Comparison of the acid–base properties of purine derivatives in aqueous solution. Determination of intrinsic proton affinities of various basic sites †

Gunnar Kampf,[a,b] Larisa E. Kapinos,[a] Rolf Griesser,[a] Bernhard Lippert *[b] and Helmut Sigel *[a]

[a] *Institute of Inorganic Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland. E-mail: Helmut.Sigel@unibas.ch*
[b] *Department of Chemistry, University of Dortmund, Otto-Hahn-Strasse 6, D-44227 Dortmund, Germany. E-mail: Lippert@pop.uni-dortmund.de*

Received (in Cambridge, UK) 26th February 2002, Accepted 19th April 2002
First published as an Advance Article on the web 28th May 2002

The acidity constants of protonated 7,9-dimethylguanine, 7-methylguanosine, 7,9-dimethylhypoxanthine, 7-methylinosine, 9-methyladenine, 1,9-dimethyladenine, 7,9-dimethyladenine and 1-methyladenosine were determined in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$). In those instances where $pK_a > 2$ potentiometric pH titrations were used for the determinations; when $pK_a < 2$, UV spectrophotometric and ^{1}H-NMR shift measurements were employed (25 °C). In these latter instances, where I is often larger than 0.1 M, the H_0 scale was applied to define the H^+ activity of the strong acid ($HClO_4$; HNO_3). A combination of the present results with values taken from our earlier work allowed us to quantify the intrinsic acidic properties in aqueous solution of the $(N1)H^{0\ or\ +}$ and $(N7)H^+$ sites *via* micro acidity constant schemes for seven purine derivatives and to calculate the tautomeric ratios regarding the monoprotonated species, that is N7–N1·H *versus* H·N7–N1 meaning that in one isomer H^+ is at the N1 site and in the other at N7. A plot of the micro acidity constants $pk^{N7-N1}_{H\cdot N7-N1}$, which quantify the acidity of the $(N7)H^+$ site, *versus* the macro acidity constants $pK_{a/(N1)H}$, which largely refer to the release of the proton from the (N1)H unit, results in a straight line for the guanine and hypoxanthine derivatives. This fact allows estimation of the micro acidity constant for any related derivative provided a value for $pK_{a/(N1)H}$ is known. The presented results are also meaningful for nucleic acids because they quantify the acid–base properties of their individual sites.

1 Introduction

The predominant tautomeric structures of the common purine nucleobases with their current atom numbering systems are shown in Fig. 1. These structures and the proton binding sites of the nucleobases were established nearly five decades ago (see ref. 1), and (macro) acidity constants for the release of protons, mostly determined by potentiometric pH titrations, are known.[1–4] However, the insight that the intrinsic proton affinities in aqueous solution, as quantified by so-called micro acidity constants, are needed to understand the chemistry of nucleobases, *e.g.*, their metal ion-binding properties, is of much more recent date and mostly due to the work of Martin,[1,5] who also provided the first listing of such data.[4]

Since the basicity of the ring nitrogens of N9-substituted purines, and only these are considered here, decreases in the order N1 > N7 > N3,[4,6,7] the acidity of the $(N7)H^+$ site in an adenine residue can only be measured under conditions where the N1 site also carries a proton, and of course, the positive charge of $(N1)H^+$ facilitates the deprotonation of $(N7)H^+$. Clearly, for many sophisticated comparisons one would like to know the (micro) acidity constant of $(N7)H^+$ under conditions where N1 is free. Of similar interest is the acidity of the zwitterionic guanine residue, where N7 carries the proton and N1 a negative charge. It is evident that these micro acidity constants can be derived usually only by indirect procedures,[1] *e.g.*, based on methylation [5] or metal ion binding [4] at certain sites.

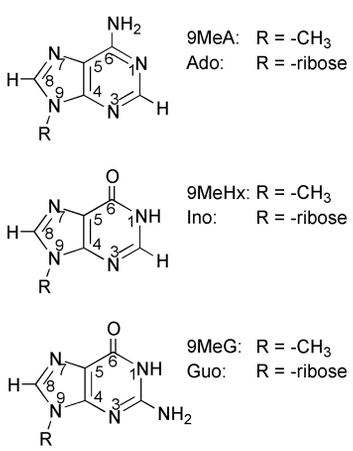


Fig. 1 Chemical structures of representative purine derivatives considered in this study: 9-methyladenine (9MeA) and adenosine (Ado), 9-methylhypoxanthine (9MeHx) and inosine (Ino), as well as 9-methylguanine (9MeG) and guanosine (Guo).

We employ here purine derivatives which are methylated at various sites. For example, in 7,9-dimethylguanine (7,9DiMeG$^+$) the methyl group at N7 resembles the $(N7)H^+$ site and its effect on the release of the proton bound to $(N1)^-$. Knowledge of the pK_a of the (N1)H unit in 7,9DiMeG$^+$ and application of the properties of a cyclic system, *i.e.*, developing

† Electronic supplementary information (ESI) available: Figures S1 (UV absorption spectra of 9-methyladenine), S2 (spectra of 1,9-dimethyladenine), S3 (plot of absorption *versus* H_0/pH for 1,9-dimethyladenine), S4 (spectra of 1-methyladenosine), S5 (absorption *versus* H_0/pH for 1-methyladenosine), S6 (spectra of 7,9-dimethyladenine), S7 (absorption *versus* H_0/pH for 7,9-dimethyladenine), S8 (absorption *versus* H_0/pH for $N^{6'}$,$N^{6'}$,N^9-trimethyladenine), as well as S9, S10, S11 and S12 with the micro acidity constant schemes for 9-ethylguanine, guanosine, 9-methylhypoxanthine, and inosine, respectively. See http://www.rsc.org/suppdata/p2/b2/b202023h/

 DOI: 10.1039/b202023h

This journal is © The Royal Society of Chemistry 2002

a so-called microconstant scheme,[8–10] allow calculation of the micro acidity constant of the $(N7)H^+$ site of N7-protonated and N1-deprotonated 9-methylguanine ($9MeG^{\pm}$). Analogously the intrinsic proton affinity of N7 in neutral 9-methyladenine (9MeA) may be quantified. In the present study the micro acidity constants for seven purine derivatives are presented.

2 Experimental

2.1 Materials

The purine derivatives 7,9-dimethylhypoxanthine (7,9DiMeHx), 7-methylinosine (7MeIno), 7,9-dimethylguanine (7,9DiMeG), 7-methylguanosine (7MeGuo), 1-methyladenosine [$(1MeAdo - H)^{\pm}$; see the final paragraph in Section 2.2], and 7,9-dimethyladenine perchlorate ($7,9DiMeA^+$) were purchased from Chemogen, Konstanz, Germany. 9-Methyladenine (9MeA) and 1,9-dimethyladenine perchlorate ($1,9DiMeA^+$) were synthesized following known procedures.[11,12] All the other chemicals like HNO_3 or NaOH were from commercial sources like those given in refs. 13 and 14. The solutions were also prepared as described there.

2.2 Potentiometric pH titrations

The pH titrations were carried out with a Metrohm E536 potentiograph connected to an E535 dosimat and a 6.0222.100 macro glass electrode. The buffer solutions (pH = 4.00, 7.00, and 9.00 based on the NBS scale; now NIST) used for calibration were also from Metrohm AG, Herisau, Switzerland. The acidity constants were calculated with a Pentium desk computer connected to an Epson Stylus 1500 printer (data) as well as a Hewlett-Packard Deskjet 1600CM printer (curves) by a curve-fitting procedure using a Newton–Gauss non-linear least-squares fitting program. The direct pH meter readings were used in the calculations; *i.e.*, the acidity constants are so-called practical, mixed or Brønsted constants.[13] Their negative logarithms given for aqueous solutions at $I = 0.1$ M ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values;[13] for further details see ref. 14.

The acidity constant $K^H_{7,9DiMeG}$ of $7,9DiMeG^+$ was determined by titrating 50 mL of aqueous 0.5 or 0.4 mM HNO_3 (25 °C; I = 0.1 M, $NaNO_3$) in the presence and absence of 0.3 mM ligand under N_2 with 1 mL 0.03 M NaOH. The experimental data, *i.e.*, the differences between such a pair of titrations were evaluated as previously[15] within a pH range determined by about 2 and 98% neutralization for the equilibrium $7,9DiMeG^+/(7,9DiMeG - H)^{\pm}$. The final result given in Table 1 (Section 3.1) is the average of 12 independent pairs of titrations.

The acidity constants K^H_{7MeGuo} of $7MeGuo^+$, K^H_{7MeIno} of $7MeIno^+$, and $K^H_{7,9DiMeHx}$ of $7,9DiMeHx^+$ were determined exactly as described above. The final results in Table 1 (Section 3.1) are the averages of 8, 6, and 12 independent pairs of titrations, respectively.

The acidity constant $K^H_{H(9MeA)}$ of $H(9MeA)^+$ was measured by titrating 50 mL of aqueous 1 mM HNO_3 (25 °C; I = 0.1 M, $NaNO_3$) in the presence and absence of 0.3 mM of the ligand under N_2 with 2 mL 0.03 M NaOH. The experimental data, *i.e.*, the differences between such a pair of titrations, were evaluated within a pH range determined by about 13 and 98% neutralization for the equilibrium $H(9MeA)^+/9MeA$. The final result listed in Table 1 (Section 3.1) is the average of 10 independent pairs of titrations.

The acidity constant K^H_{1MeAdo} of $1MeAdo^+$ for the deprotonation of the exocyclic amino group (eqn. (8)) was determined by titrating 25 mL of aqueous 0.6 mM HNO_3 (25 °C; I = 0.1 M, $NaNO_3$) in the presence and absence of 0.4 mM ligand under N_2 with 1 mL 0.03 M NaOH. The differences between such a pair of titrations were evaluated[15] as described above in the pH range corresponding to about 2 and 98% neutralization for the equilibrium $1MeAdo^+/(1MeAdo - H)^{\pm}$. The final result given under entry 15 of Table 1 in column 6 (Section 3.1) is the average of 3 independent pairs of titrations (see also Section 3.2).

2.3 Spectrophotometric measurements

The acidity constants $K^H_{H_3(9MeA)}$ and $K^H_{H_2(9MeA)}$ of $H_3(9MeA)^{3+}$, $K^H_{H_2(1,9DiMeA)}$ and $K^H_{H(1,9DiMeA)}$ of $H_2(1,9DiMeA)^{3+}$, $K^H_{H_2(7,9DiMeA)}$ and $K^H_{H(7,9DiMeA)}$ of $H_2(7,9DiMeA)^{3+}$, and $K^H_{H_2(1MeAdo)}$ and $K^H_{H(1MeAdo)}$ of $H_2(1MeAdo)^{3+}$ were determined by spectrophotometry.[16] The UV-spectra (sample beam: $HClO_4$, $NaClO_4$ and [9MeA] = 0.031–0.036 mM, or $[1,9DiMeA^+]$ = 0.018–0.028 mM, or $[7,9DiMeA^+]$ = 0.035–0.039 mM, or $[1MeAdo^+]$ = 0.032–0.044 mM; reference beam: $HClO_4$ and $NaClO_4$) were recorded with a Cary 3C spectrophotometer connected to a Pentium desk computer using 2-cm quartz cells in aqueous solution at 25 °C in dependence on pH. The ionic strength was adjusted to I = 0.1 M ($NaClO_4$) when $[HClO_4] < 0.1$ M; no adjustment was made where $[HClO_4] \geq 0.1$ M. The pH was adjusted with $HClO_4$ and measured with a Metrohm 713 digital pH meter using a Metrohm 60216100 (PC) glass electrode in the pH range ≥ 1; lower pH values (≤ 1) were obtained by calculating the H^+ activity of the $HClO_4$ (H_0 scale) in these solutions as described in Section 2.5. Each solution was individually prepared.

A typical example of an experimental series is shown in Fig. S1 of the Supplementary Information for 9-methyladenine (9MeA). Plots of the absorption at various wavelengths *versus* H_0/pH give the expected 'titration' curves as seen in Fig. 2 of Section 3.2, the evaluation of which (done as described for ^{1}H-NMR shift measurements)[17,18] with the aforementioned computer equipment (Section 2.2) provides the acidity constants of $H_3(9MeA)^{3+}$ ($pK^H_{H_3(9MeA)}$; eqn. (5)) and $H_2(9MeA)^{2+}$ ($pK^H_{H_2(9MeA)}$; eqn. (6)). The final result given in Table 1 in Section 3.1 for $pK^H_{H_2(9MeA)}$ is the average of 5 independent experimental series; the final value for $pK^H_{H_3(9MeA)}$ is the average of the evaluations at the various wavelengths of two experiments (see also Fig. 2).

Figures S2 (spectra) and S3 (evaluation) in the Supplementary Information provide the data of a typical experimental series carried out with 1,9-dimethyladenine ($1,9DiMeA^+$) which allowed measurement of the pK_a values for $H_2(1,9DiMeA)^{3+}$ and $H(1,9DiMeA)^{2+}$. The final results listed in Table 1 are the averages of 2 and 5 series of experiments, respectively.

A representative example of an experimental series for 1-methyladenosine ($1MeAdo^+$) is shown in Fig. S4 and its evaluation in Fig. S5. From both figures it is evident that the deprotonation of $(N7)H^+$ in $H(1MeAdo)^{2+}$ ($pK^H_{H(1MeAdo)}$) could be determined with high precision, whereas $pK^H_{H_2(1MeAdo)}$ for the release of H^+ from the $(N3)H^+$ site in $H_2(1MeAdo)^{3+}$ must be considered as an estimate for two reasons: (i) the number of data points at $H_0 < -2$ is relatively limited, and (ii) the spectra at about 205 nm appear as somewhat blurred, if compared, *e.g.*, with the spectral series of 1,9DiMeA (Fig. S2). Possibly $H_2(1MeAdo)^{3+}$ is slowly decomposing; in fact, for $H_3(Ado)^{3+}$ it is known[6] that hydrolytic cleavage of the glycosidic bond occurs. However, the estimate is still expected to provide the correct order of magnitude for the $(N3)H^+$ deprotonation in $H_2(1MeAdo)^{3+}$ since the result was reproducible. The final results in Table 1 are the averages of 4 independent experiments for the two acidity constants.

The protonation of 7,9-dimethyladenine ($7,9DiMeA^+$) is also reflected in UV-spectral changes. A typical experimental example is shown in Fig. S6 with its evaluation in Fig. S7. These data provide the pK_a values for the monoprotonated $H(7,9DiMeA)^{2+}$ and for the diprotonated $H_2(7,9DiMeA)^{3+}$ species (see also Section 2.4). The average results of 2 and 3 independent experimental series are $pK^H_{H_3(7,9DiMeA)} = -(2.63 \pm 0.21)$ and $pK^H_{H(7,9DiMeA)} = 0.51 \pm 0.07$, respectively. The final

result in Table 1 for $pK^{H}_{H(7,9DiMeA)}$ is the average of the spectrophotometric and the NMR (see below) measurements.

2.4 ^{1}H-NMR shift measurements

During the spectrophotometric experiments with 7,9-dimethyladenine (7,9DiMeA$^+$) we also investigated the stability of the compound in 10 M $HClO_4$, which appears to be stable under these conditions; only after 6 hours is a small alteration in the absorption observed which might be attributed to a decomposition (< 5%). Therefore, and because we wanted to determine one of the constants measured *via* UV spectrophotometry also by another method, we carried out ^{1}H-NMR experiments in D_2O by measuring the chemical shift of the various protons in dependence on pD (25 °C). The pD was adjusted with DNO_3 and to the pH meter reading (pH*) (for the instrument see Section 2.3) a value[19] of 0.40 was added to obtain the final pD of the solution. *I* was adjusted to 0.1 M with $NaNO_3$ at pD > 1 and the corrected pH-meter reading was used in the evaluation; at pD < 0.4 the activity (D_0) of DNO_3 was calculated as described in Section 2.5. According to information provided by Metrohm AG, which we gratefully acknowledge, pH values in aqueous HNO_3 solutions may be measured with the glass electrode until pH about 0.

The ^{1}H-NMR shift spectra were recorded on a Bruker AC200 (200.13 MHz) instrument using [7,9DiMeA$^+$] ≃ 9 mM. The center peak of the tetramethylammonium ion triplet was used as internal reference. However, all measured chemical shifts were converted to the sodium 3-(trimethylsilyl)propane-1-sulfonate reference by adding 3.18 ppm (D_2O).[20]

The experimental data were evaluated as described.[17,18] A typical example of an experimental series is shown in Fig. 3 (see Section 3.2). The handicap with these NMR experiments is that a value for $pK^{D}_{D_2(7,9DiMeA)}$ could not be measured because in ref. 21 H_0 values are listed only until −1.99 which corresponds to [HNO_3] = 7.0 M. Therefore, we used eqn. (1)

$$pK_{a/H_2O} \cdot 1.015 = pK_{a/D_2O} - 0.45 \quad (1)$$

to transfer[22] the spectrophotometric result $pK^{H}_{H_2(7,9DiMeA)}$ = −2.63 (see Table 1), valid for H_2O as solvent, into an acidity constant valid for D_2O as solvent, *i.e.* $pK^{D}_{D_2(7,9DiMeA)}$ = −2.22. This value was then kept constant in the evaluations (see Fig. 3) of 2 experimental series which gave on average the result $pK^{D}_{D(7,9DiMeA)}$ = 0.95 ± 0.07 for the deprotonation of D(7,9DiMeA)$^{2+}$. Application of eqn. (1) gives then for H_2O as solvent $pK^{H}_{H(7,9DiMeA)}$ = 0.49 ± 0.07 in excellent agreement with the spectrophotometric measurements.

The final result in Table 1 in Section 3.1 for $pK^{H}_{H(7,9DiMeA)}$ is the average of five independent experimental series, *i.e.* of three spectrophotometric (see the last paragraph in Section 2.3) and two NMR measurements.

2.5 Values of "pH" for solutions containing high concentrations of $HClO_4$ or DNO_3

To obtain well defined limiting UV absorptions ($HClO_4$; Section 2.3) or limiting chemical shifts (DNO_3; Section 2.4) for certain protonation states of various ligands studied, it was necessary to record spectra at pH < 1 or pD < 1, respectively. Because the activity coefficients of perchloric acid and nitric acid in higher concentrations differ significantly from 1, these deviations have to be taken into account in the determination of the "pH" values of the solutions used.

To circumvent the indicated difficulties we applied the H_0 acidity function, originally conceived by Hammett and Deyrup,[21,23] which provides a quantitative measure of the acidity (H^+ activity) of a solution and which is derived from a series of overlapping indicators. There is a convenient listing of H_0 values *versus* the molar concentration of several strong acids in aqueous solution at 25 °C;[21] for $HClO_4$ H_0 values are tabulated up to 10.0 M and for HNO_3 up to 7.0 M. We carried out the necessary interpolations with the spline-function of proFit 5.1.2 from QuantumSoft, Zürich, Switzerland. In the legends of the various figures the calculated H_0 values are always given in the pH range below 1 (at pH > 1 the pH-meter reading is used) and in parentheses the concentration of $HClO_4$ (or DNO_3) present in the solutions is added.

The same procedure was applied for the calculation of H_0 values for HNO_3 and it was assumed that by adding 0.40 to the H_0 scale (as was done with the pH-meter readings; see the first paragraph of Section 2.4), D_0 values are obtained which hold for the employed concentrations of DNO_3. That this assumption is reasonable is confirmed by the results obtained for the pK_a value of monoprotonated 7,9DiMeA$^+$ in H_2O (UV spectrophotometry) and also *via* D_2O (^{1}H-NMR), if transformed to H_2O; the two results agree excellently (Sections 2.3 and 2.4).

3 Results and discussion

All experiments were carried out under conditions where the self-association of the nucleobase derivatives considered in this study (Fig. 1) is expected to be negligible.[24,25] The potentiometric pH titrations for the determination of the macro acidity constants of the various purines were made with approximately 0.3 mM solutions, which means that more than 99% of the ligands are expected to be present in the monomeric form.[26] For the spectrophotometric measurements even lower concentrations were employed. Of course, for charged species like 7,9-dimethyladenine, 7,9DiMeA$^+$, self-association is expected to be further diminished due to charge repulsion; indeed, for this ligand spectrophotometric (0.035–0.039 mM) and ^{1}H-NMR shift experiments, which had to be carried out with higher concentrations (*ca.* 9 mM), gave within the error limits the same result for the acidity constant of H(7,9DiMeA)$^{2+}$.

3.1 Acidity constants of 7,9DiMeG$^+$, 7MeGuo$^+$, 7,9DiMeHx$^+$ and 7MeIno$^+$

These nucleobase derivatives carry a methyl group at N7 and are therefore positively charged as long as (N1)H is not deprotonated. We did not attempt to measure the protonation of N3, since this is possible only at very low pH,[7] but the release of the proton from (N1)H was determined by potentiometric pH titrations in aqueous solution (25 °C; *I* = 0.1 M, $NaNO_3$). This deprotonation is defined by equilibrium (2a) where G represents the four mentioned guanine derivatives.

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

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

The results are given in entries 1–9 of Table 1 together with the acidity constants of several common guanine and hypoxanthine derivatives, in which N7 is without a substituent and thus, may be protonated; the corresponding pK_a values have previously been measured by us[26–30] under the conditions mentioned above. The first proton is here released from (N7)H$^+$ [eqn. (3)] and the second one from (N1)H [eqn. (4)].[4] If one defines GI = 9MeG, 9EtG, Guo, 9MeHx or Ino (see Table 1), the following equilibria (3a) and (4a) apply:

$$H(GI)^+ \rightleftharpoons GI + H^+ \quad (3a)$$

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

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

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

Table 1 Negative logarithms of the acidity constants of some purine-nucleobase derivatives in aqueous solution [a, b]

No.	Purine	Protonated species	pK_a for (N3)H$^+$	pK_a for (N7)H$^+$	pK_a for (N1)H$^{0\ or\ +}$	Ref.
1	9-Methylguanine	H(9MeG)$^+$		3.11 ± 0.06	9.56 ± 0.02	27
2	9-Ethylguanine	H(9EtG)$^+$		3.27 ± 0.03	9.57 ± 0.05	27
3	7,9-Dimethylguanine	7,9DiMeG$^+$			7.22 ± 0.01	—
4	Guanosine	H(Guo)$^+$		2.11 ± 0.04	9.22 ± 0.01	26
5	7-Methylguanosine	7MeGuo$^+$			7.01 ± 0.01	—
6	9-Methylhypoxanthine	H(9MeHx)$^+$		1.87 ± 0.01	9.21 ± 0.01	27
7	7,9-Dimethylhypoxanthine	7,9DiMeHx$^+$			6.46 ± 0.01	—
8	Inosine	H(Ino)$^+$		1.06 ± 0.06 [c]	8.76 ± 0.03	26
9	7-Methylinosine	7MeIno$^+$			6.20 ± 0.01	—
10	9-Methyladenine	H$_3$(9MeA)$^{3+}$	−2.83 ± 0.30 [d]	−0.64 ± 0.06 [d]	4.10 ± 0.01	—
11	$N^{6'}$,$N^{6'}$,N^9-Trimethyladenine	H$_3$(TriMeA)$^{3+}$	−2.7 [e]	−0.77 ± 0.13 [f]	4.18 ± 0.04	29
12	1,9-Dimethyladenine	H$_2$(1,9DiMeA)$^{3+}$	−2.72 ± 0.38 [d]	−0.79 ± 0.10 [d]	(9.1) [g, h]	—
13	7,9-Dimethyladenine	H$_2$(7,9DiMeA)$^{3+}$	−2.63 ± 0.21 [d]	—	0.50 ± 0.08 [i]	—
14	Adenosine	H$_3$(Ado)$^{3+}$	— [j]	−1.50 ± 0.15 [k]	3.61 ± 0.03 [l]	—
15	1-Methyladenosine	H$_2$(1MeAdo)$^{3+}$	−4.02 ± 0.28 [d, m]	−1.55 ± 0.10 [d]	(8.69 ± 0.03) [h]	—
16	Adenine	H$_3$(Ade)$^{3+}$	−4.2	−0.4 [n]	4.2	6
17	Purine	H$_3$(Pur)$^{3+}$	<−6	−1.7 [n]	2.4	6

[a] The error limits given, if nothing else is mentioned, are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] So-called practical, mixed or Brønsted constants are listed (see also Section 2.2). If nothing else is mentioned the constants were determined by potentiometric pH titrations (25 °C; I = 0.1 M, $NaNO_3$). In those instances where the above pK_a value carries a negative sign I was considerably higher than 0.1 M (see Sections 2.3–2.5, the legends for the figures, and the text in Section 3.3). [c] Measured by NMR; ref. 28. [d] Measured by UV spectrophotometry at 25 °C and I = 0.1 M ($NaClO_4$) if [$HClO_4$] < 0.1 M (see Section 2.3). The values at pH > 1 were measured with an electrode, lower ones were calculated based on the H_0 scale (see Section 2.5). [e] Estimate based on the average of entries 10, 12 and 13. [f] In ref. 29 is published $pK^H_{H_3(TriMeA)} = -0.75 \pm 0.20$; reevaluation of the spectrophotometric data[29] (three experiments, one of which is shown in Fig. S8 of the Supplementary Information) by taking into account the H_0 scale for $HClO_4$ and by keeping $pK^H_{H_3(TriMeA)} = -2.7$ constant leads to the value given above. [g] This value is from ref. 30. [h] This value refers to the deprotonation of the (C6)NH_2 group [eqn. (8)]; see text in Section 3.2. [i] This value is the average of the results from the spectrophotometric and NMR measurements (see Section 2.4; final paragraph). [j] Ado decomposes under these conditions.[6] [k] Average of the values given in refs. 5 ($pK^H_{H_3(Ado)} = -1.56$) and 6a (−1.4); the error limit is estimated. [l] From ref. 17. [m] Estimate; see the second to the last paragraph in Section 2.3. [n] There is an N7,N9 dichotomy for H$^+$ binding.[6,7]

3.2 Acidity constants of $H_3(9MeA)^{3+}$, $H_2(1,9DiMeA)^{3+}$, $H_2(7,9DiMeA)^{3+}$ and $H_2(1MeAdo)^{3+}$

The acidity constants of all these acids were measured spectrophotometrically with the exception of the acidity constant for the (N1)H$^+$ site of H(9MeA)$^+$ which was determined by potentiometric pH titrations. The species $H_3(9MeA)^{3+}$ is protonated at N3, N7 and N1. The protons are released in this order[6] giving rise to the following three equilibria, where A represents 9MeA:

$$H_3(A)^{3+} \rightleftharpoons H_2(A)^{2+} + H^+ \quad (5a)$$

$$K^H_{H_3(A)} = [H_2(A)^{2+}][H^+]/[H_3(A)^{3+}] \quad (5b)$$

$$H_2(A)^{2+} \rightleftharpoons H(A)^+ + H^+ \quad (6a)$$

$$K^H_{H_2(A)} = [H(A)^+][H^+]/[H_2(A)^{2+}] \quad (6b)$$

$$H(A)^+ \rightleftharpoons A + H^+ \quad (7a)$$

$$K^H_{H(A)} = [A][H^+]/[H(A)^+] \quad (7b)$$

In Fig. 2 a representative example for the evaluation of one series of spectrophotometric measurements for 9MeA is shown in the H_0/pH range −6 to 2; these data allow an estimation for $K^H_{H_3(A)}$ [eqn. (5)] and the determination of $K^H_{H_2(A)}$ [eqn. (6)]. The corresponding absorption spectra are shown in Fig. S1 of the Supplementary Information and the final results for 9MeA regarding eqns. (5)–(7) are given in entry 10 of Table 1.

The $H_2(1,9DiMeA)^{3+}$ species carries protons only at the N3 and N7 sites since N1 is methylated; consequently, in this case only eqns. (5) and (6) apply. An example of the spectrophotometric determination of the two acidity constants is given in Figs. S2 and S3 of the Supplementary Information. Similarly, N3 and N7 in $H_2(1MeAdo)^{3+}$ are protonated and N1 is also methylated; hence again, eqns. (5) and (6) are the relevant reactions (for an experiment and its evaluation see Figs. S4 and S5 of the Supplementary Information). The results for

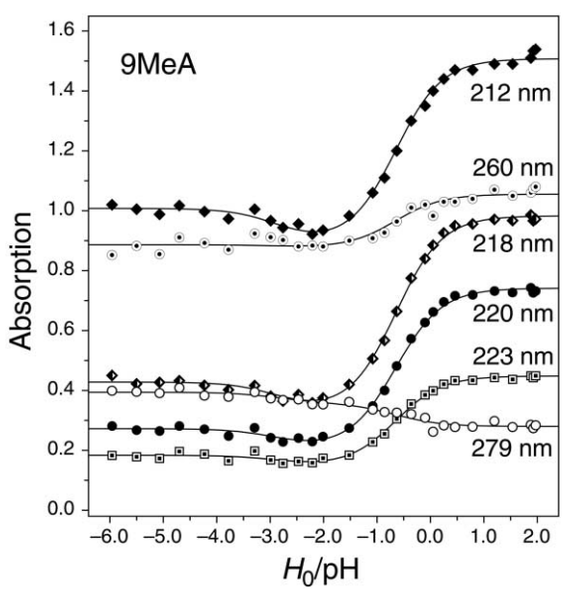


Fig. 2 Evaluation of the dependence of the UV absorption of 9-methyladenine ([9MeA] = 0.034 mM) at 212, 218, 220, 223, 260 and 279 nm on the activity of H$^+$ in aqueous solution (see Fig. S1) by plotting the absorption *versus* H_0/pH (see Section 2.5). The evaluation of this experiment led to the weighted mean $pK^H_{H_3(9MeA)} = -2.85 \pm 0.36$ (3σ) and to the following individual acidity constants at the mentioned wavelengths: 212 nm, $pK^H_{H_3(9MeA)} = -(0.59 \pm 0.04)$; 218 nm, $-(0.66 \pm 0.03)$; 220 nm, $-(0.67 \pm 0.03)$; 223 nm, $-(0.68 \pm 0.04)$; 260 nm, $-(0.41 \pm 0.14)$; 279 nm, $-(0.60 \pm 0.19)$ (1σ), which gives the weighted mean $pK^H_{H_2(9MeA)} = -(0.65 \pm 0.09)$ (3σ) for this experiment. The solid curves shown are the computer-calculated best fits at the mentioned wavelengths through the experimental data points obtained at H_0 −5.96 ([$HClO_4$] = 10.23 M), −5.50 (9.61 M), −5.07 (9.03 M), −4.70 (8.51 M), −4.23 (7.86 M), −3.78 (7.22 M), −3.29 (6.59 M), −2.99 (6.20 M), −2.76 (5.88 M), −2.47 (5.42 M), −2.21 (4.97 M), −2.01 (4.58 M), −1.52 (3.61 M), −1.08 (2.65 M), −0.86 (2.17 M), −0.63 (1.69 M), −0.36 (1.20 M), −0.10 (0.84 M), 0.05 (0.60 M), 0.25 (0.36 M), 0.46 (0.24 M), 0.79 (0.12 M), and pH 1.20, 1.54, 1.88, 1.93 and 1.97 (from left to right; see Section 2.5) by using the mentioned average results (25 °C; I = 0.1 M, $NaNO_3$, except in those solutions where [$HClO_4$] > 0.1 M).

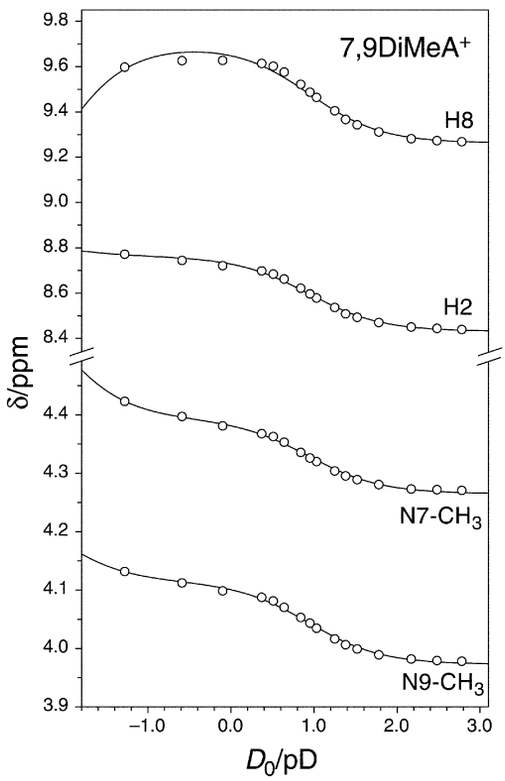


Fig. 3 Variation of the chemical shifts of the aromatic and aliphatic protons of 7,9-dimethyladenine ([7,9DiMeA] ≃ 9 mM) in D_2O in dependence on D_0/pD (Section 2.4). The evaluation of this experiment led to the following acidity constants for the various protons: H8, $pK^{D}_{D(7,9DiMeA)}$ = 1.05 ± 0.05; H2, 0.97 ± 0.04; N7–CH_3, 0.94 ± 0.04; N9–CH_3, 0.89 ± 0.03 (1σ) which gives the weighted mean $pK^{D}_{D(7,9DiMeA)}$ = 0.94 ± 0.10 (3σ). The value $pK^{D}_{D_2(7,9DiMeA)}$ = −(2.22 ± 0.21), taken from the spectrophotometric measurements (see Section 2.4), was kept constant in this evaluation. The solid curves shown are the computer-calculated best fits through the experimental data points measured for the various protons at D_0 −1.28 ([DNO_3] = 5.47 M), −0.59 (2.90 M), −0.10 (1.61 M), and pD 0.37, 0.51, 0.64, 0.84, 0.95, 1.03, 1.25, 1.38, 1.52, 1.78, 2.17, 2.48 and 2.78 (left to right; see Section 2.5) by using the mentioned average pK_a values (25 °C; I = 0.1 M, $NaNO_3$, except in those solutions where [DNO_3] > 0.1 M).

these two adenine derivatives are given in entries 12 and 15 of Table 1, respectively.

Finally, the acidity constants of H_2(7,9DiMeA)$^{3+}$ refer chargewise to the deprotonation reactions (5) and (6), but it needs to be emphasized that in reaction (6) the (N1)H^+ site is deprotonated because N7 is methylated; reaction (7) does, of course, not occur. These two acidity constants for the deprotonation of the (N3)H^+ and (N1)H^+ units were determined not only spectrophotometrically (see Figs. S6 and S7 of the Supplementary Information) but the one for (N1)H^+ also by ^{1}H-NMR shift measurements. An example of the latter is shown in Fig. 3 and the averaged results are listed in entry 13 of Table 1. To conclude, the acidity constants, in part taken from the literature,[5–7,17,29,30] for the protonated adenine derivatives are listed in entries 10–16 of Table 1. Entry 17 contains the values[6,7] for threefold protonated purine.

Compounds like 1,9DiMeA$^+$ and 1MeAdo$^+$ are known to be able to lose a proton from the (C6)NH_2 group.[30,31] The resulting zwitterionic species tautomerizes then to the uncharged imino form.[30] For 1MeAdo$^+$ we have measured this deprotonation equilibrium by potentiometric pH titration [eqn. (8)] because the pK_a values given in the literature span the wide range from 8.25 (ref. 30) to 9.3 (ref. 32) with several intermediate values around 8.55 (ref. 33).

$$1MeAdo^+ \rightleftharpoons (1MeAdo - H) + H^+ \qquad (8a)$$

$$K^{H}_{1MeAdo} = [(1MeAdo - H)][H^+]/[1MeAdo^+] \qquad (8b)$$

Our result, which we consider as reliable, is given in parentheses in the sixth column of Table 1 under entry 15. Application of this value (pK^{H}_{1MeAdo} = 8.69) to the evaluation procedure described in ref. 30 for the calculation of K_T, *i.e.* the dimensionless constant quantifying the tautomeric equilibrium between the imino and amino forms, together with $pK^{H}_{H(Ado)}$ = 3.61 (entry 14), gives for (1MeAdo − H)^0 $K_T = 10^{5.08}$ showing that the imino form is strongly favored; a result in agreement with previous conclusions,[30] and also with $K_T = 10^{5.0}$ obtained for (1,9DiMeA − H)^0 from $pK^{H}_{1,9DiMeA}$ = 9.1 (Table 1, entry 12)[30] and $pK^{H}_{H(9MeA)}$ = 4.10 (entry 10).

3.3 Comparison of the acidity constants of some purine derivatives

The acidity constants given in Table 1 for the common nucleobase derivatives are in the expected order and agree well with previous listings.[2,4] An early determination[34] for the release of the proton from 7-methyl-9-ethylguanine$^+$ (pK_a = 7.3; 20 °C; I close to 0.1 M) is also in fair agreement with the present value for 7,9DiMeG$^+$ (pK_a = 7.22; Table 1, entry 3). Since many acid–base properties of nucleobase residues were discussed previously,[4,27] only some new aspects are considered below.

Previously, several pK_a values for the deprotonation of positively charged (N)H^+ sites have been measured at 25 °C and an ionic strength of I = 1.0 M ($NaClO_4$); *i.e.*, $pK^{H}_{H(Guo)}$ = 2.33,[35] $pK^{H}_{H(Ino)}$ = 1.39,[35] $pK^{H}_{H(9MeA)}$ = 4.45,[36] and $pK^{H}_{H(Ado)}$ = 3.86.[37] These values are larger by ΔpK_a = 0.22, 0.33, 0.35, and 0.25, respectively, compared to the corresponding ones listed in entries 4, 8, 10, and 14 of Table 1, which refer to 25 °C and I = 0.1 M ($NaNO_3$). This means, the release of the proton from an (N)H^+ site is retarded on average by about 0.3 pK unit [exactly 0.29 ± 0.09 (3σ)] at the higher ionic strength. This observation is meaningful because it allows the transfer of a given result to another ionic strength. For example, for the deprotonation of 7MeIno$^+$ we measured now at 25 °C and I = 0.1 M ($NaNO_3$) pK^{H}_{7MeIno} = 6.20 ± 0.01 (Table 1, entry 9); hence, for the conditions of I = 1.0 M and 25 °C one estimates pK^{H}_{7MeIno} = (6.20 ± 0.01) + (0.29 ± 0.09) = 6.49 ± 0.09 and indeed, this result is in fair agreement with pK^{H}_{7MeIno} = 6.57 measured previously[5] at I = 1.0 M ($NaClO_4$) but at a temperature of only 21 °C.

The release of the first proton from H_2(TriMeA)$^{2+}$, *i.e.*, from the (N7)H^+ site, has been determined previously and the corresponding acidity constant was expressed by using the concentration of H^+; the result[29] $pK^{H}_{H_2(TriMeA)}$ = −0.75 ± 0.20 is in surprisingly good agreement with the present recalculation ($pK^{H}_{H_2(TriMeA)}$ = −0.77 ± 0.13; Table 1, entry 11) employing the H_0 scale (see Fig. S8 of the Supplementary Information). This result should not distract from the existing caveat if very low pK_a values are compared; reliable comparisons are only possible with values obtained by the same evaluation method.

In the above context it is satisfying to see that $pK^{H}_{H_2(Ado)}$ = −1.50 ± 0.15 (Table 1, entry 14) and $pK^{H}_{H(1MeAdo)}$ = −1.55 ± 0.10 (entry 15) are identical; indeed, in both instances the proton is released from the (N7)H^+ site in species which have overall the same charge but in one instance the hydrogen at (N1)H^+ is replaced by a methyl group. This exchange without altering significantly the acid–base properties at another nearby site is possible because of the close similarity of the electronegativity of H and CH_3. This fact is also meaningful regarding the evaluation of micro acidity constants as described in Sections 3.4 and 3.5.

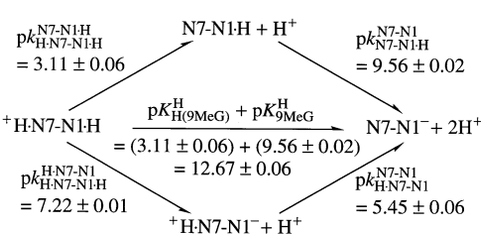
Considering the above it is not astonishing that deprotonation of (N3)H^+ in the equally charged H_3(9MeA)$^{3+}$, H_2(1,9DiMeA)$^{3+}$ and H_2(7,9DiMeA)$^{3+}$ species occurs within the error limits with the same pK_a (Table 1, column 4 of entries 10, 12, 13). Replacement of a methyl by the ribose residue at N9 leads to an acidification for all corresponding purine derivatives listed in Table 1. This is especially evident if the $pK_{a(N3)H}$ and

$pK_{a(N7)H}$ values are compared for $H_2(1,9DiMeA)^{3+}$ with $H_2(1MeAdo)^{3+}$ (entries 12 and 15); *i.e.*, $\Delta pK_{a(N3)H} = -(4.02 \pm 0.28) - (-2.72 \pm 0.38) = -1.3 \pm 0.5$ and $\Delta pK_{a(N7)H} = -(1.55 \pm 0.10) - (-0.79 \pm 0.10) = -0.76 \pm 0.14$. The two acidifications are significant and possibly even of a similar order (see the error limits).

Substitution of a hydrogen by an amino group at C6 in purine to give adenine enhances the basicity of all N sites (Table 1, entries 16, 17), yet the additional replacement of a hydrogen by a methyl group at C9 to give 9-methyladenine has little effect on the basicity of N1 (entry 10). The comparison of the acidity constants of the same two compounds (entries 10, 16) for their $H_3(A)^{3+}$ and $H_2(A)^{2+}$ species (columns 4, 5) indicates that N9 methylation only slightly (if at all) reduces the basicity of N7 but enhances that of N3 significantly.[38,39]

3.4 Intrinsic acid–base properties of guanine and hypoxanthine derivatives

The acidity constants (Table 1) discussed in the preceding section are macro constants which apply to the overall properties of a compound. Now we attempt to quantify the intrinsic acid–base properties of a given site by applying micro acidity constants. Fig. 4 summarizes the equilibrium scheme for



$$K^{H}_{H(9MeG)} = k^{N7\text{-}N1\cdot H}_{H\cdot N7\text{-}N1\cdot H} + k^{H\cdot N7\text{-}N1}_{H\cdot N7\text{-}N1\cdot H} \quad (a)$$

$$\frac{1}{K^{H}_{9MeG}} = \frac{1}{k^{N7\text{-}N1}_{N7\text{-}N1\cdot H}} + \frac{1}{k^{N7\text{-}N1}_{H\cdot N7\text{-}N1}} \quad (b)$$

$$K^{H}_{H(9MeG)} \cdot K^{H}_{9MeG} = k^{N7\text{-}N1\cdot H}_{H\cdot N7\text{-}N1\cdot H} \cdot k^{N7\text{-}N1}_{N7\text{-}N1\cdot H} = k^{H\cdot N7\text{-}N1}_{H\cdot N7\text{-}N1\cdot H} \cdot k^{N7\text{-}N1}_{H\cdot N7\text{-}N1} \quad (c)$$

Fig. 4 Equilibrium scheme for 9-methylguanine (9MeG) (Table 1, entry 1) defining the micro acidity constants (k) and showing their interrelation with the measured macro acidity constants (K) and the connection between N7–N1·H and $^+$H·N7–N1$^-$ and the other species present. In N7–N1·H and $^+$H·N7–N1$^-$ the proton is bound to N1 or to N7, respectively; $^+$H·N7–N1·H is also often written as H(9MeG)$^+$. The arrows indicate the direction for which the acidity constants are defined. Use of the value measured for 7,9DiMeG$^+$, $pK^{H}_{7,9DiMeG} = 7.22 \pm 0.01$ (Table 1, entry 3), for the microconstant $pk^{H\cdot N7-N1}_{H\cdot N7-N1\cdot H}$ permits calculation of the other microconstants with equations (a), (b) and (c). The error limits of the various constants were calculated according to the error propagation after Gauss; they correspond to three times the standard error (see Table 1; footnote *a*).

H(9MeG)$^+$ defining the microconstants (k) and giving their interrelation with the macro acidity constants (K) according to the definitions provided in the lower part of Fig. 4 by following known routes.[8] H(9MeG)$^+$ may release one proton from (N7)H$^+$ and one from (N1)H and therefore we rewrite H(9MeG)$^+$ as $^+$H·N7–N1·H; since deprotonation may occur at either site, we define the monoprotonated forms as N7–N1·H and $^+$H·N7–N1$^-$. Fig. 4 shows that there are four unknown micro acidity constants but only three independent equations interrelating them with the macroconstants; this means, one of the microconstants needs to be obtained independently.

Fig. 4 reveals that the micro acidity constants in the upper pathway are identical with the measured acidity constants because the release of the proton from (N7)H$^+$ is complete before deprotonation at (N1)H begins. If we apply the justified assumption (see Section 3.3) that the release of the proton from the (N1)H site in 7,9DiMeG$^+$ (Table 1, entry 3) represents well the corresponding situation for the deprotonation of $^+$H·N7–N1·H/9MeG it holds $pk^{H\cdot N7-N1}_{H\cdot N7-N1\cdot H/9MeG} = pK^{H}_{7,9MeG} = 7.22 \pm 0.01$. Use of this value in the lower pathway at the left in Fig. 4 allows us now to calculate according to the properties of a cyclic system the microconstant for the release of the proton from (N7)H$^+$ with N1$^-$ being unprotonated, *i.e.* $pk^{N7-N1}_{H\cdot N7-N1} = 5.45 \pm 0.06$.

The acidity constants for the release of the proton from (N1)H in 9MeG and 9EtG are identical within their error limits (Table 1; entries 1, 2) and therefore one may assume that a positive charge at N7 also has the same effect on the (N1)H site in both compounds. Hence, by following the procedure described above and by using $pk^{H\cdot N7-N1}_{H\cdot N7-N1\cdot H/9EtG} = pK^{H}_{7,9MeG} = 7.22 \pm 0.01$ as well as the macroconstants given in Table 1 (entry 2) one obtains for $pk^{N7-N1}_{H\cdot N7-N1/9EtG} = 5.62 \pm 0.06$. The complete microconstant scheme is given in Fig. S9 of the Supplementary Information.

By combining the macro acidity constants measured for H(Guo)$^+$ and 7MeGuo$^+$ (Table 1, entries 4 and 5) and by applying the described procedure, one calculates for the intrinsic micro acidity constants of the (N7)H$^+$ site with N1$^-$ being free, $pk^{N7-N1}_{H\cdot N7-N1/Guo} = 4.32 \pm 0.04$. Similarly, from the values for H(9MeHx)$^+$ and 7,9DiMeHx$^+$ (entries 6 and 7) one obtains $pk^{N7-N1}_{H\cdot N7-N1/9MeHx} = 4.62 \pm 0.02$. The corresponding microconstant schemes are provided in Figs. S10 and S11 of the Supplementary Information, respectively.

For obtaining the micro acidity constant for the (N7)H$^+$ site of Ino$^{\pm}$ the acidity constant of 7-methylinosine (7MeIno$^+$) is employed, *i.e.* $pk^{H\cdot N7-N1}_{H\cdot N7-N1\cdot H/Ino} = pK^{H}_{7MeIno} = 6.20 \pm 0.01$ (Table 1, entry 9). As seen in Fig. S12 of the Supplementary Information, for the release of the proton from the $^+$H·N7–N$^-$ species of inosine $pk^{N7-N1}_{H\cdot N7-N1/Ino} = 3.62 \pm 0.07$ follows from the cyclic system. Overall it is evident that all the various protonated inosine sites are more acidic than their guanosine counterparts (*cf.* Figs. S10 and S12 or in Table 1, entries 4, 5, 8, 9).

3.5 Micro acidity constants for 9-methyladenine

At first sight one may proceed as described in Section 3.4 for 9-methylguanine; *i.e.*, one may employ the value measured for 7,9-dimethyladenine, $pK^{H}_{H(7,9DiMeA)} = 0.50 \pm 0.08$ (Table 1, entry 13) as an estimate for $pk^{H\cdot N7-N1}_{H\cdot N7-N1\cdot H/9MeA}$, which quantifies the release of the proton from (N1)H$^+$, N7 being still protonated. This reasoning is used in the lower pathway of the microconstant scheme shown in Fig. 5 giving the result $pk^{N7-N1}_{H\cdot N7-N1} = 2.96 \pm 0.10$.

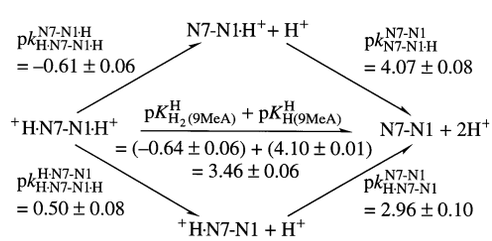


Fig. 5 Micro acidity constant scheme for 9-methyladenine (9MeA), where $^+$H·N7–N1·H$^+$ represents $H_2(9MeA)^{2+}$ (Table 1, entry 10). The micro acidity constants (k) and their interrelation with the measured macro acidity constants (K) are defined as described in the legend for Fig. 4. Use of the value measured for H(7,9DiMeA)$^{2+}$, $pK^{H}_{H(7,9DiMeA)} = 0.50 \pm 0.08$ (Table 1, entry 13), for the microconstant $pk^{H\cdot N7-N1}_{H\cdot N7-N1\cdot H}$ permits calculation of the other microconstants in the scheme (see text in Section 3.5).

However, in developing the upper pathway in Fig. 5 one cannot simply use the macroconstant $pK^{H}_{H_2(9MeA)} = -0.64 \pm 0.06$ because it is relatively close to the microconstant $pk^{H\cdot N7-N1}_{H\cdot N7-N1\cdot H} = 0.50 \pm 0.08$; *i.e.*, the buffer regions of the two deprotonation reactions overlap somewhat. Therefore, the

Table 2 Calculated micro acidity constants for the (N1)H and (N7)H sites of several purine derivatives together with the ratio quantifying the formation of the isomers. The measured macro acidity constants $pK_{a(N1)H}$, which mainly characterize the deprotonation of (N1)H are given for comparison. The values apply to 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

Purine	$pK_{a(N1)H}$[b]	$pk^{N7-N1}_{N7-N1\cdot H}$	$pk^{N7-N1}_{H\cdot N7-N1}$	$\frac{[N7-N1\cdot H]}{[H\cdot N7-N1]}$[c]
9MeG	9.56 ± 0.02	9.56 ± 0.02	5.45 ± 0.06	12 900 ± 1 900
9EtG	9.57 ± 0.05	9.57 ± 0.05	5.62 ± 0.06	8 900 ± 1 600
Guo	9.22 ± 0.01	9.22 ± 0.01	4.32 ± 0.04	79 400 ± 7 500
9MeHx	9.21 ± 0.01	9.21 ± 0.01	4.62 ± 0.02	38 900 ± 2 000
Ino	8.76 ± 0.03	8.76 ± 0.03	3.62 ± 0.07	138 000 ± 24 200
$H(9MeA)^+$	4.10 ± 0.01	4.07 ± 0.08	2.96 ± 0.10	12.9 ± 3.8
$H(Ado)^+$	3.61 ± 0.03	3.59 ± 0.14[d]	2.20 ± 0.17[d]	24.5 ± 12.4

[a] The error limits (3σ) (see footnote *a* of Table 1) of the derived data were calculated according to the error propagation after Gauss. [b] From column 6 of Table 1. [c] See eqn. (9). [d] Calculated with $pK^H_{H(Ado)} = 3.61 \pm 0.03$ and by assuming that $pK^H_{H_2(Ado)} = pK^H_{H(1MeAdo)} = -1.55 \pm 0.10$ (see Table 1, entries 14 and 15, and the terminating paragraph of Section 3.5).

equation analogous to eqn. (a) given in the lower part of Fig. 4 has to be used for obtaining a value for $pk^{N7-N1\cdot H}_{H\cdot N7-N1\cdot H/9MeA}$ ($= -0.61 \pm 0.06$) which appears at the left in the upper pathway of Fig. 5. This result allows us now to complete the upper cycle in the scheme of Fig. 5.

Tentatively, one may also use the above results for 9MeA to estimate a value for the adenosine system, *i.e.*, for $pk^{N7-N1}_{H\cdot N7-N1/Ado}$. The effect of the replacement of the methyl by the ribose residue at N9 on the deprotonation of $(N7)H^+$ is estimated by $\Delta pK_{a/N7} = pK^H_{H(1,9DiMeA)} - pK^H_{H(1MeAdo)} = -(0.79 \pm 0.10) - (-1.55 \pm 0.10) = 0.76 \pm 0.14$ (from entries 12 and 15 of Table 1).[40] Hence, one obtains for $pk^{N7-N1}_{H\cdot N7-N1/Ado} = pk^{N7-N1}_{H\cdot N7-N1/9MeA} - \Delta pK_{a/N7} = (2.96 \pm 0.10) - (0.76 \pm 0.14) = 2.20 \pm 0.17$. This estimate involves negative pK_a values and these are difficult to measure with lower error limits. On the other hand, as discussed in Section 3.3, it is comforting that the pK_a values for the monodeprotonation of $H(1MeAdo)^{2+}$ and $H_2(Ado)^{2+}$ agree so well with each other. Furthermore, the value estimated here for the H·N7–N1/Ado species also fits nicely into the overall picture as discussed in Section 4 in the terminating paragraph.

4 Conclusions

The micro acidity constants derived in this study for the (N1)H and (N7)H sites of purine derivatives are useful and significant quantities (Table 2; columns 3, 4). For example, they define the intrinsic acid–base properties in aqueous solution of the individual sites of nucleic acids (at least in a relative sense). Furthermore, we intend to use such values in comparisons with the acidity constants of Pt^{2+} complexes involving these nucleobase derivatives as ligands.[41]

In addition, based on these micro acidity constants one may calculate the ratio R^* which quantifies the formation degree of the isomeric species having the proton in one tautomer at N1 and in the other at N7 [eqn. (9)]:

$$R^* = \frac{[N7-N1\cdot H]}{[H\cdot N7-N1]} = \frac{k^{N7-N1}_{H\cdot N7-N1}}{k^{N7-N1}_{N7-N1\cdot H}} \qquad (9)$$

The corresponding results are listed in the final column of Table 2. Though R^* is rather sensitive to relatively small changes in the p*k* values involved, it is evident that for the guanine and hypoxanthine derivatives the isomer with the proton at N1 strongly dominates, with a formation degree of practically 100% allowing only the formation of traces of the zwitterionic species $^+H\cdot N7-N1^-$. For the adenine derivatives the situation is somewhat less one-sided; here both tautomers are formed in appreciable amounts, *e.g.*, monoprotonated 9-methyladenine occurs to about 93% as the $N7-N1\cdot H^+$ isomer and to about 7% in the $^+H\cdot N7-N1$ form. The corresponding isomeric distribution for adenosine is 96% *versus* 4%. However, for the guanine, hypoxanthine and adenine derivatives it always holds that replacement of the N9 methyl group by a ribose residue favors further the isomer with the proton at N1.

The previously[4] estimated isomeric ratios for guanosine, inosine and adenosine are larger, *i.e.* the N7–N1·H isomer appears as even more favored. The reason for the discrepancy is that the previously employed pK_a values referred to different ionic strengths (mainly to $I = 0.1$ and 1 M). Now the complete evaluations are based on values valid for $I = 0.1$ M (and 25 °C). However, the trends are identical in both studies; the dominance of the N7–N1·H isomer decreases in the order Ino > Guo > Ado. Furthermore, from the results in Table 2 it is evident that the given order is of a more general nature because hypoxanthine > guanine > adenine holds for the N7–N1·H isomer of all the derivatives studied.

A further interesting point is to be noted. If one plots the micro acidity constants, $pk^{N7-N1}_{H\cdot N7-N1}$, which quantify the intrinsic acidic properties of the (N7)H site, in dependence on the macro acidity constants, $pK_{a(N1)H}$, which quantify largely the release of the proton from the (N1)H site, one obtains a straight line based on the data of the first five entries of Table 2 as seen in Fig. 6. This means, the values for the guanine and hypoxanthine derivatives fall on a straight line (correlation coefficient $R = 0.976$) which is defined in eqn. (10):

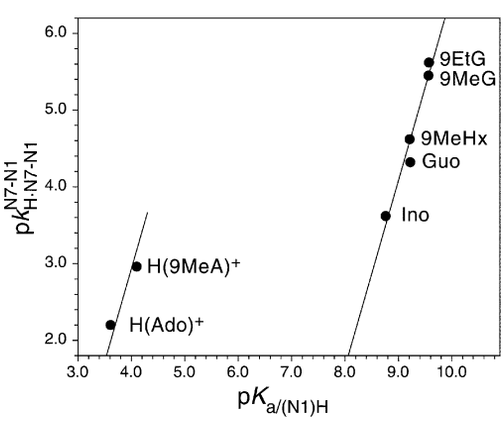


Fig. 6 Plot of the micro acidity constant, $pk^{N7-N1}_{H\cdot N7-N1}$, which defines the intrinsic acidity of the (N7)H site of purine derivatives, in dependence on the macro acidity constant $pK_{a(N1)H}$, which quantifies largely the release of the proton from the (N1)H site. The plotted values are taken from columns 2 and 4 of Table 2. The parameters of the straight line seen at the right hand side, the data points being due to the guanine and hypoxanthine derivatives, are given in eqn. (10). For the meaning of the line at the left see the terminating paragraph in Section 4.

$$pk^{N7-N1}_{H\cdot N7-N1} = (2.43 \pm 0.31)\, pK_{a(N1)H} - (17.77 \pm 2.87) \qquad (10)$$

This relation allows us to obtain estimates for the micro acidity constants of guanine and hypoxanthine derivatives if values for $pK_{a(N1)H}$ are known.

From the data points due to 9-methyladenine and adenosine it is evident that adenine derivatives behave differently. At present not enough data are yet available to define well a straight line. However, it is interesting to see that the values for $H(9MeA)^+$ and $H(Ado)^+$ fall close to the line drawn through these two data points with the slope of eqn. (10). This may be taken as a further indication that the micro acidity constant estimated in Section 3.5 for $^+H{\cdot}N7–N1/Ado$ is of a reasonable order.

Acknowledgements

We thank Professor Dr R. Bruce Martin (University of Virginia, Charlottesville) for helpful suggestions and Mrs Rita Baumbusch and Mrs Astrid Sigel for competent technical assistance in the preparation of this manuscript. This study was supported by the Swiss National Science Foundation (H.S.), the Swiss Federal Office for Education & Science within the COST D20 programme (H.S.), by the Deutsche Forschungsgemeinschaft (B.L.) and the Fonds der chemischen Industrie (B.L.).

References

1 R. B. Martin, *Acc. Chem. Res.*, 1985, **18**, 32–38.
2 R. B. Martin and Y. H. Mariam, *Met. Ions Biol. Syst.*, 1979, **8**, 57–124.
3 R. M. Smith, A. E. Martell and Y. Chen, *Pure Appl. Chem.*, 1991, **63**, 1015–1080.
4 R. B. Martin, *Met. Ions Biol. Syst.*, 1996, **32**, 61–89.
5 S.-H. Kim and R. B. Martin, *Inorg. Chim. Acta*, 1984, **91**, 19–24.
6 (*a*) R. L. Benoit and M. Fréchette, *Can. J. Chem.*, 1984, **62**, 995–1000; (*b*) R. L. Benoit and M. Fréchette, *Can. J. Chem.*, 1985, **63**, 3053–3056.
7 J. E. Šponer, J. Leszczynski, F. Glahé, B. Lippert and J. Šponer, *Inorg. Chem.*, 2001, **40**, 3269–3278.
8 R. B. Martin, *Met. Ions Biol. Syst.*, 1979, **9**, 1–39.
9 H. Sigel, S. S. Massoud and R. Tribolet, *J. Am. Chem. Soc.*, 1988, **110**, 6857–6865.
10 B. Song, R. K. O. Sigel and H. Sigel, *Chem. Eur. J.*, 1997, **3**, 29–33.
11 E. G. Talman, W. Brüning, J. Reedijk, A. L. Spek and N. Veldman, *Inorg. Chem.*, 1997, **36**, 854–861.
12 T. Itaya, F. Tanaka and T. Fujii, *Tetrahedron*, 1972, **28**, 535–547.
13 H. Sigel, A. D. Zuberbühler and O. Yamauchi, *Anal. Chim. Acta*, 1991, **255**, 63–72.
14 G. Kampf, M. S. Lüth, J. Müller, A. Holý, B. Lippert and H. Sigel, *Z. Naturforsch., Teil B*, 2000, **55**, 1141–1152.
15 L. E. Kapinos, B. Song and H. Sigel, *Inorg. Chim. Acta*, 1998, **280**, 50–56.
16 L. E. Kapinos, B. Song and H. Sigel, *Chem. Eur. J.*, 1999, **5**, 1794–1802.
17 R. Tribolet and H. Sigel, *Eur. J. Biochem.*, 1987, **163**, 353–363.
18 C. A. Blindauer, A. Holý, H. Dvořáková and H. Sigel, *J. Chem. Soc., Perkin Trans. 2*, 1997, 2353–2363.
19 P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188–190.
20 Average from the values given in refs. 20a and 20b: (*a*) P. R. Mitchell and H. Sigel, *J. Am. Chem. Soc.*, 1978, **100**, 1564–1570; (*b*) R. Tribolet, R. Malini-Balakrishnan and H. Sigel, *J. Chem. Soc., Dalton Trans.*, 1985, 2291–2303.
21 M. A. Paul and F. A. Long, *Chem. Rev.*, 1957, **57**, 1–45.
22 R. B. Martin, *Science (Washington, D. C.)*, 1963, **139**, 1198–1203.
23 L. P. Hammett and A. J. Deyrup, *J. Am. Chem. Soc.*, 1932, **54**, 2721–2739.
24 (*a*) H. Sigel, *Biol. Trace Elem. Res.*, 1989, **21**, 49–59; (*b*) K. H. Scheller, F. Hofstetter, P. R. Mitchell, B. Prijs and H. Sigel, *J. Am. Chem. Soc.*, 1981, **103**, 247–260.
25 O. Yamauchi, A. Odani, H. Masuda and H. Sigel, *Met. Ions Biol. Syst.*, 1996, **32**, 207–270.
26 H. Sigel, S. S. Massoud and N. A. Corfù, *J. Am. Chem. Soc.*, 1994, **116**, 2958–2971.
27 B. Song, J. Zhao, R. Griesser, C. Meiser, H. Sigel and B. Lippert, *Chem. Eur. J.*, 1999, **5**, 2374–2387.
28 N. A. Corfù and H. Sigel, *Eur. J. Biochem.*, 1991, **199**, 659–669.
29 C. Meiser, B. Song, E. Freisinger, M. Peilert, H. Sigel and B. Lippert, *Chem. Eur. J.*, 1997, **3**, 388–398.
30 B. Lippert, H. Schöllhorn and U. Thewalt, *Inorg. Chim. Acta*, 1992, **198–200**, 723–732.
31 J. Elguero, C. Marzin, A. R. Katritzky and P. Linda, *The Tautomerism of Heterocycles, Advances in Heterocyclic Chemistry, Suppl. 1*, Academic Press, New York, 1976, p. 20.
32 A. Kettani, M. Guéron and J.-L. Leroy, *J. Am. Chem. Soc.*, 1997, **119**, 1108–1115.
33 (*a*) T. Fujii, T. Itaya and T. Saito, *Chem. Pharm. Bull.*, 1975, **23**, 54–61; (*b*) M. Dreyfus, G. Dodin, O. Bensaude and J. E. Dubois, *J. Am. Chem. Soc.*, 1977, **99**, 7027–7037; (*c*) D.-L. Hoo and B. McConnell, *J. Am. Chem. Soc.*, 1979, **101**, 7470–7477; (*d*) B. McConnell and D. Politowski, *Biophys. Chem.*, 1984, **20**, 135–148.
34 P. D. Lawley and P. Brookes, *Biochem. J.*, 1963, **89**, 127–138.
35 H. Lönnberg and P. Vihanto, *Inorg. Chim. Acta*, 1981, **56**, 157–161.
36 J. Arpalahti and E. Ottoila, *Inorg. Chim. Acta*, 1985, **107**, 105–110.
37 H. Lönnberg and J. Arpalahti, *Inorg. Chim. Acta*, 1980, **55**, 39–42.
38 The difference of $\Delta pK_a = 1.4 \pm 0.4$ [$= -(2.83 \pm 0.30) - (-4.2 \pm 0.3$; error estimated)] appears to be real and cannot be attributed to the fact that in refs. 6, 7 (scale of ref. 39) and in the present study (scale of ref. 21) somewhat different H_0 scales were used. These scales begin to differ only at $[HClO_4] > 8.5$ M. At this $HClO_4$ concentration the two values for H_0, −4.69 and −4.71, are still practically identical; at $[HClO_4] = 9.0$ M the scale of ref. 39 is lower by $\Delta H_0 = 0.13$ (−5.18 *versus* −5.05), at $[HClO_4] = 9.5$ M by 0.24 and at $[HClO_4] = 10.0$ M by 0.50. Since the deviations became significant only at $[HClO_4] = 9.0$ M with $H_0 \simeq -5.1$, the pK_a value of −4.2 should not have been affected significantly.
39 K. Yates and H. Wai, *J. Am. Chem. Soc.*, 1964, **86**, 5408–5413.
40 It may be noted that the correction factor given in the text is within the error limits identical with the following one which is based on entries 10 and 14 of Table 1: $\Delta pK_{a/N7} = pK^H_{H(9MeA)} - pK^H_{H(Ado)} = -(0.64 \pm 0.06) - (-1.50 \pm 0.15) = 0.86 \pm 0.16$.
41 R. Griesser, G. Kampf, L. E. Kapinos, S. Komeda, B. Lippert, J. Reedijk and H. Sigel, results to be published.