PRACTICAL PROBLEMS

PROBLEM 1 (Practical)

Synthesis of the Dipeptide *N*-acetyl-*L*-prolinyl-*L*-phenylalanine Methyl Ester (Ac-*L*-Pro-*L*-Phe-OCH_3)

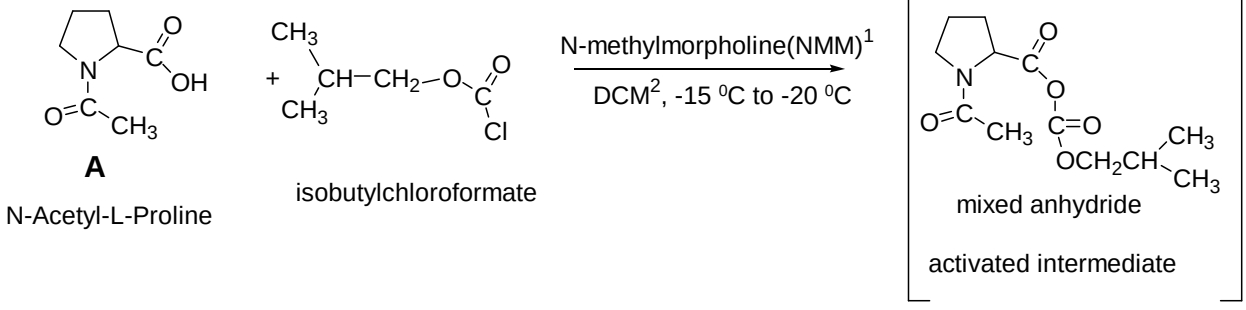
Introduction

Peptide synthesis is now a well-refined art and many of their synthetic procedures can be readily adapted to the elementary laboratory. Interest in peptides, always high, has heightened even more with the recent discovery of the importance of the so-called “opiate” peptides as well as of other biological active peptides.

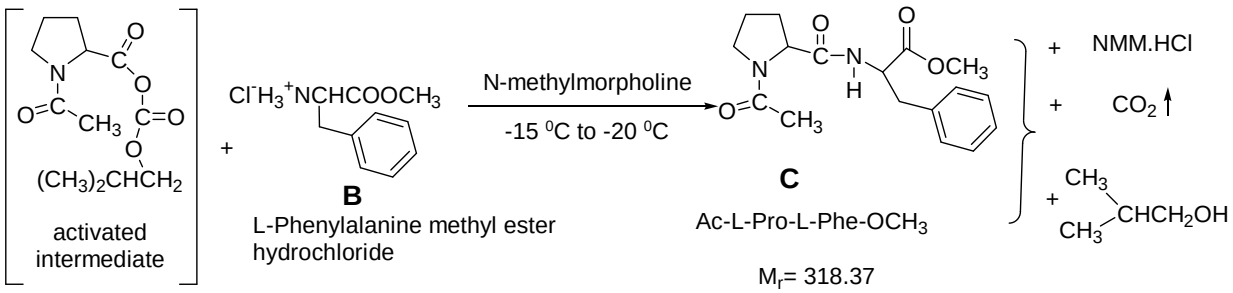
In this experiment the one-pot procedure for synthesizing the title dipeptide from its components, suitably protected amino acids, is described.

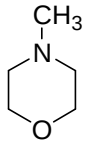
Reactions

STEP 1



STEP 2



[1] *N*-methylmorpholine (NMM) = 

[2] DCM = Dichloromethane

Procedure

STEP 1

Place the 1.50 g (0.0095 mol) sample of *N*-acetyl-*L*-proline (labelled AcPro), which you have been given, into a 50-cm^3 round-bottom flask. Add 20 cm^3 dichloromethane (labelled DCM) in the graduated cylinder. Use some of the 20 cm^3 DCM to wash out the AcPro vial and add the remaining DCM also into the round-bottomed flask. Plug the flask with a septum, clamp it loosely to a support stand and cool it to –15 ℃ to –20 ℃ in the ice/sodium chloride cold bath provided by the supervisor. Allow approximately 5 minutes for cooling. Add 1.2 cm^3 (0.0109 mol) of *N*-methylmorpholine (labelled NMM) to the flask, by means of a syringe. Then, slowly add 1.5 cm^3 (0.0116 mol) isobutyl-chloroformate (labelled IBCF) to the flask by means of a second syringe. During the addition, swirl the reaction mixture gently by hand, and continue swirling for another 10 min. The temperature should remain in the range –20 ^{o} to –15 ^{o}C.

STEP 2

Remove the septum and quickly add all the *L*-phenylalanine methyl ester hydrochloride (2.15 g, 0.0100 mol), (labelled $HCl{\cdot}H_2NPheOCH_3$) using the polypropylene powder funnel. Plug the flask again with the septum. Immediately add 1.2 cm^3 (0.0109 mol) of *N*-methylmorpholine (labelled NMM) using a third syringe, while the reaction mixture is swirled by hand. *ATTENTION: Leave the needle part of the syringe in the septum for the remainder of the reaction*. Allow the reaction to proceed for 60 min at –15 ℃ to –20 ℃, swirling periodically by hand.

During this waiting period you are highly advised to start working on the Analytical Chemistry experiment.

After 60 min at –20 ^{o}C to –15 ^{o}C, remove the 50 cm^3 round-bottomed flask from the ice/sodium chloride bath and place the flask in the 250 cm^3 beaker and let it warm up to

room temperature. Transfer the contents of the flask into the 50 cm^3 separating funnel by means of the glass funnel. Rinse the flask with a small amount of dichloromethane (3 – 5 cm^3), which is in a vial (labelled DCM). Wash the organic layer successively with two 20 cm^3 portions of 0.2 M aqueous HCl solution, two 20 cm^3 portions of 1 % aqueous

$NaHCO_3$ solution (read caution comment in next paragraph) and finally one 10 cm^3 portion of saturated solution of sodium chloride (labelled brine).

Important

After each washing allow the separating funnel to stand for enough time, so that the two phases separate completely. Also, take into consideration that the organic phase (DCM) is always the lower layer and contains the product. All the aqueous washings are collected in the same Erlenmeyer flask (empty if necessary).

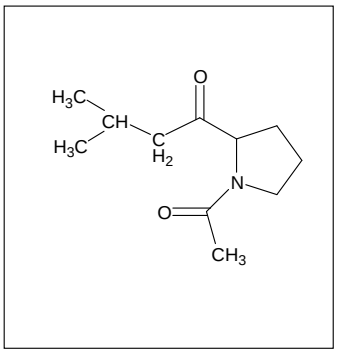
CAUTION: Keep in mind, also, that during washing with 1 % $NaHCO_3$, the CO_2 liberated is exerting pressure on the separating funnel stopper, so be sure to let the gas out through the stopcock before and after each shaking, while holding the funnel upside down.

Before continuing, wash the glass funnel, the 50 cm^3 cylinder and the 50 cm^3 round-bottom flask with water and then dry them with acetone. Your supervisor will show you where to dispose of the water and the acetone.

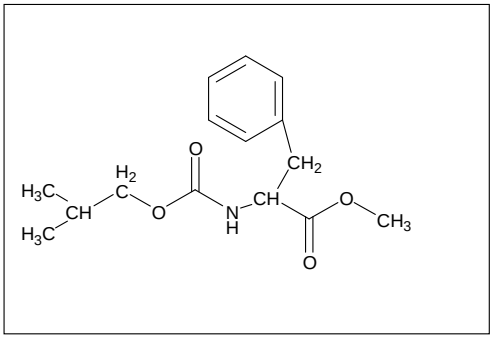
Pour the organic layer into a clean 50 cm^3 Erlenmeyer flask. Add the anhydrous sodium sulphate, which is in a vial labelled Na_2SO_4, to the Erlenmeyer flask containing the organic layer. The organic phase should become clear. Filter it through the cleaned and dried funnel, whose stem you have previously stuffed with a small piece of cotton to trap any solids, into the cleaned and dried 50 cm^3 round-bottom flask. Rinse the Erlenmeyer flask with a small amount of dichloromethane (3 – 5 cm^3). Removal of the organic solvent is done under reduced pressure, using a rotary evaporator apparatus. This will be done for you by a laboratory supervisor, who will add 20 cm^3 of diethylether to the residue in your flask, which will cause precipitation of your product. After cooling for 5 minutes in the ice bath, scrape the walls of the flask with a spatula, filter by suction the crystallized dipeptide through a fritted glass funnel. Wash twice with diethylether (5 cm^3 each time).

Leave the product on the filter under suction for at least 3 minutes. Then collect it on weighing paper, weigh it in the presence of a supervisor and then transfer it into a sample vial and label it with your student code. Write the mass of your product (**C**) on the label and on your answer sheet (on the next page).

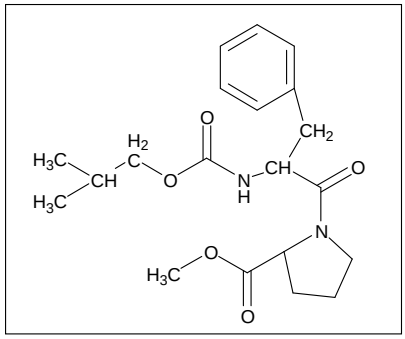
During the reaction between the phenylalanine methylester **B** and the activated mixed anhydride intermediate (step 2) the formation of the desired dipeptide product **C** is usually accompanied by a by-product the correct structure of which is one of the three structures **I**, **II**, **III** given below. Circle the Roman numeral corresponding to the correct structure.



I



II



III

TLC- Analysis

You have two Eppendorfs, one empty and one with a tiny amount of substance **B**. Put a small amount of **C** into the empty Eppendorf, and dissolve both **B** and **C** in a few drops of methanol. Use the supplied capillary tubes to apply small samples of these solutions to the TLC plate. Develop the TLC plate with a solution of chloroform-methanol-acetic acid (7 : 0.2 : 0.2) as eluant. The appropriate amount of eluant has been placed in the proper vial by the supervisor.

After the elution, analyze the TLC-plate using a UV-lamp. Clearly mark the starting line, solvent front and the UV-active spots.

Draw the diagram in the box on the answer sheet. Determine the R_f values.

Finally place the TLC-plate in a small plastic bag with a sealing strip and put it in an envelope provided by the supervisor. Write your student code on the envelope.

The examination committee will check the quality of the *N*-acetyl-*L*-prolinyl-*L*-phenylalanine methyl ester that you have prepared by determining its angle of optical rotation and consequently its specific rotation, $[a]^t_{D,}$ using an accurate polarimeter apparatus.

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SOLUTION

The following values were required to be written in the Answer Sheet

- Mass of Ac-*L*-Pro-*L*-Phe-OCH_3 obtained (product **C**).
- The yield of Ac-*L*-Pro-*L*-Phe-OCH_3 **C.**
- The TLC diagram with indication of the base line and the front of the solvent.
- R_f value of *L*-phenylalanine methyl ester hydrochloride (material B) and that of R_f value of Ac-*L*-Pro-*L*-Phe-OCH_3 (product C).

- Conclusions from the TLC analysis:
  Compound **C:**
  - Is pure,
  - Contains some **B**,
  - Contains several contaminants,
  - No conclusion.

- Specific rotation of the dipeptide Ac-*L*-Pro-*L*-Phe-OCH_3 C (to be measured later by the examination committee)
  $[a]_D^T$ = 45 °

- The correct structure of the by-product: **II.**