Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 13, pp. 1523-1524, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00266-1

ANTIBODY CATALYZED HYDROLYSIS OF A PHOSPHOTRIESTER

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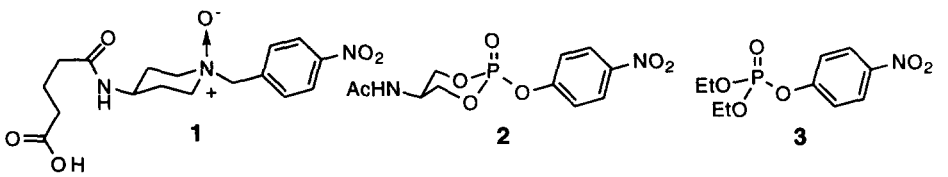
Abstract: An antibody (15C5) raised against *N*-oxide hapten **1** catalyzed the hydrolysis of phosphotriester **2**, with a k_{cat} of 2.65 x 10^{-3} min^{-1}, K_m of 87 μM, and displayed multiple turnovers.

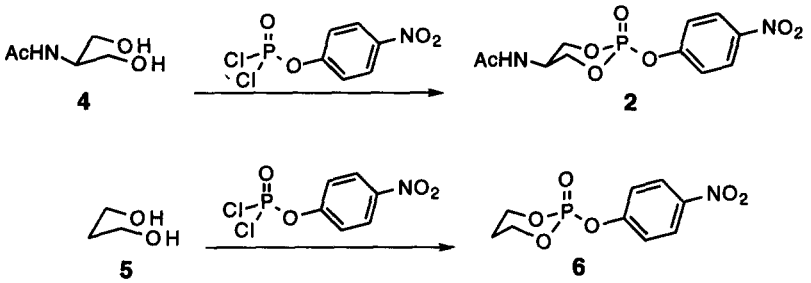
Phosphate triesters are essentially irreversible inhibitors of the enzyme acetyl cholinesterase and, as such, have been used widely as insecticides.[1] Unfortunately, as a result, phosphotriesters are also the class of compounds most often responsible for the poisoning of agricultural workers.[1] Antibodies have a long history of clinical use in treating poisonings.[2] We chose to investigate whether catalytic antibodies might be used successfully to decompose phosphotriesters.[3] As a part of that research, 25 monoclonal antibodies were raised against *N*-oxide hapten **1**. One of these antibodies (3H5) is capable of hydrolyzing the phosphotriester insecticide paraoxon **3**.[4] A second antibody catalyst (15C5), which is capable of mediating the hydrolysis of *N*-acetyl serinol-*p*-nitrophenyl phosphate **2**, was also obtained.



1 **2** **3**

The hydrolysis of phosphate triesters is first order in hydroxide ion and is believed to proceed through an in-line displacement mechanism that passes through a trigonal bipyramidal transition-state with the nucleophile and leaving groups in the apical positions.[5-7] Designing a hapten that would accurately mimic such a transition-state would require a negatively charged, trigonal bipyramidal molecule. Unfortunately, such compounds are usually unstable.[8] As a result, we chose to investigate whether a "bait and switch"[9-11] approach using *N*-oxide **2** as the hapten would induce antibodies capable of hydrolyzing phosphotriesters such as **2** and **3**. In this strategy, the partial negative charge on oxygen is expected to induce antibody residues capable of stabilizing the developing negative charge of the transition state, while the partial positive charge on nitrogen induces complementary negatively charged groups in the antibody binding pocket which may be able to act by general base catalysis.

Hapten **1** was prepared in four steps from piperidone. The synthesis is described elsewhere.[4] Substrate **3** was prepared by acylating serinol, then reacting diol **4** with dichloro-*p*-nitrophenyl phosphate. Phosphotriester **6** was prepared in similar fashion by reacting 1,3-propanediol **5** with dichloro-*p*-nitrophenyl phosphate.



Screening of a panel of 25 monoclonal antibodies generated against **1** showed that one (15C5) was a catalyst for the hydrolysis of **2**. Subsequent reactions to characterize this antibody were carried out in 50 mM bicine buffer at pH 8.1 at 25.0 °C and monitored by following the change in UV absorbance at 402 nm. Antibody 15C5 displayed turnover of **2** and followed Michaelis-Menten kinetics with a k_{cat} value of 2.65 x 10^{-3} min^{-1} and K_m of 87 μM. The value of k_{uncat} was 2.10 x 10^{-5} min^{-1}. The hydrolysis of **3** and **6** however, were not accelerated appreciably by 15C5 indicating that the *N*-acetyl group plays a significant role in binding of the substrate.

The current results provide support for the theory of using *N*-oxides to generate catalysts for the hydrolysis of phosphotriesters. The differences in substrate specificity between antibody 15C5 and 3H5 indicate that recognition of very small portions of haptens can be crucial in binding small molecules.

Acknowledgment. We gratefully acknowledge financial support from the Alfred P. Sloan Foundation and the N.I.H. (GM48351). We thank Julie Coakley for technical support in raising antibodies.

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(*Received in USA* 14 *May* 1996; *accepted* 4 *June* 1996)