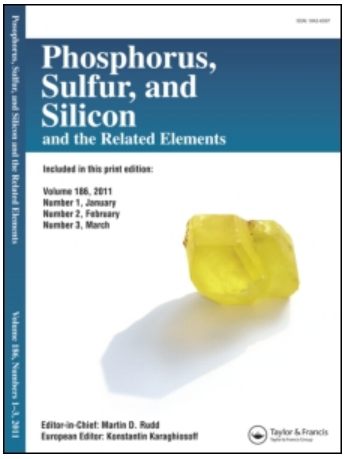

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SYNTHESIS OF PENTACOORDINATE PHOSPHORUS HAPTENS FOR CATALYTIC ANTIBODY PRODUCTION. BAIT AND SWITCH CONCEPT

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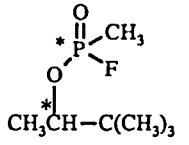
Abstract: Our objective is to obtain monoclonal antibodies which will catalyse the hydrolysis of phosphorus-based nerve agents. Our syntheses followed three strategies. The first was synthesis of stable pentacoordinate phosphoranes as haptens for the production of monoclonal catalytic antibodies based on the transition state for phosphonate hydrolysis. The second approach is based upon the potential addition of water to the phosphorus atom of a tetracoordinate fluorine containing phosphate to yield an intermediary pentacoordinate system. In the third approach the "bait and switch" concept was used.

INTRODUCTION Our research effort has been directed towards obtaining a monoclonal catalytic antibody against the nerve agent soman (1). The toxic effects of soman (1) result from irreversible inhibition of acetylcholinesterase(AChE) caused by phosphonylation of the active serine unit. [1] Blockage of the AChE activity results in continuous firing of nerves in the affected cholinergic systems leading to fatality by a number of causes the most prominent being respiratory collapse. [2a-c] Strategies for protection against soman (1) use pretreatment with a reversible cholinesterase inhibitor such as carbamate as in the case of physostigimine, in which case the carbamylated enzyme protects a pool of AChE from phosphonylation by soman (1).[3a-e] Another approach involves use of pyridinium oximes nucleophiles to dephosphonylate the AChE ester.[4a-b] A novel approach towards protection against soman (1) is creation of a monoclonal antibody against this molecule, which could act as a scavenger, or as a catalyst for its hydrolysis.[5a-b] Soman (1) possesses two chiral centers, one at carbon and the other at phosphorus. The various stereoisomers show different rates of inhibition of cholinesterases and overall toxicity.[6,7]



C_RP_R, C_RP_S, C_SP_R, C_SP_S,

1

PRESENT RESEARCH

a) Unsymmetrically Substituted Chiral Monocyclic Oxyphosphoranes.

Our design concept for generation of a hapten for production of a catalytic monoclonal antibody against soman (1) is based upon the mechanism of hydrolysis of soman (1), i.e. addition of water to the tetracoordinate tetrahedral phosphorus to yield a pentacoordinate intermediate or transition state analog. Figure 1 shows the pentacoordinate intermediate and the bridging functionality necessary for stabilization of the phosphorane.

In Figure 2 this design concept is translated into a transition state hapten which possesses all the requisite structural features.

Figure 1

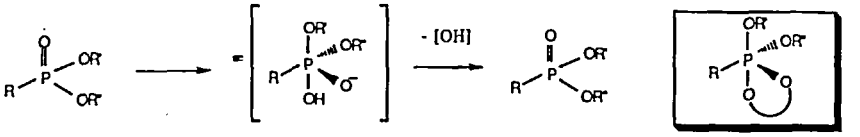
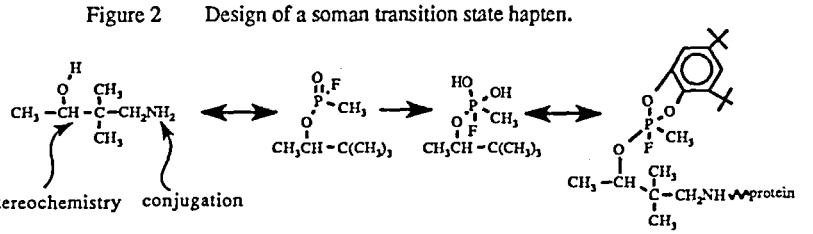
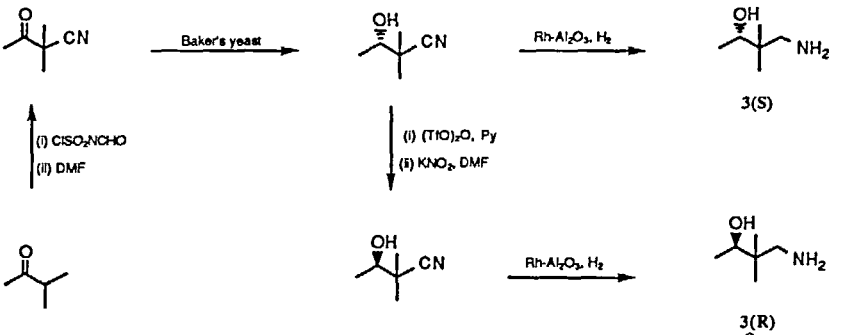


Figure 2 Design of a soman transition state hapten.

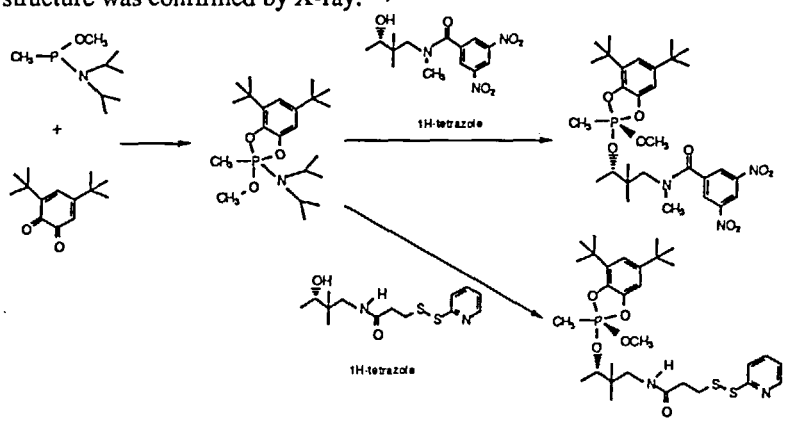


The synthesis of the stereoisomeric 2-aminomethyl-3(*R*) and (*S*)-hydroxybutanes was carried out as follows:



Bakers yeast reduction of the ketone yielded the *S* alcohol[8] and inversion of configuration of the neopentyl type alcohol using appropriate methodology was carried out.[9] The P-F bond of soman as well as the phosphorane in Fig. 2 was substituted by a P-OCH_3. Thus the *R* and *S* alcohols were each phosphonylated using $CH_3P(O)(OCH_3)Cl$ and all four diastereomers separated and characterized by X-ray crystallography. These then were correlated with the separate stereoisomers of soman using NMR spectroscopy.[10]

The synthesis of the pentacoordinate phosphorane hapten is shown below, and the structure was confirmed by X-ray.[11,12]



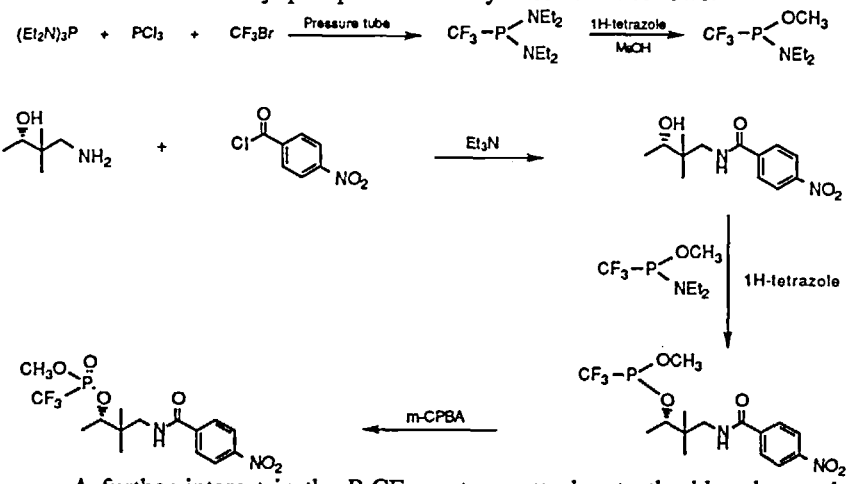
The phosphorane hapten was conjugated to the three carrier proteins BSA, KLH and PTG at three hapten:carrier ratios.

(b)Fluorine-Containing Haptens

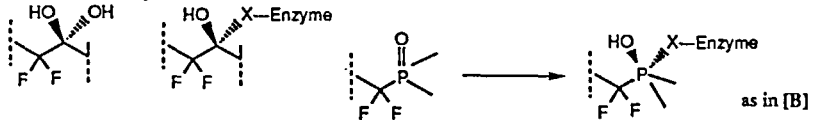
Trifluoromethyl Phosphoranes.

Unsymmetrically substituted monocyclic oxyphosphoranes discussed above were stable in neutral and in basic aqueous solution but were unstable under aqueous acidic conditions. This instability led to the formation of a phosphonate from the phosphorane presumably via initial protonation of the phosphorus-oxygen bond. Replacement of CH_3-P by CF_3P was reasoned to decrease the stability of the phosphonium intermediate.

The trifluoromethyl phosphorane was synthesized as follows:

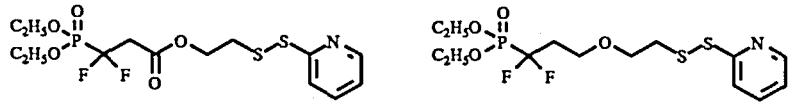


A further interest in the P-CF_3 systems attaches to the idea that a phosphonate possessing the P-CF_3 might be prone to hydration to yield a pentacoordinate phosphorane of the type $(RO)_2$ P-$CF_3(OH)_2$. This approach is based upon analogy with hydration of difluoromethylene ketones.



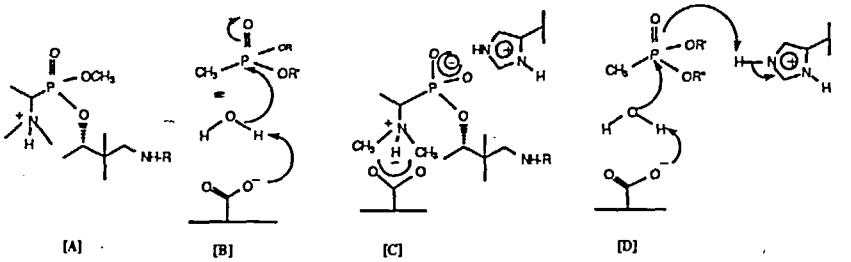
[A] [B]

The following fluorinated haptens were synthesized:



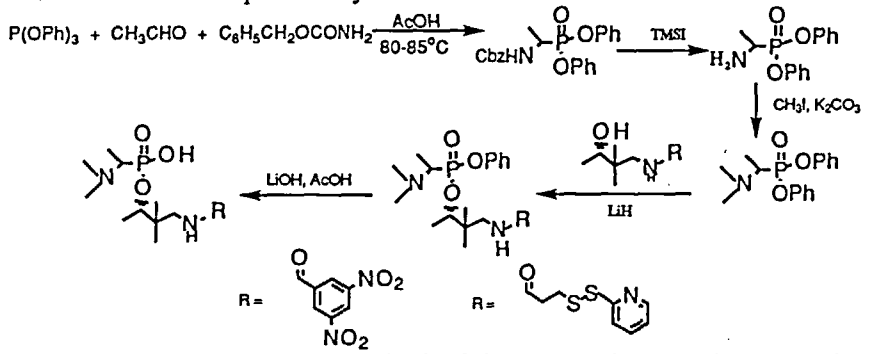
(c) Bait and Switch

Enzymatic catalysis involves binding of a substrate, in order to reduce entropy and also arrange reaction centers in the substrate with complementary reactive groups at the active site of the enzyme. Not only is the distance between the reaction center of the substrate and the complementary group on the enzyme critical, but also stereochemistry and orientation of interacting orbitals. In the case of hydrolysis of a phosphonate intermediates B and D may intervene. Hapten A may induce the complementary acid-base centers as in C.



[A] [B] [C] [D]

The bait and switch hapten was synthesized as follows:



The various haptens synthesized in this project are being carried through immunological studies.

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