EXPERIMENT 6
DYES & DYEING
PART 1: PREPARATION OF PARA RED AND RELATED AZO DYES
PART 2: GRIGNARD SYNTHESIS OF TRIPHENYLMETHANE DYES: CRYSTAL VIOLET AND MALACHITE GREEN

Reading Assignment: ***Smith*** sections 20.9–20.10, 20.13, 22. 13, 25.13, 25.15, Chapter 18.

Pre-lab Questions, Week 6:

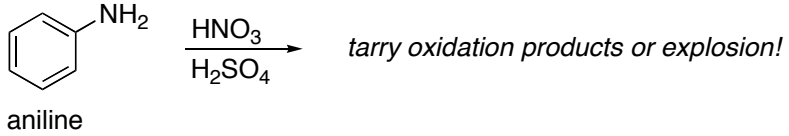
1) What is the role of sulfuric acid in the nitration step?
2) What is the role of the HCl in Part B? (see ***Smith***, section 22.13)
3) Why does addition of NH_4OH at the end of part B result in precipitation of the product, *p*-nitro aniline?

Pre-lab Questions, Week 7:

1) Anhydrous conditions are essential for the success of a Grignard reaction. To illustrate this point, calculate the mass of water it would take to completely destroy the *p*-dimethylaminophenylmagnesium bromide that you will synthesize. What is the volume of this amount of water, and what are the products of this hydrolysis reaction?
2) What is the role of the iodine?

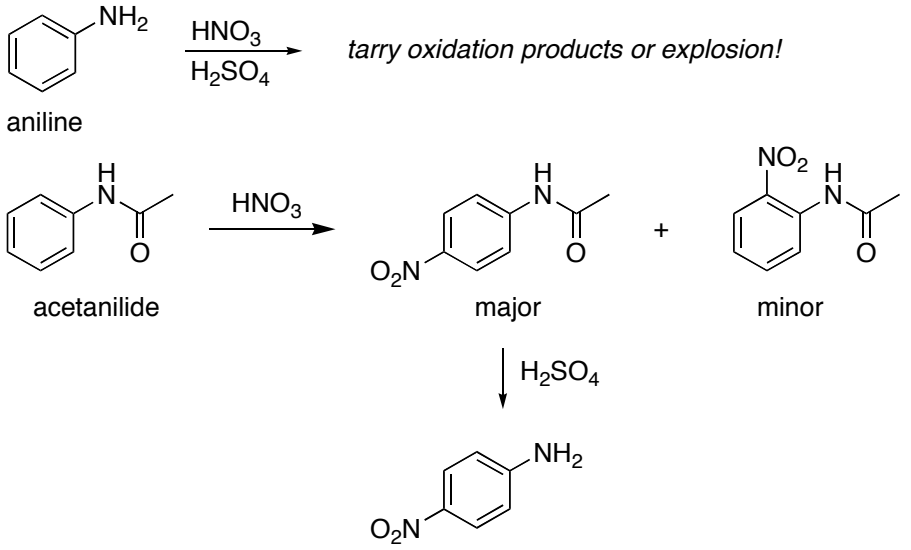
In **Part 1** of this experiment, you will be preparing *p*-nitroaniline, an aromatic amine, by the nitration of acetanilide and subsequent hydrolysis of the nitration product, *p*-nitroacetanilide. You will then diazotize *p*-nitroaniline and use the diazonium salt to synthesize *para* red and other related azo dyes.

A major synthetic route to the aromatic amines is reduction of aromatic nitro compounds, which in turn can be prepared by the nitration of a wide variety of aromatic compounds. Direct nitration of aniline with nitric acid, however, leads to tar-like oxidation by-products, and a possible explosion hazard.

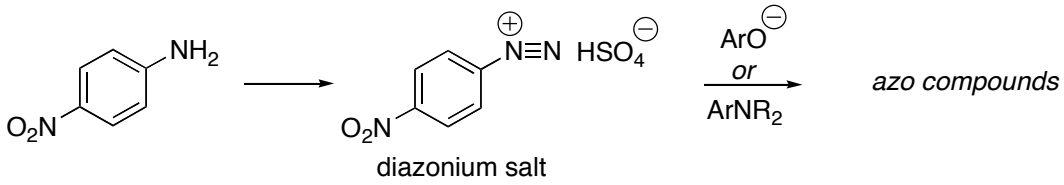


aniline

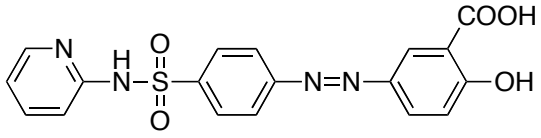
This problem can be avoided by nitrating a less reactive derivative of aniline, acetanilide:



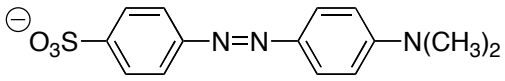
Aromatic amines are important derivatives because they can easily be converted to other derivatives *via* diazonium salts. Diazonium salts are very effective in coupling two aromatic compounds together as azo compounds.



Derivatives of azo compounds are very important as dyes and pharmaceutical agents. Some typical azo compounds:

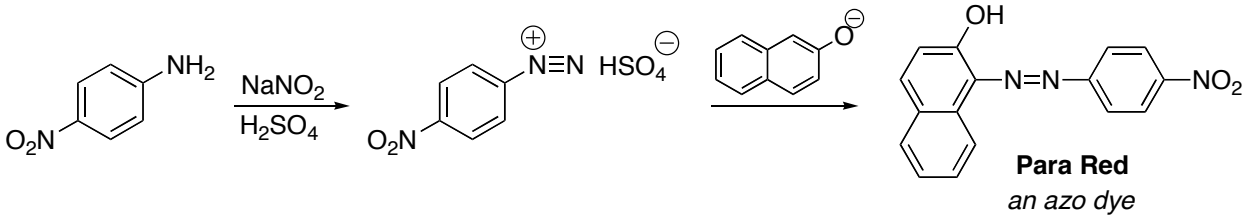


Sulfaslazine
(a sulfa drug used in the treatment of colitis)

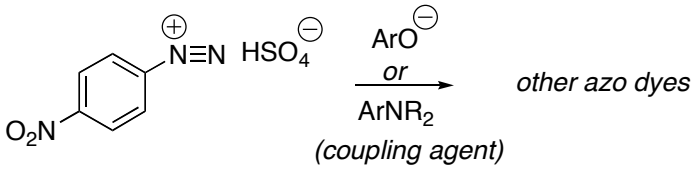


Methyl Orange
(A dye used as an acid-base indicator)

Diazonium salts are formed by the reaction of nitrous acid (HNO_2) with a primary amine. Because nitrous acid is unstable, it is formed in situ by the reaction of sodium nitrite and a strong acid such as hydrochloric or sulfuric acid. When the diazonium salt is treated with the conjugate base of 2-naphthol, the oxygen function on the naphthalene ring strongly activates the 1-position toward electrophilic attack by the positively charged diazonium ion, leading to displacement of the proton.

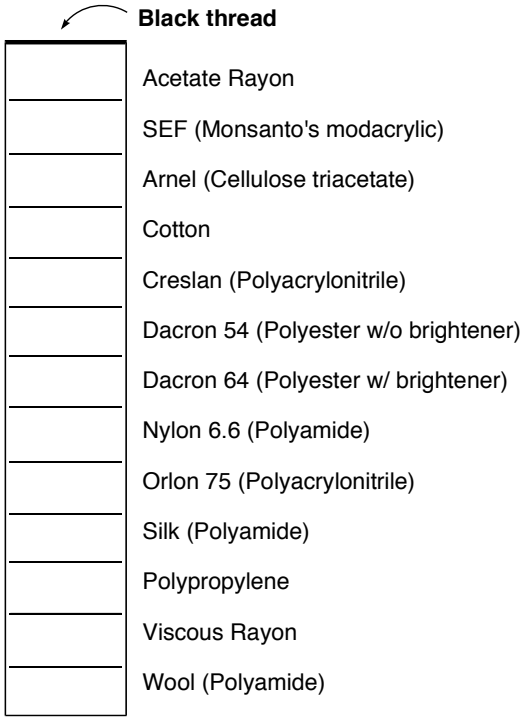


By varying the coupling agent (an activated aromatic compound), different colors of azo dyes can be created:

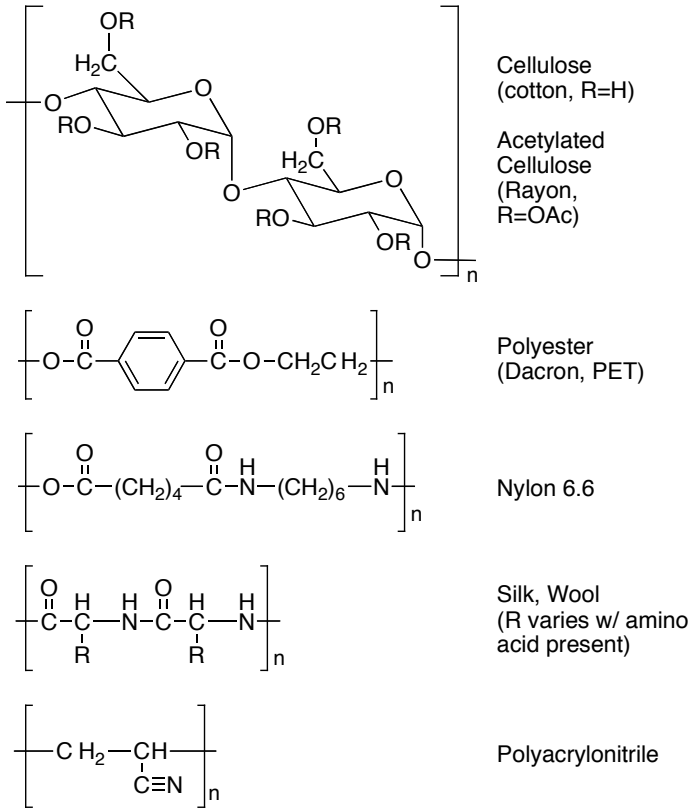


In part **1d** of the experiment, you will be dyeing a multifiber fabric with you synthesized azo dye. Cloth can be dyed by several different processes. In the ***direct process***, the dye is dissolved in water, the solution is heated, and the cloth is immersed in the hot solution. The dye molecules attach themselves to the cloth fibers by direct chemical interactions. In the ***ingrain process***, a dye is synthesized right inside the fiber. The cloth is immersed in a solution of one of the components, allowed to dry, then immersed in a solution of the other component to develop the color. Many azo dyes do not bond well to fibers such as cotton. Using the ***ingrain process***, the reactants can diffuse into the pores of the cloth, but the larger dye molecules are trapped inside the fiber. We will be doing both direct process, and ingrain process dyeing. You will be dying on a special fabric known as Multifiber Fabric 43. It includes 13 different fibers woven into small strips, so that you can see how the dyes take to different fibers. Because the fibers contain different functional groups, there will be a great variation in intensity and hue among the different fibers. You will also see a difference in variation in intensity and hue in your ingrain and directly dyed fabrics.

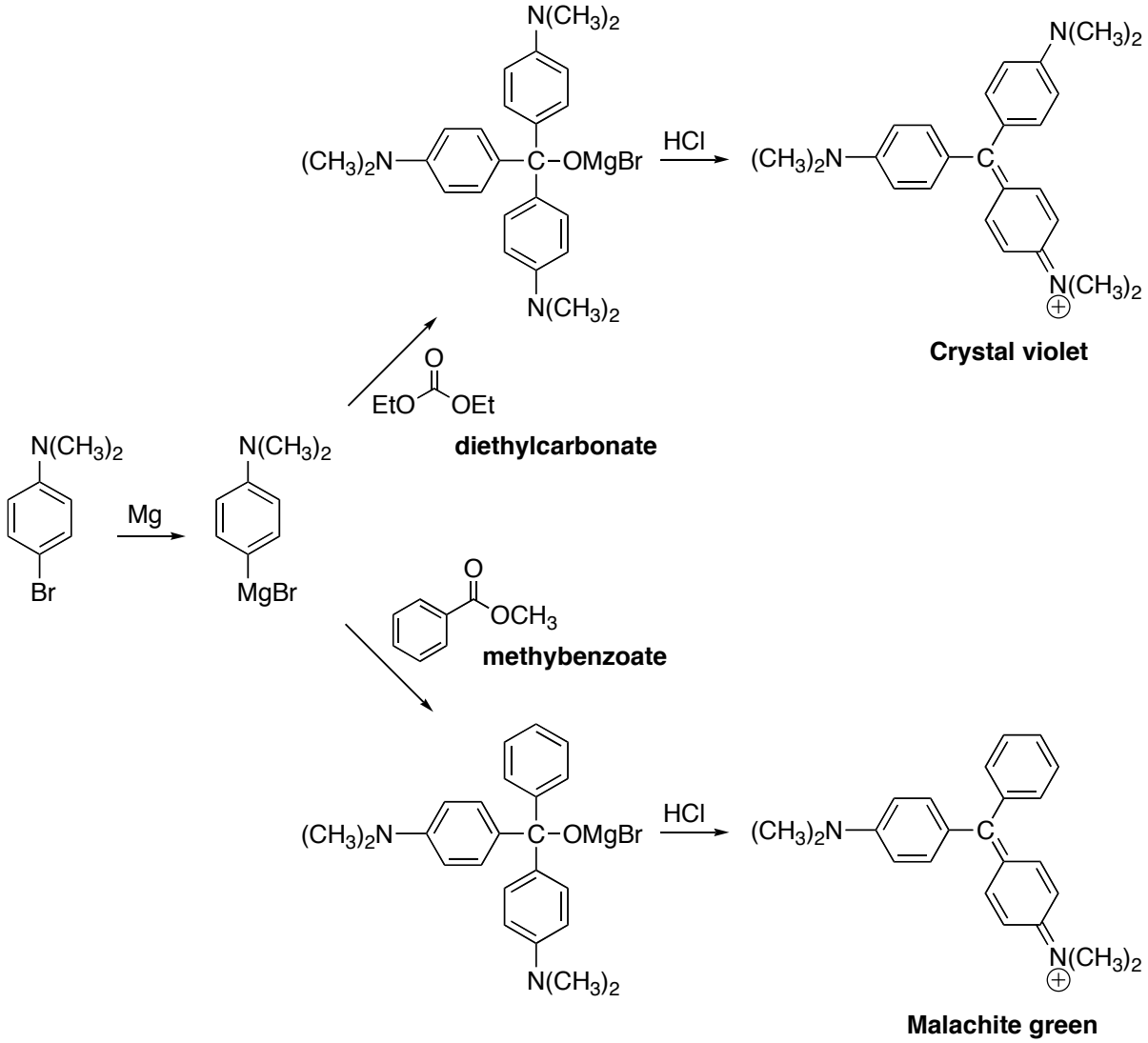
Multifiber Fabric 43



Polymeric Structure for Natural and Man-made Fibers



In **Part 2** of the experiment, you will be using a Grignard synthesis to prepare one of two triphenylmethane dyes, crystal violet, and malachite green. Crystal violet is formed by the Grignard reaction between *p*-dimethylaminophenylmagnesium bromide and diethylcarbonate. Malachite green is formed by treating the same Grignard reagent with methylbenzoate. An important aspect of both malachite green and crystal violet is their extensive conjugation, which is responsible for the colors of these two dyes. The positive charge is extensively delocalized onto all three aromatic rings, and the *para*-dimethylamino groups.



Procedure:

Complete the synthesis and purification of *para*-nitroaniline in **Week 6**. In **Week 7**, set up your Grignard reaction at the beginning of your lab section, and while refluxing, you can begin synthesizing the azo dye, which is made from the *para*-nitroaniline which you synthesized in week 6. All dying will be done **Week 7**. Wear old clothes because **Week 7** is a messy lab! You will choose one coupling agent for your azo dye, ***and*** either malachite green or crystal violet for your triphenylmethane dye. **You will work individually in week 6, and in 2-person teams in week 7!**

All aminobenzenes are potentially toxic.

The MSDS for Acetanilide Contains The Following Information:

- - - - - - - - - - HAZARDS IDENTIFICATION - - - - - - - - -

LABEL PRECAUTIONARY STATEMENTS
TOXIC (USA)
HARMFUL (EU)
HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED.
IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.
IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY (SHOW THE LABEL WHERE POSSIBLE).
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.
AFTER CONTACT WITH SKIN, WASH IMMEDIATELY WITH PLENTY OF WATER.
WEAR SUITABLE PROTECTIVE CLOTHING, GLOVES AND EYE/FACE PROTECTION.

- - - - - - - - - - FIRST-AID MEASURES- - - - - - - - - -

IN CASE OF CONTACT, IMMEDIATELY FLUSH EYES WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES.
IN CASE OF CONTACT, IMMEDIATELY WASH SKIN WITH SOAP AND COPIOUS AMOUNTS OF WATER.
IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN.
IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN.
WASH CONTAMINATED CLOTHING BEFORE REUSE.

All nitrobenzenes are potentially toxic.

The MSDS for *p*-Nitroacetanilide Contains The Following Information:

- - - - - - - - - - HAZARDS IDENTIFICATION - - - - - - - - -

LABEL PRECAUTIONARY STATEMENTS
IRRITANT
IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.
WEAR SUITABLE PROTECTIVE CLOTHING.

- - - - - - - - - - FIRST-AID MEASURES- - - - - - - - - -

IN CASE OF CONTACT, IMMEDIATELY FLUSH EYES WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES.
IN CASE OF CONTACT, IMMEDIATELY WASH SKIN WITH SOAP AND COPIOUS AMOUNTS OF WATER.
IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN.
IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN.
WASH CONTAMINATED CLOTHING BEFORE REUSE.

Week 5:

Part 1a: Synthesis of *p*-nitroacetanilide

Weigh 3.1 grams of acetanilide onto a piece of weighing paper, transfer to a mortar, and grind the acetanilide until it is a fine powder. Weigh 3.00 g (0.0222 mol) of freshly ground acetanilide into a 50 mL Erlenmeyer flask. Add 5.5 mL glacial acetic acid (100% CH_3CO_2H), then carefully pour 5.5 mL of concentrated sulfuric acid (96% H_2SO_4) into the mixture while stirring it with a short stirring rod until the acetanilide dissolves. Clamp the reaction flask in a 250 mL beaker containing a mixture of ice and rock salt. Cool the contents to 5°-10°C. While it is cooling, Prepare a solution of 2.0 mL of concentrated nitric acid (70% HNO_3) and 1.3 mL concentrated sulfuric acid (in that order) in a 13 X100 mm test tube. Carefully stir the acid mixture with a Pasteur pipette, and cool in an ice bath for several minutes. With a Pasteur pipette, add about 1/4 of the acid mixture at a time followed by a three minute interval. Stir after each addition. After the last addition, stir the mixture occasionally, and keep it cold for ten minutes. Remove the reaction vessel from the ice, allow it to slowly reach room temperature, then allow it to stand at room temperature for 5-8 minutes with occasional stirring.

Pour the reaction mixture into 25 mL of cold water with 15 g of ice in a 100 mL beaker. The solid that precipitates is a mixture of *o*- and *p*-nitroacetanilide. Cool in an ice bath at least 5 minutes, then collect the solid by vacuum filtration and wash with three 10-mL portions of ice water. TLC a small portion of the product, using 7:3 Hex:EtOAc. Recrystallize the solid from 95% ethanol to remove any remaining *o*-nitroacetanilide. Do another TLC, spotting your crude and recrystallized product on the same plate. Include a drawing of this TLC plate with your lab report. Determine the m.p. and mass of your recrystallized product. Calculate your percent yield.

Part 1b: Hydrolysis of *p*-nitroacetanilide: Synthesis of *p*-nitroaniline

Transfer the *p*-nitroacetanilide into a 25 mL round-bottom flask. Add 3mL water, 1 mL concentrated hydrochloric acid, and a small boiling stone to the flask. Fit the flask with a condenser, and reflux for 25 minutes. Allow the golden-yellow solution to cool, then cool it thoroughly in an ice bath, and in a hood, add 1 mL of concentrated ammonium hydroxide to precipitate the yellow *p*-nitroaniline. Check with pH paper to be sure the solution is basic. Cool the suspension on ice, filter the yellow product with vacuum filtration, rinse the remaining solid from the flask with a minimum amount of ice water, and allow the crude product to suck dry for at least 5 minutes. Remove the boiling stone, and determine the yield of crude product based on acetanilide. TLC a small portion of the *p*-nitroaniline, spotting *p*-nitroacetanilide on the same plate. Include a drawing of this TLC plate with your lab report

Week 6: *NOTE: Set up Grignard reaction (Part 2)* ***first*** *before preparing azo dyes!*

Part 1c: Preparation of an Azo Dye

You will mix your diazonium salt *(made from your p-nitroaniline)* with one of the following coupling components. Choose one of the following (something different than you neighbor - we want a good variety of colors.)

Coupling Agents

Phenols	**M.W.**	**Amines**	**M.W.**
phenol	*94.1*	*N*-methylaniline	*107.2*
1-naphthol	*144.2*	*N,N*-dimethylaniline	*121.2*
2-naphthol	*144.2*	aniline	*93.1*

Mix 1.38g (10.0 mmole) of *p*-nitroaniline with 8.0 mL of 3*M* HCl *(If you don't have enough p-nitroaniline from week 6, use additional p-nitroaniline provided by your TA.)* Heat the solution gently to dissolve. Cool the solution to 5° C in an ice/water or ice/salt bath with manual or magnetic stirring. The amine salt may precipitate as you cool the solution, but will diazotize satisfactorily if the solution is well stirred. Continue to stir as you add 10 mL of *freshly prepared* 1*M* sodium nitrite at a rate slow enough that the temperature remains below 10°C during the addition. Accurately divide the solution into two equal parts (**a1** and **a2**), keeping both parts cold in an ice water bath. Go to ***step A*** if the coupling component is a phenol, or to ***step B*** if the coupling component is an amine.

Step A. Coupling with a Phenol: Dissolve or suspend 10.0 mmole of the phenol in 20 mL of 1*M* NaOH, and cool the solution in an ice/water bath. (*Take care - avoid contact with the phenol - do not breathe its dust or vapors*.) Accurately divide the solution into two equal parts (**b1** and **b2**). Slowly add diazonium salt **a1** to coupling component solution **b1**, with stirring, and leave the mixture in the ice bath for 15 minutes or more until crystallization is complete (keep solution **a2** cold and save it *and* part **b2** for dying by the ingrain process.) If little or no colored solid appears, adjust pH with dilute HCl or NaOH to induce coupling. Collect the azo dye by vacuum filtration**,** washing it on the filter with cold water.

Step B. Coupling with an Amine: Dissolve or suspend 10.0 mmole of the aromatic amine in 10 mL of 1*M* HCl, and cool the solution in an ice/water bath. Accurately divide the solution into two equal parts (**b1** and **b2**). Slowly add diazonium salt **a1** to coupling component solution **b1**, with stirring, and leave the mixture in the ice bath for 15 minutes or more (keep solution **a2** cold and save it *and* part **b2** for dying by the ingrain process.) Neutralize the solution to litmus with 3*M* aqueous sodium carbonate, then allow it to stand in the cold bath until crystallization is complete. If little or no colored solid appears, adjust pH with dilute HCl or NaOH to induce coupling. Collect the azo dye by vacuum filtration**,** washing it on the filter with cold water.

Part 1d: Dyeing

Dyeing cloth by the Ingrain Process:

Dilute coupling component **b2** to 50 mL with water, and soak a piece of clean cloth in it for 2-3 minutes. Remove the cloth with a pair of stirring rods, blot it between towels to remove most of the water, and hang to dry. Dilute diazonium salt **a1** to 50 mL with **ICE** water, insert the dry cloth, and agitate the solution for a few minutes with a stirring rod to dye the cloth uniformly. If your coupling component was an aromatic amine, dip the cloth briefly into a little 3*M* sodium carbonate solution. Remove the cloth and dry as before. Turn in the labeled dyed cloth with your report.

Dyeing the cloth with Direct Dyeing

Use your dye for direct dying by suspending 0.5 g of the dye in 100 mL hot water and acidifying the mixture with a few drops of concentrated sulfuric acid. Immerse the test strip in the mixture for 5 minutes or more. Remove the cloth, rinse it with water, and let it dry. Adjusting the pH of the dyeing mixture with dilute HCl or NaOH may give better results in some cases. Turn in the labeled dyed cloth with your report, and compare the results obtained with ingrain dying *vs*. direct dying.

Part 2: Grignard Synthesis of Crystal Violet or Malachite Green

The Grignard reagent is *very* water sensitive. All glassware used for this experiment ***must be dry***. Prepare a drying tube filled with anhydrous calcium chloride. Take a 25 mL round bottom flask, magnetic stirring bar, and reflux condenser from the drying oven and assemble your glassware while hot! *The stockroom may have already placed hot glassware into a dessicator and allowed it to cool, in which case, get glassware from the dessicator and assemble quickly*. Place the reflux condenser on the round bottom and the drying tube on top of the reflux condenser and secure with Keck clips. When the apparatus has cooled to room temperature, add 1.25 g of 4-bromo-*N*,*N*-dimethylaniline, 13 mL anhydrous THF, 0.20g magnesium turnings *(break one or two magnesium turnings in half before adding to expose fresh metal surface)*, and 1 or 2 small crystals of iodine. Work quickly so that minimal amounts of air *(which contains a lot of water)* enters the system. After attaching water line to the condenser, swirl the reaction mixture, then heat in a 70° - 75°C water bath. Maintain a gentle reflux for 30 minutes, swirling the flask every 5 minutes during the heating period. The initial dark color fades and is replaced by a grayish solution typical of Grignard reagents. Cool the reaction flask in a beaker of tap water until it reaches room temperature.

For malachite green: Weigh 0.105 g of methyl benzoate into a small Erlenmeyer flask. Add 0.5 mL anhydrous THF to the vial. **For crystal violet:** Weigh 0.15 g diethyl carbonate into a small Erlenmeyer flask. Add 0.5 mL of anhydrous THF to the vial.
Remove the condenser. Using a Pasteur pipette, add the ester solution drop-wise to the reaction flask with stirring. After the addition is complete, replace the condenser and heat the reaction mixture under reflux for 5 minutes. Swirl the flask occasionally while heating it. Cool the flask to room temperature.

Dyeing test samples

Put on gloves before starting the dye synthesis. Pour the reaction mixture into a 100 mL beaker. Slowly add 2.5 mL 5% HCl solution to the beaker with stirring; some bubbling will occur as the residual magnesium reacts with the acid.

Dip a fabric test strip into the dye solution, and leave it in for at least 1 minute. Remove the sample, rinse it with tap water into another beaker, and blot dry. The dye solution is very concentrated, so intense color should be produced on some of the fiber types in the test strip, depending on how well the particular type of fiber accepts the dye. Record the types of fabrics you tested, and describe any variations in intensity observed for the different fibers. Compare you results with those of another student who synthesized the alternative dye. Compare and contrast the results obtained with your azo dye. Do some fibers more readily accept azo dyes than triphenylmethane dyes?

Allow the samples to dry and attach them to your report. Keep them in a small plastic bag because the dye may rub off an anything the samples touch.

Cleanup: the dye solution should be poured into the waste container labeled "Dye Solution." Dye stains on glassware can be removed with a few milliliters of 6M HCl, followed by washing with water. Neutralize the acid washings with sodium carbonate before pouring them into the container for aqueous inorganic waste. DO NOT PLACE CLEANED GLASSWARE BACK INTO DESSICATOR! The stockroom will take care of baking cleaned glassware for the next lab section.

Post-lab Questions, Week 6:

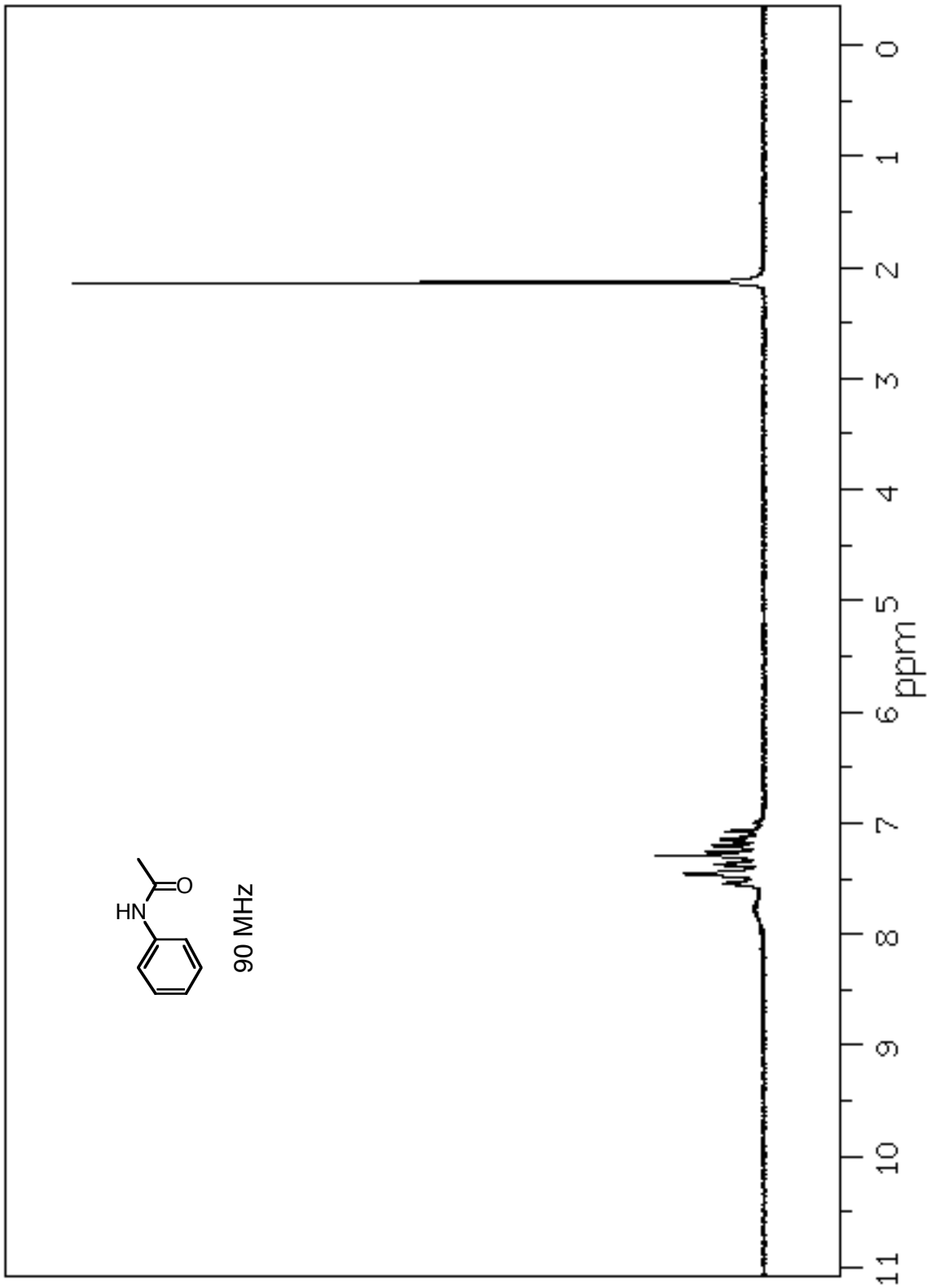
1) Draw the full mechanism for the nitration step. Include all of the important resonance structures that stabilize the intermediate.
2) Why does nitration only occur once (rather than giving di- and/or tri-nitro products?
3) Why not simply use aniline to make 4-nitro aniline? What advantage does the acetyl protecting group give? (there are several)

4) Why is the substitution of the aromatic ring in the nitrated product obvious from inspection of the ^{1}H NMR spectrum? (see page 14)
5) Can IR be used to determine the substitution pattern of the aromatic ring? (assume that the products have never been made before)

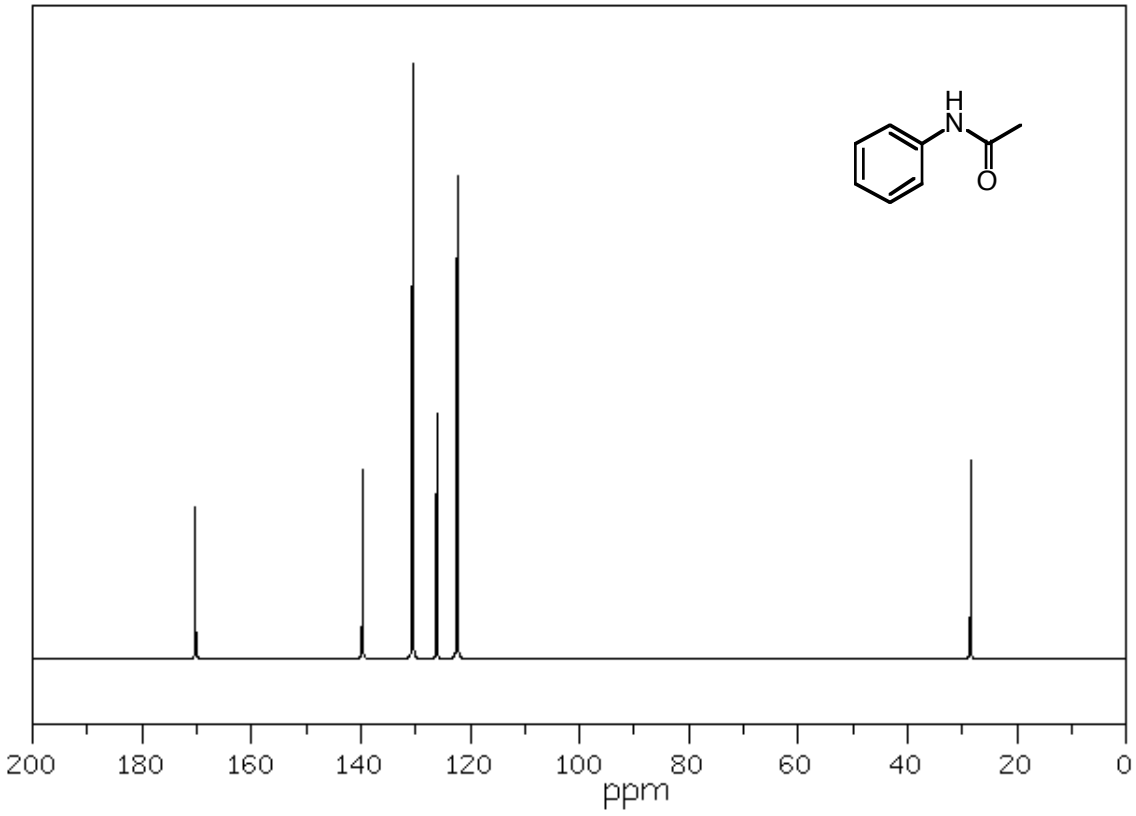
Post-lab Questions, Week 7:

1) Why does it require three moles of the Grignard reagent for each mole of ester in the synthesis of crystal violet, but only two moles of the Grignard reagent are required for each mole of ester in the synthesis of malachite green?
2) What precipitate formed when the ester was added to a solution of your Grignard reagent?
3) Provide a mechanism for formation of an azo dye from your diazonium salt and your coupling agent.

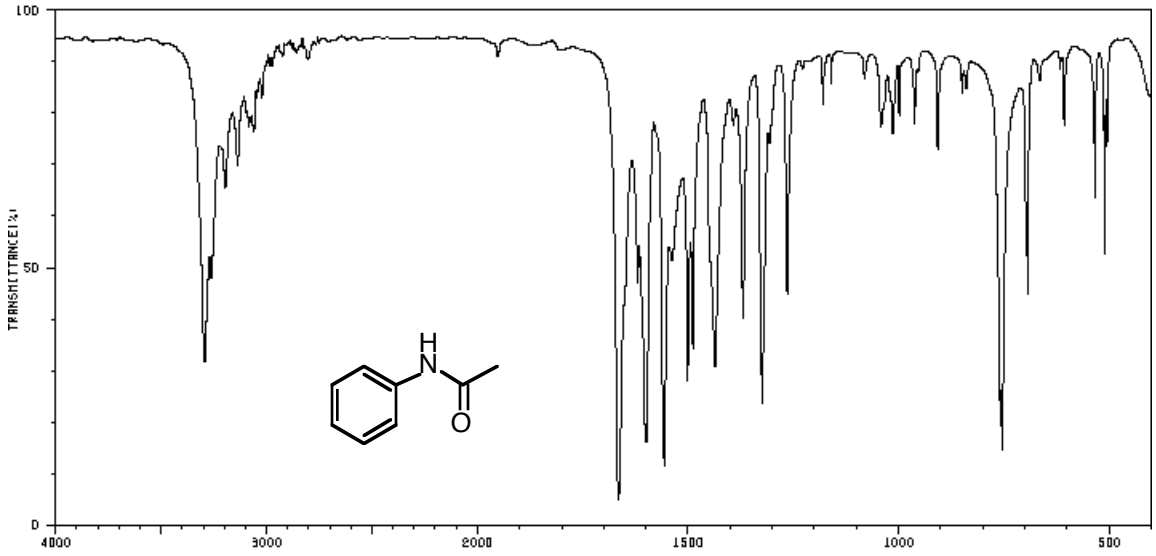
^{1}H NMR Spectrum



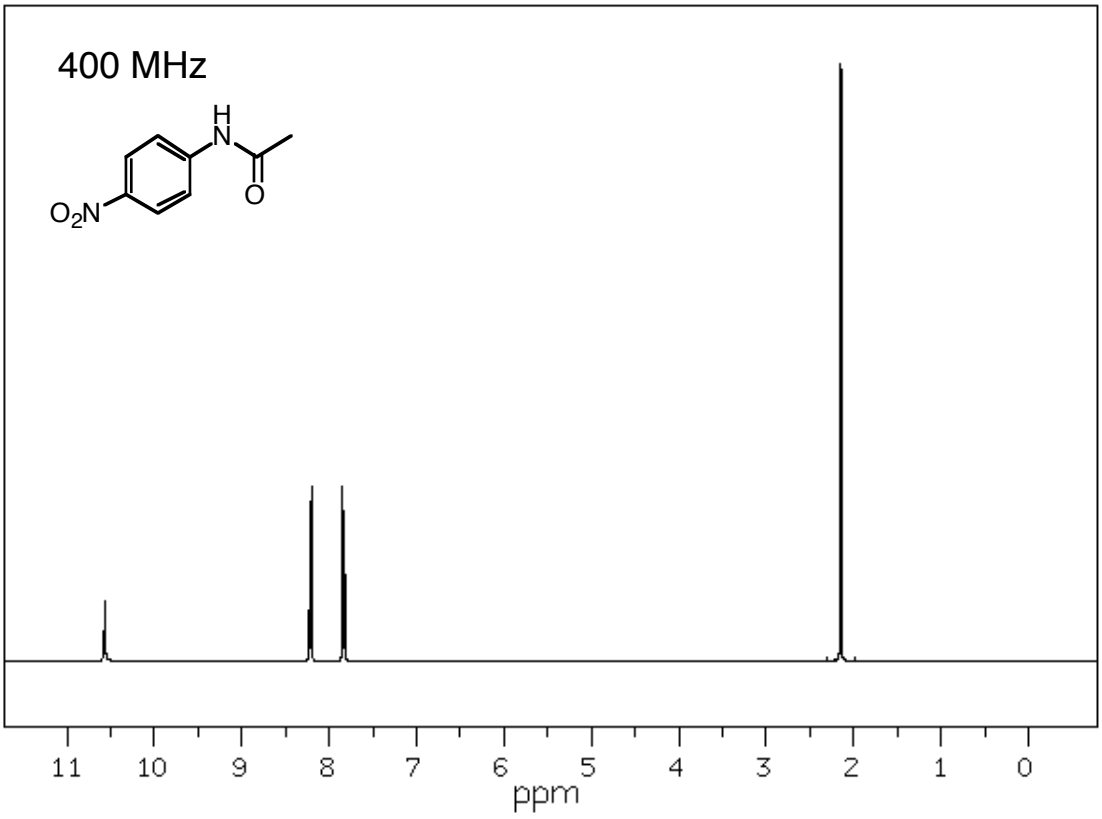
^{13}C NMR Spectrum



Infrared Spectrum



^{1}H NMR Spectrum



Infrared Spectrum