Supporting Information

Perimidines Synthesis: a Simple and Effective Experiment of Organic Chemistry for Undergraduate Students

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CONTENTS

1. Chemical Hazards

All reagents, products, and solvents should be managed according to the guidelines provided in their respective Material Safety Data Sheets (MSDS) for chemicals on the Chemical Book Website [1] Students participating in these experiments must use personal protective equipment (PPE), such as nitrile gloves, lab coats, and eye protection. In addition, students must carry out their experiments inside a well-ventilated hood. Regarding the hazards associated with the chemicals, the following are noted: The reagent 1,8-diaminonaphtalene is harmful if swallowed, may cause an allergic skin reaction, is possibly carcinogenic and is toxic to the environment; benzaldehyde is acutely toxic by ingestion and inhalation, and it can cause serious irritation to the eyes; perimidines are reported as irritant agents in contact with the skin, eyes and they may be irritant to the respiratory system; the solvent hexane is irritant, flammable, and presents a danger to health and the environment; similarly, ethyl acetate solvent is flammable and irritant. Considering these factors, the reagents must be handled inside a fume hood. In addition, all waste produced in the lab should be disposed of in an appropriate container.

2. Information about the experiments

Organization

Initially, the students participated in a brief theoretical introduction to the laboratory’s techniques and the concepts to be explored in the class, among them, carbonyl addition and elimination reactions, TLC analysis, reaction extraction, recrystallization, melting point and spectroscopy techniques (NMR, IV and UV-Vis). After this, the students read the practical class script together with the instructor to clarify any questions about the procedures. Students were organized in pairs due to the limitations of the laboratory. Two groups were in charge of the synthesis using water as the solvent and the two other ones using glycerol. Then the students carried out the stoichiometric calculations necessary to perform the reaction.

Is important to note that all materials and reagents used in this experimental class can be obtained from commercial sources since most of them are simple, economically viable and easy to handle.

Lab Session 1

In the first class, 158.2 mg of macerated 1,8-diaminonaphthalene (MW = 158.20 g/mol, 1.0 mmol) was added to a 25 mL round-bottom flask, along with 2 mL of solvent (water or glycerol) and a magnetic stirring bar. The mixture is kept under magnetic stirring at room temperature until the 1,8-diaminonaphthalene dissolves. Later, 101.0 µL of benzaldehyde (MW = 106.12 g/mol, d = 1.05 g/mL, 1.0 mmol) was added. The reaction was kept under stirring and reagent consumption and was monitored by TLC using ethyl acetate/hexane (2:8) as the eluent. The detection methods used were UV light and iodine vapor.

After the reaction was completed, 5 mL of ethyl acetate and 10 mL of distilled water were added to the flask while agitation was maintained for a few minutes. Subsequently, the mixture was transferred to a separatory funnel and organic compounds were extracted with ethyl acetate (2 x 5 mL). The combination of the collected organic phase was dried with anhydrous sodium sulfate, filtered, and the crude product concentrated under a rotary evaporator resulting in a reddish-colored oily product.

The purification of the product was carried out by dissolving the reaction product in 10 mL of ethanol, which was then poured into a beaker containing 30 mL of cold distilled water and ice. After the precipitate was formed, the mixture was filtered using a pre-weighted filter. The filter is then left inside the hood at room temperature to dry.

Lab Session 2

In the second class, the students calculated the yield of the reaction weighing the filter that was left to dry. After that, the product was scraped with a scraper and collected in a small bottle so that the determinations could be applied. The purity of the product was determined by TLC analysis and melting point. The TLC analysis (Figure S1) was performed by applying product **3** and the reagents (**1** and **2**) in a chromatographic plate, using ethyl acetate/hexane (2:8) as the eluent and UV-light and iodine vapor as a visualization method. Furthermore, the retention factor (Rf) of product **3** and of the reagents was calculated.

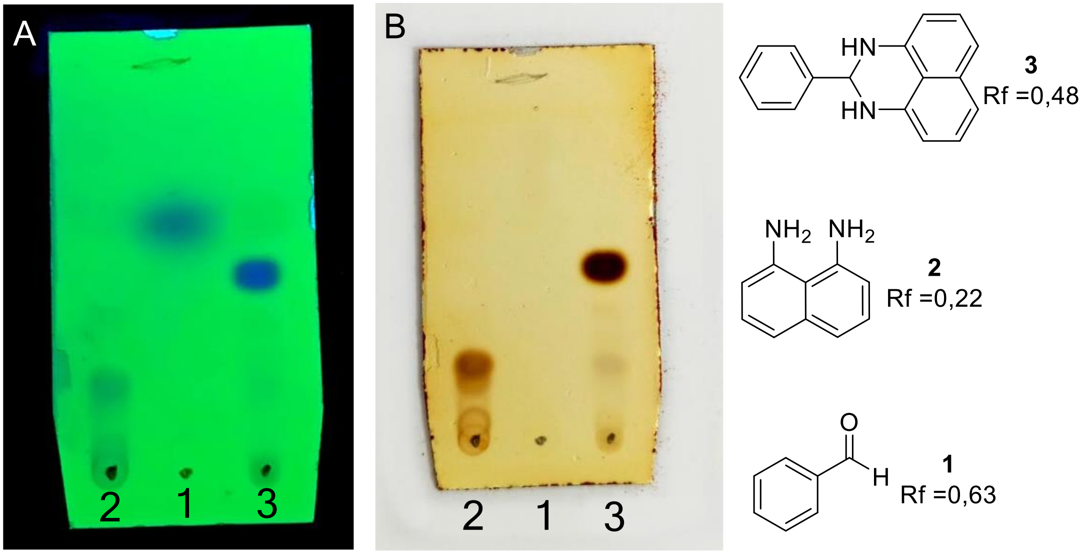


Figure S1. (A): UV-light visualization method; (B): iodine vapor visualization method.

The melting point (m.p.) was determined using the capillary method. The sample was placed inside a capillary, which was attached to a thermometer. The thermometer, along with the capillary, was then inserted into a Thiele tube containing mineral oil. The system was heated slowly utilizing a Bunsen burner. The m.p. was recorded at the point when the sample started to melt and when it was completely consumed.

After this, the product was characterized by spectroscopic techniques of NMR ^{1}H, NMR ^{13}C and FTIR. For NMR analyses, 30 mg of perimidine **3** was added in an NMR tube and dissolved in 0.5 mL of deuterated chloroform ($CDCl_3$). Thus, the ^{1}H and ^{13}C were obtained (15 min) and their signals were analyzed on the MestreNova software.

The analysis of the NMR signals was carried out with the students, as described below. In ^{1}H NMR it was possible to observe all signals of the aromatic hydrogens in the chemical shifts region between 7.62 and 6.49 ppm. In 5.44 ppm, a singlet with a relative integral for 1 H, related to the CH of the product may be observed, which confirms that the condensation reaction was successful since this carbon comes from the carbonyl group of the benzaldehyde. Finally, in 4.51 ppm it is possible to observe a broad singlet, with a relative integral for 2 H, referent to hydrogens linked to the nitrogens of the perimidine. In the upfield region, it is possible to observe some signals related to the solvents used in the extraction step of the reaction and a water residue; however, even though the product contained some impurities, it is important to show students the need to obtain a pure, dry compound. After this discussion, the ^{13}C spectra was analyzed, and it was possible to observe the 11 signals related to all carbons of the molecule, due to the equivalence of some carbons of the aromatic system. However, is important to notice the signal of alkyl

CH in 68.53 ppm related to a condensation of the carbonyl group. The ^{1}H and ^{13}C NMR spectrais shown in Figures S5 and S6, respectively.

The infrared behavior of the compound[1] was analyzed using Fourier transform infrared (FTIR) spectroscopy in attenuated total reflectance (ATR), as shown in Figure S7. The discussion of the spectra will focus on providing an example of the expected conclusions, which will be presented to the students and debated with them by the lecturer. Vibrational modes of amines are identified by the band at 3382 and 3358 cm^{-1}, attributed to the stretching mode ν(N-H). Additionally, the band at 3038 cm^{-1} corresponds to the stretching vibration ν(=C-H) of sp^2-hybridized carbons. Moreover, the bands 1595 and 1480 cm^{-1} correspond to the aromatic vibration modes of the ν(C=C). Furthermore, the presence of a monosubstituted aromatic ring is confirmed by the out-of-plane (oop) bending vibrations γ(=C-H) at 755 and 706 cm^{-1}, in addition to the overtone bands at 1911, 1826 and 1721 cm^{-1}. Another important consideration when analyzing the spectra is the absence of the band at 1700-1660 cm^{-1}, which corresponds to the stretching ν(C=O) of aromatic aldehydes.

The UV-Vis spectroscopy analysis was performed in order to identify and discuss the main absorption bands of the compound and to calculate the molar absorptivity coefficient (ε). The molar absorptivities were calculated according to the Lambert-Beer law (Equation S1), through several successive absorbance measurements at a given wavelength (233.5, 333.5 and 345.0 nm), for solutions of product 3 in ethanol at different concentrations (0.00459 at 0.0642 mmol.L^{-1}). With this data, it is possible to plot the graphics of absorbance against perimidine concentration (Figure S2-S4) to determine the linear regression (R^2) and the values of absorptivity molar at 233.5, 333.5 and 345.0 nm (Table S1).

$$A = \varepsilon . b . C \tag{S1}$$

where A is absorbance, ε is the molar absorptivity coefficient, b is the length of the light path and C is concentration.

Table S1. Value of the molar absorptivity coefficient and R of compound 3.

λ (nm);	**ε (10^4 L·mol^{-1}·cm^{-1})**	**R^2**
233.5	4.869	0.9971
333.5	1.343	0.9979
345	1.373	0.9980

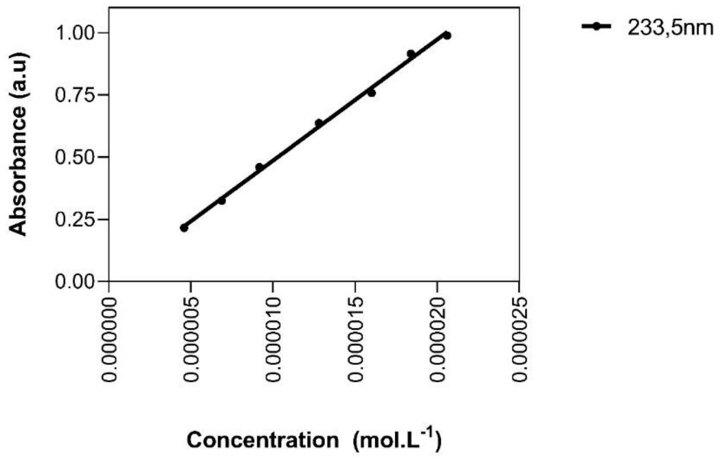


Figure S2 – Absorbance plotted against perimidine **3** concentration at 233.5 nm. The black line represents the linear fit. The coefficient of molar absorptivity was 4.869 x 104 L.mol^{-1}.cm^{-1} (R^2 = 0.9971).

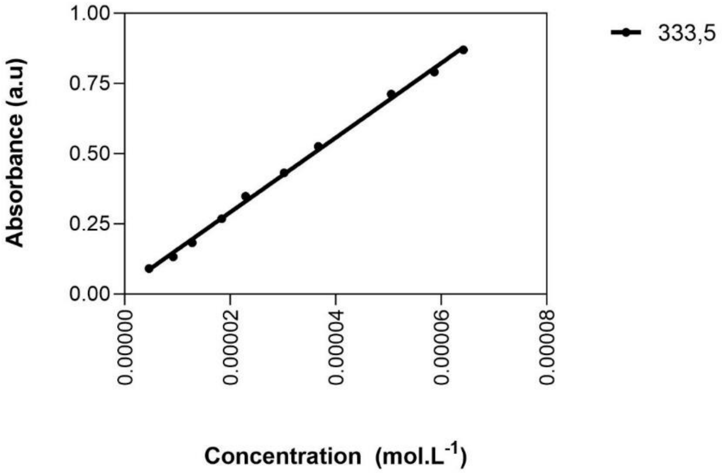


Figure S3 – Absorbance plotted against perimidine **3** concentration at 333.5 nm. The black line represents the linear fit. The coefficient of molar absorptivity was 1.343 x 10^4 L.mol^{-1}.cm^{-1} (R^2 = 0.9979).

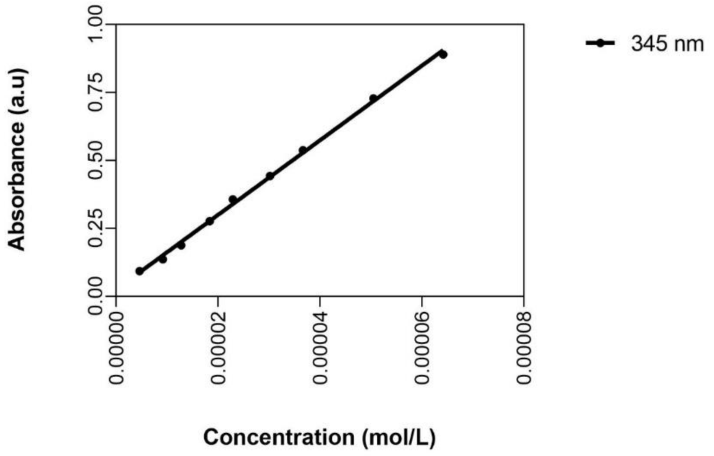


Figure S4 – Absorbance plotted against perimidine **3** concentration at 345.0 nm. The black line represents the linear fit. The coefficient of molar absorptivity was 1.373 x 10^4 L.mol^{-1}.cm^{-1} (R^2 = 0.9980).

3. Spectral data, ^{1}H NMR, ^{13}C NMR and FT-IR spectra for compound 3

2-phenyl-2,3-dihydro-1H-perimidine (**3**) [2]: *m.p.*: 128 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.62-7.23 (m, 9H); 6.50 (d, *J* = 6.5 Hz, 2H); 5.44 (s, 1H); 4.51 (bs, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 142.2, 140.2, 135.0, 129.7, 129.0, 128.0, 127.0, 118.0, 113.6, 106.0, 68.5.

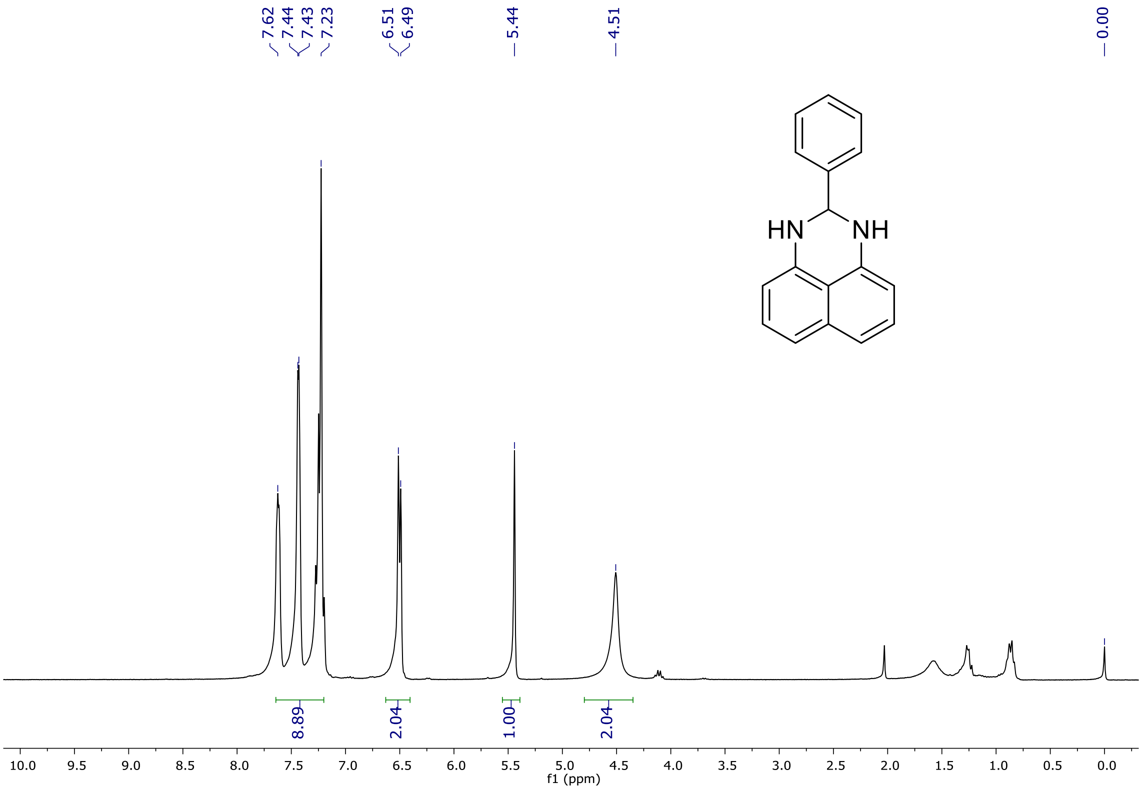


Figure S5 – ^{1}H NMR of product **3** ($CDCl_3$, 300 MHz).

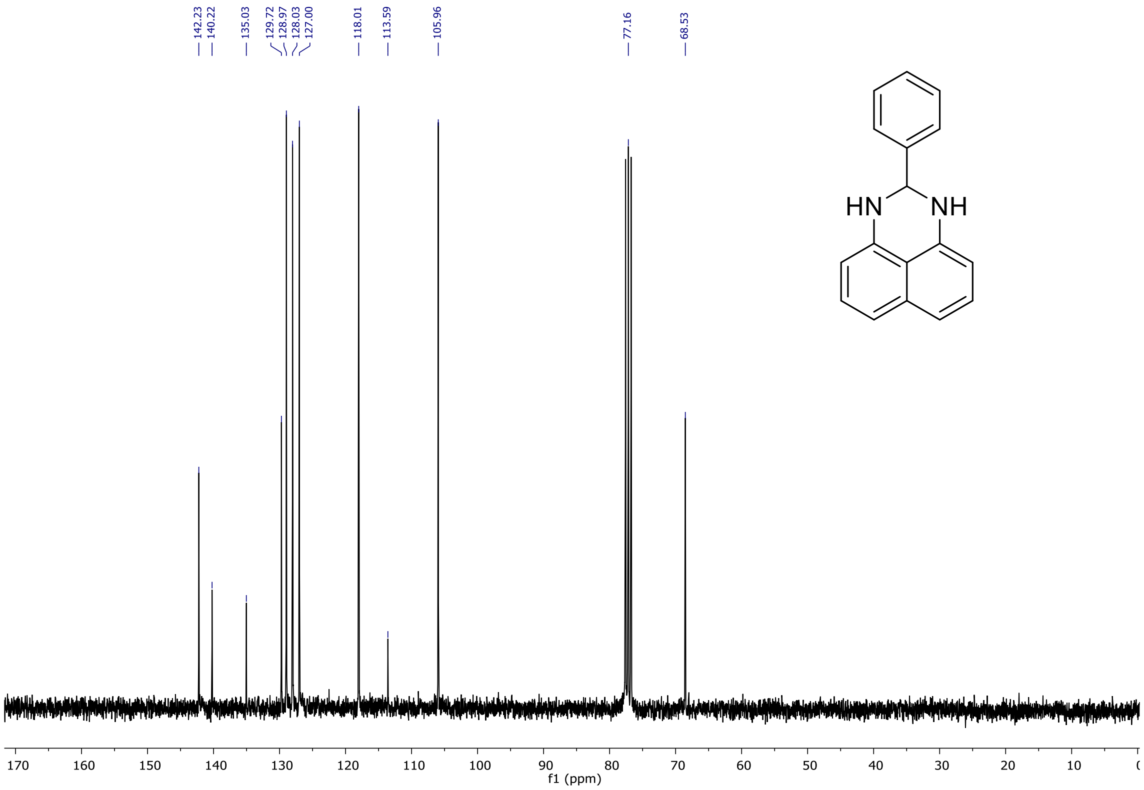


Figure S6 – ^{13}C NMR of product **3** ($CDCl_3$, 75 MHz).

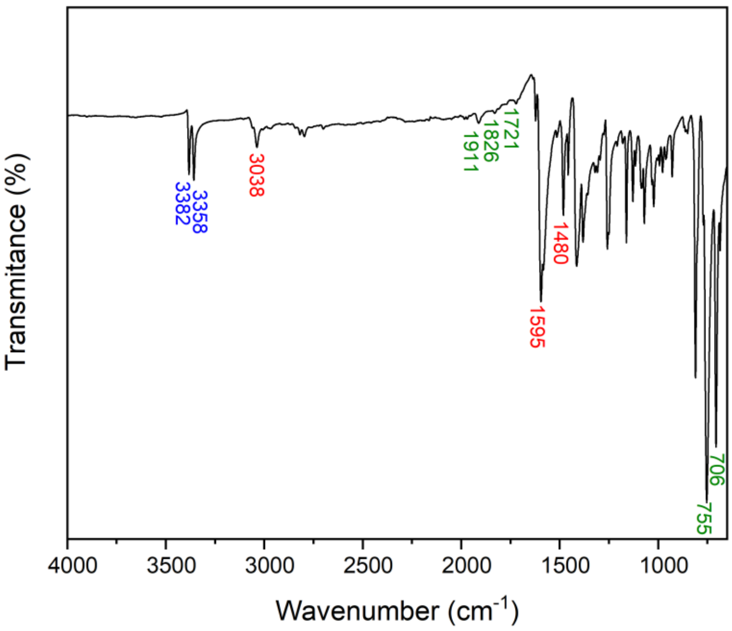


Figure S7 – Infrared spectra of product **3**.

4. Average time spent on each stage of the experiment

Table S1. Average time spent on each stage of the experiment.

Lab Session	Activity	Time (min)
1	Pre-experiment questionnaire	15
1	Introduction to theory and stoichiometric calculations	15
1	Preparation and reaction execution	60
1	Extraction and rotary evaporation	60
1	Recrystallization	30
2	Yield calculation and Rf determination (TLC)	30
2	Melting point determination	20
2	Analysis of ^{1}H and ^{13}C NMR and IR	60
2	UV-Vis analysis and absorptivity molar calculation	70

5. Pre-experiment questionnaire

1) What is thin layer chromatography (TLC)? What is Rf? Cite one use of TLC.

R: Thin layer chromatography (TLC, is an analytical technique used to separate the components of a mixture. The method relies on the differential affinity of these components for two phases: a stationary phase, which is a thin layer of an adsorbent like silica gel coated onto a plate, and a mobile phase, a solvent or solvent mixture that ascends the plate via capillary action. To quantify the migration of each component, the retention factor (Rf) is calculated, which is the ratio of the distance traveled by the substance to the total distance traveled by the solvent. One of the most common laboratory uses for TLC is to monitor the progress of a chemical reaction, allowing one to visualize the consumption of reactants and the formation of products over time.

2) What is the basic principle of the (discontinuous) solvent extraction process?

R: The principle of solvent extraction is the separation of substances from a mixture based on its differential solubility in two immiscible solvents, such as water and an organic solvent.

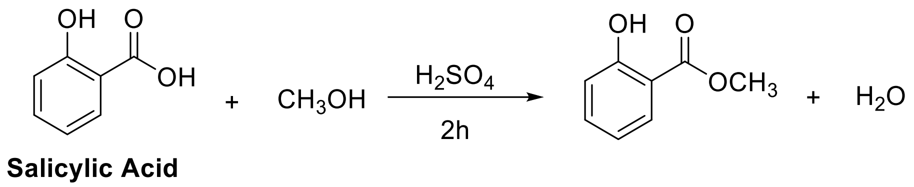
3) What do you understand by recrystallization?

Recrystallization is a method to purify a solid. The idea is to dissolve the impure solid in a hot solvent until it's saturated and then let the solution cool down slowly. As it cools, the pure compound forms clean crystals, while the impurities stay dissolved in the liquid.

4) How would you confirm the identity and purity of an organic compound?

R: Confirming the identity and purity of an organic compound requires a combination of physical and spectroscopic methods, such as measuring the melting point, TLC analysis, infrared (IR) and NMR spectroscopy and mass spectrometry (MS)

5) Methyl salicylate is a natural product found in many plant species. In the pharmaceutical industry, it is commonly used for cosmetic and analgesic purposes. In the laboratory, it can be synthesized by the following reaction:



In an experiment for the synthesis of methyl salicylate, 7.0 g of salicylic acid and 20 mL of absolute methanol were added. At the end of the process, 6.1 g of methyl salicylate was obtained. Calculate the yield of this reaction. **Data:** Salicylic acid: Molar mass = 138.12 g/mol; Methanol: Molar mass = 32.04 g/mol, Density = 0.792 g/ mL; Methyl Salicylate: Molar mass = 152.14 g/mol.

R: To calculate the percent yield for the synthesis of methyl salicylate, one must first determine the limiting reagent of the reaction. We calculate the number of moles of each reactant, starting with 7.0 g of salicylic acid (molar mass = 138.12 g/mol), which corresponds to approximately 0.05068 mol. For methanol (molar mass = 32.04 g/mol), we use its density (0.792 g/mL) to find the mass of 20 mL, which is 15.84 g, corresponding to about 0.4944 mol. Since the reaction proceeds in a 1:1 molar ratio, salicylic acid, being present in the smaller molar amount, is the limiting reagent. The theoretical yield is the maximum mass of product that can be formed, calculated from the limiting reagent. Thus, 0.05068 mol of salicylic acid would theoretically produce 0.05068 mol of methyl salicylate (molar mass = 152.14 g/mol), which equates to 7.71 g. The percent yield is the ratio of the experimentally obtained mass (6.1 g) to the theoretical yield. Therefore, the reaction yield is (6.1 g / 7.71 g) × 100%, which results in approximately 79.1%.

6. Satisfaction research of the students

1) The experimental procedure provided was relevant for carrying out the experiments.

a. I totally disagree (0,0%) *[1]
b. I disagree a little (0,0%)
c. I neither agree nor disagree (0,0%)
d. I somewhat agree (0,0%)
e. I totally agree (62,5%)

2) In my opinion, the experiment was completed satisfactorily.

a. I totally disagree (0,0%)
b. I disagree a little (0,0%)
c. I neither agree nor disagree (0,0%)
d. I somewhat agree (20,8 %)
e. I totally agree (41,7 %)

3) I consider that the experiments developed are relevant to the discipline of Experimental Organic Chemistry.

a. I totally disagree (0,0%)
b. I disagree a little (0,0%)
c. I neither agree nor disagree (0,0%)
d. I somewhat agree (0,0%)
e. I totally agree (62,5%)

4) The experiments developed helped me learn about experimental techniques frequently used in Organic Chemistry.

a. I totally disagree (0,0%)
b. I disagree a little (0,0%)
c. I neither agree nor disagree (0,0%)
d. I somewhat agree (0,0%)
e. I totally agree (62,5%)

5) I suggest that this experimental class continues to be developed for other students of the Experimental Organic Chemistry discipline.

a. I totally disagree (0,0%)
b. I disagree a little (0,0%)
c. I neither agree nor disagree (0,0%)
d. I somewhat agree (0,0%)
e. I totally agree (62,5%)

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[1] Reported as absolute number followed by percentage

*(number of students, % of total students)

7. Questionnaire for discussion of the experiment (Report)

1) List some applications of the perimidines.

2) What is the purpose of using thin layer chromatography (TLC) during the reaction? What is the retention factor (Rf) of the product obtained?

3) What is the purpose of water/glycerol in the reaction? Why was the reaction extracted (batch extraction) with ethyl acetate and water?

4) Research the melting point of the synthesized 2-Phenyl-2,3-dihydro-1 *H* -perimidine. Does the experimentally determined melting point agree with the literature data? Explain the result obtained.

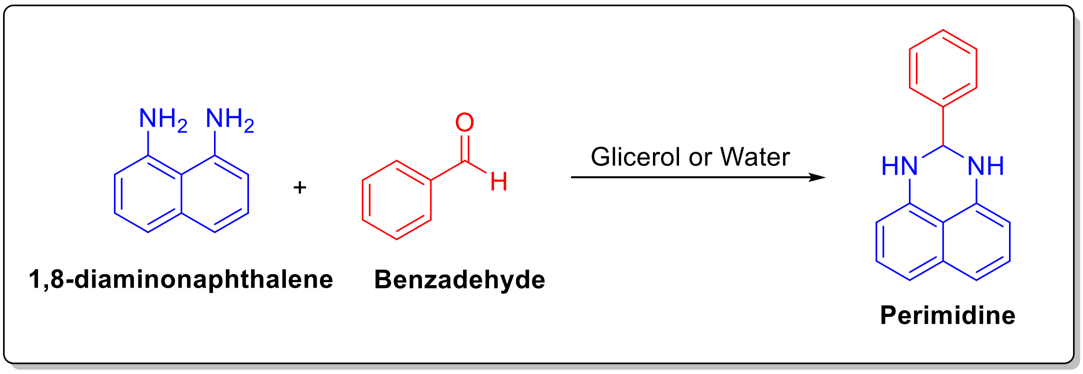
5) Based on the experimental results, calculate the reaction yield.

6) Provide the mechanism for obtaining perimidine through the condensation reaction between benzaldehyde and 1,8-diaminonaphthalene.

8. Practical class script for the first session

1. Synthesis of perimidines

Nitrogen-containing heterocyclic compounds are prevalent in nature and exhibit a wide range of biological properties. Perimidines are heterocyclic compounds containing two nitrogen atoms, which enhance the delocalization of π electrons in aromatic rings and provide a characteristic reactivity. These compounds exhibit a range of biological properties, including antifungal, antimicrobial, and antitumor activity. In addition, they have been extensively used as dyes for polymers. One of the main synthetic routes for the preparation of perimidine derivatives is from the condensation reaction of 1,8-diaminonaphthalenes with different carbonyl compounds, such as aldehydes (Scheme 1).



Scheme 1. Condensation reaction for the synthesis of perimidine in glycerol or water.

2. List of reagents

- 1,8-Diaminonaphthalene
- Benzaldehyde
- Anhydrous sodium sulfate
- Glycerol
- Anhydrous ethanol
- Ethyl acetate
- Hexane

3. List of materials

- Stirring plate
- Universal support
- Claw (pincer and ring)
- Micropipette 20 – 200 μ L
- Magnetic bar
- Capillaries
- Metal tweezers
- Watch glass
- 6 test tubes
- 50 and 100 mL beakers
- Glass rod
- Separating funnel
- Simple funnel
- Filter paper
- 2 100 mL Erlenmeyer flasks

- Chromatographic plates
- Iodine vapor tank
- UV lamp
- Ice bath container
- Pauster pipettes
- 25 and 100 mL round bottom flask
- 15 mL test tubes
- Scrapers

4. Experimental Procedure

Synthesis of perimidine in GLYCEROL

In a 25 mL round bottom flask add 0.158 g of powdered 1,8-diaminonaphthalene and 2 mL of glycerol. Stir to solubilize. Then add 101.0 µL of benzaldehyde. Keep the reaction under magnetic stirring and monitor the consumption of reagents by thin layer chromatography (TLC).

Synthesis of perimidine in WATER

In a 25 mL round bottom flask add 0.0791 g of powdered 1,8-diaminonaphthalene and 5 mL of distilled water. Stir to solubilize. Then add 60.6 µL of benzaldehyde. Keep the reaction under magnetic stirring and monitor the consumption of reagents by thin layer chromatography (TLC).

Procedure for thin layer chromatography

Separate chromatographic plates cut to the appropriate size for use. Apply aliquots of the reaction and reagents (benzaldehyde and 1,8-diaminonaphthalene) using a capillary to approximately 1.0 cm above the lower edge. Whenever applying each sample, wash the capillary with ethyl acetate.

Prepare the elution chamber by adding a hexane/ethyl acetate solution (8:2) to a 50 or 100 mL beaker. Be careful that the amount of solvent in the beaker does not exceed the level of the sample application point. Then, cover the beaker with a watch glass.

Place the prepared plates in the elution chamber and monitor the elution until the solvent reaches approximately 1.0 cm before the end of the plate. Use an iodine vapor chamber and ultraviolet light (254 nm) to analyze the results.

Discontinuous extraction

After the reaction is complete, add 10 mL of ethyl acetate and 10 mL of water to the flask and continue stirring for a few more minutes. Then transfer the mixture to a separatory funnel. Close the funnel with a suitable stopper. Hold the stopper with one hand and the stopcock with the other. Position the flask upside down at a 45° angle and shake it in circular motions. Remember to release the pressure occasionally. Attach the funnel to the ring clamp and wait for the phases to separate. Separate the organic (upper) phase from the aqueous (lower) phase in two Erlenmeyer flasks. Add the aqueous phase back to the funnel and repeat the extraction process two more times.

Dry the organic phase by adding anhydrous sodium sulfate. Filter the mixture through pleated filter paper and collect the filtrate in a 100 mL flask. Finally, concentrate the crude product by evaporating the solvents with a rotary evaporator.

Recrystallization

Dissolve the product in the flask with 10 mL of ethanol. Then pour this mixture into a beaker containing 30 mL of ice-cold distilled water (with a few pieces of ice). Filter the mixture through previously weighed pleated filter paper. After drying, weigh the product obtained and determine the reaction yield.

9. Practical class script for the second session

1. Determination of purity and identity of the perimidine

The melting temperature or melting point is a well-defined physical property for pure substances. Thus, it can be used to analyze the identity and purity of compounds. However, it cannot be considered a sufficient parameter for characterization, since different substances can have the same melting point.

The purity of a compound can also be analyzed by thin layer chromatography (TLC) through the absence or presence of secondary compounds on the chromatoplate. To analyze the identity of the product, the Retention factor (Rf) obtained can be compared with the literature data. However, as with the melting point, this analysis cannot be considered sufficient for characterization.

For a greater degree of reliability of the purity and chemical structure of organic compounds, more robust complementary techniques can be used, such as Nuclear Magnetic Resonance (NMR), Infrared (IR) and Mass Spectrometry (MS).

2. List of reagents

• Glycerin	• Ethyl acetate	• Hexane

3. List of materials

• Thiele tube	• Universal support and clamp	• UV lamp
• Thermometer	• Glass sweep	• 100 mL beaker
• Capillary tubes	• Rubber stopper	• Metal tweezers
• Bunsen burner	• Chromatographic plates	• Watch glass
• Rubber band	• Iodine vapor tank	• 3 test tubes

4. Experimental procedure

Thin layer chromatography (TLC)

Perform TLC with an aliquot of the purified product following the procedure described in the previous lesson. Use ethyl acetate/hexane (2:8) as the eluent. After elution, check using an ultraviolet lamp and an iodine vapor chamber to see if there are any compounds other than the desired product on the chromatoplate. Calculate the Retention factor (Rf) for the product. The Rf value of a compound is equal to the distance traveled by the compound divided by the distance traveled by the solvent front, both measured from the point of application.

Melting point determination

Initially, using a capillary with one end closed; introduce the sample through the open end. The capillary should then be dropped into a glass rod, in order to compact the solid at the bottom of the capillary. Using a latex rubber band, connect the capillary parallel to a thermometer so that the substance is at the same height as the bulb. Position the thermometer so that the bulb is completely submerged in the glycerin. Then, using a Bunsen burner, begin heating the glycerin. Heating should be gradual, and the material of the capillary should be carefully observed. Note the temperature at which the first drop of liquid forms and the temperature at which the last crystals of solid disappear. Compare the result with data from the literature.

Spectroscopy characterization

The NMR, IV and UV-Vis analyses depend on the equipment and the location where the analyses are performed since each spectroscopy technique laboratory has its own rules of use.

10. References

(1) *ChemicalBook*. https://www.chemicalbook.com/ (accessed 2025-03-17).

(2) Ann Harry, N.; Mary Cherian, R.; Radhika, S.; Anilkumar, G. A Novel Catalyst-Free, Eco-Friendly, on Water Protocol for the Synthesis of 2,3-Dihydro-1H-Perimidines. *Tetrahedron Lett.* **2019**, *60*, 150946. https://doi.org/10.1016/j.tetlet.2019.150946.