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## Graphical Abstract

### Nerve agent simulant detection by using chromogenic triaryl methane cation probes

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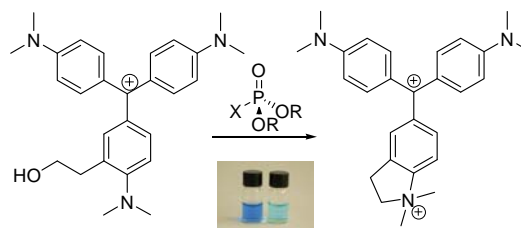
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# Nerve agent simulant detection by using chromogenic triaryl methane cation probes

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## Introduction

Many Nerve agents are highly toxic phosphoric acid esters, and are structurally related to the organophosphates family. These compounds have rapid, severe effects on human and animal health when used in gas, aerosol or liquid. The effects of nerve agents are mainly due to their ability to inhibit the action of acetylcholinesterase, a critical central nervous system enzyme.<sup>1</sup> Nerve agents are considered to be among the most dangerous chemical warfare agents (CWAs).<sup>2</sup> In addition, their easy production, their extreme toxicity and their possible use in terrorist attacks, emphasize the need to detect these odourless and colourless chemicals via the development of reliable and accurate methods.<sup>3,4</sup> Current protocols for monitoring nerve agents are based on the use of biosensors,<sup>5,6</sup> ion mobility spectroscopy (IMS),<sup>7</sup> electrochemistry,<sup>8,9</sup> microcantilevers,<sup>10,11</sup> photonic crystals,<sup>12,13</sup> optical-fibre arrays,<sup>14</sup> etc. As an alternative to these, the development of easy-to-use fluorogenic and chromogenic probes has proved useful for signalling these derivatives in different media and has kindled interest in recent years.<sup>4,15-19</sup> Moreover, colorimetric sensing is particularly attractive as it requires low-cost widely used instrumentation, and also offers the possibility of detecting these target analytes “to the naked eye”.

We have recently shown an interest in developing chemical probes capable of signalling the presence of nerve agent simulants following different sensing paradigms.<sup>20,21</sup> Moreover, given the high toxicity of nerve agents Sarin, Soman and Tabun, the related compounds DCP and DCNP have been typically used as models for the design of indicators and sensing systems as they have a similar reactivity, but lack severe toxicity (Figure 1).

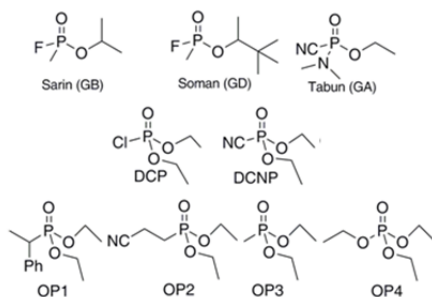


Figure 1: Nerve agents and simulants

To design the probes, we followed the well-established paradigm that relies on the use of dyes containing suitable reactive sites which, upon reaction with target guests, result in a colorimetric event. The use of specific reactions to generate the observable changes ensures the selectivity of chemodosimeters. In addition, the adequate election of the dyes gives rise to probes that shows high sensitivity. Probably, the most common chemical reaction involving nerve agents takes place directly at the phosphorus atom; in fact, the P-X bond is easily broken by nucleophiles such as water or hydroxyl ions. Given the versatility of this approach, we designed chromogenic probes **1** and **2** (see Figure 2), whose structures contain nucleophilic moieties (able to react with nerve agents) connecting triaryl methane cations dyes.

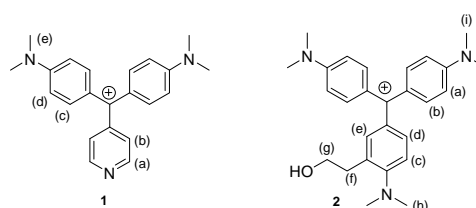
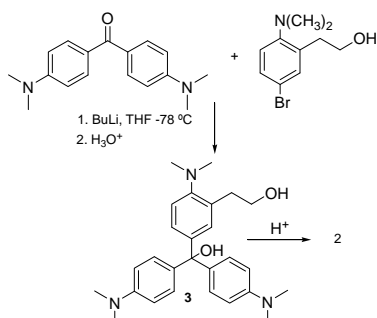


Figure 2: Chromogenic triaryl methane cations

Triaryl methane cations dyes present the advantage of being more stable when they are compared with the triarylcarbinol compounds previously used in our laboratory. In fact, triarylcarbinol derivatives can be dehydrated in a very small yield under many different conditions giving rise to colour changes in the sensor when solutions are kept for long times.

## Results and discussion

Compound **1** is the well-known 4-Pyridine Green cation and **2** is a modification of the Crystal Violet dye whose structure contains an additional 2-hydroxyethyl moiety. The former contains a pyridine group capable of undergoing phosphorylation reactions. The latter holds a 2-(2-(dimethylamino)phenyl)ethanol moiety (see Scheme 1), which is known to give reactions with phosphonate substrates to further undergo rapid intramolecular N-alkylation to yield a quaternary ammonium salt.<sup>20-22</sup> We expected, upon reaction with certain organophosphorous (OP) substrates, the transformation of pyridine **1** into the corresponding pyridinium salt. The stronger electron withdrawing character of the pyridinium salt would result in a modulation of the push-pull character on the dye and in a colour shift. Similarly the phosphorylation of the hydroxyl group in compound **2** that is followed by an intramolecular S<sub>N</sub>2 reaction would transform the tertiary amine into a quaternary ammonium salt with similar changes that these described for compound **1**.

Scheme 1: Synthesis of cromoreagent **2**

### Detection studies

First, reactivity of **1** and **2** was tested with DCP and DCNP (see Scheme 2) in **acetonitrile: water** (25:75 v/v) buffered at pH 5.5 with MES (2-morpholinoethanesulfonic acid) 0.1 mol dm<sup>-3</sup>. **These conditions were chosen for two reasons: (i) to guaranty that the observed changes were due to the expected reaction and not to a mere acid-base process and (ii) to ensure the probe solubility.**

In this medium, compound **1** displays an intense absorption band at 635 nm, typical of triaryl cations. Addition of either DCP or DCNP resulted in a bathochromic shift of 20 nm. This change in colour is consistent with the phosphorylation of the pyridine, and gives rise to a pyridinium salt showing a stronger electron withdrawing character (when compared with pyridine)<sup>23,26</sup> (see Figure 2). In order to assess the proposed mechanism, <sup>1</sup>H NMR studies were carried out. Addition of DCNP to the CD<sub>3</sub>CN solutions of chromoreagent **1** induced clear changes in the <sup>1</sup>H NMR spectrum. Thus, H<sub>b</sub> underwent a downfield shift of  $\Delta\delta = 0.13$  ppm, whereas the variation of the H<sub>a</sub> shifts was almost negligible. In the dimethylaniline ring, H<sub>c</sub> and H<sub>d</sub> were downfield shifted ( $\Delta\delta = 0.03$  ppm and  $\Delta\delta = 0.02$  ppm respectively). The signals corresponding to the methyl groups underwent no chemical shift variation (Figure 3). The changes observed were in agreement with the proposed formulation.

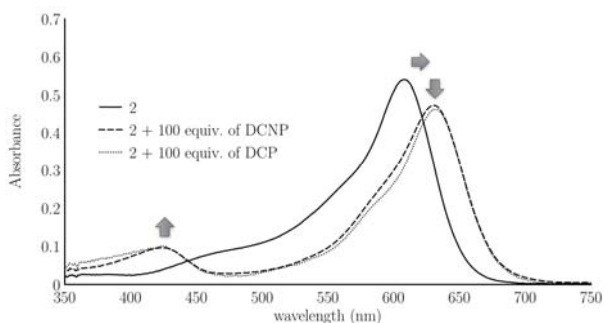
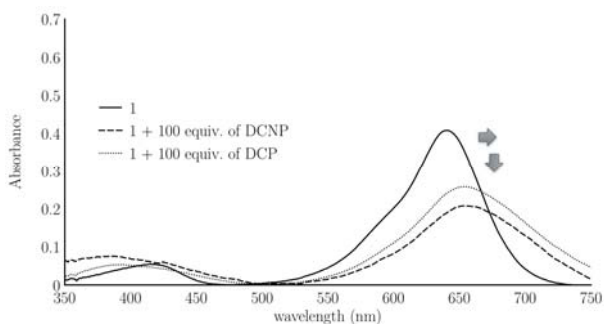
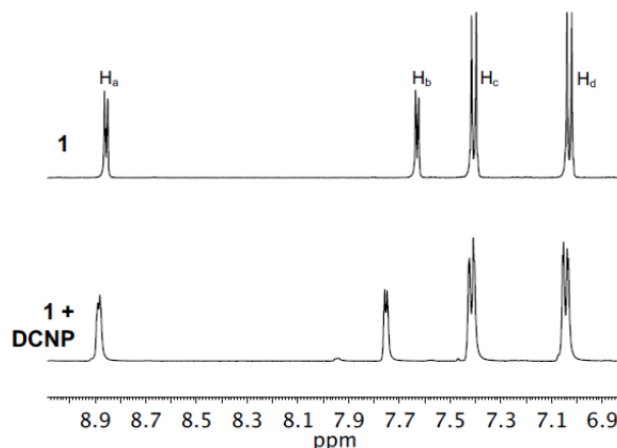


Figure 3: (up) UV spectrum of **1** ( $1 \times 10^{-5}$  mol dm<sup>-3</sup> in buffered acetonitrile:water (25:75 v/v) solutions (pH 5.5, MES 0.1 mol dm<sup>-3</sup>)) free and upon addition of 100 equiv of DCNP and DCP; (bottom) UV spectrum of **2** ( $1 \times 10^{-5}$  mol dm<sup>-3</sup> in buffered acetonitrile:water (25:75 v/v) solutions (pH 5.5, MES 0.1 mol dm<sup>-3</sup>)) free and upon addition of 100 equiv of DCNP and DCP.

Figure 4. <sup>1</sup>H NMR spectra of ligand **1**: free and in the presence of 36 equiv. of DCNP (aromatic region)

Compound **2** ( $1 \times 10^{-5}$  mol dm<sup>-3</sup> in acetonitrile:water (25:75) buffered at pH 5.5 using MES 0.1 mol dm<sup>-3</sup>) showed an absorption band at 603 nm, which underwent a bathochromic shift at 627 nm, along with the appearance of a new band at 427 nm upon addition of DCNP or DCP (Figure 2). This colour modulation from marine blue to turquoise (see Figure 5) was consistent with the intramolecular cyclisation process in which the donor character of *N,N*-dimethylanilino moiety drastically diminished upon the formation of the corresponding quaternary ammonium salt (scheme 2).

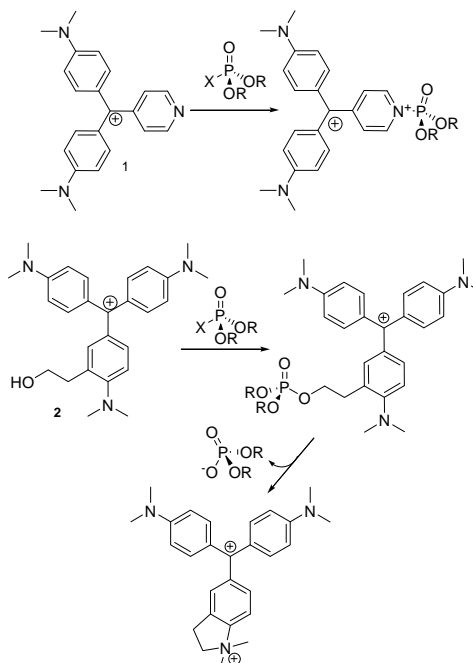
Scheme 2: Reactivity of **1** and **2** with DCP and DCNP



Figure 5: (left) Probe **2** ( $1 \times 10^{-5}$  mol dm<sup>-3</sup> in buffered acetonitrile:water (25:75 v/v) solutions (pH 5.5, MES 0.1 mol dm<sup>-3</sup>)) and (right) **2** ( $1 \times 10^{-5}$  mol dm<sup>-3</sup> in buffered acetonitrile:water (25:75 v/v) solutions (pH 5.5, MES 0.1 mol dm<sup>-3</sup>)) upon addition of 100 equiv of DCNP. More pronounced colour changes (from marine blue to turquoise) can be perceived on the real sample when compared to the picture.

In order to demonstrate the mechanism involved in the observed chromogenic response, *N,N*-dimethylanilino compound was also prepared following a different synthetic path via the reaction of **2** with *p*-toluenesulfonyl chloride in the presence of sodium carbonate in acetonitrile. This resulted in the corresponding tosylated derivative that underwent spontaneous cyclisation to give the cyclised product. The formation of this final derivative was easily detected in the <sup>1</sup>H-NMR spectra of the final product; as expected, more marked changes in the signals of dye **2** in the presence of DCNP or DCP were observed for the hydrogen atoms in the five-membered ring; i.e., H<sub>c</sub> was shifted 0.13 ppm downfield whereas H<sub>d</sub> and H<sub>e</sub> were upfield shifted at 0.05 and 0.08 ppm, respectively. In addition, very minor or insignificant shift changes were observed for H<sub>f</sub> and H<sub>g</sub>.

#### Selectivity and detection limits

Other studies also confirmed that **1-2** were unable to react with other organophosphorus derivatives (i.e., OP1-OP4) (see Figure 4) under similar conditions, indicating that the sensing chromogenic reaction was selective for the detection of simulants DCP and DCNP. The detection limits for probes **1** and **2** were  $3.2 \times 10^{-3}$  mol dm<sup>-3</sup> and  $2.1 \times 10^{-3}$  mol dm<sup>-3</sup> for DCP and  $8.0 \times 10^{-3}$  mol dm<sup>-3</sup> and  $4.1 \times 10^{-3}$  mol dm<sup>-3</sup> for DCNP, respectively. These values are similar to those obtained with other chemodosimeters based on a similar approaches.<sup>21</sup>

#### Kinetic studies

In order to gain a better understanding of the reaction, the reactivity between compounds **1** and **2** and DCP and DCNP in buffered acetonitrile-water (75:25 v/v) solutions (pH 5.5, MES 0.1 mol dm<sup>-3</sup>) was studied. For each probe a battery of experiments were carried out using different concentrations of simulant. These concentrations were always in an excess to considerer pseudo first-order conditions. Monitoring in each case the changes in the absorbance intensity at 655 nm and 627 nm for **1** and **2**, respectively, and by plotting  $\ln[(A_f - A)/A_f]$  versus time (where  $A_f$  was the final absorbance and  $A$  was the absorbance at a given time) allowed us to determine the observed rate constant ( $k_{obs}$ ) for the reaction of the nerve agent simulants with **1** and **2**. From these data, the constant rates for the studied reactions were evaluated and from them the corresponding half-life time ( $t_{1/2} = \ln 2/k$ ) were calculated. Table 1 summarises the results obtained. Additionally, as an example, Figure 6 shows the increase in the absorbance at 627 nm for dye **2** in the presence of 300 equiv of DCNP in buffered acetonitrile:water 25:75 v/v mixtures.

**Table 1.** Kinetic data for Ligands **1** and **2**

Ligand	DCP		DCNP	
	$k$ [s <sup>-1</sup> ]	$t_{1/2}$ [s]	$k$ [s <sup>-1</sup> ]	$t_{1/2}$ [s]
<b>1</b>	91.97	<0.01	21.74	0.03
<b>2</b>	>100	<0.01	1.36	0.51

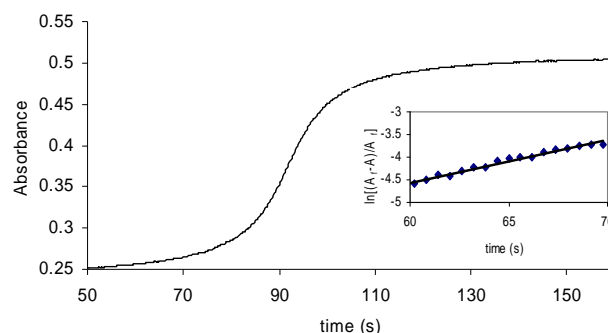


Figure 6: Absorption changes at 627 nm upon the reaction of **2** with DCNP (300 equiv.). The inset shows the pseudofirst-order kinetic plot achieved after an induction time of 40 s).

The rate constants were 0.016 s<sup>-1</sup> and 0.001 s<sup>-1</sup> for **1** in the presence of DCP and DCNP, whereas the  $k$  values for **2** were higher; i.e., 0.095 s<sup>-1</sup> and 0.035 s<sup>-1</sup> for DCP and DCNP, respectively. Regarding the latter probe, in systems which employ similar cyclisation paradigms for the detection of nerve gases simulants, it has been reported that the reaction of alcohol with the phosphorous atom to form the phosphate ester intermediate is slow when compared with intramolecular cyclisation.<sup>21</sup>

#### Conclusions

Two new triarylmethyl cations (**1** and **2**) have been reported as probes for the colorimetric sensing of nerve agent simulants. The reaction of **1** with DCP and DCNP results in phosphorylation at the pyridine, which induces a bathochromic shift in the UV-Vis spectrum. Compound **2** also shows a bathochromic shift in the presence of nerve agent stimulants; in this case, however, the phosphorylation of the hydroxyl group was followed by intramolecular cyclisation. In both cases, a modulation in the electronic donor properties of the nitrogen atoms occurs and results in colour changes which can be observed to the naked eye. It is remarkable that chemosensors **1** and **2** react only with DCP and DCNP, which show similar chemical structures to the Sarin, Soman and Tabun nerve agents, whereas **1** and **2** remain silent in the presence of other organophosphorous derivatives such as OP-1-4. The simple preparation of these derivatives and their selective response make the preparation of other probes using a similar combination of nucleophilic groups (i.e., pyridine and hydroxyl moieties) with different dyes feasible. Further studies in this line are being carried out.

#### Experimental section

THF was distilled from Na/benzophenone under Ar prior to use. All other reagents were commercially available, and were used without purification. Silica gel 60 F<sub>254</sub> (Merck) plates were used for TLC. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded on a Bruker 500 MHz spectrometer. Chemical shifts are reported in ppm units with tetramethylsilane as an internal standard. High resolution mass spectra were recorded in the positive ion mode

on a VG-AutoSpec mass spectrometer. UV-vis absorption spectra were recorded using a 1 cm path length quartz cuvette on a Shimadzu UV-2101PC spectrophotometer. All measurements were carried out at 293 K (thermostated).

#### Synthesis of cations **1** and **2**

The synthesis of **1** was carried out following procedures described in the literature,<sup>23</sup> whereas compound **2** was prepared using the pathway described in Scheme 1. The synthesis involved the use of the previously reported 2-[4-bromo-2-(N,N-dimethylamino)phenyl]ethanol.<sup>24,25</sup> 200 mg (0.82 mmol) of bromide was dissolved under argon atmosphere in 8 ml of dry THF. The solution was cooled down to -78 °C, and then 1.23 ml (1.97 mmol) of a 1.6 M butyl lithium solution was added dropwise. After 15 min, 219.37 mg (0.82 mmol) of 4,4'-(dimethylamino)benzophenone dissolved in 7 ml of dry THF were added. Reaction was kept overnight. After quenching the remanent butyl lithium with water, the organic solvent was partially removed. The mixture was dissolved in 10 ml of MeOH, and was heated to 50 - 60 °C during 20 min after adding 0.4 ml of a 70 % HClO<sub>4</sub> solution. MeOH was then evaporated, and the remaining oil was partitioned in a 40 ml DCM:H<sub>2</sub>O 1:1 mixture. The aqueous layer was extracted 5 times with portions of 40 ml of DCM, and the combined organic layers were dried with MgSO<sub>4</sub>. After evaporating the solvent, the product was purified by column chromatography using (DCM to DCM:MeOH, 97:3) as eluent to yield a 34% of a blue solid. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.35 (d, J = 8.6 Hz, 4H), 7.17 (d, J = 2.7 Hz, 1H), 7.11 (dd, J = 8.5, 2.7 Hz, 1H), 7.00 (d, J = 8.5, 1H), 6.81 (d, J = 9.0 Hz, 4H) 3.87 (t, J = 6.5, Hz, 2H), 3.28 (s, 12H), 2.99 (s, 6H), 2.98 (t, J = 6.6 Hz, 2H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 178.04, 158.83, 156.30, 140.54, 139.82, 135.89, 132.04, 131.74, 127.01, 118.35, 112.99, 62.71, 44.01, 40.71, 35.37. HRMS calcd for C<sub>27</sub>H<sub>34</sub>N<sub>3</sub>O [M]<sup>+</sup>: 416.2702 found 416.2707.

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#### Supplementary Material

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