24. Qualitative Organic Analysis – Identification of an Unknown

A. Introduction

Over the course of the organic I and II laboratories you have discovered a number of different reactions used to prepare organic molecules and techniques to characterize these compounds. With advances in mass spectrometry and NMR spectroscopy, the necessity to characterize molecules via chemical means has largely fallen to the wayside. It is still, however, important to have an understanding of the chemical techniques that can be used to classify and identify organic molecules. Additionally, the technical skill and problem solving ability employed in the qualitative analysis of an organic unknown is an important proficiency for any student completing the organic chemistry laboratory course. This experiment culminates the laboratory techniques that you have learned this semester and requires you to put the various pieces of information together to identify an unknown organic molecule.

For this experiment, you will be provided two organic unknowns. Each unknown may be an aldehyde, ketone, carboxylic acid, amine, phenol, or alcohol. Next, you will subject each of these unknowns to a number of physical tests to help narrow down the possible compounds. An IR spectrum will help in identifying the important functional groups. Chemical tests will then be performed to confirm the presence of the hypothesized functional group. Once you have your unknown down to one or two possibilities, you will then prepare derivatives to confirm your identification. You will be provided with tables of possible compounds from which your unknown will have been chosen.

As you collect the various data and perform the specified chemical tests, you should record your results and observations in your laboratory notebook. Additionally, you should fill out an unknown report sheet. This experiment will span over two lab periods. You will perform parts B and C during the first lab period and then complete part D (preparation of derivatives) during the second lab period.

B. Physical Properties and Preliminary Data

1. Basic Properties

Assess your unknown for the most basic properties. Is it a solid or a liquid? What color is it? Does it have a characteristic odor?

2. Melting Point/Micro Boiling Point

If the unknown is a solid, you should obtain the melting point range. Keep in mind that while the unknowns are supposed to be pure, if they do contain any impurities, the melting point will be depressed and the melting range will be broader than expected. In this event, you may need to recrystallize some of your sample. See your TA for guidance.

If the unknown is a liquid, then you will need to determine its micro boiling point. You first determined a micro boiling point in the organic I lab (see Chem 235 experiment 9 – Hydroboration-Oxidation). You can also obtain the refractive index of a liquid sample. A

molecule's refractive index is the extent to which light is bent when it is passed through a sample. The refractive index, *n*, does not have any units and depends on the ambient temperature and wavelength of light being used. Your TA will show you how to operate the refractometer.

3. Solubility

Using the solubility flow chart shown in figure 1, you should test the solubility of your unknown in water, acidic solution, and basic solution. The solubility will give important insight as to the functional group present in your molecule.

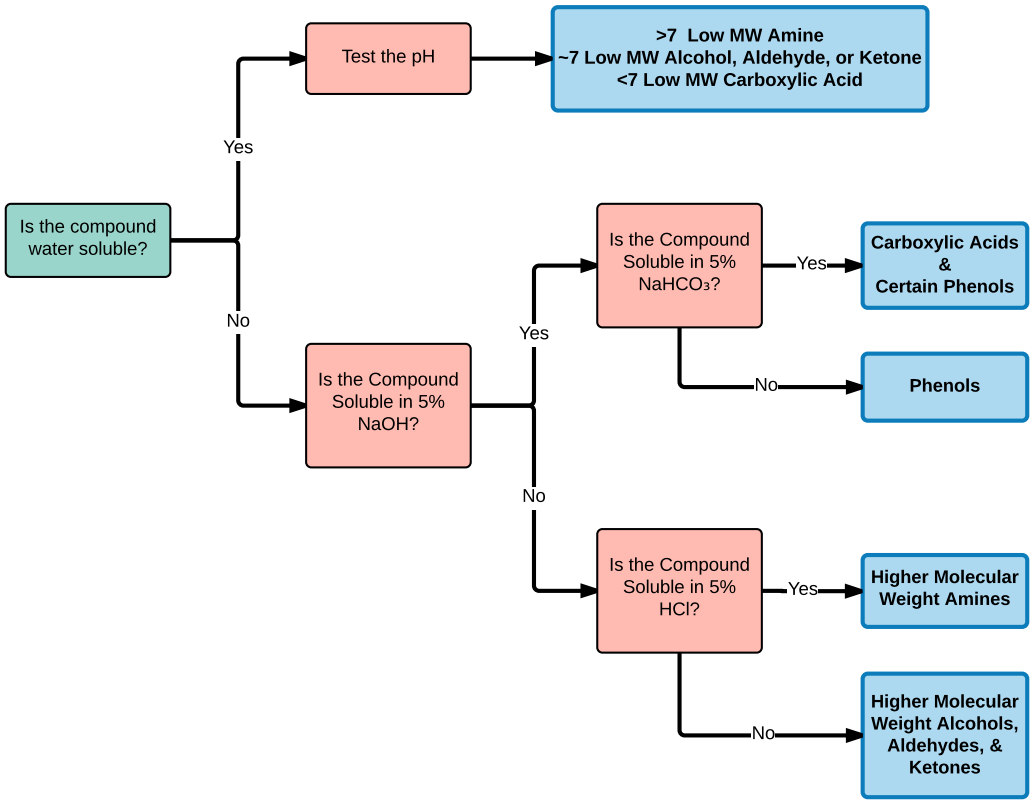


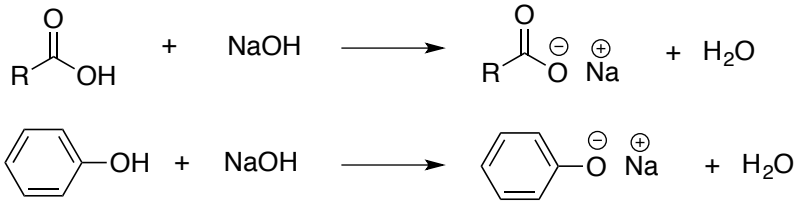
Figure 1. Solubility Flow Chart

To test the water solubility, add 2 drops of your liquid or 10-20 mg of your solid unknown to a small test tube. Add water in 0.1 mL portions up to a total volume of 0.5 mL stirring well with each addition. If your sample does not dissolve then is it insoluble in water. Keep in mind the "**rule of 4"** where a molecule needs 1 polar functional group for every 4 carbon atoms for it to be water-soluble. If the unknown is water soluble, test the pH of the solution to help narrow down the identity of its functional group.

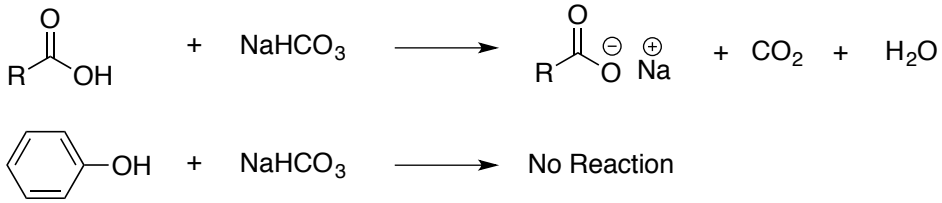
If the compound is not water soluble, then you should test the solubility in basic and acidic solutions. Most amines are soluble in hydrochloric acid because they form water-soluble ammonium salts.

$$R{-}NH_2 \quad + \quad HCl \longrightarrow R{-}\overset{\oplus}{N}H_3 \ \overset{\ominus}{Cl}$$

Relatively acidic functional groups such as phenols and carboxylic acids are soluble in aqueous NaOH because water-soluble phenoxide and carboxylate salts are formed.



Because carboxylic acids are more acidic than phenols (pKa 5 vs 10), they are soluble in the weaker base, aqueous sodium bicarbonate while phenols typically are not. If your unknown is soluble in NaOH, you should then test its solubility in aqueous $NaHCO_3$ to help determine whether it is a phenol or carboxylic acid.



To test the solubility, add two drops of liquid or 10-20 mg of solid to a small vial containing a screw cap. Add ~15 drops of water then add the reagent (aqueous HCl, NaOH, or $NaHCO_3$) dropwise with shaking and observe whether or not the unknown dissolves.

4. IR Spectroscopy

Record the IR spectrum of your unknown and use the spectrum to look for the presence or absence of particular functional groups. Don't get caught in the trap of making a 100% confirmation based on IR. Remember that various factors can influence the IR stretching frequency (i.e. conjugation lowers the C=O stretching frequency). Some key functional group stretches are shown in the table below. See experiment 8 to review IR spectroscopy.

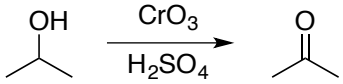
Alcohol/Phenol O-H Stretch	3200-3500 cm^{-1} (broad, strong)
Carboxylic Acid O-H Stretch	2500-3300 cm^{-1} (broad, variable intensity)
Carbonyl C=O Stretch	1650-1850 cm^{-1} (strong)
Amine N-H Stretch	3300-3500 cm^{-1} (medium intensity)

C. Chemical Tests

Once you have performed the analysis from part B, you should have a relatively good idea as to the functional group present in each of your unknowns. The following chemical tests listed in this section should be used to confirm the identity of your functional group. Only do the tests that are necessary. Do not aimlessly perform these chemical tests. For example, if your IR spectrum does not indicate an alcohol, then there is no reason to perform the Jones Oxidation.

1. Test for Alcohols

Jones Oxidation: Primary and secondary, but not tertiary alcohols are oxidized using Jones reagent (CrO_3/H_2SO_4). During the oxidation, Cr(VI) [orange] is reduced to Cr(III) [green]. A color change from orange to green is a positive test.

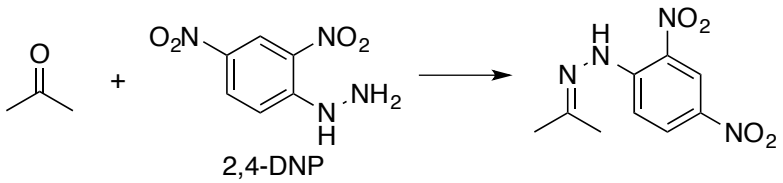


In a small test-tube, dissolve one drop of liquid unknown or ~10 mg of solid unknown in 5 drops of acetone. Add one drop of the Jones reagent and stir the mixture. A positive test should be visualized within 5 seconds. You may also want to run a blank test on the acetone solvent to ensure it does not contain any oxidizable impurities that will give a "false positive" test.

Beware: In addition to primary and secondary alcohols, aldehydes also give a positive test when subjected to the Jones Oxidation.

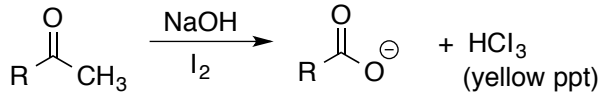
2. Tests for Ketones

2,4-Dinitrophenylhydrazine (DNP Test): When mixed with the DNP reagent, ketones (and aldehydes) react to form a hydrazone product, which should appear as a yellow, orange, or dark red solid.



In a small test-tube dissolve 2 drops of liquid or 20 mg of solid unknown in 10 drops of ethanol. Add 10 drops of DNP reagent and mix the solution. For a positive test, a yellow, orange, or dark red precipitate will be observed after 3-5 min. If a precipitate is not observed, try heating the solution at 40-50 °C for a few minutes, but do not evaporate off the ethanol.

Iodoform Test: The iodoform test is exclusive for methyl ketones. If you believe you have the ketone functionality, you can further narrow down your structure via the iodoform test. A positive test yields iodoform (CHI_3), which is a bright yellow solid and confirms the presence of a $-COCH_3$ group.

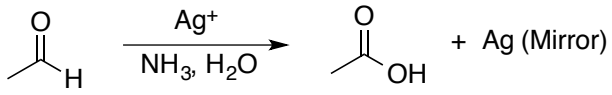


Add 2 drops of liquid or 20 mg of solid unknown to a small test-tube. Add ~1 mL of 10% aqueous NaOH. You may need to add 10-15 drops of 1,2-dimethoxyethane if your unknown is insoluble in the solution. Next, add I_2/KI solution dropwise. With a positive test, the purple color of iodine will disappear and a yellow solid will precipitate out. You may need to warm the solution in a 60 °C water bath for ~5 min to affect the reaction.

Caution: Alcohols with a methyl group attached to the alcohol carbon can also give a positive iodoform test. This is because the reagent can oxidize the alcohol to a methyl ketone.

3. Test for Aldehydes

Tollens' Test: Aldehydes are oxidized to carboxylic acids in the presence of Ag(I). The silver byproduct from this reaction leaves a shiny silver mirror on the walls of the flask.



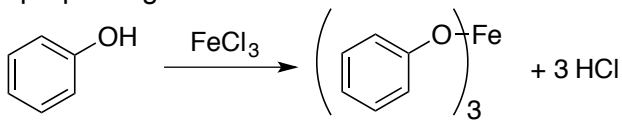
Be sure to use a new test-tube for this reaction. To the test-tube, add 1 mL of 5% $AgNO_3$. Next add 2 drops of 10% aqueous NaOH and stir the mixture. A silver oxide precipitate will be observed. Add 15% NH_3 solution dropwise with stirring until the precipitate dissolves. Add two drops of liquid unknown or 10-15 mg of solid unknown and mix the solution well. Allow the mixture to stand undisturbed for 10 min. If a positive test is not observed, heat in a 40 °C water bath for 5 min. The Tollens' reagent must be prepared fresh and used within 15 min. When finished, promptly dispose of the waste in the designated waste container.

Jones Oxidation: Will give a positive chemical test with aldehydes because aldehydes are oxidized to carboxylic acids with the Jones reagent (see procedure in part 1).

DNP Test: Will give a positive chemical test with aldehydes (see procedure in part 2).

4. Test for Phenols

Iron(III) Chloride Test: Most phenols will give intensely colored complexes with $FeCl_3$. Colors range from red to blue to purple to green.



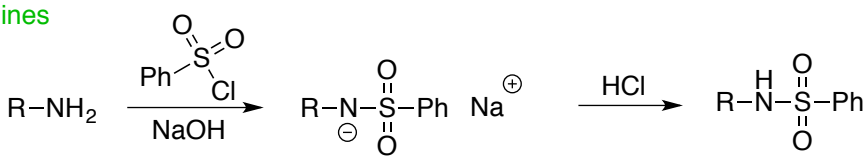
intensely colored complex

In a small test-tube dissolve one drop of liquid unknown or 10 mg of solid unknown in 10 drops of methanol. Next, add 1-2 drops of $FeCl_3$ solution and mix well. A red, blue, purple, or green solution is a positive test.

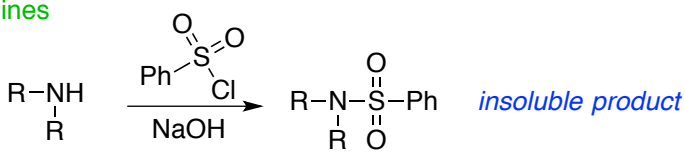
5. Test for Amines

Hinsberg Test: This test will allow for the differentiation of primary, secondary, and tertiary amines. Primary and secondary amines react with benzensulfonyl chloride in aqueous NaOH. In the case of a primary amine, a soluble salt is formed. To further confirm, HCl can be subsequently added; formation of a precipitate gives a positive test. Secondary amines react with the reagent to give an insoluble product. Finally, tertiary amines do not react with benzenesulfonyl chloride.

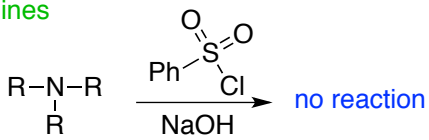
1° amines



2° amines



3° amines



Add 2 drops of liquid or 20 mg of solid unknown to a small vial followed by 3 drops of benzenesulfonyl chloride. Next, add 1 mL of 10% aqueous NaOH solution. Place the screw cap onto the vial and shake it for 3 min. Check the pH with litmus. If the solution is not basic, add more aqueous NaOH. If there is undissolved material, heat the solution in an 80 °C water bath for 10 min. If after heating, a precipitate remains, then you have a secondary amine. If the reaction mixture is clear or slightly cloudy, you have either a primary amine or a tertiary amine. Add aqueous HCl. If a precipitate forms, you have a primary amine. If no precipitate forms, you have a tertiary amine.

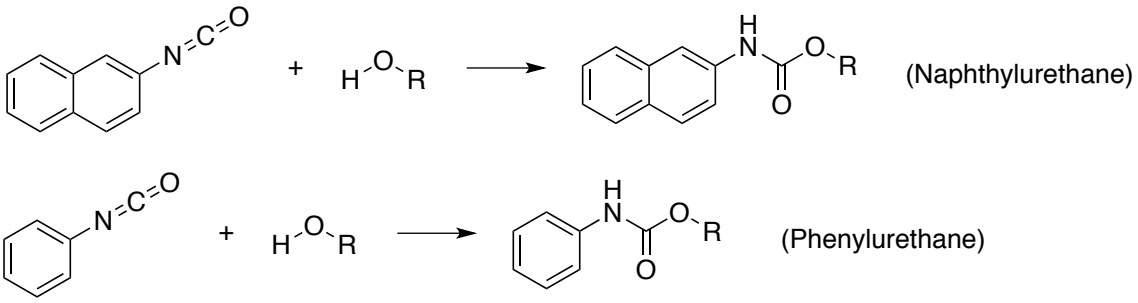
D. Preparation of Derivatives

Based on the suspected identity of the functional group in your unknown, prepare 1 or 2 derivatives. If the melting points of your prepared derivatives match the literature melting point values in the data tables, then you can confirm the identity of your unknown.

1. Alcohol Derivatives

Naphthylurethanes & Phenylurethanes

Upon reaction with isocyanates, alcohols can be converted to carbamates (also called urethanes). Naphthylurethanes and phenylurethanes are often crystalline solids that can be easily characterized. This reaction works well for primary and secondary, but not tertiary alcohols.

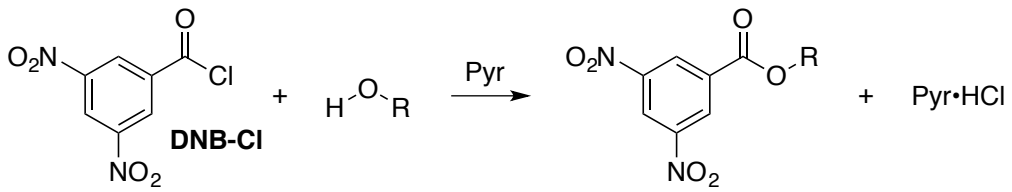


Based on the suspected identity of your unknown alcohol, calculate the mass of 1.2 mmol of alcohol and add this amount to a 5 mL conical reaction vial. Next, add either 170 mg of 1-naphthylisocyanate or 120 mg of phenylisocyanate. Stir the solution at room temperature for 5 min. If no reaction occurs, heat the reaction in hot water bath at 80 °C for 10 min. Allow the reaction mixture to cool and then add 2 mL of hexane or heptane. The solid should crystallize out. You may need to initiate crystallization if it does not readily occur. Collect the crystals via Hirsch filtration. Recrystallize the product using hot heptane. If there are insoluble impurities, you will need to perform a hot filtration to remove these impurities.

Note: If you suspect your unknown is a secondary alcohol, you may need to add 15 mg of $AlCl_3$ catalyst prior to the heating step.

3,5-Dinitrobenzoates

DNB chloride reacts readily with alcohols in the presence of a base such as pyridine to provide DNB esters, which are typically crystalline solids. Since DNB chloride, like most acid chlorides, isn't incredibly stable, it must be prepared by reacting DNB-acid with $SOCl_2$.



Add 100 mg of DNB-Acid (3,5-dinitrobenzoic acid) to a 5 mL conical reaction vial. Next, add 5 drops of pyridine followed by 7 drops of thionyl chloride ($SOCl_2$). Equip the vial with a spin vane and condenser. Heat the mixture with stirring to 130 °C. Once the solid disappears, heat the solution for an additional 10 min. Allow the vial/condenser to cool to the touch and remove the condenser. Next add 10 drops of liquid unknown or 100 mg of solid unknown. Re-attach the condenser and heat the solution at 130 °C for 10 min with stirring. Allow the reaction mixture to cool and add 3 mL of water. Collect the solid product via Hirsch filtration washing with cold water followed by 2-3 mL of 5% aqueous K_2CO_3. Recrystallize the solid using a minimum amount of hot ethanol.

2. Phenol Derivatives

Naphthylurethanes & Phenylurethanes

Follow the same procedure as outlined in part 1. Add 2 drops of pyridine to accelerate the reaction. Attach a condenser to the reaction vial and heat it to 130 °C for 15 min.

3,5-Dinitrobenzoates

Follow the same procedure as outlined in part 1. The reaction with phenols is slower so you will need to heat the reaction for 30-60 min.

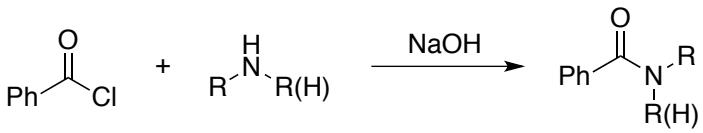
Bromination

Because the phenol OH activates the aromatic ring, phenol derivatives undergo rapid bromination to add bromine at the open *ortho* and *para* positions (see experiment 16).

Add 75 mg of unknown phenol to a conical reaction vial followed by 2 mL of water. You may need to add 0.5-1 mL of 1,2-dimethoxyethane as a co-solvent if your unknown is not water-soluble. Next, add 20% Br_2 in AcOH dropwise with stirring until a red-brown color persists, indicating the reaction is complete. Add $NaHSO_3$ solution dropwise to reduce excess bromine. Collect the product via Hirsch filtration washing the solid with cold water. Recrystallize the solid product from a minimum amount of hot ethanol.

3. Amine Derivatives

Benzamides (for primary and secondary amines)

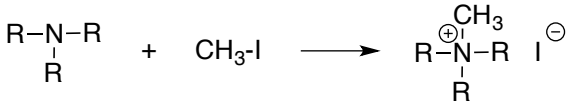


In a small screw cap vial, mix 100. mg of unknown amine and 1.0 mL of 10% NaOH solution. Add 0.40 mL of benzoyl chloride dropwise with swirling. Cap the vial and shake it for 5 min. Next, add 3 M HCl dropwise until the solution reaches a pH of 8. Collect the solid by Hirsch filtration washing the solid with cold water. Recrystallize the solid from a minimum amount of hot ethanol or 1:1 ethanol-water solution.

Benzenesulfonamides (for primary and secondary amines)

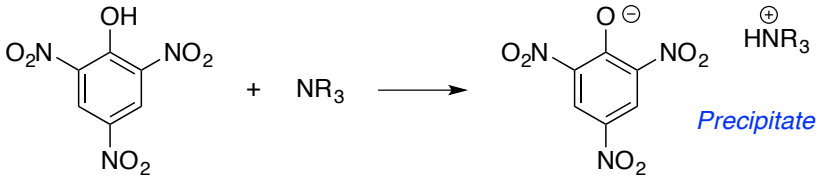
Use the procedure for the Hinsberg test in part C5. Scale the reaction up by a factor of 5 such that approximately 100 mg of unknown is used. The solid product can be recrystallized from hot ethanol.

Methiodides (for tertiary amines)
Tertiary amines react with iodomethane to provide quaternary ammonium salts.



In a small screw cap vial, combine 100 mg of unknown amine with 1 mL of 2 M methyl iodide in THF. Cap the vial and swirl. After a few min, crystals should appear. Cool the solution in an ice bath then collect the crystals via Hirsch filtration washing with ice-cold THF. Recrystallization is typically not necessary.

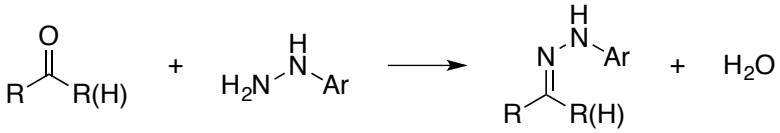
Picarates
Trinitrophenol (picric acid) is quite acidic and will readily donate its phenol proton to an amine forming ammonium salt.



In a 5 mL conical reaction vial, dissolve 100 mg of unknown amine in 2 mL of ethanol. Next, add 2 mL of saturated picric acid in ethanol. Heat the mixture to boiling in a boiling water bath then let it cool to room temperature. Collect the resulting crystals via Hirsch filtration washing the crystals with cold ethanol. If necessary, the solid can be recrystallized from ethanol.

4. Aldehyde and Ketone Derivatives

2,4 –DNP
Aldehydes and ketones react with 2,4-dinitrophenylhydrazine to form a 2,4-dinitrophenylhydrazone (DNP) product.

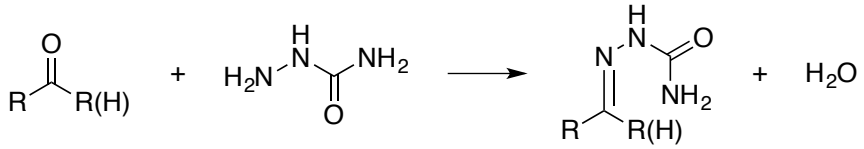


Note: Acetone reacts with the hydrazine reagent to give a DNP product so be sure your equipment does not contain residual wash acetone.

Add 200 mg (1.0 mmol, 1 equiv) of 2,4-dinitrophenylhydrazine to a 25 mL Erlenmeyer flask followed by 4 mL of ethanol and 0.4 mL of concentrated hydrochloric acid. Warm the solution with swirling until the solid dissolves. Next, add 1.2 mmol of your unknown aldehyde or ketone (calculate the necessary amount based on the suspected identity of your unknown). Heat the mixture to boiling with swirling for 2 min. Add water dropwise until the solution becomes slightly cloudy. Allow the solution to cool to room temperature. Collect the solid via Hirsch filtration washing the solid with cold 1:1 EtOH/H_2O. The solid can be recrystallized from hot ethanol or a mixed ethanol/water solvent system.

Semicarbazones

Aldehydes and ketones react with amines to form imines. When the amine is part of a semicarbazide (H_2N-NH-CO-NH_2), the resulting product is called a semicarbazone.

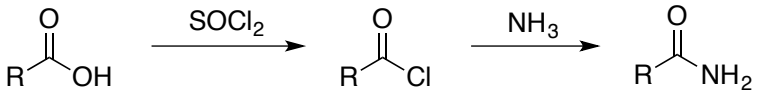


In a small test tube containing a spin vane, mix 100 mg of semicarbazide hydrochloride, 150 mg of sodium acetate, 1 mL of water, and 1 mL of ethanol. Add 100 mg or 10 drops of your unknown and stir the solution. Add additional ethanol if the solution is cloudy. Stir the mixture for 2 min then place it in an ice bath for 5 min. If crystals do not form, heat the solution in a boiling water bath for 3-5 min then cool the solution at which time crystals should form. Collect the solid via Hirsch filtration washing with ice-cold water. The solid can be recrystallized from ethanol or a ethanol-water mixture.

5. Carboxylic Acid Derivatives

Amides

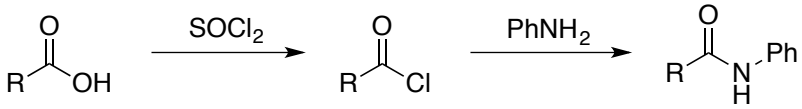
Amides can be prepared from carboxylic acids by first converting the carboxylic acid to an acid chloride and then subsequently reacting the acid chloride with an amine.



To a 5 mL conical reaction vial, add 100 mg of your unknown carboxylic acid followed by 0.5 mL of thionyl chloride. Attach a condenser and heat the solution to 80 °C for 15-20 min. Remove the condenser and continue to heat the solution for 5 min to evaporate excess thionyl chloride. Remove the vial from heat and allow the solution to cool to room temperature.

Add 4 mL of ammonium hydroxide solution to a 25 mL Erlenmeyer flask and cool the solution in an ice bath. Next, add the acid chloride slowly with stirring. Collect the solid via Hirsch filtration washing with ice-cold water. The solid can be recrystallized from water or an ethanol/water mixture.

Anilides



To prepare the anilide derivative, first prepare the acid chloride of your carboxylic acid unknown as described in the above procedure. To the vial containing the acid chloride, add 1 mL of pyridine followed by 200 mg of aniline. Heat the solution in a boiling water bath for 5 min. Pour the reaction mixture into an Erlenmeyer flask containing 7 mL of cold water and swirl the mixture well. Cool the solution in an ice bath then collect the solid product via Hirsch filtration. Wash the solid with cold dilute acetic acid followed by cold water. The solid can be recrystallized from ethanol or an ethanol/water mixture.