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Exploration of Fluorinated α,β-Dehydroamino Acids and their Structure

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Honors Thesis

EXPLORATION OF FLUORINATED α,β-DEHYDROAMINO ACIDS AND THEIR STRUCTURE

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ABSTRACT

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Bachelor of Science

This thesis explores the synthesis of fluorinated α,β-dehydroamino acids, specifically a fluorinated dehydrovaline derivative. Previous work has been done on the equivalent dehydrovaline derivative without fluorine present and this work builds toward the fluorinated version with the goal of comparing the two structurally. The synthesis presented here pulls from previous synthetic strategies employed for dehydrovaline while also exploring the synthetic impact of the electronegative fluorine atoms.

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Introduction

In organic chemistry, much of what we do is inspired by our observations of nature. For example, our synthetic targets are often natural products and we regularly seek inspiration from the biosynthetic pathway that the organism itself uses to make the product. Even some of the completely novel molecules we make are designed to mimic naturally occurring compounds or fit into a known protein binding site. And nowhere is the natural inspiration more prevalent than in the pharmaceutical industry.

By looking at our own biology and that of other organisms we can take advantage of millennia of evolution to help us increase our knowledge of pharmaceutical chemistry. Every species has developed its own ways of fighting infections and regulating internal problems in order to survive, so there is plenty of material to pull from. One prevalent type of molecule involved in these processes is the peptide. Peptides are present in everything from antimicrobial defenses to cell signaling in our bodies, so understanding them better should open up new opportunities for peptide-based drugs.

There are, however, some downsides to peptide pharmaceuticals and despite all their potential only a few have made their way into mainstream use. The main problem is that our digestive systems are very good at breaking down proteins and that includes peptide pharmaceuticals. This means that these peptides are not viable oral pharmaceuticals without some sort of protection mechanism. Researchers have tried to circumvent this problem by administering the drugs intravenously, by inhaling, through the nose, or even by coating them in a liposome.[1] Unfortunately, most of these methods have proven impractical and new strategies are needed if we are to take full advantage of the wealth of options nature has given us.

Researchers have also experimented with various methods of modifying the peptides themselves to increase proteolytic resistance. Proteases, the enzymes responsible for cutting up peptides, can attack peptides much more easily if the peptide is unfolded than if it is folded,[2] so finding a way to keep peptides in their folded conformation should increase their half-life in the body. Many methods for doing this have been proposed such as peptoids,[3] β and α/β peptides,[4] stapled peptides,[5] hydrogen bond surrogates,[6] γAA-peptides,[7] D-peptides,[8] α,α-disubstituted amino acids,[9] N-amino pep-tides,[10] and β-turn mimics.[11] More methods are still needed since peptide-based pharmaceuticals still have not reached large-scale commercial usage.[12] Additionally, since modifications could easily change peptide structure and perhaps render them useless, it is important that we find more methods so that we can effectively stabilize a wide variety of peptides.

The method that our lab is exploring to stabilize peptides is α,β-dehydroamino acids (ΔAAs) (Figure 1). ΔAAs are not included in the canonical 20 amino acids but there are some organisms that use them. For example, our lab's interest in them stems from our work in synthesizing Yaku'amide A (Figure 2), a ΔAA-containing peptide from the sea sponge *Ceratopsion.*[13] Our synthesis of this anticancer peptide[14] has inspired us to explore more fully the application and synthesis of ΔAAs. In other work, we have also been exploring new methods for converting these β-hydroxy amino acids into ΔAAs.[15]

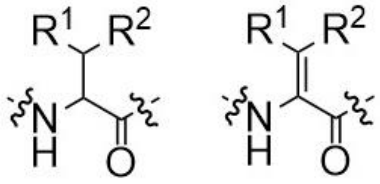


Figure 1. Regular amino acid (left) and α,β-Dehydroamino acid (right)

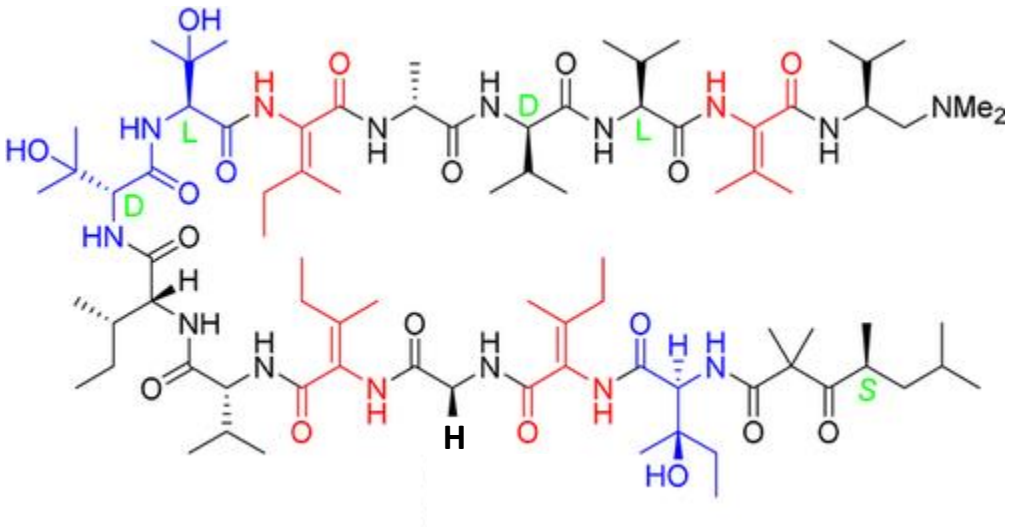


Figure 2. Yaku'amide A

We have also studied how ΔAAs stabilize peptides. The main mechanism by which ΔAAs reduce peptide susceptibility to proteolysis is by maintaining the peptide in its folded conformation. Allylic strain (Figure 3) between the R group and the peptide backbone is the main force responsible for holding the peptide's conformation since it favors folded conformations by destabilizing unfolded conformations. In particular, ΔAAs stabilize turns. We have shown this in our lab through work with β turns[16] and incipient 3-10 helices.[17] In both cases we included ΔAAs at key positions in the turns then tested the model peptides in a proteolysis assay. Results showed that some of the ΔAAs we tried did increase how long the peptides lasted in our assay, with ΔVal (Figure 4) being the most effective. Surprisingly, ΔEnv (Figure 5) was not as effective although it was bulkier. This is presumably due to its increased flexibility.

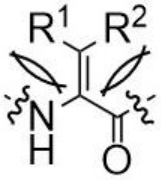


Figure 3. $A_{1,3}$ Strain

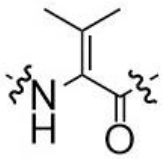


Figure 4. ΔVal

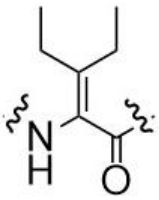


Figure 5. ΔEnv

My work specifically has been with hexafluoro ΔVal (Figure 6). We recently began exploring fluorinated versions of the ΔAAs that proved most effective in previous experiments to see what impact the fluorination would have on peptide structure. We hypothesized that since fluorine is very small, it is unlikely to affect the allylic strain aspect of peptide stabilization. Its high electronegativity, however, could influence the electron density of the molecule in new and interesting ways. Initial molecular modeling simulations gave us predicted Ramachandran plots of ΔVal and F_6ΔVal (Figure 7) with the two plots having clear differences in their preferred ϕ angles (horizontal axis). The ϕ (psi) angle represents the rotation around the bond between the α-carbon and amide nitrogen (Figure 8), so an angle closer to zero as predicted in the ΔF_6-Val Ramachandran plot would imply a more planar structure than seen in ΔVal. This gave us the justification to begin work synthesizing and testing F_6ΔVal compounds.

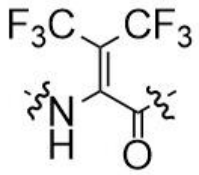


Figure 6. ΔF_6-Val

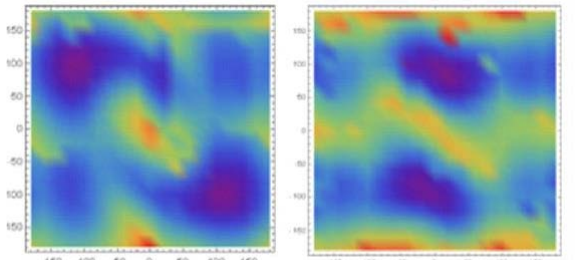


Figure 7. Ramachandran Plots
(blue = low energy, red = high energy)

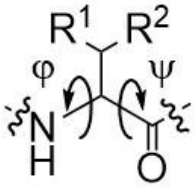


Figure 8. φ and ψ angles

We hope this work will expand the options that medicinal chemists have for making effective peptide-based pharmaceuticals. If F_6ΔVal can impart the same

proteolytic stability that ΔVal can but in different secondary structures, then we will have successfully provided new options for stabilizing peptide drugs.

Synthesis

My main synthetic goal for this project was a $F_6\Delta$Val derivative (Figure 9) that could be used as a model for some initial structural tests. This molecule was identified as a target since ΔVal was the most effective of the normal ΔAAs. The acyl group on one end and dimethyl amide on the other end allow us to examine the structural effects of the hexafluoro addition in the context of an amino acid capped by amide bonds. We planned to perform x-ray crystallography studies as well as NMR structural studies on the target molecule and then compare it to data from previous studies on ΔVal[18] to see how the hexafluoro addition changed the structure.

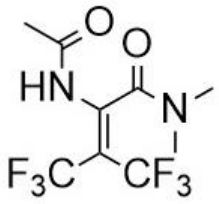
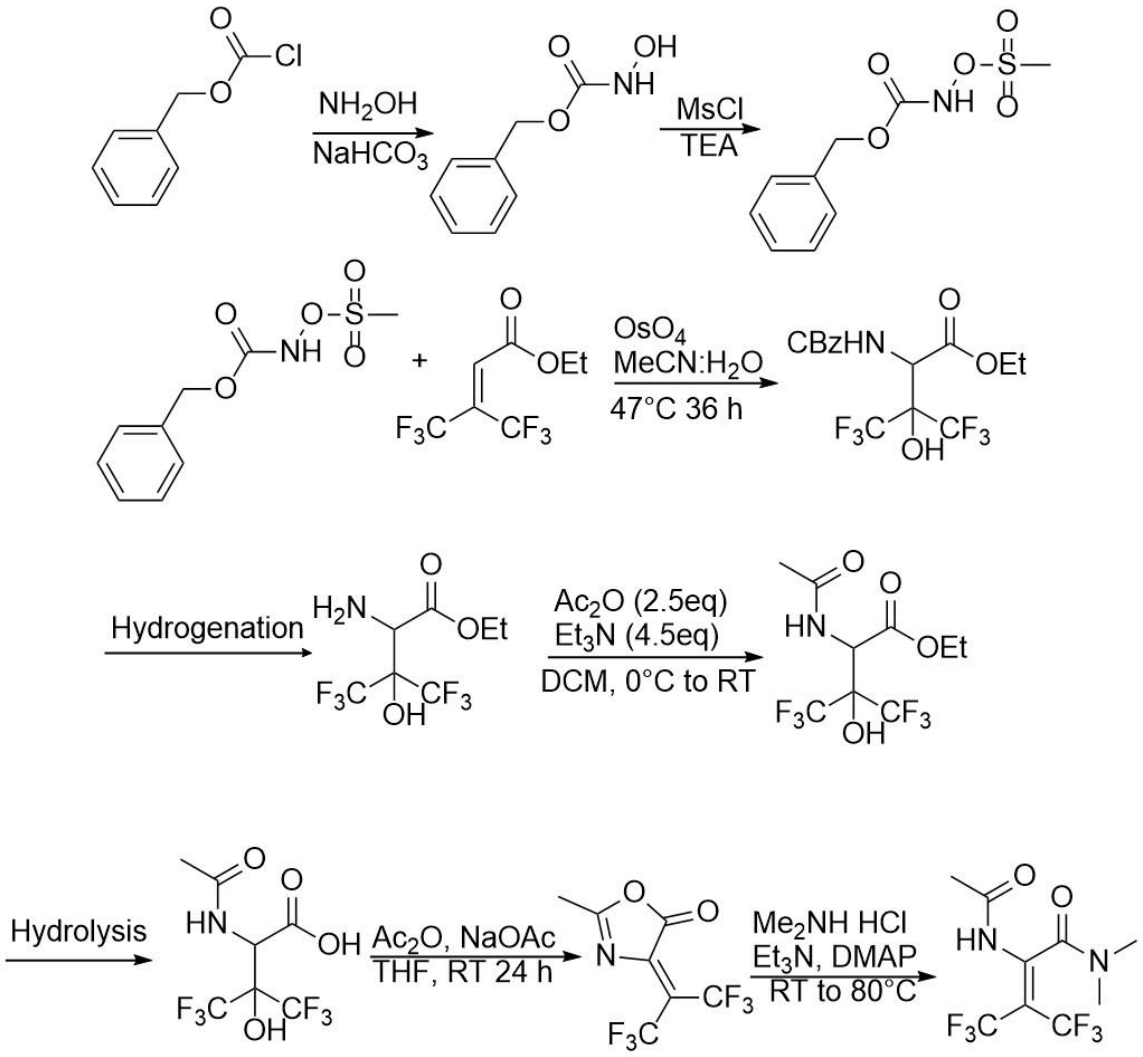


Figure 9. My Target

In order to synthesize this compound, we decided on following the general strategy shown in Scheme 1. It begins with the synthesis of a hydroxycarbamate that gets turned into a mesyloxycarbamate. These carbamates are then used in an aminohydroxylation reaction to simultaneously install our β-hydroxy group and our CBz protected N-terminus. From here the molecule is hydrogenated to deprotect the amine, then acylated to complete the N-terminus. Next the ester is hydrolyzed in preparation for an azlactone formation reaction. This azlactone is then opened with a dimethylamine to give the final product.



Scheme 1. Planned Synthesis of ΔF_6-Val for X-ray Crystallography Studies

The first two steps, carbamate synthesis, are fairly common in our lab since the mesyloxycarbamate is key to the aminohydroxylation reaction that gives us β-hydroxy amino acids that can be turned into ΔAAs. First though, we need to form the hydroxycarbamate (Figure 10). This is done by protecting a hydroxylamine with benzylchloroformate in the presence of sodium bicarbonate. The crude product is then recrystallized by dissolving it in dichloromethane and then adding hexanes and leaving it in the refrigerator. If the product is especially pure, hexanes alone can sometimes be

enough to cause it to crystallize. This reaction routinely gave yields of over 80%, with the best yield being 96%.

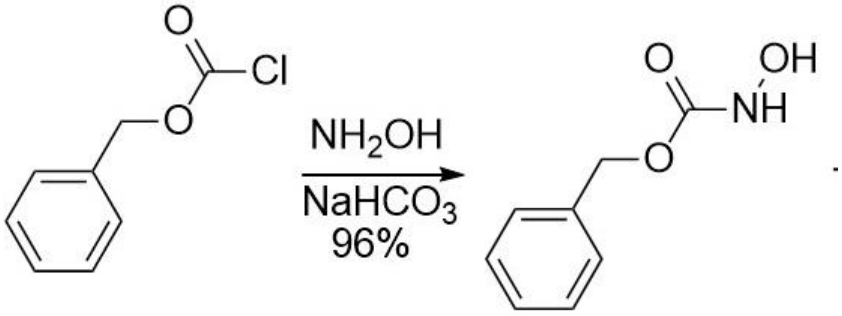


Figure 10. Hydroxycarbamate

The second step in creating the mesyloxycarbamate is mesylating the hydroxycarbamate (Figure 11). This reaction was a bit more problematic than the first but still worked more often than not. This reaction is achieved by deprotonating the hydroxycarbamate under basic conditions, then adding the mesylchloride dropwise at low temperature. The mesylchloride is added cold and slow because it is possible to form the dimesylated product (Figure 12) under certain conditions. For example, in one of my earlier attempts at this reaction I miscalculated how much mesylchloride to add and conducted the reaction with excess mesylchloride, giving almost exclusively the useless dimesylated carbamate.

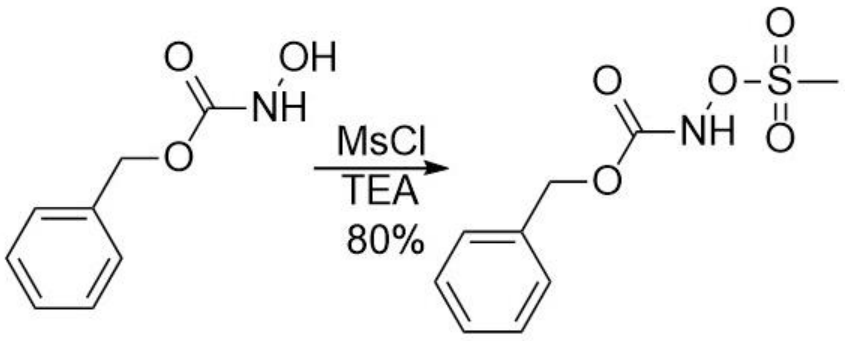


Figure 11. Mesyloxycarbamate

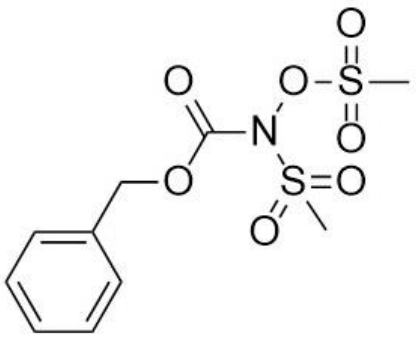


Figure 12. Dimesylated Compound

Once the product is formed, it is purified in much the same way as the hydroxycarbamate. This recrystallization is more complicated, though, as the impurities present will come out alongside the product as an oil if not enough dichloromethane or too much hexanes are used. Due to the difficulty of recrystallization and the nature of the reaction itself, my yield was lower than that of the hydroxycarbamate, generally around 50-60%, although a particularly good run gave a yield of 80%. Since starting materials are easy to come by and we perform these reactions on multigram scales, lower yields are not a major issue.

With the mesyloxycarbamate in hand I could then perform the aminohydroxylation reaction (Figure 13). This is one of the central reactions our lab does because it produces the β-hydroxy amino acids that we use in other projects and that we dehydrate to form the ΔAAs I work with. The reaction involves an addition of a CBz protected amine and a hydroxy group across the double bond of a commercially available enoate. This all happens at 47°C with an osmium catalyst. Purification of the crude product is straightforward since there are no major byproducts formed. An initial low yield on the aminohydroxylation led me to increase the amount of mesyloxycarbamate

used to 1.4 equivalents which increased later yields somewhat. Final yields were generally in the 40-50% range.

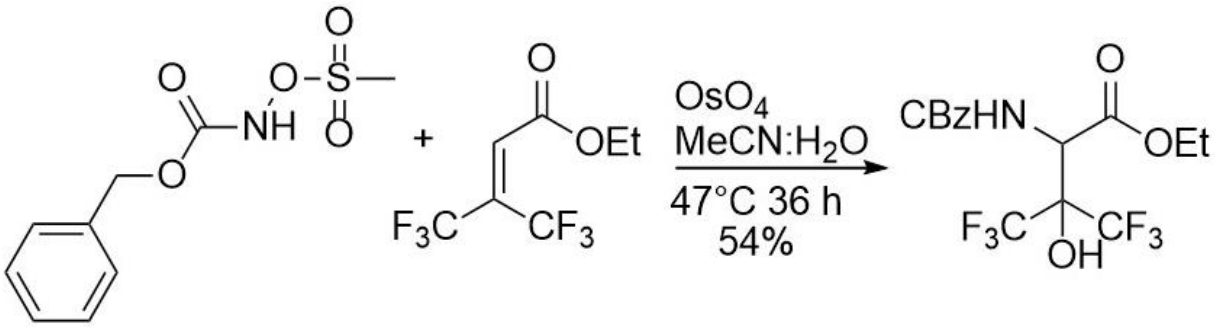


Figure 13. Aminohydroxylation

The next step, hydrogenation (Figure 14), was the first one that gave me any real trouble. Normally our method for removing CBz protecting groups is a 3-day process with Pd/C under 600 psi H_2. When I first got to this point though, our lab had recently lost one of our two hydrogenation vessels so the remaining one was in high demand and I tried a new procedure. This method uses Pd/C and ammonium formate for 4 hours although, unlike the 3 day procedure, it does require running a column. This method worked great on the β-hydroxy valine compound I was also working with at the time and regularly gave over 90% yield, so I expected it would work well with my hexafluoro β-hydroxy valine. Unfortunately, it did not. Over a few tries I managed to get a maximum of 13% yield and even then it was not totally pure. I eventually went back to the 3-day procedure which worked well and gave 88% yield after filtering through Celite.

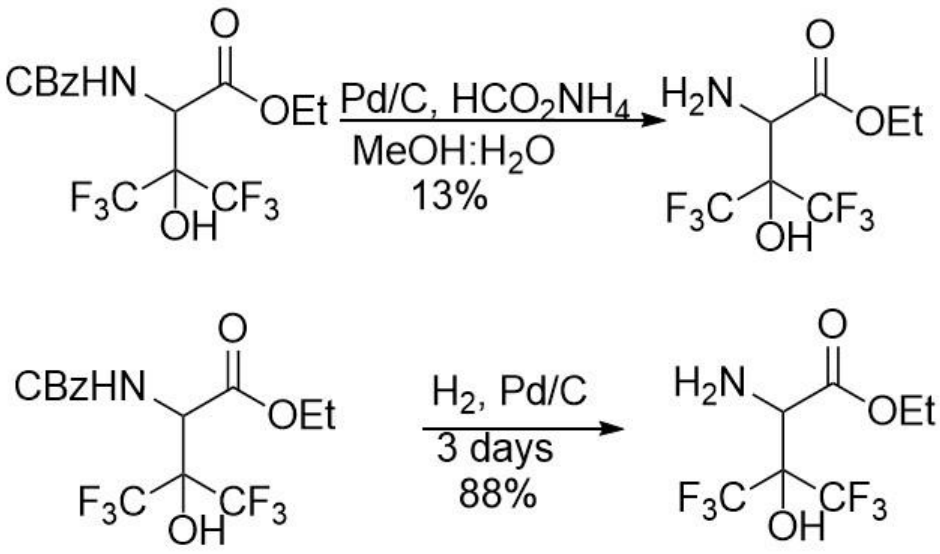


Figure 14. Hydrogenation Methods

Acylation worked immediately but with a minor complication (Figure 15). By treating the deprotected compound with triethylamine and acetic anhydride for 4 hours I hoped to install an acyl group on the N-terminus. After running a column I only saw one spot on the TLC so I assumed I had purified the acylated product, but when I acquired NMR spectra I saw two compounds in the FNMR. After analyzing FNMR, HNMR and HMBC I concluded that the β-hydroxy group was also acylated in the byproduct. Since the diacylated compound was difficult to separate from the desired product by column and because the next step, hydrolysis, should remove the extra acyl group, I decided to continue without additional purification.

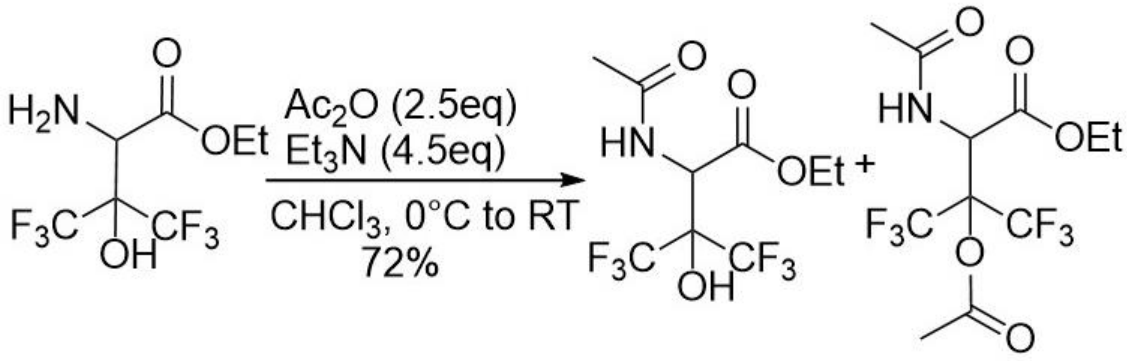


Figure 15. Acylation

Hydrolysis (Figure 16) is where things hit a wall. I first tried a base-catalyzed hydrolysis with lithium hydroxide, but my initial attempt gave a crude mixture that showed no fluorine in the NMR so we hypothesized that the compound may have undergone a retro aldol reaction. The next attempt still had fluorine present and no ethyl peaks in the HNMR but was otherwise still very messy. I took it through the next two steps but was unable to find anything resembling the final product so I tried a different method. Our lab has also regularly used trimethyl tin hydroxide for hydrolysis and so I tried that. Unfortunately, the results were very similar. NMR spectra showed that some runs were able to remove the ethyl peaks but there were far more byproducts than we expected from this reaction. I still took the resulting mixture through the final steps but did not see any promising results.

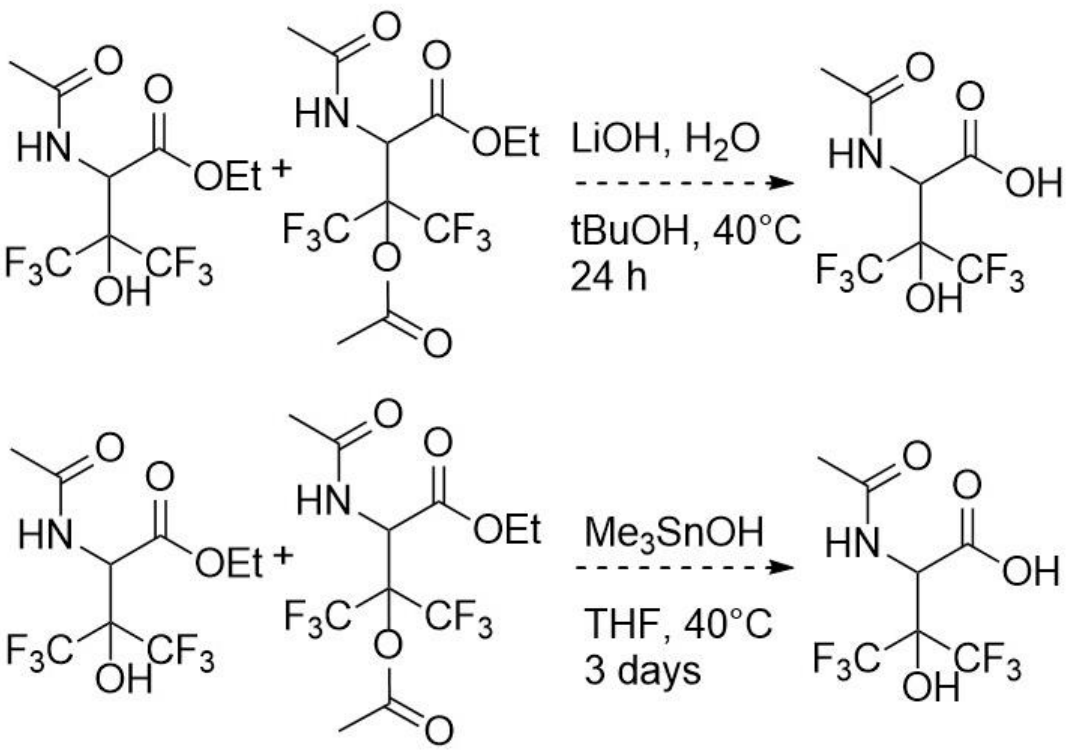


Figure 16. Hydrolysis

The final two proposed steps involved an azlactone ring formation and opening (Figure 17). This is a common method our lab has used on other projects to simultaneously form an amide bond and create the α,β-double bond. We form the azlactone with acetic anhydride and sodium acetate for 24 hours, then open it without purification with DMAP, triethylamine and an amine nucleophile (in my case dimethylamine). This step has worked well on other compounds in the past so I hoped to use this reaction to see if my compound had been successfully hydrolyzed. Since this step forms the key double bond, it should cause a significant shift in the FNMR spectrum that I could use to determine if the reaction worked. FNMR of the crude product showed exactly the same peaks as the hydrolysis product, so I concluded that the hydrolysis had failed.

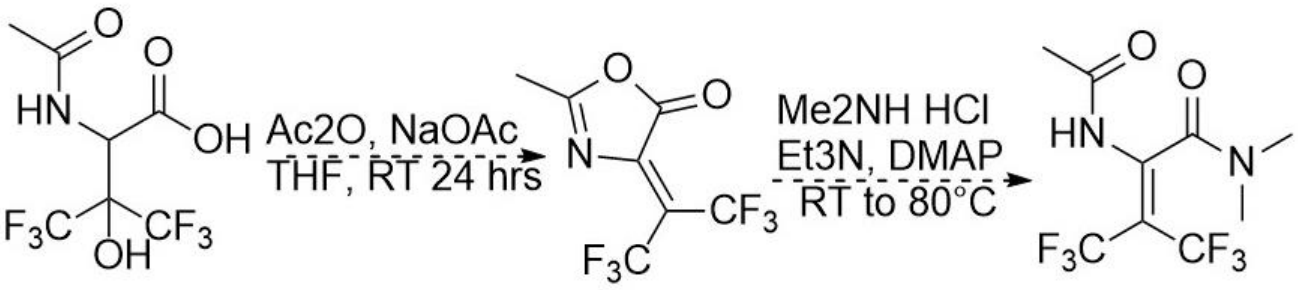


Figure 17. Azlactone Formation and Opening

Conclusion/Results

As the project currently stands, I was unable to fully synthesize and characterize my target compound. I was, however, able to make solid progress toward my target and along the way synthesize and characterize some novel compounds. The aminohydroxylation product and acylation product in particular were new advances from this project.

In addition to the new compounds, I also introduced our lab to fluorine NMR. FNMR has been incredibly useful in this project since it allowed me to monitor major changes to my compound without having to worry about contaminants. It also helped me identify ratios of products to byproducts since each compound had predictable double peaks. Finally, it would make identifying my final product easier since forming the double bond should cause a large shift in the FNMR.

Moving forward, I plan to try a modified synthetic strategy. Since the hydrolysis was the problematic step, I will proceed as originally planned up through the acylation step. Then from there I will dehydrate the compound to form the α,β-double bond. We have been working on new dehydration methods in our lab so I have plenty of options to try. From there I can attempt hydrolysis again in a different context, hopefully allowing it to work, then proceed to attach the dimethylamine with traditional coupling methods.

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