Inorg. Chem. **2004**, 43, 1311–1322

Inorganic Chemistry

Article

Metal Ion-Binding Properties of (1*H*-Benzimidazol-2-yl-methyl)phosphonate ($Bimp^{2-}$) in Aqueous Solution.$^{\perp}$ Isomeric Equilibria, Extent of Chelation, and a New Quantification Method for the Chelate Effect

María José Sánchez-Moreno,[†,‡] Alfonso Fernández-Botello,[†,§] Raquel B. Gómez-Coca,[†] Rolf Griesser,[†] Justyn Ochocki,[||] Andrzej Kotynski,[||] Juan Niclós-Gutiérrez,[‡] Virtudes Moreno,[§] and Helmut Sigel*,[†]

Inorganic Chemistry, Department of Chemistry, University of Basel, Spitalstrasse 51,
CH-4056 Basel, Switzerland, Department of Inorganic Chemistry, Faculty of Pharmacy,
University of Granada, Campus de Cartuja, s/n, E-18071 Granada, Spain,
Inorganic Chemistry Department, Faculty of Chemistry, University of Barcelona,
E-08028 Barcelona, Spain, and Department of Bioinorganic Chemistry, Faculty of Pharmacy,
Medical University, Muszyňskiego 1, PL-90151 Łódź, Poland

Received May 29, 2003

The acidity constants of the 2-fold protonated (1*H*-benzimidazol-2-yl-methyl)phosphonate, $H_2(Bimp)^{\pm}$, are given, and the stability constants of the $M(H;Bimp)^+$ and M(Bimp) complexes with the metal ions $M^{2+} = Mg^{2+}$, Ca^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} have been determined by potentiometric pH titrations in aqueous solution at $I = 0.1$ M ($NaNO_3$) and 25 °C. Application of previously determined straight-line plots of log $K^{M}_{M(Bi-R)}$ versus p$K^{H}_{H(Bi-R)}$ for benzimidazole-type ligands, Bi–R, where R represents a residue which does not affect metal ion binding, proves that the primary binding site in the $M(H;Bimp)^+$ complexes is (mostly) N3 and that the proton is located at the phosphonate group; outersphere interactions seem to be important, and the degree of chelate formation is above 60% for all metal ion complexes studied, except for $Zn(H;Bimp)^+$. A similar evaluation based on log $K^{M}_{M(R-PO_3)}$ versus p$K^{H}_{H(R-PO_3)}$ straight-line plots for simple phosph(on)ate ligands, $R-PO_3^{2-}$, where R represents a residue which cannot participate in the coordination process, reveals that the primary binding site in the M(Bimp) complexes is (mostly) the phosphonate group with all metal ions studied. In this case, the formation degree of the chelates varies more widely in dependence on the kind of metal ion involved, i.e., from 17 ± 11% to nearly 100% for Ba(Bimp) and Cu(Bimp), respectively. For all the $M(H;Bimp)^+$ and M(Bimp) systems, the intramolecular equilibria between the isomeric complexes are evaluated in a quantitative manner. The fact that for $Bimp^{2-}$ the metal ion affinity of the two binding sites, N3 and PO_3^{2-}, can be calculated independently, i.e., the corresponding micro stability constants become known, allows us to present for the first time a method for the quantification of the chelate effect solely based on comparisons of stability constants which carry the same dimensions. This effect is often ill defined in textbooks because equilibrium constants of different dimensions are compared, which is avoided in the present case. For the M(Bimp) complexes, it is shown that the chelate effect is close to zero for Ba(Bimp) whereas for Cu(Bimp) it amounts to about four log units. This method is also applicable to other chelating systems. Finally, considering that benzimidazole as well as phosphonate derivatives are employed as therapeutic agents, the potential biological properties of Bimp, especially regarding nucleic acid polymerases, are briefly discussed.

1. Introduction

Benzimidazole, sometimes also addressed as 1,3-dideazapurine,[1] and its derivatives often serve as model compounds for purines because of the structural similarities.[2] For this reason and also because the benzimidazole residue is a constituent[3] of vitamin B_{12}, the biological properties[4] of benzimidazoles as well as their potential use as therapeutics[5]

---

[*] To whom correspondence should be addressed. E-mail: Helmut.Sigel@unibas.ch. Fax: ++41-61-267 1017.

[†] University of Basel.

[‡] University of Granada.

[§] University of Barcelona.

[||] Medical University of Łódź.

10.1021/ic030175k CCC: $27.50

have been and still are studied extensively. Similarly, the phosphonate group may in many instances well represent[6,7] a phosphate residue, and as a consequence also in this case, the biological properties of many phosphonate derivatives and their possible use as drugs were investigated[8,9] including such simple compounds[10] like phosphonoformate or phosphonoacetate which show antiviral properties.[11]

The mentioned observations initiated the synthesis[12] of (1*H*-benzimidazol-2-yl-methyl)phosphonate (Bimp^{2-}; Figure 1), a molecule which combines a benzimidazole residue with a phosphonate group. To allow meaningful biological studies, the acid−base properties of Bimp^{2-} need to be known, and therefore, these were studied very recently.[12] However, considering that the biological actions of many phosphate derivatives[13] and consequently also of their phosphonate analogues[14,15] involve metal ions, we decided to study also the metal ion binding properties of Bimp with the view to make predictions and to better understand later on possible biological effects.

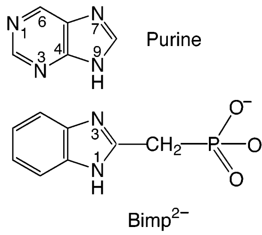

Figure 1. Chemical structures of purine and of (1*H*-benzimidazol-2-yl-methyl)phosphonate (Bimp^{2-}), which may also be addressed as 1,3-dideaza-8-phosphonomethyl-purine.

There is, however, a further very appealing aspect from a coordination chemical point of view, which makes Bimp^{2-} an interesting ligand to study; that is, the individual metal ion binding properties of the N3 site[16,17] and of the phosphonate group[6,18,19] have been well characterized, and therefore, their combination into a six-membered chelate should now provide insights into the chelate effect (see section 3.7). Moreover, there are metal ions which prefer O donors and others that favor N donors.[20,21] How are these different properties reflected in the stability and structure of the M^{2+}/Bimp complexes in solution? We selected therefore as metal ions Mg^{2+}, Ca^{2+}, and Ba^{2+}, which prefer O donors,[20] and Cu^{2+}, Zn^{2+}, and Cd^{2+} which favor N donors,[20,21] as well as Mn^{2+} and Co^{2+} which have more intermediate properties.[21] Indeed, the results presented now show that the tendency to form chelates may differ significantly from metal ion to metal ion; i.e., it is more pronounced in the present case for the "softer" metal ions.

2. Experimental Section

2.1. Materials and Equipment. The disodium salt of 1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (Na$_2$H$_2$EDTA) and the

---

[⊥] Further abbreviations (see also Figure 1) and definitions are the following: Bi, benzimidazole; Bi−R, benzimidazole derivative with a noninteracting residue R; (d)ATP^{4-}, (2′-deoxy)adenosine 5′-triphosphate; En, ethylenediamine = 1,2-diaminoethane; *I*, ionic strength; K_a, general acidity constant; L, general ligand; M^{2+}, general divalent metal ion; MeP^{2-}, methylphosphonate; PMEA^{2-}, dianion of 9-[2-(phosphonomethoxy)ethyl]-adenine; R−PO$_3^{2-}$, simple phosphate monoester or phosphonate ligand with R representing a noninteracting residue. Species written without a charge either do not carry one or represent the species in general (i.e., independent of their protonation degree); which of the two possibilities applies is always clear from the context. In formulas such as M(H;Bimp)$^+$, the H$^+$ ion and Bimp^{2-} are separated by a semicolon to facilitate reading, yet they appear within the same parenthesis to indicate that the proton is at the ligand without defining its location.

(1) (a) Seela, F.; Bourgeois, W. *Synthesis* **1989**, 912−918. (b) Seela, F.; Wenzel, T. *Helv. Chim. Acta* **1995**, *78*, 833−846.

(2) (a) Guckian, K. M.; Morales, J. C.; Kool, E. T. *J. Org. Chem.* **1998**, *63*, 9652−9656. (b) Morales, J. C.; Kool, E. T. *Nat. Struct. Biol.* **1998**, *5*, 950−954.

(3) Pratt, J. M. In *Handbook on Metalloproteins*; Bertini, I., Sigel, A., Sigel, H., Eds.; M. Dekker, Inc.: New York, 2001; pp 603−668.

(4) (a) Tamm, I.; Folkers, K.; Shunk, C. H. *J. Bacteriol.* **1956**, *72*, 59−64. (b) Genieser, H.-G.; Winkler, E.; Butt, E.; Zorn, M.; Schulz, S.; Iwitzki, F.; Störmann, R.; Jastorff, B.; Døskeland, S. O.; Øgreid, D.; Ruchaud, S.; Lanotte, M. *Carbohydr. Res.* **1992**, *234*, 217−235. (c) Morales, J. C.; Kool, E. T. *J. Am. Chem. Soc.* **1999**, *121*, 2323−2324. (d) White, A. W.; Almassy, R.; Calvert, A. H.; Curtin, N. J.; Griffin, R. J.; Hostomsky, Z.; Maegley, K.; Newell, D. R.; Srinivasan, S.; Golding, B. T. *J. Med. Chem.* **2000**, *43*, 4084−4097. (e) Skalitzky, D. J.; Marakovits, J. T.; Maegley, K. A.; Ekker, A.; Yu, X.-H.; Hostomsky, Z.; Webber, S. E.; Eastman, B. W.; Almassy, R.; Li, J.; Curtin, N. J.; Newell, D. R.; Calvert, A. H.; Griffin, R. J.; Golding, B. T. *J. Med. Chem.* **2003**, *46*, 210−213.

(5) (a) Murray, M.; Ryan, A. J.; Little, P. J. *J. Med. Chem.* **1982**, *25*, 887−892. (b) Trivulzio, S.; Colombo, R.; Rossoni, G.; Caironi, E. *Pharmacol. Res. Commun.* **1988**, *20*, 975−982. (c) Ersan, S.; Nacak, S.; Noyanalpan, N.; Yesilada, E. *Arzneimittelforschung* **1997**, *47*, 834−836. (d) Valdez, J.; Cedillo, R.; Hernandez-Campos, A.; Yepez, L.; Hernandez-Luis, F.; Navarrete-Vazquez, G.; Tapia, A.; Cortes, R.; Hernandez, M.; Castillo, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2221−2224. (e) Hauel, N. H.; Nar, H.; Priepke, H.; Ries, U.; Stassen, J.-M.; Wienen, W. *J. Med. Chem.* **2002**, *45*, 1757−1766. (f) Lalezari, J. P.; Aberg, J. A.; Wang, L. H.; Wire, M. B.; Miner, R.; Snowden, W.; Talarico, C. L.; Shaw, S.; Jacobson, M. A.; Drew, W. L. *Antimicrob. Agents Chemother.* **2002**, *46*, 2969−2976.

(6) Sigel, H.; Chen, D.; Corfù, N. A.; Gregáň, F.; Holý, A.; Strašák, M. *Helv. Chim. Acta* **1992**, *75*, 2634−2656.

(7) Sigel, H.; Kapinos, L. E. *Coord. Chem. Rev.* **2000**, *200−202*, 563−594.

(8) Ochocki, J.; Graczyk, J. *Pharmazie* **1998**, *53*, 884−885.

(9) (a) Holý, A.; Votruba, I.; Masojídková, M.; Andrei, G.; Snoeck, R.; Naesens, L.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **2002**, *45*, 1918−1929. (b) Holý, A.; Günter, J.; Dvořáková, H.; Masojídková, M.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1999**, *42*, 2064−2086.

(10) Song, B.; Chen, D.; Bastian, M.; Martin, R. B.; Sigel, H. *Helv. Chim. Acta* **1994**, *77*, 1738−1756.

(11) (a) De Clercq, E. *Collect. Czech. Chem. Commun.* **1998**, *63*, 480−506. (b) Zhang, Y.; Schols, D.; De Clercq, E. *Antiviral Res.* **1999**, *43*, 23−35.

(12) Sánchez-Moreno, M. J.; Gómez-Coca, R. B.; Fernández-Botello, A.; Ochocki, J.; Kotynski, A.; Griesser, R.; Sigel, H. *Org. Biomol. Chem.* **2003**, *1*, 1819−1826.

(13) (a) Klevickis, C.; Grisham, C. M. *Met. Ions Biol. Syst.* **1996**, *32*, 1−26. (b) Crowley, J. D.; Traynor, D. A.; Weatherburn, D. C. *Met. Ions Biol. Syst.* **2000**, *37*, 209−278. (c) Rusnak, F. *Met. Ions Biol. Syst.* **2000**, *37*, 305−343. (d) Fatemi, N.; Sarkar, B. *Inorg. Chim. Acta* **2002**, *339*, 179−187. (e) Lawson, D. M.; Smith, B. E. *Met. Ions Biol. Syst.* **2002**, *39*, 75−119. (f) Sigel, R. K. O.; Pyle, A. M. *Met. Ions Biol. Syst.* **2003**, *40*, 477−512.

(14) Sigel, H. *Pure Appl. Chem.* **1999**, *71*, 1727−1740.

(15) Sigel, H.; Song, B.; Blindauer, C. A.; Kapinos, L. E.; Gregáň, F.; Prónayová, N. *Chem. Commun.* **1999**, 743−744.

(16) Kapinos, L. E.; Song, B.; Sigel, H. *Chem. Eur. J.* **1999**, *5*, 1794−1802.

(17) (a) Kapinos, L. E.; Holý, A.; Günter, J.; Sigel, H. *Inorg. Chem.* **2001**, *40*, 2500−2508. (b) Kapinos, L. E.; Sigel, H. *Eur. J. Inorg. Chem.* **1999**, 1781−1786. (c) Kapinos, L. E.; Song, B.; Sigel, H. *Z. Naturforsch.* **1998**, *53b*, 903−908.

(18) (a) Sigel, H. *Coord. Chem. Rev.* **1995**, *144*, 287−319. (b) Sigel, H. *J. Indian Chem. Soc.* **1997**, *74*, 261−271 (P. Ray Award Lecture).

(19) (a) Da Costa, C. P.; Song, B.; Gregáň, F.; Sigel, H. *J. Chem. Soc., Dalton Trans.* **2000**, 899−904. (b) Sigel, H.; Da Costa, C. P.; Song, B.; Carloni, P.; Gregáň, F. *J. Am. Chem. Soc.* **1999**, *121*, 6248−6257. (c) Song, B.; Sajadi, S. A. A.; Gregáň, F.; Prónayová, N.; Sigel, H. *Inorg. Chim. Acta* **1998**, *273*, 101−105.

(20) Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201−208.

(21) Martin, R. B. *Inorg. Chim. Acta* **2002**, *339*, 27−33.

nitrate salts of Na^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} (all pro analysi) were from Merck KGaA, Darmstadt, Germany. The same lot of (1*H*-benzimidazol-2-yl-methyl)phosphonic acid, i.e., $H_2(Bimp)^{\pm}$ was used as before, the synthesis of which is described in ref 12. All the other reagents including the ultrapure CO_2-free water and the buffers needed for calibration of the potentiograph were the same as those used recently.[12]

The titer of the NaOH used for the titrations was established with potassium hydrogen phthalate, and the concentrations of the M^{2+} stock solutions were determined by potentiometric pH titrations via their EDTA complexes by measuring the equivalents of protons liberated from $H(EDTA)^{3-}$ upon complex formation.

The equipment, including the computers, was the same as that used before.[22,23]

2.2. Determination of the Stability Constants of the Complexes. For the potentiometric pH titrations, the same conditions were used as described recently[12] for the determination of the acidity constants $K^{H}_{H_2(Bimp)}$ (eq 1) and $K^{H}_{H(Bimp)}$ (eq 2) of $H_2(Bimp)^{\pm}$ and $H(Bimp)^-$, respectively, one proton being at the benzimidazole ring and the other at the phosphonate group. This means that 50 mL of aqueous 0.59 mM HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) was titrated in the presence and absence of 0.26 mM $Bimp^{2-}$ under N_2 with 1 mL of 0.03 M NaOH, but now for the determination of the stability constants $K^{M}_{M(H;Bimp)}$ (eq 3) and $K^{M}_{M(Bimp)}$ (eq 4) of the $M(H;Bimp)^+$ and M(Bimp) complexes, respectively, $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$ (25 °C; $I = 0.1$ M). The M^{2+}/ligand ratios employed were close to 115:1 (Mg^{2+}, Ca^{2+}, Ba^{2+}), 80:1 (Mg^{2+}, Ca^{2+}, Ba^{2+}), 50:1 (Mg^{2+}, Mn^{2+}, Zn^{2+}), 48:1 (Co^{2+}), 25:1 (Mn^{2+}, Co^{2+}), 15:1 (Zn^{2+}), 13:1 (Co^{2+}, Cd^{2+}), 12:1 (Zn^{2+}), 6:1 (Cd^{2+}), 5:1 (Co^{2+}), 4:1 (Cd^{2+}, Zn^{2+}), 2.6:1 (Cu^{2+}), and 1.5:1 (Cu^{2+}).

The differences in NaOH consumption between a corresponding pair of titrations, as indicated above, were used for the calculations. The stability constants were calculated with a curve-fitting procedure using a Newton−Gauss nonlinear least-squares program by taking into account the species,[24] H^+, $H_2(Bimp)^{\pm}$, $H(Bimp)^-$, $Bimp^{2-}$, M^{2+}, $M(H;Bimp)^+$, and M(Bimp), and by collecting the experimental data every 0.1 pH unit from about 3% complex formation of $M(H;Bimp)^+$ to a neutralization degree of about 90% with respect to the $H(Bimp)^-$ species, or until the beginning of the hydrolysis of $M(aq)^{2+}$ which was evident from the titrations without ligand.

The evaluation of the experimental data for the Zn^{2+}/Bimp system was hampered because both protons of $H_2(Bimp)^{\pm}$ are released upon complex formation in a single step; i.e., no stability constant could be obtained for the monoprotonated $Zn(H;Bimp)^+$ complex. Indeed, by neglecting this species in the fitting procedure, an excellent fit of the experimental data was obtained. To gain an estimate for the upper limit of the stability constant for the $Zn(H;Bimp)^+$ complex, we kept this constant fixed beginning with $\log K^{Zn}_{Zn(H;Bimp)} = 1$ and increased this value then step by step by 0.1 log unit; at $\log K^{Zn}_{Zn(H;Bimp)} \geqslant 2.1$ the fit of the experimental data became clearly poorer, and the value for $\log K^{Zn}_{Zn(Bimp)}$ started then also to become significantly different compared to the result obtained by neglecting the $Zn(H;Bimp)^+$ species in the calculation. Hence, the result of this estimation is $\log K^{Zn}_{Zn(H;Bimp)} \leqslant 2.1$. As an example it may be added that by keeping $\log K^{Zn}_{Zn(H;Bimp)} = 2.1$ and $\log K^{Zn}_{Zn(Bimp)} = 5.79$ (see the result in Table 1 in section 3.1) constant, one calculates for the experimental conditions of the Zn^{2+}/Bimp ratio of 15:1 a maximal formation degree of 4.6% for the $Zn(H;Bimp)^+$ species, whereas the formation degree of Zn(Bimp) amounts at pH 5.5 already to 92.6%.[25]

For all M^{2+}/Bimp systems it holds that the results showed no dependence on the excess of M^{2+} used in the experiments. The final results given for the stability constants of the complexes are always the averages of at least four, usually five, independent pairs of titrations.

3. Results and Discussion

Purine derivatives are well-known to undergo self-association due to stacking of their aromatic-ring systems.[26,27] The same may be surmised for benzimidazole derivatives. Therefore, all potentiometric pH titrations (25 °C; $I = 0.1$ M, $NaNO_3$), the results of which are summarized below, were carried out at ligand concentrations smaller than 0.3 mM. Under these conditions, self-stacking is negligible; e.g., with the self-association constant $K = 15\ M^{-1}$ (which holds for adenosine),[28] one calculates that in a 1 mM solution about 97% of the species are present in their monomeric form.[29] Since the $H(Bimp)^-$ and $Bimp^{2-}$ species carry negative charges at their phosphonate residue, this will give rise to repulsion,[30] and thus, their stacking tendency will be further reduced. This guarantees that in the present study the properties of monomeric species are evaluated.

3.1. Stability Constants of M^{2+} Complexes Formed with Bimp. $Bimp^{2-}$ (see Figure 1) accepts three protons, two at the phosphonate residue and one at N3 of the benzimidazole moiety, giving rise to $H_3(Bimp)^+$. However, the release of the first of the three protons occurs[12] from the $P(O)(OH)_2$ group with $pK^{H}_{H_3(Bimp)} = 1.5 \pm 0.2$; this reaction is thus not of relevance for this study which was carried out in the pH range 3.8−8.2. The same is true for the deprotonation of the neutral benzimidazole residue, i.e., the release of the proton from the (N1/N3)H site,[12] which occurs with $pK^{H}_{Bimp} = 14.65 \pm 0.12$. Hence, we have to consider only the two deprotonation steps defined in eqs 1 and 2 of the net zero-charge $H_2(Bimp)^{\pm}$ species:

$$H_2(Bimp)^{\pm} \rightleftharpoons H(Bimp)^- + H^+ \qquad (1a)$$

$$K^{H}_{H_2(Bimp)} = [H(Bimp)^-][H^+]/[H_2(Bimp)^{\pm}] \qquad (1b)$$

---

(22) Kampf, G.; Kapinos, L. E.; Griesser, R.; Lippert, B.; Sigel, H. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1320−1327.

(23) Blindauer, C. A.; Sjåstad, T. I.; Holý, A.; Sletten, E.; Sigel, H. *J. Chem. Soc., Dalton Trans.* **1999**, 3661−3671.

(24) Sigel, H.; Griesser, R.; Prijs, B. *Z. Naturforsch*. **1972**, *27B*, 353−364.

(25) (a) The likely reason for the low stability of the protonated $Zn(H;Bimp)^+$ species and the high stability of the Zn(Bimp) complex, if compared, e.g., with the stabilities of the corresponding Co^{2+} complexes, is the reduction of the coordination number (CN) of Zn^{2+} in going from $Zn(H;Bimp)^+$ (CN = 6) to Zn(Bimp) (CN = 4 or 5) (for details see ref 25b). (b) Sigel, H.; Martin, R. B. *Chem. Soc. Rev*. **1994**, *23*, 83−91.

(26) (a) Sigel, H. *Pure Appl. Chem*. **1998**, *70*, 969−976. (b) Yamauchi, O.; Odani, A.; Masuda, H.; Sigel, H. *Met. Ions Biol. Syst*. **1996**, *32*, 207−270.

(27) (a) Corfù, N. A.; Sigel, H. *Eur. J. Biochem*. **1991**, *199*, 659−669. (b) Sigel, H.; Corfù, N. A. *Eur. J. Biochem*. **1996**, *240*, 508−517.

(28) Tribolet, R.; Sigel, H. *Biophys. Chem.* **1987**, *27*, 119−130.

(29) (a) Removal of nitrogens from the purine-ring system does not alter the stacking tendency significantly, as the examples of tubercidin (=7-deazaadenosine)[29b] or of its monophosphate[30] show. The stacking tendency of these two deaza compounds corresponds to that of adenosine[28,29b] and of its monophosphate,[30] respectively. (b) Ji, L.-n.; Corfù, N. A.; Sigel, H. *J. Chem. Soc., Dalton Trans*. **1991**, 1367−1375.

(30) Tribolet, R.; Sigel, H. *Eur. J. Biochem*. **1987**, *163*, 353−363.

Table 1. Logarithms of the Stability Constants of $M(H;Bimp)^+$ (Equation 3) and M(Bimp) Complexes (Equation 4) as Determined by Potentiometric pH Titrations in Aqueous Solution, Together with the Negative Logarithms of the Acidity Constants (Equations 5 and 6) of the $M(H;Bimp)^+$ Complexes (25 °C; $I = 0.1$ M, $NaNO_3$)[a,b]

M^{2+}	$\log K^{M}_{M(H;Bimp)}$	$\log K^{M}_{M(Bimp)}$	$pK^{H}_{M(H;Bimp)}$
Ba^{2+}	0.75 ± 0.06	1.35 ± 0.05	6.81 ± 0.08
Ca^{2+}	1.06 ± 0.07	1.77 ± 0.06	6.70 ± 0.09
Mg^{2+}	1.02 ± 0.15	2.09 ± 0.06	6.34 ± 0.16
Mn^{2+}	1.97 ± 0.07	4.18 ± 0.06	5.20 ± 0.09
Co^{2+}	2.31 ± 0.08	4.92 ± 0.05	4.80 ± 0.10
Cu^{2+}	4.21 ± 0.08	7.68 ± 0.05	3.94 ± 0.10
Zn^{2+}	$\leqslant 2.1$[c]	5.79 ± 0.04	$\leqslant 3.7$
Cd^{2+}	2.83 ± 0.06	5.54 ± 0.09	4.70 ± 0.11

[a] The acidity constants[12] for $H_2(Bimp)^{\pm}$ are $pK^{H}_{H_2(Bimp)} = 5.37 \pm 0.02$ [deprotonation of the $(N3)H^+$ site] and $pK^{H}_{H(Bimp)} = 7.41 \pm 0.02$ [deprotonation of the $P(O)_2(OH)^-$ group];[b] so-called practical, mixed, or Brønsted constants are given (see ref 32).[12] [b] The error limits given are three times the standard error of the mean value (3σ) or the sum of the probable systematic errors, whichever is larger. The error limits of the derived data, in the present case for column 4, were calculated according to the error propagation after Gauss. [c] Regarding this upper limit, see sections 2.2 and 3.1.[25]

$$H(Bimp)^- \rightleftharpoons Bimp^{2-} + H^+ \qquad (2a)$$

$$K^{H}_{H(Bimp)} = [Bimp^{2-}][H^+]/[H(Bimp)^-] \qquad (2b)$$

The corresponding acidity constants are $pK^{H}_{H_2(Bimp)} = 5.37 \pm 0.02$ and $pK^{H}_{H(Bimp)} = 7.41 \pm 0.02$ as determined recently.[12] These constants refer to the release of the protons from the $(N3)H^+$ site and the $P(O)_2(OH)^-$ group, respectively.[12]

Since benzimidazole[16] and methylphosphonate[6] are known to form complexes with divalent metal ions (M^{2+}), one has to expect that not only M(Bimp) complexes exist but that also monoprotonated species $M(H;Bimp)^+$ may form. These two complex equilibria are defined in eqs 3 and 4:

$$M^{2+} + H(Bimp)^- \rightleftharpoons M(H;Bimp)^+ \qquad (3a)$$

$$K^{M}_{M(H;Bimp)} = [M(H;Bimp)^+]/([M^{2+}][H(Bimp)^-]) \qquad (3b)$$

$$M^{2+} + Bimp^{2-} \rightleftharpoons M(Bimp) \qquad (4a)$$

$$K^{M}_{M(Bimp)} = [M(Bimp)]/([M^{2+}][Bimp^{2-}]) \qquad (4b)$$

Indeed, the experimental data of the potentiometric pH titrations can be completely described by considering eqs 1−4, provided the evaluation is not carried into the pH range where hydroxo complexes form. The occurrence of this latter reaction was easily identified from titrations in the absence of ligand (see section 2.2). It needs to be added further that complex formation of Zn^{2+} with $H_2(Bimp)^{\pm}$ takes place in a single step; i.e., the two protons from $H_2(Bimp)^{\pm}$ are released simultaneously, and therefore, no hint for the formation of a $Zn(H;Bimp)^+$ species (eq 3) was observed. It was only possible to estimate the upper limit of the stability constant for this complex (see section 2.2).[25]

For the metal ions Mg^{2+}, Ca^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, and Cd^{2+}, the formation of the $M(H;Bimp)^+$ complex according to equilibrium 3a could be proven. Hence, in these instances also reaction 5 needs to be considered:

$$M(H;Bimp)^+ \rightleftharpoons M(Bimp) + H^+ \qquad (5a)$$

$$K^{H}_{M(H;Bimp)} = [M(Bimp)][H^+]/[M(H;Bimp)^+] \qquad (5b)$$

However, the corresponding acidity constant (eq 5b) is connected with the other equilibrium constants[31] already mentioned and can thus be calculated with eq 6:

$$pK^{H}_{M(H;Bimp)} = pK^{H}_{H(Bimp)} + \log K^{M}_{M(H;Bimp)} - \log K^{M}_{M(Bimp)} \qquad (6)$$

The results obtained for equilibria 3a, 4a, and 5a are listed in columns 2, 3, and 4 of Table 1, respectively.[32]

3.2. Preliminary Evaluation of the Stability and Structure of the $M(H;Bimp)^+$ and M(Bimp) Complexes. To obtain a first impression of the contribution of the two binding sites present in Bimp, i.e., the N3 site and the phosphonate group (Figure 1), to the measured stabilities of the complexes, it will be helpful to know the metal ion binding affinity of the two isolated sites. The N3 site of Bimp is certainly well represented by the ligating qualities of benzimidazole (Bi) itself, especially as the two acidity constants of $(N3)H^+$, $pK^{H}_{H_2(Bimp)} = 5.37 \pm 0.02$ and $pK^{H}_{H(Bi)} = 5.63 \pm 0.01$,[12,16] are relatively close to each other. Similarly, the acidity constants $pK^{H}_{H(Bimp)} = 7.41 \pm 0.02$ and $pK^{H}_{H(MeP)} = 7.53 \pm 0.01$, which refer to the deprotonation of monoprotonated[12] $Bimp^{2-}$ and methylphosphonate (MeP^{2-}; $CH_3PO_3^{2-}$),[6] show that the latter ligand is a good representative of the phosphonate group in $Bimp^{2-}$. Therefore, the relevant and previously measured stability constants of the M(Bi) and M(MeP) complexes are summarized in Table 2.

A comparison of the constants listed in Tables 1 and 2 allows the following conclusions: (i) From the values in Table 2, it follows that the main binding site, i.e., the one which contributes most to the stability of the M(Bimp) complexes, is the phosphonate group; for this group the stability constants span a difference in complex stability of 2.2 log units as follows from the values for the M(MeP) complexes. (ii) This contrasts with the span of 3.6 log units

Table 2. Logarithms of the Stability Constants[a] of the M^{2+} 1:1 Complexes Formed with Benzimidazole (Bi)[b] and with Methylphosphonate (MeP^{2-}; $CH_3PO_3^{2-}$)[c] in Aqueous Solution at 25 °C

M^{2+}	$\log K^{M}_{M(Bi)}$	$\log K^{M}_{M(MeP)}$
Ba^{2+}	-0.33 ± 0.20	1.29 ± 0.02
Ca^{2+}	-0.18 ± 0.21	1.64 ± 0.03
Mg^{2+}	0.05 ± 0.03	1.86 ± 0.02
Mn^{2+}	0.76 ± 0.05	2.48 ± 0.02
Co^{2+}	1.59 ± 0.01	2.24 ± 0.02
Cu^{2+}	3.26 ± 0.03	3.49 ± 0.05
Zn^{2+}	1.61 ± 0.02	2.60 ± 0.03
Cd^{2+}	2.10 ± 0.02	2.90 ± 0.03

[a] Error limits: see footnote *b* of Table 1. [b] From ref 16; $pK^{H}_{H(Bi)} = 5.63 \pm 0.01$; $I = 0.5$ M, $NaNO_3$. The fact that these equilibrium constants refer to $I = 0.5$ M and the others considered in this study to $I = 0.1$ M has no significant influence on any of the comparisons made since benzimidazole as well as the benzimidazole residue are uncharged ligating sites. [c] From ref 6; $pK^{H}_{H(MeP)} = 7.53 \pm 0.01$; $I = 0.1$ M, $NaNO_3$.

---

(31) Sigel, H. *Eur. J. Biochem.* **1968**, *3*, 530−537.
(32) Sigel, H.; Zuberbühler, A. D.; Yamauchi, O. *Anal. Chim. Acta* **1991**, *255*, 63−72.

for the N3 site as follows from the constants for the M(Bi) complexes; hence, here the affinity differences between the various metal ions are much larger. Interestingly, in the case of Cu^{2+} the affinity difference between the N3 and the PO_3^{2-} sites is relatively small, i.e., 0.23 ± 0.06 log units only. (iii) Since metal ion binding to a ligand must lead to an increased acidity of potentially present acidic protons,[23,33−35] a comparison of $pK^{H}_{H_2(Bimp)} = 5.37$ with the $pK^{H}_{M(H;Bimp)}$ values in Table 1 shows that in the $M(H;Bimp)^+$ complexes of Mg^{2+}, Ca^{2+}, Ba^{2+}, and most likely also Mn^{2+} (since the value of 5.20 is only slightly lower than 5.37), the proton must be located at the phosphonate group. This then suggests that the metal ion is at the N3 site. (iv) For the corresponding complexes of Co^{2+}, Cu^{2+}, and Cd^{2+}, the same conclusion is reached, but it is based on a different comparison: The stability constants $K^{M}_{M(H;Bimp)}$ are larger for Co^{2+} and Cu^{2+} than the stability constants for the corresponding M(Bi) and M(MeP) complexes which means that the metal ion must be at N3 and the proton at the phosphonate group because only in this way an enhanced stability, as observed, can be achieved by involving the $P(O)_2(OH)^-$ group in chelate formation. Similarly, if Cd^{2+} were at the phosphonate group and H^+ at N3, then repulsion should occur, and the measured stability constant for $Cd(H;Bimp)^+$ should be smaller than the one found for Cd(MeP). However, the two constants are within the error limits identical, which again means that Cd^{2+} actually is at N3 and H^+ at the phosphonate residue.

To conclude, the above analysis shows that in all $M(H;Bimp)^+$ complexes studied, the metal ion is (overwhelmingly) coordinated at N3 and the proton at the phosphonate group. Indeed, this conclusion will be further substantiated in sections 3.3 (final paragraph) and 3.5 (micro constants).

3.3. Quantitative Evaluation of the Stability Enhancement in the $M(H;Bimp)^+$ and M(Bimp) Complexes. The above conclusions mean that a quantitative evaluation of the stability situation for the two types of complexes is justified. This can be done by applying $\log K^{M}_{M(L)}$ versus $pK^{H}_{H(L)}$ plots; such plots should lead to straight lines, as defined by eq 7, for families of related ligands:[36]

$$\log K^{M}_{M(L)} = m \cdot pK^{H}_{H(L)} + b \qquad (7)$$

In fact, the slopes *m* and the intercepts *b* with the *y*-axis have been determined[6,16] and tabulated for benzimidazole-type[16] and also for phosph(on)ate-type ligands.[6,7,18a,37] Hence, the straight reference lines for benzimidazole-type complexes may be applied to evaluate further the properties of the $M(H;Bimp)^+$ species, and the phosph(on)ate-type lines for the stabilization of the M(Bimp) complexes.

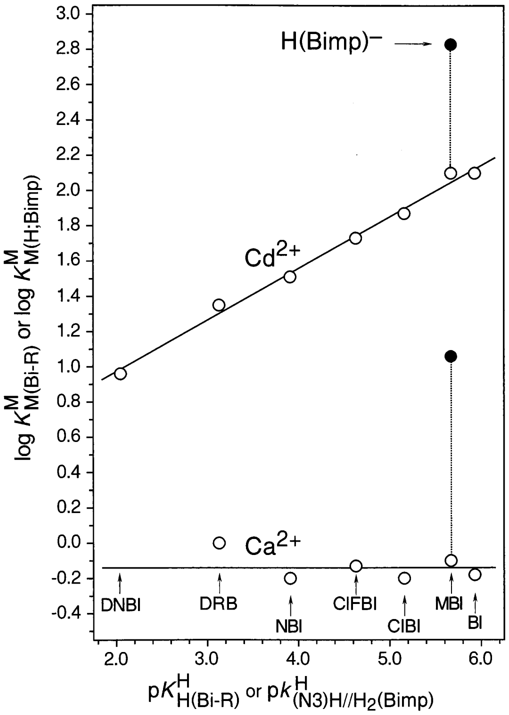

Figure 2. Evidence for an enhanced stability of the Ca^{2+} and Cd^{2+} (●) 1:1 complexes formed with monoprotonated $Bimp^{2-}$, i.e., $H(Bimp)^-$, based on the $\log K^{M}_{M(Bi-R)}$ versus $pK^{H}_{H(Bi-R)}$ plots for the 1:1 complexes of Ca^{2+} and Cd^{2+} with the following simple and sterically unhindered benzimidazole-type ligands (Bi−R; ○): 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 points are from ref 16 (where appropriate the micro acidity constants were applied; see section 2.2 in ref 16) (25 °C; *I* = 0.5 M, $NaNO_3$). The least-squares straight reference line for the $Cd(Bi-R)^{2+}$ complexes is drawn through the corresponding seven data sets; the parameters for eq 7 are listed in table 3 of ref 16. For the $Ca(Bi-R)^{2+}$ complexes $\log K^{Ca}_{Ca(Bi-R)} = -0.14 \pm 0.15$ holds in the given pK_a range.[16] The points due to the equilibrium constants for the $M^{2+}/H(Bimp)$ systems (●) are based on the data listed in Table 1 and the micro acidity constant $pk^{H}_{(N3)H/H_2(Bimp)}$ given in eq 8 (25 °C; *I* = 0.1 M, $NaNO_3$). The vertical dotted lines emphasize the stability differences to the corresponding reference lines; these differences equal $\log \Delta_{M/H(Bimp)}$ as defined in section 3.3 by eq 9, and the values are listed in column 4 of Table 3.

However, before such an analysis is carried out, it is important to note that $H_2(Bimp)^{\pm}$ is a "symmetrical" acid; i.e., the formation of the monoprotonated $H(Bimp)^-$ species can be achieved in two ways, either by releasing the proton from $(N1)H^{0.5+}$ or from $(N3)H^{0.5+}$ because both sites are equivalent and deprotonation of either site will give the same product. Hence, the deprotonation reaction is favored[16] by a factor of 2, and therefore, $H_2(Bimp)^{\pm}$ is more acidic than is actually the case if the intrinsic acidity/basicity of the two nitrogens is considered. However, it is this intrinsic basicity of an N site which determines the metal ion affinity. Hence, for plots of the mentioned kind or for applications of eq 7, the intrinsic micro acidity constant as defined in eq 8

$$pk^{H}_{(N3)H/H_2(Bimp)} = pK^{H}_{H_2(Bimp)} + 0.30 = (5.37 \pm 0.02) + 0.30 = 5.67 \pm 0.02 \qquad (8)$$

needs to be considered.

Application of the mentioned micro acidity constant leads to the results shown in Figure 2 for two examples of $\log K^{M}_{M(Bi-R)}$ versus $pK^{H}_{H(Bi-R)}$ plots. It is evident that in both

---

(33) Sigel, H.; Lippert, B. *Pure Appl. Chem.* **1998**, *70*, 845−854.
(34) Song, B.; Zhao, J.; Griesser, R.; Meiser, C.; Sigel, H.; Lippert, B. *Chem. Eur. J.* **1999**, *5*, 2374−2387.
(35) Griesser, R.; Kampf, G.; Kapinos, L. E.; Komeda, S.; Lippert, B.; Reedijk, J.; Sigel, H. *Inorg. Chem.* **2003**, *42*, 32−41.
(36) Martin, R. B.; Sigel, H. *Comments Inorg. Chem.* **1988**, *6*, 285−314.
(37) Sigel, H.; Song, B. *Met. Ions Biol. Syst.* **1996**, *32*, 135−205.

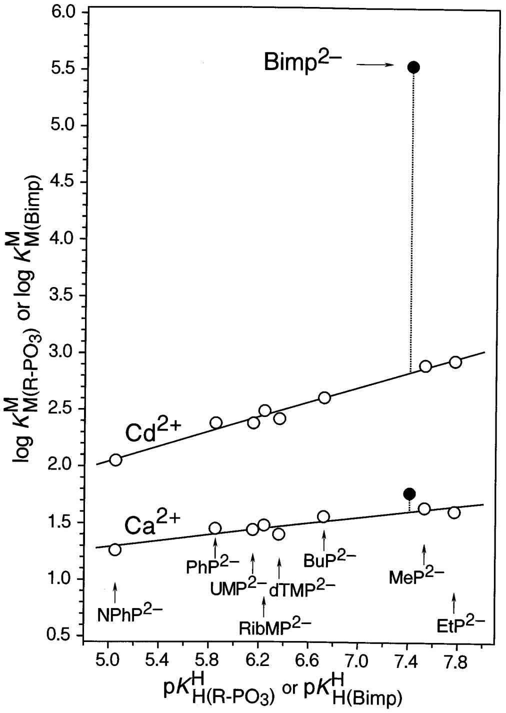

Figure 3. Evidence for an enhanced stability of the Ca^{2+} and Cd^{2+} (●) 1:1 complexes of $Bimp^{2-}$ based on the relationship between $\log K^{M}_{M(R-PO_3)}$ and $pK^{H}_{H(R-PO_3)}$ for the 1:1 complexes of Ca^{2+} and Cd^{2+} with some simple phosphate monoester and phosphonate ligands ($R-PO_3^{2-}$; ○): 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate (PhP^{2-}), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5-monophosphate ($RibMP^{2-}$), thymidine (=2′-deoxyribosylthymine) 5′-monophosphate ($dTMP^{2-}$), butyl phosphate (BuP^{2-}), methanephosphonate (MeP^{2-}), and ethanephosphonate (EtP^{2-}) (from left to right). The least-squares straight reference lines are drawn through the corresponding eight data sets, which are taken for the phosphate monoesters from ref 38 and for the phosphonates from ref 6; the parameters for these reference lines according to eq 7 are listed in table 5 of ref 6.[7,18a,37] The points due to the equilibrium constants for the M^{2+}/Bimp systems (●) are given in Table 1. All the plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$). The vertical dotted lines emphasize the stability differences to the corresponding reference lines; these differences equal $\log \Delta_{M/Bimp}$ as defined in section 3.3 by eq 9, and the values are listed in column 7 of Table 3.

instances, i.e., for $Ca(H;Bimp)^+$ and for $Cd(H;Bimp)^+$ as well, an enhanced complex stability is observed and this is indicative for the formation of chelates[36] involving in the present case the monoprotonated $P(O)_2(OH)^-$ group.

A similar plot is constructed in Figure 3 for $M(R-PO_3)$ complexes[6,38] which is the basis for stability constant comparisons with the M(Bimp) species. It is evident that also here for the two examples, i.e., the Ca(Bimp) and Cd(Bimp) complexes, an increased complex stability is observed. However, in contrast to what is seen in Figure 2, now the stability of the Ca^{2+} complex is only slightly increased (about 0.15 log unit) whereas that of the Cd^{2+} complex is dramatically enhanced (about 2.7 log units).

The mentioned observation agrees with the much larger affinity of Cd^{2+}, compared with the one of Ca^{2+}, toward N donors. In other words, chelate formation of the phosphonate-bound Cd^{2+} with N3 of the benzimidazole residue is much more pronounced than is the case with Ca^{2+}. That the stability enhancement for the same two metal ions in Figure 2 is much more similar is in accord with the fact that the affinity of the Ca^{2+} and Cd^{2+} ions toward $CH_3PO_3^{2-}$ (Table 2) differs only by a factor of about 18, and that it will certainly be even smaller toward a $P(O)_2(OH)^-$ residue, whereas the affinity toward N3 of benzimidazole differs for the same two metal ions by a factor of nearly 200.

A quantitative evaluation analogous to what is seen in Figures 2 and 3 is given for all metal ions studied here in Table 3, where the experimental data (expt) are listed in columns 2 and 5 for the $M(H;Bimp)^+$ and M(Bimp) complexes, respectively. The corresponding calculated stability constants (calcd) based on the $pK^{H}_{H(L)}$ values and the straight lines (eq 7)[6,16] are given in columns 3 and 6. Formation of the stability differences, as defined in eq 9

$$\log \Delta_{M/L} = \log K^{M}_{M(L)expt} - \log K^{M}_{M(L)calcd} \tag{9a}$$

$$= \log K^{M}_{M(L)} - \log K^{M}_{M(L)op} \tag{9b}$$

in a general way (the terms of eq 9b are analogous to those in eq 9a; see also sections 3.4 and 3.6), leads to the values listed in columns 4 and 7 of Table 3. It is evident that the stability enhancements observed for the M(Bimp) complexes vary much more than is the case for the $M(H;Bimp)^+$ species. However, the observed enhanced complex stabilizations, as defined by $\log \Delta_{M/H(Bimp)}$ and $\log \Delta_{M/Bimp}$ (in analogy to eq 9), which also correspond to the vertical dotted lines seen in Figures 2 and 3, are indicative of chelate formation in all instances, though this needs to be evaluated further as done below.

Yet, one aspect is immediately evident without any further evaluation and needs to be emphasized here: The stability enhancement $\log \Delta_{M/H(Bimp)}$ is more pronounced for the $Mn(H;Bimp)^+$ complex than for the $Co(H;Bimp)^+$ complex (see Table 3, column 4). This observation supports further the conclusion of section 3.2 that in the $M(H;Bimp)^+$ species M^{2+} is at N3 and the proton at the phosphonate group because it is a longstanding experience[6,7,18,20,37] that Mn^{2+} has a larger affinity toward phosph(on)ate groups than Co^{2+}. Consequently, there is a drop in the stability enhancement from $Mn(H;Bimp)^+$ to $Co(H;Bimp)^+$. Of course, for the M(Bimp) complexes, where the phosphonate group is the primary binding site, the situation is reversed; here, chelation occurs with N3, and consequently, the Irving−Williams order is followed[20] by the $\log \Delta_{M/Bimp}$ differences since the affinity of the phosphonate-bound Co^{2+} toward this nitrogen atom is larger than that of the correspondingly bound Mn^{2+} (cf. the $\log \Delta_{M/Bimp}$ values in column 7 of Table 3).

3.4. Further Evaluations of the $M(H;Bimp)^+$ Systems and Extent of Chelate Formation. In the comparisons made in Figure 2 and also in those via $\log \Delta_{M/H(Bimp)}$ (Table 3, column 4), the attractive effect of the negatively charged $P(O)_2(OH)^-$ residue on the binding of metal ions at N3 has been ignored since the calculations according to eq 7 are based on simple benzimidazole-type complexes.[39] However, the mentioned negative charge, which is from N3 only four bonds or five atoms away, should facilitate M^{2+} binding at N3 even without a direct metal ion interaction.[39]

---

(38) Massoud, S. S.; Sigel, H. *Inorg. Chem.* **1988**, *27*, 1447−1453.

Table 3. Stability-Constant Comparisons for the $M(H;Bimp)^+$ and M(Bimp) Complexes Between the Measured Stability Constants (expt) and the Calculated Stability Constants (calcd) Based on the Basicity (pK_a Values) of the N3 Site and the Phosphonate Group of Bimp, Respectively, and the Reference-Line Equations (Equation 7) Determined Earlier for a Pure Benzimidazole-type[16] or Pure Phosphonate-type[6] Coordination (25 °C; $I = 0.1$ M, $NaNO_3$)[a,b]

M^{2+}	$\log K^{M}_{M(H;Bimp)}$ expt	$\log K^{M}_{M(H;Bimp)}$ calcd[c]	$\log \Delta_{M/H(Bimp)}$	$\log K^{M}_{M(Bimp)}$ expt	$\log K^{M}_{M(Bimp)}$ calcd[d]	$\log \Delta_{M/Bimp}$
Ba^{2+}	0.75 ± 0.06	−0.2 ± 0.2	0.95 ± 0.21	1.35 ± 0.05	1.27 ± 0.04	0.08 ± 0.06
Ca^{2+}	1.06 ± 0.07	−0.14 ± 0.15	1.20 ± 0.17	1.77 ± 0.06	1.61 ± 0.05	0.16 ± 0.08
Mg^{2+}	1.02 ± 0.15	0.02 ± 0.05	1.04 ± 0.16	2.09 ± 0.06	1.81 ± 0.03	0.28 ± 0.07
Mn^{2+}	1.97 ± 0.07	0.74 ± 0.05	1.23 ± 0.09	4.18 ± 0.06	2.45 ± 0.05	1.73 ± 0.08
Co^{2+}	2.31 ± 0.08	1.57 ± 0.04	0.74 ± 0.09	4.92 ± 0.05	2.21 ± 0.06	2.71 ± 0.08
Cu^{2+}	4.21 ± 0.08	3.15 ± 0.04	1.06 ± 0.09	7.68 ± 0.05	3.43 ± 0.06	4.25 ± 0.08
Zn^{2+}	⩽2.1	1.56 ± 0.04	⩽0.54	5.79 ± 0.04	2.54 ± 0.06	3.25 ± 0.07
Cd^{2+}	2.83 ± 0.06	2.05 ± 0.04	0.78 ± 0.07	5.54 ± 0.09	2.84 ± 0.05	2.70 ± 0.10

[a] Error limits: see footnote *b* of Table 1. [b] The values given in columns 2 and 5 are from columns 2 and 3 of Table 1, respectively. All these values refer to aqueous solutions with $I = 0.1$ M whereas the straight reference lines for the benzimidazole-type coordination were determined for $I = 0.5$ M; however, since N3 of benzimidazole is an uncharged binding site, this difference in I does not affect the results, and any influence is certainly within the given error limits. [c] Calculated by considering the micro acidity constant given in eq 8: $pk^{H}_{(N3)H/H_2(Bimp)} = 5.67 \pm 0.02$. [d] Calculated with $pK^{H}_{H(Bimp)} = 7.41 \pm 0.02$.

A detailed analysis of such an effect has previously been made[10] for the $^{+}H_3N{-}CH_2{-}PO_3^{2-}{\cdot}M^{2+}$ complex, which is derived from (aminomethyl)phosphonate. Here the repulsive effect of the positively charged ammonium group on M^{2+} binding at the PO_3^{2-} group was considered; with opposite sign this effect, this time promoting, describes also the situation in $M^{2+}{\cdot}{}^{-}O(O)C{-}P(O)_2^{-}(OH)$, which is derived from phosphonoformate.[10] In both instances, the charge separation is by three bonds or four atoms and the absolute effect amounts to 0.38 ± 0.09 log units.[10] In our case of $H(Bimp)^-$, where the proton resides at the PO_3^{2-} group, the separation is by four bonds or five atoms; therefore, we reduce the effect conservatively by only 0.08 log units, but at the same time we increase the error limit generously and use 0.30 ± 0.15 log units for describing the promoting charge effect of the $P(O)_2(OH)^-$ group which a metal ion experiences upon binding at N3 *without* interacting directly with the monoprotonated phosphonate group. Two things should be noted: (i) The value of 0.30 log units is rather an upper limit which means that the percentages calculated below for the formation degrees of the chelated isomers are rather lower limits. (ii) Due to the significant enlargement of the error limit (±0.15), it is safe to assume that the true charge effect is within the given limits, i.e., between 0.15 and 0.45 log units.

Hence, the total stability enhancements defined by eq 9, i.e., $\log \Delta_{M/H(Bimp)}$, and as listed in column 4 of Table 3 minus the mentioned charge effect, lead to the stability enhancement, $\log \Delta^*$ (eq 10), which is actually due to the chelation in the $M(H;Bimp)^+$ species:

$$\log \Delta^* = \log \Delta_{M/H(Bimp)} - (0.30 \pm 0.15) \qquad (10)$$

These values are listed in the third column of Table 4.

Knowledge of the effect solely due to the chelation, i.e., of $\log \Delta^*$, allows us now to assess the position of the intramolecular equilibrium 11, where we define $(M{\cdot}Bimp{\cdot}H)^{+}_{op}$ as the species with M^{2+} at N3 and H^+ at PO_3^{2-}, and $(M{\cdot}Bimp{\cdot}H)^{+}_{cl}$ as the closed or chelated species in which the (N3)-coordinated M^{2+} also interacts with the $P(O)_2(OH)^-$ group:

Table 4. Extent of Chelate Formation in $M(H;Bimp)^+$ Complexes (Equilibrium 11) as Calculated from the Stability Enhancement $\log \Delta^*$ (Eq 10) and Quantified by the Dimensionless Equilibrium Constant K_I^* (Equations 12 and 13) and the Percentage of the Chelated Isomer (Equation 14) in Aqueous Solution (25 °C; $I = 0.1$ M, $NaNO_3$)[a,b]

M^{2+}	$\log \Delta_{M/H(Bimp)}$	$\log \Delta^*$	K_I^*	$\%(M{\cdot}Bimp{\cdot}H)^{+}_{cl}$
Ba^{2+}	0.95 ± 0.21	0.65 ± 0.26	3.47 ± 2.67	78 ± 13
Ca^{2+}	1.20 ± 0.17	0.90 ± 0.23	6.94 ± 4.21	87 ± 7
Mg^{2+}	1.04 ± 0.16	0.74 ± 0.22	4.50 ± 2.78	82 ± 9
Mn^{2+}	1.23 ± 0.09	0.93 ± 0.17	7.51 ± 3.33	88 ± 5
Co^{2+}	0.74 ± 0.09	0.44 ± 0.17	1.75 ± 1.08	64 ± 14
Cu^{2+}	1.06 ± 0.09	0.76 ± 0.17	4.75 ± 2.25	83 ± 7
Zn^{2+}	⩽0.54	⩽0.24	⩽0.74	⩽42
Cd^{2+}	0.78 ± 0.07	0.48 ± 0.17	2.02 ± 1.18	67 ± 13

[a] Error limits: see footnote *b* of Table 1. [b] The values given in column 2 are from column 4 in Table 3.

$$(M{\cdot}Bimp{\cdot}H)^{+}_{op} \rightleftharpoons (M{\cdot}Bimp{\cdot}H)^{+}_{cl} \qquad (11)$$

The corresponding dimensionless equilibrium constant K_I^* is defined by eq 12:

$$K_I^* = [(M{\cdot}Bimp{\cdot}H)^{+}_{cl}]/[(M{\cdot}Bimp{\cdot}H)^{+}_{op}] \qquad (12)$$

Application of previous routes[6,7,36,40] allows to calculate values for K_I^* according to eq 13

$$K_I^* = \frac{K^{M}_{M(H;Bimp)}}{K^{M}_{M(H;Bimp)op}{}^{*}} - 1 = 10^{\log \Delta^*} - 1 \qquad (13)$$

where $K^{M}_{M(H;Bimp)}$ represents the measured stability constant (Table 3, column 2) and $K^{M}_{M(H;Bimp)op}{}^{*}$ the one calculated for the open isomer by taking into account the charge effect (cf. also eqs 9 and 10). The percentage of the closed species occurring in equilibrium 11 follows from eq 14:

---

(39) It should be noted that the $pk^{H}_{(N3)H/H_2(Bimp)}$ value according to eq 8 used in these calculations contains the effect of the $P(O)_2(OH)^-$ residue since $pK^{H}_{H_2(Bimp)}$ is measured in its presence. Hence, only the attractive effect between a M^{2+} at N3 and the uncoordinated $P(O)_2(OH)^-$ group needs in addition to be taken into account.

(40) (a) Sigel, H.; Massoud, S. S.; Tribolet, R. *J. Am. Chem. Soc.* **1988**, *110*, 6857−6865. (b) Sigel, H.; Massoud, S. S.; Corfù, N. A. *J. Am. Chem. Soc.* **1994**, *116*, 2958−2971.

$$\% \ (\mathrm{M{\cdot}Bimp{\cdot}H})^{+}_{\mathrm{cl}} = 100{\cdot}K^{*}_{\mathrm{I}}/(1 + K^{*}_{\mathrm{I}}) \tag{14}$$

The corresponding results for the $\mathrm{M(H;Bimp)^+}$ systems are summarized in columns 4 and 5 of Table 4.

The formation degree of the chelated $(\mathrm{M{\cdot}Bimp{\cdot}H})^{+}_{\mathrm{cl}}$ species is surprisingly high, varying for all metal ions only between about 65% and 85% (Table 4, column 5), the only exception being the $\mathrm{Zn^{2+}}$ system.[25] This result is astonishing since it means that the alkaline earth ions as well as the 3d transition ions including $\mathrm{Cd^{2+}}$ behave quite alike; in fact, on average the percentage for the closed species for the seven measured examples amounts to 78 ± 10% (3σ), and within the error limits all individual values are identical with this average. There are two explanations for this observation: (i) Chelate-ring formation is largely driven by the entropy gain associated with the release of a water molecule, and this would be constant for all the metal ions; or (ii), more favored by us, chelate formation of the N3-bound $\mathrm{M^{2+}}$ with the $\mathrm{P(O)_2(OH)^-}$ residue occurs to a large part in an outersphere fashion. Furthermore, it had previously been concluded[16,17a] that the alkaline earth ions form outersphere complexes with N3 of benzimidazoles and that this is the reason why the stability of their complexes is rather independent of $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H(Bi-R)}}$ (see also Figure 2); in contrast, the divalent 3d ions interact preferably in an innersphere manner with N3.[16,17a] Consequently, one may suggest that the alkaline earth ions form to the larger part outersphere species with both binding sites of $\mathrm{H(Bimp)^-}$, and this is also in agreement with the stability of these complexes, i.e., $\log K^{\mathrm{M}}_{\mathrm{M(H;Bimp)}} \simeq 1$ (Tables 1 and 3, columns 2).

3.5. A More Detailed Appraisal of the Structural Aspects Regarding the $\mathrm{Cu(H;Bimp)^+}$ Species. In section 3.2, it was concluded that in the $\mathrm{M(H;Bimp)^+}$ species the metal ion must be at N3 and the proton at the phosphonate group. This was further substantiated in the other sections, especially in section 3.4, where it was proven that chelate formation is large for all $\mathrm{M(H;Bimp)^+}$ systems (Table 4, column 5), and of course, chelates can only be formed if the proton is located at the phosphonate group. However, one may still ask: Is there an isomer, possibly in small concentrations, which carries the proton at N3 and $\mathrm{M^{2+}}$ at the phosphonate group?

Considering that from all the metal ions studied here, only $\mathrm{Cu^{2+}}$ has an approximately equal affinity toward N3 and the $\mathrm{PO_3^{2-}}$ group (see Table 2) and that it is also the $\mathrm{Cu(H;Bimp)^+}$ complex, which has with $\mathrm{p}K^{\mathrm{H}}_{\mathrm{Cu(H;Bimp)}} = 3.94 \pm 0.10$ (Table 1) an acidity constant which is not only significantly below $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H(Bimp)}} = 7.41 \pm 0.02$, but also significantly below $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H_2(Bimp)}} = 5.37 \pm 0.02$, it is this complex for which the possible existence of an isomer with $\mathrm{M^{2+}}$ at the phosphonate group and the proton at N3 is highest, and therefore, this analysis is carried out for $\mathrm{Cu(H;Bimp)^+}$. Of course, the indicated isomer can exist only in an "open" form, and we abbreviate it as $(\mathrm{H{\cdot}Bimp{\cdot}Cu})^{+}_{\mathrm{op}}$. The chelated isomer is symbolized as above in section 3.4 by $(\mathrm{Cu{\cdot}Bimp{\cdot}H})^{+}_{\mathrm{cl}}$. The corresponding open isomer of equilibrium 11 is ignored in the analysis because it occurs only in minor amounts; in fact, it may be recalled that the formation degrees of the chelated isomers listed in column 5 of Table 4 are rather lower limits as discussed in section 3.4.

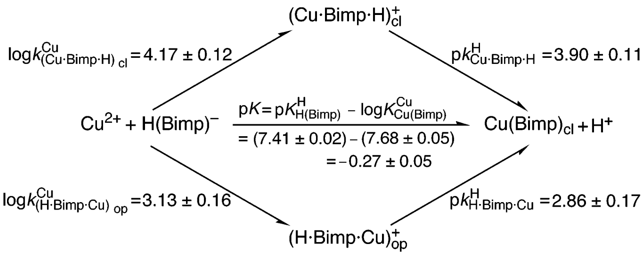

Figure 4. Equilibrium scheme showing the interrelation between the monoprotonated and chelated $(\mathrm{Cu{\cdot}Bimp{\cdot}H})^{+}_{\mathrm{cl}}$ species (cf. equilibrium 11 and the top of the scheme), and the isomer having the proton at the N3 site of the benzimidazole residue and $\mathrm{Cu^{2+}}$ at the phosphonate group, $(\mathrm{H{\cdot}Bimp{\cdot}Cu})^{+}_{\mathrm{op}}$ (bottom), together with the other species in equilibrium with these two isomers. The scheme also defines the micro constants (k) and gives their interrelation with the macro constants (K); the arrows indicate the direction for which the constants are defined. The macro constants are known from the experiments (Table 1), and the micro constant $\log k^{\mathrm{Cu}}_{\mathrm{(H{\cdot}Bimp{\cdot}Cu)op}} = 3.13 \pm 0.16$ (lower part; left) has been estimated as described in section 3.5.[42] From this value[10,41] and $1/K^{\mathrm{H}}_{\mathrm{Cu(H;Bimp)}} = (1/k^{\mathrm{H}}_{\mathrm{Cu{\cdot}Bimp{\cdot}H}}) + (1/k^{\mathrm{H}}_{\mathrm{H{\cdot}Bimp{\cdot}Cu}})$ follows $1/k^{\mathrm{H}}_{\mathrm{Cu{\cdot}Bimp{\cdot}H}} = (1/K^{\mathrm{H}}_{\mathrm{Cu(H;Bimp)}}) - (1/k^{\mathrm{H}}_{\mathrm{H{\cdot}Bimp{\cdot}Cu}}) = (1/10^{-(3.94\pm0.10)}) - (1/10^{-(2.86\pm0.17)}) = 10^{(3.94\pm0.10)} - 10^{(2.86\pm0.17)} = 10^{(3.90\pm0.11)}$, which is given in the upper part at the right side. The two other micro constants follow from the properties of cyclic systems.

The micro constant scheme seen in Figure 4 defines the two possible pathways leading from $\mathrm{Cu^{2+}}$ and $\mathrm{H(Bimp)^-}$ to Cu(Bimp) and $\mathrm{H^+}$, where Cu(Bimp) exists to nearly 100% in the form of the chelated isomer as will be shown in section 3.6 (see Table 5, vide infra); this "closed" species is abbreviated as $\mathrm{Cu(Bimp)_{cl}}$. The equilibrium scheme of Figure 4, which is related to an earlier similar problem,[10] connects the micro constants with the macro constants; the latter ones have been measured (Table 1). Since there are four unknown micro constants but only three independent equations, which interrelate the macro with the micro constants (see, e.g., ref 41), one of the micro constants needs to be estimated. We use $\log K^{\mathrm{Cu}}_{\mathrm{Cu(Bimp)calcd}} = \log K^{\mathrm{Cu}}_{\mathrm{Cu(Bimp)op}} = 3.43 \pm 0.06$ (Table 3, column 6), which quantifies the stability constant of the open isomer with only a phosphonate−metal ion coordination in the Cu(Bimp) system, and reduce this stability constant by the charge effect which results if N3 of the benzimidazole residue is protonated; this effect amounts to 0.30 ± 0.15 log units as estimated in section 3.4. Hence, we obtain for the micro stability constant $\log k^{\mathrm{Cu}}_{\mathrm{(H{\cdot}Bimp{\cdot}Cu)op}} = \log K^{\mathrm{Cu}}_{\mathrm{Cu(Bimp)op}} - (\text{charge effect}) = (3.43 \pm 0.06) - (0.30 \pm 0.15) = 3.13 \pm 0.16$.[42] This value appears at the left side in the lower part of the scheme in Figure 4, and now all the other micro constants can be calculated (see legend of Figure 4).

---

(41) Song, B.; Sigel, R. K. O.; Sigel, H. *Chem. Eur. J.* **1997**, *3*, 29−33.

(42) This value is an upper limit for the micro stability constant $k^{\mathrm{Cu}}_{\mathrm{(H{\cdot}Bimp{\cdot}Cu)op}}$ because the effect of $\mathrm{(N3)H^+}$ on the release of the proton from $\mathrm{P(O)_2(OH)^-}$ has been ignored and this would give a somewhat lower $\mathrm{p}K_{\mathrm{a}}$ value. If taken into account in the calculation with the straight reference line, a value a bit lower than the employed 3.43 log units for the stability constant of $\mathrm{Cu(Bimp)_{op}}$ would result. This means, however, the ratio R considered in eq 15 would even become larger; hence, all the presented conclusions are correct.

Table 5. Extent of Chelate Formation in M(Bimp) Complexes (Equilibrium 16) as Calculated from the Stability Enhancement (see Figure 3) log $\Delta_{\text{M/Bimp}}$ (Equation 9) and Quantified by the Dimensionless Equilibrium Constant K_I (Equation 17 which is analogous to Equation 13) and the Percentage of the Chelated Isomer $\text{M(Bimp)}_{\text{cl}}$ (analogous to Equation 14) in Aqueous Solution (25 °C; $I = 0.1$ M, $NaNO_3$)[a,b]

M^{2+}	log $\Delta_{\text{M/Bimp}}$	K_I	% $\text{M(Bimp)}_{\text{cl}}$
Ba^{2+}	0.08 ± 0.06	0.20 ± 0.17	17 ± 11
Ca^{2+}	0.16 ± 0.08	0.45 ± 0.27	31 ± 13
Mg^{2+}	0.28 ± 0.07	0.91 ± 0.31	48 ± 8
Mn^{2+}	1.73 ± 0.08	52.7 ± 9.9	98.1 ± 0.4
Co^{2+}	2.71 ± 0.08	512 ± 94	99.81 ± 0.04
Cu^{2+}	4.25 ± 0.08	17800 ± 3300	99.994 ± 0.001
Zn^{2+}	3.25 ± 0.07	1780 ± 290	99.94 ± 0.01
Cd^{2+}	2.70 ± 0.10	500 ± 115	99.80 ± 0.05

[a] Error limits: see footnote *b* of Table 1. [b] The values given in column 2 are from column 7 in Table 3.

The availability of the various micro constants in Figure 4 allows now to answer the above question. The ratio, *R*, of the chelated isomer, $(\text{Cu·Bimp·H})^{+}_{\text{cl}}$ (which also occurs in equilibrium 11), to the open phosphonate-bound species with the proton at N3, $(\text{H·Bimp·Cu})^{+}_{\text{op}}$, follows from eq 15 and the application of the corresponding micro constants:

$$R = \frac{[(\text{Cu·Bimp·H})^{+}_{\text{cl}}]}{[(\text{H·Bimp·Cu})^{+}_{\text{op}}]} = \frac{k^{\text{Cu}}_{(\text{Cu·Bimp·H})\text{cl}}}{k^{\text{Cu}}_{(\text{H·Bimp·Cu})\text{op}}} \tag{15a}$$

$$= \frac{10^{(4.17\pm0.12)}}{10^{(3.13\pm0.16)}} = 10^{(1.04\pm0.20)} \tag{15b}$$

$$= \frac{11}{1}\left(\frac{17}{1}; \frac{7}{1}\right) \tag{15c}$$

$$= \frac{92\%}{8\%}\left(\frac{94\%}{6\%}; \frac{88\%}{12\%}\right) \tag{15d}$$

The values given in parentheses in eqs 15c and 15d are the upper and lower limits which follow from the error limit of ±0.20 which appears in eq 15b. The result of eq 15d confirms the conclusions of sections 3.2 and 3.4 that the proton is mainly located at the phosphonate group and that the species with the proton at N3 and M^{2+} at the phosphonate group occurs only in small amounts. As indicated above, for complexes with metal ions other than Cu^{2+}, the likelihood for the formation of species with the proton at N3 is even smaller.

3.6. Extent of Chelation in the M(Bimp) Complexes. In section 3.2, in the context of Figure 3 we have seen that for several metal ions a dramatic stability enhancement for their M(Bimp) complexes is observed, if the experimentally measured constants are compared with those calculated on the basis of the basicity of the phosphonate group in Bimp^{2-}. This stability enhancement has to be attributed[36] to chelate formation of the phosphonate-coordinated metal ion with N3 of the benzimidazole residue; this means that there must be an "open" isomer in which M^{2+} is only bound to the PO_3^{2-} group and a "closed" or chelated species where in addition N3 is involved. This intramolecular equilibrium is expressed in eq 16:

$$\text{M(Bimp)}_{\text{op}} \rightleftharpoons \text{M(Bimp)}_{\text{cl}} \tag{16}$$

Of course, the position of equilibrium 16 is quantified by the dimensionless constant K_I, defined in eq 17

$$K_I = \frac{[\text{M(Bimp)}_{\text{cl}}]}{[\text{M(Bimp)}_{\text{op}}]} = \frac{K^{\text{M}}_{\text{M(Bimp)}}}{K^{\text{M}}_{\text{M(Bimp)op}}} - 1 = 10^{\log\Delta_{\text{M/Bimp}}} - 1 \tag{17}$$

for which values may be calculated via log $\Delta_{\text{M/Bimp}}$ (eq 9) in analogy to eq 13 (see Table 3, columns 5−7), and the formation degree of $\text{M(Bimp)}_{\text{cl}}$ is obtained in analogy to eq 14. The corresponding results are summarized in Table 5.

From the results listed in column 4 of Table 5, it is evident that the formation degree of the chelated species varies dramatically from a few percent for Ba(Bimp) to nearly 100% in the case of Cu(Bimp). Of course, the stability enhancement log $\Delta_{\text{M/Bimp}}$, connected with these formation degrees, is a reflection of the change in free energy which occurs upon chelate formation;[7] in the case of the Ba(Bimp) system, the change in $\Delta G^{\circ}_{(25\,°\text{C})}$ amounts only to about −0.5 kJ/mol whereas for the Cu(Bimp) system it equals −24 kJ/mol. Hence, the formation of a small percentage of a chelated species, e.g., of 20%, involves only a rather insignificant change in free energy. Clearly, the tremendous differences observed between the various metal ions originate in their different affinity toward N donors, which is low for the alkaline earth ions and quite large for ions such as Cu^{2+} or Zn^{2+}.[20,21]

3.7. A New View on the Chelate Effect and Its Quantification. The so-called "chelate effect" refers to the enhanced stability of a complex formed by a ligand offering two or more donor atoms,[43] thus giving rise to the formation of chelate rings, in comparison to the stability of a complex formed by monodentate ligands with the same kind of donor atoms, as is exemplified in eqs 18 and 19:

$$\text{Ni}^{2+} + 3\,\text{En} \rightleftharpoons \text{Ni(En)}_3^{2+} \tag{18a}$$

$$\beta^{\text{Ni}}_{\text{Ni(En)}_3} = \frac{[\text{Ni(En)}_3^{2+}]}{[\text{Ni}^{2+}][\text{En}]^3} \tag{18b}$$

$$\text{Ni}^{2+} + 6\text{NH}_3 \rightleftharpoons \text{Ni(NH}_3)_6^{2+} \tag{19a}$$

$$\beta^{\text{Ni}}_{\text{Ni(NH}_3)_6} = \frac{[\text{Ni(NH}_3)_6^{2+}]}{[\text{Ni}^{2+}][\text{NH}_3]^6} \tag{19b}$$

There are (still many and also new) textbooks in which the overall stability constants, β, of equilibria 18a and 19a or of similar systems are compared, and then, it is concluded that log $\beta_{\text{chelate}} >$ log $\beta_{\text{monodentate}}$. This result is then attributed to the chelate effect. However, this definition and quantification is improper,[44,45] because β values with different dimensions are compared; e.g., for $\beta^{\text{Ni}}_{\text{Ni(En)}_3}$ (eq 18b) the dimension is evidently M^{-3} and for $\beta^{\text{Ni}}_{\text{Ni(NH}_3)_6}$ (eq 19b) it is M^{-6}. Even

---

(43) Martell, A. E.; Hancock, R. D.; Motekaitis, R. J. *Coord. Chem. Rev.* **1994**, *133*, 39−65.

(44) Jameson, R. F. In *An Introduction to Bio-inorganic Chemistry*; Williams, D. R., Ed.; Thomas: Springfield, Illinois, 1976; pp 33−35.

(45) Fraústo da Silva, J. J. R. *J. Chem. Educ.* **1983**, *60*, 390−392.

Table 6. Quantification of the Chelate Effect According to Equation 21 for Several M(Bimp) Complexes Based on the Intrinsic Metal Ion Affinity of the Individual Binding Sites, i.e., of the N3 Site (log $k^{\mathrm{M}}_{\mathrm{M\cdot N3/Bimp}}$) and the $\mathrm{PO_3^{2-}}$ Group (log $k^{\mathrm{M}}_{\mathrm{PO_3\cdot M/Bimp}}$) (Equation 20) of $\mathrm{Bimp^{2-}}$. The Values for log *Chelate* (Column 6; Equation 21) Are a Measure for the Chelate Effect in the Various M(Bimp) Species (Aqueous Solution; 25 °C; $I \simeq 0.1$ M, $\mathrm{NaNO_3}$)[a]

$\mathrm{M^{2+}}$	log $k^{\mathrm{M}}_{\mathrm{M\cdot N3/Bimp}}$ [b]	log $k^{\mathrm{M}}_{\mathrm{PO_3\cdot M/Bimp}}$ [c]	log $K^{\mathrm{M}}_{\mathrm{M/Bimp/expected}}$ [d]	log $K^{\mathrm{M}}_{\mathrm{M(Bimp)}}$ [e]	log *Chelate*
$\mathrm{Ba^{2+}}$	−0.2 ± 0.2	1.27 ± 0.04	1.28 ± 0.04	1.35 ± 0.05	0.07 ± 0.06
$\mathrm{Ca^{2+}}$	−0.14 ± 0.15	1.61 ± 0.05	1.62 ± 0.05	1.77 ± 0.06	0.15 ± 0.08
$\mathrm{Mg^{2+}}$	0.03 ± 0.05	1.81 ± 0.03	1.82 ± 0.03	2.09 ± 0.06	0.27 ± 0.07
$\mathrm{Mn^{2+}}$	0.80 ± 0.05	2.45 ± 0.05	2.46 ± 0.05	4.18 ± 0.06	1.72 ± 0.08
$\mathrm{Co^{2+}}$	1.62 ± 0.05	2.21 ± 0.06	2.31 ± 0.05	4.92 ± 0.05	2.61 ± 0.07
$\mathrm{Cu^{2+}}$	3.27 ± 0.08	3.43 ± 0.06	3.66 ± 0.05	7.68 ± 0.05	4.02 ± 0.07
$\mathrm{Zn^{2+}}$	1.64 ± 0.06	2.54 ± 0.06	2.59 ± 0.05	5.79 ± 0.04	3.20 ± 0.06
$\mathrm{Cd^{2+}}$	2.13 ± 0.07	2.84 ± 0.05	2.92 ± 0.04	5.54 ± 0.09	2.62 ± 0.10

[a] Error limits: see footnote *b* of Table 1. [b] Calculated with $\mathrm{p}k^{\mathrm{H}}_{\mathrm{H\cdot Bimp}} = 5.97 \pm 0.15$ (see text in section 3.7) and the straight-line equations given in ref 16; the relatively large error of the $\mathrm{p}k^{\mathrm{H}}_{\mathrm{H\cdot Bimp}}$ value is considered in the error limits listed above. [c] From column 6 of Table 3; see text in section 3.7. [d] Calculated according to eq 20. [e] From column 3 of Table 1.

worse, by an adequate choice of the units of concentration (e.g., mol/mL instead of mol/L) the "effect" can actually be inverted.[44] Unfortunately, there is up to now no simple way to quantify the chelate effect.

For the ligand $\mathrm{Bimp^{2-}}$ (Figure 1), which is in the center of this study, it has been shown in section 3.6 that its complexes exist to a certain extent (eq 16) in the form of chelates (Table 5). Furthermore, for this ligand the unique opportunity exists to quantify the metal ion affinity of the N3 site and the $\mathrm{PO_3^{2-}}$ group individually (section 3.3). The sum of these two intrinsic micro stability constants, which has the dimension $\mathrm{M^{-1}}$ like the experimentally measured constant (eq 4), provides the expected metal ion affinity of the two individual binding sites in $\mathrm{Bimp^{2-}}$ (eq 20):[46,47]

$$K^{\mathrm{M}}_{\mathrm{M/Bimp/expected}} = \frac{[(\mathrm{M\cdot N3/Bimp})] + [(\mathrm{PO_3\cdot M/Bimp})]}{[\mathrm{M^{2+}}][\mathrm{Bimp}]} \quad (20a)$$

$$= k^{\mathrm{M}}_{\mathrm{M\cdot N3/Bimp}} + k^{\mathrm{M}}_{\mathrm{PO_3\cdot M/Bimp}} \quad (20b)$$

The micro stability constants of eq 20b can be calculated by applying the appropriate acidity constants of the $\mathrm{(N3)H^+}$ and $\mathrm{P(O)_2(OH)^-}$ sites of $\mathrm{Bimp^{2-}}$ to the existing plots of log $K^{\mathrm{M}}_{\mathrm{M(L)}}$ versus $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H(L)}}$ according to eq 7 (cf. Figures 2 and 3); i.e., the previously determined[6,16] parameters for benzimidazole-type ligands (N3) and phosph(on)ate-type ligands ($\mathrm{PO_3^{2-}}$) are known.[6,16]

In section 3.3 we have seen that the intrinsic acidity of the $\mathrm{(N3)H^+}$ site in $\mathrm{H_2(Bimp)^{\pm}}$ (better written as $\mathrm{^+H\cdot Bimp\cdot H^-}$) is described by the micro acidity constant $\mathrm{p}k^{\mathrm{H}}_{\mathrm{(N3)H/H_2(Bimp)}} = 5.67 \pm 0.02$ (eq 8). However, this value refers to a species which still carries a proton at the phosphonate group; a change from $\mathrm{P(O)_2(OH)^-}$ to $\mathrm{PO_3^{2-}}$ will indirectly enhance via electrostatic effects the basicity of the N3 site somewhat, and this change needs to be taken into account. We estimate for this charge effect $\Delta \mathrm{p}K_{\mathrm{a}} = 0.30 \pm 0.15$ in accord with the reasonings given in section 3.4 and assume for the micro acidity constant of the $\mathrm{(N3)H^+}$ site in $\mathrm{^+H\cdot Bimp^{2-}}$ (the proton being at N3 with $\mathrm{PO_3^{2-}}$ free), $\mathrm{p}k^{\mathrm{H}}_{\mathrm{H\cdot Bimp}} = (5.67 \pm 0.02) + (0.30 \pm 0.15) = 5.97 \pm 0.15$. Application of this acidity constant (including its large error limit) to the equations (eq 7) for the straight reference lines[16] gives the micro stability constants listed in column 2 of Table 6.

The calculation of the second term in eq 20b is much simpler, since the micro acidity constant for the deprotonation of $\mathrm{Bimp\cdot H^-}$, the proton being at the $\mathrm{PO_3^{2-}}$ group, is practically identical[12] with the macro acidity constant $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H(Bimp)}} = 7.41 \pm 0.02$; application of the straight-line equations[6,7,37] leads to the results already listed in column 6 of Table 3; hence, log $K^{\mathrm{M}}_{\mathrm{M(Bimp)calcd}}$ = log $k^{\mathrm{M}}_{\mathrm{PO_3\cdot M/Bimp}}$. These values are given (again) in column 3 of Table 6, and now, the sums according to eq 20 may be calculated; these results are listed in column 4 of Table 6. Since now the expected stability constants, log $K^{\mathrm{M}}_{\mathrm{M/Bimp/expected}}$, for M(Bimp) complexes without chelate formation but based on the $\mathrm{M^{2+}}$ affinity of the two binding sites are known, the chelate effect can be defined according to eq 21, i.e., by comparing the expected stability constant with the one actually measured (eq 4):

$$\log \mathit{Chelate} = \log K^{\mathrm{M}}_{\mathrm{M(Bimp)}} - \log K^{\mathrm{M}}_{\mathrm{M/Bimp/expected}} \quad (21)$$

The values for the three terms which appear in eq 21 are listed in columns 6, 5, and 4 of Table 6, respectively.

Comparison of the log *Chelate* values in column 6 of Table 6 reveals that the chelate effect differs dramatically for the various M(Bimp) complexes; for Ba(Bimp) the effect is close to zero whereas for Cu(Bimp) it amounts to about 4 log units.

---

(46) One might be tempted to argue here that instead of eq 20b an approach via ΔG should be employed: Since at 25 °C the interrelation between $\Delta G°$ and an equilibrium constant, K_{eq}, is given by $\Delta G° = -5.71 \times \log K_{\mathrm{eq}}$,[47] the sum of the logarithms of $k^{\mathrm{M}}_{\mathrm{M\cdot N3/Bimp}}$ and $k^{\mathrm{M}}_{\mathrm{PO_3\cdot M/Bimp}}$ should describe the expected stability ($\Delta G°$) for the chelate and comparing this sum with the experimentally measured stability constant, log $K^{\mathrm{M}}_{\mathrm{M(Bimp)}}$, should then give the "chelate effect", log *Chelate*(*G*). This approach leads to the following equation:

$$10^{\log \mathit{Chelate}(G)} = \frac{[\mathrm{M(Bimp)}]}{[\mathrm{M^{2+}}][\mathrm{Bimp^{2-}}]}\,\frac{[\mathrm{M^{2+}}][\mathrm{Bimp^{2-}}]}{[\mathrm{M\cdot N3/Bimp}]}\,\frac{[\mathrm{M^{2+}}][\mathrm{Bimp^{2-}}]}{[\mathrm{PO_3\cdot M/Bimp}]} = \frac{[\mathrm{M(Bimp)}][\mathrm{M^{2+}}][\mathrm{Bimp^{2-}}]}{[\mathrm{M\cdot N3/Bimp}][\mathrm{PO_3\cdot M/Bimp}]}$$

Since this latter equation necessarily defines the position of an equilibrium, namely of $\mathrm{M\cdot N3/Bimp + PO_3\cdot M/Bimp \rightleftharpoons M(Bimp) + M^{2+} + Bimp^{2-}}$, it is evident that such a definition for the chelate effect is *not* reasonable (in contrast to eqs 22 and 23; see the corresponding discussion in the text).

(47) Sigel, H.; Kapinos, L. E. *Coord. Chem. Rev.* **2000**, *200−202*, 563−594.

Of course, the observed chelate effect parallels the formation degrees of the chelates as discussed in section 3.6, the values of which are listed in column 4 of Table 5. Since the $\Delta H°$ values, which reflect the binding strength between M^{2+} and a ligating site, are not expected to vary much for a given metal−ligand bond be it in a monodentate or a chelated species, one may conclude in accord with earlier suggestions that the chelate effect is mainly an entropy effect,[48] though bond distortion in a chelate may affect $\Delta H°$ as well.[45] Clearly, for a detailed answer calorimetric measurements are needed for each system to reveal the exact ΔH and ΔS contributions.

At this point it may be helpful to investigate the implications of eq 21 a bit more in detail. From this equation follows eq 22:

$$10^{\log \mathit{Chelate}} = K^{M}_{M(Bimp)}/K^{M}_{M/Bimp/expected} \tag{22a}$$

$$= K^{M}_{M(Bimp)}/(k^{M}_{M\cdot N3/Bimp} + k^{M}_{PO_3\cdot M/Bimp}) \tag{22b}$$

$$= \frac{[M(Bimp)]}{[M^{2+}][Bimp^{2-}]} \times \frac{[M^{2+}][Bimp^{2-}]}{([M\cdot N3/Bimp] + [PO_3\cdot M/Bimp])} \tag{22c}$$

$$= \frac{[M(Bimp)]}{([M\cdot N3/Bimp] + [PO_3\cdot M/Bimp])} \tag{22d}$$

According to this definition (eq 22) $10^{\log \mathit{Chelate}}$ is the dimensionless equilibrium constant which quantifies the position of equilibrium 23:[49]

$$(M\cdot N3/Bimp + PO_3\cdot M/Bimp) \rightleftharpoons M(Bimp) \tag{23}$$

For log *Chelate* = 0 in eq 21, the ratio

$$\frac{[M(Bimp)]}{([M\cdot N3/Bimp] + [PO_3\cdot M/Bimp])}$$

in eq 22d equals one, and this means, as it should be, that no chelates exist and that all M(Bimp) species are present as monodentatally coordinated M·N3/Bimp and PO_3·M/Bimp complexes.

For all situations where log *Chelate* > 0, the ratio will be larger than 1 and this then means that chelated M(Bimp) species exist. For example, for log *Chelate* = 0.3 a value of 2/1 follows for the ratio namely

$$\frac{[M(Bimp)]}{([M\cdot N3/Bimp] + [PO_3\cdot M/Bimp])} = \frac{2}{1} = \frac{1+1}{1}$$

i.e., 50% of all M(Bimp) complexes exist in the form of chelates (it should be remembered here (see eq 4) that [M(Bimp)] encompasses all complex species present). In the case that log *Chelate* = 1 the ratio equals 10/1 giving

$$\frac{[M(Bimp)]}{([M\cdot N3/Bimp] + [PO_3\cdot M/Bimp])} = \frac{10}{1} = \frac{9+1}{1}$$

and showing thus that 90% of the M(Bimp) species are present in the form of chelates. Finally, in the case that log *Chelate* = 2 the ratio is 100/1

$$\frac{[M(Bimp)]}{([M\cdot N3/Bimp] + [PO_3\cdot M/Bimp])} = \frac{100}{1} = \frac{99+1}{1}$$

meaning that from all M(Bimp) species 99% are present as chelates. In other words, eqs 21 and 22 represent a method to quantify the chelate effect by putting the formation degree of the chelated species in relation to their monodentate complexes.[50]

4. Conclusions

The acyclic nucleotide analogue 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) is an antivirally active compound[9,11] which resembles (d)ATP in its diphosphorylated state; indeed, it is recognized by polymerases as a substrate but after its insertion into the growing nucleic acid chain this is terminated due to the lack of a 3′-hydroxy group. It has been suggested[14] that the ether oxygen of the aliphatic chain, (N9)adenyl−CH_2CH_2−O−CH_2−PO_3^{2-}, is crucial in facilitating the $M(P_\alpha)$-binding mode which is needed to achieve the reactive $M(P_\alpha)$-$M(P_\beta,P_\gamma)$ location of the two M^{2+} ions at the triphosph(on)ate chain. In fact, in the M(PMEA) complexes of Mg^{2+}, Ca^{2+}, Mn^{2+}, and Zn^{2+}, a weak, but significant, interaction with the ether oxygen takes place.[14] If one compares this situation with $Bimp^{2-}$ then one is forced to conclude that Bimp most likely will not be antivirally active, or via a different mechanism than PMEA, because with the exception of Mg^{2+} and Ca^{2+} the "back binding" to N3 of a phosphonate-coordinated M^{2+} appears as being too strong to facilitate the proper orientation[14] of metal ions in the active-site cavity of a polymerase; in other words, the strong chelation is expected to act as a "trap". Of course, this does not mean that in the end no useful biological activity can be discovered for Bimp; it only means that mechanistically it is expected to behave differently than PMEA.

However, the coordinating properties of $Bimp^{2-}$ and of $H(Bimp)^{-}$ are most intriguing in their own right since they give rise to various chelated species involving the N3 site and the phosphonate group, including such where the phosphonate group also carries a proton (section 3.4). The coordination chemistry of this ligand system is very rich and varies from metal ion to metal ion (section 3.6) which is fascinating with regard to the chelate effect which could be quantified in this case by a new method (section 3.7) due to the previous characterization of the individual metal-ion binding properties of the N3 site[16] and the phosphonate

---

(48) (a) Wiberg, N. *Holleman-Wiberg. Lehrbuch der Anorganischen Chemie*; Walter de Gruyter: Berlin, 1985; p 975. (b) Huheey, J. E. *Anorganische Chemie. Prinzipien von Struktur und Reaktivität* (translated from *Inorganic Chemistry. Principles of Structure and Reactivity*); Reuter, B., Sarry, B., Eds.; Walter de Gruyter: Berlin, 1988; pp 579−580.

(49) Of course, the two monodentatally bound species, M·N3/Bimp and PO_3·M/Bimp, are also in equilibrium with each other; in fact, the position of this equilibrium is defined by the ratio of the two micro constants given in eq 20b, the values of which are listed in Table 6.

(50) Evidently, the same method may also be applied to chelates formed with, e.g., ethylenediamine or glycinate.

group,[6,7] i.e., by employing the intrinsic micro stability constants. It is evident that the same method may also be applied to other chelating systems.[50]

Acknowledgment. The encouragement received during the completion of this study by Professors Harry B. Gray (California Institute of Technology) and R. Bruce Martin (University of Virginia) is gratefully acknowledged. We would also like to thank Mrs. Rita Baumbusch and Mrs. Astrid Sigel (Basel) for their competent technical assistance in the preparation of this manuscript, and Professor Bernhard Lippert (Dortmund, Germany) for his interest in this study within the frame of COST D20. This study was supported by the Swiss National Science Foundation (H.S.) and within the COST D20 program by the Swiss Federal Office for Education and Science (H.S.) as well as by the University of Granada, Spain (J.N.-G.), the University of Barcelona, Spain (V.M.), and the Medical University of Lódź, Poland (J.O.; Grant 502-13-849). M.J.S.-M. is grateful for support received from the University of Basel during her leave from the University of Granada, Spain.

IC030175K