CRITICAL REVIEW

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Metal ion complexes of antivirally active nucleotide analogues. Conclusions regarding their biological action

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Acyclic nucleoside phosphonates (ANPs), *i.e.*, analogues of (2′-deoxy)nucleoside 5′-monophosphates, have been studied during the past 15 years for their potential as antiviral drugs. One of these compounds, 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA; *Adefovir*) was recently approved in the form of its bis(pivaloyloxymethyl)ester (*Adefovir dipivoxil*) for use in hepatitis B therapy, a disease evoked by a DNA virus. Diphosphorylated PMEA^{2-}, *i.e.*, PMEApp^{4-}, is initially recognized by nucleic acid polymerases as an excellent substrate, but after insertion in the growing nucleic acid chain, this is terminated due to the lack of a 3′-hydroxy group. Based on the metal ion-binding properties of PMEApp^{4-} it can be explained why the ether oxygen in the aliphatic chain, R–CH_2–O–CH_2–PO_3pp^{4-}, is compulsory for a useful biological activity. Consequently, this *critical review* presents an overview on the coordination chemistry of various ANPs and correlates this to their biological properties.

1 General considerations

Nucleotides and their metal ion complexes play a key role in all aspects of metabolism (*e.g.*[1–5]);† two typical examples are adenosine 5′-triphosphate (ATP^{4-}) and guanosine 5′-triphosphate[6] (GTP^{4-}; see Fig. 1).[7–10] ATP is likely the most wellknown nucleotide, it is very versatile and in the form of metal ion complexes (mostly of Mg^{2+})[11–13] it is at the crossroad of many biological reactions;[14] it is not only involved in biosynthesis (*e.g.*, of nucleic acids)[15,16] but also in a vast majority of cellular activities.[2,14] ATP is generally regarded as an intracellular energy donor,[17] but it is also recognized as an important neurotransmitter,[18–21] a property which is in part possibly interlinked with its ability to form stacks;[22] it can mediate fast ligand-gated synaptic transmission at nerve-nerve synapses,[18,21] and it appears that Zn^{2+} has a physiological role in the regulation of the excitatory action of ATP on mammalian neurons.[19] Indeed, calculations show, in accord with the central metabolic role of ATP, that in one day an average person will make an amount of ATP equivalent to the body weight, and of course, the same amount is used in cellular activity.[14]

The other example of similar prominence is GTP (Fig. 1) which is utilized by so-called G-proteins in such diverse processes[23] as cellular signaling,[24,25] protein synthesis,[26] vesicular trafficking,[27] ion channel regulation,[28] signal transduction,[24,29] nerve growth[30] or exocytosis.[31] GTP hydrolysis is also essential for the insertion of nickel into hydrogenases[32] or the synthesis of activated sulfate,[33] which is an essential step in the metabolic assimilation of sulfur. Metal ions, mostly Mg^{2+} (*e.g.*[12,34,35]) but also Mn^{2+} (*e.g.*[3,36,37]) or Zn^{2+} (*e.g.*[38,39]) are needed for the reactions.

Considering the central role of nucleotides in crucial metabolic processes, as indicated above, it is no surprise to find that attempts to exploit nucleotide analogues as drugs are old. For example, the antiviral activity of benzimidazole derivatives was first described in 1947.[40] Indeed, the close similarity in shape between purine and benzimidazole has long been recognized;[41] this similarity is already evident from the fact that the latter compound may also be addressed as 1,3-dideazapurine (for an example see Fig. 2),[42] and indeed, 1-β-D-ribofuranosylbenzimidazole derivatives had been tested for their antiviral activity against the influenza B virus,[41,43] the herpes simplex virus[44] and several strains of polio virus[44] already in the fifties and sixties of the last century (see also ref. 45).

However, the above type of (attempted) "chemical warfare" is nothing new; in Nature it occurs for a long time since many microorganisms produce antibiotics that kill competing species in

† Abbreviations: Listed below are only those abbreviations which are not given or do not logically follow from the definitions provided in the legends of Figs. 1, 4, 8 and 9. ANP^{2-}, acyclic nucleoside phosphonate; ANPpp^{4-}, diphosphorylated ANP^{2-}; (d)NMP^{2-}, (2′-deoxy)nucleoside 5′-monophosphate; *I*, ionic strength; K_a, general acidity constant; L, general ligand; M^{2+}, general divalent metal ion; NTP^{4-}, nucleoside 5′-triphosphate.

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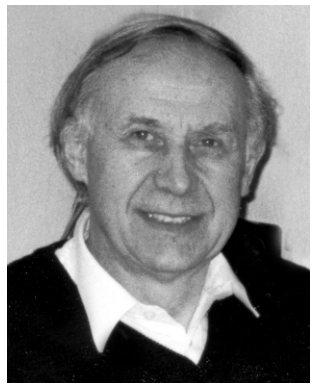

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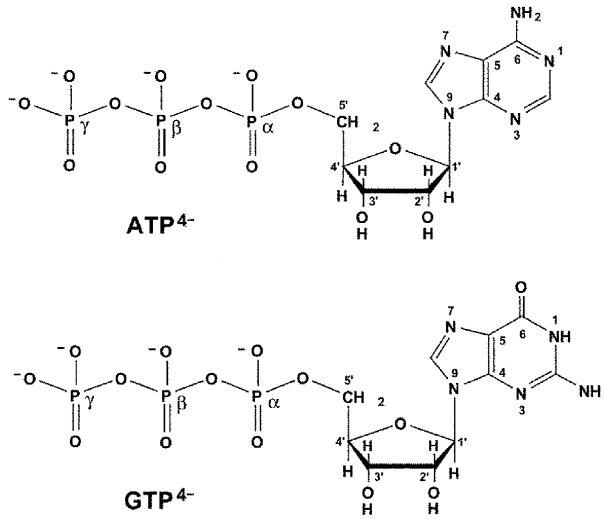


Fig. 1 Chemical structures of adenosine 5′-triphosphate (ATP^{4-}) and guanosine 5′-triphosphate (GTP^{4-}) in their dominating *anti* conformation.[7–10] In the 2′-deoxynucleoside 5′-triphosphates ($dNTP^{4-}$), $dATP^{4-}$ and $dGTP^{4-}$, which are employed in DNA synthesis catalyzed by polymerases, the 2′-hydroxy group is replaced by a hydrogen atom.

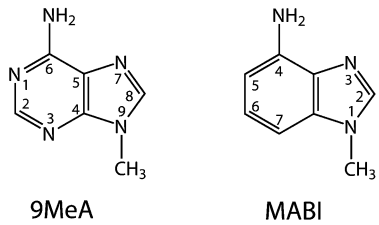


Fig. 2 Chemical structures of 9-methyladenine (9MeA) and 4-aminobenzimidazole (MABI), which may also be addressed as 6-amino-9-methyl-1,3-dideazapurine.

the same environmental niche.[46] There are many types of antibiotics[46–48] but among these are also nucleotide analogues[46,47] like tubercidin, which is nothing else but 7-deazaadenosine (*cf.* also Fig. 2) and which is produced by moulds and fungi.[47] Tubercidin was first isolated[49] from the culture broth of *Streptomyces tubercidicus* in 1957, its structure described[49] in 1961 and later proven by total synthesis[50] and X-ray crystal structure analysis.[51] Of course, tubercidin and its derivatives (their metal ion-binding properties have been studied and compared with those of the corresponding adenosine derivatives)[52–55] were also studied for their potential as therapeutic agents, indeed, these compounds are not only antibacterial but also antiviral agents,[56] they are active against some forms of cancer[57] and widely used in enzymatic studies[58] still today;[59] *e.g.*, tubercidin inhibits glycolytic enzymes in *Trypanosoma brucei*.[59]

Since viral infections belong to the most significant causes of illnesses[60,61] the above indicated long standing attempts to develop antiviral agents is understandable and, of course, the advent of the human immunodeficiency viruses (HIV-1 and HIV-2)[62] spurred this type of research further. Among the synthetic antiviral agents, artificial nucleotide analogues belong to the most promising ones,[45] and so-called acyclic nucleoside phosphonates (ANP^{2-}), which can be considered as analogues of (2′-deoxy)nucleoside 5′-monophosphates ($(d)NMP^{2-}$),† gained increasing interest over approximately the past 15 years.[63–66] In this class of antivirally active compounds the ribose phosphate residue is replaced by an aliphatic chain with a phosphonate group, to be more precise, by a phosphonomethyl ether residue as an isopolar equivalent of the α-phosphoryl group (see Fig. 4 in Section 2).[67]

Many of the nucleotide-based antivirals act *via* viral DNA polymerases or reverse transcriptases, in the case of retroviruses, and therefore, it may at this point be helpful to consider the replicative cycle of a virus. For HIV, a retrovirus, the cycle can be divided into the ten steps[61,68,69] depicted in Fig. 3: (*i*) Virus adsorption at the surface of the cell; (*ii*) virus-cell fusion; (*iii*) uncoating of the virus inside the cell; (*iv*) reverse transcription, *i.e.* conversion of the single-stranded viral RNA genome to the double-stranded proviral DNA which is subsequently (*v*) integrated into the cellular DNA genome; (*vi*) proviral DNA replication; (*vii*) proviral DNA transcription to viral messenger RNA; (*viii*) viral messenger-RNA translation to viral precursor proteins; (*ix*) viral maturation involving proteolytic cleavage, myristoylation and glycosylation; and (*x*) budding, *i.e.* release of the assembled virus. All these steps may be considered as targets for chemotherapeutic interventions with the viral replication cycle and for most of them inhibitors are known.[68,69]

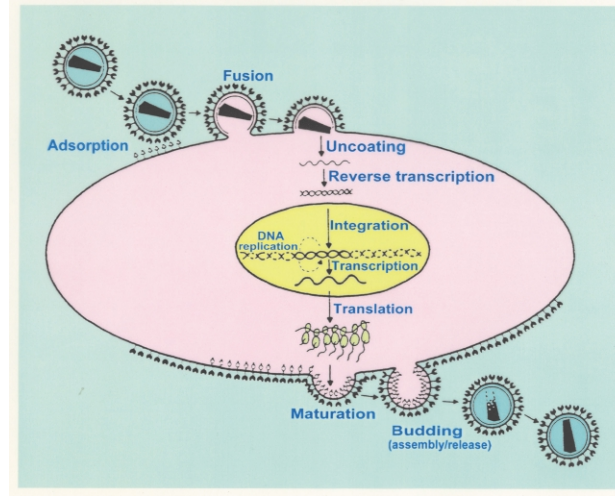


Fig. 3 The ten essential steps of the replicative cycle of human immunodeficiency virus (HIV), a retrovirus. The above figure is based on information provided in ref. 61, 68 and 69 as well as figures shown in ref. 61 and 68.

In a first approximation one may say that the situation for DNA viruses, an example is the hepatitis B virus (HBV), is similar, though here, of course, no reverse transcription and usually also no integration into the host DNA is needed, since the virus carries its own DNA.[61] Here the information transfer follows the order DNA → RNA → protein, whereas for retroviruses it is RNA → DNA → RNA → protein (Fig. 3).[61] There are seven classes of viruses, if based on their genetic material.[61] All have in common that nucleic acid polymerases are needed for their replication. This means, directed by (single-stranded) DNA or RNA templates these enzymes synthesize the complementary nucleic acids by the use of (d)NTP-metal ion complexes as substrates, under release of diphosphate (pyrophosphate).[2,46,47] Finally, it needs to be emphasized that an isolated virus is as dead as is a sodium-chloride crystal; only once inside its target cell it becomes highly vivacious by using the machinery of the invaded cell to multiply itself to large numbers.

2 Properties of 9-[2-(phosphonomethoxy)ethyl]adenine. An example of an antiviral acyclic nucleoside phosphonate

As indicated above, many of the conventional antivirals act *via* viral DNA polymerases or reverse transcriptases and many of these agents are nucleoside analogues like adenine arabinoside, azidothymidine or acyclovir [= 9-(2-hydroxy-ethoxymethyl)guanine] which have to be transformed into the active metabolites, *i.e.* nucleoside triphosphate analogues, by the consecutive action of nucleoside and nucleotide kinases.[68,69] However, after their monophosphorylation base- or sugar-modified nucleoside ana-

logues, and this also applies of course to administered nucleotide analogues with a monophosphate-ester residue, undergo enzyme-catalyzed dephosphorylation during their passage through the cellular membrane or in blood plasma[70] due to the wide occurrence of non-specific dephosphorylation enzymes capable of splitting any phosphoric acid-ester bond disregarding the nature of the alcohol component;[67] clearly, this renders therapeutic application of such anti-metabolites inefficient. This difficulty is circumvented by applying analogues with a phosphonate residue,[67,70] *i.e.* the phosphorus-oxygen ester bond is replaced by a phosphorus-carbon bond which is nondegradable. Furthermore, the initial phosphorylation step is also unnecessary in this case,[68–70] and only a diphosphorylation is required to obtain the active triphosph(on)ate derivative.

One of these nucleotide analogues with a phosphonate group, or more precisely, a phosphonomethyl ether residue, is 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA),[64] which is also known as *Adefovir*[68,69] and which may be considered as an analogue of (2′-deoxy)adenosine 5′-monophosphate ((d)AMP^{2-}) (see Fig. 4).[7–9,71]

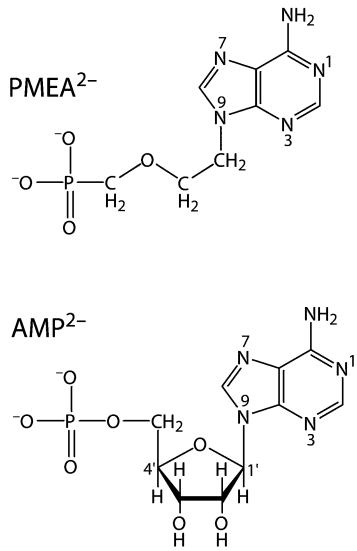


Fig. 4 Chemical structure of the dianion of 9-[2-(phosphonomethoxy)ethyl]adenine (= PMEA^{2-} = *Adefovir*) and of its parent nucleotide adenosine 5′-monophosphate (AMP^{2-}), which is shown in its dominating *anti* conformation.[7–9] The orientation of PMEA^{2-} in solution[71] is similar to the *anti* conformation of AMP^{2-}. Substitution of the ether oxygen in PMEA^{2-} by a CH_2 unit gives 4-(adenin-9-yl)butylphosphonate, known as 3′-deoxa-PMEA^{2-} (= dPMEA^{2-}; see Section 4).

It is interesting to note that ^{1}H-NMR studies indicate that PMEA^{2-} occurs in aqueous solution to some extent in an orientation similar[71] to the *anti* conformation of AMP^{2-},[7–9] *i.e.* the phosph(on)ate group is close to H8. However, the structures of the metal ion complexes of these two ligands are rather different; AMP^{2-} forms macrochelates with divalent metal ions (M^{2+}) by an interaction of the phosphate-coordinated metal ion with N7 of the adenine residue,[52,72–74] whereas for the M(PMEA) complexes five-membered chelates involving the phosphonate-bound metal ion and the ether oxygen atom (see Fig. 4) are important.[75–77]

This AMP analogue, PMEA, has remarkable antiviral properties.[65,68,69] It is active against herpes-, hepadna- and retroviruses,[78] induces apoptosis in human leukemia cell lines[79] and exhibits cytostatic activity in rat and mouse carcinomas and sarcomas.[80] The oral prodrug of PMEA, that is, its bis(pivaloyloxymethyl)ester, also named *Adefovir dipivoxil* or *Preveon*[65] (Fig. 5), is being evaluated in patients infected with human immunodeficiency viruses (HIV-1 and HIV-2).[68,69,81] This easily hydrolyzable diester facilitates cellular uptake, *i.e.* PMEA is released inside cells.[82]

Furthermore, *Adefovir dipivoxil* was very recently, *i.e.* in the second part of 2002, approved by the US Food and Drug Administration (FDA) for use in hepatitis B therapy;[83] in March

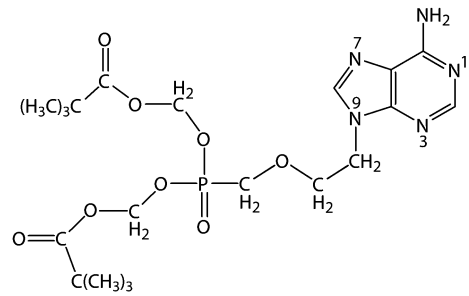


Fig. 5 Chemical structure of the bis(pivaloyloxymethyl)ester of PMEA, *i.e.* its oral prodrug form, also known as *Adefovir dipivoxil*, or *Preveon Hepsera*.[65,68,69]

2003 it was also granted *Community Marketing* for the "treatment of chronic hepatitis B in adults" under the name *Hepsera* by the European Agency for the Evaluation of Medicinal Products (EMEA).[84] This is a significant progress considering that about 5% of the world's human population is chronically infected with the hepatitis B virus (HBV)[85] and thus, at an increased risk of developing cirrhosis, hepatocellular carcinoma or decompensated liver disease.[85] Moreover, it was recently also discovered that the same prodrug possesses antiarthritic properties.[86]

The remainder of this account will mainly focus on the metal ion-binding properties of PMEA^{2-} (Fig. 4) since these are clearly interlinked with its potent antiviral properties.

3 Mechanistic considerations and the role of metal ions

The nucleotide analogue PMEA interferes with nucleic acid transcription; it is first diphosphorylated to PMEApp^{4-},[87] an analogue of (2′-deoxy)adenosine 5′-triphosphate ((d)ATP^{4-}), and in this form it serves as a substrate for the nucleic acid polymerase.[68,69] This means, it is incorporated into the growing nucleic acid chain, which is then terminated due to the lack of a 3′-hydroxy group.[88,89]

Here it should be recalled that DNA polymerases utilize two metal ions in catalysis,[15,90] and that a common mechanism for all DNA polymerases was proposed based on crystal structure studies.[16,90] Similarly, RNA polymerases also contain several Zn^{2+} and Mg^{2+} ions.[39,91] All these enzymes use nucleoside 5′-triphosphates as substrates only in the form of metal ion (mostly Mg^{2+}) complexes.[92] As indicated already above, PMEApp^{4-} is initially a substrate for several polymerases,[67,89,93] and indeed, an excellent one. 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.[67,93]

Knowing from kinetic studies,[94] as well as from the indicated crystal structure studies, that for the activation of a nucleoside 5′-triphosphate (NTP^{4-}) two metal ions have to be coordinated to the triphosphate chain[54,94] 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 of the (d)NTP^{4-}, it was recently concluded[95–97] 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)[98] and the possibility that a coordinated metal ion (*e.g.* Mg^{2+}) can form a five-membered chelate with the ether oxygen.[76] 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 favor PMEApp^{4-} over dATP^{4-}.[96] Fig. 6[99,100] depicts the structure of the two metal ion-bearing PMEApp^{4-}-intermediate proposed to occur in the active site of the nucleic acid polymerase.

It should be emphasized that the mechanistic proposal of Fig. 6 is based on comprehensive kinetic experiments[99,101] carried out mainly on the metal ion-facilitated hydrolysis of ATP and other triphosphates, *i.e.* the transfer of the terminal γ-phosphate group to

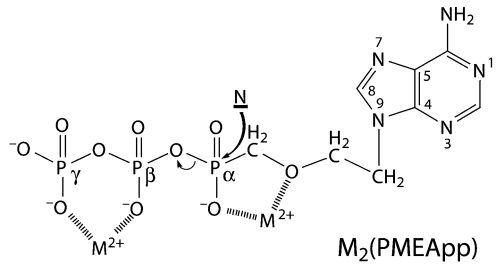


Fig. 6 Simplified structure of the M_2(PMEApp) intermediate ready for the attack of a nucleophile (N) and on its way to the transition state in a nucleic acid polymerase.[96] Both metal ions are anchored[94,99] to amino acid side chains (often carboxylate groups of aspartate or glutamate units[100] of the enzyme). Of course, the adenine moiety may be replaced by any other nucleobase residue and the nucleophile N may in addition interact with M^{2+} at the α-phosphate group.

water. It turned out that for an effective promotion two metal ions need to be coordinated to the triphosphate chain;[94,99] coordination of one Mg^{2+} alone rather prevents the reaction whereas in a combination with Cu^{2+} or Zn^{2+} synergism occurs and the system becomes highly reactive.[102] Combination of all results lead to the conclusion[94] that binding of two metal ions in a M(α,β)-M(γ)-type fashion, which occurs at a triphosphate chain without any additional enforcement by a third partner (enzyme), gives rise to transphosphorylations and it is this metal ion pattern which is relevant for kinases and related enzymes; this coordination pattern is depicted in the upper part of Fig. 7. Of course, one of the two

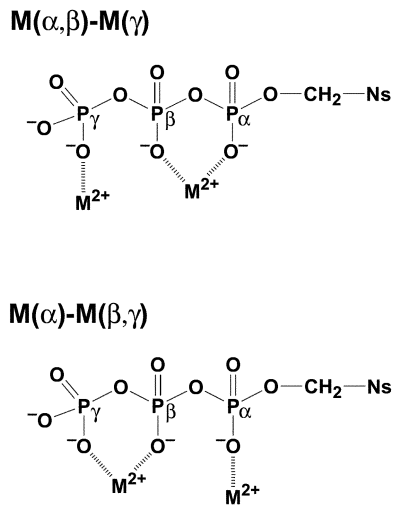


Fig. 7 Simplified structures of two M_2(NTP) complexes: Once with an M(α,β)-M(γ)-coordination mode (upper part) relevant for transphosphorylations (kinases, *etc.*), and once with an M(α)-M(β,γ)-type mode (lower part) relevant for the transfer of a nucleotidyl unit as catalyzed by polymerases (see also Fig. 6) (CH_2–Ns = nucleosidyl residue).

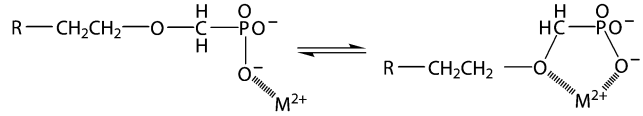
metal ions could be replaced by an ionic interaction, *e.g.* with an arginyl group, and a reactive intermediate would still result,[94,103] especially under conditions of a low polarity, *i.e.* in a hydrophobic environment.[104] The M(α)-M(β,γ)-type coordination pattern,[94,96] which is shown in the lower part of Fig. 7, needs to be enforced by the enzyme (see legend of Fig. 6); this pattern, which facilitates the break between P_α and P_β, allows the transfer of a diphosphoryl or a nucleotidyl group, the latter being relevant for nucleic acid polymerases.

The described transphosphorylation mechanism was confirmed years later by an X-ray structural study[105] of *Escherichia coli* phosphoenolpyruvate carboxykinase. Similarly, X-ray structural studies of nucleic acid polymerases also confirmed the involvement of two metal ions and mechanisms similar to the one indicated in the lower part of Fig. 7 (see also Fig. 6) were proposed.[90,100] The crucial step in the polymerase reaction indicated above is to force a metal ion into the α-position of the triphosphate chain[96] of an (d)NTP (Fig. 7). Here $PMEApp^{4-}$ being initially an excellent substrate, as said above, has an advantage over $dATP^{4-}$ because of the formation of a five-membered chelate involving the ether oxygen (see also below).[76] At the same time the formation of such a five-membered chelate should make the transfer of a phosphoryl group more difficult. Indeed, there are indications in the literature, given unfortunately without experimental details,[106] that PMEApp is a somewhat poorer substrate than ATP for ATPases because for these, like for kinases,[105] a M(α,β)-M(γ) metal ion-coordination pattern is desirable.[94] Further support for the presented view comes from the knowledge that the ether oxygen responsible for chelate formation (see Section 4) is compulsory for an antiviral activity; replacement by a methylene group[67] or by sulfur,[107] or shifting of the position of this oxygen within the alkyl chain leads to inactive products.[67,93]

4 Metal ion-binding properties of $PMEA^{2-}$ in comparison with those of AMP^{2-} and $dPMEA^{2-}$

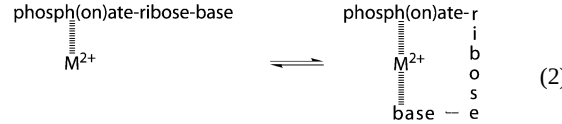
4.1 General comparisons and evaluation procedure

The dimensions of $H_2(PMEA)^{\pm}$ are known from an X-ray structure study[108] and they are similar to those of AMP as is the orientation of these two compounds in aqueous solution;[71] it is thus not surprising that PMEA mimics AMP well in certain reactions.[54,109] However, the metal ion-binding properties of the two ligands differ considerably. For M(PMEA) complexes the intramolecular equilibrium 1



(1)

involving the ether oxygen is of great significance,[75,76] whereas for M(AMP) complexes, especially of those with the divalent ions of the second half of the 3d series including Zn^{2+} and Cd^{2+}, macrochelate formation due to the interaction of the phosphate-coordinated metal ion with N7 is of relevance.[52,72–74] Of course, this is also true for the complexes of the carba analogue of PMEA, *i.e.* for 9-(4-phosphonobutyl)adenine, which is also known as 3′-deoxy-PMEA (= dPMEA) or 4-(adenin-9-yl)butylphosphonate (see Fig. 4).[110] Hence, for the M(AMP) and M(dPMEA) complexes the intramolecular equilibrium 2 needs to be considered:



(2)

The primary binding site, *i.e.* the site which has the largest impact on complex stability, is for the mentioned cases the phosph(on)ate group. However, any additional interaction of a phosph(on)ate-coordinated metal ion with another site in the ligand must be reflected in an increased complex stability.[111] This is proven for several examples (PA^{2-} = $PMEA^{2-}$, $dPMEA^{2-}$ and AMP^{2-}) in Fig. 8,[74,75,110] where plots of log $K^{M}_{M(R-PO_3)}$ *versus* $pK^{H}_{H(R-PO_3)}$ are shown for simple phosphate monoesters[112] and phosphonates;[75] 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. 3,

$$\log K^{M}_{M(R-PO_3)} = m \cdot pK^{H}_{H(R-PO_3)} + b \qquad (3)$$

have been tabulated,[75,76,113,114] *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.

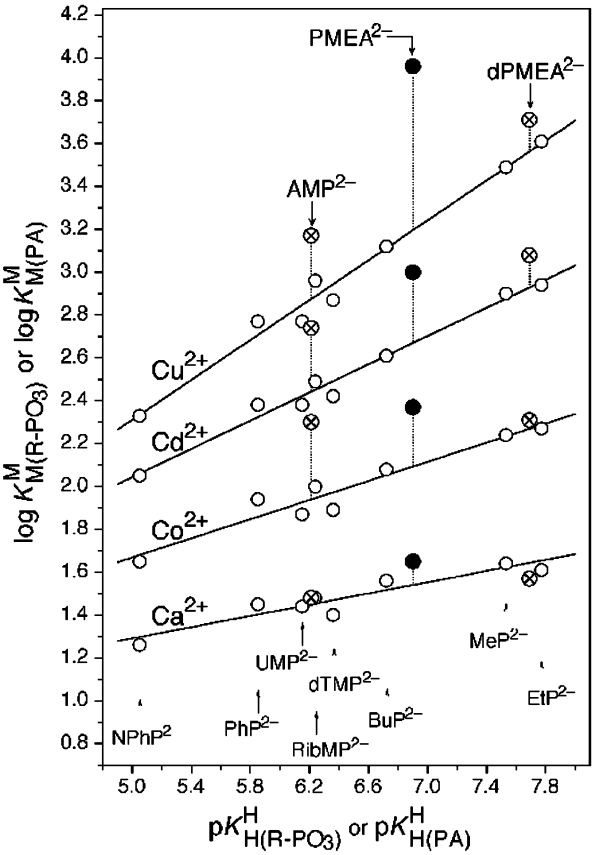


Fig. 8 Evidence for an enhanced stability of several M(PMEA) complexes (●), together with the data points for the corresponding metal ion complexes of $dPMEA^{2-}$ and AMP^{2-} (⊗) (PA^{2-} = $PMEA^{2-}$, $dPMEA^{2-}$ and AMP^{2-}) based on the relationship between log $K^{M}_{M(R-PO_3)}$ and $pK^{H}_{H(R-PO_3)}$ 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 ($dTMP^{2-}$), *n*-butyl phosphate (BuP^{2-}), methanephosphonate (MeP^{2-}), and ethanephosphonate (EtP^{2-}) (from left to right). The least-squares lines (eqn. 3) are drawn through the corresponding 8 data sets (○) taken from ref. 112 for the phosphate monoesters and from ref. 75 for the phosphonates. The points due to the equilibrium constants for the M^{2+}/PMEA (●), M^{2+}/dPMEA (⊗) and M^{2+}/AMP (⊗) systems are from references 75, 110 and 74, respectively. The vertical broken lines emphasize the stability differences from the reference lines; they equal log $\Delta_{M/L}$ as defined in eqn. 4. All the plotted equilibrium constants refer to aqueous solutions at 25 °C and I = 0.1 M ($NaNO_3$).

It is evident that the indicated procedure allows now a comparison between the experimentally (exp) measured stability constants and those expected on the basis of the acid-base properties of the considered ligand (L), *i.e.* of its phosph(on)ate group; clearly, the calculated values reflect the stability constants of the 'open' (op) isomers indicated in equilibria 1 and 2. The differences between the mentioned stability constants are defined in eqn. 4:

$$\log \Delta_{M/L} = \log K^{M}_{M(L)exp} - \log K^{M}_{M(L)op} \quad (4)$$

Of course, a positive value for log $\Delta_{M/L}$ reflects the extent of the formation of the 'closed' (cl) species in equilibria 1 and 2 and it can easily be proven that the position of these intramolecular equilibria is defined by eqn. 5:[52,72,111,114]

$$K_I = \frac{[M(L)_{cl}]}{[M(L)_{op}]} \quad (5a)$$

$$= \frac{K^{M}_{M(L)exp}}{K^{M}_{M(L)op}} - 1 = 10^{\log \Delta_{M/L}} - 1 \quad (5b)$$

Knowledge of K_I allows then to calculate the formation degree or percentage of the closed species according to eqn. 6:

$$\% M(L)_{cl} = 100 \cdot K_I/(1 + K_I) \quad (6)$$

4.2 Macrochelate formation with N7 in M(AMP) and M(dPMEA) species

Since the situation regarding the M(AMP) complexes, where macrochelate formation occurs with certainty *via* N7,[52] is unequivocal and since the same has been concluded for the M(dPMEA) complexes,[110] these shall be discussed first. It is evident that the vertical distance of a given data point to its reference line (dotted lines) seen in Fig. 8 corresponds to the stability increase log $\Delta_{M/L}$ as defined in eqn. 4. Comparison of the situation in Fig. 8 for the M(AMP) and M(dPMEA) complexes reveals that the stability increases for the M(AMP) complexes of Co^{2+}, Cu^{2+} and Cd^{2+} are more pronounced than those of the corresponding M(dPMEA) complexes meaning that macrochelate formation is more important for the former; there is also clearly no hint for a Ca^{2+} adenine-ring interaction in the Ca(AMP) and Ca(dPMEA) complexes.

The above qualitative considerations are confirmed by the quantitative evaluations summarized in Table 1. Application of these data to eqns. 5 and 6 reveals that the formation degree of the macrochelated or closed species amounts[110] to 21 ± 15% (error limit 3σ), 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 2 is zero within the error limits (see the log $\Delta_{M/dPMEA}$ values in Table 1). In the M(AMP) systems macrochelate formation is clearly more pronounced, *e.g.* it amounts[74] to 75 ± 4%, 50 ± 7% and 50 ± 8% for Ni(AMP)$_{cl}$, Cu(AMP)$_{cl}$ and Cd(AMP)$_{cl}$, respectively; Co(AMP)$_{cl}$ occurs with 56 ± 7%, Zn(AMP)$_{cl}$ with 44 ± 12% and Mg(AMP)$_{cl}$ with 13 ± 10%, but in the latter case the N7 interaction is outersphere, *i.e.* with a water molecule between Mg^{2+} and N7.[73,74,113]

Table 1 Comparison of the stability enhancements defined by eqn. 4 for M(dPMEA) and M(AMP) complexes (aqueous solution; 25 °C; I = 0.1 M, $NaNO_3$)[a,b]

M^{2+}	log $\Delta_{M/dPMEA}$	log $\Delta_{M/AMP}$
Mg^{2+}	−0.03 ± 0.05	0.06 ± 0.05
Ca^{2+}	−0.07 ± 0.07	0.03 ± 0.06
Sr^{2+}	−0.06 ± 0.06	0.02 ± 0.04
Ba^{2+}	−0.07 ± 0.07	0.02 ± 0.06
Mn^{2+}	−0.04 ± 0.06	0.07 ± 0.05
Co^{2+}	0.04 ± 0.08	0.36 ± 0.07
Ni^{2+}	0.10 ± 0.08	0.61 ± 0.06
Cu^{2+}	0.16 ± 0.09	0.30 ± 0.06
Zn^{2+}	0.1 ± 0.2	0.25 ± 0.09
Cd^{2+}	0.15 ± 0.11	0.30 ± 0.07

[a] The error limits (3σ) of derived data, in the present case for columns 2 and 3, were calculated according to the error propagation after Gauss. The error limits of the log K values on which the above data are based (eqn. 4) correspond to three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] The values in columns 2 and 3 are from references 110 and 74, respectively.

4.3 Five-ring chelation involving the ether oxygen in M(PMEA) complexes

The reason for the observed stability enhancements in Fig. 8 for all M(PMEA) complexes is best evaluated by considering complexes of ligands of the kind PME-R (= R–CH_2–O–CH_2–R–PO_3^{2-}) where R offers no accessible binding sites for metal ions,[115] *e.g.* where R = CH_3.[75] The corresponding log $\Delta_{M/L}$ values are summarized in Table 2: In column 2 the values for the M(PMEA) and in column 3

Table 2 Stability enhancements as defined by eqn. 4 for M(PMEA) and M(PME-R) complexes and their comparison according to eqn. 7 (aqueous solution; 25 °C; I = 0.1 M, $NaNO_3$)[a,b]

M^{2+}	log $\Delta_{M/PMEA}$	log $\Delta_{M/PME\text{-}R}$	Δ log Δ
Mg^{2+}	0.16 ± 0.05	0.16 ± 0.04	0.00 ± 0.06
Ca^{2+}	0.11 ± 0.07	0.12 ± 0.05	−0.01 ± 0.09
Sr^{2+}	0.07 ± 0.05	0.09 ± 0.05	−0.02 ± 0.07
Ba^{2+}	0.08 ± 0.06	0.11 ± 0.05	−0.03 ± 0.08
Mn^{2+}	0.21 ± 0.08	0.19 ± 0.06	0.02 ± 0.10
Co^{2+}	0.28 ± 0.07	0.20 ± 0.06	0.08 ± 0.09
Ni^{2+}	0.30 ± 0.07	0.14 ± 0.07	0.16 ± 0.10
Cu^{2+}	0.77 ± 0.07	0.48 ± 0.07	0.29 ± 0.10
Zn^{2+}	0.30 ± 0.10	0.29 ± 0.07	0.01 ± 0.12
Cd^{2+}	0.33 ± 0.06	0.30 ± 0.05	0.03 ± 0.08

[a] Regarding the error limits see footnote *a* of Table 1. [b] The values in columns 2 and 3 are from references 75 and 115, respectively.

for the M(PME-R) systems are given. Formation of the difference Δ log Δ according to eqn. 7,

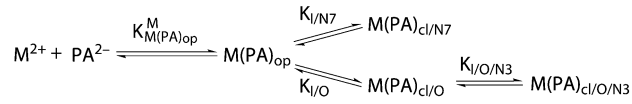
$$\Delta \log \Delta = \log \Delta_{M/PMEA} - \log \Delta_{M/PME\text{-}R} \quad (7)$$

leads to the results listed in column 4 of Table 2. In most instances these differences are zero within the error limits, thus proving for the M(PMEA) complexes of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Zn^{2+} and Cd^{2+} that only equil. 1 operates and that the increased complex stabilities are solely due to the formation of the 5-membered chelates involving the ether oxygen (= $M(PMEA)_{cl/O}$).[75,96] For example, the formation degrees for the $M(PMEA)_{cl/O}$ species with Ca^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+} amount[75] to 22 ± 13% (3σ), 31 ± 8%, 38 ± 11% and 50 ± 12%, respectively, thus proving the importance of the M^{2+}/ether-oxygen interaction on which the arguments discussed in Section 3 are based. It needs to be emphasized that the changes in free energy (ΔG^0) connected with equilibrium 1 are small;[114] for example, a log $\Delta_{M/L}$ value of 0.3 log units which corresponds to a formation degree of the chelated species of about 50% amounts energy-wise only to $\Delta G°_{(25\,°C)} = -1.71$ kJ mol^{-1}; for log $\Delta_{M/L}$ = 0.1, which corresponds to about 20% of the closed species, the energy difference is actually only −0.57 kJ mol^{-1}.

4.4 Metal ion-adenine interactions in certain M(PMEA) species

Only the Ni(PMEA) and Cu(PMEA) complexes, as well as possibly the Co(PMEA) complex (see Table 2), show a stability contribution which goes beyond that of a $M(PMEA)_{cl/O}$ isomer, thus indicating that in these instances the adenine residue also participates in metal ion binding.[75,76,96] With regard to the arguments given in Section 3 this is of no relevance because the different structures in solution for the M(AMP) and the M(PMEA) complexes for the biologically relevant metal ions are unequivocal from the results presented above.

However, for reasons of completeness the additional M^{2+}-adenine interaction occurring in some M(PMEA) complexes (see also ref. 116) shall also be summarized shortly by concentrating on Cu(PMEA) because for this system the effect is most pronounced (Δ log Δ = 0.29 ± 0.10; see Table 2). Already at an early stage[75] it was recognized, based on steric considerations,[76] that N3 is of relevance and that the $Cu(PMEA)_{cl/O}$ isomer with its 5-membered chelate ring can easily undergo a further chelation by forming a 7-membered ring with N3, thus leading to the $Cu(PMEA)_{cl/O/N3}$ isomer. Indeed, by studying[117] the complex stabilities of the N1-, N3- and N7-deaza derivatives of PMEA and by carrying out ^{1}H-NMR line broadening measurements, the importance of the N3–Cu^{2+} interaction could be confirmed. Once the dPMEA data became available also the N7–Cu^{2+} interaction, $Cu(PMEA)_{cl/N7}$, could be quantified[110] and combination of all these results allowed then to solve the four-isomer problem summarized in the equilibrium scheme 8, where $PMEA^{2-}$ = PA^{2-} (see also refs. 116 and 118):



(8)

The following results were obtained:[110] 17 ± 3% (3σ) are present as $Cu(PMEA)_{op}$ and in total 83 ± 3% exist in the form of closed isomers, $Cu(PMEA)_{cl/tot}$, of these are 34 ± 10%, 7.7 ± 5.3% and 41 ± 12% present as $Cu(PMEA)_{cl/O}$, $Cu(PMEA)_{cl/N7}$ and $Cu(PMEA)_{cl/O/N3}$, respectively, indicating that the species involving N3 is the most important one, yet at the same time these results also show that the species involving the ether oxygen amount in total to 75%.

The finding that N3 may also bind metal ions, despite its relatively low basicity,[119] provided a suitable primary binding site is available, is of general interest not only for nucleotides but also for nucleic acids, since N3 is exposed to the solvent in the minor groove of DNA.[9] In fact, more and more evidence accumulates for such N3 interactions in the solid state[120] and in solution with kinetically inert metal ions,[121] as well as with labile metal ions.[117,122–124]

5 Properties of other acyclic nucleoside phosphonates

5.1 Antivirally active 2-(phosphonomethoxy)ethyl derivatives closely related to PMEA

Considering the success of PMEA as an antiviral agent, it is not surprising that a large number of such acyclic nucleoside phosphonates (ANP^{2-}) has been synthesized and tested for their biological activity.[65,66] Indeed, replacement of the adenine residue (see Fig. 4) by a 2,6-diaminopurine moiety gives 9-[2-(phosphonomethoxy)ethyl]-2,6-diaminopurine (PMEDAP), a compound which inhibits DNA viruses and retroviruses and also possesses selective antitumor properties.[65] Similarly, replacement of the adenine by a guanine residue leads to the 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG) counterpart, which is a potent antiviral and exhibits powerful antitumor activities as well (see also refs in 66).[65]

In accord with the above mentioned biological activities and the conclusions of Section 3 regarding nucleic acid polymerases and the desired coordination pattern of $ANPpp^{4-}$ species is the observation that the formation degrees of the 5-ring chelates involving the ether oxygen in equilibrium 1 with $PMEDAP^{2-}$ for the biologically relevant metal ions Mg^{2+}, Ca^{2+}, Mn^{2+} and Zn^{2+} amount[125] to 32 ± 8%, 24 ± 11%, 34 ± 9% and 60 ± 10%, respectively; these percentages are within the error limits identical with those determined for the corresponding M(PMEA) systems (see Section 4.3). This is also true[126] for the analogous complexes of $PMEG^{2-}$. It is no surprise that in both instances[125,126] metal ions like Co^{2+}, Ni^{2+} and Cu^{2+} do *not* interact with N3, as it is the case with $PMEA^{2-}$ (Section 4.4), in addition to the ether oxygen but that they interact with N7 of the purine moiety since it is well known[55] that an amino group in *ortho* position to an N site strongly inhibits binding of these metal ions, and both ligands have a $(C2)NH_2$ group neighboring N3. This observation is interesting from a coordination chemical point of view, but not of relevance with regard to the antiviral properties of these compounds, since the ether oxygen remains accessible.

5.2 Structural alterations which lead to ANPs devoid of an ether-oxygen interaction and consequently also of an antiviral activity

The observations made with the PMEA analogue, in which the methylene group between the phosphorus and the ether oxygen (see Fig. 4) is replaced by an ethylene group giving 9-[2-(2-phosphonoethoxy)ethyl]adenine (PEEA) fits well into the described picture. Clearly, in principle $PEEA^{2-}$ is able to form 6-membered chelates

with the ether oxygen, yet, it is well known that these are less stable than 5-membered ones,[111] and indeed, Mg^{2+}, Ca^{2+} and Mn^{2+} do not form such chelates and from the biologically relevant metal ions only Zn^{2+} interacts possibly very weakly with the ether oxygen of $PEEA^{2-}$.[127] In fact, these M(PEEA) complexes reflect the properties of the corresponding complexes[128] of the carba analogue 3′-deoxy-PEEA [= dPEEA = 9-(5-phosphonopentyl)adenine], which shows, as one might expect, no useful biological activity,[128] and PEEA is devoid of such an activity as well.[129] Needless to emphasize that the carba analogue of PMEA, *i.e.* dPMEA (see Section 4.2), also has no antiviral activity.[67]

A further interesting observation results from the attempt to combine the anticancer properties of Pt(II) complexes[130] with the antiviral ones of PMEA (Section 2); this led to the synthesis of a compound in which diethylenetriamine (= Dien = 3-azapentane-1,5-diamine) bound to Pt(II) is also coordinated to N7 of PMEA giving (Dien)Pt(PMEA-*N7*).[131] However, charge repulsion by the $(Dien)Pt^{2+}$ unit at N7 reduces the overall stability of the complexes by about 0.4 log units[131] and totally suppresses chelate formation according to equilibrium 1 for the complexes with Ca^{2+}, Mg^{2+} and Mn^{2+} though it allowed some (about 30%) for the $Zn[(Dien)Pt(PMEA\text{-}N7)]^{2+}$ species. Hence, in accord with the conclusions of Section 3 no biological activity is expected and indeed, the test revealed no activity of (Dien)Pt(PMEA-*N7*) against DNA viruses or retroviruses and also no cytostatic activity was discovered.[131] For the isomeric compound (Dien)Pt(PMEA-*N1*), with $(Dien)Pt^{2+}$ at N1 of $PMEA^{2-}$,[132] the situation is similar; the stability of the complexes is reduced and no chelate formation according to equilibrium 1 was discovered[132] for Mg^{2+} and Ca^{2+} (Mn^{2+} was not studied), only with Zn^{2+} a minor fraction (about 30%) occurred.

That the quaternary 1-[2-(phosphonomethoxy)ethyl] derivative of 2,4-diaminopyrimidine ($PMEDAPy^-$; see Fig. 9) does neither exhibit any antiviral activity against DNA viruses or retroviruses nor any cytostatic activity[133] is no surprise if one considers the coordination chemistry of this ligand:[134] The stability of all complexes studied is reduced due to charge repulsion by about 0.4 log units and most important, no hint for any chelate formation involving the ether oxygen was observed for any of the studied metal ion complexes. The corresponding charge effect connected with an inhibition of the ether-oxygen interaction was also found[135] for the structurally related complexes of *O*-phosphonatomethylcholine, $(CH_3)_3N^+–CH_2CH_2–O–CH_2–PR–PO_3^{2-}$.

5.3 Other factors affecting biological activity

Surprisingly, 1-[2-(phosphonomethoxy)ethyl]cytosine (PMEC; see Fig. 9), which is closely related to the highly potent antiviral (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (HPMPC; Fig. 9), also known as *Cidofovir*[78,136] and which is used in the treatment of cytomegalovirus-induced retinitis[137] and several other virus-induced diseases,[138] is devoid of a significant and useful activity against the viruses tested,[65–67] despite the fact that the biologically relevant metal ions form with $PMEC^{2-}$ chelates[115] according to equilibrium 1 in the same extent as does $PMEA^{2-}$. On the other hand it needs to be noted that $PMECpp^{4-}$ is not totally inactive; it also inhibits *in vitro* DNA synthesis by DNA polymerases and reverse transcriptases but it is not as effective as for example $PMEApp^{4-}$ or $PMEDAPpp^{4-}$.[65–67,93,96] This then indicates that other points, aside from the possibility to form the M(α)-M(β,γ)-coordination pattern discussed in Section 3, are import. As summarized previously,[96] one of these is most likely the anchoring process of the substrate in the active site cavity of the enzyme. Here the nucleobase residues play certainly an important role *via* their hydrogen bonding and stacking properties. As far as PMEC and HPMPC are concerned, one is tempted to suggest that the hydroxy methyl residue of HPMPC, *i.e.* of its diphosphorylated product, facilitates initially the anchoring process by hydrogen bonding with its OH group (in addition, incorporation of HPMPC into the growing nucleic acid chain does not immediately lead to chain termination due to the OH group).[78] Of course, the parent compound (2′-deoxy)cytidine 5′-triphosphate can form such a hydrogen bond *via* its sugar moiety. It may be further added in this context that from comparisons of the metal ion-binding properties of $PMEA^{2-}$ and $HPMPA^{2-}$ [= dianion of (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine] it may be concluded[139] that the additional presence of the hydroxy methyl group in $HPMPA^{2-}$ (or $HPMPC^{2-}$) does not significantly affect the position of equilibrium 1.

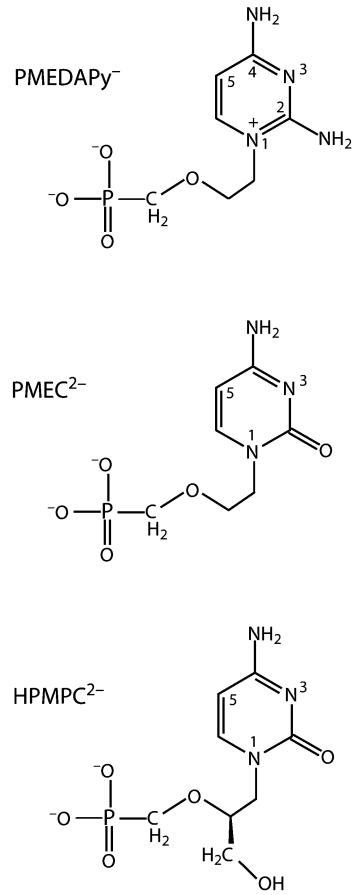


Fig. 9 Chemical structures of the anions of {[2-(2,4-diaminopyrimidinio)-ethoxy]methyl}phosphonate, sometimes also, not quite correctly, named as 1-[2-(phosphonomethoxy)ethyl]-2,4-diaminopyrimidine (= $PMEDAPy^-$), as well as of 1-[2-(phosphonomethoxy)ethyl]cytosine ($PMEC^{2-}$) and (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine ($HPMPC^{2-}$).

That the anchoring and related processes are important is also borne out from the properties of the 1-, 3- and 7-deaza analogues of PMEA. The formation degree of the 5-membered chelates in their Mg^{2+} complexes (only these[124] and those with Cu^{2+} (*cf.*[117]) were studied) corresponds to that found with Mg(PMEA) but all three deaza compounds are devoid of any significant antiviral activity.[124,140] Hence, it appears likely[124] that the differences in physiological activity between PMEA and its deaza analogues originate in their different properties regarding the formation of hydrogen bonds, which can be very important in recognition reactions.[141] *Inter alia*, such might be displayed not only in the anchoring process but also during transport (PMEA transport over the cellular membrane depends on the cell line)[142] or during the subsequent activation *via* phosphorylation.[87,143]

The stacking properties, which may also be important for anchoring processes, depend on the nucleobase residues. Since these residues are the same in the acyclic nucleoside phosphonates and in their parent nucleotides, it is not surprising that in studies of mixed ligand complexes, where the stacking properties were probed with 2,2′-bipyridine or 1,10-phenanthroline (= Arm), it was found that the formation degrees of the stacked species in

M(Arm)(ANP) and in their corresponding M(Arm)(NMP) systems are comparable.[128,134,144]

Since the solution-equivalent or intrinsic dielectric constant in the active-site cavity of enzymes is expected to be reduced,[145] *i.e.* the solvent polarity is decreased compared to water, studies of the complexes of the ANPs in solvents with a reduced polarity would be desirable. However, so far only very few data are available,[146,147] but they prove that the overall stability of phosph(on)ate complexes increases dramatically, *e.g.* by going from water as solvent to a 50% aqueous-dioxane mixture, whereas the position of equilibrium 1 is only little affected;[147] this is different again for equilibrium 2.[146] Hence, it appears that polarity changes in an active site are more relevant for the overall stability of M(ANP) complexes and thus for the binding and release of substrates and products, but less so for the structure of the complexes involving the ether oxygen.

6 Conclusion

The results summarized in this account explain why $PMEApp^{4-}$ and some other closely related $ANPpp^{4-}$ derivatives are initially better substrates for viral polymerases than the parent $(d)NTP^{4-}$ nucleotides. The reason is that the formation of the M(α)-M(β,γ)-coordination mode needed for the reactive state in the active-site cavity of the enzyme is favored (Section 3), compared to that of the (d)NTPs, due to the formation of 5-membered chelates involving the ether oxygen (equilibrium 1); consequently, all those ANP derivatives which do not form these chelates are devoid of an antiviral activity. The lesson thus is that in devising antivirally active therapeutics, which aim for the polymerase chain reaction as the target for viral inhibition should be chemically constructed such that a metal ion interaction at the P_α group of the diphosphorylated derivative is favored.

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