A Zinc(II) Complex of 1,5,9-Triazacyclododecane ([12]aneN$_3$) as a Model for Carbonic Anhydrase

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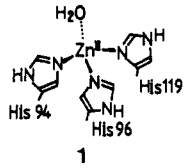
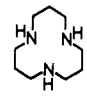
Abstract: Among macrocyclic tri- and tetraamines tested, a 12-membered triamine, [12]aneN$_3$, is the most appropriate ligand that mimics the ligand field surrounding Zn^{II} in carbonic anhydrases. In its 1:1 Zn^{II}L complex, the H_2O bound at the fourth coordination site deprotonates with the pK_a value of 7.30 at 25 °C, I = 0.1 ($NaClO_4$), almost the same value being reported for the Zn^{II}-enzymes. The resulting hydroxo complex is precipitated as a trimer from pH 8 aqueous solution, which with a formula of $[Zn^{II}L(OH)]_3(ClO_4)_3 \cdot HClO_4$ has been analyzed by X-ray crystal study. The crystals of $(\mathbf{11})_3 \cdot (ClO_4)_3 \cdot HClO_4$, $C_{27}H_{67}N_9O_{19}Cl_4Zn_3$, are trigonal, space group $R3c$ with six molecules of **11** in the unit cell of dimensions a = 22.103 (1) Å, c = 16.514 (2) Å. Anion binding affinity to the Zn^{II}L complex is determined by pH titration to have an order of OH^- (log K = 6.4) ≫ CH_3COO^- (2.6) > SCN^- (2.4) > I^- (1.6) > Br^- (1.5) > F^- (0.8), which is almost comparable with the anion inhibition order and magnitude reported for carbonic anhydrase activities. Moreover, like the Zn^{II}-enzymes, the $[Zn^{II}L(OH)]^+$ species catalyzes methyl acetate hydrolysis and acetaldehyde hydration, where the Zn^{II}-bound OH^- commonly acts as a nucleophile to the carbonyl carbons. The plots of these rate constants vs pH in either case show the kinetic pK_a values of Zn^{II}L(OH_2) to be nearly the same as the thermodynamically obtained values of 7.3 at 25 °C and 7.9 at 0 °C. Various outstanding properties of Zn^{II} in enzymes (over other metal ions such as Co^{III}), which contribute to its biological significance, have been well demonstrated by the present macrocyclic triamine complex behaviors.

Introduction

Reaction mechanisms of hydrolytic metalloenzymes (e.g., carbonic anhydrase (CA), carboxypeptidase, phosphatase) and the role of the metal ions in their active centers have constantly been interesting bioinorganic subjects.[1,2] As one of the approaches, various types of metal complexes have been designed to account for or mimic the functions played by the central Zn^{II} ions, a typical central metal ion. Thus far the most successful model complexes have employed cobalt(III)[3-7] or copper(II) ions,[8-10] which however, are not common in those enzymes. On the other hand, only a few models with zinc(II) complexes have been reported.[11-15] Because of the lack of good experimental facts about the inherent acid properties of Zn^{II}, some of the chemical feasibility of the conclusions and propositions derived from enzymatic studies on the role of the metal ion and the catalysis mechanisms remain yet to be verified. To cite a few instances, while the pK_a value of ~7.5 has been assigned to deprotonation of the water bound to Zn^{II} in CA (see **1**),[16,17] there is no Zn^{II} complex known to date that



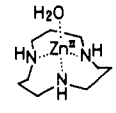
1
Carbonic Anhydrase
active center



2
[12]aneN$_3$

has a dissociable H_2O with a pK_a as low as ~7.5. Or while anion inhibition of CA activities is explained by occupation of the H_2O binding site,[18,19] there has been no chemical analogy with the past model complexes.

Herein, we present a 12-membered macrocyclic triamine 1,5,9-triazacyclododecane (**2**, [12]aneN$_3$) that can reproduce the simplest and yet the closest environment known so far of the active metal center of CA. Many biochemical phenomena involving the active metal ion are reconstructed with a Zn^{II}-**2** complex **3**, serving to bridge a gap between the enzymological facts and Zn^{II} coordination chemistry.



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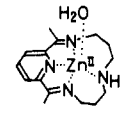

4

In 1975, a macrocyclic tetraamine Zn^{II} complex **4** was introduced as a CA model.[11,12] Although this model drew intriguing and very convincing pictures about the essential role of Zn^{II} ions, the fatal drawbacks of **4** were its Zn^{II} being N_4 coordinated (against N_3 coordinated in CA) and the higher pK_a value of the bound H_2O being 8.7 (against ~7.5 in CA).

To demonstrate the special merit of **2**, we have made com-

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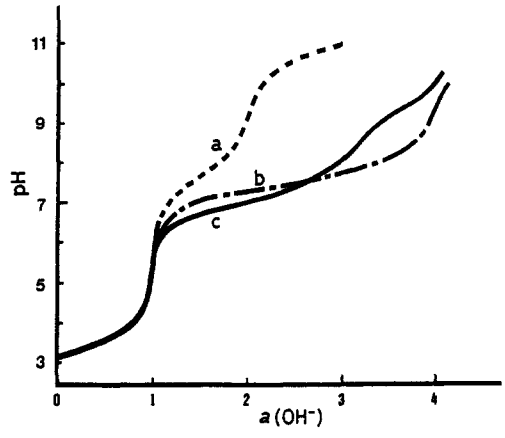
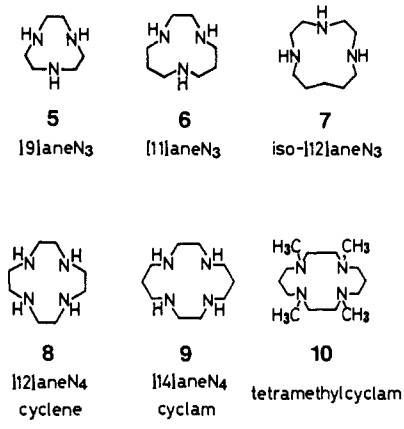


Figure 1. Titration curves for the [12]aneN$_3$ **2** system. (a) 1 mM [**2**·3H$^+$]$^{3+}$ at I = 0.10 ($NaClO_4$) and 25 °C; (b) **a** + 1 mM $Zn^{II}SO_4$; (c) 1 mM [**2**·3H$^+$]$^{3+}$ + 1 mM $Zn^{II}SO_4$ + 0.20 M NaSCN at 25 °C.

parative studies using the relevant macrocyclic polyamine ligands **5**–**10**.



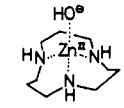
Experimental Section

General Methods. ^1H and ^{13}C NMR spectra were obtained on a JEOL GX-400 spectrometer (400 MHz, 25 °C). IR and UV spectra were recorded on a Shimadzu FTIR-4200 and a Hitachi U-3200 spectrophotometer, respectively.

All reagents and solvents used were of analytical grade. Macrocyclic polyamine ligands **2** and **5**–**8** as the free form were synthesized by the Richmann–Atkins procedure.[20] Acetonitrile (CH_3CN) was distilled over calcium hydride and stored in a dark bottle with 10% (v/v) molecular sieves (4 Å). Acetaldehyde was freshly distilled under nitrogen atmosphere and stored in the dark below 5 °C. 4-Nitrophenyl acetate was recrystallized from dry diethyl ether.

Potentiometric Titrations. A typical pH-metric determination is as follows: An aqueous solution (50 mL) of the ligand **3** (1.00 mM) with 3 equiv of $HClO_4$ (3.00 mM) in the absence [for determination of ligand protonation constants (log K_n)] or presence of equivalent Zn^{II} ion [for determination of K(ZnL) and deprotonation constants of Zn^{II}-bound H_2O in Zn^{II}L complexes] was titrated with 0.100 M NaOH aqueous solution (see Figure 1 for L = **2**). The calibration method of the electrode system at I = 0.10 and 0.20 was identical with that used previously.[21,22] In order to study the following anion coordination effect on Zn^{II} complexation, $NaClO_4$ (ClO_4^- is well-known as a very weak donor) was used as supporting electrolyte. The titration data were treated by a mass balance method, which was identical with that previously reported for the Zn^{II}–[10]aneN$_3$ complex[22] and the phenol-pendant [12]aneN$_3$ complex.[23] Protonation constants (log K_n) of [12]aneN$_3$ reported earlier were 12.60, 7.57, and 2.41 (I = 0.1 at 25 °C),[24] and our determined values are 12.6 ± 0.1, 7.50 ± 0.02, and 2.4 ± 0.1 [I = 0.2 ($NaClO_4$) at 25 °C]. Protonation constants and Zn^{II} complex formation constants for other macrocyclic polyamines were reported previously.[24-26]

Synthesis of (Zn^{II}–[12]aneN$_3$(OH))$_3$·$(ClO_4)_3$·$HClO_4$, (11)$_3$$(ClO_4)_3$·$HClO_4$. A solution of **2** (150 mg, 0.88 mmol) in 10 mL of EtOH was added to $Zn(ClO_4)_2$·$6H_2O$ (326 mg, 0.88 mmol) in EtOH (10 mL) at room temperature. After 30 min, the resulting white powder was filtered and then recrystallized from water. Colorless crystals of (**11**)$_3$$(ClO_4)_3$·$HClO_4$

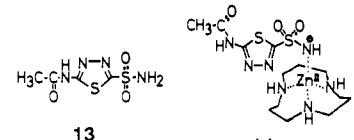


11

were obtained in ca. 40% yield. ^1H NMR (D_2O at pD 10, DSS as reference): δ 1.64 (3 H, ttd, J = 2.2, 8.8, 16.6 Hz, CCHC), 2.03 (3 H, ttd, J = 2.2, 7.8, 16.6 Hz, CCHC), 2.89 (6 H, ddd, J = 2.2, 8.8, 13.4 Hz, NCHC), 3.20 (6 H, ddd, J = 2.2, 7.8, 13.4 Hz, NCHC). This ^1H NMR chart was presented as a supplementary material. IR (KBr pellet): 3500 (br), 3125 (s), 2890, 1480 (br), 1275, 1144 (s), 1117 (s), 1088 (s), 1047, 1032, 972, 891, 638 (s), 626 (s) cm^{-1}. Anal. Calcd for $C_{27}H_{66}N_9O_3Zn_3(ClO_4)_3$·$HClO_4$: C, 27.96; H, 5.82; H, 10.87. Found: C, 28.28; H, 5.41; N, 10.64.

The crystals of (**11**)$_3$$(ClO_4)_3$·$HClO_4$ (11.6 mg, 0.1 mmol) were dissolved in 100 mL of H_2O (at 25 °C, I = 0.1) and titrated with 0.10 M NaOH. An inflection (pH ca. 8) was observed at a titration point of 1 equiv of OH^- consumption (0.1 mmol), supporting the [3(Zn^{II}–OH) (this is inert to NaOH) + $HClO_4$] form in each Zn^{II} trimer.

Isolation of (Deprotonated Acetazolamide–Zn^{II}–[12]aneN$_3$)·ClO_4, 14·ClO_4. A solution of $Zn^{II}(ClO_4)_2$·$6H_2O$ (74 mg, 0.20 mmol) and acetazolamide (**13**; 44 mg, 0.20 mmol) was added dropwise to **2** (34 mg)



13
acetazolamide

14

in 10 mL of CH_3CN. After filtration of the mixture, the filtrate was concentrated slowly at room temperature. Crystals of **14**·ClO_4 were obtained as colorless needles in 40% yield. ^1H NMR (CD_3CN, TMS as reference): δ 2.25 (3 H, s, CH_3CO), 1.66, 1.94 (6 H, m, CCH_2C), 2.83, 3.51 (12 H, m, NCH_2C). UV (in CH_3CN): 263 nm (ε 9800). IR (KBr pellet): NH, 3260 (s); SO_2NH, 3170, 3015; CO, 1703; SO_2 (asym), 1305; SO_2 (sym), 1151; ClO_4^-, 1123, 1100, 1094 cm^{-1}. Anal. Calcd for $C_{13}H_{26}N_7S_2O_3Zn$·ClO_4: C, 28.02; H, 4.70; N, 17.59. Found: C, 28.39; H, 4.77; N, 17.52.

Calculation of Anion Complexation Constant. The measurement of the anion complexation constants $K(A^-)$ for CH_3COO^-, SCN^-, I^-, Br^-, and F^- were conducted by the potentiometric titration method. The ionic strength of 0.20 (using $NaClO_4$) was used to prepare solutions containing high concentrations (up to 200 mM) of anions. Under such conditions, smaller $K(A^-)$ values [with respect to large $K(OH^-)$] could be measured. A typical titration curve in the presence of a large excess of SCN^- (0.20 M) is represented in Figure 1c. The following equilibria and equations were considered to take place in the buffer region [3 < a < 4] of the titration curves.

$$ZnL + H_2O \rightleftarrows ZnL(OH^-) + H^+;\ K_{OH} = [ZnL(OH^-)][H^+]/[ZnL] \quad (1)$$

$$ZnL + A^- \rightleftarrows ZnL(A^-);\ K(A^-) = [ZnL(A^-)]/[ZnL][A^-] \quad (2)$$

$$C_{ZnL} = [ZnL] + [ZnL(A^-)] = [ZnL](1 + K(A^-)[A^-]) \quad (3)$$

$$C_{ZnL} = (1 - a')C_L \quad (4)$$

$$[ZnL(OH^-)] = a'C_L \quad (5)$$

where $a' = a - 3$ and C_L is a total concentration of [**12**]aneN$_3$.

When eqs 2–5 were substituted into eq 1, we obtained the simplified eqs 6 and 7. A plot of the left-hand side of eq 7 against $1/[H^+]$ gives

$$[a'/(1 - a')][H^+] = K_{OH}/(1 + K(A^-)[A^-]) = K' \quad (6)$$

$$a'/(1 - a') = K'(1/[H^+]) \quad (7)$$

a straight line with zero intercept and a slope for K'. Furthermore, a plot

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of $1/K'$ values against anion concentration (0–0.20 M) gives a linear line with an intercept of $1/K_{OH}$ (see eq 8 derived from eq 6). Finally, we can

$$1/K' = [K(A^-)/K_{OH}][A^-] + 1/K_{OH} \quad (8)$$

obtain $K(A^-)$ values as the slope × K_{OH}. Typical plots for SCN^- and Br^- complexes are presented as supplementary material. The anion complexation constant log $K(OH^-)$ of 6.4 ± 0.1 is calculated from the K_{OH} value of 4.0×10^{-8} and pK_w (13.78).

Kinetics of Acetaldehyde Hydration. The hydration rate of acetaldehyde in aqueous solution was measured by following the disappearance of the UV absorption at 278 nm (ϵ 16.1 mol^{-1} cm^{-1}), exactly as described in Woolley's procedure for the M^{II} complex-promoted catalytic hydration of acetaldehyde.[11,12,27] The hydration reaction was so rapid at 25 °C that it was measured at 0 °C, where the hydration reaction was practically followed by a syringe injection method. It was necessary to constantly sweep dry nitrogen gas to prevent condensation of water vapor in the spectrophotometer chamber. A solution of 50 mM Good's buffer [TAPS (pH > 8), HEPES (8 > pH > 7), MES (pH < 7)] was used at I = 0.10 (using $NaClO_4$) and 0 ± 0.5 °C. These buffers had little influence on the catalytic hydrolysis reaction (against the Zn^{II} complex **11**). The reactions were carried out under pseudo-first-order conditions with a large excess of the acetaldehyde over the catalysts, where the rate constants k_{obs} (s^{-1}) were obtained by a log plot method. A typical procedure for a kinetic measurement was as follows: After rapid injection of 3.6 M acetaldehyde in dry CH_3CN (to a final concentration of 35 mM) into the appropriate pH buffer solution containing 0.5 mM Zn^{II}–[12]aneN$_3$, where the final CH_3CN concentration was 10% (v/v) (the reference experiment did not contain the catalyst), the UV absorption decay was recorded immediately and was generally followed for at least 2 half-lifes (e.g., ca. 3 min at pH 8). Addition of the trace amount of Zn^{II} complexes had no appreciable effect on the hydration–dehydration equilibrium of acetaldehyde ($[CH_3CH(OH)_2]/[CH_3CHO]$ = 2.3).[28] A plot of k_{obs} vs the Zn^{II} complex concentration (0.1–1 mM) at a given pH gave a straight line, and then we determined the slope as the second-order rate constant k_{cat} (M^{-1} s^{-1}) and the intercept as the conditional rate constant (s^{-1}) in the absence of catalysts. A typical plot with the Zn^{II}–[12]aneN$_3$ complex was presented as supplementary material.

Kinetics of Methyl Acetate Hydrolysis. The hydrolysis reaction of methyl acetate was followed by a pH-stat method under pseudo-first-order conditions [$[CH_3COOCH_3]$ = 0.050–1.00 M and [Zn^{II} complex] = 0.50–5.0 mM, at 25.0 ± 0.1 °C and I = 0.10 ($NaClO_4$)] until one catalytic turnover had been achieved. A solution of 25.0 mM NaOH was used to keep the pH constant with an automatic titrator (Kyoto Electronics AT-117) in a nitrogen atmosphere. A plot of the hydrolysis rate (consumption of NaOH) vs Zn^{II} complex concentration at a given pH gave a straight line, and then we determined the slope/$[CH_3COOCH_3]$ as the second-order rate constants k_{Ac} (M^{-1} s^{-1}).

The methyl protons of the reaction products (CH_3COO^- and CH_3OD) in the Zn^{II}-promoted reactions were identified by ^1H NMR analysis in buffered D_2O solution [7.5 < pD < 8.5 (50 mM Tris), 8.5 < pD < 10.5 (50 mM borate)] at 25 °C, where no side product such as Zn^{II}-bound acetate complex was detected.

Kinetics of 4-Nitrophenyl Acetate Hydrolysis. The hydrolysis rate of 4-nitrophenyl acetate in aqueous solution was measured by following the increase in the 400-nm absorption of the released 4-nitrophenolate. The reaction solution was maintained at 25.0 ± 0.1 °C and the ionic strength was adjusted to 0.10 with $NaClO_4$. HEPES buffer (50 mM) was used. The typical procedure was as follows: After 4-nitrophenyl acetate and Zn^{II}-[12]aneN$_3$ (both the final concentrations of 1.0 mM) in 10% CH_3CN solution at appropriate pH (the reference experiment did not contain the catalyst) were mixed, the UV absorption decay was recorded immediately and was followed generally until 2% decay of 4-nitrophenyl acetate (i.e., ca. 10 min at pH 7.5). We determined the second-order rate constant k_{NP} (M^{-1} s^{-1}) by the same initial slope method used for the methyl acetate hydrolysis determination.

Crystallographic Study. A colorless crystal with dimensions 0.3 × 0.3 × 0.3 mm^3 of **(11)**$_3$$(ClO_4)_3$·$HClO_4$ was used for data collection. The lattice parameters and intensity data were measured on a Rigaku AFC-5 diffractometer with graphite monochromated Cu Kα radiation. The structure was solved by the heavy atom method and refined anisotropically by using absorption-corrected data to give R = 0.049, R_w = 0.071 for 1257 independent observed reflections. The crystals of **(11)**$_3$·(ClO_4)·$HClO_4$, $C_{27}H_{67}N_9O_{19}Cl_4Zn_3$, are trigonal, space group $R3c$ with six molecules of **11** in the unit cell of dimensions a = 22.103 (1) Å, c = 16.514 (2) Å. The space group was suggested to be $R3c$ or $R\bar{3}c$, the former being verified after the structure refinement. All the hydrogen atoms could not be located in a difference electron density map. The molecular structure is illustrated in Figures 2 and 3. Selected bond lengths and bond angles around the Zn^{II} and intermolecular hydrogen bonds are presented in Tables I and II, respectively. Crystal data and data collection parameters, atomic positional parameters with standard deviations, bond lengths, and bond angles are given as supplementary material. The temperature factors of the carbon atoms are larger than those of the remaining atoms of the complex ion, some of which exhibit a large anisotropy. We assumed a disordered structure of the ring, but could not confirm it on the difference electron map. Therefore, the bond lengths and angles relevant to the carbon atoms are less reliable.

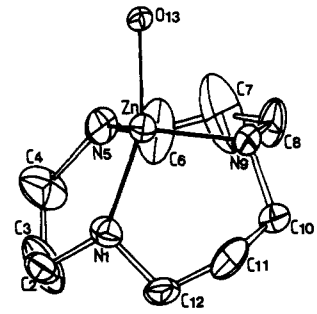

Figure 2. ORTEP drawing of **11**. Atoms are drawn with 30% probability ellipsoids.

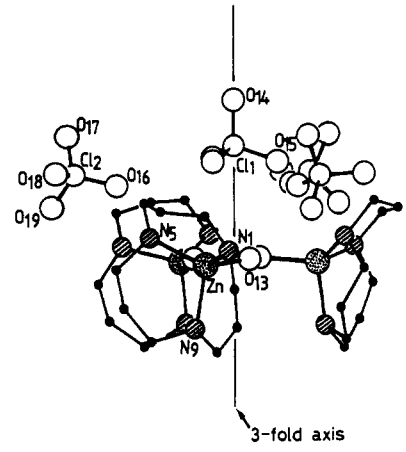

Figure 3. Molecular structure of **(11)**$_3$$(ClO_4)_3$·$HClO_4$ with the 3-fold axis.

Table I. Bond Distances and Bond Angles around the Zn^{II} of **(11)**$_3$$(ClO_4)_3$·$HClO_4$[a]

	Bond Distances, Å		
Zn–N(1)	2.004 (9)	Zn–N(5)	2.007 (9)
Zn–N(9)	2.042 (9)	Zn–O(13)	1.944 (5)
	Bond Angles, deg		
N(1)–Zn–N(5)	104.3 (4)	N(1)–Zn–N(9)	105.2 (4)
N(1)–Zn–O(13)	127.2 (3)	N(5)–Zn–N(9)	107.0 (4)
N(5)–Zn–O(13)	109.2 (3)	N(9)–Zn–O(13)	102.5 (3)

[a] ESD in parentheses.

Table II. Intermolecular Hydrogen Bonds for **(11)**$_3$$(ClO_4)_3$·$HClO_4$[a]

proton donor···acceptor		distance, Å
O(13)···O(13′)		2.256 (7)
N(1)···O(16)	$[^2/_3 + x, {}^1/_3 + x - y, {}^{-1}/_6 + z]$	3.09 (1)
N(5)···O(16)	$[^2/_3 - x + y, {}^{-2}/_3 + y, {}^{-1}/_6 + z]$	3.15 (1)
N(5)···O(18)	$[^2/_3 - x + y, {}^{-2}/_3 + y, {}^{-1}/_6 + z]$	3.10 (2)
N(9)···O(14)	$[1 - y, 1 - x, {}^1/_2 + z]$	3.08 (1)

[a] ESD in parentheses.

Results and Discussion

Deprotonation Constants (pK_a) of the Zn^{II}-bound H_2O in Macrocyclic Polyamines. These, along with the $Zn^{II}L$ complexation constants [K(ZnL)], were determined by pH-metric titrations of 1:1 $Zn^{II}SO_4$ and H_nL^{n+} in aqueous solutions (I = 0.10)

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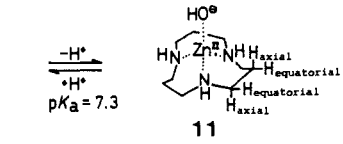
Table III. Zn^{II} Complexation Constants [log K(ZnL)][a] and Deprotonation Constants (pK_a) of Zn^{II}-bound H_2O

ligand	log K(ZnL)[a]	pK_a at 25 °C	pK_a at 0 °C
2	8.41 ± 0.02[b] (8.75)[c]	7.30 ± 0.02[b] (7.51)[c]	7.89 ± 0.03[b]
7	7.01 ± 0.02[b]	7.34 ± 0.02[b]	
6	10.41[c]	8.20[c]	
8	16.2[d]	8.02 ± 0.03[b]	8.54 ± 0.03[b]
9	15.5[d]	9.77 ± 0.05[b]	
10	10.4[e]	8.36[e]	

[a] K(ZnL) = [Zn^{II}L]/[Zn^{II}][L] (M^{-1}) at 25 °C. [b] This work, at I = 0.10 ($NaClO_4$). [c] I = 0.1 (KNO_3), from ref 24. [d] I = 0.2 ($NaClO_4$), from ref 25. [e] I = 0.1 ($NaNO_3$), from ref 26.

Scheme I



11

at 25 and 0 °C (see Figure 1b for **2**). All the results are summarized in Table III. The K(ZnL) and the pK_a values obtained here for **2** are in good agreement with the literature values. For comparison, we have also measured pK_a values for the Zn^{II} complexes with **7** and macrocyclic tetraamines **8** and **9**.

The most interesting observation in this study is that the Zn^{II} ions in larger sized macrocyclic triamines **2** and **7** possess the most acidic nature, with pK_a values of 7.3 (Scheme I), while Zn^{II} ions in macrocyclic tetraamines are less acidic, with pK_a values of >8. It is readily conceivable that in general the fewer the coordinating donors, the higher the affinity for the remaining donor atoms, which accounts for the above observation. Among the N_3 systems, the less stable macrocyclic complexes yield lower pK_a values. We have attempted to measure the pK_a value for the [9]aneN_3 complex. However, at pH >7 precipitations started and the pK_a could not be determined [a similar observation was reported by Zompa at I = 0.1 (KNO_3) and 25 °C].[24] Among the N_4 series, the H_2O–Zn^{II}–**8** showed the most facile deprotonation, while the H_2O attached to Zn^{II}–**9** is an extremely weak acid. Such a large discrepancy probably is caused by the difference in the N_4 complex structures: the smaller 12-membered N_4 **8** would yield a folded *cis*-N_4 configuration,[29] while the larger 14-membered N_4 **9** would yield a square-planar N_4 configuration, as earlier determined by X-ray crystal analysis.[30] The H_2O could bind more strongly to an "open" site of the *cis*-cyclene complex than to closed sites of the *trans*-cyclam complex. In the tetramethylcyclam **10**,[26] Zn^{II} would stay above the N_4 plane with one open coordination site and hence is better exposed to H_2O, resulting in its strengthened acidity.

The pK_a value of 7.3 with **2** is appreciably low compared with the pK_a value of 9.0 for free (solvated) Zn^{II} ion in aqueous solution.[31] It is also much lower than the 8.7 reported for the earlier CA model complex **4**.[11] Most interesting of all is the fact that this value is almost comparable to the pK_a value ~7.5 for CA.[16] Although the deprotonation in CA may be aided by an adjacent imidazole and a hydrophobic environment around the Zn^{II} ion,[1c,32] our study demonstrated that such a low pK_a value can be attained with Zn^{II} complexes of appropriate triamine ligands without assistance from the neighboring base and a hydrophobic environment. We thus predicted that macrocyclic triamine complexes can be better mimics of CA than **4**.

X-ray Crystal Structure of a Zn^{II}–Hydroxo Complex of (11)$_3$(ClO_4)$_3$·$HClO_4$. From an aqueous solution of $Zn^{II}SO_4$ and equimolar **2** titrated with 0.10 M NaOH solution to ca. pH 8 (see Figure 1b), colorless crystals gradually precipitated out as the solution was concentrated. The same complex could be obtained in larger quantity by reaction in EtOH (see Experimental Section). The elemental analysis (C, H, N) and its potentiometric titration suggested the formula (**11**)$_3$(ClO_4)$_3$·$HClO_4$. The final support for the trimeric structure of **11** comes from an X-ray analysis.

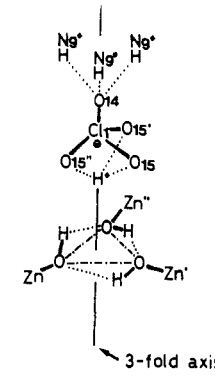


Figure 4. Proposed hydrogen bond network in (**11**)$_3$(ClO_4)$_3$·$HClO_4$ around the 3-fold axis.

Figures 2 and 3 show the numbering scheme in the Zn^{II} complex. There are three [Zn^{II}([12]aneN_3)(OH)]$^+$ ions around a crystallographic 3-fold axis and one $HClO_4$ resides on this axis, which passes through Cl(1) and O(14) (see Figure 3). With Z = 6 in the cell of space group $R3c$ there is only one-third a [(**11**)$_3$·$HClO_4$]$^{3+}$ ion in the asymmetric unit, and the other trimer exists at a neighboring position on the 3-fold axis. The 3+ charge of the unit is balanced by three perchlorate anions in the crystal. Unfortunately, all the hydrogen atoms were not located due to the large thermal vibration. However, one could indirectly guess positions of the H atoms involved in hydrogen bondings.

A schematic representation of the hydrogen bonds around the 3-fold axis is shown in Figure 4. The three Zn^{II}-bound oxygens O(13), O(13′), O(13″) make an extremely small equilateral triangle with the O···O separation of 2.256 (7) Å (see Figure 4). The O···O distances are much shorter than the range for regular linear strong hydrogen bond distances (O···HO) of 2.4–2.8 Å.[33,34] This fact is accounted for by extremely strong hydrogen bonding between the three Zn^{II}-bound OH anions, which might offer a model for the hydrogen bond network that plays a critical role in the CA active center.[35]

The O(14) atom on the 3-fold axis is hydrogen bonded to three macrocyclic nitrogens HN(9) (in the other trimer) with an average distance of 3.08 (1) Å. The Cl(1)–O(14) and –O(15) bond lengths of 1.478 (9) and 1.479 (9) Å are longer than an average distance of 1.43 Å for the counter perchlorate ion [Cl(2)O_4^-] or that of free ClO_4^- anion in other M^{II} complexes (1.38–1.40 Å).[36,37] These results suggest a likely location for the H atom ($HClO_4$) as being just on the 3-fold axis with strong hydrogen bonding to the three O(15) atoms (see Figure 3). It is considered that the amphoteric nature of the Zn^{II} ion renders the solid-state trimeric Zn^{II}–OH hydrogen bonds so stable that even a proton (bound to ClO_4^-) cannot break them.

The zinc atom has a slightly distorted tetrahedral coordination environment. The average angles of O–Zn^{II}–N and N–Zn^{II}–N are 114 and 105°, respectively. The Zn^{II}–OH bond length is extremely short 1.944 (5) Å, which is shorter than Zn^{II}–N lengths of the average 2.02 Å. Furthermore, this Zn^{II}–O bond length is much shorter than the Zn^{II}–Br^- bond length of 2.36 Å in the

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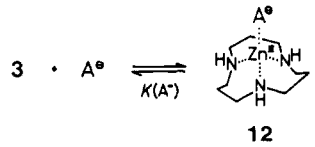
Scheme II



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previously reported [Zn^{II}[12]aneN$_3$(Br)]$^+$ complex.[38] In a predicted tetrahedral Zn^{II} coordinate environment of CA,[39] the Zn^{II}–OH and Zn^{II}–N(imidazole) distances are 1.96 and 2.1 Å, respectively, which are not far off from our values.

This trimeric (**11**)$_3$(ClO$_4$)$_3$·HClO$_4$ structure in crystals is easily torn to two basic Zn^{II}–OH species and one neutral Zn^{II}–OH$_2$ species in aqueous solution, as established titrimetrically (see Experimental Section). Since the symmetrical ^{1}H NMR signal of **11** and only one pK_a value (7.3) to **11** were observed, it is reasonable to assume that the Zn^{II}–OH species **11** acts as a mononuclear complex in aqueous solution.

Macrocyclic [12]aneN$_3$ Configuration of the Zn^{II}–OH Complex 11 in Aqueous Solution. Of the ^{1}H NMR spectrum in D$_2$O solution at 25 °C, the free ligand **2** showed only two types of signals [6 H (quintet) and 12 H (triplet)] for two different methylene protons (NCH$_2$C and CCH$_2$C). This fact indicates that the macrocyclic conformation changes very rapidly. On the other hand, the D$_2$O solutions (1–10 mM) of the above crystalline (**11**)$_3$(ClO$_4$)$_3$·HClO$_4$ and of **11** complex prepared in situ from **2** and equimolar Zn^{II}(ClO$_4$)$_2$·6H$_2$O at pD 10 have given identical ^{1}H NMR spectra, which present four types of multicoupled proton signals, distinctively assignable to the axial and equatorial protons of NCH$_2$C and CCH$_2$C (see Experimental Section). This fact supports a notion that in solution, the Zn^{II} complex is very inert (i.e., little Zn^{II}–N dissociation) and the macrocyclic configuration in the Zn^{II} complex **11** is rigid, with the three propylene groups adopting the same chair configuration as shown by the crystal structure. This ^{1}H NMR pattern does not change during the following catalytic reactions.

Anion and Acetazolamide Affinity to Zn^{II}[12]aneN$_3$ Complex. Since some chemical analogies have been found between CA and **3**, we searched for further common properties. The first test was whether Zn^{II} in our model complex **3** has a similar trend of affinity for anions or acetazolamide as found for CA, which has been explained as the cause of inhibition.[40] In order to determine the anion binding and also the constants K(A$^-$) for 1:1 Zn^{II}L–anion complex **12**, we have conducted the pH-metric titration of 1 mM Zn^{II} and 1 mM [12]aneN$_3$·3H$^+$ in the presence of a large excess A$^-$ (see Figure 1c for 0.20 M SCN$^-$). From the comparison of the titration curves Figure 1b and c at lower pH (<7) a possible Zn^{II}L–SCN complex in the presence of excess SCN$^-$ is formed, but at pH >7 the Zn^{II}L–OH species is more stable than the Zn^{II}L–SCN species. Elaborate calculations (see Experimental Section) of the titration curves have established the following equilibrium (Scheme II) and the affinity constants K(A$^-$). Table IV summarizes the log K(A$^-$) values, along with the literature K(A$^-$) values for CA.[18,19]

It is quite remarkable to note that the order and magnitude of K(A$^-$) values are quite common with those reported for CA and that OH$^-$ possesses the strongest affinity to Zn^{II} among the anions. The former fact accords with the 1:1 anion–Zn^{II} active-site complexation in CA, as proposed.[18,19] An extrapolation of the latter fact will be that the weaker binding bicarbonate anion, formed from CO$_2$ on Zn^{II} of CA, tends to be replaced by the stronger binding OH$^-$, thereby the catalytic cycle goes on smoothly. Unfortunately, the affinity of bicarbonate to our Zn^{II} complex **3** coupld not be determined due to its rapid decomposition to CO$_2$. For comparison, the 1:1 anion binding constants for hydrated Zn^{II}(H$_2$O)$_n$ are cited in Table IV, which illustrate different behaviors from **3**.

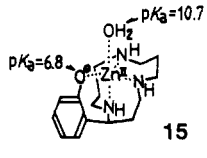
Table IV. Anion Complex Stability Constants log K(A$^-$) at 25 °C and I = 0.20 (NaClO$_4$)[a]

anion	3	Zn^{II}–CA		Zn^{II}(H$_2$O)$_n$
OH$^-$	6.4 ± 0.1	6.5[b]	6.5[c]	5.0[d]
CH$_3$COO$^-$	2.6 ± 0.1	2.1[b]	1.5[c]	0.9[e]
SCN$^-$	2.4 ± 0.1	3.2[b]	2.8[c]	0.7[e]
I$^-$	1.6 ± 0.1	1.2[b]	2.1[c]	−1.5[e]
Br$^-$	1.5 ± 0.1	1.1[b]	1.8[c]	−0.6[e]
F$^-$	0.8 ± 0.1	−0.1[b]		0.8[e]
HCO$_3^-$		1.6[b]		0.3[e]

[a] K(A$^-$) = [complex–A$^-$]/[complex][A$^-$] (M^{-1}). [b] Calculated values using inhibition activities of bovine CA in 4-nitrophenyl acetate hydrolysis, from ref 18. [c] Calculated values using inhibition activities of bovine CA in CO$_2$ hydration, from ref 19. [d] At 25 °C and I = ca. 0, from ref 31. [e] At 25 °C and I = 1.0, from ref 31.

Acetazolamide (**13**) is a strong inhibitor of CA,[18,40] the postulated mechanism being that **13** strongly binds with the active metal center as an anionic donor (like the above anions). We therefore treated **13** with equivalent Zn^{II} and **2** in CH$_3$CN and discovered crystalline precipitates, whose characterization with elemental analysis, IR, UV, and ^{1}H NMR spectral measurements (see Experimental Section) has indeed indicated a 1:1 **13**–(Zn^{II}–[12]aneN$_3$) complex **14** wherein the sulfonamide is deprotonated. In previous attempts to prepare such metal–**13** complexes the donor site was often not the deprotonated N$^-$ but the thiadiazole N (e.g., M = Ni^{II}).[41] Since good crystals could not be obtained for X-ray study, the exact complex structure **14** is somewhat open to question (e.g., whether the deprotonated acetazolamide is a monodentate with N$^-$ binding or a bidentate with N$^-$ and O donors). At any rate, it is of great interest to point out that the pK_a value of 7.42 at 25 °C and I = 0.1 (NaClO$_4$) for acetazolamide (determined pH-metrically) is almost identical with that for the H$_2$O bound to Zn^{II}–[12]aneN$_3$. Thus, the deprotonation of the sulfonamide with simultaneous coordination is conceivable and such a model complex seems to verify the reactivity of acetazolamide with Zn^{II} in CA. In order to determine the binding affinity of acetazolamide anion, we attempted to measure its complexation constant (like other anions), but the low solubility of acetazolamide in aqueous solution blocked our efforts.

Phenol is also an inhibitor of CA.[18] Earlier, we reported a phenol-pendant [12]aneN$_3$ complex **15** with Zn^{II}.[37] Its crystal



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structure is trigonal bipyramidal with an extremely short phenolate–Zn^{II} bond distance (1.93 Å). The H$_2$O bound to Zn^{II} (distance 2.22 Å) has a pK_a value of 10.7, indicating a greatly reduced (from 7.3 of **3**) acidity of Zn^{II} due to the phenolate (pK_a = 6.8) coordination. Such a 5-coordinate structure with a strong inhibitor also may account for the loss of the CA nucleophilic activity at physiological pH.

Hydration of Acetaldehyde with Zn^{II}–[12]aneN$_3$ Complex. CA is also an effective catalyst for the hydration of acetaldehyde (eq 9). This kinetic study at 0 °C is much easier than the hydration

$$CH_3CHO + H_2O \underset{k_-}{\overset{k_+}{\rightleftharpoons}} CH_3CH(OH)_2;\ k_{obs} = k_+ + k_-\ (s^{-1}) \quad (9)$$

of CO$_2$, since the former reaction could be studied in the ultraviolet by following the decrease in the carbonyl band at 278 nm. Our procedure with the Zn^{II} model complex is identical with the one reported with CA[42] and **4**.[27]

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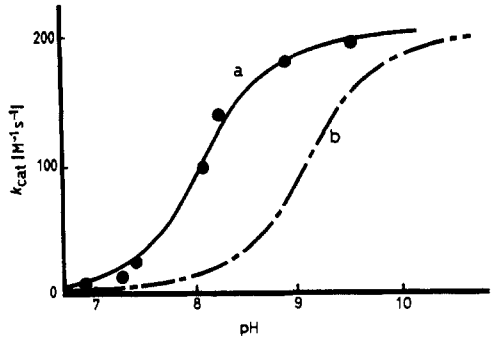
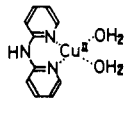


Figure 5. Second-order rate constants (k_{cat} [M^{-1} s^{-1}]) of acetaldehyde hydration as a function of pH. (a) With Zn^{II}–[12]aneN$_3$ complex; (b) with **4**, data collected from ref 11. pK_a values from these curves are 8.0 and 9.1, respectively.

For the determination of the catalytic second-order rate constant k_{cat} (M^{-1} s^{-1}), an initial concentration of acetaldehyde was kept constant at 35 mM. The reaction followed good pseudo-first-order kinetics both in the presence and in the absence of the trace amount of catalysts (<1 mM). The hydration process is reversible and its equilibrium constant at 0 °C is 2.3.[28] The hydration of acetaldehyde is general-acid–general-base catalyzed, and hence the control rates (only in the absence of the Zn^{II} complex) were always determined. A plot of apparent first-order rate constant k_{obs} (s^{-1}) vs [total Zn^{II} complex] at a given pH gave a straight line with an intercept, the slope of which was defined as k_{cat} (M^{-1} s^{-1}). This procedure was followed at seven different pH values (Figure 5a). The resulting pH–rate profile reveals a point of inflection at pH ca. 8, strongly suggesting that this is the same as the deprotonation constant pK_a of 7.9 (at 0 °C) determined thermodynamically, and hence, the Zn^{II}–OH species must play a critical role in the catalytic activity. The same pH–rate profiles for CA and **4** gave inflection points of 7.0[28] and 9.1[11] (see Figure 5b), respectively, each being identical with the pK_a value of the Zn^{II}-bound H_2O. These three data suggest the common importance of the deprotonated Zn^{II}–OH species. The maximum rate constant (k_{cat}) of 200 M^{-1} s^{-1} is similar to 196 M^{-1} s^{-1} reported for **4**,[11] which is ca. one-seventh the value for bovine CA (1.4×10^3 M^{-1} s^{-1}),[42] suggesting a similar catalytic efficiency by the model Zn^{II}–OH species. The major difference between **3** and **4**, however, lies in the lower pK_a value for our model, which is closer to the behavior of CA.

Thus, our CA model is kinetically proven to be a better model, too.

Hydrolysis of Methyl Acetate with Zn^{II}–[12]aneN$_3$ Complex. Although metal complex catalyzed hydrolysis of activated esters has been well studied,[43-45] hydrolysis of unactivated esters under mild conditions (e.g., pH 7 and 25 °C) has been rarely achieved. Very recently, only a few complexes such as **16**[10] and **17**[6] have



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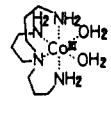

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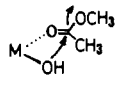

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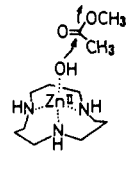

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succeeded in catalytic hydrolysis of methyl acetate, where the

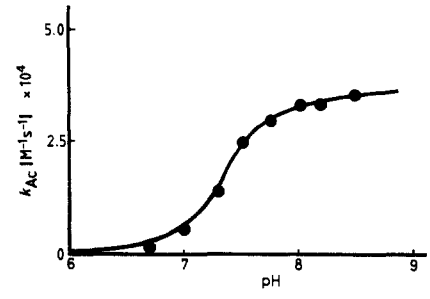
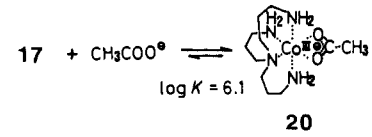


Figure 6. Second-order rate constants (k_{Ac} [M^{-1} s^{-1}]) of methyl acetate hydrolysis as a function of pH with the Zn^{II}–[12]aneN$_3$ complex. pK_a value from this curve is 7.3.

Scheme III



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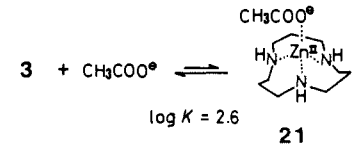
Scheme IV



21

hydrolysis involves coordination of the ester to one of the two vacant sites, followed by the intramolecular attack of M–OH on the coordinated ester (**18**). Since CA was also demonstrated to act as an esterase,[46,47] we have investigated the catalytic efficiency in the methyl acetate hydrolysis.

Catalytic activity was indeed discovered in neutral water at 25 °C. The catalyzed hydrolysis of methyl acetate (0.05–1.00 M) with Zn^{II}–[12]aneN$_3$ complex (0.5–5 mM) was monitored by the pH-stat method. The hydrolysis was checked with ^{1}H NMR to see the acetate and methanol CH_3 groups. The catalytic turnover time with our system is ca. 60 min at [CH_3COOCH_3] = 1 M, pH 8, and 25 °C, which is to be compared with 23 min with the Cu^{II} complex **16** at [CH_3COOCH_3] = 1 M, pH 7.0, and 25 °C[10] and 34 min with the Co^{III} complex **17** at [CH_3COOCH_3] = 1 M, pH 7.6, and 25 °C.[6] The somewhat slower rate with the Zn^{II} complex may arise from the inability of Zn^{II} to form the 5-coordinate intermediate **18**, necessitating going through a less efficient bimolecular reaction mechanism **19**. As predicted, when the Zn^{II}(cyclam) complex was used, ester hydrolysis was too slow to be observed even at pH 9.

The second-order rate constants K_{Ac} (M^{-1} s^{-1}) were obtained at various pH. The rate–pH profile (Figure 6) is sigmoidal, as in the case for acetaldehyde hydration, and the kinetically obtained pK_a of 7.3 is the same as the thermodynamic pK_a. Like the hydration reaction, this fact indicates the rate-determining step as involving attack of Zn^{II}–OH to the ester carbonyl group. As anticipated, the CA inhibitors deactivated **11**: e.g., Br^- and SCN^- (both at 0.20 M) lowered k_{Ac} to $^1/_3$ and $^1/_{10}$ [compared at 0.20 M ($NaClO_4$)], respectively.

Our catalysis system has another major difference from the previous Co^{III} complex model **17**, where the product complex with a bidentate, acetate **20** is stable enough to be isolable with the complexation constant log K of 6.1 (Scheme III).[6] On the other hand, in our Zn^{II} model, log K for the monodentate, acetate complex **21** (Scheme IV) is smaller 2.6, as listed in Table IV. In this respect, the Zn^{II} system is more favorable for catalytic turnover

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Table V. Second-Order Rate Constants k_{NP} (M^{-1} s^{-1}) for Hydrolysis of 4-Nitrophenyl Acetate at 25 °C

nucleophile	k_{NP}	pK_a (H_2O)
11	4.1×10^{-2} [a]	7.3
$(NH_3)_5Co^{III}OH$	1.5×10^{-3} [b]	6.4
bovine carbonic anhydrase	4.0×10^{2} [c]	7.5
OH^-	9.5[d]	15.5

[a] At pH 8.2 (50 mM HEPES buffer) and I = 0.10 ($NaClO_4$). [b] From ref 3. [c] pH 8.9, from ref 46. [d] From ref 48.

cycles due to the more facile liberation of the product anion.

Comparison of the Nucleophilicity with Other Hydroxo Species. The aforementioned acetaldehyde hydration and methyl acetate hydrolysis reactions have established that the Zn^{II}–OH species is acting as a nucleophile toward the substrates. In order to compare the intermolecular nucleophilicity of the Zn^{II}–OH species with that of the well studied Co^{III}-bound hydroxide species ($(NH_3)_5Co^{III}$–OH),[3] where neither has an extra vacant site for the prior ester coordination, we ran a kinetic study of 4-nitrophenyl acetate hydrolysis. Here again, the plots of the second-order rate constant vs pH gave a sigmoidal curve like the previous two reaction cases, and its midpoint pH corresponded almost to the pK_a value of 7.3. The resulting second-order rate constant k_{NA} for **11** is summarized in Table V.

It is concluded that as a nucleophile the Zn^{II}–OH is ~1 order of magnitude more reactive than $(NH_3)_5Co^{III}$–OH toward the same ester substrate. The same direct nucleophilic mechanism was proposed for the Co^{III}–OH reaction.[3] Taking into consideration the charge and ligand field difference of the metal ions, this order of nucleophilic efficiency would be reasonable. Note the pK_a of 6.4 for $(NH_3)_5Co^{III}$–OH_2 vs 7.3 for **3**. Compared with free OH^- ion,[48] the Zn^{II}-bound OH^- ion is ~250 times less reactive in 4-nitrophenyl acetate hydrolysis reaction. However, if the pK_a values of 15.5 and 7.3 for free H_2O and Zn^{II}–OH_2, respectively, are considered, the latter would make a more effective nucleophile at neutral pH.

Conclusion

Zinc(II) complex **3** of a symmetrical 12-membered macrocyclic triamine [12]aneN$_3$ **2** has been shown to be the most suitable model for the active site of carbonic anhydrase (CA), where Zn^{II} is bound by three histidine imidazole residues and by a water or hydroxide as the fourth ligand. The pK_a value of 7.3 (at 25 °C and I = 0.1) and inhibitor (such as anions and acetazolamide) binding mode reported for CA are reproduced for the first time with **3**. Zn^{II}–[12]aneN$_3$(OH) **11** is isolable as a trimer, whose X-ray study has proven that the unit **11** takes a 4-coordinate, tetrahedral structure. The extremely short Zn^{II}–OH bond distance of 1.94 Å suggests a strong affinity of Zn^{II} for OH^- and accounts for the extremely low pK_a of 7.3. Such strong affinity of **3** to OH^- ion surpasses any other anions including CA-catalyzing reaction products, CH_3COO^- (from ester substrates) or possibly HCO_3^- (from hydration of CO_2). This fact, characteristic of the Zn^{II} ion, is very favorable for the catalytic cycles. In the two CA-catalyzing reactions, acetaldehyde hydration and ester hydrolysis, the second-order kinetics and the plots of the rate constant vs pH point to a common reaction mechanism involving the direct nucleophilic attack of Zn^{II}–OH at the carbonyl carbons. Although these rates are much slower than those with CA, the reaction mechanism is the same as the one currently accepted for CA. In comparison of the metal-bound OH nucleophilicity toward 4-nitrophenyl acetate, the Zn^{II}–OH is ca. 10 times more powerful than the previously reported $(NH_3)_5Co^{III}$–OH.[3] It is concluded that the amphoteric Zn^{II}-bound OH retains stronger nucleophilicity than the acidic Co^{III}-bound OH. We have found that the nucleophilic power of the Zn^{II}–OH also effects phosphate ester hydrolysis, which will be reported elsewhere. Finally, further structural modification of the basic structure of **2** (e.g., attachment of intramolecular bases to aid proton transfers or of other substituents for substrate recognition or for polarity change, etc.) is likely to yield even closer CA and other Zn^{II}-containing hydrolytic enzyme models, which is currently under investigation in our laboratory.

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Supplementary Material Available: ^1H NMR chart of **11** in D_2O solution, plots of $1/K'$ vs concentration of anion (SCN^- and Br^-) and of k_{obs} vs Zn^{II} complex concentration, and tables of anisotropic temperature factors, crystal data and data collection summary, final fractional coordinates and equivalent isotropic temperature factors, bond distances, and bond angles (9 pages); listing of observed and calculated structure factors for the Zn^{II}[12]aneN$_3$–OH complex (9 pages). Ordering information is given on any current masthead page.

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Sulfonamidoglycosylation of Glycals. A Route to Oligosaccharides with 2-Aminohexose Subunits†

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Abstract: Reactions of glycals with the combination iodonium di-*sym*-collidine perchlorate (**5**) and benzenesulfonamide (**6**) afforded, stereoselectivity, 2-β-iodo-1-α-sulfonamidohexoses. This process was demonstrated with D-glucal, D-galactal, and D-allal. Treatment of these products with strong base apparently generated a C_1–C_2 sulfonylaziridine. A 2-α-sulfonamide-1-β-linked disaccharide was produced when this reaction was carried out with excess base in the presence of a glycosyl acceptor.

Glycosides of N-acylated 2-amino-2-deoxy saccharides are important subunits of many glyconjugates such as glycoproteins,[1] chitin,[2] and heparin.[3] Several elegant methods have been employed to generate[4] and couple[5] 2-amino-2-deoxy carbohydrates. A particularly interesting method was recently disclosed by Leblanc, Fitzsimmons, and co-workers.[6] Their procedure involved the cycloaddition of an azodicarboxylate to the double bond of

† We dedicate this paper in memory of Dr. Brian Fitzsimmons for his studies on the azaglycosylation of glycals.

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