UNIT 13

Amino Acids and Peptides

Structure

13.1 INTRODUCTION

This is the first unit of Block 4 of this course. In this Unit, you will study about the structure and chemistry of amino acids and peptides.

Amino acids are the compounds which contain both an amino group and a carboxy group in their molecules. They constitute a particularly important class of bifunctional compounds as they are the building blocks of proteins. Here, you will first study about the amino acids and then the structure and synthesis of peptides will be described.

We will begin this unit with a discussion on the structure and physical properties of common amino acids. Then, we will explain the zwitterionic nature of amino acids. This will be followed by description of the synthesis of the amino acids.

Then, the structure of peptides will be illustrated. The synthesis of peptides using N-protection, C-protection and C-activating groups will be discussed in

detail. You will also study about Merrifield solid - phase synthesis which is a versatile technique for the synthesis of peptides. Finally, the methods of laboratory detection of amino acids will be explained.

Expected Learning Outcomes

After studying this unit and having the experiments performed, you should be able to:

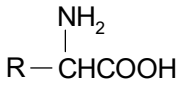
- list some amino acids and write their structures;
- give the common and IUPAC names of amino acids;
- discuss the zwitterionic nature of amino acids;
- explain the isoelectric point and importance of electrophoresis;
- give various methods of preparation of 2- amino acids;
- discuss the reactions of amino acids;
- briefly explain the structure of peptides;
- describe the methods of synthesis of peptides using N-protection, C-protection and C-activating groups;
- discuss Merrifield solid-phase synthesis and highlight its importance; and
- explain the laboratory detection of amino acids.

13.2 Structure and Physical Properties of Amino Acids

Before studying the chemistry of amino acids, let us first understand their structure.

13.2.1 Structure of Amino Acids

The general structure of a 2-amino acid can be represented as follows:

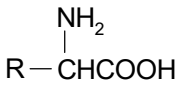
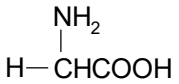
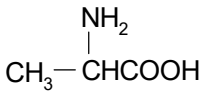
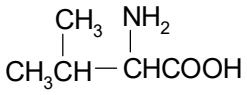
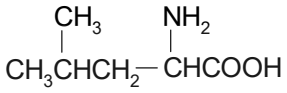
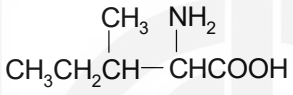
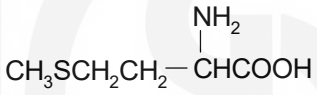
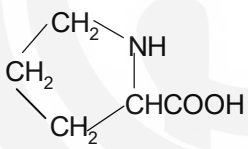
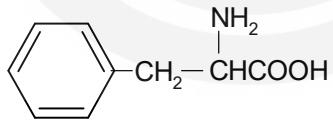
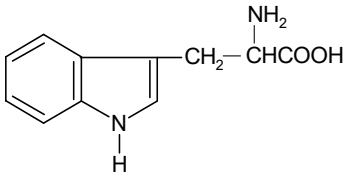
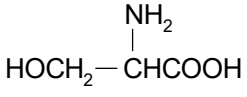
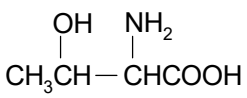
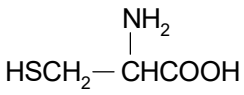


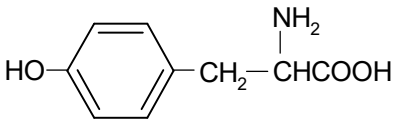
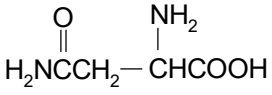
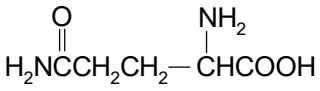
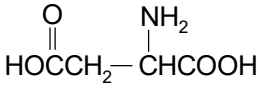
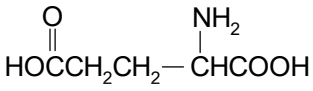
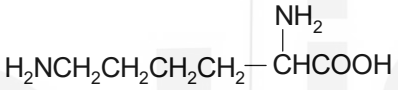
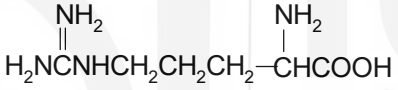
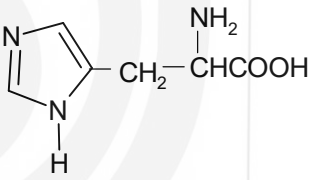
These are more often referred as *α*-amino acids.

While several hundred different amino acids are known to occur naturally, 20 of them deserve special mention as they are mainly present in proteins. These amino acids are listed in Table 13.1. As given in the Table, for amino acids trivial names are commonly used.

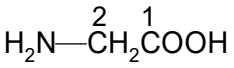
The convention to use a three letter code, as an abbreviation, for each amino acid is also given in the table. These abbreviations are particularly useful in designating the sequence of amino acids in peptides and proteins. The last column of the table given below also shows a single letter code to denote the amino acids. You will study more about peptides and proteins in Unit 14.

Table 13.1: Physical Properties of Amino Acids

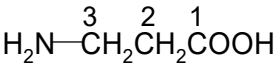
Amino Acid, 	**Name**	**Abbreviation Three letter Code**	**Abbreviation One letter Code**
	glycine	Gly	G
	alanine	Ala	A
	valine	Val	V
	leucine	Leu	L
	isoleucine	Ile	I
	methionine	Met	M
	proline	Pro	P
	phenylalanine	Phe	F
	tryptophan	Trp	W
	serine	Ser	S
	threonine	Thr	T
	cysteine	Cys	C

	tyrosine	Tyr	Y
	asparagine	Asn	N
	glutamine	Gln	Q
	aspartic acid	Asp	D
	glutamic acid	Glu	E
	lysine	Lys	K
	arginine	Arg	R
	histidine	His	H

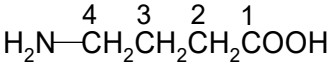
Amino acids can be classified as α, β, γ,… etc., depending upon the location of the amino group on the carbon chain containing the carboxy function. Some examples are illustrated below:



2-aminoethanoic acid
(α-amino acid)



3-aminopropanoic acid
(β-amino acid)



4-aminobutanoic acid
(γ-amino acid)

Thus, the amino acids listed in Table 13.1 are α-amino acids or 2-amino acids. Now, answer the following SAQ.

SAQ 1

Give one example each for a 2-amino acid which contains the following in the side chain:

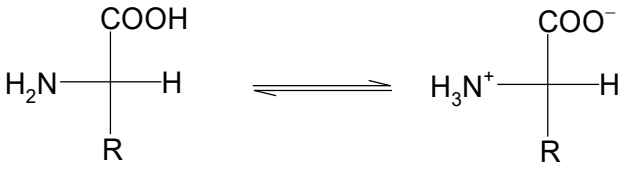
i) Sulphur

ii) An aromatic ring

iii) A carboxyl group

13.2.2 Zwitterionic Nature

Because amino acids contain both carboxy and amino groups in their molecules, they are *amphoteric* in nature, i.e. they behave both as acids and bases. Amino acids actually exist as *inner salts*, called **zwitterions**. A zwitterionic structure is possible for amino acids because the amino group is basic in nature and can accept a proton from the acidic carboxy group. A zwitterion can be represented as shown below.



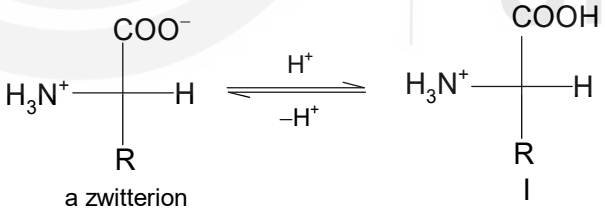
a zwitterion

The highly polar nature of zwitterion allows the formation of strong crystal lattices similar to the ionic compounds. Amino acids, therefore, resist conversion from solid to liquid state and *do not melt* but decompose on heating.

The zwitterionic nature is also reflected in their higher solubility in water and low solubility in nonpolar solvents. In addition to the above observations, large dipole moments also indicate the zwitterionic nature of amino acids.

13.2.3 Isoelectric Point and Electrophoresis

Let us now study the zwitterionic form of amino acids in more detail. You can see in the zwitterion shown above that the amino group is protonated and the carboxy group exists as the carboxylate anion. Thus, the acidic group is a substituted ammonium ion and the basic group is the carboxylate anion. As a result, *in strongly acidic medium* i.e., at low pH, the carboxylate group will be protonated to yield the following species, I.

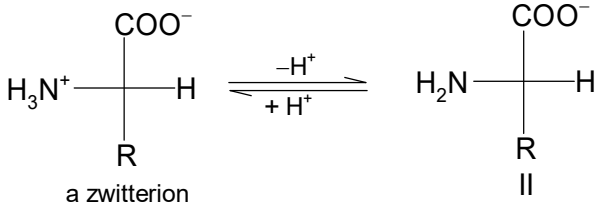


a zwitterion

I

species present in strongly acidic medium

Let us next consider the species present in *strongly basic medium*, i.e. at higher pH of the solution. Under these conditions, the proton will be removed from the $^{+}NH_3$ group to yield the following species.

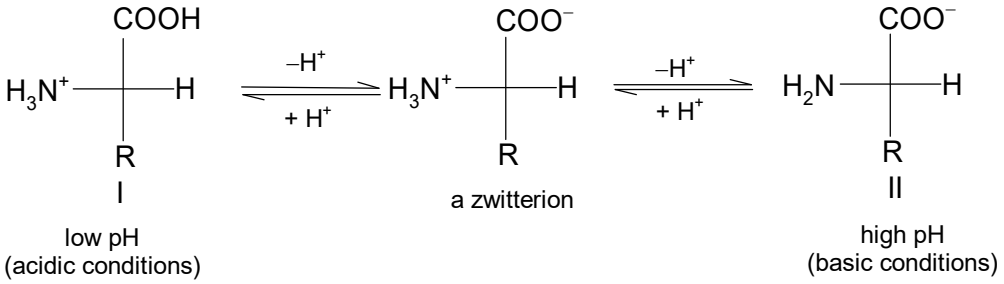


a zwitterion

II

species present in strongly basic conditions

Thus, we can write a combined equation for the acid-base behavior of the acids as shown below.



You can see that at low pH, species I has a net positive charge and has two acidic sites ($^+NH_3$ and COOH). On the other hand, at high pH, species II has a net negative charge and has two basic sites (NH_2 and COO^-).

It is clear from the above equation that the amino group will be first protonated and then the carboxylate anion. Also at some intermediate pH, the amino acid exists as a zwitterion *with no net charge*. The pH at which this occurs is known as **isoelectric point**, pH_i of the amino acid. At this pH, the amino acid is stationary in an electric field, i.e., it migrates neither to the negative pole nor to the positive pole because the charges on it are balanced.

At low pH, there are two acidic sites in the amino acid I and therefore, it has two pK_a values. The pK_a value corresponding to the more acidic site is referred to as pKa_1 and that corresponding to the less acidic site as pKa_2. Thus, for the simplest amino acid, glycine, we can write the two equilibria as follows:

$$\overset{+}{H_3}NCH_2COOH + H_2O \underset{}{\overset{pK_{a1} = 2.4}{\rightleftharpoons}} \overset{+}{H_3}NCH_2COO^- + H_3O^+$$

$$\text{and } \overset{+}{H_3}NCH_2COO^- + H_2O \underset{}{\overset{pK_{a2} = 9.8}{\rightleftharpoons}} H_2NCH_2COO^- + H_3O^+$$

At this stage you can compare the pKa_1, with pKa of ethanoic acid which is equal to 4.76. This leads to the conclusion that due to the electron withdrawing nature of the protonated amino group, the acidity of amino acid is increased as compared to ethanoic acid. Table 13.2 lists the pK_a values and pH_i of some amino acids.

Table 13.2: pK_a and pH_i values of some amino acids

Amino acid	pKa_1	pKa_2	pH_i
Glycine	2.34	9.60	5.97
Alanine	2.34	9.69	6.00
Valine	2.32	9.62	5.96
Leucine	2.36	9.60	5.98
Isoleucine	2.36	9.60	6.02
Methionine	2.28	9.21	5.74
Proline	1.99	10.60	6.30
Phenylalanine	1.83	9.13	5.48
Tryptophan	2.83	9.39	5.89
Asparagine	2.02	8.80	5.41
Glutamine	2.17	9.13	5.65
Serine	2.21	9.15	5.68
Threonine	2.09	9.10	5.60

You can verify from Table 13.2 that

$$pH_i = \frac{pKa_1 + pKa_2}{2}$$

The amino acids having acidic and basic side chains are characterised by three pK_a values. The third pK_a value, i.e., pKa_3 reflects the nature of the functional group present in the side chain.

Electrophoresis

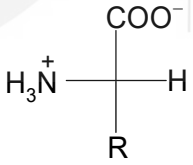
It is a technique which involves the migration of charged particles using an electric field. The charged particles have different mobilities depending upon their shape, size and charge. Thus, different amino acids and in turn, the peptides and proteins which are made up of these amino acids, can be separated using electrophoresis. Since different amino acids and peptides (or proteins) have different acidic and/or basic groups, they have different isoelectric points. Thus, at a particular pH, in a solution, they move towards the cathode or the anode at different rates.

In this way, electrophoresis can be used for the separation and analysis of various amino acids, peptides or proteins when present in a mixture.

13.2.4. Stereochemistry of Amino Acids: Optical Activity

With the exception of 2-aminoethanoic acid (glycine), the 2-amino acids have at least one chiral centre.

According to the older D, L system of specifying the configuration (discussed in Sec. 11.3, Unit 11, Block 3 of BCHCT-131 Course), the 2-amino acids derived from animals or higher plants were found to have L configuration, i.e., they have the same relative configuration as L-glyceraldehyde. Thus, we can write the following Fischer projection formula of an L amino acid.



an L amino acid

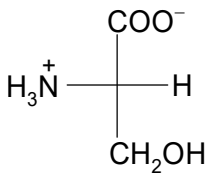
Enzymes use up one enantiomer preferentially.

On the basis of your knowledge about assigning the absolute configuration, can you attempt the following SAQ?

SAQ 2

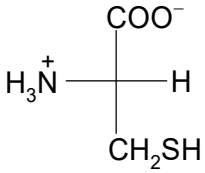
What is the absolute configuration (*R* or *S*) of the following amino acids?

a)



L-serine

b)



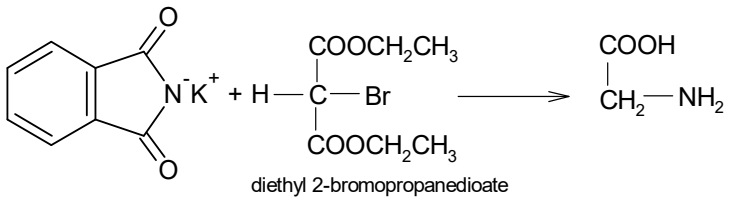
L-cysteine

Having learnt about some general aspects of the structure of amino acids, let us now focus our attention on the synthesis of 2-amino acids.

13.3 SYNTHESIS OF 2-AMINO ACIDS

2-Amino acids can be synthesised by using the methods given in Table 13.3.

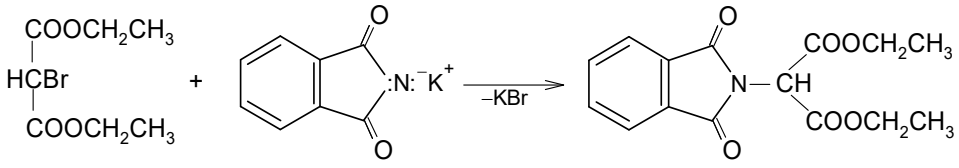
Table 13.3: Methods of Synthesis of 2-Amino Acids

1.	**By *N*-alkylation of 1,2-benzenedicarboxylic imide (phthalimide) anion**  diethyl 2-bromopropanedioate 2-substituted amino acids can also be prepared.
2.	**From aldehydes : Strecker Synthesis** 
3.	**From 2-halo acids** 

13.3.1 Gabriel Phthalimide Synthesis

This method is a modification of the Gabriel synthesis of amines which has been discussed in Unit 11, Sec. 11.4.

It involves the *N*-alkylation of 1,2-benzenedicarboxylic imide (phthalimide) anion with diethyl 2-bromopropanedioate as shown below:

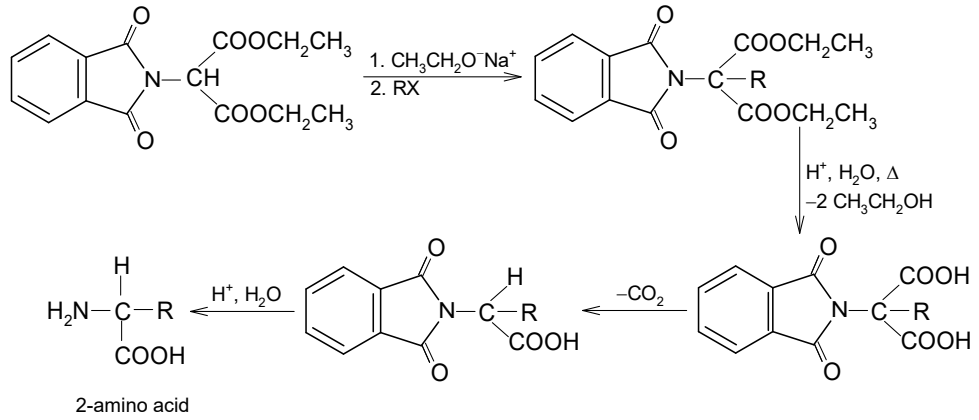


diethyl 2-bromo-propanedioate (diethyl 2-bromo-malonate)

potassium 1,2-benzene-dicarboxylic imide (potassium phthalimide)

N-alkylated product (85%)

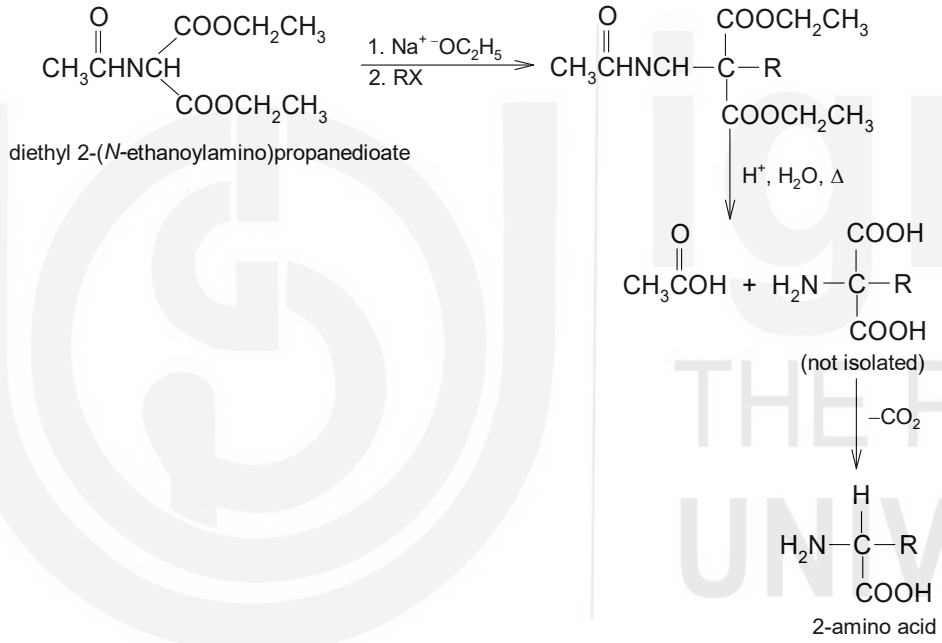
The advantage of this method is that the alkylated product obtained in the above reaction can be further alkylated to yield a variety of substituted amino acids by the following sequence of reactions.



Diethyl 2-bromopropanedioate can be prepared by the bromination of diethyl propanedioate.

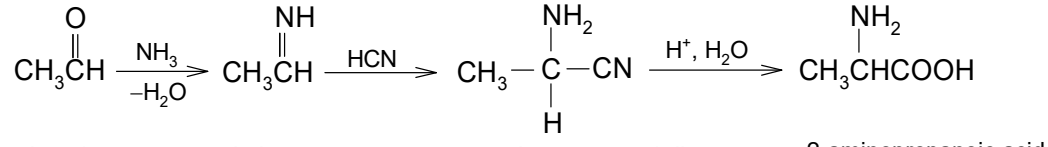
Thus, we can get a variety of amino acids depending upon the nature of R.

A variation of the above mentioned method utilises diethyl 2-(*N*-ethanoylamino)propanedioate instead of the imide derivative. The sequence of reactions involved is shown below:



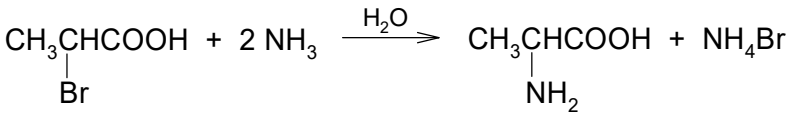
13.3.2 Strecker Synthesis

It was pointed out in sub-Sec. 14.4.1, Unit 14, Block 3 of BCHCT-133 Course that aldehydes on reaction with hydrogen cyanide yield a cyanohydrin. But, when the same reaction is carried out in the presence of ammonia, the first step is probably the initial formation of an imine from the reaction of the aldehyde with ammonia. The addition of hydrogen cyanide to the imine furnishes the corresponding 2-amino nitrile which on acidic or basic hydrolysis yields the 2-amino acid. This is also known as **Strecker synthesis**. The sequence of reactions involved in this synthesis is given below.



13.3.3 From 2-Halo Acids

In Unit 9, Sec. 9.5, you have studied that 2-halo acids can be obtained from carboxylic acids using the Hell-Volhard-Zelinsky reaction. These 2-halo acids on nucleophilic substitution by NH_3 yield 2-amino acids as shown below:



2-bromopropanoic acid

2-aminopropanoic acid (65-70%)

Enzymes preferably use one enantiomer

It is also worthwhile to mention here that the amino acids obtained by synthesis using the methods discussed above are racemic mixtures. Enantiomerically pure amino acids can be obtained by resolution of the racemic mixtures or by chiral synthesis using enzymes.

SAQ 3

Which alkyl halide will you use for the synthesis of methionine in the Gabriel phthalimide synthesis?

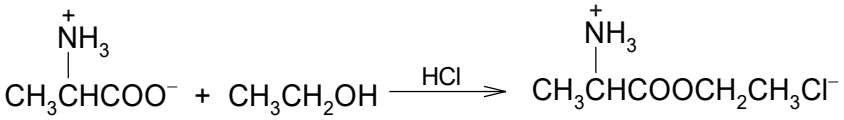
13.4 REACTIONS OF AMINO ACIDS

Amino acids undergo many of the reactions characteristic of the amino and carboxy groups. For example, a typical reaction of carboxy group is esterification and that of the amino functional group is alkanoylation. Let us now study these reaction in detail.

1. Esterification

Methyl, ethyl and benzyl esters are used as intermediates in the synthesis of peptides.

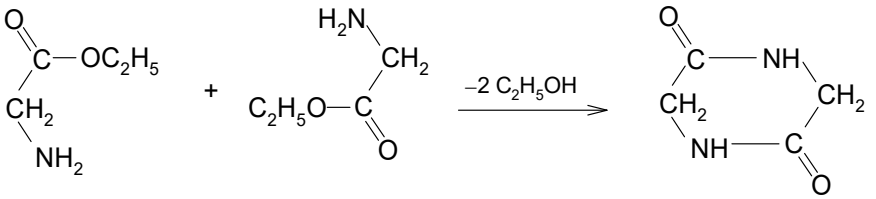
The carboxy group of an amino acid can be esterified in the normal way using excess of an alcohol under acidic conditions.



(90-95%) hydrochloride salt of amino acid ester

Neutralisation of the hydrochloride with alkali yields the ester.

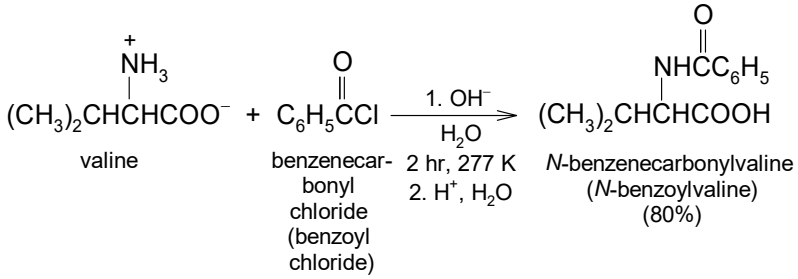
Esters of amino acids undergo intermolecular cyclisation to yield cyclic amides shown below:

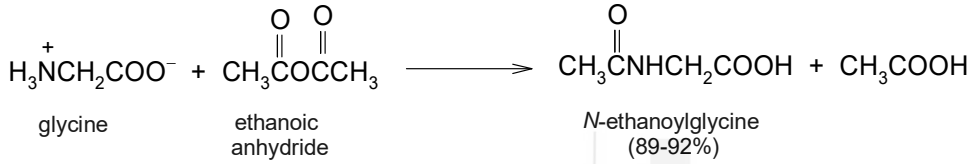


2,5-diazacyclohexane-1,4-dione (diketopiperazine)

2. Alkanoylation of Amino Acids

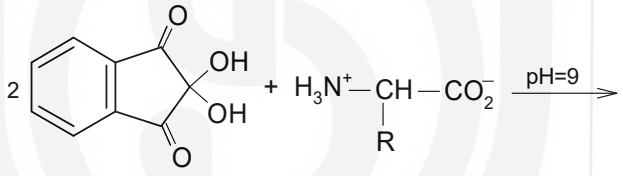
Alkanoylation of the amino group of an amino acid is carried out under basic conditions so that the free amino form is present in substantial concentration. Alkanoylation can be carried out by alkanoyl halides (acid chlorides) or carboxylic acid anhydrides. The product is finally obtained by acidifying the reaction mixture.

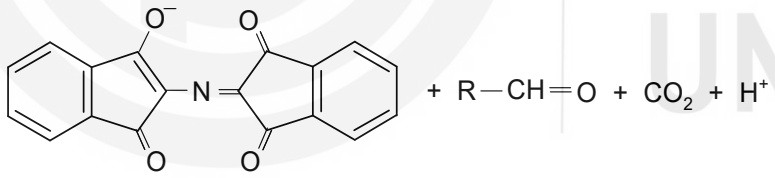




3. Reaction with Ninhydrin

When the aqueous solution of a 2-amino acid is treated with triketohydrindene hydrate (ninhydrin), a blue-violet colour is obtained.





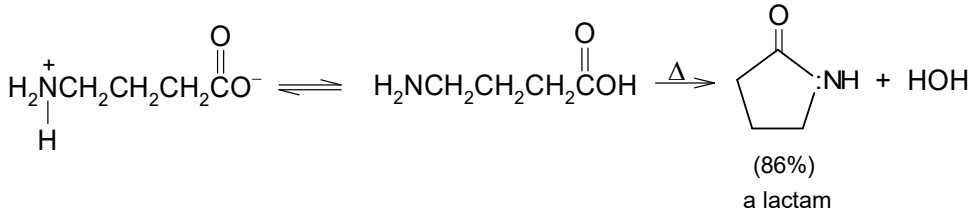
Ninhydrin test is given by amino acids containing primary amino group.

The blue-violet coloured compound formed is also known as *Ruhemann's purple.*

This is an important reaction used in the detection of small amounts of amino acids.

4. Formation of Lactones

Some amino acids undergo cyclisation to yield cyclic amides, called **lactams**. See Sec. 10.7, Unit 10 for nomenclature of lactams.



5. Formation of Peptides

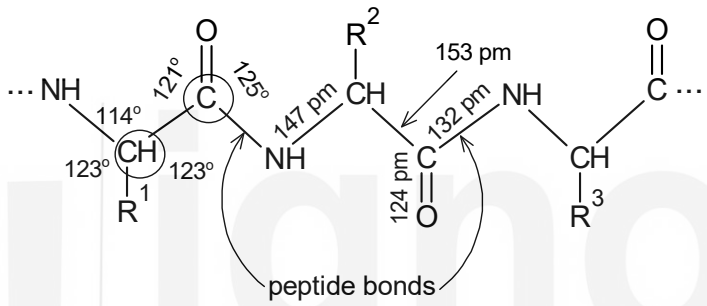
In addition to the above reactions, amino acids constitute the structural units of peptides and proteins about which you will study in the forthcoming sections.

But before studying the formation of peptides, you must be curious to know about the structure of peptides.

13.5 STRUCTURE OF PEPTIDES

Peptides are biologically important polymers in which 2-amino acids are joined by the amide linkages, formed by the reaction of the carboxy group of one amino acid with the amino group of another amino acid. These amide linkages are also called **peptide bonds.** The general structure of a peptide is shown below:

The detailed structure of peptides will be dealt in Unit 14.



General Peptide Structure

Peptides can be classified as **dipeptides, tripeptides** and **tetrapeptides,** depending on whether the number of amino acids is **two, three** or **four,** respectively. Peptides containing up to 50 amino acids are called **polypeptides.** *Bradykinin* is an important naturally occurring nonapeptide which is present in blood plasma and is involved in the regulation of blood pressure.

Remember that a three letter code to represent the amino acids was given in Table 13.1, Sec. 13.2 of this Unit.

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

bradykinin

13.6 SYNTHESIS OF PEPTIDES

The synthesis of peptides poses a challenge because even if you want to synthesise a dipeptide from the starting amino acids A and B, we get a mixture of following four dipeptides:

A-A, B-B, A-B and B-A

The amino acids in a peptide are also known as amino acid residues.

For example, the reaction of glycine and alanine will give the following four dipeptides:

Glycyl glycine , Alanyl alanine, Glycyl alanine and Alanyl glycine

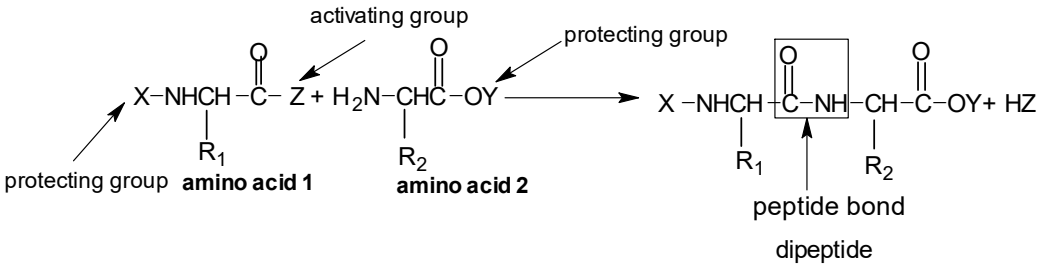
Here, the convention is to write the N- terminal amino acid on the left and the C- terminal amino acid on the right.

Therefore, for the selective synthesis of a particular peptide, we have to *protect*

i) the N-terminal of one amino acid and

ii) the C-terminal of the other amino acid, and then

iii) combine or react the two protected amino acids to give the *desired dipeptide* using the carboxy group activation .



The higher peptides can be synthesised by extending the chain of the dipeptide either from the N-terminus side or the C-terminus side. For that we have to deprotect and remove the protecting groups X or Y and repeat the above sequence using the third amino acid.

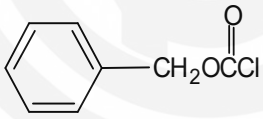
Let us now understand the protection and deprotection of amino- and carboxy- groups.

13.6.1 Protection and Deprotection of the Amino Group

The protection of amino group reduces its nucleophilicity. The following reagents and groups are used for the protection of the amino group:

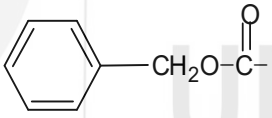
i) N-protection by Benzyloxycarbonyl and *tert*-Butoxycarbonyl groups

Reagent



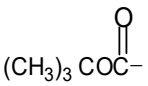
Phenylmethyl chloroformate
(Benzyloxycarbonyl chloride)

Protecting group



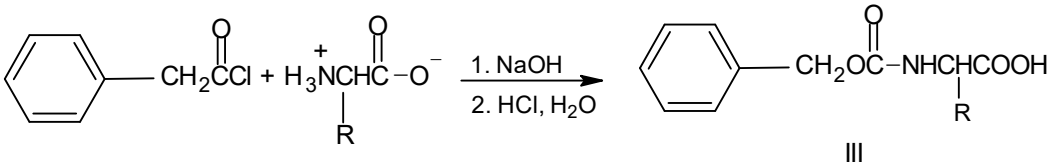
Benzyloxycarbonyl
(Cbz group or –Z group in older literature)

bis (1,1-dimethylethyl) dicarbonate
di-*tert*-butyl dicarbonate



tert-butoxycarbonyl
(Boc-group)

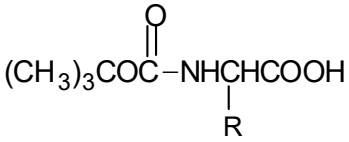
The protection of the amino group of an amino acid by benzyloxycarbonyl group can be represented as shown below:



III

(Z-protected amino acid)
(a carbamate)

Similarly, we can write for the protection by Boc group and get the Boc- protected amino acid.

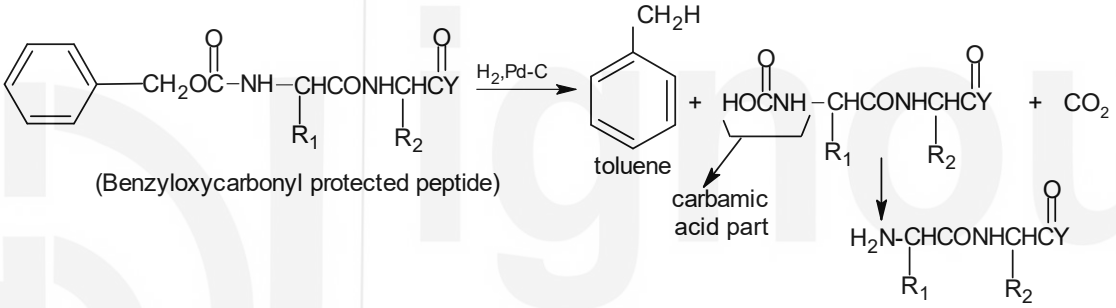


(Boc-protected amino acid)

When required, after the formation of the required dipeptide, the deprotection of the protected amino group can be carried out as follows:

1. By hydrogenolysis

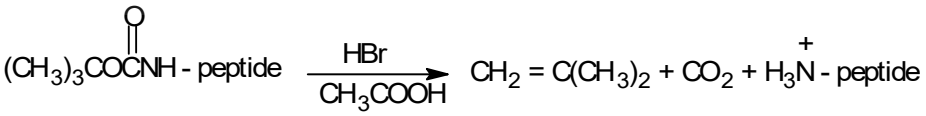
The Boc benzyloxycarbonyl group can be removed from the Cbz- protected amino acid by reaction with H_2 in the presence of a transition metal catalyst. The reaction gives toluene and the carbamic acid which on instantaneous decarboxylation gives the unprotected amino group in the peptide.



2. By treatment with acid in mild conditions

The Boc carbamate group is stable in dilute base but it can be removed in *mild acidic conditions* which do not affect the peptide bond. The deprotection of Boc- protected amino group can be done by treatment with HBr in CH_3COOH or HCl.

Thus,

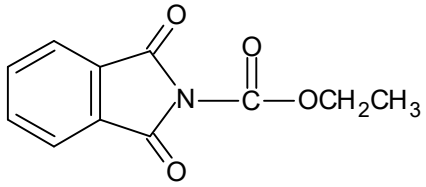


ii) N-protection by Phthaloyl (Phth) Group

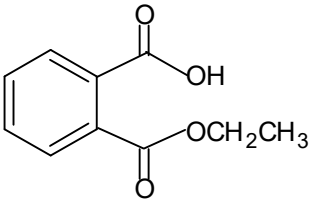
The reaction of 1, 2-dicarboxylic acid anhydrides with amino acids yields imides. Imides being stable to acids and hydrogenolysis, are good protecting groups as they can be used in variety of synthetic conditions. Phthalic acid derivatives can, thus, be used for N-protection.

Earlier, phthalic anhydride was used as phthaloylating agent which involved fusion with the amino acid. These conditions were harsh and caused racemisation. Even the use of solvents such as benzene, dioxane etc. could not eliminate the problem of racemisation.

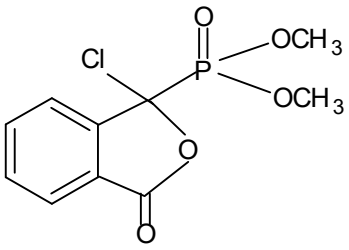
The protection of NH_2 group can be done by the following phthaloylating reagents:



N-(ethoxycarbonyl) phthalimide

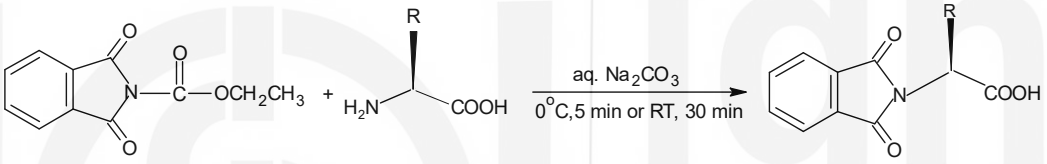


monoethylphthalate



3-chloro-3-(dimethoxyphosphoryl)isobenzofuran-1(3*H)*-one

The phthaloylation using the above reagents can be carried out under mild conditions and the racemisation does not occur under these conditions. This is shown below:



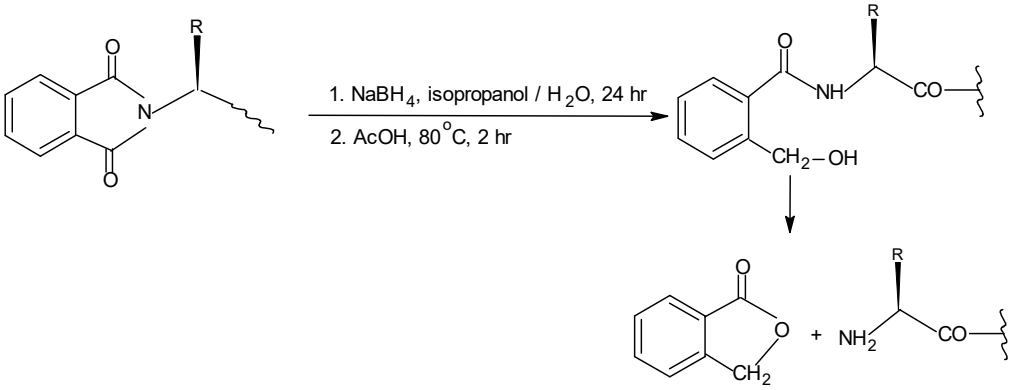
Deprotection

The phthaloyl group can be removed by the following methods:

i) **By hydrazinolysis**

It involves treatment with hydrazine hydrate under reflux conditions using methanol or ethanol as the solvent.

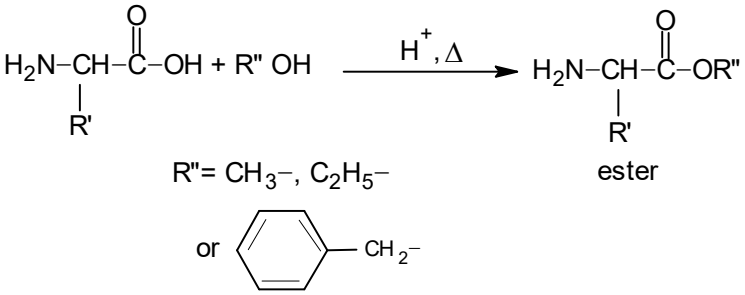
ii) **Reductive opening o**f the ring followed by acid- catalysed lactonisation of the formed hydroxyl compound.



13.6.2 Protection and Deprotection of the Carboxyl Group

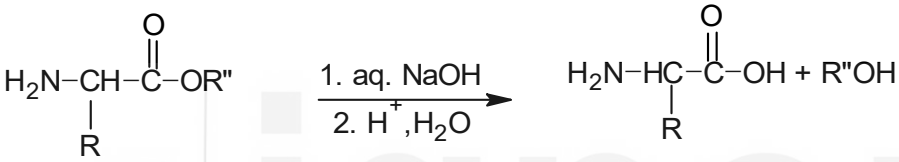
The carboxyl group of the amino acids can be *protected* by converting it to an ester. The methyl, ethyl or benzyl esters can be prepared.

Remember that methyl and ethyl esters can be conveniently prepared by Fischer esterification.



Deprotection: The ester group can be converted back to the carboxyl group by the following methods at appropriate stage of the peptide synthesis.

i) Hydrolysis using aqueous base



But this method is not preferred because base can cause racemisation.

ii) The benzyl esters can be also deprotected by the following methods:

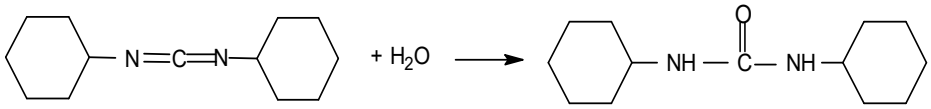
a) Hydrogenolysis with H_2 using Pt or Pd as the catalyst, or

b) Treatment with HBr in acidic conditions in acetic acid.

13.6.3 Peptide Bond formation using Carboxy Activating Groups

The most commonly used reagent for this purpose is dicyclohexylcarbodimide (DCC).

DCC is the anhydride of *N*, *N*-dicyclohexylurea (DCU). DCC on reaction with water gets converted to DCU

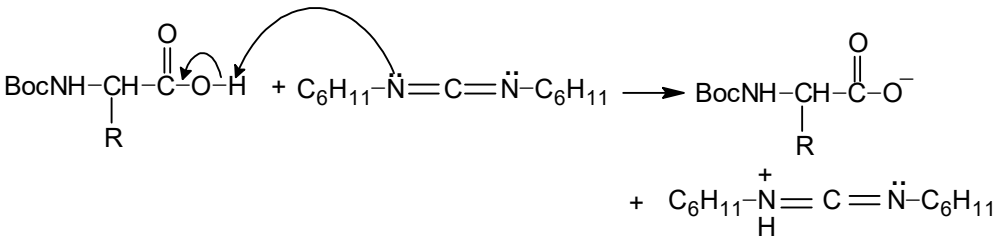


1, 3-Dicyclohexylcarbodiimide
DCC

N, *N'*-Dicyclohexylurea
DCU

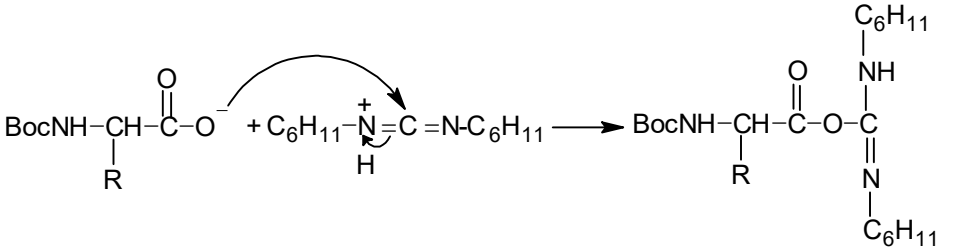
DCC activates the carbonyl group of the N-protected amino acid by taking away a proton in the first step.

Step 1



Step 2

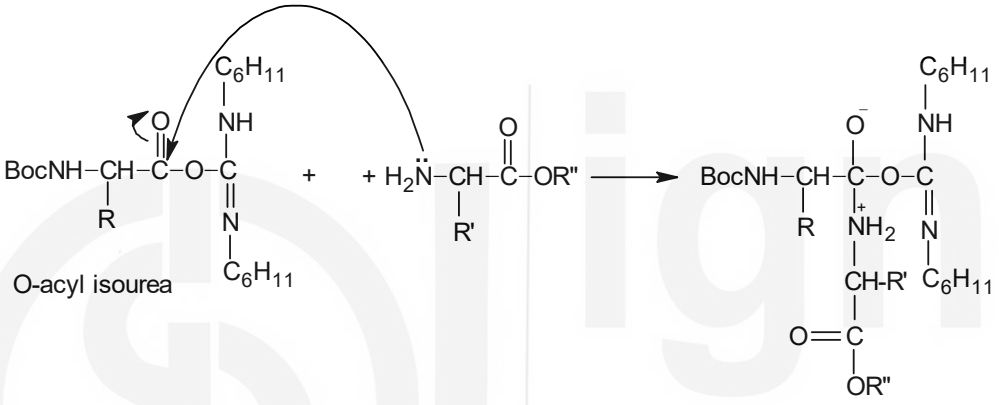
In the next step, the carboxylate ion attacks the carbon of the C=N bond to yield an *O-acyl isourea.*



O-acyl isourea

Step 3

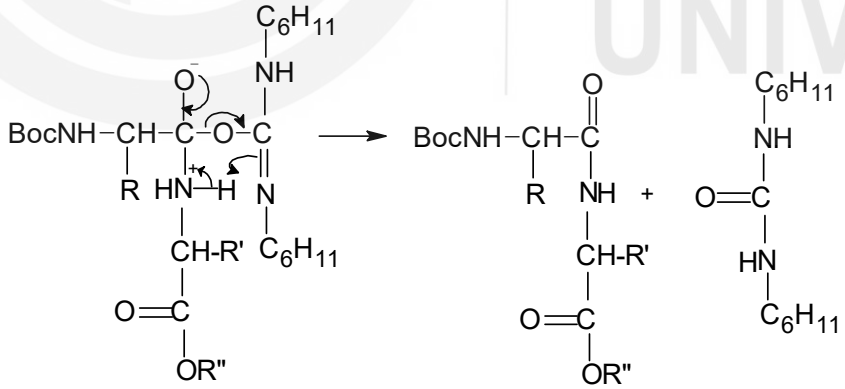
Nucleophilic addition of amino group of the carboxyl protected amino acid to the carbonyl group of O-acyl isourea.



O-acyl isourea tetrahedral intermediate

Step 4

Regeneration of carbonyl group and displacement of DCC as DCU.



tetrahedral intermediate dipeptide DCU

The tetrahedral intermediate breaks down to give the dipeptide and *N, N*'-dicyclohexylurea. Hence, we have synthesised a dipeptide which is having both the amino end and the carboxy end protected with their respective protective groups.

If we want of synthesise a tripeptide from this, we have to deprotect the amino group and react it with another N-protected amino acid and repeat the rest of the steps. Alternatively, to get a dipeptide from it, we can deprotect both the protecting groups present at the amino end and the carboxy end.

Having understood the above sequence of steps to be followed for the synthesis of a dipeptide, answer the following SAQ.

SAQ 4

Write the steps for the preparation of the peptide Gly-Ala.

SAQ 5

What could be the major problems associated with the above discussed method of synthesis of peptides?

SAQ 6

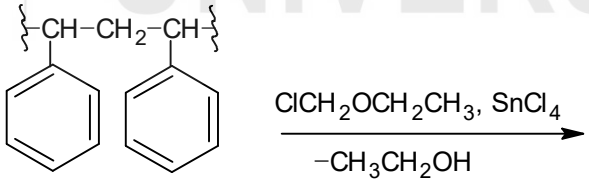
Write all the possible structures of the dipeptides formed by the amino acids glycine and valine.

13.7 MERRIFIELD SOLID PHASE SYNTHESIS

Robert Bruce Merrifield
(15th July, 1921- 14th May, 2006)

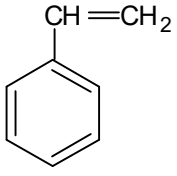
He was awarded Nobel Prize in Chemistry for the year 1984.

A major advancement in the syntheses of polypeptides was introduced by R. Brace Merrifield in 1962. This technique is also known as **polymer supported** synthesis and uses a polymer. Merrifield used a polystyrene in which about 5% of the phenyl groups carried a chloromethyl (-CH_2Cl) group in their *para* positions. This functionalisation of the phenyl ring can be done by Friedel -Crafts alkylation as given below:

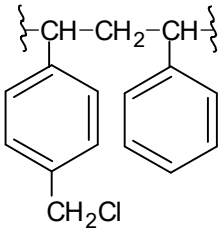


polystyrene

Polystyrene is a polymer of styrene which is ethenylbenzene.



styrene

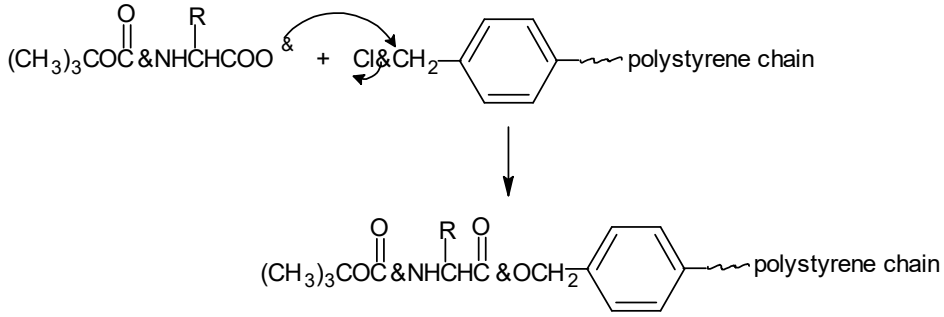


functionalised polystyrene

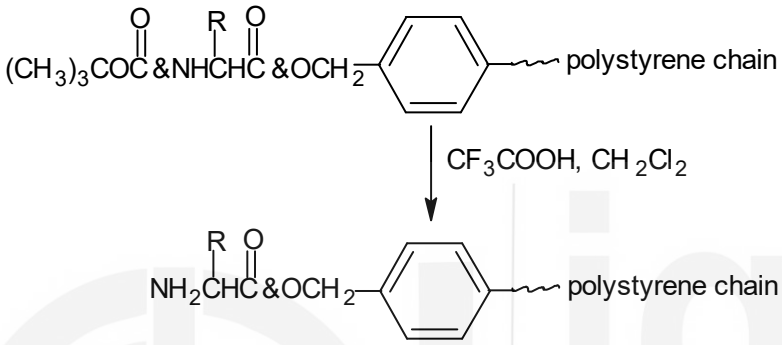
The chloromethyl groups are reactive towards nucleophilic substitution reactions.

The solid phase synthesis of a dipeptide involves the following steps:

Step 1: The amino protected amino acid is attached to the polystyrene chain.

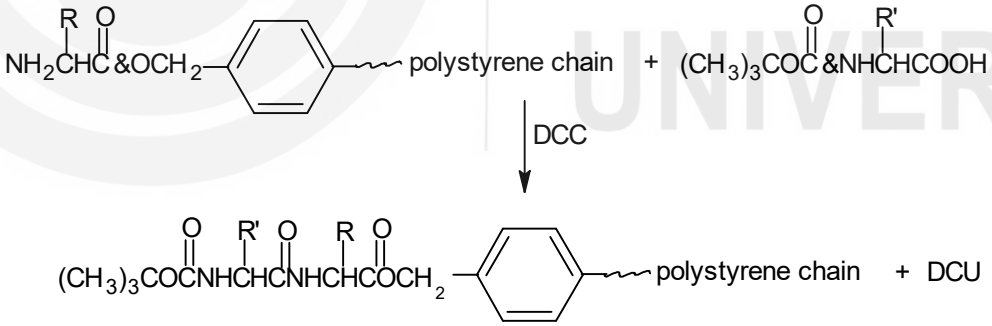


Step 2: The deprotection of the amino terminus is done by treatment with trifluoroacetic acid.



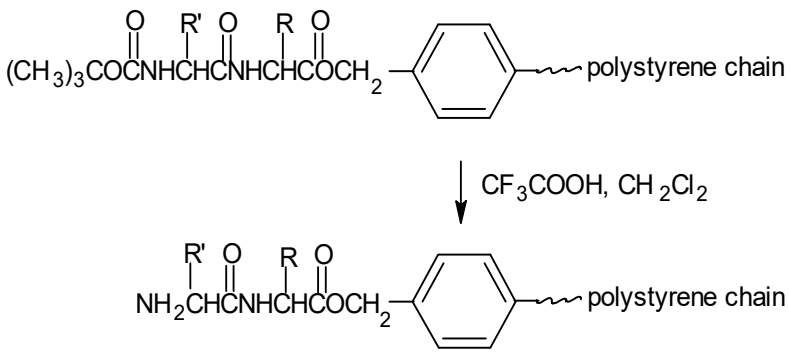
Step 3: Coupling with second N-protected amino acid is done using DCC.

The peptide (or amide) bond formation takes place in solution similar to the previous method discussed in last sub-section. Here, the difference is that the dipeptide formed is attached to the insoluble resin.

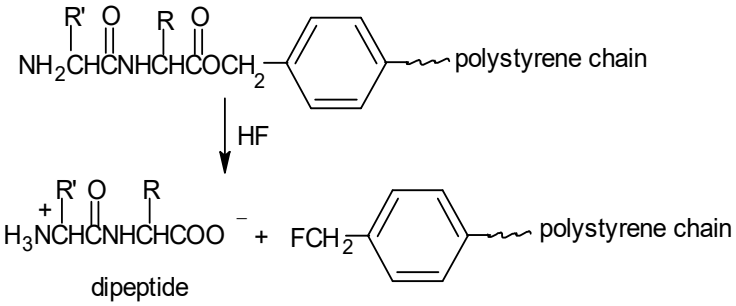


The DCU formed is removed by washing.

Step 4: Deprotection of the amino terminus of dipeptide



Step 5: If we want to stop at the dipeptide stage and do not wish to synthesise higher peptides, the benzyl ester bond is broken using HF to release the dipeptide from the polymer chain.



Alternatively, if we wish to synthesise higher peptides, then coupling with third N-protected amino acid is done using DCC .This is followed by deprotection of the amino protected group, as explained above in Steps 3 and 4, respectively. These steps, i.e. Steps 3 and 4, are repeated till we get the desired peptide; and then finally, Step 5 is performed to get the product.

You have now studied *two methods* of synthesis of peptides. At this stage, you may be curious to know which one is more advantageous. Obviously, the solid phase synthesis has the following advantages over the previous method.

The polymer beads carrying the peptide chain are insoluble in the solvents used in the synthesis. Hence, the product can be purified by simply filtering and washing to remove the excess reagent and the byproducts.

In 1969, the *enzyme ribonuclease* was synthesised by Merrifield using solid support. He designed a machine by which the steps required for the synthesis could be performed *automatically.* This made the synthesis of peptides much simpler and less time consuming.

The synthesis of ribonuclease required the addition of 124 N-protected amino-acids and coupling reactions and 11,931 operations. The automated machine made it possible in much shorter time and the intermediate stages of isolation were also not required.

Similarly, synthesis of insulin involving 51 amino acids in two separate chains comprising more than 5000 operations was achieved only in few days using the automated procedure.

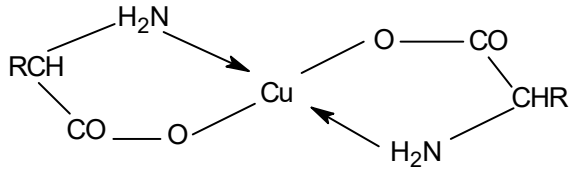
Automation in the protein synthesis has helped in the confirmation of the structure of polypeptides obtained by degradation of chains. Also, new proteins and polypeptides can be easily syntheised which are useful and have better activity as compared to the ones available in nature. These new synthetic options find applications in the field of medicine and biology.

13.8 LAB DETECTION OF AMINO ACIDS

The amino acids can be tested in the laboratory by the following methods.

1. Complexation with Cu^{2+} Ions

Aqueous solution of amino acids on addition of copper sulphate solution gives a deep blue colour. The complex formed has the following structure:



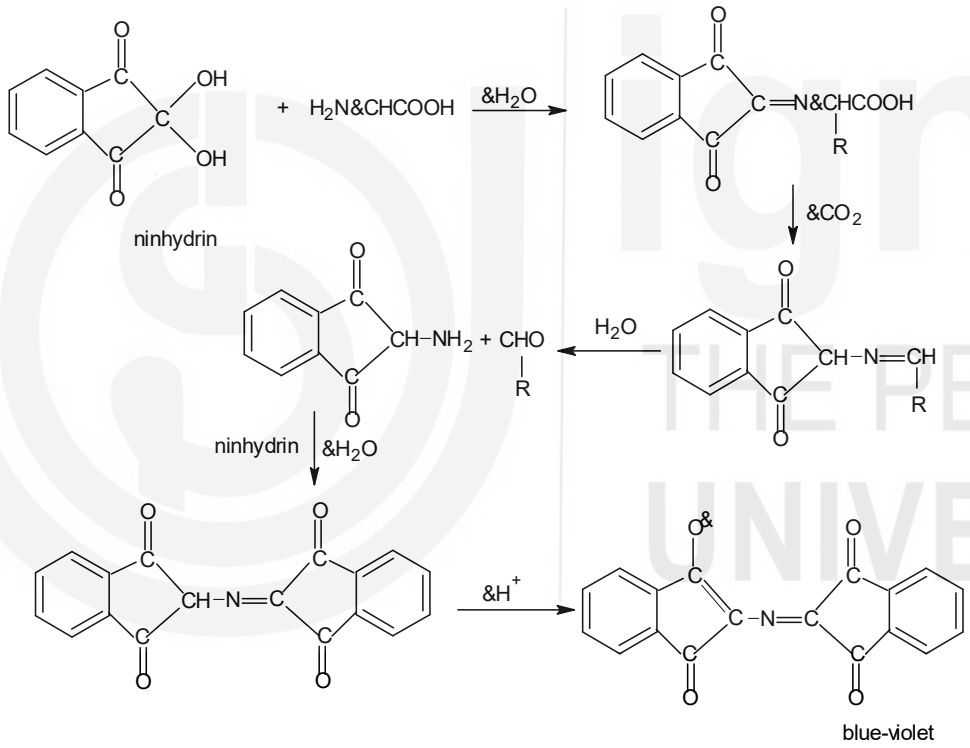
2. Ninhydrin Test

In Sec. 13.4 above, you have read about the reaction of amino acids with ninhydrin.

Ninhydrin was accidentally discovered by Siegfried Ruhemann in 1910. He was an German- English chemist .The reaction of amino acids and ninhydrin was observed by him in the same year.

Ninhydrin is a white solid soluble in water, ethanol, and acetone etc. It reacts with ammonia, primary and secondary amines, and peptides to form a blue-purple coloured compound which is also known as **Ruhemann's purple**.

The mechanism of the formation of the blue colour is given below:



Siegfried Ruhemann
4th January,1859– September, 1943)

This is a very sensitive test and is also given by some *β*-amino acids (3- amino acids) and some peptides as well as proteins, especially on warming.

Ninhydrin is also used as a reagent for the detection of latent fingerprints.

13.9 SUMMARY

In this Unit, you have studied that

- 20 amino acids occurring in proteins are L amino acids.
- The convention to use a three letter code, as an abbreviation, for each amino acid. The single letter code are also used to denote the amino acids.
- Amino acids exist as inner salts called *zwitterions.*
- At isoelectric point, pH_i , the amino acid is stationary in an electric field, i.e., it migrates neither to the negative pole nor to the positive pole because the charges on it are balanced.

- 2- Amino acids can be synthesised from potassium 1,2-benzenedicarboxylic imide, aldehydes (Strecker synthesis) and 2-halo carboxylic acids.
- The reactions of amino acids include the usual reactions of the carboxy and the amino group. For example, they undergo esterification (characteristic of the carboxy group) and alkanoylation (characteristic of the amino group) reactions.
- Amino acids give a blue-violet color with ninhydrin.
- In peptides, 2-amino acids are joined by the amide linkages which are formed by the reaction of the carboxy group of one amino acid with the amino group of another amino acid. These amide bonds are called peptide bonds.
- For the selective synthesis of a particular peptide, we have to *protect* i) the N-terminal of one amino acid and ii) the C-terminal of the other amino acid, and then iii) combine or react the two protected amino acids to give the *desired dipeptide* using the carboxy group activation.
- Various protecting groups such as benzyloxycarbonyl, *tert*-butoxycarbonyl and phthaloyl groups are used for protecting the amino group of the amino acids.
- The polymer supported solid phase synthesis is advantageous because the product can be purified by simply filtering and washing to remove the excess reagent and the byproducts.
- Amino acids can be detected in the laboratory by complexation with Cu^{2+} ions and ninhydrin test.

13.10 TERMINAL QUESTIONS

1. Which properties of amino acids indicate their zwitterionic nature?
2. Write the structures of leucine and isoleucine.
3. Name the amino acids which contain a basic group in the side chain.
4. Outline the Strecker synthesis of tyrosine.
5. Which protecting groups can be used for the protection of amino group of the amino acid?
6. How can benzyloxycarbonyl group be removed from the N-protected amino acid/peptide?

13.11 ANSWERS

Self-Assessment Questions

1. i) Methionine or cysteine

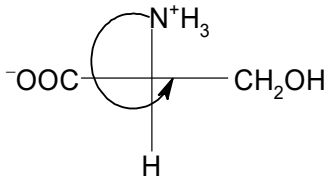
   ii) Phenylalanine or Tyrosine or Tryptophan

   iii) Glutamic acid or Aspartic acid

2. a) The order of priority of substituents is

$-\overset{+}{N}H_3 > -COO^- > -CH_2OH > -H$

The given Fischer projection can be converted to another Fischer projection given below by interchanging twice two substituents.



Overlooking H and moving from the substituents of highest priority to lower priority, the direction is anticlockwise, so the configuration is *S*.

b) Similarly, we can arrive at the configuration by following the steps given in 2 a) above and get the configuration as *R*.

3. $ClCH_2CH_2SCH_3$

4. $(CH_3)_3COCNHCH_2COOH$ (Boc–Gly) + $H_2NCH(CH_3)COCH_2C_6H_5$ (Ala–$OCH_2C_6H_5$) $\xrightarrow{DCC}$ $(CH_3)_3COCNHCH_2CNHCH(CH_3)COCH_2C_6H_5$ (Boc–Gly–Ala–$OCH_2C_6H_5$)

$\downarrow$ 1. H^+, H_2O
$\downarrow$ 2. H_2, Pd–C

$H_3\overset{+}{N}CH_2CNHCH(CH_3)COO^-$ (Gly–Ala) + $C_6H_5-CH_3$ + CO_2 + $CH_2=C(CH_3)_2$

5. It involves a large number of steps such as protection, activation, coupling and deprotection. This is time consuming and involves purification at each step. The overall yield obtained is also low.

6. i) $H_2NCH_2CONHCH(C(CH_3)_2)COOH$ ii) $H_2NCH(C(CH_3)_2)CONHCH_2COOH$

iii) $H_2NCH_2CONHCH_2COOH$ iv) $H_2NCH(C(CH_3)_2)CONHCH(C(CH_3)_2)COOH$

Terminal Questions

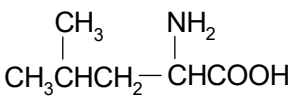
1. i) They form strong crystal lattices like ionic compounds.

ii) They decompose and not melt on heating.

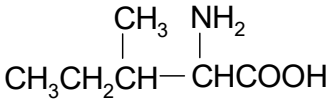
iii) They are more soluble in water as compared to non-polar solvents.

iv) They have large dipole moments.

2. Structures of leucine and isoleucine are given below.

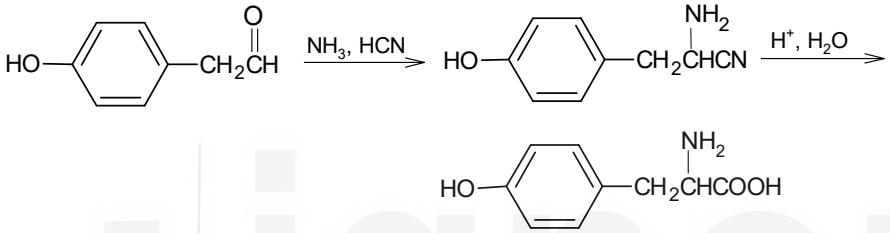


leucine



isoleucine

3. Lysine, arginine and histidine

4. 

tyrosine

5. Benzyloxycarbonyl, *tert*-butoxycarbonyl and phthaloyl groups are some such protecting groups.

6. By hydrogenolysis i.e. reaction with H_2 in the presence of a transition metal catalyst.

UNIT 14

STRUCTURE OF PEPETIDES AND PROTEINS

Structure

14.1 INTRODUCTION

In the previous unit, i.e. Unit 13, you studied about the chemistry of amino acids as well as the structure and synthesis of peptides. We will further elaborate on the structure of peptides and proteins in this unit.

Proteins are large polypeptides containing from about 50 to more than 8,000 amino acids per molecule. They have diverse biological functions. Proteins catalyse and regulate various reactions occurring in our body as enzymes and hormones. As skin and hair, they give outer covering to our body, and as muscles they help in movement. In the form of antibodies, they protect us from various diseases. The oxygen present in the air we breathe, is transported by the protein *hemoglobin***.** The nucleoproteins in the genes supply and transmit the genetic information in cell division. In addition, proteins also provide structural support in combination with other substances. After having an idea about the importance of proteins, you must be curious to know about the structure of peptides and proteins.

We will begin this unit with a discussion on the overview of structure of peptides and proteins and explain different levels at which their structure is understood. We will then elaborate on the details of the primary structure.

Here, we will keep our focus on the determination on the primary structure and explain the methods used for this purpose.

Expected Learning Outcomes

After studying this unit and having the experiments performed, you should be able to:

- explain the primary, secondary, tertiary and quaternary structures of peptides and proteins;
- explain the primary, secondary, tertiary and quaternary structures of peptides and proteins;
- highlight the important features of α-helix and β-pleated sheet arrangements of amino acids in a polypeptide;
- describe the methods used for the determination of the primary structure of peptides and proteins; and
- arrive at the primary structure of simple peptides from the data obtained by their hydrolysis and end group analysis.

14.2 OVERVIEW OF PRIMARY, SECONDARY, TERTIARY AND QUATERNARY STRUCTURES OF PROTEINS

You are now aware about the importance of peptides and proteins. In this section, you will study about what we broadly mean about their structure.

Since peptides and proteins contain a number of amino acids linked together, the first step in the determination of their structure requires a knowledge about which amino acids are present and how they are linked together as well as any *disulphide links* present in them. The order in which the amino acids are joined is called the **primary structure.**

The terms α and β refer to two characteristic X-ray diffraction patterns. The α-type pattern was associated with right handed helix and β-type with the pleated sheet.

In a right handed helix, the chain turns in clockwise direction.

Let us now study their secondary structure, i.e., the spatial arrangement of the peptide chains.The description of the conformational relationship of the nearest amino acids of a peptide is called its **secondary structure**. Two conformational arrangements called ***α*-helix** and ***β*-pleated sheet** are particularly stable. The *α-helix* conformation is shown in Fig. 14.1.

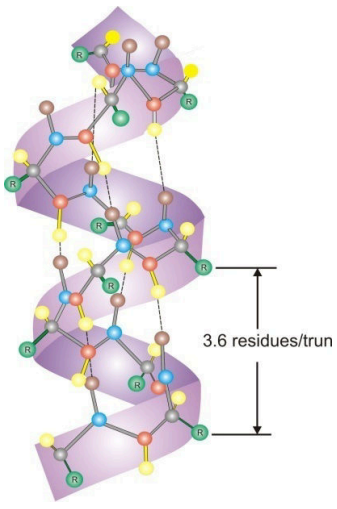


Fig. 14.1: α-helix.

In this the polypeptide chain forms a right handed coil having 3.6 amino acids per turn. This allows hydrogen bonding between each carbonyl oxygen and an amide hydrogen which stabilises the conformation. α-Helix is important in structural proteins such as α-keratins which constitute proteins of skin, nails, hair and feathers.

The β-pleated sheet is shown in Fig.14.2 a). The adjacent chains in the β-pleated structure may be aligned either parallel of anti-parallel to each other as shown inFig.14.2 b).

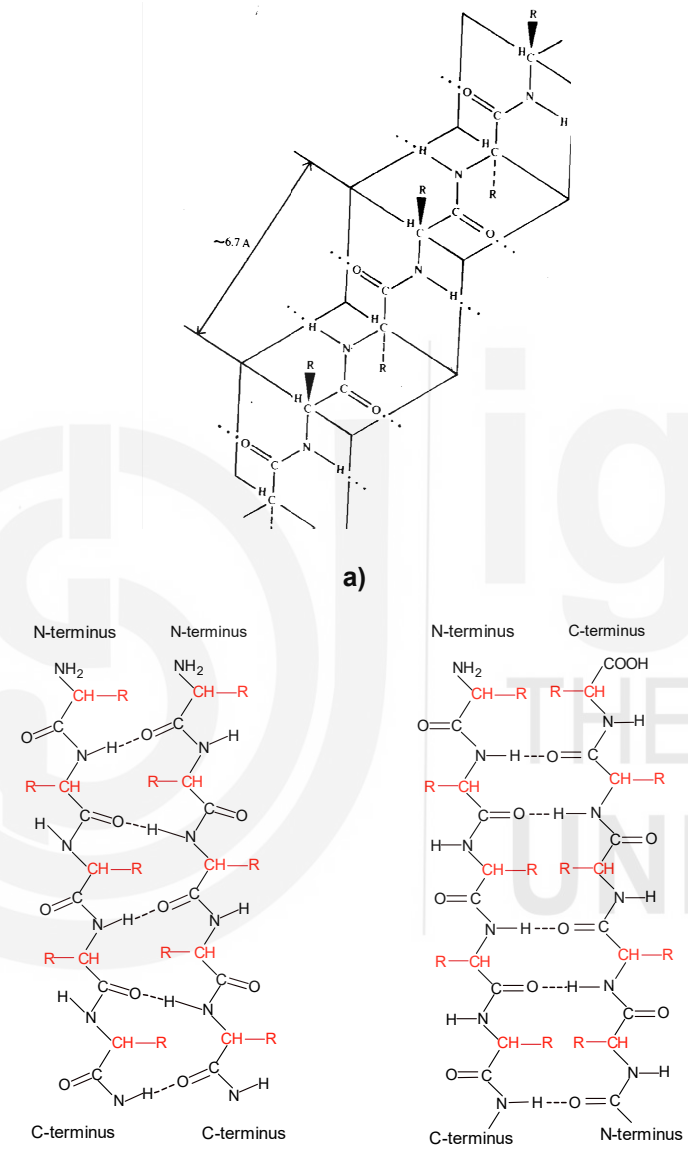


Fig. 14.2: a) The β-pleated sheet in a polypeptide and b) parallel and anti-parallel arrangement ofβ-pleated sheets

Note that the hydrogen bonds are formed between the carbonyl oxygen and the amide hydrogen of *two chains* in β-pleated sheet. You can see that the side chains alternate above and below the planes of the pleated sheets.

In *β*-pleated sheet structure, there are two possibilities: *parallel β*-pleated sheets or *antiparallel β*-pleated sheets. In *parallel β* pleated sheet arrangement, polypeptide chains run in the same direction (i.e. from N- to C-terminus) whereas in *antiparallel β*-pleated sheet, neighbouring chains

extend in opposite directions, see Fig.14.2 b). The hydrogen bonding present in the two types of arrangements is different which makes antiparallel arrangement more stable than the parallel one.

Tertiary structure refers to further folding of the peptide chain leading to its three-dimensional shape. Folding affects the physical and biological properties. Tertiary structure depends upon a variety of factors such as hydrogen bonding, van der Waals forces and electrostatic forces. A protein adopts the tertiary structure in such a way that favourable interactions are maximised and the unfavourable ones are minimised.

The side chains of some amino acids are *hydrophilic* and in others, these are *hydrophobic*. In the body, in an *aqueous solution,* the hydrophilic side chains orient towards the water while the hydrophobic ones turn away from the water (towards inside of the protein). These interactions create the looping and folding of the protein.

The tertiary structure of a protein is important in the sense that it defines an **active** site wherein a substrate can bind (fit) as lock and key. This allows the specificity of enzyme action. The tertiary structure of **fibrous proteins** show a **super helix** in which several α-helices are coiled, see Fig. 14.3.

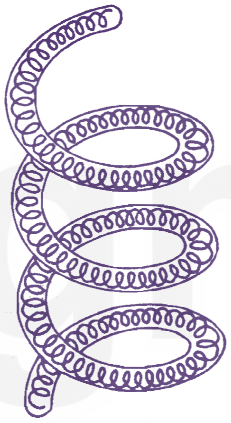


Fig. 14.3: A super helix: helix within a helix.

Pronounced folding is observed in **globular proteins** in which the tertiary structure allows them to be spherical. The globular proteins perform a crucial role in chemical transport.

Hemoglobin is present in the red blood cells and carries oxygen throughout our body.

The tertiary structure of a single polypeptide chain present in hemoglobin is illustrated below in Fig.14.4.

Fig.14.4: Tertiary structure of a single polypeptide chain of hemoglobin.

In sickle cell anemia, a mutation results in the change in one amino acid in the primary structure which leads to changes in the secondary, tertiary and quaternary structures of hemoglobin.

All proteins do not have a quaternary structure. Some proteins such as hemoglobin have a **quaternary structure** in which *two* or *more* peptide chains combine to form an assembly. The manner in which these subunits are organised is referred to as the quaternary structure of the protein. Hemoglobin, contains four polypeptide chains where two chains are alpha and two chains are beta chains. The quaternary structure of polypeptides present in hemoglobin is shown below in Fig. 14.5.

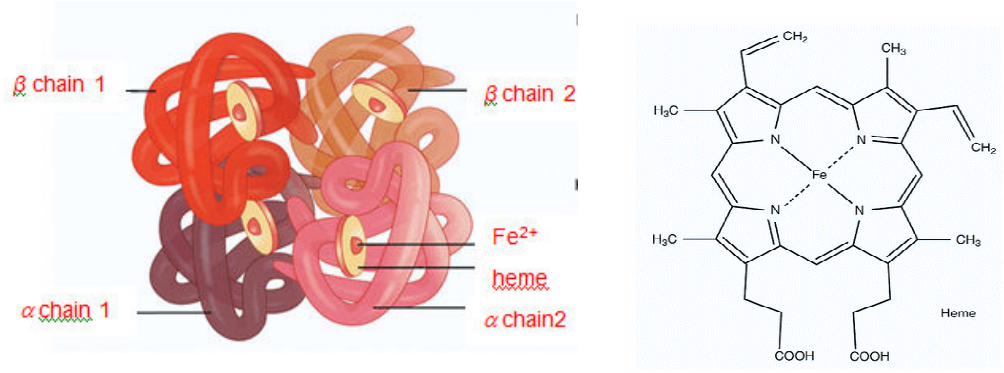


Fig.14.5: Quaternary structure of polypeptides present in hemoglobin.

Photo Credits:
https://commons.wikimedia.org/wiki/File:1904_Hemoglobin.jpg

SAQ 1

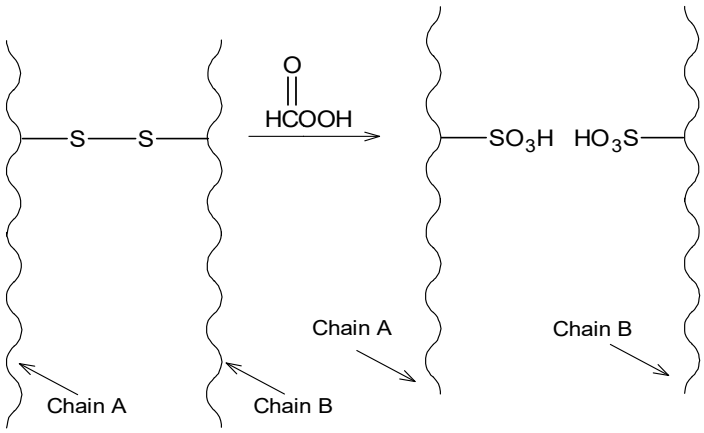
The artificial sweetener, aspartame is as partylphenylalanine methyl ester. Write its structure.

SAQ 2

What is meant by the primary structure of a peptide or a protein?

14.3 DETERMINATION OF PRIMARY STRUCTURE OF PEPTIDES AND PROTEINS

Determination of the primary structure involves oxidation of the disulphide bridges linking the chains in peptides or proteins to sulphonic acids using peroxymethanoic acid, as shown below:



Disulphide links hold peptide chains together in a protein molecule.

Proteins can be degraded into peptides by **partial hydrolysis** using either dilute hydrochloric acid or enzymes. Peptides on **complete hydrolysis** by heating with 6 N HCl for 24 hours yield a mixture of all the amino acids present in it. This mixture is then separated, taking advantage of the acid-base properties of amino acids, in an apparatus known as **amino acid analyser.**

The separation involves ion-exchange chromatography. This analysis, thus, provides information about the amino acids present and their relative amounts.

Frederick Sanger
(13th August 1918-19thNovember 2013)

He was awarded two Nobel prizes in Chemistry. One in the year 1958 and the other in 1980. He shared the second Nobel prize with Walter Gilbert (one-fourth to each), for their contributions related to the determination of base sequences in nucleic acids, and Paul Berg (one-half) for his work on recombinant DNA.

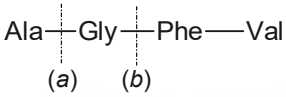
The *sequence of amino acids present in a peptide* chain can be determined either by analysing the products of ***partial hydrolysis*** or by ***end group analysis.*** Let us understand these methods with the help of examples.

14.3.1 Partial Hydrolysis

In a particular case, partial hydrolysis of a tetrapeptide containing Ala, Gly, Phe and Val yielded a tripeptide Gly-Phe-Val and a dipeptide Ala-Gly.

$$\text{Tetrapeptide} \xrightarrow{\text{hydrolysis}} \text{Gly—Phe—Val} + \text{Ala—Gly}$$

Since the dipeptide shows that Ala is linked to Gly, the amino acids in the tetrapeptide are linked in the following sequence:



In the above structure, cleavage at (*a*) gives the tripeptide and that at (*b*)gives the dipeptide.

14.3.2 End Group Analysis

Let us first understand what is an end group? By convention, peptide structures are written in such a way that the amino group is at the left and the carboxy group is at the right. Thus, the amino end is called the **N-terminus** and the carboxy end is called the **C-terminus,** the end groups being amino and carboxy groups.

We will now discuss how to identify the N-terminus and C-terminus of a peptide.

1. N-Terminal Identification by Degradation

The following two methods are used for this purpose:

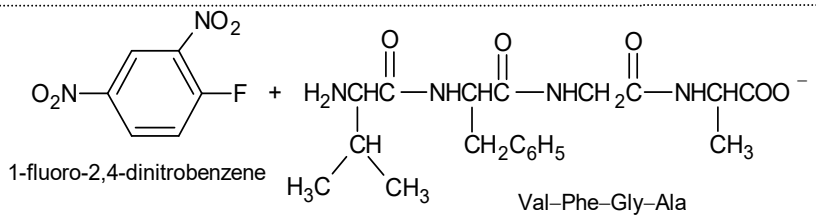
i) Sanger Method

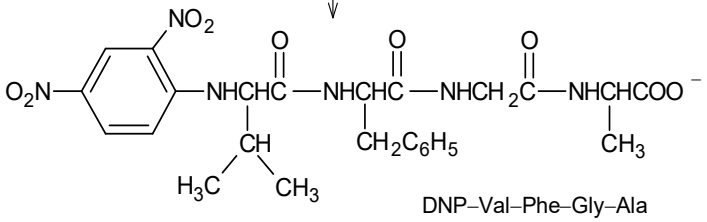
ii) Edman degradation

The amino groups of all the amino acids, except the N-terminal amino acid, are involved in the amide bond formation. Therefore, the amino group of the N-terminalamino acid is free and can react as a nucleophile.

The **Sanger method** of identifying N-terminal amino acid involves the reaction of the free amino group in the peptide with l-fluoro-2,4-dinitrobenzene to yield a peptide in which the N-terminal nitrogen is tagged with a 2,4-dinitrophenyl group. After complete hydrolysis of the peptide, the tagged amino acid is identified by chromatographic methods.

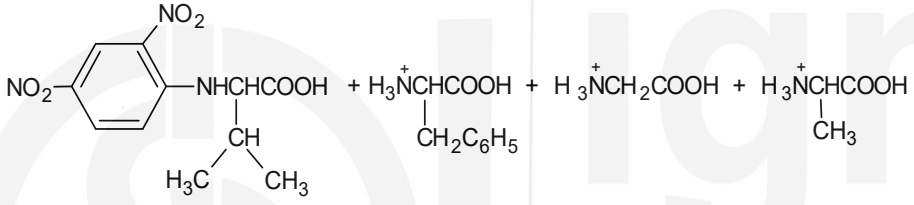
This procedure is illustrated below.





Acid hydrolysis cleaves the amide bonds of the 2,4-dinitrophenyl-labeled peptide, giving the 2,4-dinitrophenyl-labeled *N*-terminal amino acid and a mixture of unlabeled amino acids.

H^+, H_2O



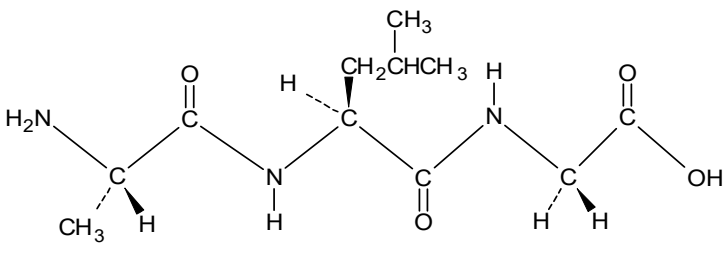
Sanger utilised this method in the determination of sequence of amino acids in insulin and was awarded Nobel Prize in 1958 for this pioneering work.

Insulin is a protein which acts as a hormone that regulates sugar content in blood.

The drawback of this method is that complete degradation is required after the polypeptide is once tagged.

A more useful method is by the **selective removal** of the tagged terminal amino acid and leaving the remaining chain intact so that it can be again tagged with the reagent. One such method which enables identification of one amino acid in the sequence at a time is the *Edman degradation*.

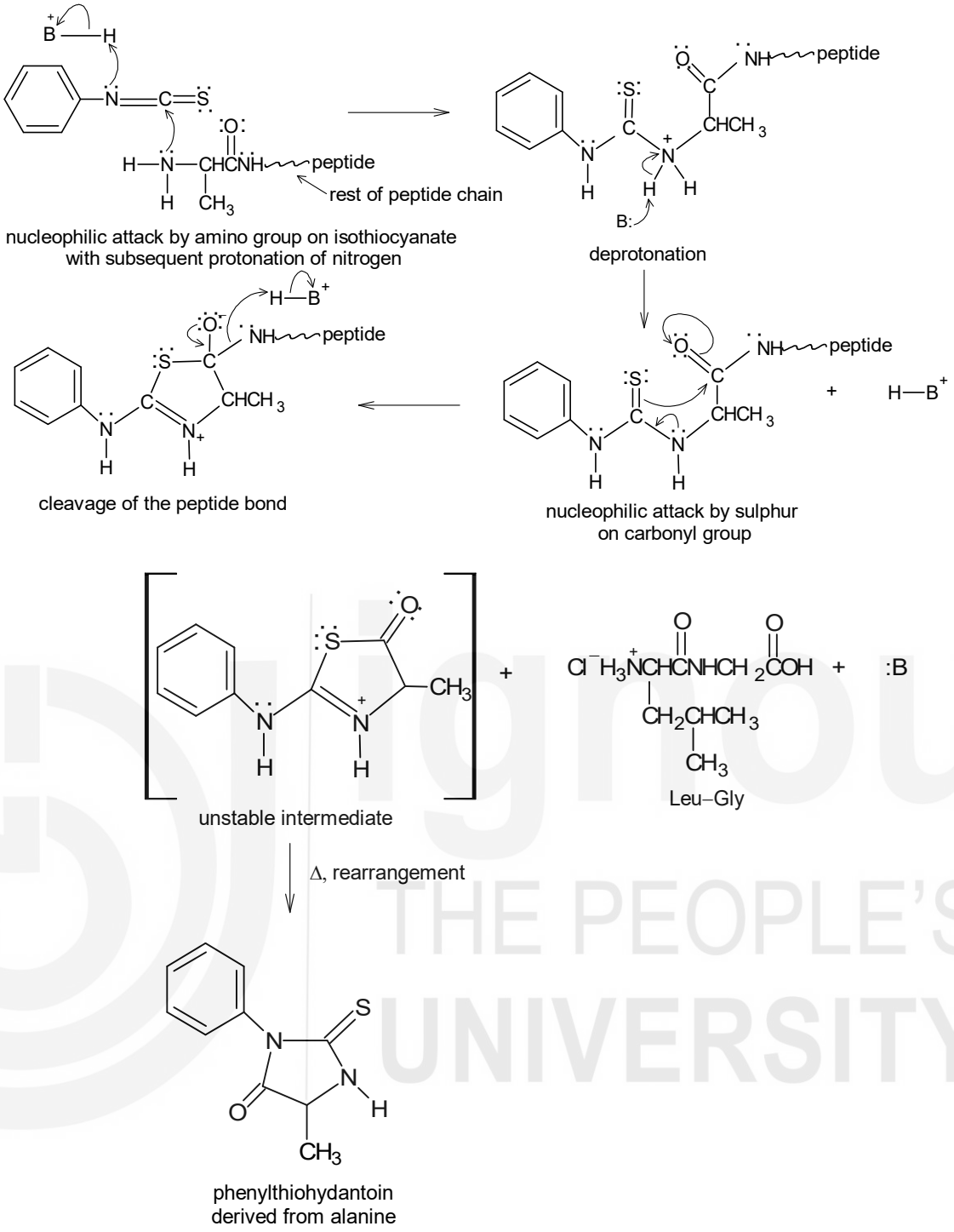
In **Edman degradation**, the terminal amino group adds to phenyl isothiocyanate, $C_6H_5N=C=S$ to yield a *thiourea derivative*. Treatment with mild acid gives the tagged amino acid as a *phenylthiohydantoin* and the remainder of the peptide chain. The tagged amino acid is identified and the remaining peptide is again subjected to Edman degradation. This sequence is repeated till all the amino acids in the peptide are identified. An example of Edman degradation using the peptide Ala-Leu-Gly is shown below:



Edman degradation of a peptide

Ala-Leu-Gly peptide

Pehr Victor Edman (14th April 1916- 19th March 1977)



2. C-Terminal Identification with Carboxypeptidase Enzymes

The amino acid present at the C terminus is identified by **enzymatic hydrolysis.** The enzymes which are used are called **peptidases** or **proteases.** Thus, **carboxypeptidases** sequentially cleave the C-terminus amino acids and the amino acids so cleaved are identified with time to know the sequence.

Certain peptidases which allow controlled hydrolysis by cleaving certain specific amide bonds, can also be used in sequence analysis. For example, *trypsin,* present in intestine, catalyses the hydrolysis of the peptide bonds involving carboxy groups of lysine or arginine. Similarly, *chymotrypsin* allows cleavage of the amide bonds of amino acids containing aromatic groups in their side chains, namely phenylalanine, tyrosine and tryptophan.

SAQ 3

Write the structure of the phenylhydantoin formed by valine when present in the dipeptide Val-Gly.

SAQ 4

A tripeptide contains the following sequence of amino acids:

Met-Leu-Phe.

Which reagent will you use for identification of amino acid present at its C-terminus?

14.4 SUMMARY

In this Unit, you have learnt that

- The order in which the amino acids are joined is *called the primary structure.*
- The description of the conformational relationship of the nearest amino acids of a peptide is called its *secondary structure*.
- Two conformational arrangements called *α-helix* and *β-pleated sheet are particularly stable.*
- *Tertiary structure* refers to further folding of the peptide chain leading to its three-dimensional shape. Folding affects the physical and biological properties.
- The manner in which *two* or *more* peptide chains combine to form an assembly is called the *quaternary structure of the protein*.
- Proteins can be degraded into peptides by *partial hydrolysis* using either dilute hydrochloric acid or enzymes.
- Peptides on *complete hydrolysis* by heating with 6 N HCl for 24 hours yield a mixture of all amino acids present.
- The sequence of amino acids present in a peptide chain can be determined either by analysing the products of *partial hydrolysis*or by *end group analysis*.
- The following methods are used for N-Terminal identification by degradation:
  - Sanger Method
  - Edman degradation
- The amino acid present at the C terminus is identified by *enzymatic hydrolysis.* The enzymes which are used are called *peptidases*or *proteases.*

14.5 TERMINAL QUESTIONS

1. A heptapeptide contained three units of alanine, two units of glycine and one unit each of serine and valine. It yielded the following mixture of tripeptides and dipeptides on partial hydrolysis:

   Ala-Gly-Ala, Ala-Val-Ser, Ser-Ala and Ala-Gly

   What is the primary structure of the heptapeptide?

2. Which type of structure governs the overall the three-dimensional folding of a polypeptide?

3. Why are globular proteins spherical in shape?

4. Why do some proteins have quaternary structure while the other do not have?

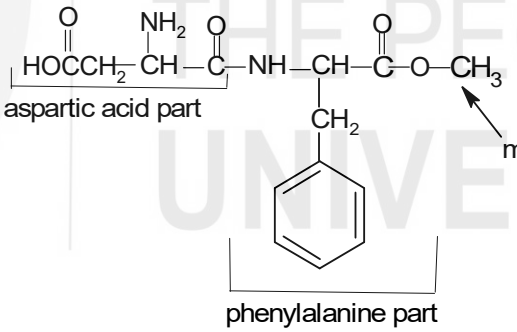
5. How is the degradation of a polypeptide by Sanger method different from the Edman degradation?

6. Illustrate different levels of structure of polypeptides and proteins using a diagram.

14.6 ANSWERS

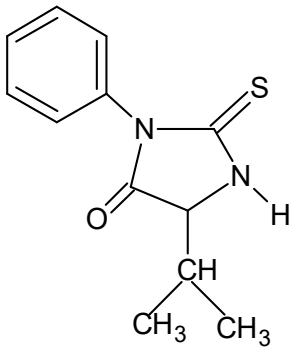
Self Assessment Questions

1. It is a dipeptide with the methyl ester. So the structure is



2. The sequence of amino acids present in a peptide or protein indicates its primary structure.

3. Phenylhydantoin formed by valine has the following structure:



4. Chymotrypsin

Terminal Questions

1. Ala-Gly-Ala-Val-Ser-Ala-Gly or Ala-Val-Ser-Ala-Gly-Ala-gly

2. Tertiary structure

3. Pronounced folding in globular proteins due to their tertiary structure make them spherical.

4. Because quaternary structure of a protein results only when two or more peptide chains are present in it.

5. In Sanger method, we can identify the amino acid present at N-terminal of the polypeptide whereas in Edman degradation, the sequence in which the amino acids are present can be identified.

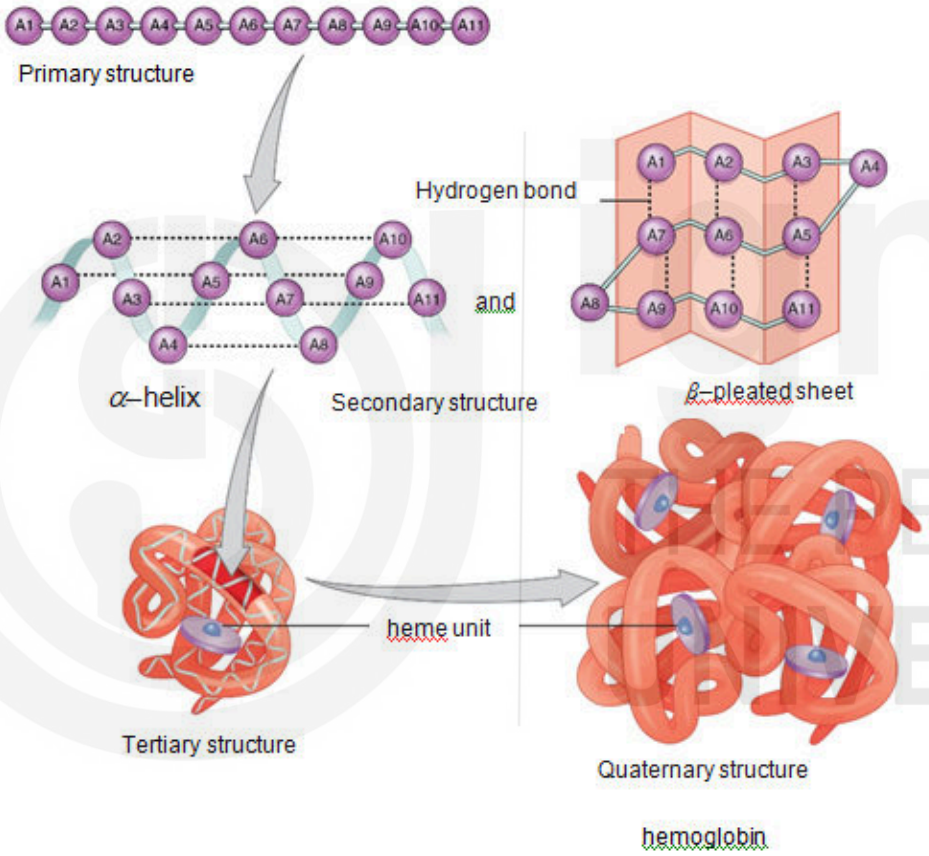
6. 

Photo Credit: https://commons.wikimedia.org/wiki/File:225_Peptide_Bond-01.jpg

UNIT 15

CARBOHYDRATES-I: MONOSACCHARIDES

Structure

15.1 INTRODUCTION

In the last two units of this Block, i.e. Units 13 and 14, you have studied about the chemistry of amino acids and structure of peptides and proteins.

In this unit and in the next unit, you will study about another such category of compounds viz. *carbohydrates.* **Carbohydrates**, **proteins**, **nucleic acids** and **fats** occur in almost all organisms and play an important and primary role in metabolic processes. These natural products are called **primary metabolites**.

We will begin this unit with a discussion on the importance and classification of carbohydrates which will give you an idea about their vast variety and diverse roles. Here, in this unit, we will keep our focus on *monosaccharides only* and discuss about *disaccharides* and *polysaccharides* in the next unit. The general properties including the reactions exhibited by monosaccharides will be explained. Then, we will describe how the structures of monosaccharides are represented. We will specifically elaborate on the structures and absolute configuration of the familiar monosaccharides-glucose and fructose.

Finally, the methods of ascending and descending of carbon chains in monosaccharides will be discussed.

Expected Learning Outcomes

After studying this unit and having the experiments performed, you should be able to:

- classify a carbohydrate as a monosaccharide, oligosaccharide (disaccharide, trisaccharide and so on) or a polysaccharide;
- give some examples of monosaccharides and classify them as an aldose or a ketose;
- explain some general reactions exhibited by monosaccharides;
- write the structures of various monosaccharides in different representations such as Fischer projections and Haworth projections;
- explain mutarotation; and
- discuss the methods of ascending and descending of carbon chains in monosaccharides.

15.2 CLASSIFICATION OF CARBOHYDRATES

Carbohydrates received their name because of their general formula $C_x(H_2O)_y$, according to which they appear to be **hydrates of carbon.**

Carbohydrates are wide spread in nature. In plants, they constitute up to80% of the dry weight. Carbohydrates occurring in plants include **cellulose** (which gives structural support to plants), **starch** (which serves as the reserved energy source) and **sugars** (like sucrose and glucose).

Sugars are crystalline substances having sweet taste and are soluble in water.

Glucose is an essential constituent of blood in higher animals and occurs in polymeric form as glycogen, in liver and in muscles. Carbohydrates also occur in adenosine triphosphate which is involved in biological energy storage and transport systems. They are also present in the nucleic acids which control the production of enzymes and the transfer of genetic information.

In nature, carbohydrates are synthesised by a process called **photosynthesis.** In this process, sunlight impinging on chlorophyll present in the green plants is absorbed and the photochemical energy, thus obtained is used to convert carbon dioxide and water into carbohydrates and oxygen. The overall process can be represented as follows:

$$x\,CO_2 + y\,H_2O \xrightarrow{\text{sunlight, chlorophyll}} C_x(H_2O)_y + x\,O_2$$

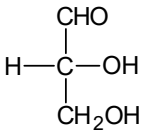
Carbohydrates are polyhydroxy aldehydes and ketones and substances which hydrolyse to polyhydroxy aldehydes and ketones.

The simplest carbohydrates are called **sugars** or ***saccharides*****,** (Latin: *Saccharum,* sugar). Carbohydrates can be classified as **monosaccharides, oligosaccharides** and **polysaccharides.**

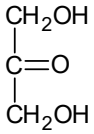
Let us now study about the monosaccharides in detail.

Monosaccharides

Monosaccharides are the simplest carbohydrates which cannot be hydrolysed into smaller and simpler carbohydrates. A monosaccharide may be further classified as an **aldose** or a **ketose**if it contains an *aldehyde*or a *keto*group, respectively. The simplest monosaccharides are 2,3-dihydroxypropanal, an aldose(glyceraldehyde)and1,3-dihydroxypropanone, a ketose. Their structures are as shown below:



2,3-dihydroxypropanal
(an aldose)



1,3-dihydroxypropanone
(a ketose)

Depending upon the number of carbon atoms in the chain, sugars are called **trioses** (3 carbons), **tetroses**(4 carbons), **pentoses**(5 carbons), **hexoses**(6 carbons) and so on. Therefore, 2, 3-dihydroxypropanal is an **aldotriose**and 1, 3-dihydroxypropanone is a **ketotriose.**

It was pointed out in Sec.11.3, Unit11 of Block 3 of BCHCT-131 Course that monosaccharides can be classified as D or L depending upon whether the position of the hydroxyl group on carbon next to primary alcoholic group is *right* or *left i*n the Fischer projection of the molecule projected vertically in such a way that the aldehyde function is on the top. The structures of aldoses belonging to D-family are given below in Fig.15.1.

Most of the naturally occurring sugars belong to D family.

Remember that (+) and (–) signs indicate the optical activity.

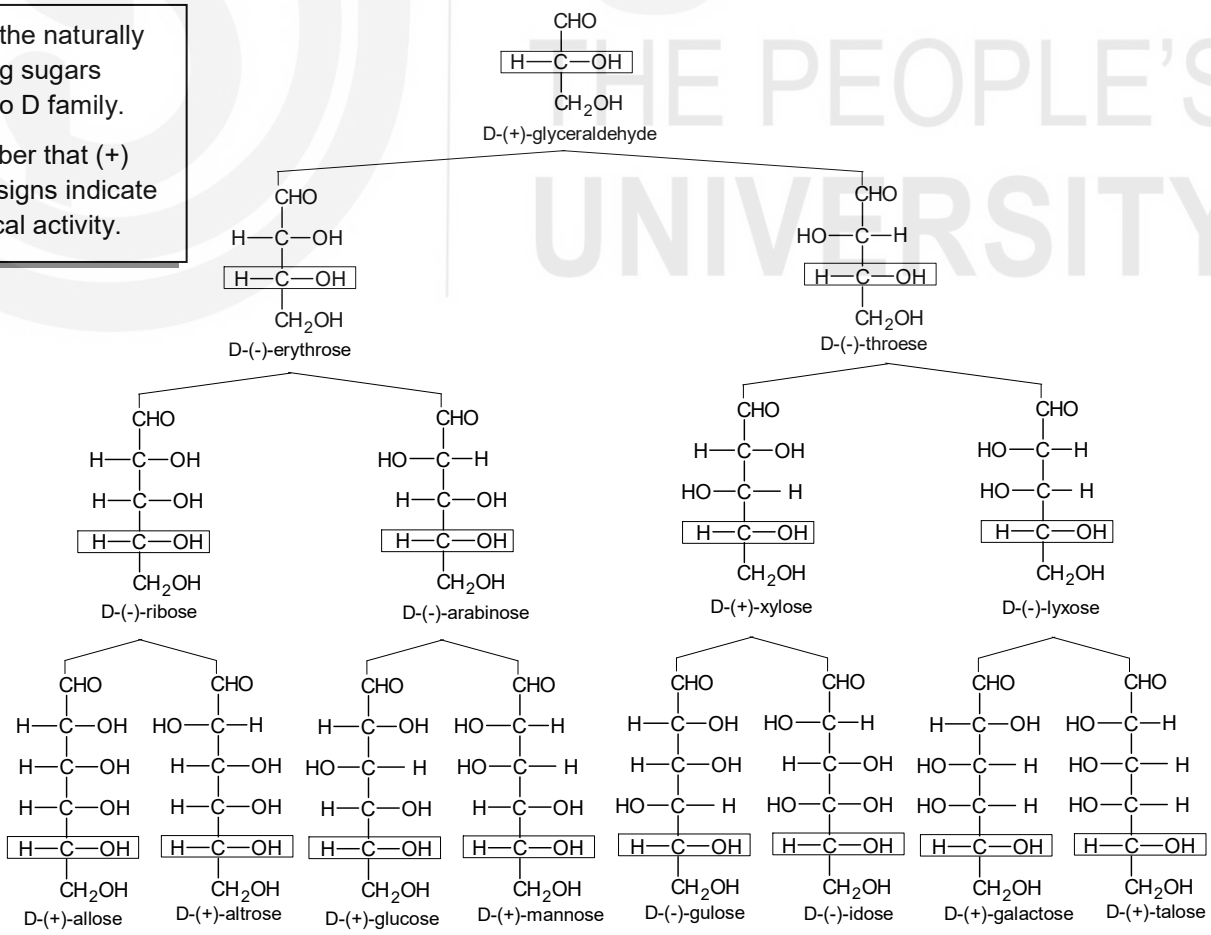


Fig.15.1: **The D-family Aldoses**

Each of the D sugars shown above has an enantiomeric L-counterpart.

As in the case of D-aldoses shown in Fig.15.1, the Fischer projections of monosaccharides belonging to D-ketose series are shown in Fig.15.2.

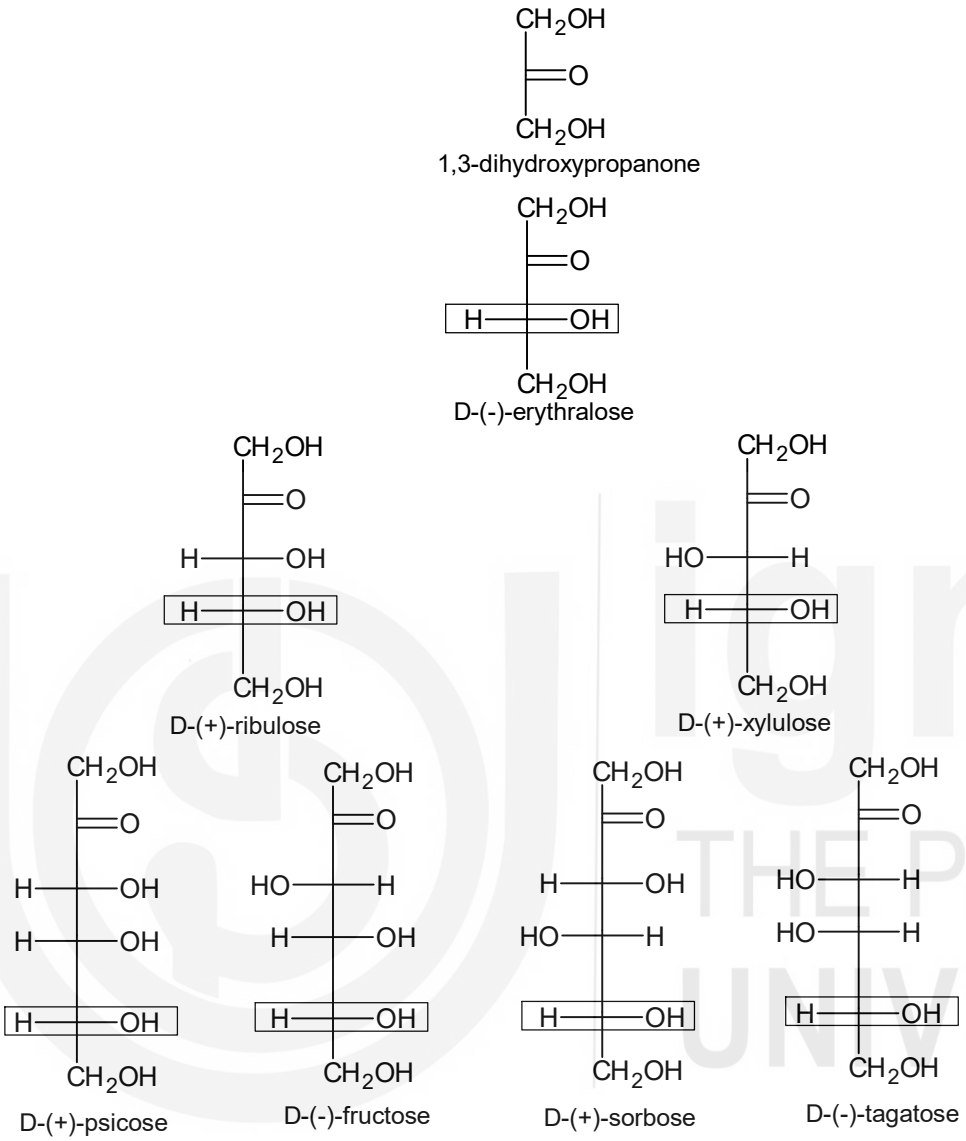


Fig.15.2: The D-ketoses

Having seen the large variety in the structures of monosaccharides, let us understand their general properties.

But before that answer the following SAQ to check your understanding.

SAQ 1

Fill in the blanks in the following with the words given below:

five, pentose, hexose, four, ketose, aldose

i) Ribose is a……..while fructose is a …….

ii) Xylulose is a ……having a …….carbon chain.

iii) D (-)-erythrose is a……having…….. carbon atoms in the chain.

15.3 GENERAL PROPERTIES

Tollens reagent is freshly prepared by first adding a few drops of dilute NaOH and then aqueous ammonia to aqueous silver nitrate solution. Initially, the brown solid silver oxide forms which dissolves on addition of aqueous ammonia to yield diammine silver (I) complex, $[Ag(NH_3)_2]^+$ in the clear solution.

Fehling's solution A is made by dissolving 69.28 g $CuSO_4.5H_2O$ in water and making solution to 1 litre volume.

Fehling's solution B is prepared by dissolving sodium potassium tartrate, $C_4H_4O_6NaK.4H_2O$, Rochelle salt (346g) and sodium hydroxide (120 g) in water and making the solution up to 1 litre volume.

Both Fehling's solution A and B are mixed in equal volume and used as **Fehling's solution.**

Benedict's reagent is a solution of copper sulphate pentahydrate, sodium hydroxide and tartaric acid.

Benedict's reagent contains copper sulphate (173 g), sodium carbonate (100 g) and sodium citrate (173 g) in 1000 ml of solution in water. Sodium carbonate keeps the solution alkaline while sodium citrate i.e. citrate ions, complex with Cu (II) ions and stop their conversion to Cu (I) ions on storage.

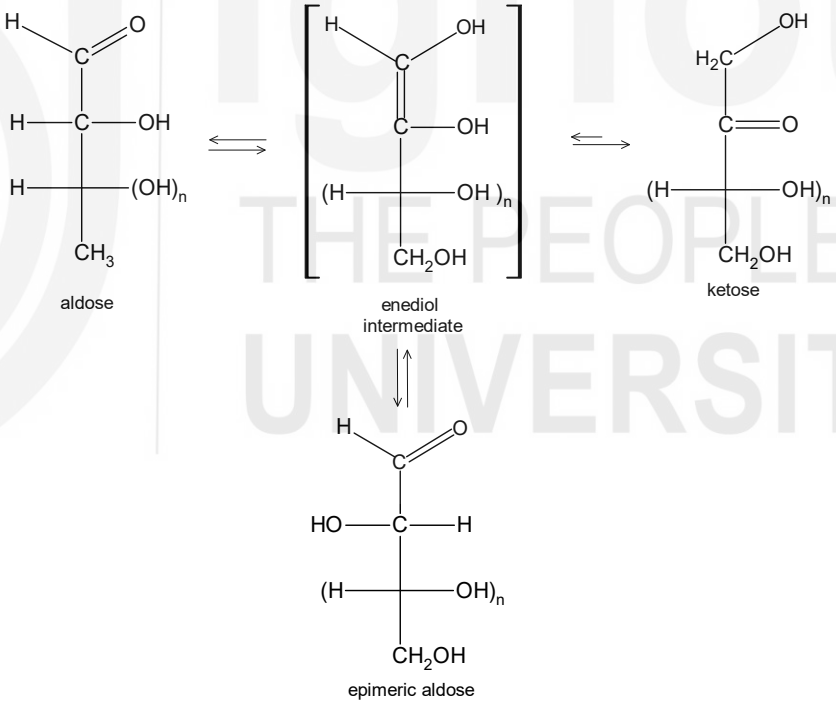
Monosaccharides are colourless crystalline solids which are soluble in water. The exhibit the following reactions:

Reactions of Monosaccharides

1. **With Tollens reagent-** containing $[Ag^+(NH_3)_2^-OH]$**.** Tollens reagent is reduced by aldoses and *α*-hydroxyketones. The corresponding monosaccharide gets oxidised to the aldonic acid. The silver formed is this reaction gets deposited on the walls of the test tube forming a *silver mirror*.

2. **With Fehling's Solution** (which contains a cupric tartrate complex in alkaline solution) and **Benedict's reagent** (which contains a cupric ion complexed with citrate ion).

   Aldoses and ketoses (*α*-hydroxyketones) reduce both Fehling's solution and Benedict's solution. Hence, the inital blue colour of the complex present in these solutions changes to brick red due to the formation of Cu_2O. In alkaline medium, aldoses undergo isomerisation reactions and equilibrium exists between an aldose, enediol form, ketose and isomeric (epimeric) aldose.



Carbohydrates which contain the hemiacetal group give positive Tollens and Benedict's tests and hence, these are called *reducing sugars.*

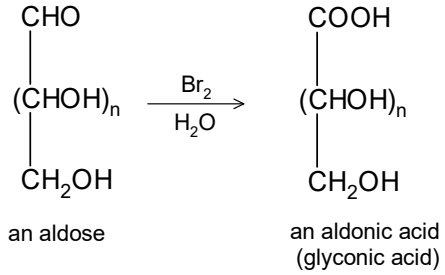
This is so because in the aqueous solution, there is an equilibrium between the hemiacetal and the open chain structure of the sugar. It is the open chain form which gets oxidised and the equilibrium keeps on shifting to give more of the open chain form.

However, formation of an *acetal* linkage does not allow such equilibrium and those sugar glycosides which contain *acetal* linkages instead of hemiacetals do not give positive tests with Tollens' reagent and Benedict's reagent are called *non-reducing sugars.*

You will study more about reducing and non-reducing nature of sugars when you study the structures of disaccharides in Unit 16.

3. Reaction with Bromine Water

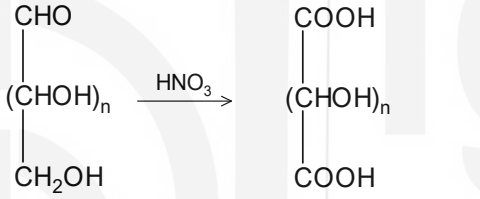
Since bromine water is mildly acidic with pH 6.0, no isomerisation takes place when a monosaccharide is oxidised with it to yield an *aldonic acid* in which only the -CHO group gets oxidised to -COOH group.



an aldose — an aldonic acid (glyconic acid)

4. Reaction with Nitric Acid

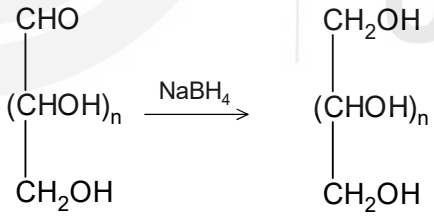
The reaction of an aldose with dil. nitric acid, which is a stronger oxidising agent than bromine water, oxidises both -CHO and -CH_2OH groups and yields an *aldaric acid* as the product.



an aldaric acid — a dicarboxylic acid

5. Reduction of Monosaccharides

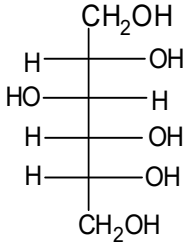
Both aldoses and ketoses can be reduced using sodium borohydride or H_2 and Pt catalytic reduction to the corresponding alcohols.



an aldose — an alditol

The *alditol* formed by D-glucose is called D-glucitol, commonly also known as D-sorbitol. Can you write its structure?

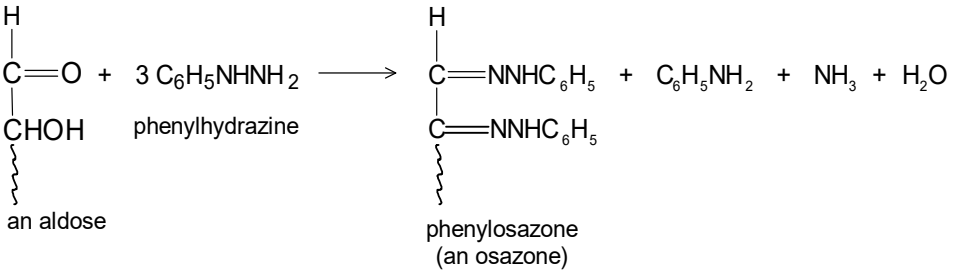
Yes, it is like this.



D-glucitol
(D-sorbitol)

6. Reaction with Phenylhydrazine

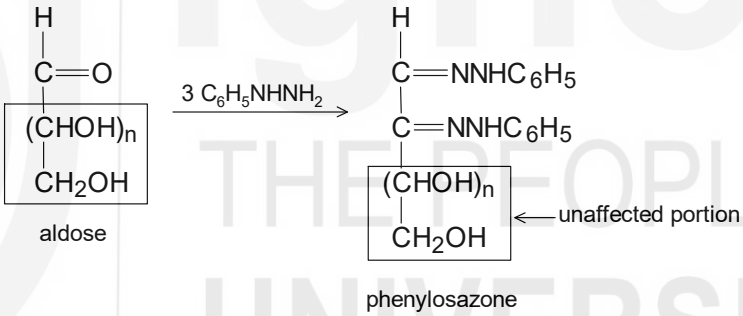
You have already studied in your second semester course, BCHCT-133, that aldehydes react with phenylhydrazine to give phenylhydrazones. Monosaccharides also react with *phenylhydrazine* (3 molar equivalents) to yield a *phenylosazone.*



Note that two pheylhydrazone groups are present at C-1 andC-2 in the phenylosazone. The third molecule of phenylhydrazine is used to oxidise the -CHOH group to C=O group and gets converted to aniline and ammonia which are present in the products.

Osazones being solids, could be easily isolated and purified. Their crystalline forms helped in their identification also.

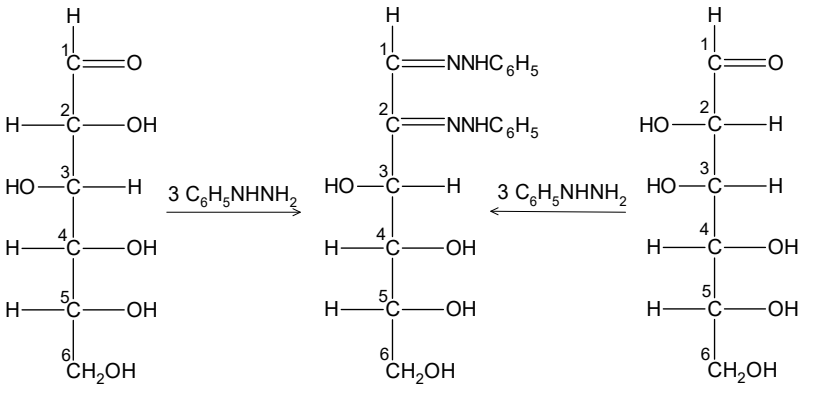
You can also see that in a phenylosazone,



C-2 carbon is no more a chiral centre. Hence, irrespective of the starting aldose having a different configuration at C-2, *same phenylosazone should result* provided the configuration at other carbon atoms is the same.

> The crystals of the osazones are very characteristic e.g. osazone of maltose forms sunflower shaped crystals while that of lactose forms has powder puff-shaped.
>
> The crystals of the osazones of galactose are rhombic-plate shaped while those of osazone of glucose are needles arranged in a broom stick

Thus, D-(+)-glucose and D-(+)-mannose which differ in configuration only at C-2 result in the same phenylosazone as shown below:



This reaction of D-(+)-glucose and D-(+)-mannose leading to the same phenylosazone was studied by Emil Fischer who then concluded that these two monosaccharides have same configuration at C-3, C-4 and C-5 carbon atoms; and they differ in their configuration at C-2 carbon atom only. Hence, D-(+)-glucose and D-(+)-mannose are *epimers*.

Many glycosides occur naturally e.g. anthocyanins, saponins, vanillin *etc.*

7. Formation of Glycosides

Monosaccharides may exist in open chain form as well as in cyclic pyranose or furanose form. The cyclic form arises from intramolecular addition of alcoholic group to the aldehyde or keto group of an aldose or a ketose.This has been discussed in detail later.

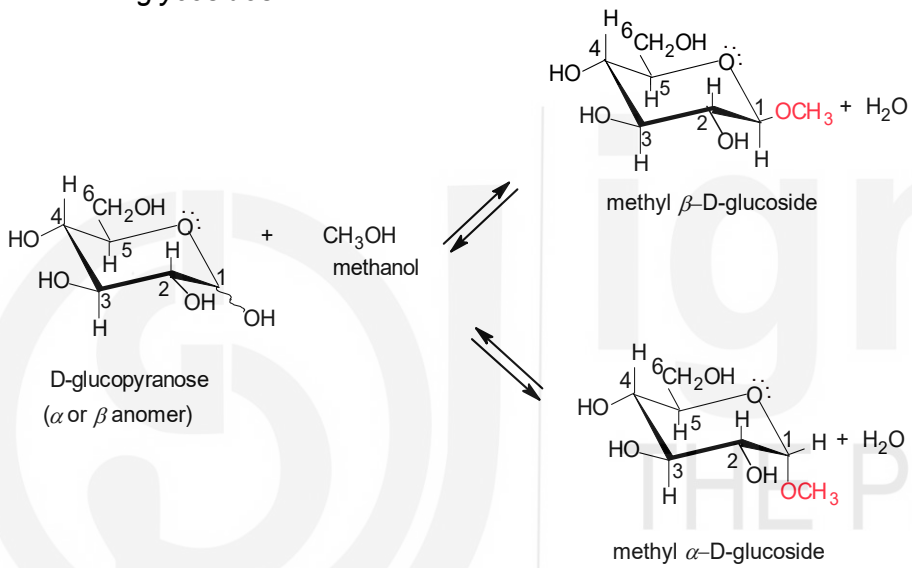
The name **pyranose** is derived from pyran, a six-membered cyclic ether.

pyran

Similarly, **furanose** is derived from furan which is a five-membered cyclic ether.

furan

You know that an anomeric -OH group is present in the cyclic form of monosaccharides. This can react with alcohols and form derivatives which are called *glycosides*.

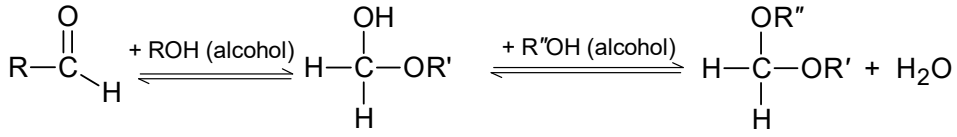


As shownhere, D-(+)-glucose forms two glycosides on treatment with CH_3OH and HCl gas.

The formation of the glycoside linkage converts a *hemiacetal* of the starting monosaccharide to an ace*tal* in the *glycoside.*

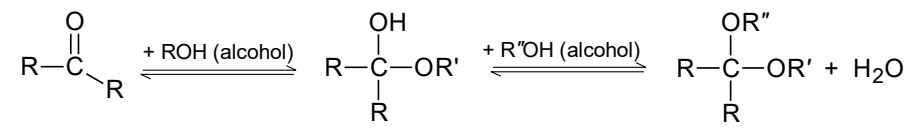
A *glycose* having *six* membered ring is known as *pyranose* while its *glycoside* is called *pyranoside*. Similarly, a *glycose* with a *five* membered ring is known as *furnanose* while its *glycoside* is called *furanoside*.

You have studied in earlier course (BCHCT-133)that in an *acetal,*two -OR(or-OR') groups are linked to a carbon atom.



Glycosides formed by glucose are called *glucosides*.

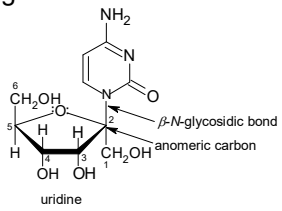
Similarly, a *hemi-ketal* and *ketal* is formed if the starting material is a ketone.



In glycosides, as the anomeric -OH group is replaced by -OR group; they are called *O*-glycosides. However, if the -OH group is substituted by -NR'R", the glycoside is called *N*-glycoside. Similarly, in *C*-glycosides, -OH group is replaced by -CRR'R".

Remember that a keto group is present in fructose which is numbered as C-2 and it is this *C-2 carbon* which is the *anomeric carbon* in the cyclic form of fructose.

An example of an *N*-glycoside is uridine whose structure is given below:

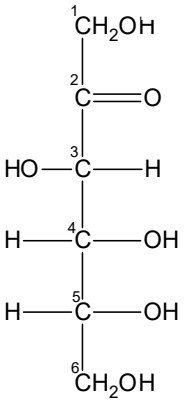


uridine

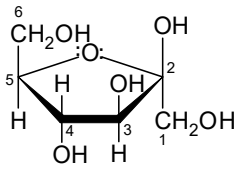
Glycosides formed from fructose are called fructosides.

Glycosides are acetals; hence, they are stable in basic solutions. While in acidic solutions, they hydrolyse and

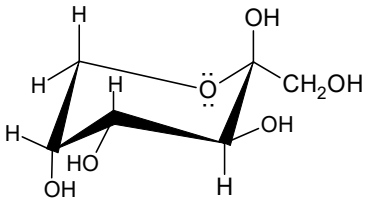
The formation of the acetal linkage by substitution leads to loss of properties such as mutarotation, reduction *etc.* associated with the hydroxyl group present at the anomeric carbon.



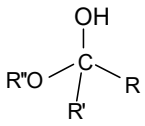
Fischer projection
D-fructose



Haworth projection
β-D-fructofuranose

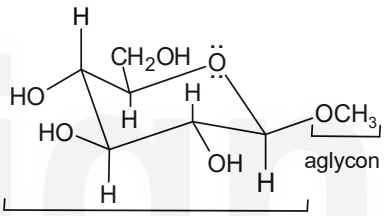


β-D-fructopyranose



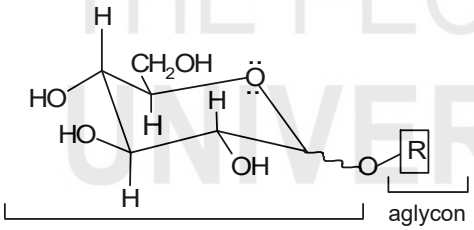
Hemi-ketal

In a glycoside, the carbohydrate part is called *glycon* while the non-carbohydrate part is known as *aglycon.* Thus, in methyl *β*- D-glucoside referred above, the *glycon* and *aglycon* parts can be shown as follows:

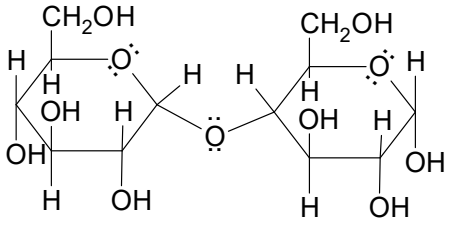


methyl β-D-glucoside

Instead of CH_3OH, the aglycon part can come from other alcohols, glycerol, phenol, steroid etc.



If the aglycon part is formed another monosaccharide, then the formation of glyosidic bond between two monosaccharides yields a **disaccharide.**You will study about disaccharides in detail in the next unit, i.e. Unit 16.

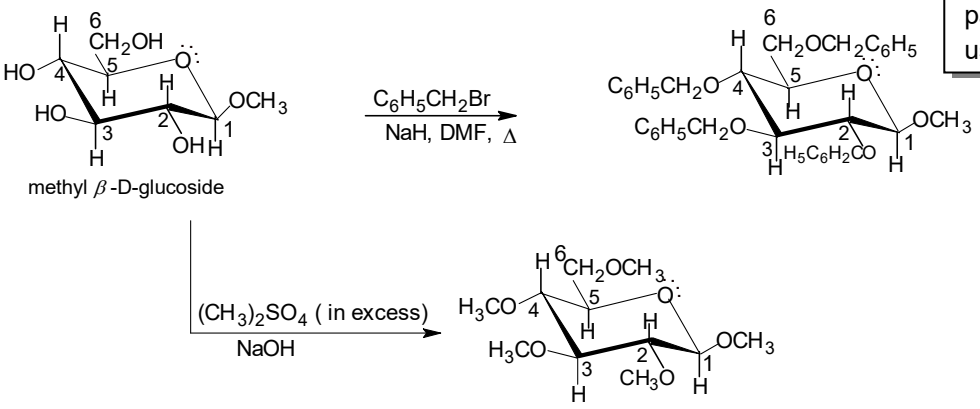


4-(α-D-glucopyanosyl)-D-glucose
maltose

Since more than one -OH group is present in the monosaccharide acting as the aglycon, the linkages resulting from these hydroxyl groups lead to different isomeric compounds.

These glycosides played an important role in the determination of the structure and (relative) configurations of monosaccharides. They were further used for various reactions for example, the -OH groups of the glycoside could be converted to benzyl, methyl, silyl ethers *etc.*

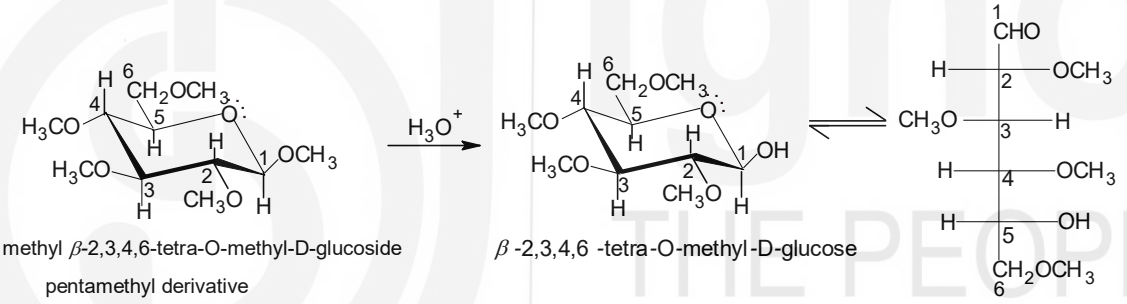
For removing the benzyl groups to get back the -OH groups, hydrogenolysis using palladium catalyst is used.



methyl β-D-glucoside

methyl β-2,3,4,6-tetra-O-methyl-D-glucoside
pentamethyl derivative

When such a pentamethyl derivative is hydrolysed in aqueous acidic medium, the acetal linkage breaks to give back -OH group; however, the rest of the other $-OCH_3$ groups remain unaffected.



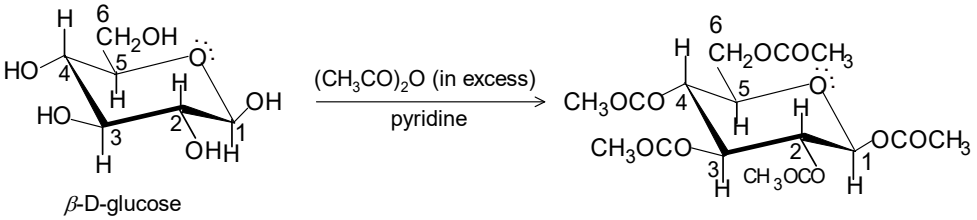
methyl β-2,3,4,6-tetra-O-methyl-D-glucoside
pentamethyl derivative

β-2,3,4,6-tetra-O-methyl-D-glucose

The resulting hemiacetal or the cyclic form of the tetra-O-methyl derivative is in equilibrium with its open chain form. This reaction was the basis of structure elucidation indicating that the C-5 hydroxyl of D-glucose was involved in the formation of the hemiacetal pyranose form.

8. Formation of Esters

One more important reaction used in the structure elucidation of monosaccharides is their conversion to acetate esters. All the hydroxyl groups of the monosaccharide get converted to the acetate groups on treatment with excess of ethanoic (acetic) anhydride in a weak base.



β-D-glucose

penta-O-acetyl-β-D-glucose

The formation of pentacetate of glucose indicated that it contained five hydroxyl groups when its structure was being elucidated.

After understanding the reactions exhibited by monosaccharides, let us study the structure of glucose and fructose in detail.

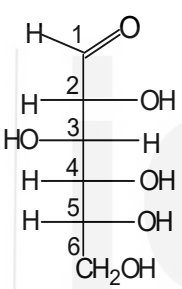
It is worthwhile to attempt the following SAQ at this stage.

SAQ 2

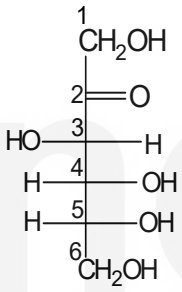
Write the product formed by the oxidation of D-glucose with dil. HNO_3.

15.4 STRUCTURE OF GLUCOSE AND FRUCTOSE

As given above in Figs.15.1and 15.2, the open chain structures of D-(+)-glucose and of D-(-)-fructose are as follows:



D-(+)-glucose

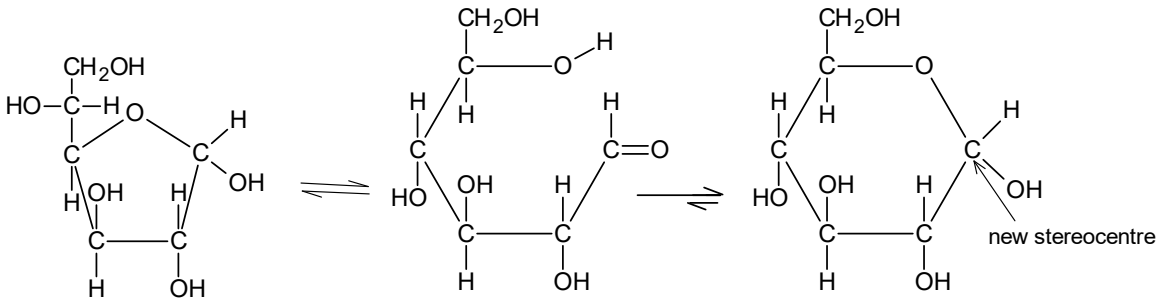


D-(-)-fructose

You are aware from sub-Sec. 14.4.1, Sec. 14.4, Unit 14, Block 3 of BCHCT-133 Course that aldehydes and ketones form hemiacetals or hemiketals with alcohols. Since sugars are hydroxycarbonyl compounds, they are capable of forming intramolecular cyclic hemiacetalsand hemiketals.

While in principle, any one of the hydroxyl group could add to the carbonyl function; the formation of a six-membered ring is preferred, although five-membered rings are also formed.

This is shown below in case of glucose.



D-(+)-glucofuranose
less stable

D-(+)-glucose

D-(+)-glucopyranose
more stable

Cyclic Hemiacetal Formation by Glucose

Similar cyclic structures are possible for fructose also. You will study about them in the next section.

15.5 CONFIGURATION OF MONOSACCHARIDES

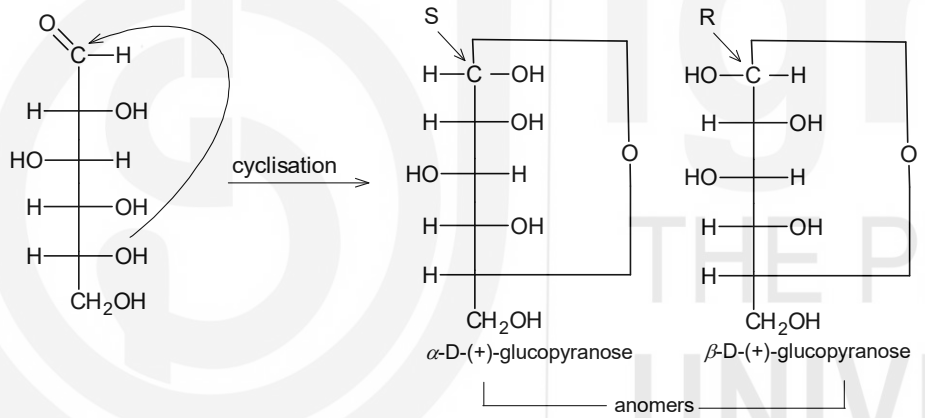
The configuration of monosaccharides refers to the spatial arrangement of different groups present in them. Let us now explore about this with the examples of glucose and fructose in more detail.

15.5.1 Absolute Configuration of Glucose and Fructose

The absolute configurations are described by assigning the configuration to each stereocentre in the molecule. For example, D-(+)-glucose is *(2R, 3S, 4R, 5R)*-2,3,4,5,6-pentahydroxyhexanal.

You may recall that *R, S* system of assigning configurations was discussed in Unit 11, Block-3 of BCHCT-131Course.

You can see that the formation of a hemiacetal (or a hemiketal) turns the carbonyl carbon into a new stereocentre. This leads to **two** new compounds which are diastereomers having different configurations at C-1. Such isomers are called **anomers**and the hemiacetal or hemiketal carbon (C-1) is called the **anomeric carbon.** The two anomers are differentiated by the Greek letters αandβ. Thus, we can call the anomers of glucose asα-D-(+)-Glucopyranose andβ-D-(+)-Glucopyranose. These anomers are represented below in the modified Fischer projections using elongated lines to indicate the new bonds formed.



Note that in β-anomer the OH at C-1 is written to the left and that in the α-anomer is written to right.

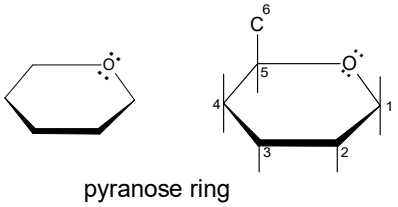
Since these modified Fischer projections do not give the correct picture of the molecule in terms of bond lengths, Haworth introduced an alternate projection formula called **Haworth projections.**

In Haworth projections, the cyclic ether is written as a *planer pentagon* or a *hexagon* having the anomeric carbon on the right and the ether oxygen at the top. The substituents located above and below the ring are joined by vertical lines to the ring carbons.

Norman Haworth
(19 March 1883-
19 March 1950)

The English Chemist who received the Nobel Prize in 1937 for his work in carbohydrate chemistry.

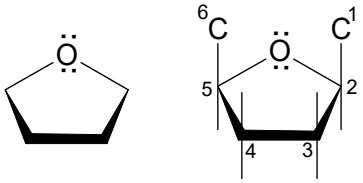
Note that in Haworth projections for the *pyranose* form in standard orientation, the C-1 which is the *hemiacetal carbon*, is written in the *right* side and the *ring oxygen* is written in the *upper right corner*. The numbering is then done in the clockwise manner.



pyranose ring

Glucose exists mainly in the pyranose form whereas fructose forms an equilibrium mixture of pyranose and furanose forms in the ratio 70:30.

Similarly, for the *furanose* structure, the *hemiacetal*carbon is placed at *the right side top* position and the *oxygen is placed at the centre at the top most position* as shown below along with the numbering of carbon atoms.



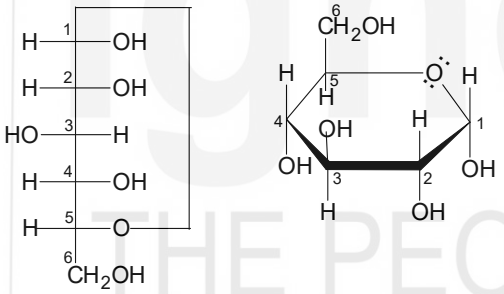
furanose ring

In aqueous solutions of monosaccharides, the pyranose form dominates, but in biomolecules, the furanose from occurs commonly.

While showing the glycosidic linkages, these rings can be rotated suitably. Let us write the Haworth projections for*α*-D-glucopyranose and *β*-D-glucofuranose from their Fischer projections.

In *α*-D-anomer, -OH group on C-1 is on the right in the Fischer projection and it is shown down in the cyclic structure in Haworth projection and reverse is true for *β*-D-anomer.

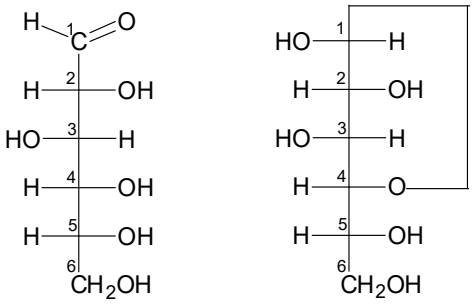
For *α*–D-glucopyranose, the substituents on the *right hand side* in the Fischer projection are shown *down* in the Haworth projection while the -CH_2OH bearing carbon, i.e. carbon number 6, is placed at the *up position* linked to C-5. Also note that in *α-form*, the *-OH group at C-1 is at the right side in the Fischer projection.*



Fischer projection Haworth projection

α-D-glucose

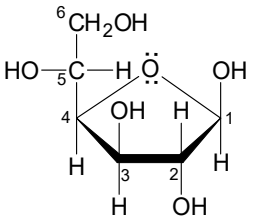
Similarly, for *β-D-glucofuranose,* we first draw the Fischer projection of D-glucose. The five membered ring containing the acetal linkage is formed by -OH group at the C-4 carbon atom. Since the form is *β*, the *-OH group at C-1 is at the left side* in the Fischer projection.



D-glucose

Fischer Projection

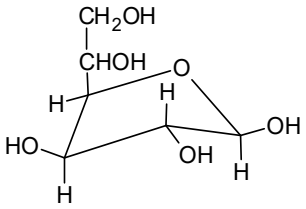
Again, the Haworth projection is written by drawing the furanose ring and placing the *right hand side* substituents of the *Fischer Projection* on the *down side* in the Haworth projection.



β-D-glucofuranose

Haworth projection

The envelope conformation of the furanose form can be represented as shown below.



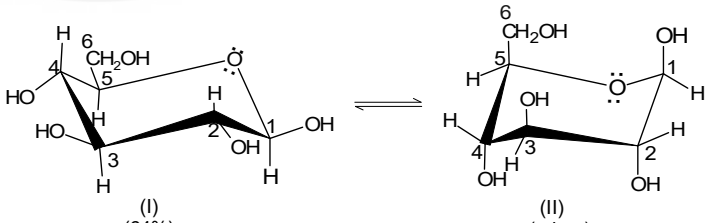
β-D-glucofuranose

Earlier, in 1909, according to an American Chemist C.S. Hudson, in D-sugars, the most dextrorotatory of *α/β* anomers is called *α* and the other one is named as *β.* Similarly, for L-sugars, the more levorotatory anomer is named as *α*-anomer while the lesser levorotatory anomer is known as *β*-anomer.

But later, a system based on absolute configuration has been adopted wherein the anomer having the hemiacetal hydroxyl on the same side as the hydroxyl group reacting with the aldehyde or keto group (*cis*) is called *α-anomer* while the anomer in which the hemiacetal hydroxyl is on opposite side, i.e. *trans* is called *β-anomer*.

From Haworth projections of D-(+)-Glucose, we can write its conformations. Since its pyranose form is a six-membered ring in which C-O-C bond angle is III°, its chair conformations can be written similar to the cyclohexane molecule.

Now two alternate chair forms are possible, *e.g.* for *β*-D-(+)-glucopyranose. We can write them as follows:



(I)
(64%)
(C1)
$^4C_1$ conformation

(II)
(minor)
(1C)
$^1C_4$ conformation

β-D-(+)-glucopyranose

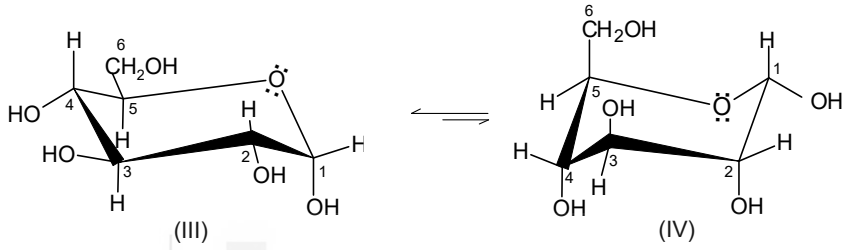
In structure (I), carbon number 1 is *down* whereas carbon number 4 is *up,* so it is written as **$^4C_1$ conformation** and is called **C1 conformation**. However, in structure (II), C-1 carbon is *up* and C-4 carbon is *down*; therefore, it is written as $^1C_4$ conformation and is also called 1C conformation.

Note that C-6 carbon atom bearing the primary hydroxyl group (-CH_2OH) is *axial* or *up* in the 1C conformation structure (II) whereas it is *equatorial* in C1 conformation structure (I).

You can also see in structure (I) that all bulky –OH groups are in *equatorial* positions; therefore, this conformation is *lower in energy* and hence,*more stable* due to lower interactions between the bulky groups as compared to the conformation shown in structure (II).

As a result, the C1 conformation shown above in structure (I), predominates and occurs to the extent of 64% in the aqueous solution of D-Glucopyranose; however, the conformation shown in structure (II) is present in the minor form.

The other conformer which is present to the extent of 36% is *α*-D-glucopyranose in $^4C_1$ conformation as shown in structure (III) below while its $^1C_4$ conformation, structure (IV), is present in minor amount in the solution.

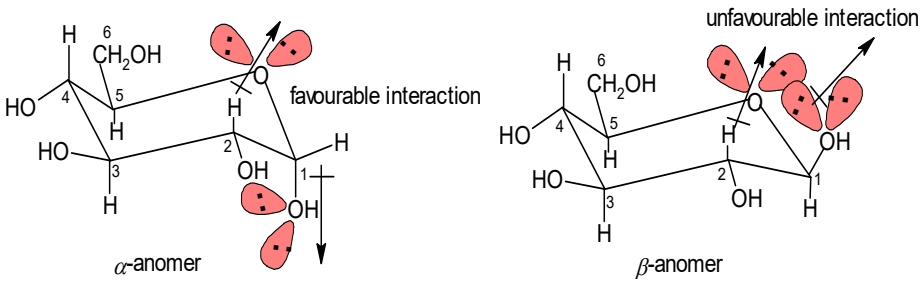


$^4C_1$ conformation *α*-D-(+)-glucopyranose $^1C_4$ conformation

You can see in structure (III) that -CH_2OH group is occupying the *equatorial* position alongwith -OH groups at C-2,C-3 and C-4 carbon atoms. Remember that the *-OH group at C-1 has to be axial as it is the α-D-(+)-glucose.*

An interesting fact about *β-D-(+)-glucose*is that amongst all D-aldohexoses, only this is the one which can have a conformation shown by structure (I) in which *all bulky groups occupy equatorial positions.* Hence, it is *the most abundant form in nature*.

The formation of *α-anomer* can be explained on the basis of *anomeric effect*. The lone pair -lone pair interactions are u*nfavourable* between the oxygen of anomeric -OH and the ring oxygen atom in the *β*-anomer whereas these interations or repulsions are reduced when the -OH group occupies the *axial position in α-anomer*.



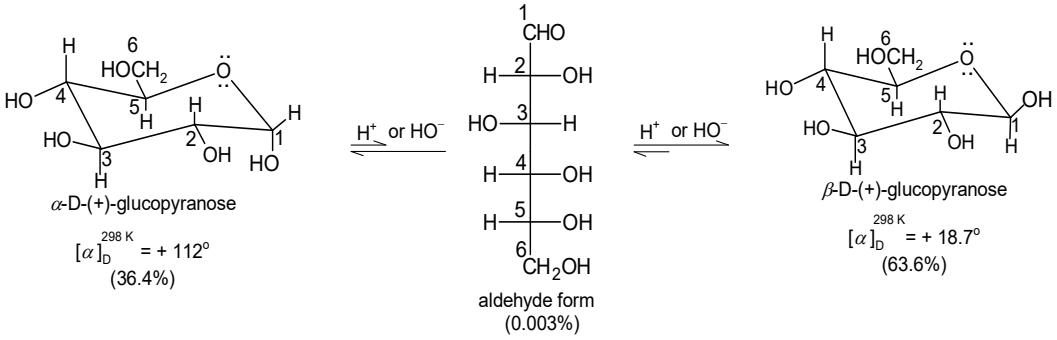
Mutarotation is catalysed by acids and bases.
In Latin, *mutare* means to **change**.

The anomeric effect is more prominent in non-polar solvents and decreases with the inecrase in the polarity (dielectric constant) of the solvent such as water.

15.5.2 Mutarotation

Both *α*-D-(+)-glucopyranoseand *β*-D-(+)-glucopyranose are optically active but differ widely in their optical rotations. The *α*-D-(+)-glucopyranose has the specific rotation of $[\alpha]_D^{298\,K} = +112^o$ whereas *β*-D-(+)-glucopyranose has

$[\alpha]_D^{298\,K} = +18.7^o$. When dissolved in water, the optical rotation of the solution gradually changes with time until it reaches an equilibrium value of +52.7°. This is because α-D-(+)-glucopyranose rapidly establishes an equilibrium with a small amount of the open-chain aldehyde form which in turn undergoes renewed and reversible ring closure to the β-anomer.



This interconversion is called **mutarotation**and was first observed in 1846. It involves change of configuration at one stereocentre in a compound having more than one such centres.

SAQ 3

What is the relationship between α-D-(+)-Glucose and β-D-(+)-Glucose?

SAQ 4

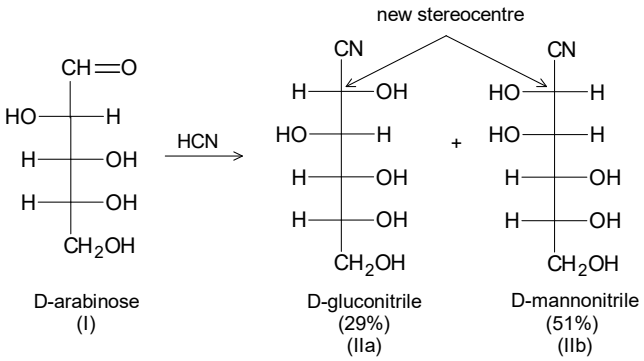
What are anomers?

15.6 ASCENDING AND DESCENDING OF CHAINS IN MONOSACCHARIDES

We will focus our attention on the methods which are used to convert one aldose to another aldose which has either *one more carbon atom* in the chain or *one less carbon atom* in the chain. The former conversion is called *ascending* or *lengthening of carbon chain* whereas the latter is known as descending of carbon chain. Let us first study the method for ascending the carbon chain.

Kiliani-Fischer Synthesis

Kiliani showed that cyanohydrins obtained from aldoses could be hydrolysed to aldonic acids.



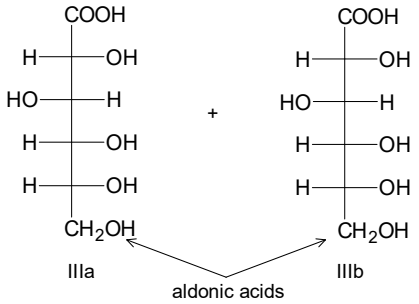
Heinrich Kiliani
University of Freiburg
(30th October 1855-
25th February 1945)

In this method, an aldose is treated with hydrogen cyanide to give the cyanohydrins. Let us understand this with the example of D-arabinose (I).

Note here that the formation of the cyanohydrins has led to the generation of a new stereocentre; therefore, the product formed is a mixture of diastereomers (IIa and IIb) which are obtained in different amounts. These two diastereomers are *epimers* and they differ in configuration at C-2 carbon.

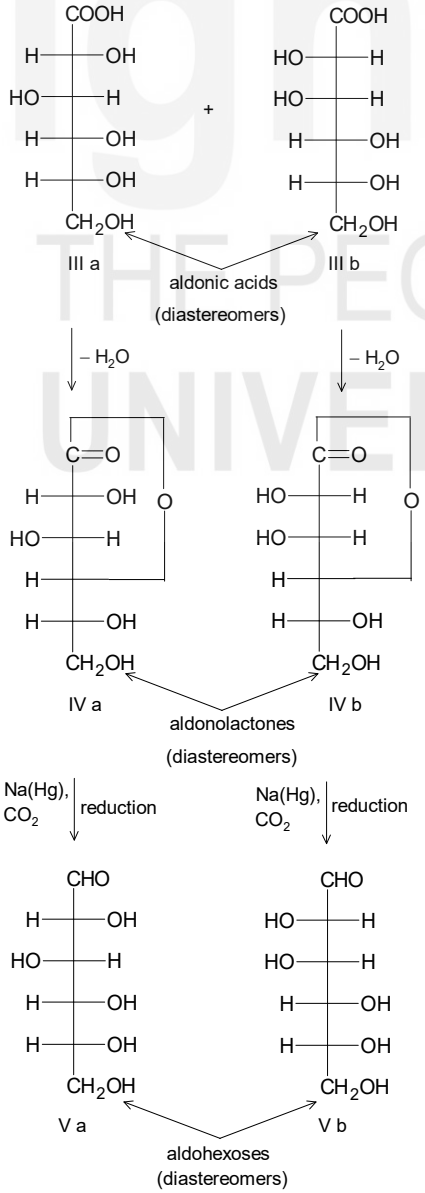
The hydrolysis of the above mixture of cyanohydrins yields a mixture of aldonic acids (IIIa and IIIb) as given below.

The mixture of aldonic acids is separated at this stage.

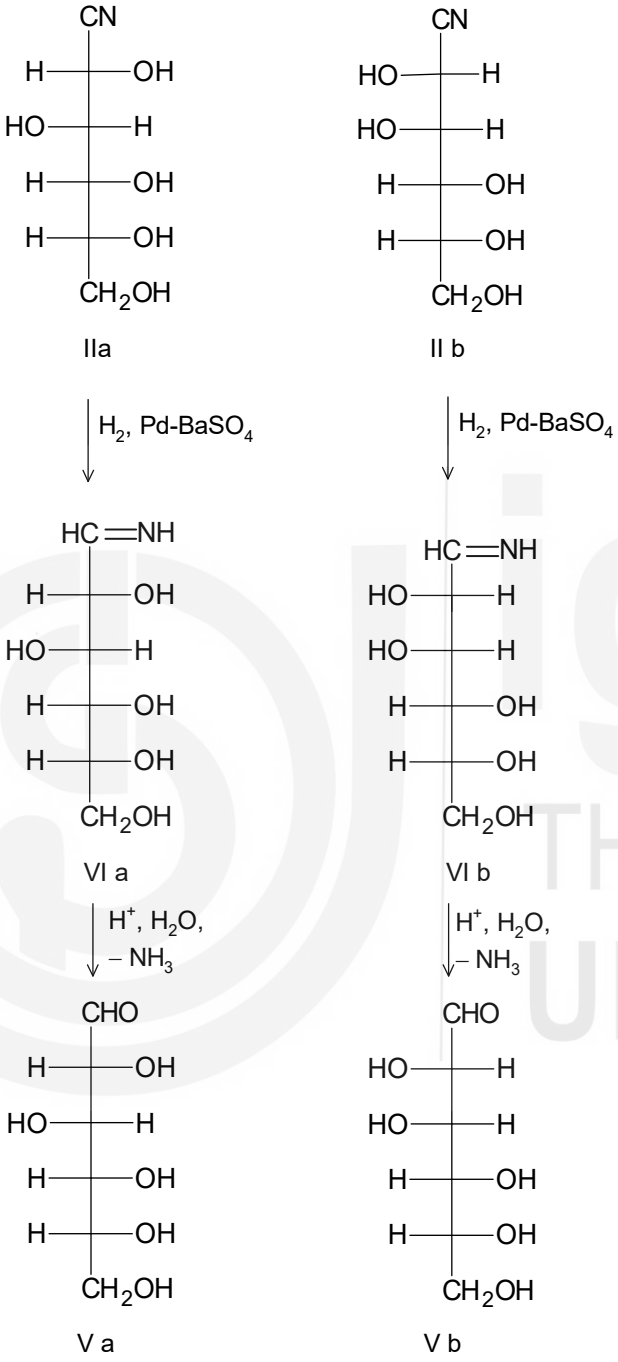


Fischer developed a method for the conversion of aldonic acids to aldoses by the formation of lactones which were reduced to the aldose.

The –OH groups present at γ and δ positions can form lactones. The γ lactone is formed is more stable.



The cyanohydrins obtained (IIa and IIb) in Kiliani-Fischer synthesis outlined above could be separated. As an alternative these on catalytic reduction in aqueous acid yield the aldoses which have one more carbon atom in their chain as compared to the starting aldose.



After understanding how to increase the chain length of an aldose by one carbon atom, let us study how we can decrease the chain length of an aldose by one carbon atom.

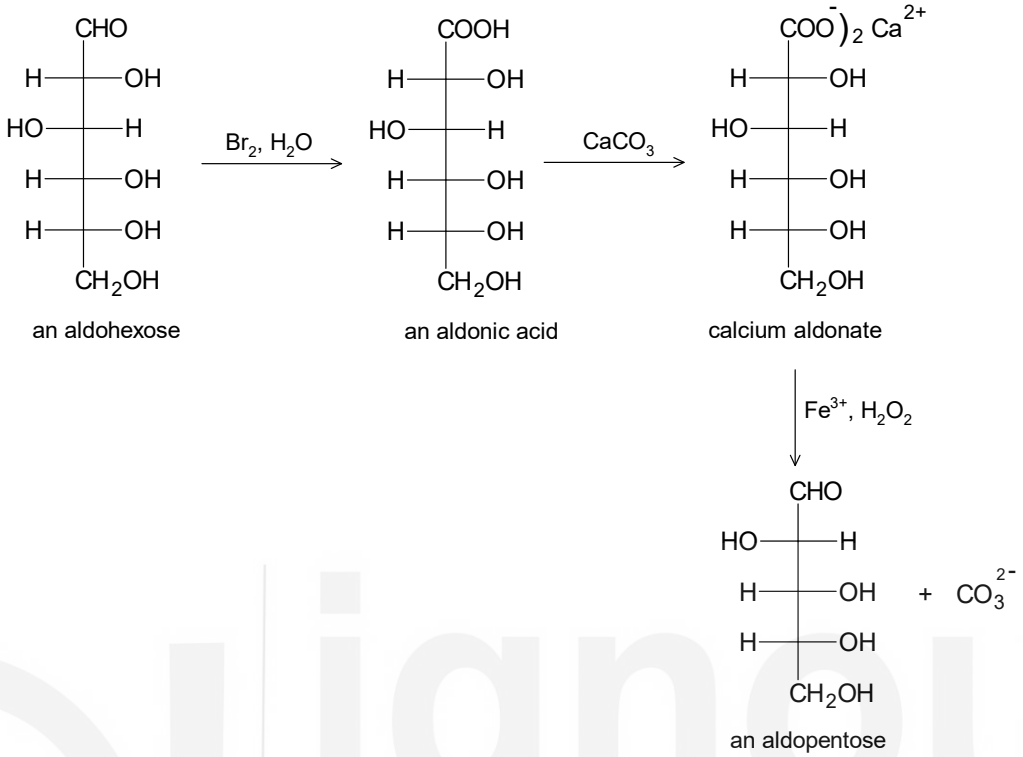
One method used for this purpose is **Ruff degradation**. It involves oxidative decarboxylation. The aldose whose length is to be shortened is first oxidised to the aldonic acid by bromine water. The calcium salt of the aldonic acid is oxidised by hydrogen peroxide in the presence of ferric salt which gives the desired aldose and the carbonate ion.

Prof. Otto Ruff

(12th December, 1871-17th September, 1939)

University of Danzig, Germany

Let us understand these steps by the example of conversion of an aldohexose to an aldopentose.



SAQ 5

What products will be obtained by Kiliani-Fischer synthesis of D-(+)-glyceraldehyde?

15.7 SUMMARY

- Carbohydrates constitute an important class of compounds which have diverse functions.
- Some common examples of carbohydrates are cellulose which gives structural support to plants, starch which serves as the reserved energy source and sugars such as sucrose and glucose. Glucose is an essential constituent of blood in higher animals.
- *Monosaccharides* are the simplest carbohydrates which cannot be hydrolysed into smaller and simpler carbohydrates.
- A monosaccharide may be further classified as an *aldose* or a *ketose* if it contains an *aldehyde* or a *keto* group.
- Depending upon the number of carbon atoms in the chain, sugars are called *trioses*(3 carbons), *tetroses*(4 carbons), *pentoses* (5 carbons), *hexoses*(6 carbons), and so on.
- Monosaccharides undergo a variety of reactions such as oxidation, reduction and formation of osazones and glycosides.

- Formation of hemiacetal (or hemiketal) turns the carbonyl carbon into a new stereocentre. This leads to *two*new compounds which are diastereomers having different configurations at C-1. Such isomers are called *anomers* and the hemiacetal or hemiketal carbon (C-1) is called the *anomeric* carbon.
- The structures of monosaccharides can be represented by Fischer projections and Haworth projections.
- *Mutarotation* involves change of configuration at one stereocentre in a compound having more than one such centres.
- In monosaccharides, ascending of carbon chain can be done by*Kiliani – Fischer synthesis* and descending of carbon chain can be done by *Ruff degradation***.**

15.8 TERMINAL QUESTIONS

1. Draw the Haworth projection for D-(+)-xylose and D-(+)-glucose.
2. Write the Fischer projection of D-(+)-xylose and D-(+)-xylulose.
3. Write the product formed by the oxidation of D-(+)-glucose with bromine water.
4. Which compound will form same phenylosazone as D-(+)-galactose?
5. Write the Haworth projection of *α*-D-fructofuranose.
6. Draw the possible chair conformations of *β*-D-(+)-glucose. Which one of these conformations is more stable and why?
7. Name the product formed by Ruff degradation of D-(+)-glucose. Which other aldose will give the same product on Ruff degradation?

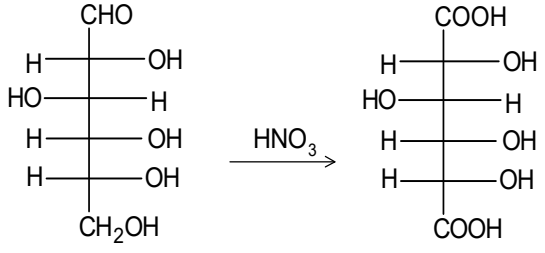
15.9 ANSWERS

Self-Assessment Questions

1. i) Ribose is a pentose while fructose is a hexose.

   ii) Xylulose is a ketose having a five carbon chain.

   iii) D-erythrose is an aldose having four carbon atoms in the chain.

2. 

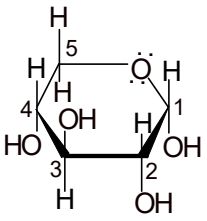
D-glucose D-glucaric acid

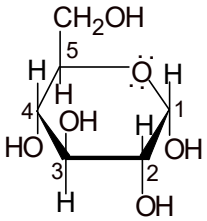
3. They are diastereomers or anomers.

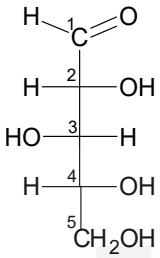
4. The diastereomeric pair of monosaccharides which differ in configuration at C-1 carbon atom are called anomers.

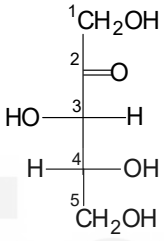
5. D-(+)-erythrose and D-(+)-threose.

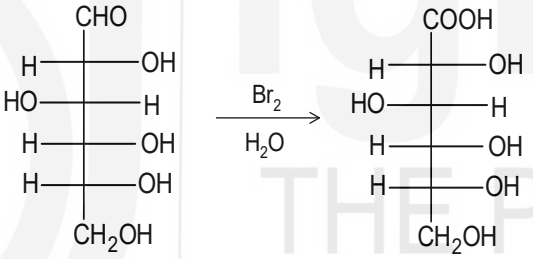
Terminal Questions

1. 
D-(+)-xylose

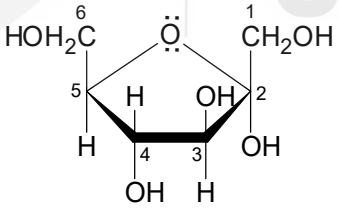

D-(+)-glucose

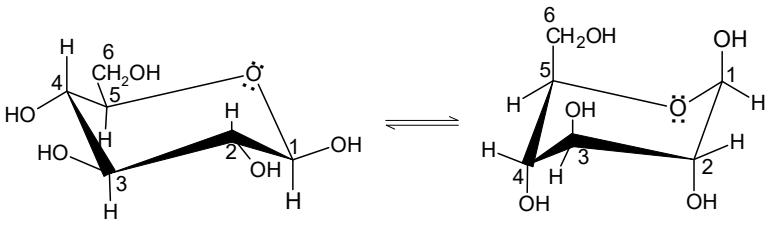
2. 
D-(+)-xylose


D-(+)-xylulose

3. 
D-(+)-glucose D-(+)-gluconic acid

4. D-(+)-talose

5. 
α-D-fructofuranose

6. 
equatorial bulky groups axial bulkyl groups

The first conformation is more stable because all bulky substituents are equatorial.

7. D-(+)-arabinose. D-(+)-mannose will also give the same pentose.

UNIT 16

CARBOHYDRATES-II: DISACCHARIDES AND POLYSACCHARIDES

Structure

16.1 INTRODUCTION

In Unit 15 on monosaccharides, you have studied about the basic aspects of carbohydrates and monosaccharides. Therein, we have discussed the important monosaccharides i.e. glucose and fructose in detail. Having understood the basic aspects of monosaccharides and how to write Fischer and Haworth projections of monosaccharides, let us now widen our window to the study of other important classes of carbohydrates, i.e., disaccharides and polysaccharides.

You already know that *disaccharides* contain *two* monosaccharides linked to each other. In this unit, we will be discussing about some important disaccharides such as maltose, cellobiose, lactose and sucrose. In addition to these, we will also elaborate on the structure and importance of starch and cellulose which are *polysaccharides* containing many glucose monosaccharide units linked to each other.

Expected Learning Outcomes

After studying this unit and having the experiments performed, you should be able to:

- define disaccharides and polysaccharides;

- ❖ give examples and importance of some common disaccharides and polysaccharides;
- ❖ explain glyosidic linkage;
- ❖ write the structures of disaccharides such as maltose, cellobiose, lactose and sucrose;
- ❖ name the above disaccharides and discuss their important;
- ❖ properties; and
- ❖ describe the structural features and importance of polysaccharides such as starch and cellulose.

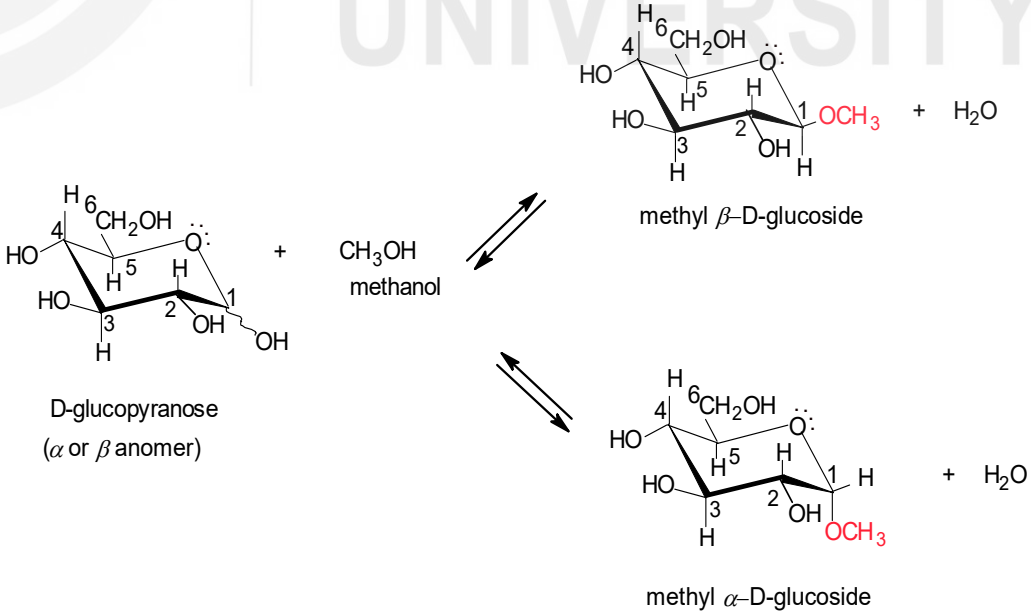
16.2 DISACCHARIDES

You already know that **disaccharides** are the carbohydrates formed by the linkage of two monosaccharide units and on hydrolysis they yield *two* molecules of monosaccharides. These two monosaccharide units can be *same* or *different*, as you will study in case of different disaccharides discussed in this section.

The structure, properties and biological roles of carbohydrates can be easily understood by their three dimensional shapes. Hence, we will be emphasising on the type of linkages joining the monosaccharides.

Let us now refresh some of the concepts you have learnt in Unit 15. This will help you to understand the forthcoming discussion on writing of structures and nomenclature of disaccharides. Now, carefully revise the following points:

- An anomeric –OH group is present in the cyclic form of monosaccharides. This can react with alcohols and form derivatives which are called *glycosides*.



- In a glycoside, the carbohydrate part is called *glycon* while the non-carbohydrate part is known as *aglycon.* Thus, in methyl D-glucosides (*α*-and *β*-) referred above, the glycon parts are shown in the black colour while the aglycon parts are indicated by the red colour.

- The formation of the glycoside linkage converts a *hemiacetal* of the starting monosaccharide to an ace*tal* in the *glycoside.* Remember that in an *acetal* two –OR (or –OR') groups are linked to a carbon atom.
- *Glycosides are acetals; hence*, *they are stable in basic solutions. While in acidic solutions, they* hydrolyse and produce a sugar and the alcohol.
- A *glycose* having *six* membered ring is known as *pyranose* while its *glycoside* is called *pyranoside*. Similarly, a *glycose* with a *five* membered ring is known as *furanose* while its *glycoside* is called *furanoside*.
- Glycosides formed by glucose are called *glucosides*.
- Instead of CH_3OH, aglycon part can be derived from other alcohols, glycerol, phenols, steroid etc. If the aglycon part is *another monosaccharide*, then the formation of glyosidic bond between two monosaccharides yields a **disaccharide.**
- Thus, the monosaccharides can link to each other through the *glycosidic linkage.*

Having refreshed the above points, let us also understand what is meant by the term reducing and non-reducing sugars before proceeding to the study of disaccharides.

Reducing and Non-reducing Sugars

Carbohydrates which contain the hemiacetal group give positive Tollens, Fehling and Benedicts tests and hence, these are called *reducing sugars.* This is so because in an aqueous solution, there is an equilibrium between the hemiacetal and the open chain structure of the sugar. It is the open chain form which gets oxidised and the equilibrium keeps on shifting to give more of open chain form.

The formation of the acetal linkage by substitution leads to loss of properties such as mutarotation, reduction *etc.* associated with the hydroxyl group.

However, formation of an *acetal* linkage does not allow such equilibrium and those sugars i.e. glycosides which contain *acetal* linkages instead of hemiacetal linkages, do not give positive tests with Tollens' reagent, Fehling reagent and Benedict's reagent and are called *non-reducing sugars.*

You will study more about *reducing* and *non-reducing* nature of sugars when you study the structures of disaccharides.

Having refreshed the above knowledge, let us know focus our attention on some very interesting disaccharides which are very commonly used.

16.2.1 Maltose

The hydrolysis of starch using the enzyme *diastase* yields maltose as one of the products. It has molecular formula $C_{12}H_{22}O_{11}$ and is dextrorotatory in nature. Some of its properties are listed below:

- One mole of maltose on acid-catalysed hydrolysis or hydrolysis using the enzyme *maltase* (from yeast) gives *two* moles of D-(+)-glucose.

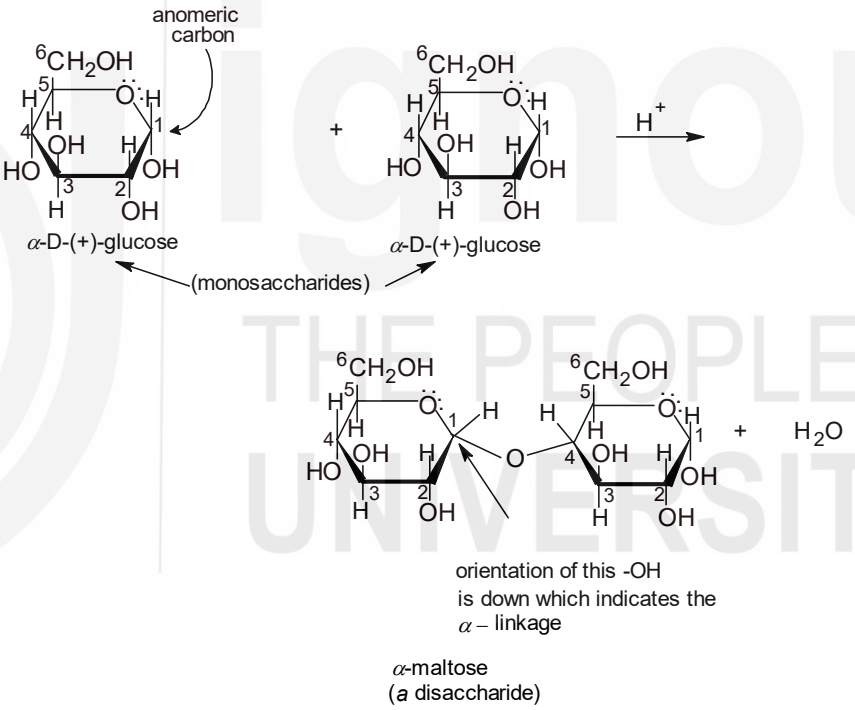
Maltase hydrolyses only *α*-glycosidic linkages and not *β*-ones. Therefore, in (+)-maltose, two D-(+)-glucose units are linked to each other by *α*-glycoside link in which the *α*-anomeric –OH of one glucose unit is linked to the –OH group of the other glucose unit.

- Maltose reduces Tollens reagent and Fehling's solution and is called a *reducing sugar*. This indicates that one monosaccharide unit in the glucoside exists in the hemiacetal form. The presence of one hemiacetal is also indicated by the bromine water oxidation of maltose to maltonic acid, which is a monocarboxylic acid.

Note that the details of these reactions are beyond the scope of this course and you will study them at a higher level.

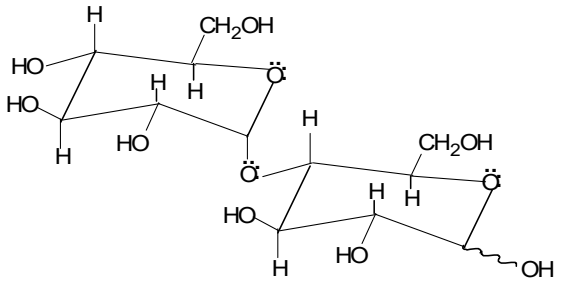
- The methylation of maltonic acid followed by hydrolysis and methylation of maltose followed by hydrolyses indicated that the –OH group at C-4 of one glucose unit is involved in the formation of glycosidic linkage and the reducing hemiacetal linkage is present in the pyranose ring.

All these facts led to the following structure of maltose. Please also understand the numbering of the carbon atoms shown below:



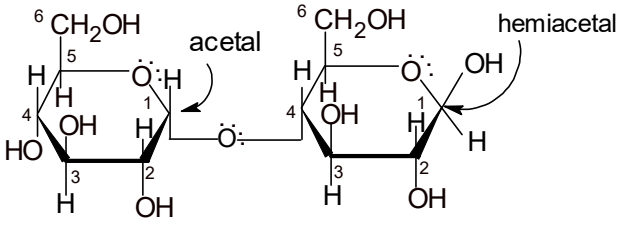
The type of linkages present here are same as those present in amylopectin, about which you will study in the later section i.e. Sec.16.3.

The –OH group at C-1 can be represented by a wavy line as shown below which indicates that it can take either *α*- or *β*- orientation.

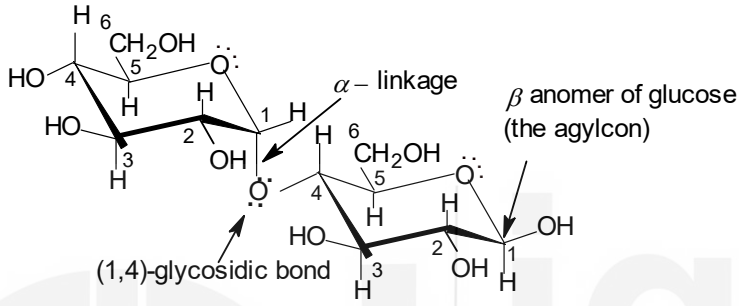


Thus, according to the position of the –OH group we get two anomeric forms *i.e*., *α-anomer* and *β-anomer.* The specific rotations of these *α*-(+) and *β*-(+) forms being, +168^o and +112^o at 25^o C, respectively.

The structure of *β-anomer* of maltose is given below along with its name:



4-O-(α-glucopyranosyl)-β-D-glucopyranose
maltose



4-O-(α-glucopyranosyl)-β-D-glucopyranose
maltose

For understanding how to arrive at the names of disaccharides, let us study the recommendations given below. But before that you can answer the following SAQs.

SAQ 1

Does maltose show mutarotation?

SAQ 2

Write the structure of maltonic acid.

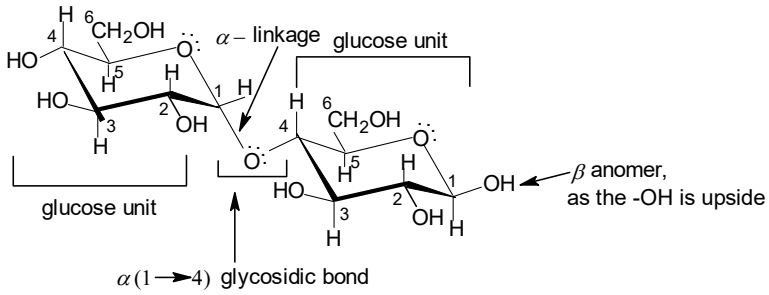
Nomenclature of Disaccharides:

As per the Recommendations (1996) of Joint Commission of IUPAC and International Union of Biochemistry and Molecular Biology, a *disaccharide* in which a *free hemiacetal is present* can be named as a *glycosylglycose*. The locants of the glycosidic linkage and the anomeric descriptor being mentioned in the name. The locants are given in brackets between the glycosyl and glycose units in *one way of nomenclature* related to oligosaccharides. The *other way* is to give the locants before (*in front of*) the glycosyl prefix, similar to names given to glycosides as discussed earlier. Hence, according to these two ways, maltose can be named as:

i) *α*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranose

[*α*-D-Glc*p* (1→4)-*β*-D-Glc*p*] or

ii) 4-O-*α*-D-glucopyranosyl-*β*-D-glucopyranose



The second method of nomenclature is used by Chemical Abstracts Service. *Also note that O-locant is not used in the first way of naming.*

In the first way of nomenclature, you can also see that a shorthand form of notation is given below the name in square brackets. Such a shorthand can be easily correlated with the name given above for maltose.

You will see later that this shorthand representation is quite useful when higher oligosaccharides (or *polysaccharides*) are named.

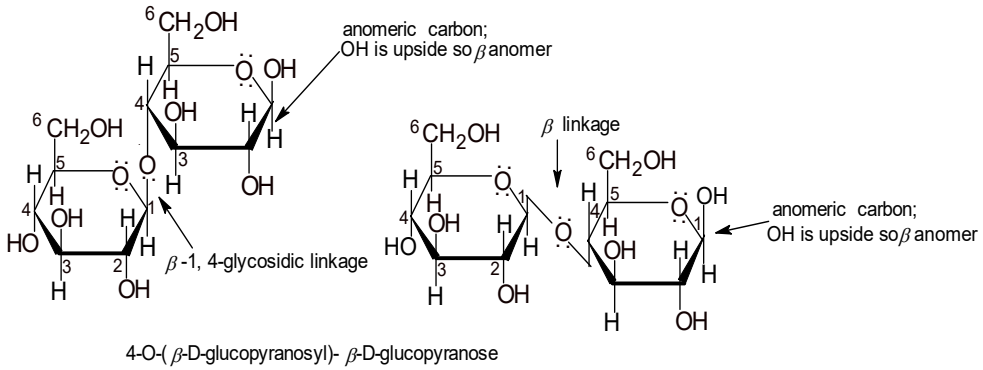
16.2.2 Cellobiose

Cellobiose is obtained by the partial hydrolysis of cellulose. It has molecular formula $C_{12}H_{22}O_{11.}$ The cellobiose shows the properties similar to (+)-maltose discussed above.

On acid-catalysed hydrolysis, it yields two molecules of D-(+)-glucose. It is a reducing sugar and a sequence of reactions such as oxidation, methylation and hydrolysis indicated that a glucoside linkage is present between the two glucose rings in their pyranose forms, involving–OH group at C-4 carbon atom.

It is hydrolysed by the enzyme *emulsin* which hydrolyses on *β*-glucoside linkages; hence two glucose units are linked by a *β*-glucoside linkage in (+)-cellobiose. Cellobiose is not hydrolysed by *α*-glucosidase enzyme maltase.

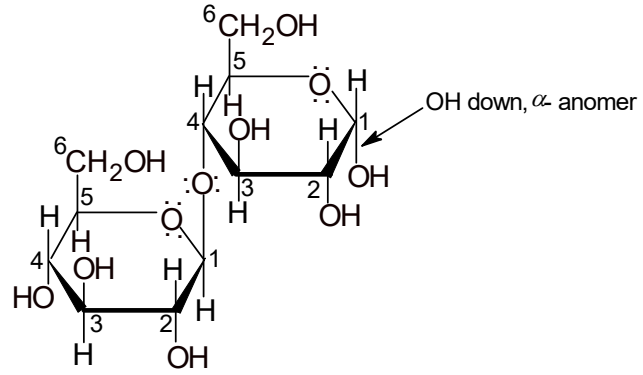
You can show the *β* linkage in the following two ways:



4-O-(*β*-D-glucopyranosyl)- *β*-D-glucopyranose

Cellobiose also shows mutarotation; hence it can exist as *α*- and *β*-anomers. In the above two structures, the *β*-anomer has been shown as the hydroxyl group at C-1 anomeric carbon atom is *up*.

If we have to write the structure of *α*-anomer, we will write the anomeric –OH at C-1carbon atom down and this is shown below:



4-O-(*β*-D-glucopyranosyl)- *α*-D-glucopyranose

Let us next study another disaccharide, lactose. But before that attempt the following SAQ.

Lactose in milk gets broken by the enzyme *lactase* to glucose and galactose in small intestine. Many people have *lactose intolerance* because not enough lactase is produced in their bodies; the reasons for that may be different. In such people, lactose on reaching to colon mixes with bacteria and gets fermented which results in symptoms like gas, diarrhoea and bloating.

SAQ 3

What is the name of *β*-anomer of (+)-cellobiose?

16.2.3 Lactose

Lactose occurs in human milk, cow's milk and milk of other mammals as well. Its molecular formula is $C_{12}H_{22}O_{11}$.Similar to the disaccharides studied above, it is also a *reducing sugar* and undergoes *mutarotation*.

The acidic hydrolysis of (+)-lactose yields equal amounts of D-(+)-glucose and D-(+)-galactose. It is also hydrolysed by *emulsin* enzyme which hydrolyses *β*-glycosidic linkages; therefore, lactose is formed by *β*-glycosidic linkage between D-(+)-glucose and D-(+) galactose.

Lactose exhibited the following reactions:

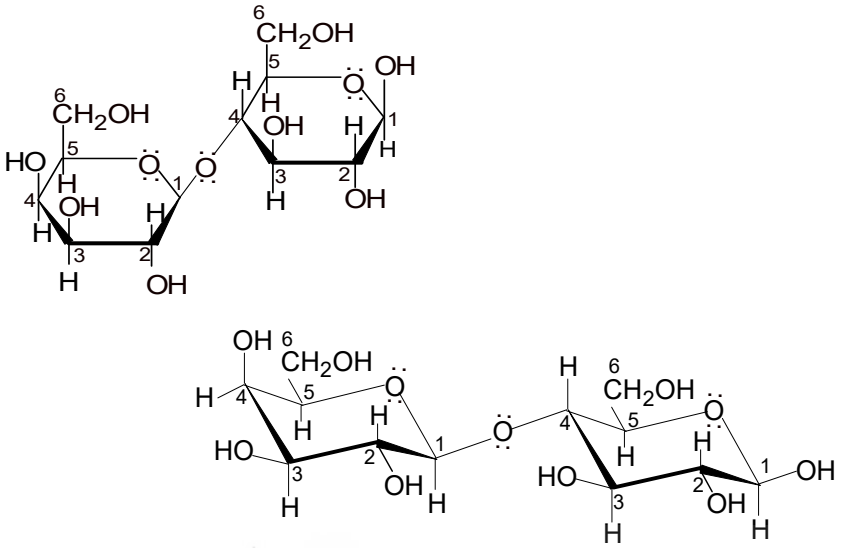
- Osazone formation and its hydrolysis
- Oxidation followed by hydrolysis
- Oxidation, methylation and hydrolysis.

The details of these reactions are beyond the syllabus and you will study them at a higher level.

These reactions indicated that

- the two monosaccharide units - glucose and galactose exist in pyranose forms in lactose.
- the galactose forms *β*-glycosidic linkage with the hydroxyl group at C-4 of glucose unit. The anomeric –OH of the glucose, being free, exhibited oxidation and osazone formation. The mutarotation showed by lactose was also due to this anomeric -OH group.

D-(+)-glucose and D-(+)-galactose differ in their configurations at carbon number 4 only. Thus, the structure of *β*-anomer of lactose can be written as follows:



β–lactose

4–O–(*β*–D–galactopyranosyl)–*β*–D–glucopyranose

A *galactoside* is the glycoside of *galactose*. Refer to naming of glycosides again.

You can see in the above structure that a D-galactosyl unit is linked to the hydroxyl group present at C-4 of the glucose. As the *anomeric -OH of galactose* is linked to the -OH group at C-4 of glucose, lactose is a *galactoside not a glucoside*.

SAQ 4

Write the Howarth projection for *α*-lactose. Also give its name.

SAQ 5

What is the other way to write the name of *α*-anomer of lactose?

16.2.4 (+)–Sucrose

(+)-Sucrose is the most common table sugar with which you come across daily. This is the most widely occurring disaccharide which is obtained from sugarcane and sugar beets.

Sucrose has molecular formula is $C_{12}H_{22}O_{11}$ and on acid-catalysed hydrolysis its one mol yields *one* mol of D-(+)-glucose and *one* mol of D-(+)-fructose.

Honey contains *invert sugar* where honeybees use*invertase*enzyme for hydrolysis.

An interesting property of sucrose is that it itself is dextrorotatory while its products of hydrolysis show net levorotation due to the greater value of levorotation by D-(-)-fructose. The resulting mixture is called *inverted sugar* or *invert sugar*.

	(+) - sucrose	hydrolysis *invertase* enzyme →	D- (+) - glucose + D- (-) -fructose
specific rotation $[\alpha]$	$+66.5^{o}$		$+52.7^{o}$ $\quad -92.4^{o}$

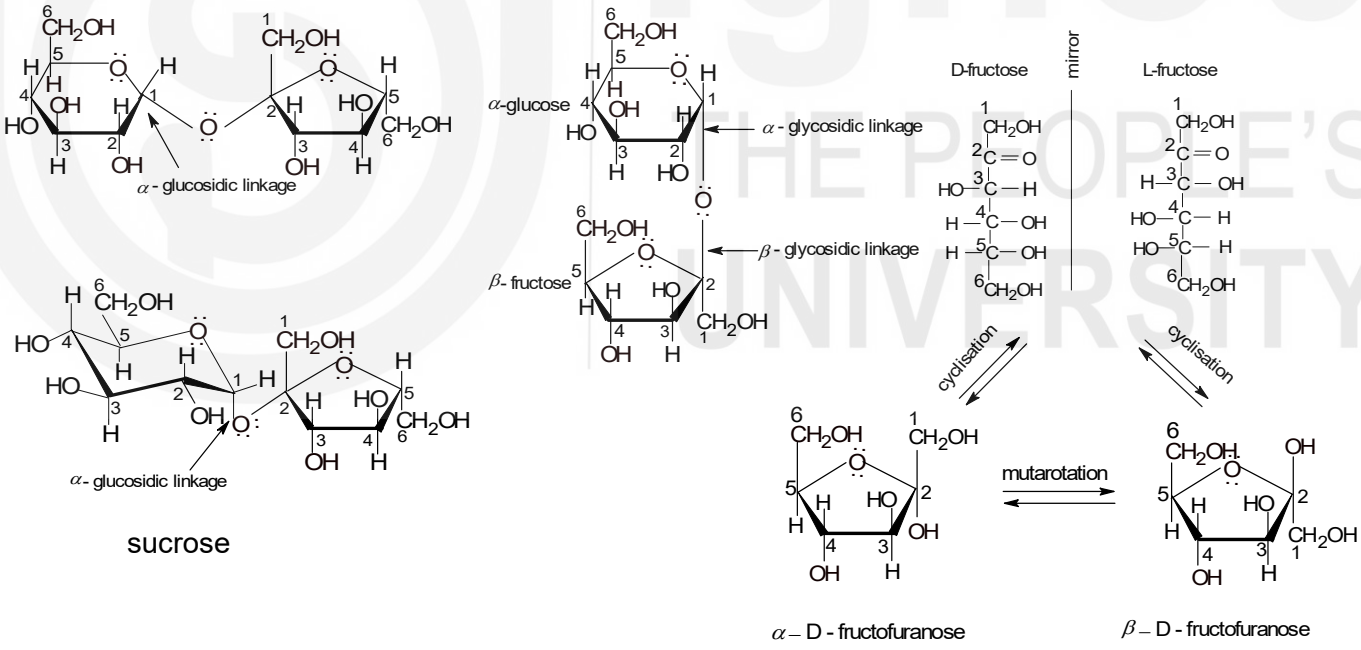
It does not reduce Tollens reagent and Fehling's solution. Thus, it is *a non-reducing sugar.* It does not form an osazone and does not show mutarotation which indicates absence of free aldehyde or ketone group which can form a hemiacetal.

Thus, the glycosidic linkage is present between the C-1 of glucose and C-2 of fructose to give the acetal linkage.

Sucrose is hydrolysed by *α-glucosidase* enzyme obtained from yeast and *not* by *β-glucosidases*. *Thus, there is α-configuration at the glucosidic (glycosidic) linkage*.

Similarly, the hydrolysis by an enzyme *sucrase* which hydrolyses only *β-fructofuranoside* linkages indicates that fructoside portion is linked by *β*-glycosidic linkage to the hydroxyl group at the anomeric carbon, i.e. C-1 of the glucose portion.

Thus, in sucrose, the glucose portion in its pyranose form is linked by *α-glycosidic* linkage to fructose portion in its furanose form by *β-glycosidic* linkage. Hence, the following structure emerges for sucrose.



sucrose

The disaccharides obtained by the formation of glycosidic linkages between the hydroxyl groups of *two anomeric groups* of two monosaccharide units are named as ***glycosyl glycosides,*** according to the recommendations of the Joint Commission referred above. According to the recommendations, *for choosing the parent glycoside, the aldose is preferred over the ketose*; therefore, sucrose is named as *β*-D-fructofuranosyl-*α*-D-glucopyranoside but *not* as *α*-D-glucopyanosyl-*β*-D-fructofuranoside.

Also note a double headed arrow is placed between 2 and 1 in the shorthand name and think why this is so.

Thus, the name of sucrose in shorthand notation can be written as

[*β*-D-Fruc*f*-(2↔1)-*α*-D-Glc*p*]

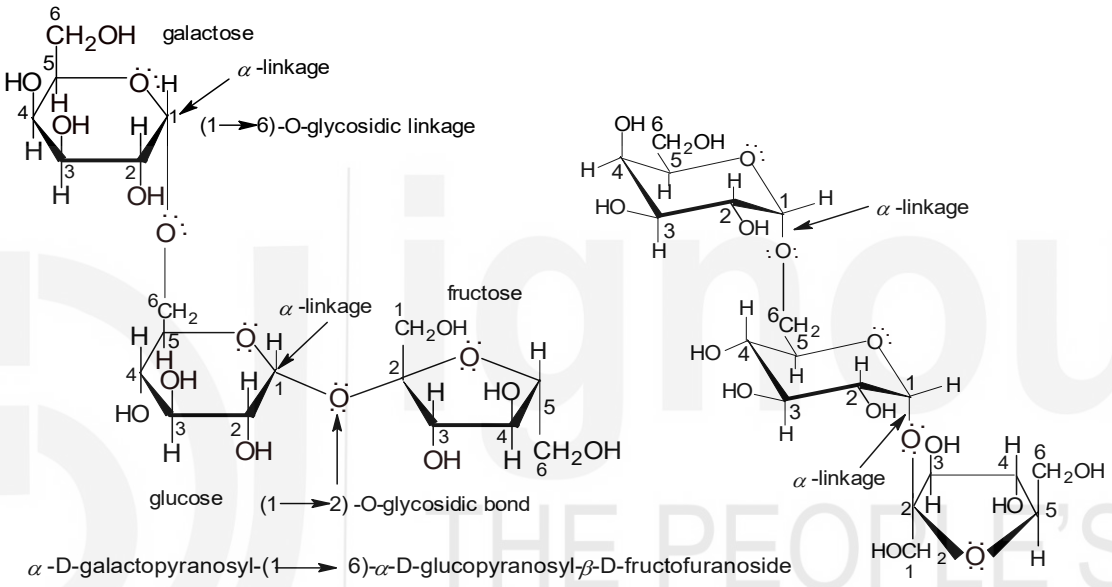
Before studying the polysaccharides in the next section, it would be worthwhile to know the nomenclature of *oligosaccharides.*

Nomenclature of Oligosaccharides

i) *Oligosaccharides without a free hemiacetal group*

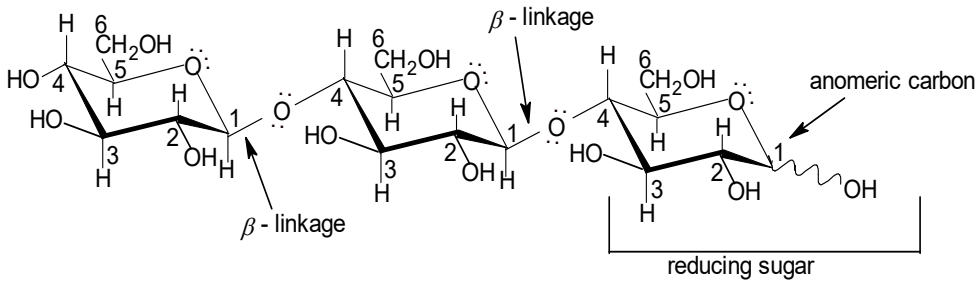
Such oligosaccharides can be named as *glycosylglycosyl glycoside* or *glycosylglycosylglycoside,* where the parent chain will be decided by rules of nomenclature as listed in the ***Recommendations 2-carb-2.1.***

Alternatively, an *end to end sequential method* is used for naming, e.g. as in case of raffinose shown below:



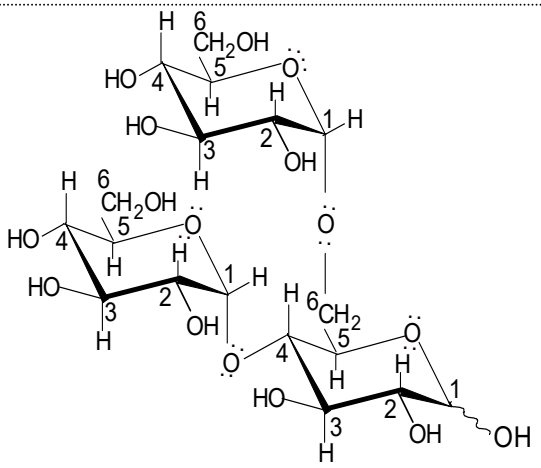
ii) *Oligosaccharides with a free hemiacetal group*

The name of such oligosaccharides is given as *glycosyl[glycosyl]$_n$ glycose*. The *glycose* or the reducing sugar being written on the *right* or *down stream* portion.



In case of branched chains, the *longest* chain is regarded as the *parent chain*. If two chains of equal length are possible, then the chain having *lower locants* for the branch point is chosen as the parent chain.

The terms designating the branches are enclosed in square brackets. An example to illustrate this is shown below:



α-D-gulcopyranosyl-(1 ⟶ 4)-[α-D-glucopyranosyl-(1 ⟶ 6)-D-glucopyranose
(isopanose)

Ref: gmul.accuk/sbcs/iupac/2carb/37.html#373

16.3 POLYSACCHARIDES

Polysaccharides are naturally occurring polymers which contain hundreds or thousands of monosaccharide units. The polysaccharides formed from a single monosaccharide are called *homopolysaccharides* while those formed from more than one type of monosaccharide are called *heteropoly-saccharides.* There are three important polysaccharides formed from the *same* monomer, i.e. glucose. These are *starch, cellulose* and *glycogen*.

These homopolysaccharides have different structures, linkages and properties. While starch and cellulose form food reserves and structural material, respectively in plants; glycogen is a carbohydrate reserve in animals.

Similar to the approach followed for disaccharides, here also, you will study the structures of starch and cellulose. The knowledge of linkages present between them will help you to understand how their properties and functions are related to their structure.

The homopolysaccharides are also called *glycans.* Those ending in the name of the monosaccharide is replaced by *an* ending in the polysaccharide. Thus, starch, cellulose and glycogen are called *glucans* (from gluc*ose*). Similarly, the homopolysaccharides of *galactose* are known as *galactans.* Let us now study about starch and cellulose in detail.

SAQ 6

What is general formula for starch, cellulose and glycogen?

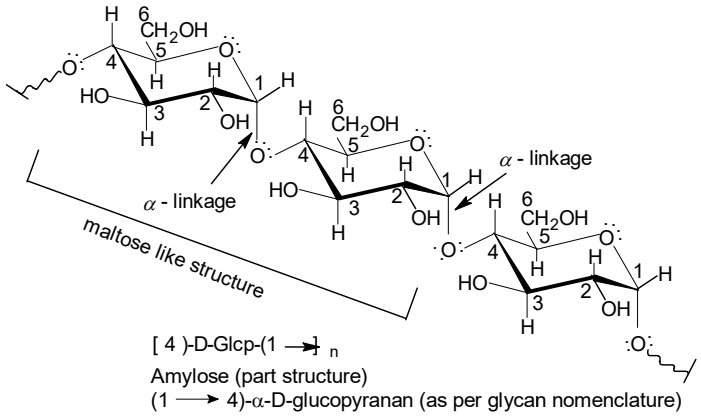
16.3.1 Starch

The major sources of starch are corn, potatoes, wheat, rice etc. which are used as sources of carbohydrates in food by humans. It occurs in the roots, tubers and seeds of plants as microscopic granules. These granules are insoluble in cold water but they swell in hot water, burst and release the water-soluble fraction called *amylose* which constitutes about 10-20%. The rest 70-80% water insoluble portions is called *amylopectin.*

In the name, the linkages between the carbon atoms forming glycosidic bonds precede the symbols designating the configuration of sugars.

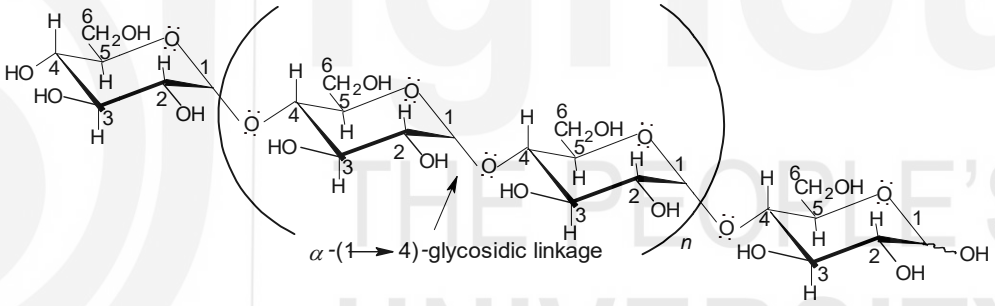
Amylose on hydrolysis yields only (+)-maltose which further hydrolyses to yield D-(+)-glucose. This indicates that amylose contains D-(+)-glucose units

linked to each other by *α*-glycosidic bonds; similar to the one you studied above in case of (+)-maltose. *Remember that* the *C-1 anomeric hydroxyl group forms a glycosidic bond with* the *-OH group at C-4 of the other glucose unit*. Thus, a part structure of amylose can be represented as shown below:



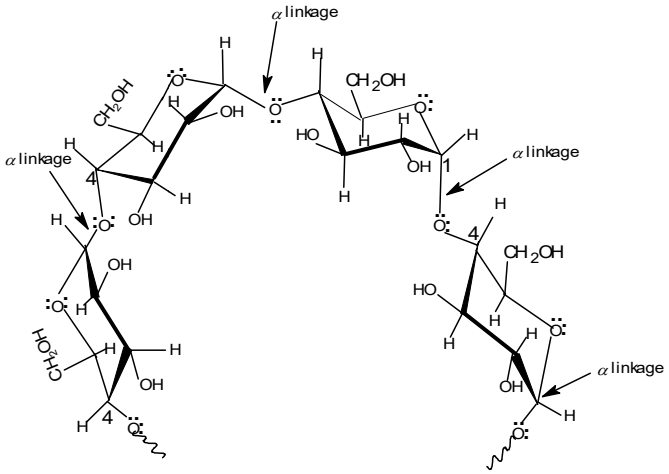
Ref: Fig. ctri.in/modifiedstarches/intriomodifiedstarches.aspe

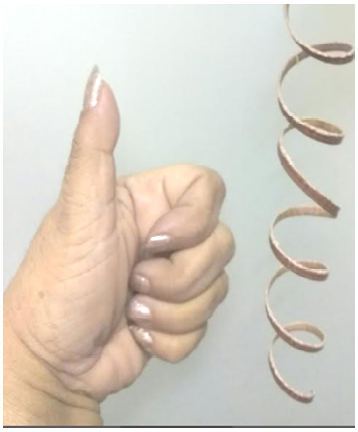
The next question then arises, how many such D-(+)-glucose units are joined together to form a chain. The studies about physical and chemical properties have shown that chains contain more than 1000 units to 4000 units and the molecular weight ranges between 1,50,000-6,00,000. Such a chain is shown below:



Thus, there is almost no branching and these chains are folded in a left handed helical structure, as given below in Fig.16.1.

The helical structure gives a compact shape to amylose molecules.





left handed helix

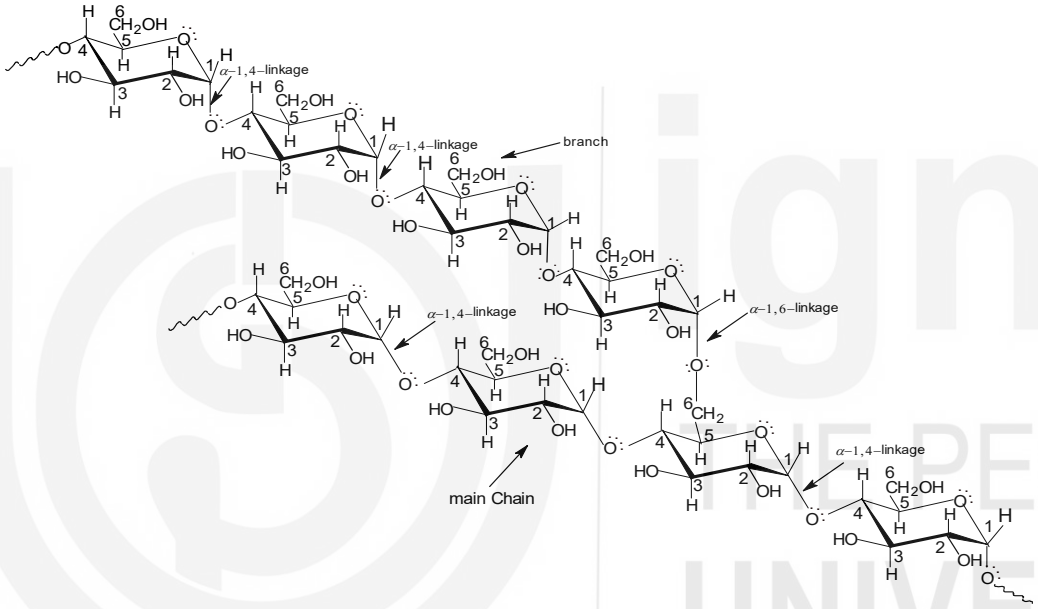
Fig. 16.1: Amylose: helical structure, left handed helix

SAQ 7

What kind of interactions are responsible for helical structure?

Similarly, the hydrolysis and other reactions along with the molecular weight determination studies of **amylopectin** showed that it has a branched structure. Its molecular weight ranges between 1 to 6 million.

Thus, amylopectin contains D-(+)-glucose units linked by $\alpha(1\rightarrow4)$ glycosidic linkages similar to that in amylose but in addition, it has several branches which have 20-25 glucose units. Such branches are linked by $\alpha(1\rightarrow6)$ linkages, i.e. C-1 hydroxyl of the branch forms a glycosidic linkage with the hydroxyl group at C-6 position of the main chain. This is shown below in Fig. 16.2 illustrating the part structure of amylopectin.



Glycogen, the energy source present in the cells of animals has highly branched structure. It has branches at every 6th unit. It also has high molecular weight, i.e. about 100 million.

Fig. 16.2: Amylopectin (part structure).

Thus, amylopectin has highly branched structure having several hundred short chains of 20-25 D-glucose units which are interconnected. This is shown below in the Fig. 16.3.

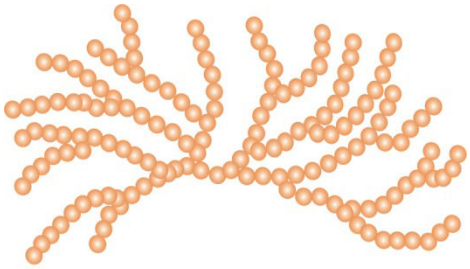


Fig. 16.3: Branched Structure of Amylopectin.

SAQ 8

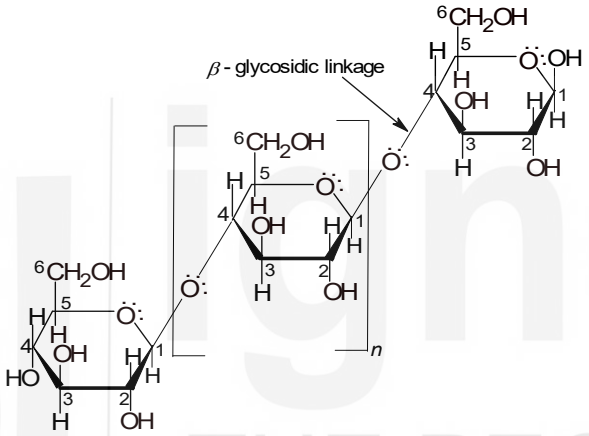
Write the part structure of amylopectin in Haworth projections.

16.3.2 Cellulose

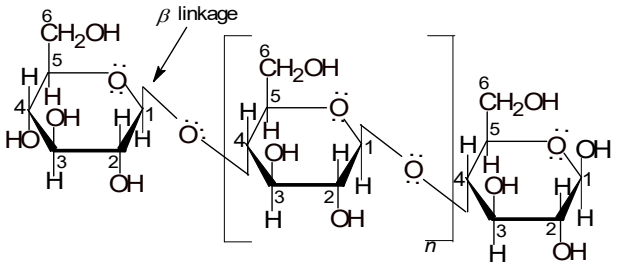
Cotton is almost pure cellulose.

Cellulose forms the main structural material in plants and gives them the rigidity. For humans, the important uses of cellulose include the structural material in the form of wood for buildings, cotton and rayon for making clothes, paper and packaging. Some of the derivatives of cellulose include *cellulose trinitrate* (gun cotton) which is used in explosives and *cellulose xanthate* from which cellulose can be regenerated to give *rayon* fibre. The treatment of regenerated cellulose with glycerol softens it and yields the *cellophane* sheets.

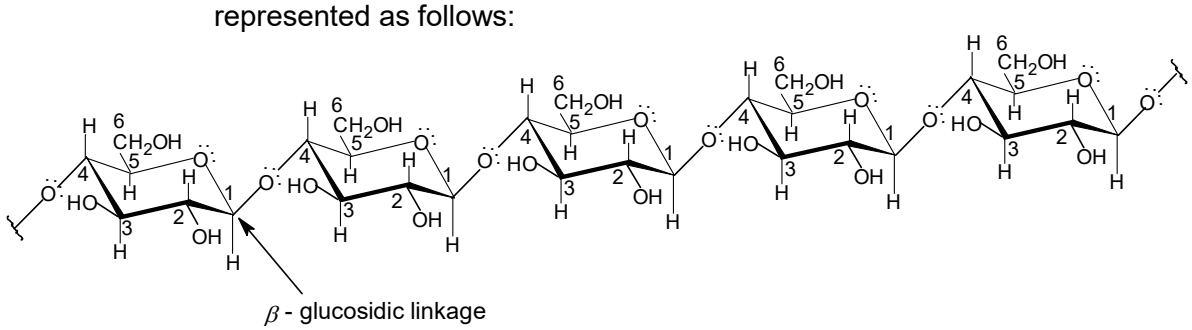
Cellulose is tasteless and insoluble in water. The molecular weight for cellulose ranges between 2,50,000 to 1,000,000 or even greater than this. Various chemical reactions and analysis by physical methods indicated that in cellulose, the D-glucopyranoside units are linked by *β*-glycosidic linkages at 1→4 positions forming long unbranched chains. The structure thus formed can be written as follows:



Note that in the *β*-glycosidic linkage, as shown in the Haworth structure above, -OH group at C-1 of one D-(+)-glucose unit which is shown *upside,* forms the linkage with the -OH group at C-4 of another D-(+)-glucose molecule which is placed *down*. This is clearly evident in the part structure of cellulose shown above. Alternatively, *β*-glycosidic linkages are also represented in the way they are shown in the following part structure of cellulose.

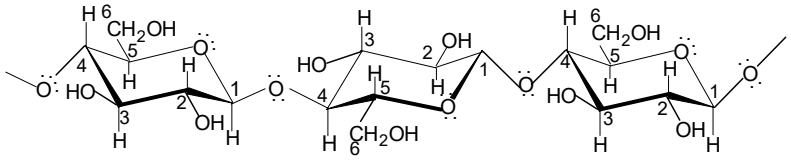


When represented as the chain conformation, the above structure can be represented as follows:



Another way of drawing the above part structure is by turning alternate pyranose rings of above structure by 180° to give the following representation for cellulose.

Scienceinschool.org/content/cellulose-trees-treats



Note that by turning the alternate ring, the positions of -OH and -CH_2OH groups do not change and they remain at equatorial position in both the representations. You can also see that in the above structure, -OH groups on alternate rings are placed on either side of the chain and hence, are uniformly distributed on both the sides of the chain. When such long chains lie side by side, these -OH groups form hydrogen bonds as shown below in Fig. 16.4.

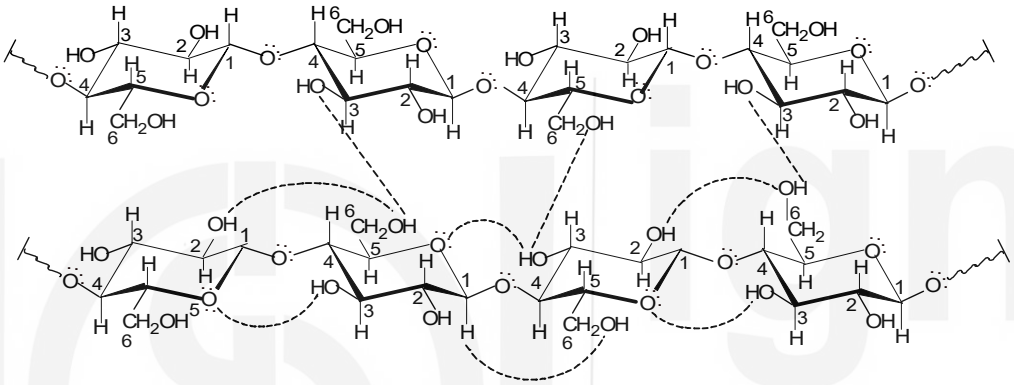


Fig.16.4: Hydrogen bonding between the chains

Many such chains, say about 40, lie laterally to form a sheet. The hydrogen bonding takes place between the hydroxyl groups across the sheets also. Such sheets stack and give a rigid, highly insoluble material which forms cell wall in plants.

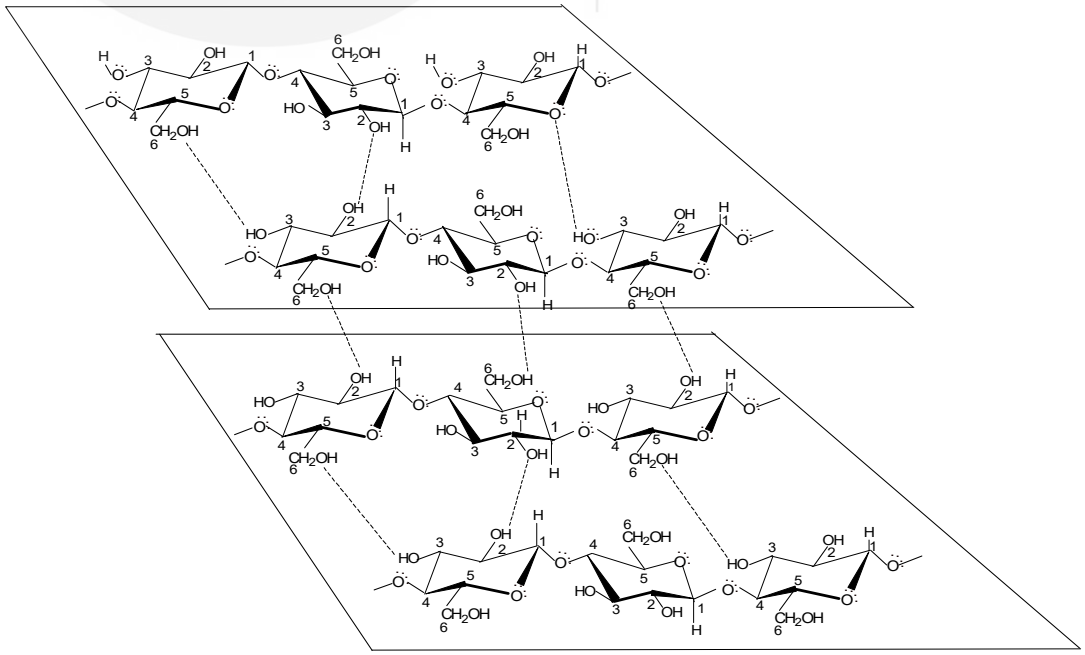


Fig: 16.5: Sheets showing H-bonding between chains.

The side by side arrangement of chains form bundles which twist to give **rope like structures in fibres.**

An interesting fact about cellulose is that humans cannot use cellulose as food whereas animals such as cows and termites can. The reason behind this is that humans do not have enzymes which can break $\beta(1\rightarrow4)$ linkages whereas symbiotic bacteria in some animals (as mentioned above) provide such enzymes to them.

16.4 SUMMARY

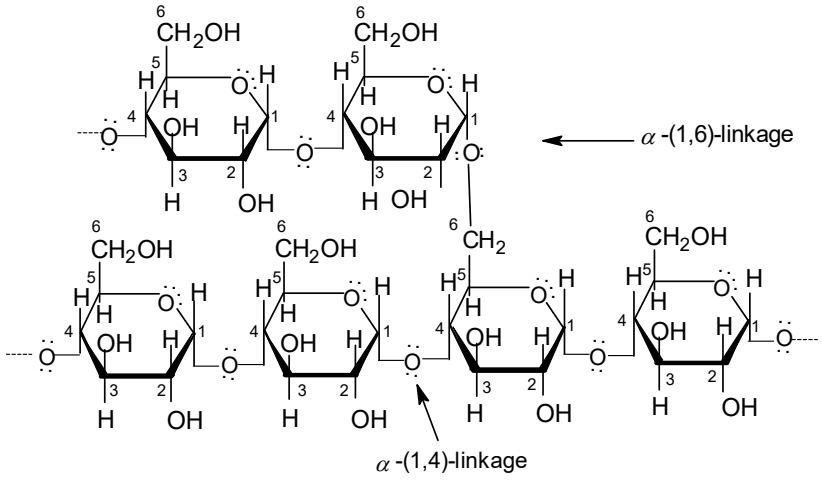
In this unit, you have learnt the following important concepts.

- Glycosides are formed when the anomeric -OH of a monosaccharide reacts with alcohols, phenols or other monosaccharide unit.
- Glycosides are named according to the monosaccharide forming them, i.e. glycoside of *glucose* is known as a *glucoside* and that formed by *fructose* is called *fructoside* and so on.
- Disaccharides are formed when two monosaccharide units are linked by a glycosidic bond.
- Disaccharides may be formed by the combination of the same monosaccharides or by two different monosaccharides.
- Maltose has two D-(+)-glucose units linked by an α-$(1\rightarrow4)$ linkage and it is a reducing sugar;
- In cellobiose, the two D-(+)-glucose units are joined by a β-glycosidic linkage.
- Lactose, present in the milk of mammals has a D-glactose unit linked to a D-glucose unit by a β-glycosidic linkage. It is also a reducing sugar.
- Sucrose, the table sugar is a non-reducing sugar.
- In sucrose the D-(+)-glucose unit is linked by α-glucosidic bond to a fructofuranoside unit which is linked by the β-glycosidic bond.
- Polysaccharides, called*glycans* are important both for plants and animals; their examples being starch, cellulose and glycogen.
- Starch consists of amylose (10-20%) and amylopectin (80-90%).
- Both amylose land amylopectin have D-(+)-glucose units linked to each other by α-$(1\rightarrow4)$ glycosidic linkages. The difference between the amylose and amylopectin is that the amylose does not have any branches but amylopectin has branching at C-6 -OH group to which the glucose unit of the branch is joined by the α-glycosidic linkage.
- Amylose can form the helical structure but amylopectin is having a branched structure and has no helices.
- The pictorial representation of amylose, amylopectin and glycogen can be shown as given below.

- Cellulose forms the structural material in plants which gives them rigidity.
- Cellulose is also a polymer of D-(+)-glucose unit in which these units are linked by *β*-(1→4) linkages. The resulting long chains lie side by side to form bundles which have H-bonding between different chains.Many such bundles form sheets. These sheets are stacked one over the other to form the structural materials in plants.
- Thedifferent properties of the disaccharides and polysaccharides arise due to their different structural features.Thus, their functions can be correlated to their properties and structures.

16.5 TERMINAL QUESTIONS

1. Write the structure of *α*-anomer of (+)-maltose and give its name.
2. Draw the structures for the conversion of *α*-form of maltose to *β*-form of maltose.
3. Write the *β*-anomer of (+)-cellobiose in chair form.
4. Write the chair representation of *α*-lactose. Also give its name.
5. Why is a double headed arrow placed between 2 and 1positions in the shorthand name of sucrose?
6. How are amylose and amylopectin different from each other?
7. What is the difference between amylose and cellulose?
8. Name the monosaccharide which forms cellulose. What type of linkages joins these monosaccharide units?
9. Which carbohydrate is represented by the following part structure?

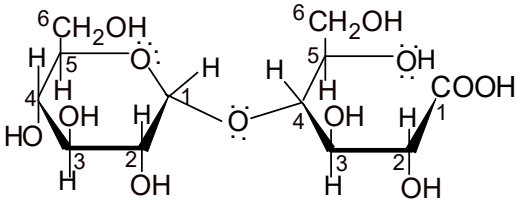


16.6 ANSWERS

Self-Assessment Questions

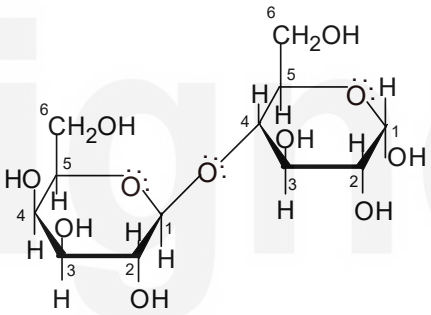
1. Yes

2. Structure of maltonic acid



3. 4-O-(*β*-D-glucopyranosyl)-*β*-D-glucopyranose

4. Howarth projection for *α*-lactose:



β-D-galactopyranosyl-(1→4)-*α*-D-glucopyranose

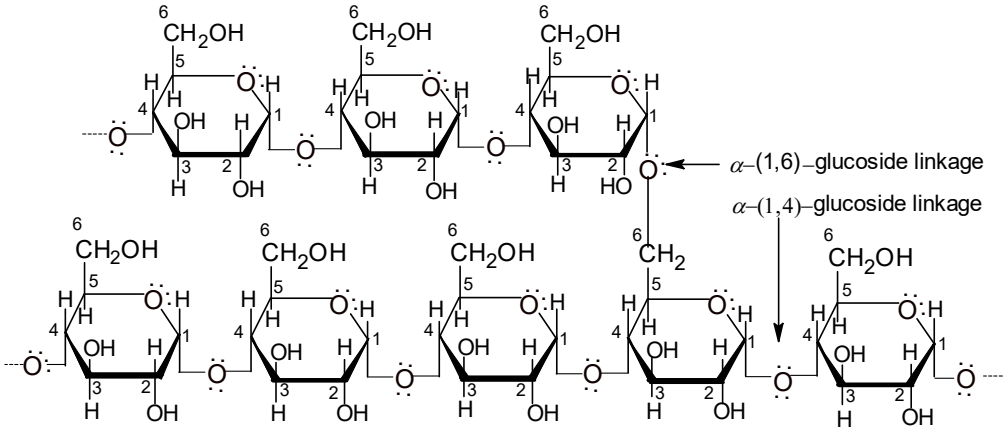
5. *β*-D-galactopyranosyl-(1→4)-*α*-D-glucopyranose

   or *β*-D-Gal*p*-(1→4)-*α*-Glc*p*.

6. $(C_6H_{10}O_5)_n$– since all of them contain the hexose, D-(+)-glucose.

7. The hydrogen bonding between the hydroxyl groups stabilises the helical structure.

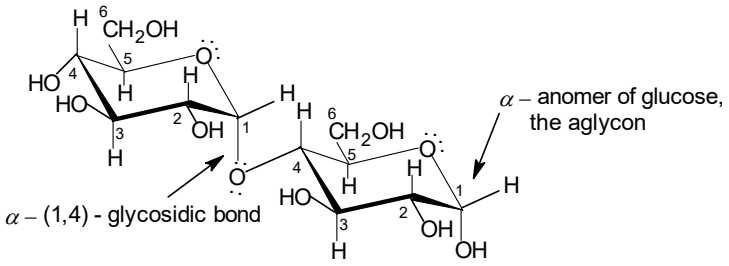
8. part structure of amylopectin in Haworth projections:



Structure of amylopectin

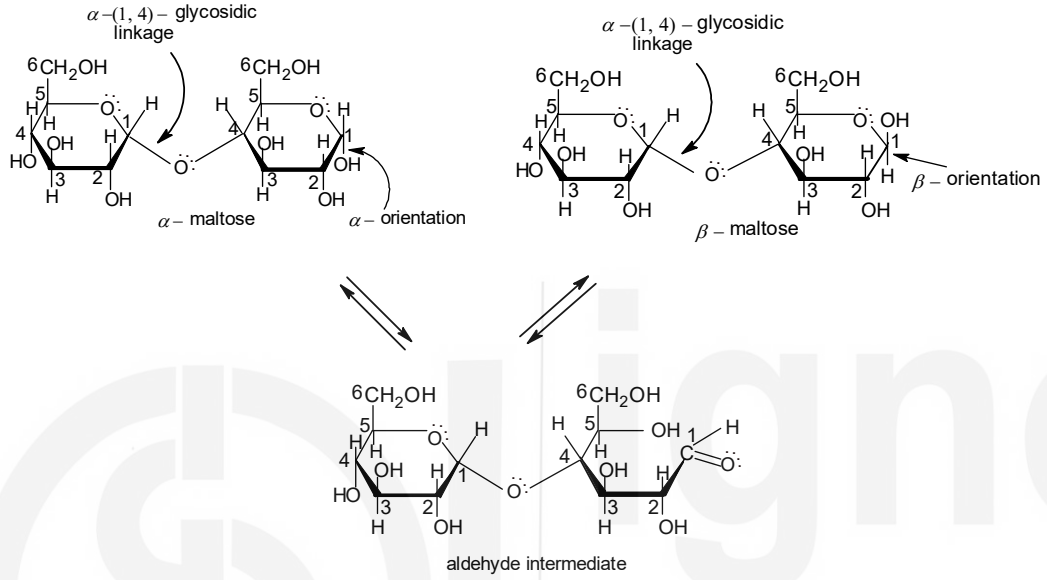
Terminal Questions

1.

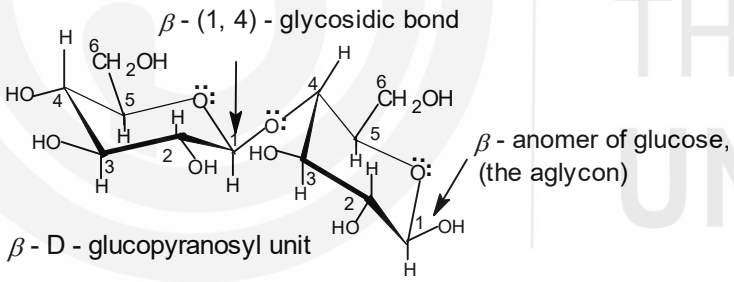


Name: 4-O-(α-D-glucopyranosyl)-α-D-glucopyranose

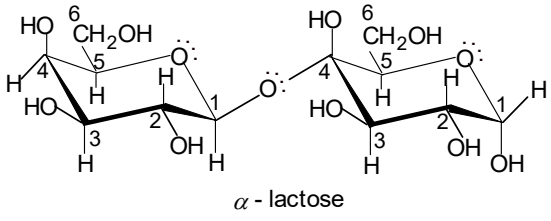
2.



3.



4.



β-D-galactopyranosyl-(1 ⟶ 4)-α-D-glucopyranose

5. Because these positions are linked by the glycosidic bonds formed by the anomeric hydroxyls placed at these positions of the respective monosaccharide units linked in that order.

6. Amylose has D-(+)-glucose units linked by α-(1→4) glycosidiclinkages with no branches. But, in amylopectin, branches occur at every 20-25 units and are linked by α-(1→6) linkages.

7. In amylose, D-(+)-glucose units are linked by α-(1→4) glycosidiclinkages while in cellulose, these units are linked by β-(1→4) glycosidic linkages.
8. D-(+)-glucose units. These units are linked by β-(1→4) linkages.
9. The structure represents the part structure of amylopectin.

References:

1. Useful website for polysaccharides – starch and cellulose

   pslc.ws/macrog/starlose.htm