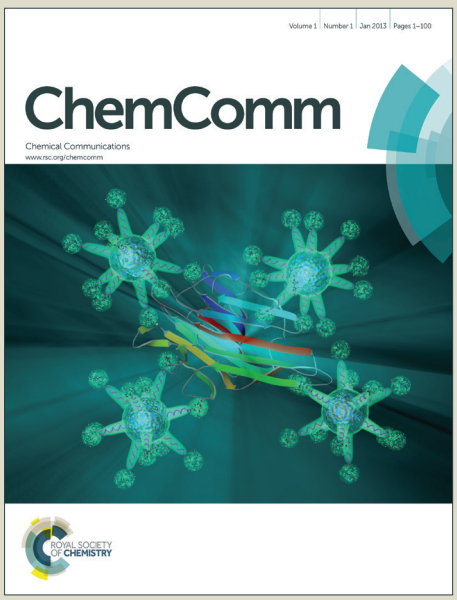
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

ChemComm

COMMUNICATION

A Near Infrared Colorimetric and Fluorometric Probe for Organophosphorus Nerve Agent Mimic by Intramolecular Amidation

Received 00th January 20xx,
Accepted 00th January 20xx

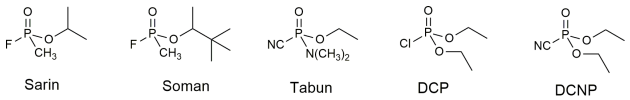
DOI: 10.1039/x0xx00000x

www.rsc.org/

Xiao-Xiao Hu,[a] Yue-Ting Su,[a] Yun-Wei Ma,[a] Xin-Qi Zhan,[b] Hong Zheng,*[a] and Yun-Bao Jiang[a]

A near infrared probe for sensitive colorimetric and fluorimetric detection of nerve agent mimic, DCP and DCNP, was reported based on the activating of a carboxylic acid group by the mimics to conduct an intramolecular amidation reaction in heptamethine chromophore, where its absorption or excitation maximum wavelength could be greatly red-shifted by attenuating the electron-donating ability of the amine group in the bridgehead site of heptamethine cyanine.

Nerve agents (Scheme 1) such as Tabun (GA), Sarin (GB), and Soman (GD) are highly reactive volatile organophosphorus (OP). These OP nerve agents are the basis of chemical weapons and can easily enter the body through inhalation or through the skin in the form of a gas, aerosol, or liquid. They are essentially phosphorylating agents that act as powerful inhibitors of acetylcholinesterase, resulting in acetylcholine accumulation in the synaptic junctions, which hinders muscle relaxation. [1] Due to the threat of a chemical attack with these OP reagents, the development of sensitive and rapid detection methods is of particular importance. To date, a variety of detection methods have been developed, such as electrochemistry, [2] optical-fibre arrays, [3] microcantilevers, [4] enzyme based biosensors[5] and gas chromatography-mass spectrometry. [6] These detection systems have, in general, suffered from at least one of the following disadvantages: poor portability, complicated operation, low sensitivity or low selectivity. Colorimetric and fluorometric methods based on molecular probes are low-cost systems capable of performing qualitative or quantitative analysis with operational simplicity; thus, the development of novel probes that respond to nerve agents has gained intense interest in recent years. The export



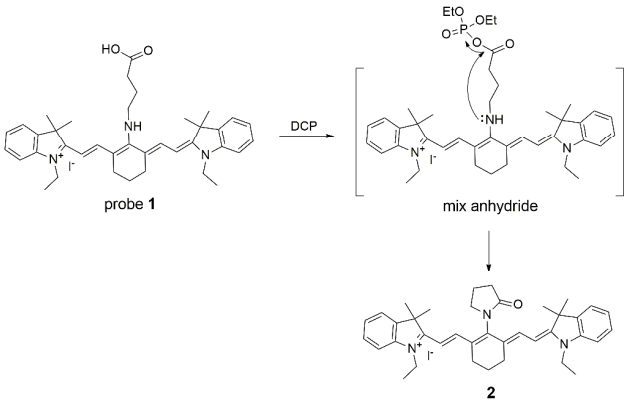
Scheme 1 Nerve Agents and Some of Their Mimics

of the spectroscopic signals of these probes is clearly based on the mechanism of the addition of a phosphoryl group to an aliphatic or aromatic hydroxyl group, [7] an oxime group, [8] an organo-lanthanide complex, [9] an amine group, [10] a pyridine group, [11] a spirobenzopyran group, [12] a hydroxamic acid, [13] or a carbonyl group. [14]

In this study, we report new available progress in this field with a heptamethine cyanine as a near-infrared (NIR) chromo- or fluorophore. Heptamethine cyanine has a rigid chlorocyclohexenyl ring in a polymethine chain, and the bridgehead chloride atom provides an ideal site for further modification with aliphatic amino substitutions. [15] These dyes typically have large molar absorption coefficients and emission in the NIR region between approximately 750-900 nm, where spectral interference caused by nonspecific absorption or the fluorescence of biological or environmental samples is generally lower than in the visible range; therefore, the great shift of the spectrum in the NIR region is expected to allow a high-sensitivity measurement to be achieved. On the other hand, the maximum absorption or excitation wavelength of an aliphatic amine-substituted heptamethine cyanine, however, could be drastically red-shifted by attenuating the electron-donating ability of the amine group towards the heptamethine chromophore, and in such an amine-substituted heptamethine cyanine, turning the amino substituent into an amide substituent would be competent for this job. [16] To achieve this goal, the carboxylic acid group can be effectively activated to conduct an amidation reaction by means of the in-situ generated mixed anhydride through the reaction of the carboxylic acid with a phosphorylating agent. [17] Based on these considerations, we herein developed a heptamethine cyanine probe **1**, which is easily prepared by a one-step reaction of chloro-heptamethine cyanine with 4-aminobutyric acid, with

[a]*Department of Chemistry, College of Chemistry and Chemical Engineering, and the MOE Key Laboratory of Physical Laboratory of Spectrochemical Analysis & Instrumentation, Xiamen University, Xiamen, 361005, China. E-mail: hzheng@xmu.edu.cn Fax: +86 592 2186731*
[b]*Basic Medicine Department, Medical College of Xiamen University, Xiamen, 361102, China*

† Electronic Supplementary Information (ESI) available: Synthesis and characterization of probe **1**, product **2**, and supplementary spectral results. See DOI:



Scheme 2 Propesed nechanism of the sensing for DCP

the expectation of strong chromogenic and fluorescent response to the OP nerve agent mimic, via the conversion of the 4-aminobutyric acid substituent into a valerolactam group, affording the NIR product **2** (Scheme 2). In here, diethyl chlorophosphate (DCP) and diethyl cyanophosphonate (DCNP) is tested as a model for studying these OP nerve agents due to their similar reactivity while lower toxicity. [7a-d,7f,8e]

The viability of this design was validated by reactions between probe **1** and DCP. The effect of some organic base （ 100 equiv） on the reaction was tested first. Upon reaction of DCP (10.0 equiv) with probe **1** (1.0 μM) in an acetonitrile solution containing various species of base, the NIR fluorescence was monitored (λex = 760 nm). The fluorescence of the reaction system containing DBU, triethylamine, N,N-diisopropylethyl amine (DIPEA), proton sponge, pyridine, and 4-dimethylaminopyridine (DMAP), respectively, was in comparison with that of the reaction system containing no base (the "None" item, Figure S6, ESI†). The results showed that the fluorescence signal rose in the presence of almost all tested bases except DBU, and DMAP induced the strongest enhancement. This behavior suggested that, DMAP in here acts not only as a base towards the carboxylic acid but also as an efficient acylation catalyst for the thereafter generated mixed anhydride, and the latter is its most famous characteristics in acylation reaction. [18]

Then a detailed study on the response characteristics of probe **1** toward DCP was carried out in acetonitrile with DMAP (50 equiv.), and the absorption spectra of probe **1** in the presence of varying DCP concentrations were recorded. As shown in Figure 1, the solution of probe **1** alone (1.0×10^{-6} M) showed a band centered at 615 nm (ε = 54000 $cm^{-1}\cdot M^{-1}$), consistent with the blue color of the solution. Upon the addition of DCP, a 174 nm red shift of the absorption maximum was found. This new peak located at 784 nm made the color of the resultant solution change from blue to light green (Fig. 1), allowing for "naked-eye" detection. To further evaluate the reaction between the probe and the concentration of DCP, titrations with different concentrations of DCP were conducted. As shown in Figure S7 in ESI†, the

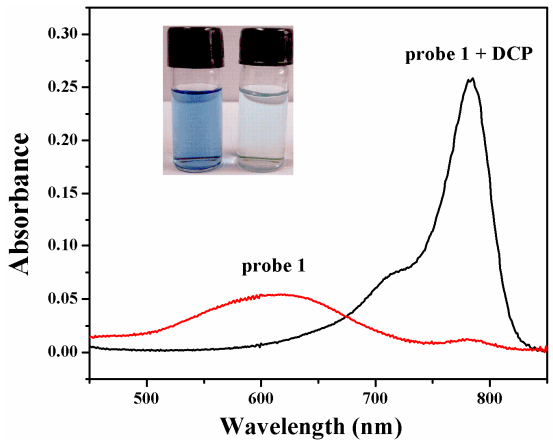


Fig. 1 The absorption spectra of probe **1** (1.0 μM) in CH3CN solution containing DMAP (50.0μM) before and 15 min after adding of DCP (10.0 μM).

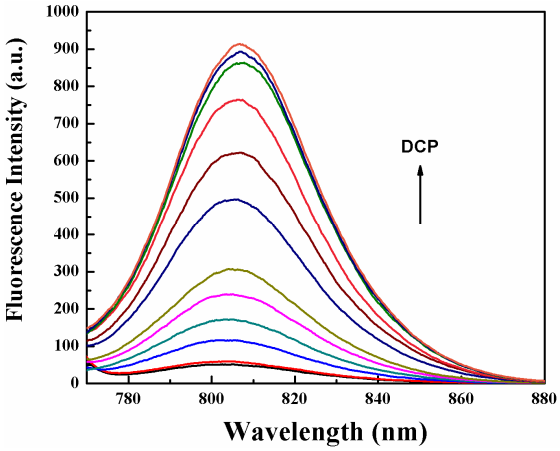


Fig. 2 NIR fluorescence enhancement of probe **1** (1.0 μM) in CH3CN solution containing DMAP (50.0 μM) vs. concentrations of DCP. [DCP] = 0, 0.1, 0.3, 0.5, 0.7, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0,7.0 μM, respectively. λex = 760 nm.

absorbance at 784 nm increased with increasing DCP concentrations between 0.30 and 9.0 μM.

The reaction of probe **1** with DCP also produced noticeable changes in the fluorescence emission. The maximum excitation wavelength of probe **1** in an acetonitrile solution was at 620 nm with an emission peak at 750 nm (Φ_{fl} = 0.064 in acetonitrile, Figure S8, ESI†). Upon the addition of DCP, the resulting maximum excitation at 790 nm was greatly red shifted to the NIR region with relatively little change in the maximum emission wavelength, which was located at 807 nm. Therefore, when monitored using an excitation wavelength at 760 nm, the fluorescence titration curve revealed that the fluorescence intensity at 807 nm increased with increasing concentrations of DCP (Fig. 2). Moreover, we found that the fluorescence could show an appropriate response even when the concentration of DCP was as low as 0.1 μM. The limit of detection (LOD) of probe **1** for DCP was 0.136 nM (R^2 = 0.98) according to the nonlinear approach (Figure S9, ESI†), [19] which suggested that the probe had a good sensitivity towards DCP.

The above experiments revealed the great spectral changes arising from the reaction of probe **1** with DCP, which can be ascribed to the forming of product **2** ($\varepsilon_{784\ nm}$ = 257000 cm^{-1} • M^{-1}, Φ_{fl} = 0.075 in acetonitrile). Product **2** was confirmed by its high-resolution mass spectra (HR-MS): Calcd. for $C_{38}H_{46}N_3O^+$: 560.36408(M^+); found: 560.36321; and ^1H NMR spectra (Fig. S4 and S5, ESI†). In product **2**, the electron-withdrawing carbonyl group in the valerolactam moiety attenuated the electron-donating ability of the amino group toward the heptamethine chromophore, this attenuation effect led to obvious red shifts of both the maximum absorption and excitation wavelengths, just as demonstrated in our previous design consideration. Meanwhile, the time-dependence of fluorescence was also evaluated at two DCP concentration levels, e.g., 1.0×10^{-5} and 1.0×10^{-6} M (Fig. S10, ESI†). The results showed that upon the reaction of probe **1** with DCP, the fluorescence of the two tested solutions increased remarkably, and both were close to the maximum intensity in the first 10 min. No changes in the fluorescence were detected in the absence of DCP.

Meanwhile, the kinetic responses of the probe were also tested against similarly reactive and volatile toxic industrial acylating agents that are generally available, e.g., $SOCl_2$ or oxalyl chloride, which may also pose a potential threat to public order. The presence of both induced negligible changes in the kinetic profile. In addition, acids, including p-toluene sulfonic acid and HCl, could not induce a spectral red shift of the detection system, thus excluding interference from acids (Fig. S11, ESI†).

Probe **1** at 1.0×10^{-6} M had less reaction with DCNP compared to with DCP (Fig. S12, ESI†). However, when its concentration was elevated to 10.0×10^{-6} M, probe **1** showed strong response to DCNP even when the concentration of DCNP was as low as 0.5 μM (F. S13～14, ESI†), with LOD being 2.23 nM (R^2 = 0.98) by the nonlinear approach. Meanwhile, in this case, probe **1** also worked well at the presence of 0.10 μM DCP with LOD being 0.661 nM (R^2 = 0.97, Fig S15, ESI†).

The detection towards DCP vapor with sensor-loaded filter paper was also studied. A small piece of filter paper was immersed in a CH_3CN solution that contains probe **1** (3.0×10^{-4} M) and DMAP (6.0×10^{-3} M). The filter paper was then air-dried, and a small amount of sensor dispersed on the paper make it blue. The sensor-loaded filter paper was placed in a seal vial containing some drops of DCP in CH_2Cl_2 solution (after complete evaporation, the total DCP vapor was *ca.* 15 ppm in the vial). As a control, another sensor-loaded filter paper was placed in a seal vial containing only air. It was found that in vial containing DCP vapor, the blue color of the filter paper was almost disappeared, whereas the color of the control filter paper had no change (Fig S16, ESI†).

In summary, in compound **1**, the carboxylic acid group can be activated by the nerve agent mimic to act as an acylating agent to induce an effective intramolecular amidation, and the resulting large red shift in the NIR spectra clearly indicates that compound **1** can be used as a chromogenic and fluorogenic sensor for nerve agent mimics with good sensitivity. In addition, the probe has the advantages of a facile synthesis, high productivity, fast response, obvious color change, and NIR turn-on signal. However, it is worth noting that the methods reported here may not have the same detection sensitivity for the mimetic agents and real OP nerve agents, given their somewhat different chemical structures. Currently, there are few methods developed for the real agents, including some SERS-based techniques for Tabun and VX with LODs between 1%～5%,[20] a nanocrystal-based photoluminescent method for GB, GD, VX, and RVX with a LOD of 0.12 nM, [21] and μ-PADs-based colorimetric method for Sarin and Soman with LOD of 7.5 mM and 2.5 mM, [22] respectively. Hence, a contribution of our research is the development of a probe with novel mechanisms showing enough sensitivity for the potential practical application. The principle of this study may also provide the basis for a new design strategy of chromo- or fluorogenic chemosensors for more reactive toxic targets.

This work has been supported by the National Natural Science Foundation of China (No.21435003), Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT, No. IRT13036), and NFFTBS (No. J1310024). We thank Prof. Bai Kang Pei for help with language editing of the manuscript.

Notes and references

1 S.M. Somani, *Chemical Warfare Agents,* Academic, San Diego,1992.
2 a) A. R. Hopkins and N. S. Lewis, *Anal. Chem.* 2001, **73**, 884; b) O. A. Sadik, W. H. Land Jr. and and J. Wang, *Electroanalysis.* 2003, **15**, 1149; c) F. Wang, H. Gu and T. M. Swager, *J. Am. Chem. Soc.* 2008, **130**, 5392.
3 (a) M. T. McBride, S. Gammon, M. Pitesky, T.W. OBrien, T. Smith, J. Aldrich, R. G. Langlois, B. Colston and K. S. Venkateswaran, *Anal. Chem.* 2003, **75**, 1924; (b) L. Song, S. Ahn and D. R. Walt, *Anal. Chem.* 2006, **78**, 1023. (c) M. J. Aernecke and D. R. Walt, *Sens. Actuators B.* 2009, **142**, 464.
4 Y. Yang, H.-F. Ji and T. Thundat, *J. Am. Chem. Soc.* 2003, **125**, 1124.
5 (a) J. J. Gooding, Anal. Chim. Acta. 2006, **559**, 137; (b) F. Arduini, A. Amine, D. Moscone and G. Palleschi, *Microchim.Acta.* 2010, **170**, 193.
6 W. E. Steiner, S. J. Klopsch, W. A. English, B. H. Clowers and H. H. Hill, *Anal. Chem.* 2005, **77**, 4792.
7 (a) K. A. Van Houten, D. C. Heath and R. S. Pilato, *J. Am. Chem. Soc.* 1998, **120**, 12359. (b) S.-W. Zhang and T. M. Swager, *J. Am. Chem. Soc.* 2003, **125**, 3420. (c) T.J. Dale and R. Rebek, Jr., *J. Am. Chem. Soc.* 2006, **128**, 4500. (d) A. M. Costero, S. Gil, M. Parra, P. M. Mancini, R. Martinez-Manez, F. Sancenon and S. Royo, *Chem.Commun.* 2008, **44**, 6002. (e) A. M. Costero, M. Parra, S. Gil, R. Gotor, P. M. Mancini, R. Martínez-Máñez, F. Sancenón and S. Royo, *Chem. Asian J.* 2010, **5**, 1573. (f) E. Climent, A. Marti,; S. Royo, R. Martinez-Manez, M. D. Marcos, F. Sancenon, J. Soto, A. M. Costero, S. Gil and M. Parra, *Angew. Chem., Int. Ed.* 2010, **122**, 6081. (g) R. Gotor, A. M. Costero, S. Gil, M. Parra, R. Martínez-Má ñez and F. Sancenón, *Chem. Eur. J.* 2011, **17**, 11994. (h) F. Nourmohammadian, T. Wu and N. R. Branda, *Chem. Commun.* 2011, **47**, 10954. (i) X. Wu, Z. Wu and S. Han, *Chem. Commun.* 2011, **47**, 11468. (j) A. M. Costero, M. Parra, S. Gil, R. Gotor, R. MartínezMañez, F. Sancenón and S. Royo, *Eur. J. Org. Chem.* 2012, 4937. (k) A. Barba-Bon, A. M. Costero, S. Gil, A. Harriman and F. Sancenón, *Chem. Eur. J.* 2014, **20**, 6339. (l) S. E. Sayed, L. Pascual, A. Agostini, R. Martinez-Manez, F. Sancenon, A. M. Costero, M. Parra, and

S. Gil, *ChemistryOpen*, 2014, **3**, 142. (m) G. W. Jonathan and T. M. Swager, *ACS Macro Lett.* 2015, **4**, 138.

8 (a) K. J. Wallace, J. Morey, V.M. Lynch and E. V. Anslyn, *New J. Chem.* 2005, **29**, 1469. (b) K. J. Wallace, R. I. Fagbemi, F. J. Folmer-Andersen, J. Morey, V. M. Lynth and E. V. Anslyn, *Chem. Commun.* 2006, **42**, 3886. (c) T. J. Dale and J. Rebek, Jr., *Angew. Chem.,Int. Ed.* 2009, **48**, 7850. (d) I. Walton, M. Davis, L. Munro, V. J. Catalano, P. J. Cragg, M. T. Huggins and K. J. Wallace, *Org. Lett.* 2012, **14**, 2686. (e) Y.J. Jang, O. G. Tsay, D. P. Murale, J. A. Jeong, A. Segev and D. G. Churchill, *Chem. Commun.* 2014, **50**, 7531.

9 D. Knapton, M. Burnworth, S. J. Rowan and C. Weder, *Angew. Chem. Int. Ed.* 2006, **45**, 5825.

10 (a) F. Ilhan, D. S. Tyson and M. A. Meador, *Chem. Mater.* 2004, **16**, 2978. (b) S. Bencic-Nagale, T. Sternfeld and D. R. Walt, *J. Am. Chem. Soc.* 2006, **128**, 5041. (c) B. D. de Grenu, D. Moreno, T. Torroba, A. Berg, J. Gunnars, T. Nilsson, R. Nyman, M. Persson, J. Pettersson, I. Eklind and P. Wästerby, *J. Am. Chem. Soc.* 2014, **136**, 4125.

11 S. Royo, A. M. Costero, M. Parra, S. Gil, R. Martínez-Máñez and F. Sancenón, *Chem. Eur. J.* 2011, **17**, 6931.

12 S. Goswami, S. Das and K. Aich, *RSC Adv.* 2015, **5**, 28996-29001.

13 (a) S. Han, Z. Xue, Z. Wang and T. B. Wen, *Chem. Commun.* 2010, **46**, 8413. (b) D. Pardasani, V. Tak, A. K. Purohit and D. K. Dubey, *Analyst.* 2012, **137**, 5648.

14 (a) W. Xuan, Y. Cao, J. Zhou and W. Wang, *Chem. Commun.* 2013, **49**, 10474. (b) Z. Lei and Y. Yang, *J. Am. Chem. Soc.* 2014, **136**, 6594.

15 X. Peng, F. Song, E. Lu, Y. Wang, W. Zhou, J. Fan and Y. Gao, *J. Am. Chem. Soc.* 2005, **127**, 4170.

16 K. Kiyose, S. Aizawa, E. Sasaki, H. Kojima, K. Hanaoka, T. Terai, Y. Urano and T. Nagano, *Chem.-Eur. J.* 2009, **15**, 9191.

17 J. P. Collman, T. Kodadek, S. A. Raybuck, J. I. Brauman and L. M. Papazian, *J. Am. Chem. Soc.* 1985, **107**, 4343.

18 (18) (a) W. Steglich and G. Höfle, *Angew. Chem. Int. Ed.* 1969, **8(12)**, 981. (b) G. Höfle, W. Steglich, H. Vorbrüggen, *Angew. Chem. Int. Ed.* **1978**, **17**, 569. (c) E.F.V. Scriven, *Chem. Soc. Rev.* 1983,**12,**129. (d) ULF. Ragnarsson and L. Grehn, *Acc. Chem. Res.* 1998, **31**, 494.

19 (19) A. Hakonen and N. Stromberg，*Analyst*, 2012, **137**, 315.

20 (20) A. Hakonen, P.O. Andersson, M.S. Schmidt, T. Rindzevicius, and M. Käll, *Anal.Chim.Acta*., 2015, DOI: 10.1016/j.aca.2015.04.010

21 (21) T. Yu, J. S. Shen, H. H. Bai, L. Guo, J. J. Tang, Y. B. Jiang and J. W. Xie, *Analyst*, 2009, **134**, 2153.

22 (22) D. Pardasani, V. Tak, A. K. Purohit and D. K. Dubey, *Analyst*, 2012, **137**, 5648.