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# Neutral 1,3-Diindolylureas for Nerve Agent Remediation

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Nerve gases are a class of warfare agent. Chemically, they can be described as organophosphonates that contain good leaving groups. These compounds are highly toxic to both humans and animals by inhibition of acetylcholinesterase.[1] As chemical weapons, they are classified as weapons of mass destruction by the United Nations. Even though production and stockpiling of these agents was outlawed by the Chemical Weapons Convention of 1993, their use in terrorist attacks makes the development of new sensing systems and the design of new remediation products of prime importance.[2] In relation to the latter, there has been an increased interest in the chemistry of nerve agents and in the development of methods of neutralization of these chemicals. Stockpiles of nerve agents should be destroyed and the safety as well as the environmental impact of the destruction process is of crucial concern. Remediation procedures involve the conversion of nerve agents to less toxic products. Examples under development include nerve-agent oxidation through the use of supercritical water,[3] caustic bleaching, incineration, and bioremediation.[4] However these procedures show certain limitations. For instance, supercritical water has proved effective in oxidizing organophosphorous-based nerve agents. However, this procedure is associated with high energy costs. The use of bleach requires solubilization of nerve agents in large quantities of solvent, which must be dealt with later in the process. In the case of bioremediation, free or immobilized enzymes are used. But the stability of these enzymes requires cold-chain handling and the extension of small lab-scale procedures to pilot-scale or field-scale procedures has not been widely employed. This makes high-temperature incineration the only currently approved technique for the destruction of stored nerve agents. Therefore efforts to develop new remediation methods are under investigation[5] and chemical processes that can effectively detoxify stored nerve agents are in great demand. Alternative approaches tested involve the use of catalysts able to enhance the hydrolysis of nerve agents. Even though some metal-catalyzed hydrolysis strategies have been developed,[6] examples of organocatalysts in this context are very rare and include, for instance, iodosylcarboxylates promoting the hydrolysis of G-series nerve agents.[7]

Taking the above issues into account, and as a part of our interest in these chemicals, it was in our aim to test the possible use of supramolecular interactions as a route to the design of supramolecular-based organocatalysts for nerve-agent remediation. In fact, host-guest chemistry has already found many applications in catalysis[8] and the construction of catalysts that employ supramolecular forces has recently become a powerful tool.[9] In particular, we wanted to study the use of supramolecular receptors for organophosphorous derivatives that may enhance the hydrolysis rate of these compounds through a suitable combination of coordinative forces and enhancement of the electrophilic character of the phosphorous atom. As a proof-of-concept we report herein the catalytic remediation effect of a family of 1,3-diindolylureas and thioureas (compounds **1**–**4**, Figure 1) as potential receptors for nerve-agent destruction.

The choice of neutral 1,3-diindolylureas and thioureas was based in their known properties to selectively coordinate phosphate anions in polar solvent mixtures and in the solid state through NH···O hydrogen-bonding interactions.[10] We expected that the corresponding formation of complexes with nerve agents might lead to an enhanced polarization of the P=O bond, which might result in an enhancement of the rate of hydrolysis. In fact, while this research was under development, the interaction between 1,3-diindolylureas and the nerve agent Soman was demonstrated.[11] However, stud-

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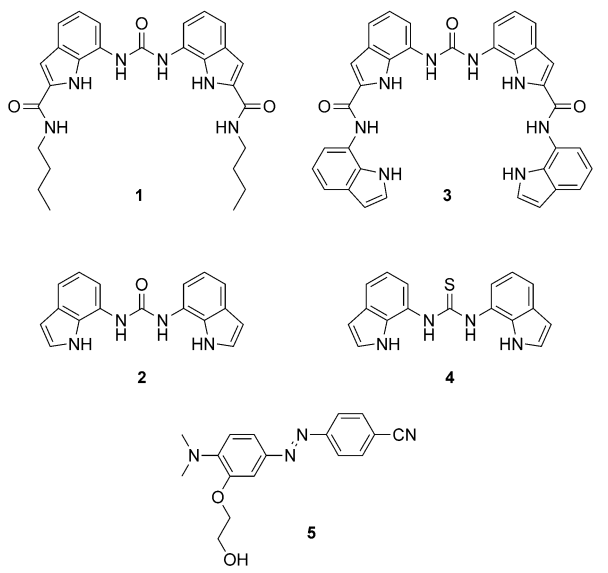



Figure 1. Catalysts **1**–**4** used for nerve-agent destruction and structure of indictor **5**.

ies on the possible use of this interactions in remediation has not been explored. Remediation studies have been carried out here with the safer-to-handle simulants diethylchlorophosphate (DCP) and diethylcyanophosphonate (DCNP, Figure 2). These compounds have similar reactivity, but reduced toxicity compared with the corresponding chemical warfare agents Sarin, Soman, and Tabun.

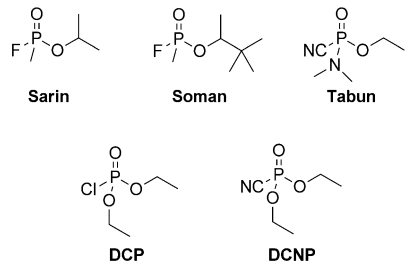



Figure 2. Chemical structures of nerve agents and the simulants DCP and DCNP.

As a first step, $^1$H NMR experiments were carried out to verify the formation of complexes between the receptors and the simulants. Studies in [D$_6$]acetone with receptors **1**–**4** and DCNP clearly demonstrated the existence of interactions. This was reflected in shifts of the NH resonances of the urea and indole groups and of the CH resonance at the C6 position of the indole when **1**–**4** were mixed with DCNP. Figure 3 shows the aromatic region of the NMR spectra corresponding to receptors **1** and **4** (see the Supporting Information for spectra of compounds **2** and **3**). Titration experiments demonstrated that under these conditions 1:2 and 1:1 (receptor/DCNP) complexes were formed with receptors **1** and **3** or **2** and **4**, respectively (see Figure 4). Considering these stoichiometries and by using the software package

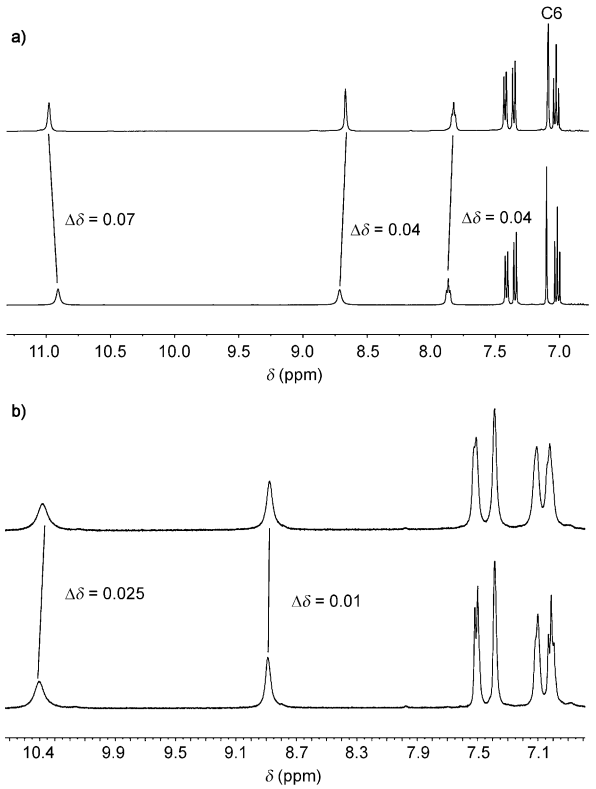



Figure 3. a) $^1$H NMR (aromatic region) in [D$_6$]acetone corresponding to free compound **1** (bottom) and compound **1** in the presence of 2 equivalents of DCNP (top). b) $^1$H NMR (aromatic region) corresponding to free compound **4** (bottom) and compound **4** in the presence of 1 equivalent of DCNP (top).

WinEQNMR[12] the corresponding association constants were determined (see Table 1).

Table 1. Stoichiometry and association constants of compounds **1**–**4** with DCNP in [D$_6$]acetone.

| Compound | Complex stoichiometry | Association Constant |
|---|---|---|
| **1** | 1:2 | 881 ± 90 |
| **2** | 1:1 | 4.4 ± 0.3 |
| **3** | 1:2 | 1743 ± 35 |
| **4** | 1:1 | 10.1 ± 0.9 |

The stability constants revealed a clear relation between the number of possible interactions for each receptor, the stoichiometry of the complexes, and the strength of the interaction. For instance, for receptors **1** and **3**, which have a larger number of binding sites, the formation of 1:2 (receptor/DCNP) complexes was favored, whereas receptors **2** and **4**, with a lower number of coordination sites, were only able to form 1:1 species. Moreover, as expected **1** and **3** showed larger association constants with DCNP than **2** and **4**. In addition when the coordination constant of DCNP with **1** was compared with that of **3**, the latter was to a greater extent due to the presence of more NH groups. Additionally the higher value of the stability constant determined for **4** com-

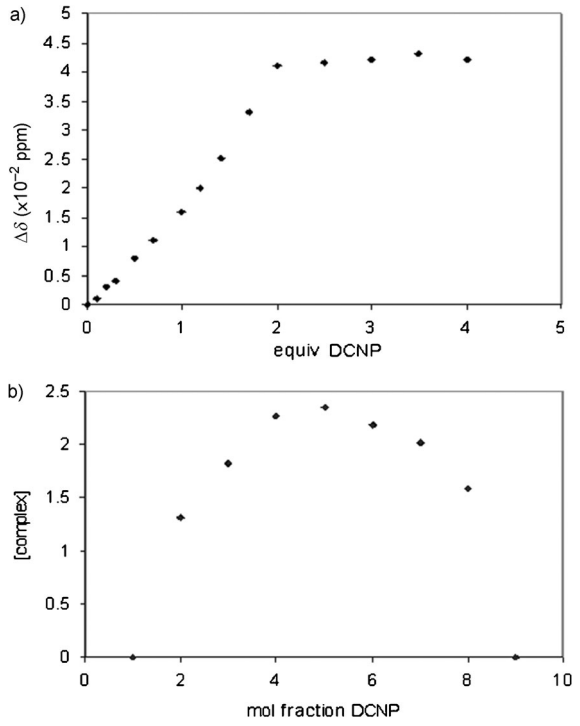



Figure 4. a) $^1$H NMR titration binding curve for compound **1** plus DCNP in [$D_6$]acetone for which $[\mathbf{1}]_{initial} = 5.80 \times 10^{-2}$ M; 293 K. b) Job plot for the DCNP–**4** system in [$D_6$]acetone for which $[\mathbf{4}] + [DCNP] = 4.23 \times 10^{-2}$ M; 293 K.

pared with **2** can be attributed to the presence of more acidic protons in the thiourea group in **4** than for the same moiety in **2**. In addition, we also tested the interaction of receptor **4** and DCNP as a model simulant in [$D_6$]acetone/$D_2O$ (85:15), which is similar to the medium in which the hydrolysis experiments were carried out (see below). In particular, NMR studies in this solvent mixture clearly demonstrated that formation of complexes still occurred in this competitive medium (see the Supporting Information).

After the demonstration of the formation of complexes of **1**–**4** with DCNP, remediation experiments were carried out in buffered acetone/water (80:20 v/v) solutions. A potential problem with hydrolysis of these agents is that they can form particles that might, to some extent, protect them from hydrolysis and may complicate the analysis of the results. Therefore light-scattering studies in the presence and absence of compounds **1**–**4** were performed, however no formation of such particles was observed under the conditions used in this study.

The detection of the hydrolysis of the nerve-gas simulants (DCP or DCNP) in the presence of the corresponding organocatalyst (**1**–**4**) was based on detecting acid generated upon hydrolysis of the simulant with an appropriate indicator.[13] When the hydrolyzed simulant generates enough protons to outstrip the leveling effect of the buffer, indicator **5** (2(*E*)-5-(2-(4-cyanophenyl)diazenyl)2-(dimethylamino)phenoxy)ethanol, Figure 1) changes the color of the solution from yellow to colorless. These color changes were detected with the naked eye. Control UV and $^1$H NMR experiments demonstrated that there was no interaction between the indicator and compounds **1**–**4**.

In a typical experiment, two series of samples (3 mL each) of indicator **5** ($10^{-5}$ M in 1:3 v/v acetonitrile/2-morpholinoethanesulfonic acid ($10^{-1}$ M) solution at pH 5.6) were used. In addition, two DCNP (or DCP) solutions were also prepared. An uncatalyzed solution contained 10% (v/v) DCNP (or 5% (v/v) DCP) in acetone/water (80:20 v/v), whereas the analogous catalyzed solution, had the corresponding catalyst plus 10% (v/v) of DCNP (or 5% (v/v) DCP) in acetone/water (80:20 v/v). The time at which these solutions were prepared was noted and considered $t = 0$. The temperature during these experiments was maintained at 25 °C. At regular intervals (1 min), uncatalyzed and catalyzed solutions (100 μL) were added to the corresponding indicator solutions and the color was observed. Changes in color for the different aliquots could eventually be related to the hydrolysis of the nerve-gas simulants. Although this procedure does not allow determination of the absolute hydrolysis rate, it permits calculation of the enhancement in the hydrolysis for the catalyzed reaction in relation to the uncatalyzed one. The variation of the simulant hydrolysis rate was determined as $\Delta\nu = 100 - [(\nu_{cat} \times 100)/\nu_{uncat}]$ for which $\nu$ is the time at which the color change occurred. Studies were performed with two different concentrations of catalysts (0.001 and 0.005 equiv). The results are summarized in Table 2.

Table 2. Variation of the hydrolysis rate of the simulants DCP and DCNP in acetone/water (80:20 v/v) in the presence of organocatalysts **1**–**4**. Data is given as the percentage of enhancement of the hydrolysis rate compared with the hydrolysis of the simulants in the absence of catalyst.

| Catalyst | 0.001 equiv[a] | | 0.005 equiv[a] | |
|---|---|---|---|---|
| | DCP | DCNP | DCP | DCNP |
| **1** | 44.6±0.4% | 17±2% | 30±1% | 6.3±0.2% |
| **2** | 13±1% | 6.4±0.3% | 14.8±0.2% | 6.3±0.4% |
| **3** | 27±2% | 19.5±0.6% | 29.5±0.5% | 6.4±0.2% |
| **4** | 9.8±0.5% | 6.7±0.3% | 12.7±0.2% | 11.3±0.4% |

[a] Equivalents of catalyst relative to the amount of simulant.

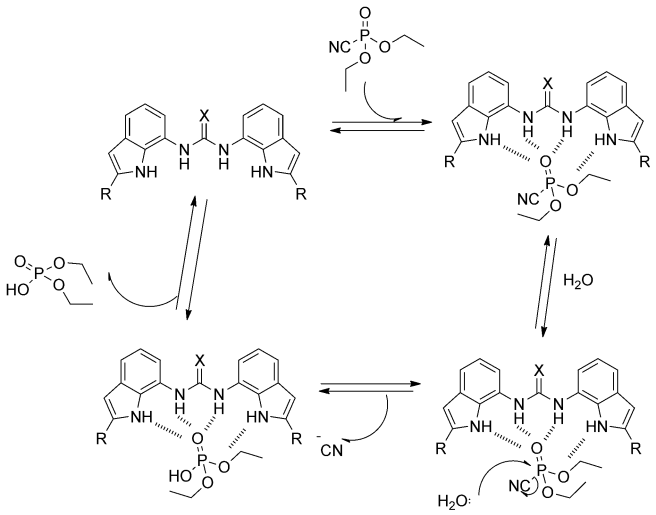
The data clearly shows that there is an enhancement in the hydrolysis rate of the nerve-agent simulants in the presence of the corresponding supramolecular organocatalysts. Additional conclusions can also be drawn from these data. For instance, for all four catalysts, the enhancement in the rate for DCP hydrolysis was higher than that for DCNP. These results are most likely related to the fact that $Cl^-$ is a better leaving group than $CN^-$. In addition, it can be also concluded that the catalytic effect was higher when there were a larger number of hydrogen-bond donors present in the receptor. Thus, **1** and **3** catalyzed the hydrolysis more effectively than **2** and **4**. These data strongly support the hypothesis that the catalytic mechanism includes simulant complexation by increasing the electrophilic character of the P atom and enhancing the final nucleophilic attack of water, which results in the formation of the corresponding organo-

phosphate derivative and HCl or HCN. After hydrolysis, the new generated species, which are less hydrophobic, are released from the catalyst, which is now able to start the catalytic process again. This influence of the hydrophobic effect in the binding site can be also observed if we compare the results obtained with receptors **1** and **3**. Thus, even though **3** showed higher association constants than **1**, its catalytic effect was poorer due to its lower hydrophobic character in the catalytic center. Moreover, the presence of 1:2 complexes of **1** and **3** may also favor the existence of some autocatalytic mechanisms similar to those shown in hydrolysis studies with V-type nerve agents.[14] This suggestion is supported by the experiments carried out at different concentrations. Thus, experiments at other concentrations (from 0.0005 to 0.01 equiv of catalyst) showed that the increase in catalyst concentration did not always lead to better results and, in some cases, lower hydrolysis rates were observed. This effect was more pronounced with **1** and **3** for which a larger amount of receptor would give rise to lower concentrations of the 1:2 complex and as a consequence the autocatalytic effect decreases. With receptors **2** and **4**, which form 1:1 complexes, this effect was not observed (see the Supporting Information).

Finally, the influence of the temperature was also considered and studies with **1** and DCNP were carried out to monitor the hydrolysis enhancement from 10 to 50 °C. An increase in the hydrolysis rate was observed. However, a similar increase of the hydrolysis was also found in the corresponding uncatalyzed samples (see the Supporting Information).

In conclusion, we have observed that neutral 1,3-diindolylureas and thioureas (**1**–**4**) are useful supramolecular organocatalysts in remediation of nerve-agent simulants. To the best of our knowledge, this is the first time that supramolecular-based organocatalysts have shown catalytic effect in remediation processes for nerve agents, such as organophosphorous derivatives. In addition this method shows several advantages compared with other previously described procedures for the elimination of organophosphorous hazards (see the Supporting Information). An enhancement of the hydrolysis rate to near 45 % in some cases was observed in the presence of submolar concentrations of the receptors. The formation of complexes between the receptors and DCP and DCNP suggests that the catalytic mechanism involves complexation of the nerve-gas simulants in a hydrophobic environment provided by the receptors, enhancement of the electrophilic character of the P atom, and the final nucleophilic attack of water that results in the formation of the corresponding less toxic organophosphate derivatives (see Scheme 1). Moreover our findings suggest that other hydrogen-bond-donor derivatives that are able form complexes with organophosphorous compounds can also enhance the hydrolysis rates of these hazardous compounds. By considering the large number of hydrogen-bond-donor derivatives known in the supramolecular field, this observation opens new routes towards the possibility of designing other organocatalysts based on supramolecular coordinative concepts for new effective remediation procedures of nerve agents.




Scheme 1. The proposed catalytic cycle involving catalysts **1**–**4** and DCNP. The cycle involves 1) complexation or the nerve-gas simulants with catalysts **1**–**4**, 2) enhancement of the electrophilic character of the P atom due to coordination, 3) nucleophilic attack of water, and 4) formation of the corresponding less toxic organophosphate derivative.

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