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Fluorescent Organometallic Sensors for the Detection of Chemical-Warfare-Agent Mimics**

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Organophosphates are toxic species commonly found in both pesticides and chemical-warfare agents whose rapid and severe effects on human and animal health lie in their ability to block the action of acetylcholinesterase, a critical central-nervous-system enzyme.[1] As a consequence, intense research efforts have been directed to develop sensitive and selective schemes for the detection of these compounds.[2] A variety of approaches has been investigated for sensors, including the use of enzymatic assays,[3] molecular imprinting and lanthanide luminescence,[4] colorimetric methods,[5] surface acoustic waves,[6] organic fluorescent compounds,[7] gas chromatography–mass spectrometry,[8] and interferometry.[9] In general, each of these methods suffers from at least one undesirable limitation, such as limited selectivity, low sensitivity, operational complexity, lack of portability, and/or the difficulties of real-time monitoring.[4b, 7a]

One of the most convenient and simplest means of chemical detection is the generation of an optical event, such as a change in absorption or fluorescence color, in the presence of an analyte of interest.[10] It was previously shown that lanthanide complexes, with characteristically narrow excitation and emission bands, intense fluorescence, and long excited-state lifetimes, are well suited to be used as fluorescence-based chemical sensors.[11] For example, Eu^{3+} complexes can exhibit intense luminescence in the presence of an appropriate UV-light-absorbing ligand through the so-called “antenna effect”.[12] This process involves optical absorption by the ligand, followed by ligand-to-metal energy transfer and results in metal-ion-based fluorescence. Any analyte, which can act as a competitive binder for the Eu^{3+} center, can “switch off” the Eu^{3+}-based emission and potentially restore the emission of the “free” ligand. On the other hand, many metal ions do not lend themselves to metal-based emission,

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for example Zn^{2+} or La^{3+}; in these cases, the ligand-based fluorescence may be quenched or shifted to a different wavelength upon complexation. Taking advantage of these processes and exploiting the known binding affinity of organophosphates to lanthanide ions,[4,6a,12c] we present herein a most versatile sensor platform (Figure 1) that combines high selectivity and sensitivity with ease of signal transduction. The approach relies on the fact that 2,6-bis(1′-methylbenzimidazolyl)pyridine (Mebip) ligands are highly fluorescent and have been shown[13] to act as "antenna" for Eu^{3+} ions. Furthermore, we have developed Mebip ligands and complexes in which ligand–metal energy transfer is absent. We show herein that such ligand-based emission can be designed to occur at different wavelengths upon metal ion (de-)complexation. Mixing and matching consciously designed ligands and carefully selected metal ions allows the formation of sensor complexes which can exhibit different selectivity and optical response.

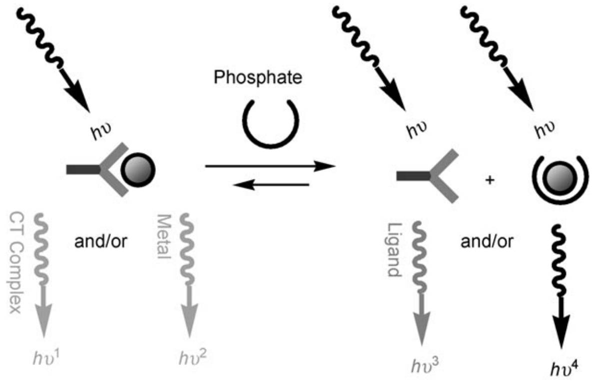
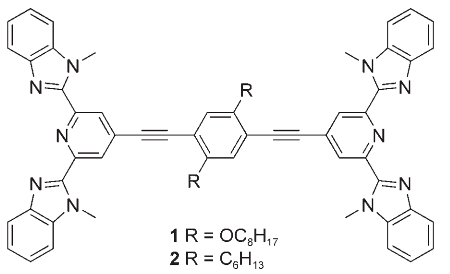
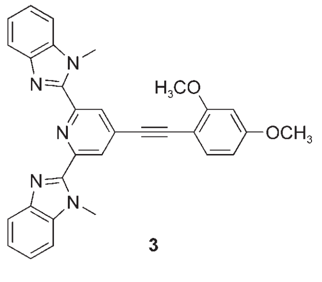


Figure 1. Schematic representation of the mechanism utilized for the detection of metal-ion-coordinating analytes.

In our ongoing efforts to develop metallo-supramolecular materials,[14] we previously reported the synthesis of the ditopic Mebip-functionalized ethynylene **1** and its self-assembly with $Zn(ClO_4)_2$. The metal-ion coordination results in large bathochromic shifts of both the absorption and fluorescence spectra.[14b] We now show that the addition of one equivalent of $[La(NO_3)_3]\cdot 6H_2O$ to ethynylene **1** produces a similar effect: the maximum of the emission band λ_{max} is shifted by 97 nm upon complexation (Table 1; all experiments were conducted in $CHCl_3/CH_3CN$ (9:1, v/v)).[15] The large redshift of the ligand emission upon La^{3+} binding is consistent with either an intraligand charge transfer (CT), which results from the electron-rich arylethynylene core to the electron-poor metal-bound Mebip moieties,[14b,16] or a narrowing of the π–π* transition upon metal binding, on account of stabilization of the π* molecular orbital.[17] In both mechanisms, the electron density on the ligand is a key factor in the large bathochromic shift. This behavior is evident by comparison of the **1**/[**1**·La^{3+}] system with **2**/[**2**·La^{3+}]: In ligand **2**, the electron density of the core component was decreased by replacing the alkoxy functionality with alkyl substituents, thus resulting in a significantly smaller red shift (31 nm) upon binding to La^{3+} (Table 1).



1 R = OC_8H_{17}
2 R = C_6H_{13}



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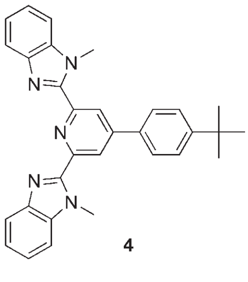

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Table 1: Emission and UV/Vis absorption data for solutions of the free ligands **1**–**4** and their corresponding complexes (compound/M^{n+} = 1:1).

Ligand/ complex[a]	Ligand emission λ_{max} [nm][b]	Complex emission λ_{max} [nm][b]	Ligand absorbance λ_{max} [nm][d]	Complex absorbance λ_{max} [nm][d]
1/[**1**·La^{3+}]	448	545	395	427
2/[**2**·La^{3+}]	397	428	347	365
3/[**3**·La^{3+}]	420[c]	493[c]	341	378
3/[**3**·Zn^{2+}]	420[c]	570[c]	341	391
3/[**3**·Eu^{3+}]	420[c]	578, 593, 614[c]	341	383
4/[**4**·La^{3+}]	375	413	337	356

[a] All measurements were made in $CHCl_3/CH_3CN$ (9:1, v/v) solutions at concentrations of 0.025 mM. [b] Excitation at 320 nm. [c] Excitation at 360 nm. [d] Lowest-energy transition.

Having demonstrated the ability to readily control the extent to which the fluorescence of the Mebip ligands changes upon metal binding, we refined our sensory system for the detection of competitive coordinating organophosphates. To avoid any potential complications associated with multiple metal–ligand interactions and self-assembly/polymerization, we designed and synthesized fluorescent Mebip ligands with only one binding site. Different ligands (**3** and **4**) were prepared with the objective of investigating the influence of the electron density of the ligand on the fluorescence color change that occurs upon binding to La^{3+} or Zn^{2+} ions and on the sensing abilities of these complexes. In addition, these ligands were complexed to Eu^{3+} ions to study sensor complexes that utilize well-defined lanthanide-based emission. Dialkoxyarylethynylene **3** contains an electron-rich aromatic group, whereas the electron-donating nature of the *tert*-butyl aryl moiety in **4** can be expected to be significantly less. Compounds **3** and **4** were prepared through Sonogashira and Suzuki coupling reactions, respectively. The ligands were complexed to the La^{3+} center in a 1:1 ligand/M^{n+} ratio, and the resulting emission and absorption spectra were recorded. Figure 2a shows that upon binding with La^{3+} the emission spectrum of ligand **3** experiences a significant bathochromic shift ($\Delta\lambda_{max} = 73$ nm, Table 1), which is manifested in a pronounced change of the emission color (blue to green), and the fluorescence intensity is significantly decreased. At the same time, a large change of the lowest-energy UV/Vis absorption band ($\Delta\lambda_{max} = 37$ nm, Table 1) is observed. As expected, much smaller changes are observed in the emission ($\Delta\lambda_{max} = 38$ nm, Table 1) and UV/Vis absorption spectra ($\Delta\lambda_{max} = 19$ nm, Table 1) for ligand **4**.

The readily available triethyl phosphate was used as a mimic for phosphate-based chemical-warfare agents to determine the organophosphate-sensing capability of the 1:1 [**3**·La^{3+}] and [**4**·La^{3+}] complexes.[7b,18] In addition, tri-*o*-tolyl phosphate (TOTP) was employed to investigate the selectivity of the phosphate sensing. The phosphates were titrated into dilute solutions of each complex in $CHCl_3/CH_3CN$ (9:1, v/v; 25 μM). In the case of the aliphatic phosphate, an instantaneous blue shift of the emission spectra and increase of the fluorescence intensities are observed, thus indicating the release of "free" ligand (Figure 2), which is consistent with the phosphate binding to the La^{3+} ions. As expected, the largest spectral changes are observed at low analyte concentrations, with the effect leveling off at a concentration of approximately 10 mM phosphate for both complexes. Plotting the relative fluorescence intensity, defined as the (intensity at a given analyte concentration − intensity of the initial 1:1 complex)/intensity of the free ligand × 100, at the λ_{max} value of the free ligand as a function of analyte concentration yields a sensory response for each complex (Figure 2, insets). The importance of bestowing the ligand with electron-donating substituents is readily evident as a visible color change was observed for [**3**·La^{3+}] at a concentration of 1.8 mM phosphate, whereas the sensory response of [**4**·La^{3+}] could only be monitored spectrophotometrically at a similar concentration of phosphate. Interestingly, the addition of the aromatic phosphate TOTP yielded only very small fluorescent responses when added to [**3**·La^{3+}] (Figure 2a). Even at a very large concentration of TOTP (17.5 mM), only a very small intensity increase and blue shift of the emission can be detected (Figure 2a, inset), thus indicating the virtual absence of the free ligand and pointing to very weak competitive binding. A very similar response was observed for water at comparably high concentrations (Figure 2a, inset), thus demonstrating that this sensor system shows excellent selectivity for the detection of alkyl phosphates over bulky aromatic phosphates and water.

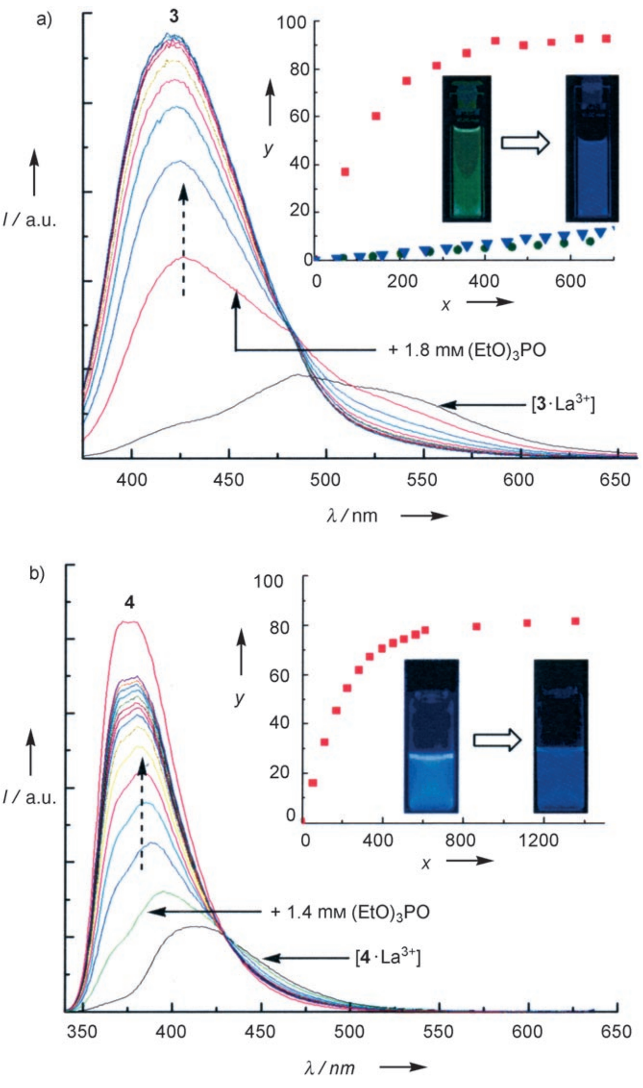

Figure 2. Photoluminescence (PL) spectra acquired upon titration of a) [**3**·La$(NO_3)_3$] and b) [**4**·La$(NO_3)_3$] (0.025 mM) in $CHCl_3/CH_3CN$ (9:1, v/v) with $(EtO)_3PO$. The inset (a) shows the relative emission at 420 nm (*y*) as a function of the ratio of analyte ($(EtO)_3PO$, red square; TOTP, blue triangle; and H_2O, green circle) to [**3**·La$(NO_3)_3$] (*x*). The inset (b) shows the relative emission at 375 nm (*y*) as a function of the ratio of $(EtO)_3PO$/[**4**·La$(NO_3)_3$] (*x*). The pictures illustrate the fluorescence color of the initial sensor complex solutions, as well as those after adding $(EtO)_3PO$ to the saturation point.

Having established the high selectivity of these Me bip·La^{3+} complexes for trialkyl phosphate over triaryl phosphate moieties and water, La^{3+} was replaced with Eu^{3+} and Zn^{2+} to further explore the influence of different metal ions on the sensory capacity of the resulting complexes. The [**3**·Zn^{2+}] complex fluoresces yellow and displays an even larger bathochromic shift of the emission band ($\Delta\lambda_{max} =$ 150 nm, Table 1) than [**3**·La^{3+}]. By contrast, the [**3**·Eu^{3+}] complex displays emission characteristics that are dominated by a red, metal-based fluorescence, whereas the ligand-based emission is fully suppressed. Both complexes were exposed to increasing amounts of $(EtO)_3PO$. Again the relative fluorescence intensity at a wavelength of 420 nm was plotted as a function of analyte concentration to yield the sensory response for each complex (Figure 3a). The sensory response of [**3**·Eu^{3+}] was observed to behave in a similar manner to that of [**3**·La^{3+}], thus indicating that the binding of La^{3+} and Eu^{3+} to **3** is comparable. However, we should point out that the visual effect is much more pronounced in the case of the [**3**·Eu^{3+}] complex on account of the suppression of the intense Eu^{3+}-based emission.

With the objective of determining the sensitivity of these new sensor complexes, triethyl phosphate titrations were conducted with [**4**·Eu^{3+}] in a fashion similar to those reported above but with the addition of smaller aliquots of the analyte. The experiments yielded a spectroscopically observable increase of the fluorescence intensity (corresponding to free ligand) at analyte concentrations of as low as 20 μM—a level

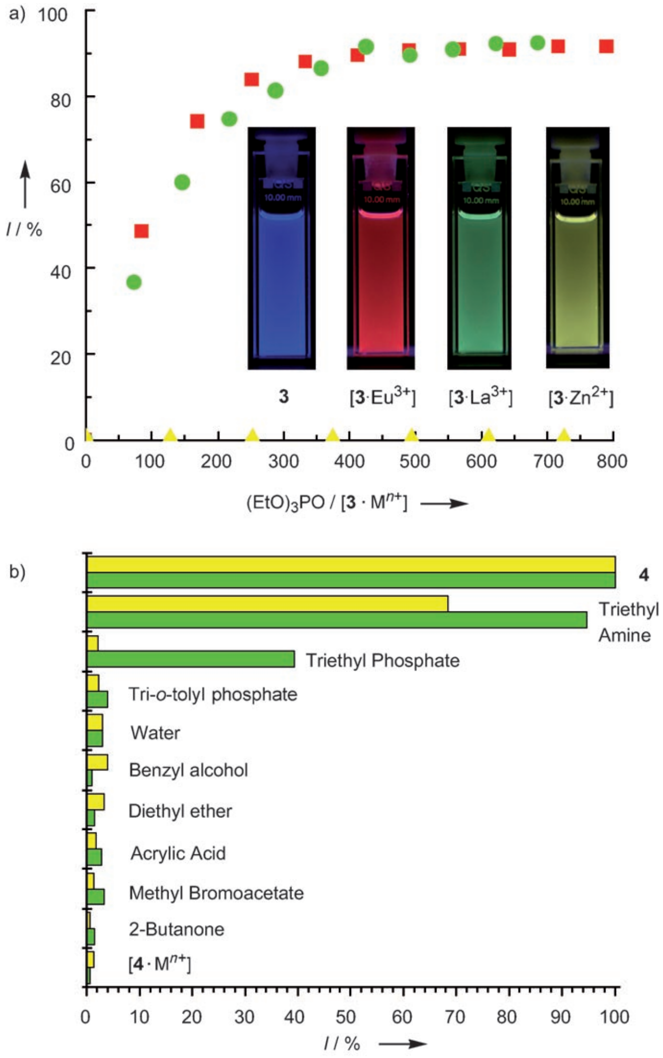

Figure 3. a) Relative emission at 420 nm as a function of the ratio of $(EtO)_3PO$ to the 1:1 metal/ligand complexes [**3**·MX_n] (0.025 mM, M = Eu^{3+}, La^{3+}, and Zn^{2+}) in $CHCl_3/CH_3CN$ (9:1, v/v). b) Relative fluorescence intensity (% relative to the intensity of the free ligand) of solutions of [**4**·$La(NO_3)_3$] and [**4**·$Zn(ClO_4)_2$] upon exposure to common organic compounds. Experiments were performed at a complex concentration of 0.025 mM and analyte concentrations of 2.5 mM in $CHCl_3/CH_3CN$ (9:1, v/v).

commensurate with current organophosphate biosensors.[18] In contrast to its lanthanide counterparts, [**3**·Zn^{2+}] showed no increase of "free"-ligand fluorescence intensity upon addition of even large amounts (19 mM) of $(EtO)_3PO$ (Figure 3a). The absence of a response for [**3**·Zn^{2+}] illustrates the stronger binding of Mebip to Zn^{2+} ions relative to Ln^{3+} ions and/or a preference of the phosphate for interaction with Ln^{3+} ions, thus clearly demonstrating the importance of carefully tailoring the strength of the metal–ligand interaction to an appropriate level.

One important aspect in the design of effective organophosphate sensors is to impart a high degree of analyte specificity, which is necessary to avoid false positive readings.[7a,b,20] To determine the extent to which our complexes would respond to various common organic species, 25 μM solutions of [**4**·La^{3+}] and [**4**·Zn^{2+}] were exposed to a variety of compounds (2.5 mM). As can be seen from the data compiled in Figure 3b, under these conditions both complexes show effectively no response to ketones, esters, organic acids, ethers, alcohols, water, and bulky aromatic phosphates, thus imparting a great degree of selectivity to these sensors. Apart from the trialkyl phosphate, the only other investigated compound that elicited a response was triethylamine. Importantly, although [**4**·La^{3+}] shows a response to both of these analytes, [**3**·Zn^{2+}], as discussed above, and equally [**4**·Zn^{2+}], do not show the presence of aliphatic phosphates but do respond to amines. Thus, using different combinations of metal ions and Mebip ligands, one is allowed to readily differentiate between aliphatic phosphates and amines. This point is illustrated clearly by the sensor array shown in Figure 4a. The array demonstrates the ability to detect the presence of aliphatic over aromatic phosphates, which is dictated by the nature of the binding of the lanthanide and Mebip ligand. In addition, the use of Zn^{2+} complexes, such as [**1**·Zn^{2+}] for example, which detect only amines, provides the ability to single out the latter and prevent amine-driven false positive readings.

To facilitate practical devices, the vapor-phase detection of organophosphates is desirably accomplished by the use of a solid-state sensor.[20,21] The first experiments to address this need involved hydrophobic silica particles treated with a solution of [**4**·Eu^{3+}] to yield (after drying) a pink fluorescent powder (Figure 4b). The fluorescence color of the powder

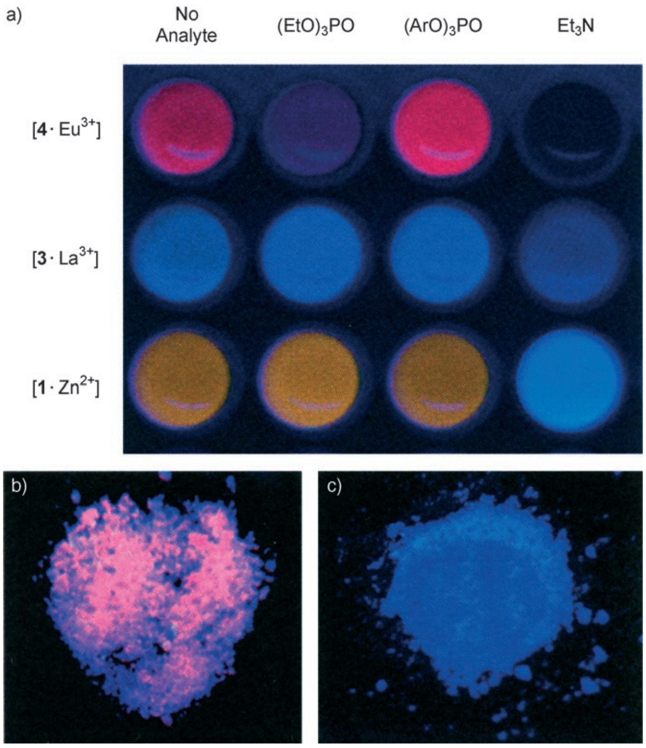

Figure 4. Pictures taken under excitation at 365 nm of a) a sensor array, which illustrates the selective detection of $(EtO)_3PO$ and Et_3N by 1:1 metal ion complexes (experiments were carried out in $CHCl_3/CH_3CN$ (9:1, v/v) with a complex concentration of 0.025 mM and analyte concentrations of 2.5 mM); b) hydrophobic silica particles coated with [**4**·Eu^{3+}]; c) hydrophobic silica particles coated with [**4**·Eu^{3+}] after exposure to $(EtO)_3PO$ vapor for 2 h at 60 °C.

changed slowly upon exposure to $(EtO)_3PO$ vapor (on account of the low vapor pressure of this analyte, as confirmed by the instantaneous response from exposure of the solid-state sensor to $(EtO)_3PO$ in liquid form) from pink to blue (Figure 4c), which is indicative of the decomplexation of [**4**·Eu^{3+}] and the formation of "free" ligand **4**.

In summary, we have developed a modular sensory system that utilizes a multimetal/multiligand-based approach. The judicious design of fluorescent ligands and the careful selection of metal/ligand combinations allowed us to create a very simple system that allows the selective detection of aliphatic organophosphates with good sensitivity. By tailoring the nature of the metal–ligand interactions, it should be possible to further enhance the sensitivity of these systems on the one hand and tailor their selectivity towards different analytes on the other.

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