Amino acids, Peptides and Proteins

The Proteins are essentially polypeptide macromolecules formed from α-amino acids of L-configuration linked by peptide (-CONH) links. The α-carbon atom can produce two optical isomers (viz: D- and L-isomers). Only the L-isomers are the constituents of Proteins. Twenty different naturally occouring amino acids are involved in protein formation. The sequence of amino acid residues would determine the Chemical and Physical properties of Proteins.

Although the amino acids are commonly shown as containing an amino group and a carboxyl group, $NH_2CHRCOOH$, certain properties, both Physical and Chemical, are not consistent with this structure.

i. In contrast to amines and carboxylic acids, the amino acids are non volatile crystalline solids which melt with decomposition at fairly high temperature.

ii. They are insoluble in non-polar solvents like Pet-ether, benzene or ether and are appreciably soluble in Water

iii. Their aqueous solutions behave like solutions of substances of high dipole moment.

iv. Acidity and basicity constants are ridiculously low for -COOH and NH_2- groups.

All these properties are quite consistent with a dipolar ion structure for the amino acids.

$$H_3\overset{+}{N}-CH(R)-COO^-$$

dipolar ions

The Physical properties - melting point, solubility, high dipole moment - are just what would be expected of such a salt.

The acid-base properties also become understandable when it is realized that the measured K_a actually refers to the acidity of an ammonium ion, $R\overset{+}{N}H_3$

$$H_3\overset{+}{N}CHRCOO^- + H_2O \rightleftharpoons H_3O^+ + H_2NCHRCOO^-$$
(I) (II)

$$K_a = \frac{[H_3O^+]\,[H_2NCH_2COO^-]}{[{}^+H_3NCHRCOO^-]}$$

Similarly and K_b actually refers to the basicity of a carboxylate ion, $RCOO^-$

$$H_3\overset{+}{N}CHRCOO^- + H_2O \rightleftharpoons H_3\overset{+}{N}CHRCOOH + OH^-$$
(I) (III)

$$K_b = \frac{[H_3\overset{+}{N}CHRCOOH]\,[OH^-]}{[H_3\overset{+}{N}CHRCOO^-]}$$

* How does zwitter ionic character of an amino acid change with pH of the medium? (3)

When the solution of an amino acid is made alkaline, the dipolar ion (I) is converted into anion (II), the stronger base, hydroxide ion, removes a proton from the ammonium ion and displaces the weaker base, the amine.

$$H_3\overset{+}{N}CHRCOO^- + OH^- \rightleftharpoons H_2NCHRCOO^- + H_2O$$

Strong acid — Strong base — Weaker base — Weaker acid

When the solution of an amino acid is made acidic, the dipolar ion (I) is converted ~~in the~~ into the cation (III); the stronger acid, H_3O^+, gives up a proton to the carboxylate ion, and displaces the weaker carboxylic acid.

$$R\overset{+}{N}H_3CHRCOO^- + H_3O^+ \rightleftharpoons H_3\overset{+}{N}CHRCOOH + H_2O$$

Isoelectric point of amino acids:

What happens when a solution of an amino acid is placed in an electric field depends upon the acidity or basicity of the solution.

$$H_2N\text{-}CHR\text{-}COO^- \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} H_3^+N\text{-}CHR\text{-}COO^- \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} H_3^+N\text{-}CHR\text{-}COOH$$

(II) (I) (III)

In quite alkaline solution anions (II) exceed cations (III), and there is a net migration of amino acid toward the anode. In quite acidic solution, cations (III) are in excess, and there is a net migration of amino acid toward the cathode. If II and III are exactly balanced, there is no net migration; under such conditions any one molecule exists as a positive ion and as a negative ion for exactly the same amount of time, and any small movement in the direction of one electrode is subsequently canceled by an equal movement back toward the other electrode. The hydrogen ion concentration of the solution in which particular amino acid does not migrate under the influence of an electric field is called isoelectric point of that amino acid.

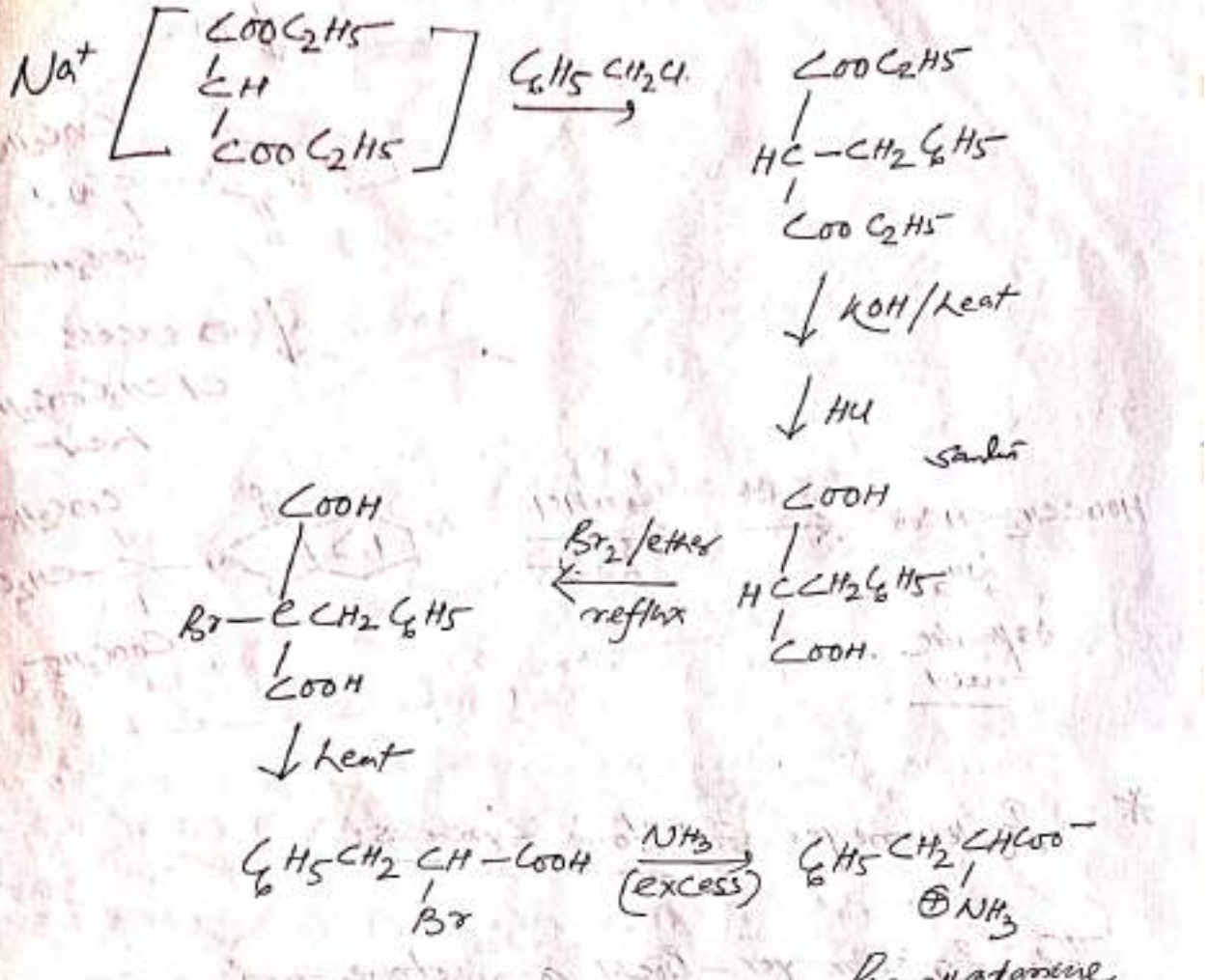
Preparation of amino acids:

Of the many methods that have been developed for synthesizing amino acids, we shall here use only one: amination of α-halo acids. Considered in its various modifications, this method is probably the most generally useful.

Sometimes an α-chloro or α-bromo acid is subjected to direct ammonolysis with large excess of concentrated aqueous ammonia. For example

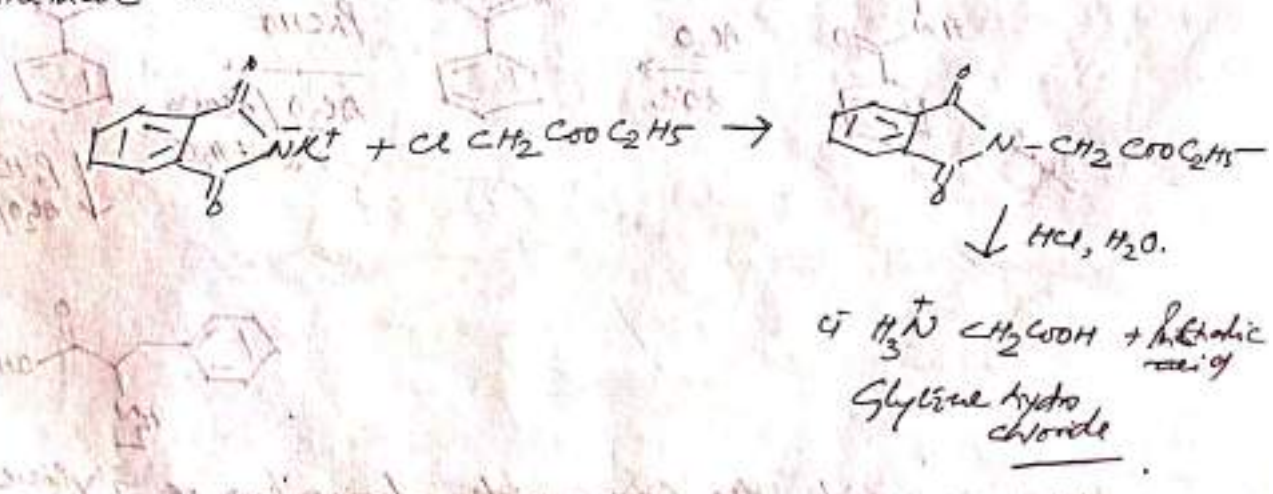
$$CH_3CH_2COOH \xrightarrow{P/Br_2} CH_3\underset{Br}{\underset{|}{C}}H\text{-}COOH \xrightarrow{NH_3} CH_3\underset{NH_2}{\underset{|}{C}}H\text{-}COOH$$

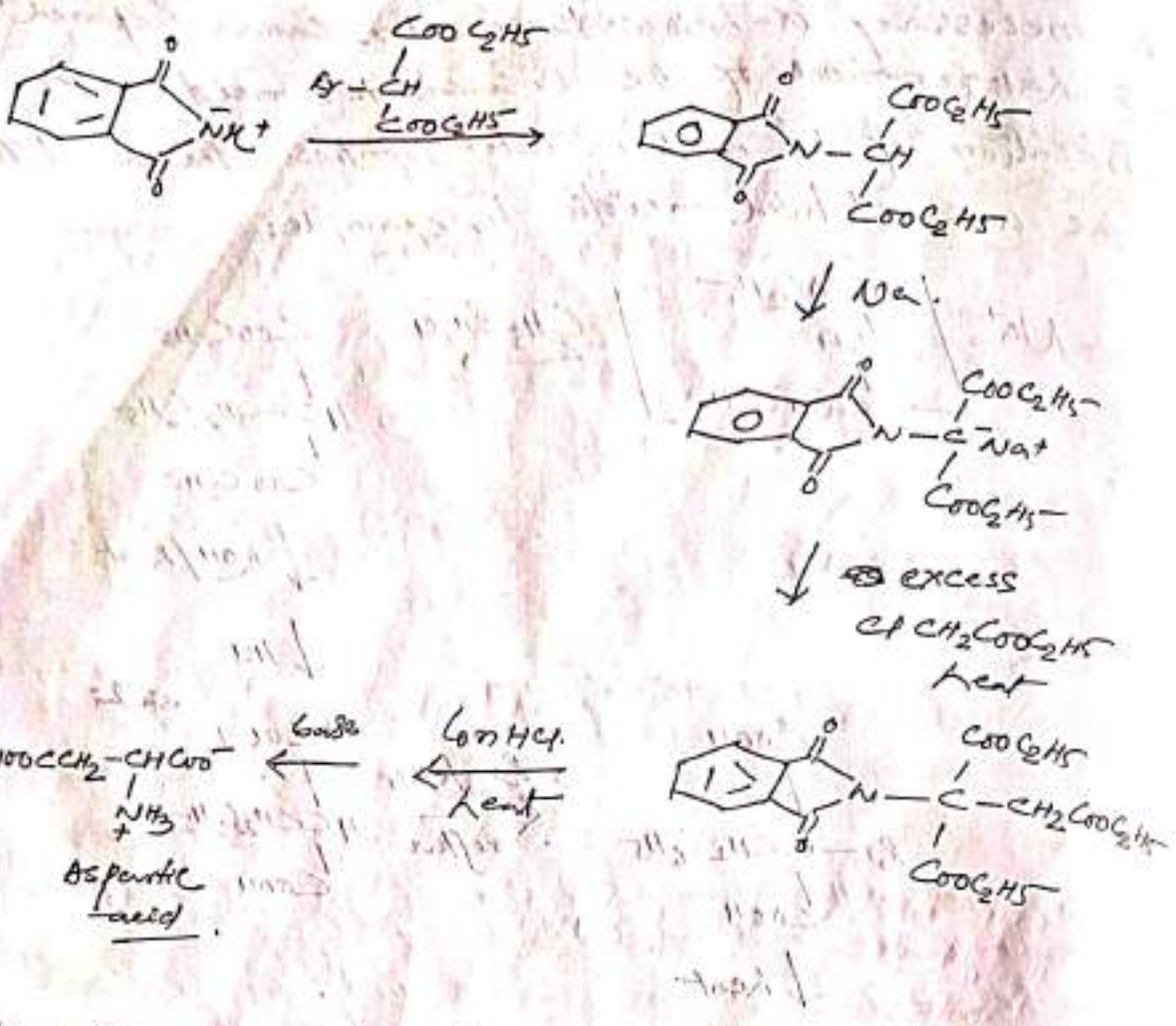
The necessary α-halo acids or esters can be prepared by direct halogenations of the unsubstituted acids or by modification of the malonic ester synthesis, the usual route to the unsubstituted acids. For example:



Phenylalanine

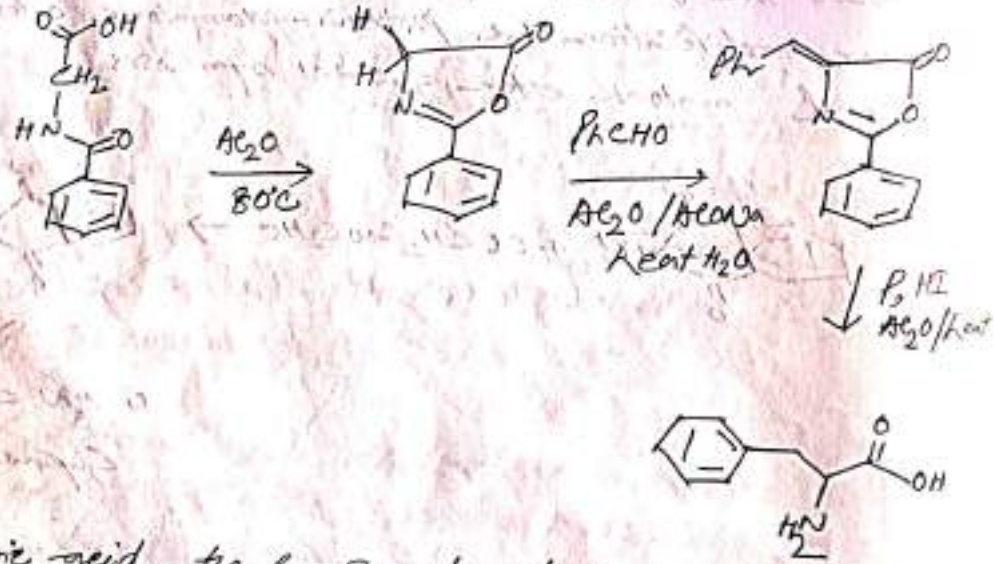
Better yields are generally obtained by the Gabriel phthalimide synthesis the α-halo ester are used instead of α-halo acids. A further modifications the phthalimidomalonic ester method, e's –
Combined malonic ester – Gabriel synthesis





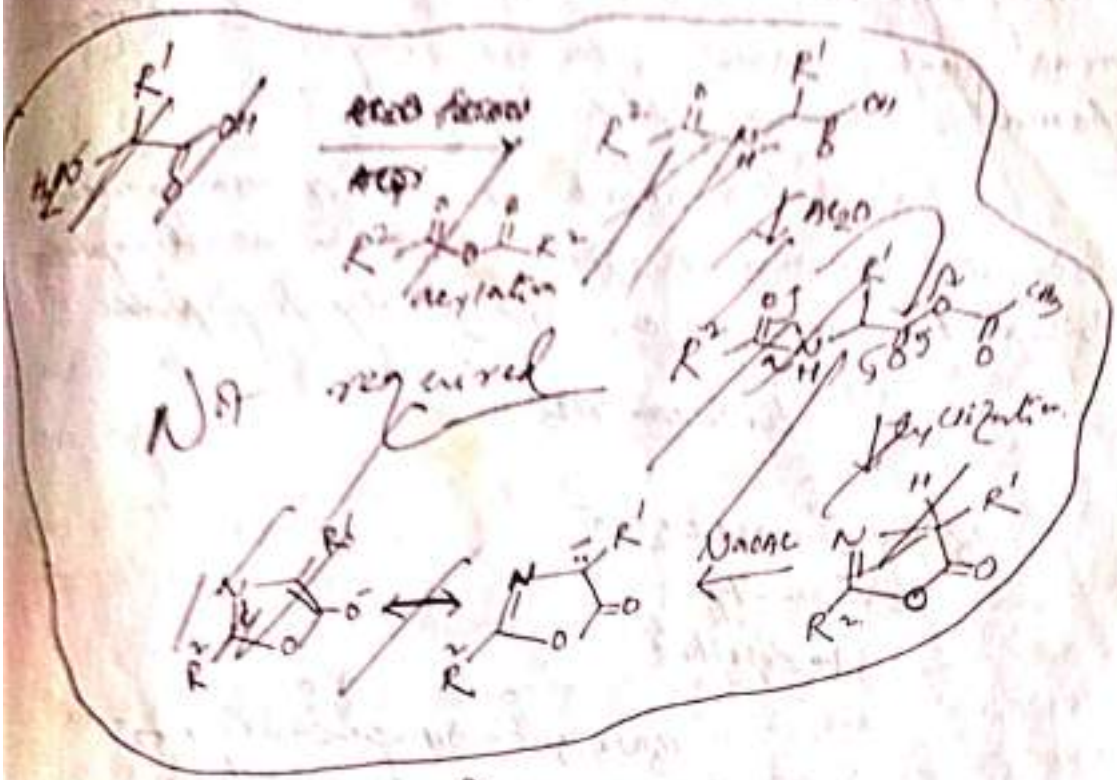
* Erlenmeyer azalactone Synthesis:

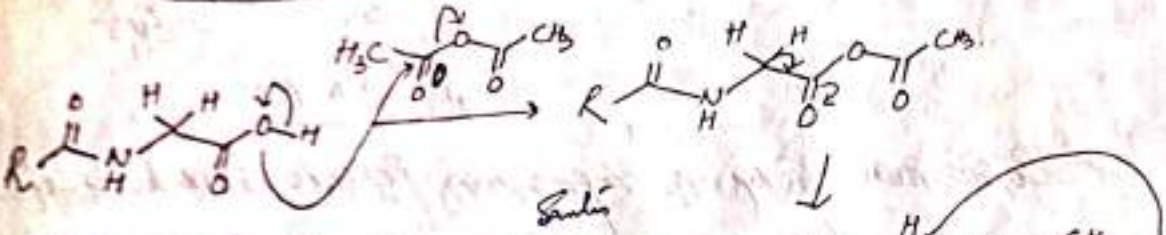
The Erlenmeyer – Plöchl azalactone and amino acid Synthesis, is a series of chemical reactions which transform an N-acyl Glycine to various other amino acids via an oxazolone and an azalactone

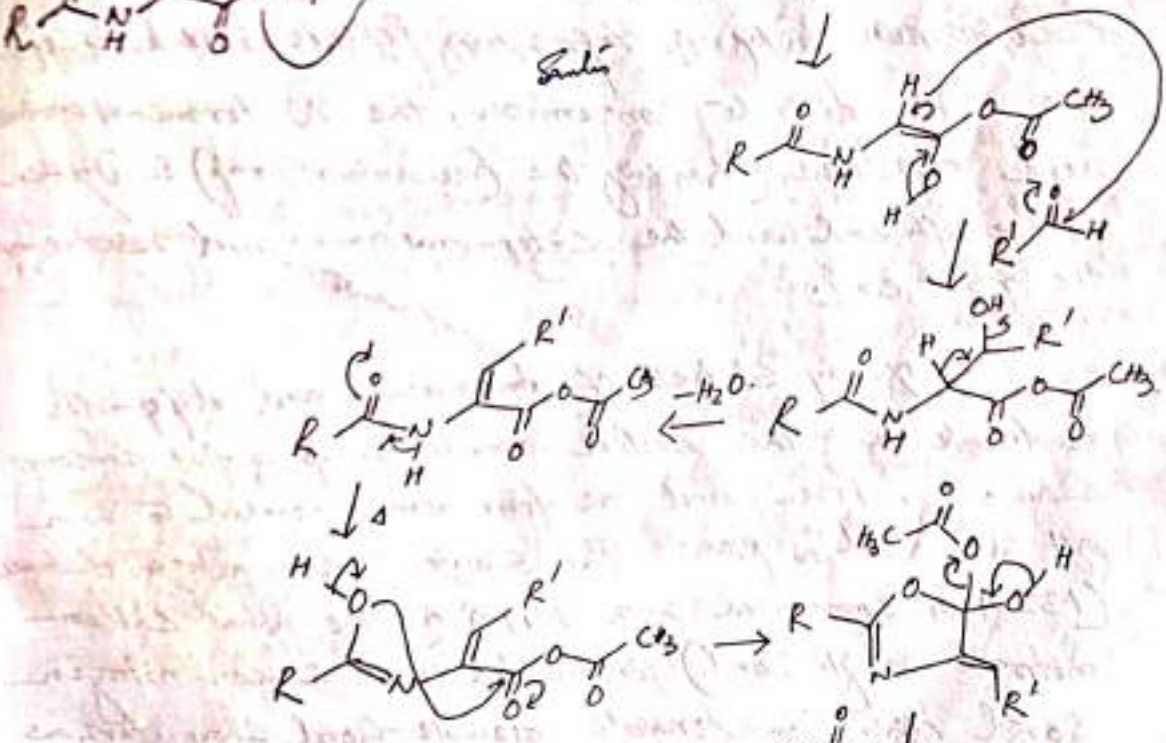


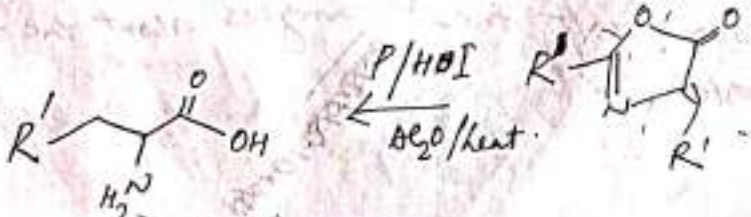
Hippuric acid, the benzamide derivative of Glycine cyclizes in the presence of acetic anhydride, Condenses to give 2-Phenyl Oxazolone. This intermediate also has two acidic protons and reacts with benzaldehyde

acetic anhydride and Sodium acetate to a so called azlactone. This compound on reduction gives racemic phenyl alanine.







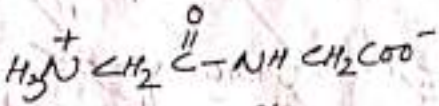


Peptides:

Peptides are amides formed by interaction between amino groups and carboxyl groups of amino acids. The amino group, $-NHCO-$, in such compounds is often referred to as the peptide linkage.

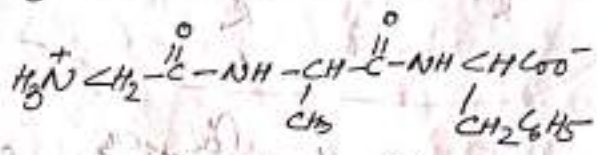
Depending upon the number of amino acid residues per molecule, they are known as dipeptides, tripeptides and so on, and finally polypeptides.

For example



Gly-Gly

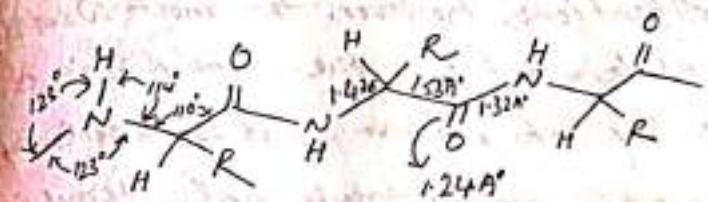
A dipeptide



Gly-Ala-Phe

A convenient way of representing peptide structures is used. According to convention, the N-terminal amino acid residue (having the free amino group) is written at the left end and the C-terminal amino acid residue at the right end.

X-ray studies of amino acids and dipeptides indicate that the entire amide group is flat. Carbonyl carbon, nitrogen, and the four atoms attached to them all lie in a plane. The short carbon-nitrogen distance (1.32 Å as compared with 1.47 Å for the usual carbon-nitrogen single bond) indicates that carbon-nitrogen bond has considerable double-bond character; as a result the angles of the bonds to nitrogen are similar to the angles about the trigonal carbon atom.

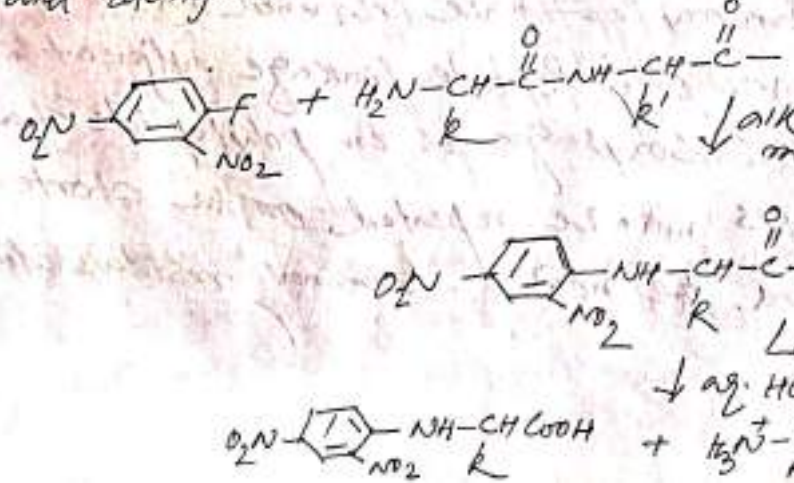


Determination of Structure of Peptides:

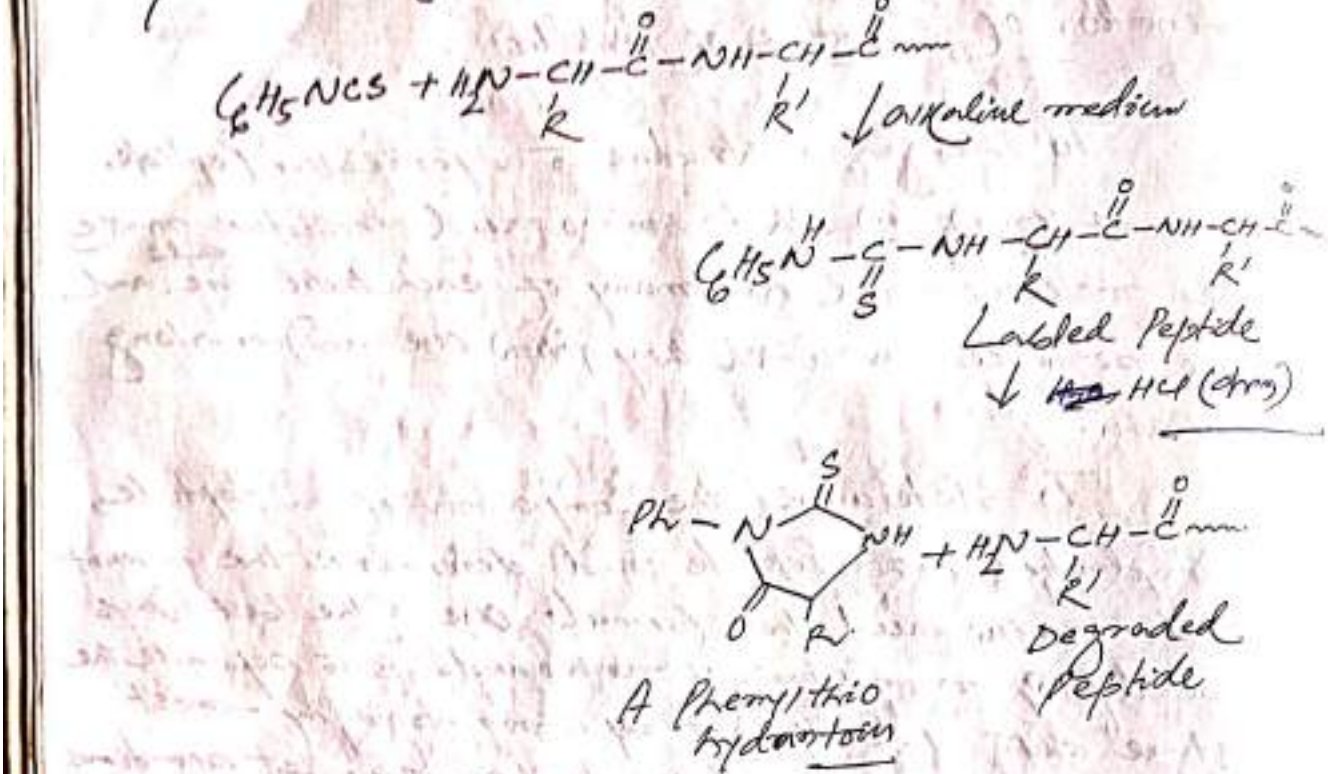
To assign a structure to a particular peptide, one must know (a) what amino acid residues make up the molecule and how many of each there are, and (b) the sequence in which they follow one another along the chain.

To determine the composition of a peptide, one hydrolyzes the peptide and determines the amount of each amino acid thus formed. One of the best ways of analyzing a mixture of amino acids is to separate the mixture into its component by chromatography — most commonly by ion-exchange chromatography, but sometimes after conversion into methyl esters, by gas chromatography.

A very successful method of identifying the N-terminal residue make use of 2,4-dinitrofluorobenzene (DFNB), which undergoes nucleophilic substitution by the free amino group to give an N-dinitrophenyl (DNP) derivative. The substituted peptide is hydrolyzed to the component amino acids, and the N-terminal residue, labeled by the 2,4-dinitrophenyl group, is separated and identified.



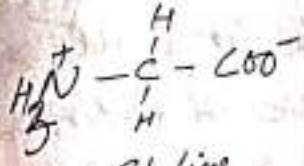
In its various modifications, however, the most widely used method of N-terminal residue analysis is the one introduced by 1950 by Pehr Edman. This is based upon the reaction between an amino groups and Phenyl isothiocyanate to form a substituted thiourea.



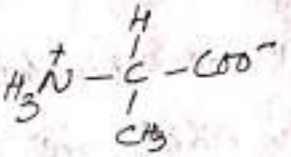
Mild hydrolysis with HCl selectively removes the N-terminal residue as the phenyl thiohydantoin which is then identified. The great advantage of this method is that it leaves the rest of the peptide chain intact, so that the analysis can be repeated and the new terminal groups of the shortened peptide identified.

* One successful method of determining the C-terminal residue has been enzymatic rather than chemical. The C-terminal residue is removed selectively by the enzyme Carboxy Peptidase (obtained from the Pancreas) which cleaves only peptide linkage adjacent to free alpha-carboxyl groups in polypeptide chains. The analysis can be repeated on the shortened peptide and the new C-terminal residue identified and so on.

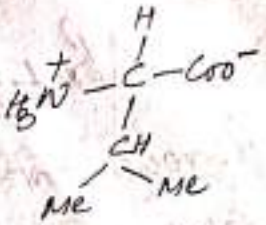
Amino acids:



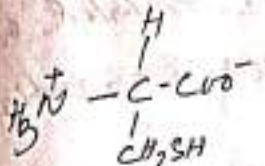
Glycine



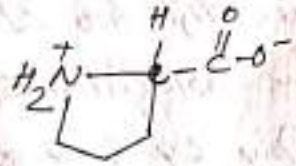
Alanine



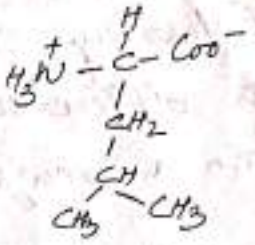
Valine



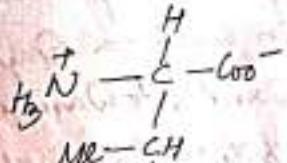
Cysteine



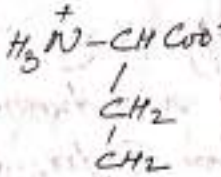
Proline



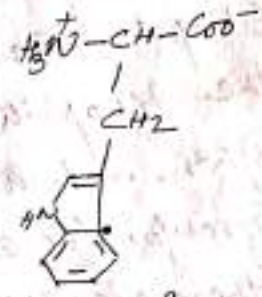
Leucine



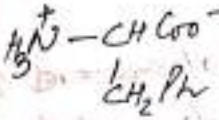
Isoleucine



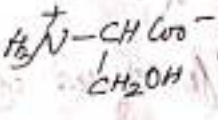
Methionine



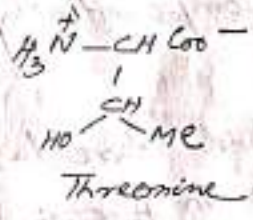
Tryptophan



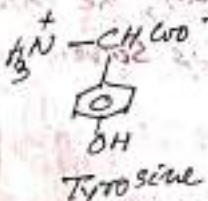
Phenylalanine



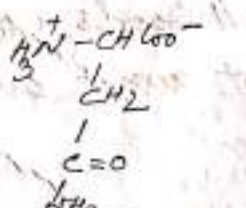
Serine



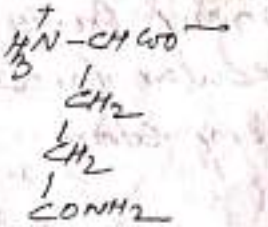
Threonine



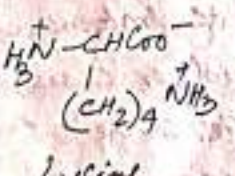
Tyrosine



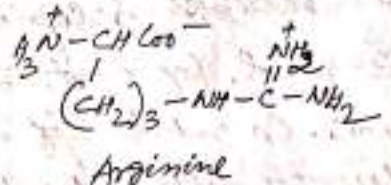
Asparagine



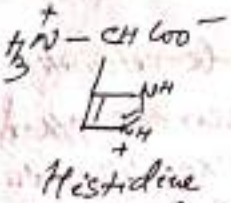
Glutamine



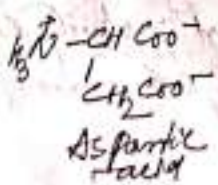
Lysine



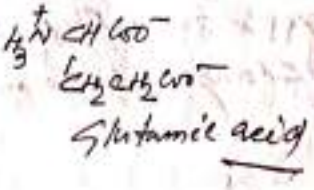
Arginine



Histidine



Aspartic acid



Glutamic acid

Proteins:

Proteins are divided into two broad classes: fibrous proteins, which are insoluble in water, and globular proteins, which are soluble in water or aqueous solⁿ of acids, bases or salts. The difference in solubility between the two classes is related to a difference in molecular shape, which is indicated in a rough way by their names.

Molecule of globular proteins are folded into compact units that often approach spheroidal shapes. The folding takes place in such a way that the lipophilic part are turned inward, toward each other, and away from water; hydrophilic parts - charged groups, for example - tend to stud the surface where they are near water. Areas of contact between molecules are small and intermolecular forces are comparatively weak.

Molecules of fibrous proteins are long and thread-like, and tend to lie side by side to form fibers; in some cases they are held together at many points by H-bonds. As a result, the intermolecular forces that must overcome by solvent are very strong.

Denaturation:

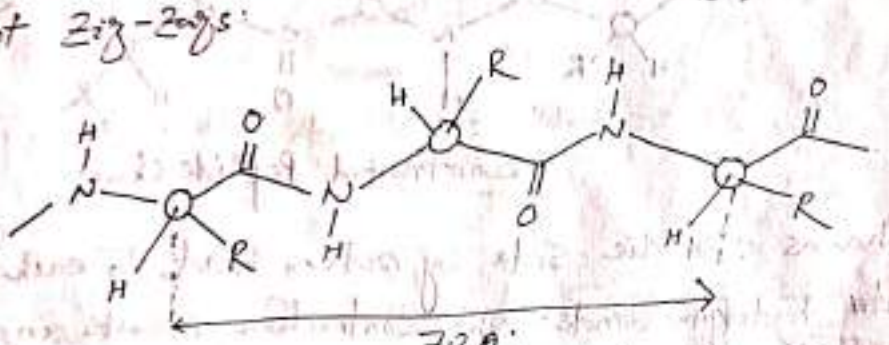
Irreversible precipitation of proteins, called denaturation, is caused by heat, strong acid or bases, or various other agents. Coagulation of egg white by heat, for example, is denaturation of the protein egg albumin. Denaturation causes a fundamental change in a protein - in particular destroying any physiological activity. Denaturation appears to involve changes in secondary structure of protein

Essential amino acids

An essential amino acid, or indispensable amino acid, is an amino acid that cannot be synthesized de novo (from scratch) by the organism and thus must be supplied in its diet. The nine amino acids humans cannot synthesize are phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine and histidine

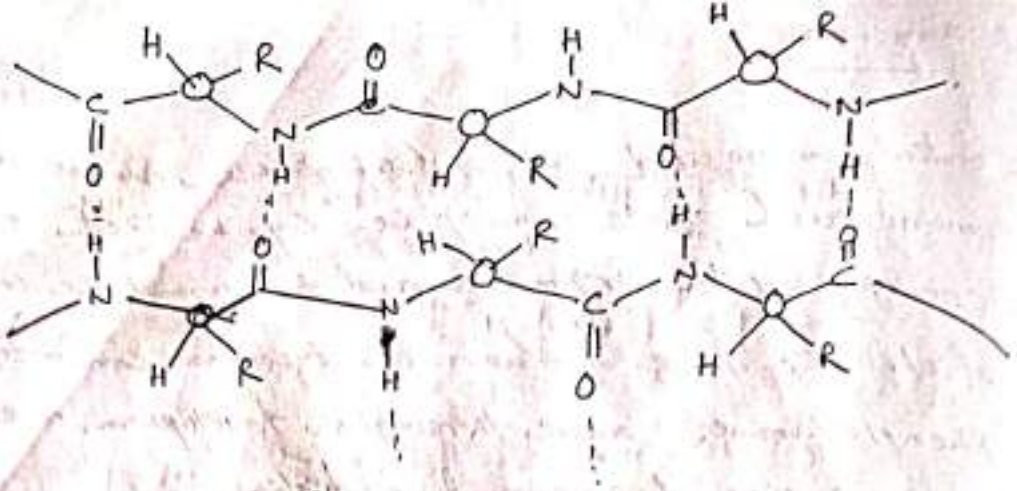
Secondary Structure of Proteins:

It is convenient to consider a structure of a protein in which peptide chains are fully extended to form flat zig-zags.



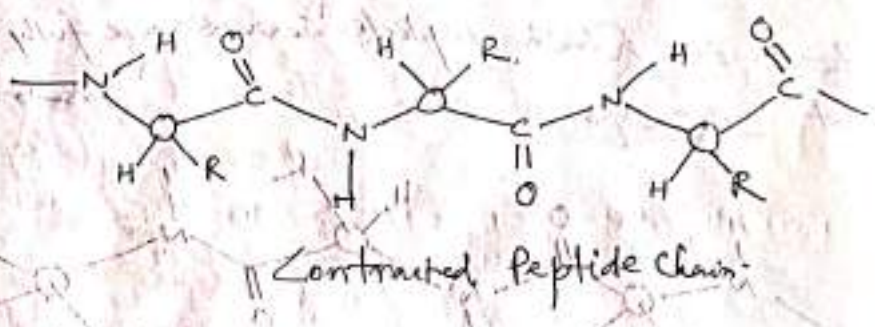
Extended peptide chain

These chains lie side by side to form a flat sheet. Each chain is held by hydrogen bonds to the two neighboring chains. This structure has a repeat distance of 7.2 Å, the distance between alternate amino acid residues. (Notice that alternate side chain lie on the same side of the sheet. However, bending between side chains makes this idealized flat structure impossible, except perhaps for synthetic polyglycine)



Hypothetical flat sheet structure for a protein

Room can be made for small or medium-sized side chains by a slight contraction of the peptide chains



Contracted peptide chain

The chains still lie side by side, held to each other by ~~hydrin~~ hydrogen bonds. The contraction results in a pleated sheet, with a somewhat shorter distance between alternate amino acid residue. Such a structure called the beta arrangement proposed for silk fibroin.

When the side chain are quite large they are best accommodated by a quite different kind of structure. Each chain coiled to form a helix. Hydrogen bonding occurs between different parts of the same chain and holds the helix together. For α-Keratin Pauling has proposed a helix in which there are 3.6 amino acid residue per turn. Models show that this 3.6-helix provides room for the side chains and allows all possible hydrogen bonds to form. It accounts for the repeat distance of 1.5 A°, which is the distance between amino acid

along the axis of the helix. To fit into this helix, all the amino acid residues must be of the same configuration, of course, they are; furthermore, their L-configuration requires the helix to be right-handed, as shown. The α-Helix as it is called, is of fundamental importance in the Chemistry of Proteins.

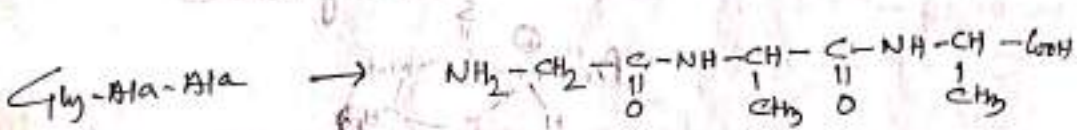
Alpha Helix Structure of Protein

* How would you differentiate chemically between the following two peptides?

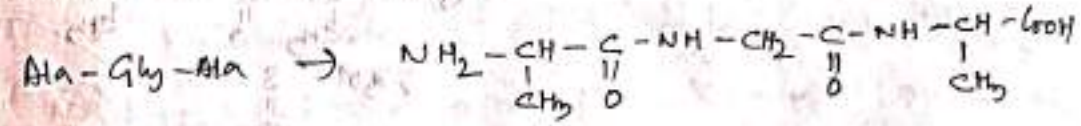
Gly-Ala-Ala and Ala-Gly-Ala.

Ans

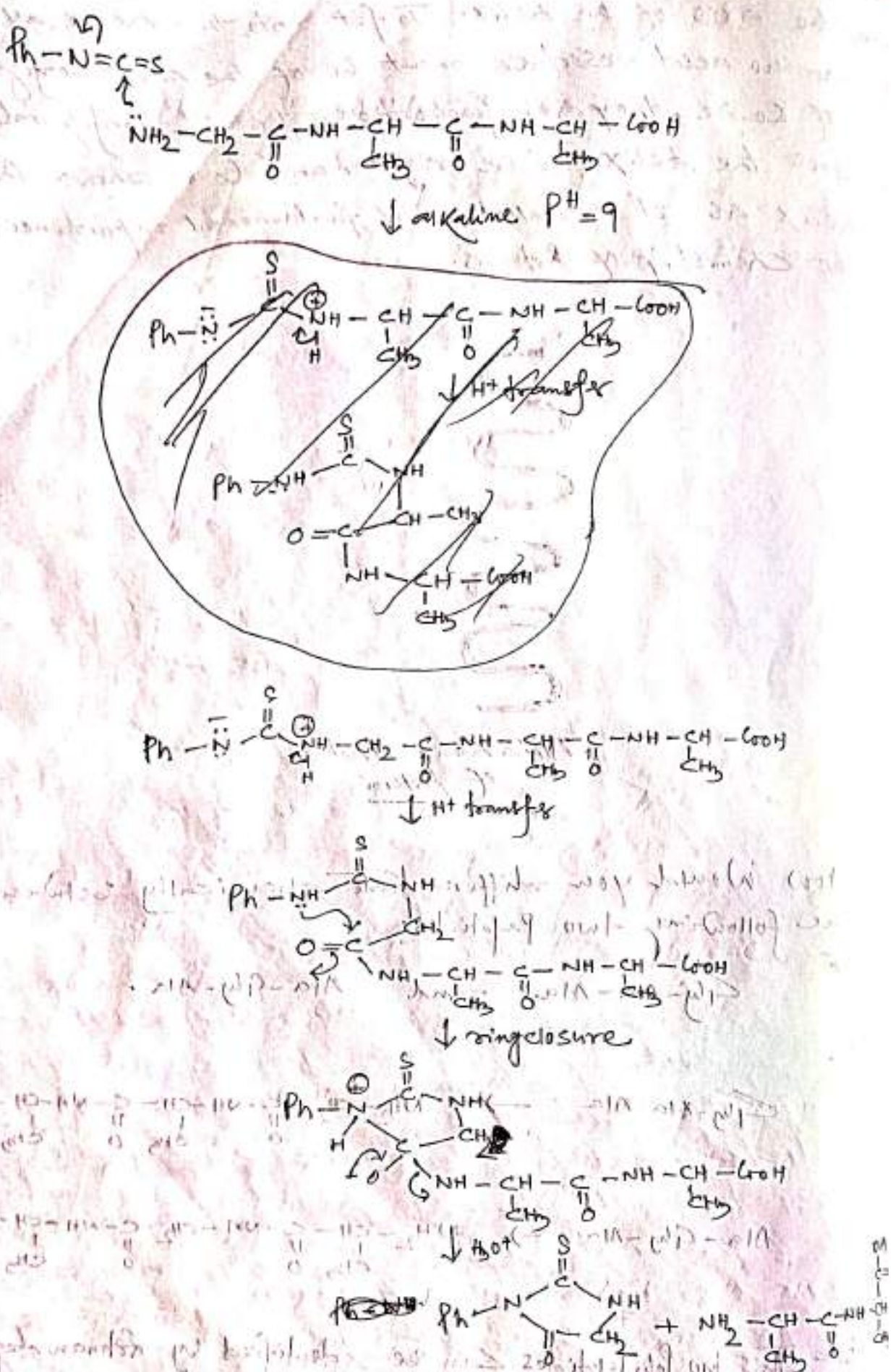
Gly-Ala-Ala →



Ala-Gly-Ala →



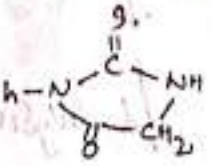
This two polypeptides can be identified by Edman degradation. This involves a nucleophilic addition of the free NH_2 groups of the polypeptide to the C=N of Phenyl isothiocyanate in a mildly basic medium (pH=9). The Product then undergoes a ring-closure reaction.

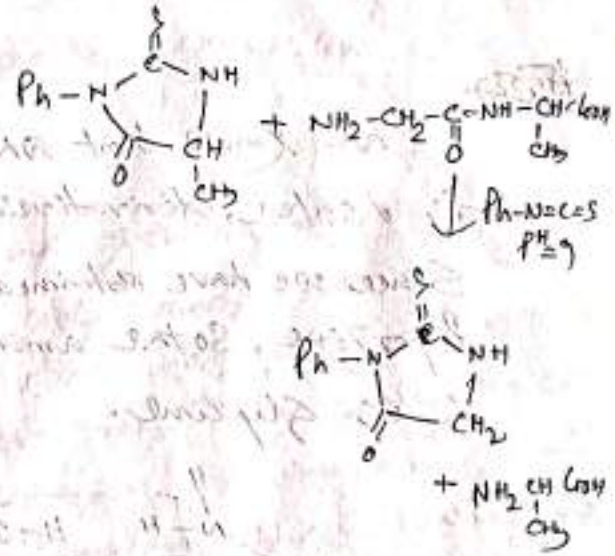


Thus in this reaction, the N-terminal amino acid unit forms a Phenylthiohydantoin and detach itself from the rest of the peptide which does not decompose but remain intact with all its sequences. The N-terminal unit can be identified by Komparing the Phenylthio

hydantoin so formed with standard Phenyl thiohydantoins. Since the residual polypeptide chain remain intact, the residual chain can further be subjected to the Edmann degradation and all the amino acids of the starting peptide can be identified sequentially.

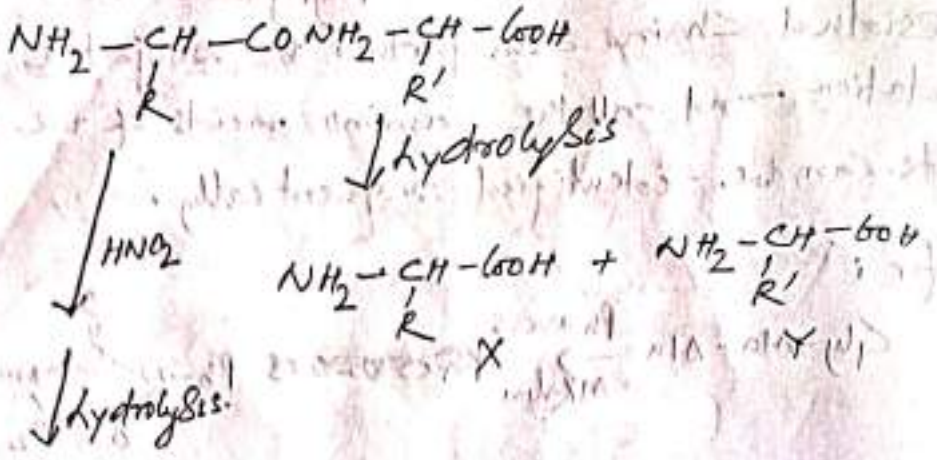
Thus for;

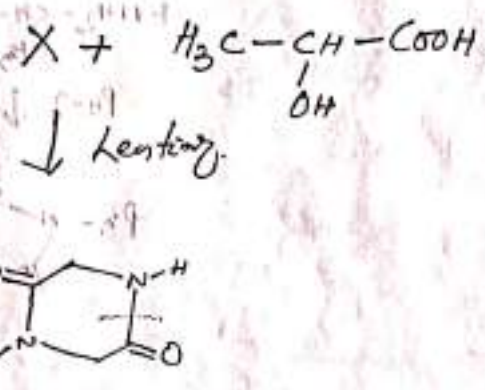
Gly-Ala-Ala $\xrightarrow[\text{Alkali}]{Ph-N=C=S}$ 

Ala-Gly-Ala $\xrightarrow[PH=9]{Ph-N=C=S}$ 

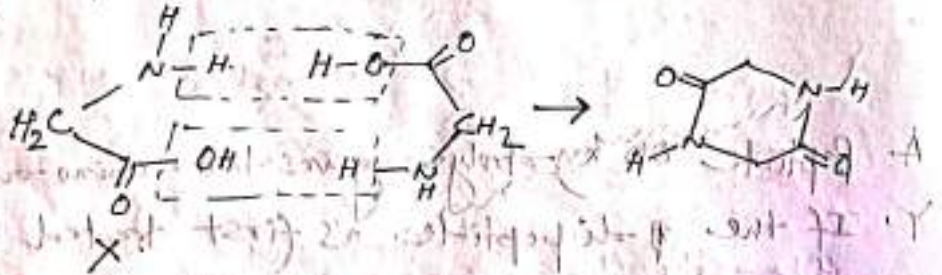
* A peptide on hydrolysis gives two amino acids X and Y. If the dipeptide is first treated with HNO_2 and then hydrolysis is carried out, X and Lactic acid are obtained. X on heating gives 2,5 diketo Piperazine, as shown below. Identified X and Y and write their sequence in dipeptide.

Ans.

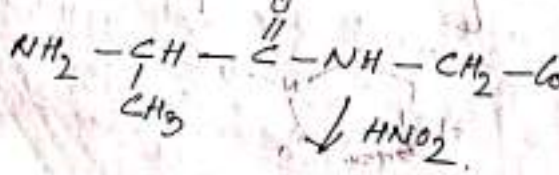


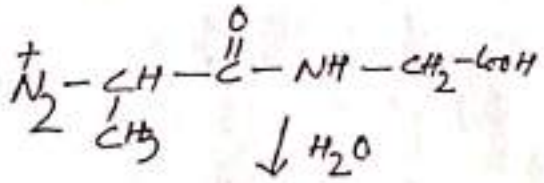


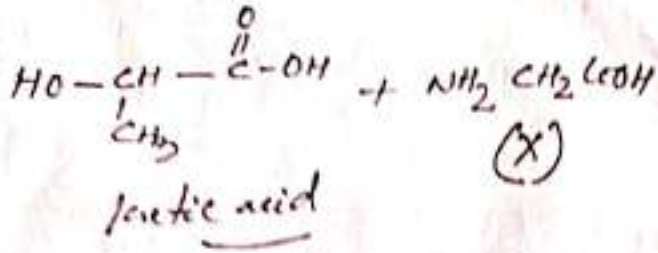
(b) We know that when a neutral α-amino acid is heated, derivatives of diketopiperazines are obtained. Since we have obtained aforesaid 2,5-diketo piperzine, so the amino acid is Glycine i.e X is Glycine.



Since dipeptide when treated with HNO_2 followed by hydrolysis gives Glycine (X) and lactic acid so the peptide is,



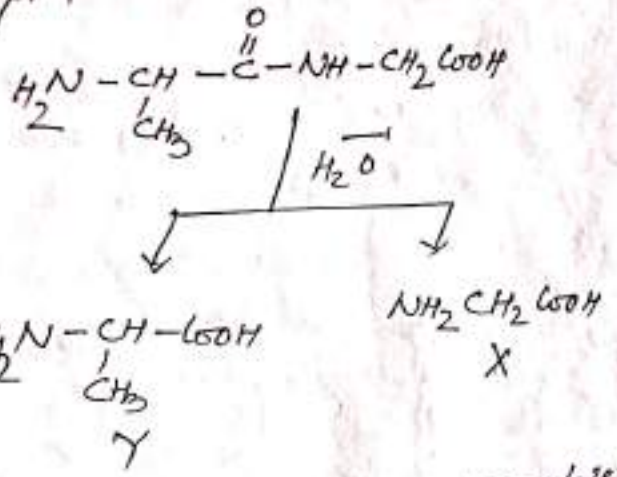




Lactic acid

So Y is Alanine → $H_2N-CH(CH_3)-COOH$

and the dipeptide is



* Define Prosthetic Group and give example?

A Prosthetic group is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The Prosthetic group may be organic such as a Vitamin, Sugar or lipid or inorganic such as metal ion, but is not ~~Compound~~ composed of amino acids.