Total Synthesis Project: Synthesis and study of Warfarin Analogues

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 process known as blood coagulation (clotting). 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 is designed to orient you to the project and to give you background and approximate goals for each week of the project. It is valuable to revisit this document and reread it each week as well as to realize the goals are approximate and you may have to modify them given setbacks or shortcuts you may experience when doing the project. The document is divided into weeks and you have to scroll to the appropriate week referred to on the schedule.

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General

The goal of this project is to carry out a total synthesis of Warfarin (this is a practice synthesis) and at least one analogue (a compound that has a small structural difference and might have a different biological activity). In this project, you will work 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 locate 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 procedures located and transferred into your notebook, a specific weekly work plan in your notebook, with reactions written out in structural and procedural form, old procedures reviewed and written out and diagrams drawn of needed apparatus. This notebook should be such (and kept by each student in the group), that it is essential to writing the paper and that another group could pick it up and follow it. As I would say in the fall, you should also have visualized what you are going to do so that you really have an idea of what you are going to do before you get into lab. You also have the responsibility to understand what you are doing – which means you should know the theory behind your practice before you get into lab and if you don’t, you should ask. The notebooks will regularly reviewed by TAs. It is a substantial part of your grade. 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. Drafts of these sections are due on specific days and will not be accepted in any other form than paper. Unless, a notebook is submitted to a TA, all written work must be submitted into the steel mailbox of MNS on time. All lab quizzes will be administered by the library and must be obtained and submitted to the library on time. There are no further submissions of work as attachments or as photos etc. It is way to difficult to administer and materials get lost. If you do not submit to the mailbox, we are not responsible for it.

Overall Plan

The overall plan for the synthesis will involve making and purifying coumadin and a coumadin analogue and two different, alpha, beta unsaturated ketone analogues (different groups will make different compounds) using the aldol reaction. The purification of the aldol products will involve column chromatography. The purified aldol product will be coupled to 4-hydroxycoumarin using a chiral catalyzed 1,4-addition (Michael addition) in multiple combinations, yielding a several warfarin analogues. Again: The overall syntheses are convergent syntheses utilizing alpha, beta unsaturated ketone analogues and 4-hydroxycoumarin analogues which are coupled through conjugate addition using a rather sophisticated chiral catalysis to form warfarin analogues. The overall goal is to create compounds that are warfarin analogues that could potentially have altered biological activity compared to the parent, warfarin, the well known anticoagulant.

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:

\[
\begin{align*}
4\text{-hydroxycoumarin} + \text{alpha, beta unsat. Ketones} & \rightarrow \text{Catalysts/solvents} \rightarrow 2 \text{warfarin analogues} \\
4\text{-hydroxycoumarin analogue} + \text{alpha, beta unsat. ketones} & \rightarrow \text{Cat. /solv.} \rightarrow 2 \text{additional warfarin analogues}
\end{align*}
\]

A. Aldol Reaction Producing alpha, beta-Unsaturated Ketones needed for Warfarin Synthesis

During the first week, you and your group should be prepared to do the following activities. This means you should have the reactions 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. Your notebook should be set up according to the acronym pneumatic given in class, repeatedly in the webbook and posted prominently on the side of every hood in the lab. I Make Molecules Productively During Daytime. Remember, Observations are part of Data. Visualize your work ahead of time. Have a checklist/plan for work.
Aldol synthesis: The following is the compound that is needed for the synthesis of warfarin, however, other aromatic aldehydes will be substituted in the procedure. It is expected you will try this synthesis with two substituted aldehydes such as p-methoxybenzaldehyde as shown below the standard synthesis.

\[
\text{benzaldehyde} + \text{acetone} \xrightarrow{\text{KOH}} \text{E-4-phenyl-3-butene-2-one}
\]

\[
\text{4-methoxybenzaldehyde} + \text{acetone} \xrightarrow{\text{KOH}} \text{4-methoxyphenyl-3-butene-2-one}
\]

In lab lecture beginning this week, it is expected that you will have gone over the aldol reaction and will have also reviewed this reaction in your text. 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. Use the index if necessary. When you come to lab you should have the reaction, the scaled reaction procedure (with a full reference) and the mechanism written in your lab book (this is the blow by blow description of the reaction showing intermediates and using arrow formalism). You should have reviewed any old techniques and drawn any useful diagrams. Every student in the lab should be doing this. Also all data should be recorded by all students in the notebook (including observations). I Make Molecules Productively During Daytime.

B. 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 should try to do two versions of the reaction with acetone this week – two different aldehydes than benzaldehyde.
You can do this procedures at 5-10 times the given scale. Note: This is the procedure for piperonaldehyde (look up structure). It has to be adapted to two different aldehydes in lab in terms of grams of the aldehyde and the overall scale. You will be directed in different groups, to use different aldehydes. 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.

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 as described for the first procedure. 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 desiccator. This sheet will be about the size of a playing card, 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 and estimating is good and OK. 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 a 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

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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 heated with a hot air gun for about one minute. Be careful with the stain as it contains strong acids.

General Information about the stain and the compounds we are making: Warfarin and derivatives stain purple, 4-phenyl-3-buten-2-one (and analogues) stains red and 4-hydroxycoumarin does not stain at all (white). Acetone is volatile and evaporates off the plate? This is very useful to tell what is going on through the entire project. What would you expect on the first week? What are the standards for the aldol reaction? How would you make up the standards?

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

Additionally, you should begin reading about how anticoagulents such as coumadin (warfarin) work in the human body. Save the references for your paper. You will be given a useful packet to help you understand background. Please use this information.

Summary of Week one:

1. Lab notebook set up properly for two aldols.
2. Aldehydes chosen in lab and masses of aldehydes adjusted accordingly.
3. Attended lecture on overall project, aldol reaction and column chromatography.
4. You will be prepared to discuss the aldol mechanism in discussion with instructor/TAs.
5. Experimentally you will carry out the two aldol reactions to prepare two analogues of 4-phenyl-3-buten-2-one and study them by TLC.

Week Two

During the second week, you and your group should be prepared to do the following activities. This means you should have the reactions 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. Visualize your work ahead of time. Have a checklist/plan for work. Remember, the lab notebook for a new reaction is set up according to IMMPDD – data includes observations and TLC results. Please note that the notebook should be kept chronologically, entering data on a new page as it comes up in the notebook.

General

There are many possible conjugate addition (Michael) 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) → 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 → 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. 1 to practice – starting next week.

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

Procedures, Possible Plans:

A. You should plan to carry out the first step of both of the two syntheses of 4-hydroxycoumarin a and analogue this week as described below.

B. This week you should plan to synthesize another aldol (a different one) if you did not carry out two syntheses last week. 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.

C. This week you should also complete the purification and characterization of one of the aldols (synthesis of alpha, beta unsaturated ketones) you attempted (the one you did last week). When you arrive at lab, allow your aqueous filtrate or supernatant from an aldol reaction done last week to come to room temperature and filter out any crystal that has 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 a requirement of the project that you purify one of the aldols by column chromatography. This practice column is essential to success in the project and for some people it is the only way to purify the obtained aldol alpha, beta-unsaturated ketone products.

Synthesis of 4-Hydroxycoumarin Analogues

A. To prepare for the second week you should look up the procedure we are going to use to make 4-hydroxycoumarin and 4-hydroxycoumarin analogues (the other half of the structure we need to make warfarin analogues) and record the procedure in an understandable way in your notebook. You will likely set up this reaction first given the four hour reaction time and then at four hours cap it and we will freeze it and you complete the procedure during week two. The paper is a paper by Gao, W-T et. al and it was in Synthetic Communications in 2010. Plan to work on two or three times the scale given. To do this procedure the first week, it must be set up very quickly. Otherwise you should do it next week.
Synthesis of 4-hydroxycoumarin analogues.

Start trying to figure out the mechanism of the reaction, though Dr. Nerz will go over it in lab lecture earlier in the week. You need to (every person in the group) to write the reaction in your notebook, the mechanism in your notebook, the scaled, procedure, the reference for the procedure, the review of any new or old techniques and useful diagrams. All data should be recorded by all students in the notebooks. Notebooks are worth a substantial part of the grade and should be taken very seriously. They will be evaluated every week. Our most basic expectation is that you will be highly prepared, that you will take responsibility for your preparation and that your notebooks will be such that another student could take your notebook and work from it.

Some guidelines:

a. This a change, but each group will make one analogue at four times the scale written. This reaction should be done at three to four times the written scale. The other should be an analogue. The analogue, will be either a di-t-butyl analogue or a mono-t-butyl analogue. You will pick an analogue when you come to lab. Groups will share their compounds with others. We may make more in future weeks, but if things go well, we should have sufficient compound.

b. To facilitate these rather easy but inconvenient reactions, I will have oil baths preset at 100 °C so you don’t have to spend too much time adjusting temperature. We will leave these baths on all day, every day. Please do not change the settings unless you discuss it with your instructor (Dr. Nerz or Porello).

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. Use your 25 mL Erlenmeyer for the reflux and a small stir bar.

d. The reactions have to heat for three hours with Meldrum’s acid. At the end of the time, the Eaton’s reagent is normally added and then the reaction is heated for several more hours and then quenched with water. The resulting crystals are isolated by vacuum filtration and then re-crystallized as per the procedure in the paper you looked up.

e. In an effort to facilitate your experience, I and students last year did the reaction in a sequence by carrying out the 100 degree heating and then freezing the reaction overnight and then finishing with the 70 degree heating of the reaction at another time (successfully). So we have decided to do the 100 degree heating and then carefully cap, label and freeze your reactions until next week. Leave your stir bar in!!!

f. It is imperative that your round-bottom be meticulously labeled and that you have a beaker ready as this lab could easily run over. NDNSLT!!!!! Complete tape labels!!! Please see the side of your hood if you need to review labels.
Purification and Characterization of alpha, beta-Unsaturated Ketones (aldols)

As stated earlier, you might find that some of your crystallized material is pure by TLC and can be directly analyzed by melting point, IR and proton NMR, however, some product mixtures must be purified by column chromatography (they are not adequately pure by crystallization) and all students will have some crude material that is amenable to column chromatography. It is required that all students run such a column this or next week.

A Note About the TLC Solvent

It is sufficient 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 if methanol is used in the TLC chamber?

Once you have establish the purity of your product mixtures by TLC, any that are not pure should be purified by column chromatography. It is possible that the product of the reaction or the oil initially isolated is at least partially contaminated with some starting material or by-product. Note also, there is often some of the corresponding carboxylic acid in reactions utilizing aldehydes as aldehydes are prone to auto-oxidation to carboxylic acids. In this case, the by-products are analogues of 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. Note plastic backed plates require special gentle heating – as in a drying oven or lightly heated with heat gun.

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=EytuRMS1154

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. Column will have a stopcock, if it does not attach one.
6. Pour ca. six centimeters of dichloromethane (or other solvent) into the column. This is the eluting solvent.
7. 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. Open the stopcock and let the solvent run 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 with the stopcock.

8. 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 stopcock to control the flow and to stop the flow when necessary.

9. 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.

10. Carefully allow the solvent to drain out to just above the sand layer. Use the stopcock to stop things when necessary!!!

11. The amount of silica gel can be used to separate -.1 to 0.2 grams of material.

12. 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).

13. 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.

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

15. 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).

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

17. After doing this, fill the column with the solvent and begin running the column at a slow rate (use stopcock 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.

18. 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.

19. 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.

20. 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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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.
3. Start the 4-hydroxycoumarin and 4-hydroxycoumarin analogues syntheses immediately upon coming to lab if you intend to start this.  
   If you only did one aldol the first week, start another aldol.  Very important.
4. Isolate any crystals from your aqueous filtrate or supernatant from last week’s aldol reaction or reactions.
5. Establish purity of these crystals using TLC.  Make sure you have mastered TLC.
6. Possibly: Run your column to purify some of your cruder aldol product.  This may or may not be done this week.  Combine fractions containing your product and rotavap it to dryness.
7. Possibly 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. Start working out the mechanism for this synthesis in your notebook.
9. Start reading about how Warfarin and warfarin derivatives work.  Write down your references for future paper.
10. Leave compounds to dry for future melting point, IR, NMR analysis.

Note: this is a highly ambitious schedule and it is completely possible the first two weeks could take three weeks (or a bit more).  It is unlikely anyone will run spectra until next week.

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Week III

1. Repeat synthesis of 4-hydroxy coumarin analogue if you have had a reaction that did not work.  If you have not done this reaction at all, you should do it this week and you need to carry out the cyclization step with Eaton’s reagent to finish any 4-hydroxy coumarin analogues you may have started last week.  Very important because these are hard.  Also they take a long time – so you have to start any 4-hydroxy coumarin work when you arrive – do either the first step or second step (wherever you are) first thing in lab.  The cyclization takes four hours.  Please talk to your instructor if someone from your group can’t quench the Eaton’s step after four hours.

2. Work on purifying and characterizing aldol reactions (measure mass, run IR spectra, run NMR spectra), make up aldol and purify it, if need be.  If you have not run the column you need to run the practice column on one of the aldols.  In general, you should be working on making sure your aldols are pure by growing crystals and running columns.  Before you proceed to the Michael Additions (the last step of the syntheses), both your aldol products have to be pure as do the 4-hydroxy coumarin analogues.  You may have to also repeat reactions to succeed.  If products appear to be pure by TLC, please start characterizing by melting point, IR and proton NMR.

Summary of Week III.

1. If time permits, purify the product from the Pechman Synthesis.
2. If first step was not carried out, carry out the first step of the Pechman Synthesis using Meldrum’s Acid.  If either of these are being done, they have to be started at the beginning of lab.
3. While the reaction of choice is being carried out, work on purifying and characterizing aldol products.

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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 focus on how to write the research paper (start working on it now) and mechanisms involved in the synthesis. This specific week will focus on the paper writing, using another real example, the mechanism of the synthesis of the 4-hydroxycoumarin derivatives and the beginning of the catalysis mechanism. There will only be two more weeks of lab lecture on this project. There will be more info on paper writing, more mechanism and possibly some carbon NMR.

2. First thing in lab, you need to finish your 4-hydroxycoumarin analogue reactions. In this area, you are entering relatively uncharted territory. As we attempted with the first part of the sequence, the oil baths will already be set at 70 degrees. Please do not change the voltage without first speaking with your instructor or TA. Also, please note that the voltage setting is not the temperature and when the temperature is off by a few degrees, one does not change the voltage radically to induce the change. One can adjust it slightly and wait or add small amounts of cool or hot sand. Think about cooking – if you were cooking on your stove and you were say cooking an egg and the temperature was a little too hot would you turn the stove off? If the temperature was just a bit too cool, would you turn the heat up all the way? Of course not!!! Realize the voltage control is exactly the same sort of devise you have on an electric stove.

   It is very important that you should finish your 4-hydroxycoumarin analogue reactions by adding the Eaton’s reagent and heating with stirring for the time indicated in your paper. Note: Eaton’s reagent though viewed by chemists as a relatively safe dehydrating agent, it is still comprised of P₂O₅ and p-toluenesulfonic acid. You have to handle it with care! If fact we are going to provide vials that contain three mL of Eaton’s reagent for you to add to your reactions. Use the number of vials needed for your scale. Please, simply return the vials after you have added it to your TA. After the two or three hour period at seventy degrees using a reflux apparatus with stirring (use the stir bar that is already in the apparatus), the reaction is quenched by adding water. I feel you should add about 5-10 mL of cool water. At this point crystals should form which should be isolated by micro-vacuum filtration and then re-crystallized as described in your paper. Use a light hand with the re-crystallizing solvent and remember it is micro-scale (no large equipment). Please keep track of which compound is which and exactly which analogue you made. You may not be finished this part at the end of lab. It could be your compounds are at the quenched point or you could have the purified material. It is a not a race and what I do is make suggestions.

3. While the reaction is heating you can do whatever you need to do. This might include characterizing your aldol analogues (getting mass, IR, NMR). Purifying aldol analogues if needed. It would be nice if each group has two aldol analogues though it is not necessary. You do not need a tremendous amount to proceed. I could see people moving forward with a milligram or two. We have methods to use the material over and over – e.g. NMR samples can be rota-vapped down and potentially used in reactions.

4. Start a practice conjugate addition coupling to make Warfarin itself. 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 smallest 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 look this procedure up before lab, write it in your notebook, write the reactions, mechanisms, diagrams and review of any procedures.

The Green Synthesis of Warfarin.

![Chemical Structure](image)

4-hydroxycoumarin aldol product

warfarin

Note: the above is the method we are going to use to synthesize all analogues and it will have to be adapted to fit the molecular weights of the 4-hydroxycoumarin analogue and/or 4-phenyl-3-buten-2-one analogues used. Scaling has to be done on site unless it is the practice reaction.

Labelling!!!!!!! Label your reaction: printed names, date, name of reaction, lab section and a code that all students have recorded in notebook.

b. Run TLC of starting materials above as a baseline. Develop your TLC in dichloromethane. 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.

Continuing as always this week, TAs will be looking at your notebook and giving you a grade. Please prepare well for lab. Preparation means you have written out everything you plan to do, you have all the procedures scaled, you have all apparatus diagramed, and all techniques reviewed (for example we should no longer be explaining to you how to run a tlc, a rotavap or an IR spectrum. Some of you would benefit from visualizing what you are going to do the night before you do it or get with your group and talk it through. Review you tubes if necessary.

Well prepared is not simply copying procedures into your notebook. It is also extremely important with all the reagents that are out at this point that you read labels very carefully. It is dangerous to randomly mix reagents!!!

Also note again – these are just suggestions as to where you might be and what you might be working on. Increasingly, students will be in different places. It is not a competition. Some projects will encounter more snafus and others will run smoothly.

Also please note that the TAs and instructors will have less time to go over nmrs with you. You have to run them in about ten minutes and really more along. We have a lot of spectra to run. The TAs or instructor may have to run some of the spectra.
This week your group will be given a card with a clean up task on it. Please complete this task before leaving. By the way, the lab has generally been left in terrible condition on most days. Please clean up after yourself, do not hoard equipment in your locker and wipe down your area completely, turn off lights in your area and put all equipment away. By the way, the very large old clamps do not go in the bins on the benches. For those who feel their lockers are raided, we pick up hundreds and hundreds of pieces of glassware and wash them (yes, I spend inordinate amounts of time washing glassware on weekends) every week. A lot of equipment and glassware is left out every day. Please thoroughly clean nmr tubes.

Summary of Potential work for Week IV

1. Attend lab lecture.
2. Finish syntheses of 4-hydroxycoumarin and 4-hydroxycoumarin analogues. (Eaton's Reagent Step).
3. Purify 4-hydroxycoumarin and 4-hydroxycoumarin products by re-crystallization?
4. Work on purifying and characterizing aldol products, getting them ready for Warfarin synthesis.
5. Carry Out Practice Warfarin Synthesis and Run baseline TLC.
6. Look up references for procedures, record in notebook, read about compounds you are making.

Week V

1. This week’s lecture will explain how the catalyst works.
2. In this week’s lab you need to work on purifying your 4-hydroxycoumarin and your t-butyl analogue using re-crystallization. First, you should isolate your crude material from the two reactions, if you have not done so yet. Don’t filter if you don’t have crystals. Don’t throw anything out until we have explored all options. The products are acids (phenols) so it is possible you could extract them out with base and then precipitate them with acid. So don’t give up if you have no crystals. Try to outline this idea. If you pull the proton from the phenol, you will have the conjugate base which is water soluble. Then it is possible we could precipitate the product with acid. We might also extract the compound out with dichloromethane. So, please do not give up or rush things. Please discuss your reaction with your instructor or TA before filtering or throwing anything away.
3. Once you have crystals, you can rinse the crude crystals with cold water. The crude compounds have to be re-crystallized using ethanol. Since the reactions were done on a very small scale, the re-crystallizations must be done on a very small scale. Use your smallest Erlenmeyer, a boiling stone, and heat gently. It may only take one or two mL of ethanol. Again, do not give up and do not overheat.
4. Once you obtain crystals from the two reactions, you should dry them and obtain data to prove you made the compounds. You need to measure masses (tare and measure all masses on analytical balance), melting points, IR and NMR. Remember a good way to work micro-scale is to first measure the NMR, then use the NMR sample to make the film on the IR and then rotavap the NMR sample down in a tared (using analytical balance) vial and get the melting point (if possible). Obtain the mass of the remaining material and set up your warfarin reactions directly in the vial. We can show you how to do this.
5. To the extent that you have products from the 4-hydroxycoumarin and 4-hydroxycoumarin analogues, you should start your Warfarin reactions. These should involve the four possible crosses of your two alpha, beta
unsaturated ketones (aldol products) with the two 4-hydroxycoumarin products. If you were unsuccessful with your reactions, you can limit your crosses or talk to us about what to do. For example, if you did not isolate any 4-hydroxycoumarin, we do have a supply of that you can use. If any groups have any extra t-butyl-4-hydroxycoumarine analogues, they might share some with other groups.

6. So you might set up four micro-scale warfarin reactions. We have done these on as small a scale as 1/5th of the scale written in the smaller of the two procedures. As indicated earlier, these would be set up in vials just as your warfarin practice reaction, adjusting quantities based on the molecular weights of the compounds being used. Please sit down and do the calculations before labs. Remember, the reactions have to be all done in the same molar proportion, but the compounds being used in making the analogues have different molecular weights than those in the actual Warfarin reaction. Again, your possibilities are as follows. All compounds should be made by you unless, you had some sort of synthetic problems. We might want to use a double amount of catalyst (on the scale you are working) to speed up reactions given our limited amount of time.

Alpha, beta-unsaturated ketone 1 with 4-hydroxycoumarin \( \rightarrow \) Warfarin analogue 1
Alpha, beta-unsaturated ketone 2 with 4-hydroxycoumarin \( \rightarrow \) Warfarin analogue 2
Alpha, beta-unsaturated ketone 1 with 4-hydroxycoumarin analogue \( \rightarrow \) Warfarin analogue 3
Alpha, beta-unsaturated ketone 2 with 4-hydroxycoumarin analogue \( \rightarrow \) Warfarin analogue 4

7. When you get the reactions started, you should run a baseline TLC with UV and p-anisaldehyde staining as a baseline for each reaction.
8. Next week you will take the best of these four and separate them via column chromatography. You will likely run two columns only and follow the other reactions via TLC next week.
9. In any spare time you have, start working on your paper. Please go to the library and get a copy of a recent total synthesis paper from the Journal of Organic Chemistry and print it out or download it on your computer.
10. Please start working on your introduction. What are the major components of an introduction. We have gone over this three times in class. Normally Introductions include info on why the compound is of interest (its biological activity in this case) stated fairly briefly with citations. In some cases, there will be some inclusion of where the compound was isolated from and any structural issues that may still exist (such as needing to establish the stereochemistry). The authors will summarize prior synthesis and then state the significance of their synthesis.

The following are just some general comments regarding coumadins. Please refer to the packet I have given you.

**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.

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 anti-coagulents 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.

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 anti-coagulant 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.

11. You might also start working on your experimental section. Here are some general comments.

Some useful information for paper writing

Write in third person, passive voice. Follow the style of the journal.

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 CDCl$_3$
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-oxobutyl)chromen-2-one.

**Summary of Possible Work for week V.**

1. Finish synthesis of 4-hydroxycoumarin and analogues by isolating and purifying by re-crystallization.
2. Characterize any compounds that are ready by NMR, IR and MP. Determine yields.
3. Start as many microscale reactions making warfarin reactions as possible. Obtain a baseline TLC of each and visualize using both UV and p-anisaldehyde stain.
4. Work on paper introduction and experimental section in intervening times.

Remember – always tare on analytical balance, record all data, label all spectra, label all vials and flasks clearly, keep good records in your notebooks. Always prepare well.

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**Week VI 2014**

Lab lecture will be a little more on coumadin, I will finish the catalysis mechanism and I will talk a little about the nmr of coumadins that are very interesting and complex and will fit in well with our discussion of sugars.

This week you should take all warfarin analogue reactions you have done and evaluate them for product using TLC (eluting solvent dichloromethane or dichloromethane with a few drops of methanol) with visualization using the UV lamp and with the p-anisaldehyde stain. Please run the TLC with standards and few people ran baselines. Then the one or two best analogue reactions – based on yields and amount of warfarin product will be separated by column chromatography using dichloromethane or a solution of dichloromethane with methanol (use very little methanol – less than 5 percent if you choose to use it, the column can be run without it). Use your column skills, review the video, the info given in prior weeks. You may have to cut down on the silica if your yield is very low. I would say for 200 mg use about 7 grams of silica and then cut it down proportionately. A very small.

If each group is doing two columns you must be efficient. You have to divide the work and use the time saving tips. Meaning, you must do you test plate while you are running the columns, you must continuously run the column, keep the column full so it runs at high speed. You must start honing in on the product while the column in running.

Any product may be characterized at a later date – you can submit your nmr samples in vials and I will run them over the weekends and evenings. We do not want to take time from the grad students for this week, so we will have a limited amount of nmr time each day next week (we have been sort of hogging it.) I will try to get these done as efficiently as possible each evening and over weekends. If you want anything run now, please give it to me in a labelled vial. I will be running many spectra this weekend. Please relinquish all nmr tubes from your drawers. The tubes are in terrible condition and we have very few. I have included below the info about writing the paper. For editing by me or your instructor, drafts are due by April 17 and the paper is due the 25. All we want in draft is one experimental section and your intro on google doc or paper form.
Possible work for Week VI

1. Lecture will cover more on warfarins, the rest of mech for catalyst and some info about the spectrum of warfarin which is complex.
2. Evaluate green warfarin reaction by TLC.
3. Decide which one or two to pursue, but report all TLC data for future reference.
4. Run up to two simultaneous columns, using the efficiency tips above.
5. Try to get samples in concentrated form by end of lab.
6. Come in to run Ir and melting points, mass etc. submit samples in timely fashion in vials for Dr. Nerz to run.
7. Please look up the spectrum of Warfarin – look at spectrum in the Green Synthesis of Warfarin paper you have and look for spectra on web for you analysis.
8. Start working on paper. Come to lab with a vision of what you are doing.
9. More info on project and paper.

Under construction I will add more on intro and discussion. Next week.

The goal of the project was described above – making a library of compounds (some known) 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 or the 4-hydroxycoumarin compound? 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 two of their reactions. They may be semi-synthesis as about fifty percent of the 4-hydroxycoumarin products and analogues were over heated and or just not isolated. Though we tested this a number of times, this was new for the class and the very experimental, uneven results were not unexpected. We actually learned a lot from this run and your work will result in modifications next year. It is very important that you report this regardless of your success or failure. Please include it in your report as we will use the data. I intend to write a paper on the total synthesis this summer so please give me any and all info. VERY IMPORTANT. You will be included as authors if you wish.

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 a formally written section (to the extent it is possible for each compound you made, even if it was not utilized) One paragraph for each of the aldol products (an analogue of E-4-phenyl-3-buten-2-one), one for any 4-hydroxycoumarins you made and characterized (to the extent you did) one for each warfarin analogue reaction completed, any just followed by TLC can be discussed in the results and discussion section. These paragraphs should have your procedure in third person passive voice and all the data for the compound in journal style. Your procedure, on your scale with your modifications should be included, 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.

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 including what you have finished by the end of April 25 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.

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