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LETTER

1- Nitronyl nitroxide pyrene as a new off-on fluorescent chemosensor for Cu²⁺

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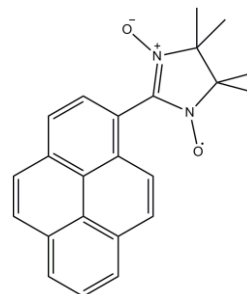
1-Nitronyl nitroxide pyrene (1) has been investigated as a new off-on fluorescent chemosensor for metal ions in CH₃CN. Compound 1 shows high selectivity and sensitivity to Cu²⁺ with a detection limit as low as 3.60×10^{-7} M. A coordination mechanism between compound 1 and Cu²⁺ is well proved by UV-Vis and ESR results.

Recognition of heavy and transition metal ions by fluorescent chemosensors has received considerable attentions due to not only the important function of heavy and transition metal ions, but also their potential toxicity to the environment and biological systems.^{1,2} Cu²⁺, the third most abundant heavy metal ion in human body after Fe²⁺ and Zn²⁺, involves in various physiologic processes in organisms.² Although, Cu²⁺ is an indispensable trace element in human body, excessive levels of Cu²⁺ can damage organisms.³ Therefore, detection of Cu²⁺ in a selective and rapid way is of toxicological and environmental concern.^{2,4}

In recent years, several chemosensors based on rhodamine,⁵ naphthalimide,⁶ pyrene,⁷ BODIPY,⁸ porphyrin,⁹ bis(2-pyridylmethyl)amine (DPA),¹⁰ coumarine,¹¹ have been studied for detection of Cu²⁺. However, in some cases, the fluorescent chemosensors for Cu²⁺ showed “turn-off” manner in emission spectra due to the fluorescence-quenching nature of paramagnetic Cu²⁺.^{9,12} Such fluorescent “turn-off” sensors is less preferable than those “turn-on” types, since they may give false positive results caused by other quenchers in real samples and are undesirable for analytical purposes.¹³ Furthermore, the sensitivity of some chemosensors is not high enough to distinguish between Cu²⁺ and other ions, such as Fe²⁺, Pb²⁺ and Hg²⁺.¹⁴ Although some good off-on chemosensors were established for Cu²⁺,¹⁵ designing fluorescent “turn-on” sensors with high selectivity for Cu²⁺ is still very challenging,¹⁶ especially with a low cost visual detection method.

To improve fluorescent intensity of a chemosensor upon binding Cu²⁺, the designed receptor unit should has a maximal fluorescence quenching effect, whereas this effect is minimized in the metal-bound state.^{7c,13}

Nitronyl nitroxides, known as stable organic radicals, were initially discovered by Ullman in 1970's and have been widely used as spin probes in recent years.¹⁷ In 1991, p-nitrophenyl nitronyl nitroxide was found to be a bulk ferromagnet.¹⁸ Since then, nitronyl nitroxides were intensively investigated as components of organic magnets. In fact, nitronyl nitroxides are efficient fluorescence quenchers as electron donors. The fluorescence of these spin labeled fluorophores can be switched on when the paramagnetic nitroxide is converted to a diamagnetic species such as hydroxylamine or O-alkyl hydroxylamine, which also causes decay of the ESR signal.¹⁹ Moreover, nitronyl nitroxides can also coordinate with metal ions.²⁰ Up to now, some fluorophore-nitroxide radical hybrid compounds have been developed for detection of metal ions,²¹ nitric oxide radicals,²² superoxide radicals,²³ vitamin C²⁴ and H⁺.²⁵



Scheme 1 The structure of compound 1

Herein, compound **1** (1-nitronyl nitroxide pyrene) was reported as a new fluorescent chemosensor for detecting Cu^{2+} . It showed significant “off-on” fluorescence accompanied with color changes from purple to bright yellow.

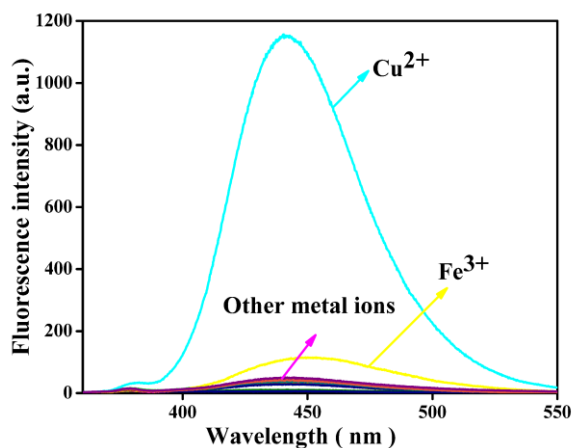


Fig. 1 Fluorescence spectra of **1** (1.0×10^{-5} mol/L) upon addition of perchlorate salts of Ba^{2+} , Li^{+} , Na^{+} , Fe^{3+} , Fe^{2+} , Mn^{2+} , Mg^{2+} , Cu^{2+} , Hg^{2+} , Ag^{+} , Pb^{2+} , Cd^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} (10.0 equiv., respectively) in CH_3CN with excitation at 340 nm.

Compound **1** was synthesized according to the literature¹⁶. The selectivity of compound **1** toward various metal ions was investigated in CH_3CN by using their perchlorate salts. All metal ions were tested after incubation individually with compound **1** for 5 min. at room temperature. As depicted in Figure 1, a weak fluorescence signal was detected for the metal ion free solution at 378 nm. Interestingly, addition of 10.0 equiv. of Cu^{2+} resulted in a remarkable increase of fluorescence intensity at 444 nm. But no significant changes of the fluorescence intensity were observed when compound **1** mixed with other metal ions such as Li^{+} , Na^{+} , Ba^{2+} , Fe^{3+} ,²⁶ Fe^{2+} , Mn^{2+} , Mg^{2+} , Cu^{2+} , Ag^{+} , Pb^{2+} , Cd^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} and even Hg^{2+} which is well known to strongly interfere with detection of Cu^{2+} .¹³

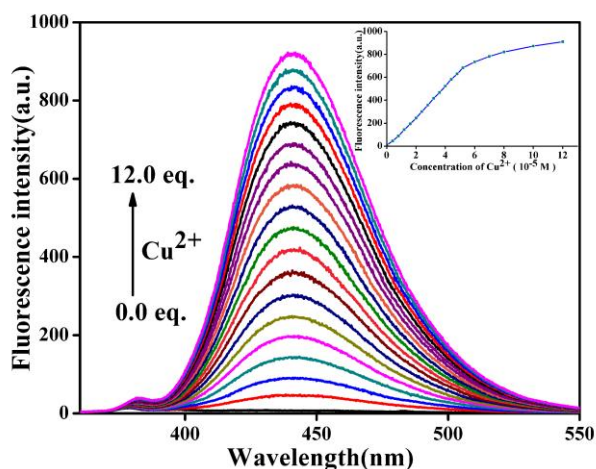


Fig. 2 Fluorescence spectra of compound **1** (1.0×10^{-5} mol/L) in CH_3CN titrated with Cu^{2+} (0.0–12.0 equiv., $\lambda_{\text{ex}} = 340$ nm). Inset: Changes in fluorescence intensity of the solution measured at 444 nm.

To examine the sensitivity of compound **1** towards Cu^{2+} , Fluorescence titration of Cu^{2+} (Fig. 2) was carried out by using a solution of 10 μM of compound **1** in CH_3CN . A new band centered at 444 nm was achieved through gradually adding Cu^{2+} to the solution of compound **1**. The significant increase of fluorescence intensity of compound **1** at 444 nm might be led by the inhibition of PET from nitronyl nitroxide to pyrene unit. This inhibition may be caused by the coordination or REDOX between Cu^{2+} and nitronyl nitroxide. The fluorescence response of compound **1** toward Cu^{2+} was calculated to cover a linear range from 8.0×10^{-7} to 5.2×10^{-5} M (Fig. 2, inset), with a detection limit as low as 3.60×10^{-7} M (based on $\text{S/N} = 3$).

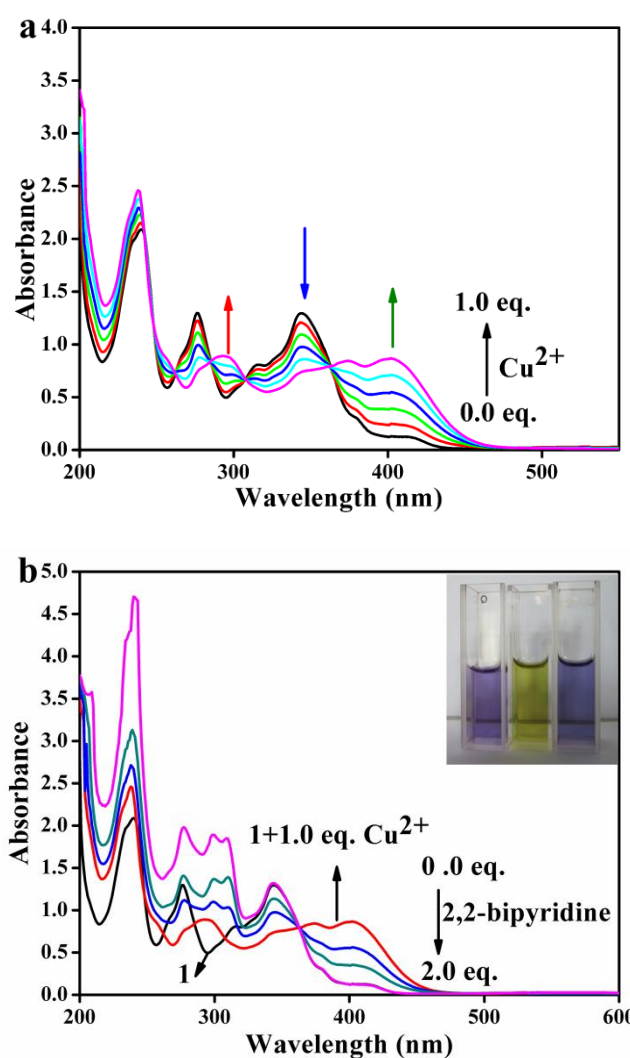


Fig. 3 (a) UV-Vis absorption spectra of compound **1** (5.0×10^{-5} mol/L) in CH_3CN with continuous addition of Cu^{2+} (0.0, 0.2, 0.4, 0.6, 0.8, 1.0 equiv.); (b) UV-Vis absorption spectra of **1**- Cu^{2+} with addition of 2,2'-bipyridine (0, 0.5, 1.0, 2.0 equiv.). Inset: Solution of compound **1**; compound **1** + 1.0 equiv. of Cu^{2+} ; compound **1** + 1.0 equiv. of Cu^{2+} + 2.0 equiv. of 2, 2'-bipyridine (from left to right).

The absorption spectra of compound **1** with Cu^{2+} was also investigated by UV-Vis to identify the mechanism of action between Cu^{2+} and compound **1**. As is shown in Figure 3a, obvious changes were caused by adding various amount of Cu^{2+} to the solution of compound **1**. The absorbance at 345 nm and 276 nm decreased sharply, accompanied with a marked increase of absorbance at 402 and 295 nm, which is responsible for the color change from purple to bright yellow. Four well-defined isosbestic points at 363 nm, 308 nm, 285 nm and 262 nm were also displayed. It indicated the presence of a unique complex in equilibrium in the solution. More interestingly, changes of absorbance of compound **1** at 345 nm can be reversed by subsequent titration of 2, 2'-bipyridine (Fig. 3b) accompanied with the color's recovery, which is likely due to a stronger coordination between Cu^{2+} and 2,2'-bipyridine.

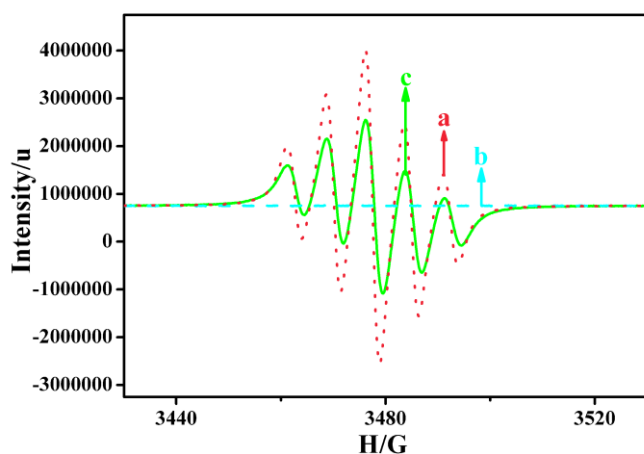


Fig. 4 ESR spectra of compound **1** (1.0×10^{-3} mol/L, a), compound **1** + 2.0 equiv. of Cu^{2+} (b) and compound **1** + 2.0 equiv. of Cu^{2+} + 10.0 equiv. of 2,2'-bipyridine (c) in CH_3CN .

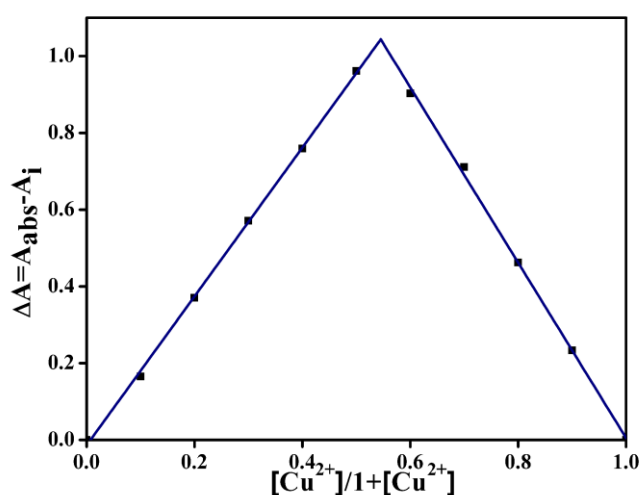


Fig. 5 Job's Plot of **1** with Cu^{2+} obtained by UV-Vis measurements ($\lambda = 372$ nm). Total concentration of **1** and metal ions is 1.0×10^{-4} mol/L.

There are two kinds of interaction – redox and coordination – between Cu^{2+} and compound **1**. For better understanding of the main interaction between them, ESR experiment was performed. As Figure 4 displayed, before adding Cu^{2+} , compound **1** exhibited expected five ESR principal lines (a, Fig. 4). The ESR spectra of compound **1** was completely decayed in the presence of 2.0 equiv. of Cu^{2+} . This result indicated that the decay of ESR signal might be caused by the elimination of the magnetic interaction of the unpaired electron with the two equivalent ^{14}N nuclei of the imidazoline ring (b, Fig. 4). Additionally, this ESR decayed effect caused by Cu^{2+} can be decreased by subsequently addition of 2, 2'-bipyridine (c, Fig. 4).

Thus, the ESR results strongly support the proposed coordination mechanism inferred from the fluorescence and UV-Vis results. Furthermore, the job-plot measurements were carried out at 372 nm to quantify the complexation ratio between compound **1** and Cu^{2+} (Fig. 5). The molar fraction of $[\text{Cu}^{2+}]/[\text{1}] + [\text{Cu}^{2+}]$ is 0.545 which indicates the coordinated ratio between Cu^{2+} and **1** is about 1:1.

In summary, a new fluorescence enhancement chemosensor for Cu^{2+} based on nitronyl nitroxide is developed. It displays high selectivity and sensitivity to Cu^{2+} with a detection limit as low as 3.60×10^{-7} M in CH_3CN . The chemosensor **1** shows reversible absorption response caused by Cu^{2+} and subsequent addition of 2, 2'-bipyridine, accompanied with color changing from purple to bright yellow and then changing to purple. A coordination mechanism is well proved to exist between compound **1** and Cu^{2+} by UV-Vis and ESR results. The development of these fluorophore-nitroxide sensors opened more opportunities to design new “off-on” fluorescent chemosensors.

Experimental

Synthesis of Compound **1**

1.48 g (10 mmol) of 1, 1, 2, 2-tetramethyl-1, 2-dihydroxylaminoethane and 2 mL of triethyl orthoformate was added to a solution of 2.3 g (10 mmol) of 1-pyrenecarboxaldehyde in 50 mL of absolute methanol and 50 mL of absolute benzene. The solution was stirred under reflux for 12 hours. The solvent was removed in vacuo, the resulting solid was used for the next step. A solution of the solid in 50 mL of methanol was stirred with 15 g of lead dioxide for 3 hours at room temperature. After filtration and evaporation of the solvent, the precipitate was purified by chromatography ($\text{SiO}_2, \text{CH}_2\text{Cl}_2/\text{Ethyl acetate}$), followed by crystallization from $\text{CH}_2\text{Cl}_2/\text{petroleum ether}$ afforded **1** as a purple solid. m.p.: 202–203°C. Anal. obsd(calcd) for $\text{C}_{23}\text{H}_{21}\text{N}_2\text{O}_2$: C, 76.89(77.29); N, 7.75(7.84); H, 5.90(5.92). IR (KBr, cm^{-1}) 1630, 1601, 1451, 1405, 1363, 1213, 1171, 1139, 848. FAB-MS 359(M + 2). UV/Vis(CH_2Cl_2) λ_{max} 349, 317.5, 241.5, 279.5 nm. ESR(methanol): 5 lines, $g = 2.006$, $\alpha_N = 0.75$ mT.

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- 26 A weak fluorescence enhancement was detected when Fe³⁺ and CH₃COOOH (Figure S1) was added separately to the solution of **1** in CH₃CN. According to the results, Fe³⁺ and organic redox agents will interfere with recognition of Cu²⁺ to some extent.