# COURSE : SC202 (CHEMISTRY)

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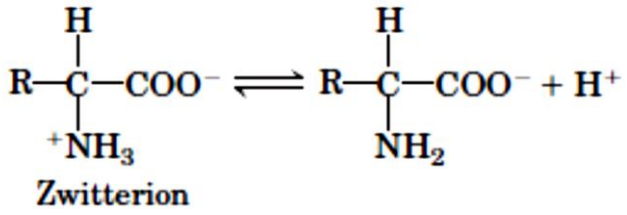
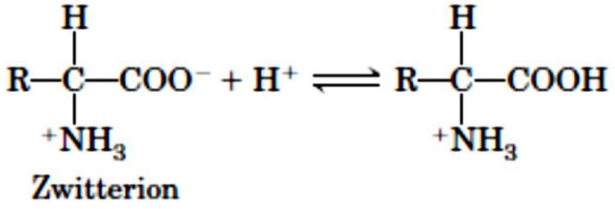
LECTURE-9

DATE: 28.1.2021

When an amino acid is dissolved in water, it exists in solution as the dipolar ion, or **zwitterion** (German for "hybrid ion") A zwitterion can act as either an acid (proton donor):




or a base (proton acceptor):




Substances having this dual nature are **amphoteric** and are often called **ampholytes** (from "amphoteric electrolytes").

In an acidic solution, the -COO- group is protonated to a free -COOH group, and the molecule has an overall positive charge. As the pH is raised, the -COOH loses its proton at about pH 2. This point is called pKa1, the first acid dissociation constant. As the pH is raised further, the – NH  group loses its proton at about pH 9 or 10. This point is called pKa1, the second acid-dissociation constant. Above this pH, the molecule has an overall negative charge.

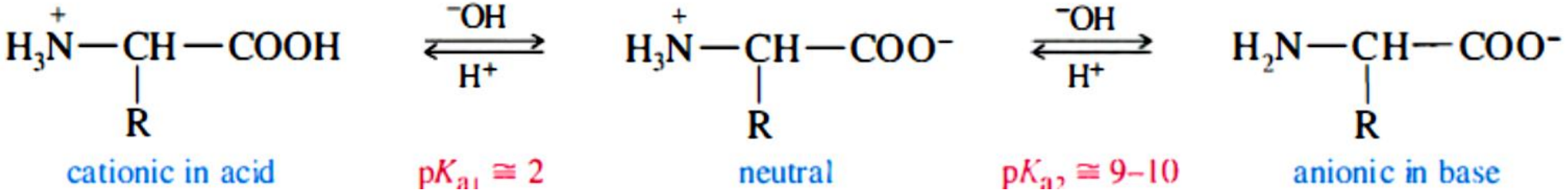
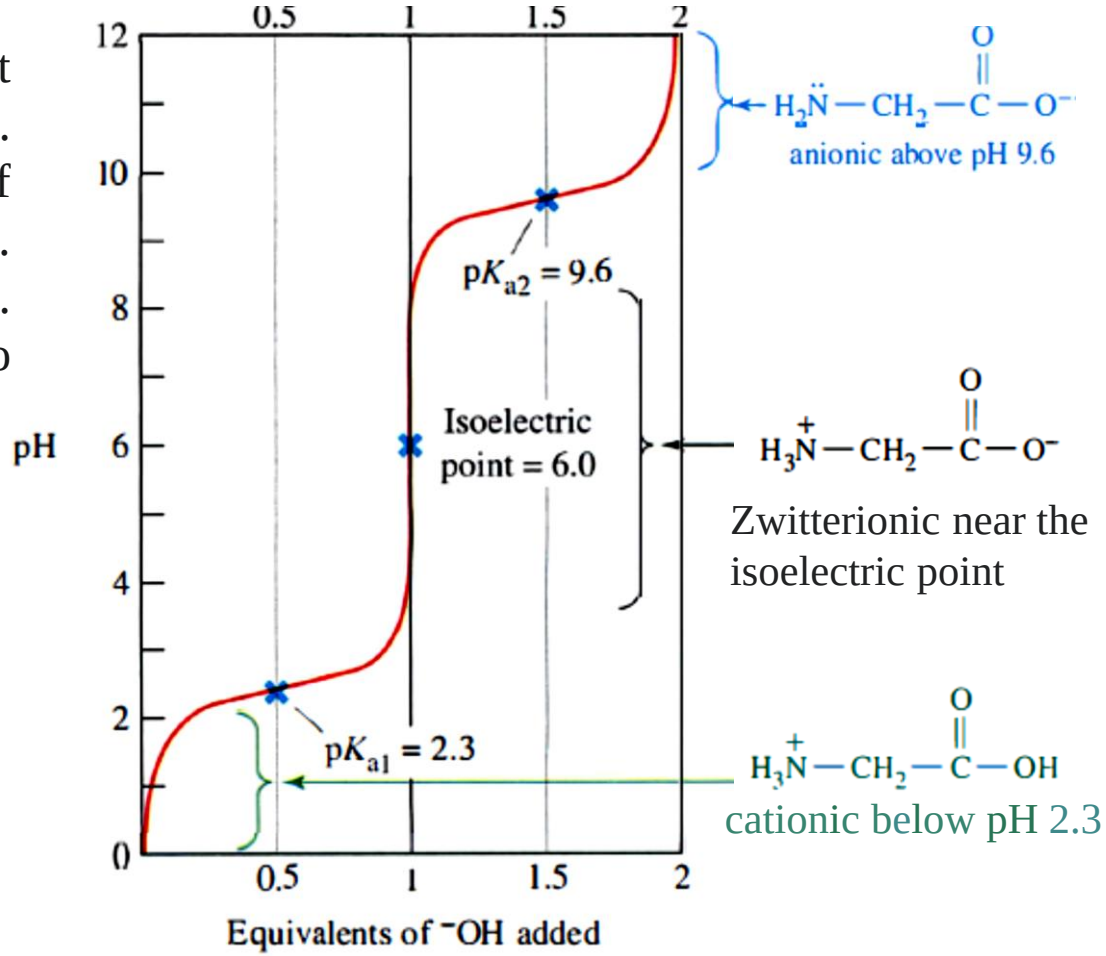
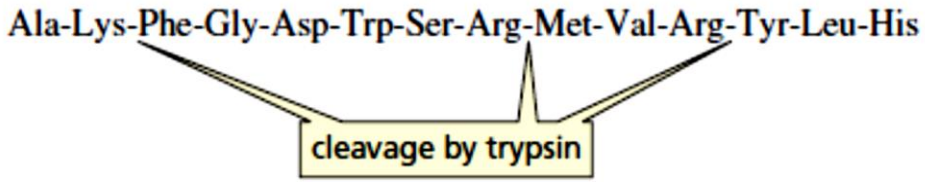


Figure 1 shows a titration curve for glycine. The curve starts at the bottom left, where glycine is entirely in its cationic form. Base is slowly added, and the pH is recorded. At pH 2.3, half of the cationic form has been converted to the zwitterionic form. At pH 6.0, essentially all the glycine is in the zwitterionic form. At pH 9.6, half of the zwitterionic form has been converted to the basic form.

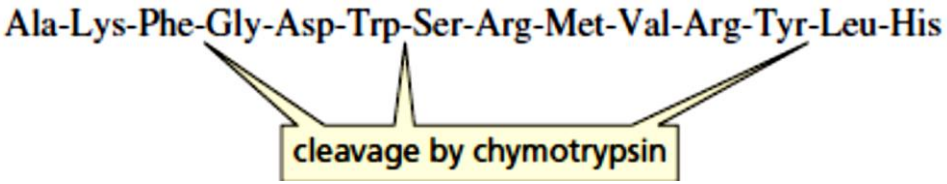


## Cleavage of the specific peptide bonds

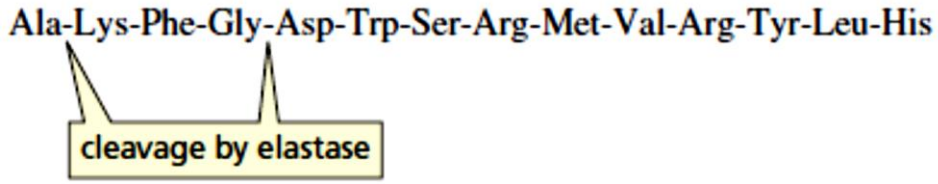
Trypsin, for example, catalyzes the hydrolysis of the peptide bond on the C-side of only arginine or lysine residues.



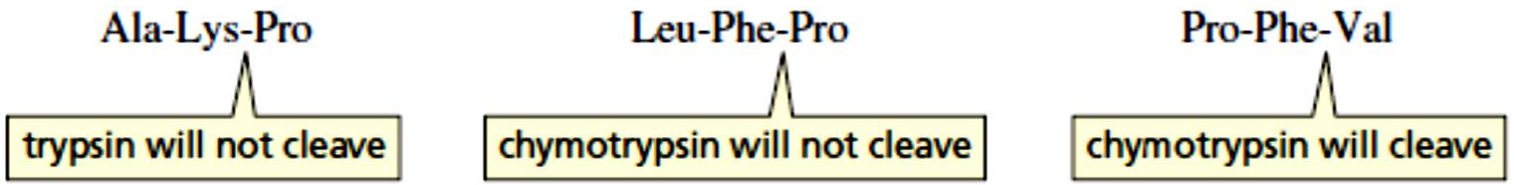

Chymotrypsin catalyzes the hydrolysis of the peptide bond on the C-side of amino acids that contain aromatic six-membered rings (Phe, Tyr, Trp).



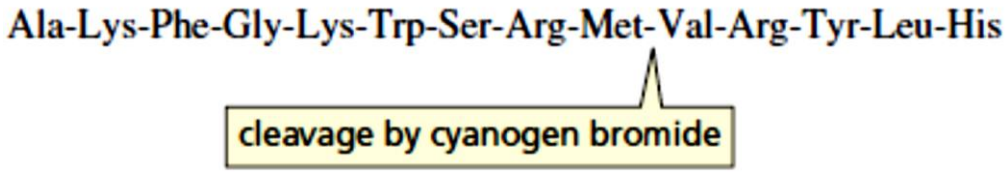

Elastase catalyzes the hydrolysis of peptide bonds on the C-side of small amino acids (Gly, Ala). Chymotrypsin and elastase are much less specific than trypsin



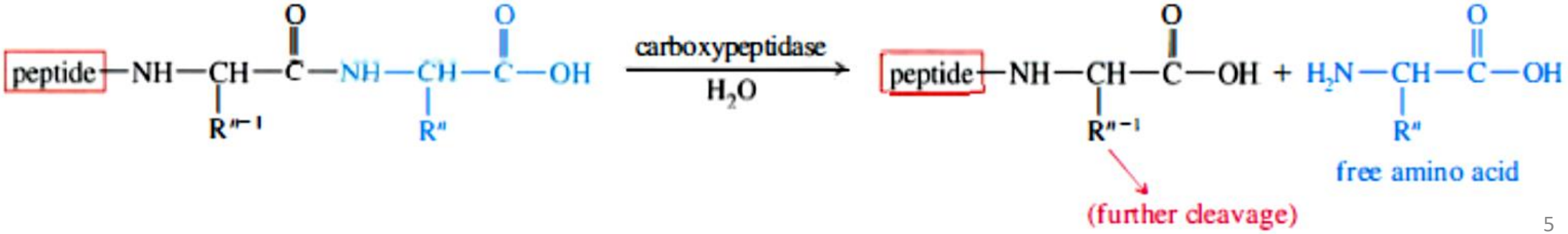
None of the exopeptidases or endopeptidases that we have mentioned will catalyze the hydrolysis of an amide bond if proline is at the hydrolysis site.




Cyanogen bromide causes the hydrolysis of the amide bond on the C-side of a methionine residue.

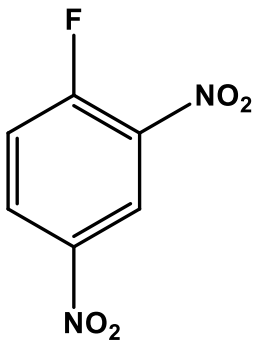



The C-terminal amino acid can be identified using the enzyme carboxypeptidase which cleaves the C-terminal peptide bond. The products are the free C-terminal amino acid and a shortened peptide. Further reaction cleaves the second amino acid that has now become the new C terminus of the shortened peptide. Eventually, the entire peptide is hydrolyzed to its individual amino acids.

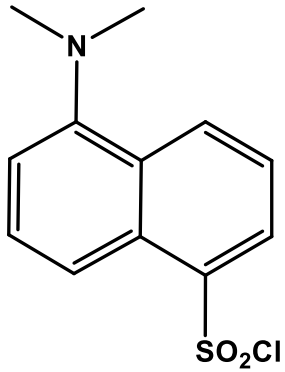


# Summary

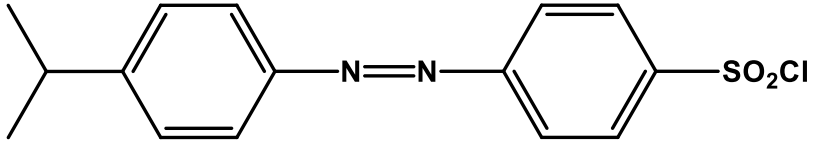
1. Reagents used to identify the N-terminal amino acid residue




1-fluoro-2,4- dinitrobenzene (FDNB)

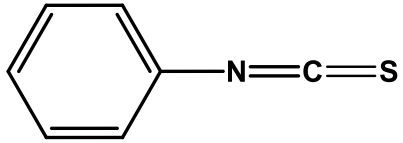



dansyl chloride




dabsyl chloride

phenyl isothiocyanate (PITC), more commonly known as **Edman's reagent**



## 2. Reagents used to identify the C-terminal amino acid residue

| Reagent | Specificity |
|---|---|
| Cyanogen bromide | Hydrolyzes on the C-side of Met |
| Carboxypeptidase A | removes the C-terminal amino acid (not Arg or Lys) |
| Carboxypeptidase B | removes the C-terminal amino acid (only Arg or Lys) |
| Trypsin | Hydrolyzes on the C-side of Arg and Lys |
| Chymotrypsin | Hydrolyzes on the C-side of amino acids that contain aromatic six-membered rings(Phe, Tyr, Trp) |
| Elatase | Hydrolyzes on the C-side of small amino acids (Gly and Ala) |

*Cleavage will not occur if Pro is on either side of the bond to be hydrolyzed.

**Problem 1:** A nonapeptide undergoes partial hydrolysis to give peptides whose amino acid compositions are shown. Reaction of the intact nonapeptide with Edman's reagent releases PTH-Leu. What is the sequence of the nonapeptide?

| | | | |
|---|---|---|---|
| a. Pro, Ser | c. Met, Ala, Leu | e. Glu, Ser, Val, Pro | g. Met, Leu |
| b. Gly, Glu | d. Gly, Ala | f. Glu, Pro, Gly | h. His, Val |

N-terminal amino aci is Leu. Fragment (g) tells us that Met is next to Leu and fragment (c) tells us that Ala is next to Met. Now we look for a fragment that contains Ala. Fragment (d) contains Ala and tells us that Gly is next to Ala. From fragment (b), we know that Glu comes next. Glu is in both fragments (e) and (f). Fragment (f) has only one, so from fragment (f), we know that Pro is the next amino acid. Fragment (e) tells us that the next amino acid is Val, and fragment (h) tells us that His is the last (C-terminal) amino acid.

Thus, the amino acid sequence of the nonapeptide is Leu-Met-Ala-Gly-Glu-Pro-Ser-Val-His

**Problem 2:** A decapeptide undergoes partial hydrolysis to give peptides whose amino acid compositions are shown. Reaction of the intact decapeptide with Edman's reagent releases PTH-Gly. What is the sequence of the decapeptide?

| | | | |
|---|---|---|---|
| a. Ala, Trp | c. Pro, Val | e. Trp, Ala, Arg | g. Glu, Ala, Leu |
| b. Val, Pro, Asp | d. Ala, Glu | f. Arg, Gly | h. Met, Pro, Leu, Glu |

**Problem 3:** Explain why the Edman degradation is usually preferred over the Sanger method.

**Problem 4:** Show where trypsin and chymotrypsin would cleave the following peptide.

Tyr-Ile-Gln-Arg-Leu-Gly-Phe-Lys-Asn-Trp-Phe-Gly-Ala-Lys-Gl y-Gln-Gln . $NH_2$

**Problem 5:** Determine the primary structure of an octapeptide from the following data:

Acid hydrolysis gives 2 Arg, Leu, Lys, Met, Phe, Ser, Tyr.

Carboxypeptidase A releases Ser.

Edman's reagent releases Leu.

Cyanogen bromide forms two peptides with the following amino acid compositions:

1. Arg, Phe, Ser  2. Arg, Leu, Lys, Met, Tyr

Trypsin forms the following two peptides and two amino acids:

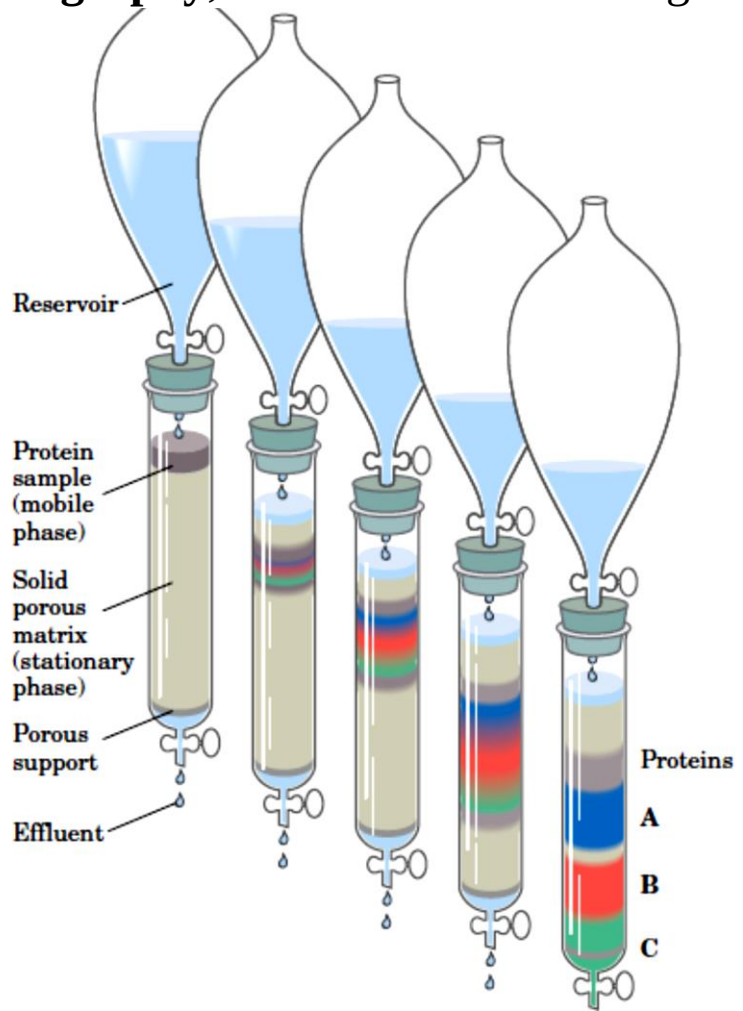
1. Arg  3. Arg, Met, Phe

2. Ser  4. Leu, Lys, Tyr

# Purification of Proteins

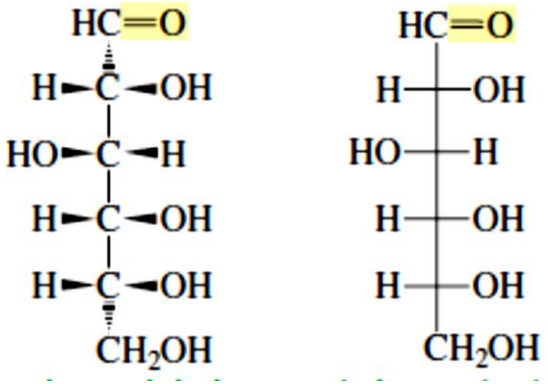
1. The most powerful methods for fractionating proteins make use of **column chromatography.** A porous solid material with appropriate chemical properties (the stationary phase) is held in a column, and a buffered solution (the mobile phase) percolates through it. The protein-containing solution, layered on the top of the column, percolates through the solid matrix as an ever-expanding band within the larger mobile phase. Individual proteins migrate faster or more slowly through the column depending on their properties. For example, in **cation-exchange chromatography**, the solid matrix has negatively charged groups. In the mobile phase, proteins with a net positive charge migrate through the matrix more slowly than those with a net negative charge, because the migration of the former is retarded more by interaction with the stationary phase. The two types of protein can separate into two distinct bands.

2. A modern refinement in chromatographic methods is **HPLC,** or **high-performance liquid chromatography.** HPLC makes use of high-pressure pumps that speed the movement of the protein molecules down the column, as well as higher-quality chromatographic materials that can withstand the crushing force of the pressurized flow. By reducing the transit time on the column, HPLC can limit diffusional spreading of protein bands and thus greatly improve resolution.

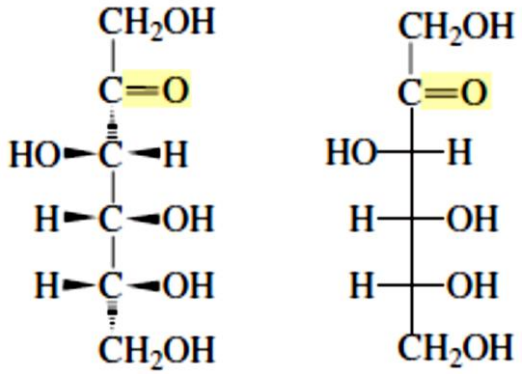


# Carbohydrates

**Carbohydrates** are polyhydroxy aldehydes such as D-glucose, polyhydroxy ketones such as D-fructose, and compounds such as sucrose that can be hydrolyzed to polyhydroxy aldehydes or polyhydroxy ketones. The chemical structures of carbohydrates are commonly represented by wedge-and-dash structures or by Fischer projections.




D-glucose




D-fructose

There are two classes of carbohydrates: *simple carbohydrates* and *complex carbohydrates*.

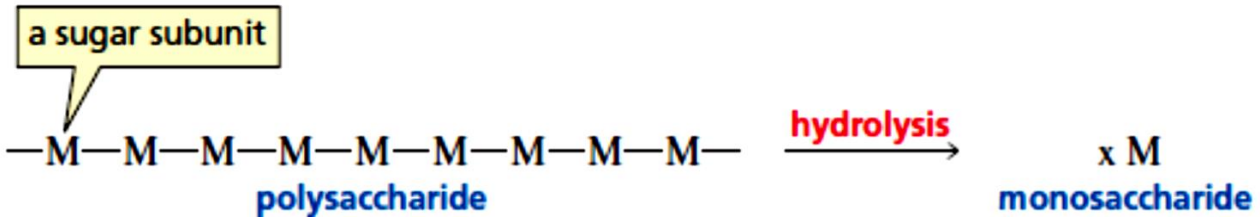
**Simple carbohydrates** are **monosaccharides** (single sugars), whereas

**complex carbohydrates** contain two or more sugar subunits linked together.

**Disaccharides** have two sugar subunits linked together, **oligosaccharides** have three to 10 sugar subunits

(*oligos* is Greek for "few") linked together, and

**Polysaccharides** have more than 10 sugar subunits linked together.

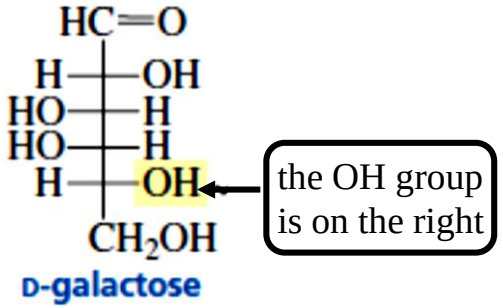


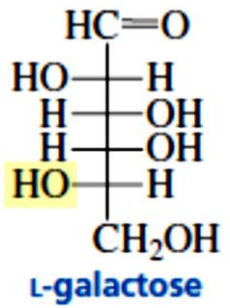

A *monosaccharide* can be a polyhydroxy aldehyde such as D-glucose or a polyhydroxy ketone such as D-fructose. Polyhydroxy aldehydes are called **aldoses** ("ald" is for aldehyde;"ose" is the suffix for a sugar), whereas polyhydroxy ketones are called **ketoses**.

Monosaccharides with three carbons are **trioses**, those with four carbons are **tetroses**, those with five carbons are p**entoses**, and those with six and seven carbons are **hexoses** and **heptoses**, respectively.

## The D and L Notation

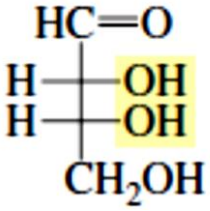
Galactose has four asymmetric carbons (C-2, C-3, C-4, and C-5). *If the OH group attached to the bottom-most asymmetric carbon (the carbon that is second from the bottom) is on the right, then the compound is a D-sugar. If the OH group is on the left, then the compound is an L-sugar.* Almost all sugars found in nature are D-sugars.



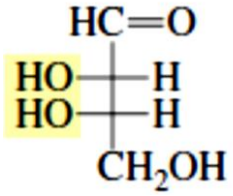



## Configurations of Aldoses

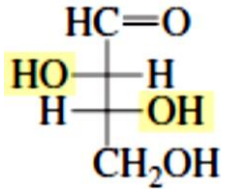
Aldotetroses have two asymmetric carbons and therefore four stereoisomers. Two of the stereoisomers are D-sugars and two are L-sugars. The names of the aldotetroses—erythrose and threose



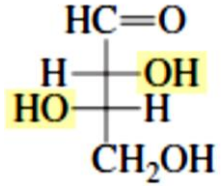

D-erythrose




L-erythrose

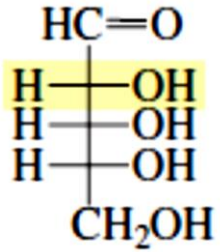



D-threose

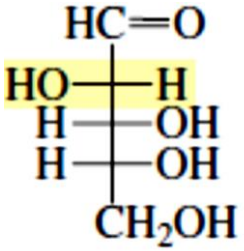



L-threose

Diastereomers that differ in configuration at only one asymmetric carbon are called **epimers**. For example, D-ribose and D-arabinose are C-2 epimers (they differ in configuration only at C-2), and D-idose and D-talose are C-3 epimers.

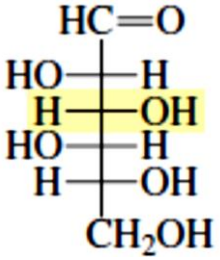



D-ribose

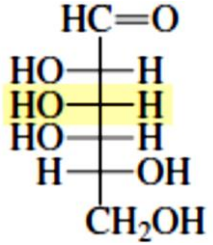



D-arabinose

C-2 epimers

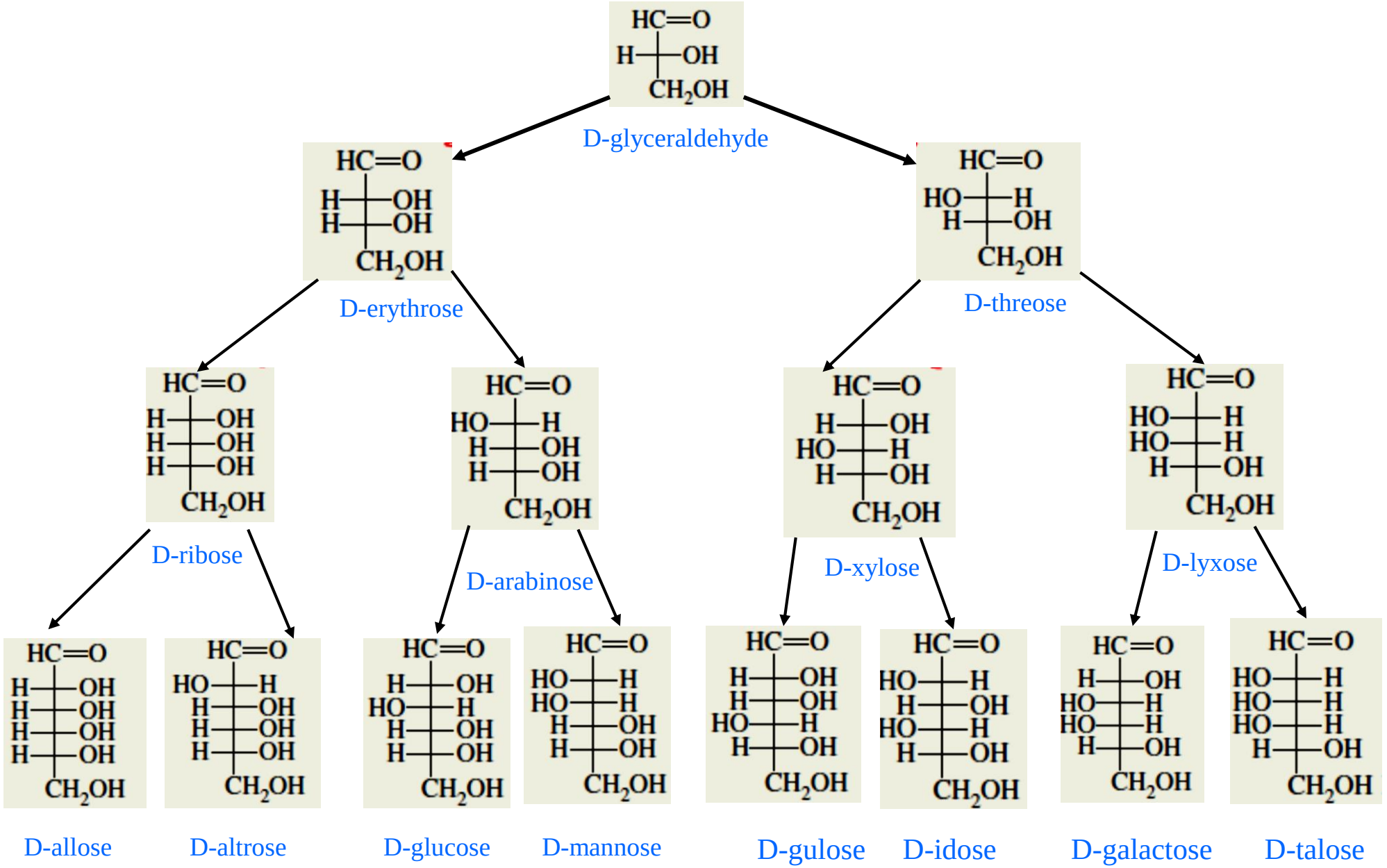



D-idose

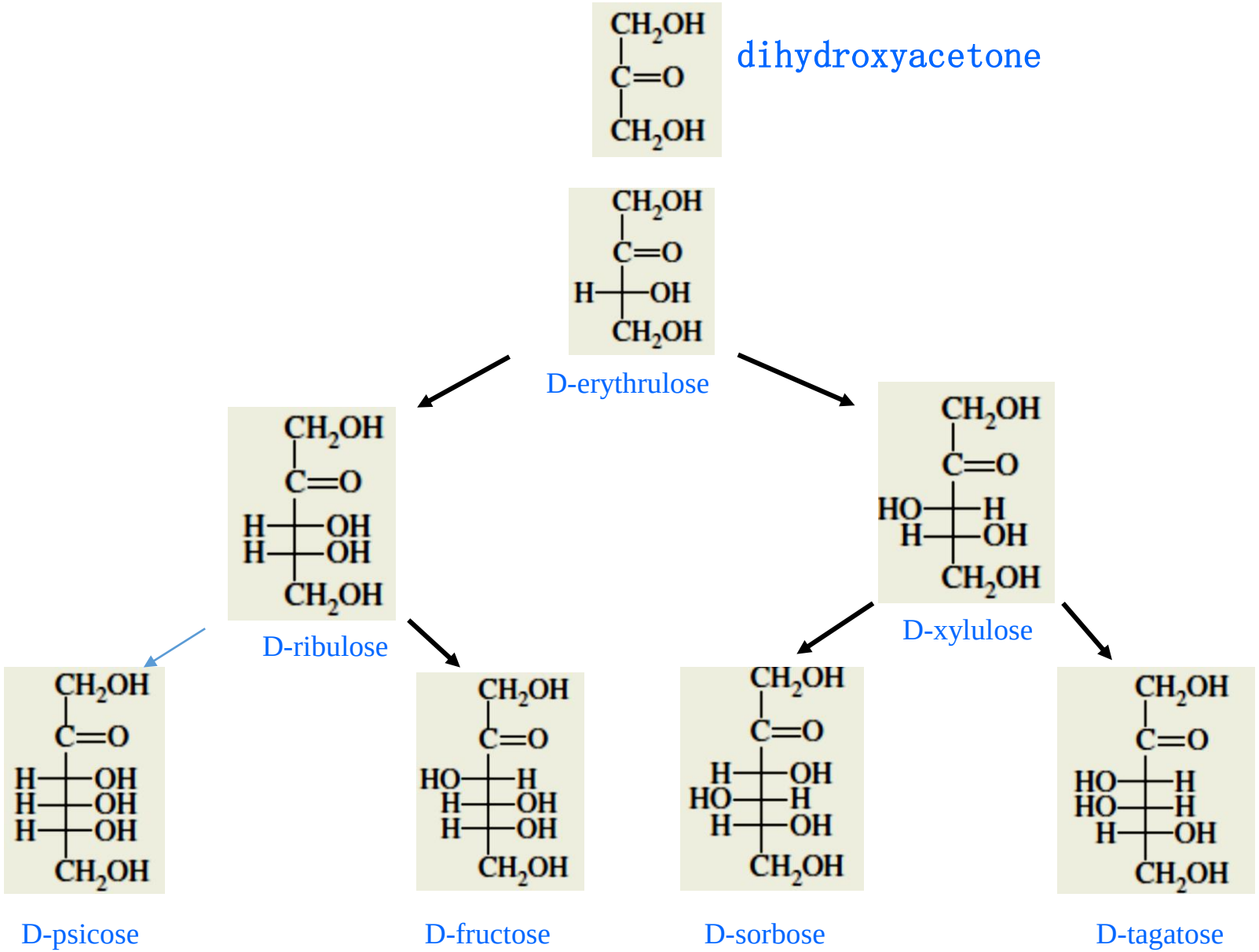



D-talose

C-3 epimers

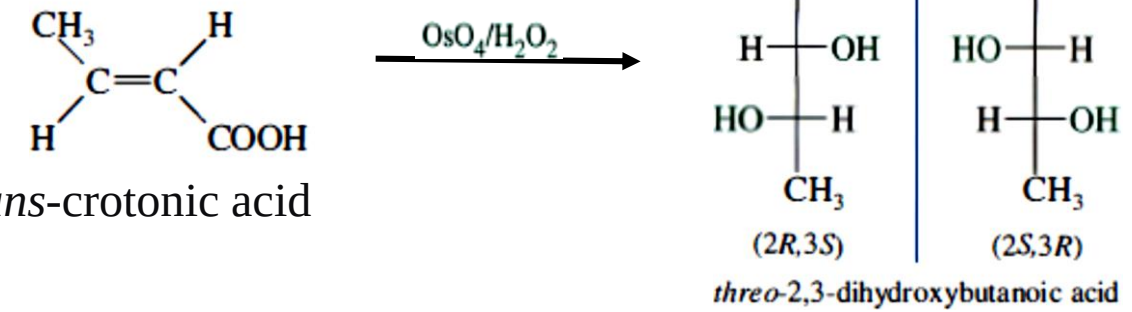

D-glyceraldehyde
D-erythrose
D-threose
D-ribose
D-arabinose
D-xylose
D-lyxose
D-allose
D-altrose
D-glucose
D-mannose
D-gulose
D-idose
D-galactose
D-talose

# Configurations of D-Ketoses



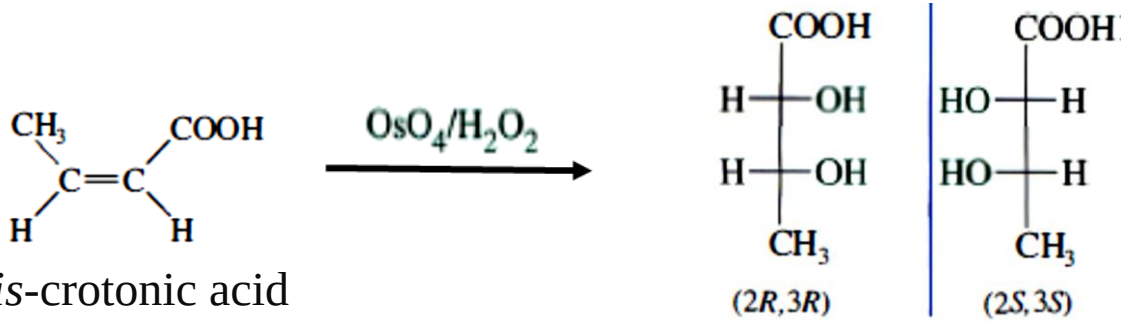
## Erythro and Threo Diastereomers

A diastereomer is called erythro if its Fischer projection shows similar groups on the same side of the molecule. It is called threo if similar groups are on opposite sides of the Fischer projection.



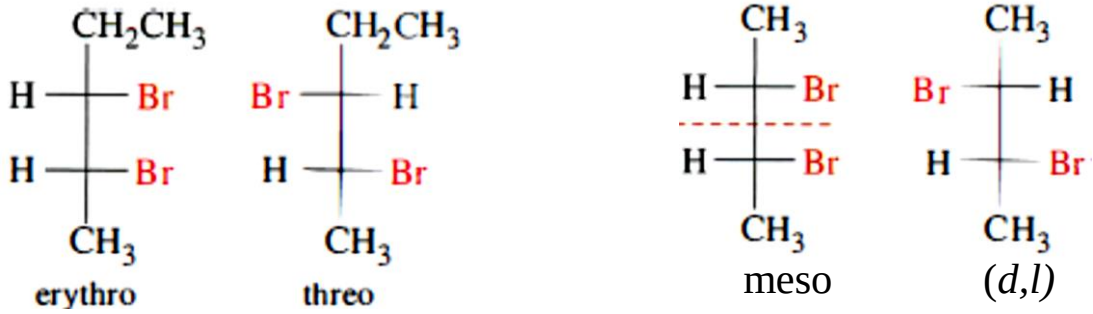

*trans*-crotonic acid

*threo*-2,3-dihydroxybutanoic acid




*cis*-crotonic acid

*erythro*-2,3-dihydroxybutanoic acid



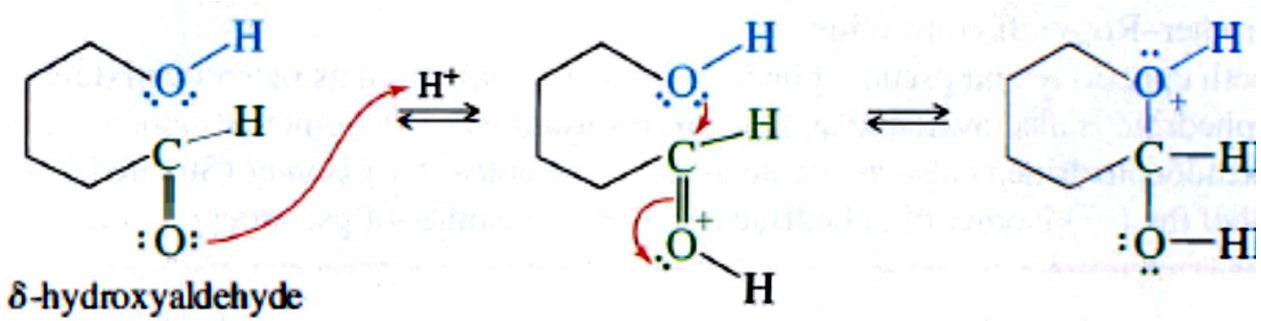

The terms erythro and threo are generally used only with molecules that do not have symmetric ends. In symmetric molecules such as 2,3-dibromobutane and tartaric acid, the terms meso and (d,l) are preferred because these terms indicate the diastereormer.

## Cyclic Structures of Monosaccharides

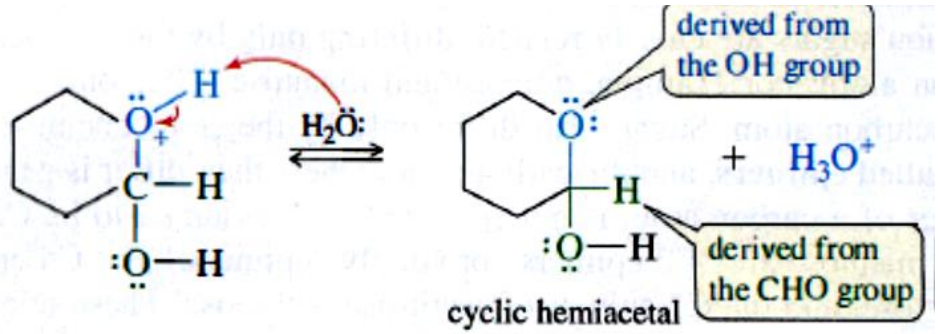
If the aldehyde group and the hydroxyl group are part of the same molecule, a cyclic hemiacetal results. Cyclic hemiacetals are particularly stable if they result in five- or six-membered rings.

Step 1: Protonation of the carbonyl

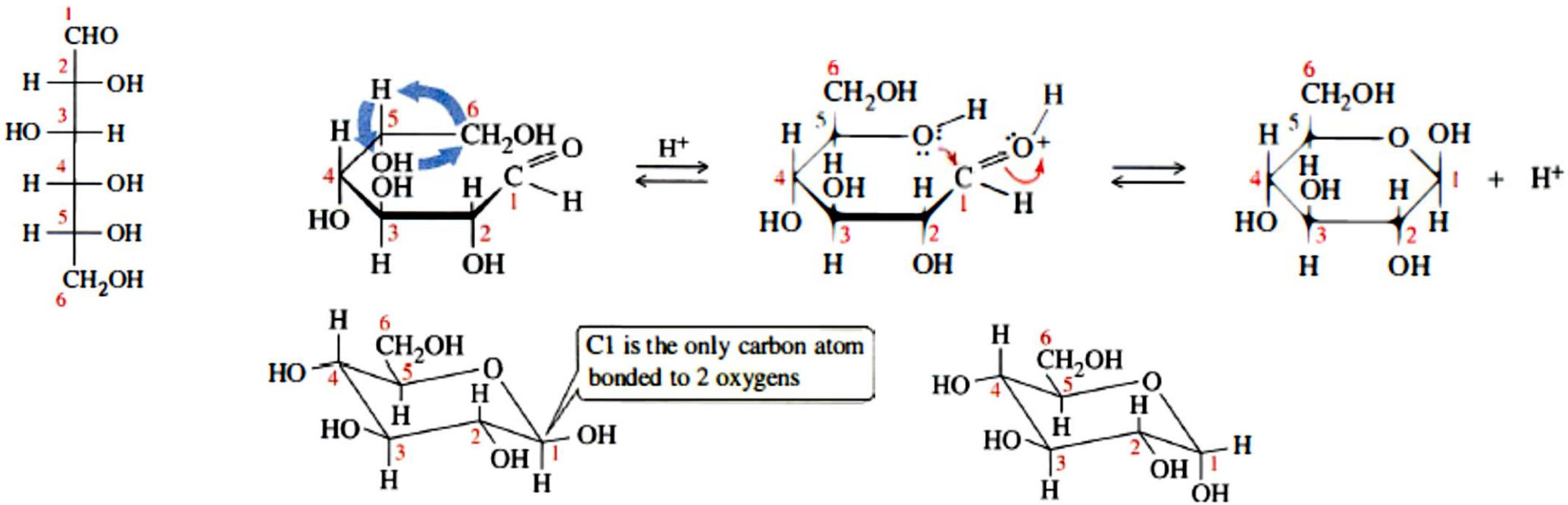
Step 2: The OH group adds as a nucleophile.



## Step 3: Deprotonation gives a cyclic hemiacetal.




- Glucose exists almost entirely as its cyclic hemiacetal form.

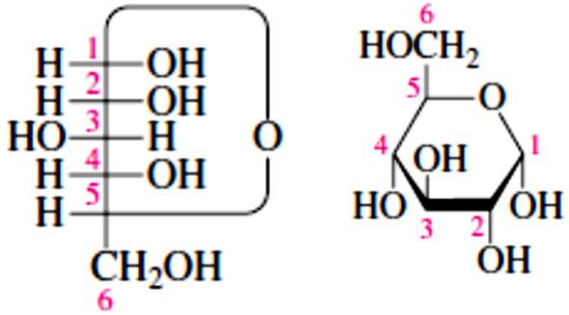


# Haworth projection

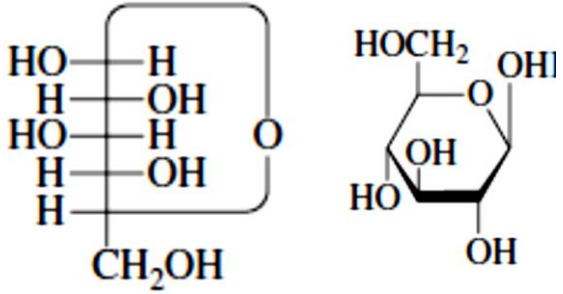
Fischer projections are not the best way to show the structure of a cyclic sugar, because of how the bond is represented. A somewhat more satisfactory representation is given by a **Haworth projection**.

1. In a Haworth projection of a D-pyranose, the six-membered ring is represented as flat and is viewed edge on.
2. The ring oxygen is always placed in the back right-hand corner of the ring, with the anomeric carbon (C-1) on the right-hand side and the primary alcohol group drawn *up* from the back left-hand corner (C-5).
3. Groups on the *right* in a Fischer projection are *down* in a Haworth projection, whereas groups on the *left* in a Fischer projection are *up* in a Haworth projection

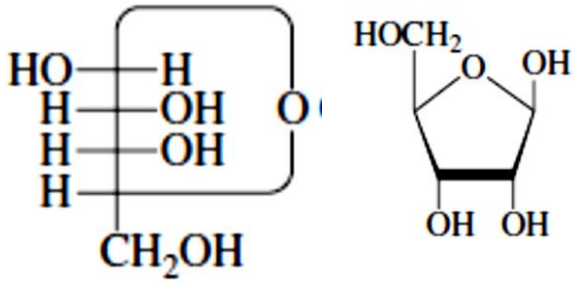
The Haworth projection of a D-furanose is viewed edge on, with the ring oxygen away from the viewer. The anomeric carbon is on the right-hand side of the molecule, and the primary alcohol group is drawn *up* from the back left-hand corner.




α-D-glucopyranose




β-D-glucopyranose




β-D-ribofuranose