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COMMUNICATIONS

Published on Web 03/17/2006

Fluorescent Sensors for Organophosphorus Nerve Agent Mimics

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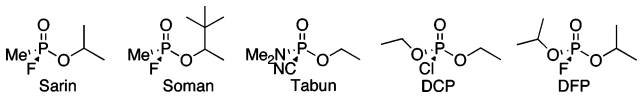
The ease of production and extreme toxicity of organophosphorus (OP)-containing nerve agents underscores the need to detect these odorless and colorless chemicals. The volatile agents including Sarin, Soman, and Tabun are inhibitors of serine proteases, most



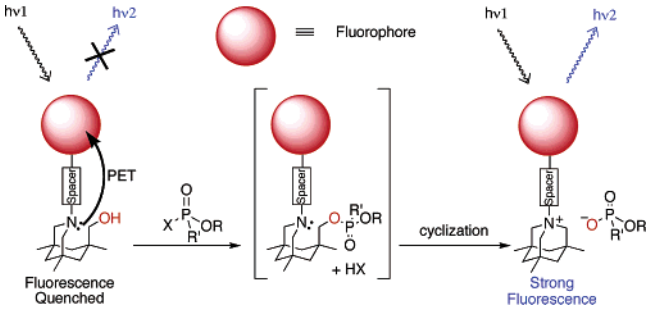
notably acetylcholinesterase, and can be lethal within minutes when inhaled. Their toxicity arises from the nucleophilic attack of the hydroxyl group of a serine residue at the active site of the enzyme on the electrophilic phosphorus. The esterification of the alcohol with the phosphorus renders the enzyme inoperative, allowing a buildup of acetylcholine in the cholinergic synapses that effectively paralyzes the central nervous system.[1] Detection methods for OP nerve agents have been developed based on the use of enzymes,[2] interferometry,[3] and fluorescence.[4] These systems suffer from limitations such as slow response times, operational complexity, and limited portability. We report here a versatile sensor that combines a functional chemical device that triggers easily read responses with a range of fluorescent labels.

Investigations using Kemp's triacid[5] derivatives revealed a particularly promising candidate for an OP nerve agent sensor; these investigations located a primary alcohol in very close proximity to a tertiary amine. Acylation of the alcohol facilitated a rapid intramolecular N-alkylation reaction to relieve strain and produce a quaternary ammonium salt.[6] We extrapolated this reactivity to the conversion of the primary hydroxyl group to a phosphate ester upon reaction with an OP nerve agent, the reaction that is the basis for their toxicity. We expected that a suitable fluorophore appended near the amine would be quenched via photoinduced electron transfer (PET) and increased fluorescence would be observed upon conversion to its ammonium salt (Scheme 1). Many optical sensors employ PET to modulate the fluorescence intensity of a molecule.[7] Amines are frequently utilized as quenchers for PET as one of their nonbonding electrons is readily transferred to a wide variety of fluorescent species including polycyclic aromatic hydrocarbons,[9] coumarins,[10] and fluorescein derivatives.[11] Sensors of this type have been used to detect pH changes,[12] metal ions,[13] and anions.[14]

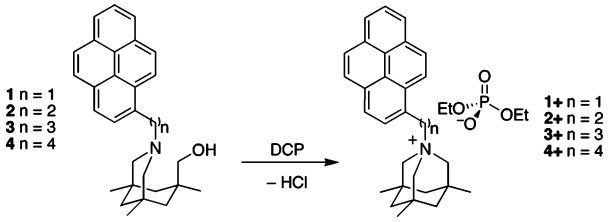
A series of compounds **1**−**4** were synthesized that contain pyrene as the fluorescent acceptor (see Supporting Information). Saturated aliphatic chains ranging from one (**1**) to four methylene units (**4**) were employed to determine how the spacer linking the fluorophore and the amine would affect the efficiency of the sensor. Pyrene was used as the fluorophore as it is available in many easily manipulated derivatives and it is known to accept electrons from tertiary amines in PET processes.

All four alcohols (**1**−**4**) were reacted individually with diethylchlorophosphate (DCP), an available albeit less toxic mimic for the reactivity of OP nerve agents (Scheme 2). All of the compounds reacted cleanly with one equivalent of DCP to produce the

Scheme 1. Fluorescent Sensor Mechanism



Scheme 2. Conversion of the Tertiary Amines **1**−**4** to the Ammonium Salts **1**+−**4**+ Using DCP



azaadamantane quaternary ammonium salts **1**+−**4**+ as observed by TLC and confirmed by NMR. Monitoring the 1:1 stoichiometric reaction in situ by ^{1}H and ^{31}P NMR revealed the first step, the reaction of alcohol **1** with DCP to form the phosphate ester intermediate, to be slow relative to the cyclization to **1**+. We studied the reaction under pseudo-first-order conditions with a large excess of DCP and determined an apparent rate constant for the reaction. With varying equivalents of DCP (from 25 to 150) we calculated the second-order rate constant for the reaction of **1** with DCP: $k = 0.07\ M^{-1}\ min^{-1}$.

The fluorescence emission spectra of the purified ammonium salts were obtained to compare their fluorescence intensities with those of the corresponding alcohols (Figure 1). Although the fluorescence spectrum of each salt was compared with that of its corresponding alcohol, in Figure 1 they have all been normalized to the fluorescence of **1** to allow for easy comparison between the four spectra. The fluorescence intensity of **1** at $\lambda_{em} = 378$ nm was found to increase 22-fold upon cyclization to **1**+. ($\Phi_{fl}(\mathbf{1}) < 0.005$; $\Phi_{fl}(\mathbf{1}+) = 0.10$) As observed for intramolecular electron transfer,[15] the quenching efficiency of the tertiary amine compounds **1**−**4** decreases as more methylene units are added between the amine and the fluorophore. The fluorescence intensity of **4** only displays an increase of 1.1-fold.

The response of the compounds could be conveniently observed with the naked eye. A small piece of filter paper was immersed in a CH_2Cl_2 solution of **1** (1 mg/mL) and then air-dried, leaving a small amount of the sensor dispersed on the paper. The paper emitted light-blue fluorescence when exposed to a handheld UV lamp set to 365 nm (Figure 2: left circle). The sensor-loaded filter

10.1021/ja057449i CCC: $33.50

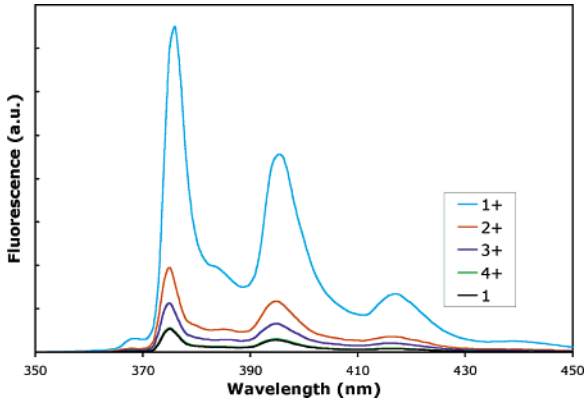


Figure 1. Fluorescence emission spectra of purified compounds **1**, **1**+, **2**+, **3**+, and **4**+ in MeOH (10^{-5}M). The emission intensity of each salt has been normalized to the emission of **1**. Excitation wavelength = 340 nm.

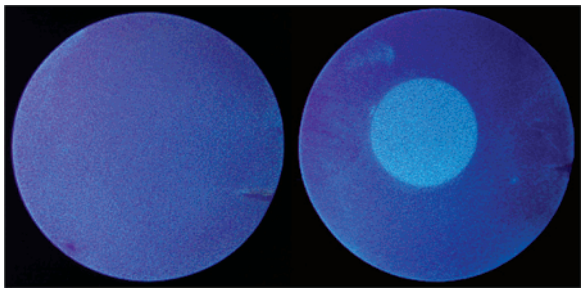


Figure 2. (Left) Filter paper with a thin layer of sensor **1** emitting blue fluorescence. (Right) Filter paper after exposing center portion to 10 ppm DFP vapor for 5 s. Both are irradiated at 365 nm with a UV lamp.

paper was placed across the top of a vial containing 10 ppm DFP vapor for a timed exposure period before being removed and observed again under the 365-nm UV hand lamp. The test was performed with varying exposure times, and a photograph showing the results after 5 s of exposure is shown in Figure 2, right circle. The increased fluorescence intensity is clearly observed in the center portion that was exposed to the DFP vapor.

The sensor design is modular and not limited to pyrene because the fluorophore is not involved in the reaction with the phosphorylating agent; it only responds to the quaternization event. An ingenious sensor devised by Swager operates by *creating* a fluorophore in response to phosphorylating agents.[4] The reactive module presented here can be deployed with many existing fluorophores, provided the energetics for electron transfer are favorable. For example, the amino alcohol module was attached to 6,7-dimethoxycoumarin. The emission spectra before and after exposure to DCP reveal a 20-fold intensity enhancement using this longer-wavelength fluorophore (Figure 3). (Note: fluorescence spectra are obtained on the purified compounds. Φ_{fl}(**alcohol**) = 0.007; Φ_{fl}(**cyclized salt**) = 0.064.) Additional sensors utilizing polyaromatic fluorophores perylene and coronene are given in the Supporting Information.

In summary, a series of small-molecule fluorescent sensors for the detection of OP nerve agents were constructed. The pyrene-based compound containing the shortest spacer between the

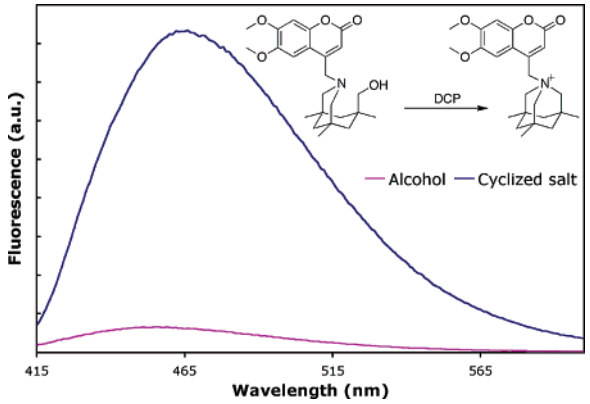


Figure 3. Fluorescence emission spectra of dimethoxycoumarin-based sensor in MeOH (10^{-5}M). Excitation wavelength = 410 nm.

fluorescent acceptor and the amine donor, one methylene unit, provides the most significant increase in fluorescence intensity upon reaction with the nerve agent mimic DCP. The response is observed visually within seconds using a handheld UV lamp. The versatility of the system with other fluorescent species allows a considerable range of absorption and emission wavelengths to be accessed.

Acknowledgment. We thank the Skaggs Institute for Chemical Biology for financial support, and Profs. Tim Swager and Fraser Hof for helpful discussions. T.J.D. is a Skaggs Predoctoral Fellow.

Supporting Information Available: Additional fluorescent sensors, detailed descriptions of experimental methods, synthetic procedures, characterization of new compounds, and additional absorption and emission spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA057449I