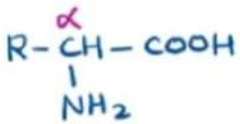
→ Amino acids:—

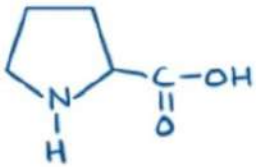
→ Introduction:-

- Amino acids represent one of the most important classes of naturally occuring compounds, because they formed fundamental structural units of proteins.
- All naturally occuring amino acids have an amino group located at α-position with respect to the carboxylic group.

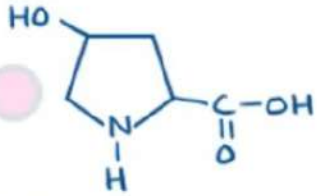


An α-amino acid

- Proline and hydroxyproline are exceptions as in these molecules the amino group forms a part of pyrrolidine ring.



Proline



Hydroxyproline

- In addition to carboxylic and amino groups, some amino acids contain benzene or heterocylic ring system, phenolic or alcoholic group or even sulphur.

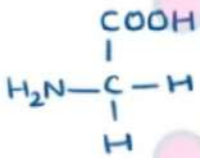
→ Structure and stereochemistry:-

- The structure of a typical amino acids contains a chirality centre at the α-carbon.

- The three dimensional and plane projection formulas of a typical α-amino acids are shown below:-

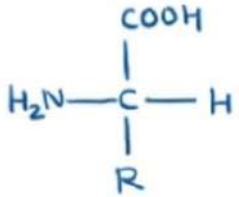
or

- All amino acids are optically active except Glycine. because Glycine does not contain chiral centre.

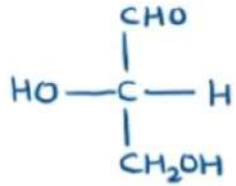


Glycine

- All the naturally occuring α-amino acids belong to L-series. because they have same relative configuration as L-Glyceraldehyde



L-Amino acid



L-Glyceraldehyde

- Most of amimo acids have S-configuration.

→ Amino acids:-

→ Acid Base behaviour: Dipolar nature:→

- When we added an amine in carboxylic acid, it gives ammonium salt of carboxylic acid. This takes place by the transfer of a proton from the carboxylic group to the amino group.
- In case of amino acids, both these groups are part of the same molecule. In this situation the proton transfer occurs internally to give an internal salt called "dipolarion" or "zwitterion".

An α-amino acid — Zwitter ion

- Evidence in favour of the dipolar ionic structure:-
- Because of the dipolar ionic structure, amino acids show the following typical properties:-

(i) They are highly soluble in water and insoluble in common organic solvents.

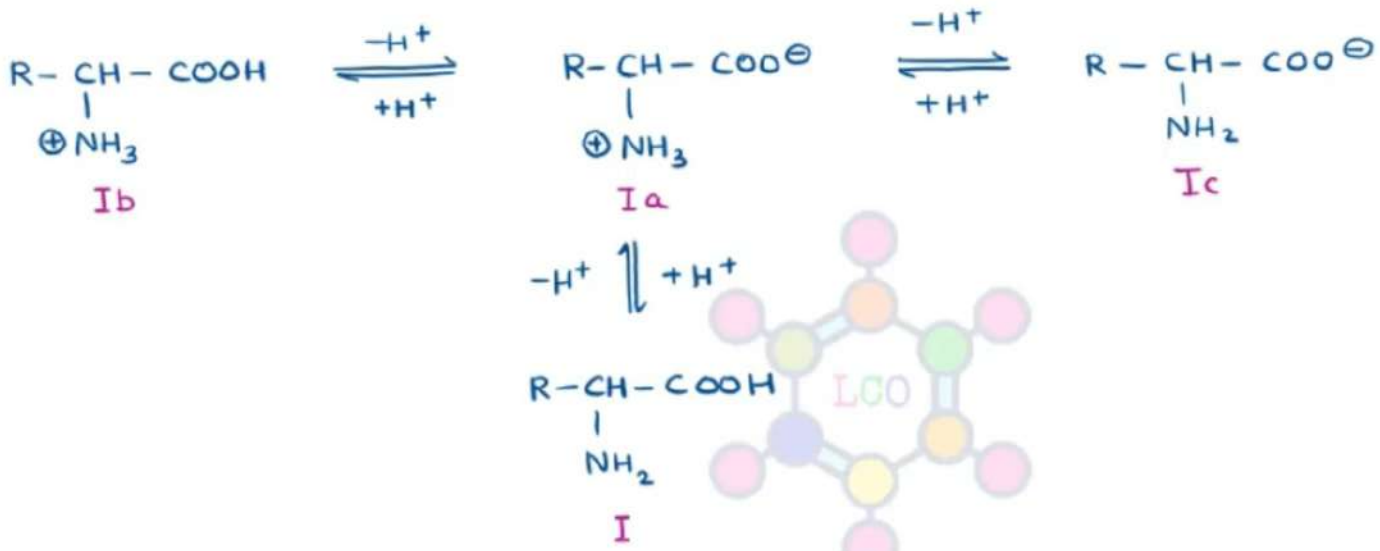
(ii) They melt at temp. above 473 K and that too with decomposition.

(iii) Their aqueous solutions behave like the solution of substace having dipole moment.

(iv) The spectral studies do not show the presence of free $-NH_2$ and $-COOH$ groups but indicate the presence of $-\overset{\oplus}{N}H_3$ and $-COO^-$ groups.

(v) Because of dipolar nature they are amphoteric, i.e., they can accept a proton from stronger acid as well as donate a proton to a stronger base.

- In aqueous solutions, the amino acids exist in various species which are in equilibrium with each other. Water acts both as an acid and as a base.



- The position of equilibrium depends upon the pH of the solution. At lower pH (*i.e.* in acidic solution) the conjugate base (Ib) predominates while at higher pH (*i.e.* in alkaline solution) the conjugate base (Ic) preodominates.

→ Amino acids:→

→ Nomenclature:→

- The common names of all amino acids are given on the basis of their source or on the basis of their special properties.

e.g. Glycine → sweet taste → (Greek, Glykys, sweet)

Leucine → It was isolated for the first time from muscle fibers → (Greek, leucos, white)

Tyrosine → obtained from caseine → (Greek, tyros, cheese)

Cystine → obtained from urinary calculi → (Greek, kystis, bladder)

- The names of the amino acids are generally written in their abbreviated forms.

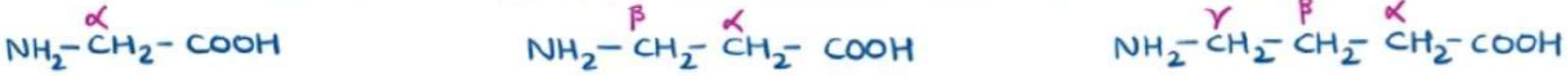
e.g. Glycine → gly

Alanine → ala

→ Classification:→

→ On the basis of position of amino group with respect to carboxylic group.

- Amino acids are classified as α-, β-, γ- amino acid etc. For example-



α-aminoacetic acid β-aminopropanoic acid γ-aminobutyric acid

→ On the basis of relative number of amino and carboxylic groups in their molecule.

(i) Neutral amino acids:→ contain equal number of amino and carboxylic groups.

e.g. Glycine, alanine, etc.

(ii) Acidic amino acids:→ Contain more carboxylic groups as compared to amino groups
- Carboxylic group may be present as free carboxylic group or in the form of amido group.
  e.g. aspartic acid, glutamic acid, asparagine, etc.

(iii) Basic amino acids:→ Contain more amino groups as compared to carboxylic groups.
  e.g. lysine, arginine, etc.

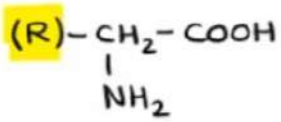
→ On the basis of requirement to the human body:-

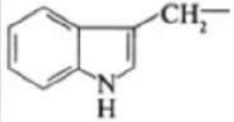
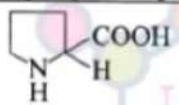
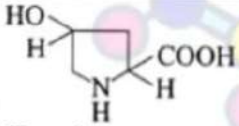
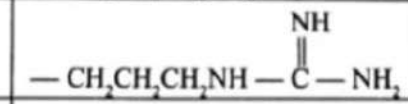
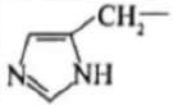
(i) Non-essential amino acids:→
- The amino acids, which can be synthesised in the body are known as non-essential amino acids

(ii) Essential amino acids:→
- The amino acids which can not be synthesised in the body are known as essential amino acids. These amino acids must be obtained through diet.

Name	Structure (R)	Abbreviation	Isoelectric point
Neutral amino acids			
1. Glycine	— H	Gly	5.97
2. Alanine	— CH_3	Ala	6.02
3. Valine*	— $CH(CH_3)_2$	Val	5.97
4. Leucine*	— $CH_2CH(CH_3)_2$	Leu	5.98
5. Isoleucine*	— $CH(CH_3)CH_2CH_3$	Ile	6.02
6. Tyrosine		Tyr	5.67

Table:- some common amino acids



7. Phenylalanine*	$C_6H_5 — CH_2 —$	Phe	5.48
8. Serine	$— CH_2OH$	Ser	5.68
9. Threonine*	$— CH(OH)CH_3$	Thr	5.60
10. Cysteine	$— CH_2SH$	CysH	5.02
11. Cystine	$— CH_2 — S — S — CH_2 —$	Cys-cys	5.06
12. Methionine*	$— CH_2CH_2SCH_3$	Met	5.06
13. Tryptophan*		Trp	5.88
14. Asparagine	$— CH_2CONH_2$	Asn (or $AspNH_2$)	5.41
15. Glutamine	$— CH_2CH_2CONH_2$	Gln (or $GluNH_2$)	5.70
16. Proline	 (Complete structure)	Pro	6.30
17. Hydroxyproline	 (Complete structure)	Hyp	6.33
Acidic amino acids			
18. Aspartic acid	$— CH_2COOH$	Asp	2.98
19. Glutamic acid	$— CH_2CH_2COOH$	Glu	3.22
Basic amino acids			
20. Lysine*	$— CH_2CH_2CH_2CH_2NH_2$	Lys	9.74
21. Arginine*		Arg	10.76
22. Histidine*		His	7.59

* Essential amino acids

→ Isoelectric point of amino acids:-

- The pH at which a particular amino acid exists as a dipolar ion is called the isoelectric point (pI).
- The isoelectric point of amino acids have no additional acidic or basic groups and is exactly half of the sum of pKa value of $-COOH$ group and pKa value of $-\overset{\oplus}{N}H_3$ group.

$$\text{Isoelectric point (pI) of a neutral amino acid} = \frac{pK_a \text{ of } -COOH + pK_a \text{ of } -\overset{\oplus}{N}H_3}{2}$$

Example:- Isoelectric point of Glycine (NH_2-CH_2-COOH).

- In aqueous solution, glycine exists in the following species which are in equilibrium with each other

$$\underset{\text{Ib}}{\overset{\oplus}{N}H_3-CH_2-COOH} \underset{+H^{\oplus}}{\overset{-H^{\oplus}}{\rightleftharpoons}} \underset{\text{Ia}}{\overset{\oplus}{N}H_3-CH_2-COO^-} \underset{+H^{\oplus}}{\overset{-H^{\oplus}}{\rightleftharpoons}} \underset{\text{Ic}}{NH_2-CH_2-COO^-}$$

conjugate acid — conjugate base

- Now when the conjugate acid of glycine is titrated against alkali and a graph is plotted with number of equivalents of base against pH of the solution there appear two equivalence points.

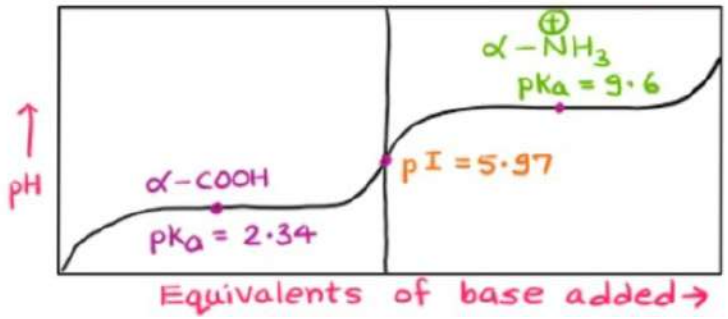
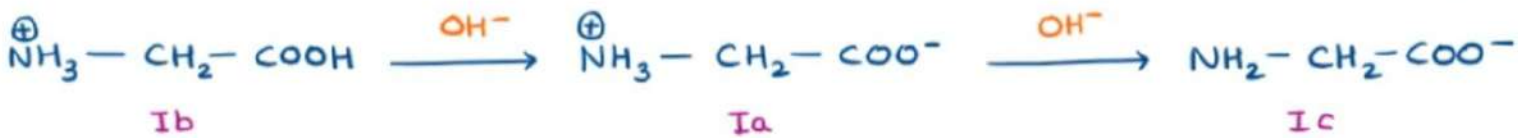


fig:- Titration curve of Glycine

- The first equivalence point indicates the change of Ib into Ia which corresponds to pKa of α-carboxylic group (2.34) and is several units lower than that of an ordinary carboxylic acids.



- The second equivalence point represents the change from dipolar form Ia to basic form Ic corresponding to the pka of a $\alpha-\overset{+}{N}H_3$ group (9.60) and is one unit lower than that of aliphatic ammonium ions.
- After the addition of one equivalent of NaOH there is a steep rise in pH of solution and the curve takes the shape as shown in fig. This point corresponds to pH 5.97 at which the acid exists as dipolar ion. This point is called isoelectric point of Glycine at which the sum of charges of all the molecules of amino acid is zero.

$$\text{Isoelectric point of Glycine} = \frac{2.34 + 9.60}{2} = 5.97$$

- Isoelectric point can also be defined as the pH at which the concentration of Ib is exactly equal to that of Ic.
- In general, pI of amino acids lie between 4 and 8 when they exist as dipolar ion.
- Isoelectric point is characteristic property of amino acids. If an electric current is passed through the aqueous solution of amino acid at pH lower than pI, the amino acid will migrate towards cathode. Similarly at higher pH it will migrate towards anode. But at pH equal to pI there will no migration because of the existance of amino acid as dipolar ion.

- Isoelectric point may also be defined as the pH at which amino acid does not migrate when its aqueous solution placed under the influence of an electric field.
- The solubility of amino acid is minimum at it isoelectric point, and this property has been quite useful in the separation of amino acids from mixtures.

→ Electrophoresis :→

- Electrophoresis is a technique of separation and purification of compounds on the basis of differential movement of charged particle in an electrical field.
- This technique is used for separation of different amino acids obtained on hydrolysis of a protein.

→ Methodology :→

- A paper strip or a suitable plastic or cellulose acetate plate is used in this technique.
- In paper electrophoresis, the mixture of amino acids is place in the form of spot at the center of the paper strip.
- The strip is then soaked with an aqueous buffer of a particular pH. The pH of the buffer depends upon the isoelectric points of the amino acids to be separated.
- The two ends of the filter paper are dipped into the buffer solution in which the electrodes are dipped.
- When a electric field is applied, different amino acids migrate towards cathode or anode at different rates depending upon their isoelectric points and charge density. As a result, amino acids can be separated. The following changes occur:

(i) The amino acids, whose isoelectric point is the below pH of the buffer, start moving towards anode because they exist mainly in the anionic form.

(ii) The amino acids, whose isoelectric point is the above pH of the buffer, start moving towards cathode because they exist mainly in the cationic form.

(iii) The amino acids, whose isoelectric point corresponds to the pH of the buffer, do not migrate from the origin because they have no net charge.

- After the separation is over, the paper strip is dried and sprayed with a developer such as ninhydrin when different amino acids become visible.

→ Example:- separation of mixture of Glycine (pI = 5.97), Aspartic acid (pI = 2.98) and lysine (pI = 9.7).

- The pH of buffer solution is 5.97
- At this pH, aspartic acid will move towards andoe, lysine will move towards cathode while glycine will not migrate at all and hence remains at the origin point.

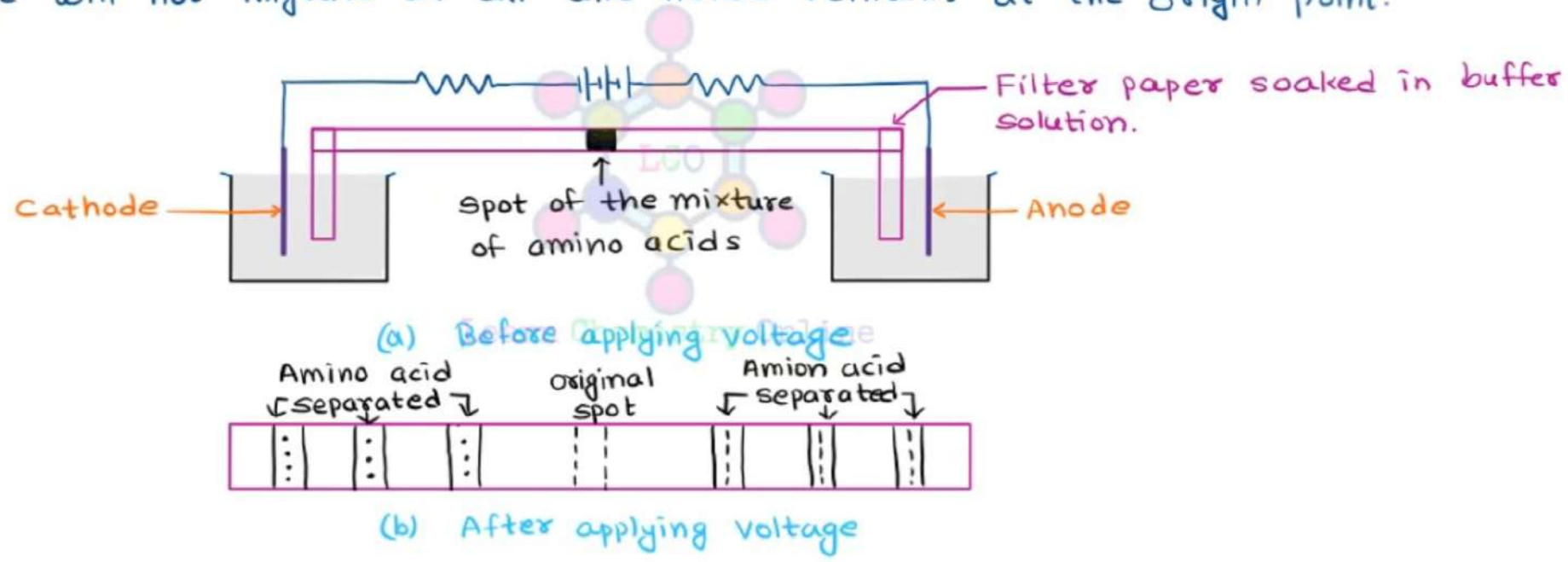


fig:- separation of amino acid mixture by paper electrophoresis.

→ Amino acids:-

→ Methods of preparation:→

1. From proteins:→

- Proteins from various sources are extracted with water, saline, acid, alkali and ethanol.
- These extracts so obtained are subjected to dialysis to free from ionic impurities.
- They they are evaporated to dryness.
- The residue is hydrolysed by following methods-

(a) Acidic medium:- By refluxing with 6N HCl for several hours.

(b) Alkaline medium:- By refluxing with 5N $Ba(OH)_2$.

(c) Proteolytic enzymes:- Since, in acidic or basic mediums, some of the amino acids are destroyed. So, the hydrolysis of these proteins is better accomplished by the use of certain proteolytic enzymes. This process, though quite slow, but does not involve destruction of any of the constituent amino acids.

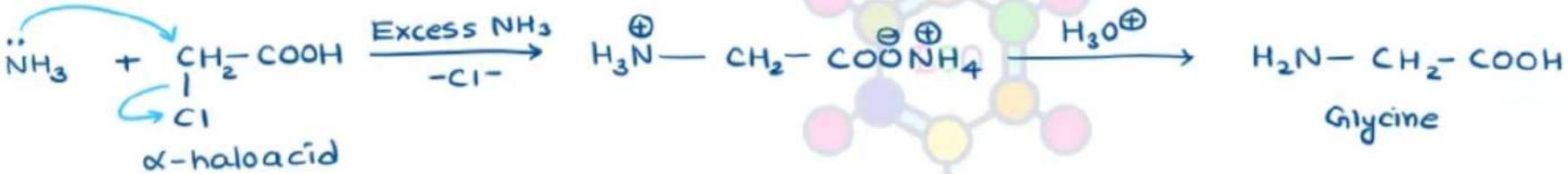
- The mixture of amino acids obtained after hydrolysis is esterified and the mixture of esters is separated by fractional distillation.
- Another method is Dakins procedure. In this method, butanol-water mixture is used for selective extraction of amino acids. The residue is then separated by using special reagents such as phosphotungstic acid.
- The separation of amino acids may also achieved by electrophoresis, ion exchange, chromatography and paper chromatography.

2. From α-halogenated acids or esters :→

- The easiest methods for the synthesis of α-amino acids involves the introduction of an amino group through nucleophilic displacement in α-haloacids or their esters by variety of nitrogen nucleophiles.
- The α-haloacids or esters are obtained by Hell-Volhard-Zelinsky reaction or through malonic ester synthesis.
- some important methods are listed below-

(a) Direct amination of α-halogenated acids :→

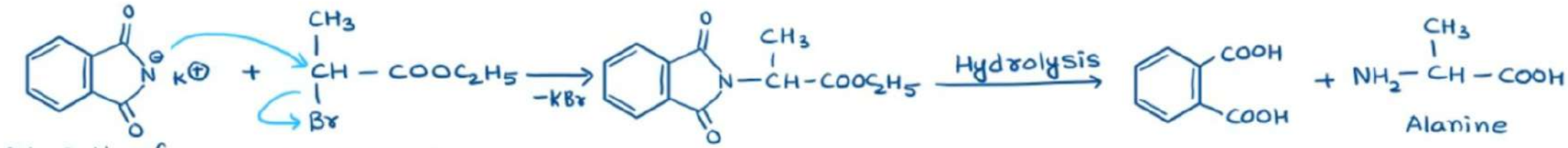
- Nucleophilic substitution reaction.



α-haloacid

(b) Gabriel phthalimide synthesis :→

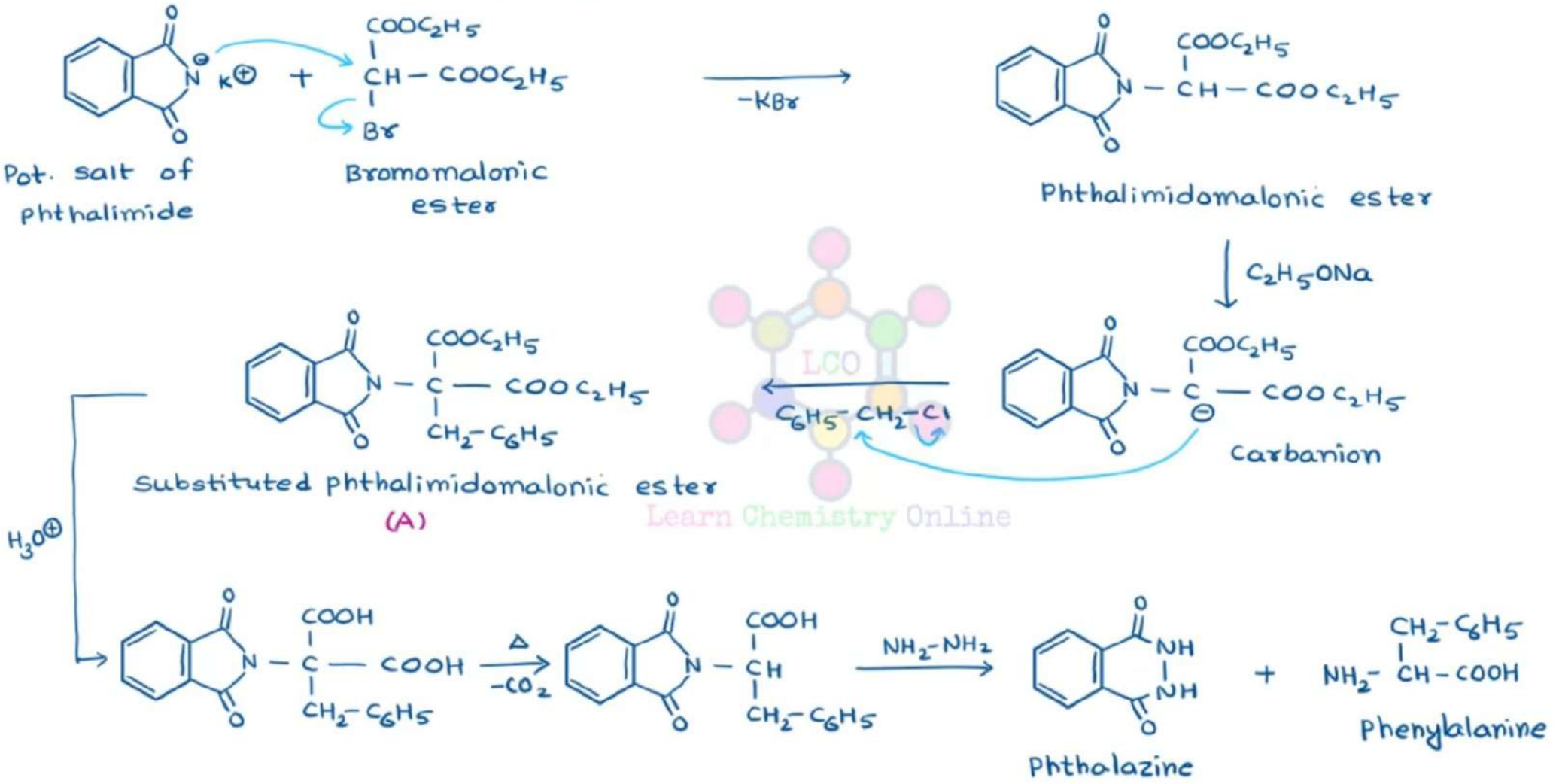
- Phthalimide is a very good reagent for the introduction of a primary amino group into the molecule.
- This reagent has widely been used for the synthesis of α-amino acids.



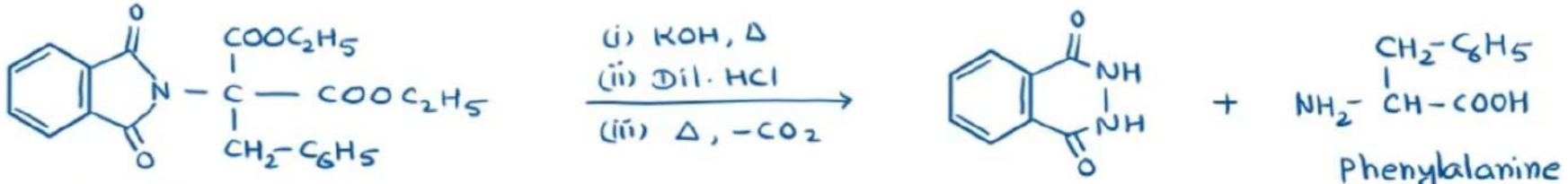
Pot. salt of Phthalimide

α-Bromoester

(c) Phthalimidomalonic ester synthesis :→



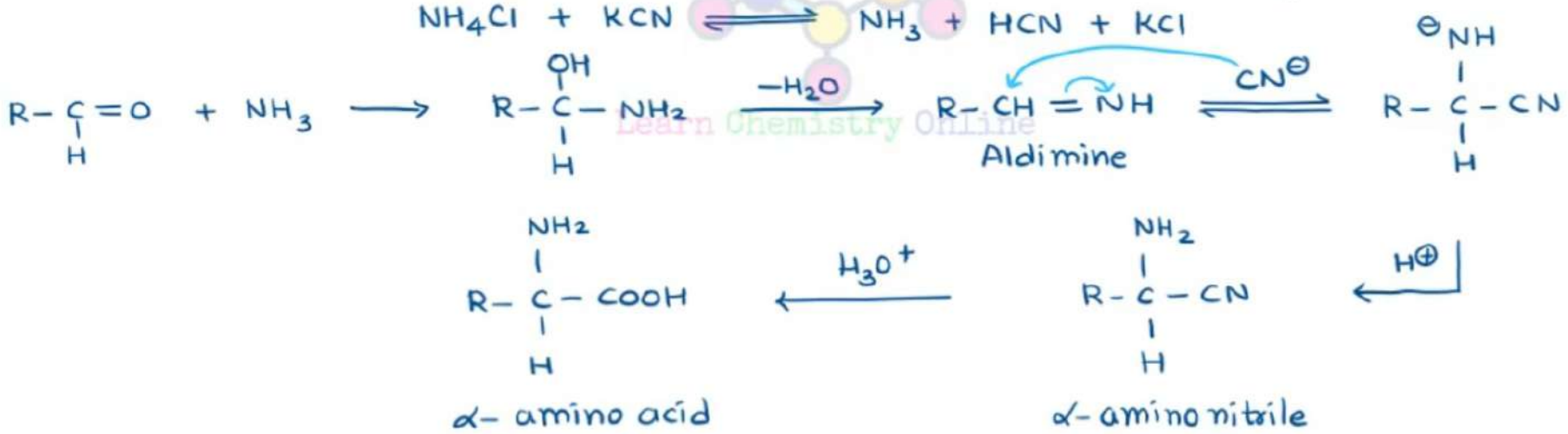
- Alternatively, base hydrolysis of phthalimidomalonic ester (A) followed by decarboxylation gives the same α- amino acid.



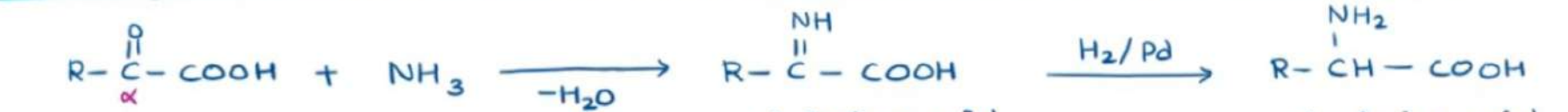
Substituted phthalimidomalonic ester (A)

3. Strecker Synthesis :→

- This is one of the most convenient method for synthesis of α- amino acids and involves the reaction between an aldehyde, ammonium chloride and potassium cyanide.



4. Koop synthesis: Reductive condensation of α- keto acids with ammonia :→

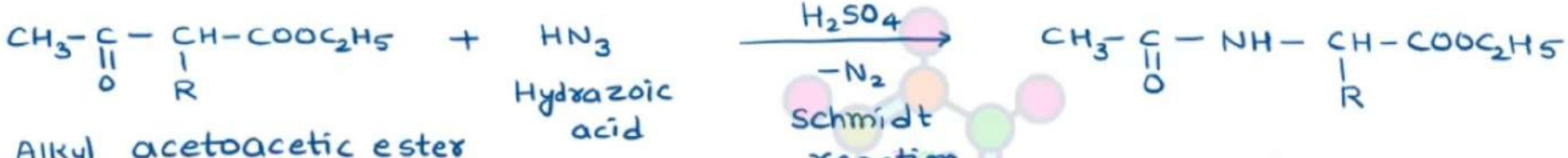


α- keto acid

α- Imino acid

α- Amino acid.

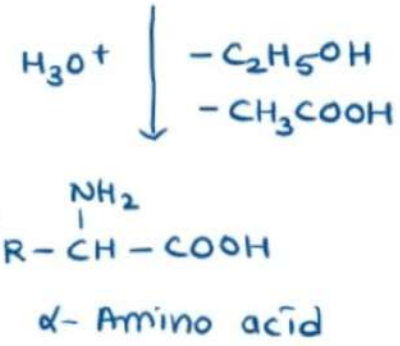
5. Schmidt synthesis :→



Alkyl acetoacetic ester

Hydrazoic acid

Schmidt reaction

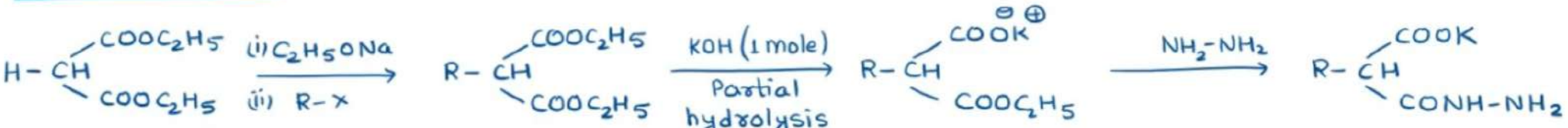


α- Amino acid

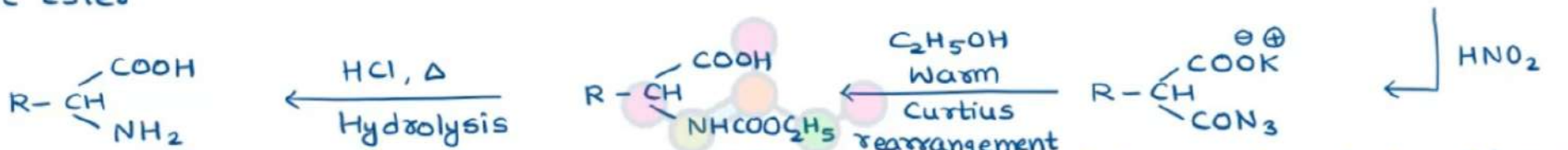
→ Amino acids :→

→ Methods of preparation :→

6. Curtius reaction :→



Malonic ester



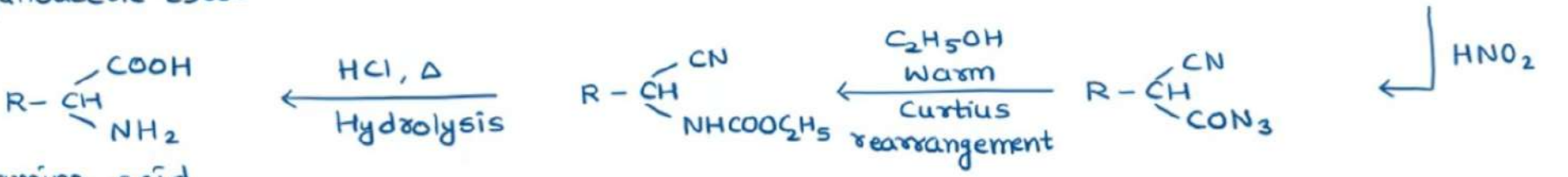
α- amino acid

Urethane

Pot. salt of malonic acid monoazide

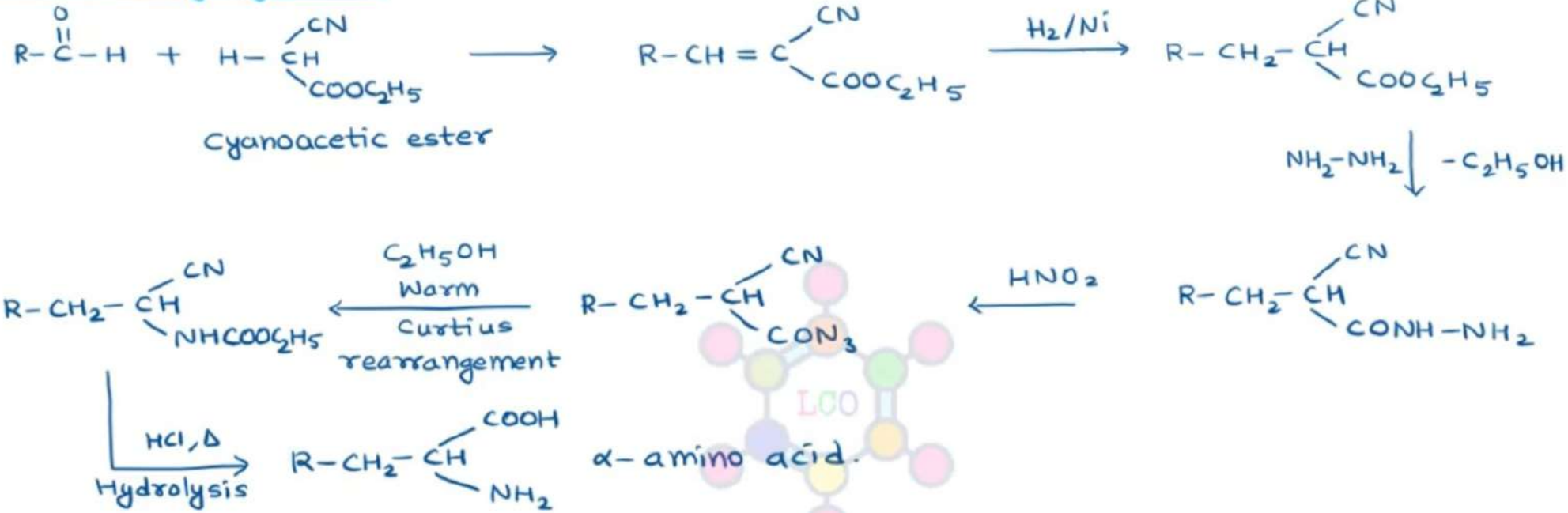
- Cyanoacetic ester can also be used in place of malonic ester.

cyanoacetic ester

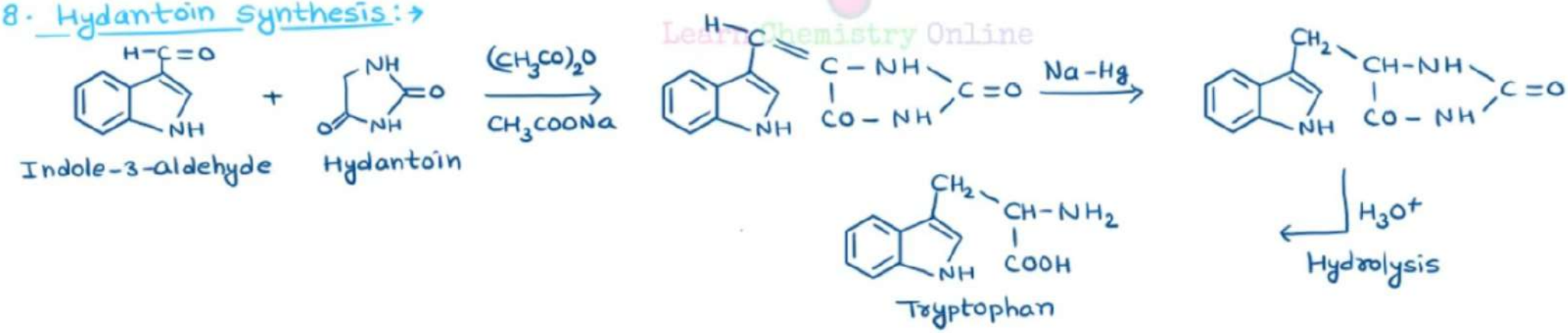


α- amino acid

7. The Darapsky synthesis :→



8. Hydantoin synthesis :→



9. Reduction of oximes and hydrazones of α-keto acids:→

→ $CH_3-C(=O)-CH(R)-COOC_2H_5$ + $R'ONO$ $\xrightarrow{H^+}$ $R-C(=NOH)-COOC_2H_5$ + CH_3COOH + $R'OH$

Alkyl acetoacetate — Alkyl nitrite — oxime

oxime $\xrightarrow{H_2/Pd}$ $R-CH(NH_2)-COOC_2H_5$

$R-CH(NH_2)-COOC_2H_5$ $\xrightarrow[\text{Hydrolysis}]{H_3O^+}$ $R-CH(NH_2)-COOH$

α-amino acid

→ $CH_3-C(=O)-CH(R)-COOC_2H_5$ + $C_6H_5-N^{\oplus}\equiv N\,Cl^{\ominus}$ $\longrightarrow$ $R-C(=NNHC_6H_5)-COOC_2H_5$ + CH_3COOH

Alkyl acetoacetate — Diazonium salt — phenylhydrazone

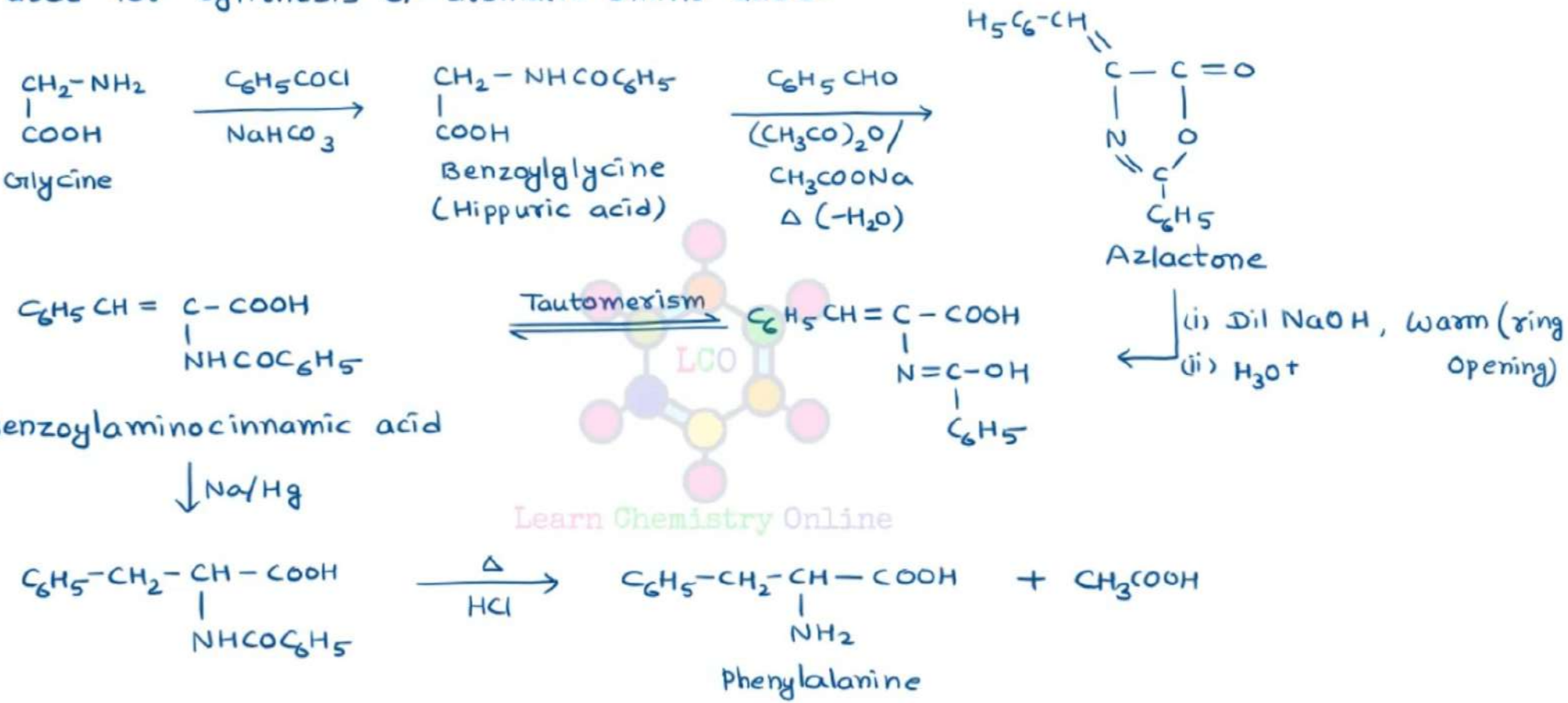
phenylhydrazone $\xrightarrow[CH_3COOH]{Zn/}$ $R-CH(NH_2)-COOC_2H_5$ + $C_6H_5NH_2$

$R-CH(NH_2)-COOC_2H_5$ $\xrightarrow[\text{Hydrolysis}]{H_3O^+}$ $R-CH(NH_2)-COOH$

α-amino acid

10. Erlenmeyer azlactone synthesis:→

- It is used for synthesis of aromatic amino acids.



→ Amino acids: –

→ Physical properties of α-amino acids:-

- Amino acids are colourless salt like crystalline solids.
- They have high melting points and decompose before melting.
- They are soluble in polar solvents but insoluble in organic solvents.
- Their aqueous solution exhibit dipole moments.

→ Chemical reactions :→

- Amino acids show three types of chemical reactions-

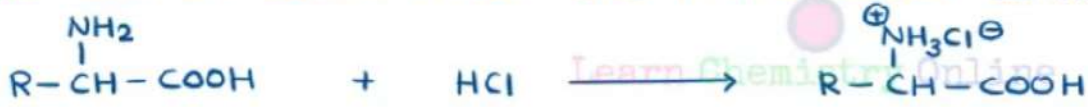
Ⓐ Reactions due to amino group. Ⓑ Reactions due to carboxylic group.

Ⓒ Reactions due to both carboxylic and amino group.

Ⓐ Reactions due to amino group.

1. Basic nature:-

- Amino acids act as weak bases and hence react with strong mineral acids to form salts.



2. Alkylation : Formation of Betaine:-

Betaine
(N,N,N- trimethylglycine)
zwitterion like character

3. Action of nitrous acid :-

Hydroxy acid

- This reaction forms a basis of well-known Van Slyke method for estimation of amino acids.

4. Reaction with nitrosyl chloride or bromide :-

α- halo acid

5. Acylation :-

- Acylamino acids are obtained upon treatment with acid chlorides and anhydrides.

Acetylglycine

Benzoylglycine

- These products do not exist in dipolar form and are useful intermediates in peptide synthesis.

6. Action of formaldehyde : Formol titration:-

$CH_2O + {}^{\oplus}NH_3-CH_2-COO^{\ominus} \longrightarrow CH_2=NCH_2COOH$

Methyleneglycine

OR

$2CH_2O + {}^{\oplus}NH_3-CH_2-COO^{\ominus} \longrightarrow (CH_2OH)_2NCH_2COOH$

Dimethylolglycine

- This process is simply a nucleophilic addition of on amino group to the carbonyl group of formaldehyde. This treatment " masks" the amino group and hence carboxylic group can be easily treated with alkali (Sorensen formol titration).

7. Reaction with Sanger's reagent:-

- 2,4-dinitrofluorobenzene (DNFB) is know as Sanger's reagent.

2,4-dinitrophenylamino acid

- This reaction is highly useful in determining the N-amino acid residue in a peptide or protein.

(B) Reactions due to carboxylic group:→

- Amino acids show acidic character due to presence of a carboxylic group. This becomes evident by evolution of CO_2 when an aqueous solution of amino acid is treated with $NaHCO_3$.

1. Acidic nature:-

- Amino acids react with base to form salts.

2. Esterification:-

Amino acid hydrochloride

Amino acid ester hydrochloride

3. Formation of acid chlorides:-

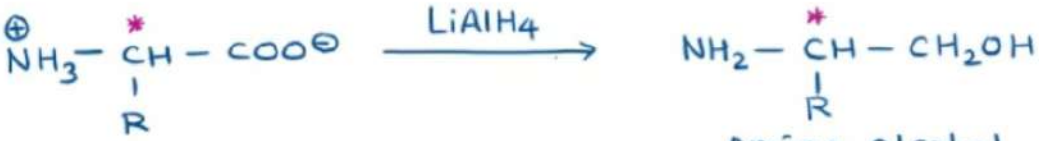
- Conversion of amino acids into corresponding acid chlorides is quite difficult because direct treatment of amino acids with PCl_5, $SOCl_2$ etc. fails to give the chlorides due to presence of amino group.
- To overcome this difficulty, amino acids are first converted into acyl derivatives and then treated with $SOCl_2$.

Acylderivative of amino acid

4. Decarboxylation:-

Amine

6. Reduction:-



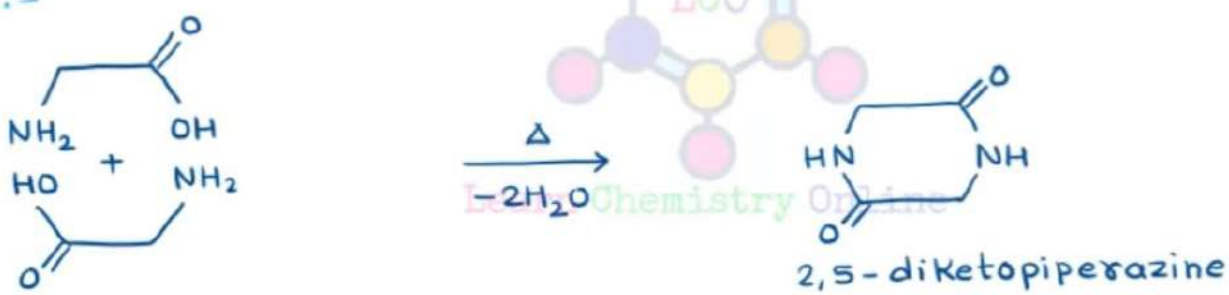
- The product formed without any loss of optical activity.

© Reactions due to both carboxylic and amino groups:-

1. Effect of heat:-

- Depending upon relative position of amino and carboxylic groups, amino acids show different behaviour on heating as discussed below-

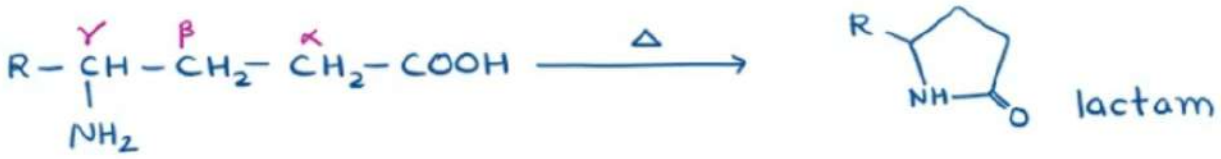
(i) α - Amino acids:-



(ii) β - Amino acids:-

(iii) γ- and δ- Amino acids:-

- These amino acids on heating form cyclic amides called lactams.

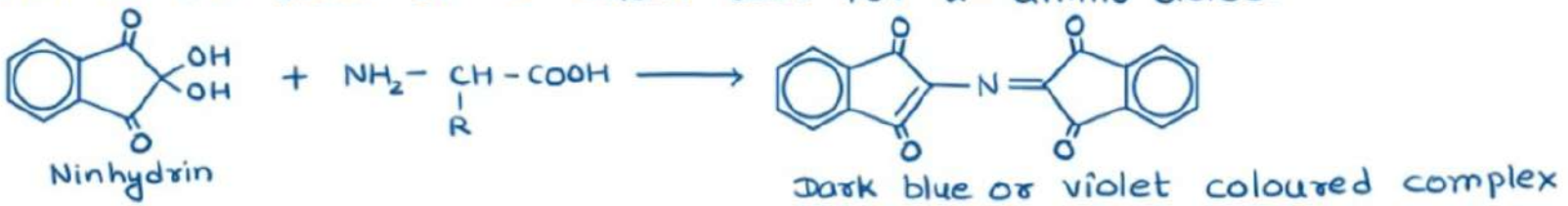


- Lactams show a special type of tautomerism called lactam - lactim tautomerism.

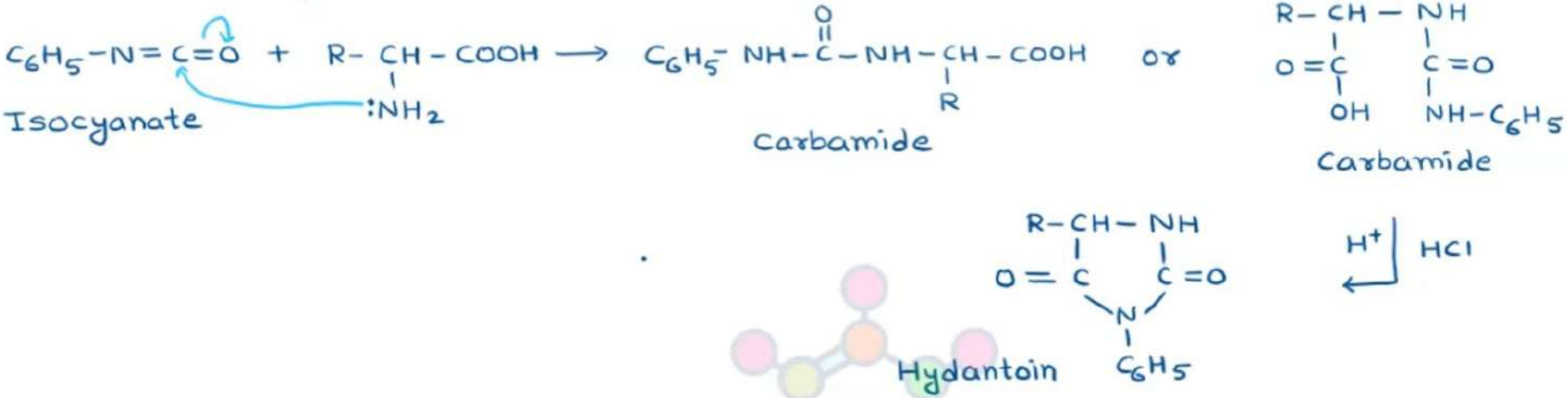
- If the amino group is present farther along the chain, linear polymeric amide is the product

2. Ninhydrin reaction:-

- Ninhydrin reacts with amino acid to give dark blue or violet coloured complex.
- This reaction is the basis of a colour test for α- amino acids.



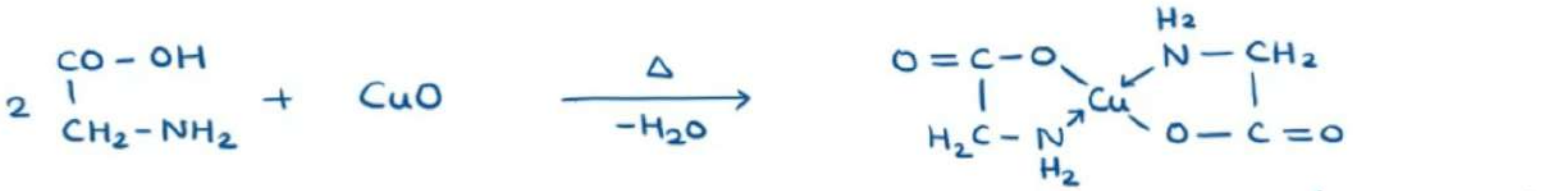
3. Formation of hydantoins:-



4. Formation of metal chelates:-

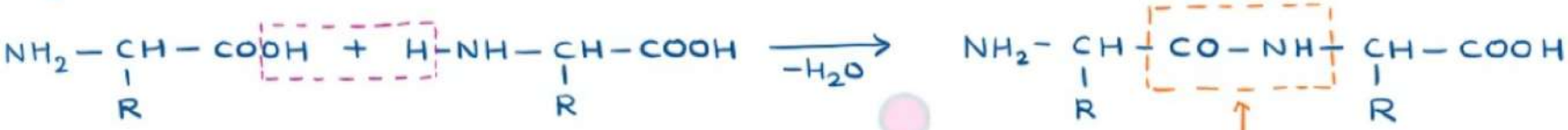
- Like other corboxylic acids they also form metal salts. But their salts with heavy metals are highly coloured because of the chelate ring structure.

Example.



→ Peptides:-

- Peptides are the polyamides formed by the condensation of amino group of one amino acid with the carboxylic group of the other.
- Secondary amides having –CO–NH– linkage commonly referred as peptide bond or peptide linkage.



A dipeptide

→ Classification of peptides:→

- Depending upon the number of amino acid residues per molecule they are referred as dipeptides, tripeptides, tetrapeptides and polypeptides.
- proteins are also polyamides.
- The compounds having molecular weight of 10,000 or less are called polypeptides while the compounds which have molecular weight higher than 10,000 are called proteins.

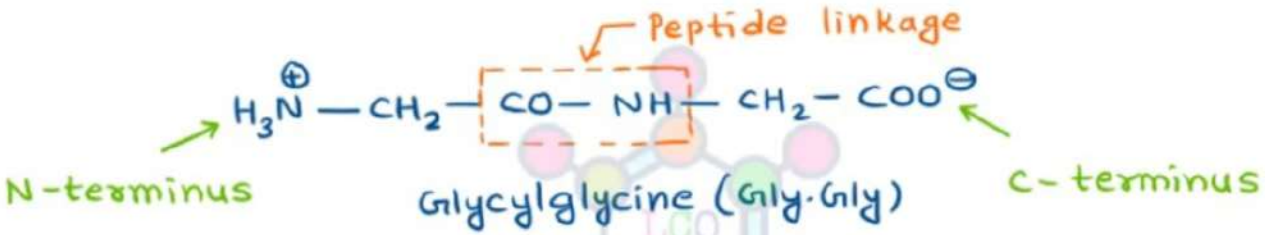
→ Nomenclature of Peptides:→

- The one end of peptide which have free amino group is called N-terminus and other end of peptide which have free carboxylic group is C-terminus.
- By convention, peptides are always written with N-terminus on left side and the C-terminus on the right side.

- They are named from N-terminus to C-terminus through the sequential listing of the names of amino acids. This is done by replacing the ending -ine by -yl in the names of all amino acids excepting the C-terminus.
- The names of polypeptides are abbreviated by using three letter abbreviation for amino acids.

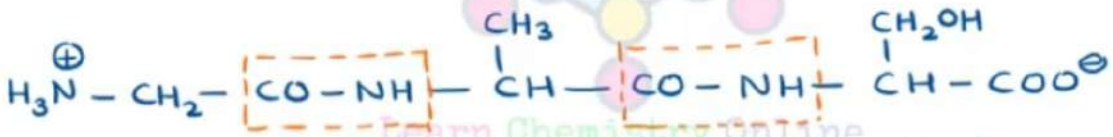
Example

→ A dipeptide



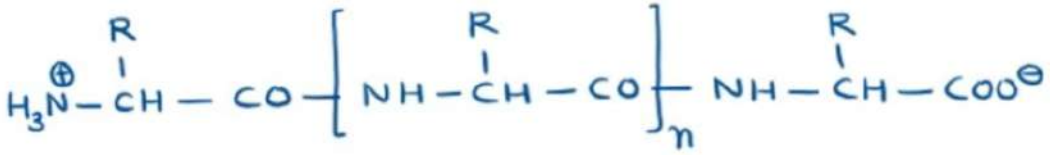
Glycylglycine (Gly·Gly)

→ A tripeptide



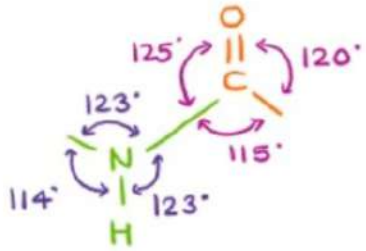
Glycylalanylserine (Gly·Ala·Ser)

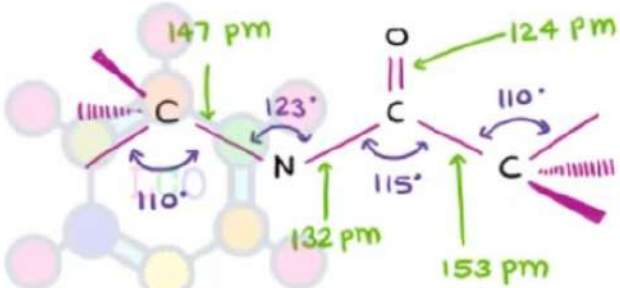
→ A polypeptide



→ Geometry of peptide linkage :→

- Studies on structure determination of peptides have revealed that the amide group is flat and the carbonyl and amino groups lie in one plane having H of NH and O of CO trans with respect to each other
- X-Ray studies (done by Linus Pauling) of peptides show that the C-N bond length of $-CO-NH-$ is 132 pm which is shorter than the usual 147 pm showing slight double bond character.

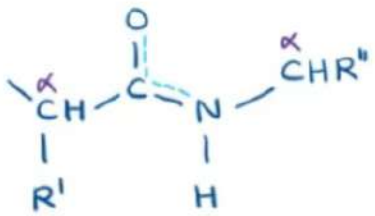




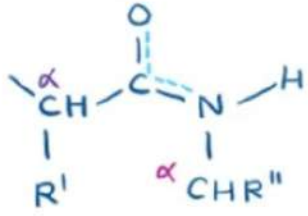
- Since, carbon-nitrogen has 50% double bond character, therefore the lone pair on the N-atom in the peptide bond is delocalised over C=O group as shown below-

Resonating structure of a peptide bond

- Due to double bond character in C-N bond, the rotation about C-N bond is restricted. Therefore the peptide bond can show geometrical isomerism.



trans (more stable)



cis (less stable)

- Trans form is more stable than cis form because of much larger steric repulsion between R' and R" groups. Thus, the atoms forming peptide bond lie in a plane with O and H atoms in trans orientation.
- Free rotation of a peptide can occurs only around the bonds joining the nearly planar amide groups to α-carbons. Hence, conformation of a protein molecule or the polypeptide chain can be described in terms of angle between R'-CH-NH bonds and the angle between R'-CH-CO bonds. These angles are called Ramchandran angle. (on the name of G.N.A. Ramchandran, Indian bio-physicist)

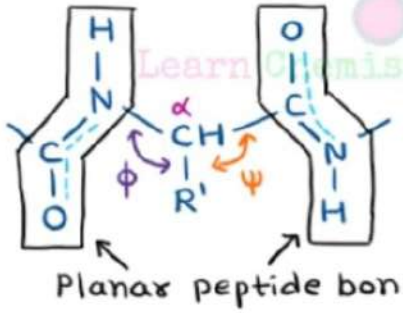


Fig:- Model of segment of a polypeptide showing planar peptide bonds in boxes and Ramachandran angles of rotation (ϕ & ψ) around α-carbon

→ Peptide synthesis :→

– Synthesis of peptide is important from two viewpoints –

(i) The synthesised peptides are identical with the natural one.

(ii) Synthetic peptides are useful model compounds for understanding the structural features of proteins.

– Peptides are polyamides and can be synthesised by the stepwise condensation in which the amino group of one amino acid is condensed with carboxylic group of a second amino acid. The condensation reaction takes place until a desired polypetide chain is obtained.

– The process of peptide synthesis is complicated because self condensation of two molecules of same amino acids take place. To overcome this difficulty the amino group or carboxylic groups are proctected or blocked by converted into substituted amino or carboxylic group.

– The peptide synthesis consists of three steps –

(a) Protection of amino or carboxylic group

(b) Condensation

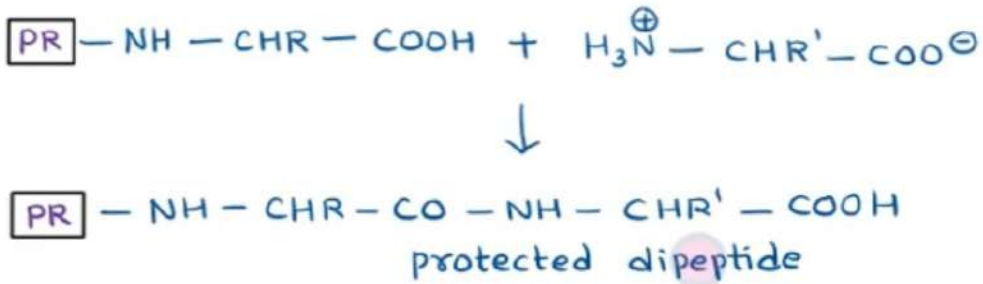
(c) Removal of protecting group.

(A) Peptide synthesis by protecting amino group :–

(a) Protection of amino group of amino acid :–

– This is done by treating the amino acid, which will form N-termius of the peptide with a protecting agent. This agent condenses with amino group of acid and forms N- protected amino acid.

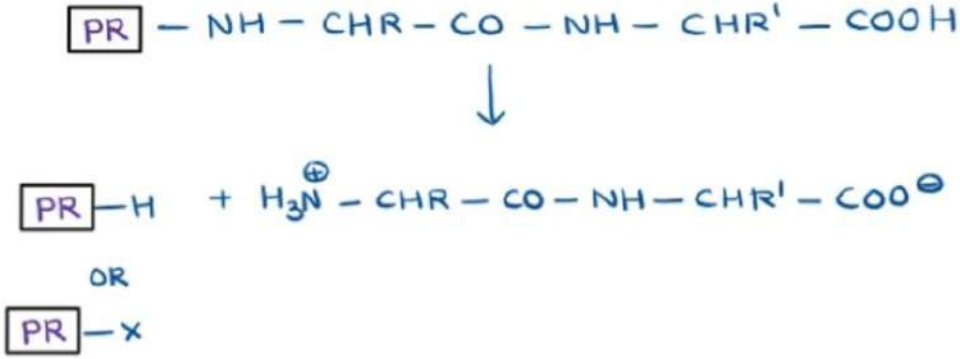
(b) Condensation of the carboxylic group of the N-protected amino acid with the amino group of the second amino acid:-



This step is repeated several time using desired amino acids one by one till the protected polypeptide of appropriate length is obtained.

(c) Removal of the protecting group:-

- The protecting group is removed using any usual method taking care to chose such reagents that do not affect the peptide chain.

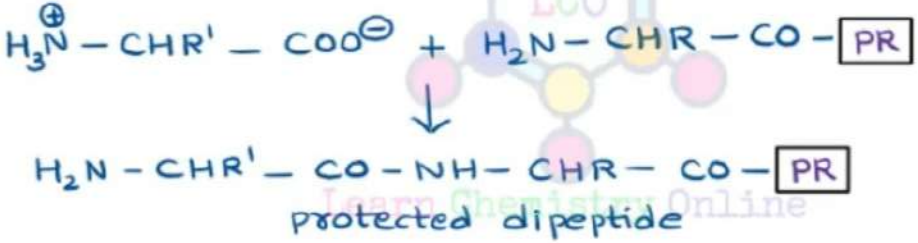


(B) Peptide synthesis by protecting carboxylic group:-

(a) Protection of the carboxylic group of amino acid:-

- This is done by treating amino acid, which will form the C-terminus of the peptide, with protecting agent. This agent condenses with carboxylic group of this acid and forms C-protected amino acid.

(b) Condensation of the amino group of the protected amino acid with carboxylic group of the other amino acid:-



This step is repeated several time to obtain desired protected polypeptide.

(c) Removal of protecting group:-

- The protected group is removed using any of the usual methods taking care to choose such reagents that do not affect the peptide chain.

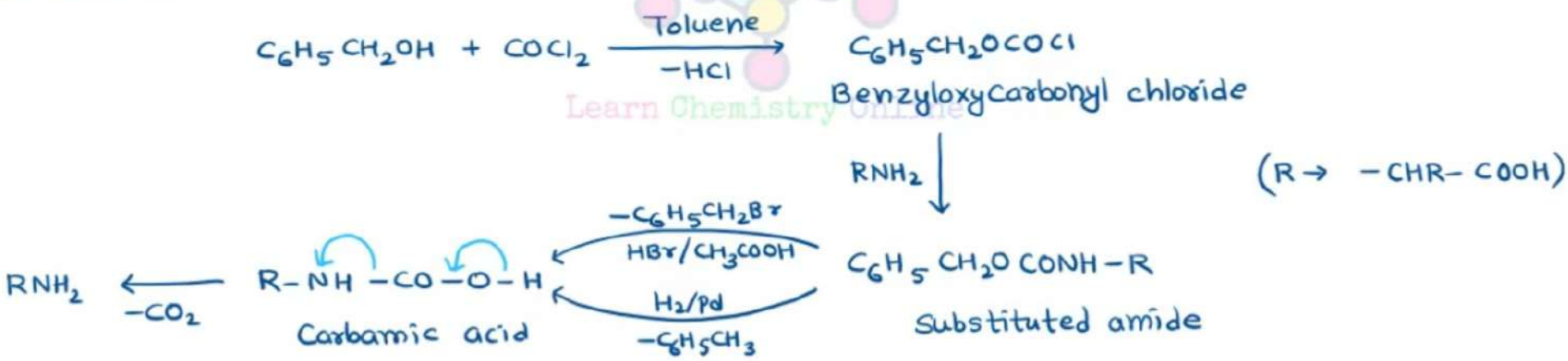
→ The protecting agents:→

- A number of protecting agents have been developed for synthesis of peptides.
- A good protecting agent is that which can react with the amino or carboxylic easily and can be removed, at the end of the synthesis, without affecting the peptide bond.
- Some important protecting agents are-

(A) N-terminus protecting agents:-

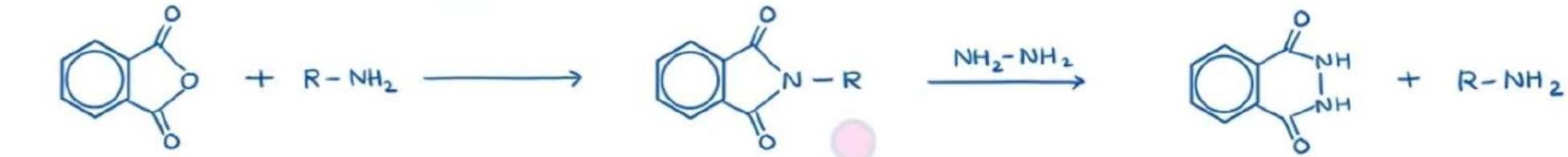
(i) Carbobenzoxy chloride or Benzyloxycarbonyl chloride ($C_6H_5CH_2OCOCl$):-

- It is an ester as well as acid chloride of carbonic acid (H_2CO_3).
- It is obtained by treating benzyl alcohol with carbonyl chloride (phosgene).
- It reacts with amino compound to form corresponding substituted amide. This amide on catalytic reduction or treatment with hydrobromic acid in acetic acid, regenerates the amine via carbamic acid.



(ii) Phthalic anhydride :→

- The acylation of free amine group can be done by treating it with phthalic anhydride.
- The resulting N-substituted phthalimide on treatment with hydrazine forms phthalazine and librates the amine.



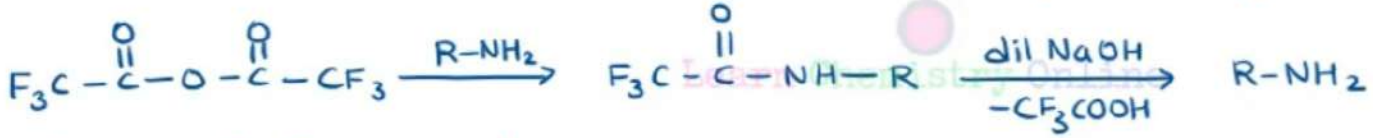
R → -CHR-COOH

N-Substituted phthalimide

Phthalazine

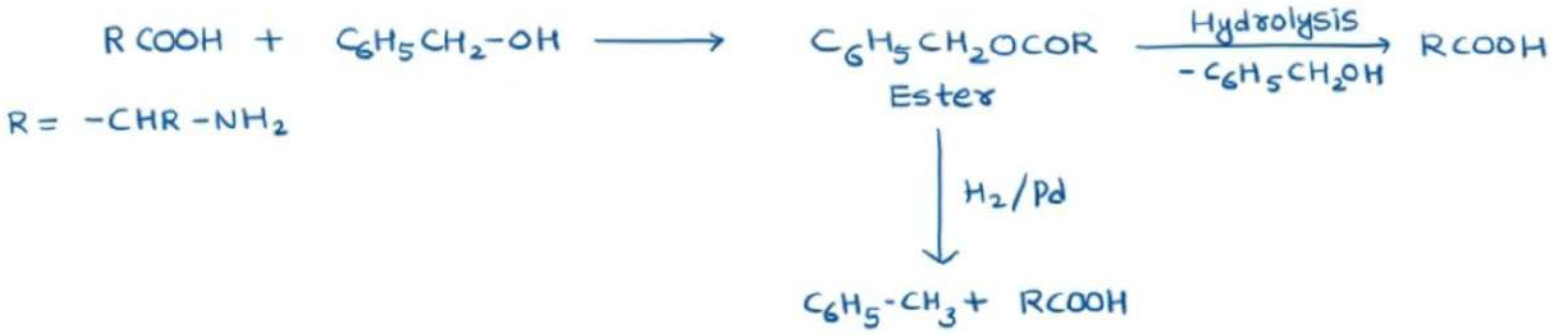
(iii) Aliphatic anhydrides:-

- Trifluoroacetic anhydride is used for trifluoroacetylating the amino group.
- The amide so obtained is converted into amine by treating it with dil. NaOH.



(B) C-terminus protecting agents:-

- Esterifaction of carboxylic group with benzyl alcohol is most common method of protecting this group.
- The ester is so obtained is converted into the free carboxylic acid either by catalytic reduction or hydrolysis of dilute alkali.



→ Classical peptide synthesis :→

- For the formation of a peptide bond, activation of free carboxylic or amino group of the protected amino acid or peptide is necessary. This activation can be done by converting the protecting species into the corresponding acid chloride before the condensation step.
- Explame:- Synthesis of glycylalanine (dipeptide)

(a) Protection of amino group of glycine by treating it with carbobenzoxy chloride:-

$C_6H_5CH_2OCOCl + H_2N-CH_2-COOH \longrightarrow C_6H_5CH_2OCO-HNCH_2COOH$

Carbobenzoxy chloride — Glycine — carbobenzoxyglycine

(b) Activation of carboxylic group of carbobenzoxyglycine:-

$C_6H_5CH_2OCO-HNCH_2COOH \xrightarrow[-HCl, -SO_2]{SOCl_2} C_6H_5CH_2OCO-HNCH_2COCl$

corresponding acid chloride

(c) condensation of corresponding acid chloride with alanine

$$C_6H_5CH_2OCO-HNCH_2COCl + H_2N-\underset{\displaystyle CH_3}{\underset{|}{CH}}-COOH \xrightarrow[-HCl]{} C_6H_5CH_2OCO-HNCH_2CONH-\underset{\displaystyle CH_3}{\underset{|}{CH}}-COOH$$

carbobenzoxyglycylalanine

(d) Removal of protecting group

$$C_6H_5CH_2OCO-NHCH_2CONH-\underset{\displaystyle CH_3}{\underset{|}{CH}}-COOH \xrightarrow[-C_6H_5CH_3,\ -CO_2]{H_2/Pd} H_2NCH_2CONH-\underset{\displaystyle CH_3}{\underset{|}{CH}}-COOH$$

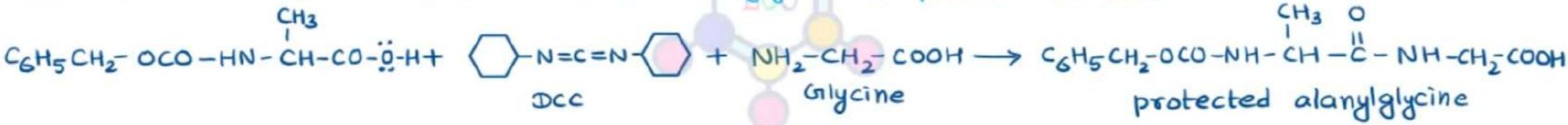
Glycylalanine (Gly-Ala)

→ Modification of classical peptide synthesis:→

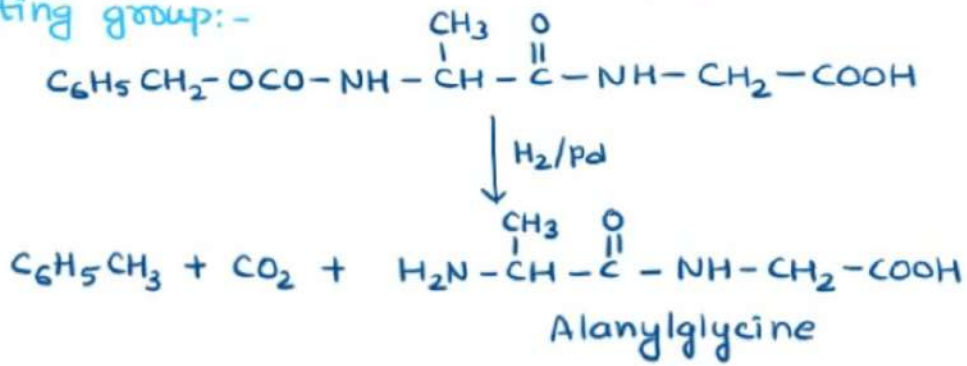
- Step (b) of the classical peptide synthesis can be avoided by treating the protected amino acid with the second molecule of free amino acid in presence of dicyclohexylcarbodiimide (DCC).
- DCC is a dehydrating agent which is used for direct conversion of acids into esters and amides.
- Example:- Synthesis of Alanylglycine

(a) Protection of amino group of alanine:-

(b) Condensation of protected amino acid with glycine in presence of DCC.



(c) Removal of protecting group:-



→ Solid phase peptide synthesis :→

- Classical peptide synthesis has some limitations such as -

(i) Method work well for smaller molecules such as having less than 10 amino acids residues but larger molecules are difficult to synthesise.

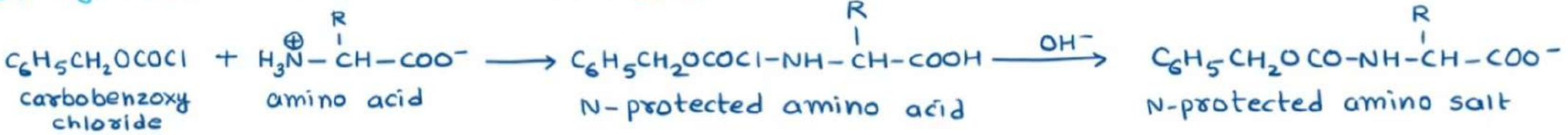
(ii) time consuming.

(iii) Poorer yields.

- To overcome these limitations, Merrifield and co-workers (1964) developed a new technique known as solid-phase peptide synthesis or Merrifield solid-phase peptide synthesis or modern method.
- With the help of this method, the enzyme ribonuclease (containing 124 amino acid residues) in time span of only six weeks. This is why this technique is also referred to as protein making machine.
- The main feature of this technique consist of using insoluble polymer supports such as halomethyl derivative of polystyrene (Abbreviation $PS-CH_2X$) in presence of SnX_4.
- Solid-phase peptide synthesis involves following steps :-

(i) Synthesis of halomethyl derivative of polystyrene :-

(ii) Synthesis of N-protected amino salt :-



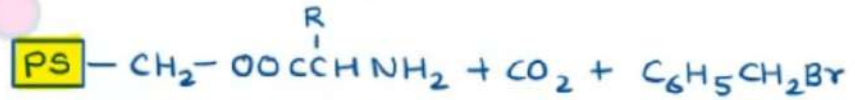
(iii) Reaction of N-protected amino salt with halometyl derivative of polystyrene :-

- This reaction involves nucleophilic displacement of halide by carboxylate ion take place at the benzylic center of polymer support.

* DMF → N,N-dimethylformamide

N-terminal protected amino acid derivative

dil. HBr / CH_3COOH — Removal of protecting group



polystyrene bound amino acid derivative

(iv) Condensation of polystyrene bound amino acid derivative with N-protected amino acid:-

N-protected amino acid

* DCC → dicyclohexylcarbodiimide

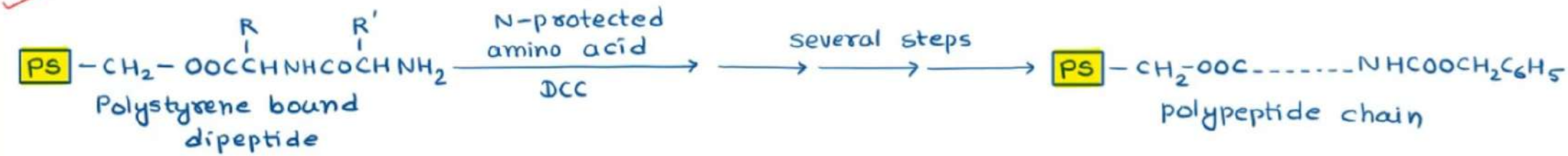
DCC* catalysed condensation

polystyrene bound N-protected dipeptide derivative

dil. HBr — Removal of protecting group

Polystyrene bound dipeptide

(v) Repeatation of condensation steps:-



Polystyrene bound dipeptide

polypeptide chain

(vi) Removal of polystyrene substrate:-

polypeptide chain

→ Advantages of peptide synthesising machine:-

- This solid-phase technique has now been automated. computer compatible peptide synthesising machines have following advantages-

(i) High yield

(ii) Side products can be easily washed away with negligible loss of the desired product.

(iii) Purification of the products after each coupling is not necessary since insoluble polymer bound amino acid or peptide is thoroughly washed with suitable solvents to remove excess of the reagents.

(iv) Since the whole process is automated, it saves a lot of time.

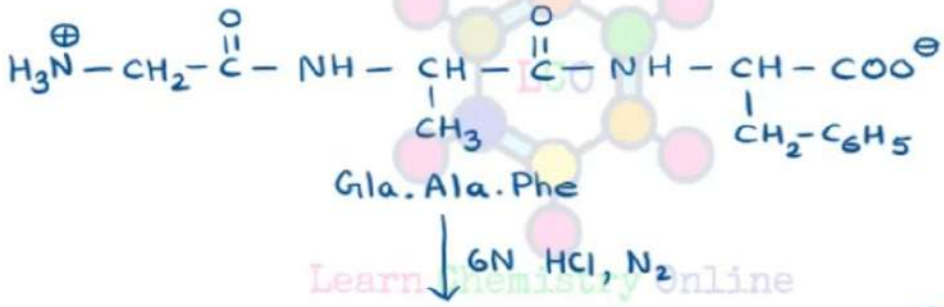
* Merrifield recevied the Nobel Prize for this work.

→ Peptide structure determination :→

- Structure of peptide can be estabilised by determining the number of amino acids constituting the peptide chain and sequence of these amino acids in the peptide chain.

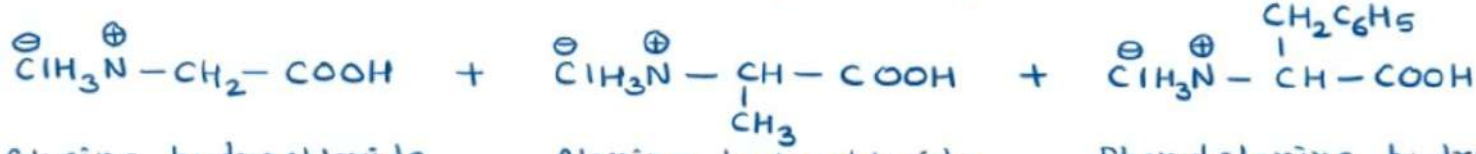
1. Analysis of peptide chain :→

- Hydrolysis of the peptide with 6N HCl under nitrogen atomsphere releases all amino acid except tryptophan which, being a pyrrole, is destroyed by this procedure. The presence of amino acid can be determined by hydrolysis of polypeptide with 2N alkali. However, this procedure, destroys arginine, cystine, serine and threonine and racemises many amino acids. very little racemisation occurs in acidic medium.



Gla. Ala. Phe

↓ 6N HCl, N_2



Glycine hydrochloride Alanine hydrochloride Phenylalanine hydrochloride

- For estimating the amount of each amino acid from a known weight of the given peptide, following methods are used-

(i) Ion-exchange chromatography (ii) pH dependent precipitation (iii) Electrophoresis

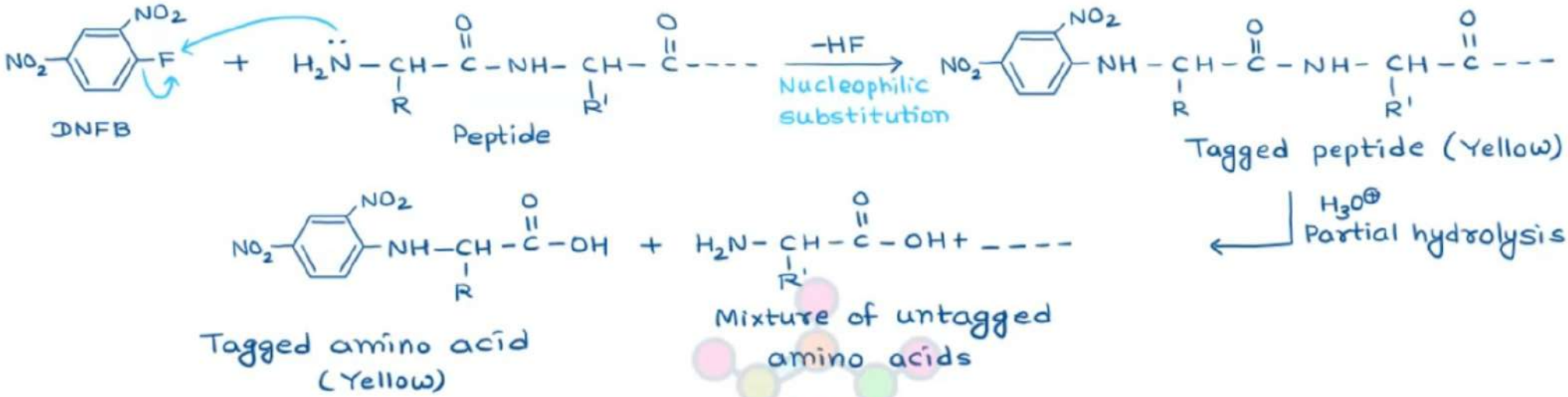
2. Sequence of amino acids: End group analysis:→

- The sequence of amino acids in a peptide chain is determined by end group analysis (terminal residue analysis) coupled with partial hydrolysis.
- The peptides (and proteins) are bifunctional molecules having free amino and carboxylic groups at the end of the chains which are termed as N-terminus and C-terminus, respectively.
- These terminals are detected by using some very specific reagents. When the peptide is treated with a reagent then reagent reacts with a particular terminus to form a tagged peptide.
- The tagged peptide releases tagged amino acid on partial hydrolysis (catalytic or enzymatic) and tagged amino acid is then identified.
- The remaining peptide is tagged and subjected to partial hydrolysis again to yield another tagged amino acid which is again identified.
- This process is repeated till all the tagged amino acid are identified, thus, giving an idea of the amino acid sequence.
- Depending upon the nature of terminus, two types of tagging agents have been developed.

(a) N-Terminal residue analysis:-

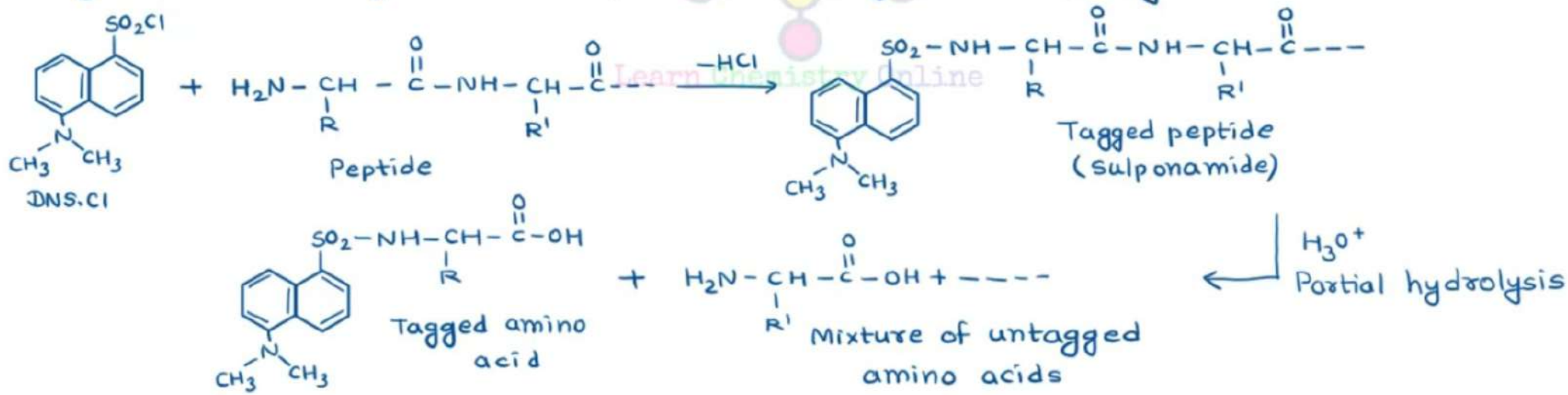
(i) Sanger's method:→

- The most important tagging agent for N-terminus, is 2,4-dinitrofluorobenzene (DNFB)
- DNFB is developed by Frederick Sanger who got Nobel Prize for determinating structure of Insulin. Insulin is a peptide hormone that controls blood sugar level.



(ii) Dansyl method:-

→ Reagent → 5-dimethylamino-1-naphthalenesulphonyl chloride (dansyl chloride or DNS.Cl)



- The Dansyl method is better than Sanger's method because

(i) The corresponding sulphonamides are resistant to the action of acids as compare to the dinitrophenyl substituted amino acids.

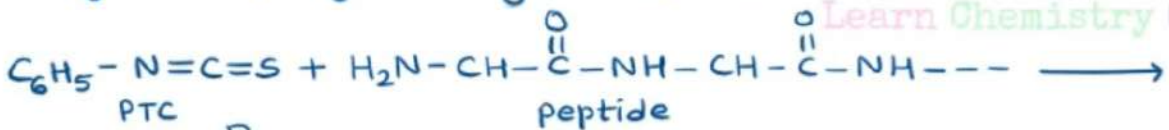
(ii) The dansyl group is highly fluoresent. Therefore, it allows the estimation of dansyl amino acid even at a very lower concentration levels.

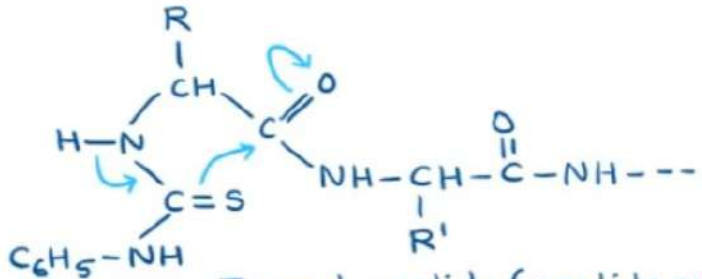
- Limitation :-

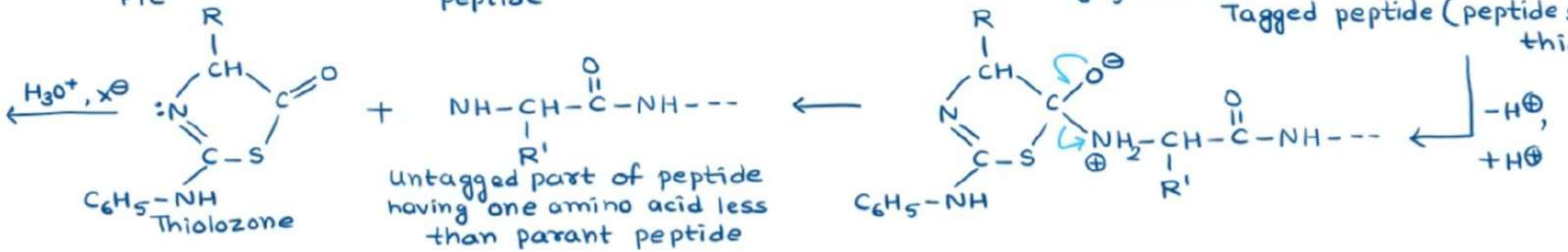
- Both the methods described above have a limitation that the hydrolytic cleavage bring about the complete breakdown of the peptide to the component amino acids, and therefore, the amino acid sequence cannot be easily determined. However, selective enzymatic or partial chemical hydrolysis to smaller fragments followed by fresh N-terminal tagging and hydrolysis has been sucessfully employed for this purpose.

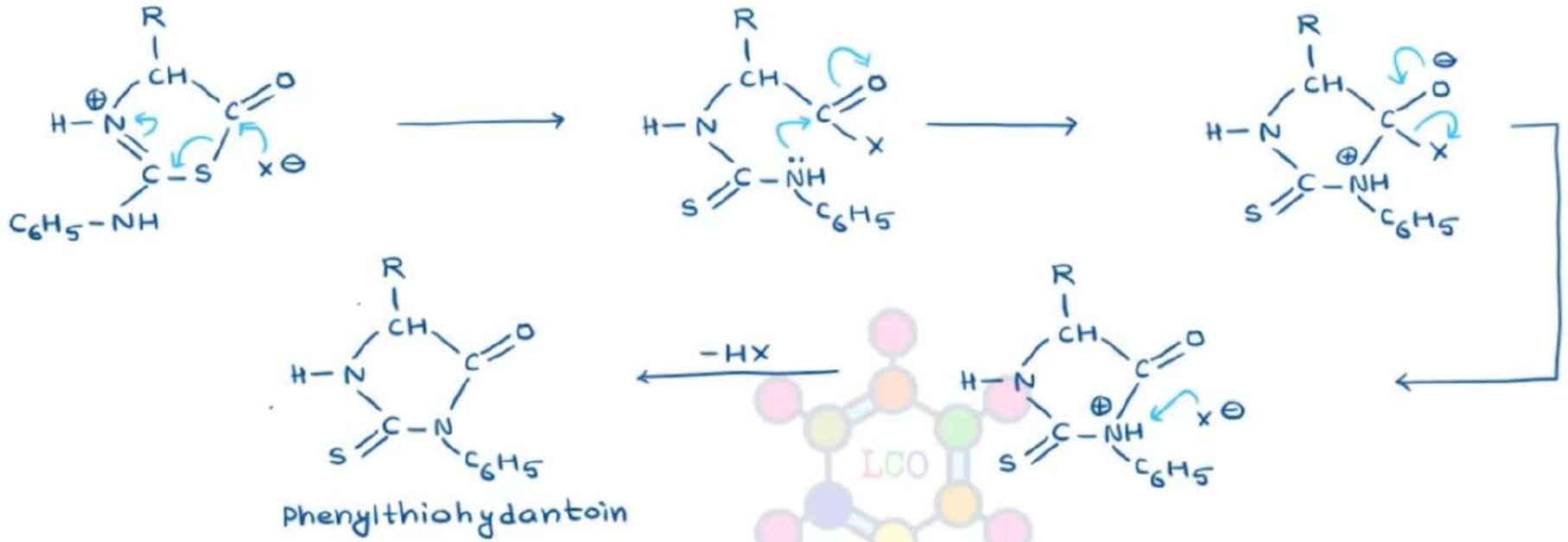
(iii) Edman's method :-

- Developed by Pehr Edman and modified by several workers.
- Reagent → phenylisothiocyanate (PTC)









Phenylthiohydantoin

- The above process can be automatically repeated on the protein molecule upto nearly 40 amino acid residues.

(b) C-Terminal residue analysis :-

- The chemical method for C-terminal analysis have not been so sucessful as those used for N-terminal residue analysis. Two methods used for this purpose:-

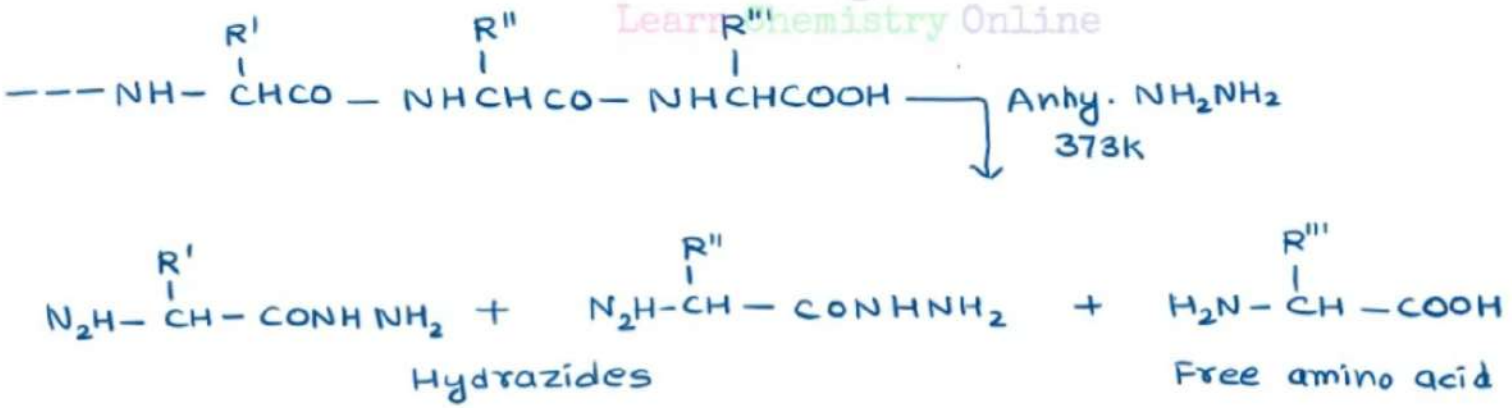
(i) Enzymatic method: Selective hydrolysis :-

- Cetrain enzymes are used for specific cleavage of peptides.
- They are also peptides but they are used in very small amounts so that they do not interfere with the peptide analysis.

- Two enzymes, carboxypeptidase A and B (isolated from swine pancreas) are used for the catalytic cleavage of only that peptide bond which has a free carboxylic group at C-terminus.
- This give rise to free amino acid and shortened peptide which has a new C-terminus. The new peptide is again selectively cleaved to yield a new amino acid and further shortened peptide with another new C-terminus. This process repeats itself step by step till the whole peptide chain cleaves.
- Example:- In the peptide NH_2- A-B-C-D-E-COOH, after certain time interval when the peptide is only partially hydrolysed, a mixture of amino acids A, B, C, D and E is obtained. The relative amounts of these acids will be in the order E>D>C>B>A. The sequence of of amino acids determined on the basis of quantitative analysis.

(ii) Hydrazinolysis method:-

- In this method all amino acid except C-terminus are converted into hydrazides when treated with hydrazine at 373K.



→ Proteins :→

- Protein → Greek word → proteios → first
- Proteins are complex natural polymers and are rated first among the organic compounds essential for growth and maintenance of life.
- They are present in almost all living cells and found in almost every part of every plant and animal.
- In human beings, they are the main constituents of muscles, skin hair, nails, tendons, arteries and connecting tissues.
- In briefly, each living shell is made up of thousand of different proteins.
- The proteins present in different plants and animals and even the proteins present in different tissues of a particular part of plant or animal are different from one another in the composition and biological functions.

→ Characteristics of Proteins :→

1. Elemental composition :→

- The proteins are polyamides of α-amino acids. They generally contains C, H, N, O and S.
- The percentage of these elements varies with the source.
- The range of these elements are-

  C → 45-55% H - 5.5-9% O → 12-30% N → 10-32% S → 0.2-0.3%

  Sometimes they also contains P, I and traces of metals such as Fe, Cu, Zn and Mn.

2. High molecular weight :→

- They have high molecular weights, generally above 10,000 and may run up to millions.

3. Amphoteric nature:-
- Like amino acids, proteins also have amino and carboxylic group terminal and are therefore, amphoteric in nature.

4. Isoelectric points:-
- The behave as both cations and anion under the influence of electric field. Therefore, they also have isolectric points characteristic of amino acids.

5. Optical activity:-
- All proteins are optically active because of the presence of chirality centers at α-position of amino acid residues.

6. Physical state:-
- Proteins are colourless and tasteless molecules. They are soluble in water and their solutions are colloidal in nature. They can be purified by crystallisation.
- Proteins are sensitive to heat, acids, alkalies and many organic solvents.

7. Denaturation:-
- When protein is coagulated or precipited by the change in temp of their aqueous solution or addition of acids or alkali then this process is called denaturation of proteins.
- It is an irreversible process. However, in certain cases, original properties of proteins can be restored by slow cooling if denaturation has been affected by heat. Such process is called renaturation or annealing of the proteins.

8. Colour reactions:-

- Proteins are characterised easily by the following colour reactions-

(i) Ninhydrin reaction:-

- When proteins treated with pyridine solution of ninhydrin, then they give colours ranging from deep blue to violet pink, or even red in some cases.

(ii) Biuret test:-

- This test is characteristic of all compounds having peptide linkage and indicates the presence of peptide linkage in proteins.
- When alkaline solution of protein is treated with a drop of aqueous copper sulphate, bluish voilet colour is obtained.

(iii) Millon's reaction:-

- Millon's reagent → $HgNO_3$ in HNO_3 containing a little HNO_2
- This test is characteristic of phenols and of only those proteins which have phenol group i.e. those having tyrosine unit.
- When such protein is treated with Millon's reagent, a white precipitate is obtained in cold which changes to red on heating

(iv) Xanthoproteic test:-

- Protein upon treatment with nitric acid give yellow or orange colour. This is why our skin turns yellow when it comes in contact with nitric acid.

(v) Heller's test:-

- When conc. HNO_3 is poured along the sides of a test tube containing protein solution, a ppt is appears at the junction of two layers. This test is commonly used for detecting albumin in urine.

→ Classification of proteins :→

(A) Classification on the basis of their structures and features :-

(i) Fibrous proteins :→

- They are threadlike shape and form fibres.
- They are generally insoluble in water and other common solvents but soluble in strong acid or alkaline solutions.
- e.g. (a) Keratin – found in hair, skin, nails etc.
  (b) Myosin – a protein forming muscular fibre.

(ii) Globular proteins :→

- They are spherical is shape.
- They are soluble in water and dilute acids or alkalies.
- e.g. (a) Insulin – found in pancreas
  (b) Albumin – Egg white

(B) Classification on the basis of hydrolysis products :-

(i) Simple proteins :-

- They are simple polyamides which give only amino acids on hydrolysis.
  (a) Albumins (water soluble proteins, e.g., egg albumin, serum albumin etc.)
  (b) Globulins (water insoluble but soluble in dilute salts, acids or alkali, e.g. serum globulin, tissue globuline etc.)

(c) Glutelins (Insoluble in water or salt solution but soluble in dilute alkalies or acids, e.g., gluetin from wheat and oxyzerin from rice)

(d) Albuminoids or scleroproteins (water insoluble but soluble in strong acid or alkali solutions, e.g., keratin, fibronin etc.

(ii) Conjugated proteins:→

- They are proteins which have some non-protein residue attached to the protein molecules.
- Such non-proteins moieties are called the prosthetic groups.
- The main functions of prosthetic group is to control the specific biological action of proteins.
- They are sudivided into following -

(a) Chromoproteins (having coloured prosthetic group, e.g., haemoglobin has porphin nucleus as the prosthetic group)

(b) Nucleoproteins (they contain nucleic acid as prosthetic group)

(c) Glycoproteins (they contain sugar residue as prosthetic group)

(d) Phosphoproteins (they have phosphoric acid residue as prosthetic group)

(e) Lipoproteins

(iii) Derived proteins:→

- These are lower proteins or peptides and are formed by degradation of proteins or enzymes.
- e.g. denaturated proteins, proteoses, peptones and polypeptides

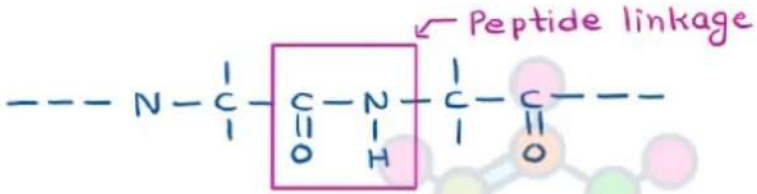
Proteins ⟶ Proteoses ⟶ Peptones ⟶ Polypeptides

→ Structure of proteins and levels of protein structure:-

- Structure and shape of proteins can be studied at four different levels:-

(1) Primary structure:→

- Primary structure of proteins contain one or more polypeptide chains and each polypeptide has amino acids linked with each other in a specific sequence. This sequence is called primary structure of protein.



- In primary structure of proteins every third atom of the peptide chain contains a group referred to a 'side chain' which is characteristic of the amino acid present.

example

$-H$ → Glycine	$-CH_2C_6H_5$ → Phenylalanine
$-NH_2$ → Lycine	$-COOH$ → Aspartic acid

- The difference in number of acidic or basic side chains is responsible for separation of proteins by electrophoresis.
- The procedure for determination of number of amino acid molecules and its sequence is same as peptides.
- Any change in the sequence of amino acids creates a different proteins.

(2) Secondary structure :→

- In these type of structures, peptide backbone can interact itself.
- These interactions depend upon size of the 'side chain'.
- Depending upon the size of group R, following different structures are possible-

(a) Flat sheet :→

- If the case of proteins having side chains of smaller size (say H), the peptide chains are regarded as fully extended in zig-zag manner with alternate side chains being on the same side situated at a fixed distance.
- Such extended peptide chains lie side by side and each one is joined to two neighbouring chains through hydrogen bonds. This results in the formation of flat sheet.

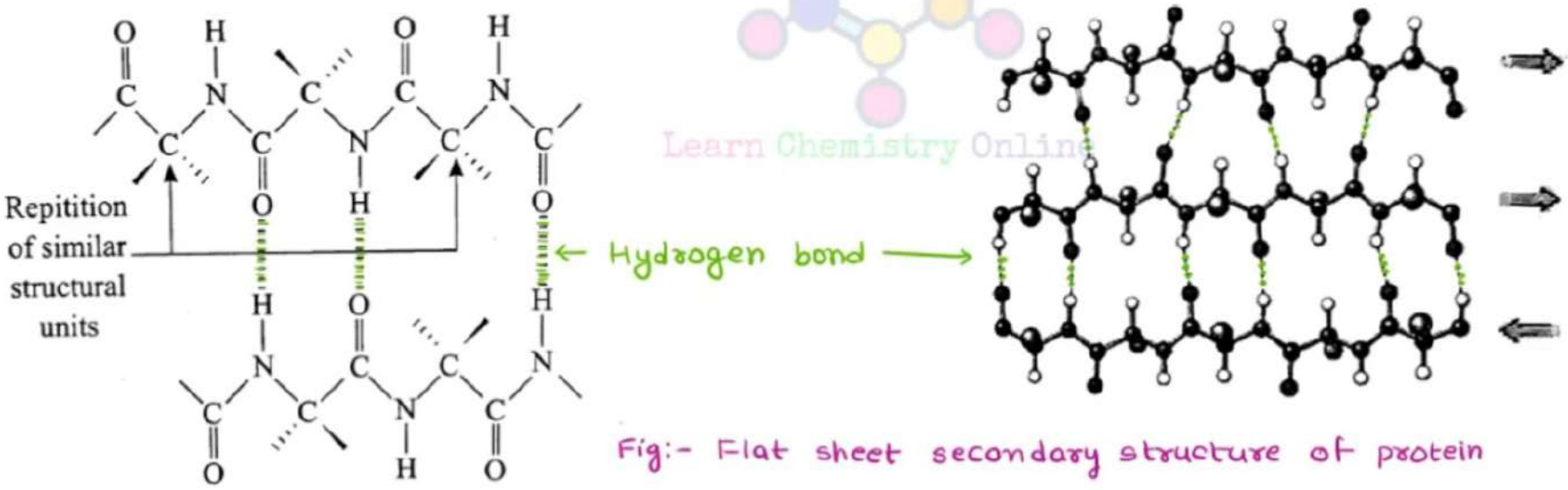


Fig:- Flat sheet secondary structure of protein

(b) Pleated sheet :→

- If the side chain is of moderate size, the peptide chain contracts a little in order to accommodate them. This results in the pleated sheet structure or the β-arrangement of the protein.
- The distance between alternate chains becomes short and they are held together by hydrogen bonds.

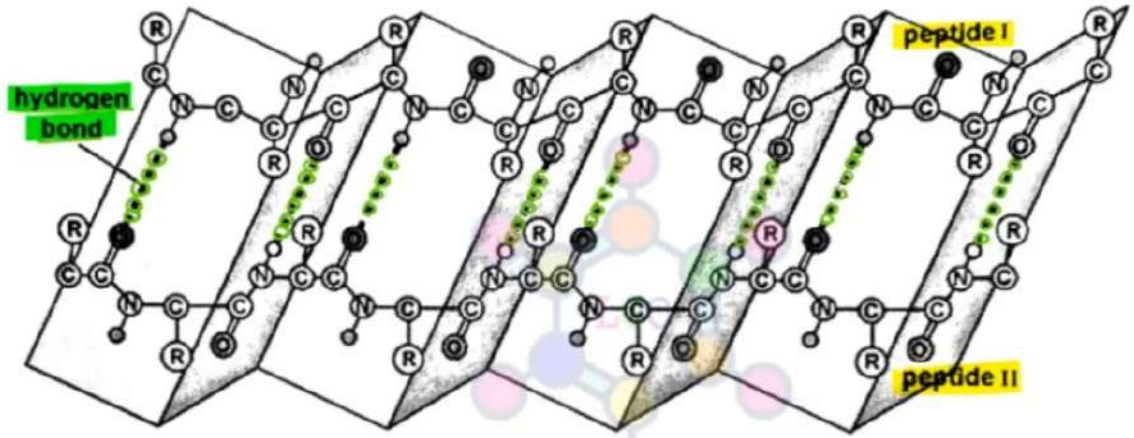


Fig:- The β-pleated sheet of protein

(c) α-Helical structure:→

- If the side chains are quite large, then whole peptide chain is coiled in particular helical form known as α-helix.
- As all the naturally occuring amino acids have L-configuration, all protein helices have right handed.

- Each amide group is hydrogen bonded with every third amide in either direction along the chain.
- 3.6 amino acid units form one turn of helix and the side chains extend out away from the axis of this helix.

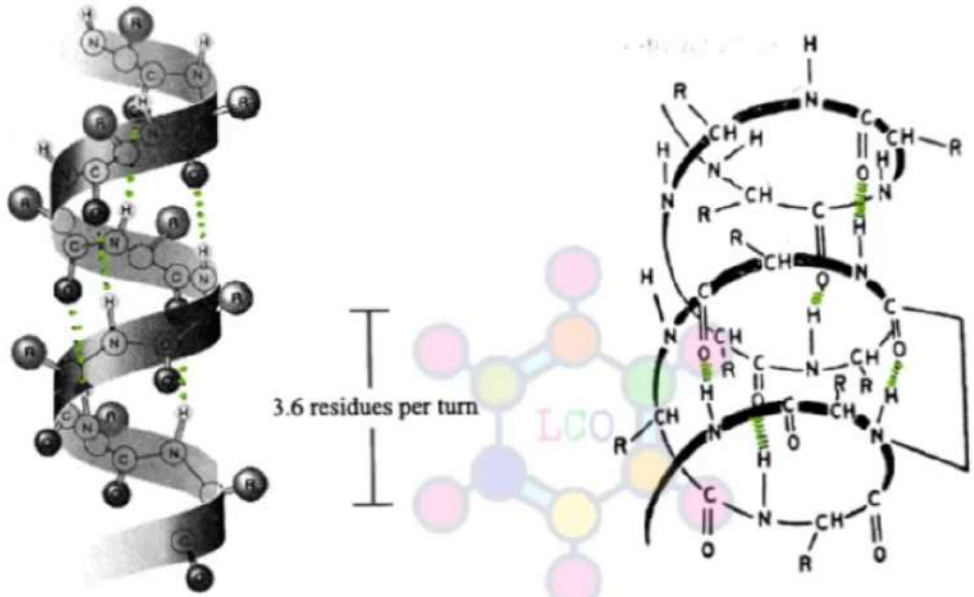


Fig:- A right handed α- helical structure of a typical protein

(3) Tertiary structure:→

- The tertiary structure of protein constitutes a three-dimensional shape due to further folding of polypeptide chain having compact, highly complex structure which is characteristic of a particular protein.

- Tertiary structures of protein contain following bonding interaction between the amino acid chains-

(a) Very weak Van der Waals forces → e.g. interaction between tryptophan and phenylalanine residues.

(b) Additional hydrogen bonds → Apart from usual ones. e.g. hydrogen bonding between side chains of serine and histidine.

(c) Intramolecular salt like dipolar bonding → e.g. between 'extra' carboxylic and amino groups of aspartic acid and lysine, respectively.

(d) Disulphide covalent bonding → e.g. between two cysteine residues.

- The tertiary structure folds the entire protein molecule in a particular shape and stablises the protein molecule. This particular type of folding is called native conformation of protein.
- Globular and Fibrous shape are tertiary structures of proteins. Globular proteins are spherical in shape and Fibrous proteins have rod-like shape.

(4) Quaternary structure:→

- Some of the proteins are composed of two or more polypeptide chains. These are called sub-units.
- The spatial arrangement of these subunits with respect to each other is known as quaternary structure.
- Example:- Myoglobin (contain haeme unit as prosthetic group)

Fig:- Diagramatic representation of myoglobin molecule.

→ Protein denaturation/renaturation :→

- Proteins are sensitive to heat, mineral acids, alkalies etc.
- When soluble form of proteins are heated or treated with mineral acids, they undergo coaguation or precipitation to given fibrous protein which are insoluble in water. The coagulated protein are called denatured proteins and this process is called denaturation of proteins.
- This coagulation also results in the loss of the biological activity of the proteins.
- Chemically, denaturation does not change the primary structure but brings about change in the secondary and tertiary structures of proteins.

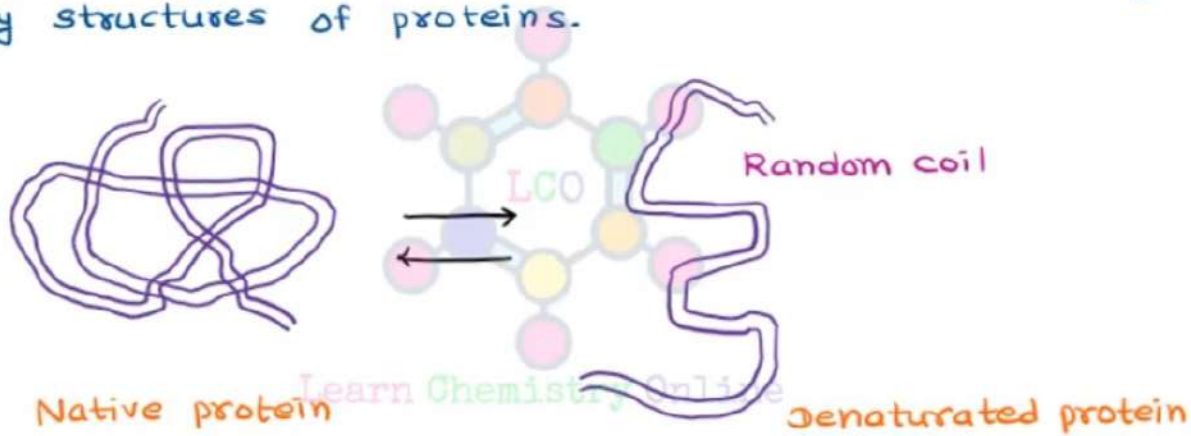


Fig:- Denaturation of globular protein

- Examples of denaturation of proteins are:-

(a) Coagulation of albumin present in egg white:-

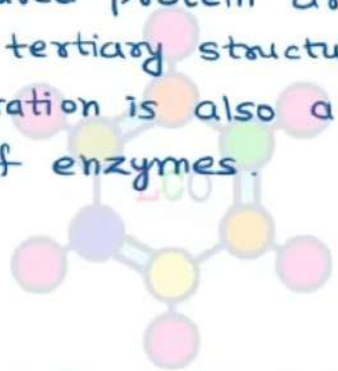
- The soluble globular protein (albumin) present in the egg is denatured when the egg is boiled hard resulting in the formation of insoluble fibrous proteins.

(b) Coagulation of milk:-

- When milk is heated with an acid (lemon juice or tartaric acid) the denaturation of milk leads to formation of cheese. During this denaturation, the globular milk protein (lactalbumin) becomes fibrous.
- Earlier, denaturation, was considered to be irreversible process. However, now it has been shown that in some cases, the process is actually reversible. The reverse process is called renaturation.

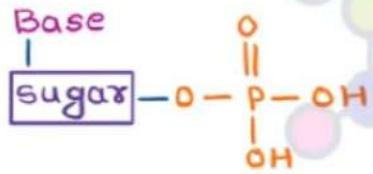
Example:- When temp. and pH of denatured protein are bought back to conditions which stabilize native protein, secondary and tertiary structures of the proteins are restored.

Consequently, renaturation is also accompanied by recovery of the biological activity particulary in case of enzymes.



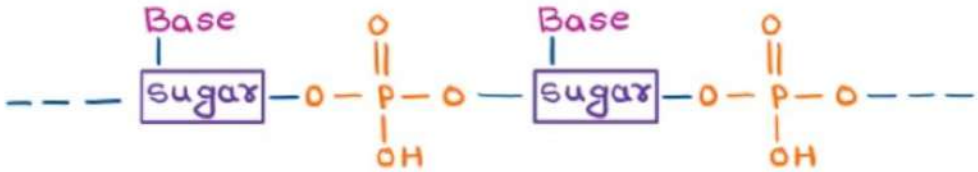
→ Nucleic acids:→

- Nucleic acids are class of biopolymers composed of long chains of repeating monomeric units called as nucleotides.
- Each nucleotide is made up of three constituents-
  (a) A pentose (ribose or deoxyribose)
  (b) A heterocylic base (a purine or a pyrimidine)
  (c) Phosphoric acid
- A typical nucleotide may be represented as-



- The polymeric chain of nucleic acid is formed by joining 3'-OH group of one nucleotide to 5'-OH of the nucleotide through a phosphodiester bond. This constituents the "backbone structure" of nucleic acids.
- A molecule containing two nucleotide units joined together is called a dinucleotide.
- A molecule containing three to ten nucleotide units is called as oligonucleotide.
- A molecule containing more than ten nucleotide units is called polynucleotide.
- In the polymeric chain one end has a free 5'- phosphate & other end has free 3'-OH group.

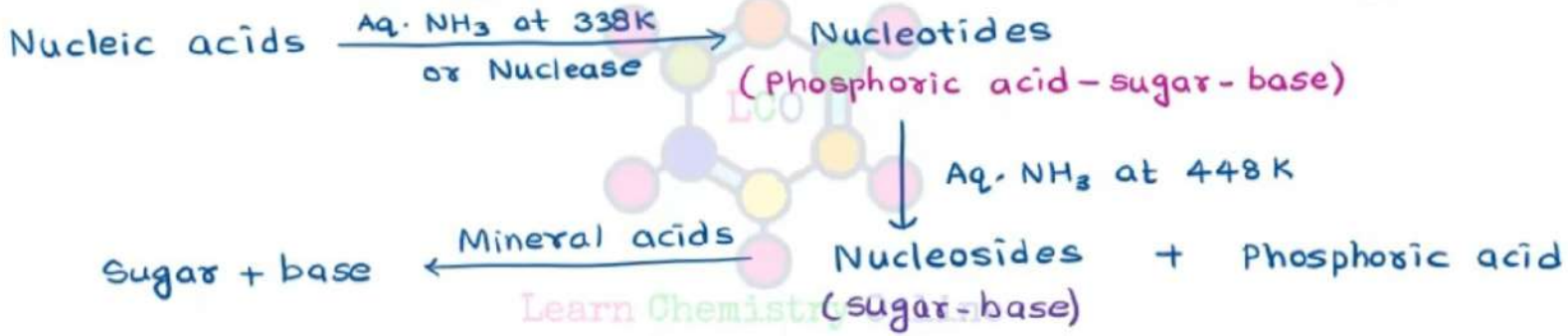
- Polynucleotides may be represented as-



- The first nucleic acid was isolated from the nucleus of puss cell and was found to be acidic in nature. so, this class of macromolecules is called nucleic acid.
- Nucleic acids are the genetic material of the cells and are responsible for transmission of hereditary effects.
- They also responsible for biosynthesis of proteins.
- The genetic information coded in nucleic acids controls the structure of all proteins including enzymes and thus governs the entire metabolic activity in the living organism.

→ Constituents of nucleic acids:→

- Hydrolysis of nucleic acids give two different types of products under different conditions.
- When nucleic acids treated with aqueous ammonia at 388K or in the presence of enzyme nuclease, they give nucleotides. In nucleotides, molecular ratios, of sugar, base and phosphoric acid is 1:1:1.
- The nucleotide upon further treatment with aqueous ammonia at elevated temp. (448K) librate phosphoric acid. Thus, produced a another class of compounds called as nucleosides, in which molecular ratio of sugar and base is 1:1.
- The nucleosides upon further hydrolysis with mineral acids affords free sugar and bases.

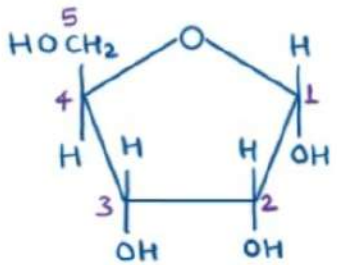


- Complete hydrolysis of all nucleic acids produce a mixture of three different types of compounds-
  (i) Sugar (ii) Phosphoric acid (iii) Base

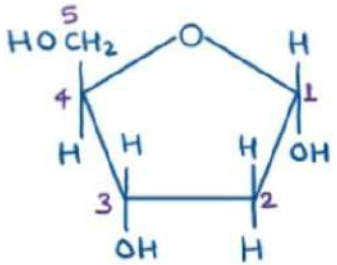
(i) Sugar:-

- Two pentose sugar, isolated by the hydrolysis of nucleic acids are D-(-)-ribose and 2-deoxy-D-(-) ribose.
- Both these sugars are present in furanose form.

- In these sugars, the C_1 carbon is anomeric carbon due to which both these sugars are exist in both α- and β- forms.



α- D-(-)- Ribose (In RNA)

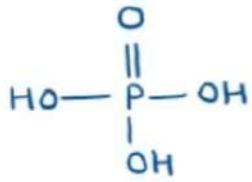


α- 2-Deoxy- D-(-)- ribose (In DNA)

Fig:- Furanose forms of D-Ribose and 2-Deoxy-D-ribose

(ii) Phosphoric acid:→

- It has three ionisable -OH groups with pKa values of 2.1, 7.2 and 12.3.
- It can form mono, di or triphosphoesters with alcohols.



(iii) Base:→

- Two different types of heterocyclic nitrogenous bases have been isolated by the hydrolysis of nucleic acids.

(a) Purines (b) Pyrimidines

(a) Purines:-

- Adenine (A) and Guanine (G)

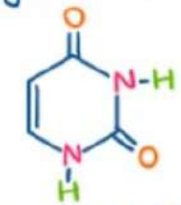
Adenine (A)
(6-Aminopurine)

Guanine (G)
(2-Amino-6-oxopurine)

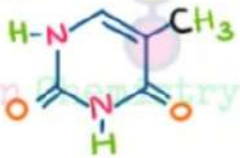
First isolated from guano (bird manure), hence the name

(b) Pyrimidines :→

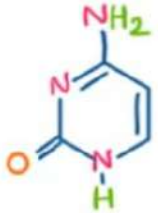
- Uracil (U), Thymine (T) and Cytosine (C).



Uracil (U)
2,4-dihydroxypyrimidine
or Pyrimidinedione



Thymine (T)
5-Methyl-2,4-dihydroxypyrimidine
or methylpyrimidinedione

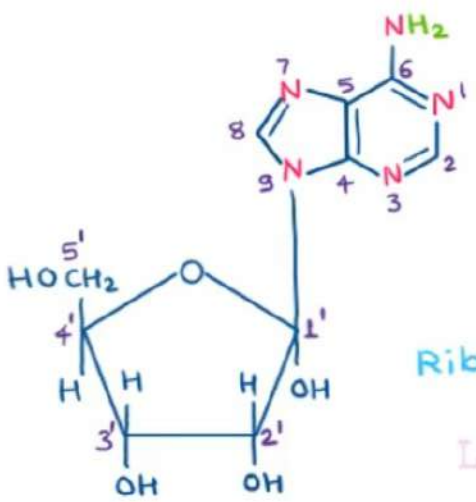


Cytosine (C)
2-Oxo-4-aminopyrimidine
or aminopyrimidinone

- DNA contains four bases → Adenine, Guanine, Cytosine and Thymine. (AGCT)
- RNA contains four bases → Adenine, Guanine, Cytosine and Uracil . (AGCU)

→ Nucleosides and nucleotides (Ribonucleosides and ribonucleotides):→

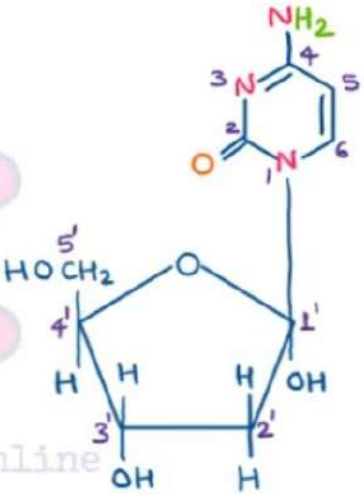
- Nucleosides contain only two constituent molecules of nucleic acids, i.e. a pentose sugar and a nitrogenous base.
- During formation of nucleosides, N-1 of pyrimidine base or N-9 of the purine base is linked to C-1 of sugar by a β-N-glycosidic linkage.
- Example:-



Adenosine
(9-β-D-ribofuranosyladenine)

Adenine

Ribose



Cytosine

2-Deoxyribose

2'-Deoxycytidine
(1-β-D-deoxyribofuranosylcytosine)

- Thus, depending upon the type of sugar present, nucleosides can be classified in the following categories-

(i) Ribonucleosides (containing ribose)

(ii) Deoxyribonucleosides (containing deoxyribose)

- Examples:-

Base	Abbreviation	Nucleoside	Deoxyribonucleoside
Adenine	A	Adenosine	2′-Deoxyadenosine
Guanine	G	Guanosine	2′-Deoxyguanosine
Cytosine	C	Cytidine	2′-Deoxycytidine
Thymine	T	Thymidine	Deoxythymidine
Uracil	U	Uridine.	—

→ Nucleotides:→

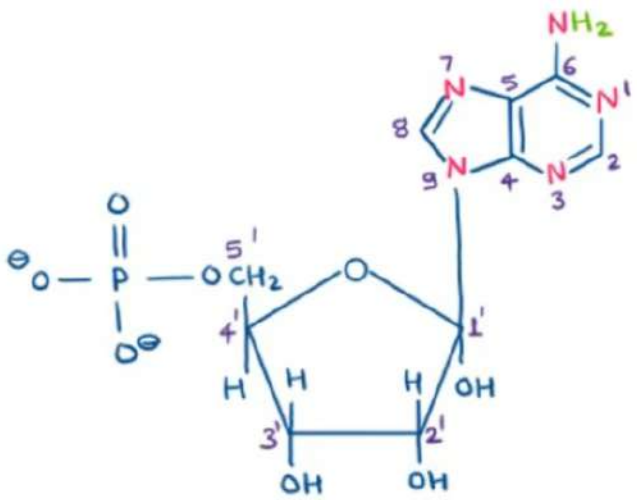
- Nucleotides contain three constituent molecules of nucleic acids, ie. pentose sugar, nitrogenous base and phosphoric acid.
- Nucleotides are nucleosides having either the 5- or 3-OH group bondend as ester to phosphoric acid. In other words, nucleotides are nucleoside monophosphate.
- Like nucleosides, nucleotides can be classified into two categories depending upon the type of pentose sugar.

(i) Ribonucleotides (containing ribose) and (ii) Deoxyribonucleotides (containing deoxyribose)

-Examples:-

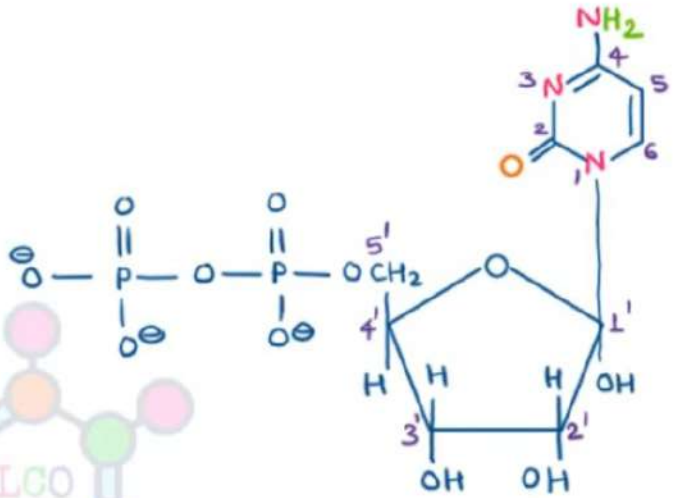
Base	Nucleotide	Abbr.	Deoxynucleotide	Abbr.
Adenine	Adenosine 5′-phosphate	AMP	2-Deoxyadenosine 5′-phosphate	dAMP
Guanine	Guanosine 5′-phosphate	GMP	2-Deoxyguanosine 5′-phosphate	dGMP
Cytosine	Cytidine 5′-phosphate	CMP	2-Deoxycytidine 5′-phosphate	dCMP
Thymine	—	—	Thymidine 5′-phosphate	dTMP
Uracil	Uridinel 5′-phosphate	UMP	—	—

- Structure and names of some typical nucleotides are as under-



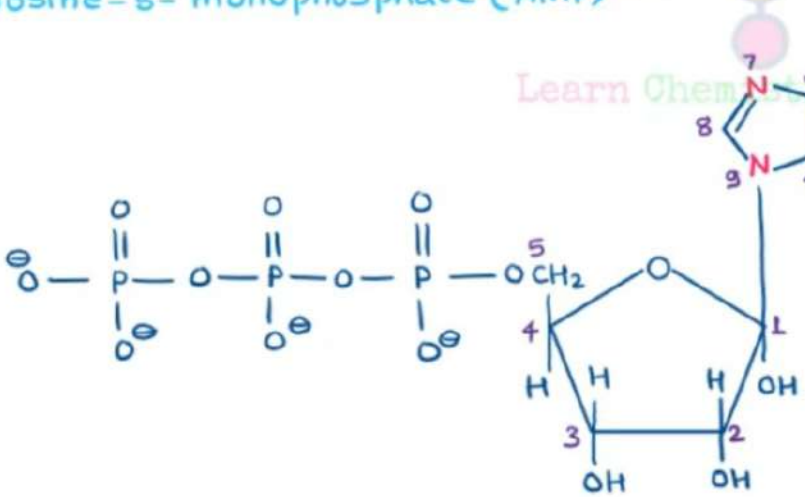
Adenylate

Adenosine-5'- monophosphate (AMP)



Uridylate

Uridine-5'- diphosphate (UDP)



Adenosine-5'- triphosphate (ATP)

(Energy rich molecule or energy storing molecule)

→ Types and functions of nucleic acids:→

→ Types of nucleic acids:→

- Depending upon the type of sugar present, nucleic acids are classified into following categories-

(i) Deoxyribonucleic acids (DNA) → Contain 2'-deoxy-D-(-)-ribose

(ii) Ribonucleic acids (RNA) → Contain D-(-)-ribose

- Some important points of difference between DNA and RNA are given in following table:-

Deoxyribonucleic acid (DNA)	Ribonucleic acid (RNA)
1. The sugar present in DNA is 2-*deoxy* *D*–(–)–*ribose*.	1. The sugar present in RNA is *D*–(–)–*ribose*.
2. DNA contains cytosine and *thymine* as pyrimidine bases and, guanine and adenine as purine bases.	2. RNA contains cytosine and *uracil* as pyrimidine bases and, guanine and adenine as purine bases.
3. DNA has double stranded α-helical structure.	3. RNA has single stranded α-helical structure.
4. DNA chiefly occurs in nucleus of the cell.	4. RNA mainly occurs in cytoplasm of the cell.
5. DNA molecules are very large; their molecular weights may vary from 6 milliion to 16 million.	5. RNA molecules are relatively small with molecular weights ranging from 20,000 to 40,000.
6. DNA has the unique property of replication.	6. RNA usually does not replicate.
7. DNA controls the transmission of hereditary effects.	7. RNA controls the synthesis of proteins.

→ Functions of nucleic acids:→

(I) Functions of DNA:→

- It acts as a template for RNA which carries out the protein synthesis. i.e. DNA controls the synthesis of proteins.
- It controls the entire structral and functional make up of the cell.
- It has unique property of self replication. The self replication is the ability to build another molecule of its own kind and hence responsible for passing heredity trait from one generation to another.
- When exposed to X-Rays, UV rays, Y-rays and some chemicals, DNA undergoes mutation. Muntation means that there a slight change occurs in the sequence of nitrogenous bases along the DNA strands. This change is reflected in the subsequent generations.

(ii) Functions of RNA:→

- It controls the synthesis of proteins. There are three types of RNA molecules in a cell which controls the biosynthesis of specific proteins.

(a) messenger RNA (m-RNA):- The messenger RNA is a complementary copy of one strand of DNA which carries the message of DNA for specific protein synthesis when required.

(b) ribosomal RNA (r-RNA):- The ribosomal RNA provides the site for the protein synthesis in the cytoplasm but it does not carry any message of DNA.

(c) transfer RNA (t-RNA):- The transfer RNA is a small molecule and it transfered activated α-amino acids to the site of protein synthesis. There are 22 t-RNA molecules specific for one amino acid.

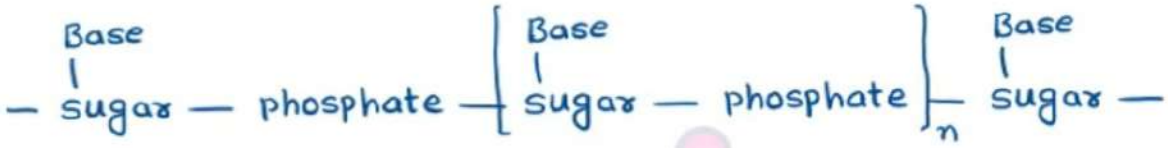
- RNA controls the process of learing and memory storages.

→ The double helical structure of DNA:→

- DNA have primary, secondary and high level structures:-

(1) Primary structure:→

- The primary structure of nucleic acid can be represented by the following structure:-



(2) Secondary structure:→

- Qualitative analysis of bases in the DNA hydrolysate (E. charagoff) revealed that the base composition in DNA varied from one species to the other, but in all cases the molar ratios of adenine to thymine and guanine to cytosine are 1:1. In other words, the total number of moles of purines are equal to the total number of moles of pyrimidines, i.e. A+G = C+T. However, the (A+T)(C+G) ratio differes from species to species. e.g. it is 1.52 in man and 0.93 in E.Coli.
- Based upon analytical data and X-Ray diffraction studies, Watson and Crick proposed a double helical structure of DNA.
- This model not only explained the base equivalence but other important characteristics such as replication of DNA.

- Salient features of Watson-Crick model are-

(i) The DNA molecule consist of two polynucleotide strands coiled around a common axis in the form of a right handed helix. These strands run in opposite directions, i.e. from 5'-terminal to 3'-terminal in case of one strand and from 3'- terminal to 5'- terminal in the other strand.

(ii) The backbone of each strand consist of sugar-phosphate units which are found on the periphery of the helix. Base are present in centre. The planes of bases are orthogonal to the axis of the helix or to the planes of sugar- phosphate chains.

(iii) The base pair of the two strands are held together through hydrogen bonds between specific base pairs. This pairing always occurs between adenine and thymine and between guanine and cytosine. The guanine-cytosine base pairing takes place through three hydrogen bonds (G≡≡≡C) whereas adenine-thymine base pairing takes place through two hydrogen bonds (A==T).

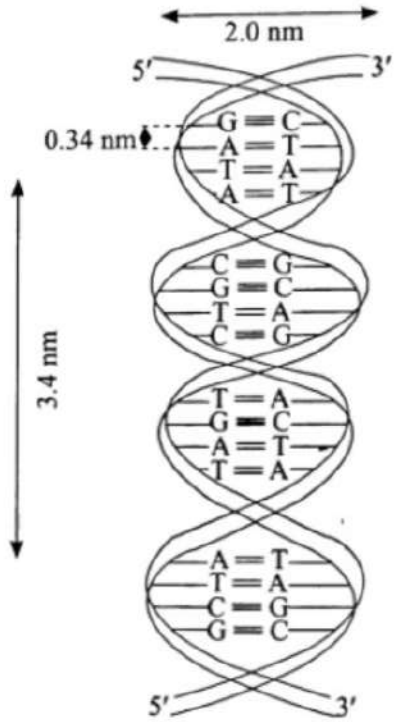


Fig:- The double α-helical structure of DNA.

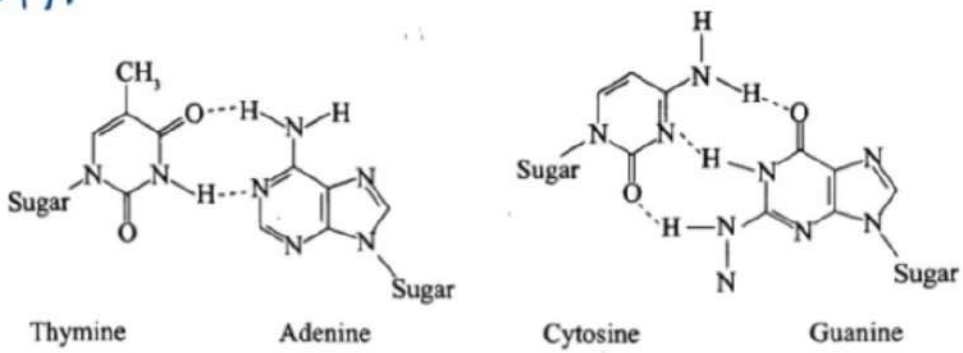


Fig:- pairing of complementary bases

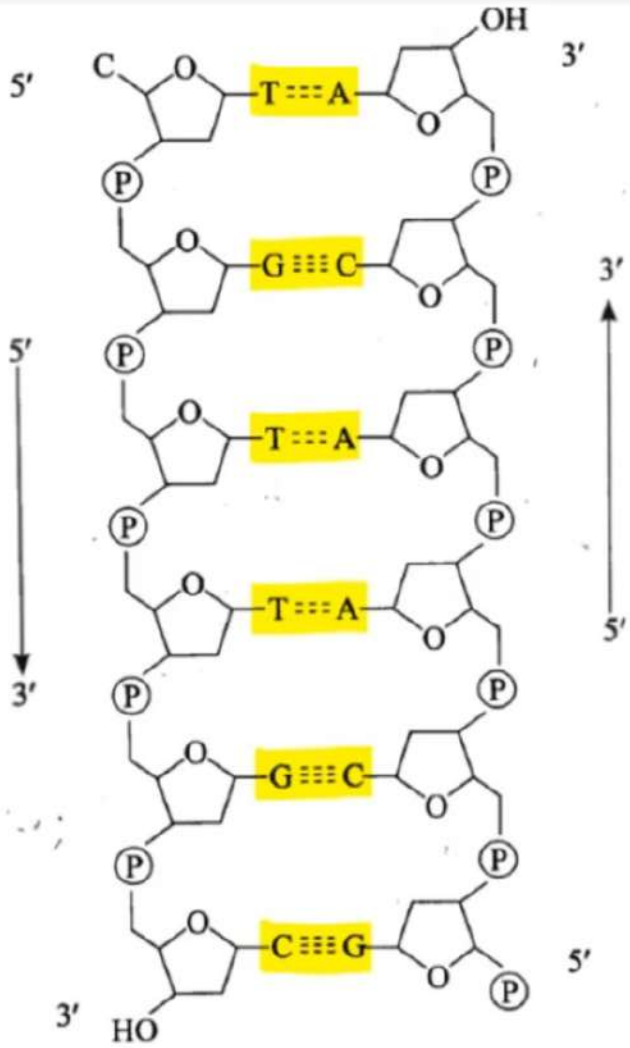


Fig:- Complementary base pairing in DNA A==T and G===C

- The two strands of the double helix are complementary and not identical because the sequence of one strand automatically fixes that of the other due to the base-pairing principle.
- The distance between two adjacent base pairs is 0·34 nm.
- The distance between any two successive turns of the helix is 3·4 nm.
- The diameter of helix is about 2·0 nm.
- On heating, two strands of DNA separates from each other. This is called melting. However, on cooling these again hybridise. This is called annealing.
- The temperature at which the two strands separate completely is called its melting temperature (T_m) which is specific of each specific sequence.
- Unlike DNA, RNA has single strand.