A Novel Biomimetic Zinc(II)−Fluorophore, Dansylamidoethyl−Pendant Macrocyclic Tetraamine 1,4,7,10-Tetraazacyclododecane (Cyclen)

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Abstract: On the basis of the chemical principle of carbonic anhydrase (CA)−aromatic sulfonamide inhibitor interaction, a dansylamidoethyl−pendant cyclen (1-(2-(5-(dimethylamino)-1-naphthalenesulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane, HL) has been synthesized as a novel type of zinc(II)−fluorophore. The new ligand HL forms very stable complexes (ML) with zinc(II), cadmium(II), and copper(II) at physiological pH. The potentiometric and spectrophotometric pH-titration study disclosed the 1:1 metal(II) complexes stability constants log K(ML) (= log([ML]/[M][L])) to be 20.8 ± 0.1 for ZnL, 19.1 ± 0.1 for CdL, and >30 for CuL. The crystalline zinc(II) complex ZnL was isolated from aqueous solution at pH 7. The X-ray crystal study of ZnL disclosed a five-coordinate, distorted square-pyramidal structure with the deprotonated dansylamide N^- coordinating at the apical site. Crystals of the monoperchlorate salt of ZnL ($C_{22}H_{35}N_6O_6SClZn$) are orthorhombic, space group $Pna2_1$ (no. 33) with a = 23.777(3) Å, b = 12.744(5) Å, c = 9.092(3) Å, V = 2755(2) Å^3, Z = 4, R = 0.032, and R_w = 0.047. The zinc(II) complex shows a maximum UV absorption band (λ_{max}) at 323 nm (ϵ 5360) at 25 °C in aqueous solution. The fluorescent maximum and the quantum yield (Φ) of ZnL vary with the solvent: at 528 nm (Φ = 0.11) in H_2O, 496 nm (0.53) in MeOH, 489 nm (0.60) in EtOH, and 484 nm (0.44) in CH_3CN. Demetalation of ZnL with excess amount of EDTA yielded the metal-free ligand HL, which in pH 7.3 aqueous solution has an excitation and a weak emission fluorescence at 330 nm (ϵ 4950) and 555 nm (Φ = 0.03), respectively. The copper(II) ion, to the contrary, completely quenches the fluorescence. The crystalline copper(II) complex CuL (λ_{max} 306 nm, ϵ 7630 in H_2O) was isolated as its monoperchlorate salt. The zinc(II)-dependent fluorescence with 5 μM HL at pH 7.3 is quantitatively responsive to 0.1−5 μM concentration of zinc(II), which is unaffected by the presence of mM concentration of biologically important metal ions such as Na^+, K^+, Ca^{2+}, and Mg^{2+}. The new ligand HL forms a far more stable 1:1 zinc(II) complex than any previous zinc(II)-fluorophore and is evaluated as a new zinc(II)-fluorophore.

Introduction

Quantitative analysis of trace zinc(II) ion with a selective analytical reagent has become extremely important for environmental and biological applications.[1] While remarkable development has been made for other biologically important divalent metal ions such as Ca^{2+} with quite a few selective fluorophores such as Quin-2, 1,2-bis(1-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, etc.,[2] there have been few zinc(II)-selective analytical reagents available.

While working on elucidation of intrinsic properties of zinc(II) ion in the catalytic sites of zinc enzymes (in particular carbonic anhydrase, CA[3,4]) by means of macrocyclic complexes (e.g., zinc(II) complexes with [12]aneN_3 (1,5,9-triazacyclododecane) **1**, and cyclen (1,4,7,10-tetraazacyclododecane) **2**),[5] we have discovered unique acid properties of zinc(II) ion.[6] One of the most outstanding zinc(II) properties is a strong affinity to aromatic sulfonamides, as illustrated by formation of the strong bonds between zinc(II) and the deprotonated sulfonamide N^- anions at physiological pH.[4c] Thus, for the first time a

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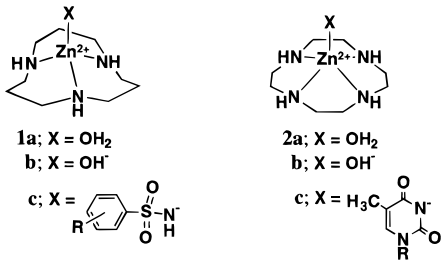
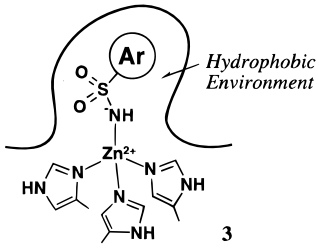
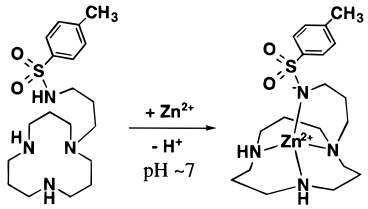
chemical model (**1c**) was presented to account for aromatic sulfonamide anions being strong ligands for zinc(II) at the active center of carbonic anhydrase and acting as strong inhibitors (see **3**).[7] Our zinc enzyme models **1** and **2** form stable 1:1 ternary complexes with various weak acids such as aromatic sulfonamides (**1c**)[3,4c] and thymine derivatives (**2c**)[6] in aqueous solution,





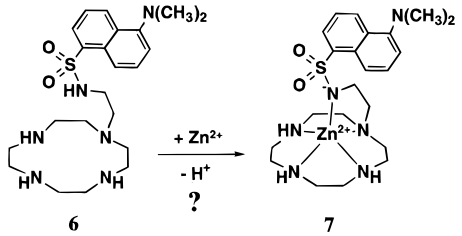
Bonding of Aromatic Sulfonamides to Zinc(II) in Carbonic Anhydrase

which results from the Zn^{2+}-OH^- species generated with pK_a = 7.3 (for **1a** ⇄ **1b** + H^+) and 7.9 (for **2a** ⇄ **2b** + H^+) acting as bases to dissociate the acidic protons. We further demonstrated that Zn^{2+}−tosylamidopropyl[12]aneN$_3$ **4** yielded a stable four-coordinate, tetrahedral complex **5** under physiological pH, where the sulfonamide N^- anion strongly binds to zinc(II) at the fourth coordination site.[4c]

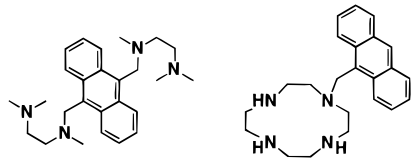


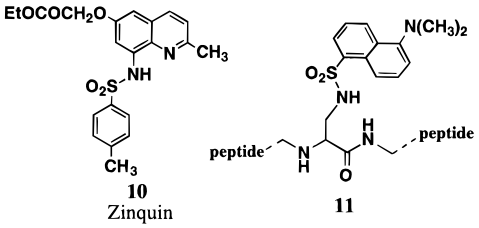
On the basis of these earlier findings, we now have designed a dansylamidoethyl-pendant cyclen **6** (1-(2-(5-(dimethylamino)-1-naphthalenesulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane, HL) that might serve as a novel type of zinc(II)-fluorophore by forming a highly fluorescent 1:1 complex **7** (ZnL) with zinc(II) ion at physiological pH. The reason why we have adopted cyclen instead of [12]aneN$_3$ is because cyclen forms a much more stable zinc(II) complex (K(ZnL) = [ZnL]/[Zn^{2+}][L] = $10^{15.3}$ M^{-1})[5e] than [12]aneN$_3$ (K(ZnL) = $10^{8.4}$ M^{-1})[4b] in aqueous solution at 25 °C.

In 1967, Chen and Kernohan used 5-(dimethylamino)-naphthalene-1-sulfonamide ("dansylamide") as a fluorescent probe to investigate a possible sulfonamide−CA complex **3**.[8] Using this chemical principle, a CA-based fiber optic zinc(II)−



biosensor was devised in 1993,[9] where the specific recognition of zinc(II) ion by apocarbonic anhydrase (possibly as in the form of **3**) is quantitatively assessed by enhancement in the fluorescence of the inhibitor dansylamide. In 1988, chelation-enhanced fluorescence (CHEF) was reported for 9,10-bis-(trimethylethylethylenediamine)anthracene **8** with zinc(II) in acetonitrile solution.[10] Later, **8** was extended to macrocyclic system (e.g., **9**),[11] which showed CHEF with zinc(II) and cadmium(II) at pH 12 in aqueous solution. However, the metal complexes were not isolated for full characterization in these CHEF systems. Recently, a new aromatic sulfonamide fluorophore **10** ("zinquin") for zinc(II) ion, which is chemically more relevant to ours (**6**), was reported.[12] Another new type of fluorescent chemosensor for zinc(II) ion, **11**, has very recently been reported, where a zinc finger-mimetic synthetic polypeptide attached with a dansylamide function, upon binding with zinc-(II) ion, is susceptible to peptide folding to shield the fluorescent reporter from H_2O and increase the emission intensity.[13]





Herein we present the synthesis, characterization, and assessment of our newly designed macrocyclic chelate **6** as a zinc-(II)−fluorophore in comparison to the previous systems and a new reference dansylamide fluorophore **12** (1-(dansylamido)-2-(dimethylamino)ethane). The present study also lends the first chemical proof for the zinc(II)-bound deprotonated dansylamide being a major factor contributing to the great enhancement in

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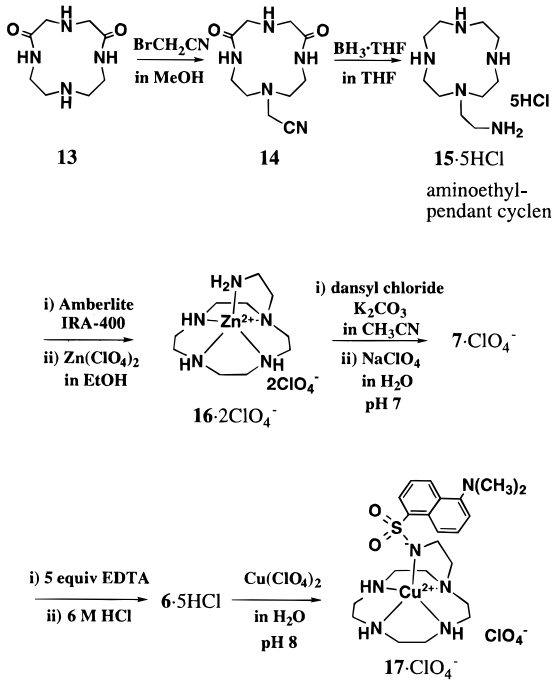
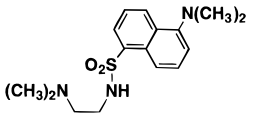
Scheme 1



fluorescence in the CA−dansylamide complex, in addition to the hydrophobic environment in CA (see **3**).[7,8]



12

Results and Discussion

Synthesis of Dansylamidoethyl−Pendant Cyclen 6, Its Zinc(II) Complex 7, Copper(II) Complex 7, and Reference 12. The aminoethyl−pendant cyclen **15** was synthesized by reaction of dioxocyclen **13**[14] with bromocyanomethane (to **14**) followed by BH_3·THF reduction of carbonyl and cyano groups (see Scheme 1). Because direct reaction of **15** with dansylchloride yielded many products (e.g., polydansyl compounds), **15** was initially converted to a zinc(II) complex **16** as its diperchlorate salt. The isolated aminoethyl−pendant cyclen zinc(II) complex was treated with equimolar dansyl chloride in acetonitrile at room temperature to obtain the desired zinc(II) complex of dansylamidoethyl−pendant cyclen **7** (ZnL, L is monodeprotonated ligand), which was purified as its monoperchlorate salt and isolated as colorless prisms in 74% yield. To our knowledge, this is the first isolation of a dansylamide N^--bound zinc(II) complex. Demetalation of **7** to dansylamidoethyl−pendant cyclen **6** (HL) was achieved by treatment with excess EDTA (5 equiv) in H_2O. The zinc(II)-free ligand was purified as its 5 HCl salt, as colorless prisms in 60% yield. Treatment of **6**·5HCl with equimolar $Cu(ClO_4)_2$ in aqueous solution at pH 8 yielded blue crystals of dansylamidoethyl−pendant cyclen copper(II) complex **17** (CuL), as its monoperchlorate salts. The reference dansylamide derivative **12** was synthesized by treating dansylchloride with *N*,*N*-dimethylethylenediamine and purified as its dihydrochloric acid salt.

Protonation and Complexation Constants of Dansylamidoethyl−Pendant Cyclen HL 6. The protonation constants (K_n) of **6** (HL) were determined by potentiometric and spectrophotometric pH titrations of **6**·5HCl (1 mM) against 0.10 M NaOH with $I = 0.10$ ($NaNO_3$) at 25 °C. A typical pH titration curve is shown in Figure 1a, which shows dissociation of six protons at $0 < eq(OH^-) < 6$. The titration data were analyzed for the acid−base equilibria 1 and 2, where a_{H^+} is the activity of H^+. Table 1 summarizes the protonation constants (K_{1-6}) as logarithmic values in comparison with the reference values. The six protonation constants are assigned according to Scheme 2, where HL (in aqueous solution) is a zwitterionic form of **6**. This assignment came from the following facts: (i) Pendantless cyclen (1,4,7,10-tetraazadodecane) has two large protonation constants log K_1 of 11.04 and log K_2 of 9.86, while the remaining two are below 2 (see Table 1).[5e] (ii) The UV absorption spectral change for **6** at pH 2.8−7.3 and 25 °C with $I = 0.10$ ($NaNO_3$) (see Figure 2a−c) is almost the same as those for dansylamide and **12** (see UV data in Experimental Section), leading to the assignment of the protonation constants log K_4 of 4.03 to $ArN(CH_3)_2 + H^+ \rightleftarrows ArNH^+(CH_3)_2$. (iii) The UV absorption change of **6** at pH 7.3−12.4 (Figure 2c−e) is almost

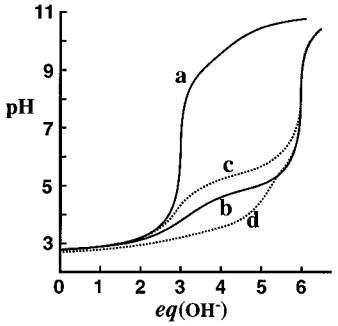
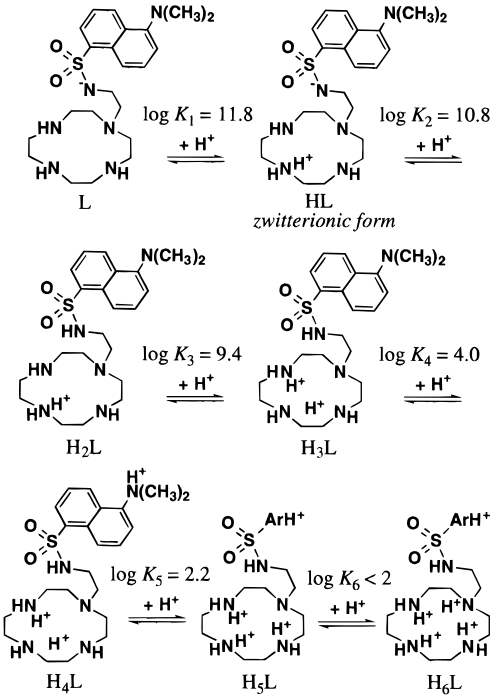


Figure 1. Typical titration curves for dansylamidoethyl−pendant cyclen **6**·5HCl at 25 °C with $I = 0.10$ ($NaNO_3$): (a) 1.0 mM **6**·5HCl; (b) a + 1.0 mM $ZnSO_4$; (c) a + 1.0 mM $CdSO_4$; (d) a + 1.0 mM $CuSO_4$. $eq(OH^-)$ is the number of equivalents of base added.

Scheme 2



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(15) The two protonation constants of dansylamide were estimated spectrophotometrically to be 9.9 ± 0.1 for $ArSO_2NH^- + H^+ \rightleftarrows ArSO_2NH_2$ and 3.8 ± 0.1 for $ArN(CH_3)_2 + H^+ \rightleftarrows ArNH^+(CH_3)_2$ at 25 °C with $I = 0.10$ ($NaNO_3$): $\lambda_{max} = 316$ nm (ϵ 5820) for monoanionic form at pH 12.1, 327 nm (5085) for neutral form at pH 7.3, 285 nm (8090) for monocationic form at pH 2.3.

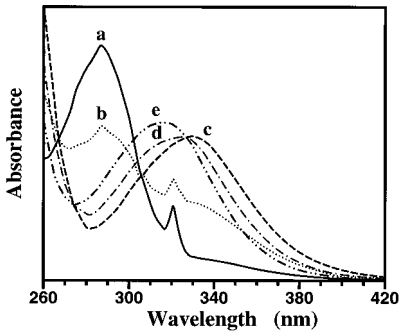


Figure 2. UV-pH profile for **6** (1 mM) at 25 °C with $I = 0.10$ ($NaNO_3$): (a) pH 2.8, $\lambda_{max} = 287$ nm (ϵ 8070); (b) pH 3.6; (c) pH 7.3, 330 nm (4950); (d) pH 10.8; (e) pH 12.4, 317 nm (5300).

the same as those for dansylamide[15] and **12**. Accordingly, log K_2 of 10.77 was assigned to $ArSO_2N^- - + H^+ \rightleftarrows ArSO_2NH-$ for **6**. The potentiometric pH titration for **12** (1 mM) disclosed the protonation constants log K_1 of 10.94 for $ArSO_2N^- - + H^+ \rightleftarrows ArSO_2NH-$, log K_2 of 8.33 for $(CH_3)_2NR + H^+ \rightleftarrows (CH_3)_2NH^+R$, and log K_3 of 3.57 for $ArN(CH_3)_2 + H^+ \rightleftarrows ArNH^+(CH_3)_2$ under the same conditions (see Table 1). Accordingly, at physiological pH, **6** is present mostly as a diprotonated cyclen species (H_3L) with a neutral form of the dansylamidoethyl−pendant.

$$L + H^+ \rightleftarrows HL\ (\mathbf{6}) \quad K_1 = [HL]/[L]a_{H^+} \tag{1}$$

$$H_nL + H^+ \rightleftarrows H_{n+1}L \quad K_{n+1} = [H_{n+1}L]/[H_nL]a_{H^+} \quad (n = 1-5) \tag{2}$$

The zinc(II), cadmium(II), and copper(II) complexation equilibria were determined by potentiometric pH titration of **6**·5HCl (1 mM) in the presence of an equimolar amount of metal(II) (M) at 25 °C with $I = 0.10$ ($NaNO_3$). The titration curves (Figure 1b−d) revealed the formation of stable 1:1 metal-(II) complexes (ML) with deprotonated sulfonamide N^- coordination at physiological pH, a conclusion being derived from the observation of the neutralization break at $eq(OH^-) = 6$. Since the equilibration for zinc(II) complexation at pH below 6 was extremely slow, a minimum of 1 h separated each titration point. Further deprotonation or precipitation of metal(II) hydroxide was not observed at $eq(OH^-) > 6$, indicating that all the complexes ML remains stable up to pH 12. With these titration results, formation of the ML complexes (**7**, **17**, and **18**) and their monoprotonated species **19a**−**c** (MHL) were taken into consideration for the pH titration analysis. The complexation constants K(ML) and protonation constants K(MHL) are defined by eqs 3 and 4. The final structural assignment of ML complex containing the dansylamide N^--coordinating metal-(II) bonds came from X-ray crystal structure analysis of ZnL **7** and similar UV (excitation bands) and IR spectrophotometric results for **7** and **17** (see below). Compared to **6**, the reference dansylamide derivative **12** had much weaker complexation abilities with those metal(II) ions under the titration conditions

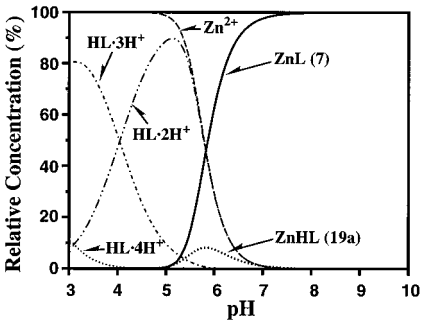
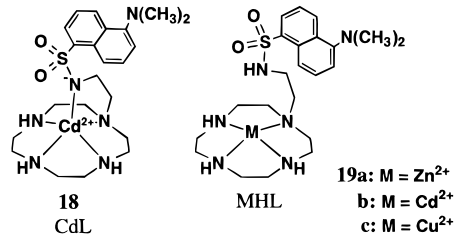


Figure 3. Distribution diagram for the zinc(II) species in 1 μM **6**/1 μM zinc(II) system as a function of pH at 25 °C.

(i.e., 25 °C, $I = 0.10$ ($NaNO_3$), [L] = [M] = 1 mM) as indicated by metal(II) hydroxide precipitation around neutral pH.

$$L + M \rightleftarrows ML\ (\mathbf{7}, \mathbf{17}, \text{or } \mathbf{18}) \quad K(ML) = [ML]/[L][M] \tag{3}$$

$$ML + H^+ \rightleftarrows MHL\ (\mathbf{19}) \quad K(MHL) = [MHL]/[ML]a_{H^+} \tag{4}$$



The obtained logarithmic values of K(ML) and K(MHL) are shown in Table 1, along with the reference values for the pendantless cyclen and tosylamidopropyl−pendant [12]aneN_3. The present N_5-coordinate ML complexes **7**, **17**, and **18** are much more stable than the corresponding N_4-coordinate metal-(II) complexes with cyclen[5e,16] or tosylamidopropyl−pendant [12]aneN_3 (for zinc(II) complexes, planar **2a**, and tetrahedral **5**, respectively). The copper(II) complex **17** is so stable that the complexation constant (log K(CuL) > 30) could not be accurately calculated.[17] The cadmium(II) complex **18** (log K(CdL) = 19.1) is less stable than **7** (log K(ZnL) = 20.8), which is intuitively evident from the complexation equilibrium for CdL at higher pH than that for ZnL in Figure 1 (parts b and c).[18] On the basis of the obtained K(ZnL) and K(ZnHL) values, the distribution of various complexes in solution was calculated as a function of pH at [total zinc(II)] = [total ligand] = 1 μM, as shown in Figure 3. It is remarkable to find that **6** sequesters almost 100% of zinc(II) in the form of ZnL even at μM concentrations of zinc(II) and **6** and at physiological pH. This is one of the most outstanding properties of the new ligand **6** and will be extremely advantageous when using **6** to quantify traces amount of zinc(II) ion in environment and biological systems. In earlier zinc(II) analyses, the complexations were neither strong (e.g., with **9**[11] or CA[9]) nor stoichiometric (e.g., a mixture of ZnL and ZnL_2 complexes with zinquin[12,19]).

(16) Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1978**, 1081−1085.

(17) Earlier, we reported that 2-(2-hydroxyphenyl)-1,5,9-triazacyclododecane (HL′) forms stable complexes (ML′) with zinc(II) and copper(II), which are surrounded by a phenolate O^- and three secondary amines. The log K(ML′) (= log([ML]/[M][L′])) values are 12.6 for ZnL′ and 18.4 for CuL′ at 25 °C. In comparison to the stability constants for tosylamidopropyl−pendant [12]aneN_3 **4**, the O^--bound ZnL′ is less stable than the N^--bound ML (**5**) (log K(ZnL) = 14.7), while the copper(II) complex CuL′ is more stable than CuL (log K(CuL) = 16.9). This fact is similarly related to the stronger affinity of acidic zinc(II) ion for N^- anion than that for O^- anion. Kimura, E.; Yamaoka, M.; Morioka, M.; Koike, T. *Inorg. Chem.* **1986**, *25*, 3883−3886.

(18) It is worth noting that a 16-membered pentaamine [16]aneN_5 (1,4,7,10,13-pentaazacyclohexadecane, L) forms a more stable 1:1 five-coordinate cadmium(II) complex (log K(CdL) = 18.1) than the corresponding zinc(II) complex (log K(ZnL) = 17.9) at 25 °C: Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1978**, 1081−1085. However, when the deprotonation of a secondary amide is involved for five-coordination with a 16-membered monooxo[16]aneN_5 (14-oxo-1,4,7,10,13-pentaazacyclohexadecane, HL), zinc(II) forms a more stable five-coordinate complex (log $K(MH_{-1}L) = [ML]a_{H^+}/[M][HL] = 2.2$) than cadmium(II) (log $K(MH_{-1}L)$ = 1.1): Kimura, E.; Koike, T.; Shiota, T.; Iitaka, Y. *Inorg. Chem.* **1990**, *29*, 4621−4629.

(19) The structures of these zinquin complexes are unknown.

Table 1. Comparison of the Protonation Constants[a] and Zinc(II) Complexation Constants[b] for Cyclen, Tosylamidopropyl−Pendant[12]aneN$_3$ **4**, Dansylamidoethyl−Pendant Cyclen **6**, and **12** at 25 °C with $I = 0.10$ ($NaNO_3$)

	cyclen	**4**	**6**	**12**
log K_1	11.04[c]	12.2[e]	11.80 ± 0.03	10.94 ± 0.03 [f]
log K_2	9.86[c]	11.23[e, f]	10.77 ± 0.02 [f]	8.33 ± 0.02
log K_3	<2[c]	6.40[e]	9.37 ± 0.02	3.57 ± 0.05
log K_4	<2[c]	2.75[e]	4.03 ± 0.03	
log K_5			2.2 ± 0.1	
log K_6			<2	
log K(ZnL)	15.3[c]	14.7[e]	20.8 ± 0.1	
log K(ZnHL)			5.0 ± 0.1 [f]	
log K(CdL)	14.3[d]		19.1 ± 0.1	
log K(CdHL)			5.4 ± 0.1 [f]	
log K(CuL)	24.8[d]	16.9[e]	>30	
log K(CuHL)			5.6 ± 0.1 [f]	

[a] $K_n = [H_nL]/[H_{n-1}L]a_{H^+}$. [b] K(ML) = [ML]/[M][L], K(MHL) = [MHL]/[ML]a_{H^+}. [c] From ref 5e at 25 °C with $I = 0.1$ ($NaNO_3$). [d] From ref 16 at 25 °C with $I = 0.2$ ($NaClO_4$). [e] From ref 4c at 25 °C with $I = 0.1$ ($NaClO_4$). [f] For $ArSO_2N^- + H^+ \rightleftarrows ArSO_2NH$−.

In the present study, we have demonstrated the extremely facile deprotonation of the dansylamide in **19a** (i.e., the smallest log K(MHL) value of 5.0, see Table 1) below physiological pH. This fact is certainly due to the proximity of the dansylamide group to the acidic zinc(II). Thus, our new zinc(II) complex **7** chemically lends support to the dansylamide deprotonation (despite its pK_a ca. 10) toward zinc(II) binding in the active center of carbonic anhydrase at physiological pH.[8] The pK_a value of 7.9 for Zn-OH_2 (**2a**) $\rightleftarrows$ Zn-OH^- (**2b**) + H^+ is much lower than that of >11 for (Cu^{2+}-cyclen)-$OH_2 \rightleftarrows$ (Cu^{2+}-cyclen)-OH^- + H^+,[20] which is reflected in the higher protonation constant log K(CuHL) of 5.6 in the present study.

An X-ray Crystal Structure of Dansylamidoethyl−Pendant Cyclen Zinc(II) Complex (7·ClO_4). A colorless prism of **7**·ClO_4 for X-ray crystallographic study was obtained by slow recrystallization of **7**·ClO_4 from H_2O/MeOH solution at room temperature. The elemental analysis (C, H, N), ^{1}H NMR, and IR data suggested a water free formula, ZnL·(ClO_4). The crystal structure provided unequivocal evidence for the intramolecular coordination of the pendant dansylamide N^- anion, which is shown as an ORTEP drawing with 30% probability thermal ellipsoids in Figure 4. Selected crystal data and collection parameters are displayed in Table 2.

A somewhat distorted square pyramid (or one may view it as a trigonal bipyramid) coordination is evident with the cyclen moiety of four nitrogens (N1, N4, N7, and N10) and a unidentate sulfonamide N^- anion N15. The dansylamide oxygen atoms do not seem to have direct interaction with zinc(II). The Zn−N^- bond distance of 1.969(4) Å is much shorter than the average Zn−N(cyclen) bond distance of average 2.13 Å, indicating strong interactions between the N^- anion and zinc(II). These facts are compatible with very strong acidic properties of zinc(II) in favor of dansylamide N^- anion over neutral nitrogen and O^- anion donors.[12] In a similar fashion, the Zn−N^-(tosylamide) bond (1.925 Å) is shorter than Zn−N([12]aneN$_3$) bonds (average 2.03 Å) in our earlier four-coordinate complex **5**.[4c]

Absorption and Emission Spectra of the Isolated Free Ligand 6, Zinc(II) Complex 7, and Copper(II) Complex 17. The UV absorption and emission spectra of the free ligand **6**, zinc(II) complex **7**, and copper(II) complex **17** at 25 °C with $I = 0.10$ ($NaNO_3$) are compared in Figure 5a−f, where the metal-free ligand **6** is almost in the diprotonated form H_3L^{2+} (see above) and the metal(II) complexes are in the dansylamide-deprotonated ML form. The absorption spectrum of H_3L^{2+} having a neutral dansylamide−pendant (λ_{max} = 330 nm, ϵ 4950) does not change from those for neutral dansylamide (λ_{max} = 327 nm, ϵ 5080, at pH 6.2) and the reference **12**·H^+ with a neutral dansylamide (λ_{max} = 330 nm, ϵ 4620, at pH 6.9). By comparison of Figure 5a−c, the UV absorption spectra of the dansylamide−pendant in the metal(II) complexes are shifted toward shorter wavelengths, which is due to the dansylamide deprotonation, as found for the free ligand **6** in alkaline solution (see Figure 2c−e).

Despite the similar UV spectra, the fluorescence emission spectra dramatically varied for H_3L^{2+}, ZnL **7**, and CuL **17** at pH 7.3 (1.0 mM HEPES buffer) and 25 °C with I = 0.10

(20) Kimura, E.; Koike, T. unpublished result.

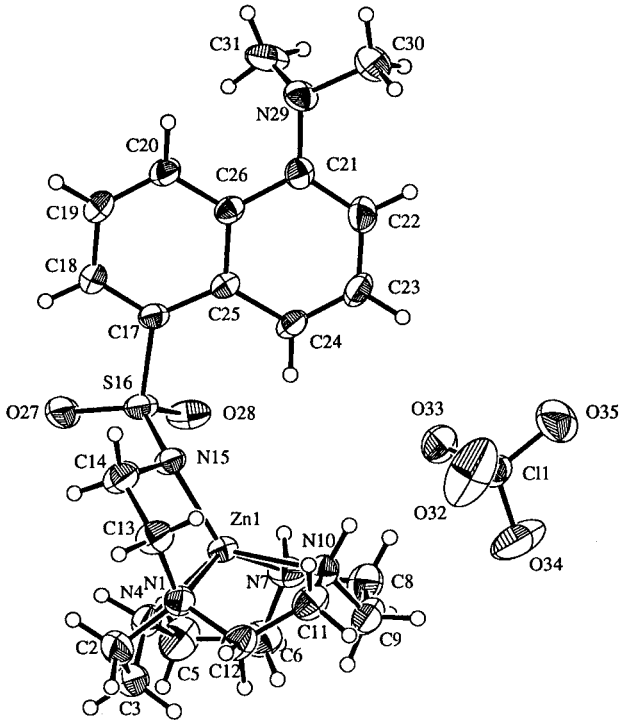


Figure 4. ORTEP drowing (30% probability) of **7**·ClO_4. Selected bond distances (Å): Zn−N1 2.155(4), Zn−N4 2.148(6), Zn−N7 2.085(6), Zn−N10 2.145(5), Zn−N15 1.969(4). Selected bond angles (deg): N1−Zn−N4 82.5(2), N1−Zn−N7 140.9(2), N1−Zn−N10 80.9(2), N1−Zn−N15 85.2(2), N4−Zn−N7 82.6(2), N4−Zn−N10 131.4(2), N4−Zn−N15 109.4(2), N7−Zn−N10 82.4(2), N7−Zn−N15 133.9(2), N10−Zn−N15 114.2(2).

Table 2. Selected Crystallographic Data for **7**·ClO_4

formula	$C_{22}H_{25}N_6O_6SClZn$
formula wt	612.45
crystal color, habit	colorless, prismatic
crystal system	orthorhombic
space group	*Pna*2$_1$ (no. 33)
lattice parameters	
a (Å)	23.777(3)
b (Å)	12.744(5)
c (Å)	9.092(3)
V (Å^3)	2755(2)
Z value	4
D_{calc} (g cm^{-3})	1.476
μ(Cu Kα) (cm^{-1})	32.47
temperature (°C)	23.0
scan type	$\omega-2\theta$
scan rate (deg min^{-1}, in ω)	32, 16, 8 for each 2θ shell (4 < 70 < 100 < 120°)
$2\theta_{max}$ (deg)	120.1
structure solution	direct method
refinement	full-matrix least-squares
no. reflns meased	2379
no. obsvns ($I > 3.00\sigma(I)$)	2040
residuals: R, R_w	0.032, 0.047

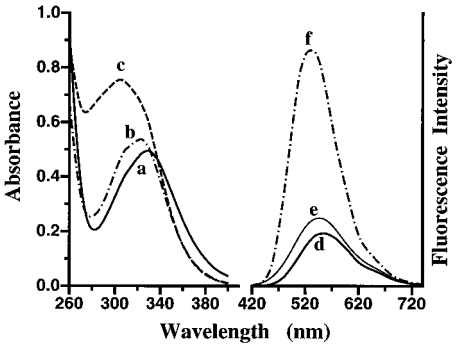


Figure 5. UV absorption spectra at 25 °C and pH 7.3 (10 mM HEPES buffer) with $I = 0.10$ ($NaNO_3$): (a) 0.10 mM H_3L (**6**·$2H^+$), $\lambda_{max} =$ 330 nm (ϵ 4950); (b) 0.10 mM ZnL (**7**), 323 nm (5360); (c) 0.10 mM CuL (**17**), 305 nm (7550). Fluorescence spectra by 330 nm excitation at 25 °C with $I = 0.10$ ($NaNO_3$): (d) 5 μM H_3L (**6**·$2H^+$), $\lambda_{max} = 555$ nm, pH 7.3 (1.0 mM HEPES buffer); (e) 5 μM L (monoanionic form of **6**), 548 nm, pH 12.5 (nonbuffer); (f) 5 μM ZnL (**7**), 528 nm, pH 7.3 (1.0 mM HEPES buffer). 5 μM CuL (**17**) has no fluorescence under the same conditions.

($NaNO_3$). While dansylamide−pendant deprotonation of H_3L^{2+} (at pH 7.3) to L (at pH 12.5) accompanies only 25% increase in the fluorescence intensity (Figure 5e), the dansylamide−pendant deprotonation with zinc(II) increases the fluorescence intensity of **6** (5 μM) by 4.9-fold (see Figure 5d,f). To the contrary, dansylamide deprotonation with copper(II) completely quenches the fluorescence. The quantum yields of the free ligand **6** (H_3L^{2+}) and the zinc(II) complex **7** (ZnL) in pH 7.3 (HEPES buffer) at 25 °C with $I = 0.10$ ($NaNO_3$) are 0.03 and 0.11, respectively. A blue shift of the fluorescence of **6**·$2H^+$ ($\lambda_{max} = 555$ nm) occurred upon the zinc(II) complexation into **7** ($\lambda_{max} = 528$ nm). Moreover, the fluorescence spectra of **7** ($\lambda_{max} = 528$ nm) in H_2O moves toward shorter wavelengths in organic solvents: 496 nm in MeOH, 489 nm in EtOH, and 484 nm in CH_3CN. The hydrophobic effect by these nonaqueous solvents is also manifested by greater quantum yields, $\Phi = 0.53$ in MeOH, 0.60 in EtOH, and 0.44 in CH_3CN. Similar fluorescence blue shift and fluorescence intensity enhancement were reported for the dansylamide complexation with carbonic anhydrase (CA): the fluorescence of free dansylamide in water has a peak emission at 580 nm with $\Phi = 0.06$ upon excitation at 320 nm, while the CA−dansylamide complex has an emission maximum at 468 nm with $\Phi = 0.84$.[8] The larger blue shift and greater fluorescence enhancement were accounted for by both the hydrophobic environment and the deprotonation of dansylamide at the active center of CA.

With Imperiali's peptide chemisensor **11** (1.4 μM) in the absence and presence of 1.5 μM zinc(II) ion, the dansylamide−pendant emission occurs at 560 and 525 nm, respectively, upon excitation at 333 nm.[13] The blue shift was much smaller than that observed for the CA−dansylamide complex (from 580 to 468 nm). The fluorescence intensity of the zinc(II)-saturated **11** was only 5.6 times greater than the ligand. Those facts are almost the same as those for our zinc(II) fluorophore **6** in aqueous solution.

Effect of Various Metal Ions on the Fluorescence of Dansylamide−Pendant Cyclen 6 and Comparison with Previous Zinc(II) Sensors. The fluorescence changes of dansylamide−pendant cyclen **6** (5 μM) upon addition of various metal ions (5 μM) at pH 7.3 (1.0 mM HEPES buffer) and 25 °C with $I = 0.10$ ($NaNO_3$) are summarized in Table 3. The addition of various concentrations of zinc(II) ion results in increased emission upon excitation at 330 nm as shown in Figure 6.[21] The response (at 528 nm) is linear between 0.1 and 5 μM,

Table 3. Comparison of the Relative Fluorescence Intensity of 5 μM Dansylamidoethyl−Pendant Cyclen **6**·$2H^+$ in the Presence of Various Additives at 25 °C and pH 7.3 (1.0 mM HEPES Buffer)

none	1.0[a]	KNO_3	1.0[a]
$ZnSO_4$	4.9[a,b]	$LiNO_3$	1.0[a]
$Zn(NO_3)_2$	4.9[a]	$MgSO_4$	1.0[a]
$Zn(ClO_4)_2$	4.9[a]	$CaSO_4$	1.0[a]
$Cd(NO_3)_2$	5.1[a]	$CoSO_4$	0.8[a]
$AgNO_3$	2.7[a]	$MnSO_4$	1.0[a]
$CuSO_4$	0[a]	$Fe(ClO_4)_3$	1.0[a]
$HgCl_2$	0.4[a]	$Fe(ClO_4)_2$	1.0[a]
$Pb(NO_3)_2$	0.8[a]	$NiSO_4$	1.0[a]
5 μM $Zn(ClO_4)_2$			5.1[c,d]
5 μM $Zn(ClO_4)_2$ + 0.1 M KNO_3			5.1[d]
5 μM $Zn(ClO_4)_2$ + 0.1 M $MgSO_4$			5.3[d]
5 μM $Zn(ClO_4)_2$ + 5 mM $CaSO_4$			5.3[d]

[a] At [additive] = 5 μM with $I = 0.10$ ($NaNO_3$). [b] The value is the same as that for 5 μM **7** with $I = 0.10$ ($NaNO_3$). [c] The value is the same as that for 5 μM **7** without supporting electrolyte. [d] Without supporting electrolyte.

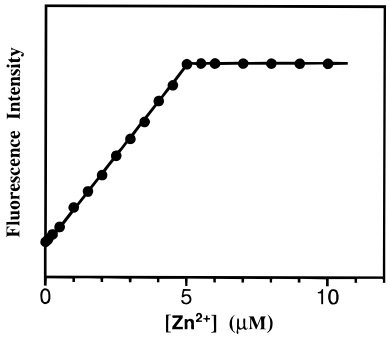


Figure 6. Fluorescence emission response of dansylamidoethyl−pendant cyclen **6** (5 μM) to increasing levels of zinc(II) at 25 °C and pH 7.3 (1.0 mM HEPES buffer) with $I = 0.10$ ($NaNO_3$). The increase in fluorescence intensity at 528 nm is linear between 0.1 and 5 μM zinc(II).

until it reaches a 1:1 [L]/[zinc(II)] ratio, indicating that the increase in fluorescence is stoichiometric, totally due to the ZnL complex formation, and, moreover, once formed ZnL is so stable that even at nanomolar concentrations, the 1:1 complex does not dissociate. The zinc(II)-dependent fluorescence was unaffected by the presence of two times or more excess amount of zinc(II) ion. Cadmium(II) and silver(I) ions display a similar linear fluorescence enhancement behavior, although silver(I) is not as effective as zinc(II) and cadmium(II). A similar linear fluorescence titration curve for zinc(II) was reported for carbonic anhydrase with dansylamide,[8] until the [dansylamide]/[CA] ratio reached 1 (see **3**). Since its binding (K = [dansylamide−CA]/[dansylamide][CA] = 4×10^6 M^{-1})[8] is not so strong, the stoichiometric 1:1 complex formation occurred only at [zinc(II)] ≥ 10 μM. For another comparison, the fluorometric titration of zinc(II) ion with zinquin **10** has been carried out under the conditions used for our **6**. The sensitivity in the fluorescence intensity was almost the same, but the linearity in [zinc(II)] diminished as [zinc(II)] increased due to the formation of both 1:1 and 2:1 zinquin−zinc(II) complexes with much smaller stepwise binding constants of 2.7×10^6 M^{-1} and 1.2×10^7 M^{-1}.[12d] Accordingly, relatively higher concentration of zinquin should be used to obtain linear response to zinc(II) ion.

(21) The ZnL complexation rate was followed by monitoring the 528-nm emission of ZnL at 25 °C and pH 7.3 (1.0 mM HEPES buffer) with $I = 0.10$ ($NaNO_3$). A second-order dependence on the concentration of Zn^{2+} (50 and 100 μM) and **6**·$2H^+$ (2 and 5 μM) was observed and the rate constant was estimated to be $(1.7 \pm 0.1) \times 10^2$ M^{-1} s^{-1}.

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

On the other hand, copper(II) linearly diminishes the fluorescence emission until complete quenching at [copper(II)]/[**6**·2H$^+$] ratio = 1:1. Other quenching metal ions (*e.g.*, open-shell, paramagnetic Co^{2+}, or easily reducible Hg^{2+}, Pb^{2+}) that bind tightly to **6** also cause the intramolecular quenching, although the effects were not so dramatic as copper(II). Copper(II) ion forms the extremely stable five-coordinate complex CuL **17**, which accounts for the most dramatic quenching effect by copper(II) ion.

When the binding interaction was not strong, as demonstrated by 5 μM concentration of Fe^{2+}, Fe^{3+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, or K^+ in aqueous solution, there was no effect on the fluorescence. The fluorescence intensity of 5 μM zinc(II) complex **7** is almost unaffected by the presence of large excess amounts of Na^+, K^+, Mg^{2+}, and Ca^{2+} ions at pH 7.3 (1.0 mM HEPES buffer) (see Table 3).

Conclusion

A newly synthesized dansylamidoethyl−pendant cyclen HL **6** has been proven to be a suitable zinc(II)−fluorophore. The potentiometric pH titration has established the stable 1:1 complexation ML with zinc(II), copper(II), and cadmium(II) at physiological pH, where the dansylamide is deprotonated as a strong N^- donor. The dansylamide-deprotonated complex ZnL **7** was isolated, and its five-coordinate square-pyramidal structure was confirmed. This is the first isolation of a dansylamide anion-coordinating zinc(II) complex, which allowed a full characterization of the fluorescence properties, as a chemical model for the previously suspected dansylamide−carbonic anhydrase complex. The enhanced acidic properties of zinc(II) in a cyclen complex is responsible for the deprotonation of the dansylamidoethyl−pendant in a pH 7 aqueous solution. The resulting stable bonding $ArSO_2N^-$−Zn in **7** increases the fluorescence intensity by 4.9-fold. By changing the solvent, the quantum yield of **7** in emission increases from 0.11 (in H_2O) to 0.60 (in EtOH). The fluorescence of 5 μM **6** increases linearly with [zinc(II)] = 0.1−5 μM and, until the [**6**]/[zinc(II)] ratio reaches 1:1. The cadmium(II) complex **18** similarly emits fluorescence. On the other hand, copper(II) ion that yields an analogous five-coordinate (dansylamide-deprotonated) complex CuL **17** as the zinc(II) complex **7** completely quenches the fluorescence. Other quenching metal ions include easily reducible Hg^{2+}, Pb^{2+} or paramagnetic Co^{2+}. The zinc(II)-dependent fluorescence is unaffected by the presence of mM concentrations of the important biological metal cations Na^+, K^+, Ca^{2+}, and Mg^{2+}. Since Cd^{2+}, Hg^{2+}, or Pb^{2+} are not present to a significant extent and Cu^{2+} is strongly bound to amino acids, peptides, or proteins in ordinary biological systems, **6** may be useful as a new type of zinc(II) fluorophore.

Experimental Section

General Information. All reagents and solvents used were purchased at the highest commercial quality and used without further purification. All aqueous solutions were prepared using deionized and distilled water. Tetrahydrofuran (THF) was distilled over $LiAlH_4$. Acetonitrile (CH_3CN) was distilled over calcium hydride. A reference fluorophore zinquin **10** was kindly provided by Prof. P. D. Zalewski.[12] A Good's buffer HEPES (2[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, pK_a = 7.6 at 20 °C) was purchased from Dojindo.

UV and visible spectra were recorded on a Hitachi U-3500 spectrophotometer at 25.0 ± 0.1 °C. Fluorescence spectra were obtained with a Shimadzu RF-5000 spectrofluorometer at 25.0 ± 0.1 °C. Quantum yield was determined by comparison of the integrated corrected emission spectrum of a standard quinine. Excitation at 330 nm was used for quinine in 0.10 M H_2SO_4, and its quantum yield was assumed to be 0.54.[8] IR spectra were recorded on a Shimadzu FTIR-4200 spectrophotometer at 25 ± 2 °C. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra at 35.0 ± 0.1 °C were recorded on a JEOL JNM LA500 spectrometer. 3-(Trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid sodium salt in D_2O and tetramethylsilane in $CDCl_3$ were used as internal references for ^{1}H and ^{13}C NMR measurements. Elemental analysis was performed on a Perkin Elmer CHN Analyzer 2400. Thin-layer (TLC) and silica gel column chromatographies were performed using Merck Art 5554 (silica gel) TLC plate and Fuji Silysia Chemical FL-100D (silica gel), respectively.

Synthesis of Aminoethyl−Pendant Cyclen Pentahydrochloride, 15·5HCl. A MeOH solution (100 mL) of 2,6-dioxo-1,4,7,10-tetraazacyclodecane (**13**) (1.5 g, 7.5 mmol)[14] and 0.5 equiv of bromoacetonitrile (0.45 g, 3.75 mmol) was refluxed for 1 day. The reaction mixture was evaporated to dryness. The residue was purified by silica gel column chromatography (eluent; CH_2Cl_2/MeOH/28% aqueous NH_3 = 10:1:0.1) followed by crystallization from EtOH to yield 10-(cyanomethyl)-2,6-dioxo-1,4,7,10-tetraazacyclododecane (**14**) as colorless prisms (0.75 g) in 70% yield): mp 198−199 °C; TLC (eluent; CH_2Cl_2/MeOH/28% aqueous NH_3 = 5:1:0.1) R_f = 0.36. IR (KBr pellet) 3333, 3275, 2973, 2951, 2907, 2836, 2240, 1640, 1549, 1466, 1449, 1437, 1420, 1358, 1306, 1285, 1175, 1142, 1067, 856, 826, 783, 693, 606 cm^{-1}. The ^{1}H and ^{13}C NMR assignments were obtained by 1D (^{1}H and ^{13}C) and 2D (NOESY and HMBC) NMR experiments in D_2O. ^{1}H NMR: δ 2.75 (4H, t, J = 5.6 Hz, HC-9,11), 3.33 (4H, s, HC-3,5), 3.34 (4H, t, J = 5.6 Hz, HC-8,12), 3.83 (2H, s, HC-1′). ^{13}C NMR: δ 38.8 (C-8,12), 43.9 (C-1′), 53.3 (C-9,11), 57.0 (C-3,5), 120.1 (CN), 177.5 (CO). Anal. ($C_{10}H_{17}N_5O_2$) C, H, N: Calcd, 50.2, 7.2, 29.3. Found, 50.6, 7.3, 29.7.

To a suspension of dioxo macrocycle **14** (1.6 g, 6.7 mmol) in dry THF (32 mL) was slowly added a THF solution (100 mL) of 1 M BH_3−THF complex at 0 °C. The mixture was stirred at room temperature for 2 h and then heated at 60 °C for 1 day. After decomposition of the excess amount of hydroborane complex with water at 0 °C, the solvent was evaporated. After the residue had been dissolved in 6 M aqueous HCl (100 mL), the solution was refluxed for 3 h. The mixture was washed with CH_2Cl_2 (50 mL × 2). After evaporation of the solvent, the residue was passed through an anion exchange column of Amberlite IRA-400 eluting with water to yield the aminoethyl−cyclen **15** as a colorless oil. Crystallization of **15** from 12 M aqueous HCl afforded colorless prisms of the pentahydrochloride salt **15**·5HCl·2H_2O (1.8 g) in 67% yield: dec 214 °C; TLC (eluent; CH_2Cl_2/MeOH/28% aqueous NH_3 = 2:2:1) R_f = 0.2. IR (KBr pellet) 3456, 3009, 2968, 1611, 1576, 1493, 1480, 1443, 1426, 1391, 1375, 1358, 1283, 1096, 1082, 1067, 1002, 974, 934, 787, 600, 500 cm^{-1}. ^{1}H NMR (D_2O, pD = 11): δ 2.50 (2H, t, J = 6.2 Hz), 2.56−2.61 (12H, m), 2.67 (2H, t, J = 6.2 Hz), 2.74 (4H, t, J = 5.2 Hz). ^{13}C NMR (D_2O, pD = 11): δ 38.7, 43.9, 44.9, 45.5, 51.5, 56.8. Anal. ($C_{10}H_{34}N_5OCl_5$) C, H, N: Calcd, 27.7, 7.9, 16.2. Found, 27.7, 7.9, 16.1.

Synthesis of Aminoethyl−Pendant Cyclen Zinc(II) Complex, 16. An aqueous solution (10 mL) of **15**·5HCl (0.92 g, 2.3 mmol) was passed through an anion exchange column of Amberlite IRA-400 eluting with water to obtain the free ligand as a colorless oil. After the oil had been dissolved in EtOH (20 mL), $Zn(ClO_4)_2$·6H_2O (0.89 g, 2.4 mmol) was added. The solution was stirred at 60 °C for 2 h. After the solvent was evaporated, the residue was crystallized from water to yield colorless prisms of **16**·$(ClO_4)_2$ (0.90 g) in 83% yield: dec 264 °C; TLC (eluent; CH_3CN/MeOH/10% aqueous NaCl = 2:2:1) R_f = 0.2. IR (KBr pellet) 3440, 3301, 3268, 3223, 2917, 2882, 1647, 1474, 1142, 1120, 1092, 959, 627 cm^{-1}. ^{1}H NMR (D_2O): δ 2.74−2.86 (10H, m), 2.95−3.10 (10H, m). ^{13}C NMR (D_2O): δ 41.1, 45.5, 45.6, 46.7, 46.8, 47.39, 47.41, 53.15, 53.18, 53.24. Anal. ($C_{10}H_{25}N_5O_8Cl_2Zn$) C, H, N: Calcd, 25.0, 5.3, 14.6. Found, 25.1, 5.3, 14.7.

Synthesis of Dansylamidoethyl−Pendant Cyclen Zinc(II) Complex, 7. To a dry CH_3CN solution (35 mL) of **16**·$(ClO_4)_2$ (0.80 g, 1.7 mmol) and K_2CO_3 (0.23 g, 1.7 mmol) was slowly added a dry CH_3CN solution (100 mL) of dansyl chloride (0.45 g, 1.7 mmol). The reaction mixture was stirred at room temperature for 1 day. After the solvent had been evaporated, the residue was dissolved in 0.5 M aqueous $HClO_4$ (20 mL). After the aqueous solution had been washed with CH_2Cl_2 (25 mL × 8), the solution pH was adjusted to 7 with a saturated aqueous $NaHCO_3$ solution to obtain dansylamidoethyl−pendant cyclen zinc-

(II) complex **7** as white precipitate. Crystallization of the solid from 0.1 M aqueous $NaClO_4$/MeOH afforded **7**·ClO_4 as colorless prisms (0.76 g) in 74% yield: TLC (eluent; MeOH/10% aqueous NaCl = 1:1) $R_f = 0.36$. IR (KBr pellet) 3455, 3277, 2894, 2874, 1576, 1480, 1453, 1395, 1304, 1258, 1138, 1099, 984, 837, 791, 637, 625, 579 cm^{-1}. ^1H NMR (CD_3CN): δ 2.64−2.68 (6H, m, CH_2), 2.78−2.84 (4H, m, CH_2), 2.85 (6H, s, CH_3), 2.95−3.01 (10H, m, CH_2), 7.22 (1H, d, $J = 7.6$ Hz, ArH), 7.52 (1H, t, $J = 7.2$ Hz, ArH), 7.54 (1H, dd, $J = 7.6$ and 7.9 Hz, ArH), 8.13 (1H, d, $J = 7.2$ Hz, ArH), 8.42 (1H, d, $J = 7.2$ Hz, ArH), 8.44 (1H, d, $J = 7.9$ Hz, ArH). ^{13}C NMR (CD_3CN): δ 43.5, 44.6, 45.6, 45.8, 46.7, 51.1, 54.5, 115.8, 121.8, 124.6, 128.0, 128.2, 129.0, 130.9, 131.2, 140.7, 152.7. Anal. ($C_{22}H_{35}N_6O_6SClZn$) C, H, N: Calcd, 43.1, 5.8, 13.7. Found, 43.0, 5.7, 13.5.

Synthesis of Dansylamidoethyl−Pendant Cyclen Pentahydrochloride, 6·5HCl. An acidic aqueous solution (0.25 M HNO_3, 20 mL) of **7**·ClO_4 (0.64 g, 1.0 mmol) and 5 equiv of EDTA (ethylenediaminetetraacetic acid, 1.46 g, 5.0 mmol) was stirred at room temperature for 1 h. The solution pH was adjusted to 7 with saturated aqueous $NaHCO_3$ at room temperature. The solution was extracted with CH_2Cl_2 (100 mL × 10). After the combined organic layers had been dried over anhydrous Na_2SO_4, the organic solvent was evaporated. The oily residue was crystallized from 6 M aqueous HCl to yield colorless prisms of (1-(2-(5-(dimethylamino)-1-naphthalenesulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane pentahydrochloric acid salt, **6**·5HCl·$(H_2O)_3$ (0.41 g) in 60% yield: dec 158 °C; TLC (eluent; MeOH/10% aqueous NaCl = 1:1) $R_f = 0.4$. IR (KBr pellet) 3440, 2992, 2747, 1626, 1458, 1324, 1177, 1144, 1096, 1074, 1051, 795, 586 cm^{-1}. The ^1H and ^{13}C NMR assignments were obtained by 1D (^1H and ^{13}C) and 2D (^1H COSY, HMQC, and HMBC) NMR experiments in D_2O. ^1H NMR: δ 2.72 (2H, t, $J = 5.3$ Hz, HC-13), 2.97−3.28 (16H, m, CH), 3.03 (2H, t, $J = 5.3$ Hz, HC-14), 3.56 (6H, s, CH_3), 7.97 (1H, dd, $J = 7.9$ and 8.9 Hz, HC-23), 7.98 (1H, dd, $J = 7.6$ and 8.5 Hz, HC-19), 8.15 (1H, d, $J = 7.9$ Hz, HC-22), 8.42 (1H, d, $J = 7.6$ Hz, HC-18), 8.51 (1H, d, $J = 8.5$ Hz, HC-20), 8.83 (1H, d, $J = 8.9$ Hz, HC-24). ^{13}C NMR: δ 44.0 (C-14), 44.5, 45.1, 47.3, 49.9 (C-30,31), 51.4 (C-2,12), 55.0 (C-13), 122.7 (C-22), 128.6 (C-26), 128.8 (C-20), 129.4 (C-24), 130.1 (C-23), 131.3 (C-19), 131.6 (C-17), 133.2 (C-18), 137.9 (C-25), 141.5 (C-21). Anal. ($C_{22}H_{47}N_6O_5SCl_5$) C, H, N: Calcd, 38.6, 6.9, 12.3. Found, 38.6, 6.7, 11.9.

Synthesis of Dansylamidoethyl−Pendant Cyclen Copper(II) Complex, 17. To an aqueous solution (10 mL) of **6**·5HCl·$(H_2O)_3$ (0.84 g, 1.1 mmol) was slowly added $Cu(ClO_4)_2$·$6H_2O$ (0.41 g, 1.1 mmol). The solution pH was adjusted to 8 with 1 M NaOH at 50 °C. After cooling the solution to room temperature, dansylamidoethyl−pendant cyclen copper(II) complex **17** was obtained as a blue precipitate. Crystallization of the solid from 2-propanol/H_2O afforded **17**·ClO_4 as blue prisms (0.49 g) in 73% yield: TLC (eluent; MeOH/10% aqueous NaCl = 1:1) $R_f = 0.56$. Visible absorption maximum (λ_{max}) 588 nm (ϵ 294) at pH 2.6, 633 nm (ϵ 207) at pH 7.8. IR (KBr pellet) 3456, 3296, 2932, 2876, 2838, 1578, 1478, 1456, 1395, 1248, 1227, 1144, 1090, 978, 943, 833, 791, 625, 579 cm^{-1}. Anal. ($C_{22}H_{35}N_6O_6SClCu$) C, H, N: Calcd, 43.3, 5.8, 13.8. Found, 43.4, 5.9, 13.7.

Synthesis of 2-(Dimethylamino)-1-(5-(dimethylamino)-1-naphthalene-sulfonamido)ethane Dihydrochloric Acid Salt, 12·2HCl. To a dry CH_3CN solution (5 mL) of 1-amino-2-(dimethylamino)ethane (0.10 g, 1.1 mmol) was slowly added a dry CH_3CN solution (50 mL) of dansyl chloride (0.30 g, 1.1 mmol). The reaction mixture was stirred at room temperature for 3 h. After evaporation of the solvent, the residue was dissolved in 0.1 M aqueous HCl (20 mL). The aqueous solution was washed with CH_2Cl_2 (20 mL × 5), and the pH adjusted to 10 with 0.1 M NaOH. After extraction of the aqueous solution with CH_2Cl_2 (20 mL × 10), the combined organic layers were concentrated. The residue was crystallized from 6 M aqueous HCl/2-propanol to yield 2-(dimethylamino)-1-(5-(dimethylamino)-1-naphthalenesulfonamido)-ethane dihydrochloric acid salt (**12**·2HCl) (150 mg) in 34% yield: dec. 154 °C; TLC (eluent; CH_2Cl_2/MeOH/28% aqueous NH_3 = 10:1:0.1) $R_f = 0.69$; $\lambda_{max} = 315$ nm (ϵ 5580) at pH = 12.2, 330 nm (4620) at pH = 6.9, 287 nm (8170) at pH 2.2 at 25 °C with $I = 0.10$ ($NaNO_3$). IR (KBr pellet) 3416, 3069, 2824, 2704, 1638, 1512, 1464, 1439, 1393, 1333, 1206, 1177, 1157, 1144, 1094, 1059, 1047, 1015, 988, 934, 826, 785, 669, 646, 585, 532, 461 cm^{-1}. ^1H NMR (D_2O): δ 2.94 (6H, s, CH_3), 3.27−3.31 (4H, m, CH_2), 3.45 (6H, s, CH_3), 7.927 (1H, dd, $J =$ 7.9 and 8.7 Hz, ArH), 7.929 (1H, dd, $J = 7.3$ and 8.7 Hz, ArH), 8.04 (1H, d, $J = 7.9$ Hz, ArH), 8.41 (1H, d, $J = 7.3$ Hz, ArH), 8.51 (1H, d, $J = 8.7$ Hz, ArH), 8.72 (1H, d, $J = 8.7$ Hz, ArH). ^{13}C NMR (D_2O): δ 40.6, 46.0, 49.5, 59.5, 122.1, 128.1, 129.2, 129.5, 129.7, 131.4, 131.6, 133.6, 137.0, 143.4. Anal. ($C_{16}H_{25}N_3O_2SCl$) C, H, N: Calcd, 48.7, 6.4, 10.7. Found, 48.5, 6.5, 10.4.

Potentiometric pH Titration. The pH-meter (Horiba F-16) and electrode system (a pH-glass electrode and a double-junction reference electrode) was daily calibrated as follows:[16] An aqueous solution (50 mL) containing 4.00 mM of HCl and 96 mM of $NaNO_3$ ($I = 0.10$) was prepared under an argon atmosphere (>99.999% purity) at 25.0 ± 0.1 °C, and then the first pH value (pH_1) was read. After 4.0 mM of 0.10 M NaOH (>99% purity) was added to the acidic solution, the second pH value (pH_2) was read. The corresponding theoretical pH values to pH_1 and pH_2 are calculated to be $pH_1' = 2.481$ and $pH_2' = 11.447$, respectively, using K_w ($= a_{H^+} \cdot a_{OH^-}$) $= 10^{-14.00}$, K_w' ($[H^+][OH^-]$) $= 10^{-13.79}$, and f_{H^+} (a_{H^+}/H^+) $= 0.825$. The correct pH values (pH = $-\log a_{H^+}$) can be obtained using the 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 potentiometric pH titrations of **6**·5HCl (1 mM) were carried out in the presence or absence of equimolar MSO_4 (M = Cu^{2+}, Zn^{2+}, and Cd^{2+}) with $I = 0.10$ ($NaNO_3$), and at least three independent titrations were performed. The protonation constants ($K_n' = [H_nL]/[H_{n-1}L][H^+]$) of **6** and **12** and metal complexation constants (K(ML) = [ML]/[L][M]) and K(MHL)′ = [MHL]/[ML][H^+]) were determined by means of the pH-titration program BEST.[22] The σ pH fit values defined in the program are smaller than 0.005 for K_n', and 0.05 for K(ML) and K(MHL)′. The species distribution values (%) against pH (= −log[H^+] + 0.084) were obtained using the program SPE.[22] The mixed protonation constants K_n and K(MHL) are derived from K_n' and K(MHL)′ using [H^+] = a_{H^+}/f_{H^+}.

Crystallographic Study. A colorless prismatic crystal, 0.45 × 0.25 × 0.20 mm of **7**·ClO_4 was used for data collection. The lattice parameters and intensity data were measured on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation and a rotating anode generator at 23.0 °C. The structures was solved by direct methods (SIR92) and expanded using Fourier techniques (DIRDIF94). All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 2040 observed reflections ($I > 3.00\sigma(I)$) and 335 variable parameters and converged with R ($= \sum||F_o| - |F_c||/\sum|F_o|$) $= 0.032$ and R_w ($= (\sum w(|F_o| - |F_c|)^2/\sum wF_o^2)^{0.5}$) $= 0.047$.

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Supporting Information Available: Tables of crystallographic parameters, atomic coordinates, equivalent isotropic temperature factors, anisotropic temperature factors, bond distances, and bond angles in CIF format for **7**·ClO_4. See any current masthead page for Internet access instructions.

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