44th National Chemistry Olympiad

University of Leiden

Leiden

PRACTICAL TEST

Assignment booklet

Tuesday June 13, 2023

SCHEIKUNDE OLYMPIADE

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Universiteit Leiden

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Directions/resources

- This practical test consists of two integrated parts:
  - The synthesis of Hantzsch ester and Hantzsch pyridine;
  - The determination of the molar absorptivity (extinction coefficient) of Hantzsch ester at 400 nm.

- The practical test ends after 4 hours. During this time:
  - the attached answer sheets need to be completed;
  - all questions must be answered.

- After the practical test, when you have handed in everything, the glassware still needs to be cleaned and tidied up.

- The maximum score for the practical test is 80 points.

- The score is determined by:
  - practical skills, working clean, safety maximum 20 points
  - results of the determinations and answers to the questions maximum 60 points

- Required tools: (graphic) calculator, ruler/protractor and Binas or ScienceData.

- First read the introduction and all assignments before you start working.

- Write the answers to the questions in the boxes on the answer sheets provided. If you don't have enough space, you can ask for extra paper.

Additional:

- This is a test; it is not permitted to consult with other participants.
- If you have a question, you can ask the supervisor.
- If something is wrong with your glassware or equipment, report it to the supervisor as soon as you discover it. Don't borrow someone else's things!

Sequence of experiments

This test consists of two experiments.

Experiment 1 consists of two parts: the preparation of the so-called Hantzsch ester (**synthesis part 1**) and the oxidation of Hantzsch ester to the pyridine form (**synthesis part 2**).
In **Experiment 2,** the molar absorptivity (extinction coefficient) of Hantzsch ester at 400 nm is determined.

To ensure that there is sufficient time in **Experiment 1** to synthesize, isolate and dry the crystalline Hantzsch pyridine and then measure everyone's NMR spectrum, you must start **synthesis part 2** immediately after starting **synthesis part 1**. You will receive pre-prepared Hantzsch ester for **synthesis part 2.** You will also receive pre-prepared Hantzsch ester for **Experiment 2.**

You will be asked several times to wait a few minutes, for example until crystals have formed. Make good use of this time by answering questions or starting **Experiment 2**. It is best to take a short break at step 13 of **synthesis part 2**.

Setups:

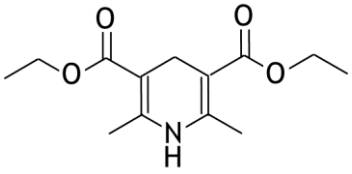
In the fumehood there are two setups ready for the two reactions and a setup for filtration. You can use the same Büchner flask several times for the three filtrations, each time with a clean filter.
Setup 1 is for **synthesis part 1** where a hot plate is used in combination with a magnetic stirrer. The temperature and stirring speed have already been set correctly, both the hot plate and the magnetic stirrer must be turned on.
Setup 2 is for **synthesis part 2.** This involves first stirring only at room temperature; the stirring speed is already set correctly. Only the hot plate is used in the recrystallization step; the temperature is already set correctly.

Experiment 1 Synthesis of Hantzsch ester and oxidation to the pyridine form — 46 points

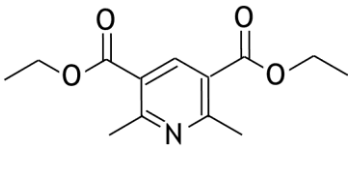
Introduction

Hantzsch ester is a particularly versatile molecule. It is an analogue of the biological reducing agent NADH and as such can be easily oxidized to a pyridine. It is used, among other things, as a hydride donor in hydrogenation transfer reactions. Derivatives of Hantzsch ester also find use as a drug for blocking Ca^{2+} ion channels, thereby lowering blood pressure.

The structural formulas of Hantzsch ester and the pyridine form are as follows:

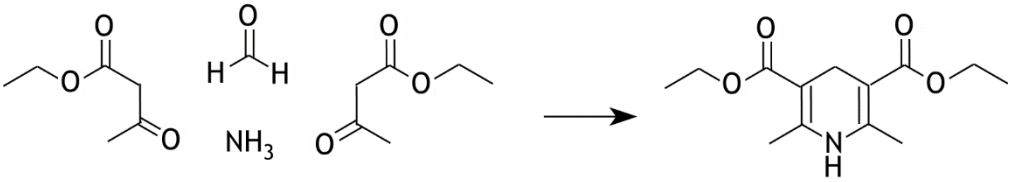


Hantzsch ester



Hantzsch pyridine

The synthesis of Hantzsch ester is a so-called multi-component condensation reaction: multiple reactants react simultaneously (at the same time) or sequentially (one after the other) in the same solution. Schematically this can be shown as follows:



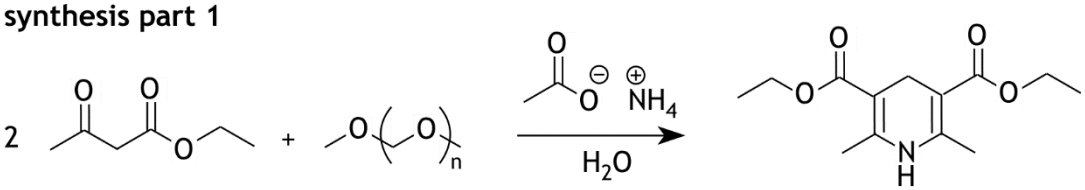
multicomponent condensation reaction

In the synthesis performed in this experiment, the polymer paraformaldehyde is used as the source of the super-electrophilic methanal (formaldehyde) and ammonium acetate is used as the source of ammonia.

The synthesis proceeds via tautomerization of a ß-keto ester (ethyl acetoacetate) to its nucleophilic enol form.

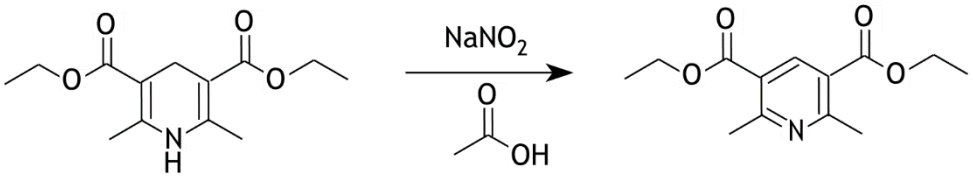
The schemes below summarize **synthesis part 1** and **synthesis part 2**:

synthesis part 1



ethyl acetoacetate paraformaldehyde Hantzsch ester

synthesis part 2



Hantzsch ester Hantzsch pyridine

Chemicals and safety

<u>Ethyl acetoacetate</u> ▪ Formula: $C_6H_{10}O_3$ ▪ CAS No.: 141-97-9 ▪ Molar mass: 130.14 g mol^{-1} ▪ Density: 1.029 g mL^{-1} ▪ Boiling point: 181 °C ▪ No H/P sentences	<u>Sodium nitrite</u>  ▪ Formula: $NaNO_2$ ▪ CAS No.: 7632-00-0 ▪ Molar mass: 69.00 g mole^{-1} ▪ H272, H301, H319, H400 ▪ P210, P220, P264, P273, P301+P310, P305+P351+P338
<u>Paraformaldehyde</u>  ▪ Formula: $[CH_2O]_n$ ▪ CAS No.: 30525-89-4 ▪ Molar mass: 30.03 g mol^{-1} (monomer) ▪ Density: 0.984 g mL^{-1} ▪ H228, H302 + H332, H315, H317, H318, H335, H341, H350 ▪ P210, P280, P301+P312+P340, P305+P338+P351, P308+P313	<u>Ethanol (absolute)</u>  ▪ Formula: C_2H_6O ▪ CAS No.: 64-17-5 ▪ Molar mass: 46.07 g mol^{-1} ▪ Density: 0.789 g mL^{-1} ▪ Boiling point: 78 °C ▪ H225, H319 ▪ P210, P233, P240, P241, P242, P305+P351+P338
<u>Ammonium acetate (1.0 M aq.)</u> ▪ Formula: $C_2H_7NO_2$ ▪ CAS No.: 631-61-8 ▪ Molar mass: 77.08 g mol^{-1} ▪ No H/P sentences	<u>Hantzsch ester (product 1)</u> ▪ Formula: $C_{13}H_{19}NO_4$ ▪ CAS No.: 1149-23-1 ▪ Molar mass: 253.29 g mol^{-1} ▪ No H/P sentences
<u>Acetic acid</u>  ▪ Formula: $C_2H_4O_2$ ▪ CAS No.: 64-19-7 ▪ Molar mass: 60.05 g mol^{-1} ▪ Density: 1.049 g mL^{-1} ▪ Boiling point: 118 °C ▪ H226, H314 ▪ P210, P233, P240, P280, P303+P361+P353, P305+P351+P338	<u>Hantzsch pyridine (product 2)</u>  ▪ Formula: $C_{13}H_{17}NO_4$ ▪ CAS No.: 1149-24-2 ▪ Molar mass: 251.28 g mol^{-1} ▪ H315, H319, H335 ▪ P261, P264, P271, P280, P302+P352, P305+P351+P338

Materials (synthesis part 1)

- 50 mL round bottom flask with stir bar and septum
- 25 mL measuring cylinder
- 5.0 mL measuring pipette + pipette balloon
- Büchner filter
- watch glass with filter paper
- 100 mL volumetric flask + stopper

Materials (synthesis part 2)

- 50 mL round bottom flask with stir bar
- 25 mL measuring cylinder (2x)
- 10 mL measuring cylinder (2x)
- 100 mL beaker
- 25 mL beaker
- watch glass
- glass filter (2x)
- weighing bottle containing 625 mg sodium nitrite

Materials (general)

- stir bar catcher (magnetic bar)
- tweezers
- spatula
- pH paper
- pan for ice bath
- squeezing bottle with demineralized water
- sample vial

Synthesis part 1

1. In the 50 mL round bottom flask with stir bar in setup 1, there is 450 mg paraformaldehyde (this yields 15.0 mmol methanal).
2. Measure approximately 18 mL of 1.0 M ammonium acetate (around 18 mmol) in a measuring cylinder and add it to the round bottom flask.
3. Using a measuring pipette, measure 4.0 mL of ethyl acetoacetate (32 mmol) and add it to the round bottom flask.
4. Turn on the magnetic stirrer and allow the reaction to stir for at least 1.5 hours at 65 °C with a rubber septum lightly placed on the flask.

Now perform **synthesis part 2.**
After you have completed **synthesis part 2,** do the following actions (from **synthesis part 1**) below.

5. After stirring for at least 1.5 hours:
   - switch off the magnetic stirrer;
   - remove the flask from the stand and place it in an ice bath;
   - let the flask sit on ice for 10 minutes.
6. Weigh the filter paper and write down the mass.
7. Filter the cold reaction mixture using a vacuum pump over a Büchner filter with filter paper. Rinse the flask with a small amount of demineralized water and use it to wash the solids on the filter. Allow the solid to dry on the filter for another 10 minutes with the pump switched on.
8. Remove the filter paper from the Büchner filter with tweezers and place it on the watch glass. Let this dry on air for as long as possible.

Now perform **Experiment 2.**
After running **Experiment 2**, perform the final steps (from **Synthesis Part 1**) below.

9. Determine the mass of the filter paper with product.
10. Accurately weigh approximately 10 mg of your self-prepared Hantzsch ester and record the exact weighed mass and transfer it to a 100 mL volumetric flask and fill it with ethanol. Shake vigorously to dissolve everything.
11. Now determine the absorbance (extinction) at 400 nm of this solution, as you did in **Experiment 2, using holder '5' of the carousel.**

Synthesis part 2

1. In the 50 mL round bottom flask with stir bar in setup 2, there is 1.14 g of Hantzsch ester.
2. Measure approximately 15 mL of acetic acid into a measuring cylinder and add it to the flask.
3. Place the flask in the stand and stir the reaction mixture.
4. While the reaction mixture is being stirred, add the 625 mg of sodium nitrite from the weighing bottle to the round bottom flask in small portions over a period of 5 minutes.
5. Place the round bottom flask on a cork ring and remove the stir bar from the flask with the stir bar catcher. Rinse the stir bar with demineralized water in a 100 mL beaker.
6. Add approximately 40 mL of demineralized water to the beaker and let it cool briefly in an ice bath. Also allow approximately 10 mL of demineralised water to cool in a measuring cylinder in the ice bath.
7. Pour the reaction mixture into the beaker and rinse the flask with a small amount of demineralized water from the squeezing bottle.
8. Neutralize the contents of the beaker with aqueous ammonia (25%). You need about 18 mL. Check with pH paper (pH 7 to 8).
9. Using vacuum filtration, filter the solid through a glass filter and rinse with cold water from the measuring cylinder. Allow the solid to dry for a few more minutes on the glass filter with the vacuum pump switched on.
10. Determine the mass of the 25 mL beaker, collect the solid in the beaker and determine the mass of the beaker filled with the solid.

Recrystallization:

11. Add 6 mL of ethanol and heat the beaker, with the hot plate at 120 °C, until the crystals have dissolved. Place a watch glass on the top of the beaker. Swirl occasionally to homogenize the mixture well.
12. Remove the beaker from the hot plate and let it cool to room temperature with the watch glass on top. Swirl the beaker occasionally.
13. Place the beaker on ice for another 10 minutes. Also place 10 mL of ethanol in a graduated cylinder on ice.
14. Filter the resulting crystals through a glass filter using vacuum filtration.
15. Rinse the beaker with cold ethanol and wash the crystals on the filter. Allow the vacuum pump to draw air through the crystals for a few more minutes.
16. Determine the mass of the empty sample vial, transfer the crystals to the sample vial and determine the mass of the sample vial filled with crystals.
17. Submit the sample vial for NMR analysis.

Questions for Experiment 1 – write the answers on the answer sheet

1 Write down:
- The mass of the empty 25 mL beaker (**synthesis part 2**, point 10)
- The mass of the 25 mL beaker filled with solid (Hantzsch pyridine) (**synthesis part 2**, point 10)
- The mass of the empty sample vial (**synthesis part 2**, point 16)
- The mass of the sample vial filled with crystals (**synthesis part 2**, point 16)
- The mass of the clean filter paper (**synthesis part 1**, point 6)
- The mass of the filter paper with product (Hantzsch ester) (**synthesis part 1**, point 9)
- The mass of Hantzsch ester added to the 100 mL volumetric flask (**synthesis part 1**, point 10)
- The absorbance of the solution of Hantzsch ester at 400 nm (**synthesis part 1**, point 11) 4

2 Calculate the percentage yield of Hantzsch pyridine after recrystallization. 9

3 Consider the ^{1}H NMR spectrum of Hantzsch pyridine made from your product. Assign all signals associated with Hantzsch pyridine and justify each one. 5

4 The conversion of Hantzsch ester to the pyridine form is a redox reaction. The equation of the half-reaction of the oxidizer is $HNO_2 + H^+ + e^- \rightarrow H_2O + NO$.
Give the equation of the half-reaction of the reducing agent (Hantzsch ester), use molecular formulas for the organic substances, and give the total reaction equation. 2

5 Calculate the percentage yield of Hantzsch ester. 13

6 Calculate the purity of the synthesized Hantzsch ester in percent based on the absorbance. You can assume that any contaminants present will not absorb. 3

Experiment 2 Determination of the molar absorptivity (extinction coefficient) ε of Hantzsch ester at 400 nm 34 points

Introduction

Hantzsch ester contains a conjugated system that gives the compound a color. The absorption maximum of Hantzsch ester is at 371 nm. The Lambert-Beer law describes the absorbance (extinction) A of light of a certain wavelength according to:

$A = \varepsilon c l$

Herein is:

- ε the molar absorptivity (extinction coefficient)
- c the concentration (in mol L^{-1}) of the solute
- l the length of the path of the light (in cm)

In this experiment the ε is determined at 400 nm using a calibration series.

Chemicals and safety

- absolute ethanol (see experiment 1)
- Hantzsch ester (see experiment 1)

Materials

- 100 mL volumetric flask
- 25 mL volumetric flask (4x)
- volumetric pipettes (4x)
- Pasteur pipettes with balloon
- 100 mL measuring cylinder
- funnel
- 50 mL beaker for liquid waste
- cuvettes, l = 1.00 cm

The determination of the molar absorptivity via UV-VIS

To determine the molar absorptivity of Hantzsch ester, a stock solution with an accurately determined concentration is first made. A dilution series is made from this stock solution. The absorbance (*A*) of each of the solutions from the dilution series at 400 nm is then determined against a blank. A calibration curve is created by plotting the measurement results in a diagram. We use the Genesys 10s spectrophotometer.

Preparation of the stock solution:

- Accurately weigh approximately 25 mg of the supplied Hantzsch ester on a weighing paper and record the exact weighed mass.
- Transfer this to the 100 mL volumetric flask and fill the volumetric flask to 100 mL with ethanol.
- Stopper the volumetric flask and shake vigorously until all the substance is dissolved. Please note: this may take several minutes.

Dilution series:

Label the four 25 mL volumetric flasks '1' to '4'.

- Make four solutions according to the table below.
  Use volumetric pipettes.
  If necessary, pre-rinse the pipettes and empty them into the liquid waste beaker.
  Fill the volumetric flask with ethanol to 25 mL each time and homogenize the solution.

Dilution no.	Volumetric flask (mL)	Volumetric pipette (mL stock solution)
1	25	1.00
2	25	4.00
3	25	7.00
4	25	10.0

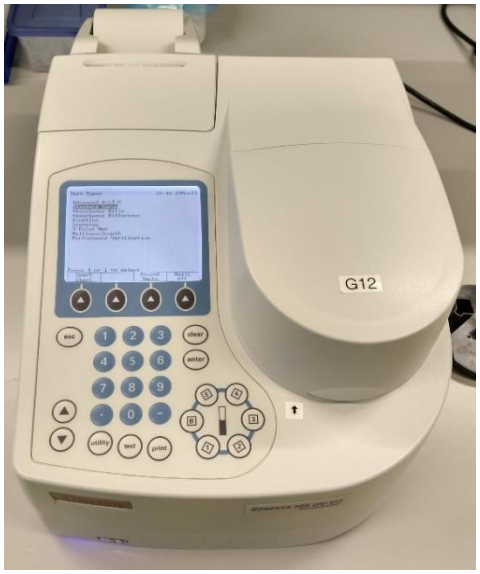


Figure 1: the Genesys 10s spectrophotometer

Measuring the absorbances:

Use the Genesys 10s spectrophotometer. The correct setting has already been selected. You fill the cuvettes each time using Pasteur pipettes and fill them with one volume of a Pasteur pipette.

- To measure the blank, fill a cuvette with absolute ethanol only, place it in the spectrophotometer carousel at position 'B' and press 'Measure Blank'.
- Fill a new cuvette with the first dilution and place it in the spectrophotometer at position '1' of the carousel.
- Repeat the actions until the other three dilutions have also been placed in the correct position ('2' to '4').
- Press the buttons '1' to '4' in succession and copy the absorbances that appear on the screen.
- Leave the cuvettes in the carousel!

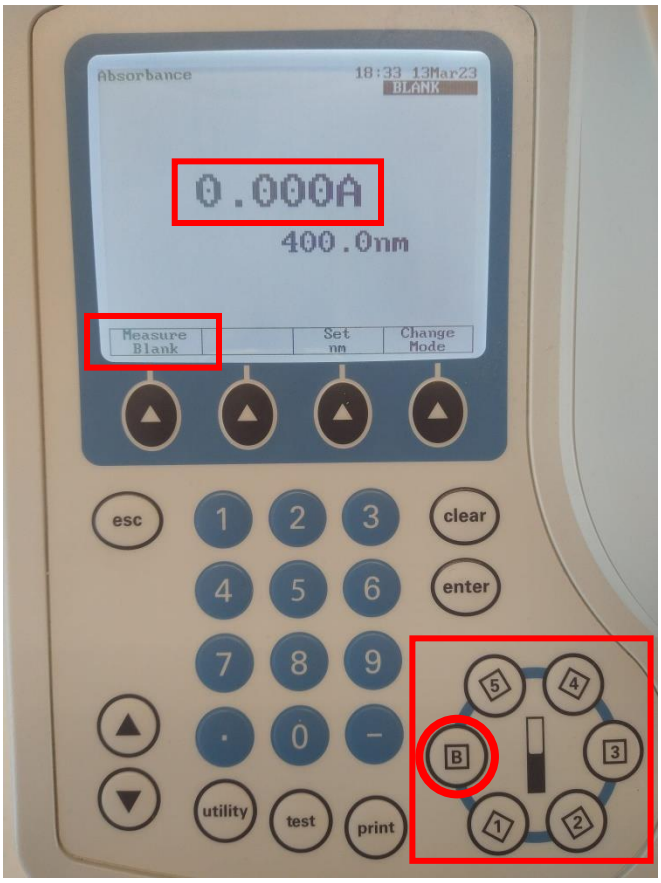


Figure 2: The screen and control buttons of the Genesys 10s.

Questions for Experiment 2 – write the answers on the answer sheet

7 Write down:
- the mass of Hantzsch ester used to make the stock solution
- the measured absorbances. 2

8 Calculate the concentration in mol L^{-1} of Hantzsch ester in the stock solution and in the four solutions of the dilution series. 6

9 Plot the measured absorbances at 400 nm against the concentrations in mol L^{-1} of Hantzsch ester. 4

10 Calculate the molar absorptivity at 400 nm. 12