Metal Ion-Binding Properties of 1-Methyl-4-aminobenzimidazole (=9-Methyl-1,3-dideazaadenine) and 1,4-Dimethylbenzimidazole (=6,9-Dimethyl-1,3-dideazapurine). Quantification of the Steric Effect of the 6-Amino Group on Metal Ion Binding at the N7 Site of the Adenine Residue[§]

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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 1-methyl-4-aminobenzimidazole (MABI) or 1,4-dimethylbenzimidazole (DMBI) were determined by potentiometric pH titrations in aqueous solution (25 °C; $I = 0.5$ M, $NaNO_3$). Some of the stability constants were also measured by UV spectrophotometry. The acidity constants of the species $H_2(MABI)^{2+}$ and $H(DMBI)^{+}$ were determined by the same methods, some twice. Comparison of the stability constants of the $M(MABI)^{2+}$ and $M(DMBI)^{2+}$ complexes with those calculated from $\log K^{M}_{ML}$ versus pK^{H}_{HL} straight-line plots, which were established previously for sterically unhindered benzimidazole-type ligands (=L), reveals that the stabilities of the $M(MABI)^{2+}$ and $M(DMBI)^{2+}$ complexes are significantly reduced due to steric effects of the C4 substituents on metal ion binding at N3. This effect is more pronounced in the $M(DMBI)^{2+}$ complexes. Considering the steric equivalence of methyl and (noncoordinating) amino groups (as they occur in adenines), it is concluded that the same extent of steric inhibition by the $(C6)NH_2$ group is to be expected on metal ion binding at N7 with adenine derivatives. The basicity of the amino group in MABI is significantly higher than in its corresponding adenine derivative. Indeed, it is concluded that in the $M(MABI)^{2+}$ complexes chelate formation involving the amino group occurs to some extent. The formation degrees of these "closed" species are calculated; they vary for the complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} between about 50 and 90%. The stability of the $M(MABI)^{2+}$ and $M(DMBI)^{2+}$ complexes with the alkaline earth ions is very low but unaffected by the C4 substituent; this probably indicates that in these instances outersphere complexes (with a water molecule between N3 and the metal ion) are formed.

1. Introduction

The similarity in shape between benzimidazoles and purines has attracted the attention of researchers for a long time, and this similarity is also reflected in the fact that benzimidazole is often called 1,3-dideazapurine. Therefore, it is no surprise that the biological properties of benzimidazole and its derivatives were, and still are, intensively studied, and e.g. the mutagenic properties of these compounds have been known for a long time.[1]

4-Amino-1*H*-benzimidazole (4ABI) was synthesized about 50 years ago,[2] its glycosylation properties were studied,[3] and 4ABI derivatives, i.e., adenine analogues, were tested, e.g. for their effect on gastric acid secretion[4] as well as for their antiviral activity.[5] 4-Amino-1-(β-D-ribofuranosyl)benzimidazole, or 1,3-dideazaadenosine, was first prepared[6] more than 30 years ago, and its cytotoxicity against KB cells III turned out to be minor.[6] Its 3′,5′-cyclic monophosphate proved effective in activating cAMP-dependent protein kinases.[7] The 2′-deoxy derivative, i.e., 1,3-dideaza-2′-deoxyadenosine, was also synthesized,[8] and oligonucleotides containing the corresponding 4-aminobenz-

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[§] Abbreviations (see also Figure 1, and legend for Figure 4): 4ABI, 4-amino-1*H*-benzimidazole; 4A1RBI, 4-amino-1(β-D-ribofuranosyl)benzimidazole (=1,3-dideazaadenosine); AMP^{2-}, adenosine 5′-monophosphate; cAMP, adenosine-3′,5′-cyclic-monophosphate; L, general ligand, but especially DMBI or MABI (Figure 1); M^{2+}, general divalent metal ion; pK_a, negative logarithm of a general acidity constant. Species which are given 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 versions applies is always clear from the context.

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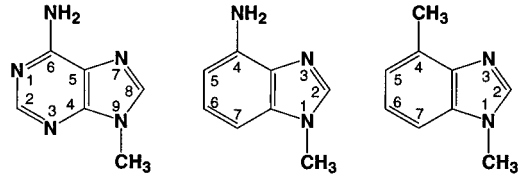

Figure 1. Chemical structures of 9-methyladenine (9MeA), 1-methyl-4-aminobenzimidazole (=MABI = 9-methyl-1,3-dideazaadenine), and 1,4-dimethylbenzimidazole (=DMBI = 6,9-dimethyl-1,3-dideazapurine).

imidazole unit were prepared.[9] It appears that 4-amino-1*H*-benzimidazole interacts with regular nucleoside residues within a Watson−Crick duplex structure, most likely by vertical stacking.[9]

Considering that many reactions in which nucleobase derivatives are involved, especially those with nucleotides,[10] also depend on the presence of metal ions, it is surprising to find, considering the above-mentioned studies, that metal ion complexes of 4-aminobenzimidazole(s) have apparently not been studied.[11−13] This contrasts with the various attempts that have been made to quantify the metal ion-binding properties of the adenine residue.[14−17] Indeed, from studies with tubercidin (=7-deazaadenosine), it is well kown that the 6-amino group considerably inhibits binding of metal ions at N1.[18] Recently it has been further concluded[17] that the 6-amino group also gives rise to steric hindrance for metal ion binding at N7, and for Cu^{2+} it was estimated that this reduces its N7 affinity by about 0.6 log unit.[17] We are attempting now to put this observation on a more quantitative basis by studying 1-methyl-4-aminobenzimidazole (MABI), also named 9-methyl-1,3-dideazaadenine (Figure 1), and by comparing the stability constants of its 1:1 complexes formed by Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} with the $\log K^{M}_{ML}$ versus pK^{H}_{HL} straight-line plots, determined recently for benzimidazole-type ligands with a sterically unhindered N3 position.[19]

However, it was clear to us right from the beginning that there might be a problem with the ligand 1-methyl-4-aminobenzimidazole because the basicity of its amino group was expected to be relatively high compared to that in adenines. For example, the protonated amino group in 4-amino-1*H*-benzimidazole[2] has a pK_a value of 1.5 whereas the same group in 9-methyladenine[20] has a $pK_a < -0.5$ (Figure 1). This difference of more than 2 log units in basicity of the amino groups could possibly give rise to a participation of the amino group in metal ion binding in $M(MABI)^{2+}$ complexes, thus somewhat blurring its inhibitory effect. The direct participation of the amino group in metal ion binding has never been observed in complexes of adenines (see the citations given in ref 21).[22] Therefore, we also included in our study the ligand 1,4-dimethylbenzimidazole (DMBI, Figure 1), since from our earlier studies[18] with tubercidin, 2-methylpyridine (α-picoline), and 2-aminopyridine, it is known that the inhibitory effect of a nonbonding $-NH_2$ and a $-CH_3$ group on metal ion binding are equal. This agrees with the recent conclusion about the shape complementarity of the adenine and the 4-methylbenzimidazole residues.[23]

Finally, it needs to be emphasized that an investigation of the metal ion-binding properties of 1,4-dimethylbenzimidazole, which may also be named 6,9-dimethyl-1,3-dideazapurine (Figure 1), is justified in its own rights since 4-methyl-1*H*-benzimidazole(s) show(s) biological activity,[24] e.g. its 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl) derivative is an excellent inhibitor of HIV-1 reverse transcriptase in an in vitro assay.[25] Furthermore, the 2′-deoxyribonucleoside of 4-methyl-1*H*-benzimidazole was recently shown to be inserted into DNA by the Klenow fragment of DNA polymerase I,[23,26] and its pairing properties were studied.[27] The X-ray crystal structure has also confirmed that this artificial nucleoside is a close steric match for 2′-deoxyadenosine.[27]

2. Experimental Section

2.1. Materials. The ligands 1-methyl-4-aminobenzimidazole and 1,4-dimethylbenzimidazole were prepared as described in section 2.2. The aqueous stock solutions of the ligands were freshly prepared daily just before the experiments by dissolving the compounds in deionized, ultrapure (MILLI-Q185 PLUS; from Millipore S.A., 67120 Molsheim, France) CO_2-free water.

The perchlorate salts needed for the spectrophotometric measurements were obtained from Fluka AG, Buchs, Switzerland. The perchloric acid (70%) was from Merck AG, Darmstadt, Germany. All the other chemicals were the same as used previously,[28] and the stock solutions and titer determinations were also made as previously described.[28]

2.2. Synthesis of 1,4-Dimethylbenzimidazole and 1-Methyl-4-aminobenzimidazole. 2.2.1. 1,4-Dimethylbenzimidazole. A mixture of 4-methyl-1*H*-benzimidazole[29] (660 mg), acetone (50 mL), potassium carbonate (5 g), and methyl iodide (2 g) was refluxed with stirring for 3.5 h. The suspension was filtered, and the residue was washed with acetone (3 × 50 mL). The combined filtrates were evaporated in vacuo, and the residue was chromatographed on a silica gel column (150 g) successively with chloroform and a chloroform−methanol mixture (95:

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5). The product was finally purified by preparative HPLC on a reversed phase (RP-18) column with water, and by crystallization of the residue from ether/petroleum ether. Yield, 52%; mp 78 °C (literature[30] 78 °C). ^{1}H NMR: 8.00 s, 1H; 7.35 d, 1H; 7.18 t, 1H; 7.02 d, 1H (arom protons); 3.90 s, 3H (1-CH_3); 2.59 s, 3H (4-CH_3).

2.2.2. 1-Methyl-4-nitrobenzimidazole. Methyl iodide (20 mL) was added to the stirred solution of 4-nitro-1*H*-benzimidazole[5] (4.9 g, 30 mmol) in methanol (100 mL) and 1 M sodium methoxide in methanol (40 mL), and the mixture was stirred overnight at room temperature. The mixture was concentrated in vacuo, and the residue was chromatographed on a column (150 mL) of Dowex 1X2 (acetate form). Elution with water (UV absorption of the eluate was monitored at 254 nm) gave the fraction of the product with retention. After evaporation in vacuo, the residue was codistilled with ethanol (2 × 50 mL) and crystallized from ethanol/petroleum ether. Yield, 2.7 g, 50.8%; mp 166−167 °C (literature[31] 168 °C). For $C_8H_7N_3O_2$ (177.2) calculated 54.24% C, 3.98% H, 23.72% N; found 54.37% C, 4.02% H, 24.00% N. ^{1}H NMR: 8.50 s, 1H (H-2); 8.05 d, 2H (H-5, H-7); 7.46 t, 1H, J = 8.1 Hz (H-6); 3.94 s, 3H (1-CH_3).

2.2.3. 4-Amino-1-methylbenzimidazole. 1-Methyl-4-nitrobenzimidazole (2.5 g, 14 mmol) in methanol (200 mL) and concentrated hydrochloric acid (1.5 mL) was hydrogenated over 10% palladium-on-charcoal catalyst (0.5 g) at room temperature with slight hydrogen overpressure until the disappearance of the starting material (6 h). The mixture was filtered over Celite, washed with methanol, made alkaline with concentrated aqueous ammonia, and evaporated in vacuo. The residue was applied to a column (150 mL) of Dowex 50X8 (H^+-form), washed with water until the acidity of the eluate decreased, and then eluted with 2.5% ammonia in 20% aqueous methanol. The UV-absorbing fraction of the eluate was evaporated in vacuo, the residue was codistilled with ethanol (2 × 25 mL) and crystallized from ethanol/petroleum ether. Yield, 1.6 g, 77.6%; mp 125 °C (literature[32] 125−127 °C). For $C_8H_9N_3$ (177.2) calculated 65.29% C, 6.16% H, 28.55% N; found 65.43% C, 6.02% H, 28.80% N.

2.3. Potentiometric pH Titrations. The instrumentation, including the desk computers, was the same as described recently,[28] except that for several experiments the DMS−Titrino 716 (Metrohm AG, Herisau, Switzerland) connected with an IBM-compatible desk computer with a Pentium processor and a Hewlett-Packard Desk Jet 1600C Color Smart printer was used. The data obtained with the different equipment agreed within the error limits.

The acidity constants determined are so-called practical, mixed, or Brønsted constants.[33] 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.[33] 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.

2.3.1. Determination of the Acidity Constants. The acidity constant $K^H_{H(DMBI)}$ of $H(DMBI)^+$ was mainly determined by titrating 25 mL of 3.6 mM HNO_3 (25 °C, I = 0.5 M, $NaNO_3$) in the presence and absence of 1.6 mM 1,4-dimethylbenzimidazole, or 15 mL of 6 mM HNO_3 in the presence and absence of 2.7 mM DMBI under N_2 with 1 mL of 0.1 M NaOH; the differences in NaOH consumption between such a pair of titrations were used in the calculations. This acidity constant was also measured twice by titrating 50 mL of 1.8 mM HNO_3 in the presence and absence of 0.8 mM 1,4-dimethylbenzimidazole with 1 mL of 0.1 M NaOH; another three experiments were carried out by titrating 50 mL of 0.54 mM HNO_3 in the presence and absence of 0.3 mM 1,4-dimethylbenzimidazole under N_2 with 1 mL of 0.032 M NaOH (25 °C; I = 0.5 M, $NaNO_3$). These different conditions allowed for a wide variation of the metal-to-ligand ratios (see section 2.3.2).

The acidity constants $K^H_{H_2(MABI)}$ and $K^H_{H(MABI)}$ of $H_2(MABI)^{2+}$ were determined by titrating 25 mL of 2.2 mM HNO_3 (25 °C, I = 0.5 M, $NaNO_3$) in the presence and absence of 1.1 mM 1-methyl-4-aminobenzimidazole under N_2 with 1 mL of 0.06 M NaOH. To reach a lower pH and thus, to obtain a larger formation degree of the $H_2(MABI)^{2+}$ species, we also titrated 10 mL of 25 mM HNO_3 (25 °C, I = 0.5 M, $NaNO_3$) in the presence and absence of 4 mM MABI with 2.5 mL of 0.1 M NaOH.

The acidity constant $K^H_{H(DMBI)}$ was calculated as described previously[28] by taking into account H^+, HL^+, and L species (the formation degree of HL^+ varied between 97 and 3%). Values for the acidity constants $K^H_{H_2(MABI)}$ and $K^H_{H(MABI)}$ were obtained[28] by considering H^+, H_2L^{2+}, LH^+, and L. The highest formation degree that was reached under the experimental conditions for the $H_2(MABI)^{2+}$ species was about 20%. The final result for $K^H_{H_2(MABI)}$ is the average of the values from 18 independent pairs of titrations. In all other instances the results for the acidity constants are the averages of at least 30 independent pairs of titrations.

2.3.2. Determination of the Stability Constants. The stability constants $K^M_{M(DMBI)}$ and $K^M_{M(MABI)}$ of the $M(DMBI)^{2+}$ and $M(MABI)^{2+}$ complexes, respectively, were determined under the same conditions as the acidity constants of the corresponding ligands, but $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ (25 °C, I = 0.5 M).

For most metal ion systems, i.e., with Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}, the titrations in the presence and the absence of DMBI and MABI were made with $[M(NO_3)_2]$ = 0.1667 M, i.e., the M^{2+}:DMBI ratios were 556:1, 208:1, 104:1, and 62:1 for [DMBI] = 0.3 mM, 0.8 mM, 1.6 mM, and 2.7 mM, respectively. In the case of MABI, the M^{2+}:L ratio was 152:1 ([MABI] = 1.1 mM). These conditions had to be used because of the relatively low stability of all these complexes. The use of at least two different DMBI concentrations for each metal ion allowed to measure the stability constants $K^M_{M(DMBI)}$ with at least two different M^{2+}:DMBI ratios. In the case of the MABI complexes with Zn^{2+} and Cd^{2+}, $[M(NO_3)_2]$ = 0.0833 M (i.e., M^{2+}:L = 76:1) was also employed in order to have two different M^{2+}:L ratios for at least two $M(MABI)^{2+}$ complexes.

For the Cu^{2+}/DMBI system, $[Cu(NO_3)_2]$ = 0.1667 M and [DMBI] = 0.3 mM were used (ratio 556:1) as well as $[Cu(NO_3)_2]$ = 0.0417 M (ratio 52:1) and 0.0208 M (M^{2+}:L = 26:1) with [DMBI] = 0.8 mM. In the case of the Cu^{2+}/MABI system, $[Cu(NO_3)_2]$ = 0.0208 M (ratio 19:1) and 0.0104 M (ratio 9.5:1) were employed ([MABI] = 1.1 mM).

All experiments were carried out with $[M^{2+}] \gg [L]$, i.e., $[M^{2+}]_{total} \cong [M^{2+}]_{free}$, and therefore the stability constants $K^M_{M(DMBI)}$ and $K^M_{M(MABI)}$ could be calculated via the apparent acidity constant K'_a as described previously.[28,34] The results showed no dependence on the excess of M^{2+} or the ligand concentration used in the experiments. The final results given for the stability constants, $K^M_{M(DMBI)}$ and $K^M_{M(MABI)}$, are the averages from usually five or more independent pairs of titrations.

The stability of the Ca^{2+}, Sr^{2+}, and Ba^{2+} complexes was very low, and consequently, the buffer depression in all experiments was very small; e.g., for the DMBI system ΔpK_a varies only between 0.036 for Sr^{2+} and 0.053 Ba^{2+}. Therefore the error limits for these complexes are rather large.

2.4. Spectrophotometric Measurements. The acidity constants $K^H_{H_2(MABI)}$ and $K^H_{H(MABI)}$ of $H_2(MABI)^{2+}$ and $H(MABI)^+$, respectively, were also determined by spectrophotometry (Table 1, footnote *c*). The UV spectra of MABI (0.10 mM) (Figure S1 in the Supporting Information) were recorded in aqueous solutions (25 °C, at pH ≥ 0.3, I = 0.5 M, $NaClO_4$) with a Varian Cary 3C spectrophotometer, connected to an IBM-compatible desk computer (OS/2 system) and an EPSON Stylus 1500 printer, and also with a Perkin-Elmer (Lambda 2) UV−vis spectrophotometer, connected to an IBM-compatible desk computer and an EPSON 1000 printer, by using 1-cm quartz cells. The pH values below 1 are defined by the negative logarithm of the $HClO_4$ concentration used; the pH values above 1 were adjusted and measured as described in ref 19 by using the same equipment. A typical experimental series is shown in Figure S1 and its evaluation (it was

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Table 1. Negative Logarithms of the Acidity Constants of Protonated 1-Methyl-4-aminobenzimidazole ($H_2(MABI)^{2+}$, Eqs 2 and 3) and 1,4-Dimethylbenzimidazole ($H(DMBI)^+$, Eq 3) Together with Related Data[a,b]

no.	protonated ligand (L)	$pK^H_{H_2L}$	pK^H_{HL}
1	1-methyl-4-aminobenzimidazole;[c] $H_2(MABI)^{2+}$	1.33 ± 0.10	5.28 ± 0.04
2	4-amino-1*H*-benzimidazole;[d] $H_2(4ABI)^{2+}$	1.5	5.3
3	1,3-dideazaadenosine;[e] $H_2(A1RBI)^{2+}$	0.7	4.5
4	adenosine; $H_2(Ado)^{2+}$	−1.5[f]	3.61 ± 0.03[g]
5	9-methyladenine;[h] $H_2(9MeA)^{2+}$	−0.37 ± 0.06	4.10 ± 0.01
6	1,4-dimethylbenzimidazole;[i] $H(DMBI)^+$		5.78 ± 0.02
7	4-methyl-1*H*-benzimidazole;[j] $H(4MBI)^+$		5.65
8	1-methylbenzimidazole;[k] $H(1MBI)^+$		5.67 ± 0.01
9	benzimidazole;[k] $H(BI)^+$		5.63 ± 0.01

[a] It should be noted that the $pK^H_{H_2L}$ values of entries 1−3 refer to the deprotonation of the (C4)NH_3^+ group, and those of pK^H_{HL} for entries 1−3 and 6−9 refer to that of the (N3)H^+ site of benzimidazoles (see Figure 1). For the adenine derivatives (entries 4, 5; see also Figure 1) the first proton is released from (N7)H^+ ($pK^H_{H_2L}$) and the second one from (N1)H^+ (pK^H_{HL}). If nothing else is mentioned, the data refer to aqueous solutions at 25 °C and $I = 0.5$ M, $NaNO_3$. So-called practical (or mixed) constants[33] are listed (see section 2.3) as far as our own work is concerned. [b] The error limits given are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [c] This work; the values determined by spectrophotometry are $pK^H_{H_2(MABI)} = 1.28 \pm 0.13$ and $pK^H_{H(MABI)} = 5.24 \pm 0.11$ (25 °C, $I = 0.5$ M, $NaClO_4$).[a,b] [d] From ref 2, conditions unknown. [e] From ref 9, conditions unknown; A1RBI = 4-amino-1(β-D-ribofuranosyl)benzimidazole. [f] From refs 17 and 38; 25 °C; I is high. [g] From ref 39. [h] From ref 20a. [i] This work. [j] From ref 40; 25 °C, $I \approx 0.002$ M (= c). [k] From ref 19.

analyzed as before)[19] is provided in Figure 2. The final results, given in footnote *c* of Table 1, for $K^H_{H_2(MABI)}$ and $K^H_{H(MABI)}$ are the averages of three independent experiments (see also legend of Figure 2).

The stability constants $K^M_{M(MABI)}$ of $Ni(MABI)^{2+}$ and $Cd(MABI)^{2+}$ were also determined by recording UV difference spectra between 190 and 330 nm with the instrumentation mentioned above. As a typical example of an experimental series, the difference spectra of one of the Cd^{2+}/MABI measurements are shown in Figure S2 of the Supporting Information, and their evaluation is provided in Figure 3. These spectra were recorded in the 230−330 nm range (see Figure S2) because the $Cd(ClO_4)_2$ solution itself absorbs in the UV range below 235 nm. In the case of the $Ni(ClO_4)_2$ solution, the self-absorption occurs only below 200 nm; therefore the absorption change observed for the Ni^{2+}/MABI system around 220 nm could also be used in the determination of the stability constant, $K^{Ni}_{Ni(MABI)}$; i.e., this stability constant was determined via the differences $\Delta\Delta A_{246-220} = \Delta A_{246} - \Delta A_{220}$, $\Delta\Delta A_{278-286} = \Delta A_{278} - \Delta A_{286}$, and $\Delta\Delta A_{246-266} = \Delta A_{246} - \Delta A_{266}$ (compare with Figure 3). All evaluations were made analogously as described for ^1H NMR shift data[35] by employing a curve-fitting procedure that used a Newton−Gauss nonlinear least-squares program. The concentration range used for both $M(ClO_4)_2$ systems was within 0.014−0.1667 M (25 °C, $I =$ 0.5 M, $NaClO_4$) (see also Figure 3). The pH of the solutions was adjusted in all experimental series to one of the following two values, 6.06 ± 0.02 or 6.46 ± 0.02. The measured apparent stability constants

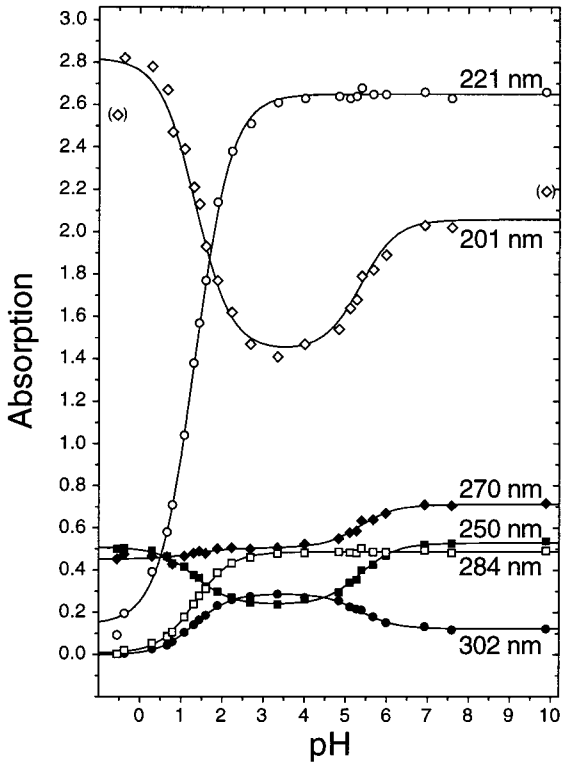

Figure 2. The UV absorption spectra of 1-methyl-4-aminobenzimidazole (MABI) in aqueous solution, as shown in Figure S1 of the Supporting Information (23 pH values), were evaluated at 201 (◇), 221 (○), 243, 250 (■), 258, 270 (◆), 284 (□), and 302 (●) nm as a function of pH. The evaluations at 243 and 258 nm are not shown because of space limitations, but they are similar to the one at 250 nm. The evaluations at 201, 243, 250, 258, and 302 nm furnished both acidity constants of $H_2(MABI)^{2+}$, whereas the evaluations at 221 and 284 nm provided only a value for $pK^H_{H_2(MABI)}$ and the one at 270 nm a value for $pK^H_{H(MABI)}$. All these evaluations led to the average results $pK^H_{H_2(MABI)} = 1.34 \pm 0.03$ and $pK^H_{H(MABI)} = 5.36 \pm 0.09$ (3σ) for this experiment (25 °C, at pH ≥ 0.3, $I = 0.5$ M, $NaClO_4$). The solid curves shown are the computer-calculated best fits for the various wavelengths through the experimental data points obtained at pH −0.54, −0.37, 0.30, 0.67, 0.80, 1.09, 1.31, 1.45, 1.60, 1.89, 2.24, 2.69, 3.35, 4.01, 4.84, 5.11, 5.27, 5.40, 5.68, 5.99, 6.93, 7.59, and 9.88 (from left to right) by using the mentioned averages of the two acidity constants. In the evaluation at 201 nm, the points at the lowest and the highest pH (−0.54 and 9.88) were not taken into account in the calculations but they are shown to provide a complete picture. The final results given in section 3.1 (Table 1, footnote *c*) are the averages of three independent experiments.

were then corrected for the competition of the proton by means of eq 1:[36,37]

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

The final results, given in section 3.2 (see also footnotes *d* and *e* of Table 2), are the averages of two independent series of experiments with six evaluations in total in the case of $Ni(MABI)^{2+}$; for $Cd(MABI)^{2+}$ three independent series of experiments, with nine evaluations in total, were performed.

3. Results and Discussion

3.1. Acid−Base Properties of 1,4-Dimethylbenzimidazole (DMBI) and 1-Methyl-4-aminobenzimidazole (MABI). Knowing that 4-aminobenzimidazole[2] may be protonated at the amino

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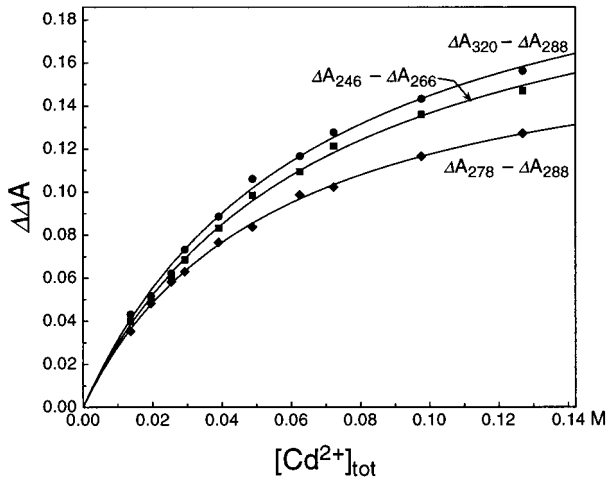

Figure 3. Evaluation of the UV absorption difference spectra of 1-methyl-4-aminobenzimidazole (0.1 mM), seen in Figure S2 (Supporting Information), via the differences $\Delta\Delta A_{320-288}$ (●), $\Delta\Delta A_{278-288}$ (◆), and $\Delta\Delta A_{246-266}$ (■) as a function of the total $Cd(ClO_4)_2$ concentration present in aqueous solutions at pH = 6.06 ± 0.02 (25 °C, I = 0.5 M, $NaClO_4$) for log K_{app} and $\Delta\Delta A_{max}$. The solid curves represent the computer-calculated best fits of the experimental data points (see also section 2.4) which lead to K_{app} = 14.9 ± 0.9, 18.3 ± 0.6, and 14.8 ± 0.8 (1σ), and $\Delta\Delta A_{max}$ = 0.242 ± 0.008, 0.182 ± 0.003, and 0.229 ± 0.006 (1σ) for the calculations with $\Delta\Delta A_{320-288}$, $\Delta\Delta A_{278-288}$, and $\Delta\Delta A_{246-266}$, respectively. The weighted mean of the logarithmic results gives log K_{app} = 1.202 ± 0.089 (3σ); this apparent stability constant needs to be transformed with eq 1 into the pH-independent constant to give log $K^{Cd}_{Cd(MABI)}$ = (1.202 ± 0.089) + 0.067 = 1.27 ± 0.09 (3σ). The final value given in section 3.2 is the average of three independent experiments.

group in the low pH region, the same was expected for MABI. Considering further the well-known proton affinity of N3 of benzimidazoles,[19] the two-fold protonated $H_2(MABI)^{2+}$ species should form, and therefore, in the present study the following two equilibria needed to be taken into account:

$$H_2L^{2+} \rightleftharpoons HL^+ + H^+ \quad (2a)$$

$$K^H_{H_2L} = [HL^+][H^+]/[H_2L^{2+}] \quad (2b)$$

$$HL^+ \rightleftharpoons L + H^+ \quad (3a)$$

$$K^H_{HL} = [L][H^+]/[HL^+] \quad (3b)$$

Of course, with DMBI only the (N3)-protonated $H(DMBI)^+$ species can form and therefore, only equilibrium 3 is of relevance. The acidity constants of the two mentioned compounds were determined by potentiometric pH titrations (aqueous solution; 25 °C, I = 0.5 M, $NaNO_3$). The results are given in entries 1 and 6 of Table 1, together with some related data.[2,9,17,19,20,38−40]

Since the release of the first proton from $H_2(MABI)^{2+}$ occurs with $pK^H_{H_2(MABI)}$ = 1.33 (Table 1), the conditions needed for the potentiometric pH titrations do not allow to reach a large degree of formation of $H_2(MABI)^{2+}$. Therefore, we have also endeavored to determine the corresponding acidity constants by UV spectrophotometry by measuring the absorption spectra of MABI, as a function of pH. Because of the absorption of NO_3^- in the UV range, the pH was now adjusted with $HClO_4$, and the ionic strength with $NaClO_4$. A representative experiment with its set of spectra is shown in Figure S1 of the Supporting Information, and a partial evaluation of these experimental data is given in Figure 2. The overall result from three experimental series is $pK^H_{H_2(MABI)}$ = 1.28 ± 0.13 and $pK^H_{H(MABI)}$ = 5.24 ± 0.11 (25 °C, I = 0.5 M, $NaClO_4$); these results are in excellent agreement with the constants measured by potentiometric pH titrations (Table 1).

Comparison of the various constants summarized in Table 1 allows us to make the following conclusions: (i) Substitution of the hydrogen at N1 by a methyl group (Figure 1) has little effect on the acid−base properties of N3 of benzimidazoles (cf. entries 1, 2 and 8, 9). (ii) Similarly, replacement of the hydrogen at C4 by a methyl group is not of relevance (entries 7 and 9) whereas the substitution of H or CH_3 at C4 by an amino group makes the latter compound more acidic at the $(N3)H^+$ site by $\Delta pK_a \simeq 0.4$ (cf. entries 6−9 with 1, 2). (iii) Of utmost interest is the replacement of N1 and N3 in adenines by CH units to give the 1,3-dideazaadenines, i.e., benzimidazole derivatives; comparison of entry 4 with 3, and entry 5 with 1, shows that removal of the two ring nitrogens does not only alter the sites of protonation (see Table 1, footnote *a*), but in addition it leads to a large decrease in acidity (i.e., an increase in basicity) by $\Delta pK_a \simeq 2$, as far as $pK^H_{H_2L}$ is concerned. (iv) As one might expect, the indicated substitutions have a small effect on the pK^H_{HL} values ($\Delta pK_a \simeq 1$) which concern the deprotonation of $(N)H^+$ ring sites, i.e., of $(N1)H^+$ in the adenines and $(N3)H^+$ in the benzimidazoles.

3.2. Stability Constants of $M(DMBI)^{2+}$ and $M(MABI)^{2+}$ Complexes. The stability constants of the metal ion complexes formed with DMBI and MABI were determined via potentiometric pH titrations (25 °C, I = 0.5 M, $NaNO_3$). The experiments were carried out with the metal ion concentrations in large excess to the ligand concentration, and therefore only the formation of 1:1 complexes, according to equilibrium 4, needed to be considered:

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

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

This means that the experimental data of the potentiometric pH titrations may be completely described by taking into account the equilibria 3a and 4a (the role of eq 2 is insignificant) if the evaluation is not carried into the pH range where hydroxo complex formation occurs. The results are summarized in columns 2 and 5 of Table 2. To the best of our knowledge,[11−13] none of the listed stability constants has been determined before.

The determination of stability constants by potentiometric pH titration 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 be measured with high precision if the pK_a values are in an easily accessible pH range. However, due to the special properties of the $M(MABI)^{2+}$ complexes of the transition metal ions (see section 3.4) we considered it necessary to determine, at least for a few cases, the stability of the complexes by an independent method. We did this by UV spectrophotometry for the complexes of Ni^{2+} and Cd^{2+}.

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(39) Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1987**, *163*, 353−363.
(40) (a) Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution*; IUPAC, Butterworth: London, 1965; p 266. (b) Davies, M. T.; Mamalis, P.; Petrow, V.; Sturgeon, B. *J. Pharm. Pharmacol.* **1951**, *3*, 420−430.

Table 2. Comparison of the Logarithms of the Stability Constants of the 1:1 Complexes (Eq 4) Formed between Several Divalent Metal Ions (M^{2+}) and 1,4-Dimethylbenzimidazole (DMBI) or 1-Methyl-4-aminobenzimidazole (MABI) as Determined by Potentiometric pH Titrations (exptl) in Aqueous Solution with the Calculated (calcd) Stability Constants Based on the Acidity of the $(N3)H^+$ Site of the $H(DMBI)^+$ and $H(MABI)^+$ Species[a,b] and the Straight-Line Equations 5a to 5g for Sterically Unhindered Benzimidazole-Type Ligands (Ref 19) (25 °C, $I = 0.5$ M, $NaNO_3$). The Steric Effects Exerted by the 4-Methyl or 4-Amino Group on Metal Ion Binding at N3 of Benzimidazole-Type Ligands are Expressed by log $\Delta_{M/L}$ (eq 7)[b]

	$\log K^{M}_{M(DMBI)}$			$\log K^{M}_{M(MABI)}$		
M^{2+}	exptl	calcd[c]	log $\Delta_{M/DMBI}$	exptl	calcd[c]	log $\Delta_{M/MABI}$
Ba^{2+}	-0.11 ± 0.15	-0.2 ± 0.2[c]	0.09 ± 0.25	-0.20 ± 0.18	-0.2 ± 0.2[c]	0.00 ± 0.27
Sr^{2+}	-0.28 ± 0.19	-0.2 ± 0.15[c]	-0.08 ± 0.24	-0.11 ± 0.15	-0.2 ± 0.15[c]	0.09 ± 0.21
Ca^{2+}	-0.20 ± 0.22	-0.14 ± 0.15[c]	-0.06 ± 0.27	-0.07 ± 0.14	-0.14 ± 0.15[c]	0.07 ± 0.21
Mg^{2+}	-0.04 ± 0.12	0.02 ± 0.05	-0.06 ± 0.13	-0.02 ± 0.10	0.01 ± 0.05	-0.03 ± 0.11
Mn^{2+}	-0.10 ± 0.06	0.76 ± 0.05	-0.86 ± 0.08	0.13 ± 0.13	0.67 ± 0.05	-0.54 ± 0.14
Co^{2+}	0.09 ± 0.06	1.59 ± 0.04	-1.50 ± 0.07	0.57 ± 0.04	1.50 ± 0.04	-0.93 ± 0.06
Ni^{2+}	0.16 ± 0.06	2.02 ± 0.04	-1.86 ± 0.07	1.37 ± 0.06[d]	1.92 ± 0.04	-0.55 ± 0.07
Cu^{2+}	2.26 ± 0.05	3.20 ± 0.04	-0.94 ± 0.06	2.49 ± 0.06	3.00 ± 0.04	-0.51 ± 0.07
Zn^{2+}	0.36 ± 0.08	1.59 ± 0.04	-1.23 ± 0.09	0.62 ± 0.03	1.44 ± 0.04	-0.82 ± 0.05
Cd^{2+}	0.72 ± 0.06	2.08 ± 0.04	-1.36 ± 0.07	1.27 ± 0.04[e]	1.93 ± 0.04	-0.66 ± 0.06

[a] Acidity constants: $pK^{H}_{H(DMBI)} = 5.78 \pm 0.02$ and $pK^{H}_{H(MABI)} = 5.28 \pm 0.04$ (Table 1; entries 1 and 6).[b] [b] For the error limits see footnote *b* of Table 1; the error limits of the derived data, in the present case of log $\Delta_{M/L}$, were calculated according to the error propagation after Gauss. [c] See ref 19 and eq 6. [d] By spectrophotometry (25 °C, $I = 0.5$ M, $NaClO_4$), $\log K^{Ni}_{Ni(MABI)} = 1.46 \pm 0.09$.[b] [e] By spectrophotometry, $\log K^{Cd}_{Cd(MABI)} = 1.28 \pm 0.09$ (see Figure 3).[b]

The spectral alterations which occur for MABI upon complex formation are small, but they can be well characterized by recording difference spectra. Figure S2 of the Supporting Information provides a representative set of such spectra which show the effect of increasing amounts of Cd^{2+} on MABI. These experimental data were evaluated for the differences of the differences at ($\Delta A_{320} - \Delta A_{288}$), ($\Delta A_{246} - \Delta A_{266}$), and ($\Delta A_{278} - \Delta A_{288}$) by a curve-fitting procedure (section 2.4), which is shown in Figure 3.

The difference spectra of Figure S2 were recorded at pH 6.06; since $pK^{H}_{H(MABI)} = 5.28$ (Table 1, entry 1), a part of the ligand still exists in its protonated form. Hence, only so-called apparent stability constants are obtained; these need to be corrected for the competition between proton and metal ion binding by eq 1 (section 2.4).[36,37] Corresponding experiments were made for the Ni^{2+}/MABI system. The results of several experimental series (see section 2.4) are $\log K^{Ni}_{Ni(MABI)} = 1.46 \pm 0.09$ and $\log K^{Cd}_{Cd(MABI)} = 1.28 \pm 0.09$ for the $Ni(MABI)^{2+}$ and $Cd(MABI)^{2+}$ complexes, respectively. The results of the spectrophotometric measurements and of the potentiometric pH titrations (Table 2) are evidently in excellent agreement, thus confirming the correctness of the data in Table 2.

The error limits of the stability constants determined for the ML^{2+} complexes of Ca^{2+}, Sr^{2+}, and Ba^{2+} are rather large (Table 2, columns 2 and 5), because of the instability of these complexes and the connected small buffer depression (section 2.3). This observation fits into the trends that are usually observed with ligands of this kind;[19,41] i.e., the stabilities of the complexes of the alkaline earth ions are much lower than those of the complexes of the divalent 3d transition ions; for the latter complexes the Irving−Williams series[42] is clearly followed.

3.3. Evaluation of the Steric Inhibition of the $(C4)CH_3$ or $(C4)NH_2$ Group on Metal Ion Binding at the N3 Site. The previously established[19] correlation between (N3)-benzimidazole basicity and the stability of the corresponding M^{2+} complexes that formed with several simple and sterically unhindered benzimidazole-type ligands (see legend of Figure 4) allows a quantitative evaluation of the indicated steric effects. In this previous work,[19] it was shown that the $\log K^{M}_{ML}$ versus pK^{H}_{HL} plots resulted in straight lines. A least-squares treatment of the experimental data for seven metal ions and benzimidazole-type ligands led to the straight-line equations[19] summarized below (error limits: 1σ):

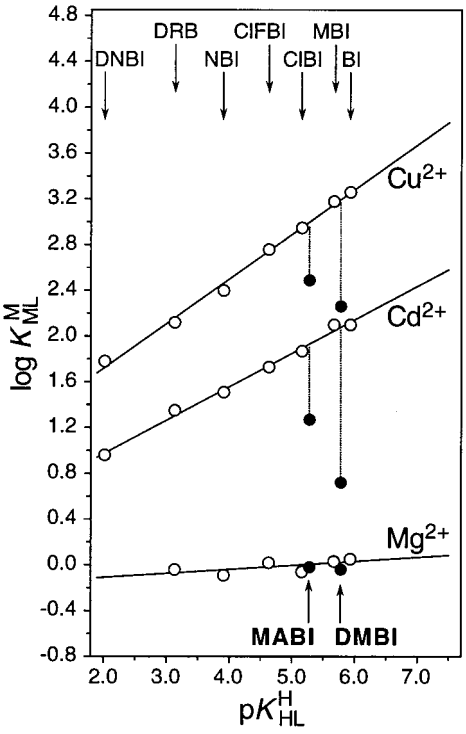

Figure 4. Evidence for an unaffected stability of the Mg^{2+} and for a reduced stability of the Cd^{2+} and Cu^{2+} (●) 1:1 complexes of 1,4-dimethylbenzimidazole (DMBI) and 1-methyl-4-aminobenzimidazole (MABI), based on the $\log K^{M}_{ML}$ versus pK^{H}_{HL} plots for the 1:1 complexes of Mg^{2+}, Cd^{2+}, and Cu^{2+} with the following simple and sterically unhindered benzimidazole-type ligands (○): 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); the data pairs are from ref 19 (where appropriate the micro acidity constants were applied; see section 2.2 in ref 19). The least-squares straight-reference lines are drawn according to the eqs 5a, 5e, and 5g (see also Table 3 of ref 19). The vertical broken lines emphasize the stability differences to the corresponding reference lines. The data pairs for the M^{2+}/DMBI and M^{2+}/MABI systems are from Tables 1 and 2. All plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.5$ M ($NaNO_3$).

$$\log K^{Mg}_{MgL} = (0.035 \pm 0.020)pK^{H}_{HL} - (0.179 \pm 0.095) \quad (5a)$$

$$\log K^{Mn}_{MnL} = (0.182 \pm 0.017)pK^{H}_{HL} - (0.287 \pm 0.081) \quad (5b)$$

$$\log K^{Co}_{CoL} = (0.186 \pm 0.011)pK^{H}_{HL} + (0.513 \pm 0.048) \quad (5c)$$

$$\log K^{Ni}_{NiL} = (0.201 \pm 0.011)pK^{H}_{HL} + (0.860 \pm 0.052) \quad (5d)$$

$$\log K^{Cu}_{CuL} = (0.391 \pm 0.013)pK^{H}_{HL} + (0.936 \pm 0.057) \quad (5e)$$

$$\log K^{Zn}_{ZnL} = (0.284 \pm 0.010)pK^{H}_{HL} - (0.055 \pm 0.045) \quad (5f)$$

$$\log K^{Cd}_{CdL} = (0.293 \pm 0.011)pK^{H}_{HL} + (0.385 \pm 0.050) \quad (5g)$$

The error limits of log stability constants calculated with given pK^{H}_{HL} values and eqs 5a−5g are ±0.051 (Mg^{2+}), ±0.045 (Mn^{2+}), ±0.042 (Co^{2+}), ±0.042 (Ni^{2+}), ±0.042 (Cu^{2+}), ±0.039 (Zn^{2+}), and ±0.039 (Cd^{2+}) log unit (3σ), respectively, in the pK_a range of about 2−6 (see Figure 4) (aqueous solution; 25 °C, $I = 0.5$ M, $NaNO_3$; see Tables 3 and 4 in ref 19).

For the benzimidazole-type complexes of Ca^{2+}, Sr^{2+}, and Ba^{2+} in the pK_a range 3−6, no correlation between complex stability and the basicity of N3 was observed. In these instances, the following results were obtained[19] in the mentioned pK_a range (error limits: 3σ):

$$\log K^{Ca}_{CaL} = -0.14 \pm 0.15 \quad (6a)$$

$$\log K^{Sr}_{SrL} = -0.2 \pm 0.15 \quad (6b)$$

$$\log K^{Ba}_{BaL} = -0.2 \pm 0.2 \quad (6c)$$

The above achievements now allow one to calculate the stability constant for a pure and sterically unaffected coordination of a metal ion to N3 of a benzimidazole-type ligand with the known acidity constant of any $(N3)H^+$ site. These calculated constants, $\log K^{M}_{ML/calcd}$, may then be compared with the experimental values, $\log K^{M}_{ML/exptl}$, according to eq 7:

$$\log \Delta_{M/L} = \log K^{M}_{ML/exptl} - \log K^{M}_{ML/calcd} \quad (7)$$

From the three examples seen in Figure 4, for plots of $\log K^{M}_{ML}$ versus pK^{H}_{HL}, it is evident that there are complexes in which the substituent at C4 exerts a significant effect on the stability of the complexes, e.g., on those of Cu^{2+} or Cd^{2+} with MABI or DMBI, whereas corresponding complexes such as those with Mg^{2+} show the stability expected on the basis of the basicity of N3. Application of the pK^{H}_{HL} values of $H(MABI)^+$ and $H(DMBI)^+$ (Table 1) to eqs 5a to 5g, as well as the use of eqs 6a to 6c, gives the results listed in columns 3 and 6 of Table 2 for the $M(DMBI)^{2+}$ and $M(MABI)^{2+}$ complexes, respectively. The now possible application of eq 7 provides the differences listed in columns 4 and 7, which are a direct reflection of the steric inhibition caused by the C4 substituent.

Several conclusions are immediately evident from the results provided in Table 2: (i) The $\log \Delta_{M/L}$ values for the Ba^{2+}, Sr^{2+}, Ca^{2+}, and Mg^{2+} complexes of DMBI and MABI are zero within the error limits. This means, the C4 substituent does not exert a steric hindrance, and most likely indicates that in these instances outersphere complexes are formed, with the complexes having a water molecule between N3 and M^{2+}. (ii) In the case of the ML complexes of the divalent transition metal ions, including Zn^{2+} and Cd^{2+}, the $\log \Delta_{M/L}$ values are strongly negative, varying between about −0.5 and −1.9 log units, thus proving a significant steric effect of the C4 substituent on metal ion binding at N3. (iii) A careful comparison of the $\log \Delta_{M/L}$ values between columns 4 and 7 for the complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} reveals that the steric effect in the $M(MABI)^{2+}$ complexes is about 0.3−1.3 log units less pronounced.

The observation mentioned last can only mean that, if one considers the steric equivalence of a methyl and an amino group as discussed in section 1, the relatively high basicity of the amino group of MABI leads to a contribution of this group in metal ion binding; this problem will be discussed further in section 3.4 below. For the present, it needs to be emphasized that only the $\log \Delta_{M/L}$ values obtained for the M(DMBI) complexes provide a correct reflection of the steric effect of a methyl or a "nonparticipating" amino group at C4 on metal ion binding at N3 of benzimidazole-type ligands (cf. the structures in Figure 1).

In the past, it has been repeatedly observed[16−19,43] that the $\log K^{M}_{ML}$ versus pK^{H}_{HL} plots for sterically unaffected ligands and for those with a constant steric effect, e.g., pyridine-type ligands as compared to 2-methylpyridine-type ligands,[18] lead to straight lines which are parallel to each other, i.e., they have the same slope m within the error limits (usually 2σ). One such example, which is meaningful to the present situation, is shown in Figure 5 for the Zn^{2+} complexes of imidazole- and benzimidazole-type ligands; the slopes m of the two straight lines are identical within the error limits (1σ), $m_{Zn/imidazoles} = 0.296 \pm 0.012$ (see ref 28) and $m_{Zn/benzimidazoles} = 0.284 \pm 0.010$ (see ref 19).

From Figure 5 it is evident, and this is a general observation also valid for other M^{2+},[19,28] that the Zn^{2+} complexes of the benzimidazole-type ligands have a reduced stability compared to those of the imidazole-type ligands; in other words annelation, i.e., the fusion of a benzene ring to the 4,5 positions of imidazole, leads to a steric inhibition. Having recognized this, it is no additional surprise that a methyl substituent at C4 affects complex formation at the N3 site even more, as is also seen in Figure 5, and a further parallel reference line, defined by the Zn^{2+}/DMBI data pair, is anticipated for 4-methyl(-1-substituted)-benzimidazole-type ligands. The corresponding combination of the results given in eqs 5 and 6 with those listed in column 4 of Table 2 leads then to the straight line parameters defined in Table 3 for complexes of 4-methyl(-1-substituted)benzimidazole-type ligands.

3.4. Evidence for Chelate Formation Involving the Amino Group in $M(MABI)^{2+}$ Complexes. The occurrence of a smaller steric inhibition by the $(C4)NH_2$ group compared to that of the $(C4)CH_3$ substituent is evident from Figure 4, and as discussed already in section 3.3, this observation may be attributed to the participation of the amino group in metal ion binding. Indeed, from the structure of MABI seen in Figure 1, it is evident that with this ligand, a metal ion coordinated to N3 may also form a chelate by binding in addition to the $(C4)NH_2$ group. Hence, the stability increase observed, e.g. for $Zn(MABI)^{2+}$, if compared to the reference line defined by $Zn(DMBI)^{2+}$ and as expressed by the vertical arrow seen in Figure 5, is a reflection

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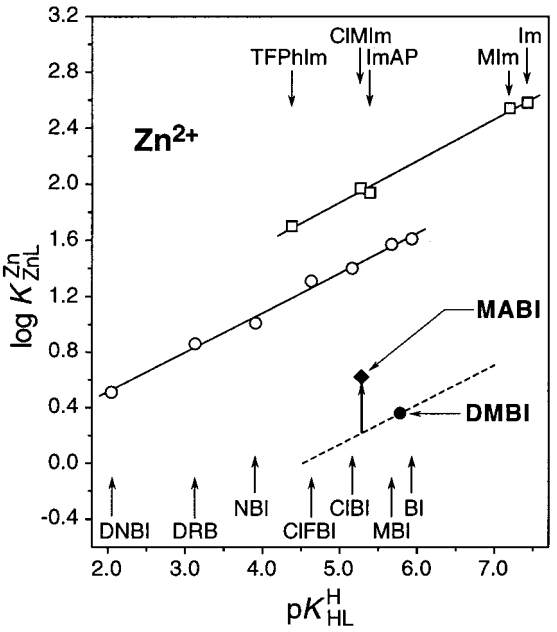

Figure 5. Evidence for a reduced stability of Zn^{2+} 1:1 complexes of benzimidazole-type ligands (○) compared with those of imidazole-type ligands (□), based on the relationship log K^{Zn}_{ZnL} versus pK^{H}_{HL}. The reduced stability reflects the steric inhibition due to annelation. The insertion of a methyl substituent at C4 leads to a further steric effect, as one might expect, and as is evidenced by the Zn^{2+}/DMBI data pair (●) (data from Table 2). The enhanced complex stability of Zn(MABI)$^{2+}$ (◆) relative to that of Zn(DMBI)$^{2+}$ is emphasized by the vertical arrow (see section 3.4). 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) (see ref 28). For the benzimidazole-type ligands, see the legend of Figure 4. All plotted equilibrium constants refer to aqueous solutions at 25 °C and I = 0.5 M ($NaNO_3$).

of this chelate formation. This means that an intramolecular equilibrium exists between an "open" (op), only (N3)-coordinated, and a chelated or "closed" (cl) species (eq 8):

$$M(MABI)^{2+}_{op} \rightleftharpoons M(MABI)^{2+}_{cl} \quad (8a)$$

$$K_I = [M(MABI)^{2+}_{cl}]/[M(MABI)^{2+}_{op}] \quad (8b)$$

Whether the closed species consist of five-membered chelates, of semi-chelates, i.e., with a water molecule between M^{2+} and the NH_2 group, or of a mixture of the two types of chelates cannot be concluded with certainty from the present study. Though, considering the high formation degrees of the closed species (see below), it appears likely that with the transition metal ions the five-membered chelates are playing a role.

Application of the straight-line parameters given in Table 3 with $pK^{H}_{H(MABI)} = 5.28$ (Table 1) allows one to calculate the stability constant of the open complex, in which the NH_2 group exerts its full steric effect, and to define the stability increase (as expressed by the vertical arrow in Figure 5) due to chelate formation by comparing it with the measured value according to eq 9:

$$\log \Delta^{*}_{M/MABI} = \log \Delta^{*} = \log K^{M}_{M(MABI)} - \log K^{M}_{M(MABI)/op} \quad (9a)$$

$$= \log K^{M}_{M(MABI)/exptl} - \log K^{M}_{M(MABI)/calcd} \quad (9b)$$

Table 3. Straight-Line Correlations[a] for M^{2+}-4-Methyl(-1-substituted)benzimidazole-type Complex Stabilities and N3 Site Basicities, for Aqueous Solutions at 25 °C and I = 0.5 M ($NaNO_3$)[b,c]

no.	M^{2+}	m	b	3 × SD[d]
1	Ba^{2+}	0	−0.2	0.2
2	Sr^{2+}	0	−0.2	0.15
3	Ca^{2+}	0	−0.14	0.15
4	Mg^{2+}	0.035	−0.239	0.14
5	Mn^{2+}	0.182	−1.147	0.09
6	Co^{2+}	0.186	−0.987	0.08
7	Ni^{2+}	0.201	−1.000	0.08
8	Cu^{2+}	0.391	−0.004	0.07
9	Zn^{2+}	0.284	−1.285	0.10
10	Cd^{2+}	0.293	−0.975	0.08

[a] Straight-line equation: $y = mx + b$, where x represents the pK^{H}_{HL} value of any N3-protonated 4-methyl(-1-substituted)benzimidazole derivative and y the calculated stability constant (log K^{M}_{ML}) of the corresponding ML^{2+} complex (eq 4). [b] The data for entries 1−3 are based on the information given with eqs 6a−6c. [c] For entries 4−10, the slopes m are taken from eqs 5a−5g and the stability difference log $\Delta_{M/DMBI}$ (Table 2, column 4) was subtracted from the corresponding values for b in these equations to obtain the b values given above, valid for the complexes formed with 4-methyl(-1-substituted)benzimidazole-type ligands. [d] SD = standard deviation (see ref 19). The values given above for 3 × SD (3σ) are considered as reasonable error limits in the pK^{H}_{HL} range 2−6. The above values were calculated (via the error propagation according to Gauss) from the error limits given in the text in section 3.3 following eqs 5a−5g and the error limits listed in column 4 of Table 2 for log $\Delta_{M/DMBI}$.

Table 4. Evidence for Chelate Formation in $M(MABI)^{2+}$ Complexes Based on the Stability Constants Determined by Potentiometric pH Titrations (exptl) for $M(MABI)^{2+}$ Complexes (Eq 4) and Their Comparison with Those Calculated (calcd) for a Sole Monodentate M^{2+}-(4-Methylbenzimidazole)-type N3 Coordination. The Corresponding Stability Differences log $\Delta^{*}_{M/MABI}$ (Eq 9), as well as the Intramolecular and Dimensionless Equilibrium Constants K_I (Eqs 8, 10) and the Percentages of the Closed Species (Eq 11) are Given. The Data Apply to Aqueous Solutions at 25 °C and I = 0.5 M ($NaNO_3$)[a]

	log $K^{M}_{M(MABI)}$				
M^{2+}	exptl[b]	calcd[c]	log $\Delta^{*}_{M/MABI}$	K_I	%M $(MABI)^{2+}_{cl}$
Ba^{2+}	−0.20 ± 0.18	−0.2 ± 0.2	0.00 ± 0.27		n.e.[d]
Sr^{2+}	−0.11 ± 0.15	−0.2 ± 0.15	0.09 ± 0.21		n.e.
Ca^{2+}	−0.07 ± 0.14	−0.14 ± 0.15	0.07 ± 0.21		n.e.
Mg^{2+}	−0.02 ± 0.10	−0.05 ± 0.14	0.03 ± 0.17		n.e.
Mn^{2+}	0.13 ± 0.13	−0.19 ± 0.09	0.32 ± 0.16	1.09 ± 0.76	52 ± 17
Co^{2+}	0.57 ± 0.04	0.00 ± 0.08	0.57 ± 0.09	2.72 ± 0.77	73 ± 6
Ni^{2+}	1.37 ± 0.06	0.06 ± 0.08	1.31 ± 0.10	19.42 ± 4.70	95 ± 1
Cu^{2+}	2.49 ± 0.06	2.06 ± 0.07	0.43 ± 0.09	1.69 ± 0.57	63 ± 8
Zn^{2+}	0.62 ± 0.03	0.21 ± 0.10	0.41 ± 0.10	1.57 ± 0.62	61 ± 9
Cd^{2+}	1.27 ± 0.04	0.57 ± 0.08	0.70 ± 0.09	4.01 ± 1.03	80 ± 4

[a] For the error limits see footnote *b* of Table 2. [b] From column 5 of Table 2. [c] Calculated with $pK^{H}_{H(MABI)} = 5.28$ (Table 1, entry 1) and the straight-line parameters listed in Table 3. [d] n.e. = no evidence for chelate formation within the error limits.

The equivalence of the various terms in eq 9 is evident, and the corresponding results are listed in columns 2, 3, and 4 of Table 4.

Following previous routes,[44,45] the application of the log $\Delta^{*}_{M/MABI}$ values (Table 4, column 4) allows one to define the position of equilibrium 8a; i.e., the intramolecular equilibrium constant, K_I, as defined in eq 8b, follows from eq 10:

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

Knowledge of K_I allows then the calculation of the percentage of the closed isomer occurring in equilibrium 8a:

$$\%M(MABI)_{cl}^{2+} = 100 \cdot K_I/(1+K_I) \quad (11)$$

The use of eqs 10 and 11 leads to the results that are summarized in columns 5 and 6 of Table 4.

The results of Table 4 allow for several interesting conclusions to be drawn. (i) There is no evidence for the formation of closed or chelated species for the $M(MABI)^{2+}$ complexes of the alkaline earth ions; this is in accord with the known[36] low affinity of these metal ions toward amino groups. (ii) The formation degree of the closed species of the $M(MABI)^{2+}$ complexes of the transition metal ions is quite pronounced; that it is smallest for $Mn(MABI)^{2+}$, about 50%, is a reflection of the known[36] relatively low affinity of Mn^{2+} for NH_2 groups. (iii) That the largest formation degree of the closed species is observed with $Ni(MABI)^{2+}$, and not with $Cu(MABI)^{2+}$, is most likely partially due to the Jahn−Teller distorted coordination sphere of Cu^{2+}, which allows only weak interactions at apical positions;[46] this means, in a first approximation, that a (N3)-bound MABI ligand has only two more (equatorial) sites available for further binding whereas the octahedral coordination sphere of Ni^{2+} offers for the same situation four further sites. In addition, it could be that the mentioned semi-chelate is especially favored with Ni^{2+}.[47]

4. Conclusions

This study proves that a methyl substituent at C4 significantly inhibits metal ion binding at N3 of benzimidazole-type ligands. Considering the steric equivalence of methyl and amino groups (see section 1), it is evident that in adenine residues, in which the (C6)NH_2 group shows no affinity for metal ions (see section 1), it has a corresponding steric inhibitory effect on metal ion binding at N7. This means, by taking into account previously[18] obtained results, that the steric effects of the (C6)NH_2 group on metal ion binding at the N1 and the N7 sites (see Figure 1) of adenines are now well defined. Whether the indicated semi-chelates involving hydrogen bonding of the (C6)NH_2 group to a metal-coordinated water molecule are of any relevance for an (N7)-bound metal ion remains to be seen.

A further interesting result is the observation that the amino group of 1-methyl-4-aminobenzimidazole, which has in its monoprotonated form a pK_a of 1.3 (Table 1) being thus more than 2 log units more basic (see sections 1 and 3.1)[20b] than the NH_2 group of the corresponding adenine derivative, may participate in metal ion binding. Indeed, for the $M(MABI)^{2+}$ complexes of the second half of the 3d metal ion series, this gives rise to an intramolecular equilibrium between (N3)-bound isomers and chelated species; the formation degree of the latter reaches significant concentrations with about 50−95%. However, the binding site which determines, to the larger part, the overall stability of these complexes is still the N3 site.

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Supporting Information Available: Figures S1 and S2 showing the effect of pH and of $[Cd^{2+}]$, respectively, on the UV absorption of MABI; the evaluation of these spectral series is given in Figures 2 and 3, respectively, in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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(47) (a) Of course, the relatively large value log $\Delta^*_{Ni/MABI} = 1.31 \pm 0.10$ (Table 4, column 4) also has its origin in the rather low stability of the $Ni(DMBI)^{2+}$ complex (log $K^{Ni}_{Ni(DMBI)} = 0.16 \pm 0.06$; Table 2, column 2). Hence, one could argue (following the indications of one of the reviewers) that the rather rigid and highly symmetrical coordination sphere of Ni^{2+} is more sensitive to any (small) differences in the steric requirements of the CH_3 and NH_2 groups (though we have no evidence for such a difference), than it is the case with the other metal ions which have somewhat more adaptable coordination spheres. Following this argument further, one could then use the factor of 2 (=4/2; see also ref 47b, section 6), which favors the coordination of the amino group in $Ni(MABI)^{2+}$ compared with that of the same group in $Cu(MABI)^{2+}$, and then one obtains as a lower limit for the stability enhancement due to chelate formation log $\Delta^*_{Ni/MABI} = 0.43$ (Cu^{2+} value; Table 4, column 4) + 0.30 (statistical value) = 0.73. From this, the limiting values $K_I \geq 4.37$ and % $Ni(MABI)^{2+}_{cl} \geq 81$ follow. Such a "statistical" difference of 0.3 log unit has been observed previously[45,47b] for the Ni(AMP) and Cu(AMP) complexes with regard to N7 binding of the already phosphate-coordinated metal ions (see ref 47b). Thus, in any case, chelate formation is most pronounced with $Ni(MABI)^{2+}$. However, it needs to be emphasized again that the experimentally measured stability constant for $Ni(DMBI)^{2+}$ (Table 2, column 2) is correct; we double-checked this value, which is the average of 10 independent pairs of titrations. (b) Sigel, H.; Massoud, S. S.; Tribolet, R. *J. Am. Chem. Soc.* **1988**, *110*, 6857−6865.