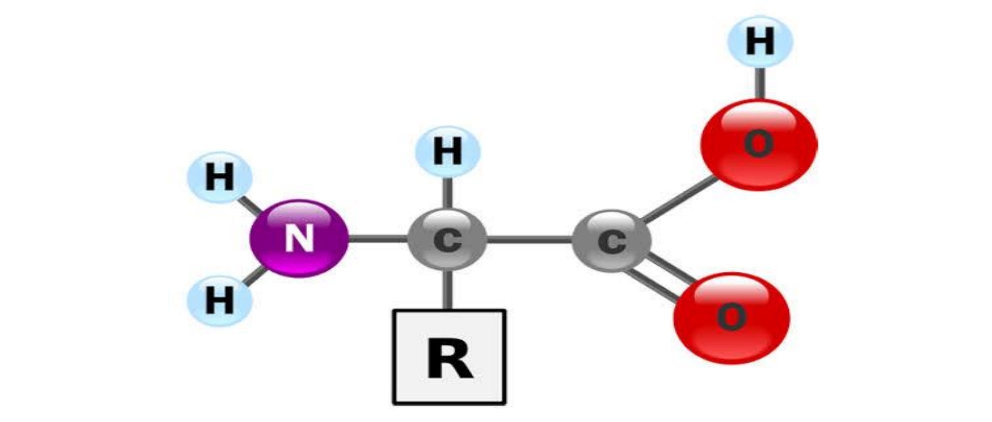
COURSE CODE: BCH 201

COURSE TITLE: GENERAL BIOCHEMISTRY I

NO OF UNITS : 3



COURSE CONTENT

Water: physical and chemical properties in relation to its biological role, water balance. acidity and alkalinity, pH and PKa values and their effects on cellular activities. Buffers. Biochemical applications of pH, pKa and Buffers. Chemistry/structures of carbohydrates. Chemistry of amino acids, proteins and their derivatives; methods of isolation and identification. Primary, secondary, tertiary and quaternary structures of proteins; determination and biochemical applications of these structures

INTRODUCTION

Proteins are among the most important biomolecules

Living cells produce a vast array of proteins

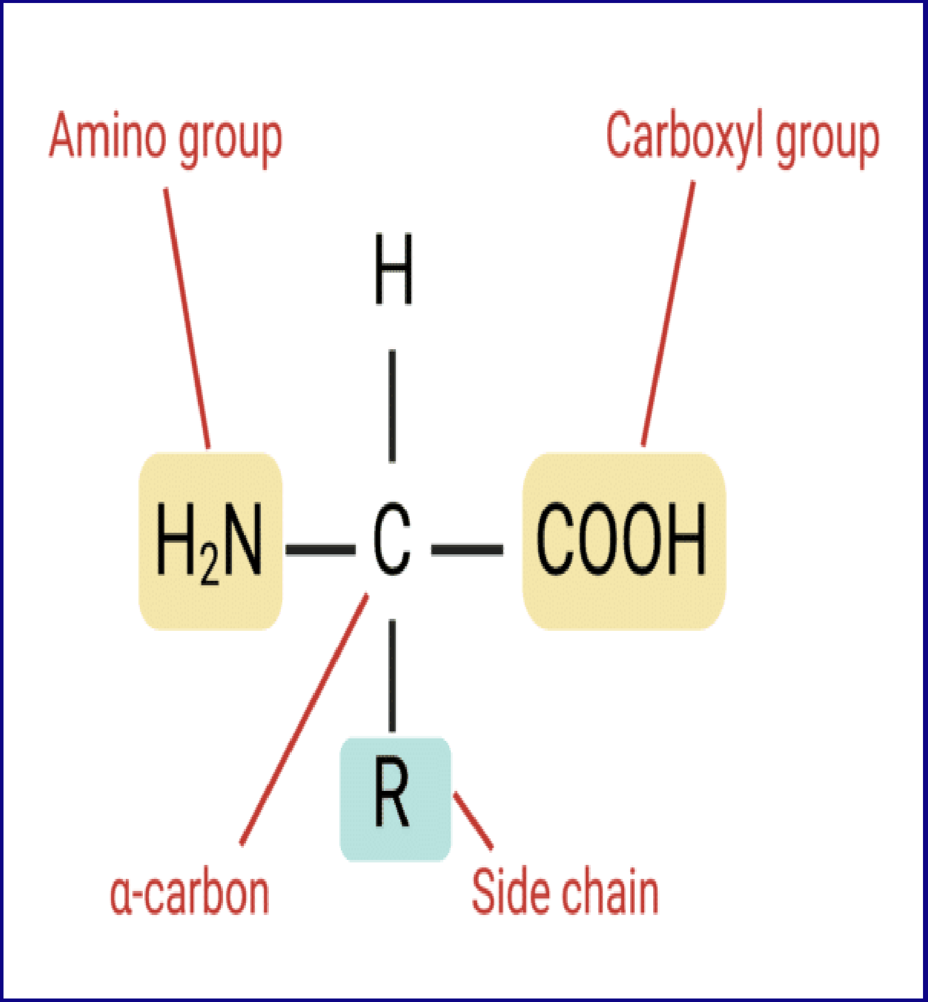
Proteins perform a variety of biological functions

Amino acids are the building blocks of proteins

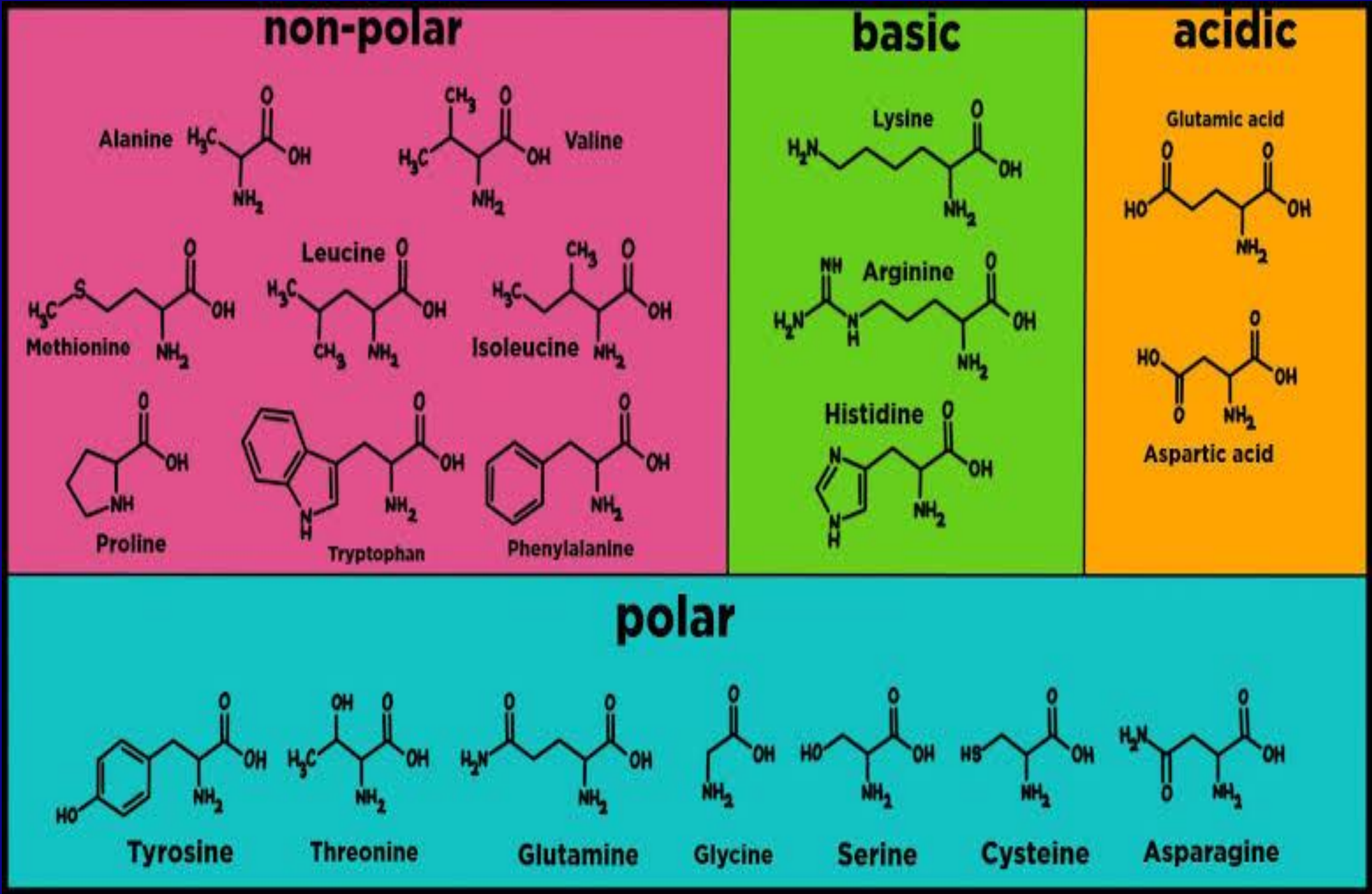
Chemistry of Amino Acids

Amino acids are a class of organic compounds containing

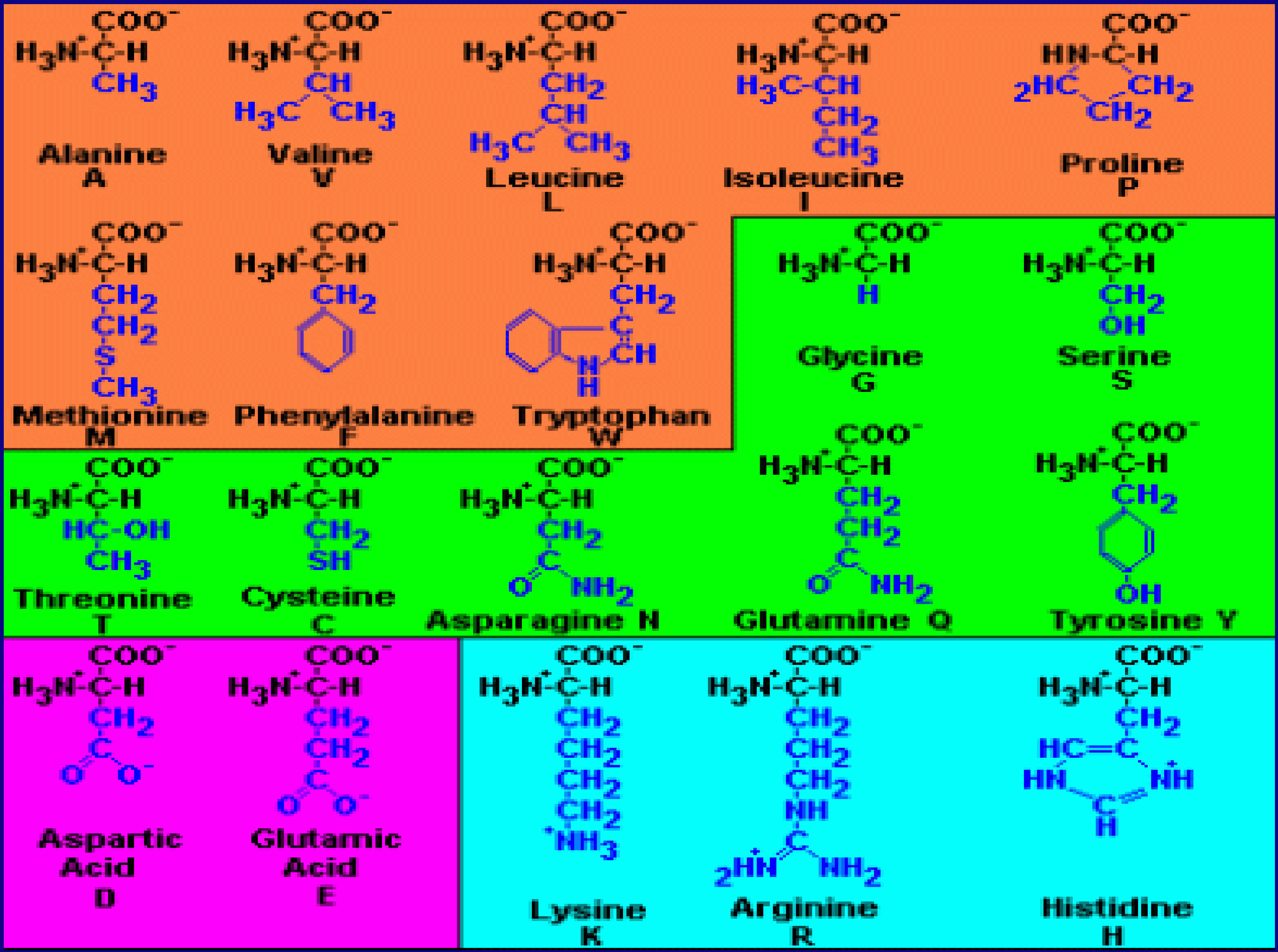
- a **basic amino group ($-NH_2$),**
- **an acidic carboxyl group (-COOH)** and
- **an organic alkyl group** as side chain as basic structural components



- Amino acids are building monomers of proteins
- Amino acids may be **proteinogenic** (protein creating) or **non-proteinogenic** (non-protein creating)
- Some amino acids can be synthesized in the body (**non-essential**) while some cannot be synthesized (**Nutritionally *essential***).
- Over 300 amino acids occurring in nature, each differ but only 22 participate in protein formation



STRUCTURE OF THE FIRST 20TH AMINO ACIDS


Alanine A
Valine V
Leucine L
Isoleucine I
Proline P
Methionine M
Phenylalanine F
Tryptophan W
Glycine G
Serine S
Threonine T
Cysteine C
Asparagine N
Glutamine Q
Tyrosine Y
Aspartic Acid D
Glutamic Acid E
Lysine K
Arginine R
Histidine H

Structure and function of a protein depend upon the:

- Nature of amino acids present in it
- Sequence in which the amino acids are present
- Spatial relationship of amino acids with one another

Amino acids

Carboxylic acids having one or more amino groups

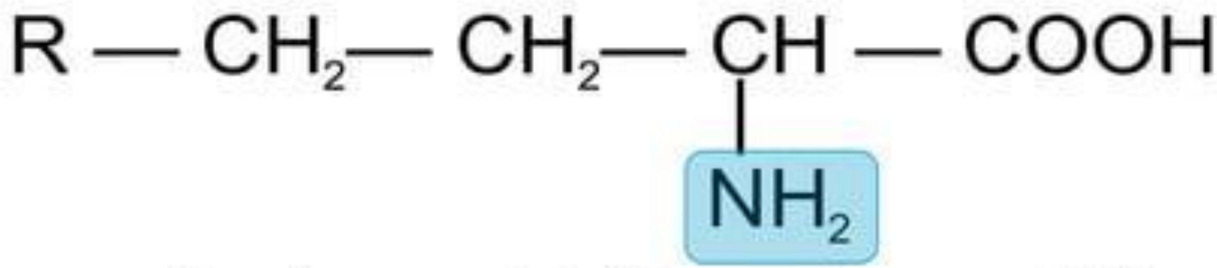
Carbon atoms are numbered 1, 2, 3 etc starting from the carboxyl group

Also named α, β, γ etc starting from carbon atom next to the carboxyl group

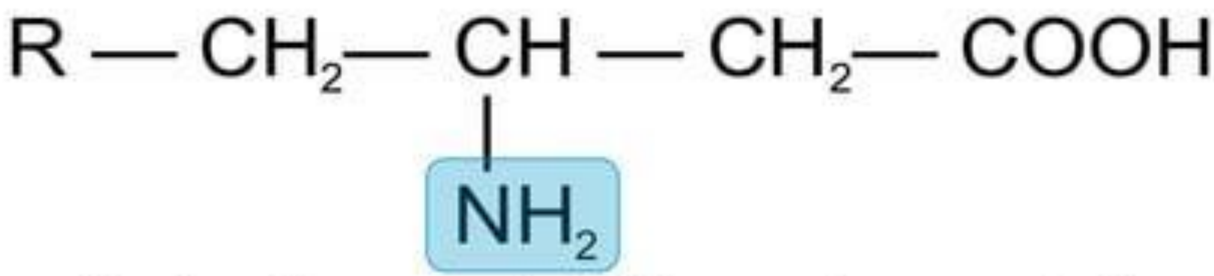
$$
\overset{4}{\underset{\gamma}{R - CH_2}} - \overset{3}{\underset{\beta}{CH_2}} - \overset{2}{\underset{\alpha}{CH_2}} - \overset{1}{COOH}
$$

The amino group may be attached to any of the carbon atoms next to the carboxyl group

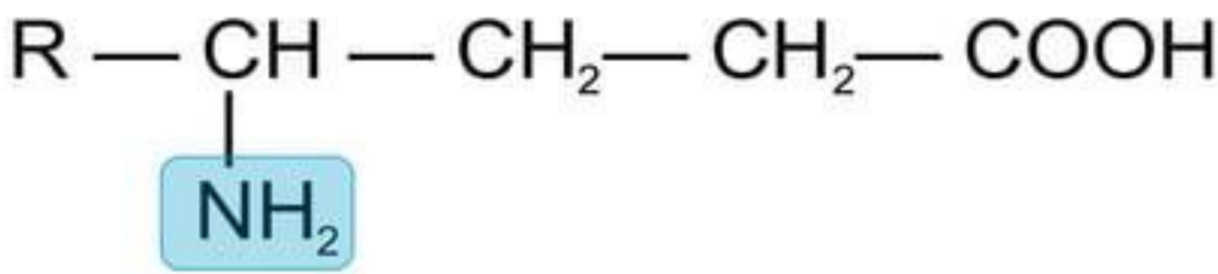
The amino acid is accordingly known as α-amino acid (2-amino acid), β-amino acid (3-amino acid), γ-amino acid (4-amino acid) etc



α-Amino acid (2-amino acid)



β-Amino acid (3-amino acid)



γ-Amino acid (4-amino acid)

Proteins are made up of only α-amino acids

The α-amino acids contain at least one asymmetric carbon atom

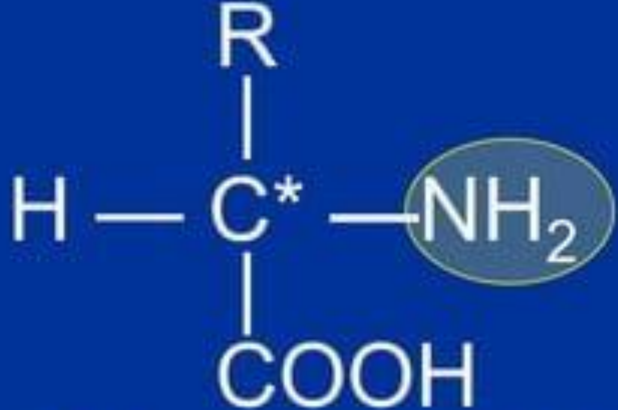
Therefore, they exhibit stereo-isomerism and optical isomerism

The amino acid can exist as a D-isomer and an L-isomer

D- and L- Isomers

Amino group is present on right hand side of α-carbon* in D-amino acids, and on left hand side in L-amino acids

All the amino acids found in proteins are L- Isomer except glycine



D-Amino acid

L-Amino acid

Glycine is the only amino acid that has no asymmetric carbon atom and, hence, no stereoisomers

Threonine, isoleucine, hydroxylysine and hydroxyproline have two asymmetric carbon atoms, and hence four stereoisomers each

Chirality in amino acids

All Amino acids except glycine exhibit chirality as a compound because they have at least one chiral carbon atom (a carbon with 4 different attachment)

Proteins are made up of only L-isomers of α-amino acids

Only 20 amino acids are used to synthesize proteins

These are known as standard amino acids

Nomenclature and symbol of amino acids

- The international Union of Biochemistry and Molecular Biology has officially designated **three letters abbreviation** and **one letter symbol** for easier identification and representation of all the proteinogenic amino acids

- For example, amino acid Alanine was designated “**Ala**” as it three letters code representation and letter “**A**” as one letter code

Nomenclature and symbol of amino acids

Amino Acid	Three Letter Code	One Letter Code
Alanine	Ala	A
Arginine	Arg	R
Aspartic Acid	Asp	D
Asparagine	Asn	N
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Classification of Amino acids

Generally, amino acids are classified on the basis of the following criteria:

- Chemical nature of the amino acid in the solution
- Chemical structure of the side chain of the amino acids
- Nutritional requirement of amino acids
- Metabolic product of amino acids
- Polarity of the side chain of the amino acids
- Functional group of the side chain

Classification based on the chemical nature of the amino acid in the solution

On the basis of chemical nature of amino acids in solution, all amino acids are classified into three groups namely:

- Acidic amino acids e.g. glutamic and aspartic acid
- Basic amino acids e.g. lysine, arginine and histidine
- Neutral amino acids. glycine and alanine

Classification according to polarity

The side chain of the amino acid may be polar or non-polar

Accordingly, the amino acids may be polar or non-polar amino acids

Polar and non-polar amino acids

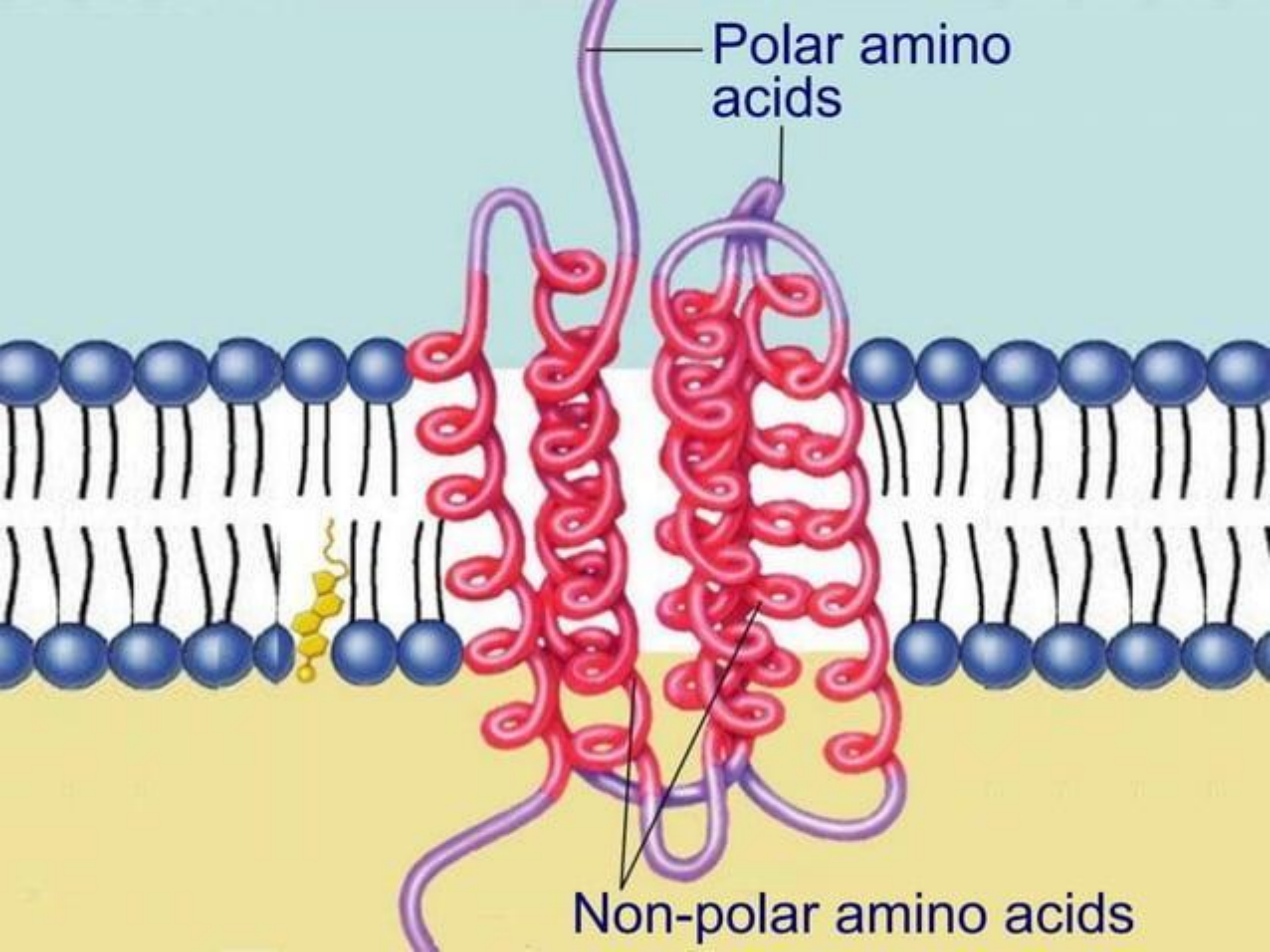
Polar amino acids	Non-polar amino acids
• Have ionizable groups in side chain • Include glycine, serine, threonine, cysteine, aspartate, glutamate, asparagine, glutamine, tyrosine, lysine, arginine and histidine	• Have no ionizable groups in side chain • Include alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan and proline

However, non-polar amino acids are not absolutely non-polar

They are less polar and less soluble in polar solvents than the polar amino acids

In trans-membrane proteins:

- Non-polar amino acids are generally embedded in the lipid bilayer
- The polar amino acids are present outside or inside the membrane


Polar amino acids
Non-polar amino acids

Classification according to nature of side chain

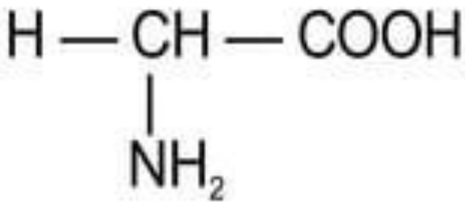
The side chains of amino acids may differ in chemical nature

The amino acids may be divided into several groups on this basis

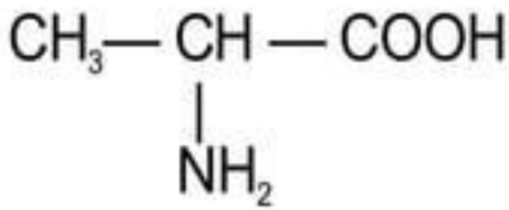
According to chemical nature of side chains, amino acids can be divided into those having:

- Aliphatic side chains
- Side chains having hydroxyl group
- Side chains containing sulphur
- Side chains having acidic groups or their amides
- Side chains having basic groups
- Side chains containing aromatic rings
- Imino group

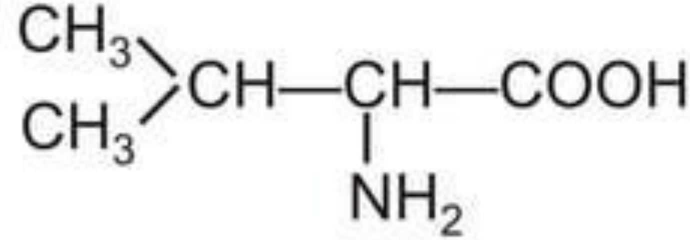
1. Amino acids with aliphatic side chains



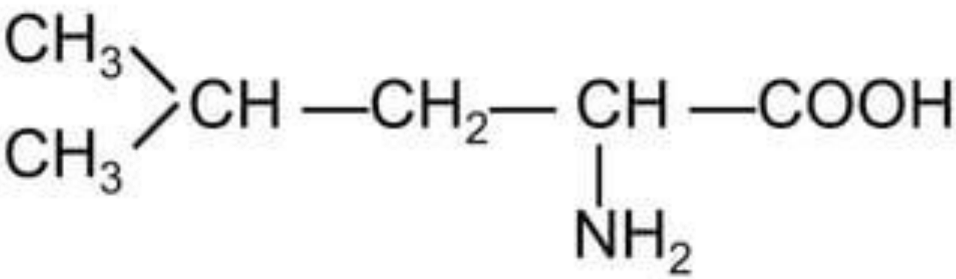
Glycine (Gly or G)



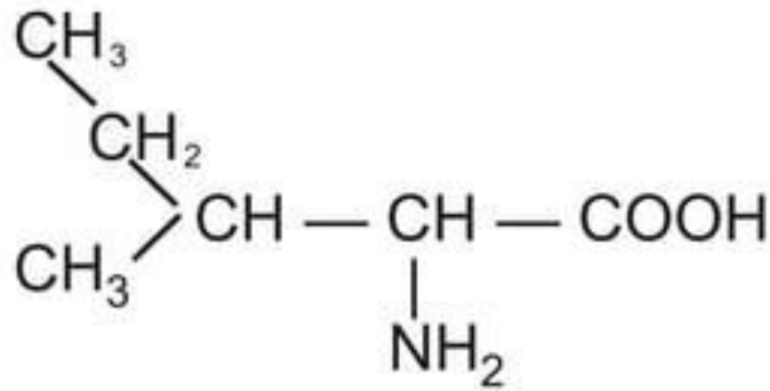
Alanine (Ala or A)



Valine (Val or V)

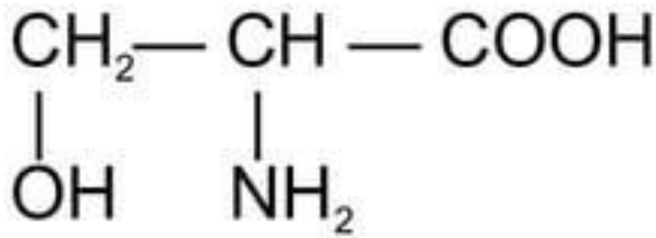


Leucine (Leu or L)

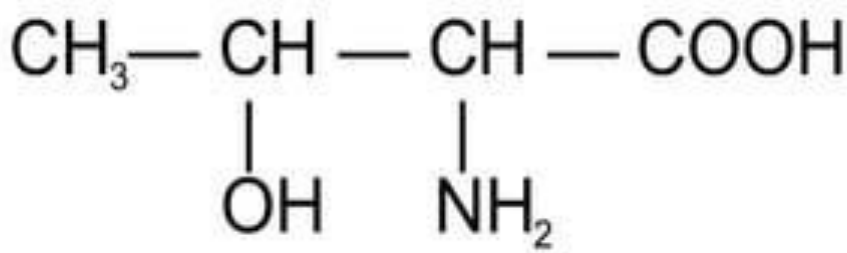


Isoleucine (Ile or I)

2. Amino acids with side chains having hydroxyl group

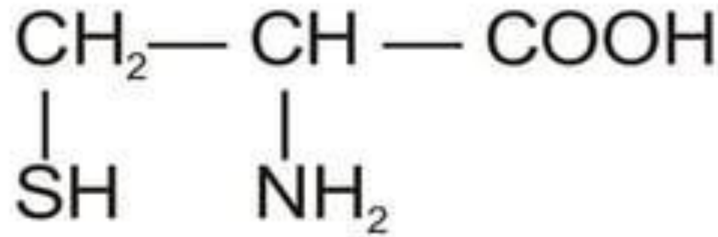


Serine (Ser or S)

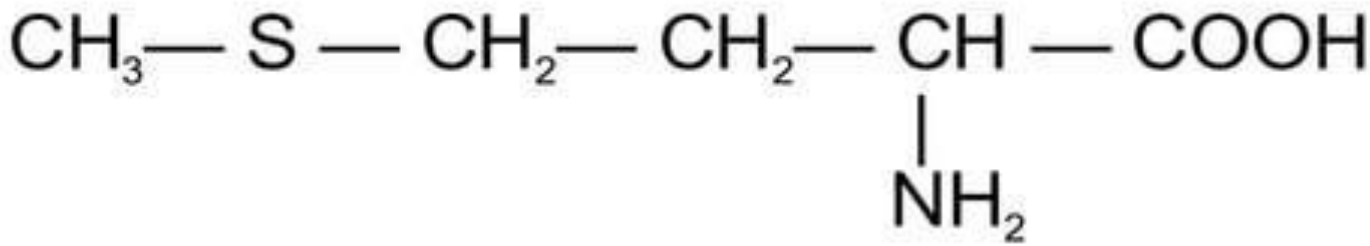


Threonine (Thr or T)

3. Amino acids with side chains containing sulphur

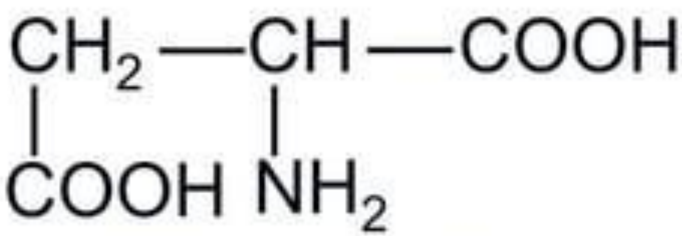


Cysteine (Cys or C)

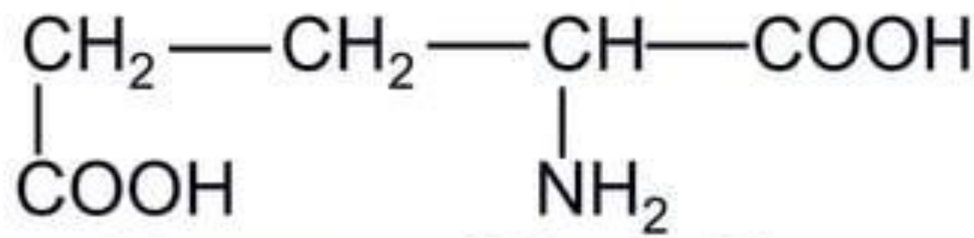


Methionine (Met or M)

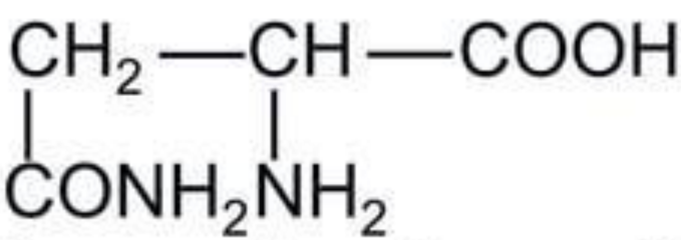
4. Amino acids with side chains having acidic groups or their amides



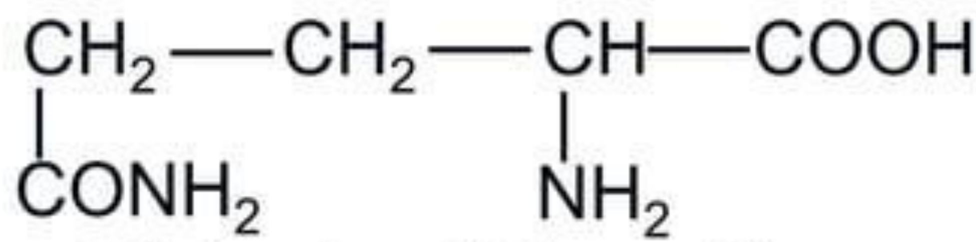
Aspartate (Asp or D)



Glutamate (Glu or E)

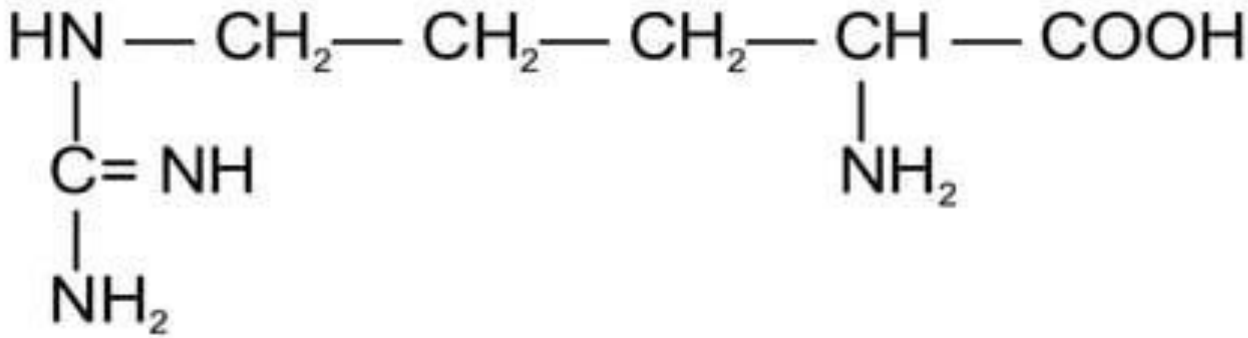


Asparagine (Asn or N)

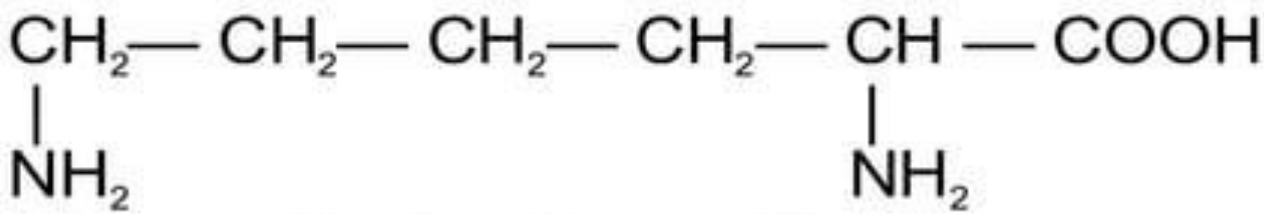


Glutamine (Gln or Q)

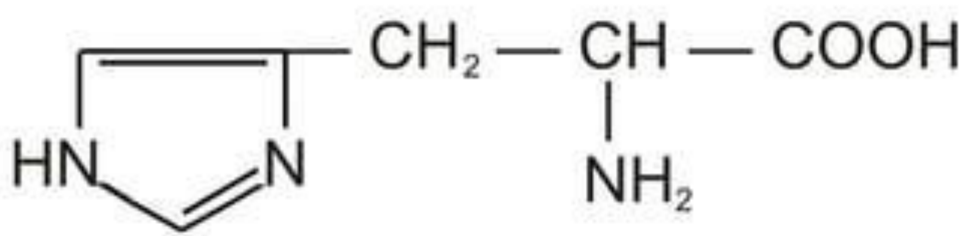
5. Amino acids with side chains having basic groups



Arginine (Arg or R)

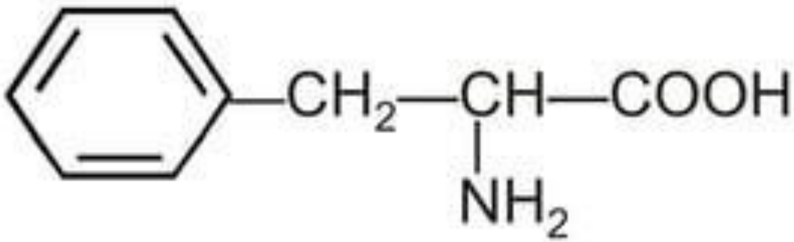


Lysine (Lys or K)

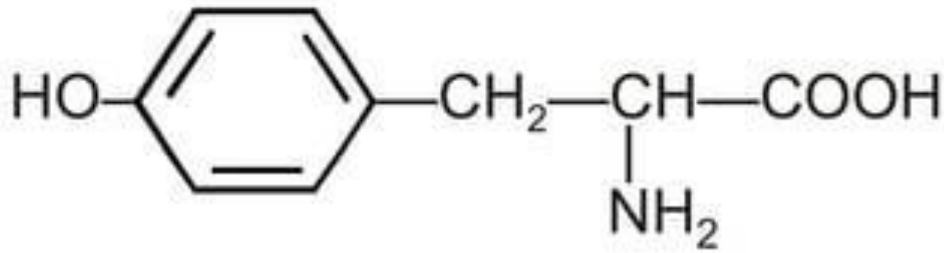


Histidine (His or H)

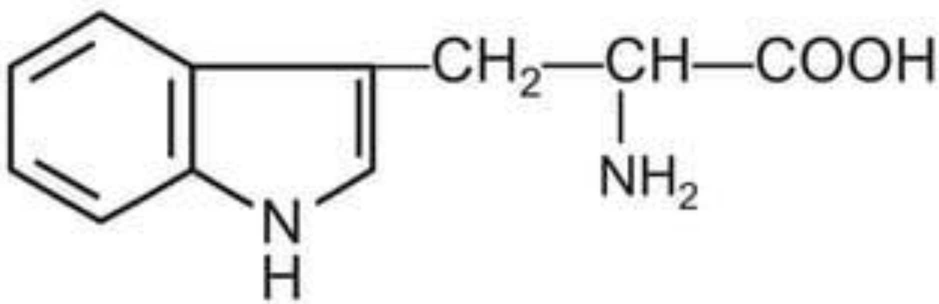
6. Amino acids with side chains containing aromatic rings



Phenylalanine (Phe or F)

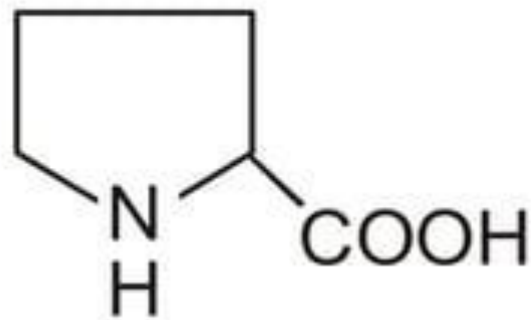


Tyrosine (Tyr or Y)



Tryptophan (Trp or W)

7. Imino acids



Proline (Pro or P)

Classification according to nutritional importance

According to nutritional importance, amino acids may be divided into:

- Essential amino acids
- Semi-essential amino acids
- Non-essential amino acids

Essential amino acids

Proteins are synthesized from 20 standard amino acids

All the standard amino acids are equally important for protein synthesis

However, some of these amino acids can be synthesized in our body

Nutritionally essential or indispensable amino acids

- This group of amino acids cannot be synthesized by the body hence, they are required to be supply through diet as they are required for proper growth and maintenance of individual.
- Presently there are eight amino acids classified as essential amino acids, these include:

Valine, **I**soleucine, **L**eucine, **L**ysine, **M**ethionine, **P**henylalanine, **T**hreonine, **T**ryptophan.

- Their deficiency leads to impaired protein synthesis

Semi-essential amino acids

Synthesis of semi-essential amino acids is below their requirement in childhood

Hence, they must be provided in the diet of children

Arginine and histidine are semi-essential amino acids

Non-essential amino acids

Endogenous synthesis of non-essential amino acids can meet our requirement

Hence, their presence in the diet is not essential

Glycine, alanine, serine, cysteine, aspartate, glutamate, asparagine, glutamine, tyrosine and proline are non-essential amino acids

Nutritionally non-essential amino acids

- These are amino acids that can synthesized in the body in order to meet the metabolic demand in a living system since they cannot supply through diet.

- **Examples includes glycine, alanine, serine, cysteine, aspartate, asparagine, glutamate, glutamine, tyrosine and proline.**

Classification based on their metabolic fate

A. Glycogenic amino acids

The carbon skeleton of this group of amino acids can serve as precursors for the synthesis of glucose or glycogen. e.g. Glycine, alanine, serine, cysteine, aspartic acid, aspargine, glutamic acid, glutamine, proline, histidine, arginine, methionine, threonine, and valine.

B. Ketogenic amino acids

The carbon skeleton of this group of amino acids are ketogenic in nature (Ketone), and can serve as precursors for the synthesis of ketone bodies. Examples are leucine and lysine

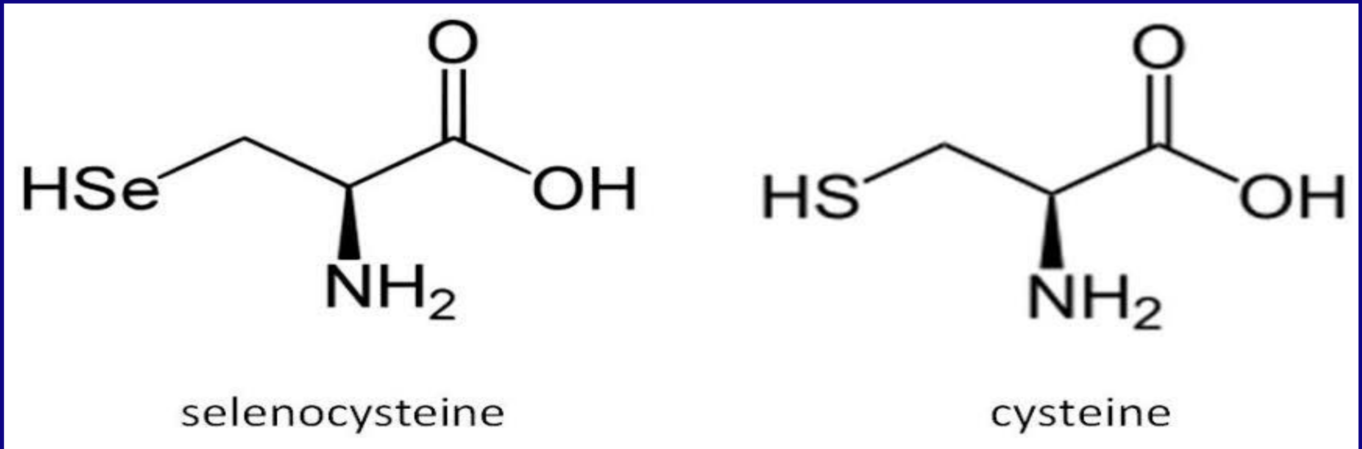
- **Glycogenic and ketogenic amino acids**

The carbon skeleton of For this group has dual functions, as it can be serve as precursor of for synthesis both glucose and ketone bodies.

Example includes isoleucine, phenylalanine, tryptophan and tyrosine

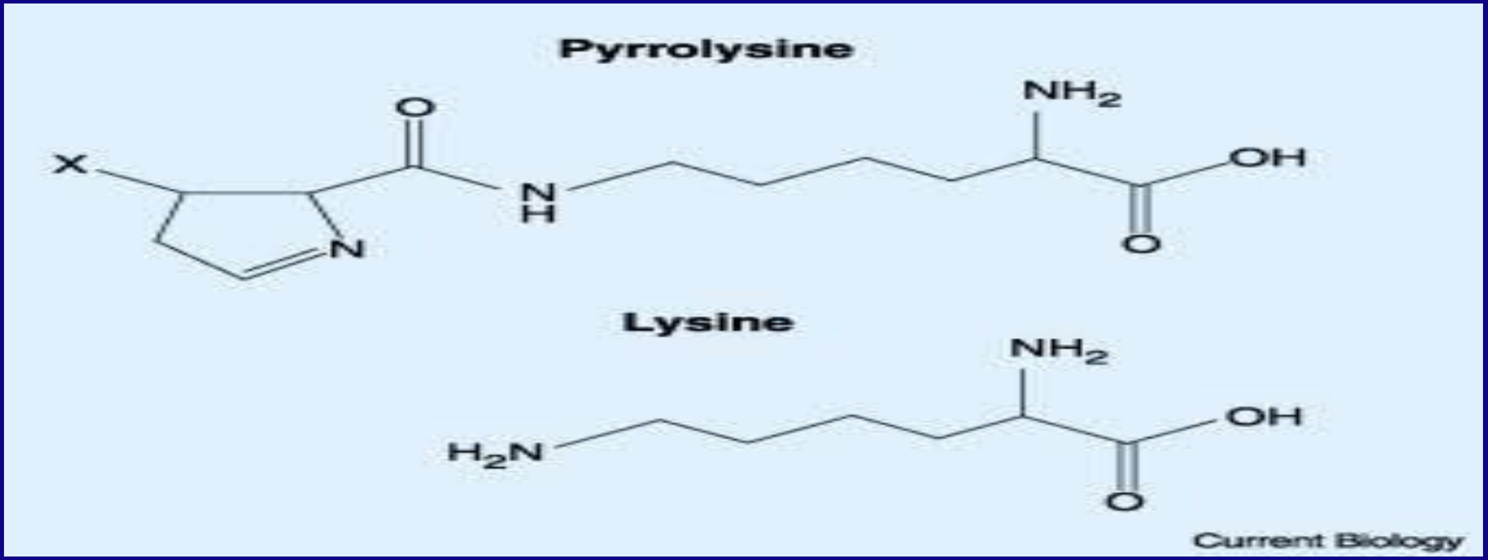
Selenocysteine

- This amino acid was discovered by biochemist Thressa Stadtman in 1974 was recently tagged as the 21st amino acid.
- Synthesized from cysteine during translation process by replacement of it sulphur atom with selenium.
- Found at the active sites of some enzymes/proteins e.g glutathione peroxidase and glycine reductase
- The IUPBMB has officially assigned three later symbol SEC and one letter symbol U for this amino acid



Pyrrolysine

- It is the 22nd amino acid formed by modification of the amide group in lysine with 4-methylpyrroline-5-carboxylate group as shown below
- It is exist in some protein present in methanogenic archea and in some bacteria



Non-standard amino acids

Besides the 20 standard amino acids, some other L-α-amino acids are also found in human beings

These are either intermediates or products of various metabolic pathways

NON-STANDARD AMINO ACIDS

- Besides the 20 standard amino acids present in the protein structure, there are several other amino acids which are biologically important

These includes

✓The amino acid derivatives found in proteins

✓Non-protein amino acids

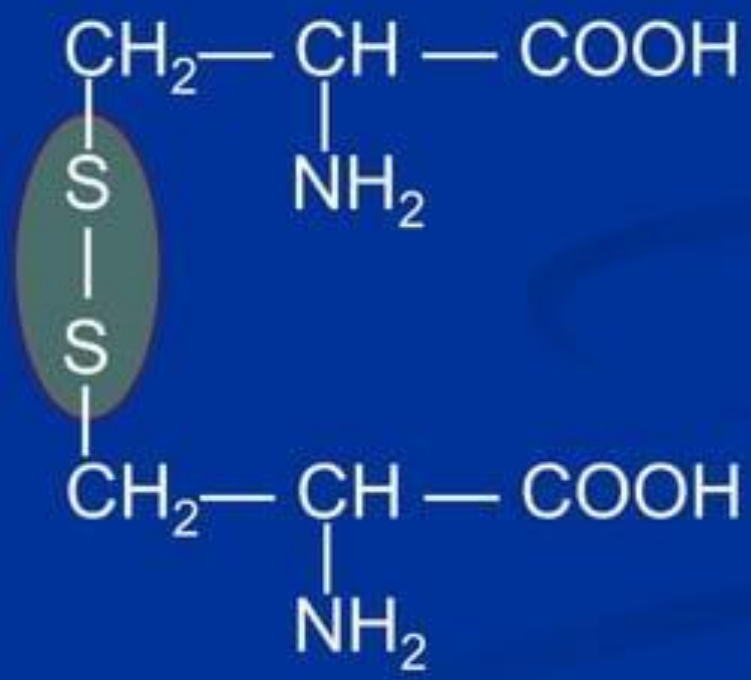
✓D-amino acids.

The amino acid derivatives found in proteins

- Some of these amino acids undergo specific modification after the protein synthesis occurs.

- This modification results in formation of derivatives which are very important for protein structure and functions. Examples include
  - ✓Cystine
  - ✓γ-Carboxyglutamic acid is found in certain plasma proteins involved in blood clotting
  - ✓4-hydroxyproline and 5-hydroxylysine which exist in abundant in structural protein Collagen

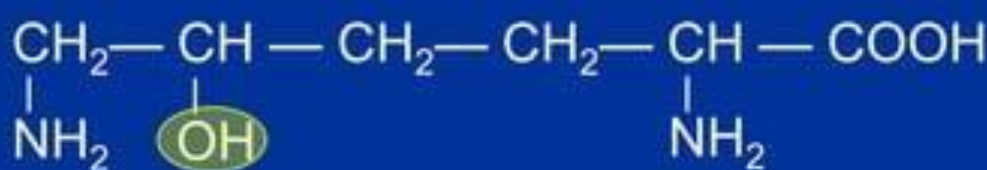
Two cysteine residues in a protein may be linked through their –SH groups to form a cystine residue



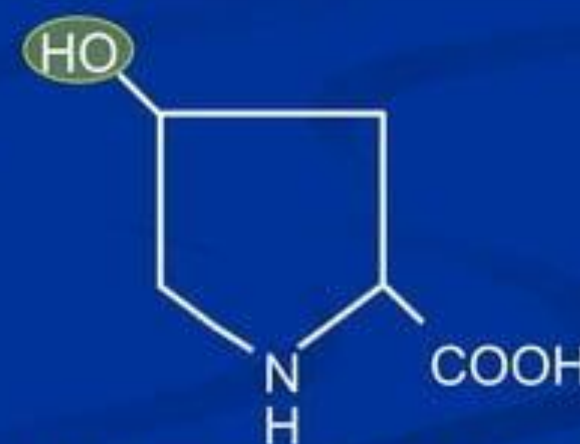
Cystine

Lysine and proline can be hydroxylated after their incorporation into proteins

They are converted into hydroxylysine and hydroxyproline respectively



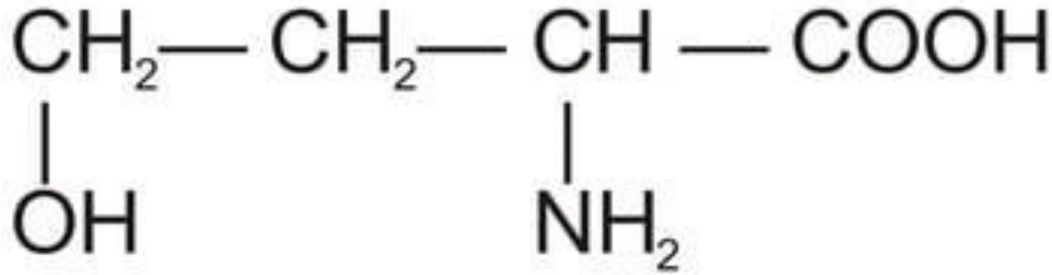
Hydroxylysine (Hyl)



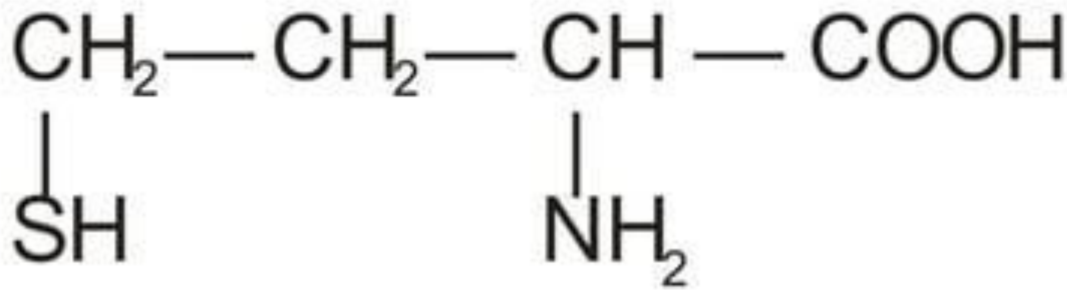
Hydroxyproline (Hyp)

The non-standard L-α-amino acids are:

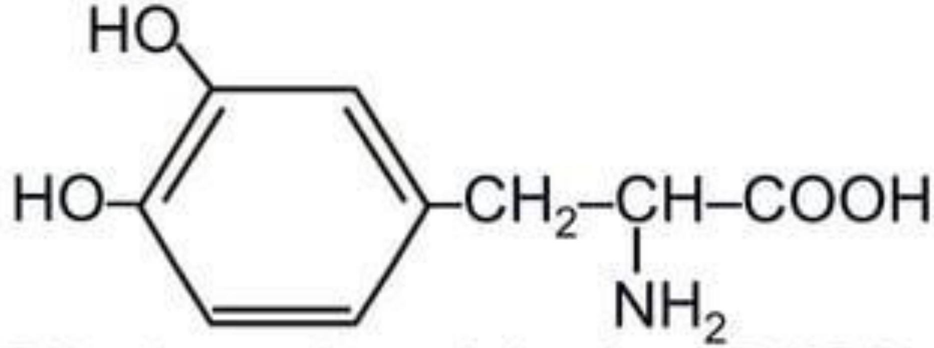
- Homoserine
- Homocysteine
- Dihydroxyphenylalanine (DOPA)
- Mono-iodo-tyrosine (MIT)
- Di-iodo-tyrosine (DIT)
- Tri-iodo-thyronine (T_3)
- Tetra-iodo-thyronine (T_4)
- Ornithine
- Citrulline
- Argininosuccinic acid



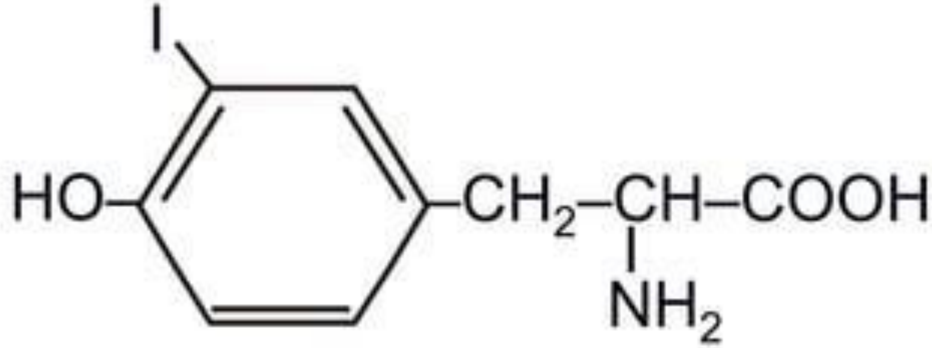
Homoserine



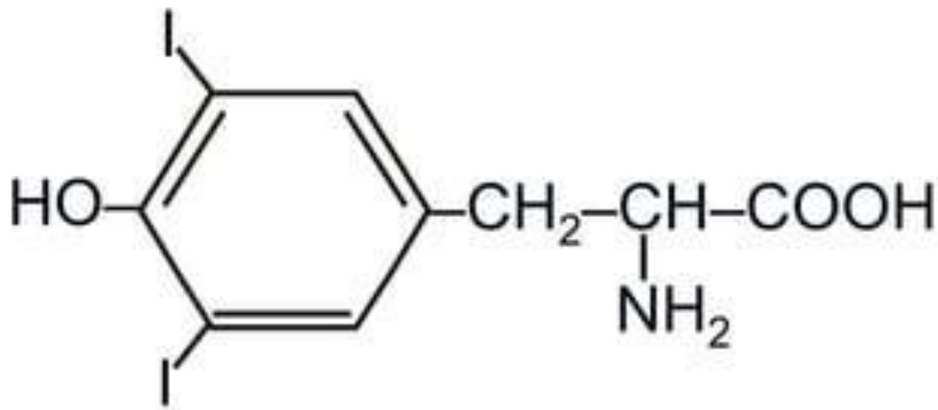
Homocysteine



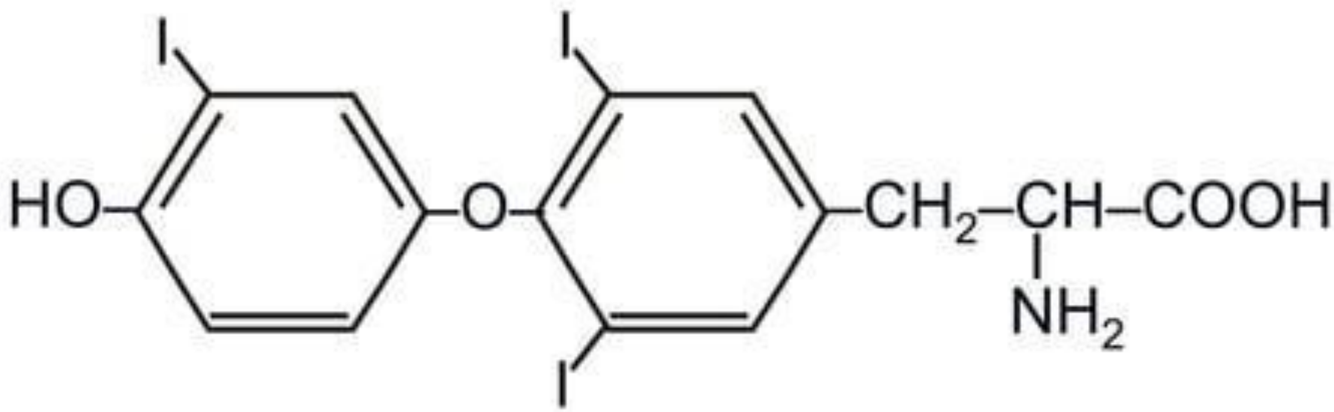
Dihydroxyphenylalanine (DOPA)



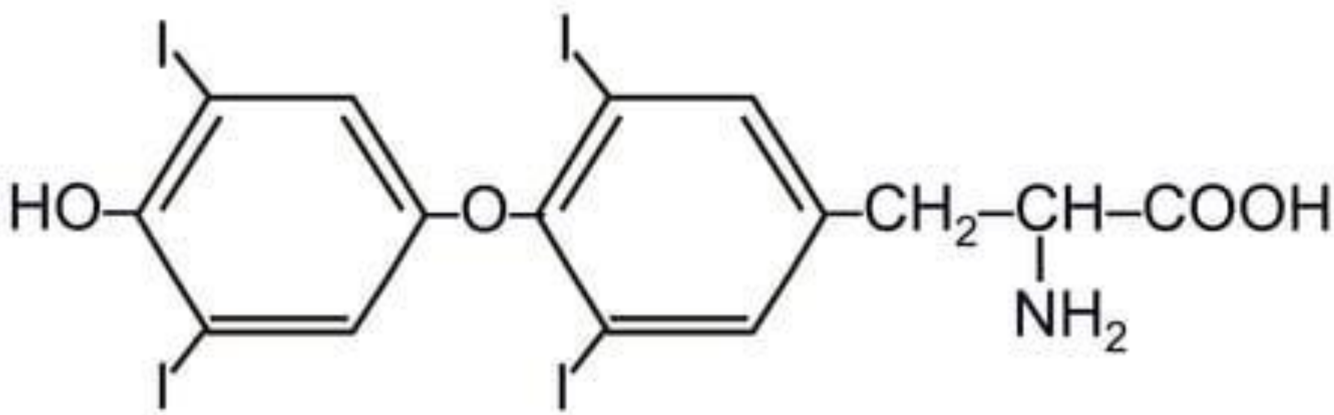
Mono-iodo-tyrosine (MIT)



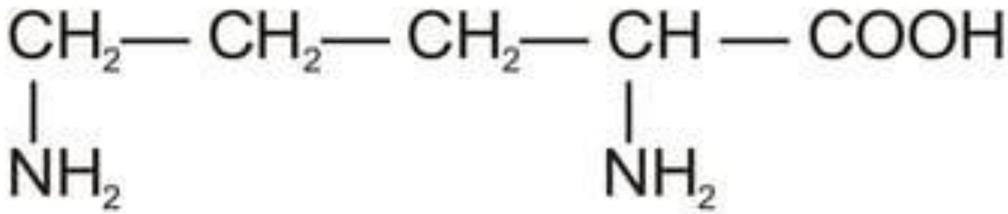
Di-iodo-tyrosine (DIT)



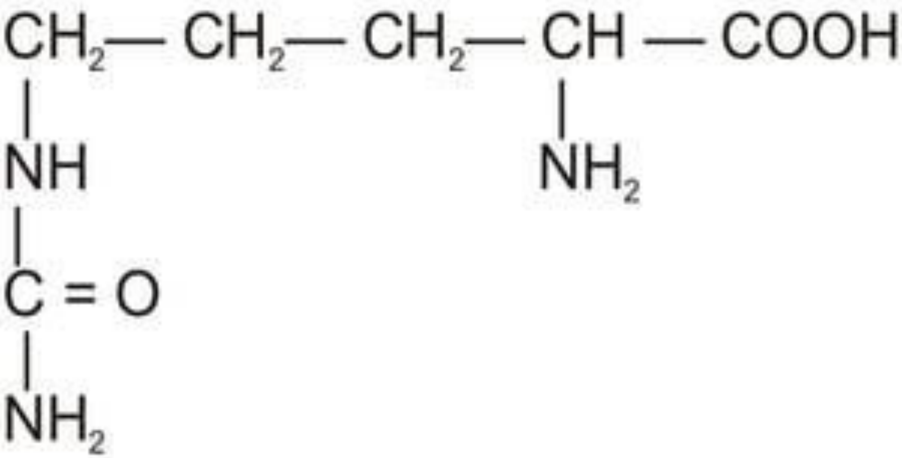
Tri-iodo-thyronine (T_3)



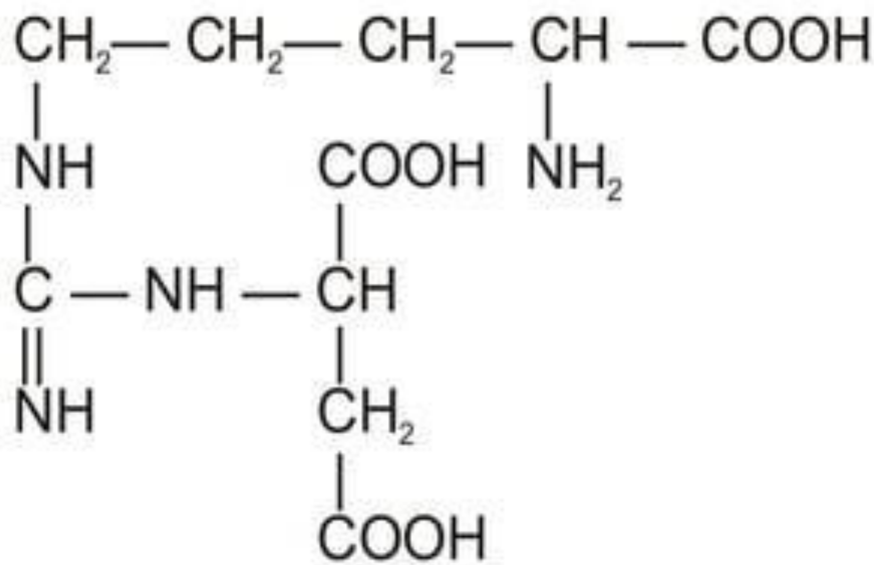
Tetra-iodo-thyronine (T_4)



Ornithine



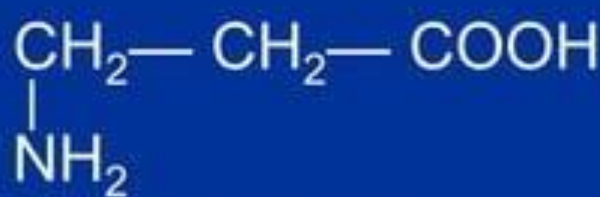
Citrulline



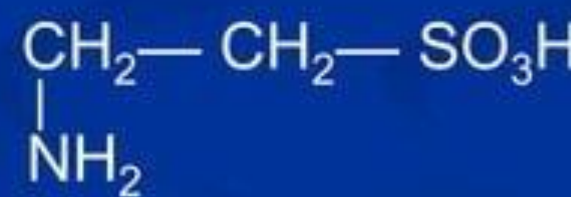
Argininosuccinic acid

Some non-α-amino acids are also present in human beings

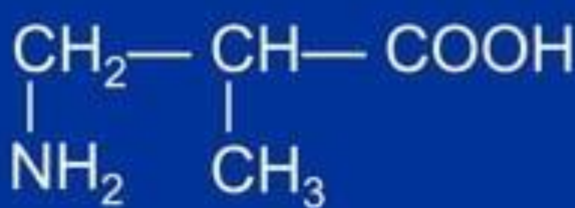
Examples are β-alanine, taurine, β-amino-isobutyric acid, γ-aminobutyric acid etc



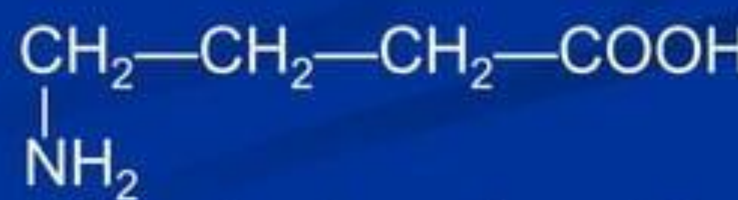
β-Alanine



Taurine



β-Aminoisobutyric acid



γ-Aminobutyric acid

β-Alanine is a constituent of pantothenic acid, acyl carrier protein and coenzyme A

Taurine is a neurotransmitter, and is a constituent of bile acids and bile salts

β-Aminoisobutyric acid is a metabolite of some pyrimidines

γ-Aminobutyric acid (GABA) is a neuro-transmitter

General Properties of Amino acids

Physical properties

- Amino acids are colorless, crystalline solid.
- All amino acids have a high melting point greater than 200^{o}C
- Solubility: They are soluble in water, slightly soluble in alcohol, and dissolve with difficulty in methanol, ethanol, and propanol.
- On heating to high temperatures, they decompose.
- All amino acids (except glycine) are optically active

Chemical properties

- Amphoteric nature
- Formation of peptide bonds
- Reactions of carboxyl and amino groups
- Reactions of sulphydryl groups
- Reactions used for protein sequencing
- Reactions for identification of specific amino acids

Amphoteric nature

All amino acids have at least one carboxyl and one amino group

Both these groups are ionizable

$$R\text{–}COOH \rightleftharpoons R\text{–}COO^- + H^+$$

$$R\text{–}NH_2 + H^+ \rightleftharpoons R\text{–}NH_3^+$$

R–COOH and R–NH_3^+ are acidic forms as they can donate hydrogen ions (protons)

R–COO^- and R–NH_2 are conjugate bases as they can accept hydrogen ions

Thus, amino acids can act as acids (proton donors) as well as bases (proton acceptors)

Therefore, amino acids are said to be amphoteric in nature

They can behave as acids as well as bases depending upon the pH of the medium in which they are present

In an alkaline medium, the carboxyl group is dissociated while ionization of amino group is suppressed

The amino acid, therefore, behaves as an acid in an alkaline medium

In an acidic medium, the amino group is ionized while ionization of carboxyl group is suppressed

Thus, the amino acid acts as a proton acceptor (base) in an acidic medium

$$R{-}\underset{\displaystyle NH_2}{\underset{|}{C}}H{-}COOH + H^+ \rightleftharpoons R{-}\underset{\displaystyle NH_3^+}{\underset{|}{C}}H{-}COOH$$

There is a certain pH at which both carboxyl and amino groups are ionized

The carboxyl group is un-protonated and the amino group is protonated

$$\begin{array}{c} R - \underset{\displaystyle NH_3^+}{\underset{|}{CH}} - COO^- \end{array}$$

This form of amino acid is known as a zwitterion

Both carboxyl and amino groups are ionized in a zwitterion, yet it is electrically neutral as a whole

It does not move in an electric field

The pH at which an amino acid exists in the zwitterion form is known as its isoelectric pH or isoelectric point (i.e.p.)

Isoelectric pH is constant for every amino acid

Solubility of the amino acid is the least at its isoelectric pH

Completely un-dissociated form of amino acids is shown frequently for the sake of simplicity

It does not exist in solution at any pH

Strength of an acid depends on the degree to which it dissociates and liberates protons

Strength of acids is generally expressed in terms of their dissociation constants

Since amino acids are very weak acids, their strength is expressed in terms of pK pK is the negative log of dissociation constant

The pK of the α-carboxyl groups of amino acids is around 2.1

The pK of the α-amino groups is around 9.8

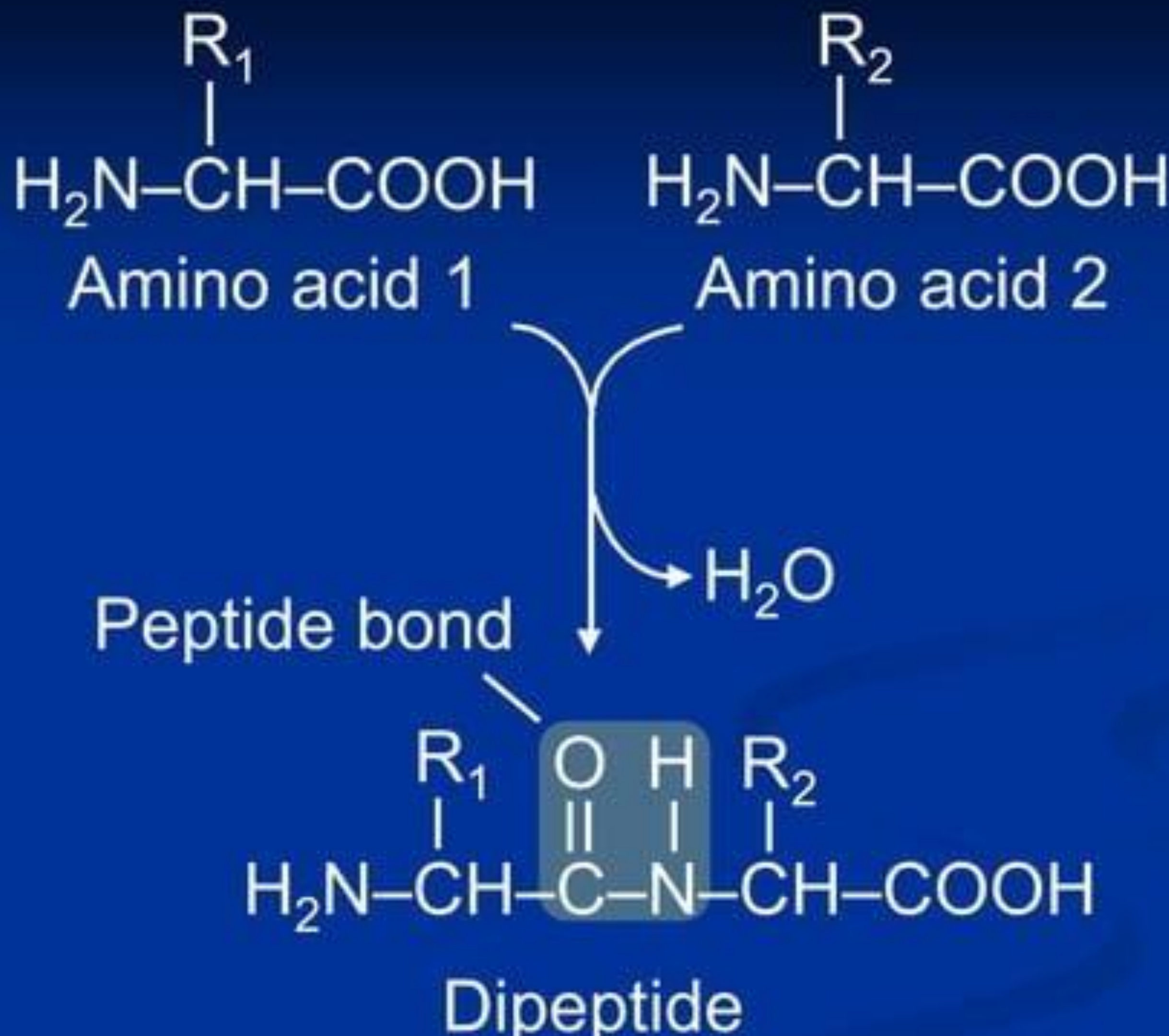
This means that $R–NH_3^+$ form is weaker than the R–COOH form as acid

Formation of peptide bonds

One of the most important reactions of amino acids

The bond by which amino acids are linked with each other in peptides and proteins

Formed between carboxyl group of one amino acid and amino group of another amino acid


R_1
$H_2N–CH–COOH$
Amino acid 1
R_2
$H_2N–CH–COOH$
Amino acid 2
H_2O
Peptide bond
R_1 O H R_2
$H_2N–CH–C–N–CH–COOH$
Dipeptide

Formation of peptide bonds in living cells is a complex process

The reaction occurs in stages, and requires the presence of enzymes, coenzymes and other factors

The peptide has a free $-NH_2$ group at one end and a free $-COOH$ group at the other

The former is known as the amino end and the latter as the carboxyl end

H_2N-AA AA AA AA AA AA AA AA-COOH

↑ Amino end

↑ Carboxyl end

Amino end is also known as N-terminus and the carboxyl end as C-terminus

All peptides and proteins possess an N-terminus and a C-terminus

H_2N-AA AA AA AA AA AA AA AA-COOH

↑ N-Terminus

↑ C-Terminus

Reactions of carboxyl and amino groups

The carboxyl and amino groups of amino acids can undergo their usual reactions such as:

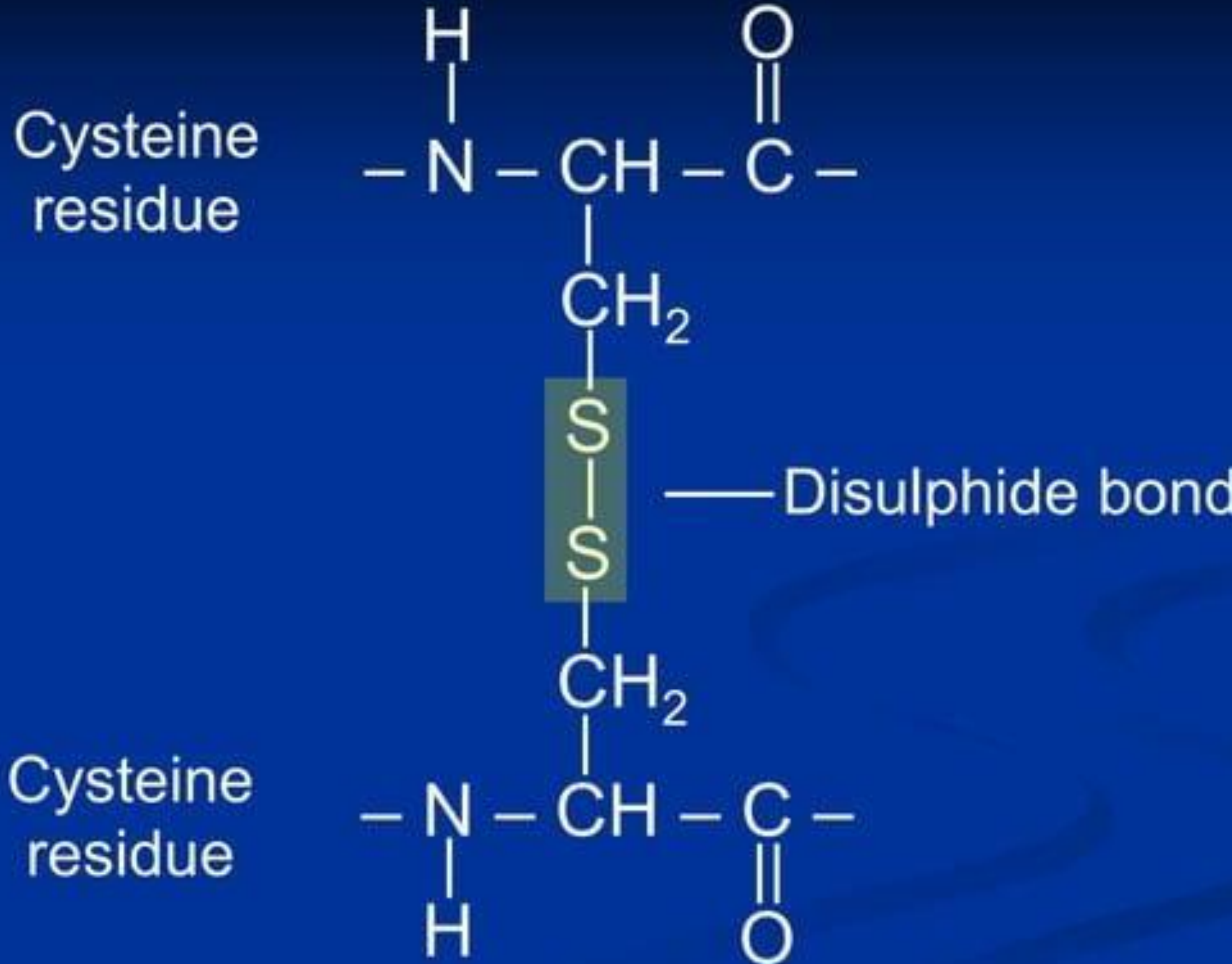
- Acylation
- Esterification
- Formation of salts

Reactions of sulphydryl groups

The sulphydryl (–SH) group of cysteine can undergo reversible oxidation and reduction

Disulphide bonds can be formed between the –SH groups of two cysteine residues

The –SH groups are essential for the biological activity of many proteins


Cysteine residue
Disulphide bond
Cysteine residue

Reactions for determining amino acid sequence of proteins

Determination of amino acid sequence is important for elucidating the structures of proteins

Generally, the N-terminal amino acid is tagged with some reagent

The tagged amino acid is split off by hydrolysis, and is identified

The reaction is, then, repeated with the new N-terminal amino acid and so on

Complete sequence of amino acids can, thus, be determined

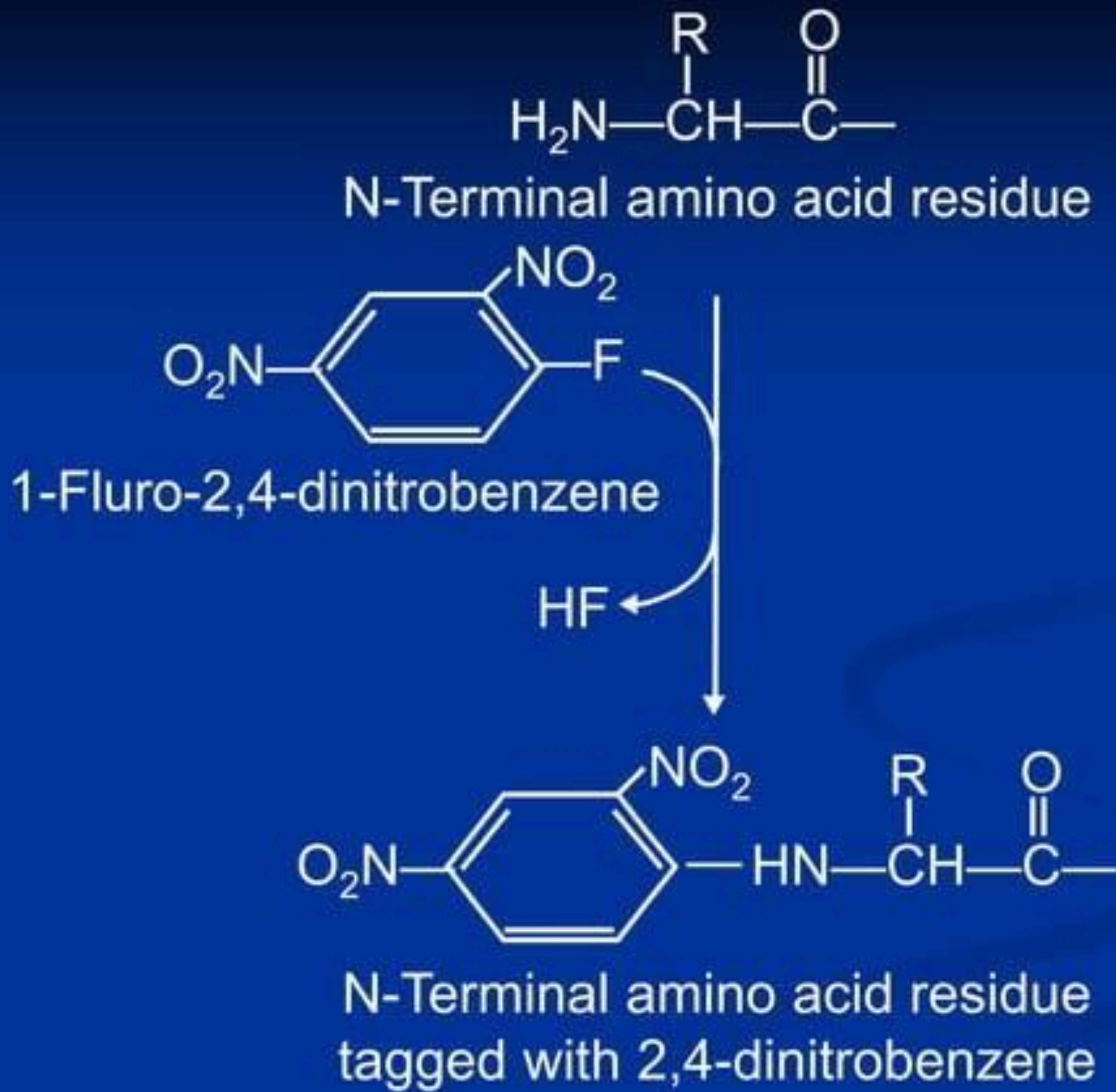
Common reactions used for determining amino acid sequence are:

- Sanger's reaction
- Edman's reaction

Sanger's reaction

The amino group of the N-terminal amino acid residue is tagged with 1-fluoro-2,4-dinitrobenzene (Sanger's reagent)

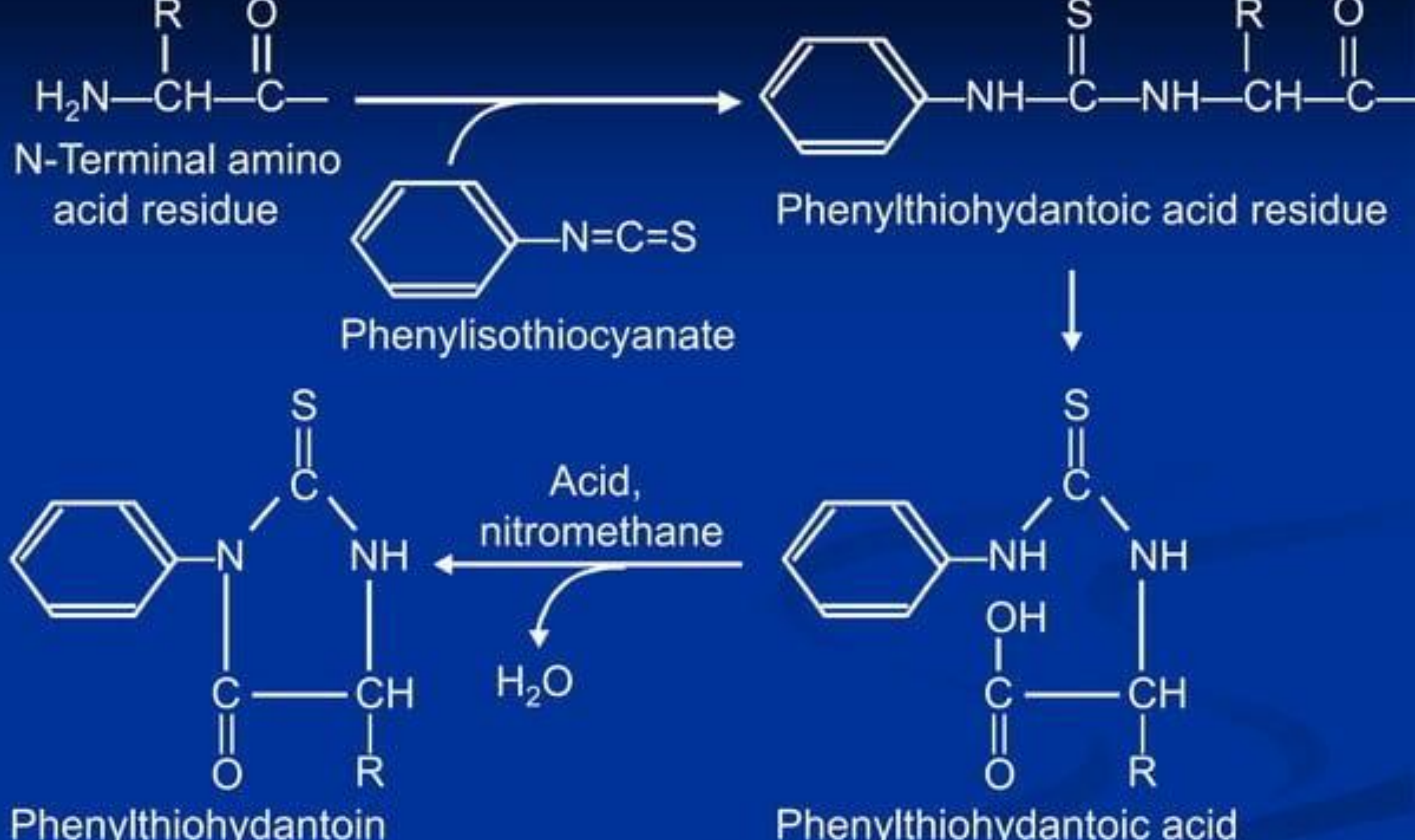
The tagged amino acid is split off and identified


R
O
H_2N—CH—C—
N-Terminal amino acid residue
NO_2
O_2N
F
1-Fluro-2,4-dinitrobenzene
HF
NO_2
R
O
O_2N
—HN—CH—C—
N-Terminal amino acid residue
tagged with 2,4-dinitrobenzene
RCG

Edman's reaction

Amino group of N-terminal amino acid residue is tagged with phenylisothiocynate to form phenylthiohydantoic acid

The latter is converted into phenylthio-hydantoin in the presence of an acid and nitromethane


N-Terminal amino acid residue
Phenylisothiocyanate
N=C=S
Phenylthiohydantoic acid residue
Acid, nitromethane
H2O
Phenylthiohydantoin
Phenylthiohydantoic acid
RCG

Reactions for identification of specific amino acids

These reactions are used for qualitative detection and/or quantitative measurement of amino acids

These reactions are given by free amino acids as well as amino acids present in peptides and proteins

Some important reactions of amino acids are:

- Ninhydrin reaction
- Xanthoproteic reaction
- Millon-Nasse reaction
- Aldehyde reaction
- Hopkins-Cole reaction
- Sakaguchi’s reaction
- Lead sulphide reaction

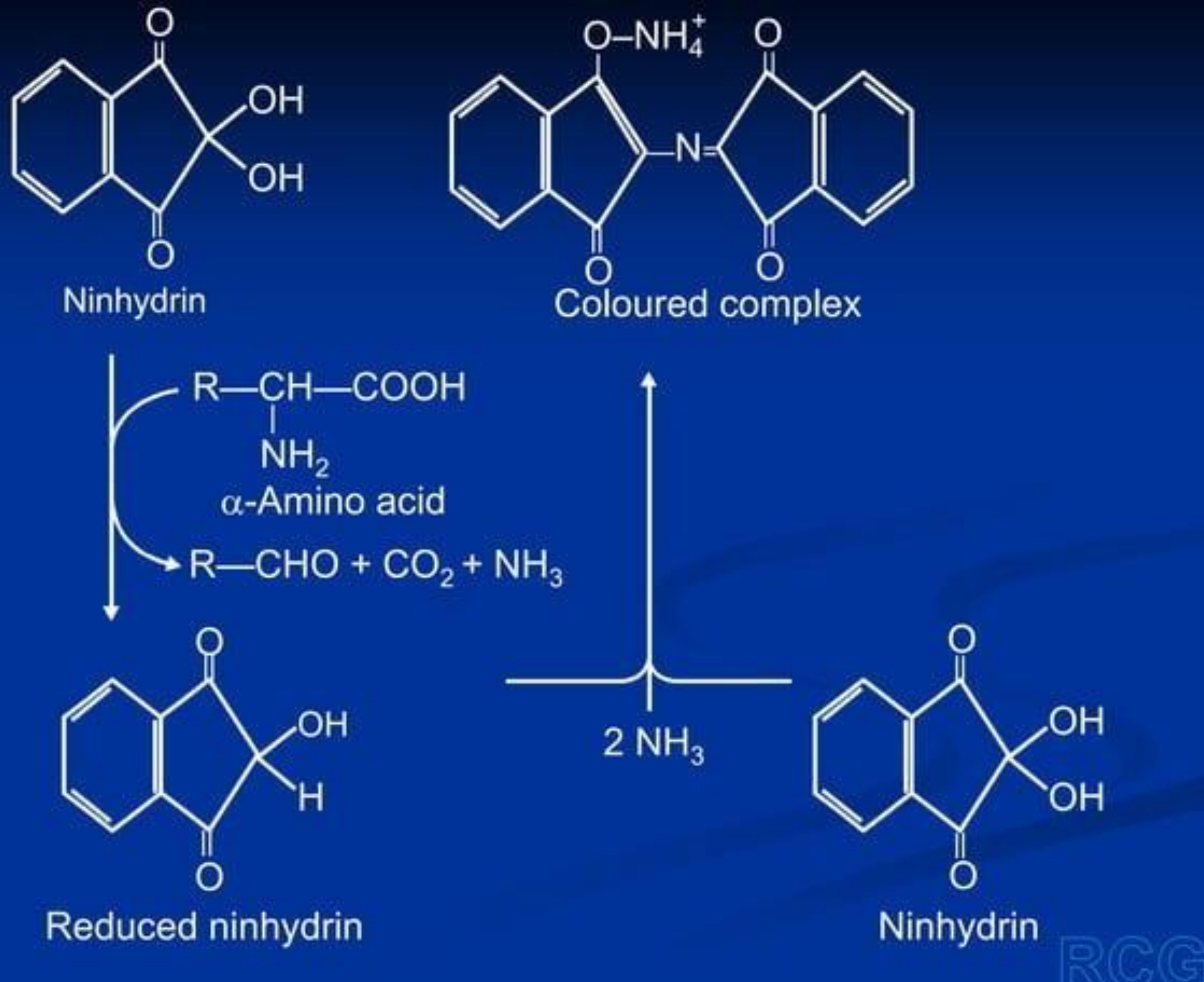
Ninhydrin reaction

This reaction is given by all amino acids and peptides

The α-amino acids react with two molecules of ninhydrin

A blue-purple coloured complex is formed (proline gives a yellow colour)

The reaction occurs in stages


Ninhydrin
Coloured complex
R—CH—COOH
NH2
α-Amino acid
R—CHO + CO2 + NH3
2 NH3
Reduced ninhydrin
Ninhydrin
RCG

Xanthoproteic reaction

This reaction is given by aromatic amino acids (phenylalanine, tyrosine and tryptophan)

On boiling with concentrated HNO_3, the phenyl groups are converted into nitrophenyl groups

These ionize on addition of an alkali, and impart an orange colour to the solution

Millon-Nasse reaction

This reaction is given by the 3,5-unsubstituted hydroxyphenyl group of tyrosine

Mercuric sulphate and nitrous acid cause mercuration and nitration or nitrosation of the hydroxyphenyl group of tyrosine

This produces a red colour

Aldehyde reaction

This reaction is given by the indole ring of tryptophan

The indole ring reacts with formaldehyde, in the presence of sulphuric acid, to form a violet complex

Hopkins-Cole reaction

This is similar to the Millon-Nasse reaction

The aldehyde here is glyoxylic acid (HOOC–CHO)

This reacts with the indole ring of tryptophan in the presence of sulphuric acid to form a violet complex

Sakaguchi's reaction

This reaction is given by the guanidino group of arginine

In a basic medium, α-naphthol reacts with the guanidino group forming a complex

On adding sodium hypobromite/hypochlorite, the complex is oxidized to a red coloured product

Lead sulphide reaction

This reaction is given by sulphur-containing amino acids, cysteine and cystine

On boiling with sodium hydroxide, the sulphur present in these amino acids is released in the form of sodium sulphide

This reacts with lead acetate to form a brown or black precipitate of lead sulphide

Methionine contains sulphur but it does not give lead sulphide reaction

Sulphur present in methionine is not released on boiling with sodium hydroxide

Thank you