ChemComm

Chemical Communications

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: W. Liu, Z. Chen, L. Yang, H. Y. Au-Yeung and W. Jiang, *Chem. Commun.*, 2019, DOI: 10.1039/C9CC04603H.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/chemcomm

ROYAL SOCIETY OF CHEMISTRY View Article Online
DOI: 10.1039/C9CC04603H

Journal Name

COMMUNICATION

Molecular Recognition of Organophosphorus Compounds in Water and Inhibition of Their Toxicity to Acetylcholinesterase

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

DOI: 10.1039/x0xx00000x

www.rsc.org/

Wei-Er Liu,[a,b] Zhao Chen,[b] Liu-Pan Yang,[b] Ho Yu Au-Yeung,*[,a] and Wei Jiang *[,b]

The molecular tubes with hydrogen bonding donors in their deep hydrophobic cavity are able to selectively bind organophosphorus compounds in water through hydrogen bonding and the hydrophobic effect. They can also be used as a fluorescent sensor for nerve agent simulants and to reduce the toxicity of paraoxon to acetylcholinesterase.

Organophosphorus compounds[1] are commonly used as pesticides and nerve agents. They have been listed by the United States Environmental Protection Agency as acute, highly toxic agents to bees, wildlife, and human beings.[2] The widely-accepted mechanism of organophosphates' toxicity is through irreversibly binding and blocking acetylcholinesterase (AChE).[3] This leads to the accumulation of acetylcholine, resulting in dangerous effects such as paralysis and convulsions. There are expensive and nonportable analytical methods for detecting toxic organophosphorus compounds.[4] However, a cheaper and portable method relying on spectroscopy is still urgently needed.

Many reaction-based detection methods have been explored.[5] But a supramolecular method based on noncovalent interactions would be more appealing even for the catalytic destruction[6] or the recognition-based removal[7] of toxic organophosphorus compounds. This has been explored with many macrocyclic receptors. For example, deep cavitands,[5c] cyclodextrins,[6a,8] calixarenes,[6a,9] and molecular baskets[7,10] have been reported to be able to encapsulate some of the nerve agent simulants. This recognition ability was further used for their catalytic destruction and removal from water. Structurally, toxic organophosphorus compounds, including nerve agents, feature both polar phosphate groups and hydrophobic side chains (Fig. 1a, left). In general, the aforementioned macrocyclic receptors are designed to encapsulate these organophosphorus compounds mainly through the hydrophobic effect. Thus, the recognition selectivity relies only on the size and shape complementarity between the guests and the cavities. A more hydrophobic molecule with similar size and shape will surely compete for the hydrophobic cavity, which may significantly interfere with

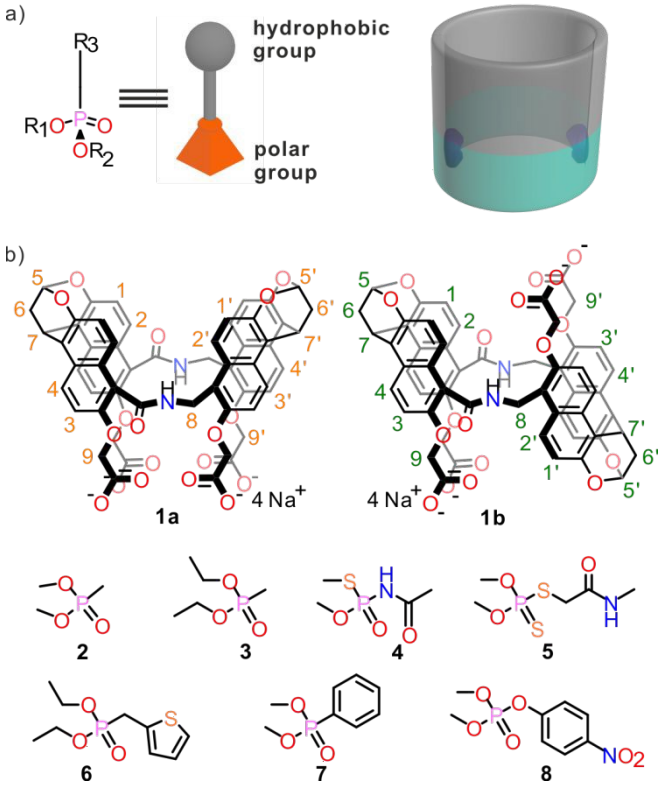


Fig. 1 (a) Cartoon representation of organophosphorus compound and the molecular tube; (b) Chemical structures of molecular tubes **1a** and **1b** and organophosphorus compounds involved in this research.

[a] Department of Chemistry, The University of Hong Kong, Hong Kong, China. E-mail: hoyuay@hku.hk
[b] Shenzhen Grubbs Institute and Department of Chemistry, Southern University of Science and Technology, Shenzhen, 518055, China. E-mail: jiangw@sustech.edu.cn
Electronic Supplementary Information (ESI) available: Experimental procedures and all the NMR spectra. See DOI: 10.1039/x0xx00000x

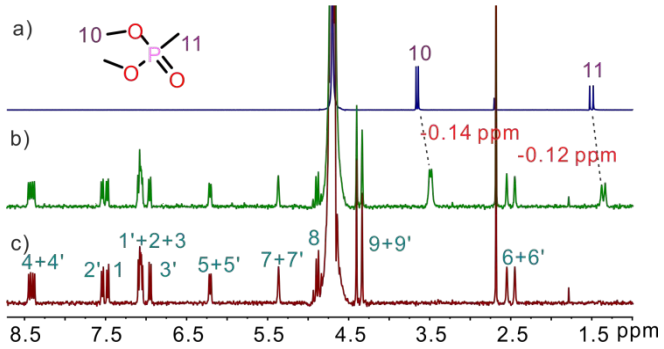


Fig. 2 ^{1}H NMR spectra (400 MHz, D_2O, 0.5 mM, 298 K) of (a) **2**, (c) **1b**, and (b) their equimolar mixture.

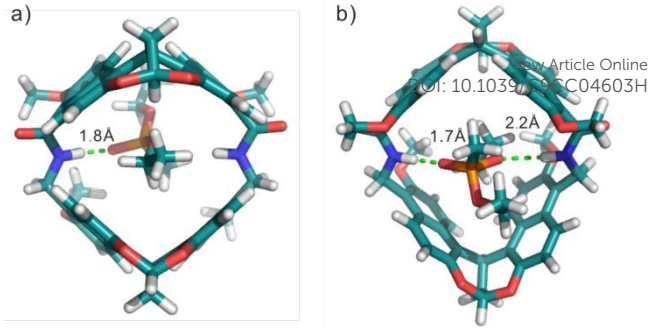


Fig. 3 Energy minimized structures of (a) **3@1a** and (b) **3@1b** computed at the Semiempirical PM06 level of theory by Spartan'14 (Wavefunction, Inc.). The peripheral feet were shortened to methyl groups for convenience.

the sensing or inhibit the catalytic destruction or removal of the organophosphates.

With these aforementioned receptors containing only a hydrophobic cavity, the head phosphate groups with hydrogen bonding acceptors are not appropriately addressed in the recognition. An ideal receptor would interact with the phosphate group and the hydrophobic side chain through hydrogen bonding and the hydrophobic effect, respectively. In this way, a much higher selectivity and higher binding affinity may be achieved. This would be good for high-selectivity sensing and destruction/removal, and reduce the probability of false response.

Recently, we reported a pair of *endo*-functionalized molecular tubes (**1a** and **1b**, Fig. 1b) which contain hydrogen bonding donors in their hydrophobic cavities.[11] These molecular tubes are able to selectively recognize highly hydrophilic molecules in water by using hydrogen bonding and the hydrophobic effect. The cavity features of the molecular tubes are rather complementary to organophosphorus compounds (Fig. 1a, right). Therefore, we wondered whether these molecular tubes can recognize organophosphorus compounds in water. Herein, we report that the molecular tubes not only can strongly bind organophosphorus compounds (Fig. 1c) in water, but also work as a fluorescent sensor and even inhibit the toxicity of paraoxon to AChE.

Organophosphate **2** is the simplest organophosphorus compound and only contains the phosphate head group and minimal hydrophobic side chains (methyl groups). We started with **2** to test the ability of molecular tubes **1a** and **1b** in binding organophosphorus compounds in water. As shown in Fig. 2, the protons of **2** undergo obvious upfield shift in the 1:1 mixture with **1b** when compared to free **2**. This suggests that **2** should be bound inside the cavity and thus experiences the shielding effect of the four naphthalenes of **1b**. Similar binding behaviour was observed for other organophosphorus compounds by using ^{1}H NMR (Figs. S1-S13) and ^{31}P NMR (Fig. S14) spectra. Job's plot and molar ratio titration plot (Fig. S15) support the binding to be in a 1:1 stoichiometry. The association constants of all the organophosphorus compounds with **1a** and **1b** have been determined in D_2O by NMR titrations (Figs. S16-S41) and are listed in Table 1.

Table 1 Association constants (M^{-1}) of **1a** and **1b** with organophosphorus compounds in water at 25 °C as determined by NMR titrations (400 MHz, D_2O)[a]

	2	**3**	**4**	**5**
1a	31±7	530±20	-[b]	45±6
1b	340±70	1500±60	130±20	280±30
	6	**7**	**8**	
1a	1500±200	15000±600	28000±2000	
1b	1400±200	3800±300	850±10	

[a] The concentration of the hosts is fixed at 0.2 mM. [b] The association constant is too small (< 10 M^{-1}) to be determined.

Generally speaking, the *anti*-configured molecular tube **1b** is a much better host than **1a** for guests **2** – **5**; while **1a** binds more strongly than **1b** to **7** and **8**. With increasing the hydrophobicity of the phosphonates, the association constants become higher. These molecular tubes can not only bind to model organophosphorus compounds, such as **2**, **3**, **6**, and **7**, but also show high binding affinities to real pesticides, such as acephate (**4**), dimethoate (**5**), and paraoxon (**8**). The binding constants reach 10^4 M^{-1} for **7** and **8**.

The binding affinities of the molecular tubes are generally higher than those of the aforementioned molecular receptors. Cyclodextrins show the best binding to diphenyl methylphosphonate with K_a = 700 M^{-1};[8] Badjić's molecular baskets have a binding constant of 447 M^{-1} to **2**[7b], 154 M^{-1} to **3**,[10a] and 8891 M^{-1} to **7**.[7b] The molecular baskets and **1b** are comparable in the binding to **2**, but the association constant of molecular tubes to guests **3** and **7** are significantly higher.

Both hydrogen bonding and the hydrophobic effect are known to operate in the molecular recognition of these molecular tubes.[11c] But further experiments were performed to confirm their roles in the recognition of these organophosphorus compounds. Firstly, **1b** was titrated with **3** in 9:1 H_2O/D_2O (Fig. S42). NH protons of the host are visible in this solvent and were observed to shift upfield with adding more **3**. This suggests that hydrogen bonds between **1b** and **3** are slightly weaker than those between **1b** and the encapsulated water.[11c] Thus, the hydrophobic effect through releasing the "high-energy" cavity water is the major driving force;[12] but after the guest is encapsulated in the cavity, hydrogen bonding should also operate and contribute to the binding. This is supported by molecular computation. As shown in Fig. 3, hydrogen bonds are indeed formed between the oxygen atoms of **3** and the NH protons of the hosts. Only one hydrogen bond is formed in the case of **1a**; while two hydrogen bonds are observed for **1b**. This may be due to the

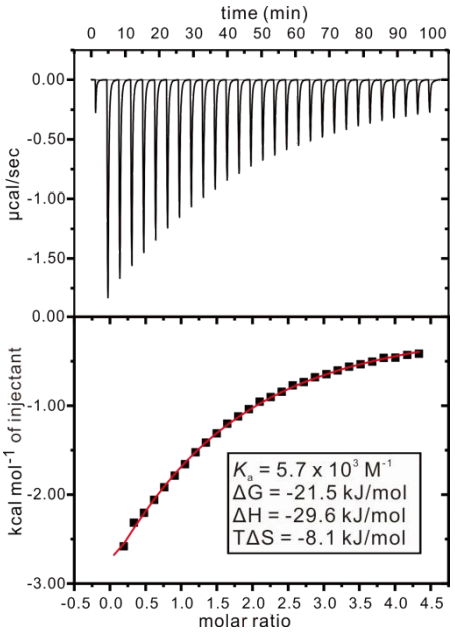


Fig. 4 ITC titration plots (heat rate versus time and heat versus guest/host ratio) of **1a** with **7** in H_2O at 298 K.

twisted geometry in **1a** which is not favourable to form two hydrogen bonds with the phosphonates. This may also explain why **1b** is a better receptor for small organophosphorus compounds. But **1a** has a more well-defined hydrophobic cavity and thus binds better to the organophosphorus compounds with a large hydrophobic group.

Isothermal titration calorimetry (ITC) experiments were performed for the binding of **1a** to **7** (Fig. 4). The binding is largely driven by enthalpic contribution (ΔH = −29.6 kJ/mol) with an unfavourable entropy (−TΔS = 8.1 kJ/mol). This is in drastic contrast to other macrocyclic receptors. For example, the binding of Badjić's molecular baskets[10] is dominated by entropic contribution with minor or even unfavourable enthalpic contribution (for example, for guest **7**, ΔH = −4.4 kJ/mol; −TΔS = −14.4 kJ/mol)[10c]. A classic hydrophobic effect was invoked to explain this. In our case, the large enthalpic contribution may be originated from the release of "high-energy" cavity water[12] and hydrogen bonding between the host and the guest shielded in the hydrophobic cavity. Consequently, these results support that hydrogen bonding and the hydrophobic effect are cooperatively the driving forces for the selective binding of the molecular tubes to organophosphorus compounds.

The efficient binding of the molecular tubes to the toxic pesticides (**4** and **5**) and paraoxon (**8**) encouraged us to test their application as a fluorescent sensor and as an inhibitor to protect the function of AChE.

The molecular tubes are fluorescent because they contain four naphthalenes and often show fluorescent enhancements upon guest binding.[11] We have demonstrated that they can be used as a fluorescent sensor for the detection of persistent environmental contaminants in water[11a,11g] and for monitoring the hydrolysis kinetics of nonfluorescent esters.[11f] However, addition of most of these organophosphorus compounds does not obviously change (**1** - **5**) or quenches (**8**, Fig. S43) the

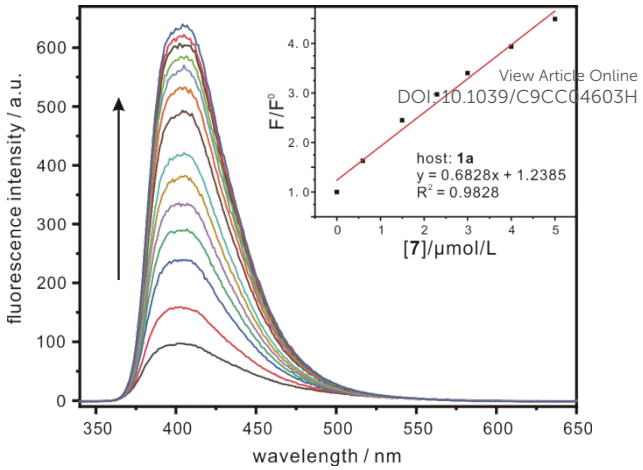


Fig. 5 Fluorescence spectra of **1a** (1.0×10^{-5} M) when titrated with **7** (0-0.019 mM) in deionized H_2O at 25 °C. Inset: the LOD and the detection range of **7**.

fluorescence of the molecular tubes. Binding of **6** (Fig. S44) and **7** (Fig. 5) however significantly enhances the fluorescence of **1a**. The limit of detection (LOD, 3δ/slope) and the detection range for **7** were determined to be 2.3 µmol/L and 2.3-5 µmol/L, respectively. Consequently, these molecular tubes may be used as a turn-on fluorescent sensor for certain toxic organophosphorus compounds such as nerve agents.

Nerve agents and some organophosphorus compounds are toxic to human and animals because they inhibit the function of AChE by covantly linking to the serine residue (Fig. 6). Thus, AChE losts its function in cleaving acetylcholine into choline and acetate. Accumulation of acetylcholine in the body causes the symptons of paralysis and convulsions, and eventually leads to death.

A known method to eliminate the toxicity of nerve agents is to use butyrylcholinesterase or paraoxonase-1 enzymes as bioscavengers to covalently trap the nerve agents, followed by their removal from the bloodstream[13]. However, this method requires significant amount of the enzymes and is thus very expensive. By using the efficient binding of **1a** to paraoxon **8**, we wondered whether **1a** can be used as an noncovalent scavenger to prevent the toxicity of paraoxon **8** to AChE.

By following the literature procedure,[14] in vitro experiments at 37 °C were performed to test the effect of **1a** in preventing the toxicity of paraoxon (**8**) to AChE (Figs. 6, 7, and S45-S51). The bioactivity of acetylcholinesterase (AChE) under several

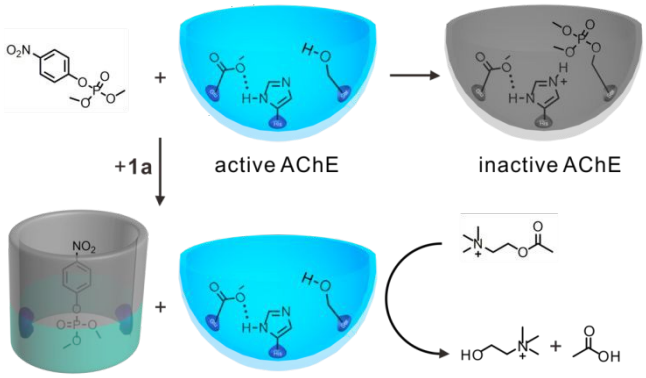


Fig. 6 The toxicity mechanism of paraoxon **8** to AChE (top) and the inhibition mechanism of **1a** to the toxicity of paraoxon **8** (bottom).

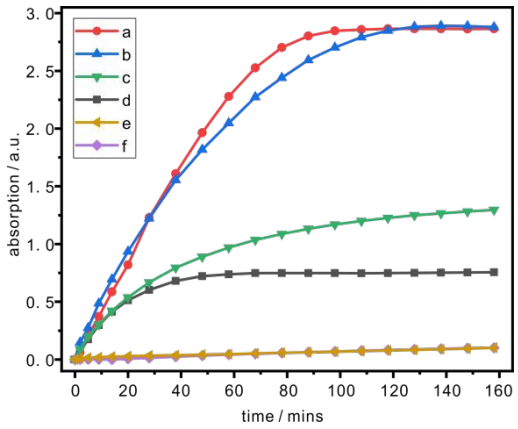


Fig. 7 In vitro enzymatic experiments (37 °C, UV-vis absorption monitored at 412nm) to demonstrate the prevention of paraoxon's toxicity to AChE by **1a**. a) AChE + substrate + indicator; b) 1 eq. **1a** + AChE + substrate + indicator; c) 5 eq. **1a** + AChE + substrate + indicator + 1 eq. paraoxon; d) 1 eq. **1a** + AChE + substrate + indicator + 1 eq. paraoxon; e) AChE + substrate + indicator + 1 eq. paraoxon; f) 1 eq. **1a** + substrate + indicator. Substrate: AICl; indicator: DTNB.

conditions were detected with the help of the indicator 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and the substrate acetylthiocholine iodide (AICl). Figs. 7a, 7b, and 7f show that one equivalent of molecular tube **1a** has little influence on the hydrolysis of AICl by AChE. But the existence of one equivalent of paraoxon **8** completely shut down the activity of AChE (Fig. 7e). In the presence of one equivalent of **1a**, the function of AChE can be restored to some extent even when adding paraoxon **8** (Figs. 6 and 7d). But this is only effective when adding **1a** before **8** (Figs. S52-54). This is in line with the fact that **8** irreversibly blocks to the catalytic site of AChE. Increasing the amount of **1a** to five equivalents does not significantly improve the restoration (Fig. 7c). This may be due to the following reasons: **1a** can also bind AICl (K_a = 7300 M^{-1}, Figs. S55-S57) and thus the existence of a large amount of **1a** would also inhibit the hydrolysis of AICl by AChE. Nevertheless, AICl will be slowly released from the cavity of **1a** and undergo hydrolysis catalysed by AChE. Indeed, the hydrolysis kinetics did not reach a plateau even after 160 min (Fig. 7c) when all other experiments have been finished.

In summary, we report that the molecular tubes with hydrogen bonding donors in their deep hydrophobic cavity can strongly bind organophosphorus compounds in water by using hydrogen bonding and the hydrophobic effect. These organophosphorus compounds include the toxic pesticides dimethoate, acephate and paraoxon. The binding affinities are generally stronger than other molecular receptors only with a non-functionalized hydrophobic cavity. The binding is enthapically driven, in contrast to the dominated entropic contribution for other molecular receptors. In addition, the molecular tubes show fluorescent enhancement to certain organophosphorus compounds, permitting their use as fluorescent sensors to toxic organophosphorus compounds such as nerve agents. Finally, the in vitro enzymatic experiments suggest that the molecular tubes may be used as a non-covalent scavenger to reduce the toxicity of paraoxon to acetylcholinesterase. This may be extended to other toxic organophosphorus compounds, such as nerve agents and pesticides. Consequently, these molecular tubes should be useful in combating chemical warfare agents.[3]

View Article Online
DOI: 10.1039/C9CC04603H

This research was financially supported by the National Natural Science Foundation of China (Nos. 21772083, 21822104), the Shenzhen Science and Technology Innovation Committee (Nos. JCYJ20180504165810828 and KQJSCX 20170728162528382), and the Shenzhen Nobel Prize Scientists Laboratory Project (C17783101). We thank SUSTech-MCPC for instrumental assistance.

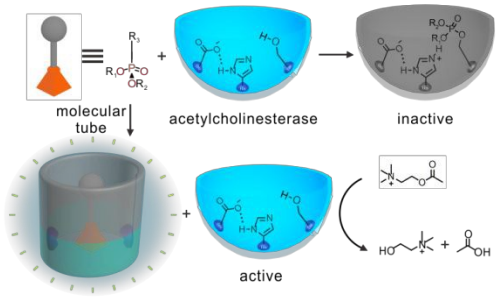
Conflicts of interest

There are no conflicts to declare.

Notes and references

1 R. C. Gupta, *Toxicology of Organophosphate and Carbamate Compounds*, Elsevier Academic Press, New York, **2006**.
2 https://www.epa.gov/sites/production/files/documents/rmpp_6thed_ch5_organophosphates.pdf
3 Y. J. Jang, K. Kim, O. G. Tsay, D. A. Atwood and D. G. Churchill, *Chem. Rev.*, 2015, **115**, PR1.
4 S. S. Aleksenko, P. Gareil and A. R. Timerbaev, *Analyst*, 2011, **136**, 4103.
5 (a) T. J. Dale and J. Rebek Jr, *J. Am. Chem. Soc.*, 2006, **128**, 4500; (b) T. J. Dale and J. Rebek Jr., *Angew. Chem., Int. Ed.*, 2009, **48**, 7850; (c) D. Ajami and J. Rebek Jr., *Org. Biomol. Chem.*, 2013, **11**, 3936; (d) X. Sun and E. V. Anslyn, *Angew. Chem. Int. Ed.*, 2017, **56**, 9522; (e) X. Sun, S. D. Dahlhauser and E. V. Anslyn, *J. Am. Chem. Soc.*, 2017, **139**, 4635; (f) S.-W. Zhang, T. M. Swager, *J. Am. Chem. Soc.*, 2003, **125**, 3420.
6 a) M. Zengerle, F. Braundhuber, C. Schneider, F. Worek, G. Reiter and S. Kubik, *Beilstein J. Org. Chem.*, 2011, **7**, 1543; (b) C. Schneider, A. Bierwisch, M. Koller, F. Worek and S. Kubik, *Angew. Chem., Int. Ed.*, **2016**, 55, 12668.
7 (a) S. E. Border, R. Z. Pavlovic, Z. Lei and J. D. Badjić, *J. Am. Chem. Soc.*, 2017, **139**, 18496; (b) S. E. Border, R. Z. Pavlovic, Z. Lei, M. J. Gunther, H. Wang, H. Cui and J. D. Badjić, *Chem. Commun.*, 2019, **55**, 1987.
8 B.-L. Poh and W. Saenger, *Spectrochim. Acta.*, 1983, **39**, 305.
9 C. Tudisco, P. Betti, A. Motta, R. Pinalli, L. Bombaci, E. Dalcanale and G. G. Condorelli, *Langmuir*, 2012, **28**, 1782.
10 (a) Y. Ruan, H. A. Taha, R. J. Yoder, V. Maslak, C. M. Hadad and J. D. Badjić, *J. Phys. Chem. B*, 2013, **117**, 3240; (b) S. Chen, Y. Ruan, J. D. Brown, J. Gallucci, V. Maslak, C. M. Hadad and J. D. Badjić, *J. Am. Chem. Soc.*, 2013, **135**, 14964; (c) S. Chen, Y. Ruan, J. D. Brown, C. M. Hadad and J. D. Badjić, *J. Am. Chem. Soc.*, 2014, **136**, 17337.
11 (a) G.-B. Huang, S.-H. Wang, H. Ke, L.-P. Yang and W. Jiang, *J. Am. Chem. Soc.*, 2016, **138**, 14550; (b) L.-L. Wang, Z. Chen, W.-E. Liu, H. Ke, S.-H. Wang and W. Jiang, *J. Am. Chem. Soc.*, 2017, **139**, 8436; (c) H. Yao, H. Ke, X. Zhang, S.-J. Pan, M.-S. Li, L. P. Yang, G. Schreckenbach and W. Jiang, *J. Am. Chem. Soc.*, 2018, **140**, 13466; (d) G.-B. Huang, W.-E. Liu, A. Valkonen, H. Yao, K. Rissanen and W. Jiang, *Chin. Chem. Lett.*, 2018, **29**, 91; (e) H. Ke, L.-P. Yang, M. Xie, Z. Chen, H. Yao and W. Jiang, *Nat. Chem.*, 2019, **11**, 470; (f) L.-M. Bai, H. Zhou, W.-E. Liu, H. Chai, L.-P. Yang, W. Yan, W. Zhang, H.-H. Yang and W. Jiang. *Chem. Commun.* 2019, **55**, 3128; (g) L.-M. Bai, H. Yao, L.-P. Yang, W. Zhang and W. Jiang. *Chin. Chem. Lett.* 2019, **30**, 881.
12 (a) F. Biedermann, W. M. Nau and H.-J. Schneider, *Angew. Chem. Int. Ed.*, 2014, **53**, 11158; (b) P. S. Cremer, A. H. Flood, B. C. Gibb and D. L. Mobley, *Nat. Chem.*, 2018, **10**, 8.
13 Nachon, X. Brazzolotto, M. Trovaslet and P. Masson, *Chem.-Biol. Interact.*, 2013, **206**, 536.
14 G. L. Ellman, K. D. Courtney, V. Andres Jr. and R. M. Featherstone. *Biochem. Parmacol.*, 1961, **7**, 88.

View Article Online
DOI: 10.1039/C9CC04603H



The *endo*-functionalized molecular tubes are able to recognize toxic organophosphorus compounds in water. They can be used as their fluorescent sensor and as an inhibitor to reduce the toxicity of paraoxon to acetylcholinesterase.