Tetrahedron Letters 49 (2008) 6141–6144

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Metal ion-induced FRET modulation in a bifluorophore system

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ARTICLE INFO

Article history:
Received 17 June 2008
Revised 2 August 2008
Accepted 7 August 2008
Available online 26 August 2008

Keywords:
FRET
Fluorescence
Heavy metal ion effect

ABSTRACT

A new fluorogenic sensor **1** capable of illustrating metal ion-triggered FRET changes has been synthesized. Based on the FRET changes, **1**, bearing coumarin-fluorescein moieties, provides a selective sensor for Cu^{2+} and Hg^{2+} ions for experiments implemented in aqueous media with a chloride counter anion.

Selective detection and sensing of transition metal ions has received increasing attention in supramolecular chemistry because of their significant importance in chemical, biological, and environmental processes.[1] In particular, numerous research groups have developed superior fluorescent chemosensors for detection and recognition of Hg^{2+} and Cu^{2+} due to their high toxicity.[2,3] Hg^{2+} can be converted into methylmercury by bacteria, which can affect human health via marine products.[4a] Methylmercury can cause mercurial poisoning, bringing about problems in the central nervous system such as Minamata disease.[4b] Conversely, copper is an essential element in the human body and plays an important role in facilitating iron uptake, thus copper deficiency can induce anemia-like disease.[5] Nevertheless, overdose of copper in body tissue can cause Wilson's disease-like symptoms.[6] With these biological reasons, sensing copper ions and toxic mercury ions in living systems is extremely important.

For the quantitative analysis of these metal cations, many analytical methods, including atomic absorption spectrometry (AAS),[7] ion selective electrodes (ISE),[8] and flame photometry,[9] have been applied. However, they require high cost, large amounts of sample, and do not allow continuous monitoring. Fluorometric sensors have the considerable advantages of simplicity, selectivity, sensitivity, and response time. Most fluorometric sensors, therefore, have been developed to adopt photo-physical changes produced upon metal-cation complexation, including photo-induced electron transfer (PET),[10] photo-induced charge transfer (PCT),[11] excimer/exciplex formation[12] and extinction, and fluorescence resonance energy transfer (FRET).[2d,3a,i,13,14] Among them, FRET is known to be sensitive, selective, and adaptable to a wide variety of systems, but is still at a limited number. FRET arises from an interaction between a pair of fluorophores in their excited states where the excited photon is non-radiatively transferred to the acceptor unit. Therefore, the FRET requires spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor.[15,16]

Although many successful fluorescent chemosensors for Hg^{2+} and Cu^{2+} have been developed, most of them have a couple of problems in practical application, such as low water solubility, slow response time, and low selectivity. In particular, for biological application, it is essential that the target molecule be soluble in water. With these concerns in mind, we now report on rationally designed coumarin-fluorescein **1** capable of showing FRET modulation by the addition of Hg^{2+} or Cu^{2+} ions in 90% aqueous media.

Synthesis of the target molecule and its precursors is described in Scheme 1. 1,7-Diethylamino coumarin (**3**) and *N*-(2,7-dichlorofluorescein)lactam (**4**) were prepared according to the procedures reported earlier.[17,18] Condensation of **3** with **4** for 24 h gave **1** in 78% yield.[19] **1** was well characterized by ^1H NMR, ^{13}C NMR, FT-IR, and FAB-MS (see Supplementary data).

For optimizing conditions to make **1** adaptable to biological and environmental-friendly systems, the ratio of H_2O/DMSO for the solvent was varied. As seen in Figure 1, the fluorescence intensity at 528 nm decreases with an increasing ratio of H_2O/DMSO upon irradiation at 420 nm, corresponding to the coumarin absorption band. In a solvent system of H_2O/DMSO = 9:1, a new emission band centered at 568 nm appears while fluorescence intensity at 528 nm is markedly quenched. It should be noted that in a mixture of H_2O/DMSO (9:1), the new band of **1** at 568 nm is the FRET band

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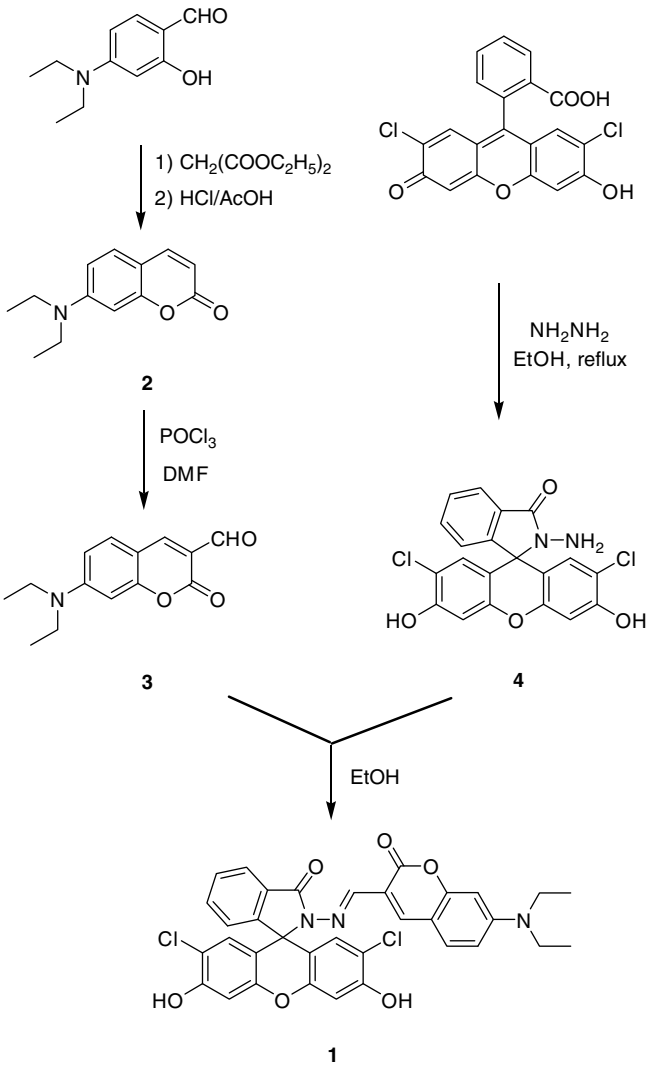
doi:10.1016/j.tetlet.2008.08.027



Scheme 1. Synthetic pathway of **1**.

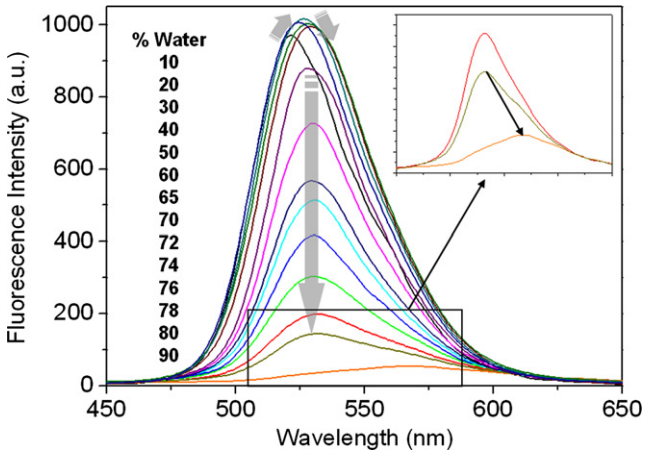


Figure 1. Fluorescence spectra of **1** (3.0 μM) in H_2O/DMSO mixture with an excitation at 420 nm. Ratios of H_2O/DMSO: 1/9; 1/4; 3/7; 2/3; 1/1; 3/2; 13/7; 7/3; 18/7; 37/13; 19/6; 39/11; 4/1; and 9/1.

generated by energy transfer from coumarin to fluorescein, induced by the water-promoted ring opening of the fluorescein cyclolactam. In water-free systems such as CH_3CN or DMSO, however, we found that the spirolactam ring of **1** remains closed upon metal cation addition (see Fig. S5).

Thus, we decided to implement all fluorescence experiments in a mixed solvent system of H_2O/DMSO = 9:1, which can be further applicable to a biological system.

To obtain an insight into the binding properties of **1** toward metal ions, we investigated the absorption and fluorescence changes upon addition of the chloride salt to a wide range of cations including Li^+; Na^+; K^+; Rb^+; Cs^+; Mg^{2+}; Ca^{2+}; Sr^{2+}; Ba^{2+}; Fe^{2+}; Co^{2+}; Ni^{2+}; Cu^{2+}; Zn^{2+}; Cd^{2+}; and Hg^{2+} in aqueous solution (H_2O/DMSO = 9:1). The UV and fluorescence emission changes are depicted in Figure 2.

Compound **1** showed 2 characteristic absorption bands centered at 453 and 490 nm for the coumarin and fluorescein units, respectively (Fig. 2a). No considerable band shift was observed upon addition of most metal cations (50 equiv) except the Hg^{2+} ion. When Hg^{2+} was added to a solution (H_2O/DMSO = 9:1) of **1**, a blue-shifted band of **1** from 453 to 448 nm was noticeable, due presumably to the ICT (Intramolecular Charge Transfer) suppression in the coumarin, driven by complexation of Hg^{2+} to the *N,N*-diethylaniline unit of the coumarin. In line with the UV spectral changes, we observed fluorescence spectral changes upon Hg^{2+} and Cu^{2+} ion addition as well (Fig. 2b). When Hg^{2+} ions are added to a solution of **1**, the FRET band centered at 568 nm declines and a new emission band at 485 nm from coumarin fluorescence appears. This is in good accordance with UV spectral changes where ICT suppression induced by Hg^{2+} complexation was elucidated (Fig. 2a). Meanwhile, in the case of Cu^{2+} binding to **1**, we noticed that

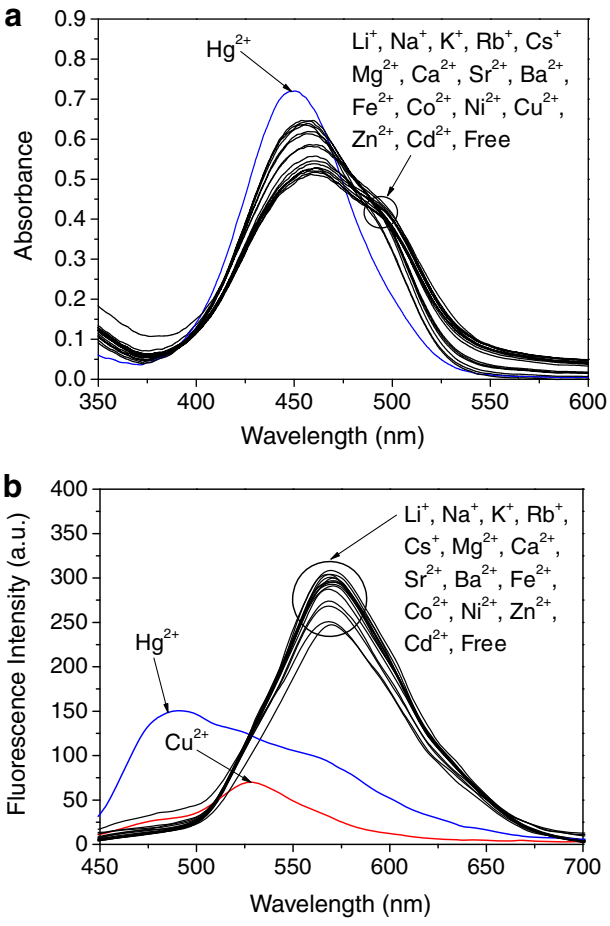


Figure 2. (a) Absorption spectra of **1** (20.0 μM) and (b) fluorescence spectra of **1** (6.0 μM) upon addition of metal cations (50 equiv) in H_2O/DMSO = 9:1 with an excitation at 420 nm.

the fluorescence is diminished and an emission at 528 nm, featuring the original coumarin band, appears. It is conceivable that the FRET is still working considerably in this system, but the fluorescence is rather quenched given the heavy metal ion effect of the copper ion.[20] Observing the different fluorescence behaviors above, we demonstrate that the binding site of **1** for Cu^{2+} is different from that for Hg^{2+}, whereby the Hg^{2+} is likely bound to the *N,N*-diethylaniline unit of the coumarin group, while the Cu^{2+} is encapsulated between 2 carbonyl units and an imine moiety. To obtain insight into the regioselective binding of **1** for metal cation, pH variation studies were conducted (see Fig. S9). At acidic conditions, pH 2, the fluorescence emission band of **1** at 485 nm gradually increased, while that at 568 nm was completely quenched, showing a similar pattern of fluorescence changes of **1** upon Hg^{2+} ion complexation. From this point of view, one can make a point that the Hg^{2+} seems to be bound to the *N,N*-diethylaniline unit of coumarin.

Figure 3 displays fluorescence changes of **1** upon addition of increasing concentrations of (a) $CuCl_2$ and (b) $HgCl_2$ in H_2O/DMSO (9:1). The fluorescence intensity gradually decreased upon addition of Cu^{2+}, up to 20 equiv, and reaches a plateau (Fig. 3a). Conversely, in a function of $[Hg^{2+}]$, a new emission band at 485 nm appears while the emission band at 568 nm disappears.

To confirm the stoichiometry between **1** and the Cu^{2+} and Hg^{2+} ions, FAB-MS analysis was conducted and its mass data are shown in Figure S4. Mass peaks at *m*/*z* 740.9, 706.0, and 641.7 corresponding to $[\mathbf{1} + CuCl]^+$, $[\mathbf{1} + Cu]^{2+}$, and free **1** are clearly observed, confirming a 1:1 complex (Fig. S4a). In a similar manner, a mass peak at *m*/*z* 877.8, corresponding to $[\mathbf{1} + HgCl]^+$, is also observed,

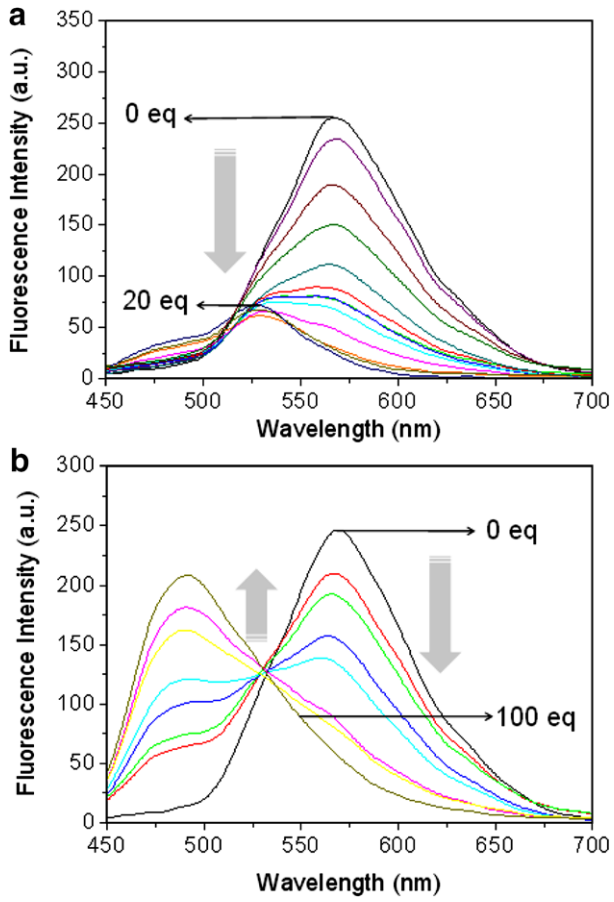


Figure 3. Fluorescence spectra of **1** (6.0 μM) with addition of various concentrations of (a) $CuCl_2$ (0, 1.2, 2.4, 3.6, 4.8, 7.2, 8.4, 12, 30, 60, and 120 μM, respectively); and (b) $HgCl_2$ (0, 12, 30, 42, 60, 120, 300, and 600 μM, respectively) in H_2O/DMSO = 9:1 with an excitation at 420 nm.

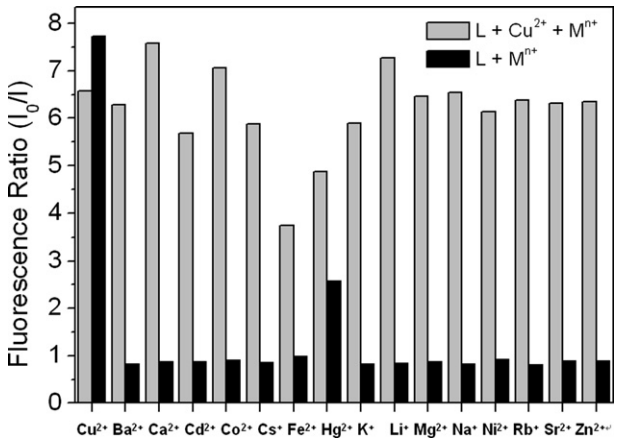


Figure 4. Fluorescence changes of **1** (6.0 μM) at 568 nm in H_2O/DMSO = 9:1 upon addition of different metal cations (120.0 μM of each) and subsequent addition of Cu^{2+} (120.0 μM).

giving solid evidence for a 1:1 complex as seen in Figure S4b. On the basis of the 1:1 stoichiometry and fluorescence titration data, the association constant of **1** was calculated to be 8.31×10^5 and $1.78 \times 10^4\ M^{-1}$ for the Cu^{2+} and Hg^{2+} ions, respectively.[21]

In order to utilize **1** as an ion-selective fluorescence chemosensor for Cu^{2+} and Hg^{2+}, the effect of competing metal ions was also investigated. As shown in Figure 4, no interference in detection of Cu^{2+} and Hg^{2+} was observed in the presence of group 1 or group 2 metals or with silver and lead ions. Thus, it is notable that **1** can be used as a Cu^{2+}- and Hg^{2+}-selective FRET sensor in the presence of most competing cations.

Conclusively, the synthesis of a new fluorescent chemosensor **1** has been achieved in a high yielding synthetic sequence. Based on the metal cation-induced FRET changes, the inclusion of coumarin-fluorescein as both spacers and cation-binding sites provides a selective sensor for Cu^{2+} and Hg^{2+} ions. The metal ion binding mechanism concerning FRET changes is demonstrated in Figure 5. When the Hg^{2+} is bound to the *N,N*-diethyl unit of coumarin, the FRET from the coumarin to the fluorescein group is diminished on account of the ICT suppression. When the Cu^{2+} ion is added to a solution of **1**-Hg^{2+}, the FRET is revived to be *On*. In copper ion complexation, **1** shows rather weaker fluorescence intensity than does metal free **1**, presumably because of the heavy metal ion effect of the Cu^{2+} ion. Since the complexation studies with respect to fluorescence changes have been undertaken in aqueous solution with chloride counter anions, the first-time synthesized compound **1** is able to be utilized in recognition of Cu^{2+} or Hg^{2+} ions in any living

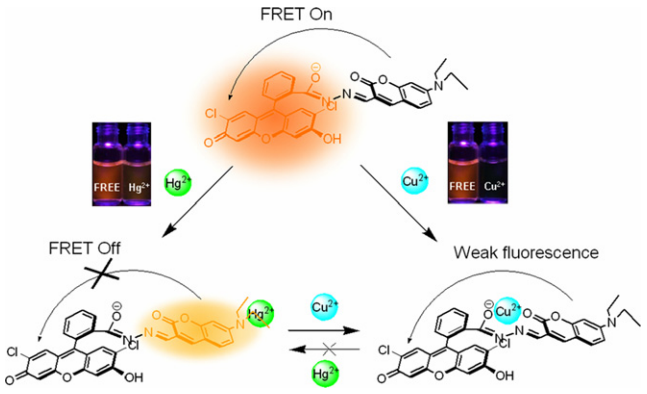


Figure 5. Cu^{2+}-induced fluorescence On→Off and Hg^{2+}-induced FRET On→Off along with visual changes upon irradiation at 420 nm.

system or in an environmentally friendly separation system for further application.

Acknowledgments

The authors wish to acknowledge the financial support of the SRC program (R11-2005-008-02001-0(2008) and the SRC Research Centre for Women's Diseases of Sookmyung Women's University.

Supplementary data

Supplementary data (NMR, FAB-MS, UV and Fluorescence spectra) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.08.027.

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19. *Preparation of N-(2,7-dichlorofluorescein)lactam-N′-7-diethylamino-3-methyl coumarin* (**1**): A portion of **4** (415 mg, 1.0 mmol) and **3** (368 mg, 1.5 mmol) was combined in absolute ethanol (20 mL). The solution was stirred at reflux conditions overnight. The reaction mixture was added to CH_2Cl_2 (200 mL) and washed with water several times. The organic layer was dried over anhydrous magnesium sulfate and evaporated in vacuo. Recrystallization in DMSO/C_2H_5OH (v/v, 1/3) gave orange **1** (501 mg) in 78% yield. Mp > 300 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.96 (s, 2H, OH), 8.83 (s, 1H, $Ar_{cou}H$), 7.96 (dd, 1H, $Ar_{fluo}H$, J = 5.58 Hz), 7.94 (s, 1H, $Ar_{cou}H$), 7.65 (ddd, 2H, $Ar_{fluo}H$, J = 6.64 Hz), 7.51 (d, 1H, $Ar_{cou}H$, J = 9.08 Hz), 7.16 (dd, 1H, $Ar_{fluo}H$, J = 6.24 Hz), 6.91 (s, 2 H, $Ar_{fluo}H$), 6.67 (dd, 1H, $Ar_{cou}H$, J = 9.06 Hz), 6.62 (s, 2H, $Ar_{fluo}H$), 6.50 (d, 1H, $Ar_{fluo}H$, J = 2.08 Hz), 3.42 (q, 4H, $-NCH_2CH_3$, J = 7.00 Hz), 1.10 (t, 6 H, $-NCH_2CH_3$, J = 7.06 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): 164.1, 161.3, 157.3, 155.0, 152.2, 151.0, 138.9, 131.8, 130.2, 129.0, 127.8, 124.3, 116.7, 112.9, 111.3, 110.3, 108.4, 104.8, 97.0, 65.1, 44.9, 13.0 ppm. IR (KBr plate, cm^{-1}): 3146, 1677, 1608. FAB MS m/z (M^+): calcd, 642.48. Found, 642.40.
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