Acid–Base and Metal-Ion-Coordinating Properties of Benzimidazole and Derivatives (= 1,3-Dideazapurines) in Aqueous Solution: Interrelation between Complex Stability and Ligand Basicity

Larisa E. Kapinos, Bin Song, and Helmut Sigel*[a]

Abstract: The stability constants of the 1:1 complexes formed between Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} (= M^{2+}) and benzimidazole-type ligands sterically unhindered at the N3 position (= L), namely, benzimidazole, 1-methylbenzimidazole, 5(6)-chlorobenzimidazole, 6-chloro-5-fluorobenzimidazole, 5(6)-nitrobenzimidazole, 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole, and 5,6-dinitrobenzimidazole (DNBI), were determined by UV/Vis spectrophotometry for DNBI and for all the other ligands by potentiometric pH titration in aqueous solutions (25 °C, $I = 0.5$ M, $NaNO_3$). The acidity constants for the monoprotonated ligands HL^+ were also measured. For the HL^+ species which are symmetric with respect to the H(N1) and H(N3) sites, the corresponding micro acidity constants are also given. Plots of $\log K^{M}_{ML}$ versus pK^{H}_{HL} (taking into account the micro acidity constants where appropriate) give straight lines. The equations for these least-squares lines allow calculation of the expected stability constant for a complex of any benzimidazole-type ligand, provided its pK^{H}_{HL} value (in the pK_a range 2–6) is known. For the stabilities of Fe^{2+} complexes with benzimidazole-type ligands an estimation procedure is described. The effect of steric inhibition resulting from annelation (the fusion of a benzene ring to C4 and C5 of imidazole), is quantified and compared to that of a methyl group in the *ortho* position to the N atom binding the metal ion. The effect of annelation is considerable for the complexes of the divalent 3d metal ions (−0.3 to −0.7 log units) but practically nonexistent for those of the alkaline earth ions, which indicates outer-sphere complex formation for the latter. This interpretation agrees with the observation that the stability of the ML^{2+} complexes of Ca^{2+}, Sr^{2+}, and Ba^{2+} is practically independent of the basicity of the benzimidazole derivative. The regression lines obtained for the complexes of the benzimidazole-type ligands now permit the determination of the extent of the steric inhibition of the $(C6)NH_2$ group on metal-ion binding at N7 of the adenine residue.

Keywords: acid–base equilibria · alkaline earth metals · imidazole derivatives · metal-ion complexes · nitrogen heterocycles · N ligands · stability constants

1. Introduction

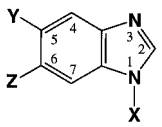
The structure and size of benzimidazole is very similar to that of the nucleobase purine; indeed, benzimidazole may also be named 1,3-dideazapurine. It is thus not surprising that many benzimidazole derivatives have been prepared to mimic purine nucleosides and nucleotides (e.g. ref. [1–3]), and a significant number of such compounds is known to be biologically active. For example, 1-(β-D-ribofuranosyl)benzimidazole 3′,5′-phosphate and analogues thereof activate cyclic adenosine 3′,5′-phosphate-dependent protein kinases.[2] Similarly, benzimidazole and a broad spectrum of derivatives can serve as substrates for purine nucleoside phosphorylase.[4] The benzimidazole residue is also often used in drug design:[5, 6] for instance, several 2-[(4-chlorophenoxy)methyl]benzimidazoles act as selective neuropeptide receptor antagonists,[3] and various substituted benzimidazoles can serve as prodrugs which, activated by acid, serve as gastric proton pump inhibitors.[7] Some benzimidazoles have shown antioxidant capacities in lipid peroxidation.[8] Another example is bis(5′-amidino-2-benzimidazolyl)methane, which is an excellent inhibitor of trypsin; the structural basis of this inhibition depends on the binding of Zn^{2+}.[9] This observation has led to the development of highly selective and Zn^{2+}-dependent inhibitors of several serine proteases.[9]

Considering the situation described above, it is surprising to find that apparently no comprehensive studies exist[10–12] which deal with the acid–base and especially the metal-ion-binding properties of benzimidazole and derivatives. This is even more startling given that enzymes employing nucleotides as substrates are generally also metal-ion-dependent,[13, 14] and indeed, this latter fact has prompted much research on the

[a] Prof. Dr. H. Sigel, Dr. L. E. Kapinos, Dr. B. Song
Institut für Anorganische Chemie, Universität Basel
Spitalstrasse 51, CH-4056 Basel (Switzerland)
Fax: (+41) 61-267-1017
E-mail: sigel@ubaclu.unibas.ch

0947-6539/99/0506-1794 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 6

interaction between metal ions and nucleotides or nucleic acids as well as their constituents,[15, 16] but not yet between nucleotide analogues,[10–12] aside from some antivirally active derivatives.[17, 18]



BI: X=Y=Z=H
MBI: X=CH_3; Y=Z=H
ClBI: X=Z=H; Y=Cl
ClFBI: X=H; Y=F; Z=Cl
NBI: X=Z=H; Y=NO_2
DRB: X=β-D-ribose; Y=Z=Cl
DNBI: X=H; Y=Z=NO_2

Figure 1. Chemical structures of benzimidazole (BI) and its derivatives considered in this study: 1-methylbenzimidazole (MBI), 5(6)-chlorobenzimidazole (ClBI), 6-chloro-5-fluorobenzimidazole (ClFBI), 5(6)-nitrobenzimidazole (NBI), 5,6-dichloro-1-(β-D-ribofuranosyl)-benzimidazole (DRB), and 5,6-dinitrobenzimidazole (DNBI).

It is the aim of the present study to correlate the acid–base and the metal-ion-binding properties of benzimidazole and the derivatives shown in Figure 1. The only compound studied in detail in this respect[19] is 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB), which is biologically very active.[20] A quantification of the indicated relations should also further our research[21, 22] dealing with the metal-ion-binding dichotomy[23, 24] of the adenine residue.

2. Results and Discussion

2.1. Acid–base properties of benzimidazoles: Benzimidazole and its derivatives shown in Figure 1 can accept one proton at N3 giving the monoprotonated species HL^+. Hence, only the deprotonation reaction of Equilibrium (1a) needs to be considered.

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

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

The acidity constants were determined by means of potentiometric pH titrations in aqueous solutions (25 °C; $I = 0.5$ M, $NaNO_3$), and the results are given in the third column of Table 1.[25] The acidity constants of monoprotonated benzimidazole and 1-methylbenzimidazole have been determined before[10–12, 26] and they agree excellently with the present results. The acidity constants of the other benzimidazole derivatives listed in Table 1 have, to the best of our knowledge, not been determined before.

Since the stability constants of the metal-ion (M^{2+}) complexes formed with 5,6-dinitrobenzimidazole (DNBI) can only be determined by spectrophotometric measurements (see Sections 2.3 and 4.3), we have endeavored also to measure the acidity constant of $H(DNBI)^+$ by the same method. The evaluation of one such experiment is shown in Figure 2. It is satisfying to note that the result, $pK^H_{H(DNBI)} = 1.72 \pm 0.06$, is almost identical with the one determined by potentiometric pH titration (Table 1, entry 7); in both instances $I = 0.5$ M, $NaNO_3$ (25 °C). Only recently[19] very similar results were also obtained for the deprotonation of $H(DRB)^+$; specifically, $pK^H_{H(DRB)} = 3.13 \pm 0.02$ (potentiometry) and 3.10 ± 0.10 (spectrophotometry) (see also entry 6 of Table 1). The excellent agreement between the results established by the two rather different experimental methods provides the necessary confidence in the spectrophotometric stability constant measurements for the $M(DNBI)^{2+}$ complexes (see Section 2.3).

For $H(MBI)^+$ we have measured the acidity constant by potentiometric pH titration also at another, somewhat lower ionic strength, $I = 0.1$ M, $NaNO_3$ (25 °C); the result,

Table 1. Negative logarithms of the acidity constants [Eq. (1)] of the protonated benzimidazole ligands (HL^+) shown in Figure 1 and as determined by potentiometric pH titrations in aqueous solutions at 25 °C and $I = 0.5$ M ($NaNO_3$) together with the corresponding micro acidity constants, pk_{ma} [Eq. (2)], when appropriate. The $pK^H_{H(Im/MIm)}$ values of protonated imidazole (Im) and 1-methylimidazole (MIm) are taken from ref. [27] and given for comparison to reveal the effect of annelation.[a, b]

	HL^+	pK^H_{HL}	pk_{ma}[c]	$pK^H_{H(Im/MIm)}$[27]	$\Delta pK_{a/annelation}$[d]
1	$H(BI)^+$	5.63 ± 0.01	5.93	7.13 ± 0.01[g]	1.50 ± 0.01
2	$H(MBI)^+$	5.67 ± 0.01	–	7.20 ± 0.02[h]	1.53 ± 0.02
3	$H(ClBI)^+$	4.86 ± 0.01	5.16	(5.27 ± 0.01)[i]	(0.41 ± 0.01)[i]
4	$H(ClFBI)^+$	4.33 ± 0.01	4.63		
5	$H(NBI)^+$	3.61 ± 0.02	3.91		
6	$H(DRB)^+$	3.13 ± 0.02[f]	–		
7	$H(DNBI)^+$	1.75 ± 0.05[e]	2.05		

[a] So-called practical (or mixed) constants[25] are listed; see Section 4.2. [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 column 6 to the right) were calculated according to the error propagation after Gauss. [c] See text in Section 2.2. [d] $\Delta pK_{a/annelation} = pK^H_{H(Im/Der)} - pK^H_{HL}$. [e] The spectrophotometric measurements gave $pK^H_{H(DNBI)} = 1.72 \pm 0.06$; this value (i.e., $pk_{ma} = 2.02$) is used in the evaluations presented in Section 2.4 because the stability constants of the $M(DNBI)^{2+}$ complexes were also determined by spectrophotometry. [f] From ref. [19]. Spectrophotometric measurements at 25 °C and $I = 0.5$ M (NaCl) gave $pK^H_{H(DRB)} = 3.10 \pm 0.10$ (3σ). [g] For $H(Im)^+$. [h] For $H(MIm)^+$. [i] The pK_a value for H(5-chloro-1-methylimidazole)$^+$ is given for a further comparison (see text in Section 2.1), and the difference should not be confused with a simple annelation effect; therefore, both values are given in parentheses.

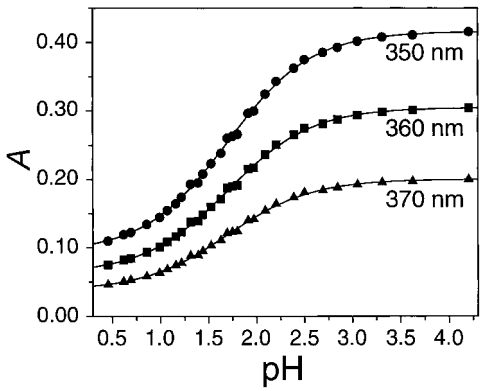


Figure 2. Evaluation of the dependence of the optical absorption of DNBI at 350 (●), 360 (■) and 370 nm (▲) on pH in aqueous solution by plotting the absorption (A) versus pH. The recorded absorption spectra (sample beam: [DNBI] = 0.10 mM, HNO_3 and $NaNO_3$ to give $I = 0.5$ M; reference beam: $NaNO_3/HNO_3$; 1 cm quartz cells) were evaluated at the three wavelengths mentioned; this led to the average result $pK^H_{H(DNBI)} = 1.70 \pm 0.01$ (3σ) for this experiment. The solid curves shown are the computer-calculated best fits for the three wavelengths through the experimental data points obtained at pH 0.46, 0.62, 0.69, 0.86, 0.99, 1.07, 1.16, 1.22, 1.31, 1.39, 1.44, 1.53, 1.63, 1.70, 1.75, 1.80, 1.91, 1.97, 2.09, 2.21, 2.39, 2.50, 2.70, 2.85, 3.05, 3.31, 3.63, and 4.21 (from left to right) by using $pK^H_{H(DNBI)} = 1.70$ (25 °C; $I = 0.5$ M, $NaNO_3$).

$pK^{H}_{H(MBI)} = 5.62 \pm 0.03$, is lower by $\Delta pK_a = 0.05 \pm 0.03$ (cf. entry 2 in Table 1) than the value measured at $I = 0.5$ M. This very slight increase in acidity is expected and in agreement with previous observations for related compounds: For example, for monoprotonated imidazole (Im), $pK^{H}_{H(Im)} = 7.13 \pm 0.01$ ($I = 0.5$ M, $NaNO_3$; 25 °C)[27] and 7.05 ± 0.02 ($I = 0.1$ M, $NaNO_3$; 25 °C),[28] that is, $\Delta pK_a = 0.08 \pm 0.02$, and for 1-methylimidazole (MIm) $pK^{H}_{H(MIm)} = 7.20 \pm 0.02$ ($I = 0.5$ M)[27] and 7.16 ± 0.01 ($I = 0.1$ M),[28d] that is, $\Delta pK_a = 0.04 \pm 0.02$. A variation of the ionic strength from 0.5 to 0.1 M results in a similar slight decrease in stability for the ML^{2+} complexes (see Section 2.4).

The data in entries 1 and 2 of Table 1 allow a further interesting comparison: Annelation (the fusion of a benzene ring to C4 and C5 of imidazole) increases the acidity of the resulting compound, that is, of $H(BI)^+$, by $\Delta pK_a = 1.5$. The corresponding acidification is observed if the values for monoprotonated 1-methylimidazole and 1-methylbenzimidazole (entry 2) are compared. These results concur with previous observations.[26, 29] That the effect of a substituent changes if it is moved from the 4,5-position in imidazole to a 5,6-position in benzimidazole is not surprising; the influence of a chloro substituent, for example, is diminished in this way (entry 3). It may be added that a similar acidification upon annelation occurs for the neutral ligands too; the formation of the anion is favored in benzimidazole ($pK^{H}_{BI} = 12.8$)[26] compared with imidazole ($pK^{H}_{Im} = 14.4$)[26] by $\Delta pK_a \simeq 1.6$.[30]

2.2. Micro acidity constants for HL^+ species symmetrical with regard to the H(N1) and H(N3) sites: For a detailed comparison of the acidity constants listed in column 3 of Table 1 for the monoprotonated HL^+ species, as well as for the evaluations to be carried out in Section 2.4, it is important to note that for the release of a proton from $H(BI)^+$ and $H(DNBI)^+$ two possibilities exist whereas there is only a single one for $H(MBI)^+$ and $H(DRB)^+$ (see Figure 1). This means the symmetric acids $H(BI)^+$ and $H(DNBI)^+$ are favored by a factor of two in their deprotonation reactions and hence for comparisons one has to add to the corresponding pK_a values 0.3 (= log 2) to obtain the micro acidity constants (pk_{ma}) which actually quantify the acid–base properties of $H(BI)^+$/BI and $H(DNBI)^+$/DNBI. This is expressed in a general form in Equation (2). The corresponding results for the two systems mentioned are listed in column 4 of Table 1 (entries 1, 7).

$$pk_{ma} = pK^{H}_{HL} + 0.3 \quad (2)$$

How is the situation for those ligands which carry no substituent at N3 but have nonequivalent substituents at C5 and C6, namely 5(6)-chlorobenzimidazole (ClBI), 6-chloro-5-fluorobenzimidazole (ClFBI), and 5(6)-nitrobenzimidazole (NBI) (Figure 1)? In these instances the probability of the release of a proton from the monoprotonated species HL^+ may not be equal for the H(N1) and H(N3) sites. However, based on linear free-energy relations it was previously concluded[31] that "the five and six tautomers of 5- (or 6-) substituted benzimidazoles are thermodynamically nearly equivalent". In agreement therewith, glycosylation of 5(6)-nitrobenzimidazole occurred such that it was concluded that "the nitro group does not perceptibly influence the glycosylation position if it is located in the 5- or 6-position of the benzimidazole moiety".[1] Therefore, we have applied Equation (2) to the pK^{H}_{HL} values of $H(ClBI)^+$, $H(ClFBI)^+$, and $H(NBI)^+$ too (see entries 3–5 in column 4 of Table 1). Indeed, the excellent fit of the data points from these asymmetric ligands in the $\log K^{M}_{ML}$ versus pK^{H}_{HL} correlations to be discussed in Section 2.4 confirms that the micro acidity constants obtained in the way described quantify the acid–base properties of these ligands well.

2.3. Stabilities of metal-ion complexes formed with benzimidazole and derivatives: All the experiments aimed to determine stability constants of metal-ion complexes were carried out with the metal-ion (M^{2+}) concentrations in excess of the concentrations of the ligands (L). This means that under these conditions only 1:1 complexes form and therefore the experimental data of the potentiometric pH titrations could be described completely by considering Equilibrium (1) given above and Equilibrium (3) for complex formation. The only

$$M^{2+} + L \rightleftharpoons ML^{2+} \quad (3a)$$

$$K^{M}_{ML} = [ML^{2+}]/([M^{2+}][L]) \quad (3b)$$

restriction is that the evaluation of the experimental data is not carried into the pH range in which hydroxo complex formation occurs; however, this was evident from the titrations of the metal-ion solutions in the absence of ligand (see Section 4.2.2). The results obtained from the potentiometric experiments are collected in columns 3–8 of Table 2.

The determination of stability constants by potentiometric pH titrations rests in principle on the observation of a depression of the buffer region of the free ligand, compared with that in the presence of metal ions. Such depressions can usually no longer be measured with a high enough precision if $pK_a < 2.5$. This is clearly the case for the $H(DNBI)^+$ system (Table 1, entry 7); hence, the stability constants for the corresponding $M(DNBI)^{2+}$ complexes had to be determined by spectrophotometric measurements. Therefore, first we measured the stability constant of the $Cu(NBI)^{2+}$ complex under the same conditions (25 °C; $I = 0.5$ M, $NaNO_3$) by spectrophotometry (see Section 4.3): $\log K^{Cu}_{Cu(NBI)} = 2.48 \pm 0.07$ agrees excellently with the potentiometric result (Table 2, column 4).

The spectral alterations which occur for DNBI upon complex formation are small, but they can be well characterized by recording difference spectra. Figure 3 provides a representative set of such spectra which show the effect of increasing amounts of Cu^{2+} on DNBI. These experimental data were evaluated at 350, 360, and 370 nm by a curve-fitting procedure (Section 4.3), which is shown in Figure 4.

The difference spectra of Figure 3 were recorded at pH 2.72; since $pK^{H}_{H(DNBI)} = 1.72$ (Table 1, footnote [e]), a part of the ligand still exists in its protonated form. Hence, only so-called apparent stability constants are obtained, which need to be corrected for the competition between proton and metal-

0947-6539/99/0506-1796 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 6

Table 2. Logarithms of the stability constants of the 1:1 complexes formed between several divalent metal ions (M^{2+}) and benzimidazole or its derivatives (L) shown in Figure 1 [Eq. (3)] as determined by potentiometric pH titrations in aqueous solutions at 25 °C and $I = 0.5$ M ($NaNO_3$).[a,b]

M^{2+}	DNBI[b]	DRB[c]	NBI	$\log K^{M}_{ML}$ for L = ClFBI	ClBI	MBI	BI
Ba^{2+}	–	-0.16 ± 0.20	-0.25 ± 0.20	-0.16 ± 0.10	-0.28 ± 0.15	-0.16 ± 0.14	-0.33 ± 0.20
Sr^{2+}	–	-0.13 ± 0.28	-0.30 ± 0.20	-0.22 ± 0.10	-0.19 ± 0.06	-0.26 ± 0.15	-0.26 ± 0.28
Ca^{2+}	–	0.00 ± 0.25	-0.20 ± 0.15	-0.13 ± 0.10	-0.20 ± 0.12	-0.10 ± 0.13	-0.18 ± 0.21
Mg^{2+}	–	-0.04 ± 0.20	-0.09 ± 0.15	0.02 ± 0.05	-0.06 ± 0.03	0.03 ± 0.08	0.05 ± 0.03
Mn^{2+}	–	0.31 ± 0.17	0.37 ± 0.07	0.57 ± 0.01	0.67 ± 0.03	0.78 ± 0.04	0.76 ± 0.05
Co^{2+}	0.84 ± 0.07	1.14 ± 0.09	1.25 ± 0.02	1.41 ± 0.04	1.47 ± 0.03	1.55 ± 0.04	1.59 ± 0.01
Ni^{2+}	1.23 ± 0.10	1.52 ± 0.07	1.63 ± 0.05	1.84 ± 0.02	1.91 ± 0.01	2.00 ± 0.02	2.00 ± 0.02
Cu^{2+}	1.78 ± 0.03	2.12 ± 0.07	2.40 ± 0.04[d]	2.76 ± 0.03	2.95 ± 0.01	3.18 ± 0.02[e]	3.26 ± 0.03
Zn^{2+}	0.51 ± 0.07	0.86 ± 0.10	1.01 ± 0.04	1.31 ± 0.02	1.40 ± 0.02	1.57 ± 0.04	1.61 ± 0.02
Cd^{2+}	0.96 ± 0.09	1.35 ± 0.10	1.51 ± 0.05	1.73 ± 0.02	1.87 ± 0.01	2.10 ± 0.06	2.10 ± 0.02

[a] For the error limits see footnote [b] of Table 1. [b] The stabilities of the $M(DNBI)^{2+}$ complexes were determined by spectrophotometry (see also Figures 3 and 4 and Sections 2.3 and 4.3). [c] From ref. [19]. [d] The spectrophotometric determinations gave $\log K^{Cu}_{Cu(NBI)} = 2.48 \pm 0.07$ (see also Sections 2.3 and 4.3). [e] Potentiometric pH titrations at $I = 0.1$ M ($NaNO_3$; 25 °C) gave $pK^{H}_{H(MBI)} = 5.62 \pm 0.03$ and $\log K^{Cu}_{Cu(MBI)} = 3.07 \pm 0.03$.

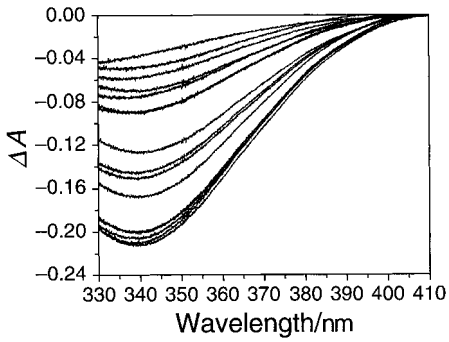


Figure 3. UV/Vis difference spectra of aqueous solutions of 0.00024 M 5,6-dinitrobenzimidazole at 25 °C in dependence on increasing concentrations of $Cu(NO_3)_2$: 0.002, 0.005*, 0.008*, 0.01*, 0.02, 0.025*, 0.035, 0.06, 0.08*, 0.10, 0.13 (an asterisk indicates that the experiment was done twice). One 1-cm quartz cell in the reference beam contained the corresponding concentration of $Cu(NO_3)_2$ plus $NaNO_3$ to maintain I at 0.5 M and the other DNBI (0.24 mM), while the sample beam contained the mixed system in one cell and water in the other. The pH was adjusted to 2.72 ± 0.02 in the two cells which contained DNBI. Evaluation of these spectra at 350, 360, and 370 nm leads to 16 points for each wavelength seen in the plots of Figure 4.

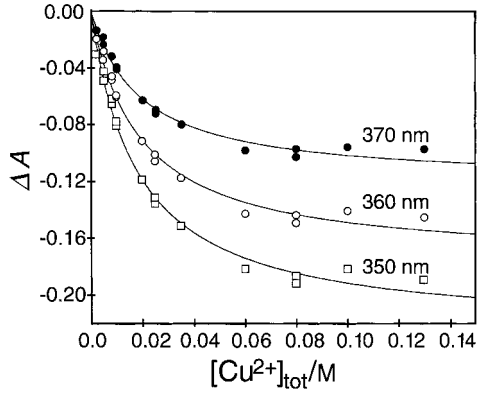


Figure 4. Evaluation of the UV/Vis absorption difference spectra of 5,6-dinitrobenzimidazole seen in Figure 3 at 350 (□), 360 (○), and 370 nm (●) in dependence on the total $Cu(NO_3)_2$ concentration present in aqueous solutions at $pH = 2.72 \pm 0.02$ (25 °C; $I = 0.5$ M, $NaNO_3$) for $\log K_{app}$ and ΔA_{max}. The solid curves represent the computer-calculated best fits of the experimental data points (see also Section 4.3) which lead to $K_{app} = 56.2 \pm 3.7$, 53.3 ± 4.1 and $53.4 \pm 4.3\ M^{-1}$ (1σ) and $\Delta A_{max} = -0.225 \pm 0.005$, -0.177 ± 0.005, and -0.121 ± 0.004 (1σ) at 350, 360, and 370 nm, respectively. The weighted mean of the logarithmic results gives $\log K_{app} = 1.737 \pm 0.055$ (3σ); this apparent stability constant needs to be transformed with Equation (4) into the pH-independent constant to give $\log K^{Cu}_{Cu(DNBI)} = (1.737 \pm 0.055) + 0.041 = 1.78 \pm 0.06$ (3σ). The final value given in Table 2 is the average of four independent experiments.

ion binding by Equation (4).[32, 33] In all instances at least three independent experiments like those shown in Figures 3 and 4

$$\log K^{M}_{ML} = \log K_{app} + \log (1 + [H^+]/K^{H}_{HL}) \quad (4)$$

were carried out. The results for the $M(DNBI)^{2+}$ complexes are given in column 2 of Table 2. For the corresponding complexes of the alkaline earth ions and Mn^{2+} no stability constants could be measured because the spectral alterations were too small and therefore not of sufficiently good reproducibility.

Of the seven metal-ion – benzimidazole systems considered in Table 2, stability constants had only been previously measured[10–12] for the $M(BI)^{2+}$ complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+}. The agreement between the earlier results and the present ones is fair to excellent. The stability constants of the $M(BI)^{2+}$ complexes with the alkaline earth ions and Zn^{2+} were now determined for the first time as well as all the other stability constants given in columns 2 and 4–7 of Table 2; the values for the $M(DRB)^{2+}$ complexes (column 3) are from our recent work.[19]

2.4. Correlation between complex stability and ligand basicity: For families of structurally closely related ligands a linear relationship between $\log K^{M}_{ML}$ and pK^{H}_{HL} is expected[34] as expressed by Equation (5). In Figure 5 the values from

$$\log K^{M}_{ML} = m \cdot pK^{H}_{HL} + b \quad (5)$$

Tables 1 and 2 for $\log K^{M}_{ML}$ versus pK^{H}_{HL} are plotted for four metal ions as examples. It is evident that the data for a given metal ion fit excellently on a straight line. It is especially satisfying to note that this also holds for the data pairs of the DNBI systems which were determined by spectrophotometry (see also Figure 7 in Section 3).

The results listed in Tables 1 and 2 provide six or seven data points for each metal ion. These were used to calculate the straight-line equations summarized in Table 3 by the least-squares procedure. From the plot of the equilibrium constants

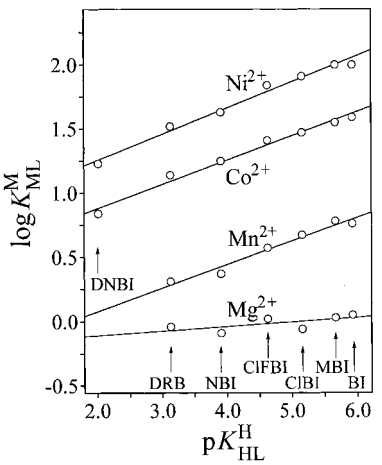


Figure 5. Relationship between $\log K^{M}_{ML}$ and pK^{H}_{HL} for the Mg^{2+}, Mn^{2+}, Co^{2+}, and Ni^{2+} 1:1 complexes of 5,6-dinitrobenzimidazole (DNBI), 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB), 5(6)-nitrobenzimidazole (NBI), 6-chloro-5-fluorobenzimidazole (ClFBI), 5(6)-chlorobenzimidazole (ClBI), 1-methylbenzimidazole (MBI), and benzimidazole (BI) (from left to right) based on the results given in Tables 1 and 2 (note that the micro acidity constants were applied when appropriate; see text in Section 2.2). The least-squares lines are drawn through the indicated seven data sets (when available); the corresponding straight-line equations are given in Table 3. Table 4 provides the differences between a given data point and its straight line. All plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.5$ M ($NaNO_3$).

of the Mg^{2+} systems in Figure 5, it is evident that the data can still be fitted rather well to a straight line. However, this line only shows a small dependence on pK^{H}_{HL}, and this is the reason for the low value obtained for the correlation coefficient R (Table 3, row 1, column 4). Such a dependence is no longer observed for the other three alkaline earth ions, Ca^{2+}, Sr^{2+}, and Ba^{2+}. From the entries in Table 2, it is evident that the corresponding results are not very precise (see also Section 4.2.2) and that for a given metal ion the stability constants, within the error limits, appear to be independent of the ligand considered. Thus, the stability constants $\log K^{M}_{ML}$ for the complexes of Ca^{2+}, Sr^{2+}, and Ba^{2+} are probably best represented in the pK^{H}_{HL} range from 3–6 by the averages -0.14 ± 0.15, -0.2 ± 0.15, and -0.2 ± 0.2 (3σ), respectively.

For the seven metal ions for which straight-line equations were calculated (see Table 3), it is interesting to determine the deviation from the least-squares line for the stability constant of each individual complex. It is satisfying to see from the results summarized in Table 4 that all deviations of the data points from their straight line are within ± 0.06 log unit.

Table 3. 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	R[c]
Mg^{2+}	0.035 ± 0.020	-0.179 ± 0.095	0.661
Mn^{2+}	0.182 ± 0.017	-0.287 ± 0.081	0.983
Co^{2+}	0.186 ± 0.011	0.513 ± 0.048	0.992
Ni^{2+}	0.201 ± 0.011	0.860 ± 0.052	0.992
Cu^{2+}	0.391 ± 0.013	0.936 ± 0.057	0.997
Zn^{2+}	0.284 ± 0.010	-0.055 ± 0.045	0.997
Cd^{2+}	0.293 ± 0.011	0.385 ± 0.050	0.997

[a] The slopes (m) and intercepts (b) for the straight reference lines from plots of $\log K^{M}_{ML}$ *versus* pK^{H}_{HL} were calculated by the least-squares procedure from the experimentally determined equilibrium constants listed in Tables 1 and 2 and by considering, where appropriate, the micro acidity constants [Eq. (2)] for the various benzimidazole systems. [b] Straight-line equation: $y = mx + b$, where x represents the pK_a value of any monoprotonated benzimidazole derivative and y the calculated stability constant ($\log K$) of the corresponding ML^{2+} complex [Eq. (5)]; the errors given with m and b correspond to one standard deviation (1σ). [c] Correlation coefficient. In the case of small values for the slope (m) the values for R are also expected to be relatively small (see Mg^{2+}).

Table 4. Logarithmic differences between the experimentally determined stability constants ($\log K^{M}_{ML}$ of Table 2) of the M^{2+} complexes for DNBI, DRB, NBI, ClFBI, ClBI, MBI, and BI (see Figure 1) and the least-squares lines of the $\log K^{M}_{ML}$ versus pK^{H}_{HL} plots (Table 3) as determined by the seven (six for Mg^{2+} and Mn^{2+}) complex systems.[a]

M^{2+}	DNBI	DRB	NBI	ClFBI	ClBI	MBI	BI	*SD*
Mg^{2+}	–	0.03	−0.04	0.04	−0.06	0.01	0.02	0.017
Mn^{2+}	–	0.03	−0.06	0.01	0.02	0.03	−0.03	0.015
Co^{2+}	−0.05	0.05	0.01	0.04	−0.02	0.00	−0.03	0.014
Ni^{2+}	−0.04	0.03	−0.01	0.05	0.02	0.00	−0.05	0.014
Cu^{2+}	0.05	−0.04	−0.06	0.01	0.00	0.03	0.01	0.014
Zn^{2+}	−0.01	0.03	−0.05	0.05	−0.01	0.01	−0.02	0.013
Cd^{2+}	−0.02	0.05	−0.02	−0.01	−0.03	0.05	−0.02	0.013

[a] The column farthest to the right gives the standard deviation (*SD*) resulting from the listed differences.

To provide a reliable error limit for any stability constant calculated with the equations of Table 3 and a given pK^{H}_{HL} value for each of the seven metal ions listed in Table 3, the standard deviation of the seven (six) data points from the relevant least-squares line was calculated (Table 4, heading *SD*). Users of the results described in this section are recommended to apply the equations of Table 3 to benzimidazole-type ligands in the pK_a range 2–6 and to consider as error limits for the calculated stability constants, $\log K^{M}_{ML}$ [Eq. (5)], two or three times the standard deviation (*SD*) given in Table 4 for the corresponding metal ion system. For calculated stability constants in the pK^{H}_{HL} range 0–2 and 6–8 the error limits given for b (intercept with the y axis) should also be taken into account.

For the 1-methylbenzimidazole system, a few experiments were also performed at $I = 0.1$ M, $NaNO_3$ (25 °C): $pK^{H}_{H(MBI)} = 5.62 \pm 0.03$ and $\log K^{Cu}_{Cu(MBI)} = 3.07 \pm 0.03$ (Table 2, footnote [e]). If one applies the value of 5.62 to the straight-line equation given in Table 3, one calculates $\log K^{Cu}_{Cu(MBI)} = 3.13 \pm 0.04$. The difference of 0.06 ± 0.05 shows that in a first approximation the results of Tables 3 and 4 can also be applied to an ionic strength of 0.1 if needed.

No stability constants were determined in this study for Fe^{2+}/benzimidazole-type ligands, despite the obvious biological significance of this metal ion, because such measurements are difficult to carry out and are rather error-prone if traces of Fe^{3+} are present or formed during the experiment. In fact, in the three stability constant compilations[10–12] not a single value is given for $Fe(BI)^{2+}$ or the Fe^{2+} complex of any of the other ligands. Therefore, we made the following estimation: According to experience[32] based on the Irving–Williams sequence,[35] one may propose the use of the average of the results obtained for the Mn^{2+} and Co^{2+} complexes; this view is

0947-6539/99/0506-1798 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 6

also supported by the results seen in Figure 6. This averaging gives the straight-line Equation (6) (25 °C; $I = 0.5$ M, $NaNO_3$). The error limit of log stability constants calculated in the pK_a

$$\log K^{Fe}_{FeL} = 0.184 \cdot pK^{H}_{HL} + 0.113 \quad (6)$$

range 2–6 is expected to be within ±0.10 log unit. Application of Equation (6) to, for example, $pk_{ma/H(BI)} = 5.93$ (Table 1) gives $\log K^{Fe}_{Fe(BI)} = 1.20 \pm 0.10$, and for $pK^{H}_{H(MBI)} = 5.67$ (Table 1) $\log K^{Fe}_{Fe(MBI)} = 1.16 \pm 0.10$ (25 °C; $I = 0.5$ M, $NaNO_3$) for the stabilities of the $Fe(BI)^{2+}$ and $Fe(MBI)^{2+}$ complexes, respectively.

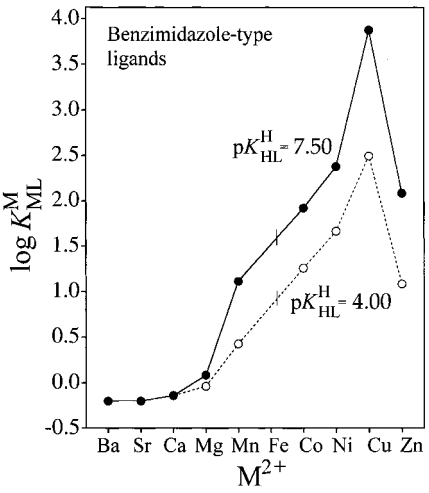


Figure 6. Logarithms of the stability constants according to the Irving-Williams sequence for the 1:1 complexes for Ba^{2+} through Zn^{2+} formed with a benzimidazole-type ligand with $pK^{H}_{HL} = 7.50$ (●) and another one with $pK^{H}_{HL} = 4.00$ (○). The corresponding stability constants, $\log K^{M}_{ML}$, were calculated with the straight-line equations given in Table 3; the values for the Ca^{2+}, Sr^{2+}, and Ba^{2+} complexes are given in the text of Section 2.4 (aqueous solution; 25 °C; $I = 0.5$ M, $NaNO_3$).

3. Conclusions

Comparison of the plots seen in Figure 7 for the complexes of Zn^{2+} and Cd^{2+} with imidazole- (□) and benzimidazole-type (○) compounds reveals that these ligands are members of two families, because the corresponding experimental data fit onto two different straight lines. This proves that the benzene ring fused to the 4,5-position of imidazole sterically inhibits metal-ion binding to N3. From Figure 7 it appears that the two straight lines for a given metal ion are approximately parallel to each other. Indeed, the slopes *m* of the regression lines for the benzimidazole-type (Table 3) and the imidazole-type ligands (Table 2 in ref. [27]) agree within a single standard deviation in most instances.

To obtain a broader basis as well as a quantification of the indicated steric effect, we have calculated the metal-ion-binding properties of a benzimidazole-type ligand to compare it with those of an imidazole-type ligand (both ligands having $pK^{H}_{HL} = 6.00$); the results are listed in columns 2 and 3 of Table 5. The steric effect of the benzene ring is evident from the stability differences listed in column 4, which were calculated according to Equation (7).

$$\Delta \log K = \log K^{M}_{M(BI\text{-type})} - \log K^{M}_{M(Im\text{-type})} \quad (7)$$

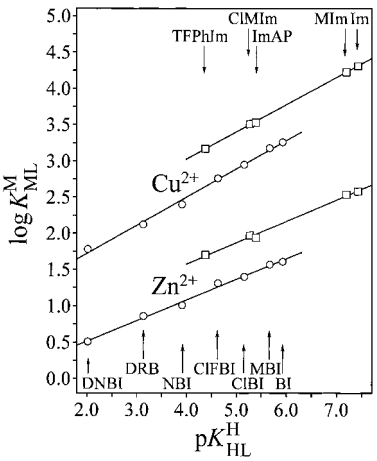


Figure 7. Evidence for a reduced stability of the Cu^{2+} and Zn^{2+} 1:1 complexes of benzimidazole-type ligands (○) compared with those of imidazole-type ligands (□), based on the relationship $\log K^{M}_{ML}$ versus pK^{H}_{HL}. The reduced stability reflects the steric inhibition due to annelation, i.e. the fusion of a benzene ring to the 4,5-positions of imidazole. The imidazole-type ligands are *N*-(2,3,5,6-tetrafluorophenyl)imidazole (TFPhIm), 5-chloro-1-methylimidazole (ClMIm), 4′-(imidazol-1-yl)acetophenone (ImAP), 1-methylimidazole (MIm), and imidazole (Im) (from left to right). For the benzimidazole-type ligands see Figure 1 and the legend of Figure 5. The least-squares lines for the benzimidazoles are drawn according to the equations given in Table 3 and those for the imidazoles according to the data in Table 2 of ref. [27]. All plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.5$ M ($NaNO_3$).

The observation of a remarkable steric effect due to annelation for the complexes of the divalent 3d metal ions but none (within the error limits) for the complexes of Ca^{2+}, Sr^{2+}, and Ba^{2+} is interesting. This indicates that binding of the latter metal ions occurs in an outer-sphere manner; this conclusion concurs with the fact that the stability of the corresponding complexes is rather independent of the basicity of the ligands involved. For Mg^{2+}, outer-sphere binding is clearly still important, whereas its role for Mn^{2+}, in the case of the ligands considered, is diminished. Hence, these results indicate that there exists principally an equilibrium between outer-sphere and inner-sphere metal-ion binding and that this equilibrium is of the inner-sphere type for Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}, and of the outer-sphere one for Ca^{2+}, Sr^{2+}, and Ba^{2+}. The different extent of steric inhibition observed for the complexes of Zn^{2+} and Cd^{2+} is probably the result of the greater bond lengths of the latter ion.

At this point it seems of interest to compare the described annelation effect, that is, the steric inhibition caused by the benzene ring fused to C4 and C5, with the steric inhibition resulting from the presence of a methyl group adjacent to N3 on the metal-ion-binding properties of imidazole-type ligands. This can be done for several metal ions by means of the published[36] stability constants for the complexes formed with 1,2-dimethylimidazole: The expected stability constants based on the basicity of N3 were calculated (calcd) and compared with the experimental (exptl) values (see columns 5 and 6 of Table 5). From column 7 it is evident that the inhibition resulting from an *ortho* methyl group on metal-ion binding at N3 of imidazole is more pronounced than that due to annelation (cf. column 4), but the inhibition of a methyl

Table 5. Extent of the steric inhibition [Eq. (7)] of metal-ion binding to N3 due to annelation, as calculated with $pK^{H}_{HL} = 6.00$ and the straight-line equations of Table 3 for benzimidazole-type ligands (BI-type) and for imidazole-type ligands (Im-type) with the corresponding data given in Table 2 of ref. [27] (25 °C; $I = 0.5$ M, $NaNO_3$).[a] The extent of the steric inhibition by a methyl group adjacent to N3, as exemplified by a methyl group at C2 of imidazole,[b] is given for comparison (see columns 5–7).

M^{2+}	$\log K^{M}_{M(BI\text{-}type)}$	$\log K^{M}_{M(Im\text{-}type)}$	$\Delta \log K$	$\log K^{M}_{M(DMIm)}$ exptl[b]	calcd[c]	$\log\Delta$[d]
Ba^{2+}	-0.2 ± 0.2	-0.3 ± 0.2	0.1 ± 0.3			
Sr^{2+}	-0.2 ± 0.15	-0.2 ± 0.15	0.0 ± 0.2			
Ca^{2+}	-0.14 ± 0.15	-0.12 ± 0.10	0.0 ± 0.2			
Mg^{2+}	0.03 ± 0.05	0.11 ± 0.05	-0.08 ± 0.07			
Mn^{2+}	0.81 ± 0.05	1.15 ± 0.02	-0.34 ± 0.05			
Co^{2+}	1.63 ± 0.04	2.18 ± 0.03	-0.55 ± 0.05	1.13 ± 0.05	2.66 ± 0.03	-1.53 ± 0.06
Ni^{2+}	2.07 ± 0.04	2.77 ± 0.03	-0.70 ± 0.05	2.15 ± 0.05	3.27 ± 0.03	-1.12 ± 0.06
Cu^{2+}	3.28 ± 0.04	3.78 ± 0.03	-0.50 ± 0.05	3.70 ± 0.05	4.61 ± 0.02	-0.91 ± 0.05
Zn^{2+}	1.65 ± 0.04	2.17 ± 0.04	-0.52 ± 0.06	1.92 ± 0.05	2.82 ± 0.04	-0.90 ± 0.06
Cd^{2+}	2.14 ± 0.04	2.39 ± 0.03	-0.25 ± 0.05			

[a] For the error limits, see footnote [b] of Table 1. [b] The ligand used is 1,2-dimethylimidazole (DMIm); the stability constants are from ref. [36]; they apply to aqueous solutions at 25 °C and $I = 0.5$ M (KNO_3). It may be added that the constants determined by these authors[36] for 1-methylimidazole (MIm) systems are in excellent agreement with our results[27] [with the exception of the stability constant for $Zn(MIm)^{2+}$, which is quoted as being 0.16 log units larger than our value; we assume that this is a hydrolysis effect]. [c] Calculated with $pK^{H}_{H(DMIm)} = 8.21$ (from ref. [36]) and the straight-line equations given in Table 2 of ref. [27] for imidazole-type complexes; the corresponding errors (3σ) are from Table 3 of ref. [27]. [d] Log $\Delta = \log K^{M}_{M(DMIm)/exptl} - \log K^{M}_{M(DMIm)/calcd}$.

group in *ortho*-imidazole-type ligands and in *ortho*-pyridine-type ligands[21, 37] seems to a first approximation to be of a comparable order.

With the described results in mind, a question arises concerning the steric inhibition caused by the 6-amino group on metal-ion binding to N7 of an adenine residue. The relevance of this question regarding biological systems is evident. We intend to address this problem by studying the stability of complexes formed with 4-amino-1-methyl-benzimidazole (= 9-methyl-1,3-dideazaadenine) and/or 1,4-dimethylbenzimidazole (= 6,9-dimethyl-1,3-dideazapurine).

4. Experimental Section

4.1. Materials: Benzimidazole (99%) was from Sigma Chemical, St. Louis (MO, USA), 1-methylbenzimidazole (99%) from Aldrich Chemical, Buchs (Switzerland), 5(6)-chlorobenzimidazole (98%) from Kasei Kogyo, Tokyo (Japan), 6-chloro-5-fluorobenzimidazole (>95%) from Maybridge Chemical, Trevillett (Tintagel, Cornwall, UK), 5(6)-nitrobenzimidazole (>98%) and 5,6-dinitrobenzimidazole were from Fluka, Buchs (Switzerland); all these substances were used as obtained; however, potentiometric pH titrations proved that they were free of any acid–base impurities. All the other chemicals, including water, were the same as used previously,[27] and the stock solutions and titer determinations were also made as described.[27]

4.2. Potentiometric pH titrations: The instrumentation, including the desk computers, was the same as described recently.[27]

The acidity constants determined are so-called practical, mixed, or Brønsted constants.[25] 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 listed pK_a values.[25] 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.

4.2.1. Determination of the acidity constants: The acidity constants K^{H}_{HL} [Eq. (1)] of $H(BI)^+$, $H(MBI)^+$, and $H(ClBI)^+$ were determined by titrating 50 mL of 1.8 mM HNO_3 (25 °C; $I = 0.5$ M, $NaNO_3$) in the presence and absence of 1.0 mM benzimidazole or one of the mentioned derivatives under N_2 with 1 mL of 0.1 M NaOH and by using the differences in NaOH consumption between such a pair of titrations for the calculations. To investigate the influence of the ionic strength, the acidity constant $K^{H}_{H(MBI)}$ of $H(MBI)^+$ was also measured at $I = 0.1$ M ($NaNO_3$; see footnote [e] of Table 2).

With ClFBI, NBI, and DNBI, more concentrated solutions had to be used because the acidity constants of their HL^+ species are lower. We titrated 25 mL of 3.6 mM HNO_3 (25 °C; $I = 0.5$ M, $NaNO_3$) in the presence and absence of 1.7 mM ClFBI, and 25 mL of 7.2 mM HNO_3 ($I = 0.5$ M, $NaNO_3$) in the presence and absence of 2.5 mM NBI with 1 and 2 mL 0.1 M NaOH, respectively. The rather low solubility of DNBI required the following conditions: 25 mL of 7.2 mM HNO_3 ($I = 0.5$ M, $NaNO_3$) were titrated in the presence and absence of 0.96 mM DNBI with 3 mL 0.07 M NaOH.

The acidity constants were calculated as described.[27] For the determination of $K^{H}_{H(DNBI)}$ only the pH range corresponding to about 60–97% neutralization for the equilibrium HL^+/L was accessible; in this case three independent titrations were made. In all the other instances the final results for the various acidity constants are the averages of 14–36 independent pairs of titrations.

4.2.2. Determination of the stability constants: The stability constants K^{M}_{ML} [Eq. (3)] of the $M(BI)^{2+}$, $M(MBI)^{2+}$, $M(ClBI)^{2+}$, $M(ClFBI)^{2+}$, and $M(NBI)^{2+}$ 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). The stability constant $K^{Cu}_{Cu(MBI)}$ of the $Cu(MBI)^{2+}$ complex was also measured at $I = 0.1$ M ($NaNO_3$; see footnote [e] of Table 2). The stability constant of $M(DNBI)^{2+}$ could not be determined (see Section 4.3) by potentiometric pH titration because $pK^{H}_{H(DNBI)} < 2$.

For the systems containing Mg^{2+}, Ca^{2+}, Sr^{2+}, or Ba^{2+}, $NaNO_3$ was always replaced completely by $M(NO_3)_2$ because of the low stability of the corresponding complexes; that is, $[M(NO_3)_2]$ was equal to 0.1667 M and consequently, M^{2+}:L ≃ 167:1 for the BI, MBI, or ClBI systems, M^{2+}:L ≃ 98:1 for ClFBI, and M^{2+}:L ≃ 67:1 for NBI.

For the other metal ions in the systems with BI, MBI, and ClBI, the following concentrations of $M(NO_3)_2$ were employed and at least two different $[M(NO_3)_2]$ were used for a given ligand: 0.1667 M (M^{2+}: L = 167:1 for Mn^{2+}, Co^{2+}, Ni^{2+}, and Cd^{2+}), 0.0833 M (M^{2+}: L = 83:1 for Mn^{2+} and Co^{2+}), 0.0417 M (M^{2+}: L = 42:1 for Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}) and 0.0208 M (M^{2+}: L = 21:1 for Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}). The same metal ion concentrations were used for the other ligand systems but now the M^{2+}:L ratios were 98:1 (Mn^{2+} and Co^{2+}), 49:1 (Co^{2+} and Zn^{2+}), 24:1 (Ni^{2+}, Zn^{2+}, and Cd^{2+}) and 12:1 (Co^{2+}, Ni^{2+}, and Cd^{2+}) for ClFBI as well as 67:1 (Co^{2+} and Mn^{2+}), 33:1 (Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}) and 17:1 (Ni^{2+}, Zn^{2+}, Cd^{2+}) for NBI; in the latter case, the lowest $[M^{2+}]$ of 0.0208 M was not employed.

In the Cu^{2+}/BI, MBI, and ClBI systems, $[Cu(NO_3)_2]$ was 10.4, 5.2, 4.2, 3.4, 2.1, and 1.7 mM, so the Cu^{2+}:L ratios were 10.4:1, 5.2:1, 4.2:1, 3.5:1, 2.1:1,

0947-6539/99/0506-1800 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 6

and 1.7:1 (at least two different ratios were used for each ligand); in the Cu^{2+}/ClFBI system $[Cu(NO_3)_2]$ was 8.3 and 4.2 mM, giving Cu^{2+}:L ratios of 4.9:1 and 2.5:1; finally, in the Cu^{2+}/NBI system $[Cu(NO_3)_2]$ was 20.8 and 10.4 mM, giving Cu^{2+}:L ratios of 8.3:1 and 4.2:1.

All the stability constants were calculated exactly as described in ref. [27].[38] The results showed no dependence on the excess of M^{2+} used in the experiments. The final results given for the stability constants, K^{M}_{ML} [Eq. (3)], are always the averages from at least six independent pairs of titrations.

The stability of the Ca^{2+}, Sr^{2+}, and Ba^{2+} complexes was always very low, and consequently the buffer depression was small; for example, for the Ba^{2+}/NBI system under the present experimental conditions it amounts to only $\Delta pK_a \simeq 0.04$. For this reason the error limits for these complexes are rather large (Table 2).

4.3. Spectrophotometric measurements: The acidity constant K^{H}_{HL} of $H(DNBI)^{+}$ was also determined by spectrophotometry (Table 1, footnote [e]). The UV/Vis spectra of DNBI (0.10 mM) were recorded in aqueous solutions (25 °C; $I = 0.5$ M, $NaNO_3$) with a Perkin Elmer (Lambda 2) UV/Vis spectrophotometer using 1 cm quartz cells. The pH of the solutions was adjusted by dotting with relatively concentrated HNO_3 or NaOH and measured with a Metrohm 654 pH meter with a Metrohm 6.0216.100 (PC) glass electrode. The spectrophotometric data were analysed (see Figure 2) with an IBM-compatible desk computer with a Pentium processor connected to an EPSON Stylus 1000ESC/P2 printer and a Hewlett–Packard Desk Jet 1600C Color Smart printer. The evaluation of the spectrophotometric measurements (Figure 2) was carried out above 330 nm because then the absorption of 0.5 M $NaNO_3$ is no longer relevant. The final result given for $pK^{H}_{H(DNBI)}$ (see Table 1, footnote [e]) is the average of three independent experiments.

The stability constants K^{M}_{ML} [Eq. (3)] of $Co(DNBI)^{2+}$, $Ni(DNBI)^{2+}$, $Cu(DNBI)^{2+}$, $Zn(DNBI)^{2+}$, and $Cd(DNBI)^{2+}$ were determined by recording difference spectra of the UV/Vis region between 330 and 420 nm (see Figure 3) with the Perkin Elmer instrument and also with a Varian Cary 3C spectrophotometer connected to an IBM-compatible desk computer (OS/2 system) and an EPSON Stylus 1500 printer.

A typical experiment for a stability-constant determination is shown in Figure 3 and its evaluation in Figure 4; the latter was carried out analogously to the evaluation described for ^{1}H NMR shift data,[39] by employing a curve-fitting procedure using a Newton–Gauss nonlinear least-squares programme. An evaluation by regression lines according to Equation (1) of ref. [33] gave the same results as the curve fit. All experiments ($[DNBI] = 0.24$ mM) were adjusted to a certain pH in the range 2.6–3.5; the measured apparent stability constants were then corrected for the competition of the proton by means of Equation (4). The concentration range used for $Cu(NO_3)_2$ was from 0.002–0.13 M; for the other $M(NO_3)_2$ systems concentrations of 0.02–0.167 M were employed.

The final results given in Table 2 (column 2) are the averages of three independent series of experiments with, in total, nine evaluations in the case of $Zn(DNBI)^{2+}$ or $Cd(DNBI)^{2+}$; five series of experiments with in total fifteen evaluations were carried out each for $Co(DNBI)^{2+}$, $Ni(DNBI)^{2+}$ and $Cu(DNBI)^{2+}$.

The stability constant of the $Cu(NBI)^{2+}$ complex was also determined spectrophotometrically to confirm the result obtained by potentiometric pH titration. The difference spectra have a similar appearance to those for the M^{2+}/DNBI systems; they were evaluated at 350, 360, and 370 nm. The conditions were $[NBI] = 0.20$ mM and $[Cu(NO_3)_2] = 0.001–0.05$ M (25 °C; $I = 0.5$ M, $NaNO_3$); four independent experiments at pH 4.02, 4.04, 4.51, and 5.02 were performed with in total 12 evaluations which led to the result given in footnote [d] of Table 2.

Acknowledgments: The competent technical assistance of Rita Baumbusch in the preparation of the manuscript and research grants from the Swiss National Science Foundation, the Swiss Federal Office for Education & Science (COST D8), and the Novartis Foundation, formerly the Ciba–Geigy Jubilee Foundation, are gratefully acknowledged.

[1] F. Seela, W. Bourgeois, *Synthesis* **1989**, 912–918.
[2] H.-G. Genieser, E. Winkler, E. Butt, M. Zorn, S. Schulz, F. Iwitzki, R. Störmann, B. Jastorff, S. O. Døskeland, D. Øgreid, S. Ruchaud, M. Lanotte, *Carbohyd. Res.* **1992**, *234*, 217–235.
[3] H. Zarrinmayeh, A. M. Nunes, P. L. Ornstein, D. M. Zimmerman, M. B. Arnold, D. A. Schober, S. L. Gackenheimer, R. F. Bruns, P. A. Hipskind, T. C. Britton, B. E. Cantrell, D. R. Gehlert, *J. Med. Chem.* **1998**, *41*, 2709–2719.
[4] I. A. Mikhailopulo, Z. Kazimierczuk, A. I. Zinchenko, V. N. Barai, V. V. Romanova, L. A. Eroshevskaya, *Nucleosides Nucleotides* **1995**, *14*, 477–480.
[5] L. Garuti, M. Roberti, T. Rossi, C. Cermelli, M. Portolani, M. Malagoli, M. Castelli, *Anti-Cancer Drug Des.* **1998**, *13*, 397–406.
[6] F. Gumus, O. Algul, *J. Inorg. Biochem.* **1997**, *68*, 71–74.
[7] W. Kromer, U. Kruger, R. Huber, M. Hartmann, V. W. Steinijans, *Pharmacology* **1998**, *56*, 57–70.
[8] B. Can-Eke, M. O. Puskullu, E. Buyukbingol, M. Iscan, *Chem. Biol. Interact.* **1998**, *113*, 65–77.
[9] B. A. Katz, J. M. Clark, J. S. Finer-Moore, T. E. Jenkins, C. R. Johnson, M. J. Ross, C. Luong, W. R. Moore, R. M. Stroud, *Nature* **1998**, *391*, 608–612.
[10] *IUPAC Stability Constants Database*, Version 3.02 (compiled by L. D. Pettit, H. K. J. Powell), Academic Software, Timble, Otley, W. Yorks (UK), **1998**.
[11] *NIST Critically Selected Stability Constants of Metal Complexes*, Reference database 46, Version 5.0 (data collected and selected by A. E. Martell, R. M. Smith), U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD (USA), **1998**.
[12] *Joint Expert Speciation System (JESS)*, Version 5.3 (joint venture by K. Murray, P. M. May), Division of Water Technology, CSIR, Pretoria (South Africa) and School of Mathematical and Physical Sciences, Murdoch University, Murdoch, WA (Australia), **1996**.
[13] Interrelations among Metal Ions, Enzymes, and Gene Expression (Eds.: H. Sigel, A. Sigel), *Met. Ions Biol. Syst.* **1989**, *25*, 1–557.
[14] J. J. R. Fraústo da Silva, R. J. P. Williams, *The Biological Chemistry of the Elements*, Clarendon, Oxford, **1991**, pp. 1–561.
[15] Recent research on this and related topics is summarized in: a) Interactions of Metal Ions with Nucleotides, Nucleic Acids, and their Constituents (Eds.: A. Sigel, H. Sigel), *Met. Ions Biol. Syst.* **1996**, *32*, pp. 1–814; b) Probing of Nucleic Acids by Metal Ion Complexes of Small Molecules (Eds.: A. Sigel, H. Sigel), *Met. Ions Biol. Syst.* **1996**, *33*, 1–678.
[16] a) H. Sigel, *Chem. Soc. Rev.* **1993**, *22*, 255–267; b) H. Sigel, B. Song, *Met. Ions Biol. Syst.* **1996**, *32*, 135–205; see ref. [15a].
[17] a) H. Sigel, *Coord. Chem. Rev.* **1995**, *144*, 287–319; b) H. Sigel, *J. Indian Chem. Soc.* **1997**, *74*, 261–271 (P. Ray Award Lecture).
[18] a) C. A. Blindauer, A. H. Emwas, A. Holý, H. Dvořáková, E. Sletten, H. Sigel, *Chem. Eur. J.* **1997**, *3*, 1526–1536; b) C. A. Blindauer, A. Holý, H. Dvořáková, H. Sigel, *J. Biol. Inorg. Chem.* **1998**, *3*, 423–433.
[19] L. E. Kapinos, B. Song, H. Sigel, *Z. Naturforsch. B* **1998**, *53*, 903–908.
[20] a) R. V. Devivar, E. Kawashima, G. R. Revankar, J. M. Breitenbach, E. D. Kreske, J. C. Drach, L. B. Townsend, *J. Med. Chem.* **1994**, *37*, 2942–2949; b) W. Schul, B. Groenhout, K. Koberna, Y. Takagaki, A. Jenny, E. M. Manders, I. Raska, R. van Driel, L. de Jong, *EMBO J.* **1996**, *15*, 2883–2892; c) S. Nekhai, R. R. Shukla, A. Kumar, *J. Virol.* **1997**, *71*, 7436–7441.
[21] H. Sigel, N. A. Corfù, L.-n. Ji, R. B. Martin, *Comments Inorg. Chem.* **1992**, *13*, 35–59.
[22] a) L. E. Kapinos, B. Song, A. Holý, H. Sigel, *Chimia* **1996**, *50*, 334; b) L. E. Kapinos, B. Song, A. Holý, M. Bastian, H. Sigel, *Chimia* **1996**, *50*, 373.
[23] R. B. Martin, *Acc. Chem. Res.* **1985**, *18*, 32–38.
[24] R. B. Martin, *Met. Ions Biol. Syst.* **1996**, *32*, 61–89; see ref. [15a].
[25] H. Sigel, A. D. Zuberbühler, O. Yamauchi, *Anal. Chim. Acta* **1991**, *255*, 63–72.
[26] J. Catalán, R. M. Claramunt, J. Elguero, J. Laynez, M. Menéndez, F. Anvia, J. H. Quian, M. Taagepera, R. W. Taft, *J. Am. Chem. Soc.* **1988**, *110*, 4105–4111.
[27] L. E. Kapinos, B. Song, H. Sigel, *Inorg. Chim. Acta* **1998**, *280*, 50–56 (M. E. Vol'pin Memorial Issue).

[28] Average value from: a) D. Banerjea, T. A. Kaden, H. Sigel, *Inorg. Chem.* **1981**, *20*, 2586 – 2590; b) N. Saha, H. Sigel, *J. Am. Chem. Soc.* **1982**, *104*, 4100 – 4105; c) L.-n. Ji, N. A. Corfù, H. Sigel, *Inorg. Chim. Acta* **1993**, *206*, 215 – 220; d) M. S. Lüth, L. E. Kapinos, B. Song, B. Lippert, H. Sigel, *J. Chem. Soc. Dalton Trans.* **1999**, 357 – 365.
[29] G. Yagil, *Tetrahedron* **1967**, *23*, 2855 – 2861.
[30] The values of pK_L^H and pK_{HL}^H differ by $\Delta pK_a \simeq 7$ (see also ref. [31]). Hence, the deprotonation of DNBI (row 7 of Table 1) to $(DNBI - H)^-$ should occur with $pK_a \simeq 8.8$ and thus should fall into a pH range accessible for potentiometric pH titrations. Consequently, we shall attempt next to measure the stability of complexes formed with 5,6-dinitrobenzimidazolate.
[31] H. Walba, R. Ruiz-Velasco, Jr., *J. Org. Chem.* **1969**, *34*, 3315 – 3320.
[32] H. Sigel, D. B. McCormick, *Acc. Chem. Res.* **1970**, *3*, 201 – 208.
[33] K. H. Scheller, T. H. J. Abel, P. E. Polanyi, P. K. Wenk, B. E. Fischer, H. Sigel, *Eur. J. Biochem.* **1980**, *107*, 455 – 466.
[34] R. B. Martin, H. Sigel, *Comments Inorg. Chem.* **1988**, *6*, 285 – 314.
[35] H. Irving, R. J. P. Williams, *Nature* **1948**, *162*, 746 – 747; H. Irving, R. J. P. Williams, *J. Chem. Soc.* **1953**, 3192 – 3210.
[36] B. Lenarcik, B. Barszcz, *J. Chem. Soc. Dalton Trans.* **1980**, 24 – 28.
[37] L.-n. Ji, N. A. Corfù, H. Sigel, *J. Chem. Soc. Dalton Trans.* **1991**, 1367 – 1375.
[38] R. K. O. Sigel, B. Song, H. Sigel, *J. Am. Chem. Soc.* **1997**, *119*, 744 – 755.
[39] H. Sigel, K. H. Scheller, V. M. Rheinberger, B. E. Fischer, *J. Chem. Soc. Dalton Trans.* **1980**, 1022 – 1028.

Received: November 11, 1998 [F1431]

  0947-6539/99/0506-1802 $ 17.50+.50/0