Experiment 2

Oxidation-Oxime synthesis; Two-Step reaction of 9-Fluorenol

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PURPOSE OF THE EXPERIMENT

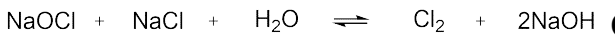
Perform thin-layer chromatography (TLC) to understand the extent of reaction by monitoring the reaction progress.

Conduct a multistep synthesis: Oxidation of 9-fluorenol and 9-fluorenone oxime synthesis.

BACKGROUND INFORMATION

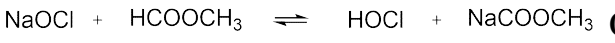
Oxidation of 9-fluorenol

At the first reaction of this experiment, 9-fluorenol (9-Hydroxyfluorene) reacts with sodium hypochlorite (bleach) which oxidizes 9-fluorenol (alcohol) to 9-fluorenone (ketone) in an acidic condition (glacial acetic acid). To use sodium hypochlorite as an oxidizing agent, you should notice that chlorine becomes a significant product in the decomposition of an aqueous bleach solution. (equation 1)

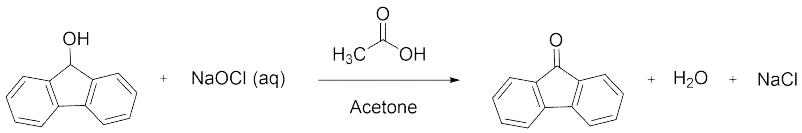


(Equation 1)

So it needs to ensure that the pH is kept below 11 by adding acetic acid. (equation 2)



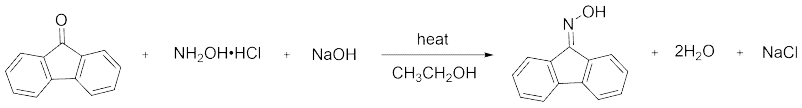
(Equation 2)



Scheme 1. Oxidation of 9-fluorenol

9-fluorenone oxime synthesis

At the second reaction of this experiment, 9-fluorenone (produ ct of the first reaction) reacts with hydroxylamine hydrochlorid e and NaOH.



Scheme 2. 9-fluorenone oxime synthesis

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is an affinity-based method used to separate compounds in a mixture. TLC is a highly versatile separation method that is widely used for both qualitative and quantitative sample analysis.

The stationary phase is a thin adsorbent material layer, usually silica gel or aluminum oxide, coated on inert plate surface, typically glass, plastic, or aluminum.

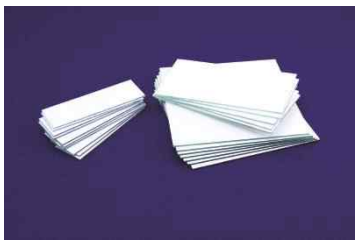


Figure 1. silica gel TLC plates

The sample is spotted onto one end of the TLC plate and placed vertically into a closed chamber (developing chamber) with an organic solvent (mobile phase). The mobile phase travels up through the plate by capillary forces and sample components migrate varying distances based on their differential affinities for the stationary and mobile phases. When the solvent reaches the top of the plate, the plate is removed from the developing chamber and dried. Separated components appear as spots on the plate. Separated components can be detected by UV lamp or staining solution.

Figure 2. Developing chamber

Figure 3. The process of TLC

The retention factor (Rf) of each component is assessed. Under the same stationary phase, mobile phase, and temperature, same substances have the same retention factor. By using this property, retention factor can be used to identify the compound of specific TLC spots. A calculation for R_f is shown in Equation 3.

$$R_f = \frac{\text{distance traveled by compound (mm)}}{\text{distance traveled by eluent front (mm)}} \quad \textbf{(Equation 3)}$$

Reference 1

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EXPERIMENT 2

Oxidation-Oxime; Two-Step reaction of 9-Fluorenol

Reagents and Properties

substance	*quantity*	*molar mass (g/mol)*	*mp (°C)*	bp (°C)	*density (g/mL)*
9-fluorenol	150 mg	182.22	152	367.5	1.151
acetone	10+ mL	58.08	-94.7	56.05	0.791
glacial acetic acid	10 drops	60.052	16	118	1.05
bleach (sodium hypochlorite)	3 mL	74.44	18	101	1.11
sodium bicarbonate ($NaHCO_3$)		84.007	228		2.2
Sodium thiosulfate ($Na_2S_2O_3$)		158.11	48.3	100	1.67
hexane	13+ mL	86.178	-96	69	0.659
anhydrous sodium sulfate		142.04	884	1,429	2.66
95% ethanol	8 mL	46.07	-114.1	78.37	0.789
2.5 M aqueous NaOH solution	1.5 mL	39.997	318	1,388	2.13
2.5 M aqueous hydroxylamine hydrochloride solution	1.5 mL	69.49			1.67
Ethyl acetate	10 mL	88.11	-83.6	77.1	0.9
Saturated sodium chloride solution (NaCl)	10 mL	58.44	801	1,465	2.16

PROCEDURE

Caution: Wear lab coats and safety goggles at all times while in the lab. Many chemicals are potentially harmful. Prevent contact with your eyes, skin, and clothing. Wearing contact lens is strictly prohibited.

Apparatus

10 mL cylinders, Funnel, Developing chamber glass, Hot plate, thermowell, 3 of 100 mL round bottom flask, UV light source, 125 mL separatory funnel, 2 of 100 mL Erlenmeyer flasks, cotton.

First Reaction

Oxidation of 9-fluorenol

A. Preparing TLC reference

1. Set thermowell on the hot plate.
2. Turn on the hot plate (60℃)

3. Mix 7 mL of hexane and 3 mL of acetone in the 10 mL cylinder
4. Pour 2.5 mL of hexane/acetone (70:30) solution into glass with a dropper. (Developing chamber)
5. Put the cover on the glass.

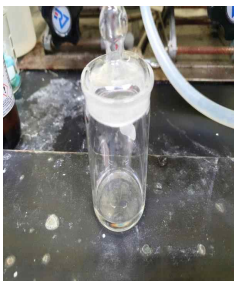


Figure 4. Developing chamber

6. Prepare 4 of 2.0 × 5.0 cm TLC silica plates.
7. Mark two lines across the plate by pencil (0.5-1.0 cm from the bottom and 0.5 cm from the top).
8. Mark the three dots on the line.
9. Label the dots to indicate where the solution will be spotted. (Starting material, Co-spot, reaction mixture)

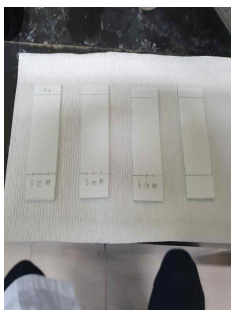


Figure 5. Prepared TLC plates

B. 9-fluorenol oxidation

1. Measure 150 mg of 9-fluorenol.

Figure 6. 150 mg of 9-fluorenol

2. Transfer 9-fluorenol to 100 mL round bottom flask using funnel.

3. Put a magnetic bar in the flask gently.
4. Add 10 mL of acetone to reaction flask.
5. Shake the reaction flask until 9-fluorenol dissolves into acetone.

Figure 7. Add 10 mL of acetone to reaction flask

6. Heat the flask (60℃) for 5 min.

Figure 8. Heat the flask with thermowell

7. Add 10 drops of glacial acetic acid to the reaction mixture.
8. Add 3 mL of bleach to the reaction mixture. [Note 1]
9. Heat the reaction flask with occasional swirling.

Note 1. Do not make bleach contact with an air for a long time.

Figure 9. Color change of the solution

10. Start a timer to record total time of reaction.

C. Monitoring the reaction with TLC

1. Right after starting the timer, take the first aliquot from the reaction mixture by capillary tube.
2. Spot the reaction mixture on the prepared TLC plate (Reaction mixture & Co-spot).
3. Write “t=0” on the top of the TLC plate with pencil.
4. Place the end of capillary tubes in the 9-fluorenol (Starting material).
5. Spot it on the TLC plate (Starting material & Co-spot).
6. Wait until the solvent of the spot evaporate.
7. Check the level of eluent in developing chamber is below the spots on TLC plate.
8. Open the cap of developing chamber.
9. Put the TLC plate in the developing chamber leaning against the wall.
10. Cover the beaker with a cap

Figure 10. Developing TLC plate

11. Remove the TLC plate from developing chamber when eluent front rises to the line.
12. Cover the developing chamber with cover again.
13. Allow eluent to be evaporated from the plate under the fume hood.
14. Examine the developed TLC plate under a UV light.
15. Use a pencil to circle visualized spots.
16. Compare circles of reaction mixture with starting material and reaction mixture.

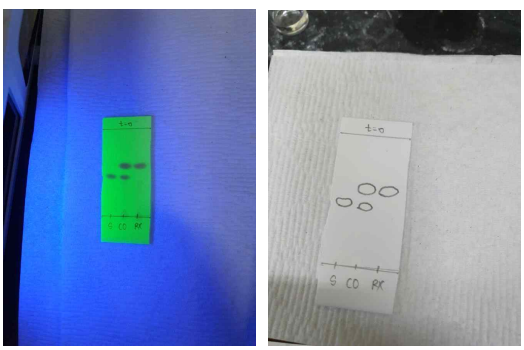


Figure 11. Examine visualized spots of TLC plate under UV light. (t=0)

17. After at least 10 min from the first aliquot, take the second aliquot from the reaction mixture.
18. Spot on the reaction mixture and co-spot label of prepared TLC plate.
19. Spot Starting material on the starting material and co-spot label.
20. Write the time on the top of the TLC plate with pencil.
21. Repeat 7-16 again to the second TLC plate.
22. If starting material still remains in the reaction mixture, add 1 mL of bleach and 3 drops of glacial acetic acid to the reaction mixture. Then repeat TLC analysis after 10 min.
23. After starting materials disappear, take TLC analysis with product sample (9-fluorenone).

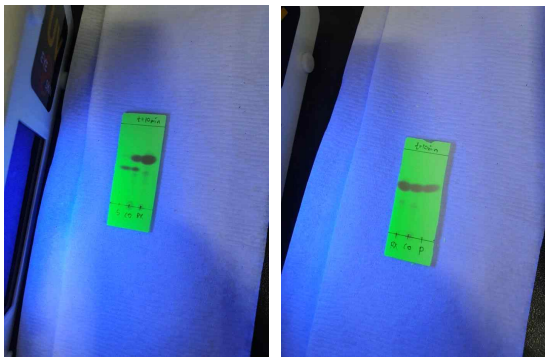


Figure 12. TLC analysis between starting material and reaction mixture (left), reaction mixture and product (right) (t=10min)

24. Cool down and remove the reaction flask from the thermowell and record the total time of the reaction.

D. Work-up

D-1. Quenching

1. Check the result solution with potassium iodide(KI)-starch paper.
2. If the color of paper change to blue-violet, add aqueous sodium thiosulfate($Na_2S_2O_3$) solution(0.6 M) to the solution.
3. Check the solution with another iodide(KI)-starch paper again.
4. Repeat 2-3 until the blue-violet color doesn't come out.
5. Check the pH of the reaction mixture using blue litmus paper
6. If it is acidic, add saturated aqueous $NaHCO_3$ solution to t he mixture to neutralize the reaction mixture.
7. Check the solution with blue litmus paper again.
8. Repeat 6-7 until the solution is not acidic.

D-2. liquid-liquid extraction

Caution: During the pressure releasing work, **don't let the end of the funnel see a person**. Erupted solution can splatter on a person.

1. Fix 125 mL separatory funnel with clamps and turn the stopcock to close the end of funnel.
2. Transfer reaction mixture into separatory funnel.

Figure 13. Transfering reaction mixture into separatory funnel.

3. Add 8 mL of hexane to the separatory funnel.
4. Cap the funnel.
5. Shake the funnel and gently open the cock with look-up to release the gases from mixing.
6. Close the cock.
7. Repeat 5-6 for 4 times.

Figure 14. Separated hexane layer (top) and aqueous layer (bottom)

8. Set 100 mL Erlenmeyer flask under the separatory funnel.
9. Open the cock until the bottom layer (aqueous layer) falls to the flask.

Figure 15. Separate two layers using a cock

10. Set another 100 mL Erlenmeyer flask under the separatory funnel.
11. Open the cock and collect remaining hexane layer in the flask.

Figure 16. Separated aqueous (Left) and hexane layer (Right)

E. Drying

1. Measure the mass of 100 mL round bottom flask.
2. Add excess amount of anhydrous sodium sulfate (Na_2SO_4) to the hexane solution.
3. Cover the funnel with a cotton.

Figure 17. Simple filter made with cotton.

4. Pour the hexane solution in a preweighed round bottom flask using cotton-covered funnel.
5. Wash remaining sodium sulfate with hexane until the color

of sodium sulfate turns back to white.

Figure 18. Filtered hexane solution

6. Evaporate the solvent with Rotary evaporator

Figure 19. Rotary Evaporator

7. Measure the mass of 100 mL round bottom flask with final yellow solid product (9-fluorenone)

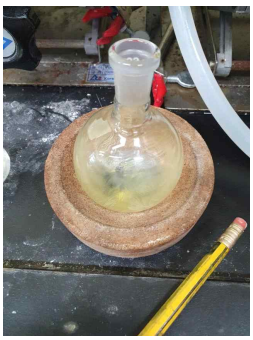


Figure 20. Final product of the first reaction (9-fluorenone)

8. Calculate percentage yield of the first reaction.

Second Reaction **9-fluorenone oxime synthesis**

F. Preparing TLC reference

1. Obtain three 2.0 × 5.0 cm TLC silica plates.
2. Mark two lines across the plate by pencil (0.5-1.0 cm from

the bottom and 0.5 cm from the top).
3. Mark the three dots on the line.
4. Name for dots to indicate where the solution will be spotted. (Starting material, Co-spot, Reaction mixture)

Figure 21. Prepared TLC plates

G. Oxime formation from 9-fluorenone

1. Set thermowell on the hot plate.
2. Turn on the hot plate (70℃)
3. Put a magnetic bar in the flask with product of the first reaction
4. Add 6 mL of 95% ethanol to the flask.
5. Stir the reaction flask until 9-fluorenone dissolves into acetone.
6. Add 1.5 mL of 2.5 M aqueous NaOH solution to the reaction mixture.
7. Add 1.5 mL of 2.5 M aqueous hydoxylamine hydrochloride solution to the reaction mixture.
8. Heat the reaction flask in the hot water bath with occasional swirling for 30-45 min.

Figure 22. Running the reaction for 30-45 min

H. Monitoring the reaction with TLC

1. Right after starting the reaction, take the first aliquot from the reaction mixture by capillary tube.
2. Spot the reaction mixtrue on the prepared TLC plate (Reaction mixture & Co-spot)
3. Place the end of capillary tubes in the 9-fluorenone solution. (Starting material).
4. Spot it on the TLC plates (Starting material & Co-spot).

Note 2. Use premade developing chamber from Step A.

5. Write reaction time on the top of the TLC plate with pencil.
6. Check that the level of eluent in developing chamber is below the spots on TLC plate. [Note 2]
7. Open the cap of developing chamber.
8. Put the TLC plate in the developing chamber leaning against the wall.
9. Cover the beaker with a cap
10. Remove the TLC plate from developing chamber when eluent front rises to the line..
11. Cover the developing chamber with cover again.
12. Allow the eluent to be evaporated from the plate under fume hood.
13. Examine the developed TLC plate under a UV light.
14. Use a pencil to circle visualized spots.
15. Compare circles of reaction mixture with starting material and reaction mixture.

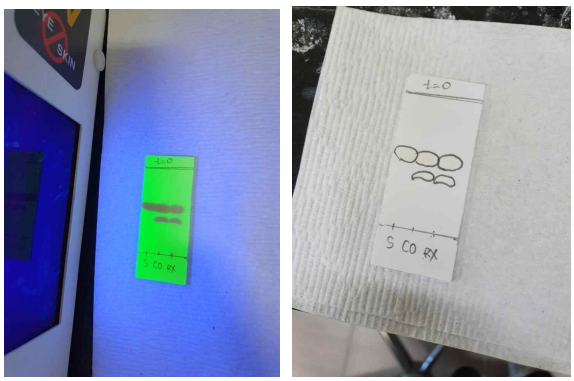


Figure 23. Examine visualized spots of TLC plate under UV light. (t=0)

16. After 30 min from the first aliquot, take the second aliquot from the reaction mixture by capillary tube.
17. Repeat 2-15 again to the second TLC plate.

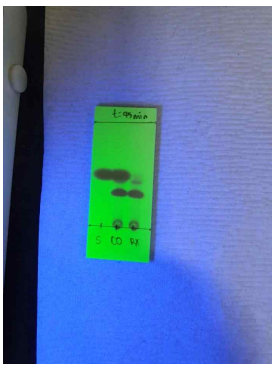


Figure 24. Examine visualized spots of TLC plate (t=30min)

18. If starting material still remains in the reaction mixture, keep running the reaction and take another TLC analysis after 15 min.

I. Work-up

I.1 liquid-liquid Extraction

Caution: During the pressure releasing work, **don't let the end of the funnel see a person**. Erupted solution can splatter on a person.

1. Remove the reaction flask from hot plate.
2. Fix the separatory funnel with clamps and turn the stopcock to close the end of funnel.
3. Add 10 mL of ethyl acetate to the reaction flask.
4. Transfer the reaction mixture into the separatory funnel.
5. Add 10 mL of saturated sodium chloride solution to the separatory funnel.
6. Cap the funnel.
7. Shake the funnel and slowly open the cock with look up to release the gases from mixing.
8. Close the cock
9. Repeat 7-8 for 4 times.
10. Set 100 mL Erlenmeyer flask under the separatory funnel.
11. Open the cock until the bottom layer (aqueous layer) falls to the flask.
12. Set another 100 mL Erlenmeyer flask under the separatory funnel.
13. Open the cock and collect remaining ethyl acetate layer in the flask.

I.2 Drying

1. Measure the mass of 100 mL Round bottom flask.
2. Add excess amount of anhydrous sodium sulfate (Na_2SO_4) to the ethyl acetate solution.
3. Cover the funnel with a cotton.
4. Pour the ethyl acetate solution in a preweighed round bottom flask using cotton-covered funnel.
5. Wash remaining sodium sulfate with ethyl acetate until the color of sodium sulfate turn back to white.

Figure 25. Filtered ethyl acetate solution

6. Evaporate the solvent with Rotary evaporator.
7. Measure the mass of 100 mL round bottom flask with final yellow solid product (9-fluorenone oxime).

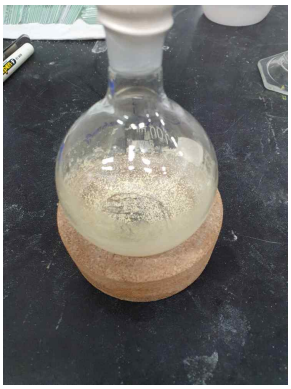


Figure 26. Final product of the second reaction (9-fluorenone oxime)

8. Calculate percentage yield of the second reaction.

J. Charaterizing the product

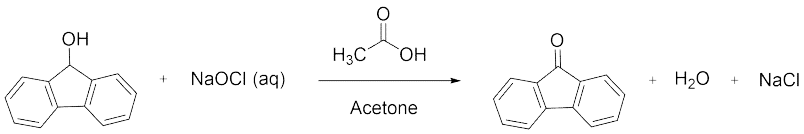
NMR analysis

Assign the structure of the product based on the ^{1}H NMR spectra provided by TA. (Use DMSO-d_6 as a solvent)

Pre-Laboratory Questions

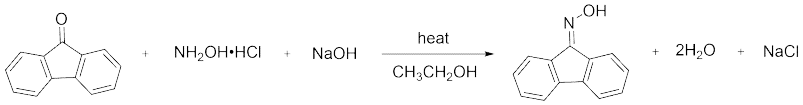
1. Summarize all the MSDS of chemicals used in this experiment.

2. Draw the mechanism of 9-fluorenol oxidation with NaOCl.



Mechanism:

3. Draw the mechanism of 9-fluorenone oxime synthesis



Post-Laboratory Questions

1. In this experiment, 9-fluorenol was used as substrate instead of cyclohexanol. Explain the benefits of using 9-fluorenol.

2. Calculate a retention factor of spots on each TLC plate. Then identify the compounds by comparing with ideal values.

3. Explain why developing chamber was kept sealed during a TLC analysis.

4. Assign the ^{1}H NMR spectra of the product (9-fluorenone oxime).