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Design and synthesis of haptens for antibody catalyzed hydrolysis of organophosphorus nerve agents.

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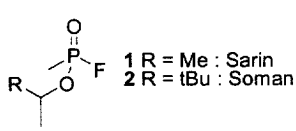
Abstract

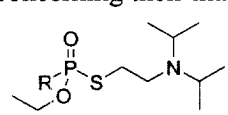
Haptens bearing phosphorane and α-hydroxyphosphinate structures have been designed and synthesized for the selection of monoclonal antibodies with phosphatase like activity in order to detoxify highly toxic organophosphorus chemical weapons such as VX.

Key words : Antibodies; Catalysis; Phosphoranes; Thiophosphates

The organophosphorus chemical weapons such as phosphonates Sarin **1** or Soman **2** and exceedingly toxic thiophosphonate VX **3** mainly act as irreversible inhibitors of acetylcholinesterase enzyme (AChE) [1]. Such compounds are relatively easy to synthesize or to handle, and the close relationship between their chemical precursors and widely used pest control agents makes their international control particularly difficult. This can be illustrated by the use of Sarin in 1995 deadly bombing in Tokyo's subway, or by the difficulties encountered by the UNO experts to prove wether or not IRAK possesses hiden amounts of VX. Therefore, many investigations have been reported lately concerning their inactivation [2].



1 R = Me : Sarin
2 R = tBu : Soman



3 R = Me : VX
4 R = Ph : PhX

Considerable efforts have been made concerning the hydrolysis of neurotoxics with exogenic or genetically modified enzymes. However, this strategy implies expensive, and time consuming processes, especially for the production and purification of large scale quantities of

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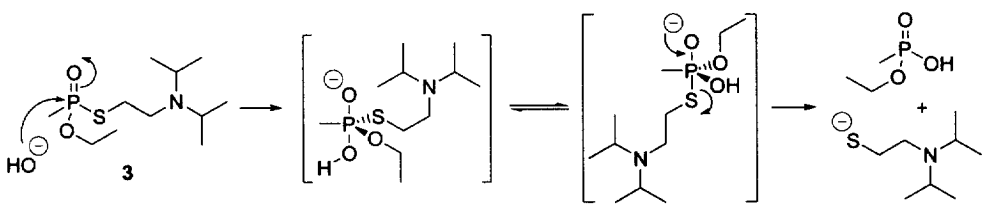
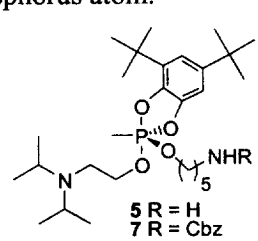
proteins. On the other hand, monoclonal antibodies production and purification techniques allow easy and costless production of antibodies. Since the pioneering work of R.A. Lerner [3] and P.G. Schultz [4], it has been confirmed that it was possible to select from the huge repertoire of immunoglobulins, tailored antibodies catalyzing given reactions (by the means of stable transition state analogs haptens). To date, despite the recent efforts [5-8], only few antibodies endowed with a phosphatase like activity have been described [9-14].

Our present studies aimed at the synthesis of stable transition state analogs haptens for the degradation of VX **3**, and, in a first set of experiments its less toxic analog PhX **4**. Immunization process requires hapten stability for at least a few days in physiological condition, namely aqueous medium around pH 7.0, in presence of excess primary amines. The hydrolysis of phosphorus esters is commonly described as a nucleophilic attack on the phosphorus atom, with pentacoordinated phosphoranes as intermediates [15,16] as examplified for VX on scheme 1. Pseudorotational interconversion of substituents through turnstile rotation processes, essential for the hydrolysis since the leaving groups are inevitably in apical position, are described as very low energy demanding processes. Thus, only the nucleophilic attack on the phosphorus atom and the formation of phosphorane can be considered as the rate limiting step for this reaction.

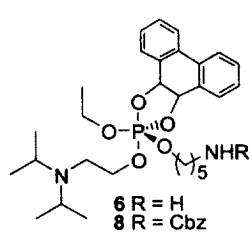


Scheme 1

A first, but sketchy and controversial publication on soman hydrolysis catalyzed by antibodies raised against haptens with a phosphorane structure [12,17], prompted us to use haptens **5** and **6** as a first stable transition state analog. Phosphoranes **7** and **8** (^{31}P NMR δ_{ppm} = -19.6 for **7** and δ_{ppm} = -46.6 for **8**) were synthesized in two steps from commercially available methyl dichlorophosphite *via* oxydative addition of the corresponding *o*-quinone on the trisubstituted phosphorus atom.[1]



5 R = H
7 R = Cbz

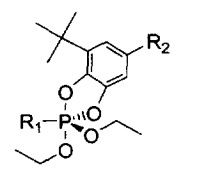


6 R = H
8 R = Cbz

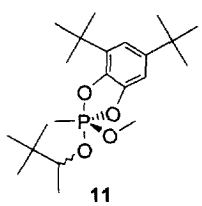
[1] Phosphite and phosphonite intermediates were formed by successive addition at -20°C of the two alcoholates in Et_2O on the dichloride, then carfully flash chromatographied with degazed eluants on desactivated (Et_3N) silicagel

Unfortunately, but as expected since phosphoranes are described to be hydrolytically unstable, **7** and **8** spontaneously decomposed in phosphonates (respectively phosphates) mixtures either when they were hydrogenolyzed or left in the presence of water or traces amount of primary and secondary amines (with 5 mol/mol eq. water, phosphorane **7** completely decomposed within 12 hours, **8** within 24 hours).

During the course of our investigations on the stability of phosphoranes, compounds **9-12** were synthesized *via* the same strategy. Phosphoranes **9** (^{31}P NMR ($CDCl_3$) δ_{ppm} = -48.0) **13** (^{31}P NMR (C_6D_6) δ_{ppm} = -46.5) and **10** (^{31}P NMR ($CDCl_3$) δ_{ppm} = -19.4), spontaneously decomposed within a few minutes in the presence of water.



9 R_1 = OEt, R_2 = H
10 R_1 = Me, R_2 = *t*-Bu



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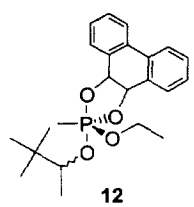

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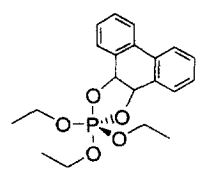
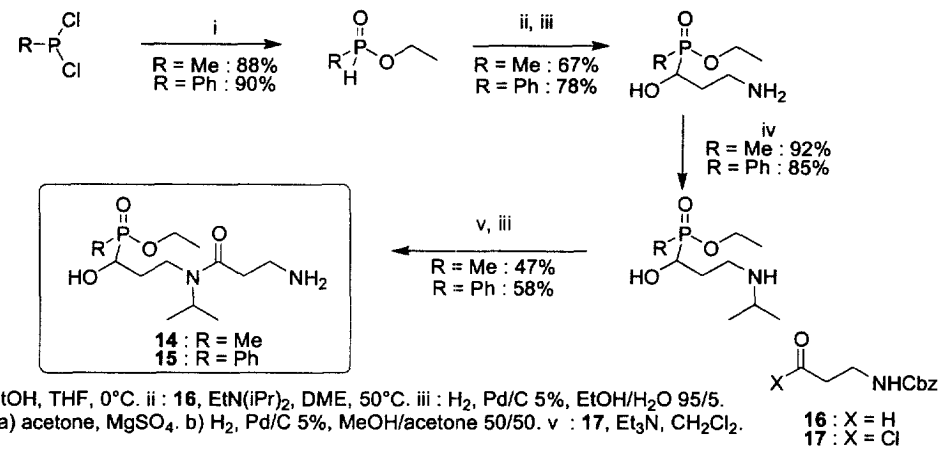


13

On the other hand, phosphoranes **11** (1/1 mixture of diastereoisomers, ^{31}P NMR ($CDCl_3$) δ_{ppm} = -16.7 and -17.5) and **12** (1/1 mixture of diastereoisomers ^{31}P NMR ($CDCl_3$) δ_{ppm} = -20.9 and -21.7), once purified on desactivated silicagel (hexanes/Et_3N 9/1), proved hydrolytically stable for more than one week. Only 50°C heating in a 1/1 acetone/water mixture enabled slow appearance of a complex mixture of phosphonates. This hydrolytic stability, which has been previouly described only for a few phosphoranes [18,19], can be related to a higher pseudorotational interconversion energy barrier due to the third bulky substituents on phosphorus. Taking into account this difficulty to obtain water stable phosphoranes with structures related to VX, and the recent discussions on hydrolysis mechanisms of this nerve agent driving back the intervention of a phosphorane intermediates [20,21], we decided to immunize mice with haptens mimicking the very first step of the hydrolysis mechanism, namely the approach of water on phosphorus. Moreover, α-hydroxyphosphinates are known to be good competitive inhibitors for phosphatase enzymes [9,22,23]. We thence chose haptens **14** and **15** for the hydrolysis of VX **3** and PhX **4**. Those haptens were synthesized from commercially available methyl and phenyl dichlorophosphines in 6 steps with 25% and 35% overall yields. The key step is the Pudovik [24] condensation of aldehyde **16** on hydrogenophosphinites. ^{31}P NMR studies then proved that haptens **14** and **15** are stable in physiological conditions. Haptens were then covalently coupled to KLH (keyhole limpet hemocyanin) for immunization, and to AChE to be used as enzymatic tracers as described earlier [25].



In this paper, we have demonstrated that use of phosphorane haptens was not possible for the antibody catalyzed hydrolysis of VX **3**, since bulky fonctionnal groups, incompatible with the stucture of VX **3**, are needed for the stabilisation of phosphoranes. We then successfully synthesized stable α-hydroxyphosphinates haptens. Results of the immunization, monoclonal antibodies production and characterisation and catalytic activity analysis will be reported in due time.

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