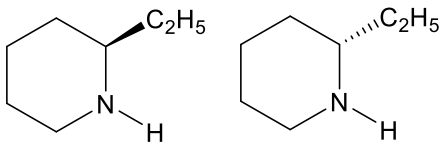
Organic chemistry practical course

Synthesis of (*R*)-2-ethylpiperidine and (*S*)-2-ethylpiperidine

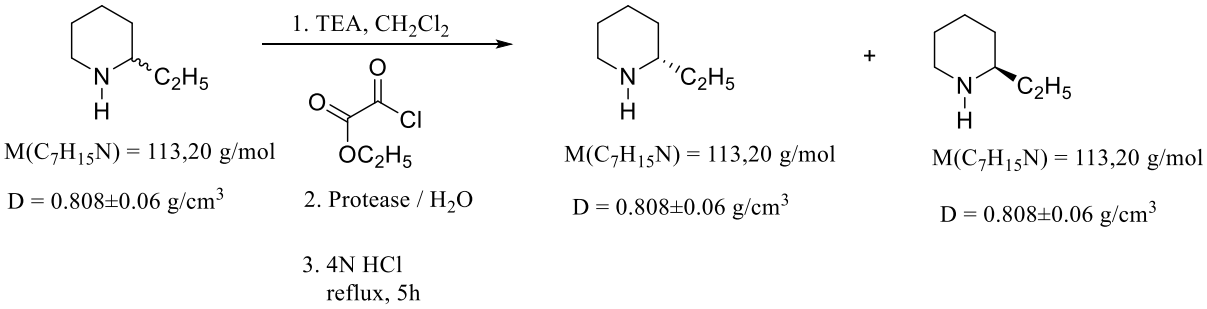
($C_7H_{15}N$)



(*R*) (*S*)

Hand over: X

I. Reaction:



Scheme 1: Reaction overview.

II. Theory: [1]

This report describes the preparation of a chiral secondary amine, namely [(*R*)-2-ethylpiperidine and (*S*)-2-ethylpiperidine] from a racemic secondary amine (2-ethylpiperidine). The preparation was achieved *via* an enzymatic hydrolysis, in which treatment of an oxalamic ester derivative (the reaction intermediate) with a specific enzyme (protease) was carried out to obtain the enantiomeric products. The oxalamic ester attached to the nitrogen of the amine group serve as a protecting group for this amine group.

Protecting groups are used in organic synthesis to temporarily protect a functional group from reacting because this functional group could interfere with another reaction.[2]

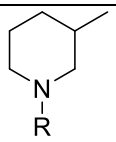
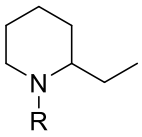
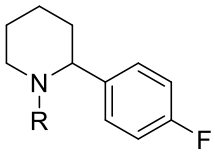
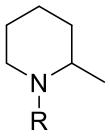
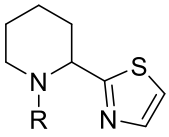
Literature [1] shows that this is an efficient method for the preparation of secondary amines with optical properties, high purity, and yields. The enzyme protease (*Aspergillus species*) has a vital role in such reactions because it ensures the resolution of the racemic mixture, thereby resulting in enantiomeric species, from which the products are obtained. Furthermore, the enzyme "Aspergillus protease" in this reaction selectively hydrolyzes the ester bond in the oxalamic derivative favouring the ease at which the oxalamic acid is formed.

Pure enantiomeric secondary amines are building blocks that are essential in the synthesis of biologically active molecules or compounds. This explains why some current drugs have some partial structures of optically pure secondary amines.

For the preparation of optically pure secondary amines through enantioselective hydrolysis of oxalamic esters of amines, different oxalamic ester derivatives of amines may require the same or a different type of protease enzyme species. This means each protease enzyme(s) has a particular desired reactivity and enantioselectivity.

Some examples are listed in Table 1 below.

Table 1: List of the corresponding enzymes for enantioselective hydrolysis of oxalamic acid esters during the production of secondary amines.

Molecule	Required Enzyme (Protease)
	*β-chymotrypsin*
	*Aspergillus species*
	*Streptomyces griseus*
	*Aspergillus species*
	*Bacillus licheniformis*

R = $COCO_2C_2H_5$

Enzymes are biological substances serving as catalyst and they regulate the rate at which chemical reactions occur without being affected by the process. In living organisms, biological activities are mainly regulated by enzymes. [3]

Enzymes are classified according to the type of action they perform on the substrate on which they act.

Typically, enzymatic reactions occur in which the free enzyme E binds to its substrate S (reactant) to form an enzyme-substrate, mostly denoted ES, which then disintegrates to form the product with the release of the free enzyme. This mechanism is called the "Lock and Key Model".[4]

III. Mechanism: [1][2][6]

This reaction has 3 major mechanistic steps.

1. Acylation of (±)-2-ethylpiperidine to obtain the racemic oxalamic acid ester.
2. Enzymatic hydrolysis of the oxalamic ester to obtain the enantiomeric intermediates.
3. Deprotection *via* workup with hydrochloric acid to obtain the products.

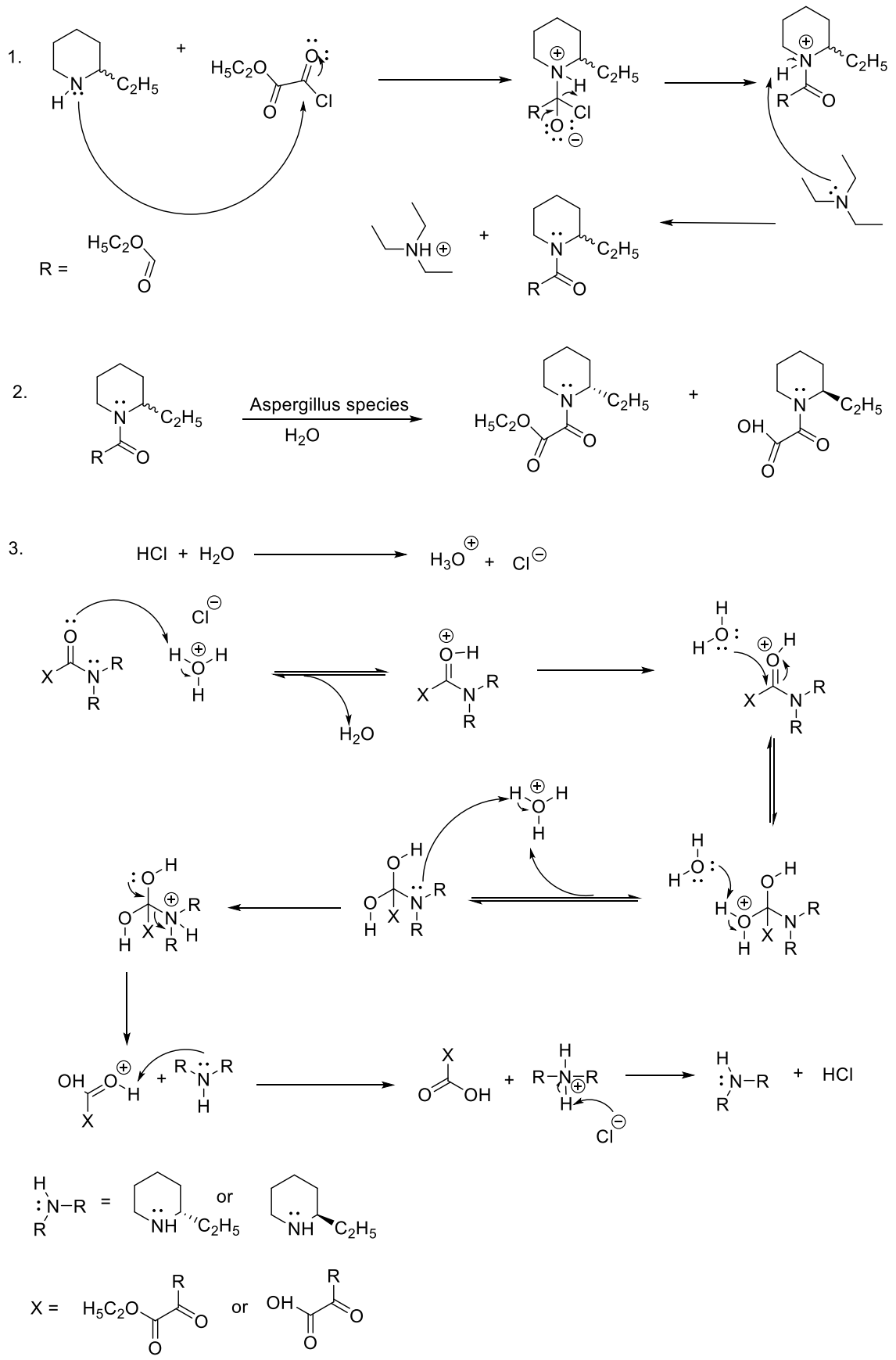
In the first step, the carbonyl centre of the acid chloride group is attacked by the electron lone pair of the nitrogen on the (±)-2-ethylpiperidine forming a sort of ammonium-oxalamate complex. An electron shift occurs on the acyl group of the complex formed leading to expulsion of the chloride ion and triethylamine accepts the proton bound to the nitrogen of the complex, replenishing the electron deficiency at the ammonium, and thus leading to the (±)-oxalamate ester.

In the second step, the (±)-oxalamate ester is hydrolyzed with water and the protease (Aspergillus species) leading to the formation of oxalamic ester and acid, respectively.

In the deprotection step, the racemic mixture is worked up in hydrochloric acid (amide hydrolysis with aqueous acid). The following events happen:

First, water reacts with hydrochloric acid (HCl) to form hydronium ions. Then, protonation of the carbonyl group of the amide bond takes place in which electrons on the oxygen atom will attack a proton on the hydronium ion. Next, nucleophilic addition of water to the formed conjugate base occurs, releasing a hydronium ion. In the next step, deprotonation of the released hydronium ion by lone pair on the nitrogen takes place, then the elimination of the amine group occurs resulting in a sort of positively charged carboxylic acid which is later deprotonated by the electrons of the cleaved amine to produce an ammonium.

The chloride ion in the medium deprotonates the ammonium to form the products.



Scheme 2: Proposed reaction mechanism.

IV. Procedure: [1]

In a round bottom flask, 5.0 mmol of 2-ethylpiperidine was dissolved in 20 mL of dichloromethane, then 20 mL (1.0 eq, 0.51 mmol) of triethylamine was added to the mixture at 0 °C. In another flask, 5.0 mmol (1.0 eq) of ethyl chlorooxoacetate were dissolved in 20 mL of dichloromethane and then was added dropwise to the previously prepared reaction mixture over a period of 30 minutes under argon (inert atmosphere). The solution was stirred for 3h at room temperature. Afterwards, the reaction mixture was washed with 1N HCl, 1N $NaHCO_3$ and brine. The organic phase was isolated and dried over $MgSO_4$ and the solvent was evaporated using a rotary evaporator to obtain the oxalamic ester.

In the next step, oxalamic ester [(±)-2-ethylpiperidine-oxo-ethyl acetate] was hydrolysed with a protease (*Aspergillus species*) to form both the oxalamic ester and acid.

In a of potassium phosphate buffer solution (27 mL, 0.1 M, pH 7.0) was dissolved 2.0 g of protease and then 3 mL (3.0 mmol) of acetonitrile was added. This mixture was given to the (±)-2-ethylpiperidine-oxo-ethyl acetate. The monitoring of this reaction was done by HPLC (high pressure liquid chromatography). After 24 h, when about 50% of conversion was reached, the reaction mixture was quenched and extracted with 30 mL of dichloromethane to obtain the organic phase which includes the oxalamic ester ((*R*)-2-ethylpiperidine-oxo-ethyl acetate).

The pH of the aqueous phase was adjusted to 4.0 and solution was extracted with dichloromethane to obtain the oxalamic acid [(*S*)-2-oxo-2-(2-propylpiperidin-1-yl)acetic acid]. Both the oxalamic acid and ester were refluxed each with 4 N HCl for 5 hours. The reaction mixture was washed with dichloromethane, the pH of the aqueous layer was adjusted to 10 and finally extracted with dichloromethane. After the evaporation of the dichloromethane, the enantiomeric amines (*R*) and (*S*) were obtained in good yields.

V. Results and Analysis:

Percentage yield$= \frac{Actual\ yield}{Theoritical\ yield} * 100$

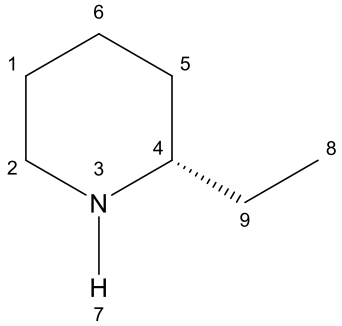
Mass of Product = $Actual\ yield$ = 0.45 g

$Theoritical\ yield$ = 0.561 g

Percentage yield = 80.21 %

- **Proton NMR Analysis**

The ^{1}H-NMR spectra for both enantiomeric products look alike.



^{1}H-NMR (300 MHz, DMSO-d$_6$): δ= 2.79 - 2.64 (m ,4H, 4-H, 2-H), 2.0 (s ,1H, 7H), 1.56 – 1.31 (m, 4H, 1-H, 5-H), 1.55 – 1.45 (m, 2H, 6-H), 1.48 (m, 2H, 9-H), 0.87 (t ,3H, 8-H) ppm. (Note: If you can state the multiplicity of the NMR signal, you are also obligated to determine the coupling constant $J_{H,H}$)

The ^{1}H-NMR spectrum is consistent with the products structure.

Furthermore, the literature discloses that this synthetic route yields product with high purity. The optical purity of the products was controlled by comparison with the commercially available pure products [1]

VI. References:

[1] Hu S., Tat D., Martinez C. A., Yazbeck D. R., Tao J., *Org. Lett.* **2005,** *7*, 4329 – 4331.

[2] Warren S., Wyatt P., *Organic Synthesis: The Disconnection Approach*, John Wiley & Sons, Inc., New York, **2008**.

[3] Shukla G., *How Stuff Works – Enzymes*, Feb 01, **2007**.

[4] Titz B., Knorr A., Sewer A., Martin F., Ivanov N.V., Talikka M., Suarez G., Peitsch M.C., Hoeng J., *Comprehensive Medicinal Chemistry III*, **2017.**

[6] Bruice P. Y., *Organic chemistry*, **2003**