

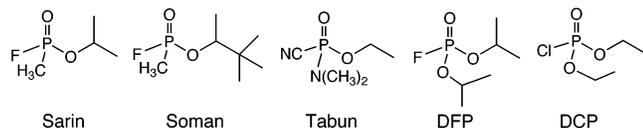
Fluorescent Detection of Chemical Warfare Agents: Functional Group Specific Ratiometric Chemosensors

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Despite several decades of research, there continues to be a need for new and improved methods for the detection of highly toxic organophosphonates.¹ Highly reactive volatile organophosphonates such as Tabun (GA), Sarin (GB), and Soman (GD) are powerful inhibitors of acetylcholinesterase, which is critical in nerve function. The interruption of this enzyme has rapid and fatal consequences to mammalian life and is the basis of chemical weapons that are often referred to as nerve gas.² Related compounds diisopropyl-fluorophosphate (DFP) and diethylchlorophosphate (DCP) have similar reactivity, but they lack the efficacy of typical nerve agents and hence are good model compounds for the design of indicators. There have been many innovations for the detection of these species, including colorimetric detection methods,³ surface acoustic wave (SAW) devices,⁴ enzymatic assays,⁵ and interferometry;⁶ however, all are plagued by at least one limitation such as slow responses, lack of specificity, low sensitivity, operational complexity, or nonportability.

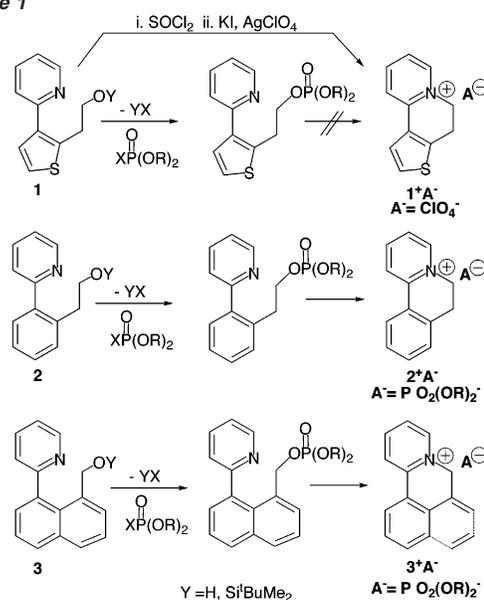


A guiding principle of our efforts in nerve gas detection is to develop methods that respond to the general reactivity that provides the basis of their toxicity. The mechanism of action of nerve agents is the reaction with a hydroxy group to form a phosphate ester at the catalytic site of acetylcholinesterase. Hence, we have sought to produce functional group specific sensors that will transduce the conversion of a hydroxyl to a leaving group such as a phosphate ester.⁷ We report herein the development of a sensitive, fluorescent ratiometric chemosensor and related compounds for the detection of nerve agents.

Our indicator designs focused on producing a new bathochromic absorption and fluorescence in response to the formation of reactive phosphate esters. To achieve this response, we have focused upon intramolecular cyclization reactions, which transform flexible nonplanar weakly conjugated chromophores into rigid planar highly delocalized systems. In addition to generating the expected spectral shifts, the transformation from a flexible chromophore to a rigid extended one will further produce a significant increase in the emission efficiency by reducing the nonradiative rate.

We began by examining thienylpyridyl and phenylpyridyl systems, **1** and **2**, respectively. As shown in Scheme 1, these compounds were expected to undergo an intramolecular nucleophilic substitution reaction upon exposure to a reactive nerve agent. The indicators in Scheme 1 were studied as both free alcohols and silylated variants, with the silicon group potentially offering specificity for P–F groups found in DFP, Sarin, and Soman. Our initial interest in **1**'s framework was due to the fact that in its cyclized form, **1⁺A⁻**, we expected a strong charge-transfer optical

Scheme 1



transition between the electron-rich thienyl group and the electron-poor pyridinium. Although intermediate phosphate esters of **1** form rapidly, they did not undergo cyclization, perhaps due to an unfavorable transition state structure. Forcing conditions produced the cyclized product **1⁺A⁻** (Scheme 1) and the desired red shift with a dramatic increase in fluorescence quantum yield (Table 1).

Table 1. Spectral Data in CH₂Cl₂

compound	abs. max. ^a (log ϵ)	em. max. ^a	quantum yield (%)
1	314 (3.88)	370	4.90
2	273 (3.89)	345	<0.01
3	291 (4.06)	385	13.1
1⁺A⁻	310, 353 (3.71, 3.85)	412	39.6
2⁺A⁻	290 (4.12)	382	52.5
3⁺A⁻	275, 340 (4.16, 3.84)	465	61.9

^a Given in nanometers. ^b Determined on the purified compounds.

In an effort to accelerate the transduction/cyclization reaction, we considered that compound **2** with a phenyl ring in place of the thiophene might provide a more favorable cyclization geometry. Indeed **2** reacts with both DFP and DCP to give the highly fluorescent cyclized product **2⁺A⁻**. As shown in Figure 1, simple protonation of the pyridine nitrogen in **2** by HCl produces a minimal fluorescence response. This specificity is due to the fact that the aromatic rings in **2** undergo geometrical (vibrational–rotational) changes in the excited state that facilitate nonradiative processes and dramatically lower the quantum yield. Hence, by design, we observe a highly efficient fluorescence only after the cyclization reaction, which eliminates rotation about the phenyl–pyridyl bond.

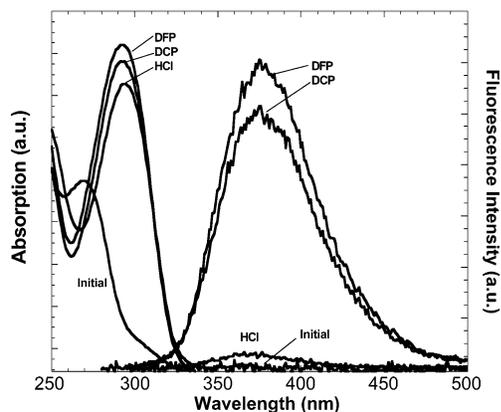


Figure 1. Absorption and emission of **2** before and after addition of DFP, DCP, and HCl (8×10^{-4} M) in dichloromethane (8×10^{-6} M).

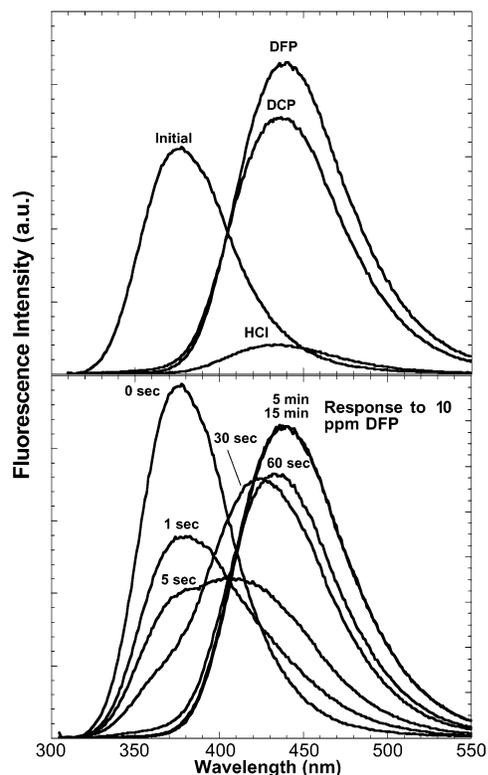


Figure 2. (Top) Emission spectra (thin film) of **3** in its initial state and after saturation with DFP, DCP, and HCl. (Bottom) Thin film emissive response to 10 ppm DFP vapor.

The sensitivity of our sensory scheme is directly related to the rate of the cyclization reaction with higher rates giving lower detection limits. Monitoring the reaction in situ by ^1H NMR analysis revealed that the reactions of DFP with **2**'s alcohol (1:1 stoichiometry) to form the intermediate phosphate ester are fast relative to the cyclization reaction. Hence, by reacting **2** (8×10^{-6} M) with a 100 \times excess of DFP (8×10^{-4} M), we find that the cyclization is rate limiting and can be studied under pseudo first-order kinetics. The emission of 2^+A^- provided a convenient concentration

measurement that proved to be kinetically well-behaved with a pseudo first-order rate constant of $k_{\text{obs}} = 0.0014 \text{ s}^{-1}$.

Although **2**'s response could be enhanced by amplification methods developed by our group,⁸ we considered the cyclization reaction to be unacceptably slow. To produce an intrinsically more sensitive indicator, we synthesized **3** that should have superior reaction kinetics due to the restricted conformation of the naphthalene group that favors cyclization and the substitutionally activated α -aryl phosphate ester intermediate. Compound **3** offered additional spectroscopic advantages such as longer wavelength emission and absorption as well as relatively strong fluorescence in its native state. The latter allows **3** to be used directly in ratiometric detection schemes. Figure 2 shows responses of **3** in a cellulose acetate film to DFP, DCP, and HCl. In this thin film, the emission wavelengths are shifted to higher energy relative to the solution values (Table 1) and occur at 375 and 438 nm for **3** and 3^+A^- , respectively, suggesting that the cellulose acetate exhibits less dielectric relaxation about a more polar excited state. The reaction rates of **3** are now sufficiently fast that detailed kinetics were not possible, and the reaction rate in CH_2Cl_2 is estimated to be $k_{\text{obs}} > 0.024 \text{ s}^{-1}$ or at least 17 times faster than **2**. This faster rate is reflected in the rapid responses of thin films to 10 ppm vapors of DFP (Figure 2, bottom). The lower quantum yields observed in Figure 2 at 1 s and 5 s are likely due to protonation of the pyridine and/or the transient intermediacy of the phosphate ester.

In summary, we have developed a highly sensitive chemosensor for chemical warfare agents. Indicator **3**'s functional group specific nature produces a response not only to chemical warfare agents but also to other similarly reactive toxic industrial chemicals (e.g., SOCl_2) that also pose a threat to homeland security.

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Supporting Information Available: Data and synthetic preparations (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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