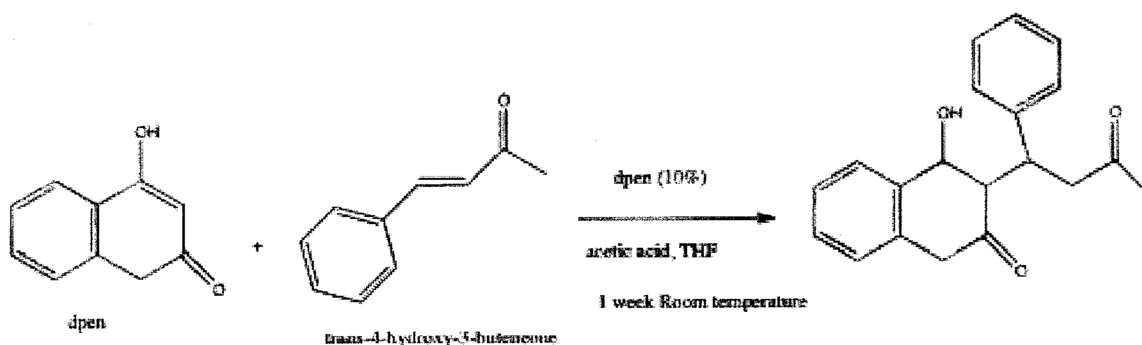
Experiment 26 – A Green, Enantioselective Synnthesis of Warfarin [1]

Warfarin (coumadin) is a commonly used anticoagulent in medicine it is also a commercial rat poison. [1] In preparation for this lab, you should read about conjugate addition (Michael addition) in your text (Chapter 22). For your own edification and enjoyment, you should read about how coumadin works as an anticoagulent in its medical application and how it works as a rat poison.

In the synthesis we are using, we will be working at microscale to produce a single enantiomer of warfarin from the dpen catalyzed conjugate addition of 4-hydroxycoumadin to trans-4-phenyl-3-buten-2one. The net reaction is shown below.



What makes it green? Primarily, the very small scale and the use of a catalytic quantity of a potentially recyclable catalyst though the principles of green chemistry are given below. [2]

The reaction is very interesting on many counts. It demonstrates two major areas of carbonyl chemistry, the formation of imines or Schiff bases and conjugate addition. The mechanism will be covered in detail in class because it is quite complicated. It will probably be covered in the second week of lab. The lab is not due until the second week of lab, but it does the formation of imines on dpen with 4-phenyl-3-buteneone which sets the group stereochemically for the conjugate addition of the 4-hydroxycoumadin. Please take detailed notes in lab. It is very important to take notes even in this age of high tech. I will probably make a move of the mechanism which will be embedded in this text. Again, see chapter 19 and 22 .

The chirality of the specific depn results in facial selectivity and the formation of a single enantiomer.

Procedure:

Toward better technique. Use the designated, clearly labeled spatula, syringe or pipet in measuring out the material. For small containers re-clamp them to the ring stand.
Do not waste material. The materials for this lab are very expensive. When you remove a cap, lay it on its back and then replace it when you are finished. The exact same cap.

You should work in groups of four for this procedure. All teams should work together and make observations together for this procedure. Don't be just an observer, get involved, give your opinion get your gloves dirty!

We will be using procedure two given below (below, but above my explanation of TLC – Thin Layer Chromatography) on a quarter of the scale. The following is very, very important. It is very important that you do not waste materials and that you only use the spatula designated for the dpen catalyst. It is .01 grams and no more. Waste of the extremely expensive catalyst will result in some people not being able to do the reaction. When finished with the catalyst, clamp it back to the ring stand and replace the cap (the actual cap).

It is very important to only use the labeled spatulas to measure out the 4-hydroxycoumadin and the trans-4-phenyl- 3-buten-2-one Please use the designated syringes for the measurement and transfer of the acetic acid and the dry THF. Please return the reagents to where you found them and place the correct cap on the bottles.

These reactions should be done in the small provided vials. Again you should be working in groups of four and you should do this reaction while experiment twenty-four is cooking. It should take no more than thirty minutes to get it started if you are prepared.

Please note again we will be following procedure no. 2 below from Harvey Mudd College on ¼ scale.[1] They very kindly gave us permission to use some of their materials. This week you will mix up the reaction and study its TLC (Thin Layer Chromatography) vs. some standards. The reaction will be allowed to react for a week after which you will continue with procedure 2 on a ¼ scale. We will measure another TLC to see how the reaction has changed over the course of a week. You

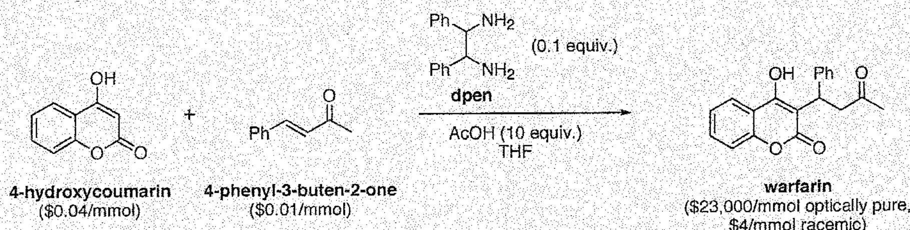
will notice below the Harvey Mudd pages, there is an explanation of TLC. Please read this explanation.

Procedure and some other introductory comments from Harvey Mudd College[1]

Student laboratory handout/instructions

A Green, Enantioselective Synthesis of Warfarin

Introduction: In this experiment, you will use (*R*,*R*)- or (*S*,*S*)-1,2-diphenylethylenediamine (dpen) to catalyze the conjugate addition of 4-hydroxycoumarin to 4-phenyl-3-buten-2-one to produce warfarin. You will also determine the stereoselectivity of the reaction using polarimetry.



Background Information: In synthesizing complex organic molecules, chemists often face the challenge of creating molecules with correctly oriented stereocenters. Although racemates can be separated in some cases, this is not always easy and the undesired enantiomer is generally discarded as waste. Thus, stereoselective reactions are highly desirable and represent an active field of research. For several decades, chiral organometallic catalysts have been the dominant tools for such transformations, while organocatalysts were largely overlooked until recently.

Warfarin, first used as a rat poison in 1948, is named after the Wisconsin Alumni Research Foundation (its patent holder) plus the suffix from coumarin. Fears about its toxicity to humans declined after an unsuccessful suicide attempt in 1951,[1] and racemic warfarin is now a frequently prescribed oral anticoagulant. Warfarin is a vitamin K antagonist, and it attenuates the biosynthesis of vitamin K-dependent blood clotting factors. The *R* and *S* enantiomers of warfarin differ in potency, metabolism and interactions with other drugs.[2] Practical, enantioselective routes to warfarin are therefore attractive for future applications of the drug, particularly for patients using other medications.[3-4]

Before you begin the experiment, decide whether you wish to perform experimental procedure 1 or 2 and whether you plan to use (*R*,*R*)- or (*S*,*S*)-dpen.

Experimental Procedure 1[4] (0.1 mmol scale with column purification):

To a ½-dram vial (without a magnetic stir bar), add 4-hydroxycoumarin (16 mg, 0.10 mmol), *trans*-4-phenyl-3-buten-2-one (15 mg, 0.11 mmol), your dpen catalyst (2.1 mg, 0.010 mmol), anhydrous tetrahydrofuran (0.2 mL), and acetic acid (57 µL, 1.0 mmol). Swirl the vial to dissolve the solids and let it sit at room temperature for one week. At completion, the previously peach or pink solution should be clear yellow.

After one week, check the progress of the reaction with thin-layer chromatography (TLC) on silica gel plates using pure CH_2Cl_2 as eluent and anisaldehyde stain. You may wish to prepare a TLC sample of commercial, racemic warfarin to compare with your product. Remove the solvent and acetic acid from the reaction vial with a stream of air. Set aside a small amount of crude product (diluted in CH_2Cl_2) as a TLC reference sample.

Prepare a microscale column on silica gel in the following way. Gently but firmly, plug a Monstr-pette® with a small amount of glass wool or cotton. Cover the glass wool or cotton with a thin layer (5 mm) of sand. Make a slurry of silica in CH_2Cl_2 appropriate for your column. Slowly rinse through the column with CH_2Cl_2 before adding the silica/CH_2Cl_2 slurry. The final height of the silica layer should be about 3 cm. Cover with another thin layer (5 mm) of sand. When the solvent has fallen to the level of the top layer of sand, add your crude product dissolved in a minimal amount of CH_2Cl_2. Using a 1% methanol/CH_2Cl_2 solvent system, obtain approximately 20 1-mL fractions. Never let the silica gel run dry. Spot every third fraction on a TLC plate. Before developing the TLC plate, check with a UV lamp that you have UV-active spots in at least some of the fractions. Check the purity of the fractions by developing the TLC plate with CH_2Cl_2. Determine the optimal fractions, combine them in a tared vial, and concentrate by rotary evaporation. Calculate the yield of product.

Table 1. Thin-layer chromatographic analysis (CH_2Cl_2 on silica gel).

compound	R_f (pure substance)	R_f (in reaction mixture)	**stain color** (using anisaldehyde)
4-hydroxycoumarin	0.00	0.00	faint white
dpen	0.00	0.00	faint white
trans-4-phenyl-3-buten-2-one	0.63	0.72	intense red
warfarin	0.12	0.34	intense purple

Experimental Procedure 2 (2.0 mmol scale with recrystallization):

To a 4- or 6-dram vial (with or without a magnetic stir bar), add 4-hydroxycoumarin (0.325 g, 2.0 mmol), *trans*-4-phenyl-3-buten-2-one (0.307 g, 2.1 mmol), your dpen catalyst (0.042 g, 0.20 mmol), anhydrous THF (4.0 mL), and acetic acid (1.14 mL, 20 mmol). Swirl the vial for a few minutes and let it sit at room temperature for one week (or, if preferred, stir magnetically). At completion, the previously peach or pink solution should be clear yellow.

After one week, check the progress of the reaction with thin-layer chromatography (TLC) using pure CH_2Cl_2 as eluent and anisaldehyde stain. You may wish to prepare a TLC sample of commercial, racemic warfarin to compare with your product. Remove the solvent and acetic acid from the reaction vial either by rotary evaporation or using a stream of air.

Dissolve the residue in a minimal amount of boiling acetone. Add boiling water dropwise until the solution becomes significantly cloudy. Heat the mixture to dissolve the crystals, adding a few drops of boiling acetone if necessary. Slowly cool the solution to room temperature and then in an ice bath. Collect the crystalline product with a Buchner funnel, carefully rinsing with a small amount of ice-cold 4:1 acetone/water. Let the crystals air-dry and calculate the yield of product.

Explanation of TLC

Please read before doing the lab.

To analyze this reaction before and after reaction we will be using a new technique called thin layer chromatography. Like gas chromatography, it involves a competition for molecules between a mobile phase and a stationary phase. In the type of thin layer chromatography we will be practicing, the stationary phase is a thin layer of very dry silica gel. Silica gel is very polar. The mobile phase is any solvent you choose to rise up the silica gel plate by capillary action. The compounds to be analyzed are applied to the lower part of the plate (the origin) and the migrating solvent is in competition for the molecules with the silica gel. Molecules that are very polar will spend more time adhering to the silica gel and will not migrate as much (low Rf). Typically the solvent is significantly less polar than the silica gel, so if the applied molecules are less polar they tend to spend more time in the solvent, migrating higher on the plate (higher Rf). The plates are run until the solvent is about a cm from the top of the plate. If the compounds are white they

need to be visualized using some sort of visualizing agent. We will be using a UV lamp. Our TLC plates are impregnated with a fluorescent compound and upon exposure to UV light will either absorb or emit light against a florescent green or orange background. When compound absorb, they will look dark blue or black in the bright background. Fluorescing compounds are pretty obvious, but will tend to be in the blue range.

Once your prepare your reaction solution, you should shake it and get a baseline TLC. The reaction should be a lovely peach color at this point. To learn more about TLC watch the following video and read the following instructions.
Each student in a group should do his or her own TLC analysis.

http://www.youtube.com/watch?v= TqGAnK9Rkw

The above video is useful in that the student is using similar equipment to what we are using and her technique is the technique I hope to emphasize, however, our compounds will be colorless, we will be applying standards (pure compounds that may be in the mixture) to the TLC plate to help identify the compounds in the reaction and we will be visualizing the compounds using a UV lamp.
I will produce another video this weekend just in case you want it. It will be fun.

1. First, set up your TLC chamber. You can do this by taking a clean, dry 400 or 600 mL beaker and adding about 5-6 mm of a 1 percent solution of methanol in dichloromethane (this is a volume;volume ratio). A large piece of filter paper should be dipped in the solution and stuck to the wall of the chamber. Put a watch glass on the top and allow the chamber to come to equilibrium for ten minutes. Every student should do this. Not a group activity.

2. Next, you need to prepare your standards. Each group can prepare one group of standards. Your standards are 4-hydroxycoumarin, 4-phenyl-3-butene-2-one, and warfarin. To prepare the standards, you need to add a spatua tip of each compound to about 1 mL of dichloromethane. At the end of this process you should have four labelled vials that your team can use and that can be used again next week.

3. Obtain a TLC plate. As the student shows in the video, draw a light pencil line about 1 to 1.5 cm from the bottom of the TLC plate. This is called the origin. Make four evenly spaced tick marks with the pencil on the line. You should be working on the silica side of the plate (the other side is shiny plastic). Note too much pressure with the pencil will scrape the silica off the plate. The plates are delicate. Avoid putting the tick marks too close to the edge of the plate. These are the locations where you will apply your standards and your reaction mixture. You can lightly label each tick mark if you desire. Make a diagram of your plate in your lab notebook.

4. Like the student in the video you will use a thin capillary pipet to apply one or two spots of each of your standards (applied to separate tick marks) and your

reaction mixture. I highly recommend blotting the pipet on a paper towel before spotting. Normally the reaction is applied on one of the more central tick marks. The goal as seen in the video is to apply a small concentrated amoun of the solution and to allow the solution to dry in each location. Two spots in a given location should be adequate. Note each spot is a different compound or mixture and a new pipet is used for each. Though it is a group lab, every student should do this.

5. After all four solutions have been applied, you will dip this plate into your TLC chamber and allow the solvent to rise by capillary action to within 1 cm of the top of the plate. The plate is leaned against the wall opposite to the filter paper, with the silica gel side out. The lid must be placed on the chamber during this elution.

6. When the solvent has risen, the plate is removed and is allowed to dry. Upon drying it is visualized using a UV lamp as described above. Be careful with the UV lamp. You can hold it over your plate, just take a pencil reach in and circle the spots you observed. They will not be retained after turing off the lamp. Record any color differences or fluorescence changes between the spots. These may help in the identification. You can calculate a Rf value for each spot. It is the distance from the origin to the center of the spot divided by the distance from the origin to the distance the solvent travelled, this should have been marked. Rf are constants for a given compound in a given solvent system under perfectly replicated conditions. They are analogous to retention times measured in gas Chromatography.

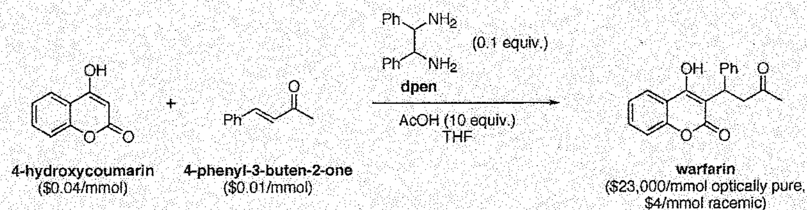
As stated in the above Harvey Mudd procedure, you will leave your reaction for a week. Next week you will run another TLC plate and note any progression of the reaction.. While your first reaction of your total synthesis is taking place you will try to recrystallize your product as described in the Harvey Mudd procedure 2 if there is any evidence of product. If we obtain the product, we may run an NMR – perhaps a carbon for fun, probably a hydrogen nmr.

Lab Write Up

- Your lab write up will consist of the worksheets given below (courtesy of Harvey Mudd), please complete these in lab during the second week. There is a long wait during the first reaction of your total synthesis project.

Student classroom handout/in-class group activity

An Enantioselective Synthesis of Warfarin



1. Draw the structures of (*R*,*R*)- and (*S*,*S*)-dpen.

2. Pick one of those stereoisomers and draw the product of its reaction with two molecules of 4-phenyl-3-buten-2-one. Let's call that intermediate **A**.

3. Using your model set, build intermediate **A** with accurate stereochemistry, remembering that π-systems often like to stack on each other (so-called π-stacking). You may need to abbreviate the phenyl rings.

4. How might acetic acid react with intermediate **A**? Draw the resulting product **B**.

5. Draw a mechanism for the reaction of 4-hydroxycoumarin with intermediate **B** to provide warfarin. Indicate the stereoisomer of warfarin you expect using your dpen catalyst.

- Drawings of your TLC plates.

- The mass of product obtained.

- The percent yield.

- The melting point of your product.

- The interpretation of any nmr spectra.

- A brief discussion of yield, purity and identity. – a one pager no more.

- Please submit only one lab report per group to Dr. Nerz. Please submit the best TLC data (one set only please).

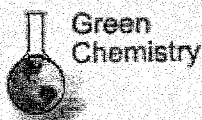
- Please try to complete as much of this lab as possible while in lab.

References:

1. **Terence C. Wong, Camille M. Sultana and David A. Vosburg** *J. Chem. Educ.*, **2010**, *87* (2), pp 194–195

2.

Green Chemistry[1]



Green Chemistry is an overarching philosophy of chemistry defined by a set of principles. The leaders of the Green Chemistry movement, P. T. Anastas and J. C. Warner define Green Chemistry as follows:

"Green chemistry, environmentally benign chemical synthesis, alternative synthetic pathways for pollution prevention, benign by design: these phrases all essentially describe the same concept. Green chemistry is the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products. Green chemistry is not complicated although it is often elegant. It holds as its goal nothing less then perfection, while recognizing that all of the advances and innovations towards this goal will contain some discrete risk."[2]

Green Chemistry principles (see below) can be applied to any field of chemistry. The focus is on minimizing the risks and maximizing the efficiency of any chemical reaction. Green Chemistry seeks to reduce and ideally eliminate pollution at its source. The principles of Green Chemistry provide a checklist for chemists to use when designing new materials, products, processes and systems. Systematic integration of these principles is key to achieving genuine sustainability for the simultaneous benefit of the environment, economy, and society.

Twelve Principles of Green Chemistry[3]

1. **Prevention**: It is better to prevent waste than to treat or clean up waste after it has been created.
2. **Atom Economy:** Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. **Less Hazardous Chemical Syntheses:** Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
4. **Design Safer Chemicals:** Chemical products should be designed to effect their desired function while minimizing their toxicity.
5. **Safer Solvents and Auxiliaries:** The use of auxiliary substances--solvents, separation agents, and others--should be made unnecessary wherever possible and innocuous when used.
6. **Design for Energy Efficiency:** Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.
7. **Use Renewable Feedstocks:** A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.
8. **Reduce Derivatives:** Unnecessary derivatization—use of blocking groups, protection/deprotection, and temporary modification of physical/chemical processes—should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.
9. **Catalysis:** Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. **Design for Degradation:** Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.
11. **Real-Time Analysis for Pollution Prevention:** Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
12. **Inherently Safer Chemistry for Accident Prevention:** Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

[1] This handout is adapted from one prepared by Kerry K. Karukstis at Harvey Mudd College.
[2] *Green Chemistry: Theory and Practice,* Paul T. Anastas and John C. Warner. New York: Oxford University Press, 1998, p.11.
[3] *Ibid,* chapter 4.