Zn^{2+} Inclusion Complexes of Endodentate Tripodands as Carbonic Anhydrase-Inspired Artificial Esterases

Rick Jairam and Pierre G. Potvin*

Department of Chemistry, York University, 4700 Keele Street, North York, Ontario, Canada M3J 1P3

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Facile syntheses are reported for three endo-tridentate tris-imidazole podands **1-3**, two of which also bear basic amino residues, designed to reproduce the active site of carbonic anhydrase. The architecture was tested for the ability to bind Zn^{2+} in a "biomimetic" fashion and for the esterase activity of the Zn^{2+} complexes. Complexation of Zn^{2+} and Co^{2+} was studied by pH-titrimetry and NMR and UV-vis spectrometry and was found to be moderate. Besides species of LM^{2+} and $^{+}HLM^{2+}$ stoichiometries, the formation of $LZnOH^{+}$ species was facilitated by inclusion, with associated pK_a values ranging from 7.6 to 8.1. Cooperativity was found in the protonation of $3ZnOH^{+}$, however, wherein both the amino vertex and the hydroxozinc moieties are protonated in a single stage with an overall pK_a 7.95 and no $3Zn^{2+}$ (or $^{+}H3ZnOH^{+}$) was detected. There was some evidence for the formation of $2Zn^{2+}$ as its tautomer, $^{+}H2ZnOH^{+}$. The catalysis of hydrolysis of *p*-nitrophenyl acetate was studied in buffered H_2O between pH 7.35 and 8.15. Even though complexation was incomplete and gave rise to two or three complexes in each case, the residual first-order catalytic rate constants owing to these complexes were obtained after discounting the catalyses owing to other species present in solution. The catalyses were attributed to $1Zn^{2+}$, $2Zn^{2+}$, and $3ZnOH^{+}$ (but not $1ZnOH^{+}$ or $2ZnOH^{+}$) with second-order rate constants 0.079, 0.098, and 0.139 M^{-1} s^{-1}, respectively, significantly higher than with several previous artificial esterases.

Introduction

Carbonic anhydrase (CA, EC 4.2.1.1) is one of several metal-containing hydrolytic enzymes and has been much studied.[1] It very efficiently catalyzes the deceptively simple interconversion between CO_2 and HCO_3^-. Its active site features a Zn^{2+} ion held in a distorted tetrahedral environment by three histidine imidazoles and an aquo ligand of low pK_a ($\approx$7.6 $\pm$ 0.6 for the human isozyme II or B).[2] It also possesses significant esterase activity and efforts to emulate its active site, as well as those of other metal-containing hydrolytic enzymes, promise to provide useful hydrolytic catalysts for a variety of substrates as well as a better understanding of the enzymic modes of action.

Much enzyme modeling effort has been devoted to demonstrating the catalytic importance of a nucleophilic Zn^{2+}-bound OH^-, but the generation of model $ZnOH^+$ complexes leads to particular difficulties. Early "biomimetic" work by the Brown[3] and Breslow[4] groups, using tris-imidazole methane derivatives, was hampered by the formation of stable, octahedral 2:1 ligand-Zn^{2+} complexes, by weak Zn^{2+} binding, which is deleterious and complicates the analysis of kinetic results, and by precipitation of hydroxides. Affixing bulky substituents on the periphery[3] solved the first problem but introduced H_2O insolubility. Provision of a more octahedral environment was achieved by "splaying out" the imidazoles, either on more extensive carbon skeletons[3,5] or by using tris-imidazole phosphines.[6] The use of nonbiomimetic tri- and tetraazamacrocylic ligands for Zn^{2+}, first by Wooley,[7] then by the Breslow[8] and Kimura[9] groups, has circumvented the earlier problems. One interesting feature to arise from the latter work is that strong binding and $ZnOH^+$ production at moderate pH do not go hand in hand. But $ZnOH^+$ production alone is insufficient to reproduce CA's fast catalysis. More elaborate models have resulted in some improvements. For instance, in relation to esterase activity, the attachment of a hydrophobic substrate binding site to the catalytic site improved the rates of phosphate ester hydrolysis by a factor of 7.[8b] In imitation of similar functional groupings in peptidases, work in the Groves[10] and Breslow[11] laboratories has demonstrated improvements in amide hydrolysis with pendant phenol or carboxylate groupings. On the other hand, a pendant serine-like hydroxyl contributed nothing.[6e]

The rate-determining step in CO_2 hydration by isozyme II (or B) of CA is actually the deprotonation leading to the basic ($ZnOH^+$) form.[12] A complex proton relay has been found to be responsible,[13] and this includes a histidine residue (His-64) that cannot be substituted by alanine without a significant loss of activity.[14] In this context, we have explored the biomimetic tris-imidazole podands **1-3** as promising ligands. Their important features include: (a) synthetic simplicity, (b) H_2O solubility, (c) an endodentate architecture, forming inclusion complexes (termed podates) that create a more hydrophobic cavity upon complexation and that promote low coordination numbers, (d) a more tetrahedral binding geometry than in tris-imidazolylmethanes or -phosphines, owing to steric interactions between the imidazoles' 2-hydrogens, and (e) amino vertices in **2** and **3** intended to emulate the proton shuttle involving His-64 in CA. The synthesis of **1** and **2** and the binding of univalent ions in $CHCl_3$ by *N*-CH_2Ph analogues has been communicated earlier.[15] Synthetic

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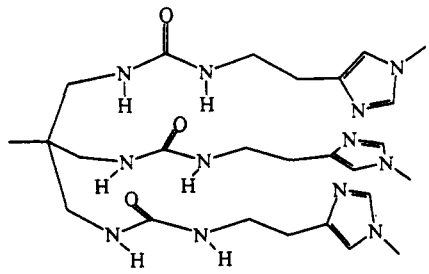

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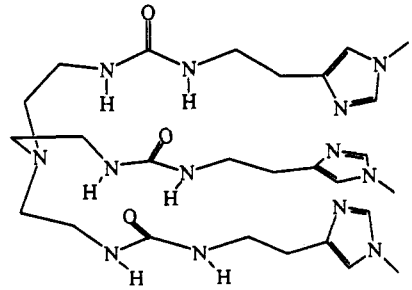

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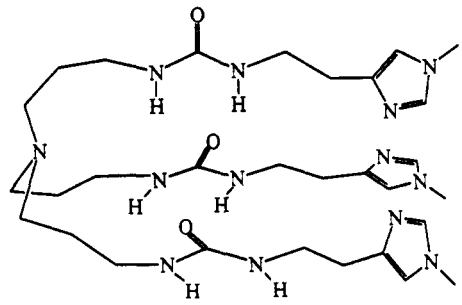

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improvements, reported herein, have been realized and also applied to the preparation of **3**. We also report on the protonation and complexation equilibria with Zn^{2+} and Co^{2+} as studied by pH-metric titration, NMR and UV–vis spectroscopy, and on a preliminary exploration of the catalytic viability of the Zn^{2+} complexes in activated ester hydrolysis.

Experimental Section

General. NMR spectra were recorded at 300 MHz (for ^1H) or 75 MHz (for ^{13}C). 2-Methyl-2-propanol or acetone was used as internal references in D_2O. Histamine was generated from the dihydrochloride salt by passage through a column of Dowex IRA-400 resin (OH^- form). Tris-(2-aminoethyl)amine (tren) (Aldrich) was used as received. 2-(Aminomethyl)-2-methylpropane-1,3-diamine (tame) was a gift from Dr. D. N. Butler. Tris(3-aminopropyl)amine (trpn) was prepared by the method of Chin et al.[16]

5-Oxo-5,6,7,8-tetrahydroimidazo[1,5-*c*]pyrimidine. Histamine (28.8 mmol) was dissolved in 10 mL of $CHCl_3$ and added to a warm, stirred solution of 28.8 mmol of *p*-nitrophenyl carbonate (Aldrich) in 150 mL of $CHCl_3$. The solution immediately discharged a bright yellow color. The urethane intermediate, a brownish oil, formed as the solution was brought to reflux, but it later redissolved. The reaction was allowed to proceed for 24 h, after which the solvent was removed, leaving a pale yellow residue. This was extracted with three 100-mL portions of Et_2O to remove *p*-nitrophenol and then suspended in a small volume of warm DMF to remove any unreacted carbonate. Filtration, followed by washing with cold 2-propanol afforded a 66% yield of the known[17] imidazopyrimidine with mp 220–222 °C. It was converted to the methiodide salt **4** by the literature procedure[18] in 89% yield. Mp 228–230 °C dec.

Podand 1. The salt **4** (1.0 mmol) was added as a solid to 0.33 mmol of tame dissolved in 8–10 mL of CH_3CN. The mixture was refluxed for 3 d, after which the solvent was removed and the residue was neutralized by stirring with a small amount of Dowex IRA-400 resin in OH^- form. Filtration and evaporation produced a clear oil which solidified after drying under high vacuum at 70 °C for several hours. The white hygroscopic solid amounted to a 90% yield and required no further purification. ^1H- and ^{13}C-NMR spectra in $CDCl_3$ have been reported earlier.[15] ^1H-NMR (D_2O): δ 7.41 (s, 3 H, H-2′), 6.80 (s, 3 H, H-5′), 3.53 (s, 9 H, NCH_3), 3.28 (t, 6 H, CCH_2CH_2NH), 2.70 (s, 6 H, CH_3CCH_2), 2.61 (t, 6 H, CCH_2CH_2NH), 0.57 (s, 3 H, CCH_3) ppm. ^{13}C-NMR (D_2O): δ 160.78 (CO), 139.05 (C-4′), 138.54 (C-2′), 118.78 (C-5′), 43.45 (CCH_2CH_2NH), 41.11 (CCH_3), 40.11 (CH_3CCH_2), 33.39 (NCH_3), 28.51 (CCH_2CH_2NH), 18.22 (CCH_3) ppm. Anal. ($C_{28}H_{42}N_{12}O_3 \cdot 2H_2O$) C, H, N.

Podand 2. The salt **4** (3.0 mmol) was dissolved in 5–8 mL dry DMSO at about 40 °C in a capped, Ar-flushed flask. Tren (1.0 mmol) was added by syringe, and the reaction mixture was heated at 75–80 °C for 2 d. The darkened reaction mixture was then cooled, neutralized as before, and concentrated under high vacuum at ca. 80 °C for several hours, followed by several rounds of azeotropic drying with portions of benzene. The glassy hygroscopic solid amounted to a 90% yield and needed no further purification. ^1H- and ^{13}C-NMR spectra in $CDCl_3$ have been reported earlier,[15] though our assignments to the methylene resonances were in error and should have followed the pattern given below. ^1H-NMR (D_2O): δ 7.42 (s, 3 H, H-2′), 6.76 (s, 3 H, H-5′), 3.56 (s, 9 H, NCH_3), 3.23 (t, 6 H, CCH_2CH_2NH), 3.06 (t, 6 H, NCH_2CH_2NH), 2.58 (t, 6 H, CCH_2CH_2NH), 2.48 (t, 6 H, NCH_2CH_2NH) ppm; ^{13}C-NMR (D_2O): δ 160.84 (C=O), 139.00 (C-4′), 138.53 (C-2′), 118.64 (C-5′), 53.95 (NCH_2CH_2NH), 40.07 (CCH_2CH_2NH), 38.15 (NCH_2CH_2NH), 33.40 (NCH_3), 28.25 (CCH_2CH_2NH) ppm. Anal. ($C_{27}H_{45}N_{13}O_3 \cdot 2H_2O$) C, H, N. With [**2**] = $[Zn(ClO_4)_2]$ = 0.01 M in D_2O. ^1H-NMR: δ 7.68 (bs, 3 H, H-2′), 7.05 (s, 3 H, H-5′), 3.66 (bs, 9 H, NCH_3), 3.16 (bm, 6 H, CCH_2CH_2NH), 2.99 (bm, 6 H, NCH_2CH_2NH), 2.61 (bm, 12 H, CCH_2CH_2NH, NCH_2CH_2NH) ppm: ^{13}C-NMR: δ 160.74 (C=O), 138.78 (C-4′), 138.04 (C-2′), 119.82 (C-5′), 54.74 (CCH_2CH_2NH), 39.5 (NCH_2CH_2NH), 37.75 (CCH_2CH_2NH), 34.53 (NCH_3), 27.78 (NCH_2CH_2NH) ppm.

Podand 3. Using the same procedure as for **1**, trpn and **4** afforded a quantitative yield of a clear oil. Upon standing in air, neat or in CH_2Cl_2 solution, a white solid deposited, mp 270–275 °C dec. Two crops were collected by trituration, filtration, and washing with CH_2Cl_2. The yield totaled 66%, based on its formulation as the hemihydrate. As such, it was insoluble in organic solvents. The liposoluble oily form could be regenerated by dissolving the solid in H_2O and then evaporating to dryness under vacuum. A similar preparation in DMSO, as with **2**, afforded impure product that needed chromatographic purification before it could be crystallized as above, with a much reduced isolated yield (30%). ^1H-NMR (D_2O): δ 7.42 (s, 3 H, H-2′), 6.77 (s, 3 H, H-5′), 3.55 (s, 9 H, NCH_3), 3.23 (t, 6 H, $NCH_2CH_2CH_2NH$), 3.00 (t, 6 H, CCH_2CH_2NH), 2.59 (t, 6 H, $NCH_2CH_2CH_2NH$), 2.42 (bt, 6 H, CCH_2CH_2NH), 1.54 (bm, 6 H, $NCH_2CH_2CH_2NH$) ppm. ^{13}C-NMR (D_2O): δ 160.88 (CO), 139.00 (C-4′), 138.52 (C-2′), 118.63 (C-5′), 50.68 ($NCH_2CH_2CH_2NH$), 40.15 (CCH_2CH_2NH), 38.46 ($NCH_2CH_2CH_2NH$), 33.42 (NCH_3), 28.28 (CCH_2CH_2NH), 26.14 ($NCH_2CH_2CH_2NH$) ppm. Anal. ($C_{30}H_{51}N_{13}O_3 \cdot 1/2H_2O$) C, H, N. With [**3**] = $[Zn(ClO_4)_2]$ = 0.01 M in D_2O. ^1H-NMR: δ 7.66 (s, 3 H, H-2′), 6.99 (s, 3 H, H-5′), 3.64 (s, 9 H, NCH_3), 3.13, 3.11, 3.08 (3bt, 18 H, $NCH_2CH_2CH_2NH$, CCH_2CH_2NH, $NCH_2CH_2CH_2NH$), 2.57 (bt, 6 H, CCH_2CH_2NH), 1.77 (bm, 6 H, $NCH_2CH_2CH_2NH$) ppm; ^{13}C-NMR: δ 160.72 (C=O), 138.72 (C-4′), 138.01 (C-2′),

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Table I. Log Formation Constants for the Indicated Species (Weighted Standard Deviations in Parentheses)

species	ligand (L) 1	2	3
HL^+	7.95 (0.039)	7.63 (0.004)	8.99 (0.013)
H_2L^{2+}	14.96 (0.009)	14.45 (0.003)	16.39 (0.020)
H_3L^{3+}	21.22 (0.006)	20.56 (0.024)	23.23 (0.015)
H_4L^{4+}		*a*	29.49 (0.020)
LZn^{2+}	3.86 (0.053)	3.71 (0.010)	*a*
$^+HLZn^{2+}$		10.63 (0.009)	12.77 (0.011)
$LZnOH^+$	−4.26 (0.030)	−3.92 (0.035)	−3.13 (0.028)

[a] Undetected (see text).

119.68 (C-5'), 50.61 ($NCH_2CH_2CH_2NH$), 39.68 (CCH_2CH_2NH), 37.07 ($NCH_2CH_2CH_2NH$), 34.28 (NCH_3), 27.89 (CCH_2CH_2NH), 24.75 ($NCH_2CH_2CH_2NH$) ppm.

pH Titrimetry. The protonations and metal binding were investigated by pH-metric titrations with NaOH of acidic aqueous solutions in the presence of Zn^{2+} or Co^{2+} and, in their absence, using equipment and data treatment already described.[19] For **2**, triplicate 3.5-mL aliquots of a solution of **2** (3.87×10^{-3} M), HCl (1.91×10^{-2} M) with and without $Zn(NO_3)_2$ or $Co(NO_3)_2$ (3.81×10^{-3} M) in 0.10 M NaCl were titrated with 0.0997 N NaOH. Alternatively, duplicate 4-mL aliquots of a solution 4.67×10^{-3} M in **2**, 2.34×10^{-2} M in HCl with and without $Zn(NO_3)_2$ (2.53×10^{-3} M) in 0.15 M KNO_3 were titrated with 0.1004 N NaOH. An identical KNO_3 solution was used for podand **1** (5.24×10^{-3} M). Similarly, triplicate 3-mL aliquots 4.71×10^{-3} M in **3**, 2.50×10^{-2} M in HCl and 0.10 M in NaCl were used, with and without $ZnSO_4$ (6.09×10^{-3} M). The protonation of SO_4^{2-} ($pK_{a1} = 1.92$) was included in the chemical model in this case. The value of pK_w and the formation constants of $ZnOH^+$ and $Zn(OH)_2$ were taken from tables.[20]

The computation by nonlinear least-squares analysis of the log formation constants given made use of data weighting by inverse variances, calculated from estimates of all sources of experimental error. The uncertainty in fitting each log formation constant to each data set was calculated according to the method of Hamilton.[21] The values reported in Table I are mean values. To obtain estimates of experimental errors, standard deviations were calculated with weighting of the deviations from the mean by those uncertainties. The chemical models that the assemblies of species in Table I constitute were tested against other possible models. When other species were included, for instance 2:1 ligand–metal complexes, one or more of the following occurred: (a) the new species' formation constants were very small; (b) the parent 1:1 complexation became very weak; (c) the associated uncertainties were very large; (d) strong correlations (>0.9) between formation constants arose; (e) the sum of the squares of the deviations of the calculated data from the experimental data became very large; (f) the Hamilton R ratio test[21] failed.

UV–vis Spectrometry. With **2**, a working solution of 73% EtOH was prepared by mixing appropriate amounts of a 0.0100 M **2** solution in EtOH with aqueous solutions of $Co(ClO_4)_2$ (0.100 M), HCl (0.100 N), and Me_4NCl (1.0 M) and topping up to 5 mL with absolute EtOH to afford final concentrations of 0.00200 M for **2** and Co^{2+}, 0.0100 M for HCl, and 0.15 M for Me_4NCl. Aliquots (0.5 mL) were titrated by syringe with 0.027 N NaOH in 73% EtOH, prepared by dilution of 0.100 N NaOH with EtOH. The absorbances measured at 610 nm were corrected for dilution. Precipitation of hydroxide occurred above pH 8. With **1** and **3**, therefore, similar titrations were carried out with acid (0.027 N HCl in 73% EtOH). With **3**, however, 0.15 M Me_4NCl failed to redissolved the blue precipitate initially produced upon mixing **3** and $Co(ClO_4)_2$ solutions. Thus, 1 mL of a 0.0100 M aqueous solution of **3** was mixed with 0.10 mL of 0.100 M $Co(ClO_4)_2$, diluted with 0.25 mL H_2O, treated with 0.329 g of solid Me_4NCl, and made up to 5 mL with absolute EtOH for a final background electrolyte concentration at 0.6 M. Parallel titrations were carried out with the same volume increments with monitoring of the pH (uncorrected for the EtOH content). Control experiments in the absence of any ligand showed no spectral changes over the same pH range.

Table II. Derived Log Constants for the Indicated Reactions

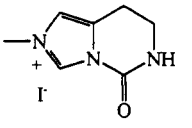
reaction	ligand (L) 1	2	3
$L + H^+ \rightarrow LH^+$	7.95	7.63	8.99
$LH^+ + H^+ \rightarrow LH_2^{2+}$	7.01	6.82	7.40
$LH_2^{2+} + H^+ \rightarrow LH_3^{3+}$	6.26	6.12	6.84
$LH_3^{2+} + H^+ \rightarrow LH_4^{4+}$			6.26
$LZnOH^+ + H^+ \rightarrow LZn^{2+}$	8.12	7.63	≤ 7.95[a]
$LZn^{2+} + H^+ \rightarrow {}^+HLZn^{2+}$		6.93	≥ 7.95[a]
$L + Zn^{2+} \rightarrow LZn^{2+}$	3.86	3.71	≤ 4.82[a]
$LH^+ + Zn^{2+} \rightarrow {}^+HLZn^{2+}$		3.01	3.78
$L + ZnOH^+ \rightarrow LZnOH^+$	5.21	5.26	6.05
$LH^+ + ZnOH^+ \rightarrow LZn^{2+}$		5.26	≤ 5.01[a]

[a] Limits calculated assuming maximum [$3Zn^{2+}$] (see text).

Ester Hydrolysis. Kinetic measurements were performed on solutions prepared in cuvettes by mixing 0.20 mL of a 0.01 M stock solution of **1**, **2** or **3** with 0.20 mL of a 0.01 M $Zn(ClO_4)_2$ solution and made up to 3 mL with 0.05 M Tris buffer previously adjusted to the desired pH with HCl or NaOH. The samples' pH were checked and the absorbance spectra were measured and stored as references in memory. The reactions were initiated by the addition of 180 μL of a 0.01 M CH_3CN solution of *p*-nitrophenyl acetate (*p*-NPOAc) via microsyringe. The absorbances (*A*) at 348 nm ($\epsilon = 5400\ M^{-1}\ cm^{-1}$), the isosbestic point between bands due to p-nitrophenol and p-nitrophenolate ion, were recorded automatically for 1800 s. The pH was redetermined and found to have negligibly deviated. Using points 100 s apart, plots of $\log_e[(A_t/\epsilon - [p\text{-NPOAc}]_0)/(A_0/\epsilon - [p\text{-NPOAc}]_0)]$ vs time were linear with regression coefficients >0.99, and the slopes provided estimates of the *pseudo*-first-order rate constants with errors ≤2%. Similar experiments were also run in the absence of ligand, in the absence of Zn^{2+}, and in buffer alone.

Results and Discussion

Synthesis. The podands **1–3** were prepared by a variant of the method previously communicated.[15] A simple change in solvent (from DMF to DMSO or CH_3CN) drastically improved the yields and the purity of the products. Small amounts of *N*-formylamines had been found in the DMF reactions, along with fair amounts of the urea derived from *N*-methylhistamine.[22] This latter product arose from alkaline hydrolysis of the *N*-methylhistamine-derived **4** during the neutralization step and



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signaled incomplete reaction. Therefore, N-formylation by DMF was likely responsible for the reduced yields in that solvent. All three podands were very hygroscopic. Solid dihydrates of **1** and **2** were obtained after prolonged drying under high vacuum while **3** crystallized as the hemihydrate.

Protonation and Complexation. The convention $\beta_{xyz} = [L_xM_yH_z^{(2y+z)+}]/[L]^x[M^{2+}]^y[H^+]^z$ is used to represent formation constants. The formation constants for the protonated ligands and those for the complexes were obtained in separate pH-metric titration experiments and are reported in Table I. Other constants, derived from these,

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are given in Table II. Three significant features of the protonations are (a) the similar magnitudes of log β_{101} for 1 and 2 and of log β_{102} for 3, indicating that the first protonation steps take place on imidazole groups of 1 and 2 and on the amino vertex of 3; (b) that only three protonations of 2 could be measured, as the inclusion of H_4**2**$^{4+}$ in the chemical model resulted in a pK_{a4} value below the experimental pH range and associated with a large uncertainty, while the other pK_a values were similar in magnitude to those of 1; thus, after protonation of the imidazoles at the extremities, the subsequent protonation of H_3**2**$^{3+}$ apparently induced strong repulsions; (c) there was little difference in the protonation constants of 2 measured in either Cl^-- or NO_3^--containing background electrolytes, while binding of one or the other anion by poly-protonated species would have been signaled by an easy fourth protonation and significant increases in the other pK_a values, relative to those of 1.

Unlike with simple, unencumbered imidazole tripods,[3,4] only 1:1 complexes formed with Zn^{2+}, even in the presence of excess ligand. This is entirely in accord with inclusion complexation and was tested by trial inclusions in the chemical models of species of other stoichiometries. The ligands were only moderate binders overall, and precipitation (presumably of hydroxides) started at pH 8.3–8.5 with consumption of 2 equiv of OH^- per equivalent of metal. However, several points of interest arose.

(a) A protonated complex, written $^+$H2Zn^{2+} to emphasize protonation on the amino vertex, could be included in the model. Conversely, 2Zn^{2+} was readily protonated, implying that the amino vertex was not involved in metal binding and that 2 acted in a tridentate fashion, as must have 1, with aquo ligands completing the coordination shell. This was supported by the small difference in stability between 2Zn^{2+} and 1Zn^{2+}. Similarly, $^+$H3Zn^{2+} could be included and the affinity of H3$^+$ for Zn^{2+} was entirely in line with the affinities of 1 and 2, indicating that the protonated vertex of H3$^+$ had little effect on the imidazole groups. Moreover, because the protonations of 2Zn^{2+}, of H1$^+$, and of H2$^+$ were all of similar magnitude, the amino vertex of 2 evidently remained sufficiently distant from the metal binding site to form a true cavity, as must also exist in 1Zn^{2+}. It was not possible, however, to determine the location of the added H^+ in $^+$H2Zn^{2+} or $^+$H3Zn^{2+}. Inprotonation would favor a larger, more open cavity and might be stabilized by H bonding to the urea carbonyls.[23]

(b) The Zn^{2+} complexes of 1 and 2 also reacted with more OH^- than was needed to fully deprotonate the ligands before precipitation (probably of $Zn(OH)_2$) occurred. Hence, 2ZnOH$^+$ and 1ZnOH$^+$ were also included in the chemical models. Conversely, the aquo complexes were readily deprotonated. In fact, the pK_a value for 2Zn^{2+} (7.63) was close to the value found with the enzyme[2] (7.6) and approached the values found with the best previous models (7.30 and 7.34 with 1,5,9- and 1,4,7-triazacyclododecanes,[9] respectively, 7.01 with a protease model[13]). Thus, in spite of only moderate metal binding strengths, the podands were clearly able to induce acidity in their Zn$(OH_2)_n^{2+}$ complexes. To our best knowledge, these are the weakest Zn^{2+} complexes to show acidity and are overall better binders of ZnOH$^+$ than of Zn^{2+}, solubilities permitting (this holds for any Zn$(OH)_2)_n^{2+}$ complex with a pK_a lower than that of Zn$(OH_2)_6^{2+}$ itself.) Thereby, we agree with the finding by Kimura et al.[9] that Zn^{2+} binding ability and Zn$(OH_2)_n^{2+}$ acidity are not directly related. As those authors surmised, this success can be attributed to a reduction in the metal's coordination number from its solvated state.

(c) Similarly, with 3, consumption of more OH^- than was necessary to completely deprotonate the podand also occurred before precipitation, and 3ZnOH$^+$ could be included in the chemical model. However, 3Zn^{2+} *was excluded entirely* (log$\beta_{110} \rightarrow -\infty$). Alternative models gave much less satisfactory fits of the data. This situation was reminiscent of cooperativity phenomena.[23,24] Thus, $^+$H3Zn^{2+} acted as a diacid, releasing two protons in a single step with an overall or average pK_a of 7.95 (i.e., $^1/_2$ log $(\beta_{111}/\beta_{11-1})$ or half the sum of the pK_a's). Certainly, the acidity of the protonated vertex was expected to increase upon binding Zn^{2+}, as was true with 2. It was therefore not inconceivable that the acidities of both the protonated vertex and the aquozinc moieties be similar, given the acidities of 1Zn^{2+} and of 2Zn^{2+}. The absence of detectable 3Zn^{2+} meant that the first deprotonation of $^+$H3Zn^{2+}, generating either 3Zn^{2+} or the titrimetrically equivalent $^+$H3ZnOH$^+$, caused an easier second deprotonation step or vice versa. The removal (or addition) of the second proton could be facilitated by proton shuttling between the vertex and the metal center, but the driving force for such cooperativity must be an unusual stabilization of 3ZnOH$^+$ relative to 3Zn^{2+} (or $^+$H3ZnOH$^+$) or a destabilization in the latter. Indeed, 3 had a significantly higher affinity for ZnOH$^+$ than had 1, 2, or H3$^+$. This may be rooted in a more favorable geometry in free 3 for binding the ZnOH$^+$ moiety than is available with the others.

The formulation of 3Zn^{2+} as $^+$H3ZnOH$^+$ raised the possibility that 2Zn^{2+} could similarly be formulated as its $^+$H2ZnOH$^+$ tautomer. Indeed, free 2 and 2ZnOH$^+$ were equally basic. Certainly, the ability to shunt a proton from the Zn$(OH_2)_n^{2+}$ moiety to the amino vertex could reduce the charge density and, in a poorly solvated cavity, would be expected to stabilize the complex. The affinity for ZnOH$^+$ by H2$^+$ was in fact the same as by free 2 for Zn^{2+}. However, 1, incapable of such stabilization, was actually a slightly better Zn^{2+} binder. Moreover, were $^+$H2ZnOH$^+$ the exclusive form present, our results would imply that (a) H2$^+$ bound ZnOH$^+$ slightly better than did 1, despite the added charge; (b) the deprotonation of the Zn$(OH_2)_n^{2+}$ moiety in $^+$H2Zn^{2+} was unusually facile (pK_a 6.91); and (c) the subsequent deprotonation of the amino vertex in the putative $^+$H2ZnOH$^+$ was counterintuitively no easier (log $(\beta_{110}/\beta_{11-1}) = 7.63$) than was that of H2$^+$ itself, in spite of the added charge. It seems, therefore, that $^+$H2ZnOH$^+$ cannot be the sole form of 2Zn^{2+}, but we cannot exclude its presence. In this regard, ^{1}H-NMR spectroscopy was informative. A D_2O solution 0.01 M in both 2 and Zn^{2+} showed broadened signals, in accord with reduced motion and/or exchange processes that were slow on the NMR time scale. The most significant complexation-induced downfield shifts occurred with the imidazolyl nuclei (0.26–0.29 ppm), as expected. (The ^{13}C spectrum was less informative, showing mostly small upfield shifts, but with no significant changes in the position of the C=O peak. This was also consistent with a tridentate mode of complexation.) However, there was also a 0.13 ppm downfield displacement of the signal due to the methylenes flanking the vertex, relative to its position with free 2 in D_2O. Assuming no isotope effect, the 2–Zn^{2+} mixture was calculated to contain some 27% 2Zn^{2+}, 18% D2$^+$, and about 5% $^+$D2Zn^{2+} when dissolved in D_2O, as well as negligible amounts of other deuterated species, whereas 2 alone in

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D_2O should have included only 3% $2D^+$. (Some differences are of course to be expected in the values of the formation constants measured in D_2O.) If, as was discussed earlier, $D2^+$ is deuterated largely at an imidazole group, then the small amount of $^+D2Zn^{2+}$, which must be deuterated at the vertex, cannot alone account for that downfield displacement. Thus, some of the $2Zn^{2+}$ probably contributed to the downfield shift in the form of $^+D2ZnOD^+$. NMR also confirmed the stability of the urea groups in the presence of Zn^{2+}.

As with CA[1] and models that enforce low coordination numbers[3], Zn^{2+} was preferred over Co^{2+} by 1 and 2: No Co^{2+} complex was detected with the former, while a significantly weaker complex formed with 2 ($\log \beta_{110} = 2.10 \pm 0.63$) that deprotonated at much higher pH with a pK_a above the useable pH range and more uncertain ($\log \beta_{11-1} = -6.85 \pm 0.44$). There were no detectable changes in the visible spectra as a result of complexation and no pH-dependence with any podand. In more alcoholic solution, complexation was enhanced. In the absence of added Cl^-, deep blue precipitates formed upon mixing the podands with $Co(ClO_4)_2$ in aqueous EtOH and these probably consisted of tetrahedral species. This stands in contrast with the results of Brown et al.,[6a,d] who found that $Co(ClO_4)_2$ with hindered tris(imidazolyl)phosphines produced spectra typical of octahedral Co^{2+}, while $CoCl_2$ had formed tetrahedral species. Visible spectra were therefore obtained in 73% EtOH containing 0.15 M $Me_4N^+Cl^-$ (0.6 M with 3) at various pH values (uncorrected for the EtOH content). At low pH, a solution of 2 and Co^{2+} was pink and the spectrum was typical of octahedral Co^{2+} ($\epsilon \approx 10$ M^{-1} cm^{-1} at 510 nm), implying no binding and protonation of 2. The absorbance at 610 nm, expected for tetrahedral or pentacoordinate Co^{2+}, increased with pH. At pH 6.2, the apparent spectrophotometric pK_a, the color was blue. The absorbance at 610 nm continued to increase (to $\epsilon \approx 100$ M^{-1} cm^{-1} overall with fully deprotonated 2) until precipitation occurred near pH 8 with excess OH^-. In the absence of ligand, no blue color developed before precipitation occurred. Similar inflections were found with 1 (at pH 6.9) and 3 (at pH 6.7) during titrations with acid. Thus, the spectral changes were associated with the simple deprotonation of ligand and no further reaction with OH^- could be detected below pH 8. According to Bertini et al.[25] and assuming that the complexes had fully formed, the maximum overall ϵ values indicated pentacoordinate Co^{2+} (50 M^{-1} $cm^{-1} < \epsilon < 200$ M^{-1} cm^{-1}). However, as the complexes may not have fully formed even in 73% EtOH, tetracoordination could not be ruled out.

Hydrolytic Catalysis. Operationally, esterase activity was studied by measurement of the rates of hydrolysis of p-nitrophenyl acetate (*p*-NPOAc) in buffered aqueous solutions at pH values between 7.35 and 8.15. A preliminary survey in unbuffered solutions at pH 7.41 with equimolar 2 and either Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+} revealed that only Zn^{2+} and Ni^{2+} exerted any cooperative effect with 2, with Zn^{2+} the clear winner. Because of this and because Zn^{2+} is the natural ion, all subsequent work therefore used this ion. Several complications arose, however. On the one hand, the complexation of Zn^{2+} by the podands was only moderate and, at the concentrations employed, much free podand and free Zn^{2+}, which are catalytically active on their own, will have persisted. Hence, control experiments were required to assess their contributions to ca-

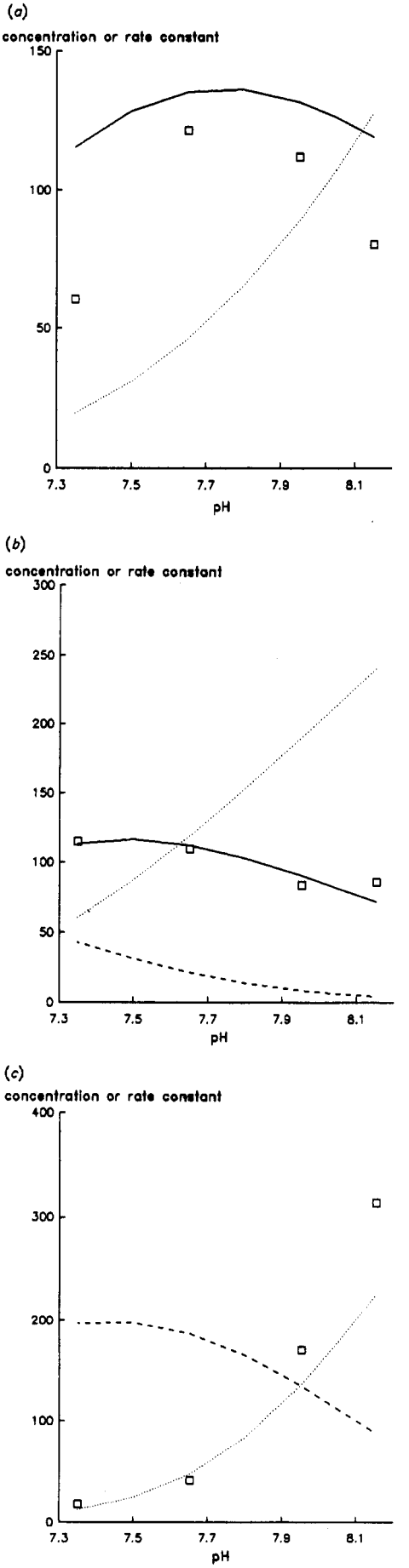

Figure 1. Variation with pH in k_{cat} (□, $\times 10^7$ s^{-1}) and in the concentrations (in μM) of $^+HLZn^{2+}$ (– –), LZn^{2+} (—) and $LZnOH^+$ (···), with (a) 1, (b) 2, and (c) 3.

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Table III. Observed Pseudo-First-Order Rate Constants k ($\times 10^6$ s^{-1}) for the Appearance of p-NPO^- from 5.66×10^{-4} M p-NPOAc and Calculated Concentrations (μM) of Complex Species. In All Cases, $[L]_{tot} = [Zn^{2+}]_{tot} = 6.3 \times 10^{-4}$ M, as Appropriate

	pH			
	7.35	7.65	7.95	8.15
k_{bgd}	12.2	17.8	24.6	35.3
k_M	15.2	27.9	57.3	79.3
k_1	15.5	22.0	36.0	43.8
k_2	24.1	32.9	46.7	68.5
k_3	21.0	25.3	45.1	63.6
k_{1+M}	23.1	39.9	63.6	74.1
$[1Zn^{2+}]$	115	135	132	119
$[1ZnOH^+]$	19.6	45.9	89.1	128
k_{2+M}	24.1	32.9	46.7	68.5
$[^+H2Zn^{2+}]$	43.0	21.3	8.59	4.32
$[2Zn^{2+}]$	113	112	90.3	72.0
$[2ZnOH^+]$	60.1	119	191	241
k_{3+M}	21.8	33.0	71.9	103
$[^+H3Zn^{2+}]$	197	186	134	88.8
$[3ZnOH^+]$	12.6	47.4	136	226

talysis. The Tris buffer employed also forms a weak Zn^{2+} complex (log K = 2.27),[20] resulting in a reduction of the total Zn^{2+} available to the podand, though the buffer concentration was thereby negligibly diminished. As well, more than one complex could be active. On the other hand, even with substrate, Zn^{2+} and podand nearly equimolar, the ester was always in excess over any one catalytically active species, and pseudo-first-order kinetics could be assumed. However, given the competitive catalyses at play, it was very difficult to demonstrate turnover by the complexes of interest. A second-order process was therefore assumed, and constant concentrations of ester, podand, and metal were used for operational simplicity, but the variations in rate with ester concentration during any one run and of catalyst concentration with pH (vide infra) were entirely in accord with this assumption.

The observed pseudo-first-order rate constants are given in Table III. When compared to the background catalysis due to the medium (k_{bgd}), accelerations were apparent with either added Zn^{2+} (k_M) or added podands (k_L) owing to catalyses by the suites of species to which these reactants give rise. In all cases, the presence of both podand and Zn^{2+} (k_{L+M}) gave faster reactions and this must reflect catalysis by one or more complexes. To assess their catalytic effect, our approach paralleled that of Brown et al.[6e] Thus, we calculated values of k_{cat}, the first-order rate constants owing to complexes after subtraction of the catalyses due to the medium and to the uncomplexed ligand and metal species, at each pH and for each podand according to

$$k_{cat} = k_{L+M} - k_{bgd} - (k_L - k_{bgd})\frac{[L]_{L+M}}{[L]_L} - (k_M - k_{bgd})\frac{[Zn^{2+}]_{L+M}}{[Zn^{2+}]_M}$$

where $[L]_{L+M}$ and $[Zn^{2+}]_{L+M}$ are concentrations of free ligand and metal calculated to persist in the podand–Zn^{2+} mixtures at any one pH, and where $[L]_L$ and $[Zn^{2+}]_M$ are the corresponding concentrations in the control reactions. These concentrations were obtained using the known log β values while taking into account buffer-containing species and assuming little change in log β values with the medium. The behavior of k_{cat} with pH (Figure 1) should then indicate which complex species are important to the catalysis. Surprisingly, the contributions from the podates of 1 and 2 decreased with increasing pH, while those of 3 increased. In fact, the k_{cat} values roughly paralleled the concentrations of $1Zn^{2+}$, $2Zn^{2+}$, and $3ZnOH^+$, respectively. This suggested that these were the most active complexes, though not necessarily the exclusive catalysts. The correlations of k_{cat} with $[1Zn^{2+}]$ or $[2Zn^{2+}]$ were only fair, leading to estimates of the second-order rate constants of 0.079 ± 0.018 (r = 0.86) and 0.098 ± 0.009 M^{-1} s^{-1} (r = 0.973), respectively. The correlation with $[3ZnOH^+]$ was excellent, leading to a value of 0.139 ± 0.007 M^{-1} s^{-1} (r = 0.992). These values compare very favorably with values measured with previous esterase models, for instance, $Co(NH_3)_5OH^{2+}$ with pK_a 6.4 (0.0015 M^{-1} s^{-1} at pH 7.456),[26] a cyclodextrin-anchored Ni^{2+} complex (0.330 M^{-1} s^{-1} at pH 5.17),[27] the $ZnOH^+$ complex of 1,5,9-triazacyclododecane of pK_a 7.3 (0.041 M^{-1} s^{-1} at pH 8.2),[9] or the $ZnOH^+$ complex of Wooley's pyridinotetrazamacrocycle with a pK_a of 8.12[7] (0.0503 M^{-1} s^{-1} at pH 8[8b]), but still fall far short of that with the enzyme (400 M^{-1} s^{-1} for bovine CA at pH 8.9)[28] or even OH^- (9.5 M^{-1} s^{-1}).[29]

Whereas the catalytic activities of 3 with Zn^{2+} and of previous Zn^{2+}-containing CA, esterase, and peptidase models appear to be tied to a biomimetic deprotonation phenomenon, those of 1 or 2 with Zn^{2+} are apparently not. All three podands should lead to very similar metal binding geometries, whether tetra- or pentacoordinate, and whether the podands completely wrap the metal or whether trigonal bipyramidal structures with equatorial imidazoles can exist. Cavity size may instead be at the root of this difference. The larger cavity in $3ZnOH^+$ may allow mechanisms less feasible with $1ZnOH^+$ or $2ZnOH^+$, be it general base catalysis or nucleophilic attack by the Zn^{2+}-bound OH^- (if the ester can approach the metal center). Similarly, a too small cavity may also prevent such catalysis by the putative tautomer $^+H2ZnOH^+$. On the other hand, less efficient general acid catalysis or Lewis acidic activation of the ester carbonyl may operate with $1Zn^{2+}$ and $2Zn^{2+}$ (but not with $3Zn^{2+}$, since it is not present) and should have operated with $^+H2Zn^{2+}$ and $^+H3Zn^{2+}$. That these latter species were apparently ineffective may reflect *out* protonations of the amino vertices which would tend to elongate and collapse the cavities. (This would also apply to $^+H2ZnOH^+$). Certainly, more work would be required to verify these hypotheses. Nevertheless, this work demonstrates that the endo-tridentate architecture is a useful one as it is able to inhibit solvation sufficiently well to promote catalytically useful Lewis acidity and to achieve moderate, biomimetic pK_a values which in one case, at least, produces a fairly good esterase. We were not able to show a kinetic role for the moderately basic amino vertex of 2, but the more basic one in 3 had a pronounced effect on the relative stabilities of its podates. While the architecture could be maintained in future catalysts, stronger metal binding will clearly be required to achieve useful levels of catalysis and synthetic work in this vein is currently underway.

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