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New 1,8-naphthyridine-based probes for the selective fluorescence signalling of toxic cadmium: synthesis, photophysical studies and molecular modelling

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Newly synthesised 1,8-naphthyridine-based molecular probes, NAP-1 and NAP-2, exhibit highly selective fluorescence responses towards the toxic cadmium over coordinatively competing Zn^{2+} and several other metal ions examined. On the one hand, NAP-1 (MeOH:H_2O, v/v 80:20, pH 7.4) exhibits ca. 1.5 order of magnitude higher stability constant for Cd^{2+} over Zn^{2+}; on the other hand, NAP-2 in MeOH offers unique selectivity only towards Cd^{2+}, exhibiting both absorbance and emission red shifts as well as fluorescence enhancement. By ^{1}H NMR analysis, the tetra-coordinated binding is indicated at least for the NAP-1 + Cd^{2+} complex and theoretical calculations reveal relatively stronger binding of Cd^{2+} over Zn^{2+}.

Keywords: 1,8-naphthyridine; fluorophore photophysical studies; fluorescent cadmium sensors; NMR analysis; molecular modelling

Introduction

The development of molecular probes for the selective recognition of metal ions of biological and environmental significance is currently one of the important goals in supramolecular chemistry. Of the many methods known for ion detection, the fluorescence modulation is finding increasing popularity due to the advantages of high sensitivity and practical conveniences (*1*). The design of optical probes typically involves connecting a selective receptor to a chromophore/fluorophore motif such that the binding event triggers photophysical perturbations in the probe, delivering easily quantifiable colorimetric, fluorimetric or ratiometric responses (*2*). With regard to the sensing strategy, several attractive photophysical processes, which include photoinduced electron transfer (PET), intramolecular charge transfer, monomer vs. excimer formations and fluorescence resonance energy transfer mechanism, are currently available to tune the optical signalling in the presence of an interacting analyte (*2a*).

Cadmium is notorious for its well-recognised acute toxic effects on human and environments. It can contaminate food chain and environment through mining and various industrial processes (*3*, *4*). The recommended levels of cadmium in drinking water is no more than 3 μg/l (*1*, *5*). Overexposures to cadmium beyond this level can cause bone, renal, and nervous system diseases, calcium metabolism disorders as well as cancers of many vital body organs (*6*). Despite its health hazards, a cadmium-dependent carbonic anhydrase has recently been detected in marine diatoms thriving in a zinc-depleted environment (*7*). Consequently, monitoring Cd uptake in human and environment is an increasingly important goal in biology and health sciences. One major limitation associated with many currently known fluorescent Cd^{2+} sensors is the competing binding from the coordinatively similar Zn^{2+} (*8*). Although receptors such as pyridyl(di)amines, quinolylamines or their derivatives in conjunction with suitable fluorophores have afforded many efficient Zn^{2+}-selective fluorescent sensors (*9*, *10*), despite extensive reports (*11*), the Cd^{2+}-selective optical probes are comparatively less common (*12*). Thus, it is a challenge to seek new chemosensor motifs which could allow the selective discrimination of Cd^{2+}, while excluding or minimising interferences of Zn^{2+} and other metal ions (*13*).

1,8-Naphthyridine core has found applications in the design of supramolecular systems, as a recognition unit for DNA bases as well as components of organic solar dyes (*14*). Surprisingly, this photoemittive heterocycle, possessing a rigid 1,3-dinucleation geometry so far, has found limited utility in the design of sensors of few selected metal ions, viz. Zn^{2+}, Cu^{2+} and Hg^{2+} (*15*). While the present work was in progress, Xiao et al. (*16*) independently reported a naphthyridine-based Cd^{2+}-selective sensor, offering fluorescence response under a bi-nuclear coordination. However, a small fluorescence amplification of approximately only 2-folds means that the sensitivity of this Cd^{2+} chemosensor, though not reported by the authors, may not be appreciable.

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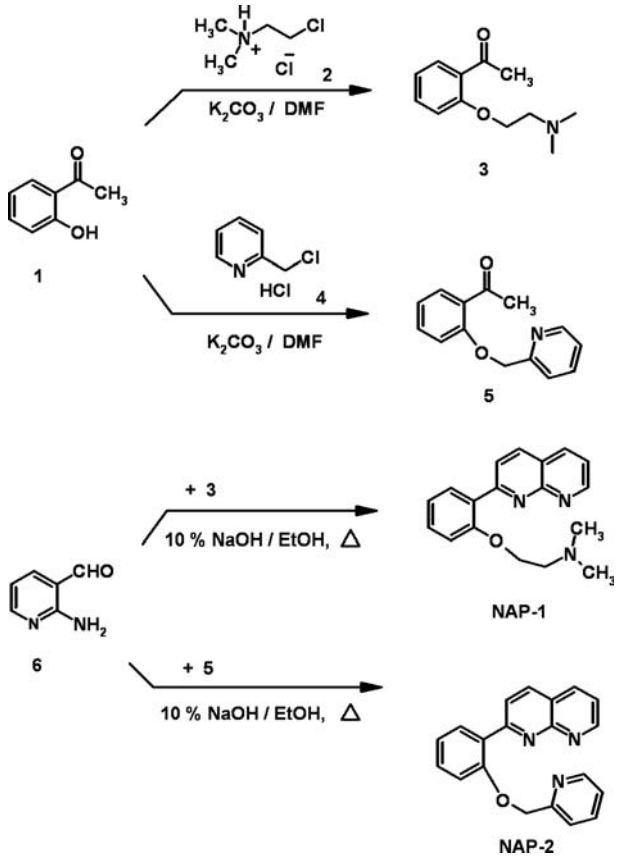
In continuation of our interest in metal ion chemosensors (*17*), presently, we presumed that optical chemosensors featuring tetra-coordination around the rigid naphthyridine core might offer improved binding characteristics with selected metal ion(s) of biological or environmental interest (*18*). With this anticipation, we have synthesised and investigated the metal ion sensitivity of two potentially tetra-dentate naphthyridine probes, designated as NAP-1 and NAP-2. Based on our photophysical studies, we find these probes to function as Cd^{2+}-selective fluorescence turn-on chemosensors with less significant or no detectable optical interferences being observed from the coordinatively competing Zn^{2+} as well as from several other biologically or environmentally relevant metal ions investigated.

Results and discussion

Synthesis

The syntheses of NAP-1 and NAP-2 were carried out in two easy steps as outlined in Scheme 1. Alkylations of 2-hydroxy acetophenone (**1**) with *N*,*N*-dimethylaminoethane hydrochloride (**2**) and 2-chloromethylpyridine hydrochloride (**4**) under the DMF/K_2CO_3 condition afforded the corresponding *O*-alkylated products **3** and **5**, respectively. The condensation of 2-aminopyridine-3-carboxaldehyde (**6**) with **3** and **5** in 10% NaOH in refluxing alcohol (*19*), followed by work-up and chromatographic purification, afforded the target products NAP-1 and NAP-2, respectively, as pale yellow solids in a reasonable yield.



Scheme 1. Synthesis of NAP-1 and NAP-2.

Optical spectral studies with metal ions

The optical sensitivities of NAP-1 and NAP-2 towards selected metal ions of biological and/or environmental relevance were evaluated via optical spectroscopic techniques. The UV–vis spectra of NAP-1 and NAP-2, carrying a common chromophore motif, displayed similar absorption behaviour with maxima located around 244 and 326 nm, attributable to the anisyl and naphthyridine chromophores, respectively. Addition of perchlorates of Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, Zn^{2+}, Mg^{2+}, Cd^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Ag^+ and Pb^{2+} up to 1000 equiv. into a solution of NAP-1 (2.83×10^{-5} M) in MeOH:H_2O (v/v 80:20), HEPES buffer, pH 7.4, did not significantly alter the absorption profile of the probe, except for the marginal reductions in the absorbance by 10–25% (see Supporting Information, available online). The absence of detectable changes in the ground state of NAP-1 means that no appreciable charge shift takes place within the chromophoric system in the presence of metal ions.

The absorption profile of NAP-2 (2.5×10^{-5} M, MeOH) was also virtually invariant to Na^+, Li^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Ag^+, Pb^{2+}, including Zn^{2+} up to 3.5×10^{-3} M, thereby implying the absence of any ground-state perturbations in the probe by these metal ions (see Supporting Information, available online). However, unlike NAP-1, the ground state of NAP-2 was detectably perturbed in the presence of Cd^{2+}. As shown in Figure 1, the spectrophotometric titration of a probe's solution (2.5×10^{-5} M in MeOH) as a function of

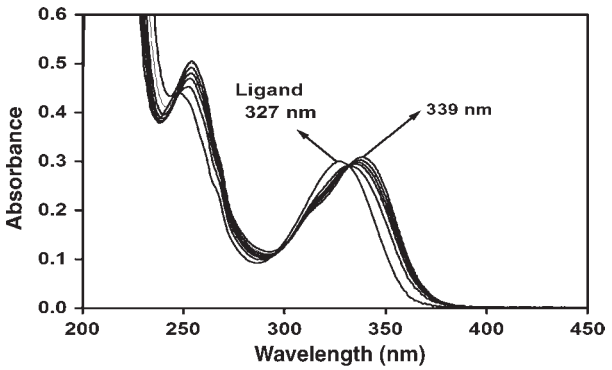


Figure 1. Spectrophotometric titration of NAP-2 (2.5×10^{-5} M) with cadmium perchlorate (0–2.84×10^{-3} M) in MeOH.

increasing Cd^{2+} (0–2.84×10^{-3} M) induced a red shift of the probe's 327 nm maximum to 339 nm. A clear isosbestic point at 332 nm indicates the formation of a well-defined equilibrium complexation. Although not yet well defined, the observed red shift of 12 nm implies the inducement of a moderate degree of intramolecular charge transfer from the donor phenoxyl ring to the acceptor naphthyridine ring upon interaction of NAP-2 with Cd^{2+}.

Excitations of NAP-1 in MeOH:H_2O (v/v 80:20) at pH 7.4 at 326 nm generated a structured emission in the range of 350–600 nm, with a maximum intensity centred at 430 nm, while the excitation of NAP-2 in MeOH at the isosbestic point at 332 nm produced a structured emission at 433 nm. The quantum yields of NAP-1 and NAP-2 were determined to be 0.005 and 0.016, respectively, with respect to anthracene as the standard. The lower quantum efficiency of NAP-1 compared to that of NAP-2 may presumably be due to PET-induced fluorescence quenching via electron transfer from the saturated nitrogen to the excited naphthyridine chromophore. Support for the involvement of the PET process in NAP-1 is obtained from the study of the changes in fluorescence intensity of NAP-1 with respect to variations in the pH. As shown in Figure 2, the fluorescence was nearly unchanged between the 11.5 and 7 pH range, thereafter gradual increases in the emission intensity at 430 nm occurred in going from the 5 to 3 pH range, giving a maximum of approximately 2-fold emission enhancements. From this result, we calculated the pK_a of 4.65 assignable to the more basic tertiary amino group. The increase in the fluorescence intensity under the acidic pH clearly supports the PET nature of the probe. A further decrease in pH below 3 changed the fluorescence only slightly. Although the protonation of the less basic naphthyridine ring is expected to take place below pH 3, the absence of significant changes in the fluorescence intensity precluded the determination of the pK_a of this ring by this method.

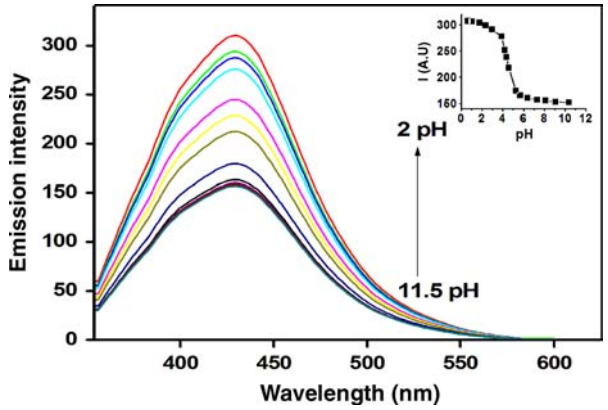


Figure 2. pH titration of NAP-1 (pH 2 to 11) by monitoring fluorescence intensity at 430 nm.

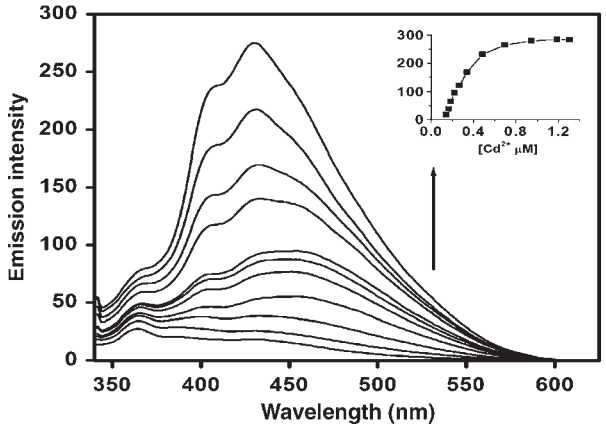


Figure 3. NAP-1 (1×10^{-6} M) on addition of cadmium perchlorate (0–1.3×10^{-4} M) in MeOH:H_2O (v/v 80:20) at pH 7.4.

Of the different metal ions examined for their fluorescence sensitivity towards NAP-1, the most remarkable fluorescence response was induced by Cd^{2+} only. As shown in Figure 3, sequential addition of Cd^{2+} to a solution of NAP-1 (1×10^{-6} M) in MeOH:H_2O (v/v 80:20) at pH 7.4 gave rise to progressive fluorescence enhancement at the same wavelength until at saturating Cd^{2+} (1.3×10^{-4} M), the emission intensity reached a maximum of 15-fold enhancement. The inset shows the linear increase in the emission intensity as a function of increasing concentration of Cd^{2+}. The probe showed no emission spectral shift, a feature which is consistent with the PET character of the probe.

In contrast to Cd^{2+}, the coordinatively competing Zn^{2+} required significantly higher concentrations in order to induce detectable fluorescence modulation. The fluorescence response of NAP-1 towards Zn^{2+}, together with the effects of subsequently added Cd^{2+}, is shown in Figure 4. First, the addition of Zn^{2+} at its saturating concentration (1.2×10^{-3} M) caused a blue shift of the probe's emission maximum from 430 to 386 nm with approximately 4-fold emission enhancement at the newly

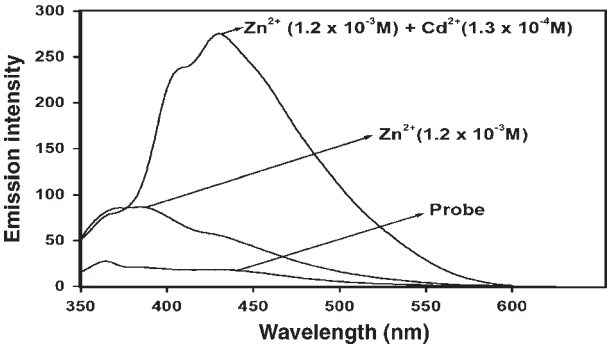


Figure 4. NAP-1 (1×10^{-6} M) on addition of Zn^{2+} (1.2×10^{-3} M) + Cd^{2+} (1.3×10^{-4} M) in MeOH:H_2O (v/v 80:20) at pH 7.4.

generated maximum. Subsequent addition of 1.3×10^{-4} M Cd^{2+} (ca. 10 times less than the saturating Zn^{2+}) into the above solution of NAP-1 + Zn^{2+} not only caused the 386 nm emission band to shift back to 430 nm, but also displayed about 15-fold emission enhancement at this emission maximum. These emission features are reminiscent of those observed when NAP-1 interacts with Cd^{2+} alone (see Figure 3). This experiment clearly demonstrates that Cd^{2+}, even in relatively lower concentrations, can completely displace Zn^{2+} from its NAP-1 chelate. Thus, we can conclude that the affinity of NAP-1 towards Cd^{2+} is relatively more pronounced than with Zn^{2+}. For other metal ions, namely Na^+, Li^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Ag^+ and Pb^{2+} investigated, no detectable fluorescence responses could be discerned up to 1.2×10^{-3} M, suggesting either very weak or no binding interactions of NAP-1 with these metal ions.

The fluorescence sensing of NAP-2 towards Cd^{2+} was next examined. As depicted in Figure 5, fluorimetric titration of NAP-2 (1×10^{-6} M in MeOH) with increasing addition of Cd^{2+} caused the probe's emission maximum at 433 nm to progressively red shift to a new location at 465 nm, with a distinct isoemissive point being observed at 403 nm. The red shift in emission band is consistent with the similar shift also observed in the absorbance of NAP-2 with Cd^{2+}. At a saturation concentration of 7.4×10^{-4} M Cd^{2+}, the fluorescence intensity at the new maximum registered approximately 5-fold increase with respect to that of the unbound probe at 433 nm. Interestingly, in contrast to NAP-1 that offers only enhanced fluorescence with Cd^{2+}, the fluorescence response of NAP-2 comprises both the change in the emission wavelength and fluorescence amplification. Consistent with the absence of any detectable absorbance changes, essentially no fluorescence responses were exerted by Na^+, Li^+, K^+, Ca^{2+}, Ba^{2+}, Mg^{2+},Co^{2+}, Ni^{2+}, Cu^{2+}, Ag^+, Pb^{2+} as well as coordinatively Zn^{2+} up to 5.3×10^{-3} M. These findings imply that, among the metal ions examined, only Cd^{2+} exhibits uniquely selective complexation with NAP-2 under both ground-state and excited-state conditions with virtually no detectable optical interferences arising from other metal ions even at relatively higher concentrations.

For the case of NAP-1, tethered with a saturated nitrogen donor, the observed fluorescence turn-on response upon Cd^{2+} binding (and to some extent with Zn^{2+}) can be attributed to a combination of the chelation-enhanced fluorescence (CHEF) effect and the metal ion-induced suppression of the PET process prevailing in the metal-free probe (*20*). However, in NAP-2, in the absence of any saturated nitrogen donor, the involvement of the PET process may be excluded. Therefore, in this case, the CHEF effect is presumably the main factor contributing to the fluorescence enhancement upon Cd^{2+} chelation.

The complexation stoichiometry of Cd^{2+} with both NAP-1 and NAP-2 was found to be 1:1 on the basis of the Job plots (see Supporting Information, available online). The apparent stability constants, log *Ks*, were calculated by applying a non-linear square regression analysis of fluorescence changes against metal ion concentrations (*21*). For NAP-1, the log *Ks* for Cd^{2+} and coordinatively competing Zn^{2+} were found to be 2.99 and 1.3, respectively. Thus, in comparison to Zn^{2+}, over 1.5 log unit, higher stability constant for Cd^{2+} signifies its superior binding interaction over Zn^{2+}. For other metal ions, the concentration-dependent fluorescence changes were too insignificant to allow a reliable measure of their log *Ks.* On the other hand, for the case of NAP-2, which interacts only with Cd^{2+}, the log *Ks* was determined to be 3.48. The detection limits of NAP-1 and NAP-2 towards Cd^{2+}, calculated from the fluorescence data (*22*), were found to be 1.51×10^{-6} M and 1.4×10^{-6} M, respectively (see Supporting Information, available online). These micromolar sensitivities compare well with many reported Cd fluorescence sensors and, as such, may be useful for environmental monitoring (*13*).

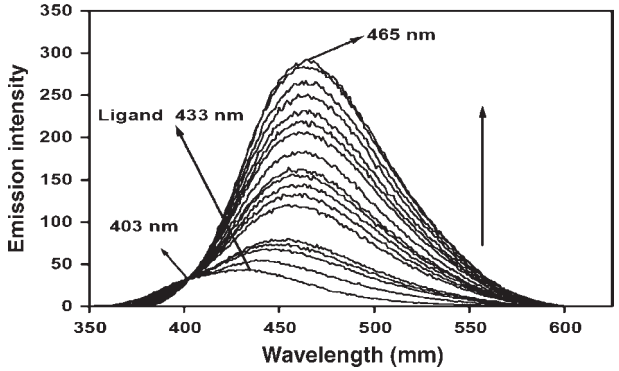


Figure 5. NAP-2 (1×10^{-6} M) on addition of cadmium perchlorate ($0–7.4 \times 10^{-4}$ M) in MeOH.

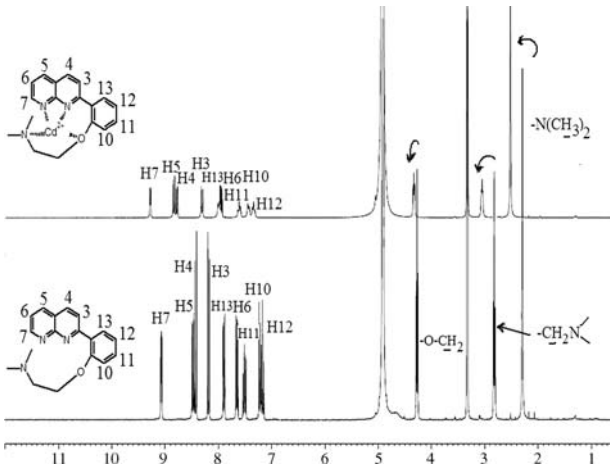


Figure 6. (a) ^{1}H NMR spectra of NAP-1 in MeOH and (b) ^{1}H NMR of NAP-1 in the presence of ca. 5 equiv. of $Cd(ClO_4)_2$.

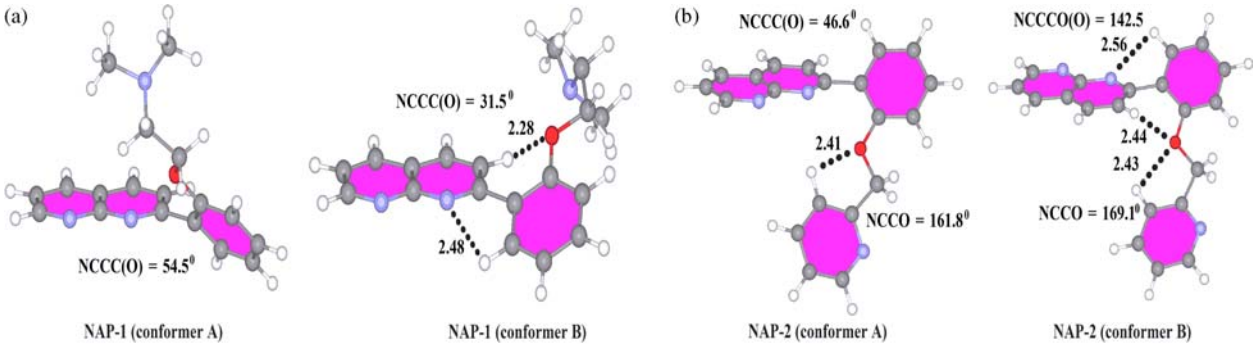


Figure 7. (a) Two conformations of NAP-1 and the NCCC(O) dihedral values (degrees) are shown. (b) Two conformations of NAP-2 and the NCCC(O) and NCCO dihedral values (degrees) are shown.

Evaluation of cadmium binding by NMR

We have also evaluated the Cd^{2+} binding interactions by ^1H NMR analysis. The ^1H NMR spectra of NAP-1 without and with Cd^{2+} are depicted in Figure 6. The assignment of various protons is based on the chemical shift positions and spin multiplicities and further supported by 2D NMR analysis (see Supporting Information, available online). It can be clearly seen that upon Cd^{2+} complexation, all the protons associated with the naphthyridine and the anisyl rings exhibit downfield shifts in the range of 0.1–0.4 δ and 0.05–0.20 δ, respectively. In addition, the side-chain protons $(CH_3)_2N—$, $>N—CH_2—$ and $—CH_2—O—$ also suffer downfield shifts by 0.20, 0.31 and 0.12 δ, respectively. These cation-induced downfield shifts of the rings and the side-chain protons clearly imply that Cd^{2+} interacts with NAP-1 via a tetra-dentate coordination. An attempt was also made to evaluate Cd^{2+} interaction with NAP-2 by ^1H NMR analysis. Although some of the protons associated with the ring systems as well as the side chain do appear downfield shifted in the presence of Cd^{2+}, individual assignments are complicated by the broad and overlapping nature of the resonances (see Supporting Information, available online). Although a clear conclusion about the binding mode in NAP-2 could not be extracted from the NMR study, we believe that the tetra-coordinated chelation is possible in this case as well.

Molecular modelling studies

To further understand metal ion interactions, we optimised the conformations of NAP-1 and NAP-2 based on the theoretical calculations using RI-BP86/def2-TZVP both under the gas phase and taking into account the solvent effects. The two most stable conformations for both NAP-1 and NAP-2 are shown in Figure 7(a) and (b), respectively. For NAP-1, the conformation designated as the conformer B can establish two weak intramolecular non-covalent interactions. Thus, the conformer B is calculated to be more stable by 3.14 kcal/mol in the gas phase and 1.98 kcal/mol in the solvent ($\varepsilon = 41.76$) compared to the conformer A lacking in any non-covalent interaction. For the case of NAP-2, the conformer B, which exhibits three weak intramolecular non-covalent interactions, is more stable by 0.89 kcal/mol in the gas phase and 1.69 kcal/mol in the solvent ($\varepsilon = 32.61$) compared to the conformer A showing only one weak intramolecular non-covalent interaction.

The most stable conformations of the complexes of Cd^{2+} and Zn^{2+} with NAP-1 and NAP-2 are depicted in Figure 8(a) and (b), respectively, and the computed binding energies are summarised in Table 1. Contrary to our experimental results, the gas-phase calculations indicate that the zinc cation should form the most stable complexes. However, when the solvent effects are taken

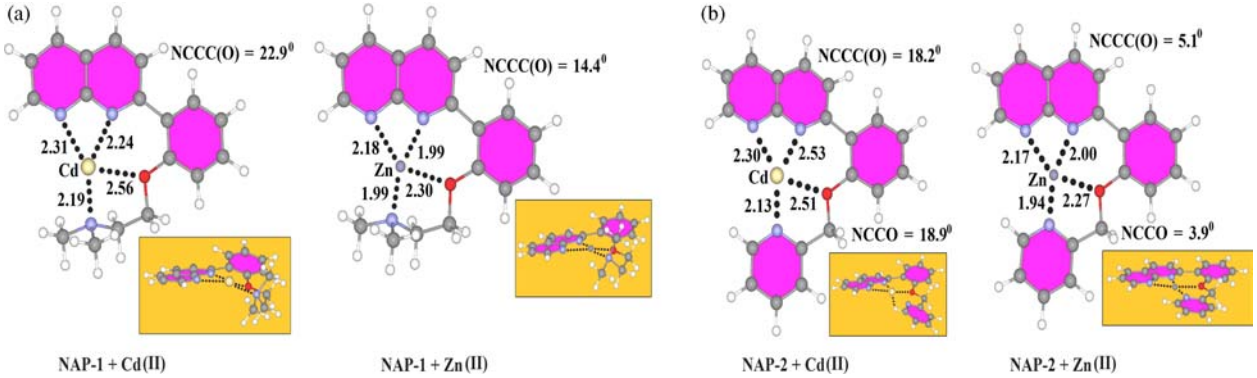


Figure 8. Optimised complexes of (a) NAP-1 and (b) NAP-2 with Cd^{2+} and Zn^{2+}.

Table 1. Binding energies (ΔE, kcal/mol), equilibrium distances (R_e, Å) and dihedral angles (°).

Probe+cation	ΔE (gas phase)	ΔE (solvent)	R_e (solvent)	NCCC(O)
NAP-1 + Cd^{2+}	−297.5	−140.7	2.31, 2.24, 2.56, 2.19	22.9
NAP-1 + Zn^{2+}	−349.4	−91.5	2.18, 1.99, 2.30, 1.99	14.4
NAP-2 + Cd^{2+}	−299.9	−139.4	2.30, 2.53, 2.51, 2.13	18.2, 18.0
NAP-2 + Zn^{2+}	−354.3	−95.7	2.17, 2.00, 2.27, 1.94	5.1, 3.9

into account, cadmium exhibits more favourable interaction than zinc, the results being in conformity with our experimental findings. From the calculations, it can also be observed that the values of the NCCC(O) and NCCO dihedral angles correlate well with the binding energies of these metal ions. It can be seen that the tetra-coordinated metal ion interaction is accompanied by certain degrees of reduction in the dihedral angles between the naphthyridine and the sphenoxyl rings. While the larger Cd^{2+} ($r+ = 0.96$ Å) imposes a lower level of reduction in the dihedral angles, the geometrical requirements of the small cation Zn^{2+} ($r+ = 0.74$ Å) for the shorter metal—N,O distance provoke sharp reductions in these dihedral angles and consequently there are higher energetic costs attached to the zinc complexation with the probes (*2a*).

Conclusion

In summary, the photoemittive 1,8-naphthyridine has been elaborated to access two weakly emissive chemosensors, NAP-1 and NAP-2, via simple synthetic procedures. Upon exposure to Cd^{2+}, NAP-1, a PET-based probe, delivers high fluorescence amplification, whereas the coordinatively competing Zn^{2+} only at significantly higher concentrations induces relatively small fluorescence enhancement. The superior affinity of Cd^{2+} is evident by its ca. 1.5 log unit higher stability constant over that of Zn^{2+}. Moreover, physiologically important metal ions, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+} and Co^{2+}, as well as Li^+, Ba^{2+}, Ni^{2+}, Ag^+ and Pb^{2+}, of some biological/environmental import do not exert measurable optical perturbations. On the other hand, NAP-2 exhibits remarkable selectivity only towards Cd^{2+}, affording the absorbance and emission red shifts as well as fluorescence turn-on signalling responses. Other metal ions, including Zn^{2+}, do not pose any interference even in relatively higher concentrations. The ^{1}H NMR analysis supports the formation of a tetra-coordinated complexation between NAP-1 and Cd^{2+}. In agreement with our experimental findings, the theoretical calculations also predict superior binding interactions of Cd^{2+} over Zn^{2+}. Although the micromolar detection offered by NAP-1 and NAP-2 towards Cd^{2+} augurs well for environmental monitoring, the present sensitivity needs to be improved to make the probes compatible for nanomolar biological tracking of Cd^{2+}. Work is currently under way to modify the ligand structures around the naphthyridine core to enhance the sensitivity towards Cd^{2+} with the possibility of physiological measurements.

Experimental and theoretical methods

General

Commercially available compounds were used without further purification. Reagents and solvents used were purchased form S.D. Fine Chemicals, Mumbai, India or Sigma-Aldrich, Bangalore, India and used as received. UV–vis spectra were recorded using Shimadzu recording spectrophotometer, model no. UV-2401PC. Fluorescence studies were carried out using Shimadzu spectrofluorometer, model no. RF-5301PC. IR spectra were recorded using Perkin-Elmer FT-IR spectrometer. ^{1}H and ^{13}C NMR were recorded on Bruker Model Avance II 300 MHz.

Theoretical methods

The geometry of all the complexes included in this study was fully optimised at the RI-PB86/def2-TZVP level of theory within the program TURBOMOLE version 5.7 (*23*). The binding energies were calculated at the same level. The optimisation of the molecular geometries has been performed without imposing symmetry constraints. We have also performed calculations in the presence of a solvent (methanol, $e = 32.61$ and MeOH:H_2O (v/v 80:20), $e = 41.76$) using the Conductor-like Screening Model (COSMO) (*24*) as implemented in TURBOMOLE.

*Preparation of compound **3***

2-Hydroxy acetophenone **1** (680 mg, 5 mmol) and *N*,*N*-dimethylaminoethane hydrochloride **2** (1.08 g, 7.5 mmol) were dissolved in dry DMF (20 ml) containing anhydrous K_2CO_3 (1.38 g, 10 mmol). The reaction mixture was stirred and heated at 100°C for 48 h. The reaction mixture was diluted with water and extracted with chloroform (3 × 50 ml). The organic extract was washed once with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude oily product was purified by SiO_2 column chromatography (eluant $CHCl_3$:MeOH, 9:1) to give the desired product **3** as a colourless oil (550 mg) in 53% yield. IR (liquid film, cm^{-1}): 3054, 2963, 2872, 1640, 1615,

1577, 1539, 1307, 1215, 840, 755. ^{1}H NMR ($CDCl_3$): δ 2.37 (s, 6H), 2.63 (s, 3H), 2.84 (t, 2H, $J = 13.5$ Hz), 4.15 (t, 2H, $J = 12$ Hz), 6.97 (q, 2H), 7.45 (t, 1H, $J = 7.2$ Hz), 7.73 (d, 1H, $J = 9$ Hz). Anal. Calcd: $C_{12}H_{11}NO_2$. C, 69.56; H, 8.21; N, 6.76; Found: C, 69.55; H, 8.23; N, 6.77.

Preparation of compound ***5***

2-Hydroxy acetophenone **1** (1.36 g, 10 mmol) and 2-chloromethyl pyridine hydrochloride HCl **4** (820 mg, 5 mmol) were dissolved in dry DMF (20 ml) containing anhydrous K_2CO_3 (2.76 g, 20 mmol). The reaction mixture was stirred and heated at 100°C for 18 h. The reaction mixture was worked up as described for the preparation of **3**. The crude oily product was purified by SiO_2 column chromatography (eluant CH_3Cl) to give the desired product **5** as a colourless oil (670 mg) in 59% yield. IR (liquid film, cm^{-1}): 3014, 2973, 1659, 1593, 1504, 1436, 1387, 1368, 1269, 1120, 745. ^{1}H NMR ($CDCl_3$): δ 2.84 (s, 3H), 5.38 (s, 2H), 7.27 (t, 1H, $J = 12.5$ Hz), 7.4 (d, 1H, $J = 6.8$ Hz), 7.45 (t, 1H, $J = 12.0$ Hz), 7.6 (m, 2H), 7.86 (t, 1H, $J = 17.0$ Hz), 7.9 (d, 1H, $J = 7$ Hz), 8.62 (d, 1H, $J = 6.4$ Hz). Anal. Calcd: $C_{14}H_{13}NO_2$, C, 74.0; H, 5.72; N, 6.16. Found: C, 73.92; H, 5.75; N, 6.20.

Preparation of NAP-1

Compound **3** (414 mg, 2 mmol) and 2-aminopyridine 3-carboxyldehyde **6** (244 mg, 2 mmol) were dissolved in ethyl alcohol (15 ml) containing ca. 50 mg of KOH. The reaction was heated to reflux under nitrogen atmosphere for 12 h. After completion of the reaction, the reaction mixture was diluted with water, the solid precipitate was filtered and air dried. The crude solid was purified by SiO_2 column chromatography (eluant CH_3Cl) to give the desired product NAP-1 as a light yellow solid (260 mg) in 44% yield. Mp = 99–101°C. IR (KBr, cm^{-1}): 3002, 2950, 2823, 2773, 1602, 1580, 1537, 1432, 1262, 871, 750. ^{1}H NMR (CD_3OD): δ 2.29 (s, 6H), 2.81 (t, 2H, $J = 10.8$ Hz), 4.28 (t, 2H, $J = 11.1$ Hz), 7.16 (t, 1H, $J = 12.6$ Hz), 7.23 (d, 1H, $J = 7.8$ Hz), 7.51 (t, 1H, $J = 12$ Hz), 7.67 (t, 1H, $J = 12$ Hz), 7.9 (d, 1H, $J = 7.2$ Hz), 8.19 (d, 1H, $J = 9$ Hz), 8.4 (d, 1H, $J = 9$ Hz), 8.48 (d, 1H, $J = 11.4$ Hz), 9.07 (d, 1H, $J = 6$ Hz). ^{13}C NMR ($CDCl_3$): 45.85, 58.11, 67.09, 112.86, 121.49, 121.54, 121.67, 124.77, 128.93, 130.99, 132.42, 135.84, 136.55, 153.28, 156.15, 156.67, 160.20. Mass *m/e* value: 294. Anal. Calcd: $C_{18}H_{19}N_2O$, C, 73.72; H, 6.50; N, 14.33. Found: C, 73.54; H, 6.57; N, 14.08.

Preparation of NAP-2

Compound **5** (227 mg, 1 mmol) and 2-aminopyridine 3-carboxyldehyde **6** (122 mg, 1 mmol) were dissolved in ethyl alcohol (10 ml) containing ca. 20 mg of KOH. The reaction mixture was heated to reflux under nitrogen atmosphere for 12 h. After completion of the reaction, the reaction mixture was concentrated by half, diluted with water and the precipitate solid was filtered. The product was purified by repeated crystallisation from $CHCl_3$: petroleum ether (50:50) to give NAP-2 (120 mg) in 41% yield. Mp = 169–171°C. IR (KBr, cm^{-1}): 1600, 1577, 1537, 1489, 1456, 1422, 1309, 1268, 1203, 1025, 845. ^{1}H NMR (CD_3OD): δ 5.38 (s, 2H), 7.25 (t, 1H, $J = 12.4$ Hz), 7.39 (d, 1H, $J = 6.7$ Hz), 7.41 (t, 1H, $J = 12.3$ Hz), 7.58 (m, 2H), 7.71 (t, 1H, $J = 12.5$ Hz), 7.85 (t, 1H, $J = 17.7$ Hz), 7.9 (d, 1H, $J = 7$ Hz), 8.30 (d, 1H, $J = 6.7$ Hz), 8.49 (d, 1H, $J = 6.7$ Hz), 8.5 (d, 1H, $J = 7.9$ Hz), 8.63 (d, 1H, $J = 6.4$ Hz), 9.15 (d, 1H, $J = 6.2$ Hz). ^{13}C NMR ($CDCl_3$): 71.17, 112.86, 121.17, 121.52, 121.76, 122.58, 124.67, 129.06, 131.01, 132.52, 135.93, 136.62, 136.83, 149.09, 153.35, 156.12, 156.23, 156.95. Mass *m/e* = 314. Anal. Calcd: C, 76.71; H, 4.80; N, 13.41. Found: C, 76.97; H, 4.69; N, 13.49.

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Supporting Information: ^{1}H NMR spectra of compounds **3** and **5**, ^{1}H NMR and ^{13}C NMR spectra of NAP-1, NAP-2, UV–vis profiles, Job's plots and detection limits of NAP-1 and NAP-2, 2D NMR of NAP-1 and ^{1}H NMR of NAP-2 with Cd^{2+}, available online.

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