Student Name:	Student Code:

Text language: English

Translator countries (if more than one):

35th International Chemistry Olympiad

Athens, Greece

Practical Examination

Tuesday, 8 July 2003

Introductory Remarks

- At all times while you are in the laboratory you should wear safety spectacles or your own spectacles if they have been approved. Use only a pipette filler bulb for pipetting. Eating of any kind of food is strictly prohibited in the laboratory.
- Participants are expected to work safely, to behave socially and to keep equipment and work environment clean. Do not hesitate to ask a laboratory assistant if you have any questions concerning safety issues.
- When you enter the laboratory, check the place of the safety shower.
- Work may only begin when the start signal is given.
- You have **5** hours to complete all of the experimental tasks, and record your results on the answer sheets. There will be a pre-warning 15 minutes before the end of your time. You must stop your work immediately after the stop command is given. A delay in doing this by 5 minutes will lead to cancellation of the current task and will result in zero points for that task.
- **This practical examination comprises two experiments. In order to use the available time efficiently, you will start working on the organic chemistry experiment up to the point where you are instructed to work on the analytical chemistry experiment. Then you will finish the work on the organic chemistry experiment.**
- Write your name and personal identification code (posted at your work station) in the appropriate box of the answer sheets.
- All results must be written in the answer boxes on the answer sheets. Data written elsewhere will not be marked. Do not write anything in the back of your answer sheets. If you need more paper for working or a replacement answer sheet, request it from the laboratory assistant.
- When you have finished the examination, you must put all papers into the envelope provided. Only papers in the envelope will be marked.
- Do not leave the examination room until you have permission to do so.
- Use only the tools provided.
- The number of significant figures in numerical answers must conform to the rules of evaluation of experimental error. The inability to perform calculations correctly will result in penalty points, even if your experimental technique is flawless.
- The examination has 3 pages of answer sheets.
- An official English-language version is available only on request.

Disposal of waste chemicals, spills, and glassware

Organic filtrates and organic washings and any other waste should be placed in the waste beaker or bottle.

Use the appropriate waste containers for disposals of chemical and other waste materials.

Broken glass should be placed in the waste bucket. There is a one-point penalty for broken glassware or replaced samples.

Cleaning up

The lab bench should be wiped clean with a wet tissue.

Organic Chemistry Experiment

Synthesis of the dipeptide *N*-acetyl-*L*-prolinyl-*L*-phenylalanine methyl ester (Ac-*L*-pro-*L*-phe-OCH_3)

Glassware and equipment

Round-bottomed flask (50 mL)	1	
Septum	1	
Support stand	1	
Clamp holder	1	
Clamp	1	
Syringe polyethylene (5 mL) + needle	3	
Polypropylene powder funnel	1	
Glass funnel	1	
Separating funnel (50 mL)	1	
Erlenmeyer flask (50 mL)	3	
Spatula	1	
Pair of forcepts	1	
Measuring cylinder (50 mL)	1	
Weighing paper	1	(Located near the balances)
Fritted glass funnel	1	
Sample vial	1	
Screw cap bottle (large) for TLC	1	
Thin layer plate (3-7 cm)	1	Located at the end of the bench
Capillary tubes for TLC (in sample tube)	2	Located at the end of the bench
Thermometer	1	
Filter flask (100 mL)	1	
Filter rubber adaptor	1	
Eppendorf	1	
Stationery (pen, pencil)		
Beaker (250 mL)	1	

Chemicals

Dichloromethane	30 mL
N-Acetyl-*L*-proline (Ac-L-Pro)	1.50 g (in a vial)
L-Phenylalanine methylester hydrochloride (HCl.*L*-Phe-OMe)	2.15 g (in a vial)
Isobutyl chloroformate	1.5 mL (Located at the end of the bench)
N-Methylmorpholine	2.4 mL
Methanol	
Sodium hydrogen carbonate ($NaHCO_3$) 1%	40 mL
Hydrochloric acid (HCl) 0.2M	40 mL
Anhydrous sodium sulfate	2 g
Cotton wool	
Diethyl ether	30 mL provided by the laboratory assistant
Wash bottle with acetone (for rinsing)	500 mL
TLC eluant (chloroform-methanol-acetic acid (7:0.2:0.2)	15 mL provided by the laboratory assistant
Ice/sodium chloride cold bath [-20^oC - -15^oC]	Provided by the laboratory assistant
Compound **B**	In Eppendorf labelled **B**

Risk and Safety Information

Acetone

Formula	C_3H_6O
Molecular weight	58.08
Melting point	–95 ^oC
Boiling point	56 ^oC
Density	0.79 g/cm^3

R11	Highly flammable
S9	Keep container in a well-ventilated place
S16	Keep away from sources of ignition
S23	Do not breathe vapour
S33	Take precautionary measures against static discharges

Hydrochloric acid

Formula	HCl
Molecular weight	36.46
Density	1.200 g/cm^3

R34	Causes burns
R37	Irritating to respiratory system
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advise
S36	Wear suitable protective clothing
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

Methanol

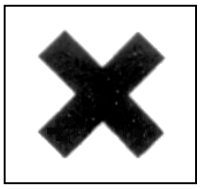
Formula	CH_4O
Molecular weight	32.04
Melting point	–98 ^oC
Boiling point	65 ^oC
Density	0.79 g/cm^3

R11	Highly flammable
R23-25	Toxic by inhalation, in contact with skin and if swallowed
R39/23/24/25	Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed
S7	Keep container tightly closed
S16	Keep away from sources of ignition-No smoking
S36/37	Wear suitable protective clothing and gloves
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

Dichloromethane

Formula	CH_2Cl_2
Molecular weight	84.93
Melting point	–97 ^oC
Boiling point	40 ^oC
Density	1.325 g/cm^3

R40	Limited evidence of a carcinogenic effect
S23-24/25	Do not breathe fumes. Avoid contact with skin and eyes
S36/37	Wear suitable protective clothing and gloves



Isobutyl Chloroformate

Formula	$C_5H_9O_2Cl$
Molecular weight	136.58
Boiling point	128.8 ^oC
Density	1.053 g/cm^3

R10	Flammable
R23	Toxic by inhalation
R34	Causes burns
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S45	In case of accident or if you feel unwell, seek medical advice immediately (show label where possible)
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection

N-Methylmorpholine

Formula	$C_5H_{11}NO$
Molecular weight	101.15
Melting point	–66 ^oC
Boiling point	115-116 ^oC/750torr
Density	0.920g/cm^3

R11	Highly flammable
R34	Causes burns
R20/21/22	Harmful by inhalation, in contact with skin and if swallowed
S16	Keep away from sources of ignition-No smoking
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S45	In case of accident or if you feel unwell, seek medical advice immediately (show label where possible)
S36/37/39	Wear suitable protective clothing, gloves and eye/face

_L_-Phenylalanine methyl ester hydrochloride

Formula	$C_{10}H_{13}NO_2.HCl$
Molecular weight	215.68
Melting point	158-162 ^oC
Density	0.920g/cm^3

_N_-Acetyl-_L_-proline

Formula	$C_7H_{11}NO_3$
Molecular weight	157.17

Diethyl ether (Ether)

Formula	$C_4H_{10}O$
Molecular weight	74.12
Melting point	-116 ^oC
Boiling point	34.6 ^oC
Density	0.706 g/cm^3

R12	Extremely flammable
R19	May form explosive peroxides
R22	Harmful if swallowed
R66	Repeated exposure may cause skin dryness or cracking
R67	Vapours may cause drowsiness and dizziness
S9	Keep container in a well-ventilated place
S16	Keep away from sources of ignition-No smoking
S29	Do not empty into drains
S33	Take precautionary measures against static discharges

Materials available for general use

Cleaning paper
Sponge
Waste container

Equipment for general use

Flash evaporator
Balance
UV lamp

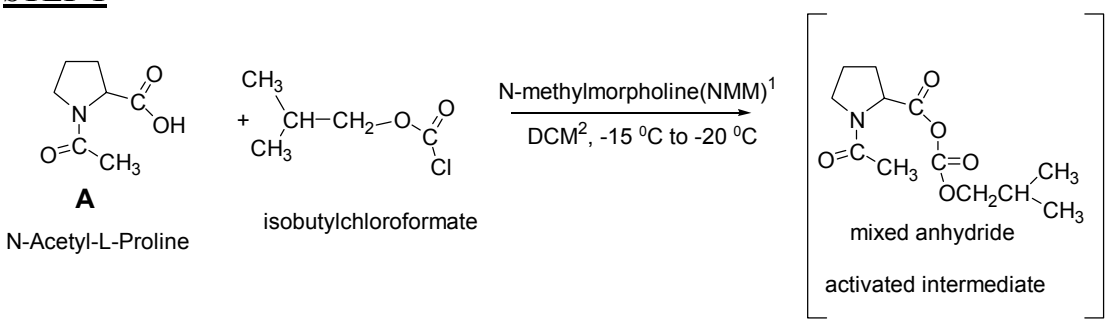
Synthesis of the dipeptide *N*-acetyl-*L*-prolinyl-*L*-phenylalanine methyl ester (Ac-*L*-Pro-*L*-Phe-OCH_3)

Introduction

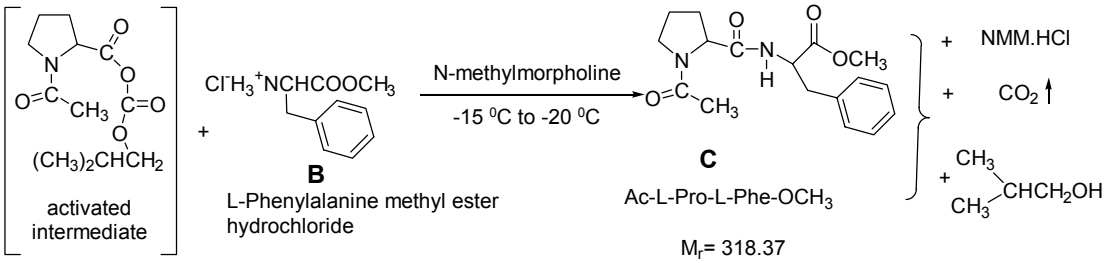
Peptide synthesis is now a well-refined art and many of their synthetic procedures can be readily adapted to the elementary laboratory. Interest in peptides, always high, has heightened even more with the recent discovery of the importance of the so-called "opiate" peptides as well as of other biological active peptides.
In this experiment the one-pot procedure for synthesizing the title dipeptide from its components, suitably protected amino acids, is described.

Reactions

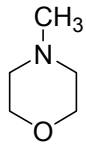
STEP 1



STEP 2



M_r = 318.37

[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-bottomed 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 °C to –20 °C 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) isobutylchloroformate (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^oC.

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 °C to –20 °C, 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^oC to -15^oC, 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-bottomed 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 sulfate, 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-bottomed 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).

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.

Student Name:	Student Code:

Answer Sheet 1

Synthesis of *N*-Acetyl-*L*-prolinyl-*L*-phenylalanine methyl ester (Ac-*L*-Pro-*L*-Phe-OCH_3)

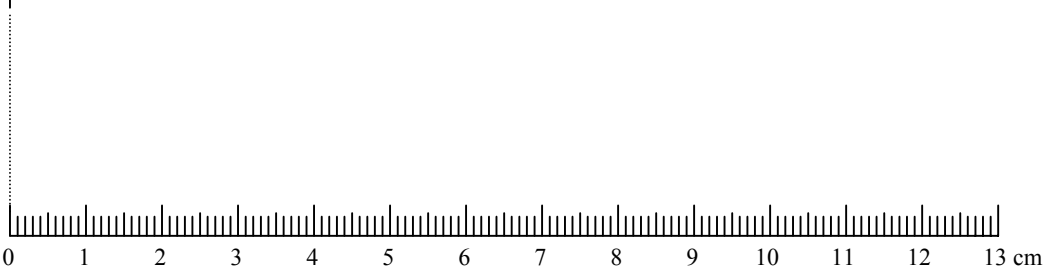
Box	1	2	3	4	5	6	7
Points	10	3	2	2	2	10	2

1 Mass of **Ac-*L*-Pro-*L*-Phe-OCH_3** obtained (product **C**): g

Calculate the yield of Ac-*L*-Pro-*L*-Phe-OCH_3 **C:**

Yield % =

2 Draw the TLC diagram



3 R_f value of ***L*-phenylalanine methyl ester hydrochloride** (material **B)**

4 R_f value of **Ac-*L*-Pro-*L*-Phe-OCH_3** (product **C)**

Student Name:	Student Code:

Answer Sheet 2

5 Conclusions from the TLC analysis:

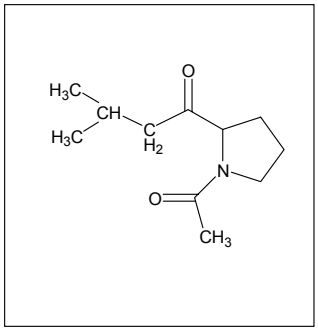
Compound **C:**

- ☐ Is pure
- ☐ Contains some **B**
- ☐ Contains several contaminants
- ☐ No conclusion

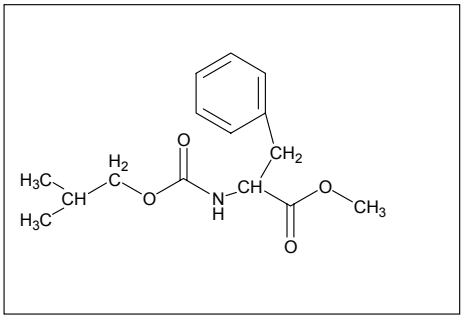
6 Specific rotation of the dipeptide Ac-*L*-Pro-*L*-Phe-OCH_3 C (to be measured later by the examination committee)

$$[a]_D^T =$$

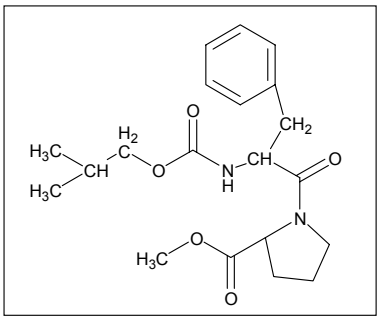
7 During the reaction between the phenylalanine methylester **B** and the activated mixed anhydride intermediate (step 2) the formation of the desired dipeptide product **C** is usually accompanied by a byproduct the correct structure of which is one of the three structures **I**, **II**, **III** given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



I



II



III

Analytical Chemistry Experiment
TITRATION OF ASCORBIC ACID WITH POTASSIUM IODATE

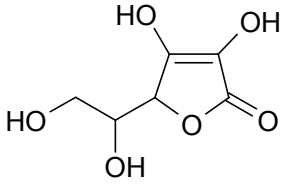
Introduction

Ascorbic acid (vitamin C, $C_6H_8O_6$, symbolized below as $AscH_2$) is a weak acid and undergoes the following dissociation steps:

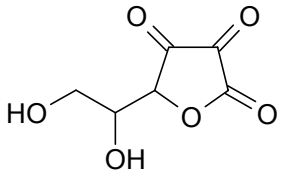
$$AscH_2 \rightleftharpoons AscH^- + H^+ \qquad K_{a1} = 6.8\times10^{-5}$$
$$AscH^- \rightleftharpoons Asc^{2-} + H^+ \qquad K_{a2} = 2.7\times10^{-12}$$

Ascorbic acid is readily oxidized to **dehydroascorbic acid** according to the half reaction:

$$C_6H_8O_6 \rightleftharpoons C_6H_6O_6 + 2H^+ + 2e^-$$



Ascorbic acid ($C_6H_8O_6$)



Dehydroascorbic acid ($C_6H_6O_6$)

A typical titrant used for the redox titration of ascorbic acid is potassium iodate, KIO_3. If the titration is carried out in 1 M HCl medium, then the reaction proceeds as follows:

$$3C_6H_8O_6 + IO_3^- \rightleftharpoons 3C_6H_6O_6 + I^- + 3H_2O$$

The end point is detected by the reaction of the first excess of iodate with iodide ions already present in the solution, producing I_2 which colours starch indicator blue:

$$IO_3^- + 5I^- + 6H^+ \rightleftharpoons 3I_2 + 3H_2O$$

Principle of the method

Ascorbic acid will be titrated by using a solution of potassium iodate of known concentration. The titration will be carried out in 1 M HCl, while starch solution will be used as indicator to detect the end point.

Solutions

1. Solution of potassium iodate of known concentration.
   Make a note here of the concentration written on the bottle:

Molarity of KIO_3 = M

2. Solution of 2 M HCl
3. Starch solution

Risk and Safety Information

Potassium iodate

Formula	KIO_3
Molecular weight	214.00
Melting point	560 ^oC
Density	3.930 g/cm^3

R8	Contact with combustible material may cause fire
R36/38	Irritating to eyes and skin
R42/43	May cause sensitisation by inhalation and skin contact

R61	May cause harm to the unborn child
S17	Keep away from combustible material
S22	Do not breathe dust
S45	In case of accident or if you feel unwell, seek medical advice immediately (show label where possible)
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection

Ascorbic acid

Formula	$C_6H_8O_6$
Molecular weight	176.13
Melting point	193^{o}C (dec.)

Glassware

1. One 50 mL burette
2. One burette stand
3. One burette clamp
4. One 250-mL volumetric flask
5. Three 250-mL conical flasks
6. One graduated cylinder (25 or 50 mL)
7. One dropper
8. One 500-mL wash bottle (polyethylene, squeeze type) with deionized water
9. One 25.00-mL pipette
10. One pipette-filling bulb

Procedure

Preparation of burette

Rinse the burette with deionized water at least three times. Rinse twice with solution of potassium iodate and fill. Record the initial volume of titrant ($V_{initial}$).

Titration of unknown sample

Obtain the unknown solution in a clean 250-mL volumetric flask. Record batch number of solution given. Dilute to the mark with deionized water and shake well. Use a pipette to transfer 25.00 mL of this solution into a 250-mL conical flask. Use a graduated cylinder to transfer 25 mL of 2 M HCl into the same flask and shake well. Add 40 drops of starch solution and titrate the solution with potassium iodate up to a permanent blue colour. Record final volume of titrant (V_{final}) (titration 1). Repeat the procedure as many times as necessary. Calculate the concentration of ascorbic acid (mg $C_6H_8O_6$/mL of solution). Each time refill the burette with solution of potassium iodate.

Student Name:	Student Code:

Results (8 points) **Answer Sheet 3**

Batch number of solution given

Titration No	$V_{initial}$ mL	V_{final} mL	V mL
Final volume			

mg $C_6H_8O_6$ / mL	

Questions

(2 points)

1. If the titration of ascorbic acid is carried out in 5 M HCl medium, then the reaction proceeds as follows:

$$C_6H_8O_6 + IO_3^- + H^+ + Cl^- \rightleftharpoons C_6H_6O_6 + ICl + H_2O$$

Balance the above reaction.

2. If V_1 and V_2 are the volumes of KIO_3 solution (titrant) required for the titration of 25.00 mL of the ascorbic acid solution given to you, in 1 and 5 M HCl, respectively, then the two volumes are related by the following relationship: (Circle the correct answer)

a. $V_2 = (3/2)\ V_1$
b. $V_2 = (2/3)\ V_1$
c. $V_2 = V_1$
d. none of the above