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PAPER

Optimized strategies to synthesize β-cyclodextrin-oxime conjugates as a new generation of organophosphate scavengers†

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A new generation of organophosphate (OP) scavengers was obtained by synthesis of β-cyclodextrin-oxime derivatives **8–12**. Selective monosubstitution of β-cyclodextrin was the main difficulty in order to access these compounds, because reaction onto the oligosaccharide was closely related to the nature of the incoming group. For this purpose, non-conventional activation conditions were also evaluated. Intermediates **5** and **7** were then obtained with the better yields under ultrasounds irradiation. Finally, the desired compounds **8–10** were obtained from **5–7** in high purity by desilylation using potassium fluoride. Quaternarisation of compounds **8** and **9** was carried out. OP hydrolytic activity of compounds **8–12** was evaluated against cyclosarin (GF) and VX. None of the tested compounds was active against VX, but these five cyclodextrin derivatives detoxified GF, and the most active scavengers **10** and **11** allowed an almost complete hydrolysis of GF within 10 min. Even more fascinating is the fact that compounds **9** and **10** were able to hydrolyze enantioselectively GF.

Introduction

Organophosphorus compounds (OP), such as pest control agents or chemical warfare nerve agents (Fig. 1) sarin, cyclosarin, soman, tabun (G-type nerve agents) or exceedingly toxic VX act as irreversible acetylcholinesterase (AChE) inhibitors.[1] This enzyme plays a key role in the central and peripheral cholinergic system and intoxication by OPs can cause life-threatening cholinergic signs and symptoms.[2] The development of new scavengers to prevent phosphylation of cholinesterases by these poisons is of paramount importance,[3] not only because poisoning by OP pest control agents is considered to cause more than 200,000 fatalities yearly,[4] but also since the Matsumoto and Tokyo subway strikes with sarin underlined the reality of a terrorist threat.[5] In order to gain access to efficient antidotes, it is necessary to develop compounds which will be able to detoxify OP compounds by rapid binding and hydrolysis. The purpose of this work is to design a new generation of scavengers exhibiting this activity. Recently, functionalized cyclo-maltoheptaose (β-CD) derivatives bearing a reactive moiety against OPs were developed as artificial enzymes.[6] This oligosaccharide can form inclusion complexes with various organic substrates, and in particular with organophosphorus poisons.[7] The OP, once "trapped" in the internal cavity of the β-CD can moreover be subjected to attack by a reactive group branched on one of the hydroxyl groups. The interest of this strategy is to take advantage of the OP binding into the oligosaccharide to force a reactive positioning of the OP towards a nucleophilic function. In order to effectively hydrolyse OPs to nontoxic metabolites, the nucleophile must specifically cleave the P-X bond (X = F, S, CN, N, depending on the nerve agent).

α-Nucleophilic groups have the ability to realize this cleavage, and oximes seem to be the most suitable.[8] Although pyridinaldoximes and pyridinium-aldoximes such as pralidoxime are

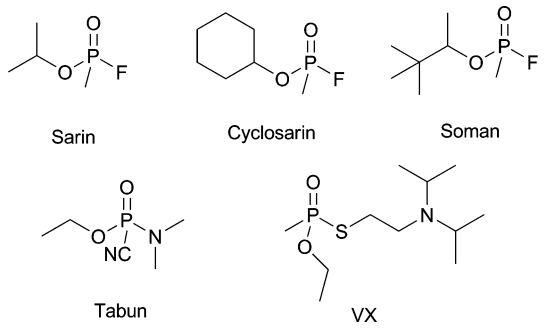

Fig. 1 Structures of chemical warfare agents.

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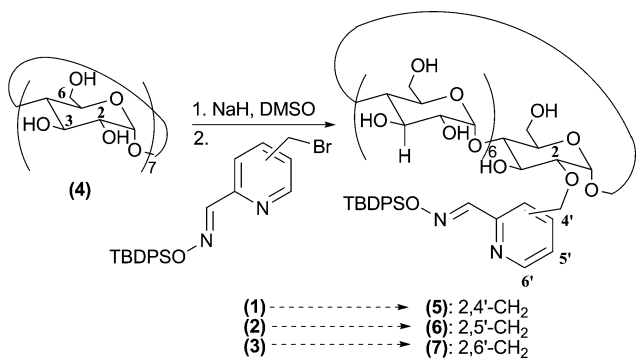
† Electronic supplementary information (ESI) available: Experimental procedures, NMR data, identification of regioisomers, NMR spectra for representative products. See DOI: 10.1039/c0ob00931h

mainly known for their effects in reactivating OP-inhibited acetylcholinesterases,[7] some data concerning a direct reaction of such oximes with nerve agents have already been published,[9] but the obtained maximal reaction velocities are too low to be applied for an *in vivo* detoxification.[10] Thus, introduction of a 2-pyridinaldoxime backbone on β-CD was also a challenge to access to a new generation of organophosphate scavengers. To modify the orientation of the nucleophilic group toward the internal oligosaccharidic cavity, the reactive group could also be grafted on the three distinct positions of β-CD, *i.e.* positions 2, 3 (upper rim of β-CD torus) and 6 (lower rim) of the glucose moieties. The secondary side of cyclodextrins is stated to be catalytically more efficient,[11,12] and therefore, modifications of this face are believed to produce valuable derivatives for enzyme mimics. If formation of an inclusion complex by the cavity of cyclodextrins played a very prominent role in determining the reaction site for the incoming group, the acidity of hydroxyl groups was also an important parameter. The basicity of hydroxyl groups are in the order 6 > 3 > 2. This feature has been exploited by using a strong base under anhydrous conditions for selective substitution at the position 2.

Results and discussion

To prepare these scavengers, the synthetic strategy consists in monosubstituting β-CD with a suitable bromomethyl pyridine derivative. The key step is then the regioselective monosubstitution of the oligosaccharidic moiety by the *tert*-butyldiphenylsilyl (TBDPS) protected 4, 5 or 6-bromomethyl pyridinaldoxime motives **1–3**.‡ The total substitution yields were deceptively low using the optimized monosubstitution method on the 2 position of the sugar we previously published in the case of benzyl halides,[13] because an unavoidable deprotection of the TBDPS group under these experimental conditions was observed, and led to poly-addition side products. These conditions, optimized for other benzyl bromides, had thus to be modified. Different coupling reactions between β-CD **4** and electrophiles **1–3** were carried out with dimethylsulfoxide as the solvent, and under basic conditions (Scheme 1).



Scheme 1 Coupling reactions using methods A–D.

The hydroxyl groups at the position 2 being the most acidic, sodium hydride has also been successfully used to selectively substitute β-CD.[14] This strong base was then tested here. In order to further improve the efficiency of the β-CD substitution, different activation processes were also evaluated (Methods A–D, Table 1), including ultrasounds or microwaves irradiation.[15,16] These non-conventional conditions already allowed a tosylation of one of the alcohols at O-6 or O-2 of CDs.[16] They were used here to directly introduce the desired functional group in the position 2 of one glucose unit of the native β-CD (Table 2).

In the case of compound **3**, heat or microwaves activation has no influence, but, sonication allowed for a substantial increase (+ 50%) of the substitution yield. The strong shockwave generated from the collapsing bubbles by sonication seemed to improve the substitution efficiency. Whatever the method A-D used, the 2-monosubstituted derivative **7** was the main isolated product, but a small amount of another regioisomer was also detected. High intensity ultrasounds irradiation led also to the best selectivity in favour of the monosubstitution against the polysubstitution observed as a side reaction. As already proved, this technique was also very advantageous in terms of reaction times for the synthesis of highly valuable CD derivatives.[16] In this case, the substitution time was considerably shortened down to three hours instead of eight hours with methods A and B. Altogether, for compound **3**, under ultrasounds activation, an acceptable 30% yield of O-2 monosubstitution product was cleanly isolated.

For compound **2**, the best isolated yield (29%) was obtained using sodium hydride in dimethylsulfoxide at room temperature. Microwaves also gave a satisfactory substitution yield of 25%. On the other hand, activation through an increase of the temperature to 70 °C led to a decrease to 7% yield. Sonication did not allow in this case a satisfactory result, because the substitution yield

Table 1 Experimental details of coupling methods between β-CD and electrophiles

Method	US[a]	MW[b]	Deprotonation step (time/temperature)	Substitution step (time/temperature)
A	—	—	14 h/r.t.	8 h/r.t.
B	—	—	14 h/70 °C	8 h/70 °C
C	+	—	1 h/r.t.	3 h/r.t. to 45 °C
D	—	+	5 min/70 °C	30 min/70 °C

[a] US = Ultrasounds; [b] MW = Microwaves

Table 2 Influence of method use on the substitution rates

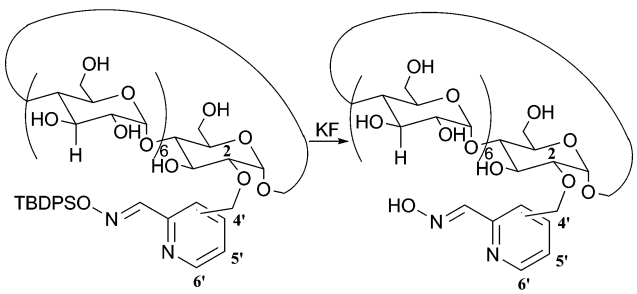
Reagent	Method	Total substitution yield (%)	Polysubstitution yield (%)	Monosubstitution yield at O-2 (%)
3	A	23	2	18
	B	24	3	18
	C	36	<1	30
	D	25	2	20
2	A	29	0	29
	B	7	0	7
	C	16	0	16
	D	25	0	25
1	A[a]	5	0	5
	A[b]	3	0	3
	C	14	0	14
	D	14	0	14

[a] The time of substitution was decreased to 90 min. [b] The time of substitution was decreased to 45 min.

‡ The corresponding procedures for the syntheses of compounds **1–3** are included in the ESI.†

reached only 16%. Contrary to what we observed with reagent **3**, the 2-modified β-CD **6** was the only isolated regioisomer. For electrophile **1** which proved to be a more unstable reagent compared to compounds **2** and **3**, standard methods only allowed isolation of the desired compound **5** with a particularly low yield, the best result leading to 5% monosubstitution yield by the method A. In this case, only activation through the use of ultrasounds or microwaves allowed an increase of the substitution yield to 14%. All these results showed that the monosubstitution reaction onto β-CD is a tricky and hardly predictable reaction, since the substitution pattern and global yield are closely related to the nature of the incoming group. In some cases, non conventional activations such as ultrasounds or microwaves irradiation were then interesting alternative methods to lead efficiently in a short time to the desired monosubstituted β-CD. Moreover, ultrasounds or microwaves did not affect significantly the regioselectivity, when a mixture of regioisomers was obtained by classical methods.

Identification of 2-monosubstituted β-CD regioisomers was carried out using a previously described methodology.[17,§] Once the protected β-CD-oximes were obtained in a sufficient yield, deprotection of compounds **5–7** was carried out using potassium fluoride to give cleanly the corresponding β-CD-oxime conjugates **8–10** (Scheme 2).

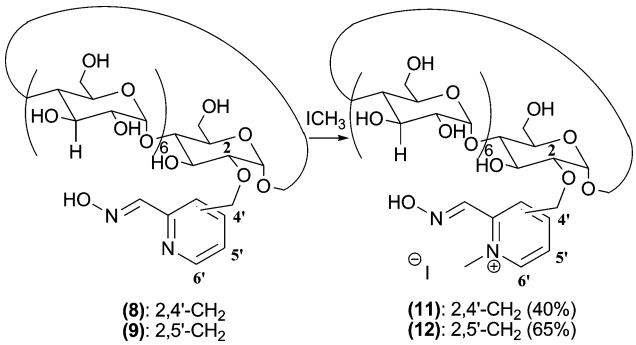


Scheme 2 Deprotection of the oxime function.

In order to increase the nucleophilic efficiency of the oximate function against organophosphorus compounds at a physiological pH, quaternarization of the pyridine nitrogen atom was also accomplished to access a nucleophilic reactive group similar to pralidoxime. Different experimental conditions tested with compound **10** by varying the amount of alkylating agent, temperatures and reaction times, did not allow the access to the corresponding quaternary derivative.

Even if alkylation was attempted with compound **3** itself before substitution by β-CD, it was not possible to introduce a methyl group on the nitrogen atom situated between two bulky groups. On the other hand, methylation of pyridinic nitrogen was successfully performed with a large excess of methyl iodide in DMF without trace of base from compound **8** and **9** to give respectively derivatives **11** and **12** (Scheme 3).

In a first set of experiments the ability of cyclodextrin derivatives to detoxify cyclosarin (GF) and VX was tested with a biological



Scheme 3 Quaternarization of the pyridine nitrogen atom in compounds **8** and **9**.

assay (remaining AChE cholinesterase activity after treatment with the nerve agent with or without pre-treatment with the scavenger).

The efficiency of **8–12** to detoxify nerve agents was tested with a single GF and VX concentration, *i.e.* 1 μM (Fig. 2). Hereby, these cyclodextrins (500 μM) were able to degrade GF in the order **10** = **11** > **9** > **8** > **12**. With **10** and **11** an almost complete GF detoxification was achieved within 10 min and the five tested cyclodextrins were able to degrade GF within 40 min completely. After a pre-treatment with free pyridine-2-aldoxime methiodide (pralidoxime) itself used as the same concentration than the scavengers **8–12**, no relevant degradation of GF was observed after 40 min.¶ These kinetic assays showed the interest to graft an oxime derivative on β-CD to improve GF-hydrolyzing efficiency. Based on the structure of compounds **8–10**, results proved that hydrolytic activity on GF was regiocontrolled. It had been already the case with β-CD-iodosobenzoate conjugates,[6a] but the fast detoxification of GF was obtained here when the linker between β-CD and the pyridinic moiety was in *meta*-position of the oxime function and just beside the cyclic nitrogen atom. Even if the linker was in *meta*-position of the oxime group, compound **8** showed the weakest activity. An intermediate activity was obtained with compound **9**, when the linker was in position 5′ of the aromatic ring. Although compound **10** was tested in a mixture with another regioisomer (respectively in an 80/20 ratio), hydrolytic assays seemed to indicate that the oxime function and nitrogen atom of

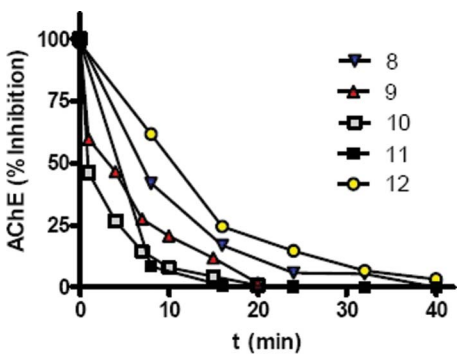


Fig. 2 Detoxification of cyclosarin (GF; 1 μM) by **8**, **9**, **10**, **11**, and **12** (500 μM). Data are given as means ± SD of two assays.

§ Accurate NMR structural determination of compound **9** is detailed in the Electronic Supplementary Information† as a specific and representative example.

¶ Kinetic course of the direct reaction of GF (1 μM) and 2-PAM (500 μM) is included in the ESI.†

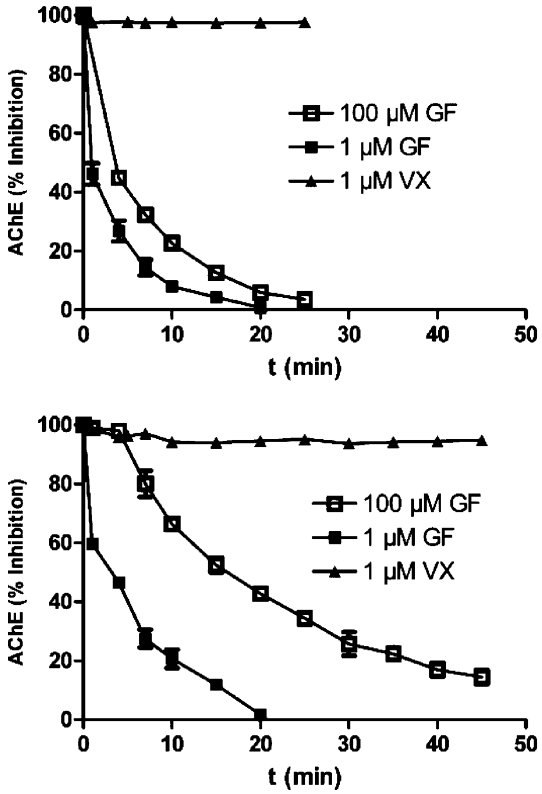


Fig. 3 Detoxification of cyclosarin (GF) and VX by **10** (top) and **9** (bottom). Data are given as means ± SD of two assays.

the ring should be both in the surroundings of GF the upper rim of β-CD. The higher activity obtained with compound **11** compared to those of compound **8** provided evidence that quaternarization of pyridinic nitrogen atom increased hydrolysis rate (through a decrease of the oxime pK_a). In the case of compound **12**, the decrease of the detoxification efficacy of GF was certainly due to the relative steric hindrance caused by the methyl group, which modifies the relative positioning of the oxime towards the nerve agent. None of the tested compounds had any detoxifying effect with VX (data shown in Fig. 4 in the case of **9** and **10**). This surprising result can be explained by a different (a non reactive) binding mode of VX in the β-CD as compared to GF.

In addition, at the given conditions, *i.e.* 500 µM cyclodextrins, **9** and **10** were able to detoxify GF in a concentration-dependent manner (Fig. 3). With both compounds 1 µM cyclosarin was totally detoxified within 20 min. The results obtained from the biological assay were further confirmed by a GC-MS quantitative analysis. The direct degradation of GF (100 µM) by **9** and **10** (500 µM) was then tested by a pH-stat procedure (Fig. 4). Incubation of GF with **9** or **10** resulted in a rapid degradation of the fluorophosphonate with a half-time of 15.9 and 20.9 min, respectively, and was faster than spontaneous hydrolysis of GF ($t_{\frac{1}{2}}$ = 74 min).

Finally, the stereoselective hydrolysis of cyclosarin (100 µM) by **9** and **10** (500 µM) was investigated by GC-MS. The results showed a moderate enantioselective hydrolysis of GF. With both compounds the degradation of the more toxic enantiomer (–)GF was faster (Fig. 5). However, the difference in the hydrolysis of both GF enantiomers was more pronounced with **10** (Table 3). As

Table 3 Kinetic constants (min^{-1}) for the hydrolysis of GF enantiomers by **9** and **10**

Compound	(–)GF	(+)GF
9	0.0656	0.0497
10	0.0925	0.0422

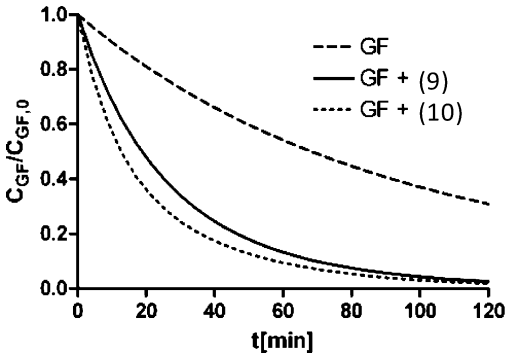


Fig. 4 Spontaneous, **9** and **10** catalyzed hydrolysis of cyclosarin.

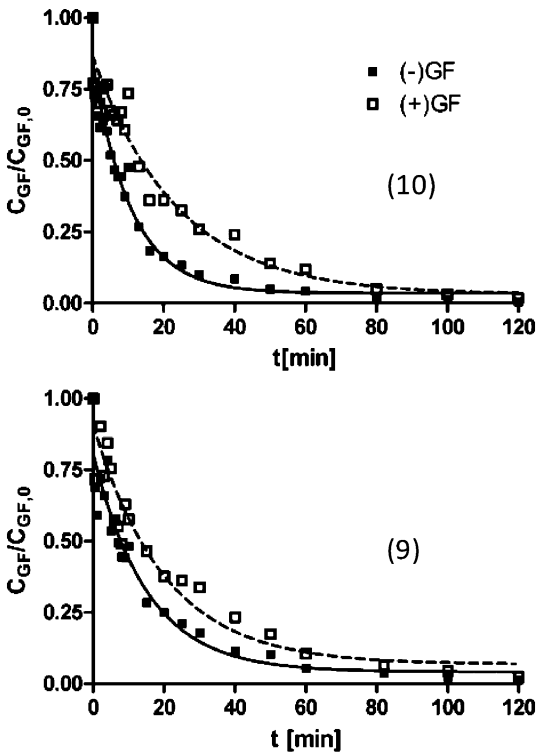


Fig. 5 Stereoselective hydrolysis of cyclosarin by **9** and **10**.

observed with native β-CD,[18] these results indicate an enantioselective effect of the investigated cyclodextrin derivatives which is reversed as compared with *in vivo* enzymatic degradation.[11]

In the design of OP scavengers, β-CD was chosen for its ability to bind a guest molecule of OP. The fact that we observed an enantioselective hydrolysis of cyclosarin by compounds **9** and **10** further proves that β-CD is involved in the hydrolysis phenomenon, and thus that β-CD acted then as a substrate binding site. It is generally assumed that the lipophilic part of the organophosphate molecule is taken up into the cyclodextrin central cavity.[19] Nevertheless, the

hydroxyl groups on the outer surface of the cyclic oligosaccharide are also able to form a complex with lipophilic compounds,[20] and a mixture of inclusion and non-inclusion complexes can coexist in aqueous solutions.[21] For this purpose, further experiments would be required to characterize the exact type of bonding between GF and the new β-CD-oxime conjugates, but such experiments are complicated by the fast kinetics of GF detoxification. They could only be undertaken with a stable analogue of GF under the conditions used for kinetic assays, thus not reflecting the precise GF complexation mode. Yet whatever the bonding process involved, a close interaction occurs, since β-CD induces an enantioselective effect, even if the substrate enantioselectivity is moderate. Further modifications of the β-CD scaffold will have to be investigated in order to improve the chiral recognition of the substrate.

Conclusions

Different β-CD derivatives monosubstituted by a 2-pyridinaldoxime moiety at O-2 were prepared. Selective monosubstitution reaction onto β-CD with functionalized and bulky aromatic groups was tricky and this key step needed refined strategies so as to obtain such compounds bearing different pyridinaldoximes on the upper rim of the oligosaccharidic torus. Functionalisation efficiency was closely related to the nature of the incoming group. The most important and pivotal factor was then the relative stability of the silyl protecting group of the electrophile. In this regard, the use of non conventional activation such as ultrasounds or microwaves irradiation constituted in some cases an interesting alternative to access the desired substitution at the O-2 position. All the synthezised oligosaccharidic compounds were able to detoxify the highly deadly chemical warfare agent GF, and hydrolysis rates of this OP were dependent on the position of the linker between β-CD and the reactive moiety. Even more fascinating is the fact that two scavengers were able to hydrolize enantioselectively GF. In the design of OP scavengers, β-CD has been chosen for its ability to bind a molecule of OP and further studies will be developed to explore structure–activity relationships.

Experimental

General

NMR spectra were recorded on either a Bruker Advance 300 instrument or a Bruker Avance DMX 500 spectrometer. Chemical shift (*d*) values are given in ppm and coupling constants (*J*) are given in Hertz. DMSO-d_6, H_2O-d_6 and $CDCl_3$ with an isotopic purity of 99.8% were purchased from Cambridge Isotope Laboratories Inc. Reactions were monitored by thin-layer chromatography (TLC) on a plate of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). Developed plates were visualised using ultraviolet light and/or by dipping the plates into a solution of malonic acid (1 g), aniline (1 mL) and orthophosphoric acid at 85% (3 mL) in ethanol (200 mL) followed by heating with a heat gun. Column chromatographies were performed on silica gel 60 (0.063–0.200 mm, E. Merck). MW-promoted reactions were carried out in a professional oven, CEM Discover. All sonochemical reactions were carried out with S120H reactor, Elmasonic. All solvents and reagents were purchased from commercial sources and used without further purification.

General procedures for substitution of β-cyclodextrin

Methods A and B. To a solution of β-cyclodextrin (0.5 g, 0.44 mmol, previously dried for 24 h under vacuum at 80 °C) in 2.5 mL of dry DMSO, was added NaH (60% in mineral oil, 18 mg, 0.44 mmol) and the mixture was stirred under argon for 14 h at room temperature (method **A**) or at 70 °C (method **B**). A solution of bromomethyl-2-pyridinealdoxime (**1–3**) (0.2 g, 0.44 mmol) in 1.5 mL of dry DMSO was then added and the reaction mixture was stirred for 8 h at room temperature (method **A**) and at 70 °C (method **B**). Acetone (250 mL) was then added and the crude product precipitated. After filtration, the solid residue was chromatographed on silica gel; solvent: ethyl acetate/isopropanol/water (12 : 7 : 4 to 8 : 7 : 4, v/v/v).

Methods C and D. To a solution of β-cyclodextrin (0.5 g, 0.44 mmol, previously dried for 24 h under vacuum at 80 °C) in 2.5 mL of dry DMSO, was added NaH (60% in mineral oil, 18 mg, 0.44 mmol) and the mixture was stirred under argon for 1 h at room temperature (method **C**) and for 5 min at 70 °C (method **D**). A solution of bromomethyl-2-pyridinealdoxime (**1–3**) (0.2 g, 0.44 mmol) in 1.5 mL of dry DMSO was then added and the reaction mixture was stirred for 3 h at room temperature (method **C**) and for 30 min at 70 °C (method **D**). Acetone (250 mL) was added and the crude product precipitated. After filtration, the solid residue was chromatographed on silica gel; solvent: ethyl acetate/isopropanol/water (12 : 7 : 4 to 8 : 7 : 4, v/v/v).

Mono-2-*O*-({2-[({[*tert*-butyldiphenylsilyl]oxy}imino)methyl] pyridin-4-yl}methyl)-cyclo-maltoheptaose 5. mp > 260 °C; IR (KBr) ν_{max}/cm^{-1} 3390 (OH), 2930 (CH), 1655 (CN); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.61 (d, *J* = 5 Hz, 1H; CH-Py), 8.56 (s, 1H, CH-aldoxime), 7.72–7.68 (m, 4H, CH-Ph), 7.64 (s, 1H, CH-Py), 7.54 (d, *J* = 5 Hz, 1H; CH-Py), 7.48–7.40 (m, 6H, CH-Ph), 5.97–5.66 (m, 13H, OH-2, OH-3), 5.05 (d, *J* = 3 Hz, 1H, H-1′), 4.85–4.80 (m, 8H, H-1, CH_2-Py), 4.52–4.45 (m, 7H, OH-6), 3.88 (t, *J* = 9 Hz, 1H, H-3′), 3.68–3.30 (m, 41H, 15H_2O), 1.11 (s, 9H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 155.6, 150.7, 149.8, 148.3, 135.0, 132.6, 130.0, 127.9, 122.9, 118.8, 101.9, 101.6, 99.8, 82.0, 81.5, 80.4, 73.0, 72.3, 72.0, 71.8, 71.0, 59.8, 26.8, 19.0; MS ES+: 1508 $(M+H)^+$, 1530 $(M+Na)^+$; HMRS calcd for $C_{65}H_{94}N_2O_{36}NaSi$: 1529.5253, found: 1529.5289.

Mono-2-*O*-({6-[({[*tert*-butyldiphenylsilyl]oxy}imino)methyl] pyridin-3-yl}methyl)-cyclo-maltoheptaose 6. mp > 260 °C; IR (KBr) ν_{max}/cm^{-1} 3400 (OH), 2930 (CH), 1655 (CN); ^{1}H NMR (300 MHz, (300 MHz, DMSO-d_6): δ = 8.65 (s, 1H, CH-Py), 8.56 (s, 1H, CH-aldoxime), 7.86 (d, 1H, *J* = 6 Hz, CH-Py), 7.72–7.65 (m, 5H, CH-Py, CH-Ph), 7.49–7.41 (m, 6H, CH-Ph), 5.97–5.67 (m, 13H, OH-2, OH-3), 5.02 (s, 1H, H-1′), 4.87–4.80 (m, 8H, CH_2-Py, H-1), 4.58–4.45 (s, 7H, OH-6), 3.85 (t, *J* = 9 Hz, 1H; H-3′), 3.70–3.25 (m, 41H, 14H_2O), 1.10 (s, 9H; CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 155.4, 150.2, 149.2, 136.6, 135.0, 132.6, 130.0, 128.9, 127.9, 120.4, 101.9, 101.6, 99.9, 82.0, 81.5, 80.1, 73.0, 72.3, 72.0, 71.7, 70.2, 59.8, 26.8, 18.9; MS ES+: 1508 $(M+H)^+$, 1530 $(M+Na)^+$; HMRS calcd for $C_{65}H_{94}N_2O_{36}NaSi$: 1529.5253, found: 1529.5295.

Mono-2-*O*-({6-[({[*tert*-butyldiphenylsilyl]oxy}imino)methyl] pyridine-2-yl}methyl)-cyclo-maltoheptaose 7. mp > 260 °C; IR (KBr) ν_{max}/cm^{-1} 3400 (OH), 2930 (CH), 1650 (CN); ^1H NMR (300 MHz, DMSO-d_6): δ = 8.54 (s, 1H; CH-aldoxime), 7.86 (t, 1H, J = 7.71 Hz, CH-Py), 7.71–7.61 (m, 4H, CH-Ph), 7.64–7.58 (m, 2H, CH-Py), 7.48–7.44 (m, 6H, CH-Ph), 5.97–5.70 (m, 13H, OH-2, OH-3), 5.12 (d, 1H, J = 3.00 Hz, H-1′), 4.91 (s, 2H, CH_2-Py), 4.86–4.82 (m, 6H, H_1), 4.68–4.48 (m, 7H, OH-6), 3.90 (t, 1H, J = 9 Hz, H-3′), 3.70–3.25 (m, 41H, 20H_2O), 1.10 ppm (s, 9H; CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 158.4, 155.4, 150.1, 137.8, 135.0, 132.6, 130.0, 127.9, 119.3, 101.9, 81.8, 81.5, 81.4, 81.3, 81.2, 80.5, 73.0, 72.3, 72.0, 71.8, 59.9, 26.86, 18.9; MS ES+: 1508 $(M+H)^+$, 1530 $(M+Na)^+$; HMRS calcd for $C_{65}H_{94}N_2O_{36}NaSi$: 1529.5253, found: 1529.5262.

General procedure for the preparation of compounds 8–10. To a solution of cyclodextrin derivative (**5–7**) (0.22 mg, 0.146 mmol) in 1.3 mL of water and 6.3 mL of methanol, was added $KF{\cdot}8H_2O$ (21 mg, 0.219 mmol). The reaction mixture was stirred for 12 h at room temperature. Methanol was distilled under reduced pressure. Water (5 mL) was added to the mixture. The solution was then washed with CH_2Cl_2 (3 × 50 mL) and the aqueous layer was concentrated to give the desired product.

Mono-2-*O*-({2-[(hydroxyimino)methyl]pyridin-4-yl}methyl)-cyclomaltoheptaose 8. mp > 260 °C; IR (KBr) ν_{max}/cm^{-1} 3300 (OH), 2930 (CH), 1655 (CN); ^1H NMR (300 MHz, DMSO-d_6): δ = 8.54 (d, 1H, J = 5 Hz, CH-Py), 8.07 (s, 1H, CH-aldoxime), 7.79 (s, 1H, CH-Py), 7.42 (d, 1H, J = 5 Hz, CH-Py), 6.05–5.60 (m, 13H; OH-2, OH-3), 5.06 (s, 1H, H-1′), 4.85–4.82 (m, 8H, H-1, CH_2-Py), 4.52–4.46 (m, 7H, OH-6), 3.88 (t, 1H, J = 9 Hz, H-3′), 3.60–3.25 (m, 41H, 6H_2O); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 152.0, 149.4, 148.9, 147.7, 122.2, 117.9, 101.8, 101.6, 99.7, 81.9, 81.5, 80.4, 73.0, 72.3, 71.9, 71.7, 71.1, 59.8; HMRS calcd for $C_{49}H_{76}N_2O_{36}Na$: 1291.4075, found: 1291.4070.

Mono-2-*O*-({6-[(hydroxyimino)methyl]pyridin-3-yl}methyl)-cyclomaltoheptaose 9. mp > 260 °C; IR (KBr) ν_{max}/cm^{-1} 3400 (OH), 2930 (CH), 1635 (CN); ^1H NMR (500 MHz, D_2O): δ = 8.49 (s, 1H, CH-Py), 8.14 (s, 1H, CH-aldoxime), 8.03 (d, 1H, J = 4 Hz, CH-Py), 7.85 (d, 1H, J = 4 Hz, CH-Py), 5.08 (d, 1H, J = 1 Hz, H-1′), 5.02–4.93 (m, 7H; H-1, CH_2-Py), 4.89 (d, 1H, J = 7 Hz, CH_2-Py), 4.04 (t, 1H, J = 5 Hz, H-3′), 3.85–3.50 (m, 40H), 3.40 (d, 1H, J = 6 Hz, H-2′); ^{13}C NMR (125.75 MHz, D_2O): δ = 151.5, 149.4 (CH-aldoxime), 148.2 (CH-Py), 137.3 (CH-Py), 134.0, 120.6 (CH-Py), 102.1–101.6 (6C, C-1), 100.2 (C'-1), 81.6–80.6 (7C, C-4), 78.9 (C'-2), 73.3–73.2 (6C, C-3), 72.8 (C′-3), 72.1–71.7 (13C, C-2,C-5), 69.9 (CH_2-Py), 60.4–60.0 (7C, C-6); HMRS calcd for $C_{49}H_{76}N_2O_{36}Na$: 1291.4075, found: 1291.4069.

Mono-2-*O*-({6-[(hydroxyimino)methyl]pyridin-2-yl}methyl)-cyclomaltoheptaose 10. mp > 260 °C; IR (KBr) ν_{max}/cm^{-1} 3400 (OH), 2930 (CH), 1635(CN); ^1H NMR (300 MHz, DMSO-d_6): δ = 11.68 (s, 1H; OH-aldoxime), 8.09 (s, 1H, CH-aldoxime), 7.86 (t, 1H, J = 8 Hz, CH-Py), 7.70 (d, 1H, J = 8 Hz, CH-Py), 7.54 (d, 1H; J = 8 Hz, CH-Py), 5.99–5.68 (m, 13H; OH-2, OH-3), 5.09 (d, 1H, J = 3 Hz, H-1′), 4.88 (s, 2H, CH_2-Py), 4.82 (s, 6H, H-1), 4.49–4.47 (m, 7H, OH-6), 3.89 (t, J = 9 Hz, 1H; H-3′), 3.70–3.22 (m, 41H, 7H_2O); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 157.8, 151.4, 148.7, 137.5, 121.6, 118.5, 101.8, 101.5, 100.0, 81.8, 81.5, 81.4, 80.4, 73.4, 73.0, 72.9, 72.4, 72.0, 71.8, 71.7, 59.9; HMRS calcd for $C_{49}H_{76}N_2O_{36}Na$: 1291.4075, found: 1291.4049.

General procedure for the preparation of compounds 11 and 12. To a solution of cyclodextrin derivative **8** or **9** (0.201 mg, 0.158 mmol) in 1.8 mL of dry DMF, was added iodomethane (0.39 mL, 6.32 mmol). The mixture was stirred for 14 h at 50 °C. After cooling to room temperature, the product was precipitated with acetone (30 mL) and filtered to obtain the desired compound as a powder.

Mono-2-*O*-({2-[(hydroxyimino)mehtyl]-1-methylpyridinium-4-yl}methyl)-cyclomalto heptaose iodide 11. IR (KBr) ν_{max}/cm^{-1} 3370 (OH), 2930 (CH), 1655 (CN); ^1H NMR (300 MHz, DMSO-d_6): δ = 8.92 (d, 1H, J = 6 Hz, CH-Py), 8.66 (s, 1H, CH-aldoxime), 8.35 (s, 1H, CH-Py), 8.08 (d, 1H, J = 6 Hz, CH-Py), 5.98–5.66 (m, 13H; OH-2, OH-3), 5.17–5.05 (m, 3H, H-1′, CH_2-Py), 4.85–4.82 (m, 6H, H-1), 4.50–4.45 (m, 7H, OH-6), 4.33 (s, 3H, CH_3), 3.90 (t, 1H, J = 9 Hz, H-3′), 3.60–3.25 (m, 41H, 23H_2O); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 158.0, 146.7, 146.1, 141.7, 124.2, 121.6, 101.9, 101.5, 99.2, 81.5, 81.0, 80.8, 73.0, 72.2, 72.0, 71.6, 69.7, 59.9, 45.8; MS ES+: 1284 $(M)^+$; HMRS calcd for $C_{50}H_{79}N_2O_{36}$: 1283.4413, found: 1283.4425.

Mono-2-*O*-({6-[(hydroxyimino)mehtyl]-1-methylpyridinium-3-yl}methyl)-cyclomalto-heptaose iodide 12. IR (KBr) ν_{max}/cm^{-1} 3380 (OH), 2930 (CH), 1655 (CN); ^1H NMR (300 MHz, DMSO-d_6): δ = 9.06 (s, 1H, CH-Py), 8.66 (s, 1H, CH-aldoxime), 8.47 (d, 1H, J = 8 Hz, CH-Py), 8.39 (d, 1H, J = 8 Hz, CH-Py), 5.99–5.55 (m, 13H; OH-2, OH-3), 5.13 (s, 1H, H-1′), 4.97 (s, 2H, CH_2-Py), 4.85–4.82 (m, 6H, H-1), 4.66–4.47 (m, 7H, OH-6), 4.34 (s, 3H, CH_3), 3.89 (t, 1H, J = 8 Hz, H-3′), 3.65–3.30 (m, 41H, 8H_2O); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 146.3, 144.9, 143.2, 141.6, 138.6, 124.3, 101.9, 101.4, 99.3, 81.6, 81.5, 81.2, 80.9, 80.4, 79.4, 73.0, 72.3, 72.0, 71.6, 68.3, 59.8, 46.3; MS ES+: 1284 $(M)^+$; HMRS calcd for $C_{50}H_{79}N_2O_{36}$: 1283.4413, found: 1283.4376.

Procedure for evaluation of the detoxification of cyclosarin and VX

Cyclosarin (GF) and VX (1 or 100 μM) were incubated with cyclodextrin derivates (500 μM) in TRIS HCl (0.1 M) buffer at pH 7.4 and 37 °C for up to 50 min. At different time points samples were taken and diluted to a nerve agent concentration of 500 nM with TRIS buffer. Afterwards, 10 μl of the solution were incubated with 90 μl AChE (erythrocyte ghosts) for 3 min at 37 °C. Then, a 10 μl aliquot was taken and the AChE activity was measured spectrophotometrically (412 nm) with a modified Ellman assay[22] to determine the residual AChE activity. The assay mixture (3.16 ml) contained 0.45 mM of acetylthiocholine iodide (ATCh) as substrate and 0.3 mM of 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) as chromogen in 0.1 M tris-(hydroxymethyl)aminomethane (TRIS) HCl (pH 7.4). AChE activities were referred to control activity and data are given as % of control. Data were corrected for spontaneous degradation of GF and VX.

General procedure for kinetic evaluation of cyclosarin hydrolysis by compounds 9 and 10

The hydrolysis of GF (100 μM) by compounds **9** and **10** (500 μM) was initially tested with pH-Stat using 155 mM NaCl at pH 7.4

and 37 °C. For the determination of stereoselective kinetics of cyclosarin hydrolysis by these cyclodextrin derivatives TRIS buffer (0.1 M) was used as incubation medium (pH 7.4 and 37 °C). GF enantiomers were determined by chiral GC-MS as described previously with minor changes.[23]

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