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Solvent effect and fluorescence response of the 7-*tert*-butylpyrene-dipicolylamine linkage for the selective and sensitive response toward Zn(II) and Cd(II) ions†

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The different binding behaviour of 7-*tert*-butylpyrene based chemosensors bearing dipicolylamine (Dpa) linkages at the 1,3-positions was investigated in various solvents for the sensing of Zn(II) and Cd(II).The potential mono-chelating ligand **L1** follows the same binding pattern in both THF and methanol-water solvent systems, exhibiting high selectivity and sensitivity for Cd(II) than Zn(II) mainly in THF solvent system. The potential bis-chelate ligand **L2** can selectively bind both Zn(II) and Cd(II) in a 1:1 ratio in THF, whereas in methanol-water (7:3) at pH = 7.0; a 1:2 binding ratio was observed. In THF, two sites of ligand **L2** can only selectively and sensitively bind one Zn(II) or Cd(II). The different complexation behaviours of **L1** and **L2** in different solvents were studied by means of fluorescence spectra and ^{1}H-NMR titration experiments in the presence of Zn(II) and Cd(II).

Introduction

The design and synthesis of molecular receptors for the detection of environmentally and biologically important species has attracted growing interest in recent years.[1] Among them, chemosensors whose fluorescence emission is sensitive to the environment and solvent media are especially important.[2–5] Many fluorescence mechanisms have also been reported based on Photoinduced Electron Transfer (PET), Intermolecular Charge Transfer (ICT), Chelation Enhanced Fluorescence (CHEF). Indeed, their application in the field of supramolecular chemistry has been elegantly illustrated.[6] In case of PET,[7] there is little or no change of the spectral shifts with changes of emission intensities, whereas both spectral shifts and intensity changes are observed for ICT[8], CHEF[9] also exhibited fluorescence enrichment with or without accompanying spectral changes.

The Zn^{2+} detection is taken into consideration both in vitro and vivo due to its importance in biological site.[10] It is an indispensable element for the human body and in many physiological and pathological processes, it performs an essential roles.[11] Its deficiency give rise to an acrodermatitis enteropathica, [12] but it is detrimental when present in excess, caused severe health problem such as superficial skin diseases, prostate cancer, diabetes and brain diseases. Unfortunately spectroscopically silent, Zn^{2+} is difficult to detect directly.[13] On the contrary, a trace amount of Cd^{2+} is highly toxic for human body. It's intake causes serious diseases such as renal dysfunction, calcium metabolism disorders, and prostate cancer.[14] Consequently, research efforts on Zn(II) based complexes have been extensively investigated, especially promising luminescent properties of benzimidazole, quinoline based Zn(II) compounds suggest that nitrogen based heterocycles are worthy of consideration. Moreover, using different solvents (DMSO, methanol, THF and DCM) in elucidating the photoluminesent and fluorescent properties provide a significant breakthrough for the characterization of zinc complexes.[15]

It is known that fluorescence quenching sometimes creates an unfavourable condition for a high signal output upon recognition of ions and also interferes with temporal separation of spectrally similar complexes with time-resolved fluorometry.[16] Thus, our main focus is to design a chemosensor that does not quench the fluorescence upon binding with a metal ion. In this regard, the PET which is responsible for fluorescence quenching is minimized in the signaling moiety upon binding and results in the enhancement of the fluorescence. Recently, some researchers utilized this most efficient and simple ligand system to develop fluorescent sensor. Ojida et al. synthesized anthracene binuclear Zn(II)-Dpa complex as an anion sensor for phosphorylated peptides.[17] On the other hand, Yoon et al. designed napthalimide-Dpa fluorescent probe based on PET and ICT that can differentiate Zn^{2+} and Cd^{2+} through green and blue fluorescence, respectively. They also reported a Dpa linkage fluorescent sensor selective for PPi based on excimer emission.[18] However, a large number of fluorescein or benzoresorufin based fluorescent sensors were also functionalized with Dpa or Dpa analague metal binding moieties which display a conserved [N_3O] binding motif to detect labile Zn in cells and neuronal tissue.[19]

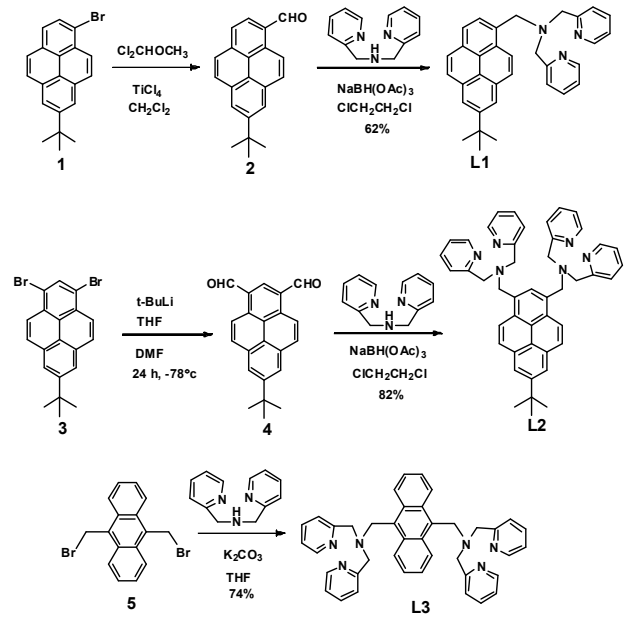
Recently, pyrene has been utilized widely as a fluorophore to detect ion pairs, cations, anions and neutral species, because of the

photoluminescence properties and chemical stabilities associated with pyrene.[20] Given this, we have developed chemosensors that contain a 7-*tert*-butylpyrene as a fluorophore moiety and dipicolylamine as a receptor moiety connected through a C–N bond. In our present work, we have established the ligands as efficient cation sensors which reveal different behaviour in different solvent systems.

The purpose of this work is to shed light on the mechanism of the different fluorescence response of receptor **L2** with Zn^{2+} and Cd^{2+} in various solvent systems. Interestingly, a 1:1 ligand to metal binding ratio was observed in case of THF for both Zn^{2+} and Cd^{2+} ions, whereas when using a methanol-water solvent system, it can selectively interact with $Cd^{2+}$and Zn^{2+} ions in a 1:2 (ligand/metal) stoichiometry. In case of methanol-water, **L2** exhibits a significant fluorescence enhancement for Zn^{2+}, which is twice that observed for the THF solvent system. However, the potentially mononuclear receptor **L1** is highly selective in coordinating with Zn(II) and Cd(II).

Results and discussions

We have designed and successfully synthesized **L1** and **L2** using the reaction pathway shown in scheme 1. The fluorogenic molecule **L2** is synthesized from 7-*tert*-butylpyrene-1,3-dicarbaldehyde by treatment with 2,2′-dipicolylamine, following which, the Schiff base is reduced by the gradual addition of $NaBH(OAc)_3$ to obtain **L2** in 82% yield. Following the same reaction pathway, the potentially mono-chelate **L1** has also been prepared from 7-*tert*-butylpyrene-1-carbaldehyde in order to compare the binding affinities for Zn^{2+} and Cd^{2+} in different solvent systems. The characterization of these compounds was confirmed by ^1H and ^{13}C NMR spectroscopy and by High-Mass spectrometry. In the absence of Zn^{2+} and Cd^{2+} ion, both



Scheme 1: Synthesis of receptors **L1, L2** and **L3.**

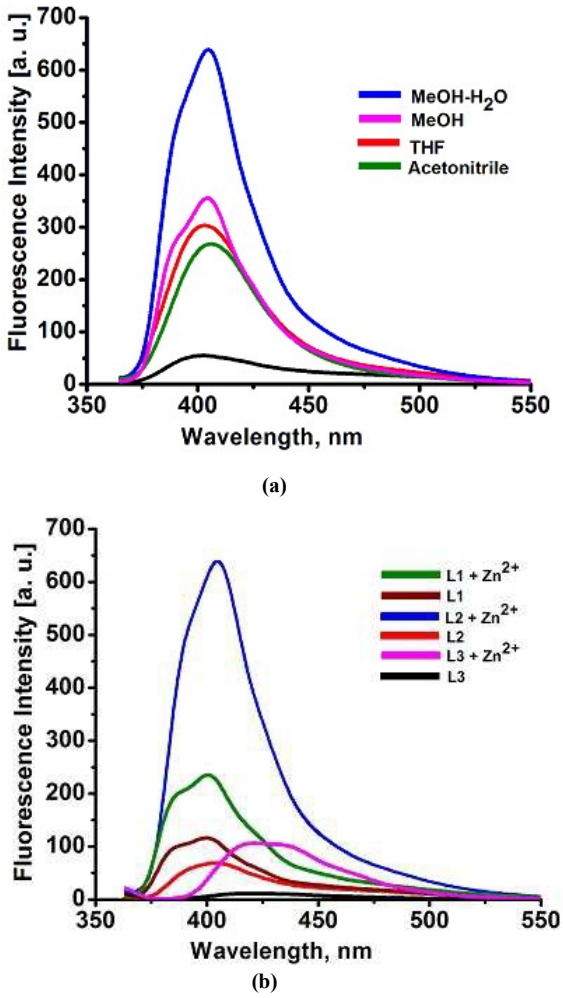


Fig. 1 (a) Fluorescence response of ligand **L2** (7 μM) upon addition of Zn^{2+} in different solvent systems with excitation at 353 nm. (b) Fluorescence spectra of **L1, L2** and **L3** in CH_3OH/H_2O (10 mM HEPES/CH_3OH = 3:7, pH = 7.0) with excitation at 347 and 353 nm respectively.

L1 and **L2** only afford weak fluorescence because of PET; the lone pair electrons from the amino group are transferred to the excited pyrenyl moiety and are presumed to quench the emission intensity of the pyrenyl fluorophore**.** After addition of $Zn^{2+}$and Cd^{2+} at small concentrations, preferential binding with dipicolylamine occurs to terminate the PET. In this way, the 7-*tert*-butylpyrene binuclear-Dpa complex exhibits a significant fluorescent enhancement for Zn^{2+} and can detect both Zn^{2+} and Cd^{2+} ions upon changing the solvent system. Addition of Zn^{2+} and Cd^{2+} ions using THF as solvent reveals a fluorescence at 402 nm. On the other hand, ligand **L2** can only detect Zn^{2+} ion with almost twice the fluorescence enhancement on changing the solvent media, *ie* methanol-water instead of THF.

As the spectroscopic properties indicate the significant influences of the solvents on the wavelength and the changes of the emission intensities,[15] the fluorescence properties of the receptor **L2** were at first investigated in different solvents (Fig. 1a) following addition of Zn^{2+}. **L2** itself exhibits very weak fluorescence. It was then found that a large fluorescence enhancement (8-fold) was observed upon addition of Zn^{2+} in methanol-water solvent.

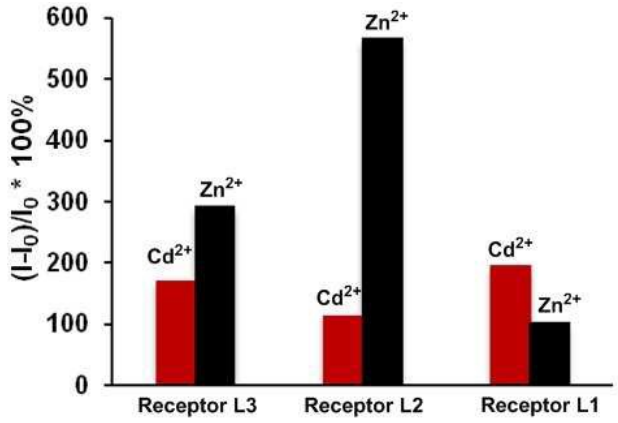


Fig. 2 Fluorescence intensity changes of receptor **L1**, **L2** and **L3** (7 μM) in CH_3OH/H_2O (10 mM HEPES/CH_3OH = 3:7, pH = 7.0) at 298K. *I* is the fluorescence intensity after addition of Zn^{2+} and Cd^{2+} (100 μM) and I_0 is fluorescence intensity for free receptor.

By contrast, in methanol, THF or acetonitrile, the fluorescence intensity increase was monitored for similar ratios and was found to be almost half that observed in methanol/water. This suggests that the addition of water has a great impact on the fluorescence enhancement in the methanol-water system.

The changes of the fluorescence emission spectrum of **L2** with Zn^{2+} using different ratios of methanol-water was also monitored, which suggested that a 7:3 ratio methanol-water solvent system was the ideal solvent media for the present work. In other words, either increasing or decreasing the amount of water present, the emission intensity decreased (Figure SI 23). The same solvent system was also used in combination with [(2,2′-dipicolylamino)]butyl]pyrene as the Zn^{2+} receptor, and pyrophosphate works as the bridging substrate for the excimer formation.[21]

However, the pH dependent changes in fluorescence spectra of L2 were also measured over the pH range 3–10. At the lower pH, two binding sites become gradually protonated and the fluorescence spectra give a strong intensity although at a very weak level (Figure SI 35).[22] It is predicted that protonation with both binding sites cannot stop PET from amino groups to pyrenyl moiety. On the other hand, at higher pH value, fluorescence intensity is gradually decreased which indicates the deprotonation states of amino moiety. In this case PET is occurred predominantly.[22] However, in presence of Zn^{2+} (2 equiv.), fluorescence intensity is significantly enhanced with increasing the pH value (Figure SI 36). This result indicates, at pH<6 the amino group is still protonated, but at pH>6, two binding sites are co-ordinated with Zn^{2+} and fluorescence intensity is sharply enhanced.[19a, 22] These data suggest that at higher pH value in MeOH/H_2O, DPA is ruled out from protonation state and by using a combination of spectrophotometric and pH titration methods, the protonated and zinc bound species can easily be identified in aqueous solution. On the basis of above findings, to determine the optimal ratio of metal-ligand complexes, titration experiment was performed in an aqueous buffer at pH 7.0.

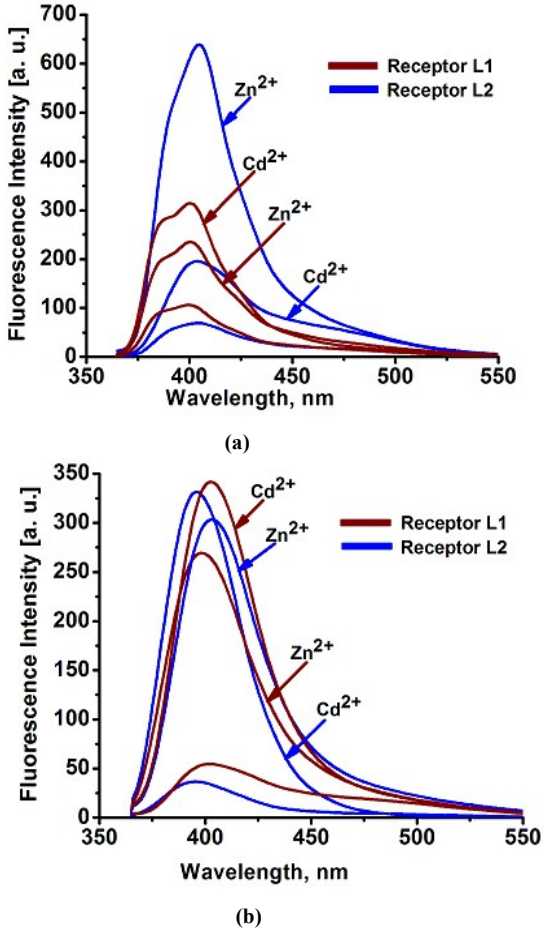


Fig. 3 Fluorescence response of ligand **L1** and **L2** (7.0 μM) in (a) CH_3OH/H_2O (10 mM HEPES/CH_3OH = 3:7, pH = 7.0) (b) THF solvent at 298 K after addition of Zn^{2+} and Cd^{2+} ion with excitation at 347 nm and 353 nm respectively.

The receptor, 9,10-bis[(2,2′-dipicolylamino)methyl]anthracence, **L3** was also synthesized from 1,8-bis(bromomethyl)anthracene in order to compare the sensitivity of **L1** and **L2** toward Zn^{2+} and Cd^{2+}.

Table 1. Association constants[a] of receptors **L1** and **L2.**

Compound	Solvent	Binding model	K_a (M^{-1})	Equilibria
L2⊃Zn^{2+}	MeOH-H_2O	1:2	3.3×10^4	L + Zn^{2+} = LZn LZn + Zn^{2+} = LZn_2
L2⊃ Zn^{2+}	THF	1:1	6.6×10^4	L + Zn^{2+} = LZn
L1⊃ Zn^{2+}	THF	1:1	5.0×10^5	L + Zn^{2+} = LZn
L2⊃ Cd^{2+}	THF	1:1	5.0×10^5	L + Cd^{2+} = LCd

[a]Measured at 27 °C by fluorescence titration experiments[25] (Figure SI. 31–34); host concentration was 7 μM.

As indicated in Fig. 1b, like **L2**, neither **L3** nor **L1** exhibit a distinct fluorescence emission after addition of Zn^{2+} (10 equiv) in methanol-water (10 mM HEPES/MeOH = 3:7, pH = 7.0). These observations suggest that in methanol-water, the ligand **L2** was highly sensitive toward the Zn^{2+} ion. Fig. 2 shows the selective fluorescence enhancement after addition of Zn^{2+} and Cd^{2+} ion. As shown in Fig. 2, receptor **L1** was more selective for Cd^{2+} ion than Zn^{2+} unlike receptors **L2** and **L3**. Fig. 3a reveals that the fluorescence emission intensity of **L2** become approximately 7 times greater than that of **L1** upon addition of 10 equiv. of Zn^{2+} and that ligand **L1** exhibits greater fluorescence enhancement than does **L2** in the presence of Cd^{2+} (10 equiv.) in methanol-water. Fig. 3b shows that in THF the ligands **L1** and **L2** exhibit similar fluorescence enhancement in the presence of Cd^{2+} and Zn^{2+} ion.

To verify the fluorescence intensity changes in different solvents, fluorescence titration experiments and job's plot were carried out. Figure 4 illustrates a gradual enhancement of fluorescence upon addition of Zn^{2+} in **L2** (7 μM) was observed at 406 nm when excited at 353 nm. The change was almost terminated after addition of 2 equiv. of Zn^{2+}, which suggested a 1:2 stoichiometry for the ligand-metal complex. This was again confirmed by the Job's plot analysis. The fluorescence intensity exhibited a maximum at the mole fraction 0.65, suggestive of 1:2 complexation. The association

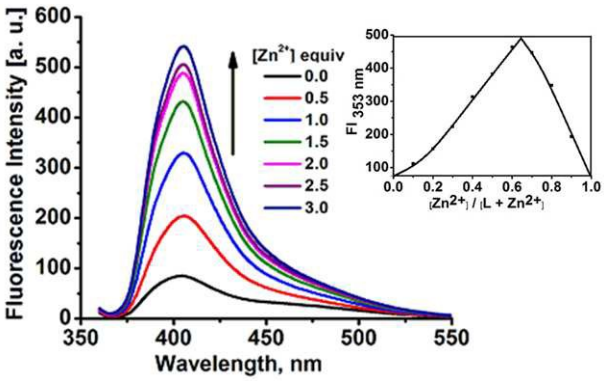


Fig. 4. Fluorescence response of ligand **L2** (7 μM) upon addition of Zn^{2+} in CH_3OH/H_2O (10 mM HEPES/CH_3OH = 3:7, pH = 7.0) at 298 K with excitation at 353 nm.

constant for the complexation of **L2** with Zn^{2+} was determined to be 3.3×10^4 M^{-1} (Fig SI 31). Figure 5a shows the fluorescence titrations of Zn^{2+} with **L1** in THF. Stepwise addition of Zn^{2+} led to an increase of the fluorescence intensity until the complete addition of 1 equiv. of Zn^{2+}. To confirm the binding sites of the sensor, the stoichiometries of L1 with Zn^{2+} were calculated using the job's plot

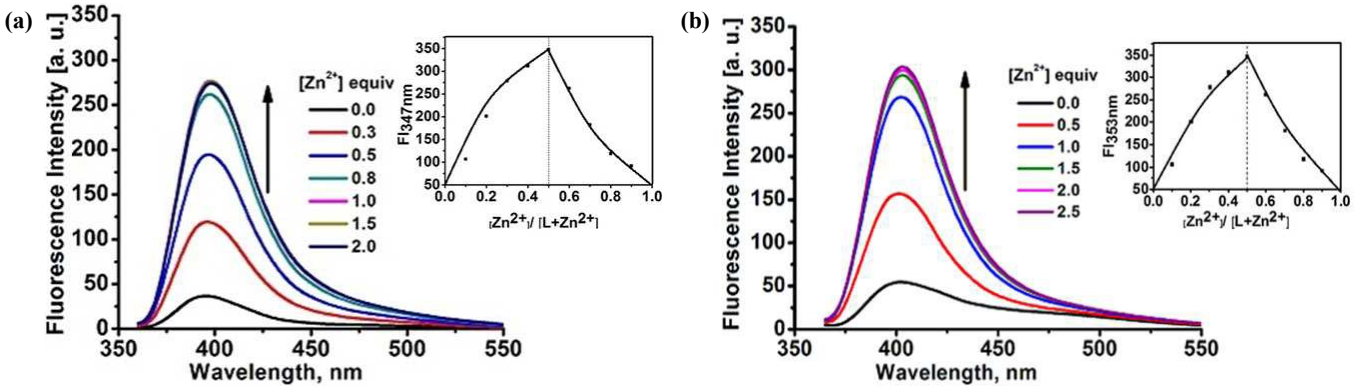


Fig. 5 Fluorescence response of (a) ligand **L1** (7 μM) (b) ligand **L2** (7 μM) after addition with Zn^{2+} in THF at 298 K. The excitation was performed at 347 nm for **L1** and 353 nm for **L2**.

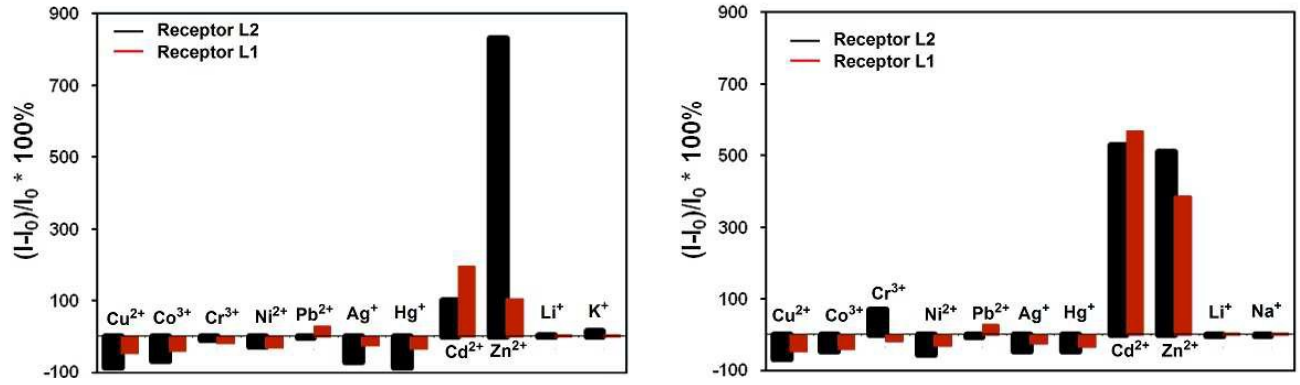


Fig. 6 Fluorescence intensity changes of ligand **L1** and **L2** (7 μM) in (a) CH_3OH/H_2O (10 mM HEPES/CH_3OH = 3:7, pH = 7.0) and (b) THF solvent at 298 K after addition of various metal ions (100 μM). *I* is the fluorescence intensity after addition of metal ions and I_0 is fluorescence intensity for free receptor.

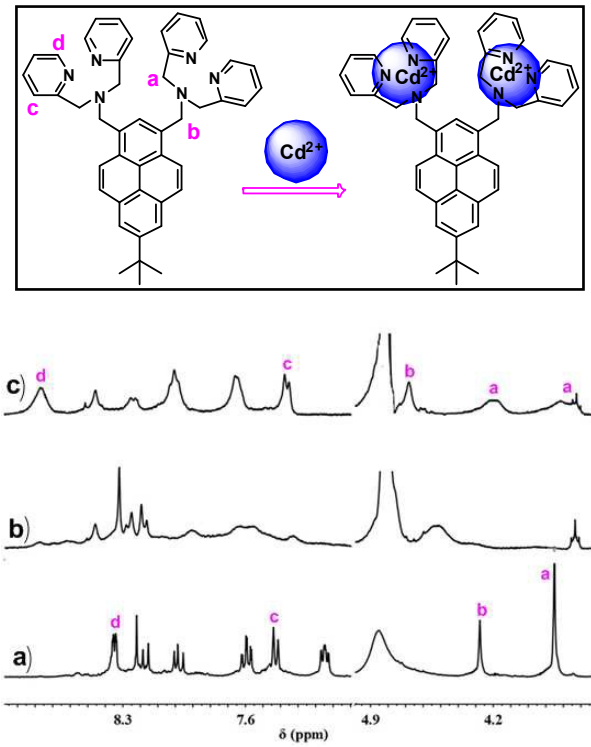


Fig. 7 Partial ^{1}H-NMR titration of **L2**/guest (H/G = 1:2); (a) Free ligand **L2** (1.5×10^{-2} M); (b) **L2**⊃Cd^{2+} (1 equiv.); (c) **L2**⊃Cd^{2+} (2 equiv.). Solvent: CD_3OD–D_2O (9:1, v/v, pD = 7.0). 300 MHz at 298 K.

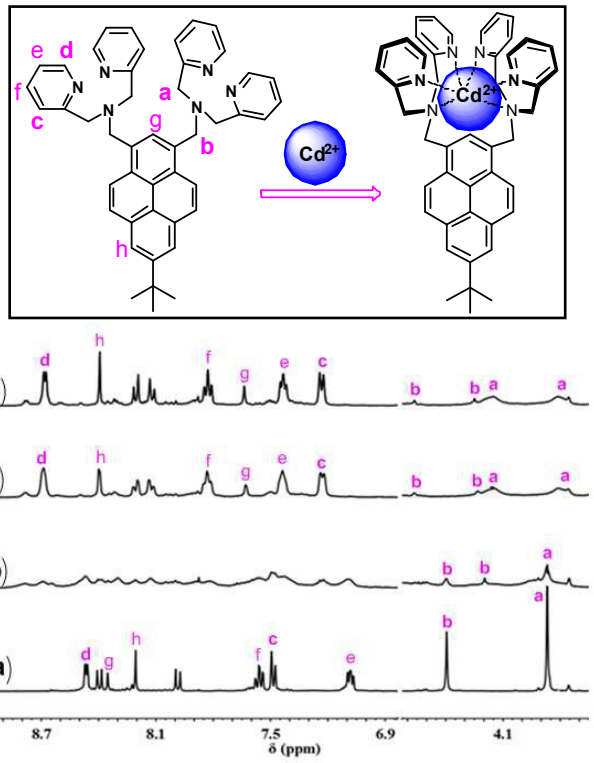


Fig. 8 Partial ^{1}H-NMR titration of **L2**/guest (H/G = 1:1); (a) Free ligand **L2** (0.5×10^{-3} M); (b) **L2**⊃Cd^{2+} (0.5 equiv.); (c) **L2**⊃Cd^{2+} (1 equiv); (d) **L2**⊃(Cd^{2+} (2 equiv.). Solvent: THF-d_8. 400 MHz at 298 K.

for which there was a maximum at 0.5 mole fraction, indicative of a 1:1 stoichiometry. Figure 5b presents the change of the fluorescence spectra of **L2** upon addition of Zn^{2+} in THF. After addition of 1 equiv. of Zn^{2+}, no obvious change occurred, which signified the 1:1 stoichiometry between **L2** and Zn^{2+}. These results indicated that ligands **L2** and **L1** exhibit similar behavior and binding toward Zn^{2+} and Cd^{2+} ions in THF. The US Environmental Protection Agency (EPA) set the maximum contaminant levels of Zn^{2+} and Cd^{2+} in drinking water at 7.6×10^{-8} and 4.5×10^{-8} M respectively.[22] Given this, the receptors **L1** and **L2** can be considered to be highly selective for the detection of $Zn^{2+}$and Cd^{2+} (Table 1). Figure 6a shows the selectivity among various metal ions. Probe **L2** exhibited high selectivity toward Zn^{2+} over (Cu^{2+}, Pb^{2+}, Ag^{+}, Hg^{+}, K^{+}, Li^{+} (as their perchlorate salts) and Co^{3+}, Cr^{3+}, Ni^{2+} including Cd^{2+} (as nitrate salts). Therefore, the affinity of **L1** was observed with each of the respective metal cations and the results implied that **L1** can selectively detect both Cd^{2+} and Zn^{2+} ions, but with a slightly higher affinity for Cd^{2+} *versus* Zn^{2+}. Figure 6b reveals that **L1** and **L2** were more sensitive toward Cd^{2+} than Zn^{2+} when using THF as solvent. By contrast, the addition of other cations (Cu^{2+}, Pb^{2+}, Ag^{+}, Hg^{+}, K^{+}, Li^{+}, Co^{3+}, Cr^{3+}, Ni^{2+}, Na^{+}, Li^{+}) showed almost no fluorescence enhancement. These results indicated that **L1** and **L2** exhibit selective emission enhancement toward Zn^{2+} and Cd^{2+} both in THF and methanol-water solvents.

On the other hand, observations for the fluorescence emissions for the **L2** (7 μM) and Zn^{2+} (100 μM) system, indicated that most of the competitive cations such as Pb^{2+}, Ag^{+}, Hg^{+}, K^{+}, Li^{+}, Co^{3+}, Cr^{3+}, Ni^{2+} Cd^{2+} caused no obvious change at higher concentration (100 μM) (figure SI 22.). However, Cu^{2+}, Ag^{+}, Hg^{+} all strongly quenched the fluorescence in the **L2**+Zn^{2+} system. These results suggested that the co-ordination of Zn^{2+} with **L2** is more selective than other metal ions, with the exception of Cu^{2+}, Ag^{+} and Hg^{+}.[21, 22]

The ^{1}H NMR spectroscopic analysis of **L2** provided further evidence of the 1:1 and 1:2 binding mode in THF and methanol-water. In methanol-water, receptor **L2** is not fully soluble in the 3:7 mixture of D_2O/CD_3OD. Therefore, a 1:9 ratio of D_2O/CD_3OD (pD = 7.0) was applied for these analysis. It is assumed that at neutral condition, there is little or no interaction occurred by protons of water with L2 in NMR titrations experiment. But at lower pH values, protons of water have interactions with two binding moieties of **L2** and at higher pH, amine N are deprotonated gradually.[19a, 22]

The ^{1}H NMR signals in methanol-water reveal the aromatic and methylene regions of **L2** (Fig. 7 and Figure SI 28). After addition of 2 equiv. of Cd^{2+} and Zn^{2+}, the proton signals of **L2**, when in the

Table 2. Chemical shift of dipicolylamine and methylene protons of free **L2** and **L2** with Zn^{2+} and Cd^{2+}.[a]

	Chemical Shift, δ_{ppm} in MeOH-H_2O (H/G = 1:2)					Chemical Shift, δ_{ppm} in THF (H/G = 1:1)		
	Free **L2**	**L2**⊃Cd^{2+}	Δδ	**L2**⊃Zn^{2+}	Δδ	Free **L2**	**L2**⊃Cd^{2+}	Δδ
Ha	3.85	3.80, 4.21	-0.05, 0.36	3.88, 4.36	0.03, 0.51	3.87	3.81, 4.16	-0.06, 0.29
H_b	4.28	4.68	0.40	4.64	0.36	4.39	4.25, 4.56	-0.14 0.17
H_c	7.42	7.38	-0.04	7.49	0.07	7.48	7.23	-0.25
H_d	8.35	8.75	0.40	8.83	0.48	8.46	8.68	0.22

[a]Δδ values are the difference of the chemical shift between **L2** and Zn^{2+} or Cd^{2+} at 27 °C.
Here, minus sign (–) denotes a shift to higher magnetic field.

presence of Zn^{2+} ion, undergo a larger downfield shift than when the Cd^{2+} ion was present. Moreover, two sets of four methylene H_a protons were split into two peaks and broadened following binding with Cd^{2+} and Zn^{2+}. The proton signals among the four pyridine rings of the two sets of Dpas are overlapped each other like pyrene ring protons and induced downfield shift which is due to the decrease of electron density by the metal-nitrogen co-ordination.[22] The H_d protons of adjacent pyrene rings underwent a significant downfield shift (δ 8.35 to 8.75 and 8.83 ppm) for Cd^{2+} and Zn^{2+} ions respectively. Furthermore, two sets of two methylene H_b protons also broadened and underwent a large downfield shift. These results suggested that two sets of dpas were equally assigned for making a co-ordination bond with two metal ions and confirmed a 1:2 ligand-metal stoichiometry.[17] The ^1H NMR analysis also revealed larger chemical shift differences for **L2** for the complexation with Zn^{2+} *versus* the Cd^{2+} ion.

In contrast, when using THF as solvent, there is no such change after addition of 1 equiv. of Cd^{2+} ion which confirmed the1:1 binding mode for the complexation of **L2** with Cd^{2+} (Fig. 8). Here, the same H_d protons of the adjacent four pyridine rings undergo a smaller downfield shift (from δ8.46 to 8.68 ppm) than in methanol-water solvent after addition of 1 equiv. of Cd^{2+}. Another three protons (H_c, H_e and H_f) also experience a downfield shift. Moreover, two sets of four methylene H_a protons are split into two broad peaks from δ 3.87 to 3.81 and 4.16 ppm following binding with Cd^{2+} akin to the methanol-water system. On the other hand, the H_g proton of the pyrene ring exhibits a large upfield shift from δ 8.35 to 7.64 ppm, and unlike the methanol-water system, the H_b protons split into two peaks from δ 4.39 to 4.25 and 4.56 ppm, which suggested that the methylene H_b and pyrene H_g protons directly contribute to the binding with the metal ion. This phenomenon is only possible when the Cd^{2+} is positioned at the centre between the two binding sites. However, in THF, addition of Zn^{2+} induces vigorous precipitation which does not allow for analysis using ^1H NMR spectra for elucidation of the binding mode. Moreover, the fluorescence spectra and Job's plot confirmed the 1:1 binding mode of a **L2**⊃Zn^{2+}complex.
The above NMR and fluorescence spectra together with the Job's plot suggested that in methanol-water solvent system, two binding sites equally co-ordinate with two metal ions. On the other hand, Zn^{2+} or Cd^{2+} is positioned between two binding sites in THF. Given the shape of THF (a five membered ring), it can lead to a pronounced pseudorotational effect which is responsible for the stable twisted conformation. It is assumed that this structural property plays an important role in the 1:1 ligand to metal binding system.

Conclusion

In conclusion, the novel fluorogenic molecules **L1** and **L2** based on 7-*tert*-butylpyrene have been synthesized. The binding of Zn^{2+} and Cd^{2+} ions at the pyrene linked dipicolylamine moieties was investigated by using fluorescence and ^1H NMR titration experiments. It was found that receptor **L1** exhibits a similar binding toward Cd^{2+} and Zn^{2+} in both solvent systems. Herein, **L1** displayed higher fluorescence sensitivity for Cd^{2+} *versus* Zn^{2+}. On the other hand, receptor **L2** exhibited different binding behaviour in different solvent systems. When the molecule was dissolved in methanol-water solvent system, it selectively detected Cd^{2+} and $Zn^{2+}$with a 1:2 (ligand:metal) binding ratio. It was noticeable that **L2** had the strongest affinity for binding with Zn^{2+} ion *versus* Cd^{2+} and all the other competitive metal ions. In contrast, using THF as solvent, Zn^{2+} or Cd^{2+} is positioned between two binding sites and followed a 1:1 binding mode. It was concluded that ligands **L1** and **L2** exhibited similar binding behaviour in THF.

General : Unless otherwise stated, all other reagents used were purchased from commercial sources and were used without further purification. Compounds **1**,[26] **3**,[23] **4**[23] and receptor **L3**[17a] were prepared following the reported procedures. All the solvents used were dried and distilled by the usual procedures before use. All melting points were determined using a Yanagimoto MP-S1. JEOL FT-300 NMR spectrometer and Varian-400MR-vnmrs400 with $SiMe_4$ as an internal reference: *J*-values are given in Hz. Mass spectra were obtained with a Nippon Denshi JMS-HX110A Ultrahigh Performance mass spectrometer at 75 eV by using a direct-inlet system.

Synthesis of Compound 2

To a mixture of 7-*tert*-butylpyrene (500 mg, 1.93 mmol), 1,1-dichloromethyl methyl ether (333 mg, 2.90 mmol) was added in CH_2Cl_2 (20 ml) at 0 °C with stirring for 15 min. A solution of $TiCl_4$ (0.53 ml, 4.8 mmol) was added drop wise to the stirred solution over 10 min. After this addition, the reaction mixture was continuously stirred for 3 h at room temperature. Then, the reaction mixture was quenched with ice water and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was washed with water (2 × 200 mL), brine (2 × 200 mL), dried over $MgSO_4$ and then evaporated. The crude product was recrystallized from hexane to obtain 7-*tert*-butylpyrene-1-carbaldehyde **2** as a yellow powder (400 mg, 72%). The ^{1}H NMR spectrum agreed with the reported values.[23] ^{1}H NMR (300MHz, $CDCl_3$): δ =1.60 (9H, s, *t*Bu), 8.06 (1H, d, *J* = 7.83 Hz, pyrene-H_5), 8.20 (1H, d, *J* = 4.83 Hz, pyrene-H_4), 8.23 (1H, d, *J* = 3.8 Hz, pyrene-H_9), 8.29 (1H, d, *J* = 9.2 Hz, pyrene-H_{10}), 8.34 (2H, d, *J* = 3.2 Hz, pyrene-$H_{6,8}$), 8.39 (1H, d, *J* = 7.9 Hz, pyrene-H_3), 9.38 (1H, d, *J* = 9.0 Hz, pyrene-H_2) and 10.78 (1H, s, *CH*O) ppm.

Synthesis of Receptor L2

To a solution of 7-*tert*-butylpyrene-1,3-dicarbaldehyde (336 mg, 1.07 mmol) in 1,2-dichloroethane (18 mL), 2,2′-dipicolylamine(436 mg, 2.18 mmol) was added drop wise. Then the mixture was stirred for 18h at 45°C. After that sodium triacetoxyborohydride (1.35 g, 6.42 mmol) was added, and the mixture was further stirred for 24h at 50°C. Then, the reaction mixture was quenched with ice water and extracted with CH_2Cl_2 (2 × 100 mL). The organic layer was washed with water (2 × 200 mL), brine (2 × 200 mL), dried over $MgSO_4$ and then evaporated. The crude product was purified by column chromatography eluting with acetone-methanol (1:1) to afford a orange gummy substance **L2** (600 mg, 82%). Mp. 65–66°C; ^{1}H NMR (300MHz, $CDCl_3$): δ = 1.55 (9H, s, *t*Bu), 3.82 (8H, s, CH_2), 4.36 (4H, s, CH_2), 7.12–7.07 (4H, m, pyridine-*H*), 7.45 (4H, d, *J* = 7.8 Hz, pyridine-*H*), 7.56 (4H, ddd, *J* = 1.8, 7.8, 15.2 Hz, pyridine-*H*), 7.99 (2H, d, *J* = 9.3 Hz, pyrene-$H_{4,10}$), 8.18 (2H, s, pyrene-$H_{6,8}$), 8.21 (1H, s, pyrene-H_2), 8.29 (2H, d, *J* = 9.2 Hz, pyrene-$H_{5,9}$) and 8.52–8.50 (4H, m, pyridine-*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 31.9, 35.1, 57.1, 60.5, 121.9, 122.2, 123.1, 123.3, 123.9, 125.4, 126.9, 129.2, 130.2, 130.8, 131.7, 136.3, 148.8, 148.9 and 159.6 ppm; HRMS: *m/z* calcd. for $C_{46}H_{44}N_6$ 681.3706; found 681.3707 [M^+].

Synthesis of receptor L1

To a solution of 7-*tert*-butylpyrene-1-carbaldehyde (225 mg, 0.79 mmol) in 1,2-dichloroethane (18 mL), 2,2′-dipicolylamine (156 mg, 0.79 mmol) was added dropwise. Then the mixture was stirred for 18h at 45°C. After that sodium triacetoxyborohydride (500 mg, 2.36 mmol) was added. The mixture was further stirred for 24h at 50°C. The reaction mixture was quenched with ice water and extracted with CH_2Cl_2 (2 × 100 mL). The organic layer was washed with water (2 × 200 mL), brine (2 × 200 mL), dried over $MgSO_4$ and then evaporated. The crude product was purified by column chromatography eluting with ethyl acetate-hexane (3:1) to afford a yellow solid (230 mg, 62%). Mp: 134–135°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.58 (9H, s, *t*Bu), 3.92 (4H, s, CH_2), 4.39 (2H, s, CH_2), 7.14–7.09 (2H, m, pyridine-*H*), 7.47 (2H, d, *J* = 7.8 Hz, pyridine-*H*), 7.60 (2H, ddd, *J* = 1.74, 7.7, 15.2 Hz, pyridine-*H*), 7.96 (2H, s, pyrene-$H_{9,10}$), 8.04 (1H, d, *J* = 9.33 Hz, pyrene-H_3), 8.07 (2H, s, pyrene-$H_{4,5}$), 8.19 (2H, dd, *J* = 1.7, 6.3 Hz, pyrene-$H_{7,8}$), 8.33 (1H, d, *J* = 9.2 Hz, pyrene-H_2) and 8.53 (2H, d, *J* = 4.9 Hz, pyridine-*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 31.9, 35.2, 57.1, 60.4, 122.0, 122.1, 122.3, 122.9, 123.3, 123.9, 124.3, 124.9, 127.2, 127.3, 127.9, 129.6, 130.5, 130.6, 131.1, 132.3, 136.4, 148.8, 148.9 and 159.6 ppm. HRMS: *m/z* calcd. for $C_{33}H_{31}N_3$ 470.2596; found 470.2596 [M^+].

Spectroscopic measurements

UV-vis spectra were recorded using a ShimadzuUV-3150UV-vis-NIRspectrophotometer. Fluorescence spectroscopic studies of compounds in solution were performed in a semimicro fluorescence cell (Hellma®, 104F-QS, 10× 4 mm, 1400 µL) with a Varian Cary Eclipse spectrophotometer. Measurements of pH were performed in methanol-water (10 mM HEPES/MeOH = 3:7). The aqueous buffer solution were prepared from 10 mM HEPES and 10 mM NaOH solution for pH 6-10 and to obtain pH 3–5, 10 mM HCl and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) solution were used. The association constants were determined by using the fluorescent titration experiment of **L1** and **L2** in a constant concentration of host receptor (7×10^{-6} M) and varying the guest concentration (0–20 × 10^{-6} M). The association constant (K_a) for the complexes of receptor **L1** and **L2** were calculated by observing the integral intensities of the complex and of free host molecules using nonlinear curve–fitting analysis according to the literature procedure.[25]

^{1}H NMR Titration Experiments

A solution of $Zn(ClO_4).6H_2O$ or $Cd(NO_3)_2{\cdot}4H_2O$ in D_2O (1.5×10^{-2} M) was added to a CD_3OD–D_2O (11:1, v/v) solution of receptor **L2** in the absence or presence of Zn^{2+} and Cd^{2+} ion in an NMR tube (300 MHz NMR). The pH of the solution was adjusted with 10 mM DCl and 10 mM K_2CO_3 solution. Similarly, a solution of $Zn(ClO_4){\cdot}6H_2O$ or $Cd(NO_3)_2{\cdot}4H_2O$ in THF-d_8 (0.5×10^{-3} M) was added to a THF-d_8 solution of **L2** (400 MHz NMR). ^{1}H NMR spectra were recorded after addition of the reactants and the temperature of the NMR probe was kept constant at 27 °C.

Supporting information: Detailed fluorescence and ^{1}H NMR titration data.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Details of the ^{1}H/^{13}C NMR spectra, ^{1}H NMR spectroscopic and UV-vis titration experimental data, the Bensei–Hilderbrand plot and Job's plot, See DOI: 10.1039/b000000x/

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