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Functionalized cyclodextrins bearing an alpha nucleophile – A promising way to degrade nerve agents

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ABSTRACT

Organophosphorus nerve agents are irreversible inhibitors of acetylcholinesterase. Current treatment of nerve agent poisoning has limited efficacy and more efficient medical countermeasures need to be developed. A promising approach is to design chemical scavengers more stable during storage and less immunogenic than bioscavengers. Furthermore, they could be produced at lowest production costs. Cyclodextrins are attractive cyclic oligosaccharides that can be used to develop chemical scavengers of organophosphorus nerve agents. Their abilities to form inclusion and non-inclusion complexes with organic substrates are useful to trap chemical warfare agents. Selective introduction of an α-nucleophile residue on the secondary face of β-cyclodextrin allowed to obtain supramolecular derivatives active against organophosphorus compounds. The degradation activity of these monosubstituted cyclodextrins was determined against paraoxon and chemical warfare agents. These tests showed that the structure of the scavengers mainly influences the interaction between the organophosphorus substrate, or its reaction products, and the cyclodextrin moiety. All the tested G-type agents were efficiently degraded. According to the binding modes of cyclosarin, some oligosaccharidic scavengers led to an enantioselective degradation of this nerve agent. These promising derivatives open the way to further investigations of new structural modifications to reach more sophisticated and efficient scavengers for prophylactic and curative medical applications.

1. Introduction

Highly toxic organophosphorus (OP) compound-based chemical warfare agents are irreversible inhibitors of acetylcholinesterase, a key enzyme in the cholinergic neurotransmis-sion [1]. Despite the signature of the Chemical Weapons Convention in 1993, large stockpiles of OP nerve agents still exist in several countries. In addition, synthesis routes of nerve agents can be found in the open literature and these compounds have been used by terrorists [2,3]. Moreover, accidental or intentional exposure of OP pesticides is a major toxicological problem, especially in developing countries [4–6]. Different strategies have already been implemented to protect civilians and particularly military personnel against the toxic effects of nerve agent exposure, but more efficient medical countermeasures need to be developed. The most recent approach consists in using enzymes [7,8] as biopharmaceutical means for preventing the toxicity of nerve agents and OP pesticides. The first generation bioscavenger, human butyrylcholinesterase (BChE) stoichiometrically binds OP agents and may prevent the distribution to target tissues [9]. Unfortunately, inadequate bio-stability, need to administer large amounts of enzyme, lack of large scale production and high production costs are major disadvantages of human BChE. To significantly avoid or reduce all these shortcomings, our project is focused on chemical scavengers able to neutralize OP molecules before they reach cholinesterases. This approach has several advantages in comparison to the bioscavenger strategy by identifying new effective and biocompatible detoxification means for use in nerve agent and pesticide poisoning. In the end, chemical scavengers may lead to various prophylactic or curative biomedical applications.

In order to build biomimetic structures, cyclodextrins are extremely interesting scaffolds and present several advantages in preparing active scavengers [10]. Cyclodextrins are cyclic oligosaccharides

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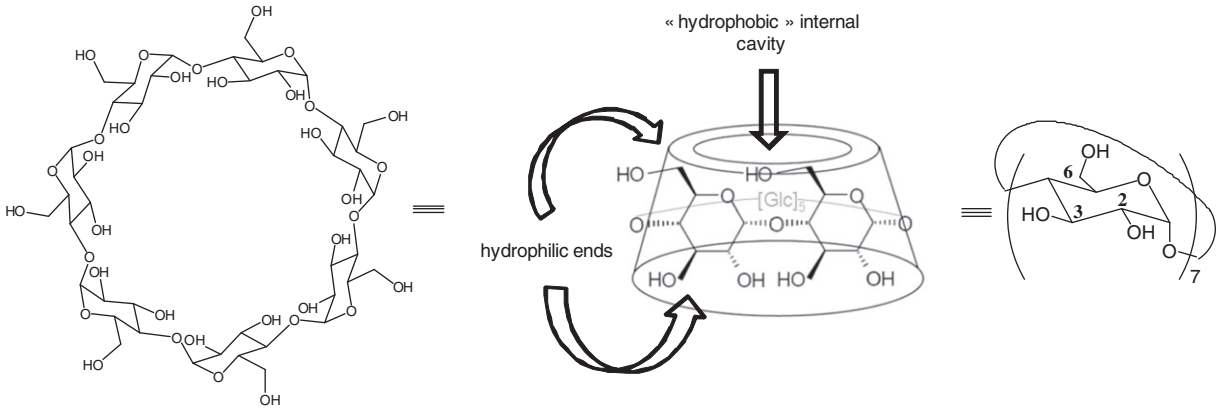

Fig. 1. Toroïdal structure of β-CD.

containing 6, 7 or 8 glucose units and they called α-, β- and γ-cyclodextrin, respectively. Each glucopyranose ring contains secondary hydroxyl groups at the 2- and 3-positions and primary hydroxyl groups at the 6-position. These hydroxyl groups induce a hydrophilic property to the upper and lower end of the molecule. By opposition, the internal cavity of cyclodextrins is rendered less hydrophilic, and it is so-called "hydrophobic cavity" (Fig. 1). This specificity is then useful to trap small organic molecules such as organophosphorus compounds. In this process, cyclodextrin is the "host" molecule admitting within its cavity "guest" molecules. Interactions between the host and the guest molecules are weak and the binding step is reversible. Moreover, cyclodextrins can also form non-inclusion complexes with other molecules by hydrogen bonds via the hydroxyl groups on the outer surface of the torus [11–13]. First tests with β-cyclodextrin (β-CD) and chemical warfare agents showed weak detoxifying effects with sarin and soman but no effect with the more stable tabun and VX [14,15]. β-CD acted obviously as a mimic enzyme to hydrolyze P–F bonds. For this purpose, the internal cavity of β-CD has better size compatibility with nerve agents compared to the two other natural α- and γ-CD [14]. Moreover, ß-CD is available in bulk quantities from commercial sources at low cost. Hence, β-CD is a suitable scaffold to develop more effective chemical scavengers.

In order to reinforce the detoxification activity and to extend the detoxification spectrum of this oligosaccharide, the presence of secondary hydroxyl groups located on the most open face of the cyclodextrin torus can be exploited for introducing functional chemical groups active against nerve agents. We decided to monofunctionalize one secondary alcohol function since pesticides such as paraoxon or parathion were included in the cyclodextrin cavity with the phosphorus end near the secondary face [16,17]. This strategy had two interests, firstly to trap the organophosphorus substrate in the inner and/or the outer of the oligosaccharide cavity and secondly to force a suitable positioning of the nerve agent towards the reactive function. For this purpose, we grafted on β-CD two different kinds of α-nucleophiles, i.e., 2-iodosobenzoate and 2-pyridinyl-aldoximate derivatives.

2. Development of β-CD derivatives as chemical scavengers

In order to access chemical scavengers, the synthetic strategy was then to selectively introduce an α-nucleophile on a secondary alcohol of β-CD through a specific linker. To study the influence of the orientation of the nucleophilic group toward the internal oligosaccharidic cavity, the linker could be grafted on various positions of the aromatic ring of the α-nucleophile. Several precursors of α-nucleophile groups were then prepared (Fig. 2).

In order to extend the possibilities of the reactive group positioning, all these α-nucleophile precursors could also be grafted on the two distinct positions of β-CD, i.e., positions 2 and 3 (upper rim of β-CD torus) of the glucose moieties. For this reason, selective mono substitution methodologies of the secondary face of β-CD were developed.

2.1. Selective grafting of the precursors on the oligosaccharidic moiety

It is well known that hydroxyl groups in position 6 of β-CD are the most nucleophilic, whereas the secondary alcohol functions are the most acidic [18]. This feature has been exploited by using a strong base for selective substitution at O-2 and O-3 playing with the respective acidity and accessibility of both hydroxyls [19,20].

Various base/solvent systems under anhydrous conditions were then tested to introduce benzyl derivatives at O-2 (Fig. 2) [21]. Only a few aprotic polar solvents such as dimethylformamide (DMF) or dimethylsulfoxide (DMSO) could be used owing to the weak solubility of native β-CD in organic media. In a general manner, the efficiency of the nucleophilic substitution was better in DMSO than in DMF due to its higher polarity. Nevertheless, the nature of the base dramatically influenced the yield and the regioselectivity of the substitution. Moreover, this tricky and hardly predictable reaction was function of the structure of the incoming group; its consecutive ability to form an inclusion complex with the cavity of the cyclodextrin but also the relative stability of the protected electrophile itself during the substitution process [22,23]. Thus, the use of non conventional activation conditions such as ultrasounds or microwaves irradiation constituted an interesting alternative to reduce the reaction time and to allow the mono functionalization at O-2 position in the case of compound **6** (Fig. 2) despite the relative instability of the silyl group [23]. Taking into account all these parameters, all the electrophiles **1**–**7** (Fig. 2) were grafted at the 2-position in satisfactory yields in this series and without a previous activation of this alcohol or/and a specific protection of primary hydroxyls groups [22,23].

Alcohols at position 3 being the least reactive hydroxyl groups in cyclodextrins, they are the most difficult to regioselectively substitute and only a few examples of 3-modified cyclodextrins have been published [24–27]. We developed a new methodology based on a temporary metal complexation which led to the modification of the reactivity of these alcohol functions in producing a distortion of the oligosaccharide cavity by a diagonal link between copper ions and oxygen atoms (at C-2 and C-3) on adjacent glucopyranose units [28]. By this process, we successfully and regioselectively grafted benzylic and allylic compounds as electrophilic reagents at O-3. Using this approach, it was then possible to mainly introduce the nucleophilic precursors **3** and **4** at O-3 [29](See Scheme 1).

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