The Deprotonation and Subsequent Sn2 Reaction of a Biologically Active Compound to Prepare an Antibacterial Compound.

Every student should research and carry out her or his own reaction. The “report” for this lab will be simply keeping a good notebook, following the guidelines shown every week on the main page of the electronic lab manual. There will be no separate notebook. Your notebook must follow the normal competents that are on the main page of the web lab book and it must have diagrams of apparatus and especially TLC. Take copious notes and observations in your notebook. You have been doing this on some level, every week, however, this will be the most like what you will be doing next semester on a week to week basis when you are doing a long term project. It is extremely important to keep an excellent notebook.

This is a very unusual lab for this semester, but it is the lab that is the most like research and it is to help prepare you for next semester, which as I have stated is largely a research project. Unlike you labs that have come directly from your lab notebooks, perhaps with slight variations. In this lab, you will look up an original organic chemistry synthesis paper and derive and adapt the procedure for our lab.

Prelab

Your pre-lab consists of the following. Go to the literature (I am sure you can find it on the web) and find this exact paper. Just using the authors name and the topic, you will be able to find it on the web.

Synthesis and antimycobacterial activity of 7-O-substituted-4-methyl-2H-2-chromenone derivatives vs Mycobacterium tuberculosis

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When you obtain the paper, you should read it and find the general procedure. You need to write a short introduction in your lab book. You will need to figure out what the main reaction is – it may require that you look up some structures. The reaction is really two the deprotonation of the chromenone which is an acidic phenol with potassium carbonate and the subsequent reaction of the resulting anion as a nucleophile with an alkyl halide. The alkyl halide we will be using is octyl bromide, though this may be varied on occasion. You will need to write the procedure in your notebook in a way that is useful to you. Break it down and write it in clear terms that make sense to you. The procedure does need to be modified a bit for it to work in our lab.

The following are the modifications. You will be doing the general procedure, using octyl bromide (bromoointment) as your substrate in the $\text{S}_\text{N}2$. We will be doing
the reaction on 1/5 the given scale (you should scale all quantities i in your notebook). Scaling to 1/5 scale means you have to divide all the quantities in the procedure to 1/5, including the solvent. You do not scale the time. The reaction still takes the same amount of time (why?). We will be using a standard reflux apparatus, this will involve a 25 mL flask for the reaction clamped to monkey bars, with a reflux condensor (water flowing through it) clamped to monkey bars, a flask heater on a platform (clamped to monkey bars) and drying tube (this is similar to what you did for the Friedel-Crafts – look back in your notes). You should draw the apparatus in your notebook. Instead of finishing the entire long lab, you will monitor the reaction by TLC and watch how it progresses. Please study at the reading on TLC linked into this lab in the on main page of the lab book (It is also in this dock) You will look at it after an hour and then after two hours by TLC. You will spot the TLC plate vs. the starting materials and run the plates up in the solvent system suggested in the paper for column chromatography. Visualization of the plate by UV will be adequate and fun. Draw diagrams in your notebook and calculate Rf values for bands. Try to use the standards to figure out what is starting to material and what is product in your product mixture. What should be happening over time to the bands? What will happen if you let it run all night. Please refer to the reading on the website. It does take a while to master TLC. Please note that we will not be completing the reaction, you will just turn in your partially converted reaction. This means we will not be doing column chromatography. But again, do note the chromatography solvents as these are suitable for your TLC. TLC is a model for column chromatography.

**Background for TLC**

It is expected that you will read up on Thin Layer Chromatography and Column Chromatography. The following is a reasonably good site for Thin Layer Chromatography.

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

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

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

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

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

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

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

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

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

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

6. **Preparing the Eluting chamber.** We will use a makeshift eluting chamber that will be either a 250 mL or 400 mL beaker with about a 0.5-1.0 cm of dichloromethane with about 1.0 mL of methanol added (eluting solvent). You need to use a piece of filter paper as diagramed below to saturate the beaker’s atmosphere with the eluting solvent. Use a watch glass to cover the chamber.
7. **Eluting the TLC plate.** Dip your TLC plate in the solvent. It is important that the solvent does not immediately touch your spots. If it does, you need to adjust the level of the eluting solvent or spot a new plate. The former is preferable. The plate should be dipped, but also resting against the wall of the beaker. The back side toward the beaker, but on the opposite side of the beaker from the filter paper. The solvent rises up the plate by capillary action. It will take about ten minutes. In this process, there is a competition for the compounds by the silica gel on the plate (very polar – the stationary phase) and the solvent system (varying degrees of polarity, but less polar than the stationary phase – this is the mobile phase). The compounds are in equilibrium between the stationary phase (the silica) and the mobile phase (the solvent). If the compound is extremely polar it will spend most of its time interacting with the silica gel and it won’t move much. If it is of very low polarity (provided it is soluble in the eluting solvent), it will spend most its time in the solvent and will move way up the plate. Compounds move to varying degrees depending on their polarity. Compounds that are the same, will elute to the same point on the plate, so your standard lanes will help you identify compounds in the reaction mixture.

8. When the solvent is about a cm. from the top of the plate, you should remove the plate and lightly mark the level of the solvent. This is called the solvent front and its level is significant. Let the solvent completely evaporate off in the hood. The spots are visualized (most of the compounds we are working with are white and are not readily visible) with a UV lamp. Be careful not to expose your hands or your neighbors to the UV lamp. The background will fluoresce as previously described and your compounds should show up as blue or black spots in the background. Lightly circle them with a pencil. After this, you can more permanently visualize the compounds with a stain such as p-anisaldehyde stain. This entails dipping your plate into the stain with forceps and then, blotting the excess stain off with a towel. The plate then needs to be baked in an oven at about 100 °C for ten minutes or heated with a hot air gun for about one minute. Be careful with the stain as it contains strong acids. Plates can also be visualized with iodine vapor and variety of other stains.

9. Calculating \( R_f \):
   The \( R_f \) value is the ration of the distance from the origin to the middle of the spot as visualized to the distance of the solvent from the origin. It is a constant for a given compound on a given type of stationary phase in a particular solvent. It is much like retention time in GC.

10. Common errors: Spotting too close to the bottom of the plate so that spot is immersed in the solvent. Engraving the plate with a pencil so that the silica gel actually comes off. Spotting too close to the edge of the plate (spots will dive into middle of plate). Not making up the solvent system carefully. Having the plate touching the sides of the chamber (this causes cross currents of solvent across the plate). Spotting too big. Not applying sample enough times to be able to visualize it.
A good practice is to look at your plate after you have spotted it, but before you run it. You might even show it to your TA. If you can't see the spots on the plate before you run it, you have not put enough on the plate. If you have any other spotting issues, they may become evident when you examine your plate in this manner.

Again. You should record the chromatography techniques in your lab note book as well as the synthetic procedure you have researched. The apparatus should be drawn. All TLC info should be recorded. Take good notes in lab lecture and when studying any references you use.

Note: This lab is designed to prepare you for research. There is more of a burden on you to organize yourself. This is a good thing.

Once again: The write up for this lab is just a really well kept notebook according to our normal acronym.