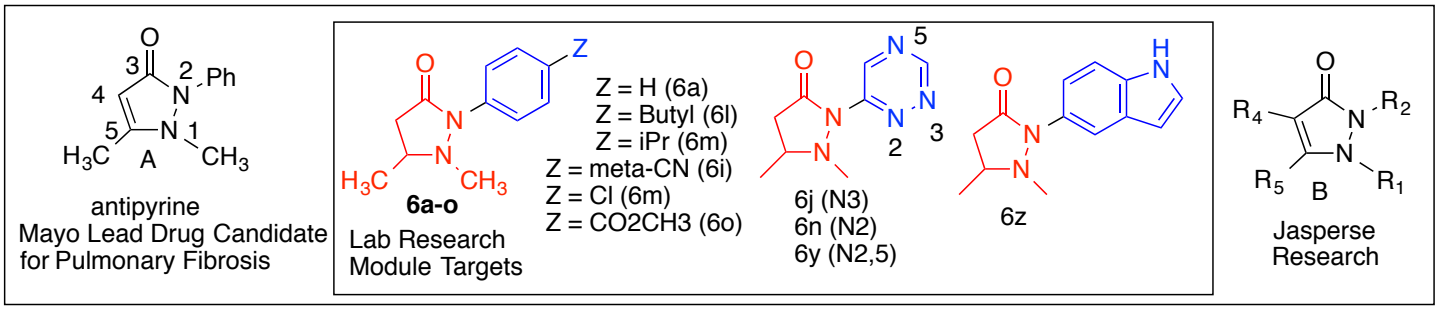
Multistep synthesis of Aryl Pyrazolidinones for Pulmonary Fibrosis Testing.

Introduction

We will begin a two-week research sequence in which we make a variety of novel "aryl pyrazolidinones", see structures **6a-z** in the figure below. None of these substances have ever been reported in the chemical literature. Each is of interest as a medicinal candidate. Half of these were first prepared, isolated, and characterized in the Jasperse research group and in Chem365 lab last spring; the other half have never been prepared, isolated, or characterized before! 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, (see structure **B**) and having them bio-screened, in hopes of both improving drug performance and improving the understanding of drug binding and mechanism. Where is there volume allowance to get bigger? Where is it necessary to include hydrophobic substituents? Where might drug performance and binding perhaps improve by inclusion of hydrophilic hydrogen-bonding heteroatoms like nitrogen or oxygen? Does addition of electron donors or withdrawers to the benzene ring make a difference?

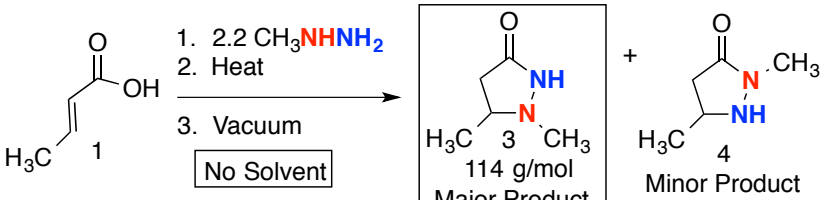


Structure **6a** differs from antipyrine in its lack of the C=C double bond. Structures **6l-z** will have not only the double-bond variation, but will also include variations in or on the aryl ring. These will differ in size (**6l, 6m, 6i, 6m, 6z**); in hydrogen-bonding capacity (oxygens in **6o;** nitrogens in **6i, 6j, 6n, 6y,** and **6z**); in whether the aryl ring is heterocyclic (pyridines **6j** and **6n**, pyrazine **6y**, indole **6z**); and in electron density (CN **6i** and CO2CH3 **6o** have strong electron withdrawers; alkyl-substituted **6l,m** have weak donors; heterocycles **6j, n, y, z** differ significantly in their electronics from a normal benzene).

Drug tests should indicate how variations in the size and nature of the Z-subsituents impact drug performance. Second, testing **6a** against antipyrine will help evaluate whether the double bond is essential. Determining whether the C=C double bond essential or irrelevant will be useful to learn. (Many single-bonded analogs are easier to synthesize, so *if* the drug doesn't even need the double bond, that could be helpful to know.)

It is beyond the scope of the Chem 365 research module for this year, but the Jasperse research group (Eli Mans) has recently developed a procedure for oxidizing structures **6a-z** to convert the C4-C5 single bond into a double bond. If the double bond is essential, convenient access to **6a-z** will indirectly access the double bonded analogs for all of them.

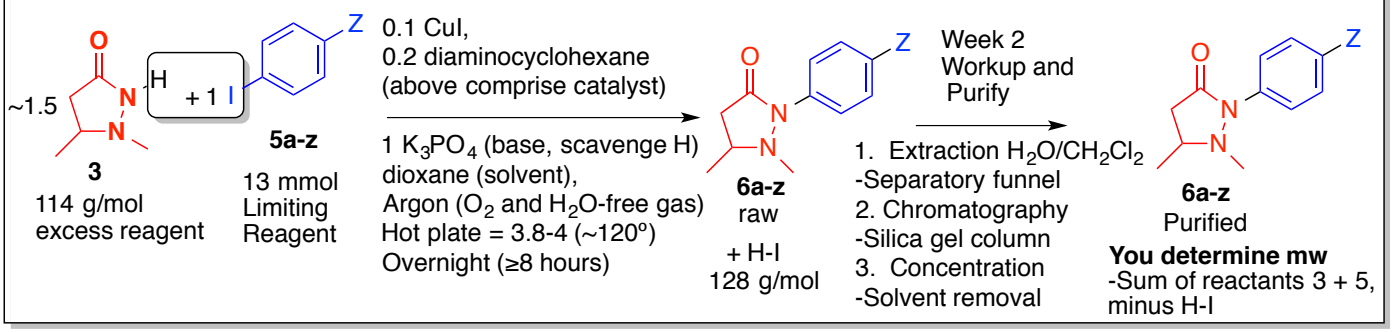
Scheme 1: Synthesis of N1,C5-Dimethyl Pyrazolidinone



The syntheses will involve two reactions. The initial reaction, illustrated in Scheme 1 on the previous page, will react methylhydrazine (CH_3NHNH_2) with "crotonic acid" (2-butenoic acid) to make the N1,C5-dimethyl substrate **3**. This product will be an oil. 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. (Many reactions of these types are included in Chem 360 Test 4 material.). Some selectivity (but how much?) for product **3** over **4** 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 excess methylhydrazine and the water byproduct.

The crude mixture of **3** and **4** will then be carried on directly through the reaction illustrated in Scheme 2. In this reaction, the N2-nitrogen will be arylated by various aryl iodides **5**. None of the molecules **6a-z** have been reported in the literature, nor been tested for medicinal use. These are truly novel molecules that have never been purified before! ☺☺ (Some were made last spring at MSUM; others have never been made before even here!).

Scheme 2: N2-Arylation Using Aryl Iodides and CuI-Diaminocyclohexane Catalyst



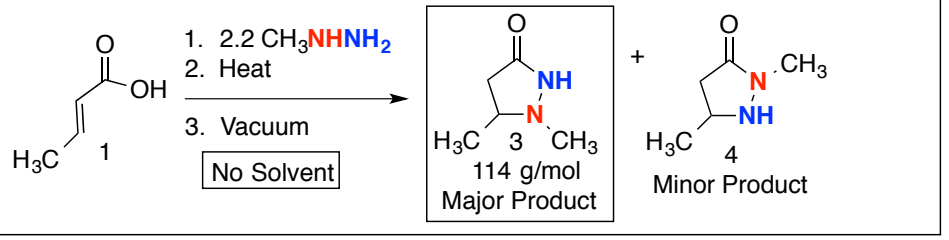
The chemistry used in Scheme 2 is not necessarily fast. Thus, students will want to start the Scheme 2 reaction at the end of the first laboratory day. That will allow hours of heating to enable completion. During the second week, students will conduct an extraction process and chromatographic workup to hopefully 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 show splitting patterns that are unfamiliar to you. This is because one alpha-H will be "cis" to the C5-methyl, 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 should 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. I hope you enjoy the research module and the ability to synthesize, isolate, and characterize something that has never ever been isolated and characterized before!

Scheme 1: Synthesis of N1,C5-Dimethyl Pyrazolidinone



- See the following page for the hypothetical mechanism.

Reagents:

1. 20.0 mmol of Crotonic acid
   - Crotonic acid: 86.0 g/mol
2. 44 mmol of Methyl Hydrazine (52.4 ml/mol)
3. 125 mL Ground-Glass Erlenmeyer flask
4. Long sized stir bar
5. Hot plate
6. Reflux Condenser
7. Vacuum Condenser and Vacuum adaptor.

Scheme 1 Procedure: Formation of Pyrazolidinone Ring Using Hydrazine

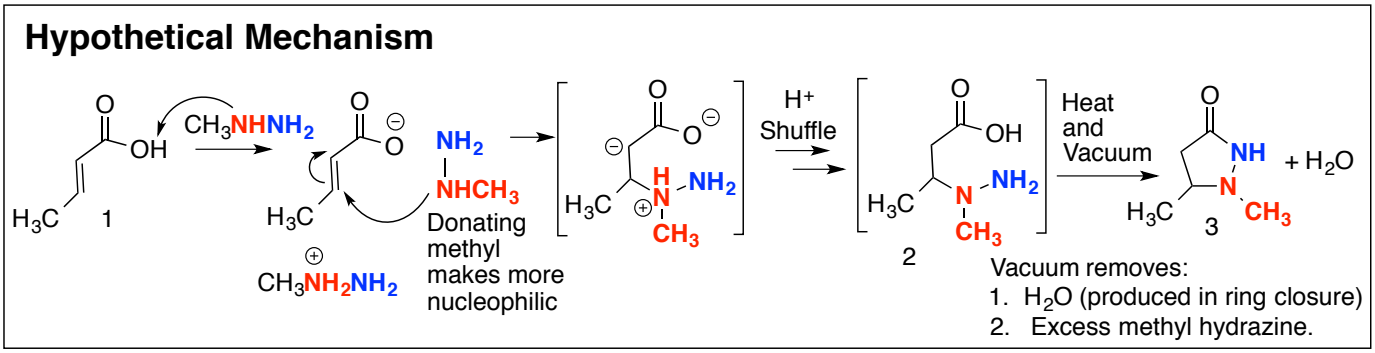
Part A. Heating/Hydrazine Addition Phase

1. Get a 125-mL ground-glass-jointed Erlenmeyer, and add a long stir-bar. **Weigh the combination and record the mass.** Write your name(s) on the flask with your sharpie.
   - You'll need this mass to calculate your product yield, so record it somewhere!
2. Weigh out 20.0 mmols of "crotonic" (2-butenoic) acid:
   - Crotonic acid, 86.0 g/mol, is a solid. Add it using a powder funnel.
3. Add a rubber septum to the flask, and poke a syringe needle through it to vent any pressure buildup.
4. Add 44 mmol (0.044 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.
5. Add a reflux condenser, with a gentle flow of water running through it, and place the septum on top to prevent oxygen exposure. (Oxygen causes some oxidative decomposition.)
6. Turn your hot-plate setting up to 8, and continue stirring for an additional 30 minutes.
7. During the stirring time, prepare and run H-NMR and GC for your aryl iodide 5.
8. If it's a liquid, use a long-stemmed pipet and draw up about ½-inch of aryl iodide into the skinny end section of the pipet; place into NMR tube; then pour in ~1.3mL of CDCl3 through the pipet. Shake.
9. During the stirring times, plan ahead. Prepare the following:
   a. Find your vacuum adapter, and plug it into the vacuum hose. This adapter 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.
   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. Calculate what the theoretical yield for your product **3** should be, given the 20.0 mmol scale.

e. 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…
f. 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 below).
g. At this stage you should also have, in addition to the product, at least two other chemicals:
    - The excess methylhydrazine
    - Water which is produced during the ring closure.
    - Probably some contaminants or side products.

- The hope is to later vacuum-distill away all or most of these extra things, without decomposing the cyclic products **3** (and side product **4)** in the process.

10. 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.
11. Let your solution (and the hot-plate) cool for at least **5 minutes** before starting Scheme 1 Part B.



Hypothetical Mechanism and discussion (for your interest): The probable mechanism involves an initial proton transfer from the acid to the methylhydrazine. Amines are basic enough to rapidly and completely deprotonate acids. Amines are also modestly nucleophilic; upon extreme heating, the amine is able to add to the alkene, to produce a resonance-stabilized enolate. Without the resonance stabilization this would not be possible. It would also not be possible without high heat: in the procedure, we use a hot-plate setting of 8, which heats the mixture to ~200ºC. The nitrogen addition is irreversible; as soon as the enolate ion forms, it probably gets protonated immediately. Structure **2** in the figure is drawn in neutral form; that isn't really accurate, because it would mostly exist in an ammonium carboxylate ionic form. But picture **2** makes it easier to visualize that ring formation releases a water molecule. The ring formation again requires high heat, but it also requires vacuum for the removal of the water as it forms.

Excess methylhydrazine is needed because the first equivalent just deprotonates the acid, and the protonated methylhydrazine would not be nucleophilic. Using 2.5 methylhydrazines ensures that there is always neutral nucleophilic methyl hydrazine available.

When the Jasperse group first attempted this reaction, I doubted it would be effective. I thought the blue NH2 nitrogen might add preferentially for steric reasons, or else that the blue and red nitrogens would add about the same and give a bad mixture of structural isomers **3** and **4**. We now believe that the electron-donating effect of the methyl group makes the methylated nitrogen more electron rich and nucleophilic, resulting in useable selectivity for desired product **3** over structural isomer **4**.

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

12. After the 5-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.
13. 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 vacuum opened**.
14. Stir/heat/vacuum for **25 minutes**. (Measured 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.
15. After the 25 minutes, slide the hot plate out from under your reaction mixture.
16. 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 all of next week's lab 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.
- **Note: The first lab report that is due next week should include only the chemistry included in Scheme 1. Even though you are beginning the Scheme 2 chemistry during week one, include ALL of the scheme 2 chemistry in a lab report that will be due the week following completion of Scheme 2 and it's workup.**

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.
- You'll need both for your lab report, but either will be enough to let you get started on Scheme 2.

17. **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.
18. Put your pipet with that small sample into an NMR tube.
19. 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.
20. 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.
21. **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
22. **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.
23. **Submit your GC-MS sample to the GC-MS queue**.
    - This will probably take a while to run.
24. **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).
25. 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.**
26. Calculate how many mmol of product you have.
27. 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?
28. **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.

Scheme 1/Week 1 Lab Report:

1. Write a standard synthesis style lab report for your Scheme 1 reaction (**1** → **3**).
2. Show all calculations. (Including any mole => mass for reactants, or mass => mole for products)
3. Include procedural details and observations as usual.
4. Calculate mass yields, and percent yields, etc., for product **3**.
5. 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**.
6. Include a note about your approximate ratio of the major product **3** to the minor product **4**. Produ
7. Include your GC-MS-3, and **print and attach mass spectra**.
8. In the experiment, what was the approximate ratio of major product 3a to minor product 4a, based on GC data?
9. In the GC, what was the retention time for the major isomer, versus the minor isomer?
10. Given the major/minor **3/4** ratio measured by GC, can you see evidence in the H-NMR for a similar mixture of major-to-minor isomers?
11. What are the chemical shifts for the N-methyl signal from **3**, versus the N-methyl signal in **4**?
12. Explain very briefly why the two α-Hydrogens in the NMR have different chemical shifts, despite being connected to exactly the same carbon?
13. 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**.
14. This should have already been in your main lab report, but for my grading convenience, for the major product **3**, list the chemicals shifts for the following signal sets in major product 3a:
    - N-methyl:
    - C-methyl.
    - β-H:
    - α-H (one of them):
    - α-H (the other one):
15. 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?
16. 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.

NMR Analysis/Interpretation, General Considerations,

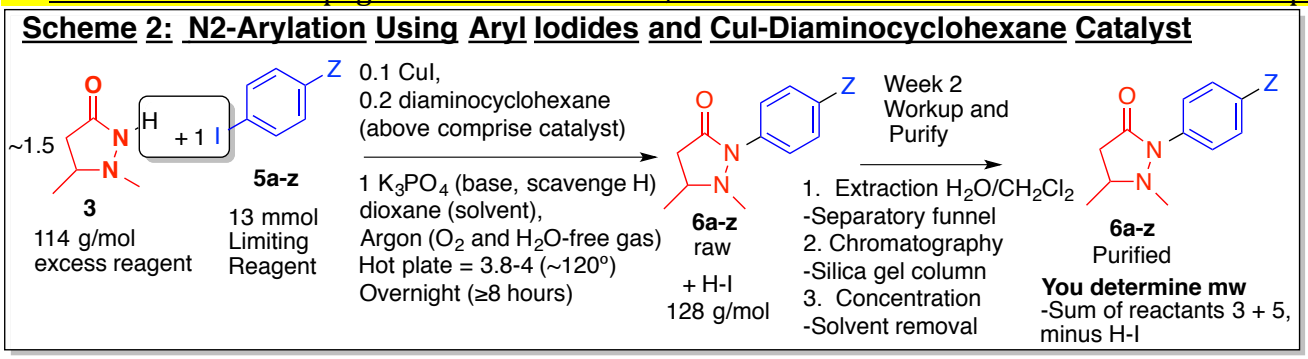
1. An **abbreviated standard summary report** will be required for 1H NMR. Draw the structure and label the different carbons. Then make a table with the actual chemical shifts, integrations, and splittings for the actual spectra, and by each one write the letter of the carbon to which it is attached. This will demonstrate that you have analyzed and understand your spectra. The two alpha-hydrogens will have difference chemical shifts.
2. **N-H hydrogens**, like OH hydrogens, are typically broad and unpredictable, and probably invisible for product **3**. Don't look for them or try to assign them in product **3**.
3. **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 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.
4. **Chirality, H-non-equivalency, and splitting**: The non-equivalence of the two α-hydrogens 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 the methyl group**.** A video talking through some of this is available.
5. **Signature signals**: Sample **3** should have a nice 3H methyl doublet in the 1's, and a nice 3H N-methyl singlet in the 2's.
6. **Nitrogen impact on chemical shift**: The impact of a nitrogen attachment on chemical shifts is 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.
7. **Chemical shift logic**:
   - For the α-hydrogens in each of **3** and **6**, they are next to a carbonyl. So we'd expect them to come in the 2's. They are also β to nitrogen, 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.
8. **Impurities/contamination**: Real products often are accompanied by many impurities. The present of impurities, can complicate NMR interpretation.

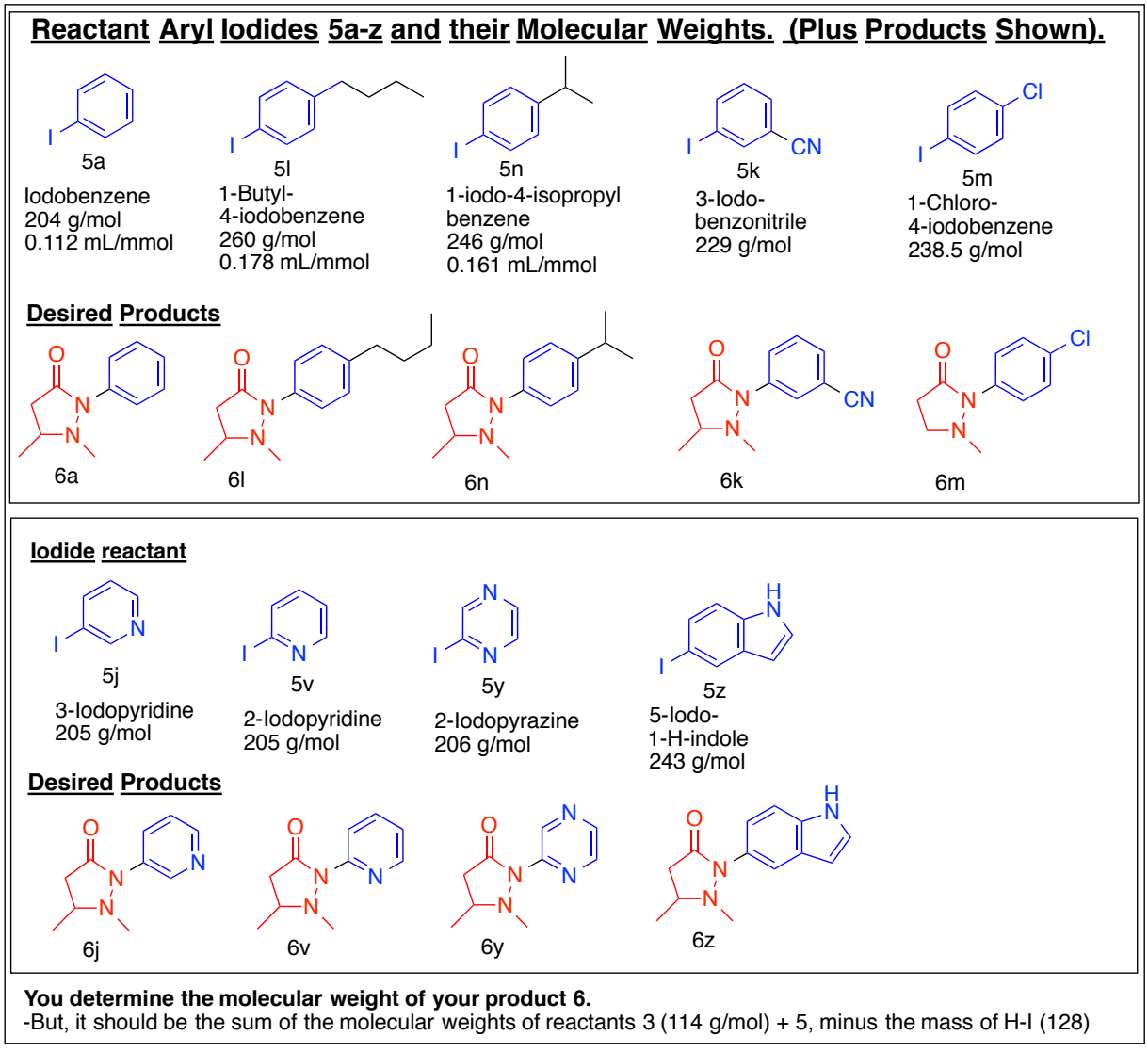
GC-MS Analysis/Interpretation

9. Draw the structure for your product on the GC-MS sheet, and write the molecular weight underneath.
10. **Retention time**? What is the retention time for your **3**? (It may be really broad, but you should still be able to get a general idea).
11. **Purity:** How pure is your **3** by GC?
    - 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 .**
12. **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. Both the major product **3** and the minor product **4** should weigh the same 114.
13. **Lab report**: In your lab report, make sure that you have not only attached the labeled GC-MS information, but that you also present the retention time and purity in your data/results/discussion section.

Scheme 2: N2-Arylation using Aryl Iodides and CuI-Diaminocyclohexane Catalyst

- Note: The next two pages are done week one, but should be included in Scheme Two lab report.





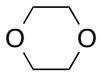
Reagents:

1. Pyrazolidinone **3**: Report how many grams and mmoles you actually produced at the end of Scheme 1. But it should be ≤20 mmol.
2. CuI: 2 mmol x 190 = 380mg
3. K3PO4: 20 mmol x 0.212 g/mmol = 4.2 g
4. Aryl iodide: 12 mmol. (you figure out how much of yours you need to add!
5. Dioxane (anhydrous): 20 mL
6. Diaminocyclohexane: 4 mmol x 0.120 mL/mmol = 0.48 mL
    - Argon atmosphere. Ask instructor help with this.

Procedure:

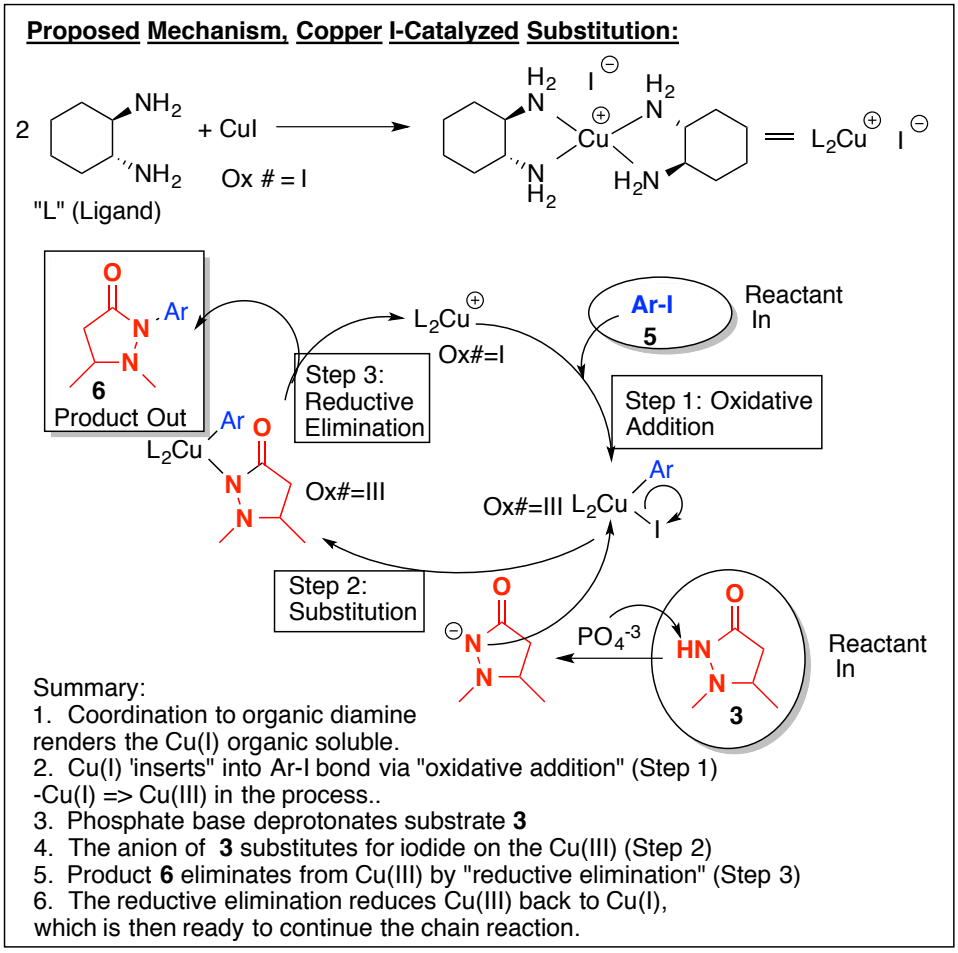
Part 1: Starting the Reaction for N-Arylation

1. Carry your flask with your reagent **3** in it, and the septum, to the balance, to weigh in any solids. Avoid air exposure, so pop the septum back in after each addition.
2. Add Copper iodide: 2 mmol x 190 = 380mg
3. Add Potasssium phosphate: 20 mmol x 0.212 g/mmol = 4.2
4. Add 12.0 mmol of your aryl iodide, whether it's a solid or a liquid.
    - Details for each one are listed on previous page.
    - For a solid, the molecular weight is used to determine how much mass to add.
    - For a liquid, use the mL/mmol value to calculate how much to add.
        - In either case, calculate for 12 mmol.
        - If uncertain, check with Dr. Jasperse.
5. Add ~20 mL of anhydrous dioxane.
    - The measurement doesn't need to be precise.
    - Get within 2 mL of 20mL, but better to act quickly than to be super precise! ☺
        - The dioxane is air/moisture sensitive. Because it a cyclic ether, it hydrogen bonds to water, so moisture from the air can dissolve in and contaminate the solvent.
        - We want it to stay as dry as possible for future users, so screw the cap back onto the dioxane bottle first, as soon as you've poured your 20mL out, before pouring your dioxane into your flask.
        - Then pour your 20-mL into your flask, and put the septum back in to exclude further air exposure.



Dioxane
1. Ether-type dissolving properties (only better)
2. Larger, so higher boiling point
   => hotter + faster reactions, but not too high-boiling to distill away at the end
3. Symmetry gives it a very simple NMR, so residual dioxane doesn't confuse spectra much

6. Purge three times with argon. The instructor will do this for you, at the purging station in the hood near the acetone waste hood.
    - The goal is to replace the air in your flask with inert argon gas, which has no water and no oxygen in it.
    - Residual oxygen is otherwise able to oxidize and destroy the Cu(I)-oxidation-state catalyst. If all of your catalyst gets destroyed, the catalytic reaction will fail!
    - The instructor will execute the gas purge for you, but if you have a partner, make sure both come along to see how this is done.
7. Bring the flask back to your hood and stir vigorously for 1 minutes to get everything mixed.
8. Add diaminocyclohexane, via syringe by puncturing through the septum.
    - 4 mmol x 0.120 mL/mmol = 0.48 mL diaminocyclohexan
    - The order of addition of the other chemicals didn't really matter. But the diamine should be added last, and only after the argon purge is complete.
    - The diaminocyclohexane attaches to the copper to make the hopefully active catalyst.
    - It also serves to get the Cu(I) ion dissolved into the organic solution. (with two diamonocyclohexanes attached, the composite catalyst now has a lot of organic character.)
9. Stir vigorously on a hot plate.
    - Set hot-plate setting to ~3.5.
10. Stay for 15 minutes to watch and record color changes or other observations. After that, you are free to go! (But make sure your flask has your name labelled on it, so it won't get confused with other students in same hood!) **YOU ARE NOW DONE WITH THE WEEK ONE CHEMISTRY**! ☺
11. Let stir hot overnight. The instructor will come in the morning and turn the heat off.
12. The mixture will then wait till next week for workup, isolation, purification, and analysis! ☺



Proposed Hypothetical Mechanism for the Cu(I)-catalyzed arylation and discussion (for your interest): The mechanism is very interesting and is **very** different from anything you've seen before. In introduction to SN1 and SN2 reactions, for example, we couldn't use aryl iodides in either of those type mechanisms.

But something very different happens here with the Cu(I). Several low-oxidation-state transition metals [Cu(I) and Pd(0)] have a capacity to do "oxidative addition" into certain aryl-halide bonds. In Step I, Cu(I) inserts into the Ar-I bond, creating new Cu-I and Cu-Ar bonds. This is formally an oxidation-reduction reaction: copper is oxidized from Cu(I) => Cu(III). The iodide and carbon are reduced; they can be viewed as anions following Step 1. The mechanistic detail of how this oxidative addition proceeds is beyond the scope of this course!

The nitrogen then substitutes onto copper in Step 2. This can be viewed as a simple SN2-type substitution. The phosphate base is strong enough to generate the resonance-stabilized nitrogen anion under the high temperatures.

After both the nitrogen and aryl groups bond to the Cu(III), those two then hook together and detach from the copper (Step 3) to make product **6**. This is termed "reductive elimination" because the Cu(III) is reduced back to Cu(I). The aryl and nitrogen, formally anionic when coordinated to the copper, are oxidized back to neutral. The mechanistic detail of this reductive elimination is again beyond the scope of this course! ☺☺

The diaminocyclohexane serves two crucial roles. First, coordination to the Cu(I) makes the complex mostly "organic" so that it becomes soluble in the dioxane solvent. Solubility of the catalyst is essential. Second, coordination enriches the electron density of the Cu(I), which makes it more reactive as a reducing agent in Step 1. In the figure, "L" is a shorthand for "ligand", which is a general term for something coordinated to a metal. So "$L_2Cu(I)$" represents two diaminocyclohexane ligands coordinated to a Cu(I).

Notice how the $L_2Cu(I)$ catalyst, shown at the top of the loop, functions as a catalyst. Following the cycle of oxidative addition-substitution-reductive elimination (Steps 1-3), the original $L_2Cu(I)$ is regenerated and can repeat the chain. Thus a stoichiometric amount is not required.

Aryl-substituted nitrogens are prolific in nature and in medicinal reagents. The ability to use catalytic arylation to attach aryl groups onto nitrogen is very powerful and useful.

You probably noticed some color changes. If you saw some blue, that would be some Cu(II), probably resulting from trace oxygen oxidizing the catalyst. As the reaction proceeds (or when you return next week), you'll probably see a lot of red/purple. That is the color of iodine, resulting from oxidation of iodide product, either by adventitious oxygen leakage through the septa, or else by reduction of something else in the mixture.

Week Two: Scheme 2 Product Workup, Isolation, Purification, Concentration, and Analysis.

Overview:

1. Remember that you will be submitting a lab report on ALL of Scheme 2. That includes the scheme 2 work and calculations and listings of chemicals and moles calculations that you did last week (page 9).

2. **Contaminants**: At this point you will have a LOT of different things in your solution mixture:
   a. **Product** (hopefully, and hopefully lots!)
   b. The dioxane solvent.
   c. Lots of potassium phosphate (or hydrogen phosphate, the conjugate acid)
   d. Iodide ions
   e. Excess reactant **3**
   f. Chemical **4**, the structural-isomer side-product from Scheme 1.
   g. Copper-iodide/diaminocyclohexane stuff.
      - These were combined to make the catalyst, so they should still be in there.
      - Some catalyst decomposition into who-knows-what occurs upon exposure to air and water.
      - I suspect that any insoluble junk (maybe a lot) is copper/diaminocyclohexane stuff?
   h. Some aryl iodide?
      - Hopefully not, because it's the limiting reagent.
      - But maybe the reaction didn't convert it all perfectly and completely?
   i. Carryover contaminants: Any junk that was present at the end of Scheme 1 is still in the soup.
   j. Contaminants in aryl iodides: the commercial aryl iodides weren't 100% pure to start with.
   k. Newly-formed contaminants = side products! (We hope not a lot, but there are probably some things other than just desired product forming.)
   - So, lots and lots of things we want to get our product away from!

3. **Isolation/Purification Plan: The Overall Plan for the Day**
   a. Part 1: **Dichloromethane extraction from water in separatory funnel**:
      - The product **6** (neutral organic) should extract out into dichloromethane,
        - although several extractions will be needed to get it all out. (With at least two nitrogens and an oxygen, the solubility in water is non-trivial. For products that have heteroatoms in or attached to the aryl ring, water solubility may be higher yet.)
      - Ionics should stay in the water. (Iodides, sodium phosphate, sodium hydrogenphosphate)
      - Starting substrate **3** will largely remain in the water. It turns out that excess reactant **3** and structural isomer **4**, which have two nitrogens and an oxygen versus only 5 carbons, are really strong hydrogen-bonders with water.
   b. **Chromatography**. The organic solution will be passed through silica gel and sodium sulfate.
      - Any insoluble junk will get stuck.
      - Any water in the organic solution will get physically absorbed on the sodium sulfate.
      - Any starting substrate **3** that didn't stay in the water will absorb tightly to the polar silica gel.
      - Any other contaminants that are significantly more polar than the product will hopefully stay absorbed on the polar silica surface.
      - Organic contaminants that are not much more polar than the product will pass through, however.
   c. **Concentration (hot and with vacuum)** to remove all solvent
      - Following filtration, the solvent needs to be removed.
      - The solvent will include lots of dichloromethane, plus a lot of dioxane which has a much higher boiling point (~100ºC) than dichloromethane, plus some methanol.
      - The combination of vacuum and strong heating should be able to distill these away.
      - The product being much heavier and less volatile should remain behind!

Workup materials:

1. Dichloromethane: ~100 mL
2. Water: 50 mL
3. silica: 15g
4. Fuller's Earth: 5g
5. Sodium sulfate: 30grams
6. 5% methanol/Dichloromethane: ~20 mL

Workup, Isolation, Purification, Concentration

1. Record observations of your solution.
2. Add 50 mL water (tap is fine)
3. Add 35 mL of dichloromethane ("DCM")
4. Stir very vigorously for ≥2 minutes to try to get the potassium phosphate solids dissolved up into the water layer.
5. Add a long stir-bar to a 250-mL ground-glass flask, and pre-weigh the combination
   - Your final yield will be determined by subtracting this mass from the flask+stir-bar+product mass.
6. Pour solution into a separatory funnel and allow it ≥2=1 minutes to settle.
7. Add another 10 mL of dichloromethane. Do not shake; this may help visualize the distinction between the aqueous layer on top versus the organic layer on the bottom.
8. Record observations.
9. Add fritted filter column to the 250-mL flask
10. Add 15 g silica to filter column
11. Add 5g of Fuller's earth.
12. Add 30 g sodium sulfate to the filter column
13. Carefully drain the lower organic layer from your separatory funnel into the filter column, so that liquid gets pulled through without boiling out and getting sucked into the vacuum tube.
    - Don't worry if there is a lot of solid insoluble stuff at the interface between the two layers.
    - It isn't desired material; it is something copper-containing.
    - Whatever comes through will just get stuck on the sodium sulfate anyway.
13. If the solution flows slowly, you can assist by attaching a vacuum hose and gently vacuuming.
14. Avoid having any of the water layer drain out.
15. Add another 20 mL DCM to rinse the original flask
16. Pour this into the sep funnel, shake, let settle, and again drain the DCM layer through column
17. Repeat steps 15 and 16 twice more (basically you're doing a 3rd and 4th extraction with DCM to make sure all of your product is extracted from the aqueous phase. This may be especially important for products with nitrogens in the aromatic ring.).
18. Take 20 mL of 5% methanol-dichloromethane (available in the hood), and rinse this directly and cautiously through the filter column.
    - Methanol is a stronger elution solvent than is DCM.
    - The purpose here is to make sure that all of your product is washed off of the silica, but hopefully without having a lot of dark-colored polar contaminants wash off besides.
    - As you do this last rinse, *if* it looks like a colored band is moving down the column and is going to come off, DO WHATEVER YOU CAN TO STOP BEFORE THAT COMES OFF!
        - Pull the vacuum hose off of the filter column,
        - Remove the filter column from the flask.
        - Turn off the vacuum.
        - I don't want dark colored bands flowing into the receiver flask, if possible!
        - Check with instructor!
19. 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.

- The Fuller's earth sometimes can absorb certain (but not all) very large dark-colors contaminants.
- 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, 40-60% yield at >90% purity is much preferred to 75% yield at 75% purity, for example.
- I'm hoping that your product **6** will be pure enough for direct drug testing. But this is research, so I don't know! ☺☺
- The amount of silica and the specific solvent is selected to hopefully allow most of the more-mobile product **6** 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!
- As you might guess, expecting the silica to selectively bind ALL of the various side chemicals, while retaining NONE of the desired product **6**, seems somewhat unlikely!

1. **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 may bubble a lot at first. Crack open the vacuum as aggressively as you can get away with without causing the mixture to foam over.
   - You may want to request the instructor to come over to get this started.
   - 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.
2. Once you've been able to safely open the vacuum fully, turn the hot plate on at a setting of 6, and heat/boil/vacuum the mixture while continuing to stir for 25 minutes.
   - Try to wipe off the frost from the walls as early as possible.
   - The mixture may be pretty thick and concentrated by the end, with limited bubbling.
   - In some cases, the material may perhaps foam up like cotton candy or taffy. With continued heating, though, usually the entrapped solvent will escape, and the material will collapses back to an oil.
3. During this time, *IF* you haven't previously run and collected an an H-NMR on the starting aryl iodide, do so. You probably already did this last week, but if not do it now.
   - You'll want to be able to compare the NMR for your final product **6** to both the starting material **3** and the starting aryl iodide **5**.
4. If you haven't previously calculated your theoretical yield, do so now.
5. 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.
6. Measure the mass of the flask with the product.
   - Subtract the original mass of the flask and stir bar in order to determine the mass of product.
   - Record the mass of product.
7. If your yield exceeds 100%, more hot vacuum is required.

8. **NMR-Sample Preparation**: Dip in with a long-stem pipet and draw up a half-to-one inch of material. Immediately place the pipet into an NMR tube, and put the septum back into the flask.
9. Add 1.2 mL of $CDCl_3$ as solvent to your NMR sample/pipet.
10. **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
11. **Submit the NMR sample**. (Print an extra copy of the un-zoomed to give to instructor!)
    - Will want to do horizontal expansion in the 1-4 and 6-9 ranges.
    - The 1-4 analysis will show whether alkyl signals from substrate have been converted to new product signals, and if cleanly or junky.

- Will want to do horizontal expansion in the 6.5-9 kind of range. This should be able to illustrate whether starting aryl iodide has been converted, and if so whether cleanly or not.

12. **Submit your GC-MS sample to the GC-MS queue**. (Print an extra copy to give to instructor!)
    - Turnover time will be about 10-12 minutes? (Dr. J can perhaps bring to class or email scans?)
    - To analyze this simply, you'll need to review what your retention time was for substrate 3.
    - You should also have a reference GC on the starting aryl iodide
    - The product should have a longer retention time than either substrate or iodobenzene.
    - You want to know the molecular weight of the product, so you can check to see whether a significant peak with matching molecular weight will be the dominant new peak. **Scheme 3**

Part Three: Cleaning Up and Collecting Products into the Collection Vials

1. Using a pipet, transfer as much as you can of your final product into a 20-mL vial with a black cap.
2. Write your sample name (**6a** or **6b** or **6c** or whatever) **and** your **student name(s)** on the vial with a sharpie.
3. Place the vial into a product-collection beaker
4. Place an extra copy of your NMR printout (no zooms required) and your GC printout next to the product-collection beakers. (I can sort them later.)
    - I'll want to look through the spectra to find the most pure samples, ones that are hopefully pure enough for Mayo submission! ☺☺.

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.)

Otherwise glassware should be washable with water and acetone.

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

1. Write a standard synthesis style lab report for your Scheme 2 reaction (**3 + 5 → 6**).
2. Show all calculations. (Including any mole => mass for reactants, or mass => mole for products)
3. Include procedural details and observations as usual.
4. Calculate mass yields, and percent yields, etc., for product **3**.
5. Include your NMRs for **6**
   - Be sure to draw your structure, and then provide an abbreviated summary report. This should include a listing of chemical shifts, integrations, and a matchup-assignment between signals and hydrogens in the molecule.
   - You can perhaps ignore splittings for the alph and beta H's, since they will likely be rounded.
6. Note: For the Scheme 2 report, include both NMR's and GC's for product **6** but also for starting material **3** and for your aryl iodide **5** too, for comparison.
   - Comparing products to reactants helps show whether they converted or not, and whether unreacted starting materials are contaminating your product.

17. 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**.
18. This should have already been in your main lab report, but for my grading convenience, for the major product **6**, list the chemicals shifts for the following signal sets in major product 3a:
    - N-methyl:
    - C-methyl.
    - β-H:
    - α-H (one of them):
    - α-H (the other one):
    - Aryl H's
    - Any additional methyl groups, etc.

NMR and GC-MS Data in the Research Module Scheme 2.

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 (some hydrogens may have very rounded signals) 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 spectra. The two alpha-hydrogens will have difference chemical shifts

   - Include integration.
   - Do not need to analyze/report the splitting (although you may do so. But in several cases H's may be very broad and rounded.)
   - 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 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**?
3. 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.
4. You should be able to recognize the presence of residual solvents. CH_2Cl_2 gives a singlet at ~5.28 ppm; dioxane in the high 3's, maybe ~3.8?
5. **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.
6. **Rounded H-signals in products 6:** 5-membered rings sometimes have energetically similar conformations that exchange at rates comparable to the NMR time scale. A result is that some of the ring-H's in products **6** will look really broad and rounded. The integrations and chemicals shifts should be appropriate, but the splitting may not be visible.
7. **Signature signals**: All of the samples will have the interesting α- and β-hydrogens. But there will be other signature peaks for other situations:
   - There should be a nice methyl doublet in the 1's, and an N-methyl singlet in the 2's.
   - Samples **6b, 6c,** and **6c** should each an additional methyl singlet in the 2's or 3's.
   - Samples **6b,d-f** should all have a pair of 2H doublets in the aryl region, due to the para substitution.
8. **Nitrogen impact on chemical shift**: The impact of nitrogen on chemical shifts is somewhat similar to but weaker than the impact of an oxygen attachment (addition factor of about +1.5-2.)
9. **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.
10. **Comparison to Other NMRs**: It may be interesting to look at how your NMR looks compared to how NMRs 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").

GC-MS Analysis/Interpretation

14. Draw the structure and write the molecular weight underneath the picture.
15. **Retention time**? What is the retention time for your **6**?
    - Product **6** should have a longer retention time than product **3**, being bigger.
16. **Purity:** How pure is your **6** by GC?
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.