*Inorg. Chem.* **2002**, *41*, 3239–3248

# Study of pH-Dependent Zinc(II)–Carboxamide Interactions by Zinc(II)–Carboxamide-Appended Cyclen Complexes (Cyclen = 1,4,7,10-Tetraazacyclododecane)

**Eiichi Kimura,*,† Teruhiro Gotoh,† Shin Aoki,† and Motoo Shiro‡**

*Department of Medicinal Chemistry, Faculty of Medicine, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima, 734-8551 Japan, and Rigaku Corporation X-ray Research Laboratory, Matsubaracho 3-9-12, Akishima, Tokyo, 196-8666 Japan*

Received January 29, 2002

To elucidate intrinsic recognition of carboxamides by zinc(II) in carbonic anhydrase (CA) (as inhibitors) and carboxypeptidase A (CPA) (as substrates), a new series of $Zn^{2+}$–carboxamide-appended cyclen complexes have been synthesized and characterized (cyclen = 1,4,7,10-tetraazacyclododecane). Two types of $Zn^{2+}$–carboxamide interactions have been found. In the first case represented by a zinc(II) complex of carbamoylmethyl-1,4,7,10-tetraazacyclododecane ($L^1$), the amide oxygen binds to zinc(II) at slightly acidic pH (to form $ZnL^1$), and the deprotonated amide $N^-$ binds to zinc(II) at alkaline pH (to form $ZnH_{-1}L^1$) with p$K_a$ = 8.59 at 25 °C and $I$ = 0.1 ($NaNO_3$), as determined by potentiometric pH titrations, infrared spectral changes, and $^{13}C$ and $^1H$ NMR titrations. The X-ray crystal structure of $ZnH_{-1}L^3$ (where $L^3$ = *N*-(4-nitrophenyl)carbamoylmethyl cyclen, p$K_a$ = 7.01 for $ZnL^3 \rightleftarrows ZnH_{-1}L^3$) proved that the zinc(II) binds to the amidate $N^-$ (Zn–$N^-$ distance of 1.974(3) Å) along with the four nitrogen atoms of cyclen (average Zn–N distance 2.136 Å). Crystal data: monoclinic, space group $P2_1/n$ (No. 14) with $a$ = 10.838(1) Å, $b$ = 17.210(2) Å, $c$ = 12.113(2) Å, $b$ = 107.38(1)°, $V$ = 2156.2(5) Å$^3$, $Z$ = 4, $R$ = 0.042, and $R_w$ = 0.038. These model studies provide the first chemical support that carboxamides are $CA^-$ inhibitors by occupying the active $Zn^{2+}$ site both in acidic and alkaline pH to prevent the occurrence of the catalytically active $Zn^{2+}$–$OH^-$ species. In the second case represented by a zinc(II) complex of 1-(*N*-acetyl)aminoethylcyclen, $ZnL^6$, the pendant amide oxygen had little interaction with zinc(II) at acidic pH. At alkaline pH, the monodeprotonation yielded a zinc(II)-bound hydroxide species $ZnL^6(OH^-)$ (p$K_a$ = 7.64) with the amide pendant remaining intact. The $ZnL^6(OH^-)$ species showed the same nucleophilic activity as $Zn^{2+}$–cyclen–$OH^-$. The second case may mimic the $Zn^{2+}$–$OH^-$ mechanism of CPA, where the nucleophilic $Zn^{2+}$–$OH^-$ species does not act as a base to deprotonate a proximate amide.

## Introduction

Carbonic anhydrase (CA, EC 4.2.1.1) and carboxypeptidase A (CPA, EC 3.4.17.1) are mechanistically the two most typical zinc(II) enzymes.[1] CA catalyzes the reversible hydration of $CO_2$ to bicarbonate ion,[2] and its active-site zinc(II) is bound to His94, His96, His119, and a water molecule. CPA catalyzes the hydrolysis of hydrophobic C-terminal amino acids from polypeptide substrates,[3] and its active-site zinc(II) is bound to His69, Glu72, His196, and a water molecule hydrogen bonding to Glu270 (Scheme 1). In a widely accepted $Zn^{2+}$−hydroxide mechanism for CA and CPA, the zinc(II)-bound waters that are generated at neutral pH are activated to attack at the electrophilic sites of

* To whom correspondence should be addressed. E-mail: ekimura@hiroshima-u.ac.jp.

† Hiroshima University.

‡ Rigaku Corporation.

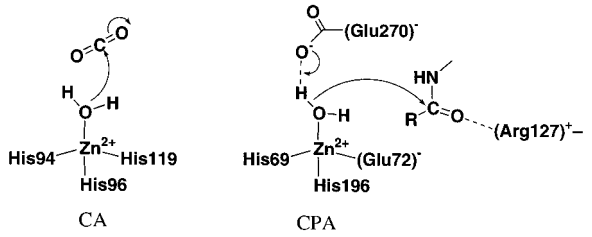
(1) (a) Walsh, C. *Enzymatic Reaction Mechanisms*; Freeman and Co.: New York, 1979. (b) Voet, D.; Voet, J. G. *Biochemistry*; Wiley & Sons: New York, 1990. (c) Lipscomb, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2375−2433. (d) Coleman, J. E. *Curr. Opin. Chem. Biol.* **1998**, *2*, 222−234.

(2) (a) Botrè, F.; Gros, G.; Storey, B. T. *Carbonic Anhydrase*; VCH: Weinheim, 1991. (b) Bertini, I.; Luchinat, C. *Acc. Chem. Res.* **1983**, *16*, 272−279. (c) Silverman, D. N.; Lindskog, S. *Acc. Chem. Res.* **1988**, *21*, 30−36. (d) Liljas, A.; Håkansson, K.; Jonsson, B. H.; Xue, Y. *Eur. J. Biochem.* **1994**, *219*, 1−10. (e) Christianson, D. W.; Flerke, C. A. *Acc. Chem. Res.* **1996**, *29*, 331−339.

(3) (a) Quiocho, F. A.; Lipscomb, W. N. *Adv. Protein Chem.* **1971**, *25*, 1−78. (b) Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62−69.

10.1021/ic020087k CCC: $22.00 
Published on Web 05/17/2002

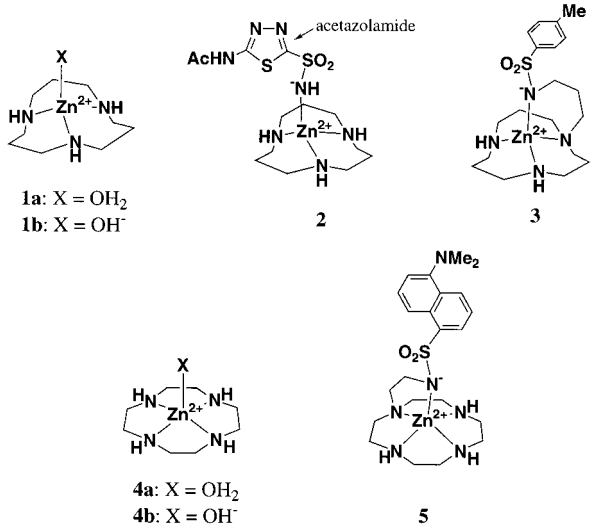
**Scheme 1**




polarized carbonyl substrates. It is interesting that carboxamides are substrates for CPA and inhibitors for CA.[4] The visible spectral study of binding of iodoacetamide ($K_i = 40$ mM, at pH 8.1) to cobalt(II)-substituted CA I suggested $N^-$ coordination of the amidate anion to cobalt(II).[4b] Until now, however, there have been few chemical models for this type of amide or amidate association with $Zn^{2+}$. More fundamentally, the p$K_a$ values of the amide $\rightleftarrows$ amidate in the vicinity of $Zn^{2+}$ were not reported.[5] On the other hand, aromatic sulfonamides, which are stronger CA inhibitors than carboxamides, are now well established to bind to zinc(II) as amidate anions at physiological conditions.[6]

How do $Zn^{2+}$ ions work differently toward the carboxamides in CA and CPA? It may be helpful to consider that the acidity of zinc(II) is probably higher in CA with the ligation of three neutral donors $(His)_3$ than in CPA with $(His)_2(Glu^-)$. This argument is compatible with a fact that the p$K_a$ of 6.8 for the zinc-bound water in the wild-type CA II is increased to $\geq$9.6 in a mutant CA II with His94 $\rightarrow$ $Asp^-$, as kinetically determined by hydrolysis of 4-nitrophenyl acetate.[7] The more acidic $Zn^{2+}$ in CA may be more favorable in attracting a carboxamide at the fourth coordination site than the less acidic $Zn^{2+}$ in CPA. Then, intrinsic properties of $Zn^{2+}$ to be questioned are (1) how do the carboxamide inhibitors and the nucleophile hydroxide compete for $Zn^{2+}$ in CA? (2) what are the p$K_a$ values for deprotonating the $Zn^{2+}$-bound carboxamides? and (3) why does the catalytically active $Zn^{2+}-OH^-$ species in CPA (see Scheme 1) not work as a base toward substrate carboxamides to turn it from a substrate to an inhibitor?

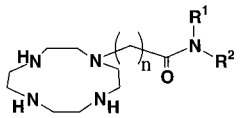
In our earlier zinc enzyme model studies, zinc(II)−1,5,9-triazacyclododecane ($Zn^{2+}-$[12]ane$N_3$) complex **1** has been shown to be one of the most suitable mechanistic models for CA in aqueous solution.[8] The water at the fourth coordination site of **1a** can be deprotonated to **1b** with a very low p$K_a$ value of 7.3 at 25 °C.[9a] Compound **1** has revealed the intrinsic role of zinc(II) of CA in catalyzing the reversible $CO_2$ hydration and the carboxyester hydrolysis.[9b] Moreover, a typical CA inhibitor acetazolamide reacted with **1** to yield 1:1 complex **2** at physiological pH, wherein the sulfonamide nitrogen of acetazolamide is deprotonated.[10] The zinc(II) ion in the [12]ane$N_3$ complex lowered the p$K_a$ value of an intramolecular sulfonamide from 11.2 to <7 to yield a very stable sulfonamidate complex **3** at neutral pH,[11] which lost the catalytic activity. Later, a zinc(II) complex of 1,4,7,10-tetraazacyclododecane (cyclen), **4a**, also produced a nucleophilic and simultaneously basic zinc(II)$-OH^-$ species (**4b**) with p$K_a$ of 7.9 at 25 °C[10] and acted as another CA model.[12] A greater advantage of the macrocyclic ligand cyclen over [12]ane$N_3$ is that the zinc(II) is more firmly held with higher thermodynamic and kinetic stability. A dansylamide-pendant cyclen was designed as a model for fluorescent dansylamide binding to CA, which made an efficient and selective $Zn^{2+}$ fluorosensor in the form of **5** (cyclen = 1,4,7,10-tetraazacyclododecane).[13] The $Zn^{2+}-$cyclen complex **4** catalytically hydrolyzed an activated carboxamide in $\beta$-lactam ring of penicillin as a model for zinc(II)-containing $\beta$-lactamase II.[14]



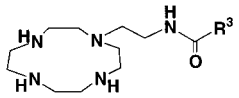

Accordingly, different modes of recognition of carboxamides by CA and CPA might be mimicked by using carboxamide-appended cyclens, such as carbamoylmethylcyclen **6** ($L^1$), *N*-benzylcarbamoylmethylcyclen **7** ($L^2$), *N*-(4-nitrophenyl)carbamoylmethylcyclen **8** ($L^3$), *N*,*N*′-diethylcar-

(4) (a) Whitney, P. L. *Eur. J. Biochem.* **1970**, *16*, 126−135. (b) Rogers, J. I.; Mukherjee, J.; Khalifah, R. G. *Biochemistry* **1987**, *26*, 5672−5679. (c) Liang, J. Y.; Lipscomb, W. N. *Biochemistry* **1989**, *28*, 9724−9733.
(5) Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385−426.
(6) (a) Eriksson, A. E.; Kylsten, P. M.; Jones, T. A.; Liljas, A. *Proteins* **1988**, *4*, 283−293. (b) Boriack, P. A.; Christianson, D. W.; Kingery-Wood, J.; Whitesides, G. M. *J. Med. Chem.* **1995**, *38*, 2286−2291.
(7) Kiefer, L. L.; Ippolito, C. A.; Christianson, D. W. *J. Am. Chem. Soc.* **1993**, *115*, 12581−12582.
(8) For review: (a) Kimura, E. In *Progress in Inorganic Chemistry*; Karlin, K. D., Ed.; John Wiley & Sons: New York, 1994; Vol. 41, pp 443−491. (b) Kimura, E.; Koike, T. In *Advances in Inorganic Chemistry*; Sykes, A. G., Ed.; Academic Press: New York, 1997; Vol. 44, pp 229−261. (c) Kimura, E.; Koike, T. In *Comprehensive Supramolecular Chemistry*; Reinhoudt, D. N., Ed.; Pregamon: Tokyo, 1996; Vol. 10, pp 429−444. (d) Kimura, E.; Koike, T. *J. Chem. Soc., Chem. Commun.* **1998**, 1495−1500. (e) Kimura, E.; Koike, T. In *Bioinorganic Catalysis*; Reedijik, J., Bouwman, E., Ed.; Marcel Dekker, Inc.: New York, 1999; pp 33−54. (f) Kimura, E.; Kikuta, E. *J. Biol. Inorg. Chem.* **2000**, *5*, 139−155. (g) Kimura, E. *Acc. Chem. Res.* **2001**, *34*, 171−179.
(9) (a) Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805−5811. (b) Zhang, X.; van Edlik, R.; Koike, T.; Kimura, E. *Inorg. Chem.* **1993**, *32*, 5749−5755.
(10) Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1991**, *113*, 8935−8941.
(11) Koike, T.; Kimura, E.; Nakamura, I.; Hashimoto, Y.; Shiro, M. *J. Am. Chem. Soc.* **1992**, *114*, 7338−7345.
(12) Zhang, X.; van Edlik, R. *Inorg. Chem.* **1995**, *34*, 5606−5614.
(13) (a) Koike, T.; Watanabe, T.; Aoki, S.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 12696−12703. (b) For review: Kimura, E.; Koike, T. *Chem. Soc. Rev.* **1998**, *27*, 179−184.
(14) Koike, T.; Takamura, M.; Kimura, E. *J. Am. Chem. Soc.* **1994**, *116*, 8443−8449.

bamoylmethylcyclen **9** ($L^4$), 2-(carbamoyl)ethylcyclen **10** ($L^5$), 2-(*N*-acetylamino)ethylcyclen **11** ($L^6$), and 2-((4-nitrobenzoyl)amino)ethylcyclen **12** ($L^7$).



**6** ($L^1$) : n = 1, $R^1 = R^2 = H$
**7** ($L^2$) : n = 1, $R^1 = H$, $R^2 = CH_2Ph$
**8** ($L^3$) : n = 1, $R^1 = H$, $R^2$ = 4-nitrophenyl
**9** ($L^4$) : n = 1, $R^1 = R^2 = Et$
**10** ($L^5$) : n = 2, $R^1 = R^2 = H$



**11** ($L^6$) : $R^3$ = Me
**12** ($L^7$) : $R^3$ = 4-nitrophenyl

## Experimental Section

**General Information.** All reagents and solvents were purchased at the highest commercial quality and used without further purification. Anhydrous acetonitrile ($CH_3CN$) was obtained by distillation from calcium hydride. All aqueous solutions were prepared using deionized and distilled water. The Good's buffers (Dojindo) were commercially available: MOPS (3-(*N*-morpholino)-propanesulfonic acid, p$K_a$ = 7.2), HEPES (*N*-(2-hydroxyethyl)-piperazine-*N*′′-2-ethanesulfonic acid, p$K_a$ = 7.5), EPPS (3-(4-(2-hydroxyethyl)-1-piperazinyl)propanesulfonic acid, p$K_a$ = 8.0), TAPS (*N*-(tris(hydroxymethyl)methylamino)-3-propanesulfonic acid, p$K_a$ = 8.4), and CHES (2-(cyclohexylamino)ethanesulfonic acid, p$K_a$ = 9.5). *N*-(4-Nitrophenyl)chloroacetamide and *N*-benzylchloroacetamide were prepared by standard procedure from the corresponding acid chloride and amine. UV spectra were recorded on a Hitachi U-3500 spectrophotometer equipped with a temperature controller unit at 25 ± 0.1 °C. IR spectra were recorded on a Horiba FTIR-710 spectrophotometer at room temperature. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR spectra at 35 ± 0.1 °C were recorded on a JEOL Delta 500 spectrometer. 3-(Trimethylsilyl)propionic-2,2,3,3-$d_4$ acid sodium salt in $D_2O$ and tetramethylsilane in $CD_3CN$ were used as internal references for $^1$H and $^{13}$C NMR measurements. The pD values in $D_2O$ were corrected for a deuterium isotope effect using pD = (pH-meter reading) + 0.40. Elemental analyses were performed on a Perkin-Elmer CHN 2400 analyzer. Thin-layer (TLC) and silica gel column chromatographies were performed using a Merck 5554 (silica gel) TLC plate and Fuji Silysia Chemical FL-100D, respectively.

**1-Carbamoylmethyl-1,4,7,10-tetraazacyclododecane Trihydrochloric Acid Salt (6·3HCl·3H$_2$O), 1-(*N*-Benzylcarbamoylmethyl)-1,4,7,10-tetraazacyclododecane Trihydrochloric Acid Salt (7·3HCl·3H$_2$O), and 1-(*N*-(4-Nitrophenyl)carbamoylmethyl)-1,4,7,10-tetraazacyclododecane Trihydrochloric Acid Salt (8·3HCl·0.5H$_2$O).** A solution of 3Boc-cyclen (3.8 g, 8.0 mmol)[15] and the corresponding 2-bromo- (or 2-chloro-) acetamide derivatives (9.6 mmol) (2-bromoacetamide for **6**, *N*-benzyl-2-chloroacetamide for **7**, or 2-chloro-*N*-(4-nitrophenyl)acetamide for **8**, respectively) in $CH_3CN$ (50 mL) was stirred in the presence of $Na_2CO_3$ (9.6 mmol) and NaI (9.6 mmol) at 80 °C under an argon atmosphere for 1 day. After insoluble inorganic salts were filtered off, the filtrate was concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography (hexane/AcOEt) to obtain **14**, **15**, or **16**. These compounds were deprotected with 4 M HCl in 1,4-dioxane for 0.5−2 h at 0 °C to room temperature, and precipitates were recrystallized from $H_2O$ to afford **6**·3HCl·3H$_2$O, **7**·3HCl·3H$_2$O, or **8**·3HCl·0.5H$_2$O.

(15) Kimura, E.; Aoki, S.; Koike, T.; Shiro, M. *J. Am. Chem. Soc*. **1997**, *119*, 3068−3076.

**6**·3HCl·3H$_2$O (58% yield): Mp 250−251 °C dec. IR (KBr): 3322, 2739, 1688, 1605, 1478, 1441, 1370, 1316, 1267, 1020 cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 3.01−3.19 (16H, m), 3.48 (2H, s). $^{13}$C NMR ($D_2O$): $\delta$ 45.26, 45.70, 47.33, 52.82, 57.77, 179.00. Anal. Calcd for $C_{10}H_{32}N_5O_4Cl_3$: C, 30.58; H, 8.21; N, 17.83. Found: C, 30.57; H, 8.13; N, 17.81. **7**·3HCl·3H$_2$O (75% yield): Mp 217−219 °C. IR (KBr): 3441, 2965, 2934, 1657, 1545, 1453, 1370, 702 cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 3.00−3.19 (16H, m), 3.51 (2H, s), 4.42 (2H, s), 7.34−7.43 (5H, m). $^{13}$C NMR ($D_2O$): $\delta$ 43.27, 43.70, 45.39, 50.81, 56.46, 128.27, 128.58, 129.85, 138.65, 174.07. Anal. Calcd for $C_{17}H_{38}N_5O_4Cl_3$: C, 42.28; H, 7.93; N, 14.50. Found: C, 42.31; H, 8.11; N, 14.51. **8**·3HCl·0.5H$_2$O (54% yield): Mp 242−244 °C dec. IR (KBr): 3001, 2643, 1701, 1614, 1597, 1555, 1505, 1410, 1343, 1300, 1258 cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 3.07−3.27 (16H, br), 3.70 (2H, s), 7.62 (2H, d, $J$ = 9.2 Hz), 8.17 (2H, d, $J$ = 9.2 Hz). $^{13}$C NMR ($D_2O$): $\delta$ 45.13, 45.69, 47.44, 52.85, 59.15, 123.28, 128.02, 146.20, 146.47, 175.21. Anal. Calcd for $C_{16}H_{30}N_6O_{3.5}Cl_3$: C, 40.99; H, 6.40; N, 17.93. Found: C, 41.17; H, 6.37; N, 17.94.

**Zinc(II) Complex of 1-Carbamoylmethyl-1,4,7,10-tetraazacyclo-dodecane, 17(ClO$_4$)$_2$.** A MeOH solution (2 mL) of **6**·3HCl·3H$_2$O (392 mg, 1.0 mmol) was mixed with 1 M NaOMe in MeOH (3 mL). After insoluble inorganic salts were filtered off, the filtrate was concentrated under reduced pressure to obtain the free ligand **6**. To an EtOH solution (10 mL) of **6** was added a EtOH (10 mL) solution of $Zn(ClO_4)_2$·6H$_2$O (372 mg, 1.0 mmol) at room temperature, and the whole was stirred for 1 h. After the solvent was evaporated, the remaining solids were recrystallized from $CH_3CN$/EtOH to obtain **17**$(ClO_4)_2$ (356 mg, 72% yield) as colorless needles (although we have not experienced the explosion of $ClO_4$ salts of zinc complexes, the standard warning of their hazards should be noted): Mp 227−228 °C dec. IR (KBr): 3237, 3127, 2924, 1686, 1422, 1327, 1144, 1115, 1090, 1013, 993, 627 cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 2.65−3.10 (16H, m), 3.31 (2H, s). $^{13}$C NMR ($D_2O$): $\delta$ 45.77, 46.27, 47.22, 55.86, 56.67, 177.69. Anal. Calcd for $C_{10}H_{23}N_5O_9Cl_2Zn$: C, 24.33; H, 4.70; N, 14.19. Found: C, 24.19; H, 4.57; N, 14.23.

**Zinc(II) Complex of Carbamoylmethylamidate, 17a(ClO$_4$)·H$_2$O.** To a solution of **17**$(ClO_4)_2$ (247 mg, 0.5 mmol) in $CH_3CN$ (10 mL) was added 0.5 mL of 1 M NaOMe in MeOH (0.5 mmol). After the reaction mixture was concentrated under reduced pressure, the remaining solids were recrystallized from $CH_3CN$ to afford **17a**$(ClO_4)$·H$_2$O (102 mg, 50% yield) as colorless needles: Mp > 270 °C. IR (KBr): 3185, 2921, 1584, 1447, 1406, 1248, 1144, 1117, 1090, 999, 627 cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 2.73−2.84 (8H, m), 2.96−3.07 (8H, m), 3.29 (2H, s). $^{13}$C NMR ($D_2O$): $\delta$ 46.31, 47.39, 48.36, 55.27, 60.26, 179 (carbonyl carbon, as determined by HMQC and HSQC). Anal. Calcd for $C_{10}H_{24}N_5O_6ClZn$: C, 29.20; H, 5.88; N, 17.00. Found: C, 29.13; H, 5.67; N, 17.04.

**Zinc(II) Complex of 1-(*N*-(4-Nitrophenyl)carbamoylmethyl)-1,4,7,10-tetraazacyclododecane, 19(ClO$_4$)$_2$·H$_2$O.** An aqueous solution (10 mL) of **8**·3HCl·0.5H$_2$O (230 mg, 0.49 mmol) was added to a 3 N NaOH aqueous solution, and the solution was extracted with $CHCl_3$ (50 mL × 5). After the combined organic layers were dried over anhydrous $Na_2SO_4$, the solvent was concentrated under reduced pressure to obtain the free ligand **8** as a yellow oil. To a EtOH (10 mL) solution of the free **8** was added $Zn(ClO_4)_2$·6H$_2$O (186 mg, 0.50 mmol) at room temperature, and the whole was stirred for 1 h. After the solvent was evaporated, the remaining solids were crystallized from $CH_3CN$/EtOH to obtain **19**$(ClO_4)_2$·H$_2$O (179 mg, 58% yield) as pale yellow needles: Mp 249−250 °C. IR (KBr): 1663, 1626, 1597, 1579, 1563, 1512, 1345, 1121, 1109, 1092, 888, 627 cm$^{-1}$. $^1$H NMR ($CD_3CN$): $\delta$ 2.78−3.09 (16H, m), 3.69 (2H, s), 7.84 (2H, d, $J$ = 9.3 Hz), 8.29 (2H,

d, $J = 9.3$ Hz), 9.48 (1H, br). $^{13}$C NMR ($CD_3CN$): $\delta$ 45.64, 45.99, 47.43, 55.81, 57.97, 121.87, 126.00, 143.40, 145.87, 174.20. Anal. Calcd for $C_{16}H_{30}N_6O_{13}Cl_2Zn$: C, 30.36; H, 4.46; N, 13.29. Found: C, 30.38; H, 4.61; N, 13.23.

**Zinc(II) Complex of *N*-(4-Nitrophenyl)carbamoylmethylamidate, 19a(ClO$_4$)·H$_2$O.** To a solution of **19**$(ClO_4)_2$·$H_2O$ (189 mg, 0.30 mmol) in $CH_3CN$ (3 mL) was added 3 mL of 0.1 M NaOH. After the reaction mixture was concentrated under reduced pressure, the remaining solids were recrystallized from $CH_3CN/H_2O$ to obtain yellow prisms of **19a**$(ClO_4)$·$H_2O$ (122 mg, 76% yield): Mp > 270 °C. IR (KBr): 3295, 1615, 1572, 1491, 1319, 1181, 1119, 1107, 955, 855, 625 cm$^{-1}$. $^1$H NMR ($CD_3CN$): $\delta$ 2.67−2.81 (8H, m), 2.97−3.05 (8H, m), 3.35 (2H, s), 7.55 (2H, d, $J = 9.3$ Hz), 8.11 (2H, d, $J = 9.3$ Hz). $^{13}$C NMR ($CD_3CN$): $\delta$ 44.46, 45.52, 47.01, 53.36, 60.49, 124.76, 125.38, 142.79, 155.71, 173.98. Anal. Calcd for $C_{16}H_{27}N_6O_8ClZn$: C, 36.08; H, 5.11; N, 15.84. Found: C, 36.00; H, 4.93; N, 15.70.

**1-(*N*,*N*-Diethylcarbamoylmethyl)-1,4,7,10-tetraazacyclododecane Tetrahydrochloric Acid Salt, 9·4HCl·H$_2$O.** To a solution of 1,4,7,10-tetraazacyclododecane (1.3 g, 7.3 mmol) in EtOH (15 mL) was slowly added a solution of *N*,*N*-diethylchloroacetamide (570 mg, 3.8 mmol) in EtOH (15 mL). The reaction mixture was stirred at 80 °C for 8 h and then reacted with $(Boc)_2O$ (6.6 g, 30 mmol) and triethylamine (3.1 g, 30 mmol) at room temperature for 8 h. After the reaction mixture was concentrated under reduced pressure, the residue was purified by silica gel column chromatography (AcOEt). After evaporation of the solvent, the residue was dissolved in MeOH (10 mL), to which was slowly added 37% aqueous HCl (10 mL) and stirred for 2 h at room temperature. After the solvents were evaporated, the residue was crystallized from MeOH to obtain **9**·4HCl·$H_2O$ (922 mg, 54% yield) as colorless prisms: Mp 188 °C dec. IR (KBr): 3002, 2820, 2762, 2672, 1642, 1576, 1478, 1458, 1431 cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 1.11 (3H, t, $J =$ 7.2 Hz), 1.18 (3H, t, $J = 7.2$ Hz), 3.02−3.15 (16H, m), 3.32−3.38 (4H, m), 3.66 (2H, s). $^{13}$C NMR ($D_2O$): $\delta$ 14.96, 15.85, 44.53, 44.71, 45.61, 45.99, 47.48, 53.33, 57.36, 174.58. Anal. Calcd for $C_{14}H_{37}N_5O_2Cl_4$: C, 37.42; H, 8.30; N, 15.59. Found: C, 37.19; H, 8.61; N, 15.39.

**1-(2-(Carbamoyl)ethyl)-1,4,7,10-tetraazacyclododecane Ditrifluoroacetic Acid Salt (10·2CF$_3$COOH).** Compound **10**·2$CF_3$-COOH was synthesized from 1,4,7,10-tetraazacyclododecane and 2-bromopropionamide using the same method as **9**·4HCl·$H_2O$ and isolated as ditrifluoroacetic acid salt. IR (KBr): 1667, 1200, 1176, 1129, 798, 720 cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 2.50 (2H, t), 2.89−3.20 (18H, m). $^{13}$C NMR ($D_2O$): $\delta$ 31.73, 42.21, 42.56, 44.72, 48.42, 48.67, 178.25. Anal. Calcd for $C_{15}H_{27}N_5O_5F_6$: C, 34.90; H, 6.08; N, 15.66. Found: C, 34.75; H, 6.21; N, 15.39.

**1-Cyanomethyl-4,7,10-tris(*tert*-butylcarbonyl)-1,4,7,10-tetraazacyclododecane, 24.** Bromoacetonitrile (2.0 g, 16.9 mmol) was reacted with 3Boc-cyclen (4.2 g, 8.9 mmol) in $CH_3CN$ (30 mL) in the presence of $Na_2CO_3$ (1.2 g, 11.3 mmol) at 80 °C under an argon atmosphere for 2 days. After insoluble inorganic salts were removed, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt) to afford **24** as a colorless amorphous solid (3.6 g, 87%). IR (KBr): 2231, 1678, 1458, 1363, 1252, 1171 cm$^{-1}$. $^1$H NMR ($CDCl_3$): $\delta$ 1.44 (18H, s, $C(CH_3)_3$), 1.47 (9H, s, $C(CH_3)_3$), 2.78−2.87 (4H, br), 3.28−3.53 (12H, m), 3.29−3.33 (2H, br). $^{13}$C NMR ($CDCl_3$): $\delta$ 28.52, 28.77, 46.50, 47.40, 49.93, 50.17, 53.98, 54.49, 79.62, 79.94, 80.25, 114.55, 155.15, 155.96, 156.12.

**1-(2-Acetylamino)ethyl-1,4,7,10-tetraazacyclododecane Tetrahydrochloric Acid Salt, 11·4HCl·H$_2$O.** A mixture of **24** (3.6 g, 7.8 mmol), Raney nickel (Aldrich (Raney 2800 Nickel), 50% slurry in water), and 1N NaOH (8.0 mL, 8.0 mmol) in EtOH (80 mL) was stirred under $H_2$ (20 atm) at room temperature for 2 days. After Raney nickel was filtered off with Celite (No. 545), the filtrate was evaporated. The remaining residue was dissolved in $CH_3CN$ (20 mL), to which was added $Na_2CO_3$ (0.79 g, 7.45 mmol) and acetyl chloride (0.78 g, 9.9 mmol). The reaction mixture was stirred at room temperature under an argon atmosphere for 4 h. After insoluble inorganic salts were filtered off, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CH_2Cl_2$/MeOH). After evaporation of the solvent, the remaining colorless amorphous solid was dissolved in MeOH (20 mL), to which 37% aqueous HCl (10 mL) was slowly added at 0 °C. After the reaction mixture was stirred for 4 h at room temperature, the solvent were evaporated, and the remaining solids were recrystallized from $H_2O$/EtOH to obtain colorless prisms of **11**·4HCl·$H_2O$ (467 mg, 14% yield): Mp 177−178 °C. IR (KBr): 1668, 1577, 1498, 1442, 1375, 1286, 1099, 950 cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 2.00 (3H, s), 2.73 (2H, t, $J = 6.0$ Hz), 2.94−2.99 (8H, m), 3.18−3.20 (8H, m), 3.34 (2H, t, $J = 6.0$ Hz). $^{13}$C NMR ($D_2O$): $\delta$ 25.18, 39.40, 44.82, 45.15, 47.36, 51.48, 55.80, 177.80. Anal. Calcd for $C_{12}H_{33}N_5O_2Cl_4$: C, 33.89; H, 7.82; N, 16.47. Found: C, 34.19; H, 8.20; N, 16.71.

**Zinc(II) Complex of *N*-1-(2-Acetylaminoethyl)-1,4,7,10-tetraazacyclododecane, 27(ClO$_4$)$_2$.** Compound **11**·4HCl·$H_2O$ (100 mg, 0.24 mmol) was passed through an anion exchange column (Amberlite IRA-400, $OH^-$ form) with water to obtain the free ligand **11**. To an EtOH solution (10 mL) of the free **11** was added Zn-$(ClO_4)_2$·$6H_2O$ (102 mg, 0.27 mmol), and the whole was stirred at room temperature for 1 h. After the solvents were evaporated, the remaining solids were crystallized from $CH_3CN$/EtOH to obtain **17**$(ClO_4)_2$ (68 mg, 55% yield) as colorless needles: Mp > 270 °C. IR (KBr): 2933, 1643, 1579, 1377, 1143, 1115, 1090, 627 cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 2.02 (3H, s), 2.81−2.99 (16H, m), 3.09−3.11 (2H, m), 3.46 (2H, t, $J = 6.7$ Hz). $^{13}$C NMR ($D_2O$): $\delta$ 22.09, 34.77, 42.66, 43.98, 44.77, 50.49, 51.58, 175.19. Anal. Calcd for $C_{12}H_{27}N_5O_9Cl_2Zn$: C, 27.63; H, 5.22; N, 13.43. Found: C, 27.70; H, 5.22; N, 13.37.

**1-(2-(4-Nitrobenzoyl)amino)ethyl-1,4,7,10-tetraazacyclododecane Trihydrochloric Acid Salt, 12·3HCl·2H$_2$O.** Compound **12** was synthesized from 3Boc-cyclen (4.3 g, 9.1 mmol) and 4-nitrobenzoyl chloride (1.86 g, 10 mmol) using the same method used for the synthesis of **11**·4HCl·$H_2O$. Recrystallization from $H_2O$ gave pale yellow needles of **12**·3HCl·$2H_2O$ (2.2 g, 48% yield): Mp 237−238 °C. IR (KBr): 1651, 1601, 1523, 1446, 1344, 1302, 877, 843, 714 cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 2.86−2.91 (6H, m), 2.98 (4H, br), 3.16−3.21 (8H, m), 3.60 (2H, t, $J = 6.1$ Hz), 8.00 (2H, d, $J = 8.9$ Hz), 8.36 (2H, d, $J = 8.9$ Hz). $^{13}$C NMR ($D_2O$): $\delta$ 39.77, 44.60, 45.07, 47.24, 51.19, 55.46, 127.02, 127.22, 131.36, 131.49, 141.76, 152.75, 171.74. Anal. Calcd for $C_{17}H_{35}N_6O_5Cl_3$: C, 40.04; H, 6.93; N, 16.49. Found: C, 40.22; H, 7.01; N, 16.36.

**Potentiometric pH Titration.** The preparation of the test solutions and the calibration method of the electrode system (Potentiometric Automatic Titrator AT-400 and Auto Piston Buret APB-410 (Kyoto Electronics Manufacturing, Co. Ltd.) with Orion Research Ross Combination pH Electrode 8102BN) were described earlier.[9−11,13−17] The theoretical pH values to $pH_1$ and $pH_2$ are calculated to be $pH_1' = 2.481$ and $pH_2' = 11.447$, using $K_w$-$(a_H a_{OH^-}) = 10^{-14.00}$, $K_w'([H^+][OH^-]) = 10^{-13.79}$, and $f_{H^+} = 0.825$. The correct pH values ($pH = -\log a_{H^+}$) can be obtained using the

(16) Shionoya, M.; Ikeda, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1994**, *116*, 3848−3859
(17) Aoki, S.; Kawatani, H.; Goto, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **2001**, *123*, 1123−1132.

following equations: $a = (pH_2' - pH_1')/(pH_2 - pH_1)$; $b = pH_2' - a \times pH_2$; pH = $a \times$ (pH-meter reading) + $b$. The calibration method provides an experimental confidence limit of ±0.01 pH unit. For the potentiometric pH titration, the experiment was carried out (0.1 N aq NaOH was used as a base) with $I = 0.10$ ($NaNO_3$ or $NaClO_4$), and at least two independent titrations were performed. Protonation constants ($K_n' = [H_nL]/[H_{n-1}L][H^+]$) of **6**−**11**, the deprotonation constants ($pK_a' = \log([ZnL]/[ZnL\text{-}OH^-(ZnH_{-1}L)][H^+])$) of zinc(II) complexes (**17**−**19**, **21**, **23**, **27**, and **28**), the metal complexation constants ($K(ZnL) = [ZnL]/[Zn^{2+}][L]$), and the succinimide anion ($A^-$) complexation constants ($K(ZnL\text{-}A^-) = [ZnL\text{-}A^-]/[ZnL][A^-]$) were determined by means of the pH-titration program BEST.[18] The mixed constants ($K_n = [H_nL]/[H_{n-1}L]a_{H^+}$ and $pK_a$) were calculated from $K_n'$ and $pK_a'$ with $[H^+] = a_{H^+}/f_{H^+}$. The speciation distribution values (%) against pH (=−log[$H^+$]; 0.084) were obtained using the program SPE.[18]

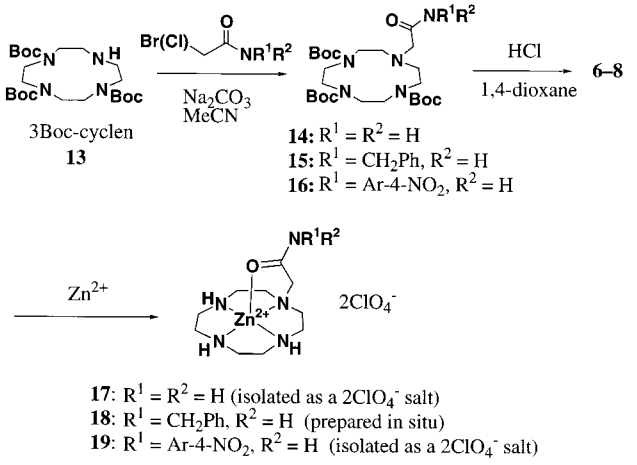
**Crystallographic Study of 19a(ClO$_4$)·H$_2$O.** A yellow prism of **19a**($ClO_4$)·$H_2O$ ($C_{16}H_{27}N_6O_8ClZn$, $M_r$ = 532.26), having approximate dimensions 0.30 × 0.10 × 0.08 mm$^3$, was mounted in a glass capillary. All measurements were made on a Rigaku RAXIS IV imaging plate area detector with graphite monochromated Mo Kα radiation ($\mu$ = 13.20 cm$^{-1}$) at −70 ± 1 °C. Indexing was performed from two oscillations which were exposed for 1 min. A total of 55 images, corresponding to 220° oscillation angles, were collected with two different goniometer settings. Exposure time was 0.20 min/°. The camera radius was 127.40 nm. Readout was performed in the 100 $\mu$m pixel mode. The structure was solved by direct methods (SIR 97) and expanded by means of Fourier techniques (DIRDIF 94). All calculations were performed with the teXsan crystallographic software package developed by the Molecular Structure Corp. (1985, 1992).

**Kinetics for 4-Nitrophenyl Acetate Hydrolysis by 16 in Aqueous Solution.** The 4-nitrophenyl acetate (NA) hydrolysis was measured by an initial slope method (following the increase in 400 nm absorption of released 4-nitrophenolate) in 10% (v/v) $CH_3CN$ aqueous solution at 25.0 ± 0.5 °C. Buffer solutions containing 20 mM Good's buffers (MOPS, pH 7.1; HEPES, pH 7.5; EPPS, pH 7.9; TAPS, pH 8.5) were used, and the ionic strength was adjusted to 0.10 with $NaClO_4$. For the initial rate determination, the following typical procedure was employed: After NA (0.5 mM) and a zinc(II) complex (0.5 mM) were mixed in the buffer solution, the UV absorption increase was immediately recorded until ~1% formation of 4-nitrophenolate, where log $\epsilon$ values for 4-nitrophenolate were 3.97 (pH 7.1), 4.12 (pH 7.5), 4.22 (pH 7.9), and 4.25 (pH 8.5) at 400 nm. The first-order rate constant $k_{obsd}$ (s$^{-1}$) was calculated from the decay slope ([4-nitrophenolate]/[NA]). The value of $k_{obsd}$/[total $Zn^{2+}$ complex] gave the second-order rate constant $k'_{NA}$ (M$^{-1}$ s$^{-1}$) for NA hydrolysis. The second-order rate constant $k_{NA}$ was determined from the maximum $k'_{NA}$ values.

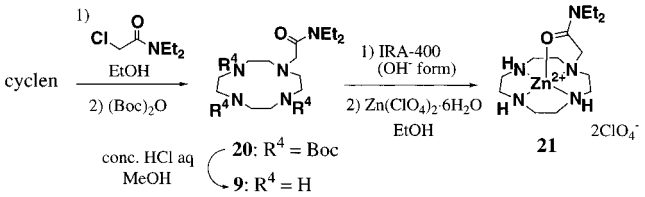
## Results and Discussion

**Design and Synthesis of New Ligands 6−12 and Their Zinc(II) Complexes.** Basically, two kinds of amide pendants were designed; one was a carboxyamide, type **6**−**10**, and the other an acylaminoethyl type **11** and **12**. The ligands **6** and **7** could produce five-membered chelates by coordination of the carbonyl oxygen or amidate $N^-$. The ligand **9** has no dissociable amide hydrogen and could form a five-membered chelate only with the carbonyl oxygen. The ligand **10** could form a six-membered chelate with coordination of the carbonyl oxygen or amidate $N^-$. The second type of ligands, **11** and **12**, may yield five-membered chelates by coordination of the amidate $N^-$, or seven-membered chelates with the carbonyl oxygen.

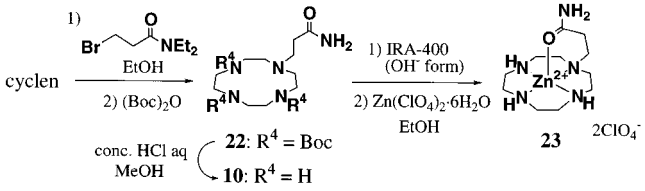
**Scheme 2**




**Scheme 3**




**Scheme 4**

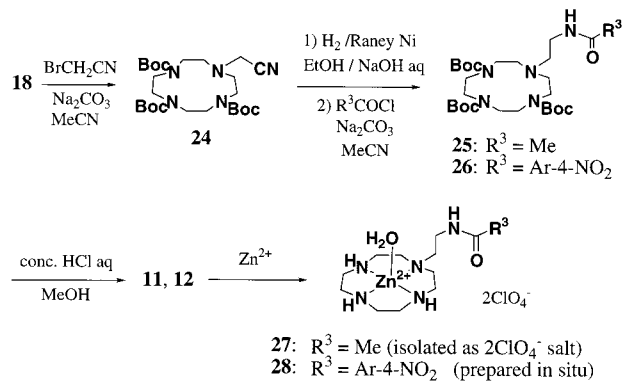



Ligands **6**−**8** and their zinc(II) complexes **17**−**19** were prepared from 1,4,7-tris(*tert*-butyloxycarbonyl)cyclen **13** (3Boc-cyclen),[15] as shown in Scheme 2. *N*,*N*-Diethylcarbamoylmethylcyclen, **9**, was synthesized via **20** by treating cyclen with *N*,*N*-diethylchloroacetamide in EtOH (Scheme 3), and its $Zn^{2+}$ complex **21** was isolated as a perchlorate salt. A similar method was used to synthesize 2-(carbamoyl)ethylcyclen, **10**, and the corresponding $Zn^{2+}$ complex **23** (Scheme 4). Acylaminoethylcyclens **11** and **12** were synthesized as shown in Scheme 5. The zinc(II) complex **27** with **11** was isolated as a perchlorate salt, while **28** with **12** was prepared in situ. For details, see the Experimental Section.

**Protonation and Zinc(II) Complexation Constants for 6−12.** The protonation constants ($K_n$) of new ligands **6**−**12** (all represented by L) were determined by potentiometric pH titrations (1.0 mM) with 0.10 M NaOH solution containing $I = 0.10$ ($NaClO_4$ or $NaNO_3$) at 25.0 °C (for a typical pH titration curve for 1 mM **8**, see the Supporting Information). The titration data were analyzed for equilibria 1, where $a_{H^+}$ is the activity of $H^+$. The log $K_1$ and $K_2$ values are

(18) Martell, A. E.; Motekaitis, R. J. *Determination and Use of Stability Constants*, 2nd ed; VCH: New York, 1992.

**Scheme 5**




separated from log $K_3$ and $K_4$ values, the trend being the same as those of *N*-methylcyclen (log $K_1 = 10.7$, log $K_2 = 9.7$, log $K_3 < 2$, log $K_4 < 2$).[16] Deprotonation of the amide hydrogens was not recognized for the free ligands **6**−**12**.

$$H_{n-1}L^{(n-1)+} + H^+ \rightleftarrows H_nL^{n+} \qquad K_n = [H_nL]/[H_{n-1}L]a_{H^+} \tag{1}$$

The 1:1 zinc(II) complexation equilibria were determined by the potentiometric pH titration of protonated ligands **6**−**12** (1.0 mM) in the presence of an equimolar amount of zinc(II) ion with $I = 0.10$ ($NaClO_4$ or $NaNO_3$) at 25.0 °C (for a typical pH titration curve for 1 mM **8** + 1 mM $Zn^{2+}$, see the Supporting Information). The titration curves revealed two distinct equilibria: the first is for the ZnL complex formation (see eq 2), and the second is for monodeprotonation from ZnL (see eq 3). The first equilibration was slow and took more than 2 h for each titration point, while the following deprotonation process was fast within 5 min at each titration point. For the monodeprotonated species, either a hydroxide-bound structure ZnL-$OH^-$ or an amidate-$N^-$-bound structure $ZnH_{-1}L$ may be considered.

$$Zn^{2+} + L \rightleftarrows ZnL \qquad K(ZnL) = [ZnL]/[L][Zn^{2+}] \tag{2}$$

$$ZnL(H_2O) \rightleftarrows ZnL(OH^-)\ (\text{or } ZnH_{-1}L) + H^+ \qquad K_a = [Zn(OH^-)\ (\text{or } ZnH_{-1}L)]a_{H^+}/[ZnL(H_2O)] \tag{3}$$

All of the obtained values for ligands **6**−**12** are summarized in Table 1, along with a reference *N*-methylcyclen ligand. For practical comparison, apparent stability constants $K_{app}$ (defined by eqs 4−6) at pH 7.0 are also included. The log $K_{app}$ values for the carbamoyl-pendant cyclens **6**−**9** are 9.8, 10.0, 9.6, and 8.9, respectively, which are larger than the values of 8.2 and 8.0 for the acetylamino-pendant cyclens **11** and **12**, or 8.6 for *N*-methylcyclen. The comparison implies that the higher stability of the former type of ZnL complexes **6**−**9** is due to the coordination of the pendant amide oxygen to form the stable five-membered chelates. A six-membered chelation by an analogous carbamoyl pendant of **10** (to **23**) is less likely, and the log $K_{app}$ remains to be 8.4. Earlier, the five-membered chelations by pendant carbamoyls were reported in **29** and **30**. In **29**, zinc(II) is 7-coordinate with four nitrogens of cyclen and three carbonyl oxygens of amides.[17] In **30**, zinc(II) is 6-coordinate with four nitrogens of cyclen and two carbonyl oxygens of amides.[19]

**Table 1.** Comparison of Ligand Protonation Constants ($K_n$),[a,b] Zinc(II) Complexation Constants ($K$(ZnL)),[c] and Deprotonation Constants (p$K_a$),[d] and Apparent Complexation Constants $K_{app}$ ($M^{-1}$) at pH 7.0[e] at 25 °C with $I = 0.10$ ($NaNO_3$ or $NaClO_4$)

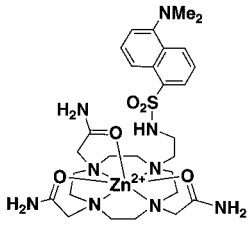
| | *N*-methylcyclen[b,f] | **6**[b,g] | **7**[b,h] | **8**[b,h] |
|---|---|---|---|---|
| log $K_1$ | 10.7 | 10.57 | 10.42 | 10.62 |
| log $K_2$ | 9.7 | 9.31 | 9.22 | 9.22 |
| log $K$ (ZnL)[c] | 15.1 | 14.4 | 14.2 | 14.0 |
| p$K_a$ [d] | 7.68 | 8.59 | 7.92 | 7.01 |
| log $K_{app}$ [e] | 8.6 | 9.8 | 10.0 | 9.6 |
| | **9**[b,g] | **10**[b,h] | **11**[b,h] | **12**[b,g] |
| log $K_1$ | 11.10 | 11.00 | 10.67 | 10.88 |
| log $K_2$ | 9.63 | 9.09 | 8.82 | 8.67 |
| log $K$ (ZnL)[c] | 15.5 | 14.5 | 13.4 | 13.3 |
| p$K_a$ [d] | 9.92 | 8.19 | 7.64 | 7.48 |
| log $K_{app}$ [e] | 8.9 | 8.4 | 8.2 | 8.0 |

[a] $K_n = [H_nL]/[H_{n-1}L]a_{H^+}$ ($M^{-1}$). Experimental errors are ±0.03. [b] For all ligands shown, log $K_3$ and log $K_4$ are <2. [c] $K$(ZnL) = [ZnL]/[Zn][L] ($M^{-1}$). Experimental errors are ±0.1. [d] $K_a = [ZnL(OH^-)\ (\text{or } ZnH_{-1}L)]a_{H^+}/[ZnL]$ (M). [e] $K_{app} = [ZnL]/[Zn]_{free}[L]_{free}$ ($M^{-1}$) at pH 7.0, where [ZnL] = $[ZnL(OH_2)] + [ZnL(OH^-)]$ and $[L]_{free}$=[L] + [HL] + $[H_2L]$ + $[H_3L]$ + $[H_4L]$. Experimental errors are ± 0.1. [f] From ref 16. [g] $I = 0.10$ ($NaClO_4$). [h] $I = 0.10$ ($NaNO_3$).

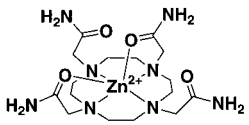
$$K_{app} = [ZnL]/[Zn]_{free}[L]_{free}\ (M^{-1}) \tag{4}$$

$$[ZnL]_{free} = [ZnL(OH_2)] + [ZnL(OH^-)] \tag{5}$$

$$[L]_{free} = [L] + [HL] + [H_2L] + [H_3L] + [H_4L] \tag{6}$$




**29**

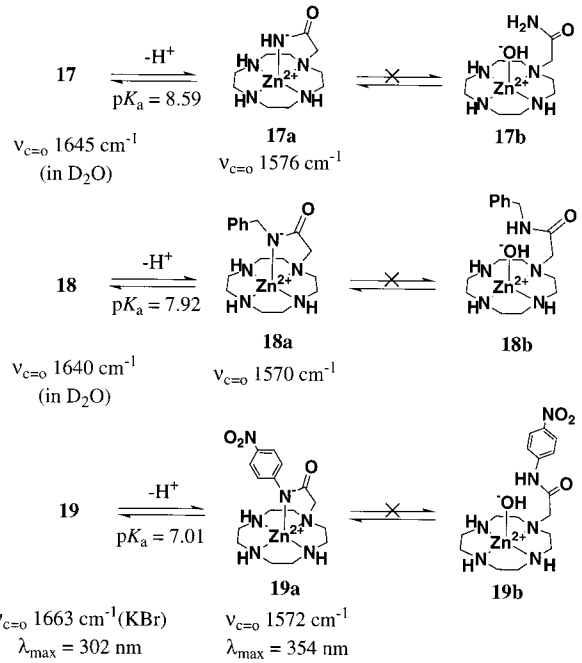



**30**

**Amide Oxygen-Coordinating ZnL (17−19) and Amidate $N^-$−Coordinating $ZnH_{-1}L$ (17a−19a) with Carbamoyl-Pendant Cyclens (L = 6, 7, and 8).** The monodeprotonation occurred in **17**, **18**, and **19** with p$K_a$ of 8.59, 7.92, and 7.01, respectively. The monodeprotonated complexes **17a** and **19a** were isolated as perchlorate salts. The deprotonated species were all assigned to the amidate $N^-$-bound zinc(II) complexes ($ZnH_{-1}L$), **17a**, **18a**, and **19a**, rather than the $Zn^{2+}$−$OH^-$ complexes, **17b**, **18b**, and **19b** (Scheme 6). The first evidence for the amidate $N^-$-coordinating structures is the remarkable lowering of the amide stretching frequency $\nu_{C=O}$: from 1686 $cm^{-1}$ (KBr pellet) or 1645 $cm^{-1}$ (in $D_2O$) for **17** to 1576 $cm^{-1}$ (in KBr pellet) or 1576 $cm^{-1}$ (in $D_2O$) for **17a**, from 1640 $cm^{-1}$ (in $D_2O$) for **18** to 1570 $cm^{-1}$ (in $D_2O$ solution) for **18a**, and from 1663 $cm^{-1}$ (KBr pellet) for **19** to 1572 $cm^{-1}$ (in KBr pellet) for **19a**. An amidate $N^-$-bound zinc(II) in **31** showed a similar $\nu_{C=O}$ at 1570 $cm^{-1}$ (in KBr

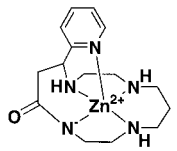
(19) Maumela, H.; Hancock, R. D.; Carlton, L.; Reibenspies, J. H.; Wainwright. *J. Am. Chem. Soc.* **1995**, *117*, 6698−6707.

**Scheme 6**




pellet) or 1577 $cm^{-1}$ (in $D_2O$).[20] Other evidence came from the UV absorption change for **19** ⇄ **19a**. The absorption maximum $\lambda_{max}$ = 302 nm ($\epsilon$ = 14500) for **19** red-shifted to 354 nm ($\epsilon$ = 14800) for **19a** in acetonitrile. The UV spectral change with an isosbestic point at 325 nm was seen in the titration of **19** (0.5 mM) with 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) (0−0.5 mM) to **19a** in acetonitrile. The $^{13}C$ NMR spectra of **19** and **19a** in $CD_3CN$ showed the carbonyl carbon signals at almost the same $\delta$ = 174 ppm, while the $^1H$ NMR spectra (in $CD_3CN$) showed the amide hydrogen at $\delta$ = 9.5 for **19** disappeared for **19a**.

The final conclusive evidence for the amidate $N^-$-bound structure was obtained by X-ray crystal analysis of **19a**. Figure 1 shows ORTEP drawing of **19a** with 50% probability thermal ellipsoids.[21] The zinc(II) ion is coordinated by four nitrogen atoms (N(1), N(4), N(7), and N(10)) of cyclen, and amidate anion nitrogen N(15). The Zn−N bond distances are 2.178(3) Å for Zn−N(1), 2.127(2) Å for Zn−N(4), 2.141(3) Å for Zn−N(7), and 2.097(3) Å for Zn−N(10), while the Zn−$N^-$(15) bond distance is 1.974(3) Å. For comparison, the reported amidate anion-bound zinc(II) complex **31** showed the Zn−$N^-$ bond distance being 2.035(4) Å, which is shorter than the Zn−N bonds (2.09−2.12 Å, where N are three secondary amines of macrocyclic ring).[20]



**31**

To examine if the electrostatically equivalent zinc(II)−$OH^-$ species (**17b** or **19b**) is present in equilibration with

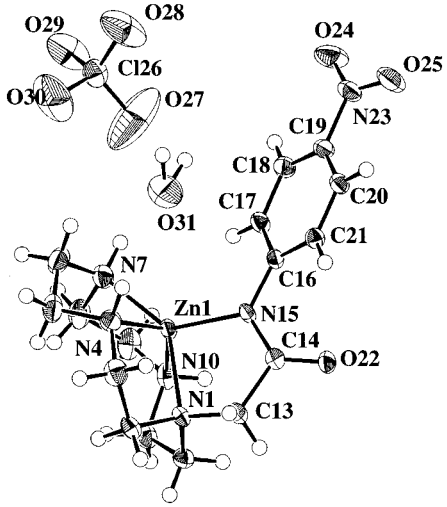


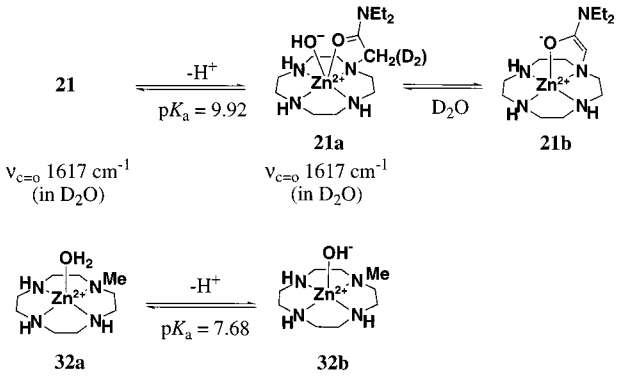

**Figure 1.** ORTEP drawing (50% probability ellipsoids) of **19a**($ClO_4$)·$H_2O$. Bond distances (Å): Zn(1)−N(15) 1.974(3), Zn(1)−N(1) 2.178(3), Zn(1)−N(4) 2.127(2), Zn(1)−N(7) 2.141(3), Zn(1)−N(10) 2.097(3). Bond angles (deg): N(1)−Zn(1)−N(4) 81.9(1), N(1)−Zn(1)−N(10) 82.7(1), N(4)−Zn(1)−N(7) 82.3(1), N(7)−Zn(1)−N(10) 83.2(1), N(1)−Zn(1)−N(15) 85.0(1).

**17a** or **19a** in solution, we have studied hydrolysis of 4-nitrophenyl acetate (NA) by **17** or **19** at pH 7.0−8.5 and 35 °C with $I$ = 0.1 ($NaNO_3$ or $NaClO_4$) and found no hydrolysis over 24 h. The lack of the nucleophilic activity indicates a negligible proportion of the nucleophilic zinc(II)−$OH^-$ species. Our conclusion is that *the reactive sites on the* $Zn^{2+}$*−cyclen* ***17−19*** *are occupied by the carboxamide oxygens in acidic pH and carboxamidato nitrogens in basic pH*. Therefore, the nucleophilic zinc(II)−$OH^-$ species could not be generated in acidic to alkaline pH in **17−19** (for the distribution diagram for **19**, see the Supporting Information). This study provided with the first chemical model of the amide inhibition of CA, just as we earlier designed **5** as a model for the sulfonamide inhibition of CA.[13]

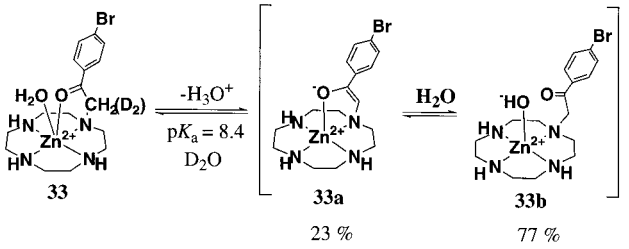
**Amide-Coordinating ZnL, 19, and a Hydroxide-Bound ZnL($OH^-$), 21b, with L = *N*,*N*-Diethylcarbamoylmethylcyclen 9.** The zinc(II) complex **21** of *N*,*N*-diethylcarbamoylmethylcyclen **9** has the highest stability constant log*K*(ZnL) of 15.5 among the carbamoyl-cyclen complexes, implying the strongest amide coordination. Despite its absence of dissociable amide hydrogen, the potentiometric pH titration of **21** showed monodeprotonation with a high p$K_a$ value of 9.92. The IR spectra of **21** and its deprotonated species (pD 11.2) showed the same $\nu_{C=O}$ (in $D_2O$) at 1617 $cm^{-1}$. The $^{13}C$ NMR spectra of **26** (pD 5.9) and its monodeprotonated species showed the carbonyl carbon signals at $\delta$ = 174 and 172, respectively. In the NA hydrolysis reaction with **21**, the catalysis was found at high pH (CHES buffer, pH 9.3). Thus, the deprotonated species was assigned to a zinc(II)−$OH^-$ complex, **21a** (Scheme 7). The p$K_a$ value of 9.92 for **21** ⇄ **21a** is much higher than that of 7.68 for the *N*-methylcyclen complex, **32a** ⇄ **32b**,[22] because of the competitive amide coordination. Moreover, we saw a H−D exchange for the methylene adjacent to the carbonyl in

(20) Kimura, E.; Koike, T.; Shiota, T.; Iitaka, Y. *Inorg. Chem.* **1990**, *29*, 4621−4629.
(21) Crystallographic data of **19a** are listed in the Supporting Information.
(22) Koike, T.; Kajitani, S.; Nakamura, I.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1995**, *117*, 1210−1219.

**Scheme 7**




**Scheme 8**

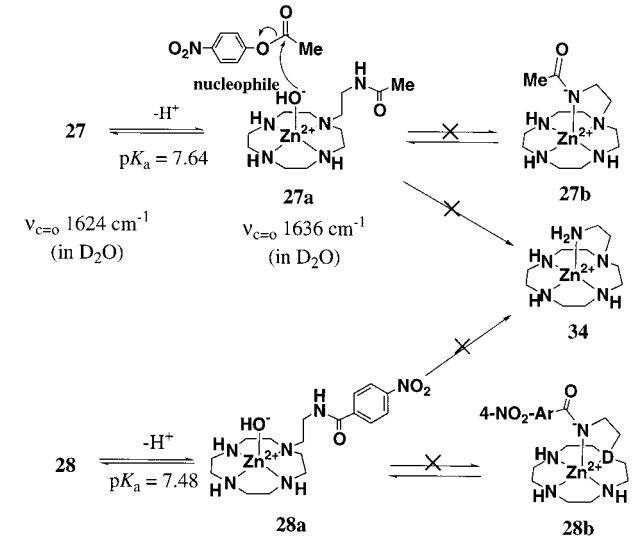



alkaline $D_2O$ solution (pD 11.2), implying equilibration with an enolate $Zn^{2+}$ complex **21b**. In a relevant carbonyl-bound $Zn^{2+}$ complex, **33**, reported as a model for zinc-containing class II aldolases (Scheme 8),[23] deprotonation occurred with lower p$K_a$ of 8.4 to yield a mixture of an enolate-bound $Zn^{2+}$ complex **33a** and a hydroxide-bound complex **33b** (in a ratio of 23%:77% at 25 °C). In analogy to **21**, the H−D exchange occurred at the methylene adjacent to the carbonyl.

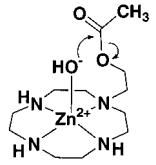
**Amide-Noncoordinating ZnL (27 and 28) and Hydroxide-Bound ZnL(OH$^-$) (27a and 28a) with L = *N*-Acetylaminoethylcyclen, 11, and *N*-Nitrobenzoylaminoethylcyclen, 12.** The lower *K*(ZnL) values with the acetylamino-pendant **11** and **12** than those with the carbamoyl-pendant cyclens **6**−**9** should reflect unfavorable seven-membered chelations with *N*-acetylamino pendants (Table 1). The deprotonation constants p$K_a$ of their zinc(II) complexes **27** and **28** were 7.64 and 7.48, values similar to 7.68 for the zinc(II)-bound water in **32a**.[23] The IR spectra showed constant $\nu_{C=O}$ (in $D_2O$) at 1624 cm$^{-1}$ (for **22**) and 1636 cm$^{-1}$ (for **23**). These IR changes differed from those found for the earlier amidate-bound zinc(II) complexes **17a**, **18a**, and **19a**. Little UV spectral changes at varing pH 7−9 disproved formation of 4-nitrobenzoylamino anion **28b**. Accordingly, the present deprotonated structures were assigned to the $Zn^{2+}$−$OH^-$ species **27a** and **28a** (Scheme 9).

If acting as a nucleophile, these $Zn^{2+}$−$OH^-$ species might attack at the intramolecular amides for hydrolysis. However, we saw no amide hydrolysis at pD 7.5−8.5 and 60 °C in 72 h, that is, no occurrence of 4-nitrocarboxylate and zinc(II) complex of aminoethylcyclen, **34**.[13a] Earlier, we found extremely fast ester hydrolysis of an acetate-pendant by the intramolecular attack of $Zn^{2+}$−$OH^-$ in **35**.[22] We then have

**Scheme 9**




tested to see the hydrolysis of external NA by **27a** at pH 7.1−8.5. The second-order rate constant $k_{NA}$ was determined to be $(4.6 \pm 0.2) \times 10^{-2}$ M$^{-1}$ s$^{-1}$, a value almost the same as $4.7 \times 10^{-2}$ M$^{-1}$ s$^{-1}$ reported for **32b**.[22] We consider that these $Zn^{2+}$−cyclen−$OH^-$ species are nucleophilic enough for carboxyesters, but not for carboxamides.



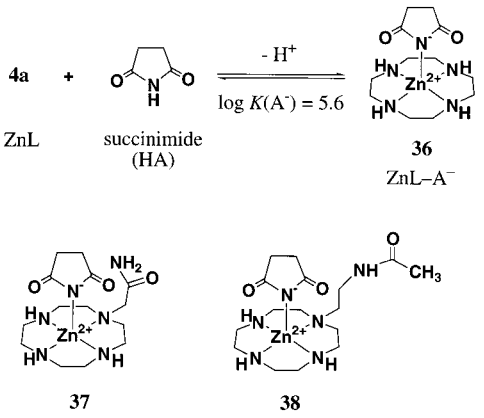
**35**

**Different Succinimide Affinity to Carbamoyl-Pendant Complex 17 and Acetylamino-Pendant Complex 25.** It is now understood that the higher p$K_a$ value (8.59) for **17** ⇄ **17a** with respect to those for **32** (=7.68) (Scheme 7) or **27** (=7.64) (Scheme 9) is due to competitive occupation of a vacant $Zn^{2+}$ site by the carbamoyl amide pendant. We then have studied whether the carbamoyl pendants may be displaced by an external succinimidate. In $Zn^{2+}$−cyclen complex **4a**, a succinimide is a strong ligand to yield 1:1 $Zn^{2+}$−cyclen−succinimidate complex ZnL−A$^-$, **36** (Scheme 10).[14] The succinimide binding constants *K*(A$^-$) (=[ZnL−A$^-$]/[ZnL][A$^-$] (M$^{-1}$), where A$^-$ is deprotonated imide N$^-$ anion species of succinimide for **17** and **27**) were determined by potentiometric pH titrations of **17** and **27** with the aid of the pH-titration program BEST.[18] The results indeed fit to the formation of ZnL−A$^-$ species **37** and **38**. The log *K*(A$^-$) value for **17** ⇄ **37** was 4.0, which is smaller than the values of 5.8 for **27** ⇄ **38** and 5.6 for **4a** ⇄ **36**.[14] It is concluded that, although carbamoyl groups bind to $Zn^{2+}$ either with oxygen or nitrogen, they can be replaced by other stronger ligands. The similar log *K*(A$^-$) values for **4a** and **27** support the proposed little coordination of the acetylamino group in **27**.

**Comparison with Other Metal Ion−Amide Complexes.** It is instructive to compare the present pH-dependent $Zn^{2+}$−

(23) Kimura, E.; Gotoh, T.; Koike, T.; Shiro, M. *J. Am. Chem. Soc*. **1999**, *121*, 1267−1274.

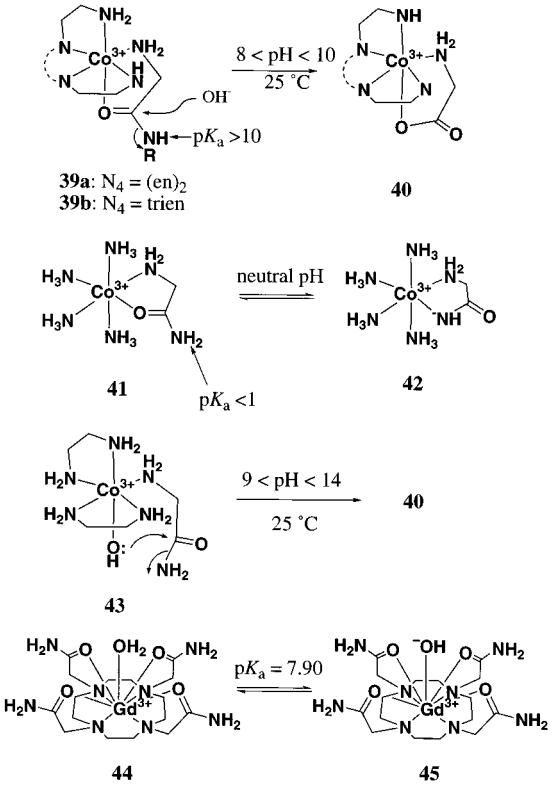
**Scheme 10**




amide binding manners with previous metal models including well studied $Co^{3+}$−tetraamine $N_4$ systems.[24−27] With respect to the $Zn^{2+}$−amide hydrogens, higher acidities (i.e., lower p$K_a$ values) for the $Co^{3+}$-bound amide hydrogens are anticipated. Surprisingly, however, the $Co^{3+}$-coordinated amide protons are much less acidic with p$K_a$ of 11.4 for $[Co^{3+}(en)_2(glyNH_2)]^{3+}$ (**39a**)[25b] and 10.1 (by kinetic measurement)[25b] or 9.4 (by potentiometric titration)[24] for $[Co^{3+}(trien)(glyglyOCH_3)]^{3+}$ (**39b**), with respect to the p$K_a$ value of 8.6 for the comparable $Zn^{2+}$−amide complex (**17**) (Scheme 11). The solvent hydroxide at pH below the p$K_a$ values (namely ∼8 < pH < ∼10) acted as an external nucleophile to attack at the $Co^{3+}$-chelated amide carbons in **39** at 25 °C to yield the hydrolysis products **40**. However, a following study[25c] indicated that hydroxide seemed to simultaneously attack at $Co^{3+}$ in $Co^{3+}(en)_2$ complexes, as monodeprotonated $Co^{3+}$−hydroxide complex *cis*-$[Co^{3+}(en)_2(glyNR_1R_2)(OH^-)]$ (**43**) was identified from pH 9∼14 solution of *cis*-$[Co^{3+}(en)_2(glyNR_1R_2)Br]$ (where $R^1 = R^2 = H$, $R^1 = H$ and $R^2 = CH_3$, $R^1 = R^2 = CH_3$). The glycine amide hydrolysis by the $Co^{3+}$−$OH^-$ in **43** was 10 times more efficient than by the external $OH^-$ in **39**. Thus, the $Zn^{2+}$−OH species in our models did not seem to be as nucleophilic as $Co^{3+}$−$OH^-$ for the intramolecular carboxamide hydrolysis. It is interesting to note that the same $Co^{3+}$-bound glycine amide hydrogen in $[Co^{3+}(NH_3)_4(glyNH_2)]$ (**41**) was extremely acidic with a p$K_a$ value of ∼0.4 to yield $[Co^{3+}(NH_3)_4(glyNH^-)]$ (**42**), which was inert to the amide hydrolysis.[25a]

In a recent work of peptide hydrolysis at very acidic pH < 2 by $Pd^{2+}$ complexes,[28] the external attack of $H_2O$ at the very electrophilic $Pd^{2+}$-bound carboxamides or the internal attack of $Pd^{2+}$−$OH_2$ at the proximate amides was proposed for the peptide hydrolysis mechanism in very acidic pH < 2. At pH > 2, the amide hydrogens were deprotonated to bind to $Pd^{2+}$. As a consequence, peptides did not undergo amide hydrolysis at neutral pH.

**Scheme 11**




In a $Gd^{3+}$ complex of tetraamide derivatives of cyclen (**44**), the deprotonation first yielded a hydroxide complex, **45**, with p$K_a$ of 7.90 at 25 °C and then the amide NH deprotonated complexes with p$K_a$ of 11.02 and 11.89.[29] Some of these lanthanide complexes promote RNA cleavage, apparently by the catalytically active lanthanide-bound hydroxide species such as **45**.[30]

## Summary and Conclusions

Two different pH-dependent $Zn^{2+}$−amide interactions in the newly designed zinc(II) complexes of carboxamide-pendant cyclens were revealed. The zinc(II)−cyclen complexes with carbamoyl-pendants **17**, **18**, and **19** possessed amide-coordinating structures at acidic to neutral pH. Monodeprotonation occurred with p$K_a$ values of 8.59 for **17**, 7.92 for **18**, and 7.01 for **19** at 25 °C, and the resulting products were all the amidate $N^-$-bound zinc(II) complexes **17a**, **18a**, and **19a**, respectively. By the preferred amidate $N^-$ formation from **17**, **18**, and **19**, nucleophilic $Zn^{2+}$−$OH^-$ species were

(24) Collman, J. P.; Kimura, E. *J. Am. Chem. Soc.* **1967**, *89*, 6096−6103.
(25) (a) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1969**, *91*, 3451−3456. (b) Buckingham, D. A.; Davis C. E.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1970**, *92*, 5571−5579. (c) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1970**, *92*, 6151−6158.
(26) For review: Sutton, P. A.; Buckingham, D. A. *Acc. Chem. Res.* **1987**, *20*, 357−364.
(27) Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145−152.
(28) (a) Chen, X.; Zhu, L.; Yan, M.; You, X.; Kostic, N. M. *J. Chem. Soc., Dalton Trans.* **1996**, 2653−2658. (b) Parac, T. N.; Kostic, N. M. *J. Am. Chem. Soc.* **1996**, *118*, 51−58. (c) Zhu, L.; Bakhiar, R.; Kostic, N. M. *J. Biol. Inorg. Chem.* **1998**, *3*, 381−391. (d) Parac, T. N.; Ullmann, G. M.; Kostic, N. M. *J. Am. Chem. Soc.* **1999**, *121*, 3127−3135. (e) Kaminskaia, N. V.; Johnson, T. W.; Kostic, N. M. *J. Am. Chem. Soc.* **1999**, *121*, 8663−8664.
(29) Aime, S.; Barge, A.; Bruce, I. I.; Botta, M.; Boward, J. A. K.; Moloney, J. M.; Parker, D.; de Sausa, A. S.; Woods, M. *J. Am. Chem. Soc.* **1999**, *121*, 5762−2771.
(30) (a) Chin, K. O. A.; Morrow, J. R. *Inorg. Chem.* **1994**, *33*, 5036−5041. (b) Amin, S.; Voss, D. A., Jr.; Harrocks, W. DeW.; Lake, C. H.; Churchill, M. R.; Morrow, J. R. *Inorg. Chem.* **1995**, *34*, 3294−3300.

not generated. It is thus chemically feasible that carboxamides strongly bind to zinc(II) both in acidic pH and basic pH as inhibitors in some $Zn^{2+}$ enzymes such as CA. In another type of $Zn^{2+}$−cyclen complexes with acetylamino pendants **11** and **12**, the carboxamides did not interact with $Zn^{2+}$, because of the unfavorable seven-membered chelation. In alkaline pH, hydroxide ion preferentially bound to $Zn^{2+}$ to yield nucleophilic zinc(II)−$OH^-$ complexes **27a** and **28a** with p$K_a$ values of 7.64 and 7.48, respectively. Hence, the $Zn^{2+}$−amidate $N^-$ species, **27b** and **28b**, were not produced. This situation may somewhat mimic the carboxamide substrate interaction to the active center of CPA. The $Zn^{2+}$−$OH^-$ species in **27a** was demonstrated to hydrolyze 4-nitrophenyl acetate, although it failed to hydrolyze the intramolecular carboxamides, as known to CPA. In our model, $Zn^{2+}$−$OH^-$ species alone was not sufficient to hydrolyze a proximate carboxamide.

**Acknowledgment.** We are grateful to the Ministry of Education, Science and Culture in Japan for financial support through a Grant-in-Aid (12470479 for E.K and 12771355 and 13557195 for S.A.). S.A. is also thankful to Asahi Glass Foundation and the Research Foundation for Pharmaceutical Sciences. T.G. thanks the Japan Society for the Promotion of Science for Graduate Scholarship.

**Supporting Information Available:** Titration curves for the 1.0 mM **8** and the 1.0 mM **8**/1.0 mM $Zn^{2+}$ mixture and speciation diagram for the 1.0 mM **8**/1.0 mM $Zn^{2+}$ mixture as a function of pH, Tables of crystallographic parameters, atomic coordinates, equivalent isotropic temperature factors, anisotropic temperature factors, bond distances, bond angles, and torsion angles in CIF format of the X-ray structure report for **19a**$(ClO_4)_2 \cdot H_2O$. This material is available free of charge via the Internet at http://pubs.acs.org.

IC020087K