### THE DIELS-ALDER REACTION

## Gilbert, J. C., Martin, S. F., Experimental Organic Chemistry, A Miniscale and Microscale Approach, 5th ed, p. 421- 427

OBJECTIVES This experiment introduces you to pericyclic reactions. The most 'famous' and common pericyclic reaction is the 4+2 cycloaddition also called Diels Alder Reaction. In Particular, you are synthesizing a bicyclic ring system, 4-cyclohexene-cis-1,2-dicarboxylic anhydride by reacting butadiene (prepared *in situ*) with maleic anhydride.

#### BACKGROUND

Certain diene compounds have the ability to react with alkenes or with themselves undergoing a concerted process resulting in the formation of a cyclic compound. The governing factors for the reaction are the reactive nature of the conjugated electronic  $\pi$ systems of the diene and the alkene, which is called a dieneophile. The supporting principles for this process are based upon orbital symmetry of the HOMO (diene) and LUMO (dienophile).<sup>i</sup> Below is a general MO diagram of this experiment. You should consult the textbook to get a more detailed understanding on MO theory and pericyclic reactions.<sup>ii</sup>



HOMO - LUMO orbital interactions

Two important practical considerations to take away from the Diels-Alder reaction are:

1) It can be a useful method in the preparation of cyclic compounds. Compound A is an intermediate toward the synthesis of Taxol, a compound extracted from a Pacific yew tree which is a known drug used for treating breast and ovarian cancers.<sup>iii</sup> Compound B is called Aldrin used as a controversial hard insecticide that has been claimed by the EPA as non biodegradable.<sup>iv</sup>



2) The stereochemistry of products is controlled specifically by the approach of the diene and dienophile during the reaction's transition state.

Abbreviated Reaction Scheme



#### **Experimental Details**

1) We will more closely follow the mini-scale procedure on page 426 of the lab textbook rather than the microscale procedure since the products obtained from the small amounts of reagents in the microscale procedure will not crystallize. Use the glassware setup on p. 426 with the *exception of the Craig drying tube*. Also be sure all glass joints are greased and seated properly.

2) We do need to scale down the amounts of the mini-scale procedure however, since we need the reaction materials to fit into our 10 ml round bottom flasks. So we will place 1.5 g of 3sulfolene, 0.93 g of finely pulverized maleic anhydride (maleic anhydride is often sold in large chunks which have to be crushed with a mortar and pestle or use the tip of scoopla press against a small beaker. If you do need to crush maleic anhydride avoid getting the maleic anhydride on you since it is an irritant and smells bad! We have obtained powdered maleic anhydride however, and you will most likely not need to crush it) and 1ml of xylenes into your 10 ml round bottomed flask. Remember to add the stir bar (not the spin vane). Then set up the reaction for reflux. We will not use a gas trap since we are all working in a hood with ventilation.

3) Your instructor will suggest a heat source for the reaction (most likely the new heat blocks). On the hot plate, place the round bottom flask directly on to a heating block and hot plate, or heating the flask in a heating mantle with some aluminum foil inside the heating mantle to supply even heating.

4) Take care to follow the instructions to heat gently until all of the solids dissolve before refluxing for the  $\frac{1}{2}$  hour. If you heat too rapidly right away you may generate the butadiene which will not be able to react with the maleic anhydride in the solid phase. The butadiene is a gas and therefore, some of the butadiene could escape the reaction flask before the maleic anhydride dissolves. This would lower your product yield significantly.

5) After you begin to reflux your reaction, you may be able to see the vapors of the sulfur dioxide leave the flask. This is ok, but if the evolution of the gas seems to be too rapid, you can lift the reaction assembly away from the heat source. Make sure that you have the reaction assembly clamped in such a way that you can easily lift the reaction assembly up and move it away from the heat source if necessary. Sometimes the solution will stop refluxing and you will have to increase the heat source to start the reflux again. Keep an eye on the reaction assembly during the  $\frac{1}{2}$  hour reflux to make sure the reflux does not stop.

6) After the reflux, crystals of the product usually form. Since this Diels-Alder product is difficult to recrystallize, you can simply cool and filter the crystals you obtained after cooling down the reaction. You may have to cool the reaction mixture before crystals form. Wash the crystals with 1 mL of cold xylenes, then with 2 - 3 mL of petroleum ether. Xylenes have a high bp ~ 136-137 °C so the lower boiling petroleum ether should displace the xylenes from your product and helping the drying process. Spread the crystals to air-dry. You should take the melting point of the Diels-Alder adduct during the same lab period.

7) Weigh the crystals, record the results in your notebook and show them to your instructor.

#### Post Lab

Things you should consider for your discussion section of your report:

- 1. Provide a detailed mechanism including orbitals (this is important for the Diels Alder Reaction!
- 2. Describe chemical requirements for the diene and the dienophile (for example, does either one requires high/low electron density).
- 3. Why did we use 3-sulfolene instead of butadiene?
- 4. If you did use butadiene do you think it would be more efficient than 3-sulfolene with the current lab set up?
- 5. Is there more than one stereochemical or structural isomer product produced from this reaction?

- 6. Why do you think we needed to minimize water content during the reaction?-this one is tricky to answer!
- 7. State the limiting reagent clearly!
- 8. Show us your calculations (equation clearly indicating what the values are) for the product yield. For your calculation please convert and use weights in grams or milligrams and not moles! You <u>do</u> <u>not need</u> to show % error calculations!
- 9. You need to provide the <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra as well as all assignments for the product. You may use the CHEM DRAW program and copy the spectra into your lab report (spectra should NOT take up more than 25% of a page each). However assignment of signals and listing of chemical shifts *must be your own.* You are NOT allowed to use the CHEM DRAW predictor. You may also look up spectra from a reliable resource for example the Aldrich Chemical Company website and cite the source in your report. Make sure your assignments in your report are organized and neat! See example below!
- 10. Format for assigning NMR chemical shifts.



Post Lab Questions

- Do Post Lab Questions from G & M textbook p. 433 434.
- #1 Use no more than 3 short sentences to answer. An accompanied diagram is OK but not required!
- # 4- Structure only
- # 8- Structures only

<sup>ii</sup>Jones, Jr, M., Fleming, S. A., Organic Chemistry, 4th ed, p. 544 – 553.

<sup>&</sup>lt;sup>i</sup>Gilbert, J. C., Martin, S. F., Experimental Organic Chemistry, A Miniscale and Microscale Approach, 5th ed, p. 421 – 424.

<sup>&</sup>lt;sup>iii</sup>Nicolaou, K. and coworkers, J. Am. Chem. Soc. 117:624 (1995)

<sup>&</sup>lt;sup>iv</sup> Solomons, G., Fryhle, C., Organic Chemistry, 7<sup>th</sup> ed, p. 615.

### **MULTISTEP PREPARATION OF 4-BROMO-2-CHLOROANILINE**

## Gilbert, J. C.; Martin, S. F. Experimental Organic Chemistry: A Miniscale and Microscale Approach, 5th edition, Cengage Learning, 2011, p. 726.

OBJECTIVES The syntheses of organic compounds of use in everyday lives, such as drugs, pesticides, polymers, insecticides, or additives for fragrances, foods, cosmetics, adhesives, detergents, typically start from simple materials and involve numerous chemical steps. They require careful planning and optimization of the overall process. Over the next three weeks, you will prepare a complex product using a multistep sequence. In particular, you are carrying out the multistep synthesis of 4-bromo-2-chloroaniline, starting from aniline. For these experiments, the product of one step is the starting material for the next step. If you are to be successful, you must have good experimental technique: you have to strive to obtain each intermediate product with good purity and yield. In general it is good practice to keep a small sample of each intermediate of a synthesis, for purposes of characterization by spectroscopic and/or physical methods (Save around 100 mg of each intermediate product).

#### BACKGROUND

As you know from the previous semester, the reaction of alkenes and alkynes with electrophiles is typified by addition products. In sharp contrast to this, aromatic compounds undergo substitution reactions in the presence of electrophiles. Typically, aromatic compounds undergo processes in which a hydrogen atom on the ring is replaced by a particular atom or functional group.

You will be carrying out reactions in which functional groups are introduced onto an aromatic ring. You will have to use your knowledge of the effects that existing substituents on the ring have on the rates and on the regiochemistry of the substitution reactions.

Study the theory of aromatic substitution (Chapter 14 in Maitland Jones' book, p. 623).<sup>i</sup> Read the relevant sections of Chapter 21 of "experimental Organic Chemistry" by Gilbert and Martin. Start at page 703.<sup>ii</sup> The synthesis for this lab is described starting in section 21.3, page 726. Notice that you will carry out the synthesis until (and including) the preparation of 4-bromo-2chloroaniline. The discussion for the preparation of acetanilide from aniline is found in the previous section (section 21.2).

#### Acetanilide and 4-bromoacetanilide (1 lab period)

The amine functional group in aniline is electron donating. It activates the ring toward electrophilic substitution. It is strongly ortho/para directing. However, it can lead to problems: a) multiple substitution; b) since it is strongly basic it can be protonated, or complexed with Lewis acids, resulting in an ammonium group that deactivates the ring toward electrophilic substitution; and c) electrophiles can react directly with the amino group. To avoid these problems the amino group is protected by acetylation (formation of an amide) at the beginning of the synthesis (Scheme 1).

The acetyl group is electron withdrawing, and it causes the formed amide to be less electron donating than the free amine. The amide group is still ortho/para directing but less reactive than aniline and multiple substitutions are much less likely to occur. Due to the steric hindrance of the acetanilide group, substitution at the ortho position is inhibited and the major product of substitution will be para.



## Scheme 1. Multistep synthesis of 4-bromo-2-chloroaniline starting from aniline

Acetanilide is obtained by acetylation from aniline according to Scheme 2. Aniline is first solubilized in the aqueous phase by reacting it with HCl and thus converting it to its hydrochloride salt. Acetic anhydride and sodium acetate are then added. The HCl/AcONa mixture forms a buffer that allows the amine to be kept in solution as the ammonium salt but allows this salt to be in equilibrium with a small amount of the free amine. The free amine reacts rapidly with the acetic anhydride to form the amide in a good yield. This is a high yield general method for the acetylation of amines.



#### Scheme 2. Acetylation of aniline

The acetamido group of acetanilide is activating, i.e. selective mono-bromination can occur under mild conditions, for example the use of bromine in acetic acid without a Lewis acid catalyst. The acetamido group is ortho/para directing, but due to its steric bulk the formation of the 4-bromo product is strongly favored.

Ortho products usually have different physical properties than the para products (mainly due to different packing behavior and potential dipoles etc.). Para products are more symmetrical; they tend to be relatively high melting solids, whereas ortho products tend to have lower melting points, and are often liquids.

#### 4-Bromo-2-chloroacetanilide (1 lab period)

4-Bromoacetanilide is slightly less reactive than acetanilide toward further electrophilic substitution. Still, it is sufficiently reactive to be mono-chlorinated by chlorine dissolved in acetic acid (Scheme 3). Notice that the formation of 4-bromo-2chloroacetanilide rather than 4-bromo-3-chloroacetanilide is proof that the acetamido group is much more strongly ortho/para directing than the bromo substituent.



Scheme 3. Formation of 4-Bromo-2-chloroacetanilide

Chlorine will be generated *in situ* rather than using dangerous chlorine gas, by reacting concentrated HCl with sodium chlorate. This is an oxidation/reduction process where a chloride ion is oxidized to chlorine gas and chlorate ion is reduced to chlorine gas (Scheme 4). You should balance the chemical equations in Scheme 4 as an exercise. This is an easy and safe way to generate precise amounts of molecular chlorine.

$$\begin{aligned} \mathrm{Cl}^{-1} & \rightarrow \frac{1}{2} \mathrm{Cl}_2 + \mathrm{e}^{-1} & \text{oxidation} \\ 6 \mathrm{H}^+ + \mathrm{ClO_3}^{-1} + 5 \mathrm{e}^{-1} & \rightarrow \frac{1}{2} \mathrm{Cl}_2 + 3 \mathrm{H}_2 \mathrm{O} & \text{reduction} \end{aligned}$$

Scheme 4. Generation of chlorine from HCl/ chlorate.

#### 4-Bromo-2-chloroaniline (1 lab period)

In this last step of the synthesis you will remove the acetyl protecting group and generate the free amine (mechanism shown in Scheme 5). The reaction is the hydrolysis of an amide.



Scheme 5. Mechanism of hydrolysis of the amide

#### **EXPERIMENTAL DETAILS**

#### 1) Acetanilide. Miniscale (Gilbert & Martin, p. 713)

SAFETY: Acetic anhydride is a lachrymator. Transfer in a hood. Do not inhale the vapors.

Apparatus: 250-mL Erlenmeyer flask, thermometer, ice-water bath, hot plate/ magnetic stirrer, vacuum filtration.

Place 100 mL of 0.4 M hydrochloric acid and a stirbar in the 250 mL Erlenmeyer flask. Add 3.6 mL of aniline. Stir the mixture and warm it to about 50 °C. Prepare a solution of sodium acetate trihydrate (6.0 g) in 20 mL of water. In a separate container measure out 4.4 mL of acetic anhydride. Add the Ac<sub>2</sub>O in one portion to the warm solution of anilinium hydrochloride with vigorous stirring (magnetic bar). Without stopping the stirring, add the solution of AcONa immediately and in one portion (You must add the AcONa within 30 sec. Each minute you lose could reduce your product yield by as much as 30%). Cool the reaction mixture to 5 °C in an ice-water bath. Keep stirring until the crystalline product completely precipitates. Collect the acetanilide by vacuum filtration. Wash it with a small portion of ice-cold water and

air-dry it. Do NOT recrystallize the product. Do not cover the product. Leave it open until you do the next step.

Calculate the percent yield of the acetanilide from aniline. Save a small amount (0.1g) for a melting point.

Densities: aniline: 1.02 g/mL; acetic anhydride: 1.08 g/mL.

If you are going to use the acetanilide for making pbromoacetanilide on the same day, remember that you will not have totally dry acetanilide. Calculate the theoretical yield of acetanilide from aniline. You CANNOT have made more than the theoretical yield even if your damp weight indicates otherwise, i.e. you cannot create matter out of nothing. So when scaling the reaction take the theoretical yield into account.

Scaling of the reaction. Calculations: divide the amount of starting material you have by the amount indicated in the text to get a factor. Then multiply all other quantities by this factor to get the appropriate new quantities. Example: Let's say the book asks for 8.1 g of acetanilide, and you have 4.5 g of acetanilide. Divide 4.5 by 8.1, this gives you a factor of 0.55. Then multiply all other quantities in the procedure by 0.55 to get the new amounts needed.

#### 2) 4-Bromoacetanilide. Miniscale (Gilbert & Martin, p. 732)

SAFETY: You must wear gloves when handling bromine! Bromine is highly toxic and can cause severe burns! Make sure you are reading the MSDS before handling bromine.

Do not leave beakers of bromine open on the counter! Cover all containers with Bromine in it until you are ready to use it. Do not take more bromine than the amount you will actually use. Put excess bromine in the bromine waste bottle. Do NOT pour bromine down the drain. Do NOT use acetone at all in this experiment. Do not use it to rinse your glassware. We do not want to produce the lachrymator  $\alpha$ -bromoacetone, formed from acetone and bromine. Wear gloves when handling glacial acetic acid.

Apparatus: A 250 mL Erlenmeyer flask, hot plate/ magnetic stirrer, vacuum filtration.

The bromine in glacial acetic acid solution (3.2 mL in 6 mL) is already prepared for you: use 9.2 mL of this solution for 8.1 g of acetanilide. Remember to scale down the amounts depending on the amount of acetanilide you have. If your acetanilide is wet remember you cannot have more than the theoretical amount of acetanilide (around 5.33 g).

Dissolve 8.1 g of acetanilide in 30 mL of glacial acetic acid in the Erlenmeyer flask containing a stirbar. Add the bromine solution to the rapidly stirred solution of acetanilide over a period of 1-2 min. Stir the mixture for about 10 min after completion of the addition. Slowly add 100 mL of ice-cold water with stirring. Add just enough ice-cold saturated aqueous sodium bisulfite to discharge the color of the mixture. Cool the mixture in an ice-water bath and collect the product by vacuum filtration. If the crude product appears yellow, wash it with aqueous sodium bisulfite. In any case, wash it well with cold water and press it as dry as possible on the filter. Do not recrystallize.

Weigh the product, calculate its yield, determine its Mp.

## 3) 4-Bromo-2-chloroacetanilide. Miniscale (Gilbert & Martin, p. 733)

SAFETY: Since you generate chlorine gas, keep the hood door as closed as possible. Methanol is flammable; use the steam cone NOT the hot plate for boiling it during the recrystallization of the final product. Wear gloves when handling concentrated hydrochloric and acetic acids (corrosive liquids). Thoroughly wash with water any areas of your skin that may come in contact with them.

Apparatus: A 250 mL Erlenmeyer flask, hot plate/ magnetic stirrer, thermometer, vacuum filtration.

Scale down the amounts as needed.

Suspend 10.7 g of 4-bromoacetanilide in a stirred solution of 23 mL of concentrated hydrochloric acid and 28 mL of glacial acetic acid in the Erlenmeyer flask. Heat the mixture gently while stirring until it becomes homogeneous. Cool the solution to 0 °C (make sure it is about 0 °C, check with a thermometer before adding the sodium chlorate solution. A warm solution or adding the sodium chlorate too fast [see below] can ruin the experiment and you will have to start over!). Prepare a solution of 2.8 g of sodium chlorate in 7 mL of water. Add the sodium chlorate solution SLOWLY over a period of 5 minutes to the stirred cold mixture; some chlorine gas should evolve. After the completion of the addition, stir the reaction mixture at room temperature for 1 hr and collect the precipitate by vacuum filtration. Wash the crude product thoroughly with ice-cold water until the washes are neutral. The product may be recrystallized from methanol.

Weigh the product and calculate its yield. Determine its melting point.

## 4) 4-Bromo-2-chloroaniline. Miniscale (Gilbert & Martin, p. 734)

SAFETY: HCl and 14N NaOH are corrosive and dangerous. Wear gloves when handling them. Thoroughly wash with water any areas of your skin that may come in contact with them. If your fingers or hands feel slippery at all after handling 14N NaOH, wash with lots of water until the slippery feeling goes away.

Apparatus: In the following procedure the amounts indicated in *Gilbert & Martin* (miniscale) where scaled down to 1/10. Use the glassware from your micro kit: a 10 mL round-bottom flask, condenser, hot plate/ magnetic stirrer, vacuum filtration.

Combine 1.12 g of crude 4-bromo-2-chloroacetanilide, 2 mL of 95% EtOH, and 1.3 mL of concentrated hydrochloric acid in the round-bottom flask. Add a stirbar and assemble the reflux apparatus.

Reflux the mixture for 0.5 hr. Combine with 9 mL of hot water. swirl the mixture to dissolve any solids completely. Pour the hot solution onto about 15 g of ice contained in a beaker. Add a stir bar. Slowly add 1.2 mL of 14N sodium hydroxide to the mixture, while stirring well. Add more ice if it has melted before the addition is complete. Check the pH of the mixture to insure that it is basic. If it is not, add more aqueous base. Collect the solid by vacuum filtration, wash it thoroughly with ice-cold water, and press it as dry as possible on the filter. You have to recrystallize the final product. Dissolve it in boiling methanol (use a steam cone, not the hot plate). Allow the solution to cool to below 40 °C and add cold water dropwise until crystallization barely begins (Should the product begin to oil out instead of crystallizing, add a few drops of methanol to redissolve the oil). Cool the mixture in an ice-water bath and collect the product by vacuum filtration. Wash the product with a small volume of ice-cold 1:1 methanol/ water.

Calculate the percent yield and take the melting point of the final product. Hand in to your instructor the recrystallized 4-bromo-2-chloroaniline in a vial.

#### 5) Analysis of the Products

Save around 100 mg of each product to take a melting point and for TLC analysis.

The melting points of each product are as follows:

- Acetanilide 114<sup>0</sup>C
- 4-bromoacetanilide 168<sup>0</sup>C

- 4-bromo-2-chloroacetanilide 151-152<sup>o</sup>C
- 4-bromo-2-chloroaniline 72<sup>o</sup>C

#### TLC Analysis:

- Use silica gel coated plates. Suggested solvent systems: dichloromethane/ methanol 9/1, or ethyl acetate.
- We have authentic samples of aniline, acetanilide, and 4-bromoacetanilide. Make dilute solutions of all the samples in dichloromethane, except for 4bromoacetanilide: here use EtOH and warm the mixture up. You can spot your acetanilide product against aniline and the authentic acetanilide.
- You can spot your 4-bromoacetanilide product against authentic samples of acetanilide and 4-bromo acetanilide.
- Although we do not have authentic 4-bromo-2chloroacetanilide samples, you can spot your 4-bromo-2-chloroacetanilide product against authentic 4bromoacetanilide to make sure the reaction took place.
- Besides running a TLC for 4-bromo-2-chloroaniline, compare the melting point of this product with the melting point of 4-bromo-2-chloroacetanilide. The great drop in melting point value from 151 °C to 72 °C is a good indication that the reaction took place.

#### POST LAB

Prepare lab reports for each of the labs. Things you should consider for your post-lab report:

1. Indicate what differences you would see in the IR and NMR spectra of the starting material and the product for each reactions.

2. For each lab period, make sure you provide detailed mechanisms of each step.

3. Discuss ortho/para versus meta directors and what the scientific reason for the directing character of a functional group is.

4. Show us your calculations (equation clearly indicating what the values are) for the product yield. For your calculation please convert and use weights in grams or milligrams and not moles! You <u>do</u> **not need** to show % error calculations!

5. You need to provide the <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra as well as all assignments for the product. You may use the CHEM DRAW program and copy the spectra into your lab report (spectra should NOT take up more than 25% of a page each). However assignment of signals and listing of chemical shifts *must be your own*. You are NOT allowed to use the CHEM DRAW predictor. Make sure your assignments in your report are organized and neat!

6. Discuss the TLC analysis in detail. Make sure you understand WHY some compound/product/starting material runs FASTER/Slower than others.

7. Why do you have to cool some reactions with ice (for example, the last step requires ice cooling).

#### POST LAB QUESTIONS

(Do not forget to write down the questions in your report before answering them).

- Acetanilide and 4-bromoacetanilide: p. 720, part B. 19, 20, 22, 25. p. 740, part B. 8, 14 (NMR only).
- **4-Bromo-2-chloroacetanilide:** p. 741, part C. 18, 21 (NMR only).
- 4-Bromo-2-chloroaniline: p. 741, part D. 22, 27.

<sup>i</sup> Jones, Jr., M.; Fleming, S. A. Organic Chemistry, 4th Edition, W. W. Norton & Co, 2010, ISBN 978-0-393-93149.
<sup>ii</sup> Gilbert, J. C.; Martin, S. F. Experimental Organic Chemistry: A Miniscale and Microscale Approach, 5th edition, Cengage Learning, 2011, ISBN 1439049149.

#### OBJECTIVES

•Synthesis of 2-allylphenol from allyl phenyl ether via a Claisen rearrangement

- •Learning the chemical transformation and mechanism of the Claisen rearrangement
- •Acid/base extraction techniques to isolate the product

•TLC to analyze the progress of the reaction and the success of the purification steps

•Characterization of the isolated final product by TLC, IR and <sup>1</sup>H NMR

#### INTRODUCTION

The Claisen rearrangement is a unimolecular reaction that converts an allyl vinyl ether into an γ,δ-unsaturated carbonyl compound. It was discovered in 1912 by the German chemist Ludwig Claisen and its mechanism elucidated in the 1960s. One fundamental feature of this reaction is that it results in the formation of a new carbon-carbon sigma bond. Due to the importance of carbon-carbon bond forming reactions in organic synthesis, the Claisen reaction has found extensive utility in natural products synthesis as well as in pharmaceutical drug discovery. For example, a variant of the classical Claisen rearrangement, the Johnson-Claisen, was used in Danishefsky's synthesis of the neurotrophic agent Merrilactone A (a neurotrophic agent promotes the growth of neurons and therefore has applications in neurodegenerative diseases like Alzheimer's and Parkinson's). Follow the bonds highlighted in blue in the intermediate in brackets to the product in Scheme 2.



allyl vinyl ether γ,δ-unsaturated carbonyl

Sheme 1. The Claisen rearrangement



## Scheme 2. The Claisen rearrangemnt in Danishefsky's synthesis of Merrilactone A

The Claisen rearrangement is an example of a [3,3]sigmatropic rearrangement. The term [3,3] refers to the numbering system of sigmatropic rearrangements. It can be derived by identifying the bond being broken in the reaction and numbering the atoms on each side of this bond until the atoms where the new bond forms is reached. A sigmatropic rearrangement proceeds through a cyclic transition state and involves the breaking and forming of sigma and pi bonds. In the case of the Claisen rearrangement, the reaction proceeds through a six-membered transition state and involves breaking the sigma bond between oxygen and carbon 1 and forming a sigma bond between carbon 3 and carbon 3 along with rearrangement of the pi system. As shown in the mechanism below, the transformation occurs via the simultaneous movement of 3 pairs of electrons much like the Diels-Alder reaction in Experiment 1.

 $\sigma$  bond broken



Scheme 3. Numbering and mechanism of the Claisen rearrangement

In this lab we will use the Claisen rearrangement to transform phenyl allyl ether (1) into 2-allyl phenol (2). The phenol results from the tautomerization of the initial product of the Claisen rearrangement to restore aromaticity as shown below. (While you've learned that keto-enol tautomerization usually favors the ketone, in this case the restoration of aromaticity provides a driving force for the formation of the enol).



Scheme 4. Synthesis of 2-allyl phenol from phenyl allyl ether

The transformation of **1** to **2** requires 6 hours of heating at reflux to reach completion. Due to time constraints, we will only reflux the reaction mixture for 2 hours. This will result in incomplete conversion of the starting material to product. Therefore, in order to isolate the product of the reaction it will be necessary to separate the product from unreacted starting material. Fortunately, this is easily done via an organic/aqueous base extraction. The product of the reaction is a phenol, which bears a proton acidic enough to be deprotonated by NaOH. The deprotonated phenol is soluble in the aqueous layer whereas the starting phenyl allyl ether does not react with NaOH and is only soluble in the organic layer. Treatment of the aqueous layer with HCl and extraction/separation with diethyl ether furnishes the neutral final product.

#### **EXPERIMENTAL SECTION**

SAFETY: HCl and 14N NaOH are corrosive and dangerous. Wear gloves when handling them. Thoroughly wash with water any areas of your skin that may come in contact with them. If your fingers or hands feel slippery at all after handling 14N NaOH, wash with lots of water until the slippery feeling goes away.

Apparatus: Magnetic stirrer/hot plate. From the micro kit: the 5 mL reaction vial, reflux condenser and spin vein.

Use 3 mL of allyl phenyl ether for the Claisen rearrangement. Put the starting material in a 5 mL reaction vial with a spin vein and equip with a condenser for reflux using a hot plate as a heat source. Heat the allyl phenyl ether to reflux and react for 2 hours. Most hot plates will require close to the maximum heat setting to reach reflux. While this reaction requires heating at reflux temperatures to proceed at a reasonable rate, you will also want to be careful not to heat the reaction too vigorously as this will result in evaporation of starting material and thus reduced yield of product.

At the end of 2 hours, the solution becomes dark brown. Remove the heat and allow the reaction to cool to room temperature. While the solution cools, prepare the solvent for TLC. Take one drop of the reaction mixture and dilute with 1 mL of ethyl acetate in a screw top vial. Save this sample for TLC analysis at the end of the experiment.

#### **Isolation Of The Product**

The product of the reaction will be separated from the starting material via organic/aqueous extractions. This process is relatively complex compared to extractions done in previous labs. Therefore, it is necessary that you fully understand what is happening chemically at each of the extraction/washing steps and their purpose. COME TO LAB WITH A FLOWCHART IN YOUR NOTEBOOK DETAILING EACH OF THESE STEPS. As a precaution against accidently throwing out a fraction containing your product, we strongly advise you to keep every aqueous and organic fraction until you are certain that you do not need them (in other words, when you have isolated the final product).

Add the reaction mixture to the 125mL separatory funnel (making sure first, that the separatory funnel is in the closed position). Then rinse the reaction vial with 10 mL of hexane and pour that into the separatory funnel. Next add 20 mL of 2 M NaOH into the separatory funnel. Shake the separatory funnel carefully, then remove and keep the aqueous layer. Remove the organic layer from the separatory funnel. Replace the aqueous layer back into the separatory funnel and wash with two 5.0 mL portions of hexane. The desired product will be in the aqueous layer so **KEEP THE AQUEOUS FRACTION.** 

Add 6M HCl to the aqueous fraction until it is acidic to reprotonate the 2-allylphenol product. You can roughly calculate how much 6M HCl you will need based on the volume of 2M NaOH used. Monitor with pH paper. (A visual clue: when the 2allylphenol reforms from its anion in the acidic solution, the solution becomes an oily white mixture)

Add 20 mL of diethyl ether to the acidic solution and transfer the mixture to a separatory funnel. Shake and allow the layers to separate. Your product will be in the organic layer. Drain out the aqueous layer. Pour the organic layer into a flask and dry with anhydrous sodium sulfate. Decant the solution into a weighed Erlenmeyer flask and use the steam cone to remove most of the ether. Stop heating when the boiling subsides. Weigh your product. Take one drop of the final product and dilute with 1 mL of ethyl acetate in a screw top vial. Save this sample for TLC analysis.

#### Analysis Of The Product

The success of the reaction and isolation procedure will be determined by TLC and the final product characterized by IR and <sup>1</sup>H NMR. Using capillary tubes, spot a TLC place with the starting material, the diluted reaction mixture that you saved, the diluted sample of the final product, and a diluted sample of authentic product (the starting material and authentic samples are already made up for TLC spotting). Develop the TLC plate in a beaker containing 5:1 hexane/ethyl acetate TLC solvent system (we will provide you with this solution). Since the starting material and the product both absorb ultraviolet light, you can use the uv light to anaylize the developed TLC plate. Compare the TLC traces of the reaction mixture before extraction with the TLC of the reaction mixture after extraction. Your lab instructor will select one sample from your section for analysis by IR spectroscopy. <sup>1</sup>H NMR spectra of the starting material and the product will be posted on Blackboard.

#### LAB REPORT

Things you should consider for your lab report:

1. Provide a detailed mechanism of the transformation of phenyl allyl ether to 2-allyl phenol.

2. Provide a flow chart describing the separation procedures used to isolate the final product.

3. Show your calculations for the product yield assuming the reaction only goes to 33% conversion (ie you only get 0.33 times the theoretical yield). You do NOT need to show % error calculations!

4. Indicate what differences you would see in the TLC and the IR & <sup>1</sup>H NMR spectra of the starting material and the product.

5. Discuss the TLC analysis in detail. Make sure you understand why the starting material and product have different  $R_f$ 's. Include a picture of your developed TLC plate with your report. The TLC plate tool in ChemDraw is useful for this.

6. Assign the most diagnostic absorbance in the IR spectrum of your product.

7. Assign the signals in the <sup>1</sup>H NMR spectrum of the product to the hydrogens in 2-allyl phenol. Make sure your assignments in your report are organized and neat!

#### Post Lab Questions

1. The "all carbon variant" of the Claisen reaction is called the Cope rearrangement (see Jones Ch. 20.7 for further discussion of the Cope). It is depicted below. Using curved arrows provide a mechanism for this transformation.



2. If both ortho positions of the allyl phenol are blocked with methyl groups a rearrangement still occurs, but the product of the rearrangement is the *p*-allylphenol. Draw a mechanism accounting for this transformation and explain why the reaction takes a different course in this case. (Hints: Follow the movement of the labeled carbon (\*) in this reaction. The mechanism you learned in Post Lab Question 1 provides additional clues)



#### REFERENCES

- 1. Sanford, E.M.; Lis, C.C.; McPherson, N.R. J. Chem. Ed. 2009, 86, 1422.
- 2. Danishefsky, S.J. et al. J. Am. Chem. Soc. 2002, 124, 2080.
- 3. Jones, Jr, M., Fleming, S. A., Organic Chemistry, 4th ed.

### THE WITTIG REACTION

## Gilbert, J. C., Martin, S. F., Experimental Organic Chemistry, A Miniscale and Microscale Approach, 5th ed, p. 601-605

OBJECTIVES This experiment is the preparation of ethyl trans-cinnamate from benzaldehyde and (carbethoxymethylene)triphenylphosphorane. The synthesis involves a demonstration of a Wittig reaction a useful tool in linking two compounds through formation of a double bond.

#### BACKGROUND

The Wittig reaction is a very important method for introducing double bonds into a molecule through a carbonyl functional group (either aldehyde or ketone). The second component of the Wittig reaction is a compound intermediate known as an ylide which is comprised of phosphonium group and an R group. The R group is the functionality that you will transfer to your carbonyl compound. It is this type of reaction that is key in the arsenal of an organic chemist since connecting molecules via carbon-carbon bonds is not trivial. The ylide is typically prepared from triphenylphosphine and an alkyl halide forming the alkyl phosphonium salt via an  $S_N 2$  reaction (Scheme 1). The hydrogens on the CH<sub>2</sub> group are acidic enough from the inductive positive charge on the phosphorus that they are readily deprotonated with a strong base such as BuLi. Even KOH can be used in certain situations. Notice that the ylide can be written in two resonance forms (Scheme 1). Phosphorus has access to d orbitals that can allow it to have 10 shared valence electrons around the nucleus.

#### Scheme 1. Formation of the Wittig reagent.



For your experiment the ylide is stable enough that it is commercially available and you do not need to prepare it. However you should familiarize yourself with the preparation and applications.

Examples of actual Wittig reactions used in organic synthesis are described in Scheme 2. The first example is the olefination of an aldehyde leading to a product intermediate for the synthesis of  $\alpha$ -cuparenone (A),<sup>1</sup> a terpenoid natural product. This reaction has been published by one of our clinical faculty members. The other example is the synthesis of fluprostenol (B),<sup>2</sup> belonging to a well known class of compounds termed prostaglandins. Fluprostenol (B) can be used as a drug for treating glaucoma patients and terminating unwanted pregnancies in animals and humans.

#### Scheme 2. Examples of Wittig reactions in the literature.







#### **EXPERIMENTAL DETAILS**

1. In a 3 ml conical vial with a magnetic spin vane, add benzaldehyde (0.5 mmol). Since benzaldehyde (MW= 106.1, density: 1.045 g/ml) is in a small quantity you may weigh it using a scale (it is 53 mg) or via the use of a micropipette if they are available (take a volume 50  $\mu$ l). If you weigh it, tare the weight of vial and spine vane before adding benzaldehyde. Next weigh 201 mg (0.57 mmol) of (carbethoxymethylene)triphenylphosphorane on weighing paper and transfer it into vial. The benzaldehyde is a reagent as well as serving as the solvent.

2. Stir the mixture for 25 minutes at room temperature. Add hexanes (3 ml) and continue to stir for 5 more minutes. Note: The mixture has a paste consistency and may be difficult to stir. It is recommended not to raise the stirring speed too high. Use a wooden stick to help unclog spin vane if there is a problem! Be patient, it does work!

3. To isolate the product from the solid you need to build a filter using a Pasteur pipette and a small piece of cotton ball. Using a wooden stick, push a small wad of cotton through the wide barrel of pipette filling about  $\frac{1}{2}$  inch in length, it does <u>not</u> have to be exact! If possible, clamp the pipette to a ring stand and underneath it have a 50 or 100 ml pre-weighed beaker for product recovery. Using a clean pipette remove the solution portion from your reaction mixture and run it through the top of your filtering pipette. During the extraction step it is OK if some of the solids are picked up, that is what the cotton wad is for. You may use additional hexanes (2 – 3 ml) to rinse out the conical vial. Be sure to run this through the filter pipette. You may add this to the rest of the original solution. 4. Now you should run the TLC before removing the solvent using the steam cone or water bath heated on a hot plate. You will perform two TLC's for your product with two different solvent systems. On each plate you will run the product standard along with your reaction. You will evaluate your TLC run with your instructor and submit your sample after removing the solvent.

TLC mobile phases:

Hexanes/ethyl acetate: 90/10

Hexanes/ethyl acetate: 95/5

5. Carefully evaporate the solvent with the steam cone or hot water bath. Leave on the steam cone or hot water bath until the boiling subsides. There will be only a small amount of liquid product left after the hexanes has evaporated. The product should smell like cinnamon.

6. Weigh the product and calculate the yield for your lab report write up. You should have a discussion about your TLC runs.

#### DISCUSSION AND POST LAB

Things you should consider for your lab report:

1. Include standard reagents table and clearly state the limiting reagent.

2. Include in your discussion the evaluation of your product by TLC.

3. As part of your write up you need to write out a step-by-step mechanism on how the ylide, (carbethoxymeth-ylene)triphenylphosphorane, reacts with benzaldehyde leading to the trans isomer of the product.

4. Let us assume you took an NMR spectrum of your product and assuming the major product is the trans isomer. How would you distinguish between the two stereoisomers using this (NMR spectroscopy) information? You may use the Chemdraw program for obtaining spectra or you can look them up elsewhere. However, the chemical shift assignments must be your own interpretation. Be as detailed and specific with your answers.

#### POST LAB QUESTIONS

Do Post Lab Questions from G & M textbook p. 612

- #2 Use no more than 2 short sentences to answer.
- # 3 Use no more than 2 short sentences to answer.
- #8

#### **R**EFERENCES

1. Kwok, T. J.; Wink, D.J.; Organometallics, **1993**, *12*, 1954. 2. Lednicer, D. Strategies for Organic Drug Synthesis and Design; Wiley Interscience: New York, 1998, p. 7

### SYNTHESIS OF AN ESTER

#### **O**BJECTIVES

• To Design and carry out a synthesis of an ester from a carboxvlic acid and an alcohol.

• To learn the chemical transformation and mechanism of the Fischer esterification.

#### INTRODUCTION

Often an organic chemist will not have at his or her disposal an exact detailed experimental procedure for the synthesis of a chemical compound. Instead the organic chemist must rely on general procedures for the desired transformation along with intuition and laboratory know-how. In this experiment, you will use such tools to design and carry out a synthesis of an ester of your choice.

Esters all have the general structure R–CO–OR'. Esters are common organic synthesis and biological materials. For example, most fats and oils are the fatty acid esters of glycerol. Volatile esters play an important role in determining the flavor and fragrance of fruit. Due to these properties esters are used extensively in the flavor and fragrance industry. Some esters and their odors are listed below.



Figure 1. Naturally occurring esters.

Ester	Fragrance	Ester	Fragrance
n-propyl acetate	pear	isobutyl propanoate	floral
isopenyl acetate	banana	propyl propanoate	pineapple
methyl salicylate	wintergreen	methyl benzoate	prune
benzyl acetate	peach	geranyl acetate	rose
butyl proponate	balsamic	3-hexyl propanoate	green apple
isobutyl propanoate	rum	ethyl propanate	fruity
n-octyl acetate	orange	ethyl benzoate	fruit
methyl anthranilate	grape	ethyl heptanoate	fruity
<b>T</b> 11 4 0			

Table 1. Some esters and their fragrances

One of the most common methods for preparing an ester is the Fischer-Spier esterification. The Fischer esterification is the acidcatalyzed synthesis of an ester from an alcohol and a carboxylic acid. It was first reported by the great German chemist Emil Fischer in 1895. The general scheme for this reaction is shown below (see Jones p. 841 for a detailed mechanism).



#### Scheme 1. The Fischer esterification.

To determine what alcohol and carboxylic acid you need in order to synthesize your ester, you have to know how to name an ester. In an ester's name, the first word is the name of the group derived from the alcohol. The second word is the name of the acid, where "-ic" has been dropped and "oate" (or "ate") has been added. For example, methyl anthranilate is synthesized from methanol (methyl) and anthranilic acid (anthranilate).



Scheme 2. Example of ester nomenclature.

#### EXPERIMENTAL SECTION

SAFETY:  $H_2SO_4$  and 1.5N NaOH are corrosive and dangerous. Wear gloves when handling them. Thoroughly wash with water any areas of your skin that may come in contact with them. If your fingers or hands feel slippery at all after handling 14N NaOH, wash with lots of water until the slippery feeling goes away.

Apparatus: Magnetic stirrer/hot plate and aluminum heating block. From the micro kit: the 10 mL round bottom flask, reflux condenser and spin vein.

A list of the alcohols and carboxylic acids that are available for use in the synthesis will be posted on Blackboard. Once you determine which ester you want to synthesize, you will need to devise the procedure for synthesizing approximately 20 mmol of the ester. We will provide you with a worksheet that will guide you through the process of designing a laboratory procedure. **PREPARE THE WORKSHEET BEFORE COMING TO LAB.** Your instructor must check your experimental procedure before you can begin the experiment.

Before writing your procedure, consider the following:

1. In this reaction, the acid and alcohol reactants are in equilibrium with the ester product (implied by the double arrow). In other words, once you reach equilibrium, there could potentially be a large amount of starting material remaining. This situation would result in a low yield of the ester. To resolve this problem, you can make use of Le Chatelier's principle. One of the consequences of Le Chatelier's principle is that we can drive the equilibrium to the right (to the products) by having one of the reactants in excess (in effect, putting pressure on the left side). Usually, a threefold molar excess of one of the reagents is sufficient to drive the equilibrium far to the right in an esterification reaction.

2. Note that sulfuric acid ( $H_2SO_4$ ) is written over the equilibrium arrow. This notation means that sulfuric acid is used as a catalyst for this reaction; it will accelerate the rate at which the product is formed. Because a catalyst is not consumed during the course of a reaction, you need to use only a small amount of acid (3 or 4 drops) for it to be effective.

3. Heating a reaction is another method used to increase the rate of the reaction. When heating a reaction it is necessary to make sure neither the starting materials nor the product evaporate from the reaction mixture. Use of a reflux condenser allows for heating without allowing the reagents or the product to evaporate.

4. Your product will need to be purified at the end of the reaction. Remember that you are adding one of the reagents in excess. You must consider how to remove the excess starting material. Most esters are not water-soluble. If your excess reagent is water soluble, you can separate it from your product by "washing" the reaction mixture with water. Place the cooled reaction mixture in a separatory funnel with approximately 10mL of ether. Add water to the funnel and shake gently. The excess water-soluble reagent will be in the water layer and separated from the ester. If neither reagent is water-soluble you should add excess acid. The carboxylic acid can easily be converted into a water-soluble carboxylate ion by washing with aqueous NaOH instead of with water.

5. Once you have developed a synthesis, create a reagent table for your reaction. For this you need to find out the structures, physical properties, amount used, etc, of all your reagents and products. This table will give you information on the reflux temperature (what components has the lowest boiling point), how to separate your product from excess reagents (what are the solubilities), and so on.

#### Build off of the following general procedure. This procedure will vary depending on what ester you are synthesizing and what starting material you are using in excess!

a. You will choose an ester to synthesize. Looking at your ester data sheet, add the required amount of the alcohol and carboxylic acid needed for your ester synthesis into a 10mL round bottomed flask. Add 3 or 4 drops of concentrated sulfuric acid, add a few boiling chips and set up the apparatus for a reflux assembly.

b. Start to heat your set-up and start timing when the solution in the round bottom flask begins to boil. (the solution is said to be refluxing when it is boiling) Allow the solution to boil for 15 minutes.

c. Cool the solution to room temperature, then add 20mL of diethyl ether to the round bottomed flask, and carefully add this solution to your 125mL separatory funnel. Add 10 mL of 1.5N NaOH to the separatory funnel. Carefully shake and remove the aqueous layer leaving the organic solution in the separatory funnel.

d. At this point the procedure varies slightly depending which reagent you used in excess. Either wash twice with 15 mL of water or once with 15 mL of 1.5 N AoH.

e. Pour the organic layer into a flask and dry with anhydrous sodium sulfate. Decant the solution into a weighed beaker and use the steam cone to evaporate the ether. Stop heating when the boiling subsides (stops bubbling). Weigh your product.

Once the lab is complete, be sure to note the fragrance of your synthesized ester as well as the fragrances of the esters synthesized by your classmates. You'll want to make sure you've evaporated off all of the ether, as ether is pretty unpleasant to inhale! It often helps to add water and ice (add about a half cup of ice and roughly 20mL of water to the beaker and stir) to the ester to enhance the detection of the ester's odor.

#### LAB REPORT

Things you should consider for your lab report:

1. Provide a detailed mechanism of the transformation of the carboxylic acid and alcohol to an ester.

2. Provide a full detailed procedure. Explain your reasoning for designing the keys steps of the procedure. Your procedure should be clear enough that any randomly chosen person trained in organic chemistry could repeat your experiment.

3. Provide a flow chart describing the separation procedures used to isolate the final product.

4. Show your calculations for the product yield. You do NOT need to show % error calculations!

5. Propose how you could use either <sup>1</sup>H NMR or IR to determine whether or not you prepared your product. Think about the differences you would see in the <sup>1</sup>H NMR and IR spectra of the starting materials and the product.

#### Post Lab Questions

Do Post Lab Questions from Gilbert & Martin, p. 658.

#5 parts a. and b.

#7

#### **References**

Jones, Jr, M.; Fleming, S.A., Organic Chemistry, 4th ed.
Gilbert, J.C.; Martin, S.F., Experimental Organic Chemistry, 5<sup>th</sup> ed.

## THE ALDOL CONDENSATION REACTION AND CHARACTERIZATION OF UNKNOWN ALDEHYDES AND KETONES

## Gilbert, J. C: Martin, S. F. Experimental Organic Chemistry: A Miniscale and Microscale Approach. 5<sup>th</sup> Edition, Cengage Learning, 2011. p 620.

Jones, M.; Fleming, S. A. Organic Chemistry, 4th ed. Norton Publishers; New York. 2011. p 965.

OBJECTIVES: To characterize an unknown aldehyde and ketone using a variety of chemical reactions and characterization techniques. To synthesize a crossed aldol condensation product using an unknown aldehyde and ketone. To obtain and utilize <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopy to determine the original aldehyde and ketone that were utilized to synthesize the aldol condensation product. To understand the mechanism for an aldol condensation and how the starting materials affect the products formed in the reaction. Finally, to understand why specific reagents and reaction conditions are utilized for the aldol condensation reaction.

#### TIME ALLOCATED: TWO LAB PERIODS

The first period will be used to complete the synthetic reactions and the second to complete all the characterization tests that are required.

#### Reaction Scheme

Aldol Condensation



Scheme 1. General reaction equation for an aldol condensation

#### Background Aldol Condensation

In a carbonyl compound, the carbon that is adjacent to the carbonyl group (C=O) is known as the  $\alpha$ -carbon and as a result the hydrogens that are bonded to this carbon are known as  $\alpha$ -hydrogens.  $\alpha$ -Hydrogens of ketones, aldehydes and esters are more acidic than those in alkanes and alkenes. This is a result of (1) the electron-withdrawing inductive effect of the carbonyl group and (2) that the negative charge present in a deprotonated ketone, aldehyde or ester (known as an enolate anion, Figure 1) is stabilized through resonance delocalization. In organic synthesis, enolate anions are important nucleophiles that are utilized to form new carbon-carbon bonds.





When aldehyde or ketone containing compounds with acidic  $\alpha$ hydrogens are treated with a strong base (ex. NaOH, NaOEt) an enolate anion can be formed readily. This enolate anion, which is a nucleophile, can then add to another carbonyl group resulting in the formation of a  $\beta$ -hydroxyaldehyde or  $\beta$ -hydroxyketone (Scheme 1). This condensation reaction is known as an aldol reaction. Once the  $\beta$ -hydroxyaldehyde or  $\beta$ -hydroxyketone product is formed they can be dehydrated, under the same conditions as the initial aldol reaction, to form an  $\alpha$ ,  $\beta$ -unsaturated aldehyde or ketone (i.e. carbon-carbon double bond in conjugation with a carbonyl group) (Scheme 1).

The initial base-catalyzed aldol reaction is highly reversible and therefore a limited amount of the aldol product is formed at equilibrium. However, the equilibrium constant for the formation of the  $\alpha$ ,  $\beta$ -unsaturated aldehyde or ketone is considerable larger, and therefore under vigorous reaction conditions that can promote further dehydration, high yields of the  $\alpha$ ,  $\beta$ -unsaturated aldehyde or ketone can be achieved.

In this experiment you will complete a crossed aldol reaction, in which you will be given two unknowns. One of these unknowns will be an aldehyde and the other will be a ketone, thus resulting in the formation of a crossed aldol condensation product. For a crossed aldol condensation experiment to be successful to produce significant final yields, one of the two reactants should have no  $\alpha$ -hydrogens, thereby preventing self-condensation of either the aldehyde or ketone.

In this experiment as "chemists" you will encounter a variety of experimental issues that you will need to solve in order to complete the synthesis and determine the structure of your final aldol product and the unknown aldehyde and ketone starting materials.

#### Experimental

Lab Period 1: Aldol Condensation Reactions

In this reaction you will determine the structure of your unknown aldehyde and ketone, by utilizing an aldol condensation reaction. The experimental details are described below as "Aldol Condensation Procedure" and were developed from the Gilbert and Martin text (pp. 620) and other aldol reaction procedures. The samples that you synthesize and collect from these experiments will be analyzed using <sup>1</sup>H NMR, and <sup>13</sup>C NMR spectroscopies. You will be provided with two unknowns, "Unknown A and Unknown B", you will not know which is the aldehyde and which is the ketone. The steps below will guide you in evaluating your unknowns so that you can attempt to correctly identify them using the lists provided.

The general experimental procedure for an aldol reaction is outlined below. This procedure is

Aldol Condensation Procedure 1:

 Prepare a sodium hydroxide solution by dissolving 0.1 g of sodium hydroxide in 1 mL of water in a large test tube. You may need to heat the mixture slightly to allow for dissolution, but remember to cool the solution back to room temperature before you proceed with the experiment (Be sure to calculate the molarity of this solution). To this NaOH solution add an additional 3 mL of 95% ethanol.

- 2) To the large test tube with the sodium hydroxide (at room temperature) solution, place 1 mL or 1 g of Unknown A and 1 mL or 1 g of Unknown B. Gentle shake the test tube to dissolve the reactants.
- Leave the reaction to sit at room temperature for approximately 30 minutes swirling the reaction mixture every few minutes.
- 4) Dilute the reaction mixture with 15 mL of water and stir thoroughly to break up any lumps, this also encourages the product to form as a solid. Once the reaction is well mixed cool on ice for 10-15 min (it may require longer depending on unknown approx 30 min) and collect the crystals by suction filtration.

<u>Note</u>: Different aldehydes and ketones react with a wide range of speeds, and you may need to adjust your conditions in order to optimize the formation of a product. As the product separates from solution color and milkiness may appear. Eventually after time course crystals may develop. Be sure to record all observations and changes you made to induce crystal formation.

Sometime the product will form as an oily mixture (usually a result of excess NaOH) and there are several techniques that can be used to promote a complete solid product including:

- Freezing the mixture on an ice bath for longer than 10 to 15 min, usually 30 min.
- Warming the mixture to 40 C and then adding enough ethanol to allow the oil to dissolve. Often when it cools crystals will form.
- Finally try to extract the mixture with methylene chloride and wash 3 times repeatedly with water to remove the excess NaOH, dry with sodium sulfate and evaporate off the methylene chloride on a steam bath (a residue should result). If you still get oil, find your final weight and submit it to your clinical in a vial.
- 5) Transfer the collected crystals into a beaker, add approximately 30 mL of water and stir them thoroughly, then collect the crystals by suction filtration again.
- 6) Test the final drop of the filtrate with litmus paper. If the filtrate is still basic, continue washing with water until it is neutral. Be sure to dry your sample as much as possible.
- 7) Set aside a small amount of the crude crystals in a collection vial for TLC analysis and melting point, which will be completed in lab period 2.
- 8) Transfer the dried, crude product to a small, dry Erlenmeyer and recrystallize. This depends on your particular product and yield. One method is to use some ethyl acetate or ethanol and warm on a steam cone to boiling.

<u>Note:</u> If the solution is cloudy this indicates that water is present. To remove the water, add an additional 5 mL of ethyl acetate or ethanol, then add a small amount of anhydrous sodium sulfate. Let stand for 2-3 min. At this point remove the anhydrous sodium sulfate using gravity filtration and concentrate your solution back to the original amount of ethyl acetate and finish the recrystallization. 9) Collect your product, determine the final mass/percent yield and set aside a small amount for TLC and melting point analysis which will both be completed in lab period 2. Submit the remainder of your purified product to your TA for NMR.

<u>Note:</u> At the end of the first lab period you must provide your adjunct with your sample from the aldol condensation reaction so that the NMR analyses can be completed.

Lab Periods 1 and 2: Characterization

Since you are being provided with two unknowns (Unknown A and Unknown B), this experiment also involves several different chemical techniques that will be used to further characterize your unknown aldehyde and ketone.

<u>NMR</u>: A <sup>1</sup>H NMR and <sup>13</sup>C NMR will be completed and returned to you during the second lab period. After analyzing and assigning the NMRs you will be able to use this information to help you figure out the original aldehyde and ketone that created that aldol condensation product.

<u>TLC:</u> Since all students will have different unknowns you will need to determine a suitable solvent to dissolve your aldol condensation product, a suitable TLC eluting solvent system and which method should be used to visualize your TLC plates (staining, UV, etc.). In lab you will be provided with a variety of solvents to do this, but it is up to you to determine and explain your optimum TLC conditions.

General TLC Procedures:

- Prepare a dilute solution of your purified crystals using a small amount of the purified product you set aside from week 1 in a small test tube. You will only need to add two to three drops of your chosen solvent to the crystals to prepare this solution.
- Spot each of your samples (Procedure 1, Procedure 1 crude, Unknown A and Unknown B) onto your TLC plate.
- 3) Elute each plate with your chosen solvent system
- 4) Visualize and calculate the respective  $R_f$  for all spots

<u>Physical constants:</u> The melting point of a solid, the boiling point/freezing of a liquid and the density can be determined

General Physical Constants Procedures:

Melting point of a solid, or boiling point of a liquid, sometimes you can get the freezing point of a compound. If you have a high boiling liquid (over 200C°) you might try to get a freezing point by cooling the liquid in an ice or ice-salt bath. If you have a low melting solid, sometimes it's easier to melt the solid, and allow it to recrystallize measuring the temperature while it crystallizes to get the freezing point. Keep in mind that although this is rare, sometimes a high boiling liquid might be listed as a low melting solid and a low melting solid could be listed as a high boiling liquid.

Boiling points should be taken by distilling the unknown sample. To check this result you can check the boiling point by adding about 1 inch of the unknown in a large test tube, add a boiling stone, and then clamp the test tube into a heating mantle attached to a variable power supply. When the liquid is vigorously boiling, insert your thermometer, and wait for the temperature to equilibrate. Check this boiling point with that of the distillation process. Although this test tube process for finding the boiling point may not be as accurate as the distillation method, it is rarely way off from the literature value since the thermometer is actually in the liquid.

Taking the density of a solid is not practical, and is too imprecise to be of any use. The density of a liquid sample should be more accurate, but may not be of much use here since we are using similar compounds, whose density may not vary much. The easiest way to get the density is to weigh an empty 10mL graduated cylinder, then add as much liquid unknown as possible (up to 10mL would be best). Now re-weigh and you have the weight per volume, or density.

<u>Solubility Tests</u>: Can provide you with some useful information about your unknown.

Solubility Test Procedures:

If your compound dissolves in water, it is probably a lower molecular weight compound, or one that has additional polar functional groups. Remember that both carboxylic acids and phenols are both soluble in dilute NaOH (1.5M). However in general, only carboxylic acids are soluble in the weaker base and sodium carbonate, phenols are generally not soluble in sodium carbonate. Compounds that are soluble in dilute HCl (1.5M) usually contain the amine functional group.

<u>Classification Tests</u>: We will look at various classification tests to determine the presence of functional groups. We will first study the classification tests pertaining to our particular unknown; aldehydes and ketones, and we will briefly look at classification tests for some other functional groups (alcohols, alkenes, alkyl halides, nitro compounds, amines, etc.).

Classification Test Procedures:

- 1) Tests for aldehydes:
  - a. Tollens test (p 795 & 859)

 $\text{RCHO} + 2\text{Ag}(\text{NH}_3)_2^+ + 2\text{OH}^- \rightarrow 2\text{Ag} + \text{RCO}_2^- + \text{NH}_4^+ + \text{H}_2\text{O}$ 

b. Chromic Acid Test (p 860 & 877)

> Sometimes both the Tollens and Chromic Acid Test may give a false positive test with ketones. Always take the strongest, or the faster positive test as the aldehyde.

- 2) Tests for Methyl Ketones (iodoform p 862): When doing this test, continue to add enough iodine reagent until either the solution forms a yellow precipitate, or until the decolorization stops and the solution turns brownish with no precipitate. You may need much more than 3mL or iodine for this.
- 3) Test for Alkenes
  - a. Bromine in dichloromethane (p 867)bromine decolorizes (red-orange) when it reacts with alkenes. (colorless).
  - b. Baeyer test-alkenes (p 868) in the presence of permanganate (which is purple) will react to form diols, leaving the resulting manganese dioxide brown.
  - c. Note that your aldol products may contain double bonds.
- 4) Test for alkyl halides

Note that both the silver nitrate test (p 869), and the sodium iodine in acetone test (p 870) will produce precipitates with the appropriate alkyl halides. Please remember that aryl halides will not give a positive test with these reagents.

- 5) Test for Phenols
  - a. Ferric chloride test (p 884)-phenolic compounds in the presence of ferric chloride generally produce colored complexes (ex. Red, blue, purple).
  - b. Note the general solubility of phenols in dilute sodium hydroxide, but NOT sodium carbonate.
- 6) Test for carboxylic acids: The only test we have available to us is the solubility test. Where generally carboxylic acids are soluble in sodium carbonate, where phenols are not.
- 7) Test for amines: The only test we have available for amines is the solubility test. Most amines are soluble in dilute HCl.
- 8) Test for nitro compounds: In the ferric hydroxide test (p 895) for nitro compounds, ferrous hydroxide (blue) is added to the nitro compound, the nitro compound is reduced to an amine, causing oxidation of the ferrous hydroxide, producing ferric hydroxide which is brown, and usually a brown precipitate forms.

Report

You only have to hand in ONE lab report for both lab periods. This report should be 8 (eight) pages long NOT just four. It will be worth 30 points NOT 15.

In your report there are several important components that must be included. Please use the following as a guideline on how to write your report.

- Provide the structure and IUPAC name of your unknown aldehyde and ketone
- Provide the percent yield for the aldol condensation product (you will only be able to determine this once you identify the unknown aldehyde and ketone)
- Provide a clear discussion describing your logic on how you determined/decided on your particular unknown aldehyde and ketone from the list provided and the data that you collected.
- Construct a concise table with all the experimental data that you collected for the unknowns (product melting points, R<sub>f</sub> values, physical constants, solubility tests classification tests, etc)
- Draw and assign the structure of your aldol condensation product from the <sup>1</sup>H NMR and <sup>13</sup>C NMR analysis
- Discuss and provide the mechanism for the aldol condensation reaction
- Carefully draw the TLC plate obtained (MUST USE CHEM DRAW) for the aldol condensation product against the unknown aldehyde and ketone.

- Explain the TLC results, calculate the  $R_f$  values, indicate which solvent(s) were used, why they were used, and indicate which visualization method(s) were used.
- Using your results from the TLC how many compounds do you see in the reaction mixture? Predict reasonable side products that can be formed in the aldol condensation
- What are you able to conclude about the purity of your recrystallized aldol condensation producs from TLC? MP?
- Discuss any procedural changes that were made (if needed) when synthesizing the aldol condensation product and why these changes were needed.

Post Lab Questions

Do post lab question from the G & M textbook p. 622

#5, #6, #7

References:

Jones, M.; Fleming, S. A. Organic Chemistry, 4<sup>th</sup> ed. Norton Publishers; New York, 2011.

Gilbert, J. C.; Martin, S. F. Experimental Organic Chemistry: A miniscale and microscale approach, 5<sup>th</sup> ed; Cengage Learning, 2011

### LUMINOL AND CHEMILUMINESCENCE

Gilbert, J. C., Martin, S. F., Experimental Organic Chemistry, A Miniscale and Microscale Approach, 5th ed, p. 688-697

OBJECTIVES This experiment is the preparation of 3aminophthalhydrazide (also known as luminol) a compound that exhibits chemiluminescence. In the second part of the experiment, you will demonstrate the chemiluminescence of your product with hydrogen peroxide and an iron catalyst.

#### BACKGROUND

Chemiluminescence is a process where a compound absorbs a photon of energy (essentially light) with a specific wavelength  $(\lambda)$  promoting an electron from the ground state to a higher energy state. As that electron drops back to the ground state also called relaxation, energy is released as light which is the effect luminescence. In general, two possible pathways to emit light exist: fluorescence and phosphorescence. If a compound is fluorescent or phosphorescent depends whether the emission comes from the singlet or the triplet state. The Jablonski diagram (Figure 1) explains the general physical background of light emission.



Figure 1. Jablonski diagram<sup>i</sup>

An example of this process found in nature is how fireflies give off light, which is known as bioluminescence. This involves the compound Luciferin, which undergoes oxidation with  $O_2$  catalyzed by an enzyme, luciferase, followed by decarboxylation yielding light (Scheme 1).



Scheme 1. Decarboxylation of Luciferase

You will synthesize a luminescent compound: luminol and investigate the chemiluminescent characteristics. The synthesis of luminol involves two steps (Scheme 2):

1) Double condensation of 3-nitrophthalic acid with hydrazine (Eq. 1) forming a diamide.

2) The reduction of nitro group of the resulting 3nitrophthalhydrazide with sodium dithionite leading to luminol (Eq. 2).



Scheme 2. Synthesis of Luminol

One interesting aspect about the chemiluminescence part of the experiment (Scheme 3) is that a base is required to start the photochemistry process followed by oxidation of luminol forming a somewhat unstable moiety ultimately leading to sequential destruction of the initial bicyclic structure. However, if you are successful during the two-step synthesis of luminol you will be rewarded with a demonstration of a wonderful light show, so for this lab get your cell phone cameras ready!



Scheme 3. Chemiluminescence of Luminol

#### EXPERIMENTAL DETAILS

#### Step 1: Preparation of 3-nitrophthalhydrazide Eq. 1

Combine 200 mg of 3-nitrophthalic acid with 0.4 mL of an 8% solution (use micropipette if available or about 15 drops from the bottle's dropper) of hydrazine in a 5 mL conical vial. <u>No condenser needed.</u> Gently heat the mixture until the solid dissolves. Add 0.6 ml of triethylene glycol and a boiling stone. Clamp the vial (uncapped) on the hot plate/heating block combo, and clamp your thermometer into the vial or heating block small port. Bring the solution to a rigorous boil to drive out the water formed during the reaction. Your temperature should read 110-120 °C at this point (you may have to turn the hot plate setting to high to get the solution to rise above 120 °C and boil).

After the  $H_2O$  has evaporated, the temperature should rise to 215-220 °C. It may take some time to reach this higher temperature. You can insulate the vial with aluminum foil.

Maintain the temperature at 215-220 °C for about 2 minutes. Then remove the vial from the hot plate and allow the solution to cool to 100 °C. While the solution is cooling, bring 10 mL of  $H_2O$  to boiling using a small flask (50 ml would ideal). Add 3 mL of this boiling water to the reaction mixture. Stir the contents of the conical vial with a glass rod, cool the mixture to room temperature, and collect the 3-nitrophthalhydrazine by vacuum filtration using your Hirsch funnel.

Step 2: Preparation of 3-aminophthalhydrazide Eq. 2

Add the damp solid 3-nitrophthalhydrazide back into the conical vial and add 1mL of 3M aqueous NaOH. Mix until the solid dissolves. Add 0.6 g of sodium hydrosulfite to the solution and heat the mixture to just below boiling  $(98 - 100^{\circ}C)$  for 5 minutes. Watch out for bumping.

Note: we may have anhydrous sodium hydrosulfite instead of the sodium hydrosulfite dehydrate. The anhydrous sodium hydrosulfite can react violently with water, so add the 0.6 g *careful-ly* to the solution. We did not see any signs of an exothermic reaction upon addition of the sodium hydrosulfite, but add the hydrosulfite slowly and carefully.

Heat the mixture to just below boiling for 5 minutes (watch out for bumping). Add 0.4 mL of glacial acetic acid to the reaction mixture, cool the vial in cold water, and then collect the luminol by vacuum filtration and weigh it. No other purification is necessary! Besides you will not obtain much product.

#### **Chemiluminescence**

You and your locker partner have samples of luminol. Combine both of these samples in a 50 mL Erlenmeyer flask with 2 mL of aqueous 3M NaOH and 18 mL of water. This is solution A.

For solution B, add 4 mL of 3 % aqueous potassium ferricyanide, 4 mL of 3 % aqueous hydrogen peroxide and 32 mL of water into a second 50 ml Erlenmeyer flask.

Working in a low light environment, draw some solution A into a Pasteur's pipet and draw some solution B into a second Pasteur's pipet. Drain both pipets in a clean Erlenmeyer flask. Try to control the drainage of the pipets so that you get as much light as possible. Let your instructor see your results.

Notes

- 1. You can stir the mixture with a glass rod or wooden stick to help to dissolve the solid. Do not use a pipet to stir since the solid can get caught in the pipet tip.
- 2. As the temperature rises to 210 220 °C the solution takes on a deep yellow or amber color.
- 3. The 3-nitrophthalhydrazide crystals should be bright yellow.

- 4. We actually added 0.5 g of sodium hydrosulfite to 0.5 -1.0 mL of water to check for an exothermic reaction. We then added the sodium hydrosulfite/water solution to the 3-nitrophthalhydrazide and 3M NaOH mixture. We feel this step is not necessary however.
- 5. The solution turns dark brown upon addition of the 3M NaOH to the 3-nitrophthalhydrazide. When the glacial acetic acid is added, the solution becomes bright yellow again.
- 6. In the text, students are asked to pour all of solution A and solution B into a flask. The chemiluminescence occurs rapidly and goes away rapidly when the solutions are mixed this way. Using pipet amounts of solution mixes should allow the students to control the amounts of light produced better.

#### LAB REPORT

Things you should consider for your lab report:

1) Table of Reagents and state the limiting reagent.

2) Include a correct drawing of Scheme 20.3 (G & M textbook p. 691) as part of your discussion (did you notice the typographical error?).

3) As you are redrawing the scheme try to get a better understanding of the proposed mechanism of what luminol is going through to give blue light as it undergoes chemilumenscence. Describe the absorption and emission of Luminol in detail (including wavelength etc.). Using *your own words* and ideas make a brief summary of this phenomena as part of your discussion.

4) Include a copy of and compare IR, <sup>1</sup>H and <sup>13</sup>C NMR spectra for 3-nitrophthalhydrazide and 3-aminophthalhydrazide as part of your discussion.

#### Post Lab Questions

Do Post Lab Questions 3, 5 and 6 from G & M (p. 696) textbook.

#### **REFERENCES**

<sup>&</sup>lt;sup>i</sup> http://www.shsu.edu/~chm\_tgc/chemilumdir/JABLONSKI.html

### SYNTHESIS OF NYLON AND PHOTOLITHOGRAPHY

# Taken from: Kimberly L. Berkowski, Kyle N. Plunkett, Qing Yu, and Jeffrey S. Moore "Introduction to photolithography: Preparation of microscale polymer silhouettes." *Journal of Chemical Education* 2005, 82, 1365-1369

OBJECTIVES This experiment will introduce you to polymer science. You will perform two experiments: 1) a synthesis of a polymeric molecule, nylon, using a step-growth strategy. Second, you will explore the concepts behind microchip construction via photolithography by a simple laboratory experiment using polymer chemistry.

#### **BACKGROUND FOR NYLON SYNTHESIS**

A polymer is a large molecule, often called macromolecule, that is based on repeating units (monomers). These monomers can either be polymerized in a step-wise fashion (called step polymerization) or in a linear fashion via a chain polymerization. You have seen chain-polymerizations when discussing radical reactions. Other chain-polymerization mechanisms include anionic and cationic polymerizations as well as catalyst-mediated polymerizations. The driving force of step polymerization is the reaction between two complementary functional groups. Think about basic carbonyl chemistry (ester and amide formation) regarding the chemistry behind step-polymerizations. These functionalities may either be on the same monomer (an A-B monomer), or two difunctional monomers may be used (A-A and B-B monomers).

This lab will focus on step-growth polymerization. In the Nylon part of the lab, you will carry out a step-polymerization using two difunctional monomers.



Scheme 1. Synthesis of Nylon 6,6

Nylon is a polyamide – a polymer containing amide bonds – that can be made by a step-growth polymerization. It was first synthesized in 1935 by Carothers at DuPont. Nylon-6,6 was the first commercially available polyamide, synthesized from hexanedioic acid (a 6 carbon diacid) and 1,6-hexanediamine (a 6 carbon diamine).

The strength of Nylon fibers can be attributed to hydrogen bonds, which is very similar to proteins. When chains of the polymer are laid out, the nitrogen in the amide can act as an H-bond donor to a nearby carbonyl group. This force makes commercial fibers of nylon and silk (a protein) very strong.



**EXPERIMENTAL DETAILS** 

You will carry out two experiments. Start with the photolithography experiment and do the Nylon synthesis during the stirring and heating period of the photolithography experiment.

#### Synthesis of Nylon

For today's lab, you will be synthesizing nylon-6,10. To facilitate the reaction under mild conditions, you will be using an acid chloride instead of the acid. This will give HCl as a byproduct instead of H<sub>2</sub>O. You will need to add a base to neutralize the HCl formed. If not, the HCl produced could cause a competing acidbase reaction with the diamine (write this acid base reaction in your lab report). You will use decanedioyl chloride (sebacoyl chloride – a 10 carbon diacid chloride) and 1,6-hexanediamine (a 6 carbon diamine) for the polymerization.

1) Pour 10 mL of a 5% solution of 1,6 hexanediamine in water into a 100 mL beaker. To this, add 10 drops of 20% NaOH. Mix this thoroughly to ensure homogeneity.

2) CAREFULLY add 10 mL of the 5% decanedioyl chloride solution in  $CH_2Cl_2$  to the beaker. A polymer film will appear immediately at the interface. There are several ways to retrieve this polymer film (they are all cool): a) Use a pipette to slowly add one solution to the other. b) Pour the aqueous solution carefully down a glass rod to form a layer on top of the organic layer. c) Use a separatory funnel placed along the side of the beaker to slowly add the aqueous layer on top of the organic layer. If you add the solutions too fast, the polymerization will just make one large blob and you will need to start over.

3) When the layers have formed, use a copper wire or wooden stick to pull the polymer up (roll it up on the stick). Carefully remove the polymer thread and allow it to dry. You can either a) Wrap the polymer thread around a soda or soup can and wind it up, or b) carefully have one person hold the polymer (WITH GLOVES) while another carefully pulls it from solution.

4) Show your instructor the polymer created before you leave.

**BACKGROUND FOR THE PHOTOLITHOGRAPHY EXPERIMENT** (THIS IS TAKEN DIRECTLY FROM THE ABOVE CITED ARTICLE WITH THE PERMISSION FROM THE AUTHORS. YOU SHOULD LOOK AT THIS ARTICLE AS PART OF THE LAB PREPARATION)

Much of today's information technology relies on microchips developed by the semiconductor industry. These microchips are the brains behind most electronic devices such as personal computers, cell phones, and CD players. Although microchip function is typically described in terms of physics and engineering, the underlying process for the fabrication of these complex electronic devices, photolithography, is rooted in polymer chemistry.

Developed in 1959, photolithography is a process that uses high-intensity light and a photomask to prepare a polymer network on a silicon wafer. The polymer network is formed through a physical change to a photoresist, which contains a light-sensitive compound and a mixture of polymers that becomes soluble or insoluble when exposed to UV light. The patterned polymer network acts as a guide for the chemical etching of the silicon wafer, much like the way a canyon channels a river's flow and causes it to carve away the underlying soil. The terms given to the different types of photoresists, positive and negative, depict the resulting polymer "image" that is displayed. A positive photoresist is comprised of an insoluble polymer that degrades into a soluble polymer when exposed to UV light, while a negative photoresist is composed of monomers or polymers that polymerize or crosslink to form insoluble polymers upon UV exposure.

Principle of Photolithography



Scheme 2. Basic design of photolithography

The main steps of photolithography involving a negative photoresist are shown in Scheme 2. A thin layer of photoresist is spin-coated onto the surface with a typical thickness of 1 micron, which is approximately 1/70 the thickness of a human hair (a).

A photomask with the desired structural feature is positioned above the photoresist, and UV light is focused onto the photoresist through the photomask (b). The light that passes through the photomask (clear area) causes the photoresist to polymerize and thus become insoluble. The area that is covered and protected by the dark area of the photomask does not polymerize and can therefore be dissolved away (c). After a polymer pattern is present on the SiO<sub>2</sub> substrate, all of the SiO<sub>2</sub> that was not covered with the patterned polymer is etched away (d). Finally, the remaining polymer is removed from the surface of the wafer, resulting in a SiO<sub>2</sub> layer that is a negative image of the photomask (e).

#### EXPERIMENTAL DETAILS

#### This experiment uses a negative photoresist technique.

<u>1. Preparation of photoresist.</u> A pre-polymer mixture is prepared by combining the monomers, isobornyl acrylate (3.5 g) and Bis-GMA (2.0 g), into an amber vial containing the photoinitiator DMPA (0.18 g). The mixture is sonicated for 30 min or until a homogeneous solution is formed (in between, do the Nylon synthesis). For blue, red, or yellow pre-polymer solutions, a milligram of oil blue, oil red, or fluorescent yellow 3G, respectively, is added to the mixture and subsequently sonicated for 5 minutes. A vortex mixer or prolonged, vigorous shaking and stirring may be used if a sonicator is unavailable (you will not have a sonicator so stir it and shake it now and then). Both, the monomers and the photoinitiator are light sensitive, therefore an amber glass vial fitted with a screw-on eye dropper is recommended for easy storage and application. However, you do NOT have this <sup>(D)</sup>. A clear vial wrapped with aluminum foil is also suitable.

<u>2. Preparation of the photomask.</u> **IMPORTANT!** This needs to be done at home **BEFORE** you enter the lab to carry out the experiment. Designs for photomasks are created using Microsoft Paint, Microsoft Word, Adobe Photoshop or a similar program. The use of Microsoft Word is described as an example. In Microsoft Word, a 3 x 3" black box is formed and used as a background template for all photomask designs. Six boxes should be prepared on one page for efficient use of transparency film. To design a text-containing photomask, a text box is created and formatted for white characters. This text box is then centered inside the 3 x 3" black box so that at least 0.5" of black is present on all sides of the text. To design a picture containing photomask, paste a white picture design into the 3 x 3" black box. The photomasks are printed onto standard transparency film using a black and white printer at a resolution of 600 dpi or higher.

<u>3. Preparation of polymer patterned glass slide</u>. A transparency slide is cut into fourths to prepare equal pieces of 5.5" x 4.25" (Scheme 3).

a.) A transparency film is placed flat on a table and two coverslips  $(22 \times 50 \text{ mm No. } 1)$  are placed at the top and bottom of the transparency. These coverslips act as spacers between the transparency and the glass slide that is patterned.

b.) Approximately 15-30 drops of the pre-polymer solution are applied to the middle of the transparency, about 2.5" from each coverslip.

c.) A glass slide  $(75 \times 50 \times 1 \text{ mm})$  is then placed in the center of the transparency, allowing its top and bottom to rest on the spacer coverslips. Upon placing the glass slide on the coverslips, the pre-polymer solution flows to uniformly cover the entire area.

d.) After the entire space between the transparency and the glass slide is filled by pre-polymer solution, a photomask is placed on top of the glass slide  $(75 \times 50 \times 1 \text{ mm})$  at the desired location. A second glass slide  $(75 \times 50 \times 1 \text{ mm})$  is placed on top of the photomask to keep it flat.

e.) The exposed regions of the pre-polymer solution are polymerized by holding a hand-held UV lamp (365 nm) 0.5" above the photomask for 20 seconds. Do NOT look into the UV lamp. It is dangerous to your eyes. The photomask is then removed and the glass slide is carefully peeled away from the transparency paper to expose the polymer image, which forms on the bottom of the glass slide. The non-polymerized monomer is washed off the glass slide into a beaker with ethanol and the patterned glass slide is dried by gently dabbing it with a paper towel.



Scheme 3. Fabrication Process

#### LAB REPORT

Things you should consider for your lab report: 1) Table of Reagents.

2) Include a correct arrow pushing mechanism of formation of nylon-6,10.

3) Clearly state the step by step procedures of photolithography.

4) Describe all side reactions (such as the acid base reaction stated above) including mechanisms.

#### POST LAB QUESTIONS

1) Write an arrow-pushing mechanism for the reaction.



2) The initiation of IBA polymerization uses a radical initiator.



3) Show the propagation product of IBA polymerization. initiator.

*.*0.

4) Can you make Nylon from an a\_b monomer? If yes give an example.5) How about a cyclic monomer? Can you imagine making Nylon from a cyclic monomer? If yes give an example.

## CHEMICAL TESTS FOR ORGANIC COMPOUNDS

## Gilbert, J. C: Martin, S. F. Experimental Organic Chemistry: A Miniscale and Microscale Approach. 5<sup>th</sup> Edition, Cengage Learning, 2011. Chapter 23 and Chapter 24

#### Jones, M.; Fleming, S. A. Organic Chemistry, 4<sup>th</sup> ed. Norton Publishers; New York. 2011

OBJECTIVES: The purpose of this lab is to demonstrate how different foods and other substances can contain one, some, or all of the organic compounds that are important to cells: amino acids, monosaccharides or simple sugars, fatty acids, and nucleotides.

#### **ACTIVITIES**

- 1. Make a hypothesis about the content of the samples you and your lab partners have brought from home.
- 2. For this lab, you are required to bring in samples for testing. Lack of samples will result in failure of this lab period. You will test each sample, along with the appropriate positive and negative controls, for protein, monosaccharide, and complex carbohydrate.

#### **INTRODUCTION**

All living organisms are composed of four major classes of organic compounds similar to (but not exactly the same as) the four food groups. These compounds are composed of small subunits: amino acids, monosaccharides or simple sugars, fatty acids, and nucleotides. These subunits build larger macromolecules: protein, polysaccharides like starch, triglycerides and phospholipids, and nucleic acids. All cells are made of these macromolecules. A diet consisting of a variety of different foods will provide each of these four components for our growth and metabolism.

We will be using three different tests to identify protein, monosaccharide (glucose and/or fructose), and complex carbohydrates (starch) in various foods. Monosaccharides and starch are both carbohydrates. Monosaccharides are the small organic molecules that are the subunits used to build large starch macromolecules. Try to bring enough samples so that you will get at least one positive test for monosaccharide, one for protein and one for complex carbohydrate. Can you find a sample that will test positive for all three-test items? While you will be working with your lab locker partner for this experiment, you must both bring your own separate samples. We will also do a simple test for lipids (you do not have to bring in a sample for the lipid test).

Here are a couple of ideas of what to bring: juice, milk, onion, potato, and corn, fruit (ex. apple slices) or vegetables, flour, tofu, or bread, anything that can mix with water like gelatin (jello), certain vitamin capsules or pills, or honey. You can even try bringing a strand of your hair! DO NOT BRING: oily foods, dark colored liquids or foods, uncooked beans or pasta, or other dry unhydrated food. No acidic foods like orange juice or coke/pepsi. These items will not react well with the test chemicals.

Foods will contain at least one type of organic compound. Before beginning, make a prediction about which type of compound or compounds you think your experimental sample contains. Record your prediction on your lab notebook.

You will perform three different tests on the same four samples. Each test will use a different indicator **reagent** that will change color in the presence of the particular organic compound that is being tested for. As part of the experimental method, you must include **control samples** to insure the validity of your results. A control is a test sample with a known result. If your control samples do not give you the expected result, then your experimental results are not valid and you must reevaluate your experimental set-up (maybe your test chemicals are no good).

A negative control will result in no change in color. It will either contain no sample at all or it will contain a nonreactive sample like water. For example, if you are testing for the presence of monosaccharides, the test chemical, called Benedict's solution, will remain the original color blue when mixed with water. A positive control will result in a color change indicating the presence of the compound you are testing for. For example, a 5% glucose solution will react with Benedict's solution and change it from blue to rust (brown-red). You can compare your experimental results to your control results to determine if you obtained a positive reaction.

#### **EXPERIMENTAL SECTION**

#### **To Prepare Your Samples:**

- 1. If your experimental sample is solid, chop or break it up into the smallest pieces possible using a razor blade or glass rod, whichever is appropriate. Use a pinch of sample small enough so that the material can be suspended easily in 1.5 ml of water and does not fill the bottom of the tube. Add 1.5 ml of water to your sample and mix it well.
- 2. If your sample is a liquid, add 1.5 ml of the liquid to the appropriate tube.
- 3. Each tube should have the same volume of fluid, regardless of the type of sample.

#### Part 1: Identification of Protein

Materials:

- test tube rack
- test tubes
- tape for labeling your tubes
- 0.5% CuSO<sub>4</sub>
- 10% NaOH
- albumin protein solution (egg white)
- distilled water
- experimental sample

The test chemicals used in this experiment react with the covalent bonds (amides) that link amino acids together in protein chains. In the presence of a protein, the chemicals will turn varying shades of purple.

#### **Procedure:**

- 1. Predict which organic compounds your experimental samples might contain.
- 2. Label your tubes 1 through 4
- 3. Prepare your sample following the instructions from above.
- 4. CAUTIOUSLY add 20 drops of 10% NaOH solution to each tube. Agitate the tube gently.
- 5. Add 4 drops of 0.5% CuSO<sub>4</sub> to each tube. Agitate the tubes again.
- 6. Let the tubes sit at room temperature for a few minutes until you see a color change in your positive control.
- 7. Record the results.

#### Part 2: Identification of Monosaccharides

Materials:

- hot plate
- test tube rack
- test tubes
- tape for labeling your tubes
- 400 ml beaker half filled with boiling water
- test tube clamp for picking hot test tubes
- · Benedict's solution
- 5% glucose solution
- 5% fructose solution
- 5% sucrose solution
- · distilled water
- experimental sample

Benedict's solution is a blue solution that will change color in the presence of monosaccharides, such as glucose or fructose. Benedict's changes from blue  $\rightarrow$  green  $\rightarrow$  orange  $\rightarrow$  brown, depending on the amount of monosaccharide present. Any change in color indicates the presence of monosaccharides.

#### **Procedure:**

- Set up a boiling water bath using a 400 ml beaker filled with approximately 150 ml of tap water on a hot plate. Add 2 or 3 boiling chips to the water. These will prevent boiling water from slashing up when you add your test tubes. Set the hot plate on high to get the water boiling, then reduce the temperature to a setting of "2" to keep the water simmering. The water should be boiling BEFORE you place your tubes in it.
- 2. Label your tubes 1 through 6. If you are using tape, make sure the tape is high enough on the tube so that it does not get wet in the water bath. Otherwise, the tape will fall off and you will not be able to identify your samples.

- 3. Prepare your samples as before.
- 4. Add 1.5 ml of Benedict's solution to each test tube, for a total of 3 ml.
- 5. Agitate your tubes so that the sample and Benedict's is well mixed. See instructor for demonstration.
- 6. Place the all the test tubes at the same time in the boiling water bath for 5 minutes.
- 7. Remove the tubes using the test tube clamp and record the resulting color. Do not mix the tubes again once they have been boiled. If your sample was a solid, note the color change in the immediate vicinity of your sample. The rest of the Benedict's may stay blue since a solid cannot mix well.

#### Part 3: Identification of Starch (Complex Carbohydrate)

Materials:

- test tube rack
- test tubes
- tape for labeling your tubes
- Iodine solution (IKI)
- Starch suspension
- distilled water
- experimental sample

Iodine will react with starch and turn from a yellow/brown color to a purple/black color. Only the purple/black color is an indication of starch.

#### **Procedure:**

- 1. Number your tubes 1 through 4.
- 2. Prepare your samples as before. Remember, each tube should have 1.5 ml of liquid.
- 3. Add 2-3 drops IKI to each tube. Agitate your tubes.
- 4. Record your results.

#### Part 4: Testing for lipids/fats

#### Procedure:

- 1. Place 10 drops of corn oil and 20 drops of water into a test tube. Shake the mixture and allow it to settle.
- 2. Note what you see when you mix the corn oil with water.
- 3. Explain this observation.
- 4. Add Sudan IV to the mixture and record your observations.

#### Report

- 1. Turn in your prepared Data Sheets
- 2. What is the purpose of samples containing albumin, glucose, fructose, and boiled starch solution? What is the purpose of using the water only sample in each of the experiments?
- 3. Why does sucrose not react with the Benedict's reagent?

- 4. Draw the chemical structures of a monosaccharide and amino acid of your choice. Be sure to label the name of the specific molecules you choose.
- 5. For each food you tested, list the organic compounds it contained. How do your results compare to your original predictions? Explain.

#### POST LAB QUESTIONS

Do post lab question from the G & M textbook

p. 792: #5, #14

p. 820: #7 (a)

**REFERENCES** 

Jones, M.; Fleming, S. A. Organic Chemistry, 4<sup>th</sup> ed. Norton Publishers; New York, 2011.

Gilbert, J. C.; Martin, S. F. Experimental Organic Chemistry: A miniscale and microscale approach, 5<sup>th</sup> ed; Cengage Learning, 2011