From yellow to pink using a fluorimetric and colorimetric pyrene derivative and mercury (II) ions

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FROM YELLOW TO PINK USING A FLUORIMETRIC AND COLORIMETRIC PYRENE DERIVATIVE AND MERCURY (II) IONS

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Abstract
A new simple and multifunctional fluorescent probe based on pyrene linked through an imine bond to a hydroxyphenol unit was synthesised, fully characterized and investigated in solution. Additionally, its behaviour in the presence of several ions (H\textsuperscript{+}, Zn\textsuperscript{2+}, Cd\textsuperscript{2+}, Cu\textsuperscript{2+}, Ni\textsuperscript{2+}, Pb\textsuperscript{2+}, Fe\textsuperscript{2+}, Hg\textsuperscript{2+} and Al\textsuperscript{3+}) was investigated in CH\textsubscript{2}Cl\textsubscript{2}. It was found that the absorption spectra of probe 1 shows no differences for all ions titrations except in the presence of H\textsuperscript{+} and Hg\textsuperscript{2+} where a new longer wavelength band becomes visible (inducing a colour
change from yellow to orange and pink, respectively). In the emission, a blue-shift and an enhancement of the fluorescence of the probe for all metal ions was observed with the exception of Al$^{3+}$ where no detectable changes could be observed. The probe was found to be selective for Hg$^{2+}$ ion at low concentrations (till one equivalent).

**Keywords:** Colorimetric, Fluorescence, Pyrene, Schiff-base, Mercury (II).

1. Introduction

The design of new efficient probes capable of selective recognition of guest species, such as metal ions, is still an area of huge research in biological, analytical and environmental fields [1-10]. In addition, the demand for new colorimetric probes has widely increased, mostly because it involves the detection through a less expensive technique in which the analyte is detectable by a colour change discernible by direct visual observation. Nevertheless, fluorescent techniques also offer several advantages in comparison with others, such as high sensitivity, quick response and they are non-destructive [11,12]. In fluorescent probes several mechanisms, such as, intramolecular charge transfer (ICT) [13,14], photoinduced electron transfer (PET) [15,16], metal-ligand or ligand-metal charge transfer (MLCT or LMCT) [17], excimer/exciplex formation [18], imine isomerization [19], intermolecular hydrogen bonding [20], excited-state intramolecular proton transfer (ESIPT) [21], can be responsible for the enhancement or quenching of the fluorescence upon linkage to an analyte.
Pyrene has motivated researchers from fundamental and photochemical scientific areas mainly due to its unique fluorescent properties, namely the ability of forming an excited dimer, its long fluorescence lifetime and its $I_1/I_3$ vibronic ratio dependence with the polarity of the medium [22] being widely employed for supramolecular design, but also (intra or intermolecular) exciplexes in the presence of donor/acceptor chromophores [23]. These features might be also applied to sense environmental parameters, such as temperature [24], pressure [25], or pH [26], since the change of the fluorescence intensity of the excimer mirrors changes in the surrounding media. Moreover, pyrene can also be used to detect guest molecules, such as gases ($O_2$ or $NH_3$) [27,28], organic molecules [29-31], and metals [32-38].

Metal ions have a very important role namely in the stabilization and reactivity of proteins. However, for human and environmental welfare, they must exist in optimal quantities. Otherwise, they can promote metabolic disorders, since they are easily absorbed and accumulated from the environment [39]. Therefore, the design of specific probes which selectively detect and quantify small amounts of metal ions is still a challenge. Following our previous research with pyrene units [40], we have recently reported a single pyrene probe compound containing a fluorescent flexible dye [41]. Upon addition of metal ions, shows different colours from its yellow initial, such as, dark red for $Cu^{2+}$, red orange for $Zn^{2+}$ and gold yellow for $Ag^+$, thus allowing a direct visual differentiation and detection between these metal ions [41]. It is also worthy to report a recent study where an azide derivative of pyrene has shown to demonstrate a catalytic hydrolysis (to the aldehyde derivatives) upon addition of $Hg^{2+}$; [42] this was not found with other metal cations which led to the
conclusion that this could be used as an efficient chemosensor towards mercury.

Metal ions, as Cd\(^{2+}\), Pb\(^{2+}\) and Hg\(^{2+}\) are highly toxic and pollutant and once present in water they are carcinogenic. The conversion of inorganic Hg\(^{2+}\) metal ions (by bacteria) into organic mercury compounds such as methyl- or ethyl-mercury which are highly toxic, causing several cancer diseases and motion disorders [43-47].

Recently, L. Zhang et al. developed an innovative single molecular sensor 1 for fluoride and cyanide using different signaling channels based on a phenolic Schiff base bearing a pyrene group [48]. This sensor showed a highly selective colorimetric response to fluoride based on a deprotonation process and a highly selective fluorescent response to cyanide based on a cyclization process in DMSO solution.

More recently, M. Shellaiah et al. explored the photophysical behabiuar of compound 1 in CH\(_3\)CN after the addition of different metal ions Li\(^{+}\), Ag\(^{+}\), K\(^{+}\), Na\(^{+}\), Cs\(^{+}\), Ni\(^{2+}\), Fe\(^{3+}\), Co\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\), Pb\(^{2+}\), Ca\(^{2+}\), Cr\(^{3+}\), Mg\(^{2+}\), Cu\(^{2+}\), Mn\(^{2+}\), Hg\(^{2+}\), Fe\(^{2+}\) and Ag\(^{2+}\)) in H\(_2\)O [49].

Herein we synthesized the same Schiff base pyrene derivative 1 according with the method reported recently by L. Zhang et al [48]. This compound was also characterized by elemental analysis, \(^1\)H and \(^{13}\)C-NMR, melting point, IR (infrared), MALDI-TOF-MS spectrometry, UV-Vis absorption and fluorescence spectroscopy (SS and TR) and X-ray analysis. With the aim of evaluating the influence of the solvent in the coordination properties, the sensorial ability of probe 1 towards H\(^{+}\), Zn\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Ni\(^{2+}\), Pb\(^{2+}\), Fe\(^{2+}\),
Hg$^{2+}$ and/or Al$^{3+}$ ions were explored by absorption and fluorescence spectroscopy in dichloromethane solution.

Insert Scheme 1

2. Experimental

2.1. Chemicals and starting materials

Al(NO$_3$)$_3$.6H$_2$O, Zn(CF$_3$SO$_3$)$_2$, Cd(CF$_3$SO$_3$)$_2$, Cu(CF$_3$SO$_3$)$_2$, Ni(CF$_3$SO$_3$)$_2$, Pb(CF$_3$SO$_3$)$_2$, Hg(CF$_3$SO$_3$)$_2$, and FeCl$_2$.4H$_2$O salts, fluoroboric acid (HBF$_4$), o-aminophenol and pyrene-1-carbaldehyde have been purchased from Strem Chemicals, Sigma Aldrich and Solchemar. The solvents were obtained from Panreac and Riedel-de Haen and used as received.

2.2. Physical measurements

Elemental analyses were carried out on a Fisons EA-1108 analyser in the elemental analyses Service of the University of Vigo, Spain. NMR spectra of the ligands were obtained on a Bruker spectrometer operating at frequency of 400 MHz for $^1$H and $^{13}$C-NMR using the solvent peak as internal reference, in the facilities of the University of Santiago de Compostela, Spain. Melting points were determined on a Gallenkamp apparatus and are uncorrected. Infrared spectra were recorded in KBr windows using JASCO FT/IR-410 spectrophotometer from BIOSCOPE group.
2.3. Spectrophotometric and Spectrofluorimetric measurements.

Absorption spectra were recorded on a JASCO V-650 UV-visible or on a Shimadzu 2450 UV-Vis spectrophotometers and fluorescence emission spectra on a HORIBA JOBIN YVON Fluoromax-4 or on a Horiba-Jobin-Ivon SPEX Fluorog 3-22 spectrofluorimeters. The linearity of the fluorescence emission vs. concentration was checked in the concentration used ($10^{-4}$ – $10^{-6}$ M). A correction for the absorbed light was performed when necessary. The fluorescence decays of compound 1 were obtained with picosecond resolution (ps-TCSPC) with the equipment described elsewhere [50] and were analysed using the method of modulating functions implemented by Striker et al. [51]. The experimental excitation pulse [full width at half maximum (fwhm)= 21 ps] was measured using a LUDOX scattering solution in water. After deconvolution of the experimental signal, the time resolution of the apparatus was ca. 2 ps.

The spectrometric characterizations and titrations were performed as follows: the stock solutions of compounds 1 (ca. $10^{-3}$ M) were prepared by dissolving an appropriated amount of compound in a 10 ml volumetric flask and diluting to the mark with dichloromethane. The solutions were prepared by appropriate dilution of the stock solutions still $10^{-5}$ - $10^{-6}$ M. Titrations of compounds 1 were carried out in dichloromethane by the addition of microliter amounts of standard solutions of the ions in acetonitrile. All the measurements were performed at room temperature.

The detectable amount of the metal ions were realized by a standard addition method in dichloromethane, whereas, known amounts of metal ions were added to an aliquot containing a solution of probe 1.
3. Results and Discussion

3.1. Synthesis

Compound 1 was synthesized following a simple one-pot method, by direct condensation of o-aminophenol and pyrene-1-carbaldehyde following the method reported by L. Zhang et al. [48]. The reaction pathway is shown in Scheme 2. Compound 1 was isolated as an air-stable yellow solid, ca. 80% yield. Elemental analysis data confirmed that the Schiff-base 1 was isolated in a pure form. The infrared spectrum (in KBr) shows a band at 1648 cm\(^{-1}\) corresponding to the imine bond, and no peaks attributable to unreacted amine or carbonyl groups were found. The absorption bands corresponding to the \(\gamma(\text{C=C})\) vibrations of the phenyl groups appear at 1456 cm\(^{-1}\). The MALDI-TOF-MS spectrum of compound 1 shows a peak at 322.1 m/z, corresponding to the protonated form of the ligand \([1+H]^+\). The \(^1\text{H}\) NMR spectrum shows a peak at ca. 8.68 ppm, corresponding to the imine proton, and no signals corresponding to the amine protons were found. Yellow crystals of compound 1 suitable for X-ray diffraction were obtained by slow diffusion of diethyl ether into a dichloromethane solution of this compound at room temperature (see figure SI1 and table SI1).

*Insert Scheme 2*

3.2. Photophysical characterization of compound 1

The absorption, fluorescence emission (and excitation) spectra of 1 were obtained in dichloromethane. The absorption spectra show a characteristic pyrene derivative spectra [41] with a maximum at 426 nm (red shifted compared
to the pyrene absorption spectra) and a high extinction coefficient value (112870 cm⁻¹.M⁻¹). Moreover, the emission spectra of 1 displays an unresolved and broad emission band centred at ca. 500 nm, with low fluorescence likely resulting from a photoinduced electron transfer (PET) from the nitrogen lone pair to the aromatic moiety (intramolecular PET) [52] (see figure SI2).

3.3. Sensorial ability of compound 1 towards H⁺, Zn²⁺, Cd²⁺, Cu²⁺, Ni²⁺, Pb²⁺, Fe²⁺, Hg²⁺ and Al³⁺ ions

M. Shellaiah et al. explored the photophysical behavior of compound 1 in CH₃CN after the addition of different metal ions Li⁺, Ag⁺, K⁺, Na⁺, Cs⁺, Ni²⁺, Fe³⁺, Co²⁺, Zn²⁺, Cd²⁺, Pb²⁺, Ca²⁺, Cr³⁺, Mg²⁺, Cu²⁺, Mn²⁺, Hg²⁺, Fe²⁺ and Ag²⁺) in H₂O [49]. Compound 1 showed better selectivity to Cu²⁺ upon treatment with 2.5 equiv. of this metal ion in CH₃CN. Probe 1 illustrated the fluorescence turn-on sensing towards Cu²⁺ via chelation enhanced fluorescence (CHEF) through excimer (1-1*) formation. The 2:1 stoichiometry of the sensor complex (1 + Cu²⁺) was also calculated from Job plots based on UV-Vis absorption titrations.

In that case, the solubility of compound 1 in different solvents were tested (see figure SI3). We selected CH₂Cl₂ as the preferential solvent to evaluate the sensorial ability of compound 1 for H⁺, Zn²⁺, Cd²⁺, Cu²⁺, Ni²⁺, Pb²⁺, Fe²⁺, Hg²⁺ and Al³⁺ ions by absorption and fluorescence emission spectroscopy. The results obtained in this solvent (CH₂Cl₂) could be compared with that obtained in CH₃CN [49] to evaluate the influence of the solvent in the coordination properties.
Figure 1 shows the absorption and emission spectra of compound 1 upon the addition of H\(^+\). As can be seen, in the ground state the addition of H\(^+\) induces to the appearance of a new (orange) colored band located at ca. 545 nm. As can be seen in Figure 1B and inset, a blue shift and an enhancement of the emission intensity was visualized.

The appearance of the long wavelength absorption band clearly indicates the potential of 1 as a colorimetric probe. However, this becomes clearer with the addition of metal ions. Indeed, the addition of one equivalent of transition and post-transition metal ions (Zn\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Ni\(^{2+}\), Pb\(^{2+}\), Fe\(^{2+}\), Hg\(^{2+}\) and Al\(^{3+}\)) did not induce spectral changes in the absorption spectra with the sole exception of Hg\(^{2+}\) for which a change of color from yellow to pink was detected. However, when the amount of metal ions is increased to 10 equivalents, a colorimetric behaviour is equally visualized with Cu\(^{2+}\) and Fe\(^{2+}\) (see picture 1A and B).

In which regards the emission behaviour (spectra) of 1, a blue-shift and an enhancement of the emission spectra (with maximum at 500 nm) was observed upon addition of the aforementioned metal ions (see figure 2). However, probe 1 does not show any spectral changes upon addition of the Al\(^{3+}\) metal ion (data not shown). Figure 2 shows the emission spectra of compound 1 with the incremental addition of Zn\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Ni\(^{2+}\), Pb\(^{2+}\) and Fe\(^{2+}\) metal ions with excitation at 426 nm.

*Insert Figure 2*

As an example of the colorimetric behaviour observed, figure 3 presents the absorption and emission spectra of compound 1 upon addition of Hg\(^{2+}\).
As seen with H\textsuperscript{+}, the increase of Hg\textsuperscript{2+} ion concentration (in a dichloromethane solution) caused an absorbance decrease at ca. 426 nm and an increase at ca. 540 nm. But here sharp isosbestic points were detected (Figure 3A) at 282, 310 and 345 nm and, more evident, at 465 nm. The appearance of the new band and consequently of the observed red shift of the maxima results from the interaction of these ions (H\textsuperscript{+} and Hg\textsuperscript{2+}) with the ligand; in this case, a change from yellow to pink was visualized. Moreover the reversibility of color indicates that there is no degradation of 1 under acidic media and in the presence of Hg\textsuperscript{2+}.

Taking into account the heavy metal ion nature of Hg\textsuperscript{2+}, it would be expected a CHEQ effect (Chelation Enhancement of the Quenching) in the emission spectra [53]. However, a Chelation Enhancement of the Fluorescence Emission (CHEF) at 500 nm was observed (see Figure 3B) similar to that showed for compound 1 with 2.5 equiv. of Cu\textsuperscript{2+} in CH\textsubscript{3}CN [49]. In this particular case, the formed complex emission contribution overcomes the heavy metal ion effect, which is usually coupled with non-radiative decay routes, such as the S\textsubscript{1} to T\textsubscript{1} intersystem crossing, which would have the effect of decreasing the fluorescence emission.

As mentioned above, the low fluorescence likely results from a photoinduced electron transfer (PET) from the nitrogen lone pair to the aromatic moiety. Upon cation binding the redox potential of the donor is raised and the HOMO that was involved in the PET process has now a lower energy (stabilization arising from the covalent bonding between the donor and the
metal ion, which requires both electrons). As a consequence, PET becomes impossible, and fluorescence intensity is enhanced.

In order to evaluate the potential of the proposed turn-on fluorescent probe for detecting metal ions, the detectable amount of Zn$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Hg$^{2+}$, Pb$^{2+}$ and Fe$^{2+}$ metal ions were explored by a standard addition method in dichloromethane. The analytical results are summarized in Table 1.

The association constants for metal ion interaction were determined using the HypSpec program [54] and the main results are gathered in Table 1. As expected, the highest association constant was obtained for mercury ion with a value of $\log K_{ass} = 13.11 \pm 0.02$, followed by Cu$^{2+}$, Zn$^{2+}$, Pb$^{2+}$, Ni$^{2+}$, Cd$^{2+}$ and Fe$^{2+}$ metal ions. A stoichiometry of two ligands per metal ion was postulated.

*Insert Table 1*

Figure 4 shows a graphic bar of compound 1 without metal and upon addition of 0.5 equivalents of each metal ion together (picture on top) with the respective colour changes. Similar to the association constants, the strongest interaction was observed with Hg$^{2+}$ and Cu$^{2+}$ metal ions.

*Insert Figure 4*

Picture 1 shows the colorimetric behaviour of system 1 under four different conditions A to D. Condition A shows the color of the solutions of 1 with the addition of one equivalent of metal ions; condition B with the addition of 10 equivalents; condition C with the initial addition of 10 equivalents and
Further addition of one equivalent of Hg\(^{2+}\); condition D with the initial addition of 10 equivalents of metal ions and further addition of three equivalents of proton.

*Insert Picture 1*

Taking into account the results observed, we might conclude that compound 1 is colorimetric selective for Hg\(^{2+}\) ion in low concentrations, up to one equivalent. Moreover a different palette of pink, orange and violets colours can be developed with 1 upon examining the different metal ions and protons as color modulators.

Compound 1 showed a selective fluorescence response to Cu\(^{2+}\) in CH\(_3\)CN [49]. The results obtained in CH\(_2\)Cl\(_2\) offered the advantage of using compound 1 as a colorimetric and fluorescence molecular probe for the detection of different metal ions. It was also observed that a small quantity of the different metal ions was necessary to obtained a spectrophotometric and/or spectrofluorimetric response in CH\(_2\)Cl\(_2\) solution.

### 3.4. Lifetime measurements

Further knowledge on the photophysical behavior of 1 comes from time resolved data. The fluorescence decay of compound 1 was obtained in dichloromethane with excitation at 451 nm and with emission at 500 nm, see Figure SI4.

In the case of the free ligand the decay is single exponential with a decay time of 70 ps.
In the presence of mercury, the analysis of the decays leads to a bi-
exponential fit. The 3.35 ns species is the dominant absorbing species with a
contribution of more than 98% to the total emission. It is worth noting that the
3.35 ns species (found for 1 in the presence of mercury) is much different than
the value obtained for the free ligand (70 ps) thus once more confirming that
PET is precluded.

4. Conclusions

In summary, the Schiff base pyrene derivative (1) was successfully
synthetized and fully characterized. Probe 1 shows to be colorimetric with a
change of colour from yellow to pink for Hg^{2+} metal ion at low concentration
(till one equivalent), and fluorimetric with a strong enhancement of the
emission intensity with the highest association constant of
\[ \log K_{ass} = 13.11 \pm 0.02. \]
Moreover from the starting yellow colour, a different
palette of pink, orange and violets colours can be developed with dye 1 when
examining different metal ions and protons as color modulators. The results
obtained in CH₂Cl₂ offered the advantage of using compound 1 as a
colorimetric and fluorescence molecular probe, while in CH₃CN, only a
fluorescence response was showed by probe 1.

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Supplementary data

Crystal data, the synthesis and characterization data of probe 1 are provided. Crystallographic data on the structure of 1 reported in this paper are available in the Cambridge Crystallographic Data Centre as deposition No. CCDC 993826.
References


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Captions

**Table 1.** Association constants, minimal amount of metal ions detectable by fluorescence emission for compound 1 in dichloromethane at 293 K.
Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Metal ion</th>
<th>Log $K_{ass}$ (M:L)</th>
<th>Amount detectable (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn$^{2+}$</td>
<td>12.52±0.01 (1:2)</td>
<td>1.20</td>
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<tr>
<td>Cd$^{2+}$</td>
<td>9.80±0.01 (1:2)</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>13.00±0.01 (1:2)</td>
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<tr>
<td>Ni$^{2+}$</td>
<td>10.52±0.01 (1:2)</td>
<td>2.62</td>
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<tr>
<td>Pb$^{2+}$</td>
<td>11.31±0.01 (1:2)</td>
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<tr>
<td>Fe$^{2+}$</td>
<td>9.59±0.01 (1:2)</td>
<td>13.24</td>
<td></td>
</tr>
<tr>
<td>Hg$^{2+}$</td>
<td>13.11±0.02 (1:2)</td>
<td>0.62</td>
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</tr>
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</table>
FLUORIMETRIC AND COLORIMETRIC PYRENE DERIVATIVES FOR THE TOXIC MERCURY (II) METAL ION

Daniela Pinheiro\textsuperscript{a,b}, Catherine S. de Castro\textsuperscript{a}, J. Sérgio Seixas de Melo\textsuperscript{a,*}, Elisabete Oliveira\textsuperscript{b,c,d}, Cristina Nuñez\textsuperscript{b,e,f}, A. Fernández-Lodeiro\textsuperscript{b,d}, José Luis Capelo\textsuperscript{b,d} and Carlos Lodeiro\textsuperscript{b,d,*}

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Captions

**Scheme 1.** Chemical structure of compounds 1.

**Scheme 2.** Schematic synthesis of compound 1.

**Figure 1.** Spectrophotometric (A) and spectrofluorimetric (B) titrations of compound 1 with the addition of H\(^{+}\) in dichloromethane. The inset represents the emission intensity at 500 nm (B) as a function of [H\(^{+}\)]/[1]. ([1] = 3.0×10\(^{-6}\) M, \(\lambda_{\text{exc}}(B) = 426\) nm, T = 298 K).

**Figure 2.** Spectrofluorimetric titrations of compound 1 with the addition of Zn\(^{2+}\) (A), Cd\(^{2+}\) (B), Cu\(^{2+}\) (C), Ni\(^{2+}\) (D), Pb\(^{2+}\) (E) and Fe\(^{2+}\) (F) in dichloromethane. The inset represents the emission intensity at 500 nm as a function of [Zn\(^{2+}\)]/[1] (A), [Cd\(^{2+}\)]/[1] (B), [Cu\(^{2+}\)]/[1] (C), [Ni\(^{2+}\)]/[1] (D), [Pb\(^{2+}\)]/[1] (E) and [Fe\(^{2+}\)]/[1] (F). ([1] = 3.0×10\(^{-6}\) M, \(\lambda_{\text{exc}} = 426\) nm, T = 298 K).

**Figure 3.** Spectrophotometric (A) and spectrofluorimetric (B) titrations of compound 1 with the addition of Hg\(^{2+}\) in dichloromethane. The inset represents the absorption (A) at 426 and 540 nm; and represents the emission intensity (B) at 500 nm as a function of [Hg\(^{2+}\)]/[1]. ([1]_{Abs.} = 1.20×10\(^{-5}\) M, [1]_{em.} = 3.0×10\(^{-6}\) M, \(\lambda_{\text{exc}} = 426\) nm, T = 298 K).

**Figure 4.** From bottom to top. Normalized fluorescence intensity at 488 nm of compound 1 in dichloromethane with 0.5 equivalents of metals (Zn\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Ni\(^{2+}\), Pb\(^{2+}\), Fe\(^{2+}\), Hg\(^{2+}\) and Al\(^{3+}\) = M\(^{n+}\)) added in dichloromethane; and picture of these solutions.
**Picture 1.** Naked-eye colorimetric behaviour of system 1 in four different conditions A to D. Condition A shows the naked eye colour solutions of 1 with the addition of one equivalent of metal ions; condition B with the addition of ten equivalents; condition C with the initial addition of ten equivalents and further addition of one equivalent of Hg$^{2+}$; condition D with the initial addition of ten equivalents of metal ions and further addition of three equivalents of proton.
Figure 1
Figure 2
Figure 3
$I_{\text{norm.}}$/a.u.

Ligand vs Metal ion

$1\ +Cu^{2+}\ +Hg^{2+}\ +Zn^{2+}\ +Pb^{2+}\ +Ni^{2+}\ +Cd^{2+}\ +Fe^{2+}$
or $Al^{3+}$

Figure 4
Picture 1
FROM YELLOW TO PINK USING A FLUORIMETRIC AND COLORIMETRIC PYRENE DERIVATIVE AND MERCURY (II) IONS

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Research Highlights

* Multifunctional fluorescent probe based on pyrene and linked through an imine bond to a hydroxyphenol unit.

* Colorimetric and selective for the Hg$^{2+}$ metal ion at low concentrations.

* Fluorescence enhancement in the presence of H$^+$, Zn$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Pb$^{2+}$, Fe$^{2+}$, Hg$^{2+}$ metal ions.
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EXPERIMENTAL DATA

Synthesis of molecular probe 1

Synthesis of o-aminophenol

O-Nitrophenol (0.521 g, 3.747 mmol) in absolute ethanol (50 mL) was heated under reflux with palladium-carbon (0.257 g). Hydrazine hydrate (10 mL) was added in 1 mL portions, and the mixture was refluxed until the solution had become colorless. After filtration the solution was evaporated to dryness and crystallized from hot absolute ethanol when o-aminophenol (0.297 g, 73%) was obtained. Yield 0.297 g (73%). H\(^1\) NMR (DMSO-\(d_6\)), \(\delta\) (ppm): 6.95 (t, 1H, Ar-H); 6.59-6.53 (m, 2H, Ar-H); 6.39 (d, 1H, Ar-H); 5.96 (s, 1H, NH\(_2\)); 5.12 (s, 1H, O-H). Anal. Calcd for C\(_6\)H\(_7\)NO: C, 66.05; H, 6.45; N, 12.85; Found: C, 65.95; H, 6.25; N, 12.75.

Synthesis of compound 1

A solution of o-aminophenol (0.109 g, 1 mmol) in absolute ethanol (20 mL) was added dropwise to a solution of pyrene-1-carbaldehyde (0.231 g, 1 mmol) in the same solvent (30 mL). The resulting solution was gently refluxed with magnetic stirring for ca. 2h. The initial orange colour of the solution changed quickly to yellow. The solution, kept under nitrogen atmosphere, was stirred for 4h. A yellow powder precipitate formed, which was then filtered off, washed with cold absolute ethanol and cold diethyl ether, and dried under vacuum. Compound 1: Colour: yellow. Yield 0.274 g (80%). IR (cm\(^{-1}\)): \(\gamma\)(C=N)\(_{imine}\) 1648 cm\(^{-1}\); \(\gamma\)(C=C)\(_{ar}\) 1456 cm\(^{-1}\); H\(^1\) NMR (CDCl\(_3\)), \(\delta\) (ppm): 8.68 (s, 1H, CH\(_{imine}\)); 8.47-7.68 (m, 9H, Ar-H); 7.16-6.15 (m, 4H, Ar-H); 5.30 (s, 1H, O-
H); MALDI-TOF-MS (m/z): 322.1 [1+H]+. Anal. Calcd for C_{23}H_{15}NO.H_2O: C, 81.40; H, 5.05; N, 4.15; Found: C, 81.75; H, 5.26; N, 4.55.

**X-ray Crystal Structure Determination**

Single crystals of compound 1 were analyzed by X-ray diffraction. Crystallographic data were collected on a Bruker Smart 1000 CCD diffractometer at CACTUS (University of Santiago de Compostela, Spain) at 20 °C using graphite monochromated Mo-Kα radiation (λ= 0.71073 Å), and were corrected for Lorentz and polarisation effects. Empirical absorption corrections were also applied to the crystal structure obtained [i]. Complex scattering factors were taken from the program package *SHELXTL* [ii]. The structures were solved by direct methods, which revealed the positions of all non-hydrogen atoms. All of the structures were refined on $F^2$ by a full-matrix least-squares procedure using anisotropic displacement parameters for all non-hydrogen atoms. The hydrogen atoms were located in their calculated positions and refined using a riding model. Molecular graphics were obtained with *ORTEP-3* [iii].

**RESULTS AND DISCUSSION**

**Crystallography data**

Yellow crystals of compound 1 suitable for X-ray diffraction were obtained by slow diffusion of diethyl ether into a dichloromethane solution of compound 1 at room temperature. Compound 1 was subjected to X-ray single crystal analysis, and Figure 5 shows the ORTEP view. Summary of the
crystallographic data and the structure refinement parameters is reported in Table 2.

The crystal of compound 1 belongs to the monoclinic space group P2(1)/c. As shown in Figure 5, the structure is quite planar where the unique remarkable point shows that the hydroxyl group is pointing out of the molecular plane with an angle of 114.2° (C(25)-C(20)-N(1)). It is not indicated that there are weak intermolecular π–π stacking interactions in the crystal lattice of compound 1.

**Figure SI1.** Crystal structure of compound 1. Ellipsoids are shown at the 50% probability level. Selected bond lengths (Å) and angles (°): C(17)-C(16), 1.464(9), N(1)-C(17) 1.279(8), N(1)-C(20) 1.423(9), C(20)-C(25) 1.384(9), C(25)-O(1) 1.358(9), C(25)-C(24) 1.384(11), C(13)-C(16)-C(17) 123.9(7), N(1)-C(17)-C(16) 123.6(8), C(17)-N(1)-C(20) 120.8(7), C(25)-C(20)-N(1) 114.2(8), C(21)-C(20)-N(1) 128.1(7), O(1)-C(25)-C(20) 119.9(8), O(1)-C(25)-C(24) 118.7(8).
Table SI1. Crystal data and structure refinement for compound 1.

<table>
<thead>
<tr>
<th></th>
<th><strong>Compound 1</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empirical formula</strong></td>
<td>C$<em>{50}$H$</em>{37}$N$_3$O$_2$</td>
</tr>
<tr>
<td><strong>Formula weight</strong></td>
<td>711.83</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>293(2) K</td>
</tr>
<tr>
<td><strong>Wavelength</strong></td>
<td>0.71073Å</td>
</tr>
<tr>
<td><strong>Crystal system</strong></td>
<td>Monoclinic</td>
</tr>
<tr>
<td><strong>Space group</strong></td>
<td>P2(1)/c</td>
</tr>
</tbody>
</table>
| **Unit cell dimensions** | a = 12.376(6) Å, α = 90°  
                          | b = 11.291(5) Å, β = 107.914 (7)°  
                          | c = 12.249(6) Å, γ = 90°          |
| **Volume**           | 1628.6 (13) Å$^3$                                                             |
| **Z**                | 2                                                                             |
| **Density (calculated)** | 1.452g/cm$^3$                                                           |
| **Absorption coefficient** | 0.089mm$^{-1}$                                       |
| **F(000)**           | 748                                                                           |
| **Crystal size**     | 0.34 x 0.22 x 0.18 mm$^3$                                                     |
| **Theta range for data collection** | 1.73 to 25.09°                                      |
| **Index ranges**     | -14 ≤ h ≤ 12, -10 ≤ k ≤ 13, -14 ≤ l ≤ 14                                    |
| **Reflections collected** | 5359                                                                 |
| **Independent reflections** | 2416 [R(int) = 0.0689]                                        |
| **Completeness to theta =** | 83.3% (25.09°)                       |
| **Absorption correction** | None                                                                |
| **Refinement method** | Full-matrix least-squares on F$^2$                                           |
| **Data / restraints / parameters** | 2416 / 0 / 234                      |
| **Goodness-of-fit on F$^2$** | 1.115                                                                    |
| **Final R indices [I>2sigma(I)]** | R1 = 0.1294, wR2 = 0.2471                        |
| **R indices (all data)** | R1 = 0.2392, wR2 = 0.2970                                                    |
| **Largest diff. peak and hole** | 0.204 / -0.163Å$^3$                                                |
Figure SI2. Normalized absorption, emission and excitation spectra of compound 1 in dichloromethane at 298 K.
Figure SI3. Visual color showed by compound 1 in the different solvents tested (THF, CH$_3$CN, CH$_2$Cl$_2$ and DMSO).
Figure SI4. Fluorescence decay of compound 1 in dichloromethane (A) metal free and (B) with [Hg$^{2+}$]/[1]= 1 at T= 293 K with $\lambda_{exc}$ = 451 nm and collected at $\lambda_{em}$ = 500 nm. The instrument profile curve, decay times ($\tau$), preexponential factors ($A_{i,j}$), $\chi^2$ values, weighted residuals, and autocorrelation functions (A.C.) are shown as insets.

[i] Sheldrick GM. SADABS, Program for Empirical Absorption Correction of Area Detector Data; University of Göttingen: Göttingen, Germany, 1996.

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