New 2,3-heterodisubstituted β-cyclodextrin derivatives were designed as artificial enzymes to degrade chemical warfare agents. One of them reduced the acetylcholinesterase inhibitory potential by soman faster than its monosubstituted analog.

Organophosphorus nerve agents, such as chemical warfare agents and the most powerful pesticides, are irreversible inhibitors of acetylcholinesterases, enzymes involved in the cholinergic neurotransmission. Their chemical synthesis being quite easy, these molecules are potentially attractive for terrorist groups. This potential use poses also a threat in the event of asymmetric war. Moreover, as stockpiles of nerve agents exists in many countries, waiting to be destroyed, and as developing countries still use organophosphorus pesticides and insecticides, casual exposure can occur. In the case of a chemical attack, one of the main actions is to prevent the agent dissemination and to protect rescue people to avoid over-contamination risk. A fast hydrolysis of the toxic under physiological conditions is thus required but no mild decontamination conditions have been developed. The actual strategy is to design biocompatible scavengers able to trap and degrade nerve agents. Cycloextrin derivatives are attractive compounds for decontamination of skin, mucosa, and wounds, but also for sensitive material treatment. Moreover, β-cyclodextrin and its derivatives have already been used to functionalize polymer nanofiber membranes against paroxon. Decontaminant wipes are thus conceivable. Various cyclodextrin derivatives were recently reported with encouraging activity against cyclosarin, sarin, tabun, and soman. Herein, we describe sophisticated scavengers bearing two different substituents able to cooperate to the nerve agent degradation. These substituents were introduced on the secondary side of a methylated β-cyclodextrin scaffold, which is stated to be catalytically more efficient. Moreover, soman reaction with a secondary hydroxy group of β-cyclodextrin has been already described, and modifications of the secondary face are believed to produce valuable enzyme mimics against chemical warfare.

To our knowledge, β-cyclodextrin derivatives bearing two different groups on secondary face and designed as an artificial enzymes have never been described. Introduction of iodosobenzoate and imidazole groups as α-nucleophile and proton acceptor respectively could create an acid/base dyad (Figure 1) to enhance the nucleophilic attack onto the toxic agent. We could thus mimic the active site of enzymes through a concerted hydrolytic mechanism as encountered in the catalytic triads of some hydrolases. In the present study, we showed that a synergistic effect of these groups improves the hydrolysis of soman.

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Figure 1 Schematic representation of the proposed acid/base dyad.

Novel methodologies to obtain regioselectively polysubstituted cyclodextrins were recently developed. Such approaches rely on the use of DIBAL-H to produce O-deprotected key intermediates. Thus, diol 1 (Figure 2) was obtained by a regioselective bis-de-O-methylation of permethylated β-cyclodextrin. Compound 1 is then an excellent starting material to develop new scavengers having a hydrolytic activity towards organophosphorus nerve agents. The relative positioning of the substituents on the macrocyclic structure is essential regarding the hydrolysis efficiency of organophosphorus substrates. Our attention was foremost focused on the selective introduction of the α-nucleophile and imidazole groups on the two free positions of diol 1 to access to the two scavengers 4 and 5 (Figure 2) by playing with the different reactivities of the secondary alcohols in positions 2 and 3 of β-cyclodextrin. Although the hydroxyl at O-2 is more acidic than the hydroxyl at O-3, the substitution reactions of diol 1 are hardly predictable. Only the direct regioselective substitution at O-2 by the imidazole group followed by the
introduction of the benzylic moiety at O-3 were successfully performed to give compound 3.† To access to the target molecule 2, a synthetic strategy involving tedious protection / deprotection steps was implemented (Scheme 1). The substitution at O-3 of benzylated compound 6† by the imidazole moiety led to derivative 7. The subsequent catalytic hydrogenation reaction removed both benzy1 and trityl groups, and reprotection of the imidazole was necessary before the benzylation step toward 2. Compounds 2 and 3 were finally treated with an excess of sodium periodate to give the scavengers 4 and 5 in one step.†

![Scheme 1 Synthetic pathway of compound 2.](image)

Reagents and conditions: i) 1. NaH, DMSO, Ar; 2. 4-(chloromethyl)-1-trityl-1H-imidazole. ii) 1. Pd/C (10%), H₂, P₄O₁₀, CH₃OH; 2. NEt₃, triphenylmethyl chloride, DMF, Ar. iii) 1. NaH, DMSO, Ar; 2. Methyl 5-(bromomethyl)-2-iodobenzoate.

Identification of difunctionalized regioisomers was carried out for compounds 2 and 3.† High resolution mass spectrometry (ESI-HRMS) experiments provided evidence that both compounds were disubstituted by iodobenzoate and imidazole moieties. Further comparative studies of the MS/MS spectra showed distinct fragments intensities proving the different locations of these groups on 2 and 3. The relative locations of the two groups was highlighted by 2D NMR experiments performed on compound 3. A first HSQC experiment was carried out to identify the signals of the two CH₂ groups bearing the aromatic substituents. Firstly, the two methylenic protons (4.62 ppm and 5.13 ppm) of the iodobenzoate moiety correlates with the carbon signal at 74.5 ppm. A second correlation between the methylenic protons connected to the imidazole (4.51-5.46 ppm) and the signal at 66.8 ppm was then observed. In a further step, the 3-O substitution by the benzylic group was proved by the presence of a HMBC correlation between one of the benzylic protons signal (4.62 ppm) and the carbon signal C-3 (80.0 ppm) of the glucose unit A. The location of the imidazole part was revealed by the correlation between the methylenic protons signal (4.51-4.56 ppm) and the carbon signal C-2 (79.6 ppm) of the unit B. This was further confirmed by the correlation observed between the same proton signal and the H-2 signal of the unit B in a NOESY experiment.

The degradation properties of soman (GD, Figure 3) by the new compounds 4 and 5 were next investigated by ¹H NMR experiment (Figure 4). An efficient degradation of the neurotoxic agent was observed with compound 5 while 4 and 2-iodosobenzoic acid share similar level of activity. Indeed after 15 min, compound 4 and 2-iodosobenzoic acid show a 50% degradation of GD, this level reaching 60% in presence of 5. This result can be explained by an unsuitable positioning of the imidazole and iodobenzoate groups in compound 4. Compound 5 being more potent than 4, the relative adjacent positions of the substituents on the cyclodextrin ring have obviously a determinant effect on the efficiency of the GD degradation. In the present study, the macrocyclic moiety interacts with the nerve agent contrary to the observations made in the case of cyclosarin (GF) hydrolysis by cyclodextrin derivatives bearing a hydroxamic acid substituent.²⁸ Permethylated β-cyclodextrin by itself has no effect on the degradation rate of GD (Figure 4), but it allows a stereoselective interaction with the chiral nerve agent (Figure 5). Thus, the cyclodextrin moiety in compound 5 caused a stereoselective
degradation of GD (Figure 5). This result is to be considered as the levo-rotatory isomers of GD are the most toxic.\textsuperscript{15} Compound 5 could lead to a more or less important decrease of the toxicity of the nerve agent depending on the preference for degradation of some of the stereoisomers of GD.

![Figure 4: Degradation curves of soman (GD).](image)

The degradation curves of soman at 0.4 mM in phosphate buffer (0.1 M) were obtained at pH 7 and 25°C by \textsuperscript{1}H NMR experiments. The monitoring was performed by integration of the residual soman signal (PCH\textsubscript{2}) between 1.79 and 1.83 ppm.

![Figure 5: 1\textsuperscript{H} NMR tert-butyl signal of soman (GD) at 0.4 mM in phosphate buffer 0.1 M (a), in phosphate buffer 0.1 M with 2-iodosobenzoic acid at 2 mM (b), in phosphate buffer 0.1 M with permethylated \(\beta\)-cyclodextrin at 2 mM (c), in phosphate buffer 0.1 M with compound 5 at 2 mM (d).](image)

The increased activity observed on introducing an imidazole group in the neighboring of the iodosobenzoate moiety is clearly shown comparing the degradation curves of GD (Figure 4) by scavenger 5 and its homologous permethylated structure \textsuperscript{9}f bearing only an iodosobenzoate substituent at O-3 (Figure 3). Indeed, the half-life of GD in the presence of cyclodextrin derivative 5 is 9.7 min. against 15.1 min. during the hydrolysis process with compound 9. The almost complete degradation of GD occurred after 1 h 30 min in both cases, the main degradation product formed being pinacolyl methylphosphonic acid as already observed in phosphate buffer.\textsuperscript{16}

The acetylcholinesterase inhibitory potential by GD in the presence of the different scavengers 4, 5 and 9 was investigated in a second step by an enzymatic assay (Figure 6). This test allowed the determination of the true protective efficiency of compounds against soman, the toxicity of nerve agent being non-linear concentration-dependent. The inhibitory effect of soman was decreased to 37.5% after 30 min in the presence of derivative 5 while it was higher than 90% in the presence of scavenger 4 or 2-iodosobenzoic acid. Compound 5 is thus more potent to reduce GD acetylcholinesterase inhibitory potential compared to the other molecules tested. Despite its weaker ability to degrade GD, compound 9 allowed a significant protective effect against GD, its inhibitory effect being reduced to 62.5% after 30 min.

![Figure 6: Acetylcholinesterase inhibitory potential by soman (GD). Soman (1 mM) was incubated with 2-iodosobenzoic acid (500 \(\mu\)M) or cyclodextrin derivatives 4, 5 or 9 (500 \(\mu\)M) in phosphate buffer (0.1 M) at pH 7 and 37°C. Data are means ± SD of 2 experiments.](image)

This result can be explained by a better trapping of GD by compound 9 than derivative 4, although compound 9 is less efficient to prevent the toxicity of the nerve agent than derivative 5. The new heterodifunctionalized compound 5 is then the most active scavenger against GD.

Considering all the data, these results confirmed the main influence of cyclodextrin scaffold in the protective process against GD due to its host properties to trap the nerve agent, and they showed for the first time the synergistic effects of \(\alpha\)-nucleophile and imidazole groups introduced on adjacent units of the macrocycle in order to degrade GD.

**Conclusions**

In conclusion, we have shown that it was possible to implement an OPasic cooperative process by introducing two different groups on adjacent units of a permethylated \(\beta\)-cyclodextrin scaffold. This novel strategy allowed an access to a more potent scavenger against GD. Studies to elucidate the underlying mechanisms are ongoing, and further derivatives will be prepared to obtain structure-activity relationships. Furthermore, the cyclodextrin ring affords a cooperative contribution to the mode of action by a stereoselective interaction with the nerve agent. In this regard, a faster reduction of AChE inhibition has been obtained in the case of monosubstituted \(\beta\)-cyclodextrin derivatives versus their monosubstituted methylated counterparts. The development of new artificial enzymes based on heterodifunctionalized \(\beta\)-cyclodextrin derivatives appears as a very attractive approach. Nevertheless, only a few reactions leading to difunctionalized \(\beta\)-cyclodextrin derivatives are described. Thus the development on new methodologies to introduce different substituents on the secondary face in a selective fashion is highly desirable.
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Notes and references