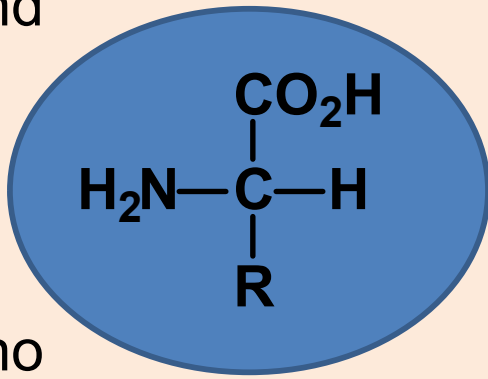
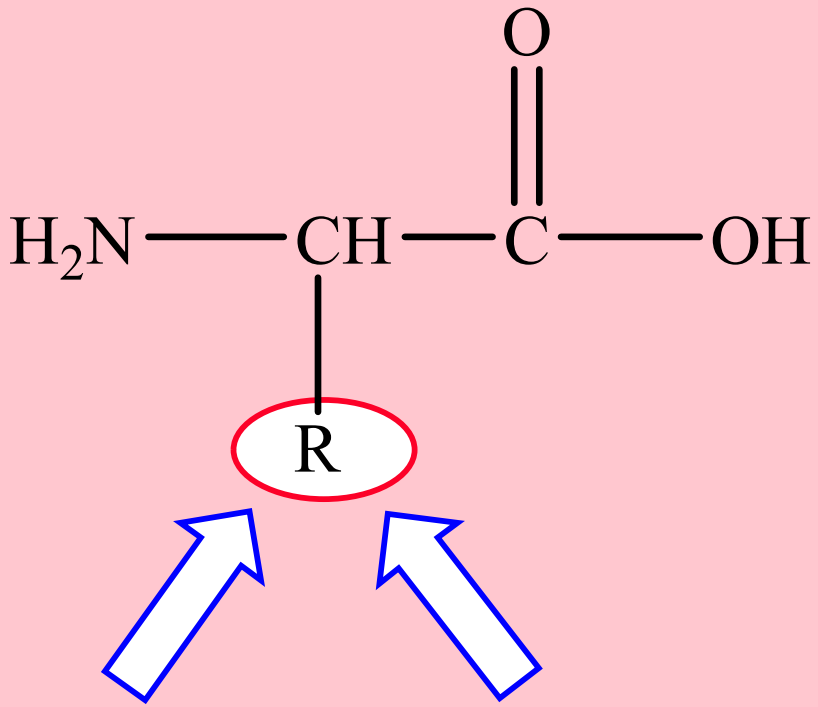
AMINO ACIDS, PEPTIDES AND PROTEINS

PRIYANKA BARUA

AMINO ACIDS

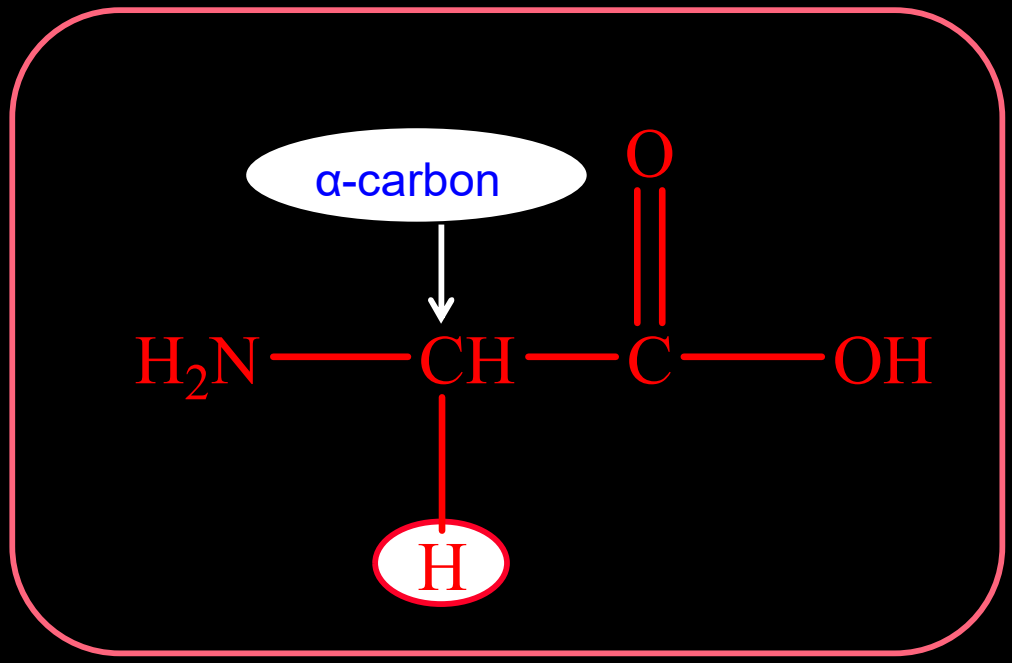


- Amino acids are compounds having one or more $–NH_2$ and one or more –COOH groups in the same molecule.
- Amino acids are building blocks of peptides and proteins.
- Peptides and proteins are polyamides containing amino acids bonded through the -COOH function of one amino acid and $–NH_2$ function of the other, in a head to tail manner.
- Hundreds of amino acids occur naturally, but 20 of them are especially important.
- These 20 amino acids are the building blocks of proteins. All are α-amino acids. These are called ‘proteinogenic’ amino acids.
- They differ in respect to the group attached to the α-carbon.



The amino acids obtained by hydrolysis of proteins differ in respect to R (the side chain).

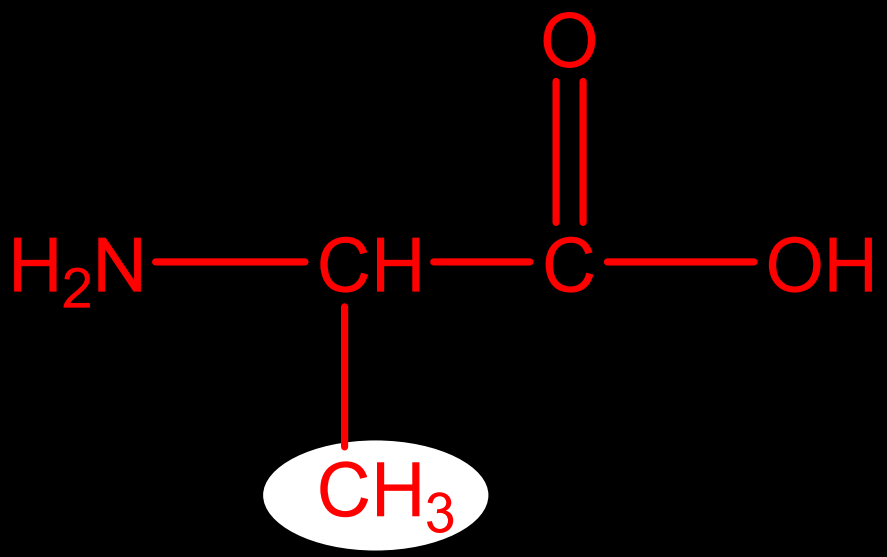
The properties of the amino acids vary as the structure of R varies.



Glycine (Gly or G)

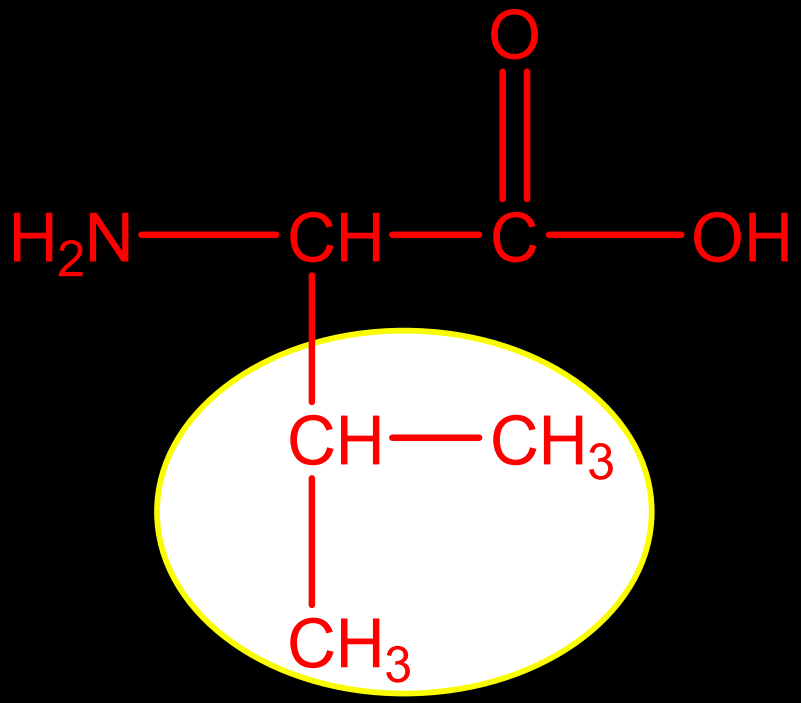
Glycine is the simplest amino acid that is achiral.

In all of the other amino acids the α-carbon is a stereogenic center.



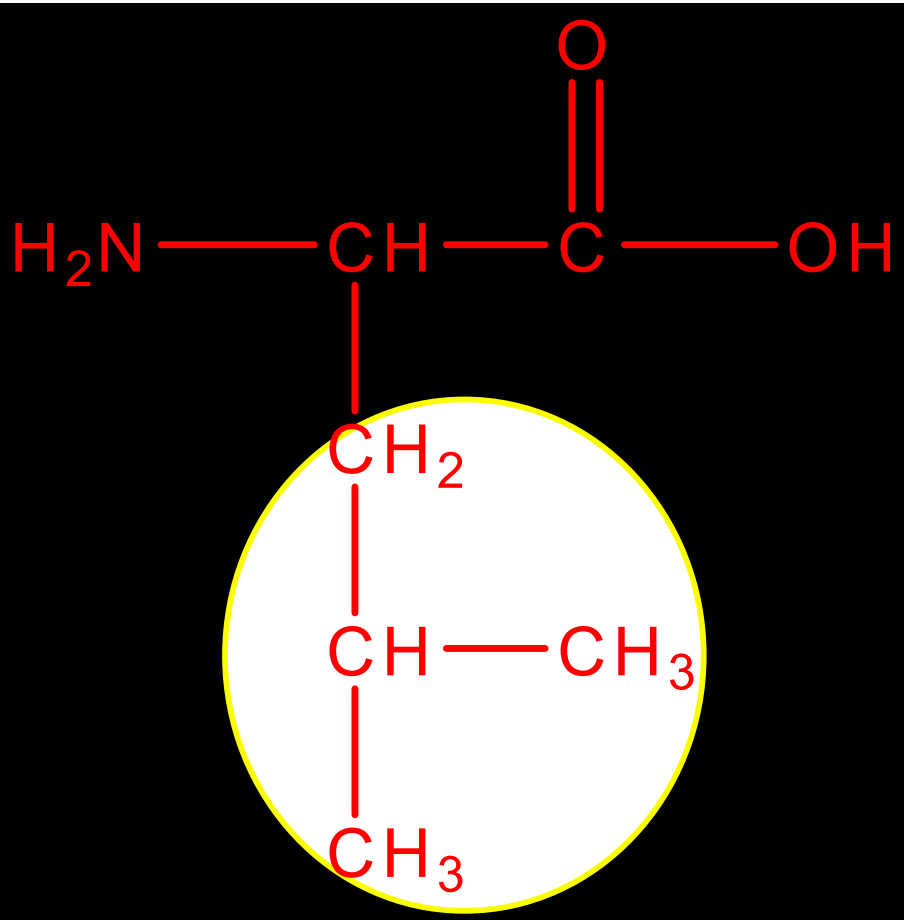
Alanine

(Ala or A)



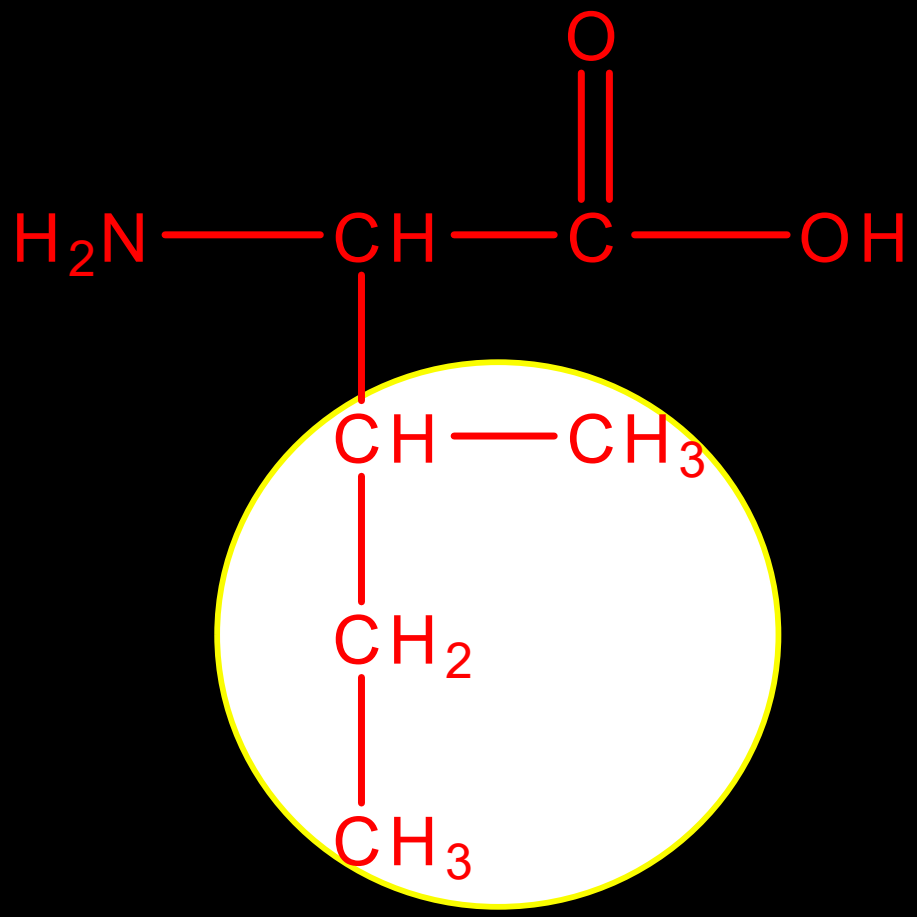
Valine

(Val or V)



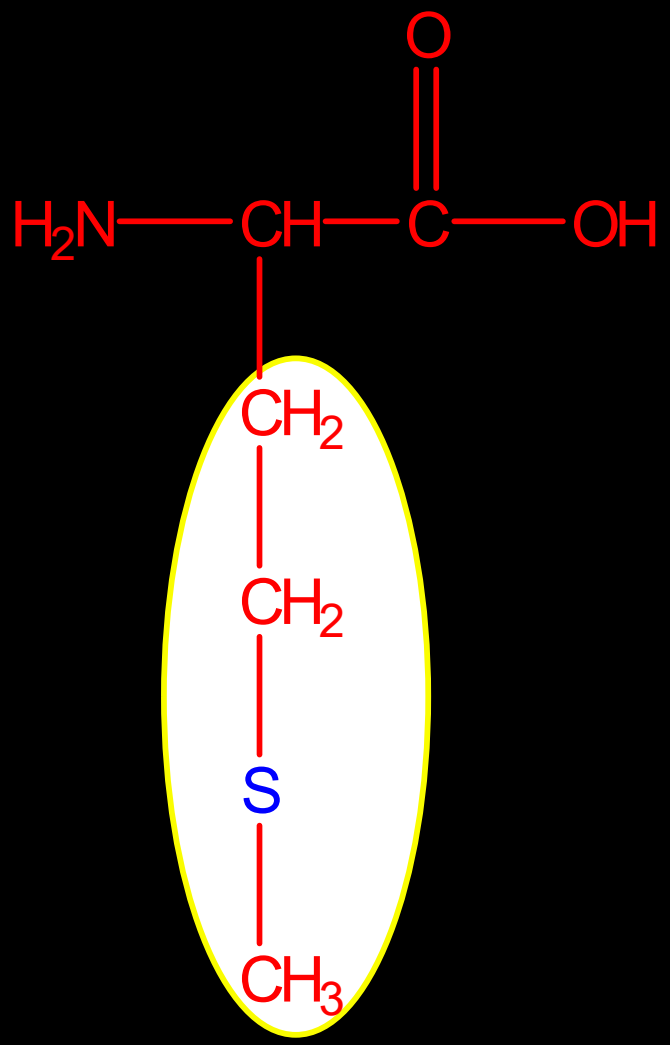
Leucine

(Leu or L)



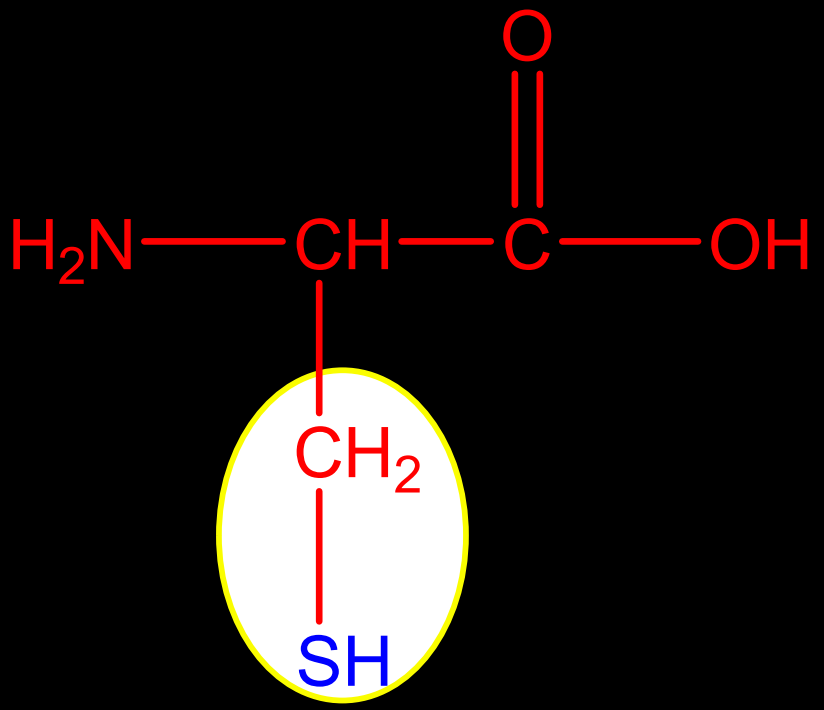
Isoleucine

(Ile or I)



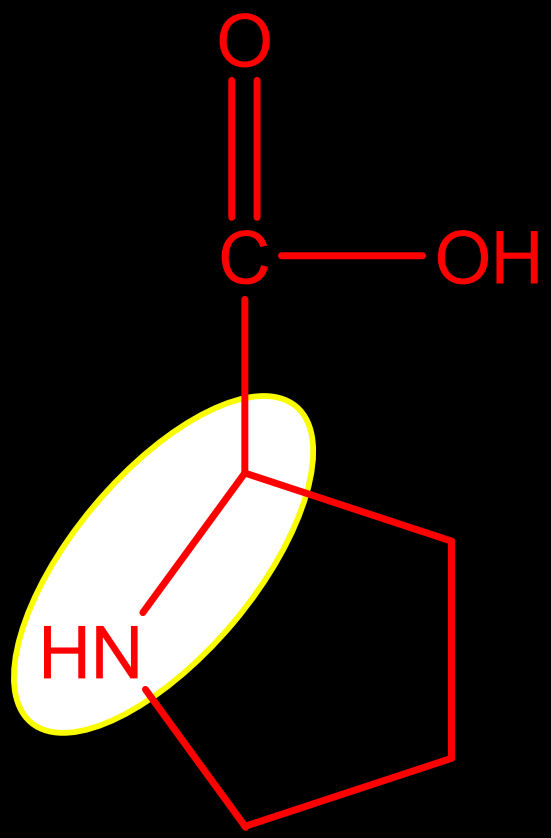
Methionine

(Met or M)



Cysteine

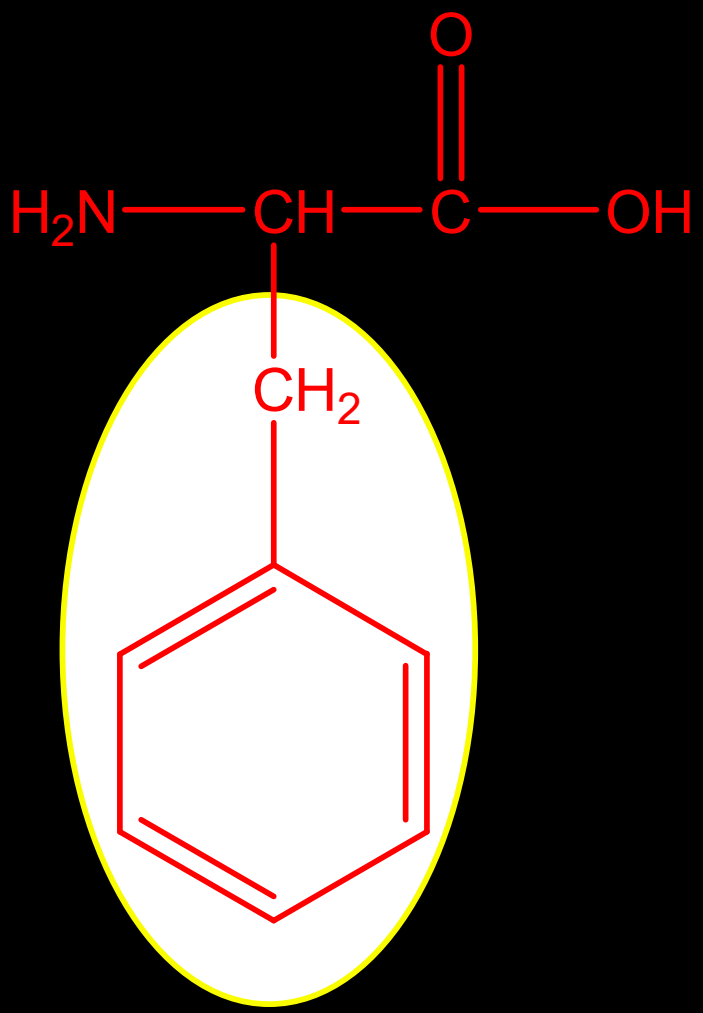
(Cys or C)



Proline

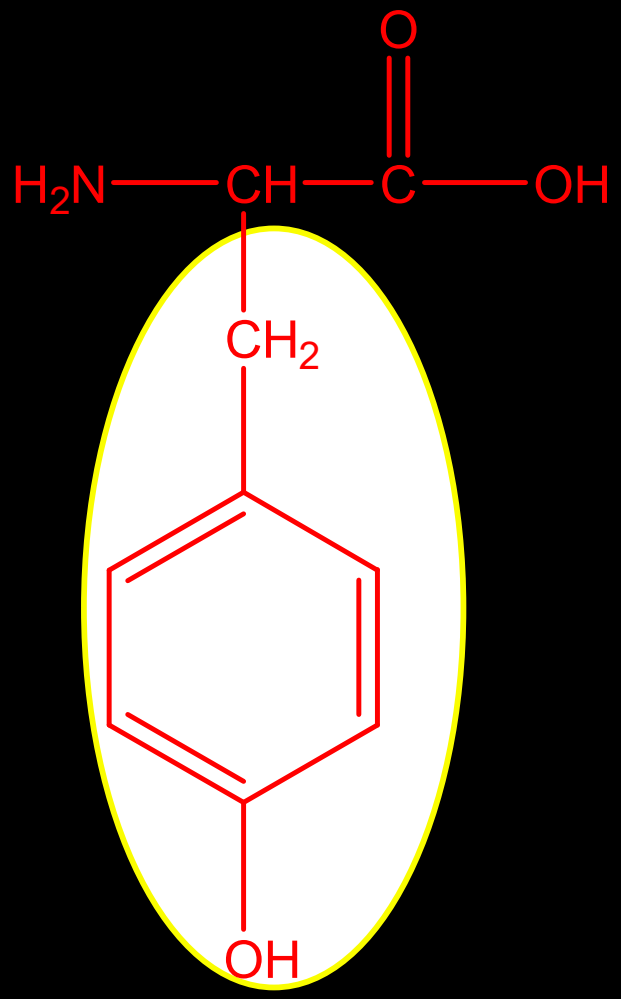
(Pro or P)

Proline is actually an “imino” acid...



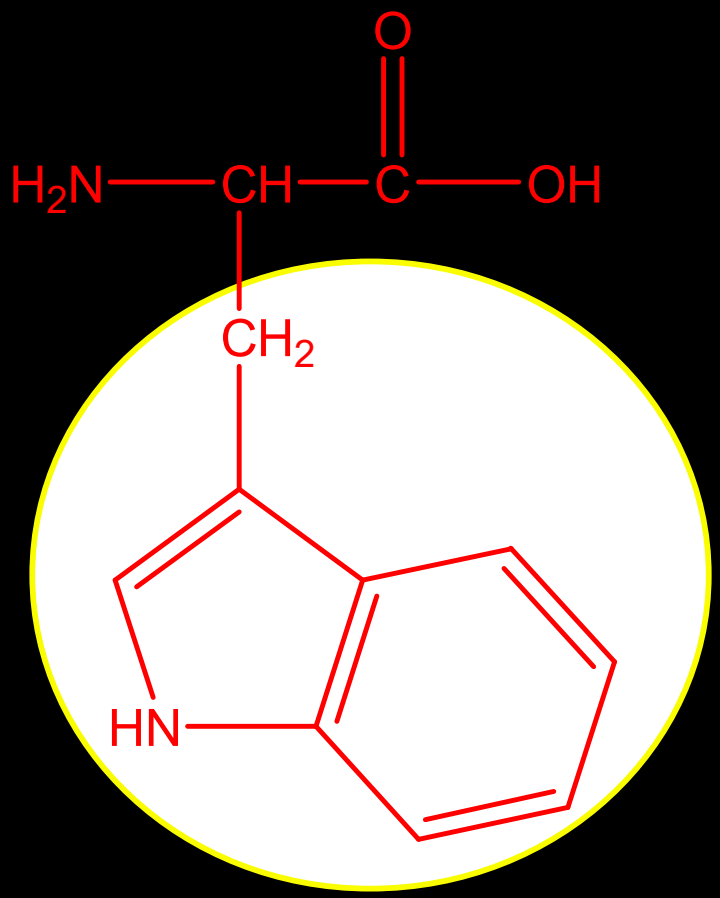
Phenylalanine

(Phe or F)



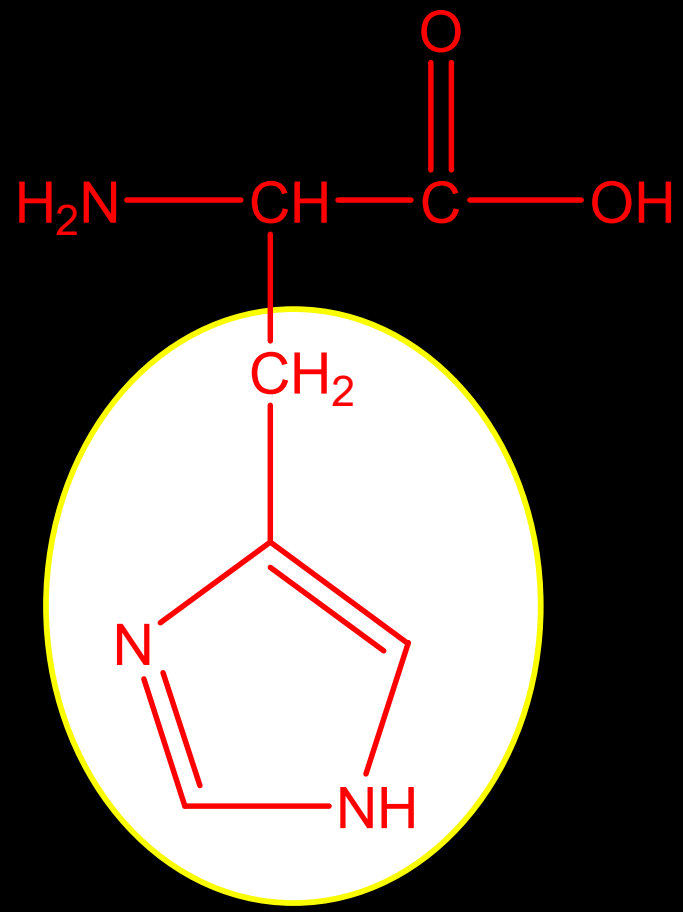
Tyrosine

(Tyr or Y)



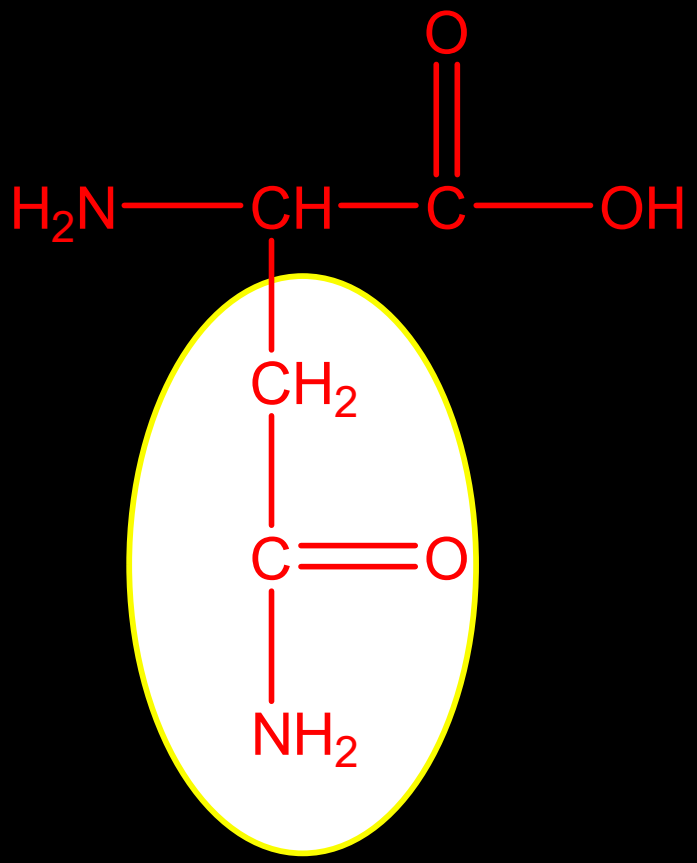
Tryptophan

(Trp or W)



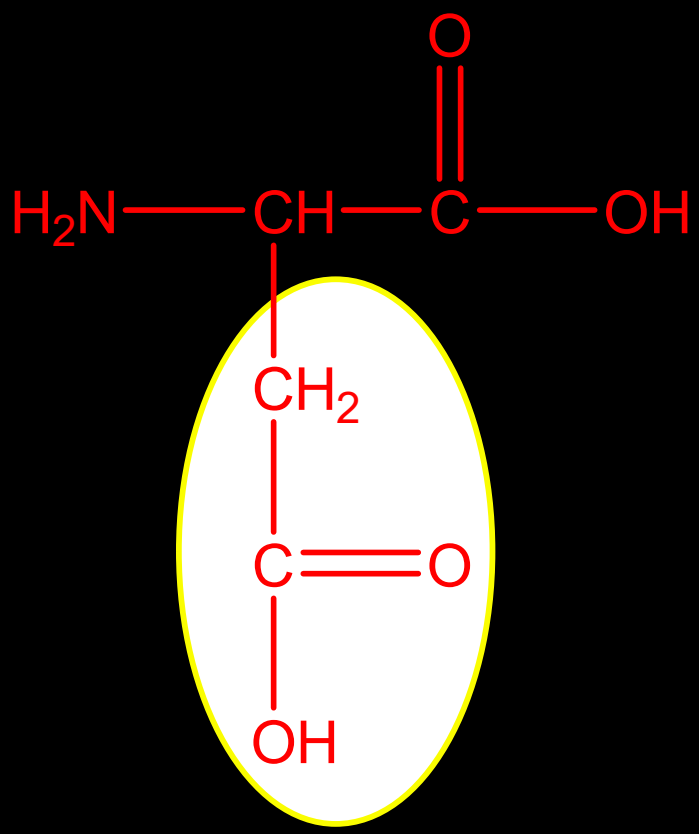
Histidine

(His or H)



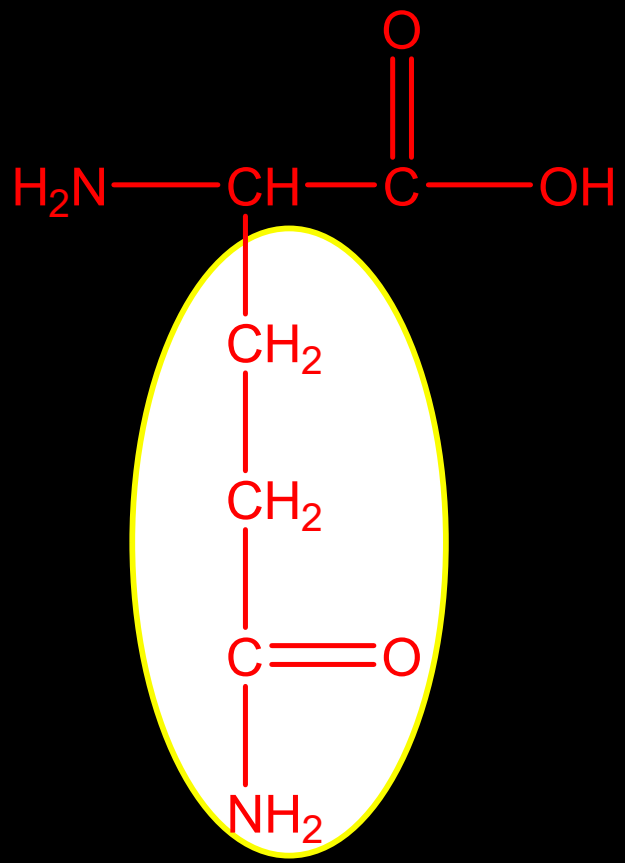
Asparagine

(Asn or N)



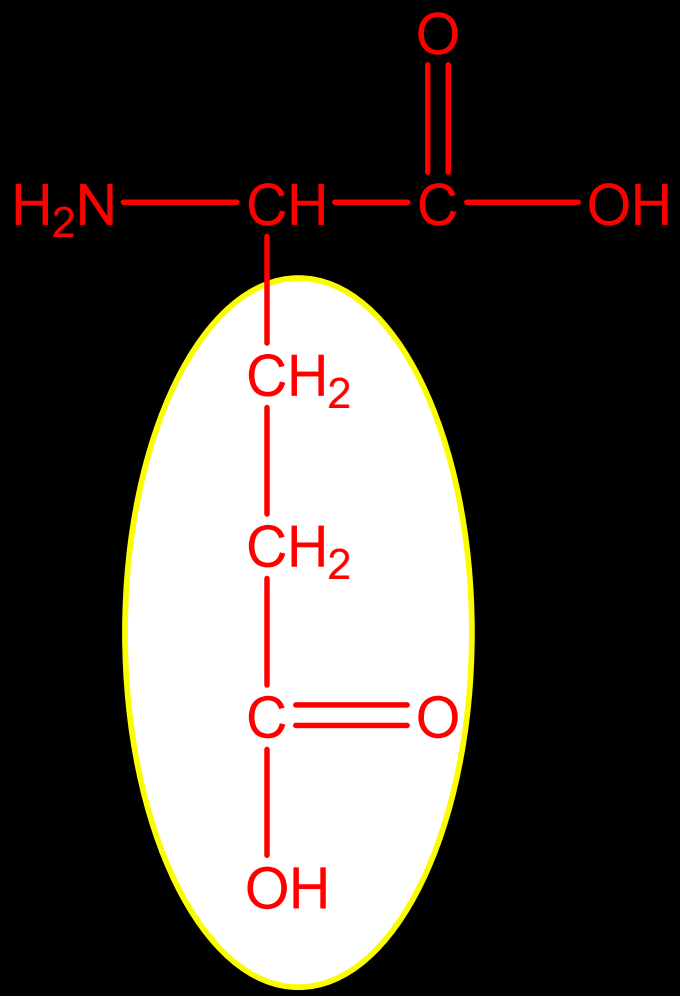
Aspartic Acid

(Asp or D)



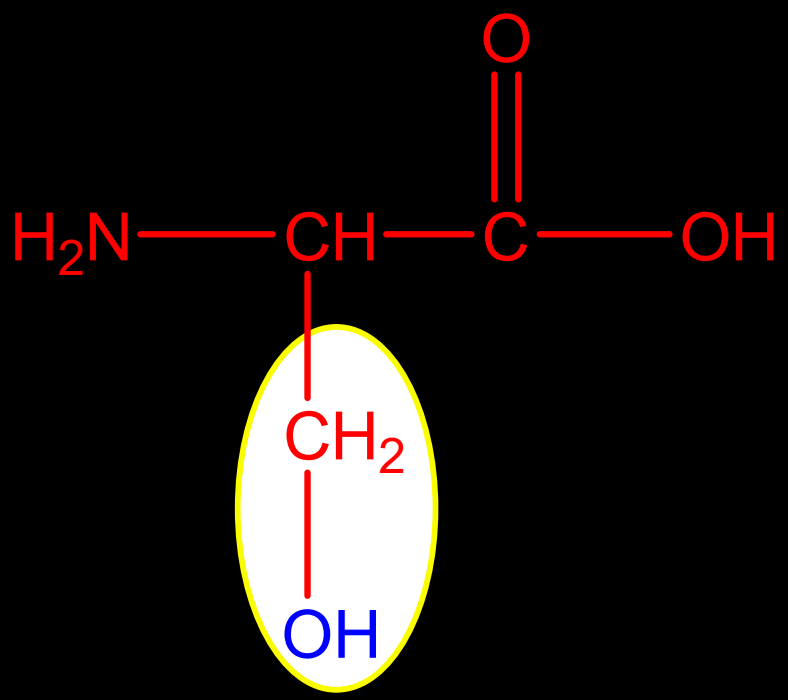
Glutamine

(Gln or Q)



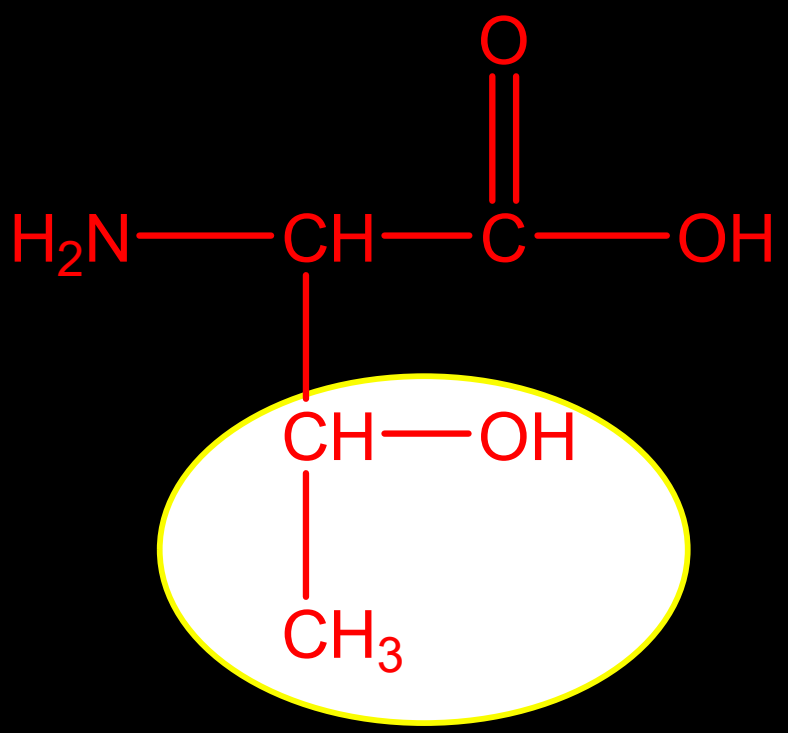
Glutamic Acid

(Glu or E)



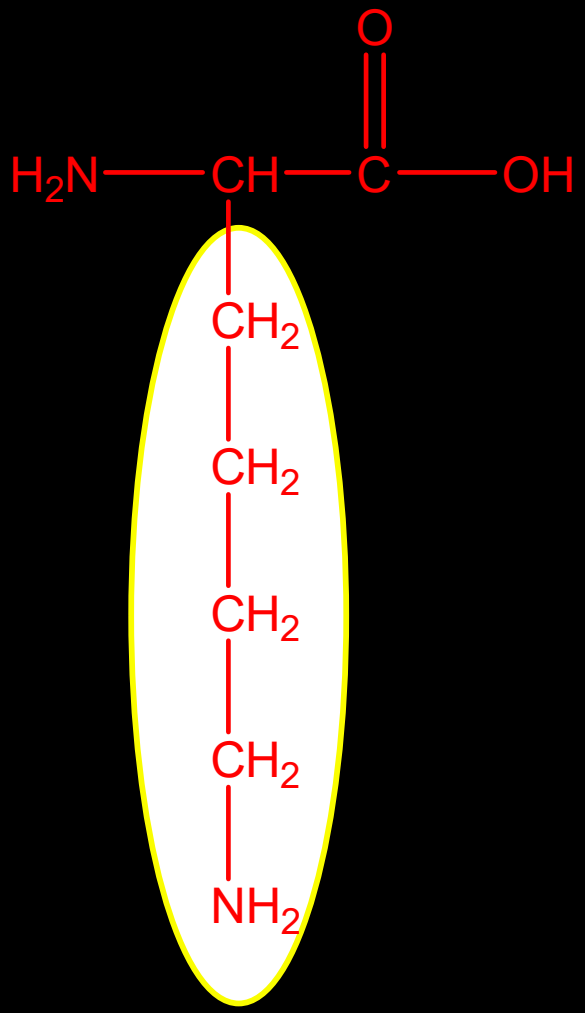
Serine

(Ser or S)



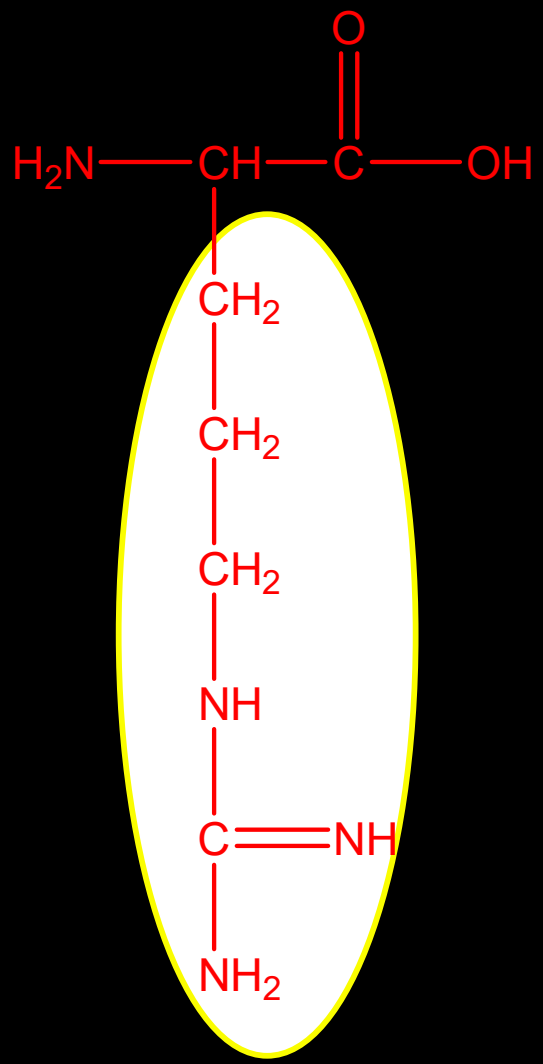
Threonine

(Thr or T)



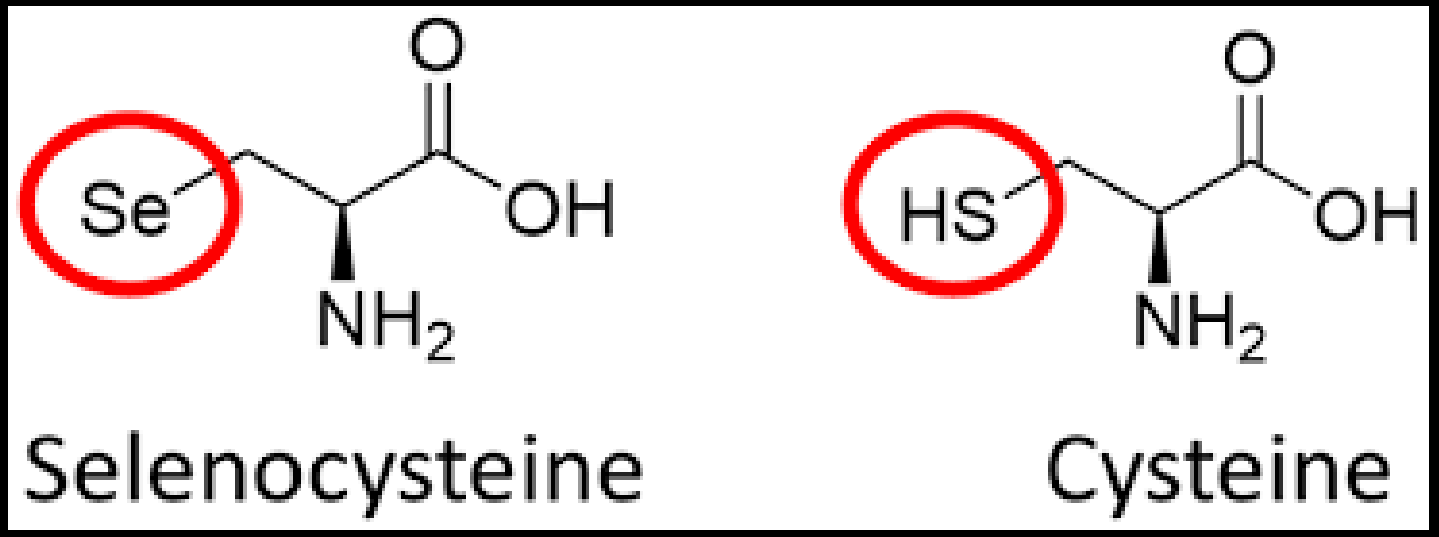
Lysine

(Lys or K)



Arginine

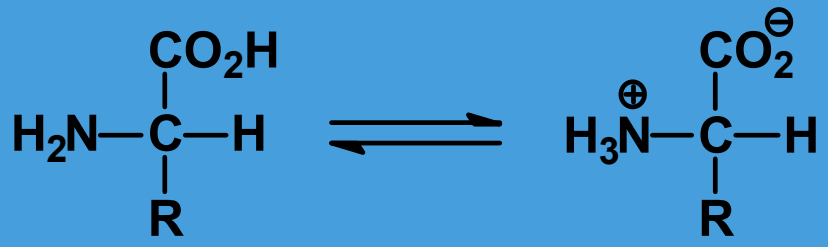
(Arg or R)



- These are found in *glutathione peroxidase*, *thioredoxin reductase*, and *iodothyronine deiodinase*.
- **Unnatural** or **unusual amino acids** are not naturally encoded amino acids. These are non-proteinogenic amino acids which can occur naturally in plant or bacteria post-translationally or, are chemically synthesized as pharmacological motifs.

Acid–Base Properties

- Since amino acids have both an acidic functionality and a basic functionality, we should expect the following equilibrium:



- In fact, the equilibrium lies to the right :

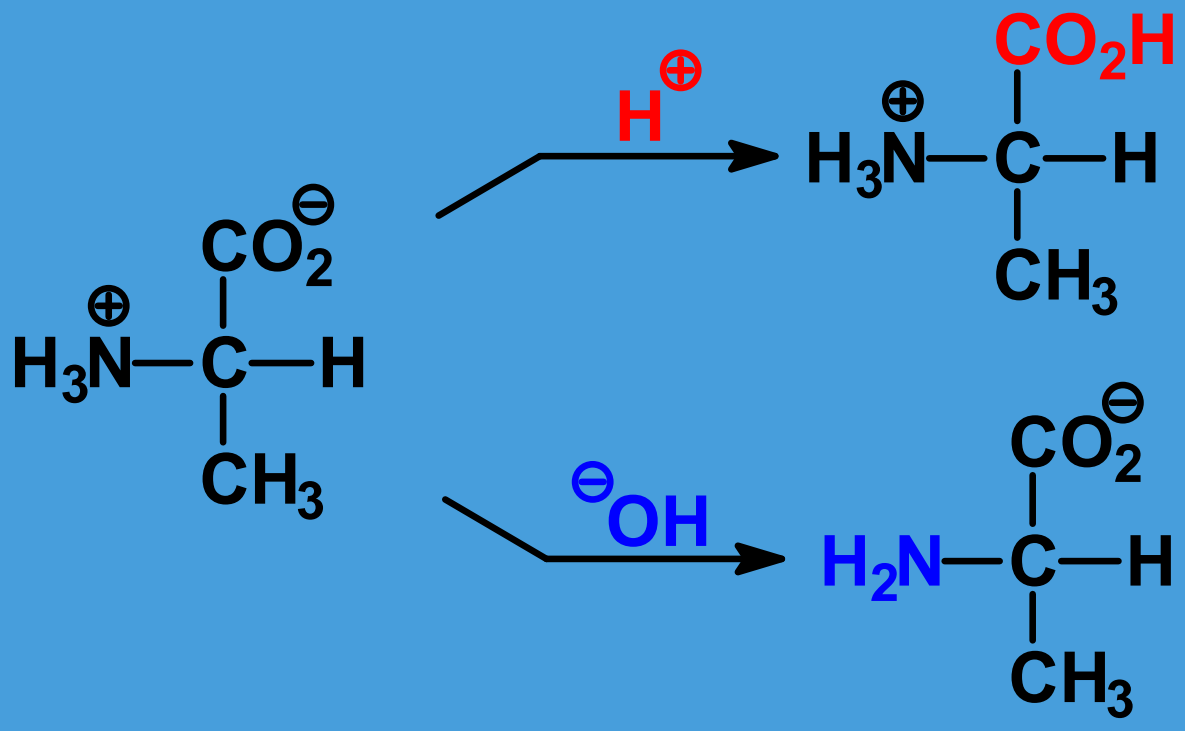
All amino acids are charged at any pH!

- In dry solid state, -COOH gp is dissociated forming a negatively charged carboxylate ion (COO^-) and amino gp is protonated forming positively charged ion (NH_3^+) forming a **Zwitter ion**

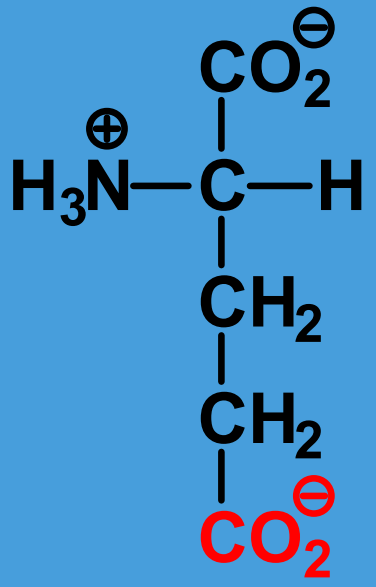
- Such species that are overall neutral molecules but contain charged ends are called ***zwitterions***

- α-amino acids owe a few of their properties to their zwitterionic form. They are: **i**) *allows it to act as both acid and base in different pH*. **ii**) *ionic form makes them high melting solids and insoluble in non-polar solvents (lipophobic)*. **iii**) *bipolar nature helps in coiling of the polypeptide chain*.

- Amino acids can react as either acids or bases:

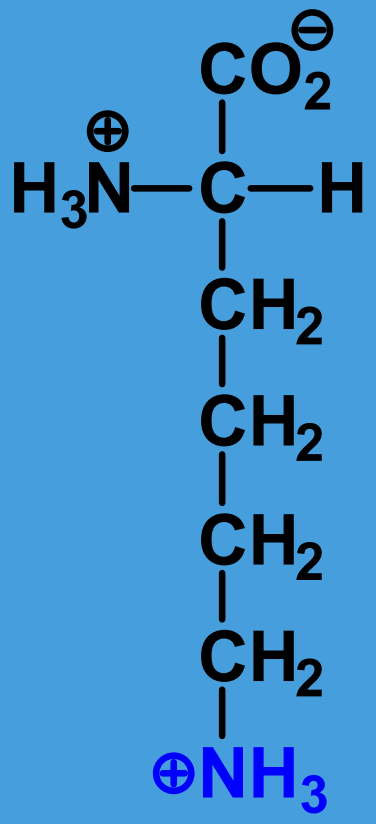


- Amino acids can also have side chains containing acidic or basic groups:



Deprotonated at physiological pH

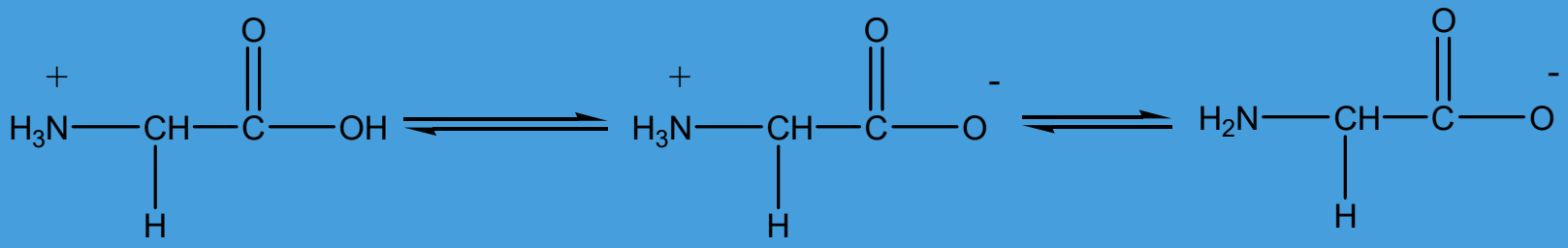
glutamic acid – acidic R



Protonated at physiological pH

Lysine –basic R

Isoelectric Point (pI)



Low pH (Highly acidic) → High pH (Highly basic)

- Isoelectric point, pI: the pH at which the majority of amino acid molecules in solution have no net charge
  - the pI for glycine, for example, falls between the pK_a values for the carboxyl and amino groups

$$pI = \frac{1}{2}\left(pK_a\ \alpha\text{–}COOH + pK_a\ \alpha\text{–}NH_3^+\right)$$

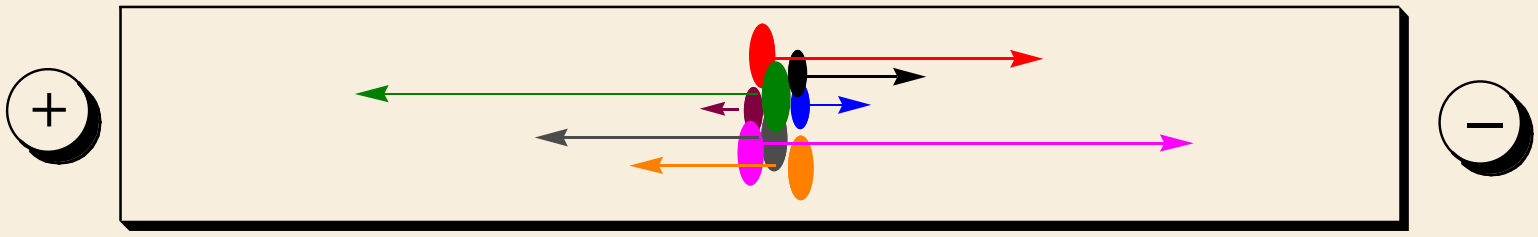
$$= \frac{1}{2}(2.35 + 9.78) = 6.06$$

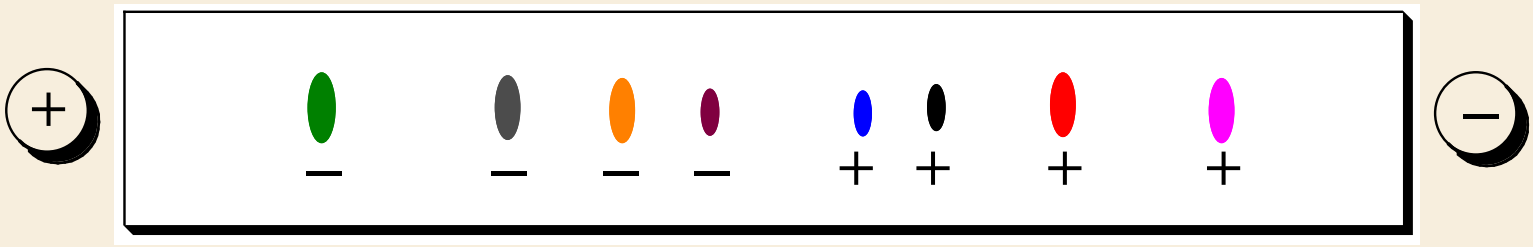
Nonpolar & polar side chains	pK_a of $\alpha-COOH$	pK_a of $\alpha-NH_3^+$
alanine	2.35	9.87
asparagine	2.02	8.80
glutamine	2.17	9.13
glycine	2.35	9.78
isoleucine	2.32	9.76
leucine	2.33	9.74
methionine	2.28	9.21
phenylalanine	2.58	9.24
proline	2.00	10.60
serine	2.21	9.15
threonine	2.09	9.10
tryptophan	2.38	9.39
valine	2.29	9.72

Acidic Side Chains	pK_a of α–COOH	pK_a of α–NH_3^+	pK_a of Side Chain	Side Chain Group
aspartic acid	2.10	9.82	3.86	carboxyl
glutamic acid	2.10	9.47	4.07	carboxyl
cysteine	2.05	10.25	8.00	sufhydryl
tyrosine	2.20	9.11	10.07	phenolic

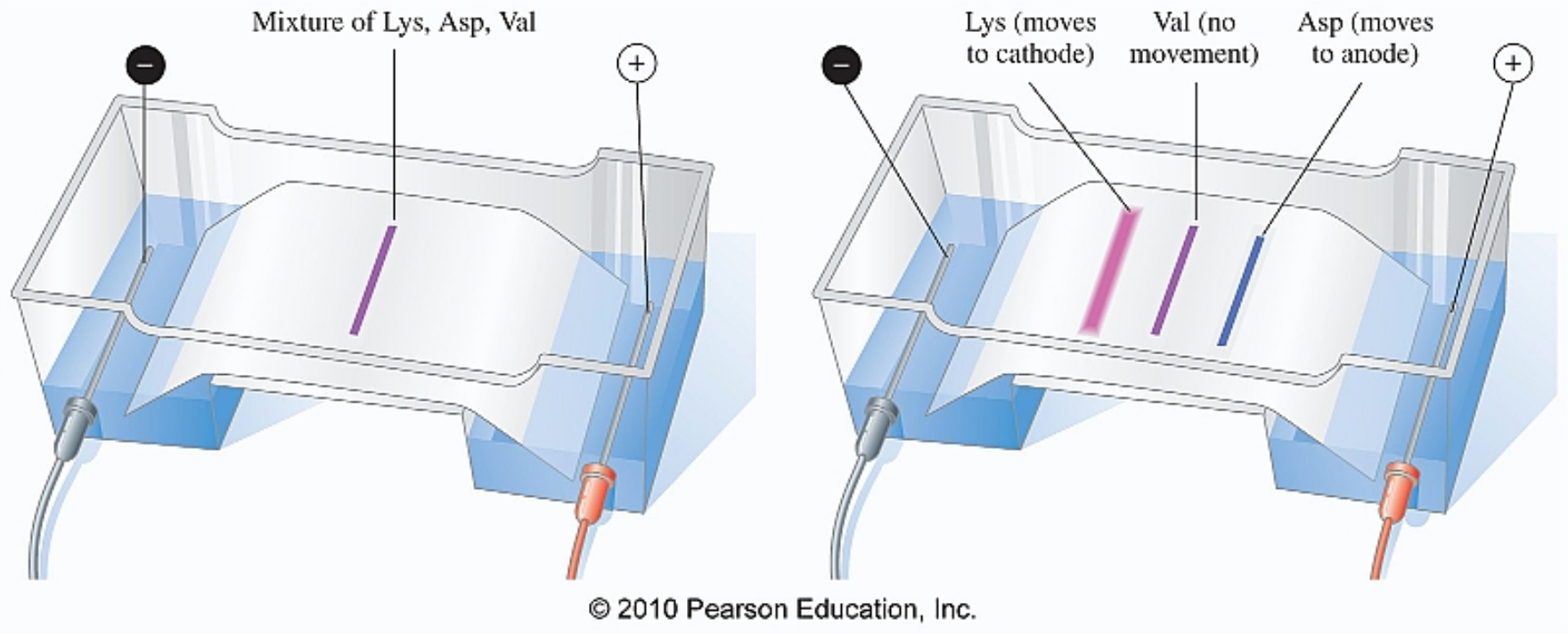
Basic Side Chains	pK_a of α–COOH	pK_a of α–NH_3^+	pK_a of Side Chain	Side Chain Group
arginine	2.01	9.04	12.48	guanidino
histidine	1.77	9.18	6.10	imidazole
lysine	2.18	8.95	10.53	1° amino

Electrophoresis: separation of polar compounds based on their mobility through a solid support under the influence of a uniform electric field.
The separation is based on charge (pI) or molecular mass.





pH = 6.0

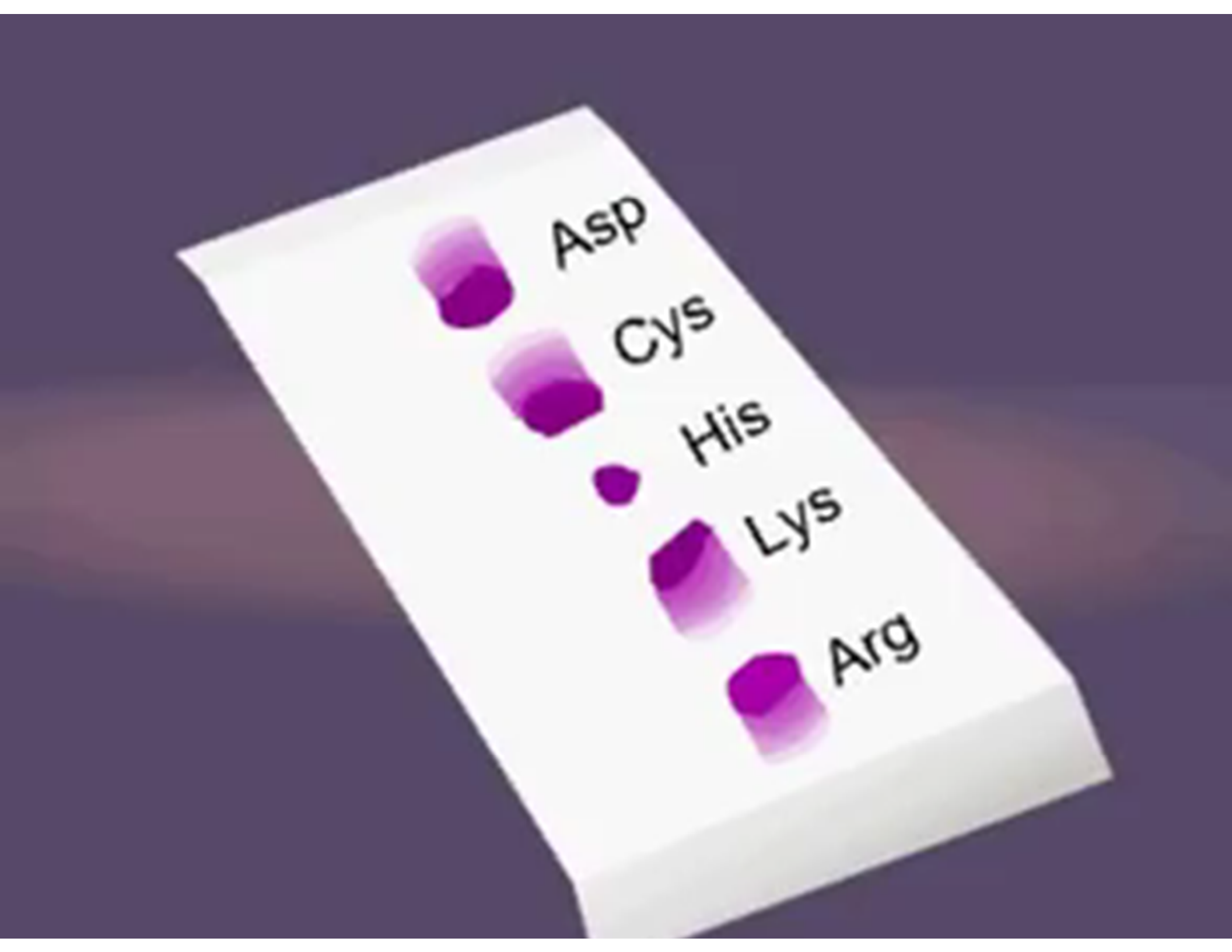


A positively charged species (pH < pI) moves toward the negative electrode; a negatively charged species (pH > pI) moves toward the positive electrode; a species with no net charge does not migrate.

lysine 9.7

aspartic acid 2.8

valine 6.0


Asp
Cys
His
Lys
Arg

Classification of amino acids

I- Chemical classification: According to number of COOH and NH_2 groups i.e. according to net charge on amino acid.

1) **Monobasic, monocarboxylic amino acids i.e. neutral or uncharged:**

i- Glycine R= H

ii- Alanine R= CH_3

iii- Branched chain amino acids: R is branched such as in:

a - **Valine** R= isopropyl gp

b- **Leucine** R= isobutyl gp

c- **Isoleucine** R = is isobutyl

iv- Neutral Sulfur containing amino acids:

e.g. Cysteine and Methionine. What is cystin?

v- Neutral, hydroxy amino acids:

e.g. Serine and Threonine

vi- Neutral aromatic amino acids:

a- Phenyl alanine : It's alanine in which one hydrogen of CH_3 is substituted with phenyl group. So it's called phenyl alanine

b- Tyrosine: - it is p-hydroxy phenyl alanine

- it is classified as **phenolic amino acid**

c- Tryptophan: as it contains indole ring so it is classified as **heterocyclic amino acid**

vii- Neutral heterocyclic amino acids:

a- Tryptophan: contains indole ring

b- Proline: In proline, amino group enters in the ring formation being α-imino gp so proline is an α-imino acid rather than α-amino acid

viii.Aspargine and Glutamine: They are amide forms of aspartate and glutamate in which side chain COOH groups are amidated.
They are classified as neutral amino acids.

2) Basic amino acids:

Contain two or more NH_2 groups or nitrogen atoms that act as base i.e. can bind proton.

At physiological pH, basic amino acids will be **positively charged**.

e.g.

a- **Lysine**

b- **Arginine:** contains guanido group

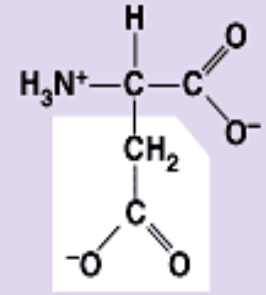
c- **Histidine:** is an example on basic heterocyclic amino acids

3) Acidic Amino acids:

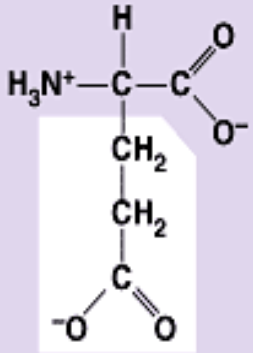
Contain two or more COOH groups that act as acid i.e. can donate proton.
At physiological pH will carry **negative charge**.
e.g. Aspartic acid (aspartate) and Glutamic acid (glutamate)

Electrically charged

Acidic

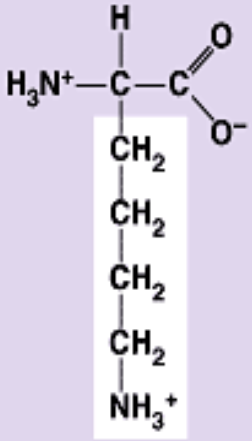


Aspartic acid (Asp)

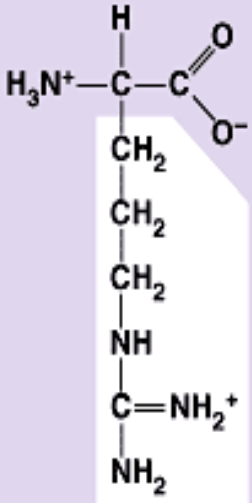


Glutamic acid (Glu)

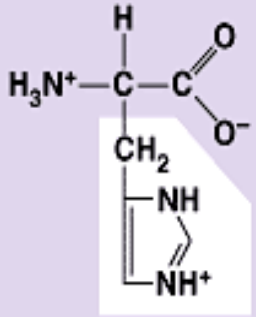
Basic



Lysine (Lys)



Arginine (Arg)



Histidine (His)

II- Classification according to polarity of side chain (R):

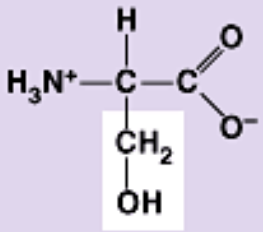
A- Polar amino acids: in which R contains polar hydrophilic group, so can form H-bond with H_2O. In those amino acids, R may contain:

1- OH group : as in serine, threonine and tyrosine
2- SH group : as in cysteine
3- amide group: as in glutamine and aspargine
4- NH_2 group or nitrogen act as a base (basic amino acids): as lysine, arginine and histidine
5- COOH group (acidic amino acids): as aspartic and glutamic .

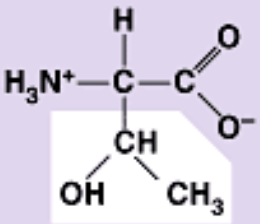
B- Non polar amino acids:

R is alkyl hydrophobic group which can't enter in hydrogen bond formation. 9 amino acids are non polar (glycine, alanine, valine, leucine, isoleucine, phenyl alanine, tryptophan, proline and methionine)

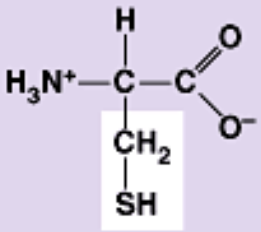
Polar



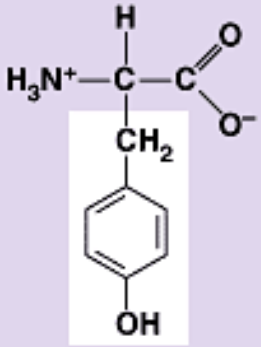
Serine (Ser)



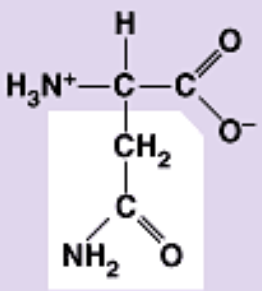
Threonine (Thr)



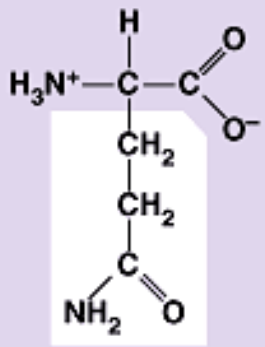
Cysteine (Cys)



Tyrosine (Tyr)

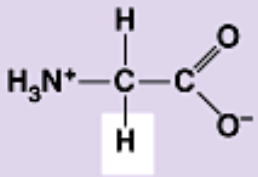


Asparagine (Asn)

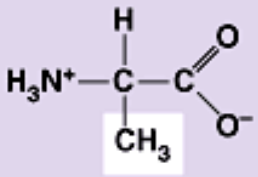


Glutamine (Gln)

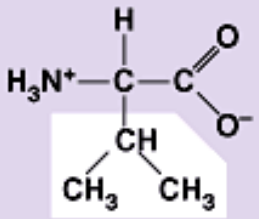
Nonpolar



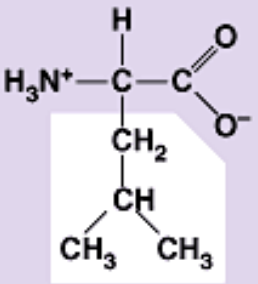
Glycine (Gly)



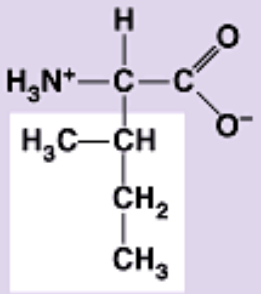
Alanine (Ala)



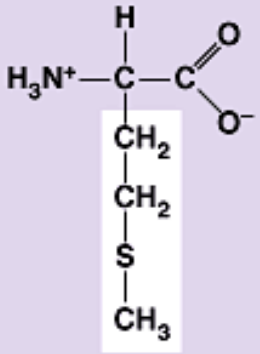
Valine (Val)



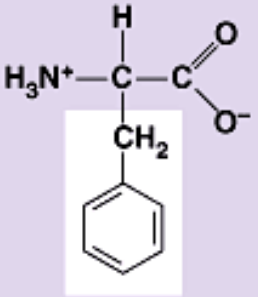
Leucine (Leu)



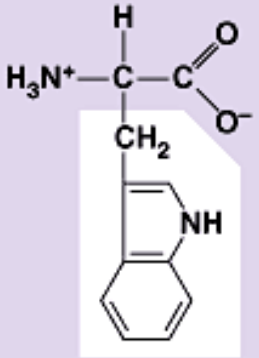
Isoleucine (Ile)



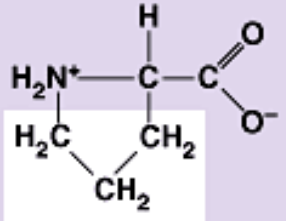
Methionine (Met)



Phenylalanine (Phe)



Tryptophan (Trp)



Proline (Pro)

III- Nutritional classification:

1- Essential amino acids: These amino acids can't be formed in the body and so, it is essential to be taken in diet. Their deficiency affects growth, health and protein synthesis.

Summary of essential amino acids:

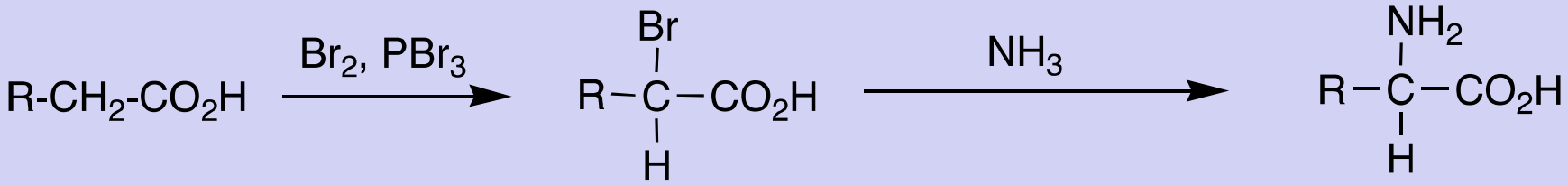
Valine Isoleucine Lysine Leucine Arginine Histidine

Methionine Tryptophan Threonine Phenylalanine

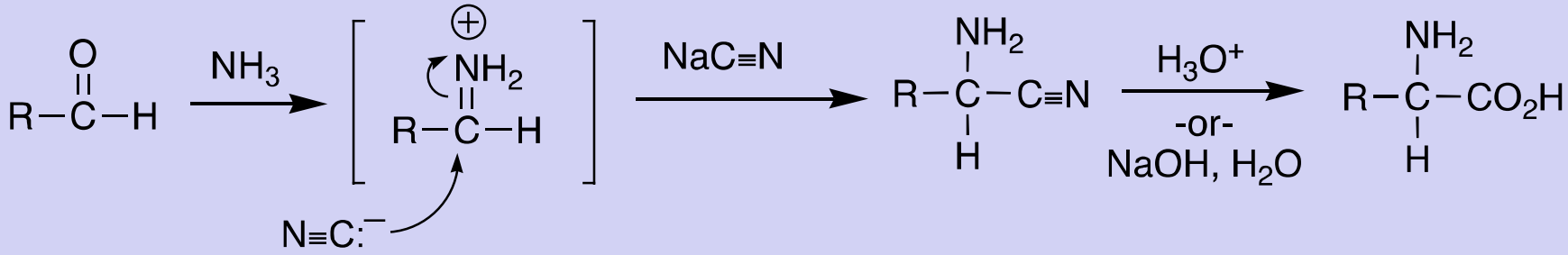
2- Non essential amino acids: These are the rest of amino acids that are formed in the body in amount enough for adults and children. They are the remaining 10 amino acids.

Synthesis of Amino Acids:

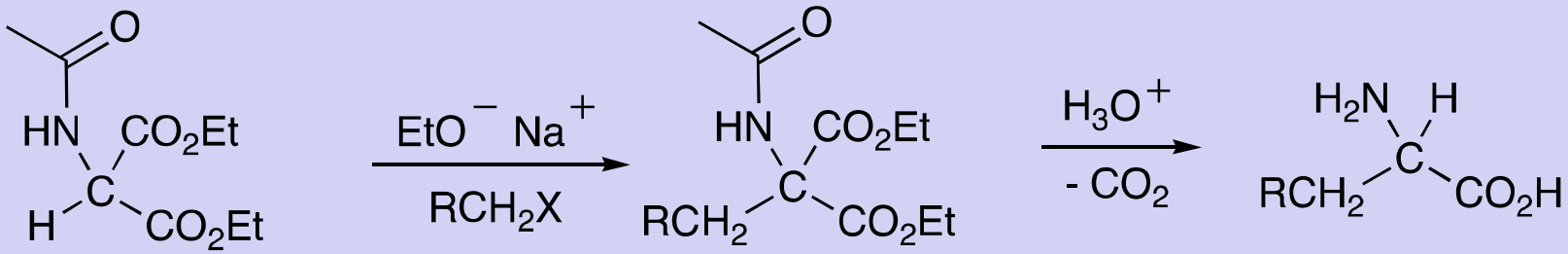
- **Nucleophilic substitution of α-halocarboxylic acids**



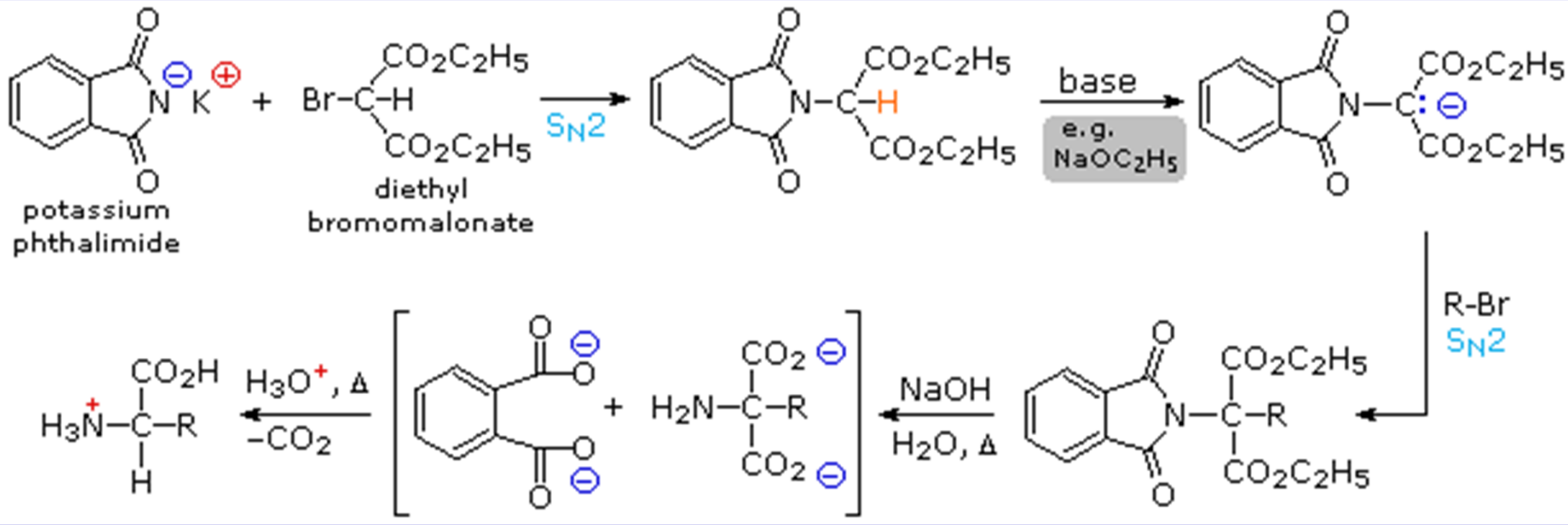
- **Strecker Synthesis**



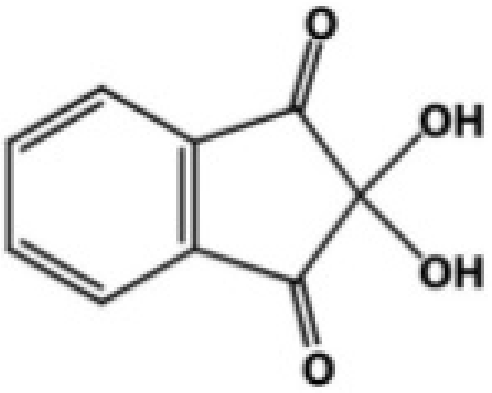
- **Synthesis from Acetamidomalonate**



Synthesis of amino acids : Gabriel phthalimide synthesis

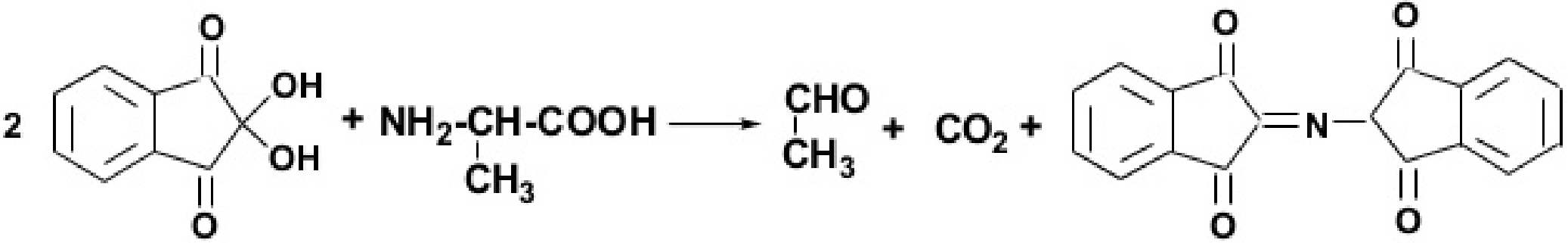


Detecting Amino Acids



Ninhydrin

Ninhydrin is the classical reagent for detecting amino acids.
Reaction requires 2-5 min at 100°C and is sensitive at the nanomole level.

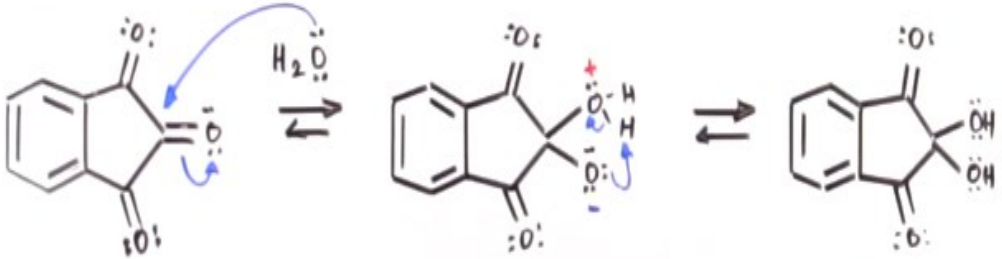


Ruhemann's Purple
570 nm

Note: The product from Pro is Yellow and absorbs at 440 nm.

Ninhydrin and Amino Acids

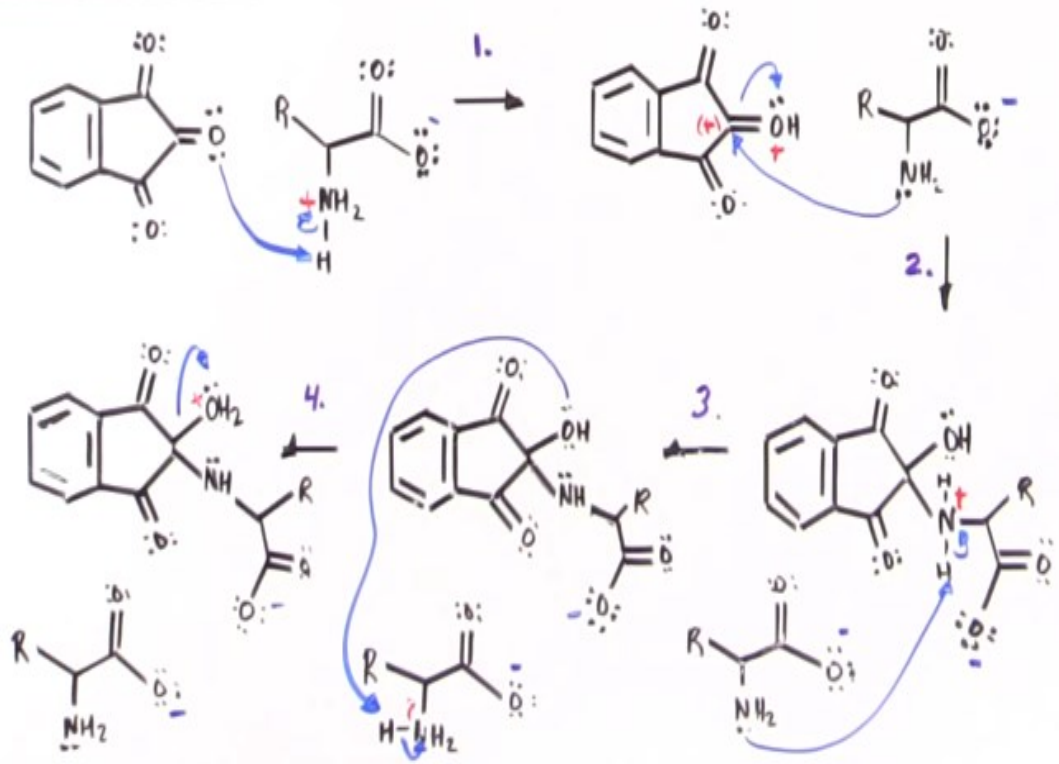
- When ninhydrin is mixed with any one of the twenty amino acids (except proline), it produces a molecule that turns the solution purple. By this method, ninhydrin can be used to detect the presence of amino acids.

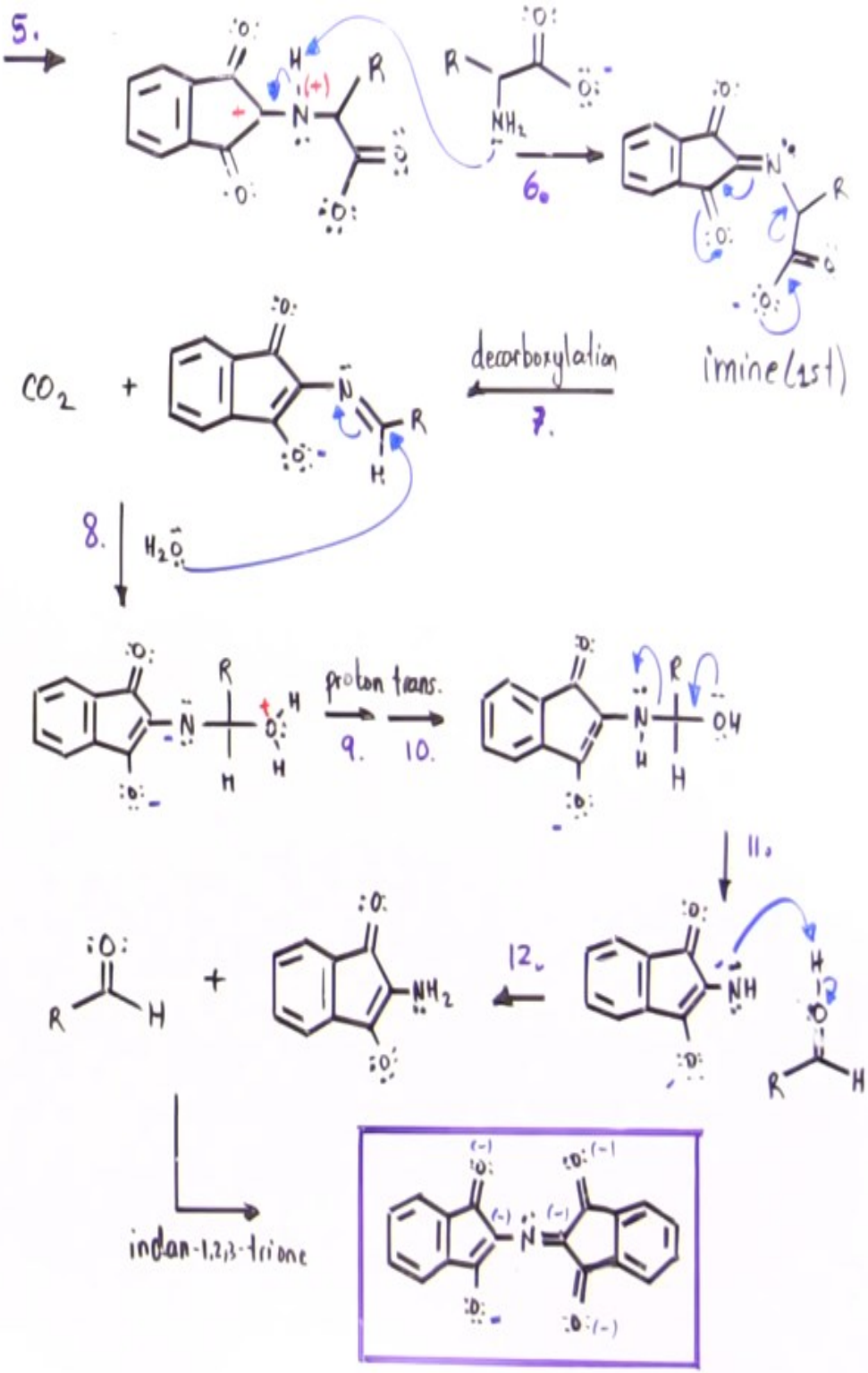


Indan-1,2,3-trione

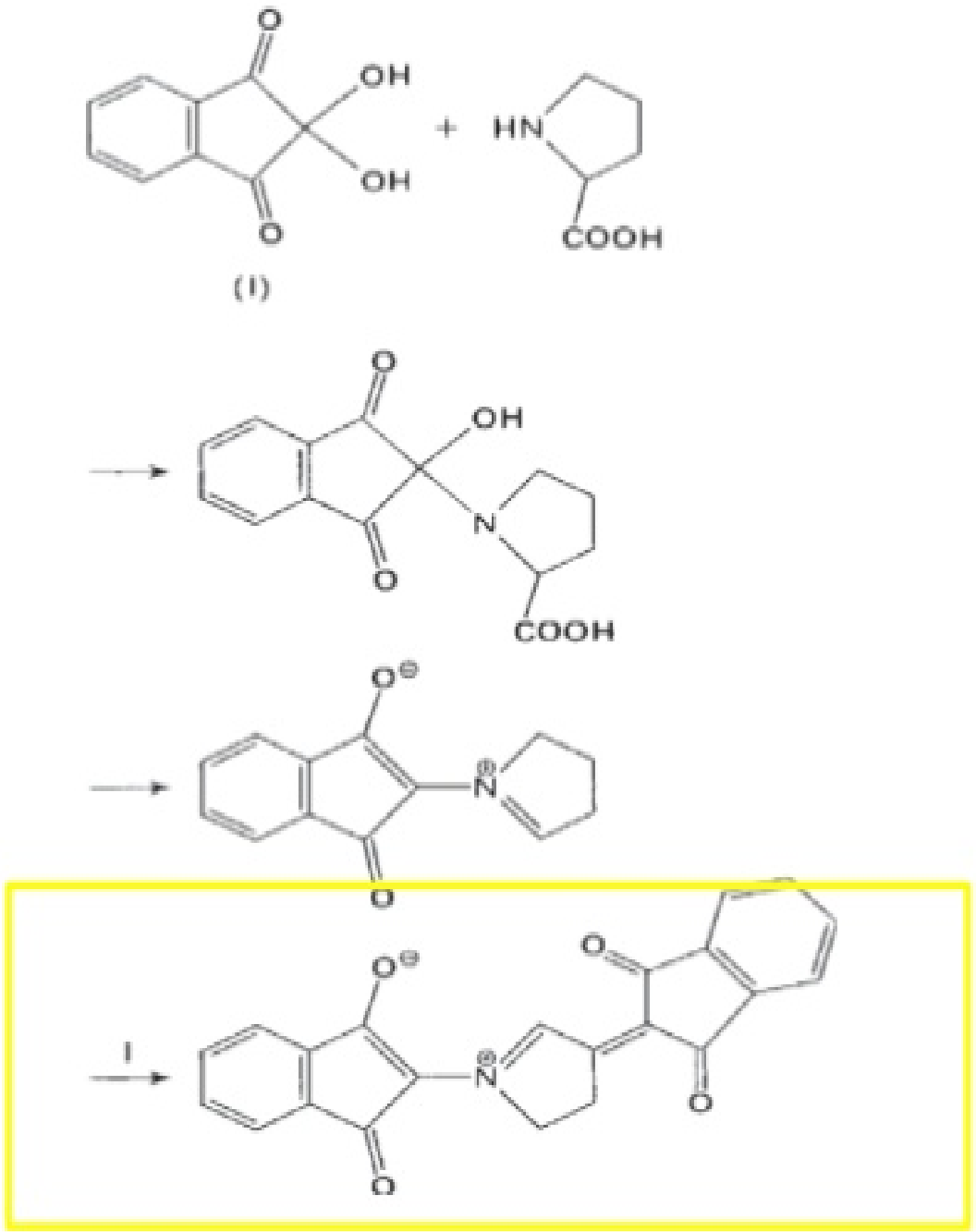
ninhydrin

Reaction Mechanism





Proline yields a yellow-colored compound with λmax= 440 nm



REACTION OF AMINO ACIDS WITH Cu (II) ions:

The biuret test, also known as Piotrowski's test, is a chemical test used for detecting the presence of peptide bonds. In the presence of peptides, a copper(II) ion forms purple-colored coordination complexes in an alkaline solution. The intensity of the color, and hence the absorption at 540 nm, is directly proportional to the protein concentration, according to the Beer–Lambert law.

Despite its name, the reagent does not in fact contain biuret (H_2N-CO-NH-CO-NH_2). The test is named so because it also gives a positive reaction to the peptide-like bonds in the biuret molecule.

In this assay, two H-atoms attached to two N-atoms are abstracted by the base. The copper(II) binds with these N^- atoms and other two N-atoms donate their lone pairs for co-ordination with Cu (II) present in the peptides of proteins. Due to its insensitivity and little interference by free amino acids, this assay is most useful for whole tissue samples and other sources with high protein concentration.

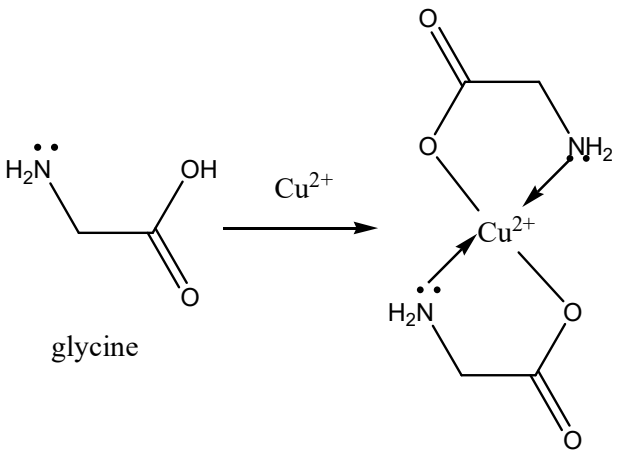


Fig: Reaction of free amino acid Glycine with Cu (II) ion

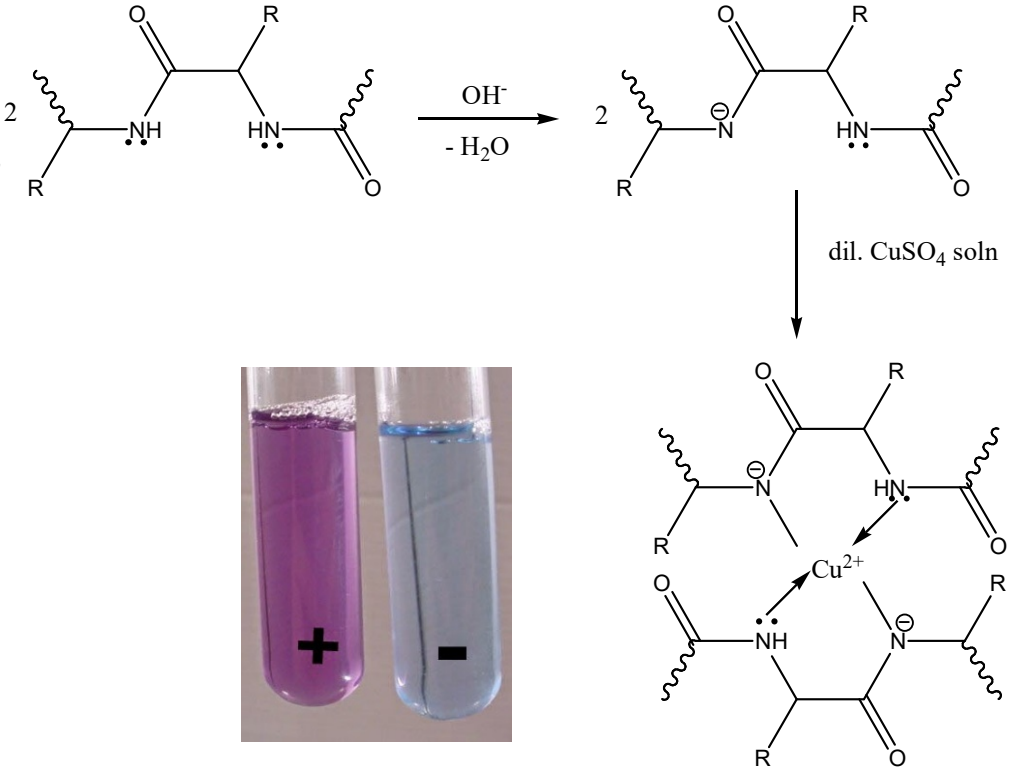


Fig.: Reaction of proteins (polymer of amino acids) with Cu(II) ions (Biuret test)

CLASSIFICATION OF AMINO ACIDS:

Based on metabolic fate: The carbon skeleton of amino acids can serve as a precursor for the synthesis of glucose(glycogenic) or fat (ketogenic) or both.

i) Glucogenic : These amino acids can serve as precursors for the formation of glucose or glycogen.
Eg. Alanine, arginine, asparagine, aspartame, cysteine, glutamine, glycine, proline, serine, histidine, methionine, threonine, valine.

ii) Ketogenic : Fat can be synthesized from these amino acids.
Eg. Leucine, lysine.

iii) Both : The four amino acids isoleucine, phenylalanine, tryptophan, tyrosine are pre-cursors for synthesis of glucose as well as fat.

Reactions of Amino Acids

1) Reactions due to amino group

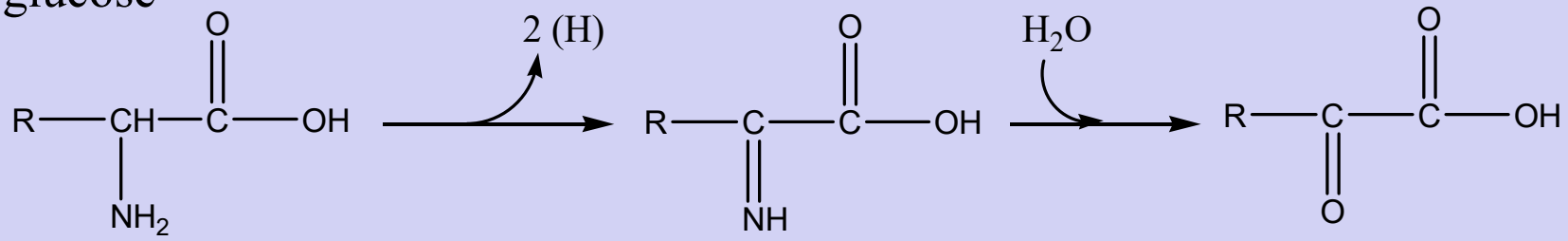
2) Reactions due to carboxyl group

3) Reactions due to both amino and carboxyl group

4) Reactions due to side chain

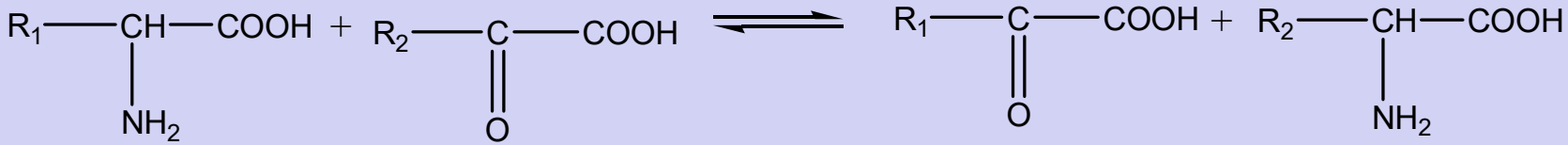
Reactions due to amino group:

i) *Oxidative deamination*: An amino gp is removed and corresponding α-keto acid is formed. It is further oxidized to other molecules like glucose



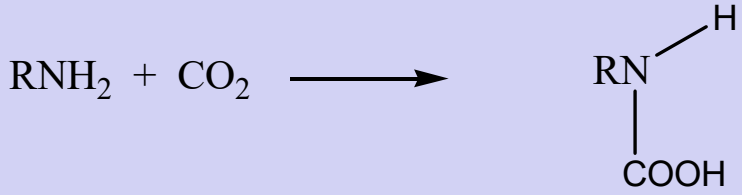
amino acid — imino acid — keto acid

ii) *Transamination:* Transfer of an amino gp from an amino acid to a keto acid to form a new amino acid and a corresponding keto acid



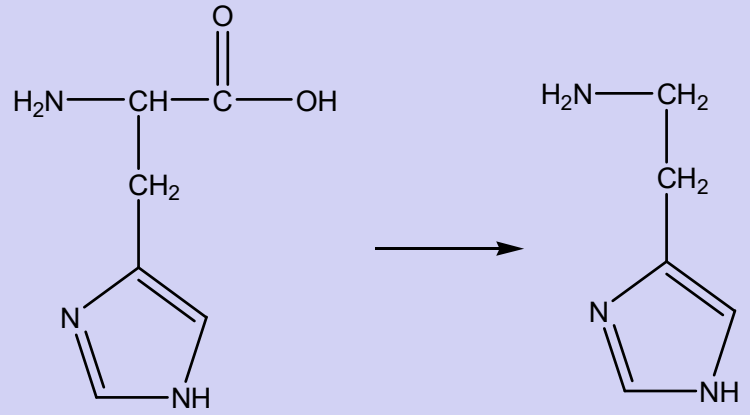
amino acid + keto acid ⇌ keto acid + amino acid

iii) *Formation of carbaimino compounds*: CO_2 binds to amino acid on the globin chain of Hb to form carbaminoHb.

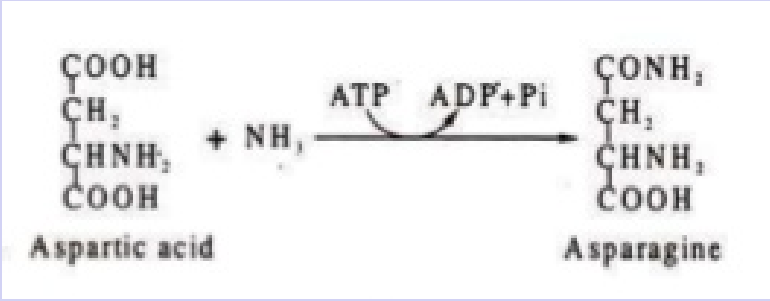


Reactions due to carboxyl group:

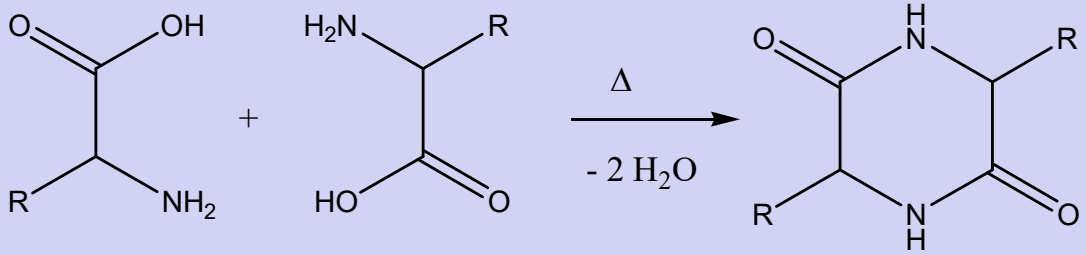
i) Decarboxylation: AA undergo α-decarboxylation to form corresponding amines.
Eg. Glutamic acid – Gamma-aminobutyric acid (GABA), Histidine-Histamine etc.



ii) Formation of amide linkage: Non α-carboxyl gp of an AA reacts with ammonia by condensation reaction to form corresponding amides



iii) Effect of heat: On heating, two molecules of AA condense by releasing 2 molecules of water to form a diketopiperazine molecule



AMINO ACIDS

DIKETOPIPERAZINE

Reactions due to side chains

1) Ester formation

- OH containing amino acids e.g. serine, threonine can form esters with phosphoric acid in the formation of phosphoproteins (figure-1)
- OH group containing amino acid can also form: Glycosides – by forming O- glycosidic bond with carbohydrate residues (figure-2)

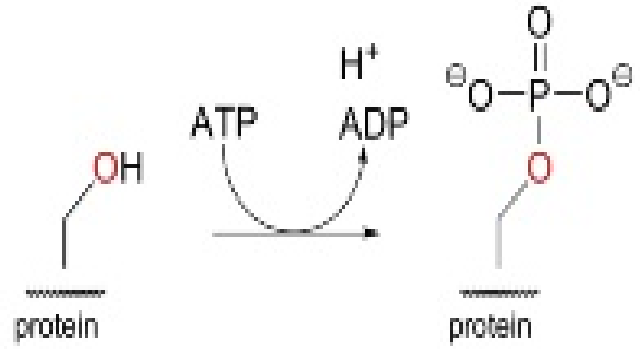


Figure-1

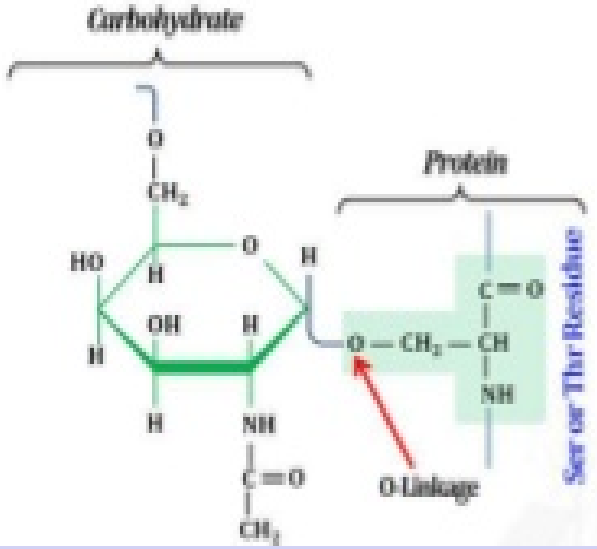
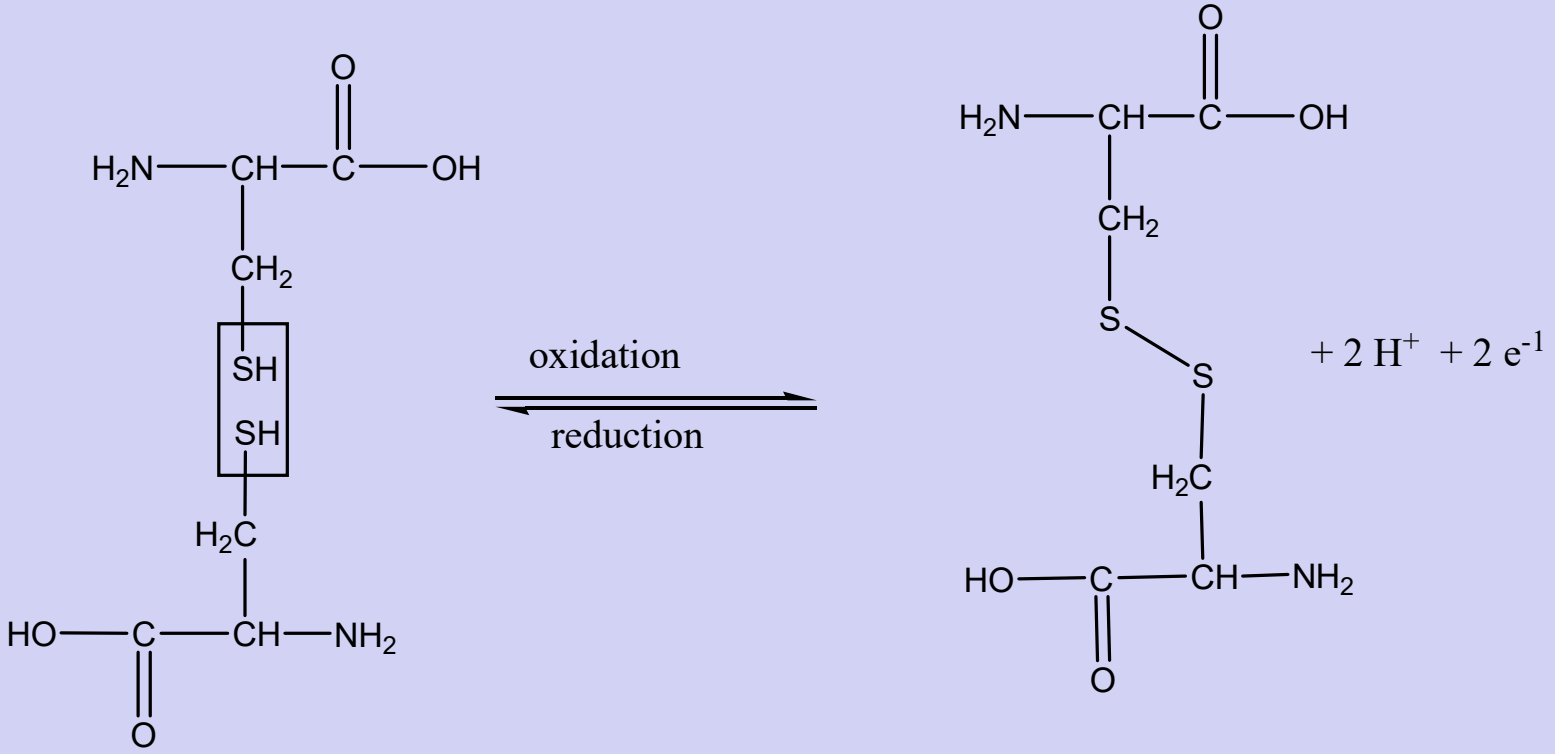


Figure-2

2. Disulfide linkage formation:

-Cysteine has a sulfhydryl group (SH) that can form a disulfide (S-S) linkage with another cysteine residue.
-The dimer is called Cystine



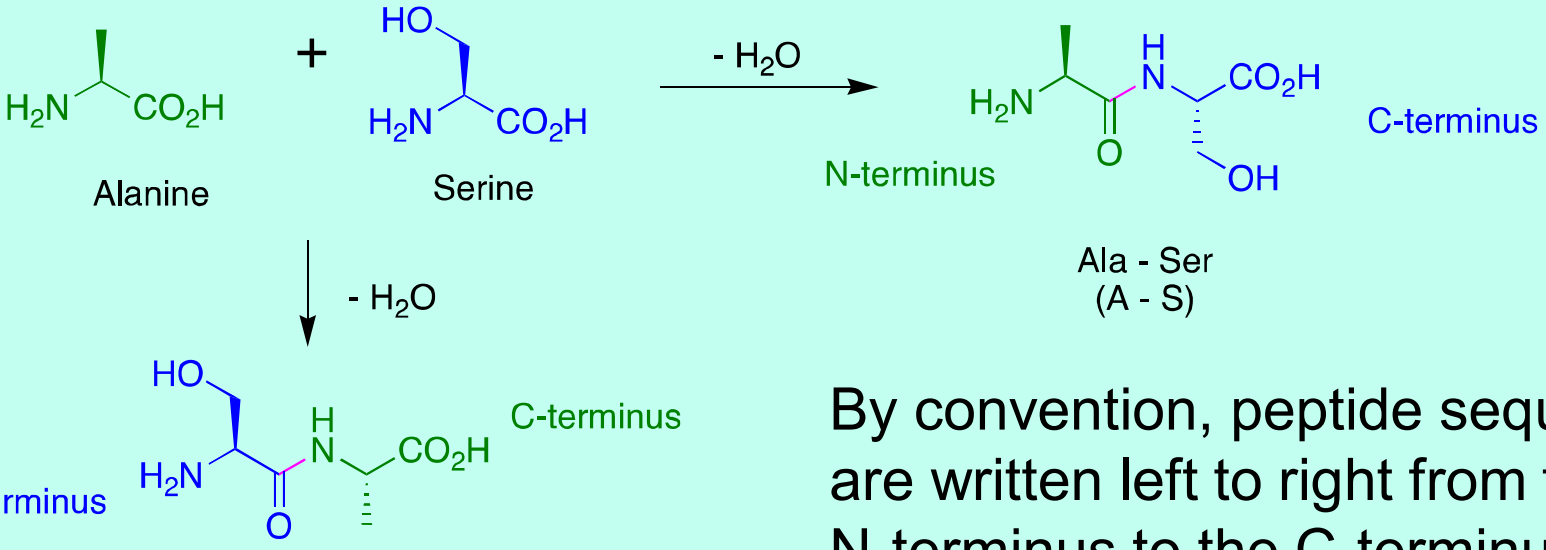
Peptides.

Proteins and peptides are polymers made up of amino acid units (residues) that are linked together through the formation of amide bonds (peptide bonds) from the carboxylate group of one residue and the amino of a second residue.

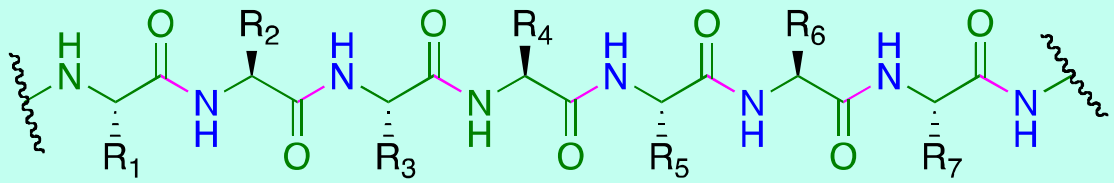
Biopolymer: the monomeric amino acids are linked through an amide bond (the carboxylic acids of one AA with the α-amino group of a second)

Peptide (< 50 amino acids)

Protein (> 50 amino acids)

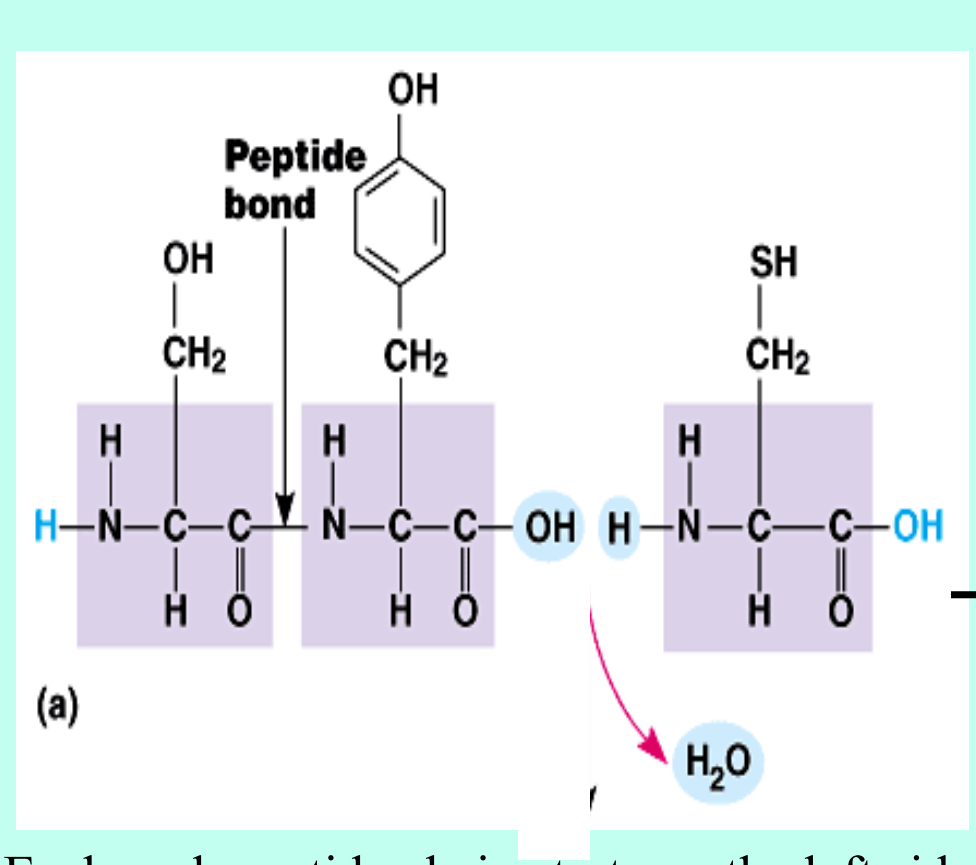


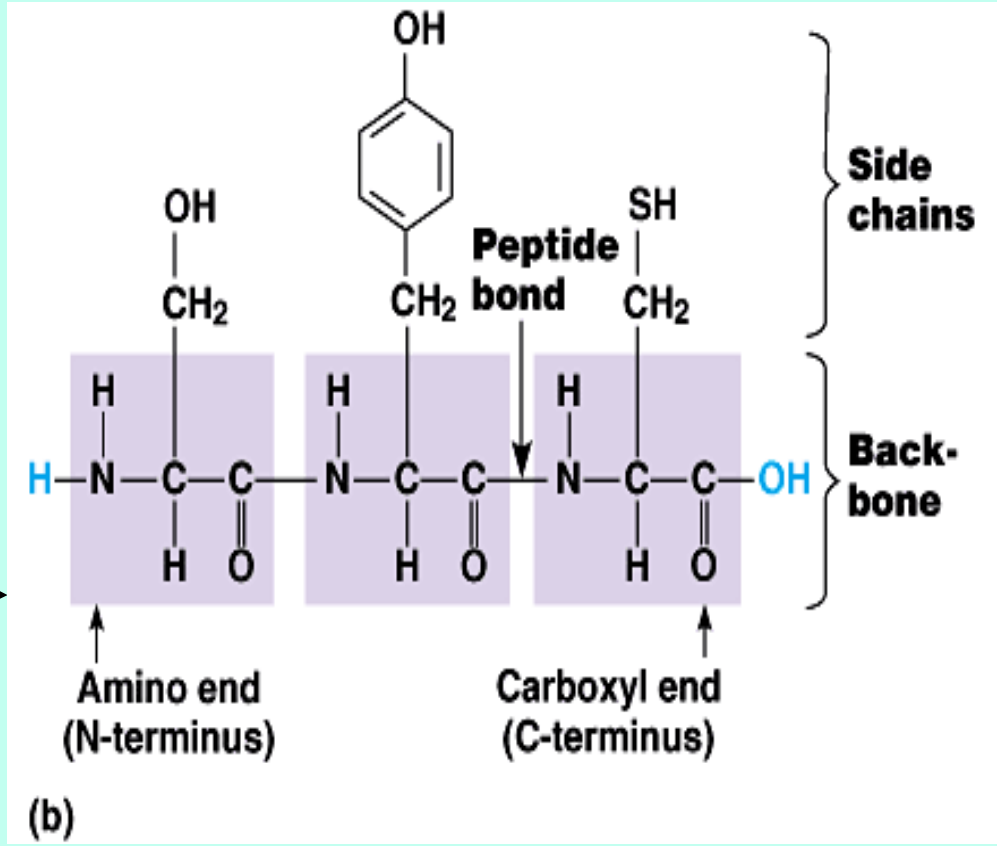
By convention, peptide sequences are written left to right from the N-terminus to the C-terminus



backbone

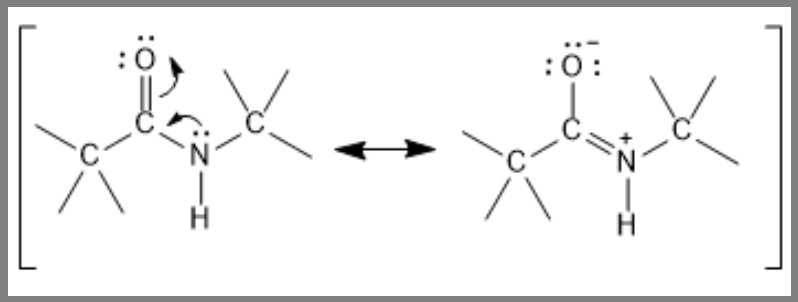
Peptide bond formation:

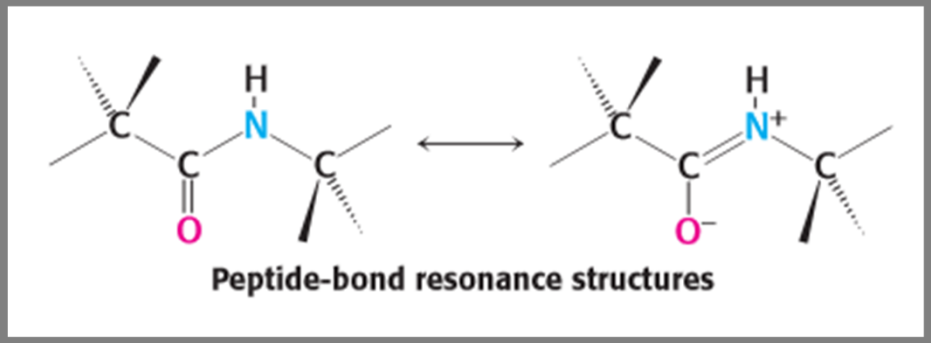


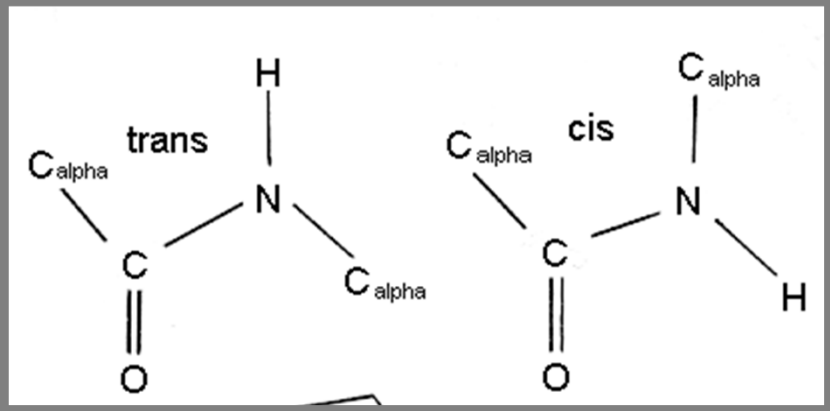


- Each polypeptide chain starts on the left side by free amino group of the first amino acid enter in chain formation . It is termed (N- terminus).

- Each polypeptide chain ends on the right side by free COOH group of the last amino acid and termed (C-terminus).







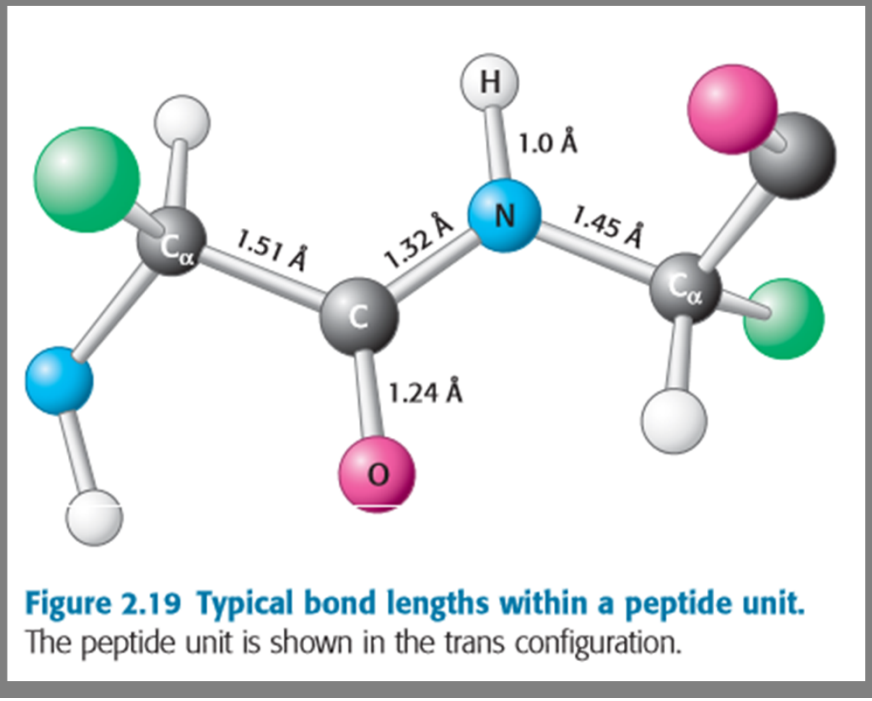
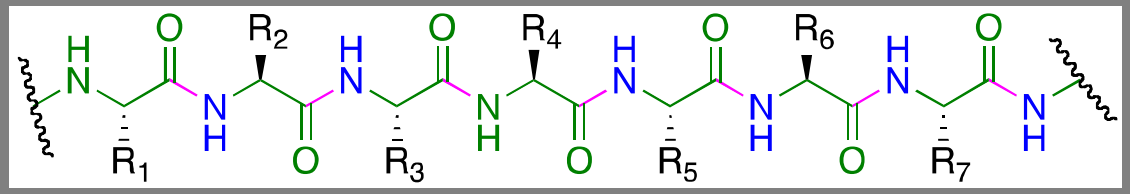
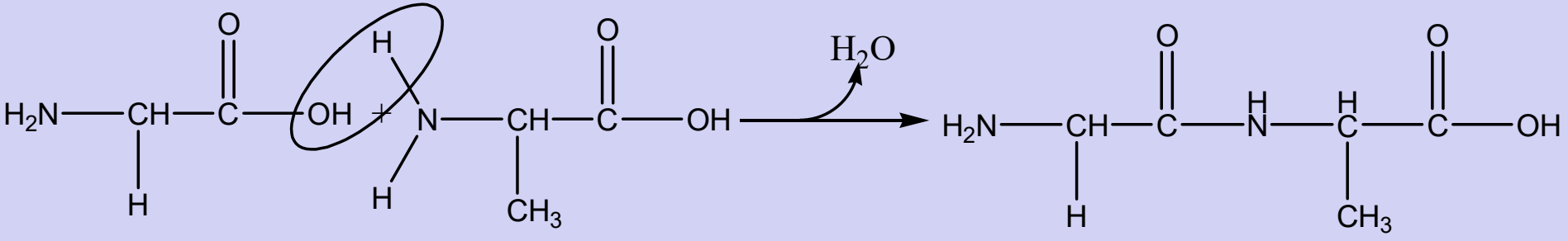


Figure 2.19 Typical bond lengths within a peptide unit.
The peptide unit is shown in the trans configuration.



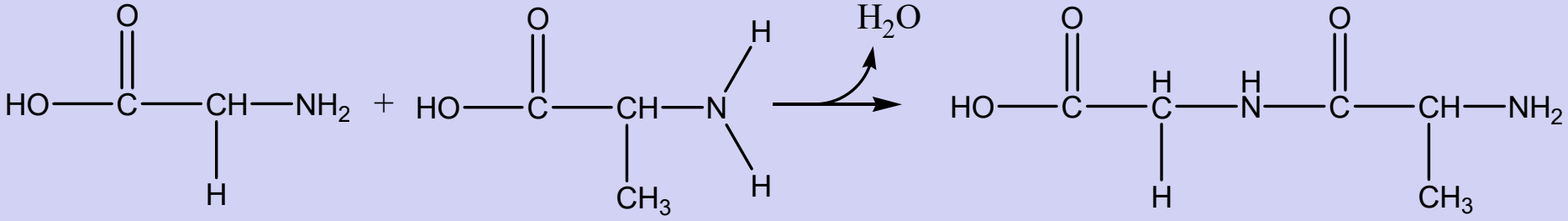
Reactions due to both amino and carboxyl group:

Formation of peptide bond: Carboxyl gp of an amino acid (head) binds with amino gp of another amino acid (tail) forming a peptide bond with the loss of one molecule of water.



glycine alanine Gly-ala dipeptide

CORRECT



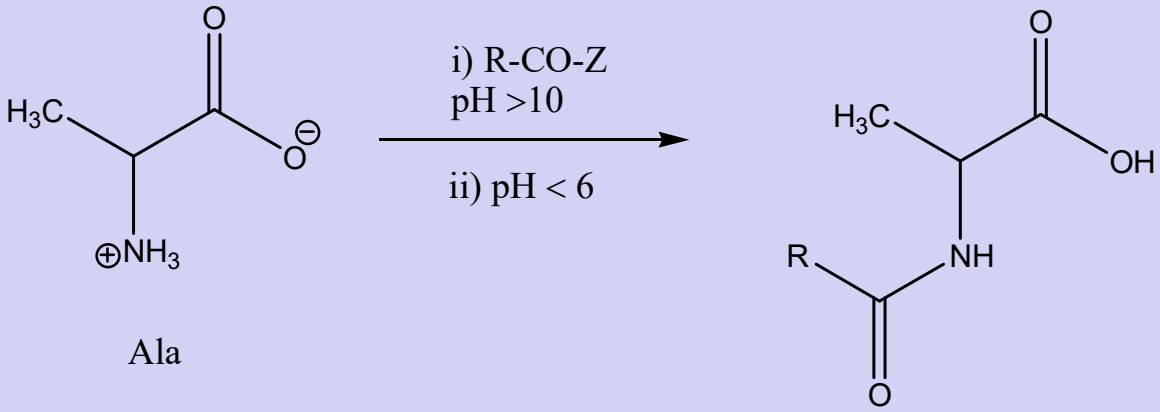
glycine alanine Gly-ala dipeptide

(Ala-gly dipeptide)

INCORRECT

Steps in peptide synthesis: The following steps are for synthesis of dipeptide Ala-gly

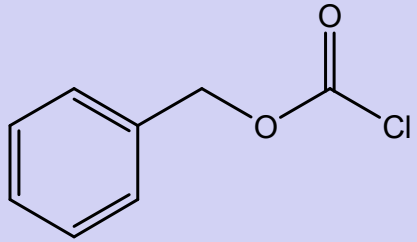
Step 1. N-protection of the first AA i.e., alanine in this example



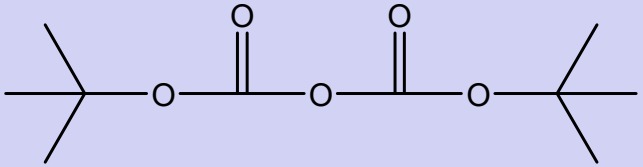
Ala

N-protected Ala

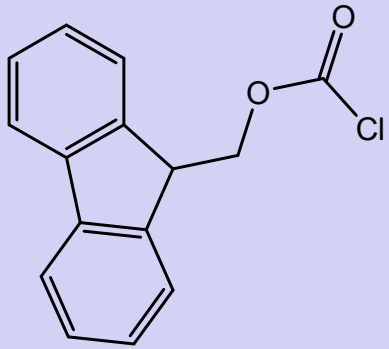
R-CO-Z :



Benzylchloroformate (Cbz)

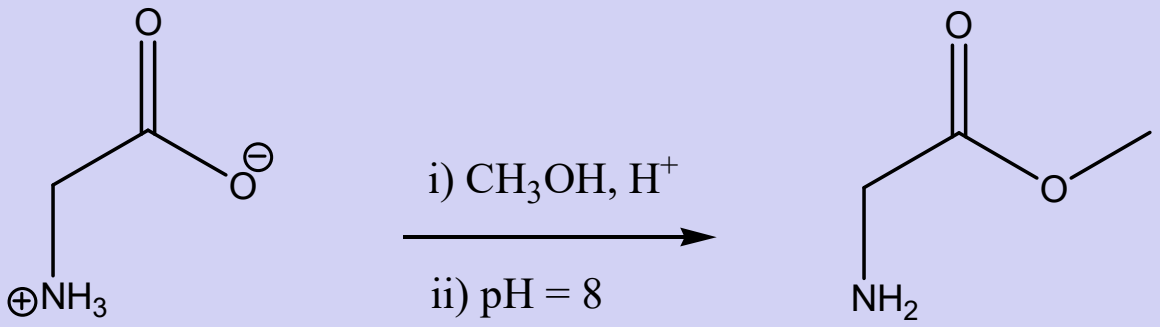


Di-tertbutyldicarbonate (BOC)



Flourinylmethoxycarbonylchloride (Fmoc)

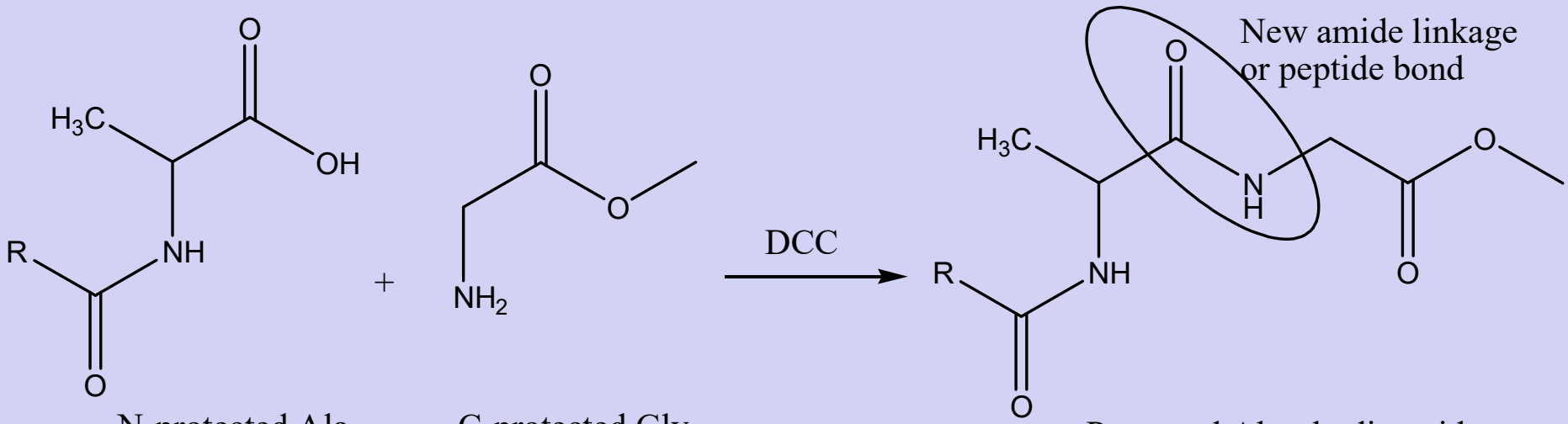
Step 2: C-protection of the second AA i.e. glycine in this example



Gly

C-protected Gly

Step 3: Amide bond synthesis with DCC

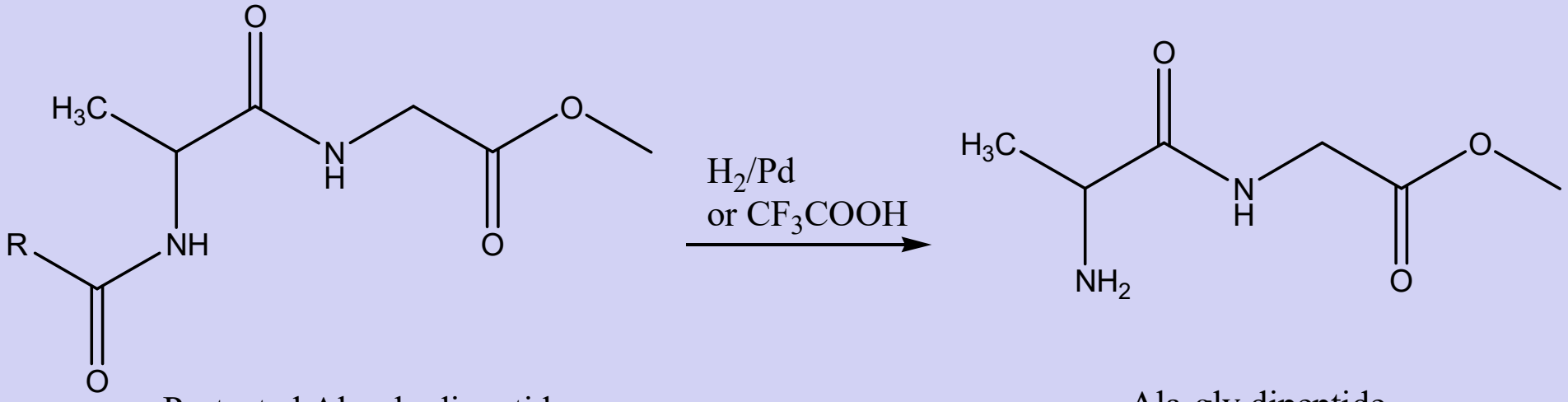


N-protected Ala

C-protected Gly

Protected Ala-gly dipeptide

Step 4: Deprotection of NH_2 and COOH: Deprotection methods are different for Cbz and BOC. H_2/Pd is used in case of Cbz and CF_3COOH is used for BOC while piperidine is used for Fmoc.

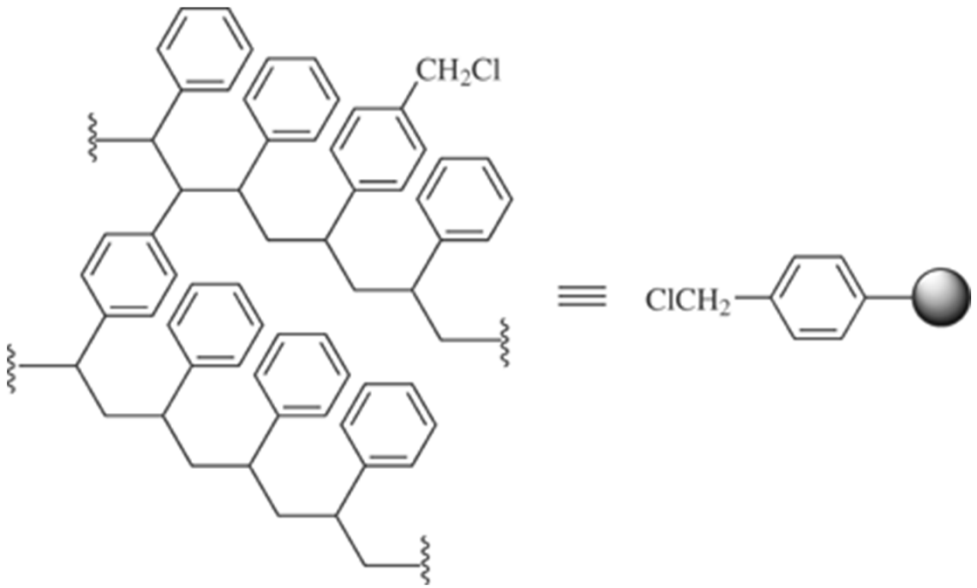


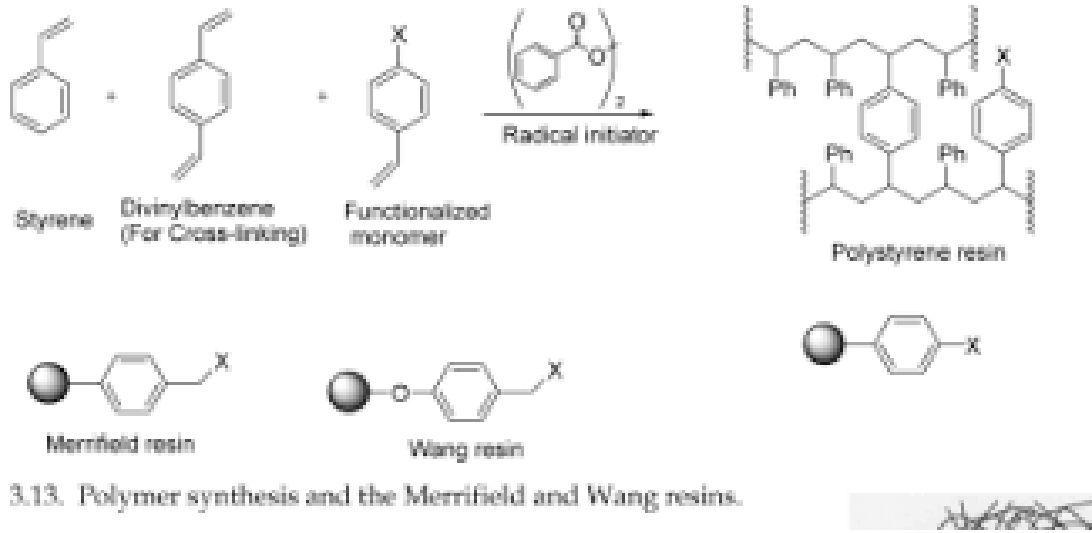
Protected Ala-gly dipeptide

Ala-gly dipeptide

In case of polypeptide synthesis, this product is made to react with an N-protected AA which results in a tripeptide which further reacts with another N-protected AA and the sequence is repeated till required polypeptide chain is achieved.

Merrifield Resin

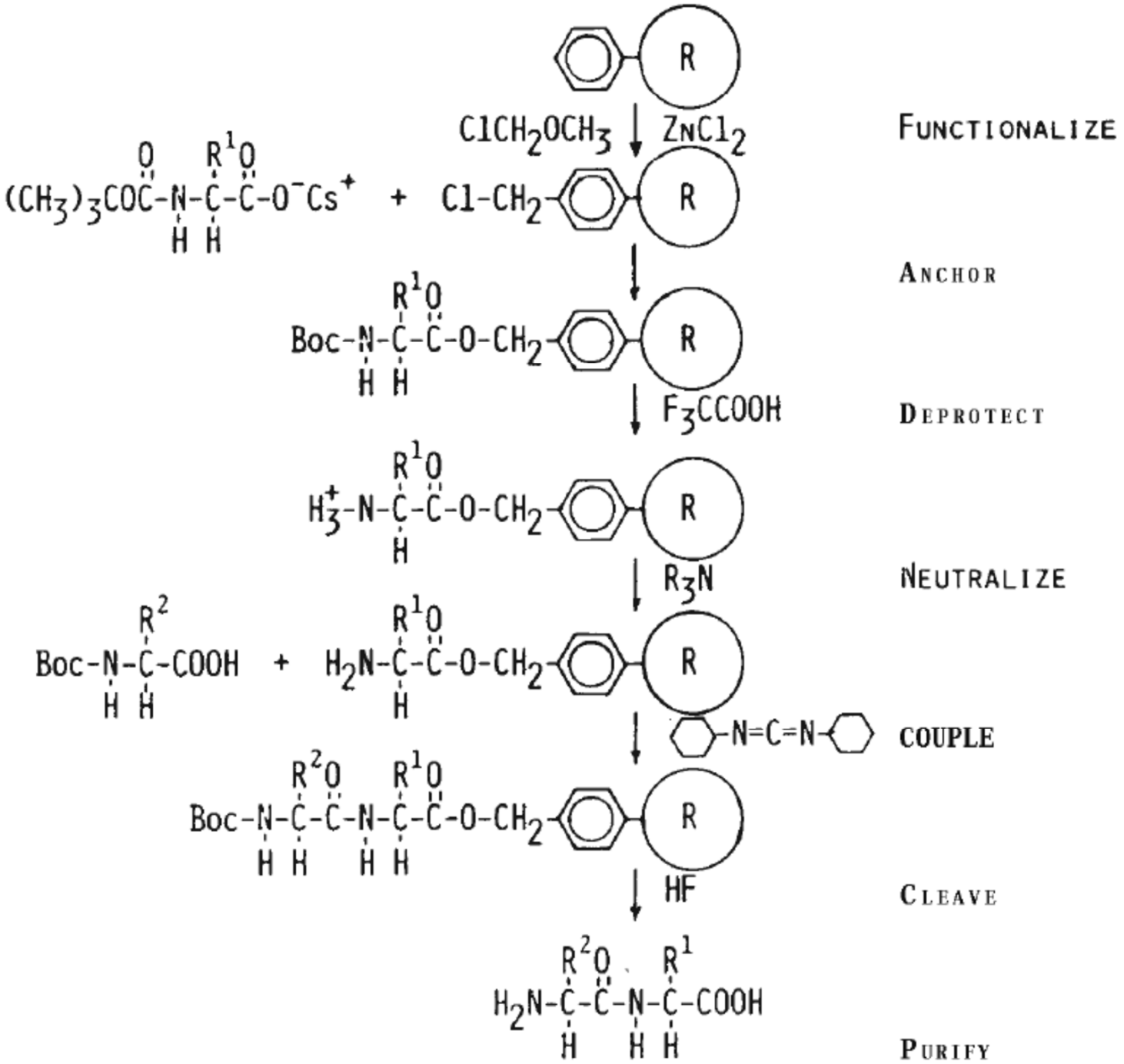




3.13. Polymer synthesis and the Merrifield and Wang resins.

The addition of each amino acid in the chain required a cycle of protection, attachment and deprotection, both along the chain and on the side-groups. Purification of the intermediate products also takes time.

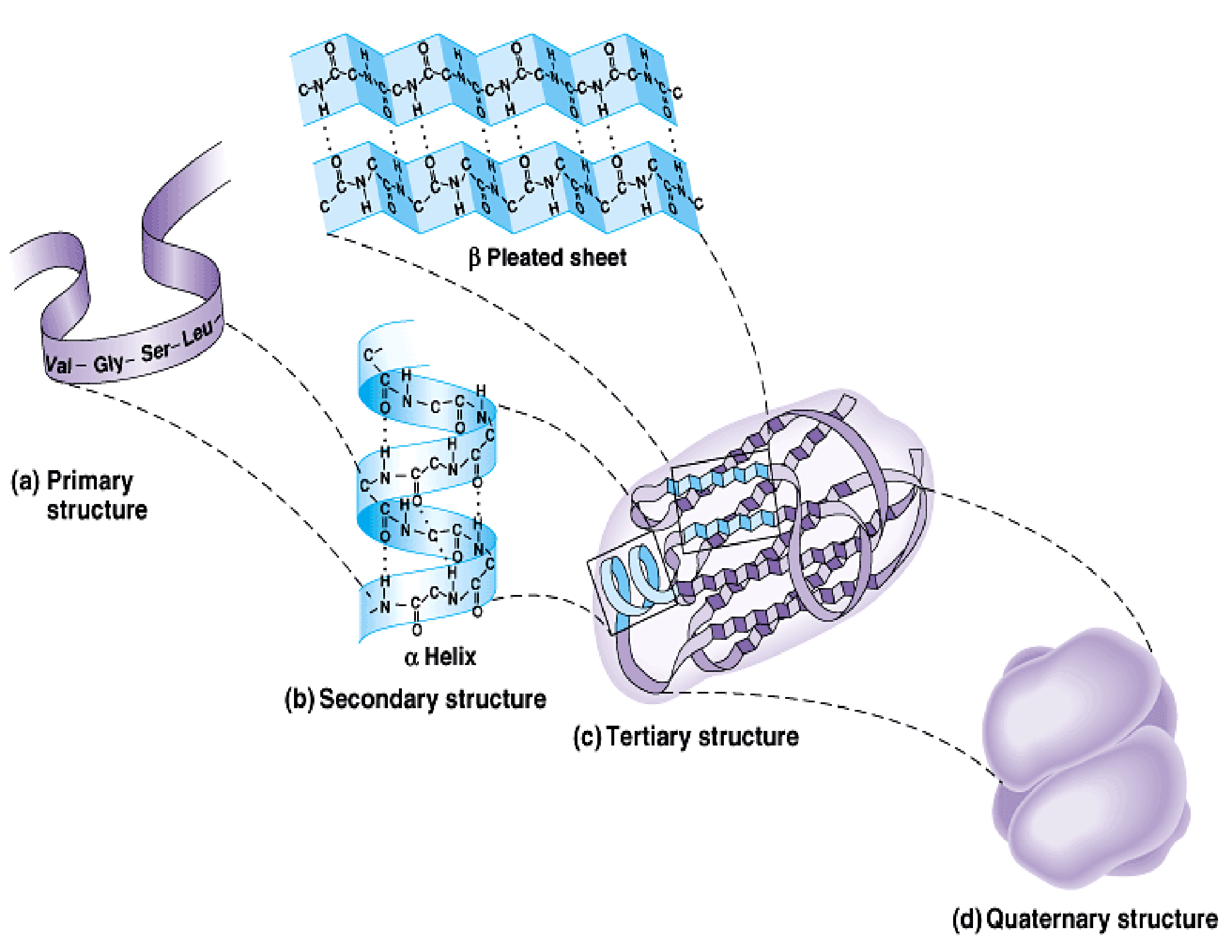
To overcome these disadvantages, Merrifield designed a synthesis where the C-terminus of an amino acid was anchored to an insoluble polymeric support (Merrifield resin). A second, protected, amino acid could then be coupled onto the first. After washing and deprotection in situ, the next amino acid could be clipped on. Hence the chain would grow, tethered to the support, removing the need for purification at each stage.



Scheme for solid-phase peptide synthesis

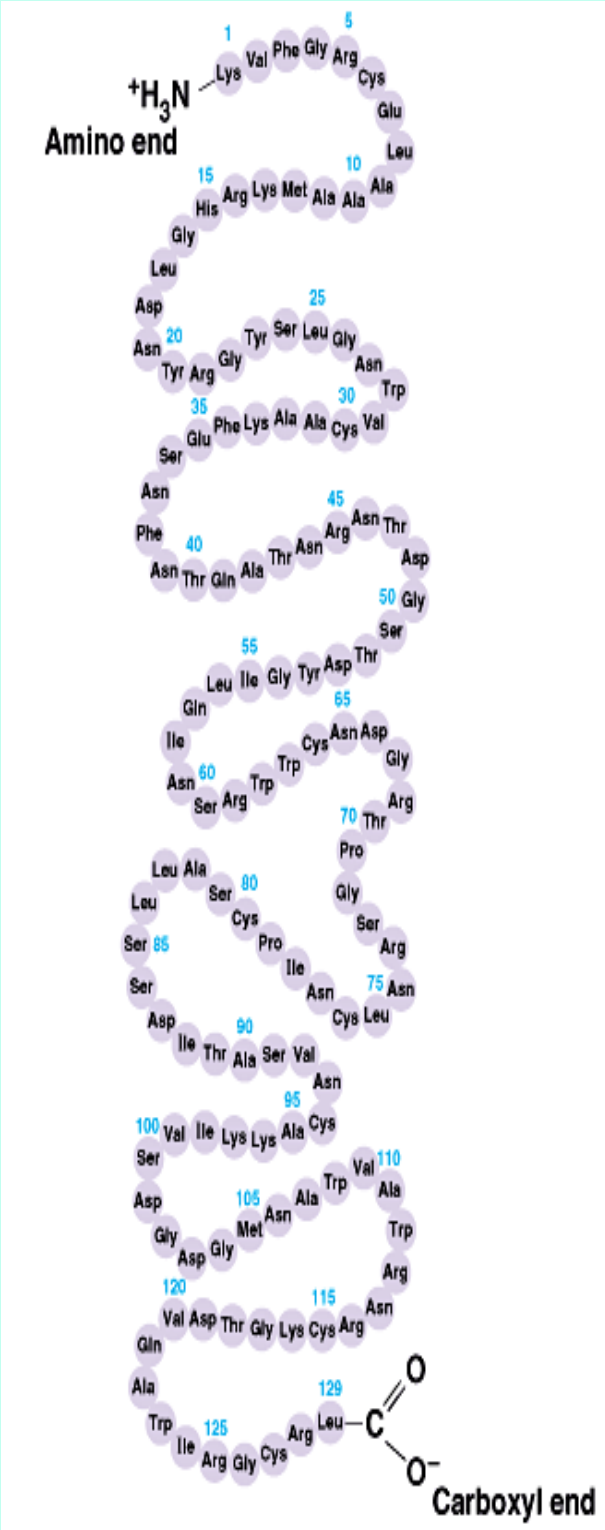
Levels of Protein Structure

- **Primary (1°) Protein Structure**
  - linear sequence of amino acids.
- **Secondary (2°) Protein Structure**
  - localized regional structures
- **Tertiary (3°) Protein Structure**
  - overall shape of proteins
- **Quaternary (4°) Protein Structure**
  - interactions between proteins


β Pleated sheet
Val – Gly – Ser – Leu
(a) Primary structure
α Helix
(b) Secondary structure
(c) Tertiary structure
(d) Quaternary structure

Primary structure:

- The **primary structure** of a protein is its unique sequence of amino acids.
  - The precise primary structure of a protein is determined by inherited genetic information.
  - At one end is an amino acid with a free amino group the (the N-terminus) and at the other is an amino acid with a free carboxyl group the (the C-terminus).

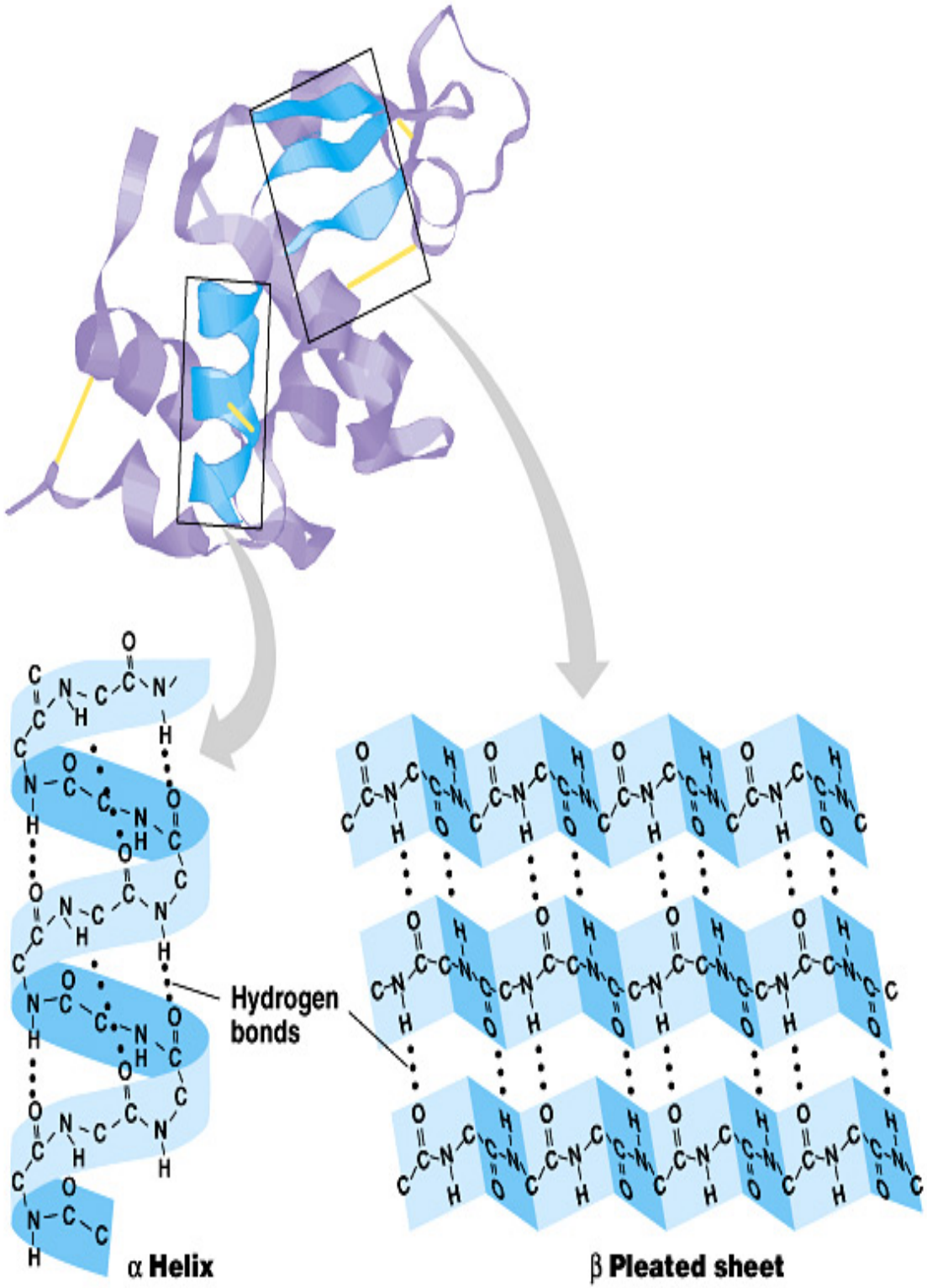


2- Secondary structure:

Results from hydrogen bond formation between hydrogen of –NH group of peptide bond and the carbonyl oxygen of another peptide bond. According to H-bonding there are two main forms of secondary structure:

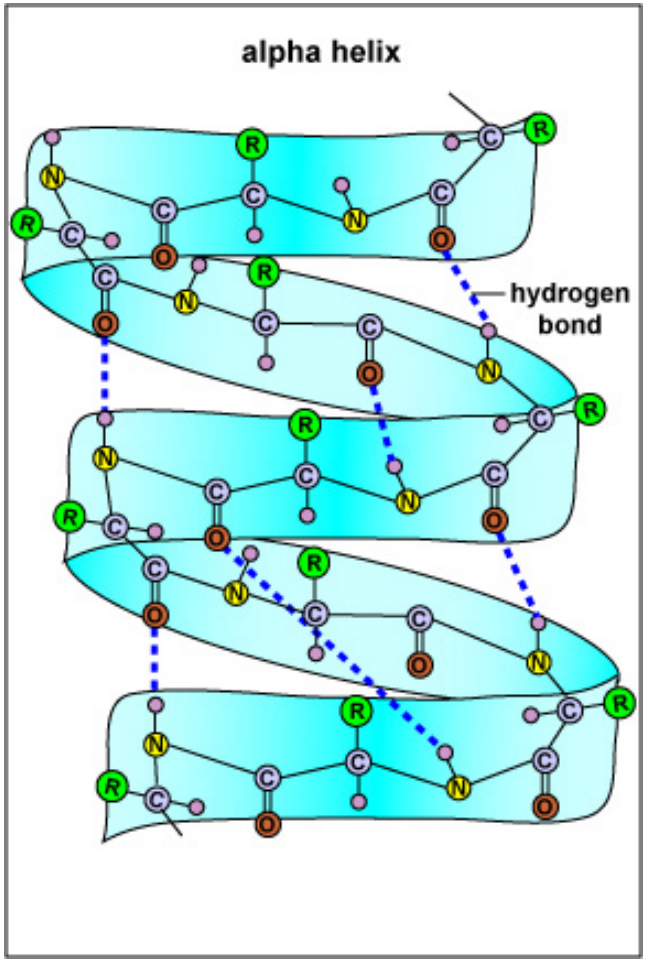
α-helix: It is a spiral structure resulting from hydrogen bonding between one peptide bond and the fourth one

β-sheets: It is another form of secondary structure in which two or more polypeptides (or segments of the same peptide chain) are linked together by hydrogen bond between H- of NH- of one chain and carbonyl oxygen of adjacent chain (or segment).

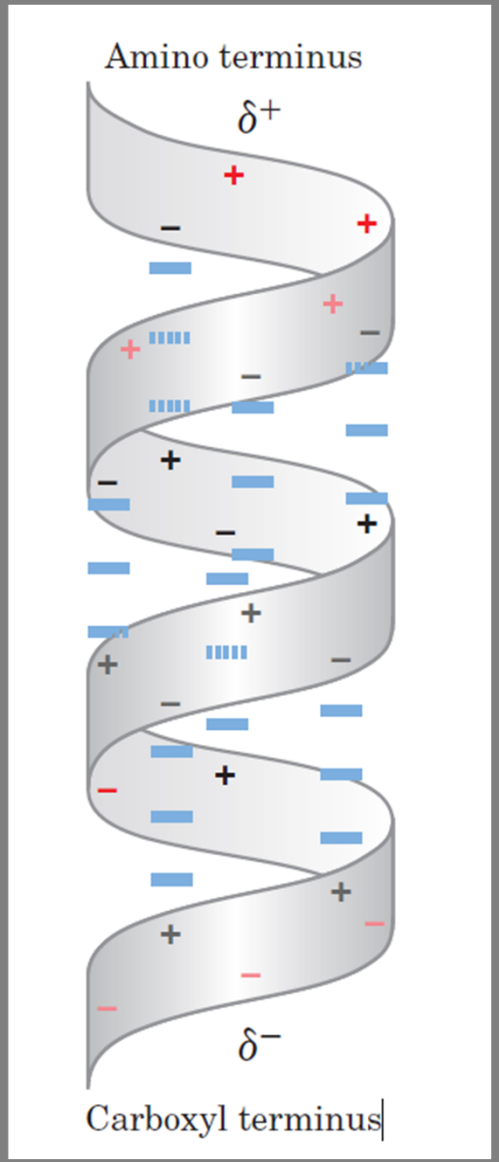


α-HELIX STABILTY:

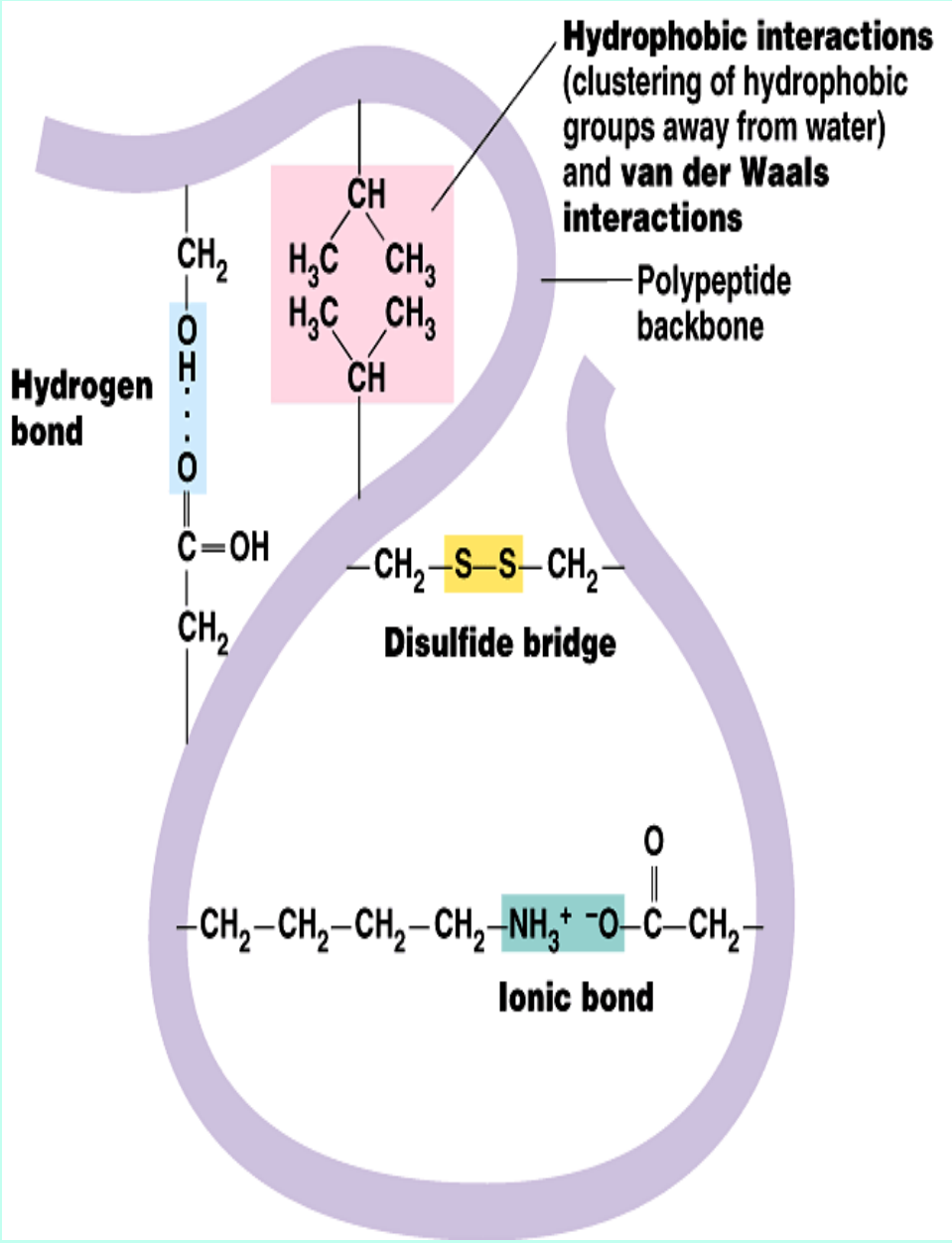
- Charged residues must not be present very close to each other. E.g., the –ve charge on the glutamic acid sidechain will repel each other very strongly and prevent the formation of helix. Also for Lys, Arg, Ser etc.
- +vely charged AAs must be placed three residues away from –vely charged AAs to result in electrostatic attraction to form an ion-pair. Aromatic sidechains also must be similarly spaced to maximise hydrophobic interaction.
- Proline leads to constraint during formation of helix. In proline, N-is part of rigid a heterocyclic ring which prevents bending of the protein backbone – introduces a kink in the helix. Also, proline has no substituent on N-atom for H-bonding. So, proline is rarely found in α-helix.
- Glycine has more conformational flexibility than other residues – the polymer coils rather than forming helix.



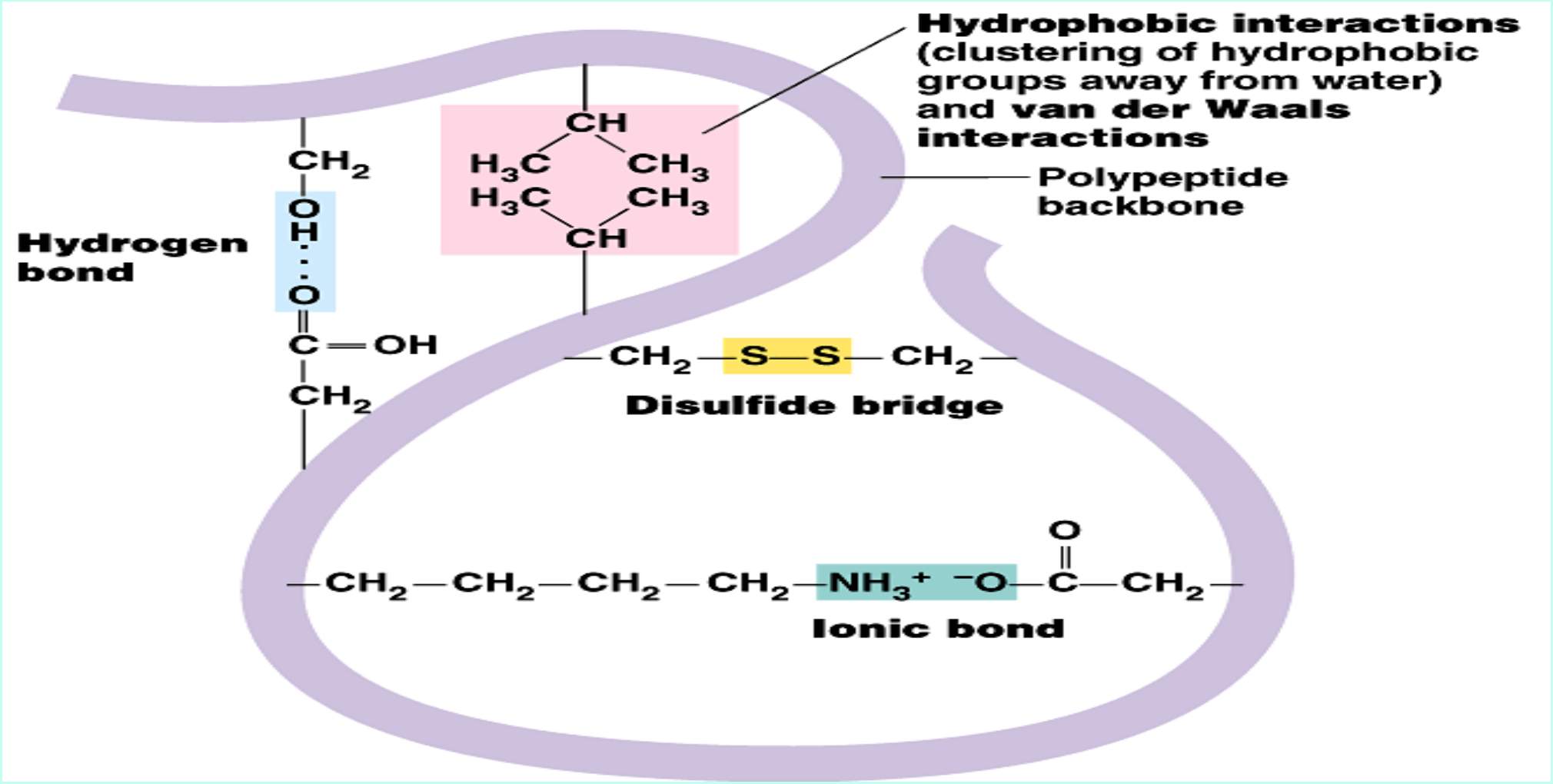
A final factor affecting the stability of an α helix in a polypeptide is the identity of the amino acid residues near the ends of the α-helical segment. A small electric dipole exists in each peptide bond (Fig. 4–2a). These dipoles are connected through the hydrogen bonds of the helix, resulting in a net dipole extending along the helix that increases with helix length (Fig. 4–6). The four amino acid residues at each end of the helix do not participate fully in the helix hydrogen bonds. The partial positive and negative charges of the helix dipole actually reside on the peptide amino and carbonyl groups near the amino-terminal and carboxyl-terminal ends of the helix, respectively. For this reason, negatively charged amino acids are often found near the amino terminus of the helical segment, where they have a stabilizing interaction with the positive charge of the helix dipole; a positively charged amino acid at the amino-terminal end is destabilizing. The opposite is true at the carboxyl-terminal end of the helical segment.



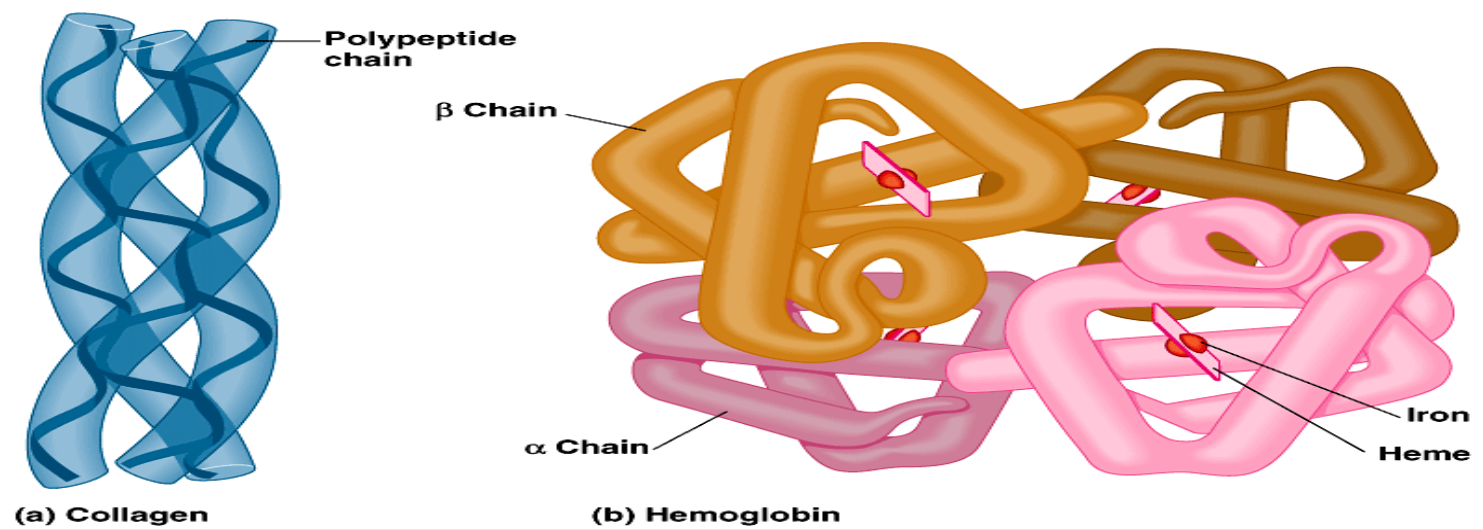
- **Tertiary structure** is determined by a variety of interactions (bond formation) among R groups and between R groups and the polypeptide backbone.
  - a. **The weak interactions** include:
  - **Hydrogen bonds** among polar side chains
  - **Ionic bonds** between charged R groups (basic and acidic amino acids)
  - **Hydrophobic interactions** among hydrophobic (non polar) R groups.



b. **<u>Strong covalent bonds</u>** include **disulfide bridges**, that form between the sulfhydryl groups (SH) of cysteine monomers, stabilize the structure.



- **Quaternary structure:** results from the aggregation (combination) of two or more polypeptide subunits held together by non-covalent interaction like H-bonds, ionic or hydrophobic interactions.
- Examples on protein having quaternary structure:
  - **Collagen** is a fibrous protein of three polypeptides (trimeric) that are supercoiled like a rope.
    - This provides the structural strength for their role in connective tissue.
  - **Hemoglobin** is a globular protein with four polypeptide chains (tetrameric)
  - **Insulin** : two polypeptide chains (dimeric)



NATIVE STRUCTURE OF PROTEINS:

The native state of a protein is its ***PROPERLY FOLDED AND ASSEMBLED FORM WITH OPERATIVE STRUCTURE AND FUNCTION***. The native state of a protein needs all four levels of biomolecular structure, with secondary to quaternary structure formed by weak interactions along the covalently-bonded backbone.

PROTEIN FOLDING:

Protein folding is the ***PHYSICAL PROCESS BY WHICH A PROTEIN CHAIN IS TRANSLATED TO ITS NATIVE THREE-DIMENSIONAL STRUCTURE***, typically a "folded" conformation by which the protein becomes biologically functional.

Protein folding is a very sensitive process that is influenced by several external factors including **electric and magnetic fields, temperature, pH, chemicals, space limitation and molecular crowding**. These factors influence the ability of proteins to fold into their correct functional forms. Any kind of misfolding or unfolding may be the cause of various diseases.

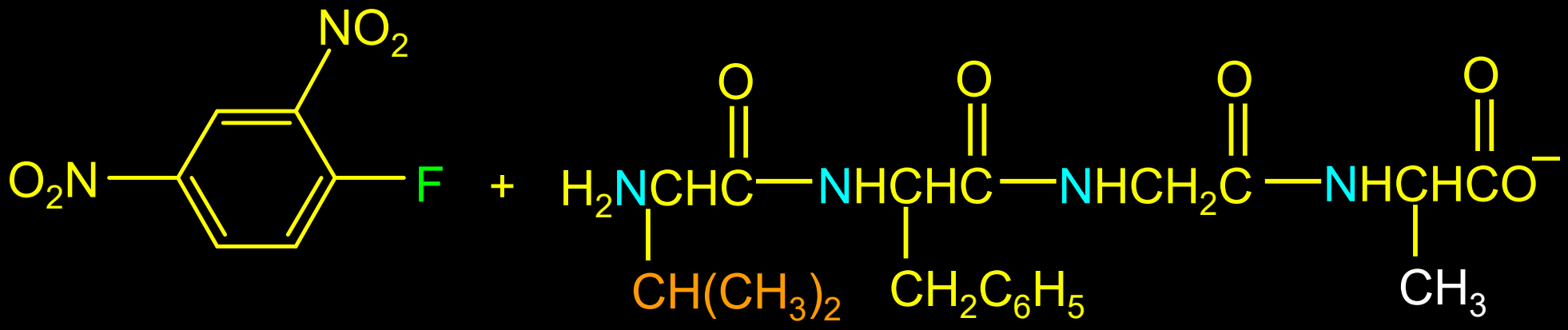
DENATURATION OF PROTEINS:

Protein denaturation takes place when the forces that maintain the secondary, tertiary, and quaternary structures of proteins are disrupted by physical or chemical agents.

Determination of Primary Structure of proteins

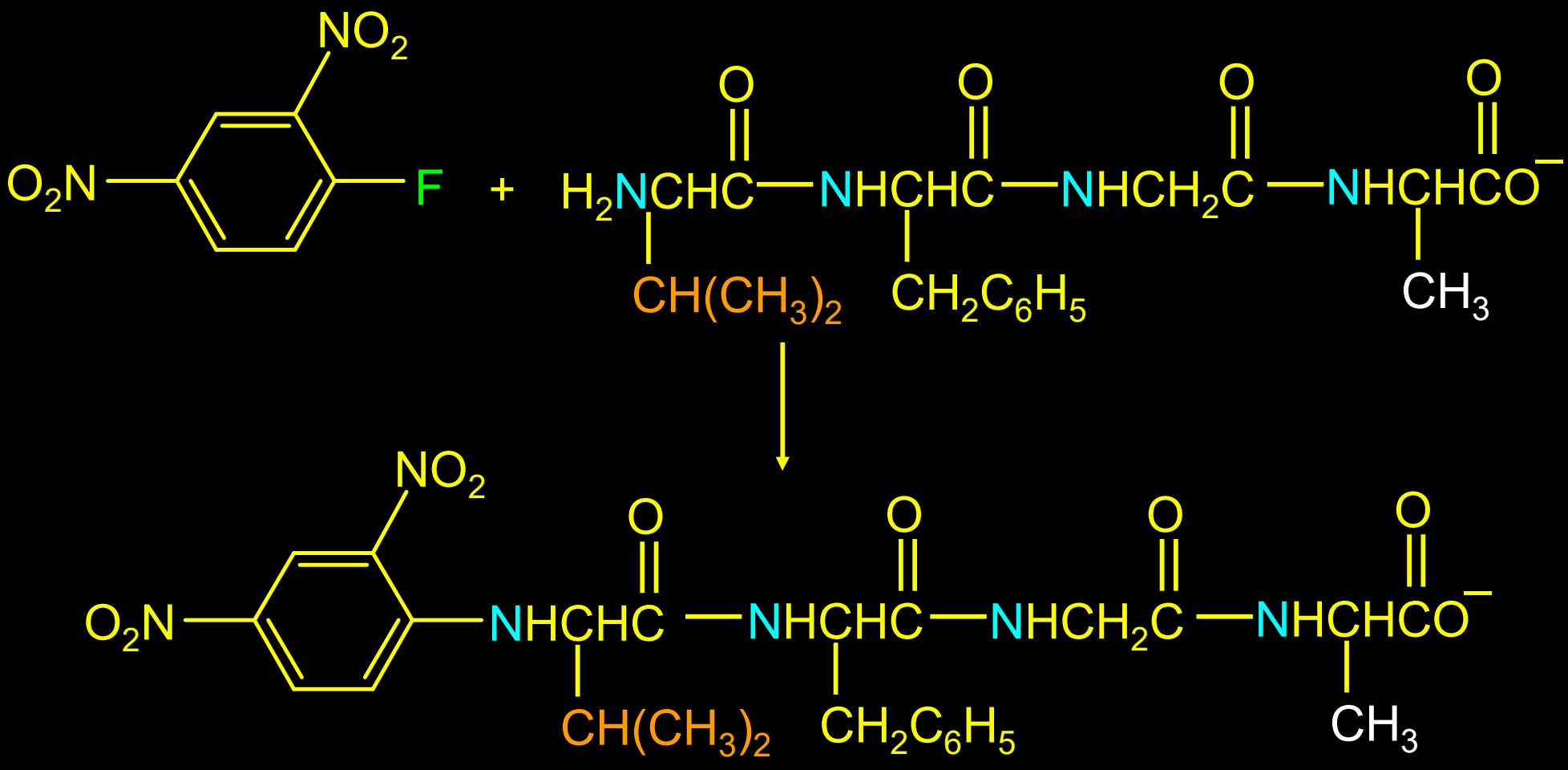
Sanger's Method

1-Fluoro-2,4-dinitrobenzene reacts with the amino nitrogen of the N-terminal amino acid.



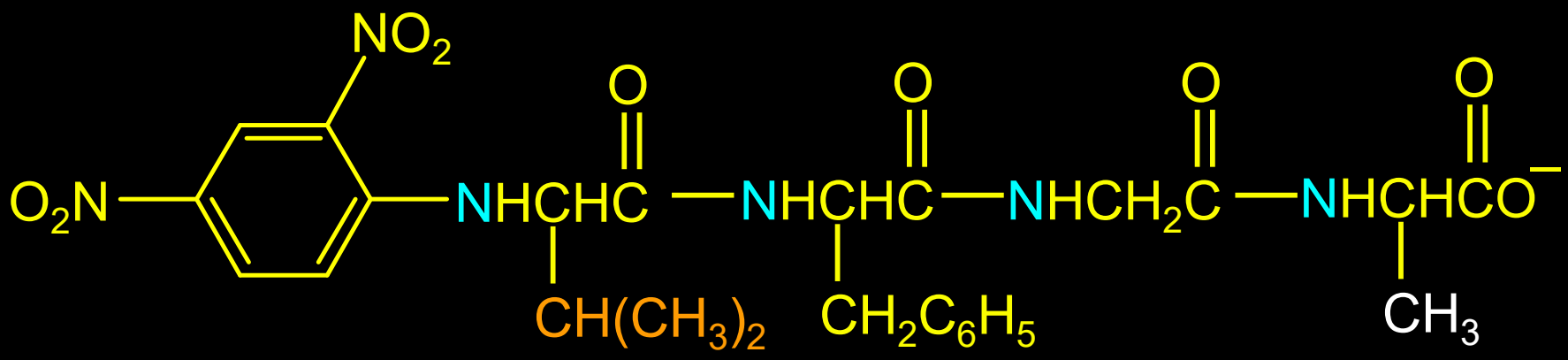
Sanger's Method

1-Fluoro-2,4-dinitrobenzene reacts with the amino nitrogen of the N-terminal amino acid.



Sanger's Method

Acid hydrolysis cleaves all of the peptide bonds leaving a mixture of amino acids, only one of which (the N-terminus) bears a 2,4-DNP group.



Sanger's Method

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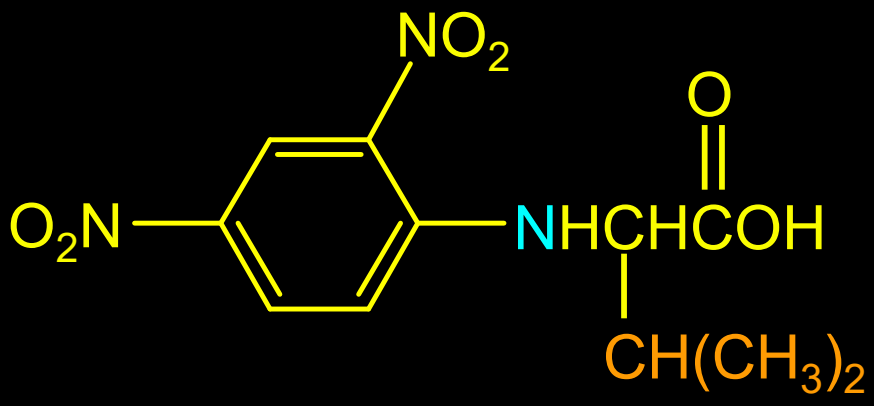
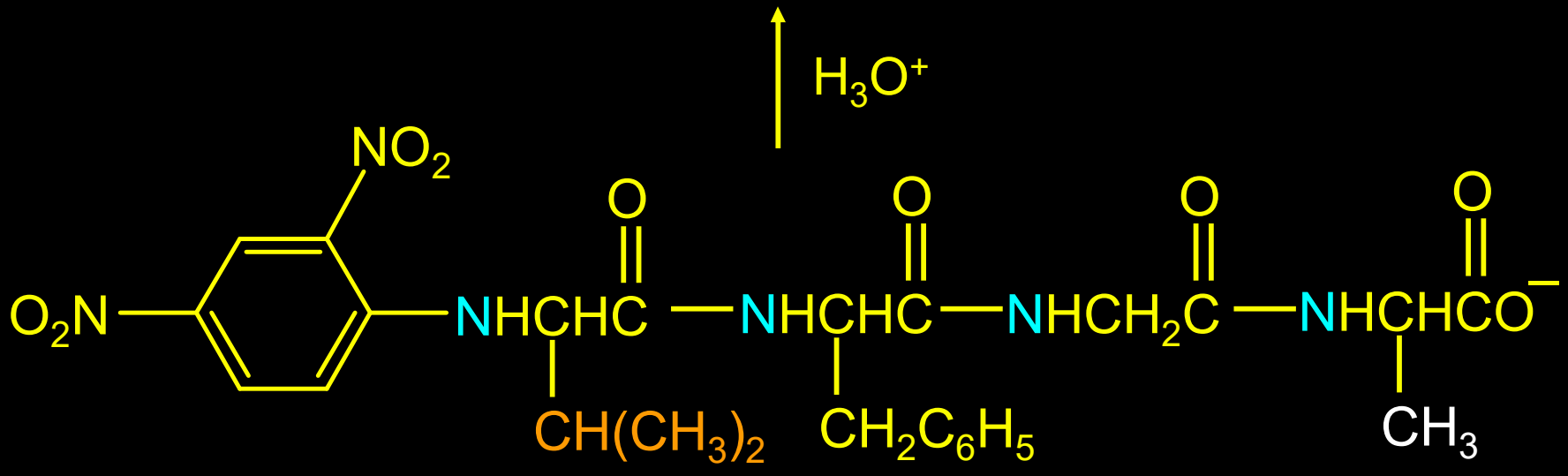
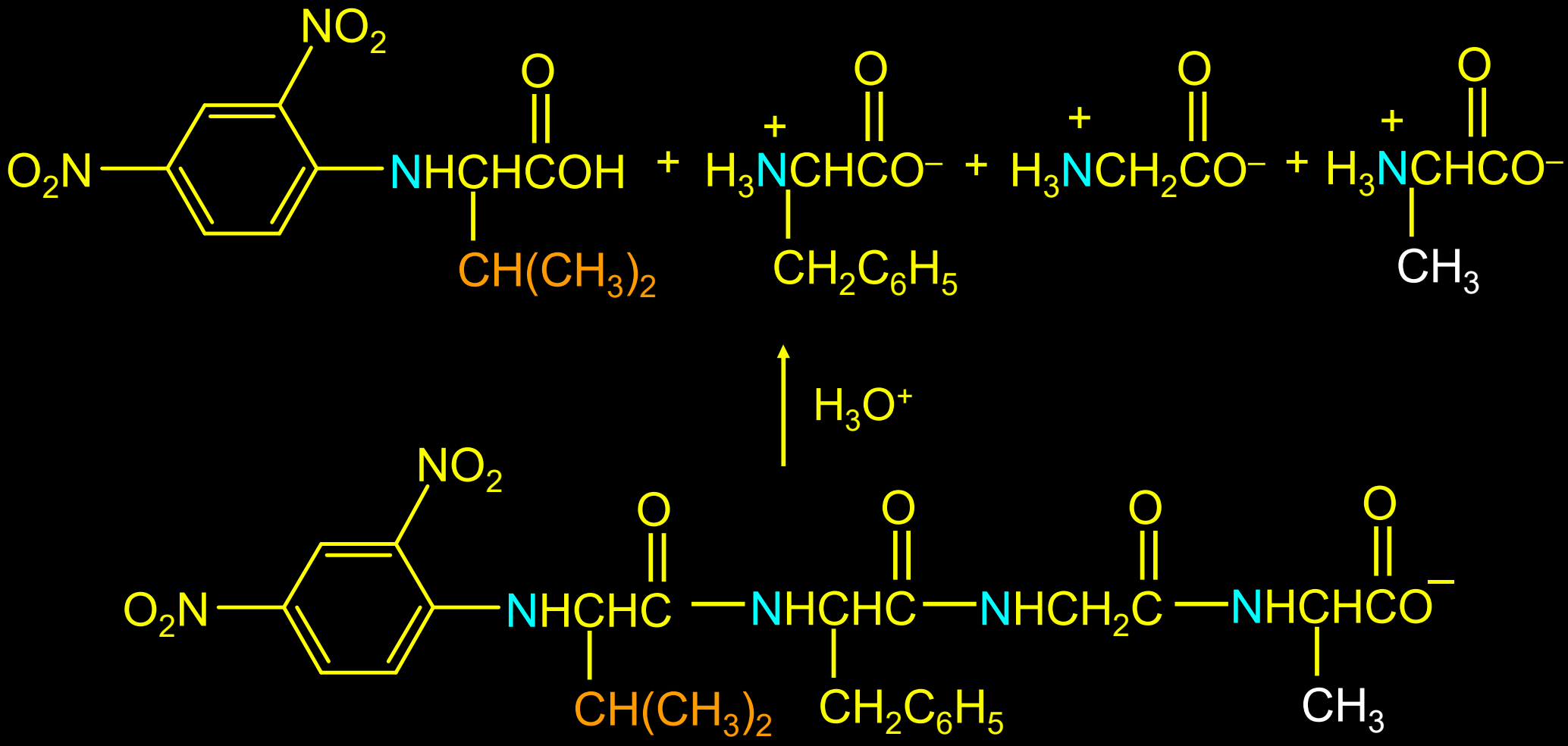
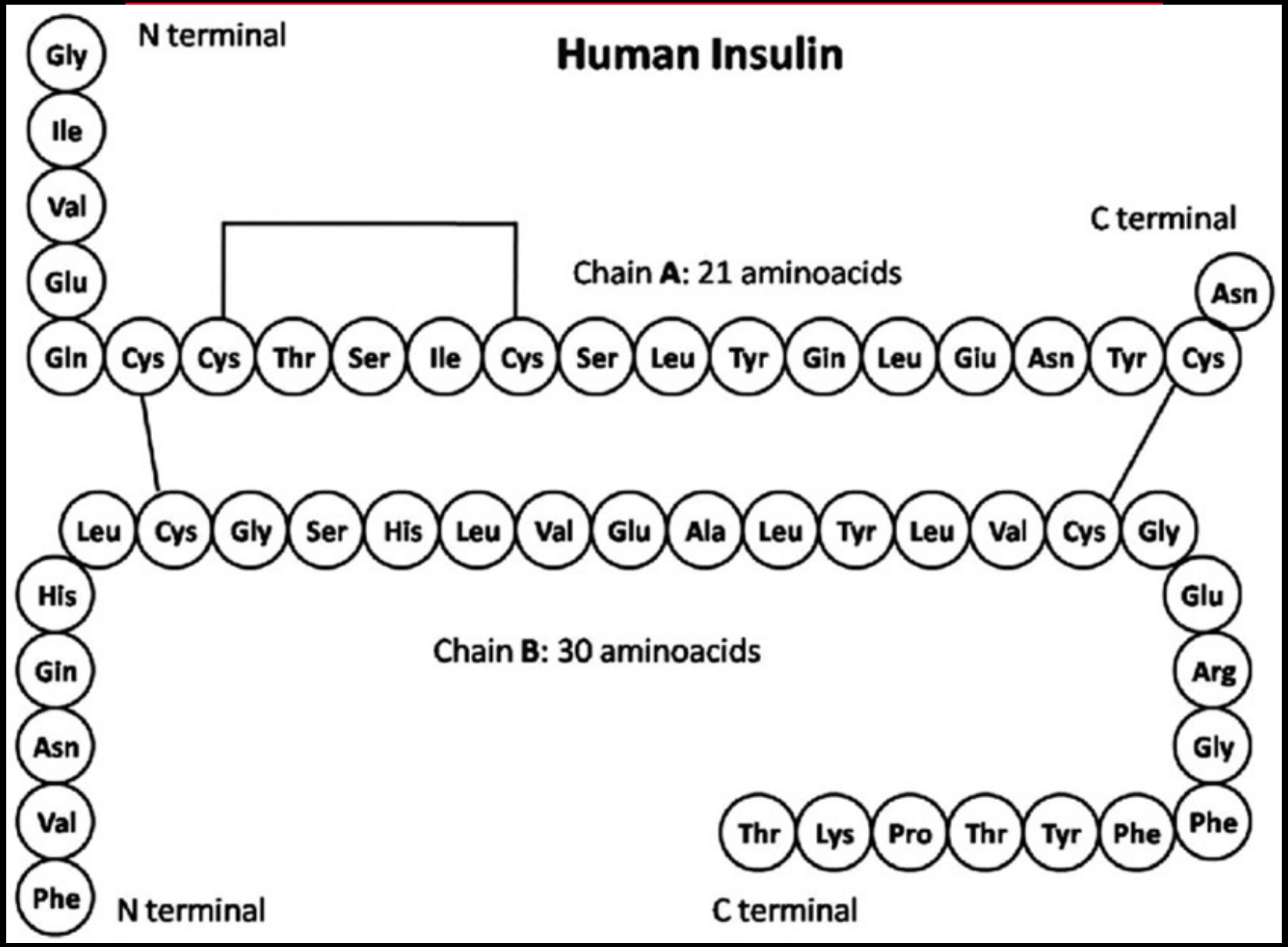




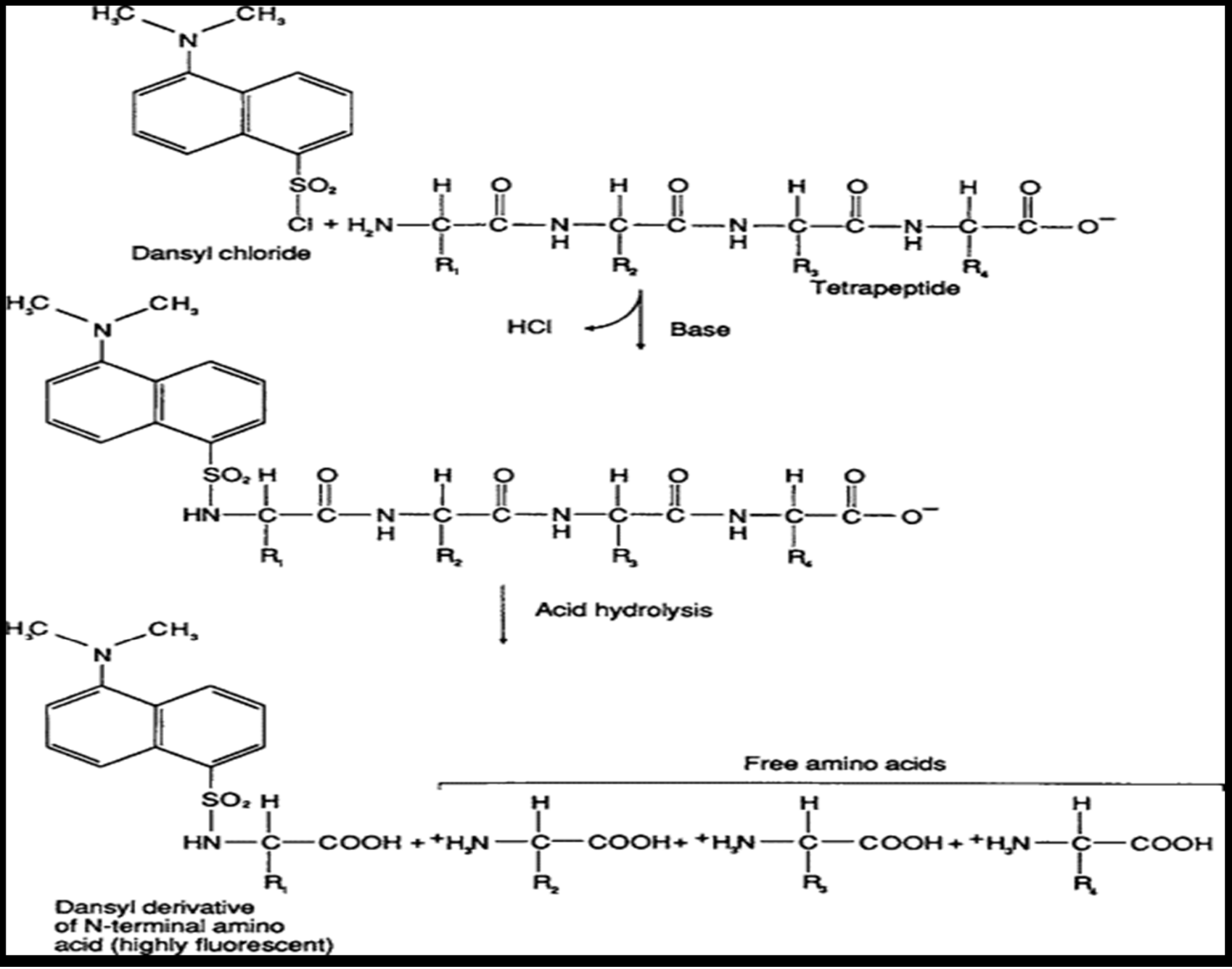
Sanger's Method

Acid hydrolysis cleaves all of the peptide bonds leaving a mixture of amino acids, only one of which (the N-terminus) bears a 2,4-DNP group.




Human Insulin
N terminal
Gly
Ile
Val
Glu
Gln
Cys
Cys
Thr
Ser
Ile
Cys
Ser
Leu
Tyr
Gln
Leu
Glu
Asn
Tyr
Cys
Asn
C terminal
Chain A: 21 aminoacids
Leu
Cys
Gly
Ser
His
Leu
Val
Glu
Ala
Leu
Tyr
Leu
Val
Cys
Gly
His
Gln
Asn
Val
Phe
N terminal
Chain B: 30 aminoacids
Glu
Arg
Gly
Thr
Lys
Pro
Thr
Tyr
Phe
Phe
C terminal

Dansyl chloride method



Partial Hydrolysis of Peptides and Proteins

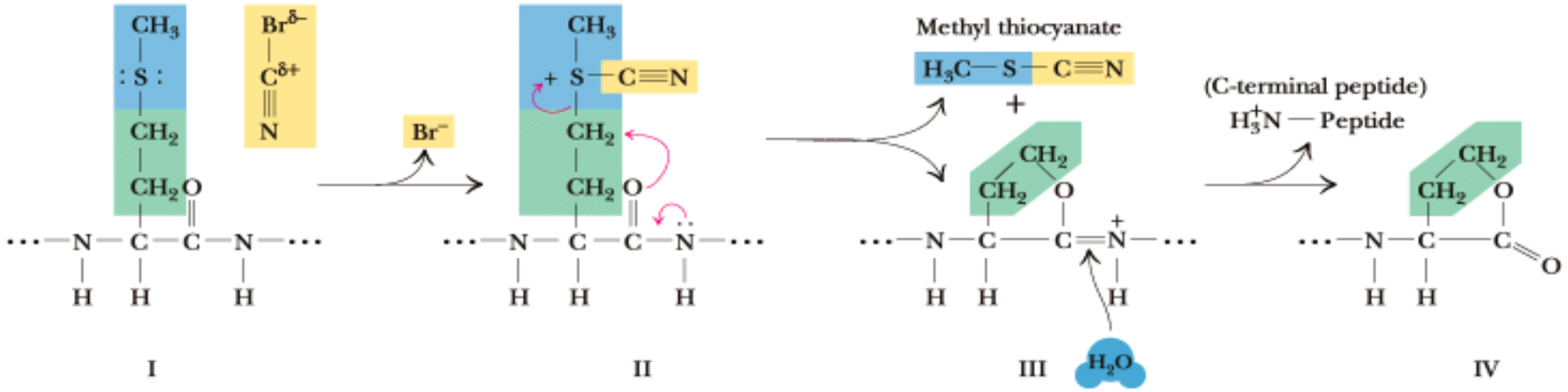
Acid-hydrolysis of the peptide cleaves all of the peptide bonds.

Cleaving some, but not all, of the peptide bonds gives smaller fragments.

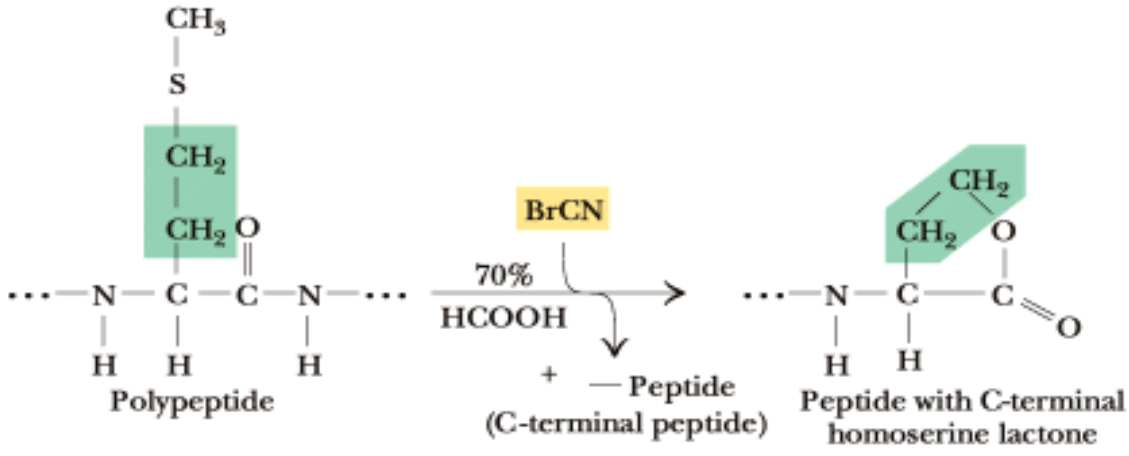
These smaller fragments are then separated and the amino acids present in each fragment determined.

Enzyme-catalyzed cleavage is the preferred method for partial hydrolysis.

Cyanogen bromide, CNBr – specific for cleavage of peptide bond of methionine on carboxyl side



OVERALL REACTION:



PEPTIDASE

Enzymes that hydrolyse the peptide bond in polypeptides.

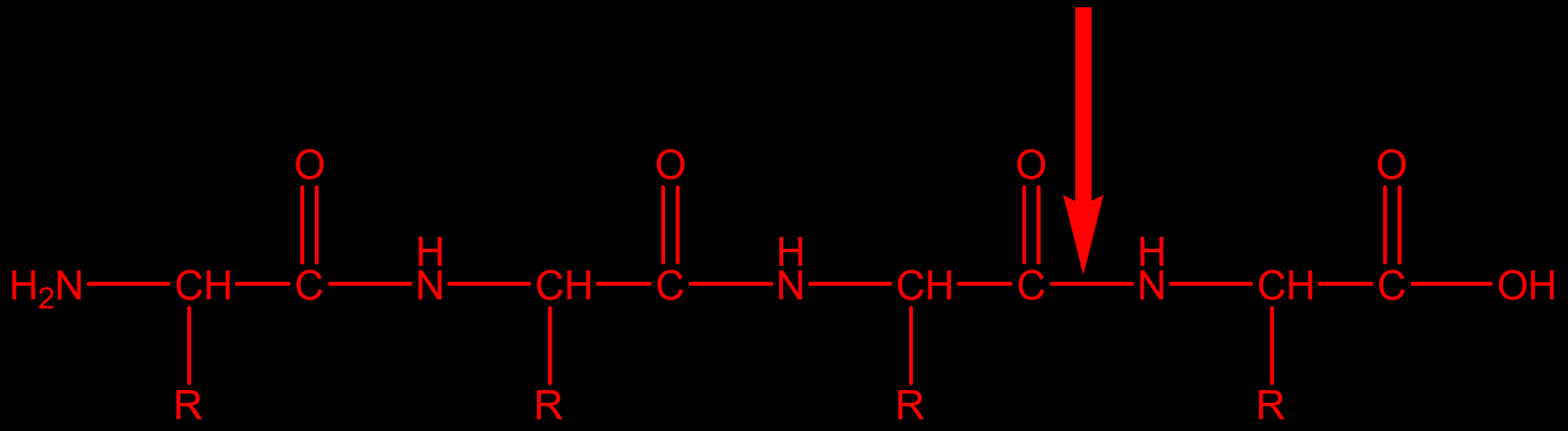
Two types of peptidase enzymes:

- **Endopeptidase** – breaks internal peptide bonds. Eg. Trypsin, chymotrypsin.
- **Exopeptidase** – breaks terminal or penultimate peptide bonds. Eg. Aminopeptidase, carboxypeptidase.

Carboxypeptidase

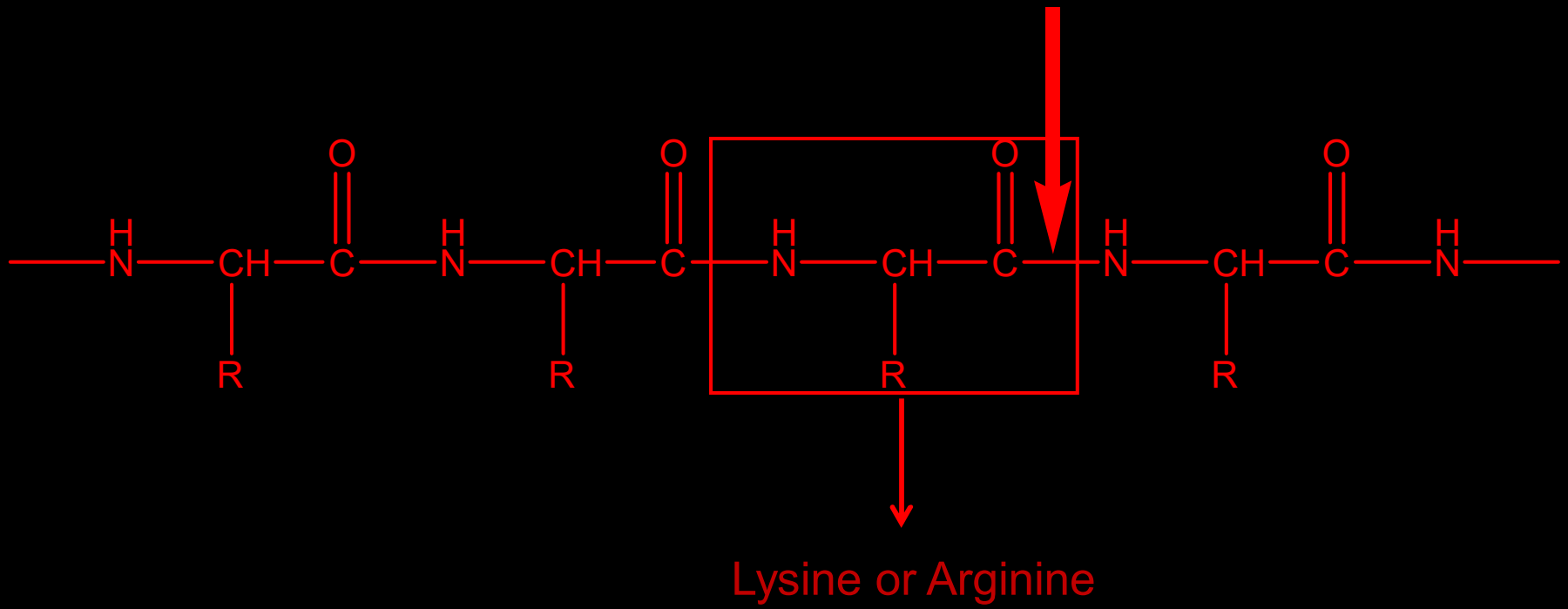
Carboxypeptidase is a *proteolytic enzyme* (catalyzes the hydrolysis of proteins).

Carboxypeptidase is selective for cleaving the peptide bond to the C-terminal amino acid.



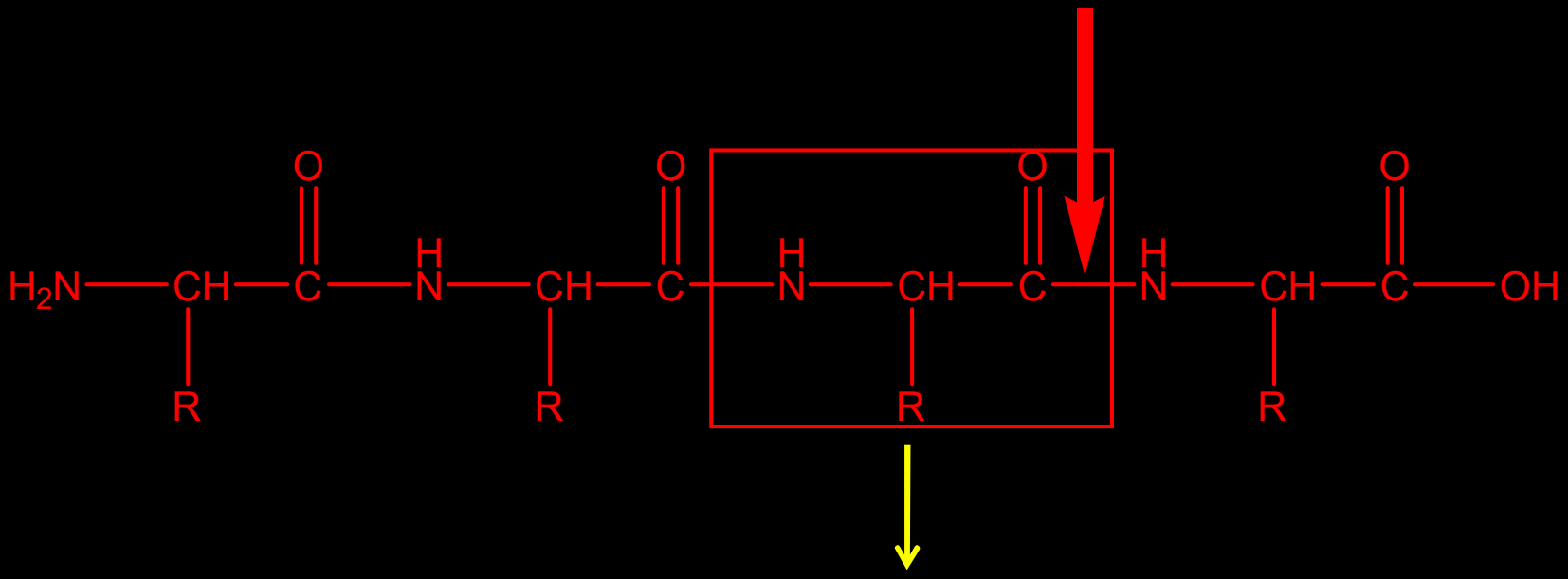
Trypsin

Trypsin is selective for cleaving the peptide bond to the carboxyl group of lysine or arginine.



Chymotrypsin

Chymotrypsin is selective for cleaving the peptide bond to the carboxyl group of amino acids with an aromatic side chain.



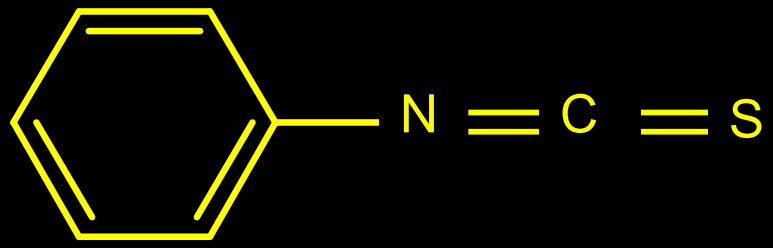
phenylalanine, tyrosine, tryptophan

Edman Degradation

1. Method for determining N-terminal amino acid.
2. Can be done sequentially one residue at a time on the same sample. Usually one can determine the first 20 or so amino acids from the N-terminus by this method.
3. 10^{-10} g of sample is sufficient.
4. Has been automated.

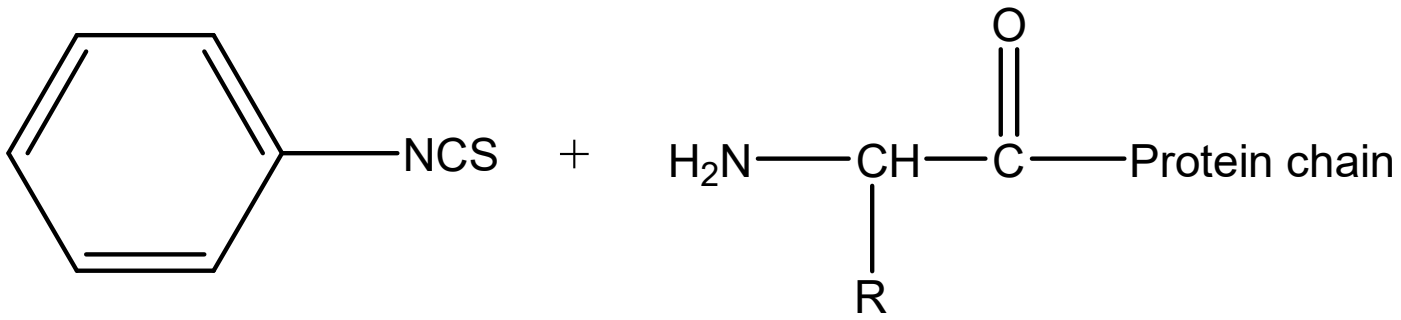
Edman Degradation

The key reagent in the Edman degradation is phenyl isothiocyanate.

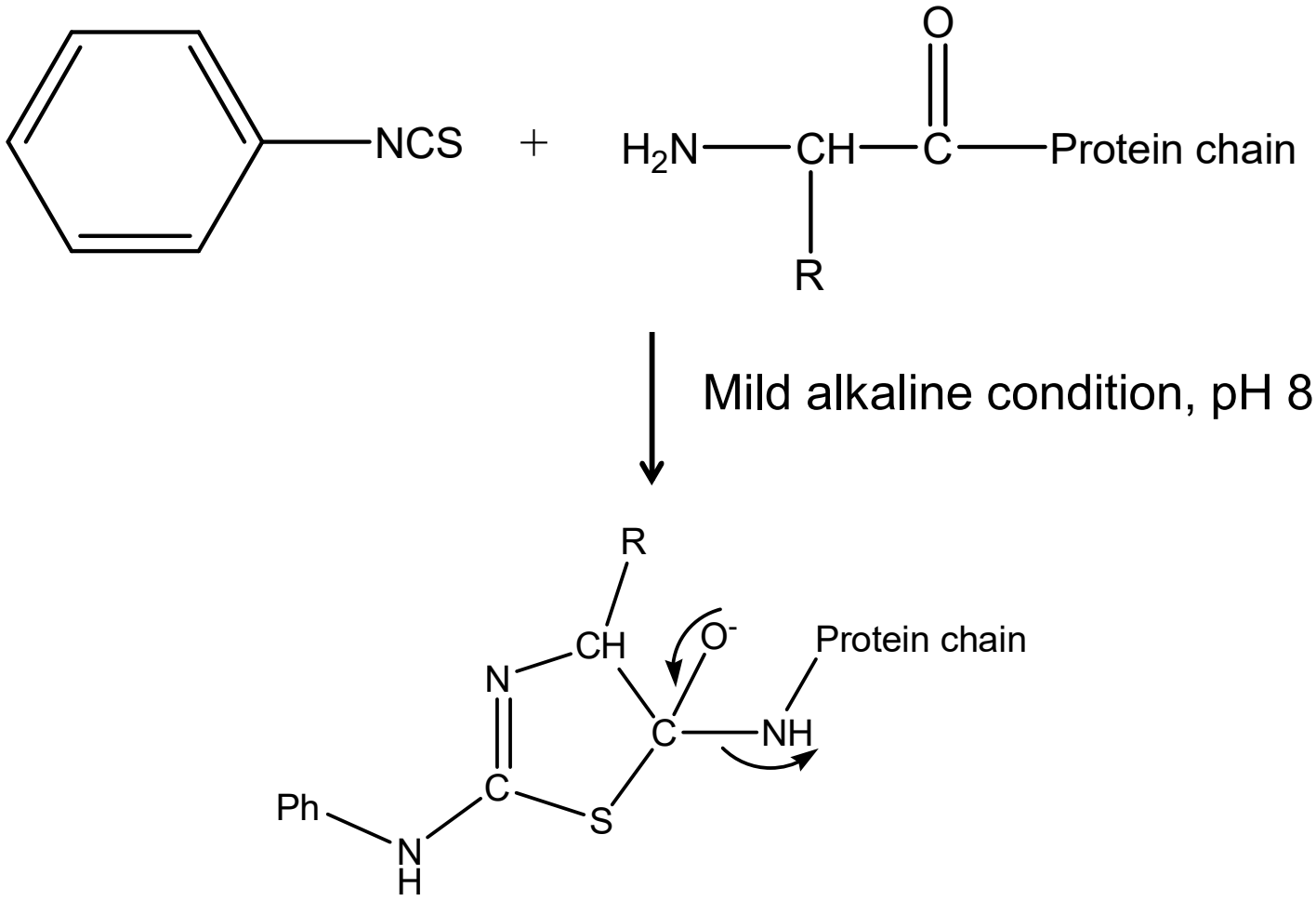


Edman Degradation

Phenyl isothiocyanate reacts with the amino nitrogen of the N-terminal amino acid.

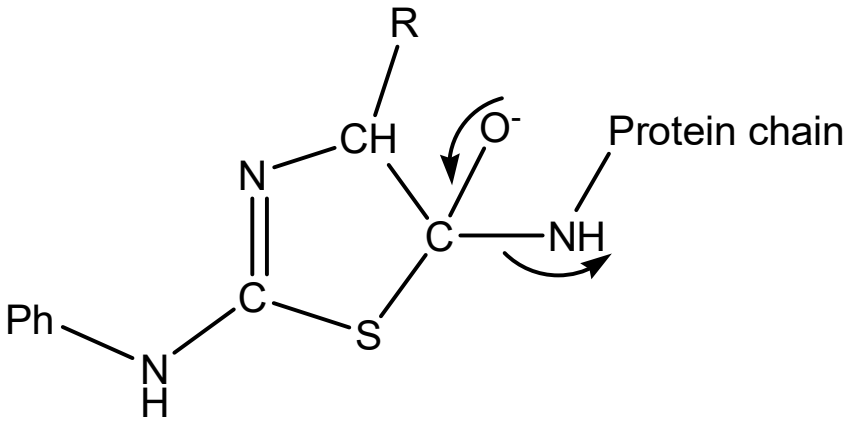


Edman Degradation

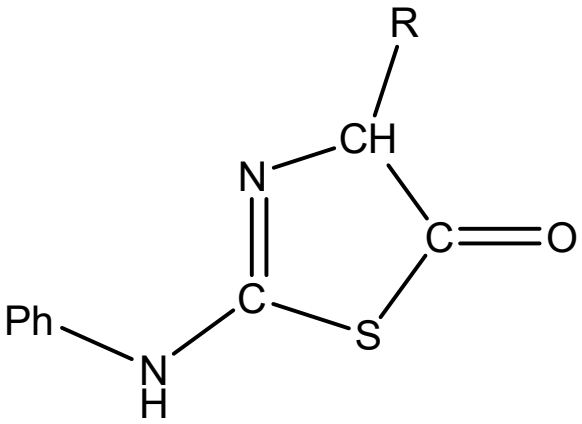


Phenylthiocarbamoyl derivative

Edman Degradation



H^+

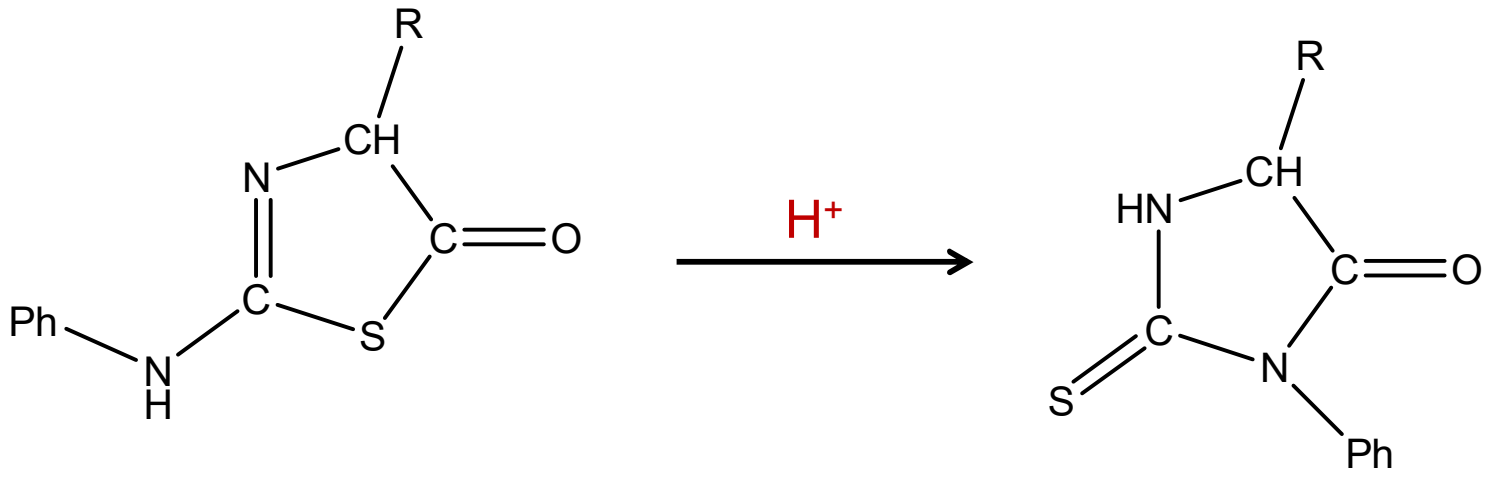


\+ Remaining Protein chain

Thiazolone derivative

Edman Degradation

The product is a thiazolone. Under the conditions of its formation, the thiazolone rearranges to a phenylthiohydantoin (PTH) derivative. This derivative maybe identified by chromatography or electrophoresis.

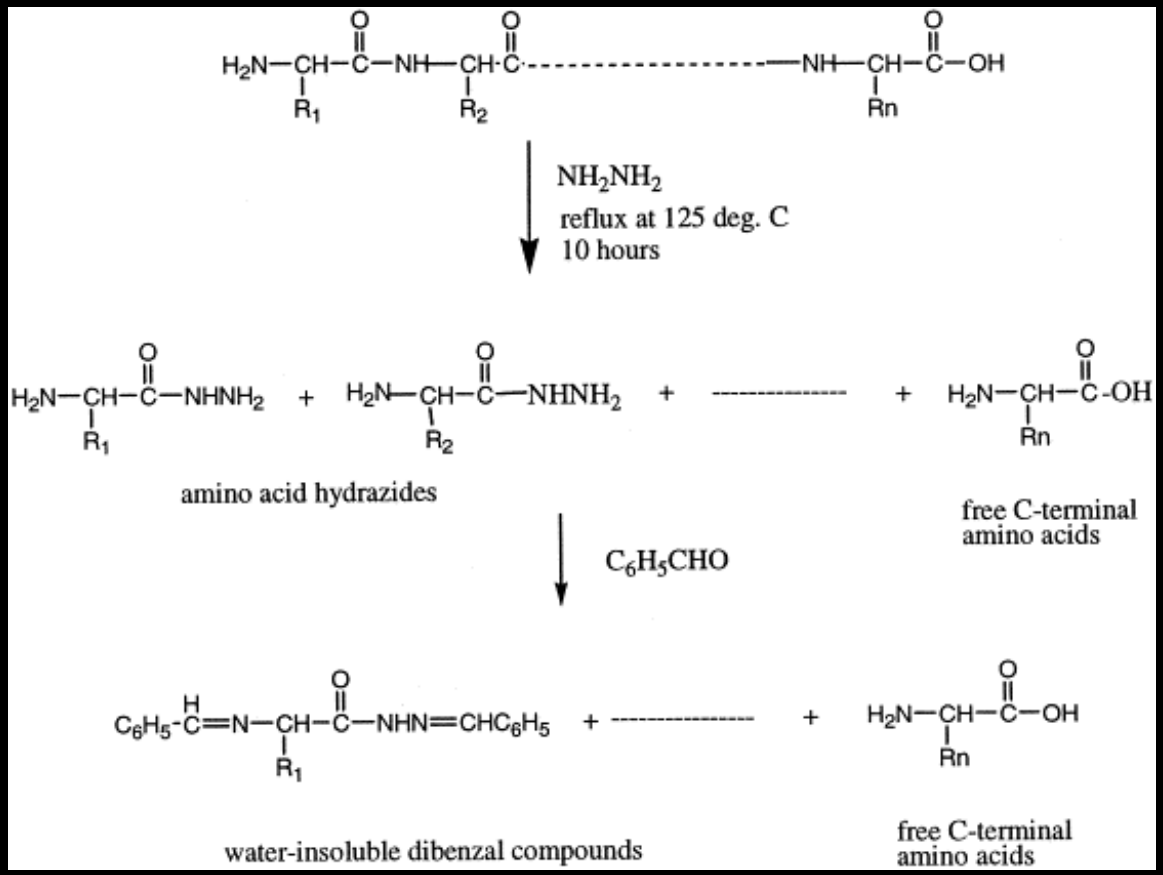


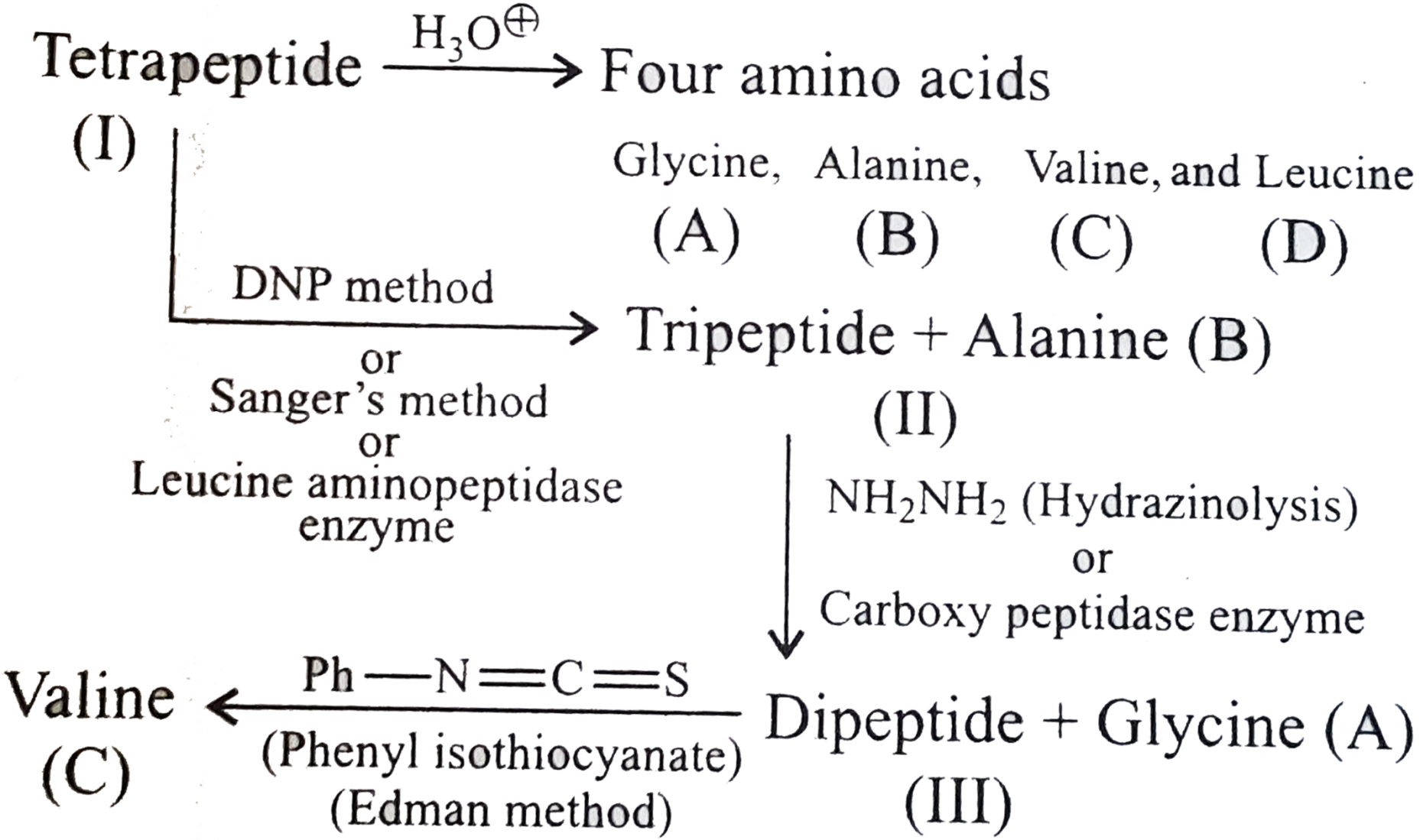
Identification of C-terminus amino acid

Reduction with $LiAlH_4$

Reaction with Carboxypeptidase

Hydrazinolysis




Tetrapeptide
$H_3O^{\oplus}$
Four amino acids
(I)
Glycine, Alanine, Valine, and Leucine
(A) (B) (C) (D)
DNP method
or
Sanger's method
or
Leucine aminopeptidase enzyme
Tripeptide + Alanine (B)
(II)
NH_2NH_2 (Hydrazinolysis)
or
Carboxy peptidase enzyme
Valine
(C)
Ph—N=C=S
(Phenyl isothiocyanate)
(Edman method)
Dipeptide + Glycine (A)
(III)