TOWARD A VACCINE AGAINST COCAINE

by

Rachel Butler

Submitted in partial fulfillment of the requirements

for Departmental Honors in

the Department of Chemistry and Biochemistry

Texas Christian University

Fort Worth, Texas

May 7, 2018

TOWARD A VACCINE AGAINST COCAINE

Project Approved:

Supervising Professor: Jean-Luc Montchamp, Ph.D.

Department of Chemistry and Biochemistry

Dr. Julie Fry, Ph.D.

Department of Chemistry and Biochemistry

Dr. Meredith Curtis, Ph.D.

Department of Biology

ABSTRACT

This project aims at preparing a compound that should prevent the effects of cocaine when injected into a human being. Certain compounds can steer the immune system into performing a chemical reaction, which in this case will be the conversion of cocaine into harmless components. The preparation of a compound possessing the required properties toward this objective is proposed. For the immune system to elicit an immune response, the substance must be of a sufficiently large size. Therefore, the preparation of such a compound requires attachment to a carrier protein or polymer so that the compound will be recognized as foreign by the immune system. We propose that introduction of this compound into the body would trigger the immune system to generate catalytic antibodies capable of degrading cocaine. Immunization should generate an immunological memory response so that even if more cocaine is introduced in the body later on, it will still be degraded into harmless components. The ultimate successful completion of this project could potentially provide a significant advance in the treatment of cocaine addiction, which is a huge cost to the healthcare system and takes a toll on many lives.

ACKNOWLEDGEMENTS

Firstly I would like to thank Dr. Jean-Luc Montchamp for not only coming up with this project idea but also for all of the help he has given me along the way. I would also like to thank Dr. Julie Fry and Dr. Meredith Curtis for all of their help in the development and finalization of this thesis. Another thank you to the Science and Engineering Research Center, the Montchamp Phosphorus Research Group, and the Honors College for providing funds to make this project a reality.

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INTRODUCTION

The overall goal of this project is to make a vaccine against cocaine. The way the vaccine will work is that when administered to a patient, the patient's immune system will respond by creating catalytic antibodies. Traditional antibodies work by indirectly destroying foreign molecules by tagging a specific antigen on a large foreign molecule for destruction by macrophages and other white blood cells. On the other hand, catalytic antibodies (or abzymes) are catalysts that work by actively destroying compounds by cleaving them. Traditionally enzymes work by lowering the activation energy of a transition state of a reaction, making the reaction occur more easily. In the case of catalytic antibodies, when a molecule is structurally similar enough to the transition state (called a transition state analog), the antibody will bind and stabilize the molecule, which lowers the activation energy enough for the reaction, which proceeds through a similar transition state, to occur. An advantage of catalytic antibodies over traditional enzymes is that the desired reaction can be selectively programmed into antibodies by using a hapten,[1] which is a small molecule that can cause an immune response when bound to a larger molecule. In the case of cocaine, this means that cocaine will be degraded into a harmless molecule in the body. The action of catalytic antibodies compared to traditional antibodies is shown in Figure 1.

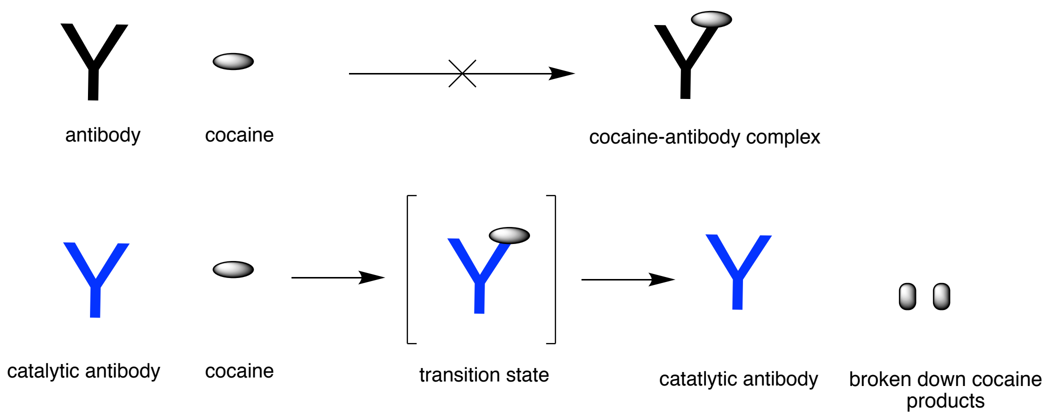
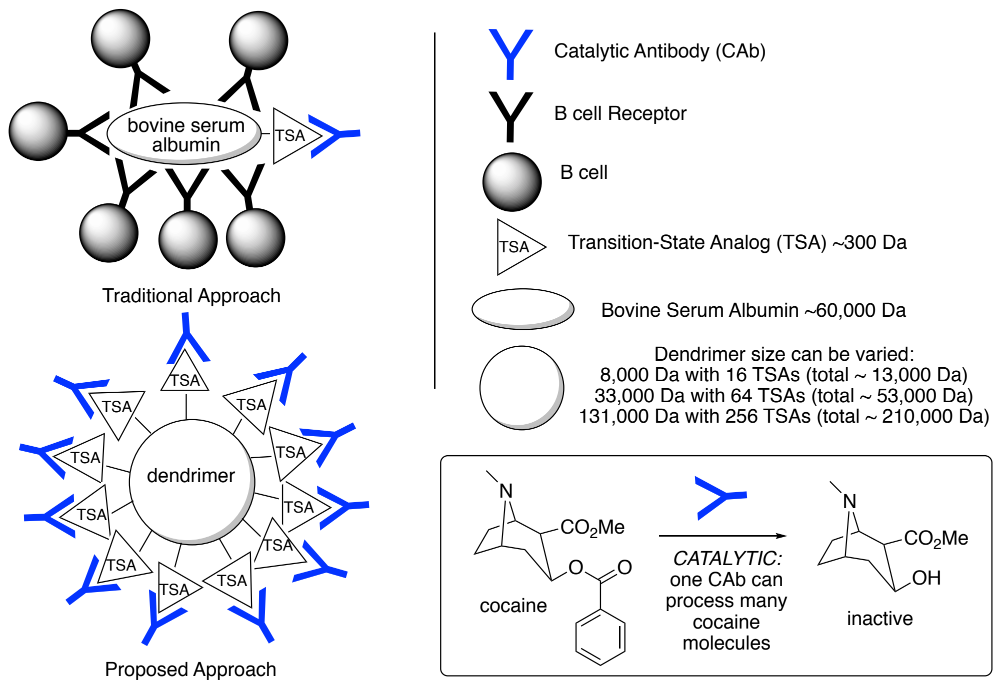


Figure 1. Comparison of traditional antibodies and catalytic antibodies, with cocaine shown as the antigen.

It is also important that the vaccine be large enough, since normally the immune system is triggered only by very large objects like bacteria, while drug molecules are very small in comparison. In past research with catalytic antibodies, the traditional approach is to attach the transition state analog to the protein bovine serum albumin.[1] In this case (shown in Scheme 1), only one transition state analog (TSA) is attached to the protein, meaning that only one catalytic antibody will bind. A stronger immune response could be elicited with many more catalytic antibodies, which is one of the benefits of this proposed project. Instead of bovine serum albumin, the TSAs will be attached to a dendrimer, which is a polymer with a highly branched structure. This not only accomplishes the large size needed, but is also better than the traditional approach in that multiple TSAs can be attached and exposed to the surface, and thus more hapten-specific B cell receptors can bind.

Also, a unique feature of catalytic antibodies is that after it hydrolyzes its substrate and releases the products, the antibody is free for more binding and hydrolysis.[2] This enables even more molecules of cocaine to be degraded at a more efficient speed. This is important because studies have shown that only a large vaccine response would provide the minimum antibody concentrations needed for useful cocaine breakdown.[3] The size of the dendrimer can also be varied, meaning that multiple variations could be synthesized and experimented with to determine which size will best be recognized by the body.



Scheme 1. Traditional approach versus proposed approach toward anti-cocaine catalytic antibodies

Before attachment to a dendrimer can occur, synthesis of a hapten must be completed. Synthesis of the hapten cannot be prepared using cocaine as the starting material, for regulatory reasons, so instead synthesis starts with nortropine. Nortropine is similar in structure to cocaine after hydrolysis (reaction shown in Scheme 1). Once the benzoyl group of cocaine has been removed, the drug is inactive. The similar structures of nortropine and debenzoylated cocaine can be seen in Figure 2. While the hapten synthesized from nortropine will be structurally simpler than cocaine, the vaccine should still perform cocaine hydrolysis because the two structures will still have very similar transition states.

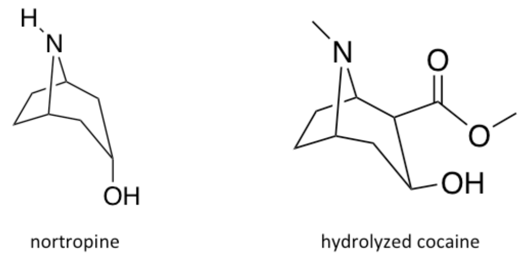
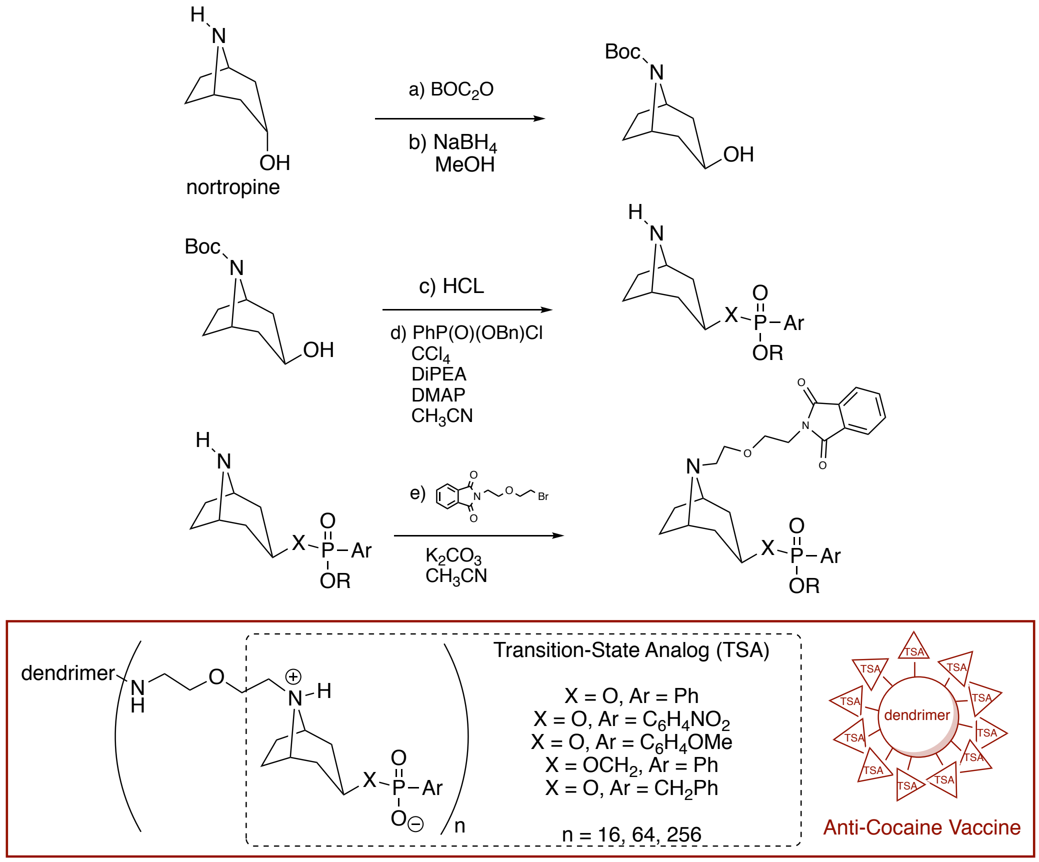


Figure 2. Difference in structure between nortropine and hydrolyzed cocaine

In order to synthesize the hapten from nortropine, several steps must be completed, each of which can be very time and labor intensive. Many of these steps have already been completed, while others have posed challenges that have not yet been resolved. The general proposed steps are shown in Scheme 2. This method has been used as a guide for the synthesis of the hapten with several modifications.

The first step of synthesis is protection of the amine group in nortropine with a Boc group to avoid interference of the amine group in other reactions. The hydroxyl group of nortropine must also be inverted to more closely match the benzoyl group in the structure of cocaine. A phosphonate ester molecule that serves as the TSA and a linker molecule, which attaches to the dendrimer, must be synthesized and attached to give the final hapten molecule.



Scheme 2. Proposed synthesis of TSAs and attachment to dendrimer to form the vaccine.

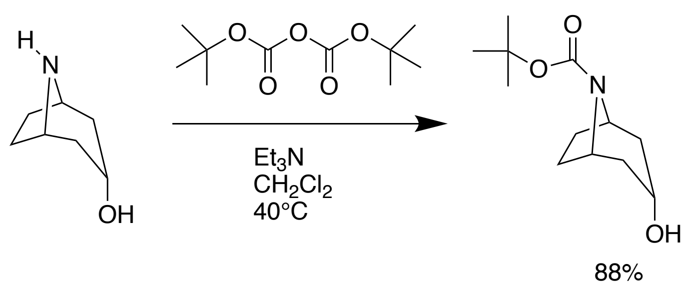
Ultimately this hapten dendrimer complex would be large enough to elicit an immune response and catalyze the chemical reaction to cleave cocaine. This complex is referred to as a vaccine because like normal vaccines, an immune response would be triggered and the host would produce the necessary catalytic antibodies to destroy cocaine. If cocaine was encountered again, the same immune response would be initiated, effectively making the patient immune to cocaine and curing addiction.

RESULTS AND DISCUSSION

This project has ultimately been done in a series of steps. The order of the steps has been investigated to determine the best method of action. Because the final molecule is very complex and there are multiple synthetic steps, finding the order in which to do the steps has proven to be one of the biggest challenges.

1. Protection of nortropine

As mentioned, synthesis cannot begin with cocaine for regulatory reasons. As seen in Figure 1, nortropine is structurally similar to cocaine and should elicit the same immune response to the vaccine. The amino group of nortropine is fairly reactive, so a protecting group is added to ensure that the amino group doesn't participate in further reactions. A Boc group, or tert-Butyloxycarbonyl, is a common amine-protecting group.[4] The reaction can be seen in Scheme 3.



Scheme 3. Protection of the nortropine amine group

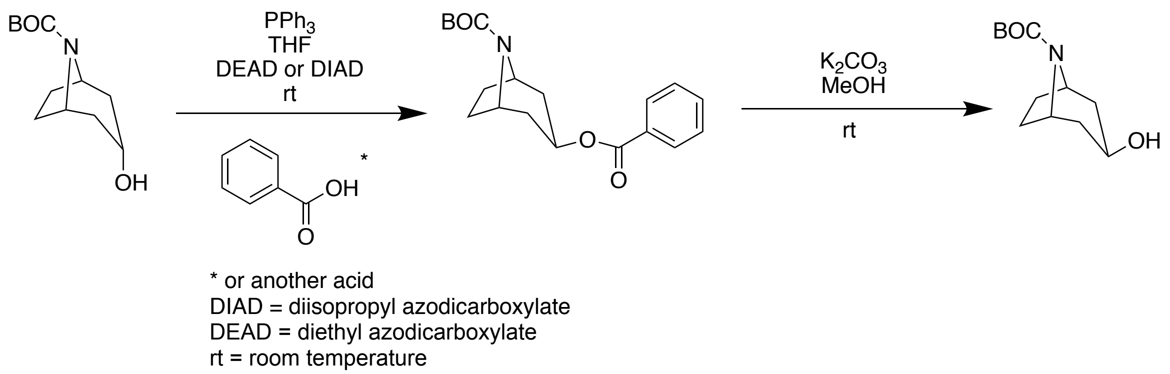
Protection of an amine with a Boc group is a commonly used reaction, which works very well, giving high yields and requiring minimal purification techniques.

2. Inversion of the alcohol

One of the main challenges in this project has been inverting the hydroxyl group of the starting material nortropine. This step is essential because as seen in Figure 1, the hydroxyl group in nortropine is in the endo (or axial) position while the group on the same carbon in cocaine is in the exo (or equatorial) position. This difference may seem small, but different isomers cause very different effects in the body. To elicit the same response as cocaine, the synthesized vaccine must be as similar as possible, especially since the CO_2Me group of cocaine is removed. This step has likely been a challenge because of the steric hindrance of the molecule, causing normal methods of alcohol inversion to be ineffective. Many methods have been attempted to give the desired product.

The first approach was to use an S_N2 reaction, which gives a product with the inverse stereochemistry of the reactant. To make the alcohol into a better leaving group, it can be turned into a toluenesulfonyl (tosyl) group. Then, in theory, an Sn2 reaction could be performed to invert the tosyl group, which could then be cleaved, leaving the alcohol in the desired exo position. However, when performed, this reaction never produced a clean enough product to proceed with the proposed steps.

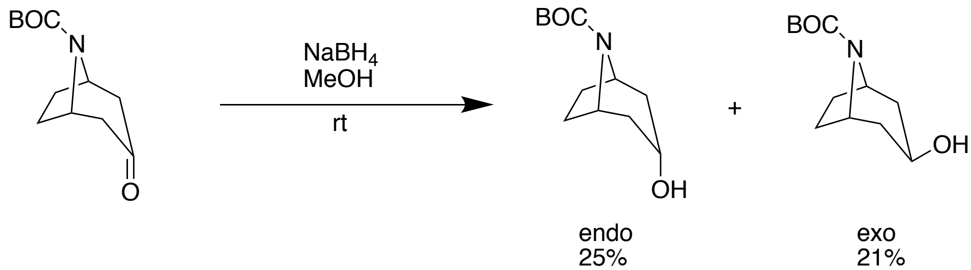
The second approach was to use the Mitsunobu reaction, which is another reaction that reverses stereochemistry, but specifically with a hydroxyl group. A proposed typical Mitsunobu reaction for a sterically hindered alcohol is shown in Scheme 4.



Scheme 4. Proposed Mitsunobu reaction[5]

The main problem with this reaction was getting a pure product. Many variations of the Mitsunobu reaction were attempted. First, directly attaching the phosphonate ester was attempted by using it in place of the acid, which would be ideal since it eliminates an extra step.[6] The acid used can also be changed. To attempt to eliminate phosphorus side products associated with triphenylphospine, diphenyl-2-pyridyl phosphinate was used instead.[7] Another modification was using 4-diphenylphosphinobenzoic acid, which serves as both the phosphine and the acid in the Mitsunobu.[8] Lastly, in an attempt to avoid side products associated with DIAD (diisopropyl azodicarboxylate), an azodicarboxylate reagent that would be easier to purify (one that was water soluble, for example) was synthesized.[9] However even with these modifications, the resulting mixture almost always contained starting material, unwanted phosphorus compounds, or other side products.

Ultimately, the method that worked was to start with the ketone nortropinone instead of nortropine and reduce it to give a mixture of exo and endo isomers. Then, silica gel chromatography can be done to separate the isomers. Although this reaction is not the most desirable since extensive purification is needed and the main product is the endo isomer, it has proven to be the best method for inversion of the alcohol due to its ability to give the desired product. This process is shown in Scheme 5.[10]

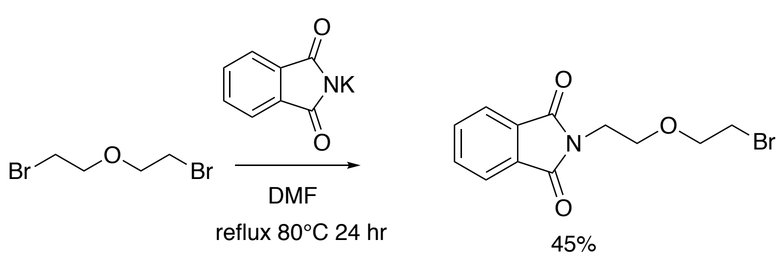


Scheme 5. Reduction of nortropinone

The reaction produces a mixture, and subsequent purification produces a clean isomer of the exo alcohol by NMR (Figure 3, experimental section). With the exo isomer successfully synthesized, the next step could be done, which was to attach the linker molecule.

3. Synthesis and attachment of linker

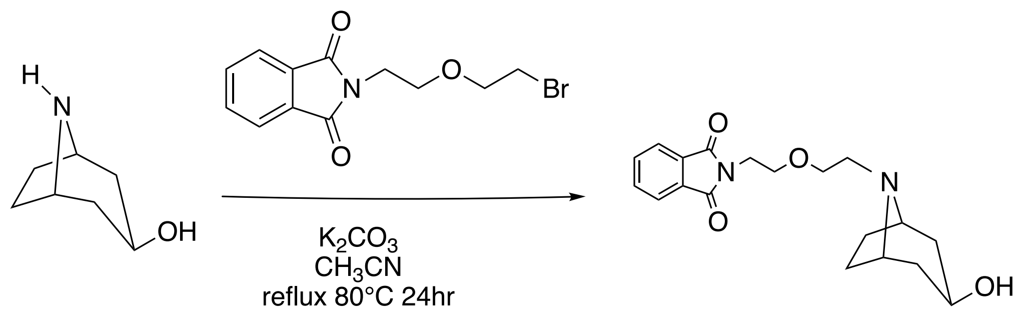
A linker molecule attached to the nitrogen of nortropine will serve as the place where the dendrimer is eventually attached in the last step of the vaccine synthesis. This reaction gives a clean product by NMR analysis (Figure 4, experimental section).



Scheme 6. Synthesis of linker molecule[11]

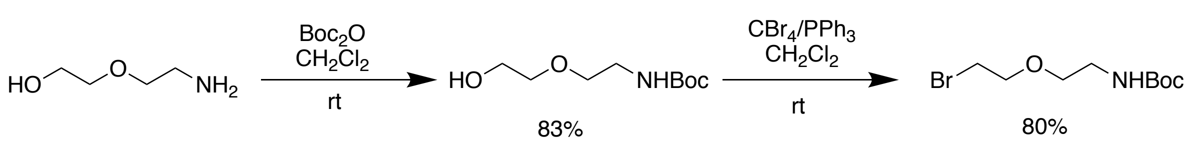
Before attachment of the linker can occur, first the Boc-nortropine molecule can be deprotected. This can be accomplished with a simple acidification under anhydrous conditions.

The amino group can then be reacted with the linker molecule under basic conditions to combine the two molecules, which is shown in Scheme 7. Because the amino group is more reactive than the hydroxyl, the OH^- should not interfere.



Scheme 7. Attachment of linker

The shown reaction likely did give the desired product, although NMR analysis showed peaks of an unknown origin in the aromatic region, which could indicate an undesired side product. An alternate linker where the pthalamide group is replaced by a Boc group might solve this problem. This linker has been successfully synthesized, and in the future could be attached to the nortropine molecule to hopefully produce a cleaner product. The synthesis of this linker is shown in Scheme 8, [12] which has given a clean product by NMR analysis (Figure 5, experimental section).



Scheme 8. Synthesis of alternate linker

4. Synthesis and attachment of the phosphinate ester

One of the most important parts of the hapten molecule is the transition state analog (TSA), which binds to the catalytic antibody and can affect how efficiently cocaine's benzoyl group is degraded. Phosphinates are the core model for TSAs in standard anti-cocaine catalytic antibody literature because they stably mimic the geometry and charge distribution of the transition state for the hydrolysis reaction.[13] Also, the Montchamp Group specializes in organophosphorus chemistry, making the phosphonate ester a good choice for a TSA. The general structure of the transition state and the desired TSA phosphonate compound is shown in Figure 2.

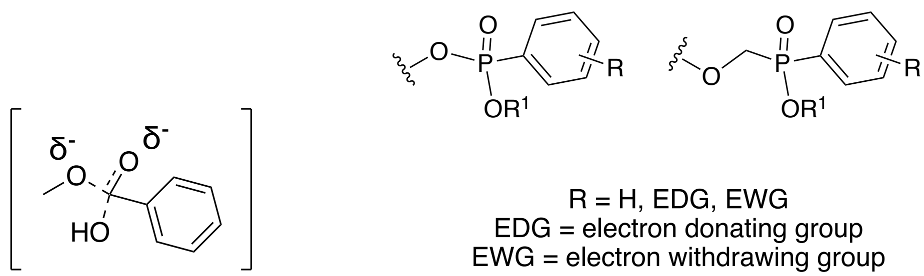
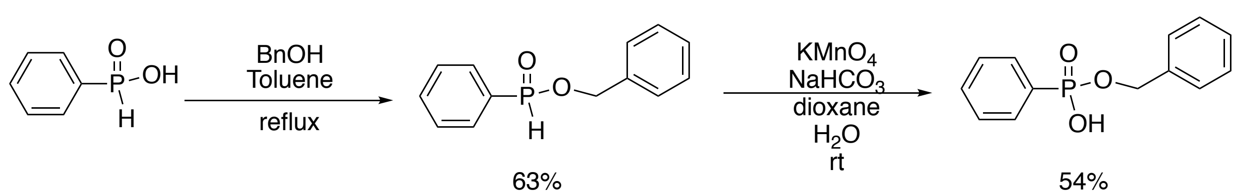


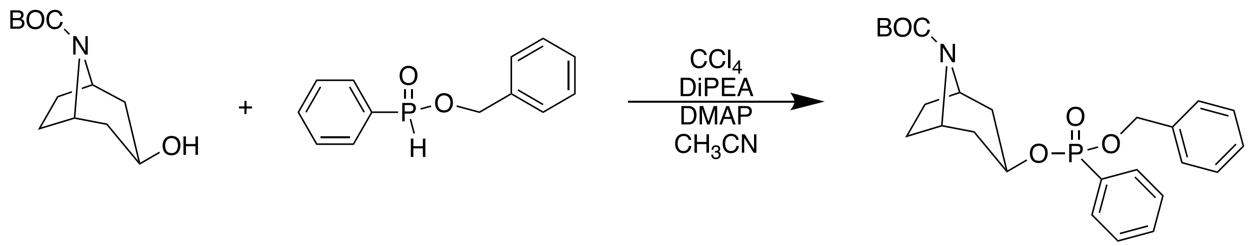
Figure 2. General structure of transition state analog and proposed phosphinate ester

Several of these compounds have been synthesized to provide a variety of options for the final molecule. As shown in Figure 2, the compound should always contain a phenyl group and an ester group, although the specific R group can be changed to alter the electronic properties. A benzyl-protected version was used for attachment experiments, the synthesis of which is shown in Scheme 9.



Scheme 9. Synthesis of benzyl phosphinate ester

The oxidized version of the molecule was synthesized for the purpose of using with the Mitsunobu reaction, although this did not end up being a successful method. Instead, the reduced form (Form 2 in Scheme 9) was attached to the nortropine molecule. This reaction was done before cleavage of the Boc group and attachment of the linker.



Scheme 10. Attachment of phosphinate ester

The shown reaction has been successful, although the order of synthesis must be further experimented with to determine the best plan to complete the vaccine molecule. For example, although it is ideal to have the amine protected for this reaction, the acidic workup may cleave the Boc group. A solution could be to attach the linker molecule first, although this reaction has not yet been perfected.

CONCLUSION AND FUTURE PLANS

Although this project has been in progress for a year and a half, there have been many roadblocks along the way that have slowed its progress. For example, much time was spent on attempting to perfect the Mitsunobu reaction, including the synthesis of many different azodicarboxylate reagents. However, the reduction reaction ultimately proved to be the better method and replaced the Mitsunobu reaction, due to the fact that it undoubtedly gave the desired molecule, even though purification was necessary. Another major set of problems has been obtaining a clean product in high enough yield to make a reaction worthwhile. Because the target molecule is very complex, there are many factors that may be interfering with reactions, such as steric hindrance and interference of side groups. All of these can lead to a very small yield, and when working with expensive chemicals, this is not ideal, even if some product is obtained. Some steps have been successful on their own, although in the future these steps will need to be combined to create the target molecule.

Once the final compound has been synthesized, the compound will be ultimately attached to a dendrimer (see Figure 1), which will be provided by the TCU Simanek group, experts in dendrimer chemistry. The size of the overall vaccine molecule can then be controlled by changing the size of the dendrimer and the number of haptens attached to each dendrimer. As previously mentioned, it is important to have a large molecule in order to trigger an immune response. Also, the more TSAs per dendrimer, the more hapten-specific B cells will bind, which could increaese the effectiveness of the immune response.

After completion of the vaccine molecule and dendrimer attachment, further biological testing can be done. This is a long-term goal but very attainable once the hurdle of synthesizing the original molecule can be completed, along with successful scaling up of the reaction.

Because cocaine creates such a burden on the current healthcare system in the United States, eventual completion of this project could have a huge potential financial impact on treating cocaine addiction. Theoretically addicts could be treated with a single dose of vaccine without the need for further injections of any kind. No such treatment for cocaine addiction currently exists and the method used to create the vaccine could hopefully be applied to that of other drugs such as heroin.

EXPERIMENTAL SECTION

Preparation of the exo alcohol:[10] into a 200 ml round bottom flask was added n-Boc nortropinone (10 g, 1 eq) and methanol (75ml). At 0°C was added sodium borohydride (2.5 g, 1.5 eq) portion wise. After addition, the reaction mixture was stirred at room temperature under N_2 for 16 h. Reaction mixture was removed from heat and the methanol was evaporated. The remaining mixture was diluted with dichoromethane (50 ml) and washed with sodium bicarbonate, then dried with magnesium sulfate. The dicholoromethane was evaporated and the mixture was purified with a silica column 2:1 ethyl acetate/hexane mixture.

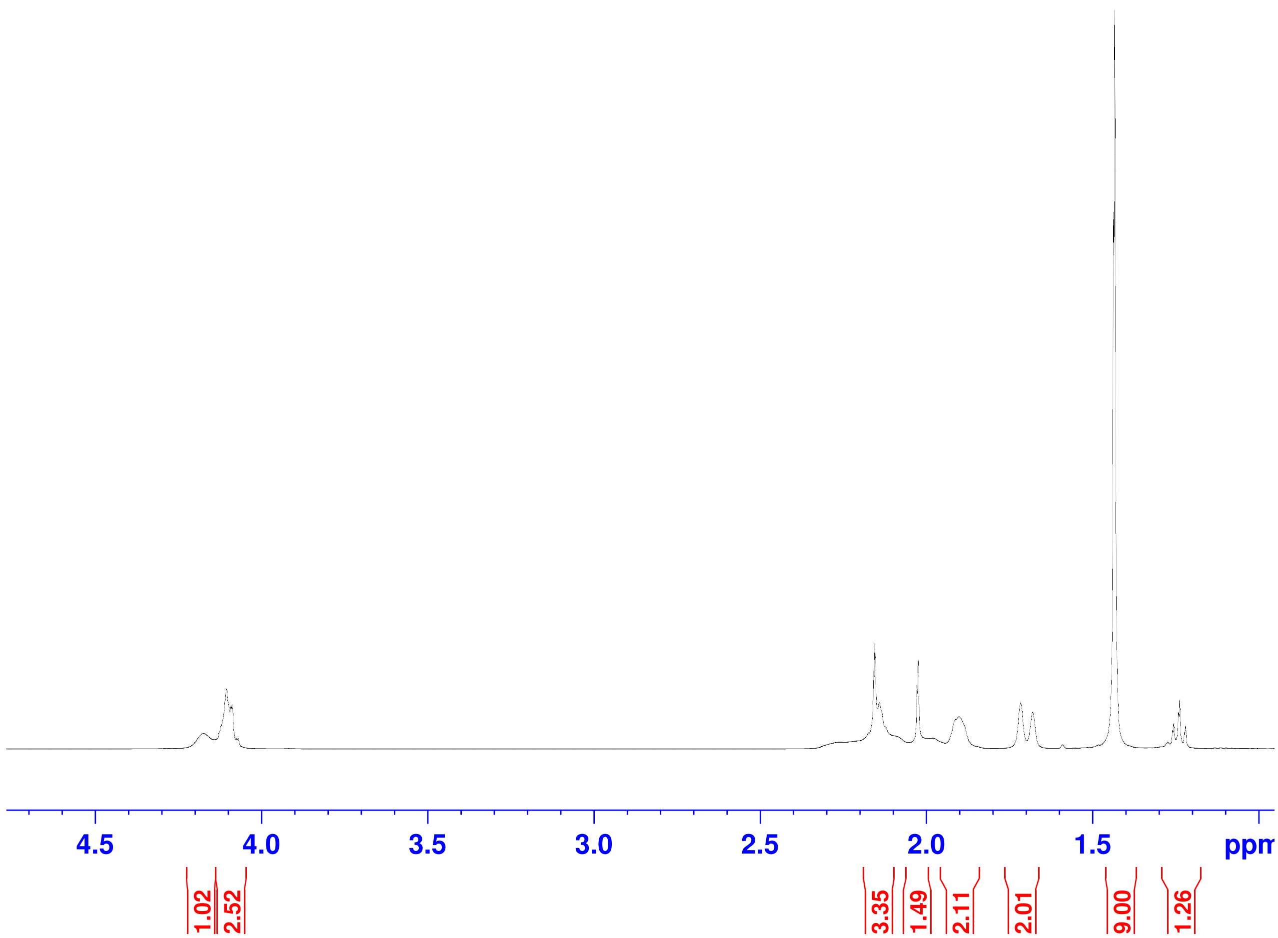


Figure 3. ^{1}H NMR of exo alcohol, solvent $CDCl_3$

Preparation of the linker molecule:[11] into a 200 ml round bottom flask was added bis(2-bromoethyl)ether (17.4 g, 2 eq), potassium pthalamide (6.9 g, 1 eq), and DMF. The reaction mixture was heated to 80°C and refluxed for 18 hours. The solvent was then evaporated and ethyl acetate was added (50 ml). The mixture was washed with sodium bicarbonate and brine then dried with magnesium sulfate. The ethyl acetate was evaporated and the mixture was purified with a silica column 2:1 hexane/ethyl acetate mixture.

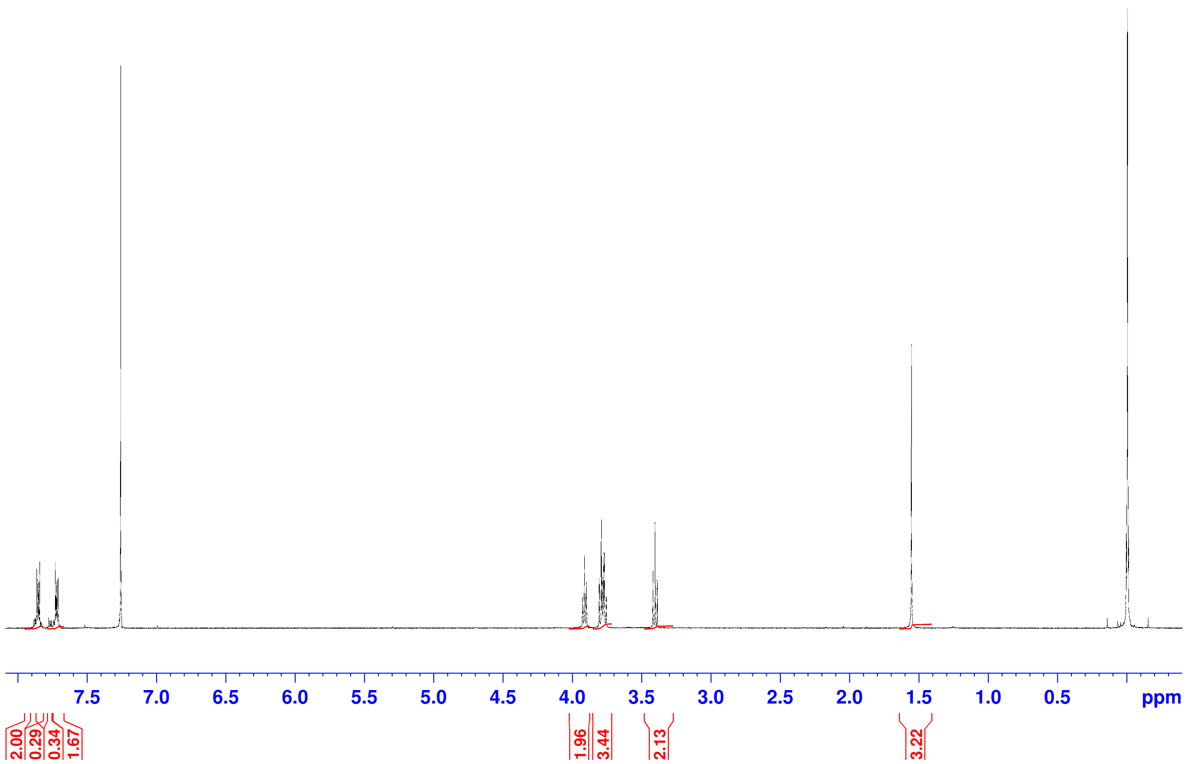


Figure 4. ^{1}H NMR of linker molecule, solvent $CDCl_3$

Preparation of the alternate linker molecule:[12] into a 100 ml round bottom flask was added dichloromethane (50 ml) and the ether molecule (8 g, 1 eq). Boc_2O (16.6 g, 1 eq) was added and the mixture was stirred at room temperature under N_2 for about 24 hours. The solvent was evaporated and the remaining mixture was purified with a silica column 2:1 ethyl acetate/hexane mixture. The product was then added to a solution of CBr_4 (21.05 g, 1 eq) and dichloromethane

(100 ml). The solution was stirred at room temperature under N_2. The solvent was evaporated and the crude product was purified with a silica column 3:1 hexane/ethyl acetate mixture.

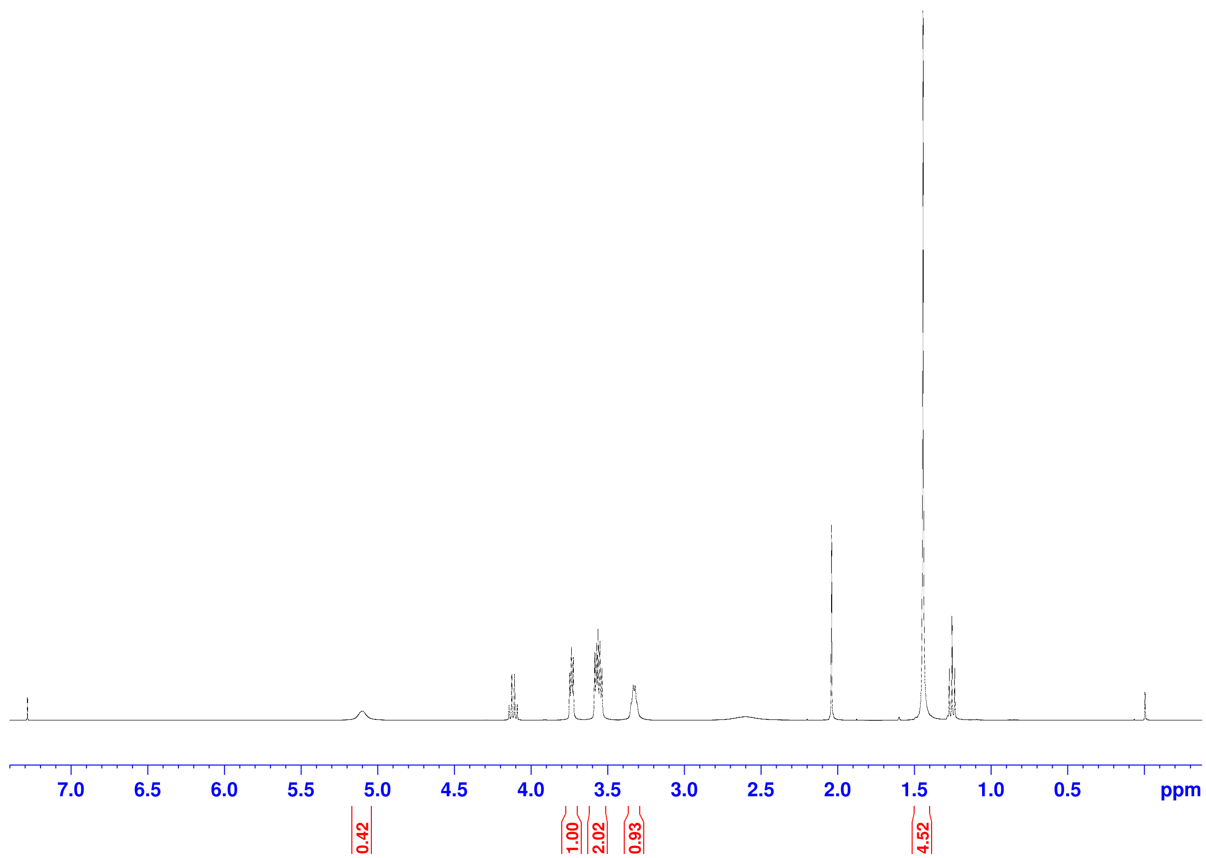


Figure 5. ^{1}H NMR of alternate linker molecule, , solvent $CDCl_3$

Preparation of benzyl phosphinate ester: into a 500 ml round bottom flask was added phenylphosphinic acid (9 g, 1 eq), benzyl alcohol (13.7 g, 2 eq), and toluene (250 ml). The flask was set up with a Dean-Stark trap under N_2 and the mixture stirred at reflux for 24 hours. The reaction mixture was then washed with sodium bicarbonate and brine, then dried with magnesium sulfate. The product was purified with a silica column 2:1 hexane/ethyl acetate mixture. To oxidize the compound to the final product, the phosphorus compound (5.9 g, 1 eq) was added to sodium bicarbonate (0.63 g, 0.3 eq) and dioxane (20 ml). Potassium permanganate

(3 g, 0.75 eq) in water (30 ml) was added dropwise. The mixture was stirred at room temperature for 24 hours. It was filtered to remove remaining solid, then acidified with HCl to a pH of 2. It was then washed with dichloromethane 3 times, then dried with magnesium sulfate to give a pure product.

Figure 6. ^{31}P NMR of benzyl phosphinate ester

Attachment of phosphinate ester: into a test tube was added Boc-nortropine (exo, 0.38 g, 1 eq), CCl_4 (0.82 g, 3.3 eq), N,N-diisopropylethylamine (0.28 g, 1.4 eq), and 4-dimethylaminopyridine (0.014 g, 0.04 eq) at 0°C. This mixture was stirred at room temperature under N_2 for 30 minutes. Next, the phosphinate ester was added (0.4 g, 1.1 eq) in acetonitrile. This was stirred at room temperature under N_2 for 2 hours. The solvent was evaporated and the mixture was washed with HCl (1 ml 1M and 4 ml H_2O) then brine, then dried with magnesium sulfate.

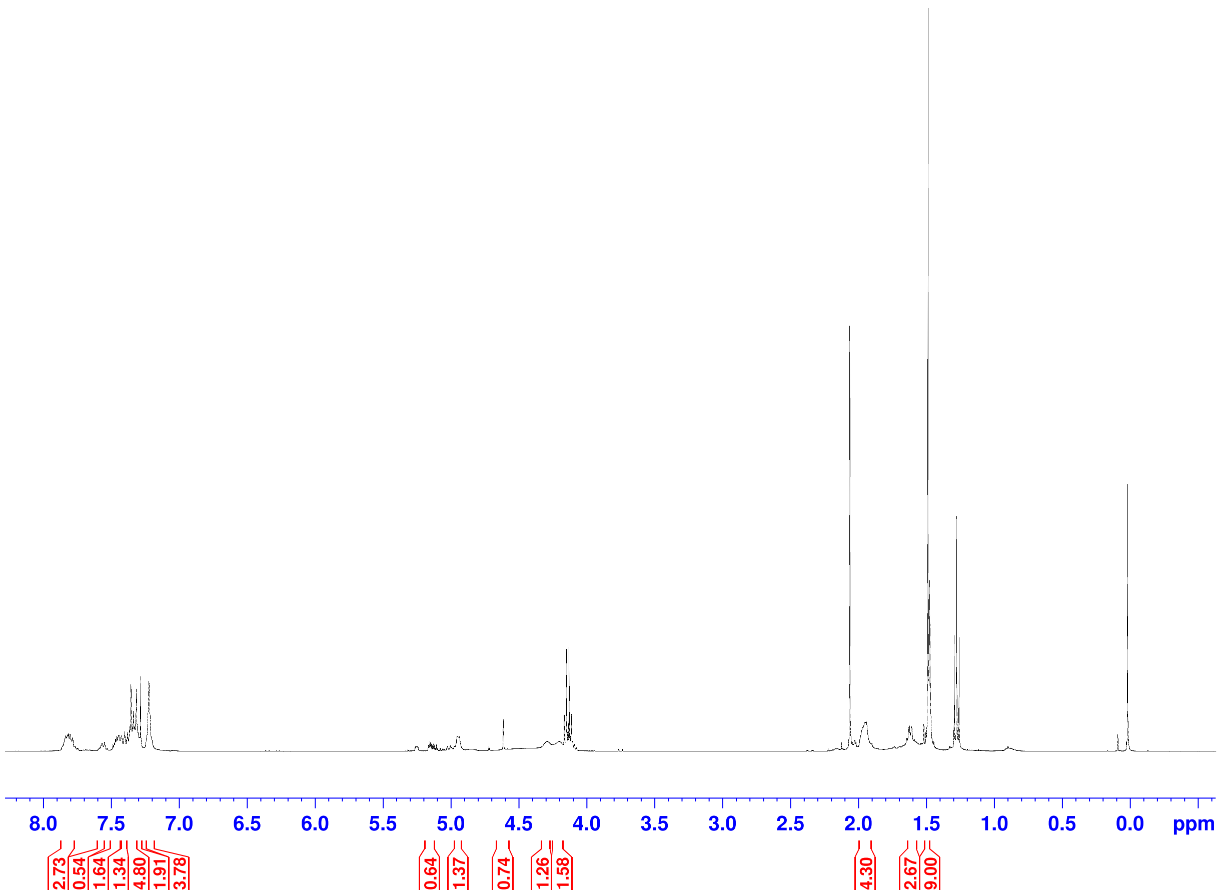


Figure 7. ^1H NMR after attachment of phosphinate ester, solvent $CDCl_3$

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