

Cite this: *Chem. Commun.*, 2011, **47**, 11468–11470

www.rsc.org/chemcomm

COMMUNICATION

Chromogenic and fluorogenic detection of a nerve agent simulant with a rhodamine-deoxylactam based sensor†

Xuanjun Wu, Zhisheng Wu and Shoufa Han*

Received 24th August 2011, Accepted 15th September 2011

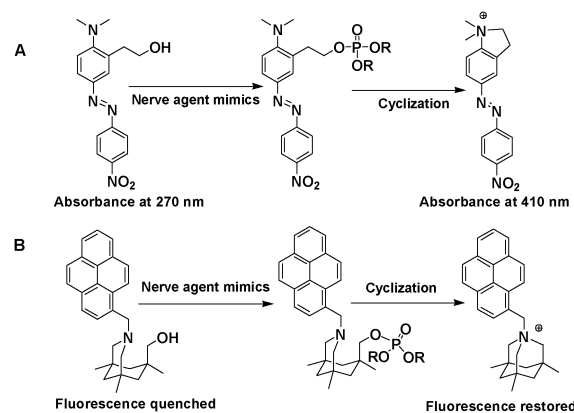
DOI: 10.1039/c1cc15250e

A chromogenic and fluorogenic detection of a nerve agent simulant was developed based on diethyl chlorophosphate triggered tandem phosphorylation and intramolecular cyclization of *N*-(rhodamine B)-deoxylactam-2-aminoethanol.

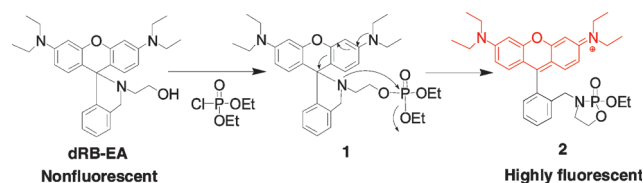
Nerve agents are a group of lethal organo-phosphates that can irreversibly inactivate acetylcholinesterase of the human nerve system.¹ The extraordinary toxicity of nerve agents can be seen from Sarin which can kill an average 70 kg man with doses as low as 0.70 mg. Historically, Sarin has been used by terrorists in Tokyo subway, leading to thousands wounded and 12 deaths.² Nerve agents are of growing concerns for homeland security due to the potential disastrous consequences from terrorist attacks, providing strong motivations to develop sensors for facile detection of nerve agents. Prior methods for detecting nerve agents,³ *e.g.* mass spectrometry or enzyme based biosensors, are limited by portability or storage/stability issues. In contrast, chemosensors, compact and portable, are advantageous as they require routine instruments and offer the possibility to detect analytes with the naked eye.

Many elegant strategies have been developed to design chemosensors that could exhibit chromogenic or fluorogenic responses in the presence of nerve agent mimics.⁴ For example, substrates containing a judiciously positioned hydroxyl moiety could undergo intramolecular transformations to generate detectable species upon phosphorylation of the hydroxyl group (Scheme 1).^{4c,e,j,m}

Herein, we report detection of a nerve agent simulant with *N*-(rhodamine B)-deoxylactam-2-aminoethanol *via* tandem phosphorylation-intramolecular cyclization. The cyclization is concomitant with opening of the deoxylactam, leading to the formation of highly fluorescent and colored species (Scheme 2). *N*-(Rhodamine B)-lactam-2-aminoethanol (referred as RB-AE), prepared by amidation of rhodamine B with 2-aminoethanol, was treated with lithium aluminium hydride in anhydrous tetrahydrofuran to give *N*-(rhodamine B)-deoxylactam-2-aminoethanol (referred as



Scheme 1 Representative chromogenic sensor^{4c} (A) and fluorogenic sensor^{4c} (B) and their detection mechanisms.



Scheme 2 Proposed fluorogenic and chromogenic reaction mechanism of the rhodamine-deoxylactam sensor (dRB-AE) with diethyl chlorophosphate.

dRB-AE) in 50% yield. dRB-AE is nonfluorescent and colorless due to the intramolecular deoxylactam (Scheme 2).

Diethyl chlorophosphate, displaying a similar chemical structure and reactivity to Sarin, was widely used as the nerve agent simulant. Upon addition of diethyl chlorophosphate, the dRB-AE solution quickly turned into red color (Fig. 1). The reaction rates of diethyl chlorophosphate with dRB-AE in selected solvents were monitored with UV-vis absorbance at 560 nm. Time course studies showed that dimethylformamide (DMF) was the preferred assay medium (ESI†, Fig. S1). The assay was also efficient in aqueous DMF containing up to 2% (v/v) of water (ESI†, Fig. S1). Kinetic analysis of the reaction between dRB-AE and diethyl chlorophosphate showed that the fast phase of the signal production (50% of the maximal fluorescence intensity) is complete at about 5 minutes (Fig. 2). The change in relative humidity is an important factor that needs to be taken into account for on-spot detection.

Department of Chemical Biology, College of Chemistry and Chemical Engineering, and the Key Laboratory for Chemical Biology of Fujian Province, Xiamen University, Xiamen, China.

E-mail: shoufa@xmu.edu.cn; Tel: +86-0592-2181728

† Electronic supplementary information (ESI) available: Synthesis and characterization of *N*-(rhodamine-B)-lactam-2-aminoethanol and *N*-(rhodamine-B)-deoxylactam-2-aminoethanol, assay procedures for diethyl chlorophosphate. See DOI: 10.1039/c1cc15250e



Fig. 1 Comparison of dRB-AE solution (1 mg ml^{-1}) in DMF containing triethylamine (TEA) (3%, v/v) before (left) and after (right) addition of diethyl chlorophosphate (100 ppm).

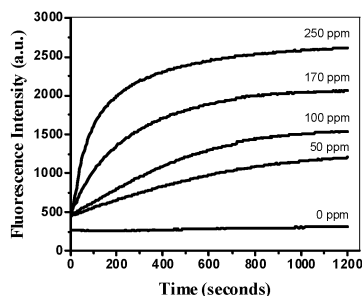


Fig. 2 Kinetic profile of the reaction between dRB-AE (1 mg ml^{-1}) and diethyl chlorophosphate in DMF. The color formation was monitored by fluorescence emission intensity at 590 nm (Ex@560 nm). The concentrations of the analyte were used as indicated.

The tolerance of water in the assay system suggests its potential utility in monitoring nerve agents under practical conditions.

To test the sensitivity of the dRB-AE based assay, diethyl chlorophosphate was added to the solution of dRB-AE (1 mg ml^{-1}) in DMF containing TEA (3%, v/v) to prepare a series of reaction solutions with various concentrations of the analyte. The assay solutions were incubated at room temperature for 10 minutes and then analyzed by fluorometry. As can be seen from Fig. 3B, the fluorescence emission intensity peaked at 590 nm increased as a function of diethyl chlorophosphate concentration. As low as 25 ppm of diethyl chlorophosphate can be detected under the assay conditions. UV-vis absorption spectra of the aforementioned titration solutions showed that the major absorption band centered at 560 nm intensified as the analyte concentration increased (Fig. 3A). The deep red color of the assay solution suggested the possibility of qualitative detection of nerve agent mimics with dRB-AE by naked eyes.

We reasoned that the genesis of fluorescence in the assay was achieved *via* nucleophilic attack of the deoxylactam amine of dRB-AE on the phosphate group of intermediate **1**, which is concomitant with opening of the deoxylactam (Scheme 2). To validate the hypothesis, RB-AE, the structural analog of dRB-AE, was tested for its efficacy to detect diethyl chlorophosphate (Scheme 3). RB-AE differs from dRB-AE in that it contains an amide moiety (lactam). We envision that the non-nucleophilic nature of the lactam could impede the intramolecular cyclization and thus prevent the formation of colored and fluorescent species in the assay solution (Scheme 3). Analysis showed that no fluorescent or colored species was produced in the reaction of RB-AE with diethyl chlorophosphate (Fig. 4), indicating that the intramolecular lactam of RB-AE remained closed under the assay conditions. The differential responses of dRB-AE relative to RB-AE highlight the essential role of the deoxylactam of dRB-AE in sensing of diethyl chlorophosphate.

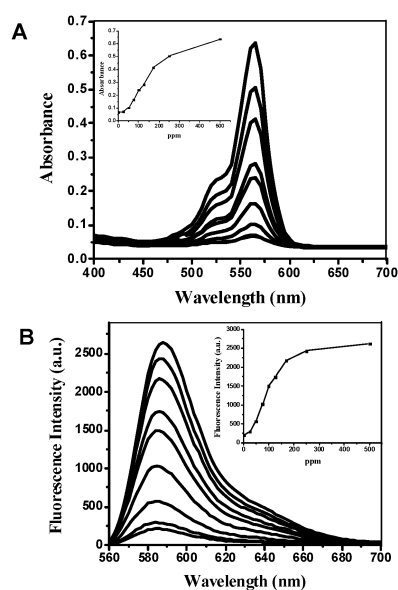
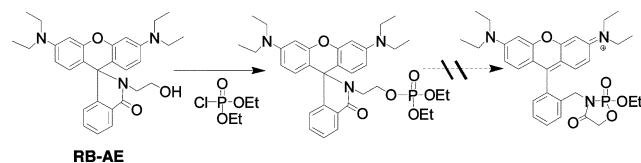


Fig. 3 Characterization of the assay sensitivity of diethyl chlorophosphate with dRB-AE by UV-vis absorption spectra (A) and fluorescence emission spectra (Ex@560 nm) (B). Analyte concentrations used from top to bottom: 500, 250, 170, 125, 100, 75, 50, 25, and 0 ppm. The insets show the titration curves by UV-vis absorbance at 560 nm (A) or by fluorescence emission intensity at 590 nm (Ex@560 nm) (B).



Scheme 3 The non-nucleophilic nature of the lactam impedes intramolecular cyclization of phosphorylated RB-AE.

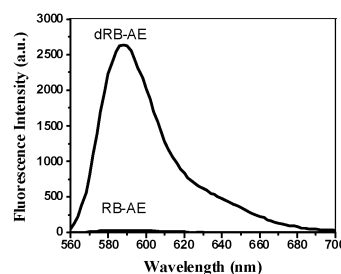


Fig. 4 Differential fluorogenic responses of dRB-AE (b) and RB-AE (a) with diethyl chlorophosphate in DMF.

To access the identity of the colored species generated in the assay which was proposed to be compound **2** (Scheme 1), the reaction solution of dRB-AE with diethyl chlorophosphate was analyzed by high resolution mass spectrometry (HRMS). A major peak located at 562.2839 was identified, which is consistent with the theoretical molecular weight of compound **2** ($\text{C}_{32}\text{H}_{41}\text{N}_3\text{O}_4\text{P}^+$; M_w : 562.2829), confirming formation of compound **2** (Fig. 5). The fluorescence excitation and emission spectra of the resultant solution were shown to be almost identical to that of rhodamine B (ESI†, Fig. S2 and S3), further supporting the formation of the rhodamine fluorophore from opening of deoxylactam in dRB-AE under the assay conditions (Scheme 2).

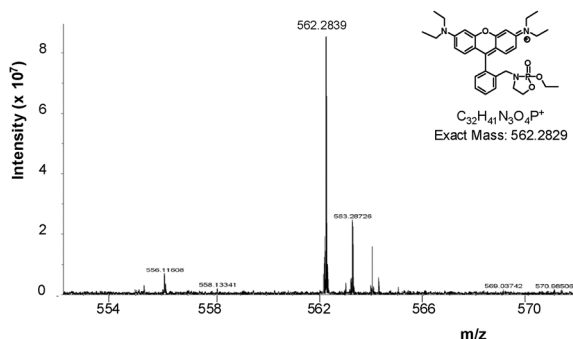


Fig. 5 HRMS confirmation of formation of compound **2** in the reaction of dRB-AE with diethyl chlorophosphate.

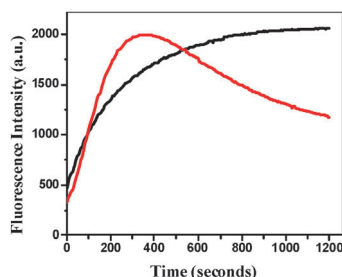


Fig. 6 Comparison of the time-dependant fluorescence emission of the dRB-AE (in black) based assay as compared to rhodamine-hydroxamate (in red). The fluorescence emission at 590 nm of the assay solutions of dRB-AE (1 mg ml⁻¹) or rhodamine-hydroxamate (1 mg ml⁻¹) in DMF containing TEA (3%, v/v) and diethyl chlorophosphate (170 ppm) was monitored as a function of time using an excitation wavelength of 560 nm.

Rhodamine-hydroxamate has been reported to detect diethyl chlorophosphate *via* Lossen rearrangement.⁵ Sensing of diethyl chlorophosphate with dRB-AE was further evaluated using rhodamine-hydroxamate as the control to compare their efficiency. The dRB-AE based assay furnished highly fluorescent and deep colored species that is suitable for visual detection. Compared to the rhodamine-hydroxamate based assay where the fluorescence emission intensity declined gradually in the late phase, the dRB-AE based assay gave highly stable fluorescence signals (Fig. 6), allowing accurate detection of nerve agents by fluorometry. It is preferential to detect the ppb level of gaseous nerve agents for practical applications. Here we show that dRB-AE is able to detect a single aliquot of diethyl chlorophosphate in DMF solution at the ppm range. We anticipate that the detection of gaseous nerve agents with dRB-AE might be

achieved by bubbling a larger volume of contaminated air into the assay solution.

In summary, a chromogenic and fluorogenic assay of a nerve agent simulant was developed based on reactive organophosphate triggered irreversible opening of the deoxylactam of dRB-AE. The assay is sensitive and exhibited improved kinetics relative to a prior sensor,⁵ allowing detection of reactive organophosphates with the aid of instruments or possibly with “naked eyes”. We anticipate that rhodamine-deoxylactams which are poised to analyte mediated opening of the intramolecular deoxylactam will be useful as the universal signal reporting platform for fluorogenic sensing of many other chemically reactive species with appropriate structural modifications.

This work was supported by grants from NSF China (20802060, 21072162), Natural Science Foundation of Fujian Province of China (2011J06004) and the Fundamental Research Funds for the Central Universities (2011121020).

Notes and references

- (a) M. Ehrich, in *Encyclopedia of Toxicology*, ed. P. Wexler, Academic Press, San Diego, CA, 1998, pp. 467–471; (b) T. C. Marrs, *Pharmacol. Ther.*, 1993, **58**, 51–66; (c) F. R. Sidell and J. Borak, *Ann. Emerg. Med.*, 1992, **21**, 865–871.
- A. Vale, *Przegl. Lek.*, 2005, **62**, 528–532.
- (a) H. H. Hill Jr. and S. J. Martin, *Pure Appl. Chem.*, 2002, **74**, 2281–2291; (b) K. Brown, *Science*, 2004, **305**, 1228–1229; (c) L. M. Eubanks, T. J. Dickerson and K. D. Janda, *Chem. Soc. Rev.*, 2007, **36**, 458–470.
- (a) M. Burnworth, S. J. Rowan and C. Weder, *Chem.–Eur. J.*, 2007, **13**, 7828–7836; (b) S. Royo, R. Martínez-Manez, F. Sancenón, A. M. Costero, M. Parra and S. Gil, *Chem. Commun.*, 2007, 4839–4847; (c) A. M. G. Costero, M. Parra, P. M. E. Mancini, R. Martínez-Mañez, F. Sancenón and S. Royo, *Chem. Commun.*, 2008, 6002–6004; (d) S. Bencic-Nagale, T. Sternfeld and D. R. Walt, *J. Am. Chem. Soc.*, 2006, **128**, 5041–5048; (e) T. J. Dale and J. Rebek, Jr., *J. Am. Chem. Soc.*, 2006, **128**, 4500–4501; (f) H. S. Hewage, K. J. Wallace and E. V. Anslyn, *Chem. Commun.*, 2007, 3909–3911; (g) D. Knapton, M. Burnworth, S. J. Rowan and C. Weder, *Angew. Chem., Int. Ed.*, 2006, **45**, 5825–5829; (h) R. Shunmugam and G. N. Tew, *Chem.–Eur. J.*, 2008, **14**, 5409–5412; (i) K. J. Wallace, R. I. Fagbemi, F. J. Folmer-Andersen, J. Morey, V. M. Lynth and E. V. Anslyn, *Chem. Commun.*, 2006, 3886–3888; (j) S. W. Zhang and T. M. Swager, *J. Am. Chem. Soc.*, 2003, **125**, 3420–3421; (k) J. M. Rathfon, Z. M. Al-Badri, R. Shunmugam, S. M. Berry, S. Pabba, R. S. Keynton, R. W. Cohn and G. N. Tew, *Adv. Funct. Mater.*, 2009, **19**, 689–695; (l) D. J. R. J. Trevor and J. Dale, *Angew. Chem., Int. Ed.*, 2009, **48**, 7850; (m) K. A. V. Houten, D. C. Heath and R. S. Pilato, *J. Am. Chem. Soc.*, 1998, **120**, 12359–12360; (n) K. J. Wallace, J. Morey, V. M. Lynch and E. V. Anslyn, *New J. Chem.*, 2005, **29**, 1469.
- S. Han, Z. Xue, Z. Wang and T. B. Wen, *Chem. Commun.*, 2010, **46**, 8413–8415.