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Selective chromo-fluorogenic detection of DFP (a Sarin and Soman mimic) and DCNP (a Tabun mimic) with a unique probe based on a boron dipyrromethene (BODIPY) dye†

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A novel colorimetric probe (**P4**) for the selective differential detection of DFP (a Sarin and Soman mimic) and DCNP (a Tabun mimic) was prepared. Probe **P4** contains three reactive sites; *i.e.* (i) a nucleophilic phenol group able to undergo phosphorylation with nerve gases, (ii) a carbonyl group as a reactive site for cyanide; and (iii) a triisopropylsilyl (TIPS) protecting group that is known to react with fluoride. The reaction of **P4** with DCNP in acetonitrile resulted in both the phosphorylation of the phenoxy group and the release of cyanide, which was able to react with the carbonyl group of **P4** to produce a colour modulation from pink to orange. In contrast, phosphorylation of **P4** with DFP in acetonitrile released fluoride that hydrolysed the TIPS group in **P4** to yield a colour change from pink to blue. Probe **P4** was able to discriminate between DFP and DCNP with remarkable sensitivity; limits of detection of 0.36 and 0.40 ppm for DCNP and DFP, respectively, were calculated. Besides, no interference from other organophosphorous derivatives or with presence of acid was observed. The sensing behaviour of **P4** was also retained when incorporated into silica gel plates or onto polyethylene oxide membranes, which allowed the development of simple test strips for the colorimetric detection of DCNP and DFP in the vapour phase. **P4** is the first probe capable of colorimetrically differentiating between a Tabun mimic (DCNP) and a Sarin and Soman mimic (DFP).

Introduction

Nerve agents are an extremely toxic class of organophosphorous compounds that are included in the group of chemical warfare agents. Nerve agents are especially dangerous because they are able to interfere with nervous system action through their reactivity with esterase enzymes in general, and with acetylcholinesterase (AChE) in particular. Nerve agents phosphorylate the serine hydroxyl group at the active site of AChE,[1] which is also involved in the binding of acetylcholine (the natural enzyme substrate). However, the interaction of the nerve agent with AChE is stronger than that with acetylcholine, and the entire process results in acetylcholine accumulation, which produces neuromuscular paralysis and eventually death.[2]

Given their easy production, extreme toxicity and possible use by military regimes and terrorist attacks, intense research efforts have been directed to develop sensitive and selective protocols to detect nerve gases. Several remarkable methodologies have been proposed, including the use of enzymatic assays,[3] ion mobility spectroscopy,[4] lanthanide luminescence,[5] electrochemistry,[6] photonic crystals,[7] micro-cantilevers,[8] optical-fibre arrays,[9] and nanomaterials such as nanotubes or nanowires[10] These protocols usually contain at least one of the following limitations: low response, lack of specificity, limited selectivity, poor sensitivity, operation complexity, non-portability, difficulties in real-time monitoring and false-positive readings.

As an alternative to these procedures, the development of chromogenic and fluorogenic probes has recently gained

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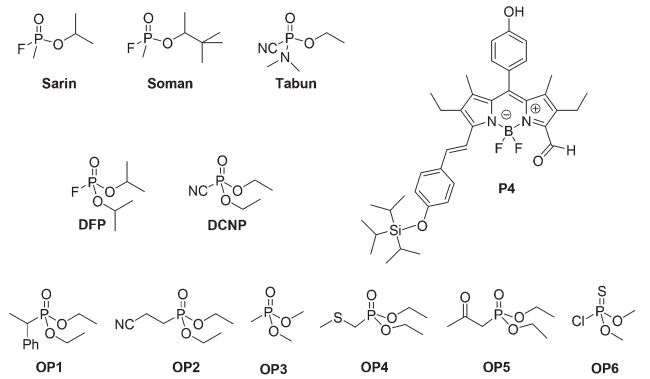
†Electronic supplementary information (ESI) available: ^{1}H-NMR, ^{13}C-NMR and mass spectra of probes **P1–P4** and aldehyde **4**, UV-visible spectra and selected photophysical parameters of probes **P1–P4** in different solvents, UV-visible spectra of **P1–P3** in the presence of DFP and DCNP, UV-visible and fluorescence titrations of **P4** with DFP and DCNP, selected photophysical parameters of probes **P1–P4** in the presence of DFP and DCNP, and UV-visible and fluorescence in the presence of organophosphates OP1–OP6. See DOI: 10.1039/c4ob01299b



Scheme 1 Chemical structures of nerve agents (Sarin, Soman and Tabun), their simulants (DFP and DCNP), selected organophosphates (OP1–OP6) and probe **P4**.

increasing interest.[11] Colorimetric detection is particularly appealing as it is low-cost and offers the possibility of detecting analytes *at site* and to the "naked-eye". In these studies, nerve gas simulants such as diethylcyanophosphate (DCNP) and diisopropylfluorophosphate (DFP), are habitually used (see Scheme 1). These compounds show similar reactivity to real nerve agents Sarin, Soman and Tabun, but lack their severe toxicity (see Scheme 1).

Most reported chromo-fluorogenic probes rely on the electrophilic reactivity of nerve gases and their binding to suitable nucleophiles. However some have certain shortcomings, *e.g.*, detection in solution, but not in the gas phase, as well as serious interference from acids. The reactions used in these probes are non-specific and the reported dye-based probes usually display the same optical response to all nerve gases and are unable to determine the specific identity of the nerve agent. Individual detection of Sarin, Soman or Tabun is proving important; for instance, poisonings with nerve agents are typically treated by administering atropine and certain oximes.[1c] Atropine antagonises the action of acetylcholine at muscarinic receptors, while oximes reactivate inhibited AChE by cleaving the ester formed by nerve gases with the serine hydroxyl group. There is some experimental evidence which indicates that some oximes are ineffective for certain nerve agents, which suggests the importance of distinguishing certain agents within this family of lethal chemicals for appropriate antidote use upon exposure to nerve agents.[12] Very few examples display the specific detection of one of the agents.[13] Indeed there are certain probes capable of selectively detecting the Tabun mimic DCNP and the Sarin and Soman mimic DFP. As far as we know, there are no reported examples able to distinguish one from the other using a unique probe.

After taking the above concept into account and following our interest in developing probes for the selective detection of nerve agents[11a,13,14] we report herein a simple molecule (**P4**, see Scheme 1) that has been designed to respond differentially to Tabun and Sarin/Soman, in addition to being insensitive to acid interference.[14c] A clear colour modulation was observed in both solution and the gas phase.

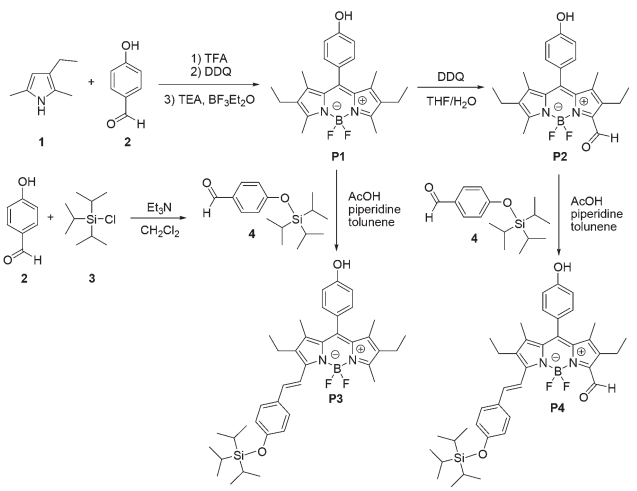
Results and discussion

Probe design

A typical chromo- and/or fluorogenic probe combines a selective analyte recognition site with a colorimetric/fluorescent reporter, which translates the binding event into a colour/fluorescence output signal. As a chromo-fluorogenic reporter, in this study we selected a boron dipyrromethene (BODIPY) core for its robustness against light and chemicals, good solubility, resistance to self-aggregation in solution, excitation/emission wavelengths in the visible spectral region (≥500 nm), and its usually high molar absorption coefficients and fluorescence quantum yields.[15] The BODIPY core in probe **P4** was also equipped with three reactive sites; *i.e.* (i) a nucleophilic phenol group (in the *meso*-position), able to react with electrophilic phosphorous atoms, such as those in nerve agents; (ii) a carbonyl group, which is a reactive site for cyanide (a specific by-product of Tabun);[16] (iii) a triisopropylsilyl (TIPS) protecting group, known to react with fluoride[17] (a specific by-product of Sarin and Soman). Moreover, all these reactions were expected to occur with significant colour and fluorescence modulation. BODIPY derivatives **P1**, **P2** and **P3**, lacking some of those reactive sites, were also prepared and used in the control experiments (see Scheme 2).

Synthesis and spectroscopic characterisation of the probe

Probe **P4** was prepared following the pathway described in Scheme 2. Firstly, compound **P1** was synthesised according to published procedures[18] by the condensation of 3-ethyl-2,4-dimethylpyrrole (**1**) with 4-hydroxybenzaldehyde (**2**) in the presence of trifluoroacetic acid (TFA) as a catalyst, followed by oxidation with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ). The boron difluoride bridging unit was introduced by treatment with boron trifluoride diethyl etherate in the presence of triethylamine. The selective DDQ oxidation of the methyl group at position 3 of compound **P1** was used to confer the corresponding aldehyde **P2**.[19] Compounds **P3** and **P4** were



Scheme 2 Synthesis of probe **P4** and compounds **P1**, **P2** and **P3**.

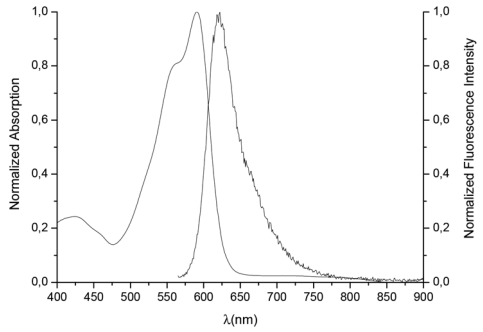


Fig. 1 Normalised absorption and fluorescence (λ_{ex} = 555 nm) spectra of probe **P4** in acetonitrile (c = 1.0 × 10^{-5} mol dm^{-3}).

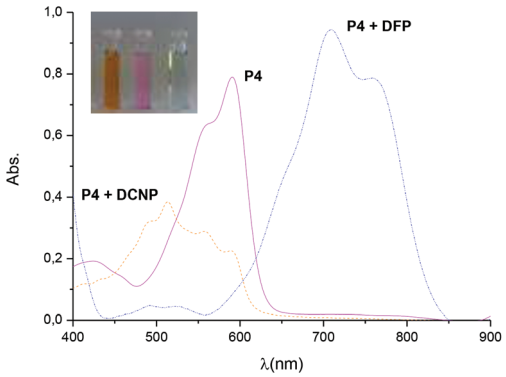


Fig. 2 Visible spectra of **P4** in acetonitrile (1.0 × 10^{-5} mol dm^{-3}) alone and in the presence of 1 eq. of DFP and DCNP. The inset shows the colour changes observed, from left to right: **P4** + DCNP, **P4** and **P4** + DFP.

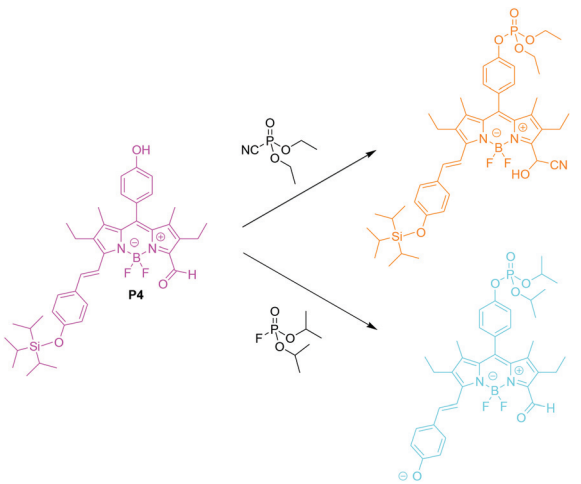
synthesised using a Knoevenagel-type[15b] reaction between **P1** or **P2** with aldehyde **4** in the presence of acetic acid and piperidine.[17]

In a first step, the spectroscopic behaviour of probe **P4** was studied. Fig. 1 shows the absorption and fluorescence spectra for **P4** in acetonitrile. The absorption profile of this derivative was reminiscent of those reported for several related BODIPY dyes,[20,21] with an intense transition at 591 nm (log ε = 4.92) and a shoulder on the short wavelength side (*ca.* 550 nm). The electronic absorption maxima at 591 nm can be attributed to the 0–0 band of a strong S_0–S_1 transition, while the second maximum or shoulder at higher energy can be attributed to the 0–1 vibrational transition. The fluorescence spectrum of **P4** displayed a small Stokes shift with good mirror symmetry (λ_{ex} = 555 nm, λ_{em} = 625 nm, ϕ = 0.52), similarly to other BODIPY dyes.[20,21] The spectroscopic characteristics of compounds **P1–P3** were also studied and are detailed in the ESI.†

Chromo-fluorogenic response against nerve agent mimics

The chromogenic behaviour of the acetonitrile solutions of probe **P4** was tested in the presence of DFP and DCNP (see Fig. 2). Addition of DCNP to **P4** induced a marked decrease in the band centred at 591 nm, and the appearance of new bands at 515 and 560 nm concomitantly with a clear colour change from bright pink to orange (see the inset in Fig. 2). In contrast, a very different chromogenic response was observed when DFP reacted with **P4**. In this case, a major bathochromic shift of the visible absorption band of **P4** from 590 to 715 nm was observed. This bathochromic shift was reflected in a colour modulation from bright pink to light blue (see the inset in Fig. 2).

The different chromogenic response observed upon the addition of DCNP and DFP to **P4** was related with the release of CN^- and F^- anions upon the phosphorylation of the phenol moiety of **P4**. Cyanide (the unique by-product from the Tabun simulant DCNP) reacted with the carbonyl group at the 3-position of the BODIPY core of **P4** to yield an electron-rich cyanohydrin (see Scheme 3) moiety. Fluoride (the unique by-product from the Sarin and Soman simulant DFP) also induced the hydrolysis of the TIPS-protective group. The deprotection reaction generated a strong intramolecular charge transfer (ICT) donor phenoxide ion in full conjugation with the BODIPY



Scheme 3 Mechanism for the chromogenic response observed for probe **P4** in the presence of DCNP and DFP.

core, which would reduce the energy gap for the S_0–S_1 transition, and thus resulted in a large red shift in absorbance.

This mechanism was confirmed with the experiments carried out with control compounds **P1**, **P2** and **P3**. The addition of both simulants to **P1** induced negligible changes in the UV-visible spectrum. This behaviour was expected as the unique phosphorylation of the nucleophilic phenol group was not anticipated to induce colour variations. For **P2**, only the addition of DCNP induced a hypsochromic shift of the absorption band from 517 nm to 489.7 nm (due to phosphorylation and the further cyanide addition to the carbonyl). With **P3**, only DFP was able to yield a bathochromic shift of the visible band from 580 to 703 nm (due to phosphorylation and the further fluoride-induced hydrolysis of the TIPS protecting group). See ESI† for additional details.

Excitation of the acetonitrile solutions of **P4** at 555 nm induced emission at 625 nm (quantum yield of 0.52) (see Fig. 1). Addition of both DCNP and DFP induced emission quenching (see ESI†) due to the phosphorylation of the

Table 1 Limits of detection for DCNP and DFP using probe **P4**

Simulant	UV-visible (ppm)	Fluorescence (ppm)
DCNP	0.91	0.36
DFP	0.95	0.40

nucleophilic phenol group (at the *meso*-position) of **P4** and the subsequent generation of electron donor moieties (a cyanohydrin with DCNP and a phenolate anion when DFP was added). Moreover, the photophysical parameters of probes **P1**–**P4** in other solvents other than acetonitrile in the presence and absence of DCNP and DFP are also found in the ESI.†

Other titration experiments of **P4** with DCNP and DFP allowed us to calculate the corresponding limits of detection (LOD) using the equation: LOD = $K \times Sb_1/S$, where $K = 3$, Sb_1 is the standard deviation of the blank solution, and S is the slope of the calibration curve.[22] Lower LOD values were found for DCNP than for DFP, which is in agreement with the trend observed using other chromogenic reagents (see Table 1).[13,23]

One fundamental study into the development of probes for nerve agents is the role played by the possible interferents or false-positive outcomes produced by other species. Bearing this concept in mind, the reactivity of probe **P4** with other organo-phosphorous compounds (OP1–OP6 in Scheme 1) and an acid medium was also studied under comparable conditions. Nevertheless, it was not possible to detect a change in absorption or in the fluorescence profiles of the probe (see ESI†). Such behaviour indicates that there was no reaction between **P4** and these particular phosphate derivatives and acids, thus rendering **P4** a suitable selective probe for the detection of nerve gases.

Encouraged by the selective response observed with **P4**, and in order to extend its applicability to real-time monitoring, we decided to take another step. In this context, two different test strips were developed for the colorimetric detection in both solution and the gas phase of the nerve agent mimics. To this end, hydrophobic polyethylene oxide films and silica gel plates containing probe **P4** were prepared (see Experimental section for details).

In a typical assay, films or plates were placed into a container holding the nerve agent simulant (30 ppm introduced as an aerosol). Alternatively, strips were exposed to acetonitrile solutions containing DCNP or DFP (10 ppm). Similar chromogenic behaviour was noted in both the solution and the gas phase (see Fig. 3). As seen, a colour modulation from purple to orange (in the presence of DCNP) and to light blue (when DFP was present) was observed. Limits of detection in the gas phase assays were determined, being lower for the polyethylene oxide membranes (1.61 ppm and 1.12 for DCNP and DFP respectively) than for silica gel plates (2.57 and 3.36 ppm for DCNP and DFP respectively).

To complete the study, the strips containing **P4** were introduced into a container with the saturated vapours of organophosphorous derivatives (OP1–OP6) and acid medium for 24 h. However, no change in the colour of the strips was observed.

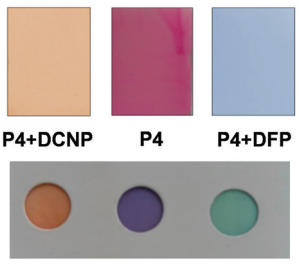


Fig. 3 Silica gel plate (up) and polyethylene oxide membrane (down) of **P4** in the presence of the DCNP and DFP vapours (30 ppm).

Conclusions

We report herein a simple probe (**P4**) based on a BODIPY derivative which is able to colorimetrically distinguish chromogenically the presence of the Tabun mimic DCNP from the Sarin and Soman mimic DFP. In particular, the reaction of probe **P4** with DCNP or DFP resulted in the phosphorylation of the phenoxy group, with the subsequent release of a fluoride (for DFP) or cyanide (for DCNP) anion. In the presence of DCNP, further cyanide nucleophilic addition of the probe to the carbonyl group resulted in a hypsochromic shift of the absorption band concomitantly with a colour change from pink to orange. In contrast when DFP was used, the released fluoride anion was able to hydrolyse the TIPS group of the probe to yield a highly coloured phenoxide anion with a charge transfer band located in the NIR region. Probe **P4** was highly selective and allowed discrimination between DFP and DCNP with remarkable sensitivity. The signalling abilities of **P4** were also retained when incorporated into silica gel plates or onto polyethylene oxide membranes, which allowed the development of simple test strips for the colorimetric detection of DCNP and DFP in the liquid or the vapour phase. To the best of our knowledge, **P4** is the first reported probe capable of showing a different colour change in the presence of the Tabun mimic DCNP and the Sarin and Soman mimic DFP. We believe that this, or similar probes, may open up new routes to help design highly selective kits for "in-the-field" real-time monitoring applications of nerve gases with operational simplicity.

Experimental section

General remarks

CH_2Cl_2 and CH_3CN were distilled from P_2O_5 under Ar prior to use. Benzene and toluene were distilled from CaH_2 under Ar prior to use. THF was distilled from Na/benzophenone under Ar prior to use. All the other solvents and starting materials were purchased from commercial sources where available, and were used without purification. Silica gel 60 F254 (Merck) plates were used for TLC. Column chromatography was

performed on silica gel (60, 40–63 μm). ^{1}H NMR (300 MHz), ^{13}C NMR spectra were determined in a Bruker AV 300 spectrometer. Chemical shifts are reported in parts per million (ppm), calibrated to the solvent peak set. High-resolution mass spectra were recorded in the positive ion mode with a VG-AutoSpec mass spectrometer.

Synthesis of P1

3-Ethyl-2,4-dimethylpyrrole (**1**, 2 mL, 14.8 mmol) and 4-hydroxybenzaldehyde (**2**, 0.86 g, 7.06 mmol) were dissolved in anhydrous CH_2Cl_2 (150 mL). Trifluoroacetic acid (27 μL, 0.35 mmol) was added and the solution was stirred at room temperature for 50 min. A solution of DDQ (2,3-dichloro-5,6-dicyano-*p*-benzoquinone, 1.6 g, 7.06 mmol) in CH_2Cl_2 was added. Stirring was continued for 50 min, followed by the addition of triethylamine (16 mL). After stirring for 30 min, $BF_3{\cdot}OEt_2$ (16 mL) was added. The mixture was stirred for 4 h at room temperature. After evaporating solvents under reduced pressure, the crude product was purified by silica gel column chromatography with AcOEt–hexane (1 : 2) to give **P1** (778 mg) as red crystals in a 28% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (dd, J = 8.8 Hz and 2.3 Hz, 2H), 6.93 (dd, J = 8.8 Hz and 2.3 Hz, 2H), 2.51 (s, 6H), 2.29 (q, J = 7.6 Hz, 4H), 1.35 (s, 6H), 0.98 (t, J = 7.6 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.17, 153.54, 140.20, 138.48, 132.71, 131.17, 129.64, 127.9, 115.62, 17.07, 14.62, 12.49, 11.85. HRMS (EI): m/z (%) calc for $C_{23}H_{28}BF_2N_2O$: 397.2263 $[M + 1]^+$ found: 397.2251. UV-Vis (CH_3CN λ_{max}/nm) 520.0; emission (CH_3CN λ_{max}/nm) 535.9.

Synthesis of P2

To a degassed solution of **P1** (200 mg, 0.50 mmol) in THF–H_2O (15/0.15 mL), a solution of DDQ (553 mg, 1.99 mmol) in THF (6 mL) was added drop-wise at 0 °C. The reaction mixture was stirred from 0 °C to room temperature overnight. The reaction mixture was concentrated under reduced pressure and then subjected to silica gel column chromatography with AcOEt–hexane (1 : 3) to give **P2** (126 mg) as orange crystals in a 60.8% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.37 (s, 1H) 7.11 (dd, J = 8.7 Hz and 2.3 Hz, 2H), 7.01 (dd, J = 8.7 Hz and 2.3 Hz, 2H), 2.71 (q, J = 7.4 Hz, 2H) 2.64 (s, 3H), 2.36 (q, J = 7.4 Hz, 2H), 1.45 (s, 3H), 1.36 (s, 3H), 1.03 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.71, 166.65, 157.86, 144.58, 142.83, 139.85, 138.10, 137.81, 136.72, 134.84, 133.41, 129.63, 126.56, 116.90, 18.05, 17.51, 14.74, 14.47, 14.08, 12.96, 11.08. HRMS (EI): m/z (%) calc for $C_{23}H_{26}BF_2N_2O_2$: 411.2055 $[M + 1]^+$ found: 411.2058. UV-Vis (CH_3CN λ_{max}/nm) 517.0; emission (CH_3CN λ_{max}/nm) 545.0.

Synthesis of 4

Triisopropylsilyl chloride (**3**, 5.8 mL, 27.06 mmol) was added to a heterogeneous mixture of 4-hydroxybenzaldehyde (**2**, 3 g, 24.6 mmol) and triethylamine (6.9 mL, 49.2 mmol) in CH_2Cl_2 (100 mL) at room temperature. After stirring for 1 h, the yellow solution was quenched by methanol and stirred for another 10 minute period. The reaction mixture was diluted with CH_2Cl_2 and washed with water and brine, and was dried ($MgSO_4$) and concentrated under reduced pressure to yield **4** (6.22 g, 91%) as a light orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.83 (s, 1H), 7.73 (d, J = 8.2 Hz, 2H), 6.92 (d, J = 8.2 Hz, 2H), 1.23 (m, 3H), 1.07 (m, 9H), 1.04 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.87, 131.40, 120.60, 18.16, 12.78. HRMS (EI): m/z (%) calc for $C_{16}H_{27}O_2Si$: 279.1780 $[M + 1]^+$ found: 279.1774.

Synthesis of P3

P1 (200 mg, 0.51 mmol) and aldehyde **4** (170 mg, 0.61 mmol) were dissolved in a mixture of toluene (140 mL), acetic acid (700 μL) and piperidine (700 μL). Any water formed during the reaction was removed azeotropically by heating in a Dean–Stark apparatus for 15 h. The reaction mixture was concentrated under reduced pressure and then subjected to silica gel column chromatography with AcOEt–hexane (1 : 2) to yield **P3** (10 mg) as purple crystals in a 30% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 16.7 Hz, 1H), 7.48 (d, J = 8.5 Hz, 2H), 7.13 (d, J = 8.5 Hz, 2H), 6.97 (m, 2H), 6.83 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 16.7 Hz, 1H), 2.59 (m, 5H), 2.32 (m, 2H), 1.64 (m, 3H), 1.37–1.22 (m, 15H), 1.13 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.70, 154.73, 146.90, 143.80, 139.44, 137.50, 137.38, 134.88, 132.23, 131.82, 131.49, 130.18, 129.91, 129.75, 128.75, 120.10, 116.10, 115.80, 115.10, 27.32, 18.32, 17.91, 17.12, 14.60, 14.12, 12.64, 11.94, 11.61. HRMS (EI): m/z (%) calc for $C_{39}H_{52}BF_2N_2O_2Si$: 657.3859 $[M + 1]^+$ found: 657.7277 UV-Vis (CH_3CN λ_{max}/nm) 580.0; emission (CH_3CN λ_{max}/nm) 600.2.

Synthesis of P4

In a 250 mL round-bottomed flask equipped with a Dean–Stark trap and a reflux condenser, aldehyde **4** (163 mg, 0.58 mmol) was dissolved in benzene (50 mL), acetic acid (400 μL) and piperidine (400 μL). The reaction mixture was stirred at reflux temperature and then a solution of **P2** (120 mg, 0.29 mmol) in benzene (40 mL) was added drop-wise. Any water formed during the reaction was removed azeotropically by heating in a Dean–Stark apparatus for 1 h. The reaction mixture was concentrated under reduced pressure and then subjected to silica gel column chromatography with AcOEt–hexane (1 : 2) to yield **P4** (40 mg) as dark purple crystals in a 20% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.46 (s, 1H), 7.69 (d, J = 16.5 Hz, 1H), 7.55 (dd, J = 8.7 Hz and 3.3 Hz, 2H), 7.50 (d, J = 16.5 Hz, 1H), 7.16 (m, 2H), 7.04 (m, 2H), 6.92 (dd, J = 10.4 Hz and 8.6 Hz, 2H), 2.69 (dq, J = 22.6 Hz and 7.5 Hz, 4H), 2.17 (s, 3H), 1.46 (s, 3H), 1.35 (s, 3H), 1.26 (m, 3H), 1.17 (s, 3H), 1.14 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.01, 158.99, 157.85, 157.14, 156.68, 143.94, 141.25, 139.65, 137.08, 134.24, 133.52, 130.01, 129.77, 129.68, 129.43, 121.20, 120.46, 116.84, 116.30, 115.92, 29.70, 18.49, 17.79, 14.35, 13.69, 12.69, 12.33, 10.77. HRMS (EI): m/z (%) calc for $C_{39}H_{50}BF_2SiN_2O_3$: 671.3652 $[M + 1]^+$ found: 671.3643. UV-Vis (CH_3CN λ_{max}/nm) 591.0; emission (CH_3CN λ_{max}/nm) 626.1.

Spectroscopic studies

All the solvents were purchased at spectroscopic grade from Aldrich Chemicals Co., used as received, and were found to be free of fluorescent impurities. Absorption and fluorescence

spectra were recorded using a Shimadzu UV-2600 spectrophotometer and a Varian Cary Eclipse spectrofluorimeter, respectively. Fluorescence quantum yields were measured at room temperature in the N_2-purged solution in relation to rhodamine B (Φ_{EtOH} = 0.49)[24] at 525 nm for **P1** and **P2**; and rhodamine 101 + 0.01% HCl (Φ_{EtOH} = 1.0)[25] at 554 nm for **P3** and **P4** as standards. The fluorescence quantum yields were calculated from eqn (1).[26] Here F denotes the integral of the corrected fluorescence spectrum, A is the absorbance at the excitation wavelength, and n is the refractive index of the medium.

$$\phi_{exp} = \phi_{ref} \frac{F\{1 - \exp(-A_{ref} \ln 10)\} n^2}{F_{ref}\{1 - \exp(-A \ln 10)\} n_{ref}{}^2} \quad (1)$$

Limits of detection measurements

Increasing quantities of the nerve agent mimics (DFP or DCNP) dissolved in acetonitrile were progressively added to a solution of **P4** (1.0×10^{-5} mol dm^{-3}) in the same solvent. The UV-visible and fluorescence spectra were recorded in 1 cm path length cells at 25 °C. Representation of Δ of absorbance or fluorescence at the appropriate wavelength *vs*. concentration of simulant allowed the limit of detection to be calculated.

Silica gel plates preparation

The strip was prepared by immersing the silica gel plate into an acetonitrile solution of **P4** (1.0×10^{-3} mol dm^{-3}) and then dried in air.

Polymeric membrane preparation

Polyethylene oxide (2 g, Mw 400 000 Dalton) was slowly added to a solution of **P4** (2 mg) in CH_2Cl_2 (40 mL). The mixture was stirred until a highly viscous mixture was formed. This mixture was poured into a glass plate (40 cm^2) and kept in a dry atmosphere for 24 h.

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