Nerve Agent Sensors

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Hydroxy Oximes as Organophosphorus Nerve Agent Sensors**

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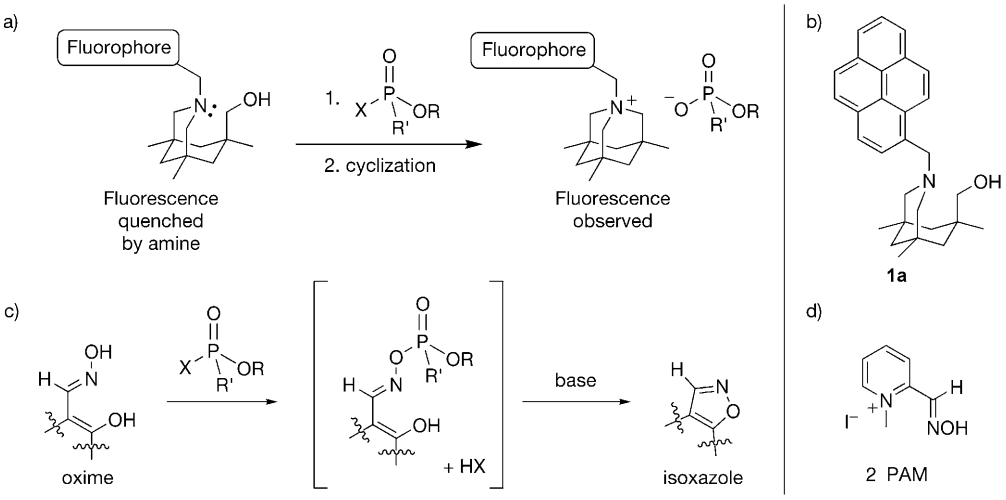
The G-series of nerve agents including Sarin, Soman, and Tabun are inhibitors of serine proteases and their primary toxicity results from the inhibition of acetylcholinesterase. They can be lethal within minutes if inhaled as the esterification of the active site serine residue by the OP agent renders the enzyme inoperative. This results in a buildup of acetylcholine in the cholinergic synapses that paralyzes the central nervous system.[1] Recent reports describe detection methods for these nerve agents using enzymes,[2] electrochemistry,[3] or luminescence,[4–6] but limitations of these systems include low sensitivity, operational complexity, and non-portability. The ease of production and extreme toxicity of these organophosphorus (OP) nerve agents underscores the need to rapidly detect and, ideally, neutralize these odorless and colorless chemicals. We report here progress towards this goal.

In previous work, a series of sensors was described in which the reaction of a primary alcohol with an OP toxin initiates an intramolecular cyclization to generate a fluorescence response (Scheme 1a).[6] While this system had the advantage of compatibility with a range of fluorophores, its response time to ppm vapor concentrations was slow (seconds). To enhance the detection rate, the alcohol was replaced with a more nucleophilic oxime group. This function is known as an excellent reagent for OP agents with rapid detection abilities,[5,7] and is even the basis for antidotes presently used against OP poisoning (e.g. 2-pyridinealdoxime (2-PAM), Scheme 1d).[8] The oxime is not without liabilities—the resultant esters with OP agents are extremely powerful inhibitors of the relevant enzymes and their destruction is paramount in any detection scheme.[9,10] To overcome some of these liabilities, we returned to a neighboring group tactic by incorporating a β-hydroxy function for cyclization and displacement of the initially formed (and likely lethal) OP ester (Scheme 1c). The resulting isoxazole becomes the source of a fluorescent signal that reports the presence and destruction of the OP agent.

Oximes possess significantly enhanced reactivity attributed primarily to the α-effect.[11] Nucleophiles such as oximes containing heteroatoms adjacent to the nucleophilic center have greater reactivity than what is predicted from their basicity. For example, oximes and phenols are oxygen nucleophiles of similar pK_a, but oximes react ca. 100 times faster with phosphorus-based electrophiles.[12] They have also been shown to react much faster with phosphorus electrophiles than do hydrazones, another α-effect function.[7]



Scheme 1. a) A previously reported[6] fluorescence detection scheme and b) the optimized sensor with this scaffold, **1a**. c) The oxime-based detection sequence: reaction of an unsaturated β-hydroxy oxime with an OP nerve agent is followed by cyclization of the OP ester to the isoxazole in the presence of a base. d) Currently employed OP agent poisoning antidote, 2-pyridinealdoxime methiodide (2-PAM).

Installing aromatic cores between the oxime and hydroxy groups served to increase the desired enol tautomer as well as enhance the practicality of the materials by shifting the absorption of the chromophore to longer wavelengths. Four oxime-based sensors, **1**–**4**, were synthesized with the aromatic cores naphthalene, pyrene, coumarin, and pyridine (Scheme 2). These were all synthesized as previously described from the corresponding salicylaldehyde derivatives.[13] The first three cores were of particular interest as they are fluorescent species, thus expanding the application of any

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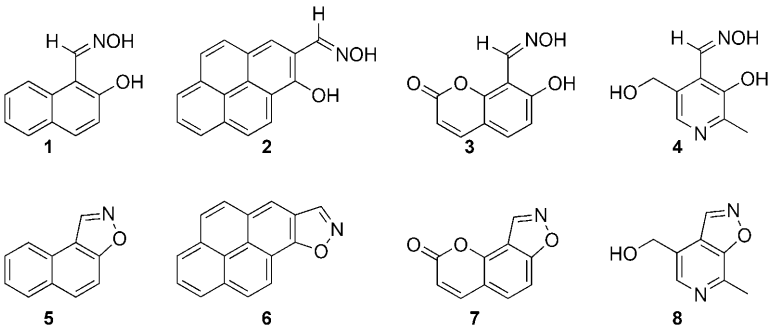

Scheme 2. Four *ortho*-hydroxy oxime targets, **1**–**4**, to be used as OP nerve agent sensors and their corresponding arylisoxazoles **5**–**8**.

formed chromogenic sensors to be used as fluorogenic sensors as well. Initially, oximes **1**–**4** were reacted with *p*-toluenesulfonyl chloride (TsCl) in the presence of an amine base to generate the desired arylisoxazoles **5**–**8** (Scheme 1).[13] As a more representative test of their utility as sensors, the oximes were found to cyclize upon reaction with diethylchlorophosphate (DCP), a reactive nerve gas mimic, under the same conditions.

The cyclization to the isoxazoles (**5**–**8**) produced measurable shifts in the absorption and emission properties, as well as altered the magnitude of both the extinction coefficients and the fluorescence intensities (see Table 1, Figure 1, and Figures S1–S3 in the Supporting Information). Cyclization of the naphthyl-based sensor **1** produced the greatest fluorescence enhancement of 62-fold—three times the enhancement observed with the alcohol-based sensor **1a**. The electronic difference between the oximes and the cyclized arylisoxazoles observed from these optical measurements confirm that the oxime cyclization is a plausible construct for a practical OP nerve agent sensor.

The detection reaction rates for oximes **1**–**4** were determined and compared with the previously developed sensor **1a**. In all cases, kinetic analysis revealed that initial nucleophilic reaction with an electrophile was rate-limiting, preceding a fast intramolecular cyclization. This result is key to the function, as a slow secondary reaction would limit the sensitivity of the oxime sensors for OP agents. Using TsCl as a generic electrophile, a rate increase of some four to five orders of magnitude was observed with the oximes compared to the alcohol **1a** (Table 2).

Table 2: Reaction rate comparison between the oxime sensors **1**–**4** and the previously reported sensor **1a**.[a]

Sensor	Relative rate	k_{obs} [min^{-1}]	$t_{1/2}$ [min]
4	132000	25.1 ± 0.1	0.028
2	31000	5.9 ± 0.1	0.12
3	14000	2.6 ± 0.2	0.27
1	10000	1.9 ± 0.1	0.36
1a	1.0	0.00019 ± 0.00001	3650

[a] Conditions: [sensor] = 0.07 mM, [TsCl] = 3 mM, [*i*Pr$_2$NEt] = 10 mM in CH_3CN. Experimental errors were calculated from repeated measurements.

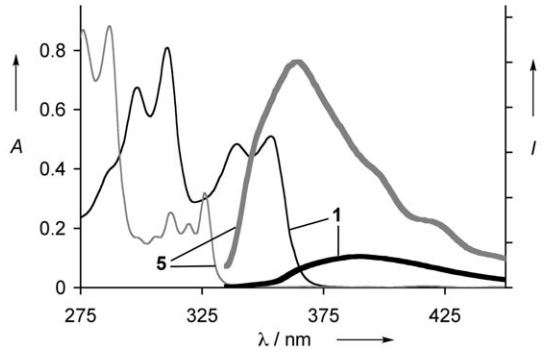

Figure 1. Absorbance (thin lines) and emission (thick lines) spectra for oxime **1** and isoxazole **5**. $\lambda_{exc} = 310$ nm; concentration = 3×10^{-5} M in CH_2Cl_2.

An additional advantage of the β-hydroxy oxime system is access to more practical water-soluble nerve agent detectors. Many of the G-series of nerve agents contain a relatively inert P–F bond and do not readily decompose under ambient aqueous conditions, a property that makes the agents such insidious chemical weapons. The hydrophobicity of the trimethylcyclohexyl scaffold renders the sensors described in Scheme 1a insoluble in water. The oxime-based compounds, however, can be easily solubilized for aqueous conditions with various cores due to the simple, modular design.

Sensor development under aqueous conditions is hampered by the instability of suitable chloride containing electrophiles (DCP is hydrolyzed in water buffered to pH 7 within a few minutes) and requires the use of more toxic nerve

Table 1: Summary of optical data measured for the cyclizations of oximes **1**–**4** to arylisoxazoles **5**–**8** (all spectra were obtained on purified compounds).

	Absorption				Fluorescence			
Cyclization	λ_{max} (oxime)	λ_{max} (isox)	$\Delta\lambda_{max}$	$\varepsilon_{isox}/\varepsilon_{oxime}$[a]	λ_{max} (oxime)	λ_{max} (isox)	$\Delta\lambda_{max}$	Fl_{isox}/Fl_{oxime}[c]
1→**5**	354 nm	326 nm	−28	0.5	392 nm	361 nm	−31	62
2→**6**	423 nm	399 nm	−24	1.8	440 nm	415 nm	−25	46
3→**7**	286 nm	300 nm	14	4.2	430 nm	435 nm	5	9
4→**8**	325 nm	< 280 nm	> 45	1.4[b]	N/A	N/A	N/A	N/A

[a] The ratio of extinction coefficients was calculated at λ_{max} for each cyclization. [b] Compound **8** does not have a $\lambda_{max} > 280$ nm; therefore the extinction coefficient at 300 nm was used. [c] The maximum fluorescence ratio at any single wavelength is tabulated.

agent mimics. Diisopropylfluorophosphate (DFP)[*] mimics the reactivity of G-series agents more accurately than chloride based electrophiles and it is not hydrolyzed by water near pH 7 at an appreciable rate, allowing for aqueous testing. We studied the reaction rates between DFP and the four sensors, **1**–**4**, in aqueous solution buffered to pH 7.5 with 10 mM HEPES buffer. Sensors **1**, **3**, and **4** reacted with DFP with similar rates, but pyrene-based sensor **2** was insoluble under these reaction conditions. The previously described trimethylcyclohexyl system was completely insoluble and direct comparisons could not be made. Instead, the rates were compared to the reaction of DFP with 2-pyridinealdoxime methiodide (2-PAM, Scheme 1 d),[10,14] a known oxime based antidote for OP poisoning, under identical conditions. The three sensors measured were all found to react faster with DFP in solution than 2-PAM (Table 3).

Table 3: Relative reaction rate comparison for the oxime sensors with DFP in aqueous solution.[a]

Compound	Relative rate	k_{obs} [min^{-1}]	$t_{1/2}$ [min]
1	1.8	0.0095 ± 0.0005	73
2[b]	N/A	N/A	N/A
3	1.9	0.010[c] ± 0.001	69
4	1.4	0.0075 ± 0.0005	92
2-PAM	1.0	0.0052 ± 0.0005	133

[a] Conditions: [oxime] = 0.07 mM, [DFP] = 3 mM, buffered at pH 7.5 with 10 mM HEPES buffer. Experimental errors were calculated from repeated measurements. [b] Oxime **2** was insoluble to the reaction conditions. [c] A slow background reaction was observed for oxime **3** in the absence of DFP.

In summary, new nerve agent sensors were developed based on the reaction of β-hydroxy oximes with OP agent mimics. The initial reaction induces a cyclization to an (aryl)isoxazole and displacement of the formed oxime–OP group gives sizable, easily-monitored optical changes. A series of sensors built on aromatic cores was synthesized, including more practical water-soluble ones. Compared to previously reported sensors, considerable reaction rate enhancements with OP mimics and greatly increased sensitivity were achieved. Additionally, any nerve agent detected is detoxified upon reaction, creating the potential construction of hybrid materials—devices that not only detect the toxins but simultaneously decompose them.

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[15] According to the Material Safety Data Sheet (MSDS) of DFP, the oral lethal dose low (LDLO) or the lowest dose at which death may occur is 2.1 $mg\,kg^{-1}$.

[*] Note: DFP is a highly toxic compound and tremendous care must be taken when handling.[15]

