Lab #7 - Reductive Amination of Vanillin with p-Toluidine

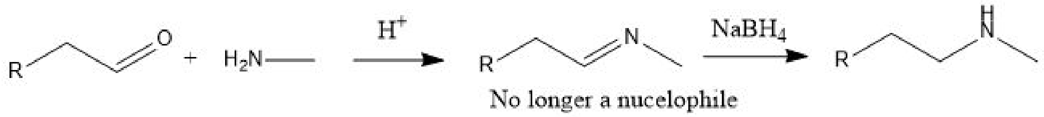
CHM 2123

Introduction:

Amine is a common class of organic compounds, which contains a nitrogen atom with a lone pair of electrons. Nitrogen is able to form three covalent bonds and, having a lone pair of electrons, it makes the amine neutral and basic. They can easily react with acids to form a positively-charged ammonium ion after protonation. A primary or secondary amine can act as a nucleophile by reacting with an electrophile to form a new covalent bond. If the amine has more alkyl substitutions, the amine is a better nucleophile because of the electron-donating ability of the alkyl groups. Even though trialkylamine is the most carbon substituted, it is not nucleophilic due to steric hindrance.

It is difficult to selectively add amines into organic compounds because of the increasing strength of the amines as a nucleophile with respect to the addition of aliphatic carbon chains. As an alkyl group is added to the nitrogen, the compound becomes the more nucleophilic product, which will react with the electrophile. As a result, multiple cross-alkylated products are formed, and it makes the single substitutions difficult to achieve from traditional nucleophilic substitutions. Reductive amination is performed to mitigate the increased nucleophilicity of the amine by reacting it with a carbonyl, such as a ketone or aldehyde, and then reducing the product with a reducing agent ,such as $NaBH_4$.$NaBH_3CN$, or $LiAlH_4$.

Scheme 1: Reductive amination allows the addition of a single amine



In the experiment, vanillin is used as the aldehyde and *p*-toluidine is used as the amine. During reductive amination, acid/base reaction will occur to activate the carbonyl centre as a good electrophile. Then the nucleophilic nitrogen will attack the carbon of the carbonyl. Following intramolecular proton transfer, the OH group becomes a good leaving group (H_2O^+). As a water molecule is eliminated, the electrons of nitrogen will form a double bond (N=C). The resulting product is known as the non-nucleophilic, imine intermediate. By adding $NaBH_4$ and ethanol (as the solvent), $NaBH_4$ will act as the reducing agent and reduce the unstable imine into the amine product. The amine product is back to being a nucleophile because it is a secondary amine and has an available lone pair to do nucleophilic attack. Acetylation of the amine product is performed by adding acetic anhydride as the electrophile and glacial acetic acid (as the solvent). The reaction is performed under heated conditions to increase the rate of reaction, which will give the reactants enough energy to overcome the activation energy. The amine will attack the carbonyl carbon on the acetic anhydride. After deprotonation and the elimination of the good leaving group, $^-OOCCH_3$, a carbonyl is regenerated and the amide product is formed. Because the amide product is very stable and non-nucleophilic (being a trialkylamine), it will not undergo further reactions.

All of these steps are carried out quickly and in one beaker, also known as the “one-pot synthesis”. Precipitation of the amide product from the solution is done by using cold water. The purity of the amide product is evaluated of the colour, TLC, and its melting point. The melting point is obtained by a melting point apparatus. A TLC with four lanes is performed, which each lane is for p-toluidine, imine and amine intermediate, and the amide product.

Mechanism:

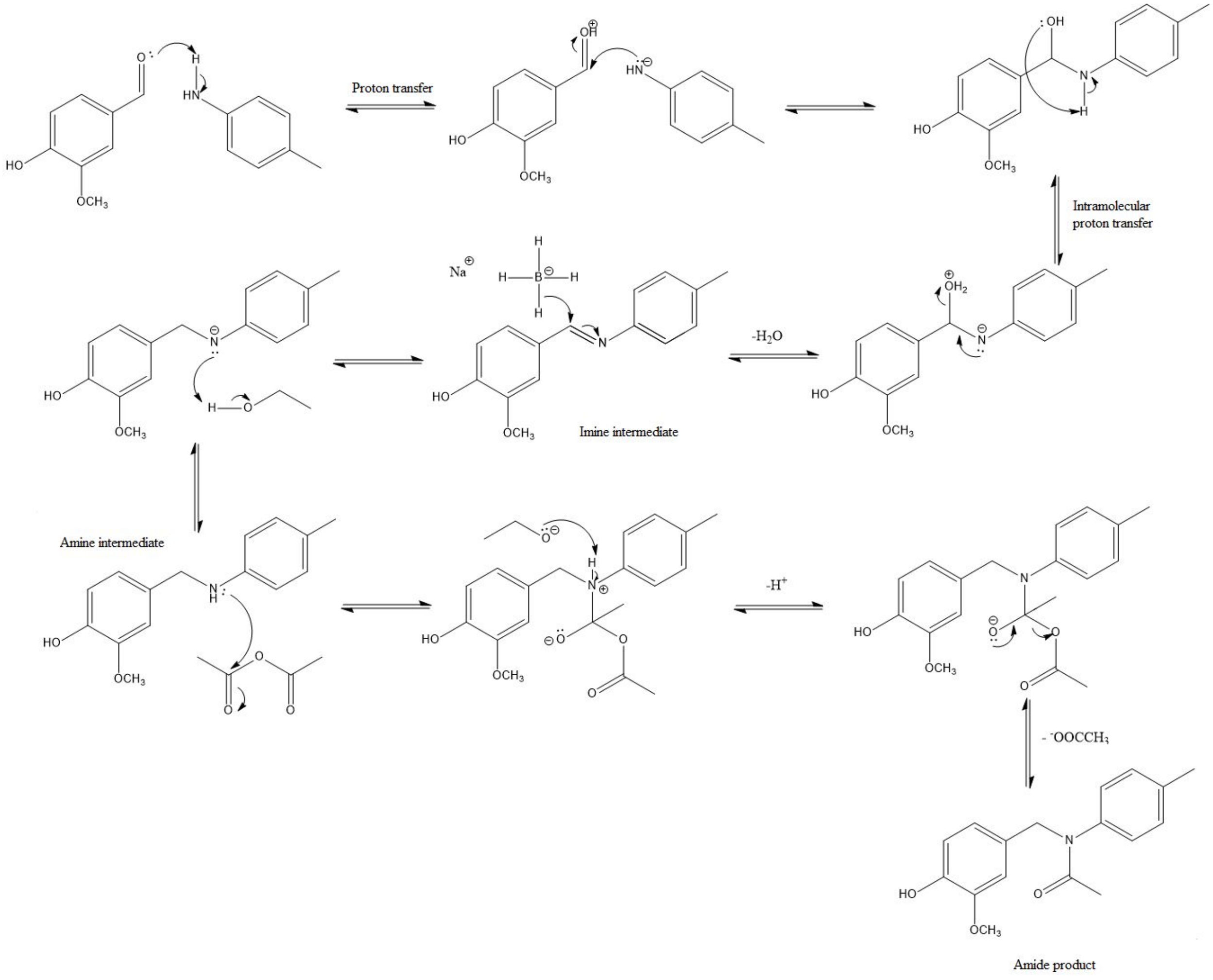


Table of Reagents

Compound	Mol. Mass (g/mol)	Quantity (g or mL)	Density (g/mL)	Mmol	Equivalent
Vanillin	152.15	0.58 g	-	3.8	1.0
p-toluidine	107.15	0.40 g	-	3.7	1.0
95% ethanol (solvent)	-	15 mL	-	-	-
$NaBH_4$	37.83	0.10 g	-	2.6	0.70
Glacial acetic acid (solvent)	-	2.0 mL	-	-	-
Acetic anhydride	102.09	2.0 mL	1.08	21	5.7

Experimental Procedure:

1. Working in the fume hood, combine 0.58 g of vanillin and 0.40 g of *p*-toluidine in a 250 mL beaker.
2. Use a Teflon stir rod and mix the solids together constantly for 5-10 minutes. A dry, bright orange powder will form as the imine intermediate. Reserve a small amount for TLC analysis.
3. In the same beaker, dissolve the imine with 15 mL of 95% ethanol.
4. Add 0.10 g of sodium borohydride in small increments to the reaction mixture.
5. Within 10 minutes, a colourless solution is an indication that the reaction is complete and the amine intermediate is formed. Using a plastic pipette, reserve a few mL of the reaction mixture for TLC analysis. First add 2.0 mL of glacial acetic acid and then 2.0 mL of acetic anhydride to the reaction solution.
6. Using a heating mantel, prepare a steam bath.Transfer the reaction into an Erlenmeyer flask by pouring the reaction mixture through a filter funnel. Rinse the beaker with ethanol to ensure complete transfer of the reaction mixture. Prepare a steam bath and let the reaction mixture heat for 5-10 minutes.
7. Cool the reaction in an ice bath. Slowly add 75 mL of cold water to induce precipitation of the amide product. If necessary, scratch the bottom of the flask to induce crystallization.
8. By vacuum filtration, isolate the white crystals from the reaction mixture. Obtain the yield of the product.
9. Run a TLC with four lanes to assess the efficiency of your reaction and purification. Separately dissolve a small amount of the starting material (*p*-toluidine), final product and the imine intermediate in ethanol, and take a TLC (60:40 hexanes: EtOAc). Include a lane for the the amine intermediate. Visualize the TLC under UV light.
10. Use a melting point apparatus to obtain the melting point range of the final product. The literature value of the amide product is 102-108 °C.

Observation and Results:

Table of Observations

Key Step	Observations
Combination of vanillin and *p*-toluidine	- Flaky and dark orange formed as the imine intermediate
Addition of 95% ethanol	- The reaction solution is clear and bright orange - Orange solid is slightly soluble
Addition of $NaBH_4$	- Solution turned to clear and bright yellow, containing the amine intermediate
Addition of acetic anhydride	- Initially, there was vigorous bubbling - Solution becomes a clear and golden solution, like the colour of ginger ale.

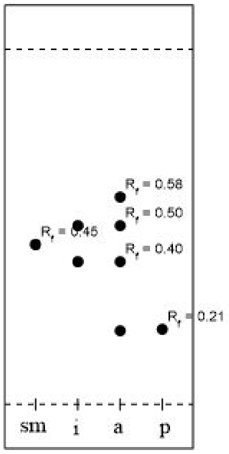
Addition of cold water	- Solution turned milky, opaque and white
Use of ice bath	- After 10 minutes, the solution remained opaque and cloudy but turned from white to beige
Final product	- Glossy and beige solid

Table of Results

Mass of amide product (g)	0.70
Melting point range of amide product (°C)	101.1-102.3
Percentage yield of amide product	65 %

TLC plates:

TLC #1
Post-filtration
Step 9



Solvent system is 60:40 Hex:EtOAc
TLCs taken after the reaction. sm= p-toluidine starting material, i= imine intermediate, a= amine intermediate, p= final product. Visualized under UV light.

Yield Calculations:

Theoretical yield: Due to 1:1 mole ratio between *p*-toluidine (limiting reagent) and the amide product,

$n_{\text{p-toluidine}} = n_{\text{amide product}} = 3.7$ mmol

Actual yield: $n_{\text{amide product}} = m_{\text{amide product}}/M_{\text{amide product}}$

$= 0.70$ g$/285.34$ g/mol

$= 2.4$ mmol

$\% \, yield = \frac{actual \, yield}{theoretical \, yield}$ X 100%= 2.4 mmol/3.7 mmol X 100%= 65 %

Discussion:

In the experiment, the amide product is produced from taking three steps of reactions because the increasing nucleophilicity of the amine from alkyl substitution must be mitigated in order to prevent the formation of multiple cross-alkylated products. *P*-toluidine and vanillin are mixed together to undergo reductive amination. First, an acid/base reaction occurs as the *p*-toluidine donates a proton to the oxygen of the carbonyl to activate the *p*-toluidine as a better nucleophile and the vanillin as a better electrophile. The electrons on the negatively charged nitrogen of *p*-toluidine attacks the carbonyl carbon. Intramolecular proton transfer occurs to form a positively charged oxygen (H_2O^+) and a negatively charged nitrogen. With the elimination of a water molecule, the electrons of the nitrogen will push in, as pi electrons, to form the C=N double bond. The formation of the imine intermediate is indicated as the dark, orange solid. It marks the end of the nucleophilic substitution of *p*-toluidine to vanillin. Reductive amination proceeds further as 95% ethanol acts as the solvent to dissolve the imine intermediate and donate protons to the imine. By adding $NaBH_4$, the borohydride ion will provide a hydride to reduce the amine by attacking the alkene carbon. The pi electrons will move from the double bond onto the nitrogen to give it a negative charge. The electrons on the nitrogen of the imine will accept a proton from the ethanol, rendering the nitrogen neutral. As a result, reductive amination is completed and the amine product is formed as the solution turned clear and bright yellow. The solution should have been clear and colourless to ensure that the reaction was complete. There was coloured impurities present in the solution, which caused the solution to turn yellow.

The reaction is carried further by adding acetic anhydride and glacial acetic acid, which acts as a solvent for the acetic anhydride. A steam bath was used to facilitate the reaction by providing the reactants with more energy to overcome the activation energy. The nucleophilic amine intermediate attacks the carbonyl centre of the acetic anhydride. The nitrogen's positive charge is neutralized due to deprotonation. The electrons on the negatively charged oxygen will collapse to reform the carbonyl because $^-OOCCH_3$ leaves as a good leaving group. As a result, the amide product is formed when the reaction mixture turns clear and golden because the product remains dissolved in the glacial acetic acid. Cold water was poured into the solution to induce precipitation because the nonpolar phenyl rings and the lack of OH groups (no hydrogen bonding) causes the amide product to precipitate out of water. With the addition of an ice bath, it increases the rate of precipitation and the solution turned white and opaque. After a few minutes, the solution remained opaque but turned off-white, which goes to show that the precipitation proceeded effectively because the formation of solid product accumulated. After vacuum filtration, the product was isolated and the crystals expectedly appeared glossy and beige. The crystals should have been white, which means the solution contain colored impurities. To prevent this, when the reaction is completed, a small amount of decolorizing carbon should be added to the hot reaction solution for the absorption and removal of the coloured impurities.

A TLC was taken and it consisted of four lanes for: the amide product, the imine and amine intermediate, and *p*-toluidine as the starting material. In the *p*-toluidine lane, the relatively low R_f value of the spot is 0.45 because the electronegative nitrogen and the hydrogens are able to hydrogen bond with the silica gel, making the spot travel slower up the plate. In the imine intermediate lane, the spot with the R_f value of 0.40 is the excess vanillin and it has a lower R_f value compared to *p*-toluidine because the presence of highly electronegative oxygens and an OH group allows for stronger hydrogen bonding between the compound and the silica gel, which makes the spot travel even slower up the plate. The spot with the higher R_f value of 0.50 is the imine intermediate because the presence of phenyl groups renders the compound nonpolar. Therefore, it interacts with the silica gel less and travels farther up the plate. In the amine intermediate lane, the spot with the low R_f value of 0.21 is the amine intermediate because the presence NH and OH groups enables for good hydrogen bonding with the silica gel, making the compound travel slower up the plate. The spot with the R_f value of 0.40 aligns with the spot in the imine lane, confirming that the spot is the excess vanillin. Because the reaction is a one-pot synthesis, it is expected that the unreacted vanillin remains in the reaction mixture. The third spot is unreacted imine when $NaBH_4$ was added because it has the same R_f value of 0.50 as the spot in the imine intermediate lane. The fourth spot with a high R_f value of 0.58 is an unknown, nonpolar impurity. In the amide product lane, the R_f value of the spot, being the amide product, is the same as the amine product at 0.21 because both compounds are similar in structure and possess similar polarity. Therefore, both compounds will interact with the silica gel similarly and travel up the plate at the same rate. Because there is one spot in the amide product lane it shows that the purification was effective and that the amide product is absent of impurities.

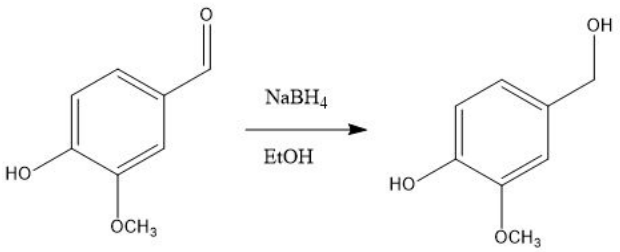
The percentage yield is 65%, which is sufficiently low and also expected. Because three reactions took place product was lost at each step as some intermediate molecules failed to react. In the TLC for the amine lane, the imine spot indicates that not all the imine reacted with the $NaBH_4$ to produce the amine intermediate. With less amine product to react with the acetic anhydride, there is less yield for the amide product. Also, there are side reactions that occurr with $NaBH_4$ and, thus, destroying the $NaBH_4$ as a reactant. $NaBH_4$ is a base sensitive to water so the water molecules, which was formed from the production of imine, can undergo a side reaction.

Scheme 2: Side reaction between sodium borohydride and water

$$NaBH_4 + H_2O \longrightarrow BH_3 + {}^{-}OH + H_2$$

As already mentioned, $NaBH_4$ may also be used up during the reduction of the unreacted vanillin. As $NaBH_4$ is used up in these side reactions, less of it is available to react with the amine intermediate.

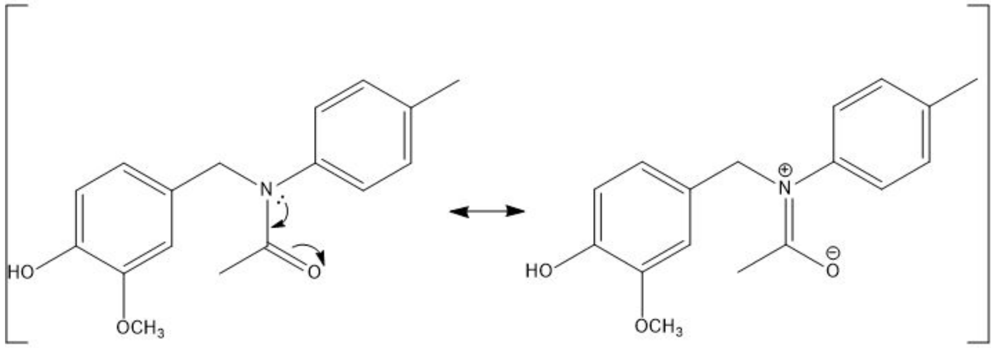
Scheme 3: Side reaction between sodium borohydride and vanillin



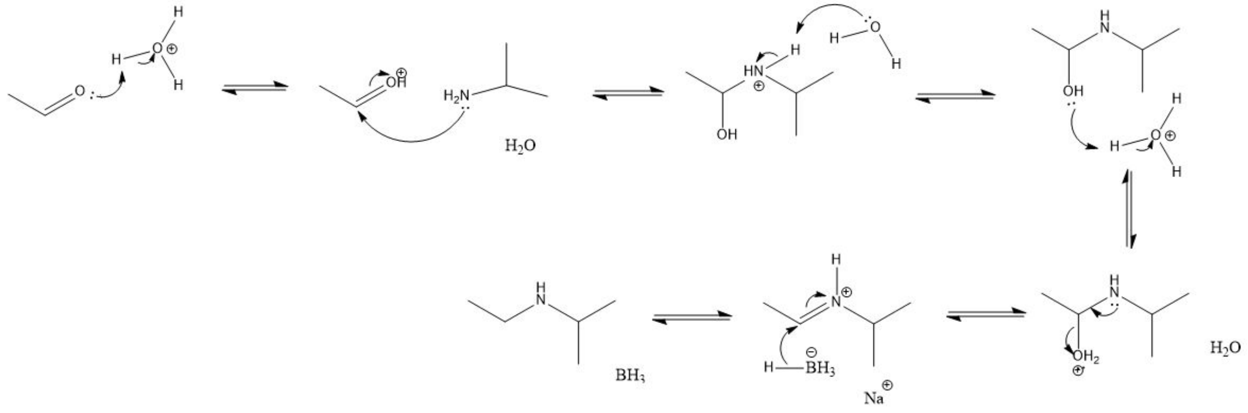
Therefore, the percentage yield of 65% is low for the amide product. As an improvement, $NaBH(OAc)_3$ can be used in the place of $NaBH_4$ to prevent side products and increase the yield of amide because it is found to be a highly selective reducing agent in reductive amination. The melting point range that was obtained by the melting point apparatus is 101.1-102.3 °C, which is lower than the literature value (102-108°C). The impurities are not from the reaction, since there was one spot in the TLC lane, so the impurities must have been appeared after the reaction. Dust particle may have landed in the solid, defected the crystal lattice, weakened the intermolecular forces and, therefore, lowered the melting point. As an improvement, multiple trials of melting point tests should have been done, using the product in the middle (which are exposed to less dust particles) to obtain more accurate readings.

Questions:

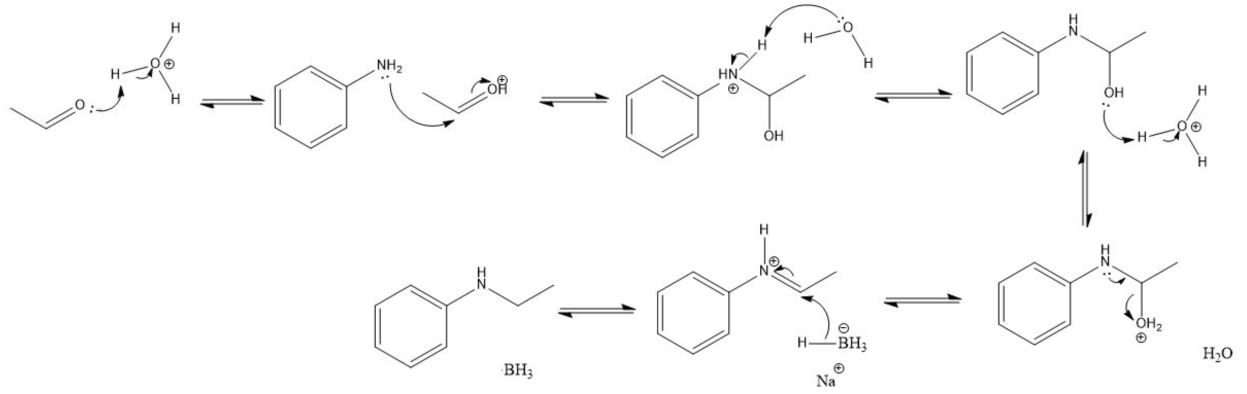
1. The experiment satisfied the green chemistry principle of minimizing the use of solvents. Because the first step of the experiment (reacting *p*-toluidine and vanillin) was solvent-free. No solvent was used and, as a result, it minimized the percentage of waste created from the reactions. Furthermore, water is a nontoxic byproduct of the first reaction and it will not harm the environment. Also, the second principle of green chemistry that the experiment satisfied is atom economy. Atom economy is the principle of which the synthetic method maximized the incorporation of the reagents in the process of producing the final product. Because the experiment called for 3.7 mmol of *p*-toluidine and 3.8 mmol of vanillin, which has a 1:1 molar ratio, the completion of the reaction barely left any unreacted vanillin. As a result, waste is reduced at the molecular level.
2. The amide product is a trialkylamine, which makes the compound less nucleophilic due to the steric hindrance caused by the alkyl groups being attached to the nitrogen. The free amine is a dialkylamine and is highly nucleophilic (being more carbon substituted without the steric hindrance) so it is more reactive. There is also resonance that delocalize the lone pair of electrons, which further decreases the nucleophilicity of the nitrogen in the amide product.



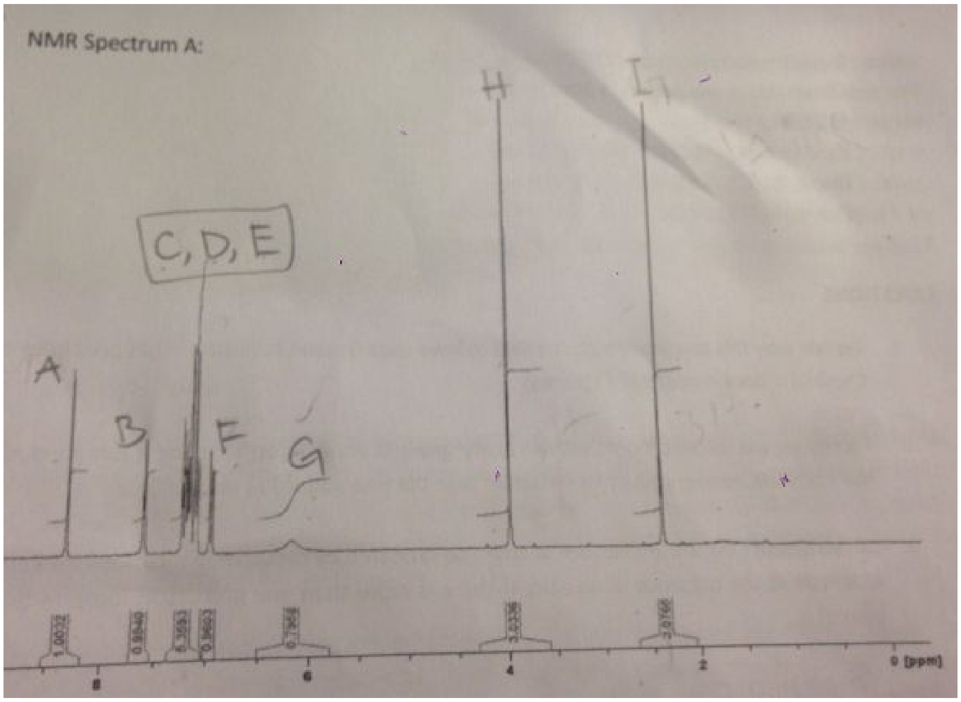
3. a)

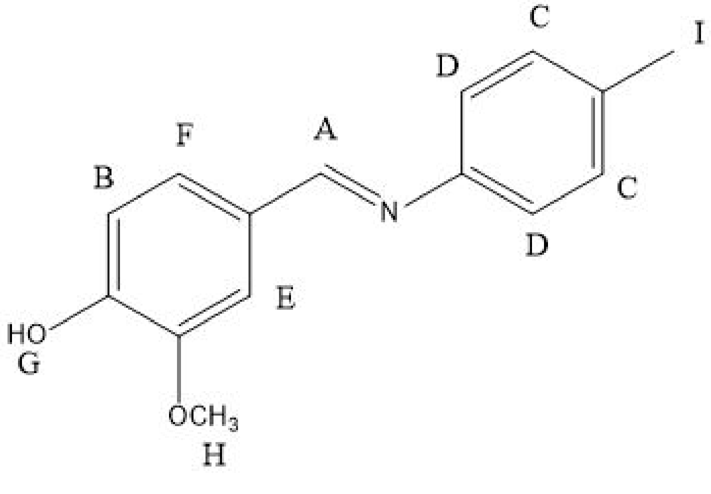


b)

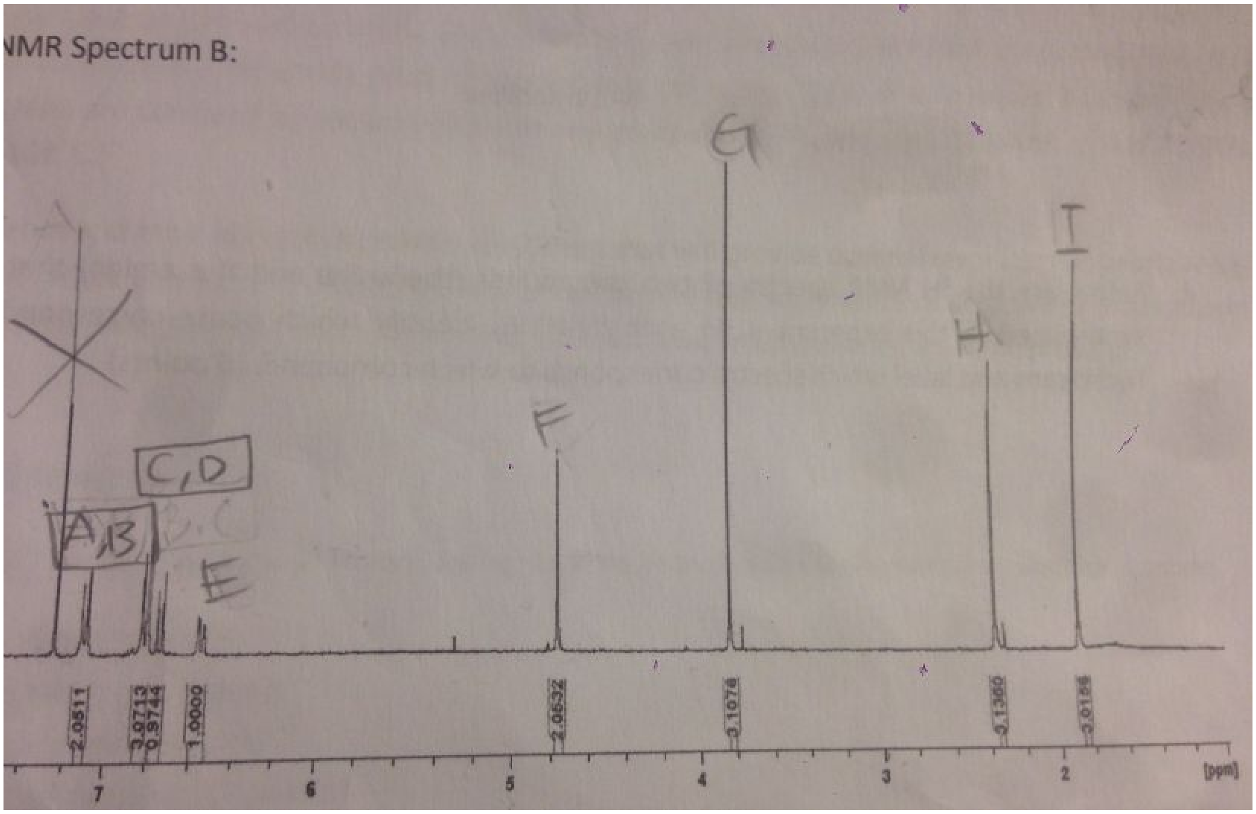


4. NMR spectrum A: Imine





NMR spectrum B: Amide



OH peak is not visible on spectrum; the 7.2 peak is the NMR solvent, CDCl3