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A Concise Colorimetric and Fluorimetric Probe for Sarin Related Threats Designed via the "Covalent-Assembly" Approach

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Supporting Information

ABSTRACT: A turn-on signal from zero background allows sensitive detection of a weak signal and is highly desired. The "covalent-assembly" probe design principle is powerful in this regard. Herein, we report an embodiment of this principle (**NA570**) for detection of Sarin related threats, based on a phenylogous Vilsmeier−Haack reaction. **NA570** bears a concise molecular construct, exhibits a colorimetric and a fluorimetric signal, and has potential for real applications.

Sarin, a weapon of mass destruction developed during WWII, acts by inhibition of acetylcholinesterase and thereby overstimulation of the central nervous system.[1] Though the possibility of military use is low with the Chemical Weapon Convention[2] in force, terroristic use of Sarin (**1**) is a real threat[3] and demands countermeasures including robust and convenient detection.[4]

Though Sarin is the active toxin, high specificity of an assay toward Sarin itself could ironically be a disadvantage, because Sarin is not stockpiled in the arsenal owing to its limited shelf life and high toxicity, but rather its two synthetic precursors, i.e., methylphosphonic difluoride (**2**) and 2-propanol are (Figure 1).[5] For this reason, Sarin and **2** are both an immediate threat.

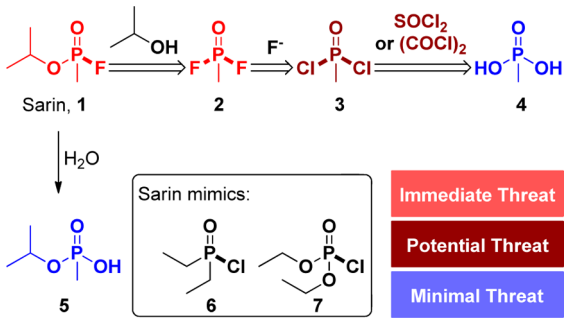

Figure 1. Chemical species related to Sarin threat.

In addition, synthesis of difluoride **2** from commercially available methylphosphonic acid (**4**) is also straightforward, i.e., through a chlorination with thionyl chloride ($SOCl_2$) or oxalyl chloride ($C_2O_2Cl_2$) followed by a halide exchange with a fluoride source like NaF or HF.[5] Thus, the presence of $SOCl_2$, $C_2O_2Cl_2$, and methylphosphonic dichloride (**3**) away from their authorized storage sites should also trigger an alarm against potential Sarin related terroristic activities. As Sarin and **2** are not available to us, diethylphosphinic chloride (**6**) and chlorodiethylphosphate (**7**) were used as mimics for Sarin, and **3** as a mimic for **2**. The fact that **3**, **6**, **7**, $SOCl_2$, and $C_2O_2Cl_2$ are all strongly electrophilic makes it chemically possible for a single probe to detect them all, and yet such a probe has not been reported to our knowledge. Methylphosphonic acid (**4**) and isopropyl methylphosphonic acid (**5**) were excluded from our list of chemical species to detect because they are not toxic and present minimal threat. Also the phosphorus atom present in reagents **4** or **5** is not prone to nucleophilic attack and requires different chemistry for detection.

While fluorescence based probes are highly sensitive and fluorimeters can be very portable for field use as demonstrated by FIDO,[6] a color change facilitating naked-eye detection could be necessary in situations where a fluorimeter is unavailable or fails unexpectedly. Therefore, a probe generating both a colorimetric and a fluorimetric turn-on signal in response to the relevant chemical species is attractive. Further, optimal detection sensitivity is achieved when a probe gives zero background signal, a feat not readily obtained through the classic dye-linker-receptor principle.[7] These considerations promote us to explore a novel design principle, which we term "covalent-assembly" (Figure 2a). The fundamental feature of the "covalent-assembly" principle is that the color-enabling push−pull conjugative backbone of a dye is split into two fragments in the probe. These two fragments are covalently assembled to restore the dye through a chemical cascade, which is triggered selectively by the analyte of interest. This feature warrants both a turn-on colorimetric and a turn-on fluorimetric signal from a zero background, hence an optimal detection sensitivity. A second important feature of the "covalent-assembly" approach is that the probes are simple in terms of structural complexity and synthesis. Synthesis of a dye with proper chemical handles is not always easy, and a multistep cascade carrying a dye scaffold is even more challenging. A "covalent-assembly" type probe employs the analyte of interest to carry out the dye synthesis *in situ*, thereby alleviates synthetic efforts.

Herein, we report our progress toward the development of a practical probe (**NA570**) fighting against Sarin related threats.

Existing probes for nerve agents generally rely on nucleophilicity of an aliphatic hydroxyl,[8] hydroxyl oxime,[9] or amine[10] toward electrophilic phosphorus atoms. However, the

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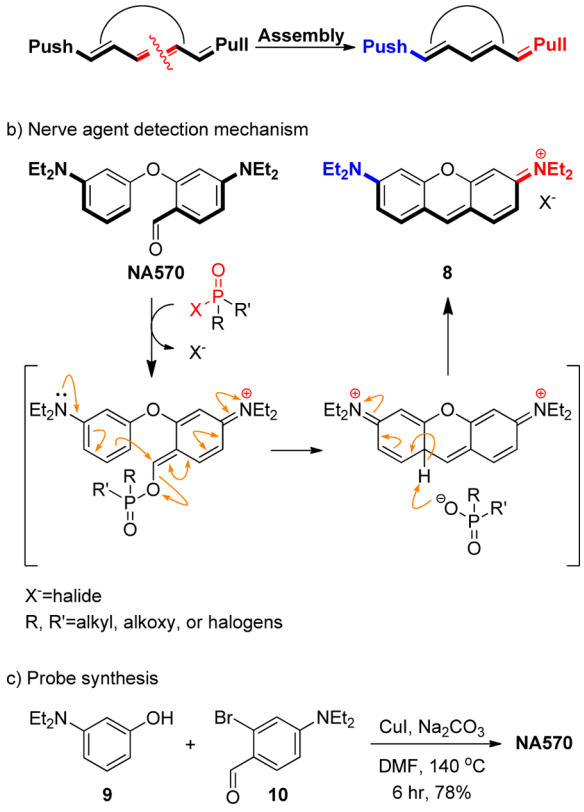

Figure 2. (a) The general working principle of molecular probes designed via the "covalent-assembly" approach. (b) The structure of **NA570** and its proposed detection mechanism. (c) Chemical synthesis of **NA570**.

existing chemistry for nerve agent recognition could not be readily coupled for use with the proposed covalent-assembly principle. Therefore, novel chemistry, i.e., a phenylogous Vilsmeier–Haack reaction, was employed in our work and lead to the design of **NA570** (Figure 2b). Highly electron deficient phosphorus centers of the nerve agents, their mimics, or precursors (vide infra) are expected to be able to activate the 4-diethylaminobenzaldehyde in **NA570** toward nucleophilic attack by the nearby diethylaniline and ultimately furnish Pyronine B (**8**), a highly absorbing and fluorescing xanthene dye (ε = 83 000 $cm^{-1}{\cdot}M^{-1}$ and ϕ = 0.64 in chlorobenzene). **NA570** was conveniently synthesized by a simple condensation of commercial 3-diethylaminophenol (**9**) and 2-bromo-4-diethylaminobenzaldehyde (**10**) in a 78% yield (Figure 2c). **NA570** is indefinitely stable if protected from light and acid. No decomposition of **NA570** was observed during the course of the project.

The viability of this design was validated by reactions between **NA570** and $PO(OEt)_2Cl$ (**7**) in a list of nonprotic solvents. Upon addition of **7** (10 equiv) into a solution of **NA570** (50 μM), the kinetics of pyronine B (**8**) formation was monitored with a fluorimeter. In chlorobenzene, dichloromethane, dichloroethane, or nitrobenzene, the fluorescence signal rose instantaneously from the baseline upon addition of **7**. The signal intensities increased steadily over the course of monitoring for 2 h (Figure 3a). In comparison, signal intensities in tetrahydrofuran, ethyl acetate, and dimethyl sulfoxide were much lower, suggesting that solvents bearing a nucleophilic oxygen atom are not an optimal working medium. On the basis of these results, chlorobenzene was chosen for further studies.

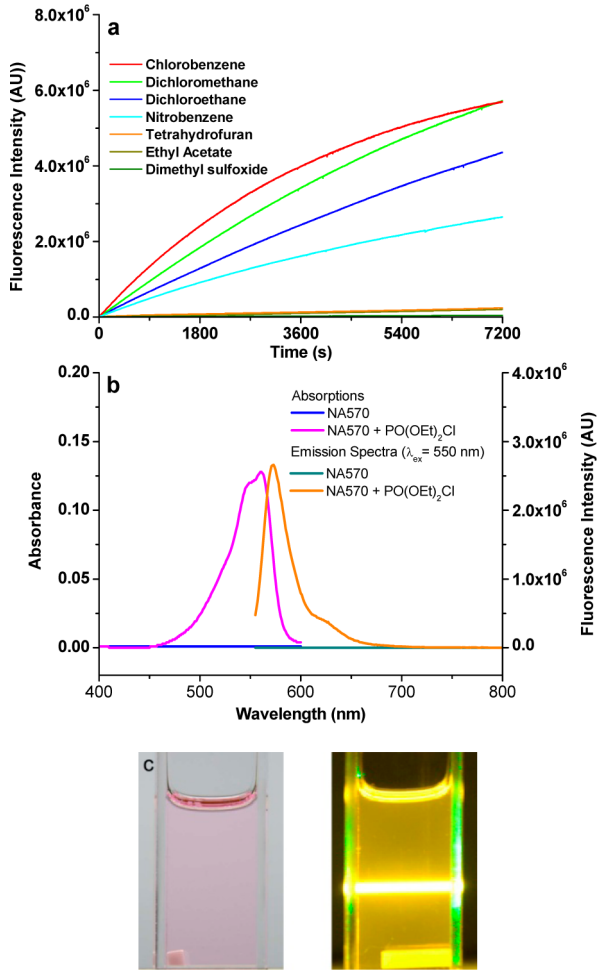

Figure 3. (a) Fluorescence enhancement upon addition of 10 equiv of $PO(OEt)_2Cl$ (**7**) into a solution of **NA570** (50 uM) in various solvents. λ_{ex} = 563 nm and λ_{em} = 573 nm. (b) The absorption and fluorescence emission spectra of **NA570** solution before and 30 min after addition of **7**. (c) The picture of the **NA570** solution 30 min after addition of **7**, taken under white light (left) and 532 laser excitation (right).

The absorption and emission spectra of the solution before and 30 min after addition of **7** were recorded in chlorobenzene (Figure 3b). Prior to the addition of **7**, the **NA570** solution displays no absorption beyond 400 nm, is colorless, and exhibits no background fluorescence with excitation at 563 nm. After 30 min, pyronine B (**8**) generated *in situ* is easily captured by its distinctive pink color with a maximum absorption at 563 nm, or by its intense yellow-orange fluorescence with a maximum emission at 573 nm (Figure 3c).

We next examined the responses of the **NA570** solution in chlorobenzene (50 μM) toward the model set of compounds indicative of immediate or potential Sarin threat: **3**, **6**, **7**, $SOCl_2$, and $C_2O_2Cl_2$. All these species (at 1 equiv) induced a fluorescence turn-on (Figure 4a). Within the first ca. 150 s, signal intensity was in the order of **7** > **6** ≈ $C_2O_2Cl_2$ > $SOCl_2$ ≈ **3**. Then, the signal enhancement from $C_2O_2Cl_2$ and **3** gradually slowed down, while the rest continued increasing steadily during the entire course of monitoring for 1800 s. In comparison, nontoxic methylphosphonic acid (**4**) did not induce any signal enhancement even at a concentration as high as 100-fold excess. Additionally, titrations showed that the signal intensities caused by these Sarin related species were

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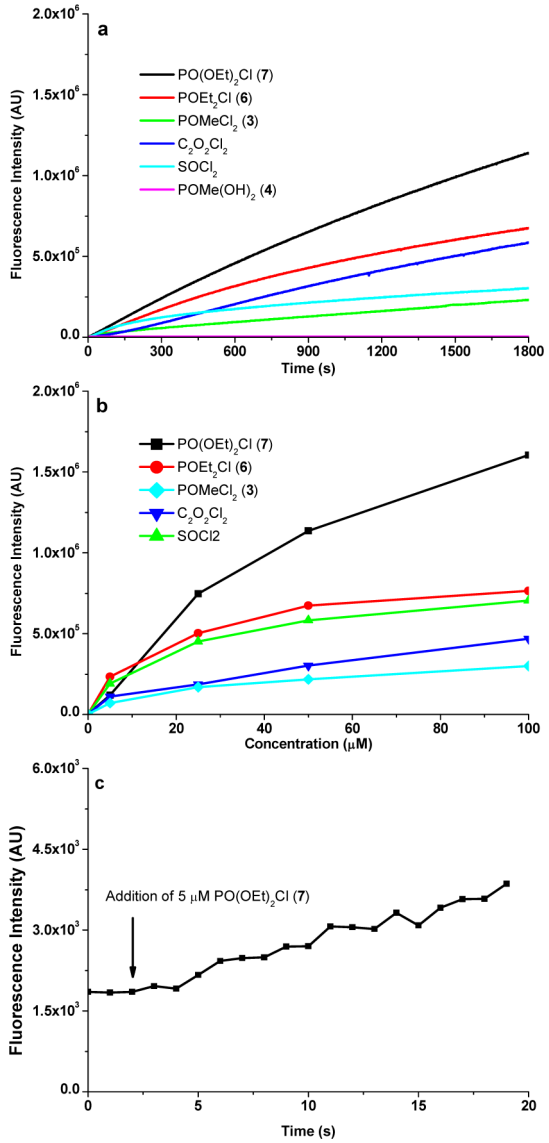

Figure 4. (a) Fluorescence enhancement (0−1800 s) of a **NA570** solution upon addition of various species (50 μM) related to Sarin threat. (b) Dose-dependent signal enhancements upon addition of various analytes. (c) Fluorescence enhancement (0−18 s) upon addition of 5 μM of **7**. The concentration of **NA570** solution is 50 μM.

dose-dependent (Figure 4b). This suggests that **NA570** has potential in not only qualitative but also quantitative measurements. Though the detection kinetics of **NA570** was slower than that reported for existing probes based on hydroxyl oxime,[9] we note that in the case of **NA570** the signal enhancement within the first 10 s is more than enough to affirmatively detect the presence of Sarin related chemical species. For example, addition of 5 μM of chlorodiethylphosphate (**7**) resulted in a fluorescence signal within 10 s that was 45 times higher than the measured noise level (Figure 4c). Though irrelevant from Sarin related threats, strong Lewis acids including $AlCl_3$, BBr_3 or $TiCl_4$ were found to also effectively induce the Pyronine B (**8**) formation from **NA570**.

Fluoro-subsituted phosphrous centers react faster than corresponding chloro analogues as has been shown previously by Pilato[8a] and Swager.[8b] Therefore, the feasibility of **NA570** for detection of the aforementioned list of reagents, especially **6** and **7**, also suggests that Sarin and its immediate precursor (**2**) should yield stronger signals and display higher sensitivity than all of the species tested in this work.

In conclusion, we have developed an optical probe (**NA570**) for detection of Sarin related threats, which we have shown to have potential for practical applications. **NA570** was designed via a novel "covalent-assembly" principle, advantages of which include compact probe structure, convenient probe synthesis, and a highly sensitive colorimetric and fluorimetric turn-on signal from zero background. We expect the covalent-assembly principle to find additional applications for a broader scope of substrates.

■ ASSOCIATED CONTENT

Supporting Information

General methods, **NA570** synthesis, ^{1}H and ^{13}C NMR, HRMS and supplementary spectral results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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