Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 2733

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Alteration of selectivity in rhodamine based probes for Fe(III) and Hg(II) ion induced dual mode signalling responses†

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Received 27th December 2011, Accepted 10th February 2012
DOI: 10.1039/c2ob07182g

The probes for metal ion induced chromo- and fluorogenic signalling responses alter their selectivity depending upon the nature of substituent as well as a function of solvent medium. 2 has shown selectivity towards Fe(III) ion, 4 towards Hg(II) ion while 3 is responsive towards both Fe(III) and Hg(II) ions.

Metal ion responsive signalling probes[1] find their wide-domain utility and impact in on-site, real time, selective analysis of various metal ions for biological, environmental, clinical monitoring and assessment.[2,3] The synthetic and operational aspects of such probes follow various methodological designs; most involve either metal ion mediated perturbation of photo-physical processes or exploitation of structure–function correlation. A probe that facilitates complexation-induced chromogenic as well as fluorogenic dual mode of signalling is advantageous in selective metal ion detection. In this regard, rhodamine based probes[1a,4] have attracted immense interest recently because of rhodamine's excellent spectroscopic properties, where the signal transduction pathway mostly exploits its contrast structure–function correlation in lactonized–delactonized conformations. As selectivity, sensitivity, reproducibility and reversibility are vital parameters to be manifested for any metal ion selective probe, the structural motif of its receptor which facilitates disposition of donor atoms in space for effective metal ion coordination plays a crucial role. An appropriate selection of donor atoms at strategic positions of the receptor to accommodate a particular ion brings about selectivity[5] in a probe. The selectivity may also be tailored by modulation of electron densities over those donor atoms, in turn, perturbation of stereo-electronic situation to tune the binding preferences. Thus, in rhodamine based signalling probes for highly selective and sensitive detection of metal ions, receptor subunits with varied substituents at donor atoms in its covalent framework may be envisaged to exhibit different selectivity towards different metal ions. We report herein the substituted aminoethyl-rhodamine based probe **2**, where a *p*-cyanobenzyl group is substituent at the donor amino end, to exhibit simultaneous enhanced absorption and emission signalling responses selectively with Fe(III) ion in MeCN. **3**, which incorporates a *p*-bromobenzyl substituent exhibits similar responses with both Hg(II) and Fe(III) ions, while **4** with a *p*-nitrophenyl substituent responds selectively in the presence of Hg(II) ion in MeCN. The physiological importance[2,3,6] and the lethal toxic effect[7,8] of Fe(III) and Hg(II) ions on human health and environment beyond requisite concentration threshold is the matter of concern, thus, promotes for development of new rhodamine based signalling probes which are capable of detecting these metal ions selectively under physiological conditions. Although many Hg(II)[9] and Fe(III)[10]-selective probes based on complexation induced delactonization of rhodamine are known, these new probes (**2–4**) not only exhibit dual mode signalling for highly selective and sensitive detection of these metal ions, but also provide a structural basis to alter selectivity as a function of attached substituent to rhodamine's 'amino-ethyl-amido' framework. Further, **2–4** exhibits both chromogenic and fluorogenic signalling responses selectively with Hg(II) ion in MeCN–H_2O medium. Hence, probe **2** which signals in both chromogenic and fluorogenic pathway switches its Fe(III) selectivity to Hg(II) ion in a dual order manner: (a) upon structural transformation to the core and (b) in a mixed organic–aqueous medium. In rhodamine based signalling systems, examples of switching in the metal ion selectivity by the same probe under different structural or functional manifestation are not much known, baring few, such as an acetylacetone coupled rhodamine-hydrazide derivative[11] switches selectivity between Fe(III) and Cu(II) ions as a function of pH, a rhodamine-phenyl urea conjugate[12] changes the Pb(II) selectivity to Hg(II) ion depending upon the medium, and signalling of rhodamine-hydrazide derivatives alter selectivity between Cu(II)–VO(II)[13] or Cu(II)–Hg(II)[14] ion pairs as a function of the mode of detection. To the best of our knowledge, no methodologies involving rhodamine based probes that impart dual mode signalling responses have been reported so far to exhibit a two-fold switching in selectivity between Fe(III) and Hg(II) ions as in **2**.

The first step of the synthesis of these probes (Scheme 1) was involved in condensation of ethylenediamine with rhodamine-B hydrochloride in EtOH to obtain **1**.[15] Its subsequent reaction

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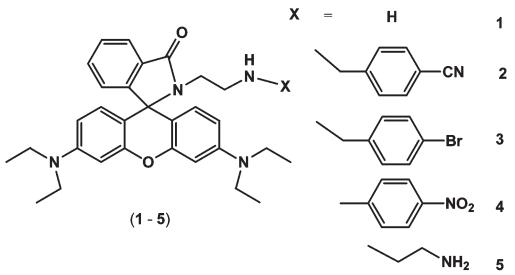
†Electronic supplementary information (ESI) available: Detailed synthetic procedure, characterization (^{1}H-, ^{13}C-NMR, ESI-MS, ESI: Fig. S26–S37) absorption and emission spectroscopic and structural data of **2–4**. CCDC 855291 (for **4**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob07182g.



Scheme 1 Pictorial representation of the probes (**1–5**).

with 4-cyanobenzaldehyde and 4-bromobenzaldehyde in EtOH followed by reduction with $NaBH_4$ afforded the reduced Schiff bases **2** and **3** respectively as desired probes. The aromatic nucleophilic substitution reaction of **1** with 4-fluoro-1-nitrobenzene resulted in **4**, which crystallizes‡ from acetonitrile through a slow evaporation technique. The diethylenetriamine appended rhodamine[16] (**5**) was synthesized and its metal ion induced signalling responses in organic–aqueous medium were compared with those of **2–4**. The characteristic peak near ~66 ppm (C_q) in ^{13}C-NMR spectrum of **1–5** in $CDCl_3$ ascertains the predominant existence of rhodamine's spirolactam conformation.

The absorption and emission spectra of **2–5** in various solvents (ESI: Fig. S1–S2†) revealed that these probes do not absorb in 500–600 nm region and exhibit a weak fluorescence (ϕ_{FT} < 0.001) in MeCN or MeCN–H_2O (1 : 1 v/v), a characteristic behaviour attributed to that of lactonized rhodamine. Apart from the high energy ligand localized absorption transitions for all probes, **4** also exhibits a solvatochromic absorption (λ_{max} = 360 nm in hexane, 40 nm red-shifted in DMSO) due to the intramolecular charge transfer[17] occurring from distal donor$_{Amino}$ to acceptor$_{Nitro}$ moiety through a π-spacer. The quenched emission in each probe is attributed to an efficient intersystem crossing occurring in the lactonized rhodamine, apart from the operative photo-induced electron transfer processes from distal Amino$_{-donor}$ groups present in their receptor moiety.

In order to investigate the metal ion induced signalling responses in these probes, various metal ions such as Na(I), K(I), Mn(II), Fe(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Pb(II), Ag(I), Cd(II) and Hg(II) were added to the probe solution in MeCN or MeCN–H_2O (1 : 1 v/v) [Fig. 1, ESI: Fig. S3†]. The colourless solution of **2** in MeCN leads to the appearance of an absorption peak at 557 nm (Fig. 1a) with a subsequent change in colour to pink selectively in presence of Fe(III) ion ($\varepsilon_{\mathbf{2}+Fe(III)}$ = 11 448 dm^3 mol^{-1} cm^{-1}), while the presence of other metal ions imparts a smaller or no change ($\varepsilon_{\mathbf{2}+M(I/II)}/\varepsilon_{\mathbf{2}}$ < 90 fold) in comparison to that with Fe(III) ion ($\varepsilon_{\mathbf{2}+Fe(III)}/\varepsilon_2$ = 1047 fold). Its metal ion induced change in emission (I_{580}) spectral pattern follows its absorption spectral behaviour in MeCN, fluoresces (ϕ_{FT} = 0.632) selectively in presence of Fe(III) ion (Fig. 2a). The absorption and emission amplifications as well as the colourless → pink colour transition of **2** in MeCN selectively in the presence of Fe(III) ions is attributed to the complexation induced delactonization of the rhodamine spiro-ring to its ring-opened amide conformation.

When measured in MeCN–H_2O (1 : 1 v/v) rather than MeCN, **2** renders such signal amplification [A_{557}(ε = 15 556 dm^3 mol^{-1} cm^{-1}) and I_{580} (ϕ_{FT} = 0.603)] selectively in the presence of Hg(II) ions (Fig. 1b). The Fe(III) ion, which resulted in enhancement in absorption and emission spectral responses of **2** in MeCN (ESI: Fig. S4†), even fails to exhibit such A_{557} and I_{580} amplification in MeCN–H_2O medium, although it exhibits an enhanced absorption at 356 nm (Fig. 1b, ε_{356} = 71 333 dm^3 mol^{-1} cm^{-1}). Thus, **2** exhibits altered signalling responses by switching the selectivity from Fe(III) to Hg(II) ions as a function of medium where the MeCN–H_2O binary mixture promotes selective Hg(II) coordination to **2** and restricts the coordination of other metal ions. A controlled experiment (ESI: Fig. S5†) was carried out to demonstrate the switching of metal ion selectivity between Fe(III) and Hg(II) ions in **2** as a function of medium. When water was added to the solution containing **2** and Fe(III)

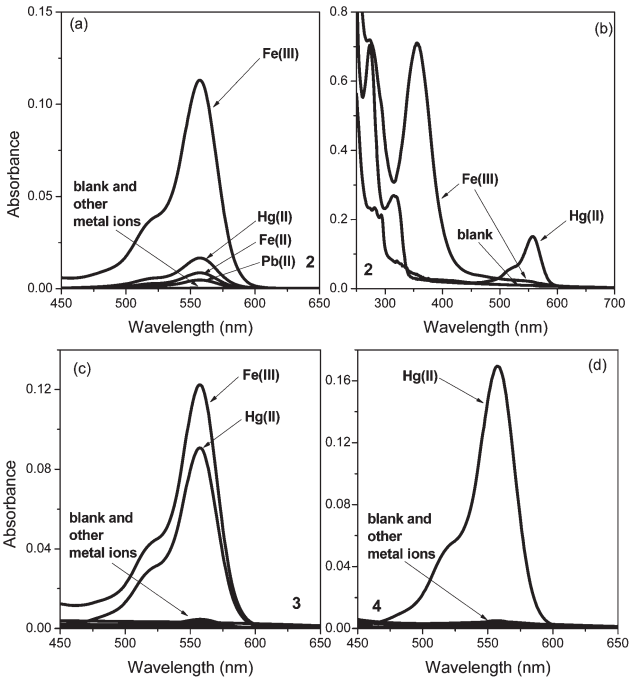


Fig. 1 Absorption spectral responses of **2** alone and in the presence of various metal ions in (a) MeCN and (b) MeCN–H_2O (1 : 1 v/v) medium. Absorption spectra of (c) **3** and (d) **4** in presence of various metal ions in MeCN. [probe] = 1 × 10^{-5} M, [M(I/II)] = 2 × 10^{-4} M in all the cases.

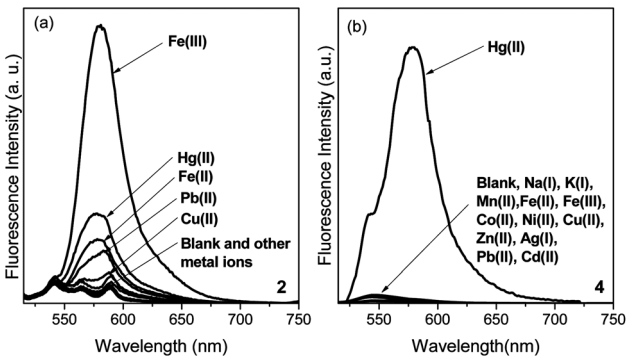


Fig. 2 Fluorescence spectral pattern of (a) **2** and (b) **4** alone and in the presence of various metal ions in MeCN. [**2** and **4**] = 0.5 × 10^{-6} M, λ_{ex} = 500 nm, RT, ex. and em. b.p. = 5 nm.

ions in MeCN (50% v/v, maintaining the concentration of **2** same), the Fe(III)-ion induced enhanced absorption and emission decreases almost to the extent of the metal free probe with pink colour of the solution disappearing. Further addition of Hg(II) to the same solution results in enhancement in A_{557}/I_{581} peaks and the reappearance of the pink colour of the solution. This infers that **2**, which exhibits preferential Fe(III) ion induced chromo- and fluorogenic signal amplification in MeCN through delactonization of the rhodamine unit, gets decomplexed with Fe(III) ions in the presence of water due to a preferential hydration of Fe(III) ions over probe-metal coordination and restores its spirolactam configuration. Addition of Hg(II) to the same solution leads to aqueous promoted selective Hg(II)-complexation to render the colourless → pink transition with enhanced A_{557} and I_{581} peaks through delactonization of the rhodamine again. Addition of other metal ions to the aqueous mediated Fe(III) decomplexed colourless solution of **2** fails to induce delactonization of the spiro ring to induce a similar effect. This establishes that **2** switches its selectivity from Fe(III) ions in MeCN to Hg(II) ions in MeCN–H_2O (1 : 1 v/v) medium.

The absorption and emission spectral pattern of **3** in MeCN in the presence of all these metal ions revealed that it does not exhibit any selectivity; it's rhodamine spiro-ring delactonizes in the presence of either Hg(II) or Fe(III) ions to exhibit enhancement in absorption (Fig. 1c) with high molar extinction coefficients ($\varepsilon_{\mathbf{3}+Hg(II)} = 9096$ and $\varepsilon_{\mathbf{3}+Fe(III)} = 12\,292$ dm^3 mol^{-1} cm^{-1}) and emission ($\phi_{FT(\mathbf{3}+Hg(II))} = 0.598$, $\phi_{FT(\mathbf{3}+Fe(III))} = 0.613$) while other metal ions fails to initiate the ring-opening process. Probe **4** absorbs ($\varepsilon_{557} = 16\,970$ dm^3 mol^{-1} cm^{-1}, Fig. 1d) and fluoresces ($\phi_{FT(581)} = 0.689$, Fig. 2b) in MeCN selectively in the presence of Hg(II) ions. Thus, the substituents attached to the distal donor amino group of the 'amino-ethyl-amido' rhodamine core alter the metal ion selective binding preferences as observed through chromogenic as well as simultaneous fluorogenic 'turn-on' signalling responses. It is worth mentioning that **3** and **4** also exhibit dual channel signalling responses selectively in the presence of Hg(II) ions in MeCN–H_2O (v/v, varied proportions) medium (ESI: Fig. S6†).

Addition of various metal ions to the colourless solution of **5** in MeCN leads[19] to a pink colour as well as amplification in its absorption and emission spectral pattern to an extent varying with the nature of the metal ions added. Although it attains maximum absorption (A_{557}, $\varepsilon_{\mathbf{5}+Hg(II)}/\varepsilon_{\mathbf{5}} = 1720$ fold) and emission ($\phi_{FT} = 0.608$) enhancement in the presence of Hg(II) ions, other metal ions also induce an appreciable spectral change ($\varepsilon_{\mathbf{5}+Pb}(II)/\varepsilon_{\mathbf{5}} = 1045$ fold, $\phi_{\mathbf{5}+Pb}(II) = 0.329$). However, **5** exhibits spectral enhancements in A_{557} ($\varepsilon = 20\,261$ dm^3 mol^{-1} cm^{-1}) and I_{580} ($\phi_{FT} = 0.643$) selectively in the presence of Hg(II) when measured in MeCN–H_2O (1 : 1 v/v) rather than in MeCN (ESI: Fig. S7†). The observed aqueous promoted Hg(II)-selectivity of **5** over other metal ions is consistent with other amino-[(ethylamino)$_{1,3,4}$]-derivatized rhodamine probes[18] under similar conditions. Although, the signalling responses of a diethylenetriamine attached rhodamine-G based probe[20] has been reported to exhibit Fe(III) selectivity, the signal perturbation in the presence of Hg(II) ions has not been evaluated in that case. Therefore, another unit of aminoethyl-substitution to the aminoethyl-rhodamine core as in **5** exhibits a preferential Hg(II) ion induced dual mode 'turn-on' signalling in MeCN and exhibits

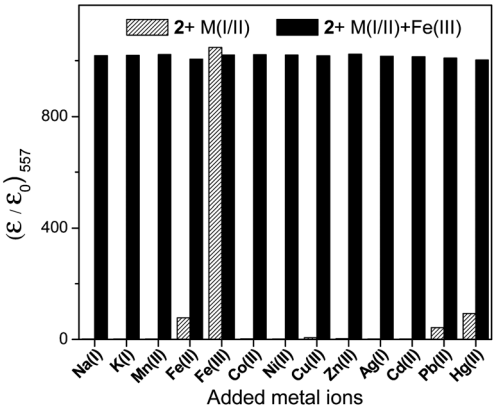


Fig. 3 Extent of absorption enhancement of **2** in the presence of other metal ions prior and after the addition of Fe(III) ions in MeCN. [**2**] = 1×10^{-5} M, [M] = 2×10^{-4} M.

here similar responses selectively in the presence of Hg(II) ions in organic–aqueous mixed medium. In comparison, the *p*-nitrophenyl substitution as in **4** selectively promotes Hg(II)-coordination to delactonize rhodamine's spiro-ring only, to exhibit such dual mode signalling process in both solvent conditions.

As the cross-sensitivity of any probe towards different metal ions limits its utility in selective detection of a metal ion, it has also been investigated with these probes (ESI: Fig. S8–S11†). Addition of other competitive metal ions to the solution containing **4** with Hg(II) ions in MeCN or MeCN–H_2O (v/v, varied proportions) does not perturb its absorption and emission spectral pattern. On the contrary, the lowered absorption and quenched emission of the individual solutions containing **4** and metal ions other than Hg(II) immediately results in A_{557} and I_{580} spectral enhancement along with the colourless solutions turning to pink upon addition of Hg(II) ions to the same extent of that of **4** alone in the presence of Hg(II) ions. This infers its high selectivity towards Hg(II) ions and the non-interference of other competitive metal ions. A similar experiment with **2** in MeCN ascertains its Fe(III) ion selective dual mode signalling responses (Fig. 3). Further, the time course studies as a function of spectral changes in absorption [$(A/A_0)_{557}$] and emission [$(I_F/I_0)_{580}$] of these probes with corresponding metal ions which selectively delactonize their rhodamine unit suggest that the probe–metal complexation occurs in almost 1 min and remains constant for at least 12 h in each case.

Further, the absorption/fluorescence spectral pattern of **2**–**4** in the 400–650 nm window does not exhibit any appreciable change with pH variation in 4.0–10.0 range. The spectral response of these probes in the absence and presence of Hg(II) in varying pH reveals that they yield stable complexation induced amplified signals in the same region without any interference from protons. However, the spectroscopic signal intensity of the metal free probes increase with a decrease in pH values (ESI: Fig. S12†) under more acidic conditions (pH < 4.0), yet it was found to be responsive towards corresponding specific metal ion coordination. Nevertheless, the signal stability of these probes and their complexes over a wide pH range indicate their potential utility in complicated/biological systems.

The plot of absorption spectral responses of **2** as a function of molar fraction of added Fe(III) ion in MeCN (continuous variation method) establishes its 1 : 1 (L : M) complexation stoichiometry. Similar plots (ESI: Fig. S13†) for absorption spectral responses of **3** with either Hg(II) or Fe(III), and that of **4** with Hg(II) also ascertain their stoichiometry of complexation to be 1 : 1 (L : M). The fluorescence spectral pattern of the titration of **4** (0.6 μM) with Hg(II) in MeCN–H_2O (1 : 1 v/v, pH = 7.0) revealed that the emission I_{580} increases with increasing Hg(II) concentration gradually up to 5 equiv. of Hg(II) and remains constant even up to addition of 25 equiv. of Hg(II). A further increase in Hg(II) concentration leads to an expected decrease in emission (I_{580}). The absorption and emission titration studies for other probes also follow similar spectroscopic trends (ESI: Fig. S14–S19†). From the fluorescence spectral changes (I_F/I_0) of these probes as a function of concentrations of added metal ions (Fig. 4), the association constants (k_a) of complexation are determined[21] to be 5.81×10^5 M^{-1} for **2** ⊂ Fe(III), 3.829×10^5 M^{-1} for **3** ⊂ Fe(III), 1.226×10^5 M^{-1} for **3** ⊂ Hg(II), 8.746×10^4 M^{-1} for **4** ⊂ Hg(II) and 8.527×10^4 M^{-1} for **5** ⊂ Hg(II) complexes respectively. The k_a were also determined through absorption spectral changes of similar titrations of these complexes, which are consistent (ESI: Table ST1†) with those obtained through fluorescence spectral changes (the correlation factor log [k_a(fluorescence)/k_a(absorption)] are found to be in 0.08–1.60 range).

The reversibility in metal ion induced absorption and emission signal responses of these probes were evaluated with subsequent addition of ammonium salts of various counter anions in MeCN : H_2O (1 : 1 v/v) medium (ESI: Fig. S20–S22†). The Hg(II)-induced enhancement in absorption (A_{557}) and emission (I_{580}) of **4** significantly decreases almost to that of the metal free probe **4** and the pink coloured solution turns colourless within 1 min in the presence of AcO^- anions. In the case of **4** ⊂ Hg(II), addition of other counter anions exhibits a negligible decrease in spectral responses and the colour of the solution remained pink for at least 12 h, except for I^- which also reduces the spectral pattern substantially though not to the same extent of AcO^- ions. The optical spectral responses of Hg(II) ⊂ **3** and Hg(II) ⊂ **5** complexes, however, get perturbed by many anions to an extent depending upon the probe–metal and metal–anion interactions. Apart from anions, **2–4** also regenerate their initial spirolactam state upon subsequent addition of chelating agents such as EDTA and ethylenediamine to these complexes in solution (ESI: Fig. S23†). Further addition of Hg(II) ions to a colourless solution of anion (AcO^-) mediated Hg(II)-decomplexed **4** ($\phi_{FT} \leq 0.002$) results in the reappearance of the pink colour and exhibits enhanced A_{557} and I_{580} peaks almost to the extent of those upon initial Hg(II) addition to **4**. This establishes its reusability as a probe for selective Hg(II) ion detection.

The X-ray diffracted crystallographic structure of **4** (Fig. 5) revealed that attachment of the electron-withdrawing 4-nitrophenyl group has lowered the degree of pyramidalization of the donor N_{amino}-atom (N4) almost to a planar conformation. Further, the N4–C31 bond (1.357(3) Å) distance is much shorter than the normal C–N bond (average N–C bond length: 1.450 Å) due to delocalization of electron density from N4 to the attached aromatic π-system inducing a partial double bond character and imposing a structural rigidity in the electron-withdrawing substituent, which is consistent with the ICT transition[17] of the D-π-A unit. The structural aspect of a signalling probe provides vital information toward the thermodynamics of metal binding and their coordination module. The variation in the size and spatial orientation of the chelation cavity of 'amino-ethyl-amido'-rhodamine core upon attachment of different substituents might have contributed significantly towards the stereo-electronic situation of the probe–metal coordination and hence, altered its metal ion selectivity preferences. Unfortunately, all attempts to get the crystals of other probes remained unsuccessful. Moreover, the crystal structures are favoured by the packing forces rather than by the intrinsic differences in free energies, thus, the probes might have different geometries in solution and may not be helpful in comparing their structures in solid states to understand the spatial orientation of the molecules. Hence, the geometrically optimized structures of **2–4** obtained by density functional theory (DFT) calculations[22] at B3LYP level were compared in order to verify the effect of substituent induced

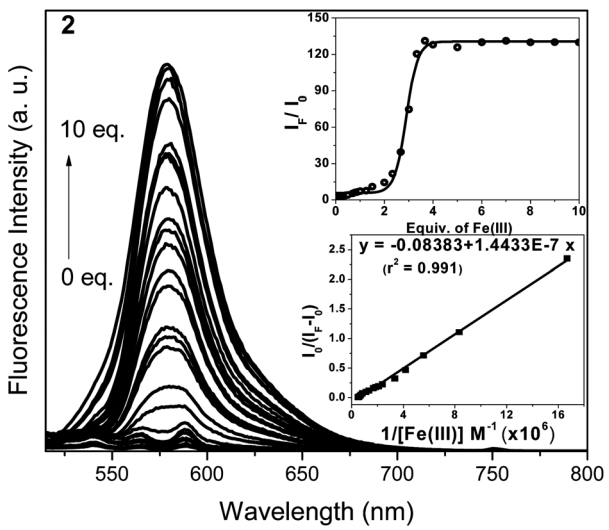


Fig. 4 Fluorescence spectral pattern of **2** as a function of added Fe(III) ions in MeCN. [**2**] = 1×10^{-6} M, λ_{ex} = 500 nm, RT, ex. and em. b.p. = 5 nm. (Inset, top) Change in fluorescence intensity (I_F/I_0) of **2** as a equivalents of Fe(III) ion added and (bottom) corresponding linear regression plot of $I_0/(I_F - I_0)$ *vs.* 1/[Fe(III)] M^{-1}.

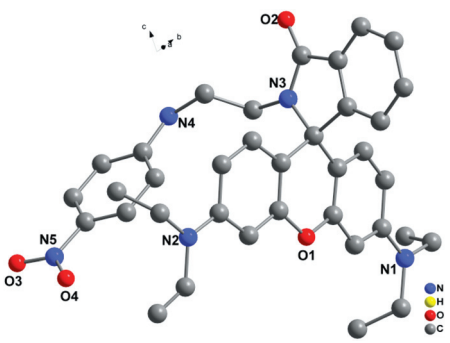


Fig. 5 Perspective view of the X-ray crystallographic structure of **4** (the hetero-atoms numbering scheme, H-atoms are omitted for clarity).

structural modification to the chelation cavity comprising donor atoms on altered complexation preferences of these substituted 'amino-ethyl-amido'-rhodamine framework (ESI: Fig. S24–S25, Table ST2–ST3†). The sum of angles around N_{donor}–atom (N4) to which the substituent is attached infers to its non planar geometry in **2** (346.56°) and **3** (346.54°), while to a lower degree of pyramidalization in **4** (359.99°) and is consistent with that of the X-ray diffracted crystallographic structure of **4**. The shorter N4–C31 bond distance in **4** (1.378 Å), in comparison to that in **2** (1.464 Å) and **3** (1.462 Å), suggests that attachment of 4-nitrophenyl induces a partial double bond character and restricts the rotation around it to impose a structural rigidity on **4**. Apart from spatial orientation, the non-bonded distances among the donor atoms, which constitute the chelation cavity, provide vital information towards effective and preferential metal ion coordination through modulation of the extent of orbital overlap. The non-bonded $O_{amido}(O2)\cdots N_{amino}(N4)$ and $N_{amido}(N3)\cdots N_{amino}(N4)$ distances, which vary depending upon the nature of the substituent, are found to be 4.462 Å and 3.822 Å respectively in **2**, 4.491 Å and 3.820 Å respectively in **3**, while a larger distance of 4.557 Å and 4.533 Å respectively is found in **4**. A smaller cavity dimension could accommodate only a smaller cation, hence, **2** preferentially binds to Fe(III) ions, which has a comparatively smaller ionic radius. The larger non-bonded distances, the electron and charge densities over donor atoms, and the structural rigidity of **4** suggests that Hg(II) ions favorably fits into its chelation-cavity and therefore it exhibits Hg(II)-selective signalling responses. The preliminary optimized geometries of these probes, which are presumed to follow a pre-organizational approach prior to complexation, establish their preferential coordination pattern among various metal ions.

In summary, the substituted aminoethyl-rhodamine based probe has been demonstrated here to switch its selectivity from Fe(III) to Hg(II) ion in a dual order. First, the *p*-cyanobenzyl-attached probe **2**, which exhibits Fe(III) selective fluorogenic and chromogenic signal amplification in MeCN through complexation-induced rhodamine delactonization, alters its selectivity to Hg(II) ions in MeCN–H_2O medium. Second, the Fe(III) selective dual signalling responses of **2** in MeCN switches its selectivity to Hg(II) ions when the electron density over the distal N_{amino} donor drifts away upon attachment of a *p*-nitrophenyl-group as in **4**. Similar methodological investigations of structural modifications might lead, in principle, to the design of dual mode signalling probes for selective detection of other metal ions. Further, the mixed aqueous–organic medium has shown here to promote Hg(II) selectivity in these probes as reflected by their signalling responses and other metal ions failing to bind under similar conditions; observations infer a competition between metal–ligand interaction and hydration of those metal ions, however, the mechanistic approach for understanding this process properly in rhodamine based signalling probes still remains elusive. We are presently working along this direction.

The authors wish to thank the Council of Scientific and Industrial Research, New Delhi for the financial support under CSIR-EMPOWER scheme (CSIR-IMMT-OLP-021) for this work and UGC, New Delhi for a senior research fellowship to B. Biswal.

Notes and references

‡Crystal data for **4**: $C_{36}H_{39}N_5O_4$; M_w = 605.72; needle-shaped; pale-yellow crystals, triclinic, space group $P\bar{1}$, a = 9.225(2) Å, b = 12.144(3) Å, c = 14.886(4) Å, α = 74.73(1), β = 78.29(2), γ = 83.99(4), U = 1572.9(6)Å^3, T = 296(2) K, Z = 2, μ(Mo K_α) = 0.085 mm^{-1}, F (000) = 644, ρ_{calc} = 1.279 mg m^{-3}, 7814 reflection data with 414 parameters, 5517 [$I \geq 2\ \sigma(I)$] unique reflections used in calculations. The final R_1 = 0.0771, wR_2 = 0.2389, S = 1.040.

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