Stabilities and Isomeric Equilibria in Solutions of Monomeric Metal-Ion Complexes of Guanosine 5′-Triphosphate (GTP^{4-}) and Inosine 5′-Triphosphate (ITP^{4-}) in Comparison with Those of Adenosine 5′-Triphosphate (ATP^{4-})**

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Abstract: Under experimental conditions in which the self-association of the purine-nucleoside 5′-triphosphates (PuNTPs) GTP and ITP is negligible, potentiometric pH titrations were carried out to determine the stabilities of the $M(H;PuNTP)^-$ and $M(PuNTP)^{2-}$ 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+} ($I = 0.1$ M, 25 °C). The stabilities of all $M(GTP)^{2-}$ and $M(ITP)^{2-}$ complexes are significantly larger than those of the corresponding complexes formed with pyrimidine-nucleoside 5′-triphosphates (PyNTPs), which had been determined previously under the same conditions. This increased complex stability is attributed, in agreement with previous ^{1}H MNR shift studies, to the formation of macrochelates of the phosphate-coordinated metal ions with N7 of the purine residues. A similar enhanced stability (despite relatively large error limits) was observed for the $M(H;PuNTP)^-$ complexes, in which H^+ is bound to the terminal γ-phosphate group, relative to the stability of the $M(H;PyNTP)^-$ species. The percentage of the macrochelated isomers in the $M(GTP)^{2-}$ and $M(ITP)^{2-}$ systems was quantified by employing the difference $\log K^{M}_{M(PuNTP)} - \log K^{M}_{M(PyNTP)}$; the lowest and highest formation degrees of the macrochelates were observed for $Mg(ITP)^{2-}$ and $Cu(GTP)^{2-}$ with $17 \pm 11\%$ and $97 \pm 1\%$, respectively. From previous studies of $M(ATP)^{2-}$ complexes, it is known that innersphere and outersphere macrochelates may form; that is, in the latter case a water molecule is between N7 and the phosphate-coordinated M^{2+}. Similar conclusions are reached now by comparisons with earlier ^{1}H MNR shift measurements, that is, that $Mg(GTP)^{2-}$ ($21 \pm 11\%$), for example, exists largely in the form of an outersphere macrochelate and $Zn(GTP)^{2-}$ ($68 \pm 4\%$) as an innersphere one. Generally, the overall percentage of macrochelate falls off for a given metal ion in the order $M(GTP)^{2-} > M(ITP)^{2-} > M(ATP)^{2-}$; this is in accord with the decreasing basicity of N7 and the steric inhibition of the (C6)NH_2 group in the adenine residue. Furthermore, although the absolute stability constants of the previously studied M(GMP), M(IMP), and M(AMP) complexes differ by about two to three log units from the present $M(PuNTP)^{2-}$ results, the formation degrees of the macrochelates are astonishingly similar for the two series of nucleotides for a given metal ion and purine-nucleobase residue. The conclusion that N7 of the guanine residue is an especially favored binding site for metal ions is also in accord with observations made for nucleic acids.

Keywords: macrochelates · metal-ion complexes · nucleic acids · nucleotides · stability constants

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[**] Abbreviations and definitions (see also Figure 1). AMP^{2-}, adenosine 5′-monophosphate; GMP^{2-}, guanosine 5′-monophosphate; Guo, guanosine; I, ionic strength; IMP^{2-}, inosine 5′-monophosphate; Ino, inosine; M^{2+}, divalent metal ion; NMP^{2-}, nucleoside 5′-monophosphate; NTP^{4-}, nucleoside 5′-triphosphate; $PuNTP^{4-}$, purine-nucleoside 5′-triphosphate; $PyNTP^{4-}$, pyrimidine-nucleoside 5′-triphosphate; Thy, thymidine [= 1-(2′-deoxy-β-D-ribofuranosyl)thymine]; Urd, uridine. Species written in the text without a charge either do not carry one or represent the species in general (i.e., independent from their protonation degree); which of the two possibilities applies is always clear from the context. In formulas like $M(H;NTP)^-$, the H^+ and NTP^{4-} are separated by a semicolon to facilitate reading; yet they appear within the same parenthesis to indicate that the proton is at the ligand without defining its location (see, e.g., footnotes to Table 2).

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Introduction

Virtually all reactions of the nucleoside 5′-triphosphates (NTPs) involve metal ions, usually Mg^{2+}.[1] Much recent activity centers on the so-called G-proteins, which utilize guanosine 5′-triphosphate (GTP^{4-}) in such diverse processes as cellular signalling, protein synthesis, vesicular trafficking, and synaptic fusion.[2, 3] G-proteins regulate ion channels,[4, 5] affect the metabolism of Ca^{2+},[5, 6] and participate in signal transduction[7] and exocytosis,[8] etc.[3] Metal ions, mostly Mg^{2+}[5, 9] but also Mn^{2+}[10] or Zn^{2+}[11] are needed for the reactions.[12] For example, the elongation factor Tu (EF-Tu), which takes part in the synthesis of polypeptides in cells, forms a complex with Mg^{2+}, GTP, and aminoacyl-tRNA that interacts with the codon-programmed ribosome; this results in the binding of aminoacyl-tRNA to the ribosomal acceptor site, the hydrolysis of GTP, and the release of EF-Tu·Mg·GDP.[13] GTP hydrolysis is also essential for the insertion of nickel into hydrogenases[14] or the synthesis of activated sulfate,[15] which is an essential step in the metabolic assimilation of sulfur.

Many of the enzymes involved in the transcription and replication of nucleic acids contain Zn^{2+}.[12, 16] The NTPs serving as substrates for DNA and RNA polymerases also have to be present as complexes of divalent metal ions,[12, 17] and there are indications[18] that N7 of ATP might interact with Zn^{2+} in a RNA polymerase during the catalytic process.[19] Hence, understanding the solution properties of metal ion–nucleotide complexes[20, 21] is a precondition for appreciating their role in enzymic reactions.[22]

In this paper we report stability constants of the complexes formed between Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} (M^{2+}) and GTP or its analogue ITP, inosine = 2-deaminoguanosine (Figure 1).[23–26] Despite some early measurements,[27] no comprehensive set of stability data is available,[28–31] and several of the constants have now been determined for the first time. More importantly, the formation of protonated complexes is now always considered, and the locations of the metal ions and the proton in these complexes are defined.

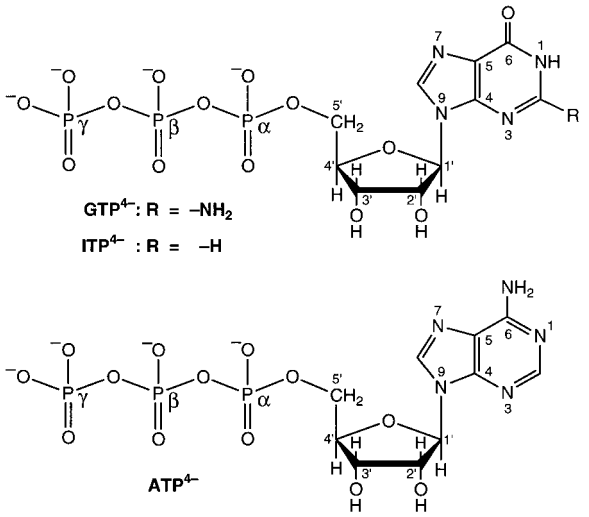
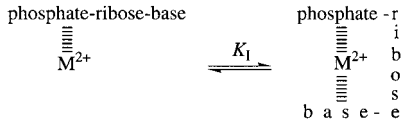


Figure 1. Chemical structure of guanosine 5′-triphosphate (GTP^{4-}), inosine 5′-triphosphate (ITP^{4-}), and adenosine 5′-triphosphate (ATP^{4-}) in their dominating *anti* conformation.[23–26]

Earlier, in a comprehensive paper,[32] we reported and evaluated stability constants for interactions of eight divalent metal ions with ATP, CTP, UTP, or dTTP. About 10% of the $Mg(ATP)^{2-}$ complex exist as a macrochelate, as indicated in Equilibrium (1), and larger percentages occur with divalent 3d



(1)

transition metal ions.[32, 33] The macrochelate involves primary binding of metal ions at the triphosphate chain and an additional interaction at N7 of the purine nucleobase.[21, 27, 34–36] Since the basicity of the N7 site increases in the nucleosides in the order adenosine < inosine < guanosine,[37, 38] larger amounts of macrochelate are expected for guanine over adenine nucleotides for all metal ions. Indeed, M(GMP) complexes[21, 39] exhibit a substantially greater degree of formation of macrochelates than M(AMP) species.[21, 40] A comprehensive evaluation of the extent of macrochelate formation is given now for $M(GTP)^{2-}$ and $M(ITP)^{2-}$, and these results are compared with earlier ones[32, 33] for $M(ATP)^{2-}$. Most importantly, however, the stability constants reported now provide the long-needed reliable basis for detailed future biochemical studies.

Results and Discussion

In the present study, great care was taken to make measurements under conditions in which no self-association of the nucleotides or their complexes occurs.[26, 35, 41] Most measurements were made in solutions with a nucleotide concentration of 0.5 mM; this guaranteed[35, 39] that it was indeed the properties of the monomeric species that were being studied.

Stability constants of the purine-nucleoside 5′-triphosphate complexes $M(PuNTP \cdot H)^-$ and $M(PuNTP)^{2-}$: Potentiometric titrations were conducted at pH > 3.0—in fact, mostly at pH > 3.5—so that deprotonation of $H_2(NTP)^{2-}$ is not a main factor [Eq. (2)].

$$H_2(NTP)^{2-} \rightleftharpoons H(NTP)^{3-} + H^+ \quad (2a)$$

$$K^H_{H_2(NTP)} = \frac{[H(NTP)^{3-}][H^+]}{[H_2(NTP)^{2-}]} \quad (2b)$$

In the pH range up to about neutrality, the main ionization was from the triphosphate-bound proton in $H(NTP)^{3-}$ [Eq. (3)]; since this proton is at the γ-phosphate, we designated this species $(NTP \cdot H)^{3-}$.

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

$$K^H_{H(NTP)} = \frac{[NTP^{4-}][H^+]}{[H(NTP)^{3-}]} \quad (3b)$$

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In the presence of metal ions, in addition to Equilibria (2a) and (3a), two more equilibria involving M^{2+} coordination, primarily at the triphosphate group, need to be considered:

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

$$K^{M}_{M(NTP\cdot H)} = \frac{[M(NTP\cdot H)^{-}]}{[M^{2+}][(NTP\cdot H)^{3-}]} \quad (4b)$$

$$M^{2+} + NTP^{4-} \rightleftharpoons M(NTP)^{2-} \quad (5a)$$

$$K^{M}_{M(NTP)} = \frac{[M(NTP)^{2-}]}{[M^{2+}][NTP^{4-}]} \quad (5b)$$

These two Equilibria, (4a) and (5a), are connected by phosphate deprotonation in the complex through Equilibrium (6a):

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

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

Values for $pK^{H}_{M(NTP\cdot H)}$ may be calculated from Equation (7):

$$pK^{H}_{M(NTP\cdot H)} = pK^{H}_{H(NTP)} + \log K^{M}_{M(NTP\cdot H)} - \log K^{M}_{M(NTP)} \quad (7)$$

Table 1 lists results for the two stability constants [Eqs. (4) and (5)] and the $pK^{H}_{M(PuNTP\cdot H)}$ values for proton loss from the protonated $M(GTP\cdot H)^{-}$ and $M(ITP\cdot H)^{-}$ complexes, according to Equations (6) and (7). Some of the equilibrium constants that appear in Table 1[42] have been determined before,[27a, 28–31] and those for the Mg^{2+} and Ca^{2+} complexes are in fair agreement with the present results. However, the few previous data[27a, 28–30] for the GTP^{4-} and ITP^{4-} complexes of the divalent 3d transition metal ions vary widely and are, in general, somewhat lower because the formation of the $M(PuNTP\cdot H)^{-}$ complexes has not always been considered.

This is the first time that comprehensive sets of stability constants are presented for the $M(PuNTP\cdot H)^{-}$ and $M(PuNTP)^{2-}$ complexes of GTP and ITP that allow detailed comparisons. The constants for the M^{2+}/GTP systems with Sr^{2+}, Ba^{2+}, Ni^{2+}, or Cd^{2+}, and those for the M^{2+}/ITP systems with Sr^{2+}, Ba^{2+}, or Cd^{2+} have not been determined before.[28–30]

An important conclusion follows immediately from Table 1. Compared to $pK^{H}_{H(NTP)} = 6.50 \pm 0.05$ (footnote [a] in Table 1)[33, 42] for the last triphosphate-bound proton in $(GTP\cdot H)^{3-}$ and $(ITP\cdot H)^{3-}$, the final column in Table 1 indicates that a metal ion bound at the triphosphate chain acidifies this proton by about 0.8 to 2.8 log units. The conclusion that, in the $M(PuNTP\cdot H)^{-}$ species, the proton is at the phosphate chain follows therefore from a comparison of the $pK^{H}_{H_2(GTP)}$ (= 2.94) and $pk^{ITP\cdot H}_{H\cdot ITP\cdot H}$ (= 1.89) values (Table 1, footnote [a]),[42] due to $(N7)H^{+}$ deprotonation, with the values for $pK^{H}_{M(PuNTP\cdot H)}$ (Table 1, column 5). The latter values are considerably higher and, hence, H^{+} cannot be located in these complexes at the nucleobase residue. Furthermore, since $pK^{H}_{M(PuNTP\cdot H)} \geq 3.7$ (see Table 1), H^{+} must be located on the γ-phosphate as this is the only basic triphosphate site at $pH > 3$.[32, 42] That the maximum in the distribution curves seen in Figure 2 for the $M(GTP\cdot H)^{-}$ species, with $M = Mg^{2+}$ or Zn^{2+}, occurs in the pH region 3 to 5 confirms the conclusion that the proton must be at the γ-phosphate group.[43]

Table 1. Logarithms of the stability constants of $M(PuNTP\cdot H)^{-}$ [Eq. (4)] and $M(PuNTP)^{2-}$ complexes [Eq. (5)] of the purine-nucleoside 5′-triphosphates (PuNTPs) GTP and ITP, as determined by potentiometric pH titrations in aqueous solution, together with the negative logarithms of the acidity constants [Eqs. (6) and (7)] of the corresponding $M(PuNTP\cdot H)^{-}$ complexes at 25 °C and $I = 0.1$ M ($NaNO_3$ or $NaClO_4$).[a–c]

$PuNTP^{4-}$	M^{2+}	$\log K^{M}_{M(PuNTP\cdot H)}$	$\log K^{M}_{M(PuNTP)}$	$pK^{H}_{M(PuNTP\cdot H)}$[d]
GTP^{4-}	Mg^{2+}	2.6 ±0.3	4.31 ± 0.04	4.8 ±0.3
	Ca^{2+}	2.6 ±0.3	3.96 ± 0.03	5.15 ± 0.3
	Sr^{2+}	2.65 ± 0.2	3.55 ± 0.04	5.6 ±0.2
	Ba^{2+}	2.65 ± 0.2	3.41 ± 0.03	5.75 ± 0.2
	Mn^{2+}	3.36 ± 0.16	5.36 ± 0.03	4.50 ± 0.16
	Co^{2+}	3.50 ± 0.05	5.34 ± 0.05	4.66 ± 0.07
	Ni^{2+}	3.69 ± 0.05	5.42 ± 0.04	4.77 ± 0.07
	Cu^{2+}	4.6 ±0.2	7.38 ± 0.08	3.7 ±0.2
	Zn^{2+}	3.45 ± 0.25	5.52 ± 0.05	4.45 ± 0.25
	Cd^{2+}	3.92 ± 0.08	5.82 ± 0.05	4.60 ± 0.10
ITP^{4-}	Mg^{2+}	2.4 ±0.25	4.29 ± 0.04	4.6 ±0.25
	Ca^{2+}	2.4 ±0.25	3.93 ± 0.05	4.95 ± 0.25
	Sr^{2+}	2.3 ±0.25	3.42 ± 0.10	5.35 ± 0.3
	Ba^{2+}	2.3 ±0.25	3.28 ± 0.09	5.5 ±0.3
	Mn^{2+}	3.1 ±0.3	5.21 ± 0.06	4.35 ± 0.3
	Co^{2+}	3.0 ±0.3	5.08 ± 0.07	4.4 ±0.3
	Ni^{2+}	3.0 ±0.4	5.01 ± 0.10	4.45 ± 0.4
	Cu^{2+}	3.9 ±0.4	6.71 ± 0.10	3.65 ± 0.4
	Zn^{2+}	3.1 ±0.3	5.32 ± 0.06	4.25 ± 0.3
	Cd^{2+}	3.55 ± 0.25	5.62 ± 0.05	4.4 ±0.25

[a] Acidity constants[b] from ref. [42]: $pK^{H}_{H_2(GTP)} = 2.94 \pm 0.02$ [Eq. (2)], $pK^{H}_{H(GTP)} = 6.50 \pm 0.02$ [Eq. (3)]; $pK^{H}_{H_2(ITP)} = 2.19 \pm 0.05$ [Eq. (2)], $pK^{H}_{H(ITP)} = 6.47 \pm 0.02$ [Eq. (3)]. The micro acidity constant for the release of the proton from the (N7)H^{+} site in $(H\cdot ITP\cdot H)^{2-}$ [one proton is at N7 and one at the γ phosphate] was calculated as $pk^{ITP\cdot H}_{H\cdot ITP\cdot H} = 1.89 \pm 0.07$; for $(H\cdot GTP\cdot H)^{2-}$ $pK^{H}_{H_2(GTP)} \simeq pk^{GTP\cdot H}_{H\cdot GTP\cdot H}$ (for details see ref. [42]). [b] The errors given are *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The error limits of the derived data, in the above case of $pK^{H}_{M(PuNTP\cdot H)}$ were calculated according to the error propagation after Gauss. [c] Many of the values given in the third column are estimates as is evident from the large error limits. [d] These values were calculated with Equation (7) by using the acidity constants given in [a] and the stability constants listed above.

The above conclusion disagrees with one that places the proton on the β-phosphate and the Mg^{2+} ion "preferentially at the terminal" γ-group in $Mg(ATP\cdot H)^{-}$.[44] We think that, with the γ-phosphate group monoprotonated, all three phosphate units exhibit comparably weak basicities, and that it is therefore unlikely that there is a single predominant location for the metal ion in the $(PuNTP\cdot H\cdot M)^{-}$ species in solution; that is, α,β,γ as well as β,γ and α,β chelates may be expected to occur in equilibrium, depending on the geometry of the coordination sphere of the metal ion involved.[43]

Stabilities of pyrimidine-nucleoside 5′-triphosphate complexes: To be able to evaluate the $M(PuNTP\cdot H)^{-}$ and $M(PuNTP)^{2-}$ complexes with regard to the position of macrochelate formation in Equilibrium (1), it is necessary to know the stabilities of those complexes in which the metal ion is coordinated only to the triphosphate chain. It has been shown[32, 35] that, in the PyNTP systems, the metal ions indeed bind only in this way, the single exception being the $Cu(CTP)^{2-}$ complex (for details see Section 8 in ref. [32]). The average values of the previous set[32] of six metal ions for

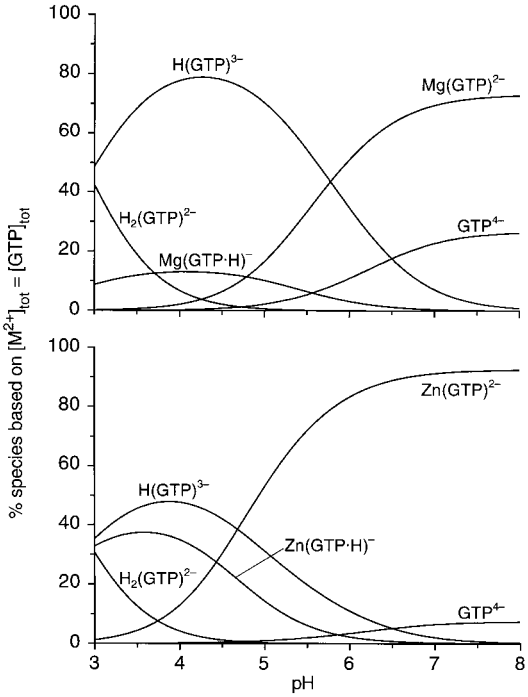

Figure 2. Comparison of the effect of pH on the concentration of the species present in an aqueous solution of GTP and Mg^{2+} (upper part) or Zn^{2+} (bottom part). The results are given as the percentage of the total M^{2+} present (= total GTP). The calculations were carried out with the potentiometrically determined acidity and stability constants (Table 1) for concentrations of 5×10^{-4} M for each reactant at $I = 0.1$ M and 25 °C. These conditions are close to those used in the experiments (see Experimental Section). In the Zn^{2+}/GTP system at pH > 7.5 the complexes $Zn(GTP\text{-}H)^{3-}$ [$pK^{H}_{Zn(GTP)} = 8.4$][27b] and $Zn(GTP{-}H)(OH)^{4-}$ [$pK^{H}_{Zn(GTP-H)(H_2O)} = 9.5$][27b] are also becoming important.

their complexes with UTP, dTTP, and CTP [except the one for $Cu(CTP)^{2-}$][32] are listed in entries 5–10 of Table 2. To complement this previous set, the Sr^{2+} and Ba^{2+} systems with UTP and CTP were now also studied, and the evaluations for the Mg^{2+} and Ca^{2+} systems with UTP were repeated. These new results are summarized in footnotes [d], [e], and [f] of Table 2. The average results valid for the $M(PyNTP \cdot H)^-$ and $M(PyNTP)^{2-}$ complexes of Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} are given in entries 1–4 of Table 2.

The stability constants given in Table 2 for metal-ion binding to a standard pyrimidine-nucleoside 5′-triphosphate (i.e., for complexes in which no nucleobase backbinding occurs that results in macrochelate formation [Eq. (1)])[32, 35] now allow for comparisons with M^{2+}/PuNTP systems. A rough comparison of the stability constants of the $M(PyNTP)^{2-}$ complexes for the transition metal ions (Table 2) with those of the corresponding $M(GTP)^{2-}$ and $M(ITP)^{2-}$ complexes (Table 1) reveals that the stabilities of these $M(PuNTP)^{2-}$ species are larger, and this immediately suggests macrochelate formation in the purine-nucleotide complexes, as already described for ATP^{4-}.[32] In accord with this the acidity of the $M(PyNTP \cdot H)^-$ species (Table 2, column 5) is slightly higher than that of the $M(PuNTP \cdot H)^-$ complexes (Table 1, column 5).

In addition, the stability constants of Table 2 show the usual trends for phosphate complexes: complex stability of the

Table 2. Logarithms of the stability constants of $M(PyNTP \cdot H)^-$ [Eq. (4)] and $M(PyNTP)^{2-}$ complexes [Eq. (5)] of pyrimidine-nucleoside 5′-triphosphates (PyNTPs) as determined by potentiometric pH titrations in aqueous solution, together with the negative logarithms of the acidity constants [Eqs. (6) and (7)] of the corresponding $M(PyNTP \cdot H)^-$ complexes at 25 °C and $I = 0.1$ M ($NaNO_3$ or $NaClO_4$).[a]

No.[b]	M^{2+}	log $K^{M}_{M(PyNTP\cdot H)}$	log $K^{M}_{M(PyNTP)}$	$pK^{H}_{M(PyNTP\cdot H)}$[c]
1	Mg^{2+}	2.3 ±0.2[d]	4.21 ± 0.04[e]	4.6 ±0.2
2	Ca^{2+}	2.2 ±0.2[d]	3.84 ± 0.05[e]	4.85 ± 0.2
3	Sr^{2+}	2.15 ± 0.2[f]	3.34 ± 0.05[f]	5.3 ±0.2
4	Ba^{2+}	2.1 ±0.2[f]	3.18 ± 0.04[f]	5.4 ±0.2
5	Mn^{2+}	2.70 ± 0.12	4.93 ± 0.03	4.27 ± 0.13
6	Co^{2+}	2.55 ± 0.24	4.76 ± 0.03	4.3 ±0.25
7	Ni^{2+}	2.51 ± 0.25	4.50 ± 0.03	4.5 ±0.25
8	Cu^{2+}	2.80 ± 0.08	5.86 ± 0.03	3.44 ± 0.10
9	Zn^{2+}	2.73 ± 0.09	5.02 ± 0.02	4.21 ± 0.10
10	Cd^{2+}	2.89 ± 0.06	5.07 ± 0.03	4.32 ± 0.08

[a] For the error limits see footnote [b] of Table 1. [b] The values for entries 5–10 are for log $K^{M}_{M(PyNTP\cdot H)}$ = log $K^{M}_{M(UTP\cdot H)}$ from Table II of ref. [32] (but now an error limit according to footnote [b] of Table 1 is given; 3σ) and those for log $K^{M}_{M(PyNTP)}$ are the averages listed in Table IV of ref. [32] (with 3σ). [c] Calculated with Equation (7) by using the average value[33, 42] $pK^{H}_{H(NTP)} = 6.50 \pm 0.05$ and the constants listed above. [d] Based on log $K^{Mg}_{Mg(UTP\cdot H)} = 2.3 \pm 0.25$ and log $K^{Ca}_{Ca(UTP\cdot H)} = 2.2 \pm 0.25$, estimated now (the earlier values in ref. [32] are too large), and by taking also into account the constants given for log $K^{M}_{M(H;CTP)}$ in Table II of ref. [32]. [e] Based on log $K^{Mg}_{Mg(UTP)} = 4.21 \pm 0.05$ or log $K^{Ca}_{Ca(UTP)} = 3.82 \pm 0.05$, which were calculated in this work, as well as on the values listed in Table II of ref. [32] for log $K^{M}_{M(dTTP)}$ and log $K^{M}_{M(CTP)}$ by using the number of titrations (Table II of ref. [32]) as weighting factors. [f] These values are based on the following results which were determined in this work: log $K^{Sr}_{Sr(UTP\cdot H)} = 2.1 \pm 0.3$ and log $K^{Sr}_{Sr(UTP)} = 3.38 \pm 0.06$; log $K^{Sr}_{Sr(H;CTP)} = 2.24 \pm 0.15$ and log $K^{Sr}_{Sr(CTP)} = 3.30 \pm 0.04$; log $K^{Ba}_{Ba(UTP\cdot H)} = 2.0 \pm 0.3$ and log $K^{Ba}_{Ba(UTP)} = 3.20 \pm 0.06$; log $K^{Ba}_{Ba(H;CTP)} = 2.15 \pm 0.18$ and log $K^{Ba}_{Ba(CTP)} = 3.15 \pm 0.05$. For the location of the proton in the $M(H;CTP)^-$ species see Section 6 in ref. [32].

alkaline earth ions decreases with increasing radii. For the divalent 3d metal ions, the long-standing experience[45] that the stabilities of phosphate–metal ion complexes do not strictly follow the Irving–Williams[46] sequence is confirmed. The observed stability order for the PyNTPs (Table 2), in accordance with that for phosphate monoesters[47] and diphosphate monoesters,[48] is $Ba^{2+} < Sr^{2+} < Ca^{2+} < Mg^{2+} < Ni^{2+} < Co^{2+} < Mn^{2+} < Cu^{2+} > Zn^{2+} < Cd^{2+}$. The situation for the PuNTPs (Table 1) is somewhat blurred due to the participation of the N7 of the purine moiety in metal ion binding (see the next two sections).

Proof of an enhanced stability of the $M(PuNTP \cdot H)^-$ and $M(PuNTP)^{2-}$ complexes: As described previously,[20, 21, 32–36, 39, 40] purine-nucleotide complexes may adopt two families of conformations: an open form in which the metal ion is only phosphate-coordinated, designated $(NTP \cdot M)^{2-}$ for nucleoside 5′-triphosphates, and a closed form, designated $(N \cdot M \cdot TP)^{2-}$ indicating that the triphosphate-bound metal ion forms a bridge to N7 of the purine nucleobase in a macrochelate according to Equilibrium (1). The corresponding dimensionless intramolecular equilibrium constant K_I is defined in Equation (8):

$$K_I = \frac{[(N \cdot M \cdot TP)^{2-}]}{[(NTP \cdot M)^{2-}]} \quad (8)$$

The observed overall stability for the purine-nucleotide complexes (Table 1, column 4) is the sum of the individual

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stability constants for the open and closed forms [see also Eq. (5b)]:

$$K^{M}_{M(NTP)} = \frac{[(NTP{\cdot}M)^{2-}] + [(N{\cdot}M{\cdot}TP)^{2-}]}{[M^{2+}][NTP^{4-}]} \quad (9a)$$

$$= K^{M}_{(NTP{\cdot}M)} + K^{M}_{(N{\cdot}M{\cdot}TP)} \quad (9b)$$

The stability constant for the open form [Eq. (10)] is taken from the values in Table 2 for the complexes of the standard $PyNTP^{4-}$ species that do not form macrochelates.

$$K^{M}_{(NTP{\cdot}M)} = \frac{[(NTP{\cdot}M)^{2-}]}{[M^{2+}][NTP^{4-}]} \quad (10)$$

Combining the last two equations with the definition of K_I [Eq. (8)] one obtains[40, 49] Equation (11):

$$K^{M}_{M(NTP)} = K^{M}_{(NTP{\cdot}M)}\,(1 + K_I) \quad (11)$$

This equation shows that the observed overall stability is equal to the stability constant for the open form augmented by the factor $(1 + K_I)$, which includes a contribution from the closed form.

We define the difference in the logarithms of the observed overall stability constants of the $M(PuNTP)^{2-}$ complexes and those of the $M(PyNTP)^{2-}$ species as given in Equation (12):

$$\log \Delta_{M(PuNTP)} = \log K^{M}_{M(PuNTP)} - \log K^{M}_{M(PyNTP)} \quad (12a)$$

$$= \log K^{M}_{M(NTP)} - \log K^{M}_{(NTP{\cdot}M)} \quad (12b)$$
$$= \log \Delta$$

The equality of the various terms in Equations (12a) and (12b) is evident. It is furthermore clear that an equation for the monoprotonated $M(PuNTP{\cdot}H)^{-}$ complexes can be written analogously:

$$\log \Delta_{M(PuNTP{\cdot}H)} = \log K^{M}_{M(PuNTP{\cdot}H)} - \log K^{M}_{M(PyNTP{\cdot}H)} \quad (13)$$

The values according to Equations (13) and (12) are summarized in Table 3 for the GTP and ITP systems. In all instances $\log \Delta_{M(PuNTP)}$ values larger than zero are observed. It is remarkable that the corresponding values for the monoprotonated $M(PuNTP{\cdot}H)^{-}$ complexes (Table 3, column 5) are quite similar, in fact mostly identical within the error limits, to those for $M(PuNTP)^{2-}$. This shows that intramolecular chelate formation occurs to about the same extent in both types of complexes. However, because of the large error limits of the $\log \Delta_{M(PuNTP{\cdot}H)}$ values, we shall concentrate the further evaluation on the $M(PuNTP)^{2-}$ systems.

Extent of macrochelate formation in $M(PuNTP)^{2-}$ systems: From the combination of Equations (11) and (12b) follows Equation (14):[40, 49]

$$K_I = \frac{K^{M}_{M(NTP)}}{K^{M}_{(NTP{\cdot}M)}} - 1 \quad (14a)$$

$$= 10^{\log \Delta} - 1 \quad (14b)$$

Knowledge of K_I allows the percentage of the closed or macrochelated form to be calculated as given by Equation (15):

$$\%\ (N{\cdot}M{\cdot}TP)^{2-} = 100\,K_I/(1 + K_I) = \%\ M(PuNTP)^{2-}_{cl} \quad (15)$$

Table 4 lists the results of $\log \Delta_{M(PuNTP)}$, K_I, and $\%\ M(PuNTP)^{2-}_{cl}$ for the GTP^{4-} and ITP^{4-} complexes with each of the ten metal ions studied. The previous results for $M(ATP)^{2-}$ complexes[32] are given in a revised form for comparison.

The situation in which $\log \Delta_{M(PuNTP)}$ and K_I [Eqs. (12) and (14)] both equal zero, which indicates that no macrochelate

Table 3. Comparison of the stability constants of the $M(PuNTP{\cdot}H)^{-}$ and $M(PuNTP)^{2-}$ complexes with those of the corresponding $M(PyNTP{\cdot}H)^{-}$ and $M(PyNTP)^{2-}$ species having only M^{2+}-phosphate coordination together with the resulting stability differences $\log \Delta_{M(PuNTP{\cdot}H)}$ [Eq. (13)] and $\log \Delta_{M(PuNTP)}$ [Eq. (12)] (25 °C; $I = 0.1$ M, $NaNO_3$ or $NaClO_4$).[a]

$PuNTP^{4-}$	M^{2+}	$\log K^{M}_{M(PuNTP{\cdot}H)}$[b]	$\log K^{M}_{M(PyNTP{\cdot}H)}$[c]	$\log \Delta_{M(PuNTP{\cdot}H)}$	$\log K^{M}_{M(PuNTP)}$[d]	$\log K^{M}_{M(PyNTP)}$[e]	$\log \Delta_{M(PuNTP)}$
GTP^{4-}	Mg^{2+}	2.6 ±0.3	2.3 ±0.2	0.3 ±0.35	4.31 ± 0.04	4.21 ± 0.04	0.10 ± 0.06
	Ca^{2+}	2.6 ±0.3	2.2 ±0.2	0.4 ±0.35	3.96 ± 0.03	3.84 ± 0.05	0.12 ± 0.06
	Sr^{2+}	2.65 ± 0.2	2.15 ± 0.2	0.5 ±0.3	3.55 ± 0.04	3.34 ± 0.05	0.21 ± 0.06
	Ba^{2+}	2.65 ± 0.2	2.1 ±0.2	0.55 ± 0.3	3.41 ± 0.03	3.18 ± 0.04	0.23 ± 0.05
	Mn^{2+}	3.36 ± 0.16	2.70 ± 0.12	0.66 ± 0.20	5.36 ± 0.03	4.93 ± 0.03	0.43 ± 0.04
	Co^{2+}	3.50 ± 0.05	2.55 ± 0.24	0.95 ± 0.25	5.34 ± 0.05	4.76 ± 0.03	0.58 ± 0.06
	Ni^{2+}	3.69 ± 0.05	2.51 ± 0.25	1.2 ±0.25	5.42 ± 0.04	4.50 ± 0.03	0.92 ± 0.05
	Cu^{2+}	4.6 ±0.2	2.80 ± 0.08	1.8 ±0.2	7.38 ± 0.08	5.86 ± 0.03	1.52 ± 0.08
	Zn^{2+}	3.45 ± 0.25	2.73 ± 0.09	0.7 ±0.25	5.52 ± 0.05	5.02 ± 0.02	0.50 ± 0.05
	Cd^{2+}	3.92 ± 0.08	2.89 ± 0.06	1.03 ± 0.10	5.82 ± 0.05	5.07 ± 0.03	0.75 ± 0.06
ITP^{4-}	Mg^{2+}	2.4 ±0.25	2.3 ±0.2	0.1 ±0.3	4.29 ± 0.04	4.21 ± 0.04	0.08 ± 0.06
	Ca^{2+}	2.4 ±0.25	2.2 ±0.2	0.2 ±0.3	3.93 ± 0.05	3.84 ± 0.05	0.09 ± 0.07
	Sr^{2+}	2.3 ±0.25	2.15 ± 0.2	0.15 ± 0.3	3.42 ± 0.10	3.34 ± 0.05	0.08 ± 0.11
	Ba^{2+}	2.3 ±0.25	2.1 ±0.2	0.2 ±0.3	3.28 ± 0.09	3.18 ± 0.04	0.10 ± 0.10
	Mn^{2+}	3.1 ±0.3	2.70 ± 0.12	0.4 ±0.3	5.21 ± 0.06	4.93 ± 0.03	0.28 ± 0.07
	Co^{2+}	3.0 ±0.3	2.55 ± 0.24	0.45 ± 0.4	5.08 ± 0.07	4.76 ± 0.03	0.32 ± 0.08
	Ni^{2+}	3.0 ±0.4	2.51 ± 0.25	0.5 ±0.45	5.01 ± 0.10	4.50 ± 0.03	0.51 ± 0.10
	Cu^{2+}	3.9 ±0.4	2.80 ± 0.08	1.1 ±0.4	6.71 ± 0.10	5.86 ± 0.03	0.85 ± 0.10
	Zn^{2+}	3.1 ±0.3	2.73 ± 0.09	0.35 ± 0.3	5.32 ± 0.06	5.02 ± 0.02	0.30 ± 0.06
	Cd^{2+}	3.55 ± 0.25	2.89 ± 0.06	0.65 ± 0.25	5.62 ± 0.05	5.07 ± 0.03	0.55 ± 0.06

[a] For the error limits see footnote [b] of Table 1. [b] From column 3 of Table 1. [c] From column 3 of Table 2. [d] From column 4 of Table 1. [e] From column 4 of Table 2.

Table 4. Increased complex stability, $\log \Delta_{M(PuNTP)}$ [Eq. (12)], and extent of chelate formation [Eq. (1)] in the $M(GTP)^{2-}$, $M(ITP)^{2-}$, and $M(ATP)^{2-}$ complexes; as quantified by the dimensionless equilibrium constant K_I [Eqs. (8) and (14)] and the percentage of $M(PuNTP)^{2-}_{cl}$ [Eq. (15)] for aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$ or $NaClO_4$).[a]

PuNTP^{4-}	M^{2+}	$\log \Delta_{M(PuNTP)}$[b]	K_I	% $M(PuNTP)^{2-}_{cl}$
GTP^{4-}	Mg^{2+}	0.10 ± 0.06	0.26 ± 0.17	21 ± 11
	Ca^{2+}	0.12 ± 0.06	0.32 ± 0.18	24 ± 10
	Sr^{2+}	0.21 ± 0.06	0.62 ± 0.22	38 ± 9
	Ba^{2+}	0.23 ± 0.05	0.70 ± 0.20	41 ± 7
	Mn^{2+}	0.43 ± 0.04	1.69 ± 0.25	63 ± 3
	Co^{2+}	0.58 ± 0.06	2.80 ± 0.53	74 ± 4
	Ni^{2+}	0.92 ± 0.05	7.32 ± 0.96	88 ± 1
	Cu^{2+}	1.52 ± 0.08	32.11 ± 6.10	97 ± 1
	Zn^{2+}	0.50 ± 0.05	2.16 ± 0.36	68 ± 4
	Cd^{2+}	0.75 ± 0.06	4.62 ± 0.78	82 ± 2
ITP^{4-}	Mg^{2+}	0.08 ± 0.06	0.20 ± 0.17	17 ± 11
	Ca^{2+}	0.09 ± 0.07	0.23 ± 0.20	19 ± 13
	Sr^{2+}	0.08 ± 0.11	0.20 ± 0.30	17 ± 21
	Ba^{2+}	0.10 ± 0.10	0.26 ± 0.29	21 ± 18
	Mn^{2+}	0.28 ± 0.07	0.91 ± 0.31	48 ± 8
	Co^{2+}	0.32 ± 0.08	1.09 ± 0.38	52 ± 9
	Ni^{2+}	0.51 ± 0.10	2.24 ± 0.75	69 ± 7
	Cu^{2+}	0.85 ± 0.10	6.08 ± 1.63	86 ± 3
	Zn^{2+}	0.30 ± 0.06	1.00 ± 0.28	50 ± 7
	Cd^{2+}	0.55 ± 0.06	2.55 ± 0.49	72 ± 4
ATP^{4-}	Mg^{2+}	0.08 ± 0.05[c]	0.20 ± 0.14	17 ± 10
	Ca^{2+}	0.07 ± 0.06[c]	0.17 ± 0.16	15 ± 12
	Mn^{2+}	0.08 ± 0.08[d]	0.20 ± 0.22	17 ± 15
	Co^{2+}	0.21 ± 0.09[d]	0.62 ± 0.34	38 ± 13
	Ni^{2+}	0.36 ± 0.06[d]	1.29 ± 0.32	56 ± 6
	Cu^{2+}	0.48 ± 0.04[d]	2.02 ± 0.28	67 ± 3
	Zn^{2+}	0.14 ± 0.06[d]	0.38 ± 0.19	28 ± 10
	Cd^{2+}	0.27 ± 0.04[d]	0.86 ± 0.17	46 ± 5

[a] For the error limits see footnote [b] of Table 1. [b] From column 8 of Table 3. [c] The values for $\log \Delta_{M(ATP)}$ for the Mg^{2+} and Ca^{2+} systems are based on $\log K^{M}_{M(ATP)}$ of Table II of ref.[32] (but with 3σ) and the revised values for $\log K^{M}_{M(PyNTP)}$ given in Table 2 in column 4. [d] These values are from Table IV of ref.[32] but now an error of 3σ is given.

forms, does not occur in Table 4. The greatest amount of macrochelate occurs with Cu^{2+}, followed (usually) by Ni^{2+}. For the four alkaline earth metal ions, the closed or macrochelated forms are lower in percentage than those of the transition metal ions; this is especially true for $M(ITP)^{2-}$ and $M(ATP)^{2-}$. For $M(GTP)^{2-}$, Ba^{2+} forms the macrochelate to about 40%, whereas that of Mg^{2+} occurs only to about 20%. A similarly high degree of formation is observed for Ba(GMP).[21] This may have to do with the availability of (C6)O in GTP^{4-}, allowing the formation of outersphere hydrogen bonds with a coordinated water molecule.[39]

For the six transition metal ions in Table 4, substantial amounts of macrochelate occur; for example, up to 97% macrochelate and only 3% open forms are observed for $Cu(GTP)^{2-}$. Generally, for a given metal ion, the percentage of macrochelate falls off in the order $(G \cdot M \cdot TP)^{2-} > (I \cdot M \cdot TP)^{2-} > (A \cdot M \cdot TP)^{2-}$. This order corresponds to the decreasing N7 basicity of GTP and ITP (see ref. [42]). The N7 basicities of the inosine and adenosine residues are comparable,[37] but M^{2+} binding at the N7 site of adenosine is hampered by the steric influence of the $(C6)NH_2$ group.[38] Hence, the above order is understandable and also in accord with the observations made for the complexes of nucleoside 5′-monophosphates.[21, 39]

Conclusions

Comparison of the present results obtained for the $M(GTP)^{2-}$ and $M(ITP)^{2-}$ complexes, also including the ones for $M(ATP)^{2-}$ (Table 4), with those obtained earlier[21, 39] for M(GMP), M(IMP), and M(AMP) (see data in ref. [21]) reveals that the formation degrees of the macrochelates are astonishingly similar for a given metal ion and purine-nucleobase residue. This is somewhat surprising considering that the triphosphate complexes are more stable than the monophosphate ones by about two to three log units.[48] On the other hand, it indicates that, for the extent of macrochelate formation, mainly the properties of the N7 site are responsible, at least as long as the coordination sphere of the metal ion considered is not yet saturated.

From potentiometric pH titrations only overall (global) stability constants can be obtained, and hence, different types of macrochelates cannot be distinguished. What is measured is the concentration of all complexes, including the sum of all possible macrochelated isomers. However, from the previous studies of $M(ATP)^{2-}$ complexes, it is well known that (at least) two types of macrochelates can form:[32, 33] one in which the phosphate-coordinated metal ion binds innersphere to N7 of the adenine residue and one in which this interaction is of an outersphere type, that is, with a water molecule between N7 and M^{2+} (see Figure 6 in ref. [20b]). A similar situation also occurs for $M(GTP)^{2-}$ and $M(ITP)^{2-}$. ^{1}H MNR shift experiments of the corresponding Mg^{2+} systems[35] gave no indication of macrochelate formation, which is proved to occur by the present results and in accord with those for $Mg(ATP)^{2-}$; hence, it must be concluded that $Mg(GTP)^{2-}_{cl}$ and $Mg(ITP)^{2-}_{cl}$ are of an outersphere type, and this is most probably also true for the other alkaline earth ions. From an early line-broadening study[50] of the Mn^{2+}/ITP system, it follows that at least some innersphere binding occurs with N7. Comparisons of the present results for the Zn^{2+} and Cd^{2+} complexes of GTP^{4-} and ITP^{4-} with those of a ^{1}H MNR shift study[35] indicate that macrochelate formation for the $M(GTP)^{2-}_{cl}$ and $M(ITP)^{2-}_{cl}$ species with these two metal ions is largely innersphere with N7 (Figure 3). It is evident that further detailed studies, either by NMR and/or spectrophotometry, are desirable to reveal the ratios of the macrochelated isomers for other metal ions as well. In addition, it should be noted that the (C6)O carbonyl group may also participate in outersphere metal ion binding as discussed in detail[39] for M(GMP) complexes.

The above conclusions concerning the M(NMP) and $M(NTP)^{2-}$ complexes showing that N7 of the guanine residue (see also Table 4) is an especially favored metal ion binding site are confirmed by observations made with nucleic acids. For example, *cis*-$(NH_3)_2Pt^{2+}$ interacts preferably with guanine-N7 sites of DNA.[51–53] It follows from observations made with nucleic acids[25, 54] including ribozymes,[55] that this also applies to labile metal ions like Mn^{2+} or Zn^{2+}.

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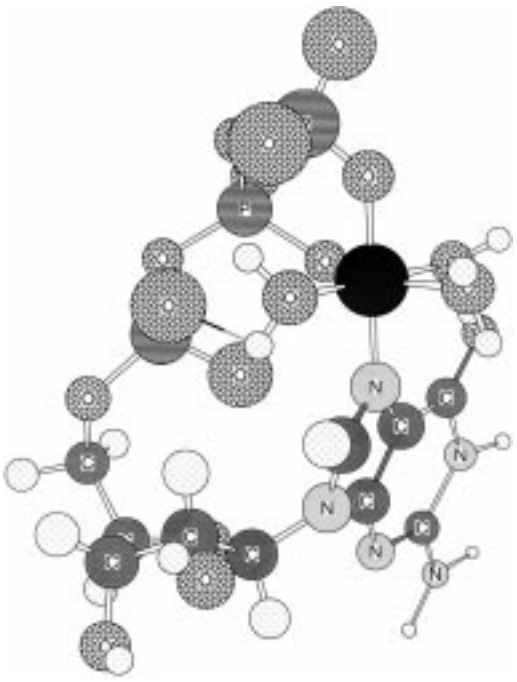

Figure 3. Simplified structure for one of the macrochelated innersphere $M(GTP)^{2-}$ isomers with a hexacoordinating metal ion; all unlabeled spheres are hydrogen atoms. The depicted M^{2+}-triphosphate coordination follows an earlier suggestion.[23] If intramolecular direct M^{2+}-N7 coordination occurs, then it is sterically more favorable to have a water molecule between M^{2+} and the α-phosphate group, though a simultaneous innersphere binding of both N7 and the α-phosphate group in complexes of purine-NMPs is possible.[39] Of course, other isomers that differ in the phosphate coordination are possible, for example, direct β,γ-phosphate and N7 coordination, which leaves the α group free. In the above structure, the equatorial positions of an octahedral coordination sphere could also have been used, thus giving rise to further isomers. In the present context, we only distinguish between macrochelated and open species, in which the metal ion is only coordinated to the phosphate, as it is expressed in Equilibrium (1). The above structure was drawn with the program Chem3D Pro (Version 5.0) from Cambridge Scientific Computing Inc.; the minimized and relaxed form is shown.

Experimental Section

Materials: The nitrate salts of Na^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}, HNO_3, NaOH (Titrisol), and potassium hydrogen phthalate (all pro analysi) were obtained from Merck AG, Darmstadt, Germany. All other reagents were the same as used recently.[42]

The titers of the NaOH solutions used for the titrations were established with potassium hydrogen phthalate. The exact concentrations of the stock solutions of the divalent metal ions were determined by using their EDTA complexes.

Potentiometric pH titrations: All the equipment used, including the potentiograph and computers, was the same as described, and the experiments were done as before.[42] The acidity constants (see ref. [42]) are so-called practical, mixed, or Brønsted constants.[56] 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 from the given pK_a values.[56a] This conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[56] No conversion is necessary for the stability constants of the metal ion complexes. The *differences* in NaOH consumption between solutions with and without ligand[56] (see below) are evaluated.

All experiments with the NTPs were done in such a way that dephosphorylation, which is metal ion promoted,[57] was kept to a minimum (see also next section).[58] There was no difference between the results of the re-evaluated data obtained from earlier experiments carried out in the presence of $NaClO_4$ (cf. ref. [27a]) and the present ones in which $NaNO_3$ was used as background electrolyte. The titrations for the Sr^{2+} and Ba^{2+} systems with UTP or CTP were carried out by a single person. All other measurements were performed independently by two (or even three) persons with intervals of years.

Determination of the stability constants: The stability constants $K^{M}_{M(NTP\cdot H)}$ [Eq. (11)] and $K^{M}_{M(NTP)}$ [Eq. (12)], in which $M^{2+} = Mg^{2+}$, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}, were determined for GTP and ITP under the same conditions as described for the acidity constants,[42] that is, two sets of conditions were used: i) $[HNO_3] = 10^{-3}$M, $[NTP] = 5 \times 10^{-4}$M, volume 50 mL, $[NaOH] = 0.05$M (1.5 mL), $I = 0.1$M ($NaNO_3$); ii) $[HClO_4] = 1.6 \times 10^{-3}$M, $[NTP] = 1.2 \times 10^{-3}$M, volume = 25 mL, $[NaOH] = 0.05$M (1 mL), $I = 0.1$M ($NaClO_4$). The titrations were done pairwise under N_2, that is, in the presence and absence of NTP (see above). Part of $NaNO_3$ or $NaClO_4$ was always replaced by $M(NO_3)_2$ or $M(ClO_4)_2$ ($I = 0.1$M, $NaNO_3$ or $NaClO_4$; 25 °C), the M^{2+}/NTP ratio being 1:1 throughout.

The stability constants of the Sr^{2+} and Ba^{2+} complexes of CTP and UTP [Eqs. (11) and (12)] were determined with $[HNO_3] = 1.9 \times 10^{-3}$M, $[NTP] = 5 \times 10^{-4}$M, volume = 50 mL, and $[NaOH] = 0.05$M (2 mL); the M^{2+}/NTP ratios again being 1:1 ($I = 0.1$M, $NaNO_3$; 25 °C).

As divalent metal ions promote the dephosphorylation of NTPs, although with a different effectiveness,[57] the two reactants were only mixed in the last minute before the titration, which was usually completed within 15 minutes. In this way, dephosphorylation of the nucleoside 5′-triphosphates was minimized. The stability constants $K^{M}_{M(NTP\cdot H)}$ (see also below) and $K^{M}_{M(NTP)}$ were calculated for each pair of titrations with the equipment used previously[42] and a curve-fitting procedure by taking into account the species H^+, $H_2(NTP)^{2-}$, $H(NTP)^{3-}$, NTP^{4-}, M^{2+}, $M(NTP\cdot H)^-$, and $M(NTP)^{2-}$.[59] The data were collected every 0.1 pH unit from either the lowest pH that could be reached in the experiment or from a formation degree of about 5% for $M(NTP)^{2-}$ to either the beginning of the hydrolysis of M^{2+}_{aq} (e.g., with Cu^{2+} or Zn^{2+}), which was evident from the titrations without ligand, or the beginning of the formation of $M(NTP{-}H)^{3-}$ complexes (e.g., with Ca^{2+} and GTP), or a formation degree corresponding in total to about 90% for $H(NTP)^{3-}$.

The stability constants $K^{M}_{M(NTP\cdot H)}$ could only be determined for the M^{2+}/CTP and, in part, for the M^{2+}/GTP systems because $pK^{H}_{H_2(NTP)}$ is very low, especially for ITP and UTP. Therefore, for these systems only estimates, with relatively large errors, for the stabilities of $M(NTP\cdot H)^-$ were possible; these are in part based on our experience with related ligands.[21, 32, 48] Since the value of $K^{M}_{M(NTP\cdot H)}$ can have some effect on the results for $K^{M}_{M(NTP)}$, this effect was considered in the error limits of the latter constants, especially in the case of the ITP complexes, by keeping $K^{M}_{M(NTP\cdot H)}$ constant—once with the lower and once with the upper error limit—and by repeating the calculations for $K^{M}_{M(NTP)}$. However, this effect is not overwhelming because the degree of formation of the $M(NTP\cdot H)^-$ species is relatively low in the pH range where that of the $M(NTP)^{2-}$ complexes is high.

The final stability constants in the tables are the results from the averages of at least eight independent pairs of titrations, on average ten titration pairs were carried out for each system.

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