Comparison of the Extent of Macrochelate Formation in Complexes of Divalent Metal Ions with Guanosine (GMP^{2-}), Inosine (IMP^{2-}), and Adenosine 5'-Monophosphate (AMP^{2-}). The Crucial Role of N-7 Basicity in Metal Ion–Nucleic Base Recognition

Helmut Sigel,* Salah S. Massoud,[1] and Nicolas A. Corfù

Contribution from the Institute of Inorganic Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland

Received October 18, 1993⊛

Abstract: The stability constants of the 1:1 complexes formed between Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} and IMP^{2-} or GMP^{2-} were determined by potentiometric pH titration in aqueous solution (25 °C; I = 0.1 M, $NaNO_3$) and evaluated together with the constants previously determined for the corresponding AMP^{2-} complexes. The experimental conditions were selected such that self-association of the nucleotides (NMP^{2-}) and their complexes was negligibly small; i.e., the monomeric M(NMP) and M(NMP-H) complexes were studied. On the basis of recent measurements with simple phosphate monoesters and phosphonate derivatives, $R\text{-}MP^{2-}$, where R is a noncoordinating residue (Sigel, H. et al. *Helv. Chim. Acta* **1992**, *75*, 2634), it is shown that in all the M(NMP) complexes of IMP^{2-} and GMP^{2-} a base interaction of the phosphate-coordinated metal ion occurs. The various formation degrees of the resulting M(NMP) and M(NMP-H) macrochelates are determined. To be able to relate the formation degree of the M(NMP) macrochelates with the basicity of N-7, the acidity constants of monoprotonated inosine and guanosine were also measured and the microacidity constant scheme for $H_3(IMP)^+$ was elaborated. Plots of the log stability increases determined for the M(NMP) complexes by comparison with calculated data for a sole metal ion–phosphate coordination versus the negative log of the microacidity constants of the H^+(N-7) site of the *monoprotonated* nucleosides, including adenosine, reveal that the extent of macrochelate formation is mainly determined by the basicity of N-7. However, these same plots also indicate that in the M(IMP) and M(GMP) complexes formed with Co^{2+}, Ni^{2+}, and Cd^{2+} an additional outer-sphere coordination to O-6 is likely. Macrochelation with the alkaline earth ions (formation degree <30%) is suggested to be largely of an outer-sphere type. The structures of the mentioned macrochelates, the evidence for inner-sphere versus outer-sphere binding of the metal ions, and the various intramolecular equilibria are discussed. Finally, it is emphasized that this kind of knowledge also improves our understanding of the selective recognition of metal ions by nucleic acids.

Derivatives of orthophosphoric acid, mainly as esters and anhydrides, are widely distributed in living systems.[2] They are part of the so-called phosphate–sugar backbone of DNA and RNA; in the form of nucleotides they occur as coenzymes and intermediates. The most prominent among the purine nucleotides is certainly adenosine 5'-triphosphate (ATP^{4-}) with its immense role in the transfer of biochemical energy.[3]

Though often ignored, wherever nature uses a phosphate derivative, there are also metal ions present. These are not only needed to counterbalance the negative charge of the phosphate derivative, but they also affect the structure of these derivatives in solution.[4] The best known example, probably due to the early suggestion of Szent-Györgyi,[5] is the formation of macrochelates with certain purine nucleotides by coordination of the metal ion not only to the phosphate residue but also to the nucleic base moiety.[6,7]

Indeed, e.g., for purine nucleoside 5'-monophosphates various suggestions about the formation of macrochelates[8-11] have finally led, by several methods, to the definite proof[12-15] of a direct metal ion binding to the phosphate group and also to N-7 of the purine residue. For several adenosine 5'-monophosphate (AMP^{2-}) complexes in aqueous solution this was proven by a direct comparison with tubercidin 5'-monophosphate ($TuMP^{2-}$ = 7-deaza-AMP^{2-}) complexes.[15,16] Any kind of metal ion–base backbinding or macrochelate formation must lead to an enhanced complex stability[17] compared to that expected for the phosphate group alone.[15,18] In fact, $TuMP^{2-}$, where N-7 of AMP^{2-} is replaced

⊛ Abstract published in *Advance ACS Abstracts*, March 1, 1994.

(1) Work done in Basel during a leave from the University of Alexandria, Egypt.

(2) Westheimer, F. H. *Science* **1987**, *235*, 1173-1178.

(3) Boyer, P. D. *Biochemistry* **1987**, *26*, 8503-8507.

(4) Sigel, H. *ACS Symp. Ser.* **1989**, *402*, 159-204.

(5) Szent-Györgyi, A. In *Enzymes: Units of Biological Structure and Function*; Gaebler, O. H., Ed.; Academic Press: New York, 1956; pp 393-397.

(6) (a) Martin, R. B.; Mariam, Y. H. *Met. Ions Biol. Syst.* **1979**, *8*, 57-124. (b) Martin, R. B. *Met. Ions Biol. Syst.* **1988**, *23*, 315-330.

(7) (a) Sigel, H. *Eur. J. Biochem.* **1987**, *165*, 65-72. (b) Sigel, H. *Chimia* **1987**, *41*, 11-26.

(8) (a) Sigel, H. *Experientia* **1966**, *22*, 497-499. (b) Sigel, H.; Erlenmeyer, H. *Helv. Chim. Acta* **1966**, *49*, 1266-1274. (c) Sigel, H.; Scheller, K. H. *Eur. J. Biochem.* **1984**, *138*, 291-299. (d) Massoud, S. S.; Liang, G.; Tribolet, R.; Sigel, H. *Rec. Trav. Chim. Pays-Bas* **1987**, *106*, 207.

(9) (a) Frey, C. M.; Stuehr, J. *Met. Ions Biol. Syst.* **1974**, *1*, 51-116. (b) Thomas, J. C.; Frey, C. M.; Stuehr, J. E. *Inorg. Chem.* **1980**, *19*, 505-510.

(10) (a) Taylor, R. S.; Diebler, H. *Bioinorg. Chem.* **1976**, *6*, 247-264. (b) Peguy, A.; Diebler, H. *J. Phys. Chem.* **1977**, *81*, 1355-1358. (c) Nagasawa, A.; Diebler, H. *J. Phys. Chem.* **1981**, *85*, 3523-3528. (d) Diebler, H. *J. Mol. Catal.* **1984**, *23*, 209-217.

(11) Mariam, Y. H.; Martin, R. B. *Inorg. Chim. Acta* **1979**, *35*, 23-28.

(12) (a) Reily, M. D.; Marzilli, L. G. *J. Am. Chem. Soc.* **1986**, *108*, 8299-8300. (b) Reily, M. D.; Hambley, T. W.; Marzilli, L. G. *J. Am. Chem. Soc.* **1988**, *110*, 2999-3007. (c) Alessio, E.; Xu, Y.; Cauci, S.; Mestroni, G.; Quadrifoglio, F.; Viglino, P.; Marzilli, L. G. *J. Am. Chem. Soc.* **1989**, *111*, 7068-7071. (d) Torres, L. M.; Marzilli, L. G. *J. Am. Chem. Soc.* **1991**, *113*, 4678-4679.

(13) (a) Green, M.; Miller, J. M. *J. Chem. Soc., Chem. Commun.* **1987**, 1864-1865 (correction: **1988**, 404). (b) Evans, D. J.; Green, M.; van Eldik, R. *Inorg. Chim. Acta* **1987**, *128*, 27-29. (c) Orton, D. M.; Green, M. *J. Chem. Soc., Chem. Commun.* **1991**, 1612-1614.

(14) Kuo, L. Y.; Kanatzidis, M. G.; Marks, T. J. *J. Am. Chem. Soc.* **1987**, *109*, 7207-7209.

(15) Sigel, H.; Massoud, S. S.; Tribolet, R. *J. Am. Chem. Soc.* **1988**, *110*, 6857-6865.

0002-7863/94/1516-2958$04.50/0

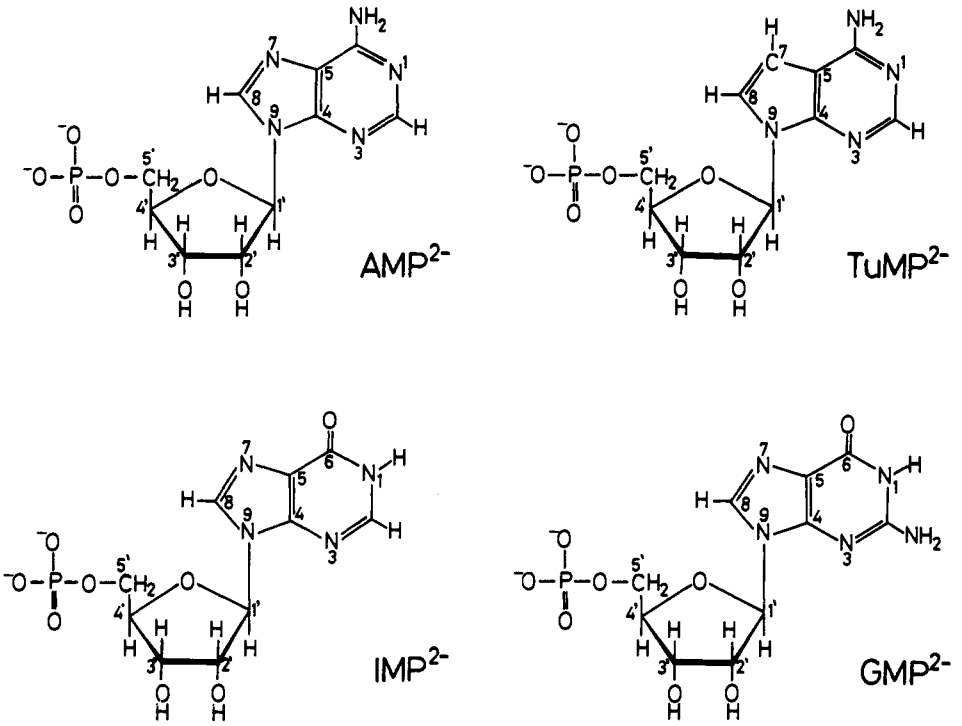


Figure 1. Chemical structure of adenosine 5′-monophosphate (AMP^{2-}), tubercidin 5′-monophosphate ($TuMP^{2-}$ = 7-deaza-AMP^{2-}), inosine 5′-monophosphate (IMP^{2-}), and guanosine 5′-monophosphate (GMP^{2-}) in their dominating anti conformation.[6a,19]

by a CH unit (see Figure 1),[6a,19] behaves, in contrast to AMP^{2-}, as a simple phosphate monoester;[15] this also confirmed that macrochelate formation with N-1 is not possible for steric reasons (see Figure 1).

These results have led to the following question: Which are the coordinating properties of the related purine nucleotides (Figure 1), i.e., of inosine 5′-monophosphate (IMP^{2-}) and guanosine 5′-monophosphate (GMP^{2-})? In contrast to the situation with AMP^{2-}, stability constants of metal ion complexes of IMP^{2-} and GMP^{2-} in aqueous solution have hardly been measured so far.[20] We had as a long-standing aim the intention to determine the stability constants of the M(IMP) and M(GMP) complexes, where M^{2+} = Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}. As indicated, the key question regarding the structure of these complexes in solution was, does macrochelate formation occur? And if so, does its extent depend on the basicity of N-7 of the purine moiety? It is evident that only an answer to this second question will facilitate an understanding of the driving forces which lead to the formation of these macrochelates.

The main obstacle for an evaluation of stability data of the indicated kind for M(AMP), M(IMP), and M(GMP) has been the fact that the basicity of N-7 in AMP^{2-} cannot directly be measured because N-1 is more basic than N-7 (Figure 1). By a complicated procedure, which involved a large number of equilibrium constants, the basicity of N-7 in *neutral* adenosine has now recently indirectly been estimated.[21] The basicity of N-7 in the hypoxanthine and guanosine moieties can be measured,[22] and thus we are finally in the position to search for a relation between N-7 basicity and the extent of macrochelate formation in the complexes of the mentioned purine nucleoside 5′-monophosphates. Such an evaluation, which of course reflects the metal ion binding properties of N-7 in these purines, is also meaningful for nucleic acids. It is well-known[23,24] that *cis*-Pt-$(NH_3)_2^{2+}$ preferably binds to the guanine and *not* to the adenine moieties of single- or double-stranded DNA. Why?

1. Experimental Section

1.1. Materials. The disodium salts of IMP^{2-} and GMP^{2-} were from Sigma Chemical Co., St. Louis, MO, and from Serva Feinbiochemica GmbH, Heidelberg, FRG. The results collected for each of the two NMPs from the two sources did not significantly differ. The aqueous stock solutions of the NMPs were freshly prepared daily, and the pH was adjusted to about 8.2 for GMP and to about 7.9 for IMP. The exact concentrations of the NMPs were determined each time.

Inosine and guanosine were from Serva Feinbiochemica GmbH, Heidelberg, Germany, and from Fluka AG, Buchs, Switzerland, respectively. All the other reagents were the same as used previously.[18] The preparation of the solutions and the determination of their exact concentrations were also carried out as before.[18]

1.2. Potentiometric pH Titrations. The pH titrations were carried out with a Metrohm E536 potentiograph, E655 dosimat, and 6.0202.100 (JC) combined single-junction macro glass electrodes. The buffer solutions (pH 4.64, 7.00, 9.00; based on the NBS scale, now U.S. National Institute of Standards and Technology (NIST)) used for calibration were also from Metrohm AG, Herisau, Switzerland. The direct pH-meter readings were used in the calculations of the acidity constants; i.e., these

(16) Abbreviations: Ado, adenosine; AMP^{2-}, ADP^{3-}, and ATP^{4-}, adenosine 5′-mono-, -di-, and -triphosphate (see also Figure 1); CMP^{2-}, cytidine 5′-monophosphate; Dien, diethylenetriamine; GMP^{2-}, GDP^{3-}, and GTP^{4-}, guanosine 5′-mono-, -di-, and -triphosphate; Guo, guanosine; *I*, ionic strength of a solution; IMP^{2-}, IDP^{3-}, and ITP^{4-}, inosine 5′-mono-, -di-, and -triphosphate; Ino, inosine; L, general ligand with an undefined charge; M^{2+}, general divalent metal ion; NMP^{2-}, nucleoside 5′-monophosphate; Ns, (uncharged) nucleoside; $R\text{-}MP^{2-}$, phosphate monoester (R may be any organic residue, e.g., phenyl or nucleosidyl; in some instances also phosphonate derivatives are included in this abbreviation); TMP^{2-}, thymidine 5′-monophosphate (=thymine 2′-deoxyribosyl 5′-monophosphate); $TuMP^{2-}$, tubercidin 5′-monophosphate (=7-deaza-AMP^{2-}); UMP^{2-}, uridine 5′-monophosphate.

(17) Martin, R. B.; Sigel, H. *Comments Inorg. Chem.* **1988**, *6*, 285–314.

(18) Massoud, S. S.; Sigel, H. *Inorg. Chem.* **1988**, *27*, 1447–1453.

(19) Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1987**, *163*, 353–363.

(20) Smith, R. M.; Martell, A. E.; Chen, Y. *Pure Appl. Chem.* **1991**, *63*, 1015–1080.

(21) Sigel, H.; Corfù, N. A.; Ji, L.-n.; Martin, R. B. *Comments Inorg. Chem.* **1992**, *13*, 35–59.

(22) Corfù, N. A.; Sigel, H. *Eur. J. Biochem.* **1991**, *199*, 659–669.

(23) (a) Lepre, C. A.; Lippard, S. J. In *Nucleic Acids and Molecular Biology*; Eckstein, F., Lilley, D. M. J., Eds.; Springer-Verlag: Berlin, Heidelberg, 1990; Vol. 4. (b) Lippard, S. J. *Pure Appl. Chem.* **1987**, *59*, 731–742. (c) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153–1181.

(24) (a) Reedijk, J. *Inorg. Chim. Acta* **1992**, *198–200*, 873–881. (b) Reedijk, J.; Fichtinger-Schepman, A. M. J.; van Oosterom, A. T.; van de Putte, P. *Struct. Bonding* **1987**, *67*, 53–89.

constants are so-called practical, mixed, or Brønsted constants.[25a] Their negative logarithms given for aqueous solutions at $I = 0.1$ M and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 log unit;[25a] this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[25,26] No conversion is necessary for the stability constants of the metal ion complexes.

It should be emphasized that the ionic product of water (K_w) and the mentioned conversion term do *not* enter into the calculations because we evaluate the *differences* in NaOH consumption between solutions with and without ligand[25] (see also below).

1.3. Determination of the Acidity Constants of $H(Ns)^+$. The acidity constants K^H_{Ns} of inosine and guanosine were determined by titrating 50 mL of aqueous 0.1 mM HNO_3 and $NaNO_3$ ($I = 0.1$ M; 25 °C) in the presence and absence of 0.9 mM nucleoside under N_2 with 1 mL of 0.1 M NaOH, and by using the differences in NaOH consumption between two such titrations for the calculations. To be able to measure $K^H_{H(Ns)}$ (and also K^H_{Ns}) of $H(Ino)^+$ and $H(Guo)^+$ the aqueous solutions were 15 mM in HNO_3 and 4.5 mM in Ns; in these cases 20-mL solutions were titrated with 2 mL of 0.2 M NaOH ($I = 0.1$ M, $NaNO_3$; 25 °C). By using less HNO_3 and more NaOH, titrations up to pH 11.7 were performed and this allowed the limiting value for $K^H_{(Ns\cdot H)}$ for the further deprotonation of $(Ino\text{-}H)^-$ and $(Guo\text{-}H)^-$ at the ribose residue to be obtained; i.e., $pK^H_{(Ns\cdot H)} > 12.0$.

The constants were calculated with a Hewlett-Packard Vectra 60PC desk computer connected with a 7475A plotter and a Brother M 1509 printer by a curve-fit procedure using a Newton–Gauss nonlinear-least-squares program within the pH range corresponding (where possible) to 3–97% of neutralization of the considered species. The results given in Section 2.3 are the averages of usually 5 independent pairs of titrations.

1.4. Determination of the Acidity Constants of $H_2(NMP)^\pm$. The acidity constants $K^H_{H(NMP)}$ of $H(IMP)^-$ and $H(GMP)^-$ were determined by titrating 50 mL of aqueous 0.54 mM HNO_3 and $NaNO_3$ ($I = 0.1$ M; 25 °C) in the presence and absence of 0.3 mM NMP^{2-} under N_2 with 1 mL of 0.03 M NaOH, and by using the differences in NaOH consumption between two such titrations for the calculations. For IMP 16 independent pairs of titrations were made and for GMP 26. These same experiments also yielded values for K^H_{IMP}, as well as for $K^H_{H_2(GMP)}$ and K^H_{GMP}.

In addition, 4 independent pairs of titrations by employing 50 mL of aqueous 0.324 mM HNO_3 and 0.3 mM IMP^{2-} using 2 mL of 0.03 M NaOH resulted in additional values for $K^H_{H(IMP)}$ and K^H_{IMP}. The titration of 15-mL solutions containing 31.7 mM HNO_3 and 6 mM IMP^{2-} with 2.5 mL of 0.2 M NaOH gave 4 values each for $K^H_{H_2(IMP)}$ and $K^H_{H(IMP)}$; two more such values were obtained from titrations of 10-mL solutions containing 47.5 mM HNO_3 and 22.5 mM IMP^{2-} with 2.5 mL of 0.2 M NaOH. Hence, the final results (see Section 2.3) for $K^H_{H_2(IMP)}$, $K^H_{H(IMP)}$, and K^H_{IMP} are the averages of 6, 26, and 20 titration pairs, respectively ($I = 0.1$ M, $NaNO_3$; 25 °C). The individual results showed no dependence on the concentration of IMP employed in the various experiments.

Similarly, another 6 values for $K^H_{H_2(GMP)}$, together with those for $K^H_{H(GMP)}$, were obtained from titration pairs based on 50 mL of 1.08 mM HNO_3 and 0.3 mM GMP^{2-} by using 2 mL of 0.03 M NaOH. From two more titration pairs with 2 mL of 0.03 M NaOH employing 50 mL of 0.324 mM HNO_3 and 0.3 mM GMP^{2-} additional values for $K^H_{H(GMP)}$ and K^H_{GMP} were determined. The final results (see Section 2.3) for $K^H_{H_2(GMP)}$, $K^H_{H(GMP)}$, and K^H_{GMP} are the averages of 27, 34, and 28 independent experiments ($I = 0.1$ M, $NaNO_3$; 25 °C). No dependence of the results on the GMP concentration was observable.

All these acidity constants were calculated as indicated in Section 1.3. The titrations for IMP and GMP were done independently by 2 and 3 persons, respectively; this also applies for the stability constants of the complexes in the following section.

1.5. Determination of the Stability Constants. The conditions for the determination of the stability constants $K^M_{M(NMP)}$ of the binary M(NMP) complexes ($I = 0.1$ M, $NaNO_3$; 25 °C) were the same as given in the first paragraph of Section 1.4 for $K^H_{H(NMP)}$ of $H(IMP)^-$ and $H(GMP)^-$ (i.e., 0.54 mM HNO_3 and 0.3 mM NMP^{2-}), except $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$. With Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} [$M(NO_3)_2$] was 0.0333 M, i.e., $NMP:M^{2+} = 1:111$; for Mg^{2+} and Ca^{2+} in addition [$M(NO_3)_2$] = 0.0267 M was used ($NMP:M^{2+} = 1:89$). For Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} [$M(NO_3)_2$] was 0.0167 M ($NMP:M^{2+} = 1:56$), for Co^{2+} and Ni^{2+} it was in addition 0.0133 M (1:44), and for Mn^{2+}, Zn^{2+}, and Cd^{2+} it was in addition 0.0083 M (1:28). For Cu^{2+} [$M(NO_3)_2$] was 3.33 and 1.67 mM, i.e., the NMP to Cu^{2+} ratios were 1:11 and 1:5.6. For each metal ion system at least 6 or 8 independent pairs of titrations were made with IMP or GMP, respectively.

The stability constants $K^M_{M(NMP)}$ were computed for each pair of titrations by taking into account the species H^+, $H_2(NMP)^\pm$, $H(NMP)^-$, NMP^{2-}, M^{2+}, and M(NMP).[27] Throughout, the data were collected (every 0.1 pH unit) from about 5% complex formation to a neutralization degree of about 85% or to the beginning of the formation of $M(NMP\text{-}H)^-$ species or of the hydrolysis of $M(aq)^{2+}$; the latter was evident from the titrations without NMP. The values calculated individually for log $K^M_{M(NMP)}$ showed no dependence on pH or on the excess amount of M^{2+}.

In those cases were $M(aq)^{2+}$ did not hydrolyze before the onset of the formation of $M(NMP\text{-}H)^-$ (i.e., for Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, and Cd^{2+}), the experimental data were also analyzed with a curve-fitting procedure[28] by taking into account in addition $M(NMP\text{-}H)^-$ and $(MNP\text{-}H)^{3-}$. In this way $K^M_{M(NMP)}$ was again determined, but this time together with the acidity constant $K^H_{M(NMP)}$ of the M(NMP) species. The values for $K^M_{M(NMP)}$ obtained in the two evaluation procedures agreed excellently. Regarding the accuracy of the values for $K^H_{M(NMP)}$ see footnote *d* in Table 2 of Section 2.5.

1.6. A Caveat. As described above, $K^M_{M(NMP)}$ was calculated by taking into account the NMP species $H_2(NMP)^\pm$, $H(NMP)^-$, NMP^{2-}, and M(NMP), and there was no dependence of the results on pH or the excess amount of M^{2+}. Hence, our experimental data provide no hint for the formation of the protonated complex $M(H\cdot NMP)^+$. However, it may well remain undetected as the concentration of this species under our conditions ($[NMP]_{tot} = 0.3$ mM) is certainly low and as its deprotonation is expected to occur in the same pH range we are evaluating for the reaction $M^{2+} + H(NMP)^- \rightleftharpoons M(NMP) + H^+$ ($pH \geq 4.0$). In fact, the stabilities of several $M(Ns)^{2+}$ complexes, where Ns = guanosine,[29,30] inosine,[10c,29,30] or adenosine,[21] were previously determined and thus the existence of $M(H\cdot NMP)^+$ with the metal ion (mainly) at N-7 has to be assumed and indeed $Ni(H\cdot IMP)^+$ was shown to occur[10c] at 15 °C and $I = 0.2$ M ($NaClO_4$).

To be able to evaluate the possible effect of the formation of $M(H\cdot NMP)^+$ on our results we estimated the stability constants log $K^M_{M(H\cdot NMP)}$ from the known log $K^M_{M(Ns)}$ versus $pK^H_{H(N\cdot 7/Ns)}$ relationships (cf. Table IV in ref 30b) by using the acidity constants of the present work (Table 1) and by adding between 0.4 and 0.6 log unit to account for the charge effect[25b] of the $-P(O)_2(OH)^-$ group (see also Table 3 in ref 31); i.e., log $K^M_{M(H\cdot NMP)}$ = log $K^M_{M(Ns)}$ + (0.4 to 0.6). Analysis of the experimental data with a curve-fitting procedure[28] by keeping the estimated values for log $K^M_{M(H\cdot NMP)}$ constant resulted in all cases also in an excellent fit but in various instances in slightly larger values for log $K^M_{M(NMP)}$ than those obtained by the procedure described in Section 1.5. These results are given in the following lines where the first value in parentheses refers to the newly calculated constant log $K^M_{M(NMP)}$ and the second to the employed estimate for log $K^M_{M(H\cdot NMP)}$: (i) M^{2+},GMP for Mn^{2+} (2.42/0.4), Co^{2+} (2.87/1.4), Ni^{2+} (3.36/1.7), Cu^{2+} (3.74/2.2), Zn^{2+} (2.76/1.1), and Cd^{2+} (3.10/1.5); (ii) M^{2+},IMP for Co^{2+} (2.68/1.1), Ni^{2+} (3.07/1.4), Cu^{2+} (3.44/1.7), Zn^{2+} (2.57/0.7), and Cd^{2+} (2.95/1.2); (iii) M^{2+},AMP for Cu^{2+} (3.16/1.3). For all the other 18 M^{2+},NMP systems not listed, like Ba^{2+},GMP or Ni^{2+},AMP, the results given in Table 2 (vide infra) remain practically unaffected by taking into account an estimate for log $K^M_{M(H\cdot NMP)}$; i.e., the differences between the newly calculated values for log $K^M_{M(NMP)}$ and the constants listed in Table 2 are ≤ 0.04 log unit.

To conclude, there is a high probability that the complexes $M(H\cdot NMP)^+$ are actually existing, but we cannot prove this with our experimental data: working with higher reactant concentrations would enhance the formation degree of the $M(H\cdot NMP)^+$ species but also create a conflict with regard to the self-association (see Section 2.1). As our experimental data from the potentiometric pH titrations can be excellently fitted without $M(H\cdot NMP)^+$, we are using the results of the calculation described in Section 1.5 and these are listed in Table 2. In this way it

(25) (a) Sigel, H.; Zuberbühler, A. D.; Yamauchi, O. *Anal. Chim. Acta* **1991**, *255*, 63–72. (b) Bastian, M.; Sigel, H. *J. Coord. Chem.* **1991**, *23*, 137–154.

(26) Irving, H. M.; Miles, M. G.; Pettit, L. D. *Anal. Chim. Acta* **1967**, *38*, 475–488.

(27) Griesser, R.; Sigel, H. *Inorg. Chem.* **1970**, *9*, 1238–1243.

(28) Sigel, H.; Griesser, R.; Prijs, B. *Z. Naturforsch. B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1972**, *27B*, 353–364.

(29) Lönnberg, H.; Vihanto, P. *Inorg. Chim. Acta* **1981**, *56*, 157–161.

(30) (a) See Table III in ref 30b. (b) Kinjo, Y.; Tribolet, R.; Corfù, N. A.; Sigel, H. *Inorg. Chem.* **1989**, *28*, 1480–1489.

(31) Sigel, H.; Chen, D.; Corfù, N. A.; Gregáň, F.; Holý, A.; Strašák, M. *Helv. Chim. Acta* **1992**, *75*, 2634–2656.

is also guaranteed that we are not overestimating the formation degree of the macrochelates (Table 4, vide infra) and thus we remain on the safe side in our interpretations. Finally, it needs to be emphasized that the overall appearance of Figure 4 is *not* affected if the above constants would be used for the plots; this would only lead to a slight vertical shift of some of the data points, especially for the IMP and GMP systems, and hence to slightly steeper slopes of some of the straight lines.

2. Results and Discussion

2.1. Some Considerations on the Self-Association of Purines. Purine derivatives undergo self-association due to stacking of their nucleic base–ring systems.[32] Indeed, the equilibrium constants according to the isodesmic model for an indefinite noncooperative self-association[32b] have been determined in aqueous solution for IMP^{2-} and GMP^{2-} as K = 1.4 and 1.3 M^{-1}, respectively.[33] Assuming K = 2 M^{-1} one calculates that in 0.3 mM solutions (Section 1.5) more than 99% of the NMP^{2-} species are present in their monomeric form. In fact, the limiting value of 99% is still obtained if K would equal 16 M^{-1} (a constant close to that measured for adenosine:[32a] K = 15 M^{-1}); this conclusion is important because metal ion coordination is known to promote the self-association of nucleotides.[32,34,35] Even in the experiments (Section 1.3) with the uncharged inosine (K = 3.3 M^{-1})[34b] or guanosine (K = 8 M^{-1})[34b] the monomeric species exceed 98%.

Only in a few of the experiments carried out for the determination of $K^{H}_{H_2(IMP)}$ (Section 1.4), with IMP concentrations of up to 22.5 mM, some self-association might play a role. However, for $H_2(IMP)^{\pm}$ this is expected not to be larger than the one determined earlier for $H(Ino)^+$/Ino (K = 1.9 M^{-1});[34b] even for this most unfavorable case still more than 92% of the species are present in their monomeric form. Hence, all the results presented below apply to monomeric species.

2.2. Definition of the Equilibrium Constants Measured. Inosine and guanosine are used below for various comparisons; therefore, their acid–base properties were measured. Both nucleosides (Ns) may accept a proton at N-7 and release one from the H(N-1) site;[6a] hence, the following two equilibria have to be considered:

$$H(Ns)^+ \rightleftharpoons Ns + H^+ \quad (1a)$$

$$K^{H}_{H(Ns)} = [Ns][H^+]/[H(Ns)^+] \quad (1b)$$

$$Ns \rightleftharpoons (Ns\text{-}H)^- + H^+ \quad (2a)$$

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

For the ribose moiety of both nucleosides $pK^{H}_{(Ns\cdot H)} > 12.0$ (Section 1.3); hence, this deprotonation was not considered further in the present study.

The nucleoside 5′-monophosphates (NMP^{2-}) shown in Figure 1 are tribasic species; they may bind two protons at the phosphate group and one at the purine moiety. In a first approximation one may conclude (see also Sections 2.3 and 2.4) that $H_3(IMP)^+$ and $H_3(GMP)^+$ release their first proton from $-P(O)(OH)_2$, the second one from H^+(N-7), and the third one again from the phosphate; a fourth proton is released in the alkaline pH range from H(N-1) (Section 2.3). These steps are expressed by the following equilibria:

$$H_3(NMP)^+ \rightleftharpoons H_2(NMP)^{\pm} + H^+ \quad (3a)$$

$$K^{H}_{H_3(NMP)} = [H_2(NMP)^{\pm}][H^+]/[H_3(NMP)^+] \quad (3b)$$

$$H_2(NMP)^{\pm} \rightleftharpoons H(NMP)^- + H^+ \quad (4a)$$

$$K^{H}_{H_2(NMP)} = [H(NMP)^-][H^+]/[H_2(NMP)^{\pm}] \quad (4b)$$

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

$$K^{H}_{H(NMP)} = [NMP^{2-}][H^+]/[H(NMP)^-] \quad (5b)$$

$$NMP^{2-} \rightleftharpoons (NMP\text{-}H)^{3-} + H^+ \quad (6a)$$

$$K^{H}_{(NMP)} = [(NMP\text{-}H)^{3-}][H^+]/[NMP^{2-}] \quad (6b)$$

For completeness it should be added that equilibria 3–5 apply also to $H_3(AMP)^+$ and $H_3(TuMP)^+$ (see Figure 1), but the second proton (eq 4) is released from the H^+(N-1) site.

The experimental data of the potentiometric pH titrations of the M^{2+}/NMP systems may be completely described by considering equilibria 4 (which actually plays only a very minor role), 5, and 7 (see also Section 1.6), as long as the evaluation is not carried into the pH range where $M(NMP\text{-}H)^-$ or hydroxo complexes form (Section 1.5).

$$M^{2+} + NMP^{2-} \rightleftharpoons M(NMP) \quad (7a)$$

$$K^{M}_{M(NMP)} = [M(NMP)]/([M^{2+}][NMP^{2-}]) \quad (7b)$$

In those systems, where deprotonation of H(N-1) in M(IMP) or M(GMP) takes place before the onset of the hydrolysis of $M(aq)^{2+}$, equilibrium 6 was also considered and then the constant for the following reaction could be determined as well (see Section 2.5).

$$M(NMP) \rightleftharpoons M(NMP\text{-}H)^- + H^+ \quad (8a)$$

$$K^{H}_{M(NMP)} = [M(NMP\text{-}H)^-][H^+]/[M(NMP)] \quad (8b)$$

2.3. The Stepwise Acidity Constants of $H_3(IMP)^+$ and $H_3(GMP)^+$ in Comparison with the Constants of Related Species. Locations of the Protons. The results obtained via potentiometric pH titrations for the various acidity constants of the four-proton donors $H_3(IMP)^+$ and $H_3(GMP)^+$ (eq 3–6) are listed in Table 1, together with the pK_a values for $H_3(AMP)^+$, $H(RibMP)^-$, $H(Ino)^+$, and $H(Guo)^+$.[15,18,19,22] The given acidity constants agree well, as far as available, with those in a recent data compilation,[20] i.e., for $H_2(GMP)^{\pm}$, $H(GMP)^-$, and $H(IMP)^-$. There is also satisfactory agreement with other listings (giving for the pK_a values only a single digit after the decimal point).[6,36]

It should be added that for the lower limit of the deprotonation of a hydroxyl group of the ribose residue in $(Ino\text{-}H)^-$ or $(Guo\text{-}H)^-$ $pK^{H}_{(Ns\cdot H)} > 12.0$ (25 °C; I = 0.1 M, $NaNO_3$) was obtained (Section 1.3). This result contrasts somewhat with a recent study[37] in which $pK^{H}_{(Ino\text{-}H)} = 11.65$ is given for 37 °C at I = 0.15 M

(32) (a) Sigel, H. *Biol. Trace Elem. Res.* **1989**, *21*, 49–59. (b) Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Prijs, B.; Sigel, H. *J. Am. Chem. Soc.* **1981**, *103*, 247–260.

(33) Neurohr, K. J.; Mantsch, H. H. *Can. J. Chem.* **1979**, *57*, 1986–1994.

(34) (a) Scheller, K. H.; Sigel, H. *J. Am. Chem. Soc.* **1983**, *105*, 5891–5900. (b) Corfù, N. A.; Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1990**, *191*, 721–735.

(35) (a) It may be emphasized that in a 1 mM solution only about 97% of adenosine (K = 15 M^{-1})[32a] exists in the monomeric form. Hence, experiments aiming for the properties of monomeric nucleotide species should not be carried out in concentrations higher than 10^{-3} M.[4,7] In fact, often even lower concentrations, e.g., with $Zn(ADP)^-$ below 0.3 mM, are required.[34a] Unfortunately, these facts are often ignored; e.g., in an NMR study[35b] of Co^{2+} adenine nucleotides concentrations between 0.06 and 0.3 M were employed rendering all the structural conclusions referring to monomeric complexes, including those for Co(AMP), invalid. (b) Leroy, J. L.; Guéron, M. *J. Am. Chem. Soc.* **1986**, *108*, 5753–5759.

(36) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32–38.

(37) Tauler, R.; Cid, J. F.; Casassas, E. *J. Inorg. Biochem.* **1990**, *39*, 277–285.

Table 1. Negative Logarithms of the Acidity Constants of the 3-Fold Protonated Nucleoside 5'-Monophosphates Considered in This Study (Figure 1) as Well as of the Related Monoprotonated Nucleosides and D-Ribose 5'-Monophosphate As Determined by Potentiometric pH Titrations in Water at 25 °C and I = 0.1 M ($NaNO_3$)[a,b]

acid	$pK^H_{H_3(NMP)}$ (eq 3)	$pK^H_{H_2(NMP)}$ or $pK^H_{H(Ns)}$ (eqs 1, 4)	$pK^H_{H(NMP)}$ or $pK^H_{H(RibMP)}$ (eq 5)	pK^H_{NMP} or pK^H_{Ns} (eqs 2, 6)
$H_3(AMP)^+$	0.4 ± 0.2[a,c]	3.84 ± 0.02[d,e]	6.21 ± 0.01[e]	
$H_3(IMP)^+$	0.45 ± 0.25[f]	1.30 ± 0.10	6.22 ± 0.01	9.02 ± 0.02
$H_3(GMP)^+$	0.3 ± 0.2[g]	2.48 ± 0.04	6.25 ± 0.02	9.49 ± 0.02
$H(RibMP)^-$			6.24 ± 0.01[h]	
$H(Ado)^+$		3.61 ± 0.03[d,i]		
$H(Ino)^+$		1.06 ± 0.15[j]		8.76 ± 0.03[k]
		1.06 ± 0.06[a,j]		
$H(Guo)^+$		2.11 ± 0.04[j]		9.22 ± 0.01[k]
		2.11 ± 0.10[a,j]		

[a] These values were determined by ^{1}H-NMR shift experiments.[19,22] [b] The range of error given with the constants is 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [c] The unrounded value that follows from the calculations in ref 19 is 0.42 ± 0.2; this value is used in the text in Section 2.4 regarding Figure 2. [d] This value refers to the deprotonation of the H^+(N-1) site of the adenine residue; all the other values in this column refer (largely/see Figure 2) to the deprotonation of a H^+(N-7) site. The micro acidity constant of the H^+(N-7) site of monoprotonated adenosine has been estimated:[21] $pk^H_{H(N\cdot7/Ado)} = -0.2 \pm 0.3$ (see also Section 2.9). Regarding the location of all the protons in the various species see also the text in Sections 2.2 to 2.4. [e] From ref 15. [f] Rounded value of the result (0.43 ± 0.24) given in Figure 2. [g] See text in Section 2.4. [h] From ref 18. [i] From ref 19. [j] In the case of $H(Ino)^+$ the value (with its error limits) determined by ^{1}H-NMR shift experiments (25 °C; I = 0.1 M, $NaNO_3$)[22] is the more reliable one whereas for $H(Guo)^+$ the value determined by potentiometric pH titrations is recommended. [k] $pK^H_{H(Ns\cdot H)} > 12.0$ (Section 1.3).

(KNO_3). Despite the somewhat different experimental conditions this literature value appears as being rather low. In any case, the limit $pK^H_{(Ns\cdot H)} > 12.0$ determined now agrees with previous data[38] for 25 °C on the deprotonation of ribose hydroxyl groups.

Comparison of the acidity constants listed in Table 1 for the $H_2(IMP)^\pm$ and $H_2(GMP)^\pm$ species with those given for the monoprotonated forms of D-ribose 5'-monophosphate and inosine or guanosine shows that the first proton in $H_2(NMP)^\pm$ is released (mainly) from H^+(N-7), the next from the $-P(O)_2(OH)^-$ group, and the last from the neutral H(N-1) site; these assignments agree with previous conclusions.[6a] Furthermore, if one assumes that the first proton from $-P(O)(OH)_2$ in $H_3(GMP)^+$ has an acidity constant similar to that of $H_3(AMP)^+$ (pK_a = 0.4) it is evident that the buffer regions of the individual deprotonation steps of $H_3(GMP)^+$ are separated by about 2 or more log units; hence, there is no significant overlap of the buffer regions and the macro acidity constants are identical with the micro acidity constants of the individual sites.

This may be different for $H_2(IMP)^\pm$, because $pK^H_{H_2(IMP)} = 1.30 \pm 0.10$ (Table 1) could well contain a contribution from the release of the first proton from $-P(O)(OH)_2$ if its pK_a is close to 0.4. Therefore, a micro acidity constant[39] for reaction 9,

$$(H\cdot IMP\cdot H)^\pm \rightleftharpoons (IMP\cdot H)^- + H^+ \qquad (9)$$

where $(H\cdot IMP\cdot H)^\pm$ represents a species that carries one proton each at N-7 and the phosphate group, is estimated based on the following reasoning: The *difference* between the pK_a values for the deprotonation of H^+(N-7) in the guanine and hypoxanthine moieties is expected to be independent of the presence of a phosphate residue and should be the same if calculated via the pK_a values of the two nucleosides, Ino and Guo, or via those of the two NMPs, though the acidity constants themselves

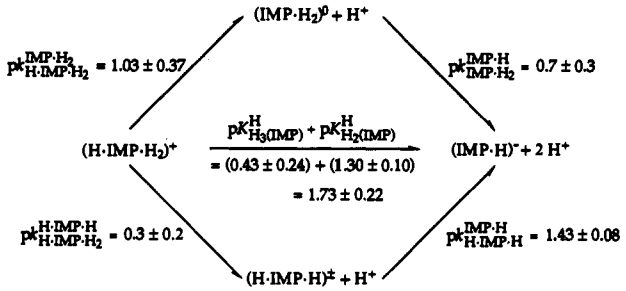


Figure 2. Equilibrium scheme defining the micro acidity constants (k) and showing their interrelation with the macro acidity constants (K) and also the interrelation between $(IMP\cdot H_2)^0$ and $(H\cdot IMP\cdot H)^\pm$ and the other species present. In $(IMP\cdot H_2)^0$ both protons are bound to the phosphate group, while in $(H\cdot IMP\cdot H)^\pm$ one proton is at the N-7 site and the other at the phosphate residue (Figure 1); $(H\cdot IMP\cdot H_2)^+$ is also often written as $H_3(IMP)^+$, it carries one proton at N-7 and the two others at the phosphate group. The arrows indicate the direction for which the acidity constants are defined. For the origin of the various constants see the text in Section 2.4.

are different of course. Hence, $\Delta pK_a = pK^H_{H(Guo)} - pK^H_{H(Ino)} = (2.11 \pm 0.04) - (1.06 \pm 0.06) = 1.05 \pm 0.07$, and therefore $pk^{IMP\cdot H}_{H\cdot IMP\cdot H} = pK^H_{H_2(GMP)} - \Delta pK_a = (2.48 \pm 0.04) - (1.05 \pm 0.07) = 1.43 \pm 0.08$; this value describes now the position of equilibrium 9. This microacidity constant is close to the macroconstant, $pK^H_{H_2(IMP)} = 1.30 \pm 0.10$ (Table 1), indicating that the contribution from the deprotonation of $-P(O)(OH)_2$ to this macroconstant is not very pronounced; but still, this result requires a more rigorous evaluation which is given in Section 2.4.

2.4. Micro Acidity Constant Scheme for $H_3(IMP)^+$ and Estimation of the Macro Acidity Constant for $H_3(GMP)^+$. Figure 2 summarizes the equilibrium scheme for $H_3(IMP)^+$ defining the microconstants (k) and giving their interrelation with the macro acidity constants (K). In principle it would be possible with the estimate given in Section 2.3 for $pk^{IMP\cdot H}_{H\cdot IMP\cdot H}$ and the values for the macroconstants to calculate values for the other microconstants in Figure 2 by following known routes.[15,39] However, as the error limits of some of the acidity constants employed are rather large we prefer in the present case to make sophisticated estimates for further equilibrium constants.

A consideration of the equilibria in Figure 2 shows that one of the steps for which a micro acidity constant is needed is for the deprotonation of $(IMP\cdot H_2)^0$, this means, for the release of the first proton from $-P(O)(OH)_2$ of an NMP which carries a neutral nucleic base residue. Such a value has previously been given for $H_2(UMP)$, i.e. $pK^H_{H_2(UMP)} = 0.7 \pm 0.3$,[18] and this value is considered as a good estimate for $pk^{IMP\cdot H}_{H\cdot IMP\cdot H}$.

In a similar manner an estimate for the macro acidity constant of $H_3(GMP)^+$ may be obtained: In the anti conformation the H^+(N-7) site of $H_3(GMP)^+$ is somewhat closer to the $-P(O)(OH)_2$ group than the H^+(N-1) site in $H_3(AMP)^+$ (see Figure 1), and therefore, the first mentioned site should have a slightly stronger acidifying effect on the $-P(O)(OH)_2$ group. Indeed, this *distance* effect is given by $\Delta\Delta pK_a = \Delta pK_{a/N\cdot7} - \Delta pK_{a/N\cdot1}$, where $\Delta pK_{a/N\cdot7} = pK^H_{H_2(GMP)} - pK^H_{H(Guo)} = (2.48 \pm 0.04) - (2.11 \pm 0.04) = 0.37 \pm 0.06$ (Table 1) and $\Delta pK_{a/N\cdot1} = pK^H_{H_2(AMP)} -$

(38) (a) Christensen, J. J.; Rytting, J. H.; Izatt, R. M. *J. Chem. Soc. B* **1970**, 1643–1646, 1646–1648. (b) Christensen, J. J.; Rytting, J. H.; Izatt, R. M. *Biochemistry* **1970**, *9*, 4907–4913.

(39) Martin, R. B. *Met. Ions Biol. Syst.* **1979**, *9*, 1–39.

(40) (a) This conclusion is confirmed by ^{1}H-NMR shift measurements of Martin et al.[40b] in D_2O at 34 °C and I = 0.5 M (KNO_3) for $D_2(GMP)^\pm$ (pK_a = 2.37 ± 0.02) and $D_2(IMP)^\pm$ (pK_a = 1.3 ± 0.1); these values are not corrected[40b] for D_2O but due to the NMR method employed they represent microconstants. Transformation with a published relation[40c] of these values to H_2O as solvent gives $pK^H_{H_2(GMP)} = pk^{GMP\cdot H}_{H\cdot GMP\cdot H} = 2.29 \pm 0.02$ and $pk^{IMP\cdot H}_{H\cdot IMP\cdot H} = 1.23 \pm 0.10$; these pK values are 0.19 and 0.20, respectively, lower than our values ($pK^H_{H_2(GMP)} = 2.48 \pm 0.04$ (Table 1); $pk^{IMP\cdot H}_{H\cdot IMP\cdot H} = 1.43 \pm 0.08$ (Figure 2)), which is in accordance with the higher temperature and larger ionic strength employed by Martin et al. However, it is most satisfying that their difference $\Delta pK_a = pK^H_{H_2(GMP)} - pk^{IMP\cdot H}_{H\cdot IMP\cdot H} = 1.06 \pm 0.10$ agrees excellently with our corresponding value, i.e. $\Delta pK_a = 1.05 \pm 0.09$. (b) Scheller, K. H.; Scheller-Krattiger, V.; Martin, R. B. *J. Am. Chem. Soc.* **1981**, *103*, 6833–6839. (c) Martin, R. B. *Science* **1963**, *139*, 1198–1203.

$pK^{H}_{H(Ado)} = (3.84 \pm 0.02) - (3.61 \pm 0.03) = 0.23 \pm 0.04$ (Table 1), and hence $\Delta\Delta pK_a = (0.37 \pm 0.06) - (0.23 \pm 0.04) = 0.14 \pm 0.07$. On the basis of the acidity constant for $H_3(AMP)^+$ one obtains $pK^{H}_{H_3(GMP)} = pK^{H}_{H_3(AMP)} - \Delta\Delta pK_a = (0.42 \pm 0.2) - (0.14 \pm 0.07) = 0.3 \pm 0.2$; this value is listed in the second column of Table 1.

However, considering that we are now having a pK_a for the release of H^+ from $-P(O)(OH)_2$, which is affected by a protonated H^+(N-7) site, we also obtained thus an estimate for the microconstant $pk^{H\cdot IMP\cdot H}_{H\cdot IMP\cdot H_2}$, quantifying the deprotonation of $(H\cdot IMP\cdot H_2)^+$, and therefore this value is also given at the left in the lower part of Figure 2.

Evidently, the sum of $pK^{H}_{H_3(IMP)} + pK^{H}_{H_2(IMP)}$ in Figure 2 can now be calculated ($=1.73 \pm 0.22$). This value in turn furnishes together with $pK^{H}_{H_2(IMP)} = 1.30 \pm 0.10$ (Table 1) a value for the macro acidity constant $pK^{H}_{H_3(IMP)}$ ($=0.43 \pm 0.24$) which quantifies the acidity of $H_3(IMP)^+$. The rounded result is also listed in the second column of Table 1.

On the basis of the mentioned sum of $pK^{H}_{H_3(IMP)} + pK^{H}_{H_2(IMP)}$ a value for $pk^{IMP\cdot H_2}_{H\cdot IMP\cdot H_2}$ (see the upper left part of Figure 2) may now also be calculated. It is most satisfying to note that $pk^{IMP\cdot H_2}_{H\cdot IMP\cdot H_2} = 1.03 \pm 0.37$ excellently agrees with $pK^{H}_{H(Ino)} = 1.06 \pm 0.06$ (Table 1); clearly, in $(H\cdot IMP\cdot H_2)^+$ and $H(Ino)^+$ deprotonation of the H^+(N-7) site of the hypoxanthine moiety occurs in both cases from an otherwise neutral species and therefore the corresponding acidity constants should be identical. This result is of a 2-fold importance: (i) It puts the basicity considerations regarding N-7 in connection with the structure of the M(NMP) complexes in solution in Section 2.9 on solid grounds. (ii) It indicates further that the micro acidity constants summarized in Figure 2 are probably more reliable than their error limits (3σ) are indicating.[40]

Finally, one may attempt to estimate the ratio R of the 2-fold protonated and isocharged species $(H\cdot IMP\cdot H)^{\pm}$ and $(IMP\cdot H_2)^0$ (Figure 2), which carry one proton at N-7 and one at the phosphate or both protons at the phosphate, respectively:

$$R = \frac{[H\cdot IMP\cdot H]}{[IMP\cdot H_2]} = \frac{k^{H\cdot IMP\cdot H}_{H\cdot IMP\cdot H_2}}{k^{IMP\cdot H_2}_{H\cdot IMP\cdot H_2}} = \frac{10^{-0.3\pm0.2}}{10^{-1.03\pm0.37}} = 10^{0.73\pm0.42} =$$

$$5.37 \pm 2.63$$

This means, the species $(H\cdot IMP\cdot H)^{\pm}$ is dominating and occurs to about 70–90%, while $(IMP\cdot H_2)^0$ forms only to about 10–30%. Certainly, this result is only an estimation, but still it proves (i) that both tautomeric forms of $H_2(IMP)$ occur simultaneously in appreciable amounts and (ii) that the $(H\cdot IMP\cdot H)^{\pm}$ species dominates and therefore largely determines $pK^{H}_{H_2(IMP)}$ as concluded already at the end of Section 2.3.

2.5. Stability and Acidity Constants of M(IMP) and M(GMP) Complexes. The results obtained from the potentiometric pH titrations (Section 1.5) for the complexes formed between the alkaline earth ions and several of the divalent metal ions of the 3d series as well as Zn^{2+} or Cd^{2+} and IMP^{2-} or GMP^{2-} according to equilibrium 7 and the stability constants determined earlier[15] for the M(AMP) complexes are listed in Table 2.[41] This allows an important conclusion already at this point: Evidently complex stability increases for all the metal ions considered in the series M(AMP) < M(IMP) < M(GMP). As for the M(AMP) complexes with Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} it has been proven previously,[15] based on stability comparisons with M(TuMP) complexes (see Figure 1), that macrochelates are indeed formed including N-7; the above series demonstrates that also in all the M(IMP) and M(GMP) complexes base backbinding of the phosphate-coordinated metal ion occurs. A more quantitative evaluation of this observation is given in Section 2.7.

(41) Massoud, S. S.; Sigel, H. *Bull. Chem. Soc. Ethiop.* **1988**, *2*, 9–14.

Table 2. Logarithms of the Stability Constants of M(AMP), M(IMP), and M(GMP) Complexes (Eq 7) Together with the Negative Logarithms of the Acidity Constants for Some of the M(IMP) and M(GMP) Complexes (Eq 8) As Determined by Potentiometric pH Titrations in Aqueous Solution at 25 °C and I = 0.1 M ($NaNO_3$)[a]

M^{2+}	$\log K^{M}_{M(AMP)}$ [b]	$\log K^{M}_{M(IMP)}$	$\log K^{M}_{M(GMP)}$	$pK^{H}_{M(IMP)}$	$pK^{H}_{M(GMP)}$
Mg^{2+}	1.60 ± 0.02	1.67 ± 0.02	1.70 ± 0.02	8.65 ± 0.05	9.02 ± 0.15
Ca^{2+}	1.46 ± 0.01	1.50 ± 0.01	1.53 ± 0.01[c]	8.62 ± 0.04	9.01 ± 0.15
Sr^{2+}	1.24 ± 0.01	1.32 ± 0.02	1.36 ± 0.02	8.61 ± 0.04	9.02 ± 0.11
Ba^{2+}	1.17 ± 0.02	1.28 ± 0.02	1.32 ± 0.02	8.61 ± 0.07	9.02 ± 0.15
Mn^{2+}	2.23 ± 0.01	2.31 ± 0.02	2.39 ± 0.02	8.21 ± 0.09	8.58 ± 0.13
Co^{2+}	2.23 ± 0.02	2.59 ± 0.01	2.72 ± 0.02	7.69 ± 0.03	8.16 ± 0.17
Ni^{2+}	2.49 ± 0.02	2.91 ± 0.03	3.13 ± 0.03	6.95 ± 0.25[d]	6.7 ± 0.4[d]
Cu^{2+}	3.14 ± 0.01	3.38 ± 0.02	3.61 ± 0.04	*e*	*e*
Zn^{2+}	2.38 ± 0.07[f]	2.54 ± 0.02	2.69 ± 0.02	*e*	*e*
Cd^{2+}	2.68 ± 0.02	2.88 ± 0.02	2.98 ± 0.02[c]	7.45 ± 0.15	7.91 ± 0.10

[a] The error limits are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger (see also Section 1.6). [b] These results are taken from Table VI in ref 15. [c] Previously also given in ref 41. [d] The acidity constants for Ni(IMP) and particularly Ni(GMP) are only estimates. Especially the result for Ni(GMP) seems to depend on the total concentration used in an experiment (see Section 1.5); this might indicate that also $Ni_2(GMP)^+$ species form to some extent where one Ni^{2+} could be coordinated to the phosphate group and the other to the N-1 deprotonated guanine residue. [e] Due to hydrolysis of $M(aq)^{2+}$ no value could be measured (see Section 1.5). [f] Regarding experimental difficulties in the determination of this value see ref 15.

Just like IMP^{2-} and GMP^{2-} also the M(IMP) and M(GMP) complexes can dissociate a proton from their H(N-1) site (see Figure 1). This deprotonation is defined in eq 8 and the appropriate acidity constants are given in columns 5 and 6 of Table 2. On the basis of these constants as well as on the acidity constants for NMP^{2-} (eq 6) and eq 10,

$$\log K^{M}_{M(NMP\text{-}H)} = \log K^{M}_{M(NMP)} + pK^{H}_{NMP} - pK^{H}_{M(NMP)} \quad (10)$$

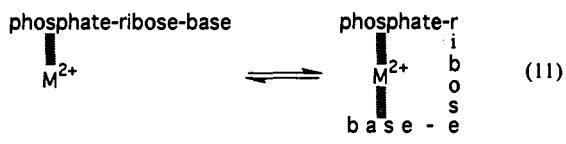
$K^{M}_{M(NMP\text{-}H)} = [M(NMP\text{-}H)^-]/([M^{2+}][(NMP\text{-}H)^{3-}])$ of the $M(IMP\text{-}H)^-$ and $M(GMP\text{-}H)^-$ complexes may be calculated; however, to do this is left to the interested reader.

So far practically none of the stabilities of the IMP and GMP complexes in Table 2 have been determined before.[20] The values given in the literature[42] for $Cu(GMP\text{-}H)^-$ and $Zn(GMP\text{-}H)^-$ were calculated without considering the formation of Cu(GMP) and Zn(GMP), which are definitely formed as the results of Table 2 show, and therefore are not acceptable. In the same study[42] values for Co(GMP) and Ni(GMP) are given, but they are 0.78 and 0.36 log unit, respectively, lower than our values listed in Table 2; the reason for this discrepancy is most probably the calculation procedure applied in ref 42.

However, the stability constant determined earlier by Nagasawa and Diebler[10c] for the Ni(IMP) complex at 15 °C and I = 0.2 M ($NaClO_4$), i.e. $\log K^{Ni}_{Ni(IMP)} = 2.96$, if transformed to the conditions of 25 °C and I = 0.1 M as given in ref 20, i.e. $\log K^{Ni}_{Ni(IMP)} = 2.91$, is identical with the value determined now (see Table 2). This is a most satisfying result, especially with regard to the criticism expressed in footnote 43. In addition, Taylor and Diebler[10a] determined by *spectrophotometry* a value for Ni(AMP), i.e. $\log K^{Ni}_{Ni(AMP)} = 2.48 \pm 0.04$, at 25 °C and I = 0.1 M ($NaClO_4$), i.e. under conditions analogous to ours ($NaNO_3$), which again is within the error limits identical with our value (2.49 ± 0.02; Table 2).

2.6. Proof of an Enhanced Stability for the M(IMP) and M(GMP) Complexes. We have already noted the increase in stability in the series M(AMP) < M(IMP) < M(GMP). Indeed, any macrochelation, as described previously for several M(AMP) complexes,[15] must be reflected in an enhanced complex stability.[15,17,31] Of course, such macrochelates will hardly form to 100%; i.e., equilibrium 11 must be considered:

(42) Reddy, P. R.; Adharani, T. K. *Indian J. Chem.* **1990**, *29A*, 1002–1007.



(11)

The formation degree of the macrochelated or "closed" species, which we designate as $M(NMP)_{cl}$, is independent of the total complex concentration because the intramolecular equilibrium constant K_I, as defined by eq 12, where $M(NMP)_{op}$ refers to the "open" species in equilibrium 11, is dimension-less:

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

Taking this into account equilibrium 7a may be rewritten as below:

$$M^{2+} + NMP^{2-} \rightleftharpoons M(NMP)_{op} \rightleftharpoons M(NMP)_{cl} \quad (13)$$

The corresponding equilibrium constant is then defined by eq 14:

$$K^M_{M(NMP)} = \frac{[M(NMP)]}{[M^{2+}][NMP^{2-}]} = \frac{([M(NMP)_{op}] + [M(NMP)_{cl}])}{[M^{2+}][NMP^{2-}]} \quad (14)$$

This expression contains as one term the stability constant of the open isomer shown in equilibrium 11, which is defined in eq 15:

$$K^M_{M(NMP)_{op}} = [M(NMP)_{op}]/([M^{2+}][NMP^{2-}]) \quad (15)$$

It is evident that any breakdown of the values for $K^M_{M(NMP)}$, which has to reflect the contribution of the various terms necessary for a further interpretation, requires that values for $K^M_{M(NMP)_{op}}$, which cannot directly be measured, are obtainable; in contrast $K^M_{M(NMP)}$ (eqs 7 and 14) is experimentally accessible. However, the existence of a linear relationship between log K^M_{ML} and pK^H_{HL} is well-known.[17] The corresponding relationship between metal ion–phosphate or –phosphonate coordination and phosphate or phosphonate group basicity was recently established by plotting log $K^M_{M(R\cdot MP)}$ versus $pK^H_{H(R\text{-}MP)}$ for a series of simple phosphate monoesters and phosphonate derivatives ($R\text{-}MP^{2-}$).[31] The parameters of the resulting straight reference lines (least squares) for the complexes of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} are summarized in Table 3. This achievement now allows the calculation of the stability constant for a pure phosphate coordination of a metal ion with the known acidity constant of any monoprotonated phosphate residue.

Table 3. Base Line Correlations for M^{2+}–Phosphate or –Phosphonate Complex Stabilities and Phosphate or Phosphonate Group Basicities (25 °C; I = 0.1 M, $NaNO_3$)[a,b]

M^{2+}	m	b	SD
Mg^{2+}	0.208 ± 0.015	0.272 ± 0.097	0.011
Ca^{2+}	0.131 ± 0.020	0.636 ± 0.131	0.016
Sr^{2+}	0.082 ± 0.016	0.732 ± 0.102	0.012
Ba^{2+}	0.087 ± 0.016	0.622 ± 0.107	0.013
Mn^{2+}	0.238 ± 0.022	0.683 ± 0.144	0.017
Co^{2+}	0.223 ± 0.026	0.554 ± 0.167	0.019
Ni^{2+}	0.245 ± 0.023	0.422 ± 0.147	0.017
Cu^{2+}	0.465 ± 0.025	–0.015 ± 0.164	0.019
Zn^{2+}	0.345 ± 0.026	–0.017 ± 0.171	0.020
Cd^{2+}	0.329 ± 0.019	0.399 ± 0.127	0.015

[a] The slopes (m) and intercepts (b) for the straight lines from plots of log $K^M_{M(R\text{-}MP)}$ versus $pK^H_{H(R\cdot MP)}$ were calculated[31] from the equilibrium constants determined earlier for six simple phosphate monoesters (4-nitrophenyl phosphate, phenyl phosphate, *n*-butyl phosphate, D-ribose 5′-monophosphate, uridine 5′-monophosphate, and thymidine 5′-monophosphate)[18] and two simple phosphonates (methyl phosphonate and ethyl phosphonate).[31] The column at the right lists the standard deviations (SD) resulting from the differences between the experimental and calculated values for the mentioned eight ligand systems.[b] [b] The above data are abstracted from Tables 5 and 6 in ref 31. Straight-line equation: $y = mx + b$, where x represents the pK_a value of any phosphate monoester or phosphonate ligand and y the calculated stability constant (log K) of the corresponding M(R-MP) complex; the errors given with m and b correspond to 1 standard deviation (1σ). The listed SD values (column at the right) times 2 or 3 are considered as reasonable error limits for any stability constant calculation in the pK_a range 5–8.

Three examples for plots of log $K^M_{M(R\text{-}MP)}$ versus $pK^H_{H(R\text{-}MP)}$ are shown in Figure 3. The data measured earlier[15] for M(TuMP) fit for all three examples on the reference line in accordance with the previous conclusion that N-7 is the site responsible for macrochelation in the M(AMP) complexes.[15] Indeed, the values for Zn(AMP) and Cu(AMP) are clearly above the reference lines. This is also true for all the M(IMP) and M(GMP) complexes with Mg^{2+}, Zn^{2+}, and Cu^{2+}. This nicely proves the enhanced complex stability of these M(NMP) species. In each case the vertical distance between the point due to a certain M(NMP) complex and the base line is a reflection of its increased stability. Exactly the same observation can be made for all the other M(IMP) and M(GMP) complexes listed in Table 2; hence, the intramolecular equilibrium 11 is truly operating.

2.7. Extent of Macrochelate Formation in Solutions of M(IMP) and M(GMP) Complexes. With the results depictured in Figure 3 in mind it is evident that values for the intramolecular equilibrium constant K_I (eq 12) have to be the aim. Indeed, combination of eqs 12, 14, and 15 leads to eq 16 which may be rearranged[15,17] to yield a further definition for K_I (eq 17) in which the stability difference log Δ is defined by eq 18:

$$K^M_{M(NMP)} = K^M_{M(NMP)_{op}} + K_I K^M_{M(NMP)_{op}} = K^M_{M(NMP)_{op}}(1 + K_I) \quad (16)$$

$$K_I = \frac{K^M_{M(NMP)}}{K^M_{M(NMP)_{op}}} - 1 = 10^{\log\Delta} - 1 \quad (17)$$

$$\log \Delta = \log \Delta_{M(NMP)} = \log K^M_{M(NMP)} - \log K^M_{M(NMP)_{op}} \quad (18)$$

The equilibrium constant K_I can now be calculated via eqs 17 and 18 as the values for $K^M_{M(NMP)}$ are known (Table 2) and those for $K^M_{M(NMP)_{op}}$ may be calculated with the acidity constants of $H(IMP)^-$ and $H(GMP)^-$ ($pK^H_{H(R\text{-}MP)}$; Table 1) and the reference line equations listed in Table 3. The vertical distances indicated by dotted lines in Figure 3 are identical with the stability differences log $\Delta_{M(NMP)}$ as defined in eq 18. Clearly, the reliability

(43) In the present context a recent IUPAC publication[20] entitled "Critical Evaluation of Stability Constants for Nucleotide Complexes with Protons and Metal Ions" has to be mentioned. This compilation is very helpful to find access to the literature regarding equilibrium constants and (in part) their connected enthalpy changes. However, great care should be exercised with regard to the advice given in this publication, i.e. the values which are *recommended* and those *not recommended*. To give just two examples: (i)... "The values of... (references) ... are tentatively recommended for Cd(CMP), for Cd(UMP), and for Cd(TMP). The value of ... (reference) ... for Cd(GMP) *is much larger than the above values* and is not recommended". Of course, the apparent discrepancy arises because in the Cd^{2+} complexes with the pyrimidine nucleoside 5′-monophosphates the metal ion coordinates only to the phosphate group (see also Figure 3 in Section 2.6), whereas in Cd(GMP) a macrochelate is formed[41] with a formation degree of 70 ± 4% (see also Table 4; Section 2.7) which is responsible for a stability increase of 0.52 ± 0.06 log units (= log $\Delta_{Cd(GMP)}$; eq 18, vide infra) compared to the expectation for a sole Cd^{2+}–phosphate group coordination. (ii) Similarly, one reads in ref 20: ... "The value ... (reference) ... for Ni(IMP) *seems high* relative to the other Ni^{2+} complexes". This criticism refers exactly to this Ni^{2+} complex mentioned above for which two groups, i.e. ref 10c and Table 2, independently determined the same stability constant. Indeed, Ni(IMP) is by 0.96 ± 0.06 log units (=log $\Delta_{Ni(IMP)}$; eq 18, vide infra) more stable than expected for a pure Ni^{2+}–phosphate coordination, which means that the formation degree of the macrochelate involving also the hypoxanthine moiety reaches 89 ± 1% (Table 4; Section 2.7). These two examples (and there are more) clearly demonstrate the most unfortunate fact that any user of the mentioned compilation[20] has to make her/his own judgement in selecting stability constants to prevent being misguided.

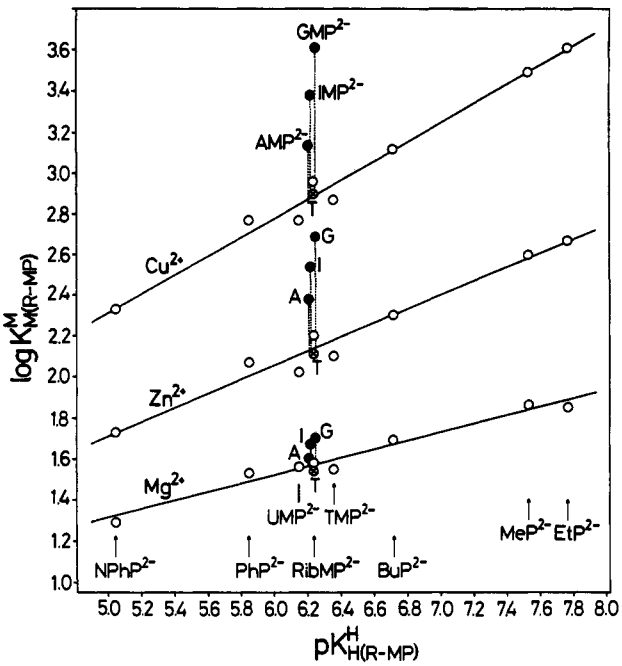


Figure 3. Evidence for an enhanced stability of several M(AMP), M(IMP), and M(GMP) complexes (●), based on the relationship between log $K^{M}_{M(R\cdot MP)}$ and $pK^{H}_{H(R\cdot MP)}$ for the 1:1 complexes of Mg^{2+}, Zn^{2+}, and Cu^{2+} with some simple phosphate monoester or phosphonate ligands ($R\text{-}MP^{2-}$): 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate (PhP^{2-}), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5′-monophosphate ($RibMP^{2-}$), thymidine 5′-monophosphate (TMP^{2-}), *n*-butyl phosphate (BuP^{2-}), methyl phosphonate (MeP^{2-}), and ethyl phosphonate (EtP^{2-}) (from left to right) (○). The least-squares lines are drawn through the corresponding eight data sets, which are taken for the phosphate monoesters from ref 18 and for the phosphonates from ref 31; the equations for these base lines are given in Table 3. The points due to the equilibrium constants for the NMP systems (●) (AMP^{2-} = A; IMP^{2-} = I; GMP^{2-} = G) are based on the data listed in Tables 1 and 2. The vertical dotted lines emphasize the stability differences to the corresponding reference lines; these differences equal log $\Delta_{M(NMP)}$ as defined in Section 2.7 by eq 18. All points for the complexes with $TuMP^{2-}$ (=T) (⊗) fall within the error limits on the reference lines; the log stability constants of the M(TuMP) complexes are plotted versus the microconstant $pk^{TuMP}_{TuMP\cdot H}$ = 6.24 and these data are taken from Table 3 and Figure 2 of ref 15, respectively. All the plotted equilibrium constant values refer to aqueous solutions at 25 °C and I = 0.1 M ($NaNO_3$).

of any calculation for K_I (eq 17) depends on the accuracy of the difference log $\Delta_{M(NMP)}$ which becomes the more important the more similar the two constants in eq 18 are. Therefore, only well-defined error limits allow a quantitative evaluation of the extent of a possibly formed macrochelate. Finally, if K_I is known, the percentage of the closed or macrochelated species occurring in equilibrium 11 follows from eq 19.

$$\%\ M(NMP)_{cl} = 100K_I/(1 + K_I) \qquad (19)$$

Application of this procedure[15,17] yields the results of Table 4. The values in column 5 for log $\Delta_{M(NMP)}$ confirm that the stability increase follows the series M(AMP) < M(IMP) < M(GMP) for all 10 metal ions studied and this is also reflected from the formation degrees for $M(NMP)_{cl}$ listed in column 7. There are substantial percentages of macrochelates formed for all the M(IMP) and M(GMP) species, including the complexes of the alkali earth ions. This latter point is remarkable and the most apparent difference to the M(AMP) complexes where if at all only traces of macrochelates are formed.

The result for Mg(AMP) is just at the limit of the accuracy of the data; without knowing the formation degrees of the closed species in Mg(IMP) and Mg(GMP) one would probably conclude that $Mg(AMP)_{cl}$ is nil. Yet, with this knowledge one is tempted to conclude that traces of $Mg(AMP)_{cl}$ exist. This would then be a result similar to that obtained for $Mg(ATP)^{2-}$ which occurs to 11 ± 6% as a macrochelate in which most probably the metal ion interacts outer-sphere, i.e. via a water molecule, with N-7.[7a,44] This latter point indicates that the structures of the $M(NMP)_{cl}$ species assembled in Table 4 need further discussion (see Sections 3.2–3.5).

It may be emphasized that the formation degree of $Ni(AMP)_{cl}$ given in Table 4 (72 ± 3%) agrees excellently with the result of Taylor and Diebler, i.e. 69%, measured under conditions corresponding to ours (25 °C; I = 0.1 M, $NaClO_4$), but via a completely different method,[10a,d] i.e. the temperature-jump relaxation technique. At lower temperature and higher ionic strength, Diebler et al. obtained for $Co(AMP)_{cl}$ 70% (8 °C; I = 0.2 M, $NaClO_4$)[10b,d] and for $Ni(IMP)_{cl}$ 94% (15 °C; I = 0.2 M, $NaClO_4$);[10c,d] due to the different conditions both formation degrees are somewhat larger than the corresponding values in Table 4, i.e. ca. 50% for $Co(AMP)_{cl}$ and 89% for $Ni(IMP)_{cl}$.

2.8. Evidence for Macrochelation in Several M(IMP-H)⁻ and M(GMP-H)⁻ Complexes. What happens to a macrochelated species $M(NMP)_{cl}$ if H(N-1) in IMP^{2-} or GMP^{2-} is deprotonated? Of course, also in this case any interaction in addition to the phosphate group coordination must be reflected in an increased stability. Therefore, it has also to be the aim here to determine the difference between the measured (overall) stability constants and the (usually) calculated constants that quantify the stability of the "open" species in equilibrium 11. This request is generally expressed in eq 20a (which is analogous to eq 18) and for the present case of the $M(NMP\text{-}H)^{-}$ complexes in eq 20b.

$$\log \Delta^{*} = \log K_{expll} - \log K_{calcd} \qquad (20a)$$

$$\log \Delta^{*}_{M(NMP\text{-}H)} = \log K^{M}_{M(NMP\cdot H)} - \log K^{M}_{M(NMP\text{-}H)_{op}} \qquad (20b)$$

Since log $K^{M}_{M(NMP\text{-}H)}$ is connected with $pK^{H}_{M(NMP)}$ (eq 8) via eq 10, log $\Delta^{*}_{M(NMP\cdot H)}$ (eq 20b) can also be expressed by eq 21a and this can easily be transformed into eq 21b, as well as into the corresponding ΔpK_a differences given in eq 21c.

$$\log \Delta^{*}_{M(NMP\cdot H)} = pK^{H}_{M(NMP)_{op}} - pK^{H}_{M(NMP)} \qquad (21a)$$

$$= (pK^{H}_{NMP} - pK^{H}_{M(NMP)}) - (pK^{H}_{NMP} - pK^{H}_{M(NMP)_{op}}) \qquad (21b)$$

$$= \Delta\, pK_a - \Delta\, pK_{a/op} \qquad (21c)$$

The experimentally based differences $pK^{H}_{NMP} - pK^{H}_{M(NMP)}$ (see eqs 6 and 8) which refer to the first term in eq 21b are listed in the fourth column of Table 5. Hence, ΔpK_a of eq 21c is defined; what is needed next is a value for $\Delta pK_{a/op}$. In other words, we would like to know to which extent the proton at H(N-1) is acidified by a divalent metal ion that solely coordinates to the phosphate group and that does not interact with the base. It is clear that even in this open form a (most probably small) electrostatic repulsion between the phosphate coordinated M^{2+} and the H(N-1) site is expected.

Such a charge effect has previously been estimated for TuMP species in which the same distance exists between the phosphate group and N-1 as in IMP and GMP (Figure 1). A corresponding estimation[25b] was made for orotidinate 5′-monophosphate, where the distance between the phosphate and carboxylate groups is also very similar to that between the phosphate and N-1 sites in purine NMPs. In both cases[15,25b] the charge effect resulted in 0.40 log unit. Hence, we consider as a good estimate with a conservative error limit for $\Delta pK_{a/op} = pK^{H}_{NMP} - pK^{H}_{M(NMP)_{op}}$ (see eq 21c and the second term in eq 21b) a value of 0.40 ± 0.10 and thus log $\Delta^{*}_{M(NMP\cdot H)}$ as defined in eq 21c can be calculated (see Table 5, column 5). Application of the results for log

(44) Sigel, H.; Tribolet, R.; Malini-Balakrishnan, R.; Martin, R. B. *Inorg. Chem.* **1987**, *26*, 2149–2157.

Table 4. Comparison of the Measured Stability, $K^{M}_{M(NMP)}$, of the M(NMP) Complexes[a] of AMP^{2-}, IMP^{2-}, and GMP^{2-} with the Calculated Stability, $K^{M}_{M(NMP)_{op}}$, for an Isomer with a Sole Phosphate Coordination of M^{2+},[b] and Extent of the Intramolecular Chelate Formation (Eq 11) in the M(NMP) Complexes in Aqueous Solution at 25 °C and I = 0.1 M ($NaNO_3$)

NMP^{2-}	M^{2+}	log $K^{M}_{M(NMP)}$ (eqs 7, 13, 14, 16)[a]	log $K^{M}_{M(NMP)_{op}}$ (eq 15)[b]	log $\Delta_{M(NMP)}$ (eq 18)[c]	K_I (eqs 12, 17)	% $M(NMP)_{cl}$ (eqs 11, 19)
AMP^{2-}	Mg^{2+}	1.60 ± 0.02	1.56 ± 0.03	0.04 ± 0.04	0.10 ± 0.09	0 (9 ± 8/<19)
	Ca^{2+}	1.46 ± 0.01	1.45 ± 0.05	0.01 ± 0.05	0.02 ± 0.12	0 (2 ± 11/<15)
	Sr^{2+}	1.24 ± 0.01	1.24 ± 0.04	0.00 ± 0.04	0.00 ± 0.09	0 (0 ± 9/<11)
	Ba^{2+}	1.17 ± 0.02	1.16 ± 0.04	0.01 ± 0.04	0.02 ± 0.11	0 (2 ± 10/<15)
	Mn^{2+}	2.23 ± 0.01	2.16 ± 0.05	0.07 ± 0.05	0.17 ± 0.14	15 ± 10
	Co^{2+}	2.23 ± 0.02	1.94 ± 0.06	0.29 ± 0.06	0.95 ± 0.28	49 ± 7
	Ni^{2+}	2.49 ± 0.02	1.94 ± 0.05	0.55 ± 0.05	2.55 ± 0.44	72 ± 3
	Cu^{2+}	3.14 ± 0.01	2.87 ± 0.06	0.27 ± 0.06	0.86 ± 0.26	46 ± 8
	Zn^{2+}	2.38 ± 0.07	2.13 ± 0.06	0.25 ± 0.09	0.78 ± 0.38	44 ± 12
	Cd^{2+}	2.86 ± 0.02	2.44 ± 0.05	0.24 ± 0.05	0.74 ± 0.22	42 ± 7
IMP^{2-}	Mg^{2+}	1.67 ± 0.02	1.57 ± 0.03	0.10 ± 0.04	0.26 ± 0.10	21 ± 7
	Ca^{2+}	1.50 ± 0.01	1.45 ± 0.05	0.05 ± 0.05	0.12 ± 0.13	11 ± 10
	Sr^{2+}	1.32 ± 0.02	1.24 ± 0.04	0.08 ± 0.04	0.20 ± 0.12	17 ± 9
	Ba^{2+}	1.28 ± 0.02	1.16 ± 0.04	0.12 ± 0.04	0.32 ± 0.14	24 ± 8
	Mn^{2+}	2.31 ± 0.02	2.16 ± 0.05	0.15 ± 0.05	0.41 ± 0.18	29 ± 9
	Co^{2+}	2.59 ± 0.01	1.94 ± 0.06	0.65 ± 0.06	3.47 ± 0.63	78 ± 3
	Ni^{2+}	2.91 ± 0.03	1.95 ± 0.05	0.96 ± 0.06	8.12 ± 1.22	89 ± 1
	Cu^{2+}	3.38 ± 0.02	2.88 ± 0.06	0.50 ± 0.06	2.16 ± 0.46	68 ± 5
	Zn^{2+}	2.54 ± 0.02	2.13 ± 0.06	0.41 ± 0.06	1.57 ± 0.37	61 ± 6
	Cd^{2+}	2.88 ± 0.02	2.45 ± 0.05	0.43 ± 0.05	1.69 ± 0.33	63 ± 5
GMP^{2-}	Mg^{2+}	1.70 ± 0.02	1.57 ± 0.03	0.13 ± 0.04	0.35 ± 0.11	26 ± 6
	Ca^{2+}	1.53 ± 0.01	1.45 ± 0.05	0.08 ± 0.05	0.20 ± 0.14	17 ± 10
	Sr^{2+}	1.36 ± 0.02	1.24 ± 0.04	0.12 ± 0.04	0.32 ± 0.14	24 ± 8
	Ba^{2+}	1.32 ± 0.02	1.17 ± 0.04	0.15 ± 0.04	0.41 ± 0.15	29 ± 7
	Mn^{2+}	2.39 ± 0.02	2.17 ± 0.05	0.22 ± 0.05	0.66 ± 0.21	40 ± 7
	Co^{2+}	2.72 ± 0.02	1.95 ± 0.06	0.77 ± 0.06	4.89 ± 0.86	83 ± 2
	Ni^{2}	3.13 ± 0.03	1.95 ± 0.05	1.18 ± 0.06	14.14 ± 2.03	93 ± 1
	Cu^{2+}	3.61 ± 0.04	2.89 ± 0.06	0.72 ± 0.07	4.25 ± 0.87	81 ± 3
	Zn^{2+}	2.69 ± 0.02	2.14 ± 0.06	0.55 ± 0.06	2.55 ± 0.52	72 ± 4
	Cd^{2+}	2.98 ± 0.02	2.46 ± 0.05	0.52 ± 0.05	2.31 ± 0.41	70 ± 4

[a] Values taken from Table 2. [b] Calculated with $pK^{H}_{H(NMP)}$ = 6.21, 6.22, and 6.25 for $H(AMP)^-$, $H(IMP)^-$, and $H(GMP)^-$ (Table 1), respectively, and the reference-line equations of Table 3; the error limits correspond to 3 times the SD values given in the column at the right in Table 3. [c] The errors given here and in the other two columns at the right were calculated according to the error propagation after Gauss by using the errors listed in the two columns to the left.

Table 5. Extent of Intramolecular Macrochelate Formation (Eq 11) in Several $M(NMP-H)^-$ Complexes of $(IMP-H)^{3-}$ and $(GMP-H)^{3-}$ As Expressed by the Dimensionless Equilibrium Constant K^{*}_{I} (Analogous to Eq 12) and the Percentage of $M(NMP-H)^-$ (Analogous to Eq 19) in Aqueous Solution at 25 °C and I = 0.1 M ($NaNO_3$)

NMP^{2-}	M^{2+} in $M(NMP-H)^-$	$pK^{H}_{M(NMP)}$ (eq 8)[a]	ΔpK_a cf. [b]	log $\Delta^{*}_{M(NMP\cdot H)}$ (eq 21c)[c]	K^{*}_{I} cf. [d]	% $M(NMP-H)_{cl}^-$ cf. [e]
IMP^{2-}	Mg^{2+}	8.65 ± 0.05	0.37 ± 0.05	−0.03 ± 0.11	0	0 (<17)
	Ca^{2+}	8.62 ± 0.04	0.40 ± 0.04	0.00 ± 0.11	0	0 (≤22)
	Sr^{2+}	8.61 ± 0.04	0.41 ± 0.04	0.01 ± 0.11	0	0 (≤24)
	Ba^{2+}	8.61 ± 0.07	0.41 ± 0.07	0.01 ± 0.12	0	0 (<26)
	Mn^{2+}	8.21 ± 0.09	0.81 ± 0.09	0.41 ± 0.13	1.57 ± 0.77	61 ± 12
	Co^{2+}	7.69 ± 0.03	1.33 ± 0.04	0.93 ± 0.11	7.51 ± 2.16	88 ± 3
	Ni^{2+}	6.95 ± 0.25	2.07 ± 0.25	1.7 ± 0.3	49.1 ± 34.6	98 ± 1
	Cd^{2+}	7.45 ± 0.15	1.57 ± 0.15	1.17 ± 0.18	13.8 ± 6.1	93 ± 3
GMP^{2-}	Mg^{2+}	9.02 ± 0.15	0.47 ± 0.15	0.07 ± 0.18	0.17 ± 0.49	0 (15 ± 35)
	Ca^{2+}	9.01 ± 0.15	0.48 ± 0.15	0.08 ± 0.18	0.20 ± 0.50	0 (17 ± 34)
	Sr^{2+}	9.02 ± 0.11	0.47 ± 0.11	0.07 ± 0.19	0.17 ± 0.51	0 (15 ± 37)
	Ba^{2+}	9.02 ± 0.15	0.47 ± 0.15	0.07 ± 0.18	0.17 ± 0.49	0 (15 ± 35)
	Mn^{2+}	8.58 ± 0.13	0.91 ± 0.13	0.51 ± 0.16	2.24 ± 1.19	69 ± 11
	Co^{2+}	8.16 ± 0.17	1.33 ± 0.17	0.93 ± 0.20	7.51 ± 3.92	88 ± 5
	Ni^{2+}	6.7 ± 0.4	2.8 ± 0.4	2.4 ± 0.4	250 ± 231	99.6 ± 0.4
	Cd^{2+}	7.91 ± 0.10	1.58 ± 0.10	1.18 ± 0.14	14.1 ± 4.9	93 ± 2

[a] Values taken from Table 2. [b] $\Delta pK_a = pK^{H}_{NMP} - pK^{H}_{M(NMP)}$ (first term in eq 21b); it was calculated with pK^{H}_{IMP} = 9.02 ± 0.02 and pK^{H}_{GMP} = 9.49 ± 0.02 (Table 1) and the data in the column to the left. The error limits (3σ) were calculated according the error propagation after Gauss; this holds also for the other columns to the right. [c] log $\Delta^{*}_{M(NMP\cdot H)} = \Delta pK_a - \Delta pK_{a/op} = \Delta pK_a - (0.40 \pm 0.10)$ (eq 21c); see also text in Section 2.8. [d] $K_I = [M(NMP-H)_{cl}^-]/[M(NMP-H)_{op}^-]$; this definition is analogous to eq 12. [e] Calculated in analogy to eq 19.

$\Delta^{*}_{M(NMP-H)}$ to the second part of eq 17 yields values for K^{*}_{I} and % $M(NMP-H)_{cl}$ (in analogy to eqs 12 and 19) which are listed in columns 6 and 7 of Table 5.

A comparison of the results in columns 5–7 of Table 5 for the M(IMP-H) and M(GMP-H) complexes with the corresponding ones for the M(NMP) species given in columns 5–7 in Table 4 provides immediately two striking observations: (i) There is no indication for a significant macrochelation in any of the M(NMP-H) complexes of the alkaline earth ions; the acidification of these ions on H(N-1) corresponds to the expected electrostatic effect. This result contrasts with that obtained for the corresponding M(NMP) complexes where "closed" species form. The given conclusion appears as unequivocal for the $M(IMP-H)^-$ species, but holds most probably also for $M(GMP-H)^-$; if at all, there are only traces of macrochelates formed in these cases. (ii) Macrochelate formation in $M(IMP-H)^-$ and $M(GMP-H)^-$ with Mn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, and Zn^{2+} is quite pronounced, i.e. it is larger than in the undeprotonated M(NMP) complexes. In addition, for a given 3d metal ion there is hardly a significant difference in base backbinding between the complexes of $(IMP-H)^{3-}$ and $(GMP-H)^{3-}$, whereas the corresponding $M(IMP)_{cl}$ and $M(GMP)_{cl}$ species form to a different extent.

Points (i) and (ii) together strongly suggest that macrochelate formation of a phosphate-coordinated M^{2+} in $M(NMP-H)^-$ occurs

to a N site of the base moiety. Clearly, the detailed structure of these species warrants further discussion (see Section 3.6).

2.9. Relation between N-7 Basicity and Extent of Macrochelate Formation in M(NMP) Complexes. Considering that in solution the anti conformation is dominating for AMP^{2-}, IMP^{2-}, and GMP^{2-} (Figure 1) there remains only N-7 for a purine backbinding. Indeed, for AMP^{2-} this has been proven, aside from various other methods,[12-14] for aqueous solutions via stability comparisons with the TuMP complexes (see Figure 3).[15]

In addition, a careful NMR study[12a,b] in D_2O for IMP^{2-} or GMP^{2-} and *cis*-$Pt(NH_3)_2^{2+}$ verified that a macrochelate is formed by simultaneous binding of Pt^{2+} to the phosphate and N-7 sites. Indeed, metal ion binding to N-7 in IMP^{2-} and GMP^{2-} complexes is also well-known from the solid state.[45-48] Hence, there can be no doubt that the macrochelate described in Section 2.7 involves this site for all three mentioned NMPs. Consequently, the extent of its formation is expected to depend on the basicity of N-7 in the corresponding purine residues, and therefore a plot of the stability increase log $\Delta_{M(NMP)}$ (eq 18) versus the pK_a of the H^+-(N-7) site in these three NMPs should result in a straight line.

The difficulty in carrying out the indicated evaluation is that the most basic site of the adenine moiety in $H(AMP)^-$ is N-1 and *not* N-7. This means, the protonation reaction of the N-7 site in the presence of an unprotonated, and hence neutral, N-1 site cannot directly be measured. However, recently we have succeeded in estimating via a complicated procedure in an indirect way the micro acidity constant for the H^+(N-7) site of *monoprotonated* adenosine:[21] $pk^H_{H(N\text{-}7/Ado)} = -0.2 \pm 0.3$. In inosine and guanosine no competition between the proton affinity of N-7 and another site exists; i.e., the measured macroconstants are identical with the corresponding microconstants: $pK^H_{H(Ino)} = pk^H_{H(N\text{-}7/Ino)} = 1.06 \pm 0.06$ and $pK^H_{H(Guo)} = pk^H_{H(N\text{-}7/Guo)} = 2.11 \pm 0.04$ (Table 1).

The given pK_a values for H^+(N-7) in the nucleosides reflect in a relative sense also the basicity of the N-7 site in AMP^{2-}, IMP^{2-} (see Section 2.4), and GMP^{2-} because in all three cases the distance between the phosphate group and N-7 is identical. Hence, one may plot, as shown in Figure 4, the stability differences log $\Delta_{M(NMP)}$ (Table 4) as defined in eq 18 for the three M(NMP) complexes of a given metal ion in dependence on the micro acidity constants, $pk^H_{H(N\text{-}7/Ns)}$, of the corresponding nucleosides.

For all ten metal ion systems studied in a first approximation within the error limits of the data straight lines are observed, unequivocally proving that the formation degree of the macrochelates (equilibrium 11) as summarized in Table 4 depends on the basicity of the N-7 site. However, various finer details inherent in Figure 4 also warrant consideration (see Sections 3.1–3.5).

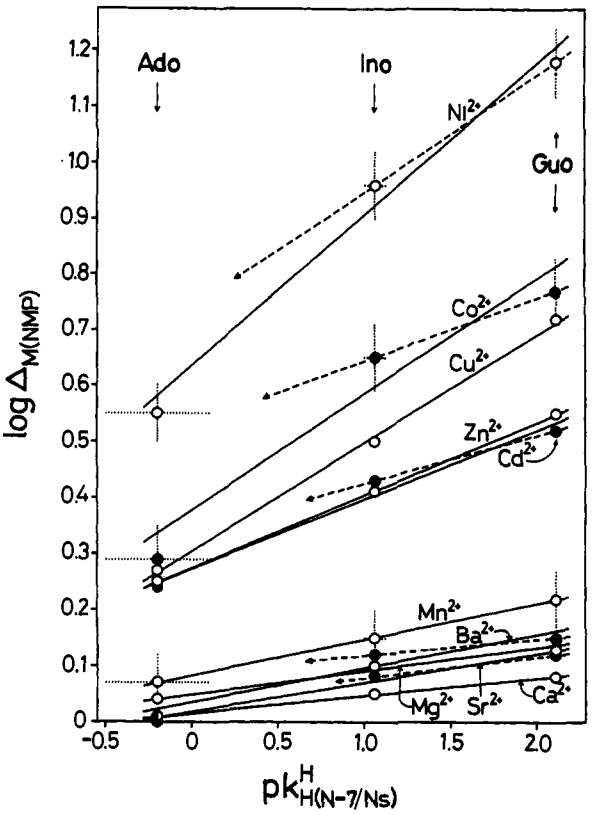


Figure 4. Relationship between log $\Delta_{M(NMP)}$ (eq 18) for the Ni^{2+} (○), Co^{2+} (●), Cu^{2+} (○), Zn^{2+} (○), Cd^{2+} (●), Mn^{2+} (○), Ba^{2+} (●, ◐), Mg^{2+} (○), Sr^{2+} (●), or Ca^{2+} (○, ◐) [from top to bottom] 1:1 complexes of AMP^{2-}, IMP^{2-}, or GMP^{2-} and $pk^H_{H(N\text{-}7/Ns)}$ of the corresponding nucleosides (Ns), adenosine (Ado), inosine (Ino), and guanosine (Guo). The values for log $\Delta_{M(NMP)}$ are from Table 4 and those for $pk^H_{H(N\text{-}7/Ns)}$ are given in the text of Section 2.9 (25 °C; I = 0.1 M, $NaNO_3$). With the data points for the Ni^{2+}, Co^{2+}, and Mn^{2+} systems also error bars (dotted lines) corresponding to the error limits (3 σ; Tables 1 and 4) are inserted. The straight broken lines for the Ni^{2+}, Co^{2+}, Cd^{2+}, Ba^{2+}, or Sr^{2+} systems are based in each case only on the two data points for Ino and Guo; their relevance is discussed in the text of Section 3.3.

3. Discussion of Further Structural Factors

3.1. Short Summary of Some Solid-State Results. From a huge amount of X-ray crystal structure data[45-47] the following observations are of relevance in this context.

The purine NMPs AMP^{2-}, IMP^{2-}, and GMP^{2-} form a family of isostructural complexes[47] with the general formula $[M(NMP)(H_2O)_5]$ with metal ions such as Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Cd^{2+}.[45-47] In all cases a square-pyramidally hydrated metal ion is bonded to N-7 of the purine residue which completes an octahedral coordination sphere around the metal ion, which is not directly bound to the phosphate group but interacts with it through the formation of two hydrogen bonds. In the cases of IMP^{2-} and GMP^{2-} with most metal ions a further hydrogen bond is formed between one of the metal ion coordinated water molecules and O-6 of the base residue. A direct five-membered N-7/O-6 chelate is normally not observed;[47-49] evidently the N-7/O-6 bite is too wide[48] to easily accommodate a metal ion [the angle (C-6)–(C-5)–(N-7) is about 132°][49] (regarding the situation with anionic purine derivatives see Section 3.6).

Direct metal ion–(N-7) interactions are also known[46,47] for Li^+, Na^+, Mg^{2+}, Ca^{2+}, and Sr^{2+} while for example in Ba(AMP)·$7H_2O$ Ba^{2+} and AMP^{2-} are interlinked only via hydrogen bonds.[50] Clearly, these observations for the solid state have to be applied with care to aqueous solutions. For an aqueous solution it is certainly not feasible to postulate a direct Na^+–(N-7) binding, while on the other hand from kinetic studies[10] of Co(AMP), Ni(AMP), and Ni(IMP) inner-sphere binding to the phosphate group *and* N-7, giving thus rise to inner-sphere macrochelates, is known to occur in agreement with various other results[12-14] including stability measurements.[15]

3.2. The Properties of the M(IMP) and M(GMP) Complexes with Cu^{2+}, Zn^{2+}, and Mn^{2+} Are in Accordance with a Pure N-7 Base Backbinding in Aqueous Solution. With the reasonings of Section 3.1 in mind a closer inspection of Figure 4 is appropriate.

(45) (a) Gellert, R. W.; Bau, R. *Met. Ions Biol. Syst.* **1979**, *8*, 1–55. (b) Terrón, A. *Comments Inorg. Chem.* **1993**, *14*, 63–88.
(46) Aoki, K. Crystal Structures of Metal Ion–Nucleotide Complexes. In *Landolt-Börnstein; Band I: Nukleinsäuren; Teilband b: Kristallographische und strukturelle Daten II*; Saenger, W., Ed.; Springer Verlag: Berlin, Heidelberg, New York, London, Paris, and Tokyo, 1989; pp 171–246.
(47) Aoki, K. Nucleosides, Nucleotides and Metal Ions. In *Bioactive Molecules; Volume 8: Metalloproteins*; Otsuka, S., Yamanaka, T., Eds.; Elsevier: Amsterdam, Oxford, New York, and Tokyo, 1988; pp 457–490.
(48) Sletten, E. In *The Purines—Theory and Experiment; The Jerusalem Symposia on Quantum Chemistry and Biochemistry*, IV; The Israel Academy of Sciences and Humanities: Jerusalem, 1972; pp 160–169.
(49) (a) Frommer, G.; Preut, H.; Lippert, B. *Inorg. Chim. Acta* **1992**, *193*, 111–117. (b) Sletten, E. In *Metal–Ligand Interactions in Organic Chemistry and Biochemistry, Part I*; Pullman, B., Goldblum, N., Eds.; D. Reidel Publ. Comp.: Dordrecht-Holland, 1977; pp 53–64.
(50) Sternglanz, H.; Subramanian, E.; Lacey, J. C., Jr.; Bugg, C. E. *Biochemistry* **1976**, *15*, 4797–4802.

Evidently the data points of the three Cu(NMP) systems *exactly* fit on the straight line proving that in this case macrochelate formation is solely governed by the basicity of N-7. Indeed, for steric reasons probably only an apical water molecule could form a hydrogen bond to O-6; if this occurs at all such an interaction would hardly contribute anything to the stability of the complex. This agrees with the (polymeric) solid-state structure[51] of $[Cu_3(GMP)_3(H_2O)_8]\cdot 4H_2O$ where the metal ion binds to N-7 and phosphate oxygens, i.e. no indirect interaction with O-6 occurs.

Similarly, the equilibrium data for the Zn(NMP) systems also yield a perfect straight line (Figure 4). This means again the size of log $\Delta_{Zn(NMP)}$ is solely determined by the basicity of N-7 in agreement with the distorted tetrahedral coordination sphere of Zn^{2+} seen[52] in $[Zn(IMP)(H_2O)]_n$ which precludes a participation of O-6.[46] In fact, the isostructural Cu^{2+} complex[46,53] has O-6 positioned at an apical Cu^{2+} distance of 3.10 Å which should be compared with the M^{2+}–(N-7) distance of 1.99 and 2.00 Å for the Zn^{2+} and Cu^{2+} complexes, respectively.[46,52,53]

The dominance of an N-7 inner-sphere interaction in the M(NMP) complexes of Cu^{2+} and Zn^{2+} is also supported by considerations based on the steepness of the slopes of the straight lines seen in Figure 4 (cf. the discussion in Section 3.4).

However, more surprising may appear the perfect straight line observed also for the Mn(NMP) complexes (especially in view of the other examples discussed below) because Mn^{2+} prefers an octahedral coordination sphere, yet the equilibrium data (Figure 4) give no hint for an (indirect) Mn^{2+}–(O-6) interaction because the stability differences between Mn(AMP), Mn(IMP), and Mn(GMP) can solely be explained by the basicity differences of the N-7 site in the various purines. In fact, these solution observations agree with an X-ray structural study[54] of $[Mn(GMP)(H_2O)_5]\cdot 3H_2O$ which shows a direct Mn^{2+}–(N-7) binding; five waters complete the octahedral coordination sphere and two of them form hydrogen bonds to phosphate oxygens. There is no hydrogen bond of one of the other Mn^{2+}-coordinated water molecules to O-6 mentioned in ref 54!

Despite this very satisfying result for the Mn(NMP) species two caveats are necessary: (i) Among the divalent 3d ions Mn^{2+} shows the smallest formation degree of the macrochelates; it varies only between about 15 and 40%, corresponding to a stability increase based on a pure phosphate coordination of 0.07–0.22 log unit (Table 4); i.e., a weak or only partially occurring Mn^{2+}–(O-6) interaction would hardly show up. (ii) As Mn^{2+} generally prefers O over N donor sites[55] one must also consider outer-sphere binding to N-7 (for further comments see Section 3.4), a species known[7a,44] to occur with $Mn(ATP)^{2-}$, and this of course would further diminish any irregular stability difference between Mn(AMP) and Mn(IMP) or Mn(GMP).

3.3. Evidence for an Additional Indirect M^{2+}–(O-6) Interaction in M(IMP) and M(GMP) with Co^{2+}, Ni^{2+}, and Cd^{2+}. The larger stability increase in log $\Delta_{M(NMP)}$ (eq 18) by about 0.28 log unit (which corresponds to a factor of 2) for Ni(AMP) compared with Cu(AMP) agrees with statistical considerations.[15] However, the corresponding comparison for Cu(IMP) and Cu(GMP) with Ni(IMP) and Ni(GMP) reveals an even larger but *identical* stability increase, i.e. of 0.46 log unit each, for the latter two complexes (Table 4); as a consequence the slope of the Ni^{2+} line in Figure 4 is steeper than that of the Cu^{2+} line. Furthermore, the three data points for the Ni(NMP) complexes therefore only poorly fit on a straight line; the same is true for the Co^{2+} complexes.

Moreover, the broken lines in Figure 4 drawn through the data points of IMP^{2-} and GMP^{2-} for the two metal ions, if extended toward the corresponding M(AMP) complexes, indicate that Ni(AMP) and Co(AMP) are "too unstable". In other words, the stability increase of Ni(IMP), Ni(GMP), Co(IMP), and Co(GMP) appears to be beyond that expected for the increased N-7 basicity; hence, a further intramolecular interaction in solution, namely a hydrogen bond formation from a metal ion bound water molecule to O-6, may exist. The same effect, though less pronounced, is also seen for Cd(IMP) and Cd(GMP).

The indicated outer-sphere interaction of Ni^{2+} with O-6 via a hydrogen bond is indeed confirmed by an ultraviolet resonance Raman study of 2′-deoxyriboguanosine 5′-monophosphate in dilute (5 mM) aqueous solutions.[56] Furthermore, the presented stability differences for aqueous solutions are in perfect agreement with solid-state studies: Co^{2+} (refs 57 and 58), Ni^{2+} (refs 58 and 59), and Cd^{2+} (ref 60) are those metal ions that show strong hydrogen bonds to O-6 in $[M(IMP)(H_2O)_5]$ and $[M(GMP)(H_2O)_5]$ units. It need not be emphasized that the M^{2+}–(N-7) interaction in solution for all these metal ions (as in the solid state) is mainly of an inner-sphere type (see also Section 3.4).

Overall it is amazing to note the close agreement between the present results for aqueous solutions and the earlier observations for the solid state. On the one hand we could find no evidence for a M^{2+}–(O-6) interaction in the IMP^{2-} and GMP^{2-} complexes of Cu^{2+}, Zn^{2+}, and Mn^{2+} (Section 3.2) in accord with X-ray structural studies,[51-54] yet on the other hand, we find such evidence (as described above) for the complexes with Co^{2+}, Ni^{2+}, and Cd^{2+}, again in agreement with crystal structure results.[46,47,57-61]

3.4. Structure of the Macrochelates Formed by the Alkaline Earth Ions in the M(NMP) Complexes Together with Some General Considerations. The interpretation of the results for the complexes with the alkaline earth ions is somewhat less straightforward because macrochelation is always small and therefore further individual, even smaller, effects are hardly detectable. Certainly, in some instances macrochelates are clearly formed (Table 4), but even in the most favorable case, i.e. for Ba(GMP) (log $\Delta_{Ba(GMP)} = 0.15 \pm 0.04$; eq 18), only to about 30% (eq 11).

For Ba(AMP) no macrochelate formation is recognizable at all in aqueous solution. This agrees with the known low affinity

(51) (a) Aoki, K.; Clark, G. R.; Orbell, J. D. *Biochim. Biophys. Acta* **1976**, *425*, 369–371. (b) Sletten, E.; Lie, B. *Acta Crystallogr.* **1976**, *B32*, 3301–3304. (c) Clark, G. R.; Orbell, J. D.; Aoki, K. *Acta Crystallogr.* **1978**, *B34*, 2119–2128.

(52) De Meester, P.; Goodgame, D. M. L.; Jones, T. J.; Skapski, A. C. *Biochim. Biophys. Acta* **1974**, *353*, 392–394.

(53) Clark, G. R.; Orbell, J. D.; Waters, J. M. *Biochim. Biophys. Acta* **1979**, *562*, 361–364.

(54) De Meester, P.; Goodgame, D. M. L.; Jones, T. J.; Skapski, A. C. *Biochem. J.* **1974**, *139*, 791–792.

(55) Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201–208.

(56) Perno, J. R.; Cwikel, D.; Spiro, T. G. *Inorg. Chem.* **1987**, *26*, 400–405.

(57) (a) De Meester, P.; Goodgame, D. M. L.; Jones, T. J.; Skapski, A. C. *C. R. Acad. Sci. Ser. C* **1974**, *C279*, 667–669. (b) Poojary, M. D.; Manohar, H. *J. Chem. Soc., Dalton Trans.* **1986**, 309–312.

(58) (a) Aoki, K. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 1260–1271. (b) Gellert, R. W.; Shiba, J. K.; Bau, R. *Biochem. Biophys. Res. Commun.* **1979**, *88*, 1449–1453.

(59) (a) De Meester, P.; Goodgame, D. M. L.; Skapski, A. C.; Smith, B. T. *Biochim. Biophys. Acta* **1974**, *340*, 113–115. (b) Clark, G. R.; Orbell, J. D. *J. Chem. Soc., Chem. Commun.* **1974**, 139–140.

(60) Aoki, K. *Acta Crystallogr.* **1976**, *B32*, 1454–1459.

(61) (a) At this point one might argue that in M(AMP) complexes an analogous hydrogen bond could be formed; this means, a hydrogen of the exocyclic amino group at C-6 could interact with an oxygen of a water molecule from the coordination sphere of the N-7 bonded metal ion and that this interaction might be comparable to the one described above involving O-6 in the M(IMP) and M(GMP) complexes. Indeed, from X-ray crystal structure studies of 9-methyladenine complexes a few such examples are known;[61b-d] however, our results clearly show (Figure 4) that in aqueous solution this interaction either does not exist at all or is too weak to give rise to an effect comparable to that described above in Section 3.3 for the hydrogen bonds involving O-6 as, e.g., in Ni(IMP). The only other way one could argue further along this line is that in the Cu(NMP) complexes of AMP, IMP, and GMP, as well as in the other examples discussed in Section 3.2, the two kinds of hydrogen bonds are of a comparable strength and that therefore fortuitously straight lines result as seen in Figure 4; we consider such an assumption as highly improbable. Though not of real relevance for the present context one may add that 6-aminopurine derivatives, coordinated via N-7 to the metal ion in the bis(acetylacetonato)(nitro)cobalt(III) unit,[61e,f] are located in crystals such that the exocyclic amino group at C-6 can form a bifurcated hydrogen bond system with two oxygen atoms of the coordinated anionic acetylacetonates. (b) Kistenmacher, T. J.; Marzilli, L. G.; Szalda, D. J. *Acta Crystallogr.* **1976**, *B32*, 186–193. (c) Sletten, E.; Ruud, M. *Acta Crystallogr.* **1975**, *B31*, 982–985. (d) Szalda, D. J.; Kistenmacher, T. J.; Marzilli, L. G. *Inorg. Chem.* **1975**, *14*, 2623–2629. (e) Sorrell, T.; Epps, L. A.; Kistenmacher, T. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1977**, *99*, 2173–2179. (f) Sorrell, T.; Epps, L. A.; Kistenmacher, T. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1978**, *100*, 5756–5763.

of Ba^{2+} for N donor sites.[55] In solid $Ba(AMP)\cdot 7H_2O$ Ba^{2+} interacts with N-7 only via a hydrogen bond;[50] in fact, all the Ba^{2+} interactions with AMP^{2-} are of an outer-sphere type in this solid.[50] In solution the stability of Ba(AMP) is solely determined by the metal ion affinity of the phosphate group. Figure 4 and Table 4 show that this changes for Ba(IMP) and Ba(GMP); i.e., the presence of O-6 is evidently crucial for the observation of a somewhat increased complex stability. Considering further that the large Ba^{2+} shows the most pronounced macrochelate formation among the M(IMP) and M(GMP) complexes, with the alkaline earth ions it appears that the entire Ba^{2+}–base interaction occurs outer-sphere.

Sr^{2+} shows exactly the same characteristics as described for Ba^{2+} (Figure 4). There is no indication for macrochelation in Sr(AMP), while in Sr(IMP) and Sr(GMP) it occurs to approximately 20% showing again the necessity of O-6. In this connection the results of an X-ray crystal structure study[62a] with $Sr(IMP)\cdot 6.5H_2O$ are of interest: there are two molecules in an asymmetric unit;[46,62a] both Sr^{2+} are heptacoordinated, one Sr^{2+} showing a hydrogen bond to O-6 with the other showing a direct N-7 and an indirect O-6 interaction. However, the bond length for Sr^{2+}–(N-7) of 2.79 Å compared with an average value of 2.57 Å for the six oxygens of the water molecules also present in the same coordination sphere indicates a very weak metal ion–N-7 interaction.

$Ca(IMP)\cdot 6.5H_2O$ contains also two molecules in an asymmetric unit and the coordination modes of the two heptacoordinated Ca^{2+} ions are remarkably similar to those described for the Sr^{2+} ions:[46,62b,c] the Ca^{2+}–(N-7) bond length amounts to 2.73 Å, a value to be compared with the average of the six Ca^{2+}–OH_2 bonds of 2.42 Å. Hence, one may conclude that the slight stability increases observed in aqueous solution for the Ca^{2+} and Sr^{2+} complexes with IMP^{2-} and GMP^{2-} ($\log \Delta_{M(NMP)} = 0.05$ to 0.12; Table 4) are mainly due to an outer-sphere interaction with O-6.

Macrochelation in Mg(AMP) is just at the limit of detection (Table 4). However, its combination with the data for Mg(IMP) and Mg(GMP) yields a perfect straight line in Figure 4; this indicates a rather strict dependence on the N-7 basicity and provides evidence that for Mg(NMP) backbinding is governed by N-7 which does of course not exclude some interaction with O-6 in Mg(IMP) and Mg(GMP).[63] As Mg^{2+} is a strongly hydrated ion, we are convinced that these base interactions are largely of an outer-sphere type. This agrees with two X-ray structure studies[64,65] of the hexanucleotide CpGpCpGpCpG, which forms a left-handed double helix (Z-DNA); from a total[64,65] of five Mg^{2+} (= 1+4) four interact with bases:[46,64,65] two $Mg(H_2O)_5^{2+}$ units form a (long) bond to N-7 of a guanine residue,[64,65] and also two hexahydrated Mg^{2+} ions each form hydrogen bonds to two O-6 sites and one N-7 site from two guanine residues, whereas an additional $Mg(OH)_6^{2+}$ is not attached to the surface of any duplex.[65] It is evident that in these solids Mg^{2+} outer-sphere interactions dominate.

To conclude, we do not think that in any of the macrochelated M(NMP) species of the alkaline earth ions a direct M^{2+}–(N-7) interaction occurs to a significant degree. This conclusion is further supported by the steepness of the slopes of the straight lines seen in Figure 4: They are close to 0.04 demonstrating a low dependence on the basicity of N-7, a property in accord with an outer-sphere interaction. In contrast, the slopes for the Cu^{2+}, Co^{2+}, and Ni^{2+} systems are between about 0.2 and 0.3 and those for the Zn^{2+} and Cd^{2+} systems are close to 0.13; these numbers reflect a rather strong dependence on N-7 basicity indicating that the inner-sphere interactions with N-7 are dominating (see also Sections 3.2 and 3.3). The slope for the Mn(NMP) complexes equals only 0.07, indicating a large portion of outer-sphere N-7 interaction as already suggested in Section 3.2.

The above conclusion regarding the outer-sphere interactions of the base moieties in the M(NMP) complexes of the alkaline earth ions disagrees with claims based on Fourier transform infrared spectroscopy in aqueous solutions of Mg(AMP) (ref 66) and Mg(GMP) (ref 67), where an inner-sphere Mg^{2+}–(N-7) interaction is proposed. However, our interpretation of the presented experimental data is in accordance with recent ^{1}H-NMR shift measurements in D_2O solutions containing GMP^{2-} as well as Mg^{2+}, Ca^{2+}, Sr^{2+}, or Ba^{2+};[68] i.e., practically no chemical shift for H-8 was observed. This is exactly what one would expect considering (i) that the NMR technique will be sensitive mainly to perturbations on the purine ring which are due to inner-sphere coordination of a metal ion and (ii) that the formation degree of the macrochelate in all these M(NMP) species is below 30% (Table 4). Moreover, ^{1}H-NMR shift experiments also provided no indication for any direct M^{2+}–(N-7) interaction in the Mg^{2+} systems with ADP^{3-}, IDP^{3-}, or GDP^{3-} (ref 34a) and ATP^{4-}, ITP^{4-}, or GTP^{4-} (ref 32b) though other evidence has led to the conclusion[7a,44] that about 10% of $Mg(ATP)^{2-}$ exist in aqueous solution in the form of a macrochelate with an *outer-sphere* Mg^{2+}–(N-7) interaction.

3.5. Summary Remarks on the Solution Structure of the Solely Metal Ion–Phosphate Coordinated Species and of the Macrochelates in the M(NMP) Complexes. The results summarized in Table 4 make it clear that in solution besides the macrochelate a purely phosphate-coordinated species also always occurs. This phosphate–metal ion binding mode has been discussed previously[18,69] and it was concluded[18] that the dominating binding mode is a monodentate, inner-sphere phosphate oxygen coordination (possibly together with a six-membered "semi-chelate" ring involving a metal ion coordinated water molecule and a hydrogen bond) which agrees with a more recent examination[70] of phosphate–metal ion interactions in the solid state: metal ions preferentially display a unidentate, out-of-plane coordination stereochemistry!

However, one has to add that in an infrared study[71] on aqueous solutions with methyl phosphate it was concluded that Cu^{2+} and Zn^{2+} bind inner-sphere to the phosphate, while for Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, and Ni^{2+} various amounts of outer-sphere species also occur; a temperature jump study[72] of Ni^{2+}–methyl phosphate indicated about 50% outer-sphere species but with a rather large error limit. Hence, we only conclude that most probably some outer-sphere phosphate-coordination also occurs in the M(NMP) complexes[73] and that the formation degree of these species strongly depends on the kind of metal ion considered. In fact, this conclusion agrees with the slopes listed in Table 3 for the $\log K^{M}_{M(R\cdot MP)}$ versus $pK^{H}_{H(R\text{-}MP)}$ plots of simple phosphate monoester and phosphonate ligands: The more the inner-sphere binding mode dominates, the more one expects a dependence on the phosphate group basicity; indeed the slopes decrease in the series

(62) (a) Brown, E. A.; Bugg, C. E. *J. Cryst. Mol. Struct.* **1980**, *10*, 19–30. (b) Brown, E. A.; Bugg, C. E. *Acta Crystallogr.* **1980**, *B36*, 2597–2604. (c) Einspahr, H.; Bugg, C. E. *Met. Ions Biol. Syst.* **1984**, *17*, 51–97.

(63) Indeed, if a straight line is drawn through the data points for Mg(AMP) and Mg(GMP) in Figure 4 the point for Mg(IMP) is then placed somewhat above this line; certainly, the corresponding difference is small but it may hint for a weak contribution of O-6.

(64) Wang, A. H.-J.; Quigley, G. J.; Kolpak, F. J.; van der Marel, G.; van der Boom, J. H.; Rich, A. *Science* **1981**, *211*, 171–176.

(65) Gessner, R. V.; Quigley, G. J.; Wang, A. H.-J.; van der Marel, G. A.; van der Boom, J. H.; Rich, A. *Biochemistry* **1985**, *24*, 237–240.

(66) Tajmir-Riahi, H.-A. *Biochim. Biophys. Acta* **1990**, *1087*, 49–54.

(67) Theophanides, T.; Polissiou, M. *Magnesium* **1986**, *5*, 221–233.

(68) Tajmir-Riahi, H.-A. *Biopolymers* **1991**, *31*, 101–108.

(69) Sigel, H. *Chem. Soc. Rev.* **1993**, *22*, 255–267.

(70) Alexander, R. S.; Kanyo, Z. F.; Chirlian, L. E.; Christianson, D. W. *J. Am. Chem. Soc.* **1990**, *112*, 933–937.

(71) Brintzinger, H. *Helv. Chim. Acta* **1965**, *48*, 47–54.

(72) Brintzinger, H.; Hammes, G. G. *Inorg. Chem.* **1966**, *5*, 1286–1287.

(73) Clearly, in an M(NMP) complex in the open form (eq 11) the metal ion will coordinate to the phosphate group in the same manner as a simple phosphate monoester. Should macrochelate formation in M(NMP) species alter the phosphate inner-sphere/outer-sphere ratio to some extent, then the percentage of the total closed form calculated for the corresponding M(NMP) complex (Table 4; of course, this holds also for the results of Table 5) would still be correct, as any alteration of the phosphate inner-sphere/outer-sphere ratio would be on account of the stability increase, i.e. $\log \Delta_{M(NMP)}$ (eq 18; Tables 4 and 5), on which the calculation for the extent of macrochelation is based.

$Cu^{2+} > Zn^{2+} \sim Cd^{2+} > Ni^{2+} \sim Mn^{2+} \sim Co^{2+} \gtrsim Mg^{2+} > Ca^{2+} > Sr^{2+} \sim Ba^{2+}$. Most probably there is a large degree of inner-sphere phosphate complexation with the divalent 3d ions and Zn^{2+} or Cd^{2+} while for the alkaline earth ions, especially Sr^{2+} and Ba^{2+}, the outer-sphere coordination may dominate.

Considering the structures of the macrochelates in the M(IMP) and M(GMP) complexes there are the following six possibilities, aside from those which also apply to M(AMP) species:[15] (i) The metal ion coordinates inner-sphere to the phosphate group, to N-7, and also to O-6. (ii) It coordinates outer-sphere to the phosphate group and inner-sphere to both N-7 and O-6. (iii) Binding is inner-sphere to the phosphate group and also to N-7 but outer-sphere, i.e. via a water molecule, to O-6. (iv) Coordination is inner-sphere to the phosphate group and outer-sphere to both N-7 and O-6.[74,75] (v) The metal ion coordinates outer-sphere to the phosphate group and also to O-6 but inner-sphere to N-7. (vi) All sites, i.e. the phosphate group, N-7, and O-6, are outer-sphere bound.

Structures (i) and (ii) are considered as being too strained (see Sections 3.1 and 3.6) and therefore as being of negligible importance. Structure (iii) with the inner-sphere coordination of the phosphate group and of N-7 and an outer-sphere O-6 interaction is considered as the dominating species for the divalent 3d ions, as well as for Zn^{2+} and Cd^{2+} (see Sections 3.2 and 3.3, and the kinetic results[10c,d] for Ni(IMP)); certainly, structures (iv) and (v) may occur in equilibrium in these cases also to some extent. Structure (iv) is considered as being important for the Mg(NMP) species with possibly (low) amounts of structure (vi) in equilibrium. In other words, structure (vi) with all sites being outer-sphere bound will play an increasing role from Mg^{2+} via Ca^{2+} and Sr^{2+} to Ba^{2+}, for which it is dominating. Finally, in certain instances, e.g. with Cu^{2+}, Zn^{2+}, and Mn^{2+} (Section 3.2), the discussed structures have also to be viewed *without* the outer-sphere interaction of O-6. In fact, this latter situation has previously been discussed in detail for the M(AMP) complexes; cf. ref 15.

3.6. Solution Structure of the Macrochelates Formed with the M(NMP-H) Species. How is the structure of the M(NMP) macrochelates affected by deprotonation of the H(N-1) site? Some help in finding an answer for this question may be gained by noting that in the Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, or Cd^{2+} complexes formed with xanthosinate, where N-1 is deprotonated, the coordination of M^{2+} still occurs to a significant extent at N-7, though the N-1 bound isomer also exists in equilibrium.[30b] The question then is, does a metal ion bound to N-7 also interact with O-6, which is expected to carry part of the negative charge created upon deprotonation of H(N-1)? Indeed, some evidence for O-6 participation in metal ion coordination follows from a ^{13}C-NMR study[76] of guanosine and inosine under basic conditions in dimethyl sulfoxide as solvent.

However, the answer to the above question is still not trivial[48] due to the large N-7/O-6 bite [the (N-7)–(C-5)–(C-6) angle is about 132°].[49,77] For example, the crystal structure analysis[77] of *trans*-tetraaquabis(xanthosinato)zinc(II) dihydrate shows a direct coordination of Zn^{2+} to N-7 but an outer-sphere interaction, i.e. hydrogen bond formation via a metal ion bound water molecule, to O-6. In other words, this structure corresponds to that described in preceding sections for the metal ion interaction of hypoxanthine and guanine moieties with an un-ionized H(N-1) site.

That the above observation for $Zn(xanthosinate)_2$ has to do with the wide N-7/O-6 bite is evident from crystal structures of complexes of pterin derivatives[78] and of the anionic 8-hydroxyquinoline,[79] where N,O chelation occurs. Here the bite between the N and O donor sites is smaller because the angle around the carbon (in the position analogous to that of C-5 in the purines, but now between two six-membered rings) is close to 120° and this allows the formation of unstrained five-membered rings. On the other hand, that in a purine system a (N-7)–(O-6) chelation can be enforced is evident from complexes of Pt^{IV} or Ti^{III} with the anion of theophylline (1,3-dimethyl-2,6-dioxopurine).[80] Especially with titanocene, $(\eta^5\text{-}C_5H_5)_2Ti^+$,[80b] the two remaining sites in the approximate tetrahedron of the Ti sphere exhibit a comparable bond length; i.e., Ti–(N-7) is 2.211 Å and Ti–(O-6) 2.278 Å. However, the N-7/O-6 bite imposes an (O-6)–Ti–(N-7) angle of 79.6° and thus the Ti–(N-7) bond lies at a 25° angle from the expected N-7 lone-pair direction [Ti–(N-7)–(C-5) = 104.6° and Ti–(N-7)–(C-8) = 154.2°] and this reduces the (N-7)–(C-5)–(C-6) angle from 131.6° to 125.2°.[80b] Of further interest in this context are Cu^{2+} complexes also formed with the anion of theophylline or derivatives thereof:[81] In the case with the shortest Cu^{2+}–(O-6) distance[81b] N-7 is equatorially coordinated with a bond length of 2.00 Å, while O-6 occupies an apical position with a distance of 2.83 Å. The (N-7)–(C-5)–(C-6) angle is reduced here[81b] to 129° (compared with the normally observed 132°),[49,77,82] a value very close to those measured in Cd^{2+} complexes of neutral and anionic 6-mercaptopurine,[83] but still somewhat larger than that found (126°) in [(9-methyl-6-thiopurine)$CuCl_2$]·H_2O.[49b,84]

Considering these observations and the preferred anti conformation of IMP^{2-} and GMP^{2-} (Figure 1) we believe that in the $M(IMP\text{-}H)^-$ and $M(GMP\text{-}H)^-$ macrochelates formed with Mn^{2+}, Co^{2+}, Ni^{2+}, and Cd^{2+} (see Table 5) in aqueous solution the metal ion is bound to N-7 and also to O-6 probably with an equilibrium involving outer-sphere and inner-sphere binding of O-6. In fact, the isomer with inner-sphere binding to both sites is possibly important which would also explain the apparent lack of macrochelated $M(NMP\text{-}H)^-$ species of the alkaline earth ions (Table 5). Their affinity for N sites is low and upon deprotonation solvation of the O-6/N-1 site certainly becomes more pronounced and hence the M^{2+} ions cannot compete effectively for binding. Finally, one should add that space-filling molecular models indicate that inner-sphere binding of N-7 *and* O-6 requests outer-sphere binding of the phosphate group in the formation of the macrochelates; otherwise the general points summarized in Section 3.6 for the M(NMP) complexes also hold here for the $M(NMP\text{-}H)^-$ species.

4. General Conclusions

The present study demonstrates the impressive ambivalent properties of IMP^{2-} and GMP^{2-} in interactions with metal ions. Thus, intramolecular equilibria between isomers are an inherent property of their complexes in solution. These complexes should never be viewed as being of a static, rigid structure! The energy differences, $\Delta G°$, between isomers (see the log $\Delta_{M(NMP)}$ values in Tables 4 and 5) are in many instances on the order of about

(74) Outer-sphere binding, i.e. hydrogen bond formation, is well-known not only for O-6 (cf.[45-47]) but also for N-7; regarding the latter site see, e.g., refs 46, 50, and 75.

(75) (a) Poojary, M. D.; Begum, N. S.; Manohar, H.; Bau, R. *J. Chem. Soc., Chem. Commun.* **1985**, 821–822. (b) Begum, N. S.; Poojary, M. D.; Manohar, H. *J. Chem. Soc., Dalton Trans.* **1989**, 1507–1512. (c) Farmer, P. J.; Cave, J. R.; Fletcher, T. M.; Rhubottom, J. A., Jr.; Walmsley, J. A. *Inorg. Chem.* **1991**, *30*, 3414–3420.

(76) Marzilli, L. G.; de Castro, B.; Solorzano, C. *J. Am. Chem. Soc.* **1982**, *104*, 461–466.

(77) Quirós, M.; Salas, J. M.; Sánchez, M. P.; Alabart, J. R.; Faure, R. *Inorg. Chem.* **1991**, *30*, 2916–2921.

(78) (a) Kohzuma, T.; Masuda, H.; Yamauchi, O. *J. Am. Chem. Soc.* **1989**, *111*, 3431–3433. (b) Burgmayer, S. J. N.; Stiefel, E. I. *Inorg. Chem.* **1988**, *27*, 4059–4065. (c) Fischer, B.; Strähle, J.; Viscontini, M. *Helv. Chim. Acta* **1991**, *74*, 1544–1554.

(79) Yamanouchi, K.; Huneke, J. T.; Enemark, J. H.; Taylor, R. D.; Spence, J. T. *Acta Crystallogr.* **1979**, *B35*, 2326–2330.

(80) (a) Lorberth, J.; El-Essawi, M.; Massa, W.; Labib, L. *Angew. Chem.* **1988**, *100*, 1194–1195; *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1160–1161. (b) Cozak, D.; Mardhy, A.; Olivier, M. J.; Beauchamp, A. L. *Inorg. Chem.* **1986**, *25*, 2600–2606.

(81) (a) Szalda, D. J.; Kistenmacher, T. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1976**, *98*, 8371–8377. (b) Colacio, E.; Suárez-Varela, J.; Dominguez-Vera, J. M.; Avila-Rosón, J. C.; Hidalgo, M. A.; Martín-Ramos, D. *Inorg. Chim. Acta* **1992**, *202*, 219–224.

(82) Nonella, M.; Hänggi, G.; Dubler, E. *J. Mol. Struct. (Theochem.)* **1993**, *279*, 173–190.

(83) Dubler, E.; Gyr, E. *Inorg. Chem.* **1988**, *27*, 1466–1473.

(84) Sletten, E.; Apeland, A. *Acta Crystallogr.* **1975**, *B31*, 2019–2022.

1 kJ/mol or even below,[4,17] allowing an easy transformation from one isomer into the other, in case a certain isomer should be consumed in a chemical or biochemical reaction.

For AMP, IMP, and GMP the metal ion recognition of the N-7 site depends strongly on its basicity (Figure 4). This result is of a general nature and applies also to polynucleotides and nucleic acids. For example, for all ten metal ions studied here the affinity for metal ion binding, be it inner-sphere or outer-sphere, is always larger for N-7 of the hypoxanthine residue compared with N-7 of the adenine residue. The basicity difference between the two sites amounts to about 1.3 log units (see Section 2.9) and the apparent stability constants for the Ni^{2+} complexes of poly(A) and poly(I) are log K = 3.92 (ref 85) and 4.30 (ref 85a), respectively; hence, with a slope of 0.3 (see Figure 4) for the log K versus pK_a plot the observed stability difference is excellently explained.

To make a more general application, we assume a log K versus pK_a slope of about 0.3–0.5 for a given metal ion and consider the basicity of N-7 in the adenine residue versus that in the guanine residue which differs by $\Delta pK_a \simeq 2.3$ (see Section 2.9); hence, this leads to a discrimination by a factor of approximately 10 for this metal ion, i.e. the affinity of the guanine N-7 is about 10 times larger than that of the adenine N-7. Amazingly, this discrimination ratio is close to that observed[86] for Pt(ethylenediamine)$^{2+}$ in its affinity toward N-7 in the guanine and adenine residues of DNA after an incubation time of 16 h (which may mean that the thermodynamic equilibrium is not yet completely reached due to the kinetic inertness of Pt^{2+});[86] similarly, platinum coordination to GpG is favored over that to ApG by a factor of 9.[87] It should also be noted in this context[88] that the reactivity of $Pd(R_4en)(H_2O)_2^{2+}$, where R_4en = N-substituted ethylenediamine (R = H, CH_3, CH_3CH_2), toward NMPs decreases within the series GMP > IMP > AMP.[88a] Hence, the above approximation suggests that the preferred binding of the *cis*-$Pt(NH_3)_2^{2+}$ drug to the N-7 of guanine over that of adenine in DNA is largely a basicity effect. It may be added that base pairing renders N-1 of adenine and N-3 of cytosine less accessible for Pt^{2+} binding, while it leaves the N-7 of guanine and adenine exposed in the major groove.[23a] Furthermore, the high affinity of the N-7 of guanine in DNA does not only hold for Pt^{2+},[23,24,89] but—not surprising because basicity is crucial—is also true for other, especially labile, metal ions,[69] like Mn^{2+} (ref 90a), Co^{2+} (ref 90b), Cu^{2+} (ref 90b), or Zn^{2+} (ref 90a,c). That Mg^{2+} interacts with double-stranded poly(dG-dC) electrostatically as a mobile outer-sphere "cloud" whereas Ni^{2+} coordinates to more than one binding site at the polynucleotide, presumably to guanine–(N-7) and a phosphate group,[91] is also in line with the expectations based on the present study.

The order of NMPs in terms of their nucleophilicity toward *cis*- or *trans*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ or *cis*-$[Pt(NH_3)_2Cl_2]$ is GMP > AMP ≫ CMP ≫ UMP.[92a] This order agrees with that observed for *cis*- or *trans*-$[Pt(NH_3)_2Cl_2]$ as well as for $[Pt(NH_3)_3Cl]^+$ with deoxynucleosides, i.e. deoxyguanosine > deoxyadenosine > deoxycytidine ≫ thymidine.[92b] However, the suggestion[92a] that the order "shows no relation to the basicity for protons and is, in fact, the inverse of this order" is not valid; N-7 of the guanine residue is by $\Delta pK_a \simeq 2.3$ more basic than N-7 of the adenine residue (Section 2.9) and the access to N-3 in the cytosine residue is sterically considerably inhibited by the neighboring *o*-amino group;[93] clearly, the uracyl or thymine residues become accessible for metal ions only after deprotonation of H(N-3); hence, the order of the given series is in accordance with the basicity properties of their members. Indeed, of special interest in this context are results of Martin et al.[40b] obtained for the micro stability constants for N-7 coordination in the Pd(Dien)(NMP) complexes with AMP^{2-}, IMP^{2-}, and GMP^{2-}; a plot of log $k^{Pd(Dien)}_{Pd(Dien)(N\text{-}7/NMP)}$ versus $pk^{H}_{H(N\text{-}7/Ns)}$ (i.e. analogous to Figure 4) yields a straight line with a slope close to 0.8 proving that the $Pd(Dien)^{2+}$ binding to N-7 is also governed by its basicity in the various nucleobase residues.[94]

To conclude, studies of the present kind improve our understanding of not only the coordination chemistry of nucleotides but also—and maybe even more desirable—of single- and double-stranded nucleic acids.

Acknowledgment. The competent technical assistance of Ms. Rita Baumbusch, as well as the performance of some of the potentiometric pH titrations by her and by Mr. O. Kauk during his advanced laboratory course in Inorganic Chemistry, the permission for a leave of absence of S.S.M. from the University of Alexandria, Egypt, the helpful suggestions and/or provisions of relevant literature by Professors and Drs. Hartmut Diebler, Erich Dubler, Berthold Fischer, Stephen J. Lippard, R. Bruce Martin, Luigi G. Marzilli, Jan Reedijk, Einar Sletten, and Bin Song, the support toward the costs of the nucleotides by the Stiftung der Portlandcementfabrik Laufen (H.S.), and a research grant from the Swiss National Science Foundation (H.S.) are gratefully acknowledged.

(85) (a) Diebler, H.; Secco, F.; Venturini, M. *Biophys. Chem.* **1987**, *26*, 193–205. (b) Dhathathreyan, K. S.; Diebler, H. *Ber. Bunsenges. Phys. Chem.* **1991**, *95*, 880–886.

(86) Eastman, A. *Pharmacol. Ther.* **1987**, *34*, 155–166.

(87) Laoui, A.; Kozelka, J.; Chottard, J.-C. *Inorg. Chem.* **1988**, *27*, 2751–2753.

(88) (a) Hohmann, H.; Hellquist, B.; van Eldik, R. *Inorg. Chem.* **1992**, *31*, 1090–1095. (b) Suvachittanont, S.; Hohmann, H.; van Eldik, R.; Reedijk, J. *Inorg. Chem.* **1993**, *32*, 4544–4548.

(89) (a) Yohannes, P. G.; Zon, G.; Doetsch, P. W.; Marzilli, L. G. *J. Am. Chem. Soc.* **1993**, *115*, 5105–5110. (b) Hambley, T. W. *Comments Inorg. Chem.* **1992**, *14*, 1–26. (c) Pullman, A.; Zakrzewska, C.; Perahia, D. *Int. J. Quantum Chem.* **1979**, *16*, 395–403.

(90) (a) Frøystein, N, A.; Davis, J. T.; Reid, B. R.; Sletten, E. *Acta Chem. Scand.* **1993**, *47*, 649–657. (b) Gao, Y.-G.; Sriram, M.; Wang, A. H.-J. *Nucleic Acids Res.* **1993**, *21*, 4093–4101. (c) Jia, X.; Zon, G.; Marzilli, L. G. *Inorg. Chem.* **1991**, *30*, 228–239.

(91) Schoenknecht, T.; Diebler, H. *J. Inorg. Biochem.* **1993**, *50*, 283–298.

(92) (a) Mansy, S.; Chu, G. Y. H.; Duncan, R. E.; Tobias, R. S. *J. Am. Chem. Soc.* **1978**, *100*, 607–616. (b) Inagaki, K.; Tamaoki, N.; Kidani, Y. *Inorg. Chim. Acta* **1980**, *46*, L93–L95.

(93) (a) Ji, L.-n.; Corfù, N. A.; Sigel, H. *J. Chem. Dalton Trans.* **1991**, 1367–1375. (b) Kinjo, Y.; Ji, L.-n.; Corfù, N. A.; Sigel, H. *Inorg. Chem.* **1992**, *31*, 5588–5596.

(94) (a) Though it should be pointed out that the labile complexes studied in the present work may not be as susceptible to steric effects as inert species, one may also mention here the observation[12d] that $Rh^{III}(tren)(H_2O)_2^{3+}$ [tren = tris(aminoethyl)amine] readily reacts in aqueous solution at 60 °C with guanosine and inosine but not with adenosine (1:1 ratio; 0.056 M). These differences in reactivity were explained[12d] ... "by the nature of the tren-nucleobase interactions, which are unfavorable and repulsive between tren and the exocyclic 6-NH_2 group of Ado but favorable for H-bonding interactions between tren and the 6-oxo group of Guo and Ino". Certainly, hydrogen bond formation with O-6 may have an influence (Section 3.3) but the basicity results presented now (Table 1) indicate as the main reason for the mentioned observation the fact that the N-7 sites of Ino and Guo are by factors of 25 ($\Delta pK_a \simeq 1.3$; Section 2.9) and 250 ($\Delta pK_a \simeq 2.3$), respectively, more basic than that of Ado. Of further interest is that $Rh(tren)(H_2O)_2^{3+}$ reacts with 5′-AMP^{2-} to form an (N-7)–inner-sphere and a (phosphate)–outer-sphere macrochelate[12d] (see also point (v) in Section 3.5 as well as the final paragraph in the same section); this observation confirms the stability-promoting charge effect of the phosphate group on metal-ion binding to N-7 (see Table 3 in ref 31 and also Section 1.6) as well as the known[94b,c] directing effects of 2′-, 3′-, and 5′-phosphate groups regarding nucleobase interactions.[12d,94b,c] The analogous outer-sphere macrochelate is also formed with 5′-GMP^{2-}; both complexes transform to (N-7)–(phosphate)–inner-sphere macrochelates with half-lives of about 24 and 5 h, respectively. The origin of the different reactivities toward inner-sphere macrochelate formation is not clear; we suggest that the different charge densities on the N-7 nitrogens of the adenine and guanine residues, to which Rh^{3+} is bound, lead to a somewhat different labilization of the remaining $Rh(H_2O)^{3+}$ site in the outer-sphere macrochelates. (b) Massoud, S. S.; Sigel, H. *Eur. J. Biochem.* **1989**, *179*, 451–458. (c) Massoud, S. S.; Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1990**, *187*, 387–393.