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Design and Synthesis of A Caged Zn^{2+} Probe, 8-Benzenesulfonyloxy-5-*N,N*-dimethylaminosulfonylquinolin-2-ylmethyl-pendant 1,4,7,10-Tetraazacyclododecane, and Its Hydrolytic Uncaging upon Complexation with Zn^{2+}

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8-Benzenesulfonyloxy-5-*N,N*-dimethylaminosulfonylquinolin-2-ylmethyl-pendant cyclen (BS-caged-L^4, BS = benzenesulfonyl) was designed and synthesized as a "caged" derivative of a previously described Zn^{2+} fluorophore, 8-hydroxy-5-*N,N*-dimethylaminosulfonylquinolin-2-ylmethyl-pendant cyclen (L^4) (cyclen = 1,4,7,10-tetraazacyclododecane). In the absence of metal ions and in the dark, BS-caged-L^4 (10 μM) showed negligible fluorescence emission at pH 7.4 (10 mM HEPES with $I = 0.1$ ($NaNO_3$)) and 25 °C (excitation at 328 nm). Addition of Zn^{2+} induced an increase in the UV/vis absorption of BS-caged-L^4 (10 μM) at 258 nm and a significant increase in fluorescence emission at 512 nm. These responses are results from the formation of $Zn(H_{-1}L^4)$ by the hydrolysis of the sulfonyl ester at the 8-position of the quinoline unit promoted by the Zn^{2+}-bound HO^-. Improvement of cell membrane permeation in comparison with L^4 is also described.

Introduction

The zinc ion (Zn^{2+}) is one of ubiquitous essential trace elements in natural biological systems. Zn^{2+} is now recognized as one of the most important cations in catalytic centers and structural cofactors of many Zn^{2+}-containing enzymes and DNA-binding proteins (e.g., transcriptions factors).[1] Although most Zn^{2+} is tightly bound to enzymes and proteins, free zinc pools exist in some tissues such as the brain,[2] pancreas,[3] liver,[4] and prostate.[5] The intracellular

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concentration of free Zn^{2+} is regulated at subfemtomolar orders by Zn^{2+} transporters and other systems.[6] Because Zn^{2+} is spectroscopically silent due to its d^{10} electron configuration, many fluorescence sensors for detection of Zn^{2+} in tissues, cells, and sample solutions have been reported.[7–15] For example, it has been revealed that Zn^{2+} is one of important cofactors in the regulation of apoptosis by a study utilizing a Zn^{2+} fluorescence sensor, Zinquin.[8,16]

Until now, it has been reported that 1,4,7,10-tetraazacyclododecane (cyclen, L^1) forms a stable Zn^{2+} complex **1** (ZnL^1) in aqueous solution at neutral pH[17,18] and is a promising platform for Zn^{2+} fluorophores (Scheme 1).[7] For example, a dansylamidoethyl-pendant cyclen **3** (L^2) forms a stable Zn^{2+} complex **4** ($Zn(H_{-1}L^2)$) in aqueous solution at μM order concentrations (K_d for **4** is 8 pM at pH 7.4), resulting in a 5-fold enhancement in fluorescence emissions (quantum yields of emission (Φ_F) increase from 0.03 to 0.11 upon complexation with Zn^{2+}) (Scheme 1).[19] We also reported that L^2 is a more stable indicator of apoptosis than is Zinquin and that L^2 alone can detect early stage apoptotic

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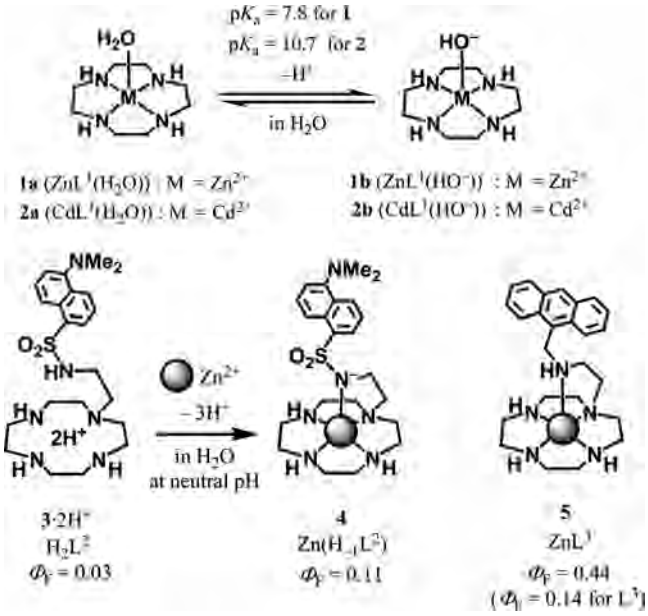
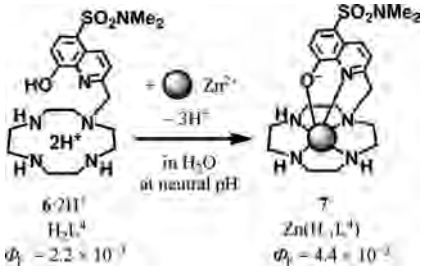
Scheme 1



Scheme 2



cells.[20] Subsequently, the emission of an anthrylmethyl-cyclen (L^3) was demonstrated to increase about 3.1 times upon formation of Zn^{2+} complex **5** (ZnL^3) at pH 7.4 (K_d for **5** at pH 7.4 is 20 pM).[21] It was found that L^3 unselectively responds to Zn^{2+} and Cd^{2+} (5-fold increase in emission for Zn^{2+} and Cd^{2+}), whereas L^2 responds to Zn^{2+}more strongly than Cd^{2+} (3.5- and 1.6-fold for Zn^{2+} and Cd^{2+}, respectively).

Recently, we reported a Zn^{2+} fluorophore **6** (L^4) having a 8-hydroxy-5-*N,N*-dimethylaminosulfonylquinoline moiety (Scheme 2).[22] It was found that **6** forms a very stable Zn^{2+} complex **7** ($Zn(H_{-1}L^4)$) in aqueous solution (K_d = 8 fM at pH 7.4), resulting in a 17-fold enhancement of the emissions at 512 nm (excitation at 338 nm). It should be noted that **7** is much more stable at slightly acidic pH to alkaline pH than **4** and **5**, enabling the determination of Zn^{2+} concentrations in a wide pH range (pH 5−8). In addition, Zn^{2+}complexation of **6** occurs in milliseconds, allowing quick trapping and detection of Zn^{2+}. It was also observed that the fluorescent response of **6** (L^4) to Zn^{2+} (17-fold increase) was smaller than its response to Cd^{2+} (43-fold increase). This phenomenon was attributed to the stronger Lewis acidity of Zn^{2+} than that of Cd^{2+}, as supported by the smaller pK_a value (7.8) of Zn^{2+}-bound H_2O of the Zn^{2+}-cyclen complex (for **1a** ⇔ **1b**) than 10.7 of Cd^{2+}-bound H_2O of the Cd^{2+}-cyclen **2** (CdL^1) (for **2a** ⇔ **2b**). A stain of Zn^{2+}-loaded and apoptotic cells with **6** (L^4) was inconclusive, possibly due to the low cell membrane permeability of **6**. These data prompted us to improve its Zn^{2+}/Cd^{2+} selectivity and cell permeability.

The most popular modification ("caging") to improve in cell-permeability of fluorescent metal sensors is esterification, as seen in acetoxymethyl (AM) esters of Ca^{2+} fluorophores such as fura-2[7a] and quin-2.[23] On the other hand, most examples of caged biomolecules, whose biological activity was temporarily masked, are reactivated by photoirradiation.[24] So far, there is no example of caged compounds that can be uncaged upon complexation with specific metal ions. On the basis of these backgrounds, we first synthesized some caged Zn^{2+} probes bearing acyl groups on the 8-quinolinol of **6**, on the assumption that acyl groups would be hydrolyzed by Zn^{2+}-bound HO^- in their Zn^{2+} complexes, as previously described.[18,25] However, it was found that acylated **6** was easily hydrolyzed (within several hours) in the absence of Zn^{2+} in neutral aqueous solution. Accordingly, we synthesized a caged L^4 having a benzenesulfonyl moiety on an 8-hydroxy group of quinolinol, **8** (BS-caged-L^4), which was assumed to be stable in the absence of Zn^{2+} (Scheme 3). It is very likely that **8** would form a Zn^{2+} complex **9a** (Zn(BS-caged-L^4)(H_2O)), where Zn^{2+}-bound H_2O is deprotonated to give **9b** (Zn(BS-caged-L^4)(HO^-)) (pK_a was estimated to be 7−8). We hypothesized that Zn^{2+}-bound HO^- in **9b** would hydrolyze a sulfonyl ester to yield **7** ($Zn(H_{-1}L^4)$). Since the pK_a value (10.7) for Cd^{2+}-cyclen **2** (CdL^1) is much higher than that for Zn^{2+}-cyclen **1** (ZnL^1) (pK_a = 7.8),[22] it was postulated that hydrolysis of the sulfonyl group at 8-OH in the Cd^{2+}−**8** complex would be very slow at neutral pH, where the Cd^{2+}-bound HO^- form is scarcely produced. In this manuscript, the synthesis of a "sulfonyl-caged" Zn^{2+} probe **8** and the results of its "hydrolytic uncaging" upon complexation with Zn^{2+} or Cd^{2+} are described. Improvement in cell membrane permeability of **8** will also be described.

Experimental Section

General Information. All reagents and solvents were purchased at the highest commercial quality and used without further

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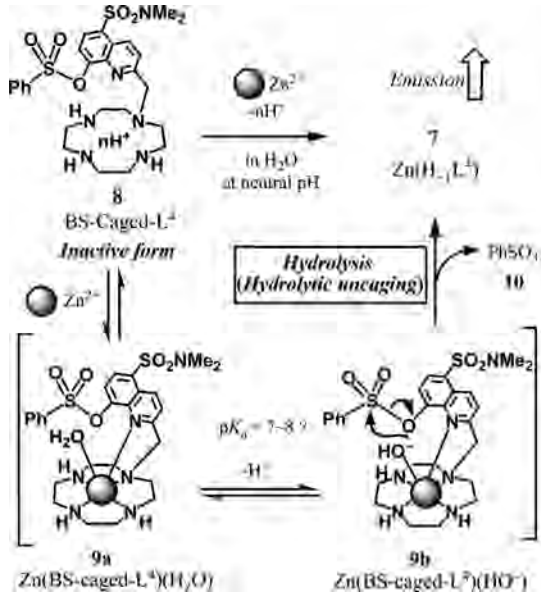
Scheme 3



purification. $ZnSO_4 \cdot 7H_2O$, and $3CdSO_4 \cdot 8H_2O$ were purchased from Kanto Chemical Co. Ltd. Anhydrous acetonitrile (CH_3CN) was obtained by distillation from calcium hydride. All aqueous solutions were prepared using deionized and distilled water. Buffer solutions (CAPS, pH 12.0, 11.5, 11.0, 10.5, and 10.0; CHES, pH 9.5 and 9.0; TAPS, pH 8.4 and 8.0; HEPES, pH 7.8, 7.6, 7.4, and 7.0; MES, pH 6.5 and 6.0) were used, and the ionic strengths were adjusted with $NaNO_3$. The Good's buffer reagents (Dojindo) were commercially available: MOPS (3-(*N*-morpholino)propanesulfonic acid, $pK_a = 7.2$), HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid, $pK_a = 7.6$), EPPS (3-(4-(2-hydroxyethyl)-1-piperazinyl)propanesulfonic acid, $pK_a = 8.0$), TAPS (*N*-(tris(hydroxymethyl)methylamino)-3-propanesulfonic acid, $pK_a = 8.4$), CHES (2-(cyclohexylamino)ethanesulfonic acid, $pK_a = 9.5$), CAPS (3-(cyclohexylamino)propanesulfonic acid, $pK_a = 10.4$). Melting points were measured on a Büchi 510 melting point apparatus and listed without corrections. UV spectra were recorded on a Hitachi U-3500 spectrophotometer and JASCO UV/vis spectrophotometer V-550, and fluorescence (excitation and emission) spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer and JASCO FP-6500 spectrofluorometer at 25 ± 0.1 °C. The quantum yield (Φ) of fluorescence was determined by comparison with the integrated corrected emission spectrum of a standard quinine sulfate, whose quantum yield in 0.1 M H_2SO_4 was assumed to be 0.55 (excitation at 366 nm). IR spectra were recorded on a Horiba FTIR-710 spectrophotometer at room temperature. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra at 35 ± 0.1 °C were recorded on a JEOL Lambda 400 spectrometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a JEOL Always 300 spectrometer. 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSP) sodium salt was used as an external reference for ^{1}H and ^{13}C NMR measurements in D_2O. 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.

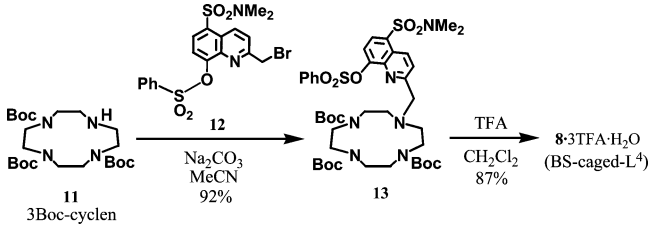
1-(8-Benzenesulfonyloxy-5-*N*,*N*-dimethylaminosulfonylquinolin-2-ylmethyl) 1,4,7,10-Tetraazacyclododecane·3(TFA) salt (8·3(TFA) salt). A solution of trifluoroacetic acid (0.4 mL, 1.39 mmol) in CH_2Cl_2 (1 mL) was added to a solution of **13** (30 mg, 0.11 mmol)[22] in CH_2Cl_2 (1 mL), and the whole mixture was stirred at room temperature for 17 h. The reaction mixture was concentrated under reduced pressure and azeotroped with toluene, and the remaining residue was recrystallized from Et_2O/EtOH to afford a **8**·3TFA salt as a colorless powder (26 mg, 87% yield): mp 159–160 °C. IR (KBr): ν = 3436, 1684, 1200, 1153, 1057, 951, 857, 795, 718 cm^{-1}. ^{1}H NMR (400 MHz, D_2O/external TSP): δ = 2.82 (s, 6H), 3.07–3.30 (br, 16H), 4.18 (s, 2H), 7.43 (d, 1H, J = 8.4 Hz, ArH), 7.63 (t, 1H, J = 8.4 Hz, ArH), 7.78 (d, 1H, J = 8.8 Hz, ArH), 7.81(t, 1H, J = 7.6 Hz, ArH), 7.87 (d, 2H, J = 8.4 Hz, ArH), 8.15 (d, 2H, J = 8.0 Hz, ArH), 9.11 (d, 1H, J = 8.8 Hz, ArH). ^{13}C NMR (100 MHz, D_2O/external 1,4-dioxane): δ = 36.3, 41.1, 41.5, 43.8, 48.3, 57.6, 120.7, 124.5, 125.0, 128.0, 129.8, 130.3, 132.3, 134.6, 135.3, 141.1, 147.6, 159.5, 162.2, 162.6 ppm. Anal. Calcd for $C_{32}F_9H_{41}N_6O_{12}S_2$: C 41.03, H 4.41, N 8.97. Found: C 40.92, H 4.58, N 9.40.

Determination of Concentrations of 3, 6, and 8 in HeLaS3 Cells. HeLaS3 cells were maintained in Dulbecco's modified eagle medium (DMEM) (Sigma) supplemented with 10% FBS (Sanko Junyaku, Japan), 100 μg/mL streptomycin, and 100 units/mL penicillin. HeLaS3 cells were seeded into 60-mm dishes. HeLaS3 cells (3×10^6 cells/6 cm dish) were treated with 50 μM fluorophores (**3**, **6**, or **8**) in culture medium for 0.5 h in a humidified atmosphere of 5% CO_2 at 37 °C. The cells were then washed twice with phosphate-buffered saline (PBS) to remove extracellular fluorophore. Then, all of the cells in a dish were collected, centrifuged (400 *g*, 4 °C, for 5 min) in a 1.5-mL microtube, and then washed with PBS. The cells were resuspended with 60 μL of PBS, and the volume of the suspension containing cells was determined (x μL) by pipetting with a micropipette. This suspension was centrifuged (400 *g*, 4 °C, for 5 min), and the volume of supernatant was determined (y μL) by utilizing a micropipette to calculate total cell volume ($x - y$ μL). To this cell suspension, an aqueous solution (100 μL/dish) containing 2% sodium dodecyl sulfate (SDS) and 40 mM dithiothreitol (DTT) was added. The reaction mixture (80 μL/dish) was ultrasonicated and diluted with 0.55 mM Zn^{2+} in 10 mM HEPES (pH 7.4) with I = 0.1 ($NaNO_3$) (220 μL/dish) to obtain the sample solution (final volume is 300 μL/dish containing 0.4 mM Zn^{2+}). Fluorescent emission spectra of the sample solutions were then measured on a JASCO FP-6500 spectrofluorometer (excitation at 330 nm for **3** and 328 nm for **6** and **8**) to determine the concentration of a Zn^{2+}-probe complex in each sample solution (z μM). Intracellular concentrations of fluorophores were calculated according to eq 1. For **8**, sample solutions were incubated before undertaking emission spectra at 55 °C for 20 h for complete uncaging (hydrolysis).

$$[\text{fluorophore}]_{\text{in\_cell}} = [z \times (300/80) \times (100 + x - y)]/(x - y) \quad (\mu\text{M}) \tag{1}$$

To obtain working curves, HeLaS3 cells (3×10^6 cells/6 cm dish) incubated in the absence of fluorophore were treated as described above to obtain sample solutions (10 mM HEPES (pH 7.4) with I = 0.1 ($NaNO_3$)) containing 0.4 mM Zn^{2+}. To these sample solutions, aliquots of aqueous solution of each fluorophore (**3**, **6**, or **8**) at several given concentrations were added (final concentrations of fluorophore = 0−0.3 μM) and emission spectra were measured. Negligible enhancement in emission of these fluorophores was observed without Zn^{2+}. It was confirmed that the emission intensities of **6** and **8** (5 μM) after reaction with Zn^{2+} (5 μM) in 10 mM HEPES (pH 7.4) with I = 0.1 ($NaNO_3$) in the presence of 500 μM DTT were almost same as those in the absence of DTT.

Scheme 4



Results and Discussion

Synthesis of Caged Zn^{2+} Fluorophore 8 (BS-caged-L^4). A benzenesulfonyl-caged ligand **8** (BS-caged-L^4) was synthesized as shown in Scheme 4. Reaction of 3Boc-cyclen **11**[26] with 8-benzenesulfonyloxy-2-bromomethyl-5-*N*,*N*-dimethylaminosulfonylquinoline **12** gave **13**.[22] Three Boc groups of **13** were removed with trifluoroacetic acid (TFA) in CH_2Cl_2 to afford **8** (BS-caged-L^4) as a TFA salt.

Hydrolysis of Sulfonyl-Caged L^4. Hydrolysis of **8** (BS-caged-L^4) in the presence of Zn^{2+} was examined by ^1H NMR measurements in D_2O at pD 7.4 (100 mM HEPES). Figure 1a and b show that **8** (1 mM) negligibly changes in the absence of Zn^{2+} in 12 h. It was found that **8** was stable at acidic pH, but was slowly hydrolyzed at basic pH (pH ~12) (data not shown). Figure 1c is ^1H NMR spectrum of 1 mM **8** immediately (within 10 min) after addition of 1 mM Zn^{2+}, indicating that Zn^{2+} complex **9** (Zn(BS-caged-L^4)) was formed quantitatively.[27] After incubation at 25 °C for 40 h, new ^1H signals emerged, as shown in Figure 1d and e, coinciding with the ^1H NMR spectra of **7** (Zn($H_{-1}L^4$)) (Figure 1f). The ^1H signals in dashed boxes of Figure 1e correspond to those of $PhSO_3^-$ (**10**) (Figure 1g). These data implied that sulfonyl ester of **8** is slowly hydrolyzed to afford **7** (Zn($H_{-1}L^4$)) and **10**.

Hydrolytic reaction of **9** (Zn(BS-caged-L^4)) at pH 7.4 (10 mM HEPES with $I = 0.1$ ($NaNO_3$)) was followed by UV absorption spectra. The dashed curve (a) in Figure 2 is a UV absorption spectrum of metal-free **8** (50 μM). Upon addition of 50 μM Zn^{2+}, curve a changed to curve b in 15 min,[27] corresponding to the Zn^{2+} complex **9** (Zn(BS-caged-L^4)). After incubation periods of 4, 16, and 24 h, UV spectra of **9** (50 μM) changed to curves c−e with isosbestic points at 274 and 328 nm, suggesting that **7** (Zn($H_{-1}L^4$)) was the major reaction product. Indeed, absorption maxima of curves c, d, and e (258 and 335 nm) agreed well with those of **7** (Figure 2f).

Formation of **7** (Zn($H_{-1}L^4$)) by hydrolysis of **9** at pH 7.4 (10 mM HEPES with $I = 0.1$ ($NaNO_3$)) resulted in the enhancement in its fluorescent emission, as displayed in Figure 3. Curve a is an emission spectrum of metal-free **8** (5 μM), showing almost silent emission. After incubation of a mixture of 5 μM **8** with 5 μM Zn^{2+} for 8, 16, and 24 h, emission spectra changed to curves b, c, and d, respectively, whose emission maxima agreed well with that of **7** (curve e

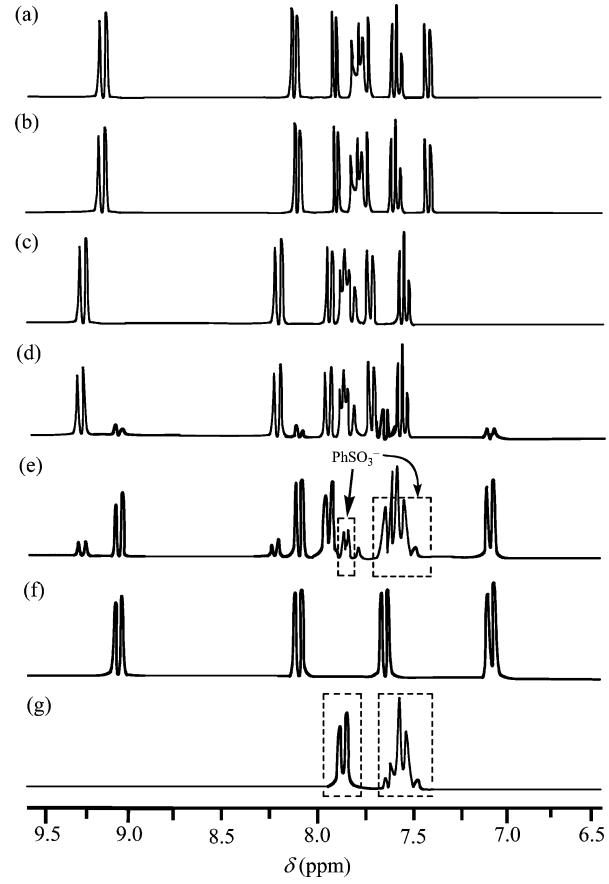


Figure 1. Hydrolysis of **8** (1 mM) in D_2O at pD 7.4 and 25 °C followed by ^1H NMR spectra (aromatic region). (a) ^1H NMR spectrum of **8** in D_2O without Zn^{2+}, (b) 12 h after being dissolved in D_2O without Zn^{2+}, (c) a mixture of 1 mM **8** + 1 mM Zn^{2+} (**9** is formed in situ) immediately after the addition of Zn^{2+}, (d) a mixture of 1 mM **8** + 1 mM Zn^{2+} after an incubation at 25 °C for 1 h, (e) a mixture of 1 mM **8** + 1 mM Zn^{2+} after an incubation at 25 °C for 40 h, (f) **7** (1 mM) in D_2O at pD 7.4, and (g) $PhSO_3^-$ (**10**) in D_2O at pD 7.4.

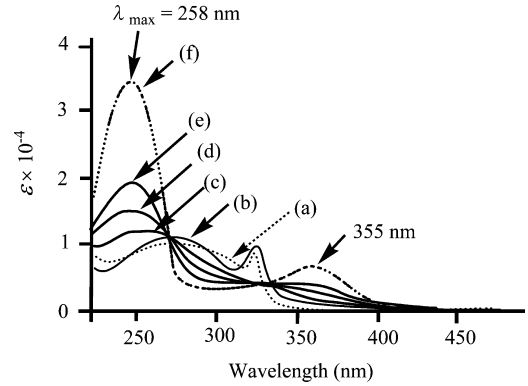


Figure 2. Change in UV absorption spectra of 50 μM **8** due to its hydrolysis upon complexation with Zn^{2+} (50 μM) in 10 mM HEPES (pH 7.4) with $I = 0.1$ ($NaNO_3$) at 25 °C. (a) UV spectra of **8** before addition of Zn^{2+}, (b) UV spectra of **9** immediately after addition of 50 μM Zn^{2+} to **8**, (c) 4 h after addition of Zn^{2+}, (d) 16 h after addition of Zn^{2+}, (e) 24 h after addition of Zn^{2+}, and (f) UV spectrum of 50 μM **7** (Zn($H_{-1}L^4$)) in 10 mM HEPES (pH 7.4) with $I = 0.1$ ($NaNO_3$) at 25 °C.

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

(27) The UV spectral change of **8** upon addition of Zn^{2+} (50, 250, and 500 μM) at pH 7.4 (10 mM HEPES with $I = 0.1$ ($NaNO_3$)) and 25 °C was followed by UV spectra. As summarized in Figure S1 in the Supporting Information, the formation of **9** completes in 15 min. The second rate constant (k_2) for Zn^{2+} complexation of **8** at pH 7.4 and 25 °C was determined to be $k_2 = 3.4 \times 10$ $M^{-1}\cdot s^{-1}$. This value was smaller than that ($k_2 = 1.3 \times 10^2$ $M^{-1}\cdot s^{-1}$) for Zn^{2+} complexation of noncaged probe **6**, possibly because the protecting (benzenesulfonyl) group at 8-quinolinol moiety disturbs chelation of the quinolinol nitrogen to Zn^{2+}.

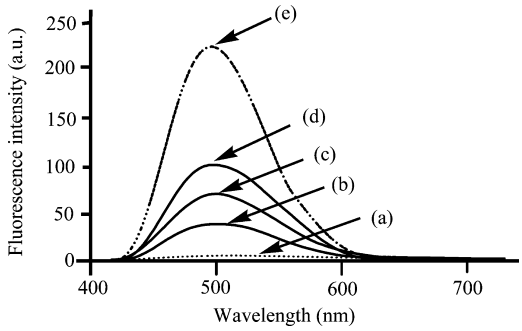


Figure 3. Change in fluorescence emission spectra (quick scan) of 5 μM **8** upon complexation with 5 μM Zn^{2+} in 10 mM HEPES (pH 7.4) with I = 0.1 ($NaNO_3$) at 25 °C (excitation at 328 nm). (a) Emission spectra of **8** before addition of Zn^{2+}, (b) 8 h after addition of Zn^{2+}, (c) 16 h after addition of Zn^{2+}, (d) 24 h after addition of Zn^{2+}, and (e) emission spectra of 5 μM **7** ($Zn(H_{-1}L^4)$). A.u. is arbitrary unit.

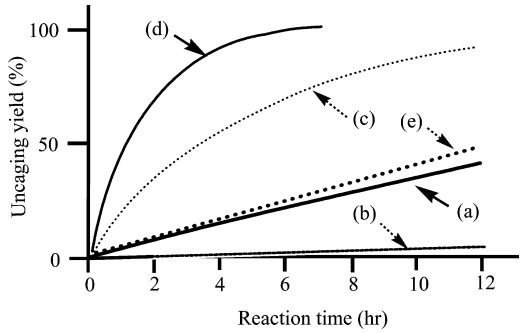


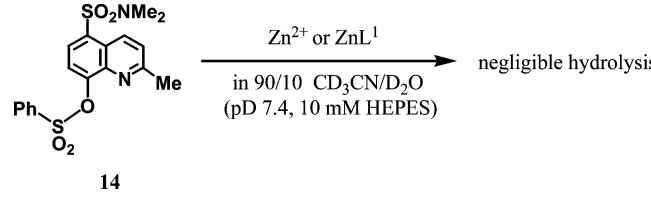
Figure 4. Time-course of hydrolytic uncaging of **8** (50 μM) in the presence of Zn^{2+} (50 μM) in 10 mM HEPES (pH 7.4 with I = 0.1 ($NaNO_3$)) at 25 (a), 5 (b), 40 (c), and 55 °C (d). (e) Hydrolytic uncaging of **8** (50 μM) in the presence of Cd^{2+} (50 μM) at pH 7.4 and 25 °C.

for 5 μM **7**). A quantum yield for fluorescence emission of **8** before addition of Zn^{2+} was almost none and changed to 8.4×10^{-3} after incubation with 5 μM Zn^{2+} at 25 °C for 24 h. Emission intensities of **8** (5 μM) after incubation at 37 °C in the presence of 0–5 μM Zn^{2+} were almost proportional to the added Zn^{2+}. Esterase (EC 3.1.1.1 from porcine liver, Sigma) had negligible effect on hydrolysis of **8**.

It should be noted that emission spectra of **8** were obtained by a quick scanning of the emission wavelength (500−1000 nm/min), because the sulfonate group is cleaved by UV irradiation. The quantum yield for photolysis was $\Phi = 2.3 \times 10^{-5}$. Because BS-caged-$L^4$ and its photoproduct L^4 exhibit almost no emission, we consider that photoreaction of BS-caged-L^4 scarcely hampers quantification of Zn^{2+}. Detail about photolysis of **8** will be reported elsewhere.

Curve a in Figure 4 indicates that ca. 30% of **8** is hydrolyzed upon complexation with Zn^{2+} at pH 7.4 and 25 °C in 12 h, and curve c shows ca. 90% hydrolysis of **8** in 12 h at 40 °C. For comparison, 8-benzenesulfonyloxy-5-*N,N*-dimethylaminosulfonyl-2-methylquinoline **14** (1 mM),[12b] which has no cyclen unit, was scarcely hydrolyzed in the presence of Zn^{2+} (1−5 mM) or ZnL^1 complex **1** (1 mM) in 90/10 CD_3CN/D_2O (10 mM at pD 7.4) at 25 °C (Scheme 5), as observed by ^1H NMR and UV (data not shown). Therefore, it is very likely that hydrolysis of the benzenesulfonate moiety of **8** is facilitated by Zn^{2+}−HO^- species in

Scheme 5



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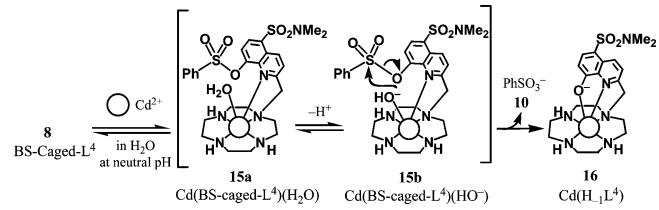
Scheme 6



9b (Scheme 3).[28] On the basis of the estimation that **9b** exists in ca. 28% at pH 7.4 based on the pK_a value (7.8) for Zn^{2+}-bound H_2O of Zn^{2+}−cyclen complex **1** (ZnL^1) (Scheme 1)[29] and that the equilibria between **9a** and **9b** is very fast, the pseudo-first-order reaction rates, k_1 (eq 1), for the hydrolysis of **9** at 25 °C (Figure 4a), 5 °C (Figure 4b), 40 °C (Figure 4c), and 55 °C (Figure 4d) were determined to be $(3.1 \pm 0.1) \times 10^{-5}$, $(5.0 \pm 0.1) \times 10^{-6}$, $(16 \pm 1) \times 10^{-5}$, and $(63 \pm 4) \times 10^{-5}$ sec^{-1}, respectively.[30] Further analysis will be discussed below.

Curve e in Figure 4 implies that the hydrolysis rate of the Cd^{2+} complex **15** (Scheme 6) at pH 7.4 and 25 °C is almost the same as that of the corresponding Zn^{2+} complex **9** under the same conditions. This result was unexpected, because the pK_a value (10.7) of Cd^{2+}-bound H_2O in **2** (and **15**) is greater than that (7.8) of Zn^{2+}-bound H_2O in **1** (and **9**), as described in the Introduction. Assuming that only 0.05% of **15** exists as the Cd^{2+}-bound HO^- species (**15b**, Cd(BS-caged-L^4)(HO^-)) at pH 7.4 (calculated based on the pK_a value of Cd^{2+}−cyclen **2** (CdL^1) of 10.7), k_1 values for the formation of $Cd(H_{-1}L^4)$ complex **16** (Scheme 6) at 25, 40, and 55 °C were determined to be $(2.3 \pm 0.2) \times 10^{-2}$, $(14 \pm 1) \times 10^{-2}$, and $(57 \pm 1) \times 10^{-2}$ sec^{-1}, respectively. These values were larger than those for the hydrolysis of **9**.[31] Negligible acceleration was observed in the hydrolysis of **8** upon addition of Fe^{2+}, Fe^{3+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+},

(28) It was described that hydrolysis of aryl esters of sulfonic acids occurs in S_N2-like reaction. Christman, D. R.; Oae, S. *Chem. Ind. (London)* **1959**, 1251–1252.

(29) (a) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; Dover Pub. Inc.: New York, 1969. (b) Bruice, T. C.; Benkovic, S. J. *J. Am. Chem. Soc.* **1963**, *85*, 1–8.

(30) These values are much smaller than the second rate constants (k_2) for formation of **7** from **6** and Zn^{2+} under the same conditions (e.g. $k_2 = 3.4 \times 10\ M^{-1}\cdot s^{-1}$ at 25 °C, see above).

(31) Vahrenkamp et al. reported the hydrolytic reactions of reactive derivatives of phosphate triesters ($PO(OPh)_2$(O-4NP) and PO(O-rib)(O-4NP) (rib = 2,3-isopropylidene-5-methylribosyl and 4NP = 4-nitrophenyl), acetate (4NPA), and thioester (MeCO(S-4NP)) by pyrazolylborate−Zn^{2+} hydroxide complexes(a) Vahrenkamp, H. *Acc. Chem. Res.* **1999**, *32*, 589–596. (b) Rombach, M. Maurer, C. Weis, K. Keller, E. Vahrenkamp, H. *Chem.−Eur. J.* **1999**, *5*, 1013–1027. The activation energies (E_a) for hydrolysis of these substrates by pyrazolylborate−Zn^{2+} hydroxide complexes were reported to be 12−17 kcal·mol^{-1}.

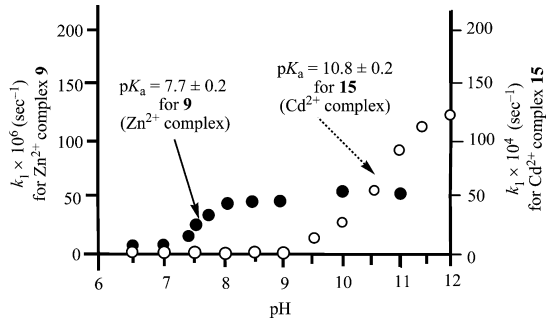


Figure 5. Profile of the pH rate for hydrolytic uncaging of Zn^{2+}−**8** complex **9** (closed circles) and Cd^{2+}−**8** complex **15** (open circles) in Good's buffer with $I = 0.1$ ($NaNO_3$) at 25 °C ([**9**] = [**15**] = 50 μM).

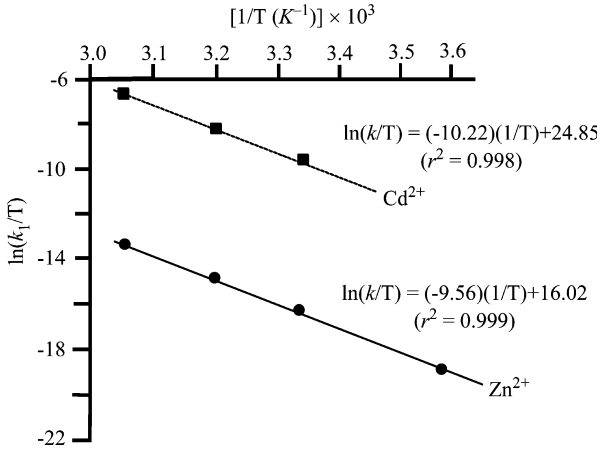


Figure 6. $1/T-\ln(k_1/T)$ profile for the hydrolysis of Zn^{2+}−(BS-caged-L^4) complex **9** and Cd^{2+}− (BS-caged-L^4) complex **15** in 10/90 CH_3CN/10 mM HEPES (pH 7.4) with $I = 0.1$ ($NaNO_3$) ([**9**] = [**15**] = 50 μM).

Al^{3+}, Mg^{2+}, Ca^{2+}, and K^+ in 10 mM HEPES (pH 7.4 with $I = 0.1$) at 25 °C.

The pH-rate profile for hydrolysis of **9** (50 μM) gave a sigmoidal curve displayed in Figure 5 (closed circles). From this curve, the kinetic pK_a value for the Zn^{2+}-bound H_2O in **9** was estimated to be 7.7 ± 0.2, which is in good agreement with the pK_a value of 7.8 for Zn^{2+}−cyclen (Scheme 1). Open circles illustrate the pH profile for hydrolysis of Cd^{2+} complex **15** (50 μM), giving a kinetic pK_a value of ca. 10.8 ± 0.2, which agrees with the pK_a value for the Cd^{2+}-bound H_2O in **2** of 10.7 (Scheme 1). It was confirmed by ^1H NMR experiments and UV absorption titrations that **8** (BS-caged-L^4) (50 μM) forms Cd^{2+} complex (**15**) quantitatively at pH 6−11 (data not shown). Therefore, we concluded that the Zn^{2+}-bound HO^- in **9b** and Cd^{2+}-bound HO^- in **15b** (Scheme 6) act as nucleophiles to hydrolyze sulfonyl esters, as we hypothesized in Scheme 3.[28]

Activation Parameters for Hydrolysis of Zn^{2+} and Cd^{2+} Complexes of 8 (BS-Caged-L^4). Figure 6 shows Eyring's plot (the $1/T-\ln(k/T)$ profile) for hydrolysis of Zn^{2+}−**8** complex (**9**) and Cd^{2+}−**8** complex (**15**), from which the Gibbs activation energy, $\Delta G^{\ddagger}$, the enthalpy of activation, $\Delta H^{\ddagger}$, and the entropy of activation, $\Delta S^{\ddagger}$, were calculated.[31] As summarized in Table 1, $\Delta H^{\ddagger}$ values for the hydrolysis of Zn^{2+} complex **9** and Cd^{2+} complex **15** are almost identical, supporting the finding that hydrolysis of **9** and **15** proceeds in a similar mechanism (nucleophilic attack of metal-bound HO^- to sulfur atom). On the other hand, the $(-T\Delta S^{\ddagger})$ value for the hydrolysis of **15** was considerably smaller than that of **9**, resulting in the smaller $\Delta G^{\ddagger}$ value for hydrolysis of **15** than that of **9**. These facts suggest that transition states for hydrolysis of Cd^{2+} complex **15b** are more flexible than those of Zn^{2+} complex **9b**, possibly due to larger ionic radius of Cd^{2+} than that of Zn^{2+}.[32,33]

Table 1. Activation Parameters for Hydrolysis of Zn^{2+} Complex **9** and Cd^{2+} Complex **15** of BS-Caged-L^4 in 10 mM HEPES (pH 7.4 with I = 0.1 ($NaNO_3$))[a]

metal	$\Delta G^{\ddagger}$ (kcal·mol^{-1})	$\Delta H^{\ddagger}$ (kcal·mol^{-1})	$(-T\Delta S^{\ddagger})$ (kcal·mol^{-1})[b]
Zn^{2+} (**9**)	24 ± 0.2	19 ± 0.1	4.6 ± 0.1
Cd^{2+} (**15**)	19 ± 0.2	20 ± 0.1	−0.6 ± 0.1

[a] [**9**] = [**15**] = 50 μM. [b] T = 298 K.

Scheme 7

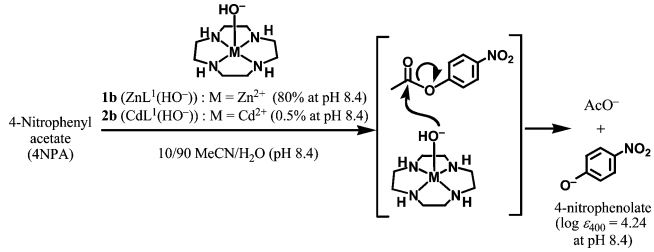


Table 2. Activation Parameters for Hydrolysis of 4NPA (100 μM) in the Presence of ZnL^1 (**1**) and CdL^1 (**2**) in 10/90 CH_3CN/10 mM TAPS (pH 8.4 with I = 0.1 ($NaNO_3$))[a]

promotor	$\Delta G^{\ddagger}$ (kcal·mol^{-1})	$\Delta H^{\ddagger}$ (kcal·mol^{-1})	$(-T\Delta S^{\ddagger})$ (kcal·mol^{-1})[b]
ZnL^1 (**1**)	15 ± 0.2	9 ± 0.1	5.9 ± 0.1
CdL^1 (**2**)	12 ± 0.2	10 ± 0.1	2.2 ± 0.1

[a] [**1**] = [**2**] = 100 μM. [b] T = 298 K.

To further compare the nucleophilicities of Zn^{2+}-bound HO^- and Cd^{2+}-bound HO^-, we checked the hydrolysis of 4-nitrophenyl acetate (4NPA) in the presence of Zn^{2+}−cyclen **1** (ZnL^1) and Cd^{2+}−cyclen **2** (CdL^1) in 10/90 MeCN/10 mM TAPS (pH 8.4 with $I = 0.1$ ($NaNO_3$)) at 25 °C (Scheme 7 and Figure S2 in the Supporting Information). Assuming that the Zn^{2+}−(HO^-) species of **1** (**1b**) and the Cd^{2+}−(HO^-) species of **2** (**2b**) exist in 80% and 0.5% at pH 8.4, respectively, based on the pK_a values of **1** and **2** (Scheme 1), the k_2 values for **1b** and **2b** were determined to be (0.31 ± 0.03) $M^{-1}\cdot s^{-1}$ and (39 ± 1) $M^{-1}\cdot s^{-1}$, respectively, at 25 °C.[34] From Eyring's plots for hydrolysis of 4NPA promoted by **1b** and **2b** (Figure S3 in the Supporting Information), the $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $(-T\Delta S^{\ddagger})$ values were obtained. As listed in Table 2, the $(-T\Delta S^{\ddagger})$ value for the hydrolysis of 4NPA by **2b** was smaller than that by **1b**, supporting more flexible

(32) Martell, A. E.; Hancock, R. D. *Metal Complexes in Aqueous Solutions*; Plenum Press: New York, 1996.

(33) As suggested by the reviewer, we don't exclude the possibility that the larger activation energy for the complex of **8** with Zn^{2+} is due to greater reorganization (release of H_2O molecules) in the transition state in comparison with that of Cd^{2+} complex, because Zn^{2+} has a spatially more extended hydration shell than that of Cd^{2+}.

(34) The k_2 value for the **1**-catalyzed hydrolysis of 4NPA at pH 7.4 was reported to be (0.46 ± 0.01) $M^{-1}\cdot s^{-1}$), which fairly agrees with the obtained value in this work. (a) Kimura, E.; Kodama, Y.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1995**, *117*, 8304–8311. (b) Koike, T.; Inoue, M.; Kimura, E.; Shiro, M *J. Am. Chem. Soc.* **1996**, *118*, 3091–3099.

Scheme 8

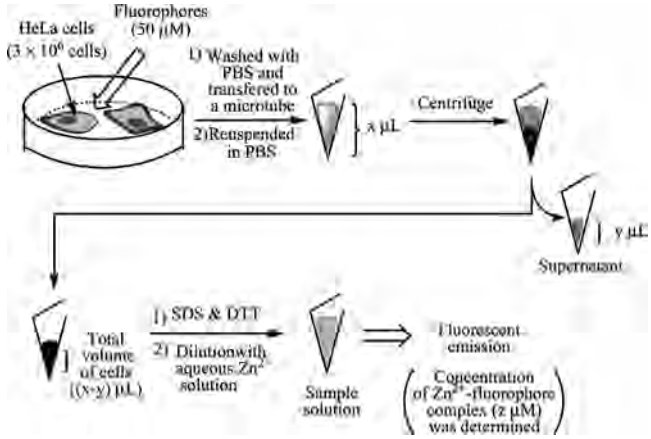


Table 3. Estimated Intracellular Concentrations (μM) of Zn^{2+} Fluorophores in HeLa Cells

	3 (L^2)[a]	**6** (L^4)	**8** (BS-caged-L^4)[a]
intracellular concentrations (μM)	6 ± 1	2± 1	12 ± 3

[a] HeLa cells were incubated with Zn^{2+} fluorophores for 0.5 h.

transition states of $Cd^{2+}-HO^-$-promoted hydrolysis of 4NPA than those of $Zn^{2+}-HO^-$-promoted hydrolysis.

Comparison of Intracellular Concentration of Zn^{2+} Fluorescent Probes (L^2 and L^4) and BS-Caged-L^4. Intracellular concentrations of **3** (L^2), **6** (L^4), and **8** (BS-caged-L^4) were estimated utilizing HeLaS3 cells, as described in the Experimental Section and summarized in Scheme 8. HeLaS3 cells (3×10^6 cells/6 cm dish) were incubated in DMEM containing 50 μM fluorophores (**3**, **6**, or **8**) at 37 °C. Then, all of the cells in a dish were collected, centrifuged, and then washed with PBS. After the volume of the suspension with PBS was determined (x μL) with a micropipette and centrifuged, the volume of the resulting supernatant was determined (y μL) and the total cell volume was calculated ($x - y$ μL). After addition of SDS and DTT, the cell suspensions were ultrasonicated and diluted with 0.55 mM Zn^{2+} to obtain the sample solution. Finally, fluorescent emission spectra of the sample solutions were measured (excitation at 330 nm for **3** and 328 nm for **6** and **8**) to determine the concentration of a $Zn^{2+}-$probe complex (z μL). Intracellular concentrations of the fluorophore were calculated according to eq 1 described in the Experimental Section. As summarized in Table 3, the intracellular concentration of **8** (BS-caged-L^4) was greater than the concentrations of **3** and **6** (L^4), implying that the efficiency of the cell−membrane permeability of **6** was considerably improved by caging.

Conclusion

The purpose of this study was to design and synthesize a new caged Zn^{2+} fluorophore **8** (BS-caged-L^4) that can be uncaged upon complexation with Zn^{2+}. It was revealed that Zn^{2+} complex **9** (Zn(BS-caged-L^4)) was slowly hydrolyzed by Zn^{2+}-bound HO^- of **9b** (Scheme 3) in aqueous solution at neutral pH, where metal-free **8** is not hydrolyzed. It was unexpected that the hydrolysis of $Cd^{2+}-$**8** complex **15** undergoes at the same reaction rates as **9** at pH 7.4, possibly due to of higher reactivity of Cd^{2+}-bound HO^- in **15b** than that of Zn^{2+}-bound HO^- in **9b.** To our knowledge, **8** (BS-caged-L^4) is the first example of caged compounds that can be uncaged upon complexation with specific metal ions. Modification of **8** to improve hydrolytic reactivity is now underway.

So far, quantitative evaluation of the cellular uptake of small molecules including fluorescent probes has remained elusive.[35] The cell permeability of caged compound **8** was compared with that of parent compound **6** (L^4), demonstrating an improvement in cell permeability. These results provide new information for the design of caged metal sensors and for the quantitative detection of small molecules such as therapeutic and diagnostic agents in living systems.

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Supporting Information Available: Figures S1−S3 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(35) Quite recently, the intracellular uptake of Ru(II) complexes were determined by utilizing flow cytometry. Puckett, C. A.; Barton, J. K. *J. Am. Chem. Soc.* **2007**, *129*, 46–47.