A phosphorus-supported coumarin-containing ligand as a fluorescence probe for detection of Cu(II) and Ag(I) ions

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A phosphorus-supported fluorescent probe \( \text{N}_3\text{P}_3(\text{O}_2\text{C}_{12}\text{H}_8)_2[\text{N(Me)N}=\text{CHR}]_2 \) (RCHO = 7-diethylamino-coumarin-3-aldehyde) has been synthesized by the condensation of \( \text{gem-N}_3\text{P}_3(\text{O}_2\text{C}_{12}\text{H}_8)_2[\text{N(Me)NH}_2]_2 \) with 7-diethylamino-coumarin-3-aldehyde. This compound has been shown to be an excellent fluorescence-based chemosensor of Cu\(^{2+}\) and Ag\(^{+}\) ions in aqueous solution.

**Keywords**: Fluorescence, Chemosensors, Probes, Phosphorus ligands, Cyclophosphazene, Hydrazides, Copper, Silver

In recent years, there has been an increased research interest in the development of fluorescent chemosensors \(^{1-3}\) that show high specificity and selectivity towards the detection of metal ions. The design of chemosensors consists of incorporating them with suitable binding sites and signaling subunits. Interaction of the binding sites with metal ions should result in a detectable response in the signaling unit such as change in fluorescence emission intensity (for a fluorescence-based chemosensor). Chemosensors based on changes in fluorescence emission are of particular interest in view of the good detection limits that can be achieved using this technique. Several types of fluorescence-based chemosensors for various types of metal ions are known in literature \(^{4-8}\).

We have been working on phosphorus-supported ligand systems for some time now \(^9\). For example, using \( (\text{S})\text{P[N(Me)N}=\text{CHC}_6\text{H}_4–2–\text{OH}]_3 \) \( (\text{LH}_3) \) we were able to assemble novel neutral trinuclear derivatives \( (\text{L}_2\text{M}_3) \) using divalent metal ions \(^{10}\). On the other hand, using trivalent metal ions afforded mononuclear compounds \(^{10}\). Modification of the ligand to \( (\text{S})\text{P[N(Me)N}=\text{CHC}_6\text{H}_4–2–\text{OH}–3–\text{OMe}]_3 \) allowed us to prepare several \( 3d–4f \) assemblies \(^{11}\), some of which were shown to possess interesting magnetic properties including single-molecule magnet behaviour \(^{12}\). More recently we have also shown that this ligand design could be further modulated such that their metal complexes possessed third-order NLO properties \(^{13}\).

In view of the above-mentioned versatility of phosphorus-supported ligands, it was of interest to examine if we could modify these systems such that they can function as fluorescence-based chemosensors. Accordingly, we have recently shown that pyrene-containing phosphorus-supported ligands, \( \text{PhP(O)}[\text{N(Me)N}=\text{CHPy}] \) and \( (\text{S})\text{P[N(Me)N}=\text{CHPy}]_3 \) (Py = pyrenyl) are excellent sensors for Cu\(^{2+}\), while \( \text{PhP(O)}[\text{N-(Me)N}=\text{CHPy}]_2 \) can be used for the specific detection of Mg\(^{2+}\) in solution \(^{14}\). We also investigated the coumarin-containing compounds, \( \text{PhP(O)}[\text{N(Me)N}=\text{CHR}]_3 \), \( (\text{S})\text{P[N(Me)N}=\text{CHR}]_3 \) and \( \text{N}_3\text{P}_3[\text{N(Me)N}=\text{CHR}]_6 \) (RCHO = 7-diethylamino-coumarin-3-aldehyde) \(^{15}\). We observed that compounds where C=N isomerization is prevented completely, upon metal binding, such as compounds containing one or two coumarin-containing arms are effective sensors for Cu\(^{2+}\), while those containing multiple arms are not \(^{15}\). We hypothesized that this could be a general strategy for designing fluorescence-based chemosensors based on this family of compounds. To test this, we have now prepared a cyclophosphazene derivative, \( \text{N}_3\text{P}_3(\text{O}_2\text{C}_{12}\text{H}_8)_2[\text{N(Me)N}=\text{CHR}]_2 \) (I) (RCHO = 7-diethylamino-coumarin-3-aldehyde) that contained only two coumarin-containing arms. In accordance with our expectation, this ligand was found to be an effective fluorescence-based chemosensor in aqueous solution for the detection of Cu\(^{2+}\) and Ag\(^{+}\). These results are reported in this paper.
Materials and Methods

Steady-state absorption and emission spectra were recorded on a spectrophotometer (Perkin-Elmer Lambda 20) and a fluorimeter (Varian Cary Eclipsed) respectively with a 10 mm quartz cell at 25 °C. Melting point was measured using a JSGW melting point apparatus and is uncorrected. $^1$H NMR spectrum was obtained on a JEOL-JNM Lambda 400 spectrometer operating at 400 MHz in CDCl$_3$ solution. $^{31}$P$^1$H NMR and $^{13}$C NMR spectra were obtained in CDCl$_3$ solutions on a JEOL-Delta2 500 spectrometer operating at 202.5 MHz and 125.0 MHz respectively. The chemical shifts are referenced with respect to TMS for $^1$H and 85 % H$_2$PO$_4$ for $^{31}$P NMR. Electrospray-Ionization High-Resolution mass spectra (ESI-HRMS) were recorded on a Micromass Quattro II triple quadruple mass spectrometer. The ESI capillary was set at 3.5 kV and the cone voltage at 40 V. 4-Diethylamino salicylaldehyde, diethyl malonate, 2,2'-dihydroxy biphenyl, hexachlorocyclo-triphosphazene, piperidine and metal salts such as Cu(ClO$_4$)$_2$·6H$_2$O, Pb(ClO$_4$)$_2$·xH$_2$O, Hg(ClO$_4$)$_2$·xH$_2$O, Ag(ClO$_4$)$_2$·H$_2$O, LiClO$_4$ and Na(ClO$_4$)$_2$·H$_2$O, were purchased from Sigma-Aldrich, USA, while the hydrated perchlorate salts, Zn(ClO$_4$)$_2$·6H$_2$O Cd(ClO$_4$)$_2$·4H$_2$O, Ni(ClO$_4$)$_2$·6H$_2$O, Mg(ClO$_4$)$_2$·6H$_2$O and Ca(ClO$_4$)$_2$·4H$_2$O were prepared from their carbonate salts with perchloric acid. gem-$\text{N}_3	ext{P}_3\text{O}_4\text{C}_{12}\text{H}_8$]$_2$[N(Me)NH$_2$]$_2$]$_{16}$ and 7-diethylamino-coumarin-3-aldehyde$^{17}$ were prepared using reported procedure. Solvents were received from SD Fine Chemicals (India) and were purified prior to use.

Fluorescence quantum yields were determined by comparing the emission intensity of the sample with that of fluorescein (φ = 0.85) in 0.1 N NaOH using Eq. (1),

$$\phi_U = \phi_R(F_UA_U/F_RA_R) \left(\frac{n_1/n_R}{n_1/n_R}\right)^2$$  \hspace{1cm} (1)

where $\phi_U$ and $\phi_R$ are the fluorescence quantum yields of the sample and the reference, $F_U$ and $F_R$ are the area under the fluorescence spectra of the sample and the reference, $A_U$ and $A_R$ are the absorbance of the sample and the reference (at the excitation wavelength), and $n_1$ and $n_R$ are the refractive indices of the solvent used for the sample and the reference respectively$^{18}$.

Stability constants ($K_a$) were obtained from the fluorescence titration data. The linear fit of the fluorescence intensity data at a particular wavelength for 1:1 complexation as evident by Job’s plot was obtained by Eq. (2).

$$I = \frac{I_0 + I_C \left(1 + K_a \left[Cu^{2+}\right]\right)}{1 + K_a \left[Cu^{2+}\right]}$$  \hspace{1cm} (2)

In Eq. (2), $I$ and $I_0$ are the fluorescence intensity of ligand in the presence and absence of metal ions respectively, while $I_C$ is the fluorescence intensity of the solution of (I) in the presence of metal ions$^{19}$.

Synthesis of (I)

7-Diethylamino-coumarin-3-aldehyde (0.15 g, 0.862 mmol) was dissolved in hot methanol (30 mL) and to it a methanolic solution of gem-$\text{N}_3	ext{P}_3\text{O}_4\text{C}_{12}\text{H}_8$]$_2$[N(Me)NH$_2$]$_2$] (0.225 g, 0.431 mmol) was added dropwise. The reaction mixture was stirred at 50 °C for 6 h. A yellow precipitate was obtained. This was filtered, washed with hot methanol and then recrystallized from a CHCl$_3$/n-hexane mixture at 0 °C to obtain (I) (0.343 g, 76 %). M. pt. 192-195 °C. FTIR (KBr) (ν/cm$^{-1}$): 3432(b), 2971 (m), 2928 (m), 1712 (s), 1601 (s), 1519 (s), 1475(s), 1410 (s), 1228 (s), 1134(m), 967 (s), 906 (s), 755 (s). $^1$H NMR (CDCl$_3$, 400 MHz, 24.5 °C, TMS) δ (ppm) = 1.13 (t, 12H, -CH$_3$), 3.32 (q, 8H, -CH$_2$), 3.26 (d, 6H, -NCH$_2$); $^3$J(H-3$^3$P) = 8.8 Hz, 6.4 (s, 2H, aromatic), 7.1 (m, 2H, aromatic), 7.12-7.47 (m, 20H, aromatic), 7.75 (s, 2H, -CH). 8.1 (s, 2H, CH=N). $^{13}$C NMR (CDCl$_3$, 125 MHz, 23 °C, TMS) δ (ppm) = 148.42, 137.25, 129.66, 129.05, 125.86, 122.06, 115.32, 109.28, 97.11, 44.85, 32.22, 12.46. $^{31}$P NMR (202.5 MHz, CDCl$_3$, 21 °C, TMS) δ (ppm) = 20.1 (t, $P$(N(Me)N=CHR), 26.7 (d, $P$(O=C$_2$H$_2$)), $^3$J(P-N-P) = 70.9 Hz. Anal. for C$_{54}$H$_{52}$N$_{14}$O$_4$P$_3$: ESI-HRMS: m/z (%); [M+H]$^+$ = Calcd 1048.3230 (100); Found 1048.3232 (100).

Results and Discussion

Synthesis of the cyclophosphazene ligand (I) was carried out by following a three-step protocol (Scheme 1). The first step involves the modification of the multitopic compound, $\text{N}_3	ext{P}_3\text{Cl}_6$ (which contains six reactive centres) into gem-$\text{N}_3	ext{P}_3\text{Cl}_2\text{O}_4\text{C}_{12}\text{H}_8$]$_2$, which now contains only two reactive P-Cl bonds$^{20}$. Reaction of this compound with N-methylhydrazine proceeds in a regiospecific manner to afford gem-$\text{N}_3	ext{P}_3\text{O}_4\text{C}_{12}\text{H}_8$]$_2$[N(Me)NH$_2$]$_2$]. The reactive –NH$_2$ groups in the latter are amenable to further elaboration and accordingly condensation of this compound with 7-diethylamino-coumarin-3-aldehyde (RCHO) afforded $\text{N}_3	ext{P}_3\text{O}_4\text{C}_{12}\text{H}_8$]$_2$[N(Me)N=CHR]$_2$ (I).
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Fig. 1—Absorption spectra of (1) and after addition of ten equivalents of various metal perchlorates in HEPES buffer. [water:DMSO = 9:1 v/v; 10 mM; pH = 7.4].

Fig. 2—Fluorescence spectra of (1) (10.0 µM) and after addition of metal perchlorates (100.0 µM) in HEPES buffer. [water:DMSO = 9:1 v/v; 10 mM; pH = 7.4].
in excellent yields (Scheme 1). (I) is soluble in a wide range of organic solvents and is quite stable in solution, as evidenced by the presence of a strong [M+H]+ peak (1048.32) in its ESI-HRMS spectrum recorded under a positive ion mode. NMR (1H, 13C and 31P) and IR spectroscopic data are consistent with the structure of (I).

**Absorption spectra**

The absorption spectrum of (I) in HEPES buffer (water:DMSO = 9:1, v/v; 10 mM; pH = 7.4) is characterized by peaks both at high and low energies [[(I), λmax (ε); 277 (5.4 × 10^4), 426 (3.0 × 10^4)] (Fig. 1). Interaction of (I) with various metal ions (Ag⁺, Ca²⁺, Cd²⁺, Cu²⁺, Hg²⁺, Li⁺, Mg²⁺, Na⁺, Ni²⁺, Pb²⁺, Zn²⁺) causes a decrease in the absorption maxima to some extent. However, this effect is neither specific nor selective, and therefore, cannot be used as a diagnostic criterion for the detection of any metal ions.

**Emission spectra**

The fluorescence spectrum of (I) (λexc = 413 nm) shows a broad band at 528 nm in the HEPES buffer (water:DMSO = 9:1 v/v; 10 mM; pH = 7.4) solution. Interaction of (I) with various metal ions shows a significant fluorescence quenching in the presence of Cu²⁺ and Ag⁺ (Fig. 2). The extent of fluorescence quenching enhances as the concentration of the metal ion increases. It was found that the addition of metal ions also shifts the wavelength of emission of (I)

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>Fluorescence Quenching Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>1.0</td>
</tr>
<tr>
<td>(I)-Ag</td>
<td>0.4</td>
</tr>
<tr>
<td>(I)-Ca</td>
<td>0.9</td>
</tr>
<tr>
<td>(I)-Cd</td>
<td>0.8</td>
</tr>
<tr>
<td>(I)-Cu</td>
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</tr>
<tr>
<td>(I)-Hg</td>
<td>0.9</td>
</tr>
<tr>
<td>(I)-Li</td>
<td>0.8</td>
</tr>
<tr>
<td>(I)-Mg</td>
<td>0.8</td>
</tr>
<tr>
<td>(I)-Na</td>
<td>0.8</td>
</tr>
<tr>
<td>(I)-Ni</td>
<td>1.0</td>
</tr>
<tr>
<td>(I)-Pb</td>
<td>0.8</td>
</tr>
<tr>
<td>(I)-Zn</td>
<td>0.8</td>
</tr>
</tbody>
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*aFluorescence intensity ratio: fluorescence intensity of (I)/fluorescence intensity of (I) in presence of 10 equivalents of the metal ion.*

Fig. 3—Fluorescence spectra of (I) (10.0 μM) with increasing concentrations of (a) Cu²⁺ and (b) Ag⁺ in HEPES buffer. [water:DMSO = 9:1 v/v; 10 mM; pH = 7.4].

Fig. 4—Job’s plot for determination of the stoichiometry of (a) (I)-Cu(II) and (b) (I)-Ag(I).
from 528 nm to 520 nm (fluorescence intensity changes from 34.1 a.u. to 14.6 a.u) in the case of Cu$^{2+}$ and from 528 nm to 513 nm (fluorescence intensity changes from 34.1 a.u. to 13.38 a.u) in the case of Ag$^{+}$ (Fig. 3). The quantum yield of (1) (0.031) decreases to 0.0018 for Cu$^{2+}$ and to 0.0016 for Ag$^{+}$ ions after adding ten equivalents, respectively of these metal ions. The fluorescence quenching experienced by (1) after adding various metal ions is given in Table 1.

The fluorescence quenching on interaction of ligands with metal ions seems to depend on the efficiency of binding. Thus, (1) binds Cu$^{2+}$ and Ag$^{+}$ more effectively. The respective binding constants for Cu$^{2+}$ and Ag$^{+}$ complexes are $1.8 \times 10^4$ L mol$^{-1}$ and $1.51 \times 10^4$ L mol$^{-1}$. This is determined from the fluorescence titration data as a function of metal ion concentration for 1:1 complexation; the latter is confirmed by the Job’s plot (Fig. 4). A tentative scheme for the mode of binding of (1) with the metal ions, Cu$^{2+}$ or Ag$^{+}$ is shown in Scheme 2. The maximum fluorescence quenching for Cu$^{2+}$ and Ag$^{+}$ with (1) occurs at a L:M ratio of 1:10. Further addition of Cu$^{2+}$ or Ag$^{+}$ does not change the extent of quenching. The interference effect of other metal ions has also been tested by adding other metal salts to a solution containing a mixture of (1) and Cu$^{2+}$. The results obtained for these experiments reveal that the fluorescence quenching of (1) (upon addition of Cu$^{2+}$) is not affected by the presence of other metal ions, and hence, this effect can be used for the detection of Cu$^{2+}$ (Fig. 5). A similar situation is also found for Ag$^{+}$. It must be pointed out that while the fluorescence quenching experienced by (1) upon addition of Cu$^{2+}$ is probably the result of the paramagnetic nature of the latter, the fluorescence quenching influence of Ag$^{+}$ is not fully understood.

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