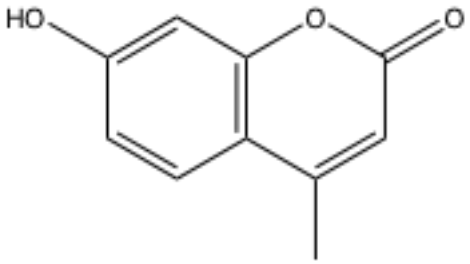
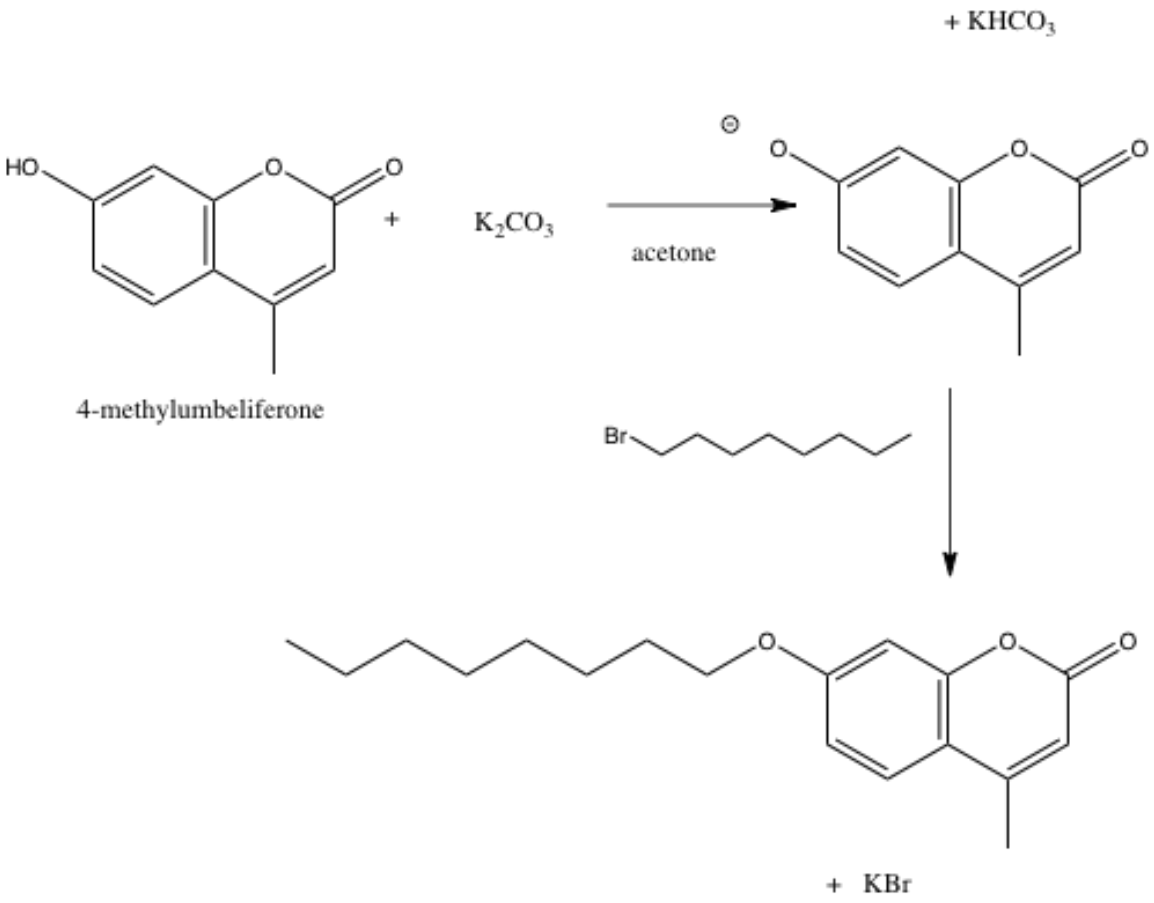
The S_N2 Reaction: Preparation of an Anti-Bacterial Compound

This investigation involves a synthesis of various 7-O-alkylated-4-methylumbelliferones that demonstrates organic acid base chemistry and a modern S_N2 reaction. This lab is also being used to introduce you to Thin Layer Chromatography (TLC). 4-Methylumbelliferone itself is a compound of known biological activity and medicinal application. [1,2] 4-methylumbelliferone analogues (compounds synthesized from 4-methylumbelliferone or related compounds) can also exhibit biological activity. [2] It happens that alkylation of the 7-hydroxy group of 4-methylumbelliferone results in compounds that have activity against various bacteria and may have other useful activities.[2]

The chemistry sequence involved in this synthesis includes a deprotonation, followed by a classic S_N2 reaction. To understand this chemistry, consider the structure of 4-methylumbelliferone illustrated below. 4-methylumbelliferone's structure includes a phenolic hydrogen. Phenols are relatively acidic from an organic chemistry perspective (Why? Consider the conjugate base.). The first step in the reaction sequence involves deprotonating the phenol group with potassium carbonate. The resulting phenoxide acts as a reasonably strong nucleophile on a variety of primary halides (1-bromooctane is shown in the sequence below), resulting in alkylation of the 7-oxygen. Think about the parameters that favor S_N2, i.e., strong nucleophile, primary substrate with good leaving group. Consider the reaction sequence given below and see if you can write the arrow formalism that corresponds to the mechanism.



4-methyl-umbelliferone



Reaction Sequence

The lab will be carried out in pairs, but each student should do his or her own background research on the reaction and keep his or her own notebook. The "report" for this lab involves keeping a good notebook for the two weeks as you have been doing for the entire semester. One lab report is adequate for each group. The lab report should be written as guided by the rubric at the end of this lab. Though some parts of the report and your notebook interrelate, keeping a notebook is a separate and distinct activity from writing the report. Your notebook must follow the normal components that are listed on the main page of the web lab book. It should have diagrams of apparatus and there should be diagrams of important TLC plates. Take copious notes and record extensive observations in your notebook. You have been doing this on some level, every week, however, this experience will be the most like what you will be engaged in next semester in terms of being a longer term project, that has a synthetic, medicinal and biological theme. It is extremely important to keep an excellent notebook.

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

You should record the thin layer chromatography techniques in your lab notebook 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.

This will be explained to you, but the most important thing to know about thin layer chromatography is that it is not unlike gas chromatography. There are stationary and mobile phases and the compounds are separated on the basis of their relative affinity for the two phases. As described below, the silica gel is the stationary phase and the solvent that moves up the plate by capillary action is the mobile phase. The silica is extremely polar and the solvent or solvent system is of lower polarity. After the compounds are applied to the origin of the plate and are exposed to the eluting solvent, they enter into an equilibrium between the silica gel and the solvent. Compounds that are less polar, rise higher as they spend proportionately more time in the mobile phase. Compounds that are more polar, spend more time in the stationary phase and tend to be nearer the origin. Ionic compounds often remain at the origin when using silica gel as the stationary phase. Since many organic compounds are not colored, they need to be visualized. There are many ways to visualize compounds. We will be using UV light. The compounds will appear as dark or fluorescent blue spots on a glowing green or orange background.

Thin Layer Chromatography – General Instructions

1. Obtain a Thin Layer Chromatography (TLC) sheet from the dessicator. This sheet will be about half 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 that is impregnated in the silica gel. When exposed to UV light, the plates will look similar to glow sticks and necklaces kids have in the summer. When not exposed to the UV lamp, one side is white and sort of rough, the other 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. For most reactions, standards consist of a small solution of the pure organic starting materials in a suitable solvent. To make standards, take a spatula tip of the organic starting material to be used as a standard and add it to a one or two dram vial. Then add about 1 mL of dichloromethane to dissolve the individual starting material (standard). These vials should be labeled and can be capped and saved for use in future weeks. Though it may seem uncomfortable, it is normal for chemists to estimate amounts when making standards. At various intervals you will also have to make a solution of your reaction mixture/crude product and eventually, your pure product. If it is liquid, you should measure a drop or two and dissolve it in about 1 mL of dichloromethane in a vial. Again, the solutions are just approximate. The goal is to study the components of the reaction versus the starting materials. New spots observed after elution and visualization are probably a new product or products, whereas spots seen at the same position as the starting material standards indicate un-reacted starting materials which correspond to either no reaction or an incomplete reaction.

4. The standards and the reaction mixture should be spotted on the TLC plate as indicated on the diagram below. It is important 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). 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 very useful to look at the plate under the UV light before eluting it to see if there is enough material on the plate. In this experiment, the spot should be a fluorescent blue.

5. It is very important to spot about a cm up from the bottom of the plate, to keep the spots about a cm apart and to spot about a cm in from the sides of the plate.

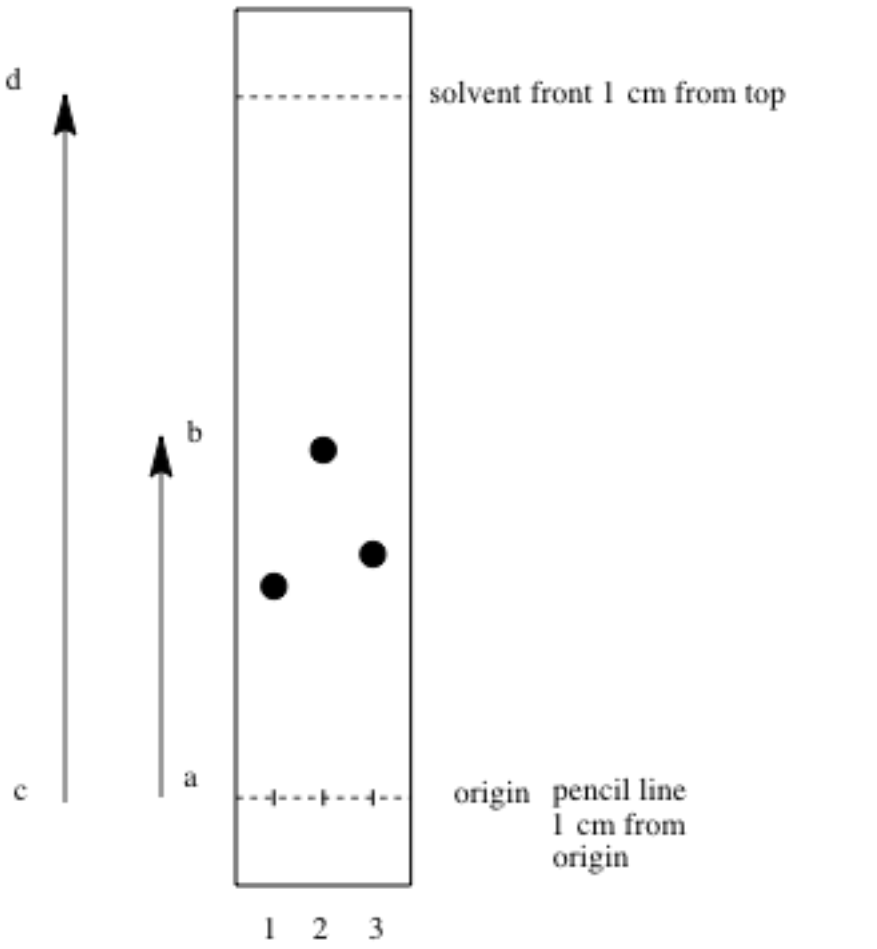


Diagram of TLC Plate

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 (or a chamber equilibrated with ethylacetate: hexanes, 10:90). 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.

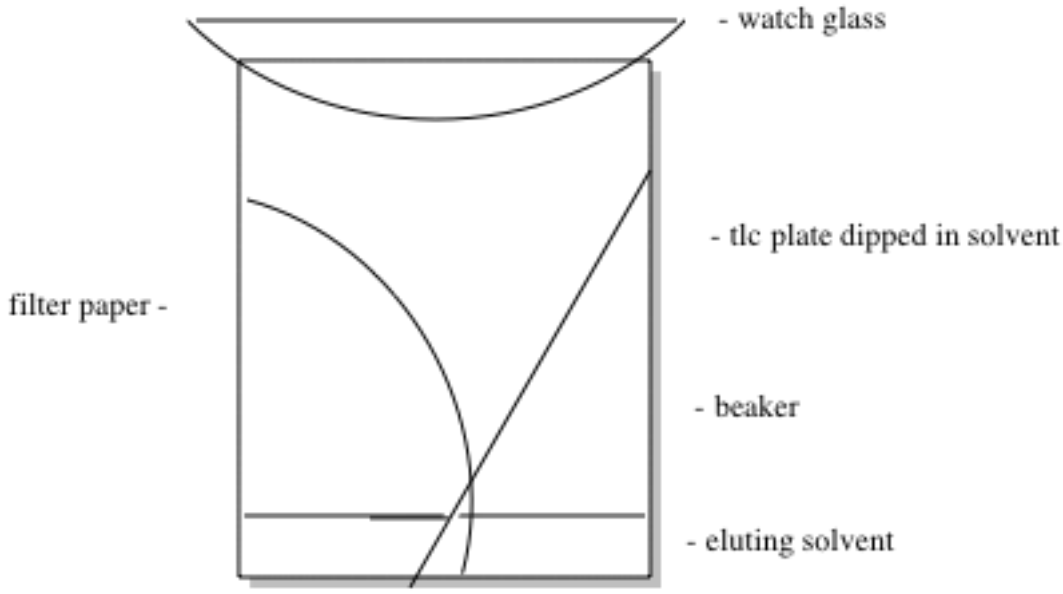


Diagram of TLC Chamber

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 backside 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 five to ten minutes. As described earlier, 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. Plates can also be visualized with iodine vapor and variety of stains, but this is not necessary in this lab.

9. Calculating R_f :
   The R_f value is the ratio 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.

    As mentioned earlier, a good practice is to look at your plate under the UV lamp 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.

Prelab

Your pre-lab consists of the following tasks. Go to the literature (Scifinder Scholar or other data base) and obtain a copy of the second paper cited at the end of this lab. Read this paper carefully. Start constructing the Introduction for your paper in the normal way, but consider this paper when giving the big picture goal of the lab. Follow the rubric given below. Study the procedures in the paper. Correlate the procedure in the paper with the newly developed procedure below. Think about what is happening at each step. Prepare your notebook according to the special acronym given multiple times on the main page of the laboratory manual. Though the report will be written in pairs, all students should engage in this preparation.

Procedure for the S_N2 reaction.

Week one:

Use normal, top loading balances during first week. The following operations refer to groups of two this year, though it is very important that all students be involved in all operation and obtain hands on experience. Please take turns with procedures that are repetitive.

1. Obtain a new, explosion proof two dram vial (brand new) and a micro-stir bar (very small stir bar) from your instructor or TA. Stir bars must be returned to the instructor and may be retrieved by the instructor at certain points in the lab. They are very small. Please be careful and keep track of them through the lab. It is very important because we only have enough and they must be shared, lab to lab. Please be particularly mindful of the stir bar as you complete the lab. They disappear if you sneeze or they can easily be lost down the drain

2. Weigh 0.09 grams of 4-methyl-umbelliferone and carefully add it to the vial. It is good to weigh it into a small weighing dish and then transfer the material into the vial. It will transfer easily. It is also a good idea to put a piece filter paper under the vial to catch any material that misses vial.

   Note: we don't start reactions over, we salvage.

3. Weigh 0.17 grams of potassium carbonate and add it to the vial. Use the same procedure as that for the substrate in 2 above. If you overshoot a little, it will not hurt the reaction.

4. Add 1 mL of high purity acetone to the vial (use the liquid in the hood, not in your squeeze bottle). Use a small graduated cylinder and a pipet to transfer the acetone into the vial. It is not a bad idea to clamp the vial at this point so you do not knock it over.

5. At this point the stir bar should be in the vial. Tightly cap your vial with the special cap that comes with your vial .and label it near the top of the vial with a small piece of tape or a sharpie. The label markings must be brief but distinct – perhaps group name and date. Record the label name in your notebook. Clamp the vial over a normal stir plate and allow it to stir for thirty minutes. The reaction mixture should turn yellow over this interval.

6. While your reaction is stirring, your TA or instructor will demonstrate TLC basics for you.

7. After thirty minutes, the reaction should have a yellow precipitate and a colorless or lightly yellow supernatant liquid. At this point , bring vial back to balance and add 0.10 grams of 1-bromooctane (or .07 g of 1-bromobutane – you will have a choice) to the vial. This entails pipeting the liquid into the vial, drop by drop. Each drop is approximately 0.01 grams. You can weigh a liquid, but you have to be careful. If you overshoot it a bit, you should not dump the reaction. It can still be used.

   Remember we do not start reactions over, we salvage.

8. Analyze your reaction using TLC. Study the general reading above and any youtube videos associated with this lab. Your instructor or TA will have gone over this with you. Make sure to spot relevant standards. Visualization will involve only UV light (no stain). This TLC is your baseline, showing which compounds are present in your sample before the reaction is really going.

9. Screw the special cap back on the reaction vial and place it in the blue pie block on the pie reactor. Allow the reaction to stir and to react for approximately 24 hours at 65 degrees celcius. You are allowed to check the progress of the reaction using TLC at mid-lab and/or toward the end of the lab period. When you look at the plate under the lamp think about what is going on and what the spots represent compared to the standards. Make sure you understand the concept of reaction progress on a TLC plate. Reaction progress is the disappearance or reduction in intensity/size of the reactant spots and the appearance of new product spots at different locations. New product spots have different R_f. This takes a while to understand. How would a complete reaction be indicated on a TLC plate?

10. What is a pie-block reactor? – it is simply a very sophisticated hotplate and stirrer. The blocks are designed to perfectly fit various vials sizes and provide precisely controlled temperature and easy stirring. It is amazing how these factors improve the outcome of reactions. This is how reactions are done in industry – you are doing reactions in a modern, efficient way!

    At the approximate 24 hour mark, your instructor or TA will remove your stir bar from your vial and place your vial in a safe place until your next lab period. Note, if a reaction has lost a lot of volume, it can be reconstituted with acetone to the 1 mL level.

11. Use any down time during lab to work on your lab report with your partner – see the rubric below. There will be one lab report per pair. Lab is a great time to get lab reports done. You may be tired, and feel like going to get a coffee (this is ok too), but if you get most of your lab done when you have the full lab staff at your disposal, you will be happy in a couple weeks. Try to get as much done as possible.

Week 2

Use analytical balances for any weighing you need to do. These are in the central room – see TA or Instructor to learn how to use.

1. Allow the reaction to cool completely and carry out a TLC analysis. Even if not complete, you should add ten drops of water and ten drops of 3M NaOH to the vial . What is the purpose of the base? Add two to three mL of

dichloromethane to the vial. At this point you should observe two layers in the vial. What is the purpose of this step? Can you write the chemistry? Think about extraction theory. Which layer should favor the product, which the alkylated-4-methylumbelliferone? Obtain and add a micro stir bar. Allow the reaction to stir for 30-45 minutes clamped over a regular stirrer at room temperature.

2. After 30-45 minutes, allow the extraction to settle and you should observe two layers. You need to figure out which layer is the organic layer (dichloromethane layer). Really think about this step and relate it to the caffeine lab you carried out several weeks ago. This process is a mini-extraction. What is going on here? Once again, which molecule or molecules should favor solubility in the organic layer (dichloromethane).

3. Pipet out the lower layer whichever it is and place it in a separate vial. If this is awkward for you, you can pipet out the top layer. Make sure everything is labeled and stable (clamp or put vial in beaker). Keep the layer that remains in the original vial in the original vial.

4. Remember: we don't start over, we salvage. If you have saved the layers the reaction is salvageable. Take your time and think. Clamp vials so you do not knock them over. Always try to salvage if anything goes wrong. Save everything and label.

5. Take the vial holding aqueous layer and re-extract it with two mL of dichloromethane. This is a backwash step. This means you will add dichloromethane to the vial holding the aqueous layer and stir it for 30-45 minutes. Depending on which vial is holding your aqueous layer, you may have to move the stir bar. After the stirring time, allow the layers to separate and pipet out the layer that is the organic layer. Again, you may choose to pipet a different layer, but you still need the organic layer. Add this organic layer to the other organic layer. This is a micro procedure similar to the extractions you carried out on tea.

6. Retrieve your stir bar and return it to your instructor (wherever it may be).. Add a tiny amount of sodium sulfate to the combined organic layer (it is the dichloromethane layer – it should contain your product). Tiny means about two millimeters high on the bottom of vial.

7. Carry out a final TLC analysis of your product. Does the product mixture look pure by TLC?

8. If it is reasonably pure, you should continue. If not, you can discuss alternative strategies with your instructor. It is possible to run the compound through a pipet with a glass wool plug and a little silica gel.

Discuss this with your instructor. It may not be practical depending on your yield.

9. The solution should be pipeted into a tared (on the analytical balances in the central room) 2 or 4 dram vial and rotavapped to dryness using the special vial adaptors. When dry the vial should be weighed again on the analytical balances to determine yield.

   Talk to your TA or instructor before using the rotavaps and the analytical balances. Bumping is a major problem with vials. It is good to start the rotation with the vent open and no heat of the vial. Then the vent can be closed and the pressure lowered slowly. Allow the rotary evaporation to occur without heating till the vial has ice on it. Then gentle lower it into the bath at 35 degrees celcius. Venting will be needed if the vial bubbles and the trap may need to be cleaned frequently.

10. The mass and IR of the compound should be obtained this week.
    Your TA or instructor will help you with the IR. In the future, we will do NMR and GCMS of the product, but for this year, you should focus on understanding the TLC and the IR analysis.

Lab Report (One per pair of students)

I. Introduction (20 points)
   One paragraph.
   What is significance of molecules being made (include references)? (8 points)
   How are molecules being made – what is significance of chemistry? (10 points)
   Reference for procedure (2 points)

II. Main Reaction (10 points)
   This is the net reaction and should be straight forward from the material given in the lab.

III. Mechanism (30 points)
   The outline of the mechanism is given in this document, students need only fill in the arrows.

IV. Raw Data
   Mass of product (6 points)
   Physical state and color (4 points)
   TLC results – representative plates of reaction partially finished and finished with R_f values tabulated. (12 points)
   IR Spectrum (12 points)

V. Processed DATA
Interpretation of IR (12 points)
Calculation of percent yield (6 points)

VI. Discussion
Proof of Identity of compound using physical state, TLC results, IR. (12 points)
Yield - why yield is less than 100 percent, realistic reasons why it is below 100 percent.
(12 points)
Purity- realistic reasons purity is less than 100 percent using spectra, TLC (12 points)

VII. References (6 points)

VIII. Quality of results (TAs only – 20 points)

Total: 174 points

References for procedure :

References

[1] Nagy, N., Kuipers, H. F., Frymoyer, A. R., Ishak, H. D., Bollyky, J. B., Wight, T. N., & Bollyky, P. L. (2015). *Frontiers in Immunology*, *6*, 123.

[2] Yee, S. W.; Shah, B. ; Simons, C. *Journal of Enzyme Inhibition and Medicinal Chemistry* **2005** *20*, 109-113.