

# Characterization of the Interaction of Nerve Agent Mimics with Selected Synthetic Receptors

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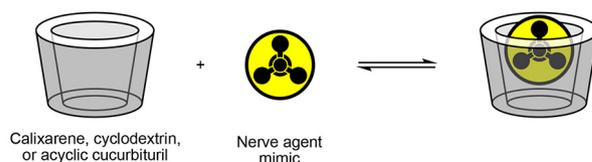
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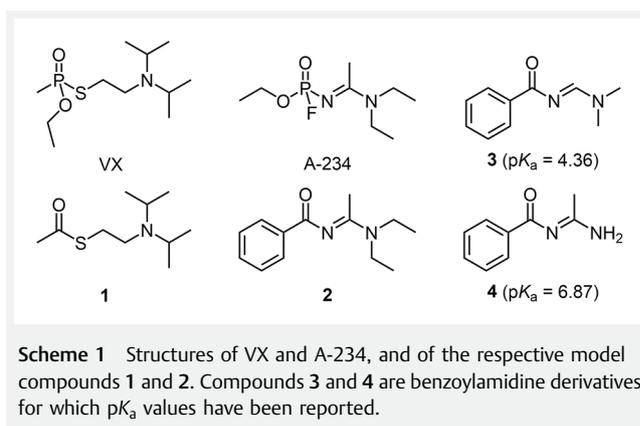
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**Abstract** Qualitative NMR spectroscopic and quantitative calorimetric binding studies were performed to characterize the interaction of non-toxic mimics of the V-type nerve agent VX (O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate) and the Novichok nerve agent A-234 (ethyl (1-(diethylamino)ethylidene)phosphoramidofluoridate) with a series of receptors in 100 mM aqueous phosphate buffer at pH 7.4 and 37 °C. These investigations provided information about the preferred geometry with which the nerve agent mimics are included into the receptor cavities and about the stability of the complexes formed. According to the results, the positively charged VX mimic prefers to bind to cation receptors such as sulfonatocalix[4]arene and an acyclic cucurbituril but does not noticeably interact with cyclodextrins. While binding to the acyclic cucurbituril is stronger than that to calixarenes, the mode of inclusion into the sulfonatocalix[4]arene cavity is better suited for the development of scavengers that bind and detoxify V-type nerve agents. The neutral Novichok mimic, on the other hand, only interacts with the acyclic cucurbituril with a strength required for scavenger development. These binding studies thus provided guidelines for the further development of nerve agent scavengers.

**Key words:** nerve agents, sulfonatocalixarenes, cyclodextrins, acyclic cucurbiturils, binding studies

## Introduction

On August 20, 2020, Russian opposition politician Alexei Navalny fell into a coma after drinking a cup of tea at a Siberian airport. He was transported to Berlin, where he was treated for nerve agent poisoning.<sup>2</sup> A-234 (ethyl (1-(diethylamino)ethylidene)phosphoramidofluoridate) (Scheme 1), the substance used in the incident, was also involved in the attempt to kill former Russian spy Sergei Skripal.<sup>3</sup> It belongs to a family of toxic organophosphates that were secretly developed in the former Soviet Union between the 1970s and 1990s



and are now known as fourth-generation nerve agents or Novichoks.<sup>4</sup> Structurally, most Novichoks are phosphoramidates, with a characteristic amidine moiety.<sup>4</sup>

Like other nerve agents, Novichoks primarily act by inhibiting the enzyme acetylcholinesterase (AChE), thus inducing a cholinergic crisis that can ultimately lead to death.<sup>5</sup> Although the Skripal and Navalny cases demonstrated that Novichok poisonings can be treated with established therapies,<sup>6</sup> there are concerns that these strategies could fail in certain cases and that Novichoks may not only target AChE.<sup>4</sup> Alternative treatment options could therefore be useful, one of which involves the administration of a scavenger that detoxifies the nerve agent before AChE inhibition occurs. Since the degradation of Novichoks has mainly been studied computationally or under conditions incompatible with an *in vivo* use,<sup>7</sup> it is so far unclear whether detoxification is possible under physiological conditions. In the context of our work on the development of low-molecular-weight scavengers,<sup>8</sup> we therefore became interested in these nerve agents.

The working principle of a scavenger involves the initial complexation of the nerve agent by a suitable receptor unit.<sup>9</sup> Examples of receptors that have been shown to complex nerve agents are cyclodextrins,<sup>10</sup> calixarenes,<sup>8f,11</sup> cyclic or acyclic cucurbiturils (aCBs),<sup>8h</sup> cavitands,<sup>12</sup> molecular baskets,<sup>13</sup> naphthotubes,<sup>14</sup> coordination cages,<sup>15</sup> or anion recep-

tors.<sup>16</sup> Once bound to the scavenger, the nerve agent should react with an appropriately placed nucleophilic group that mediates the detoxification. Based on these concepts, functionalized cyclodextrins were developed that rapidly detoxify G-type nerve agents, particularly those with a hydrophobic residue that can be included into the cyclodextrin cavity.<sup>8fa-d,10,17</sup> A sulfonatocalix[4]arene derivative was shown to bind V-type nerve agents and mediate detoxification,<sup>8f</sup> but receptors that interact with Novichoks under physiological conditions are still unknown. We therefore sought for receptors that interact with a nontoxic analog of A-234 under the conditions of the detoxification assay (aqueous buffer at pH 7.4 and 37°C). In this context, the binding of an analog of the V-type nerve agent VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate) to the same receptors was also studied. These studies showed that receptors that bind V-type nerve agents do not necessarily also interact with Novichoks. Moreover, of the receptors studied, only aCBs<sup>18</sup> appear to be suitable for developing scavengers that not only bind but also detoxify these nerve agents.

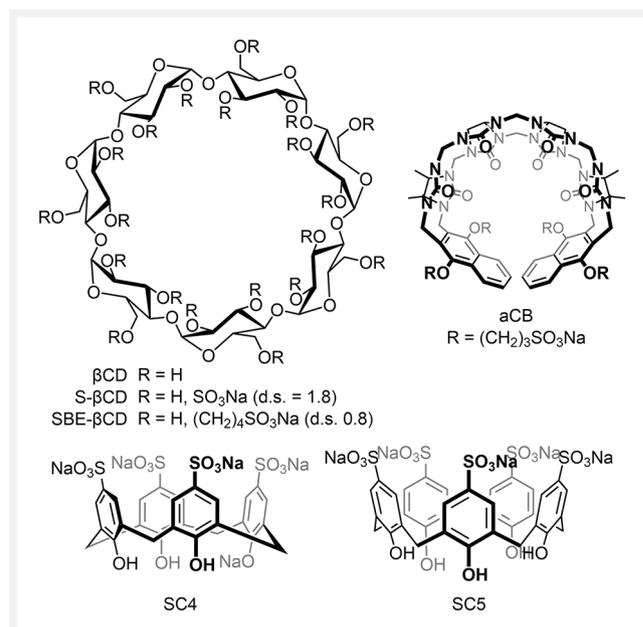
## Results and Discussion

The structures of the nerve agents A-234 and VX are shown in Scheme 1. VX contains a tertiary amino group in a side chain, rendering this nerve agent protonated and therefore cationic at physiological pH ( $pK_a = 8.6$ ).<sup>19</sup> A-234 contains a phosphorylated amidine and although amidines are typically strong bases, phosphorylation can be expected to cause a significant reduction of the  $pK_a$  value. The reason is that the electron-withdrawing nature of the substituent destabilizes the protonated form of the respective nitrogen base. While, to the best of our knowledge,  $pK_a$  values of such phosphorylated amidines have not yet been reported, those of analogs of the model compound we used in the binding studies confirm the above assumption (*vide infra*).

We used *S*-(2-(diisopropylamino)ethyl)ethanethioate **1**, which contains the same side chain as VX but an acyl group in place of the methylphosphonic acid group, as a nontoxic mimic for the V-type nerve agent. This compound was prepared by treating thioacetic acid with *N*-(2-chloroethyl)-*N*-isopropylpropan-2-amine.<sup>20</sup> As a model for the characteristic amidine moiety of A-234, we used *N*-(1-(diethylamino)ethylidene)benzamide **2**, which was prepared in a two-step sequence.<sup>21</sup> The reaction between acetonitrile and diethylamine in the presence of  $AlCl_3$  initially afforded *N,N*-diethylacetimidamide,<sup>22</sup> which was reacted further with benzoylchloride analogously to a described procedure to obtain **2**.<sup>23</sup> Structural studies indicated that such acylated amidines preferentially exist in the tautomeric form shown in Scheme 1, with the benzoyl group at the imino nitrogen atom.<sup>23</sup> In addition, density functional theory studies sug-

gested that the *E*-configured **2** is more stable than the corresponding *Z*-isomer (see the Supporting Information). The  $pK_a$  value of **2** has not yet been reported, but the  $pK_a$  values of the close structural analogs **3** and **4** (Scheme 1) are known.<sup>23</sup> They amount to 4.36 and 6.87, respectively, confirming that the benzoylation of the amidine group, which typically has a  $pK_a$  of ca. 12,<sup>24</sup> causes a substantial reduction of basicity. Model compound **2** can therefore be assumed to primarily exist in the neutral form at pH 7.4.

Since scavenger development requires receptors that allow the introduction of substituents along the cavity with which a bound nerve agent can react, we focused in this work on compounds that allow further functionalization (Scheme 2). Accordingly, we chose three cyclodextrin derivatives, namely,  $\beta$ -cyclodextrin ( $\beta$ CD) and the sulfated  $\beta$ CD (*S*- $\beta$ CD) and sulfobutylether- $\beta$ CD (*SBE*- $\beta$ CD). The two substituted cyclodextrins were selected to assess whether their anionic nature allows overcompensating the repulsive interactions between **1** and the slightly positive electrostatic potential along the inner cavity surface of  $\beta$ CD, which render cyclodextrins not well suited for cationic guests.<sup>25</sup> All cyclodextrins were commercially available, with the average degrees of substitution (number of substituents per glucose unit) amounting to 1.8 for *S*- $\beta$ CD and 0.8 for *SBE*- $\beta$ CD according to elemental analysis (see the Supporting Information). Suitable sulfonatocalix[4]arene derivatives have previously been demonstrated to mediate VX detoxification in aqueous buffered solution,<sup>8f</sup> but the stability of the respective complexes was only determined in water or at a slightly acidic pH.<sup>8h</sup> It was therefore interesting to evaluate the affin-



Scheme 2 Structures of the receptors  $\beta$ CD, *S*- $\beta$ CD, *SBE*- $\beta$ CD, SC4, SC5, and aCB.

ity of **1** to SC4 under conditions that more closely resemble those of the detoxification assay. The aCB was included in the study for a similar reason: although its affinity to **1** had previously been determined,<sup>8h</sup> the conditions differed from those used here. The larger sulfonatocalix[5]arene SC5 was considered as a potential basis for a VX scavenger for the first time. Whether these receptors bind to A-234 or a structurally related analog was unknown prior to this work. Compounds SC4, SC5, and aCB were synthesized by using reported procedures (see the Supporting Information). The composition of all receptors was determined by elemental analysis to ensure that the solutions used in the quantitative binding studies had accurate concentrations.

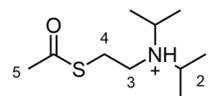
NMR spectroscopic binding studies were initially performed to assess whether the nerve agent mimics interact with the chosen receptors. To this end, solutions of a substrate and a receptor in D<sub>2</sub>O buffered with NaH<sub>2</sub>PO<sub>4</sub>/NaHPO<sub>4</sub> (100 mM) to pD 7.4<sup>26</sup> were prepared containing a 2:1 receptor/substrate ratio. The <sup>1</sup>H NMR spectra of the resulting mixtures were recorded and compared with the spectra of the respective free substrates. Since considerable amounts of hydrolysis products were detected when solutions of **1** were stored for a prolonged time (see the Supporting Information), the respective stock solutions were freshly prepared prior to each measurement.

To illustrate the outcome of such a binding study, the spectra obtained for **1** and SC4 are depicted in Figure 1. These spectra showed that the presence of SC4 caused a shielding of all protons of **1**. The effect was strongest for protons near the cationic head group, while the resonances of protons at the opposite end of **1** were much less affected. The observed signal shifts suggested that the alkyl groups

close to the cationic center of **1** preferentially entered the calixarene cavity, likely due to favorable electrostatic interactions with the surrounding sulfonate groups. The other parts of the molecule remained close to the opening, suggesting that the phosphorus atom of a nerve agent – bound to SC4 in a similar manner – should be available for the reaction with a functional group arranged at the wider cavity opening, as indeed observed.<sup>8f</sup>

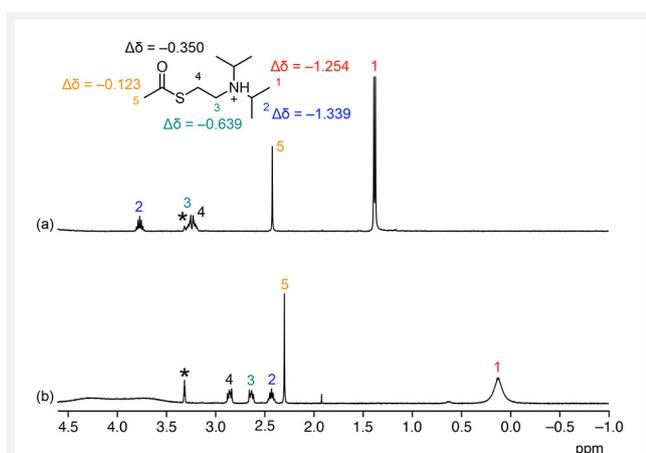
The signal shifts observed for **1** in the presence of the other receptors are summarized in Table 1 (see the Supporting Information for the individual spectra). This table shows that SC5 caused a more pronounced shielding of the protons of **1** than SC4, consistent with the larger cavity diameter of the five-membered calixarene. The pronounced shielding of protons 3 and 4, in particular, indicated that the central part of **1** was deeply incorporated into the cavity of SC5.

**Table 1** Complexation-induced shifts in ppm of the signals of **1** in the <sup>1</sup>H NMR spectrum in the presence of different receptors<sup>a</sup>



H	$\Delta\delta$ (SC4)	$\Delta\delta$ (SC5)	$\Delta\delta$ (aCB)	$\Delta\delta$ ( $\beta$ CD)	$\Delta\delta$ (S- $\beta$ CD)	$\Delta\delta$ (SBE- $\beta$ CD)
1	-1.254	-1.343	-0.800	-0.003	+0.014	+0.005
2	-1.339	-1.781	-1.338	-0.033	+0.011	-0.034
3	-0.639	-1.573	-1.361	-0.052	+0.002	-0.036
4	-0.350	-1.030	-1.316	-0.023	+0.008	-0.022
5	-0.123	-0.160	-0.707	-0.007	+0.020	-0.004

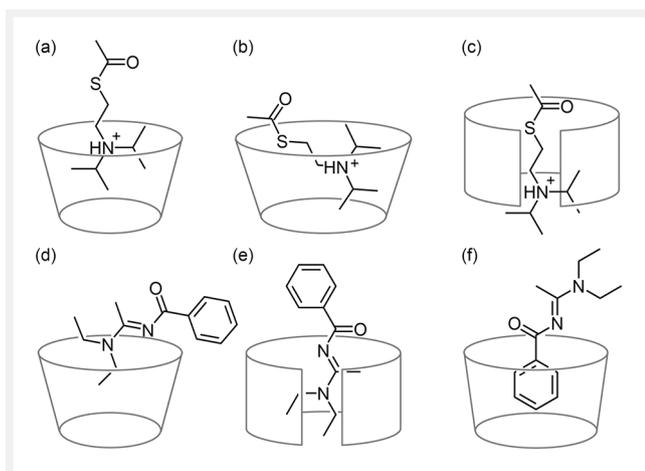
<sup>a</sup> c(**1**) = 2 mM, c(receptor) = 4 mM, solvent: 100 mM phosphate buffer in D<sub>2</sub>O (pD 7.4) containing 0.1 vol% CD<sub>3</sub>OD as an internal standard.



**Figure 1** Sections of the <sup>1</sup>H NMR spectra of **1** (2 mM) in the absence (a) and presence (b) of SC4 (4 mM) in 100 mM phosphate buffer in D<sub>2</sub>O at pD 7.4. The signals of **1** are assigned and the extents to which they shift specified. The asterisk marks the signal of CD<sub>3</sub>OD that was used as an internal standard.

In the case of aCB, the protons 3 and 4 were more strongly shielded than those at both ends of the molecule, suggesting that the cylindrical shape of this receptor allowed **1** to thread through the cavity with the end groups arranged at the two openings. This binding mode is consistent with that derived in earlier studies.<sup>8h</sup> To illustrate the preferred orientations of **1** in the cavities of SC4, SC5, and aCB, the NMR spectroscopy-derived complex geometries are schematically depicted in Figure 2.

Expectedly, the aliphatic nature of the three cyclodextrins resulted in much smaller effects on the proton resonances. In the case of  $\beta$ CD, a minor shielding of the protons of **1** was observed, but since the  $\beta$ CD signals did not move at all, an interaction was unlikely. The result was similar for SBE- $\beta$ CD, while S- $\beta$ CD caused the signals of **1** to move slightly downfield, suggesting that there could be an interaction. The overall extents to which the signals of **1** shifted in the presence of S- $\beta$ CD were small, however.



**Figure 2** Schematic representations of the preferred orientations of **1** in the cavities of SC4 (a), SC5 (b), and aCB (c), and of **2** in the cavities of SC4 (d), aCB (e), and  $\beta$ CD (f) according to the  $^1\text{H}$  NMR spectroscopic binding studies.

Analogous NMR spectroscopic studies were performed with **2**. In the presence of SC4, the signals of **2** moved upfield in the NMR spectrum, but not as strongly as those of **1** (Table 2). Importantly, the protons of the two ethyl groups were shielded to different degrees, indicating that the amidine moiety was only partially incorporated into the SC4 cavity in the complex (Figure 2d). The minor effects on the aromatic signals of **2** moreover suggested that the phenyl group remained outside the cavity.

The signal shifts induced by SC5 were only minor, likely because **2** only loosely interacted with this host. By contrast,

aCB had a pronounced effect on the spectrum of **2**, causing most signals to move upfield and to broaden. The strongest shielding was observed for the signals of the amidine moiety, but the effects extended to the aromatic signals. Of these signals, one moved upfield while the other two moved downfield, indicating that the phenyl group resided at the cavity opening with protons 5 and 6 arranged close to the carbonyl groups along the rim (Figure 2e).

The effects of the cyclodextrins on the  $^1\text{H}$  NMR spectrum of **2** were again much less pronounced. In this case, no evidence for complex formation was found for S- $\beta$ CD while the signal shifts observed in the presence of the other two cyclodextrins and, in the case of  $\beta$ CD, also the shielding of cyclodextrin protons located within the cavity accounted for an interaction (the SBE- $\beta$ CD signals were too broad to clearly detect complexation-induced shifts). The movement of the signals of protons of 4 and 5 in opposite directions, which was also observed in other cases,<sup>27</sup> suggested that complex formation likely involved the incorporation of the phenyl group of **2** into the cyclodextrin cavity (Figure 2f). This assumption was confirmed by a ROESY NMR spectrum, which showed crosspeaks between signals of the aromatic protons of **2** and those of the protons of the  $\beta$ CD glucose units residing inside the cyclodextrin cavity, while crosspeaks between these cyclodextrin signals and aliphatic protons of **2** were absent. The ROESY NMR spectrum also allowed deriving the preferred orientation of **2** in the cyclodextrin cavity (Figure 2f; see the Supporting Information).

The stability of the complexes between the different host/guest pairs was estimated quantitatively at 37 °C by using isothermal titration calorimetry (ITC). Stability constants were derived from the obtained binding isotherms by using the one-site binding model. For isotherms that had a sigmoidal shape (typically for complexes with a  $\log K_a > 3$ ), the stoichiometry factor  $n$  was fitted in the regression analyses. Values of ca. 1 were observed for  $n$  in these cases, indicating that 1 : 1 complexes were formed. If binding was weaker,  $n$  was fixed to 1 when performing the nonlinear regression analyses. The results of all measurements are summarized in Table 3.

Table 3 shows that SC4, SC5, and aCB exhibited a pronounced affinity for the cationic **1** at 37 °C in 100 mM phosphate buffer. In comparison with the  $\log K_a$  values previously obtained at 25 °C in a slightly less concentrated and more acidic buffer for the complexes of **1** with SC4 and aCB, binding was ca. 0.5 orders of magnitude weaker under the conditions used here, but still substantial.<sup>8h</sup> Complex formation of all of these receptors was exothermic and accompanied by adverse entropic terms, as frequently observed for binding processes in water.<sup>28</sup> This thermodynamic signature indicated that the release of cavity water was likely an important driving force of complex formation.<sup>29</sup> By contrast, the heat changes observed in the titrations with the three cyclodextrins were small and did not allow a reliable estimation

**Table 2** Complexation-induced shifts in ppm of the signals of **2** in the  $^1\text{H}$  NMR spectrum in the presence of different receptors<sup>a</sup>

H	$\Delta\delta$ (SC4)	$\Delta\delta$ (SC5)	$\Delta\delta$ (aCB)	$\Delta\delta$ ( $\beta$ CD)	$\Delta\delta$ (S- $\beta$ CD)	$\Delta\delta$ (SBE- $\beta$ CD)
1	-0.487/ -0.154	-0.073/ -0.102	-1.723/ -1.677	+0.014/ +0.014	+0.004/ +0.001	+0.034/ +0.018
2	-0.421/ -0.164	-0.136/ -0.095	-0.6/ -0.6 <sup>b</sup>	n. d./ +0.034	0/ +0.003	n. d./ n. d.
3	-0.106	-0.073	-0.707	+0.005	+0.002	+0.014
4	-0.009	-0.034	-0.144	+0.025	+0.002	+0.053
5	-0.007	-0.036	+0.327	-0.061	-0.001	-0.046
6	n. d. <sup>c</sup>	-0.032	+0.200	+0.001	+0.003	+0.014

<sup>a</sup>  $c(\mathbf{2}) = 2$  mM,  $c(\text{receptor}) = 4$  mM, solvent: 100 mM phosphate buffer in  $\text{D}_2\text{O}$  (pD 7.4) containing 0.1 vol%  $\text{CD}_3\text{OD}$  as an internal standard; <sup>b</sup> approximate shifts, signal overlap prevented an exact estimation; <sup>c</sup> n. d. = not detectable due to signal overlap.

**Table 3** Thermodynamic parameters of complexes of **1** and **2** with different receptors at 37 °C

Substrate	Receptor	log $K_a$	$\Delta H^\theta$ <sup>a</sup>	$T\Delta S^\theta$ <sup>a</sup>
<b>1</b>	SC4	3.77 ± 0.04 <sup>b</sup>	-33.7 ± 0.2	-11.3 ± 0.4
	SC5	3.69 ± 0.04	-32.5 ± 0.6	-10.6 ± 0.8
	aCB	4.61 ± 0.01	-36.0 ± 0.3	-8.7 ± 0.3
	$\beta$ CD		n. e. <sup>c</sup>	
	S- $\beta$ CD		n. e.	
	SBE- $\beta$ CD		n. e.	
<b>2</b>	SC4	2.09 ± 0.06	-54.2 ± 5.5	-41.8 ± 5.9
	SC5		n. e.	
	aCB	3.70 ± 0.04	-47.7 ± 2.2	-25.7 ± 2.4
	$\beta$ CD	2.45 ± 0.06	-17.6 ± 2.3	-3.1 ± 2.7
	S- $\beta$ CD		n. e.	
	SBE- $\beta$ CD	2.64 ± 0.06	-11.3 ± 1.7	+4.4 ± 2.0

<sup>a</sup> In kJ · mol<sup>-1</sup>; <sup>b</sup> the standard deviations were calculated from the results of three independent measurements; <sup>c</sup> n. e. = not estimated because too small heat changes prevented an accurate quantification of complex stability.

of binding constants. Although weak interactions could not be completely ruled out, also in light of the NMR spectroscopic results, ITC suggested that efficient scavengers for V-type nerve agents on the basis of cyclodextrins, even negatively charged ones, are unlikely to be accessible.

Assuming that the observed log  $K_a$  values are comparable to those of nerve agent complexes, the pronounced affinities of SC4, SC5, and aCB qualify all three receptors for use in scavenger development. Under the typical conditions of the assay established to determine the detoxification activity (500  $\mu$ M receptor and 10  $\mu$ M nerve agent),<sup>8d</sup> for example, 75% of the nerve agent should be complexed if the calixarenes are present, while the degree of complexation should even amount to 95% in the presence of aCB. Large fractions of the nerve agents are therefore bound by the receptors, ensuring their rapid detoxification when using appropriately functionalized derivatives. Not only complex stability controls scavenger activity, however, but also the orientation of the nerve agent within the receptor cavity. In this regard, the deep inclusion that likely occurs in the cavities of SC5 and aCB could be detrimental because a functional group at the cavity opening that should serve to mediate detoxification may not be able to reach the nerve agent. Preliminary work indeed demonstrated that functionalized aCB and SC5 derivatives detoxify VX much less efficiently than the known SC4 analog. The results of the binding studies therefore suggest that SC4 is likely the best candidate among the investigated receptors to develop scavengers for V-type nerve agents.

The Novichok analog **2**, on the other hand, only interacted with the cyclic cucurbituril to a significant degree. The lack of a positive charge at physiological pH obviously renders **2** unsuitable to form strong complexes with typical cation re-

ceptors such as SC4 or SC5. In the case of SC4, binding was observed, consistent with the <sup>1</sup>H NMR spectroscopic results, but complex stability was significantly smaller than that of the corresponding complex of **1**. It is important to note in this context that ITC is usually not well suited to quantify the stability of weakly stable complexes because the respective binding isotherms lack the characteristic sigmoidal shape. In particular, extrapolating the data to estimate the heat of complex formation can be inaccurate. The thermodynamic parameters obtained for the complex between SC4 and **2** therefore probably overestimate the actual enthalpy and entropy of complex formation. The signs are reliable, however, indicating again that formation is associated with a substantial solvent reorganization.<sup>29</sup>

The binding of **2** to SC5 and S- $\beta$ CD was too weak to allow a meaningful analysis of the data, but the other two cyclodextrins formed complexes that were slightly more stable than that of SC4. However, complex formation in these cases involved the incorporation of the phenyl residue rather than the amidine moiety of **2** into the cyclodextrin cavity according to the NMR spectroscopic results. As a consequence, the binding strength resembles that of  $\beta$ CD complexes with benzene derivatives.<sup>25</sup> The affinity of cyclodextrins for A-234 can therefore be expected to be not very pronounced, rendering only aCB a suitable basis for the development of Novichok scavengers among the receptors studied. The substantial affinity of aCB for **2** can be attributed to the fact that the complex formation of cucurbiturils is driven to a much larger extent by the release of cavity water than those of other receptors.<sup>29</sup> As a consequence, also neutral substrates can efficiently be bound. In case the deep inclusion of the nerve agent into the aCB cavity turns out to be disadvantageous, the search for Novichok scavengers should be directed at other receptors whose binding in water benefits from solvent effects. In this respect, cyclophane-based receptors including the naphthotubes studied by Jiang's group could be an option.<sup>14</sup>

## Conclusions

Binding studies involving model compounds for nerve agents and different receptors provided guidelines for the development of nerve agent scavengers. Unsurprisingly, scavengers for V-type nerve agents should be based on cation-binding receptors such as calixarenes and cucurbiturils, while cyclodextrins, even negatively ones, are much less suitable. Besides cation affinity, which should be sufficiently high to ensure a substantial degree of complexation (log  $K_a$  > 3 in buffered solution at 37 °C), a proper binding geometry is also important to ensure that the nerve agent and a nucleophilic group at the receptor opening are well preorganized for the reaction. In this respect, SC4 derivatives are

superior to aCB and SC5 derivatives because of a more suitable mode of binding.

With respect to Novichok nerve agents, only receptors that can efficiently complex neutral compounds in water are suitable for scavenger development. At the moment, aCBs are a promising option due to the pronounced affinity of aCB for the A-234 mimic **2** under the conditions of the detoxification assay. However, the binding mode might not be optimal, so that the binding studies will probably have to be extended to other receptor types in the future.

## Supporting Information

Supporting Information for this article is available online at <https://doi.org/10.1055/a-1939-6455>.

## Conflict of Interest

The authors declare no conflict of interest.

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- (20) The synthesis of **1** was performed as described elsewhere.<sup>8h</sup> M. p. = 114 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 11.69 (s, br, 1 H, NH), 3.70–3.59 (m, 2 H, iPrCH), 3.51–3.46 (m, 2 H, CH<sub>2</sub>S), 3.08–3.03 (m, 2 H, CH<sub>2</sub>N), 2.36 (s, 3 H, AcCH<sub>3</sub>), 1.57 (d, J = 6.7 Hz, 6 H, iPrCH<sub>3</sub>), 1.47 (d, J = 6.6 Hz, 6 H, iPrCH<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>): δ = 195.7, 54.8, 46.6, 30.6, 25.9, 18.7, 17.2 ppm; MS (ESI/TOF<sup>+</sup>) m/z (%): 204.1 (100) [M–Cl]<sup>+</sup>, 401.3 (14) [M<sub>2</sub>–CH<sub>3</sub>CO + H–Cl]<sup>+</sup>, 443.3 (59) [M<sub>2</sub>–Cl]<sup>+</sup>; Anal. Calcd for C<sub>10</sub>H<sub>22</sub>ClNOS: C, 50.09; H, 9.25; N, 5.84; S, 13.37. Found C, 49.90; H, 9.25; N, 5.82; S, 13.32.
- (21) *N,N*-Diethylacetimidamide was prepared as described.<sup>22</sup> This compound (0.50 g, 4.38 mmol) was dissolved in dichloromethane (10 mL). Triethylamine (8.76 mmol, 1.21 mL) was added dropwise at 0 °C. After 15 min, benzoyl chloride (4.82 mmol,

0.56 mL) was added, and the resulting mixture was stirred at room temperature for 48 h. The solution was washed with saturated aqueous NaHCO<sub>3</sub> (5 mL). The solvent was evaporated, and the residue was dissolved in water (20 mL). The solution was extracted with dichloromethane (5 × 50 mL), the combined organic layers were dried over MgSO<sub>4</sub>, and the solvent was removed. The residue was purified by column chromatography [SiO<sub>2</sub>, *n*-hexane/ethyl acetate, 1: 1 (v/v)]. Yield: 0.73 g (3.33 mmol, 76%), yellow oil; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.00–7.98 (m, 2 H, PhH<sup>2,6</sup>), 7.51–7.46 (m, 1 H, PhH<sup>4</sup>), 7.43–7.39 (m, 2 H, PhH<sup>3,5</sup>), 3.58 (q, <sup>3</sup>J = 7.0 Hz, 2 H, EtCH<sub>2</sub>), 3.44 (q, <sup>3</sup>J = 7.1 Hz, 2 H, EtCH<sub>2</sub>), 2.26 (s, 3 H, CH<sub>3</sub>), 1.18 (t, <sup>3</sup>J = 7.0 Hz, 6 H, EtCH<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 174.0, 164.4, 137.9, 131.1, 128.8, 127.9, 42.9, 42.5, 16.7, 13.7, 12.5 ppm; MS (ESI/TOF<sup>+</sup>) *m/z* (%): 219.14 (100) [M + H]<sup>+</sup>; Anal. Calcd for C<sub>13</sub>H<sub>18</sub>N<sub>2</sub>O: C, 71.53; H, 8.31; N, 12.83; found C, 71.80; H, 8.52; N, 12.64.

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