Solution properties of antiviral adenine-nucleotide analogues. The acid–base properties of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA)† and of its N1, N3 and N7 deaza derivatives in aqueous solution

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The pD dependence of the ^{1}H NMR chemical shifts of the aromatic and aliphatic hydrogens of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) and of its 1-, 3- and 7-deaza derivatives have been measured in D_2O at 25 °C (I = 0.1 mol dm^{-3}, $NaNO_3$; at pD < 1 I increases to 0.3 mol dm^{-3}) in order to determine the sites of protonation as well as the acidity constants. The most basic site in all these PMEAs (= PM) is the phosphonate group, -PO_3^{2-}, followed by N1 in PMEA, 3- and 7-deaza-PMEA. In 1-deaza-PMEA the formation of $H_2PM^{\pm}$ occurs by protonation of N3. Further protonation in strongly acidic medium is possible with all four PMEAs. All acidity constants measured in D_2O have been transformed to H_2O as solvent: p$K^H_{H_4PM} \simeq 0$ is due to deprotonation of H^+(N7), where appropriate; p$K^H_{H_3PM} \simeq 1.1$ to 1.3 is due to -P(O)$(OH)_2$; p$K^H_{H_2PM} \simeq 4.1$ to 6.6 is due to H^+(N1) or H^+(N3); and p$K^H_{HPM} \simeq 6.9$ to 7.8 is due to -$P(O)_2(OH)^-$. Determination of p$K^H_{H_2PM}$ and pK^H_{HPM} by potentiometric pH titrations in water (H_2O; I = 0.1 mol dm^{-3}, $NaNO_3$; 25 °C) give the same results. As in various instances the buffer regions of two successive equilibria are overlapping, a micro acidity constant scheme has been developed and the constants for the various sites calculated; it is concluded, *e.g.* that about 80% of the H(7-deaza-PMEA)$^-$ species carry the proton at the phosphonate residue and 20% at N1. The ^{1}H NMR data indicate that the PMEAs in the form PM^{2-} occur to some extent in an orientation similar to the *anti* conformation of 5′-AMP^{2-}; *i.e.* the phosph(on)ate group is close to H8. For H(3-deaza-PMEA)$^-$ the monoprotonated phosphonate group is in the vicinity of H2 in a hydrophobic region and it is suggested that this is the reason for the relatively high pK_a value of about 7.8 compared with p$K_a \simeq 6.9$ to 7.0 for HPM^- of the other PMEAs. Finally, the acid–base properties of the PMEAs are compared with those of 5′-AMP and of tubercidin 5′-monophosphate (= 7-deaza-5′-AMP).

Introduction

9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA), an acyclic 5′-AMP analogue, is a potent prodrug with selective antiviral activity against herpes simplex viruses (HSV) and retroviruses, including human immunodeficiency viruses (HIV-1 and HIV-2).[1–3] The structure of $PMEA^{2-}$ is shown in Fig. 1 together with that of 5′-AMP^{2-}.[4,5] Of course, PMEA is actually more closely related to 2′-deoxy-5′-AMP or even 2′,3′-dideoxy-5′-AMP, but for those latter adenine nucleotides less information is available and therefore we use mostly 5′-AMP for comparisons (see also ref. 6). In Fig. 1 (phosphonomethoxy)ethane (PME) is also depicted, which represents well the properties of the corresponding residue of PMEA.

In contrast to many other nucleotide analogues, PMEA carries a phosphonate group which mimics well the phosphate residue of a nucleoside monophosphate but cannot be dephosphorylated like the latter.[7] This is important because enzyme-catalysed dephosphorylations often render therapeutic applications of such antimetabolites inefficient. PMEA, if

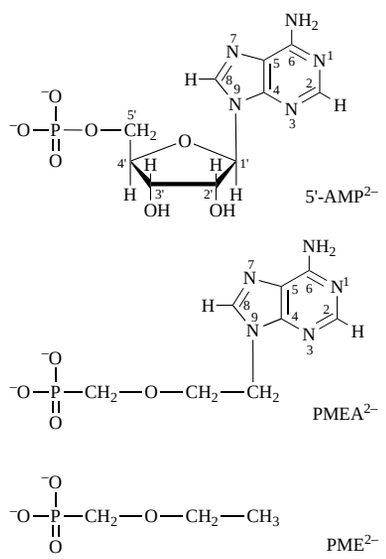


Fig. 1 Chemical structure of the dianion of 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$) in comparison with the structures of adenosine 5′-monophosphate (5′-AMP^{2-}) and the dianion of (phosphonomethoxy)ethane (PME^{2-} = ethoxymethanephosphonate). 5′-AMP^{2-} is shown in its dominating *anti* conformation.[4,5]

† *Abbreviations and definitions:* see Figs. 1 and 2; HPMPA, (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine; I, ionic strength; M^{2+}, divalent metal ion; PM^{2-}, $PMEA^{2-}$ or any of its twofold negatively charged deaza derivatives; 5′-$TuMP^{2-}$, tubercidin 5′-monophosphate (= 7-deaza-5′-AMP^{2-}). In mathematical expressions and the Tables 1-, 3- and 7-deaza-PMEA are partly written as 1d-, 3d- and 7d-PMEA. The expression 'protonation' is used throughout this study for the addition of H^+ or D^+ (= $^2H^+$) to a basic site, *i.e.* independent of the kind of hydrogen isotope. However, which isotope is considered in a given equilibrium is always clearly defined. Species which are given in the text without a charge either do not carry one or represent the species in general (*i.e.* independent from their protonation degree); which of the two versions applies is always clear from the context.

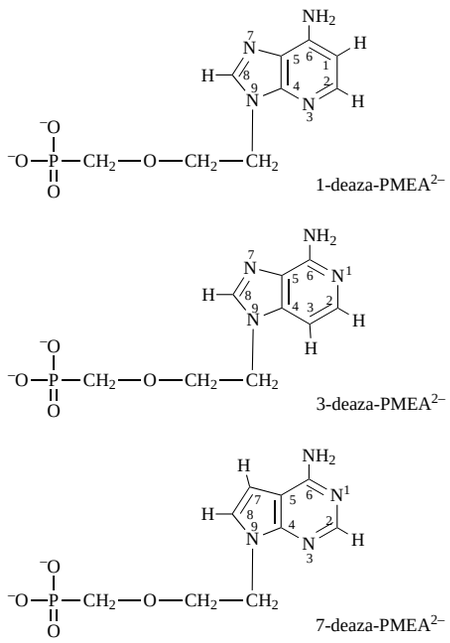


Fig. 2 Structures of the deaza analogues of $PMEA^{2-}$: 1-deaza-$PMEA^{2-}$, 3-deaza-$PMEA^{2-}$ and 7-deaza-$PMEA^{2-}$. Although the nomenclature for the deazaadenine compounds after IUPAC is 3-*H*-imidazo[4,5*b*]pyridine-7-amine (1-deazaadenine), imidazo[4,5*c*]-pyridine-4-amine (3-deazaadenine) and pyrrolo[2,3*d*]pyrimidine-4-amine (7-deazaadenine), the trivial names and the numbering system for purines are retained in the present study in order to facilitate the comparison with the parent compound, $PMEA^{2-}$, and other adenine derivatives.

taken up by a biological system, is phosphorylated by cellular enzymes to its di-phosphoryl derivative which then acts as a substrate for DNA polymerase or reverse transcriptase and exerts its antiviral activity as a DNA chain terminator.[8]

Structure–activity studies[9] of PMEA-like compounds revealed that deletion of the ether oxygen in the aliphatic chain or its replacement by other groups[7,9b,10] as well as alterations in the length of the chain[9a,b] lead to loss or considerable reduction in biological activity. There are also indications[11] that substrate or inhibitor activities of nucleosides and nucleotides as well as of their analogues might be correlated with their preferred structure in solution; therefore, the evaluation of thermodynamic and conformational parameters is one of the key issues in understanding the mechanism of a biological process.

The above mentioned cellular enzymes are metal ion dependent[12] and this has led us to study in detail the metal ion-coordinating properties of PMEA[13,14] and its 1-, 3- and 7-deaza derivatives[15] (Fig. 2) and to evaluate the various isomeric solution structures of the resulting complexes. A precondition for this is an understanding of the acid–base properties of these potential ligands.

Therefore, the pD dependency of the ^{1}H NMR chemical shifts of the various hydrogens of PMEA and the deaza derivatives mentioned were measured in D_2O in order to determine the different protonation sites of these nucleotide analogues together with their acidity constants. As the various nitrogens are systematically deleted in the deaza compounds (Fig. 2), it should be possible to elucidate their influence on the acid–base properties of PMEA (Fig. 1). Some of the acidity constants of PMEA and its derivatives were also measured independently in aqueous solution by potentiometric pH titration.

The deazaadenine derivatives of PMEA also deserve interest in their own right and not only because of their relationship to PMEA. The replacement of a nitrogen by a methine group in a nucleobase alters the steric demands of such a residue only very slightly and deazapurine derivatives often show antimetabolic character[16] and biological activity, *e.g.* tubercidin and its 5′-monophosphate ($TuMP^{2-}$ = 7-deaza-5′-AMP^{2-}) are active against bacteria, viruses and also some forms of cancer.[17] The 1- and 3-deaza analogues of the PMEA-relative (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (HPMPA) are highly active against cytomegalovirus (CMV) and varicella zoster virus (VZV), yet the corresponding PMEA analogues are devoid of such activity. None of these compounds inhibited other DNA viruses tested (HSV-1, HSV-2, vaccinia virus) or retroviruses (HIV, Moloney sarcoma virus).[2,18,19]

Experimental

Materials

PMEA and 1-, 3- and 7-deaza-PMEA were synthesized according to published procedures.[18,20] Tetramethylammonium nitrate was purchased from Fluka AG (Buchs, Switzerland) and $NaNO_3$, HNO_3, NaOH (Titrisol), potassium hydrogen phthalate and 1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetraacetate (Na_2H_2-EDTA) (all *pro analysi*) from Merck AG (Darmstadt, Germany). D_2O (>99.8% D), NaOD (>99.9% D) and DNO_3 (>99% D) were from Ciba-Geigy AG (Basel, Switzerland). The buffers used for pH calibration (pH 4.64, 7.00 and 9.00; based on the NBS scale, now NIST) were from Metrohm AG (Herisau, Switzerland).

The titre of the NaOH for the potentiometric pH titrations was determined with potassium hydrogen phthalate. The aqueous stock solutions of the PMEAs were freshly prepared daily using distilled CO_2-free water and their concentrations were determined *via* the potentiometric pH titrations (see below).

^{1}H NMR spectroscopy

The ^{1}H NMR spectra of the PMEAs were recorded at 25 °C with a Varian VXR-400 Fourier transform spectrometer operating at 399.96 MHz with D_2O as solvent, using the central peak of the tetramethylammonium ion (0.0018 mol dm^{-3}) triplet as internal standard and assigning to this peak a chemical shift value of 3.174 ppm[21] (*cf.* also ref. 17*c*), thus applying the ppm scale based on sodium 3-(trimethylsilyl)propane-1-sulfonate (for details and justification see refs. 21 and 22). With a spectral width of 6000 Hz and 32 000 data points a resolution of about 0.0005 ppm is obtained. No further sensitivity enhancement and no exponential multiplication was performed.

The pD of the solutions was measured with a Metrohm EA 6.0216.100 micro glass electrode connected to a Metrohm 605 digital pH meter. The calibration was performed with the above mentioned aqueous buffer solutions and the final pD of the D_2O solutions was obtained by adding 0.40 to the pH meter reading.[23] The desired pD of a solution was adjusted by dotting with relatively concentrated NaOD or DNO_3 on a thin glass rod.

The ionic strength, *I*, of the solutions at pD ⩾ 1 was adjusted to 0.1 mol dm^{-3} with $NaNO_3$. In the more strongly acidic solutions, *i.e.* pD < 1, the ionic strength reached a maximum value of $I = 0.3$ mol dm^{-3}.

The ^{1}H NMR signals of the hydrogens of PMEA were assigned as previously;[20b] the same is true for 1-deaza-PMEA,[18] 3-deaza-PMEA (*i.e.* by analogy with 3-deaza-HPMPA)[24] and 7-deaza-PMEA (*i.e.* by analogy with tubercidin and TuMP).[5,17c,25] To obtain sharp signals, some EDTA was added to the solutions to sequester paramagnetic metal ion impurities;[26] *i.e.* based on the concentrations of PMEA and the deaza-PMEAs which were 5×10^{-3} and 7×10^{-3} mol dm^{-3}, respectively, the solutions also contained 5% EDTA.

The AMPs, like other purines,[21,22,25–27] are known to undergo self-stacking[5,28] and this also has to be expected for the acyclic adenine derivatives studied here. By using the self-association constant of 5′-AMP^{2-}, $K = 2.1 \pm 0.3$ mol^{-1} dm^3,[5] one calculates for the experimental conditions mentioned that at least 97% of the PMs are present as monomers and this guarantees that the

acid–base properties described below refer to the monomeric species.

All experimental data regarding the dependence of the ^{1}H NMR chemical shifts on pD were analysed with a personal computer (80–486 processor) connected to a Hewlett-Packard 7475A plotter and a Brother M-1724 L printer by using a Newton–Gauss nonlinear least-squares method. The applied curve-fit program is based on the general eqn. (1),[5] where PM^{2-}

$$\delta_{obsd} = \frac{\delta_{PM} + \delta_{HPM}\,10^{(pK^{H}_{HPM} - pH)} + \delta_{H_2PM}\,10^{(pK^{H}_{H_2PM} + pK^{H}_{HPM} - 2pH)}}{1 + 10^{(pK^{H}_{HPM} - pH)} + 10^{(pK^{H}_{H_2PM} + pK^{H}_{HPM} - 2pH)}} \quad (1)$$

represents either $PMEA^{2-}$ or one of its deaza derivatives. $pK^{H}_{H_2PM}$ and pK^{H}_{HPM} are the negative logarithms of the acidity constants of H_2PM and HPM^-, respectively, which are protonated forms of PM^{2-}; δ_{obsd} is the observed chemical shift, and δ_{H_2PM}, δ_{HPM} and δ_{PM} are the chemical shifts of the species H_2PM, HPM^- and PM^{2-}, respectively. Depending on the solvent (H_2O or D_2O), all H in eqn. (1) may have to be replaced by D. Furthermore, as $PMEA^{2-}$ and part of its deaza derivatives can accept up to four protons forming H_4PM^{2+}, eqn. (1) needs to be properly extended by two more pK_a values, but for the sake of clarity only the expression for two pK_a values is given. In the pD range of about 0.5 to 11 for each PM between 22 and 25 measurements were made.

Eqn. (1) and its extensions were always applied to the chemical shifts of all hydrogens of a compound. From these results (pK^{H}_{HPM}, *etc.*) the weighted means were calculated as far as possible (Tables 1 and 2, *vide infra*), and used to obtain for each hydrogen the final chemical shifts (Table 3, *vide infra*).

Potentiometric pH titrations

The pH titration curves for the determination of the acidity constants in H_2O were recorded with a Metrohm E 536 potentiograph connected with a Metrohm E 535 dosimat and a Metrohm 6.0202 100 (NB) combined macro glass electrode. Calibration was carried out with the buffers mentioned above and the direct pH readings were used in the calculation of the acidity constants; *i.e.* these constants are so-called practical, mixed or Brønsted constants.[29] Their negative logarithms given for aqueous solutions at $I = 0.1$ mol dm^{-3} ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values;[29] this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[29,30]

The constants $K^{H}_{H_2PM}$ and K^{H}_{HPM} of H_2PM were determined by titrating under N_2 30 cm^3 of an aqueous 1.35×10^{-3} mol dm^{-3} HNO_3 in the presence and absence of the 4×10^{-4} mol dm^{-3} PMEA derivative (present in the stock solution as PM^{2-}) with 1.5 cm^3 of 0.03 mol dm^{-3} NaOH ($I = 0.1$ mol dm^{-3}, $NaNO_3$; 25 °C). As the difference in NaOH consumption between pairs of solutions, *i.e.* with and without ligand,[29] is evaluated, the ionic product of water (K_w) and the above mentioned conversion term do not enter into the calculations.

The acidity constants were calculated with the above mentioned computer facility by a curve-fitting program applying a Newton–Gauss nonlinear least-squares fitting procedure between about 3 and 97% neutralization with respect to the equilibria $H_2PM^{\pm}/HPM^-$ and HPM^-/PM^{2-}. The lower pK_a values, *i.e.* $pK^{H}_{H_4PM}$ and $pK^{H}_{H_3PM}$, cannot be reached under the present experimental conditions. The results given in columns 4 and 5 of Table 5 (*vide infra*) are in each case the averages of at least eight pairs of independent titrations. The concentrations of the PMs employed here are even smaller than those in the ^{1}H NMR experiments; hence, the results are not influenced by self-stacking interactions[13a] and apply to the monomeric PMs.

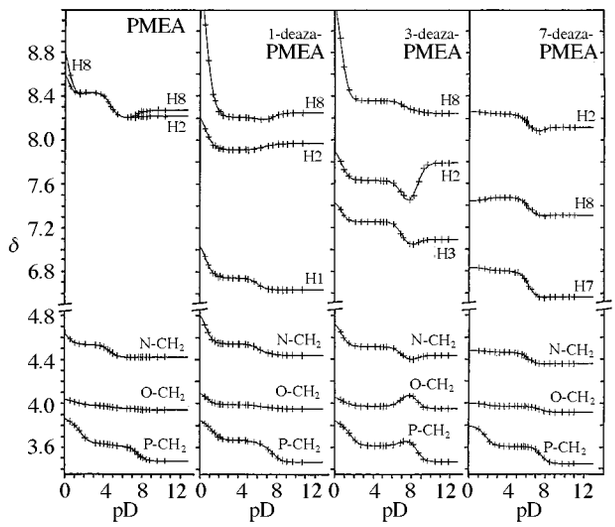


Fig. 3 Variation of the chemical shifts for all aromatic and aliphatic protons with pD for 0.005 mol dm^{-3} PMEA and 0.007 mol dm^{-3} 1-deaza-PMEA, 3-deaza-PMEA or 7-deaza-PMEA in D_2O. The spectra were measured on a Varian VXR 400 Fourier transform spectrometer operating at 399.96 MHz (25 °C; at pD ⩾ 1 $I = 0.1$ mol dm^{-3}, $NaNO_3$; at pD < 1 I increased to 0.3 mol dm^{-3}), using the central peak of internal $(CH_3)_4N^+/NO_3^-$ (0.0018 mol dm^{-3}) as standard and assigning to this peak a chemical shift value of 3.174 ppm thus transforming all data to the ppm scale based on sodium 3-(trimethylsilyl)propane-1-sulfonate. The curves are the computer-calculated best fits [see eqn. (1)] of the experimental data using the averaged pK_a values given in Table 2; the resulting shifts are listed in Table 3.

Results and discussion

The pD dependence of proton chemical shifts of the PMs in D_2O

In Fig. 3 the pD dependence of chemical shifts are shown for all hydrogens of PMEA and its deaza derivatives. The assignment of the various resonance signals to their hydrogens was unequivocally possible in accordance with the literature.[5,17c,18,20b,24,25]

Replacement of a purine nitrogen by a methine group as in 1-, 3- and 7-deaza-PMEA results in an increased electron density and thus in an upfield shift of the signal of the adjacent aromatic proton (Fig. 3); the influence on the second aromatic hydrogen is only small and its chemical shift is close to that of the corresponding proton of the parent compound, *i.e.* PMEA. For example, replacement of N1 or N3 by CH as in 1- or 3-deaza-$PMEA^{2-}$, respectively, gives rise to an upfield shift of the signals of H2, whereas those of H8 remain rather unaffected, being close to the one in $PMEA^{2-}$. The signal of the new hydrogen, *i.e.* of H(C1) or H(C3), appears at even higher field than those of H2 or H8 because it has no nitrogen atom as a direct neighbour. Consequently, H2 of 7-deaza-PMEA occurs at approximately the same shift position as the H2 of PMEA, whereas H8, which now has H(C7) as a neighbour, is considerably upfield shifted. As expected, the aliphatic hydrogens of the (phosphonomethoxy)ethyl residue (Figs. 1 and 2) have very similar positions in all four PMs (Fig. 3).

It is also worth noting that the two aromatic protons H2 and H8 of PMEA have the same chemical shifts in the pD range of about 3.2 to 6.5 (Fig. 3). In most other adenine derivatives, like 9-methyladenine,[31] adenosine[5] or all the AMPs,[5,28] the signal of H8 appears downfield of the one of H2, yet the phenomenon mentioned was also reported for 9-{2-[bis(β-chloroethyl)-phosphono]ethyl}adenine,[32a] 9-[4-(phosphono)butyl]adenine[32b] and some related compounds.[32b] There are even some examples of adenine-nucleotide analogues for which the signal of H8 appears upfield of H2.[32c] At pD < 3.2 and pD > 6.5 the resonance signals for H2 and H8 of PMEA are separated. That the signal, which appears more downfield, is due to H8 follows from comparison with the signals of the deaza-PMEAs and

Table 1 Negative logarithms of the acidity constants for $D_3(PMEA)^+$, D_3(1-deaza-PMEA)$^+$, D_3(3-deaza-PMEA)$^+$ and D_3(7-deaza-PMEA)$^+$ as determined by the ^{1}H NMR shift experiments in D_2O shown in Fig. 3 (I = 0.1 mol dm^{-3}, $NaNO_3$; 25 °C)[a]

Protonated species	H[b]	$pK^D_{D,PM}$ [eqn. (3)]		$pK^D_{D_2PM}$ [eqn. (4)]		pK^D_{DPM} [eqn. (5)]	
		$pK_{a/ind}$	$pK_{a/av}$	$pK_{a/ind}$	$pK_{a/av}$	$pK_{a/ind}$	$pK_{a/av}$
$D_3(PMEA)^+$	H8	1.719 ± 16.150		4.719 ± 0.042		7.425 ± 0.093	
	H2	1.750 ± 0.654		4.689 ± 0.038		7.208 ± 0.392	
	N-CH_2	1.004 ± 0.646	1.687 ± 0.064	4.700 ± 0.044	4.720 ± 0.046	6.199 ± 1.225	7.659 ± 0.180
	O-CH_2	1.653 ± 0.106		4.835 ± 0.076		8.071 ± 0.133	
	P-CH_2	1.691 ± 0.030		5.009 ± 0.145		7.664 ± 0.025	
D_3(1d-PMEA)$^+$	H8			6.182 ± 0.526		7.505 ± 0.289	
	H2			6.695 ± 0.508		7.733 ± 1.734	
	H1			6.028 ± 0.251	6.048 ± 0.107	7.028 ± 5.394	7.734 ± 0.084
	N-CH_2		1.58 ± 0.14[c]	6.005 ± 0.080		7.735 ± 0.235	
	O-CH_2			5.973 ± 0.250		7.669 ± 0.301	
	P-CH_2			6.671 ± 0.209		7.778 ± 0.134	
D_3(3d-PMEA)$^+$	H8	1.475 ± 7.900		6.949 ± 0.073		8.760 ± 0.186	
	H3	1.488 ± 7.991		7.010 ± 0.038		8.443 ± 0.122	
	H2	1.394 ± 2.976		7.147 ± 0.069	7.055 ± 0.050	8.527 ± 0.048	8.480 ± 0.054
	N-CH_2	1.595 ± 1.739	1.701 ± 0.137	7.053 ± 0.045		8.428 ± 0.112	
	O-CH_2	1.537 ± 0.840		7.155 ± 0.071		8.408 ± 0.069	
	P-CH_2	1.707 ± 0.140		7.288 ± 0.133		8.456 ± 0.058	
D_3(7d-PMEA)$^+$	H8	1.728 ± 0.347		6.619 ± 0.037		8.331 ± 0.504	
	H7	1.970 ± 0.437		6.169 ± 0.031		8.193 ± 0.525	
	H2	2.233 ± 0.434		6.152 ± 0.043	6.164 ± 0.074	7.692 ± 0.150	7.507 ± 0.177
	N-CH_2	1.937 ± 0.404	1.756 ± 0.065	6.163 ± 0.037		8.167 ± 0.573	
	O-CH_2	1.733 ± 0.176		5.018 ± 0.794		7.463 ± 0.052	
	P-CH_2	1.750 ± 0.040		6.328 ± 0.565		7.508 ± 0.041	

[a] The evaluations for the individual protons ($pK_{a/ind}$) are listed; all values are the results obtained from the free variation of the pK_a values (including the chemical shifts of the various species). The error limits given with $pK_{a/ind}$ are the standard deviations resulting from the fitting procedure. The average pK_a values, $pK_{a/av}$, are the weighted means of the individual results; their errors also correspond to the standard deviation (see also Table 2). [b] N-CH_2, O-CH_2 and P-CH_2 denote the corresponding protons of the (phosphonomethoxy)ethyl residue (see Figs. 1 and 2). [c] No reasonable pK_a value could be obtained in this case by a free variation of all parameters. However, by fitting the experimental data with various constant values, it became immediately evident that the above given constant (within its error limits) fits the experimental values best (see also second section).

also from the various pD dependencies of the shift positions which will be discussed below.

Published ^{1}H NMR spectra of adenosine (δ_{H8} = 8.322 ppm), 2′-AMP^{2-} and 3′-AMP^{2-} reveal[5] that a phosphate group at the 2′ or 3′ position of the ribose ring only has a small influence on the chemical shift of H8 which occurs at 8.364 and 8.362 ppm, respectively, whereas a phosphate group at the 5′ position of the sugar, giving 5′-AMP^{2-}, leads to a deshielding of H8 and a downfield shift to 8.605 ppm.[5] In contrast, replacement of the 5′-ribosyl phosphate residue by the (phosphonomethoxy)ethyl moiety gives PMEA^{2-} and leads to a slight shielding of H8 and a shift position at 8.274 ppm (Table 3, *vide infra*). This indicates that the phosph(on)ate group in 5′-AMP^{2-} as well as in PMEA^{2-} is in the neighbourhood of H8, thus giving rise to electrical field effects which affect the shift of H8.[5,33]

The variation of the chemical shifts of all hydrogens with pD follows that expected in the range of pD *ca.* 0 to 6; *i.e.* deprotonation leads to an increase of electron density and thus, the signals are shifted to higher field (*i.e.* to smaller ppm values). At pD > 6 in various instances so-called[5,34] 'wrongway shifts' are observed; these will be discussed in the fifth section. For the present it is only important to note that in Fig. 3, *e.g.* for the 7-deaza-PMEA system clearly three 'turning points' are seen, *i.e.* at pD about 7.5, 6 and 2; hence, there must be three pK_a values. Similarly, for PMEA four such points exist, namely at pD about 7.5, 4.5, 2 and 0.5; the latter two become visible only if the shifts of H8 and P-CH_2 are compared. The pK_a value responsible for the shift of the P-CH_2 signal in the pD range from 0 to 3 is clearly higher than the one responsible for the downfield shift of the signals of H8 and H2.

Definition of the acidity constants and their determination from ^{1}H NMR shift measurements

As mentioned above, in the pD range 0 to 10 four different acidity constants occur for the PMEA system. Indeed, adenine may accept in total three protons:[35] the first one at N1 (pK_a = 4.2),[35] a second one at N7 ($pK_a \simeq -0.4$)[35] and a final one at N3 ($pK_a \simeq -4.2$)[36] in very strong acids; for the pD range considered here only the first two are relevant. Similarly, a $-PO_3^{2-}$ residue binds two protons.[13a] Hence, overall PMEA^{2-} accepts four protons giving the four-protonic acid, $H_4(PMEA)^{2+}$. Thus, the following four equilibria, eqns. (2)–(5),

$$H_4PM^{2+} \rightleftharpoons H_3PM^+ + H^+ \quad (2a)$$

$$K^H_{H_4PM} = [H_3PM^+][H^+]/[H_4PM^{2+}] \quad (2b)$$

$$H_3PM^+ \rightleftharpoons H_2PM + H^+ \quad (3a)$$

$$K^H_{H_3PM} = [H_2PM][H^+]/[H_3\mathbf{PM}^+] \quad (3b)$$

$$H_2PM \rightleftharpoons HPM^- + H^+ \quad (4a)$$

$$K^H_{H_2PM} = [HPM^-][H^+]/[H_2PM] \quad (4b)$$

$$HPM^- \rightleftharpoons PM^{2-} + H^+ \quad (5a)$$

$$K^H_{HPM} = [PM^{2-}][H^+]/[HPM^-] \quad (5b)$$

where PM^{2-} is the symbol for PMEA^{2-} or one of its deaza derivatives, need to be considered.

Application of eqn. (1) and of its analogues to the chemical shifts seen in Fig. 3 *via* a curve-fit procedure is straightforward for 7-deaza-PMEA. As seen in the lower part of Table 1 each of the six resonances due to the various hydrogens of 7-deaza-PMEA (Figs. 2 and 3) furnishes a result for three different pK_a values (*cf.* the last paragraph of the previous section).

The shift difference between two differently protonated forms depends to a first approximation on the distance of the hydrogen being considered from the site of the acid–base reaction. A hydrogen close to the reaction site will sense the change in electron density strongly and its resonance signal will undergo a large shift. The larger such a shift the more exact is the calculated pK_a value and the smaller its error limit. Therefore, the

Table 2 Summary of the results of the negative logarithms of the acidity constants for the deuterated, D_4PM^{2+}, or protonated, H_4PM^{2+}, species of $PMEA^{2-}$, 1-deaza-$PMEA^{2-}$, 3-deaza-$PMEA^{2-}$ and 7-deaza-$PMEA^{2-}$ as determined from ^1H NMR shift measurements in D_2O (see also Table 1) together with the site which is deprotonated (25 °C; $I = 0.1$ mol dm^{-3}, $NaNO_3$; for $pK^D_{D_4PM}$ $I \simeq 0.3$ mol dm^{-3}, see Experimental)[a]

Solvent	pK_a	Deprot. site	$D_4(PMEA)^{2+}$ or $H_4(PMEA)^{2+}$	D_4(1d-PMEA)$^{2+}$ or H_4(1d-PMEA)$^{2+}$	D_4(3d-PMEA)$^{2+}$ or H_4(3d-PMEA)$^{2+}$	D_3(7d-PMEA)$^{2+}$ or H_3(7d-PMEA)$^{2+}$
D_2O	$pK^D_{D_4PM}$ [eqn. (2)]	D^+(N7)	0.1 ± 0.5	0.5 ± 0.3	0.6 ± 0.4	—
	$pK^D_{D_4PM}$ [eqn. (3)]	-P(O)$(OD)_2$	1.69 ± 0.13	1.58 ± 0.28	1.70 ± 0.27	1.76 ± 0.13
	$pK^D_{D_4PM}$ [eqn. (4)]	D^+(N1)	4.72 ± 0.09	6.05 ± 0.21[b]	7.06 ± 0.10	6.16 ± 0.15
	pK^D_{DPM} [eqn. (5)]	-P$(O)_2(OD)^-$	7.66 ± 0.36	7.73 ± 0.17	8.48 ± 0.11	7.51 ± 0.35
H_2O[a]	$pK^H_{H_4PM}$ [eqn. (2)]	H^+(N7)	−0.35	0.05	0.15	—
	$pK^H_{H_4PM}$ [eqn. (3)]	-P(O)$(OH)_2$	1.22	1.11	1.23	1.29
	$pK^H_{H_4PM}$ [eqn. (4)]	H^+(N1)	4.21	5.52[b]	6.51	5.63
	pK^H_{HPM} [eqn. (5)]	-P$(O)_2(OH)^-$	7.10	7.17	7.91	6.96

[a] The error limits are twice the standard error or the sum of the probable systematic errors, whichever is larger (see also second section). The pK_a values for H_2O as solvent were calculated with eqn. (6) (see sixth section); here the same error limits apply as given for the corresponding constants valid for D_2O as solvent. [b] Deprotonation of the nucleobase moiety in D_2(1-deaza-PMEA)$^{\pm}$ occurs at the D^+(N3) site (see fourth section).

weighted means for the various series of individual results were always calculated (Table 1).

Variation of all parameters connected with four different pK_a values, as is the case with PMEA and its 1- and 3-deaza derivatives (see end of the first section), did not lead to satisfying results because the chemical shifts going downfield at the very low pD values (Fig. 3) do not reach a limiting δ_{D_4PM} value and consequently the lowest pK_a is poorly defined. Moreover, the two lowest protonation equilibria (2) and (3) are overlapping. However, application of the curve-fit procedure only to the pD range > 1 gave clearcut results for three pK_a values for PMEA and 3-deaza-PMEA (*cf.* Table 1).

Since the limiting shift for D_4PM^{2+} is poorly defined, the curve-fit procedure could not be used, but by systematically varying $pK^D_{D_4PM}$ [eqn. (2)] a value could be estimated for which on the one hand the shift differences due to the chemical shifts of D_3PM^+ and D_4PM^{2+} had to be of a reasonable size (*i.e.* $\Delta\delta < 2$ ppm) and on the other the fit of the experimental data points had to be satisfactory, *i.e.* the error square sum should not increase unreasonably. This procedure has led to the estimated results listed in the first row of Table 2.

For 1-deaza-PMEA the simultaneous variation of three pK_a values did not lead to a satisfying result for $pK^D_{D_4PM}$; therefore, values for $pK^D_{D_4PM}$ and $pK^D_{D_4PM}$ were estimated by systematically using different values, applying the criteria defined above, until those were found that best fit the experimental data.

The acidity constants according to equilibria (2*a*) through (5*a*) for the four PMs are summarized in Table 2. This means the averaged values, $pK_{a/av}$, of Table 1 and the estimated values, determined as indicated above, are all listed with their error limits in the upper part of Table 2 for D_2O as solvent. In the lower part the corresponding acidity constants, transformed to H_2O as solvent,[37] are given; these values will be discussed in the sixth section.

Application of the acidity constants assembled in Table 2 to all experimental data points for a given compound results in the computer-calculated best fits, this means, the solid curves seen in Fig. 3. Table 3 contains the chemical shifts of the hydrogens of the free PM^{2-} species and of their various protonated forms, which were obtained by this same calculation. The error limits also contain the errors of the applied acidity constants (twice the standard error). In a few instances unreasonably large errors are obtained for the calculated chemical shifts because a large error was attributed to estimated pK_a values to be on the safe side. The extreme example is $\delta_{D_4PM} = 9.19 \pm 0.98$ ppm for $D_4(PMEA)^{2+}$ (Table 3, column 3, first entry); from the dependence of the chemical shift of H8 on pD it is evident (Fig. 3) that the shift value at pD ~ 0 can never be as low as $\delta = 8.21$ ppm as would be permitted by the above error limit. This means, even if in a few instances the error limits are exaggerated, the approximate sizes of the listed chemical shifts are correct (see Fig. 3).

With the known chemical shifts for the various protonated species (Table 3) one may calculate the shift differences, $\Delta\delta$, that a particular hydrogen in one of the PMs experiences upon a particular acid–base reaction. These differences (Table 4) will help to identify the various sites at which an acid–base reaction occurs.

Attribution of the acidity constants to their sites of deprotonation

Since protonation leads commonly to downfield shifts[38] for hydrogen signals which are close to the proton-binding sites ^1H NMR shift experiments, like those shown in Fig. 3, not only provide acidity constants, but also furnish information about the sites at which an acid–base reaction occurs.

In the fully deprotonated PM^{2-} species (Figs. 1 and 2) the most basic site is the -PO_3^{2-} residue which readily accepts a proton.[13a] Indeed, the hydrogen signals in P-CH_2 are shifted downfield more upon protonation for all PMs (see column 6 of Table 4); hence, the values given in Table 2 for pK^D_{DPM} have to be attributed to the deprotonation of -P$(O)_2(OD)^-$ [eqn. (5)].

The next protonation step occurs[5,35,39] at N1 and therefore significant downfield shifts are observed for H2 upon protonation of D(PMEA)$^-$, D(3-deaza-PMEA)$^-$ and D(7-deaza-PMEA)$^-$ (Table 4, column 5), leading to their zwitterionic form $D_2PM^{\pm}$ [eqn. (4)]. That H8 is also sensitive to acid–base reactions occurring at N1 is not surprising as electron density changes are easily transmitted through the π system of aromatic rings to more distant places as is observed with other adenine derivatives.[5,40] A nice example is D(7-deaza-PMEA)$^-$ in which H(C7) (replacing N7) and H8 of the imidazole ring experience, upon protonation of N1 in the pyrimidine ring, a very significant downfield shift despite the fact that they are relatively distant from N1.

Further protonation leads to $D_3\mathbf{PM}^+$ and this reaction occurs again at the phosphonate residue as the downfield shifts for the hydrogens of P-CH_2 demonstrate (Table 4, column 4); the $pK^D_{D_3PM}$ values close to 1.7 (Table 2, row 2) are due to the loss of the first proton from -P(O)$(OD)_2$ [eqn. (3)].

The formation of D_4PM^{2+} could only be observed for PMEA, 1- and 3-deaza-PMEA, all of which have an N7 atom, and accordingly the most pronounced downfield shift is observed for H8 (Table 4, column 3). This site attribution agrees with results obtained for H_2(adenine)$^{2+}$ ($pK_a \simeq -0.4$),[36] H_2(N6′,N6′,N9-trimethyladenine)$^{2+}$ ($pK_a \simeq -0.75$)[40] and also with the fact that D_3(7-deaza-PMEA)$^+$ does not accept a further proton in the pD range of this study (*cf.* Fig. 3). Hence, the values listed for $pK^D_{D_4PM}$ [eqn. (2)] in the first row of Table 2 refer to the deprotonation of the D^+(N7) site.

N3 is never protonated in the species discussed up to now, and again, this agrees with the previous experience that deprotonation of H_3(adenine)$^{3+}$ already occurs[36] with $pK_a \simeq -4.2$ and such extremely acidic conditions are not reached in the

Table 3 ^{1}H NMR chemical shifts (in ppm) of the various hydrogens for the different protonated (*i.e.* deuterated) and free forms of PMEA, 1-deaza-PMEA, 3-deaza-PMEA and 7-deaza-PMEA as determined in D_2O from the experiments shown in Fig. 3 (25 °C; $I = 0.1$ mol dm^{-3}, $NaNO_3$; for $\delta_{(D_4PM)}$ $I \simeq 0.3$ mol dm^{-3})[a]

PM	H	$\delta_{(D_4PM)}$	$\delta_{(D_3PM)}$	$\delta_{(D_2PM)}$	$\delta_{(DPM)}$	$\delta_{(PM)}$
PMEA	H8	9.194 ± 0.983[b]	8.384 ± 0.038	8.436 ± 0.016	8.199 ± 0.017	8.274 ± 0.013
	H2	8.779 ± 0.494	8.393 ± 0.023	8.433 ± 0.013	8.200 ± 0.008	8.220 ± 0.006
	N-CH_2	4.726 ± 0.237	4.540 ± 0.011	4.535 ± 0.006	4.420 ± 0.005	4.422 ± 0.002
	O-CH_2	4.060 ± 0.076	4.019 ± 0.009	3.984 ± 0.003	3.957 ± 0.003	3.941 ± 0.003
	P-CH_2	3.895 ± 0.112	3.834 ± 0.031	3.636 ± 0.005	3.610 ± 0.024	3.470 ± 0.020
1d-PMEA	H8	9.909 ± 0.656	8.368 ± 0.122	8.206 ± 0.006	8.179 ± 0.019	8.248 ± 0.009
	H2	8.293 ± 0.162	7.949 ± 0.035	7.991 ± 0.004	7.942 ± 0.011	7.968 ± 0.004
	H1	7.125 ± 0.157	6.784 ± 0.035	6.740 ± 0.005	6.634 ± 0.017	6.632 ± 0.004
	N-CH_2	4.897 ± 0.142	4.568 ± 0.026	4.539 ± 0.004	4.459 ± 0.012	4.432 ± 0.003
	O-CH_2	4.125 ± 0.056	4.009 ± 0.016	3.986 ± 0.002	3.964 ± 0.005	3.948 ± 0.002
	P-CH_2	3.870 ± 0.059	3.791 ± 0.061	3.662 ± 0.005	3.613 ± 0.021	3.462 ± 0.008
3d-PMEA	H8	9.508 ± 0.630	8.343 ± 0.124	8.357 ± 0.008	8.276 ± 0.020	8.244 ± 0.009
	H3	7.467 ± 0.139	7.242 ± 0.030	7.248 ± 0.005	7.003 ± 0.020	7.089 ± 0.005
	H2	7.957 ± 0.219	7.641 ± 0.054	7.627 ± 0.008	7.348 ± 0.039	7.791 ± 0.015
	N-CH_2	4.769 ± 0.138	4.526 ± 0.029	4.512 ± 0.003	4.372 ± 0.012	4.433 ± 0.003
	O-CH_2	4.067 ± 0.064	3.990 ± 0.021	3.969 ± 0.004	4.112 ± 0.016	3.944 ± 0.006
	P-CH_2	3.853 ± 0.069	3.775 ± 0.066	3.612 ± 0.005	3.693 ± 0.021	3.463 ± 0.008
7d-PMEA	H8		7.440 ± 0.012	7.473 ± 0.008	7.300 ± 0.026	7.311 ± 0.005
	H7		6.833 ± 0.013	6.804 ± 0.011	6.554 ± 0.035	6.566 ± 0.006
	H2		8.259 ± 0.009	8.239 ± 0.008	8.053 ± 0.026	8.119 ± 0.007
	N-CH_2		4.479 ± 0.006	4.463 ± 0.005	4.353 ± 0.016	4.360 ± 0.003
	O-CH_2		4.000 ± 0.005	3.969 ± 0.001	3.961 ± 0.012	3.913 ± 0.004
	P-CH_2		3.794 ± 0.008	3.604 ± 0.004	3.609 ± 0.036	3.447 ± 0.013

[a] The chemical shifts were calculated by applying the values of the acidity constants given in Table 2, together with their error limits, to the experimental data; the resulting δ *versus* pD curves are seen in Fig. 3. The range of errors given with the calculated shifts is twice the standard deviation. N-CH_2, O-CH_2 and P-CH_2 denote the corresponding protons of the (phosphonomethoxy)ethyl residue (see Figs. 1 and 2). [b] Regarding this exaggerated error limit see the text towards the end of the second section. The same comment as given there also applies to a few other instances for which the error limit of pK_a was estimated.

Table 4 Shift differences, $\Delta\delta$ (in ppm), as they result from the increasing deprotonation of the species beginning with H_4PM^{2+} for the various hydrogens of the protonated and free forms of PMEA, 1-deaza-PMEA, 3-deaza-PMEA and 7-deaza-PMEA, as calculated from the chemical shifts listed in Table 3[a]

PM	H	$\Delta\delta_4 = \delta_{(D_4PM)} - \delta_{(D_3PM)}$	$\Delta\delta_3 = \delta_{(D_3PM)} - \delta_{(D_2PM)}$	$\Delta\delta_2 = \delta_{(D_2PM)} - \delta_{(DPM)}$	$\Delta\delta_1 = \delta_{(DPM)} - \delta_{(PM)}$
PMEA	H8	0.81 ± 0.98[b]	−0.052 ± 0.041	0.237 ± 0.023	−0.075 ± 0.021
	H2	0.39 ± 0.49	−0.040 ± 0.026	0.233 ± 0.015	−0.020 ± 0.010
	N-CH_2	0.19 ± 0.24	0.005 ± 0.013	0.115 ± 0.008	−0.002 ± 0.005
	O-CH_2	0.041 ± 0.077	0.035 ± 0.009	0.027 ± 0.004	0.016 ± 0.004
	P-CH_2	0.06 ± 0.12	0.198 ± 0.031	0.026 ± 0.025	0.140 ± 0.031
1d-PMEA	H8	1.54 ± 0.67	0.162 ± 0.122	0.027 ± 0.020	−0.069 ± 0.021
	H2	0.34 ± 0.17	0.038 ± 0.035	−0.031 ± 0.012	−0.026 ± 0.012
	H1	0.34 ± 0.16	0.044 ± 0.035	0.106 ± 0.018	0.002 ± 0.017
	N-CH_2	0.33 ± 0.14	0.029 ± 0.026	0.080 ± 0.013	0.027 ± 0.012
	O-CH_2	0.116 ± 0.058	0.023 ± 0.016	0.022 ± 0.005	0.016 ± 0.005
	P-CH_2	0.079 ± 0.085	0.129 ± 0.061	0.049 ± 0.022	0.151 ± 0.022
3d-PMEA	H8	1.17 ± 0.64	−0.014 ± 0.124	0.081 ± 0.022	0.032 ± 0.022
	H3	0.23 ± 0.14	−0.006 ± 0.030	0.245 ± 0.021	−0.086 ± 0.021
	H2	0.32 ± 0.23	0.014 ± 0.055	0.279 ± 0.040	−0.443 ± 0.042
	N-CH_2	0.24 ± 0.14	0.014 ± 0.029	0.140 ± 0.012	−0.061 ± 0.012
	O-CH_2	0.077 ± 0.067	0.021 ± 0.021	−0.143 ± 0.016	0.168 ± 0.017
	P-CH_2	0.078 ± 0.095	0.163 ± 0.066	−0.081 ± 0.022	0.230 ± 0.022
7d-PMEA	H8		−0.033 ± 0.014	0.173 ± 0.027	−0.011 ± 0.026
	H7		0.029 ± 0.017	0.250 ± 0.037	−0.013 ± 0.036
	H2		0.021 ± 0.012	0.186 ± 0.027	−0.067 ± 0.027
	N-CH_2		0.016 ± 0.008	0.110 ± 0.017	−0.007 ± 0.016
	O-CH_2		0.027 ± 0.005	0.007 ± 0.012	0.049 ± 0.013
	P-CH_2		0.190 ± 0.009	−0.004 ± 0.036	0.161 ± 0.038

[a] The error limits correspond to twice the standard deviation; they were calculated according to the error propagation after Gauss by taking into account the error limits given in Table 3. [b] This error limit is somewhat exaggerated as a consequence of the oversized error limit in Table 3 for $\delta_{D_4(PMEA)}$; see also footnote *b* in Table 3 and the text towards the end of the second section.

present study. Hence, the basicity of the various ring nitrogens of the adenine residue decreases in the order N1 > N7 > N3, which agrees with earlier experimental[34–36,40] and theoretical[41] investigations.

Further protonation of phosphonate-monoprotonated 1-deaza-PMEA occurs at N3!

The only species not considered in the previous section is D_2(1-deaza-PMEA)$^{\pm}$. Of course, the first proton is bound at the phosphonate residue and the second one, as there is no N1 present, can be only at N3 or NH_2(C6) because N7 is only protonated at a very low pD ($pK^D_{D_4PM} = 0.5 \pm 0.3$; Table 2) as discussed above and $pK^D_{D_2PM}$ in the present case is close to 6 (Table 2, row 3).

If protonation of the adenine moiety would occur at N3, one would expect a downfield shift of the H2 signal. However, the H2 signal experiences a small 'wrongway' (*cf.* next section) upfield shift upon protonation! The shift of H8 is practically

not affected by this acid–base reaction (Table 4, column 5) which agrees with the conclusion that this protonation occurs in the pyrimidine and not in the imidazole ring. The only aromatic hydrogen of D(1-deaza-PMEA)$^-$, the signal of which experiences a downfield shift upon protonation, is H1 which is next to H_2N(C6); hence, one might be tempted to conclude that protonation occurs at the 6-amino group and not at N3.

On the other hand, 4-aminopyridine (like 2-aminopyridine[42] and 3-aminopyridine[43]) is protonated at the pyridine nitrogen and not at the amino group.[43] The preferential protonation of the pyridine nitrogen over the amino group is also supported by the pK_a values of H(pyridine)$^+$ and H(aminobenzene)$^+$ which are 5.3 (ref. 44) and 4.7 (ref. 45), respectively, in aqueous solution.

A careful consideration of the structure of 1-deaza-PMEA (Fig. 2) and of the shifts that the signals of the hydrogens of D(1-deaza-PMEA)$^-$ experience upon protonation (Tables 3 and 4) reveals that (*i*) H1 is separated by two carbon atoms from the nitrogen in the 6-amino group and the same is also true for the distance to N3, and (*ii*) the downfield shift of H1 is, within its error limits, the same as the one for the hydrogens of (N9)-CH_2 (Table 4, column 5), which are three atoms away from N3 (Fig. 2), yet much farther from H_2N(C6). This then suggests that N3 is the site of protonation! Indeed, this conclusion agrees with a recent ^{13}C NMR study[46] of 1-deazaadenosine in acidic DMSO solution.

Why is the chemical shift of H2 hardly affected? We believe that two opposite effects are operating. On the one hand a downfield shift due to protonation at N3 and on the other an upfield shift resulting from a shielding effect of the phosphonate group (which is a well known[5,34] property of the phosphate group; see next section), and that the latter slightly dominates, leading, overall, to the small observed upfield shift of −0.03 ppm for the signal of H2 (column 5 of Table 4). This must mean that the monoprotonated phosphonate group of 1-deaza-PMEA is to some extent in the vicinity of H2, which is also confirmed by the 'wrongway' upfield shifts of −0.07 and −0.03 ppm observed for H8 and H2, respectively, upon protonation of the phosphonate group (Table 4, column 6). In other words, -$P(O)_2(OD)^-$ resides at least partially above (or below) the 1-deazaadenine moiety influencing both H8 and H2, or, alternatively, one fraction of the monoprotonated phosphonate residue is close to H8 and another one close to H2.

'Wrongway' shifts and conclusions on structures in solution of various species of the PMs

The variation of the chemical shifts with pD results, for most aliphatic PM hydrogens, in the expected downfield shifts upon protonation (see third section). However, monoprotonation of the -PO_3^{2-} group initiates for most of the aromatic hydrogens an upfield shift (Fig. 3; Table 4). This behaviour is well known for H8 of purine nucleoside 5′-monophosphates;[34] *e.g.* it has been concluded[5] that 5′-AMP exists in the *anti* conformation (see Fig. 1) wherein the phosphate residue is relatively close to H8 and exerts a shielding effect upon its protonation, resulting in a so-called[34] 'wrongway' upfield shift.

Analogously, the monoprotonated phosphonate group of D(PMEA)$^{2-}$ is shielding H8, thus giving rise to a wrongway upfield shift of its NMR signal. In order to influence H8 the phosphonate group has to be in its vicinity;[33] we conclude that D(PMEA)$^-$ adopts a conformation which places the phosphonate group close to H8 (= '*anti*-like') and which is similar to the one seen in Fig. 1 for 5′-AMP^{2-}. This conclusion is also in accord with a crystal structure study of the H_2(PMEA)$^\pm$ zwitterion.[6] The fact that the upfield shift of H8 ($\Delta\delta = -0.075$ ppm; column 6 of Table 4) for PMEA^{2-} upon protonation is smaller than the one ($\Delta\delta = -0.142$ ppm)[5] for 5′-AMP^{2-} may then be explained by a lower population of this conformer in the case of PMEA; a result which is understandable due to the higher flexibility of the (phosphonomethoxy)ethyl residue compared to the more rigid ribose ring. In addition, the small upfield shift ($\Delta\delta = -0.02$ ppm; Table 4) observed for H2 upon monoprotonation of the phosphonate residue in PMEA^{2-} probably indicates that there is also a conformer with a smaller population in which the phosphonate residue is relatively close to H2, *i.e.* one might say that a '*syn*-like' conformation is adopted.

Overall, the same conclusions hold for 1-deaza-PMEA (see previous section); the conformer with the monoprotonated phosphonate residue close to H2 most possibly occurs in a somewhat larger proportion than with H(PMEA)$^-$.

In 7-deaza-PMEA monoprotonation of the -PO_3^{2-} residue causes within the error limits no alteration of the chemical shifts of H8 and H7 (see column 6 in Table 4), giving no direct evidence for an '*anti*-like' conformation (but also see below). However, the same protonation reaction produces for H2 a wrongway upfield shift of −0.067 ppm (Table 4, column 6) indicating that -$P(O)_2(OD)^-$ is now close to H2, *i.e.* that in part a '*syn*-like' conformation is adopted.

The 3-deaza-PMEA system is most interesting: no wrongway shift for H8 is observed, but upon formation of D(3-deaza-PMEA)$^-$ a very large wrongway upfield shift of the signal of H2 occurs ($\Delta\delta = -0.443$ ppm; Table 4) together with much smaller upfield shifts for H3 ($\Delta\delta = -0.086$ ppm) and N-CH_2 ($\Delta\delta = -0.061$ ppm; Table 4, column 6). Hence, there is a conformer with a large population in which -$P(O)_2(OD)^-$ is close to H2 and we conclude that the dominating conformation of D(3-deaza-PMEA)$^-$ is rather different from that of D(PMEA)$^-$. Furthermore, unexpected wrongway upfield shifts are also observed for the aliphatic hydrogens of O-CH_2 and P-CH_2 upon protonation of N1 leading to D_2(3-deaza-PMEA)$^\pm$. This can hardly be due to the phosphonate group, but rather to the aromatic ring current and its magnetic anisotropy which leads to a shielding of the hydrogens in O-CH_2 and P-CH_2 and which consequently have to be neighbouring the aromatic rings. Finally, the identity of the chemical shifts, δ, of the aliphatic hydrogens O-CH_2 and P-CH_2 in 3-deaza-PMEA^{2-} and in the other three compounds (see Table 3) indicates that all PM^{2-} species exist to a certain extent in an '*anti*-like' conformation as concluded in the first section on the basis of the absolute δ values.

The most unusual result of the above analysis is the high population of the conformation of D(3-deaza-PMEA)$^-$ in which the monoprotonated phosphonate group is close to H2. That this residue of 3-deaza-PMEA finds itself in a special environment is confirmed by its high stability, *i.e.* by the high basicity of the -PO_3^{2-} group (see next section). The high stability of the -$P(O)_2(OH)^-$ residue cannot be explained by a hydrogen bond to N1 as this is sterically not feasible; therefore, this group is evidently located in an environment with a reduced 'effective' relative permittivity (dielectric constant) (compared to that of the solvent) which inhibits the separation of the oppositely charged species, *i.e.* D^+ *versus* -PO_3^{2-}.

Acidity constants of the protonated PMs in aqueous (H_2O) solution

As nucleotides and their analogues are usually studied in water, the acidity constants determined by ^{1}H NMR shift measurements in D_2O should be transferred for this solvent by application[37] of eqn. (6), which proved to give excellent results for

$$pK_{a/H_2O} = (pK_{a/D_2O} - 0.45)/1.015 \qquad (6)$$

the acid–base reactions of ATP[47] and the adenosine monophosphates.[5] It may be useful to recall that the term generally used to transform pH-meter readings into pD values[23] and the term applied to calculate[37] pK_a values from measurements in D_2O for H_2O as solvent cancel each other to a large extent in many instances (see, *e.g.* Table 3 in ref. 5 and footnote 11 in ref. 48).

Table 5 Comparison of the negative logarithms of the acidity constants, valid for H_2O as solvent (25 °C; $I = 0.1$ mol dm^{-3}, $NaNO_3$), for $H_2(PMEA)^{\pm}$, H_2(1-deaza-PMEA)$^{\pm}$, H_2(3-deaza-PMEA)$^{\pm}$ and H_2(7-deaza-PMEA)$^{\pm}$ as determined *via* ^{1}H NMR shift measurements and potentiometric pH titrations. For further comparison the corresponding values are also listed for H_2(5′-TuMP)$^{\pm}$ and H_2(5′-AMP)$^{\pm}$ (*cf.*[a])

	^{1}H NMR		Potentiometric titration	
Protonated species	$pK^H_{H_2PM}$; eqn. (4) H^+(N1)	pK^H_{HPM}; eqn. (5) -P(O)$_2$(OH)$^-$	$pK^H_{H_2PM}$; eqn. (4) H^+(N1)	pK^H_{HPM}; eqn. (5) -P(O)$_2$(OH)$^-$
$H_2(PMEA)^{\pm}$	4.21 ± 0.09	7.10 ± 0.36	4.16 ± 0.02[b]	6.90 ± 0.01[b]
H_2(1d-PMEA)$^{\pm}$	5.52 ± 0.21[c]	7.17 ± 0.17	5.49 ± 0.02[c]	7.03 ± 0.02
H_2(3d-PMEA)$^{\pm}$	6.51 ± 0.10	7.91 ± 0.11	6.61 ± 0.02	7.83 ± 0.01
H_2(7d-PMEA)$^{\pm}$	5.63 ± 0.15	6.96 ± 0.35	5.62 ± 0.02	7.00 ± 0.01
H_2(5′-TuMP)$^{\pm}$	5.25 ± 0.06[d]	6.32 ± 0.20[d]	5.28 ± 0.02[e]	6.32 ± 0.01[e]
H_2(5′-AMP)$^{\pm}$	3.82 ± 0.02[d]	6.29 ± 0.04[d]	3.84 ± 0.02[e]	6.21 ± 0.01[e]

[a] The values in columns 2 and 3 are from the final two rows in Table 2; the error limits are from rows 3 and 4 of the same Table. The error limits given with the results from the potentiometric pH titrations correspond to three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] From ref. 13(*a*). [c] Deprotonation of the nucleobase moiety in H_2(1-deaza-PMEA)$^{\pm}$ occurs at the H^+(N3) site (see fourth section). [d] From ref. 5. [e] From ref. 49.

Application of eqn. (6) to the acidity constants for D_2O listed in the upper part of Table 2 gives the results for H_2O summarized in the lower part. In this solvent, the deprotonation of the H^+(N7) site as well as of the -P(O)(OH)$_2$ group also occurs with very low pK_a values, *i.e.* in a pH range which is hardly accessible by potentiometric pH titrations. However, deprotonation of H^+(N1) and -P(O)$_2$(OH)$^-$ of the twofold protonated PMs occurs in the ideal pH range of *ca.* 4–8. Therefore, we applied this method to the two mentioned reactions [eqns. (4) and (5)], which are, from a biochemical point of view, also the important ones. The corresponding results are listed in Table 5 together with those of the ^{1}H NMR shift experiments as well as the acidity constants for the H_2(5′-AMP)$^{\pm}$ and H_2(5′-TuMP)$^{\pm}$ species.[49] The results obtained by the two very different determination methods agree excellently within their error limits.

The results of Table 5 allow many comparisons; a few will be considered. The pK^H_{HPM} values [eqn. (5)] for H(PMEA)$^-$, H(1-deaza-PMEA)$^-$ and H(7-deaza-PMEA)$^-$ are all close to 7 and a comparison with the value due to H(PME)$^-$ (Fig. 1), $pK^H_{H(PME)} = 7.02 \pm 0.01$,[13a] shows that the nucleobase residue hardly affects deprotonation of -P(O)$_2$(OH)$^-$. However, for H(3-deaza-PMEA)$^-$ $pK^H_{H(3d\text{-}PMEA)} = 7.83 \pm 0.01$; here the nucleobase increases the basicity of -PO$_3^{2-}$ dramatically. This has to be attributed to the special conformation evaluated in the previous section for H(3-deaza-PMEA)$^-$: its monoprotonated phosphonate group is close to H2 and evidently in a hydrophobic environment which causes a low local 'effective' relative permittivity (compared with the one of bulk water) rendering the separation of H^+ from -PO$_3^{2-}$ more difficult, a known effect.[50]

Replacement of an electron-withdrawing aromatic-ring nitrogen atom by a methine group in a nucleobase should increase the basicity of the remaining ring nitrogens. Indeed, all deaza compounds have a higher $pK^H_{H_2PM}$ value than the parent compound $H_2(PMEA)^{\pm}$ (Table 5). The difference between the 1- and 3-deaza-PMEAs on the one hand and 7-deaza-PMEA on the other is mainly a distance effect; in the first two compounds a nitrogen has been replaced within the same ring where protonation occurs, whereas in 7-deaza-PMEA the nitrogen is removed from the imidazole ring, but protonation occurs at N1 of the pyrimidine ring. The N7 removal exerts the same effect in the PMs as in the AMPs:

$$\Delta pK_a = pK^H_{H_2(5'\text{-}TuMP)} - pK^H_{H_2(5'\text{-}AMP)} =$$
$$(5.28 \pm 0.02) - (3.84 \pm 0.02) = 1.44 \pm 0.03$$

and

$$\Delta pK_a = pK^H_{H_2(7d\text{-}PMEA)} - pK^H_{H_2(PMEA)} =$$
$$(5.62 \pm 0.02) - (4.16 \pm 0.02) = 1.46 \pm 0.03$$

By far the highest $pK^H_{H_2PM}$ value is observed for H_2(3-deaza-PMEA)$^{\pm}$, *i.e.* $pK^H_{H_2(3d\text{-}PMEA)} = 6.61 \pm 0.02$ (Table 5); therefore, it is interesting that 2-aminopyridine, which structurally resembles the proton-accepting part of the nucleobase in 3-deaza-PMEA (Fig. 2), has in its monoprotonated state a similar acidity constant,[42] *i.e.* $pK_a = 6.96$.

The acid–base effect initiated by the replacement of a phosphate monoester residue by a phosphonate group is illustrated by the following comparison (*cf.* also Table 5):

$$\Delta pK_a = pK^H_{H(PMEA)} - pK^H_{H(5'\text{-}AMP)} =$$
$$(6.90 \pm 0.01) - (6.21 \pm 0.01) = 0.69 \pm 0.01$$

and

$$\Delta pK_a = pK^H_{H_3(PMEA)} - pK^H_{H_3(5'\text{-}AMP)} =$$
$$(1.22 \pm 0.13;\ \text{Table 2}) - (0.4 \pm 0.2;\ \text{refs. 5, 47}) = 0.82 \pm 0.24$$

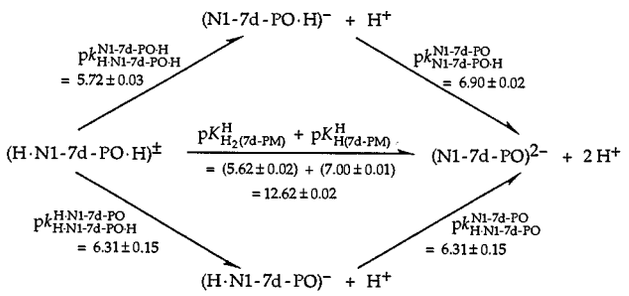
This result indicates that the effect of the C–P bond on the deprotonation reaction of the -P(O)$_2$(OH)$^-$ and -P(O)(OH)$_2$ groups is comparable.

Micro acidity constant scheme for H_2(7-deaza-PMEA)$^{\pm}$ and evaluation of various overlapping equilibria *via* their microconstants

For $H_2(PMEA)^{\pm}$ and H_2(5′-AMP)$^{\pm}$ the constants pK^H_{HPM} and $pK^H_{H_2PM}$ are more than two log units apart (Table 5), and therefore, there is practically no overlap between equilibria (4) and (5). However, the difference $pK^H_{H(7d\text{-}PMEA)} - pK^H_{H_2(7d\text{-}PMEA)}$ for H_2(7-deaza-PMEA)$^{\pm}$ equals only $\Delta pK_a = 1.38 \pm 0.02$ (Table 5), and hence, in this case equilibria (4) and (5) are overlapping. This means, before the release of the first proton from H_2-(7-deaza-PMEA)$^{\pm}$ is completed some 7-deaza-PMEA^{2-} is already formed. For a correct quantification of the intrinsic basicities (or acidities) of the proton-binding sites, the micro acidity constants need to be calculated. This evaluation is carried out in analogy to a previous treatment made for H_2(5′-TuMP)$^{\pm}$.[49]

In Fig. 4 the equilibrium scheme defining the micro acidity constants (k) is summarized giving their interrelation with the macro acidity constants (K) for the deprotonation of H_2(7-deaza-PMEA)$^{\pm}$, which is written as (H·N1-7d-PO·H)$^{\pm}$ indicating that one proton is located at N1 and the other at the -PO$_3^{2-}$. There are three independent equations (*a*), (*b*) and (*c*), but four unknown microconstants;[51] however, by using the constant of H(PMEA)$^-$, which is well separated from its next pK_a value, for $pk^{N1\text{-}7d\text{-}PO}_{N1\text{-}7d\text{-}PO\cdot H}$ the other three microconstants can be calculated. The results are shown on the arrows in Fig. 4.

The calculated microconstant, $pk^{H\cdot N1\text{-}7d\text{-}PO}_{H\cdot N1\text{-}7d\text{-}PO\cdot H} = 6.31 \pm 0.15$



(*a*) $K^{H}_{H_2(7d\text{-}PM)} = k^{N1\text{-}7d\text{-}PO\cdot H}_{H\cdot N1\text{-}7d\text{-}PO\cdot H} + k^{H\cdot N1\text{-}7d\text{-}PO}_{H\cdot N1\text{-}7d\text{-}PO\cdot H}$

(*b*) $\frac{1}{K^{H}_{H(7d\text{-}PM)}} = \frac{1}{k^{N1\text{-}7d\text{-}PO}_{N1\text{-}7d\text{-}PO\cdot H}} + \frac{1}{k^{N1\text{-}7d\text{-}PO}_{H\cdot N1\text{-}7d\text{-}PO}}$

(*c*) $K^{H}_{H_2(7d\text{-}PM)} \cdot K^{H}_{H(7d\text{-}PM)} = k^{N1\text{-}7d\text{-}PO\cdot H}_{H\cdot N1\text{-}7d\text{-}PO\cdot H} \cdot k^{N1\text{-}7d\text{-}PO}_{N1\text{-}7d\text{-}PO\cdot H}$

$= k^{H\cdot N1\text{-}7d\text{-}PO}_{H\cdot N1\text{-}7d\text{-}PO\cdot H} \cdot k^{N1\text{-}7d\text{-}PO}_{H\cdot N1\text{-}7d\text{-}PO}$

Fig. 4 Equilibrium scheme defining the micro acidity constants (k) and showing their interrelation with the macro acidity constants (K) and also the connection between (N1-7d-PO·H)$^-$ and (H·N1-7d-PO)$^-$ and the other species present in the corresponding 7-deaza-PMEA system. In (N1-7d-PO·H)$^-$ the proton is bound to the phosphonate group, and in (H·N1-7d-PO)$^-$ it is at N1 of the 7-deazaadenine residue (Fig. 2); (H·N1-7d-PO·H)$^\pm$ is also often written as H_2(7-deaza-PMEA)$^\pm$; it carries a proton at N1 and the phosphonate group. The arrows indicate the direction for which the acidity constants are defined. By using for the microconstant $pk^{N1\text{-}7d\text{-}PO}_{N1\text{-}7d\text{-}PO\cdot H}$ the value measured for H(PMEA)$^-$, $pK^{H}_{H(PMEA)} = 6.90 \pm 0.01$ (see Table 5; in the above scheme the error limit was doubled to be on the safe side), the other microconstants can be calculated with equations (*a*), (*b*) and (*c*) shown above. The error limits of the various constants were calculated according to the error propagation after Gauss; the limits correspond to three times the standard error (see Table 5). For further details see text in the seventh section.

(see lower arrow at the left in Fig. 4), carries a rather large error limit (calculated *via* the error propagation after Gauss) despite the fact that the constants given on the arrows in the upper part of Fig. 4 have relatively small errors. Considering that the mentioned microconstant is calculated *via* equation (*a*) of Fig. 4, *i.e.* by $k^{N1\text{-}7d\text{-}PO\cdot H}_{H\cdot N1\text{-}7d\text{-}PO\cdot H} = K^{H}_{H_2(7d\text{-}PMEA)} - k^{H\cdot N1\text{-}7d\text{-}PO}_{H\cdot N1\text{-}7d\text{-}PO\cdot H} = 10^{-(5.62\pm0.02)} - 10^{-(5.72\pm0.03)}$, the large error is no longer surprising because the calculation involves a small difference resulting from two rather similar numbers. This analysis also demonstrates that for a micro acidity constant evaluation macro acidity constants with well defined error limits are needed, otherwise the calculation may become misleading. The fact that the values on the left and right arrows in the lower part of Fig. 4 are the same is coincidental (see below).

However, despite the handicap concerning the error limits, one may estimate the ratio R of the monoprotonated and isocharged species, (N1-7d-PO·H)$^-$ and (H·N1-7d-PO)$^-$, which carry the proton at the phosphonate group or at N1 of the nucleobase residue, respectively:

$$R = \frac{[(\text{N1-7d-PO}\cdot\text{H})^-]}{[(\text{H}\cdot\text{N1-7d-PO})^-]} = \frac{k^{N1\text{-}7d\text{-}PO\cdot H}_{H\cdot N1\text{-}7d\text{-}PO\cdot H}}{k^{H\cdot N1\text{-}7d\text{-}PO}_{H\cdot N1\text{-}7d\text{-}PO\cdot H}} = \frac{10^{-5.72\pm0.03}}{10^{-6.31\pm0.15}} =$$

$$10^{0.59\pm0.15} = 3.89 \simeq \frac{4}{1} = \frac{80}{20}\left(\frac{85}{15}; \frac{73}{27}\right)$$

The values in parentheses are the upper and lower limits, respectively, as they follow from the error propagation. The species (N1-7d-PO·H)$^-$ dominates with about 80%, while (H·N1-7d-PO)$^-$ forms to about 20% only. Certainly, this result is an estimation, but it still proves (*i*) that both isomeric forms of H(7-deaza-PMEA)$^-$ occur simultaneously in appreciable amounts and (*ii*) that (N1-7d-PO·H)$^-$ dominates and thus largely determines $pK^{H}_{H(7d\text{-}PMEA)}$ as concluded in the third section and indicated in Tables 2 and 5 by the given site attributions.

Evidently the site attributions are strictly correct only when two subsequent pK_a values are separated by $\Delta pK_a > 2$. Moreover, by ^{1}H NMR shift measurements micro acidity constants are determined because a given site feels mainly only its own protonation/deprotonation reaction. However, as in all instances $\Delta pK_a > 1$ for two successive pK_a values, the results also resemble closely the corresponding macro acidity constants, as is further proved by the data in Table 5.

The same analysis can also be carried out for H_2(1-deaza-PMEA)$^\pm$ where $pK^{H}_{H(1d\text{-}PMEA)} - pK^{H}_{H_2(1d\text{-}PMEA)}$ equals 1.54 ± 0.03. By again using the acidity constant of H(PMEA)$^-$ and concluding that $pk^{N3\text{-}1d\text{-}PO}_{N3\text{-}1d\text{-}PO\cdot H} = pK^{H}_{H(PMEA)} = 6.90 \pm 0.02$, all the other values can be calculated in the way described above as follows:

$$pk^{N3\text{-}1d\text{-}PO\cdot H}_{H\cdot N3\text{-}1d\text{-}PO\cdot H} = pK^{H}_{H_2(1d\text{-}PMEA)} + pK^{H}_{H(1d\text{-}PMEA)} - pk^{N3\text{-}1d\text{-}PO}_{N3\text{-}1d\text{-}PO\cdot H} =$$
$$(5.49 \pm 0.02) + (7.03 \pm 0.02) - (6.90 \pm 0.02) = 5.62 \pm 0.03$$

$$k^{H\cdot N3\text{-}1d\text{-}PO}_{H\cdot N3\text{-}1d\text{-}PO\cdot H} =$$
$$K^{H}_{H_2(1d\text{-}PMEA)} - k^{N3\text{-}1d\text{-}PO\cdot H}_{H\cdot N3\text{-}1d\text{-}PO\cdot H}\ [\textit{cf.}\ \text{eqn. } (a) \text{ in Fig. 4}] =$$
$$10^{-5.49\pm0.02} - 10^{-5.62\pm0.03} = 10^{-6.08\pm0.12}$$

$$pk^{H\cdot N3\text{-}1d\text{-}PO}_{H\cdot N3\text{-}1d\text{-}PO\cdot H} = 6.08 \pm 0.12$$

$$pk^{N3\text{-}1d\text{-}PO}_{H\cdot N3\text{-}1d\text{-}PO} = pK^{H}_{H_2(1d\text{-}PMEA)} + pK^{H}_{H(1d\text{-}PMEA)} - pk^{H\cdot N3\text{-}1d\text{-}PO}_{H\cdot N3\text{-}1d\text{-}PO\cdot H} =$$
$$(5.49 \pm 0.02) + (7.03 \pm 0.02) - (6.08 \pm 0.12) = 6.44 \pm 0.12$$

These data again allow us to calculate the ratio R for the isocharged species, (N3-1d-PO·H)$^-$ and (H·N3-1d-PO)$^-$, which carry the proton at the phosphonate group and at N3 (see fourth section) of the nucleobase residue:

$$R = \frac{[(\text{N3-1d-PO}\cdot\text{H})^-]}{([\text{H}\cdot\text{N3-1d-PO})^-]} = \frac{k^{N3\text{-}1d\text{-}PO\cdot H}_{H\cdot N3\text{-}1d\text{-}PO\cdot H}}{k^{H\cdot N3\text{-}1d\text{-}PO}_{H\cdot N3\text{-}1d\text{-}PO\cdot H}} = \frac{10^{-5.62\pm0.03}}{10^{-6.08\pm0.12}} =$$

$$10^{0.46\pm0.12} = 2.88 \simeq \frac{3}{1} = \frac{75}{25}\left(\frac{79}{21}; \frac{69}{31}\right)$$

The values in parentheses are again the upper and lower limits. For H(1-deaza-PMEA)$^-$ the species with the proton at the phosphonate group dominates over the one with the proton at N3 of the nucleobase (fourth section).

Also for H_2(3-deaza-PMEA)$^\pm$ the deprotonation equilibria (4) and (5) are clearly overlapping because $\Delta pK_a = 1.22 \pm 0.02$ (Table 5), but in this case a microconstant evaluation is presently not appropriate because no reasonable estimate for one of the four unknown microconstants exists. The estimate used above is not suitable for $pk^{N1\text{-}3d\text{-}PO}_{N1\text{-}3d\text{-}PO\cdot H}$. However, as the mentioned ΔpK_a value of 1.22 ± 0.02 is relatively close to the one of the H_2(7-deaza-PMEA)$^\pm$ system ($\Delta pK_a = 1.38$), the ratio R for the monoprotonated species, (N1-3d-PO·H)$^-$ and (H·N1-3d-PO)$^-$, presumably will be similar; *i.e.* about 80% of H(3-deaza-PMEA)$^-$ will occur as (N1-3d-PO·H)$^-$, where the proton is at the phosphonate group.

Finally, the equilibria due to the deprotonations of the H$^+$(N7) site [eqn. (2)] and the -P(O)(OH)$_2$ group [eqn. (3)] are somewhat overlapping (Table 2) but no micro acidity constant evaluation is attempted because the error limits of the various constants are too large.

Conclusions

The similarities and differences between PMEA^{2-} and 5′-

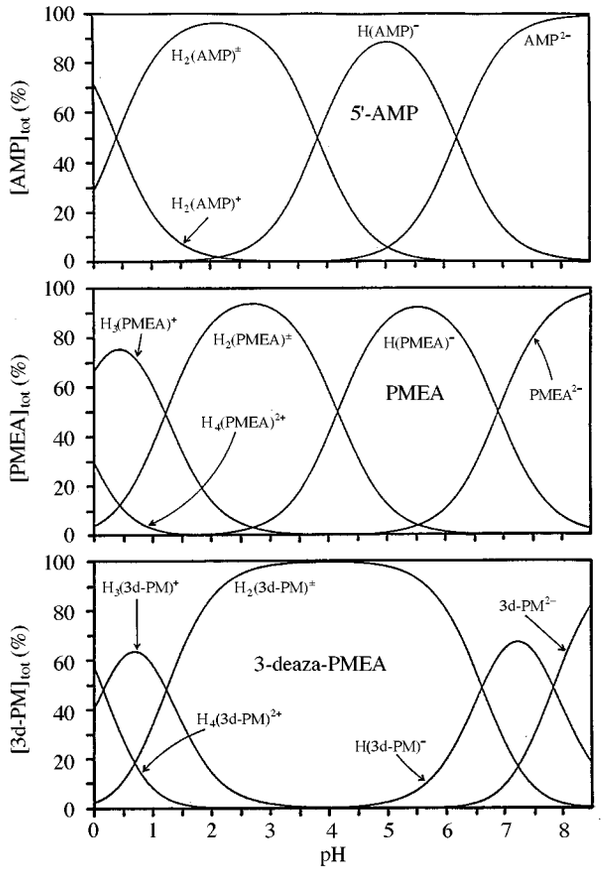


Fig. 5 Effect of pH on the concentration of the species present in a diluted aqueous solution of 5′-AMP (top), PMEA (middle) or 3-deaza-PMEA (bottom part) (25 °C; $I = 0.1$ mol dm^{-3} at pH > 1, $NaNO_3$). The results are plotted as the percentage of the total 5′-AMP, PMEA or 3-deaza-PMEA present. The calculations are mainly based on the acidity constants obtained *via* the potentiometric pH titrations as listed in Table 5, but $pK^{H}_{H_3(5'\text{-AMP})} = 0.4$ is from refs. 5 and 49, and $pK^{H}_{H_4(PMEA)} = -0.35$, $pK^{H}_{H_3(PMEA)} = 1.22$, $pK^{H}_{H_4(3d\text{-PMEA})} = 0.15$ and $pK^{H}_{H_3(3d\text{-PMEA})} = 1.23$ are from Table 2.

AMP^{2-} with respect to their metal ion-coordinating properties have recently been discussed.[13a,14] The main difference originates in the formation of five-membered chelates which involve the phosphonate group and the ether oxygen of the (phosphonomethoxy)ethyl chain of $PMEA^{2-}$ (Fig. 1). This ether oxygen interaction, not possible in M(5′-AMP) complexes, provides an additional stability contribution for the M(PMEA) species, including those of Mg^{2+} and Ca^{2+}, due to the relatively pronounced affinity of these alkaline earth ions towards O donor sites.[13a,14] This ether oxygen also seems to be essential for the antiviral activity of PMEA.[7,9b,10]

In aqueous solution an '*anti*-like' conformation is significant for all free PMEAs (PM^{2-}) (first and fifth sections) and this may be one of the reasons why $PMEA^{2-}$ can act as a mimic of 5′-AMP^{2-} and of 2′-deoxy-5′-AMP^{2-} in certain enzymic reactions; for these latter nucleotides the *anti* conformation dominates (Fig. 1). On the other hand, there are subtle differences between the acid–base properties of 5′-AMP^{2-}, $PMEA^{2-}$ and its deaza derivatives (Table 5). Also the various conformations of the protonated species in solution differ (see fifth section): in monoprotonated HPM^- for some PMEA derivatives an '*anti*-like' conformer, with the phosphonate group close to H8, occurs and for others, especially for H(3-deaza-PMEA)$^-$ a '*syn*-like' conformer exists with the phosphonate group close to H2.

The above-mentioned structural differences are the reason why 3-deaza-$PMEA^{2-}$ is more basic than $PMEA^{2-}$ by a factor of about 8 ($\Delta pK_a \simeq 0.9$; Table 5), which itself is more basic than 5′-AMP^{2-} by a factor of about 5 ($\Delta pK_a \simeq 0.7$; Table 5); this latter difference originates in the properties of a phosphonate *versus* a phosphate monoester group.

Because all these differences are somehow reflected in the physiological pH region, Fig. 5 was prepared to show the formation degree of the free and the various protonated forms of 5′-AMP, PMEA and 3-deaza-PMEA. At pH 7.5 5′-AMP^{2-} strongly dominates with a formation degree of about 95% (based on $[AMP]_{tot}$); the analogous $PMEA^{2-}$ species still occurs with about 80%, but now also approximately 20% of $H(PMEA)^-$ is present. Yet, as far as metal ion binding is concerned, both systems do not differ much, as the $-PO_3^{2-}$ residue is for the larger part in both cases available for metal ion coordination; *i.e.* there is very little competition with the proton for $-PO_3^{2-}$ binding. However, for 3-deaza-PMEA the situation is different. Here, at pH 7.5, the monoprotonated H(3-deaza-PMEA)$^-$ species dominates with about 63% and even two-fold protonated H_2(3-deaza-PMEA)$^{\pm}$ still exists with a formation degree of about 7%, whereas the fully deprotonated 3-deaza-$PMEA^{2-}$ occurs with only 30%. Hence, for this deaza-PMEA derivative metal ion binding is hampered in the physiological pH range due to the strong competition of the proton.

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References

1 (*a*) R. Pauwels, J. Balzarini, D. Schols, M. Baba, J. Desmyter, I. Rosenberg, A. Holý and E. De Clercq, *Antimicrob. Agents Chemother.*, 1988, **32**, 1025; (*b*) E. De Clercq, A. Holý and I. Rosenberg, *Antimicrob. Agents Chemother.*, 1989, **33**, 185.
2 A. Holý, *Isopolar Phosphorus-Modified Nucleotide Analogues*, in *Advances in Antiviral Drug Design*, ed. E. De Clercq, JAI Press Inc., Greenwich CT, USA, 1993, vol. 1, p. 179.
3 C.-C. Tsai, K. E. Follis, A. Sabo, T. W. Beck, R. F. Grant, N. Bischofberger, R. E. Benveniste and R. Black, *Science*, 1995, **270**, 1197.
4 R. B. Martin and Y. H. Mariam, *Met. Ions Biol. Syst.*, 1979, **8**, 57.
5 R. Tribolet and H. Sigel, *Eur. J. Biochem.*, 1987, **163**, 353.
6 C. H. Schwalbe, W. Thomson and S. Freeman, *J. Chem. Soc., Perkin Trans. 1*, 1991, 1348.
7 A. Holý, *Il Farmaco*, 1991, **46** (Suppl. 1), 141.
8 (*a*) S. A. Foster, J. černý and Y.-c. Cheng, *J. Biol. Chem.*, 1991, **266**, 238; (*b*) J. Neyts and E. De Clercq, *Biochem. Pharmacol.*, 1994, **47**, 39.
9 (*a*) E. De Clercq, T. Sakuma, M. Baba, R. Pauwels, J. Balzarini, I. Rosenberg and A. Holý, *Antiviral Res.*, 1987, **8**, 261; (*b*) A. Holý, E. De Clercq and I. Votruba, *ACS Symp. Ser.*, 1989, **401**, 51; (*c*) A. Holý, I. Rosenberg, H. Dvořáková and E. De Clercq, *Nucleosides Nucleotides*, 1988, **7**, 667.
10 A. Holý, I. Votruba, A. Merta, J. černý, J. Veselý, J. Vlach, K. Šedivá, I. Rosenberg, M. Otmar, H. Hřebabecký, M. Trávníček, V. Vonka, R. Snoeck and E. De Clercq, *Antiviral Res.*, 1990, **13**, 295.
11 H. Rosemeyer, G. Tóth, B. Golankiewicz, Z. Kazimierczuk, W. Bourgeois, U. Kretschmer, H.-P. Muth and F. Seela, *J. Org. Chem.*, 1990, **55**, 5784.
12 *Metal Ions in Biological Systems*, vol. 25: *Interrelations among Metal Ions, Enzymes, and Gene Expression*, ed. H. Sigel and A. Sigel, Marcel Dekker, New York, Basel, 1989.
13 (*a*) H. Sigel, D. Chen, N. A. Corfù, F. Gregáň, A. Holý and M. Strašák, *Helv. Chim. Acta*, 1992, **75**, 2634; (*b*) D. Chen, M. Bastian, F. Gregáň, A. Holý and H. Sigel, *J. Chem. Soc., Dalton Trans.*, 1993, 1537; (*c*) D. Chen, F. Gregáň, A. Holý and H. Sigel, *Inorg. Chem.*, 1993, **32**, 5377.
14 H. Sigel, *Coord. Chem. Rev.*, 1995, **144**, 287; *J. Ind. Chem. Soc.* (P. Ray Award Lecture), 1997, **74**, 261.
15 C. A. Blindauer, A. H. Emwas, A. Holý, H. Dvořáková, E. Sletten and H. Sigel, *Chem. Eur. J.*, 1997, **3**, 1526.

16 (*a*) A. J. Bodner, G. L. Cantoni and P. K. Chiang, *Biochem. Biophys. Res. Commun.*, 1981, **98**, 476; (*b*) J. A. Montgomery and S. J. Clayton, *J. Med. Chem.*, 1982, **25**, 96; (*c*) G. Cristalli, P. Franchetti, M. Grifantini, S. Vittori, T. Bordoni and C. Geroni, *Nucleosides Nucleotides*, 1987, **6**, 381.
17 (*a*) A. Bloch, R. J. Leonard and C. A. Nichol, *Biochim. Biophys. Acta*, 1967, **138**, 10; (*b*) T. B. Grage, D. B. Rochlin, A. J. Weiss and W. L. Wilson, *Cancer Res.*, 1970, **30**, 79; C. G. Smith, L. M. Reineke, M. R. Burch, A. M. Shefner and E. E. Muirhead, *Cancer Res.*, 1970, **30**, 69; (*c*) F. E. Evans and R. M. Sarma, *Cancer Res.*, 1975, **35**, 1458.
18 H. Dvořáková and A. Holý, *Collect. Czech.*, *Chem. Commun.*, 1993, **58**, 1419.
19 H. Dvořáková, A. Holý, R. Snoeck, J. Balzarini and E. De Clercq, *Collect. Czech.*, *Chem. Commun.*, 1990, **55** (Special Issue No. 1), 113.
20 (*a*) A. Holý, I. Rosenberg and H. Dvořáková, *Collect. Czech.*, *Chem. Commun.*, 1989, **54**, 2190; (*b*) A. Holý and I. Rosenberg, *Collect. Czech.*, *Chem. Commun.*, 1987, **52**, 2801.
21 R. Tribolet, R. Malini-Balakrishnan and H. Sigel, *J. Chem. Soc.*, *Dalton Trans.*, 1985, 2291.
22 (*a*) K. H. Scheller, F. Hofstetter, P. R. Mitchell, B. Prijs and H. Sigel, *J. Am. Chem. Soc.*, 1981, **103**, 247; (*b*) H. Sigel and N. A. Corfù, *Eur. J. Biochem.*, 1996, **240**, 508.
23 P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188.
24 H. Dvořáková, A. Holý and P. Alexander, *Collect. Czech.*, *Chem. Commun.*, 1993, **58**, 1403.
25 L.-n. Ji, N. A. Corfù and H. Sigel, *J. Chem. Soc.*, *Dalton Trans.*, 1991, 1367.
26 K. J. Neurohr and H. H. Mantsch, *Can. J. Chem.*, 1979, **57**, 1986.
27 O. Yamauchi, A. Odani, H. Masuda and H. Sigel, *Met. Ions Biol. Syst.*, 1996, **32**, 207.
28 R. Tribolet and H. Sigel, *Biophys. Chem.*, 1987, **27**, 119.
29 H. Sigel, A. Zuberbühler and O. Yamauchi, *Anal. Chim. Acta*, 1991, **255**, 63.
30 H. M. Irving, M. G. Miles and L. D. Pettit, *Anal. Chim. Acta*, 1967, **38**, 475.
31 D. Lichtenberg, F. Bergman and I. Ringel, *J. Magn. Reson.*, 1972, **6**, 600.
32 (*a*) E. P. Lira and C. W. Huffman, *J. Org. Chem.*, 1966, **31**, 2188; (*b*) I. Rosenberg, A. Holý and M. Masojídková, *Collect. Czech.*, *Chem. Commun.*, 1988, **53**, 2753; (*c*) H. Dvořáková, A. Holý and I. Rosenberg, *Collect. Czech.*, *Chem. Commun.*, 1994, **59**, 2069; A. Holý and M. Masojídková, *Collect. Czech.*, *Chem. Commun.*, 1995, **60**, 1196.
33 (*a*) L. M. Jackman and S. Sternhell, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Pergamon Press, Oxford, New York, 1969, 2nd edn., p. 61; (*b*) M. P. Schweizer, A. D. Broom, P. O. P. Ts′o and D. P. Hollis, *J. Am. Chem. Soc.*, 1968, **90**, 1042.
34 R. B. Martin, *Acc. Chem. Res.*, 1985, **18**, 32.
35 R. B. Martin, *Met. Ions Biol. Syst.*, 1996, **32**, 61.
36 R. L. Benoit and M. Fréchette, *Can. J. Chem.*, 1984, **62**, 995.
37 R. B. Martin, *Science*, 1963, **139**, 1198.
38 B. E. Fischer and H. Sigel, *J. Am. Chem. Soc.*, 1980, **102**, 2998.
39 H. Rosemeyer and F. Seela, *Helv. Chim. Acta*, 1988, **71**, 1573.
40 C. Meiser, B. Song, E. Freisinger, M. Peilert, H. Sigel and B. Lippert, *Chemistry Eur. J.*, 1997, **3**, 388.
41 G. A. Walker, S. C. Bhatia and J. H. Hall, Jr., *J. Am. Chem. Soc.*, 1987, **109**, 7629.
42 M. S. Sun and D. G. Brewer, *Can. J. Chem.*, 1967, **45**, 2729.
43 H. A. Staab, *Einführung in die theoretische organische Chemie*, Verlag Chemie, Weinheim, 1966, 4. Auflage, p. 639.
44 K. Kahmann, H. Sigel and H. Erlenmeyer, *Helv. Chim. Acta*, 1964, **47**, 1754; D. Banerjea, T. A. Kaden and H. Sigel, *Inorg. Chem.*, 1981, **20**, 2586.
45 J. W. Bunting and D. Stefanidis, *J. Am. Chem. Soc.*, 1990, **112**, 779.
46 F. Seela and T. Wenzel, *Helv. Chim. Acta*, 1995, **78**, 833; see p. 838.
47 R. Tribolet and H. Sigel, *Eur. J. Biochem.*, 1988, **170**, 617; H. Sigel, K. H. Scheller and R. M. Milburn, *Inorg. Chem.*, 1984, **23**, 1933.
48 K. H. Scheller, V. Scheller-Krattiger and R. B. Martin, *J. Am. Chem. Soc.*, 1981, **103**, 6833.
49 H. Sigel, S. S. Massoud and R. Tribolet, *J. Am. Chem. Soc.*, 1988, **110**, 6857.
50 H. Sigel, R. B. Martin, R. Tribolet, U. K. Häring and R. Malini-Balakrishnan, *Eur. J. Biochem.*, 1985, **152**, 187.
51 R. B. Martin, *Met. Ions Biol. Syst.*, 1979, **9**, 1.

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