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CHEMISTRY DEPARTMENT
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ORGANIC NITROGEN COMPOUNDS [H2 only]

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Topics	**Amines**	**Amides**	**Amino Acids & Proteins**
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3 Properties of Aliphatic Amines
4 Properties of Phenylamine
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7 Preparation of Phenylamine by Reduction of Nitrobenzene
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ASSESSMENT OBJECTIVES

(a) Describe the formation of amines as exemplified by ethylamine (through amide and nitrile reduction) and by phenylamine (through the reduction of nitrobenzene)

(b) Describe the reaction of amines in the formation of salts

(c) Describe and explain the basicity of primary, secondary and tertiary amines in the gaseous phase (interpret as Lewis bases)

(d) Explain the relative basicities of ammonia, ethylamine and phenylamine in aqueous medium, in terms of their structures

(e) Describe the reaction of phenylamine with aqueous bromine

(f) Describe the formation of amides from the condensation reaction between RNH_2 and R'COC*l*

(g) Explain why an amide is neutral in terms of delocalisation of the lone pair of electrons on nitrogen

(h) Describe the chemistry of amides, exemplified by the following reactions:

  (i) hydrolysis on treatment with aqueous alkali or acid

  (ii) reduction to amines with lithium aluminium hydride

(i) Describe the acid/base properties of amino acids and the formation of zwitterions

(j) Describe the formation of peptide (amide) bonds between α-amino acids, and hence explain protein formation

(k) Describe the hydrolysis of proteins

REFERENCES

1 Introduction to Organic Chemistry by GI Brown
2 Principles of Organic Chemistry by Peter RS Murray
3 Organic Chemistry by Morrison and Boyd
4 Organic Chemistry by TW Graham Solomons

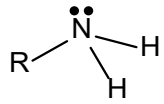
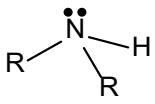
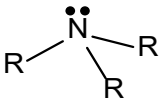
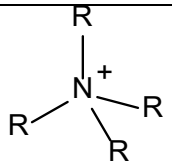
PART I: AMINES

1 Classes of Amines

☑ Amines are derivatives of NH_3. They are formed by replacing one or more hydrogen atoms by a substituent such as an alkyl or aryl group.

☑ Amines are classified into **4 groups**.

- Primary amines, RNH_2, have **one** hydrogen atom in ammonia replaced by a substituent.
- Secondary amines, R_2NH, have **two** hydrogen atoms in ammonia replaced by substituents. The two R groups can be the same or different.
- Tertiary amines, R_3N, have all **three** hydrogen atoms in ammonia replaced by substituents. The three R groups can be the same or different.
- Quaternary ammonium cations, R_4N^+, are cations with **four** substituents bonded to N. The four R groups can be the same or different.

			
1º amine	2º amine	3º amine	4º ammonium ion

2 Nomenclature of Amines

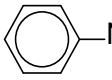
☑ Aliphatic amines are named as '**alkylamine**'

☑ Aromatic amines are named as '**phenylamine**'

☑ Quaternary ammonium cations are named as '**alkylammonium**' cations

☑ As substituents, amines are known as '**amino**'

☑ *Examples*

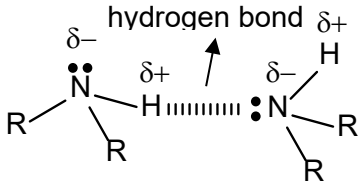
Formula	Name	Classification
$CH_3CH_2NH_2$		primary
$CH_3CH_2NHCH_3$	Ethylmethylamine *[substituents are arranged in alphabetical order]*	
$(CH_3CH_2)_3N$		tertiary
$(CH_3)_4N^+Cl^-$	tetramethylammonium chloride	
		primary
$CH_3(CH_2)_3CH(NH_2)CH_3$	2–aminohexane	
$H_2NCH_2CH_2CO_2H$		primary

3 Properties of Aliphatic Amines

Note: When comparing the boiling points of two compounds, other than the different types of intermolecular forces of attraction, it is also important to consider their relative molecular mass or molar mass. M_r or M is an indicator of the number of electrons in a molecule. A molecule with higher M_r or M has more electrons. This will lead to higher boiling points due to stronger intermolecular instantaneous dipole – induced dipole forces of attraction (**id-id**) holding the molecules in the liquid together.

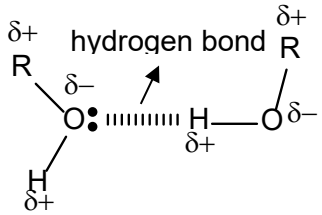
☑ Aliphatic amines have **higher** boiling points than hydrocarbons of *comparable mass* due to their ability to form intermolecular **hydrogen bonds** in addition to **id-id interactons** between the non-polar alkyl groups, whereas there are **only id-id interactions** between non–polar hydrocarbon molecules. Hence **more energy** is needed to separate amine molecules.

$CH_3CH_2CH_3$ (propane)	M_r = 44	b.p. = –42 °C
$CH_3CH_2NH_2$ (ethylamine)	M_r = 45	b.p. = 16.6 °C

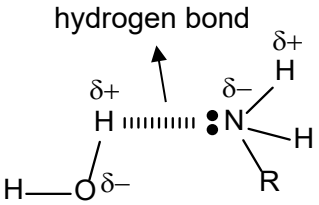


☑ Aliphatic amines have **lower** boiling points than alcohols of comparable mass. While the no. of hydrogen bonds per molecule is the same, the N–H bonds are **less polar** than O–H bonds since N is **less electronegative** than O. As such, the **hydrogen bonds** between amine molecules are **weaker** than those between alcohol molecules.

$CH_3CH_2NH_2$ (ethylamine)	M_r = 45	b.p. = 16.6 °C
CH_3CH_2OH (ethanol)	M_r = 46	b.p. = 78.5 °C



☑ Aliphatic amines are **soluble in water** as they can form **hydrogen bonds** with water molecules. Energy released can overcome the interactions between amine molecules (**hydrogen bonds and id-id interations**) and the **hydrogen bonds** between water molecules.
However, solubility **decreases** with the increase in the number of carbon atoms in the R group due to **increasing strength** of **id-id interactions** between the non-polar hydrocarbon (alkyl) chains as **number of electrons** in the molecule increass, and so **more energy** is needed to overcome the attractions between the amine molecules, leading to lower solubility.



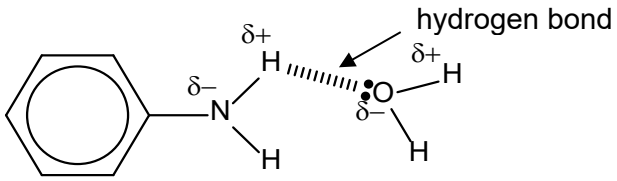
4 Properties of Phenylamine

☑ Phenylamine, also known as aniline or aminobenzene, is an aromatic primary amine. Pure phenylamine is a colourless liquid, but it darkens rapidly on exposure to light and air. It is normally a brown oily liquid.

☑ Phenylamine and methylbenzene contain similar number of electrons but phenylamine has a **higher** boiling point than methylbenzene. This is due to its ability to form **hydrogen bonds** between the amine groups in addition to **id-id** interactions between the **non-polar rings.** There are **only id-id interactions** between non–polar methylbenzene molecules. Hence **more energy** is needed to separate phenylamine molecules.

	M_r	melting point / °C	boiling point / °C
$C_6H_5NH_2$	93	–6.2	184
$C_6H_5CH_3$	92	–95	111

☑ Phenylamine is only slightly soluble in water. The energy released when phenylamine form **hydrogen bonds** with the water molecules can overcome the attractions (hydrogen bonds and id-id itneractions) between phenylamine molecules and the hydrogen bonds between water molecules. However, energy is also needed to overcome the **id-id interactions** between the large non-polar benzene ring.



Question 1 (SLS Quiz 1 / Q1)

Which of the following explain why methylamine (CH_3NH_2) is much more soluble in water than ethyl ethanoate ($CH_3CO_2CH_2CH_3$)?

1 A hydrogen bond forms between the hydrogen of the –NH group in methylamine and the hydrogen of a water molecule.

2 A hydrogen bond forms between the hydrogen of the –NH group in methylamine and the oxygen of a water molecule.

3 Methylamine is able to dissociate into hydrogen ions and methoxide ions but ethyl ethanoate is not able to dissociate.

A 1 only **B** 2 only **C** 1 & 3 **D** 2 & 3

Question 2 (SLS Quiz 1 / Q2)

Which of the following is the correct order of decreasing boiling point for compounds with comparable relative molecular mass?

A	$CH_3CH_2CH_3$	>	CH_3CH_2OH	>	$CH_3CH_2NH_2$	>	HCO_2H
B	$CH_3CH_2NH_2$	>	HCO_2H	>	CH_3CH_2OH	>	$CH_3CH_2CH_3$
C	HCO_2H	>	CH_3CH_2OH	>	$CH_3CH_2NH_2$	>	$CH_3CH_2CH_3$
D	CH_3CH_2OH	>	$CH_3CH_2NH_2$	>	HCO_2H	>	$CH_3CH_2CH_3$

5 Basic strength of Amines

Students should be able to:

- *Describe and explain the basicity of primary, secondary and tertiary amines in the gaseous phase (interpret as Lewis bases)*
- *Explain the relative basicities of ammonia, ethylamine and phenylamine in aqueous medium, in terms of their structures*

☑ Amines are bases. Basic strength of amines can be compared in 2 ways:

(1) ***availability of lone pair of electrons*** on the nitrogen [Recall: *Electron pair donors are Lewis bases*.]

(2) ***stability of the conjugate acid*** (protonated amine or alkylammonium ion) formed.

$$RNH_2 + H_2O \rightleftharpoons RNH_3^+ + OH^-$$

Lone pair of electrons on N is more available
=> amine is more basic

Protonated amine is more stable
=> more easily formed, so amine is more basic

Factors affecting the **availability of lone pair of electrons** on N for donation to an acid.

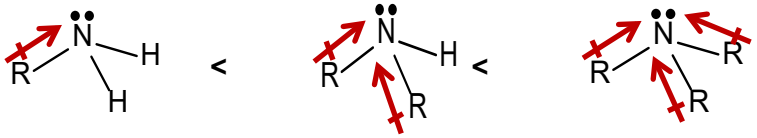
(1) Inductive effect of substituents on N:
Alkyl groups on N will exert electron–donating inductive effect thus increasing availability of electron pair.

(2) Delocalisation of electron pair on N:
Aryl groups on N will make the lone pair of electrons on N less available for donation.

(3) Steric hindrance:
Bulky groups on N can hinder donation of the lone pair of electrons on N to the approaching H^+ ion, hence making it a weaker base.

Note: It is importatnt to note the medium when comparing basicity. (See case 4)

Case 1
Relative basicity of alkylmines (aplihatic amines) in gaseous phase: **1° amine < 2° amine < 3° amine**

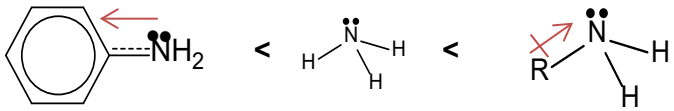


Why is this so?

- Basic strength increases with the **availability of the lone pair of electrons** on N for donation.
- Alkylamines contain alkyl groups which exert an **electron–donating inductive effect** that will **increase the availability of the lone pair of electrons** on N for donation to an acid.
  Hence, the **greater the number of alkyl groups**, the **more basic** will be the alkylamine.

Case 2

Relative basicity: **phenylamine (1° amine) < NH_3 < ethylamine (1° amine)**

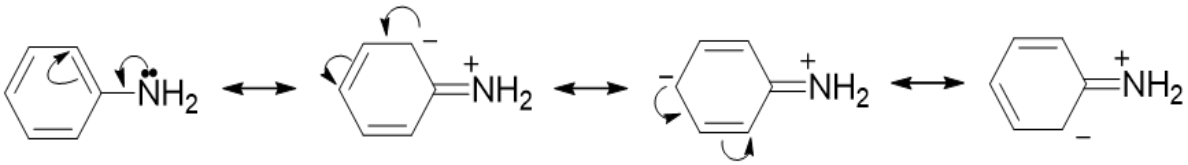


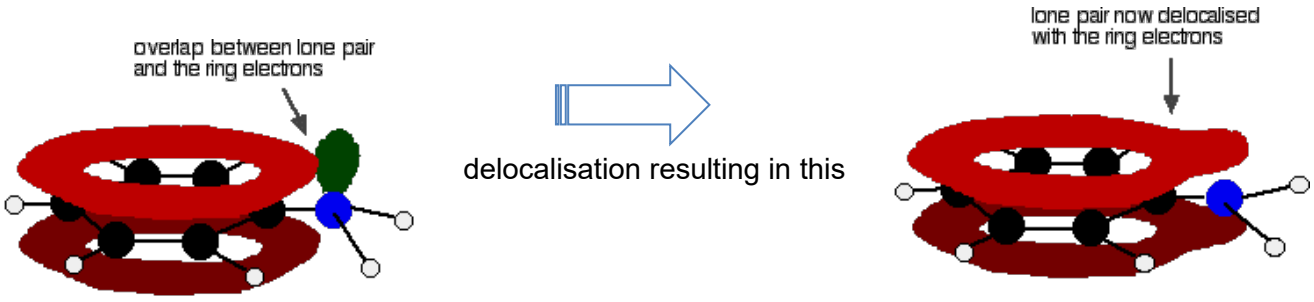
Why is this so?

- Unlike NH_3, ethylamine contains ethyl group which exerts an **electron–donating inductive effect** that will **increase the availability of the lone pair of electrons** on N for donation to an acid.
  Hence, ethylamine is **more basic** than NH_3.

- Phenylamine is a **weaker** base than NH_3 as the p orbital containing the lone pair of electrons on N overlaps with the π orbitals of the benzene ring, leading to the **delocalisation of the lone pair of electrons** into the benzene ring (also known as **mesomeric or resonance** effect). This renders them **less available** for donation to a proton.

  Electron pair flow illustration:





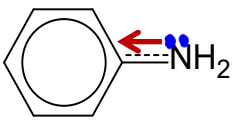
Before delocalisation of lone pair, N is tetrahedral, sp^3 hybridised.

After delocalisation of lone pair, N is trigonal planar, sp^2 hybridised.

Note: In phenylamine, N is **sp^2 hybridised** as this will allow the lone pair of electrons to be accommodated in a **p orbital** which can overlap **more effectively** with **π (or p) orbitals** of the benzene ring.

Extra information:

There are **3 effects caused by the delocalisation** of the lone pair of electrons on N into the benzene ring system:

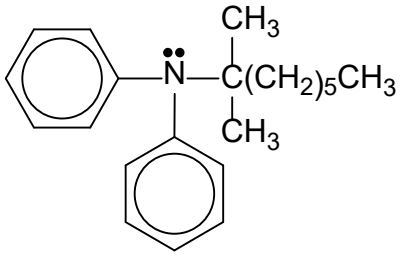


i) Makes phenylamine a **weaker base** since availability of the lone pair of electrons on N is reduced.

ii) Makes phenylamine a **weaker nucleophile** since the lone pair of electrons is less available.

iii) **Increases the electron density of the benzene ring** making the ring much more reactive than benzene itself in attracting electrophiles. Hence, phenylamine can undergo electrophilic substitution more readily than benzene. Reaction does not need lewis acid catalyst.

Case 3 (Steric effect of substituents on N)



is a very weak base.

Why is this so?

- The 3 **bulky groups** on N atom will hinder donation of its lone pair of electrons to the approaching acid causing it to be a very weak base.

Case 4

*pK_b of some bases in **aqueous medium:***

Amines	***pK*$_b$**
3^o trimethylamine, $(CH_3)_3N$	4.2
1^o methylamine, CH_3NH_2	3.34
2^o dimethylamine, $(CH_3)_2NH$	3.27

Relative basicity of the 3 amines in aqueous phase (compare to case 1):

Trimethylamine (**3°**) **<** **Methylamine** (**1°**) **<** **Dimethylamine** (**2°**)

Why is this so?

- Dimethylamine has **more methyl groups** than methylamine. Methyl group exerts an **electron–donating inductive effect** that will **increase the availability of the lone pair of electrons** on N for donation to an acid.
  Hence, dimethylamine is **more basic** than methylamine.

- Methylammmonium ion formed has **more N-H bonds** than trimethylammonium ion. Hence methylammonium ion can form **more hydrogen bonds** with water molecules (more **hydrated or solvated**) leading to greater stability, and so it is more easily formed than trimethylammonium ion. So methylamine is **more basic** than trimethylamine.

  $\mathbf{RNH_2 + H_2O \rightleftharpoons RNH_3^+ + OH^-}$
  $\mathbf{R_3N + H_2O \rightleftharpoons R_3NH^+ + OH^-}$

Question 3 (SLS Quiz 2 / Q1)
Which of the following is a weaker base than ammonia?

A $(CH_3)_2NH$ **B** CH_3NH_2 **C** $CH_3CH_2NH_2$ **D** $C_6H_5NH_2$

Question 4 (SLS Quiz 2 / Q2)
Which of the following explain why trimethylamine ($(CH_3)_3N$) is more basic than methylamine (CH_3NH_2) in gaseous phase but in aqueous solution, it is less basic than methylamine.

1. In gas phase, trimethylamine is a better electron pair donor than methylamine as it has more alkyl groups leading to greater electron–donating inductive effect.

2. In aqueous solution, the $(CH_3)_3NH^+$ ion formed is less stable than $CH_3NH_3^+$ ion as it has fewer N–H bonds available for hydrogen bonding with water molecules.

3. $(CH_3)_3NH^+$ ion is less solvated than $CH_3NH_3^+$ ion in gaseous phase.

A 1 **B** 1 & 2 **C** 2 & 3 **D** 1, 2 & 3

6 Preparation of Aliphatic Amines

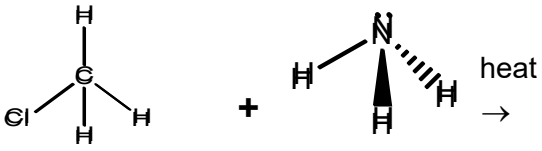
Students should be able to:
- *Describe the formation of amines as exemplified by ethylamine (through amide and nitrile reduction) and by phenylamine (through the reduction of nitrobenzene)*

6.1 Nucleophilic Substitution of Halogenoalkanes (RX)

☑ Reagents & conditions: **alcoholic NH_3** ; **heat** in a **sealed tube**

Note:
*The reaction mixture is carried out in a sealed tube even during synthesis of the amines and should **not be heated under reflux** as the ammonia would escape from the condenser as a gas.*

☑ *Examples*



$CH_3C\mathit{l}$ + NH_3 → $CH_3\mathbf{NH_2}$ + $HC\mathit{l}$

Chloromethane **Methylamine**, 1° amine

$CH_3C\mathit{l} + CH_3NH_2 \rightarrow (CH_3)_2\mathbf{NH} + HC\mathit{l}$

Dimethylamine, 2° amine

$CH_3C\mathit{l} + (CH_3)_2NH \rightarrow (CH_3)_3\mathbf{N} + HC\mathit{l}$

Trimethylamine, 3° amine

$CH_3C\mathit{l} + (CH_3)_3N \rightarrow (CH_3)_4\mathbf{N^+}C\mathit{l}^-$

Tetramethylammonium chloride, quaternary ammonium salt

Note:

- Using **excess NH_3** gives rise to **primary amines**.

- In reality, the amines produced will further react with HCl produced to form **alkylammonium** salts.
  Hence during preparation, a **strong base** such as NaOH is further added to the alkylammonium salt to obtain the amine.

  $RNH_2 + HCl \rightarrow RNH_3^+Cl^-$ alkylammonium chloride
  $RNH_3^+ + OH^- \rightarrow RNH_2 + H_2O$

- Using **excess halogenoalkane** gives rise to **higher order amines.**

6.2 Reduction of Amides

☑ Reagents & conditions: lithium aluminum hydride, **$LiAlH_4$ in dry ether**

Note:
- $NaBH_4$ **cannot** reduce amides.
- Catalytic H_2 is also **not** used.
- Lithium alumunium hydride, $LiAlH_4$ will react explosively with water or moisture to produce hydrogen gas.
  $LiAlH_4 + 4H_2O \rightarrow LiOH + Al(OH)_3 + 4H_2$

☑ *Example*

CH_3**CONH$_2$** + 4[H] $\rightarrow$ CH_3**CH_2NH_2** + H_2O

Note: A 2º amide & 3º amide will produce a 2º amine & 3º amine respectively.

6.3 Reduction of Nitriles (only 1º amines can be formed via this method)

☑ Reducing agents & conditions:
- **$LiAlH_4$ in dry ether**
- **H_2, Pt catalyst**
- **H_2, Ni catalyst, heat**

Note: $NaBH_4$ **cannot** reduce nitriles as it is too weak to do so.

☑ *Examples*

$CH_3C{\equiv}N + 4[H] \rightarrow CH_3CH_2NH_2$

Ethanenitrile **Ethylamine**

$CH_3CH_2C{\equiv}N + 4[H] \rightarrow CH_3CH_2CH_2NH_2$

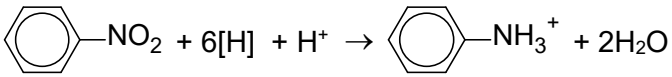
Propanenitrile **Propylamine**

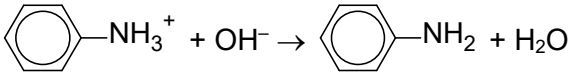
7 Preparation of Phenylamine by Reduction of Nitrobenzene

Students should be able to:
- *Describe the formation of amines as exemplified by ethylamine (through amide and nitrile reduction) and by phenylamine (through the reduction of nitrobenzene)*

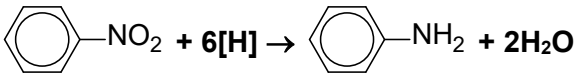
☑ Reagents & conditions:
Sn and concentrated HC*l*; heat followed by **addition of alkali** (such as NaOH)

Note: The phenylamine formed will react with acidic medium to form a salt. The addition of alkali is to form the amine from its salt.





Overall equation:



OR

[R] half-eqn: $C_6H_5NO_2 + 6H^+ + 6e^- \rightarrow C_6H_5NH_2 + 2H_2O$
[O] half-eqn: $3Sn \rightarrow 3Sn^{2+} + 6e^-$
Overall: $C_6H_5NO_2 + 6H^+ + 3Sn \rightarrow C_6H_5NH_2 + 2H_2O + 3Sn^{2+}$

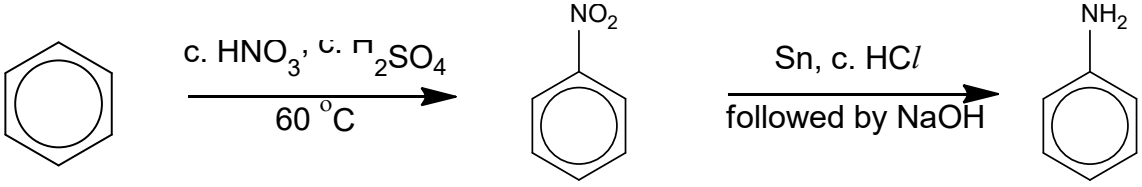
Note: Other less commonly used reduction methods include treatment with concentrated HC*l* and Zn, Fe or $SnCl_2$ under suitable conditions.

> Use the preferred ***Sn with concentrated hydrochloric acid*** when answering questions!

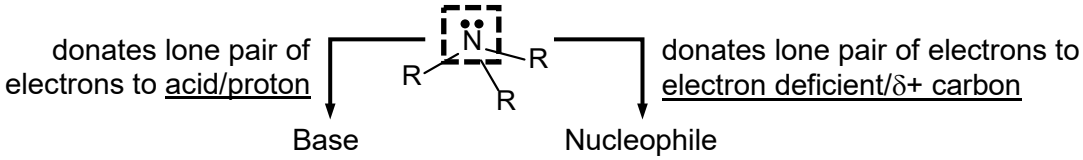
Question 5 (SLS Quiz 3 / Q1)
Nucleophilic substitution of a halogenoalkane with alcoholic KCN produced a nitrile of formula C_3H_5N. Which one of the following compounds would be produced if the same halogenoalkane was heated with excess alcoholic ammonia?

A CH_3NH_2 **B** $CH_3CH_2NH_2$ **C** $CH_3(CH_2)_2NH_2$ **D** $(CH_3CH_2)_2NH$

Question 6 (SLS Quiz 3 / Q2)
Which of the following would be used in a school laboratory to convert benzene to phenylamine?
A dilute HNO_3 followed by iron and concentrated nitric acid
B concentrated HNO_3 followed by tin and concentrated hydrochloric acid
C concentrated HNO_3 and concentrated H_2SO_4 followed by tin and concentrated hydrochloric acid
D concentrated HNO_3 and concentrated H_2SO_4 followed by tin and concentrated hydrochloric acid, and finally treatment with dilute NaOH



8 Reactions of Aliphatic Amines and Phenylamine



8.1 Neutralisation

Students should be able to:
- *Describe the reaction of amines in the formation of salts*

☑ Reagents & conditions: Any acids such as **HC*l*** or **CH_3CO_2H**

☑ *Examples*

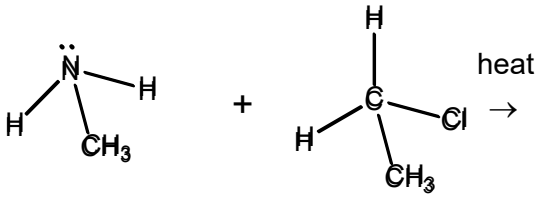
$CH_3NH_2 + HBr \rightarrow CH_3NH_3^+Br^-$
methylammonium bromide

$C_6H_5NH_2 + CH_3CO_2H \rightarrow C_6H_5NH_3^+CH_3CO_2^-$
phenylammonium ethanoate

8.2 Nucleophilic Substitution of Halogenoalkanes

☑ Reagents & conditions: **RX**; **heat**

☑ *Examples*



$CH_3NH_2 + CH_3CH_2C\mathit{l} \rightarrow CH_3NH(CH_2CH_3) + HC\mathit{l}$
ethylmethylamine

$C_6H_5NH_2 + CH_3CH_2C\mathit{l} \rightarrow C_6H_5NH(CH_2CH_3) + HC\mathit{l}$
ethylphenylamine

Note: To get 2º or 3º amine, a 1º or 2º amine is reacted with a RX respectively

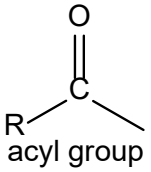
8.3 Condensation Reaction with Acyl / Acid Chloride

Students should be able to:
- *Describe the formation of amides from the condensation reaction between RNH_2 and R'COCl*

☑ A condensation reaction is a reaction in which two molecules combine to form a single molecule with loss of a small molecule such as water, H*Cl*, etc.

☑ In this reaction, amines act as nucleophiles that attack the electron-deficient carbon.

☑ In organic chemistry, the acyl group is usually derived from a carboxylic acid (IUPAC name: alkanoyl). Therefore, it has the formula RCO–, where R represents an alkyl group attached to the CO group with a single bond.

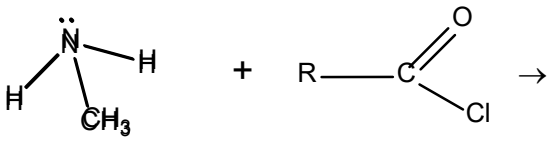


acyl group

☑ Reagents & conditions: **RCOC*l*** *(acyl or acid chloride)*

Note: heating is ***not*** required as acid chlorides undergo nucleophilic substitution and hydrolysis easily.

☑ *Examples*



CH_3NH_2 + CH_3COCl → $CH_3CONHCH_3$ + HC*l*
methylamine *ethanoyl chloride* *N–methylethanamide*

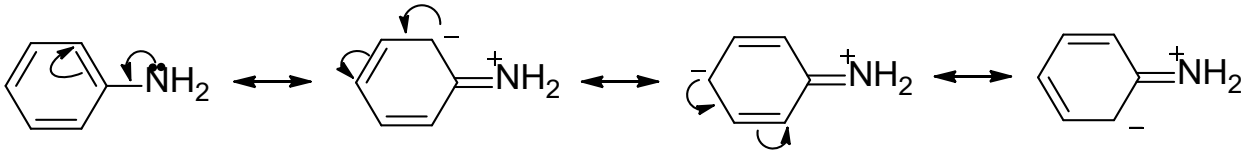
$C_6H_5NH_2$ + CH_3COCl → $CH_3CONHC_6H_5$ + HC*l*
N–phenylethanamide

9 Electrophilic Substitution of Phenylamine

Students should be able to:
- *Describe the reaction of phenylamine with aqueous bromine*

☑ Phenylamines tend to undergo **electrophilic substitution** more readily than benzene due to delocalisation of lone pair of electrons on N into the benzene ring, making it **richer** in electrons and thus more susceptible to electrophilic attack. This delocalisation is possible because of the overlap of p–orbital on N with the π orbitals of the ring.

☑ $–NH_2$ group is said to be an **activating** group. $–NH_2$ group directs substitution at 2– and 4– positions.



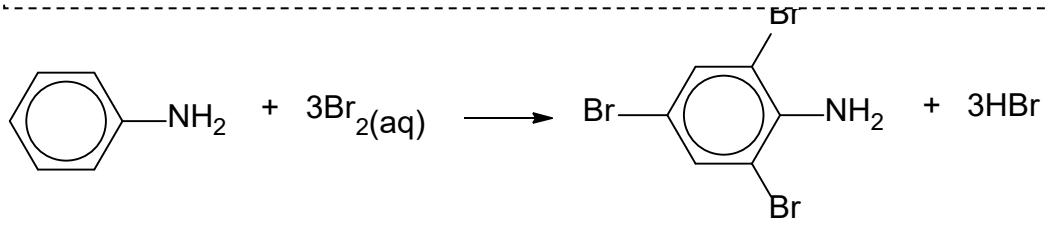
☑ **No Lewis acid catalyst** is needed and **tri–substitution** takes place readily with aqueous bromine or chlorine at room temperature.

☑ Reagents & conditions: **Br_2(aq)**

Note: No lewis acid catalyst and no heat is needed

Observations: *Decolourisation of orange $Br_2(aq)$* with formation of a *white precipitate* (2,4,6–tribromophenylamine)

P/S: This reaction is used as a ***test for phenylamine***. (Phenol reacts with aqueous bromine in the same way!)

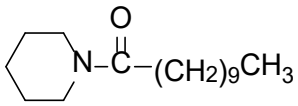


orange solution ***white precipitate***

Question 7 (SLS Quiz 4 / Q1)
When phenylamine is treated with aqueous hydrochloric acid and the solution is then evaporated, a white solid is obtained. What is the formula of the white solid?

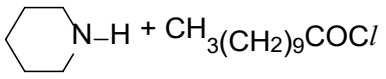
A $C_6H_5N_2^+Cl^-$ **B** C_6H_5Cl **C** C_6H_5NHCl **D** $C_6H_5NH_3^+Cl^-$

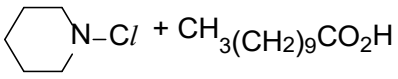
Question 8 (SLS Quiz 4 / Q2) (N13/I/29)
Acylpiperidines such as compound **X** are being studied as mosquito repellents.

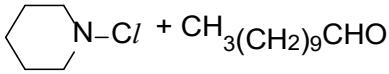


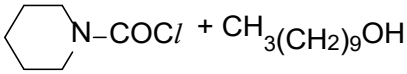
X

Which pair of compounds would produce **X** when reacted together?

A 

B 

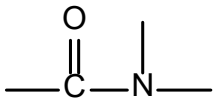
C 

D 

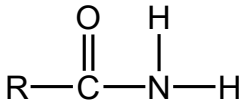
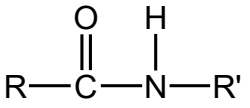
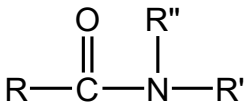
PART II: AMIDES

1 Classes of Amides

☑ Amides have the following functional group:



☑ Amides are classified as primary amides ($RCONH_2$), secondary amides (RCONHR') and tertiary amides (RCONR'R''). R, R' and R'' can be the same or different.

		
Primary amide	Secondary amide	Tertiary amide

2 Nomenclature of Amides

☑ Amides are named as 'alkanamides'.

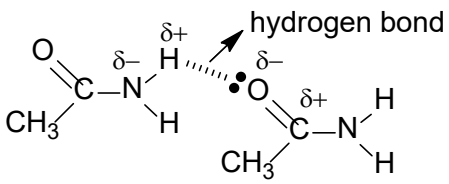
☑ *Examples*

Formula	Name	Classification
$HCONH_2$	methanamide	primary
CH_3CONH_2		
$CH_3CH_2CONH_2$	propanamide	primary
$CH_3CH_2CONHCH_3$		
$CH_3CH_2CON(CH_3)_2$	N,N–dimethylpropanamide	tertiary

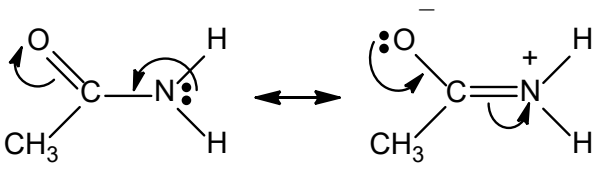
3 Properties of Amides

Students should be able to:
- *Explain why an amide is neutral in terms of the delocalisation of the lone pair of electrons on nitrogen*

☑ Amides have **relatively high** melting points and boiling points due to **intermolecular hydrogen bonds** formed between the lone pair of electrons on oxygen atom and the highly electron–deficient hydrogen atoms directly bonded to nitrogen atom.



☑ Unlike amines, amides are **not basic**. The lone pair of electrons on N is **unavailable** for donation as it is **delocalised** through interaction with π electrons of the C=O bond.



☑ Amides are **neutral** compounds.

4 Preparation of Amides by Condensation Reaction between Amines and Acyl Chorides

☑ Reagents & conditions: **concentrated NH_3** or **RNH_2** or **R_2NH**

☑ *Examples*

CH_3COCl + NH_3 → CH_3CONH_2 + HCl
ethanoyl chloride *ethanamide*

CH_3CH_2COCl + CH_3NH_2 → $CH_3CH_2CONHCH_3$ + HCl
propanoyl chloride *N–methylpropanamide*

CH_3COCl + $(CH_3)_2NH$ → $CH_3CON(CH_3)_2$ + HCl
N,N–dimethylethanamide

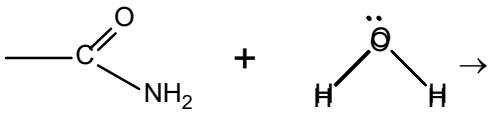
☑ 3° amines **cannot** condense with acyl chlorides as there is no H bonded to the N for condensation to occur.

5. Reactions of Amides

Students should be able to:
- *Describe the chemistry of amides, exemplified by the hydrolysis on treatment with aqueous alkali or acid*

5.1 Hydrolysis

☑ Hydrolysis is a reaction involving the splitting of water molecule. The amide bond is also split during the reaction with water.



☑ Acid or base has to be added to catalyse the reaction as water is a weak nucleophile.

5.1a Acid Hydrolysis

☑ Reagents & conditions: Any dilute acids such as **HCl(aq)** or **H_2SO_4(aq); heat**

☑ The products of acid hydrolysis of amide are <u>*carboxylic acid*</u> and <u>*ammonium salt*</u>.

☑ *Examples*

$CH_3CONH_2 + H_2O + H^+ \rightarrow CH_3CO_2H + NH_4^+$

$CH_3CONHCH_3 + H_2O + H^+ \rightarrow CH_3CO_2H + CH_3NH_3^+$

$CH_3CON(CH_3)_2 + H_2O + HCl \rightarrow CH_3CO_2H + (CH_3)_2NH_2^+Cl^-$

<u>Note</u>:
- The catalyst H^+ is consumed at the end of the reaction due to further acid-base reaction with basic NH_3 or amine produced.
- Must specify **(aq)** or **dilute** as <u>water</u> is the **<u>key reactant</u>** for hydrolysis

5.1b Base Hydrolysis

☑ Reagents & conditions: Any dilute alkalis such as **NaOH(aq)** ; **heat**

☑ The products of base hydrolysis of amide are *carboxylate salt* and *ammonia / amine*.

☑ *Examples*

***** $CH_3CONH_2 + OH^- + H_2O \rightarrow CH_3CO_2^- + H_2O + \mathbf{NH_3}$

$CH_3CONHCH_3 + OH^- \rightarrow CH_3CO_2^- + CH_3NH_2$

$CH_3CON(CH_3)_2 + NaOH \rightarrow CH_3CO_2^- Na^+ + (CH_3)_2NH$

*****This reaction is used as a ***test for primary amide*** as it produces **pungent alkaline NH_3** gas which turns **moist red litmus blue**.

Note:
- The catalyst OH^- is consumed at the end of the reaction due to further acid-base reaction with carboxylic acid produced forming salt and H_2O
- H_2O is ***cancelled out*** in the overall equation

5.3 Reduction

Students should be able to:
- *Describe the chemistry of amides, exemplified by the reduction to amines with lithium aluminium hydride*

☑ Reagents & conditions: **LiA/H_4 in dry ether**

Note:
1) $NaBH_4$ is ***not*** strong enough to reduce amides.
2) H_2 is also ***not*** used to reduce amides.

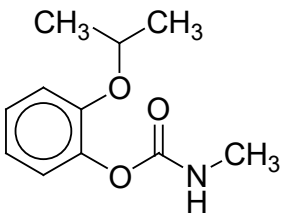
☑ *Examples*

$CH_3CONH_2 + 4[H] \rightarrow CH_3CH_2NH_2 + H_2O$
ethylamine

$CH_3CH_2CONH_2 + 4[H] \rightarrow$ ……

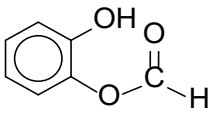
Question 9 (SLS Quiz 5 / Q1)

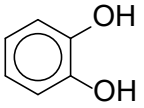
Propoxur is one of the active agents in the commercial insecticide, Baygon.

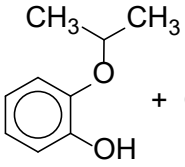


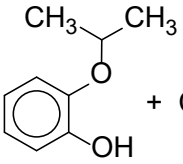
Propoxur

What are the products when it is heated with an excess of aqueous hydrochloric acid?

A  + $(CH_3)_2CHOH$ + $CH_3NH_3^+Cl^-$

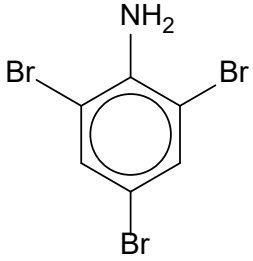
B  + $(CH_3)_2CHOH$ + CH_3NHCO_2H

C  + CO_2 + $CH_3NH_3^+Cl^-$

D  + CH_3NHCO_2H

SUMMARY

Reactions of Amines, Phenylamines and Amides by Reagents

Reactant	Reagents	Conditions	Reaction Type	Products
CH_3NH_2	HC*l*		neutralisation	$CH_3NH_3^+Cl^-$
	RX	heat	nucleophilic substitution	$CH_3NHR + HX$
	RCOC*l*		condensation	$RCONHCH_3 + HCl$
$C_6H_5NH_2$	HC*l*		neutralisation	$C_6H_5NH_3^+Cl^-$
	RX	heat	nucleophilic substitution	$C_6H_5NHR + HX$
	RCOC*l*		condensation	$RCONHC_6H_5 + HCl$
	Br_2(aq)		electrophilic substitution	 + HBr
$RCONH_2$	HC*l*(aq)	heat	acid hydrolysis	$RCO_2H + NH_4Cl$
	NaOH(aq)	heat	base hydrolysis	$RCO_2^-Na^+ + NH_3$
	$LiAlH_4$ in dry ether		reduction	$RCH_2NH_2 + H_2O$
$RCONR'_2$	HC*l*(aq)	heat	acid hydrolysis	$RCO_2H + R'_2NH_2^+Cl^-$
	NaOH(aq)	heat	base hydrolysis	$RCO_2^-Na^+ + R'_2NH$
	$LiAlH_4$ in dry ether		reduction	$RCH_2NR'_2 + H_2O$

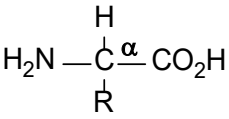
PART III: AMINO ACIDS AND PROTEINS

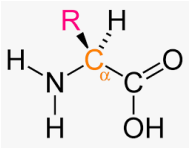
1 Structure and Nomenclature

☑ Amino acids are organic compounds that contain both an **acidic** carboxyl group, **($-CO_2H$)** and a **basic** amine group (**$-NH_2$**).

☑ Amino acids are the basic building blocks of proteins and there are a total of **20** naturally occurring **α-amino acids** (i.e. 2-aminocarboxylic acid) which make up all proteins.

☑ In α-amino acids, both functional groups are attached to the same carbon atom, known as the **α-carbon**.





☑ With the exception of glycine (where the R group is an H atom), all α-amino acids contain at least a chiral carbon (ie the α-carbon) and consequently are optically active.

🔍 **Fun Fact:**

Humans can produce 10 of the 20 amino acids. These are alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine. The others must be supplied in the food. Since the human body does not store excess amino acids for later use, unlike fat and starch, the amino acids must be in the food every day. Ingested proteins are hydrolysed into amino acids by enzymes in the digestive system.

---

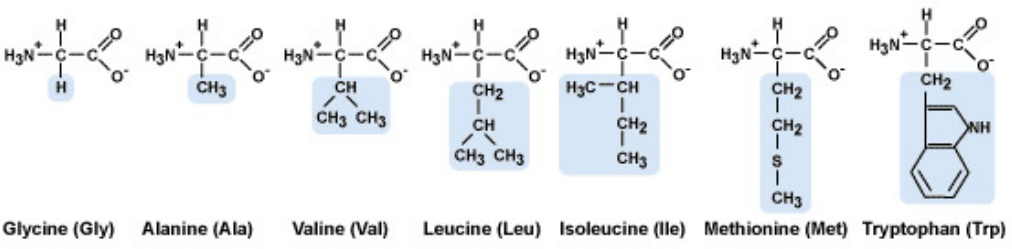
2 Classification of Amino Acids

☑ The **20** naturally occurring amino acids can be classified into **4 groups** according to the **nature** of their **R (alkyl) group**.

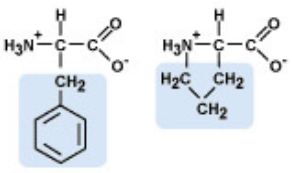
a. **Neutral** amino acids with **non-polar (hydrophobic)** R groups

These amino acids have aliphatic or cyclic hydrocarbons, or aromatic R groups which are hydrophobic (dislike water).

NONPOLAR



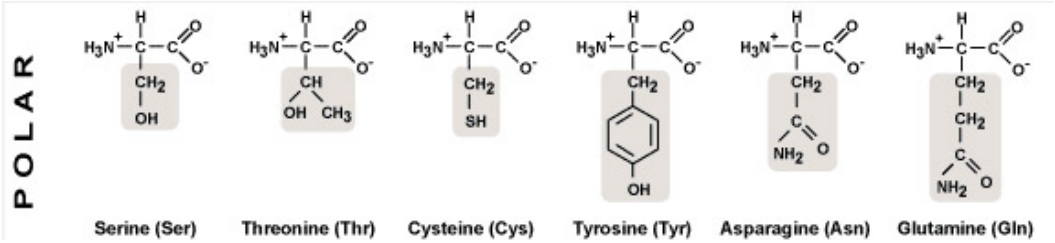
Glycine (Gly) Alanine (Ala) Valine (Val) Leucine (Leu) Isoleucine (Ile) Methionine (Met) Tryptophan (Trp)



Phenylalanine (Phe) Proline (Pro)

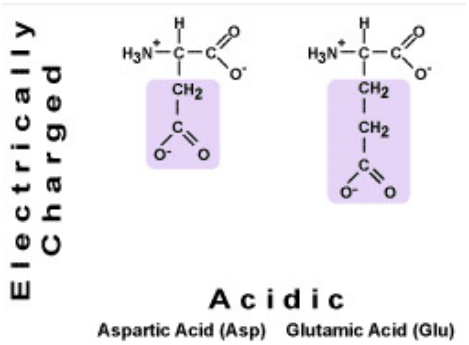
b. **Neutral** amino acids with **polar (hydrophilic; like water)** R groups

These amino acids have a variety of functional groups in the R groups but all have at least one heteroatom (N, O or S). All these amino acids, except cysteine, can form intermolecular hydrogen bonding.



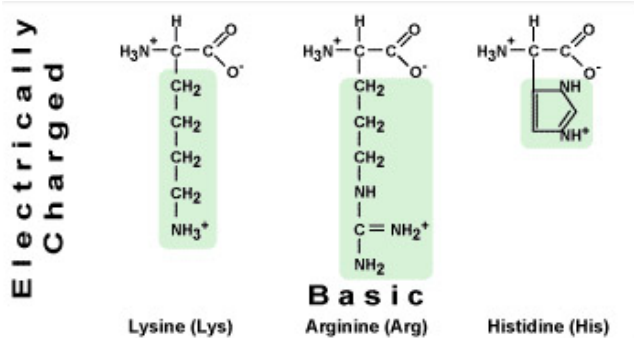
c. **Acidic** amino acids with **acidic / anionic** R groups at physiological pH (pH ≈ 7.4)

These amino acids contain acidic R groups which will be deprotonated at physiological pH.



d. **Basic** amino acids with **basic / cationic** R groups at physiological pH (pH ≈ 7.4)

These amino acids contain basic R groups which are protonated at physiological pH.

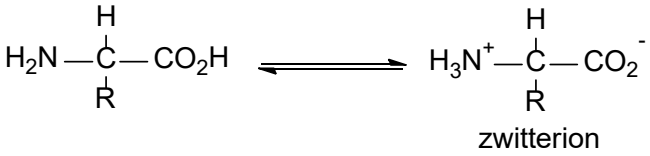


3 Acid-Base Nature of Amino Acids

Students should be able to:
- *Describe the acid/base properties of amino acids and the formation of zwitterions*

3.1 Formation of Zwitterions and their Physical Properties

☑ Amino acids are **amphoteric** as they contain **acidic –CO_2H** and **basic –NH_2** groups which can react with each other to form **zwitterions**.



zwitterion

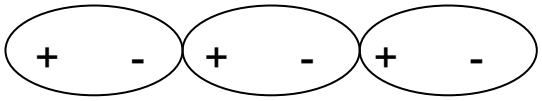
☑ A zwitterion is a **dipolar ion** with **a positive and a negative charge**, and is thus **electrically neutral**.

☑ Zwitterions are formed when the basic –NH_2 undergoes **internal acid-base reaction** with the acidic –CO_2H group.

☑ Solid amino acids contain zwitterions. Hence they are

(1) crystalline solids with **high melting points**.

↳ They have **giant ionic structures** containing dipolar ions held together by **strong electrostatic forces of attraction (ionic bonds)** between oppositely charged groups. A lot of energy is needed to separate the dipolar ions leading to a high melting point.



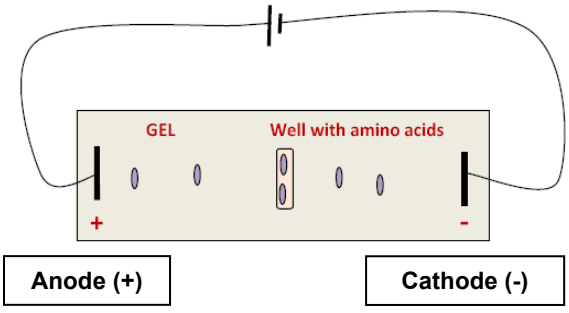
(2) **soluble in water** but **insoluble in non-polar solvents**.

↳ The ionic nature of amino acids allows it to attract water molecules strongly through **ion-dipole interactions**, thus releasing large amounts of hydration energy to overcome the **ionic bonds** between amino acids and the **hydrogen bonds** between water molecules.

↳ Amino acids are insoluble in non-polar solvents as the energy released from **ion-induced dipole attractions** between the *dipolar ions* and non-polar solvent molecules is insufficient to overcome the **ionic bonds** between the dipolar ions.

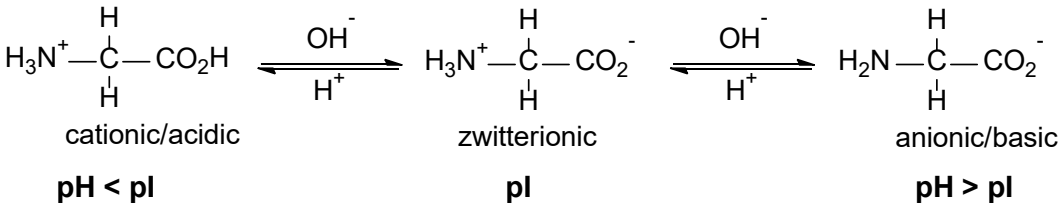
3.2 Acid-Base Properties

☑ ***Gel Electrophoresis*** is a technique for the separation of a mixture of amino acids using an electric current. In the presence of an electric current, the amino acids will migrate towards the electrodes and the rate of movement is inversely proportional to the mass of the amino acid.



☑ Depending on the **pH** of the aqueous medium, amino acids can exist as **cationic, zwitterionic or anionic.**

↳ E.g. aminoethanoic acid (Glycine)

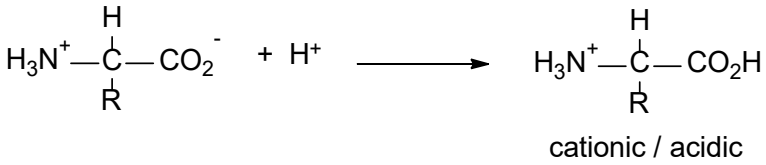


☑ The **pH** at which all amino acids exist as the **zwitterions** is called the **<u>isoelectric point</u> (pI)**.

At **pI**, the amino acids will **not** migrate in the presence of an electric field as they are **electrically neutral**.

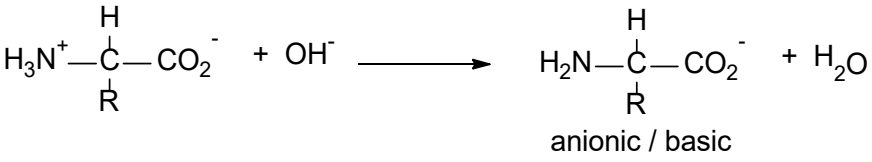
☑ When **pH < pI** of an amino acid, its weakly basic $-CO_2^-$ group (along with the basic R-group if any) will react with the H^+ ions in the medium to form **acidic cations**.

So, the **cationic amino acid** will migrate towards the **cathode (negative electrode)** in an electric field.



☑ When **pH > pI** of an amino acid, its weakly acidic $-NH_3^+$ group (along with the acidic R-group if any) will react with the OH^- ions in the medium to form the **basic anions.**

So, the **anionic amino acid** will migrate towards the **anode (positive electrode)** in an electric field.



☑ Each amino acid has a characteristic isoelectric point. The isoelectric point can be found from its titration curve. It is related to the nature of its R group too.

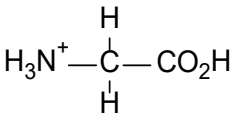
↳ <u>Amino acids with **acidic** R groups</u>
pI < 7 so that only one acidic group is deprotonated for the amino acid to be zwitterionic.
E.g. aspartic acid = 3.0, glutamic acid = 3.2

↳ <u>Amino acid with **basic** R groups</u>
pI > 7 so that only one basic group is protonated for the amino acid to be zwitterionic.
E.g. lysin = 9.7, arginine = 10.8

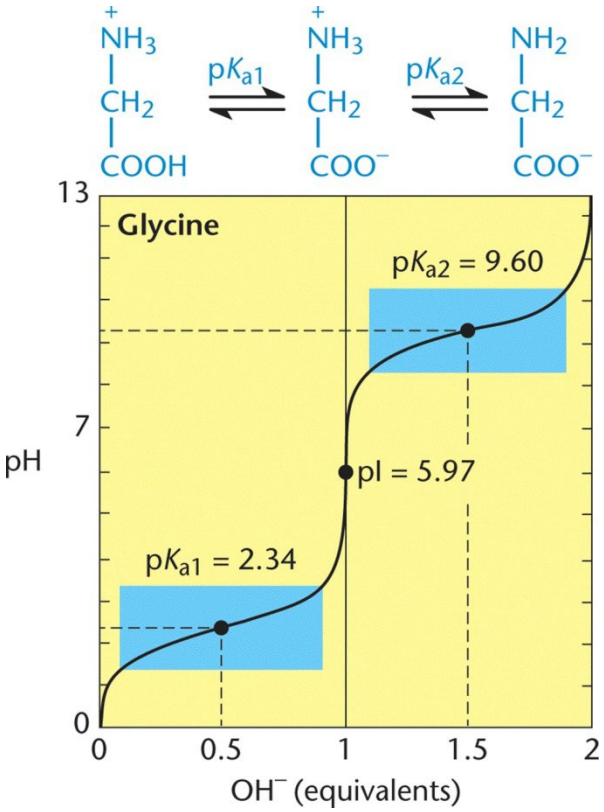
↳ <u>Amino acids with **neutral** R groups</u>
pI ≈ 7
E.g. glycine = 6.0, alanine = 6.0

3.2.1 Titration Curve of a Fully Protonated Neutral Amino Acid, Glycine $H_3N^+CH_2CO_2H$

☑ A fully protonated glycine, **Gly+** is a **dibasic** acid as it has 2 acidic groups, the **α-carboxylic acid** and the **protonated α-amine**.



☑ The graph below shows the titration curve when aqueous NaOH is added to a solution of $H_3N^+CH_2COOH$ of the same molar concentration.



☑ There are **two equivalence points** in the titration curve since a fully protonated glycine is dibasic.

Consider a weak acid, **HA**

$$HA(aq) \rightleftharpoons H^+(aq) + A^-(aq)$$

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

At mid-way of titration, $[HA] = [A^-]$

$\Rightarrow K_a = [H^+]$

$\Rightarrow -\lg K_a = -\lg [H^+]$

⇨ **pK_a = pH**

Hence, **at <u>mid-way</u> of titration pK_a = pH**

☑ Which of the two acidic groups in the fully protonated glycine is the stronger acid, i.e. has a lower pK_a value?

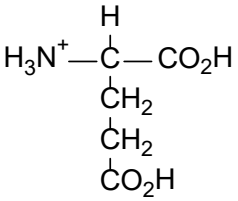
↳ Addition of OH^- to the fully protonated glycine led to **neutralisation of the α-carboxylic acid** rather than the protonated α-amine.

↳ Hence **the <u>α-carboxylic acid</u>** is **more acidic** than the **protonated α-amine,** and has a lower pK_a value.

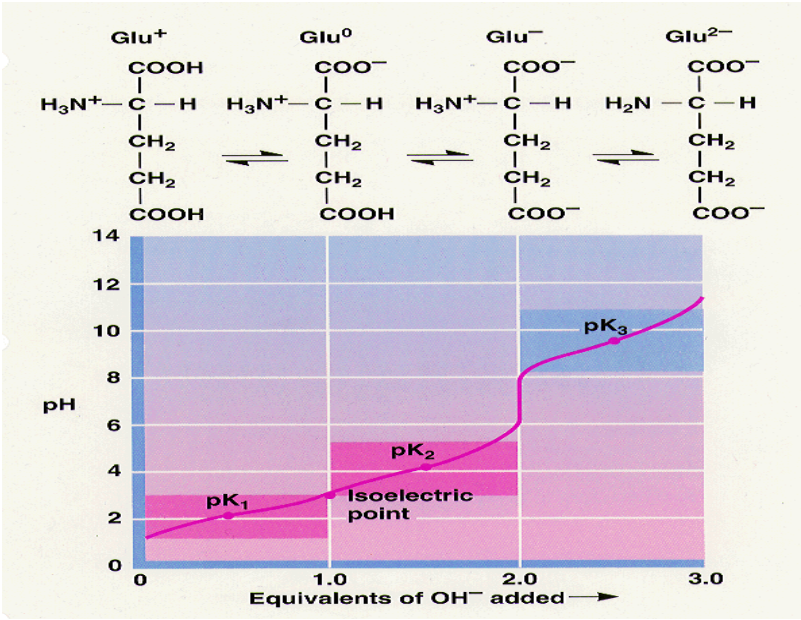
↳ The α-carboxylic acid is more acidic than protonated α-amine due to **resonance stabilisation** of the carboxylate ion produced which will not be exhibited by amine.

3.2.2 Titration Curve of a Fully Protonated Acidic Amino Acid, Glutamic Acid $H_3N^+CH(CH_2CH_2CO_2H)CO_2H$

☑ A fully protonated glutamic acid, **Glu^+** is a **tribasic** acid as it has 3 acidic groups. The 3 acidic groups are the **α-carboxylic acid**, the **alkyl-carboxylic acid** and the **protonated α-amine**.

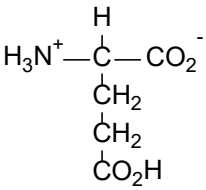


☑ The graph below shows the titration curve when aqueous NaOH is added to a solution of Glu^+ of the same molar concentration.



☑ Based on the titration curve, arrange the 3 acidic groups in order of decreasing K_a value:

↳ **α-carboxylic acid > alkyl-carboxylic acid > protonated α-amine**



↳ **Carboxylic acids** are **more acidic** than **protonated amines** due to resonance stabilisation of the carboxylate ions produced.

↳ The α-carboxylic acid is **more acidic** than the alkyl-carboxylic acid as it is **closer** to the **positive protonated α-amine** which exerts an **electron-withdrawing inductive effect**, leading to **dispersal** of negative charge on the carboxylate ion produced. Hence it is easier to be formed as it is more stable.

☑ As expected, there are **3** equivalence points.

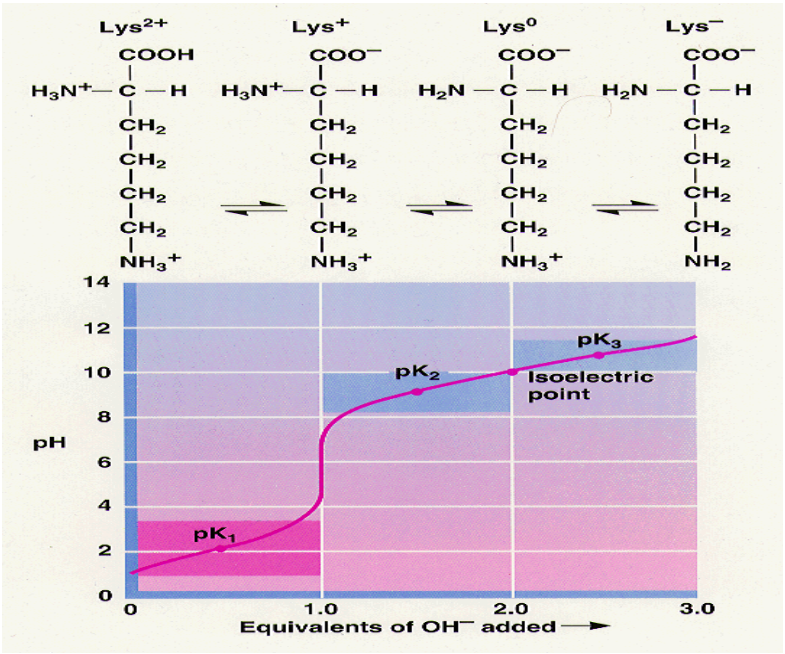
☑ From the graph, what is the approximate isoelectric point of glutamic acid?

↳ _____ **i.e. pH at 1st equivalence point**

3.2.3 Titration Curve of a Fully Protonated Basic Amino Acid, Lysine $H_3N^+CH(CH_2(CH_2)_3NH_3^+)CO_2H$

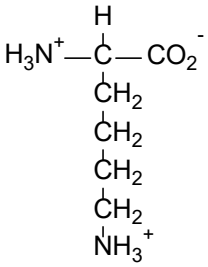
☑ A fully protonated lysine, **Lys^{2+}** is **tribasic** as it has 3 acidic groups, namely the **α-carboxylic acid**, the **protonated α-amine** and the **protonated alkyl-amine**.

☑ Below is the titration curve obtained on addition of aqueous NaOH to a solution of Lys^{2+} of the same molar concentration.



☑ Based on the titration curve, arrange the 3 acidic groups in order of decreasing K_a value:

↳ **α-carboxylic acid > protonated α-amine > protonated alkyl-amine**



↳ **Carboxylic acids** are **more acidic** than protonated amines due to resonance stabilisation of the carboxyl groups produced.

↳ The protonated α-amine group is **more acidic** than the protonated alkyl-amine as it is **closer to partially positive C in the α-carboxylate** ion which exerts an **electron-withdrawing inductive effect**, leading to **intensification** of positive charge on the protonated α-amine, thus destabilising it (i.e. easier for it to react).

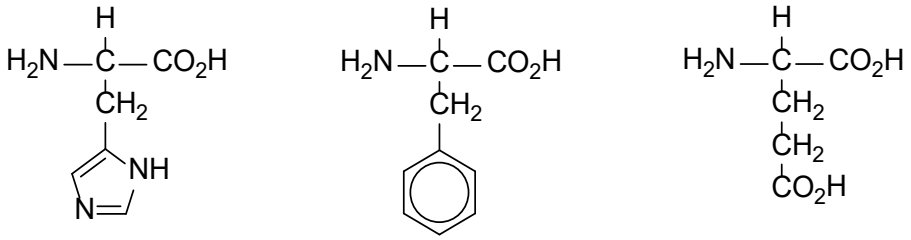
☑ As expected, there are **3** equivalence points.

☑ From the graph, what is the approximate isoelectric point of lysine? …….

_____

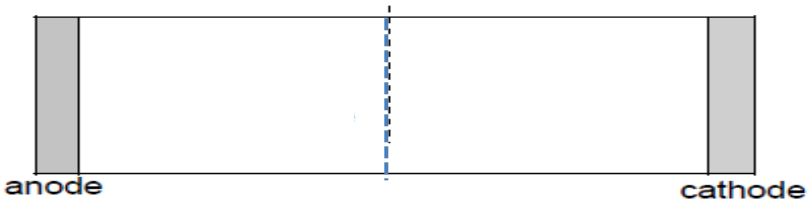
Question 10 (SLS Quiz 6)

A tripeptide, His-Phe-Glu was further hydrolysed. The resulting solution was added to an excess of a buffer solution of **pH 6.5** and placed at the centre of the plate. A potential difference was then applied across the plate.



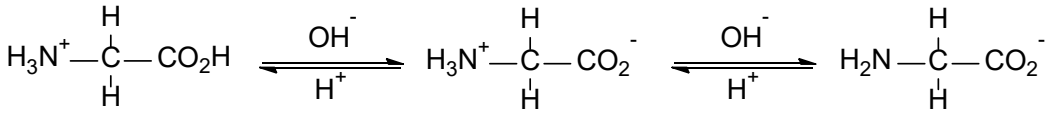
	His	Phe	Glu
Isoelectric point:	7.58	5.48	3.10

Indicate the relative positions of the amino acids in the diagram below.



Check Concept:

At **pH > pI**, amino acids are ***deprotonated/basic/anionic*** => *move towards* ***anode***
At **pH = pI**, amino acids are ***zwitterionic, electrically neutral*** => ***no movement***
At **pH < pI**, amino acids are ***protonated/acidic/cationic*** => *move towards* ***cathode***



cationic/acidic	zwitterionic	anionic/basic
pH < pI	**pI**	**pH > pI**

- **Rate** of movement is **inversely proportional** to **mass** of amino acids.
- **Higher M_r => slower, shorter distance**

Question 11 (SLS Quiz 7)

GABA, $H_2NCH_2CH_2CH_2CO_2H$, is a neurotransmitter released by red algae which encourages shellfish larvae to settle on the ocean bed.

Which statements about **GABA** are correct?

1 It is a 2-aminocarboxylic acid.
2 It is soluble in water due to zwitterion formation.
3 It migrates to the anode of an electrolytic cell at pH 12.

A 1, 2 and 3 only **B** 1 and 2 only **C** 2 and 3 only **D** 3 only

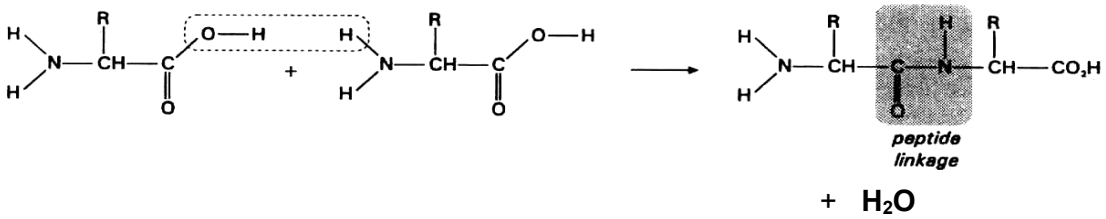
4 Peptide (Amide) bond formation

Students should be able to:

- *Describe the formation of peptide (amide) bonds between α-amino acids, and hence explain protein formation*

4.1 Condensation Reaction between Amino Acids

☑ A peptide bond/linkage is an **amide bond** formed by the elimination of a water molecule between the α-NH_2 of an amino acid and the α-CO_2H group of another amino acid (**condensation** reaction).



+ H_2O

☑ Peptides are polymers in which the individual amino acid units called residues are linked together by peptide bonds.

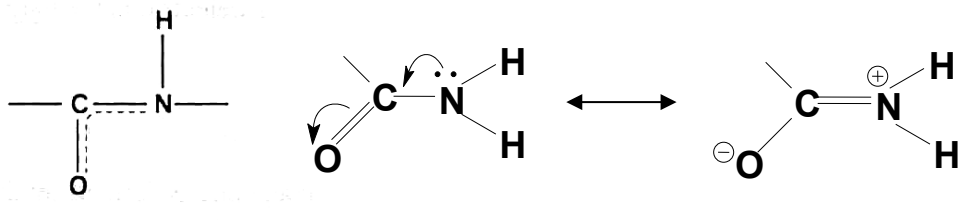
- **Dipeptides** are formed by linking **2** amino acids.
- **Tripeptides** are formed by linking **3** amino acids.
- **Polypeptides** are formed by linking 10 to 100 amino acids.
- **Proteins** are formed when polypeptides are coiled or folded into 3-dimensional structures.

☑ Peptides containing the same amino acid residues but in different order are not identical since the primary sequence reflects the relative positions of the residues linked together by peptide bonds. For example, pro-lys and lys-pro are different dipeptides.

4.2 Properties of a Peptide (Amide) Bond

☑ The peptide (amide) bond is rigid and cannot be rotated about the C-N bond. This is caused by delocalisation of π electrons in the peptide bond leading to double bond character in the C-N bond. Since the lone pair of electrons on N is now unavailable, amides are not basic.

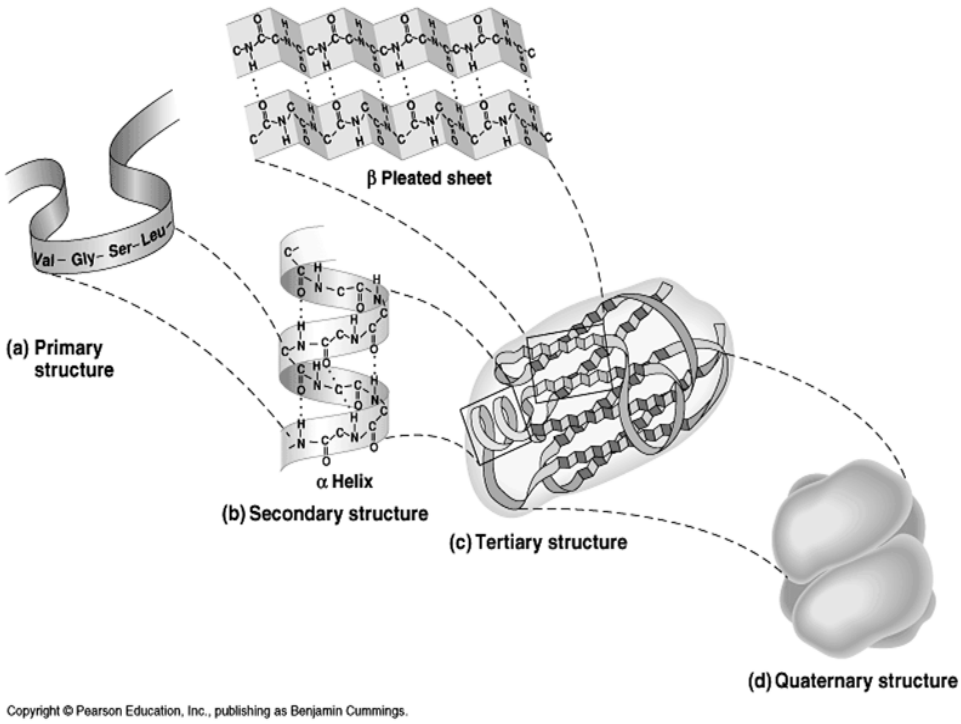
☑ Due to delocalisation, the N atom is sp^2 hybridised so that the lone pair of electrons on N is in a p-orbital which can overlap sideways with the p-orbital of C.



5 Formation of Proteins (formation of structures)

☑ Proteins are able to fulfill their functional roles by virtue of their structures caused by folding and coiling of polypeptide chains.

☑ The structure of a protein is characterised by 4 distinct levels of organisation. Here is a brief overview:



☑ The **primary structure** refers to the **sequence** of the amino acids in the polypeptide chain or chains. It is the primary structure that determines what the protein is, how it folds and its functions.

6 Hydrolysis of Protein (breaking down of structures and amide bonds)

Students should be able to:
- *Describe the hydrolysis of protein*

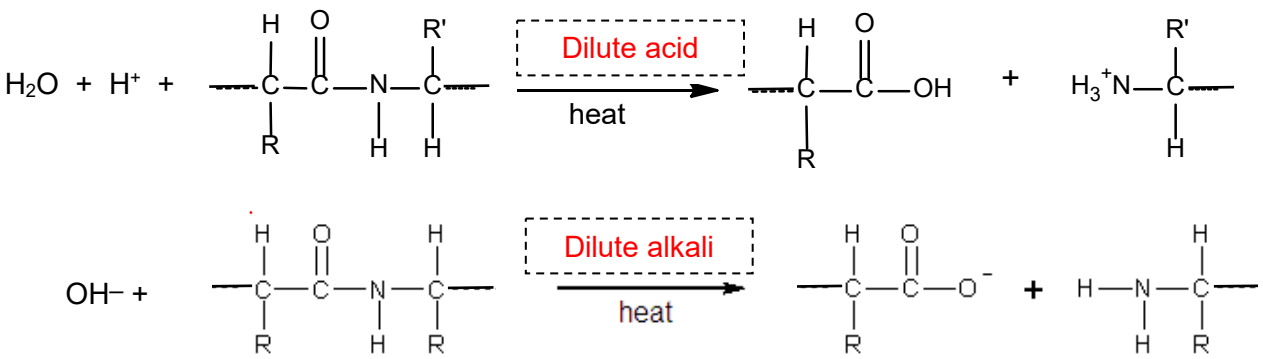
☑ The primary structure of amino acids in proteins can be obtained by a combination of terminal residue analysis and partial hydrolysis.

↳ **Partial hydrolysis** gives fragments that still retain sequence information. When these fragments are separated and identified, the overall amino acid sequence of the protein may be deduced from overlaps of the peptide sequence, just like pieces in a jigsaw puzzle.

☑ The peptide/amide bond can be split by **hydrolysis.**

6.1 Acid-Base Catalysed Hydrolysis

☑ Hydrolysis can be achieved through the use of dilute acid or alkali. Heat is needed for acidic or alkaline hydrolysis.



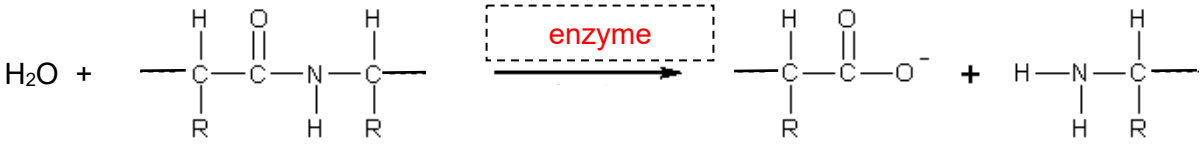
6.2 Enzymatic Hydrolysis

☑ Hydrolysis can be also be catalysed by suitable **enzymes** such as **pepsin** (in stomach) or **trypsin** (in intestine). No heat is needed.

☑ Enzymes are more selective, giving cleavage at predictable points in the chain.

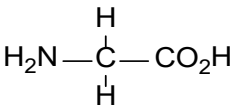
↳ Trypsin cleaves the chain at the carboxyl groups of the basic amino acids lysine and arginine.

↳ Chymotrypsin cleaves the chain at the carboxyl groups of the aromatic amino acids phenylalanine, tyrosine and tryptophan.

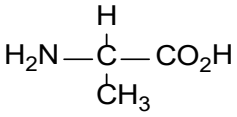


Question 12 (SLS Quiz 8)

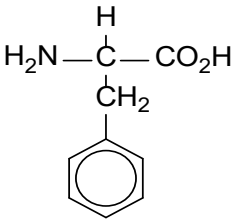
a) Draw the structural formula of a tripeptide Gly-Ala-Phe.



glycine



alanine



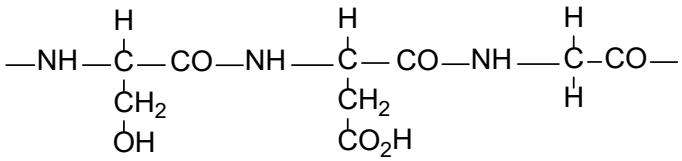
phenylalanine

By convention, the N-terminal amino acid residue (with the ***free amino group***) is written on the left side and the C-terminal amino acid residue (with the ***free carboxylic acid group***) is written on the right side.

b) Give the names (using 3-letter symbols) of possible structural isomers of this tripeptide.

Question 13 (SLS Quiz 9)

The following structure shows part of a protein molecule.



The protein was heated with dilute sulfuric acid. Draw the structural formula of each of the three amino acids produced by the reaction, labeling any chiral centres in the molecules.

Question 14 (SLS Quiz 10)

a) Deduce the primary structure of a hexapeptide from the following peptide fragments obtained from partial hydrolysis.

lys – tyr
lys – glu
asp – arg
arg – lys
lys – glu – asp

[Do not reverse the given sequence of amino acids in the fragments.]

How many amino acid residues are there in a hexapeptide? _____

The primary structure is _____

b) A proteolytic enzyme cleaves the hexapeptide at the *carboxyl group* of aspartic acid residues. Give the **sequence** of amino acid residues in the fragments obtained.
[The carboxyl group is written on the right side of each residue.]