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Novel and selective detection of Tabun mimics†

Yoon Jeong Jang,[a] Olga G. Tsay,[a] Dhiraj P. Murale,[a] Jeong A. Jeong,[a] Aviv Segev[b] and David G. Churchill*[a]

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Detection of nerve agent-related molecules based on BODIPY–salicylaldehyde oxime conjugation was studied. Fluorescence intensity of the B–SAL–OXIME species increases in the presence of DECP, whereas it decreases in the presence of DCP and DEMP (limit of detection = 997 nM). Benzonitrile formation in the novel fluorescent B–SAL–OXIME system was elucidated using model substrates.

Organophosphorus chemicals include nerve agents and pesticides and are considered as being among the most toxic chemicals to living things.[1] Nerve agents were developed to effectively harm people and decrease the military power of an opposing force.[2] Historically, nerve agents have been used during certain conflicts and/or have been stockpiled. The Chemical Weapons Convention (CWC) has placed a ban on synthesizing, stockpiling and deploying chemical warfare agents (CWAs) to prevent further casualties and reduce global dangers; however, recent news of stockpiles and utilization of nerve agents in the Syrian Arab Republic (Ghouta) showed that there remains a clear and present danger in the world regarding nerve agents.[3] The toxic mechanism involves phosphorylation and phosphonylation of the active serine–OH residues in synapses. Nerve agents bind covalently to the acetylcholinesterase active site and block the breakdown of acetylcholine in synapses. It causes an increase in acetylcholine concentration and results in a neurological imbalance in the synapse.[4] Tabun is a unique agent because of its nitrile group. Tabun includes a P–CN group, while other nerve agents of the G-series possess a P–F group. It has been suggested that this chemical group may reveal unique chemistry in chemosensing, but approaches for detection of Tabun (GA) selectively over other nerve agents are still scarce or non-existent.[1] Recently, many methods of detecting nerve agents continue to be developed and optimized, which include ion mobility spectrometry, mass spectrometry, NMR spectroscopy, and enzyme sensors.[5] These methods provide good sensitivity, but do not afford convenient access to appropriate selectivity and/or are not convenient and simplified real-time methods for the "field." Fluorescent and chromogenic sensing methods are becoming convenient and widely used for detecting nerve agents because they are very sensitive, convenient, allow for detection in real-time and are easy to assess by the unassisted eye.[6] Oximes ($R^1R^2C{=}NOH$) include aldoximes and ketoximes in which R^1 is a hydrogen or another organic group.[7] Aldoximes have been used for the treatment of nerve agents.[8] An oxime is a "super-nucleophile" and is capable of attacking the internal phosphorus at the phosphorylated serine–OH in AChE to restore the serine–OH, and thus the function of AChE.[9] BODIPY species bearing oximes have recently been explored in chemosensing (reactive oxygen species).[10] Conjugates of (a) BODIPY(4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene), a well-known fluorophore class, and (b) the salicylaldehyde core, very widely used in transition metal ligand synthetic designs, have recently been prepared. Bodipy derivatives provide high quantum yield and possess robust chemical properties and photostability and tunable solubility for use in bioimaging and chemosensing.[11,12] Herein, we extend our efforts using this conjugation for selective detection of nerve agents and their mimics.

In the present study, a new oxime-based Bodipy system (B–SAL–OXIME) was synthesized and used as a fluorescent sensor for the sensing and detection of nerve agent simulants, DCP (diethyl chlorophosphate), DEMP (diethyl methylphosphonate), and DECP (diethyl cyanophosphonate) (Fig. 1). Fluorescence emission changes of B–SAL–OXIME (compound **6**) with 0.1 M DCP, DEMP, and DECP were studied; solutions were made by 1×10^{-6} M in 0.1 mM, pH 7.4 HEPES buffer and 100 to 1200×10^{-6} M of DCP, DEMP and 2×10^{-7} M in 0.1 mM, pH 7.4 HEPES buffer and 100 to 1200×10^{-6} M of DECP. Fluorescence emission of B–SAL–OXIME with DCP and DEMP decreased (Fig. 2 and Fig. S15a and b, ESI†),

[a] *Molecular Logic Gate Laboratory, Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon, 305-701, Republic of Korea. E-mail: dchurchill@kaist.ac.kr; Fax: +82 42-350-2810; Tel: +82 42-350-2845*

[b] *Department of Knowledge Service Engineering – Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon, 305-701, Republic of Korea. E-mail: aviv@kaist.edu; Fax: +82 42-350-1610; Tel: +82 42-350-1614*

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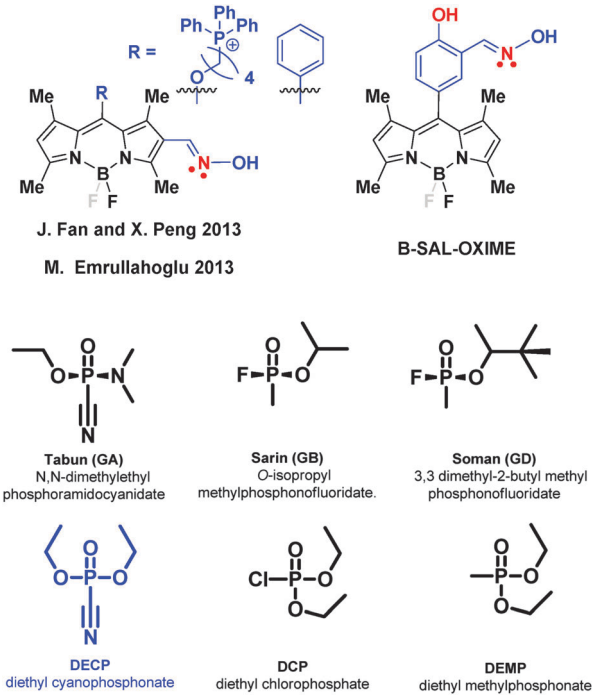


Fig. 1 Structures of probes and G-series chemical warfare nerve agents and simulants (blue color–DECP–Tabun mimic).[1]

but with DECP, intensity increased at λ_{exic} = 499 nm, λ_{emis} = 508 nm (Fig. 2a). Possible mechanisms of B–SAL–OXIME with DCP, DEMP and DECP were proposed and relate to standard nucleophilic attack pathways. Cl^- was a leaving group, departing from DCP, which together with H^+ from R = N–OH helps form R = N–O–P(O)$(OEt)_2$ (Scheme 1) causing a decrease in fluorescence intensity (Scheme 1).[13]

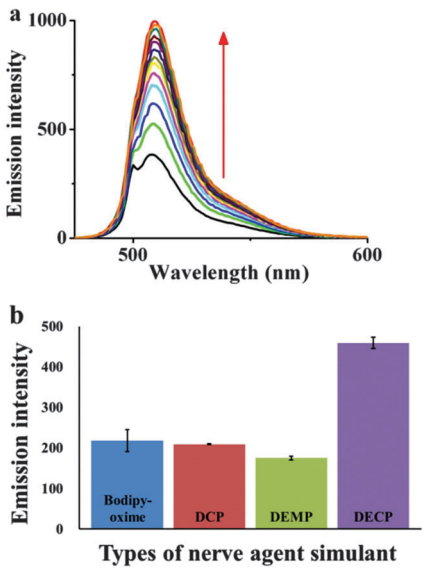
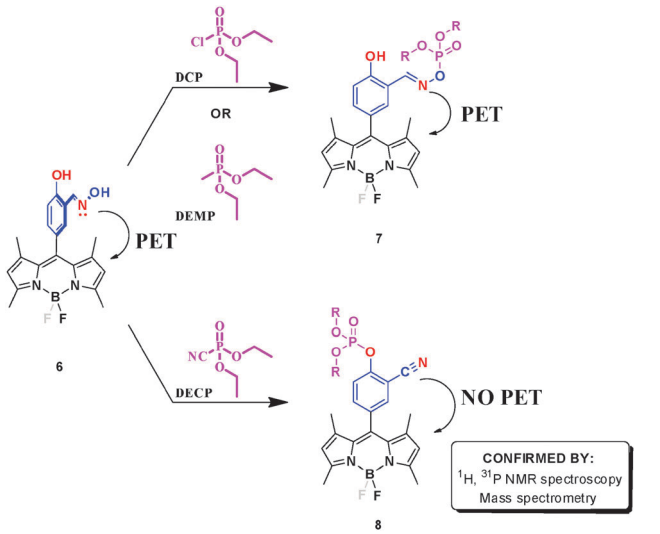


Fig. 2 (a) Emission titration spectra of B–SAL–OXIME (2×10^{-7} M in 0.1 mM, pH 7.4 HEPES buffer) with DECP (100 to 1200 μM in acetonitrile) at λ_{exic} = 499 nm, λ_{emis} = 508 nm. (b) Fluorescence intensity comparing bar graph among B–SAL–OXIME (1×10^{-6} M in 0.1 mM, pH 7.4 HEPES buffer) with 1200 μM DCP, DEMP and DECP in acetonitrile at λ_{exic} = 499 nm, λ_{emis} = 508 nm.

The ethoxy group played the role of the leaving group from DEMP to give R = N–O–P(O)(OEt)Me (Scheme 1) which causes a decrease in fluorescence intensity.[14] The fluorescence intensity of B–SAL–OXIME with DECP increases even in the presence of appreciable concentrations of DCP and DEMP. Here, cyanide (CN^-) was the leaving group to give R = N–O–P(O)$(Et)_2$. Compound 7 for this reaction could not be found by HRMS or NMR spectra (^{1}H and ^{31}P).

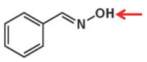
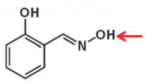
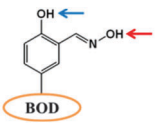
The detection limit of B–SAL–OXIME with DECP was determined to be 92.2 μM for a linear fit; but, in the case of nonlinear methods, the fitting that involves a lower LOD value also possesses a higher R^2 value (997 nM and R^2 = 0.99) (see Fig. S16, ESI† for comparative fittings).[15] This LOD (linear fit) value is not extraordinarily low. Therefore, as part of our future aims we will continue to develop new systems that possess novel modalities, or enhance newly discovered methods to tune the sensitivity, *e.g.*, conjugates with GNPs (gold nanoparticles).[15,16] B–SAL–OXIME however does have enough sensitivity in the detection of nerve agent simulants, considering the respective, relevant LD_{50} values (Table S1, ESI†).

To support the proposed pathway(s) (Scheme 1), a mechanistic study was conducted through the use of model benzene derivatives bearing the same substituents found in salicylaldehyde–oxime. Phenol, benzaldehyde oxime and salicylaldehyde oxime underwent respective reactions with DCP and DECP under basic conditions (triethylamine) in acetonitrile (Table 1 and Fig. S2, ESI†); ^{1}H NMR and ^{31}P NMR spectra were studied after purification. While kinetic differences exist between the model system and the actual probe, the model systems importantly help clarify the reactivity. ^{31}P NMR spectra results of DECP and DCP gave a singlet (δ −20.5 and 5.0, resp.) shifting to δ −5.7 and −6.0, respectively, in the presence of phenol (Fig. S12, ESI†). These data support that phenol with DECP and DCP gives Ph–O–P(O)$(OEt)_2$ functionality through nucleophilic substitution. A singlet in the ^{31}P NMR spectrum was found to be −0.1 and −0.5 in the presence of benzaldehyde oxime (Fig. S13, ESI†), and at 0.02 and 0.7 in the presence of salicylaldehyde



Scheme 1 Proposed mechanism of B–SAL–OXIME with DCP, DEMP, and DECP.

Table 1 ^{31}P NMR spectra results of B–SAL–OXIME, phenol, benzaldehyde oxime and salicyladehyde oxime with nerve agent simulants DECP and DCP

Chemicals	Structure	^{31}P NMR shift (ppm) DECP	DCP
Solvent ($CDCl_3$)		**−20.5**	**5.0**
Phenol		−5.7	−6.0
Benzaldehyde oxime		−0.1	−0.5
Salicylaldehyde oxime		0.02	0.7
Bodipy oxime		−7.1	0.3

oxime (Fig. S14, ESI†) upon treatment with DECP and DCP, respectively (Table 1). The ^{31}P NMR spectrum of B–SAL–OXIME with DECP reveals a singlet (δ −7.1), supporting that DECP is phenolate-bound in B–SAL–OXIME, and not bound to the R = NOH group. The model study with salicylaldehyde oxime revealed that dehydration occurs to give 2-hydroxyl-benzonitrile with DECP and DCP; ^{31}P NMR and ^{1}H NMR spectroscopy confirm this reaction (Fig. S14, ESI†).[17] The dehydration of oxime to nitrile occurs with DECP, in B–SAL–OXIME; the OH group in B–SAL–OXIME was also attacked by DECP wherein mass spectroscopy helps confirm this mechanism. The mass spectrum of B–SAL–OXIME with DECP was observed at *m*/*z* 524.1696; compound **8** formulated as $[C_{28}H_{30}O_{11}P_2Na]^+$ gives a calculated value of 524.1693 (Fig. S7, ESI†).

We believe that the nitrogen of the oxime is the electron donating group and the PET mechanism gives no strong signalling for compounds **6** and **7**. However, the cyano group in compound **8** works as an electron-withdrawing group and allows for strong fluorescence by inhibiting PET between the donor–acceptor units of the dyad (Scheme 1).

To assist in recognition, a logic gating treatment[18] was invoked where data were interpreted in blocks of emission intensity; these help form exclusive logic gate tiers of increasing intensity (Fig. 3). Intensity of A is 0 to 50 nm, B 50 to 100 nm, C 100 to 150 nm, D 150 to 200, E 200 to 250 nm, and F 250 to 500 nm. Each emission intensity block can be identified by a combination of levels of B–SAL–OXIME from DCP, DEMP, and DECP according to fluorescence intensity, using "AND," "OR," and "NOT" logic gates. For the 250–500 nm region, the concentration of DECP is 700 μM or greater with two different levels of fluorescence intensity. In the regions of B, C and D, the concentration of DCP may be zero; for the E region, none of the three agents may be zero. The high intensity gate for the 250–500 nm region (F) is a three-input gate based on levels of DECP and no DCP and DEMP; the 100–250 nm zone is a four-input gate; and the 0–50 nm gate is a six-input gate with DCP,

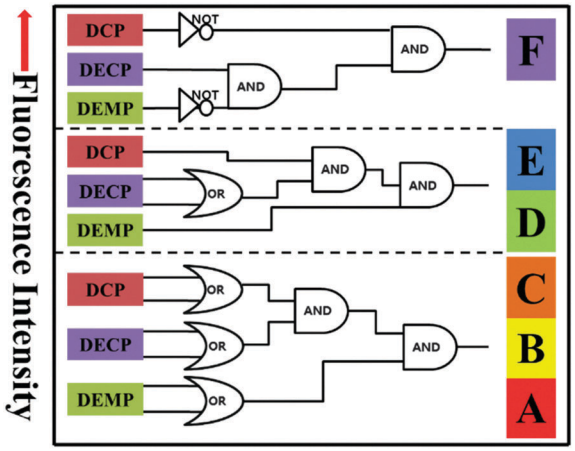


Fig. 3 Logic gate construct for B–SAL–OXIME with DCP, DEMP and DECP according to fluorescence intensity.

DEMP, and DECP, each with two different options of fluorescence intensity for 900 μM or lower.

Fluorescence change monitoring for compound **8** with metal ions (Ag^+, Ca^{2+}, Cd^+, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, K^+, Hg^{2+}, Mg^{2+}, Mn^{2+}, Na^+, Pb^{2+}, or Zn^{2+}) shows that no interference exists except for Ag^+ at concentrations equimolar to that of the organophosphonate species. A strong quenching event was found (99.7%, probe: 1×10^{-6} M in 0.1 mM, pH 7.4 HEPES buffer λ_{exic} = 499 nm, λ_{emis} = 508 nm, acetonitrile). Other trials including B–SAL–OXIME and Ag^+ or probe with DCP and Ag^+ show no change (Fig. S16 and S17, ESI†).

In conclusion, herein we introduce a novel B–SAL–OXIME probe for detecting chemical warfare nerve agent simulants. In the most straight-forward manifestation, it can be implemented as a fluorescent detection medium for the detection of DECP *over* DCP and DEMP. Fluorescence intensity of B–SAL–OXIME increased with DECP *selectively*, and *decreased* with DCP and DEMP concentrations. Models were treated with DECP and DCP and monitored by ^{1}H NMR and ^{31}P NMR spectroscopy to help interpret spectra obtained after the reaction of the B–SAL–OXIME probe with simulants. Through these model studies, B–SAL–OXIME was found to be dehydrated to the nitrile and the OH bonds to DECP leading to loss of HCl.

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