Carbohydrates and Glycobiology

CHEM 420 – Principles of Biochemistry
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Chapters 11 and 23: Voet/Voet, *Biochemistry*, 2011
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Classification of carbohydrates according to size

- monosaccharides - the fundamental "building block" units (monomers)

- oligosaccharides - comprised of monosaccharides (2-10) linked together via glycosidic bonds

- polysaccharides - comprised of monosaccharides (10-1000s) linked together via glycosidic bonds

Monosaccharide families

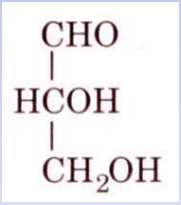
Classified according to the type of carbonyl group
and the number of carbon atoms they contain.

aldehyde: aldoses
ketone: ketoses

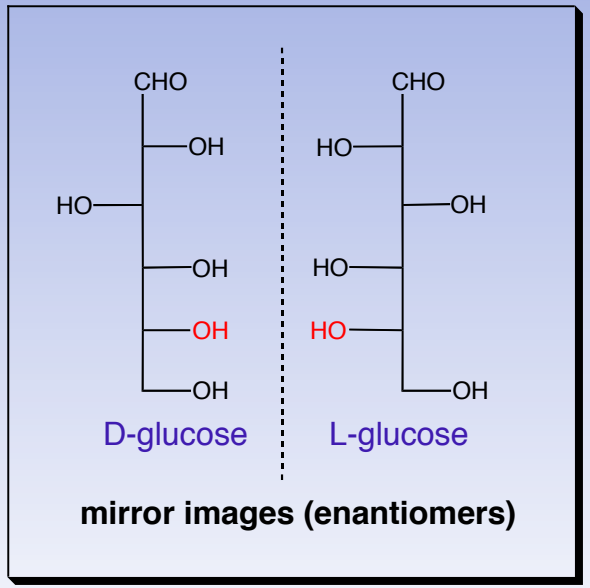
Number of carbons: triose = 3; tetrose = 4; pentose = 5; hexose = 6; heptose = 7, etc.

Convention: D-Sugars have the same configuration at their asymmetric penultimate carbon as does D-glyceraldehyde. L-Sugars are mirror images of D-sugars.

These are Fischer projections and imply specific stereochemistry at each chiral carbon.

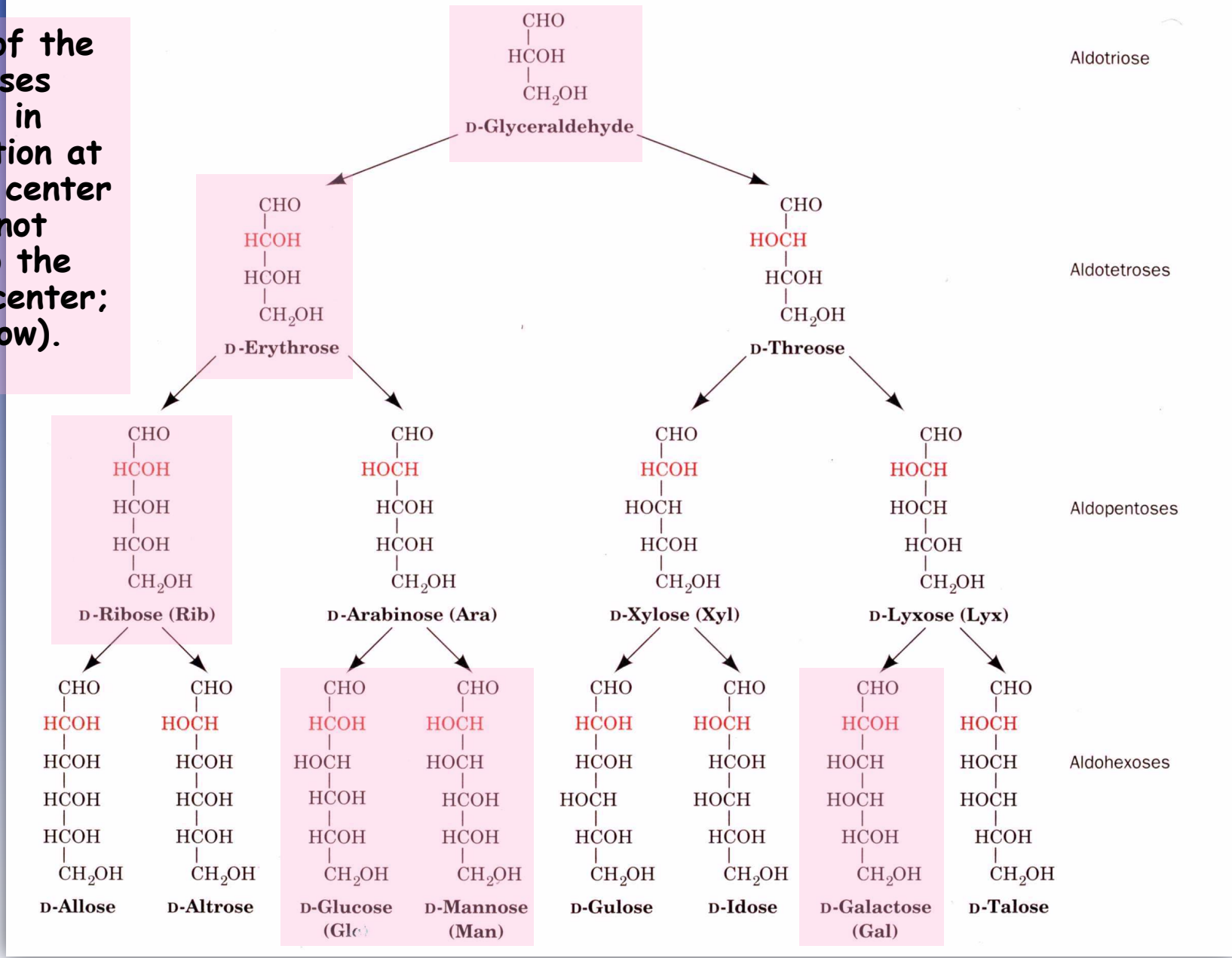


D-glyceraldehyde as reference



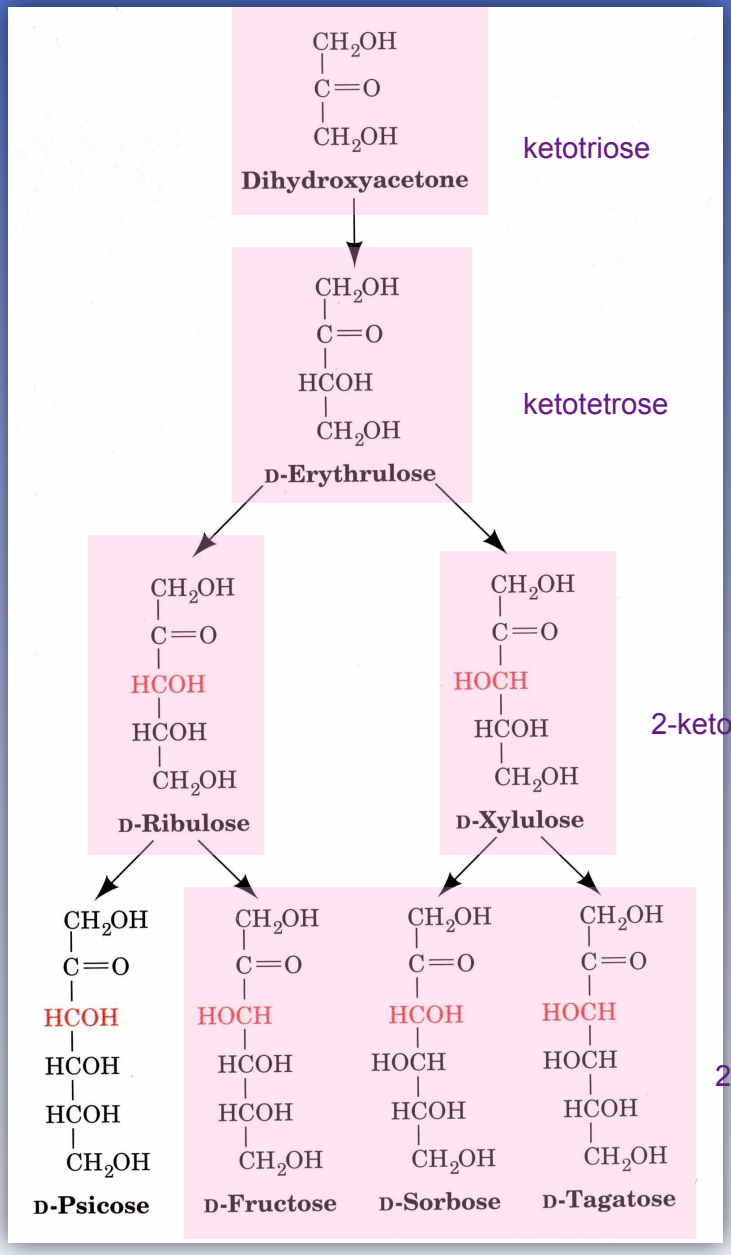
D-Sugars are more biologically abundant than L-sugars.

Epimers of the D-aldoses differ in configuration at one chiral center (does not apply to the anomeric center; see below).



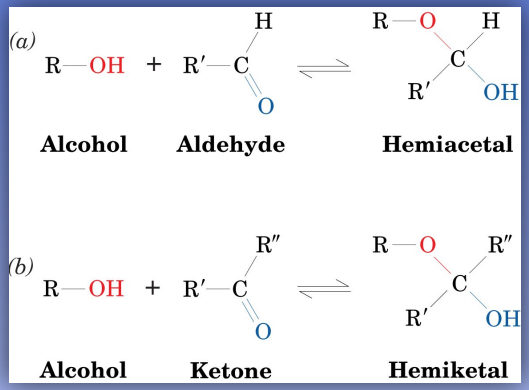
Example: an aldohexose has 4 chiral centers and 2^4 or 16 stereoisomers (8 D (shown above) and 8 L)

Epimers of D-ketoses having 3 to 6 carbons



Example: a ketohexose has 3 chiral centers and 2^3 or 8 stereoisomers (4 D and 4 L)

Monosaccharide cyclization

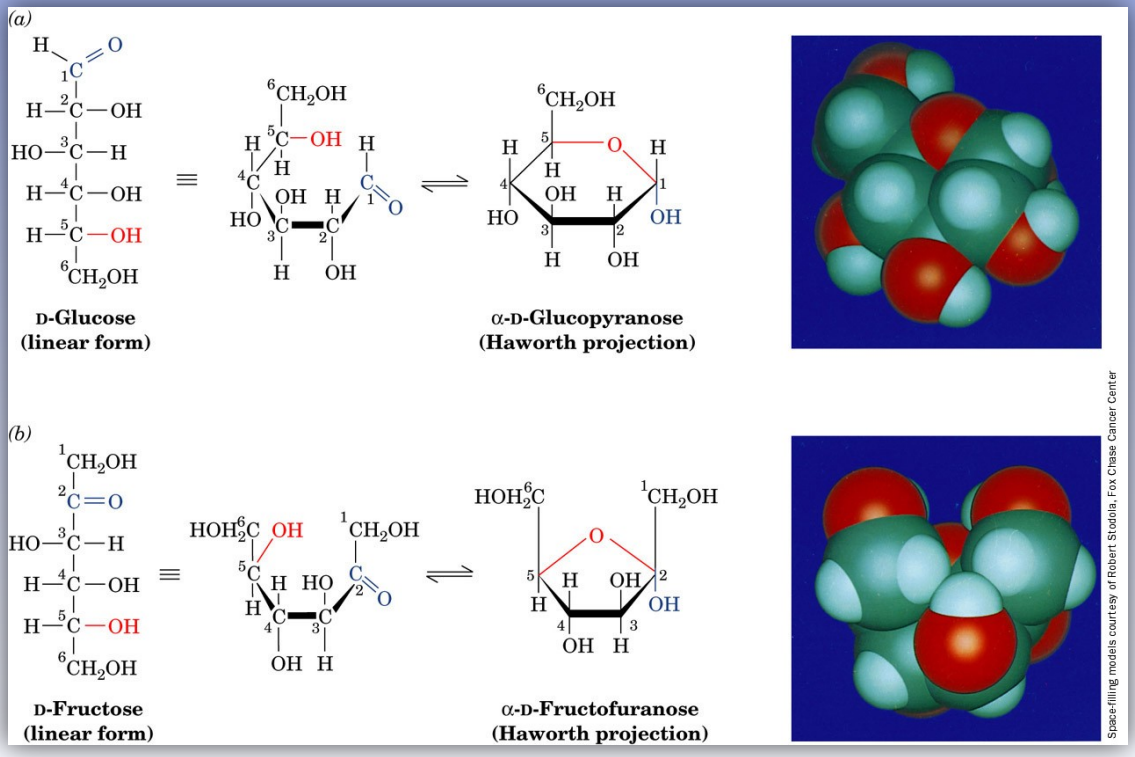


Alcohols react spontaneously and reversibly with aldehydes and ketones to form *hemiacetals* and *hemiketals*, respectively.

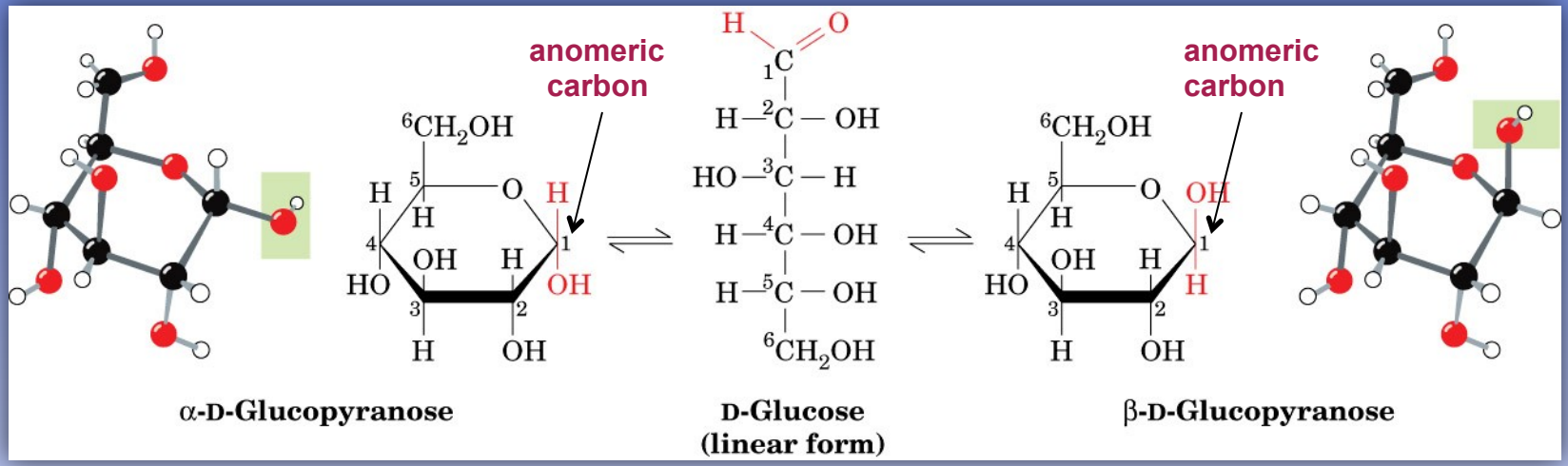
Monosaccharides undergo the same reaction intramolecularly to form cyclic structures. This cyclization reaction (anomerization) is spontaneous and reversible in aqueous solution.

6-Membered rings are known as pyranoses; 5-membered rings are known as furanoses.

Cyclic forms predominate in aqueous solutions of all monosaccharides capable of cyclization.



Formation of anomers upon cyclization



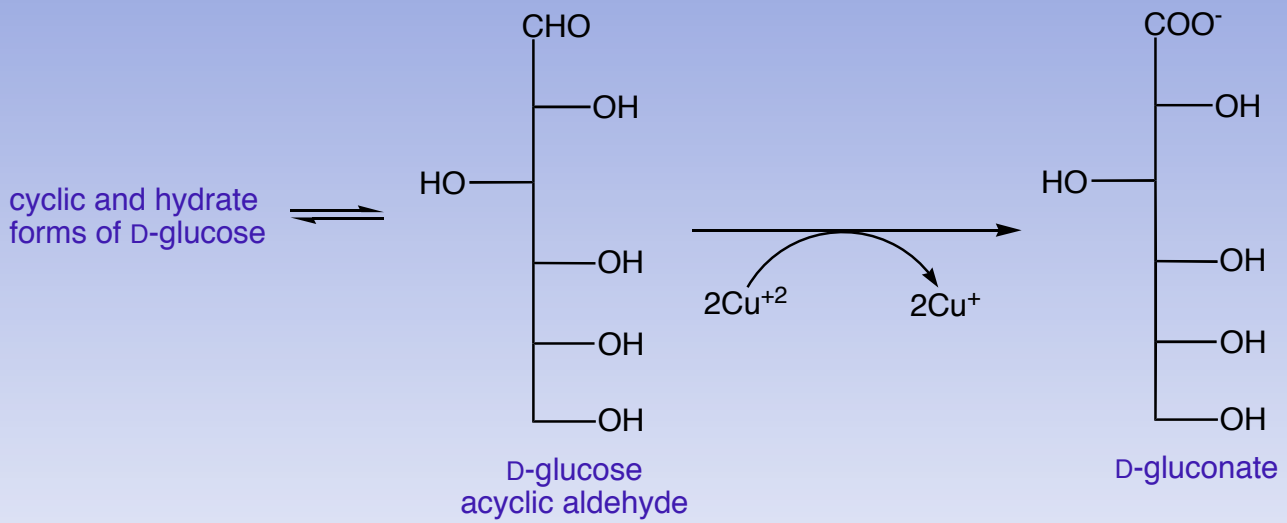
The anomeric monosaccharides, α-D-glucopyranose and β-D-glucopyranose, drawn as Fischer and Haworth projections, and as ball-and-stick models

Upon cyclization, the carbonyl carbon becomes chiral and is referred to as the anomeric carbon. In the α-form, the anomeric OH (O1) is on the opposite side of the ring from the CH_2OH group, and in the β-form, O1 is on the same side.

The α- and β-forms are referred to as anomers or anomeric pairs, and they interconvert in aqueous solution via the acyclic ("linear") form (anomerization). Aqueous solutions of D-glucose contain ~64% β-pyranose and ~36% α-pyranose.

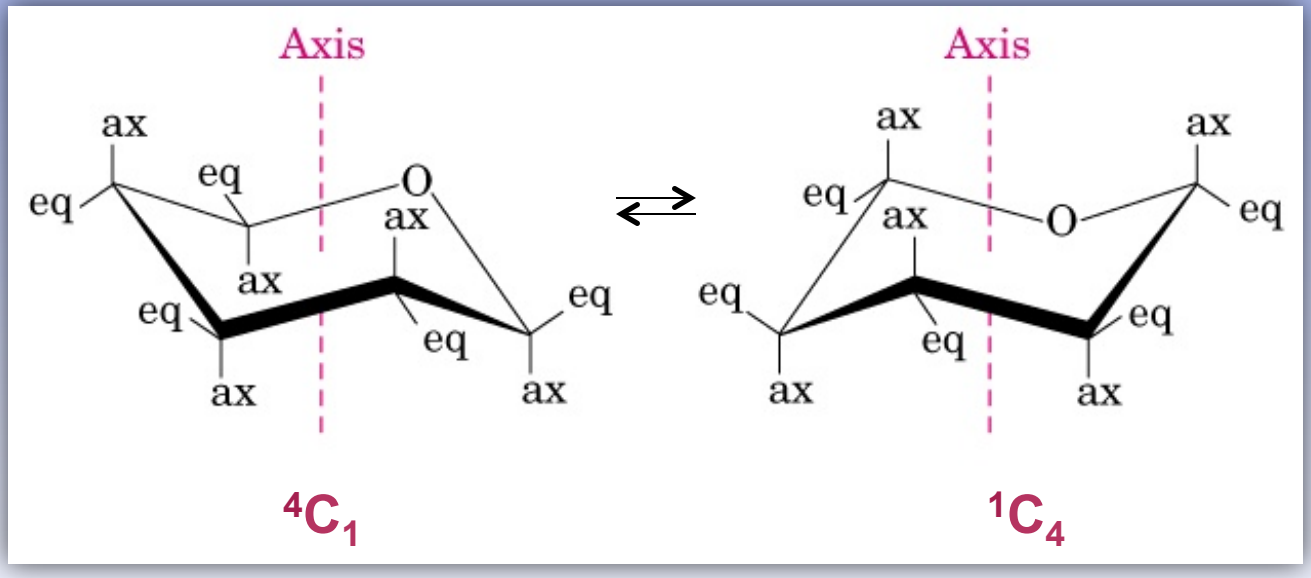
Definition of a reducing sugar

Monosaccharides that are capable of assuming a form in solution that contains a free carbonyl group can be oxidized by relatively mild oxidizing agents such as Fe^{+3} or Cu^{+2} (Fehling's reaction). The saccharide is oxidized and the reagent is reduced.

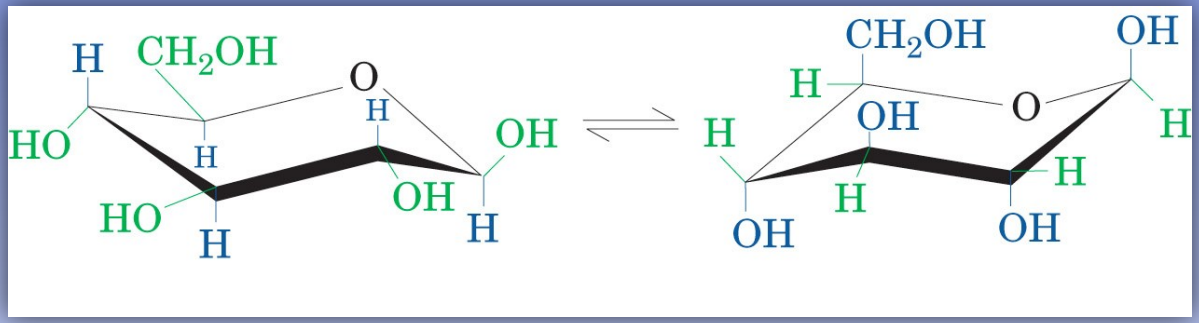


Monosaccharide ring conformations

Two chair conformations ($^4C_1$ and $^1C_4$) interconvert spontaneously in solution, and the rate of interconversion is very rapid. In general, the more stable conformation is that one that orients more of the ring substituents in **equatorial** (eq) rather than **axial** (ax) positions due to fewer steric interactions in the former.



$^4C_1$ and $^1C_4$ chair conformations of β-D-glucopyranose



$^4C_1$
more stable

5 equatorial substituents
0 axial substituents

$^1C_4$
less stable

5 axial substituents
0 equatorial substituents

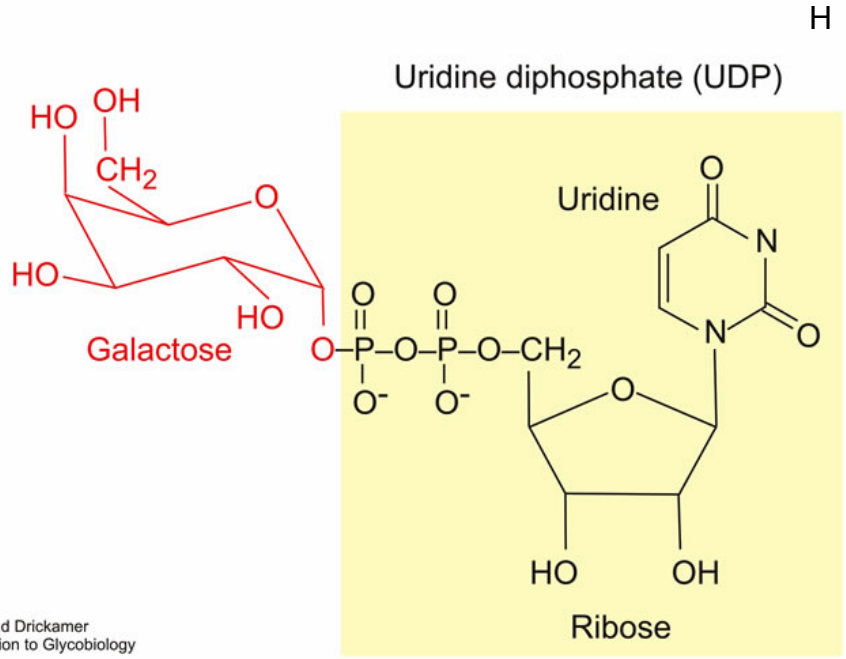
Equatorial and axial substituents exchange orientations upon ring interconversion.

Common monosaccharide modifications *in vivo*

deoxygenation	introduces hydrophobicity
amination	introduces (+) charge
N-acetylation	suppresses (+) charge
oxidation (aldonic/uronic acids)	introduces (-) charge
oxidation (osones)	introduces 2nd carbonyl carbon
reduction (alditols)	destroys carbonyl carbon
phosphorylation	introduces (-) charge
sulfation	introduces (-) charge

Many of these modifications occur *in vivo* via the participation of sugar nucleotides (nucleotide sugars).

Figure 1.11 Structure of a nucleotide sugar that can serve as a sugar donor in a glycosyltransferase reaction



Taylor and Drickamer
Introduction to Glycobiology

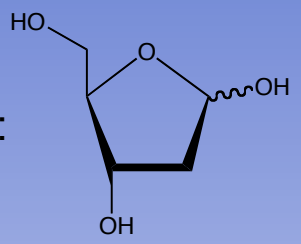
UDP-galactose

A sugar nucleotide is a "biologically activated" monosaccharide.

Sugar nucleotides are involved in sugar transformations and in the biosynthesis of complex carbohydrates (oligomers and polymers) *in vivo*. In the latter role, they serve as sugar donors in the sequential addition of monosaccharides to a growing oligomer or polymer chain catalyzed by glycosyltransferases.

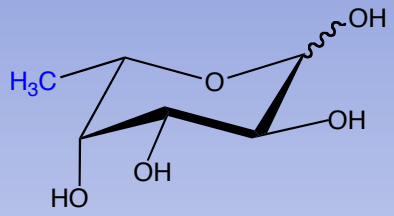
Examples of biologically important deoxysugars

2-deoxy-D-ribose (2-deoxy-D-*erythro*-pentose):



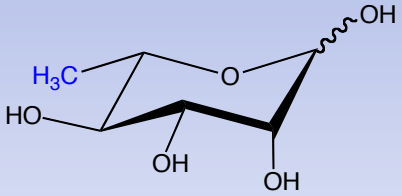
DNA

6-deoxy-L-galactose (L-fucose):



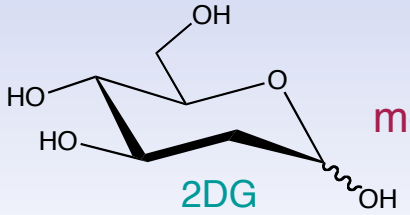
N-glycans of glycoproteins

6-deoxy-L-mannose (L-rhamnose):



bacterial polysaccharides

2-deoxy-D-glucose (2-deoxy-D-*arabino*-hexose):

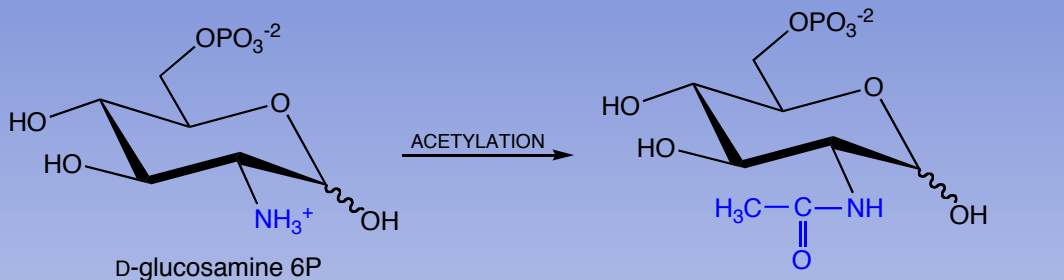


metabolic probe

2DG

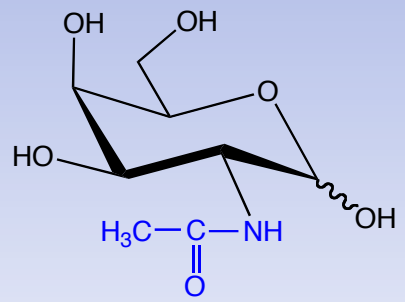
Aminosugars and *N*-acetylation

N-acetyl-D-glucosamine 6P (GlcNAc 6P)
N-acetyl-D-galactosamine (GalNAc) equivalents



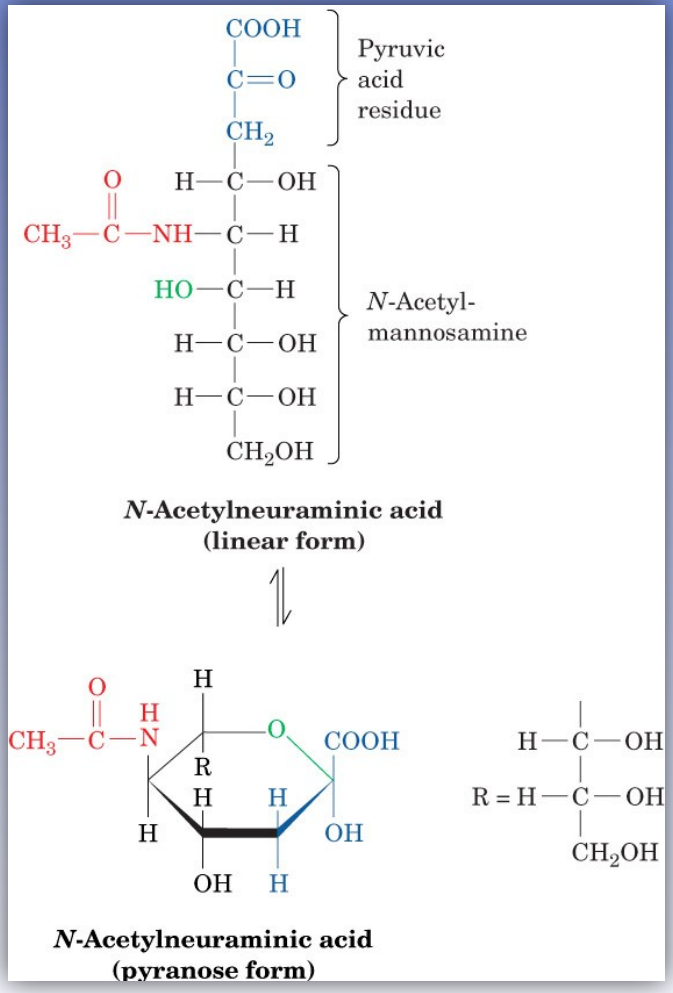
D-glucosamine 6P
(2-amino-2-deoxy-D-glucose 6P)

N-acetyl-D-glucosamine 6P (GlcNAc 6P)
(2-acetamido-2-deoxy-D-glucose 6P)



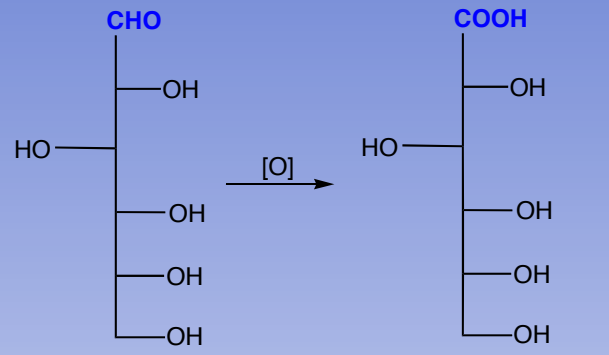
N-acetyl-D-galactosamine (GalNAc)
(2-acetamido-2-deoxy-D-galactose)

Another biologically important *N*-acetylated sugar: *N*-Acetyl-neuraminic acid (Neu5Ac)



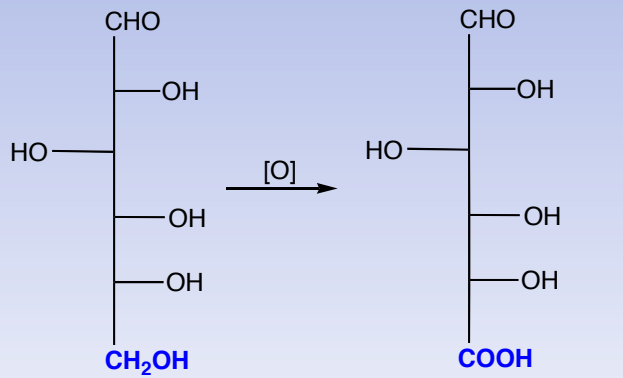
Neu5Ac is a C9 α-ketoacid derived biosynthetically from C_6 (ManNAc) and C_3 (PEP or pyruvate) precursors. Neu5Ac is a common constituent of *N*-glycans of *N*-linked glycoproteins (see below).

Oxidized Monosaccharide Derivatives



an aldose an aldonic acid

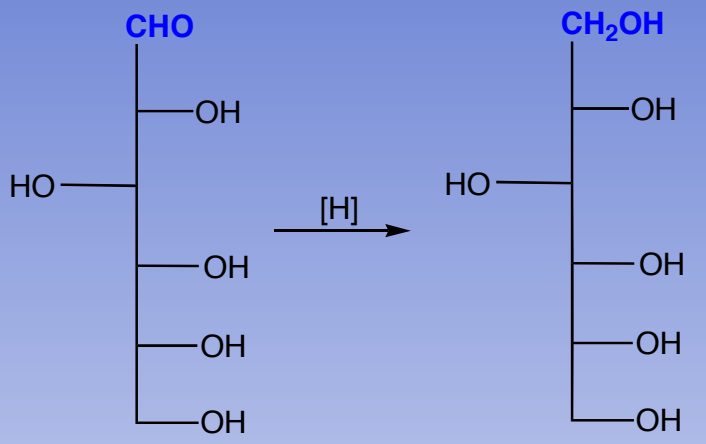
Aldonic acids: produced when C1 of an aldose is oxidized to the carboxylic acid; *e.g.,* D-glucose to D-gluconic acid; D-mannose to D-mannonic acid. Since the carbonyl (aldehydic) carbon is destroyed, aldonic acids are not reducing sugars (aldonic acids do not undergo anomerization).



an aldose an alduronic acid

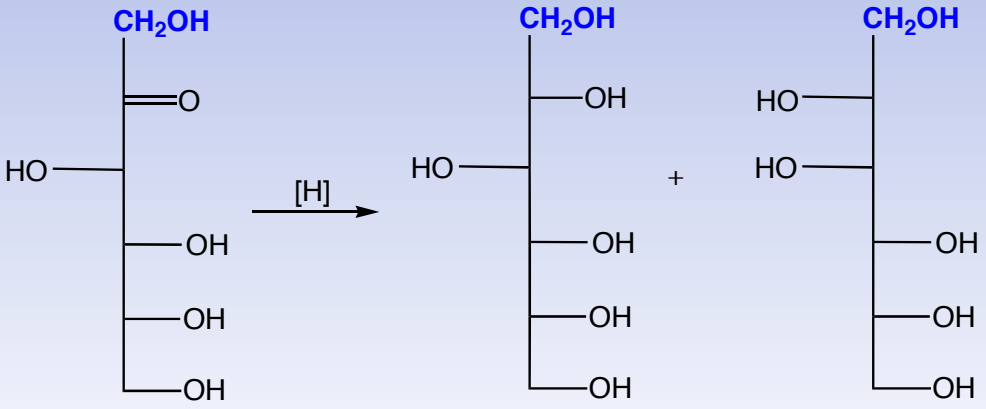
Alduronic acids: produced when the terminal primary alcohol (hydroxymethyl group) of an aldose is oxidized to the carboxylic acid; *e.g.,* D-glucose to D-glucuronic acid; D-mannose to D-mannuronic acid. Since the carbonyl (aldehydic) carbon is not destroyed, alduronic acids are reducing sugars and undergo anomerization.

Reduction of aldoses and ketoses to alditols



an aldose an alditol

Alditols: Produced from the reduction of the aldehydic carbon of an aldose or the ketone carbon of a ketose; only one product is obtained from aldose reduction, whereas two are obtained from ketose reduction. Alditols are not reducing sugars since they do not contain a carbonyl center. They are acyclic molecules. Generated *in vivo*.



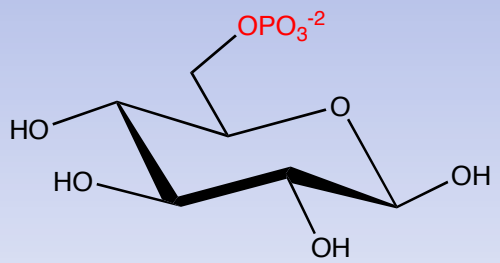
a 2-ketose two different alditols

Alditols are common chemical derivatives used to simplify the analysis of monosaccharide mixtures generated from the hydrolysis of complex oligo- and polysaccharides.

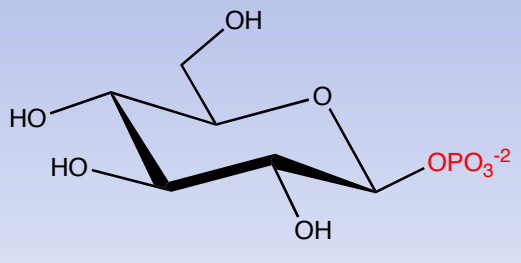
Phosphorylation: The presence of phosphomonoesters is common in saccharide metabolites. Phosphorylation inhibits diffusion of metabolites through the plasma membrane and affects chemical and biological activities. The phosphate source is usually ATP.

pK_a and $\Delta G^{o'}$ (hydrolysis) (kJ/mol) values of sugar phosphates

D-glyceraldehyde 3P	pK_1 2.1	pK_2 6.8	$\Delta G^{o'}$ ~-12
β-D-glucose 1P	pK_1 1.1	pK_2 6.1	$\Delta G^{o'}$ -20.9
β-D-glucose 6P	pK_1 0.94	pK_2 6.1	$\Delta G^{o'}$ -13.8
α-D-fructose 6P	pK_1 1.0	pK_2 6.1	$\Delta G^{o'}$ -13.8



β-D-glucose 6P
(phosphomonoester)



β-D-glucose 1P
phosphomonoester; glycosyl phosphate)

Glycosyl phosphates are produced by phosphorylation at the anomeric hydroxyl group of an aldose or ketose.

Saccharide sulfation is achieved via the sulfate donor, PAPS

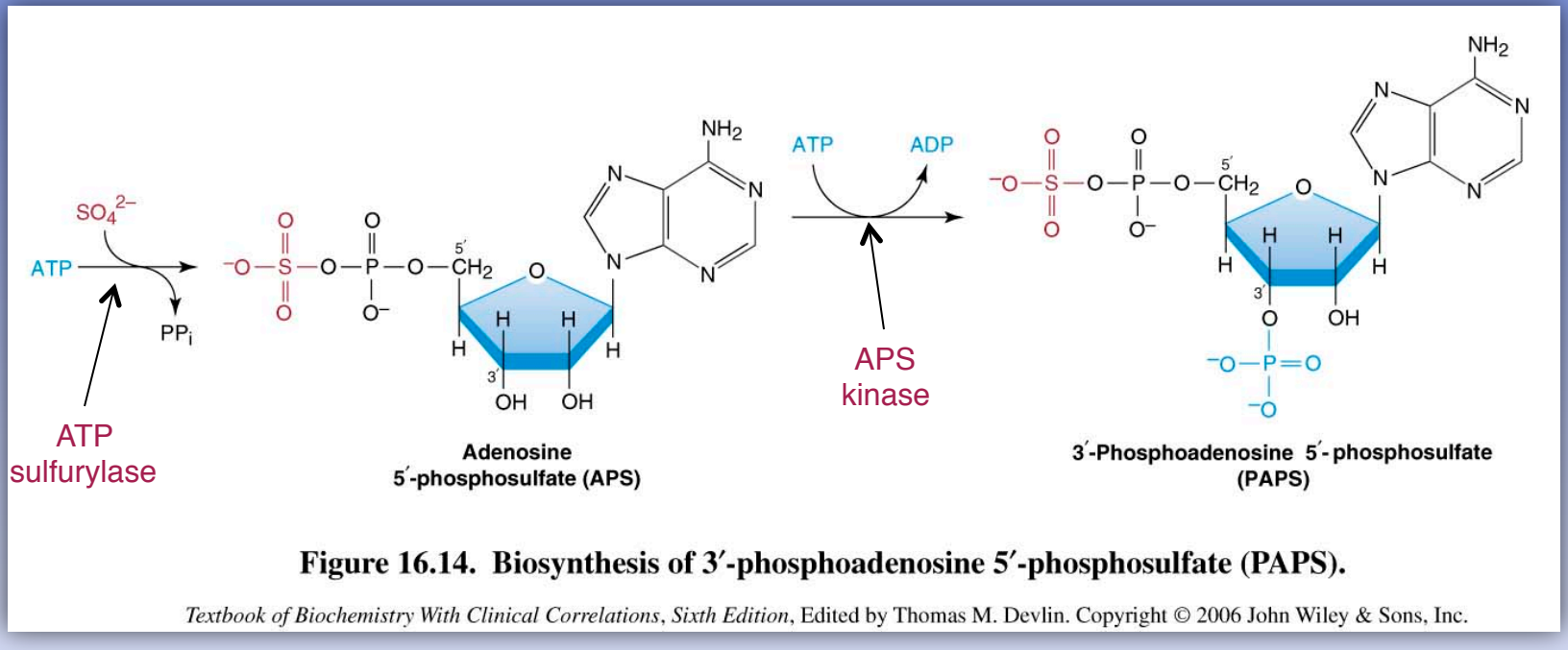
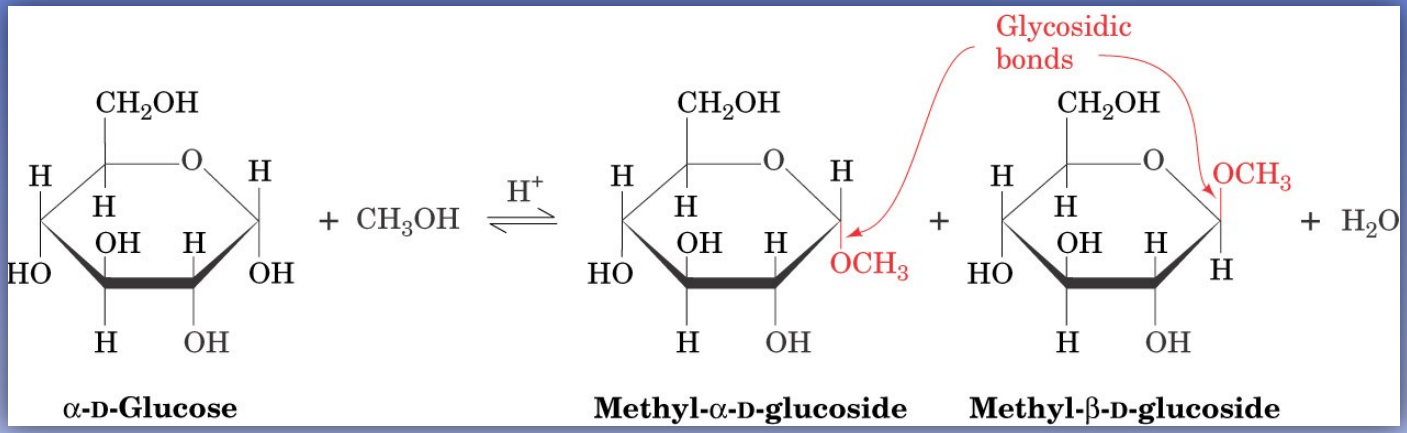


Figure 16.14. Biosynthesis of 3′-phosphoadenosine 5′-phosphosulfate (PAPS).

APS and PAPS are mixed anhydrides.

Chemical glycosylation

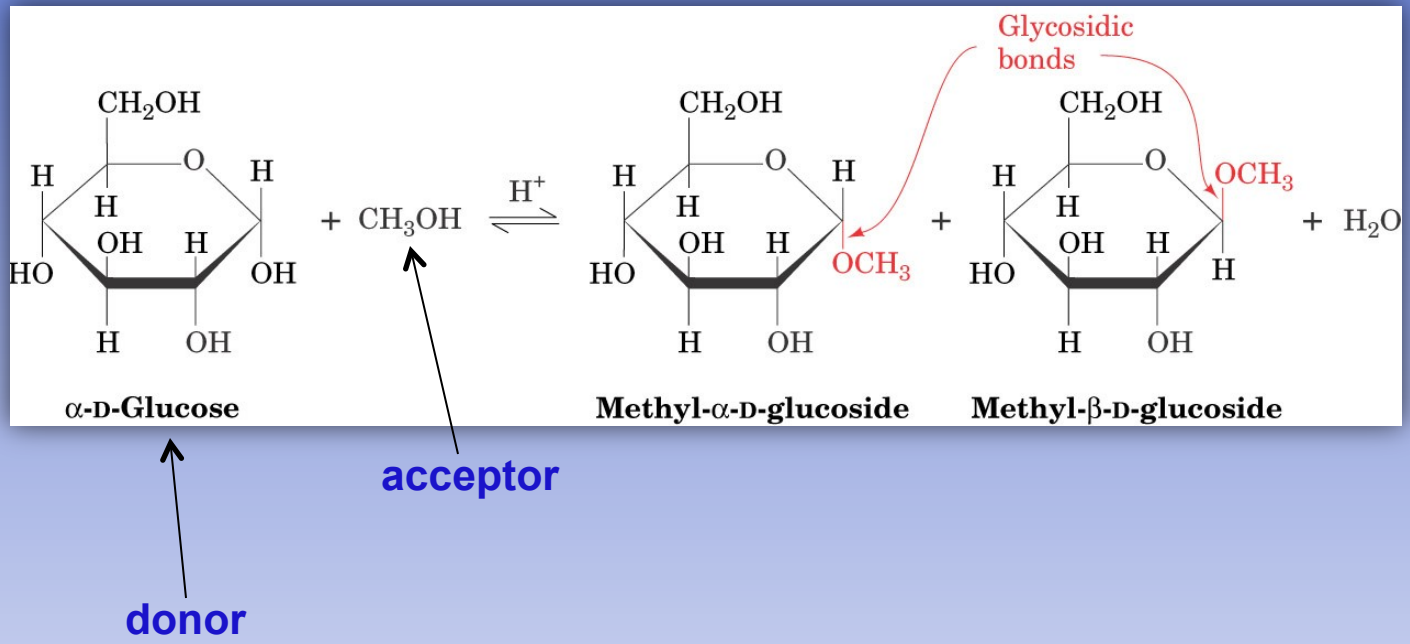


The acid-catalyzed condensation of α-D-glucopyranose in methanol solvent to form an anomeric pair of methyl D-glucopyranosides (Fischer glycosidation).

The anomeric (C1) carbon of the two pyranosides (methyl α- and β-D-glucopyranosides) is an acetal carbon, whereas the anomeric (C1) carbon of D-glucose is a hemiacetal carbon. Glycosides are not reducing sugars, and they do not undergo anomerization in solution under neutral and basic conditions.

Glycosides are always formed under acidic conditions, and are always hydrolyzed under acidic conditions. Glycosides are stable in neutral and basic solution.

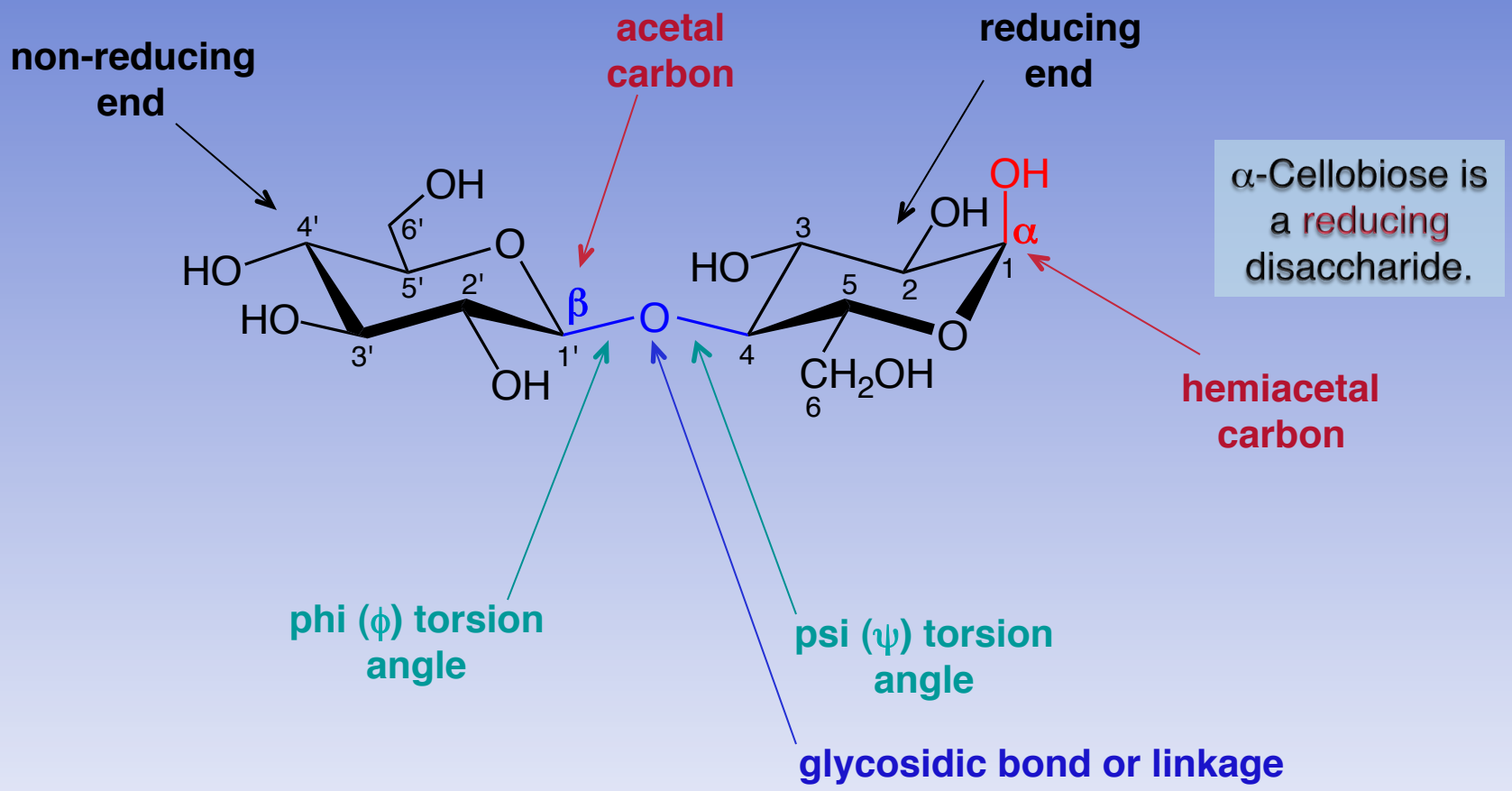
Disaccharide formation



When the alcohol functional group is supplied by another monosaccharide like D-glucose instead of methanol, a disaccharide forms. Ten different Glc-Glc disaccharides are possible since five different hydroxyl groups are present in the Glc acceptor, and the Glc donor can have the α or β anomeric configuration.

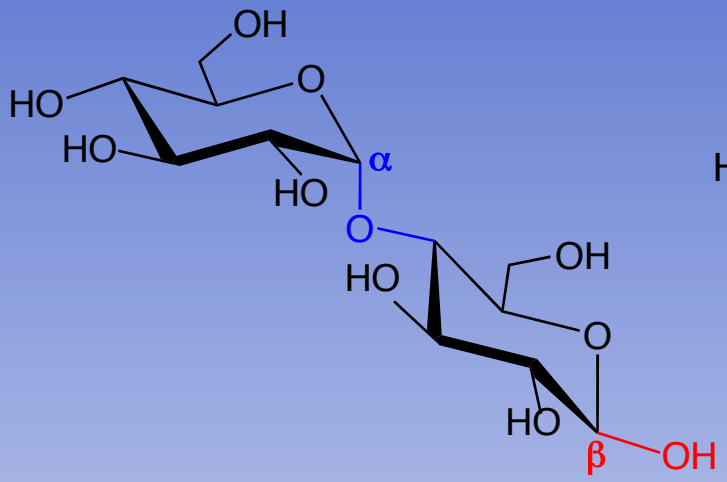
Disaccharides *in vivo* play important roles as independent sugars (*e.g.,* lactose) or occur as repeating subunits in the construction of oligo- and polysaccharides.

Nomenclature, symbolism and conventions for O-glycosidic linkages



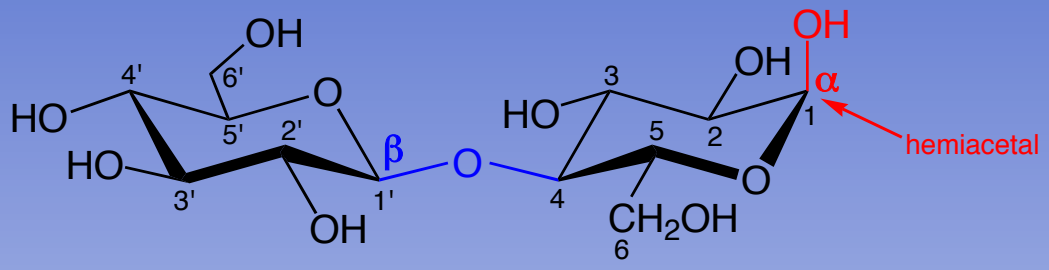
Proper name:

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



β-maltose
α-D-glucopyranosyl-(1→4)-β-D-glucopyranose
(reducing disaccharide; anomerizes in solution)

α-(1 → 4)linkage

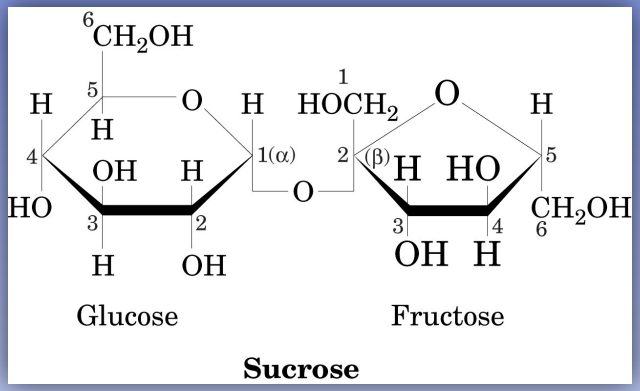


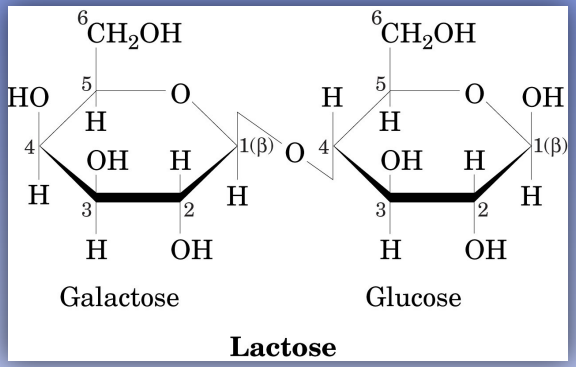
α-cellobiose
β-D-glucopyranosyl-(1→4)-α-D-glucopyranose
(reducing disaccharide; anomerizes in solution)

β-(1→ 4)linkage

Two different Glc-Glc disaccharides showing different regiochemistries and stereochemistries. Both disaccharides are reducing disaccharides.

Other common biologically important disaccharides

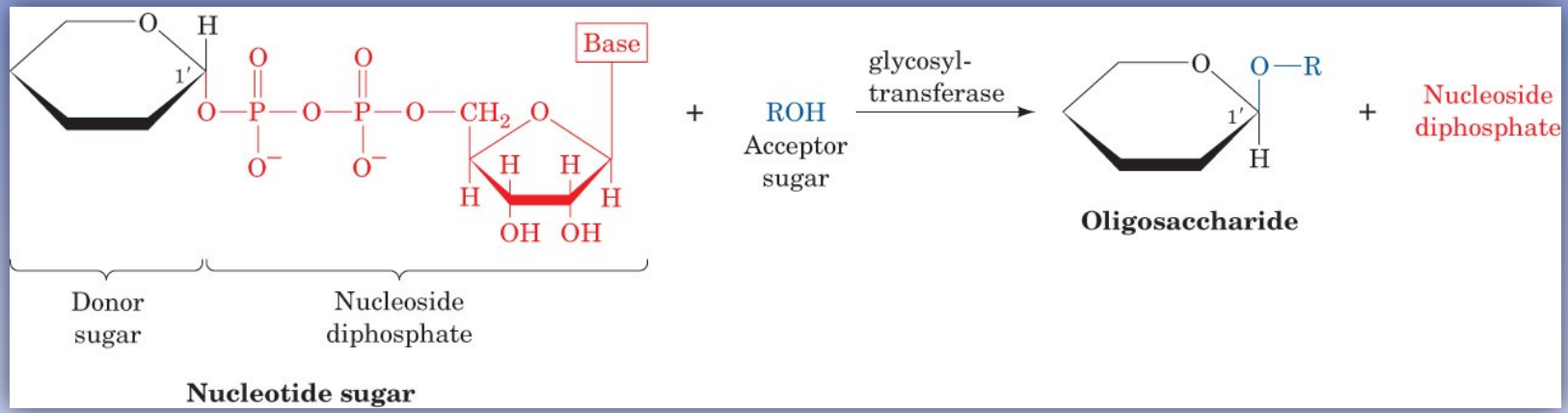




Distinguishing structural features of disaccharides

1. identities of the two monomers (monosaccharide composition)
2. linkage regiochemistry (*i.e.*, which carbons are involved in the linkage)
3. order of monomers if they are different
4. anomeric configuration of the linkage (linkage stereochemistry)

Enzyme-catalyzed synthesis of glycosidic linkages by glycosyltransferases



The major sugar nucleotides are: UDP-Glc, UDP-Gal, UDP-GlcNAc, UDP-GalNAc, GDP-Man, GDP-fucose

Hydrolysis of glycosidic linkages

Chemical methods: treatment with aqueous acid (HCl, H_2SO_4, CF_3COOH)

Enzymatic methods: use of glycosidases (glycoside bond hydrolyzing enzymes)

- Exoglycosidases: Hydrolyze glycosidic linkages involving terminal residues
- Endoglycosidases: Hydrolyze glycosidic linkages involving internal residues

Glycosidases exhibit additional specificity for the configuration of the linkage and for the configuration of the residue contributing the anomeric carbon to the linkage. Some glycosidases are also influenced by aglycone structure. Steric crowding near the linkage may protect it from hydrolysis by glycosidases.

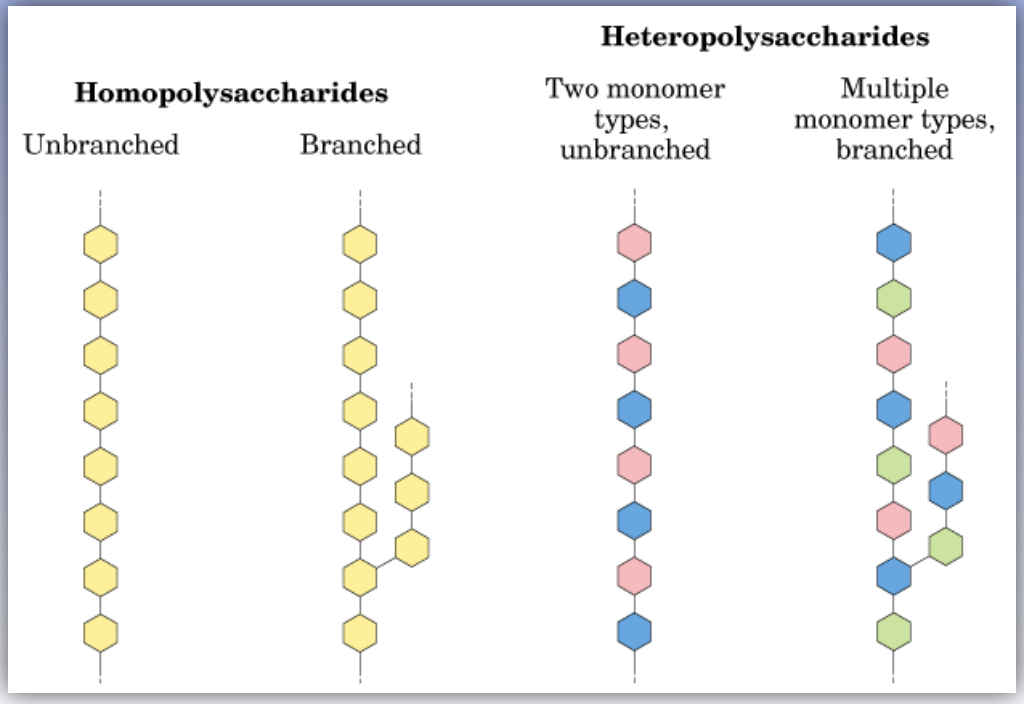
Some glycosidases and their substrate specificities

- ***endo* β-galactosidases:** cleave internal β-Gal*p* linkages
- **α-mannosidases (*exo*):** cleave terminal α-Man*p* residues
- **β-galactosidases (*exo*):** cleave terminal β-Gal*p* residues
- **β-*N*-acetylhexosaminidases (*exo*):** cleave terminal β-GlcNAc*p* residues
- **α-fucosidases (*exo*):** cleave terminal α-Fuc*p* residues
- **α-sialidases (*exo*):** cleave terminal α-NeuAc residues

Polysaccharides

Polysaccharides are formed by linking multiple monosaccharides together via *O*-glycosidic linkages. They can have molecular weights > 1 x 10^6 Da. There are two basic structural types:

- **Homopolysaccharides:** comprised of only one type of monosaccharide; linkages may not be homogeneous (examples: cellulose, starch, glycogen)
- **Heteropolysaccharides**: comprised of more than one type of monosaccharide (examples: hyaluronic acid, glycosaminoglycans)



Functions: energy storage; structural support; protective; cell identification.

Extracellular polysaccharides

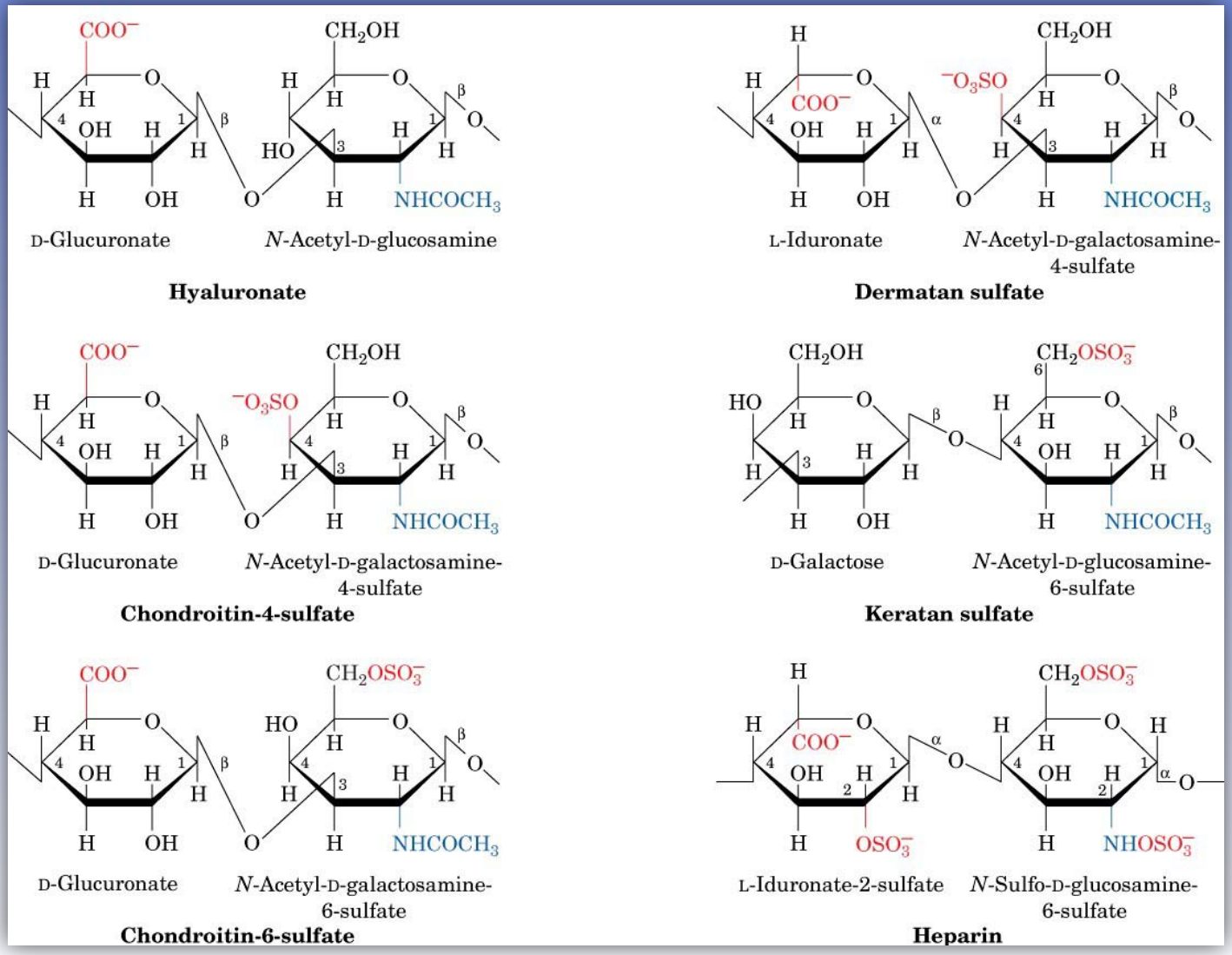
Connective tissue (cartilage, skin, tendons, blood vessel walls) consist of collagen and elastin (protein-based) fibers embedded in a viscous, gel-like matrix called ground substance.

Composed largely of glycosaminoglycans (GAGs) – the most abundant heteropolysaccharides. Unbranched structures contain derivatives of GlcNAc, GalNAc, and uronic acids (*e.g.*, D-glucuronic and L-iduronic acids); backbones consist of repeating disaccharide units.

- Highly negatively charged, due primarily to the presence of sulfate esters
- Located primarily on the surface of cells or in the extracellular space
- Extended conformation imparts high viscosity to extracellular solutions
- Low compressibility – ideal for lubricating joints
- Highly viscous and elastic
- Rigidity provides structural integrity to cells and provides passageways between cells, allowing for cell migration.

Some glycosaminoglycans are linked to core proteins in the extracellular matrix, producing proteoglycans. Proteoglycans are heterogeneous protein/polysaccharide complexes with molecular weights >10^7 Da.

GAGs are extracellular polysaccharides comprised of repeating disaccharide subunits.



Glycobiology: Definitions and terminology

Glycobiology: studies of the structures and functions of sugars attached to proteins and lipids.

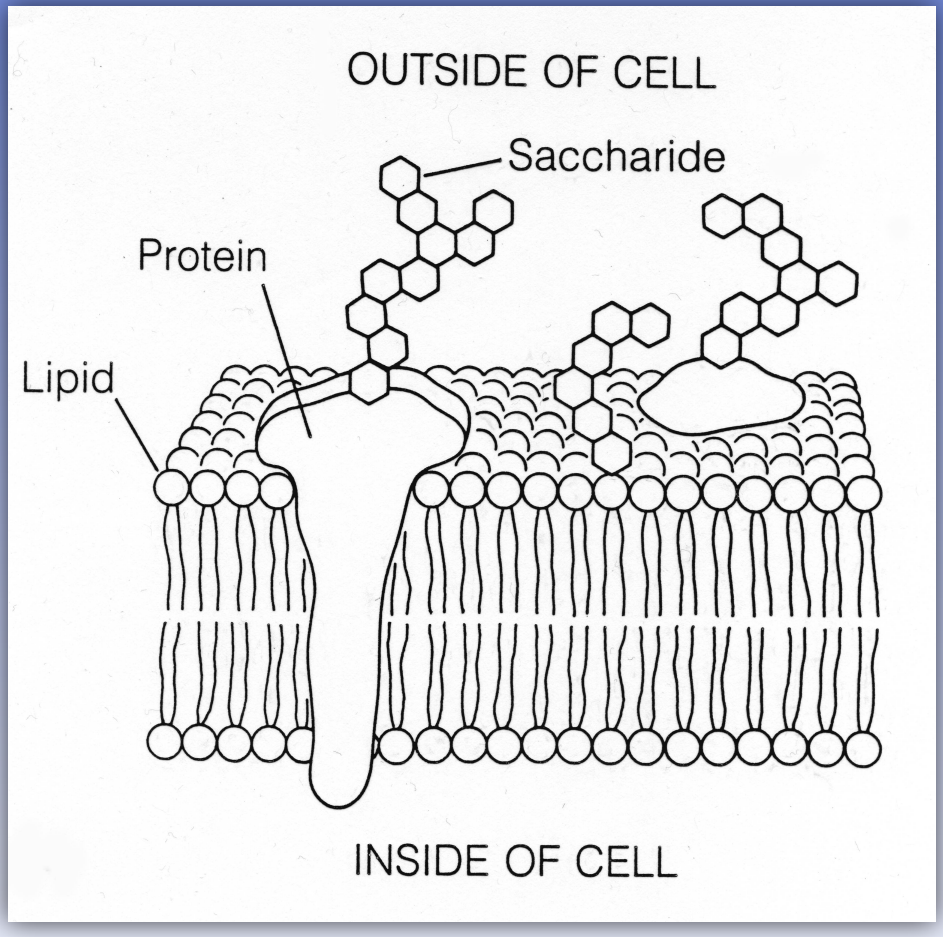
Glycoconjugates: formed when mono-, oligo- or polysaccharides are attached to proteins or lipids.

Glycoproteins and glycolipids: proteins and lipids to which carbohydrate is covalently attached; the mechanism of attachment is enzyme-catalyzed *in vivo*.

Glycan: the carbohydrate component of glycoproteins and glycolipids.

Protein glycosylation

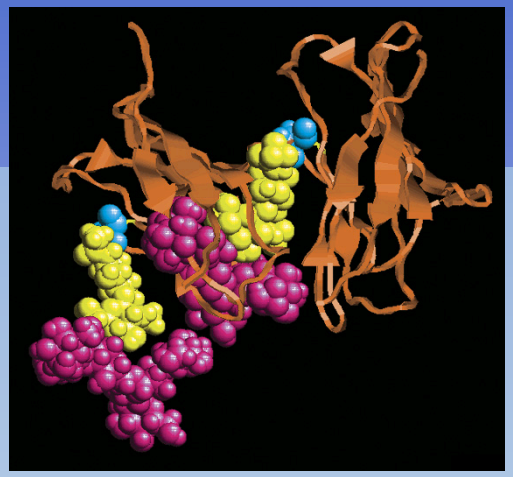
Enzyme-catalyzed covalent modification of proteins and lipids; involves specific sugar donors such as nucleotide and dolichol sugars, and glycosyltransferases; glycosylated products have specific structures and biological functions.



Glycoconjugates associated with plasma membranes (glycoproteins and glycolipids): asymmetric distribution of glycan chains on the extracellular side of the membrane.

The extracellular location allows specific glycan interactions with biomolecules, cells, viruses.

Glycoproteins



Protein glycosylation affects:

- thermodynamic stability
- biological half-life
- cellular localization
- biological activity

Protein glycosylation is controlled enzymically:

- glycosylation of a particular protein can differ by cell type, growth stage, metabolic activity, and substrate availability, resulting in different isoforms that differ by glycosylation only.
- glycosylation differences produce glycoforms characterized by their microheterogeneity (a conserved protein component but different glycan components)

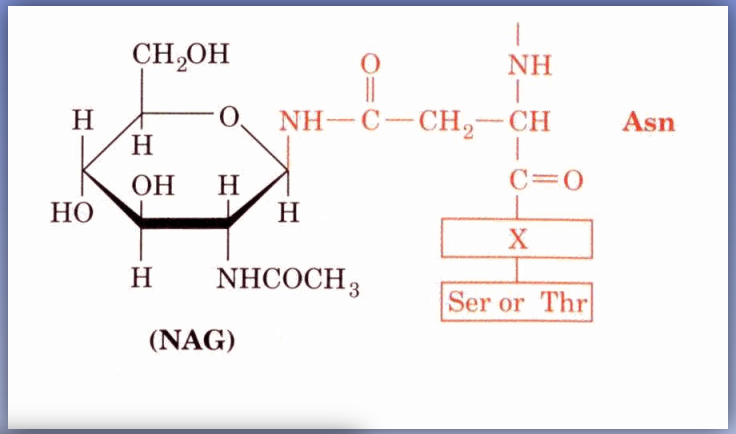
Nearly all eukaryotic secreted and membrane-associated proteins are heavily glycosylated; glycosylation is the most common post-translational modification of proteins; ~50% of proteins in the human body are glycosylated.

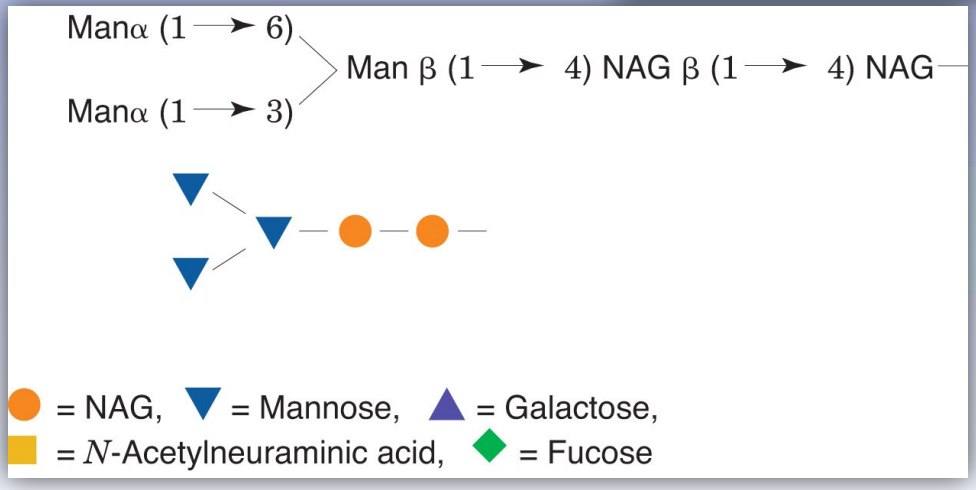
Two major forms of protein glycosylation: *N*-linked glycans and *O*-linked glycans

As a general rule, prokaryotes do not glycosylate proteins.

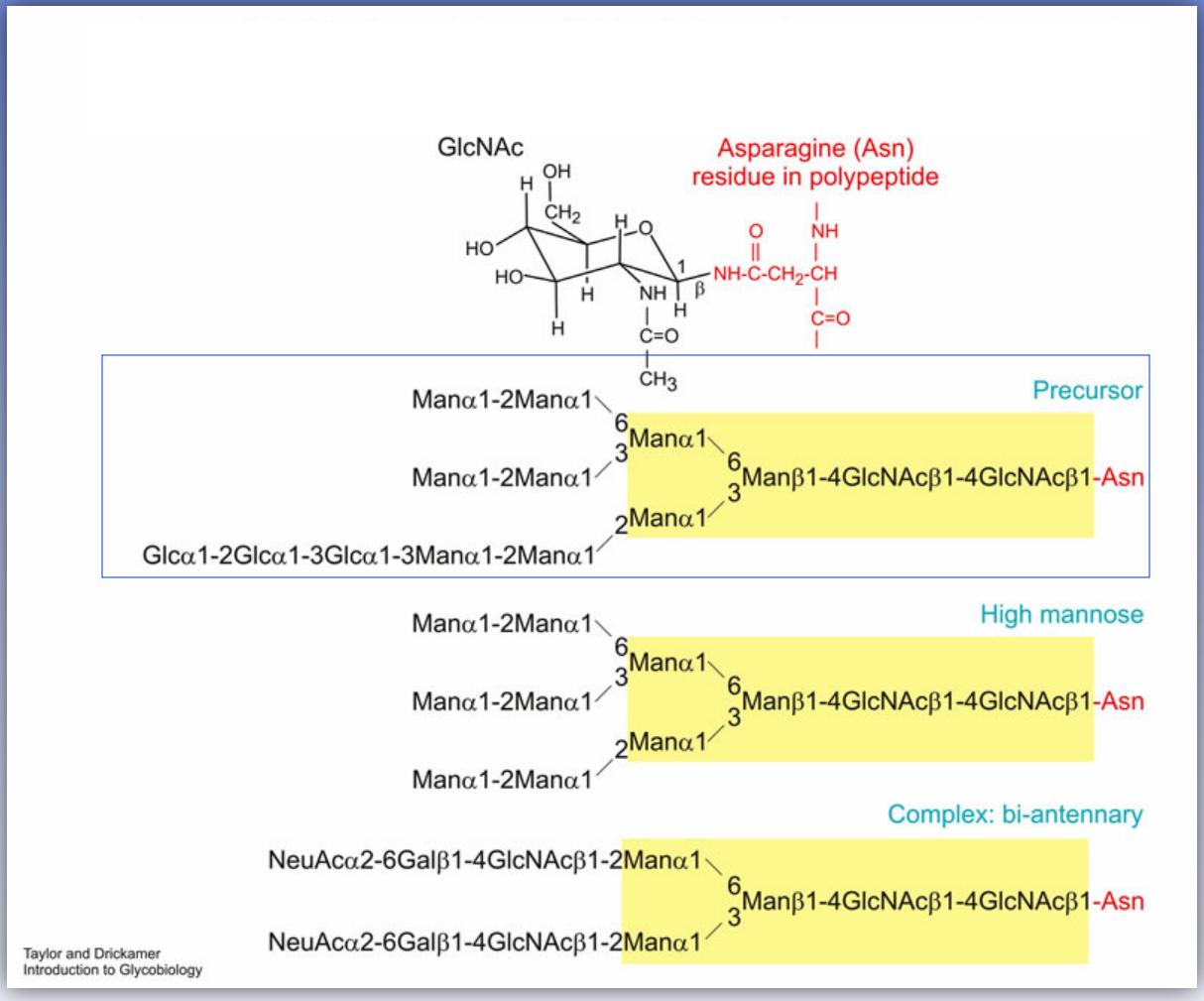
N-Linked Glycoproteins and *N*-Glycans

N-Glycosylation involves a consensus sequence:
GlcNAc is β-linked to the amide nitrogen of an Asn sidechain
Consensus tripeptide sequence = Asn-X-Ser or Thr (X ≠ Pro / Asp)





***N*-linked glycans contain a common pentasaccharide core: $(Man)_3(GlcNAc)_2$**

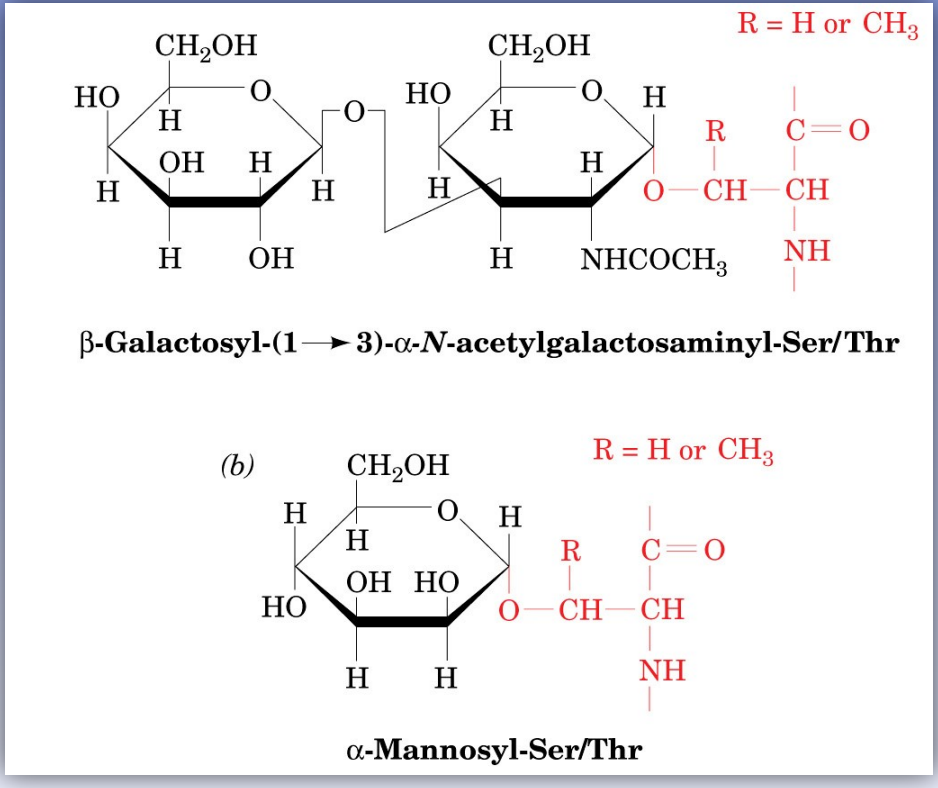


The $GlcNAc_2Man_3$ "core" pentasaccharide is common to all *N*-linked glycans. The two Man branch points in this core pentasaccharide give rise to the 1,3 and 1,6 arms of the *N*-glycan. The $GlcNAc_2Man_9Glc_3$ oligosaccharide is the biological precursor in the construction of all *N*-glycans *in vivo*.

O-Linked Glycoproteins and *O*-Glycans

O-Glycosylation

β-D-Galactopyranosyl-(1,3)-*N*-acetyl-D-galactosamine α-linked to the side-chain OH group of either Ser or Thr.



O-Glycosylation is often structural (*e.g.*, in proteoglycans and mucins). Heavy *O*-glycosylation forces the protein to adopt an extended conformation.

Biosynthetic strategy for protein *O*-glycosylation

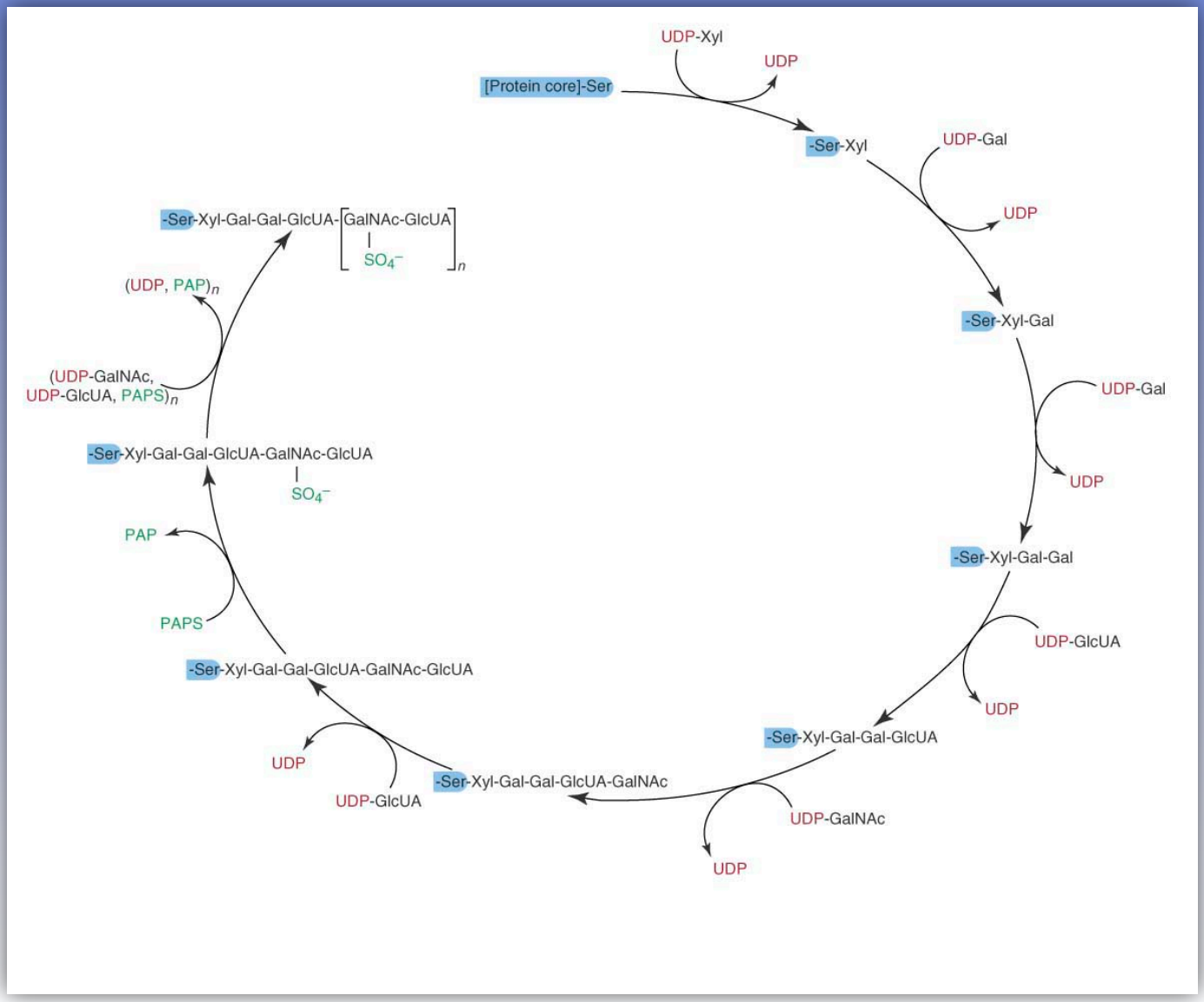
- Protein *O*-glycosylation involves glycosyltransferases analogous to those involved in protein *N*-glycosylation.

- Saccharide residues are added one at a time, starting from the initial GalNAc attached to Ser or Thr (there is no preformed core or *en bloc* transfer). There are numerous GalNAc transferases that attach the initial GalNAc to protein, each apparently displaying a unique specificity.

- There are no simple consensus sequences for *O*-glycosylation.

- *O*-Glycosylation occurs post-translationally in the Golgi.

Biosynthetic pathway for the synthesis of chondroitin sulfate proteoglycan



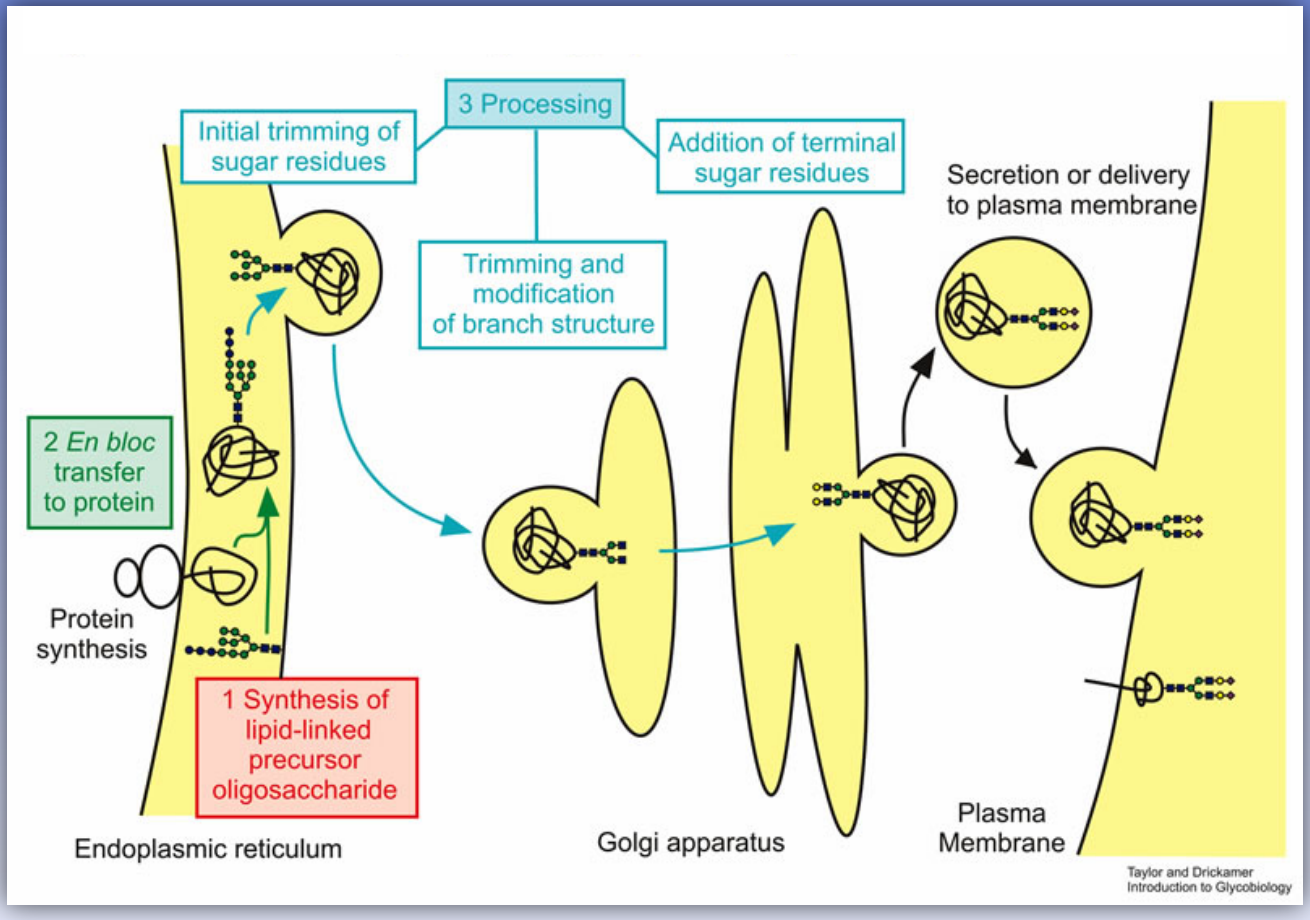
Biosynthetic route for the construction of a protein-bound chrondroitin sulfate oligosaccharide chain, showing sequential multiple additions of monosaccharide units

Biosynthetic strategy for protein *N*-glycosylation: Three stages

1. Formation of a lipid-linked precursor (parent) oligosaccharide ($Glc_3Man_9GlcNAc_2$)
2. *En bloc* transfer of the parent oligosaccharide to the polypeptide
3. Processing of the parent oligosaccharide; involves removal of some of the original saccharide residues (trimming by exoglycosidases) followed by addition of new saccharides (by glycosyltransferases) to the non-reducing termini of the glycan

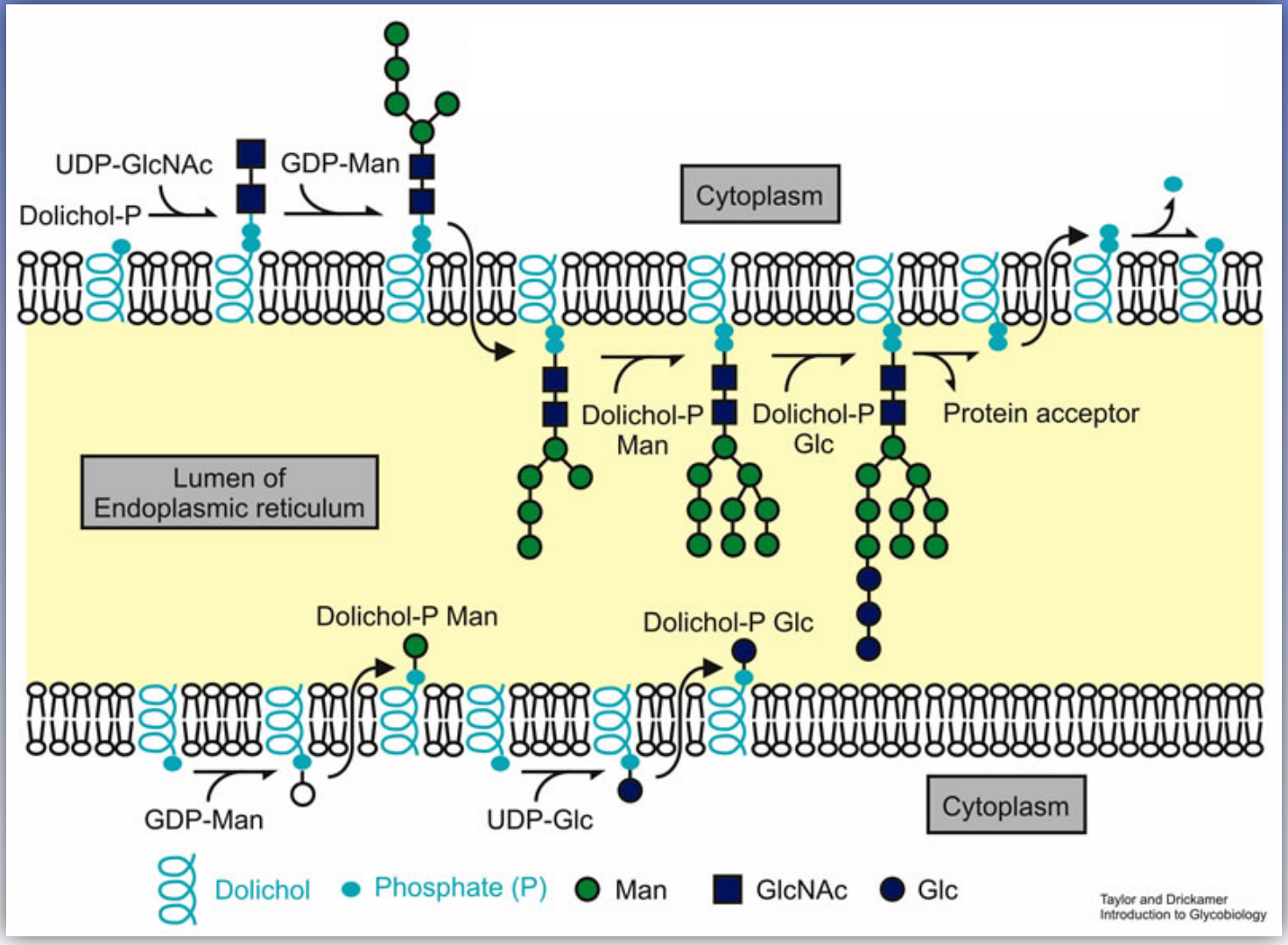
The overall process occurs intracellularly in spacially differentiated steps.

Initial attachment of an N-glycan to a protein is a co-translational event that occurs in the ER.



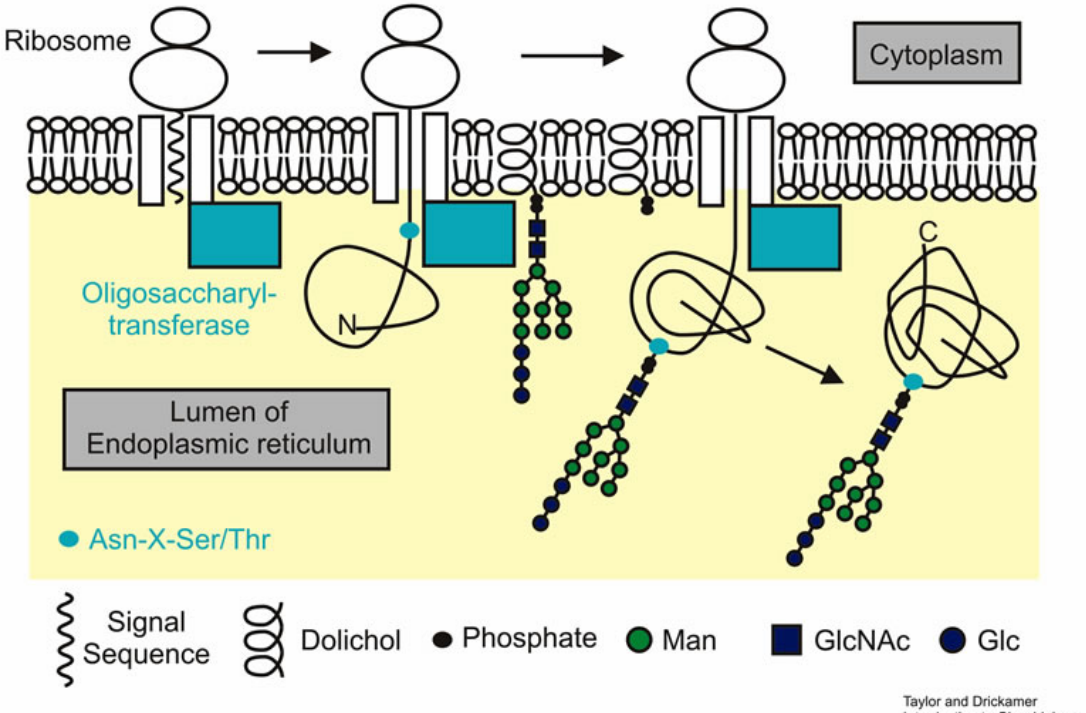
An overview of the pathway for glycoprotein biosynthesis and its intracellular location. Early stages involve glycan assembly on a glycolipid and subsequent transfer to nascent protein in the ER. Subsequent processing by glycosidases and glycosyltransferases occurs in the ER and Golgi apparatus.

Generation of the dolichol-linked oligosaccharide (glycolipid) donor (14-mer) for protein *N*-glycosylation: ER reactions



En bloc transfer of the precursor oligosaccharide ($GlcNAc_2Man_9Glc_3$) is catalyzed by oligosaccharyl transferase (OST).

Consensus sequence: Asn-Xaa-Ser or Asn-Xaa-Thr, where Xaa can be any amino acid except Pro or Asp



Co-translational addition of *N*-linked glycan to a nascent polypeptide

OST is associated with the channel through which the polypeptide is translocated to the ER lumen, so glycosylation occurs while the polypeptide is still unfolded.

N-Linked glycans are found at the surfaces of glycoproteins (not buried). Since transfer is co-translational involving presumably unfolded or partially folded protein, the mechanism for discrimination between consensus sites is unclear (*i.e.*, some consensus sequences are buried and unglycosylated).