

## 铜离子催化 DPKA 关环反应及其潜在应用

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**摘要:** 铜离子催化的氧化关环有机反应, 用于选择性荧光探测金属铜离子的存在。晶体结构和光谱分析表明, 在 DPKA 的乙腈/水混合溶剂中, 引入铜离子可以促使苯并三氮唑类荧光化合物(2)的形成, 该反应可以使得溶液的荧光明显增强。而且在滴定溶液里, 引入  $\text{Cu}^{2+}$  螯合剂 EDTA, 并不能使得溶液的荧光减弱, 表明荧光是由有机物 2 发射的。同时, 其他金属离子的引入并不能产生荧光, 而且干扰离子的存在也不能使得荧光光谱发生显著改变, 表明 DPKA 可以高选择性的检测溶液中的铜离子。研究表明, 荧光响应呈现 2 个线性关系, 区间分别在  $0.64\sim 3.2 \text{ mg}\cdot\text{kg}^{-1}$  和  $3.84\sim 7.04 \text{ mg}\cdot\text{kg}^{-1}$ 。

**关键词:** 二吡啶酮肟合物; 离子识别; 荧光; 铜离子

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## Cu<sup>II</sup>-induced Ring-formation Reaction of Di-pyridylketone Azine and Its Potential Application

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**Abstract:** A Cu-promoted ring-closed reaction of di-pyridylketone azine (DPKA) was developed in the design of a fluorescent chemodosimeter for potential selective Cu(II) detection. Structure and spectroscopic investigations demonstrated the formation of a 3H-pyrazolo[4,3-b]pyridine (2) and the “turn-on” response for Cu(II) in aqueous acetonitrile media. The introduction of  $\text{Cu}^{2+}$  chelator, such as EDTA, into the titration solution will not weaken the fluorescence, indicating the fluorescence is emitted from the organic compound (2). Two independent linear correlations exist in the range of  $0.64\sim 3.2 \text{ mg}\cdot\text{kg}^{-1}$  and  $3.84\sim 7.04 \text{ mg}\cdot\text{kg}^{-1}$ , respectively.

**Key words:** di-pyridylketone azine(DPKA); ion sensing; fluorescence; copper ion

### 0 Introduction

Since the pioneer work on new fluoroionophores for alkali and alkali-earth metal ions, much attention has been focused on the development of fluorescent chemosensors for heavy and transition metals (HTM)<sup>[1-2]</sup>. Among these metal ions, copper is an environmental pollutant but is also an essential trace element in bio-

logical systems. Although much effort has been devoted to the detection of  $\text{Cu}^{2+}$ , up to now, few fluorescent chemosensors with high sensitivity and selectivity have been reported<sup>[3]</sup>, due to its notorious fluorescence quencher via energy or electronic transfer. Hence, the design and synthesis of fluorescent enhancement upon analytic binding is a great challenge for chemists. One strategy is to reduce the communication between metal

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and fluorophore, a good case in point is Kumars sensor<sup>[4]</sup> which installed the linker nitrogen atom of the fluorophore to participate in the complexation with metal ion, successively suppressing the process of photo-induced electron transfer (PET) and storing fluorescence. Another type of fluorescence enhancement can result from metal-induced changes in the geometry or flexibility of the nonfluorescent ligand, as well as from a different availability of certain fluorophores through a certain metal-induced catalytic reaction<sup>[5]</sup>.

Although many chemodosimeters for mercury (II) were developed, the design of chemodosimeter for copper(II) with high selectivity and sensitivity still remains a challenge. Czarnik and his co-workers created a fluorescent compound (Rhodamine B) with fluorescent specific amplification for Cu(II) based on the well-known Cu<sup>2+</sup>-reactivity toward carboxylate derivative (nonfluorescent Rhodamine B hydrazine). More recently, Anslyn et al, reported a reasonably selective signal amplification for ultrasensitive molecular sensing of Cu<sup>2+</sup> using an intramolecular organometallic Hg-catalytic Heck reaction<sup>[6]</sup>. For both the Czarniks and Anslyns probes for Cu<sup>2+</sup>, the fluorescence originates from the target product of chemical reaction with specificity.

It is well-known that di-pyridylketone azine (DPKA) can be widely used depending on the solvent and pH value of the solution for the determination of first-row transition-metal ions, such as Fe(II)<sup>[7]</sup>, Co(II) and Ni(II)<sup>[8]</sup> and some other metal ions, such as Au(III)<sup>[9]</sup>, Pd(II)<sup>[10]</sup>, etc. Kinetic method for detection of low concentration of Cu(II) has also been reported for DPKA, however, a simple and conventional method to selectively differentiate copper ion(II) from other metal ion are still to be explored. Herein, we report a Cu<sup>2+</sup>-promoted catalytic organic reaction in aqueous acetonitrile solution, accomplishing fluorescent amplification with high selectivity for Cu(II).

## 1 Experimental

### 1.1 Materials and methods

Di-2-pyridyleketone was purchased from Sigma company, USA. All the other chemicals were of reagent

grade obtained from commercial sources and used without further purification. Elemental analyses (C, H and N) were carried out with a Perkin-Elmer 2400 Analyzer. IR spectra were recorded on a VECTOR 22 Bruker spectrophotometer with KBr pellets in the range of 4 000~400 cm<sup>-1</sup>. <sup>1</sup>H NMR spectra were performed on DRX500 Bruker spectrometer at 298 K with TMS as an internal reference. Electrospray mass spectra were collected on a LCQ system (Finnigan MAT, USA) with methanol as mobile phase. Dipyridylketone azine (DPKA) **1** was prepared according to the literature method<sup>[11]</sup>.

Fluorescence emission spectra were obtained using the AB series2 luminescence spectrometer. Stock solution (2×10<sup>-2</sup> mol·L<sup>-1</sup>) of the aqueous salts of K<sup>+</sup>, Li<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Fe<sup>2+</sup>, and Hg<sup>2+</sup> were prepared. Stock solutions of host DPKA (1.0×10<sup>-4</sup> mol·L<sup>-1</sup>) were prepared in buffered aqueous acetonitrile solutions. 2 mL host solutions were placed into a test tube, adding an appropriate aliquot of each metal stock solution into the host solution. For all the fluorescent measurements, the excitation wavelength was 350 nm. Excitation and emission slit widths were 8 nm and 4 nm, respectively. Optical absorption spectra were collected on a Shimadzu 3100 spectrophotometer in an aqueous acetonitrile solution (H<sub>2</sub>O-CH<sub>3</sub>CN, 40:60) at room temperature. All the spectroscopic measurements were performed at least in triplicate and averaged.

### 1.2 Synthesis of compound 3

An aqueous solution (5 mL) containing CuCl<sub>2</sub>·2H<sub>2</sub>O (0.1 mmol, 0.017 g) was added dropwise to an acetonitrile solution (15 mL) of DPKA (0.1 mmol, 0.036 g). After stirring at room temperature for 20 minutes, 5 mL aqueous solution of NaClO<sub>4</sub>·H<sub>2</sub>O (0.5 mmol, 0.07 g) was added. Green block crystal suitable for single-crystal X-ray diffraction was obtained by evaporating the resulting solution in air for several days. Yield: 74% (based on Cu). Anal. (%): calcd. for C<sub>22</sub>H<sub>20</sub>Cl<sub>2</sub>CuN<sub>8</sub>O<sub>10</sub>·H 2.93 C 38.32 N 16.26; Found: H 2.87 C 38.45 N 16.60. ESI-MS: 473.9 for [Cu(2-H)<sub>2</sub>](H<sub>3</sub>O)]<sup>+</sup>, calcd. for [Cu(2-H)<sub>2</sub>](H<sub>3</sub>O)]<sup>+</sup> 474.0.

### 1.3 X-ray Crystallography

X-ray intensity data were measured at 300 K on a Bruker SMART APEX CCD-based diffractometer (Mo- $K\alpha$  radiation,  $\lambda=0.071\ 073$  nm) using the SMART and SAINT programs.<sup>[12]</sup> Final unit cell parameters were determined by least-squares refinement of strong reflections for the compound **3**. The structure was solved by direct method and refined on  $F^2$  by full-matrix least-squares methods with SHELXTL version 5.1<sup>[13]</sup>. All of the non-hydrogen atoms except the disordered solvent molecules were refined with anisotropic thermal displacement coefficients. Hydrogen atoms were located geometrically, whereas those of solvent molecules were found on Fourier difference maps, and all the hydrogen atoms were refined in riding model. Parameters for data collection and refinement of compounds are summarized in Table 1. Further details concerning the structure determinations can be found in CCDC (**3**): 640678.

**Table 1** Crystallographic data for compound **3**

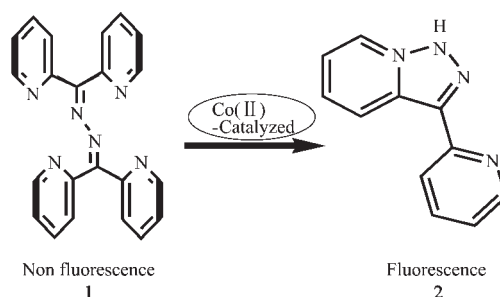
Empirical formula	Cu(C <sub>11</sub> H <sub>9</sub> N <sub>4</sub> ) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> (ClO <sub>4</sub> ) <sub>2</sub>
Formula weight	690.90
Crystal system	monoclinic,
Space group	$P2_1/c$
$a$ / nm	0.4410(13)
$b$ / nm	1.2349(2)
$c$ / nm	1.5396(3)
$\beta$ / (°)	103.984(3)
$V$ / nm <sup>3</sup>	1.3728(4)
$Z$	2
$\rho_{\text{calcd}}$ / (g·cm <sup>-3</sup> )	1.671
Good of fit ( $F^2$ )	1.023
$R_1$	0.0668
$wR_2$	0.1410

## 2 Results and discussion

On account of its simplicity and high sensitivity, fluorescence is becoming of increasing importance for chemical trace detection. Based on metallic catalytic effect on the autoxidation of the di-pyridylketone azine (DPKA), as shown in Scheme 1, we hypothesized that the nonfluorescent starting material di-pyridylketone azine (DPKA) **1** could be converted into the fluorescent pyrazolo product **2** under an optimal condition. As expected, with the introduction of  $\text{Cu}^{2+}$  into the

aqueous acetonitrile solution, the original silent fluorescence gradually aroused until the 1:1 stoichiometry (Fig.1). As shown in inset of Fig.1, two independent linear correlations exist in the range of  $0.64\sim 3.2\text{ mg}\cdot\text{kg}^{-1}$  and  $3.84\sim 7.04\text{ mg}\cdot\text{kg}^{-1}$ , with the linear correlation coefficient of 99.62% and 99.56%, respectively. Excess addition of  $\text{Cu}^{2+}$  will quench the fluorescence. Furthermore, addition of Cu(II) chelating agents, such as EDTA, does not decrease the fluorescent intensity, confirming the irreversible process, which is a characteristic of chemodosimeter rather than a sensor in the strict sense. Similar results are obtained with various copper(II) salts, such as  $\text{Cu}(\text{ClO}_4)_2$ ,  $\text{Cu}(\text{BF}_4)_2$ ,  $\text{Cu}(\text{OAc})_2$  and  $\text{CuBr}_2$ . Therefore, it appears that the counter ions have a negligible effect on the chemodosimeter. The saturation behavior of the fluorescence intensity after the addition of 1 equivalent of  $\text{CuCl}_2\cdot 2\text{H}_2\text{O}$  or other copper salts and the irreversibility of the fluorescent signaling suggests the formation of a new compound from the reaction of equivalent DPKA and Cu(II).

Solid evidence comes from the electrospray ionization mass spectrometry (ESI-MS) spectra of a  $\text{Cu}^{2+}$  titration solution of compound **1** (Fig.2). ESI-MS spectra of the ligand **1** in the presence of 1 equivalent of  $\text{Cu}^{2+}$  exhibit only a peak at  $m/z$  of 473.9, corresponding to  $[\text{Cu}(2\text{-H})_2(\text{H}_3\text{O})]^+$  (calcd. 474.09). The result further confirms the formation of ring-closed ligand **2** when the  $\text{Cu}^{2+}$  is introduced in the system. Further evidence for the exotic compound **2** comes from the independent synthesis of Cu(II) coordination compound **3** by the reaction of  $\text{CuCl}_2\cdot 2\text{H}_2\text{O}$  with DPKA in 1:1 molar ratio.



Scheme 1 Proposed Mechanism of Fluorescent Enhancement of DPKA (**1**) upon the Addition of  $\text{Cu}^{\text{II}}$ .

The reaction of di-pyridylketone azine (DPKA) **1**

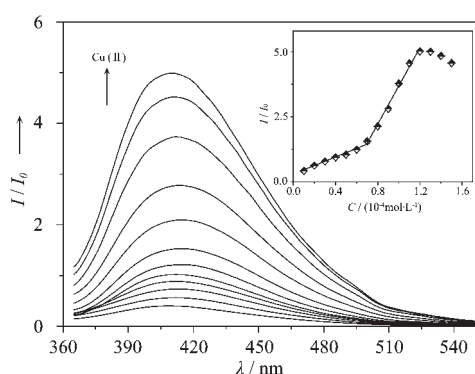


Fig.1 Fluorescent response of **1** ( $1.0 \times 10^{-4}$  mol·L $^{-1}$ ), upon increasing concentration of Cu $^{2+}$  in CH $_3$ CN/H $_2$ O (60:40). Excitation wavelength was 350 nm with 4 nm slit widths. Inset: fluorescence enhancement factor at 410 nm as a function of Cu(II) concentration.

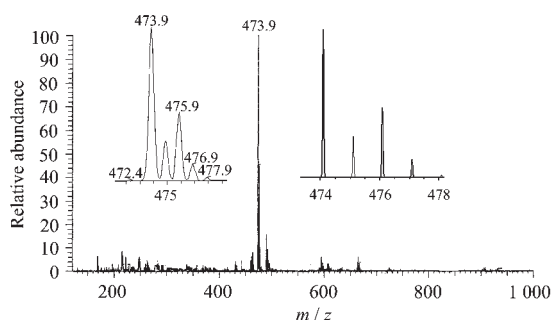
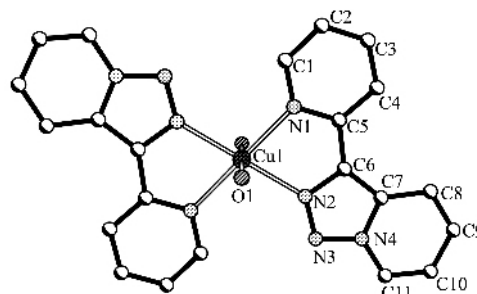


Fig.2 ESI-MS (positive) of the titration solution in the presence of Cu $^{2+}$ . Inset: Calculated (right) and observed (left) isotopic patterns for the [Cu(2-H) $_2$ (H $_3$ O)] $^{+}$  cation, indicating the formation of a 1:2 metal-ligand complex.

(0.1 mmol, 0.036 g) with CuCl $_2$ ·2H $_2$ O (0.1 mmol, 0.017 g) in acetonitrile/water (90:10) mixture at room temperature rapidly yielded dark green solution, which was allowed to stand in air, after several days, the cubic crystal suitable for single-crystal X-ray diffraction measurement was obtained in high yield (92%) with the formula of Cu(C $_{11}$ H $_9$ N $_4$ ) $_2$ (H $_2$ O) $_2$ (ClO $_4$ ) $_2$  (**3**). The analysis of the crystal structure would surprisingly reveal that the asymmetric unit consists of one molecule of 3H-pyrazolo [4,3-b]pyridine **2**, a copper atom, a coordinated water molecule, and a disordered anion ClO $_4$  $^{-}$ . As shown in Fig.3, the copper center is hexa-coordinated in a slightly distorted octahedral geometry with four pyridine nitrogen atoms occupied at the equatorial plane and two symmetric-related water molecules at

the axial positions. These results strongly support the formation of Cu-induced ring-closed product **2** in CH $_3$ CN/H $_2$ O solution via a copper-catalyzed ring-closed reaction.



Selected bond distances (nm) and bond angles( $^{\circ}$ ):

Cu1-N1 0.210 1(5), Cu1-N2 0.211 0(5),  
Cu1-O1 0.217 2(4), N2-N3 0.129 8(6),  
N2-C6 0.135 2(7), N3-N4 0.134 4(6),  
C7-N4 0.135 4(7)  
N1-Cu1-N1(A) 180.0, N1-Cu1-N2 77.3(2),  
N1-Cu1-N2(A) 102.7(2), N2-Cu1-N2(A) 180.000(1),  
N1-Cu1-O1 89.14(17), N1-Cu1-O1(A) 90.86(17),  
N2-Cu1-O1 91.77(17), N2-Cu1-O1(A) 88.23(17),  
N2-Cu1-O1(A) 88.23(17)

Symmetry code, A:  $-x, -y, 1-z$ .

Fig.3 Molecular structure of complex **3** showing the ring-closed conformation of compound **2**

The Cu-promoted catalytic reaction could be employed for Cu(II) fluorescent detection with a high selectivity. The conditions of this reaction is to be optimized and can be chosen to yield high selectivity for Cu(II) ion. A CH $_3$ CN/H $_2$ O (60:40) solution of chemodosimeter **1** (100  $\mu$ M) was prepared at pH=7. The fluorescence enhancement effects of various metal ions on **1** in CH $_3$ CN/H $_2$ O (60:40) solution were investigated under excitation at  $\lambda_{ex}$ =350 nm. The fluorescence response of **1** to various cations and its selectivity for Cu $^{2+}$  are illustrated in Fig.4. No significant spectral changes of **1** occurred in the presence of alkali or alkaline earth metals, such as Na $^{+}$ , K $^{+}$ , Mg $^{2+}$ , and Ca $^{2+}$ , and the first row transition metals Mn $^{2+}$ , Fe $^{2+}$ , Co $^{2+}$ , Ni $^{2+}$  and Zn $^{2+}$ , respectively. The competition measurements were carried out by the subsequent addition of other metal ions (5-fold excess), including alkali- and alkaline-earth ions, such as Na(I), K(I), Mg(II), and Ca(II), and transition- and heavy-metal such as Mn(II), Fe(II), Co(II), Ni(II), Zn(II), Cd(II), and even Pb(II) and Ag

(I) ions (Fig.4), to the solution of Cu(II) -containing system. The results revealed that the Cu-induced luminescence response is unaffected in the background of 10 equivalent of environmentally relevant alkali or alkaline-earth metals, such as Na(I), K(I), Mg(II), and Ca(II). In addition, among the the first-row transition-metal ions, only Fe(II) can weaken the fluorescence of the titration solution. Other transition-metal ions, such as Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, and Zn<sup>2+</sup>, do not interfere with the Cu<sup>2+</sup>-induced fluorescence increase, indicating that DPKA has a remarkable selectivity for Cu<sup>2+</sup> in aqueous/acetonitrile solution.

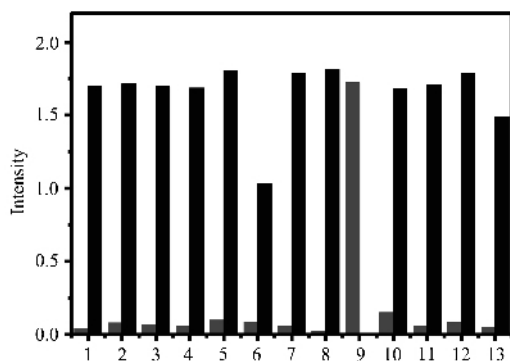


Fig.4 Selectivity for Cu<sup>2+</sup> over the metal ions of interests. 1, Na<sup>+</sup>, 2, K<sup>+</sup>, 3, Ca<sup>2+</sup>, 4, Mg<sup>2+</sup>, 5, Mn<sup>2+</sup>, 6, Fe<sup>2+</sup>, 7, Co<sup>2+</sup>, 8, Ni<sup>2+</sup>, 9, Cu<sup>2+</sup>, 10, Zn<sup>2+</sup>, 11, Cd<sup>2+</sup>, 12, Hg<sup>2+</sup>, 13, Ag<sup>+</sup>. Gray bars represent the addition of 5×10<sup>-4</sup> mol·L<sup>-1</sup> of metal ions of interest into 10<sup>-4</sup> mol·L<sup>-1</sup> solution of DPKA. Black bars represent the subsequent addition of 10<sup>-4</sup> mol·L<sup>-1</sup> CuCl<sub>2</sub> to the solution above. Excitation wavelength was 350 nm, and emission intensity was recorded at 410 nm.

The copper-induced catalytic reaction is so rapid that it can achieve the completion within ca. 50 seconds as shown in Fig.5 for the time-traced fluorescence intensity at 410 nm. When it is delayed until over 100 s, the fluorescence is partly quenched, which may result from the quenching nature of Cu<sup>2+</sup> to the fluorophore **2**. The quenching mechanism may be similar to that of some Cu(II) probes displaying fluorescence quenching for the paramagnetic nature of the copper ion.<sup>[14]</sup> The phenomenon also demonstrates that the fluorescence is emitted from the product **2** during the copper-induced catalytic reaction. From a sensi-

tivity viewpoint, it is preferable to inhibit this quenching effect to generate a more notable fluorescence enhancement. Chemical modification on the substituted group may be a good improvement. Works related to this topic are now under investigation.

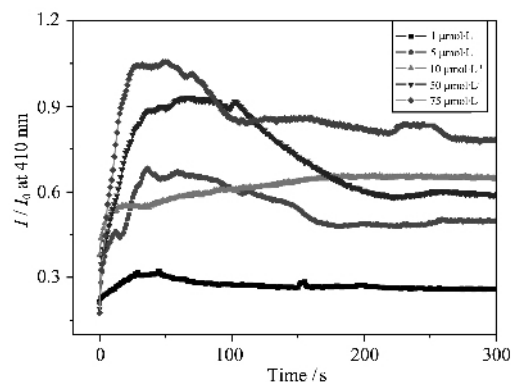


Fig.5 Time-traced fluorescence of probe **1** (10<sup>-4</sup> mol·L<sup>-1</sup>) in the presence of different amount of Cu<sup>2+</sup>. Excitation wavelength was 350 nm, and emission intensity was recorded at 410 nm.

### 3 Conclusions

In summary, the DPKA molecule can be accessible as an easy-to-prepare molecular sensing system, which utilizes an irreversible Cu<sup>2+</sup>-promoted organic reaction to form the ring-closed fluorescent product. The sensing of Cu<sup>2+</sup> can be monitored by fluorescence intensity changes that responds instantaneously at room temperature in a 1:1 stoichiometric manner to the amount of Cu<sup>2+</sup>. The selectivity of this system for Cu<sup>2+</sup> over other metal ions is remarkably high. The findings suggest that this method will serve as the potential chemodosimeters for rapidly sensing the existence of Cu<sup>2+</sup> in aqueous environments.

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