# Analysis of $[(dien)Pd]^{2+}$ binding to 8-azapurines and allopurinol by proton NMR spectroscopy

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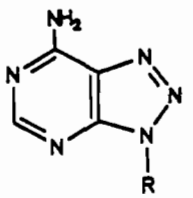
**Abstract**

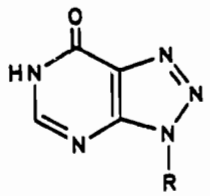
An analysis of the binding of $[(dien)Pd]^{2+}$ to 8-azapurines and 7-deaza-8-azapurines has been performed by proton magnetic resonance spectroscopy. p$K$ values were determined by non-linear least-squares for protonation equilibria, which did not give rise to an evaluable step in the average resonance value $\delta$ for the species involved. The bases studied were 8-aza-9-methyladenine (Maad), 8-aza-9-methylhypoxanthine (HMahx), 7-deaza-8-aza-9-methyladenine (Mapp) and 7-deaza-8-aza-9-methylhypoxanthine (HMall). An N1 coordinated species MB (M = $[(dien)Pd]^{2+}$, B = base) dominates in Maad solution at pH values between 2 and 6. Two $MBH_{-1}$ complexes with metal binding to N1 and N6, respectively, are observed in alkaline solution. As a result of the increase in the $pK_{a1}$ value (N1) from 2.70 to 4.43 on going from Maad to Mapp the pH range for the predomination of MB species is shifted for the latter base to higher pH values (pH > 4). Mapp solutions contain 3 MBH species at significant concentrations in the pH range 1–4 and one major and two minor MB species at higher pH values. An N1 coordinated species also dominates in HMahx solution for pH values above 5. Only one MBH complex is observed for this base (pH range 3–6 for significant concentrations). N1 is protonated so that either N3 or N7 must be the metal coordination site. Two diprotonated species $MBH_2$ and $M_2BH_2$ are present in HMall solution at significant concentrations for pH < 4. As a result of the increase in the $pK_{a1}$ value (N1) from 8.20 to 9.28 on going from HMahx to HMall the pH range for MBH complexes is shifted for the latter base to higher pH values (4–7). An N1 coordinated MB species is the only complex present in solution at significant concentrations for pH > 8.

## Introduction

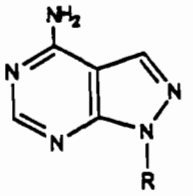
Chemical modification of the purine imidazole ring leads to profound alterations in the biological properties of the resultant bases. For instance, various 8-azapurine nucleosides, in which the 8-CH function of the parent purine has been replaced by an aza nitrogen, have been demonstrated to display effective antineoplastic properties [1]. It has been postulated that characteristic conformational changes at the glycosidic bond N9–C1′ may be mainly responsible for the mode of action of the 8-azapurines. However, it is manifest that a significant role may also be played by changes in the charge distribution within the heterocyclic base, which will be expected to influence the preferred pattern of hydrogen bonding [2]. Molecular orbital calculations have revealed that N7 and N8 in H9-tautomers of 8-azapurines carry virtually no residual charge [2, 3].



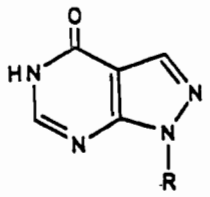
R = H, HAad
R = $CH_3$, Maad



R = H, $H_2$Ahx
R = $CH_3$, HMahx



R = H, HApp
R = $CH_3$, Mapp



R = H, $H_2$All
R = $CH_3$, HMall

The pyrazolo[3,4-d]pyrimidines (7-deaza-8-azapurines) in which the imidazole ring is replaced by

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a pyrazole ring, are isomeric with naturally occurring purines. Both pyrazolo[3,4-d]pyrimidin-4-one (HApp), allopurinol ($H_2All$) and its isomer hypoxanthine are substrates for xanthine oxidase. Enzymatic oxidation of allopurinol yields alloxanthine, which is believed to inhibit the production of uric acid by strongly binding to the reduced form of the molybdenum centre. As a result, allopurinol is sometimes administered as an antihyperuricemia drug [4]. Two models have been proposed for the binding of alloxanthine to the molybdenum centre of xanthine oxidase. Whereas N8 coordination has been postulated on the basis of EPR measurements by Hawkes *et al.* [5], an alloxanthine complex containing a Mo–N9 bond and stabilizing a N8...H–N(enzyme) interaction is assumed by Stiefel [6].

Interaction of base nitrogen atoms of these modified purines with metal cations in biological systems may be expected to lead to further alterations in the charge distribution within the heterocycles and could also influence the nature of hydrogen bonding interactions between base pairs. On account of its ability to function as a uniligating Lewis acid with minimal steric effects, the $CH_3Hg^+$ ion has proved to be a suitable cation for the characterization of binding sites for 8-azapurines [7–9] and pyrazolo-[3,4-d]pyrimidines [10, 11]. Methylmercury(II) complexes, isolated from aqueous solution in the pH range 1–10, were structurally characterized by $^1H$ NMR spectroscopy and X-ray structural analysis. These investigations indicated that, as for the natural purines, N9 is the preferred metal binding site for such modified purines. However, when N9 is blocked, either through participation in an Hg–N9 bond or through methylation, dramatic changes in the pattern of metal binding become apparent. For both Maad and HMahx further binding of $CH_3Hg^+$ cations could only be established for the pyrimidine nitrogens N1 and N3. These findings are, of course, restricted to those methylmercury(II) complexes, which could be isolated from aqueous solutions. However, they do suggest that N7 (or N8) or 8-azaadenine or 8-azahypoxanthine may not be competitive as a site for metal binding in the DNA in which the base may act. For Mapp and HMall, in contrast, the pyrazole nitrogen N8 competes as a binding site with the pyrimidine nitrogens N1 and N3. In this respect, their behaviour displays a direct analogy to that of the natural purines, in which N1 and N7 compete for metal cations [12, 13].

Reliable stability constants for metal complexes of such modified purine bases are very scarce [14]. As a result of the multiplicity of potential metal binding sites, potentiometric titrations will, as a rule, yield only macroscopic formation constants. $^1H$ NMR spectroscopy will allow the determination of individual formation constants (e.g. for metal complexes with N1 or N7 binding) if the following conditions are fulfilled. Firstly, the metal cation should be diamagnetic so that broadening and marked shifting of proton resonances does not occur. In addition, exchange of metal cations between competing binding sites should be slow, so that proton resonances may be assigned to individual complexes. Investigations carried out by Martin and co-workers [14–16] have demonstrated that these preconditions are met by the (diethylenetriamine)palladium(II) cation $[(dien)Pd]^{2+}$, which also offers the further advantage of being a uniligating ligand. The electronic and stereochemical similarities between Pd(II) and Pt(II) complexes suggest that studies with $[(dien)Pd]^{2+}$ should allow conclusions to be drawn for Pt(II) complexes, several of which (e.g. *cis*-$PtCl_2(NH_3)_2$) are effective antitumour agents.

Stability constants for the binding of $[(dien)Pd]^{2+}$ to pyrimidine and purine nucleosides have been reported by Martin and co-workers [12, 17]. They demonstrated that N1 binding is preferred in comparison to N7 binding by both adenosine ($\log K_1 = 4.5$, $\log K_7 = 3.9$) and inosine ($\log K_1 = 8.33$, $\log K_7 = 6.80$). We now report a systematic $^1H$ NMR spectroscopic study of the interaction of $[(dien)Pd]^{2+}$ with the 9-methyl derivatives of 8-azapurines and 7-deaza-8-azapurines in the pH range 2–10. Full details of the procedure adopted for the establishment of species distribution diagrams is provide in 'Experimental' and 'Discussion'. An exemplary X-ray structural analysis was also performed on [(dien)Pt-(Mapp)]$Br_2 \cdot 2H_2O$.

## Experimental

The 9-methyl derivatives of the modified purine bases were prepared as described in the literature: Maad [18], HMahx [19], Mapp [20] and HMall [20].

Stock solutions of $[(dien)PdH_2O)]^{2+}$ at molar strengths between 0.005 and 0.2 M were prepared by suspending [(dien)PdCl]Cl [21] in $D_2O$, adding 2 equiv. of $AgNO_3$ and stirring for 12 h in the dark. After filtration of the precipitate, the yellow solution was diluted with $D_2O$ to give the required molar strength and used immediately. Titrations were performed with carbonate-free 1 M NaOD for equimolar solutions of $[(dien)Pd(D_2O)]^{2+}$ and the appropriate base. The potential alteration in the total cation and base concentrations caused by the addition of NaOD was immediately compensated for by the addition of an identical volume of a $D_2O$ solution of $[(dien)Pd(D_2O)]^{2+}$ and base at double molar strength. A background ionic strength of 0.5 M $KNO_3$

was employed for all titration solutions. A combined glass electrode (Metrohm 6.0219.100) containing a saturated $KNO_3$ solution between the membrane of the internal silver chloride reference electrode and an outer membrane was used to prevent diffusion of chloride ions into the titration solution, which could lead to a precipitation of [(dien)PdCl]Cl. After addition of NaOD, the titration solution was allowed to stand until pH constancy had been achieved (at least 20 min) before samples were taken for $^1H$ NMR measurements recorded on a Bruker AM 400 spectrometer at 25 °C. All chemical shifts are reported in ppm downfield to the internal reference ($Na[Me_3SiCD_2CD_2COO]$).

Evaluation of the $^1H$ NMR spectra was performed with the computer program GLKONST written by the authors. An initial calculation of $pK_a$ values for the protonation of species $M_xB_yH_z$ (M = metal cation, B = base, H = proton), as evidenced by a definite step in the $\delta$ value, is carried out by a non-linear least-squares fit of chemical shift versus pH value. In a subsequent routine respectively $pK_a$ or $pK$ values may also be determined for protonation ($M_xB_yH_z \rightarrow M_xB_yH_{1+z}$) or possibly metal cation co-ordination ($M_xB_yH_z \rightarrow M_{1+x}B_yH_z$) equilibria, which do not give rise to an evaluable step in the average resonance value for the species involved. The program employs the Gauss–Newton algorithmus to minimise the function

$$\sum_i^I \left[ \left\{ \sum_j^J ([M_s]_{obs})_j - \sum_j^J ([M_s]_{calc})_j \right\}^2 \right]_i$$

for $I$ pH values, where $([M_s]_{obs})_j$ represents the observed concentration and $([M_s]_{calc})_j$ the calculated concentration for each of the $J$ species included in a particular refinement. Calculated species concentrations at a particular pH value are obtained from the current values of the formation constants $\beta_j$ and the total $[(dien)Pd]^{2+}$ and base concentrations $[M_t]$ and $[B_t]$ using the Newton–Raphson algorithmus. This routine is also employed to produce the species distribution diagrams reported in the 'Discussion'. The final formation constants $\beta_j$ reported in this work are average values determined for all pH values with a significant observed species concentration $([M_s]_{obs})_j$, calculated, if necessary using the refined stability constants $K_a$ or possibly $K$. Whereas the free base concentration $[B_f]$ may be determined directly by $^1H$ NMR spectroscopy, this is not the case for the free metal cation concentration $[M_f]$ as the dien proton resonances for the various species $M_xB_yH_z$ overlap with the signals for the free cation $[(dien)Pd]^{2+}$. $[M_f]$ is calculated from the difference

$$\left\{ [M_t] - \sum_j^J ([M_s]_{obs})_j \right\}$$

where $[M_t]$ is the total $[(dien)Pd]^{2+}$ concentration in the titration solution. Two reliability indices $R_1$ an $R_2$

$$R_1 = \left( \sum_i^I \left[ \left\{ \sum_j^J ([M_s]_{obs})_j - \sum_j^J ([M_s]_{calc}) \right\}_j^2 \right]_i \Big/ \sum_i^I \left[ \left\{ \sum_j^J ([M_s]_{obs})_j \right\}^2 \right]_i \right)^{1/2}$$

$$R_2 = \left( \sum_i^I \left[ \sum_j^J \left\{ ([M_s]_{obs})_j - ([M_s]_{calc}) \right\}_j^2 \right]_i \Big/ \sum_i^I \left[ \sum_j^J \{([M_s]_{obs})_j\}^2 \right]_i \right)^{1/2}$$

are provided for hypothesis testing during the process of establishing species distribution diagrams. GLKONST also allows for the refinement of $\beta$ values for species present at pH ranges for which $[B_f]$ is too small to be directly measurable by proton NMR spectroscopy. This procedure may also be applied if $[M_f]$ is too small to allow a reliable determination from the difference $\{[M_t] - \Sigma_j^J([M_s]_{obs})_j\}$.

The deprotonation of the coordinated water in $[(dien)Pd(H_2O)]^{2+}$ proceeds via a hydroxo-bridged dinuclear cation $[(dien)Pd(\mu\text{-}OH)Pd(dien)]^{3+}$, for which a formation constant $\log K_1$ of $-5.62(4)$ was reported by Martin and co-workers [17]. An acidity constant $pK_a$ of 7.74(1) for $[(dien)Pd(H_2O)]^{2+}$ was determined by the same authors. The presence of these species in the titration solution is allowed for by the program GLKONST.

### X-ray structural analysis of $[(dien)Pt(Mapp)]Br_2 \cdot 2H_2O$

0.2 mmol of Mapp was added to a suspension of 0.2 mmol [(dien)PtBr]Br in 10 ml $H_2O$. After heating with stirring for 2 h at 60 °C the clear solution was allowed to evaporate slowly at r.t. to yield colourless crystals of $[(dien)Pt(Mapp)]Br_2 \cdot 2H_2O$ (yield 65%). The compound crystallises in the triclinic space group $P\bar{1}$ with $a = 10.999(2)$, $b = 12.043(2)$, $c = 8.241(2)$ Å, $\alpha = 97.82(3)$, $\beta = 88.94(3)$, $\gamma = 64.61(1)°$, $Z = 2$, $D_{calc} = 2.19$ g cm$^{-3}$. The asymmetric unit contains two water solvate molecules. Unit cell constants were obtained from a least-squares fit to the settings of 25 reflections centered on an Enraf-Nonius CAD4 diffractometer. Intensities were collected on the dif-

fractometer in the $\omega$-scan mode with graphite-monochromated Mo K$\alpha$ radiation ($\lambda = 0.71073$ Å) for $2\theta_{max} = 50°$. From a total of 3209 independent reflections, 3023 were retained for use in the subsequent refinement after application of the rejection criterion $F_o^2 < 2\sigma(F_o^2)$. Empirical absorption corrections were applied to the reflection intensities. The structure was solved by Patterson and difference syntheses and refined by full-matrix least-squares. All non-hydrogen atoms were assigned anisotropic temperature factors. The dien hydrogen atoms were included at geometrically calculated positions with group isotropic temperature factors. Terminal reliability factors of $R = 0.057$ and $R_w = [\Sigma w(F_o - F_c)^2/\Sigma w F_o^2]^{1/2} = 0.055$ were obtained for the final refinement cycle with a last parameter shift/e.s.d. of less than 0.01. Weights were given by the expression $w = [\sigma^2(F_o) + p^2 F_o^2]^{-1}$ with $p = 0.014$. Calculations were performed with SHELX [22] and with local programs. Atom positional parameters with equivalent isotropic temperature factors are listed in Table 1, bond distances and angles to the platinum atom in Table 2. The molecular structure of the complex is depicted in Fig. 1.

TABLE 1. Atom positional parameters with equivalent isotropic temperature factors ($Å^2 \times 10^3$) for [(dien)Pt(Mapp]Br·2H$_2$O

| Atom | x/a | y/b | z/c | $U_{eq}$ |
|---|---|---|---|---|
| Pt | 0.4524(1) | 0.2759(1) | 0.3361(3) | 32(1) |
| Br1 | 0.3935(2) | 0.0042(2) | 0.2022(2) | 68(2) |
| Br2 | 0.2657(2) | 0.6823(2) | 0.2924(2) | 65(2) |
| O1 | −0.0036(13) | 0.8238(14) | 0.5173(18) | 90(14) |
| O2 | 0.0962(17) | 0.9827(14) | 0.2225(20) | 110(17) |
| N1 | 0.3190(11) | 0.3748(11) | 0.5180(12) | 36(9) |
| N3 | 0.2441(12) | 0.5617(12) | 0.7112(13) | 45(10) |
| N6 | 0.2090(12) | 0.2492(12) | 0.4710(13) | 49(11) |
| N8 | −0.0531(11) | 0.5550(12) | 0.8238(13) | 45(10) |
| N9 | 0.0326(11) | 0.6103(12) | 0.8329(13) | 43(10) |
| N11 | 0.5926(12) | 0.1550(12) | 0.4803(13) | 48(11) |
| N12 | 0.5857(10) | 0.1825(10) | 0.1556(12) | 40(9) |
| N13 | 0.3423(11) | 0.3896(10) | 0.1670(12) | 40(9) |
| C2 | 0.3278(14) | 0.4841(14) | 0.6050(15) | 44(11) |
| C4 | 0.1410(13) | 0.5362(11) | 0.7329(13) | 37(11) |
| C5 | 0.1215(13) | 0.4360(12) | 0.6660(13) | 37(11) |
| C6 | 0.2188(13) | 0.3498(14) | 0.5470(14) | 37(11) |
| C7 | −0.0002(13) | 0.4509(14) | 0.7208(16) | 40(11) |
| C9 | 0.0005(17) | 0.7287(15) | 0.9319(17) | 56(14) |
| C11 | 0.7078(15) | 0.0552(16) | 0.3663(18) | 61(15) |
| C12 | 0.7196(12) | 0.1226(16) | 0.2324(18) | 54(13) |
| C13 | 0.5657(15) | 0.2786(14) | 0.0454(16) | 52(13) |
| C14 | 0.4209(17) | 0.3336(18) | 0.0015(14) | 69(17) |

TABLE 2. Bond lengths (Å) and angles (°) to the Pt atom in [(dien)Pt(Mapp)]Br·2H$_2$O

| | | | |
|---|---|---|---|
| Pt–N1 | 1.937(10) | Pt–N11 | 2.126(12) |
| Pt–N12 | 1.918(10) | Pt–N13 | 2.109(11) |
| N11–Pt–N1 | 95.6(4) | N12–Pt–N1 | 178.1(5) |
| N12–Pt–N11 | 85.1(4) | N13–Pt–N1 | 92.9(4) |
| N13–Pt–N11 | 170.3(4) | N13–Pt–N12 | 86.3(4) |
| C2–N1–Pt | 118.0(8) | C6–N1–Pt | 119.5(10) |
| C11–N11–Pt | 108.4(8) | C12–N12–Pt | 105.7(7) |
| C13–N12–Pt | 102.7(7) | C14–N13–Pt | 106.9(8) |

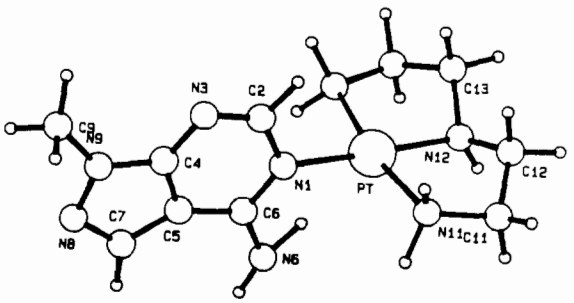


Fig. 1. Structure of the cation [(dien)Pt(Mapp)]$^+$.

## Discussion

Four separate signals for the base proton H2 of the species present in the equilibrium system Maad/[(dien)Pd]$^{2+}$ were observed for pH values between 1 and 10. (An example for the distribution of base proton chemical shifts versus pH is provided by Fig. 4 (for Mapp).) For pH values between 5 and 1, signal 4 belongs to the free base B and/or BH, in which Maad is protonated on N1. The acidity constant p$K_{a1}$ obtained from a least-squares fit chemical shift versus pH is 2.697(1) (Table 3), a value close to that of 2.80 reported for Maad by Albert [23]. The species MB(1) (signal 1) dominates at pH values below 6 (Fig. 2). In view of a marked downfield shift of H2 in MB(1) in comparison to the protonated base BH (8.73 versus 8.54 ppm at pH 1.14), it may reasonably be assumed that N1 is the [(dien)Pd]$^{2+}$ coordination site in this species. Integral values for signal 1 at pH values above 6 indicate the presence of a further complex MBH$_{-1}$(1), in which the amino

TABLE 3. Acidity constants p$K_{a1}$ and stability constants log $K_1$ for base solutions containing [(dien)Pd]$^{2+}$

| Base | p$K_{a1}$ | log $K_1$ | log $K_n$[a] | $n$ |
|---|---|---|---|---|
| Maad | 2.697(1) | 3.96(1) | 2.5(1) | 3 or 7 |
| Mapp | 4.431(1) | 3.34(6) | 1.7(1) | 3 |
| Mahx | 8.199(1) | 5.27(2) | 3.8(2) | 3 or 7 |
| Mall | 9.278(1) | 5.2(3) | | |
| Adenosine [17] | 3.89 | 4.5 | 3.9 | 7 |
| Inosine [17] | 9.06 | 8.33 | 6.80 | 7 |

[a]Stability constant log $K_n$ for the alternative coordination site $n$ in complexes MB.

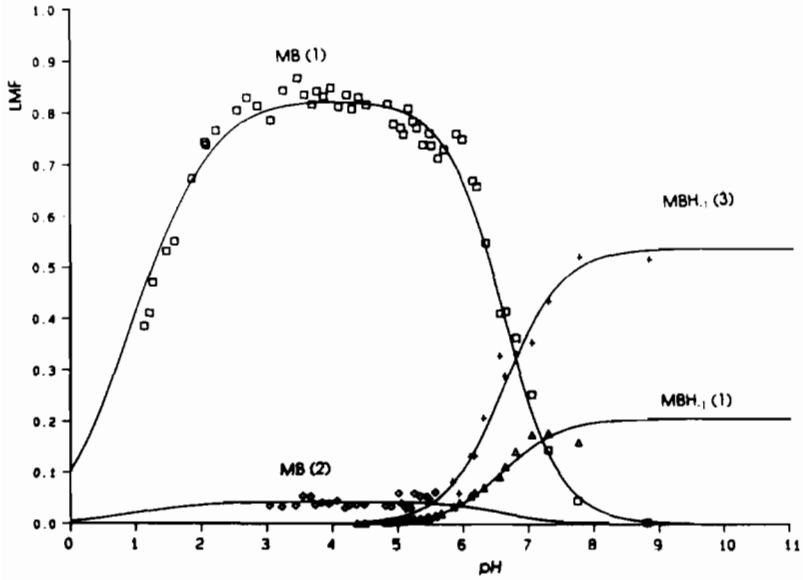


Fig. 2. Species distribution for Maad binding by $[(dien)Pd]^{2+}$ presented as ligand mole fraction (LMF) vs. pH for Maad and $[(dien)Pd]^{2+}$ at 0.005 M. The following formation constants log $\beta$ were determined (for base = $BH_{-1}$): MB(1) 10.02(1), MB(2) 8.6(1), $MBH_{-1}(1)$ 2.84(3), $MBH_{-1}(3)$ 3.23(3).

nitrogen N6 is deprotonated with N1 being retained as a coordination site. Inclusion of $MBH_{-1}(1)$ in the species distribution diagram leads to an improvement of $R_1$ from 0.054 to 0.052 and $R_2$ from 0.077 to 0.068. Refinement of the acidity constant $pK_{a6}$ for N6 yields the value 6.06(1). The remaining signals 2 and 3 may be assigned to complexes MB(2) and $MBH_{-1}(3)$. A pronounced shift of the H2 resonance in $MBH_{-1}(3)$ to higher field in comparison to $BH_{-1}$ (8.18 versus 8.37 ppm at pH 8.81) suggests that N6 is metallated in this species. Both N3 and N7 are potential metal binding sites in complex MB(2). N3 participation in a square-planar coordination sphere has been observed in $[RhCl(CO)_2(Baad)]$ (Baad = 8-aza-9-benzyladenine) [24]. Although N7 coordination of Maad was not observed for the methylmercury(II) cation [9], it was established by X-ray structural analysis [25] for $[(cod)Rh(\mu\text{-}MaadH_{-1})RhCl(cod)]_2$.

Four separate H2 resonances were also observed for species present in the equilibrium system Mahx/$[(dien)Pd]^{2+}$ in the pH range 3–11. At pH values between 11 and 6, signal 4 belongs to the free base B and/or BH, in which Mahx is protonated on N1. A least-squares fit of chemical shift versus pH yields $pK_{a1} = 8.199(1)$ for Mahx. The N1 metallated complex MB(3) (signal 3), for which a protonation at lower pH values was not observed, is predominant at pH values above 5.5. The species distribution diagram (Fig. 3) contains only one Mahx complex of the type MBH, namely MBH(2) from signal 2. N1 protonation may be assumed in this case, so that both N3 and N7 are available as potential palladium coordination sites. Inspection of the integral values for signal 2 indicate the presence of a significant concentration of a complex $MBH_2(2)$ at pH values below 4 and a minor concentration of a complex MB(2) at pH values above 6. Inclusion of $MBH_2(2)$ in the species distribution diagram (without MB(2)) leads to an improvement of $R_1$ from 0.060 to 0.047 and $R_2$ from 0.100 to 0.074. With MB(2) final $R_1$ and $R_2$ values of 0.042 and 0.066 are obtained. The remaining signal 1 may be assigned to a further complex $MBH_2(1)$. A $pK_a$ value of 2.435(6) is obtained from the least-squares refinement for the second protonation of Mahx (at either N3 or N7).

Signal pairs observed for the protons H2/H7 of complexes present in the equilibrium system Mapp/$[(dien)Pd]^{2+}$ are depicted in Fig. 4. A pronounced step in the chemical shifts of these protons is observed for the protonation of Mapp at N1 (signals 5/5'). The acidity constant $pK_{a1}$ obtained from a least-squares fit chemical shift versus pH is 4.431(1), a value close to that of 4.30 reported previously [26]. The species distribution diagram (Fig. 5) may be conveniently compared to that of the Maad/$[(dien)Pd]^{2+}$ equilibrium system (Fig. 2). As a result of the increase in the $pK_{a1}$ value from 2.699(1) to 4.431(1) on going from Maad to Mapp, the pH range for the predomination of MB species is shifted to

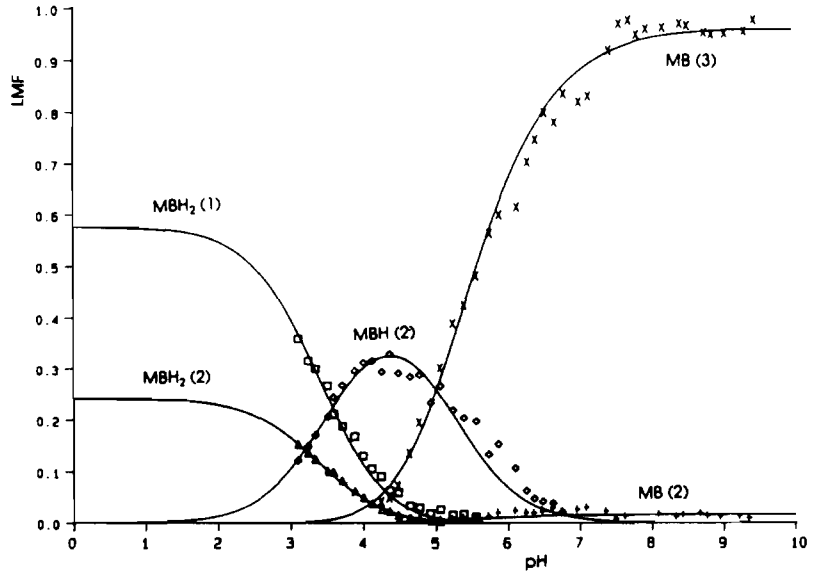


Fig. 3. Species distribution for Mahx binding by $[(dien)Pd]^{2+}$ presented as ligand mole fraction (LMF) vs. pH for Mahx and $[(dien)Pd]^{2+}$ at 0.01 M. The following formation constants log $\beta$ were determined: $MBH_2(1)$ 13.90(4), $MBH_2(2)$ 13.50(1), MBH(2) 10.31(5), MB(2) 3.8(2), MB(3) 5.27(2).

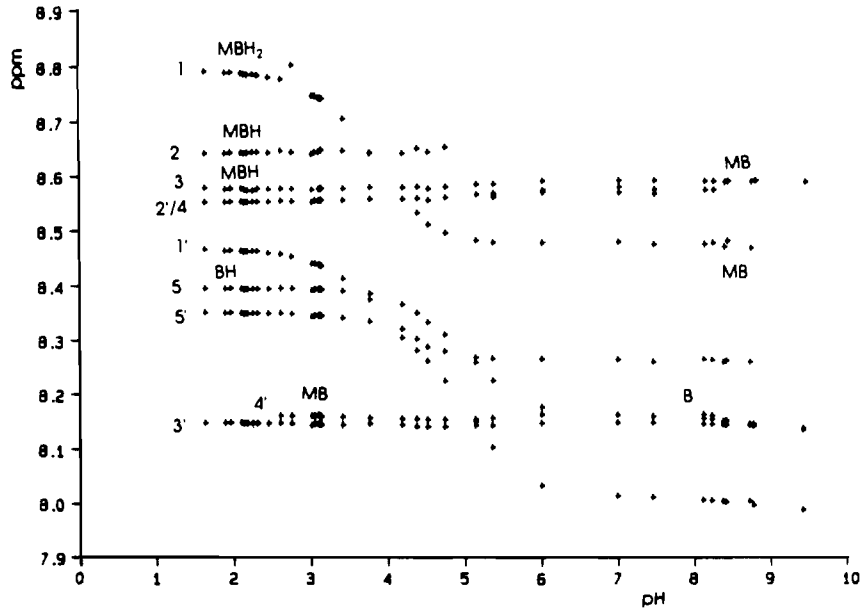


Fig. 4. Mapp H2 and H7(') chemical shifts vs. pH for an aqueous solution of Mapp (0.02 M) and $[(dien)Pd]^{2+}$ (0.02 M).

higher pH values. The coordination site for the major MB species (signal 3) may once again be assigned to N1. This site is observed in the cation $[(dien)Pt(Mapp)]^{2+}$, the structure of which was established by X-ray analysis (Fig. 1). Three different MBH complexes are observed at low pH values. Whereas both MBH(1) and MBH(3) give rise to deprotonated species MB(1) and MB(3), this is not the case for MBH(2). A definite step is observed for the chemical shifts of the signal pair 1/1' in the pH range 1.5–5.5, which suggests N1 as the protonation site of MB(1). The $[(dien)Pd]^{2+}$ coordi-

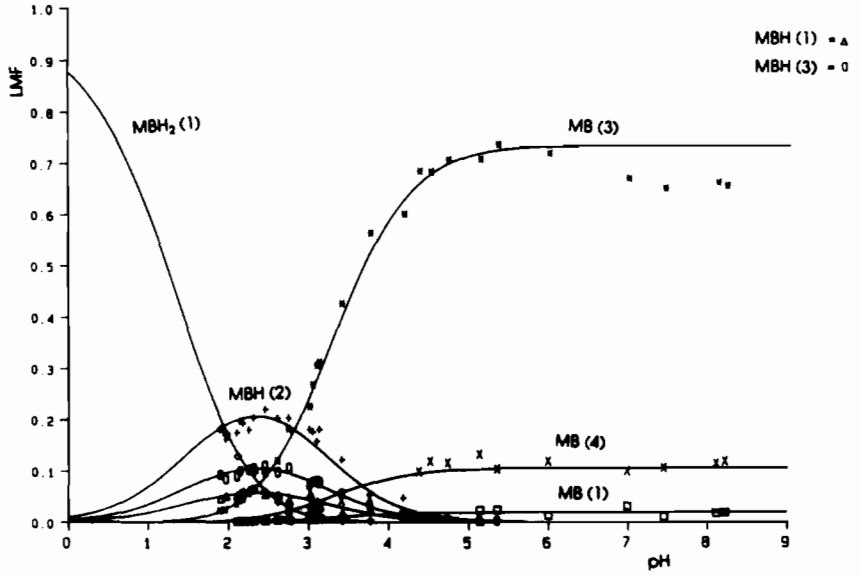


Fig. 5. Species distribution for Mapp binding by $[(dien)Pd]^{2+}$ presented as ligand mole fraction (LMF) vs. pH for Mapp and $[(dien)Pd]^{2+}$ at 0.02 M. The following formation constants log $\beta$ were determined: $MBH_2$(1) 8.01(2), MBH(1) 5.56(4), MB(1) 1.7(1), MBH(2) 6.14(6), MBH(3) 5.79(4), MB(3) 3.34(6), MB(4) 2.48(7).

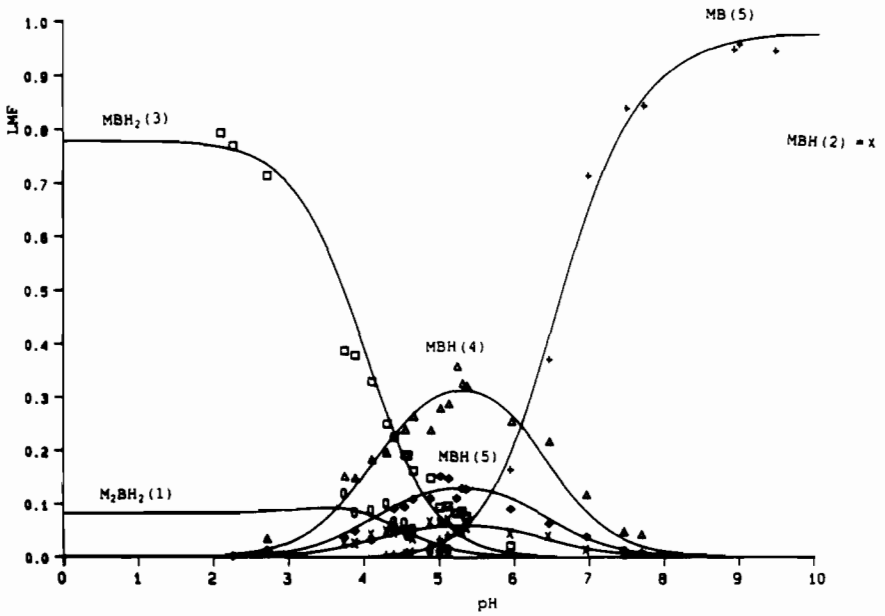


Fig. 6. Species distribution for Mall binding by $[(dien)Pd]^{2+}$ presented as ligand mole fraction (LMF) vs. pH for Mall and $[(dien)Pd]^{2+}$ at 0.02 M. The following formation constants log $\beta$ were determined: $M_2BH_2$(1) 16.96(13), MBH(2) 16.60(6), $MBH_2$(3) 15.71(12), MBH(4) 11.33(10), MBH(5) 10.92(8), MB(5) 5.2(2).

nation site may tentatively be assigned to N3 for signal 1 species MBH(1) and N8 for MBH(2). $R_1$ and $R_2$ values of 0.048 and 0.075, respectively, were obtained for the best model for the species distribution, as depicted in Fig. 5.

Five different signal pairs are observed for the protons H2/H7 of species present in the equilibrium system Mall/$[(dien)Pd]^{2+}$ for pH values between 1.3 and 10. In contrast to the other three bases studied in this work, no definite step is observed for the

base H atoms upon protonation of Mall at N1 (signals 6/6'). $R_1$ and $R_2$ values of 0.051 and 0.091, respectively, were obtained for the best model for the species distribution diagram, which is depicted in Fig. 6. Refined $pK_{a1}$ and $pK_{a8}$ values of 9.278(1) and 2.282(7) were obtained for Mall. these values may be compared with previously reported values of 9.4 and 1.8 [27]. As for Mahx, an N1 coordinated species, in this case MB(5), predominates in alkaline solution. In contrast to the analogous complex MB(3) for Mahx, MB(5) in the equilibrium system Mall/$[(dien)Pd]^{2+}$ may be protonated at N8 leading to MBH(5), which is present at considerable concentration between pH 4 and 7. Two further MBH complexes, MBH(4) and MBH(2), are observed for Mall. N8 coordination and N1 protonation may be assigned to MBH(4), which is the major species present in solution between pH 4.7 and 6.1. This coordination mode has been established by X-ray structural analysis [28] for $[RhCl(CO)_2(HMall)]$. Signals 1 and 3 belong to species $M_2BH_2$ and $MBH_2$, respectively.

Acidity constants $pK_{a1}$ and stability constant log $K_1$ for the four bases studied in this work are listed in comparison to adenosine and inosine in Table 3. The species MB with N1 coordination is predominant in neutral and alkaline solution for each of the bases. However, for both the 6-oxo-bases Mahx and Mall, the affinity for $[(dien)Pd]^{2+}$ for N1, as measured by log $K_1$, is approximately three units less than for the comparable nucleoside inosine. As a result, significant concentrations of the protonated base BH are present in acid solutions containing $[(dien)Pd]^{2+}$ for pH values between 3 and 6. The present study indicates that either N3 or N7 must be available as a secondary metal binding site for Maad and HMahx. Both N3 and N8 coordinated complexes are present in acid solutions of the 7-deaza-8-azapurines Mapp and HMall. The increase in the $pK_{a1}$ value (N1) on going from the 8-azapurines to the 7-deaza-8-azapurines leads to a shift in the pH range for the predomination of MB species to higher pH values.

## Supplementary material

Figures displaying the proton chemical shifts versus pH for aqueous solutions of $[(dien)Pd]^{2+}$ and the bases Maad (0.005 M), Mahx (0.01 M) and Mall (0.02 M) are available from the authors upon request.

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