

# Zn<sup>2+</sup> Recognition by Two New Bipyridine Ligands

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Two new bipyridine ligands with intramolecular charge transfer (ICT) transition as the lowest excited state were designed and synthesized. Upon addition of metal ions, particularly Zn<sup>2+</sup>, the absorption and emission spectra experience great changes. The binding constants towards Zn<sup>2+</sup> are  $1.27 \times 10^5$  L/mol and  $1.66 \times 10^5$  L/mol for ligands 1 and 2, respectively. The strong binding affinity and high selectivity make them attractive for application as fluorescence sensors for Zn<sup>2+</sup>.

**Keywords** bipyridine, fluorescence sensor, binding constant, intramolecular charge transfer

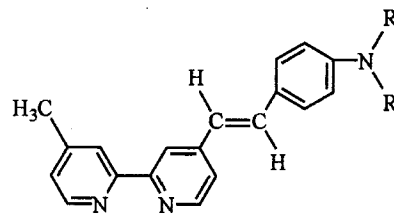
## Introduction

The high sensitivity and selectivity of molecular fluorescence or luminescence have been widely utilized in monitoring H<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, and other important cation or anion ions in biological or environmental systems. Due to the sensitive response of luminescence upon microenvironmental changes (*e.g.* polarity of solvent, pH, the presence of ions), bipyridine complexes of Re(I) and Ru(II) with metal to ligand charge transfer excited states constitute a large family of chemical sensors.<sup>1-5</sup> In these probing molecules, the co-operation of the two moieties with different functions realizes the sensing to microenvironmental changes, in which Ru(II) or Re(I) bipyridine complexes serve as luminescence signaling moiety while ion receptors such as crown ether are attached to one bipyridine ligand for binding desired ions. Considering the strong chelation ability to many metal ions, the bipyridine ligand itself can as well be developed into novel and structurally simpler metal ion probes provided the fluorescence of bipyridine ligand is sensitive to microenvironmental changes.

In this paper, two new bipyridine ligands, ligands 1 and 2 as shown in Scheme 1, are designed and synthesized. The introduction of aminostyryl at 4'-position of 2,2'-bipyridine makes the lowest energy excited states of the resultant ligands to be intramolecular charge transfer (ICT) in nature. As a result, the absorption and fluorescence emission properties of the two ligands depend strongly on the presence of metal ions, particularly zinc ion. The high sensitivity and speciality to Zn<sup>2+</sup> make them promising for application as a potential sensory

system.

Scheme 1



Ligand 1: R = CH<sub>3</sub>; Ligand 2: R = Ph

## Experimental

### Materials

4,4'-Dimethyl-2,2'-bipyridine was obtained from Aldrich. All metal salts used were of reagent grade and in their acetate forms. All solvents were freshly distilled prior to use.

Ligands 1 and 2 were synthesized by the condensation of 4,4'-dimethyl-2,2'-bipyridine with *p*-dimethylaminobenzaldehyde or *p*-diphenylaminobenzaldehyde in the presence of benzoic anhydride. The final product was purified on a silica column [eluent: chloroform:petroleum ether (1:1, V:V)]. <sup>1</sup>H NMR of ligand 1 (*d*<sub>6</sub>-acetone, 300 MHz) δ: 2.44 (s, 3H), 3.00 (s, 6H), 6.77 (d, *J* = 8.5 Hz, 2H), 7.07 (d, *J* = 16.3 Hz, 1H), 7.24 (s, 1H), 7.47—7.52 (m, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 8.31 (s, 1H), 8.54 (m, 3H). <sup>1</sup>H NMR of ligand 2 (CDCl<sub>3</sub>, 300 MHz) δ: 2.46 (s, 3H), 6.98—7.15 (m, 10H), 7.25—7.56 (m, 7H), 8.08 (s, 1H), 8.24 (d, *J* = 8.1 Hz, 1H), 8.48—8.62 (m, 3H).

### Analytical measurements

<sup>1</sup>H NMR spectra were taken on a Varian DPX-300 (300 MHz) FT-NMR spectrometer. UV-vis absorption spectra were recorded with SHIMADZU UV-1601PC spectrophotometer. Steady-state fluorescence emission spectra were obtained on a

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Hitachi F-4500 fluorescence spectrophotometer.

### Stoichiometry determination of complexes

The mole ratio method was used to determine the stoichiometric relationship of  $Zn^{2+}$  with ligands **1** and **2**.<sup>6</sup> The total ligand concentration is kept constant and the concentration of zinc ion is varied. The absorbance at the wavelength where the absorption changes most significantly is recorded against the concentration ratio of zinc ion to ligand. The discontinuities or abrupt changes of slope correspond to the stoichiometric ratio.

### Determination of binding constants

The binding constants of ligands **1** and **2** to zinc ion were measured via fluorescence intensity changes. The fluorescence intensity of the solution of zinc ion and ligand **1** or ligand **2** follows Eq. (1) if 1:1 complex of zinc ion and ligand is formed and the total concentration of ligand is held constant.<sup>6</sup>

$$I/I_0 = \{1 + (k_b/k_f) K_b [M]\} / (1 + K_b [M]) \quad (1)$$

Where  $I$  and  $I_0$  are the fluorescence intensity of the solution in the presence and absence of zinc ions, respectively.  $k_b$  and  $k_f$  represent proportionality constants connecting the intensities and concentrations of the complex formed and free ligand, respectively.  $K_b$  is the binding constant and  $[M]$  the total concentration of zinc ion added.

Eq. (1) can be transformed into Eq. (2)

$$[M] / (I/I_0 - 1) = [M] / (k_b/k_f - 1) + [1/K_b(k_b/k_f - 1)] \quad (2)$$

Then  $K_b$  can be obtained from the slope and  $y$ -intercept of the plot in terms of Eq. (2).

## Results and discussion

### The absorption and fluorescence properties of ligands **1** and **2**

Table 1 shows the lowest energy absorption maximum,

the emission maximum and the relative fluorescence quantum yield of the two ligands in different solvents. The solvents have small effect on the absorption maximum. But the fluorescence maxima undergo very large red shifts when the solvent is changed from the non-polar dioxane to the polar ethanol. The large Stokes' shifts and the significant solvatochromism effects on fluorescence emission suggest that the lowest energy excited states for ligands **1** and **2** are ICT states. Moreover, the remarkable decrease of relative fluorescence quantum yield in polar solvents is also a characteristic of ICT state.

It has been reported that the introduction of electron-donating group [*e. g.*  $-NH_2$  or  $-N(CH_3)_2$ ] in the phenyl ring of 4'-phenyl-2,2':6',2''-terpyridine raises the  $\pi_{ph}$  energy level to such an extent that  $\pi_{ph}$  becomes the HOMO.<sup>7</sup> As a result, the lowest energy absorption of the substituted terpyridine ligand is ICT transition ( $\pi_{ph} \rightarrow \pi_{tpy}^*$ ), and a large red shift of the fluorescence was observed upon polarity changes of solvents. It is believed that the similar case is occurring in ligands **1** and **2**. It is because the existence of ICT transitions that the fluorescence emission of ligands **1** and **2** depends so strongly on the microenvironmental changes such as the changes of solvent polarity, which provides the basis for their use as fluorescence sensors.

### The probing of $Zn^{2+}$ by ligands **1** and **2**

The absorption and fluorescence emission spectra of the two ligands exhibit a profound dependence on zinc ions. By adding zinc ions, the ethanol solution of ligand **1** changed from nearly colorless to yellow, the absorption band at 380 nm weakens gradually and at the same time, a new absorption band with maximum at 429 nm appears [Fig. 1 (a)]. The changes in UV-vis absorption upon addition of  $Zn^{2+}$  can be attributed to the chelation of bipyridine ligand with zinc ions. In the coordinated complex, each N atom of the bipyridine ligand donates its lone pair electrons to a vacant orbital of zinc ion to form a coordinated bond. The  $Zn^{2+}$  exists in the complex as an electron pair-acceptor and enhances the electron withdrawing ability of the bipyridine moiety of ligand **1**. Consequently, the chelation lowers the  $\pi_{tpy}^*$  energy level and stabilizes the ICT state of ligand **1**. A red shift of the absorption band or a new absorption band is thus observed.

Table 1 Photophysical properties of ligand **1** and **2** in solvents of varying polarity

		Dioxane	THF	CHCl <sub>3</sub>	Ethanol
Ligand <b>1</b>	$\lambda_{max}$ (nm, absorbance)	375	377	381	380
	$\lambda_{max}$ (nm, emission)	467	485	490	521
	$\phi_{relative}$	0.87	1.0	0.60	0.44
Ligand <b>2</b>	$\lambda_{max}$ (nm, absorbance)	383	384	391	388
	$\lambda_{max}$ (nm, emission)	471	495	501	519
	$\phi_{relative}$	0.92	1.0	0.90	0.54

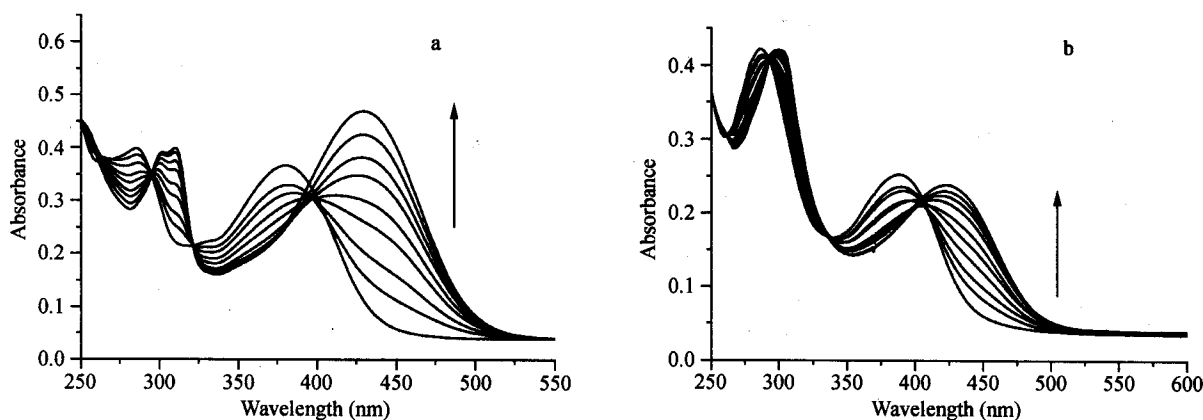


Fig. 1 Changes in the UV-vis absorption spectra of ligand 1 ( $2.05 \times 10^{-5}$  mol/L, a) and ligand 2 ( $1.25 \times 10^{-5}$  mol/L, b) upon addition of  $Zn^{2+}$  (0— $1.2 \times 10^{-4}$  mol/L for ligand 1 and 0— $5 \times 10^{-5}$  mol/L for ligand 2).

During the addition of  $Zn^{2+}$  a set of isosbestic points at 294 nm and 322 nm respectively was observed, indicating only one kind of complex is formed. It is noted that there exists a region from 385 to 405 nm where spectra intersect but isosbestic points were not maintained over the range of  $Zn^{2+}$  concentration studied. The absence of an isosbestic point in this region can be attributed to spectral dependence on the changes in solution composition caused by adding  $Zn^{2+}$ . In a separate experiment, by adding 0.1 mol/L tetra-*n*-butylammonium tetrafluoroborate as supporting electrolyte to keep the ionic strength of the solution unchanged, an isosbestic point at 402 nm was observed upon addition of  $Zn^{2+}$ . The spectral solvent dependence found only in this region is in good agreement with the assignment of ICT transition to the lowest energy absorption band. The ICT state is expected to be sensitive to the ionic strength as well as the polarity of solution.

The mole ratio method was applied to determine the stoichiometry of the complex formed between  $Zn^{2+}$  and ligand 1. Fig. 2 gives the absorbance changes at 429 nm of ligand 1 in ethanol solution when the concentration of ligand 1 was kept constant and the concentration of  $Zn^{2+}$  was increased. The stoichiometric ratio of 1:1 can be inferred from it.

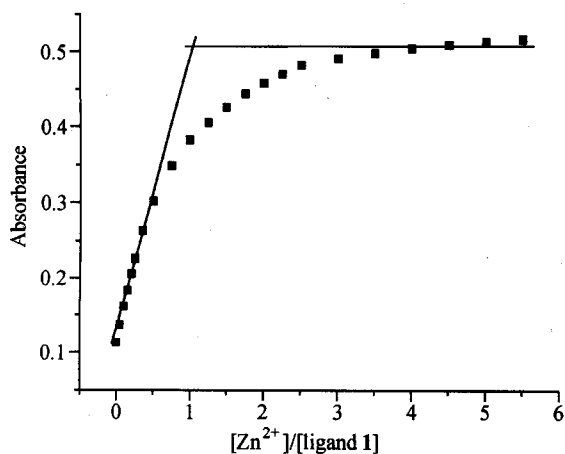


Fig. 2 Absorbance changes at 429 nm of ligand 1 upon addition of  $Zn^{2+}$ .

Ligand 2 also formed 1:1 complex with  $Zn^{2+}$  and the UV-vis absorption spectra changes upon formation of the complex is very similar to the case for ligand 1 [Fig. 1 (b)].  $Zn^{2+}$  possesses an electronic structure of  $3d^{10}$  and can chelate with three bipyridine ligands in principle, but in our experimental condition, only 1:1 complex can be observed, the residual chelation position of  $Zn^{2+}$  might be coordinated by ethanol molecules.

When excited at 380 nm, the fluorescence emission intensity of ligand 1 at 522 nm decreased with increasing the concentration of  $Zn^{2+}$  [Fig. 3 (a)]. The fluorescence

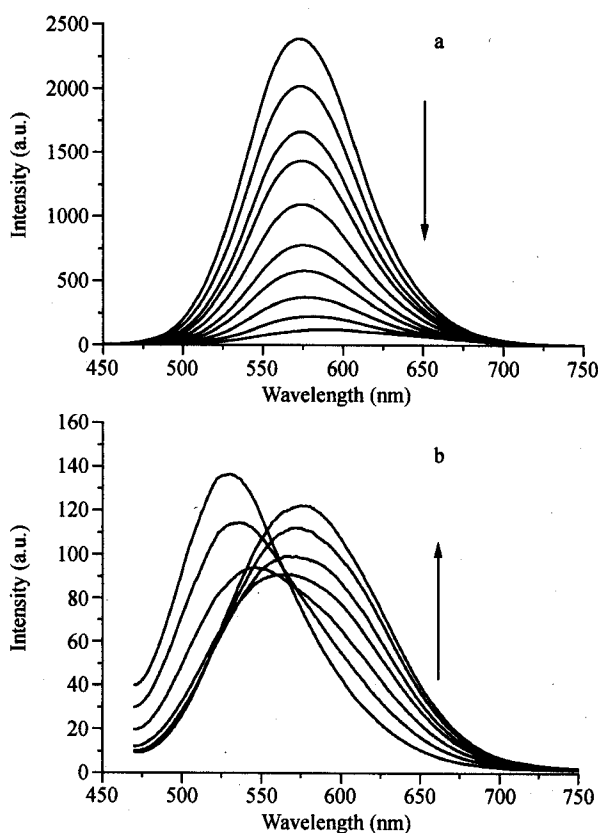


Fig. 3 Changes in the emission intensity of ligand 1 upon addition of  $Zn^{2+}$  ( $\lambda_{exc} = 380$  nm for a and 450 nm for b).

quenching could be due to the decrease of the free ligand concentration. When excited at 450 nm at which free ligands have only a small absorptivity but the absorption of  $\text{Zn}^{2+}$  complexes formed is strong enough, the emission intensity at 522 nm decreased gradually, but the emission intensity at longer wavelengths increased and at last a new emission band with maximum at 575 nm formed [Fig. 3 (b)]. This new emission band can be ascribed to the 1:1 complex of  $\text{Zn}^{2+}$  and ligand 1. This weak emission band was masked by the strong emission from the residual free ligand 1 when excitation wavelength was set at 380 nm. In practical applications of fluorescence sensors, in order to cancel out variations in intensity arising from background changes in the analytical environment, dual excitation wavelength or two emission bands monitoring is highly desirable. In this regard, the appearance of two emission bands when excited at different wavelength makes ligand 1 attractive as potential fluorescence sensor.

The fluorescence quenching data in Fig. 3 (a) were utilized to measure the binding constant of ligand 1 to  $\text{Zn}^{2+}$  according to Eq. (2). From the experimental data of  $[\text{Zn}^{2+}]/(I/I_0 - 1)$  vs.  $[\text{Zn}^{2+}]$  and their fittings to Eq. (2) (Fig. 4), a binding constant of  $1.27 \times 10^5$  L/mol was obtained. Furthermore, the very good fitting of the experimental data vindicates the 1:1 stoichiometry determined by the mole ratio approach because Eq. (2) holds only in the case where 1:1 complex is formed.

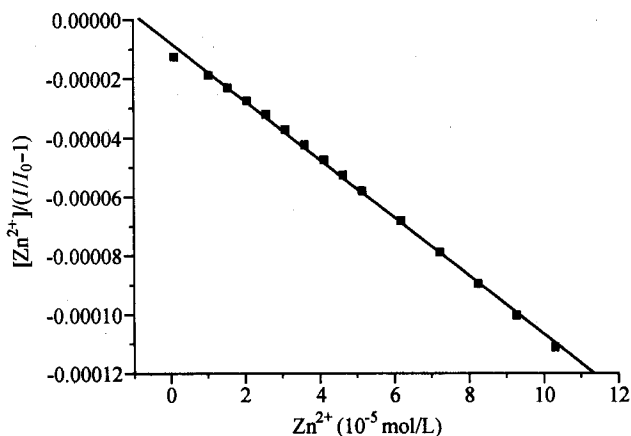


Fig. 4 The binding isotherm of ligand 1 by fitting the emission intensity excited at 380 nm to Eq. (2).

For ligand 2, the addition of  $\text{Zn}^{2+}$  caused also significant changes to the steady-state fluorescence emission as happened on ligand 1 [Fig. 5 (a) and (b)]. When excited at 390 nm, the emission intensity at 522 nm decreased gradually with the addition of  $\text{Zn}^{2+}$ . A new emission band with maximum at 567 nm appeared when excited at 450 nm. The binding constant of ligand 2 with  $\text{Zn}^{2+}$  is  $1.66 \times 10^5$  L/mol by fitting the emission data excited at 390 nm to Eq. (2).

#### The binding selectivity of ligands 1 and 2 on metal ions

The recognition to  $\text{Zn}^{2+}$  of the two ligands is unique.

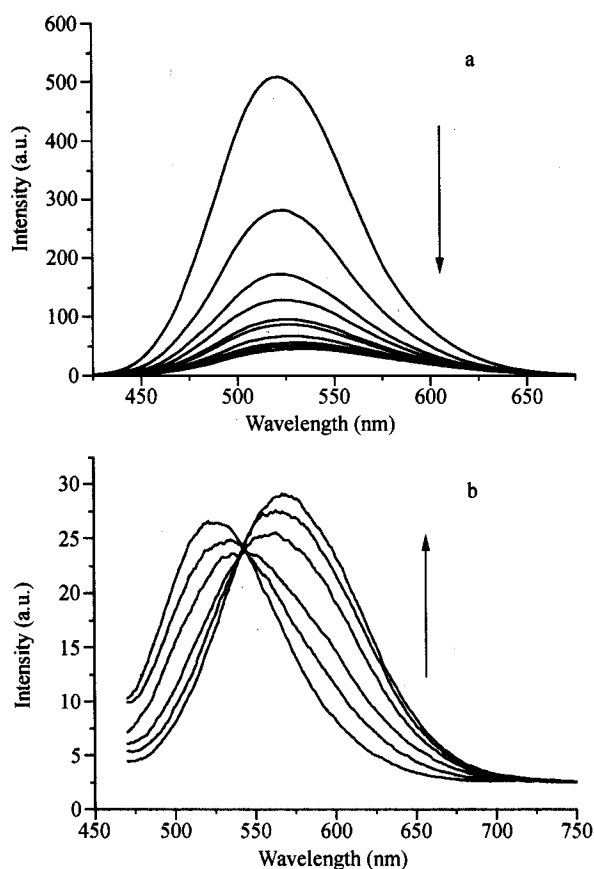


Fig. 5 Changes in the emission intensity of ligand 2 upon addition of  $\text{Zn}^{2+}$  in ethanol solutions ( $\lambda_{\text{exc}} = 390$  nm for a and 450 nm for b).

Among the metal ions investigated, the addition of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (competing ions when  $\text{Zn}^{2+}$  sensors are used in physiological studies) has no effects on the absorption and emission spectra of the two ligands perhaps due to its low affinity for bipyridine. Some transition and heavy metal ions such as  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  can cause similar changes on the absorption spectra as that of  $\text{Zn}^{2+}$ , but only quenching of the ligand emission is observed and there is no new emission band appeared. The possible reason for no observation of new emission band is that the complexes formed are nonfluorescent.<sup>8</sup>  $\text{Hg}^{2+}$ , just like  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , has no effects on the photophysical properties of the two ligands.

Fluorophores with ICT states have been considered previously for metal ion sensing by appending a cation ion receptor (usually crown ethers) onto a terminal of the fluorophores. In ligands 1 and 2, the good chelating property of bipyridine moiety to metal ions avoids the complication of introduce crown ethers and such simple structures still exhibit high sensitivity and selectivity to  $\text{Zn}^{2+}$ .

## Conclusion

The response to  $\text{Zn}^{2+}$  of the two new bipyridine-based ligands is unique among a series of metal ions studied. The absorption and fluorescence emission spectra of ligands 1 and 2 experience great changes upon addition of  $\text{Zn}^{2+}$ . The

strong binding ability and high selectivity make them attractive for application as fluorescence sensors for  $Zn^{2+}$ .

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