Inorg. Chem. **2003**, *42*, 5107−5116

Stability and Acidity Constants for Ternary Ligand-Zinc-Hydroxo Complexes of Tetradentate Tripodal Ligands

Yu-Hung Chiu and James W. Canary*

Department of Chemistry, New York University, New York, New York 10003

Received July 8, 2002

Four series of tetradentate tripodal ligands containing pyridyl, 2-imidazolyl, 4-imidazolyl, amino, and/or carboxylic groups were synthesized as hydrolytic zinc enzyme models in order to elucidate the effect of various coordination environments on zinc binding and the acidity of zinc-bound water. In aqueous solution, ligands with same charges showed a good correlation between zinc binding (log K_{ZnL}) and zinc-bound water acidity (pK_a of $LZnOH_2$); the stronger the zinc binding, the higher the pK_a. The zinc-bound water acidity decreased as pyridyl groups were replaced by carboxylate groups. However, exchanging amino groups for carboxylate groups gave no change in zinc-bound water acidity regardless of the charge of the atoms in the inner coordination sphere of the metal ion. The results are consistent with the conventional notion that negatively charged carboxylate groups inherently increase zinc binding and result in decreasing zinc-bound water acidity, but also suggest that environmental effects may modulate or dominate control of acidity.

Introduction

Among the many enzymes that utilize zinc ion as a cofactor, the zinc proteinases are the most widely studied.[1] At least seven families[2] of zinc proteinases are known, including thermolysin, astacin, serralysin, matrixin, snake venom-related, carboxypeptidase, and aminopeptidase metalloproteinases.[1b] The first five of these families are endopeptidases that contain the signature sequence HEXXH, with two zinc-ligating histidines and a glutamate that is not ligated. Thermolysin presents another ligating glutamate from another 20 amino acids along the chain. The astacins, serralysins, matrixins, and snake venom metalloproteases ligate the zinc ion by another histidine side chain six residues away (HEXXHXXGXXH consensus sequence) and usually by a tyrosine in addition to the bound aquo species. The carboxypeptidases are similar to thermolysin, but the amino acid sequence is unrelated. The aminopeptidases may have two zinc ions involved in the catalytic mechanism. Matrix metalloproteinases (MMPs) are necessary for tissue remodeling and the healing cascade.[3] Misregulated MMP activity can contribute to many disease states and conditions.[4−10]

Other zinc metallopeptidases include the classic carboxypeptidase A (CPA) and thermolysin, although these are not therapeutic targets. Inhibition of angiotensin converting enzyme (ACE) represents one of the clinically most effective means to lower blood pressure.[11] While not proteases, the carbonic anhydrases[12] (CA) possess structural and functional similarities to zinc proteases. The inner coordination

* To whom correspondence should be addressed. E-mail: james.canary@nyu.edu.

(1) (a) Auld, D. S. *Struct. Bonding* **1997**, *89*, 29−50. (b) Mock, W. L. *Zinc Proteinases*; Sinnott, M., Ed.; Academic Press: San Diego, 1998; Vol. I, pp 425−453. (c) Lipscomb, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2376. (d) Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543−551.
(2) Rawlings, N. D.; Barrett, A. J. *Methods Enzymol.* **1995**, *248*, 183−118.
(3) (a) Levy, D. E.; Lapierre, F.; Liang, W.; Ye, W.; Lange, C. W.; Li, X.; Grobelny, D.; Casabonne, M.; Tyrrell, D.; Holme, K.; Nadzan, A.; Galardy, R. E. *J. Med. Chem.* **1998**, *41*, 199−223. (b) Woessner, J., J. F. *FASEB* **1991**, *5*, 2145−2154.
(4) Wickroth, M.; Vaheri, A.; Lauharanta, J.; Sorsa, T.; Konttinen, Y. T. *J. Invest. Dermatol.* **1996**, *106*, 1119−24.
(5) Holleran, W. M.; Galardy, R. E.; Gao, W. N.; Levy, D.; Tang, P. C.; Elias, P. M. *Arch. Dermatol. Res.* **1997**, *289*, 138−44.
(6) Chandler, S.; Coates, R.; Gearing, A.; Lury, J.; Wells, G.; Bone, E. *Neurosci. Lett.* **1995**, *201*, 223−6.
(7) Ahrens, D.; Koch, A. E.; Pope, R. M.; Steinpicarella, M.; Niedbala, M. J. *Arthritis Rheumatism* **1996**, *39*, 1576−87.
(8) Witty, J. P.; Foster, S. A.; Stricklin, G. P.; Matrisian, L. M.; Stern, P. H. *J. Bone Mineral Res.* **1996**, *11*, 72−8.
(9) Folkman, J.; Shing, Y. *J. Biol. Chem.* **1992**, *267*, 10931−4.
(10) (a) Coussens, L. M.; Werb, Z. *Chem. Biol.* **1996**, *3*, 895−904. (b) Sato, H.; Takino, T.; Okada, Y.; Cao, J.; Shinagawa, A.; Yamamoto, E.; Seiki, M. *Nature* **1994**, *370*, 61−65. (c) Sato, H.; Seiki, M. *J. Biochem.* **1996**, *119*, 209−15.
(11) Inagami, T. *Essays Biochem.* **1994**, *28*, 147−164.
(12) (a) Earnhardt, J. N.; Silverman, D. N. *Isozymes of Carbonic Anhydrase*; Sinnott, M., Ed.; Academic Press: San Diego, 1998; Vol. 1, pp 483−495. (b) Lindskog, S.; Behraven, G.; Engstrand, C.; Forsman, C.; Jonsson, B.-H.; Liang, Z.; Ren, X.; Xue, Y. In *Structure−Function Relations in Human Carbonic Anhydrase II as Studied by Site-Directed Mutagenesis*; Botre, F., Gros, G., Storey, B. T., Eds.; VCH Publishers: Weinheim, 1991; pp 1−13.

10.1021/ic025855m CCC: $25.00
Published on Web 07/26/2003

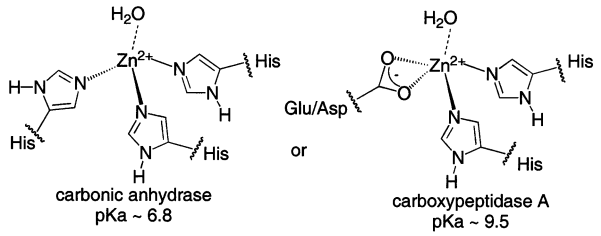

Figure 1. Active sites of hydrolytic zinc enzymes.

spheres of the catalytic zinc ions in CA and CPA are shown in Figure 1.

The currently accepted reaction mechanism for MMPs is similar to that reported for thermolysin.[13] A scheme depicting this mechanism is shown in Figure 2. The principle features include (1) coordination of the scissile amide carbonyl to the electrophilic zinc ion; (2) activation of a water molecule by the zinc ion and Glu-219 with concomitant addition to the amide carbonyl; (3) transfer of a proton to the nitrogen atom; and (4) breakdown of the tetrahedral intermediate, resulting in cleavage. It should be noted that the metal ion is five-coordinate at all stages of the reaction and that the acidic water molecule is coordinated to a pentacoordinate zinc ion except in the resting state.

There are two common motifs for the inner coordination sphere of zinc ions in these enzymes in their crystallographically observable resting states. Typically, the catalytic zinc ion is coordinated in a tetrahedral fashion with either three His or two His and one Asp/Glu, with a water molecule occupying a fourth site (e.g., Figure 1). It is generally assumed that negatively charged Glu engenders an increase in the pK_a of zinc-bound water. For example, this concept has been used to rationalize the fact that the pK_a of CA is about 6.8[14] and that of CPA is about 9.5.[15] In most cases, reported enzymatic pK_a values are determined from pH-dependent kinetic assays, as direct titration is difficult or impossible with the protein.[16] Substitution of a neutral His ligand in wild-type CAII by a negatively charged Asp or Glu showed an increase in pK_a to 8.6, although small structural changes were also noted.[14] Ab initio calculations (gas phase) also indicated that pK_a of zinc-bound water depends on the charge of the ligands, with negatively charged ligands showing reduced $Zn-OH_2$ acidity.[17]

A number of polydentate ligands have been studied as models or mimics of the zinc-coordination structures in enzymes. However, most of the ligands studied contain only nitrogen donors[18,19] or sulfur donors;[20] few attempts have been made to study anionic carboxlate ligands.[21] We are not aware of any systematic experimental data available to quantify the effect on zinc-bound water acidity induced by replacement of neutral ligands with carboxylates in model systems.

We chose to study the tetradentate tripodal ligands **1**−**15** listed in Figure 3 containing various neutral nitrogen donor and carboxylate ligands. Highly chelating, tetradentate ligands bind zinc ions strongly in aqueous solution, preventing precipitation of $Zn(OH)_2$ at high pH and allowing measurement of the [Zn(L)OH] species. In general, tetradentate ligands form 1:1 complexes with Zn(II) in aqueous solution and are less likely to aggregate at high pH compared with ligands of lower denticity.[22] Zinc complexes of tripodal ligands such as **1** form five-coordinate complexes with the ligands occupying four of the five coordination sites.[23] Each N or O donor atom of pendent group and central N atom forms a stable five-membered ring chelate with zinc ion. The five-coordinate complexes formed with aquo or hydroxo

(13) (a) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *1997*, 11359−1472. (b) Lovejoy, B.; Hassell, A. M.; Luther, M. A.; Weigl, D.; Jordan, S. R. *Biochemistry* **1994**, *33*, 8207−17.
(14) Christianson, D.; Fierke, C. A. *Acc. Chem. Res.* **1996,** *29*, 331.
(15) Zhang, K.; Auld, D. S. *Biochemistry* **1993**, *32*, 13844.
(16) Bertini, I. The Coordination Properties of the Active Site of Zinc Enzymes. In *The Coordination Chemistry of Metalloenzymes;* Bertini, I., Drago, R. S., Luchinat, C., Eds.; D. Reidel Publishing; Dordrecht, Holland, 1983; pp 1−18.
(17) Bertini, I.; Luchinat, C.; Rosi, M.; Sgamellotti, A.; Tarantelli, F. *Inorg. Chem.* **1990,** *29*, 1460.
(18) For macrocylic ligands: (a) Woolley, P. *Nature* **1975**, *258*, 677. (b) Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805. (c) Koike, T.; Takamura, M.; Kimura, E. *J. Am. Chem. Soc.* **1994**, *116*, 8443. (e) Kimura, E. *Acc. Chem. Res.* **2001**, *34*, 171.
(19) For tripodal ligands: (a) Adams, H.; Bailey, N. A.; Fenton, D. E.; He, Q.-Y. *J. Chem. Soc., Dalton Trans.* **1997**, 1533. (b) Bergquist, C.; Parkin, G. *J. Am. Chem. Soc.* **1999**, *121*, 6322. (c) Bergquist, C.; Parkin, G. *Inorg. Chem.* **1999**, *38*, 422−423. (d) Brauer, M.; Anders, E.; Sinnecker, S.; Koch, W.; Rombach, M.; Brombacher, H.; Vahrenkamp, H. *Chem. Commun.* **2000**, 647−648. (e) Canary, J. W.; Xu, J.; Castagnetto, J. M.; Rentzeperis, D.; Marky, L. A. *J. Am. Chem. Soc.* **1995**, *117*, 11545. (f) Dowling, C.; Parkin, G. *Polyhedron* **1996**, *15*, 2463. (g) Ghosh, P.; Parkin, G. *J. Chem. Soc., Dalton Trans.* **1998**, 2281. (h) Ghosh, P.; Parkin, G. *Chem. Commun.* **1998**, 413. (i) Herr, U.; Spahl, W.; Trojandt, G.; Steglich, W.; Thaler, F.; van Eldik, R. *Bioorg. Med. Chem.* **1999**, *7*, 699. (j) Jairam, R.; Potvin, P. G.; Balsky, S. *J. Chem. Soc., Perkin Trans. 2* **1999,** 363. (k) Kimblin, C.; Parkin, G. *Inorg. Chem.* **1996**, *35*, 6912. (l) Kimblin, C.; Hascall, T.; Parkin, G. *Inorg. Chem.* **1997**, *36*, 5680. (m) Kimblin, C.; Murphy, V. J.; Hascall, T.; Bridgewater, B. M.; Bonanno, J. B.; Parkin, G. *Inorg. Chem.* **2000**, *39*, 967. (n) Vahrenkamp, H. *Acc. Chem. Res.* **1999**, *32*, 589. (o) Gelinsky, M.; Vogler, R.; Vahrenkamp, H. *Inorg. Chem.* **2002**, *41*, 2560. (p) Rombach, M.; Brombacher, H.; Vahrenkamp, H. *Eur. J. Inorg. Chem.* **2002**, 153. (q) Sénèque, O.; Rager, M.-N.; Giorgi, M.; Reinaud, O. *J. Am. Chem. Soc.* **2001**, *123*, 8442. (r) MacBeth, C. E.; Hammes, B. S.; Young, V. G., Jr.; Borovik, A. S. *Inorg. Chem.* **2001**, *40*, 4733. (s) Ibrahim, M. M.; Shimomura, N.; Ichikawa, K.; Shiro, M. *Inorg. Chim. Acta* **2001**, *313*, 125. (t) Kläui, W.; Piefer, C.; Rheinwald, G.; Lang, H. *Eur. J. Inorg. Chem.* **2000**, 1549. (u) Itoh, S.; Taki, M.; Kumei, H.; Takayama, S.; Nagatomo, S.; Kitagawa, T.; Sakurada, N.; Arakawa, R.; Fukuzumi, S. *Inorg. Chem.* **2000**, *39*, 3708. (v) Xia, J.; Xu, Y.; Li, S.-A.; Sun, W.-Y.; Yu, K.-B.; Tang, W.-X. *Inorg. Chem.* **2001**, *40*, 2394.
(20) (a) Chang, S.; Sommer, R. D.; Rheingold, A. L.; Goldberg, D. P. *Chem. Commun.* **2001**, 2396. (b) Seebacher, J.; Shu, M.; Vahrenkamp, H. *Chem. Commun.* **2001**, 1026. (c) Berreau, L. M.; Allred, R. A.; Makowska-Grzyska, M. M.; Arif, A. M. *Chem. Commun.* **2000**, 1423. (d) Bridgewater, B. M.; Fillebeen, T.; Friesner, R. A.; Parkin, G. *J. Chem. Soc., Dalton Trans.* **2000**, *24*, 4494. (e) Chang, S.; Karambelkar, V. V.; Sommer, R. D.; Rheingold, A. L.; Goldberg, D. P. *Inorg. Chem.* **2002,** *41*, 239. (f) Tesmer, M.; Shu, M.; Vahrenkamp, H. *Inorg. Chem.* **2001**, *40*, 4022−29. (g) Berreau, L. M., Makowska-Grzyska, M. M.; Arif, A. M. *Inorg. Chem.* **2001**, *40*, 2212. (h) Chang, S.; Karambelkar, V. V.; DiTargiani, R. C.; Goldberg, D. P. *Inorg. Chem.* **2001**, *40*, 194. (i) Chiou, S.-J.; Innocent, J.; Riordan, C. G.; Lam, K.-C.; Liable-Sands, L.; Rheingold, A. L. *Inorg. Chem.* **2000**, *39*, 4347.
(21) (a) Abufarag, A.; Vahrenkamp, H. *Inorg. Chem.* **1995**, *34*, 2207. (b) Burth, R.; Stange, A.; Schäfer, M.; Vahrenkamp *Eur. J. Inorg. Chem.* **1998**, 1759−1764. (c) Hammes, B.; Carrrano, C. J. *Inorg. Chem.* **1999**, *38*, 4593. (d) Trösch, A.; Vahrenkamp, H. *Eur. J. Inorg. Chem.* **1998**, 827. (e) Beck, A.; Weibert, B.; Burzlaff, N. *Euro. J. Inorg. Chem.* **2001**, 521.
(22) Leporati, E. *J. Chem. Soc., Dalton Trans.* **1985**, 1605.
(23) Canary, J. C.; Allen, C. S.; Castagnetto, J, M.; Wang, Y. *J. Am. Chem. Soc.* **1995**, *117*, 8484.

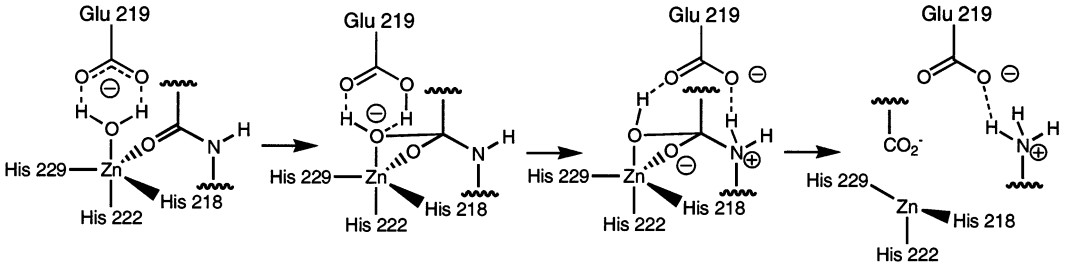

Figure 2. Chemical mechanism for cleavage of peptides by collagenase (MMP-1).[21]

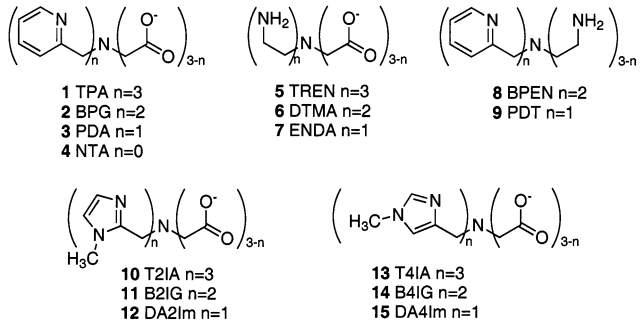

Figure 3. Ligands used in this study.

emulate the catalytically active intermediates believed to occur in the enzymatic reaction cycle.[24]

Among the ligands selected for the study, the imidazole-containing compounds are of the most obvious relevance. Due to the possible deprotonation of imidazole ligands,[25] 1-methyl-2-substituted and 1-methyl-4-substituted imidazole ligands were chosen. Amino- and pyridyl-substituted compounds were also selected to expand the scope of the study. Previous work with Zn(II) complexes of **1** had noted the unusual acidity of zinc-bound water.[26]

Experimental Section

Materials. All chemicals were of highest purity commercially available. TPA,[27a] PDA,[27b] and PDT[27c] were prepared according to the literature, and BPG[27d] and BPEN[27e] were prepared from a modification of literature procedures. Nitrilotriacetic acid (NTA) and tris(2-aminoethyl)amine (TREN) were obtained from Acros Organics.

Bis(2-pyridylmethyl)glycine (BPG), 2. A modification of the procedure by Kanamori was followed.[27d] To a 250 mL round-bottom flask was added glycine (0.75 g, 10 mmol), 2-picolyl chloride hydrochloric acid salt (3.28 g, 20 mmol), and 20 mL of water. To the stirred solution was added a 10 M NaOH aqueous solution (10 mL, 40 mmol) over a period of 10 min, and the reaction mixture was stirred at 50−60 °C for 2 h. The color changed from green to red while adding NaOH. The reaction mixture was cooled to room temperature, concentrated HCl was added to adjust the pH to 5, and the solvent was removed by lyophilization. Then 50 mL of ethanol was added to the dark red oil, resulting in the formation of white sodium chloride precipitate. The removal of sodium chloride by filtration followed by the removal of solvent with a rotary evaporator gave a red oil. Crystallization of the red oil from ethanol/diethyl ether gave 1.5 g of desired product (58% yield), mp 142−143 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.57 (d, J = 4.9 Hz, 2H), 7.66 (td, J = 7.7, 1.8 Hz, 2H), 7.24 (m, 4H), 4.11 (s, 4H), 3.63 (s, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.1, 158.5, 149.0, 137.7, 123.3, 123.1, 60.4, 58.9. Anal. Calcd for $C_{14}H_{15}N_3O_2$: C, 65.35; H, 5.88; N, 16.33. Found: C, 65.31; H, 5.93; N, 16.29. MS (MALDI-TOF, *m*/*e*): 258.2 (M + H).

***N*-(2-Pyridylmethyl)iminodiacetic acid (PDA), 3.** Iminodiacetic acid (3.27 g, 25 mmol) was added to 2.0 g (50 mmol) of NaOH in 10 mL of water and 30 mL of ethanol. To the stirred solution was added 2-picolyl chloride hydrochloric acid salt (4.1 g, 25 mmol) in 10 mL of water, followed by 2.0 g of NaOH in 5 mL of water. The reaction mixture was warmed to 70 °C and stirred for 4 h, an additional 2.0 g of NaOH pellets was added, and the solution was stirred for another hour. The solution was cooled to RT and acidified by adding concentrated HCl to pH 1.5. The yellow solid was obtained after the removal of solvent. Methanol was added to the solid to yield NaCl precipitate. Filtration and concentration yielded a white solid (32% yield). ^{1}H NMR (200 MHz, D_2O): δ 8.72−8.26 (m, 1H), 8.33−8.19 (m, 1H), 7.84−7.70 (m, 2H), 4.50 (s, 2H), 3.79 (s, 4H). ^{13}C NMR (50 MHz, D_2O): δ 176.2, 154.8, 147.1, 146.5, 128.9, 128.6, 59.8, 59.3. Anal. Calcd for $C_{10}H_{12}N_2O_4[1H_2O]$: C, 49.59; H, 5.83; N, 11.56. Found: C, 49.85; H, 5.59; N, 11.59. MS (MALDI-TOF, *m*/*e*): 225.3 (M + H).

***N*,*N*-Bis(2-pyridylmethyl)ethanediamine (BPEN), 8.** A modification of the procedure by Mandel was followed.[27e] Lysidine (1 g, 11.9 mmol) in 1 g of water was heated at 75 °C for 2 h, then added to a solution of 2-picolyl chloride hydrochloride (3.9 g, 23.8 mmol) in 10 mL of water. Aqueous NaOH (4.7 mL, 10 M, 47.6 mmol) was added slowly to the reaction mixture over a period of 1 h at 50 °C and allowed to stir for an additional 2 h. The color of the solution turned from white to pink. The cooled reaction mixture was extracted with 10 mL of chloroform three times. The chloroform layer was evaporated under reduced pressure. The red residue was dissolved in ethyl acetate and filtered through alumina in order to remove the high-polarity impurities. The product, *N*-acetyl-*N*′,*N*′-bis(2-pyridylmethyl)ethanediamine, was crystallized from ethyl acetate (1.55 g, 46% yield), mp 103−104 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.47 (d, J = 4.8 Hz, 2H), 7.55 (m, 3H), 7.27 (d, J = 8.0 Hz, 2H), 7.07 (m, 2H), 3.81 (s, 4H), 3.26 (t, 2H, J = 5.0 Hz), 2.68 (t, 2H, J = 5.0 Hz), 1.94 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.3, 159.6, 149.5, 136.9, 123.6, 122.6, 60.5, 53.0, 38.2, 23.8.

The above compound (1.0 g, 3.73 mmol) was dissolved in concentrated HCl, heated to reflux for 16 h. The mixture was evaporated to dryness. The residue was dissolved in 5 mL of water,

(24) Hancock, R. D.; Martell, A. E. *Chem. Rev.* **1989**, *89*, 1875.
(25) Hoq, M. F.; Shepherd, R. E. *Inorg. Chem.* **1984**, *23*, 1851.
(26) Anderegg, G.; Hubmann, E.; Podder, N. G.; Wenk, F. *Helv. Chim. Acta* **1977**, *60*, 123.
(27) (a) Anderegg, G.; Wenk, F. *Helv. Chim. Acta* **1967**, *50*, 2330. (b) Cox, D. D.; Benkovic, S. J.; Bloom, L. M.; Bradley, F. C.; Nelson, M. J.; Que, L., Jr.; Wallick, D. E. *J. Am. Chem. Soc.* **1988**, *110*, 2026. (c) Adams, H.; Bailey, N. A.; Fenton, D. E.; He, Q.-Y. *J. Chem. Soc., Dalton Trans.* **1997**, 1533. (d) Kanamori, K.; Kameda, E.; Kabetani, T.; Suemoto, T.; Okamoto, K.-I.; Kaizaki, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 2581. (e) Mandel, J. B.; Maricondi, C.; Douglas, B. E. *Inorg. Chem.* **1988**, *27*, 2990.

basified with concentrated NaOH solution to pH = 10, saturated with sodium chloride, and extracted six times with 5 mL of chloroform. The chloroform extract was dried over $MgSO_4$ and rotary evaporated to obtain a yellow oil (0.92 g, 100% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.45 (d, J = 4.8 Hz, 2H), 7.56 (td, J = 7.4, 1.5 Hz, 2H), 7.41 (d, J = 7.4 Hz, 2H), 7.06 (t, J = 7.0 Hz, 2H), 3.77 (s, 4H), 2.72 (t, J = 5.6 Hz, 2H), 2.59 (t, J = 5.6 Hz, 2H), 1.55 (s, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 160.2, 149.4, 136.7, 123.4, 122.4, 61.2, 57.9, 40.2. The analytical sample was obtained from recrystallization of hydrochloric acid salt, mp 117−119 °C. ^{1}H NMR (200 MHz, D_2O): δ 8.71 (d, J = 6 Hz, 2H), 8.52 (t, J = 8 Hz, 2H), 8.05 (d, J = 8 Hz, 2H), 7.95 (m, 2H), 4.28 (s, 4H), 3.28 (t, J = 6.6 Hz, 2H), 3.06 (t, J = 6.6 Hz, 2H). ^{13}C NMR (50 MHz, D_2O): δ 154.6, 150.3, 144.6, 130.3, 129.5, 58.3, 54.8, 39.5. MS (MALDI, *m*/*e*): 243.1 (M + 1). Anal. Calcd for $C_{14}H_{18}N_4$[3HCl][1H_2O]: C, 45.48; H, 6.27; N, 15.15. Found: C, 45.32; H, 6.35; N, 15.50.

***N*-(2-Pyridylmethyl)diethylenetriamine (PDT), 9.** A 3.73 g sample of 1,7-diphthaloyldiethylenetriamine[28] and 1.85 g of 2-picolyl chloride hydrochloride were dissolved in 40 mL of DMF in a 100 mL round-bottom flask. The mixture was heated at 100−110 °C overnight, then the solvent was removed. To the brown residue was added 20 mL of chloroform and 20 mL of water. The aqueous layer was extracted by 20 mL of chloroform, and the combined organic layers were dried over magnesium sulfate. The brown solid was obtained after the removal of solvent (93% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.49 (d, J = 4.4 Hz, 1H), 7.80−7.64 (m, 8H), 7.12−7.06 (m, 1H), 7.02−6.95 (m, 1H), 3.86 (s, 2H), 3.77 (t, J = 6.1 Hz, 4H), 2.85 (t, J = 6.1 Hz, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.5, 159.6, 149.1, 136.4, 134.1, 132.8, 128.6, 123.4, 122.3, 60.4, 52.5, 36.3.

The product above was added to 50 mL of concentrated HCl and heated to reflux overnight. On cooling, phthalic acid precipitated and was removed by filtration. The solvent was removed to obtain the amine-HCl salt. The analytical sample was obtained from recrystallization of the hydrochloric acid salt. ^{1}H NMR (200 MHz, D_2O): δ 8.73 (d, J = 5.5 Hz, 1H), 8.62−8.50 (m, 1H), 8.10−7.93 (m, 2H), 4.21 (s, 2H), 3.19 (t, J = 6.6 Hz, 4H), 2.94 (t, J = 6.6 Hz, 4H). ^{13}C NMR (50 MHz, D_2O): δ 160.1, 150.3, 144.5, 130.1, 129.3, 57.7, 53.6, 39.5. Anal. Calcd for $C_{10}H_{18}N_4$[3HCl][0.25H_2O]: C, 38.98; H, 7.03; N, 18.18. Found: C, 39.18; H, 7.11; N, 18.02. The free amine was obtained by washing with dilute aqueous NaOH solution. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.47 (d, J = 4.5 Hz, 1H), 7.58 (m, 1H), 7.35 (m, 1H), 7.09 (m, 1H), 3.71 (s, 2H), 2.71 (t, J = 6 Hz, 4H), 2.55 (t, J = 6 Hz, 4H). ^{13}C NMR (50 MHz, $CDCl_2$): δ 160.4, 149.5, 136.8, 123.4, 122.4, 61.5, 58.3, 40.4. MS (MALDI-TOF, *m*/*e*): 195.1 (M + H).

Tris(2-(1-methylimidazolyl)methyl)amine (T2IA), 10. 2-(Aminomethyl)-1-methylimidazole[29] dihydrochloride (800 mg, 4.35 mmol), 2-(chloromethyl)-1-methylimidazole[30] (1.45 g, 8.69 mmol), and diisopropylethylamine (3.37 g, 26.1 mmol) were dissolved in 40 mL of acetonitrile and stirred at room temperature for 5 days. The solvent was removed, and to the residue was added 10 mL of 6 M aqueous NaOH. The mixture was extracted with 20 mL of chloroform three times. The organic layer was dried over sodium sulfate, concentrated, and pump-dried to give a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.90 (s, 3H), 6.75 (s, 3H), 3.79 (s, 6H), 3.34 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 145.9, 127.8, 121.8, 49.3, 32.3. To the solid was added 3 mL of concentrated HCl, and the solvent was removed to give the amine hydrochloric acid salt, which was recrystallized from methanol/diethyl ether (40% yield), mp 262−4°C. ^{1}H NMR (200 MHz, D_2O): δ 7.43 (s, 6H), 4.27 (s, 6H), 3.79 (s, 9H). ^{13}C NMR (50 MHz, D_2O): δ 144.1, 127.8, 122.4, 51.0, 37.6 MS. (MALDI, *m*/*e*): 300.6 (M + 1). Anal. Calcd for $C_{15}H_{17}N_7$[3HCl]: C, 44.08; H, 5.92; N, 23.99. Found: C, 43.75; H, 6.08; N, 24.17.

***N*,*N*-Bis(2-(1-methylimidazolyl)methyl)glycine (B2IG), 11.** Glycine (305 mg, 4.06 mmol), 1-methyl-2-imidazolecarboxaldehyde (894 mg, 8.12 mmol), and NaOH (162 mg, 4.06 mmol) were dissolved in 80 mL of methanol and hydrogenated at atmospheric pressure over 5% palladium-charcoal overnight. Then the pH was adjusted to 1 by adding concentrated HCl. After evaporation of the filtered solution to dryness, the residue was recrystallized from ethanol to give 453 mg of yellow solid (33% yield), mp 222−4 °C. ^{1}H NMR (200 MHz, D_2O): δ 7.57 (s, 4H), 4.79 (s, 2H), 4.11 (s, 4H), 3.98 (s, 6H). ^{13}C NMR (50 MHz, D_2O): δ 171.3, 138.7, 128.5, 123.6, 50.9, 44.5, 38.3. MS (MALDI, *m*/*e*): 264.9 (M + 1). Anal. Calcd for $C_{12}H_{17}N_5O_2$[1.25H_2O]: C, 50.42; H, 6.88; N, 24.50. Found: C, 49.96; H, 6.97; N, 25.12.

***N*-(2-(1-Methylimidazolyl)methyl)iminodiacetic Acid (DA2Im), 12.** 2-(Aminomethyl)-1-methylimidazole dihydrochloride[29] (2.05 g, 11.14 mmol), chloroacetic acid (2.10 g, 22.28 mmol), and sodium hydroxide (2.67 g, 66.85 mmol) were dissolved in 60 mL of water and heated at 100−110 °C for 30 min. The solution was acidified with concentrated HCl to pH 1, and the solvent was removed. To the residue was added 100 mL of ethanol; the resulting NaCl precipitate was removed by filtration. Concentrated HCl (10 mL) was added to the ethanol solution, and the mixture was heated to reflux overnight. After removal of solvent, the residue was dissolved in 20 mL of water and basified to pH 12 by adding sodium carbonate and extracted with 50 mL of chloroform three times. The yellow oil was dissolved in 100 mL of ethyl acetate, decolorized with charcoal, and filtered through silica gel. Removal of solvent gave 1.68 g of a light yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.94 (s, 1H), 6.88 (s, 1H), 4.15 (q, 4H, J = 7.2 Hz), 4.05 (s, 2H), 3.84 (s, 3H), 3.49 (s, 4H), 1.25 (t, 6H, J = 7.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.2, 144.9, 127.5, 122.5, 61.1, 55.2, 50.9, 33.6, 14.8. MS (MALDI, *m*/*e*): 284.2 (M + 1).

The oil was dissolved in 20 mL of 2 N HCl and heated to reflux overnight. Removal of solvent gave 1.29 g of a white solid (38% yield), mp 174−5 °C. ^{1}H NMR (200 MHz, D_2O): δ 7.35 (s, 1H), 7.33 (s, 1H), 4.28 (s, 2H), 3.80 (s, 3H), 3.68 (s, 4H). ^{13}C NMR (50 MHz, D_2O): δ 171.3, 146.3, 127.6, 121.6, 59.0, 51.5, 38.0. MS (MALDI, *m*/*e*): 228.4 (M + 1). Anal. Calcd for $C_9H_{13}N_3O_4$[2HCl]: C, 36.02; H, 5.04; N, 14.00. Found: C, 35.74; H, 5.28; N, 13.68.

1-Methyl-4-imidazolecarboxaldehyde, 16. In a 250 mL round-bottomed flask was placed 2.08 g (52.1 mmol) of sodium hydride (60% oil dispersion), which was washed with hexanes (3 × 20 mL) and dried in vacuo. To the flask was added 70 mL of dry DMF, which was degassed by three cycles of evacuation and refilling with nitrogen. After the mixture was cooled to 0 °C, 5 g (52.1 mmol) of 4(5)-imidazolecarboxaldehyde in 25 mL of DMF was added. Hydrogen gas was evolved vigorously. After the reaction was completed, 8.3 g (58.5 mmol) of iodomethane was added at 0 °C. The mixture was stirred at room temperature for 3 h and was monitored by TLC. After the reaction was completed, the mixture was concentrated under reduced pressure. The residue was dissolved in 100 mL of chloroform and washed with 50 mL of water. The aqueous layer was extracted with chloroform (4 × 50 mL). The

(28) Buckingham, D. A.; Edwards, J. D.; McLaughlin, G. M. *Inorg. Chem.* **1994**, *21*, 2770.
(29) Oberhausen, K. J.; Richardson, J. F.; Buchanan, R. M.; Pierce, W. *Polyhedron* **1989**, *8*, 659.
(30) (a) Wei, N.; Murthy, N. N.; Tyeklar, Z.; Karlin, K. D. *Inorg. Chem.* **1994**, *33*, 1177. (b) Turner, R. A.; Huebner, C. F.; Scholz, C. R *J. Am. Chem. Soc.* **1949**, *71*, 2801.

combined chloroform layers were concentrated to dryness. The residue was purified by silica gel column chromatography, giving 1.82 g (35% yield) of 1-methyl-4-imidazolecarboxaldehyde, mp 62−3 °C, R_f 0.15 (silica, chloroform/methanol, 10:1, v/v). ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.83 (s, 1H), 7.58 (s, 1H), 7.52 (s, 1H), 3.76 (s, 3H). ^{1}H NMR (200 MHz, D_2O): δ 9.60 (s, 1H), 7.97 (s, 1H), 7.77 (s, 1H), 3.77 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 186.3, 143.0, 139.8, 125.9, 34.5. The isomeric byproduct 1-methyl-5-imidazolecarboxaldehyde was separated by column (R_f 0.30, silica, chloroform/methanol, 10:1, v/v). ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.77 (s, 1H), 7.79 (s, 1H), 7.64 (s, 1H), 3.95 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 179.7, 144.5, 143.6, 132.1, 34.6.

Tris(4-(1-methylimidazolyl)methyl)amine (T4IA), 13. Ammonium chloride (320 mg, 6.0 mmol) and 1-methyl-4-imidazolecarboxaldehyde (**16**) (660 mg, 6.0 mmol) were dissolved in 40 mL of methanol and hydrogenated at atmospheric pressure over 5% palladium-charcoal overnight. After evaporation of the filtered solution to dryness, the residue was added to 5 mL of concentrated hydrochloric acid and the salt was recrystallized from ethanol to give 298 mg of white solid (33% yield), mp 256−7 °C. ^{1}H NMR (200 MHz, D_2O): δ 8.66 (s, 3H), 7.42 (s, 3H), 3.88 (s, 9H), 3.84 (s, 6H). ^{13}C NMR (50 MHz, D_2O): δ 138.5, 132.8, 124.8, 50.0, 38.8. MS (MALDI, *m*/*e*): 300.7 (M + 1). Anal. Calcd for $C_{15}H_{17}N_7$·[3HCl]: C, 44.08; H, 5.92; N, 23.99. Found: C, 43.75; H, 6.08; N, 24.17.

***N*,*N*-Bis(4-(1-methylimidazolyl)methyl)glycine (B4IG), 14.** Glycine (118 mg, 1.58 mmol), 1-methyl-4-imidazolecarboxaldehyde (347 mg, 3.15 mmol), and sodium hydroxide (63 mg, 1.58 mmol) were dissolved in 40 mL of methanol and hydrogenated at atmospheric pressure over 5% palladium-charcoal overnight. After evaporation of the filtered solution to dryness the residue was recrystallized from ethanol to give 347 mg of yellow solid (66% yield), mp 225−7 °C. ^{1}H NMR (200 MHz, D_2O): δ 7.70 (s, 2H), 7.33 (s, 2H), 4.32 (s, 4H), 3.76 (s, 6H), 3.64 (s, 2H). ^{13}C NMR (50 MHz, D_2O): δ 173.7, 142.4, 132.3, 126.6, 57.0, 53.7, 36.5. MS (MALDI, *m*/*e*): 264.4 (M + 1). Anal. Calcd for $C_{12}H_{17}N_5O_2$: C, 54.74; H, 6.51; N, 26.60. Found: C, 54.42; H, 6.47; N, 26.84.

4-(Hydroxymethyl)-1-methylimidazole. 1-Methyl-4-imidazolecarboxaldehyde (1.36 g) was dissolved in 20 mL of methanol. Sodium borohydride (234 mg) was added and stirred at room temperature for 20 min, and then 5 mL of water was added. After removal of solvent, the residue was dissolved in 50 mL of chloroform, then filtered through 1 g of silica gel. A yellow solid (1.8 g) was obtained after evaporation of solvent (77% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36 (s, 1H), 6.83 (s, 1H), 4.60 (s, 2H), 3.69 (s, 3H). ^{13}C NMR (75 MHz, D_2O): δ 138.6, 131.8, 127.7, 53.0, 31.7.

4-(Chloromethyl)-1-methylimidazole, HCl Salt. At 0 °C, 1.08 g of 4-(hydroxymethyl)-1-methylimidazole dissolved in 20 mL of chloroform was added dropwise to a solution mixture of 3 mL of thionyl chloride and 5 mL of chloroform. After heating to reflux for 1 h, the solution was evaporated to dryness. The resulting solid was suspended in diethyl ether and stirred overnight. The ether was decanted, and the product was dried under vacuum to give 1.56 g of yellow solid (97% yield), 127−30 °C. ^{1}H NMR (300 MHz, CD_3CN): δ 8.58 (s, 1H), 7.39 (s, 1H), 4.61 (s, 2H), 3.82 (s, 3H). MS (MALDI, *m*/*e*): 131.8 (M + 1). ^{1}H NMR (300 MHz, D_2O): δ 8.63 (s, 1H), 7.45 (s, 1H), 4.70 (s, 2H), 3.84 (s, 3H), the compound in D_2O was quickly hydrolyzed to alcohol, δ 8.58 (s, 1H), 7.39 (s, 1H), 4.81 (s, 2H), 3.82 (s, 3H).

***N*-(4-(1-Methylimidazolyl)methyl)iminodiacetic Acid (DA4Im), 15.** 4-(Chloromethyl)-1-methylimidazole HCl salt (1.55 g, 9.32 mmol), iminodiacetic acid diethyl ester[31] (1.76 g, 9.32 mmol), and diisopropylethylamine (2.41 g, 18.63 mmol) were dissolved in 40 mL of acetonitrile and stirred at room temperature for 2 days. After the removal of solvent, the residue was dissolved in 20 mL of water, basified by adding NaOH to pH 12, then extracted with chloroform (20 mL) 10 times. The organic layer was dried over sodium sulfate, evaporated to give a yellow oil, and purified by silica column chromatography (methylene chloride/methanol, 9:1) to give 1.53 g of the desired product (58% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (s, 1H), 6.83 (s, 1H), 4.12 (q, 4H, J = 7.2 Hz), 3.84 (s, 2H), 3.61 (s, 3H), 3.55 (s, 4H), 1.22 (t, 6H, J = 7.2 Hz). MS (MALDI, *m*/*e*): 284.5 (M + 1).

The diethyl ester (1.29 g) was dissolved in 40 mL of 2 N HCl and heated to reflux overnight, then the solution was decolorized with charcoal, filtered, and dried to give 1.30 g of a light yellow solid (95% yield), mp 166−7 °C. ^{1}H NMR (200 MHz, D_2O): δ 8.76 (s, 1H), 7.74 (s, 1H), 4.67 (s, 2H), 4.16 (s, 4H), 8.83 (s, 3H). ^{13}C NMR (50 MHz, D_2O): δ 170.8, 140.4, 129.5, 124.4, 57.3, 51.5, 39.2. MS (MALDI, *m*/*e*): 228.0 (M + 1). Anal. Calcd for $C_9H_{13}N_3O_4$[2HCl][1.5H_2O]: C, 33.04; H, 5.55; N, 12.84. Found: C, 33.42; H, 5.46; N, 12.87.

Potentiometric Titrations. Potentiometric studies were conducted with a Titrino 702 autotitrator (Brinkmann Instruments). A Metrohm combined pH glass electrode (Ag/AgCl) with 3 M NaCl internal filling solution was used. All potentiometric titrations were carried out at a concentration of 4 mM, with I = 0.10 ($NaClO_4$), at 25 °C. The Zn(II) solution was standardized with primary standard EDTA in a NaOAc/HOAc buffer with 1-(2-pyridylazo)-2-naphthol as indicator. The NaOH solution was standardized against potassium hydrogen phthalate with phenolphthalein as an indicator. All solutions were carefully protected from air by a stream of nitrogen gas. The k_w value was chosen as 13.78 for 25 °C, 0.1 M $NaClO_4$. A Gran's plot using the NaOH solution found the carbonate content below the acceptable limit of 2%.[32] Ligands were isolated or purchased in neutral or protonated forms. Ligand concentrations were determined gravimetrically using formulas determined by elemental analysis. A proper amount of acid was added to ligands in the absence of zinc to determine ligand protonation constants. The ligand−zinc binding constants and zinc-bound water acidity were determined in the presence of equivalent Zn(II) ion from $Zn(ClO_4)_2$. About 100 data points were collected for each titration. The equilibrium constants were calculated using the program BEST.[29] All σ-fit values (as defined in the program) were smaller than 0.015. Species distributions were calculated using the program SPE.[29] All constants were determined using at least two independent titrations.

Conductivity Measurement. Electric conductivities were determined using a YSI Model 35 conductance meter (Yellow Springs Instrument Co., Inc., Ohio) fitted with a platinized-iridium conductivity cell (YSI 3403, cell constant K = 1.0/cm). Complex samples were dissolved in DI water at 25 °C at an initial concentration between 1×10^{-3} and 2×10^{-3} M. After measurements, the solution was diluted with DI water to 60−70% of the initial concentration. This procedure was repeated until the measurement had been done at five or more different concentrations (between 2×10^{-3} and 1×10^{-4} M). Molar conductivities λ_c were plotted versus the square root of the concentration ($c^{0.5}$), displaying a linear dependence. A standard least-squares-fit routine was used

(31) Park, H. S.; Lin, Q.; Hamilton, A. D. *J. Am. Chem. Soc.* **1999**, *121*, 8.
(32) Martell, A. E.; Motekaitis, R. J. *Determination and Use of Stability Constants*, 2nd ed.; VCH Publishers Inc.: New York, 1992.

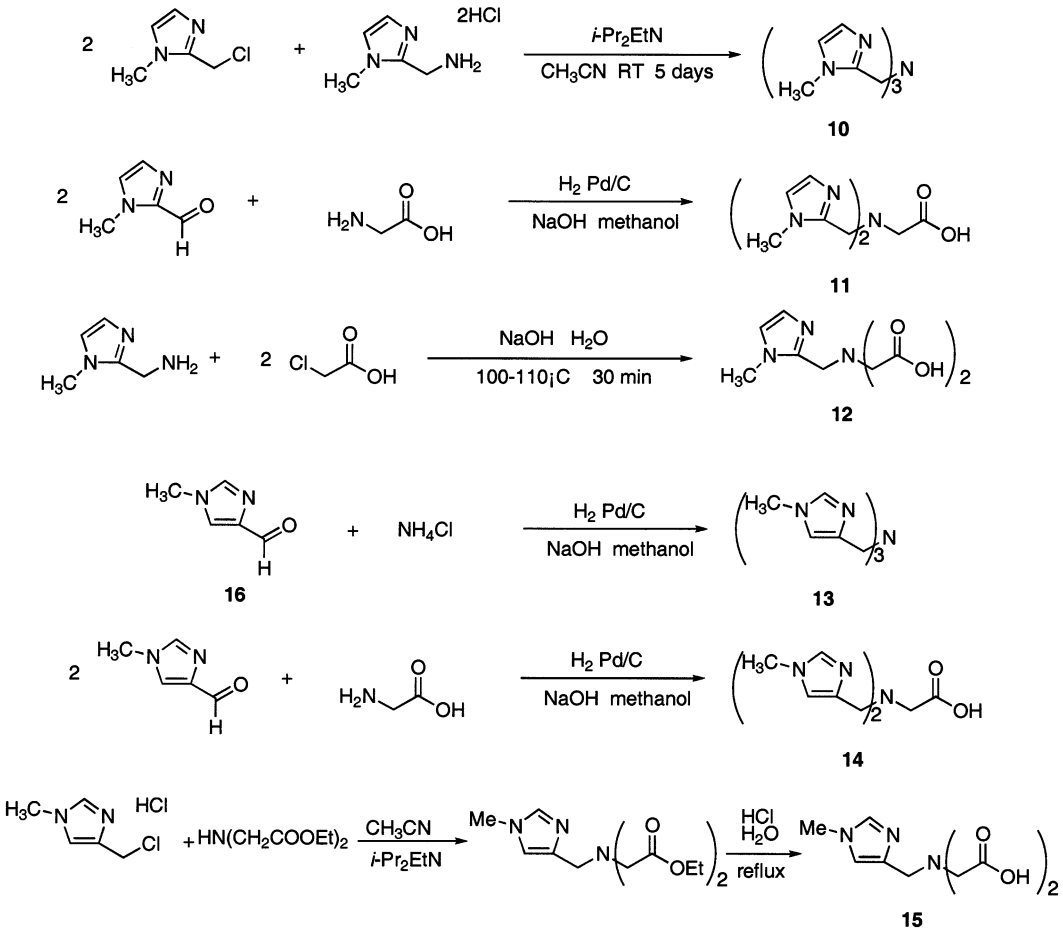

Figure 4. Syntheses of imidazole ligands.

to perform a linear extrapolation to $c^{0.5} = 0$, and the conductivities at infinite dilution Λ_0 were obtained.

Results and Discussion

Ligand Syntheses. All of the pyridyl- and amino-substituted ligands were previously known. The syntheses were followed according to or modified from literature procedures; however, high purity that might not be previously reported was necessary for potentiometric titrations. The tertiary amine tris(2-(1-methylimidazolyl)methyl)amine (**10**) was reported by Buchanan's group,[33] synthesized from the primary amine[29] and 2-(chloromethyl)-1-methylimidazole.[30] The ligand **11** was synthesized from 1 equiv of glycine and 2 equiv of aldehyde under reductive amination conditions. The ligand **12** was prepared from primary amine and 2 equiv of chloroacetic acid. For purification purposes, it was converted to the diethyl ester, then hydrolyzed in aqueous hydrochloric acid solution.

1-Methyl-4-imidazolecarboxaldehyde (**16**), the building block for 1-methyl-4-substituted imidazolyl ligands, was synthesized by methylation of 4(5)-imidazolecarboxaldehyde, and 1-methyl-5-imidazolecarboxaldehyde was also isolated as byproduct.[34] The ligands **13** and **14** were synthesized from the aldehyde **16** with ammonium chloride and glycine, respectively, by reductive amination. Reduction of aldehyde **16** followed by reaction with thionyl chloride gave 4-chloromethyl-1-methylimidazole. Reaction of the chloride with diethyl iminodiacetate followed by hydrolysis of the ester gave ligand **15**.

Potentiometric Titrations. The ligand protonation constants (K_1, K_2, and K_3) were determined from potentiometric titration of ligand·$3H^+$ (4 mM), using 0.1 M NaOH with $I = 0.1$ ($NaClO_4$) at 25 °C. A typical pH titration curve is shown in Figure 5. The 1:1 zinc complexation equilibria, including both ligand−zinc binding constants and zinc-bound water deprotonation constants, were determined from potentiometric titration of ligand·$3H^+$ (4 mM) in the presence of an equimolar amount of the Zn(II) ion under the same conditions as the titrations of ligands. A typical species distribution graph is shown in Figure 6. In addition to protonated ligand species, all ligand/zinc titration models included ZnL and ZnL(OH). For some ligands such as BPEN, ZnLH exists as a minor species in the acidic region as Figure 6 suggests. Some imidazolyl ligands contain $LM(OH)_2$ and $L_2M_2(OH)$ at high pH, indicating that under these conditions a small degree of aggregation may occur. No

(33) Oberhausen, K. J.; O'Brien, R. J.; Richardson, J. F.; Buchanan, R. M. *Inorg. Chim. Acta* **1990**, 145.

(34) Kodera, M.; Terasako, N.; Kita, T.; Tachi, Y.; Kano, K.; Yamazaki, M.; Koikawa, M.; Tokii, T. *Inorg. Chem.* **1997**, *36*, 1851.

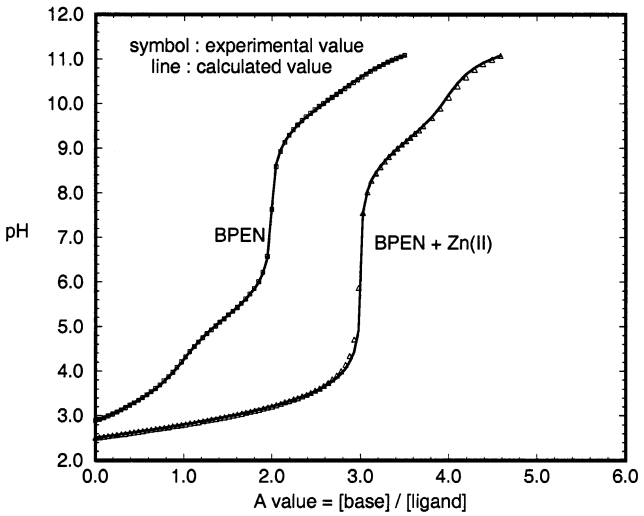

Figure 5. Titration curves of 4 mM BPEN and 4 mM BPEN and 4 mM zinc(II).

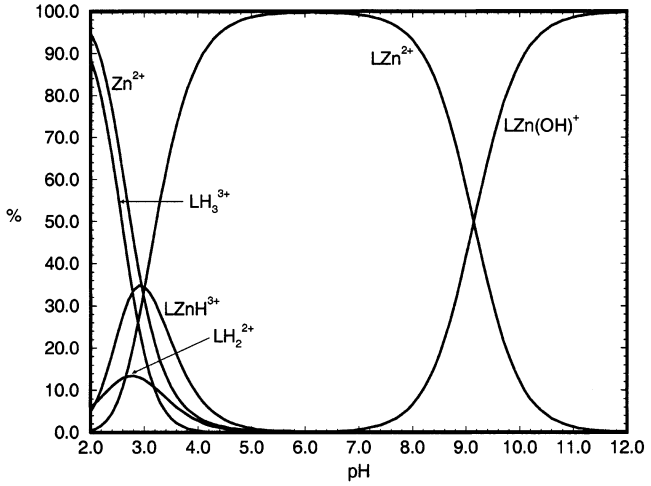

Figure 6. Species distribution curves of BPEN and zinc(II) titration.

precipitation was observed in any titration experiment described here.

The data for ligand protonation constants (K_n), ligand−metal association constants (K_{ZnL}), and pK_a of Zn-bound water, as defined in the following equations, are listed in Table 1.

$$L^{n-} + H^+ \rightleftharpoons LH^{1-n} \qquad K_1 = [HL^{1-n}]/[L^{n-}][H^+]$$

$$LH^{1-n} + H^+ \rightleftharpoons LH_2{}^{2-n} \qquad K_2 = [H_2L^{2-n}]/[LH^{1-n}][H^+]$$

$$LH_2{}^{2-n} + H^+ \rightleftharpoons LH_3{}^{3-n} \qquad K_3 = [H_3L^{3-n}]/[LH_2{}^{2-n}][H^+]$$

$$Zn^{2+} + L^{n-} \rightleftharpoons ZnL^{2-n} \qquad K_{ZnL} = [ZnL^{2-n}]/[Zn^{2+}][L^{n-}]$$

$$ZnL(OH_2)^{2-n} \rightleftharpoons ZnL(OH)^{1-n} + H^+ \qquad K_a = [ZnL(OH)^{2-n}][H^+]/[ZnL(OH_2)^{1-n}]$$

Errors represent the variance obtained from at least two independent determinations of each value. Numbers reported in parentheses are taken from the literature. Good agreement with literature values was obtained. The ligand protonation data are consistent within a series of ligands and correlate with structural features. One interesting observation is that the pK_1 for T2IA is one pK unit lower than the corresponding value for T4IA. This protonation would be expected to involve five-membered ring chelation by one imidazole ring and the tertiary nitrogen atom. The tertiary nitrogen atom in T2IA is more electron-deficient than in T4IA as a result of the greater inductive effect exerted by imidazole substituted in the 2-position.

Ligand−Zinc Binding in Aqueous Solution. Ligand−zinc binding constants listed in Table 1 follow trends expected on the basis of ligand basicity. For the ligands studied, substituent basicity follows the trend amine > imidazole > pyridine ≈ carboxylate. Thus, TREN demonstrates the strongest binding to zinc. Substituting the amino group arms of TREN for carboxylate or pyridyl groups decreases the binding. DTMA and PDT (two amino groups) have stronger binding than BPEN and ENDA (one amino group). NTA, PDA, BPG, and TPA contain no amino groups and show weakest zinc binding. Imidazole is more basic than pyridine but less basic than aliphatic amine; therefore ligands **10** and **13** have larger binding constants than TPA, but lower than TREN.

It is important to be aware that the stability constants do not reflect the metal affinity of ligands at a specific pH value. The direct comparison of these constants can be misleading. For a given complex equilibrium system, the concentration of unbound metal cation represents a direct gauge of the ligand−metal affinity with consideration of all involved equilibria. Thus, the comparison of the free metal concentration, typically referred to as pM = −log[M], allows a direct comparison of various ligands.[43] The pM depends on total ligand and metal concentration, pH, temperature, and ionic strength of the solution. Figure 7 shows calculated pZn values for solutions containing 4 mM zinc(II) and 4 mM selected ligand at various pH values under our experimental conditions. Although TREN has the largest zinc binding constant, the zinc affinity of TREN does not exceed others until pH > 9, as a result of the great proton affinity of TREN.

Thermodynamic profiles have been reported for some of the present metal complexation equilibria; examination of the NIST database[44] reveals the following thermodynamic parameters (ligand, $\Delta H°$ in kJ/mol, $\Delta S°$ in J/mol): TREN, −58.1, 82.4; NTA, −3, 191; PDA, −13, 158; TPA, −41, 70.7. Carboxylate ions immobilize water molecules; therefore on complex formation, solvent molecules are released endothermically, thus giving a larger ΔS value and more

(35) Anderegg, G. *J. Coord. Chem.* **1981**, *11*, 171.
(36) Schwazenbach, G.; Freitag, E. *Helv. Chim. Acta* **1951**, *34*, 1492.
(37) Collange, E.; Thomas, G. *Anal. Chim. Acta* **1973**, *65*, 87.
(38) Jellish, R. M. *J. Coord. Chem.* **1975**, *4*, 199.
(39) Coates, J. H.; Gentle, G. J.; Lincoln, S. F. *Nature* **1974**, *249*, 773.
(40) Motekaitis, R. J.; Martell, A. E.; Lehn, J.-M.; Watanabe, E.-I. *Inorg. Chem.* **1982**, *21*, 4253.
(41) McLendon, G.; MacMillan, D. T.; Hariharan, M.; Martell, A. E. *Inorg. Chem.* **1975**, *14*, 2322.
(42) Schwazenbach, G.; Anderegg, G.; Schneider, W.; Senn, H. *Helv. Chim. Acta* **1955**, *38*, 1147.
(43) (a) Fahrni, C. J.; O'Halloran, T. V. *J. Am. Chem. Soc.* **1999**, *121*, 11448. (b) Martell, A. E.; Hancock, R. D. *Metal Complexes in Aqueous Solutions;* Plenum Press: New York, 1996; p 150.

Table 1. Thermodynamic Data for Ligands and Zinc−Ligand Complexes

ligand	log K_{ZnL}	pK_a of L-Zn-OH_2	log K_3	log K_2	log K_1
1. TPA	(11.00 ± 0.08)[a]	(8.03 ± 0.03)[a]	(2.55 ± 0.03)[a]	(4.35 ± 0.03)[a]	(6.17 ± 0.02)[a]
2. BPG	11.4 ± 0.1	9.11 ± 0.03	2.81 ± 0.01	4.05 ± 0.01	6.96 ± 0.01
3. PDA	10.89 ± 0.07	9.62 ± 0.08	2.51 ± 0.02	2.78 ± 0.03	8.28 ± 0.02
	(10.65)[b]		(2.70)[b]	(2.70)[b]	(8.22)[b]
4. NTA	10.53 ± 0.04	10.06 ± 0.07	1.87 ± 0.05	2.53 ± 0.06	9.40 ± 0.05
	(10.45 ± 0.05)[c]		(1.97)[d]	(2.43)[d]	(9.75)[d]
5. TREN	14.42 ± 0.05	10.21 ± 0.05	8.42 ± 0.04	9.42 ± 0.07	10.13 ± 0.08
	(14.40 ± 0.01)[e]	(10.3)[f]	(8.43)[g]	(9.47)[g]	(10.17)[g]
6. DTMA[c]	(13.13 ± 0.01)[h]	(9.89 ± 0.01)[h]	(3.24)[h]	(9.59)[h]	(10.81)[h]
7. ENDA[d]	(11.93)[i]	(10.13)[i]		(5.65)[i]	(11.13)[i]
8. BPEN	12.48 ± 0.02	9.14 ± 0.01	3.17 ± 0.01	5.22 ± 0.02	9.93 ± 0.01
9. PDT	13.43 ± 0.01	9.63 ± 0.02	4.00 ± 0.01	9.27 ± 0.01	10.04 ± 0.03
10. T2IA	11.98 ± 0.01	8.72 ± 0.05	4.57 ± 0.01	6.00 ± 0.01	7.30 ± 0.01
11. B2IG	11.18 ± 0.01	8.99 ± 0.01	3.01 ± 0.01	6.26 ± 0.01	7.96 ± 0.01
12. DA2Im	10.55 ± 0.01	8.86 ± 0.03	2.72 ± 0.04	4.20 ± 0.03	8.09 ± 0.04
13. T4IA	12.47 ± 0.01	9.11 ± 0.03	5.26 ± 0.01	5.79 ± 0.01	8.36 ± 0.02
14. B4IG	11.30 ± 0.01	9.24 ± 0.02	3.16 ± 0.01	5.23 ± 0.02	8.75 ± 0.01
15. DA4Im	10.15 ± 0.03	8.49 ± 0.02		4.04 ± 0.04	8.95 ± 0.05

[a] Ref 27. [b] Ref 35. [c] Ref 36. [d] Ref 37. [e] Ref 38. [f] Ref 39. [g] Ref 40. [h] Ref 41. [i] Ref 42.

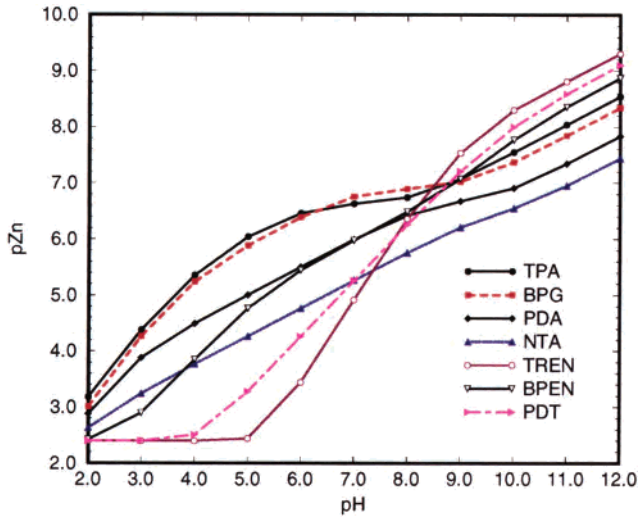

Figure 7. pM of selected ligands vs pH.

positive Δ*H* value compared with ligands containing neutral donor atoms.[35,44] There are no significant enthalpy/entropy differences between amino- and pyridyl-containing ligands. For example, TREN (3 amino groups) shows both more negative enthalpy and positive entropy driven zinc binding than TPA (3 pyridyl groups). It has been noted that pyridyl groups are unable to disperse the charge from zinc ions to solvent by hydrogen bonding,[24] resulting in a less negative enthalpy value.

Zinc-Bound Water Acidity in Aqueous Solution. Logically, Zn-OH_2 acidity should vary with the Lewis acidity of the Zn(II) ion, which in turn may be influenced by the nature and number of coordinating ligands. Some data are available for Zn(II)-polyamine complexes that indicate that in a ternary $LZnOH_2$ complex the stronger the binding of L to Zn, the less acidic the coordinated water molecule.[45] Our results further substantiate this hypothesis. Plots of pK_a of zinc-bound water against log K_{ZnL} are shown in Figures 8 and 9. Neutral ligands exhibit a linear relation with a positive slope. However, ligands containing carboxylates do not show this correlation. Indeed, Figure 8 shows that within the series NTA−ENDA−DTMA−TREN, there is little or no variation in $LZnOH_2$ acidity, although the log K_{ZnL} varies over 4 orders of magnitude. Also strikingly, the series NTA−PDA−BPG−

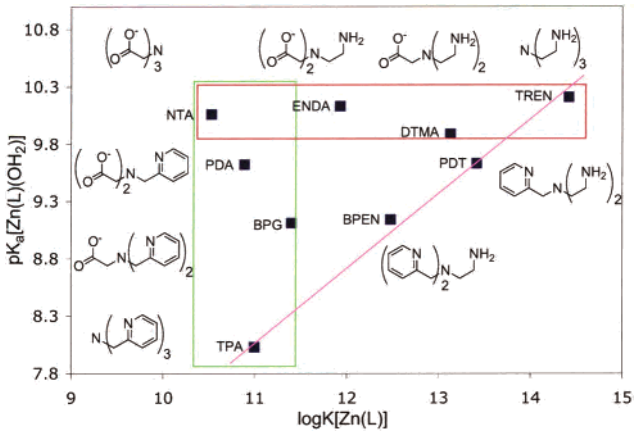

Figure 8. Relation between pK_a of zinc-bound water and ligand−zinc binding.

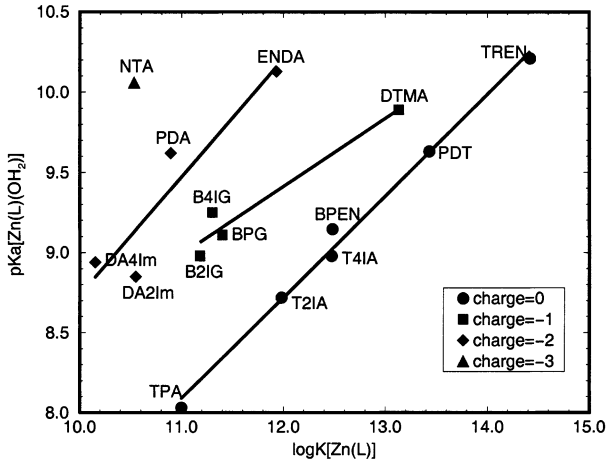

Figure 9. Relation between pK_a of zinc-bound water and ligand−zinc stability constants.

(44) Martell, A. E.; Smith, R. J.; Motekaitis, R. J. Critically Selected Stability Constants of Metal Complexes Database, Version2.0, Sep 1992.
(45) Itoh, T.; Fujii, Y.; Tada, T.; Yoshikawa, Y.; Hisada, H. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1265.

Table 2. Calculated (PM3) Heats of Formation and Enthalpy Changes (kcal/mol)

ligand	heat of formation of L (H_1)	heat of formation of L-Zn-OH_2 (H_2)	heat of formation of L-Zn-OH (H_3)	H_2-H_1	H_3-H_2
1. TPA	94.7	385.0	193.5	290.3	−191.5
2. BPG	−56.6	90.1	−26.6	146.7	−116.7
3. PDA	−148.3	−145.2	−185.9	3.1	−40.7
4. NTA	−167.0	−318.4	−283.0	−151.4	35.4
5. TREN	−6.8	280.4	77.3	287.2	−203.1
6. DTMA	−122.5	13.4	−108.3	136.0	−121.8
7. ENDA	−174.2	−187.0	−226.2	−12.8	−39.2
8. BPEN	63.2	348.1	154.4	285.0	−193.7
9. PDT	25.4	314.6	115.5	289.2	−199.1

TPA in which carboxylates and pyridine moieties are exchanged shows significant variation in $LZnOH_2$ acidity but almost no change in log K_{ZnL}.

As shown in Figure 8, TPA has the most acidic zinc-bound water of the compounds studied. Replacing one of pyridyl groups in TPA by either a carboxylate (BPG) or amino group (BPEN) results in an increase in pK_a. PDA and PDT (1 pyridyl group) have even higher pK_a's. Ligands with three carboxylate or amino groups (NTA, ENDA, DTMA, and TREN) have the largest pK_a values; surprisingly, the four ligands bear different charges and different zinc binding constants.

pK_a and K_{ZnL} data are plotted again in Figure 9, together with data for the imidazole compounds **10**−**15**. Imidazole is intermediate in donor ability between amine and pyridine; consequently imidazolyl ligands **10** and **13** are more basic than TPA, and the zinc-bound water is less acidic. However, when one pendent group was replaced by carboxylate (ligand **2**, **11**, **14**), similar zinc binding and acidity was observed. Overall, substitution of carboxylate for either 2-imidazolyl or 4-imidazolyl does not greatly impact either log K_{ZnL} or the pK_a of $LZnOH_2$. Indeed, these data do not support the notion that pK_a differences observed in enzymes with similar variation in coordination sphere are fully attributable to Zn coordination chemistry changes.[1d] Rather, the differences in enzymatic pK_a are amplified or controlled by noncovalent interactions and the electrostatic environment in the enzyme active site.[46] Our experiments were conducted in aqueous solution, whereas the polarity of the active sites of enzymes may be considerably lower, increasing the potential for contribution by electrostatic effects.[47]

In Figure 9, it is noted that for ligands with the same number of carboxylates (i.e., same charge), the $LZnOH_2$ pK_a follows a linear relationship with log K_{ZnL}. Various attempts failed to reconcile all of the data onto the same line. For example, plotting the known values of $\Delta H°$(ZnL) versus pK_a-($LZnOH_2$) does not afford a linear relationship. The data therefore suggest that ligand charge does influence ligand acidity, but other factors such as metal−ligand binding play at least as great a role. The vertical and horizontal trends indicated in Figure 8 appear to result from a fortuitous combination of contribution by ligand charge and inherent Zn/ligand binding energy, both of which have pronounced influence.

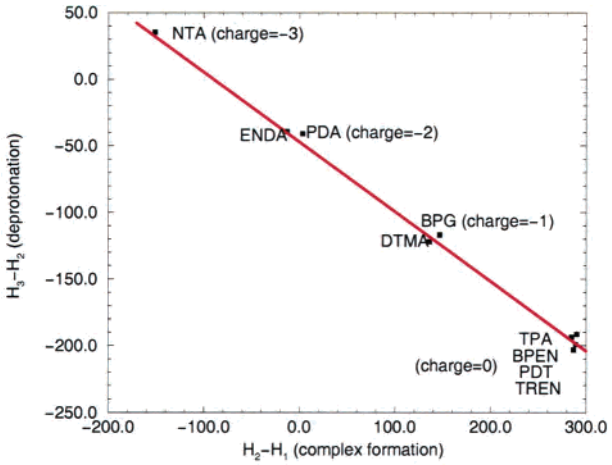

Figure 10. Relation between zinc−ligand binding energy and of deprotonation energy in the gas phase. Axis labels as defined in Table 2.

The gas-phase enthalpy of acid dissociation was calculated using the semiempirical method PM3 as implemented on SPARTAN.[48] The methodology employed follows that used in other studies.[49] The results are shown in Table 2. The zinc-bound water acidity against the zinc−ligand binding, plotted as in Figure 10, shows that in the calculation charge dominates both the metal/ligand binding enthalpy as well as acidity effects. In other words, the calculated data do not reflect differences within groups of ligands of similar charge, but are in agreement with expected differences based on ligand charge.

Species in Solutions. Among the tetradentate ligands studied, TPA has the most acidic zinc-bound water. However, the solid-state structure of TPA-zinc-hydroxide is a bis-μ-hydroxo dizinc(II) complex, which readily absorbs carbon dioxide from the air, forming a triply bridging carbonate trizinc(II) product.[27e,50] We decided to examine whether these structures persist in aqueous solution. Conductivity of three TPA zinc complexes together with KCl and $Mg(ClO_4)_2$ was measured. The electrical conductivity of $(TPAZnCl)ClO_4$ (225.6 $cm^2/\Omega\cdot mol$) is similar to the 1:2 electrolyte $Mg(ClO_4)_2$ (239.3 $cm^2/\Omega\cdot mol$), indicating that the chloride does not coordinate to zinc in aqueous solution. Therefore, the species

(46) (a) Canary, J. W.; Xu, J.; Castagnetto, J. M.; Rentzeperis, D.; Marky, L. A. *J. Am. Chem. Soc.* **1995**, *117*, 11545. (b) Bertini, I.; Luchinat, C.; Mangani, S.; Pierattelli, R. *Comments Inorg. Chem.* **1995**, *17*, 1−15.
(47) Sakurai, M.; Furuki, T.; Inoue, Y. *J. Phys. Chem.* **1995**, *99*, 17789.
(48) Spartan 5.0 Wavefunction, Inc. Irvine, CA, 19XX.
(49) Dewar, M. J. S.; Dieter, K. M. *J. Am. Chem. Soc.* **1986**, *108*, 8075.
(50) Karlin, K. D.; Murthy, N. N. *J. Chem. Soc., Chem. Commun.* **1993**, 1236.

in aqueous solution should be $(TPA\text{-}Zn\text{-}OH_2)^+$, Cl^-, and ClO_4^-. The large Λ_0 value of $[(TPAZn)_3CO_3](ClO_4)_4$ (555.8 $cm^2/\Omega\cdot mol$) indicates that the compound is a 1:4 electrolyte.

If the TPA-zinc-hydroxide complex remains a dimer in aqueous solution, it should behave as a 1:2 electrolyte; if it hydrolyzes in solution, then 2 equiv of $(TPA\text{-}Zn\text{-}OH)(ClO_4)$ 1:1 electrolyte will be formed. The observed Λ_0 value (291.3 $cm^2/\Omega\cdot mol$) is closer to twice that of the 1:1 electrolyte KCl (149.9 $cm^2/\Omega\cdot mol$); therefore, we conclude that the monomeric species $[TPA\text{-}Zn\text{-}OH]^+$ dominates in aqueous solution. This conclusion is in agreement with ^1H NMR data that show a singlet for the methylenes of the ligand, whereas two chemically unique methylenes are observed in the crystallographic structure where the zinc ions are octahedral.

Conclusion

The ligands studied here demonstrate a range of binding constants with Zn(II) ion of over 3 orders of magnitude, with differences in binding correlating with differences in basicity of ligand donor atom. All of the complexes give an additional acid titration corresponding to deprotonation of $LZnOH_2$. The acidity of the $LZn\text{-}OH_2$ group is influenced significantly by both the charge of the ligand and the binding energy of the tripod, with the latter playing a greater role in the present system. The implication for metalloenzymes is that the inner coordination sphere may play a role in $LZn\text{-}OH_2$ acidity but that this must be accentuated by the environment of the active site. Furthermore, the inherent binding of the metal ion in a protein may be one factor that the protein may utilize to control the Lewis acidity of the metal; less tightly protein-bound metals should exert greater acidity.

Acknowledgment. We thank the National Institutes of Health (GM 49170) for support of this research. We are grateful for early contributions to the project by Gregory Gabriel. Some mass spectra were obtained at the Michigan State University Mass Spectrometry Facility (supported in part by grant DRR-00480, from the Biotechnology Research Technology Program, NCRS, NIH).

IC025855M