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Synthesis of a Bifunctional Hapten Designed to Mimic both Transition State and to Induce "Bait and Switch" Catalysis

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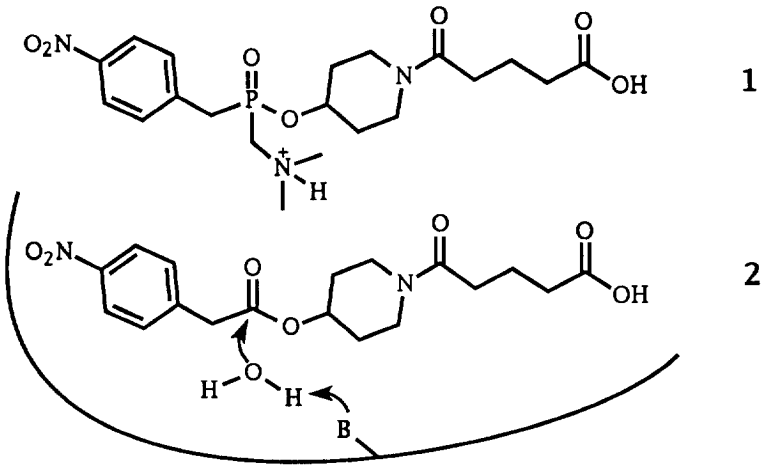
Abstract: An α-aminophosphinic ester based hapten was synthesized to mimic both transition state and to induce "bait and switch" catalysis. This bifunctional hapten was designed to test the feasibility of combining two mechanisms for inducing catalysis in a single molecule with the intent of eliciting more efficient antibody catalysts.

A major area of interest in antibody catalysis has been the acyl-transfer reaction.[1] Hapten functionality has proven to be one of the most important elements in approaches to generate antibodies to catalyze this reaction class.[2] The most successful functionality in hapten design is the phosphonate moiety, which when properly poised within a haptenic molecule effectively mimics the tetrahedral geometry of the transition state of ester hydrolysis. To expand the scope and capabilities of these hydrolytic catalytic antibodies, we have pursued new strategies in the design of haptens. One such method we have termed 'bait and switch' catalysis involves the use of haptens for explicit elicitation of particular amino acid residues within the antibody binding pocket to assist in the acyl transfer reaction. An interesting tactic to try and generate more efficient antibody catalysis would be to combine the transition state approach and our "bait and switch" methodology into one hapten structure. We anticipated such an approach would allow us to expand the chemical complexity of an antibody combining site. Herein we report the synthesis and our findings of a haptenic molecule bifunctionalized so as both transition state stabilization and "bait and switch" catalysis could have the opportunity to be operative.

The components we chose to bring together this bifunctional haptenic approach for antibody catalysis are a positively charged amino group and a tetrahedral phosphorus in the form of an α-aminophosphinic ester. The α-aminophosphinic acid moiety is an important amide bond replacement functionality which has been used to construct potent enzyme inhibitors.[3] Its success as an enzyme inhibitor derives from a compact array of stereoelectronic features that this moiety displays at ambient pH. Overall this moiety displays two unique functionalities. First, a phosphinic ester unit which can be viewed as a transition state analogue mimic. Second, a positively charged amino group for inhibitor-protein charge complementarity. Keeping these thoughts in mind, we designed a generalized haptenic molecule that would incorporate the α-aminophosphinic moiety within its structure. p-Nitrophenyl α-aminophosphinate, **1** , accomplishes this goal; it contains the bifunctional α-aminophosphinic moiety and provides us with a recognizing element (p-nitrophenyl) for antibody binding (Scheme 1).

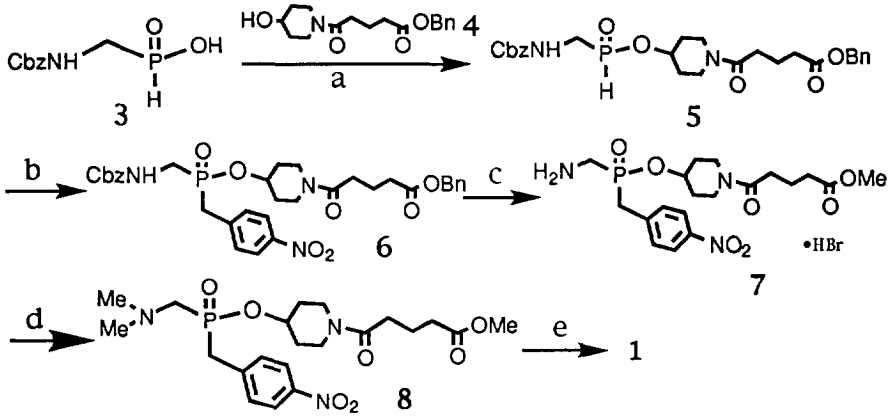


Scheme 1. Bifunctional hapten, **1**, designed to mimic both the transition state and to induce "bait and switch" catalysis for the hydrolysis of ester **2**. Curved bold line represents the antibody combining site and the chemical interaction expected if hapten **1** induces "bait and switch" catalysis. The letter B designates a basic functional group in the combining site.

Our synthesis of hapten **1** started with α-aminomethyl phosphonous acid.[4] The amino portion of this molecule was protected with a benzyloxycarbonyl (CBZ) group to give α-(benzyloxycarbonyl)aminomethyl phosphinic acid **3**.[5] DCC coupling of phosphinic acid **3** with alcohol **4**[6] yielded phosphinic ester **5** (90%); however, it should be noted that the phosphinic ester **5** is sensitive to moisture and the reaction is carried out more proficiently on a larger scale. Ester **5** was transformed into the trivalent TMS phosphonous ester by the addition of excess *N,O*-bis(trimethylsilyl)acetamide (BSA, 4 eqiv),[7] and without separation, p-nitrobenzyl bromide was added to convert this trivalent TMS ester into the desired dialkylphosphinic ester **6**.[8] Attempts to use TMSCl/TEA in place of BSA to carry out the same sequence of reactions were unsuccessful. The Cbz as well as the benzyl protecting groups were removed by treatment with hydrobromic acid. The free carboxylic acid obtained was re-esterified by treatment with HBr in methanol to provide the key intermediate aminomethylphosphinate **7** (95%). Next, the amino group of **7** was transformed into a dimethylamino functionality by reductive amination with sodium cyanoborohydide in the presence of zinc chloride.[9] This method proved useful as other commonly used conditions for reductive amination yielded a complex mixture of products. Finally, the methyl group of **8** was removed with sodium hydroxide to provide hapten **1**.[10] (Scheme 2).

The α-aminophosphinic ester **1** was coupled to keyhole limpet hemocyanin (KLH) and Bovine Serum Albumin. Monoclonal antibodies were elicited against KLH-**1** using standard hybridoma methodology.[11] A total of 27 antibody-secreting hybridomas were isolated specifically for Bovine Serum Albumin-**1** conjugate. After purification (anion exchange chromatography, affinity chromatography), the antibodies were investigated for their ability to hydrolyze **2**. Several antibodies showed rate enhancement over the background reaction, however, their overall activities were modest ($k_{cat}/k_{uncat} < 100$) and thus deemed not sufficient for

further investigation. While the observed rates were less than desirable, we would like to point out that only a small number of antibodies were sampled and thus may not provide a completely accurate assessment of the power of this bifunctional approach.



Scheme 2. Reagents and conditions: (a) DCC, THF, 90%; (b) (1) BSA, THF, (2) p-nitrobenzyl bromide, 42%; (c) (1) HBr/AcOH, (2) HBr/MeOH, 95%; (d) $NaCNBH_3$, $ZnCl_2$, 37% formaldehyde, 70%. (e) NaOH, 50%.

The synthesis outlined above enables us to obtain the desired hapten in good yield. Two points should be emphasized regarding its synthesis: (1) BSA is superior to TMSCl/TEA as a reagent to activate phosphinic esters for the Arbuzov reaction and (2) Reductive amination with sodium cyanoborohydride can be carried out efficiently and conveniently in the presence of zinc chloride. Generalization of this route to other α-aminophosphinic esters should be readily amenable due to the conciseness of our methodology.

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10. ^{1}H NMR (CD_3CN/D_2O (1/1)) δ 1.38 (m, 1H), 1.70 (quintet, 2H, *J*=7.0 Hz), 1.55-1.90 (m, 3H), 2.24-2.36 (m, 4H). 2.92 (s, 6H), 3.15-3.65 (m, 6H), 3.59 (d, 2H, *J*=17Hz), 4.60 (m, 1H), 7.51 (dd, 2H, *J*=2.3, 8.7 Hz), 8.17 (d, 2H, *J*=8.5 Hz); HRMS (FAB) *m/z* calcd for ($C_{20}H_{30}N_3O_7P$+H) 456.1900, found 456.1879.
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