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A Selective Turn-On Fluorescent Sensor for Sulfur Mustard Simulants

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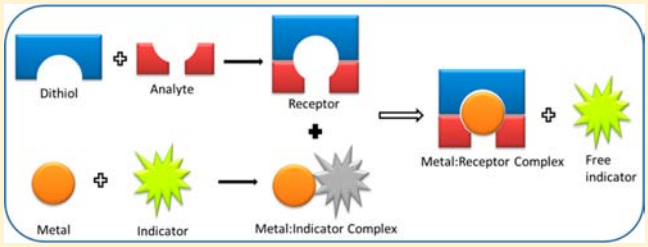
Supporting Information

ABSTRACT: A fluorescent turn-on sensor for the selective and sensitive detection of sulfur mustard simulants in water that uses a metal-ion indicator displacement assay (IDA) has been devised. In this IDA approach, a sulfur mustard simulant (the analyte) is allowed to react with a dithiol (**1**) to form a podand (**2**). This podand has a strong affinity to bind with Cd^{2+} and displaces an indicator (4-methylesculetin, **ME**) from a Cd^{2+}−indicator complex (**8**) to give a turn-on of fluorescence. The detection is rapid and highly selective, as we did not observe any interference from other electrophiles, even from the oxygen analogue of the mustard simulant. The protocol was successfully used for the detection of the simulant present on surfaces and in soil samples.



INTRODUCTION

Sulfur mustard (SM), also called mustard gas/HD (Figure 1), has frequently been used on a large scale against military and civilian targets since the beginning of the 20th century, causing millions of casualties.[1] In a biological setting, it gradually reacts with water and releases HCl, producing painful blisters on the skin, causing damage to the eyes and lungs, and sometimes leading to death. The toxicity of SM is also due in part to its ability to alkylate the guanine nucleotide in DNA.[2] In the long run, it causes carcinogenic and mutagenic effects.[3] Furthermore, there is no treatment or antidote that can arrest the basic cause of mustard gas injury. Because of its ease of preparation compared with other chemical warfare agents, the use of this agent by terrorist groups or rogue nations represents a serious threat to humankind and homeland security. Therefore, there is a significant interest in the development of sensors and detection systems for this chemical.

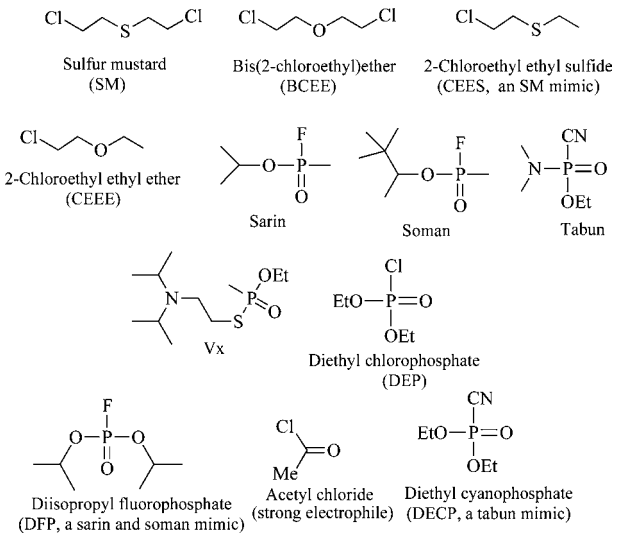


Figure 1. Various chemical warfare agents and their mimics.

Unlike the situation for nerve agents (Figure 1),[4] colorimetric or fluorescence detection methods for SM are nearly nonexistent. The detection protocols for SM can be divided into two general categories: point and standoff. The methods that use point detectors work on the principle of ion-mobility spectrometry, flame photometry, mass spectrometry, photoacoustic infrared spectroscopy, electrochemistry, and detection kits and tickets that use a variety of chemical reactions.[5] Standoff detectors use infrared remote-sensing techniques from a significant distance. Apart from these, methods based on conventional analytical techniques,[6] molecularly imprinted polymers,[7] immunochemical methods,[8] quartz crystal microbalance analysis,[9] and platinum(II) pincer complexes[10] have also been developed.[11]

The prime reason that efficient optical detection systems for SM do not exist, in contrast to the case for nerve agents, lies in the different electrophilicity of SM. The general mechanism for detection of nerve agents involves nucleophilic attack of the probe molecule on the electrophilic agent to form a phosphate ester (Figure 2), which responds in one of three ways to generate a detectable optical signal (chromogenic and/or fluorogenic): suppression of photoinduced electron transfer (PET) from the nitrogen atom to the fluorophore (Figure 2A); internal charge transfer (ICT) (Figure 2B); or intramolecular cyclization, which also results in suppression of PET (Figure 2C).[4]

In contrast, being a simple primary alkyl halide, SM is not a particularly good electrophile. However, in the presence of an ionizing solvent, its electrophilicity is greatly enhanced as a result of the formation of three-membered cationic sulfonium

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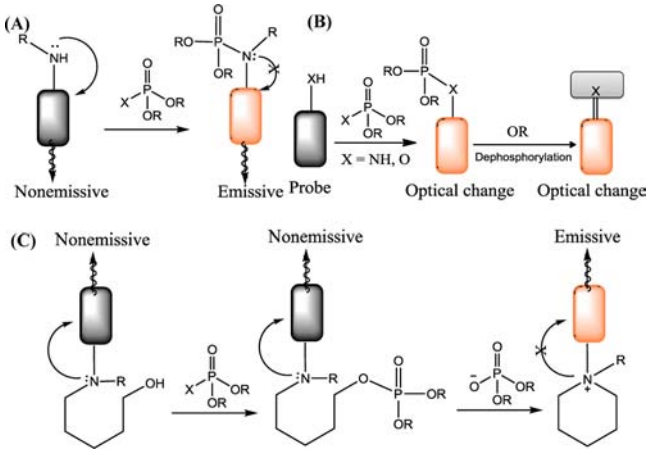
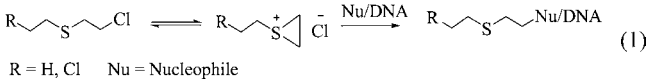


Figure 2. Three strategies for detecting phosphoryl nerve agents. (A) Phosphorylation of an amine participating in PET quenching turns on the fluorescence. (B) Chromogenic and/or fluorogenic detection via internal charge transfer (ICT). (C) Phosphorylation of a hydroxyl group proximal to an amine leads to cyclization and restoration of fluorescence.

heterocycles,[12] which can alkylate DNA and are reactive toward water (eq 1). The limited number of optical detection



(1)

techniques and the unique reactivity of SM invoked our interest in developing a rapid, selective, sensitive, and practical chromogenic and/or fluorogenic chemical sensor for this substance.

In our study, we chose 2-chloroethyl ethyl sulfide (CEES), which is known as "half mustard", as an SM simulant. CEES has the relevant chemical properties of the real agent without its associated toxicological properties. The median lethal dose (LD_{50}) values for SM are 2.4 mg/kg (rats) and 8.1−9.7 mg/kg (mice) for oral exposure and 3.4 mg/kg (rats) and 19.3 mg/kg (mice) for skin exposure.[13] CEES was introduced in World War I and is considered to have a relatively high LD_{50} of 252 mg/kg (rat oral).[14]

RESULTS AND DISCUSSION

Design Criteria. Designing a fluorescent detection method for mustard gas is challenging for two reasons. First, SM does not have any traditional molecular recognition sites, such as hydrogen-bond donor or acceptor groups. Second, there are no highly electrophilic sites, such as nerve agents possess, until the SM is placed in an ionizing solvent. Furthermore, because other chemical warfare agents are more electrophilic, they would be expected to react faster with any nucleophile, and therefore, the possibility of interference or confusion when differentiating SM from these warfare agents would be expected (Figure 1). With these issues in mind, we designed a strategy to target the three-membered cationic sulfonium heterocycle selectively, while not signaling more electrophilic agents or less active electrophiles. As discussed below, the plan was to deactivate the more electrophilic species by using high pH while employing a nucleophile that is not reactive enough to target less electrophilic species. In this manner, we planned to make a method that would give a positive signal only in the presence of SM.

A schematic illustration of the basic strategy is shown in Figure 3. The target analyte reacts with a dithiol to give a

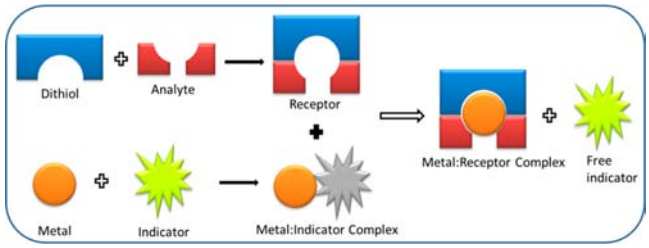
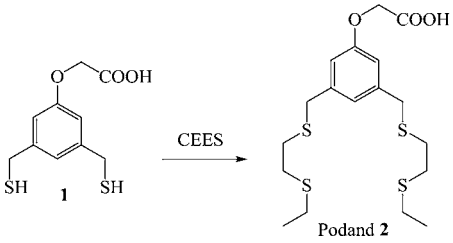


Figure 3. Schematic presentation of an approach for the detection of mustard gas.

receptor. With CEES, the receptor would be an open chain (a podand), while with real SM we anticipate that the receptor would be a macrocycle. Consequently, the analyte is incorporated into the receptor. Separately, a metal−indicator complex is prepared using a metal having a good affinity for sulfur. The indicator, when bound to the metal, is non-fluorescent. When these two components, the analyte-bound receptor and the metal−indicator complex are mixed together, the receptor binds the metal and displaces the indicator from the complex. Thus, the indicator regains its fluorescence, and the intensity can be correlated to the quantity of SM or SM simulant.

Equation 2 shows the reaction of dithiol **1** with CEES to form sulfur-based podand **2**. The carboxylic acid in **1** was



(2)

incorporated to increase the water solubility of this nucleophilic dithiol. The resulting podand was anticipated to be an excellent receptor for cadmium ion in water.[15] Moreover, the plan was to perform the reaction presented in eq 2 in hot water at pH 9, which would hydrolyze other more reactive electrophiles such as acetyl chloride and the phosphoryl nerve agents (Figure 1), thus avoiding interference by these species. Furthermore, any weakly electrophilic interferents that are simply primary halides and cannot form reactive cyclic systems, such as bis(2-chloroethyl) ether (BCCE), cannot react with **1** as quickly as SM can.

Another aspect of our strategy is the recognition that the metal-ligating ability of dithiol **1** would also lead to a displacement of an indicator from a thiophilic metal. In fact, **1** could equally or even more strongly enhance the fluorescence by stripping any indicator from Cd^{2+}. To overcome this problem, the detection of SM must necessarily involve a process of capping any unreacted **1** prior to addition of the test solution containing CEES to the metal−indicator complex (see below)

Synthesis. The synthesis of dithiol **1** was completed in five steps (Scheme 1). The esterification of 5-hydroxyisophthalic acid (**3**) with methanol in presence of H_2SO_4 afforded 5-hydroxyisophthalic acid dimethyl ester (**4**).[16a] Reduction of **4**

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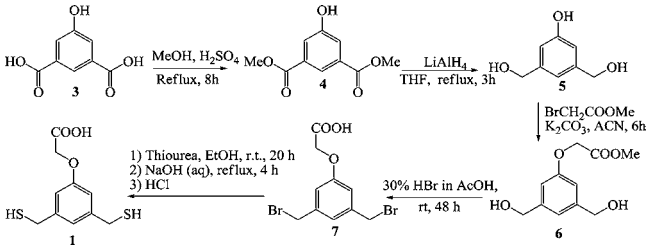
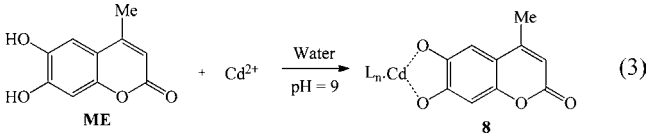
Scheme 1. Synthesis of Dithiol 1 from 5-Hydroxyisophthalic Acid



with lithium aluminum hydride gave 3,5-bis(hydroxymethyl)-phenol (**5**).[16b] The reaction of **5** with methyl bromoacetate in presence of K_2CO_3 resulted in the formation of methyl 2-[3,5-bis(hydroxymethyl)phenoxy]acetate (**6**), which upon reaction with 30% HBr in acetic acid gave 2-[3,5-bis(bromomethyl)-phenoxy]acetic acid (**7**). The reaction of **7** with thiourea followed by base hydrolysis and treatment with acid afforded 2-[3,5-bis(mercaptomethyl)phenoxy]acetic acid (**1**). The synthetic procedures for all of these steps are given in the Supporting Information.

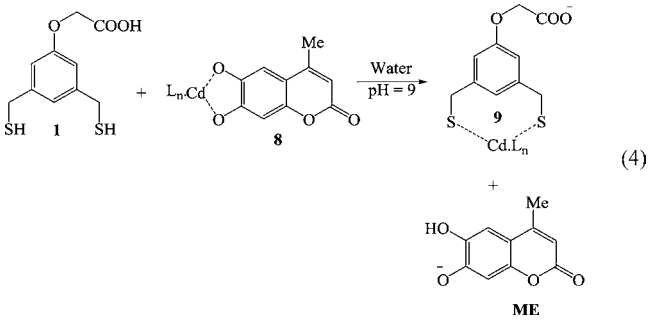
Formation of the Metal–Indicator Complex (8). 6,7-Dihydroxy-4-methylcoumarin, also known as 4-methylesculetin (**ME**), was used as an indicator. It binds with metals through its catechol moiety,[17] resulting in the quenching of its fluorescence.[18] Three heavy metal ions, Ag^+, Hg^{2+}, and Cd^{2+}, were screened for their ability both to form a stable complex with thioether-based receptor **2** and to quench the fluorescence of **ME**. Cd^{2+} was found to be suitable for nearly complete quenching (eq 3). For example, in fluorescence titrations



(3)

performed at 25 °C in an aqueous solution of **ME**, increasing the Cd^{2+} concentration led to a decrease in the indicator emission band at 460 nm (Figure 4).

Fluorescence Titrations of 1 with Complex 8. Compound **1** itself at pH 9, as well as podant **2**, should strip Cd^{2+} from Cd^{2+}–**ME** complex **8**. This was confirmed by fluorescence spectroscopy. In this control experiment, a solution of **1** (buffered at pH 9 using sodium bicarbonate/sodium hydroxide) was used to titrate a solution of **8**, resulting in the formation of Cd^{2+}–dithiol complex **9** and displacement of **ME** (eq 4). Thus, the indicator regained its fluorescence (Figure 5).



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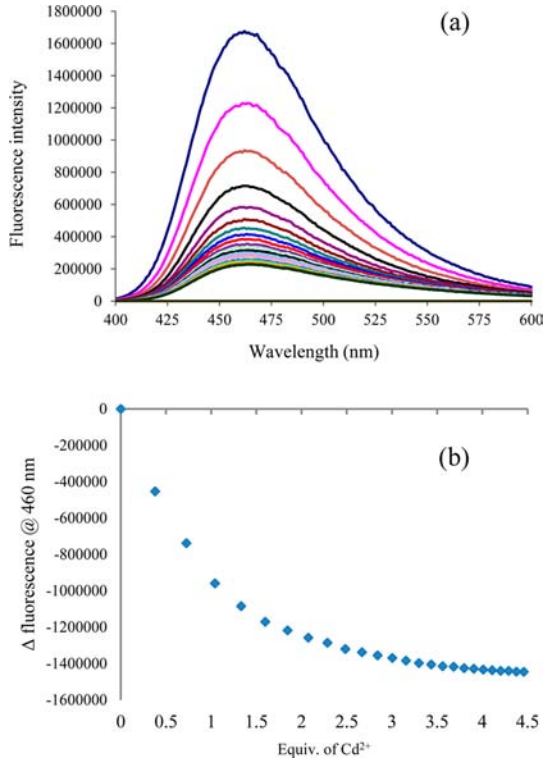

Figure 4. (a) Drop in the fluorescence intensity of **ME** (51.5 μM) at 460 nm in the presence of increasing amounts of $Cd(NO_3)_2 \cdot 4H_2O$ (titrant was 0.415 mM). The excitation wavelength was 378 nm. All of the experiments were performed at 25 °C in aqueous solution at pH 9 using a sodium bicarbonate/sodium hydroxide buffer. (b) Isotherm showing the decrease in fluorescence intensity of the **ME** solution at 460 nm with added $Cd(NO_3)_2$.

Capping of Dithiol 1. Before moving to the detection of CEES, we developed a process to cap **1**, thereby rendering it inactive in stripping Cd^{2+} from **8**. The capping agent had to meet two criteria. First, it should react with **1** very quickly under ambient conditions without reacting with water at pH 9. Second, it should withdraw electrons from the sulfur atoms to prevent the product from ligating Cd^{2+}. With these considerations, we studied various capping agents, such as 1-fluoro-2,4-dinitrobenzene, 1-bromomethyl-4-nitrobenzene, phenyl isothiocyanate, and 2,4-difluorobenzaldehyde. Although in some cases we observed capping of **1** with little change in the fluorescence intensity of **8** upon addition of the capping product, none of these reagents were quite optimal. However, using the well-known chemistry of thiol–alkyne addition,[19] in which a thiol conjugate adds to an activated alkyne to form an alkenyl sulfide, we achieved success. In the product of this reaction, the sulfur lone pair is conjugated with the resulting α,β-unsaturated ketone, thus dramatically lowering its availability for coordination with metal ions. To achieve this, we mixed 2.2 equiv of 4-phenyl-3-butyn-2-one (**10**) (8.6 mM) with **1** (4.09 mM) at 80 °C to form adduct **11**, which occurs in less than 1 min (eq 5). The formation of **11** was confirmed by mass spectrometry of the crude reaction mixture (Figure S2 in the Supporting Information). Figure 6 shows that mixing complex **8** with **1** gives a large enhancement in the fluorescence intensity. However, after addition of **10**, the intensity was nearly identical to that of complex **8** alone. As a result, we used capping of **1** to form **11** as a deactivating strategy because of the minimal ability of **11** to bind with Cd^{2+}.

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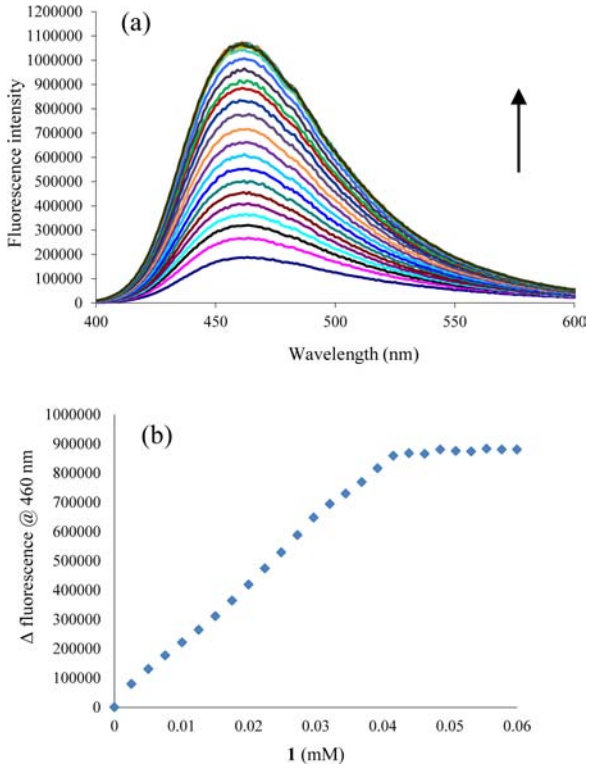
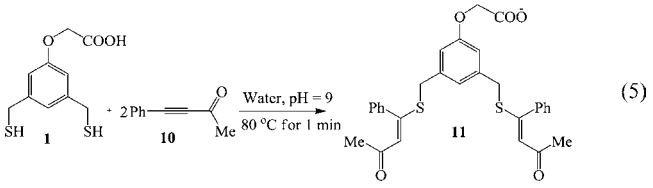


Figure 5. (a) Fluorescence titration of a solution of Cd^{2+}–indicator complex **8** [containing $Cd(NO_3)_2$ at 64.4 μM and **ME** at 26 μM] with **1** (1.02 mM). All of the experiments were performed at 25 °C in aqueous solution at pH 9 using a sodium bicarbonate/sodium hydroxide buffer. (b) Isotherm showing the increase in fluorescence intensity at 460 nm with added **1**.



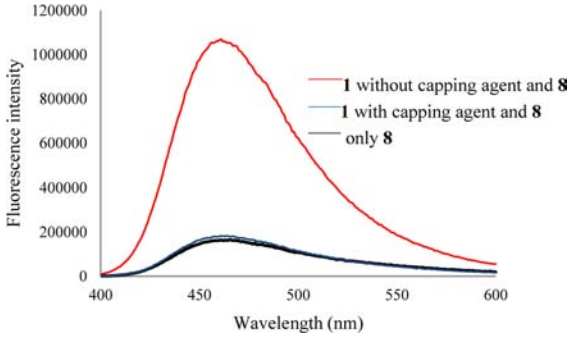


Figure 6. Fluorescence spectra of Cd^{2+}–indicator complex **8** [containing $Cd(NO_3)_2$ at 64.4 μM and **ME** at 26 μM] alone (black) or mixed with a solution of **1** (2.04 mM) before (red) or after (blue) capping with **10** (4.3 mM) to give **11**. All of the experiments were performed at 25 °C in aqueous solution at pH 9 using a sodium bicarbonate/sodium hydroxide buffer.

Detection of Mustard Simulant. To execute our approach, CEES (8.5 mM) was allowed to react with **1** (4.09 mM) in water at pH 9 for 1 min at 80 °C to a give podand **2**. The formation of **2** was confirmed by mass spectrometry (Figure S3 in the Supporting Information). Initially this reaction was carried out using 2.1 equiv of CEES (8.3 mM), and therefore, all of the thiols of **1** were converted to thioesters; thus, capping with **10** was not necessary, as shown by a fluorescence titration (Figure S1 in the Supporting Information). This solution was titrated into complex **8**, and the resulting emission spectra revealed the displacement of **ME** from **8** and restoration of the fluorescence (Figure 7). Cd^{2+} binds more strongly with the four sulfur atoms in **2** than with the catechol moiety of **ME**, thereby resulting in the formation of **12** (eq 6).

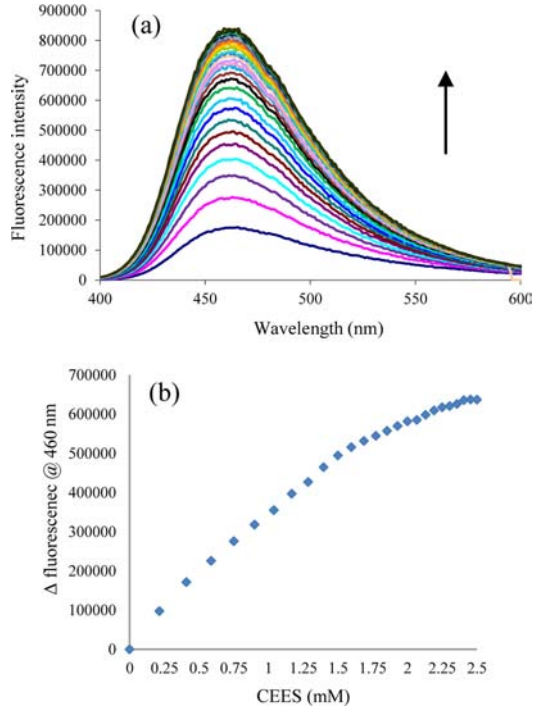
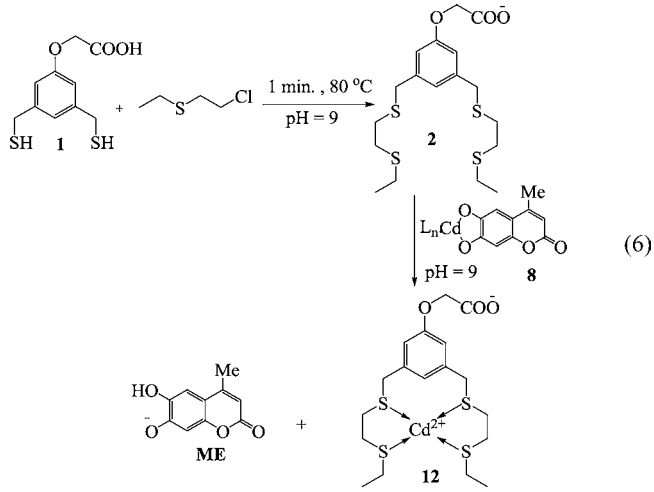


Figure 7. (a) Fluorescence titration of a solution of Cd^{2+}–indicator complex **8** (containing $Cd(NO_3)_2$ at 64.4 μM and **ME** at 26 μM) with podand **2** generated in situ from CEES (4.5 mM). All of the experiments were performed at 25 °C in aqueous solution at pH 9 using a sodium bicarbonate/sodium hydroxide buffer. (b) Isotherm showing the increase in fluorescence intensity of the Cd^{2+}–indicator solution at 460 nm with added podand solution.



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Interference Studies. To test the selectivity of our strategy, we first examined interference by 2-chloroethyl ethyl ether (CEEE), the oxygen analogue of CEES, as well as other electrophilic reagents, including the strong electrophile acetyl chloride and the nerve agent mimic diethyl chlorophosphate (DEP). Reactions of 2.2 equiv of CEEE, acetyl chloride, or DEP with **1** (4.09 mM) were carried at 80 °C for 1 min, with subsequent capping of **1** using **10** at 80 °C for another 1 min. Subsequently, the fluorescence was determined after addition of each of these solutions into a solution of complex **8**. No enchancement of the fluorescence after interaction of **8** with any of the solutions was observed (Figure 8). This may be

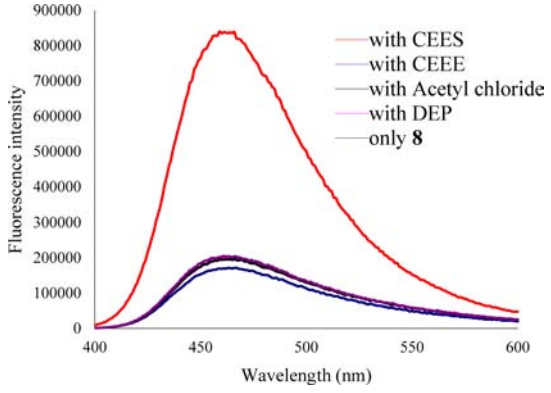

Figure 8. Fluorescence of solutions of **8** [containing $Cd(NO_3)_2$ at 64.4 μM and **ME** at 26 μM] and **1** (4.09 mM) with CEEE, acetyl chloride, or DEP (9.0 mM). The spectrum obtained with CEES and that with **8** only are shown for comparison. All experiments were performed at 25 °C in aqueous solution, pH 9, using sodium bicarbonate–sodium hydroxide as buffer.

attributed to the fact that the chlorine in CEEE is not as reactive as that in CEES[20] and that acetyl chloride and DEP hydrolyze under these conditions faster than they react with **8**.

Calibration Curve and Sensitivity. We next investigated the sensitivity of the detection system. Incrementally increasing concentrations of CEES were added to **1** (4.09 mM) using the reaction conditions as described above. After capping of **1** (vide supra), the resulting solution was added to **8**, and the emission spectra were recorded at 460 nm. The fluorescence intensity increased linearly as a function of CEES concentration from zero equiv up to 2.2 equiv (Figure 9). However, above 2.2 equiv of CEES, a plateau was obtained. Using the line below 2.2 equiv as a calibration curve, we determined the concentrations of two unknown samples of CEES (as prepared independently by the group members). A coincidental perfect agreement was found for one sample, while the other sample gave a 10% error (Figure S4 in the Supporting Information).

Analytical Applications. Mustard gas is often delivered by aerial spraying, rockets, bombs, or artillery shells. Unfortunately, it is known to be persistent in the environment and can remain active on surfaces and soil for as long as several weeks following deployment.[21] For the development of a practical detection system, it becomes mandatory to detect SM on surfaces and in soil samples. Here we describe the successful use of our system to detect the presence of CEES on a surface and in a soil sample.

CEES (8 μL), in the form of a drop, was placed on a glass surface and allowed to sit for 1 min. The liquid was then wiped up with filter paper. This paper was extracted with dichloro-

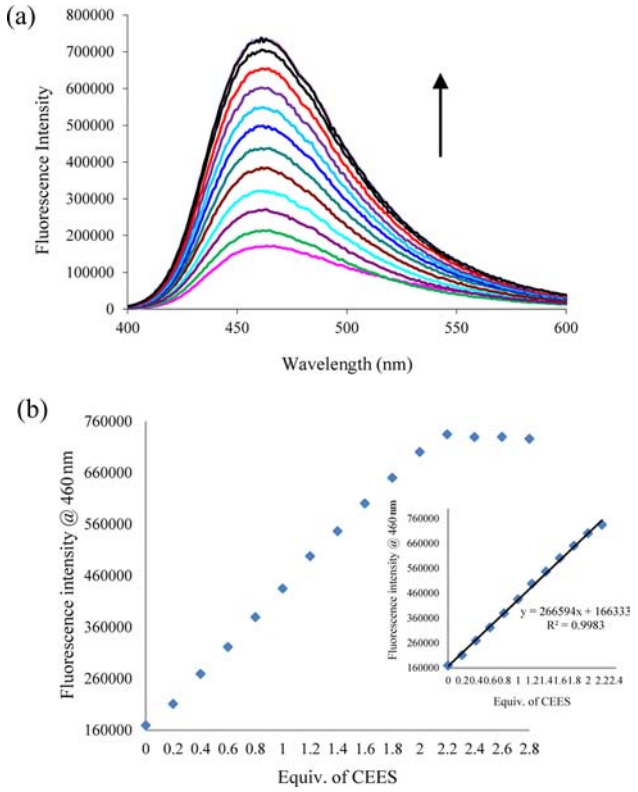

Figure 9. (a) Titration of a solution of Cd^{2+}–indicator complex **8** (metal concentration 64.4 μM and **ME** concentration 26 μM) with increasing concentration of CEES (0.2 mM in each addition). All of the experiments were performed at 25 °C in aqueous solution at pH 9 using a sodium bicarbonate/sodium hydroxide buffer. (b) Isotherm showing the increase in fluorescence intensity of **8** with added podand **2**.

methane (DCM). After evaporation of the DCM, the residue was treated with **1** at 80 °C for 1 min, and then the residue of **1** (if at all unreacted) was capped with **10**. This solution was added to **8** as described above. Figure 10a shows a large turn-on of fluorescence compared with the same treatment of paper that was not used to wipe a drop of CEES. To determine the presence of the analyte in soil, 2 g of dirt was spiked using a solution of CEES (10 μL) in diethyl ether (2 mL). The solvent was evaporated to dryness, and the soil sample was mixed with an aqueous solution of **1** followed by the capping process. Addition of this solution to complex **8** gave a large enhancement in fluorescence (Figure 10b) compared with a soil sample containing no CEES (Figure S5 in the Supporting Information).

■ SUMMARY

We have reported the first fluorescence sensing method for sulfur mustard in water. The method is rapid, highly selective, and sensitive. In our approach, we take advantage of the reactivity of three-membered cationic sulfonium heterocycles, which were found to react with dithiol **1** to form a receptor that subsequently encapsulates Cd^{2+} and thereby displaces an indicator from a Cd^{2+}–indicator complex. The unique feature of our method is the tuning of reactivity and experimental conditions in such a way that potential interferents, such as the oxygen analogue of mustard gas, as well as more electrophilic agents such as acyl chlorides and nerve agent mimics, do not interfere in the mustard gas detection. To demonstrate the

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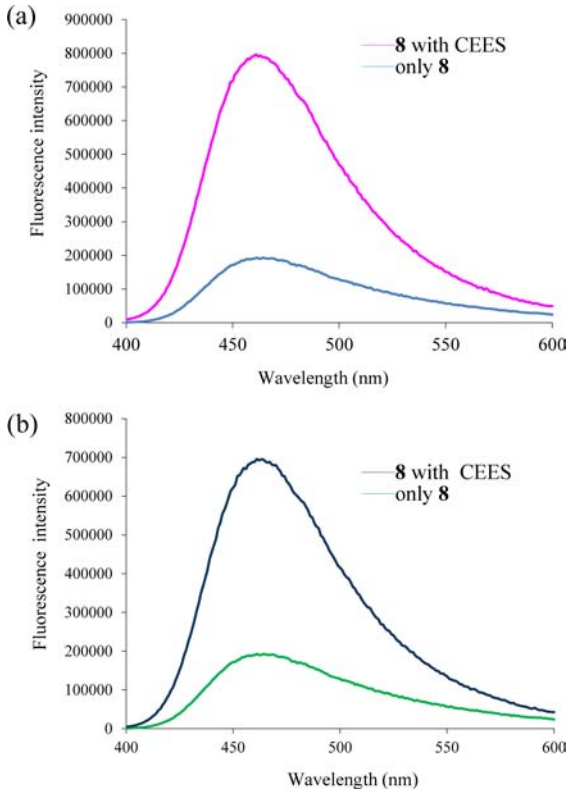
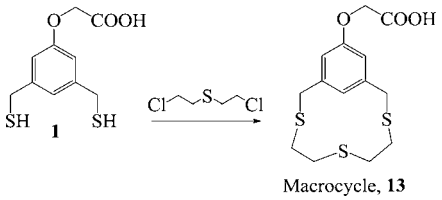


Figure 10. Fluorescence titration of Cd^{2+}–indicator complex solution [containing $Cd(NO_3)_2$ at 64.4 μM and **ME** at 26 μM] with CEES detected (a) on a surface and (b) in a soil sample. All of the experiments were performed at 25 °C in aqueous solution at pH 9 using a sodium bicarbonate/sodium hydroxide buffer.

possibility of practical applications, the presence of CEES on a surface and in a spiked soil sample was detected. The detection limit was found to be 0.2 mM, indicating that the method is sensitive enough to detect agent concentrations at or below levels that pose a health risk.

In a real-life SM assay, our strategy would likely create macrocycle **13**, with slightly modified reaction conditions such as a high dilution reaction, as presented in Scheme 2.[22] Otherwise, all of the essential aspects of the analysis are expected to remain the same.

Scheme 2. Schematic Presentation of the Proposed Reaction between Mustard Gas and 1



■ ASSOCIATED CONTENT

Supporting Information

Experimental details and additional data. 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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■ REFERENCES

(1) (a) Saladi, R.; Smith, E.; Persaud, A. *Clin. Exp. Dermatol.* **2006**, *31*, 1. (b) Shohrati, M.; Davoudi, M.; Ghanei, M.; Peyman, M.; Peyman, A. *Cutaneous Ocul. Toxicol.* **2007**, *26*, 73. (c) Szinicz, L. *Toxicology* **2005**, *214*, 167. (d) Hurst, C. G.; Smith, W. J. Chronic Effects of Acute, Low-Level Exposure to the Chemical Warfare Agent Sulfur Mustard. In *Chemical Warfare Agents: Toxicity at Low Levels*; Somani, S. M., Romano, J. A., Eds.; CRC Press: Boca Raton, FL, 2001; pp 249–264. (e) Report S-16433. United Nations Security Council: New York, 1984.

(2) Kehe, K.; Szinicz, L. *Toxicology* **2005**, *214*, 198. (b) Davis, K. G.; Aspera, G. *Ann. Emerg. Med.* **2001**, *37*, 653.

(3) (a) Wheeler, G. P. *Cancer Res.* **1962**, *22*, 651. (b) Bolt, H. M.; Laib, R. J.; Peter, H.; Ottenwälder, H. *J. Cancer Res. Clin. Oncol.* **1986**, *112*, 92. (c) Lawley, P. D. In *Chemical Carcinogens*; Searle, E. C., Ed.; American Chemical Society: Washington, DC, 1984; p 326. (d) Beranek, D. T. *Mutat. Res.* **1990**, *231*, 11.

(4) (a) Kim, K.; Tsay, O. G.; Atwood, D. A.; Churchill, D. G. *Chem. Rev.* **2011**, *111*, 5345. (b) Bencic-Nagale, S.; Sternfeld, T.; Walt, D. R. *J. Am. Chem. Soc.* **2006**, *128*, 5041. (c) Burnworth, M.; Rowan, S. J.; Weder, C. *Chem.—Eur. J.* **2007**, *13*, 7828. (d) Royo, S.; Martínez-Máñez, R.; Sancenón, F.; Costero, A. M.; Parra, M.; Gil, S. *Chem. Commun.* **2007**, 4839. (e) Costero, A. M.; Parra, M.; Gil, S.; Gotor, R.; Martínez-Mañez, R.; Sancenón, F.; Royo, S. *Eur. J. Org. Chem.* **2012**, 4937.

(5) Brletich, N. R.; Waters, M. J.; Bowen, G. W.; Tracy, M. F. *Worldwide Chemical Detection Equipment Handbook*; Chemical and Biological Defense Information Analysis Center: Aberdeen, MD, 1995.

(6) (a) Kientz, C. E. *J. Chromatogr., A* **1998**, *814*, 1. (b) Makas, A. L.; Troshkov, M. L. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2004**, *800*, 55. (c) Baumbach, J. I.; Eiceman, G. A. *Appl. Spectrosc.* **1999**, *53*, 338A. (d) Collins, D. C.; Lee, M. L. *Anal. Bioanal. Chem.* **2002**, *372*, 66.

(7) Boopathi, M.; Suryanarayana, M. V. S.; Nigam, A. K.; Pandey, P.; Ganesan, K.; Singh, B.; Sekhar, K. *Biosens. Bioelectron.* **2006**, *21*, 2339.

(8) (a) van der Schans, G. P.; Noort, D.; Mars-Groenendijk, R. H.; Fidder, A.; Chau, L. F.; de Jong, L. P. A.; Benschop, H. P. *Chem. Res. Toxicol.* **2002**, *15*, 21. (b) van der Schans, G. P.; Scheffer, A. G.; Mars-Groenendijk, R. H.; Fidder, A.; Benschop, H. P.; Baan, R. A. *Chem. Res. Toxicol.* **1994**, *7*, 408.

(9) Bunkar, R.; Vyas, K. D.; Rao, V. K.; Kumar, S.; Singh, B.; Kaushik, M. P. *Sens. Transducers J.* **2010**, *113*, 41.

(10) Wang, Q.; Begum, R. A.; Day, V. W.; Bowman-James, K. *Inorg. Chem.* **2012**, *51*, 760.

(11) (a) Murray, G. M.; Southard, G. E. *IEEE Instrum. Meas. Mag.* **2002**, *5*, 12. (b) Gripstad, B. F. O. *A Briefing Book on Chemical Warfare Agents*; Swedish National Defence Research Establishment: Stockholm, Sweden, 1992. (c) Murray, G. M.; Lawrence, D. S. In *Chemical Weapon Convention Chemicals Analysis: Sample Collection, Preparation*

and Analytical Methods; Mesilaakso, M.; Ed.; Wiley: Chichester, U.K., 2005; p 65. (d) Sun, Y.; Ong, K. Y. *Detection Technologies for Chemical Warfare Agents and Toxic Vapors*; CRC Press: Boca Raton, FL, 2005.
(12) Fruton, J. S.; Stein, W. H.; Stahmann, M. A.; Golumbic, C. *J. Org. Chem.* **1946**, *11*, 571.
(13) Vijayaraghavan, R.; Kulkarni, A.; Pant, S. C.; Kumar, P.; Rao, P. V.; Gupta, N.; Gautam, A.; Ganesan, K. *Toxicol. Appl. Pharmacol.* **2005**, *202*, 180.
(14) Sigma-Aldrich Material Safety Data Sheet Database. http://www.sigmaaldrich.com/catalog/product/aldrich/242640?lang=en&region=US (accessed April 6, 2013).
(15) (a) Tamayo, A.; Casabó, J.; Escriche, L.; Lodeiro, C.; Covelo, B.; Brondino, C. D.; Kivekäs, R.; Sillampää, R. *Inorg. Chem.* **2006**, *45*, 1140. (b) Shamsipura, M.; Sadeghi, M.; Alizadeh, K.; Bencini, A.; Valtancoli, B.; Garau, A.; Lippolis, V. *Talanta* **2010**, *80*, 2023. (c) Márquez, V. E.; Anacona, J. R. *Polyhedron* **1997**, *16*, 1931. (d) Vetrichelvan, M.; Lai, Y.-H.; Mok, K. F. *Eur. J. Inorg. Chem.* **2004**, 2086.
(16) (a) Jain, A. K.; Reddy, V. V.; Paul, A.; Muniyappa, K.; Bhattacharya, S. *Biochemistry* **2009**, *48*, 10693. (b) Li, W.; Fishkin, N. E.; Zhao, R. Y.; Miller, M. L.; Chari, R. V. J. PCT Int. Pat. Appl. WO 2010091150, 2010.
(17) (a) Katayal, M.; Singh, H. B. *Talanta* **1968**, *15*, 1043. (b) Issa, Y. M.; Omar, M. M. *Egypt. J. Chem.* **1990**, *33*, 157. (c) Zhang, L.; Dong, S.; Zhu, L. *Chem. Commun.* **2007**, 1891.
(18) Gaidamauskas, E.; Crans, D. C.; Parker, H.; Saejueng, K.; Kashemirov, B. A.; McKenna, C. E. *New J. Chem.* **2011**, *35*, 2877.
(19) (a) Hoogenboom, R. *Angew. Chem., Int. Ed.* **2010**, *49*, 3415. (b) Lowe, A. B.; Hoyle, C. E.; Bowman, C. N. *J. Mater. Chem.* **2010**, *20*, 4745.
(20) (a) Cretcher, L. H.; Pittenger, W. H. *J. Am. Chem. Soc.* **1925**, *47*, 163. (b) Cretcher, L. H.; Koch, J. A.; Pittenger, W. H. *J. Am. Chem. Soc.* **1925**, *47*, 1173.
(21) (a) Munro, N. B.; Talmage, S. S.; Griffin, G. D.; Waters, L. C.; Watson, A. P.; King, J. F.; Hauschild, V. *Environ. Health Perspect.* **1999**, *107*, 933. (b) Watson, A. P.; Griffin, G. D. *Environ. Health Perspect.* **1992**, *98*, 259.
(22) Casabo, J.; Mestres, L.; Escriche, L.; Teixidor, F.; Perez-Jimeneza, C. *J. Chem. Soc., Dalton Trans.* **1991**, 1969.