Hashemite University
Faculty of Pharmaceutical Sciences
Department of Pharmaceutical Chemistry

Practical Medicinal chemistry Manual

(131703425)

Student Name: _____

Student number: _____

Edited by:
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Course Description

Medicinal Chemistry is the study of how new drugs are developed and tested. In this course, you learn the basics of drug's synthesis, drug design and development. Medicinal chemistry requires an understanding of how chemistry, biology, mathematics and computing interact with each other to allow the scientist to effectively create new pharmaceuticals that will prevent or stop one or more disease conditions. This practical course in medicinal chemistry concerned with multistep synthesis of selected medicinal compounds.

The successful medicinal chemist is an expert organic chemist who has, or can acquire, sufficient knowledge in other disciplines to apply that knowledge to drug synthesis and design. We shall have opportunities to illustrate the dependence of medicinal chemistry on knowledge from other disciplines as we progress through this course. So our course will build on the experience gained from organic chemistry lab through the synthesis and characterization of complex molecules, the acquisition and interpretation of physical data and the investigation of chemical systems through computational techniques gained in analytical and instrumental labs. It consists of a series of laboratory-based experiments aimed at developing skills in the synthesis, safe handling and analysis of chemical substances of a range of different classes of compounds; an understanding of modern characterization techniques (e.g. chromatography, atomic and molecular spectroscopy); and the operation of instrumentation for the acquisition of kinetic, structural and thermodynamic data.

In addition to increased proficiency in standard techniques, this course provides an introduction into research-based chemistry through integrated and themed experiments. It will provide skill development in a range of techniques utilized in the modern chemistry laboratory. The subject provides experience across multiple traditional chemical disciplines whilst highlighting the importance of these disciplines in diverse 'real world' applications such as materials science and medicinal chemistry.

This Lab class is divided into two parts: the first part will focus on three synthesis cycles; multi – step Synthesis of Sulfanilamide, Benzocaine and Phenytoin, using techniques of organic compounds that an organic chemist uses daily; including crystallization, distillation, and extraction and will be will be run in groups. The second part focuses on Molecular modeling using computer software’s for drug design.

Course Objectives

1. Familiarize students with techniques commonly used in the medicinal chemistry laboratory.
2. Learn how synthesis a drug in multisteps and how get it in good yield and in pure form.
3. Demonstrate the effect of the different synthetic methodology.
4. Clarify theoretical concepts of chemical synthesis of drug molecules.
5. Working in the laboratory will give the students experience in handling and proper usage of laboratory glassware, equipment, and chemicals.
6. The students should learn how to keep an accurate and readable record of all experimental work and how to write a scientific report.
7. Students are expected at the completion of this course to master a variety of synthetic techniques including purification methods and should gain the ability to design a synthetic scheme for a proposed drug molecule.
8. Learn how to use different computer sofwares to draw & design drugs and learn how drug interact with its target.
9. Equip students with both oral and written communication skills, through your practical report and your written assignments and oral tasks, and through the communications that will be engaged in with lecturers, demonstrators and classmates, especially in the group laboratory work.

Intended Learning Outcomes (ILOs)

Successful completion of the course should lead to the following outcomes:

A. Knowledge and Understanding:

1. To understand.
2. To know the structures of different drugs.
3. To know the general laboratory safety and basic techniques.
4. To understand the principle of drug synthesis.

B. Intellectual skills (cognitive and analytical):

1. To realize any mistakes done during the assay and try to avoid it.
2. To be able to create a chemical assay for identification the quality of drug.
3. The student is expected to develop the ability to suggest suitable techniques to synthesis different drug molecules.
4. The student is expected to interpret scientific data and make sound scientific conclusions.

C. Subject specific skills

The student is expected to learn how to conduct chemical reactions within medicinal chemistry context this includes:

1. How to set up chemical instruments and tools in an experiment.
2. How to mix reactants, solvents and reagents within experimental context.
3. How to isolate and purify reaction products through (not limited to) chromatography, crystallization, distillation.
4. Identification and characterization of the final products through standard chemical procedures such as melting point, NMR, etc.

D. Transferable Skills

1. Team work.
2. Use oral communication to effectively transmit ideas and conclusions to a scientific audience.
3. Develop of problem solving and critical thinking skills.

Reading List / References:

Supplementary Textbook(s)

1	Wilson and Gisvold's Textbook of Organic, Medicinal and Pharmaceutical Chemistry, 12th Edition, 2011, Lea & Febiger.
2	Foye's Principles of Medicinal Chemistry, David A Williams, William O Foye and Thomas L Lemke, 6th Edition, 2008, Lippincott Williams & Wilkins.
3	Organic Chemistry: A Short Course. By Harold Hart, Leslie E. Craine, David J. Hart. Publisher: Houghton Mifflin College; 10th edition (January 1999) ISBN. 0395902258

Course Contents					
Week	**Credit Hours**	**ILOs**	**Topics**	**Teaching Procedure**	**Assessment methods**
1	1	A1, A3, C1, D1,	General instruction and safety rules And laboratory apparatus	Lecture+ discussion Video presentations & Animation	- Class participation
1	3	A1, A2, A3, A4, B1, B2, C1, C3, C4, B3, D3, D1	Benzocaine synthesis	Brief discussion + Video for laboratory work + Brain storming	- Class participation - Laboratory Report - Quizzes - Lab work evaluation - Assignment
2	3	A1, A2, A3, A4, B1, B2, C1, C3, C4, B3, D3, D1	Multi – step Synthesis of Phenytoin: Benzoin condensation	Brief discussion + Video for laboratory work + Brain storming	- Class participation - Laboratory Report - Quizzes - Lab work evaluation - Assignment
3	3	A1, A2, A3, A4, B1, B2, C1, C3, C4, B3, D3, D1	Multi – step Synthesis of Phenytoin: Oxidation of Benzoin to Benzil	Brief discussion + Video for laboratory work + Brain storming	- Class participation - Laboratory Report Quizzes - Lab work evaluation
4	3	A1, A2, A3, A4, B1, B2, C1, C3, C4, B3, D3, D1	Multi – step Synthesis of Phenytoin: condensation of Benzil with urea to form dilantin (Phenytoin).	Brief discussion + Video for laboratory work + Brain storming	- Class participation - Laboratory Report - Quizzes - Lab work evaluation
6	**Mid-term examination**				
7	3	A1, A2, A4, B2, B3, D1	Multi-step Synthesis of Sulfanilamide: Synthesis of Acetanilide	Brief discussion + Video for laboratory work + Brain storming	- Class participation - Laboratory Report - Quizzes - Lab work evaluation - Assignment
8	3	A1, A2, A3, A4, B1, B2, C1, C3, C4, B3, D3, D1	Multi-step Synthesis of Sulfanilamide: Synthesis of p-Acetamidobenzenesulfonyl chloride	Brief discussion + Video for laboratory work + Brain storming	- Class participation - Laboratory Report - Quizzes - Lab work evaluation - Assignment
9	3	A1, A2, A3, A4, B1, B2, C1, C3, C4, B3, D3, D1	Multi-step Synthesis of Sulfanilamide: Synthesis of p-Acetamidobenzenesulfonamide	Brief discussion + Video for laboratory work + Brain storming	- Class participation - Laboratory Report - Quizzes - Lab work evaluation - Assignment
10	3	A1, A2, A3, A4, B1, B2, C1, C3, C4, B3, D3, D1	Multi-step Synthesis of Sulfanilamide: Synthesis of p-Aminobenzenesulfonamide (Sulfanilamide)	Brief discussion + Video for laboratory work + Brain storming	- Class participation - Laboratory Report - Quizzes - Lab work evaluation - Assignment
11	3	A1, B4, D2, D3	Molecular modeling: In silico prediction of Ionization Constants of Drugs	Lecture+ discussion Video for laboratory work	- Class participation - Laboratory Report - Quizzes
12	3	A1, B4, D2, D3	Molecular modeling: SAR analysis using accelrys software	Lecture+ discussion Video for laboratory work	- Class participation - Laboratory Report - Quizzes - Lab work evaluation
14	**Final examination week**				

Grade Distribution		
Assessment	**Grade**	**Date**
1. Quiz	10%	weekly
2. Midterm exam	30%	To be arranged
3. Report	10%	weekly
4. Evaluation	10%	weekly
5. Final Examination	40%	To be arranged

Important regulations

- On average, students need to spend 3 hrs of study and preparation weekly.
- Excellent attendence is expected. According to the university policy, students who miss more than 15% of the lecture hours with or without excuse will be dismissed from the course
- At the beginning of the lab, be on time and don't leave before the end of the lab session without an accepted excuse
- If you missed a lab session, it is your responsibility to find out about any announcements or assignments you have missed
- For any clarification, please communicate your instructor at his posted office hours or by appointment
- Switch off your mobile or keep it silent throughout the lecture
- Listen well to the lab disscution and avoid side discussions, if you have a question, ask your instructor and not your collegue
- If you have any information, document your reference, if you didn't, then you broke the intellectual property rights law and the law will be applied
- Exams are scheduled to be given two times throughout the semester, your are expected to attend all. If not, make-up exams will be offered for valid reasons. It may be different from regular exams in content and format.
- Cheating, academic diconduct, fabrication and plagiarism will not be tolerated, and the university policy will be applied
- Each student is expected to familirize himself with **laboratory rules and safty precution.**

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General Instructions

A. Lab Safety Precautions

- Gloves and Eye protection Goggles must be worn at all time in the lab, regardless of what is being done. Prescription glasses (not sunglasses) are acceptable. Contact lenses provide no protection.
- Familiarize yourself with the location of fire extinguishers , safety showers, fire blankets and eye wash fountains and know how and when to use these devices.
- If chemical are splashed in or near the eye, wash immediately with clean and cold running water for 10 -15 min (remove contact lenses in case you have been wearing them). Consult a physician afterwards.
- When inserting glass tubing or thermometer into rubber stoppers, always lubricate both the glass and the whole with glycerin and protect hands with a towel.
- Never taste any compound in the laboratory.
- To determine the odor of a compound, bring the stopper of the bottle cautiously toward the nose. Do not inhale.
- Avoid any contact of chemicals with the skin, especially the face. Wash your hands as soon as possible after making transfers or other manipulations.
- When heating a test tube or carrying out a reaction in one, never point the tube toward yourself or your neighbor.
- Never heat an organic liquid directly over a flame except under a condenser.
- When refluxing a liquid, be sure that the condenser is tightly fitted. If a temperature below 95^{o}C is sufficient, use a steam bath rather than a burner.
- Before lighting a flame check to see that volatile liquids are not being poured or evaporated in your vicinity.
- Always turn a burner off as soon as you finish using it.
- As a general practice, and particularly if a burner is in use, avoid loose-fitting long sleeves and cuffs. Long hair should be tied back during laboratory work.

Lab Safety Rules

Science labs offer great opportunities for learning, teaching, and research. They also pose hazards that require proper safety precautions.

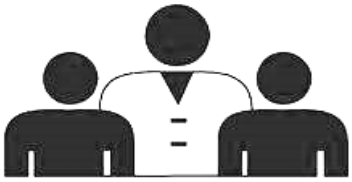
Stay safe when conducting your labs by following these guidelines.

Dress appropriately

Tie back long hair, and wear suitable gloves, goggles, and other protective equipment.

Proper supervision

Don't perform lab experiments without instructor supervision (unless given permission to do so).



Know location of emergency numbers & safety equipment

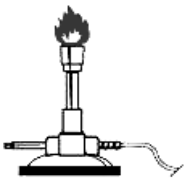
Know the location of safety equipment and emergency phone numbers (such as poison control) so you can access them quickly if necessary.

No food

Don't eat or drink in the lab—and never taste chemicals.

ID hazards

Identify hazardous materials before beginning labs.

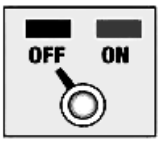


Be attentive

Be attentive while in the lab. Don't leave lit Bunsen burners unattended or leave an experiment in progress.

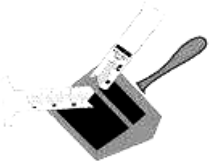
Be careful when handling hot glassware

Turn off all heating appliances when not in use. Keep flammable objects away from your workspace.



Keep a clean workspace

Don't obstruct work areas, floors, or exits. Keep coats, bags, and other personal items stored in designated areas away from the lab. Don't block sink drains with debris.



Handle glassware carefully

Properly dispose of anything that breaks. Report cuts, spills, and broken glass to your instructor immediately.

Clean up

After completing the lab, carefully clean your workspace and the equipment, and wash your hands.

- Any experiment involving the use or production of objectionable (i.e. poisonous or irritating) liquids or gases, must be performed in the hoods.

B. Laboratory rules

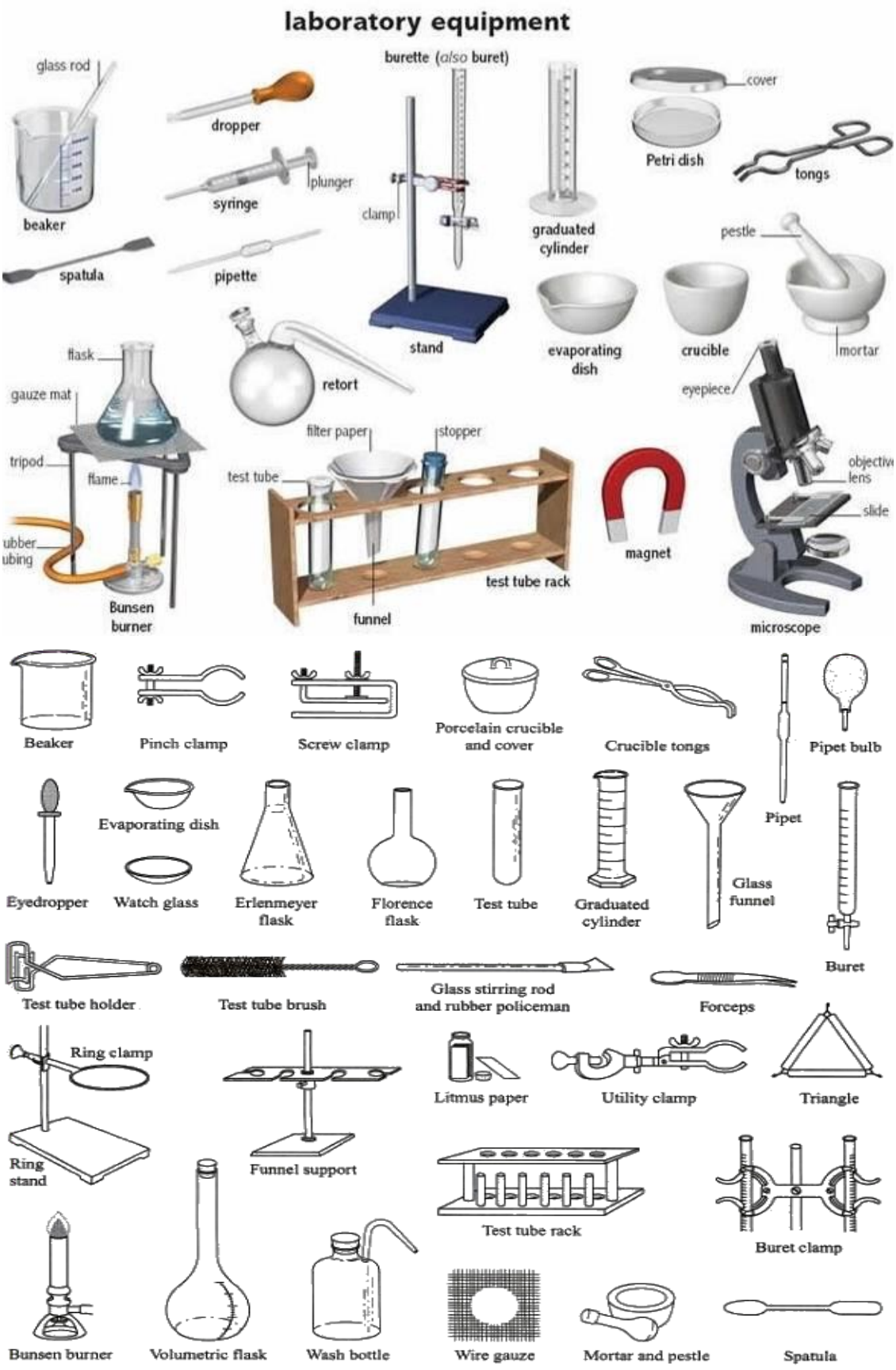
- Each student is expected to attend each lab session and to be in the laboratory on time. Those students, who come early, should wait inside the lab but never gather in the corridors.
- Each student must wear a clean and buttoned up lab coat with his\here tag name before enter your lab.
- Skirt, short clothe, sandal (open shoes) and head caps forbidden in lab.
- Smoking, Drinking, Eating or Chewing gum is prohibited in the lab.
- Each student is responsible for keeping the laboratory clean and in good order.
- Coats, book and personal belongings should be kept in your lockers. Do not bring them with you to the lab.
- All working areas and balances must be kept clean.
- Powdered drugs, greasy or waxy materials or any insoluble waste materials will block the sink if thrown in to it . Thus, dispose them properly in a waste basket. Water immiscible organic solvents and other liquids should be discarded in a designated waste – solvent can but never poured in to a sink.
- Chemicals that react vigorously with water, such as acid chlorides or alkali-metals should be decomposed in a hood in a suitable way.
- If students are assigned to work as groups, each group is expected to work quietly and independently.
- Do not carry reagent bottles to your desk.
- Never return excess materials to reagent bottles.
- Each student must bring:
  - ✓ Two hand towels
  - ✓ Sponge for disk cleaning
  - ✓ Detergent
  - ✓ Matches
  - ✓ Marker
  - ✓ Pair of gloves (nitrile gloves is preferred)
  - ✓ Calculator to each lab

You Will Be Held Responsible For Each Rule Mentioned Above, Any Violation Will Expose You To Either Being Dismissed From The Lab Or Losing Evaluation Marks Of That Particular Experiment Without Prior Notification

Common problems / easy solutions:

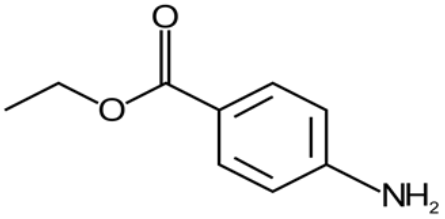
1- **NH_4OH:** Serious eye damage if contacts with eye. Very destructive to mucous membranes. Corrosive – severe burns
2- **Chlorinated solvents** such as $CHCl_3$ and DCM. Dispose in designated waste-solvent bottles found inside the hood.
3- **Chlorosulfonic acid :** Corrosive acid that may cause severe skin mburns and eye damage, vapor extremely irritating to lungs and mucous membranes and is fatal if inhaled.
4- **Alkali metals**: must always be kept covered with dry organic inert solvent such as Kerosene or paraffin oil. Remaining metal pieces, tools, knifes and spatulas must be placed in a beaker, then treated with absolute dry ethanol and leave it bubble (H2 gas) for minutes.
5- **Mercury waste** from a broken thermometer must be treated with solid sulfur powder bit never thrown in the waste basket.
6- **Ether** is very volatile and highly explosive. Keep in cold place. Dry solvent bottles on the shelves can be easily oxidized upon evaporation and leave explosive compounds. Please inform the technician.
7- You must report any **broken glass**. Don not remove yourself.
8- Do not draw any liquid with your mouth or smell any chemical.
9- **Concentrated mineral acids and bases** should always be kept at the hood and you should wear gloves when using.
10- Maintain labels on chemical containers received from manufacturers and label secondary containers. Replace old and deteriorated labels.
11- **Smelly chemicals** such as acetic anhydride, glacial acetic acid, pyridine, and benzaldehyde should be kept always in the hood and disposed in waste closed bottles.
12- Radioactive material, biological and chemical hazards should be handled and disposed in collaboration with faculty administration and local civil defense agency following special protocols.

Labs common glassware



Cycle 1

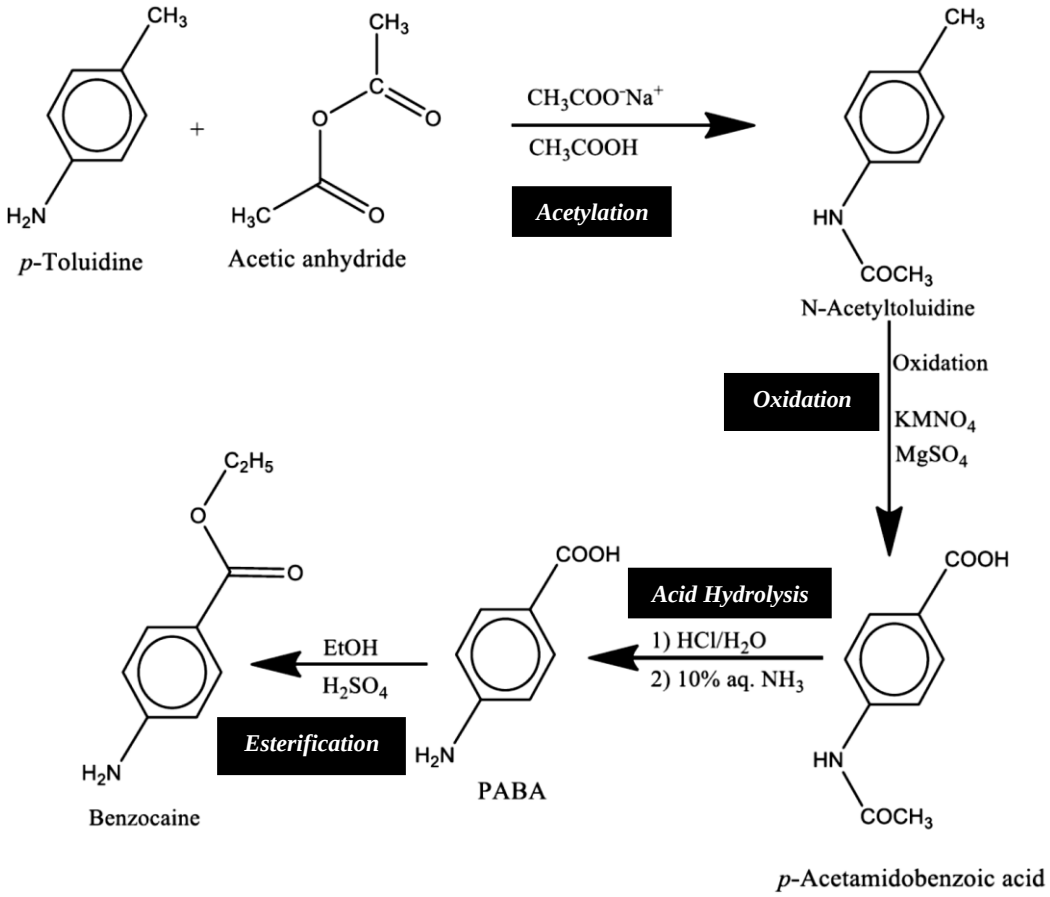
Multistep Synthesis of Benzocaine



Target Product

- Benzocaine is a local anesthetic from the ester family.
- The drug benzocaine is used in multiple forms including lotion, gel, liquid, lozenges, and sprays as a topical pain reliever.
- When Benzocaine is applied in any form, it temporarily numbs or blocks the nerve endings by inhibiting the voltage dependent Na channels on the neuron membrane, which leads to decrease in the amount of pain.

General scheme of synthesis



Four-step synthesis is used to create benzocaine:

Part 1: Synthesis of p-Acetotoluidide

Part 2: Synthesis of p-Acetamidobenzoic acid

Part 3: Synthesis of p-Amino benzoic acid (PABA)

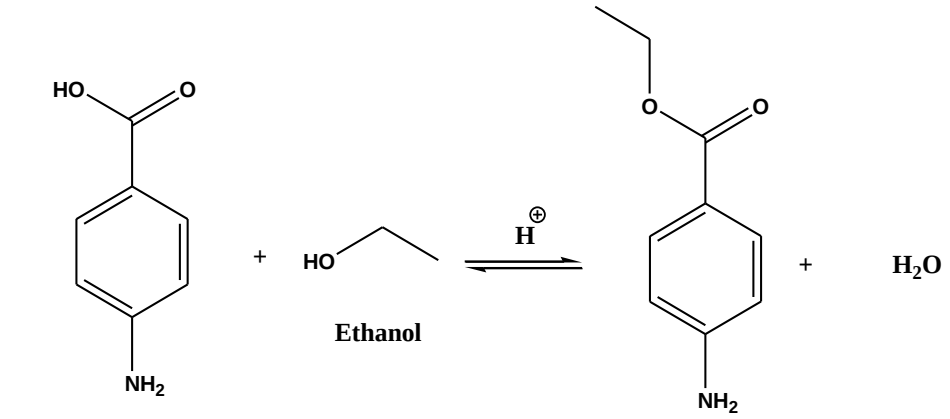
Part 4: Synthesis of Benzocaine

You will see the mechanism only for the forth steps.

Experiment 1

Multistep Synthesis of Benzocaine

Part 4: Benzocaine synthesis



p- aminobenzoic acid

PABA

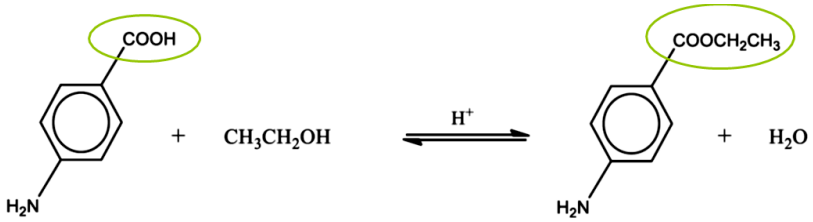
Ethyl p-aminobenzoate
(Benzocaine)

- Benzocaine is synthesized through the Fischer esterification of p-aminobenzoic acid (PABA) and ethanol, using sulfuric acid as a catalyst.
- PABA: is amphoteric that has weak acidic and weak basic properties.

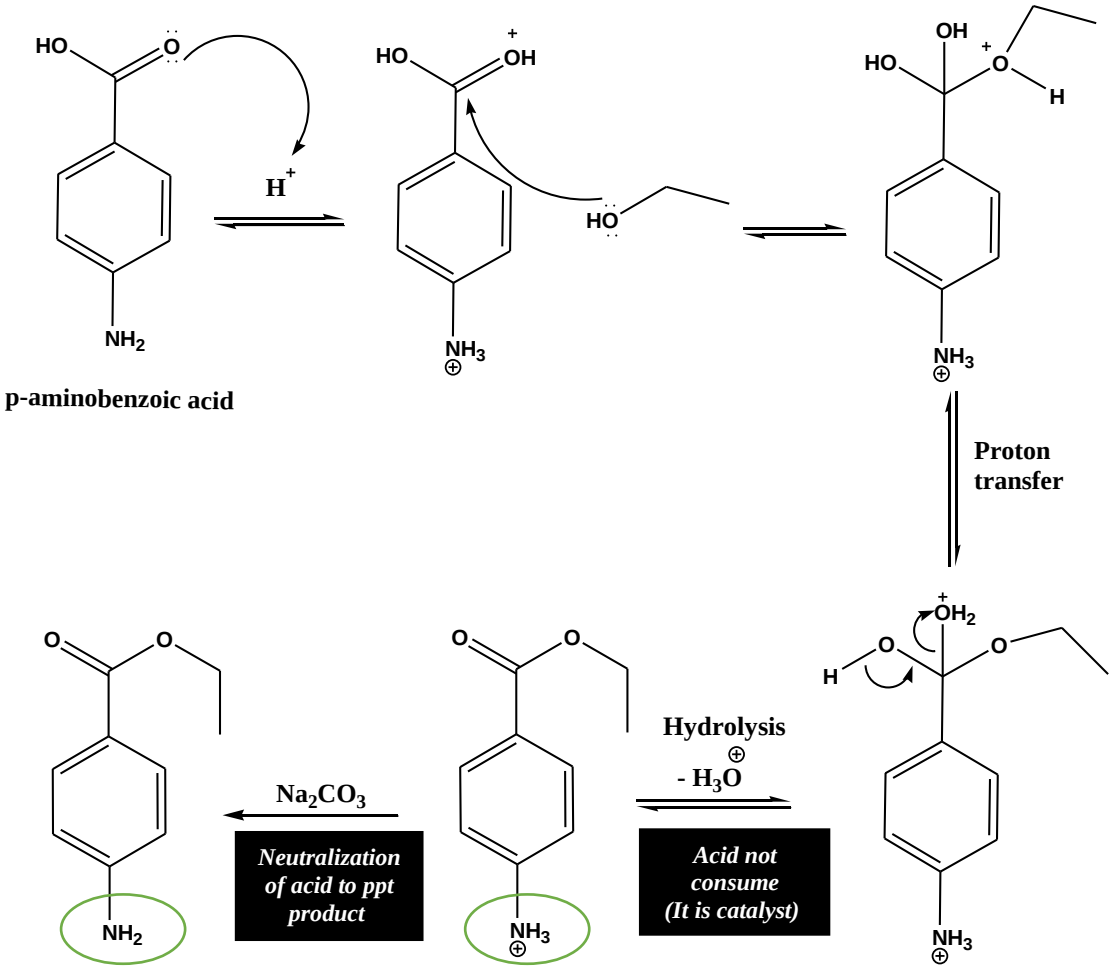
5 Assignment

5.1 Find a commercial synthesis pathway of PABA.

Mechanism



- This reaction is called **Fischer esterification reaction**: a type of condensation reaction ***(both way reaction- limited yield)***
- It is acid catalyzed *(it not consume in the reaction).*
- It proceeds very slowly in absence of strong acids as HCl or H2SO4.



<u>To increase the ester yield :</u>

- *Use excess from the reactants*
- *Remove water from reaction mixture as it is formed*

Procedure

1. In a 100 ml round-bottomed flask place 1.32 g of PABA, 10 ml of ethanol, and 1 ml of sulfuric acid (add cautiously). Add a couple of boiling chips[*] then attach a reflux condenser and heat under reflux for 1 hour.
2. Cool the solution to room temperature, neutralize with 10% sodium carbonate (foaming), and **extract**[⁂] with two 10-ml portions of dichloromethane (DCM) *[use separatory funnel in extraction]*. Then dry the combined organic layers over anhydrous magnesium sulfate *[drying agent]*.(1)
3. Remove the dichloromethane by distillation using a steam bath as a heat source.
4. Then recrystallize the residue from methanol-water [**Mixed solvent recrystallization**[‡]].(2)

(1) Add 3-4 gm of anhydrous magnesium sulfate, swirl the mixture for about 5 minutes, and then remove it by gravity filtration.

(2) Add 5 ml portions of methanol (with heating) until all the amount is dissolved, then add water drop wise until the solution becomes turbid, after that cool it in an ice bath to complete crystallization.

	Weight (gm)	**M.P**
Benzocaine		

2 *Brain storming Question*

2.1 Discus the reason behind doing extraction in DCM, and define the component for each layer.

2.2 Discus the reason behind adding drying agent for the organic layer.

[*] *Refer to appendix V: Boiling chips*

[‡] *Refer to appendix III: Recrystallization-Mixed solvent recrystallization.*

[⁂] *Refer to appendix VII: Extraction.*

pre-lab.

Report sheet (1)
Synthesis of Benzocaine

Student name:

Objectives :

Compound	MW (g/mol)	Solubility in water	Hazards
p-aminobenzoic acid			
HCl			
Benzocaine			

Find out :

1. *Mwt of Benzocaine……*
2. *The melting point of Benzocaine ……*

Draw a simple flowchart for the procedure of the exp. Or write an outline steps in your own words?

Post-lab.

Report sheet (1)
Synthesis of Benzocaine

Student name:

1. data and results:

The actual yield of Benzocaine:

The theoretical yield of Benzocaine(show detailed calculation):

The yield%:

2.chemical equations:

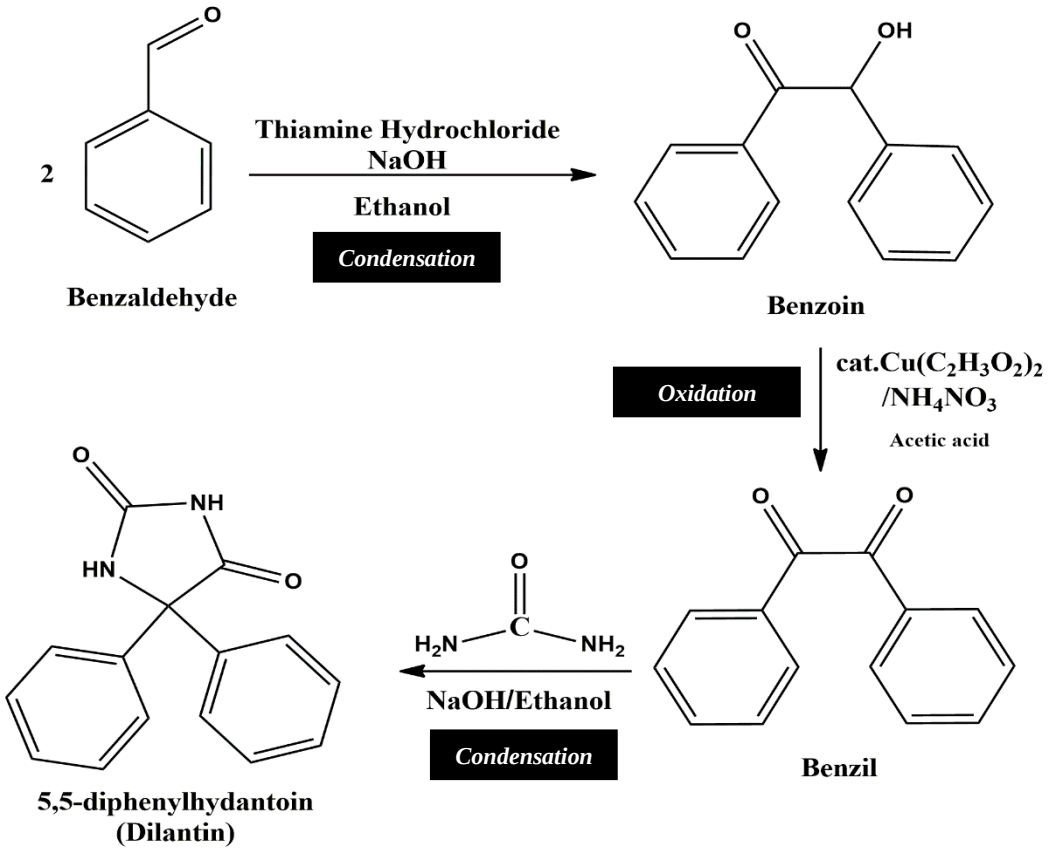
Write down the balanced chemical equations that represent the preparation of Benzocaine:

Cycle 2

Multistep synthesis of Phenytoin

- Phenytoin, sold under the brand name Dilantin among others.
- It is an anti-seizure medication.
- It is useful for the prevention of tonic-clonic seizures and partial seizures.
- Phenytoin blocks the spread of seizure activity in the brain by causing voltage dependent block of the voltage gated Na channels.
- It may also be used for certain heart arrhythmias or neuropathic pain.
- It doesn't have sedative hypnotic activity
- It can be taken intravenously or by mouth

General scheme of synthesis



Phenytoin can synthesize from benzaldehyde in three synthetic steps:

Part 1: Benzoin condensation.

Part 2: Oxidation of benzoin to benzil.

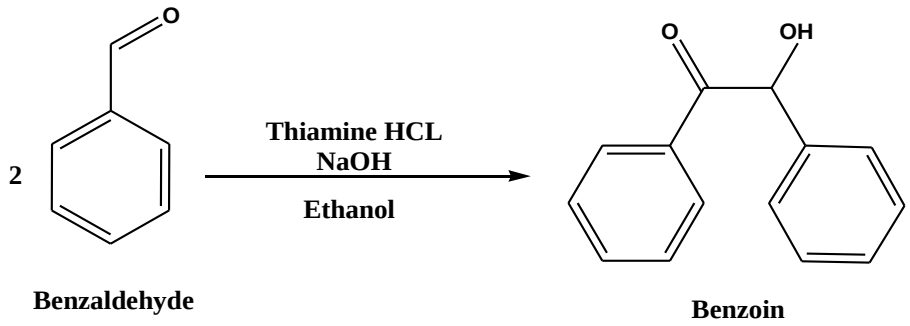
Part 3: Condensation of benzil with urea to form phenytoin.

You will see the mechanism for each steps.

Experiment 2

Multistep synthesis of Phenytoin

Part1: Benzoin Condensation



Benzaldehyde **Benzoin**

- Benzoin is produced by dimerization of 2 molecules of benzaldehyde, The benzoin condensation[§] is in fact a dimerization and not a condensation because a small molecule like water is not released in this reaction
- This reaction doesn't occur spontaneously. It needs a **catalyst** to render the aldehydic carbon acidic so that it can be deprotonated and function as a nucleophile to attack the second benzaldehyde carbon.
- Cyanide* $[K^+C \equiv N^-]$ can use as a catalytic reagent use at 75°C (give faster rate) but they are extremely poisonous.
- **Thiamine** (contains a thiazole unit) is the catalyst was used here at Room temp, (give very slow rate).

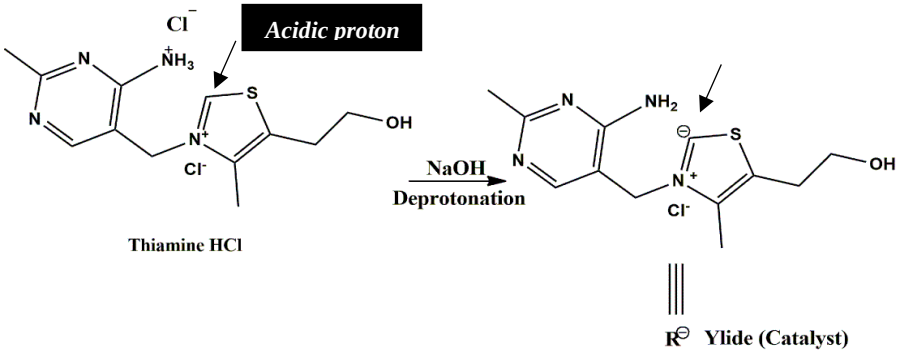
- *Thiamine (Vit B1) is non-toxic (edible) material*
- *Thiamine is heat sensitive and may decompose if heated vigorously*
- *Instead of running the reaction at elevated temperature. We will allow it to proceed closer to room temperature for at least 24 hours*

[§]***Condensation reaction:*** *a reaction in which two molecules combine to form a larger molecule, producing a small molecule such as H_2O as a by-product.*

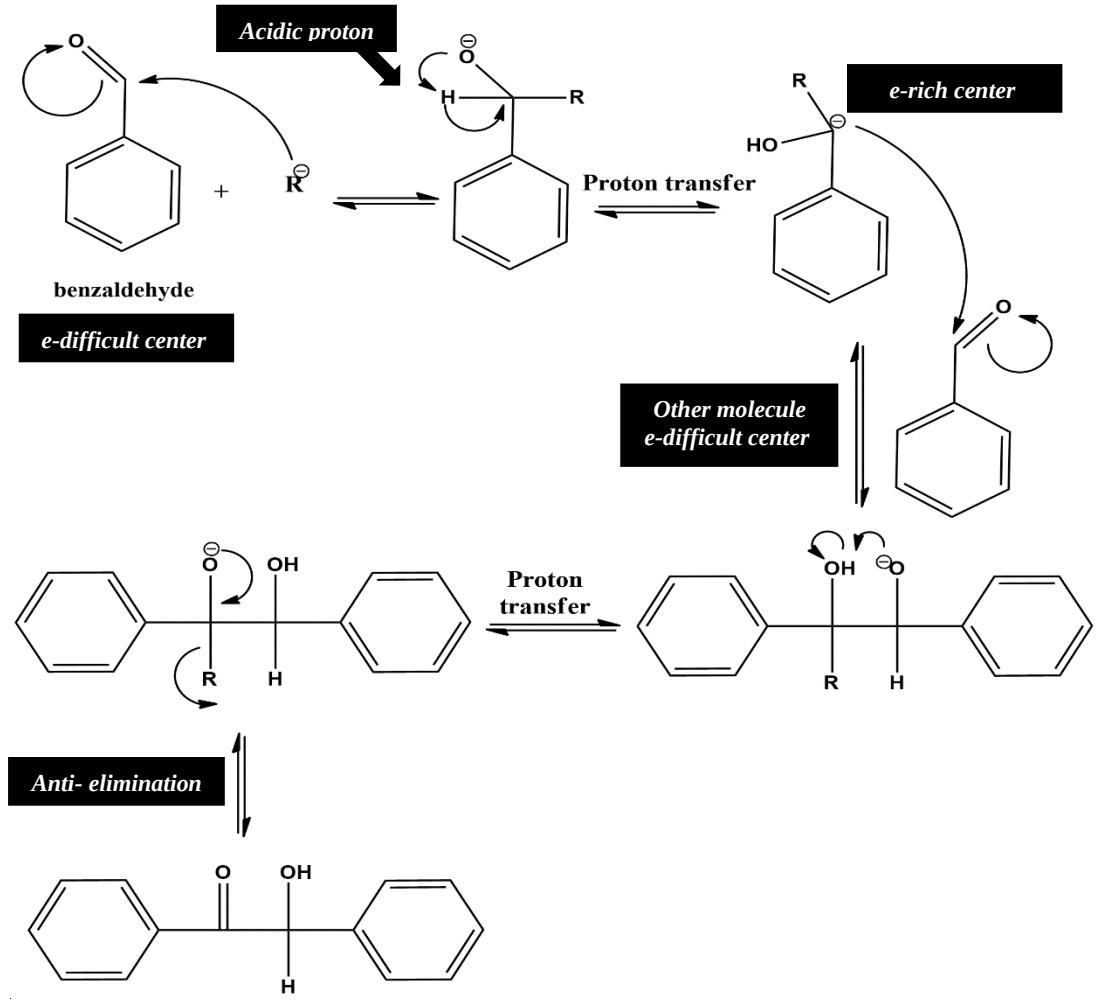
[*]*Refer to appendix VIII: Benzoin condensation using cyanide.*

Mechanism

First: Formation of the catalyst (Ylide*):



Second: The condensation reaction start with <u>umpolung</u>‡ to the carbonyl center of benzaldehyde:



Yalide: *is a species with opposite formal charges on <u>adjacent</u> atoms (both on same bond, no distance between them like in zwitter ion).*
***zwitter ion**: a molecule or ion having separate positively and negatively charged groups.*

‡***Umpolung** or **polarity inversion** in organic chemistry: is the chemical modification of a functional group with the aim of the reversal of polarity of that group. This modification allows secondary reactions of this functional group that would otherwise not be possible.*

Procedure

1. In a stoppered E. Flask, prepare a solution of 1.04 g of thiamine hydrochloride in 3 ml of water[‡].
2. When all the thiamine hydrochloride has dissolved, add 8 ml of 95% ethanol with mixing.
3. Cool the solution for a few minutes in an ice bath *[because thiamine is heat sensitive]*.
4. Very carefully and slowly add 3 ml of 10% NaOH with mixing "making sure that the temperature of the solution never rises above 20 C" *[because NaOH with water exothermic]*.
5. Add to the mixture 7 ml of pure Benzaldehyde with mixing.
6. Stopper the flask and allow it to stand at room temp at least overnight (longer period do no harm). At the end of the reaction period, the benzoin should have separated as fine crystals.
7. Cool the reaction mixture in an ice bath to complete the crystallization.
8. Collect the product by vacuum filtration and wash them thoroughly with two 7 ml portions of cold 50% ethanol and several portions of water[§] *[color change from yellow to white]*.
9. Drain well and leave your product to dry (no need for recrystallization[*]).

	Weight (gm)	**M.P**
Benzoin		

3 *Brain storming Question*

3.1 Discus the chirality of the product, does it optically active? Explain your answer.

[‡] *Water is required in this reaction BUT in specific amount:*

- *Too much water will force benzaldehyde out of the solution preventing an efficient reaction.*
- *Too little water prevents thiamine-HCl from dissolving.*

[*] Re-crystallizion an done using 95% ethanol.

[§] *Ethanol to remove excess benzaldehyde and water to get rid of thiamine.*

Pre-lab.

Report sheet (2)
Benzoin Condensation

Student name:

Objectives :

Compound	MW (g/mol)	Solubility in water	Hazards
Thiamine hydrochloride			
NaOH			
Benzaldehyde			
Benzoin			

Draw a simple flowchart for the procedure of the exp. Or write an outline steps in your own words?

Post-lab.

Report sheet (2)
Benzoin Condensation

Student name:

1. data and results:

The actual yield of Benzoin:

The theoretical yield of Benzoin (show detailed calculation):

The yield%:

2.chemical equations:

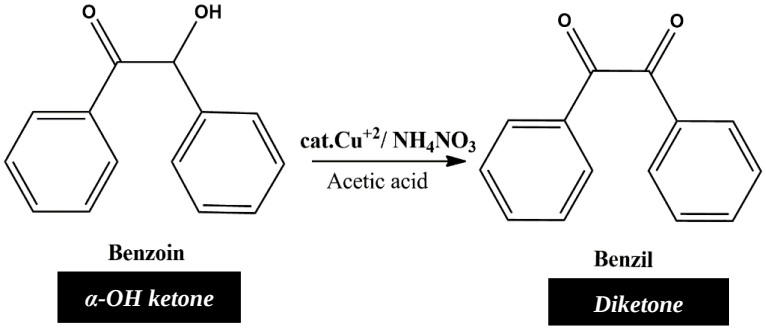
Write down the balanced chemical equations that represent the preparation of Benzoin:

Experiment 3

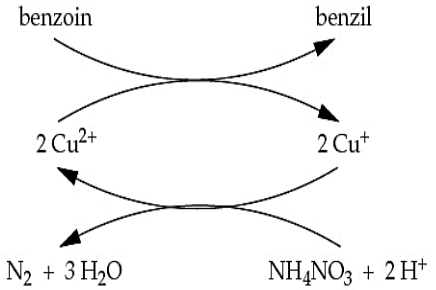
Multistep synthesis of Phenytoin

Part2: Oxidation of Benzoin to Benzil

Preparation of benzil can be done by Mild oxidation of benzoin using catalytic oxidation reaction, nitric acid or any other mild oxidizing agent, where the OH group is converted to a ketone group.



- Benzoin can be oxidized to the diketone benzyl using a Cu^{2+} salt and ammonium nitrate. Only catalytic amounts of copper(II) acetate are necessary because the Cu^{2+} is continuously recycled.

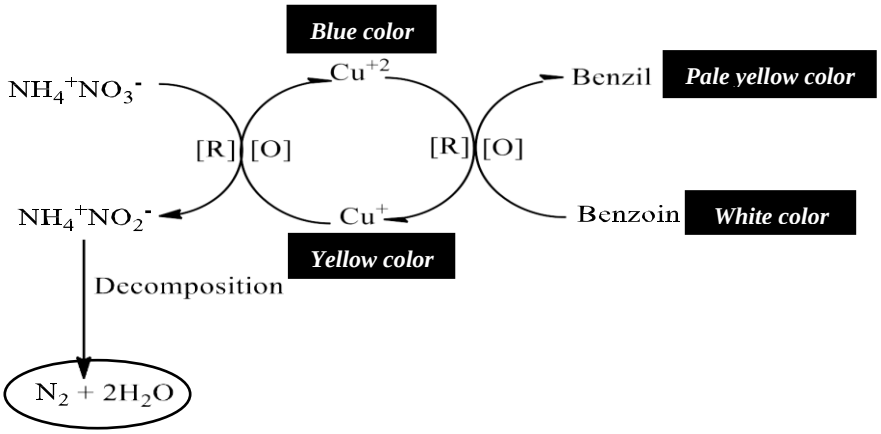


- Cupric acetate (Cu^{+2}) is used in a catalytic amount (less than 1% of stoichiometric requirement) and is continuously re-oxidized from the reduced (cuprous state Cu^{+}) by ammonium nitrate (NO_3^-) which is present in excess and is reduced to ammonium nitrite (NO_2^-) which decompose in the reaction mixture into nitrogen gas.

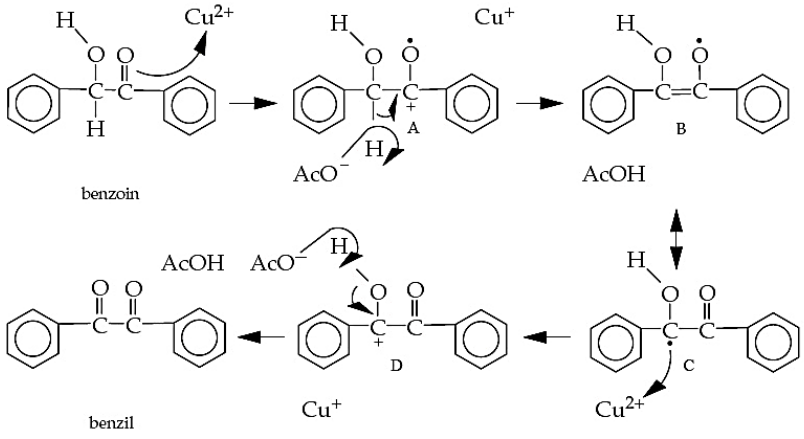
Refer to appendix IX: Oxidation of Benzoin to Benzil using nitric acid.

Mechanism

The reaction is
Coupled Oxidation; using Cu^{+2} as the catalytic transfer oxidant.



- In the first redox cycle, benzoin donates an electron to Cu^{2+}, forming Cu^{+} and benzoin redical cation A.
- The benzoin radical cation loses a proton to acetate ion (AcO^{-}), forming acetic acid(AcOH) and a resonance stabilized radical, depicted by structure B and C.
- Another redox cycle between Cu^{2+} and the radical takes place, forming a second Cu^{+} ion and cation D, which loses a proton to another acetate ion to form benzil.



- The Cu^{+} ions formed in these redox cycles are re-oxidized to Cu^{2+} by ammonium nitrate, which is present in excess. Forms ammonium nitrite (NH_4NO_2), which decomposes to nitrogen and water under the reaction conditions.

$$2\,Cu^{+} + 2\,H^{+} + NH_4NO_3 \rightarrow 2\,Cu^{2+} + H_2O + NH_4NO_2 \rightarrow 2\,Cu^{2+} + N_2\uparrow + 3\,H_2O$$

7 Assignment

7.1 Find other Mild oxidizing agents.

Procedure

1. In a round bottomed flask place 1.75 g of unrecrystallized benzoin, 5 ml of glacial acetic acid, 0.8 g of pulverized (reduced to fine particle) ammonium nitrate, and 1 ml of a 2% solution of cupric acetate.
2. Add 1-2 boiling chips, attach a reflux condenser and bring the solution to a gentle boil. As the reactants dissolve, evolution of nitrogen begins.
3. Boil the blue solution for 1.5 hr to complete the reaction.
4. Cool the solution to 50-60°C and pour it into 10 ml of ice-water mixture in a beaker *[to ppt product, since benzil insoluble in water],* while stirring it. Then Benzil separates out as yellow oil, which immediately solidifies.
5. After crystallization of benzil is complete, collect the crystals on suction filtration and wash them thoroughly with water.
6. Press the product as dry as possible on the filter.
7. *If desired, it may be purified by re-crystallize from methanol or 75% ethanol. (After dissolving the product in hot ethanol, add water dropwise to reach the cloud point and allow it to crystallize).*

	Weight (gm)	**M.P**
Benzil		

4 *Brain storming Question*

4.1 Discus the chirality of the product, does it optically active? Explain your answer.

4.2 What structural features make benzil yellow and benzoin colorless (white)?

4.3 Would you have obtained the same results for the oxidation of benzoin if the label on the copper acetate bottle had read "cuprous acetate?

pre-lab.

Report sheet (3)
Oxidation of Benzoin to Benzil

Student name:

Objectives :

Compound	MW (g/mol)	Hazards
Benzoin		
Ammonium nitrate		
Cupric acetate		
Benzil		

Find out the melting point of Benzil ?

Draw a simple flowchart for the procedure of the exp. Or write an outline steps in your own words?

Post-lab.

Report sheet (3)
Oxidation of Benzoin to Benzil

Student name:

1. data and results:

The actual yield of Benzil:

The theoretical yield of Benzil(show detailed calculation):

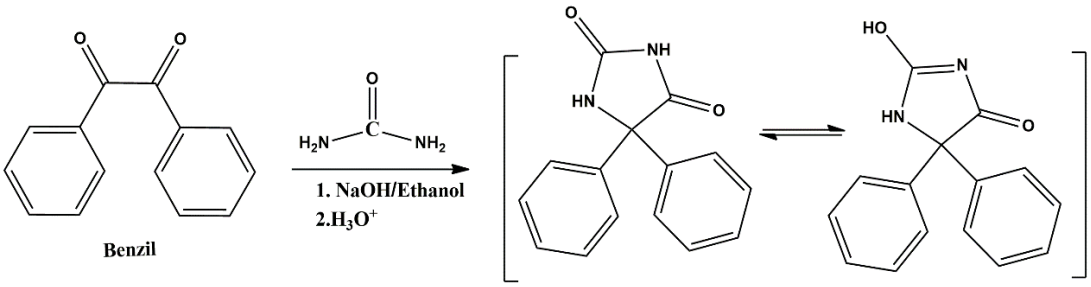
The yield%:

2.chemical equations:

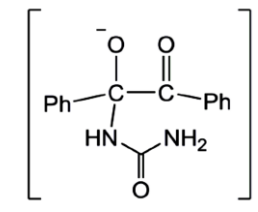
Write down the balanced chemical equations that represent the preparation of Benzil:

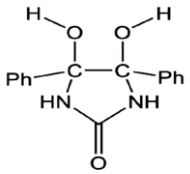
Experiment 4
Multistep synthesis of Phenytoin

Part3: condensation of Benzil with urea to form Phenytoin (Dilantin ®)



- The preparartion of phentytoin carried out by the condensation of urea with benzil in basic media (catalyst).
- The reaction involves a skeletal re-arrangement step (Benzylic acid re-arrangement), both phenyls ending up on the same carbon atom.
- The rearrangement may occur due to the stability of imide (CO-NH-CO) group that drives it or due to formation of benzyl cation (very reactive- very high-energy intermediate).
- Acidification step is required to precipitate phenytoin
- Possible intermediates:

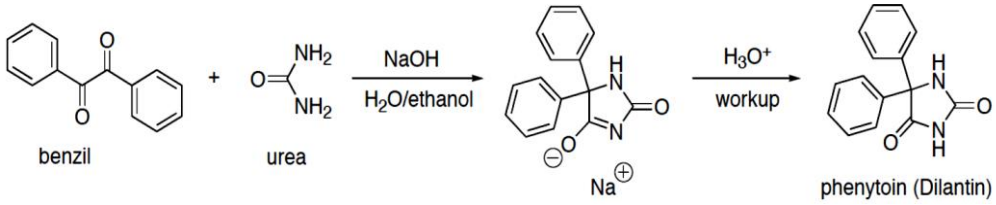

Benzilic acid intermediate


Pinacol rearrangement intermediate

4,5-diphenyl-4,5-dihydroxyl imidazolone

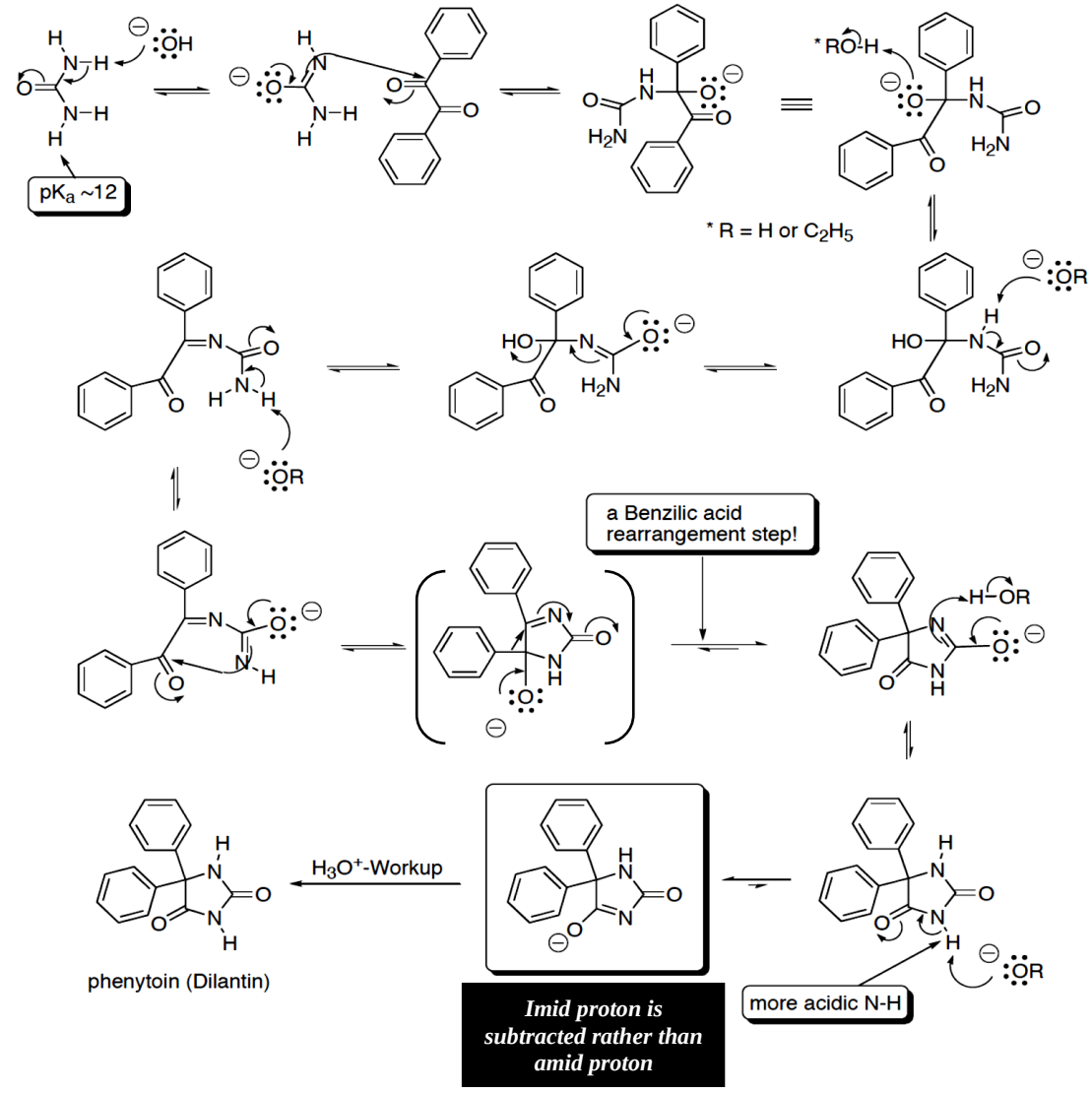
Migration reaction: is slow reactions need heat (thermodynamic reaction root)
Give a very stable product due to very high transition stat energy for the intermediate.

Mechanism



The mechanism of this reaction begins with the nucleophilic attack of a urea nitrogen atom on one of the carbonyls of benzil, then itermoleculare cyclization and end up with Benzylic acid re-arrangement
(Migration reaction).

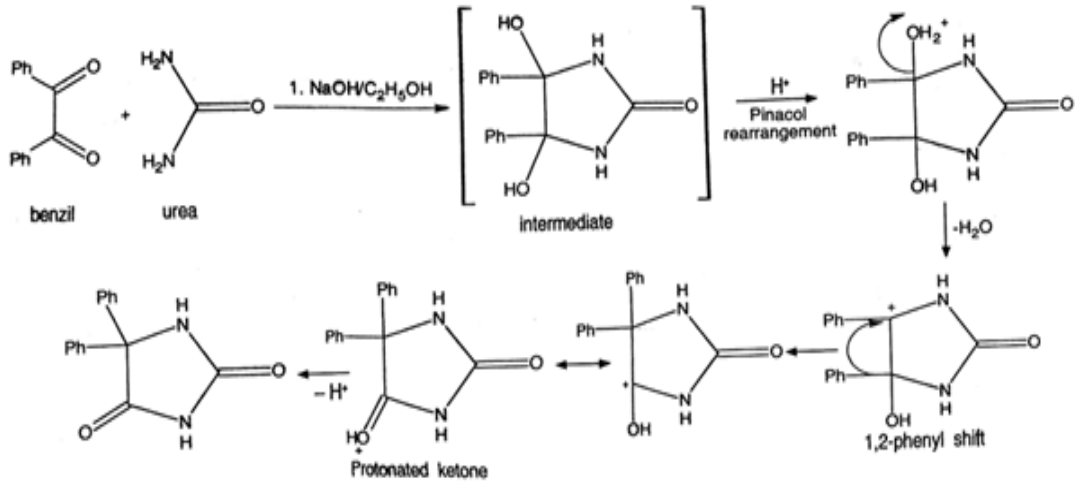
Suggested Mechanism (1)



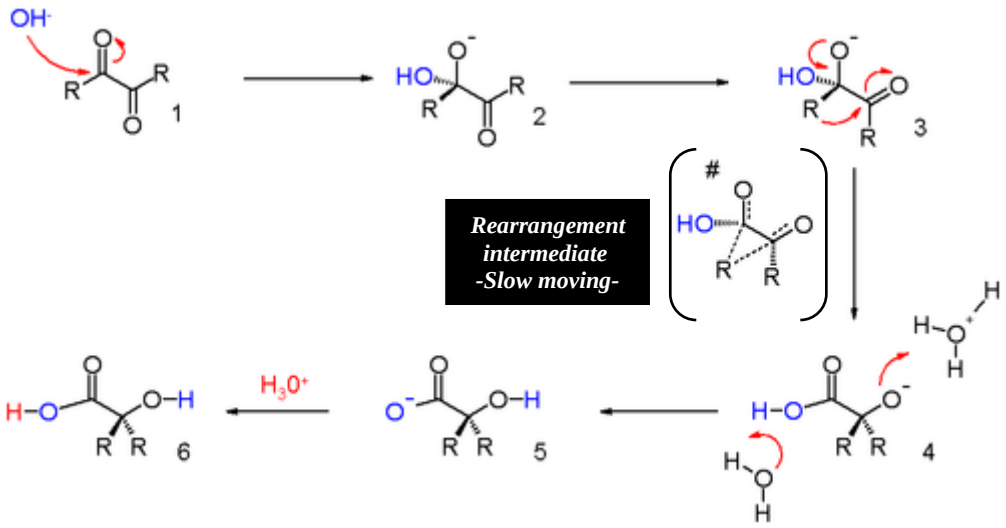
.

Suggested mechanism (2):

This reaction is proceeding via intermolecular cyclization to form an intermediate heterocyclic pinacol (4,5-diphenyl-4,5-dihydroxyl imidazolone), which on acidification yield hydantoin (phenytoin) as a result of 1,2-diphenyl shift in pinacol rearrangement reaction.



Benzylic acid re-arrangement (general mechanism)



Procedure

Because Dilantin® has significant biological activity, it is very important that protective gloves and safety goggles be worn when handling the product of this reaction!

1. In a small round bottomed flask place 400 mg of unrecrystallized benzil, 200 mg of urea, 6.0 ml of ethanol, and 1.2 ml of 30% NaOH.
2. Attach an upright condenser, add a boiling chip, and boil the mixture gently for at least one hour.
3. Cool the reaction mixture, add 10 ml of water, and filter the solution to remove a sparingly soluble side product *[pinacol side product]* that sometimes forms.
4. Acidify the filtrate with 3-4 ml of HCL (20%) to pH 2-3. Then collect the product on a suction filter, and wash it thoroughly with water.

	Weight (gm)	**M.P**
Phenytoin		

Pre-lab.

Report sheet (4)
Synthesis of Phenytoin

Student name:

Objectives :

Compound	MW (g/mol)	Solubility in water	Hazards
Urea			
NaOH			
HCl			
Phenytoin			
4,5-diphenyl-4,5-dihydroxyl imidazolone			

Find out the melting point of Phenytoin ?

Draw a simple flowchart for the procedure of the exp. Or write an outline steps in your own words?

Post-lab.

Report sheet (4)
Synthesis of Phenytoin

Student name:

1. data and results:

The actual yield of Phenytoin:

The theoretical yield of Phenytoin(show detailed calculation):

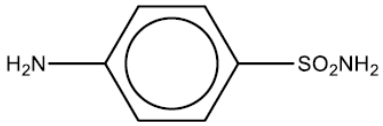
The yield%:

2.chemical equations:

Write down the balanced chemical equations that represent the preparation of Phenytoin:

Cycle 3

Multistep Synthesis of Sulfanilamide



Target Product

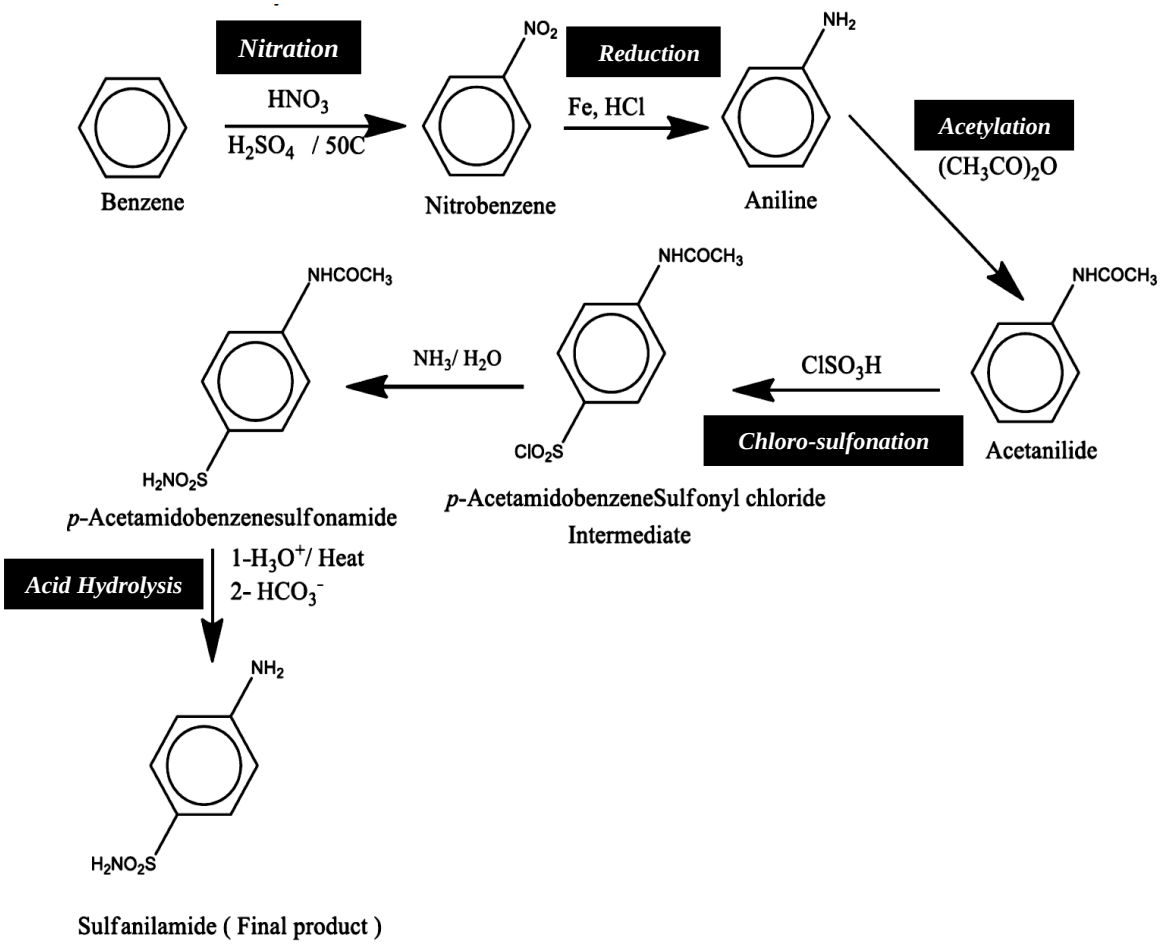
Sulfa drugs were discovered in the early 1900's and found to be active anti-bacterial agents. Sulfanilamide inhibits the formation of folic acid in bacteria, thus preventing its further growth.

- Sulfanilamides are sulfonamide synthetic anti-bacterials.
- Were the 1st successful selectively toxic antibacterial drugs.
- Used for treatment of acute uncomplicated UTI caused by E- coli , malarial infections, vaginal yeast infection and ocular infection
- Toxicity: Kidney damage (stones formation), hypersensitivity reactions.

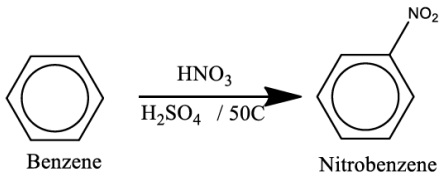
1 Assignment

1.1 Find the story for discovery of sulfonamide as anti-bacterial drug.

General scheme of synthesis



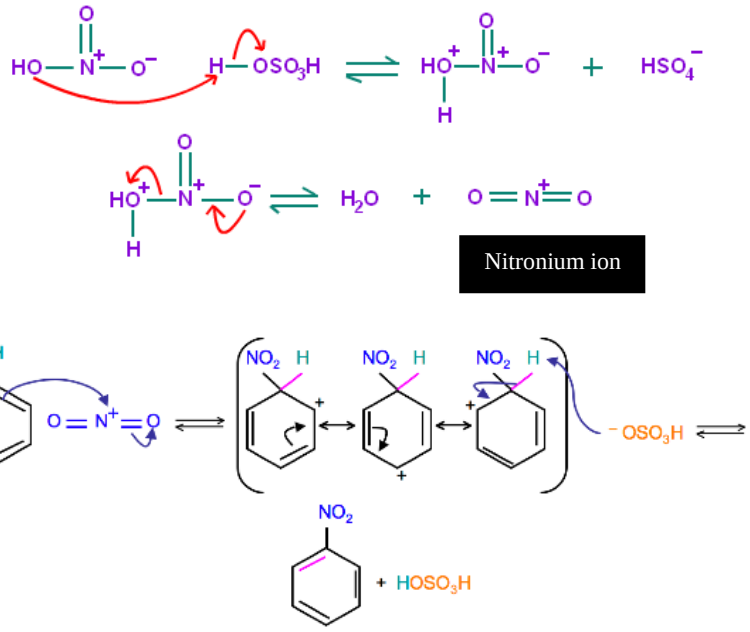
Step1: Nitration of benzene.



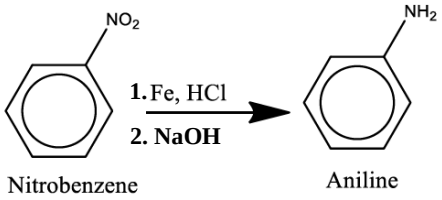
Mechanism:

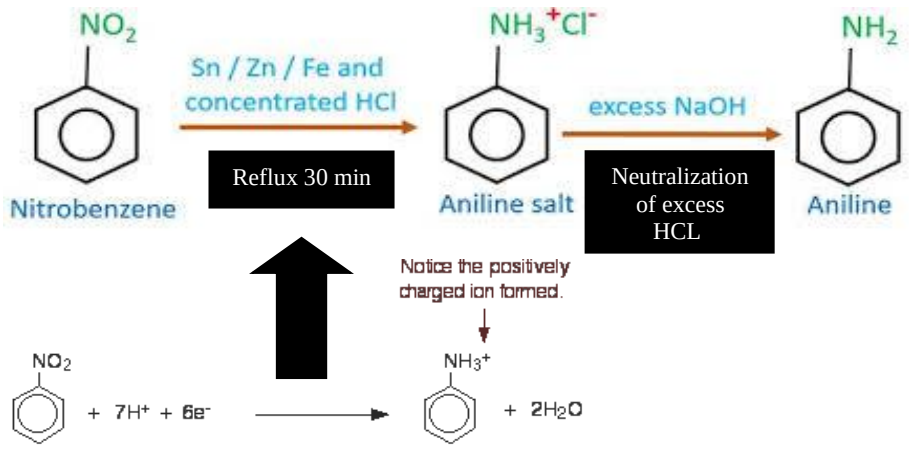
1- *Activation of nitric acid (protonation of nitric acid to form nitronium ion)*

2- *Electrophile aromatic substitution mechanism.*



Step2: Chemical reduction of Nitrobenzene to Aniline



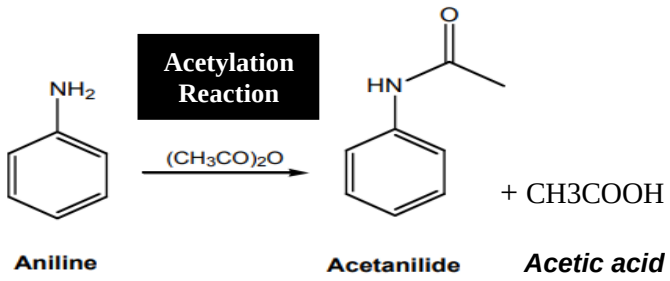


Sulfanilamide is easily synthesized <u>from aniline in four steps</u>. You will see mechanism for each steps.

Experiment 5

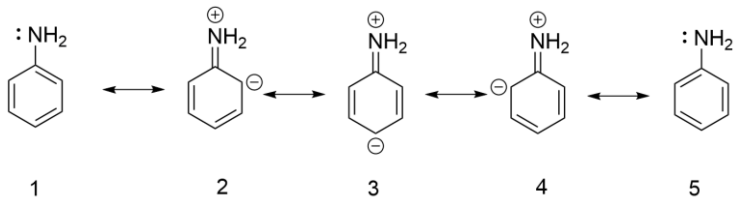
Multistep Synthesis of Sulfanilamide

Part 1: synthesis of acetanilide

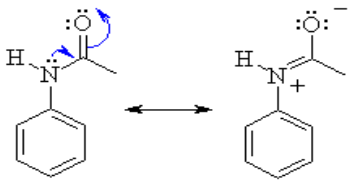


NH_2 is an electron donating group that directs the electrophilic aromatic substitution on ortho-para position (it is electron reach so good nucleophile).

Aniline resonance



Acetanilide resonance



Acetylation of NH_2- [convert amine group to amide group]

It was for:

- ✓ Protection
- ✓ Blocking ortho and para director:

- Acetyl group (Bulk) makes steric hindrance and blocks the ortho position so sulfonyl chloride group will be added to para position in the next step

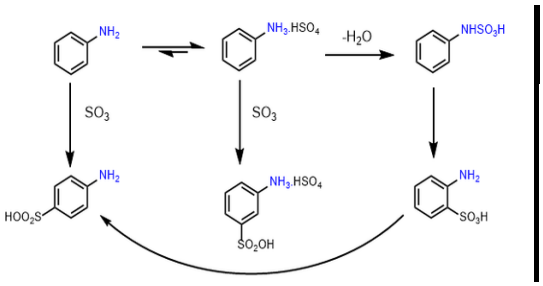


Figure 1. Sulfonation of aniline all positions (o,p, amine) are possible option

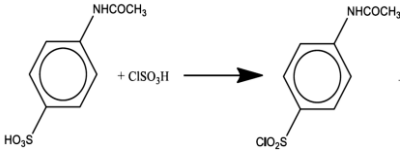


Figure 2. Sulfonation of Acetanilide Directed only to the para position

- Acetyl group will also protect from polymerization that may occur between sulfonyl chloride from one molecule and the amine group from another molecule and give polymeric material containing sulfonamide linkages.

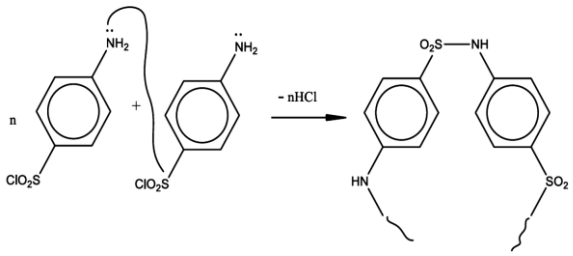


Figure 3. Polymerization

- The free amine, under the strong acid conditions of chlorosulfonation, would protonate or react with strong Lewis acids present (such as SO_3), resulting in deactivation of the ring toward chlorosulfonation and sulfonation of the free amine.

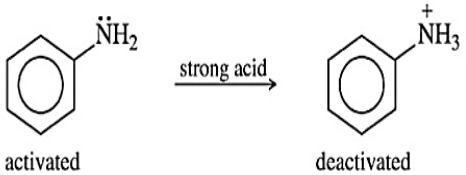
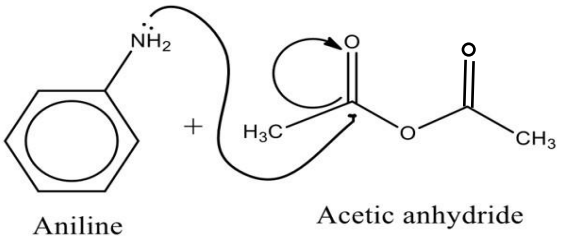


Figure 4. Protonation of aniline in acidic condition

1 Assignment

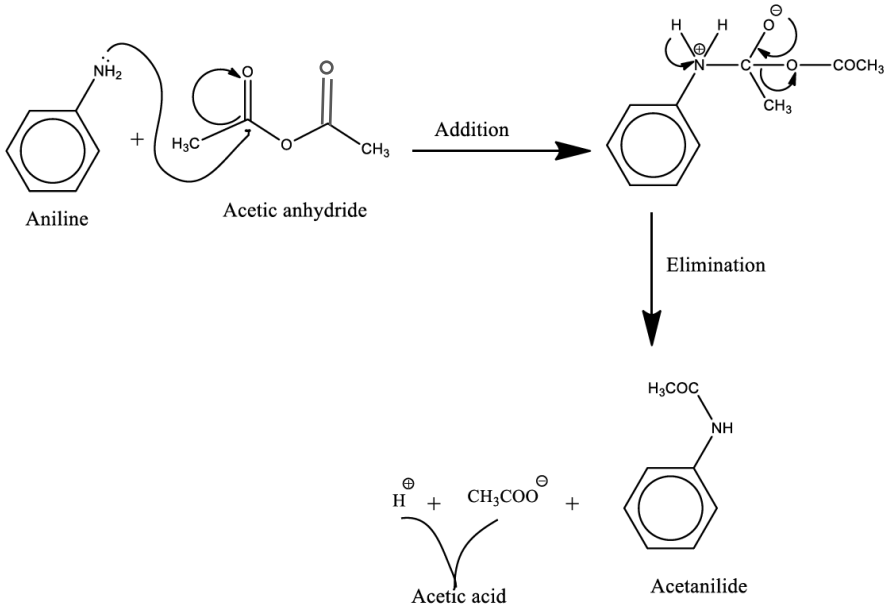
1.2 Find other amine protective groups.

Mechanism



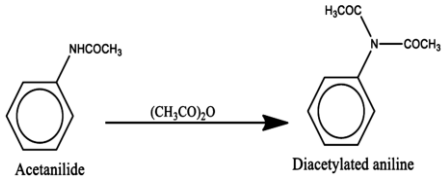
This reaction is
a nucleophilic addition-elimination reaction

- Acetic anhydride is used instead of acetic acid because its carbonyl carbon is more electrophilic than that of acetic acid so the amide formation will be faster and easier otherwise it may need reflux for 3-4 hours.



1 *Brain storming Question*

1.1 Explains in your words the following side reaction (di-acetylation of aniline) and comments on its rate? Is it faster or slower than the previous reaction? How can minimize this side reaction?



1.2 Mention another side reaction suggested to be happen during this experiment. How can minimize this side reaction?

Procedure

1. Place 16 ml of aniline, measured using a 25ml-graduated cylinder, in to a 500 ml Erlenmeyer flask.
2. Add 120 ml of water to the flask and then while **swirling** the flask add 20 ml of acetic anhydride [measure it using a dry cylinder] in **several small portions.**
3. Add 200 ml of water and boiling stones (chips)[**], and then heat until the entire solid and oil have dissolved.
4. Add about 1 gm charcoal slowly to the main hot solution. Swirl the mixture and boil gently for 5 min.
5. Filter through **hot gravity filtration**[‡] into a 500 ml Erlenmeyer flask. Have available 100 ml of boiling water for washing.
6. Cool the filtrate in an ice water bath for 15 min to complete the crystallization.
7. Filter [**cold suction filtration**[‡]**]**, then dry the crystals and determine the m.p[*].

	Weight (gm)	**M.P**
Acetanilide		

1 Brain storming Question

1.3 Justify the following:

A. Water used as solvent instead of acetic anhydride in this reaction?
B. Addition of water to aniline with swirling (shaking)? Addition of acetic anhydride in several small portion?
C. Hot filtration.

[*] *Refer to appendix II: Melting point. To measure M.P.*

[‡] *Refer to appendix III, Part II: Filtration techniques.*

[**] *Refer to appendix V: Boiling chips.*

Pre-lab.
Report sheet (5)
Synthesis of acetanilide

Student name: _____

Objectives:

Compound	MW (g/mol)	Miscibility with solvent (water)	Hazards
Aniline			
Acetic anhydride			
Charcoal			
Acetanilide			

Find out:

1. *The density of Aniline ……..*
2. *The density of Acetic anhydride*
3. *The melting point of Acetanilide …….*

Draw a simple flowchart for the procedure of the exp. Or write an outline steps in your own words?

Post-lab.

Report sheet (5)
Synthesis of acetanilide

Student name:

1. Data and results:

The actual weight (yield) of acetanilide:

The theoretical weight (yield) of acetanilide (show detailed calculation):

The yield%[§]:

2. Chemical equations:

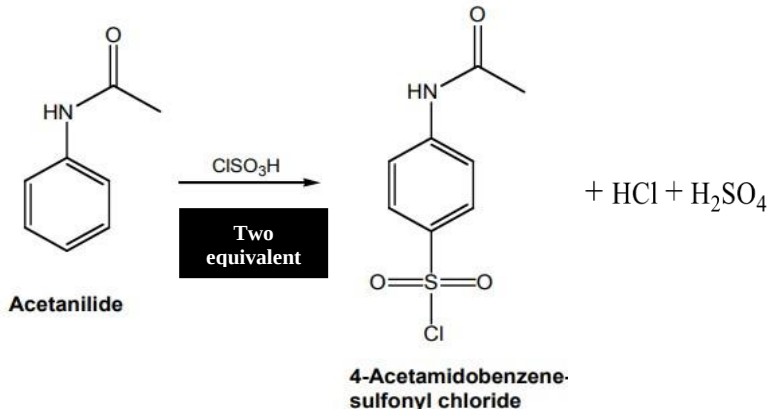
Write down the balanced chemical equations that represent the preparation of acetanilide:

[§]*Refer to appendix I: Percent Yields Calculations.*
To calculate % yield.

Experiment 6

Multistep Synthesis of Sulfanilamide

Part 2: synthesis of p-acetamidobenzenesulfonyl chloride



In This Experiment perfectly dry acetanilide is treated with chlorosulfonic acid, a highly reactive reagent. The hydrogen chloride evolved is trapped; the reaction mixture is added carefully to water; and the product, p-acetaminobenzenesulfonyl chloride, is isolated by filtration.

- This reaction is conducted without solvent
- Water serves to hydrolyze the excess chlorosulfonic acid and to stop the reaction [$ClSO_3H + H_2O \rightarrow HCl + H_2SO_4 + \Delta$]

CAUTION: Chlorosulfonic acid is a corrosive chemical and reacts violently with water. Withdraw with a pipette. Neutralize any spills and drips immediately. The wearing of gloves and handling in the hood is required.

2 *Brain storming Question*

4.4 Discus the effect of water on the product (p-acetaminobenzenesulfonyl chloride)

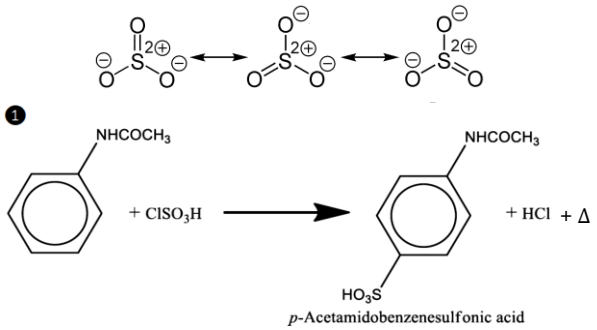
Mechanism

This reaction is more complicated than it looks at first inspection. It is happen in two steps.

Step 1: Exothermic reaction (spontaneous).

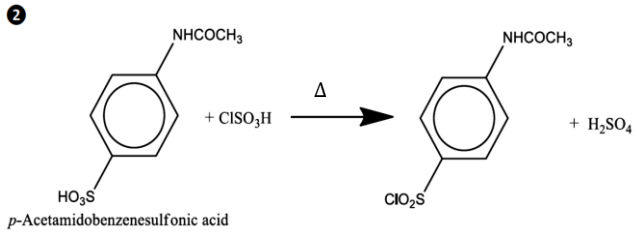
- The electrophile that initially adds to the ring is probably SO_3, forming the sulfonic acid (Ar-SO_3H). The initially formed substitution product is the sulfonic acid.
  - ✓ ***Substitution is essentially, all para due to combined electronic and steric effects.***

$$\boldsymbol{first: ClSO_3H \underset{\Delta}{\leftrightarrow} SO_3\,(g) + HCL\,(g)}$$



Step 2: Endothermic reaction (nonspontaneous).

- The sulfonic acid is then converted to 4- acetamidobenzenesulfonyl chloride (Ar-SO_2Cl) by reaction with excess chlorosulfonic acid, generating sulfuric acid as the co-product.



This reaction is:
Typical electrophilic aromatic substitution

<u>*Byproducts of this reaction*</u> : HCl (g), H_2SO_4 (g) , SO_3 (g) gas and heat (Since the overall reaction is exothermic)

Other suggested mechanism summarized in appendix VI.

Procedure

1. *To a **dry** 100 ml Erlenmeyer flask, add 40 ml of Chlorosulfonic acid* ($ClSO_3H$) carefully **while the flask in an ice bath**. *[corrosive and reacts violently with water]*
2. Add 15 g of dry **<u>finely powdered</u>** *[↑SA for rxn]* Acetanilide in **small portions** and with good mixing. *[because it is vigorous reaction]*
3. Allow the mixture to warm at room temperature and then heat the mixture on a steam bath for 30 min. (Adjust the temperature to 50 - 60°C). *[to complete the reaction –step 2]*
4. **Cool** to room temperature (using an ice bath). Place 400g of **crushed ice** and **65 ml of D.W** *[water for ease stirring and and to get read of excess $ClSO_3H$]* in a large beaker and pour the reaction mixture slowly and carefully **with stirring** *[to prevent caking – keep product suspended]* onto the ice.
5. Rinse the flask with a little of cold water and transfer this to the beaker. *[Fill the flask with water and leave it under fume hood to the end to get read of all gases out: HCL ,SO_3.]*
6. Collect the crude material by suction filtration [Cold Suction Filtration] and wash the precipitate with a small amount of cold water.
7. **Recrystallize**‡ the product from 200ml Chloroform [(1)]. The purified product should be dried in air to be used in the next lab.

   [the chloroform used in recrystallization bcz the product soluble in it at high temp and ppt at low temp]

[(1)] Dissolve the crude material in hot chloroform, and while the solution is still hot, put it in a <u>preheated separatory funnel</u> and take the <u>lower organic layer</u>. *[To remove residual water bcz the product sensitive to it]*

Evaporate part of the chloroform until reach 100ml *[keep your eyes on it will heating]* and cool the solution to R.T then in an ice bath, then collect the crystals and wash them with cold chloroform. *[Cold Suction Filtration]*

Step 1-5 under fume hood.
‡*Refer to appendix III: Recrystallization.*

	Weight (gm)	M.P
p-Acetamidobenzenesulfonyl chloride		

2 Brain storming Question

4.5 Discus cooling step (4) and use of both water and ice.

4.6 Discus is it possible to use drying agent like (MgSO4) instead of using extraction and separation step in recrystallization.

4.7 In recrystallization you used separatory funnel for sepration describe component for each layer.

4.8 In recrystallization‘Evaporate part of the chloroform until reach 100ml’. Why?

Pre-lab.

Report sheet (6)
Synthesis of p-acetamidobenzenesulfonyl chloride

Student name:

Objectives:

Compound	MW (g/mol)	Stability in water	Hazards
Chlorosulfonic acid			
Acetanilide			
Chloroform			
p-acetamidobenzenesulfonyl chloride			

Find out the melting point of p-acetamidobenzenesulfonyl chloride?

Draw a simple flowchart for the procedure of the exp. Or write an outline steps in your own words?

Post-lab.

Report sheet (6)
Synthesis of p-acetamidobenzenesulfonyl chloride

Student name:

1. Data and results:

The actual weight (yield) of p-acetamidobenzenesulfonyl chloride:

The theoretical weight (yield) of p-acetamidobenzenesulfonyl chloride (show detailed calculation):

The yield%:

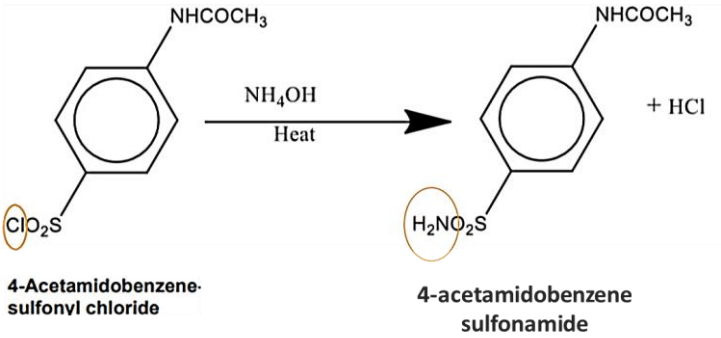
2. Chemical equations:

Write down the balanced chemical equations that represent the preparation of p-acetamidobenzenesulfonyl chloride:

Experiment 7

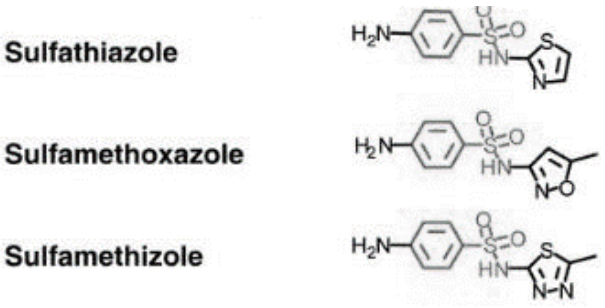
Multistep Synthesis of Sulfanilamide

Part 3: synthesis of p-acetamidobenzene-sulfonamide

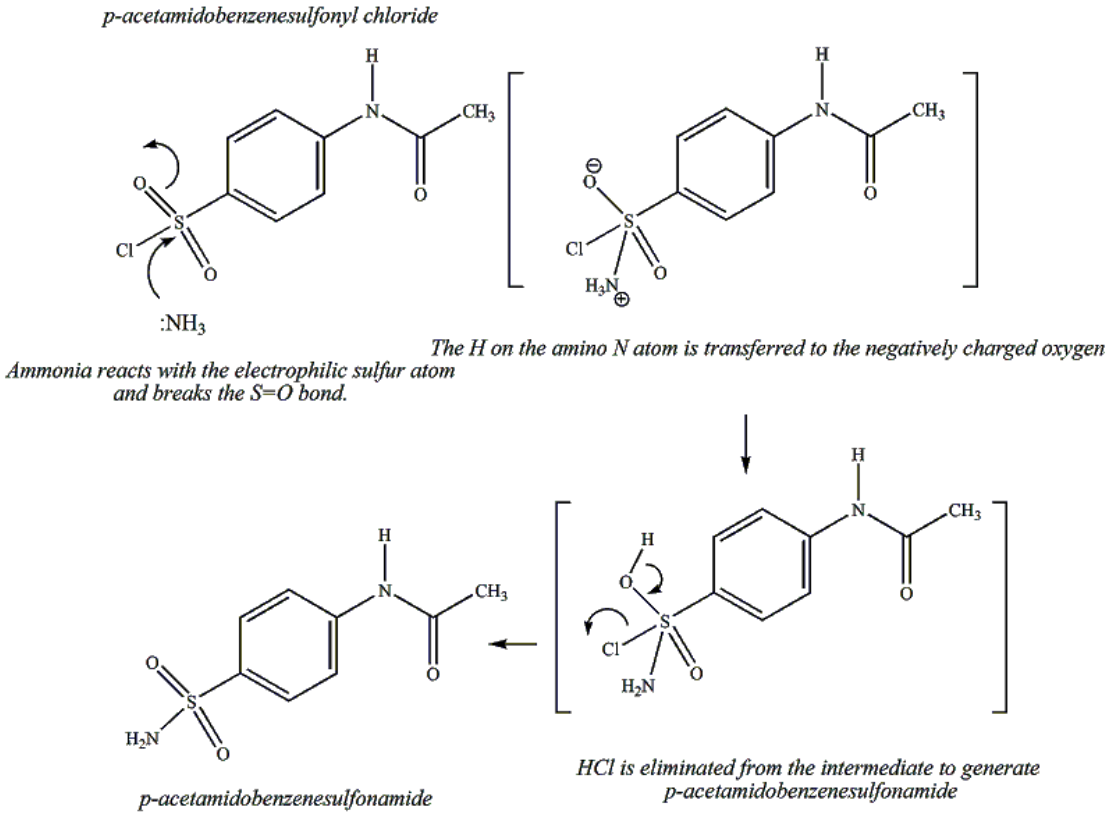


A whole family of sulfa drug is possible at this stage if any other amine RNH2 is used instead of ammonia.

Examples:



Mechanism



The reaction is
a nucleophilic acyl substitution reaction.

- The amide is produced by treatment of the sulfonyl chloride with an excess of aqueous ammonia.

$$NH_4OH\,(aq) \rightarrow NH_3\,(aq) + H_2O\,(aq)$$

- NH3 is a good nucleophile
- Excess ammonia will neutralize the produced HCl by forming a salt water soluble ($NH_4^+CL^-$) [*acid base side reaction]*.
- Sulfuric acid is used to neutralize the excess ammonium hydroxide and to decrease the solubility of our product and precipitate it.

Procedure

1. Transfer the product obtained in experiment 2 (~10g); p-Acetamidobenzenesulfonyl chloride to a 250 ml Erlenmyer flask.
2. Add 60 ml of conc. Ammonium hydroxide solution **(28 %)** and heat the mixture on an 80 ^{o}C steam bath for 30 min. *[a homogenous thick mixture produced like past due to amide intermolecular H-bonding, and heating bcz the reaction is endothermic]*
3. Cool the mixture in an ice bath and **add of sulfuric acid** (6 M) dropwise until it become acidic (pH 3-5). *[add 2ml then drop by drop]*
4. Cool the mixture again in an ice bath and collect the product by suction filtration. *[Cold Suction Filtration]*
5. Wash the crystals with a small amount of cold water and dry them.
6. If necessary, recrystallize from a small amount of hot water.

	Weight (gm)	**M.P**
p-acetamidobenzene-sulfonamide		

5 *Brain storming Question*

5.1 Discus the use of diluted ammonia instead of concentrated liquid.

3.2 Suggest other side reaction that could happen.

Pre-lab.

Report sheet (7)
Synthesis of p_acetamidobenzene-sulfonamide

Student name:

Objectives:

Compound	MW (g/mol)	Solidity in water	Hazards
p-Acetamidobenzenesulfonyl chloride			
Ammonium hydroxide			
sulfuric acid			
p_acetamidobenzene-sulfonamide			

Find out the melting point of p_acetamidobenzene-sulfonamide?

Draw a simple flowchart for the procedure of the exp. Or write an outline steps in your own words?

Post-lab.

Report sheet (7)
Synthesis of p_acetamidobenzene-sulfonamide

Student name:

1. Data and results:

The actual weight (yield) of p_acetamidobenzene-sulfonamide:

The theoretical weight (yield) of p_acetamidobenzene-sulfonamide (show detailed calculation):

The yield%:

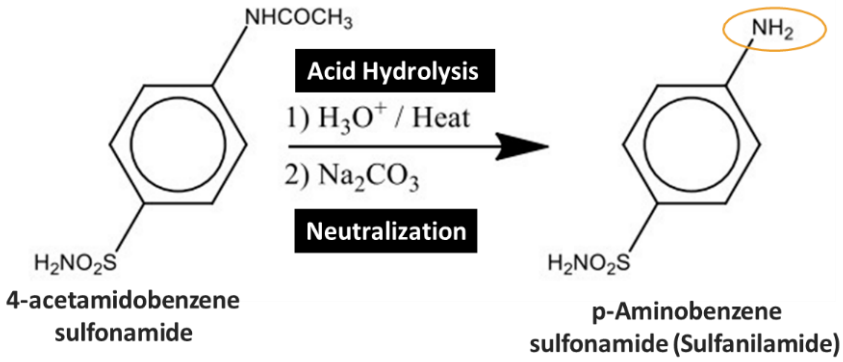
2. Chemical equations:

Write down the balanced chemical equations that represent the preparation of p_acetamidobenzene-sulfonamide:

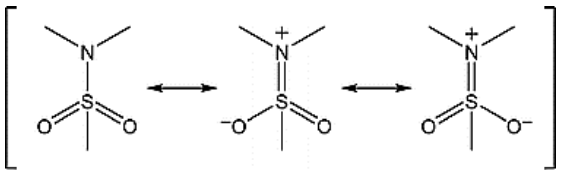
Experiment 8

Multistep Synthesis of Sulfanilamide

Part 4: Synthesis of sulfanilamide



- Sulfonamide group is slowly hydrolyzed and <u>more stable</u> in diluted acid, due to:
  - I. <u>steric hindrance</u> to the approach of water molecules on sulfur.
  - II. <u>Resonance stabilization</u>, it is more resonance stabilized than aceta-amide.

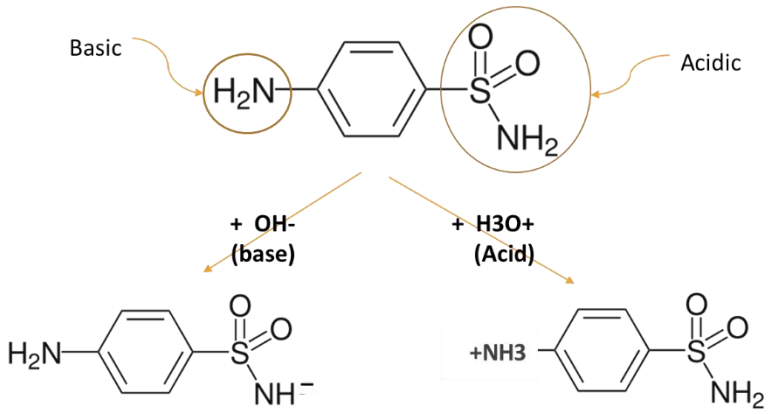


- Carboxylic amide (aceta-amide) group <u>hydrolyzes easily</u> in very diluted acid.

Amide and esters are easily hydrolyzed in both diluted acid or diluted base condition with reflux.

- Neutralization with **sodium carbonate (pH 6-7)** in order to get the free base and precipitate sulfanilamide.

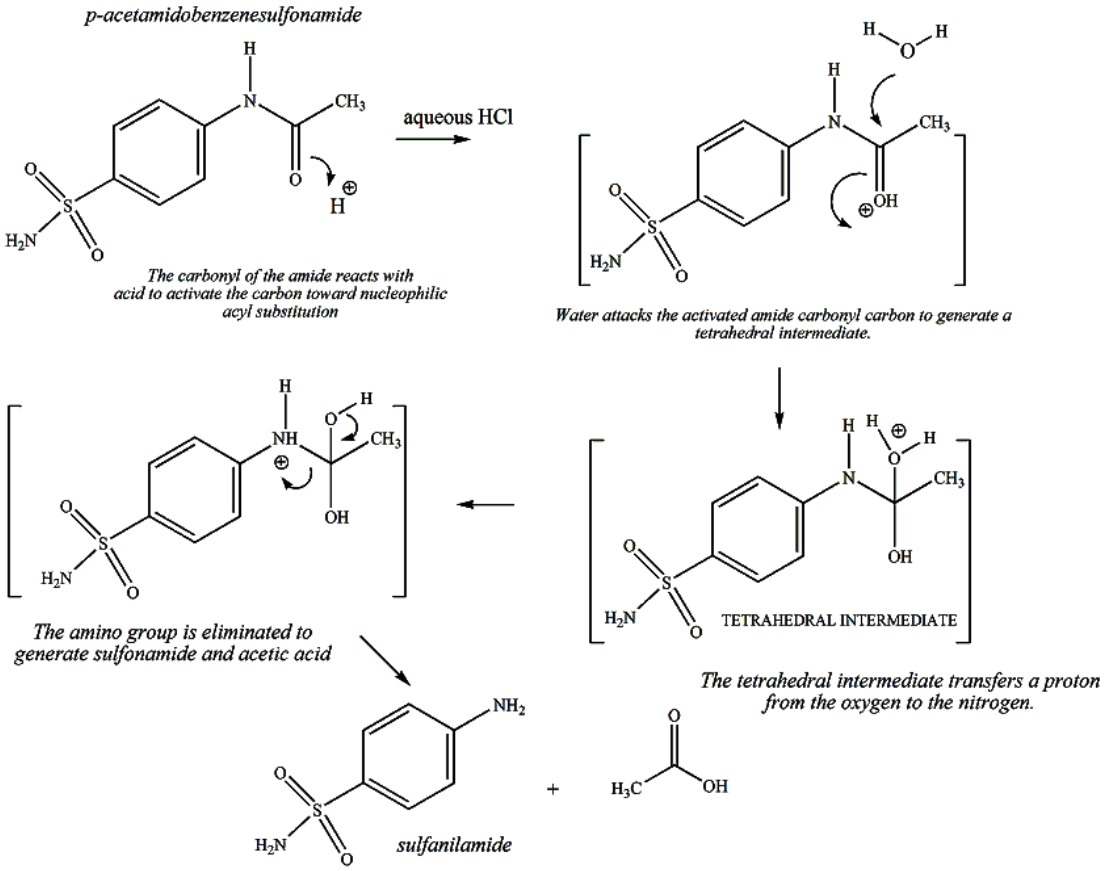
❖ Sulfanilamide is amphoteric that has weak acidic and weak basic properties.



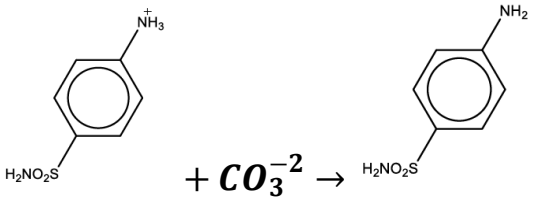
Mechanism

The reaction is
Hydrolysis reaction in diluted acid condition.

The mechanism involve proton transfer.



Sulfanilamide in acidic condition will be protonated form of amine group, so neutralization step to deprotonate it and get it free of charge to ppt.



$$HCO_3^- + H_3O^+ \rightarrow CO_{2(g)} \uparrow + H_2O$$

Procedure

1. Weigh previous product (~5g) of p-Acetamidobenzenesulfonamide and transfer it to a 100 ml round-bottomed flask.
2. Prepare a solution of dilute HCl by mixing equal volumes of conc. acid and water.
3. Add to the amide an amount of dilute acid solution **twice** [in excess] the weight of the amide. *[excess to prevent drying during reflux]*
4. Attach a **reflux*** condenser to the flask and heat at a gentle reflux for 30 minutes.
5. To the reaction mixture add equal quantity of water *(equal to dil.acid added before)* and transfer the new mixture to a 600 ml beaker. *[added water to dilute the acid]*
6. Neutralize the excess acid by the addition of small quantities of solid sodium bicarbonate (Na2CO3) until the solution is just alkaline to litmus paper (pH 6-7) and stope foaming. *[added carbonate **slowly** to ↓ foaming and prevent loss of our product and avoid extra addition]*
7. Cool the mixture in an ice bath and collect the crystals by suction filtration. Wash the crystals with a small amount of cold water.
8. Recrystallize from small amount of hot water, decolorize the solution with charcoal (if necessary) then collect the pure product and allow it to dry in air.

	Weight (gm)	**M.P**
Sulfanilamide		

6 *Brain storming Question*

6.1 Discus the reason behind using reflux.

6.2 Foaming will be resulted in this reaction, why?

6.3 In step 5, discus the effect of excess carbonate (Na_2CO_3) addition.

* *Please refer to appendix IV: Reflux and the reflux condenser. For more information.*

Pre-lab.

Report sheet (8)
Synthesis of sulfanilamide

Student name:

Objectives:

Compound	MW (g/mol)	Hazards
p-Acetamidobenzene sulfonamide		
HCl		
sodium bicarbonate		
sulfanilamide		

Find out the melting point of sulfanilamide?

Draw a simple flowchart for the procedure of the exp. Or write an outline steps in your own words?

Post-lab.

Report sheet (8)
Synthesis of sulfanilamide

Student name:

1. Data and results:

The actual weight (yield) of sulfanilamide:

The theoretical weight of sulfanilamide (show detailed calculation):

The yield%:

2. Chemical equations:

Write down the balanced chemical equations that represent the preparation of sulfanilamide:

Experiment 9
Molecular modeling

In silico prediction of Ionization Constants of Drugs

One of the basic tenets of medicinal chemistry is that biological activity is dependent on the three-dimensional placement of specific functional groups (the pharmacophore). Over the past few years, advances in the development of new mathematical models which describe chemical phenomena and development of more intuitive program interfaces coupled with the availability of faster, smaller and affordable computer hardware have provided experimental scientists with a new set of computational tools. These tools are being successfully used, in conjunction with traditional research techniques, to examine the structural properties of existing compounds, develop and quantify a hypothesis which relates these properties to observed activity and utilize these "rules" to predict properties and activities for new chemical entities. The development of molecular modeling programs and their application in pharmaceutical research has been formalized as a field of study known as computer assisted drug design (CADD) or computer assisted molecular design (CAMD).

Molecular modeling allows scientists to use computers to visualize molecules, to discover new lead compounds for drugs, or to refine existing drugs *in silico*.

"Molecular modeling" is a term for which the definition has evolved along with the capabilities of computer hard-ware and algorithms. The term referred to software capable of displaying and manipulating simple structures of molecules. As computer became faster and algorithms more

accurate, the term grew to include algorithms for calculating the structures of small molecules

Modern molecular modeling software is used to study small molecules, proteins, lipids, DNA, and non biological work.

One goal of molecular modeling is to develop a sufficiently accurate model of the system so that the physical experiment may not be necessary.

Most pharmacologically active molecules contain one or more ionizing groups, and it's well-known that knowledge of the ionization state of a drug, indicated by the pKa value, is critical for understanding many properties important to the drug discovery and development process.

The ionization state of a compound directly influences such important pharmaceutical characteristics as aqueous solubility, permeability, crystal structure, etc. Tremendous advances have been made in the field of experimental determination of pKa, in terms of both quantity / speed and quality / accuracy. However, there still remains a need for accurate in-silico predictions of pKa both to estimate this parameter for virtual compounds and to focus screening efforts of real compounds.

Methods of molecular modeling:

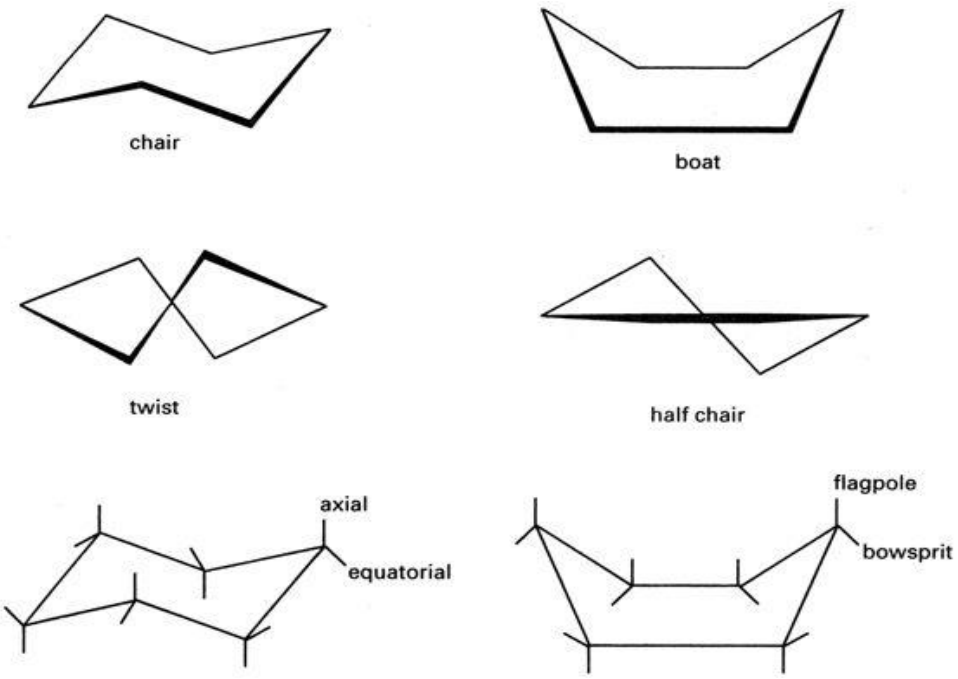
1. Quantum mechanics.
2. Molecular mechanics.

This experiment aims to teach you

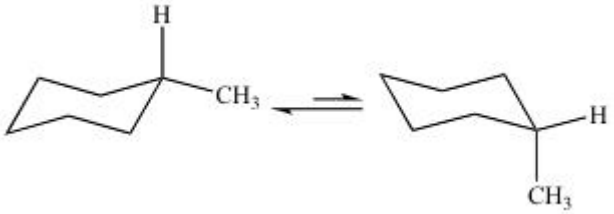
How to convert 2-D structure in to 3-D structure?

How to find the most stable conformer for a given molecule?

Conformation of cyclohexane



- The chair conformer is the most stable cyclohexane conformer consists of 6 tetrahedral carbons bonded in a ring.
- Bond angels are 109.5o with no angle strain.
- No eclipsing C-H bonds and no torsional strain (all staggered).
- The axial bonds looks like they eclipse, but the axial bonds are too far away to contain torsional strain.
- When all hydrogen atoms bonded (Cyclohexane), there is no steric strain in the chair conformer.
- Two chair forms in equilibrium



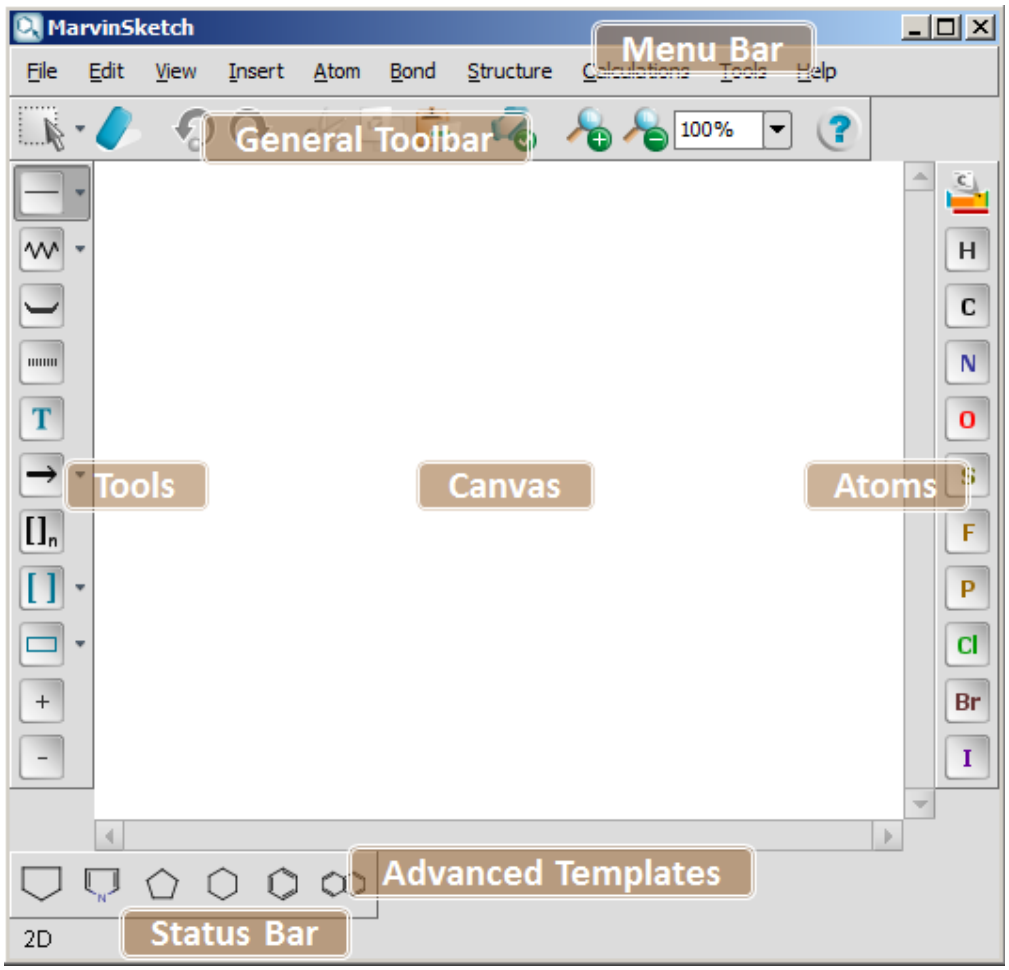
- Boat , intermediate between chairs

Software

Marvin software

Marvin is an advanced; Java based chemical editor for drawing chemical structures, queries and reactions. It has pre-loaded structure templates. It is capable of : 2-D to 3-D structural conversion ; 2-D cleaning and conformer generation ; drawing and formatting shapes , arrows and text boxes ; structure-based calculations (e.g. charg log P, etc) .

The default layout of the MarvinSketch user interface is shown in the following picture.



Opening and Saving a Molecule-File

To Open an Existing Molecule File
You can open existing molecule files (from supported file formats) by choosing File > Open on the menu bar. It will load the content of the molecule file into Marvin and discard any unsaved changes.

To Save a Molecule File

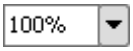
You can save the molecule to any of the supported file formats. This will allow you to open and work with this molecule later. The default behavior of the Save button is to save the molecule to the same file it was opened from, in the same format. If you want to change the file name or format, choose Save As. If you are working with a new molecule, Save will function the same as Save As.

To Save 'As Image'

The Save As Image choice in the File menu allows you to save an image of the molecule in the sketcher.

General Toolbar

	Rectangle Selection	Allows selection in rectangle mode on mouse drag.
	Lasso Selection	Allows selection in lasso mode on mouse drag.
	Structure Selection	Allows selection in structure selection mode on mouse drag. With this selection mode only whole fragments can be selected.
	Erase	Removes all structures upon selection.
	Undo	Reverses the last command or the last entry you typed.
	Redo	Reverses the action of the last Undo command.
	Cut	Removes and copies the selection to the clipboard.
	Copy	Copies the selection to the clipboard.
	Paste	Inserts the contents of the clipboard at the location of the cursor, without replacing selection.
	Check Structure	Checks and corrects chemical structures. See Structure Checker in MarvinSketch for more details.
	Zoom In	Increases the canvas's magnification.
	Zoom Out	Decreases the canvas's magnification.

	Zoom Tool	Changes the canvas's magnification to a specific value. It can also do autoscale using named values: All, Selection, Scaffold, R-groups.
	Help Contents	Shows MarvinSketch User's Guide.

Tools Toolbar

	Insert Bond	Places various bond types on the canvas.
	Insert Chain	Places a carbon chain on the canvas. The number of carbon atoms can be increased or decreased by dragging the mouse. Selection of straight or curved chain drawing is available.
	Bold Tool	Thickens the selected bond. See details on bold tool function.
	Hashed Bond Tool	Makes the selected bond hashed. It only retains single original bond type.
	Insert Text	Places a Text object on the canvas. Allows changing text properties on the appearing toolbar.
	Insert Reaction Arrow	Places various reaction arrow objects on the canvas.
	Create Group	Creates a custom abbreviation group.
	Insert Brackets	Places brackets, parentheses, chevrons or braces on the canvas.
	Insert Graphics	Places various graphical objects on the canvas.
	Increase Charge	Increases the charge of the selected atom. The number of implicit hydrogens will be adjusted if possible to accommodate the new charge. Valence errors will be highlighted in red.
	Decrease Charge	Decreases the charge of the selected atom. The number of implicit hydrogens will be adjusted if possible to accommodate the new charge. Valence errors will be highlighted in red.

Atoms Toolbar

	Periodic System	Shows periodic system and query/atom property drawing window.
	Insert Hydrogen	Places Hydrogen atom on the canvas.
	Insert Carbon	Places Carbon atom on the canvas.
	Insert Nitrogen	Places Nitrogen atom on the canvas.
	Insert Oxygen	Places Oxygen atom on the canvas.
	Insert Sulfur	Places Sulfur atom on the canvas.
	Insert Fluorine	Places Fluorine atom on the canvas.
	Insert Phosphorus	Places Phosphorus atom on the canvas.
	Insert Chlorine	Places Chlorine atom on the canvas.
	Insert Bromine	Places Bromine atom on the canvas.
	Insert Iodine	Places Iodine atom on the canvas.

Chemical Toolbar

This toolbar contains chemical functions and it is not visible by default. To make it visible, choose **View** > **Toolbars** > **Chemical**.

	Clean 2D	Calculates new 2D coordinates for the molecule.
	Clean 3D	Calculates new 3D coordinates for the molecule. Clean3D builds up conformers of fragments from which the best, i.e. the lowest energy conformer is given back. The quality of the structures is measured by a simple energy function (Dreiding type molecular mechanics).
	Convert to Aromatic Form	Transforms the molecule to aromatic representation using the transformation method set.
	Convert to Kekulé Form	Transforms the molecule to non-aromatic representation.

Simple Templates Toolbar

If you only wish to use the 6 generic template structures without additional functions, you can use the Simple Templates Toolbar. This toolbar is not visible by default. To make it visible, choose **View > Toolbars > Simple Templates**.

	Cyclopentane (house)
	Pyrrole
	Cyclopentane
	Cyclohexane
	Benzene
	Naphthalene

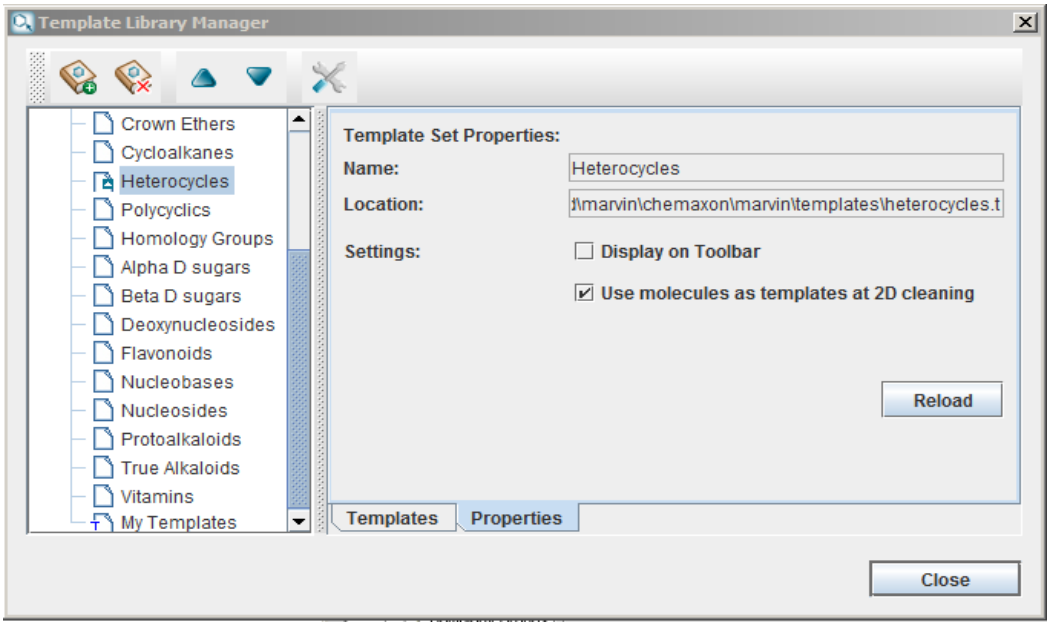
Advanced Templates Toolbar

This toolbar contains special buttons holding structure templates. Additional functions of this toolbar:

1. **The toolbar can show different template groups.**
   - General and My Templates:

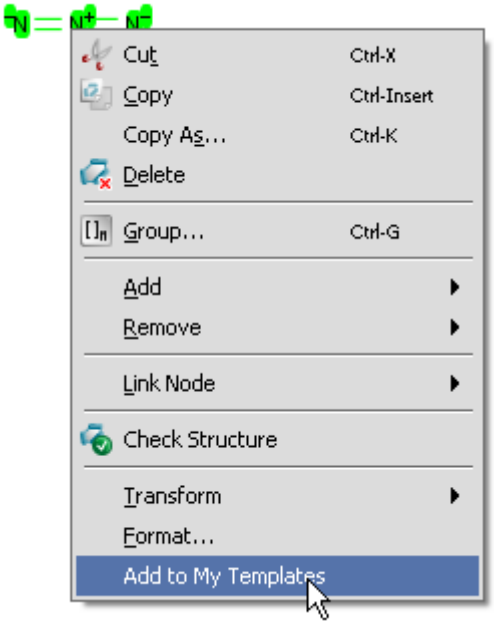
   - Crown Ethers and Bridged Polycyclics:

- To control which template sets are displayed on the toolbar, use the Properties panel in the Template Library from insert menu:



Checking the 'Use molecules as templates at 2D cleaning' checkbox will effect the structures containing that template during cleaning of the structure: the default cleaning form is overwritten by the template structure. This way, you can cutomize your drawings: add or draw a set of templates and check this option.
Any structure can be added to the My Templates group.

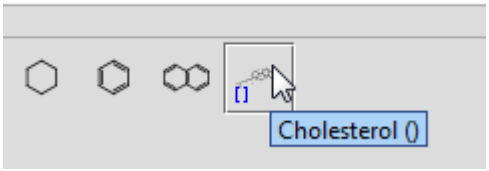
- Using the Pop-up menu



Set the name of the new template.

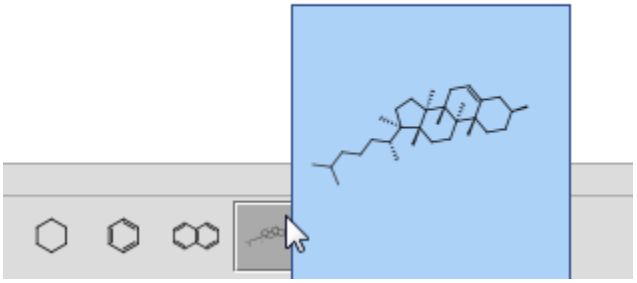
- Right-click on the template icon on the template toolbar and select **Properties**.

- Set the name and/or the abbreviation of the template in the Template Properties box.
- After that the template is identified with its name and/or abbreviation.



Templates without a name

- If the template does not have a name, hovering the cursor over its icon on the template toolbar magnifies the image on the icon. This improves the visibility of the template icon, especially for big structures.



The template can be removed from the toolbar.

- Right-click on the template icon and select **Remove** to remove the template from the toolbar and from the My Templates list.

Status Bar of MarvinSketch

The Status Bar appears at the bottom of the main frame, and unlike toolbars, it cannot be customized or moved.

The Status Bar consists of 3 parts:

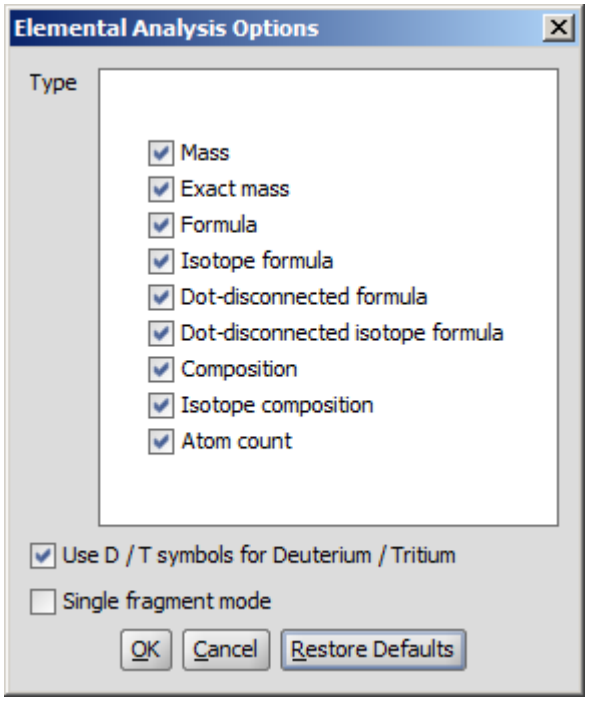
1. **Dimension Button**
   Switches between 2D and 3D modes. If the current structure is represented in 3D, then switching to 2D mode performs a 2D cleaning upon confirmation.
2. **File Status Indicator**
   This sign appears dynamically if there are unsaved modifications on the current structure, and disappears upon a Save command.

3. **Structure Checker Status**
   By default it is disabled as seen on the first image. To enable manual checking double-click on it. Right-click enables automatic checking. The status bar displays different images when there is no problem, if checking is in progress or if problems were found.
4. **Navigation Buttons**
   The Navigation Buttons appearing on the Status Bar dynamically using multipage molecular documents provide a quick way to navigate between pages.

Elemental Analysis

Basic molecular values related to the elemental composition of the molecule are calculated by the Elemental Analysis.

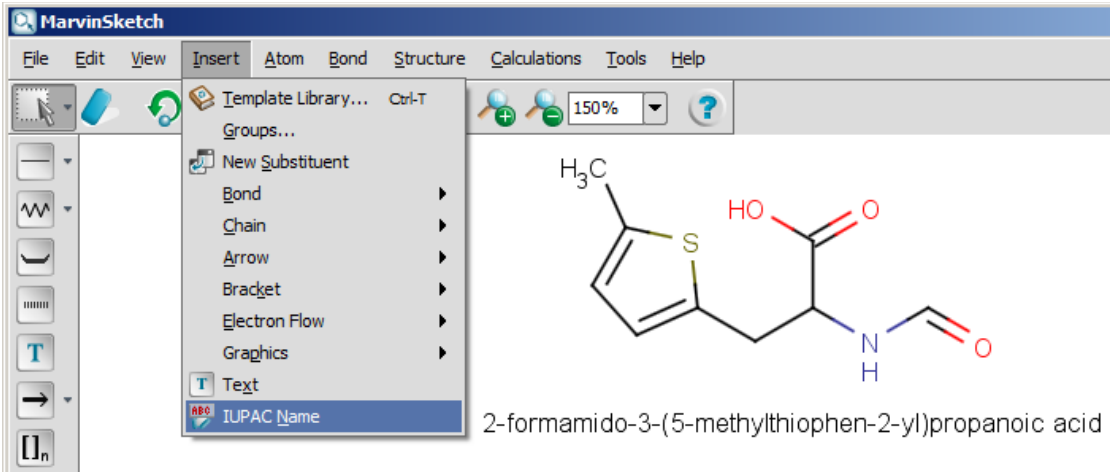
In the **Elemental Analysis Options** panel you can check different properties:



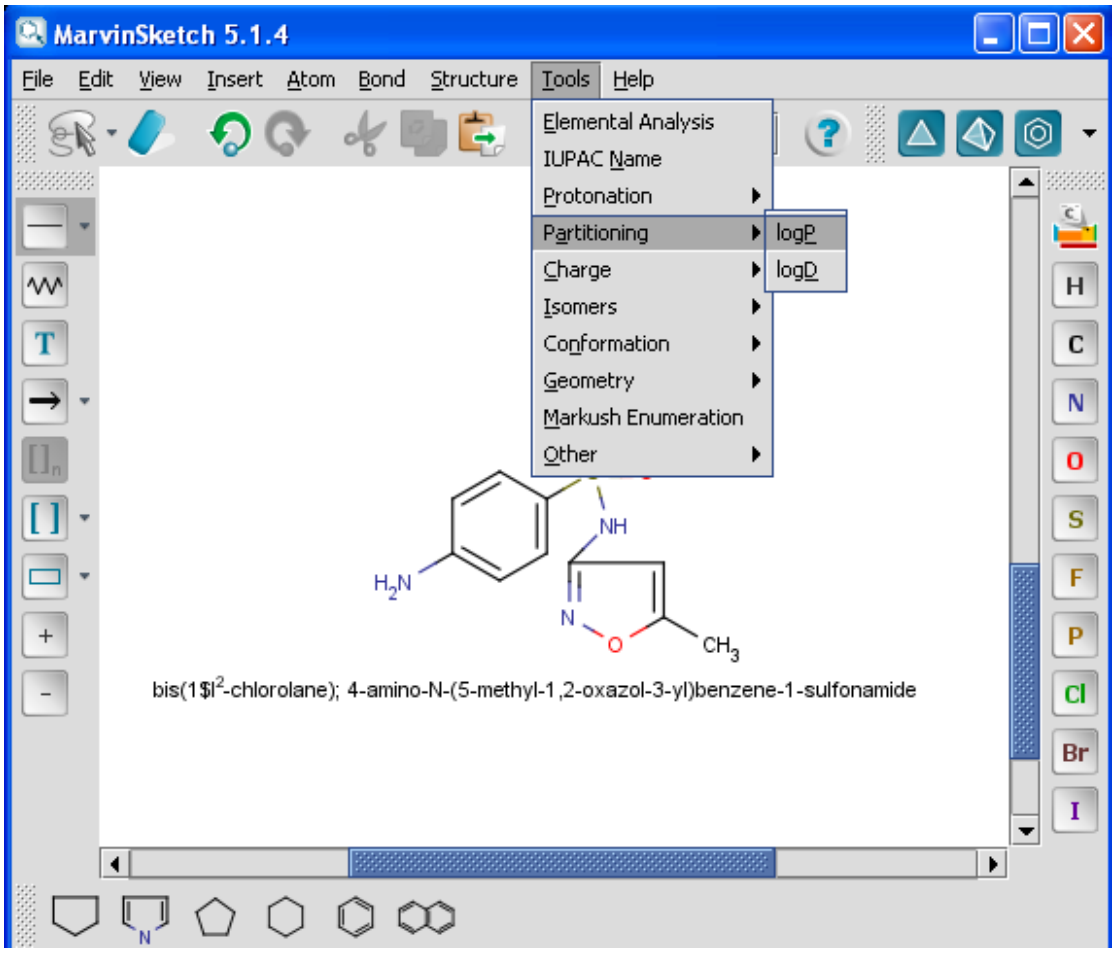
- **Type**
  - **Mass:** average molecular mass calculated from the standard atomic weights [1].
  - **Exact mass:** monoisotopic mass calculated from the weights [2] of the most abundant natural isotopes of the elements.
  - **Formula:** chemical formula of the molecule according to the Hill system [3]: the number of carbon atoms is indicated first, the number of hydrogen atoms next, and then the number of all other chemical elements subsequently, in alphabetical order. Isotopes (like Deuterium and Tritium) are not listed separately but counted together (e.g., deuterium and tritium atoms are counted as hydrogens). When the formula contains no carbon, all the elements, including hydrogen, are listed alphabetically. If the molecule contains an SRU or Repeating Unit S-group, it will be taken into account and Polymer Formula will be generated.
    Note: For polymer structures, mass, composition, and atom count calculations are not available and will return NaN, N/A, and -1, respectively.
  - **Isotope formula:** chemical formula of the molecule listing isotopes separately according to the Hill system.

- **Dot-disconnected formula:** chemical formula of the molecule(s) separating fragment formulas by dots (e.g. salts, counterions, solvent molecules etc. are present).
- **Dot-disconnected isotope formula:** chemical formula of the molecule separating fragment formulas by dots and listing isotopes separately.
- **Composition:** elemental composition given in weight percentage (w/w %) calculated from the atomic masses.
- **Isotope composition:** elemental composition listing isotopes separately (w/w %).
- **Atom count:** number of all atoms in the molecule.
- **Use D/T symbols for deuterium/Tritium:** if unchecked (default), isotopes of hydrogen are displayed in formulas as 2H and 3H, if checked, D and T symbols are used.
- **Single fragment mode:** if unchecked (default), the calculation handles unlinked molecules together (e.g. salt molecules), summing up the masses of each component, if checked, the results are displayed in a scroll window.

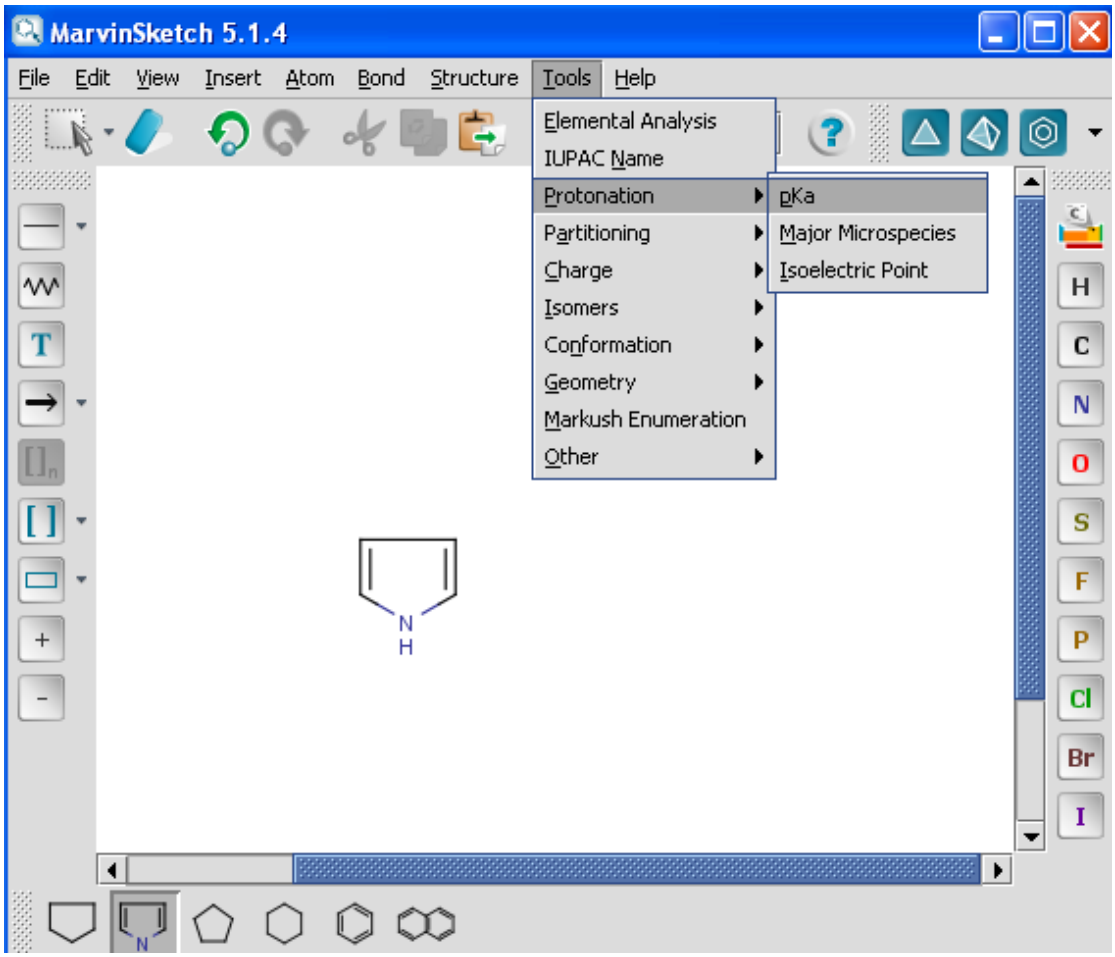
Name generator

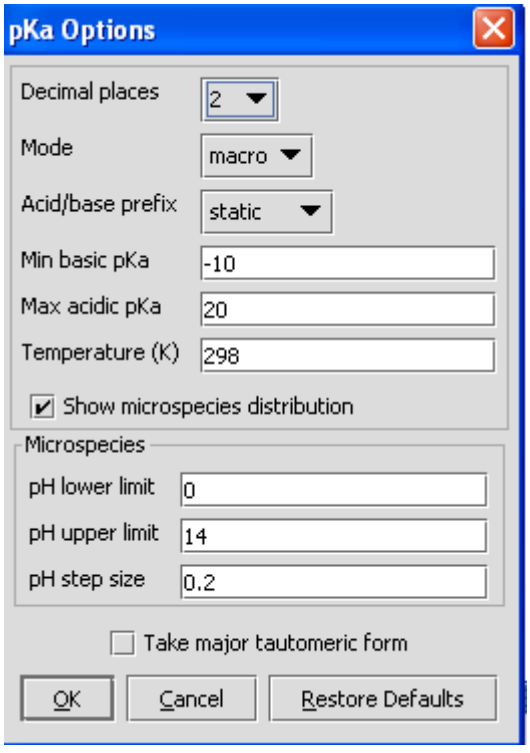


Log P/ log D


MarvinSketch 5.1.4
File Edit View Insert Atom Bond Structure Tools Help
Elemental Analysis
IUPAC Name
Protonation
Partitioning
Charge
Isomers
Conformation
Geometry
Markush Enumeration
Other
logP
logD
H
C
N
O
S
F
P
Cl
Br
I
NH
H_2N
N
O
CH_3
bis(1$l^2-chlorolane); 4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzene-1-sulfonamide

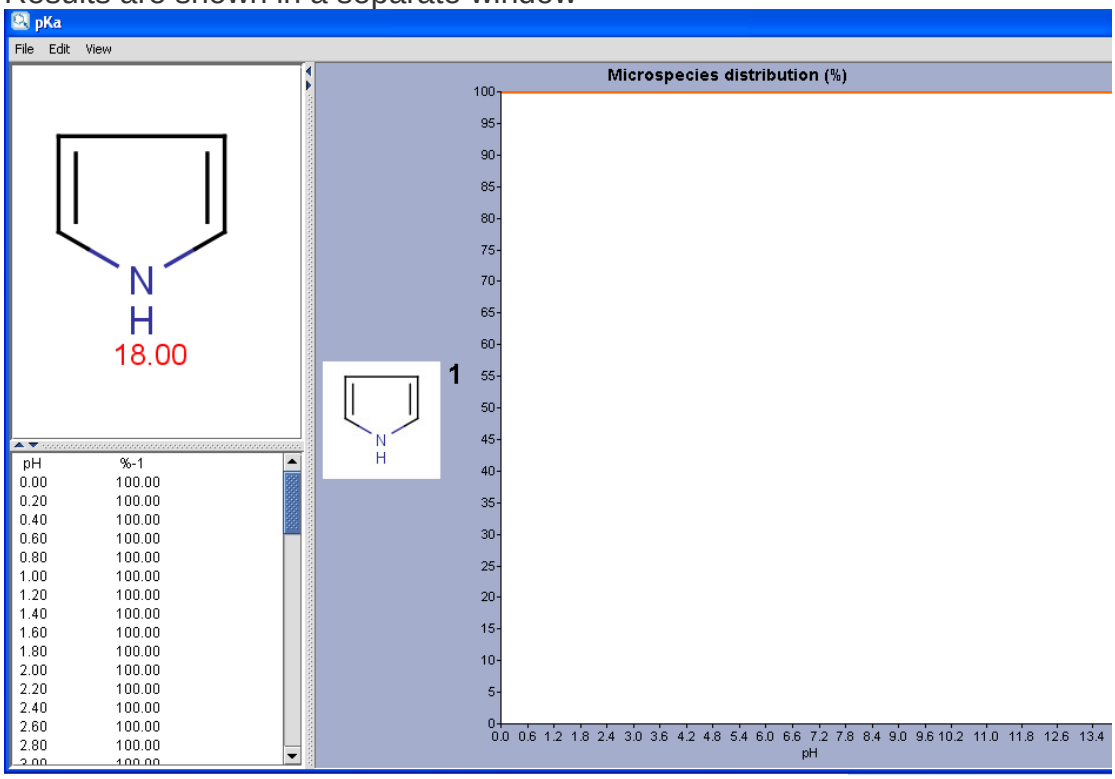
PKa


MarvinSketch 5.1.4
File Edit View Insert Atom Bond Structure Tools Help
Elemental Analysis
IUPAC Name
Protonation
Partitioning
Charge
Isomers
Conformation
Geometry
Markush Enumeration
Other
pKa
Major Microspecies
Isoelectric Point
H C N O S F P Cl Br I
N
H


pKa Options
Decimal places 2
Mode macro
Acid/base prefix static
Min basic pKa -10
Max acidic pKa 20
Temperature (K) 298
Show microspecies distribution
Microspecies
pH lower limit 0
pH upper limit 14
pH step size 0.2
Take major tautomeric form
OK
Cancel
Restore Defaults

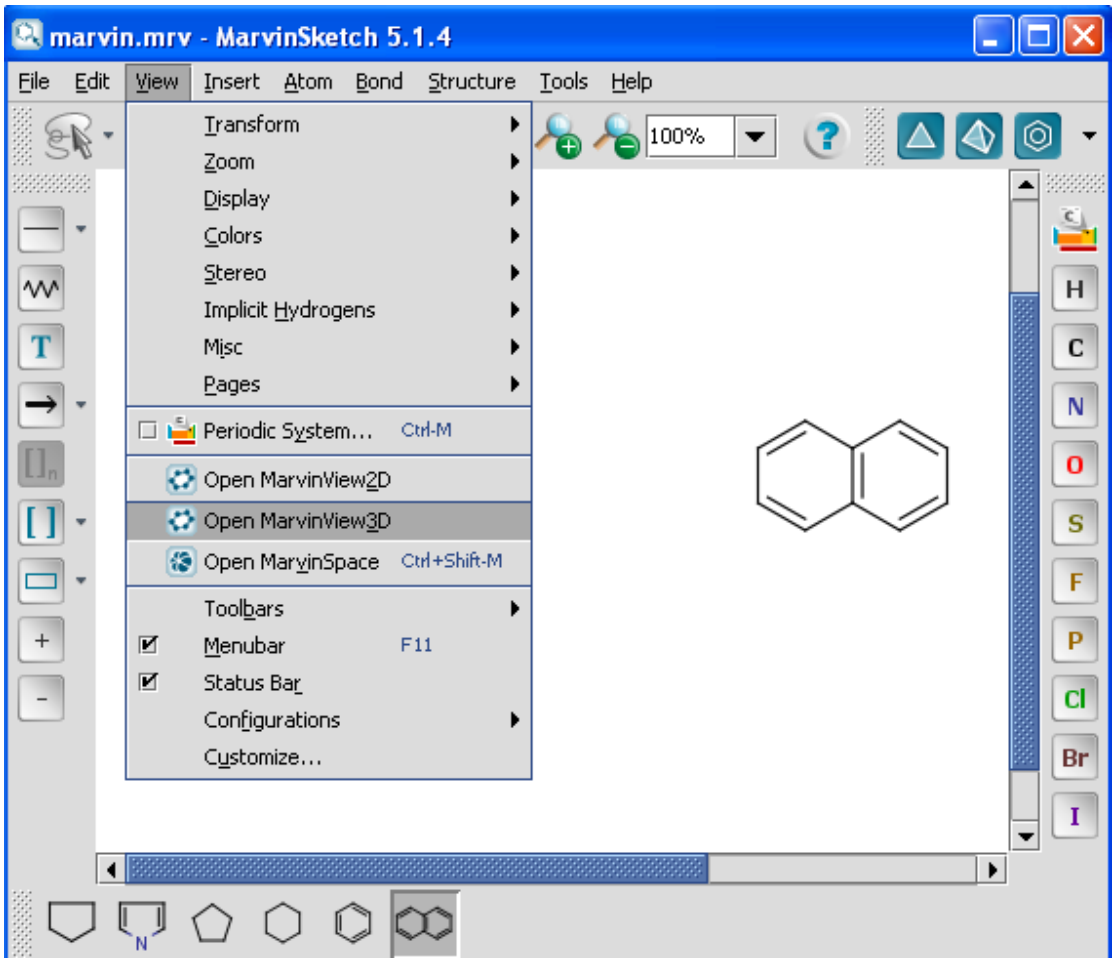
- **Decimal places:** setting the number of decimal places with which the result value is given.
- **Mode:** micro, macro: micro and macro acidic dissociation constants.
- **Acid/base prefix:**
  - **static:** submitted ionic forms are converted to their neutral forms (adding or removing protons) and their pK_a is calculated.
  - **dynamic:** the pK_a of ionic forms are calculated, not their conjugated acids or bases.
- **Min basic pK_a:** widens the calculation range because weak bases will have lower pK_a values than the default -10.
- **Max acidic pK_a:** widens the calculation range because weak acids will have higher pK_a values than the default 20.
- **Temperature:** setting the temperature in Kelvin.

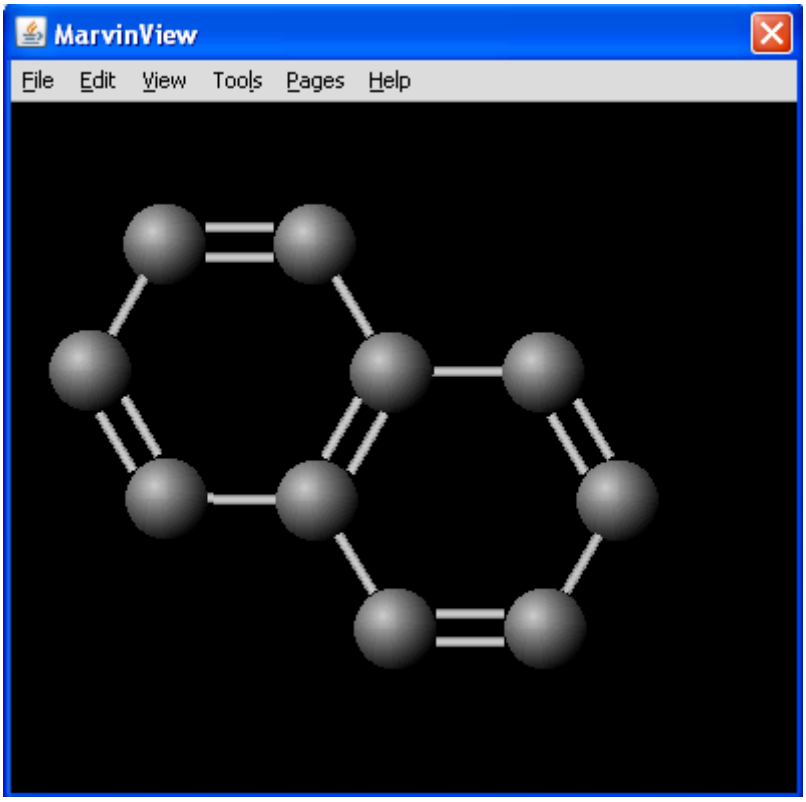
Results are shown in a separate window



2D and 3D Viewer Windows

Choosing **View >Open 2D Viewer** or **Open 3D Viewer** launches a MarvinView window containing the current molecule of MarvinSketch

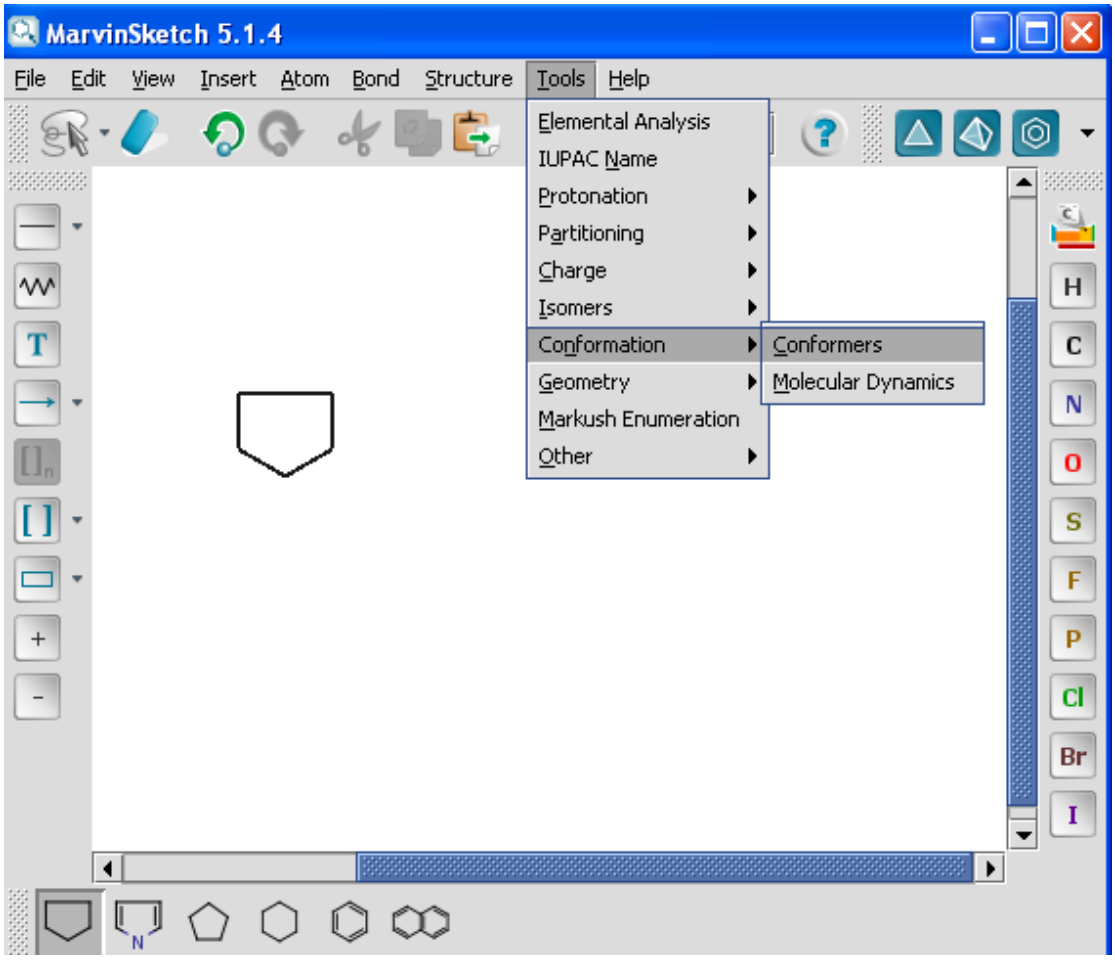


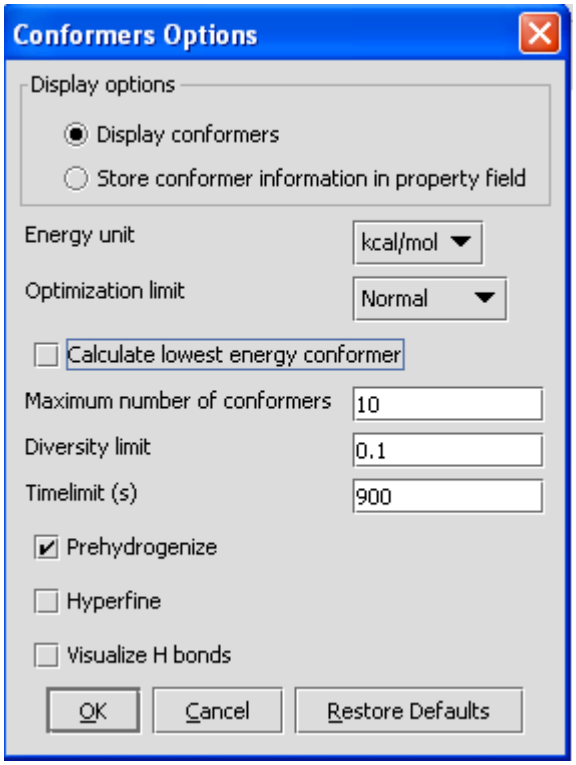


Isomers and their energies

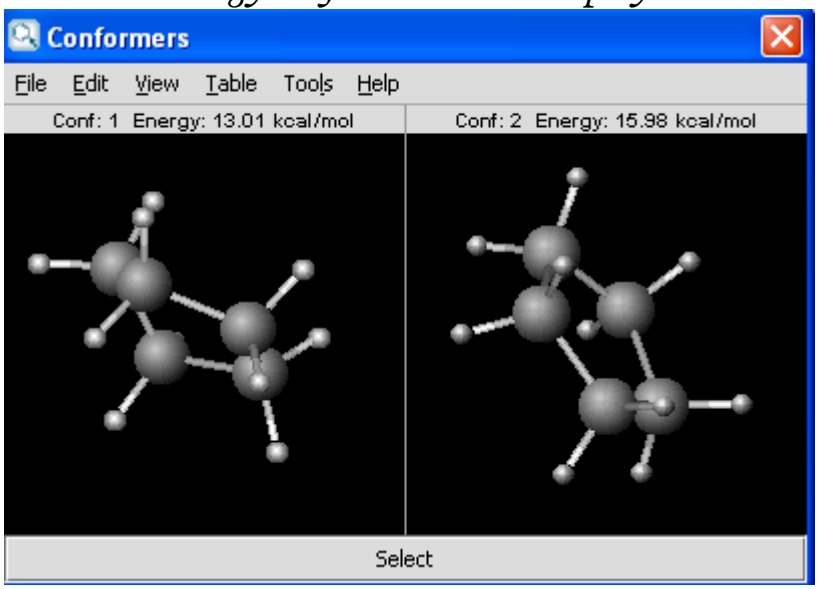
Conformers

Ex. Cyclopentane

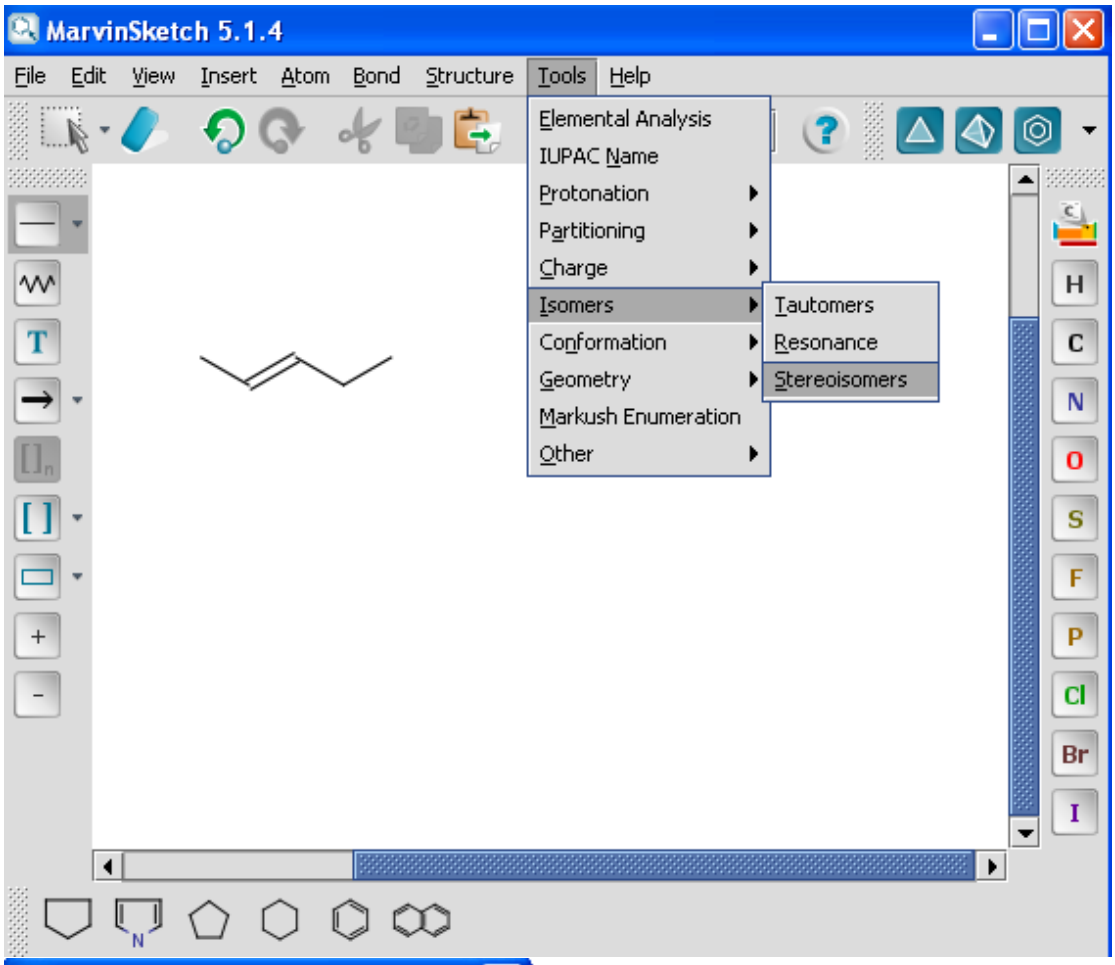


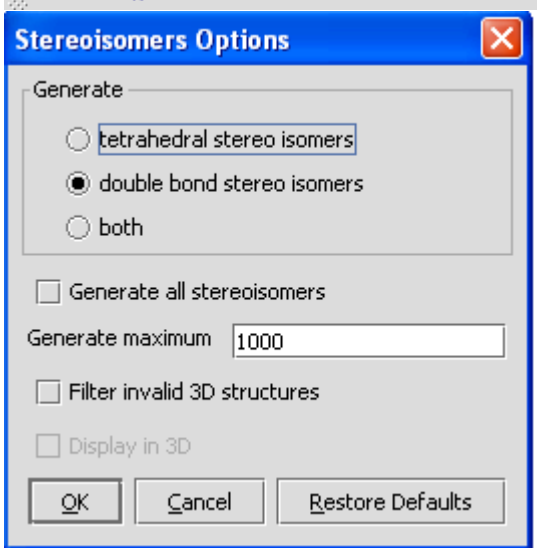


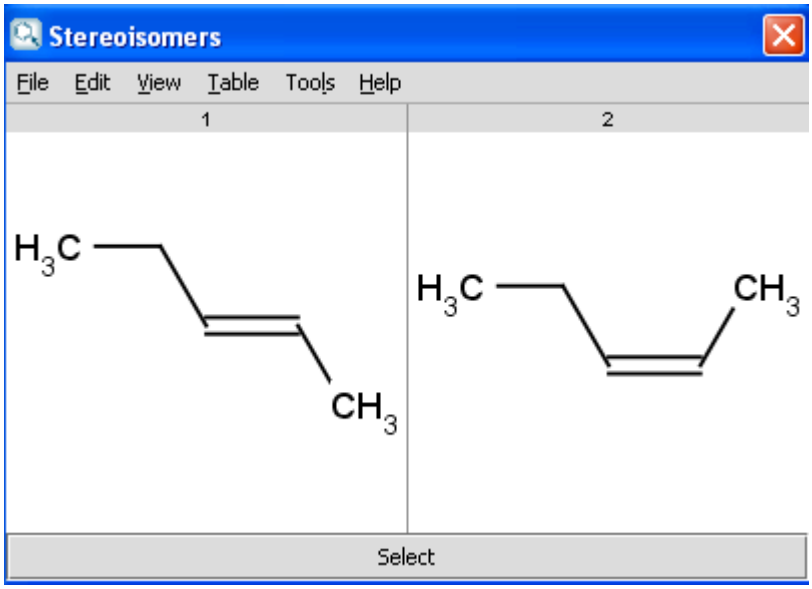
You can choose the option calculate the lowest energy conformer and just the lowest energy conformer will be displayed



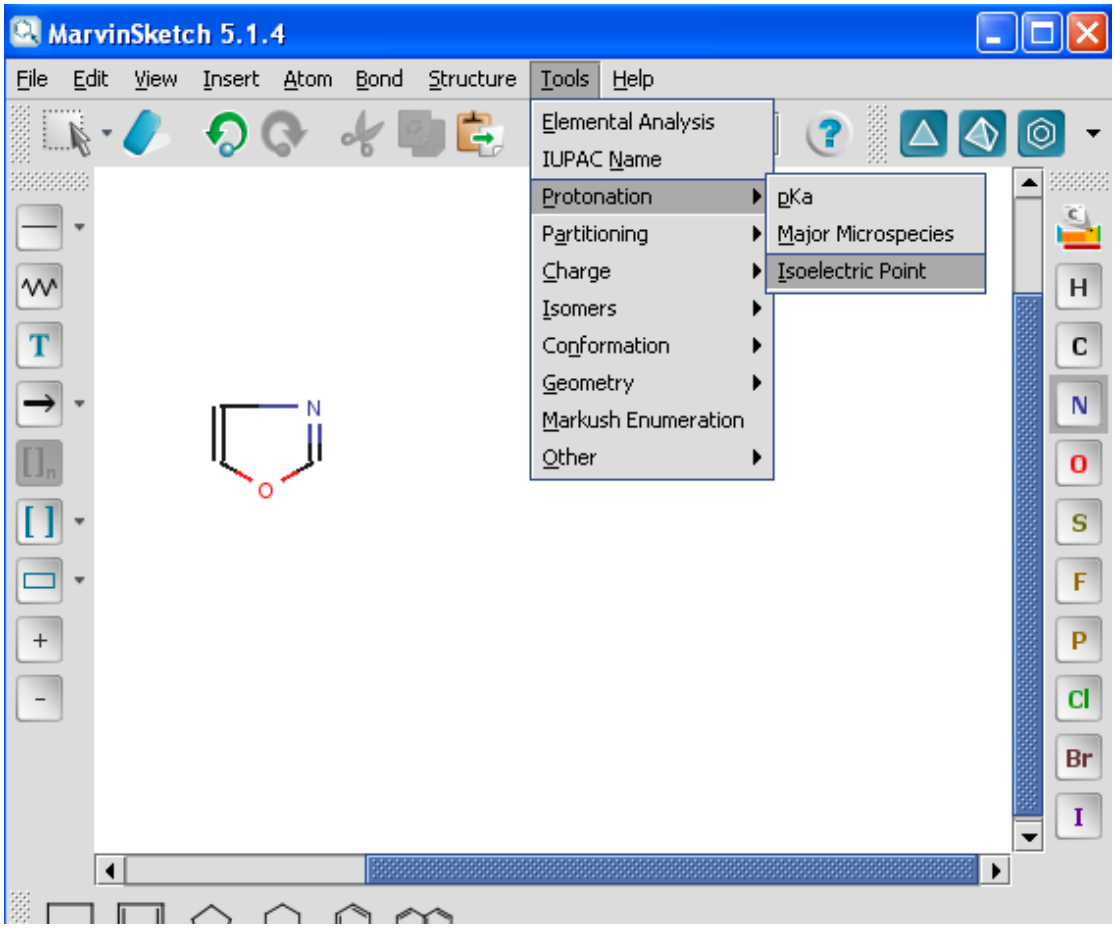
Stereoisomers(cis/trans)
Ex. 2-pentene

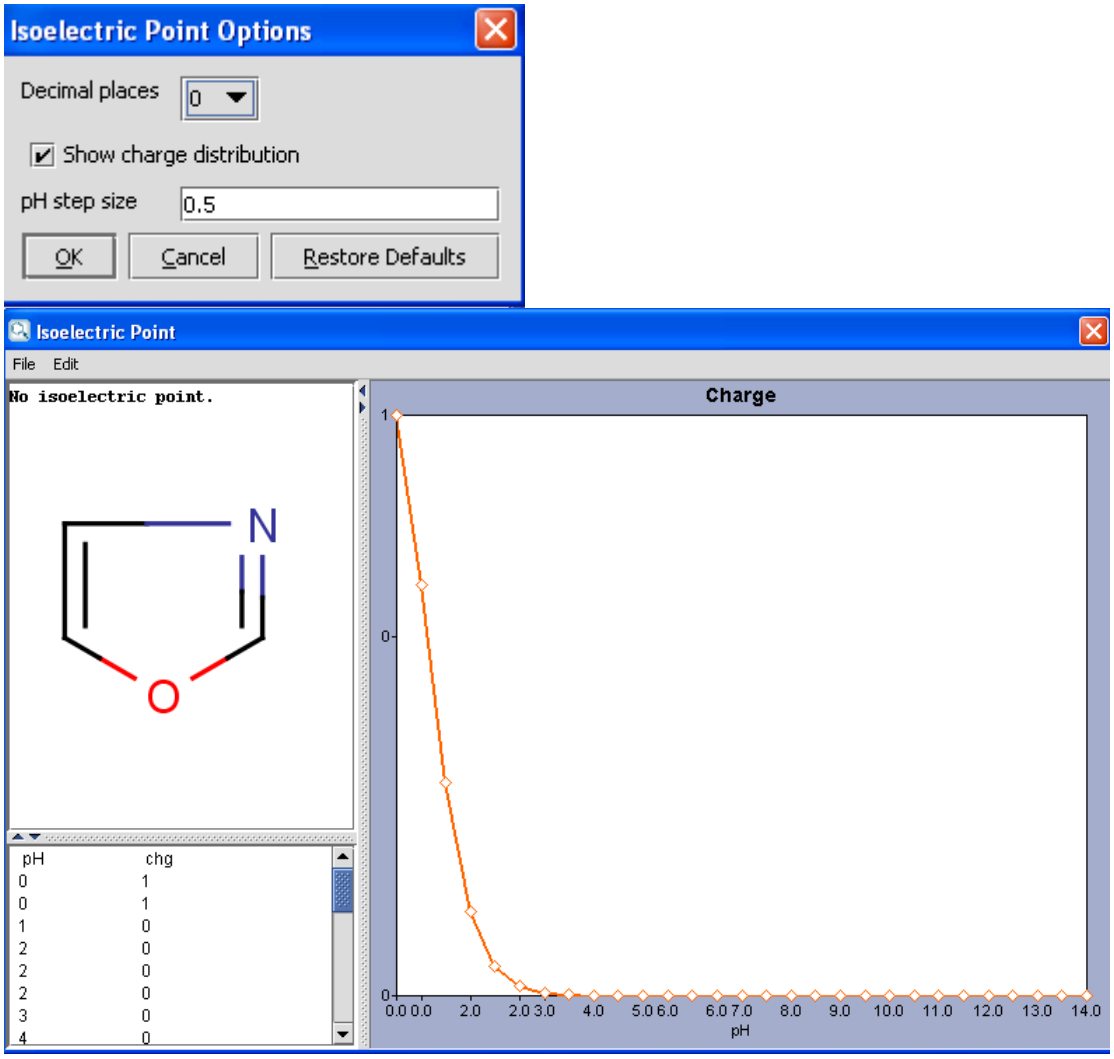




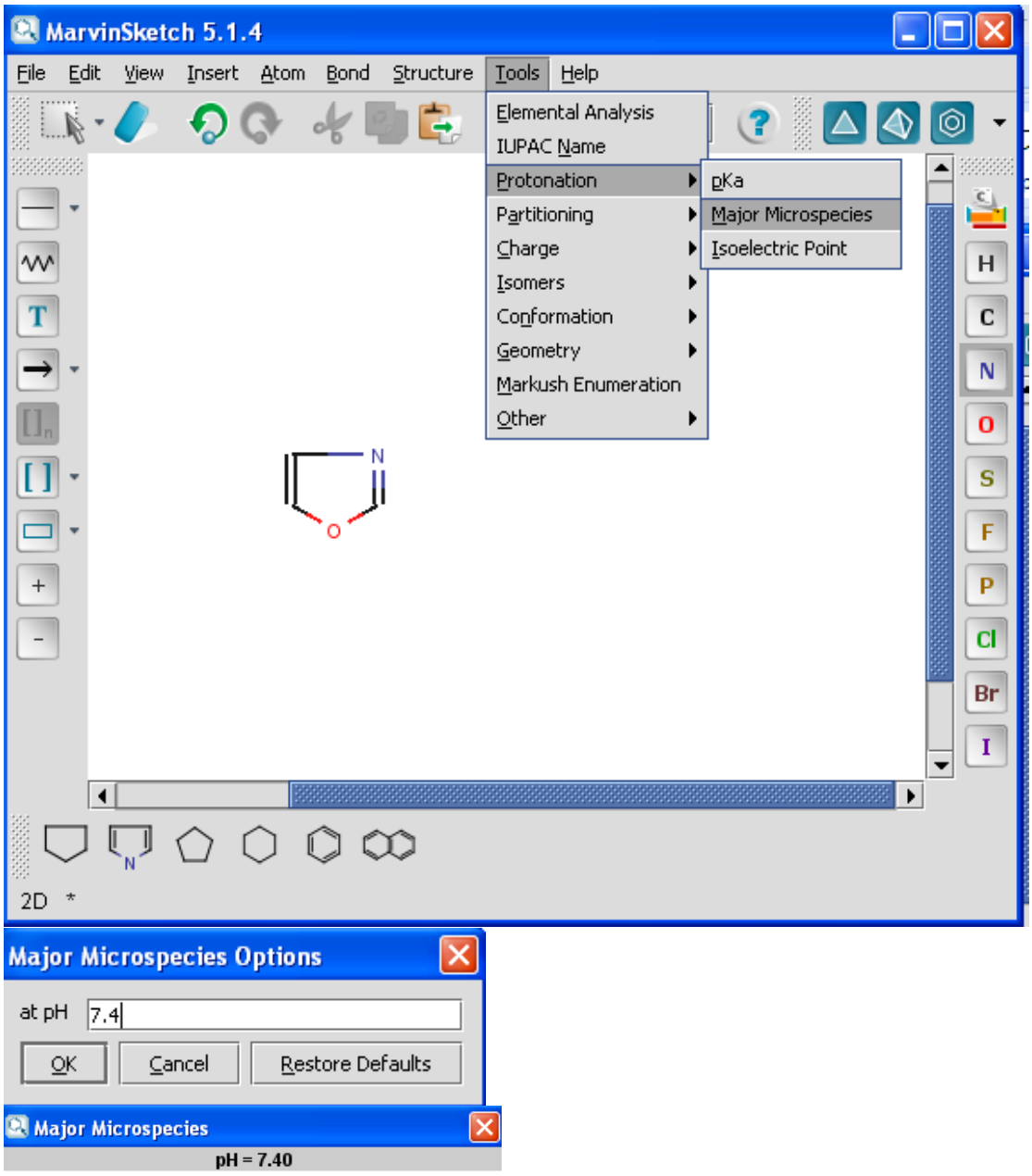


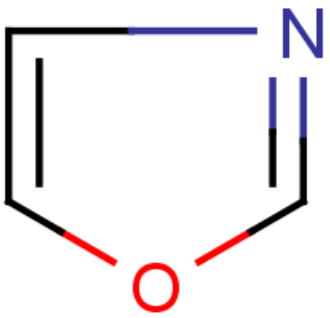
Ionization of compounds at different PH values and isoelectric point: Ex. Oxazole




Isoelectric Point Options
Decimal places
0
Show charge distribution
pH step size
0.5
OK
Cancel
Restore Defaults
Isoelectric Point
File
Edit
No isoelectric point.
N
O
pH
chg
0 1
0 1
1 0
2 0
2 0
2 0
3 0
4 0
Charge
1
0
0
0.0 0.0
2.0
2.0 3.0
4.0
5.0 6.0
6.0 7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
pH

Major microspecies at specific PH value:

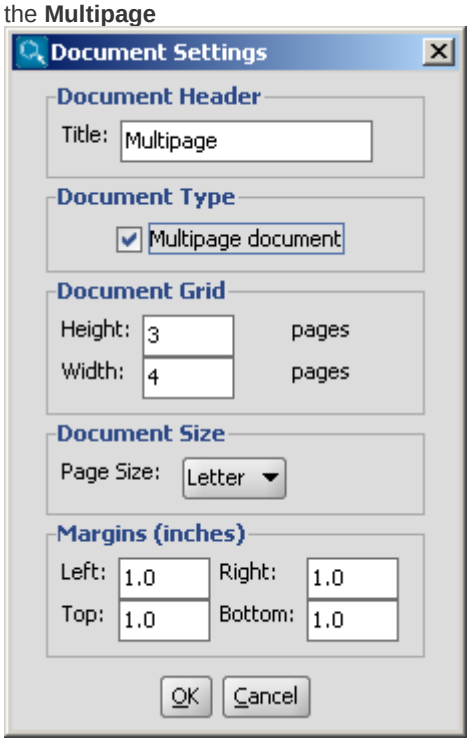




Oxazole not ionized at physiological Ph (7.4)

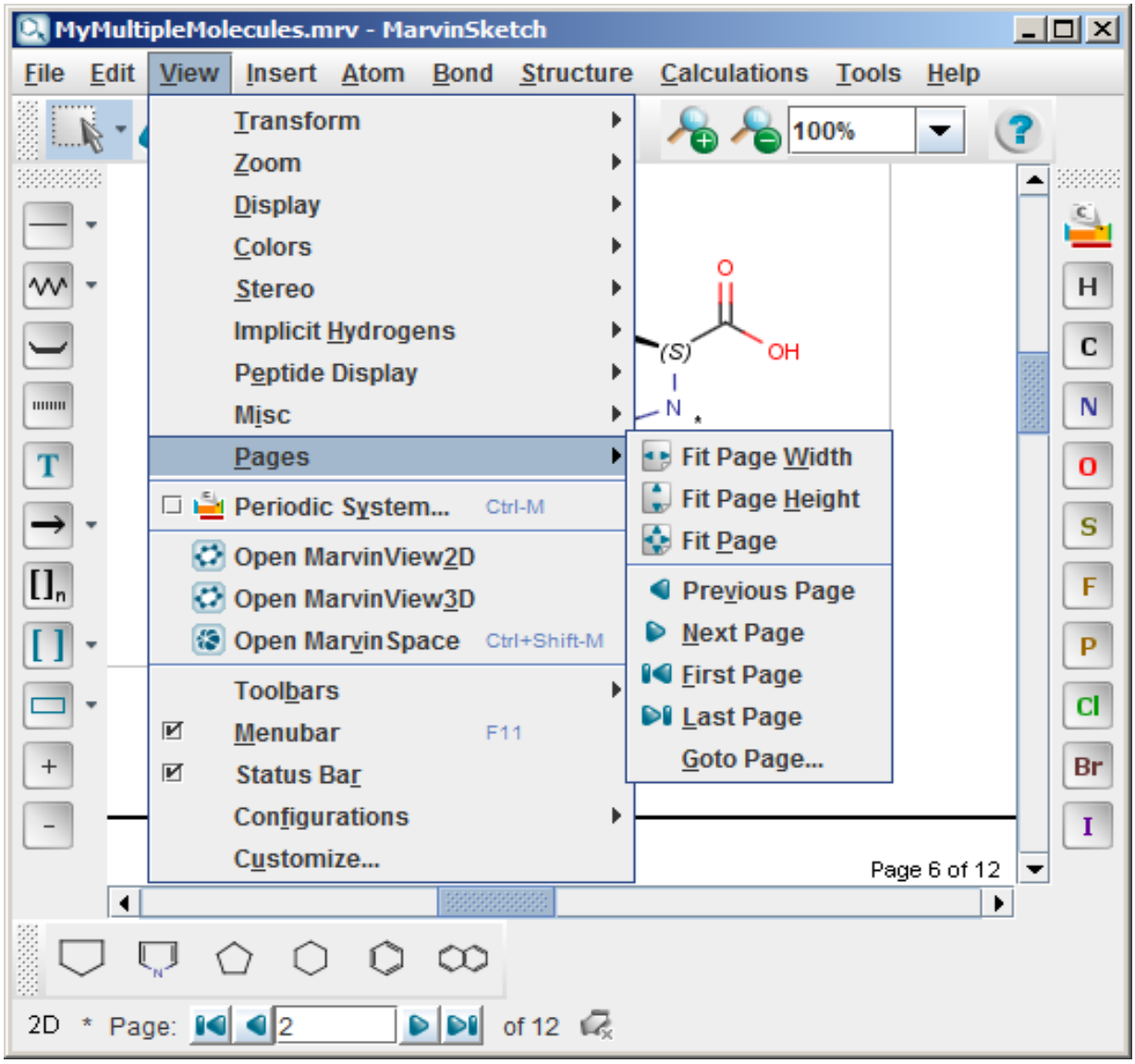
Working with Multipage Molecular Documents
How to create a multipage molecular document

Multipage molecular documents help to work with large drawings by dividing them into pages. You can create a multipage molecular document by choosing **File > Document Settings...**, then checking in the **Multipage document** checkbox.

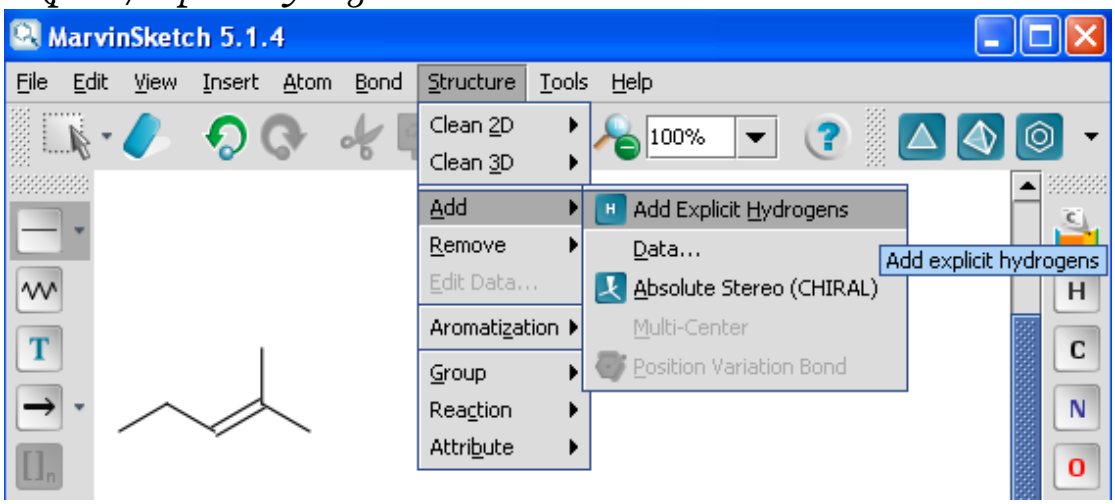


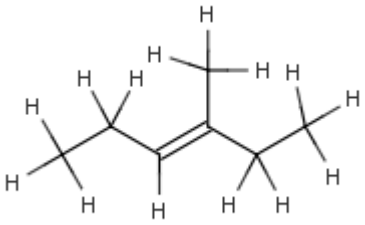
You can set the number of horizontal and vertical pages in the **Document Grid** part, and you can also define the title, the page size and the margins in the corresponding sections of this dialog window. After pushing the OK button, the following controls become automatically available:

- The items in the **View > Pages** menu are enabled
- A navigation status bar appears on the bottom of the window
- The frame of the pages appear on the canvas, while the title, the margins and the page numbers are displayed on each page



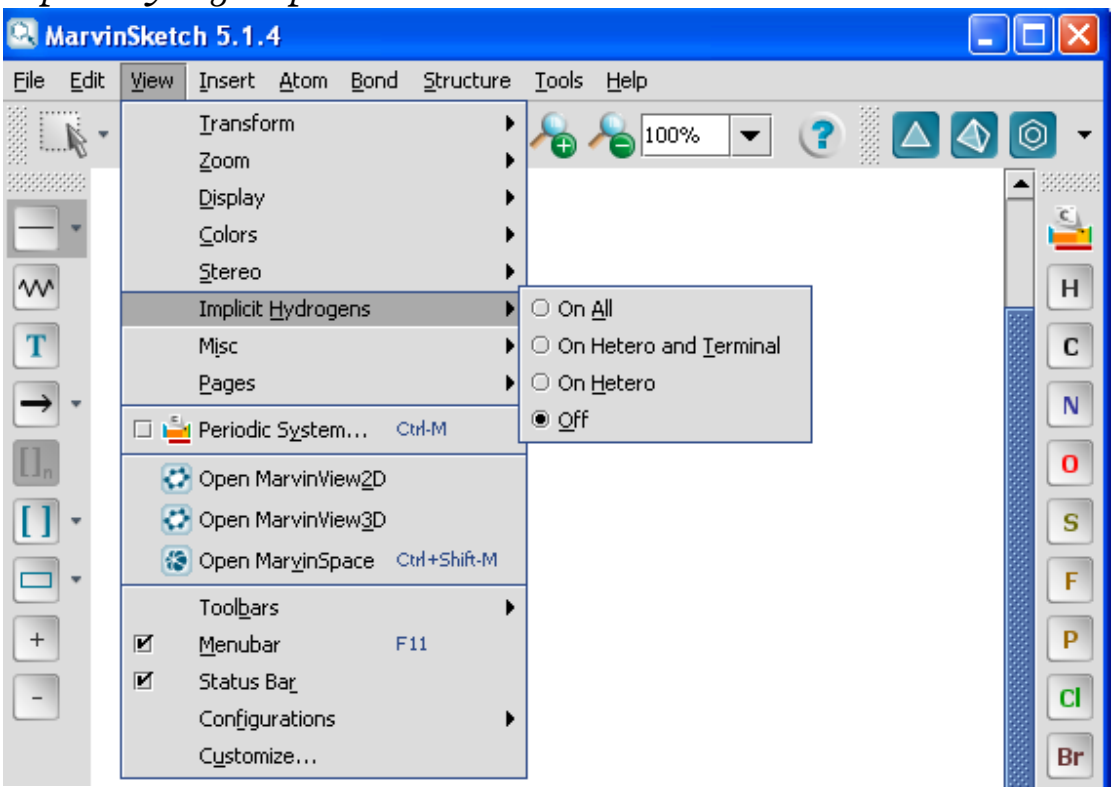
Explicit/implicit hydrogens

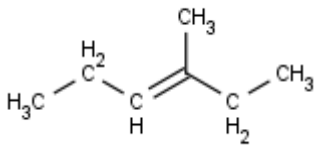




structure with explicit hydrogens

implicit hydrogen options





implicit hydrogens on all

Post-lab.

Report sheet (9)
Marvin software

Student name:

1. Draw the chemical structure of both cis- and trans-2butene, minimize them and report their energies. Which of these isomers has the lowest energy? Can you explain why?

2. Draw the structure of sulfamethoxazole .

3. Find the IUPAC name.

4. Calculate Log P value.

5. Do you think that the drug at physiological pH will be ionized or not (study the degree of ionization of the drug at different pH's).

6. Draw the chemical structure of cyclohexane (determine the shape and energy of the least conformer). Use the different visualization models.

7. Draw the chemical structure of cis- 1,2dimethylcyclohexane and trans-1,2dimethylcyclohexane (compare between the energy of the two isomers).

8. Draw the chemical structure of the compounds provided by the instructor.

Experiment 10
Molecular modeling

SAR analysis using accelrys software

Once the structure of a lead compound is known, the medicinal chemist moves on to study its structure activity relationships (SAR).

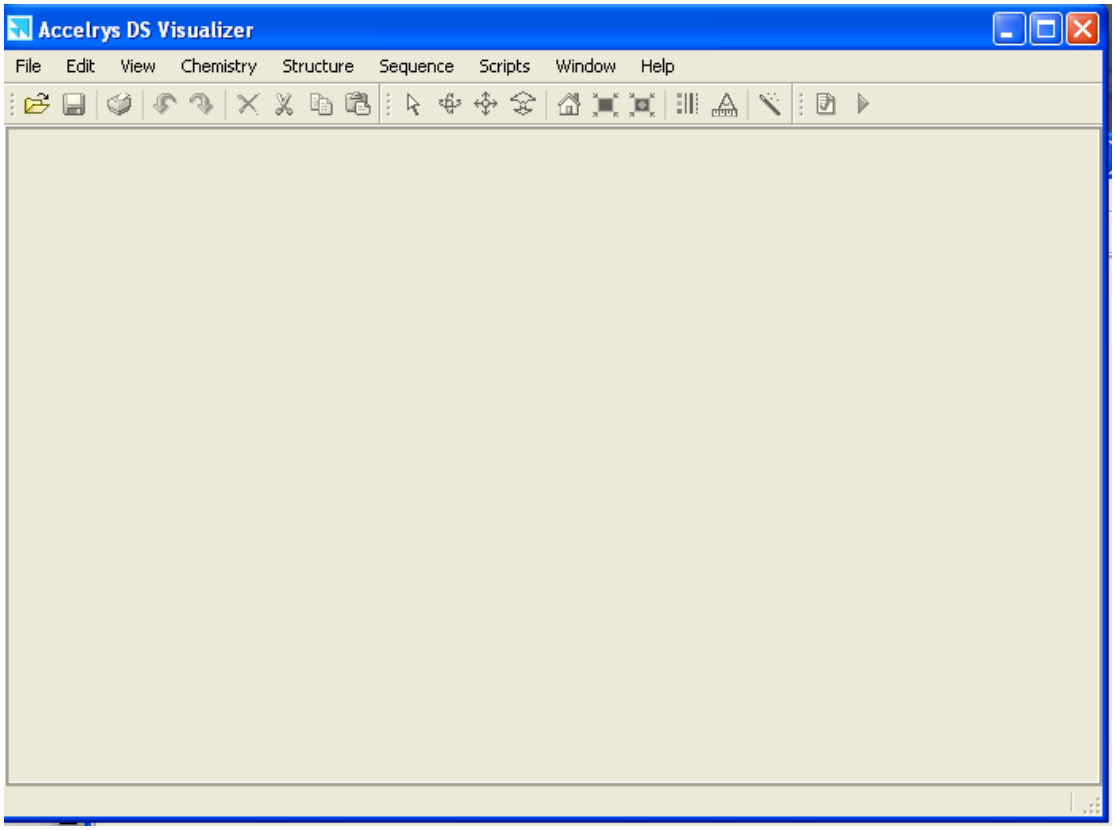
The aim here is to discover which parts of the molecule are important to biological activity and which are not. If it's possible to crystallize the target with the drug bound to the binding site, the crystal structure of the complex could be solved by x- ray crystallography, and then studied with molecular modeling soft ware to identify important binding interactions. However, this may not be possible, either because the target structure cannot be crystallized or because the target structure has not been identified. If that is the case, it will be necessary to revert to the traditional method of synthesizing a selected number of compounds that vary slightly from the original structure, then studying what effect that has on the biological activity.

In this lab, we will use the computer soft ware accelrys to study a protein binding pocket and decide the most important forces that stabilize drug protein complex.

Student must know

1. How to find binding pocket from the crystallographic image for protein.
2. How to measure the distance between two atoms.
3. Requirements for each binding force to occur between enzyme and inhibitor.

The default layout of the accelrys DS visualizer user interface is shown in the following picture.



Atom Display toolbar

The *Atom Display* toolbar contains buttons that allow you easy access to the commands on the Atom tab of the Graphics View Display Style dialog.

Toolbar button	Action	Effect
	*Atom Display Off*	Blanks selected atoms so they are not displayed on the screen.
	Line	Displays atoms using a traditional wire frame display. Nonbonded atoms are displayed as "jacks".
	Stick	Displays bonds using solid cylinders. Nonbonded atoms are displayed as "jacks" as in *Line* style.
	*Ball and Stick*	Depicts bonds using cylinders for bonds and balls for atoms.
	*Scaled Ball and Stick*	Depicts bonds using cylinders and atoms using balls scaled to the van der Waals (VDW) radii.
	*CPK*	Displays spheres sized to the van der Waals (VDW) radii.

Sketching toolbar

The *Sketching* toolbar allows you to sketch structures directly in the 3D Window by giving you access to tools to rotate torsions, to sketch atoms, chains, and rings, as well as to add text to your structures.

View toolbar

The *View* toolbar provides tools that manipulate the 3D Window or edit molecules in the document.

Select: Use the Select tool to select one or more objects using the mouse. Multiple objects can be selected by clicking with the SHIFT key down on additional objects.

Rotate: The Rotate tool is used to change the angle from which a molecule is viewed.

Translate: The Translate tool is used to move a molecule in the plane of the computer screen.

Zoom: The Zoom tool zooms the view in or out from a molecule.

Home: Undoes all rotations, returning the structure to the original orientation it was in when it was imported or created. Note that centering (Center Structure or Fit To Screen) actions are not undone.

Fit To Screen: Works in the same way as View | Fit To Screen.

Center Structure: Carries out the same operation as as View | Center.

Display Style: Accesses the same functionality as View | Display Style....

Measure: The Measure tool measures the distance, angle, or torsion between selected objects.

To measure the distance between two atoms, deselect all objects, select two atoms or one bond, and click the Measure tool. To measure the angle between three atoms, deselect all objects, select three atoms or two connected bonds, and click the Measure tool. To measure the torsion angle between four atoms, deselect all objects, select four atoms, two disconnected bonds, or three connected bonds, and click the Measure tool.

Display: The Display tool makes the object you click in either the 3D Structure View or the Hierarchy View visible, and everything else invisible.

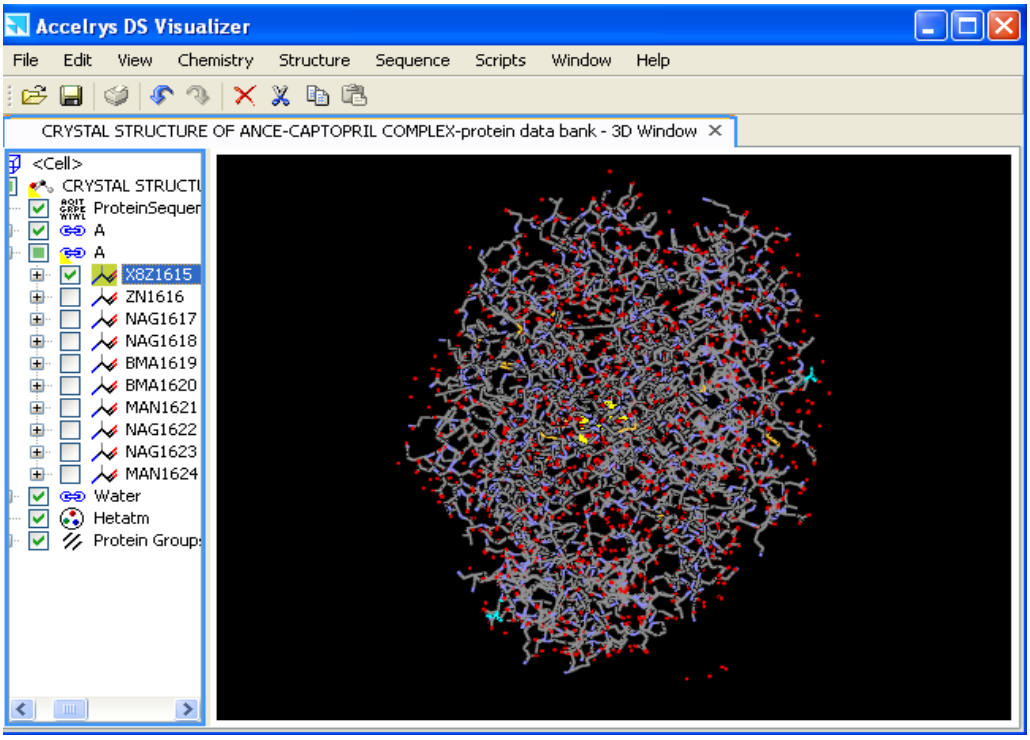
Chemistry toolbar

The *Chemistry* toolbar provides shortcuts to commands in the Chemistry menu that assist you in building molecules.

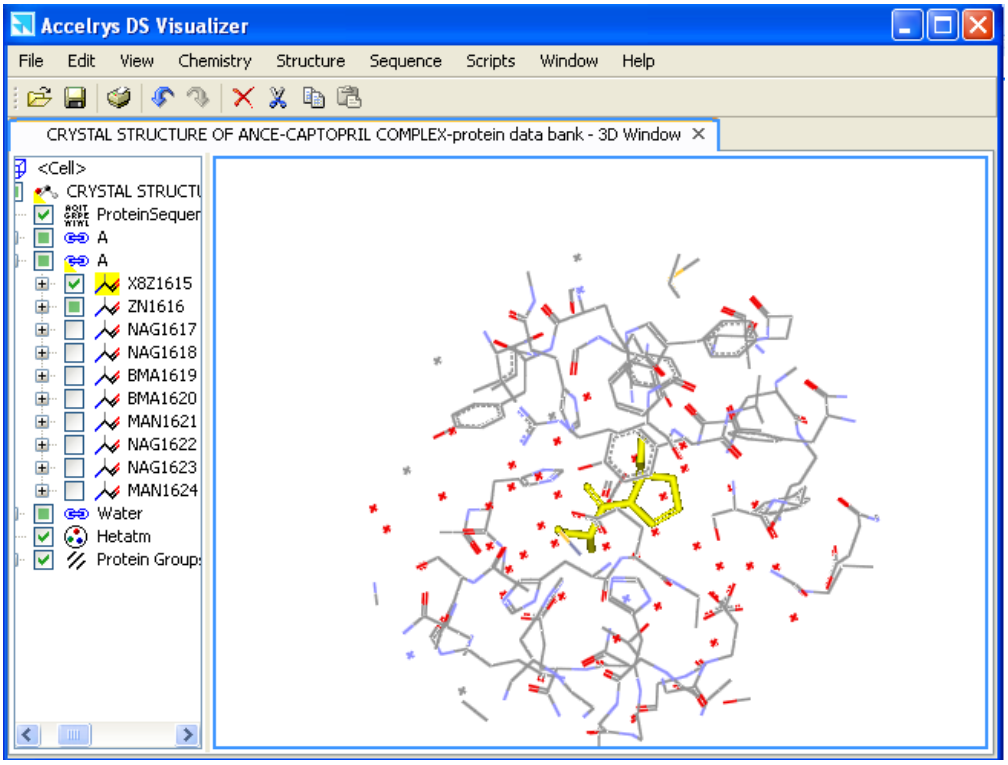
Toolbar button	Action	Effect
	Add Hydrogens	Chemistry | Hydrogens | Add
	Hide Hydrogens	Chemistry | Hydrogens | Hide
	Single Bond	Chemistry | Bond | Single
	Double Bond	Chemistry | Bond | Double
	Aromatic Bond	Chemistry | Bond | Aromatic
	Triple Bond	Chemistry | Bond | Triple
	Periodic Table	Chemistry | Element | Table...
	Clean Geometry	Structure | Clean Geometry

Ex.

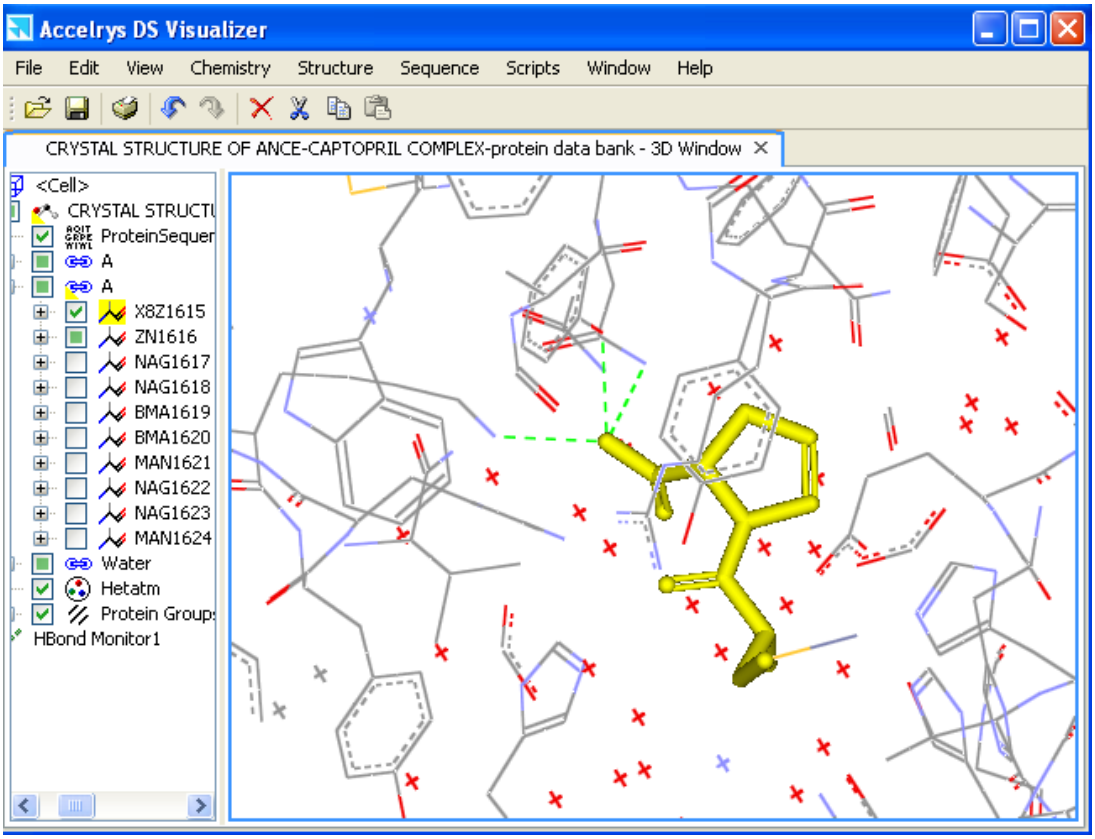
1. Open the DS visualizer.
2. From the toolbar, go to File-Open-protein data bank file-Captopril-ACE complex.



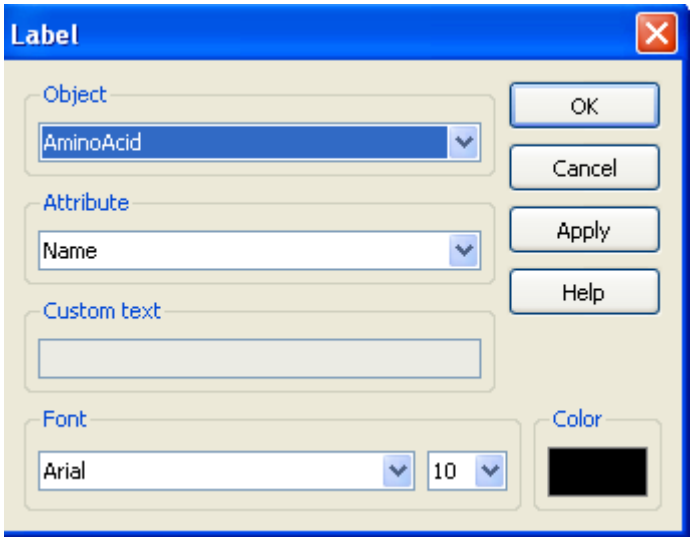
3. To change the color of the background From the toolbar, under edit open preferences then select 3D window from the left side (double click on background color and choose the color you want).
4. To identify the binding pocket, from the cell window (on the left side): Open the second A (click on +) then select captopril (code X8Z1615) .
5. From the toolbar, under Structure open Show by radius and enter the No. 10 then ok.
6. To differentiate between the amino acids in the binding pocket and the drug go to View-Display Style and select stick model.



7. Try to identify the Zn atom and identify how the drug is strongly attached to the metal atom in the binding pocket.
8. To find hydrogen bonding interactions between drug and the enzyme: select captopril (as shown above) then go to **Structure Monitor –intermolecular H-Bond**) .



9. To identify the amino acids in the binding pocket that have interactions with the ligand ,select the amino acid then right click and choose label



Choose amino acid from **object** and name from **attribute** then apply and ok and the abbreviated name of the amino acid will appear.
Or from the cell window open H bond monitor 1(click on +) and all h-bond details will appear.

10. Fill the drug enzyme interaction tables listed below:

No	Drug Functional Group	Amino acid	Type of interaction

Pre-lab.

Report sheet (10)
SAR analysis using accelrys software

Student name:

Draw the structures of the 20 standard amino acids and classify them?

Draw the structure of captopril then find out the IUPAC name?

Draw the structure of levothyroxine then find out the IUPAC name?

Post-lab.

Report sheet (10)
SAR analysis using accelrys software

Student name:

Procedure

1. Open the DS visualizer.
2. From the toolbar, go to File-Open-protein data bank file-thyroxine thyroid hormone receptor interactions.
3. To identify the binding pocket, from the cell window (on the left side): Open the second X (click on +) then select thyroxine (code T44500).
4. From the toolbar, under Structure open Show by radius and enter the No. 10 then ok .
5. To differentiate between the amino acids in the binding pocket and the drug go to View-Display Style and select stick model .
6. To find hydrogen bonding interactions between drug and the receptor: select thyroxine (as shown above) then go to Structure Monitor –intermolecular H-Bond) .
7. Fill the drug enzyme interaction tables listed below.

No	*Amino acid*	*Drug Functional Group*	*type of interaction*

Appendix I

Percent Yields Calculations

Many synthesis require more than one reaction, with each reaction yielding an isolated compound before the final product is reached. Each individual step in the sequence has a percent yield, and the total synthesis has an **overall yield** calculated from the steps yields

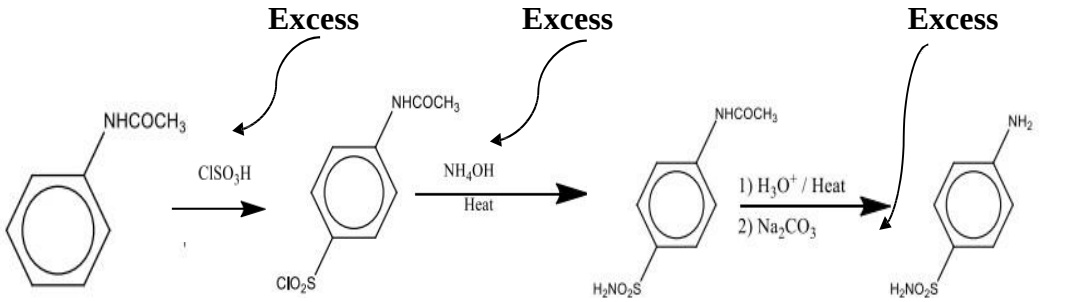
- **Limiting reagent :**
  the reagent that is present in the smallest equivalent stoichiometric amount and limits how much product will be produced. It determines the maximum amount of product(theoretical yield) that will be formed.

- **Percent yield** = $\frac{\boldsymbol{actual\ mass\ yield}}{\boldsymbol{theoretical\ yield}}$ *** 100%**

- **Overall yield =** $\frac{\%\ \boldsymbol{yield\ for\ step\ 1}}{\mathbf{100}}$ ***** $\frac{\%\ \boldsymbol{yield\ for\ step\ 2}}{\mathbf{100}}$ $*$ $\frac{\%\ \boldsymbol{yield\ for\ step\ 3}}{\mathbf{100}}$

Each individual step has a relatively high efficiency, but the overall efficiency is low. Each subsequent reaction further reduces the actual amount of product that is formed from the initial starting material.

Example (1) from cycle 1:



Acetanilide ***p-acetaminobenzene sulfonylchloride*** ***p-acetaminobenzene sulfonamide*** ***sulfanilamide***

1. You have 3 steps for this reaction, so you have to find percent yield for each step then multiply them to find the overall yield for the synthesis.

2. In this cycle the other reactants were used in excess so your main reactant will be the rate limiting reactant.

For Step 1:

Acetanilide + chlorosulfonic acid ———> p acetaminobenzene sulfonylchloride

*Amount used	15 g	excess
*No. of moles (wt/m.wt)	(15/135.17)	excess(no need to calculate)
	(0.111 moles)	excess

So rate limiting reactant is acetanilide
No. of moles of product =No. of moles of acetanilide (ratio 1:1) =0.111 moles Theoretical yield = 0.111*233.67(m.wt of product) = 25.93 g

$$\textbf{\% yield} = \frac{\boldsymbol{actual\ mass\ yield}}{\boldsymbol{theoretical\ yield}} * \textbf{100\%}$$

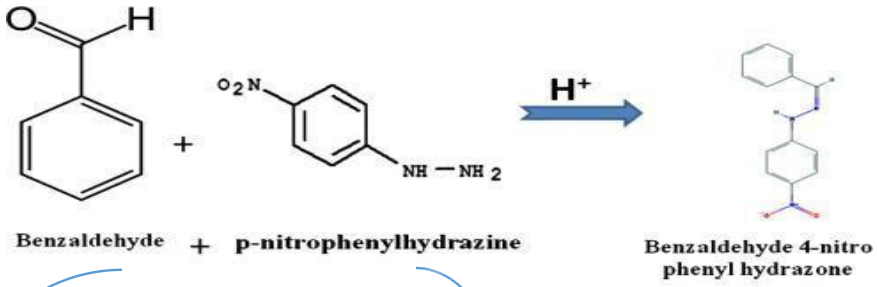
Let's assume that when you weighed your product after drying in the next lab and was 16 g (this 16 g is your actual yield)

So % yield for this step is equal to (16/25.93) *100% = 61.7%

** Make the same calculations for the second two steps , then multiply them to find the overall yield.

Example (2): (if the reactant not in excess)

Calculate the % yield if 2 ml of benzaldehyde(density =1.043, m.wt =106) were reacted with 4 ml of phenylhydrazine ((density =1.095, m.wt=108) in order to synthesize phenylhydrazone (m.wt=196)



a. Convert volumes to no. of moles

- weight (vol*density)	2*1.043 =2.080	4*1.095 =4.38
- #moles (wt/m.wt)	2.080/106 =0.019	4.38/108 =0.04

Ratios to find rate limiting reactant:

1 mol of benz. → 1 mol of hydrazone

0.019 mole → ?

= 0.019moles of hydrazine(product)

1 mol of hydrazine → 1 mol of hydrazone

0.04 mole → ?

= 0.04moles of hydrazine(product)

So benz. Is the rate limiting reactant(lower no. of moles of product).
so no. of moles of product =0.019 moles
Theoretical yield = 0.019 *196(m.wt of product)= 3.72g

Assume that you weighed your product and was equal to 2g So
%yield = 2/3.72 *100% =53.76%

Appendix II

Melting Point Measurement

A melting point is:
The temperature at which the first crystal just starts to melt until the temperature at which the last crystal just disappears. Thus the melting point (abbreviated M.P.) is actually a melting range. You should report it as such, even though it is called a melting point, for example, M.P. 147-149°C.

- It requires that the intermolecular forces that hold the solid together have to be overcome, the temperature at which melting occurs will depend on the structure of the molecule involved.
- A pure, nonionic, crystalline organic compound usually has a sharp and characteristic melting point (usually 0.5-1.0 C range).
- A mixture of very small amounts of miscible impurities will produce a depression of the melting point and an increase in the melting point range.

Generally, melting points are taken for two reasons.

1. **Determination of purity.**
   If you take a melting point of your compound and it starts melting at 60 °C and doesn't finish until 180 °G you might suspect something is wrong. A melting range greater than 2°C usually indicates an impure compound (As with all rules, there are exceptions. There aren't many to this one, though.).

2. **Identification of unknowns.**
   a. **If you have an unknown solid,** take a melting point. Many books (ask your instructor) contain tables of melting points and lists of compounds that may have a particular melting point. One of them may be your unknown. You may have 123 compounds to choose from. A little difficult, but that's not all the compounds in the world. Who knows?? Give it a try. If nothing else, you know the melting point.

   b. **Take your unknown and mix it thoroughly** with some chemical you think might be your unknown. You might not get a sample of it, but you can ask. Shows you know something. Then:
      i. **If the mixture melts at a lower temperature,** over a broad range, your unknown is NOT the same compound.
      ii. **If the mixture melts at the same temperature,** same range, it's a good bet it's the same compound. Try another one, though, with a different ratio of your unknown and this compound just to be sure. A lower melting point with a sharp range would be a special point called a eutectic mixture, and you, with all the other troubles in lab, just might accidentally hit it. On lab quizzes, this is called

"Taking a mixed melting point."
Actually, "taking a mixture melting point," the melting point of a mixture, is more correct. But I have seen this expressed both ways.

Sample preparation

Sample preparation:

- **You usually take melting points in thin,** closed end tubes called capillary tubes. They are also called melting point tubes or even melting point capillaries. The terms are interchangeable, and I'll use all three.
- **Sometimes you may get a supply** of tubes that are open on both ends! You don't just use these as is. Light a burner, and close off one end, before you start. Otherwise your sample will fall out of the tube (see "Closing Off Melting Point Tubes," following).
- **Take melting points on dry**, solid substances ONLY, never on liquids or solutions of solids in liquids or on wet or even damp solids.

Only on dry solids!

- **To help dry damp solids,** place the damp solid on a piece of filter paper and fold the paper around the solid. Press. Repeat until the paper doesn't get wet. Yes, you may have to use fresh pieces of paper. Try not to get filter paper fibers in the sample, OK?

- **Occasionally,** you may be tempted to dry solid samples in an oven. Don't—unless you are specifically instructed to. I know some students who have decomposed their products in ovens and under heat lamps. With the time they save quickly decomposing their product, they can repeat the entire experiment.

Loading the Melting Point Tube

- **Place a small amount of dry solid on a new filter paper (Fig. 1**). Thrust the open end of the capillary tube into the middle of the pile of material. Some solid should be trapped in the tube. Turn the tube over, closed end down. Remove any solid sticking to the outside. The solid must now be packed down.

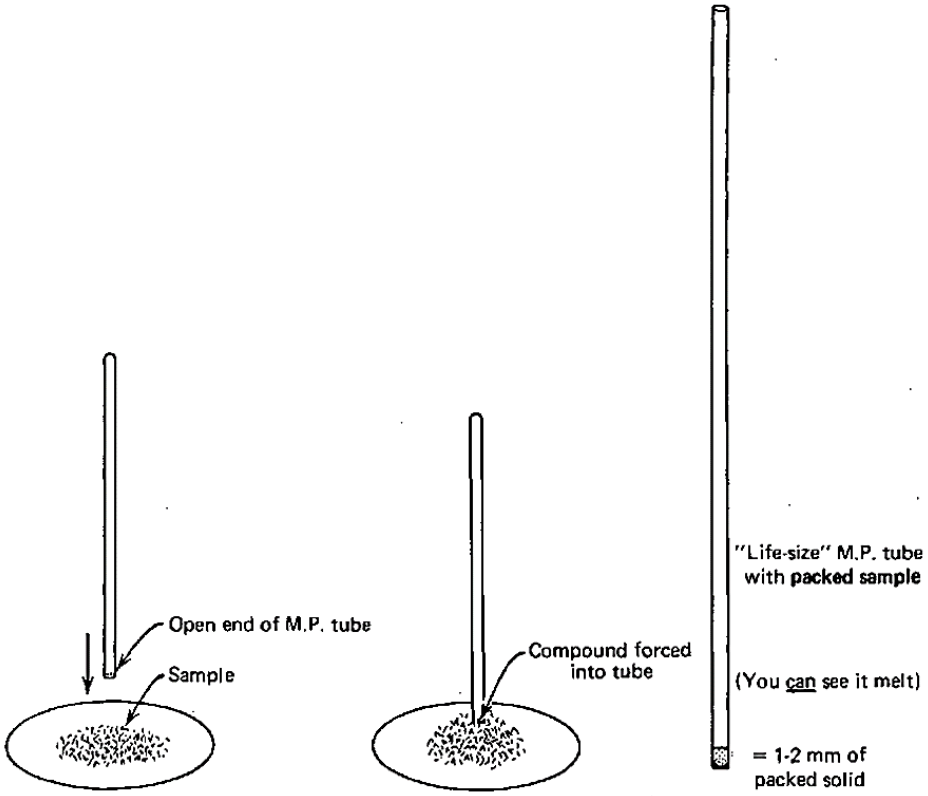


Figure1 . Loading a melting point tube.

- **Traditionally,** the capillary tube, turned upright with the open end up, is stroked with a file, or tapped on the bench top. Unless done carefully, these operations may break the tube. A safer method is to drop the tube closed end down, through a length of glass tubing. You can even use your condenser or distilling column for this purpose. When the capillary strikes the bench top, the compound will be forced into the closed end. You may have to do this several times. If there is not enough material in the M.P. tube, thrust the open end of the tube into the mound of material and pack it down again. Use your own judgment; consult your instructor.

- **Use the smallest amount** of material that can be seen to melt

Closing Off Melting Point Tubes

If you have melting point tubes that are open at both ends and you try to take a melting point with one, it should come as no surprise when your compound falls out of the tube. You'll have to close off one end, to keep your sample from falling out **(Fig.2)**. So light a burner and get a "stiff" small blue flame. SLOWLY touch the end of the tube to the side of the flame, and hold it there. You should get a yellow sodium flame, and the tube will close up. There is no need to rotate the tube. And remember, touch— just touch—the edge of the flame, and hold the tube there. Don't feel you have to push the tube way into the flame.

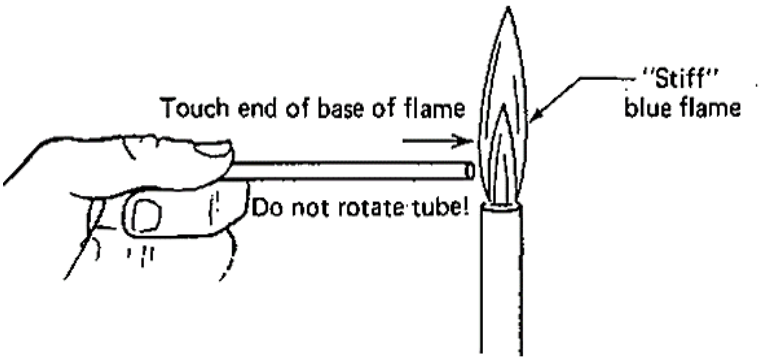


Figure2 . Closing off a M. P. tube with a flame.

Melting point hints:

1. **Use only the smallest** amount that you can see melt. Larger samples will heat unevenly.
2. **Pack down the material** as much as you can. Left loose, the stuff will heat unevenly.
3. **Never remelt any sample.** They may undergo nasty chemical changes such as oxidation, rearrangement and decomposition.
4. **Make up more than one sample**. One is easy, two is easier. If something goes wrong with one, you have another. Duplicate, even triplicate runs are common.

Never repeat melting point's measurement with the same capillary as most of organic compounds decompose or react in other ways upon heating. This resulted in new materials with new melting points.

Appendix III

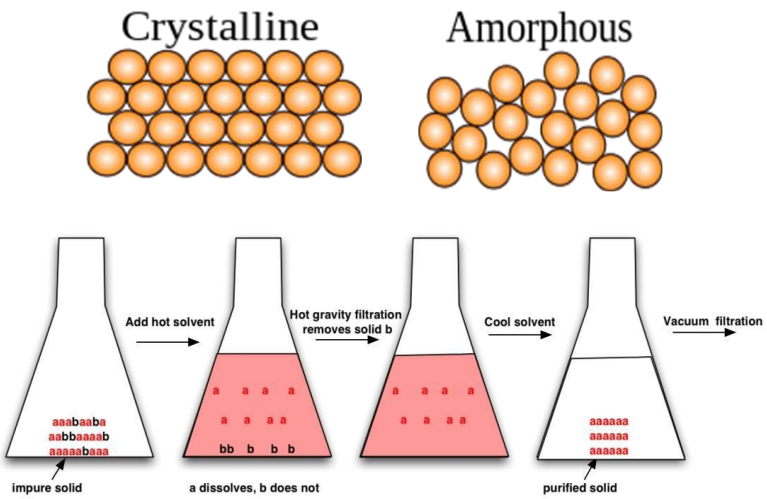
Recrystallization and filtration techniques

Part I: Recrystallization (or Crystallization):

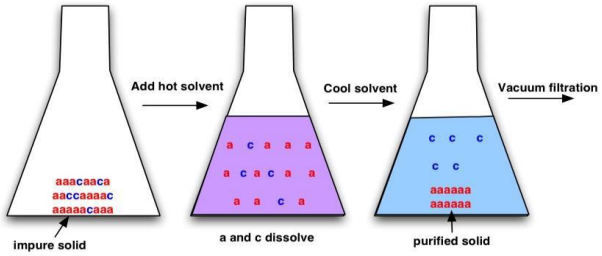
Is a technique used to **purify solids**. This procedure relies on the fact that solubility increases as temperature increases (you can dissolve more sugar in hot water than in cold water). As a hot, saturated solution cools, it becomes supersaturated and the solute precipitates (crystallizes) out.

In a recrystallization procedure, an impure (**crude**) solid is dissolved in a hot solvent. As this solution is cooled, the **pure product crystallizes out** and the **impurities stay dissolved**.

- ✓ As the compound crystallizes from the solution, the molecules of the other compounds dissolved in solution are excluded from the growing crystal lattice, giving a pure solid.
- ✓ Crystallization of a solid is not the same as precipitation of a solid.
- ✓ In crystallization, there is a slow, selective formation of the crystal framework resulting in a pure compound.
- ✓ In precipitation, there is a rapid formation of a solid from a solution that usually produces an amorphous solid, containing many trapped impurities within the solid's crystal framework.



When impurities are less soluble



When impurities are more soluble

General Recrystallization Procedure:

1) Choose an appropriate solvent(s)
   - product is very soluble in it at high temperatures (boiling point of solvent)
   - product is not soluble in it at low temperatures (only sparingly soluble in the solvent at room temperature
   - impurities are either soluble at all temps or insoluble at all temps (can be filtered off)
   - The solvent should not react with the compound being purified
   - The solvent should be volatile enough to be easily removed from the solvent after the compound has crystallized.

2) Dissolve impure solid
   - weigh out crude solid and record its mass (also take a melting point for reference)
   - add a boiling chip or boiling stick (otherwise, it may "bump" and spill)
   - use a **minimum** amount of **hot** solvent (Add a bit, heat/swirl. Not dissolved? Add more!) NOTE: IF YOU USE TOO MUCH SOLVENT, YOU WILL GET NO CRYSTALS!

3) ONLY IF NEEDED: Decolorize
   - most pure compounds are white and give colorless solutions (looks like water)
   - to remove color (really trace contaminants), add activated charcoal (adsorbs the impurities)

4) ONLY IF NEEDED: Gravity filter to remove insoluble materials (including charcoal!)
   - use fluted filter paper and a **hot, stemless** funnel

   NOTE: IF THE SOLUTION COOLS, PRODUCT WILL CRYSTALLIZE OUT & BE LOST!
   - use a <u>small amount</u> of hot solvent to rinse flask, filter

5) Crystallize solute (Finally! This is the RECRYSTALLIZATION part!)
   - cool the solution <u>slowly</u>: hot (boiling) → room temperature → 0 ˚C (put in ice water bath) NOTE: THIS GIVES LARGE, PURE CRYSTALS & LEAVES IMPURITIES IN SOLUTION
   - may need to scratch glass with a stirring rod or add a seed crystal to start crystallization

6) Collect the pure crystals

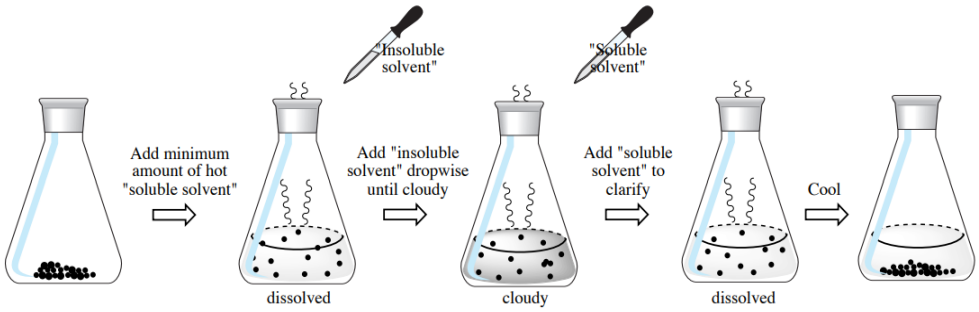
- quickest method is vacuum filtration (Büchner funnel, water aspirator and trap)
- *the impurities will stay dissolved in the solvent that is being removed* (hopefully)
- rinse the pure crystals with a small amount of **cold** solvent (don't redissolve the crystals!)
- OPTIONAL: a second crop of crystals can be obtained from the filtrate (mother liquor)

7) Analyze product
- let crystals dry thoroughly (ideally, this means overnight at room temperature or under vacuum)
- record mass (how much of your original solid did you recover? % recovery?)
- record melting point range (did you succeed in PURIFYING your solid?)

Mixed solvent recrystallization:

When no single solvent can be found that meets all of the criteria for crystallization, it may be possible to use a mixed solvent. A pair of solvents is chosen: one in which the compound is soluble (called the "**soluble solvent**"), and one in which the compound is insoluble (called the "**insoluble solvent**"). The two solvents must be miscible in one another so that their solubility with one another does not limit the proportions used. Ethanol/water, methanol/water and acetone/water are common mixed solvents used in crystallization.

To perform a crystallization using a mixed solvent, the solid to be crystallized is first dissolved in the minimum amount of hot "soluble solvent", then hot "insoluble solvent" is added dropwise until the solution becomes slightly cloudy. An additional small portion of hot soluble solvent is then added to clarify the solution, and the solution is set aside to slowly cool and crystallize. A diagram describing this process is shown in the following Figure.



Schematic for using a mixed solvent in crystallization.

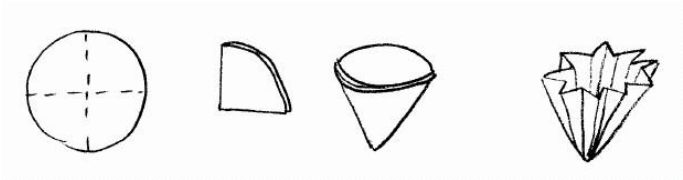
Part II: filtration techniques

A. Gravity Filtration:

Is simply putting a funnel with filter paper over a flask, pouring a liquid/solid mixture into the funnel and allowing the liquid to drip into the flask by gravity. The liquid

and any dissolved substances pass through while the solid stays behind on the filter paper (think of a drip coffee maker).

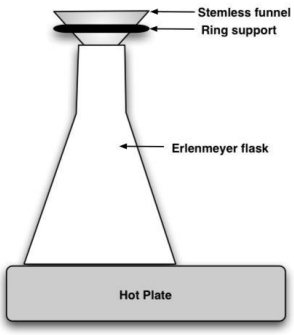
- The filter paper used in gravity filtration is a cone made by folding a round of filter paper in four and opening one leaf. Fluted filter paper works faster and can be made by folding the cone multiple times and opening it like a fan. In both cases the filter paper should be the same size as the funnel being used.



B. Hot gravity filtration

A variation of this technique is **hot gravity filtration** in which the entire setup is kept warm during the filtration process. Hot gravity filtration is needed when the desired product is soluble in hot solvent, but precipitates in cool solvent. If the solution cools during filtration, the desired product will precipitate and get trapped on the filter paper along with the insoluble impurities.

- To set up a hot gravity filtration, put an Erlenmeyer flask on a warm hotplate then lower a stemless funnel, stabilized with a ring support, until it sits on top of the flask. A stemless funnel is used because crystals can form in the cool stem of a long-stem funnel and clog it. It is important that the glass setup is warm before starting the filtration. When most of the solvent has gone though the funnel, the filter paper is washed with a few mLs of fresh, hot solvent and the setup is allowed to cool.

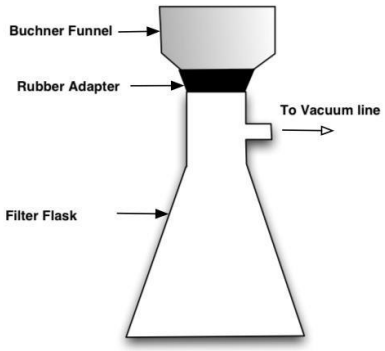


Hot gravity filtration

C. Vacuum Filtration (Suction filtration)

Vacuum or filter flasks look like Erlenmeyer flasks, but with a side arm for attachment to a **vacuum line**. To set up a vacuum filtration, clamp a filter flask to a ring stand. Connect a thick-walled vacuum hose to the side arm and to the vacuum outlet. Don't use a flimsy water hose – it will collapse under

vacuum pressure. Place a rubber adapter on top of the flask, followed by a Buchner funnel. The rubber adapter forms an air-tight seal between the funnel and the flask – do not attempt the filtration without it. Find a piece of filter paper that fits flatly in the Buchner funnel, covering all the holes, but not coming up the sides. This setup is top heavy and will tip over – **use a clamp on the filter flask**.



Vacuum Filtration
(Suction filtration)

When you are ready to filter, turn on the vacuum, wet the filter paper with a little of the solvent you recrystallized from.

– only use water if you recrystallized from water! – and pour the contents of the recrystallization flask into the Buchner funnel. Just before pouring, swirl the flask to get the crystals in suspension. Pour fast enough so that the crystals get into funnel instead of getting stuck on the sides of the flask. If crystals get stuck in the flask, you may rinse with no more than 5 mLs of cold solvent. Pull air through the filter for several minutes to help dry the crystals. If recrystallizing from water, put the filter paper with all the crystals on it in a provided envelope to dry until the next laboratory period. If recrystallizing from an organic solvent (which generally have lower boiling points and dry faster), it is usually ok to take a melting point the same lab period. We never use vacuum filtration for low boiling solvents like ethyl ether or low boiling products. A vacuum is a reduced pressure environment. Low boiling solvents boil even lower at low pressure and will vaporize and get pulled into the vacuum line.

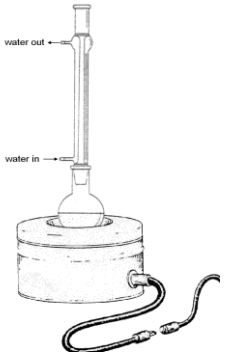
Appendix IV

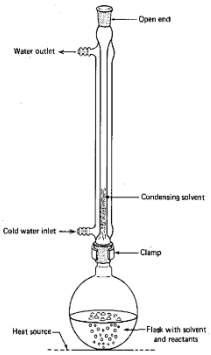
Reflux and the reflux condenser

Reflux is a chemistry lab technique that heats a solution, produces vapor, and then condenses and returns the outgoing vapor into its original mixtures. Reflux is widely used in undergraduate organic chemistry labs.

Purposes:

- to reduce the loss of solvent through evaporation
- Complete the reaction in a reasonable time





Basic Setup:

1. **Place the reagents in a round-bottomed flask.**
   The flask should be large enough to hold both the reagents and enough solvent to dissolve them, without being much more than half full.

2. **You should** now choose a solvent that
   a. **Dissolves the** reactants at the boiling temperature.
   b. **Does not react** with the reagents.
   c. **Boils at a temperature that** is high enough to cause the desired reaction to go at a rapid pace.

3. **Dissolve the reactants in the solvent**.
   Sometimes the solvent itself is a reactant. Then don't worry.

4. **Place a condenser,** upright, on the flask, connect the condenser to the water faucet, and run water through the condenser (Fig. 83). Remember —in at the bottom and out at the top.

5. **Put a suitable heat source under the flask** and adjust the heat so that the solvent condenses no higher than halfway up the condenser. You'll have to stick around and watch for a while, since this may take some time to get started. Once the reaction is stable, though, go do something else. You'll be ahead of the game for the rest of the lab.

6. **Once this is going well,** leave it alone until the reaction time is up. If it's an overnight reflux, wire the water hoses on so they don't blow off when you're not there.

Always makes sure the hoses are securely attached to the condenser.
A gentle flow of cooling water is all that is needed.
Check for leaks!

Appendix V

Boiling chips

Boiling chips:

Are small, insoluble, porous stones made of calcium carbonate or silicon carbide. These stones have pores inside which provide cavities both to trap air and to provide spaces where bubbles of solvent vapor can form. These bubbles ensure even boiling and prevent bumping and boiling over and loss of the solution.

- Always use a boiling chip when heating a solvent.
- Never add a boiling chip to a solvent which is already hot, because it can cause to solvent to boil over violently.
- If you forget to add a boiling chip before you begin, you must cool the solution before adding one to prevent product loss.
- Boiling chips cannot be re-used since the pores inside these stones become filled with liquid on cooling.

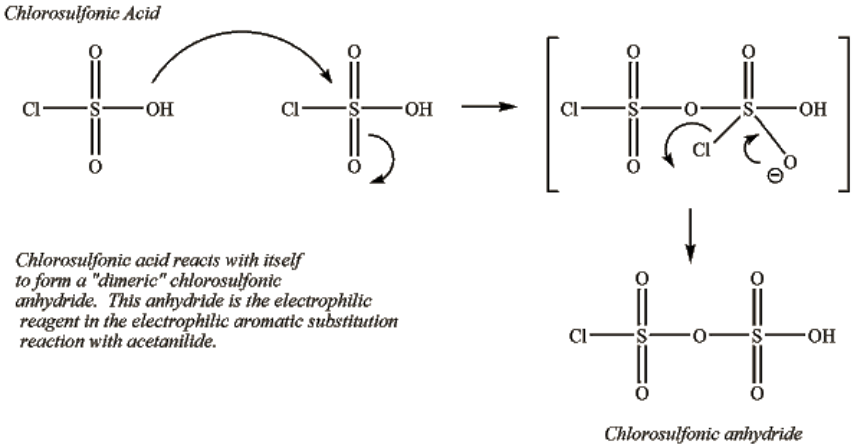
It should be placed in the distillation flask for two reasons:

1. they will prevent superheating of the liquid being distilled
2. they will cause a more controlled boil, eliminating the possibility that the liquid in the distillation flask will bump into the condenser

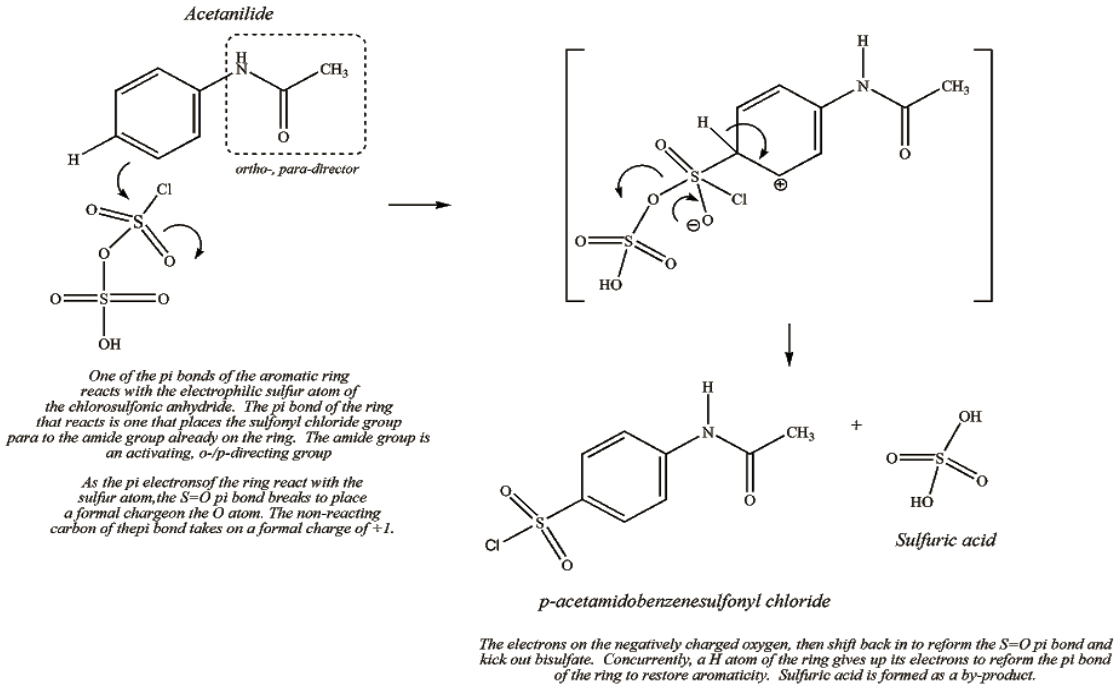
Appendix VI

Other suggested mechanism for Step2 in cycle 1

Step 1: Chlorosulfonic acid undergoes dimerization to generate the electrophilic reagent used to react with acetanilide



Step 2: Chlorosulfonic anhydride reacts with acetanilide via and electrophil ic aromatic substitution to generate p-acetamidobenzenesulfonylchloride.



Appendix VII

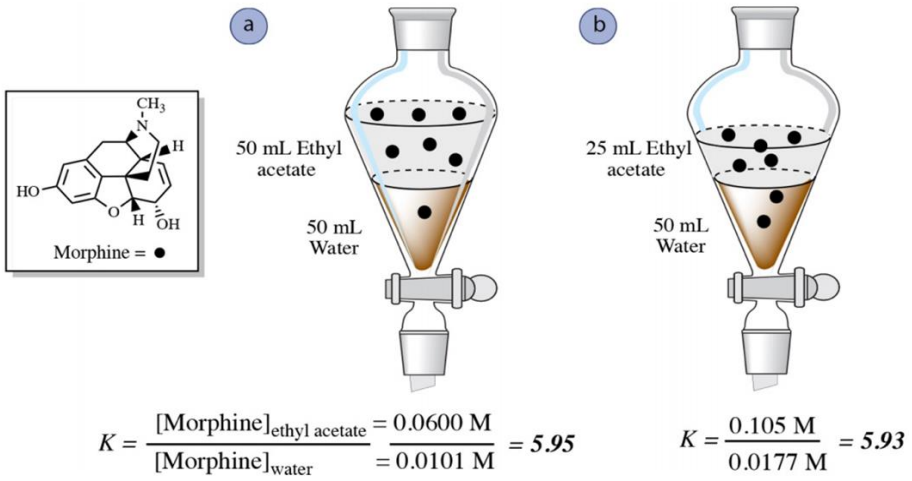
Extraction

❖ Extraction Theory:

When a solution is placed in a separatory funnel and shaken with an immiscible solvent, solutes often dissolve in part into both layers. The components are said to "partition" between the two layers, or "distribute themselves" between the two layers. When equilibrium has established, the ratio of concentration of solute in each layer is constant for each system, and this can be represented by a value KK (called **the partition coefficient or distribution coefficient**).

$$K = \frac{Molarity\ in\ organic\ phase}{Molarity\ in\ aqueous\ phase}$$

For example, morphine has a partition coefficient of roughly 6 in ethyl acetate and water.22 If dark circles represent morphine molecules, 1.00g1.00g of morphine would distribute itself .

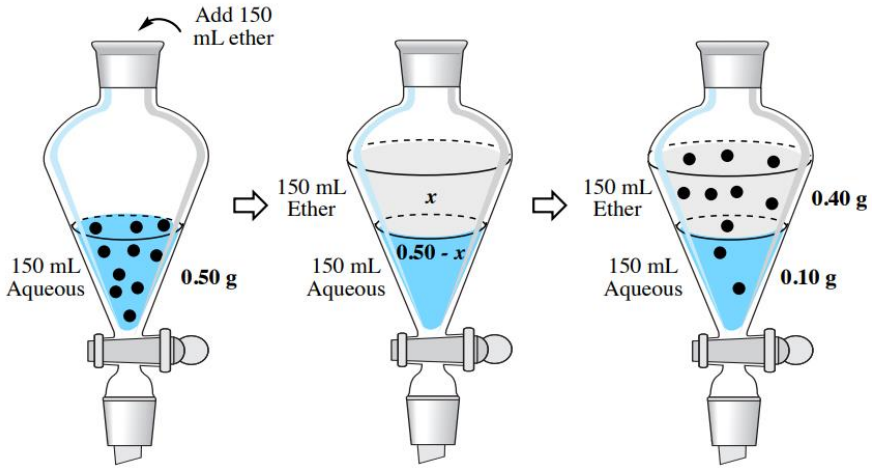


Distribution of morphine in ethyl acetate and water.

Note that with equal volumes of organic and aqueous phases, the partition coefficient represents the ratio of particles in each layer. When using equal volumes, a K of ~6 means there will be six times as many morphine molecules in the organic layer as there are in the water layer. The particulate ratio is not as simple when the layer volumes are different, but the ratio of *concentrations* always equals the K.

The partition coefficients reflect the solubility of a compound in the organic and aqueous layers, and so is dependent on the solvent system used. For example, morphine has a K of roughly 2 in petroleum ether and water, and a K of roughly 0.33 in diethyl ether and water. When the K is less than one, it means the compound partitions into the aqueous layer more than the organic layer.

❖ Quantitating Single Extraction



Single extraction of hyoscyamine *(K~4)* from water into diethyl ether.

*[0.50g0.50g **hyoscyamine in** 150mL150mL **water is to be extracted into** 150mL150mL **diethyl ether]***

$$K = \frac{organic\ solubility}{aqueous\ solubility}$$

$$K = \frac{(1.44g\ hyoscyamine/100mL\ diethyl\ ether)}{(0.354g\ hyoscyamine/100mL\ water)} = \sim 4.07$$

If " x " is the gram quantity of hyoscyamine extracted into the diethyl ether layer, then " 0.50g−x " would remain in the aqueous layer after equilibrium is established. Knowing the value of K , the value of x can be solved for using the equation below.

$$K = \frac{(x/100mL\ diethyl\ ether)}{(0.5 - x)/100mL\ water)} = \sim 4.07$$

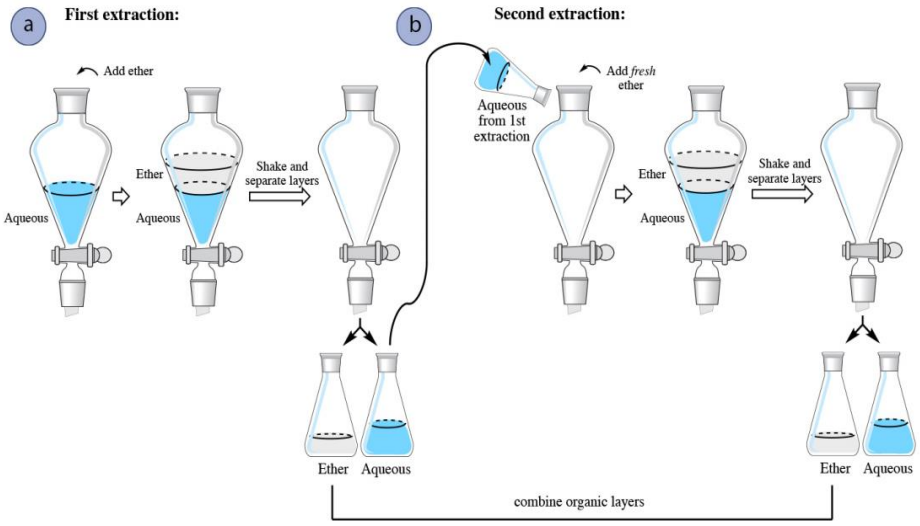
X= 0.4g

❖ Multiple Extractions

Overview of Multiple Extractions:

Depending on the partition coefficient for a compound in a solvent, a single extraction may be all that is needed to effectively extract a compound. However, more often than not a procedure calls for a solution to be extracted multiple times in order to isolate a desired compound, as this method is more efficient than a single extraction.

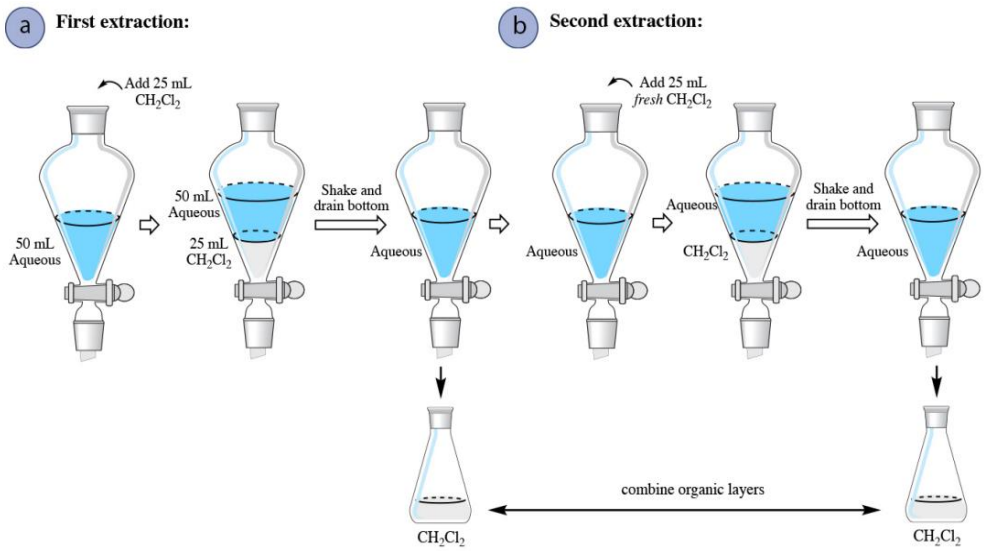
In a multiple extraction procedure, a quantity of solvent is used to extract one layer (often the aqueous layer) multiple times in succession. The extraction is repeated two to three times, or perhaps more times if the compound has a low partition coefficient in the organic solvent.



Multiple Extractions of an aqueous layer when the organic layer is on the top: a) First extraction, b) Second extraction.

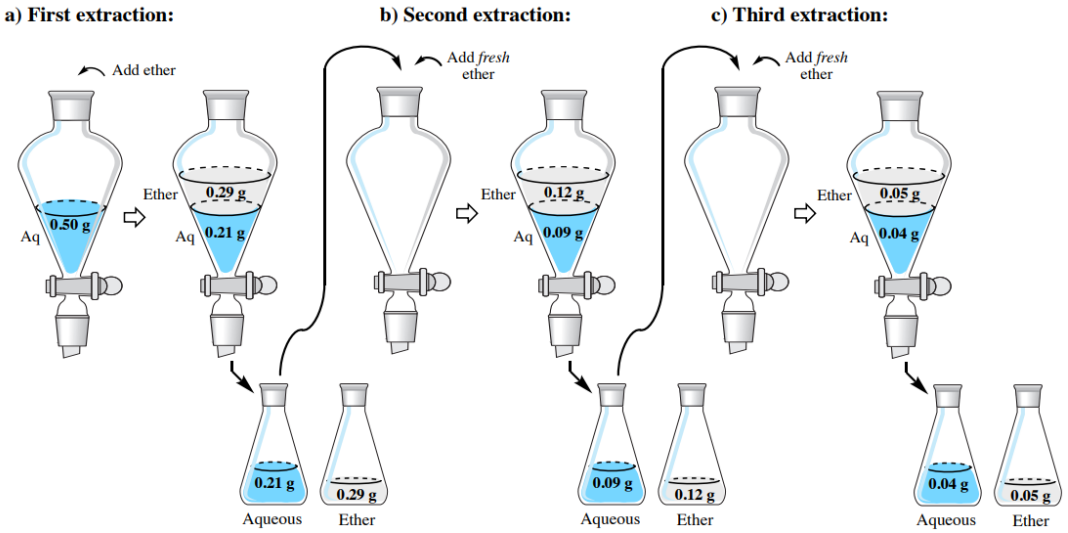
In a multiple extraction of an aqueous layer, the first extraction is procedurally identical to a single extraction. In the second extraction, the aqueous layer from the first extraction is returned to the separatory funnel, with the goal of extracting additional compound. *Since the organic layer from the first extraction had already reached equilibrium with the aqueous layer, it would do little good to return it to the separatory funnel and expose it to the aqueous layer again. Instead, fresh diethyl ether is added to the aqueous layer, since it has the potential to extract more compound*.

The process is often repeated with a third extraction, with the aqueous layer from the second extraction being returned to the separatory funnel, followed by another portion of fresh organic solvent. **In multiple extractions, the organic layers are combined together, as the goal is to extract the compound into the organic solvent**.



Multiple Extractions of an aqueous layer when the organic layer is on the bottom: a) First extraction, b) Second extraction.

❖ Quantitating Multiple Extraction

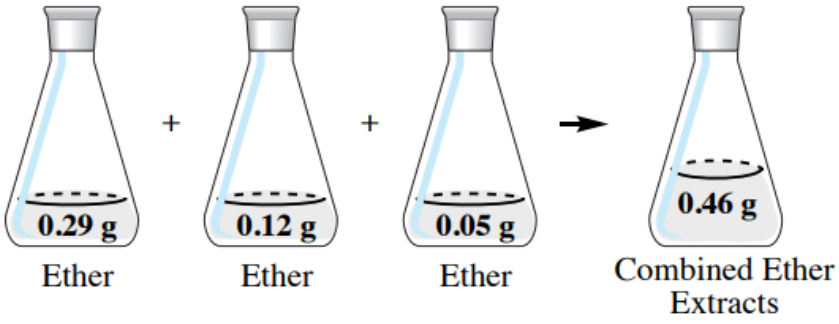


Multiple Extractions of hyoscyamine *(K~4)* from water into diethyl ether.
*[0.50g **hyoscyamine in** 150mL **water is to be extracted** into three 50mLportions of diethyl ether]**.**

$$K = \frac{(x/100mL\ diethyl\ ether)}{(\mathbf{0.5}-x)/100mL\ water)} = \sim 4.07 \ldots.\ \text{First Extract}$$

$$K = \frac{(x/100mL\ diethyl\ ether)}{(\mathbf{0.21}-x)/100mL\ water)} = \sim 4.07 \ldots.\ \text{second Extract}$$

$$K = \frac{(x/100mL\ diethyl\ ether)}{(\mathbf{0.09}-x)/100mL\ water)} = \sim 4.07 \ldots..\ \text{Third extract}$$

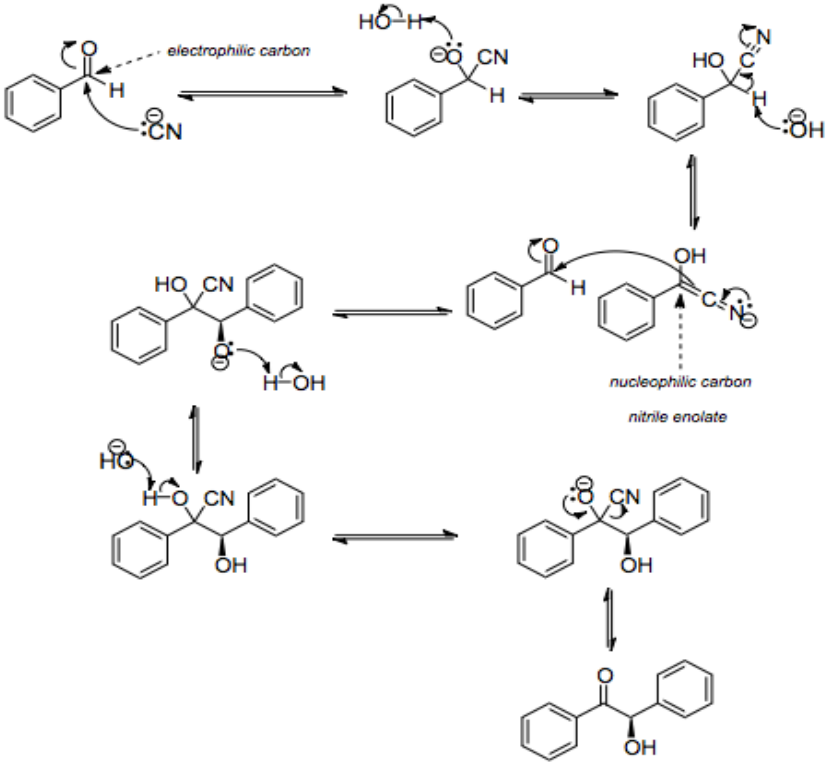


If the 50mL diethyl ether extracts are combined in this example, there would be a total of **0.46g** of hyoscyamine in the combined organic extracts. Of the 0.50g of hyoscyamine in the original aqueous layer**, 92%** of the material is extracted into the organic layer ($100\% \times \frac{0.46g}{0.50g}$). This is a greater quantity than was obtained using a single extraction of 150mLdiethyl ether, which resulted in only **0.40g** of hyoscyamine extracted **(80%).**

Appendix VIII

Benzoin condensation using cyanide

The reaction is catalyzed by nucleophiles such as a cyanide or an N-heterocyclic carbene (usually thiazolium salts). The reaction mechanism was proposed in 1903 by A. J. Lapworth. In the first step in this reaction, the cyanide anion (as sodium cyanide) reacts with the aldehyde in a nucleophilic addition. Rearrangement of the intermediate results in polarity reversal of the carbonyl group, which then adds to the second carbonyl group in a second nucleophilic addition. Proton transfer and elimination of the cyanide ion affords benzoin as the product. This is a reversible reaction, which means that the distribution of products is determined by the relative thermodynamic stability of the products and starting material.

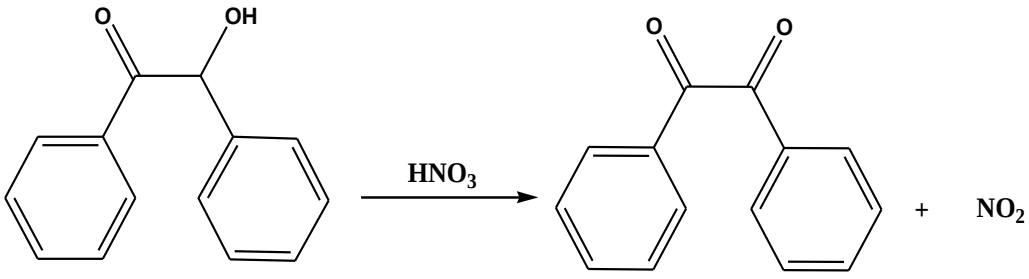


The benzoin condensation is in effect a dimerization and not a condensation because a small molecule like water is not released in this reaction. For this reason the reaction is also called a benzoin addition. In this reaction, the two aldehydes serve different purposes; one aldehyde donates a proton and one aldehyde accepts a proton. Some aldehydes can only donate protons, such as 4-Dimethylaminobenzaldehyde whereas benzaldehyde is both a proton acceptor and donor. In this way it is possible to synthesise mixed benzoins, i.e. products with different groups on each half of the product. However, care should be taken to match a proton donating aldehyde with a proton accepting aldehyde to avoid undesired homo-dimerization.

Appendix IX

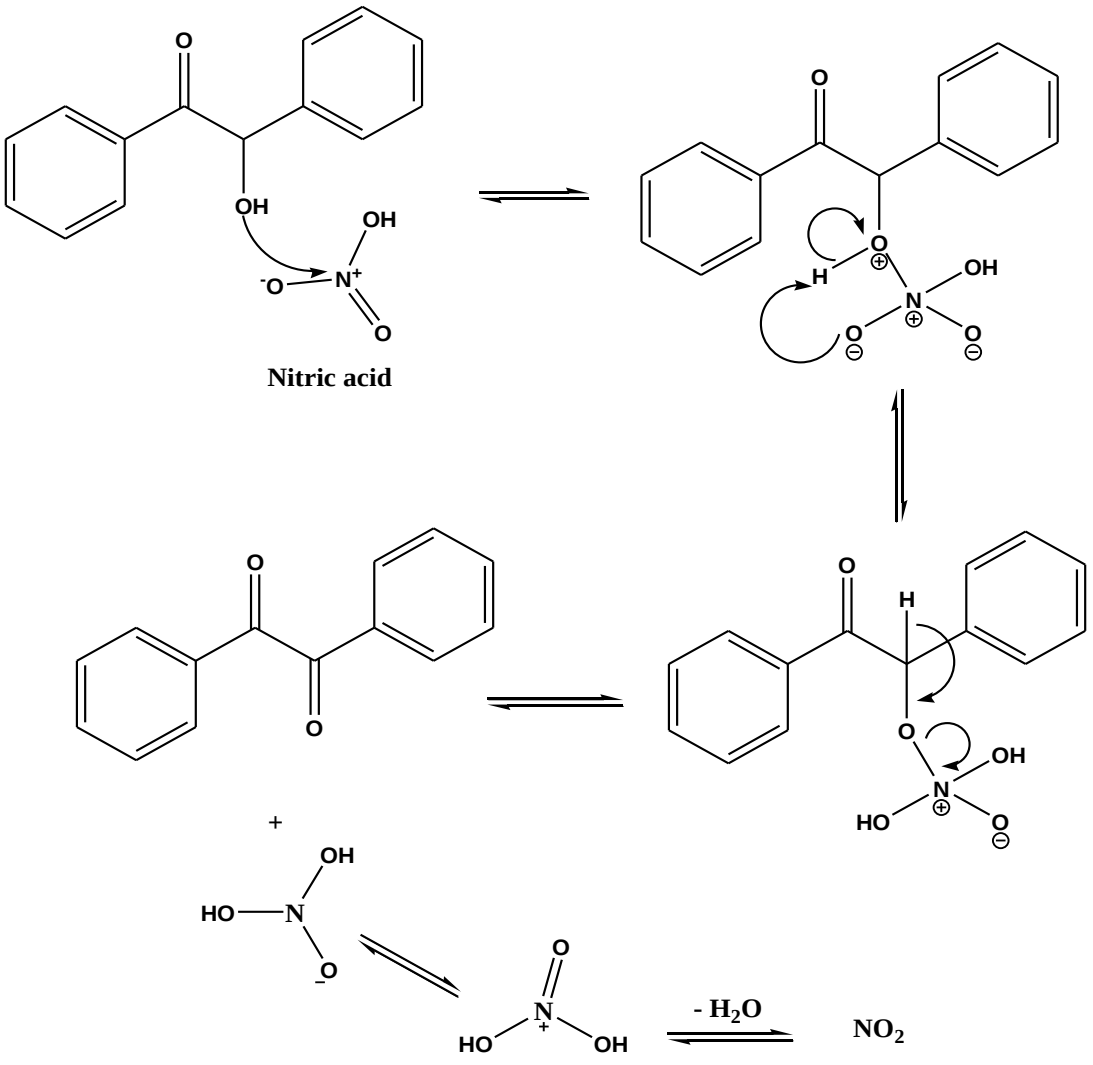
Oxidation of Benzoin to Benzil using nitric acid

Preparation of benzil can be done by the oxidation of benzoin using nitric acid, where the OH group is converted to a ketone group.



Benzoin **Benzil**

Mechanism:



Procedure

1. In a 250ml Erlenmeyer flask, mix 7.5g of benzoin and 30 ml concentrated nitric acid.
2. Place the flask on a boiling water bath in the fume hood and heat for about 1hour until the evolution of nitrogen oxides has ceased.
3. Pour the reaction mixture in about 100-150 ml of cold water in a beaker with stirring .then Benzil separates out as yellow oil, which immediately solidifies.
4. Filter on a Buchner funnel.
5. Wash with cold water to remove the nitric acid.
6. Drain well and re-crystallize from ethanol. (After dissolving the product in hot ethanol, add water dropwise to reach the cloud point and allow it to crystallize).

الجامعة الهاشمية
كلية العلوم الصيدلانية

ارشادات السلامة العامة للمختبرات

على جميع الطلاب مراعاة التعليمات التالية، لما لها من أهمية كبيرة على سلامة العامة في المختبرات:

في حال وجود اي حالة طارئة يرجي الاتصال بالأرقام التالية: 4666 ,4791, 4790.

1. عدم دخول المختبر قبل الوقت المحدد في البرنامج، وعد العمل في المختبر بدون وجود المشرف.
2. ارتداء مريول المختبر مع (tag name) قبل دخول المختبر، وارتداء والنظارات الواقية للعينين طيلة فترة تواجد في المختبر.
3. من اجل سلامتك الرجاء عدم ارتداء القبعات والملابس القصيرة كالشورت والتنورة والأحذية المكشوفة (مثل الصندل). وعدم ارتداء العدسات اللاصقة للعينين.
4. من اجل سلامتك يفضل ارتداء القفازات المخبرية أثناء عمل التجارب.
5. من اجل سلامتك على الطالب اعلام المدرس في حالته الصحية بشكل سري إذا كان يعاني من بعض الامراض التي تستدعي انتباه المدرسة كالأمراض المناعية او تناوله لأدوية تضعف جهاز المناعة او في حال كانت الطالبة حامل.
6. التعرف على الاماكن وجود الطفايات الحريق ومعرفة كيفية استخدامها وكذلك مكان وجود الدش وبطاني الحريق.
7. المحافظة على جو من المسؤولية والجد والنظام.
8. الامتناع عن الاكل والشرب والتدخين داخل المختبر.
9. عدم شم او تذوق المواد الكيمائية.
10. عدم الاسراف في استعمال المواد الكيميائية.
11. عدم تغير أماكن وجود المواد الكيميائية خصوصا المواد الموضوعة في خزانة الابخرة.
12. يجب اضافة الحامض الي الماء وليس العكس.
13. عدم فتح حنفية الغاز قبل اشعال عود الثقاب.
14. عدم جعل فوهة أنبوب الاختبار باتجاه الوجه، او باتجاه شخص اخر اثناء عمل التجارب.
15. يجب عدم تسخين المواد المتطايرة او القابلة للاشتعال على اللهب المباشر، وعدم رج الزجاجات التي تحتوي عليها.
16. يجب اجراء التفاعلات التي تنتج عنها غازات السامة في خزانة الابخرة.
17. يجب سكب كمية كافية من الماء بعد سكب الاحماض والقواعد في المغسلة.
18. التعامل مع الادوات الزجاجية بحذر لان جروحها عادة ما تكون بليغة، ووضع مادة التشحيم عند وصل أداتين ببعضها البعض.
19. عدم إلقاء النفايات الصلبة في المغاسل أو وضع فراشي التنظيف في المحاليل الحوامض أو القواعد.
20. عدم وضع الاغراض الشخصية والملابس على طاولة العمل في المختبر.
21. المحافظة على مكان العمل في المختبر نظيفا.
22. التأكد من اغلاق حنفيات الغاز والماء وغسل اليدين قبل المغادرة.