On the Metal-Ion-Coordinating Properties of the Benzimidazolate Residue in Aqueous Solution – Extent of Acidification of Benzimidazole-(N3)H Sites by (N1)-Coordinated Divalent Metal Ions

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The stability constants of the 1:1 complexes formed between Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+} or Cd^{2+} (= M^{2+}) and the anionic 5(6)-nitrobenzimidazolate [= $(NBI–H)^-$] or 5,6-dinitrobenzimidazolate [= $(DNBI–H)^-$] were determined by potentiometric pH titrations in aqueous solution (25 °C; I = 0.5 M, $NaNO_3$). The acidity constants for the deprotonation of the (N1)H sites in neutral NBI and DNBI (= L) were measured by the same method. The comparison of the stability constants determined for the $M(L–H)^+$ complexes with those calculated from log K^M_{ML} versus pK^H_{HL} straight-line plots, which were established recently for neutral benzimidazole-type ligands, reveals that the stabilities of the $M(L–H)^+$ complexes are significantly enhanced, as one might have expected due to the negative charge present on the ligands. Of course, the electron-withdrawing properties of (N3)-bound metal ions facilitate the release of the proton from the (N1)H site in the $M(NBI)^{2+}$ and $M(DNBI)^{2+}$ complexes, if compared to the situation in the free ligands. The effect of a metal ion bound to a benzimidazolate or imidazolate residue on the coordination tendency toward a further metal ion (giving rise to an imidazolate bridge) was estimated.

1. Introduction

Benzimidazole (1,3-dideazapurine) and its derivatives show biological activity[1–6] and are often employed in drug design.[1][2] For example, nitrobenzimidazoles act as substrates for the two-electron reduction catalyzed by DT-diaphorase,[3] and 5,6-dinitrobenzimidazole and related compounds are substrates for purine nucleoside phosphorylase which allows the synthesis of nucleoside analogues.[4] Other nitrobenzimidazoles are studied for their analgesic and anti-inflammatory activity[5] as well as for their antiproliferative and antiviral activity.[6] Since benzimidazole derivatives act biologically often via their nucleoside[4] and nucleotide[1] analogues, the metal-ion-binding properties of the benzimidazole residue are of general interest because such reactions usually also involve metal ions.[7–9]

In our attempts[10] to increase the understanding of the metal-ion-binding dichotomy[11] of the adenine residue, we have recently correlated[12] the acid-base and metal-ion-binding properties of a series of benzimidazoles by constructing log K^M_{ML} versus pK^H_{HL} plots which result for a given metal ion in a straight line (see Section 2.2). In this study[12] we had included 5(6)-nitrobenzimidazole (NBI) and 5,6-dinitrobenzimidazole (DNBI) because they release the proton from their monoprotonated forms, $H(NBI)^+$ and $H(DNBI)^+$, with a low pK_a, i.e. $pK^H_{H(NBI)} = 3.61$ and $pK^H_{H(DNBI)} = 1.72$,[12] which allowed us to extend the indicated correlation far into the acidic pH range. Considering that the deprotonation of monoprotonated benzimidazole, $H(BI)^+$, occurs with $pK^H_{H(BI)} = 5.63$[12] and the formation of the corresponding anion $(BI-H)^-$ with $pK^H_{BI} = 12.8$,[13] which means that the two pK_a values are separated by $\Delta pK_a \approx 7$,[13] one may expect that the pK_a values of NBI and DNBI differ by about the same extent, and thus, the formation of their anions is expected to occur in the pK_a range of about 8.5 to 10.5. The acid-base equilibrium for their neutral and anionic forms is depicted in Figure 1.

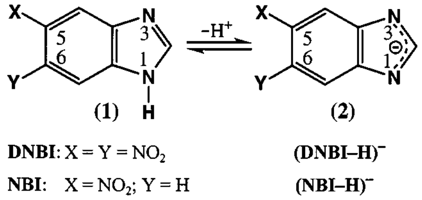

Figure 1. Chemical structures of 5(6)-nitrobenzimidazole (NBI) and 5,6-dinitrobenzimidazole (DNBI) in their neutral (**1**) and anionic forms (**2**)

A pK_a range of 8.5 to 10.5 is easily accessible for potentiometric pH titrations and therefore also suitable for the determination of stability constants. Surprisingly, no systematic study focussing on the stability of metal ion complexes in aqueous solution appears to have been carried out[14–16] for benzimidazolate or imidazolate and related ligands, even though the latter is well known to occur as a bridging residue between two metal ions in Cu,Zn superoxide dismutase[17][18] and also in a number of (low-molecular-weight) complexes studied in solution[19–21] and in the solid state;[21][22] actually quite a number of complexes formed with deprotonated imidazole derivatives and a variety of metal ions have been isolated and their properties studied (e.g.[21–23]). Interestingly, already about 60 years ago the formation of an imidazolate complex was suggested

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to occur with ferrihemoglobin[24] and the possibility to deprotonate the pyrrole nitrogen in imidazole complexes has been confirmed in the years thereafter.[25] However, with the deficit indicated above in mind we endeavored now to measure the stability constants of the complexes formed between Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, or Cd^{2+} (M^{2+}) and 5(6)-nitrobenzimidazolate or 5,6-dinitrobenzimidazolate (Figure 1).

2. Results and Discussion

2.1. Definition of Equilibria and Results

Benzimidazole and its derivatives (L) can accept one proton at N3 to give the monoprotonated species HL^+ which gives rise to the deprotonation reaction according to Equilibrium 1a.

$$HL^+ \rightleftharpoons H^+ + L \quad (1a)$$

$$K^H_{HL} = [H^+][L]/[HL^+] \quad (1b)$$

The corresponding acidity constants for $H(NBI)^+$ and $H(DNBI)^+$ have recently been determined: $pK^H_{H(NBI)} = 3.61 \pm 0.02$ and $pK^H_{H(DNBI)} = 1.72 \pm 0.06$.[12] However, as indicated already in Section 1, one expects that the proton present at N1 in the neutral form of NBI and DNBI (Figure 1) can also be ionized according to Equilibrium 2.

$$L \rightleftharpoons (L-H)^- + H^+ \quad (2a)$$

$$K^H_L = [(L-H)^-][H^+]/[L] \quad (2b)$$

Indeed, the experiments carried out now (25°C; $I = 0.5$ M,[26] $NaNO_3$) prove this to be correct; the corresponding acidity constants are $pK^H_{NBI} = 10.58 \pm 0.03$ and $pK^H_{DNBI} = 8.92 \pm 0.03$. The present acidity constant for the deprotonation of NBI is in fair accord with earlier spectrophotometric determinations which gave $pK^H_{NBI} = 10.69$ (25°C)[27] and 10.86 (25°C; $I = 0.1$ M)[13] as well as potentiometric titrations which resulted in $pK^H_{NBI} = 10.31$ (25°C)[28][29] and 11.2.[28][30]

$$\Delta pK_a = pK^H_L - pK^H_{HL} \quad (3)$$

The difference defined in Equation 3 for the NBI and DNBI systems equals $\Delta pK_{a/NBI} = (10.58 \pm 0.03) - (3.61 \pm 0.02) = 6.97 \pm 0.04$ and $\Delta pK_{a/DNBI} = (8.92 \pm 0.03) - (1.72 \pm 0.06) = 7.20 \pm 0.07$. Hence, the acidity constants of the Equilibria 1a and 2a can be determined independently for both ligands because the buffer regions are not overlapping. The ΔpK_a values of about 7 agree with previous observations made for benzimidazole derivatives.[13]

Both, the neutral species L and the deprotonated species $(L-H)^-$ may form complexes with metal ions (M^{2+}) according to Equilibria 4a and 5a.

$$M^{2+} + L \rightleftharpoons ML^{2+} \quad (4a)$$

$$K^M_{ML} = [ML^{2+}]/([M^{2+}][L]) \quad (4b)$$

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

$$K^M_{M(L-H)} = [M(L-H)^+]/([M^{2+}][(L-H)^-]) \quad (5b)$$

The stability constants for the $M(NBI)^{2+}$ complexes have recently been determined by potentiometric pH titrations and those for the $M(DNBI)^{2+}$ complexes by spectrophotometry.[12] These previous results, which are listed in column 2 of Table 1, have been taken into account in the evaluations of the potentiometric pH titrations which were carried out now to determine the stability constants of the $M(NBI-H)^+$ and $M(DNBI-H)^+$ complexes (Equation 5). The corresponding results are given in column 3 of Table 1. No values for the complexes with Cu^{2+} and Zn^{2+} could be determined because of their strong tendency to form hydroxo species.

Equilibria 4a and 5a are connected via Equilibrium 6a and the corresponding acidity constant can be calculated according to Equation 7.

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

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

$$pK^H_{ML} = pK^H_L + \log K^M_{ML} - \log K^M_{M(L-H)} \quad (7)$$

These acidity constants are listed in column 4 of Table 1. They allow to quantify the effect of a (N3)-bound metal ion on the deprotonation of the (N1)H site by defining the difference between the two relevant acidity constants according to Equation 8.

$$\Delta pK_{a/ML} = pK^H_L - pK^H_{ML} \quad (8)$$

These differences are given in column 5 of Table 1; they are of the order observed previously for imidazole complexes.[25] Since the error limits for the $\Delta pK_{a/ML}$ values are relatively large, no conclusions can be drawn for the $M(NBI)^{2+}$ and $M(DNBI)^{2+}$ complexes of Mg^{2+}, Mn^{2+} and Ni^{2+} regarding any possible differences in the acidification. However, for Co^{2+} and Cd^{2+} the acidification is clearly more pronounced in the $M(NBI)^{2+}$ complexes. This means that, relatively speaking, the deprotonation at the (N1)H site leads to a more significant stability increase in the case of the NBI complexes compared to the DNBI ones. This conclusion will also be confirmed below (see the final paragraph in Section 2.2).

2.2. Metal-Ion-Binding Properties of the Benzimidazolate Residue

To which extent does the deprotonation of the (N1)H site (see Figure 1) affect the stability of the complexes? An answer to this question may be obtained by applying the recently constructed $\log K^M_{ML}$ versus pK^H_{HL} plots, where L represents a series of benzimidazole-type ligands.[12] Three examples are shown in Figure 2 and the pertinent parameters which define the straight lines are listed in Table 2 for those metal ions considered in the present study.

Table 1. Logarithms of the stability constants of the ML^{2+} (Equation 4) and $M(L-H)^+$ complexes (Equation 5), together with the negative logarithms of the acidity constants (Equations 6 and 7) of the corresponding ML^{2+} complexes, where L = NBI or DNBI, as determined by potentiometric pH titrations in aqueous solution at 25°C and $I = 0.5$ M ($NaNO_3$)[a–c]

M^{2+}	$\log K^M_{ML}$	$\log K^M_{(ML-H)}$	pK^H_{ML}	$\Delta pK_{a/ML}$[d]
5(6)-Nitrobenzimidazole (NBI)[a]				
Mg^{2+}	−0.09 ± 0.15	0.41 ± 0.10	10.08 ± 0.18	−0.50 ± 0.18
Mn^{2+}	0.37 ± 0.07	2.22 ± 0.14	8.73 ± 0.16	−1.85 ± 0.16
Co^{2+}	1.25 ± 0.02	3.67 ± 0.17	8.16 ± 0.17	−2.42 ± 0.17
Ni^{2+}	1.63 ± 0.05	4.1 ± 0.4	8.1 ± 0.4	−2.5 ± 0.4
Cd^{2+}	1.51 ± 0.05	4.63 ± 0.16	7.46 ± 0.17	−3.12 ± 0.17
5,6-Dinitrobenzimidazole (DNBI)[a]				
Mg^{2+}	−0.11 ± 0.05[e]	0.62 ± 0.08	8.19 ± 0.10	−0.73 ± 0.10
Mn^{2+}	0.08 ± 0.05[e]	1.85 ± 0.04	7.15 ± 0.07	−1.77 ± 0.08
Co^{2+}	0.84 ± 0.07[f]	2.73 ± 0.06	7.03 ± 0.10	−1.89 ± 0.10
Ni^{2+}	1.23 ± 0.10[f]	3.31 ± 0.16	6.84 ± 0.19	−2.08 ± 0.19
Cd^{2+}	0.96 ± 0.09[f]	3.34 ± 0.17	6.54 ± 0.19	−2.38 ± 0.19

[a] Acidity constants: $pK^H_{H(NBI)} = 3.61 \pm 0.02$ (Equation 1; from ref.[12]), $pK^H_{NBI} = 10.58 \pm 0.03$ (Equation 2); $pK^H_{H(DNBI)} = 1.72 \pm 0.06$ (Equation 1; from ref.[12])[f], $pK^H_{DNBI} = 8.92 \pm 0.03$ (Equation 2). – [b] The error limits 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 present case for columns 4 and 5) were calculated according to the error propagation after Gauss. Regarding the reasons why in some instances the errors are rather large, the Experimental Section should be consulted. – [c] The values in the second column are taken from ref.[12] – [d] See Equation 8 in Section 2.1. – [e] These values were experimentally not accessible (see ref.[12]); they were calculated with the straight-line equations defined in Table 2 and the micro acidity constant $pk_{ma/H(DNBI)} = 2.02$ which follows from $pK^H_{H(DNBI)} = 1.72$; see ref.[12] – [f] These values were measured by spectrophotometry (see ref.[12]).

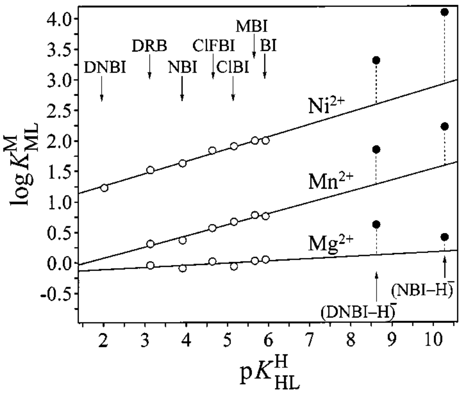

Figure 2. Evidence for an increased stability of several M^{2+} 1:1 complexes of 5,6-dinitrobenzimidazolate, $(DNBI-H)^-$, and 5(6)-nitrobenzimidazolate, $(NBI-H)^-$, (•) (see structure **2** in Figure 1), based on $\log K^M_{ML}$ versus pK^H_{HL} plots for the 1:1 complexes of Mg^{2+}, Mn^{2+} and Ni^{2+} with the following neutral benzimidazole-type ligands (o): 5,6-dinitrobenzimidazole (DNBI; see **1** in Figure 1), 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB), 5(6)-nitrobenzimidazole (NBI; see **1** in Figure 1), 6-chloro-5-fluorobenzimidazole (ClFBI), 5(6)-chlorobenzimidazole (ClBI), 1-methylbenzimidazole (MBI) and benzimidazole (BI) (from left to right). The corresponding equilibrium constants are taken from ref.[12]; note, where appropriate the micro acidity constants (pk_{am}) were applied (for details see ref.[12]); the parameters for the least-squares lines drawn through these data sets are given in Table 2; the vertical broken lines emphasize the stability differences to the corresponding reference lines (Equation 11); these differences are listed in column 4 of Table 3; the data pairs for the $M(DNBI-H)^+$ and $M(NBI-H)^+$ complexes are from Table 1 and Equations 9 and 10; all plotted equilibrium constants refer to aqueous solutions at 25°C and $I = 0.5$ M ($NaNO_3$).

Table 2. Straight-line correlations for M^{2+}-benzimidazole-type complex stabilities and benzimidazole group basicities,[a,b] for aqueous solutions at 25°C and $I = 0.5$ M ($NaNO_3$)

M^{2+}	*m*	*b*	*SD*
Mg^{2+}	0.035 ± 0.020	−0.179 ± 0.095	0.051
Mn^{2+}	0.182 ± 0.017	−0.287 ± 0.081	0.045
Co^{2+}	0.186 ± 0.011	0.513 ± 0.048	0.042
Ni^{2+}	0.201 ± 0.011	0.860 ± 0.052	0.042
Cd^{2+}	0.293 ± 0.011	0.385 ± 0.050	0.039

[a] The slopes (*m*) and intercepts (*b*) for the straight baselines from plots of $\log K^M_{ML}$ versus pK^H_{HL} were calculated[12] from equilibrium constants involving the seven (or six in the case of Mg^{2+} and Mn^{2+}) sterically unhindered ligands (L) which are named in Figure 2 (see also ref.[12]). The fourth column lists *three times* the standard deviations (*SD*) resulting from the differences between the experimental and calculated values for the seven (or six) ligand systems. – [b] The above data for *m*, *b* and *SD* are abstracted from Tables 3 and 4 in ref.[12] Straight-line equation: $y = mx + b$, where x represents the pK_a value of any monoprotonated benzimidazole-type ligand and y the calculated stability constant (log *K*) of the corresponding ML complex; the errors given with *m* and *b* correspond to one standard deviation (1 σ). The listed *SD* values (3 σ; column 4) are considered as reasonable error limits for any stability constant calculation in the pK_a range 2–6; for the error limits outside of this range see Section 2.2.

For a comparison of the stability constants determined now with these straight lines, the acid-base properties of NBI and DNBI need to be considered in detail: Once the corresponding anion $(L-H)^-$ has formed, there are two equal ways for the formation of the neutral species L (Figure 1). Consequently, the benzimidazolate derivatives appear to be more basic than they actually are. This means, micro acidity constants as defined in Equations 9 and 10 for DNBI and NBI, in which this statistical effect is considered, need to be used in any kind of acid-base comparisons.

$$pk_{ma/DNBI} = pK^H_{DNBI} - 0.3 = (8.92 \pm 0.03) - 0.3 = 8.62 \pm 0.03 \quad (9)$$

$$pk_{ma/NBI} = pK^H_{NBI} - 0.3 = (10.58 \pm 0.03) - 0.3 = 10.28 \pm 0.03 \quad (10)$$

It may be added that this reasoning is also valid (see section 2.2 of ref.[12]) for 5(6)-nitrobenzimidazole which has non-equivalent substituents at C5 and C6 (Figure 1).

Table 3. Comparison of the logarithms of the stability constants of $M(NBI-H)^+$ and $M(DNBI-H)^+$ complexes (Equation 5) as determined by potentiometric pH titrations (exptl.)[a] in aqueous solution at 25°C and I = 0.5 M ($NaNO_3$) with the calculated (calcd.) stability constants based on the basicity[b] of the benzimidazolate nitrogen atoms and the reference-line equations of Table 2.

M^{2+}	$\log K^{M}_{M(L-H)}$ exptl.[a]	$\log K^{M}_{M(L-H)}$ calcd.[b]	$\log \Delta$[c]
5(6)-Nitrobenzimidazolate, $(NBI-H)^-$			
Mg^{2+}	0.41 ± 0.10	0.18 ± 0.27	0.23 ± 0.29
Mn^{2+}	2.22 ± 0.14	1.58 ± 0.23	0.64 ± 0.27
Co^{2+}	3.67 ± 0.17	2.43 ± 0.21	1.24 ± 0.27
Ni^{2+}	4.1 ± 0.4	2.93 ± 0.22	1.2 ± 0.5
Cd^{2+}	4.63 ± 0.16	3.40 ± 0.22	1.23 ± 0.27
5,6-Dinitrobenzimidazolate, $(DNBI-H)^-$			
Mg^{2+}	0.62 ± 0.08	0.12 ± 0.16	0.50 ± 0.18
Mn^{2+}	1.85 ± 0.04	1.28 ± 0.14	0.57 ± 0.15
Co^{2+}	2.73 ± 0.06	2.12 ± 0.12	0.61 ± 0.13
Ni^{2+}	3.31 ± 0.16	2.59 ± 0.14	0.72 ± 0.21
Cd^{2+}	3.34 ± 0.17	2.91 ± 0.13	0.43 ± 0.21

[a] These values are from column 3 in Table 2. – [b] Calculated with the micro acidity constants $pk_{ma/NBI} = 10.28$ and $pk_{ma/DNBI} = 8.62$ (see Equations 9 and 10 and text in Section 2.2) and the straight-line equations given in Table 2. These error limits are estimates; see ref.[31] – [c] The error limits for these differences were calculated according to the error propagation after Gauss.

Application of the micro acidity constants of Equations 9 and 10 and of the stability constants of the $M(NBI-H)^+$ and $M(DNBI-H)^+$ complexes leads to the solid data points seen in Figure 2. Their vertical distances to the corresponding reference lines defined for neutral benzimidazole complexes reflect the effect of the negative charge of the benzimidazolates. This stability increase is defined by Equation 11, where $\log K^{M}_{M(L\text{-}H)expt1}$ represents the experimentally measured stability constant and $\log K^{M}_{M(L\text{-}H)calcd}$ the one calculated with the micro acidity constants (Equations 9 and 10) and the straight-line parameters of Table 2. The corresponding results are summarized in Table 3.[31]

$$\log \Delta = \log K^{M}_{M(L-H)expt1} - \log K^{M}_{M(L-H)calcd} \qquad (11)$$

The stability enhancements $\log \Delta$ (Equation 11), listed in column 4 of Table 3, indicate that the $M(NBI-H)^+$ and $M(DNBI-H)^+$ complexes are by about 0.2 to 1.2 log units more stable than complexes formed with neutral benzimidazoles of the same pK_a range. Comparison of these $\log \Delta$ values reveals that in the case of Mg^{2+}, Mn^{2+} and Ni^{2+} the error limits are too large for meaningful conclusions, whereas for the complexes with Co^{2+} and Cd^{2+} it is certain that the stability enhancement for $(NBI-H)^-$ is more pronounced than that for $(DNBI-H)^-$ (see Table 3). Hence, one may conclude that the slopes of $\log K^{M}_{M(L-H)}$ versus pK^{H}_{L} plots are somewhat larger than those of $\log K^{M}_{ML}$ versus pK^{H}_{HL} seen in Figure 2. Based on the stability enhancements observed for the $M(DNBI-H)^+$ complexes, which have relatively low error limits (Table 3, column 4), it appears that the extent of the stability enhancement is relatively independent of the metal ion considered.

3. Conclusions

The present study reveals that a metal ion bound to N3 of a benzimidazole residue acidifies the proton at the (N1)H site by about 0.5 to 3 pK_a units depending on the metal ion considered. The resulting benzimidazolate complexes are more stable than those formed with the corresponding neutral benzimidazoles. Clearly, it is safe to assume that this also holds for imidazole and imidazolate complexes.

How do two metal ions bound to the same imidazolate or benzimidazolate residue affect each other, as, e.g., in Cu,Zn superoxide dismutase[17][18] where an imidazolate residue bridges the two metal ions? Our results show, ignoring the one for $Mg(NBI-H)^+$ (Table 3), that the creation of a negative charge (−1) on the ligand leads on average to a stability enhancement of about 0.5−1.2 log units for the binding of the first metal ion at one of the nitrogens. Thus, in a first approximation one may assume that binding of the second metal ion at the other nitrogen atom is inhibited due to charge repulsion of the now positively (+1) charged liganding unit by approximately the same extent. Hence, an estimation of the stability constant, $\log K^{M}_{M_2(L-H)}$, for $M_2(L-H)^{3+}$ complexes is obtained by subtracting from the stability constants, $\log K^{M}_{ML}$, valid for the ML^{2+} complexes (Table 1)[12] the mentioned 0.5−1.2 log units. This then means that the stabilities of the $M(L-H)^+$ and $M_2(L-H)^{3+}$ complexes differ overall by about 1 to 2.4 log units. This conclusion also explains why under our experimental conditions no $M_2(L-H)^{3+}$ complexes were observed.

Experimental Section

1. Materials, Apparatus, and Definitions: The same reagents, experimental equipment and computers for the calculation procedures were used as previously.[12] The acidity constants determined are so-called practical, mixed or Brønsted constants.[26] Their negative logarithms given for aqueous solution at I = 0.5 M ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.03 from the pK_a values listed in the tables.[26] The ionic product of water (K_w) does not enter into the calculations because the differences in NaOH consumption between solutions with and without ligand (see below) are evaluated. The stability constants presented are, as usual, concentration constants.

2. Determination of Equilibrium Constants: The acidity constants $K^{H}_{H(NBI)}$ (see also ref.[12]) and K^{H}_{NBI} of NBI were determined by titrating 25 mL of 7.2 mM HNO_3 (I = 0.5 M, $NaNO_3$; 25 °C) in the presence and absence of 2.5 mM 5(6)-nitrobenzimidazole with 3 mL of 0.1 M NaOH. In the calculations of the two pK_a values for this ligand (L) the species H^+, HL^+, L and $(L-H)^-$ were considered.[12] Since the solubility of 5,6-dinitrobenzimidazole is low, more diluted solutions had to be used in this case, i.e., the acidity constant K^{H}_{DNBI} of DNBI was measured by titrating 25 mL of 0.15 mM HNO_3 (I = 0.5 M, $NaNO_3$) in the presence and absence of 0.24 mM DNBI with 1 mL of 0.01 M NaOH and calculated by taking into account the species H^+, L and $(L-H)^-$.[12] The final results for the acidity constants K^{H}_{L} of NBI and DNBI are the averages of 34 and 21 pairs of titrations, respectively.

The stability constants $K^{M}_{M(L-H)}$ of the $M(NBI-H)^{+}$ and $M(DNBI-H)^{+}$ complexes were determined under the same conditions as the acidity constants, but $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ ($I = 0.5$ M; 25 °C). For the systems with NBI the following concentrations of $M(NO_3)_2$ were employed: 0.1667 M (M^{2+}/L = 67:1 for Mg^{2+} and Mn^{2+}), 0.0417 M (M^{2+}/L = 17:1 for Co^{2+} and Ni^{2+}), 0.0333 M (M^{2+}/L = 13:1 for Cd^{2+}), 0.0208 M (M^{2+}/L = 8:1 for Ni^{2+} and Cd^{2+}), and 0.0104 M (M^{2+}/L = 4:1 for Ni^{2+}). Similarly, in the case of DNBI the following $M(NO_3)_2$ concentrations were used: 0.1667 M (M^{2+}/L = 695:1 for Mg^{2+} and Mn^{2+}), 0.0833 M (M^{2+}/L = 347:1 for Mn^{2+}), 0.0208 M (M^{2+}/L = 87:1 for Co^{2+} and Ni^{2+}), 0.0167 M (M^{2+}/L = 70:1 for Co^{2+}), 0.0104 M (M^{2+}/L = 43:1 for Co^{2+}), 0.00667 M (M^{2+}/L = 28:1 for Ni^{2+} and Cd^{2+}), and 0.00333 M (M^{2+}/L = 14:1 for Ni^{2+} and Cd^{2+}). The stability constants $K^{M}_{M(L-H)}$ of the $M(NBI-H)^{+}$ and $M(DNBI-H)^{+}$ complexes were calculated by taking into account the equilibrium constants determined previously for the HL^{+} and ML^{2+} species[12] and by considering now the species H^{+}, HL^{+}, L, $(L-H)^{-}$, M^{2+}, ML^{2+}, and $M(L-H)^{+}$ and by collecting the data from the titration pairs every 0.1 pH unit. The pH range from a formation degree of the complexes of about 2% to the beginning of the hydrolysis of $M(aq)^{2+}$, which was evident from the titrations without ligand, was used for the evaluations. The formation degree for the $M(DNBI-H)^{+}$ complexes reached approximately 20−45%, depending on the metal ion, which is satisfying. However, the $M(NBI-H)^{+}$ complexes formed only to about 5−14% and with Mg^{2+} even only 3.5% could be reached under the experimental conditions. This results in somewhat larger final error limits for the $M(NBI-H)^{+}$ complexes (see Table 1, vide supra). However, the results showed no dependence on the excess of M^{2+} used in the experiments; i.e., there was no indication for the formation of $M_2(L-H)^{3+}$ complexes. The final results given for the stability constants $K^{M}_{M(L-H)}$ are always the averages from at least four independent pairs of titrations.

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[31] The error limits of the calculated stability constants given in column 4 of Table 2 apply to the pK_a range 2−6, yet the data points for the $M(NBI-H)^{+}$ and $M(DNBI-H)^{+}$ complexes (Figure 2) are located at considerably higher pK_a values. Therefore, the error limits for the values calculated (calcd.) now were estimated in the following way: The error limits given with the intercepts *b* in column 3 of Table 2 reflect the error for a calculation based on $pK^{H}_{HL} = 0$; since the last experimental point of the straight line for Mg^{2+} (see Figure 2) is about three pK_a units away from the intercept at $pK^{H}_{HL} = 0$, for each pK_a unit the error limit of *b* (column 3 in Table 2) divided by 3 results. Considering that $pk_{ma/NBI} = 10.28$ is about 4.3 pK_a units larger than the last experimental point of the straight lines (Figure 2) the

mentioned value needs to be multiplied by 4.3 giving ±(0.095 : 3) × 4.3 = ±0.136 as the error limit for the calculated stability constant of the $Mg(NBI-H)^+$ complex. To be on the safe side, this value is further multiplied by 2 to give the error limit of ±0.27 which is listed in column 3 of Table 3. For the $Mn(NBI-H)^+$ complex exactly the same procedure applies. For Ni^{2+} (see Figure 2), Co^{2+} and Cd^{2+},[12] for which the last experimental point is only about two pK_a units away from the intercept, $pK^H_{HL} = 0$, the analogous calculation gives, e.g., for $Ni(NBI-H)^+$ the error limit ±(0.052 : 2) × 4.3 × 2 = ±0.22, which appears in column 3 of Table 3, and for $Ni(DNBI-H)^+$ ±(0.052 : 2) × 2.6 × 2 = ±0.14. All the other error limits given in column 3 of Table 3 were calculated correspondingly.

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