

Colorimetric and Ratiometric Fluorescent Chemosensor with a Large Red-Shift in Emission: Cu(II)-Only Sensing by Deprotonation of Secondary Amines as Receptor Conjugated to Naphthalimide Fluorophore

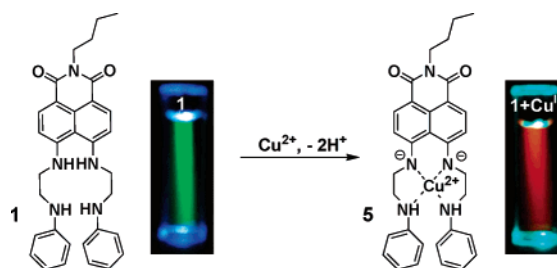
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ABSTRACT



A new fluorescent probe *N*-butyl-4,5-di[2-(phenylamino)ethylamino]-1,8-naphthalimide 1 senses only Cu(II) among heavy and transition metal (HTM) ions by means of a colorimetric (primrose yellow to pink) method with a large red-shift in emission (green to red) attributed to the deprotonation of the secondary amines as a receptor conjugated to the naphthalimide fluorophore.

Chemosensors that convert molecular recognition into highly sensitive and easily detected signals have been actively investigated in recent years. A colorimetric and ratiometric fluorescent chemosensor combines the sensitivity of fluorescence with the convenience and aesthetic appeal of a colorimetric assay.¹ In particular, ratiometric measurements have the important feature that they permit signal rationing, and thus increase the dynamic range and provide built-in correction for environmental effects.² The design of chemosen-

sors for Cu(II) is actively investigated, as this metal ion is a significant environmental pollutant and an essential trace element in biological systems.^{1a,3} However, up to now, few colorimetric and ratiometric fluorescent chemosensors for Cu(II) have been found in the literature.^{1a,4} Another issue

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related to the recognition is that of serious interference by other metal ions such as Ni(II),⁵ Ag(I),⁶ and Hg(II).^{6,7} To achieve Cu(II)-only sensing, fluorescent chemosensors require deliberate design.

We have reported a ratiometric fluorescent chemosensor of naphthalimide (**L**₁) (Figure 1) selective for Cu(II) based

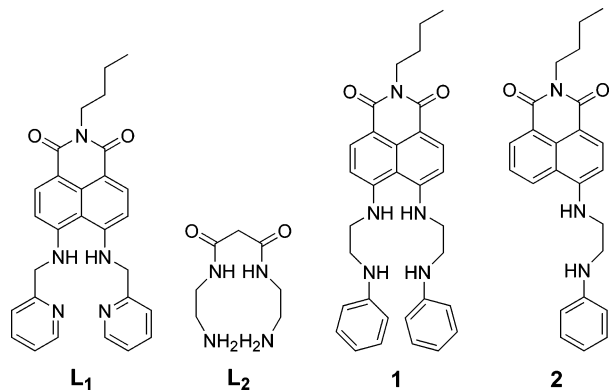


Figure 1. Structures of ligands **L**₁, **L**₂, **1**, and **2**.

on an internal charge transfer (ICT) mechanism,^{1a} which showed a 50 nm blue shift of fluorescence emission. However, a long-wavelength emission will be more useful in application, for it would overcome the disturbances induced by environmental fluorescence. A rational strategy to obtain long-wavelength fluorescence would be the deprotonation of the two secondary amines conjugated to 1,8-naphthalimide so that the electron-donating ability of the two N atoms conjugated to naphthalene ring would be greatly enhanced; as a result, red shifts in both absorption and fluorescence spectra would be expected. Gunnlaugsson et al. have reported that F[−] could deprotonate the 4-amino group of 1,8-naphthalimide and lead to a long-wavelength color change.⁸ The deprotonation of NH in amide or thiourea was believed to be the basis of F[−] sensing in recent research.⁹ In addition, Cu(II) could release the two protons on the amide nitrogen in the well-known dioxotetraamine ligands,¹⁰ in particular 1,9-diamino-3,7-diazanonane-4,6-dione (**L**₂).¹¹ In fact, the two amino groups in 4,5-di(alkylamino)-1,8-naphthalimide are very similar in structure to the two amides in **L**₂. Bearing these in mind, we designed and synthesized

N-butyl-4,5-di[2-(phenylamino)ethylamino]-1,8-naphthalimide (**1**). Because an anilino group has a lesser ability to coordinate Cu(II) compared with a pyridyl group,^{1a} the interaction between Cu(II) and the two secondary amines conjugated to naphthalimide fluorophore in **1** will be strengthened. Thus, the protons will be released more easily. It is well-known that the formed Cu(II) center is inclined to transfer an electron to the chromophore after deprotonation.¹¹ Most probably, in this case, the square planar Cu(II) complex will take part in the π -system with a naphthalene ring. These situations lead to a red-shift of the UV–vis and fluorescence spectra, from which one could sense the Cu(II) colorimetrically and ratiometrically.

Chemosensor **1** was easily synthesized by conjugating *N*-phenylethylenediamine and *N*-butyl-4-bromo-5-nitro-1,8-naphthalimide, which was prepared from 4-bromo-5-nitro-1,8-naphthalic anhydride and butylamine.^{1a} *N*-Butyl-4-[2-(phenylamino)ethylamino]-1,8-naphthalimide (**2**) was prepared for a control experiment. The influence of pH on the fluorescence of **1** was first determined by fluorescence titration in ethanol–water (60:40, v/v) solutions. The fluorescence of **1** at 518 nm remains unaffected between pH 12.0 and 2.88 ($\Phi_F = 0.0054$) and then rapidly increases nearly 116-fold from pH 2.88 to pH 1.67 ($\Phi_F = 0.63$) (Figure 2a)

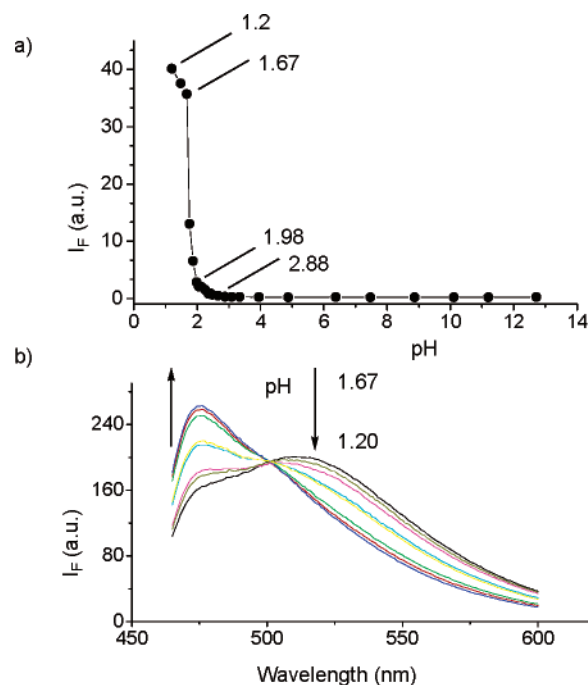


Figure 2. (a) Influence of pH on the fluorescence of **1** in the ethanol–water (60:40, v/v, 50 mM HEPES buffer, pH 7.2) solution. I_F = fluorescence intensity, excitation wavelength = 420 nm, emission wavelength = 518 nm, [**1**] = 10 μ M. (b) Fluorescent emission spectra of **1** in the ethanol–water solution from pH 1.67 to 1.20.

due to the repressed photoinduced electron transfer (PET) process from the peripheral N atoms to the fluorophore.¹² Its pK_{a1} and pK_{a2} values are 2.43 and 1.82, respectively. Very

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interestingly, when the solutions of **1** became more acidic, from pH 1.67 to 1.20, a significant decrease of the 518 nm emission and a blue-shifted emission band centered at 475 nm (Figure 2b), which was attributed to the interaction between a proton and the NH conjugated to the naphthalene ring and increased in intensity, were observed with a clear isoemission point at 501 nm. When 5 equiv Cu(II) was added to an acidic ethanol–water (60:40, v/v) solution of **1**, the absorbance at 419 nm decreased, and a longer absorption band at 509 nm occurred on titration with NaOH between pH 3.0 and 12.0 (Figure 3a). Simultaneously, the fluores-

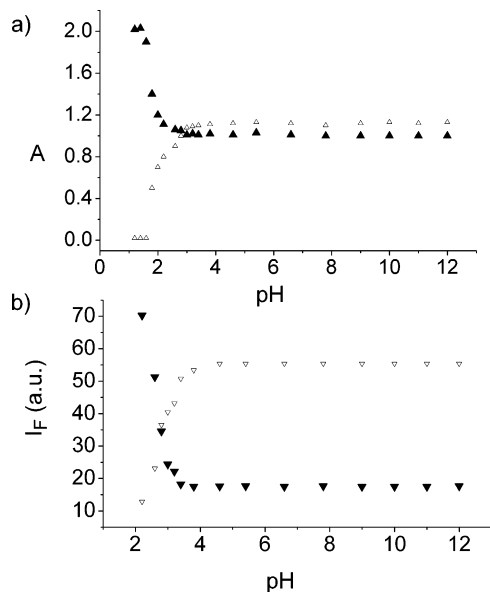


Figure 3. (a) Dependence of the absorbance of the band at 419 nm (▲) and 509 nm (△) on pH for a solution of 1 equiv of **1** and 5 equiv of Cu(II) in ethanol–water (60:40, v/v). (b) Dependence of the fluorescence intensity of the band centered at 518 nm (▼) and 592 nm (▽) on pH for a solution of 1 equiv of **1** and 5 equiv of Cu(II) in ethanol–water (60:40, v/v).

cence intensity of **1** at 518 nm decreased, and a red-shifted fluorescence emission band centered at 592 nm appeared (Figure 3b). The addition of other metal ions such as Li(I), Na(I), K(I), Mg(II), Ca(II), Fe(III), Co(II), Zn(II), Cd(II), Hg(II), Ag(I), and Pb(II) produced insignificant changes in both absorption and fluorescent spectra of **1** between pH 3.0 and 12.0. In our system, Ni(II) only shows a little interference in the absorption spectrum (Figure 4a) and quenches the fluorescence of **1** to a small extent. This means that **1** has an outstandingly high selectivity for Cu(II). Detailed studies were carried out in ethanol–water (60:40, v/v) solutions at pH 7.2 maintained with HEPES buffer (50 mM).

The absorption spectrum of **1** (10 μ M) exhibits λ_{\max} at 419 nm ($\epsilon = 19\,600\text{ M}^{-1}\text{ cm}^{-1}$) and 458 nm ($\epsilon = 12\,900\text{ M}^{-1}\text{ cm}^{-1}$). In relative terms, the absorption spectrum of **2**

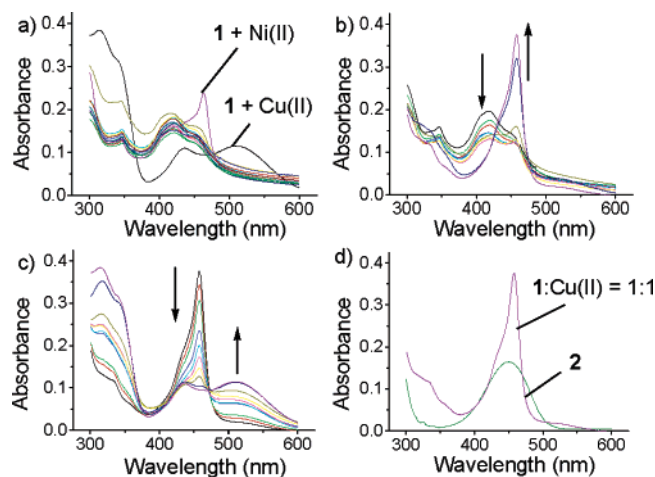


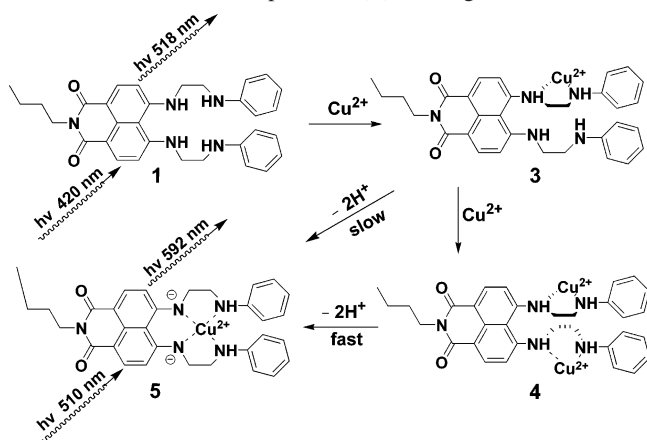
Figure 4. (a) UV–vis absorption spectra of **1** in the presence of different metal ions in ethanol–water solutions (60:40, v/v, 50 mM HEPES buffer, pH 7.2). [**1**] = 10 μ M, and the concentration of each metal ion was 50 μ M. (b) Dependence of the UV–vis absorption spectra of **1** on the concentration of Cu(II). [Cu(II)] = 0–10 μ M, [**1**] = 10 μ M. (c) Dependence of the UV–vis absorption spectra of **1** on the concentration of Cu(II). [Cu(II)] = 10–50 μ M, [**1**] = 10 μ M. (d) UV–vis absorption spectra of **1** and **2** where to the solution of **1** was added 1 equiv of Cu(II). [**1**] = [**2**] = 10 μ M.

shows only one λ_{\max} at 458 nm ($\epsilon = 16\,000\text{ M}^{-1}\text{ cm}^{-1}$). On addition of 1 equiv of Cu(II) to the solution of **1**, the absorbance at 419 nm ($\epsilon_{\min} = 10\,600\text{ M}^{-1}\text{ cm}^{-1}$) decreases sharply to its limiting value, while the one at 458 nm ($\epsilon_{\max} = 37\,600\text{ M}^{-1}\text{ cm}^{-1}$) increases prominently (Figure 4b). Furthermore, when unceasingly adding another 1 equiv of Cu(II), the absorbance at 458 nm ($\epsilon_{\min} = 9580\text{ M}^{-1}\text{ cm}^{-1}$) decreases gradually, and an absorption band at 509 nm ($\epsilon_{\max} = 11\,500\text{ M}^{-1}\text{ cm}^{-1}$) develops, which induces a color change from primrose yellow to pink (Figure 4c) with an isosbestic point at 475 nm, which should correspond to an intermediate complex.¹³ However, adding more Cu(II) leads to no significant changes in the UV–vis spectra. It is noticeable that the absorption spectrum of **1**/Cu(II) (1:1) is very similar to that of **2** (Figure 4d). It might be deduced that 1 equiv of Cu(II) or less reacts with one 2-(phenylamino)ethylamino (PEA) group in **1** first to reduce its electron-donating ability, while the other PEA is free to play a role like that in **2**. These phenomena indicated that Cu(II) possibly interacts with **1** step by step (the proposed process was shown in Scheme 1), first to form **3** [**1**:Cu(II) = 1:1], which has a similar electronic communication and dipolar interaction with **2**, and then probably to form complex **4** [**1**:Cu(II) = 1:2] corresponding to the isosbestic point¹³ with addition of another 1 equiv Cu(II), which is rapidly converted into **5** with the release of two protons on the two secondary amines conjugated to the naphthalimide fluorophore. It has been found that **3** [**1**:Cu(II) = 1:1], after 48 h, displayed the same absorption and fluorescence spectra as **5**. This means that **3**

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Scheme 1. Proposed Cu(II) Sensing Process



could also be totally and automatically transferred to **5**, and the final complex **5** could probably be a 1/Cu²⁺ adduct of 1:1 stoichiometry. This also suggests that only more than 2 equiv of Cu(II), because of the low acidity of the amines, can release the two protons on these two secondary amines immediately. To gain more insight into the role of the amount of Cu(II), 2 equiv of ethylenediamine, which had a greater affinity for Cu(II) than **1**, was added to the solution of **5**, and no obvious spectral changes were found. This fact also implied that **5** was a 1/Cu²⁺ adduct of 1:1 stoichiometry.

The emission spectra of **1** and its fluorescence titration with Cu(II) are displayed in Figure 5. The fluorescence intensity of **1** at 518 nm decreases to its limiting value after adding 1 equiv of Cu(II). As expected, when more than 1 equiv of Cu(II) was added to the solution of **1**, a red-shifted fluorescence emission band ($\Phi_F^{\max} = 0.0046$) was found centered at 592 nm, which should be ascribed to complex **5**. The two emission bands centered at 592 and 518 nm were excited at 510 and 420 nm, respectively. A slight hypsochromic effect was seen at the shorter emission band.

The probe **1** presents some outstanding advantages compared to known ratiometric fluorescent sensors for Cu(II).^{1,4} The Cu(II)-only sensing with selectivity and the large red shift in emission when Cu(II) interacts with **1**, as well as the Cu(II) detection by only one kind of fluorophore, would be more useful in practical application. It should be pointed out that the deprotonation of the two anilino groups can only be induced by Cu(II) in this case and in more favorable cases

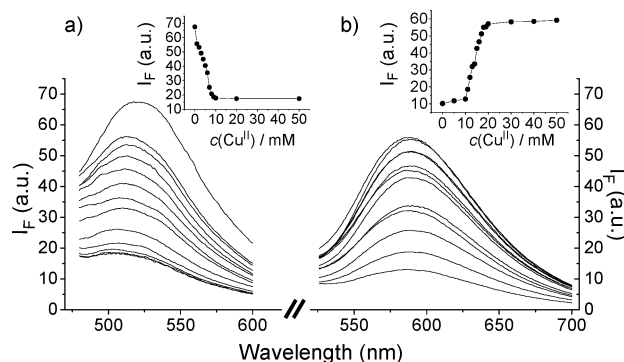


Figure 5. Changes in the emission spectra in the presence of different concentrations of Cu(II). [**1**] = 10 μM. (a) [Cu(II)] = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50 μM. Excitation wavelength was 420 nm. (b) [Cu(II)] = 0, 5, 10, 12, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50 μM. Excitation wavelength was 510 nm. Inset: Fluorescent intensity I_F as a function of Cu²⁺ concentration.

by Ni(II). Unfortunately, the probe **1** has lower sensitivity for Cu(II) and poor solubility in water. A new fluorescent probe totally soluble in water with high sensitivity and selectivity for Cu(II) will be developed in our future research.

In conclusion, we have demonstrated that probe **1** displays a colorimetric response with a large red-shift emission that is useful for the easy detection of Cu(II) ion with very high selectivity among HTM ions. The solution color change from primrose yellow to pink and the red-shift of fluorescence spectra from green to red are attributed to the deprotonation of the two secondary amines conjugated to the naphthalene ring. The design strategy and remarkable photophysical properties of the probe would help to extend the development of fluorescent probes for HTM ions.

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Supporting Information Available: Synthesis, characteristics, and spectroscopic data of **1** and **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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