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Chromo-fluorogenic BODIPY-complexes for selective detection of V-type nerve agent surrogates†

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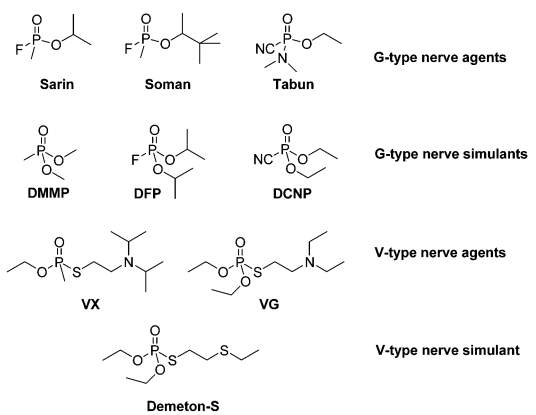
Two new Eu^{3+} and Au^{3+} BODIPY-complexes capable of chromo-fluorogenically detecting micromolar concentrations of V-type nerve agent surrogates by a simple displacement assay are described.

Nowadays, the growing fear of possible attacks with chemical warfare agents (CWAs) by military regimes and terrorist organizations has thrown up a new challenge to develop sensitive and selective devices to monitor these extremely dangerous chemicals.[1] Within CWAs, nerve agents (classified in G- and V-series, see Scheme 1) represent an extremely toxic class of organophosphorus compounds that cause incapacitation and even death through inhibition of acetylcholinesterase (AChE) activity.[2] Nerve agents phosphorylate the serine hydroxyl group in the active site of AChE that is also involved in the binding of acetylcholine (the natural substrate of the enzyme).[3] The interaction of AChE with the nerve agent is stronger than with acetylcholine, the entire process resulting in accumulation of acetylcholine that causes neuromuscular paralysis and eventually death.[2] Moreover V-gases are low-volatile liquids with high boiling points and are much more persistent and toxic than nerve agents of the G-series. For example, compared with Sarin (GB), VX is estimated to be approximately twice as toxic by inhalation, ten times as toxic by oral administration, and approximately 170 times as toxic after skin exposure.[4]



Scheme 1 Chemical structures of G-type and V-type agents and their simulants.

The most common methods for monitoring the presence of nerve agents are based on the use of biosensors,[5] ion mobility spectroscopy[6] and electrochemical methods.[7] Moreover, recently the development of fluorogenic and chromogenic chemosensors has gained increasing interest as an alternative to these instrumental procedures.[8] Chromo-fluorogenic probes are especially appealing because they allow simple visual detection *in situ* or at the site without any sample pretreatment or the use of complex equipment. However it is apparent from the literature that although a variety of chemosensors for the detection of G-type agents have been reported,[1,9] probes for V-type agents are still very rare.[10]

Due to the high toxicity of nerve agents, various comparatively innocuous and readily available molecules (usually named simulants, mimics or surrogates) are frequently used in research studies. In particular the organophosphorus insecticide, demeton-S, has been used in this work as a surrogate of the highly toxic V-type nerve agents.[11] Moreover nerve gas simulants such as diethylcyanophosphate (DCNP), diisopropylfluorophosphate (DFP) and dimethyl methylphosphonate (DMMP) are usually used as simulants of the G-type nerve agents Sarin, Soman and Tabun. All these surrogates show similar reactivity to real nerve agents but lack their severe toxicity (see Scheme 1).

Following our interest in the development of chromo-fluorogenic probes for nerve agents,[12] we show herein two new BODIPY-complexes that are able to detect selectively the

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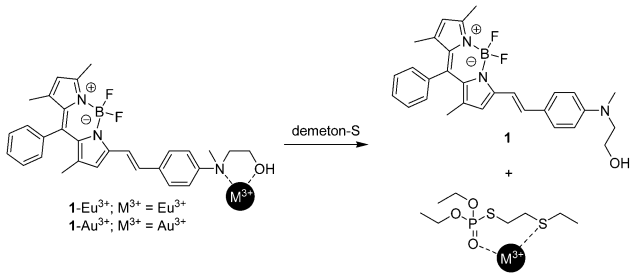

Scheme 2 Metal-complexes **1**-Eu^{3+} and **1**-Au^{3+}, and schematic outline of the sensing paradigm.

V-type nerve agent mimic demeton-S *versus* a G-type nerve agent simulant (see Scheme 2). The design of the probe is based on the use of the BODIPY derivative **1** that is known to form 1 : 1 metal complexes with trivalent cations in a bidentate coordination mode.[13] It is also known that some metal cations such as Eu^{3+} and Au^{3+} coordinate well with V-nerve agents through the formation of mono[1,14] and bidentate complexes.[10] Based on these concepts, the sensing paradigm we report herein relies on the preparation of the **1**-Eu^{3+} and **1**-Au^{3+} complexes and on a demeton-S-induced demetallation that was expected to result in a significant chromo-fluorogenic response.

Complexes **1**-Eu^{3+} and **1**-Au^{3+} were prepared by simple mixing of the corresponding metal salt with **1** and further purification by recrystallization from EtOH–water mixtures (see ESI† for details).[14c,15] Acetonitrile solutions of **1** showed an intense absorption band in the visible region centred at 600.5 nm responsible for the blue colour observed. Besides, solutions of **1** are nearly non-emissive (see Table 1), attributed to efficient ICT quenching of the excited state of the BODIPY fluorophore from the electron-donating aniline group. In contrast **1**-Eu^{3+} and **1**-Au^{3+} were pink and presented strong emission bands (at 572.8 and 573.2 nm for **1**-Eu^{3+} and **1**-Au^{3+}, respectively) due to an inhibition of the quenching process, active in **1**, upon coordination with the metal cations (see Table 1). The formation of 1 : 1 complexes between **1** and Eu^{3+} and Au^{3+} was additionally confirmed through titration experiments in acetonitrile and from Job plot studies.[1]HNMR studies were carried out to envisage the cation complexation mode (see ESI†).

As a first step, the behaviour of **1**-Eu^{3+} in the presence of demeton-S was tested in acetonitrile. Addition of increasing quantities of demeton-S induced a progressive and marked decrease of the absorption at 553 nm and the appearance of a new band at 600.5 nm with colour modulation from bright pink to blue, easily detectable by the naked eye (see Fig. 1).

Table 1 UV-vis absorption and fluorescence data of free ligand and the complexes (**1**/M^{3+} = 1 : 1)

	λ_{abs} (nm)	$\log \varepsilon$	λ_{em} (nm)	Φ^a
1	600.5	4.96	570.0	<0.001
1-Eu^{3+}	553.0	5.07	572.8	0.22
1-Au^{3+}	552.5	5.09	573.2	0.28

[a] Quantum yields were calculated using Rhodamine B in ethanol (Φ_{EtOH} = 0.49)[16] as the standard.

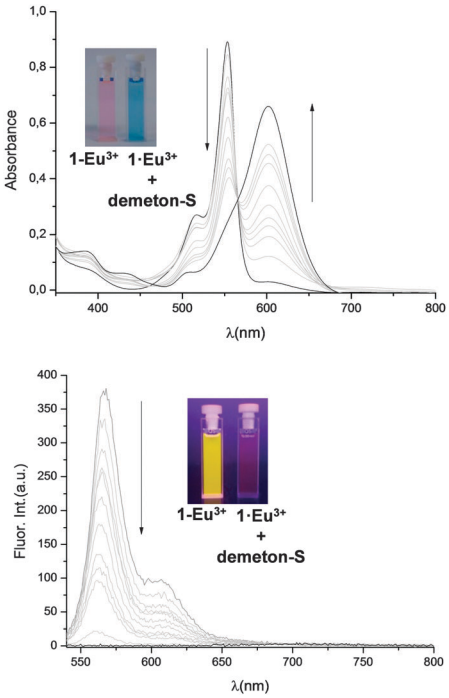

Fig. 1 Absorption (top) and emission (bottom, λ_{exc} = 530 nm) titration of **1**-Eu^{3+} (1.0×10^{-5} M in acetonitrile) obtained upon the successive addition of incremental amounts of demeton-S.

Besides, remarkable quenching of the emission band at 572.8 nm was also observed (see Fig. 1). A very similar behaviour was observed upon addition of demeton-S to acetonitrile solutions of complex **1**-Au^{3+} (see ESI†). The red-shifted bands, obtained upon addition of demeton-S to solutions of **1**-Eu^{3+} and **1**-Au^{3+}, that were identical to that of **1** alone, and the strong quenching observed were in agreement with a demetallation process as the mechanism of the observed optical modulation.

In order to further study the sensing protocol, the association constants of Eu^{3+} and Au^{3+} cations with **1** were calculated from UV-vis titration experiments by using the Specfit program.[17] Logarithms of complexation constants for **1** with Eu^{3+} and Au^{3+} for the formation of the **1**-Eu^{3+} and **1**-Au^{3+} 1 : 1 complexes were 4.1 ± 0.5 and 4.2 ± 0.4, respectively, from UV-vis titrations. Nearly similar stability constants for the formation of the complexes were determined from fluorescence experiments (*i.e.* $\log K$ = 4.6 ± 0.3 and 4.3 ± 0.4 for the formation of **1**-Eu^{3+} and **1**-Au^{3+}, respectively). On the other hand, $\log K$ for the displacement reaction (*i.e.* **1**-M^{3+} + demeton-S ⇄ **1** + demeton-S-M^{3+}) was also determined and amounted to 5.8 ± 0.5 and 6.6 ± 0.2 (from UV-vis) and 5.9 ± 0.4 and 6.7 ± 0.3 (from fluorescence) for Eu^{3+} and Au^{3+}, respectively. These studies demonstrated that Eu^{3+} and Au^{3+} showed stronger affinity for demeton-S than for **1** in agreement with the proposed sensing paradigm. Besides, Au^{3+} displayed a higher displacement association constant with demeton-S than Eu^{3+} which is tentatively ascribed to the high affinity of Au^{3+} for sulfur-containing derivatives.[18]

Moreover from titration experiments of **1**-Eu^{3+} and **1**-Au^{3+} with demeton-S limits of detection (LOD) of 13.97 and 9.17 (from UV-vis) and 12.89 and 9.08 ppm (from fluorescence) were determined for **1**-Eu^{3+} and **1**-Au^{3+}, respectively.

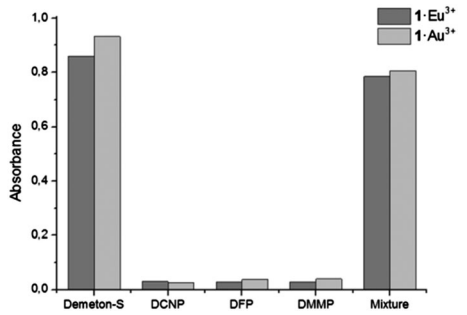

Fig. 2 Absorbance (measured at 600.5 nm) of complexes **1**-Eu^{3+} and **1**-Au^{3+} in the presence of demeton-S (100 ppm), DCNP, DFP and DMMP (500 ppm). (Mixture: DCNP + DFP + DMMP + demeton-S).

Finally, in order to verify the selectivity of the method, similar experiments with probes **1**-Eu^{3+} and **1**-Au^{3+} were performed in the presence of the G-type nerve agent mimics DCNP, DFP and DMMP (see Fig. 2). The results show that probes **1**-Eu^{3+} and **1**-Au^{3+} are highly selective to the presence of demeton-S, whereas G-type derivatives induced a very poor response even at concentrations as high as 500 ppm. The presence of G-type agents in the medium only causes negligible change in the response. Detection can even be carried out in the presence of 50% of water (see ESI†).

In summary, Eu^{3+} and Au^{3+} complexes of the BODIPY-containing derivative **1** were prepared and their utility in the selective detection of the V-type nerve agent surrogate demeton-S was demonstrated. The mechanism of detection occurs *via* the displacement of the BODIPY ligand by coordination of demeton-S with the metallic centre, resulting in colour modulation and fluorescence quenching which were easily observed by the naked eye. LOD in the 9–14 ppm range were determined. Probes **1**-Eu^{3+} and **1**-Au^{3+} are some of the very few examples reported in the literature that are able to detect V-type nerve agent mimics. Moreover **1**-Eu^{3+} and **1**-Au^{3+} are easy to prepare and we believe they may inspire further research in the design of new probes that are able to chromo-fluorogenically and selectively detect V-type *versus* G-type nerve agents.

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