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 9,10).

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 any, given that 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 mix.
   j. Newly-formed contaminants = side products! (We hope not a lot, but there are probably some things other than just desired product forming.)
   k. Aryl iodide reduction is sometimes one side-product. Some of the Aryl-I may convert to Aryl-H.

- So, lots and lots of things we want to get our product away from!

3. **Isolation/Purification Plan: The 3-Stage 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,
        - But 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 additional heteroatoms in or attached to the aryl ring, water solubility may be higher yet.)
      - Ionics should stay in the water. (Iodides, potassium phosphate, potassium hydrogen phosphate)
      - Excess starting substrate **3** will largely remain in the water. It turns out that excess reactant **3** and its structural isomer **4**, each with two nitrogens and an oxygen versus only 5 carbons, are strong hydrogen-bonders with water.
   b. **Chromatography**. The organic solution will be passed through silica gel and sodium sulfate.
      - Insoluble junk will get stuck, including copper-iodide/diaminocyclohexane residues.
      - 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. Sodium Bisulfite: 2g
3. Water: 50 mL
4. silica: 15g
5. Fuller's Earth: 5g
6. Sodium sulfate: 30grams
7. 5% methanol/Dichloromethane: ~20 mL

Workup, Isolation, Purification, Concentration

1. Record observations of your solution.
2. Weigh out 2g of sodium bisulfite, add 50 mL water (tap is fine) and shake it to dissolve the solid; then pour this into your reaction mixture from last week.
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 ≥1 minute 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.
14. Assuming the solution flows slowly, you can assist by attaching a vacuum hose and gently vacuuming.
15. Avoid having any of the water layer drain out.
16. Add another 20 mL DCM to rinse the original flask
17. Pour this into the sep funnel, shake, let settle, and again drain the DCM layer through column
18. Repeat steps 16 and 17 (basically you're doing a 3rd extraction with DCM to make sure all of your product is extracted from the aqueous phase.
19. Take 15 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. (They are impurities, and for Mayo testing, I'd rather have lower yields than contaminated products.)
    - 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!
20. 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-colored 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, but 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 collapse back to an oil.
3. During this time, prepare and run an H-NMR on the starting aryl iodide, if you haven't previously.
   - You'll want to be able to compare the NMR for your final product to both the starting material **3** from last week, 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 and record the mass of the flask with the product, and record the mass of product.
   - Subtract the original mass of the flask and stir bar in order to determine 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 the conversion is clean 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 the conversion is clean 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 aryl iodide.
    - 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. Add an additional 5mL of dichloromethane rinse and transfer what's left behind into the 20-mL vial.
3. Write your sample name (**6a** or **6b** or **6c** or whatever) **and** your **student name(s)** on the vial with a sharpie.
4. Place the labelled vial onto the center-table bench.
5. Place an extra copy of your NMR printout (no zooms required) and your GC printout next to the product vials. (I can sort them later.)
   - I'll want to look through the spectra to find the most pure samples, 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 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** → **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. Include your GC-MS for **6**.
7. 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 the reactants converted or not, and whether unreacted starting materials are contaminating your product.
18. 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**.
19. 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.
20. NMR interpretation for the aromatic hydrogens. These will be very different for a para-disubstituted arene versus a meta-disubstituted arene.
    - Para: you should get two 2H doublets. One for ortho and one for meta H's.
    - Meta: You should get 4 unequal H's. One singlet; two doublets; and one triplet. The electron-withdrawing or donating nature of the substituent will impact. Some overlap is well possible.
8. See following page for considerations that will help to understand/process the NMR and GC/MS.

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. Exact identification of each aryl H may be hard.

   - 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 ~5.3 ppm; dioxane in the high 3's, maybe somewhere in the ~3.6-3.8 area?
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 **6l** should have methyl, ethyl, and butyl groups.
   - Samples with para-substitution should have a pair of 2H doublets in the aryl region
   - Meta-substituted aryl groups should be more complex. None of the H's should be identical, and in absence of overlap you might get a singlet, a triplet, and two doublets.
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. 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.