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FULL PAPER

Synthesis and characterization of the zinc(II)-fluorophore, 5-dimethylaminonaphthalene-1-sulfonic acid [2-(1,5,9-triazacyclododec-1-yl)ethyl]amide and its zinc(II) complex

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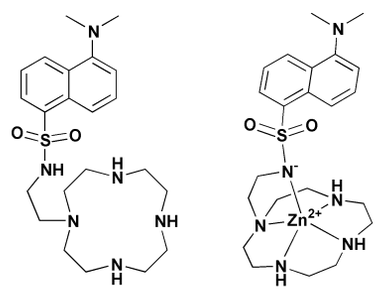
A new zinc(II)-fluorophore, 5-dimethylaminonaphthalene-1-sulfonic acid [2-(1,5,9-triazacyclododec-1-yl)ethyl]amide (HL) has been synthesized and characterized. The spectrophotometric and potentiometric pH-titration study disclosed a 1 : 1 zinc(II) complexation with a stability constant, K(ZnL) of $10^{1.3}$ (= [ZnL]a_{H^+}/[Zn^{2+}][HL]) at 25 °C with I = 0.10 (NaCl) in aqueous solution, where L is the dansylamide deprotonated ligand. The fluorescence intensity of ZnL at 538 nm (excitation at 320 nm) is 5.2 times greater than that of the ligand (HL·H^+ form) in aqueous solution at pH 7.8 and 25 °C with I = 0.10 (NaCl). The X-ray crystal analysis of the zinc(II) complex [ZnL(ClO_4)·EtOH] showed a four-coordinate zinc(II) with three nitrogen atoms of the macrocyclic triamine and the dansylamide N^- anion.

Introduction

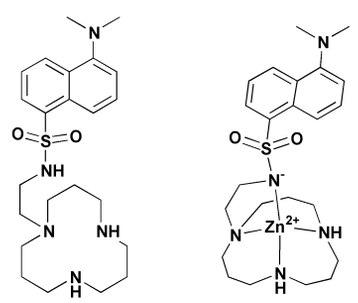
The quantitative analysis of trace amounts of the zinc(II) ion with a selective analytical reagent has become extremely important for environmental and biological applications.[1,2] While remarkable developments have been made for other biologically important divalent metal ions, in particular the calcium(II) ion, with several selective fluorophores such as Quin-2[3] and Fura-2,[4] there are few zinc(II)-selective analytical reagents available for biological research on the zinc(II) ion.[5] Currently, the most widely used probes for detecting intracellular zinc(II) ions are 8-aminoquinoline sulfonamide derivatives such as Zinquine.[6,7] Recently, fluorescein-appended zinc(II) chelates such as azacrownfluorone (ACF),[8] ZnAF,[9] and Zinpyr[10] have been developed as improved probes for understanding zinc(II) chemistry in biological systems.

While working on the elucidation of the intrinsic properties of the zinc(II) ion in the catalytic sites of zinc enzymes by use of macrocyclic polyamine complexes (*e.g.*, zinc(II) complexes with 1,5,9-triazacyclododecane ([12]aneN_3) and 1,4,7,10-tetraazacyclododecane (cyclen)), we have discovered unique acid properties of the zinc(II) ion.[11,12] One of the most outstanding zinc(II) properties is a strong affinity to aromatic sulfonamides, as illustrated by the formation of strong bonds between deprotonated sulfonamide N^- anions and zinc(II) in macrocyclic polyamines at physiological pH.[13,14] On the basis of these findings, we have designed 5-dimethylaminonaphthalene-1-sulfonic acid [2-(1,4,7,10-tetraazacyclododec-1-yl)ethyl]amide (dansylamidoethyl–cyclen, **1**) that serves as a novel type of zinc(II)-fluorophore by forming a highly fluorescent 1 : 1 complex **2** with zinc(II) at physiological pH.[15] The zinc(II) dissociation constant of **1** (K_d = 6 × 10^{-13} mol dm^{-3}) is much smaller than intracellular zinc(II) concentrations,[16] which is a drawback in the dynamic analysis of intracellular zinc(II) ions. In order to shift the analytical range to higher concentrations,[17] we have designed dansylamidoethyl–[12]aneN_3 **3** as a homologous zinc(II)-fluorophore having a larger K_d value. Herein we present the synthesis and characterization of **3** and its zinc(II) complex **4**.



1 **2**

Dansylamidoethyl-cyclen



3 **4**

Dansylamidoethyl-[12]aneN_3

Experimental

All reagents and solvents used were purchased at analytical quality and used without further purification. The supporting electrolyte NaCl, of optical grade purity, was used for micromolar fluorometric analysis. A HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was purchased from Dojindo. All aqueous solutions were prepared using deionized and distilled water. An aqueous solution of 0.100 mmol dm^{-3}

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NaOH for potentiometric pH titration was made by dilution of 10 mol dm^{-3} NaOH (Merck 6495) with decarbonated water. The 10 mol dm^{-3} NaOH solution was kept in a refrigerator below 5 °C, where Na_2CO_3 is less soluble (<1%), and can therefore be removed before raising the solution temperature. Disodium dihydrogen (ethylenedinitrilo)tetraacetate (EDTA·2Na) was used as a sequestering agent for the zinc(II) ion. UV and visible spectra were recorded on a Hitachi U-3500 spectrophotometer at 25.0 ± 0.1 °C. Fluorescence spectra were obtained with a Hitachi F-4500 spectrofluorometer at 25.0 ± 0.1 °C. Emission spectra were calibrated with a reference dye (3.0 g Rhodamine B in 1.0 dm^3 of ethylene glycol) in the range of 200–600 nm and with a standard light source (Hitachi 018-0081) in the range of 500–900 nm. Quantum yields were determined by comparison of the integrated corrected emission spectrum of a chemical standard (quinine). Excitation at 320 nm was used for quinine in 0.10 mol dm^{-3} H_2SO_4, and its quantum yield was assumed to be 0.54. IR spectra with KBr pellets were recorded on a Horiba FT-710 infrared spectrometer 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 LA500 spectrometer with a field gradient unit. Tetramethylsilane (in d_6-DMSO and $CDCl_3$) and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (in D_2O) were used as internal references for ^{1}H and ^{13}C NMR measurements. The ^{1}H and ^{13}C NMR signals were assigned on the basis of 1D and 2D (^{1}H COSY, HMQC, and HMBC) NMR experiments. Elemental analyses were performed on a Perkin Elmer CHN Analyzer 2400. Thin-layer (TLC) and column chromatographies were performed using a Merck Art. 5554 (silica gel) TLC plate and a Fuji Silysia Chemical FL-100D (silica gel), respectively.

Synthetic procedures

5-Dimethylaminonaphthalene-1-sulfonic acid [2-(1,5,9-triazacyclododec-1-yl)ethyl]amide, 3. *Method 1.* A solution of 5-dimethylaminonaphthalene-1-sulfonic acid (2-chloroethyl)-amide[18] (1.3 g, 4.2 mmol) in 30 cm^3 CH_3CN was added dropwise to a solution of 1,5,9-triazacyclododecane (1.4 g, 8.3 mmol) in 20 cm^3 CH_3CN in the presence of KI (0.70 g, 4.2 mmol) at room temperature. After the reaction mixture had been stirred overnight at room temperature, the solvent was evaporated. The residue was purified by silica gel column chromatography (eluent; CH_2Cl_2/CH_3OH/28% aqueous NH_3 = 20 : 1: 0.01) followed by crystallization from AcOEt/EtOH to yield **3**·HI as pale yellow crystals (1.45 g, 61% yield): mp 134–136 °C. TLC (eluent; CH_2Cl_2/MeOH/28% aqueous NH_3 = 10 : 1 : 0.01) R_f = 0.24. IR (cm^{-1}): 3450w, 2940m, 2830m, 1587s, 1576s, 1460w, 1316m, 1142s, 1078m, 793s, 625s, 571s. ^{1}H NMR (DMSO-d_6): δ 1.56 (4H, m, C3 and C11), 1.63 (2H, m, C7), 2.38 (2H, t, J = 6.7 Hz, C13), 2.43 (4H, m, C2 and C12), 2.71 (4H, m, C4 and C10), 2.84 (6H, s, C30 and C31), 2.89 (4H, m, C6 and C8), 2.95 (2H, t, J = 6.7 Hz, C14), 7.20 (br, NH), 7.27 (1H, d, J = 7.0 Hz, C22), 7.62 (1H, dd, J = 7.0 and 8.9 Hz, C23), 7.65 (1H, dd, J = 7.3 and 8.6 Hz, C19), 8.14 (1H, d, J = 7.3 Hz, C18), 8.30 (1H, d, J = 8.9 Hz, C24), 8.48 (1H, d, J = 8.6 Hz, C20). ^{13}C NMR (DMSO-d_6): δ 22.9 (C7), 23.7 (C3 and C11), 39.0 (C14), 45.0 (C30 and C31), 46.0 (C4 and C10), 48.6 (C6 and C8), 50.3 (C13), 52.1 (C2 and C12), 115.1 (C22), 118.9 (C24), 123.5 (C19), 127.8 (C23), 128.0 (C18), 129.0 and 129.1 (C25 and C26), 129.4 (C20), 136.0 (C17), 151.4 (C21). (The numbers of the carbon atoms are the same as those of the zinc(II) complex **4** in Fig. 4). Found: C, 48.2; H, 6.7; N, 12.2%. Calc. for $C_{23}H_{38}IN_5O_2S$: C, 48.0; H, 6.7; N, 12.2%.

Method 2. A solution of *N*-dansylaziridine[19] (0.96 g, 3.5 mmol) and 1,5-bis(*tert*-butoxycarbonyl)-1,5,9-triazacyclododecane[20] (1.1 g, 3.0 mmol) in 120 cm^3 dry CH_3CN was stirred at reflux for 5 h under a nitrogen atmosphere. After the solvent had been evaporated, the residue was purified by silica gel column chromatography (eluent; CH_3COOEt/*n*-hexane = 2 : 3) to yield 5-dimethylaminonaphthalene-1-sulfonic acid [2-(5,9-bis(*tert*-butoxycarbonyl)-1,5,9-triazacyclododec-1-yl)ethyl]-amide as a pale yellow powder (1.9 g, 2.9 mmol): TLC (eluent; CH_3COOEt/*n*-hexane = 1 : 1) R_f = 0.30; ^{1}H NMR ($CDCl_3$): δ 1.45 (18H, s, $C(CH_3)$), 1.55 (4H, q, J = 6.4 Hz, CCH_2C), 1.80 (2H, q, J = 6.9 Hz, CCH_2C), 2.29 (4H, t, J = 6.4 Hz, NCH_2), 2.40 (2H, t, J = 5.9 Hz, NCH_2), 2.89 (6H, s, NCH_3), 2.94 (2H, t, J = 5.9 Hz, NCH_2), 3.19 (4H, t, J = 6.4 Hz, NCH_2), 3.27 (4H, t, J = 6.9 Hz, NCH_2), 7.18 (1H, d, J = 7.6 Hz, ArH), 7.51 (1H, dd, J = 7.6 and 8.4 Hz, ArH), 7.56 (1H, dd, J = 8.0 and 8.4 Hz, ArH), 8.24 (1H, d, J = 8.0 Hz, ArH), 8.33 (1H, d, J = 8.4 Hz, ArH), 8.54 (1H, d, J = 8.4 Hz, ArH); ^{13}C NMR ($CDCl_3$): δ 26.3, 28.5, 40.6, 44.2, 44.8, 45.5, 49.4, 53.8, 79.6, 115.3, 118.8, 123.2, 128.5, 129.7, 130.0, 130.5, 134.7, 152.2, 156.3. To a solution of the bis(*tert*-butoxycarbonyl) intermediate (1.9 g, 2.9 mmol) in 15 cm^3 EtOH was added 12 mol dm^{-3} aqueous HCl (6.0 cm^3). The mixture was stirred at room temperature overnight. After the solvent had been evaporated, the residue was crystallized from H_2O/MeOH/EtOH to obtain colorless needles of **3**·4HCl·$3H_2O$ (0.66 g, 34% yield). TLC (eluent; CH_2Cl_2/MeOH/28% aqueous NH_3 = 10 : 1 : 0.1) R_f = 0.22. IR (cm^{-1}): 3434w, 2956m, 2794w, 1622s, 1585s, 1462s, 1432s, 1146s, 1096s, 795s, 586s, 532s. ^{1}H NMR (D_2O): δ 1.97 (4H, q, J = 5.8 Hz, C3 and C11), 2.28 (2H, q, J = 6.4 Hz, C7), 2.77 (2H, t, J = 5.9 Hz, C14), 2.84 (4H, t, J = 5.8 Hz, C2 and C12), 3.13 (2H, t, J = 5.9 Hz, C13), 3.31 (4H, d, J = 6.4 Hz, C6 and C8), 3.34 (4H, d, J = 5.8 Hz, C4 and C10), 3.56 (6H, s, C30 and C31), 7.94 (1H, dd, J = 7.9 and 8.6 Hz, C23), 7.95 (1H, dd, J = 7.6 and 8.6 Hz, C19), 8.09 (1H, d, J = 7.9 Hz, C22), 8.40 (1H, d, J = 7.6 Hz, C18), 8.50 (1H, d, J = 8.6 Hz, C20), 8.79 (1H, d, J = 8.6 Hz, C24). ^{13}C NMR (D_2O): δ 22.3 (C3 and C11), 23.2 (C7), 41.3 (C13), 45.0 (C6 and C8), 46.3 (C4 and C10), 49.9 (C30 and C31), 52.7 (C2 and C12), 55.6 (C14), 122.7 (C22), 128.6 (C26), 128.9 (C20), 129.3 (C24), 130.0 (C23), 131.3 (C19), 131.6 (C17), 133.4 (C18), 137.6 (C25), 141.7 (C21). Found: C, 42.8; H, 7.3; N, 10.5%. Calc. for $C_{23}H_{47}N_5O_5SCl_4$: C, 42.7; H, 7.3; N, 10.8%.

5-Dimethylaminonaphthalene-1-sulfonic acid [2-(1,5,9-triazacyclododec-1-yl)ethyl]amide zinc(II) complex, 4. A solution of $Zn(ClO_4)_2(H_2O)_6$ (0.37 g, 1.0 mmol) in 10 cm^3 water was added dropwise to a solution of **3**·HI (0.58 g, 1.0 mmol) and NaOH (80 mg, 2.0 mmol) in 10 cm^3 EtOH at 60 °C. After cooling the solution to room temperature, 5-dimethylaminonaphthalene-1-sulfonic acid [2-(1,5,9-triazacyclododec-1-yl)ethyl]amide zinc(II) monoperchlorate, $ZnL(ClO_4)$·EtOH was obtained as colorless needles in 80% yield. The ethanol molecule in the crystal, which was identified by ^{1}H NMR analysis, is easily removed at 50 °C and 1 mmHg pressure for 5 h (a gradual color change to white is observed). IR (cm^{-1}): 3460w, 3238s, 2950s, 2870s, 1589m, 1576m, 1452m, 1261s, 1140s, 1111s, 1092s, 995s, 804s, 638s, 627s, and 585s. ^{1}H NMR (DMSO-d_6): δ 1.55–1.65 (1H, m, C7), 1.65–1.75 (2H, m, C3 and C11), 1.90–2.10 (3H, m, C3, C7 and C11), 2.68 (2H, t, J = 5.5 Hz, C13), 2.83 (6H, s, C30 and C31), 2.80–3.00 (8H, m, NCH_2), 3.08 (2H, t, J = 5.5 Hz, C14), 3.10–3.20 (4H, m, NCH_2), 5.51 (2H, m, NH), 7.21 (1H, d, J = 7.0 Hz, C22), 7.55 (1H, dd, J = 7.0 and 8.5, C23), 7.57 (1H, dd, J = 7.3 and 8.5 Hz, C19), 8.08 (1H, d, J = 7.3 Hz, C18), 8.37 (1H, d, J = 8.5 Hz, C20), 8.43 (1H, d, J = 8.5 Hz, C24). ^{13}C NMR (DMSO-d_6): δ 23.6 (C3 and C11), 25.2 (C7), 42.0 (C14), 45.0 (C30 and C31), 49.0 (C6 and C8), 50.5 (C4 and C10), 56.1 (C2 and C12), 57.2 (C13), 114.6 (C22), 120.5 (C24), 123.5 (C19), 126.6 (C18), 126.9 (C23), 127.6 (C20), 129.1 (C17), 129.8 (C25), 139.0 (C26), 150.9 (C21). Found: C, 45.3; H, 6.0; N, 11.4%. Calc. for $C_{23}ClH_{36}N_5O_6SZn$: C, 45.2; H, 5.9; N, 11.5%.

Potentiometric pH titration

The electrode system (DKK Corporation Multi Channel Ion Meter IOL-40 with a Ross Combination pH Electrode 8102

BN) was calibrated as follows: an aqueous solution (50 cm^3) containing 4.00 mmol dm^{-3} of HCl and 96 mmol dm^{-3} of NaCl ($I = 0.10$) was prepared under a nitrogen atmosphere (>99.999% purity) at 25.0 ± 0.1 °C and then the first pH value (pH_1) was read. After 0.100 mol dm^{-3} NaOH (4.00 cm^3) was added to the acidic solution, the second pH value (pH_2) was read. The theoretical pH values corresponding to pH_1 and pH_2 are calculated to be $pH_1' = 2.481$ and $pH_2' = 11.447$, respectively, using $\log K_W$ (= $\log a_{H^+} \times a_{OH^-}$) = −14.00, $\log K'_W$ (= $\log [H^+][OH^-]$) = −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 0.50 mmol dm^{-3} **3**·4HCl were carried out in the presence or absence of 0.50 mmol dm^{-3} $ZnSO_4$ at 25.0 ± 0.1 °C with I = 0.10 (NaCl), where three independent titrations were performed. The four protonation constants ($K_n' = [HL{\cdot}nH^+]/[HL{\cdot}(n-1)H^+][H^+]$) of **3** (HL) and the zinc(II) complexation constant ($K'(ZnL) = [ZnL][H^+]/[Zn^{2+}][HL]$) were determined by means of the pH-titration program BEST.[21] The pH fit values defined in the program are smaller than 0.01 for K_n' and K'(ZnL). Relative species concentrations (%) at various pH values (pH = $-\log a_{H^+} = -\log [H^+] + 0.084$) were calculated using the program SPE.[21] Mixed constants ($K_n = [HL{\cdot}nH^+]/[HL{\cdot}(n-1)H^+]a_{H^+}$ and $K(ZnL) = [ZnL]a_{H^+}/[Zn^{2+}][HL]$) are derived from K_n' and K'(ZnL) using $[H^+] = a_{H^+}/f_{H^+}$.

Crystal structure determination

Crystal data for $ZnL(ClO_4){\cdot}EtOH$: $C_{25}H_{42}ClN_5O_7SZn$, M = 657.53, monoclinic, a = 9.1608(3), b = 14.6563(3), c = 21.6568(7) Å, β = 97.8617(8)°, U = 2880.4(1) Å^3, T = 113(1) K, space group $P2_1/n$ (no. 14), Z = 4, D_{calc} = 1.516 g cm^{-3}, μ(Mo-Kα) = 10.71 cm^{-1}, 34832 reflections measured, 8750 independent reflections (R_{int} = 0.057), no. of observations ($I > -3.00\sigma(I)$) = 8430, R = 0.066, R_w = 0.087, $R1$ = 0.041 (= $\Sigma||F_o| - |F_c||/\Sigma|F_o|$) using 5484 reflections ($I > 2.0\sigma(I)$). Measurements were made on a Rigaku RAXIS-RAPID Imaging Plate diffractometer with graphite monochromated Mo-Kα radiation. The oxygen atom of EtOH is disordered at two locations. The non-hydrogen atoms, including the ethanol oxygen atom, were refined anisotropically. Hydrogen atoms, excluding those of EtOH, were included but not refined.

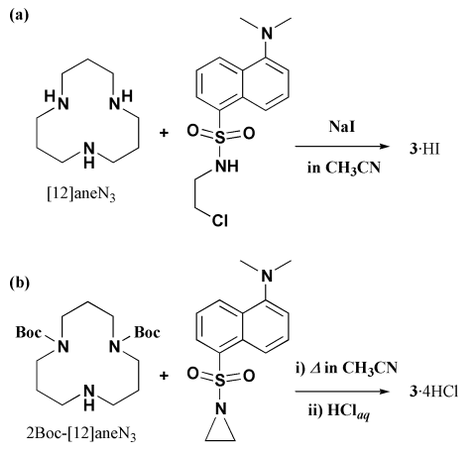
CCDC reference number 175138.

See http://www.rsc.org/suppdata/dt/b1/b110878f/ for crystallographic data in CIF or other electronic format.

Results and discussion

Synthesis of dansylamidoethyl–[12]aneN$_3$, 3 and the zinc(II) complex, 4

The ligand **3** was initially synthesized by a one-step procedure as shown in Scheme 1a. Alkylation of 1,5,9-triazacyclododecane ([12]aneN$_3$) with 5-dimethylaminonaphthalene-1-sulfonic acid (2-chloroethyl)amide[18] in the presence of an equivalent amount of NaI gave dansylamidoethyl–[12]aneN$_3$, **3** as the monohydroiodic acid salt. After the HI salt had been neutralized and deprotonated at the sulfonamide group with two equivalent amounts of NaOH, the monoanionic ligand (L) was treated with $Zn(ClO_4)_2$ in EtOH to obtain the zinc(II) complex **4** (ZnL). Recently, a convenient synthetic route for bis(dansylamidoethyl)–polyamines by use of *N*-dansylaziridine has been developed by Xue *et al.*[19] We adopted a similar procedure for the synthesis of **3** as shown in Scheme 1b. The reaction of *N*-dansylaziridine[19] and 1,5-bis(*tert*-butoxycarbonyl)-1,5,9-triazacyclododecane (2Boc–[12]aneN$_3$)[20] followed by the successive deprotection of the two *tert*-butoxycarbonyl groups by aqueous HCl gave the same ligand **3** as the tetrahydrochloric acid salt.



Scheme 1

Equilibria

The potentiometric pH titration was performed with an aqueous solution of 0.50 mmol dm^{-3} ligand (HL, **3**) at 25 °C with I = 0.10 (NaCl). A typical pH titration curve for **3**·4HCl is shown in Fig. 1a. The titration data were analyzed for four

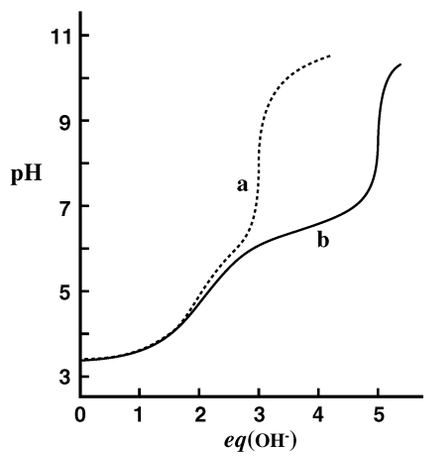


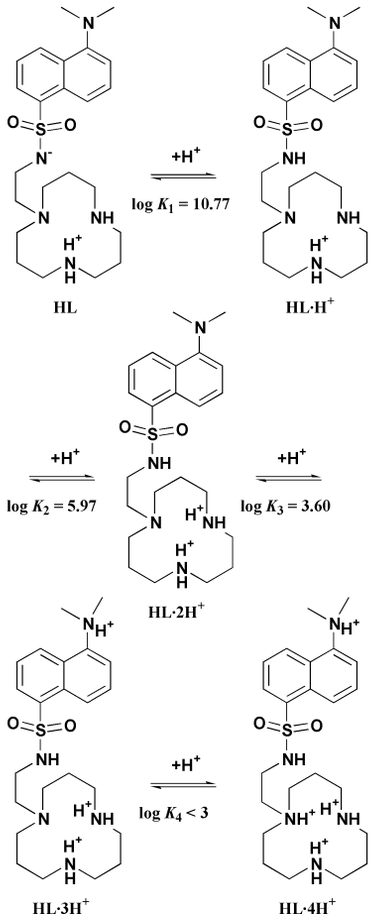
Fig. 1 Typical titration curves for dansylamidoethyl–[12]aneN$_3$, **3**·4HCl (0.50 mmol dm^{-3}) at 25 °C with I = 0.10 (NaCl) in aqueous solution: (a) in the absence of zinc(II) ions; (b) in the presence of 0.50 mmol dm^{-3} $ZnSO_4$. eq(OH^-) is the number of equivalents of base added.

protonation equilibria (1). The four protonation constants (log

$$HL{\cdot}(n-1)H^+ + H^+ \rightleftharpoons HL{\cdot}nH^+ \ (n = 1\text{–}4);\ K_n = [HL{\cdot}nH^+]/[HL{\cdot}(n-1)H^+]a_{H^+} \quad (1)$$

$K_{1\text{–}4}$) of 10.77 ± 0.03 (sulfonamide), 5.97 ± 0.02, 3.60 ± 0.03, and <3 are assigned according to Scheme 2, where HL (in solution) is a zwitterionic form of **3** (*i.e.*, $L^-{\cdot}H^+$). This assignment came from the following facts. (i) Pendant-less [12]aneN$_3$ (1,5,9-triazacyclododecane, L′) has a large protonation constant K_1 of $10^{12.6}$ mol^{-1} dm^3 (= $[L'{\cdot}H^+]/[L']a_{H^+}$), while the remaining two are below 10^8 ($K_2 = 10^{7.5}$ and $K_3 = 10^{2.4}$ mol^{-1} dm^3).[13] (ii) The UV absorption spectral changes for the dansylamide group of 0.10 mmol dm^{-3} **3** at pH 2.0–12.1 (see Fig. 2) are similar to those for dansylamide and dansylamidoethyl–cyclen **1**.[15] (iii) The homologue **1** ($ArSO_2N^-$–cyclen·H^+, zwitterionic form in aqueous solution) has almost the same protonation constant of $10^{10.8}$ mol^{-1} dm^3 for $ArSO_2N^-$– + $H^+ \rightleftharpoons ArSO_2NH$– at 25 °C.[15] At physiological pH, **3** is thus present mostly as the monoprotonated [12]aneN$_3$ species ($HL{\cdot}H^+$) with a neutral form of the dansylamide group.

The potentiometric pH titration curve of **3**·4HCl (0.50 mmol dm^{-3}) in the presence of equimolar Zn^{2+} revealed 1 : 1 zinc(II)



Scheme 2

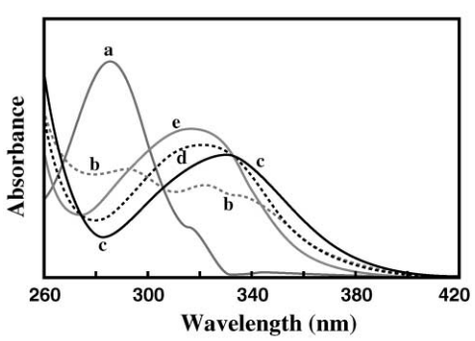


Fig. 2 UV-pH profile for **3** (0.10 mmol dm^{-3}) at 25 °C with I = 0.10 (NaCl) in aqueous solution: (a) pH 2.0, λ_{max} = 287 nm (ε = 8.3 × 10^3); (b) pH 3.9; (c) pH 7.8, λ_{max} = 328 nm (ε = 4.8 × 10^3); (d) pH 10.7; (e) pH 12.1, λ_{max} = 315 nm (ε = 5.7 × 10^3).

complexation with simultaneous dansylamide deprotonation below pH 7, a conclusion derived from the observation of the neutralization break at eq(OH$^-$) = 5 (see Fig. 1b). Further deprotonation or precipitation of $Zn(OH)_2$ was not observed at eq(OH$^-$) > 5, indicating that the zinc(II) complex ZnL **4** remains stable up to pH 12. From the analysis of the pH titration data, the complex formation constant, log K(ZnL) of 1.3 ± 0.1 was obtained (see equilibrium (2)). The dissociation constant (K_d = [free Zn^{2+}][free ligand]/[zinc(II) complex]) at pH

$$HL + Zn^{2+} \rightleftharpoons ZnL + H^+;\ K(ZnL) = [ZnL]a_{H^+}/[Zn^{2+}][HL] \quad (2)$$

7.8 is estimated to be 9 × 10^{-7} mol dm^{-3}. Although the affinity of **3** to the zinc(II) ion is much lower than that of dansylamidoethyl–cyclen **1** (K_d = 6 × 10^{-13} mol dm^{-3} at pH 7.8),[15] the dissociation constant for **4** is still in the range of intracellular zinc(II) concentrations.[6,16] A typical diagram showing species

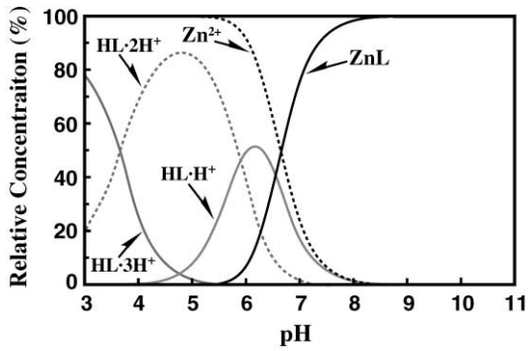


Fig. 3 Distribution diagram for a 1 : 1 mixture of zinc(II) ions and **3** (0.50 mmol dm^{-3}) as a function of pH ($-\log a_{H^+}$) in aqueous solution at 25 °C with I = 0.10 (NaCl).

distribution as a function of pH at [total zinc(II)] = [total ligand] = 0.50 mmol dm^{-3} is displayed in Fig. 3. The final structural assignment for the sulfonamide N$^-$-bound zinc(II) complex **4** comes from the X-ray crystal structure analysis presented below.

Structure of dansylamidoethyl–[12]aneN$_3$ zinc(II) complex, ZnL(ClO$_4$)·EtOH

A colorless needle crystal of the dansylamidoethyl–[12]aneN$_3$ zinc(II) complex **4**, ZnL(ClO$_4$)·EtOH having approximate dimensions of 0.30 × 0.10 × 0.05 mm was obtained by slow recrystallization from EtOH. The elemental analyses, NMR, and IR data of the EtOH-free zinc(II) complex (see Experimental section) suggested the formula ZnL(ClO$_4$). The final support for the deprotonated sulfonamide anion coordinating structure **4** with one EtOH molecule comes from an X-ray crystal analysis. The crystal structure of ZnL is shown in Fig. 4

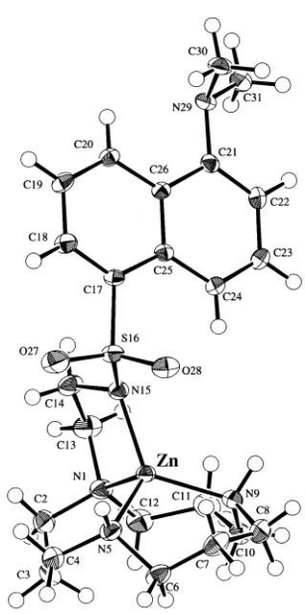


Fig. 4 Crystal structure of the dansylamidoethyl–[12]aneN$_3$ zinc(II) complex, ZnL(ClO$_4$)·EtOH. Perchlorate ion and ethanol molecule are omitted for clarity. Selected bond distances and angles: Zn–N(1), 2.064(2); Zn–N(5), 2.004(2); Zn–N(9), 2.013(2); Zn–N(15), 1.956(2) Å. N(1)–Zn–N(5), 106.70(7); N(1)–Zn–N(9), 107.71(7); N(1)–Zn–N(15), 87.43(7); N(5)–Zn–N(9), 105.11(7); N(5)–Zn–N(15), 121.60(7); N(9)–Zn–N(15), 124.29(7)°.

together with selected bond distances and bond angles relating to the coordination environment of zinc(II). The zinc(II) ion is four-coordinate with a distorted tetrahedral geometry arising from coordination by three N atoms (N1, N5, and N9) and a unidentate sulfonamide N$^-$ anion (N15). The sulfonamide oxygen, ethanol oxygen, and perchlorate oxygen atoms seem to have no direct interaction with zinc(II). The Zn–N$^-$ bond

distance of 1.956(2) Å is much shorter than the average Zn–N([12]aneN$_3$) bond distance of 2.03 Å, indicating a strong interaction between the N$^-$ anion and zinc(II). These facts are compatible with the very strong acidic properties of zinc(II) in favor of the dansylamide N$^-$ anion over neutral nitrogen and O$^-$ anions (*e.g.*, OH$^-$ and ClO_4^-).[14,15] The Zn–N$^-$ and Zn–N bonds are a little shorter than the corresponding coordination bonds in the dansylamidoethyl–cyclen zinc(II) complex **2** (Zn–N$^-$ bond distance of 1.969 Å and an average Zn–N(cyclen) bond distance of 2.13 Å).[15] The crystal structure of ZnL **4** may be viewed as a good model for the distorted tetrahedral zinc(II) coordination environment of carbonic anhydrase with the 3-(acetoxymercurio)-4-aminobenzenesulfonamide anion, which has average Zn–N$^-$ and Zn–N(imidazole) distances of *ca.* 2.0 Å.[22]

Absorption and emission spectra of the metal-free ligand 3 and the zinc(II) complex 4

The UV absorption and emission spectra of the metal-free ligand **3** (almost in HL·H$^+$ form) and the zinc(II) complex **4** at 25 °C and pH 7.8 (10 mmol dm^{-3} HEPES buffer) with I = 0.10 (NaCl) in aqueous solution are compared in Fig. 5a–d. By

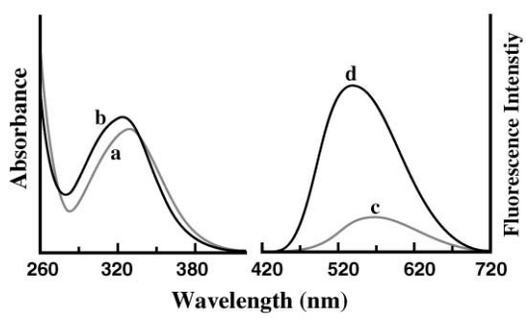


Fig. 5 UV absorption spectra at 25 °C and pH 7.8 (10 mmol dm^{-3} HEPES buffer) with I = 0.10 (NaCl): (a) 50 μmol dm^{-3} HL·H$^+$, λ_{max} = 328 nm ($\varepsilon = 4.8 \times 10^3$); (b) 50 μmol dm^{-3} ZnL, λ_{max} = 320 nm ($\varepsilon = 5.3 \times 10^3$). Fluorescence spectra by 320 nm excitation at 25 °C and pH 7.8 (10 mmol dm^{-3} HEPES buffer) with I = 0.10 (NaCl): (c) 50 μmol dm^{-3} HL·H$^+$, λ_{max} = 568 nm; (d) 50 μmol dm^{-3} ZnL, λ_{max} = 538 nm.

comparison of Fig. 5a (λ_{max} = 328 nm) and 5b (λ_{max} = 320 nm), the UV absorption spectrum of the dansylamide group in the zinc(II) complex is shifted toward shorter wavelength, which is due to dansylamide deprotonation, as found for the free ligand **3** in alkaline solution (see Fig. 2c–e).

Despite the similar UV spectra, the fluorescence intensity at 538 nm (λ_{ex} = 320 nm) of the metal-free ligand **3** (HL·H$^+$ form, 50 μmol dm^{-3}) was increased by 5.2 times upon addition of zinc(II) ions (100 μmol dm^{-3}, two equivalent amounts for complete complexation) at 25 °C and pH 7.8 (10 mmol dm^{-3} HEPES buffer) with I = 0.10 (NaCl) in aqueous solution (see Fig. 5c and 5d). In this case, the zinc(II) complex **4** was rapidly formed within 1 min, a characteristic which is convenient for biological applications. The fluorescence emission spectrum of 50 μmol dm^{-3} **4** was practically unaffected by the presence of 0.10 mol dm^{-3} Na$^+$, 0.10 mol dm^{-3} K$^+$, 5.0 mmol dm^{-3} Ca^{2+}, 10 mmol dm^{-3} Mg^{2+}, 1.0 mmol dm^{-3} Zn^{2+}, or 0.10 mmol dm^{-3} Fe^{2+} under the same conditions. On the other hand, the Cu^{2+} ion diminished the fluorescence emission until complete quenching at [**4**]/[Cu^{2+}] = 1, which is possibly due to formation of the more stable copper(II) complex CuL as previously shown with the homologous zinc(II)-fluorophore **1**.[15] The fluorescence intensity of the metal-free ligand was largely unaffected by pH change between 7 and 9. The quantum yields of the ligand (HL·H$^+$) and the zinc(II) complex **4** (ZnL) are 0.05 and 0.19, respectively, at 25 °C and pH 7.8 (10 mmol dm^{-3} HEPES buffer) with I = 0.10 (NaCl). A blue shift in the fluorescence of HL·H$^+$ (λ_{max} = 568 nm) occurred upon complexation of zinc(II) to **4** (λ_{max} = 538 nm) (see Fig. 5c and 5d). Similar fluorescence blue shifts and fluorescence intensity enhancements were reported for the dansylamide complexation with carbonic anhydrase[23] and for the zinc(II) complexation with **1**.[15]

Conclusion

A newly synthesized dansylamidoethyl–[12]aneN$_3$ **3** has been proven to be a suitable zinc(II)-fluorophore for micromolar concentrations of zinc(II) ions. Upon 1 : 1 zinc(II) complexation, the fluorescence intensity at 538 nm (320 nm excitation) increases by 5.2-fold at pH 7.8 and 25 °C with I = 0.10 (NaCl). The zinc(II)-dependent fluorescence is unaffected by the presence of physiological concentrations of important biological metal ions such as Na$^+$, K$^+$, Ca^{2+}, and Mg^{2+}. On the other hand, the Cu^{2+} ion completely quenches the fluorescence. Since the Cu^{2+} ion is strongly bound to amino acids, peptides, or proteins in ordinary biological systems,[2] **4** may be useful for the dynamic analysis of the biologically important zinc(II) ion.

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