Total Synthesis Project: Synthesis and study of Warfarin Derivatives (Analologues)

Coumadin (also called warfarin) is a blood thinner. Coumadin is a vitamin K antagonist to an enzyme called a carboxylase (it has a cofactor called vitamin K) together (the carboxylase and vitamin K) they are responsible for the carboxylation process of glutamic acid residues on Vitamin K dependent cofactors. This carboxylation is important for the formation of groups that can bind calcium and through this binding, anchor and localize the cofactors to the platelet membrane. This is part of the coagulation process of coagulation. Coumadin has a structure similar to that of vitamin K and essentially takes its place and shuts down the enzyme and associated redox needed to carboxylate these groups necessary for the coagulation process.

This document has been added on to and edited every week. I think it is important to reread it if you have not been following what is going on. There is a distinct possibility you will understand it better upon a second or third evaluation.

The goal of this project is to carry out a total synthesis of Warfarin (practice) and at least one derivative (a compound that has a small structural difference and might have a different biological activity). In this project, you will in groups of three. You will work more or less at your own pace, though we will cover certain topics in lecture or discussion each week. In addition to working out the total synthesis, you will look many of the references yourself, you will learn about the importance of the target molecules yourself, you will learn all about Thin Layer Chromatography and Column Chromatography (a method of analyzing the purity and content of product mixtures and a method to purify compounds based on TLC as a model), run more advanced NMR spectra to determine structure and possibly carry out some biological analysis of your products, such as clotting rates and/or protein binding of warfarin and your warfarin derivative.

Each week you will be given some background material to explore and you will either have a lecture or a discussion session on that material. You may work ahead of this material, but it is important that you participate in the lectures and discussions. You will be responsible for the material on a quiz and when you write a journal style paper with your group at the close of the project. There will be specific lectures on writing a journal style paper because the final goal of the project is to write a journal style paper. Though there will not be regular written assignments, it is expected that you will come into lab each week with a plan and with the assigned material prepared such that you as a group or individual could get up and discuss said material at the board. It is also important that once the lecture is given on how to write the journal style paper, that your group start writing up the parts of the paper that are ready to be written such as the introduction and the experimental section.

The overall plan for the synthesis will involve making an alpha, beta unsaturated ketone (different groups will make different compounds) using an aldol reaction and then purifying, characterizing that compound. The purified aldol product will be coupled to 4-hydroxycoumarin (possibly made in the lab) and in a separate combination, attempting an experimental coupling of an aldol product to 4-methyl-7-hydroxycoumarin (4-methyl umbelliferone) which can be made by the Pechman synthesis using resorcinol. The overall synthesis is a convergent synthesis utilizing aldol products, 4-hydroxycoumarin and/or 4-methylumbelliferone (4-methyl-7-hydroxycoumarin) which are coupled through conjugate addition using a rather sophisticated chiral catalysis to form warfarin and any derivatives. The overall goal is to create compounds that are warfarin derivatives or analogues that might have different biological activity compared to the parent, warfarin.

A large amount of this project will involve purifying and analyzing products. Please come in with an open mind, being psychologically prepared to have things fail. Come with a positive view that it is not about speed, but learning and that negative results are results. Scientists have to be very prepared, very thoughtful, very careful and yes, adventurous (in a safe way). They need to have the attitude that a
negative result is a result and to try to come up with solutions and new ideas rather than having the “I want to start over.” kind of view. Lab is not about performing a procedure perfectly. I would rather have students have some new reactions not work and learn how to trouble shoot or just understand chemistry better, than to simply execute a sequence with skill. This project is designed to be a more realistic chemistry experience, but at the same time it has some structure because of the very large number of students in the class.

The following is the overall scheme:

\[
\begin{align*}
\text{4-hydroxycoumarin} + \text{aldol product} & \rightarrow \text{warfarin or analogues} \\
\text{substituted hydroxycoumarin} + \text{aldol product} & \rightarrow \text{additional warfarin analogues}
\end{align*}
\]

**Week One**

During the first week, you and your group should be prepared to do the following three activities. This means you should have and procedures written in your lab notebook and you should have read up on the mechanism for any reactions and the theory behind any purification techniques. You should have drawn diagrams of any apparatus needed.

**A. Aldol Reaction Producing alpha, beta unsaturated ketones needed for Warfarin Synthesis**

Aldol synthesis: The following is the compound that is needed for the synthesis of warfarin, however, other aromatic aldehydes can be substituted in the procedure. It is expected you will try this synthesis and one derivative with a different aromatic aldehyde such as p-methoxybenzaldehyde as shown beneath the standard synthesis.
In the discussion session this week, it is expected that you will have gone over the aldol reaction and will be ready to discuss the procedure and mechanism at the board. The aldol reaction can be readily found in your text. If you haven’t gotten to it in class, it is still your responsibility to look it up. When you come to lab you should have the scaled reaction procedure and the mechanism written in your lab book (this is the blow by blow description of the reaction showing intermediates and using arrow formalism).

**General Procedure for Preparation of Aldols**

Choose an aldehyde (several will be available) in lab for your group. Note, the procedure is written from the perspective of piperonaldehyde, but the quantities have to be recalculated for other aldehydes. It is expected that in any class all aldehydes will be used at least by one group and that the experience will not be discussed with other groups.

You can do this procedure at 5-10 times the given scale.

1. Place 0.15 g of piperonaldehyde in a 25 mL erlenmeyer flask.
2. Add 1.00 mL of acetone and a magnetic stirrer. Stir the reaction on magnetic stir plate.
3. While the reaction is stirring, add 0.1mL of the NaOH solution which is prepared in advance by you by dissolving 0.6 g of NaOH in 1 mL of water. (What is the molarity of this solution?)
4. Cap the Erlenmeyer and allow it to stir for 45 minutes.
5. Pour the reaction into 20 mL of water.
6. At this point, you should have a cloudy solution with an oil. The oil should be centrifuged out. The oil should settle to the bottom of the centrifuge tube.
7. If any crystalline compound precipitates out, it should be isolated by vacuum filtration. The filtrate from this vacuum filtration and/or the supernatant from the centrifugation should be stored in the refrigerator until next week when more compound may be isolated by vacuum filtration.

This week the oil and any isolated compound will be studied by Thin Layer Chromatography for purity. You can start with pure dichloromethane as your eluting solvent and add methanol as needed.

**B.** You will run Thin Layer Chromatography of your isolated oil and any solid isolated, using the starting materials as standards.
It is expected that you will read up on Thin Layer Chromatography and Column Chromatography. The following is a reasonably good site for Thin Layer Chromatography.

http://orgchem.colorado.edu/Technique/Procedures/TLC/TLC.html

The following is a reasonably good site to get some background on Column Chromatography, which is a related topic and Column Chromatography may be carried out next week.

http://www.youtube.com/watch?v=EytuRMS1154

You should record the chromatography techniques in your lab note book as well as the synthetic procedure you have researched. Take good notes in lab lecture and when studying any references you use.

**Thin Layer Chromatography – A Brief Description and General Instructions**

1. Obtain a Thin Layer Chromatography (TLC) sheet from the dessicator. This sheet will be about the size of an iphone, but much thinner. One side is a very thin coating of silica gel (a very polar stationary phase) that is in a binder. The silica gel is impregnated with a fluorescent compound that will glow green or orange depending on the compound impregnated. When exposed to UV light, the plates will look sort of like those glow sticks and necklaces kids have in the summer. One side is white and sort of rough, the back side is plastic or aluminum. You will do your work on the rough side.

2. You should draw a line with a pencil very lightly about 1 cm up from the lower edge of the TLC plate. This is the origin. See the diagram below.

3. You need to prepare standards. Take a spatula tip of each of the starting materials in a vial and add about 1 mL of dichloromethane. This is sort of an estimate of the solid and solvent. You should also make a small solution of your reaction mixture/crude product. If it is liquid, you should take a drop or two and dissolve it in about 1 mL of dichloromethane. Again, the solutions are just approximate. The goal is to study the components of the reaction vs. the starting materials. New spots observed after elution and visualization indicate product, whereas spots seen at the same location as the starting material standards indicate incomplete reaction.

4. The standards and the reaction mixture should be spotted on the TLC plate as indicated on the diagram below. The idea is to use a different capillary pipet (these will be available in the lab) for each compound. You dip the capillary into the solution being spotted and it will enter the capillary via capillary action (no pun intended) and then it is a good idea to blot it a bit on a paper towel so you don’t get a big blob of liquid on the end of the capillary. Then, lightly spot the solution on its mark on the TLC plate. The idea is to spot it and get the smallest spot possible. Then reapply it several times going for a small concentrated spot. You need to let the dichloromethane evaporate between each application. You will do this for the standards and the reaction as diagramed. It may take you a couple plates to get the hang of it. It is important for all members of your group to get the hang of this technique.

5. Realize it is really important to spot about a cm up from the bottom of the plate, to keep the spots about a cm apart and to spot in from the edge of the plate about a cm as diagramed.

6. Preparing the Eluting chamber. We will use a makeshift eluting chamber that will be either a 250 mL or 400 mL beaker with about 0.5-1.0 cm of dichloromethane with about 1.0 mL of methanol added (eluting solvent). You need to use a piece of filter paper as diagramed below to
saturate the beaker’s atmosphere with the eluting solvent. Use a watch glass to cover the chamber.

7. **Eluting the TLC plate.** Dip your TLC plate in the solvent. It is important that the solvent does not immediately touch your spots. If it does, you need to adjust the level of the eluting solvent or spot a new plate. The former is preferable. The plate should be dipped, but also resting against the wall of the beaker. The back side toward the beaker, but on the opposite side of the beaker from the filter paper. The solvent rises up the plate by capillary action. It will take about ten minutes. In this process, there is a competition for the compounds by the silica gel on the plate (very polar – the stationary phase) and the solvent system (varying degrees of polarity, but less polar than the stationary phase – this is the mobile phase). The compounds are in equilibrium between the stationary phase (the silica) and the mobile phase (the solvent). If the compound is extremely polar it will spend most of its time interacting with the silica gel and it won’t move much. If it is of very low polarity (provided it is soluble in the eluting solvent), it will spend most its time in the solvent and will move way up the plate. Compounds move to varying degrees depending on their polarity. Compounds that are the same, will elute to the same point on the plate, so your standard lanes will help you identify compounds in the reaction mixture.

8. When the solvent is about a cm from the top of the plate, you should remove the plate and lightly mark the level of the solvent. This is called the solvent front and its level is significant. Let the solvent completely evaporate off in the hood. The spots are visualized (most of the compounds we are working with are white and are not readily visible) with a UV lamp. Be careful not to expose your hands or your neighbors to the UV lamp. The background will fluoresce as previously described and your compounds should show up as blue or black spots in the background. Lightly circle them with a pencil. After this, you will more permanently visualize the compounds with the p-anisaldehyde stain. This entails dipping your plate into the stain with forceps and then, blotting the excess stain off with a towel. The plate then needs to be baked in an oven at about 100 °C for ten minutes or so. Be careful with the stain as it contains strong acids. Warfarin and derivatives stain purple, 4-phenyl-3-buten-2-one stains red and 4-hydroxycoumarin does not stain at all (white). This is very useful to tell what is going on. What would you expect on the first week? If it did not occur to you earlier, 4-hydroxycoumarin and 4-phenyl-3-buten-one are the starting materials that need to be spotted on the plate, flanking the reaction mixture. What would you expect next week?

You should record the chromatography techniques in your lab note book as well as the synthetic procedure you have researched. Take good notes in lab lecture and when studying any references you use.

**Summary of Week one:**

1. Aldol net reaction in notebook with procedure.
2. You will have attended lecture on overall project, aldol reaction and column chromatography.
3. You will be prepared to discuss the aldol mechanism in discussion with instructor/TAs.
4. Experimentally you will carry out the aldol for your derivative and for warfarin without purification.
5. You will study by TLC any materials isolated from your Aldol.
6. You should run the NMR of your flutamide product.

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**Week II**
This week you should plan to do another aldol (a different one). If you are doing aldols with 4-chlorobenzaldehyde or 3-nitrobenzaldehyde, it is a good idea to cool the reaction while the reaction is running in an ice bath. This reaction can be worked up and refrigerated similarly to your reaction from last week.

This week you should also complete the purification and characterization of the first aldol you attempted (the one you did last week). When you arrive at lab, allow your aqueous filtrate or supernatant from your aldol reaction last week to come to room temperature and filter out any crystal that have formed. You should analyze this material and any material you isolated last week against your starting material standard from last week via TLC. You may discover that the new crystals require no further purification and can be analyzed directly by NMR, IR and eventually melting point.

It is sufficient to continue to use pure dichloromethane as the solvent for TLC, however, some students obtain better results (better resolution) with dichloromethane with a few drops of methanol. Methanol increases the polarity of the solution. Given this fact what would you anticipate upon visualizing the TLC plate?

Once you have established the purity of your product mixtures, any that are not pure should be purified by column chromatography. It was observed last week that nearly every solid and oil initially isolated was at least contaminated with some starting material or by-product. Note also, there is often some of the corresponding carboxylic acid in aldehydes as they are prone to auto-oxidation. Benzoic acids tend to have very short retention times (why?) and do not stain with the anisaldehyde stain.

Regarding Staining – please allow your plate to dry completely before just dipping it in the stain. Then allow it to be reasonably dry before heating with the hot gun, oven or hot plate.

Please watch the following video, but also make note of the following instructions that are more tailored to our lab. I will also be sending you a new youtube video that will be our official technique video for the lab. This will be sent to you shortly.

The following is a reasonably good site to get some background on Column Chromatography, which is a related topic and Column Chromatography may be carried out this week.

http://www.youtube.com/watch?v=EytuRM51154

Basic Instructions for Packing and Eluting a Silica Gel Gravity column.

1. A small column will be provided to your group. Clamp the column using a mini-clamp to your monkey bars. Take the cap off the top and off the bottom of the column.
2. Place a 125 mL Erlenmeyer under your column.
3. Before working on the column, prepare a test tube rack with test tubes to collect fractions from your column. Smaller test tubes are better, but we may have to use larger test tubes, due to limited equipment. Label the test tubes with numbers using the tape we have in the lab. Every test tube does not have to be labeled, but you might want to label every fifth tube.
4. Put a small amount of sand in your column (about a two or three centimeters).
5. Attach a syringe needle to the bottom of the column. Be careful.
6. Use a black rubber stopper to block the needle, by piercing the black stopper with the needle.
7. Pour ca. six centimeters of dichloromethane (or other solvent) into the column. This is the eluting solvent.
8. Weight out six or seven grams of silica gel into a 125 mL Erlenmeyer and add about 30 mL of dichloromethane. The silica gel will form a suspension in the solvent. Take the rubber stopper off the needle and after swirling the silica gel flask, start pouring the silica gel suspension into the
dichloromethane in the column. It may flow rapidly in which case it is very important to control the flow. To do so, use the column cap. By screwing on the column cap, you can slow the flow. This is tricky and requires some finesse.

9. As the column flows, the silica suspension concentrates in the bottom of the column. There will be a layer of solvent on top of the silica concentrate as the silica settles. It is very important that the column not run dry. So, use the cap and the rubber stopper to control the flow.

10. You can put a small layer of sand on top of the column by sprinkling a few millimeters of sand through the layer of solvent. It will settle on top of the settled silica gel and serve as an important barrier to keep your sample from running dry.

11. Carefully allow the solvent to drain out to just above the sand layer. Use the rubber stopper to stop things if necessary!!!

12. The amount of silica gel can be used to separate 0.1 to 0.2 grams of material.

13. Weigh out about 0.2 grams of your crude material and dissolve it in a small amount of dichloromethane (the smallest amount possible – ten drops or so).

14. Using a pipet gently add this material to the top of the column. Normally, I run it down the side of the column so it does not perturb the column bed.

15. Carefully allow this to run into the column (do not allow column to run dry).

16. Use a small amount of dichloromethane to clean the compound off the sides of the column and allow this to run in (do not allow the column to run dry).

17. Do this several times until you are confident all the sample is loaded on the column.

18. After doing this, fill the column with the solvent and begin running the column at a slow rate (use cap to regulate flow) and collect the eluent (the solvent coming off with your compounds) in two mL fractions in your test tubes in the rack. This means every two mL or so, you change to the next test tube by moving the rack.

19. The column should parallel your work with your TLC plates. The material that is on the top of the TLC plate will be eluted first (will be found in the earlier fractions off the column). Your product should come off later in the process.

20. The way you figure out what is in each fraction is by spotting four or five spots of every other fraction on a TLC plate vs. your starting material standard. Each plate could have as many as four fractions and you should put the starting material in the middle as a reference. You should elute the plates as you did last week and visualize using the UV lamp and the stain.

21. Your goal is to ascertain which fractions contain your product in a pure form. Once these fractions are determined, they should be combined in a tared flask and rotavapped to dryness. If need be you can run another column to isolate more material. Smaller roundbottoms are better.

At the end of the day, any material that is pure by TLC can be allowed to dry until next week and then studied by NMR, IR, and melting point. Remember you need masses on all your products. Keep good records in your notebook.

Regardless of your situation (meaning if the bulk of your material appears pure by TLC), you should take some of your crudest material and run a column. It is very important to get the hang of chromatography for future work.

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There are many possible coupling reactions to make warfarin-like compounds that can be attempted and we will start one or two of these next week, if we have pure material. These reactions will be started and continued next week.

1. E-4-phenyl-3-buten-2-one (out of bottle) + 4-hydroxycoumarin (out of bottle) \[\rightarrow\] warfarin – good for comparison at end of study.
2. E-4-phenyl-3-buten-2-one analogue (made by you through your first aldol) + 4-hydroxycoumarin made by you or 4-hydroxycoumarin analogue made by you next week) \[\rightarrow\] warfarin analogue.

3. E-4-phenyl-3-buten-2-one analogue (made by you through second aldol) + 4-hydroxycoumarin made by you or 4-hydroxycoumarin analogue made by you next week) \[\rightarrow\] warfarin analogue.

I could keep writing combinations, but if you think about it, other than the basic (out of the jar synthesis of warfarin), I am anticipating that you would make four other compounds, i.e., all the permutations of two aldols and two 4-hydroxycoumarins that are synthesized by you. You will create your own little library and with your class you will make a larger library (this is why it is important to try to do different things).

You will try all of these on a small scale, probably starting with no. 4 to practice – starting next week.

To prepare for next week you should look up the procedure we are going to use to make 4-hydroxycoumarin analogues (the other part we need to make warfarin analogues) and record the procedure in your notebook. You will likely carry out one or two of these procedures during week three. The paper is a paper by Gao, W-T et. al and it was in *Synthetic Communications* in 2010. We will probably work on at least three times the scale given in the procedure.

Synthesis of 4-hydroxycoumarin analogues.

Start trying to figure out the mechanism of the reaction, though Dr. Nerz will go over some of it in lecture.

Additionally, you should begin reading about how anticoagulants such as coumadin (warfarin) work in the human body. Save the references for your paper.

**Summary of Week II**

1. Attend lecture on reactions and procedures of the week.
2. Watch video on how to pack and run a chromatography column. Very important.
4. Isolate any crystals from your aqueous filtrate or supernatant from last week.
5. Establish purity of these crystals using TLC. Make sure you have mastered TLC.
6. Run your column to purify some of your crude aldol product. Combine fractions containing your product and rotavap it to dryness.
7. Start characterizing products as you isolate them by getting masses, running IR, NMR TLC.
   Note: I would like every group to run one carbon NMR over the course of the project.
8. Look up synthesis of 4-hydroxycoumarin analogue procedure and write it in your notebook. Possibly start making one?????
9. Start working out the mechanism for this synthesis in your notebook.
10. Start reading about how Warfarin and warfarin derivatives work. Write down your references for future paper.
11. Leave compounds to dry for future melting point, IR, NMR analysis.

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Week III

1 Synthesis of 4-hydroxycoumarin and analogue.

   a. Last week you should have looked up the procedure for the synthesis of 4-hydroxycoumarin and analogues. This procedure should be written in your notebook. Each group should make two. One should be 4-hydroxycoumarin itself made from phenol, Meldrums acid and Eatons reagent. This reaction should be done on three times the written scale. The other should be an analogue. The analogue, which will be either a di-t-butylnalogue or a mono-t-butylnalogue, should be done on the scale written. It is very important not to waste materials as they are expensive.

   b. To facilitate these rather easy but inconvenient reactions, I will have sand baths preset at 100 and possibly at 70 degrees celcius so you don’t have to mess around too much with temperature. We will leave these on all day, every day.

   c. The set up will involve a simple reflux and will require stirring as well as heating. As you may recall, stirring through a heater is hard, but doable.
   I would set the phenol reaction up in a 50 mL flask with reflux condenser and the analogue in a 25 mL flsk with reflux condenser.

   d. The reactions have to heat for three hours with Meldrum’s acid.
   At the end of the time, the Eaton’s reagent is added. Though I worked out a way to do this over two days, I am worried about the long delay over break, so I decided to stay late and do a bunch of work I need to do and quenc the reactions. So you will leave the reaction after having added the Eaton’s reagent at 70 degrees. Several hours later I will just add water to them and put them in the cold case till after break.
   It is imperative that your round-bottom be meticulously labelled and that you have a beaker to put it in and that you have a cap ready so I can get done fast.
   e. Then when you get back from break you, can isolate solid and recrystallize it.

2. During your three hours of heating you can work on getting your unsaturated, conjugated ketones ready for warfarin synthesis. This means purifying, running columns if need be. This means redoing aldols if need be – if you did not get enough. This means characterizing compounds, mass, IR, NMR TLC.

3. You might also want to try a warfarin reaction with 4-hydroxycoumarin and 4-phenyl-3-buten-2-one out of the bottle to practice. To prepare, you should look up the procedure for this reaction as described below.

   a. Start first conjugate addition coupling to make Warfarin. This will be your practice run and very important. You have to get the reaction to work before you start on the harder versions. To prepare for this you need to find a green synthesis of warfarin. It was carried out using 4-hydroxycoumarin and 4-phenyl-3-buten-2-one with chiral 1,2-diamino-1,2-diphenyl ethane as the catalyst. We will be doing the
small scale synthesis in THF and acetic acid as outlined below. This is a fairly recent Journal of Chemical Education article and as I recall, you have to download the procedure from supplemental materials. The reaction is done in a small vial and is very easy to set up. You must label your reactions. It is very important. It is very important that you bring the journal article to lab and write the procedure in your notebook.

The Green Synthesis of Warfarin.

4-hydroxycoumarin aldol product

warfarin

b. Run TLC of starting materials above as a baseline. Develop your TLC in dichloromethane with a couple drops of methanol. Visualize with the UV lamp as well as using the p-anisaldehyde stain that will be provided. Record all observations in notebook. What colors are the spots with p-anisaldehyde? Make sure you are recording tlc data or saving the plates. At this juncture, you should start recording Rf values. These are the distance of from the origin to the middle of the spot divided by the distance from the origin to the solvent front. Keep good notebooks. Good notebooks lead to good papers.

Starting this week, TAs will be looking at your notebook and giving you a grade of check, check plus or check minus. Please prepare well for lab.

If you do not get to this test reaction this week it is OK.

Summary of Potential work for Week III

1. Attend lab lecture, review the video on packing Columns.
2. Analyze and purify material material from last week (aldol products).
3. Do further purification?
4. Set up 4-hydroxycoumarin reactions and carry them through Eaton’s Reagent. Dr. Nerz will quench.
5. Do first Warfarin reaction (test reaction out of bottle), small scale and look at reaction by TLC.
6. Look up references for procedures, record in notebook, read about compounds you are making.

Week IV

Under Construction.

Realize you might not finish everything. At this point groups will start to not all be at the same points.
2. **Analyze compounds isolated from last week.**

We will have NMR time. You probably want to make up two samples, one from your column and one from your vacuum filtration and we will establish by nmr which is most pure. It would be good if in advance you think about what the spectra will be like for the alpha-beta unsaturated ketones should look like. You will be running solids as films or pellets. If you would like to learn how to run a pellet, please see me or a TA. You also need to run IR of both and melting points. If you have impure compound. You may need to run another column. Running columns is an art and it does get better as you go along. We found in general that the larger columns were better because you can get more solvent in and run the columns faster. Does this make sense. It can save up to an hour. You may have to pump on your compounds to make them purer. You can talk to us about that.

3. **Start first conjugate addition coupling to make Warfarin.** This will be your practice run and very important. **You have to get the reaction to work before you start on the harder versions.** To prepare for this you need to find a green synthesis of warfarin. It was carried out using 4-hydroxycoumarin and 4-phenyl-3-buten-2-one with chiral 1,2-diamino-1,2-diphenyl ethane as the catalyst. We will be doing the small scale synthesis in THF and acetic acid as outlined below. This is a fairly recent *Journal of Chemical Education* article and as I recall, you have to download the procedure from supplemental materials. The reaction is done in a small vial and is very easy to set up. You must label your reactions. It is very important. It is very important that you bring the journal article to lab and write the procedure in your notebook.

**The Green Synthesis of Warfarin.**

![Diagram of the Green Synthesis of Warfarin](image)

4. **Run TLC of starting materials above as a baseline.** Develop your TLC in dichloromethane with a couple drops of methanol. Visualize with the UV lamp as well as using the p-anisaldehyde stain that will be provided. Record all observations in notebook. What colors are the spots with p-anisaldehyde? Make sure you are recording tlc data or saving the plates. At this juncture, you should start recording Rf values. These are the distance from the origin to the middle of the spot divided by the distance from the origin to the solvent front. Keep good notebooks. Good notebooks lead to good papers.

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Week IV

At this point you have learned most of the techniques needed for the project. So the following will be simply a list of things you can be working on for the next couple weeks.

1. Lab lectures will include workshops where you write out the mechanism for the catalysis of the formation of Warfarin from coumarin compounds and aldol products by 1, 2-diamino-1,2-diphenyl ethane. You will receive a worksheet to work on with your instructor and TAs. There will only be two more weeks of lab lecture. The first week we will talk about carbon NMR, the proton nmr of warfarin and the first step in the catalysis.

2. It is important to check your Warfarin Reaction using TLC in dichloromethane with a few drops of methanol as your developing system and the UV lamp as a preliminary visualizing system and the p-anisaldehyde stain as a more permanent visualizing agent. It is up to you to decide if your reaction is complete or if it needs another week of work.

3. You need to check your 7-hydroxy-4-methylcoumarin (4-methyl umbelliferone) for purity. Measure the mass, melting point, IR and proton NMR.

4. At this point, you can decide which if any of the above microscale reactions outlined on page nine you should carry out. You have already done no. 3 (or have it in progress). If your traditional Warfarin reaction (no. 3) appears to be working you should definitely set up no. 4 – this is most likely to work well. If you can set it up on two or three times the microscale level given in the procedure (we used the small scale – do not use the large scale, it uses too much catalyst and the catalyst is expensive).

You are welcome to also do 1 and 2, but do these on the small scale since they are less likely to work well.

If you set up three microscale reactions, you should test them via TLC with UV lamp and staining against standards before you leave lab so you have a baseline. Remember to make the solutions just take a drop of your reaction mixture and put it in 10-20 drops of dichloromethane. Take a spatula tip of your starting materials and put them in 10-20 drops of dichloromethane. Note: It is important in all TLC to let the plate run up to within a cm of the top of the plate, to make small concentrated spots (see me or one of the TAs about making smaller spotting pipets), to not spot too close to the bottom or the edges, to let the plate dry before visualizing, especially before dipping in the stain. It is important to let the stain dry before baking it. When keeping a record of your TLC plates, you need to record Rf values of all important bands, especially products. This is a ratio of measurement of the height of the center of the band in question from the origin to the height the solvent rose to the origin. The Rf values for products should be included in the data you include in your journal style paper – in the experimental section.

5. How is your no. 3 reaction coming (your test of the catalyst mediated coupling of 4-hydroxy coumarin with E-4-phenyl-3-buten-2-one). Is there evidence of warfarin being present (warfarin stains bright blue or purple with the anisaldehyde stain). Does the reaction appear to be finished? Do you believe it needs more time? More catalyst? You can base these decisions on the plate and you can discuss them with myself and the other instructors. Hopefully you can start isolating this warfarin by column chromatography by next week. If you think it is finished you could run the column this week! In any event, you should work out a solvent system using TLC and dichloromethane that move the warfarin product about halfway up the plate and separates it well from its contaminants such as the starting material. This could be
accomplished by trying different combinations of methanol and dichloromethane with your product mixture vs. the standards. I hope the use and value of TLC is being understood and appreciated.

Brief Summary of Week IV (what you might be doing)
1. Testing your Warfarin Reaction via TLC to see if it is proceeding.
2. Deciding whether to let your warfarin reaction to proceed or to separate it via column chromatography today. If so, working out the chromatography conditions as described above.
3. Setting up your other crosses, 1, 2, 3 – do three on two or three times the procedure scale. We are doing the small scale reactions. The catalyst is expensive. Get baseline TLC on all.
4. Characterize your Pechman product using NMR, IR and melting point.
   If it is not clean, recrystallize it again. Without this, obviously, you can’t do crosses 1 and 2.
5. Learn how to use a flame to make smaller pipets.

Weeks V and VI

1. First a few notes sent via email.

I have been told that students do not understand what we are doing in lab. Of course, I feel bad about this.

I am going to go over the project again on Monday and Tuesday in lab lecture.

I am also going to go over the complete mechanism of the warfarin reaction.

Just briefly........

Coumadin (also called warfarin) is a blood thinner. Coumadin is a vitamin K antagonist to an enzyme called a carboxylase (it has a cofactor called vitamin K) together (the carboxylase and vitamin K) they are responsible for the carboxylation process of glutamic acid residues on Vitamin K dependent cofactors. This carboxylation is important for the formation of groups that can bind calcium and through this binding, anchor and localize the cofactors to the platelet membrane. This is part of the coagulation process of coagulation. Coumadin has a structure similar to that of vitamin K and essentially takes its place and shuts down the enzyme and associated redox needed to carboxylate these groups necessary for the coagulation process.

Please see readings outside my door.

You are making two to four different Warfarin analogs in the hope that they will exhibit different anticoagulant or blood thinning activity. Different structure may equal different function. It is totally possible that some of our compounds have not been made and are novel anticoagulents with unique activity. This is not just a repeat of some old organic lab.

The idea is that each of the four compounds you are potentially making and using have different activities in the carboxylase reaction (structure is related to activity). It could be that a given compound is more active or less active.
Does it make sense that if you vary the structure it could vary the activity of the drug. Does it make sense that if you make and purify different structures that are related to warfarin that they could be antagonists to vitamin K to different extents.

It is noteworthy that 4-methylumbelliferone is active in its own right. Yes, just making this compound is an accomplishment and can go in your paper.

Is it clear that we made warfarin itself as a model for everything? It is your TLC model, your practice for doing the chemistry to make other, possibly unique compounds, your model for column chromatography.

The idea is using the very well developed synthesis of warfarin from 4-hydroxycoumarin and 4-phenyl-3-buten-2-one as catalyzed by 2, 3-diamino-2,3-diphenylethane you are attempting that same chemistry with slightly different substrates.

By reacting 4-hydroxycoumarin with say, 4-(4-chlorophenyl)-3-butene-one in the presence of the same catalyst you might be able to make a different anticoagulant that would have exactly the same structure as warfarin except for the presence of a Cl. Some groups have nitro groups, some methoxy, etc. You are trying to generalize the reaction to make warfarin to make a slightly modified structure.

Then you carried out the Pechman - this produced 4-methylumbelliferone.

4-methylumbelliferone could be used in place of 4-hydroxycoumarin to make two more compounds.

So if adding A + B results in warfarin, where A is 4-hydroxycoumadin and B is 4-phenyl-3-buten-2-one.

Adding A+ C where C is slightly modified from B could results in a slightly modified compound that might have slightly different biological activity.

But what if you change A to 4- methyl umbelliferone? Then by reacting with compounds B and C, respectively you could create two more potential anticoagulents. Does this make any sense?

Realize it is my expectation that you probably will not complete all four compounds. You might complete A + B and A +C from beginning to end, including analysis.

The 4-methylumbelliferone reaction if started and just analyzed by tlc will probably be adequate. It is not expected or desired that you work outside your lab time.

Finally, what you are doing is new and some of it is unique.

I hope this makes some sense. I have copied some info on coumadin (warfarin) and it is outside my door. You can pick it up, it might help you with your paper and getting the relevance of what you are doing.
Finally, I am still working on this, but I am ordering anticoagulant kits and we may do some testing of our compounds just for fun for those who are interested. It does involve using a prick of blood. Surprised I did get permission from our head of safety. Cool

Regardless, it is purely voluntary.

2. Please pick up the supplementary readings outside my office – one packet per group please. If you have not been doing background reading as shown above, these should help you inform yourself and give you good models for style. Also, there is some good spectral data included for warfarin compounds.

3. I will go over the big picture again during lab lecture and I will go over the mechanisms. Please take good notes. The lecture will also be available by tegrity.

5. This week is the last week of lab lecture and it is the second to last week of organic chemistry lab. Lab will be over next week and you will end wherever you are.

6. It is your responsibility to reread this entire document so you begin to understand what you are doing.

7. This week I would imagine your Warfarin reaction – no. 4 – the original model reaction should be near finished. If it is you should have worked out a solvent system on TLC that will reasonable separate warfarin from its starting materials. This may require .5 to 1 percent methanol in the solvent. If this is the case a member of your group needs to pack a column with the correct amount of silica and solvent system and load the dried sample (see procedure, small scale ) on the column. The amount of silica used is based on the mass of the sample after blowing of the solvent with air. Again, refer to the procedure. You should have everything in your notebook at this point. We will not be providing procedures. Elute the sample into testtubes using your solvent system. Visualize with the UV lamp and the stain.

8. Some groups may need more of their original aldol product. This could be purified by column chromatography as done in the past by another member of the group.

9. Some groups may have reaction 3 completed enough that they can isolate their warfarin analogue by chromatography. This would be done analogously to warfarin itself (do this first so you get the hang of it). Many though will not be able to do this until the last week as the reaction may not be done.

10. Some will finally have 4-methylumbelliferone purified ready to react and can start reactions 1 and 2 shown above. It is expected that these reactions will not be isolated, but rather will simply be followed by tlc. Do not anticipate that you will be running columns of either of these next week unless you are ahead and have time.

11. If you 4-methylumbelliferone is not pure, try either recrystallizing it from ethanol or soaking it in a few mL of dichloromethane and vacuum filtering it. Realize 4-methylumbelliferone itself is a biologically active molecule and may very well have anticoagulative properties.
Thanks in general for working on what was almost an entirely new curriculum. We are still working out the bugs. Realize lab should be exploratory in nature and you should be thinking about and questioning what you are doing.

Your second quiz is as follows – done individually.  1. Why do you think your 1 and 2 reactions did or did not work. 2. This should be based on the TLC plate (or isolation if you have accomplished this and accompanied by full mechanism – the mechanism for the general reaction will be covered in lab lecture so take notes). Find in the literature some synthesis of a biologically active umbelliferone derivative, write out the reaction and give the reference. Due to me by the end of the course. This must be confined to one page and should take you an hour tops.

Week VI

The goal of the project was described above – making a library of compounds that may have increased biological activity or decreased activity compared with warfarin. In addition, it is of interest to test the general utility of the catalyst for catalyzing conjugate additions. Did it work when you changed the aldol component? Did it work with 7-hydroxy-4-methyllumbelliferone? Another aspect of this sort of synthesis is the utilization and demonstration of green chemistry – again extending the green chemistry to other combinations of molecules. The idea of utilizing a small amount of a catalyst at very small scale to make molecules in high yield an enantiomeric purity is very important. As you might have read in the packets I left outside my door for you, warfarin is much more active in one enantiomeric form than the other and this is a fairly common situation with drugs.

Again, my expectation is that you will write up anything you have completed in lab this week. For most people, this would be the completion of reactions “3” and “4”. The reaction of 4-hydroxycoumarin with E-4-phenyl-3-buten-2-one and the reaction of 4-hydroxycoumarin with whatever phenyl-3-buten-2-one you made. The reactions of 7-hydroxy-4-methyllumbelliferone with the two compounds (reactions 1 and 2) will probably just be part of your discussion.

I am going to get back to you with a rubric for the paper, but I see the experimental section as including a General section, and four sections corresponding to the synthesis of four compounds. One paragraph for the aldol product (an analogue of E-4-phenyl-3-buten-2-one), one for 7-hydroxy-4-methyllumbelliferone (the product of the Pechman Condensation), one for warfarin and one for your warfarin analogue or derivative (the Warfarin product derived from reaction of 4-hydroxycoumarin with your analogue of 4-phenyl-3-buten-2-one). These paragraphs should have your procedure in third person passive voice and all the data for the compound in journal style. Your procedure, but you should give a citation for the procedure your procedure was based upon. You would follow the procedure with yield, the percent yield and spectral data, etc. in paragraph format. Interpretations of peaks should be included in parentheses following spectral data.

It is important at this level for you to interpret your peaks even if it is just in the parentheses. To understand the style for a paper very similar to what you are doing, please see the packets I made available to each group (outside my door). In particular you might note the style in the paper from the journal, Angewandte Chemie. Though this is not from the Journal of Organic Chemistry, it really shows how to write the experimental section very well. Also, it gives data and names that you will find useful in writing you paper.

Again, please note that most people will probably not isolate compounds from reactions 1 and 2, that attempted reactions utilizing 7-hydroxy-4-methyllumbelliferone with the two different alpha, beta unsaturated compounds. As you will note, it becomes extremely tedious and consuming of time and space to repeatedly write names over and over. This is the reason for making a scheme in your paper.
where each compound is referred to by its number, making writing much easier. Once identified by a number, the compounds can be referred to by the number.

My expectation is that you will present in your paper what you have finished by the end of this week and that you can discuss any preliminary information regarding reactions that you have not finished. I do understand that some aldol reactions were harder than others and people are not all at the same place.

I will say though, that the reason I think the reason some are having trouble understanding what they were doing or getting the the big picture is lack of familiarity with the structures and reactions of the molecules. If you spent some time going over the compounds and reactions and learning them, the project would not seem so complicated. Really, spend an hour with the structures of your molecules and the mechanisms presented in class and it will all make a lot more sense to you. It is true that even research students, understand their projects in stages. I feel you are going through these stages and sometimes the big picture does not become apparent until a bit later.

This week I feel most of lab time will be spent running spectra, running TLCs and for some groups, separating compounds. We need to run a lot of spectra so be prepared to wait and to be very careful with your compounds. If you do not have much to do in the lab, why not spend the time working with your group on the paper? We may have to run some NMRs at other times which I or a TA will do. Since we were working at a very small scale, most people have isolated very small amounts of warfarin. For this reason, the NMR samples have to be prepared very carefully. Use the minimum amount of deuterated chloroform and keep the sample to use for IR and possibly (very unlikely), melting points. You will probably have to take all your sample up in about 1 mL of deuterated chloroform and then recycle the sample for other purposes. NMRs will probably require extra scans.

The proton and carbon NMR of warfarin is very complicated because it exists in three forms. The form written on paper and two different, diastereomeric hemi-acetals. In this way it behaves like a carbohydrate in solution. To get an idea about the complexity of warfarin NMR, please see the paper in your packet that is from the journal, Angewandte Chemie by Halland et al. Primarily we will be looking for the carbinol protons from the hemi-acetal structures that are around 1.3 ppm. Realize your analogues will be slightly different due to the groups on the benzene ring. I will also post a Carbon NMR of Warfarin on Moodle that you can look at should we run any Carbon spectra. I hope we do, but we will be limited by time.

Some useful information for paper writing

Our IR is a Perkin-Elmer FTIR Spectrum Two
All spectra were run as solid films
Our NMR is a Bruker Advance III 400 (we are operating at a central frequency of 400 MHz for proton and I believe 100 MHz for Carbon)
All spectra were run in CDCl₃
Our TLC plates were for the most part, Baker-flex Silica Gel IB2-F
Our melting point machines (the new ones) are SRS Digimelts and the melting points are reported uncorrected
The solvents we used were not purified further
R_f values should be calculated for compounds. This entails taking the ratio of the distance from the origin to the center of a spot to the origin to the solvent front.

Names and naming
Aldol product: As an example, if you have a para methoxy on your aromatic ring it would be called E-4-(4-methoxyphenyl)-3-buten-2-one. Most of the aldol products would be named similarly.

The Warfarin names can be seen in the *Angewandte Chemie* by Halland et al. that is in your packet. For example, warfarin is called 4-hydroxy-3-(3-oxo-1-phenylbutyl)-chromen-2-one. The methoxy analogue would be called 4-hydroxy-3-(1-(4-methoxyphenyl)-3-oxobuty1)chromen-2-one.