Introduction

Cancer is a peculiar burden that interests me and stimulates my thinking. It informs my chemistry and my chemistry informs my battle against cancer. So intertwined are the two, that I often wake up in the morning with chemistry ideas that will solve a cancer problem and while being treated, I often have inspirations that relate to new laboratory teaching innovations. Through my intensive study of my disease which was at first a matter of survival, I grew to respect and gain a fascination for the aberrant cells that have such a remarkable and insidious ability to evolve and survive as sub-clones after so many and increasingly specific molecular attacks. It has enhanced my scientific knowledge as I have grown to have a passion for the study of the chemistry and biology of cancer, particularly the areas of brain metastasis, the blood brain barrier, how chemical structure effects blood brain barrier penetration, natural products and their biological activities, and the design and synthesis of small molecules that have biological activity. I am also very interested in the close relationship between radiology and spectroscopy. All of these areas are relevant to the teaching of organic chemistry and biological organic chemistry.

The courage and perseverance required to fight for one's life every day, the experimental approach that must be taken with incurable metastatic cancer that is constantly changing, enabled me to live somewhat comfortably on the edge and greatly enhanced and encouraged my creative thoughts about science and medicine. This long intensive experience has given me a certain intuition and boldness to try new things in the medical world and more importantly, it decidedly extended to my science endeavors.

Shortly after the changes in the MCAT and premedical training were announced, that result in a greater emphasis on humanities and biology and less organic chemistry, an editorial was published in the New York Times by Barbara Moran,[1] a writer taking organic chemistry in the Harvard Extension School in preparation for medical school. Among her many comments, she writes…

……"Learning how to interpret the hieroglyphics is pretty easy. The hard part is learning where to draw the little arrows. To do this, you learn rules about how molecules behave…….

But the rules have many, many exceptions, which students find maddening. The same molecule will behave differently in acid or base, in dark or sunlight, in heat or cold, or if you sprinkle magic orgo dust on it and turn around three times. You can't memorize all the possible answers — you have to rely on intuition, generalizing from specific examples. This skill, far more than the details of every reaction, may actually be useful for medicine….

'It seems a lot like diagnosis,' said Logan McCarty, Harvard's director of physical

sciences education, who taught the second semester. 'That cognitive skill — inductive generalization from specific cases to something you've never seen before — that's something you learn in orgo.'

……. Sometimes, if a student has really good math skills, they can slide through physics, but you can't do that in orgo," Mr. McCarty told me, adding, "You can't slide through medical school, either…..'"

Though organic chemistry is increasingly minimized on the MCAT and in medicine, I have long been of the opinion that it is a perfect place to begin training for medical school and that one cannot understand biological chemistry without a very sound knowledge of organic chemistry.

Just before this editorial appeared, I was presented with a huge and rapidly needed change in the organic laboratory course and curriculum. I was, however, determined to maintain the important principles and techniques that I had learned and developed at Bryn Mawr and was equally determined to maintain the creative methodology and individual attention that I feel students deserve. As I have tried to indicate above, I was also undergoing a large personal transformation in the way I thought about lab teaching related to my experience with health care professionals in the medical world and my interactions with our student population. At this time, I was also beginning to develop research ideas and was implementing them with sophomore and junior chemistry majors. All these factors forced me to realize that for **all chemistry students**, the lab needed a complete transformation starting with the spring semester. I realized the last thing our students needed was some super-streamlined, feel good lab course that made life easy for me and them. Of course students need to learn facts, but most importantly, they need to learn to think and work as scientists and apply the knowledge, skills and intuitions gained to their lives. This has always been my goal, but with increased life experience, this took on a new intensity and urgency.

My philosophy about lab and life itself is in tune with that described by Atul Gawande. In his book, Better,[2] Dr. Gawande describes and advocates the positive deviant physician. In my opinion, the positive deviant physician is a scientific physician with many of the skills of a good organic chemist. In addition to minimizing complaining and asking random questions of their patients, positive deviants are always in the process of counting something (even if not carrying out formal research, a positive deviant should be organizing and analyzing the data of one's work and life). Positive deviants should be writing about their research, even if informally. They should be willing to adopt new good ideas readily and they should most importantly, be innovators. Though imperfect in my positive deviation, I can say this characterization describes my life's work in chemistry and survival of cancer. This approach especially describes my last six years. Many believe that undergraduate lab simply exits to demonstrate a reaction or concept that was

discussed in lecture the week or day before and it is not unusual for students to desire a lab to be a pretested, streamlined package that almost guarantees success and probably a false confidence. At inception, this sort of lab is basically "old news." The instructors have done the lab and the TAs know what to expect or do. This bias leads to a reduced creative experience for the students. They do not develop the courage and confidence to be positive deviants.

My new curriculum and the curriculum I am developing is designed to have students work on long term, real research projects that enable them to grow in knowledge, physical skill, analytical skill, trouble shooting ability, independent thinking and team work. My goal is to personally experience challenge and innovation every day. I hope I am training a better group of graduate students because they seem regularly surprised and challenged to innovate and inspire innovation in the students. Most importantly, though supported, the primary responsibility of researching, planning, executing and creating is transferred to the student. They are encouraged to learn to embrace change and innovation on a daily basis. If they have a good idea, they are encouraged to try it!!! These projects convert the student from the mime to the scientist who will hopefully apply what is experienced and learned when faced with problems to solve in their future lives.

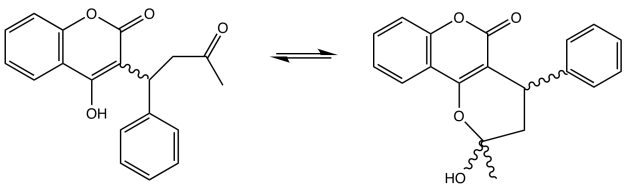
- Maryellen Nerz-Stormes, 2017

Total Synthesis Project : Synthesis and study of Coumadin Analogues

This document is designed to orient you to the total synthesis 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. It is also important to recognize the goals are approximate and you may have to modify them given setbacks or shortcuts you may experience when carrying out the project. The document is divided into weeks and you can link to the appropriate week below as referred to on the web schedule.

Natural and synthetic coumadin compounds have been found to have significant biological activity and medical application. Coumadins and related coumarins have been shown to have analgesic,[19] anti-pyretic, [20] anti-inflammatory, [21] anti-allergic, [22] anti-bacterial,[50] antifungal, [23] anti-HIV, [24]and anti-cancer [25] activities. Warfarin itself is primary well known for its clinical use as an anticoagulant (blood thinner).[26] Warfarin acts as an antagonist to vitamin K, a cofactor needed for carboxylase, an enzyme involved in the synthesis of a number of clotting factors, including prothombin. Carboxylase with its cofactor, vitamin K, is responsible for the carboxylation of glutamate side chain residues on certain clotting factors. The formation of a higher concentration of carboxylate groups near the termini of these glutamate residues, enables the clotting factors to chelate calcium ions. The calcium ions simultaneously chelate to phosphate groups at cellular surfaces, enabling coagulation. Coumadin has a structure similar to vitamin K. When it binds the vitamin K site, it shuts down the enzyme and associated oxidation/reduction needed to carboxylate these special residues necessary for the coagulation process.

In addition to the fascinating biology and medicinal properties listed above, warfarin and coumadin analogues demonstrate very interesting equilibrium in solution. Warfarin exhibits the formation of hemi-ketal anomers analogous to aqueous carbohydrates, but also in the NMR solvent, $CDCl_3$.[18] This seems a beautiful marriage of biological and organic chemistry. Not only is the warfarin NMR spectrum a wonderful example of NMR as imaging, i.e., the success of the experiment can be immediately determined by the landscape of the spectrum, but also, the isomeric products can be distinguished in various sections of the NMR.



The warfarin equilibrium forming two hemiketal anomers

As I continued to study coumadin derivatives and their vast activity, I also studied various methods by which analogues could be synthesized. In the various syntheses I studied, I saw great potential for students, even in a large course, to carry out research, synthesizing a wide variety of analogues that would have potential for future biological study and potential medicinal compounds. I saw the possibility of students truly carrying out research and gaining all the benefits outlined above and below while learning what science is and possibly having the thrill of making a new, potentially useful compound. On this basis, I formalized a warfarin research program to lay the groundwork for a second semester organic chemistry laboratory six to eight week research project that would evolve and progress each year. I worked most intensely from 2012 to 2014, working nights and weekends and hiring a research student who helped develop new steps, but also worked on making the basic steps workable and adaptable. The research was gradually introduced into the lab starting in 2012, and by the spring of 2015 was reasonably well integrated. In 2016 the project was starting to thrive as a purely synthetic project. Since 2015, student groups such as yourselves in our lab have been responsible for synthesizing increasing numbers of analogues, improving procedures and results. Many ideas have developed from this fertile and industrious environment and future publication is anticipated.

The primary goal of this project is for each student group to have the experience and excitement of planning and executing a long term research project, along with all the creativity, troubleshooting, problem solving, adaptation, salvaging and learning that such a project entails. A secondary goal for each research group is to carry out a total synthesis of up to four warfarin analogues (compounds that are structurally related to, but slightly different from warfarin.). The tertiary goal of this project is for the class to build a larger library of warfarin analogues that will most certainly contain previously unsynthesized compounds. It is hoped that ultimately, that structural variation will be linked to varied and perhaps unique biological activity and medicinal properties.

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 carrying 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 more 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 your warfarin analogues.

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. 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 and diagrams drawn of needed apparatus. You should follow the same IMMPDD format that you have used during the entire academic year. Please note that some weeks you will be entering a new procedure (IMMPDD) and some weeks you will simply be recording data and observations. 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 what you have been doing. As one TA said to me earlier this semester, "The notebook is all about recording observations." You also have the responsibility to understand what you are doing – which means you should know the theory behind your practice before you arrive in lab and if you don't, you should ask. The notebook pages will regularly reviewed by your TAs as they have been all year. Lab notebook pages are worth **8 percent** of your laboratory grade this semester, but will priceless when you are writing your paper.

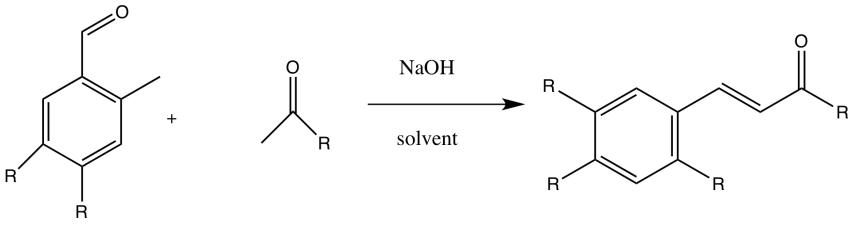
In addition to being responsible for the material from this project on a weekly basis, you will be responsible for the material when you write a journal style paper with your group at the close of the project. As noted on the second semester web schedule, there will be specific lectures on writing a journal style paper. Closely linked to those dates are due dates for rough drafts of the sections of the paper. These drafts will not be graded, but will be given timely feedback by your instructor. Please follow your specific instructor's preference for submission of drafts and the final paper.

On a small scale, you will be responsible for the material on a short quiz at the end of the course.

Overall Plan

In an effort to accomplish the goals stated earlier in this document, each research group will be utilizing variations on the following convergent synthetic plan.

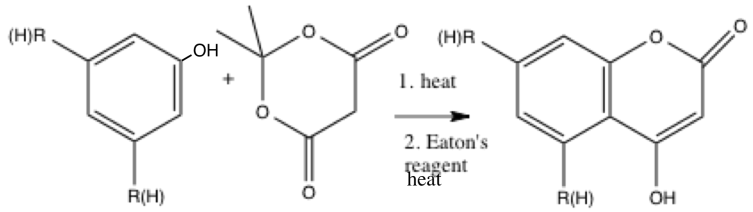
The first step is an extremely versatile, classic, thermodynamic aldol condensation reaction which constructs the coumadin analogue side chain and suggests many possibilities for variability in the analogues. We are currently working with ten to twelve aldehydes and two ketones. This creates the possibility of producing at least twenty different alpha, beta- unsaturated ketones for use in the synthesis.



aldehyde ketone alpha, beta unsaturated ketone

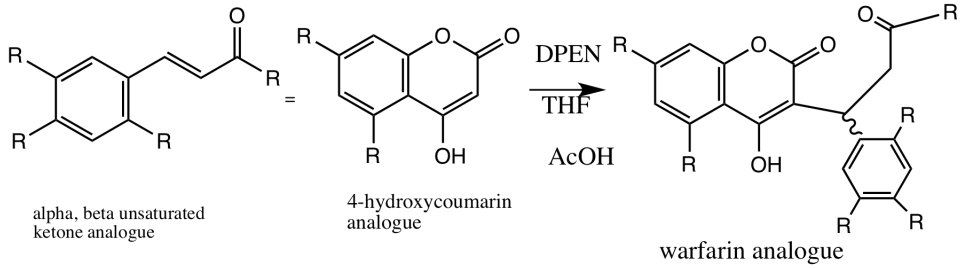
R = H or a variety of substituents. The R groups can be a any combination of these subtitutents and are subject to change as research progresses.

The parallel step in the convergent synthesis is a highly driven esterificaiton reaction using Meldrum's Acid, followed by a very interesting electrophilic aromatic substitution reaction to create the second ring of the 4-hydroxycoumarin system needed for warfarin. We will attempt this year to make up to six variations on this step.



phenol analogues Meldrum's Acid 4-hydroxycoumarin analogue

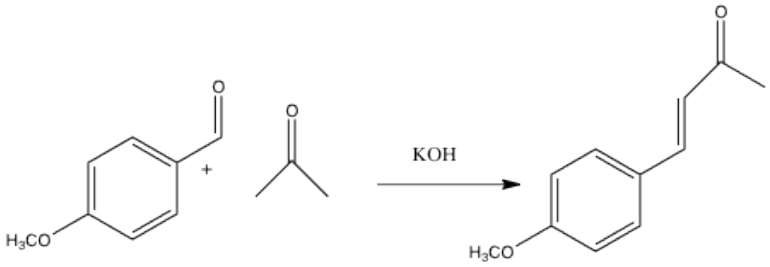
The convergence of the two components occurs in the final step which is an extremely small scale, extraordinarily elegan, t Michael (conjugate) addition utilizing a chiral catalyst that in many respects is a good model for the way enzymes work. If one considers the variations above, when the two compounds come together in the sequence below, how many compounds could be accumulated in our class library? How many aldols and how many 4-hydroxycoumarin syntheses will you need to accomplish to make up to four of the compounds in your sub- library?



alpha, beta unsaturated ketone analogue 4-hydroxycoumarin analogue warfarin analogue

R = H or a variety of substituents. The R groups can be a any combination of these subtitutents and are subject to change as research progresses.

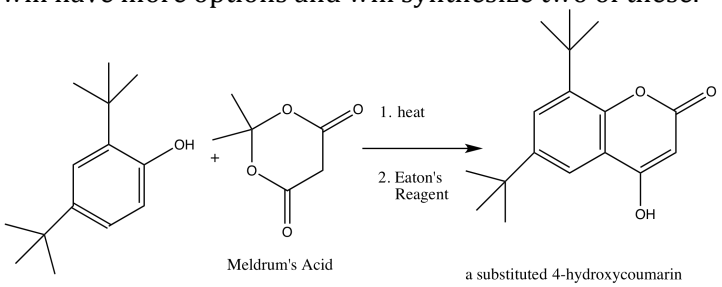
The following is a typical first step reaction, showing a single variation, but both the organic substrates can be varied and student groups are encouraged to choose from an increasing variety of substrates. Each research group will attempt to complete two of the aldol analogues, some of which have never been synthesized. Research in our lab has been completely transferred to the sophomore organic lab for this step.



4-methoxybenzaldehyde acetone 4-methoxyphenyl-3-buten-2-one

The student research groups are responsible for planning the procedure and apparatus, scaling the reactions, using observed physical changes and thin layer chromatography to make decisions about the conditions needed for the specific versions of the reaction. Students are taught how to do chromatography surrounding this reaction, but they work hard to purify their products using column chromatography and/or re-crystallization. Occasionally, the reactions generate complex product mixtures and last spring we introduced GCMS as a method to ascertain whether complex product mixtures contained the desired product. The GCMS aspect of this requires more research as to ascertain its full utility in this project. This year we will introduce Carbon-13 NMR to enhance the characterization of some of the compounds containing napthyl rings. This has already been utilized by students in the course to a small extent and will continue this spring.

An area of research for myself and my research student was developing a reasonable method for synthesizing a wide variety of 4-hydroxycoumarin analogues. My research student suggested the method outlined below,[27] which demonstrated some principles of green chemistry. The following is a typical synthesis of an analogue, but student groups will have more options and will synthesize two of these.



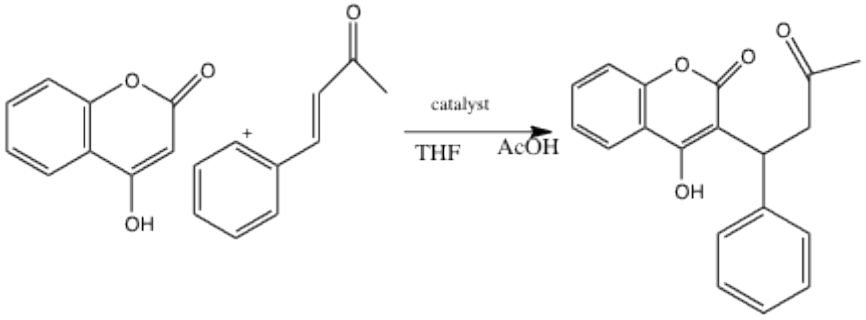
a substituted phenol Meldrum's Acid a substituted 4-hydroxycoumarin

We tested the reaction for several substrates and adapted the eight hour reaction sequence to work in a sophomore laboratory, carrying it out over two weeks using a standard reflux apparatus immersed in a temperature controlled oil bath Over the spring semester of 2016, inspired by observing the chemistry of my colleague ,Jason Schmink, I began

working with this procedure on a very small scale, in a vile, utilizing a pie-block reactor. Each student group will attempt two variations of this reaction with this new approach.

After several unsatisfactory versions of the final conjugate addition discussed above, my research student and I adapted the very simple, micro-scale, green, chiral synthesis of warfarin outlined below that appeared in the pedagogical literature [28] We attempted many combinations of the various substituted 4-hydroxycoumarins and substituted 4-phenyl-3-buten-2-ones we synthesized in our research lab, discovering the chiral catalyst (R,R or SS-1,2-diamino-1,2-diphenylethane) was amazingly versatile. Students in the sophomore lab have been remarkably successful, working at astonishingly (sometimes desperately) small scale and in some cases, with less than pure materials. The students in second semester organic chemistry have sysnthesized a number of final warfarin analogues that have not been made before, but not without some trial and tribulation. This final part of the synthetic sequence has been largely transferred to the undergraduate organic chemistry lab students. I am also interested in studying the kinetics of the catalytic process as part of the project. I plan to explore and carry out an investigation that students can carry out to explore the biological activity of their compounds. There are several possibilities utilizing commercially available 50 well kits and other simpler ideas.

What is shown is the synthesis of warfarin itself , but the reaction has worked with may variations of the two substrates.



4-hydroxycoumarin aldol product warfarin

Please come in with an open mind, being psychologically prepared to have things go wrong and sometimes fail. Scientists have to be very prepared, very thoughtful, very careful and yes, also creative and adventurous (in a safe way) to test new ideas and solve problems. . Scientists 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 , how to make it work or just understand chemistry better, than to simply execute a sequence with skill. Even better, to discover something serendipitously.

Week I

A. Aldol Reaction Producing alpha, beta-Unstaturated Ketones Needed for Coumadin Analogue 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 pneumonic given in class, repeatedly in the web book 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.

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.**

Since you will be doing two aldols, you will have to adjust calculations , based on the aldehydes and or ketones you are assigned or pick while in lab.

B. General Procedures for Preparation of Aldols

Safety Notes: Consider all compounds to be irritants that require fifteen minutes of flushing with cold water if you are exposed.
Be especially mindful of the NaOH you are working with. It is very corrosive and will dry out, make your skin itch and will ultimately give you a chemical burn. If exposed, flush the exposed area with cold water for fifteen minutes.

The p-anisaldehyde stain contains in addition to the two toxic, irritants p-anisaldehyde and ethanol, two very corrosive compounds that will cause burns to your skin and mucous membranes – sulfuric acid and acetic acid. Please wear all your gear at all times in the lab. Upon any suspected exposure flush the exposed area for fifteen minutes with cold water.

Choose an aldehyde from group A in the lab and an aldehyde from group B in the lab to react with acetone as the ketone. Alternatively, you can choose an aldehyde from group A and react it with acetophenone as the ketone. If you choose this variation ,, you must react an aldehyde from group B with acetone. We are open to other variations from the adventurous or curious, especially with acetophenone. Note, the procedures are 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 this week – two different aldehydes from groups A and B with acetone as ketone or two different aldehydes with two different ketones, A with acetophenone and B with acetone. These reactions will create your first two variants that will be built intot analogues.

You can do these procedures at 4 times the the given scale (4 mmolar). Scaling a reaction means you take all the quantities and multiply them by four, but maintain the times and temperatures. Note: This is the procedure for piperonaldehyde (look up structure) with acetone. It has to be adapted twice depending on the aldehyde being used, taking into account differing molecular weights. and the overall scale. You will be directed in different groups, to use different aldehydes and or ketones. It is generally a good idea to have an ice bath ready for all aldol reactions.

This is the general aldol procedure for a typical aldehyde with acetone. It may require adaptation beyond just the quantities.

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 Ehrlenmeyer 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 or can be isolated in other creative ways.
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 and/or any milky liquids obtained from the process should be stored in the refrigerator until next week when more compound may be isolated by vacuum filtration.
8. This week, the progress of the reaction can be monitored by TLC (similar to the flutamide reaction) vs. appropriate standards. The standards will be pre-prepared.

The following is the general aldol procedure for a typical aldehyde with acetophenone. It may require adaptation beyond just the quantities.

1. Place 0.15 g of piperonaldehyde in a 25 mL Erlenmeyer flask.
2. Add 0.12 mL of acetophenone and 1 mL of 95% ethanol into the same flask.
3. The flask may require gentle warming to dissolve the reagents.
4. Add 0.10 mL of NaOH solution (6 grams in 10 mL of water)
5. Stir the solution until it is solid or it the mixture is very cloudy.
6. Add 2 mL of ice water to the flask. If an oil forms, stir until it solidifies.
7. Add the entire mixture to a container with another 3 mL of ice water.
8. Break up the solid and isolate by vacuum filtration.
9. Using TLC, decide whether the compound is a viable candidate for column chromatography. The standards will be pre-prepared.
10. It is also possible to purify the compound by recrystallization.

C. Chromatography Background

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://orgchemboulder.com/Technique/Procedures/TLC/TLC.shtml

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.

D. Thin Layer Chromatography – A Brief Description and General Instructions

1. Obtain a Thin Layer Chromatography (TLC) sheet from the desicator. 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.

3. You may need to prepare standards for the reagents (They should be pre-made this year). 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). Note: the methanol is optional. For some product mixtures, dichloromethane is is adequate and for others small amounts of methanol are helpful - you can use anywhere from a drop to several milliliters of methanol. TLC is an art and you have to work out solvent systems experimentally. The goal is to have the desired compound to move to about the middle of the TLC plate and to be sufficiently separated from the apparent contaminants. The more methanol present, the larger the R_f of a given band. 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 ^{o}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 note book 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 I:

1. **Lab notebook set up properly for two aldols. IMMPDD – since you don't know what you are working with, you will have to do some planning with your group in the lab. ON LAB DAY.**
2. **Aldehydes chosen in lab and masses of aldehydes adjusted accordingly.**
3. **Attended lecture on overall project, aldol reactionchromatogra, GCMS and carbon NMR.**
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 their reaction progress by TLC. You will isolate or initiate the isolation of crude material.**

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Week II

A. General

During the second week, you and your research group should be prepared to do the following activities, preparations and purifications. 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.

Begin preparing for next week by thinking about how you will generate four coumadin analogues from your two aldol products and your upcoming synthesis of two hydroxycoumarin analogues. Can you draw products for four cummadin permutations. Draw these in your notebook.

If you have not already done so, you should begin reading about how anticoagulents such as coumadin (warfarin) work in the human body. Save the references for your paper.

B. Procedures, Possible Plans for Week II:

1. This week you need to isolate any new crystals or oils you isolated from your week of cooling filtrates and supernatants in the cold case over spring break. Allow the cooled liquids to come to room temperature and isolate the filtrate by vacuum filtration and isolate any oils using your creative ideas (extraction is not ruled out). Crystals tend to be very pure from this process, oils less so.

2. You should evaluate all oils and crystals isolated from both reactions via TLC for purity. Materials that appear to be pure by TLC are suitable for spectroscopic study (NMR and IR) and may be ready for the final step of the synthesis and if proved to be so, can be put away for that purpose. Material that is not pure should be subjected to column chromatography for purification. It is hoped that one of your synthesized compounds will be pure enough for spectroscopy and the other will require column chromatography. Regardless, however, every group has to purify at least one sample by column chromatography. Please study the instructions below, the linked video and please attend lab lecture and the demonstration that will be given in lab on how to run a column.

C. Purification and Characterization of alpha, beta-Unsaturated Ketones (aldols)

1. 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 nearly 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. It is a reasonably easy purification and it is excellent practice for your future purifications.

2. 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?

3. 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.

4. 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.

5. The p-anisaldehyde stain contains in addition to the two toxic, irritants p-anisaldehyde and ethanol, two very corrosive compounds that will cause burns to your skin and mucous membranes – sulfuric acid and acetic acid. Please wear all your gear at all times in the lab. Upon any suspected exposure flush the exposed area for fifteen minutes with cold water.

5. 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.

7. 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. .

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

D. 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 Ehrlenmeyer 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 one or two 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 Ehrlenmeyer 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 GCMS, 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.

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. **Isolate any crystals from your aqueous filtrate or supernatant from last week's aldol reaction or reactions.**
4. **Establish purity of these crystals using TLC. Make sure you have mastered TLC.**
5. **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.**
6. **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.**
7. **Start reading about how Warfarin and warfarin derivatives work. Write down your references for future paper.**
8. **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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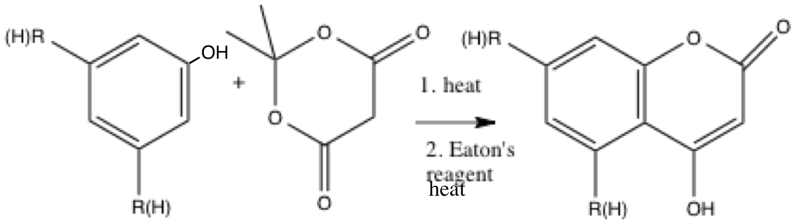
Week III

A. General Information

During the third 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.

B. Synthesis of 4-Hydroxycoumarin Analogues (the other hemisphere needed for Coumadin Analogues)

General Information - Preparation



1. To prepare for the third week you should look up the procedure we are going to use to make two, 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 three hour reaction time and then at four hours, rotavap it in the vial, cap it and freeze it, completing the reaction during week four. The paper is a paper by Gao, W-T et. al and it was in <u>Synthetic Communications</u> in 2010. Plan to work at a small scale (either the scale in the paper or twice the scale) and to carry out the reaction in a pie reactor in two dram vial with a pressure cap. . When you obtain the procedure start thinking about how this reaction could be adapted to a pie-reactor.

2. Start attempting to work 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 part of your grade, but are most important for writing your paper. They will be evaluated in the normal way every week by your TA. 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.

C. Synthesis of 4-Hydroxycoumarin Analogues
General Procedural Guidelines

1. Each group will make two analogues at the scale written or possibly, double the literature scale. There will be several choices of starting materials for analogues. You will pick your analogues when you come to lab and do the appropriate calculations. I expect students to be able to make these decisions, determine molecular weights and do reaction scaling in lab.

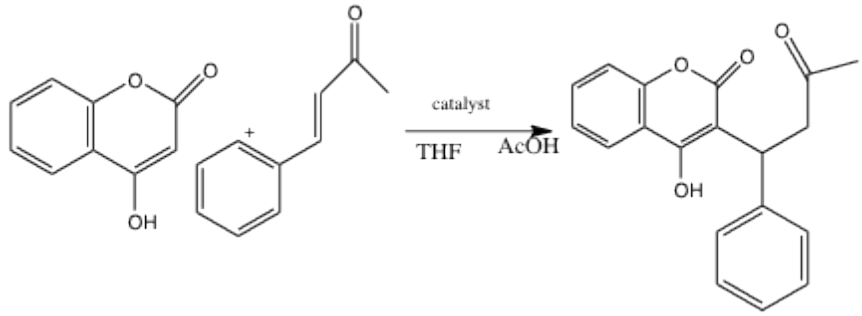
2.. The first step of the reaction will be set up in a two dram pressure cap vial with a micro stir bar. The reaction will be allowed to stir in the pie-reactor at the literature temperature, for the literature recommended time.

3. At the end of lab, the reaction can be taken out of the reactor, rotavapped, capped and frozen until the following week (ask instructor what to do about the stir bar). The following week, the appropriate amount of Eaton's reagent can be added and the reaction can be heated at the literature temperature for the recommended literature time during the next lab, once again in the pie reactor. Please do not rush these very important bond constructions.

4.. After the long Eaton's step the reaction is quenched in water and the compound is isolated and re-crystallized. See the paper.

5... It is imperative that your vials be meticulously labeled.

C. The Green Synthesis of Warfarin : General Reaction and Guidelines



4-hydroxycoumarin 4-phenyl-3-buten-2-one warfarin

1. Note: the above is the method we are going to use to synthesize all analogues and it will have to be adapted in future weeks 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.

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

3. 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.

D. A Possible Way to Organize Your Day during Week III

1. First thing in lab. If you are going to synthesize 4-hydroxycoumarin analogues, you must set up the first half of the procedure above – the part involving the phenol analogue and Meldrum's Acid, heated for four hours at 100 ^{o}C, followed by rotary evaporation at three to four times the literature scale. It takes a long time, so set it up first.

2. While the reaction is heating, you can work on other parts of the synthesis. This might include characterizing your aldol analogues (measuring mass, IR, NMR….). Working on purifying aldol analogues if needed. Possibly repeating aldols if necessary (not likely, but a possibility). We are attempting to make two analogues. They need to be pure, but you do not need a tremendous amount to proceed. Research groups have moved forward with a milligram or two. Utilize micro-techniques emphasized with flutamide to obtain the most information out of a sample – talk to us we can help you. For example, NMR samples can be rotavapped down in a tared vial and potentially used in reactions.

3**. See "C" above for this practice "warfarin" reaction. This is optional this week.** Start a practice conjugate addition coupling to make warfarin itself. **This will be your practice run and is very important. It is important to prove you can get this micro reaction to work before you attempt to apply the procedure to more complex, novel versions of the reaction.** To prepare for this practice reaction, you need to find a "green synthesis" of warfarin that 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 in a fairly recent Journal of Chemical Education article and you will need 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 if you are going to attempt it, write it in your notebook, write the reactions, mechanisms, diagrams and review of any procedures. IMMPDD

4. 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 when and how to run a TLC, when and how to use the rotavap or run an IR spectrum. Most benefit from visualizing what is going to be done the night before it is done or discussing it with the research group. Review Youtubes 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!!!

For your information, I visualize before I do anything.

5. Also note again – these are just suggestions as to where you might be and what you might be working on. Increasingly, research groups will be in different places. It is not a competition. Projects run differently and this is normal in the lab and in life.

6. We will start running spectra of aldol reactions in earnest this week. Please get used to the idea that you might not receive your spectrum until the following day or week. There are four folders outside my office, labelled with the names of the laboratory sections.

7. Work on purifying and characterizing any aldol reactions (measure mass, run IR spectra, run NMR spectra, etc.). If you have not run a column you are required 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 real (non-practice) Michael additions (the last step of the syntheses), both your aldol products have to be pure as do the 4-hydroxycoumarin 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. **Carry out the first part of the synthesis of two analogues of 4-hydroxycoumarin as described above. At the end of the lab, the reaction will be rota-vapped, carefully capped, labeled and frozen.**
2. **While the reaction of choice is being carried out, work on purifying and characterizing aldol products.**
3. **Set up practice, very small scale Warfarin reaction using DPEN catalyst. It is possible you may not do this until next week, but please do it.**
4. **Run baseline TLC against the starting materials for the practice Warfarin synthesis in dichloromethane.**

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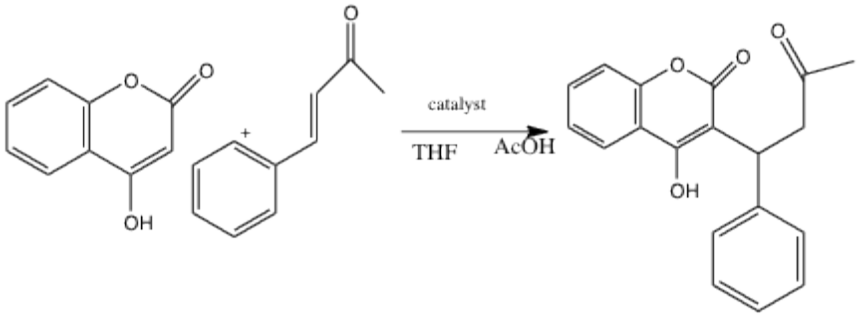
Week IV

A. General

At this point you have learned most of the techniques needed for the project. The main thrust of this week's instruction involves finishing the synthesis of your 4-hydroxycoumarin analogues and finishing other projects you might be working on to progress in your project toward the last steps. .

Lab lecture and any materials that are due is as described on the electronic schedule.

B. The Green Synthesis of Warfarin.



4-hydroxycoumarin aldol product warfarin

1. 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.

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

3. Run a 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 R_f 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

C. A Possible Plan for How to Proceed in Lab this Week

1. Finish Synthesis of 4-hydroxycoumarin Analogues (Start First)

a. Very Important - First Thing to do in Lab. It is very important that you finish your 4-hydroxycoumarin analogue reactions by adding the Eaton's reagent (this will be provided pre-measured in a vial) and then heating with stirring in the pie reactor for the same time and at the temperature indicated in the journal procedure being used for the procedure. Unless you have a stir bars frozen in your reaction vial, you will have to add a micro stir bar. Ask your instructor or TA.

b. Note: Eaton's reagent is viewed by chemists as a relatively safe dehydrating agent, however, is still comprised of P_2O_5 and p-toluenesulfonic acid. You must handle it with care! Because of this, we are going to provide vials that contain an appropriate volume of Eaton's reagent for you to add to your reactions.

c. Please return the empty vials that contained Eaton's Regent to your TA or instructor without washing. We will simply refill them for the next class.

d. At the time indicated in the journal article, the reactions are quenched in ice or cold water as indicated in the journal procedure you are using at the basis of your synthesis. A solid product should precipitate in each quench which can be isolated by vacuum filtration. Work as micro-scale chemists. If you don't have time to quench or vacuum filter before leaving, talk to your instructor or TA. The crude products can be re-crystallized this week or next week as indicated in the journal article.

e. Please be mindful of the micro-stir bar. Please return to your TA or Instructor.

2. Measure masses, melting points and IR spectra of any purified compounds.

3. Run NMR spectra of purified compounds (compounds should pass the TLC test - one band showing on plate, unique from starting material) before running NMR. Please be careful when making up NMR samples. Do not cross contaminate the deuterochloroform. If you have any doubts, please take a new pipet or syringe. You may not receive your spectrum until the next day or week. Check the files outside your instructor's offices.

4. You may have done this already…… Start a practice conjugate addition coupling to make Warfarin itself. (The basic information is given above and was included in Week III) **This will be your practice run and is very important. It is important to prove you can get this micro-reaction to work before you attempt to apply the procedure to more complex, novel versions of the reaction.** To prepare for this practice reaction, you need to find a "green synthesis" of warfarin that 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 above. This is a fairly

recent Journal of Chemical Education article and you will need 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.

Summary of Potential work for Week IV

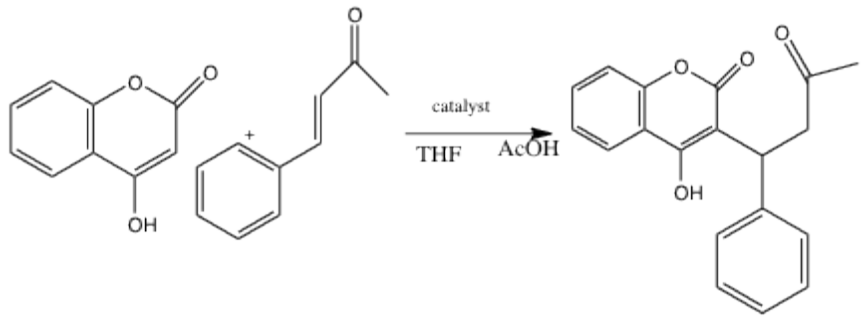
1. **Attend lab lecture.**
2. **Finish syntheses of 4-hydroxycoumarin analogues to the point of the crude product. (Eaton's Reagent Step).**
3. **Recrystallize the 4 –hydroxycoumarin analogues (very unlikely).**
4. **Work on purifying and characterizing aldol products, getting them ready for coumadin (warfarin) analogue synthesis.**
5. **Carry Out Practice Warfarin Synthesis and Run baseline TLC.**
6. **Analyze Practice Warfarin Synthesis by TLC.**
7. **Look up references for procedures, record in notebook, read about compounds you are making.**
8. **Work on and turn in any rough drafts as indicated on the general schedule.**

Week V

A. General

This week's lecture will cover the topics listed on the general lab manual schedule.

B. The Green Synthesis of Warfarin : General Reaction and Guidelines



4-hydroxycoumarin 4-phenyl-3-buten-2-one warfarin

1. Note: the above is the method we are going to use to synthesize all analogues and it will have to be adapted in this and future weeks to the

molecular weights of the 4-hydroxycoumarin analogue and/or 4-phenyl-3-buten-2-one analogues synthesized and coupled in the final reactions. Scaling has to be done on site unless it is the practice reaction.

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

3. 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 R_f 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.

4. If you have a practice reaction running you should monitor it by TLC to see if it is proceding and note how fast product is forming. You might even try isolating it by the recrystallization technique given in the paper.

C. Possible Plan to Proceed during Week V

1. Finish Purification of 4-Hydroxycoumadin Analogues

a. It is time to work on purifying your 4-hydroxycoumarin analogues, using re-crystallization. Last week or the week before, you should have isolated the crude product from the 4-hydroxycoumarin analogue reaction by precipitating it after quenching it in ice water. The desired 4-hydroxycoumarin analogue is presumably contained within the beige, brown or orange (in some cases black) solid you obtained. In regard to the crude material, don't filter if you don't have significant solid. Don't throw anything out until we have explored all options. The products are acids (conjugated enols) 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 visualize the following. If you pull the proton from the phenol, you will have the conjugate base which is water soluble. Then it is possible to 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.

b. Once you have the solid – which in most cases is a beige, brown or orange powder, you can rinse the crude crystals with cold water in the Buchner or Hirsch Funnel. Check the formation of your 4-hydroxycoumarin analogues by TLC (Use dichloromethane or dichloromethane with methanol – you can work out the proportions) using the corresponding starting material phenol as a standard.

c. The crude substituted-4-hydroxycoumarin analogue should 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 (even a vial), a boiling stone, and heat gently. It may require anywhere from two to three mL of hot ethanol to five to six mL of ethanol. Again, do not give up and do not over heat. Unfortunately, some of the yields can be small. Please be careful not to throw filtrates out or to contaminate filtrates. We may be able to isolate product from filtrate. Products can be contaminated and require another re-crystallization. This is not unusual.

d.Once you obtain crystals from the substituted 4-hydroxycoumarin analogue reactions, allow them to dry 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, possibly some C-13 NMR.

2. Working Small Scale: Remember, a good way to work micro-scale (if you have very little product) is to first measure the NMR. Next. use the NMR sample to make the film on the IR cell. Add the remaining NMR sample into a tared (using analytical balance) vial and rotavap to dryness. Measuring the melting point may not be possible, but after determining the mass of the deposited compound can be used at the basis of the synthesis of a coumadin analogue using the "green synthesis" method that is described above. (once again) Ask if you have very little of a compound, there is often a way to do the final reaction on a very small scale.

3. Working Clean: One of the biggest problems we have at this point in the synthesis is the build up of contaminants in glassware, especially round-bottoms. The contaminant after multiple transfers from dirty glassware can be in greater quantity than your micro-scale product. At this point in the synthesis, you should be working in new vials rather than round bottoms. As you know, there is a way to rotavap into vials, but you must be very careful, the trap must be clean , the vial must be new and there must be a lot of venting.

4. Aldols Continue to purify and characterize the products from your aldol reactions. Yes, this can still be going on.

5. Meeting. It is not a bad idea if you are confused at this juncture to sit down with your instructor or TA for five minutes to come up with a half-time game plan. In this meeting, you need to show your instructor or your TA your compounds, NMR spectra and masses of materials that have isolated. Your instructor or TA can give you advice as to what is finished and how to proceed.

6. Start Green Synthesis of Coumadin Analogues

a. General

To the extent that you have products from the 4-hydroxycoumarin analogues and you have pure 4-phenyl-3-butene-2-one analogues, you should begin your reactions to make warfarin analogues. These should involve the four possible reactions of your two, 4-phenylbut-3-en-2-one analogues (aldol products) with either of your two substituted 4-hydroxycoumarin analogue products. If you were unsuccessful

with any of your reactions, you can limit the reactions you attempt. Just ask and we will advise you.

b. Specifics of Getting Started. It is possible that you might set up one to four of the following proposed, four micro-scale warfarin reactions. While this is possible, it is unlikely to be ready to do all four. TALK TO YOURTA/INSTRUCTOR. IT TAKES A LOT OF TIME. A good start might be to set UP ONE OR TWO. Within the last two weeks, you should have started a practice reaction to make warfarin itself using commercial reagents. The practice reaction has presumably done on a 0.1 mmolar scale and is a good model for what you will do with your synthesized analogues. Your synthesized substituted 4-pnenylbut-2-en-2-ones and your synthesized substituted 4-hydroxycoumarins should be set up in vials just as your warfarin practice reaction, adjusting quantities based on the molecular weights of the compounds being used and scaled based on the amount of reagent in lowest quantity. Though you can set up reactions at any scale (and sometimes out of necessity have to work at very small scale, it is best to be working at two or three times the practice reaction in order to obtain a workable yield. Please sit down and do the calculations and double check the calculations before setting up a reaction. 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 DPEN Catalyzed 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.

These are the possibilities in a general format.

Alpha, beta-unsaturated ketone 1 with substituted 4-hydroxycoumarin analogue 1→ Coumadin analogue 1
Alpha, beta-unsaturated ketone 2 with substituted 4-hydroxycoumarin analogue 1→ Coumadin analogue 2
Alpha, beta-unsaturated ketone 1 with substituted 4-hydroxycoumarin analogue 2 → Coumadin analogue 3
Alpha, beta-unsaturated ketone 2 with substituted 4-hydroxycoumarin analogue 2 → Coumadin analogue 4

Note: the alpha, beta- unsaturated ketones, can be called 4-phenylbut-3-en-2-one analogues – they resulted from the aldol reactions. Most people have two of these. If you are limited to one substituted 4-hydroxycoumarin analogue, you can use commercial 4-hydroxycoumarin for two reactions.

c. Some Experimental Details

1. Please note the information given under the Green Synthesis information given in B above.

2. When, you get the reactions started, you should run a baseline TLC, visualizing with UV and p-anisaldehyde staining as a baseline for each reaction.

3.Next week you should monitor the reactions you have started and decide if any have formed warfarin products and if they are ready, you should start purifying the coumadin analogue for the best two most complete reaction. The two most ready will be decided by the amount of warfarin product observed in the TLC analysis after a week. If you are not satisfied, they can be allowed to react for another week if time avails. The purification will probably involve column chromatography. Speak with your instructor and TA about how to scale for the column.

7. Rough Draft of Introduction. If you have spare time and have not already done so, please start working on your introduction rough draft. What are the major components of an introduction? We have gone over this in class twice. Normally, Introductions include information on the source of the compound - if it was isolated from a biological source, any information on the structural proof (you need to know what you are making). They also include why the compound is important to synthesize (for example, its biological activity – is it anti-fungal, anticancer etc. ?). Introductions usually continue with a summary of how the compound was synthesized. In this case, because the compound was synthesized so many times, the summary can be more general, giving the types of reactions that have been used to make it. For example there are any number of catalyzed Michael additions that have been used, but this could be just referred to generally. Introductions end with a summary of the types of interesting reactions used in your synthesis, emphasizing why your synthesis is better. This is all written in third person, passive voice and is very succinct, using citations in footnote form for all the information summarized. Please consult the Journal of Organic Chemistry for style questions. You can also use the ACS style guide or consult your friendly librarian. This will have been discussed in lecture. Please refer to the beginning of the document and associated to understand why Coumadin and coumadin analogues are interesting to make.

Specifically, the idea is that each of the four compounds you are potentially making and using have different activities in the carboxylase reaction described above and in your reading (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 varying degrees?

Generally, it is hoped that the entire experience increased your lab skills and your ability to work independently. It is hoped your ability to reason

through procedures and trouble shoot has improved though these ideas do not belong in a formal paper.

See the end of the documents for some useful info about paper/draft writing.

Summary of Possible Work for week V.

1. **Attend Laboratory Lecture**
2. **Finish synthesis of t-butyl-4-hydroxycoumarin analogues by isolating and purifying them by re-crystallization.**
3. **Characterize any compounds that are ready by NMR, IR and MP. Determine yields.**
4. **MAYBE 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.**
5. **Work on and turn in any rough drafts as indicated by the general schedule.**

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.

Week VI

A. General

This week's lecture will cover the topics listed on the general lab manual schedule.

B. Working Small and Clean Revisited. Going forward, you need to work with new, very small vials (1 or 2 dram). The DPEN catalyzed Michael (conjugate) additions are done in small vials at room temperature. We are adapting the green synthesis paper you looked up recently using your related, but different synthesized reagents and working on a variety of scales– roughly, 0.05 mmol scale to 0.40 mmol scale. Realize we do have the capacity to rotavap in a vial. You need to be very careful when working on a micro-scale level. In extreme circumstances, this could mean you have a tiny sample in a small vial. You know the mass of the sample because you have tared the vial on an analytical balance and have measured the mass again after the sample has been deposited in the vial via rotavap. It is so small that the entire sample has to be dissolved in deuterated chloroform for the NMR measurement. This being the case, the sample has to be recovered for IR and to carry out reactions. You can apply a small amount of the NMR sample to an NaCl plate and allow the deuterochloroform to evaporate, creating a film. The remaining sample can be poured back into the original vial or split between two, tared vials (if

one wanted to do two DPEN catallyzed Michael reactions). The NMR tube should be rinsed with DCM and the rinsings added to the original vial or the reaction vials. The vial or vials can then be rotavapped to dryness and then reweighed.

B. Possible Plan to Proceed during Week VI

1. 4-hydroxycoumarin analogues. What is the situation with your substituted 4-hydroxycoumarin analogues? Do you have one or two that are pure? If not, see Dr. Nerz as she may have some spare material. Is your material still crude? If so, consider a re-crystallization with 95 percent ethanol or a base extraction followed by precipitation – see instructor. Have you run a proton NMR? If not, you should run one early in class as this is needed to proceed to final product.

I normally have a sense of where most students are at this point and it seems to me that most people are usually a bit behind in that they either have some pure or nearly pure substituted 4-hydroxycoumarin or they have their substituted coumadin crystals growing. It would be very useful to come in before lab and vacuum and rinse (with ice cold ethanol) any crystals that have formed during the week.

2. Starting DPEN catalyzed Michael Additions (conjugate additions – the green synthesis of warfarin). Regardless of situation, all research students need to set up DPEN catalyzed Michael Additions this week so they are ready for week seven (week seven is the last week. Most students have two substituted 3-buten-2-ones (but-3-en-2-ones) and they have enough to do four reactions. If not, it is OK to do fewer. If you have no substituted 4-hydroxycoumarins and we have no spare synthesized material, you can react them with commercially available 4-hydroxycoumarin that we have in the lab.

The scale of these reactions is normally dependent on how much substituted 3-buten-2-one or substituted 4-hydroxycoumarin you have (which ever is the lowest yield) and can be quite a range of scales – reasonable scales are 0.05 to 0.4 mmole. As noted above, the reactions should be done in new, small, screw cap vials that have been tared. We have adaptors so that even the smallest amounts of material can be added in solution to a tared vial and then rotavapped to dryness and reweighed. Then, the reaction is scaled according to the quantity of material in the vial.

Note that the scale of the vial syntheses that you are setting up are based on the practice green synthesis of warfarin you monitored by TLC over the past two weeks. This was done on a 0.10 mmole scale and it is a good model for your actual reactions, though you must alter masses based on the differing molecular masses of your starting materials in your analogue reactions.

A second note: it is a good idea for your final DPEN catalyzed Michael additons that will result in warfarin analogues to run a baseline TLC with DCM (with or

without methanol) as the eluting solvent. Standards can be applied to the plate if you have enough yield. The plate should be visualized using UV and then, p-anisaldehyde stain.

The practice warfarin reaction was carried out so you could have the experience and also observe how the reaction proceeds via TLC. The observations you made are very instructive when you are evaluating your final DPEN catalyzed Michael additions that result in warfarin analogues. Note: the practice reaction will not be purified, unless the final experimental reactions fail.

As written in the green synthesis paper, warfarin stains purple, the ketone reddish and the hydroxycoumarin does not stain with p-anisaldehyde stain.

3. **DPEN catalyzed Michael Additions (conjugate additions – the green synthesis of warfarin) that are in progress.** If you were fortunate enough to start any DPEN catalyzed MIchael additions last week, please check them carefully by TLC using dichloromethane (with or without methanol) (use UV light and stain to visualize) to see if you have any coumdadin analogues (warfarin analogues) are forming. If you do observe significant coumadin analogues, you should consider purifying your product via micro column. Please see your instructor about this, but note the following in 4. below.

4. Purifying Warfarin Analogues Columns will be run as you previously have in either dichloromethane, dichloromethane with perhaps 0.10 to 0.25 percent methanol or some ethyl acetate, hexane combination – we must look at your TLC to decide. The literature procedure you researched describes first isolating the crude material by blowing off the solvents using a gentle stream of air. The crude material then has to be added to a small amount of the eluting solvent before applying it to the column. If your material weighs more than 0.100 gram, I suggest using one of the medium blue columns with about four or five grams of silica gel. If you have less material, I suggest carrying out a mini-column using a large pipet or a 10 mL syringe as the column. This will require about 2.0 grams of silica gel and some instruction from your instructor/TA or another student who has done this. A few groups are experts at this procedure. In any case, please obtain some guidance before separating your warfarin products. I realize many students will not have these until week 7.

If any group is doing two columns simultaneously you must work efficiently. You have to divide the work and use time saving tips. Meaning, you should prepare the test plate while you are running the columns, you should continuously run the column, keeping the column full so it runs at high speed and should start running TLC plates of the regions of potentially overlapping bands while the column is running. The team should divide and conquer. There is a reason you are in groups.

5. Practice Warfarin. You can run another TLC and visualize in the normal way for your practice warfarin reaction. It is interesting to see if it is all warfarin at this

point. It gives you info about the rate of the reaction and the amount of catalyst you will need to get your final reactions to be complete in the remaining week.

Possible work for Week VI

1. **Attend Laboratory Lecture.**
2. **Work on purifying your substituted 4-hydroxycoumarin compound (made or borrowed) pure enough for the DPEN catalyzed warfarin reactions.**
3. **Check your practice warfarin reaction to see if it is complete via TLC analysis in DCM – visualize with UV light and the p-anisaldehyde stain.**
4. **If you did no. 3 already, don't worry about it, but don't throw out your practice reaction. It might be all you have next week.**
5. **Check your DPEN catalyzed Michael addition reactions (if any) by the same method used for the practice reaction. If you have adequate warfarin analogue, isolate it as per the green synthesis procedure and purify it by column chromatography. Consult your instructor about possibly using a mini-column or an alternative solvent system. We want to do what is most efficient for you in the waning days of our research time.**
6. **Set up any remaining or all possible DPEN catalyzed Michael additions to make final wafarin analogues. If you have no substituted coumarin material, you may substitute commercial 4-hydroxycoumarin.**
7. **Characterize any compounds still in need of characterizing.**
8. **Turn in any rough drafts as indicated in the general schedule on-line. You should be at least done with you intro rough draft and working on your experimental rough draft.**

   Words of encouragement. You only need to make one warfarin analogue to be successful. If you can only attempt two, you will probably be OK. Everyone will be OK anyway, because you have done so well and learned so much.

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Week VII

A. General

This week's lecture will cover the topics listed on the general lab manual schedule.

B. Possible Plan to Proceed during Week VII

1. **Evaluating DPEN catalyzed Michael Additions (conjugate additions – the green synthesis of warfarin) that are in progress.** First thing when you arrive, you should test all your reactions via TLC vs. standards (if you have them) using DCM as the eluent. Reminder: As usual, you have to figure out how many spots to apply to each location on the plate, but diluting the drop taken from the reaction with DCM is usually necessary prior to application. Remember you can look at the spot before you run it using the UV lamp and you should be spotting from our small (doubly open) melting point-type capillaries. Do not spot from Pasteur pipets. The spots are too big and the resolution is very poor. Always look at the plate under the lamp and show it to your instructor before staining. After it has been observed under the UV lamp, dip it in the stain. Let the stain soak in and ten use the heat gun to develop the stain.

Occasionally we will run a GCMS or perhaps an NMR of a crude reaction to see if it worked. Ask about this if your TLC is uninformative.

2. Isolating the crude coumadin analogue. When a reaction is believed to have adequate warfarin analogue product, the solvent has to be removed either by rotavapping the vial or gently blowing the solvent off with a stream of air (ask for assistance from your TA for the latter).

3. Purifying a larger scale reaction. If your reaction is larger scale (0.3 or 0.4 mmole) and appears to have a good Yield, it is possible to isolate the final warfarin analogue by recrystallizing using an acetone and water solvent system.

4. Purifying a small scale reaction. If it is smaller scale (0.2 mmmole or smaller), or your sample did not crystallize due to low yield, you should separate it by chromatography as described in the green synthesis of warfarin. As has been demonstrated numerous times in lab, you should do these column separations using a mini-column. To carry out a mini-column, one has to be ready (have all materials prepared) and should not be using more than 0.08 or 0.09 grams of material (it may mean you will use part of your sample). The sample has to be dissolved in ten to twenty drops of eluting solvent before packing the column. It is hoped that this week you can pack these columns yourself as we have now packed several columns. The procedure is also described in detail in the following. You should attempt to separate your best material – the most reacted and the material that gives a definitive blue spot after staining on TLC or spot that appears to be warfarin based on R_f or instructor experience. At most, you should do two columns and these should be run early, not late in the lab. If it turns out you have nothing that worked (this is unlikely), you will resort to separating your test warfarin reaction.

How to pack mini-column:

a. Obtain a larger pipet from your ta or instructor or a small syringe. Put a small plug of glass wool in the pipet or syringe , at the bottom. Layer a small

amount of sand (a few millimeters) over the plug). Clamp the pipet or syringe at an appropriate height for fraction collection on your monkey bars.

b. Have ready the following: a rack of 20-40 new test tubes. Also have ready, a source of DCM (about 50 mL), three or four clean, new Pasteur pipets and several bulbs. Weigh out 2 grams of silica gel and make a slurry by adding ten mL of DCM.Your sample (weighing 0.05 to 0.09 grams) should be dissolved in ten drops of DCM.

c. If the above are done, then the column can be started. You slurry pack the column to be within about 2 to 2.5 cm from the top of the pipet. Meaning the slurry when settled should be within 2 to 2.5 cm from the top of the pipet.

d. Add a mm of sand to the top of the settled silica gel.

e. Next, allow the solvent to drip down to about 1 mm from above the level
f. the sand in the column.

g. The dissolved sample should then be loaded by gentling trickling it down the side of the column in a circular motion. When the liquid is just above the silica column, rinse the sides of the column into the column with a small (about ten drops) of liquid of several times to load the residual material into the column.

h. When the liquid above the column is clear, fill the column with the eluting solvent and then start collecting fractions – they should be small fractions, about 20-40 test tubes.

4. After you isolate your compounds, you should characterize them to the best of your ability. You should obtain masses (remember to tare the vials before you rotavap the fractions), proton NMR and IR. Perhaps we will also run GCMS or Carbon-13.

Note, if we don't have time to run your NMR, we will run it at a later date. In this situation, the sample should be submitted in a very well labelled vial. The spectra will be run later in the week and will be outside my door.

Summary of Week VII

1. **Attend Laboratory Lecture**
2. **1Analyze any reactions still in process by TLC.**
3. **Remove solvents from reactions.**
4. **Weigh crude solid.**
5. **Decide whether to re-crystallize or run columns of two best reactions.**
6. **Isolate compounds by chosen method.**
7. **Characterize compounds.**

8. **Turn in any drafts as indicated in the course schedule. You should be at least at the point of turning in your rough discussion and working on your final paper.**

END NOTES

The goal of the project was described above – to synthesize a library of compounds (some known, some new that may have increased or decreased biological activity compared with warfarin. In addition, it is of interest to test the general utility of the catalyst for catalyzing conjugate additions. Did the catalyst 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 and high enantiomeric purity is very important. As you read, the two enantiomers of warfarin do not have the same biological activity.

My expectation is that you will write a paper including anything you have done in lab as of this week. For most groups, this would involve writing about the completion of two of compounds in the library. Regardless, it is very important to report all results as negative results are results. In other words, reactions that were attempted and did not yield results should at least be included in the experimental section. Eventually, if we get some good results together, the results will be published in some form and you will be credited as contributing.

You have written your introductions for the most part and I have given you feedback, so let me address the others sections. Your next section to submit is the General section, and a formally written experimental section (to the extent it is possible) for each compound you made, even if it was not utilized or gave no yield. One paragraph for each of the aldol products (analogues of E-4-phenyl-3-buten-2-one), one for your t-butyl- 4-hydroxycoumarin 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. The paragraphs should be written about your procedure, on your scale with your modifications in concise, journal language. There should be a citation for the procedure you used as the basis for your procedure. You should 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. Of course, this was described in class.
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 papers I have made available on moodle. In particular, pay attention to the Angewandte Chemie paper from 2003. Not only is it a good paper to look at

the style for interpreting peaks in the experimental section, but it has a large amount of very relevant spectral data that will help you in assigning spectra.

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 simply be referred to by the number.

Some useful information for paper writing

Write in third person, passive voice. Follow the style of the Journal of Organic Chemistry.

On the Bryn Mawr library site there is an ACS style guide which is useful – this will show you how to format your citations, including those from websites.

NMR/IR spectra and data can be found in the 2003 paper I have put on moodle, but also one can use the excellent data bases found on our Bryn Mawr Library site, including SciFinder Scholar, Reaxsis (this is a phenomenal site) and others. Check it out.

- Our IR is a Perkin-Elmer FTIR Spectrum Two
- All spectra were run as solid films or KBr pellets (so far only a few KBr pellets)
- Our NMR is a Bruker Advance III 400 (we are operating at a central frequency of 400 MHz for proton and 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 t-butyl-4-hydroxycoumarins are called 6-t-butyl-4-hydroxycoumarin, 8-hydroxycoumarin and 6, 8-ditibutyl-4-hydroxycoumarin, depending on which one you made.

The Warfarin names can be seen in the Angewandte Chemie by Halland et al. that is on moodle and has been distributed to you.[5] 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.

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