

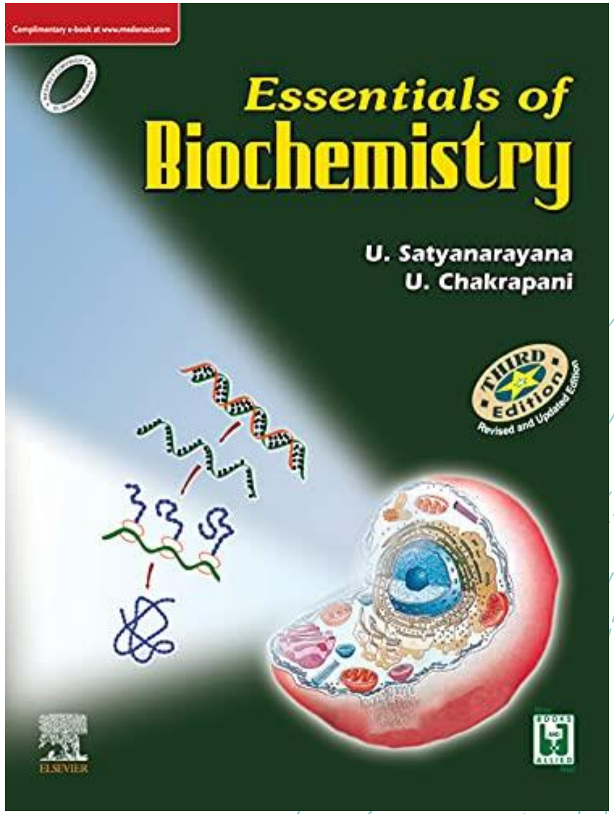

FUNDAMENTALS OF BIOTECHNOLOGY I

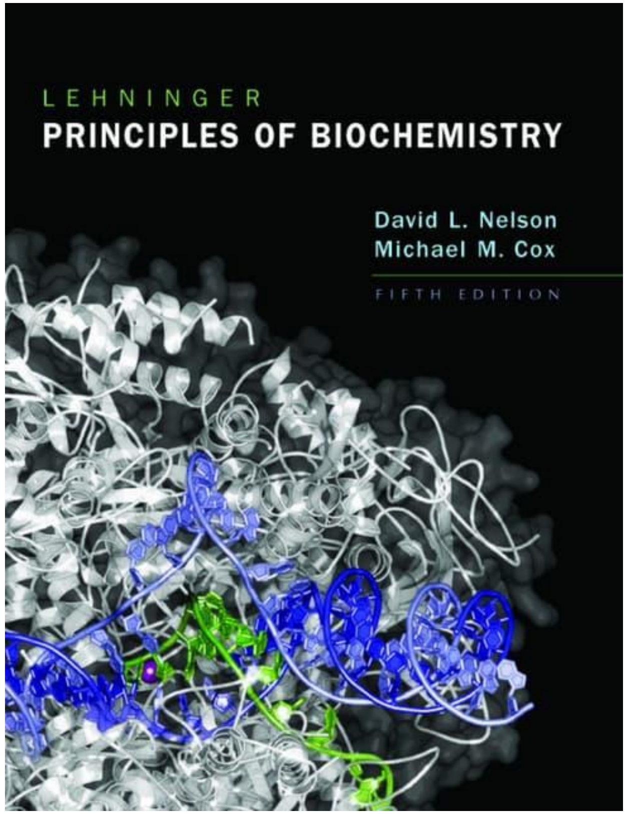
MODULE II - BIOMOLECULES

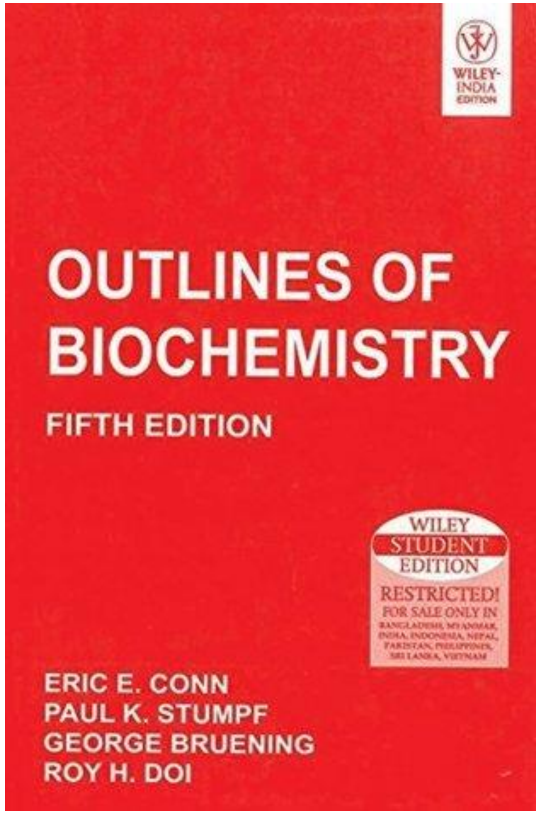
SYLLABUS

Module 2: Biomolecules
1. **Carbohydrates:** Introduction, definition, general formula & Properties. Classification of carbohydrates, Concept of glycosidic bond, Industrial applications of carbohydrates: Fermentation, Pharmaceutical and Food industry **(6 Lectures)** 2. **Introduction to Lipid Chemistry:** Definition and Biological functions of fats and Lipids. Definition of Fatty acids. Classification of Fatty acids **(4 Lectures)** 3. **Amino acids:** General introduction, Classification and structures, properties (physical & chemical), Peptide bond, Three-dimensional Structure of proteins. **(5 Lectures)**

REFERENCE BOOKS







BIOCHEMISTRY

- Biochemistry asks how the remarkable properties of living organisms arise from the thousands of different biomolecules

- Shows how collections of inanimate molecules that constitute living organisms interact to maintain and perpetuate life

- Overlaps with cell biology, genetics, immunology, microbiology, pharmacology and physiology

TABLE 1-1 Most Abundant Elements in the Human Body[a]

Element	Dry Weight (%)
C	61.7
N	11.0
O	9.3
H	5.7
Ca	5.0
P	3.3
K	1.3
S	1.0
Cl	0.7
Na	0.7
Mg	0.3

[a]Calculated from Frieden, E., *Sci. Am.* **227**(1), 54–55 (1972).

FROM MOLECULES TO LIFE

- Life matter is constructed from a limited number of elements
- Certain functional groups and linkages characterize different types of biomolecules
- Biomolecules are compounds of carbons (Organic molecules)
- Cells contain universal set of these small biomolecules

MOLECULAR COMPONENTS OF CELL

- Biological molecules are macromolecules
- Macromolecules - polymers with molecular weights above 5000 Daltons that are assembled from relatively simple monomers
- Functionally important macromolecules – Carbohydrates, lipids, proteins, nucleic acids

TABLE 1–1 Molecular Components of an *E. coli* Cell

	Percentage of total weight of cell	Approximate number of different molecular species
Water	70	1
Proteins	15	3,000
Nucleic acids		
DNA	1	1–4
RNA	6	>3,000
Polysaccharides	3	10
Lipids	2	20
Monomeric subunits and intermediates	2	500
Inorganic ions	1	20

TABLE 65.1 The major complex biomolecules of cells

Biomolecule	*Building block (repeating unit)*	*Major functions*
Protein	Amino acids	Fundamental basis of structure and function of cell (static and dynamic functions).
Deoxyribonucleic acid (DNA)	Deoxyribonucleotides	Repository of hereditary information.
Ribonucleic acid (RNA)	Ribonucleotides	Essentially required for protein biosynthesis.
Polysaccharide (glycogen)	Monosaccharides (glucose)	Storage form of energy to meet short term demands.
Lipids	Fatty acids, glycerol	Storage form of energy to meet long term demands; structural components of membranes.

CARBOHYDRATES

- Carbohydrates are the most abundant biomolecules that exist in the world
- Ultimate source of energy
- Carbohydrates are polyhydroxy aldehydes or ketones which upon hydrolysis yield these compounds
- Consists of only of carbon (C), hydrogen (H) and oxygen (O)
- Empirical formula = $C_n(H_2O)_n$

BIOLOGICAL SIGNIFICANCE OF CARBOHYDRATES

- Principal energy source - glucose
- Source of storage of energy - glycogen (in animals) and starch (in plants)
- Precursors/Intermediates in biosynthesis of fats and proteins
- Form structural and protective components – Cellulose, chitin, proteoglycans, peptidoglycans
- Associated with lipids and proteins to form surface antigens, receptor molecules, vitamins and antibiotics
- Participate in biological transport, cell-cell communication and activation of growth factors

CLASSIFICATION OF CARBOHYDRATES

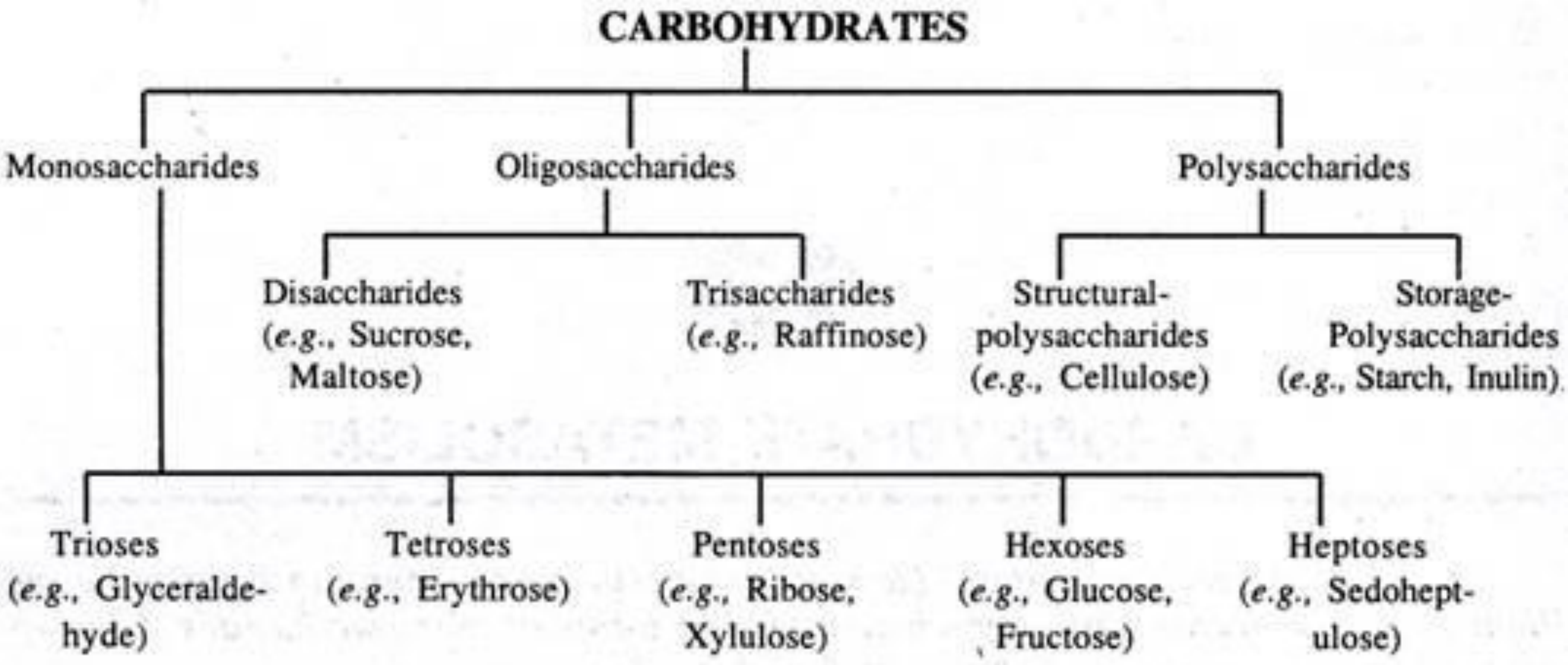
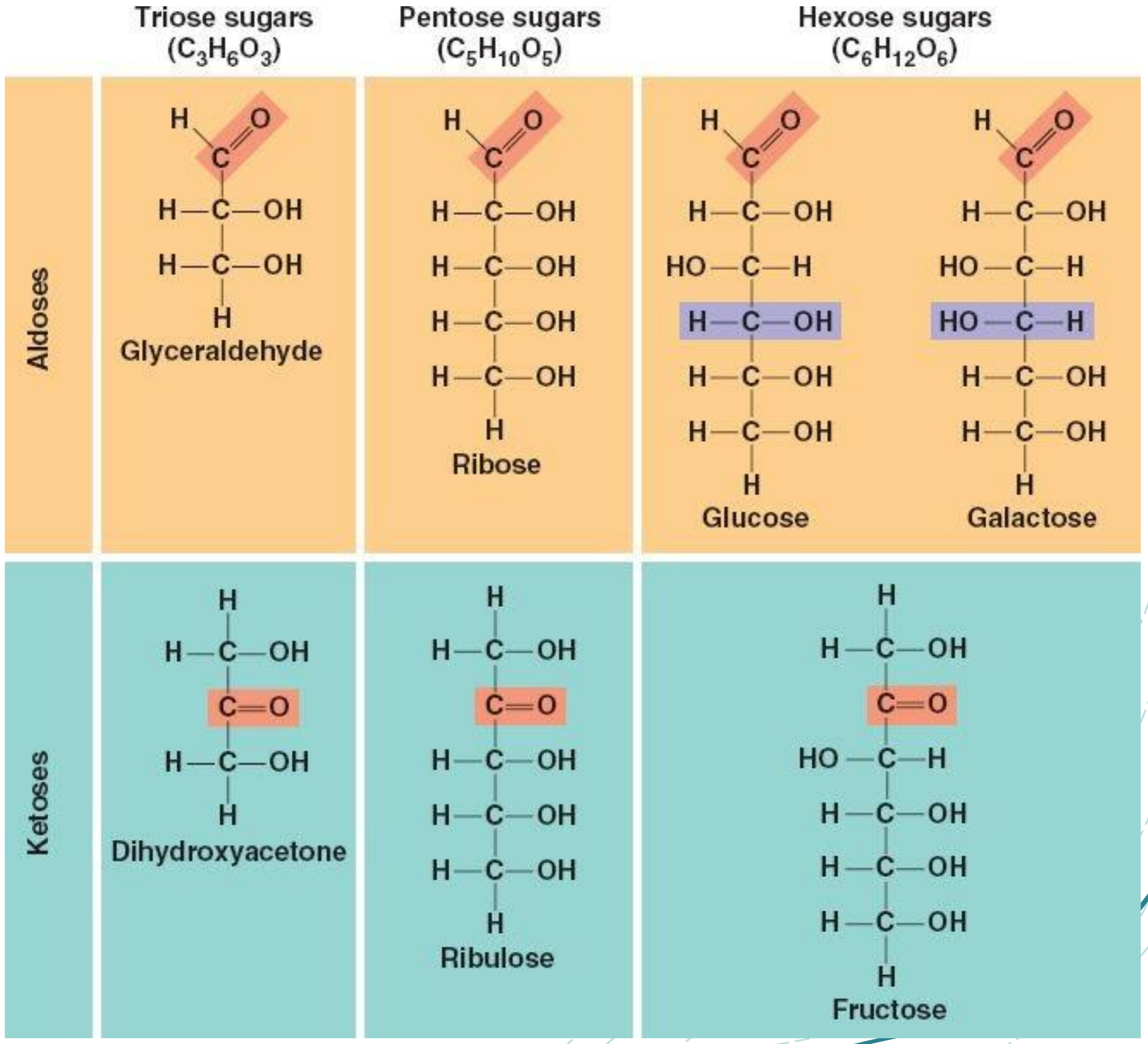


Fig. 13.1. Classification of the Carbohydrates

MONOSACCHARIDES

- Simplest group of carbohydrates - simple sugars
- Cannot be further hydrolyzed
- Colorless, crystalline solids that are soluble in water
- Possess a free aldehyde or ketone group
- Classified based on number of carbon atoms and the functional group present



OLIGOSACCHARIDES

- Sugars that yield 2 to 10 molecules of the same or different monosaccharides on hydrolysis
- Monosaccharide units are joined by glycosidic linkage
- Classified based on number of monosaccharide units
- General formula of disaccharides = $C_n(H_2O)_{n-1}$
- General formula of trisaccharides = $C_n(H_2O)_{n-2}$
- Examples: Lactose, Maltose, Sucrose

GLYCOSIDIC LINKAGE

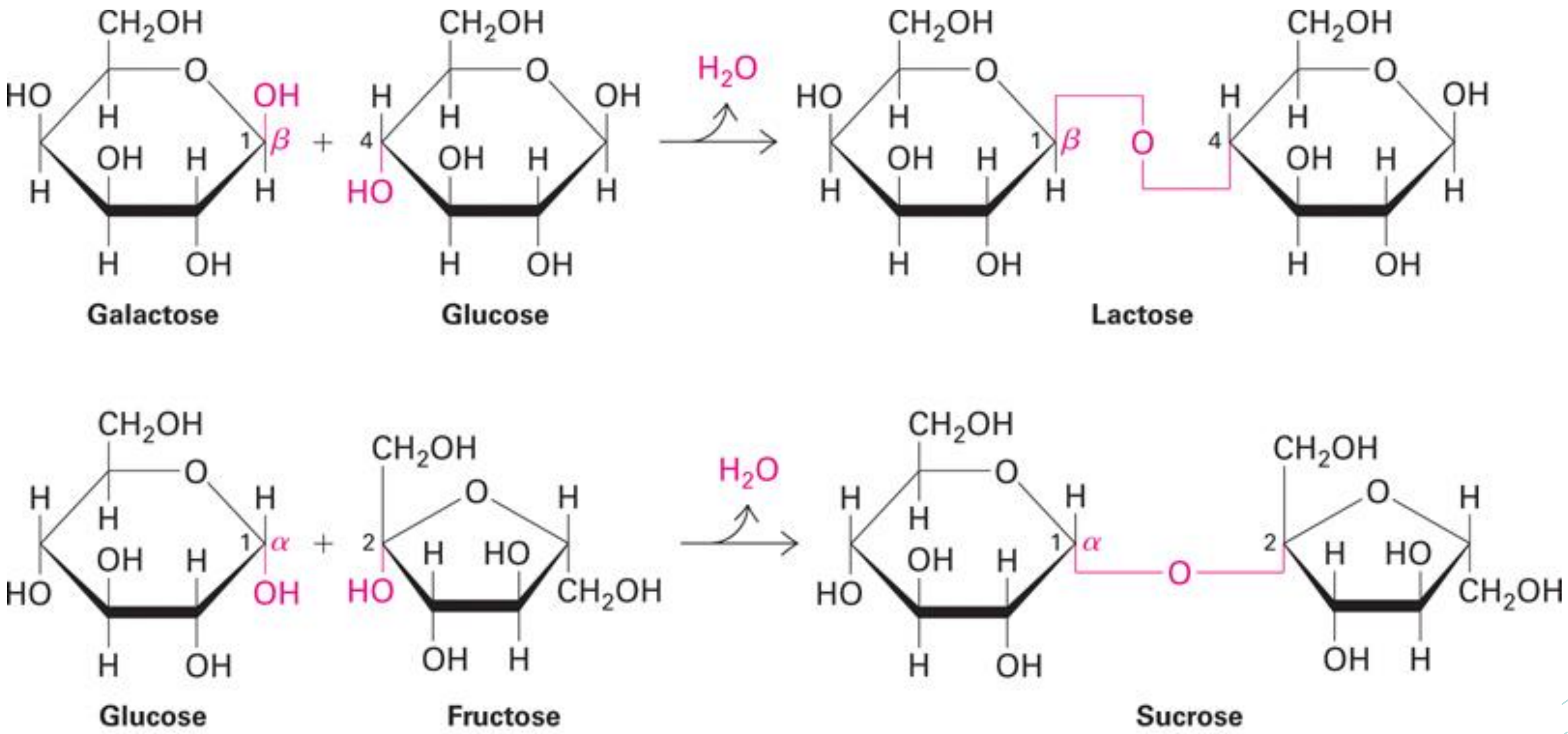
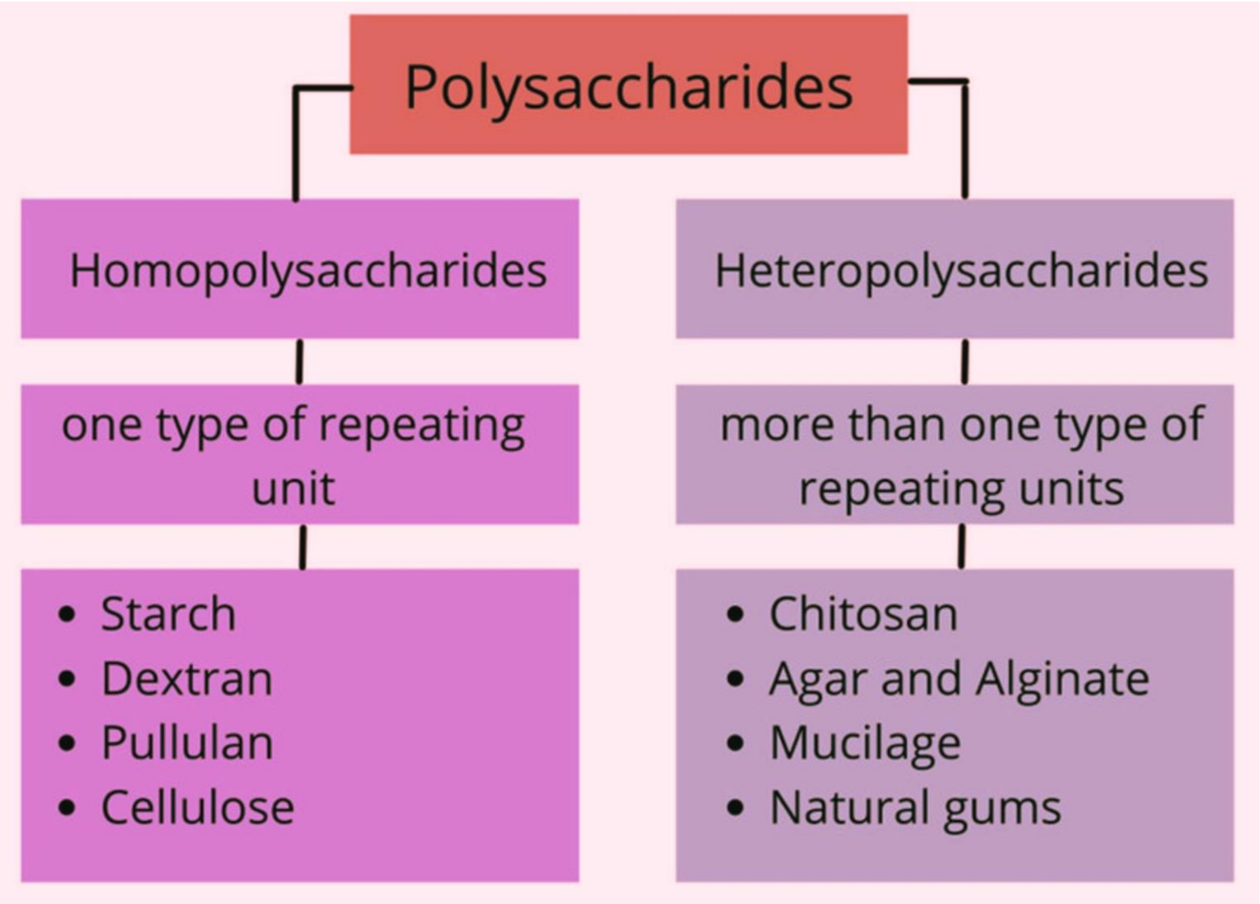
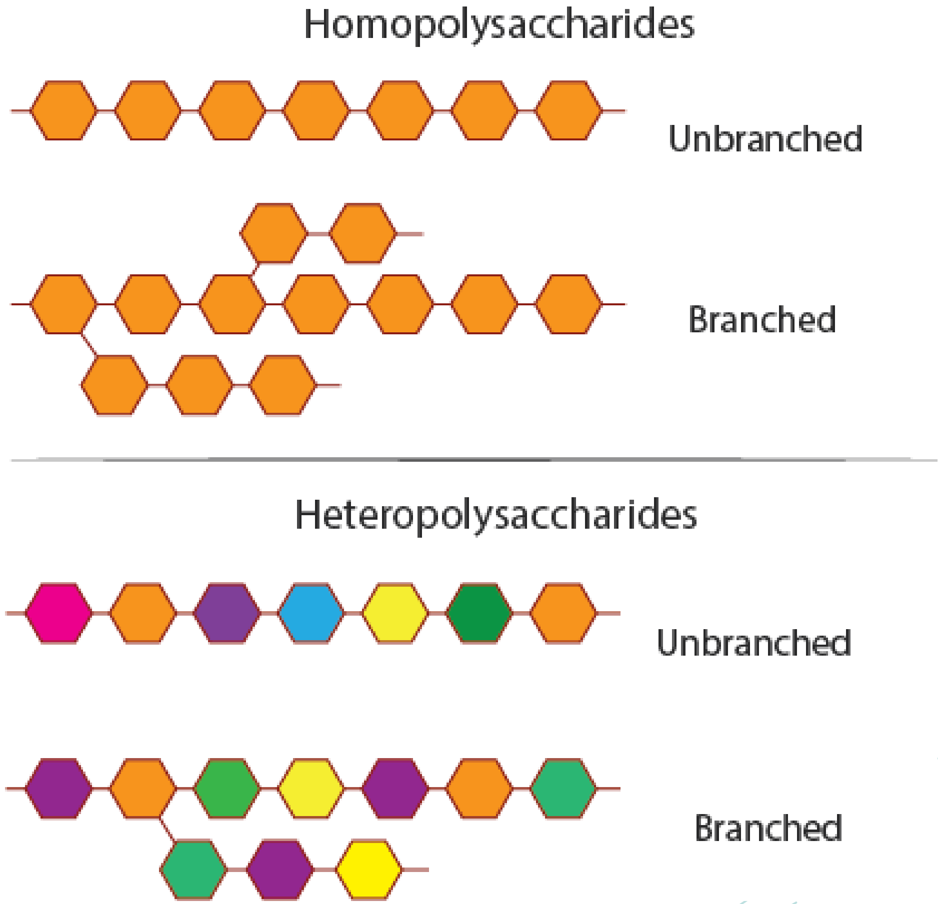


Figure 2.19
Molecular Cell Biology, Seventh Edition

POLYSACCHARIDES

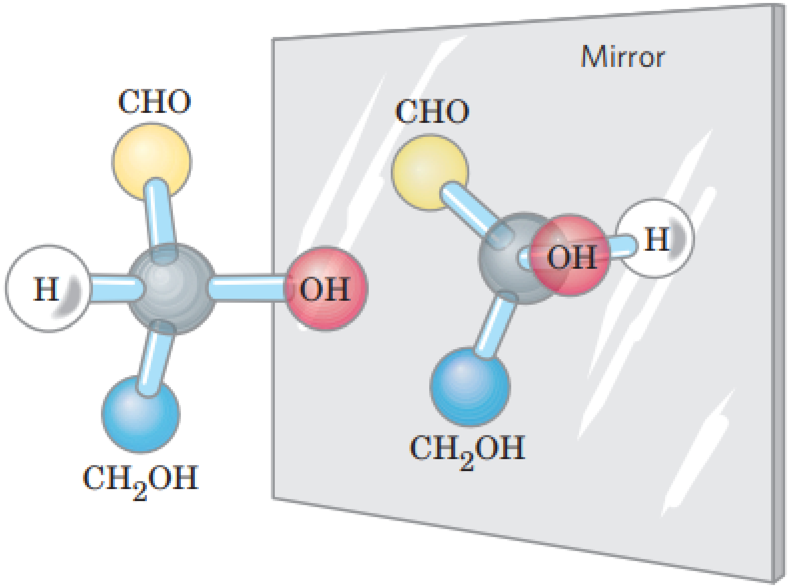
- Polysaccharides contain more than 10 monosaccharide units
- Can be hundreds of sugar units in length
- Classified depending on the type of molecules produced as a result of hydrolysis
- Polysaccharides differ from each other in
  - the identity of their recurring monosaccharide units
  - the length of their chains
  - the types of bond linking units
  - the degree of branching


Polysaccharides
Homopolysaccharides
Heteropolysaccharides
one type of repeating unit
more than one type of repeating units
• Starch
• Dextran
• Pullulan
• Cellulose
• Chitosan
• Agar and Alginate
• Mucilage
• Natural gums

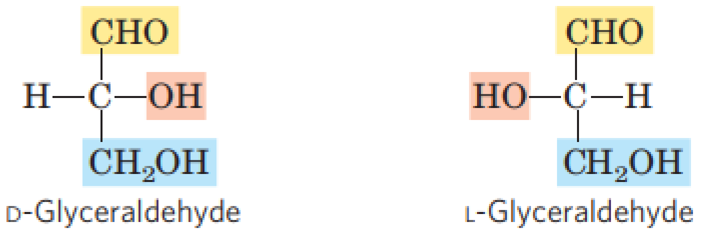

Homopolysaccharides
Unbranched
Branched
Heteropolysaccharides
Unbranched
Branched

CARBOHYDRATE STRUCTURE – FISCHER PROJECTIONS

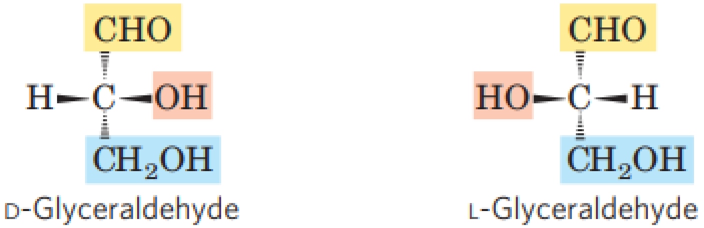
- 2-D structures
- Horizontal bonds project out of the plane of the paper, toward the reader
- Vertical bonds project behind the plane of the paper, away from the reader
- Intersection of a horizontal and a vertical line represents the central carbon atom
- First carbon (C1) is the highest priority functional group which is placed at the top



Ball-and-stick models



Fischer projection formulas

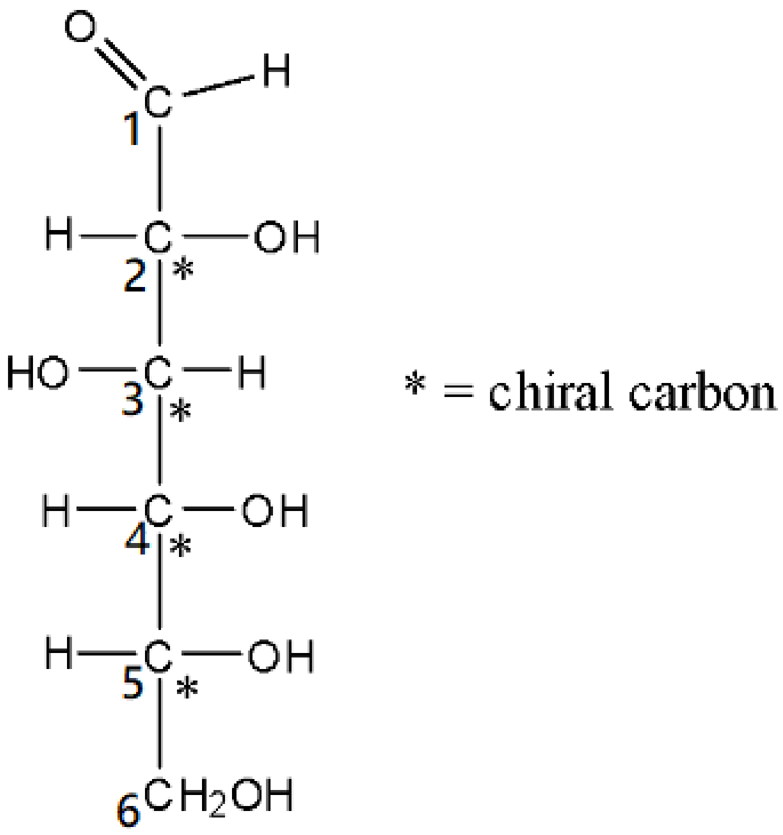


Perspective formulas

CARBOHYDRATE STRUCTURE

- **Chiral center** - an asymmetric atom in a molecule that is bonded to four different chemical species, allowing for isomerism

- **Isomerism** - a phenomenon where two or more compounds have the same chemical formula but possesses different structural formulas and different properties



D-Glucose

Isomers
Same molecular formula but different structures

Stereoisomers
Atoms are connected in the same order but differ in spatial arrangement

Enantiomers
Non-superimposable mirror images

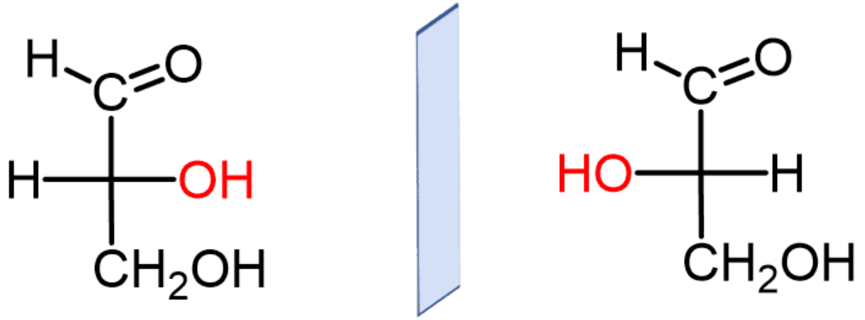
Diastereoisomers
Isomers that are not mirror images

Anomers
Isomers differ at a new carbon atom formed on ring formation

Epimers
Differ in the orientation of a -OH at only one stereocenter other than the anomeric carbon

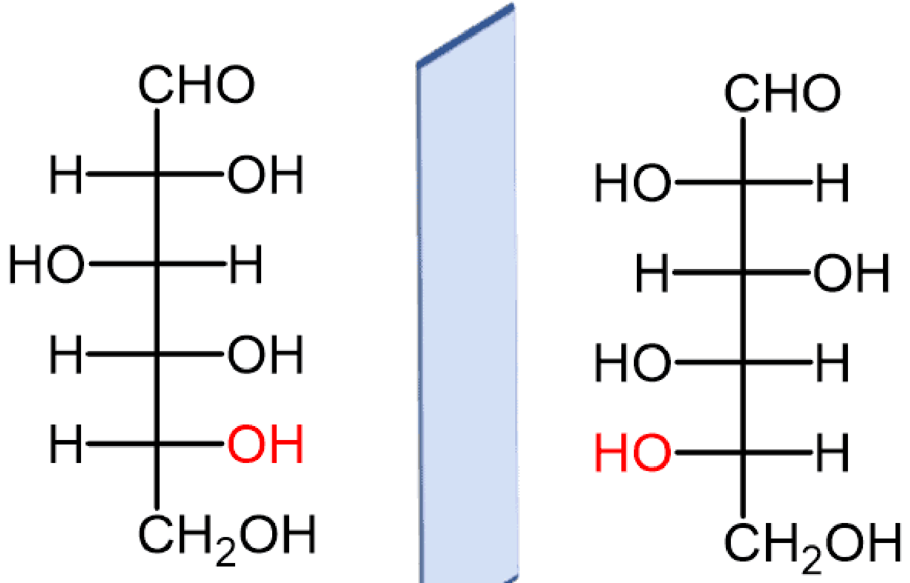
ENANTIOMERS

- Same molecular formula
- Mirror images
- Same physical and chemical properties
- EXCEPT for rotation of plane polarized light and their ability to interact with biological molecules
- Same name with different D- or L- designation



enantiomers

D-glyceraldehyde L-glyceraldehyde

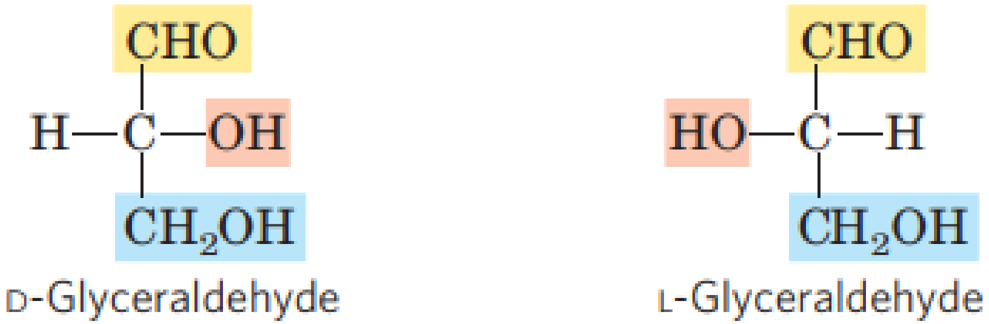


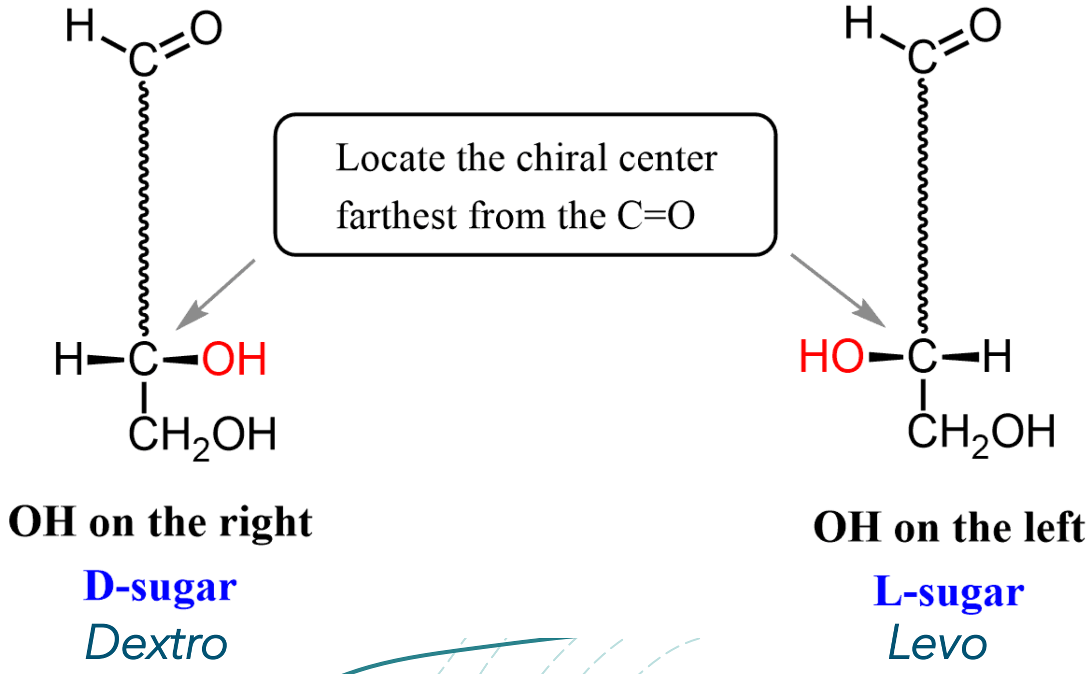
D-glucose L-glucose

Enantiomers

REFERENCE CARBON ATOM OF SUGARS

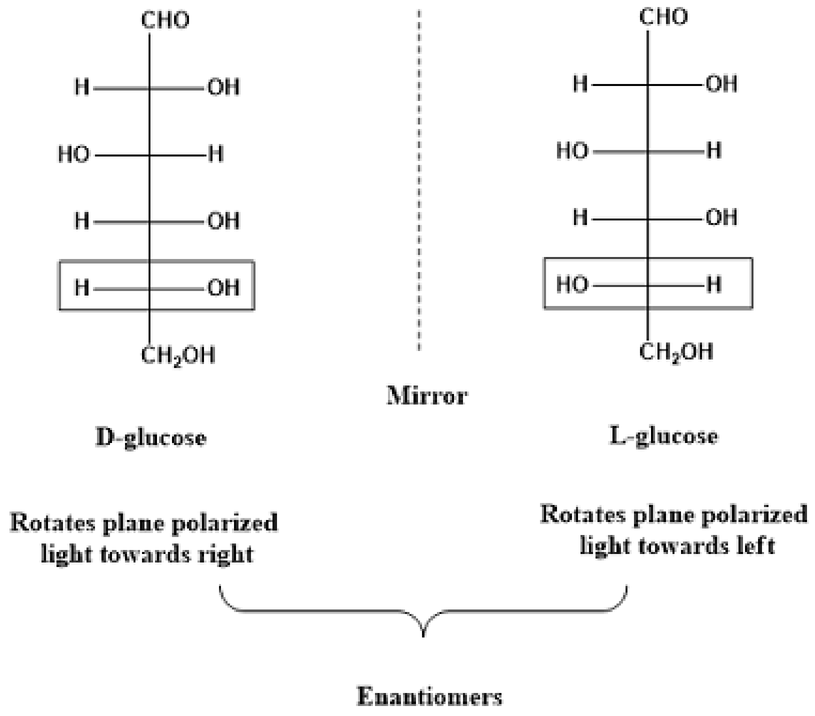
- All monosaccharides are molecules derived from glyceraldehyde by successive addition of carbon atoms
- Penultimate carbon atom is the reference carbon atom for naming the mirror images of sugars





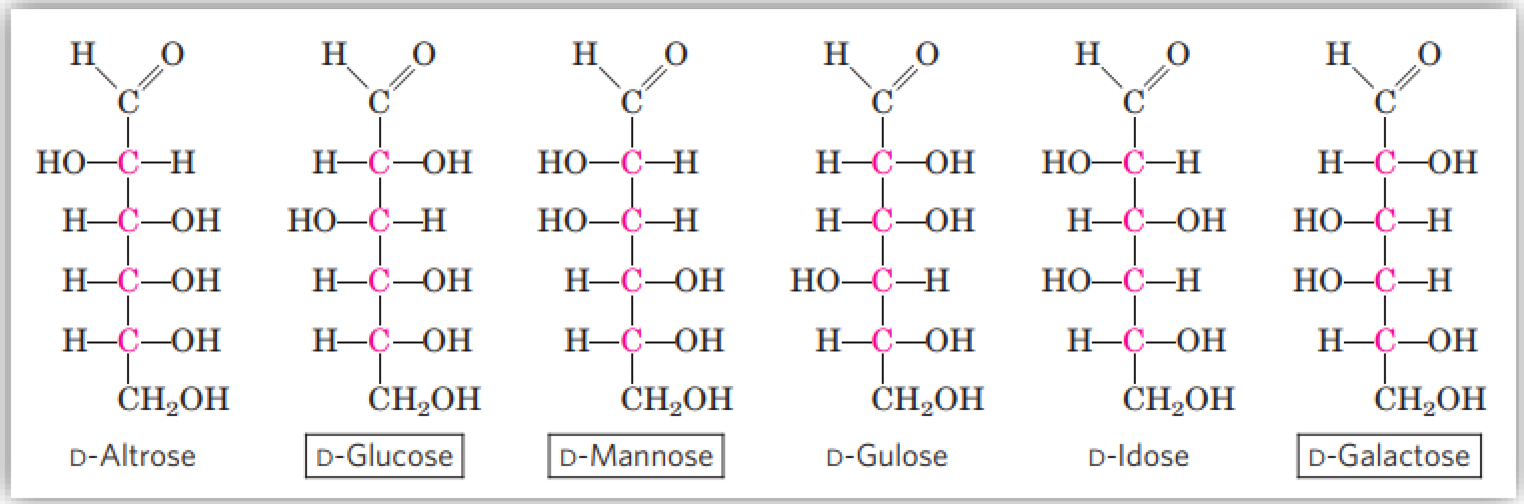
OPTICAL ACTIVITY OF SUGARS

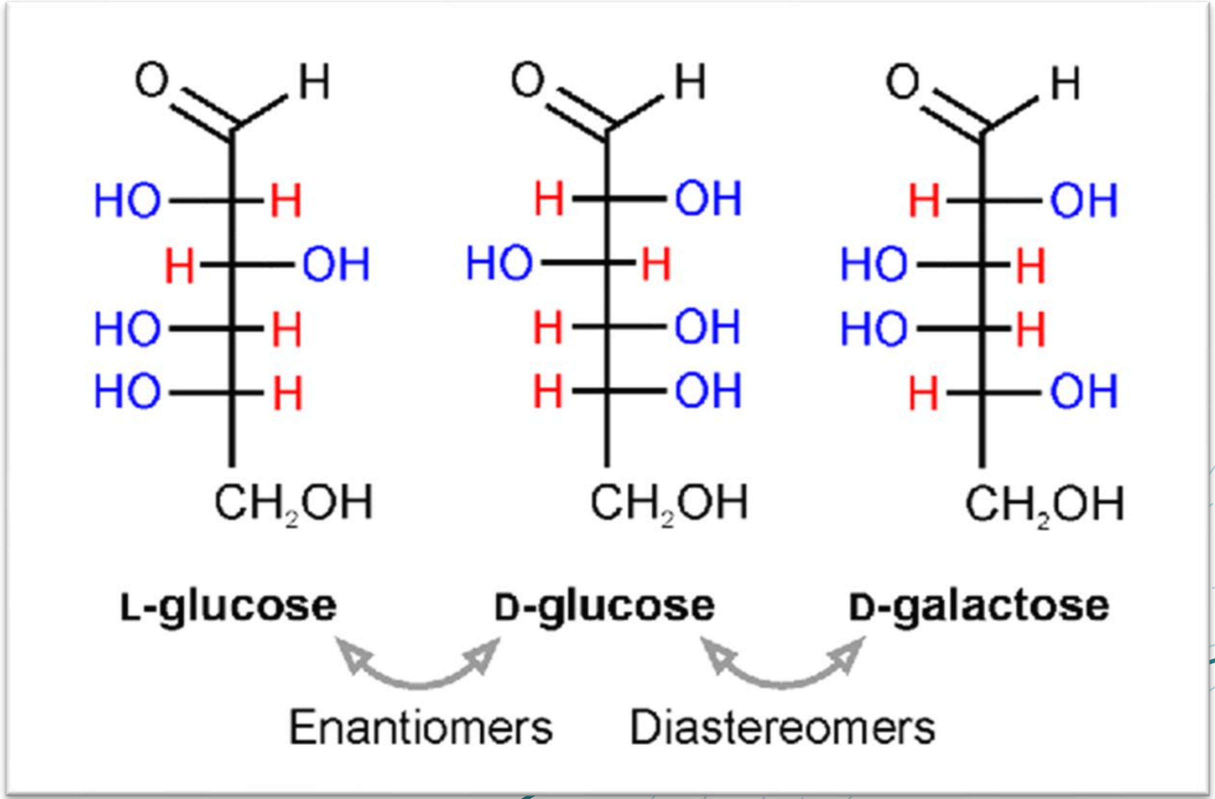
- Presence of asymmetrical carbon atom causes optical activity
- When a beam of plane polarized light is passed through a solution of carbohydrates, it will rotate the light either to right or to left
- Depending on the rotation, molecules are called
  - Dextrorotatory (+) (D) – rotates light to the right
  - Levorotatory (–) (L) – rotates light to the left
- Racemic mixture - Equimolecular mixture of optical isomers; has no net rotation



DIASTEREOMERS

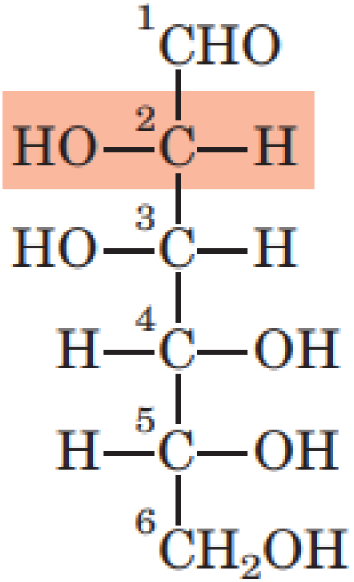
- Same molecular formula
- Not mirror images
- Different physical & chemical characteristics
- Different names
- Some diastereomers differ only at one position, while others differ at multiple stereocenters



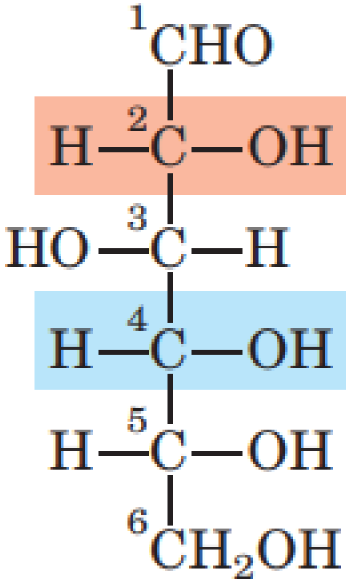


EPIMERS

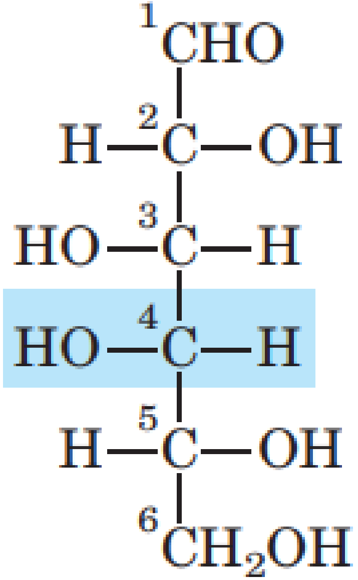
- Diastereomers that contain more than one chiral center but differ from each other in the absolute configuration at only one chiral center



D-Mannose
(epimer at C-2)



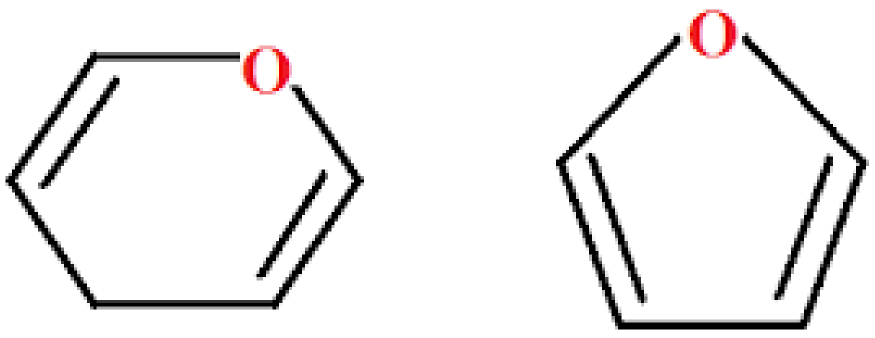
D-Glucose



D-Galactose
(epimer at C-4)

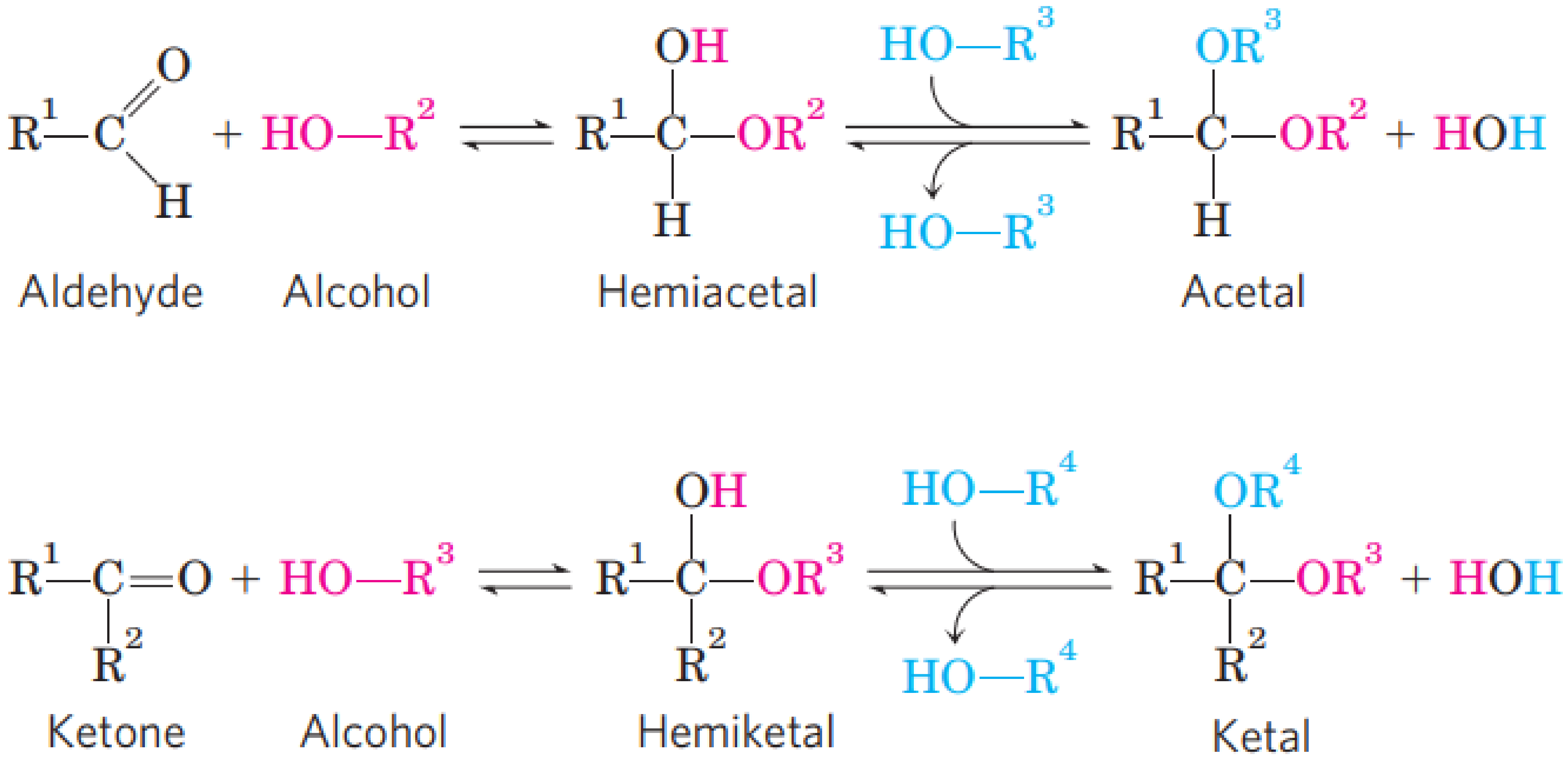
SUGAR CYCLIZATION

- In aqueous solution, aldotetroses and all monosaccharides with five or more carbon atoms in the backbone occur predominantly as cyclic (ring) structures
- Formation of covalent bond between carbonyl group and hydroxyl group along the chain

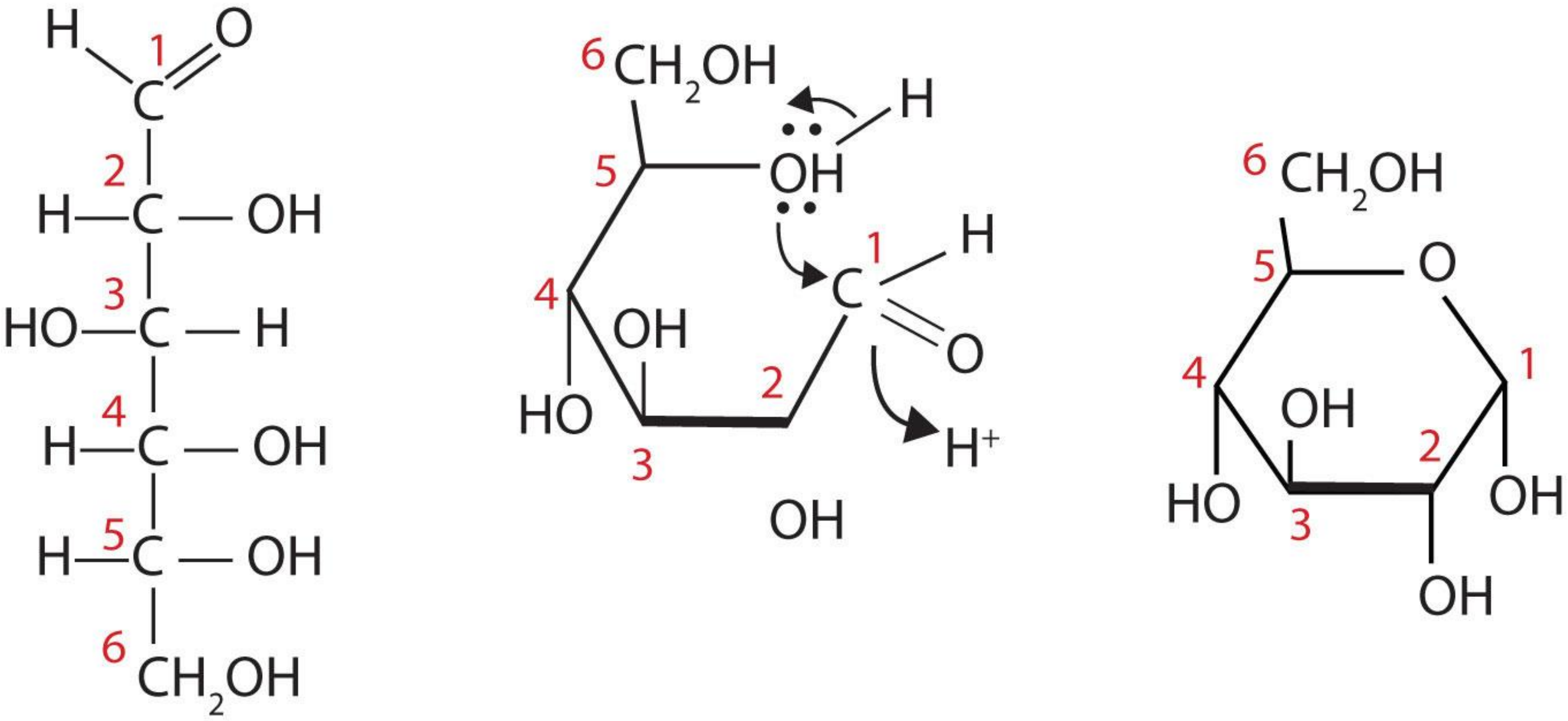


Pyran Furan

FORMATION OF HEMIACETALS AND HEMIKETALS



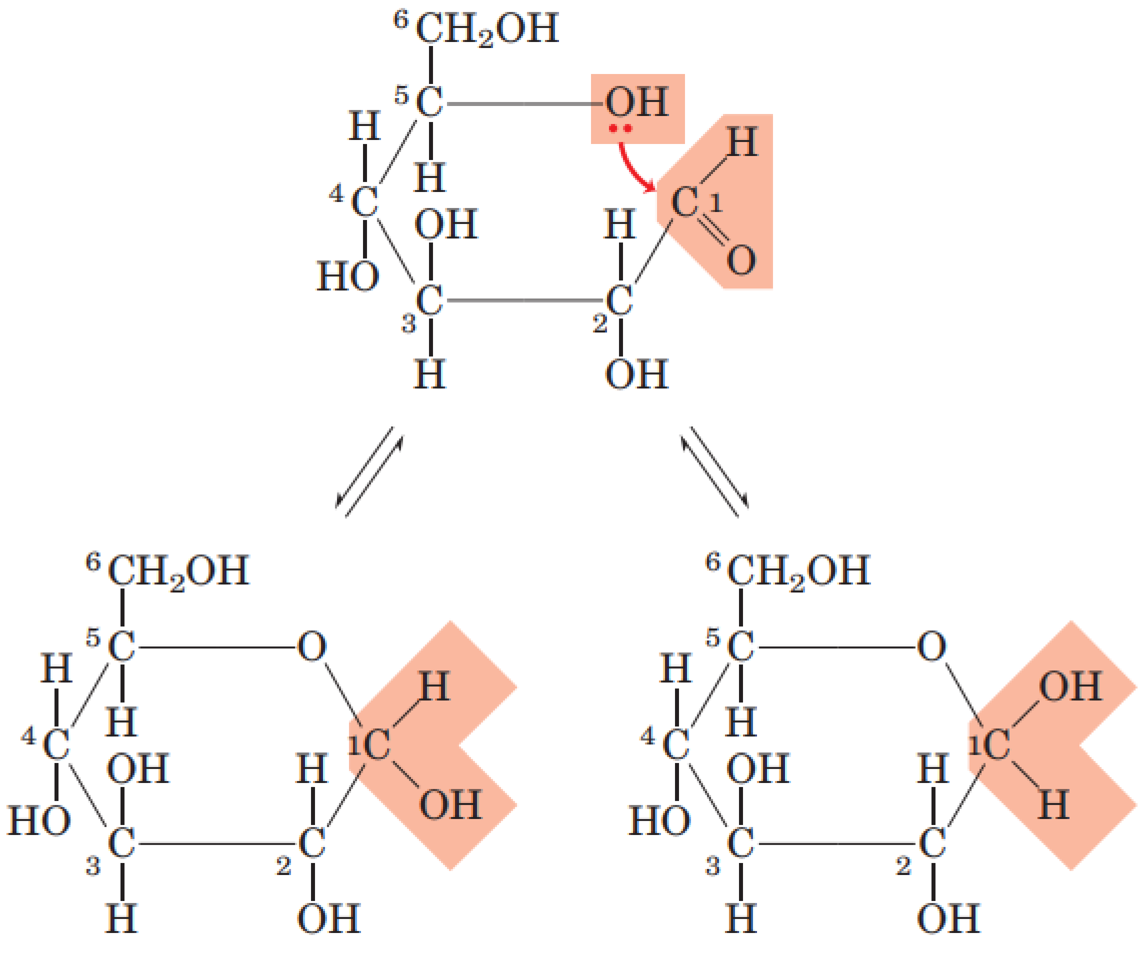
GLUCOSE CYCLIZATION



(a) Fischer projection (b) Three-dimensional representantion (c) Cyclic monosaccharide

CYCLIC FORMS OF GLUCOSE

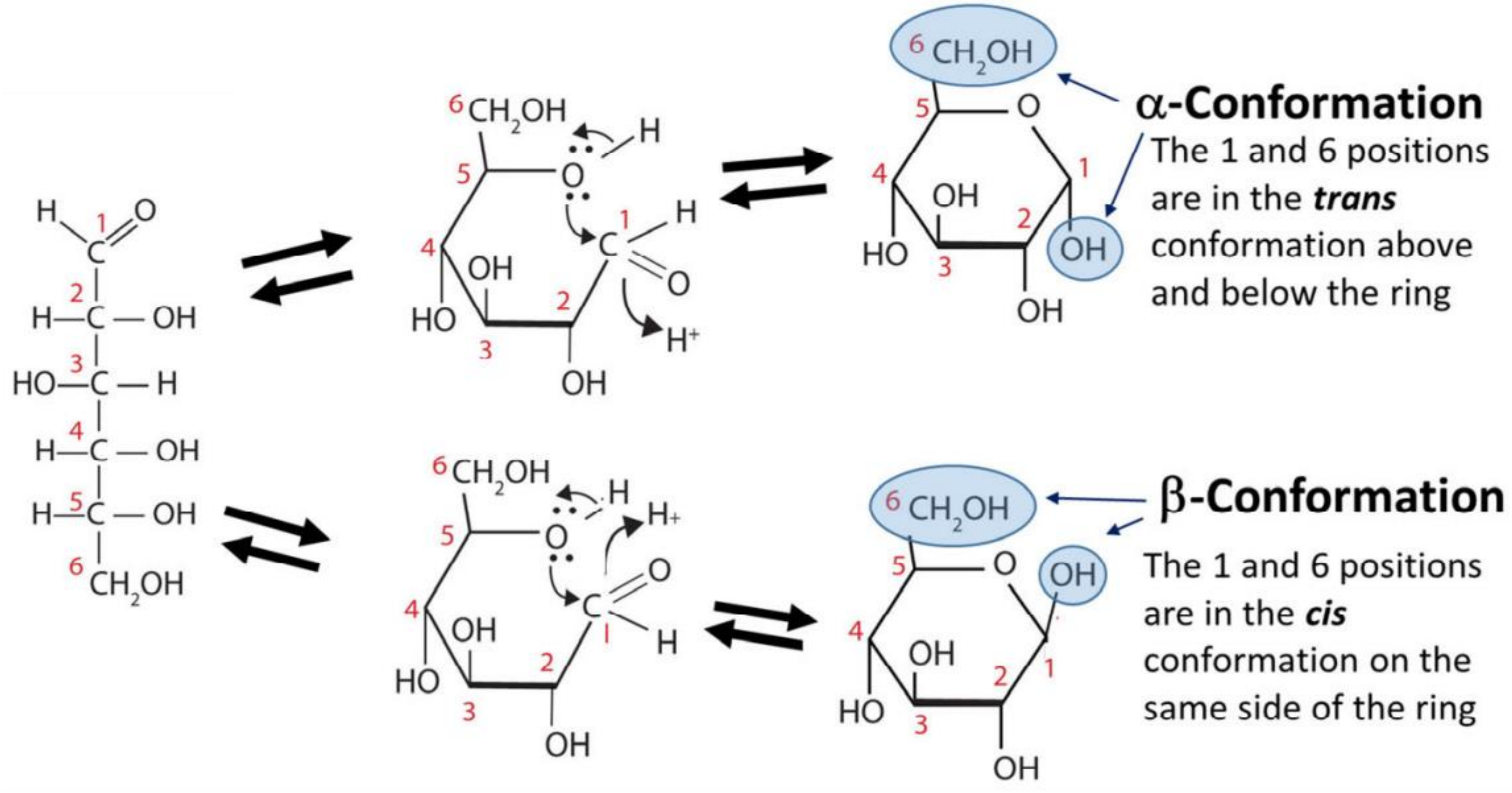
- The reaction with the first molecule of alcohol creates an additional chiral center (carbonyl carbon)
- Alcohol can add in either of two ways, attacking either the "front" or the "back" of the carbonyl carbon
- This reaction can produce either of two stereoisomeric configurations, denoted α and β



α-D-Glucopyranose

β-D-Glucopyranose

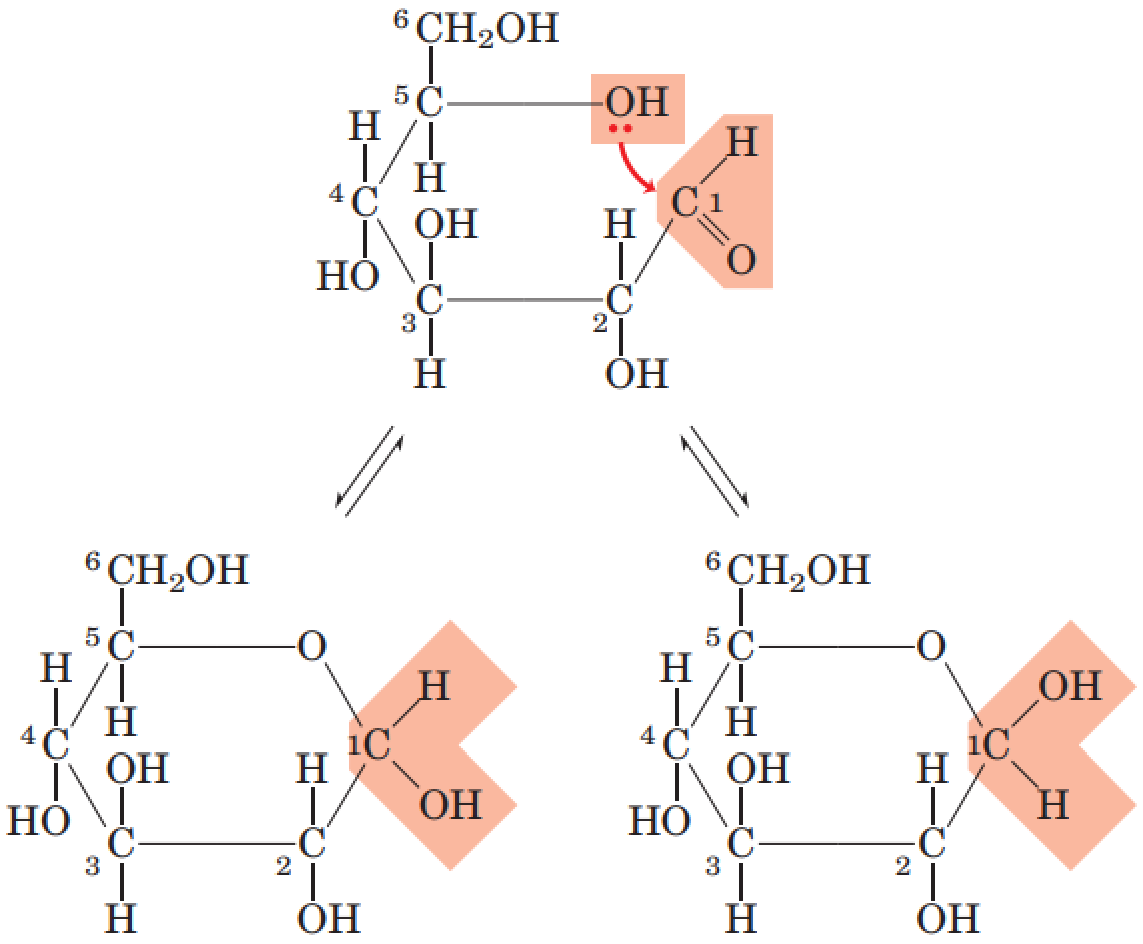
ANOMERS



α-Conformation
The 1 and 6 positions are in the ***trans*** conformation above and below the ring
β-Conformation
The 1 and 6 positions are in the ***cis*** conformation on the same side of the ring

ANOMERS

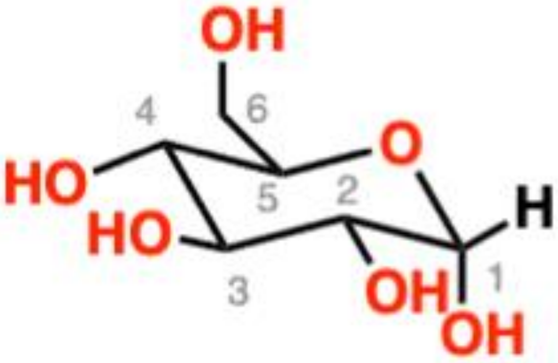
- Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom
- **Anomeric carbon** - the carbonyl carbon atom (C1 in glucose)
- **Mutarotation** - a process by which α and β anomers of D-glucose interconvert in aqueous solution



α-D-Glucopyranose β-D-Glucopyranose

MUTAROTATION

"alpha" (α) isomer:



α-D-Glucose

Specific rotation: $[\alpha]_D^{20}$ + 112°

for pure form

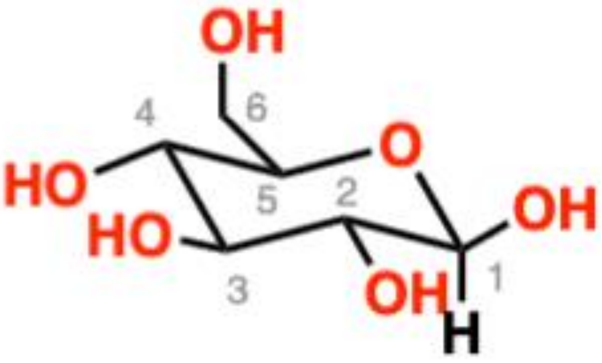
36%

dissolve in water →

Specific rotation changes over several hours until reaching a stable value of +52.5

← *dissolve in water*

"beta" (β) isomer:



β-D-Glucose

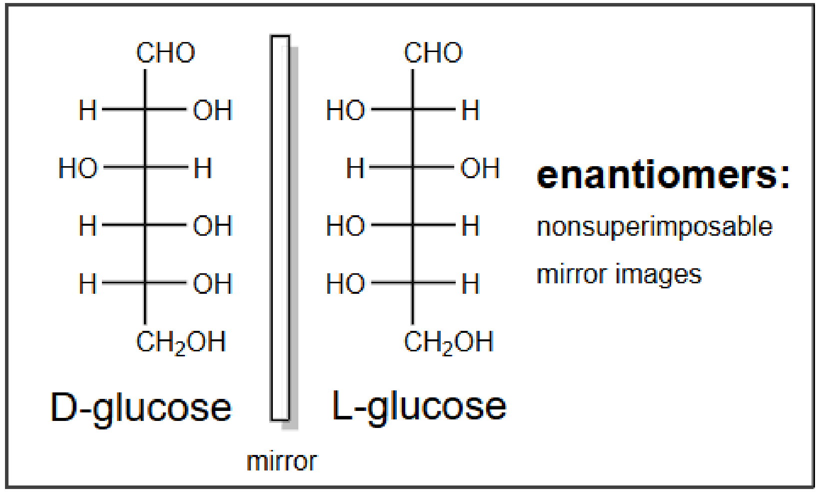
Specific rotation: $[\alpha]_D^{20}$ + 18.7°

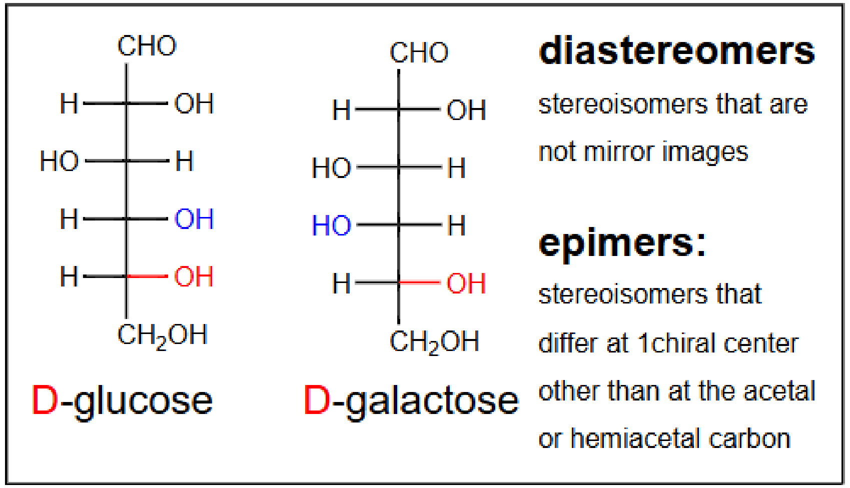
for pure form

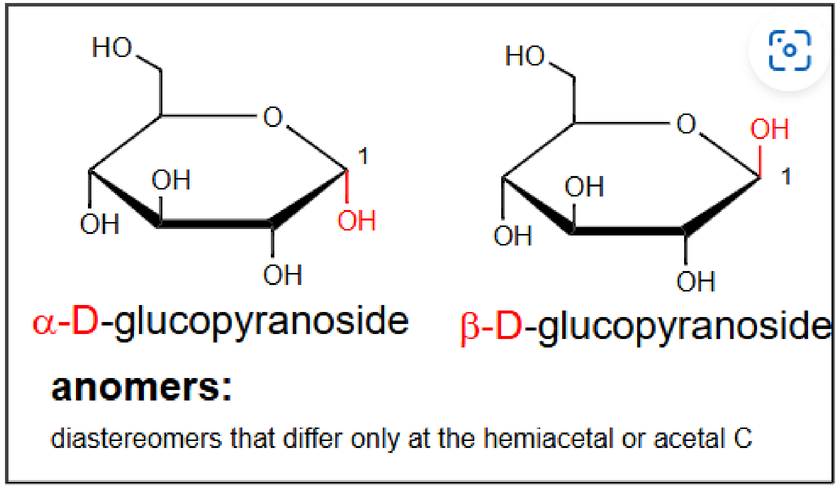
64%

PHYSICAL PROPERTIES OF CARBOHYDRATES - STEREOISOMERISM

1. Enantioisomerism
2. Optical Activity
3. Diastereoisomerism
4. Epimerism
5. Annomerism
6. Mutarotation







CHEMICAL REACTIONS OF CARBOHYDRATES

1. Reducing agent in alkaline media

- **Enediol formation** - In mild alkaline solutions, carbohydrates containing free aldehyde or keto group will tautomerize to form enediols (two hydroxyl groups are attached to the double-bonded carbon)

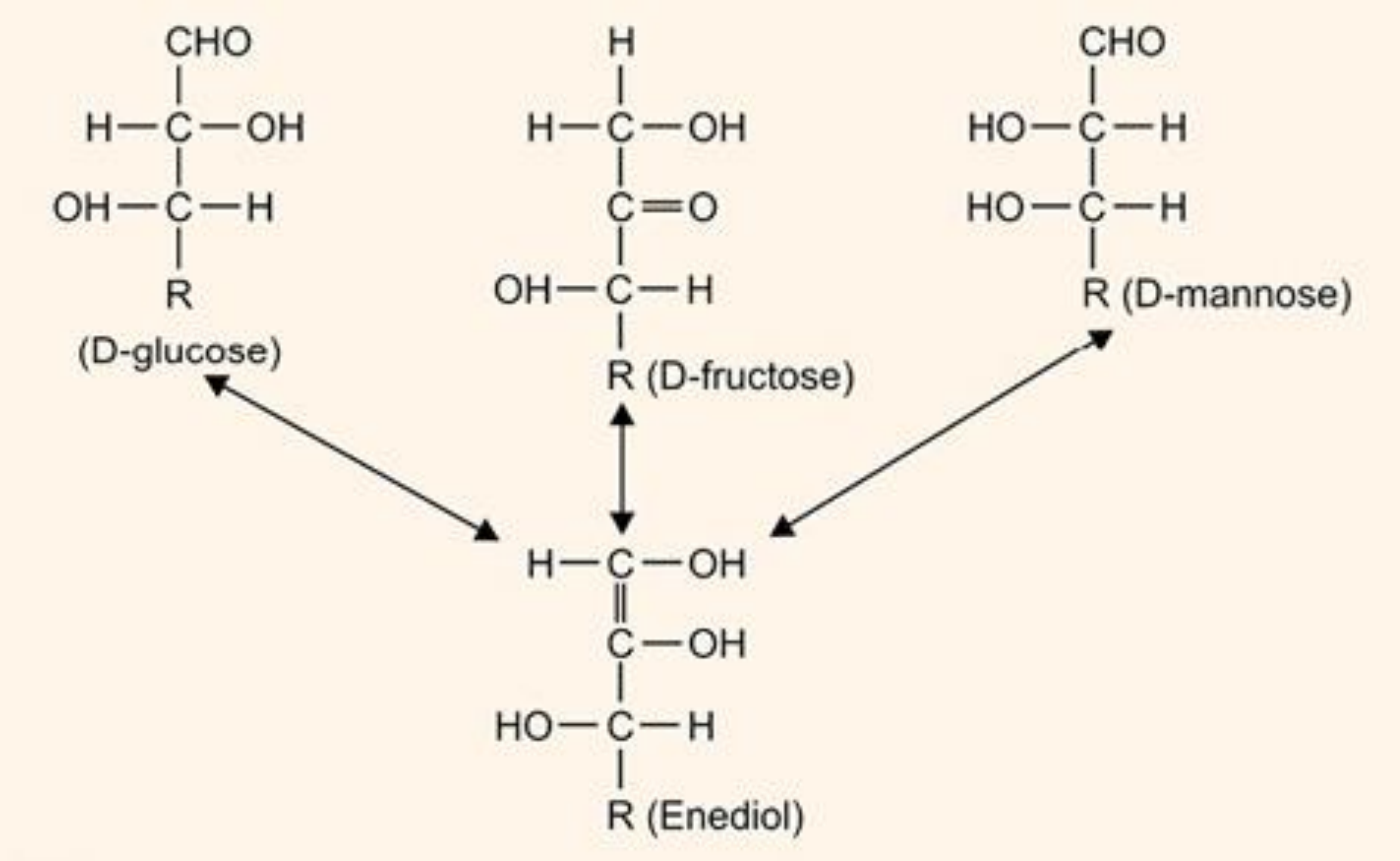
- **Tautomerization** - The process of shifting a hydrogen atom from one carbon atom to another to produce enediols

- Sugars possessing anomeric carbon atom undergo tautomerization in alkaline solutions

- Enediols are highly reactive, hence sugars in alkaline solution are powerful reducing agent

REDUCING AGENT

- In mild alkaline conditions, glucose is converted into fructose and mannose



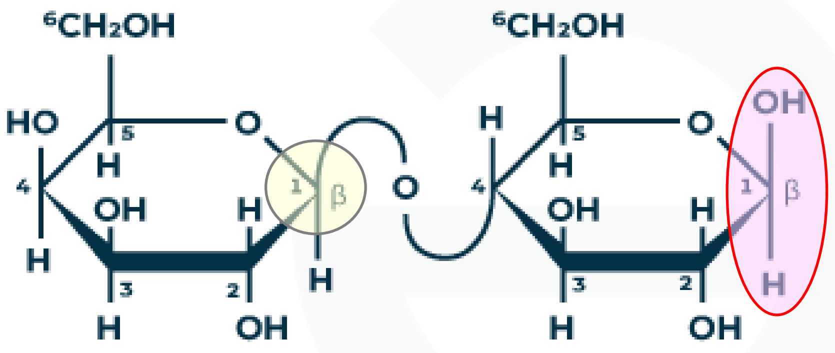
REDUCING SUGARS V/S NON-REDUCING SUGARS

Reducing Sugars

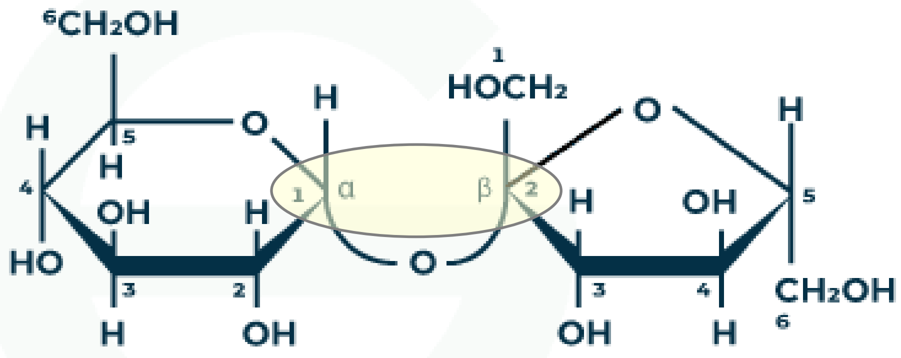
- Carbohydrates that can act as reducing agents due to presence of free aldehyde or keto group

Non-reducing Sugars

- Carbohydrates that cannot act as reducing agents due to absence of free aldehyde or keto group



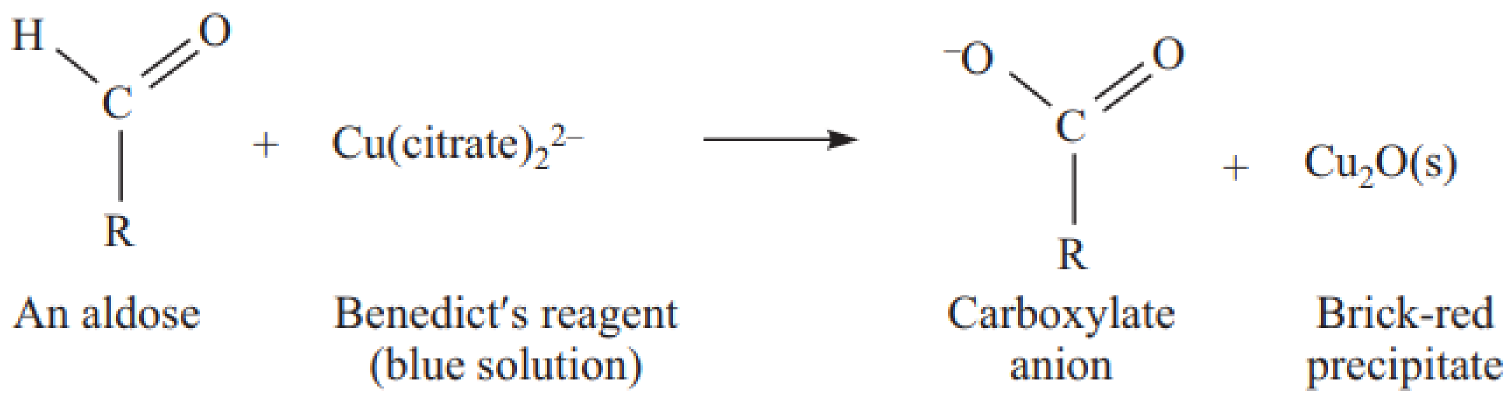
Lactose (β form)
β-D-galactopyranosyl-(1→4)-β-D-glucopyranose
Gal(β1→4)Glc



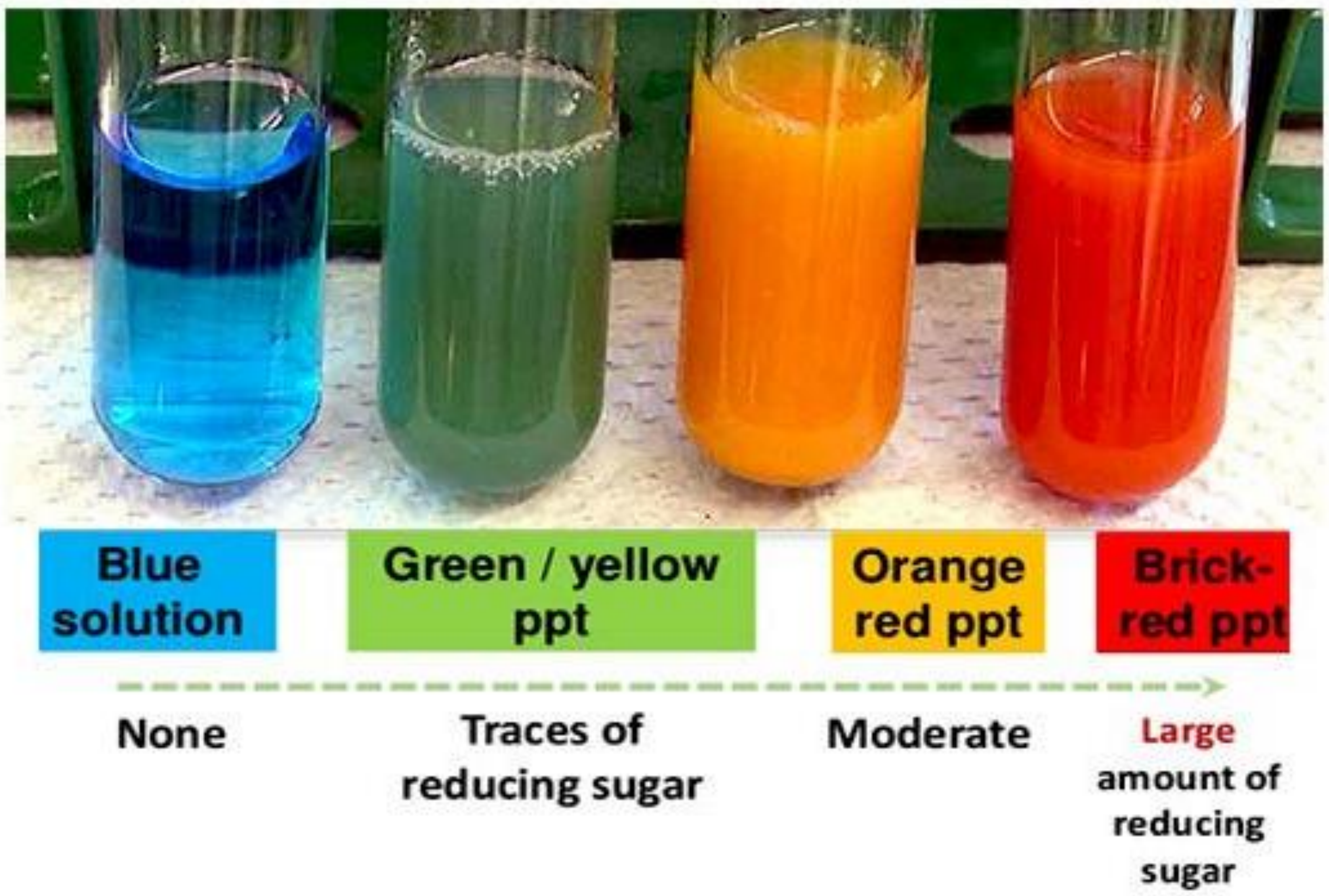
Sucrose
α-D-glucopyranosyl β-D-fructofuranoside
Glc(α1↔2β)Fru

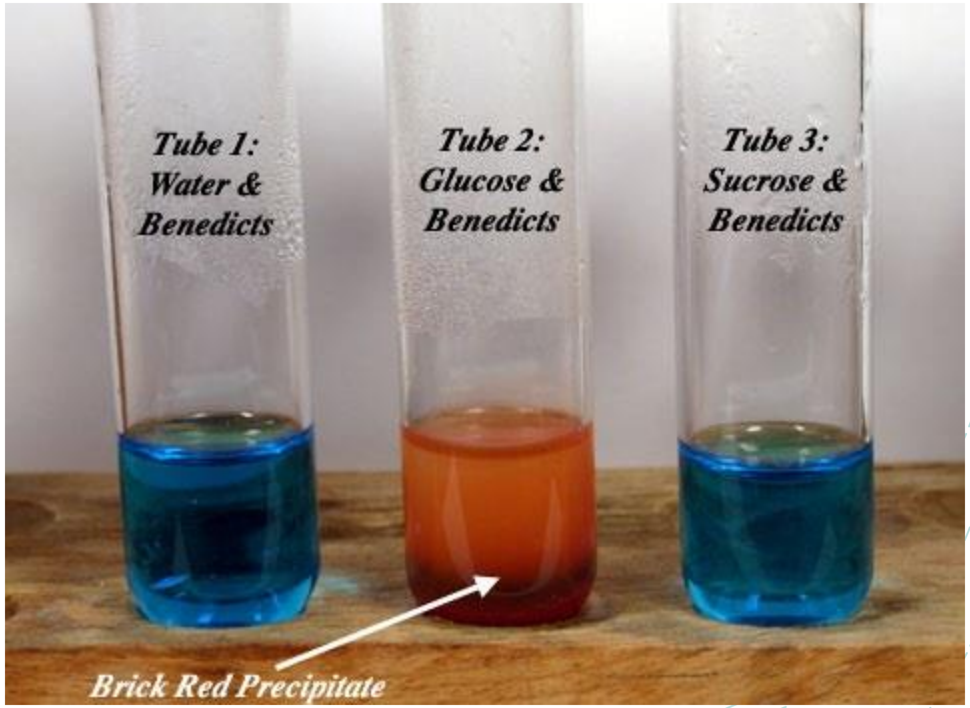
BENEDICTS REACTION

- In the laboratory, many tests are employed to identify the reducing action of sugars
- Benedict's test, Fehling's test, Barfoed’s test
- **Benedict’s reagent** contains sodium carbonate, copper sulphate and sodium citrate
- In alkaline medium, sugars form enediols which will reduce cupric ions and correspondingly the sugar is oxidized



BENEDICTS REACTION





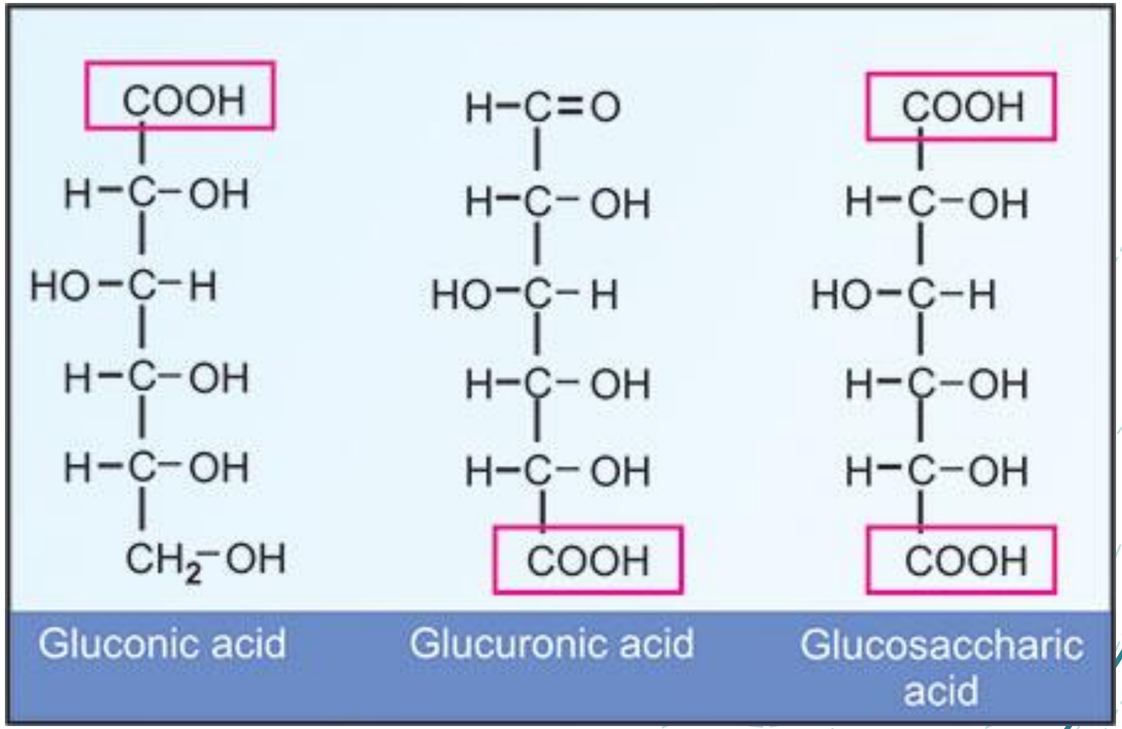
CHEMICAL REACTIONS OF CARBOHYDRATES

2. Oxidation to sugar acids

- Under mild oxidation conditions, the aldehyde group is oxidized to carboxyl group to produce aldonic acid (For eg: Glucose to Gluconic acid)

- When aldehyde group is protected and the molecule is oxidized the last carbon becomes COOH group to produce uronic acid (For eg: Glucose to Glucuronic acid)

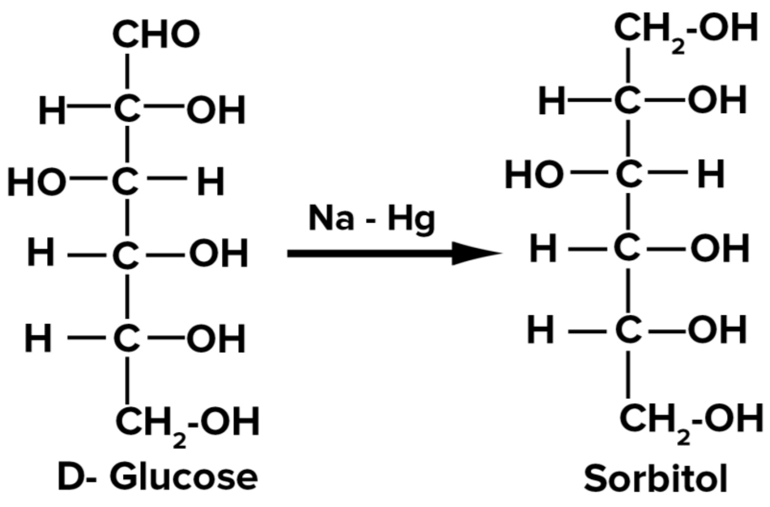
- Under strong oxidation conditions the first and last carbon atoms are simultaneously oxidized to form dicarboxyllic acids, known as saccharic acids (For eg: Glucose to Glucosaccharic acid)

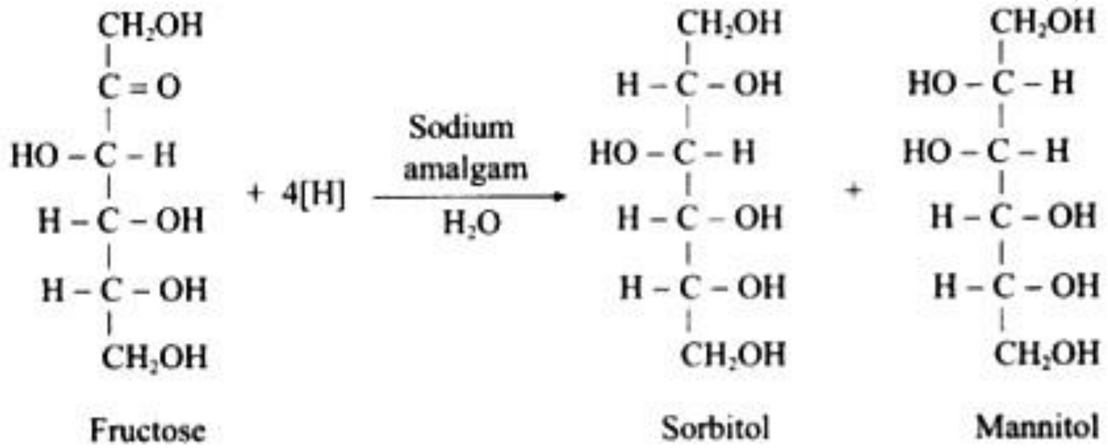


CHEMICAL REACTIONS OF CARBOHYDRATES

3. Reduction to alcohols

- When treated with reducing agents such as sodium amalgam, hydrogen can reduce sugars
- Aldose yields corresponding alcohols (For eg: Glucose forms Sorbitol)
- Ketose forms two alcohols, because of appearance of a new asymmetric carbon atom (For eg: Fructose forms sorbitol and mannitol)





CHEMICAL REACTIONS OF CARBOHYDRATES

4. Formation of:

i. Osazone with phenyl hydrazine

ii. Glycoside by condensation with alcoholic group of another substance

iii. Esters with acid

iv. Furfurals with strong acid

OSAZONE FORMATION

- All reducing sugars will form osazone with excess of phenylhydrazine when kept at boiling temperature
- Osazones are insoluable
- Each sugar will have characteristic crystal form of osazones

Needle-shaped crystals arranged like a broom
Glucososazone

Hedgehog or "pincushion with pins" or flower of "touch-me-not-plant"
Lactososazone

Sunflower-shaped or petal-shaped crystals of
Maltosazone

GLYCOSIDE FORMATION

- **Glycosides** - formed when the hemiacetal or hemiketal hydroxyl group (of anomeric carbon) of a carbohydrate reacts with a hydroxyl group of another carbohydrate or a non-carbohydrate (e.g. methyl alcohol, phenol, glycerol)
- **Glycosidic bond** - bond formed between carbohydrates
- **Aglycone** - non-carbohydrate moiety (when present)
- Examples same as studied in glycosidic linkage

ESTER FORMATION

- Hydroxyl groups of sugars can be esterified to form acetates, propionates, benzoates, phosphates, etc.

- Phosphate esters of monosaccharides are found in sugar-phosphate backbone of DNA and RNA and as intermediates in the metabolism of carbohydrates in the body (glucose-6-phosphate)

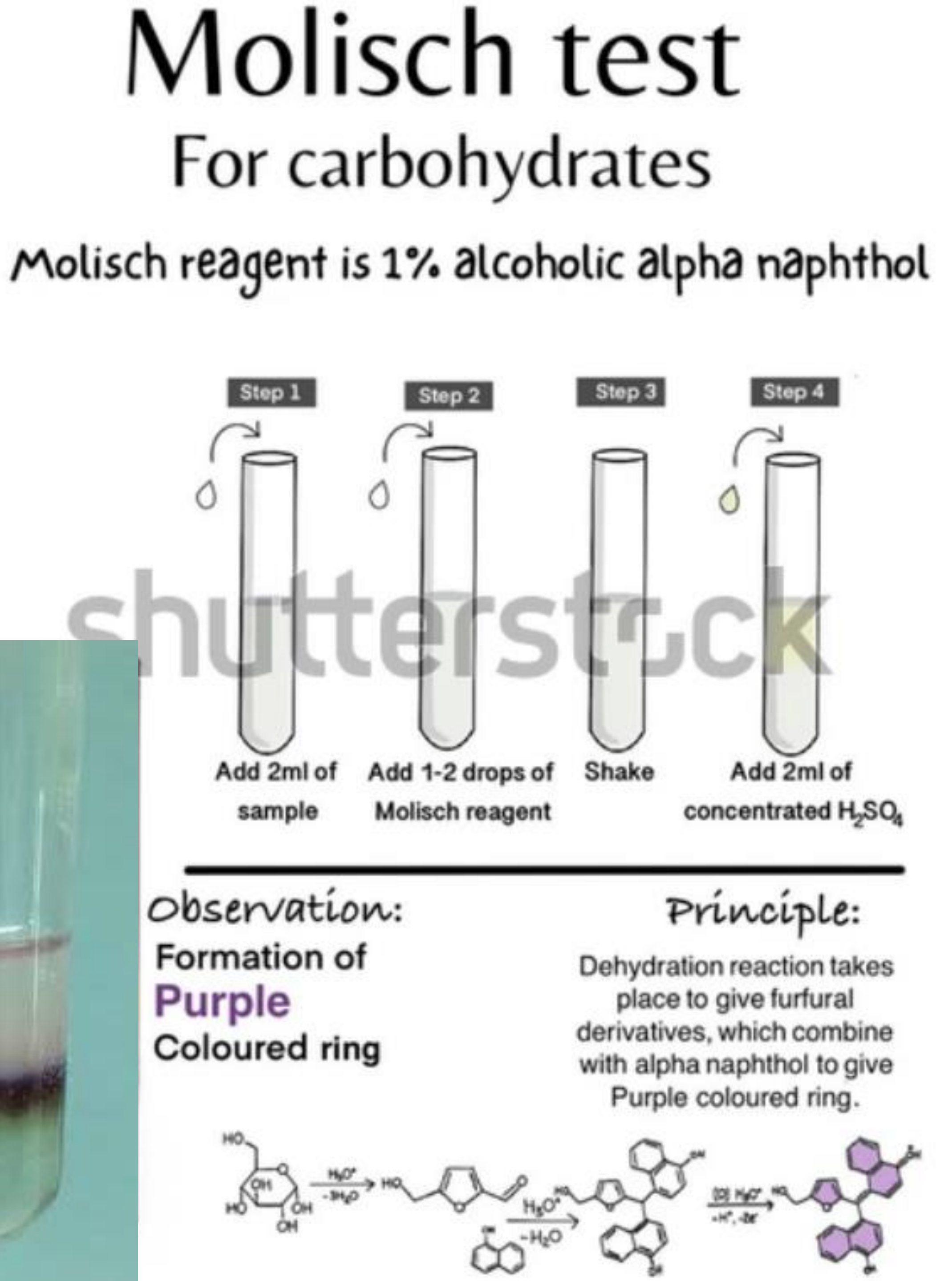
FURFURAL FORMATION

- Monosaccharides when treated with concentrated sulphuric acid undergo dehydration with removal of 3 molecules of water

- Hexoses give hydroxymethyl furfural and pentoses give furfural

- These derivatives can condense with phenolic compounds to give coloured products

- Forms the basis of Molisch test - a general test for carbohydrates

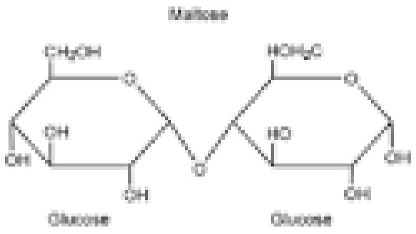
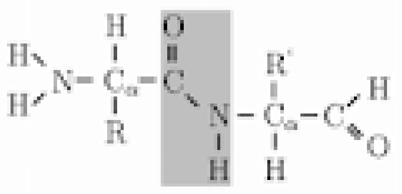
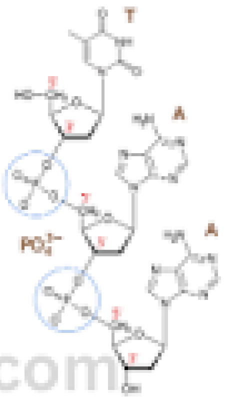
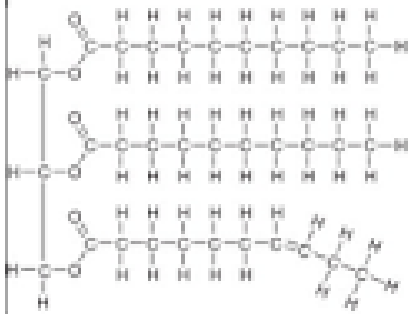


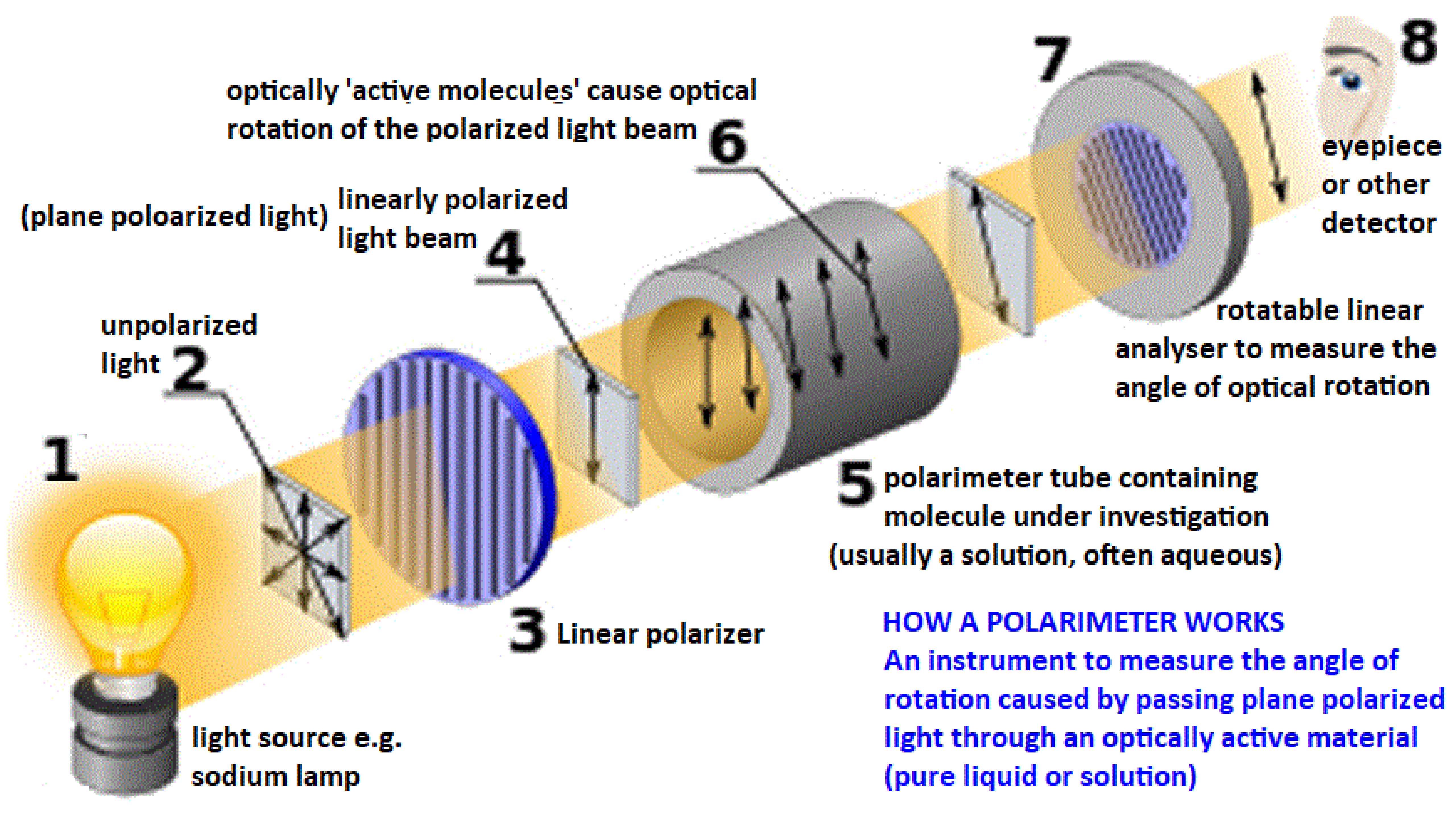
APPLICATIONS OF CARBOHYDRATES

- Fermentation
- Pharmaceuticals
- Food industry

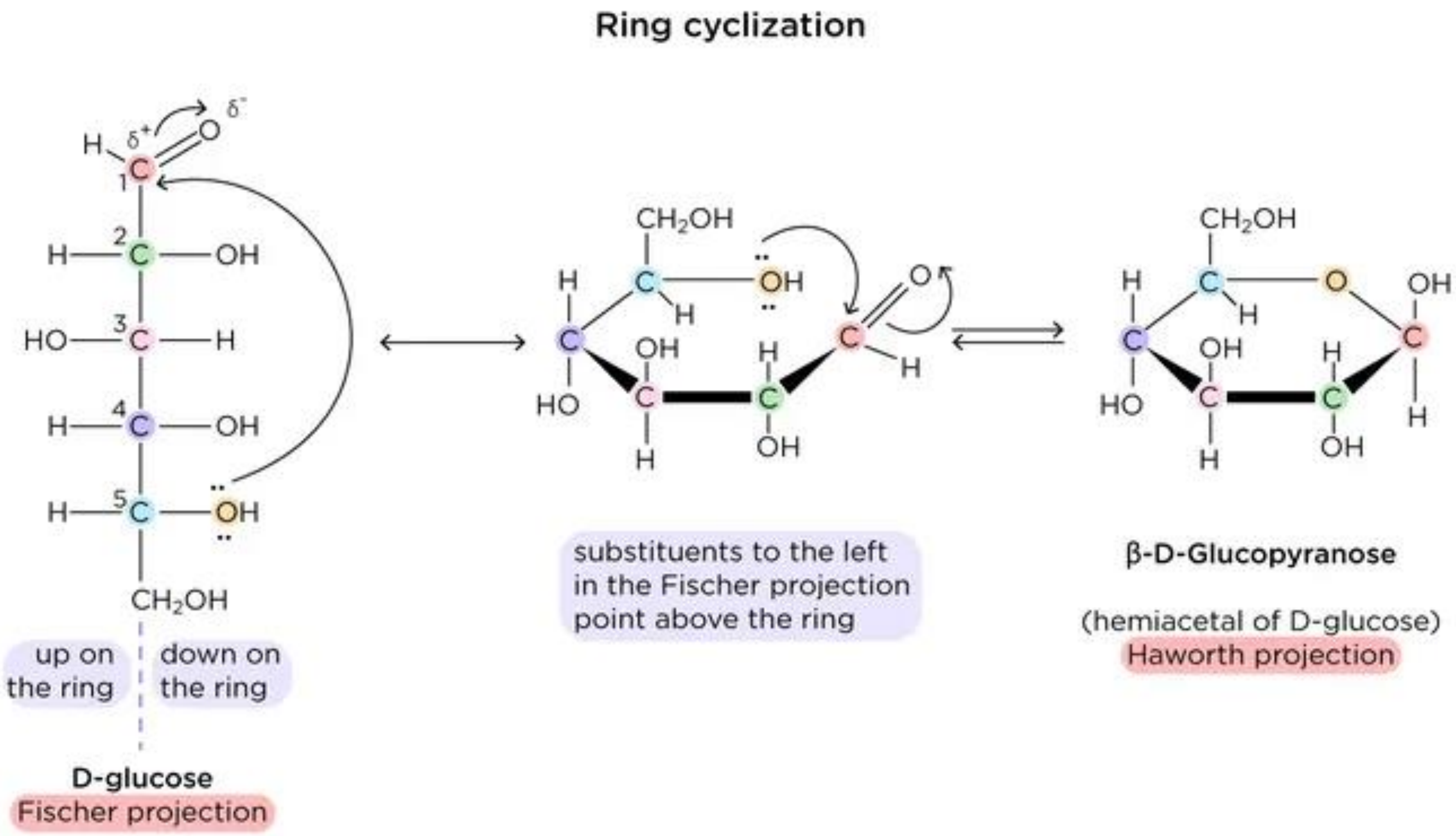
Biomolecules

COMPARISON CHART

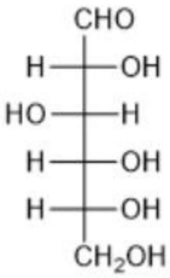
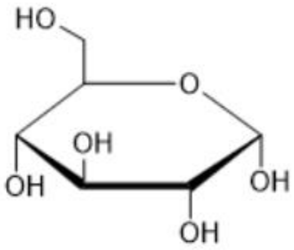
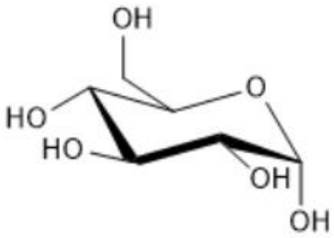
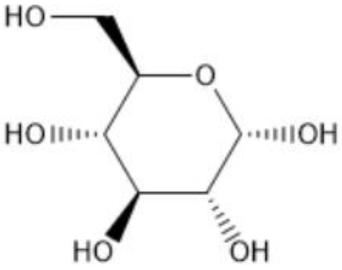
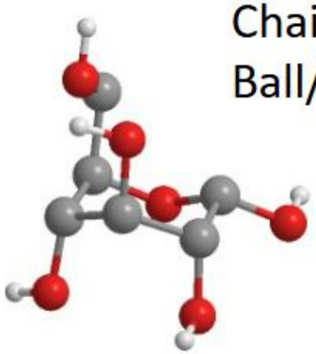
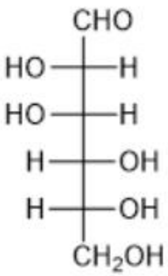
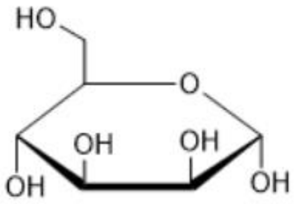
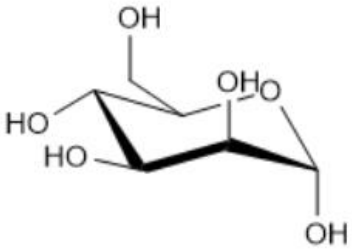
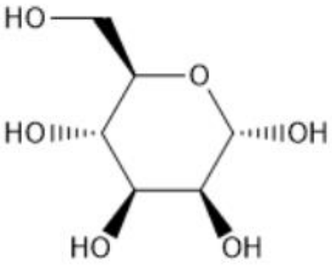
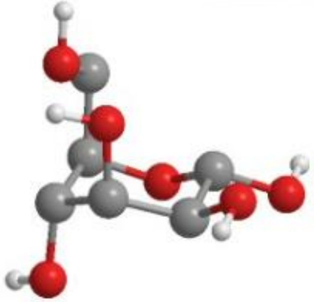
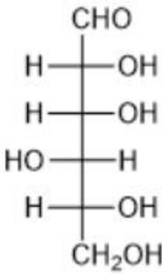
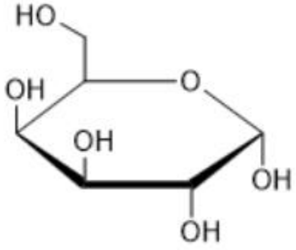
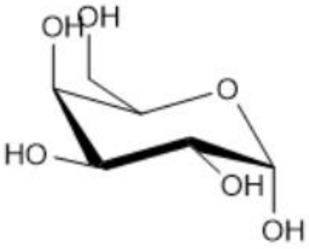
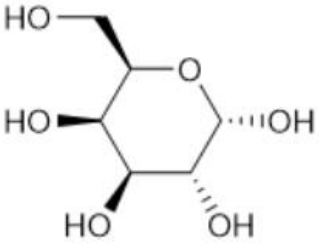
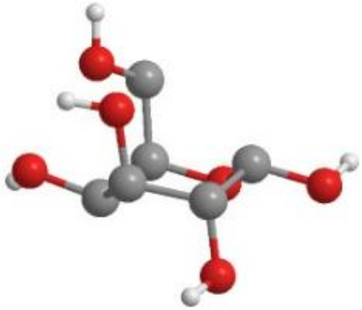
	CARBOHYDRATE	PROTEIN	NUCLEIC ACID	LIPID
Structure				
Monomers	Monosaccharides	Amino acids	Nucleotides	Fatty acids and glycerol
Primary Bond	joined by glyocosidic bond	joined by peptide bond	joined by phosphodiester bond	joined by ester bond
Elements	**C,H,O**	**C,H,O,N, S**	**C,H,O,N,P**	**C,H,O**
Function	Short term energy source Structural component Reserve food	Enzyme, structure movement, defence, hormones, Oxygen carriers	Stores genetic information	Long term energy source, insulation, biological membrane components, hormones
Examples	Starch, Glycogen, Cellulose	Insulin, Collagen, Myoglobin	DNA, RNA	Oils, Fats, Waxes



optically 'active molecules' cause optical rotation of the polarized light beam
6
7
8
eyepiece or other detector
(plane poloarized light)
linearly polarized light beam
4
unpolarized light
2
rotatable linear analyser to measure the angle of optical rotation
1
5
polarimeter tube containing molecule under investigation (usually a solution, often aqueous)
3
Linear polarizer
light source e.g. sodium lamp
HOW A POLARIMETER WORKS
An instrument to measure the angle of rotation caused by passing plane polarized light through an optically active material (pure liquid or solution)



Ring cyclization
H
δ+
O
δ-
1
C
2
H
C
OH
3
HO
C
H
4
H
C
OH
5
H
C
OH
CH2OH
up on the ring
down on the ring
D-glucose
Fischer projection
CH2OH
H
C
OH
H
O
C
H
C
OH
H
H
HO
C
C
H
OH
substituents to the left in the Fischer projection point above the ring
CH2OH
H
C
O
OH
H
C
C
OH
H
HO
C
C
H
H
OH
β-D-Glucopyranose
(hemiacetal of D-glucose)
Haworth projection

	Fischer	Haworth	Chair	Wedge/Dash	Chair Ball/Stick
α-D-Glucose					
α-D-Mannose					
α-D-Galactose					

Differences between Reducing and Non-reducing Sugars

Character	Reducing Sugar	Non-reducing Sugars
Nature	Reduce cupric ions of Fehling's reagent and Benedict's reagent to cuprous ions to produce brick-red precipitates.	Don't form brick-red precipitates with Fehling's reagent and Benedict's reagents.
Structural peculiarity	Have free carbonyl group (either aldehydic or ketonic group).	Don't have free carbonyl group.
Examples	All monosaccharides, maltose, lactose, etc.	Sucrose and all polysaccharides.

Reaction $CuSO_4 \rightarrow Cu^{++} + SO_4^{--}$

Cu^{++} + Sodium citrate $\longrightarrow$ Cupric–sodium citrate complex

Reducing sugar $\xrightarrow{Na_2CO_3}$ Enediols form of reducing sugar

Enediols + cupric: sodium citrate complex $\longrightarrow$ Cu^{+} + Mixture of sugar acids

$$Cu^{+} + OH^{-} \longrightarrow CuOH$$

$$2\,CuOH \xrightarrow{\text{Heat}} Cu_2O \downarrow + H_2O$$

Cuprous oxide Red cuprous oxide