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# A Fluorescence-Enhanced Chemodosimeter for $Fe^{3+}$ Based on Hydrolysis of Bis(coumarinyl) Schiff Base

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**Keywords:** Probes / Iron / Fluorescence / Schiff bases

Bis(coumarinyl) Schiff base **1** was designed and synthesized as a fluorescence turn-on chemodosimeter for $Fe^{3+}$. The chemodosimeter was readily synthesized in four steps from 2,4-dihydroxybenzaldehyde. The addition of $Fe^{3+}$ to chemodosimeter **1** induced about a 140-fold enhancement in fluorescence. Furthermore, chemodosimeter **1** was also highly selective to $Fe^{3+}$ over other metal ions, and most of the related metals ions exhibited negligible detection interference.



## Introduction

Fluorescent probes for metal ions have received great attention in the last few decades due to their useful applications in environment, biology and chemistry.[1–3] Iron is an essential trace element that plays significant roles in chemical and biological processes.[4,5] Therefore, the detection of trace amounts of $Fe^{3+}$ ions is critical. Indeed, enormous efforts have been devoted to the development of $Fe^{3+}$ probes. However, most of them undergo a fluorescence quenching response because of the paramagnetic nature of $Fe^{3+}$.[6] In most practical applications of fluorescent probes, fluorescence enhancement when interacting with analytes is much more desirable than fluorescence quenching. Nevertheless, the fluorescence quenching character of $Fe^{3+}$ presents a challenge to develop selective, as well as sensitive, fluorescence turn-on $Fe^{3+}$ probes. Recently, very limited cases of fluorescence-enhanced $Fe^{3+}$ sensors have been reported,[7] most of which employed the well-studied equilibrium between the fluorescence "off" spirocyclic form and the fluorescence "on" open-ring form of rhodamine derivatives.[7b–7d]

Schiff bases are important intermediates involved in many enzymatic transformations. The mechanisms of hydrolysis of Schiff bases by acids and amines have been investigated in great detail.[8] In addition, it is known that Schiff bases are also subject to metal-promoted hydrolysis.[9] On the other hand, $Fe^{3+}$ is a strong Lewis acid with a $pK_a$ of 2.83.[10] It was reported that $Fe^{3+}$ could facilitate imidazolidine and phosphate ester hydrolysis by coordinated acidic water molecules.[11] Thus, we reasoned that $Fe^{3+}$ may also hydrolyze Schiff bases in an analogous way. On the basis of this consideration, in this work, bis(coumarinyl) Schiff base **1** was designed and synthesized as the first example of a fluorescence turn-on $Fe^{3+}$ chemodosimeter. Fluorescent chemodosimeters are molecular devices that utilize abiotic receptors to achieve analyte recognition with irreversible transduction of a fluorescent signal.[12] The major difference between a chemodosimeter and a sensor is that the signaling response of the former is irreversible, whereas that of the latter is reversible. Coumarin dyes are an important class of fluorescent compounds widely used in probes because of their excellent photophysical properties.[13] It was envisioned that bis(coumarinyl) Schiff base **1** is "off" in fluorescence due to a photoinduced charge-transfer (PCT) process (from the electron-donating C=N moiety to the coumarin ring), which quenches the excited-state emission of the coumarin fluorophore.[1–3] However, upon addition of $Fe^{3+}$, bis(coumarinyl) Schiff base **1** is hydrolyzed to release highly fluorescent coumarin dye **4** (Scheme 1).

Herein, we described a fluorescence turn-on chemodosimeter for $Fe^{3+}$ based on metal-promoted hydrolysis of bis(coumarinyl) Schiff base. Chemodosimeter **1** was readily synthesized in a few steps. The addition of $Fe^{3+}$ to chemodosimeter **1** induced about a 140-fold enhancement in fluorescence intensity. Moreover, **1** was also highly selective to $Fe^{3+}$ over other metal ions, and most of the related metals ions exhibited negligible detection interference. As most transition-metal ions possess intrinsic fluorescence quenching properties, it is very challenging to construct their fluorescence-enhanced sensors.[14] However, alternatively, the design approach for this chemodosimeter should open a new avenue for the development of fluorescence turn-on chemodosimeters for other transition-metals ions by employing a particular metal-promoted reaction.

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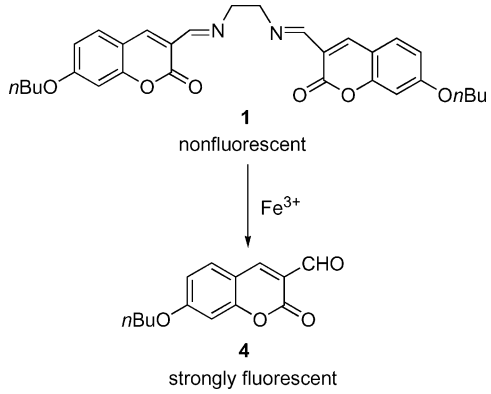
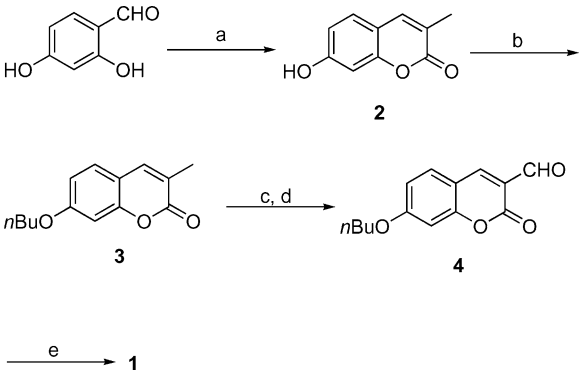



Scheme 1. Design concept of fluorescence "on" $Fe^{3+}$ chemodosimeter.

## Results and Discussion

A convenient synthetic route to chemodosimeter **1** was developed as shown in Scheme 2. Condensation of 2,4-dihydroxybenzaldehyde with propanoic anhydride in refluxing propionic anhydride gave compound **2** in modest yield,[15] which was then butylated with *n*BuBr to afford coumarin **3** in 77% yield. Reaction of **3** with an excess amount of NBS provided the dibromide intermediate, which was further hydrolyzed with NaOAc in acetic acid to afford the key intermediate, coumarin aldehyde **4** in 84% yield.[16] Finally, chemodosimeter **1** was obtained by condensation of **4** (2 equiv.) with ethylenediamine in anhydrous acetonitrile (yield: 64%). The structures of the intermediates and final product were confirmed by NMR spectroscopy, MS (ESI) and elementary analysis.




Scheme 2. Synthetic route to **1**. Reagents and conditions: (a) $CH_3CH_2COONa$, $(CH_3CH_2CO)_2O$, triethylamine, reflux, 6 h, 48.7%; (b) *n*BuBr, acetone, $K_2CO_3$, reflux, 5 h, 77.3%; (c) NBS, AIBN, $CCl_4$, reflux, 5 h; (d) $CH_3COONa$, $CH_3COOH$, reflux, 8 h, 84.4%; (e) ethylenediamine, anhydrous acetonitrile, room temp., 12 h, 63.6%

To examine the feasibility of bis(coumarinyl) Schiff base **1** as a chemodosimeter for $Fe^{3+}$, we first investigated its fluorescence character in the absence and presence of $Fe^{3+}$. As shown in Figure 1, bis(coumarinyl) Schiff base **1** (5 μM) was very stable and exhibited no fluorescence variations in the absence of $Fe^{3+}$ in $CH_3OH/H_2O$ solution (98:2). By contrast, upon treatment with $Fe^{3+}$ (12 equiv.), a marked enhancement in the fluorescence intensity was observed even after 5 min. The fluorescence intensity reached a maximum after about 50 min. Thus, an assay time of 50 min was chosen to further explore the sensitivity and selectivity of chemodosimeter **1** toward $Fe^{3+}$. Furthermore, after treatment of chemodosimeter **1** with $Fe^{3+}$ for 50 min, the addition of EDTA, an $Fe^{3+}$ chelating agent, did not reduce the fluorescence intensity, which demonstrates the irreversible nature of this hydrolysis process promoted by $Fe^{3+}$ and which is characteristic of a chemodosimeter.[12] The formation of coumarin aldehyde **4** as the hydrolysis product was confirmed by a comparative study with authentic compound **4** through excitation and emission spectra (Figure S1, Supporting Information). In addition, hydrolysis product **4** was also separated and characterized by TLC, $^1H$ NMR spectroscopy and MS analysis. In addition, 60 μM of $Fe^{3+}$ ions in $CH_3OH/H_2O$ solution (98:2) in the absence of chemodosimeter **1** did not display any fluorescence, which further confirms that the fluorescence enhancement observed is indeed from hydrolysis product **4**.

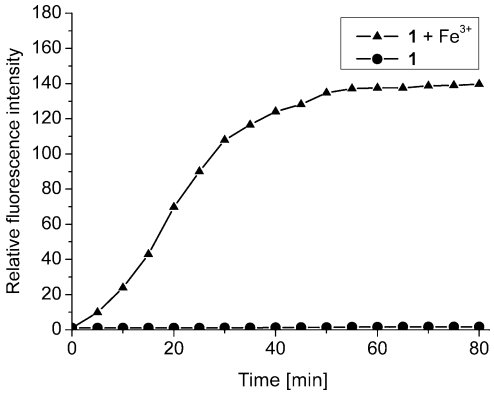



Figure 1. Reaction–time profile of chemodosimeter **1** (5 μM) in the absence (●) and presence (▲) of $Fe^{3+}$ (12 equiv.) in $CH_3OH/H_2O$ solution (98:2). The fluorescence intensity at $\lambda_{em}$ = 392 nm was plotted versus time.

Titration of **1** (5 μM) with $Fe^{3+}$ ions was performed in $CH_3OH/H_2O$ solution (98:2). Figure S2 (Supporting Information) shows the excitation spectra of **1** in the absence or presence of $Fe^{3+}$ (12 equiv.). Free **1** exhibits an excitation peak with a maximum at 337 nm, which then blueshifts to 326 nm upon addition of $Fe^{3+}$. It is noteworthy that the excitation wavelength maximum of **4** is 326 nm (Figure S1, Supporting Information), which is consistent with the formation of hydrolysis product **4** upon treatment of **1** with $Fe^{3+}$. As designed, in the absence of metal ions, only a weak broad emission band with maximum around 402 nm was observed for free **1** (Figure S3, Supporting Information). The fluorescence quantum yield of **1** was measured as 0.004 with reference to quinine sulfate (see the Supporting Information).[17] This low-fluorescence character of free **1** is apparently attributed to a PCT process (from the electron-donating C=N moiety to the coumarin ring), which quenches the excited state emission of the coumarin fluorophore.[1–3] The fluorescence response of **1** toward increasing concentrations of $Fe^{3+}$ ions is displayed in Figure 2. When

increasing concentrations of $Fe^{3+}$ ions were introduced, the emission of **1** was drastically increased with a fluorescence enhancement factor (FEF) up to 139.6 and a fluorescence quantum yield of 0.27.[18] Concomitantly, the addition of $Fe^{3+}$ ions also causes a blueshift of the maximal emission wavelength of the chemodosimeter from 402 to 392 nm (Figure S1, Supporting Information), which is in good agreement with the formation of hydrolysis product **4** upon treatment of **1** with $Fe^{3+}$. As the changes in fluorescence intensity are of such magnitude, it may be considered that the fluorescence emission is "switched off" in **1** and "switched on" upon the addition of $Fe^{3+}$.

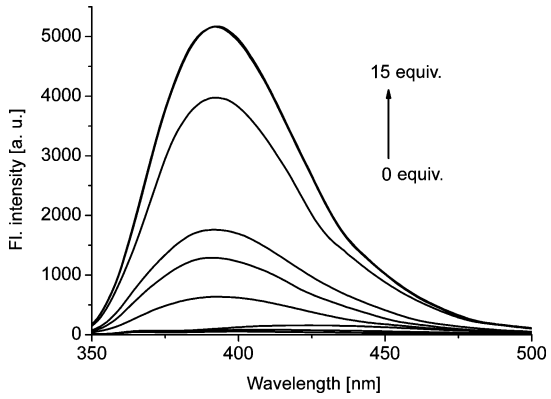



Figure 2. Emission spectra of **1** (5 μM) upon the addition of $Fe^{3+}$ (0, 0.1, 1, 3, 5, 7, 9, 12, 15 equiv.). Excitation at 330 nm.

To investigate the selectivity, representative ions such as $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Al^{3+}$, $Fe^{3+}$, $Fe^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Cu^{2+}$, $Hg^{2+}$ and $Mg^{2+}$ were treated with **1** in $CH_3OH/H_2O$ solution (98:2). Changes in the fluorescence spectra of **1** (5 μM) upon the addition of metal ions are shown in Figure 3a. Chemodosimeter **1** only exhibits negligible fluorescence variations in the presence of $Na^+$, $K^+$, $Ni^{2+}$ or $Co^{2+}$ (12 equiv.), whereas $Fe^{2+}$, $Al^{3+}$, $Cd^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Hg^{2+}$ and $Ca^{2+}$ induce a minimum fluorescence enhancement. However, $Cu^{2+}$ quenched approximately 73% of the fluorescence emission. This is attributed to a quenching effect of the $Cu^{2+}$ ions.[2,19] The FEF values of chemodosimeter **1** responding to different metal ions are shown in Figure 3b. It can be seen that $Fe^{2+}$, $Al^{3+}$, $Cd^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Hg^{2+}$ and $Ca^{2+}$ induce about 4.7-, 7.6-, 6.8-, 6.2-, 4.8-, 4.1-, 3.5- and 3.4-fold increase in fluorescence, respectively. However, $Fe^{3+}$ results in the largest fluorescence enhancement with a FEF up to 139.6-fold, which indicates that **1** is highly selective towards $Fe^{3+}$ over other metal ions tested. This could be explained by the fact that the p$K_a$ of $Fe^{3+}$ is much lower than those of other metal ions surveyed.[10]

The interference of typical alkali, alkaline-earth and transition-metal ions to the fluorescence detection of $Fe^{3+}$ was also investigated. $Fe^{3+}$ (12 equiv.) was added to chemodosimeter **1** (5.0 μM) in the presence of related metal ions (36 equiv.). Apparently, $Hg^{2+}$, $Mg^{2+}$, $Mn^{2+}$ and $Ni^{2+}$ show only very mild interference in the detection of $Fe^{3+}$ (Figure 4). By contrast, $Ca^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Fe^{2+}$, $Zn^{2+}$, $Al^{3+}$,

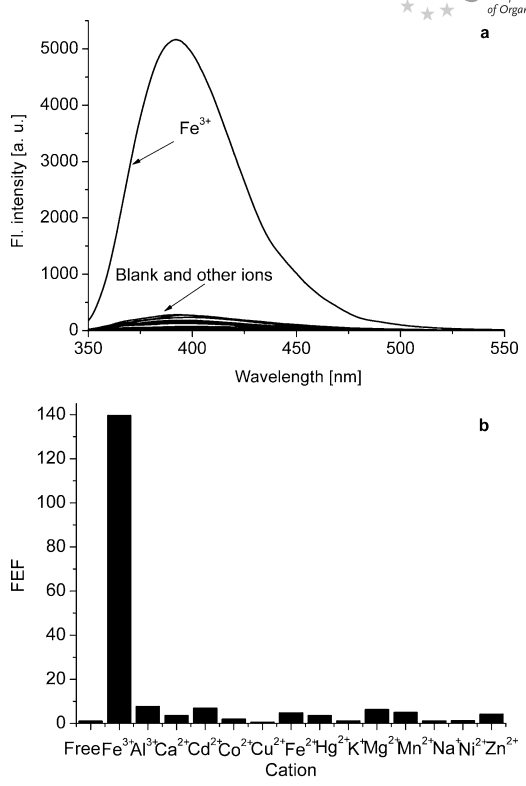



Figure 3. (a) Emission response of **1** (5 μM) to different metal ions (12 equiv.); (b) fluorescence enhancement factors (FEF) of **1** (5 μM) upon addition of the various metal ions (12 equiv.). Excitation and emission were at 330 and 392 nm, respectively..

$Na^+$ and $K^+$ have virtually no effect on $Fe^{3+}$ detection. Interestingly, when $Fe^{3+}$ (12 equiv.) was introduced to chemodosimeter **1** in the presence of $Cu^{2+}$ (36 equiv.), only 15-fold fluorescence enhancement was observed, which is much lower than that for $Fe^{3+}$ alone. This is in accordance with the quenching nature of $Cu^{2+}$.[2,19] Thus, the interference for fluorescence detection of $Fe^{3+}$ in the presence of other competing metal ions except $Cu^{2+}$ is insignificant.

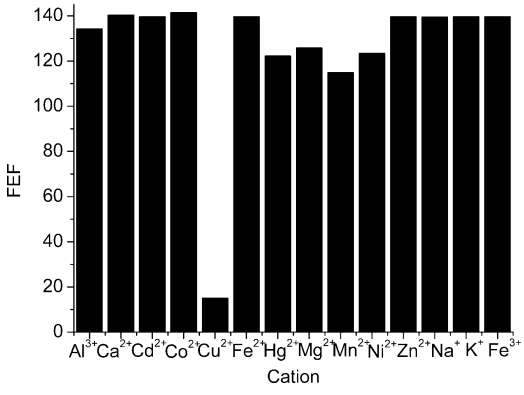



Figure 4. Fluorescence response of **1** (5 μM) to $Fe^{3+}$ (12 equiv.) in the presence of other competing metal ions (36 equiv.). Excitation and emission were at 330 and 392 nm, respectively..

## Conclusions

We described a fluorescence turn-on chemodosimeter for $Fe^{3+}$ based on metal-promoted hydrolysis of bis(coumari-

nyl) Schiff base. The chemodosimeter was readily synthesized in four steps from 2,4-dihydroxybenzaldehyde. The addition of $Fe^{3+}$ to **1** induced about 140-fold fluorescence enhancement. In addition, **1** is highly selective to $Fe^{3+}$ over other metal ions. Furthermore, most of the related metal ions exhibit negligible detection interference. Thus, **1** developed herein possess favourable features for effective applications in environmental analysis. The future efforts will focus on improving its water solubility by structural modifications so that it can also function in aqueous systems for biological applications. As most transition-metal ions possess intrinsic fluorescence quenching properties, it is very challenging to develop their sensors with fluorescence enhancement.[14] However, alternatively, the design approach for this chemodosimeter should lead to the development of fluorescence turn-on chemodosimeters for other transition-metal ions by employing a particular metal-facilitated reaction.

**Supporting Information** (see footnote on the first page of this article): Detailed experimental procedures and full characterization data for all compounds synthesized, and some spectra of the probe.

## Acknowledgments

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[18] According to Figure 2, as little as 1 equiv. of $Fe^{3+}$ is able to turn on the fluorescence (1.9-fold) and 12 equiv. of $Fe^{3+}$ can enhance the fluorescence up to 140-fold. Similar examples are well precedented. For example, see: ref.[12] and W. Jiang, Q. Fu, H. Fan, J. Ho, W. Wang. *Angew. Chem.* **2007**, *119*, 8597–8600.
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