbiol. Infect. Dis. 8 (1989) 1043.
[6] E. De Clercq, Collect. Czech. Chem. Commun. 63 (1998) 480.
[7] A. Holý, M. Buděšínský, J. Podlaha, I. Císařová, Collect. Czech. Chem. Commun. 64 (1999) 242.
[8] L. Naesens, R. Snoeck, G. Andrei, J. Balzarini, J. Neyts, E. De Clercq, Antiviral Chem. Chemother. 8 (1997) 1.
[9] (a) G. Birkuš, I. Votruba, A. Holý, B. Otová, Biochem. Pharmacol. 58 (1999) 487;
(b) G. Birkuš, I. Votruba, M. Otmar, A. Holý, Collect. Czech. Chem. Commun. 66 (2001) 1698.
[10] (a) H. Pelletier, M.R. Sawaya, A. Kumar, S.H. Wilson, J. Kraut, Science 264 (1994) 1891;
(b) H. Pelletier, M.R. Sawaya, W. Wolfle, S.H. Wilson, J. Kraut, Biochemistry 35 (1996) 12762.
[11] J.D. Crowley, D.A. Traynor, D.C. Weatherburn, Met. Ions Biol. Syst. 37 (2000) 209.
[12] R.B. Gómez-Coca, L.E. Kapinos, A. Holý, R.A. Vilaplana, F. González-Vílchez, H. Sigel, Metal Based Drugs 7 (2000) 313.

[13] G. Kampf, M.S. Lüth, L.E. Kapinos, J. Müller, A. Holý, B. Lippert, H. Sigel, Chem. Eur. J. 7 (2001) 1899.
[14] R.B. Gómez-Coca, L.E. Kapinos, A. Holý, R.A. Vilaplana, F. González-Vílchez, H. Sigel, J. Chem. Soc., Dalton Trans. (2000) 2077.
[15] C.A. Blindauer, T.I. Sjåstad, A. Holý, E. Sletten, H. Sigel, J. Chem. Soc., Dalton Trans. (1999) 3661.
[16] C.A. Blindauer, A.H. Emwas, A. Holý, H. Dvořáková, E. Sletten, H. Sigel, Chem. Eur. J. 3 (1997) 1526.
[17] H. Sigel, B. Song, C.A. Blindauer, L.E. Kapinos, F. Gregáň, N. Prónayová, Chem. Commun. (1999) 743.
[18] H. Sigel, Pure Appl. Chem. 71 (1999) 1727.
[19] H. Sigel, D. Chen, N.A. Corfù, F. Gregáň, A. Holý, M. Strašák, Helv. Chim. Acta 75 (1992) 2634.
[20] (a) H. Sigel, Coord. Chem. Rev. 144 (1995) 287;
(b) H. Sigel, J. Indian Chem. Soc. 74 (1997) 261 (P. Ray Award Lecture).
[21] (a) O. Yamauchi, A. Odani, M. Takani, J. Chem. Soc., Dalton Trans. (2002) 3411;
(b) R.K.O. Sigel, E. Freisinger, B. Lippert, J. Biol. Inorg. Chem. 5 (2000) 287.
[22] O. Yamauchi, A. Odani, H. Masuda, H. Sigel, Met. Ions. Biol. Syst. 32 (1996) 207.
[23] D. Chen, M. Bastian, F. Gregáň, A. Holý, H. Sigel, J. Chem. Soc., Dalton Trans. (1993) 1537.
[24] H. Sigel, Pure Appl. Chem. 61 (1989) 923.
[25] R.B. Gómez-Coca, L.E. Kapinos, A. Holý, R.A. Vilaplana, F. González-Vílchez, H. Sigel, J. Inorg. Biochem. 84 (2001) 39.
[26] A. Fernández-Botello, R.B. Gómez-Coca, A. Holý, V. Moreno, H. Sigel, Inorg. Chim. Acta 331 (2002) 109.
[27] H. Sigel, A.D. Zuberbühler, O. Yamauchi, Anal. Chim. Acta 255 (1991) 63.
[28] H.M. Irving, M.G. Miles, L.D. Pettit, Anal. Chim. Acta 38 (1967) 475.
[29] H. Sigel, C.P. Da Costa, B. Song, P. Carloni, F. Gregáň, J. Am. Chem. Soc. 121 (1999) 6248.
[30] A. Saha, N. Saha, L.-n. Ji, J. Zhao, F. Gregáň, S.A.A. Sajadi, B. Song, H. Sigel, J. Biol. Inorg. Chem. 1 (1996) 231.
[31] C.A. Blindauer, A. Holý, H. Sigel, Collect. Czech. Chem. Commun. 64 (1999) 613.
[32] H. Sigel, L.E. Kapinos, Coord. Chem. Rev. 200–202 (2000) 563.
[33] H. Sigel, B. Song, Met. Ions. Biol. Syst. 32 (1996) 135.
[34] H. Sigel, D.B. McCormick, Acc. Chem. Res. 3 (1970) 201.
[35] H. Irving, R.J.P. Williams, J. Chem. Soc. (1953) 3192.
[36] R.B. Martin, H. Sigel, Comments Inorg. Chem. 6 (1988) 285.
[37] S.S. Massoud, H. Sigel, Inorg. Chem. 27 (1988) 1447.
[38] (a) G. Anderegg, Helv. Chim. Acta 46 (1963) 2397;
(b) H. Irving, D.H. Mellor, J. Chem. Soc. (1962) 5222.
[39] (a) H. Sigel, Angew. Chem., Int. Ed. Engl. 14 (1975) 394;
(b) H. Sigel, in: D. Banerjea (Ed.), Coordination Chemistry-20, IUPAC, Pergamon Press, Oxford, 1980, pp. 27–45.
[40] R. Malini-Balakrishnan, K.H. Scheller, H.K. Häring, R. Tribolet, H. Sigel, Inorg. Chem. 24 (1985) 2067.
[41] S.S. Massoud, H. Sigel, Inorg. Chim. Acta 159 (1989) 243.
[42] (a) C.A. Blindauer, Inaugural Dissertation, Universität Basel, 1998.;
(b) C.A. Blindauer, A. Holý, H. Sigel, to be published.
[43] S.S. Massoud, R. Tribolet, H. Sigel, Eur. J. Biochem. 187 (1990) 387.
[44] (a) P.R. Mitchell, H. Sigel, J. Am. Chem. Soc. 100 (1978) 1564;
(b) P.R. Mitchell, B. Prijs, H. Sigel, Helv. Chim. Acta 62 (1979) 1723.
[45] Y. Fukuda, P.R. Mitchell, H. Sigel, Helv. Chim. Acta 61 (1978) 638.
[46] M.S. Lüth, L.E. Kapinos, B. Song, B. Lippert, H. Sigel, J. Chem. Soc., Dalton Trans. (1999) 357.
[47] K. Aoki, Met. Ions. Biol. Syst. 32 (1996) 91.
[48] J. Zhao, B. Song, N. Saha, A. Saha, F. Gregáň, M. Bastian, H. Sigel, Inorg. Chim. Acta 250 (1996) 185.