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Inosylyl(3′→5′)inosine (IpI$^-$). Acid–Base and Metal Ion-Binding Properties of a Dinucleoside Monophosphate in Aqueous Solution

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The acidity constants of the (N7)H$^+$ sites of inosylyl(3′→5′)inosine (IpI$^-$) were estimated and those of its (N1)H sites were measured by potentiometric pH titrations in aqueous solution (25 °C; I = 0.1 M, $NaNO_3$). The same method was used for the determination of the stability constants of the 1:1 complexes formed between Mg^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, or Cd^{2+} (= M^{2+}) and (IpI − H)$^{2-}$ and, in the case of Mg^{2+}, also of (IpI − 2H)$^{3-}$. The stability constants of the M(IpI)$^+$ complexes were estimated. The acidity constants of H(inosine)$^+$ and the stability constants of the M(Ino)$^{2+}$ and M(Ino − H)$^+$ complexes were taken from the literature. The comparison of these and related data allows the conclusion that, in the M(IpI − H) species, chelates are formed; most likely they are preferably of an N7/N7 type. For the metal ions Co^{2+}, Ni^{2+}, Zn^{2+}, or Cd^{2+}, the formation degrees of the chelates are on the order of 60−80%; no chelates could be detected for the Mg(IpI − H) complexes. It is noteworthy that the (N1)H deprotonation, which leads to the M(IpI − H) species, occurs in all M(IpI)$^+$ complexes in the physiological pH range of about 7.5 or even below.

1. Introduction

The nucleobase hypoxanthine is a minority base in RNAs, and for this reason, the dinucleoside monophosphate inosylyl(3′→5′)inosine (IpI$^-$; Figure 1; see section 5 at the end of the paper for all of the abbreviations used herein) has received early attention, and it was concluded that some intramolecular stacking between the purine residues occurs.[1] That both hypoxanthine residues have about the same properties in IpI$^-$ was concluded from the identical rates of isotopic hydrogen exchange at the C8 position.[2] The same position is sensitive toward radical attack, as was shown with guanosine-containing dinucleoside monophosphates.[3] A further observation that demonstrates the close relationship between guanine and hypoxanthine is the inhibition of a natural guanine-responsive riboswitch that also binds hypoxanthine and thus terminates transcription.[4]

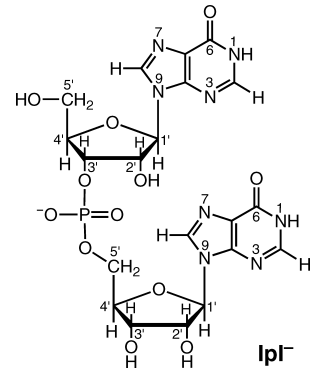

Figure 1. Chemical structure of the dinucleoside monophosphate inosylyl(3′→5′)inosine (IpI$^-$) considered in this study. The two inosine units are shown in their predominant *anti* conformation.

The fact that cisplatin cross-links in the form of *cis*-$(NH_3)_2Pt^{2+}$ two adjacent guanines via their N7 sites in DNA

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and thus acts as an antitumor agent[5–7] has led to early studies of dinucleoside monophosphates including GpG$^-$ and IpI$^-$.[8] In both instances, N7 cross-linking occurs.[8] Indeed, there is now evidence that this type of chelate formation causes a distortion of the DNA double helix that subsequently leads to apoptosis.[5–7]

The only study of IpI$^-$ that involves kinetically labile metal ions, La^{3+}, Pb^{2+}, and Zn^{2+}, was aimed toward the design of artificial ribonucleases.[9] The cleavage rates decrease in the pH range around 7 in the order UpU > ApA > IpI. This result was interpreted in the sense that metal ion-nucleobase binding is counterproductive with regard to the cleavage reaction.[9] Indeed, at pH 7 the metal ion affinity of a uracil residue[10] is much lower than that of a hypoxanthine residue.[11–14] Moreover, the given interpretation means further that the metal ion affinity of the singly negatively charged phosphate diester bridge is smaller than that of certain purine nucleobases, a conclusion in accord with experimental observations.[15,16] Overall, binding of labile metal ions to nucleic acids and their constituents is a delicate matter.[17–19]

Considering the prominent role that the hypoxanthine residue plays in the metabolism of purine nucleotides,[20] it is astonishing to find that no quantitative data on the metal ion-binding properties of IpI$^-$ are available.[21] Therefore, we have extended our previous experience with guanine and hypoxanthine residues[22–24] and studied the acid–base and metal ion-binding properties of IpI$^-$ and compared these as far as possible with those of inosine[11,12] and GpG$^-$.[15] The metal ions considered are Mg^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}.

2. Experimental Section

2.1. Materials. Nitric acid (HNO_3); disodium ethylenediamine-N,N,N′,N′-tetraacetate dihydrate ($Na_2H_2EDTA \cdot 2H_2O$); potassium hydrogen phthalate; the nitrate salts of Na^+, Mg^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} (all *pro analysi*), and sodium hydroxide (NaOH) and the pH 9.97 (for 25 °C) buffer solution (both Titrisol) were purchased from Merck AG, Darmstadt, Germany. The other buffer solutions (pH 4.00, 7.00, and 9.00) were from Metrohm AG, Herisau, Switzerland. All buffer solutions used are traceable to standard reference materials of the U.S. National Institute of Science and Technology.

All of the solutions used for the pH titrations were prepared using deionized, ultra-pure (Milli-Q185 Plus; Millipore S.A., Molsheim, France) CO_2-free water; aqueous stock solutions of IpI (see below) were freshly prepared daily. The concentrations of the NaOH solutions were determined using potassium hydrogen phthalate; those of the stock solutions of divalent metal ions were determined by potentiometric pH titrations via their EDTA complexes.

2.2. Synthesis of Inosylyl(3′→5′)inosine. The sodium salt of IpI$^-$ was synthesized by the *in-solution* phosphoramidite methodology[25] without base protection, but by using the *tert*-butyldimethylsilyl group for protection of the 2′-OH function.

The 5′-*O*-(dimethoxytrityl)-2′-*O*-(*t*-butyldimethylsilyl)inosine 3′-*O*-(β-cyanoethyldiisopropylamino)phosphoramidite was prepared in three steps from inosine by following the procedure given by Green et al.;[26] the compound was purified by silicagel column chromatography. The product was obtained as a white powder in an overall yield of 18%. ^{31}P NMR (Bruker Avance, 200 MHz; CH_2Cl_2/C_6D_6): $\delta = 150.78$ and 149.10 ppm (ref 26: $\delta = 153.35$ and 151.18 ppm).

The 2′,3′-di-*O*,*O*-benzoylinosine was prepared in three steps from inosine as described by Charubala and Pfleiderer[27] and purified by silicagel column chromatography. The product was obtained as a white crystalline powder in an overall yield of 77%. FAB-MS (Finnigan MAT 95, negative ions): *m*/*z* 474.7 (calculated *m*/*z* 476.42).

The nucleoside amidite was reacted with dibenzoylinosine under strictly anhydrous conditions in the presence of 1*H*-tetrazole (1:1.2:1 molar ratio) in a CH_2Cl_2 solution to give, after I_2/pyridine/H_2O oxidation of the intermediate phosphite, the fully protected dinucleotide. This protected product was purified by silicagel chromatography. The dimethoxytrityl group was removed by a 1 h treatment with 80% acetic acid at room temperature, whereas the base-labile substituents were removed by a 6 h storage at 55 °C in 35% aqueous ammonia diluted with ethanol (3:1, v/v).[28] The 2′-O function was finally deprotected with a 1 M tetrahydrofuran solution of tetrabutylammonium fluoride at room temperature for 12 h. The crude product was purified by ion exchange chromatography on a DEAE Sephadex A-25 (elution with a linear gradient of triethylammonium bicarbonate from 0.05 to 0.5 M). The purified dinucleoside monophosphate was then transformed into its sodium salt by passing its aqueous solution through a Dowex 50Wx8 (Na^+ form)

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column; after lyophilization, a white solid was obtained in 24% yield (based on the protected nucleoside amidite). The structure of IpI^- was confirmed by spectroscopic methods: ^{31}P NMR (D_2O) $\delta = -0.11$ ppm. FAB-MS (negative ions): *m/z* 597.3 (calculated MW 598.42 for the free acid). The compound gave a single peak on analytical RP-HPLC.

2.3. Potentiometric pH Titrations. The pH titrations were performed on a Metrohm E536 potentiograph connected to a Metrohm E665 dosimat and a Metrohm 6.0253.100 Aquatrode Plus combined macro-glass electrode. The instrument was calibrated using the buffers mentioned above. The acidity constants determined at $I = 0.1$ M ($NaNO_3$) and 25 °C are so-called practical, mixed, or Brønsted constants, which may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values.[29] The ionic product of water (K_W) and the mentioned conversion term do not enter into our calculations since the difference in NaOH consumption between solutions with and without the ligand are evaluated.[29,30] The stability constants presented are, as usual, concentration constants (for more details, see also ref 15).

2.4. Determination of the Acidity Constants. The acidity constants K^H_{IpI} and $K^H_{(IpI-H)}$ of IpI^- (eqs 3 and 4) were determined by titrating aqueous solutions (30 mL) of HNO_3 (0.05 mM; 25 °C; $I = 0.1$ M, $NaNO_3$) under N_2 with NaOH (2.0 mL, 0.02 M) in the presence and absence of IpI^-. The employed concentrations of IpI^- varied between 0.12 and 0.22 mM. The experimental data were evaluated by using a curve-fitting procedure with a Newton-Gauss nonlinear least-squares program; the difference in NaOH consumptions between the two mentioned titrations, that is, with and without the ligand, at every 0.1 pH unit was used.[15,16] The acidity constants of IpI^- were calculated in the pH range 6.9−10.0, corresponding to 2% neutralization (initial) for the equilibrium $IpI^-/(IpI - H)^{2-}$ (eq 3) and about 71% (final) for $(IpI - H)^{2-}/(IpI - 2H)^{3-}$ (eq 4). The final results for K^H_{IpI} and $K^H_{(IpI-H)}$ are the averages of the values from six independent pairs of titrations.

2.5. Determination of the Stability Constants. After each of the titrations described in the previous paragraph, the solutions were adjusted to the initial pH of around 4.3 by adding a small volume (about 0.5 mL) of 0.1 M HNO_3. Subsequently, a comparatively small volume of a solution of $M(NO_3)_2$ ($M^{2+} = Co^{2+}$, Ni^{2+}, Zn^{2+}, and Cd^{2+}) was added, and the titration was repeated. From the data obtained in the presence of M^{2+} (with and without ligand), the stability constants $K^M_{M(IpI-H)}$ of the M(IpI − H) complexes (eq 6) were calculated. The total volume of these solutions was around 33 mL, and the ionic strength I varied between 0.10 and 0.12 M. Such small variations in I had no effect on complex stability, as was evident from the experiments with $M^{2+} = Ni^{2+}$ and Zn^{2+} in which $I = 0.1$ M was used (see below). Additionally, the stability constants of the M(IpI − H) complexes ($M^{2+} = Mg^{2+}$, Ni^{2+}, and Zn^{2+}) and the $Mg(IpI - 2H)^-$ species (eq 7) were determined under the same conditions as used for the acidity constants, but $NaNO_3$ was partly ($M^{2+} = Ni^{2+}$ and Zn^{2+}) or fully ($M^{2+} = Mg^{2+}$) replaced by $M(NO_3)_2$ (25 °C; $I = 0.1$ M).

The metal-to-ligand ratios in the different titrations were 291:1 and 194:1 for Mg^{2+}; 39:1 and 20:1 for Co^{2+}; 25:1 and 13:1 for Ni^{2+}; 49:1, 37:1, and 13:1 for Zn^{2+}; and 20:1 for Cd^{2+}. It should be noted that the Cd^{2+}/IpI^- system was titrated twice with the same metal-to-ligand ratio, but different total concentrations of IpI^- and Cd^{2+} were used.

Depending on the metal ion, the evaluation of the experimental data commenced at a formation degree of the M(IpI − H) species between about 1 and 5%, while the upper limit was given by the onset of the hydrolysis of $M(aq)^{2+}$, which was evident from the titrations without the ligand. Representative examples for the pH ranges employed in the case of the M(IpI − H) complexes are 7.1–9.5 (Mg^{2+}), 6.2–7.4 (Co^{2+}), 6.1–7.2 (Ni^{2+}), 6.2–6.9 (Zn^{2+}), and 6.1–7.5 (Cd^{2+}), corresponding to maximum formation degrees of about 15% for Mg(IpI − H), 20% for Co(IpI − H), 32% for Ni(IpI − H), 33% for Zn(IpI − H), and 28% for Cd(IpI − H), respectively. For the $Mg(IpI - 2H)^-$ species, a formation degree of 44% was reached at pH 9.5 (see above). No stability constants of other $M(IpI - 2H)^-$ complexes could be obtained due to the hydrolysis of $M(aq)^{2+}$ at pH values high enough to form significant amounts of $M(IpI - 2H)^-$. The occurrence of hydrolysis is also the cause for the relatively large error limit of the stability constant for Zn(IpI − H).

For the calculation of the stability constants, the programs described previously[15,16,31,32] were used, either by taking into account or ignoring the stability constants for the formation of the $M(IpI)^+$ species (for details, see ref 15). It should be noted that the results of both evaluations were identical within their error limits. The results also showed no dependence on the excess of the metal ion concentration employed. The final results are the averages of at least two independent pairs of titrations.

3. Results and Discussion

The ligand concentrations employed in this work were close to 2×10^{-4} M or below. Hence, no self-association of IpI^- is expected.[33,34]

3.1. Acidity Constants of $H_3(IpI)^{2+}$. In theory the negatively charged phosphodiester bridge of IpI^- is able to accept a proton; however, it is also clear that this proton is released already at very low pH values. For example, for $H_2(UMP)$, $pK^H_{H_2(UMP)} = 0.7 \pm 0.3$ holds.[35] Assuming that the RO−P(O)(OH)−OR′ bridge is well represented by HO−P(O)(OH)−O(uridine), then the mentioned pK_a value is due to a situation where the nucleobase residues are uncharged. Introduction of a single positive charge should acidify the proton at the RO−P(O)(OH)−OR′ bridge, which is in accord with $pK^H_{H_3(AMP)} = 0.4 \pm 0.2$,[36] $pK^H_{H_3(GMP)} = 0.3 \pm 0.2$,[37] and $pK^H_{H_3(IMP)} = 0.45 \pm 0.25$,[37] giving on average an acidification of about 0.3 pK units. For two $(N7)H^+$ sites, as present in $H_3(IpI)^{2+}$, a larger acidification is expected; we assume it to be about 0.5 pK units; hence, $pK^H_{H_3(IpI)} = 0.2 \pm 0.3$ (error limit estimated). Since the corresponding deprotonation reaction occurs thus at a very low pH, this reaction is not considered further in this study.

Clearly, the two N7 sites of IpI^- are more basic than the phosphate diester bridge, as is evident from $M(Ino)^+$ with

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$pK^{H}_{H(Ino)} = 1.06 \pm 0.06$.[38] Therefore, a 2-fold protonation at these sites is expected, giving rise to the $H_2(IpI)^+$ species. The release of these protons is quantified by equilibria 1 and 2:

$$H_2(IpI)^+ \rightleftharpoons H(IpI)^{\pm} + H^+ \quad (1a)$$

$$K^{H}_{H_2(IpI)} = [H(IpI)^{\pm}][H^+]/[H_2(IpI)^+] \quad (1b)$$

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

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

A further release of protons is possible from the (N1)H sites (see Figure 1) of the IpI^- species:

$$IpI^- \rightleftharpoons (IpI - H)^{2-} + H^+ \quad (3a)$$

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

$$(IpI - H)^{2-} \rightleftharpoons (IpI - 2H)^{3-} + H^+ \quad (4a)$$

$$K^{H}_{(IpI-H)} = [(IpI - 2H)^{3-}][H^+]/[(IpI - H)^{2-}] \quad (4b)$$

It should be emphasized that because of the scarcity of IpI^- we could measure by potentiometric pH titrations only the acidity constants due to equilibria 3 and 4. The values for $H_2(IpI)^+$ (eqs 1 and 2) were estimated in the following way: The difference $\Delta pK_a = pK^{H}_{H(GpG)} - pK^{H}_{H(Guo)} = (2.51 \pm 0.03) - (2.11 \pm 0.04) = 0.40 \pm 0.05$ quantifies the basicity difference between GpG^- and Guo;[15] the same difference is obtained for $d(GpG)^-$ and dGuo[15] as one would expect because the relative change in basicity should be independent of the actual size of the acidity constants. Hence, by application of the acidity constant for $H(Ino)^+$, one can estimate a value for $H(IpI)^{\pm}$; that is, $pK^{H}_{H(IpI)} = pK^{H}_{H(Ino)} + \Delta pK_a = (1.06 \pm 0.06) + (0.40 \pm 0.05) = 1.46 \pm 0.08$. Further, the differences between $pK^{H}_{(IpI-H)} - pK^{H}_{IpI}$ and $pK^{H}_{H(IpI)} - pK^{H}_{H_2(IpI)}$ are expected to be identical because the extent of the (N1)H/$(N1)^-$ interaction should be the same for the $(N7)H^+$/N7 interaction, as the distances are comparable and the differences in charge are the same ($0/\pm1$). Indeed, the validity of this assumption has been proven for the GpG system.[15] Therefore, one obtains $pK^{H}_{H_2(IpI)} = pK^{H}_{H(IpI)} - [pK^{H}_{(IpI-H)} - pK^{H}_{IpI}] = (1.46 \pm 0.08) - [(9.59 \pm 0.09) - (8.64 \pm 0.03)] = 0.51 \pm 0.12$. Of course, the buffer region of this value overlaps with the one given above for $pK^{H}_{H_3(IpI)} = 0.2 \pm 0.3$.

The estimated acidity constants (eqs 1 and 2) and those determined by potentiometric pH titrations (eqs 3 and 4) for the IpI system are listed in Table 1 together with the acidity constants of several related species. Many conclusions may be drawn from the data in Table 1; a few shall be indicated:

(i) In all instances, the hypoxanthine residue is more acidic than the guanine moiety; this holds for the $(N7)H^+$ sites as well as for the (N1)H units.

(ii) The addition of a phosphate group be it single- or double-charged always increases the proton affinity of N sites of the nucleobase due to the connected charge effect.

Table 1. Negative Logarithms of the Acidity Constants for the Deprotonation of the $(N7)H^+$ and (N1)H Sites in $H_2(IpI)^+$ Species, Together with Some Related Data, as Estimated (See Text) or Determined by Potentiometric pH Titrations in Aqueous Solution (25 °C; $I = 0.1$ M, $NaNO_3$)[a]

		pK_a of the sites	
no.[b]	acids	$(N7)H^+$	(N1)H
1	$H(dGuo)^+$	2.34 ± 0.03	9.25 ± 0.02
2	$H_2[d(GpG)]^+$	$1.69 \pm 0.10/2.71 \pm 0.10$[c]	$9.37 \pm 0.03/10.39 \pm 0.07$
3	$H(Guo)^+$	2.11 ± 0.04	9.22 ± 0.02
4	$H_2(GpG)^+$	$1.49 \pm 0.03/2.51 \pm 0.03$	$9.34 \pm 0.07/10.38 \pm 0.10$
5	$H(Ino)^+$	1.06 ± 0.06	8.76 ± 0.03
6	$H_2(IpI)^+$	$0.51 \pm 0.12/1.46 \pm 0.08$[c]	$8.64 \pm 0.03/9.59 \pm 0.09$
7	$H_2(IMP)^{\pm}$	1.43 ± 0.08[d]	9.02 ± 0.02
8[e]	$H_2(GMP)^{\pm}$	2.48 ± 0.04	9.45 ± 0.02

[a] So-called practical, mixed, or Brønsted acidity constants are listed.[29] The error limits given are *3 times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The error limits of differences between constants as they appear in the text were calculated according to the error propagation after Gauss. [b] The constants in entries 1, 2, and 4 are from ref 15, those in entries 3, 5, 7, and 8 are from ref 37. The values of entry 6 were determined in this work. [c] Estimated values. The acidity constant for the release of the proton from the phosphodiester bridge was estimated to be $pK^{H}_{H_3(IpI)} = 0.2 \pm 0.3$ (see section 3.1, first paragraph); hence, the buffer regions for this and the above values overlap somewhat. [d] This value refers to the micro acidity constant, $pk^{IMP\cdot H}_{H\cdot IMP\cdot H}$, which quantifies the deprotonation of the $(N7)H^+$ site in a phosphate-monoprotonated IMP species: $(H\cdot IMP\cdot H)^{\pm} \rightleftharpoons (IMP\cdot H)^- + H^+$. The macro acidity constants for $H_2(IMP)^{\pm}$ are $pK^{H}_{H_2(IMP)} = 1.30 \pm 0.10$ and $pK^{H}_{H(IMP)} = 6.22 \pm 0.01$; the latter value refers to the final deprotonation of the phosphate group.[37] [e] The phosphate group is deprotonated with $pK^{H}_{H(GMP)} = 6.25 \pm 0.02$.[37]

(iii) For all dinucleoside monophosphates, the difference between two corresponding pK_a values (i.e., of the same two sites) is always close to 1 pK_a unit; in other words, it is somewhat larger than the expected statistical difference of 0.6 pK units,[16] and this means that the two sites "feel" each other; moreover, the two buffer regions are somewhat overlapping.

(iv) The following differences demonstrate that the relative basicity of N7 in corresponding guanine and hypoxanthine derivatives is always the same (the constants are from Table 1):

$$pK^{H}_{H(Guo)} - pK^{H}_{H(Ino)} = (2.11 \pm 0.04) - (1.06 \pm 0.06) = 1.05 \pm 0.07$$

$$pK^{H}_{H_2(GMP)} - pk^{IMP\cdot H}_{H\cdot IMP\cdot H} = (2.48 \pm 0.04) - (1.43 \pm 0.08) = 1.05 \pm 0.09$$

$$pK^{H}_{H_2(GpG)} - pK^{H}_{H_2(IpI)} = (1.49 \pm 0.03) - (0.51 \pm 0.12) = 0.98 \pm 0.12$$

$$pK^{H}_{H(GpG)} - pK^{H}_{H(IpI)} = (2.51 \pm 0.03) - (1.46 \pm 0.08) = 1.05 \pm 0.09$$

This result must mean that neither inter- nor intramolecular stacking interactions play a role in any of these derivatives, which is what, due to the positive charge at the $(N7)H^+$ site of the nucleobase residues, is expected. However, at the same time, these differences also demonstrate the internal consistency of the listed acidity constants.

(v) If the analogous situation for the (N1)H sites is considered, the following differences result (values from Table 1):

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

$$pK^{H}_{Guo} - pK^{H}_{Ino} = (9.22 \pm 0.02) - (8.76 \pm 0.03) = 0.46 \pm 0.04$$

$$pK^{H}_{GMP} - pK^{H}_{IMP} = (9.45 \pm 0.02) - (9.02 \pm 0.02) = 0.43 \pm 0.03$$

$$pK^{H}_{GpG} - pK^{H}_{IpI} = (9.34 \pm 0.07) - (8.64 \pm 0.03) = 0.70 \pm 0.08$$

$$pK^{H}_{(GpG-H)} - pK^{H}_{(IpI-H)} = (10.38 \pm 0.10) - (9.59 \pm 0.09) = 0.79 \pm 0.14$$

Here the situation is different: The first two differences describe the situation for (N1)H units without any interference of stacking because the corresponding pK_a values were obtained in very dilute solutions. In the two latter cases, the resulting differences are too large due to some intramolecular stacking interaction; this interaction is independent of the concentration, and it is evidently more pronounced in GpG than in IpI, and therefore the proton release is more inhibited in the first dinucleoside monophosphate. In fact, it is known that guanosine self-stacks by a factor of approximately 2 better than inosine.[34,39,40]

3.2. Stability Constants of M^{2+}/IpI Systems. Since the stabilities of M^{2+} complexes formed with hypoxanthines[11,12] are known not to be very stable, this property is also expected for IpI^- as the ligand. Therefore, it seemed advisable to work with a relatively large excess of M^{2+}, compared to the concentration of IpI^-, to obtain a high enough formation degree of the various complexes and thus reliable stability constants (see section 2.5). The consequence of this experimental setting is that the upper limit of the pH range that could be evaluated in the experiments was determined by the formation of M^{2+} hydroxo complexes; this pH became evident from the titrations carried out in the absence of the ligand (section 2.5).

The first complex possibly formed in significant amounts is defined by equilibrium 5; the other complexes result then from the stepwise deprotonation of the (N1)H sites, giving rise to equilibria 6 and 7:

$$M^{2+} + IpI^- \rightleftharpoons M(IpI)^+ \quad (5a)$$

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

$$M^{2+} + (IpI - H)^{2-} \rightleftharpoons M(IpI - H) \quad (6a)$$

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

$$M^{2+} + (IpI - 2H)^{3-} \rightleftharpoons M(IpI - 2H)^- \quad (7a)$$

$$K^{M}_{M(IpI-2H)} = [M(IpI - 2H)^-]/([M^{2+}][(IpI - 2H)^{3-}]) \quad (7b)$$

Of course, one may also consider the two deprotonation reactions 8 and 9

$$M(IpI)^+ \rightleftharpoons M(IpI - H) + H^+ \quad (8a)$$

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

$$M(IpI - H) \rightleftharpoons M(IpI - 2H)^- + H^+ \quad (9a)$$

$$K^{H}_{M(IpI-H)} = [M(IpI - 2H)^-][H^+]/[M(IpI - H)] \quad (9b)$$

which are interlinked[14,41] with equilibria 5−7 by the following two equations:

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

$$pK^{H}_{M(IpI-H)} = pK^{H}_{(IpI-H)} + \log K^{M}_{M(IpI-H)} - \log K^{M}_{M(IpI-2H)} \quad (11)$$

However, equilibria 7 and 9 are of relevance in the present study only for the Mg^{2+} systems; with those of Co^{2+}, Ni^{2+}, Zn^{2+}, or Cd^{2+}, the formation of hydroxo complexes occurs before the onset of equilibria 7 and 9.

The stability constants defined by equilibrium 5 could not be measured due to the scarcity of IpI^-, which prevented us from working at the low pH necessary (below 1.3; see Table 1), as this would require high IpI concentrations to measure these constants for the various $M(IpI)^+$ systems. However, on the basis of the stability constants of the $M(GpG)^+$ and $M[d(GpG)]^+$ complexes,[15] the stability constants of the $M(IpI)^+$ complexes of Mg^{2+}, Ni^{2+}, and Cd^{2+} could be estimated by taking the differences in the N7 basicity (Table 1) into account via the slopes m of log K versus pK_a plots obtained previously[42] for a series of benzimidazole-type (= 1,3-dideazapurine-type) ligands ($m_{Mg} = 0.035$; $m_{Ni} = 0.201$; $m_{Cd} = 0.293$). The corresponding estimated values for log $K^{M}_{M(IpI)}$ are listed in entries 1a, 3a, and 5a in column 3 of Table 2.

Application of $pK^{H}_{H(IpI)} = 1.46$ (Table 1) to the mentioned log K versus pK_a plots of benzimidazole-type ligands[42] allows the calculation of the expected stabilities of the corresponding Mg^{2+}, Ni^{2+}, and Cd^{2+} complexes, and these are on average 0.57 ± 0.06 log units less stable than their $M(IpI)^+$ counterparts. This equality in the stability difference also allowed estimation of the stabilities of the $Co(IpI)^+$ and $Zn(IpI)^+$ complexes by calculating the expected stability of the corresponding Co^{2+} and Zn^{2+} benzimidazole-type complexes in the indicated way and adding 0.57 log units to obtain the values for the $M(IpI)^+$ species; these results are given in entries 2a and 4a of Table 2 (column 3).

To demonstrate that the constants listed under "a" entries in Table 2 are valid results despite the estimations described in the two preceding paragraphs, we have also evaluated our potentiometric pH titration data by considering only equilibria 3 and 4 as well as 8 for the Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} systems. In the case of the Mg^{2+} system, in addition, equilibrium 9 was taken into account as already mentioned above. In other words, in this evaluation of the experimental data, the formation of the $M(IpI)^+$ complexes was ignored, yet this simplified model (section 2.5) still satisfied the

(39) Sigel, H. *Biol. Trace Elem. Res.* **1989**, *21*, 49–59.
(40) Corfù, N. A.; Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1990**, *191*, 721–735.
(41) Sigel, H. *Eur. J. Biochem.* **1968**, *3*, 530–537.
(42) Kapinos, L. E.; Song, B.; Sigel, H. *Chem.—Eur. J.* **1999**, *5*, 1794–1802.

experimental data very well because the formation degree of the $M(IpI)^+$ species is always low and independent of the H^+ concentration at pH > 3. Indeed, the stability constants determined in this way for the M(IpI − H) and Mg(IpI − 2H)$^-$ complexes, which are listed under entries labelled "b" in Table 2, are within the error limits identical with those determined by the more sophisticated model ("a" entries), though they are in all instances somewhat lower, that is, at a maximum by 0.14 log units as observed for Mg(IpI − H) (Table 2, column 4). To conclude, for any future work and comparisons, the values listed in the "a" entries of Table 2 should be used.

3.3. Comparison of the Stabilities of M^{2+}/Inosine Complexes with the Corresponding IpI Species. The acid–base properties of inosine (Ino) are defined regarding the $(N7)H^+$ and (N1)H sites (Table 1) by equilibria 12 and 13, respectively:

$$H(Ino)^+ \rightleftharpoons Ino + H^+ \quad (12a)$$

$$K^H_{H(Ino)} = [Ino][H^+]/[H(Ino)^+] \quad (12b)$$

$$Ino \rightleftharpoons (Ino - H)^- + H^+ \quad (13a)$$

$$K^H_{Ino} = [(Ino - H)^-][H^+]/[Ino] \quad (13b)$$

The neutral Ino interacts via N7 with divalent metal ions to give $M(Ino)^{2+}$ complexes (eq 14),[22] and the (N1)-deprotonated ligand reacts to yield the $M(Ino - H)^+$ species (eq 15):[14]

$$M^{2+} + Ino \rightleftharpoons M(Ino)^{2+} \quad (14a)$$

$$K^M_{M(Ino)} = [M(Ino)^{2+}]/([M^{2+}][Ino]) \quad (14b)$$

$$M^{2+} + (Ino - H)^- \rightleftharpoons M(Ino - H)^+ \quad (15a)$$

$$K^M_{M(Ino-H)} = [M(Ino - H)^+]/([M^{2+}][Ino - H]^-) \quad (15b)$$

Of course, the complex $M(Ino)^{2+}$ formed according to equilibrium 14 may lose a proton from its (N1)H site to give $M(Ino - H)^+$ according to equilibrium 16. The corresponding acidity constant, $K^H_{M(Ino)}$, is calculated[14,41] with equation 17:

$$M(Ino)^{2+} \rightleftharpoons M(Ino - H)^+ + H^+ \quad (16a)$$

$$K^H_{M(Ino)} = [M(Ino - H)^+][H^+]/([M(Ino)^{2+}] \quad (16b)$$

$$pK^H_{M(Ino)} = pK^H_{Ino} + \log K^M_{M(Ino)} - \log K^M_{M(Ino-H)} \quad (17)$$

Unfortunately, only the stability constants of the M^{2+}/Ino systems with Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} have been measured by Lönnberg and Vihanto.[12] However, by using their data and applying known procedures,[42,43] the constants for the Mg^{2+} and Cd^{2+}/Ino systems could also be estimated, as described in the footnotes of Table 3. The results of Lönnberg and Vihanto were determined at 25 °C and I = 1.0 M ($NaClO_4$); they are listed in columns 2 and 3 of Table 3. Since initial complex formation occurs with the neutral Ino species, the change in ionic strength from I = 1.0 M ($NaClO_4$) to our conditions, I = 0.1 M ($NaNO_3$), is expected to be of no significance. It may be added that the value for $Ni(Ino)^{2+}$ given by Lönnberg and Vihanto (Table 3) is in excellent accord with $\log K^{Ni}_{Ni(Ino)} = 1.15 \pm 0.13$ (15 °C; I = 1.0 M, $NaClO_4$) determined by Nagasawa and Diebler.[11]

Furthermore, Lönnberg and Vihanto[12] list $pK^H_{Ino} = 8.7$ in accord with the result given in Table 1. Hence, it is justified to apply to the stability constants listed in columns 2 and 3 of Table 3 the acidity constants given in Table 1 and to calculate via eqs 17 and 18 the results listed in columns 4 and 5 of Table 3.

$$\Delta pK_{a/N1/Ino} = pK^H_{Ino} - pK^H_{M(Ino)} \quad (18)$$

The corresponding acidity constants for the $M(IpI)^+$ complexes, $K^H_{M(IpI)}$, are listed in column 5 of Table 2, and the resulting acidifications are defined by equation 19 and given in column 6 of Table 2.

$$\Delta pK_{a/1/IpI} = pK^H_{IpI} - pK^H_{M(IpI)} \quad (19)$$

It is evident that in all instances the (N1)H deprotonation reaction occurs in or close to the physiological pH range.

The stability constants listed in columns 3 and 4 of Table 2 and columns 2 and 3 of Table 3 show the typical trend for

Table 2. Logarithms of the Stability Constants of Some M^{2+} Complexes Formed in Aqueous Solution with IpI (eqs 5–7) as Determined[a] by Potentiometric pH Titrations, Together with the Negative Logarithms of the Acidity Constants of the $M(IpI)^+$ and M(IpI − H) Species (eqs 8–11) and the Extent of the Acidification of the (N1)H Site Due to M^{2+} Complexation (25 °C; $I \approx 0.1$ M, $NaNO_3$)[b]

no.[c]	M^{2+}	$\log K^M_{M(IpI)}$	$\log K^M_{M(IpI-H)}$	$pK^H_{M(IpI)}$	$\Delta pK_{a/1/IpI}$	$\log K^M_{M(IpI-2H)}$	$pK^H_{M(IpI-H)}$	$\Delta pK_{a/2/IpI}$
1a	Mg^{2+}	0.40 ± 0.4	1.07 ± 0.09	7.97 ± 0.41	0.67 ± 0.41	1.75 ± 0.09	8.91 ± 0.16	0.68 ± 0.18
b		ignored	0.93 ± 0.11			1.69 ± 0.10	8.83 ± 0.17	0.76 ± 0.19
2a	Co^{2+}	1.35 ± 0.3	2.90 ± 0.05	7.09 ± 0.31	1.55 ± 0.31			
b		ignored	2.83 ± 0.05					
3a	Ni^{2+}	1.70 ± 0.2	3.75 ± 0.11	6.59 ± 0.23	2.05 ± 0.23			
b		ignored	3.66 ± 0.08					
4a	Zn^{2+}	0.90 ± 0.3	3.55 ± 0.25	5.99 ± 0.39	2.65 ± 0.39			
b		ignored	3.50 ± 0.25					
5a	Cd^{2+}	1.45 ± 0.3	3.40 ± 0.08	6.69 ± 0.31	1.95 ± 0.31			
b		ignored	3.36 ± 0.09					

[a] The values given in column 3 were not measured but estimated as described in section 3.2. The given error limits are generous estimates. [b] The error limits given are 3 times the standard error of the mean value (3σ) or the sum of the probable systematic errors, whichever is larger. The error limits of the derived data (columns 5, 6, 8, and 9) were calculated according to the error propagation after Gauss. [c] The values in the "b" entries have been calculated without considering the formation of the $M(IpI)^+$ complexes, while "a" entries give for each metal ion system the result of the calculations where this species was considered and where its stability constant (see footnote *a*) was kept fixed in the calculation procedure for the other stability constants (see also section 2.5).

N binding sites[44] in accord with the Irving−Williams series.[45] Furthermore, a comparison of the results given in Tables 2 and 3 reveals that the $M(Ino)^{2+}$ and $M(Ino - H)^{+}$ complexes are throughout somewhat less stable than the corresponding $M(IpI)^{+}$ and M(IpI − H) species. This observation leads then to the question of whether in the IpI complexes chelate formation occurs or if the observed increased stability is only a charge effect of the phosphate diester bridge. Considering that the stability constants for the $M(IpI)^{+}$ complexes are estimates (Table 2), we shall concentrate below only on the comparison of the stabilities of the $M(Ino - H)^{+}$ and M(IpI − H) species.

3.4. Estimate of the Extent of Chelate Formation in M(IpI − H) Complexes. Assuming that the type of metal ion binding is identical in the $M(Ino - H)^{+}$ and M(IpI − H) complexes, then their stabilities should differ only by the charge effect exercized by the negatively charged phosphate diester bridge on a divalent metal ion coordinated at N7 and possibly also interacting with (C6)O.[14,46] This 2+/− charge effect amounts to about 0.40 ± 0.15 log units, as is known[30,47] from many examples where the mentioned charges are separated by analogous distances. Hence, one may calculate expected stabilities for M(IpI − H) complexes in which only a M^{2+}-nucleobase interaction occurs, according to eq 20:

$$\log K^{M}_{M(IpI-H)calc} = \log K^{M}_{M(Ino-H)} + (0.40 \pm 0.15) \quad (20)$$

These values are listed in column 4 of Table 4, and if compared with the measured values according to eq 21, it is evident that all these differences (Table 4, column 5) are positive with the single exception of $\log \Delta_{Mg/(IpI-H)}$, which is zero within its error limits; that is, for the Mg(IpI − H) complex, no stability enhancement is observed.

$$\log \Delta_{M/(IpI-H)} = \log K^{M}_{M(IpI-H)} - \log K^{M}_{M(IpI-H)calc} \quad (21)$$

Since any stability enhancement must be attributed to an additional interaction,[48] this means that an intramolecular equilibrium between an open (op) and chelated or closed (cl) species must exist:

$$M(IpI - H)_{op} \rightleftharpoons M(IpI - H)_{cl} \quad (22)$$

Evidently, the stability of the open isomer is quantified by $K^{M}_{M(IpI-H)calc}$ (eq 20), often also addressed as $K^{M}_{M(IpI-H)op}$, whereas the measured value for $K^{M}_{M(IpI-H)}$ (eq 6) encompasses all the isomers of M(IpI − H) present in solution.

By following previous routes,[48−50] the application of the $\log \Delta_{M/(IpI-H)}$ values (eq 21; Table 4, column 5) allows

Table 3. Logarithms of the Stability Constants of $M(Ino)^{2+}$ and $M(Ino - H)^{+}$ Complexes (eqs 14 and 15) in Aqueous Solution, Together with the Negative Logarithms of the Acidity Constants of the $M(Ino)^{+}$ Species (eqs 16 and 17) and the Extent of the Acidification of the (N1)H Site Caused by the Coordinated M^{2+} (eq 18)[a−d]

M^{2+}	$\log K^{M}_{M(Ino)}$	$\log K^{M}_{M(Ino-H)}$	$pK^{H}_{M(Ino)}$	$\Delta pK_{a/N1/Ino}$
Mg^{2+}	0.0 ± 0.2[b]	0.8 ± 0.2[c]	7.96 ± 0.28	0.8 ± 0.3
Co^{2+}	0.8 ± 0.2[a]	2.1 ± 0.2[a]	7.46 ± 0.28	1.3 ± 0.3
Ni^{2+}	1.1 ± 0.2[a]	2.8 ± 0.2[a]	7.06 ± 0.28	1.7 ± 0.3
Cu^{2+}	1.3 ± 0.2[a]	4.5 ± 0.2[a]	5.56 ± 0.28	3.2 ± 0.3
Zn^{2+}	0.7 ± 0.1[a]	2.4 ± 0.1[a]	7.06 ± 0.14	1.7 ± 0.2
Cd^{2+}	0.85 ± 0.2[b]	2.6 ± 0.25[d]	7.01 ± 0.32	1.75 ± 0.3

[a] These values are from ref 12 (25 °C; $I = 1.0$ M, $NaClO_4$); those in column 2 are given with the error limits listed in ref 12, and these same error limits are now also used for the constants summarized in column 3. [b] Application of $pK^{H}_{H(Ino)} = 1.06$ (Table 1) to the log K versus pK_a plots of benzimidazole-type ligands[42] provides constants which are for Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} on average 0.13 log units smaller than the data listed in column 2; hence, this difference is added to the values obtained from these plots for the complexes of Mg^{2+} and Cd^{2+} to give the estimates listed in column 2 for $Mg(Ino)^{2+}$ and $Cd(Ino)^{2+}$. [c] Since the acidity difference, $\Delta pK_a = pK^{H}_{d(Guo)} - pK^{H}_{Ino} = (9.25 \pm 0.02) - (8.76 \pm 0.03) = 0.49 \pm 0.04$ (Table 1) is small, as is the slope for log K versus pK_a plots of Mg^{2+} complexes,[42] the value[15] $\log K^{Mg}_{Mg[d(Guo-H)]} = 0.94 \pm 0.14$ is expected to be similar to the stability of $Mg(Ino - H)^{+}$, and hence, 0.8 ± 0.2 is used above. [d] According to the *Stability Ruler*,[43] the stability for $Cd(Ino - H)^{+}$ should be close to those for $Ni(Ino - H)^{+}$ and $Zn(Ino - H)^{+}$; this has led to the above estimate given with a generous error limit.

definition of the position of the intramolecular equilibrium 22; that is, to calculate values for the dimensionless equilibrium constant K_I according to equation 23:

$$K_I = [M(IpI - H)_{cl}]/[M(IpI - H)_{op}] \quad (23a)$$

$$= \frac{K^{M}_{M(IpI-H)}}{K^{M}_{M(IpI-H)calc}} - 1 \quad (23b)$$

$$= 10^{\log \Delta_{M/(IpI-H)}} - 1 \quad (23c)$$

Once K_I is known, the formation degree or the percentage of the chelated species in equilibrium 22 follows from eq 24:

$$\%M(IpI - H)_{cl} = 100K_I/(1 + K_I) \quad (24)$$

The values for K_I and the formation degrees of the chelated species are listed in columns 6 and 7 of Table 4, respectively.

What is the structure of the closed species? Since N7 of a 5′-purine unit is in general somewhat more basic than the one of a 3′ unit,[15,51] one may assume that the initial complex formation in IpI^{-} occurs preferably at N7 of the 5′-inosine residue. This binding mode also allows an outersphere interaction with the (C6)O site, especially after deprotonation of (N1)H,[37,52] as well as a maximal electrostatic interaction with the negatively charged phosphate bridge and possibly even macrochelate formation to a certain extent, as has been suggested before for an intermediate Pt(II) complex with a related dinucleoside monophosphate[53] and as is well-known to

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Table 4. Stability Constant Comparisons[a] for the M(IpI − H) Complexes between the Measured Stability Constants $K^{M}_{M(IpI-H)}$ (eq 6),[b] which Encompass All Isomers of Equilibrium 22, and the Estimated Ones for the Open M(IpI − H)$_{op}$ Isomers, $K^{M}_{M(IpI-H)op} = K^{M}_{M(IpI-H)calc}$ (eq 20),[d] which Are Corrected[c] for the Charge Effect of the Phosphate Diester Bridge, R′−O−P(O)$_2^-$−O−R, to Give the Values Listed in Column 4,[d] and from These Follow the Stability Enhancements log $\Delta_{M/(IpI-H)}$ (eq 21), As Well As the Dimensionless Equilibrium Constants K_I (eqs 22 and 23) and the Percentages of the Closed or Chelated Isomers M(IpI − H)$_{cl}$ (eq 24) in Aqueous Solution (25 °C; I = 0.1 M, $NaNO_3$)[a]

M^{2+}	log $K^{M}_{M(IpI-H)}$ (eq 6)[b]	log $K^{M}_{M(Ino-H)}$ (eq 15)[c]	log $K^{M}_{M(IpI-H)calc}$ (eq 20)[d]	log $\Delta_{M/(IpI-H)}$ (eq 21)	K_I (eq 23)[e]	% M(IpI − H)$_{cl}$ (eq 24)[e]
Mg^{2+}	1.07 ± 0.09	0.8 ± 0.2	1.2 ± 0.25	−0.13 ± 0.27	∼0	∼0
Co^{2+}	2.90 ± 0.05	2.1 ± 0.2	2.5 ± 0.25	0.40 ± 0.25	1.51(0.41/3.47)	60(29/78)
Ni^{2+}	3.75 ± 0.11	2.8 ± 0.2	3.2 ± 0.25	0.55 ± 0.27	2.55(0.91/5.61)	72(48/85)
Zn^{2+}	3.55 ± 0.25	2.4 ± 0.1	2.8 ± 0.18	0.75 ± 0.31	4.62(1.75/10.5)	82(64/91)
Cd^{2+}	3.40 ± 0.08	2.6 ± 0.25	3.0 ± 0.29	0.40 ± 0.30	1.51(0.26/4.01)	60(21/80)

[a] For the error limits, see footnote "b" of Table 2. [b] Values from column 4 of Table 2. [c] From column 3 of Table 3. [d] See text in section 3.4 in the context of eq 20. [e] In parentheses, the lower and upper limits are given.

occur with M(IMP) and related complexes.[13,14,50,52,54] Hence, we may conclude that two types of macrochelates are possible: (i) the one indicated above between N7 and the phosphate bridge or (ii) one that involves both N7 sites of $(IpI - H)^{2-}$. Of course, both types of macrochelates may occur in equilibrium with each other, and then the formation degrees given in column 7 of Table 4 encompass both species.

Considering that Mg^{2+} does not form significant amounts of Mg(IpI − H)$_{cl}$ macrochelates, whereas the formation degrees of those species formed with Co^{2+}, Ni^{2+}, Zn^{2+}, or Cd^{2+} are high (Table 4, column 7), we favor N7/N7 macrochelates. However, in doing so, one has to answer the question why no significant amounts of macrochelates are observed for the M(GpG − H) and M[d(GpG − H)] systems.[15] If one applies the described evaluation procedure to the data of ref 15, one notes that the log $\Delta_{M/(GpG-H)}$ and log $\Delta_{M/[d(GpG-H)]}$ values are zero within their error limits. This agrees with the earlier conclusion[15] that "no hint for the formation of significant amounts of intramolecular chelates involving both N7 sites" have been found, though low concentrations could still occur. A possible explanation could be that the intramolecular stacking interaction in $(IpI - H)^{2-}$ is smaller than in $(GpG - H)^{2-}$, and in fact, this is expected.[34,38,39] Consequently, $(IpI - H)^{2-}$ is more flexible for adapting to the configuration needed in aqueous solution for an N7/N7 macrochelate which is known to form with the kinetically inert *cis*-$(NH_3)_2Pt^{2+}$ and both dinucleoside monophosphates.[8] It is important to note in this context that the amount of free energy involved to shift the situation from one side to another is very small; for example, a formation degree of 20% of a macrochelate at 25 °C corresponds only to a stability enhancement of log $\Delta_{M/ligand} = 0.1$ (eq 21) and a change in free energy of $\Delta G° = -0.57$ kJ/mol.[49]

4. Conclusions

It is evident that we are only at the brink of understanding the "next neighbor effects" on the binding of labile metal ions to dinucleoside monophosphates and thus to nucleic acids. Considering that the orientation of neighboring nucleobases appears to be governed by stacking, the next ligand to be studied is clearly ApA^- because the adenine residue stacks best, that is, the stacking tendency decreases in the order adenosine > guanosine > inosine > uridine.[34,39] Thereafter, systematic investigations of dinucleoside monophosphates like ApU^-, UpA^-, GpU^-, UpG^-, and so forth are desirable. These results together with those obtained from dinucleotides[16,55] like $pUpU^{3-}$ or $pGpG^{3-}$ will reveal the various structural elements participating in chelate formation, and they will further provide insight into their stability order for the various metal ions involved, like Mg^{2+}, Zn^{2+}, or Cd^{2+}.

However, already now it is clear that the coordination of a metal ion to N7 of IpI^- facilitates deprotonation of (N1)H to an extent that this reaction occurs in the physiological pH range of about 7.5 (see the $pK^{H}_{M(IpI)}$ values given in column 5 of Table 2). From the previous results[15] obtained for the Ni^{2+} and Cd^{2+} complexes of GpG^- or $d(GpG)^-$, that is, $pK^{H}_{M(GpG)} \approx 7.5$, it follows from the *Stability Ruler* of Martin[43] that at least for Zn^{2+} the acidification is so strong that the physiological pH range is reached. In fact, even with Mg^{2+}, where $pK^{H}_{Mg(GpG)} \approx 8.3$,[15] this is to some extent the case: At pH 7.5, about 15% exist in the (N1)-deprotonated Mg(GpG − H) form. The possible biological relevance of such guanine or hypoxanthine (N1)H deprotonations as mediated by metal ions has been discussed.[18,22,56]

5. Abbreviations (See Also Figure 1)

ApA^-, adenosylyl(3′→5′)adenosine; $d(GpG)^-$, 2′-deoxyguanylyl(3′→5′)-2′-deoxyguanosine; dGuo, 2′-deoxyguanosine; $d(pGpG)^{3-}$, 2′-deoxyguanylyl(5′→3′)-2′-deoxy-5′-guanylate; GMP^{2-}, guanosine 5′-monophosphate; GpG^-, guanylyl(3′→5′)guanosine; Guo, guanosine; IMP^{2-}, inosine 5′-monophosphate; Ino, inosine; I, ionic strength; K_a, general acidity constant; M^{2+}, general divalent metal ion; $pUpU^{3-}$, uridylyl(5′→3′)-5′-uridylate; UpU^-, uridylyl(5′→3′)uridine. Species which are given in the text without a charge either do not carry one or represent the species in general (i.e., independent of their protonation degree); which of the two possibilities applies is always clear from the context. Expressions like $(IpI - H)^{2-}$ should be read as "IpI *minus* H", meaning that the dinucleoside monophosphate IpI^- has lost a proton from one of the (N1)H sites.

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