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Metal ion-binding properties of 9-(4-phosphonobutyl)adenine (dPMEA), a sister compound of the antiviral nucleotide analogue 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), and quantification of the equilibria involving four Cu(PMEA) isomers

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Received 28th February 2000, Accepted 3rd May 2000
Published on the Web 13th June 2000

The acidity constants of the threefold protonated acyclic 9-(4-phosphonobutyl)adenine, $H_3(dPMEA)^+$, as well as the stability constants of the M(H;dPMEA)$^+$ and M(dPMEA) complexes with the metal ions M^{2+} = Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} or Cd^{2+}, have been determined by potentiometric pH titrations, in aqueous solution at I = 0.1 M ($NaNO_3$) and 25 °C. Application of previously determined straight-line plots of log $K^{M}_{M(R\text{-}PO_3)}$ *versus* p$K^{H}_{H(R\text{-}PO_3)}$ for simple phosph(on)ate ligands, R-PO_3^{2-}, where R represents a residue without an affinity for metal ions, proves that the primary binding site of $dPMEA^{2-}$ is the phosphonate group with all the metal ions studied; in fact, in most instances the stability is solely determined by the basicity of the phosphonate residue. Only for the Ni(dPMEA), Cu(dPMEA) and Cd(dPMEA) systems a stability increase due to macrochelate formation with the adenine residue occurs; the formation degrees are 21 ± 15%, 31 ± 14% and 29 ± 18%, respectively. In these three instances the additional interaction of the phosphonate-coordinated M^{2+} occurs most probably with N7; hence, $dPMEA^{2-}$ is more similar in its metal ion-binding properties to the parent nucleotide adenosine 5′-monophosphate (AMP^{2-}) than to the antivirally active and structurally more related dianion of 9-[2-(phosphonomethoxy)ethyl]-adenine ($PMEA^{2-}$). This result agrees with the observation that replacement of the ether O atom in PMEA by a CH_2 unit leads to a compound, *i.e.* dPMEA, devoid of any biological activity. In addition, use is made of the stability enhancement obtained for the Cu(dPMEA) system due to macrochelate formation to analyze the equilibria regarding the four isomeric complex species possibly formed in the Cu(PMEA) system. It is shown that a macrochelated isomer involving N7 of the adenine residue occurs with Cu(PMEA) only in trace amounts; the important isomers in this system involve the ether oxygen (formation degree *ca.* 34%) and also N3 of the adenine moiety (*ca.* 41%).

1. Introduction

Nucleotides like adenosine 5′-monophosphate (AMP^{2-})[1] and its diphosphorylated product, adenosine 5′-triphosphate (ATP^{4-}) as well as their 2′-deoxy derivatives ($dAMP^{2-}$ and $dATP^{4-}$), are at the crossroad of many metabolic processes.[2] Therefore, the idea to exploit nucleotide analogues as therapeutic agents is old. Among the many attempts, the acyclic nucleoside phosphonates proved promising[3,4] and one of the better known compounds of this class is 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), also named Adefovir.[5] The dianion of PMEA can be viewed as an analogue of AMP^{2-} (Fig. 1)[6–9] or $dAMP^{2-}$. The oral prodrug of PMEA, *i.e.* the diester bis(pivaloyloxymethyl)–PMEA (also called Adefovir Dipivoxil), is currently being evaluated in patients infected with the human immunodeficiency virus (HIV) or the hepatitis B virus (HBV).[4,5] Moreover, very recently it was discovered that the same prodrug also possesses antiarthritic properties.[10]

In the course of studies focussed on the antiviral properties of compounds closely related to PMEA, it soon became evident[3] that the ether oxygen separated from the phosphonyl group by a methylene unit is compulsory for a biological activity. For example, replacement of this oxygen by sulfur[11] or a CH_2 group[3] led to compounds devoid of any antiviral activity; similarly, shortening or lengthening of the chain between the phosphonyl group and the adenine residue resulted in inactive products;[12] the same is observed with most chain-substituted derivatives of PMEA.[13,14]

In order to be antivirally active, PMEA must be phosphorylated in the cell[15] to the diphosphate derivative, $PMEApp^{4-}$, an analogue of (d)ATP^{4-}, it is then recognized by DNA polymerases as a substrate and thus incorporated into the growing nucleic acid chain, which is then terminated due to the lack of a 3′-hydroxy group.[15,16] Indeed, $PMEApp^{4-}$ is initially an excellent substrate for several polymerases; for example, even in the presence of a 20-fold excess of dATP, *in vitro* DNA synthesis by avian myeloblastosis-virus reverse-transcriptase is depressed to 50% within 5 minutes.[3,13]

Knowing that for the activation of a nucleoside 5′-triphosphate two metal ions have to be coordinated to the triphosphate chain[17] and that for the transfer of a nucleotidyl unit one metal ion must be bound to the β,γ-phosphate groups and the other to the α-group, it was recently concluded[18,19] that $PMEApp^{4-}$ is initially a better substrate than $dATP^{4-}$ because of the higher basicity of the phosphonyl group (compared to a phosphoryl group) and the possibility of a coordinated metal ion (*e.g.*,

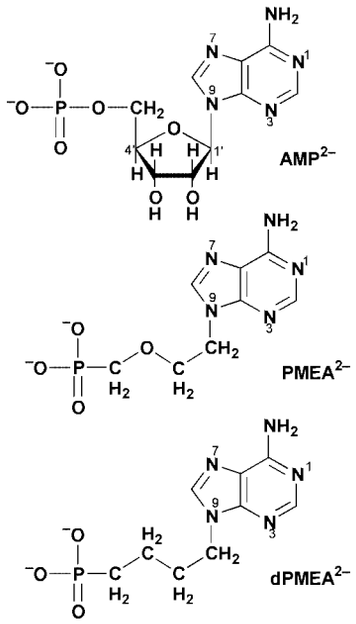


Fig. 1 Chemical structures of the dianions of 9-[2-(phosphonomethoxy)ethyl]adenine (= $PMEA^{2-}$ = Adefovir) and 9-(4-phosphonobutyl)-adenine (= $dPMEA^{2-}$ = 3′-deoxa-$PMEA^{2-}$) and of their parent nucleotide adenosine 5′-monophosphate (AMP^{2-}), which is shown in its dominating *anti* conformation.[6–8] The orientation of $PMEA^{2-}$ in solution[9] is similar to the *anti* conformation of AMP^{2-}.

Mg^{2+}) to form a five-membered chelate with the ether oxygen. These two properties facilitate the M(α)–M(β,γ) coordination pattern needed for the enzyme-catalyzed incorporation of the substrate in the growing nucleic acid chain and thus favour $PMEApp^{4-}$ over $dATP^{4-}$ (for details see ref. 19).

This conclusion is in accord with the above mentioned observation that the ether oxygen of the acyclic chain is compulsory for obtaining antivirally active nucleotide analogues. Hence, it was of interest to study the metal ion-binding properties of the carba analogue of PMEA, *i.e.* of 9-(4-phosphonobutyl)-adenine which is also known as 3′-deoxa-PMEA (dPMEA) or 4-(adenin-9-yl)butylphosphonic acid (Fig. 1). Indeed, the results of the present study show that the properties of the metal ion complexes formed with $PMEA^{2-}$ and $dPMEA^{2-}$ differ considerably.

2. Experimental

2.1 Materials

Twofold protonated 9-(4-phosphonobutyl)adenine, *i.e.* $H_2(dPMEA)^{\pm}$, was synthesized (by modification of an earlier procedure)[20] as described below in Section 2.2 with 1,4-dibromobutane, triethyl phosphite, 1,8-diazabicyclo[5.4.0]-undec-7-ene, bromotrimethylsilane and dimethylformamide, which were purchased from Sigma-Aldrich, Prague, Czech Republic. The aqueous stock solutions of the ligand were freshly prepared daily just before the experiments by dissolving the substance in deionized, ultrapure (MILLI-Q185 PLUS; from Millipore S.A., 67120 Molsheim, France) CO_2-free water and adding 2 equivalents of NaOH.

The disodium salt of 1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid (Na_2H_2EDTA), potassium hydrogen phthalate, HNO_3, NaOH (Titrisol), and the nitrate salts of Na^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} (all *pro analysi*) were from Merck AG, Darmstadt, FRG. All solutions for the potentiometric pH titrations were prepared with ultrapure CO_2-free water. Dimethylformamide and acetonitrile, used for the synthesis (Section 2.2), were also obtained from Merck; they were dried by distillation from phosphorus pentoxide and stored over molecular sieves.

The buffer solutions (pH 4.00, 7.00, 9.00 based on the NBS scale; now NIST) for calibration (Section 2.3) were from Metrohm AG, Herisau, Switzerland.

The exact concentrations of the stock solutions of the divalent metal ions were determined by potentiometric pH titrations *via* their EDTA complexes. The exact concentration of the ligand solutions was in each experiment newly determined by the evaluation of the corresponding titration pairs described below in Section 2.4.

2.2 Synthesis of 9-(4-phosphonobutyl)adenine

The first step in the synthesis of 9-(4-phosphonobutyl)adenine [= 4-(adenin-9-yl)butylphosphonic acid] was heating of a mixture of 1,4-dibromobutane (100 g, 0.46 mol) and triethyl phosphite (90 mL, 1.125 equiv.) for 2 days at 110 °C; the resulting ethyl bromide was distilled off. The portion volatile at 100 °C/2 kPa was removed. The residue was utilized in the next step without further purification.

The mixture of adenine (20.0 g, 0.148 mol), 1,8-diazabicyclo-[5.4.0]undec-7-ene (30 mL) and crude diethyl 4-bromobutyl-phosphonate (65 mL) in dimethylformamide (200 mL) was heated at 100 °C for 5 h and cooled down. The crystalline material was filtered off, washed successively with dimethyl-formamide and acetone, and recrystallized from 80% aqueous ethanol. Yield, 12.4 g (25.6%) diethyl 4-(adenin-9-yl)butyl-phosphonate. This compound (8.2 g, 25 mmol) in acetonitrile (100 mL) was stirred with bromotrimethylsilane (15 mL) and the solution was set aside overnight at ambient temperature. The volatiles were evaporated *in vacuo* and the residue co-distilled with toluene (2 × 25 mL). Water (150 mL) was added and, after 20 min, the mixture was basified with conc. aqueous ammonia and evaporated. The residue in minimum water was applied to a column of Dowex 50 × 8 (H^+-form) (250 mL) and the column was washed with water to the loss of acidity and UV-absorption of the eluate (monitored at 254 nm). Subsequent eluation of the column with diluted (1 : 10) aqueous ammonia gave a UV-absorbing eluate which was evaporated to dryness *in vacuo*. This residue in minimum water was basified with ammonia to pH 10 and applied onto a column of Dowex 1 × 2 (acetate form) prewashed with water (200 mL); the column was eluted with water (1 L) and 0.25 M acetic acid (500 mL). The resin was then stirred batchwise with 1 M formic acid (500 mL), filtered off and washed with boiling water (four 500 mL portions). The combined eluates were evaporated *in vacuo*, the residue codistilled with water (five 50 mL portions) and crystallized from water. Yield, 4.3 g (64%), mp 287 °C. For $C_9H_{14}N_5O_3P{\cdot}H_2O$ (289.2) calculated 37.37% C, 5.58% H, 24.21% N, 10.71% P; found 37.20% C, 5.44% H, 24.00% N, 11.00% P. ^{1}H-NMR (D_2O + NaOD, 500 MHz): δ 8.11 s, 1H and 8.10 s, 1H (H-2 and H-8); 4.17 t, 2H, $J(1',2') = 7.3$ (H-1′); 1.88 qnt, 2H, $J(2',1') = J(2',3') = 7.3$ (H-2′); 1.54 m, 2H and 1.44 m, 2H (H-3′ and H-4′).

2.3 Potentiometric pH titrations

The pH titration curves for the determination of the equilibrium constants in H_2O were recorded with a Metrohm E536 potentiograph connected to a Metrohm E535 dosimat and a Metrohm 6.0222.100 combined macro glass electrode. The pH calibration of the instrument was done with the buffers mentioned above. The titer of the NaOH used was determined with potassium hydrogen phthalate.

The direct pH meter readings were used in the calculations 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.[21] They may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values;[21] this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[21,22] 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 evaluated the differences in NaOH consumption between a pair of solutions, *i.e.* with and without ligand (see below in Section 2.4). The stability constants determined are, as usual, concentration constants.

All equilibrium constants were calculated by curve-fitting procedures in the way and with the equipment described recently.[23]

2.4 Determination of equilibrium constants

The acidity constants $K^{H}_{H_2(dPMEA)}$ and $K^{H}_{H(dPMEA)}$ of $H_2(dPMEA)^{\pm}$, where one proton is at the base moiety and the other at the phosphonate group, were determined by titrating 50 mL of aqueous 0.9 mM HNO_3 ($I = 0.1$ M, $NaNO_3$; 25 °C) in the presence and absence of 0.3 mM $dPMEA^{2-}$ under N_2 with 1.55 mL of 0.03 M NaOH. The differences in NaOH consumption between such a pair of titrations were used for the calculations. The pH range evaluated was determined by the lowest point of neutralization reached under these experimental conditions (*ca.* 30% for the equilibrium $H_2(dPMEA)^{\pm}/H(dPMEA)^{-}$) and the neutralization degree of about 98% for the equilibrium $H(dPMEA)^{-}/dPMEA^{2-}$. The results are the averages of 38 pairs of independent titrations.

The acidity constant for the release of the first proton from the phosphonate group in $H_3(dPMEA)^{+}$, $K^{H}_{H_3(dPMEA)}$, was determined by titrating 20 mL of aqueous 0.03 M HNO_3 ($I = 0.1$ M, $NaNO_3$; 25 °C) in the presence and absence of 1.66 mM $dPMEA^{2-}$ under N_2 with 6 mL of 0.1 M NaOH and by using the differences in NaOH consumption between such a pair of titrations for the calculations. The highest formation degree reached for $H_3(dPMEA)^{+}$ is close to 50%. The result is the average of 9 pairs of titrations.

The stability constants $K^{M}_{M(H;dPMEA)}$ and $K^{M}_{M(dPMEA)}$ of $M(H;dPMEA)^{+}$ and M(dPMEA) were determined under the same conditions as the acidity constants $K^{H}_{H_2(dPMEA)}$ and $K^{H}_{H(dPMEA)}$, but $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ ($I = 0.1$ M, 25 °C). The M^{2+}/dPMEA ratios were 111 : 1 (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}), 89 : 1 (Mg^{2+}), 56 : 1 (Co^{2+}, Ni^{2+}, Cd^{2+}), 28 : 1 (Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}), 14 : 1 (Cd^{2+}), 11 : 1 (Cu^{2+}, Zn^{2+}), 5.6 : 1 (Cu^{2+}) and 5 : 1 (Zn^{2+}).

The stability constants were calculated by considering H^{+}, $H_2(dPMEA)^{\pm}$, $H(dPMEA)^{-}$, $dPMEA^{2-}$, M^{2+}, $M(H;dPMEA)^{+}$ and M(dPMEA) and by collecting the experimental data every 0.1 pH unit from about 2% complex formation of $M(H;dPMEA)^{+}$ to a neutralization degree of about 90% with respect to the species $H(dPMEA)^{-}$, or until the beginning of the hydrolysis of $M(aq)^{2+}$, which was evident from the titrations without ligand. The maximal formation degree for the M(dPMEA) complexes varies between 30 and 70%, depending on the metal ion. However, for the Zn(dPMEA) and Cd(dPMEA) complexes only a formation degree of about 2.2% and 19% was reached, respectively; hence, the value for Zn(dPMEA) must be considered as an estimate.

The individual results for the stability constants showed no dependence on pH or on the excess of metal ion concentration used. The results are in each case the averages of at least 5 independent pairs of titration curves. However, most of the constants given for the $M(H;dPMEA)^{+}$ complexes must also be considered as estimates (see Table 2 below), since the formation degree of these species reached only about 4 to 10% in the maximum.

3. Results and discussion

The ligand 9-(4-phosphonobutyl)adenine [=4-(adenin-9-yl)butylphosphonic acid] was synthesized by modification of a described procedure:[20] 1,4-dibromobutane gave by reaction with triethyl phosphite diethyl 4-bromobutylphosphonate which was used in the following step without purification; alkylation of adenine with this synthon in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dimethylformamide[24] proceeded smoothly under formation of diethyl 4-(adenin-9-yl)butylphosphonate which, in turn, gave on transsilylation with bromotrimethylsilane followed by hydrolysis the target compound. The purification was performed by desalting on cation ion-exchanger followed by anion-exchange chromatography and crystallization from water.

Since derivatives of purines are well known to undergo self-association *via* π-stacking,[25] a concentration[26] of 3×10^{-4} M was used in this study for dPMEA in the potentiometric pH titration experiments to ascertain that only the properties of the monomeric species are quantified.

3.1 Acidity constants of $H_3(dPMEA)^{+}$

From the structure of $dPMEA^{-}$ (see Fig. 1) it is evident that this species can accept three protons, two at the phosphonate group and one at the N1 site of the adenine residue.[6] Further protonations of an adenine residue are possible at N7 and N3, but these protons are released with $pK_a < 0$,[9,27,28] and therefore, they are not considered in this study. At pH > 0 the strongest acid that can be derived in aqueous solution from $dPMEA^{2-}$ is $H_3(dPMEA)^{+}$. Hence, the following three deprotonation reactions, in which $dPMEA^{2-}$ is abbreviated as PA^{2-}, need to be considered:

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

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

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

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

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

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

Indeed, all the experimental data obtained from the potentiometric pH titrations in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$) could be excellently fitted by taking into account equilibria (1) to (3). Furthermore, in the pH range above 3.6 only equilibria (2) and (3) need to be considered. The acidity constants obtained in the present study for $H_3(dPMEA)^{+}$ are given in Table 1, together with some related data.[7,9,26,29–32]

Comparison of the values listed in Table 1 confirms the above statement that the first and third proton of $H_3(dPMEA)^{+}$ are from the phosphonate group and the second proton is released from the N1 site of the adenine residue. However, more comparisons are possible; a few are emphasized below:

(i) From the comparisons of entry 2 with 6 and of 3, 4 with 5 it follows that the protonated phosphonate groups are less acidic than a corresponding phosphate group due to the lower electronegativity of a C atom compared to an O atom.

(ii) The ether oxygen, due to its electron-withdrawing effect, makes the protonated phosphonate species of PMEA more acidic than those of dPMEA (entries 3, 4).

(iii) In fact, this acidification is equal for both the $H(PA)^{-}$ and $H_3(PA)^{+}$ species as the following comparison shows (entries 3, 4): $\Delta pK_{a/1} = pK^{H}_{H(dPMEA)} - pK^{H}_{H(PMEA)} = (7.69 \pm 0.01) - (6.90 \pm 0.01) = 0.79 \pm 0.01$ and $\Delta pK_{a/3} = pK^{H}_{H_3(dPMEA)} - pK^{H}_{H_3(PMEA)} = (1.98 \pm 0.13) - (1.22 \pm 0.13) = 0.76 \pm 0.18$.

(iv) This observation contrasts with the acid–base properties of N1 which remain unaffected by the presence of the oxygen atom in the acyclic chain (see column 4, entries 3, 4).

(v) A further remarkable observation is that the following differences are all equal within their error limits: $pK^{H}_{H(dPMEA)} - pK^{H}_{H_3(dPMEA)} = (7.69 \pm 0.01) - (1.98 \pm 0.13) = 5.71 \pm 0.13$ (entry 3), $pK^{H}_{H(PMEA)} - pK^{H}_{H_3(PMEA)} = (6.90 \pm 0.01) - (1.22 \pm 0.13) = 5.68 \pm 0.13$ (entry 4), and $pK^{H}_{H(AMP)} - pK^{H}_{H_3(AMP)} = (6.21 \pm 0.01) - (0.4 \pm 0.2) = 5.8 \pm 0.2$ (entry 5).

Table 1 Negative logarithms of the acidity constants of $H_3(dPMEA)^+$ [equilibria (1) to (3)] together with the corresponding values of some related systems in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$)[a,b]

No.	Protonated species	$pK^H_{H_3(PA)}$: $P(O)(OH)_2$	$pK^H_{H_2(PA)}$: $(N1)H^+$	$pK^H_{H(PA)}$: $P(O)_2(OH)^-$	Ref.
1	$H(9MeAde)^+$		4.10 ± 0.01		29
2	$CH_3P(O)(OH)_2$	2.10 ± 0.03		7.51 ± 0.01	30
3	$H_3(dPMEA)^+$	1.98 ± 0.13	4.17 ± 0.02	7.69 ± 0.01	—
4	$H_3(PMEA)^+$	1.22 ± 0.13[c]	4.16 ± 0.02	6.90 ± 0.01	9,[c] 31
5	$H_3(AMP)^+$	0.4 ± 0.2[c]	3.84 ± 0.02	6.21 ± 0.01	7,[c] 26
6	$CH_3OP(O)(OH)_2$	1.1 ± 0.2		6.36 ± 0.01	32

[a] The error limits given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] So-called practical, mixed or Brønsted constants are listed (see also Section 2.3). [c] Determined by ^{1}H-NMR shift measurements.

Finally, it needs to be pointed out that due to the different affinities of the phosph(on)ate groups for protons the formation degree of the free ligands, PA^{2-}, differs considerably in the physiological pH range around 7.5: Under these conditions AMP^{2-} is formed to about 95% whereas $PMEA^{2-}$ still reaches about 80%, yet $dPMEA^{2-}$ occurs only to about 40%.

3.2 Stability constants of the $M(H;dPMEA)^+$ and M(dPMEA) complexes

The experimental data of the potentiometric pH titrations (see Section 2.3) allow the determination of the stability constants defined by equilibria (4a) and (5a):

$$M^{2+} + H(PA)^- \rightleftharpoons M(H;PA)^+ \quad (4a)$$

$$K^M_{M(H;PA)} = [M(H;PA)^+]/([M^{2+}]\,[H(PA)^-]) \quad (4b)$$

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

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

Overall, equilibria (2)–(5) are sufficient to obtain excellent fitting of the titration data, provided the evaluation is not carried into the pH range where formation of hydroxo species occurs, which was evident from titrations without ligand. Of course, equilibria (4a) and (5a) are also connected *via* equilibrium (6a)

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

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

and the corresponding acidity constant [eqn. (6b)] may be calculated with eqn. (7):

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

The results are listed in Table 2; the stability constants given for the $M(H;dPMEA)^+$ complexes are only estimates since the formation degree of these species was low (see Section 2.3). The stability constants of the M(dPMEA) complexes show the usual trends. For the alkaline earth ions the stability of the complexes decreases with increasing ionic radii indicating that metal ion binding at the phosphonate group is (at least) in part inner-sphere. For the divalent 3d metal ions the long-standing experience[33,34] is confirmed that the stabilities of phosph(on)ate–metal ion complexes often do not strictly follow[23,26,31–38] the Irving–Williams sequence,[39] an observation in accord with the fact that in ligands of this kind the phosph(on)ate group is always the main binding site[23,26,36–38,40] in M(PA) complexes (see Section 3.3).

As far as the $M(H;dPMEA)^+$ complexes are concerned, it is evident that the evaluation of potentiometric pH titration data only allows the determination of their stability constants. Further information is required to detect the binding sites of the proton and the metal ion. At first one may ask where the proton is located because binding of a metal ion to a protonated ligand commonly leads to an acidification of the ligand-bound proton.[41] Indeed, the acidity constants of the $M(H;dPMEA)^+$ complexes given in column 4 of Table 2 are by about 1.2 to 2 log units smaller than $pK^H_{H(dPMEA)}$ (Table 1), but 1.4 to 2.3 log units larger than $pK^H_{H_2(dPMEA)}$. This comparison shows that the proton in $M(H;dPMEA)^+$ is bound to the phosphonate group, hence, one may tentatively assume that the metal ion is bound preferentially to the nucleobase, since a monoprotonated phosphonate group is only a weak binding site. Indeed, this suggestion agrees with evidence obtained previously for other related $M(H;PA)^+$ species.[31,40,42]

Table 2 Logarithms of the stability constants of the $M(H;dPMEA)^+$ [eqn. (4b)] and M(dPMEA) complexes [eqn. (5b)], together with the negative logarithms of the acidity constants of the protonated complexes [eqns. (6b) and (7)] in aqueous solution at 25 °C and $I = 0.1$ M, ($NaNO_3$)[a]

M^{2+}	$\log K^M_{M(H;dPMEA)}$[b]	$\log K^M_{M(dPMEA)}$	$pK^H_{M(H;dPMEA)}$
Mg^{2+}	0.3 ± 0.4	1.84 ± 0.04	6.2 ± 0.4
Ca^{2+}	0.2 ± 0.4	1.57 ± 0.05	6.3 ± 0.4
Sr^{2+}	0.1 ± 0.4	1.30 ± 0.04	6.5 ± 0.4
Ba^{2+}	0.0 ± 0.4	1.22 ± 0.06	6.5 ± 0.4
Mn^{2+}	0.4 ± 0.4	2.47 ± 0.03	5.6 ± 0.4
Co^{2+}	0.7 ± 0.3	2.31 ± 0.06	6.1 ± 0.3
Ni^{2+}	1.0 ± 0.3	2.41 ± 0.06	6.3 ± 0.3
Cu^{2+}	1.78 ± 0.13	3.72 ± 0.07	5.75 ± 0.15
Zn^{2+}	1.4 ± 0.3	2.75 ± 0.2[b]	6.3 ± 0.4
Cd^{2+}	1.4 ± 0.3	3.08 ± 0.10[b]	6.0 ± 0.3

[a] For the error limits see footnote *a* of Table 1. The error limits (3σ) of the derived data, in the present case for column 4, were calculated according to the error propagation after Gauss. [b] The constants listed for the $M(H;dPMEA)^+$ complexes are estimates (see last paragraph in Section 2.4). The experiments with Zn^{2+} were significantly hampered by precipitation; *i.e.* the pH range accessible for the evaluation of the constants was severely restricted. The same problem, though less severe, occurred also with Cd^{2+} (see also Section 2.4).

Furthermore, the stability constants of the $M(H;dPMEA)^+$ complexes are within the error limits identical with the values determined[31] for the corresponding $M(H;PMEA)^+$ species. Considering that the basicity of the N1 site in $H(dPMEA)^-$ and $H(PMEA)^-$ is also identical (see Table 1; column 4, entries 3,4) and that evidence has been provided for the $M(H;PMEA)^+$ complexes[31,40] that the metal ion is mainly located at the nucleobase residue, one may not only conclude that in the $M(H;dPMEA)^+$ complexes the proton is at the phosphonate group, but also that the metal ion is mainly at the adenine residue. The N1 *versus* N7 dichotomy for metal ion binding to the adenine residue is well known[43] though there are indications that binding to N7 dominates.[43,44] In any case, the fact that the stabilities of the $M(H;dPMEA)^+$ complexes *follow* the Irving–Williams sequence[39] (in contrast to phosph(on)ate complexes) also supports[34] the above conclusion that metal ion binding occurs preferably to a nitrogen atom.

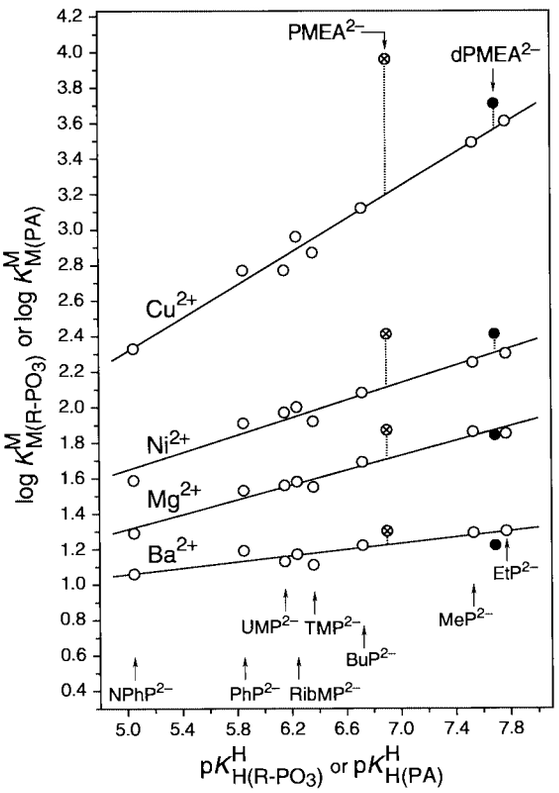


Fig. 2 Evidence for an enhanced stability of the Ni(dPMEA) and Cu(dPMEA) complexes, and for the lack of such an enhanced stability of the Ba(dPMEA) and Mg(dPMEA) species (●), together with the corresponding metal ion complexes of $PMEA^{2-}$ (⊗), which all show an enhanced stability, based on the relationship between log $K^{M}_{M(R\text{-}PO_3)}$ and $pK^{H}_{H(R\text{-}PO_3)}$ for M(R-PO_3) complexes of some simple phosphate monoester and phosphonate ligands (R-PO_3^{2-}) (○): 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate (PhP^{2-}), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5-monophosphate ($RibMP^{2-}$), thymidine [=1-(2-deoxy-β-D-ribofuranosyl)thymine] 5′-monophosphate (TMP^{2-}), *n*-butyl phosphate (BuP^{2-}), methanephosphonate (MeP^{2-}), and ethanephosphonate (EtP^{2-}) (from left to right). The least-squares lines [eqn. (8)] are drawn through the corresponding 8 data sets (○) taken from ref. 46 for the phosphate monoesters and from ref. 31 for the phosphonates. The points due to the equilibrium constants for the M^{2+}/dPMEA systems (●) are based on the values listed in Tables 1 and 2; those for the M^{2+}/PMEA systems (⊗) are from ref. 31. The vertical broken lines emphasize the stability differences from the reference lines; they equal log $\Delta_{M/dPMEA}$ as defined in eqn. (10) for the M(dPMEA) complexes. All the plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$).

3.3 Evaluation of the stabilities of the M(dPMEA) complexes

The question that arises for the M(dPMEA) complexes is: does the adenine residue also participate in metal ion binding next to the phosphonate group? Should such an additional interaction with the adenine residue occur then it has to be reflected in an increased complex stability.[45] Hence, it is necessary to define the stability of a pure $-PO_3^{2-}/M^{2+}$ interaction. This can be done by applying the previously defined[31,38] straight-line correlations which are based on log $K^{M}_{M(R\text{-}PO_3)}$ *versus* $pK^{H}_{H(R\text{-}PO_3)}$ plots for simple phosphate monoesters[46] and phosphonates;[31] these ligands are abbreviated as R-PO_3^{2-}, where R represents a non-coordinating residue. The parameters for the corresponding straight-line equations, which are defined by eqn. (8), have been

$$\log K^{M}_{M(R\text{-}PO_3)} = m \cdot pK^{H}_{H(R\text{-}PO_3)} + b \qquad (8)$$

tabulated,[26,31,36a,38] *i.e.* the slopes *m* and the intercepts *b* with the *y*-axis. Hence, with a known pK_a value for the deprotonation of a $-P(O)_2(OH)^-$ group an expected stability constant can be calculated for any phosph(on)ate–metal ion complex.

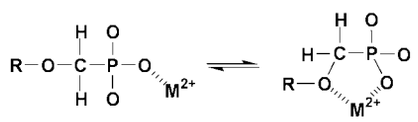
The plots of log $K^{M}_{M(R\text{-}PO_3)}$ *versus* $pK^{H}_{H(R\text{-}PO_3)}$ according to eqn. (8) are shown in Fig. 2 for the 1:1 complexes of Ba^{2+}, Mg^{2+}, Ni^{2+} and Cu^{2+}, as examples, with the data points (empty circles) of the eight simple ligand systems used[31] for the determination of the straight baselines. The four solid points refer to the corresponding M(dPMEA) complexes; those for the Ni^{2+} and Cu^{2+} species are somewhat above their reference lines, thus proving an increased stability for these two complexes, whereas the data points for the Mg^{2+} and Ba^{2+} complexes fit on the lines (or are even slightly below). In contrast, all of the data points[31] of the M(PMEA) complexes (crossed circles) are above their reference lines indicating that all these species are more stable than is expected on the basis of the basicity of the phosphonate group of $PMEA^{2-}$.

Table 3 Stability constant comparisons for the M(dPMEA) complexes between the measured stability constants (exptl; Table 2, column 3) and the calculated stability constants (calcd) based on the basicity of the phosphonate group in $dPMEA^{2-}$ ($pK^{H}_{H(dPMEA)} = 7.69$; Table 1) and the baseline equations established previously[26,31,38] [see eqn. (8) and Fig. 2] together with the stability differences log $\Delta_{M/dPMEA}$, as defined by eqn. (10). The previously determined stability enhancements, log $\Delta_{M/PMEA}$, for M(PMEA) complexes are given for comparison[31] (aqueous solution; 25 °C; $I = 0.1$ M, $NaNO_3$)[a]

	log $K^{M}_{M(dPMEA)}$			
M^{2+}	exptl	calcd	log $\Delta_{M/dPMEA}$	log $\Delta_{M/PMEA}$
Mg^{2+}	1.84 ± 0.04	1.87 ± 0.03	−0.03 ± 0.05	0.16 ± 0.05
Ca^{2+}	1.57 ± 0.05	1.64 ± 0.05	−0.07 ± 0.07	0.11 ± 0.07
Sr^{2+}	1.30 ± 0.04	1.36 ± 0.04	−0.06 ± 0.06	0.07 ± 0.05
Ba^{2+}	1.22 ± 0.06	1.29 ± 0.04	−0.07 ± 0.07	0.08 ± 0.06
Mn^{2+}	2.47 ± 0.03	2.51 ± 0.05	−0.04 ± 0.06	0.21 ± 0.08
Co^{2+}	2.31 ± 0.06	2.27 ± 0.06	0.04 ± 0.08	0.28 ± 0.07[b]
Ni^{2+}	2.41 ± 0.06	2.31 ± 0.05	0.10 ± 0.08	0.30 ± 0.07[b]
Cu^{2+}	3.72 ± 0.07	3.56 ± 0.06	0.16 ± 0.09	0.77 ± 0.07[b]
Zn^{2+}	2.75 ± 0.2[c]	2.64 ± 0.06	0.1 ± 0.2	0.30 ± 0.10[d]
Cd^{2+}	3.08 ± 0.10[c]	2.93 ± 0.05	0.15 ± 0.11	0.33 ± 0.06

[a] Regarding the error limits (3σ) see footnote *a* of Tables 1 and 2. [b] These values contain also a contribution[40] of an N3 interaction;[19,31] for Co(PMEA) see ref. 19. [c] See footnote *b* of Table 2. [d] Estimated value.

This increased stability of the M(PMEA) complexes has been proven[19,31] to be largely due to the involvement of the ether oxygen atom in metal ion binding (see Fig. 1) which gives rise to the formation of 5-membered chelates and thus, to the intramolecular equilibrium (9):[19,31,36]



(9)

In certain systems, like Ni(PMEA) and Cu(PMEA), a third isomer exists which involves in addition N3 of the adenine residue[19,31,36,40] (see also Section 3.5).

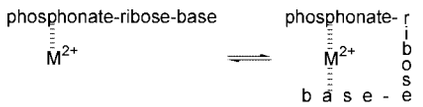
Naturally, in complexes of $dPMEA^{2-}$, whose side-chain is devoid of the ether oxygen atom (Fig. 1), equilibrium (9) cannot exist and therefore, any possibly observed stability increase has to be attributed to an interaction with the adenine residue. Stability enhancements like those seen in Fig. 2 can be quantified by the differences between the experimentally (exptl) measured stability constants and those calculated (calcd) according to eqn. (8); this difference is defined in eqn. (10)

$$\log\Delta_{M/PA} = \log K^{M}_{M(PA)_{exptl}} - \log K^{M}_{M(PA)_{calcd}} \qquad (10a)$$

$$= \log K^{M}_{M(PA)} - \log K^{M}_{M(PA)_{op}} (= \log \Delta) \qquad (10b)$$

where the expressions log $K^{M}_{M(PA)_{calcd}}$ and log $K^{M}_{M(Pa)_{op}}$ are synonymous because the calculated value equals the stability constant of the 'open' isomer, $M(PA)_{op}$ [*e.g.* equilibrium (9)], in which only a $-PO_3^{2-}/M^{2+}$ interaction occurs. In columns 2–4 of Table 3 the values for the three terms of eqn. (10) are listed. It is evident that only the log $\Delta_{M/dPMEA}$ values for

Ni(dPMEA), Cu(dPMEA) and Cd(dPMEA) are positive. The observed stability increases are small but certainly real (note that *three times* the standard error is given); hence, in these instances equilibrium (11) has to be considered:



(11)

3.4 Extent of macrochelate formation in the M(dPMEA) systems of Ni^{2+}, Cu^{2+} and Cd^{2+}

The position of the concentration-independent equilibrium (11) between the simple phosphonate-bound species, which we designate as the 'open' isomer, $M(dPMEA)_{op}$, and the macrochelated isomer involving the adenine residue, designated as the 'closed' species, $M(dPMEA)_{cl}$, is defined by the intramolecular and hence, dimensionless equilibrium constant K_I [eqn. (12)]:

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

Application of log $\Delta_{M/dPMEA}$ (Table 3, column 4) allows one to define the position of equilibrium (11) as shown previously.[26,31,38,45] For the Ni(dPMEA) system one calculates for $K_I = 10^{\log\ \Delta_{Ni/dPMEA}} - 1 = 10^{0.10 \pm 0.08} - 1 = 0.26 \pm 0.23$, for the Cu(dPMEA) system $K_I = 10^{\log\ \Delta_{Cu/dPMEA}} - 1 = 10^{0.16 \pm 0.09} - 1 = 0.45 \pm 0.30$ and for the Cd(dPMEA) system $K_I = 10^{\log\ \Delta_{Cd/dPMEA}} - 1 = 10^{0.15 \pm 0.11} - 1 = 0.41 \pm 0.36$; hence, the formation degree of the macrochelated or closed species amounts to 21 ± 15%, 31 ± 14% and 29 ± 18% for $Ni(dPMEA)_{cl}$, $Cu(dPMEA)_{cl}$ and $Cd(dPMEA)_{cl}$, respectively. For all the other M(dPMEA) systems the formation degree of the closed species in equilibrium (11) is zero within the error limits (see the log $\Delta_{M/dPMEA}$ values in Table 3). In contrast, in the M(PMEA) complexes the 5-membered chelates seen in equilibrium (9) are important for all metal ions as is evident from the log $\Delta_{M/PMEA}$ values given in column 5 of Table 3 [for details and the formation degrees of $M(PMEA)_{cl}$ see refs. 31 and 36; slightly revised values are given in ref. 19] (see also Section 3.5).

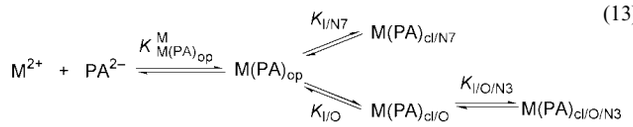
From the increased complex stabilities observed for the Ni^{2+}, Cu^{2+} and Cd^{2+} complexes of $dPMEA^{2-}$, it follows that at least for these three metal ions, which have the most pronounced affinity for N sites among the ten metal ions studied,[34] equilibrium (11) operates and that the adenine residue is involved in metal ion binding. From the three nitrogen atoms available in the purine ring, *i.e.* N1, N3 and N7 (see Fig. 1), N1 cannot be reached by a metal ion already coordinated to the phosphonate group;[36] hence, N3 and N7 remain for macrochelate formation. At this stage one cannot distinguish with certainty between these two possibilities, but we strongly favor an interaction of the phosphonate-coordinated metal ion with N7 because N7 is known to be more basic than N3.[9,27,28] Furthermore, macrochelate formation in the M(AMP) complexes occurs with certainty *via* N7;[26,47] hence, it appears that M(dPMEA) resembles in this respect the parent complexes, *i.e.* M(AMP), more than M(PMEA), though the extent of macrochelate formation is more pronounced in the M(AMP) systems[26,38] than in the M(dPMEA) species.

3.5 Lesson learnt from the properties of Cu(dPMEA) regarding the formation degree of the isomeric complexes formed in the Cu(PMEA) system. A four-isomer problem

Since it is highly likely that the enhanced complex stability observed for Cu(dPMEA) has to be attributed to macrochelate formation [eqn. (11)] of the phosphonate-bound Cu^{2+} with N7 of the adenine residue (Section 3.4), one may ask: is such a macrochelate formation involving N7 also important in the Cu(PMEA) complex system? We are concentrating the attention here on the Cu^{2+} systems since the most pronounced stability enhancement (log $\Delta = 0.16 \pm 0.09$) is observed for Cu(dPMEA) (Table 3).[48] The answer to the question raised is clearly "no" as shown below; such a species forms only in trace amounts in the Cu(PMEA) system.

It has previously been proven[40a] that three isomers are important for the Cu(PMEA) system.[19] (*i*) An 'open' isomer, $Cu(PMEA)_{op}$, in which the metal ion is solely coordinated to the phosphonate group; (*ii*) an isomer which involves the ether oxygen (see Fig. 1) as shown in equilibrium (9), designated as $Cu(PMEA)_{cl/O}$; and (*iii*) an isomer in which not only a 5-membered chelate but in addition a 7-membered one involving N3 exists, *i.e.* $Cu(PMEA)_{cl/O/N3}$.

In the present context it is important to emphasize that for steric reasons no macrochelate involving *only* N3 can be formed with $PMEA^{2-}$ and Cu^{2+}.[36a] If one tries to form such a species with molecular models, one automatically forces the ether oxygen into the coordination sphere of Cu^{2+} giving rise to the already mentioned $Cu(PMEA)_{cl/O/N3}$ isomer.[36a] Hence, we may postulate for Cu(PMEA) that the whole stability increase observed for Cu(dPMEA), if relevant for Cu(PMEA), has to be attributed to macrochelate formation [eqn. (11)] of the phosphonate-bound Cu^{2+} with N7 of the adenine residue leading to the postulation of a fourth isomer designated as $Cu(PMEA)_{cl/N7}$. With these reasonings in mind the simple equilibrium (5a) must be replaced for the Cu(PMEA) system by the rather complicated equilibrium scheme (13):



(13)

The four equilibrium constants of scheme (13) are defined by eqns. (14)–(17):

$$K^{M}_{M(PA)_{op}} = [M(PA)_{op}]/([M^{2+}][PA^{2-}]) \quad (14)$$

$$K_{I/N7} = [M(PA)_{cl/N7}]/[M(PA)_{op}] \quad (15)$$

$$K_{I/O} = [M(PA)_{cl/O}]/[M(PA)_{op}] \quad (16)$$

$$K_{I/O/N3} = [M(PA)_{cl/O/N3}]/[M(PA)_{cl/O}] \quad (17)$$

With these definitions the measured overall stability constant [eqn. (5b)] can be redefined as in eqns. (18a)–(18d):

$$K^{M}_{M(PA)} = \frac{[M(PA)]}{[M^{2+}][PA^{2-}]} \quad (18a)$$

$$= \frac{[M(PA)_{op}] + [M(PA)_{cl/N7}] + [M(PA)_{cl/O}] + [M(PA)_{cl/O/N3}]}{[M^{2+}][PA^{2-}]} \quad (18b)$$

$$= K^{M}_{M(PA)_{op}} + K_{I/N7} \cdot K^{M}_{M(PA)_{op}} + K_{I/O} \cdot K^{M}_{M(PA)_{op}} + K_{I/O/N3} \cdot K_{I/O} \cdot K^{M}_{M(PA)_{op}} \quad (18c)$$

$$= K^{M}_{M(PA)_{op}}(1 + K_{I/N7} + K_{I/O} + K_{I/O} \cdot K_{I/O/N3}) \quad (18d)$$

The connection between the overall intramolecular equilibrium constant[19,31,36] $K_{I/tot}$ and the accessible stability enhancement [eqn. (10)] is given by eqns. (19a)–(19e):

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

$$= \frac{[M(PA)_{cl/tot}]}{[M(PA)_{op}]} \quad (19b)$$

$$= \frac{[M(PA)_{cl/N7}] + [M(PA)_{cl/O}] + [M(PA)_{cl/O/N3}]}{[M(PA)_{op}]} \quad (19c)$$

$$= K_{I/N7} + K_{I/O} + K_{I/O/N3} \cdot K_{I/O} \quad (19d)$$

$$= K_{I/N7} + K_{I/O}\,(1 + K_{I/O/N3}) \quad (19e)$$

Table 4 Intramolecular equilibrium constants (K_I) for the formation of the various Cu(PMEA) isomers as defined in the equilibrium scheme (13), together with the percentages in which the isomers occur in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

No.	eqns. used	log Δ	K_I	Formation degrees in percentages	
1a	(10)	$\log \Delta_{Cu/PMEA}$	$K_{I/tot}$	% $Cu(PMEA)_{cl/tot}$	% $Cu(PMEA)_{op}$
1b	(19a), (19b)	0.77 ± 0.07[b]	4.89 ± 0.95	83 ± 3[c]	17 ± 3[d]
2a	(10), (16)	$\log \Delta_{Cu/PME\text{-}R}$	$K_{I/O}$	% $Cu(PMEA)_{cl/O}$	
2b	[analogous to (19a)] (16)	0.48 ± 0.07[e]	2.02 ± 0.49	34 ± 10[f]	
3a	(10), (15)	$\log \Delta_{Cu/dPMEA}$	$K_{I/N7}$	% $Cu(PMEA)_{cl/N7}$	
3b	see Section 3.4	0.16 ± 0.09[g]	0.45 ± 0.30	7.7 ± 5.3[h]	
4a	(17)		$K_{I/O/N3}$	% $Cu(PMEA)_{cl/O/N3}$	
4b	(19e)		1.20 ± 0.73[i]	41 ± 12[j]	

[a] Regarding the error limits (3σ) see footnote *a* of Tables 1 and 2. Note, in this table always entries (a) and (b) go together. [b] From ref. 31 [see also refs. 19, 36 and 40(*a*)]. [c] Calculated with the equation: % $Cu(PMEA)_{cl/tot} = 100 \times K_{I/tot}/(1 + K_{I/tot})$. [d] This percentage follows from 100 − % $Cu(PMEA)_{cl/tot}$. [e] From ref. 49 (see also ref. 19). [f] Calculated with $K_{I/O}$ and % $Cu(PMEA)_{op}$ by application of eqn. (16). [g] From Table 3. [h] Calculated with $K_{I/N7}$ and % $Cu(PMEA)_{op}$ by application of eqn. (15). [i] This value follows from eqn. (19e) since all the other intramolecular equilibrium constants are now known. [j] This value follows from the difference % $Cu(PMEA)_{cl/tot}$ − % $Cu(PMEA)_{cl/O}$ − % $Cu(PMEA)_{cl/N7}$; it could also be calculated with $K_{I/O/N3}$ and % $Cu(PMEA)_{cl/O}$ by application of eqn. (17).

A value for $K_{I/tot}$ can be calculated [see eqns. (10b) and (19a)] and its relation to the other three intramolecular equilibrium constants follows from eqns. (19b) and (19c). Based on the reasonable assumption[19] that the stability of the $Cu(PMEA)_{cl/O}$ isomer is well represented by that of the $Cu(PME\text{-}R)_{cl/O}$ species, where R in PME-R^{2-} is a nucleobase residue without the ability to interact with metal ions,[49] a value for $K_{I/O}$, which defines the position of equilibrium (9), is also known.[19,49] Since the stability of the $Cu(PMEA)_{cl/N7}$ isomer is assumed to be represented (but see also below in the terminating paragraph of this section) by that of the $Cu(dPMEA)_{cl/N7}$ species, as discussed and postulated above, the corresponding $K_{I/N7}$ value is also known (see Section 3.4). Hence, a value for $K_{I/O/N3}$ [eqn. (17)] can be obtained by applying eqn. (19e), and therefore the formation degree of all four isomers appearing in scheme (13) can be calculated.[19,36] The corresponding results are summarized in Table 4.

From Table 4 it is evident that the $Cu(PMEA)_{cl/O/N3}$ species with the 5- and 7-membered chelate rings dominates with a formation degree of 41 (±12)%; it is revealing to see that this result is within the error limits identical with the previously obtained 49 ± 10% where the formation of the fourth isomer, $Cu(PMEA)_{cl/N7}$, had not been taken into account.[19,31] This shows immediately that the $Cu(PMEA)_{cl/N7}$ isomer must have a low formation degree; indeed, the present calculations provide 7.7 ± 5.3% (Table 4, entry 3b) proving that it is a minority species.[50]

It needs to be emphasized that this value (7.7 ± 5.3%) must be considered as an upper limit for two reasons. The nucleotide analogue $dPMEA^{2-}$ contains a pure aliphatic chain, whereas the chain of $PMEA^{2-}$ has an ether oxygen incorporated (see Fig. 1), and this difference may have consequences: (*i*) The purely aliphatic chain of $dPMEA^{2-}$ may undergo a weak hydrophobic interaction with the aromatic imidazole-ring of the adenine residue upon "folding" of the ligand as needed for the formation of the $Cu(dPMEA)_{cl/N7}$ isomer; such an interaction would facilitate the formation of the latter species. (*ii*) The ether oxygen in the $–CH_2CH_2–O–CH_2–PO_3^{2-}$ residue of $PMEA^{2-}$ will be solvated by H_2O and it will then somewhat inhibit the "folding" process needed for the formation of $Cu(PMEA)_{cl/N7}$. Both points mean that the value $\log \Delta_{Cu/dPMEA} = 0.16 \pm 0.09$ is somewhat too large, if applied to the Cu(PMEA) system, and therefore the formation degree of about 8% calculated for the $Cu(PMEA)_{cl/N7}$ isomer may well be too large but certainly it is *not* too low. At the same time one may conclude that the present calculations indicate that the $Cu(PMEA)_{cl/N7}$ isomer is likely to be formed in trace amounts; *e.g.*, a calculation with the reduced value, $\log \Delta_{Cu/N7} = 0.1$, gives a formation degree of about 4% for the $Cu(PMEA)_{cl/N7}$ isomer.

4. Conclusions

The analysis of Section 3.5 indicates that in the Cu(PMEA) system the $Cu(PMEA)_{cl/N7}$ isomer [eqn. (11)] is likely to be formed in trace amounts. The corresponding calculations are the first example, to the best of our knowledge, of a detailed evaluation of the equilibria involving four isomeric complexes. Of course, the fact that the $Cu(PMEA)_{cl/N7}$ isomer occurs only in low concentration distinguishes this system from the situation in Cu(dPMEA) (Section 3.4) and especially in Cu(AMP).[26,38,47] In accord herewith it was concluded in Section 3.4 that the M(dPMEA) complexes resemble more the parent M(AMP) complexes than do the M(PMEA) species.

Indeed, the properties of the binary M(dPMEA) and M(PMEA) complexes differ significantly. With $PMEA^{2-}$ the ether oxygen affects the complex structures in solution [equilibrium (9)]; there is no equivalent in the M(dPMEA) systems. If it is assumed that dPMEA is transported to the cell and also diphosphorylated, like PMEA,[15,16] then it would become understandable why dPMEA lacks[3] antiviral activity. A facilitated M(α) binding (*via* the ether oxygen) is not possible[18] with $dPMEApp^{4-}$ and thus the formation of the M(α)–M(β,γ)-binding mode, which is crucial for the transfer of a nucleotidyl unit in the polymerase reaction,[19] would also not be facilitated.

As far as the correct location of dPMEA and PMEA in the active sites of enzymes is concerned, one would not expect a significant difference since the hydrogen-bonding properties of the adenine residue should be identical in both ligands. This also seems to be true for the stacking interactions with other aromatic-ring systems; the extent of stack formation in the ternary Cu(Arm)(PMEA) and Cu(Arm)(dPMEA) complexes, where Arm = 2,2′-bipyridine or 1,10-phenanthroline, is quite alike[51] indicating that this property is not significantly affected by the presence of the ether oxygen atom in the acyclic chain between the phosphonate group and the adenine residue.

Acknowledgements

The competent technical assistance of Mrs Bela Nováková and Mrs Rita Baumbusch in the synthetic work and the preparation of this manuscript, respectively, is gratefully acknowledged. This study was supported by the Swiss National Science Foundation (H. S.), the Grant Agency of the Czech Republic (203/96/K001; A. H.) and the General Health Insurance Agency of the Czech Republic (A. H.) as well as within the COST D8 programme by the Swiss Federal Office for Education and Science (H. S.) and the Ministry of Education of the Czech Republic (A. H.).

Notes and references

1 Abbreviations and definitions: see Fig. 1; ATP^{4-}, adenosine 5′-triphosphate; $dAMP^{2-}$, 2′-deoxyadenosine 5′-monophosphate; $dATP^{4-}$, 2′-deoxyadenosine 5′-triphosphate; *I*, ionic strength; M^{2+}, divalent metal ion; 9MeAde, 9-methyladenine; PA^{2-}, $dPMEA^{2-}$ or any other twofold negatively charged nucleotide or nucleotide analogue; $PMEApp^{4-}$, diphosphorylated PMEA; $R\text{-}PO_3^{2-}$, simple phosphate monoester or phosphonate ligand with R representing a non-coordinating residue (see also legend of Fig. 2). Species written without a charge either do not carry one or represent the species in general (*i.e.* independent of their protonation degree); which of the two possibilities applies is always clear from the context. In formulas like $M(H;dPMEA)^{+}$, H^{+} and $dPMEA^{2-}$ are separated by a semi-colon to facilitate reading, yet they appear within the same parenthesis to indicate that the proton is at the ligand without defining its location (see Section 3.2).

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48 It may be added that for Co(dPMEA) no enhanced complex stability is observed (Table 3); hence, the formation of macrochelated Co[(d)PMEA] species is not of relevance (see also ref. 19). Similarly, the enhanced stability of the Cd(PMEA) system [19,31,36] is solely explained by equilibrium (9); *i.e.*, there is no evidence for a nucleobase–Cd^{2+} interaction. For the Ni[(d)PMEA] systems a calculation analogous to the one carried out here for the Cu^{2+} systems could be done, but because of the smaller stability enhancements (compared to those observed with the Cu^{2+} systems) and the relatively large error limits no conclusive results are expected.

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