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A Molecular Probe for the Highly Selective Chromogenic Detection of DFP, a Mimic of Sarin and Soman Nerve Agents**

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The use of chemical-warfare (CW) agents in terrorism has proven the need for development of reliable and accurate methods to detect these lethal compounds.[1] Among CW, nerve agents are especially dangerous, and the UN classifies them as weapons of mass destruction. Nerve agents are capable of interfering with the action of the nervous system through the inhibition of acetylcholinesterase, resulting in acetylcholine accumulation in the synaptic junctions; this hinders muscles from relaxing.[2] From a chemical viewpoint, nerve agents are organophosphonates with good leaving groups. Current nerve-agents-monitoring methods are mainly based on biosensors,[3] ion mobility spectroscopy,[4] photonic crystals,[5] electrochemistry,[6] microcantilevers,[7] and optical-fiber arrays[8] and show certain limitations, such as low portability and complexity. Recently, as an alternative to these classical methods, the development of fluorogenic and chromogenic probes has proven useful for the recognition of these chemicals in solution and gas phase.[9] Chromogenic systems are especially appealing because of the use of widely available instrumentation and detection with the naked eye. Nevertheless, examples of selective and sensitive chromofluorogenic probes for detection of these derivatives are still rare. The chemosensors described in the literature make use of easily observable and measurable fluorescence and color changes, and involve photon-induced electron transfer (PET)-based processes,[10] oximate-containing derivatives,[11] molecularly imprinted polymers,[12] nanoparticles,[13] carbon nanotubes,[14] cyclization reactions in push–pull chromophores,[15] displacement assays,[16] and organic–inorganic hybrid materials.[17] In most of these studies nerve-gas simulants, such as diethylcyanophosphate (DCNP) and diisopropylfluorophosphate (DFP), are used. Because these compounds contain the same leaving groups as the real nerve agents Sarin, Soman, and Tabun, they display similar reactivity, but they lack the rigorous toxicity. Most of these reported chromofluorogenic probes rely on the electrophilic reactivity of nerve gases with suitable nucleophiles, which, in many cases, result in an ON–OFF behavior. However, despite the intrinsic interest in the design of such chromofluorogenic probes, the reported examples have certain limitations. In particular, the fact that the reactions are nonspecific and, in general, the reported dye-based probes display the same optical response to all nerve agents. However, the development of rapid methods for the individual signaling of Sarin, Soman, or Tabun may prove important. For instance, even though the emergency response protocol is similar for all nerve gases, different toxicities and experimental evidence that some antidotes are ineffective for certain nerve agents indicate the importance of distinguishing certain agents within this family of lethal chemicals.[18] Following our interest in the development of chromofluorogenic probes for nerve agents,[15,17] we report herein a simple colorimetric system that is not only able to respond to nerve-agent mimics, but is also able to distinguish DFP (a mimic of Sarin and Soman nerve gases) from other simulants and organophosphates. The signaling protocol involves chromogenic probe **1** (Scheme 1). This molecule was designed to contain two chromo–chemosensing moieties; that is, 1) a nucleophilic hydroxyl group that provides a suitable reactive site for electrophilic phosphorous atoms, such as those in nerve agents and 2) a *tert*-butyldimethylsilylether (TBDMS) group that is known to react with fluoride,[19] which is a specific byproduct to be exclusively obtained upon the phosphorylation of the OH moieties with DFP. Both reactions, that is, phosphorylation of the hydroxyl moiety and elimination of the TBDMS group, are expected to occur with a significant color modulation (see below). Derivative **1** was readily prepared from 4-bromophenol containing a TBDMS group and Michler's ketone (4,4′-bis(dimethylamino)benzo-

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[**] DFP = diisopropylfluorophosphate.

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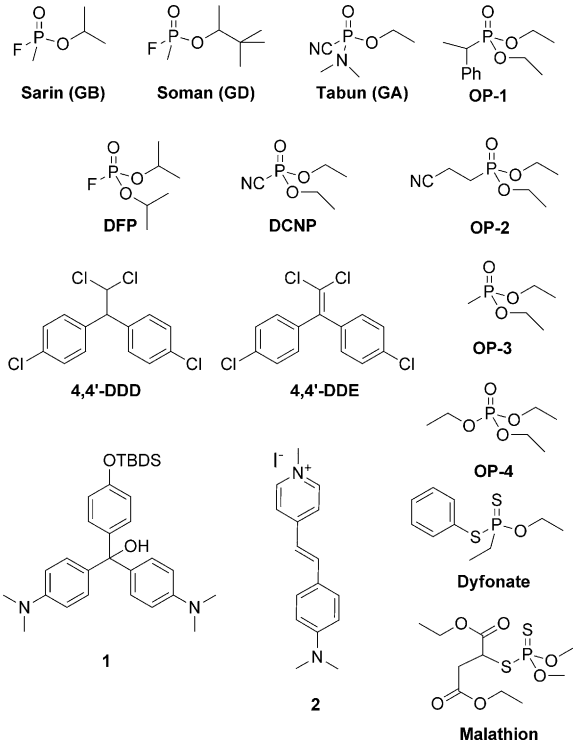


Scheme 1. Chemical structures of nerve-agents Sarin, Soman, and Tabun, their simulants (DFP and DCNP), potential interfering agents, probe **1**, and acid–base indicator **2**.

phenone) through the corresponding organolithium derivative (see the Supporting Information).

As a first step, the reactivity of **1** was tested against nerve-agent simulants DFP and DCNP in acetonitrile/water (9:1, *v/v*) mixtures ($c = 1.0 \times 10^{-5}$ mol dm^{-3}) buffered at pH 8 (HCl/borax buffer) to avoid pH changes due to a potential partial hydrolysis of the simulants. Compound **1** is colorless and displays a unique band at 266 nm. Upon the addition of 100 equivalents of DCNP, a remarkable change in the spectrum was observed after a short time (less than 30 s) with the appearance of two bands at about 342 and 608 nm (Figure 1); this resulted in a color modulation from colorless to bright blue–green (see Figure 2). This shift to higher wavelengths is consistent with a reaction between the electron-deficient phosphorus atom in DCNP and the nucleophilic hydroxyl moiety in chromo-reactant **1**. In the presence of DCNP, the hydroxyl moiety of **1** undergoes phosphorylation to yield compound **I** that quickly results in the formation of the highly conjugated salt **II** (green, see Scheme 2). To assess the mechanism involved in the observed chromogenic response, compound **II** was prepared by following a different synthetic route, which involved the treatment of an acetonitrile solution of chromo-reactant **1** with one equivalent of trifluoroacetic acid at 40 °C for 30 min. The reaction product was isolated and characterized as derivative **II**. The product obtained by following this synthetic route showed an identical ^{1}H NMR spectrum to that of the product obtained from the reaction of **1** with DCNP.

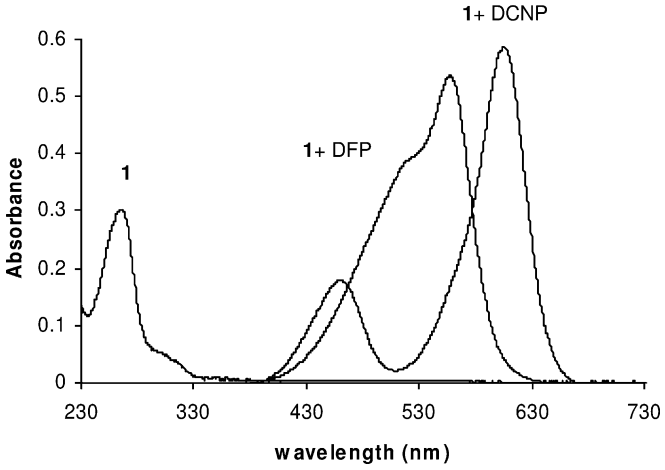
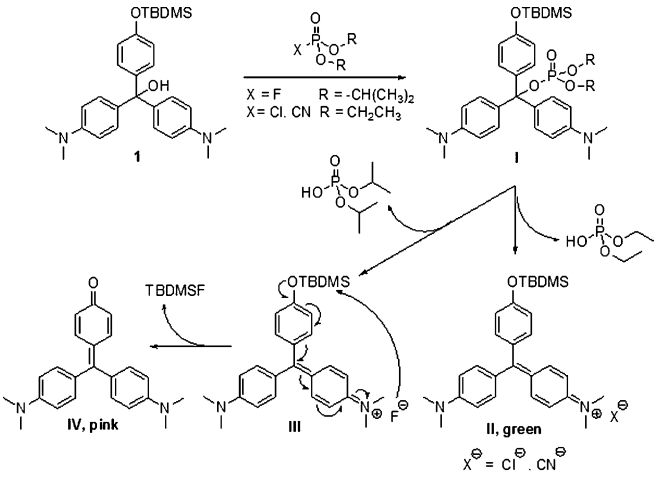


Figure 1. UV/Vis spectra of chromo-reactant **1** ($c = 1.0 \times 10^{-5}$ mol dm^{-3}) in acetonitrile/water solution only (9:1, v/v; buffered with HCl/borax at pH 8.0) and in the presence of DCNP and DFP (100 equivalents).

Figure 2. Photograph of probe **1** (1.0×10^{-5} mol dm^{-3}) in acetonitrile/water solutions (9:1, v/v; buffered with HCl/borax at pH 8.0). From left to right: **1**, **1**+DCNP, **1**+DFP.



Scheme 2. Chromogenic response mechanism for probe **1** in the presence of nerve-agent-simulants DCNP or DFP.

In contrast, a very different chromogenic behavior was observed after addition of 100 equivalents of DFP. On this occasion, the colorless solution of **1** initially became green, but then turned to pink after a few seconds due to the appearance of a band at 557 nm (see Figure 1). In this case and in mechanistic terms, after completing the first step (to yield structure **I**) and the additional dephosphorylation pro-

cess (to yield compound **III**), the fluoride anion, obtained as a byproduct of the reaction, was able to attack the silicon atom of the TBDMS moiety; this resulted in an S_N2 deprotection reaction that yielded ketone **IV** (pink, see Figure 2). To confirm the mechanism proposed, derivative **II** was treated with one equivalent of tetrabutylammonium fluoride to unequivocally yield ketone **IV** (see the Supporting Information for details). A remarkable detection limit (DL) in acetonitrile/water (9:1, *v/v*) solutions of 130 ppb for DFP was determined. The DL for DCNP was 30 ppm under similar conditions (see the Supporting Information).

In an additional step, the reactivity of **1** towards the organophosphorous (OP) compounds ($c = 160$ ppm; Scheme 1) was studied in mixed acetonitrile/water (9:1, v/v; buffered with HCl/borax at pH 8.0). In all cases, the solutions of **1** remained colorless, which indicated that no reaction occurred between **1** and these OP derivatives. The acetonitrile/water (9:1, v/v) solutions of **1** were also tested in the presence of potential interfering agents that may be present in a civilian setting, such as malathion, dyfonate, 4,4′-DDD, or 4,4′-DDE (see Scheme 1), and oxidants, such as O_3, H_2O_2, or peroxides at a concentration of 1000 ppm. No change in color was noted in the presence of any of these agents. Moreover when DCNP or DFP were added to the acetonitrile/water (9:1 v/v) solutions of **1**, containing all these potential interfering agents, color changes, such as those shown in Figure 2, were observed, indicating that these species do not interfere with the detection of these nerve-agent mimics. No changes in color were observed for the acetonitrile/water solutions of **1** in the presence of petrol or fuel oil.

As we were confident with these results, we decided to move one step further and we carried out studies with chromo-reactant **1** to develop test strips for the simple colorimetric detection of nerve-agent simulants in solution or gas phase. To test this possibility, a hydrophobic polyethylene oxide film containing 0.1 % of compound **1** and 1 % of Cs_2CO_3 was prepared as a prototype of the colorimetric probe.[20] Moreover to overcome possible false positives due to the potential presence of strong acids in solutions or as vapors, a polyurethane membrane containing 0.1 % of acid–base indicator **2** (see Scheme 1) was also prepared and included in the sensing ensemble (see Figure 3). Dye **2** was selected because it was unable to react with the simulants, yet it reacts with acids by changing the color from orange to colorless upon protonation. In a typical assay, polymers were either exposed to acetonitrile or acetonitrile/water (9:1 v/v) solutions of DFP, DCNP, HF, or HCl or placed in a container containing the nerve-agent simulants or the acids (25 ppm for DFP, DCNP and 10 ppm for HF, HCl introduced into the container as aerosols). Similar chromogenic behaviors were seen in both solution and gas phase; that is, in the presence of DFP the polyethylene oxide film containing **1** turned pink, whereas in the presence of DCNP, the film became blue–green. In both cases, the film containing **2** remained orange. Moreover, in the presence of HF and HCl, the films of **1** became pink and blue–green (as observed with DFP and DCNP, respectively) yet in this case films of **2** turned colorless due to protonation of the dye (see Figure 3). This simple ensemble, combining the reactive **1** and the acid–base dye **2**, is able to differentiate DFP (a Sarin and Soman simulant) from DCNP (a Tabun simulant) and at the same time detect the presence of possible interfering agents, including acids, such as HF.

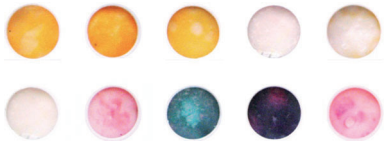


Figure 3. Polyurethane membranes of probe **1** (bottom) and the acid–base indicator **2** (top) in absence (blank) and in the presence of DFP (25 ppb), DCNP (25 ppb), HCl (10 ppm), and HF (10 ppm) vapors (from left to right).

To complete the study of potential interfering agents in the gas phase, films containing **1** were included in a flask with saturated vapors of malathion, dyfonate, 4,4′-DDD, 4,4′-DDE, and OP-1-4 derivatives for at least 24 h. The same experiments were performed in the presence of gasoline and diesel exhaust gases, which might be present in military settings. No changes in film color were observed in any of the cases. Additionally it was confirmed that the films of **1** were still able to react with DFP and DCNP in the presence of these potential interfering agents, with a similar response to that shown in Figure 3.

To summarize, we report herein a simple colorimetric probe based on a triarylmethanol derivative that is able to colorimetrically detect nerve-agent mimics and, at the same time, selectively distinguish the presence of the Sarin or Soman simulants (DFP) in either acetonitrile/water (9:1 v/v) solutions buffered at pH 8.0 or in gas phase. This probe was designed to contain two reactive sites: an OH group that provides a suitable reactive place for nerve agents and a TBDMS moiety that is able to react with fluoride, which is a specific byproduct of the phosphorylation of OH by DFP. This study indicates the possibility of developing similar systems for the selective detection of certain nerve gases that make use of the unique reactivity of the specific byproduct obtained upon the generic phosphorylation reaction induced by nerve agents. To the best of our knowledge, the system we report herein is the first probe for selective colorimetric detection of Sarin and Soman versus other nerve gases, such as Tabun.

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Keywords: chemosensors · chromogenic detection · DFP recognition · nerve-agent simulants · organophosphonates

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