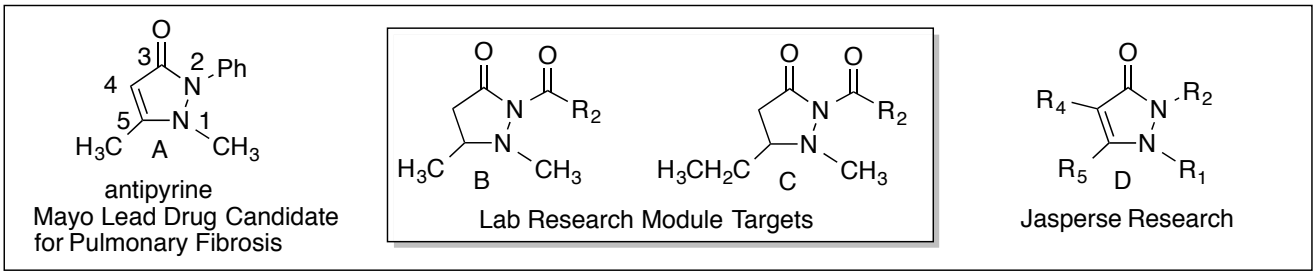
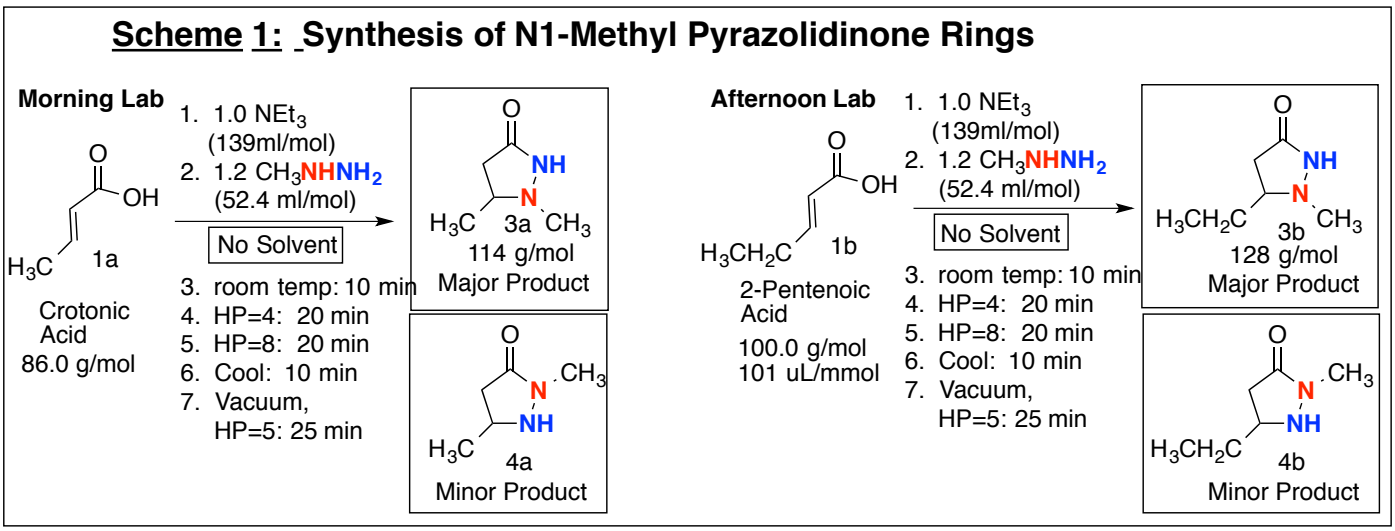
Multistep synthesis of an Acyl Pyrazolidinone

Introduction

We will begin a two-week research sequence in which we make a variety of novel "acyl pyrazolidinones", see structures **B** and **C** in the figure below. Most of these substances have never been synthesized previously, and are of interest as medicinal candidates. Research collaborators at the Mayo clinic have identified the chemical "antipyrine" (structure **A**) as a lead but non-optimized drug candidate for idiopathic pulmonary fibrosis ("IPF"). The Jasperse research group is involved in making a diverse library of analogs for antipyrine, (structure **D**) and having them bio-screened, in hopes of both improving drug performance and the understanding of drug binding and mechanism. Where is there volume allowance to get bigger? Where is it necessary to include hydrophobic substituents, versus performance and binding being improved by inclusion of hydrophilic elements?



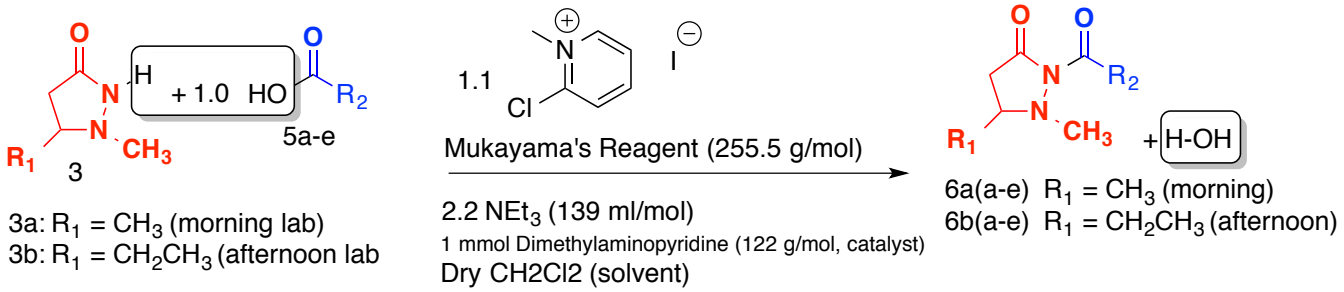
Structures "B" (targeted in the morning lab) will differ from antipyrine in three ways: it's lack of the C=C double bond, it's inclusion of the carbonyl, and variation of the R_2 group. Structures "C" (targeted in the afternoon lab) will be similar but include an ethyl group rather than a methyl group on the C5 position. First, within a given "B" or "C" series, drug tests should indicate how variations in the size and nature of R_2 impact drug performance. Second, by comparing structures with the same R_2 group between the 5-methyl "B" and 5-ethyl "C" series should indicate whether the drug receptor prefers the more hydrophobic, larger-volume 5-ethyl series or not. It is beyond the scope of the Chem 365 research module for this year, but the Jasperse research group will hopefully develop a procedure for oxidizing structures "B" and "C" to convert the C4-C5 single bond into a double bond. Drug test comparisons between the single versus double-bonded analogs should help identify whether the double bond is essential or irrelevant.



The syntheses will involve two reactions. The initial reaction, illustrated in Scheme 1, will react methylhydrazine (CH_3NHNH_2) with either "crotonic acid" (2-butenoic acid, morning lab), or 2-pentenoic acid (afternoon lab), to make the methyl and ethyl analogs **3a** and **3b**. These products will likely be oils. The mechanism probably involves amine and carboxylic acid acid-base chemistry; the involvement of amines as nucleophiles; the impact of an electron-donating methyl group; the involvement of an enolate intermediate; and thermal addition of an amine to a carboxylic acid to form an amide. Some selectivity (but how much?) for products **3a/3b** over **4a/4b** is expected, based on the expectation that the N-methyl nitrogen in methylhydrazine should be more electron rich and nucleophilic based on the electron-donor effect of the methyl substituent. The only purification will be hot vacuum distillation to remove side products triethylamine, excess methylhydrazine, and water.

The crude products **3a** and **3b** will then be carried on directly through the reaction illustrated in Scheme 2. In this reaction, the N2-nitrogen will be acylated by various carboxylic acids **5**. Of the ten products **6** whose synthesis is being proposed in this research module, only one of them (**6ab**, in which R_1 is a methyl group and R_2 is $CH{=}CHCH_3$), has been made previously, and that here at MSUM. None of the molecules **6** have been reported in the literature, nor of course been tested for medicinal use. These are truly novel molecules that have never been made before! ☺☺

Scheme 2: N2-Acylation Using Mukayama's Reagent



3a: $R_1 = CH_3$ (morning lab)
3b: $R_1 = CH_2CH_3$ (afternoon lab

2.2 NEt_3 (139 ml/mol)
1 mmol Dimethylaminopyridine (122 g/mol, catalyst)
Dry CH2Cl2 (solvent)

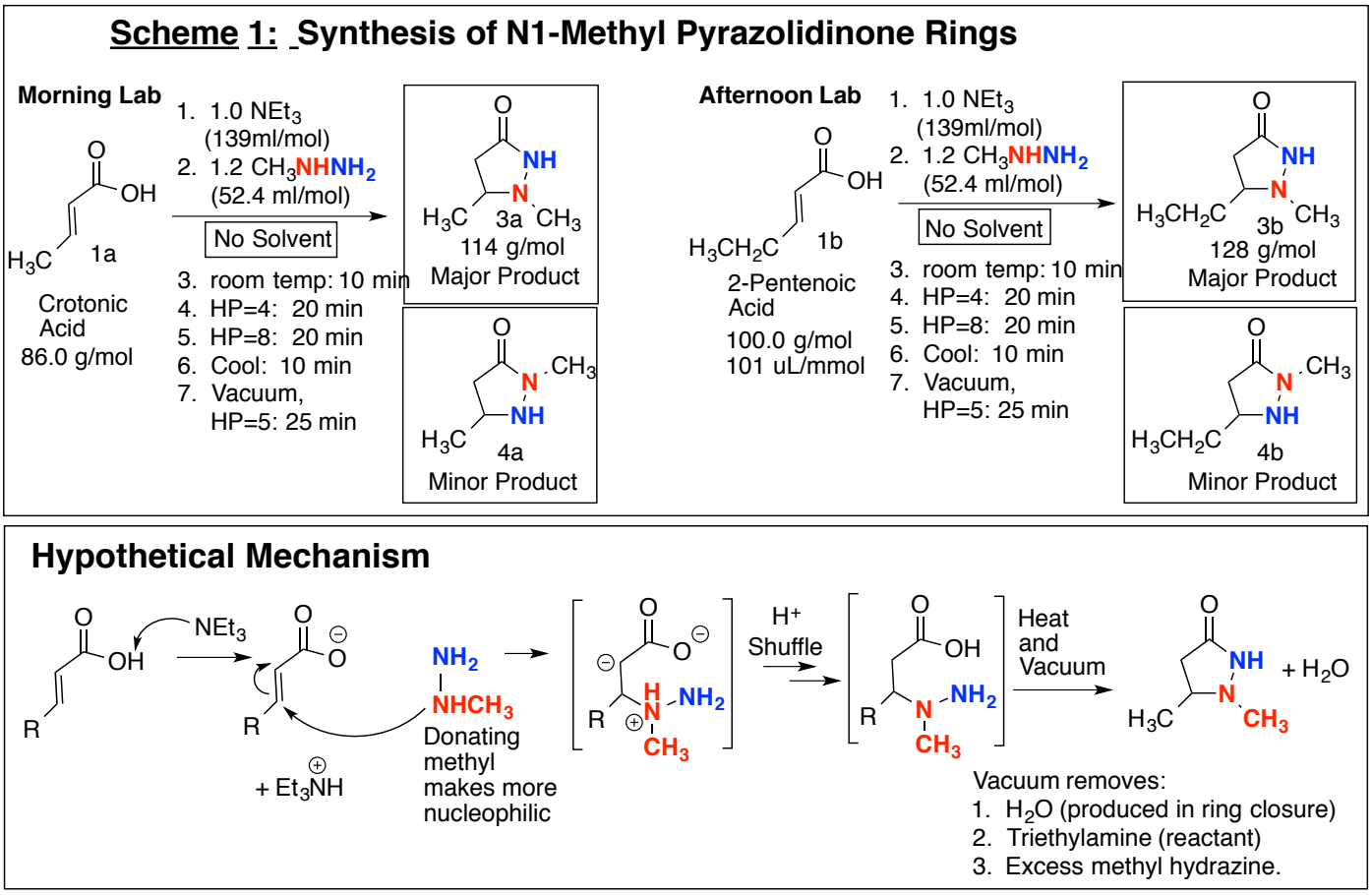
6a(a-e) $R_1 = CH_3$ (morning)
6b(a-e) $R_1 = CH_2CH_3$ (afternoon)

The chemistry used in Scheme 2 is not necessarily fast. Thus students will want to start the Scheme 2 reaction at the end of the first laboratory day. That will allow a full week to ensure completion. During the second week, they will conduct a chromatographic workup to remove the majority of side products and contaminants.

NMR and GC-MS analyses will be conducted on both product **3** and final product **6**. The chiral center in products **3** and **6** will complicate their NMR spectra. The two alpha hydrogens on the CH_2 carbon (C4) will have significantly different chemical shifts, and splitting patterns that are unfamiliar to you. This is because one alpha-H will be "cis" to the C5-methyl or ethyl group, while the other alpha-H will be trans. Being in different chemical environments, they will have different chemical shifts. A special video is available to help guide students through the NMR analyses.

Two lab reports will be required. One will cover the chemistry of Scheme 1, the other the chemistry involved in Scheme 2. Even though much of the Scheme 2 chemistry will be conducted during the end of the first week, that chemistry will be included in the Scheme 2 report which is due a week following the conclusion of the Scheme 2 workup and chromatography chemistry.

This kind of modular preparation of chemical libraries is routine in industry and medicinal chemistry. Subsequent biological testing can provide insights into the relationships between structure and activity. (Which parts of the molecule matter? Where does getting bigger or smaller help? Etc.)



Reagents:

1. 20.0 mmol of Crotonic acid (morning lab) or 2-pentenoic acid (afternoon lab)
   - Crotonic acid: 86.0 g/mol
   - 2-pentenoic acid: 100.0 g/mol, 101 mL/mol.
2. 20.0 mmol of Triethylamine (139 ml/mol)
3. 24 mmol of Methyl Hydrazine (52.4 ml/mol)
4. 125 mL Ground-Glass Erlenmeyer flask
5. Long sized stir bar
6. Hot plate
7. Reflux Condenser
8. Vacuum Condenser

Scheme 1 Procedure: Formation of Pyrazolidinone Ring Using Hydrazine

Part A. Heating/Hydrazine Addition Phase

1. Get a 125-mL ground-glass-jointed Erlenmeyer, and add a long stir-bar. **Weigh the combination and record the mass.**
   - You'll need this mass to calculate your product yield, so record it somewhere!
2. Weigh out 20.0 mmols of either "crotonic" (2-butenoic) acid (morning lab) or 2-pentenoic acid (afternoon lab):
   - Morning: Crotonic acid, 86.0 g/mol, is a solid by a balance. Add it using a powder funnel.
   - Afternoon: 2-pentenoic acid, 101 mL/mol, is a liquid. Add it using a syringe.
3. Add a rubber septum to the flask, and poke a syringe needle through it to vent any pressure buildup.

4. Add 20 mmol (0.020 mol) of triethylamine (0.139 ml/mmol) by syringe, and stir.
   - You can pull the septum out before injecting, and replace the septum following the addition.
5. Add 24 mmol (0.024 mol) of liquid methylhydrazine (0.0524 ml/mmol) via syringe while stirring.
   - You can pull the septum out before injecting, and replace the septum following the addition.
6. Stir the mixture rapidly for 10 minutes at room temperature.
7. Add a reflux condenser, with a gentle flow of water running through it, and place the septum on top to prevent oxygen exposure
8. Turn the hot plate setting to 4, and stir vigorously for another 20 minutes.
   - Make sure that your Erlenmeyer and the hot plate are in contact.
   - Make sure that the flask is not tipped and doesn't have any air-space between the hot-plate and the flask. You need direct contact for the heat to do its work in the time given. If you leave space in between, or have a tipped flask without good thermal contact, the reaction might not complete.
9. After 20 minutes, turn your hot-plate setting up to 8, and continue stirring for an additional 20 minutes.
10. During the stirring times, plan ahead. Prepare the following:
    a. Find your vacuum adapter, and plug it into the vacuum hose. This is about 1 inch long, with a ground-glass joint on one end and a 90-degree curve. The ground-glass end will plug into a reflux condenser, the tapered end into your vacuum hose.
    b. Familiarize yourself with your vacuum: With your vacuum attached to the vacuum hose, put your thumb over the end, and turn the vacuum counterclockwise to get an idea of how far you have to turn it before any vacuum actually starts to work. Then turn the vacuum back off.
       - You'll want to know this so that when you really need to apply the vacuum, you'll be able to get near the point where the vacuum engages, and then open it VERY cautiously and slightly at first.
    c. Ensure that the vacuum is turned all the way off (to the right). IF SOMEBODY HAS THEIR VACUUM OPEN TO THE AIR, THAT AIR LEAK WILL COMPROMISE EVERYBODY'S VACUUM AND NOBODY WILL GET THE LOW PRESSURE THEY NEED for the next procedure. Don't let your hood's vacuum ruin everybody's experiment!
    d. Get a glove for your left hand, so you can handle the hot glassware;
    e. Calculate what the theoretical yield for your **3** should be, given the 20.0 mmol scale.
    f. Look ahead to both subsequent stages of Scheme 1, but also to Scheme 2, which you will do today and will start shortly after you complete Scheme 1. You could do all of your Scheme 2 calculations right now…
    g. Get an empty NMR tube and stand it inside an empty Erlenmeyer, with a red cap;
    h. Get a long-stemmed pipet, with a pipet bulb to be used with NMR tube;
    i. At this stage, you should have mostly the open form of the product with a carboxylic acid (rather than the closed ring with an amide); see the Hypothetical Mechanisms figure on the previous page).
    j. At this stage you should also have, in addition to the product, at least three other chemicals:
       - Triethylamine
       - The excess methylhydrazine
       - Water which is produced during the ring closure.
       - Probably some contaminants or side products.
    - The hope is to vacuum-distill away all or most of these extra things, without decomposing the cyclic products **3** in the process.

11. After the heating is complete, a) slide the hot plate out from under your flask, b) **reduce the hot-plate setting to 5**, c) turn off your reflux condenser water, and d) detach the hose from the water source and redirect it into the drain so that most of the water in the condenser can drain out.
12. Let your solution (and the hot-plate) cool for at least **10 minutes** before starting Scheme 1 Part B.

Scheme 1 Part B: Heat/Vacuum/Ring-Closure/Amide Formation Phase

13. After the 10-minutes, attach the vacuum adapter to your reflux condenser, and then slide your hot plate back under the reaction flask. The hot plate should be set at 5 for heat. (And maybe 3-5 for stirring?)
    - If you didn't turn your hot plate down to 5 earlier, do so now and wait five minutes.
14. Crack the vacuum open, really, really carefully and gently at first (so that it doesn't cause everything to erupt and boil/foam over). As soon as the vacuum is engaged but the bubbling isn't too wild, open the vacuum until it's wide open as soon as possible (two full revolutions will more than suffice). If the mixture splatters/bubbles a lot, it may help to lower the plate slightly.
    - If instructor is available, it may be reassuring to request some help on getting the vaccum opened.
15. Stir/heat/vacuum for **25 minutes**. (Measuered from when the vacuum was first fully open.)
    - Excess vacuum heating may result in some decomposition, so avoid excess heat and time.
    - Notice that the volume should decrease as non-product chemicals boil off with the assistance of the vacuum.
    - The hot vacuum is intended to do several things:
        a. During this time, the triethylamine, excess methylhydrazine, and the water produced during ring closure should ideally be boiled away, leaving only the product and whatever organic contaminants and side-products remain.
        b. Each of the chemicals that get distilled away inhibits the ring formation. So their distillative removal, in addition to being an end in itself, also enables the ring closure to proceed more effectively.
16. After the 25 minutes, slide the hot plate out from under your reaction mixture.
17. After the flask cools a little, **turn the vacuum off**, and detach the vacuum adapter.
    - **NOTE: If you forget to turn your vacuum off, it will ruin things for everybody else because their vacuum won't work if yours is left open to the air.**
    - Pulling the vacuum adapter is easier if you grasp the hot flask with a GLOVED hand.
    - Remove the reflux condenser and replace with the septum with the syringe stuck through it.

Critical Note: Be sure to start the next reaction as described in Scheme 2 before week one is done.

- Before week one is completed, it is urgent that you get the subsequent reaction started. See Scheme 2.
- This reaction takes at least several hours after it is begun, so you don't want to be trying to both start and finish it and work it up and purify it all during the same lab period. So, if it has the whole week to complete itself, that will work out great and will allow you to waste no time!
- Plus it requires time-consuming workup. So I'd like to have next week completely available for that.
- If you don't get it started during the same lab period, you will want to/need to come in sometime at least a day before the next lab period to get it started.

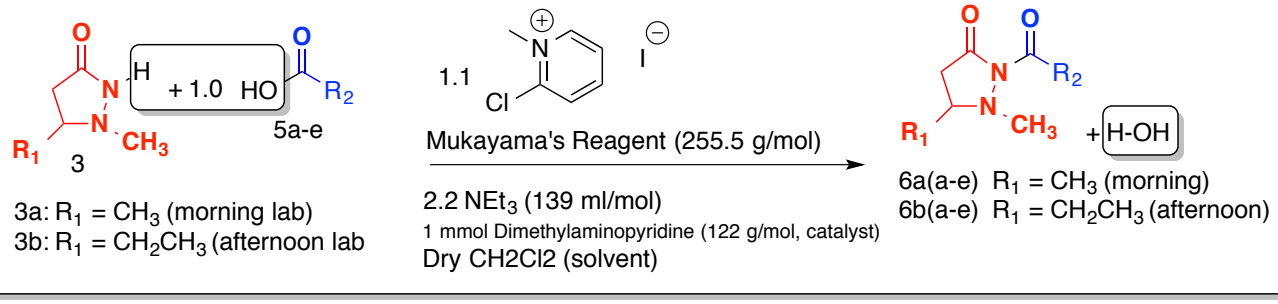
Scheme 1 Part E: Product Analysis. NMR, GC/MS, and yield. And Deciding whether You Need to Heat and Vacuum Further.

- Hopefully everything has gone reasonably well. However, this isn't certain; perhaps the ring closure and water/hydrazine removal is not sufficiently complete, in which case you'll need to do some more vacuuming. (And in which case the yield will be too high, and the NMR will be contaminated.)
- We don't want to do additional heat/vacuum time unless absolutely necessary, though, because doing so can cause some decomposition of your product.
- To decide whether you need additional heating/vacuuming, you need a quick way to assess the situation. Both the NMR and/or the % yield can tell us. If the yield significantly exceeds 100%, that represents trouble. And if the NMR shows more complexity than expected, that too means trouble.

18. **NMR and GC analysis:** dip a long-stemmed pipet into the product and draw up about a quarter-inch (1 cm) sample (finger-nail length) of your hot mixture into the skinny tip of your pipet
    - Sample should be small, both to give sharpest NMR and to minimize yield loss.
19. Put your pipet with that small sample into an NMR tube.
20. Put a septum into the top of your reaction flask to keep air out.
    - Air can cause some oxidation and decomposition of your product, particularly when it's hot.
21. Add 1.2 mL of CDCl3 into the same pipet that has the sample in it and is in the NMR tube, and attach a pipet bulb to the pipet.
22. **GC Prep**: Using the same long-stemmed pipet, draw out what solution it can reach from your NMR tube, and transfer it directly into a GC-MS vial.
    - There will still be enough solution for the NMR.
    - Basically the same solution will feed both NMR and GC-MS analyses
23. **Submit the NMR sample to the NMR queue**.
    - Students will be using the NMR both for submitting, but also for processing (printing extra copies, doing horizontal expansions, etc.) So you may need to be able to work your way between "submit" and "spectrometer" modes.
        - If not in submit mode, click "New Study" to get into submit mode. The correct experiment should automatically load when you do so, if the software is working right.
24. **Submit your GC-MS sample to the GC-MS queue**.
    - This will probably take a while to run.
25. **Mass measurement and % yield determination**. Take your flask to the balance and measure the mass.
    - Take the septum out before recording the mass; replace it immediately after recording the mass.
    - Subtract the original mass of the flask and stir bar in order to determine the mass of product.
    - Record the mass of product; you'll need it both for this week's lab report (as the product) and for next week's lab report (as the reactant).
26. Calculate the percent yield of your Scheme 1 reaction (**1 → 3)**
    - **If your yield exceeds 100%, it probably means your heat/vacuum process had problems. See instructor, in that case.**
27. Calculate how many mmol of product you have.
28. If you have the NMR, or you have the mass and use that to calculate the % yield, those will provide the information needed to determine whether or not you're good, or need more vacuum. Consult instructor?
29. **Make an extra copy of both your NMR and your GC and put the extras in your drawer**. You'll need them for your lab report for this week, but you'll also need them for your lab report next week! ☺
    - Today's product will be Scheme 2's reactant. As you go through Scheme 2, you'll want to be able to compare your NMR and GC for your product versus those of the reactant.
        - NMR: If in submit mode, click "**Cancel**" to exit Submit mode in order to enter the spectrometer/processing mode for doing additional prints or horizontal expansions.
        - In "Spectrometer" mode, must have "Zones" map displayed (96 sample nodes show). Click on little circle icon () to the upper left of the spectra-display panel, if zones map not already open.

N2-Acylation using Carboxylic Acid and Mukayama Reagent

Scheme 2: N2-Acylation Using Mukayama's Reagent

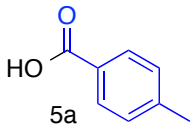


3a: $R_1 = CH_3$ (morning lab)
3b: $R_1 = CH_2CH_3$ (afternoon lab

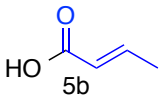
2.2 NEt_3 (139 ml/mol)
1 mmol Dimethylaminopyridine (122 g/mol, catalyst)
Dry CH2Cl2 (solvent)

6a(a-e) $R_1 = CH_3$ (morning)
6b(a-e) $R_1 = CH_2CH_3$ (afternoon)

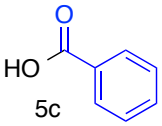
Reactant Carboxylic Acids 5a-e and their Molecular Weights



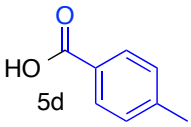
"p-Toluic acid"
4-methylbenzoic acid
136 g/mol



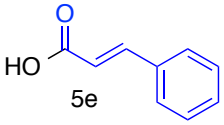
"Crotonic acid"
2-butenoic acid
86.0 g/mol



Benzoic acid
122 g/mol

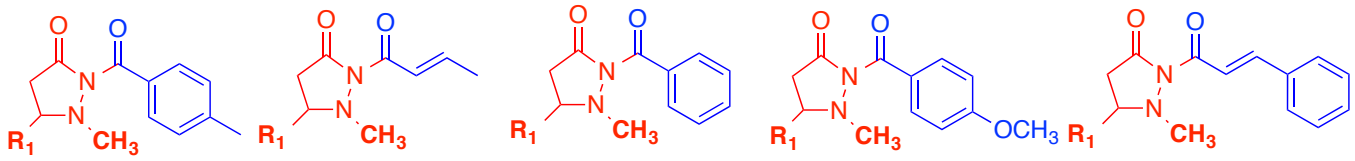


"Anisic Acid"
4-Methoxybenzoic acid
152 g/mol



"Cinnamic acid"
3-phenyl-2-propenoic acid
148 g/mol

Desired Products



6aa $R_1 = CH_3$
6ba $R_1 = CH_2CH_3$

6ab $R_1 = CH_3$
6bb $R_1 = CH_2CH_3$

6ac $R_1 = CH_3$
6bc $R_1 = CH_2CH_3$

6ad $R_1 = CH_3$
6bd $R_1 = CH_2CH_3$

6ae $R_1 = CH_3$
6be $R_1 = CH_2CH_3$

Reagents:

1. Pyrazolidinone **3**: Report how many grams and mmoles did you produce.
2. Anhydrous CH_2Cl_2: 20 mL
3. Carboxylic acid **5**: 18 mmol
4. Dimethylaminopyridine (122g/mol): 1 mmol
5. 2-chloro-1-methylpyridinium iodide (Mukayama's Reagent, 255.6 g/mol): 20 mmol
6. Triethylamine (139 mL/mol): 42 mmol
7. Ether: 60 mL
8. Either 2:1 ether/dichloromethane: 25 mL or 3:1 ether/dichloromethane: 35 mL
9. silica: 25g

N2-Acylation of N-Phenyl Pyrazolidinone Using Carboxylic Acid and Mukayama's Reagent (9)

Part 1: Starting the Reaction for N-Acylation

1. Use your ≤20 mmol of the pyrazolidione **3** that you made in Scheme 1, in the same 125-mL flask.
   - Record the mass of the flask+stir-bar that you recorded at the start of Scheme 1.
   - Record the mass of your pyrazolidinone **3** that you produced at the end of Scheme 1.
   - Record the number of mmol for pyrazolidinone **3** that you calculated at the end of Scheme 1.
   - Be sure to save NMR and GC copies for your pyrazolidinone **3**. Since that is your reactant for Scheme 2, at the end of Scheme 2 you'll want to compare the spectra for **3** versus new product **6**.
2. Add 20 mL of dry anhydrous CH_2Cl_2 and stir. (The correct bottle will have "molecular sieves" pellets to sponge up any water, and the label should say "Anhydrous" or "dry".)
3. Add your septum to exclude air.
   - It should still have a syringe needle stabbed through it to release any pressure buildup.

- Feel free to take the septum out while adding solids, but try to return it right away to exclude oxygen

4. Add 18 mmol of your carboxylic acid **5**, probably through a powder funnel assuming it's a solid.
   - Note: we're adding less than 20mmol because we're guessing not all of the 20mmol of limiting reactant in Scheme 1 converted perfectly. I'm thinking the workup and purification will be easier if the acid is limiting rather than excess. (But, who knows, I'm guessing! It's research! ☺☺)
5. Then add 1 mmol of dimethylaminopyridine (122.2 g/mol). This is a catalyst; exact amount isn't crucial.
6. Weigh and add 20 mmol of 2-chloro-1-methylpyridinium iodide (Mukayama's Reagent, 255.6 g/mol).
   - Immediately reclose the bottle from which you took the reagent, since it is moisture sensitive. If you leave it uncovered, it will go bad and subsequent users will have problems.
   - Mukayama's Reagent functions to stimulate loss of H-OH from reactants **3** and **5**.
   - The "excess" is to absorb any trace water on the glassware, from the air, or in the solvent.
7. Record all of your observations. (Is the mixture homogeneous or heterogeneous, etc.)
8. Syringe in 42 mmol of triethylamine (139 mL/mol), and attach bottle cap.
   - The NEt3 will get the reaction started.
   - Record observations. What happens? Exotherm or anything? Color change? Solubility changes?
9. Let stir for at least one ten minutes, and record observations. After ten minutes, remove the venting needle, but leave the septum in so that there will be no air exposure over the week.
10. Store in your drawer till next week!

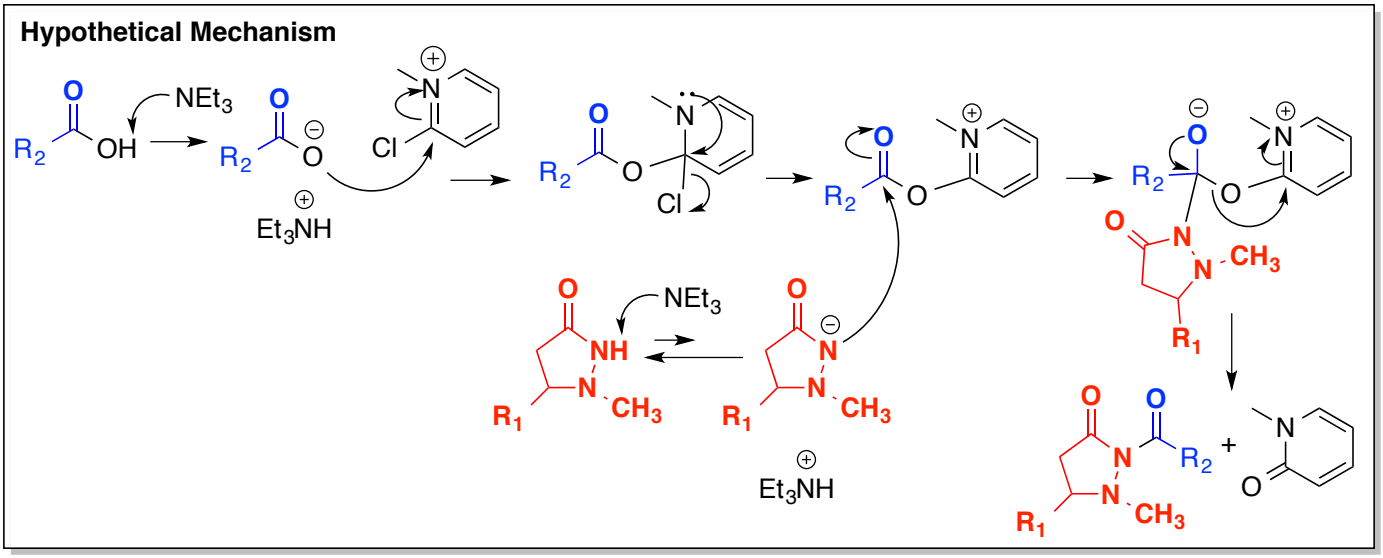
- **<u>THIS IS AN IDEAL PLACE TO STOP. MAKE SURE THERE IS A SEPTUM STOPPING YOUR FLASK, AND STASH IT IN YOUR DRAWER TILL NEXT WEEK.</u>**

- Emergency Note: If you don't get this far during week two, be sure that you come in and get the reaction set up at least a day before your lab period.

Note on Lab Reports:

- **ALL OF SCHEME I CHEMISTRY SHOULD BE INCLUDED IN THE SYNTHESIS-STYLE LAB REPORT DUE NEXT WEEK.**
- **ALL OF THE SCHEME II CHEMISTRY SHOULD BE INCLUDED IN A SINGLE LAB REPORT WHICH WILL BE DUE TWO WEEKS FROM NOW, A WEEK AFTER THE WORKUP AND PRODUCT 5 ANALYSIS IS COMPLETED.**
- **SINCE MOST OF THE REACTION AND OBSERVATIONS, AND CALCULATONS AND THEORETICAL YIELD ETC. ARE BEING DONE THIS WEEK, MAKE SURE YOU WRITE EVERYTHING UP NOW WHILE YOU HAVE THE DATA AND OBSERVATIONS IN MIND!**

Hypothetical Mechanism



Week 2, Scheme 2, Part Two: Workup/Isolation

11. Add 0.1 mL of water (The purpose is to destroy any excess, unreacted Mukayama reagent)
12. Stir vigorously for 10 minutes.
13. Add 60 mL of ether (possible labeled as either "ether" or "diethyl ether")
14. Stir vigorously for ten minutes.
15. Prepare a clean pre-weighed 250-ml ground-glass flask with a long stir-bar
16. Weigh the flask + stir-bar.
- Your final yield will be determined by subtracting this mass from the flask+stir-bar+product mass.
17. Find your fritted filter column and attach it to your flask.
    - Make sure that your flask is clamped, and within reach of a vacuum hose.
18. Weigh out 25g of silica in a large boat, and then pour it into the filter funnel. Try to shake it around to level the surface.
19. Carefully drain the organic solution onto the filter column, evenly so that the surface of the column doesn't get all pitted. (Pitting may compromise the effective length/function.)
20. Carefully/gradually open up the vacuum so that liquid gets pulled through without boiling out and getting sucked into the vacuum tube.
21. Add an additional 25 mL of 2/1 ether/dichloromethane (or else 35 mL of 3:1 ether/dichloromethane, depending on what the stockroom has prepared for us) to rinse your original reaction flask, then pour this through the silica.
    - Purpose: this extra rinse of the filter column should remove more of the desired product otherwise still absorbed on the surfaces of your flask and on the silica.
22. What is happening with this silica chromatography/filtration?
    - The silica layer is meant to adsorb as many side-products as possible, while still allowing your product to pass through the column.
    - A quick and dirty chromatography like this won't do a perfect job; probably some side-products will leach through, and possibly some desired product may remain adsorbed to the silica and lost.
    - For medicinal screening at Mayo, we are more concerned with purity than with yield. So, 50% yield at 90% purity is probably preferred to 75% yield at 75% purity, for example.
    - It's probably likely that your product **5** won't be pure enough for direct drug testing. But this is research, so I don't know! ☺☺
        - A research assistant may do flash-chromatography to further purify for drug testing at Mayo.
    - The product **5** should normally be less polar and more mobile than most contaminating side products.
    - The amount of silica and the specific solvent is selected to hopefully allow most of the more-mobile product **5** to get through, while allowing relatively little of the side products to get through. But, it's research, so we'll see how well it does!
    - Multiple side products that we are hoping to remove via the silica column:
        a. 1-methylpyridin-2(1H)-one (what the Mukayama reagent turns into, see mechanism figure.)
        b. Triethylammonium chloride and iodide (the two triethylamines take one proton from the carboxylic acid **4** and one from pyrazolidinone **3**, see mechanism figure).
        c. Dimethylaminopyridine (the catalyst).
        d. Unreacted starting material **3**, if any? (If your 20-mmol "Scheme 1" went really great, then maybe you had more mmol of pyrazolidinone **3** than the 18 mmol of carboxylic acid **4** that you added? If so, it would be nice if any trace amounts of unreacted **3** would just stick on the silica, rather than washing off and contaminating your product **5**!)
        e. Unreacted starting carboxylic acid **4**, if any? (If your 20-mmol "Scheme 1" went not-so-great, maybe you produced less mmol of pyrazolidinone **3** than the 18 mmol of carboxylic acid **4** that you added? If so, it would be nice if any trace amounts of excess carboxylic acid **4** would just stick on the silica, rather than washing off and contaminating your product **5**!)
        f. Other unknown side products, that were either produced in Scheme 2, or were produced in Scheme 1 but just carried along contaminating the Scheme 2 mixture!

- As you might guess, expecting the silica to selectively bind ALL of these various side chemicals, while retaining NONE of the desired product **5**, seems somewhat unlikely!

23. **Concentrate this solution**. Attach a reflux condenser with no water flow, and with a vacuum adaptor on top. While stirring and with no heat turned on, slowly/carefully open the vacuum. Things will bubble a lot at first. Crack open the vacuum as aggressively as you can get away with without causing the mixture to foam over.
    - Within about 2 minutes you should be able to get the vacuum fully opened.
    - Notice the condensation (and perhaps ice) that forms on the outside of the flask.
24. Once you've been able to safely open the vacuum fully, turn the hot plate on at a setting of 5, and heat/boil/vacuum the mixture while continuing to stir (setting 6?) for 20 minutes.
    - Try to wipe off the frost from the walls as early as possible.
    - The mixture should be pretty thick and concentrated by the end, with limited bubbling.
    - In some cases, the material will foam up like cotton candy or taffy. With continued heating, though, usually the entrapped solvent does escape, and the material collapses back to a thick paste.
25. After the vacuum heating, turn off your vacuum first, then turn off the heat, remove the flask from the heat, detach the vacuum hose, and remove the condenser.
26. **NMR-Sample Preparation**: Immediately, while the mixture is still hot and hopefully liquid, dip in with a long-stem pipet and draw up a quarter inch of material. Immediately place the pipet into an NMR tube. The material will probably harden/freeze as soon as it cools.
27. Add 1.2 mL of $CDCl_3$ as solvent to your NMR sample.
30. **GC Prep**: Using the same long-stemmed pipet, draw out what solution it can reach from your NMR tube, and transfer it directly into a GC-MS vial.
    - There will still be enough solution for the NMR.
    - Basically the same solution will feed both NMR and GC-MS analyses
31. **Submit the NMR sample to the NMR queue**.
    - Students will be using the NMR both for submitting, but also for processing (printing extra copies, doing horizontal expansions, etc.) So you may need to be able to work your way between "submit" and "spectrometer" modes.
        - If not in submit mode, click "New Study" to get into submit mode. The correct experiment should automatically load when you do so, if the software is working right.
32. **Submit your GC-MS sample to the GC-MS queue**.
    - This will probably take a while to run.
33. **Mass measurement and % yield determination**. Take your flask to the balance and measure the mass.
    - Take the septum out before recording the mass; replace it immediately after recording the mass.
    - Subtract the original mass of the flask and stir bar in order to determine the mass of product.
    - Record the mass of product.
34. Calculate the percent yield of your Scheme 1 reaction (**1 → 3)**
    - **If your yield exceeds 100%, it probably means your heat/vacuum process had problems. See instructor, in that case.**
    - However, it is also possible that your mass recovery will exceed your theoretical yield. That would be impossible if the material was only desired product **5**. But there were a lot of side products, and solvents, to try to remove in a short purification sequence. If the yield exceeds 100%, perhaps by a lot, that's evidence that the purification/distillation was imperfect. (Perhaps badly so….)

Scheme 3 Part Three: Cleaning Up and Pooling Products into the Collection Jars

1. Add 10 mL of dichloromethane to your flask, and try to dissolve up all of your product **5**.
   - If that doesn't succeed, try heating the mixture on a hot plate for a few minutes to facilitate solubility, and/or perhaps add an additional 5mL of dichloromethane.
2. Once the product is dissolved, simply pour the solution into an appropriately labeled collection jar.
   - Make sure you're putting your stuff into the correct jar and that is labeled well.
3. IF POSSIBLE, AND IF YOU'VE GOTTEN AN NMR FOR YOUR PRODUCT, SHOW IT TO THE INSTRUCTOR BEFORE PUTTING YOUR PRODUCT INTO THE COLLECTION JAR. IF YOUR STUFF IS JUNK FOR SOME REASON, WE DON'T WANT IT TO BE CONTAMINATING THE GOOD PRODUCT CONTRIBUTED BY OTHER STUDENTS. ☺
4. BE SURE THAT YOU'RE PUTTING YOUR PRODUCT INTO THE CORRECT CONTAINER. WE DON'T WANT ANY **5ba** GOING INTO A **5aa** CONTAINER, ETC.

Silica Waste handling: all of the solid silica and sodium sulfate in your filter column should go into a solid-waste container in the hood. Bulk may NOT go into the sink or garbage. (Trace amounts stuck on wall may.)

NMR and GC-MS Data in the Research Module.

NMR Analysis/Interpretation, General Considerations,

1. An **abbreviated summary report** process will be required. Draw the structure and label the different carbons. Then make a table with the actual chemical shifts, integrations, and splittings for the actual spectra, and by each one write the letter of the carbon to which it is attached. This will demonstrate that you have analyzed and understand your spectrum. The two alpha-hydrogens will have difference chemical shifts
   - Include integration.
   - Do analyze/report the splitting (although you may do so.)
   - Which signal is from the β-H, and which signals are from the α-H's?
   - Does your product look pretty pure? If so, there should be a fairly limited number of contaminant signal sets.
2. **Scheme 1: Does your NMR confirm that your heat-vacuum completed ring-closure, and removed water and triethylamine and methylhydrazine**? If your heat-vacuum concentration of product **3** was successful, you should NOT see a big broad lump that appears in the non-aromatic region, and you should see mostly only one N-methyl singlet. If you do have a big lump, that probably reflects residual water and/or methylhydrazine. Does it look like the hydrazine/water is gone?
3. **Scheme 2: Does your NMR confirm that your heat-vacuum process removed the solvent? Does it look like your product is relatively clean, or is there a lot of junk present? Does it confirm that you starting material 3 converted to new product 6**?
4. If you compare your NMR for product **6** versus for reactant **3**, you should be able to see whether reactant **3** is gone or is still present.
5. You should be able to recognize the presence of residual solvents. CH_2Cl_2 gives a singlet at ~5.28 ppm; diethyl ether gives a quartet in the low-to-mid 3's, and a triplet in the low 1's.
6. **Chirality, H-non-equivalency, and chemical shift**: In your NMR for both products **3** and **6**, the β-carbon will be chiral. When you have a chiral carbon, it makes the two α-hydrogens (on the CH2 group next to the carbonyl) unequal to each other. One α-H is cis and the other is trans to your β-H, so they are not in the same chemical environment. These unequal α-hydrogens usually (not always) come at significantly different chemical shifts, but both should appear in the 2's. The β-H, which is attached to a nitrogen-bearing carbon, should be further downfield, probably in the 3's. A video talking through some of this is available.
7. **Chirality, H-non-equivalency, and splitting**: The non-equivalence of the two α-hydrogens also complicates the splitting. They now are split by each other, as well as by the neighboring β-H. Plus the splitting magnitudes are different because of the differing distances. (The "other" α-H is closer than the β-H, so they don't usually provide equivalent magnetic splitting, and don't usually provide a nice triplet.) In practice, each of the two α-hydrogens will usually appear as a four-line "doublet of doublets", and the two α-hydrogens should normally combine to show eight lines. The β-H will typically look much more highly split, being split by each of the two α-H's, plus by either the methyl or ethyl group depending on whether you have **3a** or **3b.** A video talking through some of this is available.
8. **N-H hydrogens**, like OH hydrogens, are typically broad and unpredictable. In many cases you won't be able to see them at all. Don't look for them or try to assign them
9. **Signature signals**: All of the samples will have the interesting α- and β-hydrogens. But there will be other signature peaks for other situations:
   - N-methyl singlets for all samples **3** and **6.**
   - For samples **3a** and **6a**, there should be a nice methyl doublet in the 1's.
   - For samples **3b** and **6b**, there should be a recognizable ethyl group.
10. **Nitrogen impact on chemical shift**: The impact of a nitrogen attachment on chemical shifts is somewhat similar to but weaker than the impact of an oxygen attachment. In other words it has an addition factor of about +1.5-2. This will impact the chemical shift for hydrogen on the β-carbon (β

relative to the carbonyl) and the N-methyl signal. Typically the N-methyl should appear somewhere in the 2.6-3.3 range. The hydrogen on the β-carbon will probably show up somewhere in the 3's.

11. **Chemical shift logic**:
    - For the α-hydrogens in each of 3a-3e, they are next to a carbonyl. So we'd expect them to come in the 2's. They are also β to nitrogen and perhaps also an aromatic, which further pushes them a bit downfield. So typically they should fall in the high 2's or perhaps slip into the low 3's.
    - For the β-hydrogen, the β-carbon has a nitrogen (+1.5-2) so should appear in the 3's.
12. **Impurities/contamination**: Real products often are accompanied by many impurities. The present of impurities, can complicate NMR interpretation. Recognizing which signals come from the desired product and which do not is significant. And qualitatively recognizing whether a spectrum is relatively clean or is pretty contaminated is important.
    - Remember that there should be a logical integration ratio for the main H's in your actual products **3** and **6.**
    - Often there will be a variable amount of smaller signals in the baseline resulting from contaminants, side products, and product-decomposition. The less, the better.
13. **Comparison to Other NMR's**: It may be very interesting to look at how your NMR looks compared to how NMR's of other students look. It will also be very important to see how your NMR changes from structure **3** (the product of the "Scheme 1" chemistry) versus product **6** (the product of the "Scheme 2" chemistry.
    - How different is yours from different versions of **3**?
    - If you compare yours to somebody else who made the same version of **3**, how clean is yours compared to theirs?

GC-MS Analysis/Interpretation

14. Draw the structure for your specific product on each GC-MS sheet, and write the molecular weight underneath the picture. (No "R" groups; write the specific structures.)
15. **Retention time**? What is the retention time for your **3**?
    - Bigger structures will have longer retention times. Product **6** should have a longer retention time than product **3**.
16. **Purity:** How pure is your **3** by GC?
    - Many contaminants will NOT appear, since they come off fast during the solvent delay. So your purity reading will be deceptively high. NMR, which shows everything, is qualitatively more representative.
    - Often nitrogen-containing molecules, because of their basicity, get badly broadened on our GC column. So we'll have to see whether the GC's look useful for **3** or **6.**
17. **Mass Spec and Molecular Ion**: Check in the mass-spec whether there is a molecular ion peak that matches the molecular weight for your product.
18. **Lab report**: In your lab report, make sure that you have not only attached the labeled GC-MS information, but that you also discuss/present the retention time and purity in your data/results/discussion section.

Scheme 1/Week 1 Lab Report:

1. Write a standard synthesis style lab report for your Scheme 1 reaction (**1** → **3**).
2. Make sure that all structures are drawn explicitly.
   - As always for a synthesis style report, you'll want to draw out the reactants and the products. In this case, be sure you draw the **actual** reactant and product in your reaction.
   - None of your pictures should have an "R1": you should illustrate each structure with your actual R1 group drawn, whether that's methyl or phenyl or 4-methoxyphenyl or whatever.
3. Show all calculations. (Including any mole => mass for reactants, or mass => mole for products)
4. Include procedural details and observations as usual.
5. Calculate mass yields, and percent yields, etc., for product **3**.
6. Include your NMR-**3**
   - Be sure to draw your structure, and then provide an abbreviated summary report. This should include a listing of chemical shifts, integrations, and splittings, and a matchup-assignment between signals and hydrogens in the molecule.
   - **Note: you do not need to include impurities/solvents/contaminants in the abbreviated NMR summary report**.
7. Include your GC-MS-3, and **print and attach mass spectra**.
8. Include a results/data/discussion/analysis section. The analysis/discussion section needs to address what the yield information told you, and what the NMR and GC-MS data tells you about both the success and the efficiency of your reaction, and the purity of your product **3**.
9. The results/data/discussion/analysis section should summarize what the mass/yield/NMR/GC-MS data is, and what conclusions can be drawn from them. Just attaching the NMR's and GC-MS's without discussing or showing that you understand them will not be good. What is the summary for the key non-aromatic C-H hydrogens in your NMR? What is your GC-retention time? Between the NMR and the GC, did it look like the product **3** was formed successfully, and does it look reasonably clean? Or is it obviously significantly contaminated?
10. Note: Keep extra copies of your NMR and your GC-MS's. Pyrazolidinone **3** functions as the product in week one report, but it is the reactant in the week two report. So when writing up and analyzing Scheme 2, you'll need information about mass, molecular weight, structure, and mmol of your reactant **3**. You'll also need to have NMR and GC for **3** so that you'll be able to compare your product **6** to reactant **3** and tell whether the reaction really worked.

Scheme 2/Week 2 Lab Report: The same principles as described for Scheme 1 report apply.

11. Note: For the Scheme 2 report, include both NMR's and GC's for product **6** but also for starting material **3** for comparison.
12. When analyzing your NMR and GC data for product **6**, make sure you check and evaluate whether residual chemical **3** is present or not.