

Selective Fe²⁺ Ion Recognition Using a Fluorescent Pyridinyl-benzoimidazole-derived Ionophore

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Fluorescent organic molecules that respond to changes in the Fe²⁺ concentration with selectivity to other abundant di-valent metal ions will offer the ability to understand the dynamic fluctuations of the Fe²⁺ ion in interesting media. The use of **6-Br-ppmbi**, derived from 2-pyridin-2-yl-benzimidazole, for metal ion-selective fluorescence recognition was investigated. Screening of the main group and transition metal ions showed exclusive selectivity for Fe²⁺ ions even in the presence of competing metal ions. In addition, the requirement for low concentrations of probe molecules to detect certain amounts of Fe²⁺ ions make this sensor unique compared to previously reported Fe²⁺ ion sensors.

Key Words : Fluorescent chemical sensor, 6-Br-ppmbi, Fe²⁺ cation

Introduction

Effective sensors for the detection of transition metal ions have been of interest in recent years due to the essential roles of transition metal ions in the structural and functional aspects of biological systems.¹⁻³ Among the many analytic methods, many fluorescent metal ion probes have been reported because the use of fluorescence as a detecting method has distinct advantages in terms of sensitivity. A number of organic ionophores bind extremely selectively to the targeted transition metal ions. Hence, the selective and sensitive fluorescent probes for detecting metal ions can be synthesized by chemically linking the part of the ionophores to the adequate fluorophore. Upon the binding of metal ions to the ionophores, the fluorophore showed an optical change. Using such design principles, very effective fluorescent sensors for Ag⁺,⁴ Zn²⁺,⁵ Cu²⁺,⁶ Ni²⁺,⁷ Hg²⁺,^{8,9} Fe³⁺,¹⁰ etc. has been reported. In particular, Hg²⁺ and Zn²⁺ sensors are applied to image the respective metal ions in living cellular systems.

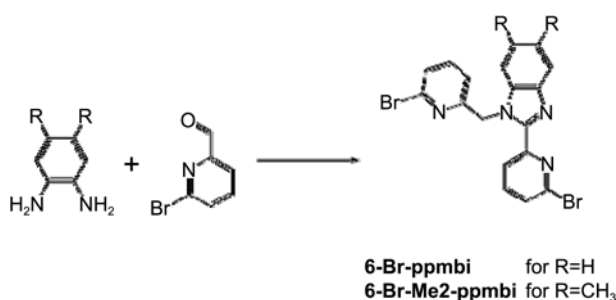
Iron is required by all living organisms owing to its potent redox chemistry and possibility to engage in catalytic activity. If the Fe²⁺ concentration is not regulated, it can lead to uncontrolled oxidative chemistry causing tissue damage and fibrosis to various organs.¹¹ Although biological iron are observed frequently in ferrous (Fe²⁺) oxidation states bound tightly by enzymes and storage proteins, the existence of labile iron within cells is controversial, resulting in high demand for selective sensors for iron.¹²⁻¹⁵ Compared to the number of fluorescent sensors for other transition metal ions, iron-specific fluorescent sensors are quite rare. Currently, a few probes for Fe²⁺ ion are available.¹⁵ Recently, "Calcein" with an EDTA-derived receptor has been reported to detect

Fe²⁺ ions with ten-fold higher selectivity for Fe²⁺ over Fe³⁺.¹⁶ On the other hand, this reagent shows significant interference from other divalent metal ions, including Cu²⁺, Ni²⁺ and Co²⁺ cations. Fluorescent sensors that directly analyze the concentration of Fe²⁺ ions over other divalent metal ions are unavailable.

Benzoimidazole and pyridine are well-known building blocks for constructing chelating ionophores for transition metal ions. In previous studies, pyridines derivatized positions with sterically hindered methyl groups have altered the binding properties to Fe²⁺ cations.¹⁷ If the bulky and electron withdrawing -Br group, instead of a methyl group, is attached to pyridine, it is possible that it would alter the binding properties of pyridine-containing ionophore to transition metal ions, and discriminate Fe²⁺ ions from other divalent metal cations. This paper reports the synthesis of 2-bromopyridinyl-benzoimidazole derivatives as well as their emission response toward Fe²⁺ ions and other metal ions.

Materials and Methods

General. All solvents and reagents were obtained from commercial suppliers and used as received without further purification. Solutions were prepared using reagent grade chemicals and doubly distilled water was used throughout. Elemental analysis for carbon, nitrogen, and hydrogen was carried out by using an EA1108 (Carlo Erba Instrument, Italy) in the Organic Chemistry Research Center of Sogang University, Korea. ¹H-NMR (300 MHz) spectra were recorded on a JEOL JNM-AL400 spectrometer. UV-Vis spectra were obtained using a Cary 3 spectrophotometer with a quartz cuvette (path length = 1 cm) and fluorescence was recorded on a Perkin Elmer LS45 fluorescence spectrometer.



Scheme 1

IR spectra were measured on a BIO RAD FTS 135 spectrometer as KBr pellets. ESI-MS spectra were measured on a Si-2/LCQ DecaXP mass spectrometer.

Synthesis of 2-(6-bromopyridin-2-yl)-1-((6-bromopyridin-2-yl)methyl)-1H-benzoimidazole (6-Br-ppmbi). To a solution of 1,2-phenylenediamine (2.16 g, 20.0 mmol) in EtOH (40 mL) was added 6-bromopyridine-2-carbaldehyde (7.60 g, 40 mmol) (Scheme 1). Stirring was continued for a further 20 h at room temperature and the precipitate was collected by filtration, washed with cold EtOH and dimethylether, dried under vacuum, and weighed (Yield: 6.05 g, 68.1%). 0.015 g (0.050 mmol) of Zn(NO₃)₂ were dissolved in MeOH (4 mL) and mixed with MeOH solution (4 mL) of **6-Br-ppmbi** ligand (0.02 g, 0.05 mmol). Slow evaporation of the diffused mixture provided suitable crystals of **6-Br-ppmbi** for X-ray analysis that were obtained in a week. Anal. Calcd for C₁₅H₁₂N₄Br₂ (444.12): C, 48.68; H, 2.72; N, 12.62. Found: C, 48.60; H, 2.84; N, 12.77%. ¹H NMR (CDCl₃, 300 MHz) δ 8.42 (d, 1H, Pyridyl-H₃), 7.86 (t, 1H, Pyridyl-H₄), 7.70 (t, 1H, CH₂-Pyridyl-H₄), 7.50, 7.38 and 7.36 (m, 6H, Benzylimidazole, Pyridyl-H₅ and CH₂-Pyridyl-H₅), 6.96 (d, 1H, CH₂-Pyridyl-H₃), 6.10 (s, 2H, CH₂). FT-IR (KBr): (cm⁻¹) = 1580 (s), 1556 (s), 1386 (s), 1329 (s), 1168 (s), 1153 (s), 1124 (s), 851 (m), 832 (s), 806 (s), 747 (s), 676 (m), 630 (m), 590 (m), 560 (m), 536 (m), 446 (m), 431 (m). ESIMS MH⁺: Calcd for C₁₈H₁₃Br₂N₄, 444.1; Found, 444.6.

Synthesis of 2-(6-bromopyridin-2-yl)-1-((6-bromopyridin-2-yl)methyl)-1H-dimethyl-benzoimidazole (6-Br-Me2-ppmbi). To a solution of 4,5-dimethylbenzene-1,2-diamine (2.72 g, 20.0 mmol) in EtOH (40 mL) was added 6-bromopyridine-2-carbaldehyde (7.60 g, 40 mmol) (Scheme 1). Stirring was continued for a further 20 h at room temperature and the precipitate was collected by filtration, washed with cold EtOH and dimethylether, dried under vacuum, and weighed (Yield: 6.72 g, 71.2%). Slow evaporation in MeOH provided suitable crystals of **6-Br-Me2-ppmbi** for X-ray analysis that were obtained in a week. Anal. Calcd for C₂₀H₁₆Br₂N₄ (472.18): C, 50.87; H, 3.42; N, 11.87. Found: C, 50.96; H, 3.47; N, 11.63. ¹H NMR (CDCl₃, 300 MHz) δ 8.38 (d, 1H, Pyridyl-H₃), 7.66 (t, 1H, Pyridyl-H₄), 7.61 (s, 1H, Benzylimidazole), 7.45 (d, 1H, Pyridyl-H₅), 7.37 (m, 2H, CH₂-Pyridyl-H₃, CH₂-Pyridyl-H₅), 7.20 (s, 1H, Benzylimidazole), 6.84 (t, 1H, CH₂-Pyridyl-H₄), 6.05 (s, 2H, CH₂), 2.48 (s, 6H, CH₃). FT-IR (KBr): (cm⁻¹) = 1579 (s), 1546 (s), 1443 (s), 1421 (s), 1326 (s), 1163 (s), 1149 (s), 1120 (s), 1068 (m),

1002 (m), 985 (s), 836 (s), 801 (s), 782 (s), 758 (s), 735 (s), 669 (m), 597 (m), 430 (m).

Synthesis of [Cu(6-Br-Me2-ppmbi)(NO₃)₂] Complex: 31.0 mg (0.1 mmol) of Cu(NO₃)₂ were dissolved in MeOH (10 mL) and mixed with MeOH solution (10 mL) of **6-Br-Me2-ppmbi** ligand (47.0 mg, 0.1 mmol). Stirring was continued for a further 10 h at room temperature. The reaction solution was then poured into 20 mL dimethylether and a dark green precipitate formed. The precipitate was filtered and washed with 2 × 20 mL dimethylether. The solid was dried under vacuum and weighed (Yield: 28.2 mg, 42.7%). For X-ray analysis, 3.3 mg (0.005 mmol) of the complex were dissolved in methylene chloride (3 mL) and carefully layered by petroleum ether (5 mL). Suitable crystals were obtained in 2 months.

X-ray Crystallographic Studies: Single crystals were mounted at room temperature on the tips of quartz fibers, coated with Partone-N oil, and cooled under a stream of cold nitrogen. Intensity data were collected on a Bruker CCD area diffractometer running the SMART software package, with Mo Kα radiation (λ = 0.71073 Å). The structures were solved by direct methods and refined on F² by using the SHELXTL software package.¹⁸ Empirical absorption corrections were applied with SADABS,¹⁹ part of the SHELXTL program package, and the structures were checked for higher symmetry by the program PLATON.²⁰ All non-hydrogen atoms were refined anisotropically. In general, hydrogen atoms were assigned idealized position and given thermal parameters equivalent to 1.2 times the thermal parameter of the carbon atom to which they are attached. Data collection and experimental details for the compounds are summarized in Table S1.

Determination of the Apparent Fe²⁺ Affinity of Ionophore 6-Br-ppmbi: The ionophore was dissolved in an aqueous solution at 0.050 μM. Upon the addition of a certain amount of Fe²⁺, the fluorescence change was measured. By assuming a 1:1 metal-to-ionophore stoichiometry, the following relationship, Eq. (1), was found between the free Fe²⁺ concentration, apparent dissociation constant K_d, and fluorescent intensity F:

$$F = ([\text{Fe}^{2+}]F_{\min} + K_d F_{\max}) / (K_d + [\text{Fe}^{2+}]) \quad (1)$$

where F_{max} = fluorescence intensity of the free ionophore and F_{min} = fluorescence intensity of the Fe²⁺-bound ionophore. Nonlinear least-squares fit analysis of the experimental intensities versus the Fe²⁺ concentrations allowed for a determination of F_{max}, F_{min}, and K_d.

Results

2-pyridin-2-yl-benzimidazole is a versatile building block for the synthesis of transition metal complexes and has been used to prepare fluorescent materials owing to the strong fluorescent properties of benzoimidazole derivatives. Compound **6-Br-ppmbi** was synthesized by the coupling of commercially available 1,2-phenylenediamine with 6-bromopyridine-2-carbaldehyde, followed by the rearrangement of resulting

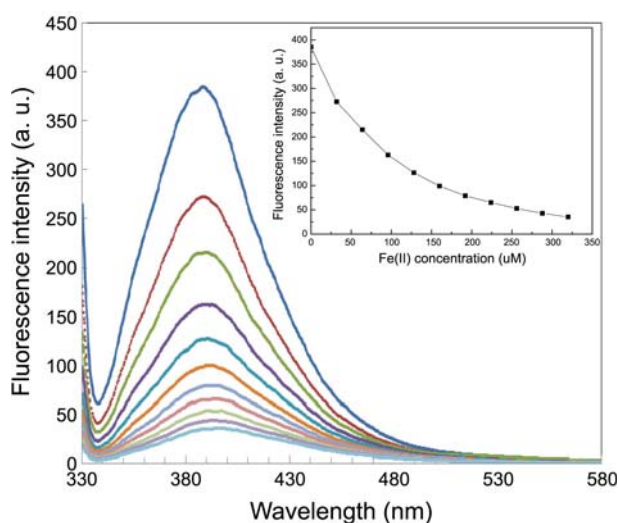


Figure 1. Fluorescence response changes of **6-Br-ppmbi** ($5.0 \times 10^{-2} \mu\text{M}$) upon titration of Fe^{2+} ion (excitation at 320 nm). (Inset) The change of fluorescence intensities at 389 nm against the amount of the added Fe^{2+} ion.

imines using Zn^{2+} catalyst, as shown in Scheme 1 and S1. Its structural features were further characterized by X-ray single crystal diffraction, showing a sterically hindered bi-dentate coordination site composed of 6-bromo pyridine and imidazole units (Fig. S1). The electronic effect of the bromo group together with steric hindrance could give the chelating unit the selective binding character to certain divalent metal ions.

As shown in Figure 1, **6-Br-ppmbi** exhibited strong fluorescent emission at 390 nm upon excitation at 320 nm. The fluorescent intensity of **6-Br-ppmbi** decreased dramatically upon the titration of Fe^{2+} ions. Saturated spectroscopic emission was reached after the introduction of more than 10000 equiv. of Fe^{2+} ions, indicating very loose binding between Fe^{2+} ions and the **6-Br-ppmbi** sensor in solution. To identify the stoichiometry of the complex with Fe^{2+} and **6-Br-ppmbi**, repeated attempts to crystallize the resulting compound was performed albeit unsuccessfully. This may originate from the poor crystalline properties of the species generated. As complementary measures, attempts to crystallize the species generated with divalent metal ions and derivatives of **6-Br-ppmbi** were made. The only complex that could be crystallized successively was the Cu^{2+} complex with **6-Br-Me2-ppmbi**, a derivative of **6-Br-ppmbi** with two additional methyl groups in benzimidazole. The octahedral copper(II) center in $[\text{Cu}(\text{6-Br-Me2-ppmbi})(\text{NO}_3)_2]$ have an N_2O_4 coordination environment with chelating terminal nitrate anion ligands (Fig. S2). As expected, 6-Br-Me2-ppmbi chelates Cu^{2+} site with 6-bromo pyridine and imidazole units. This indicates that the **6-Br-ppmbi** organic motif can generate a one to one complex with divalent metal ions.

Owing to the extremely low affinity of **6-Br-ppmbi** for Fe^{2+} ions, it was feasible to calculate the binding constant directly using Eq. (1), based on fluorescent titration, assuming 1:1 stoichiometry for Fe^{2+} ions and sensor **6-Br-ppmbi** (Fig. 1). The apparent dissociation constant of **6-Br-ppmbi** was found to be $K_d = 1.06 \times 10^2$ by a nonlinear curve fit shown in

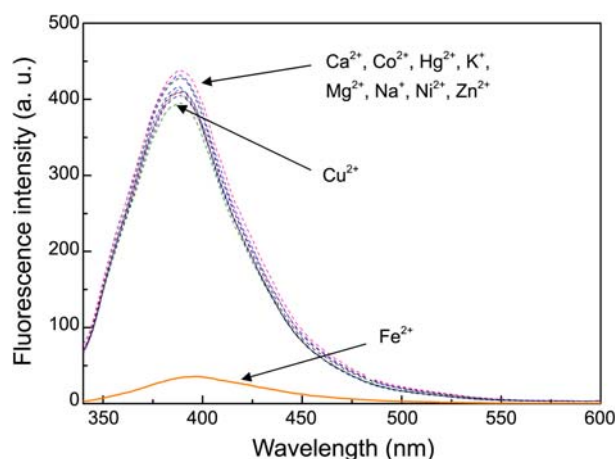


Figure 2. Fluorescent change due to binding of **6-Br-ppmbi** ($5.0 \times 10^{-2} \mu\text{M}$) with Fe^{2+} ion in comparison with other metal cations ($1.6 \times 10^2 \mu\text{M}$), including Ca^{2+} , Co^{2+} , Cu^{2+} , Hg^{2+} , K^+ , Mg^{2+} , Na^+ , Ni^{2+} , and Zn^{2+} ions.

the inset of Figure 1. This K_d value was significantly larger than that of previously reported molecular sensors, possibly due to the steric hindrance rendered by the bulky bromine in the adjacent place of the coordinating nitrogen atom in the pyridine moiety. An extremely small amount of sensor, 0.05 μM of **6-Br-ppmbi** in this case, was sufficient to detect more than 170 μM of Fe^{2+} ions, which make this molecular sensor potentially useful in environmental practice.

In contrast, the addition of **6-Br-ppmbi** to each solution of Ca^{2+} , Co^{2+} , Cu^{2+} , Hg^{2+} , K^+ , Mg^{2+} , Na^+ , Ni^{2+} , and Zn^{2+} ions caused the minor changes in the fluorescent character of **6-Br-ppmbi**, compared to the ca. 93% decrease in fluorescence intensity in the case of the addition of the Fe^{2+} (Fig. 2). These results show the highly selective response of **6-Br-ppmbi** to Fe^{2+} ions compared to other metal ions. There are two possible explanations for this unusual selectivity. One is that only Fe^{2+} could quench the fluorescence of **6-Br-ppmbi**, compared to the other ions despite the similar binding properties of the other cations with the **6-Br-ppmbi** sensor. The other one is that the selective binding affinity of **6-Br-**

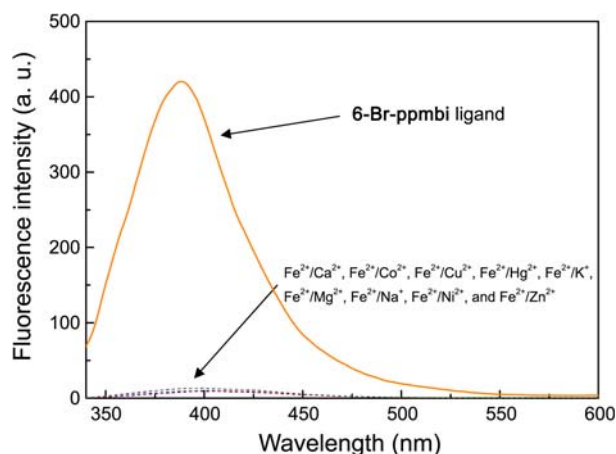


Figure 3. Fluorescence responses of **6-Br-ppmbi** ($5.0 \times 10^{-2} \mu\text{M}$) upon subsequent addition of Fe^{2+} ($3.2 \times 10^2 \mu\text{M}$) to the solution with the selected metal ions ($3.2 \times 10^2 \mu\text{M}$), respectively.

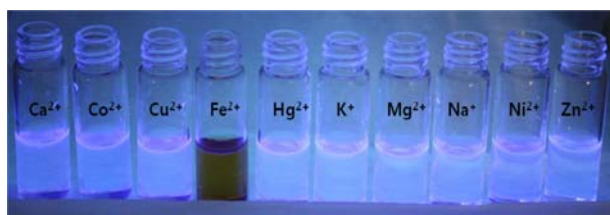


Figure 4. Digital image of the fluorescence response of **6-Br-ppmbi** upon addition of the cations in aqueous solution excited at 364 nm using UV lamp. Left to right: Ca^{2+} , Co^{2+} , Cu^{2+} , Fe^{2+} , Hg^{2+} , K^+ , Mg^{2+} , Na^+ , Ni^{2+} , Zn^{2+} ions.

ppmbi to the Fe^{2+} ion, compared to the much low affinity to the other metal cations, may also explain the observed selectivity.

The fluorescence responses of **6-Br-ppmbi** ($5.0 \times 10^{-2} \mu\text{M}$) upon the subsequent addition of Fe^{2+} ($3.2 \times 10^2 \mu\text{M}$) to solutions with the selected metal ions ($3.2 \times 10^2 \mu\text{M}$) were measured to determine the correct mechanism for the selective fluorescence quenching response to the addition of Fe^{2+} (Fig. 3). Interestingly, the fluorescence was reduced to the same level upon the addition of Fe^{2+} despite the existence of other cations at similar concentrations. This suggests that only Fe^{2+} ion binds to **6-Br-ppmbi** ligand strongly compared to the other cations. If **6-Br-ppmbi** has similar binding affinity to Fe^{2+} and other cations, the level of fluorescence in the case of the above mixed cation experiments must be higher than the one in the case of addition of Fe^{2+} cations only.

Furthermore, the fluorescent color change can be used for the naked-eye detection of Fe^{2+} ions, as shown in Figure 4. The selectivity of **6-Br-ppmbi** for Fe^{2+} over the other cations is a significant advantage compared to the other fluorescent sensors reported thus far.

Conclusions

The syntheses of water-soluble fluorescent benzimidazole molecules, which contain sterically hindered coordination moiety, are described. The fluorescence of the molecule was quenched when added to a solution of Fe^{2+} ions. The synthesized **6-Br-ppmbi** bound selectively to Fe^{2+} ion, reducing the luminescent character of its own, which makes the sensor molecule highly valuable in probing the Fe^{2+} concentration. In addition, the naked-eye detection of Fe^{2+} ions over other divalent cations and the requirement of low concentrations of sensor molecules to detect certain amounts of Fe^{2+} ions make this sensor unique compared to previously reported Fe^{2+} ion sensors.

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Supporting Information Available. Table S1 with the Summary of X-ray Crystallographic Data and Selected inter-atomic distances and angles for the Cu(II) complex. Figure S1, and S2 showing ORTEP drawing of **6-Br-ppmbi** and the Cu(II) complex. CCDC 842612 and 842613 contain the supplementary crystallographic data for the complexes. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk.

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