CHEM 537
Carbohydrate Biochemistry and Glycobiology
Part I: Monosaccharides & Their Derivatives

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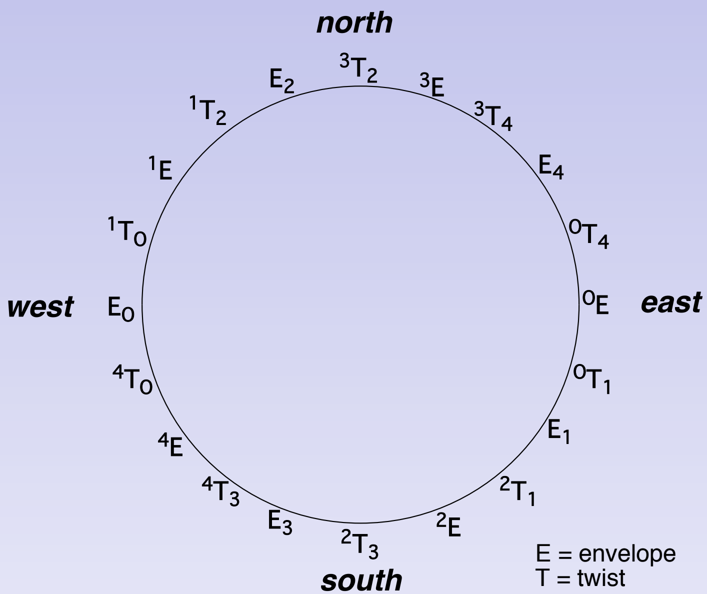
Slide Set 1c

Chapters 11 & 23: *Biochemistry*, Voet/Voet, 4th edition, 2011
Introduction to Glycobiology, Taylor/Drickhamer, 3rd edition, 2011

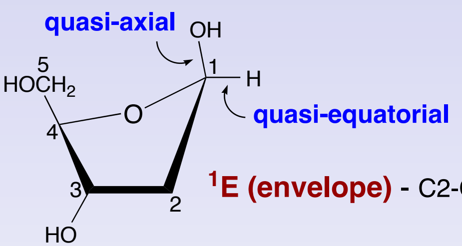
Fall 2014

Furanose conformation: pseudorotation

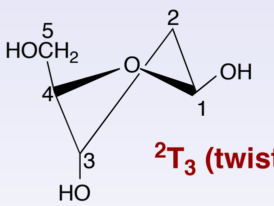


Pseudorotational itinerary of a D-aldofuranose ring

Idealized furanose conformations:
- Planar (1 form)
- Envelope = E (10 forms)
- Twist = T (10 forms)



^{1}E (envelope) - C2-C3-C4-O4 coplanar



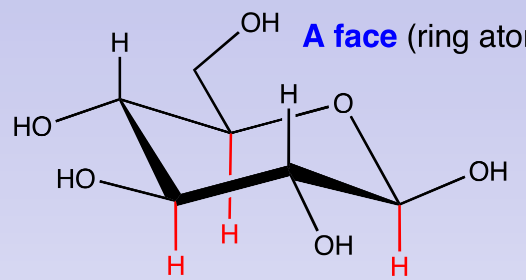
$^{2}T_{3}$ (twist) - C4-O4-C1 coplanar

Two non-planar forms of 2-deoxy-β-D-*erythro*-pentofuranose (2-deoxy-β-D-ribofuranose)

Pseudorotation provides a pathway for interconversion of all non-planar forms of furanose rings that does not involve the planar intermediate. E and T forms have different stabilities which depend on furanose ring configuration.

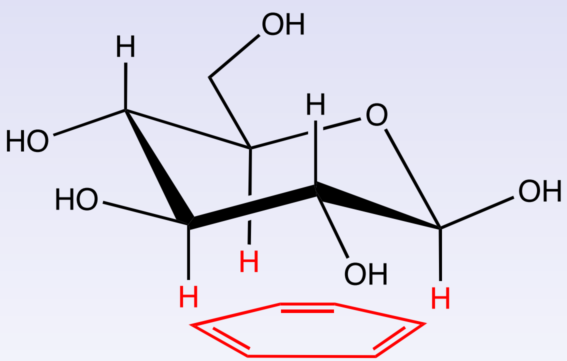
Preferred chair conformations of monosaccharides render them amphipathic; facilitates protein-saccharide recognition

all polar OH groups are arranged along molecular "edge"



A face (ring atom numbering clockwise)

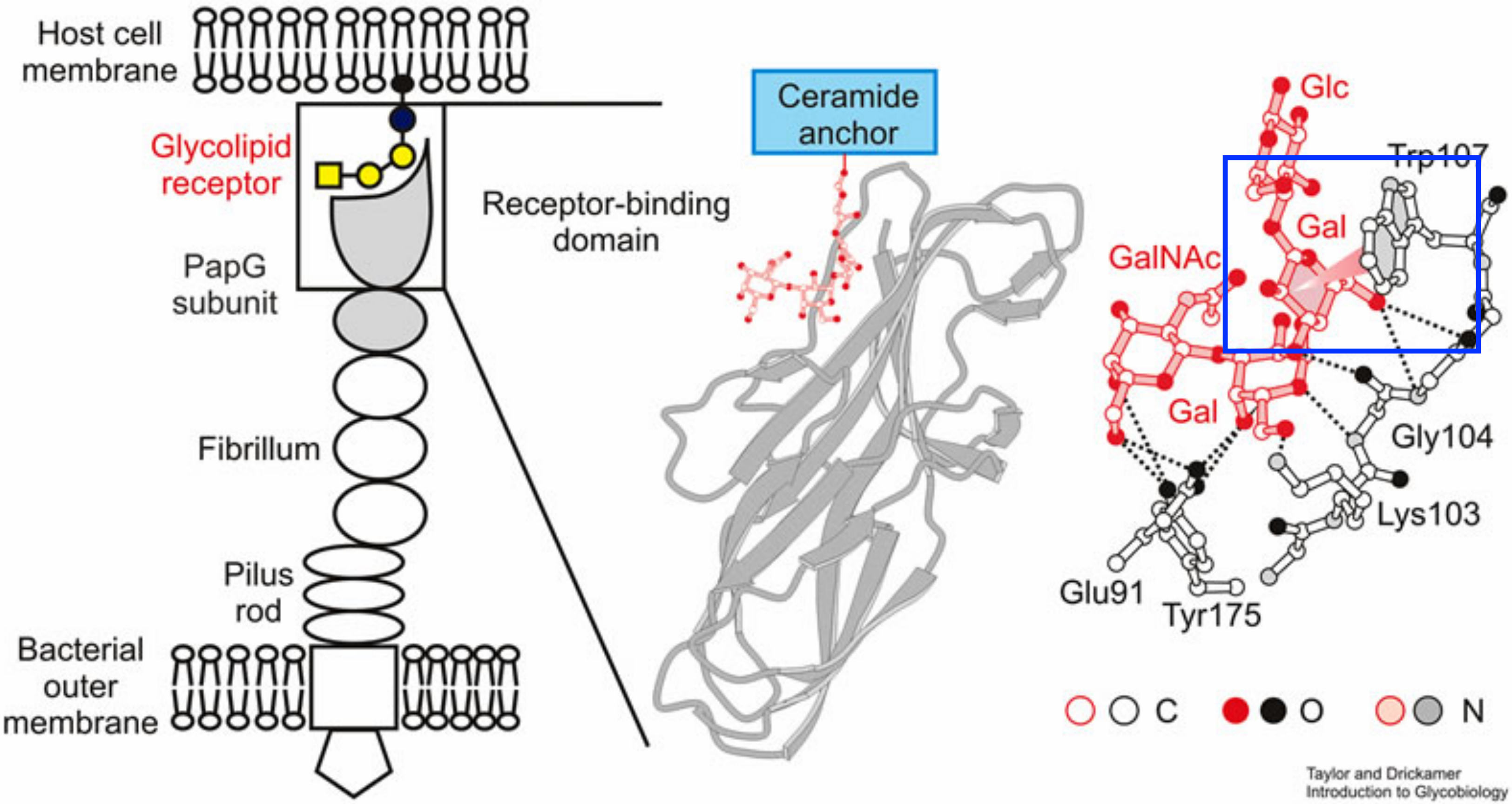
B face (ring atom numbering counter-clockwise)
(comparatively hydrophobic)



A common "recognition" (binding) motif: pyranosyl-aromatic ring "stacking" observed in the binding sites of carbohydrate-binding proteins (commonly Trp); similar interactions are possible in carbohydrate-nucleic acid recognition.

Hydrophobic aliphatic sidechains can sometimes substitute for the aromatic ring.

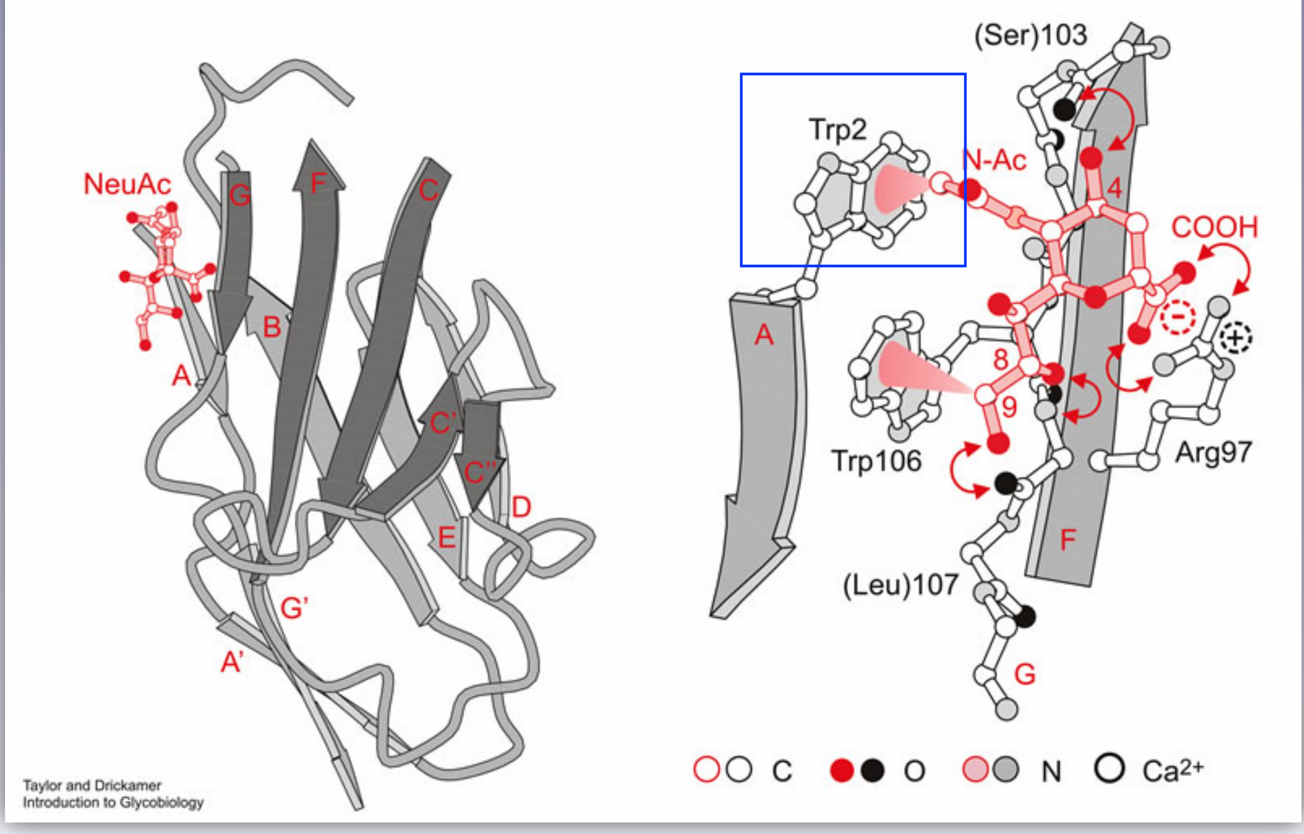
Figure 10.8 Overall structure of bacterial pilus showing subunit arrangement and the structure of the receptor-binding domain of the PapG subunit bound to a glycolipid head group oligosaccharide



Taylor and Drickamer
Introduction to Glycobiology

Carbohydrate recognition by the CRD of sialoadhesin showing ionic and Trp-*N*-acetyl interactions; another mode of protein-saccharide recognition

Figure 8.15 Overall fold and monosaccharide-binding site of the CRD from sialoadhesin

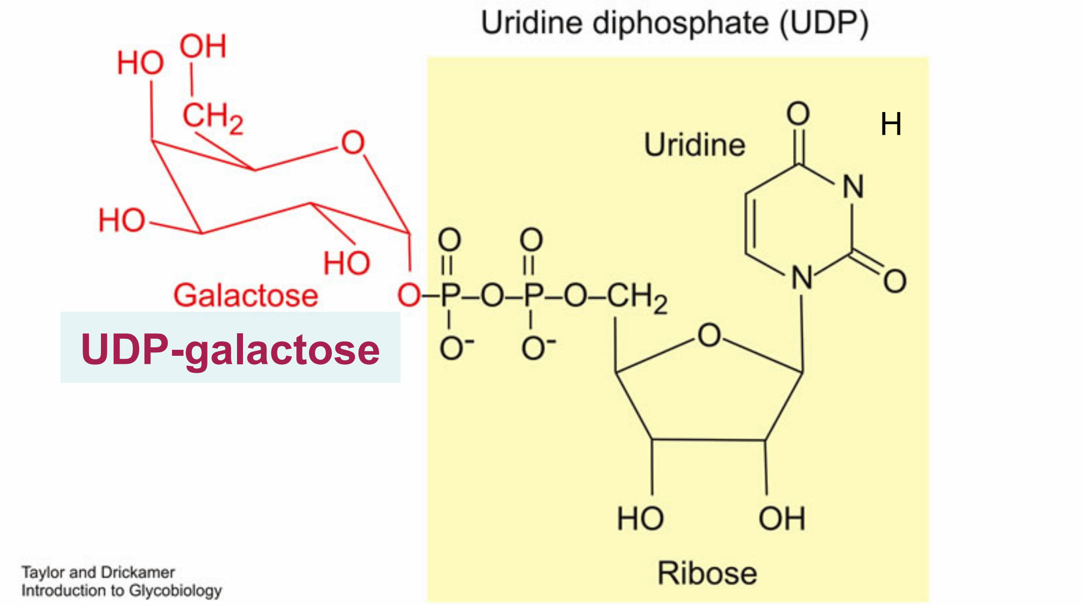


Some common monosaccharide modifications *in vivo*

❑ 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



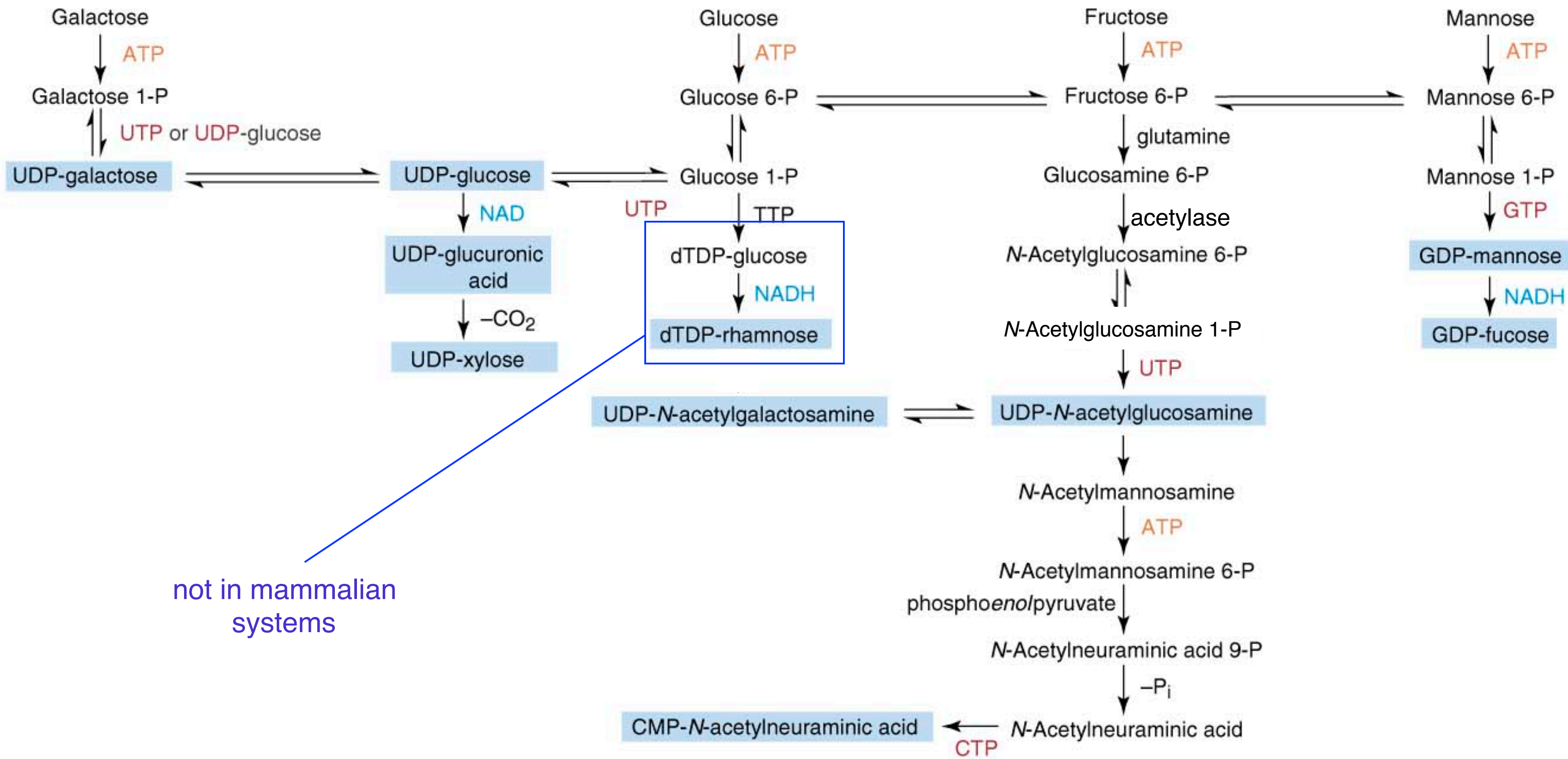
A nucleotide sugar is a "biologically activated" monosaccharide.
Sugar nucleotides are involved in sugar transformations and in the biosynthesis of complex carbohydrates (oligomers and polymers) *in vivo*. In the latter role, they serve as sugar donors in the sequential addition of monosaccharides to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

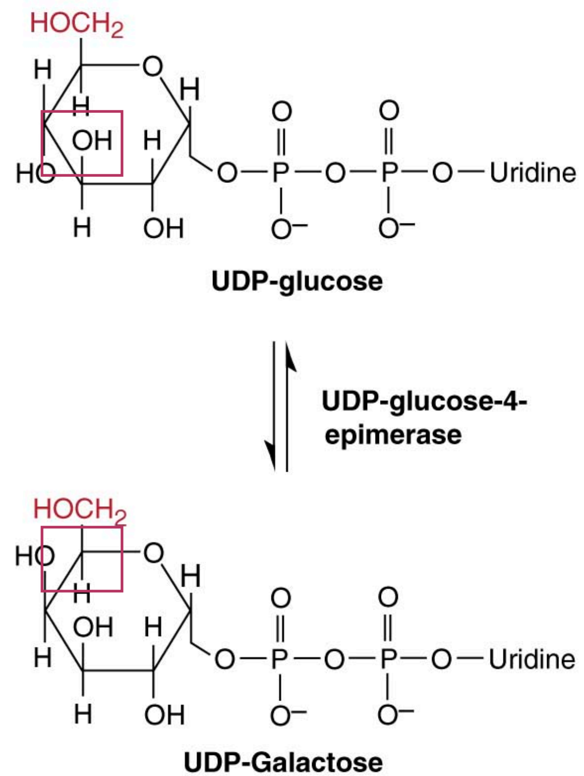
Example reaction for NDP-sugar biosynthesis

$$\text{NTP} + \text{glycosyl 1-phosphate} \underset{}{\overset{E}{\rightleftharpoons}} \text{NDP-sugar} + \text{PP}_i$$

e.g.: $$\text{UTP} + \alpha\text{-D-glucopyranosyl 1-P} \overset{E}{\rightleftharpoons} \text{UDP-glucose} + \text{PP}_i$$

Biosynthesis of nucleotide sugars and interconversion of hexoses





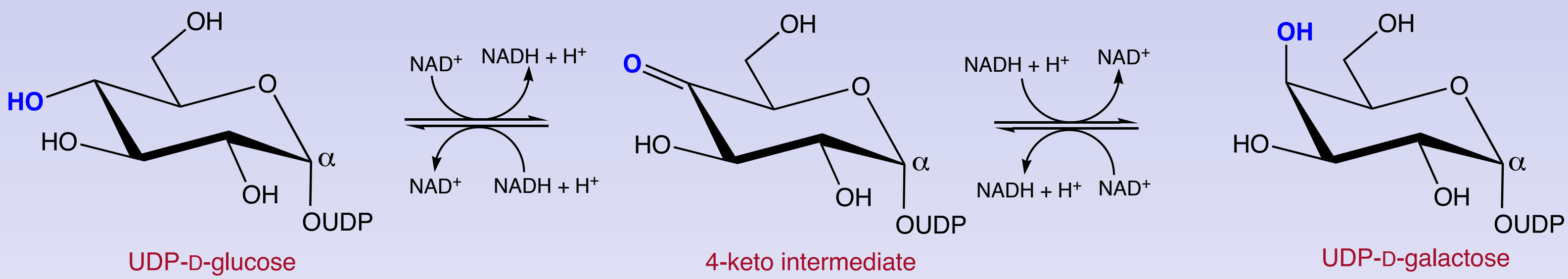
Fi

Biosynthesis of D-Gal *in vivo* occurs via C4-epimerization of UDP-Glc, followed by hydrolysis of the UDP-Gal product.

The Glc and Gal moieties of UDP-Glc and UDP-Gal are in the α-configuration.

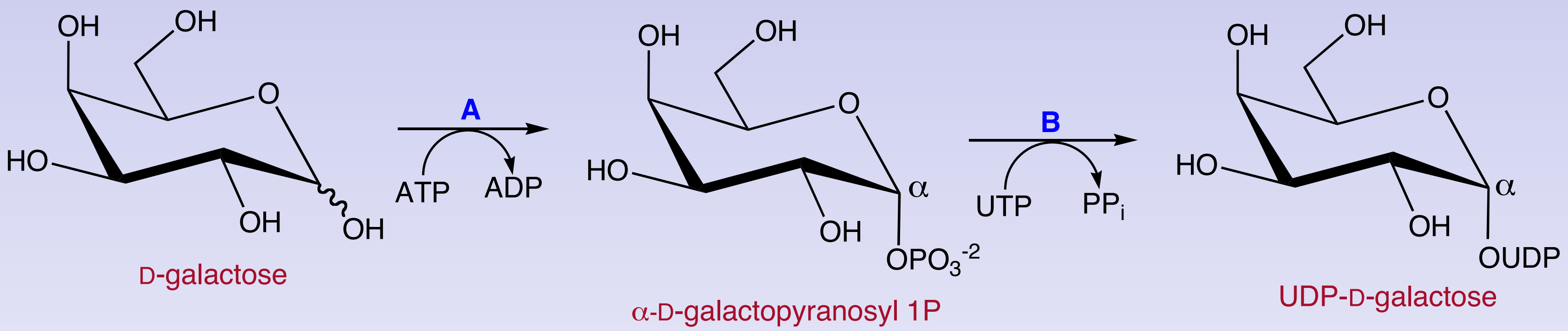
Mechanism of interconversion of UDP-glucose and UDP-galactose

UDP-glucose 4-epimerase



De novo biosynthesis of D-galactose *in vivo* requires the participation of nucleotide sugars. UDP-Gal can also be synthesized *in vivo* from free D-galactose (salvage or diet).

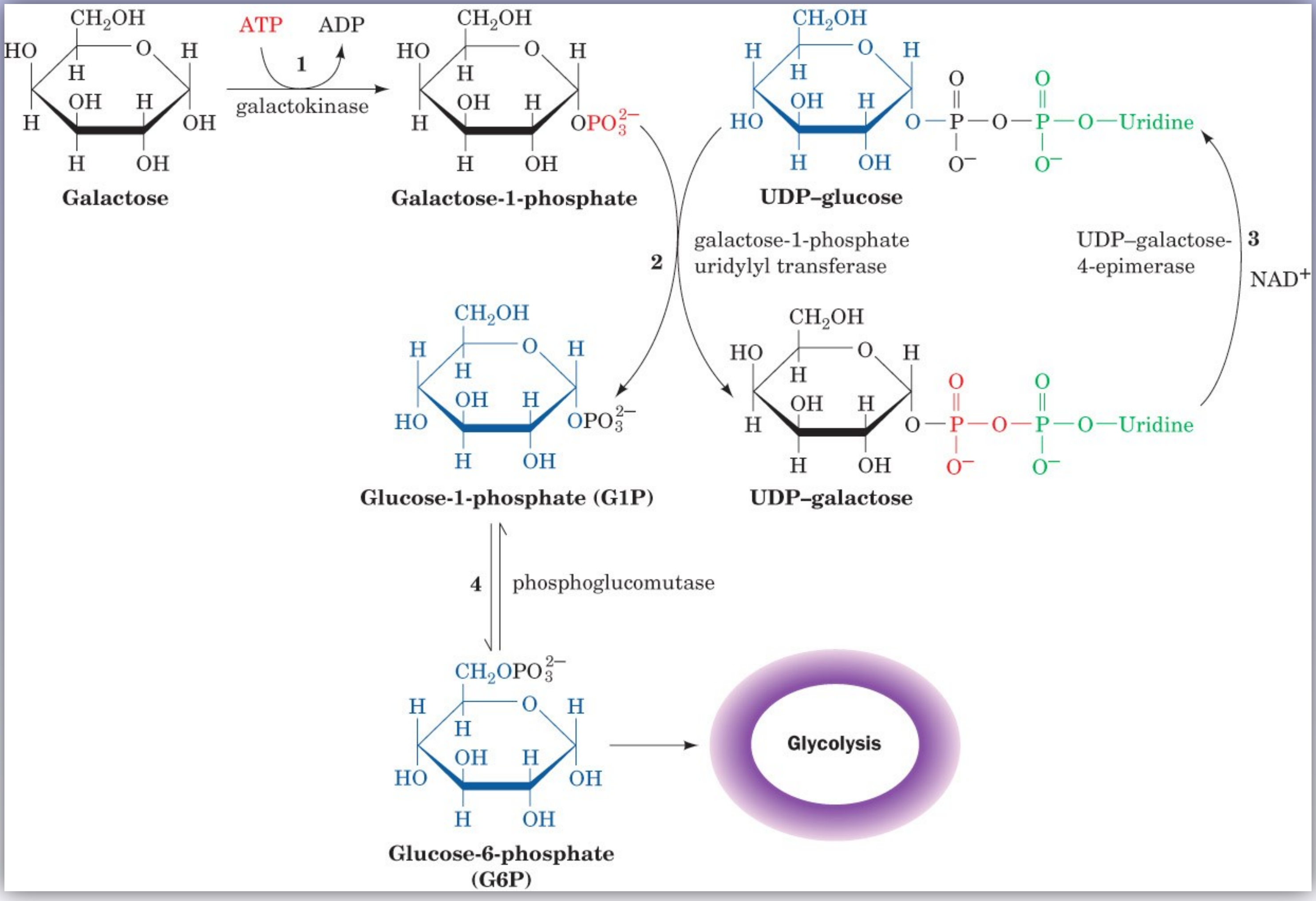
In vivo synthesis of UDP-galactose from D-galactose (salvage,diet)



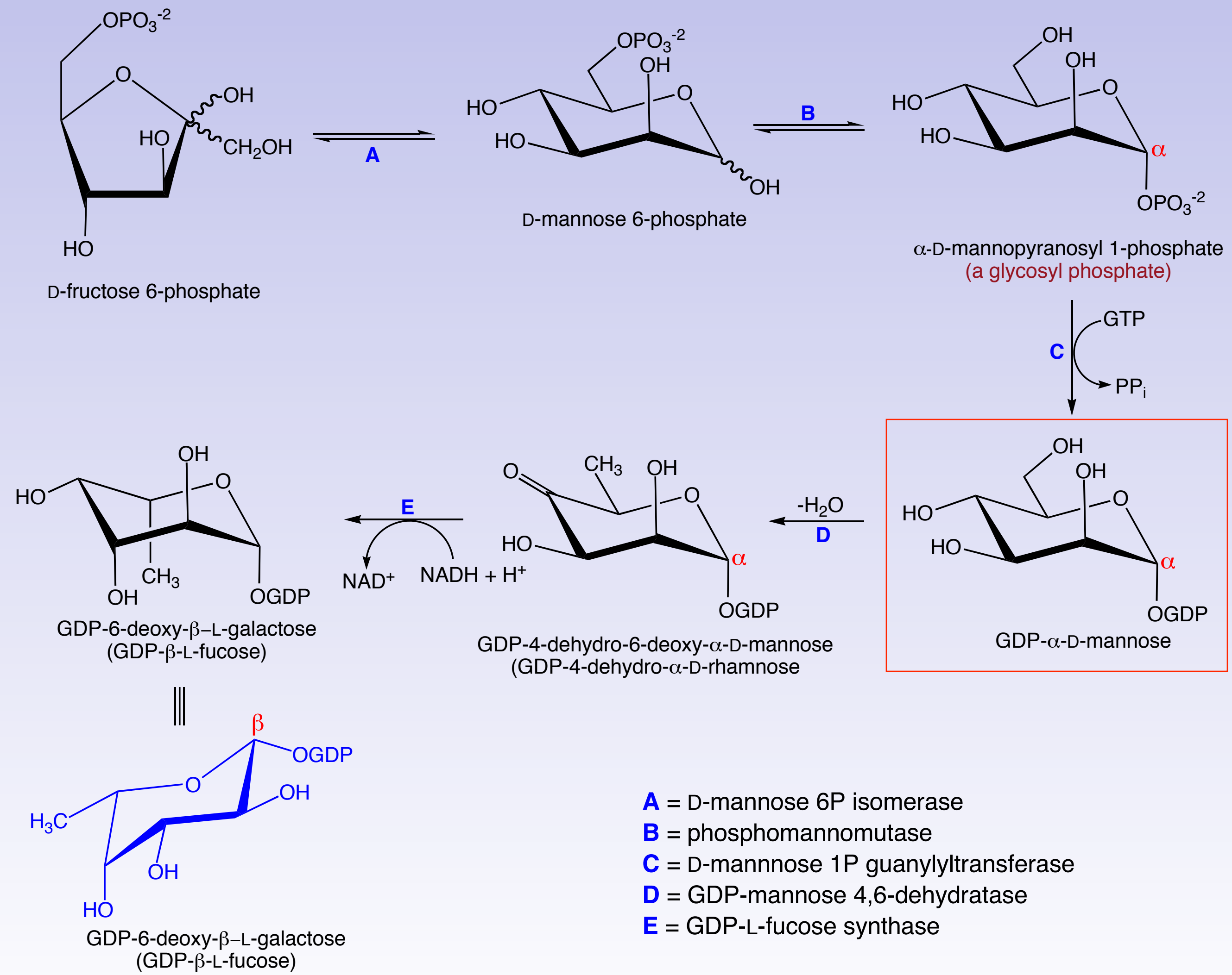
A = galactokinse

B = D-galactose 1P uridylyltransferase

Metabolism of galactose

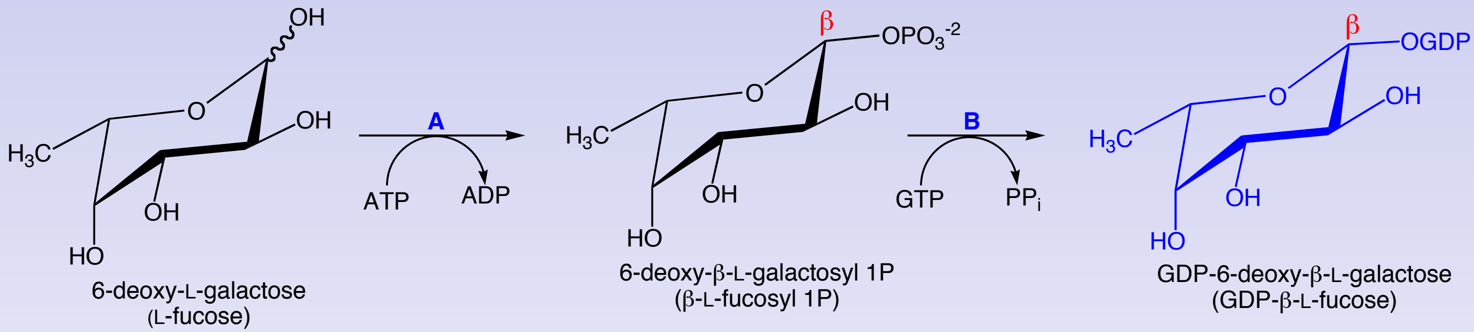


Synthesis of GDP-fucose *in vivo*



A = D-mannose 6P isomerase
B = phosphomannomutase
C = D-mannnose 1P guanylyltransferase
D = GDP-mannose 4,6-dehydratase
E = GDP-L-fucose synthase

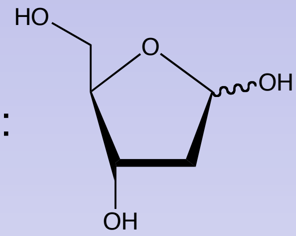
Salvage pathway for GDP-fucose biosynthesis *in vivo*



A = fucokinase
B = L-fucose 1P guanylyltransferase

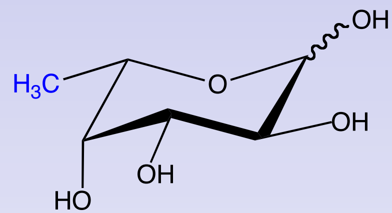
Examples of biologically important deoxysugars

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



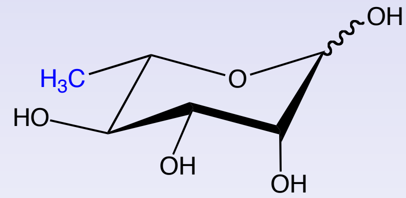
DNA

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



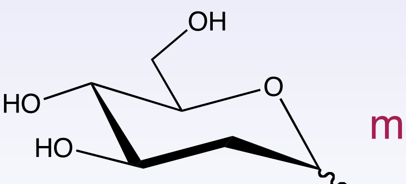
N-glycans of glycoproteins

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



bacterial polysaccharides

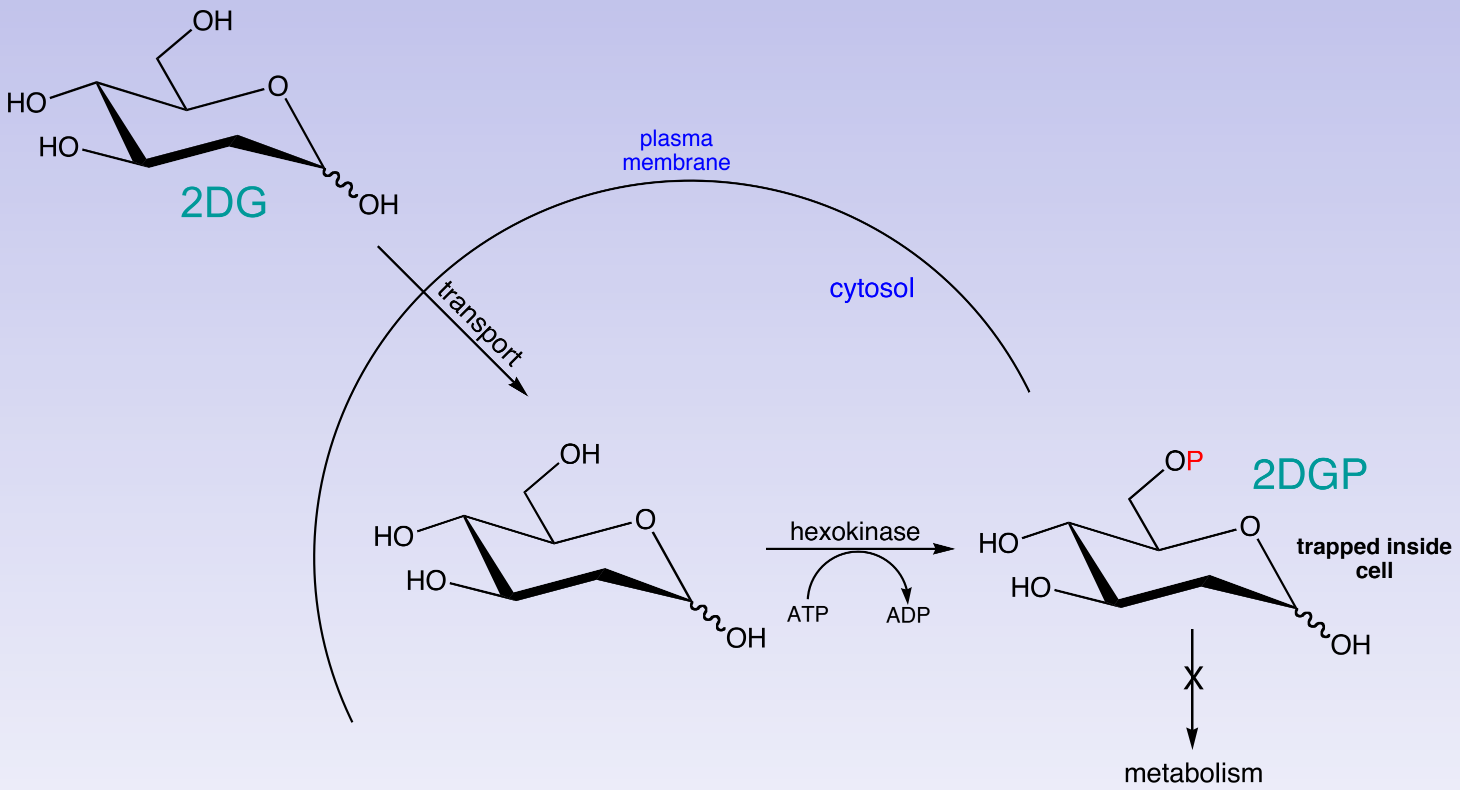
2-deoxy-D-glucose (2-deoxy-D-*arabino*-hexose):



2DG

metabolic probe

2DG as a cell viability probe



A probe of cell viability;
imaging agent if tagged

Mechanisms of deoxygenation in vivo

Case 1: Conversion of NDPs to dNDPs by ribonucleotide reductase

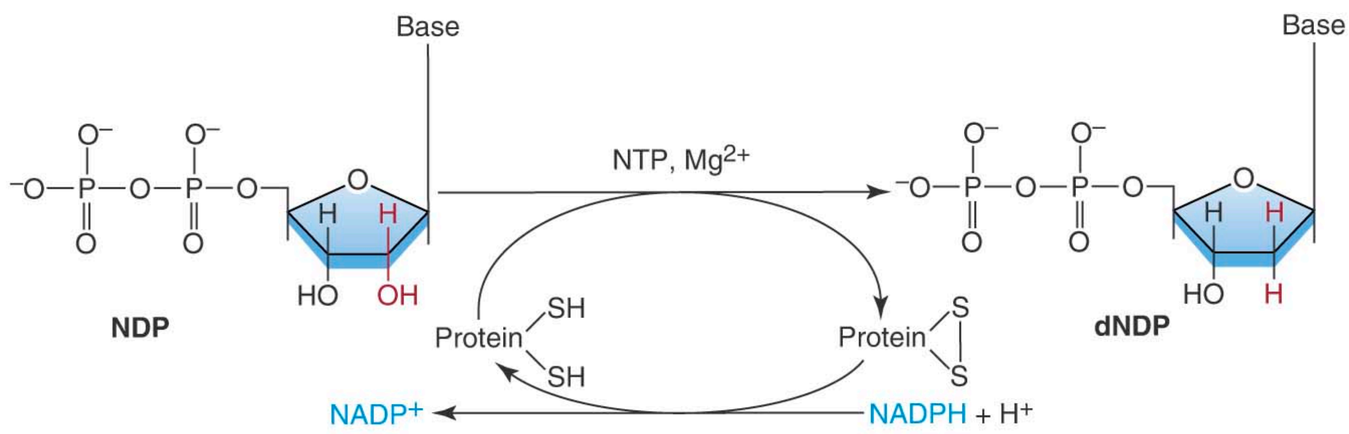
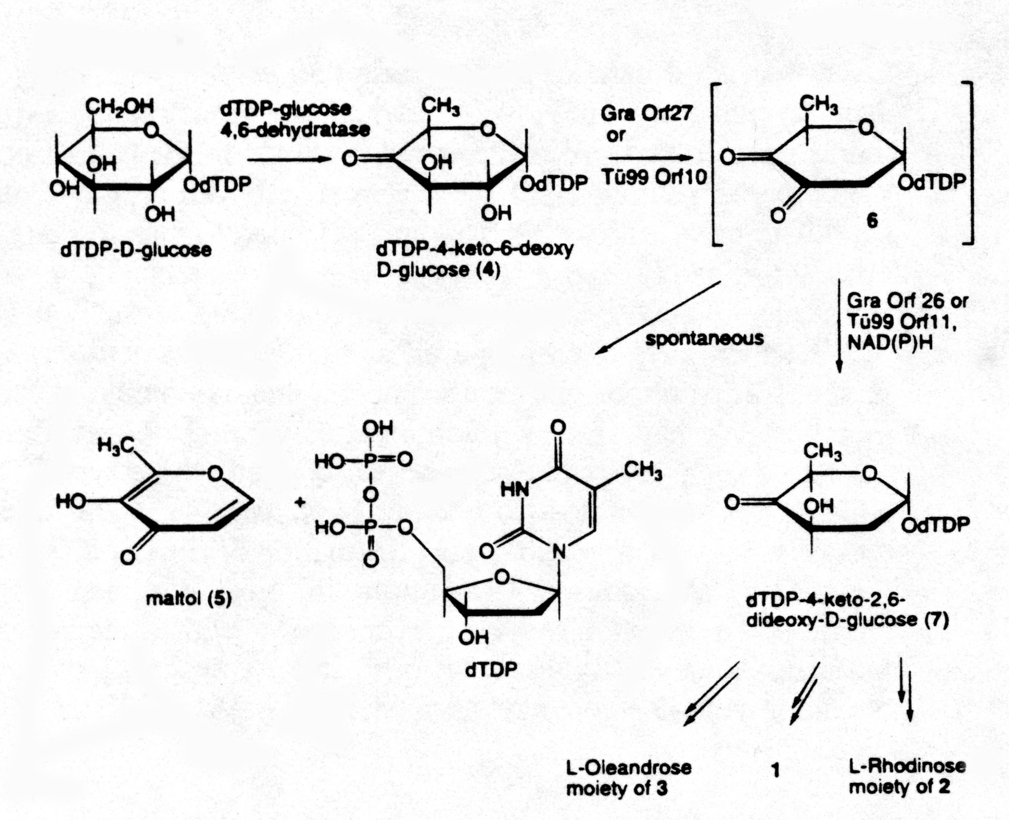


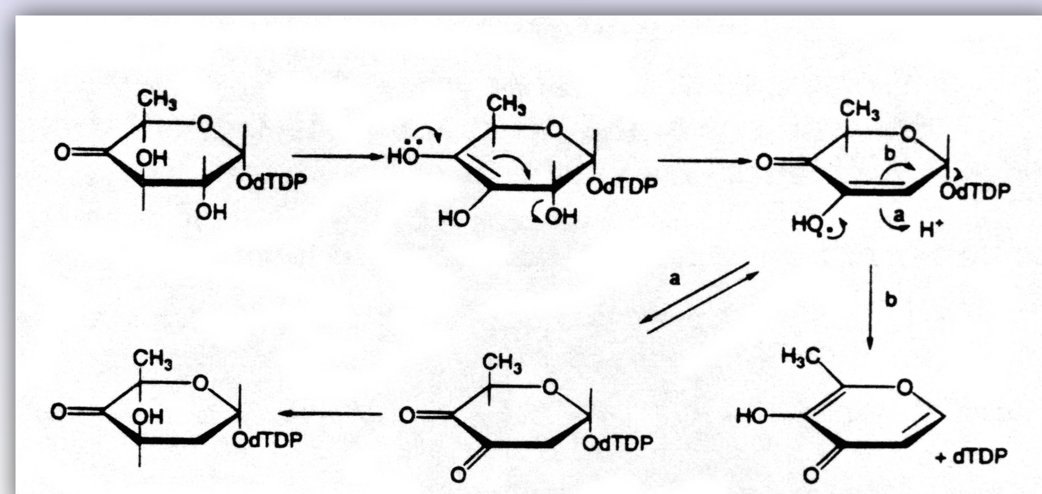
Figure 20.19. ***De novo*** **synthesis of 2′-deoxyribonucleotides from ribonucleotides.**

Mechanisms of deoxygenation in vivo



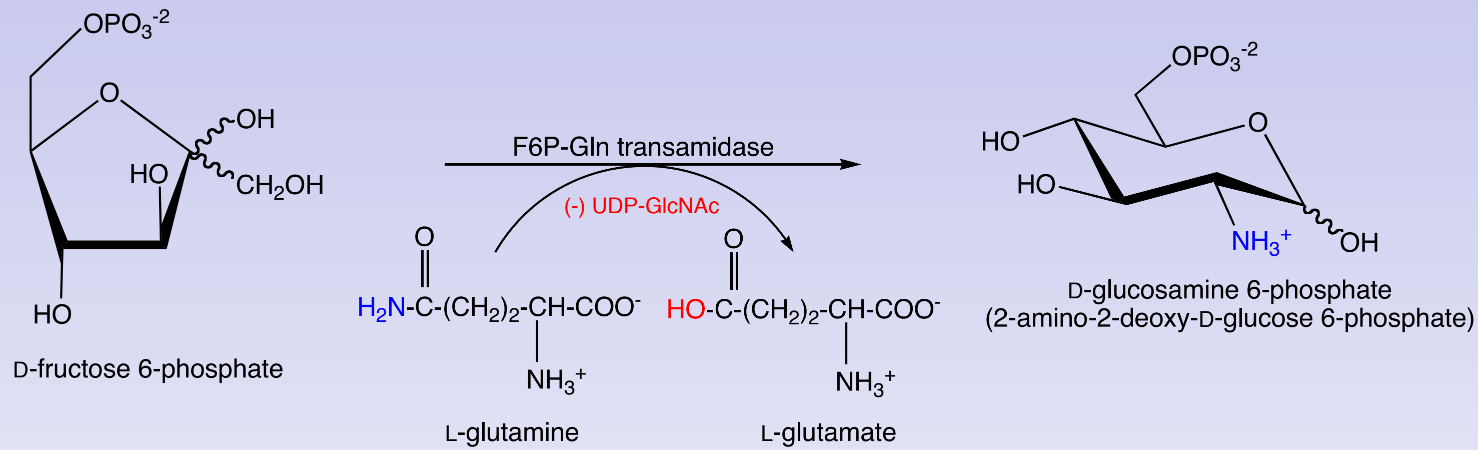
Case 2: Biosynthesis of deoxysugars in bacteria

Draeger *et al*., *JACS* **1999**, *121*, 2611-2612.



2-Aminosugars: Derived from D-fructose-6P in vivo

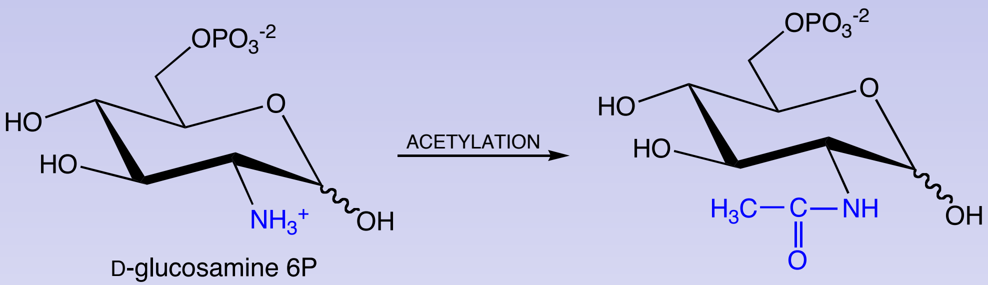
Formation of D-glucosamine-6P occurs by transamidation.



$GlcNH_2$-6P → GlcNAc-6P → GlcNAc-1P → UDP-GlcNAc → UDP-GalNAc

2-Aminosugar *N*-acetylation

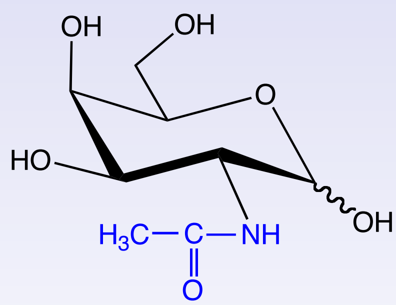
N-acetyl-D-glucosamine 6P (GlcNAc 6P)
N-acetyl-D-galactosamine (GalNAc) equivalents



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

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

For GlcNAc 6P: Acetylation of free D-glucosamine 6P generated from F6P is enzyme-catalyzed in vivo; acetyl CoA is the acetyl donor.

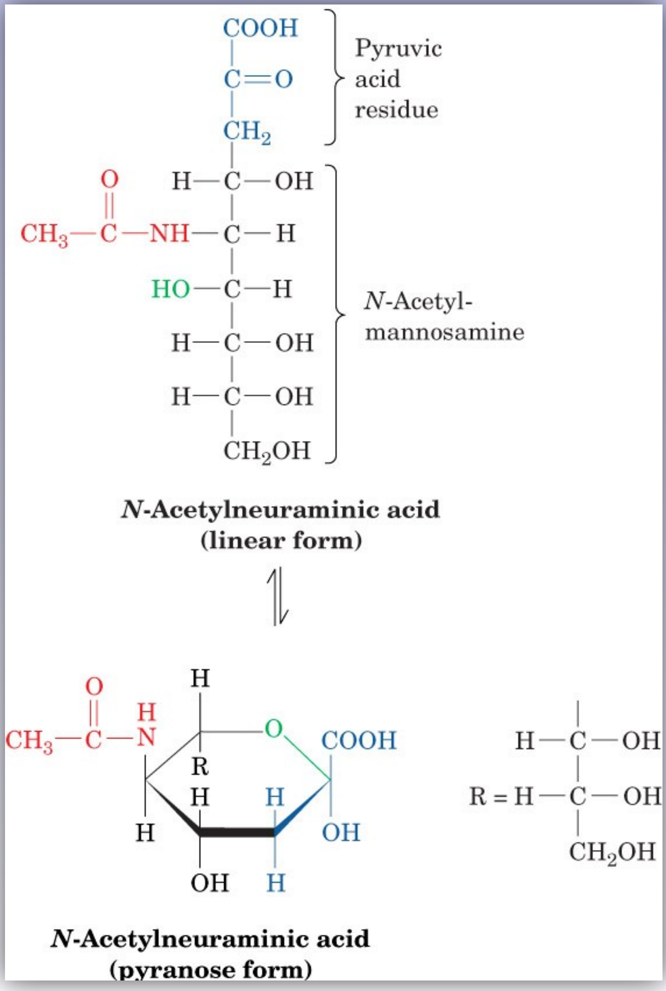


N-acetyl-D-galactosamine (GalNAc)
(2-acetamido-2-deoxy-D-galactose)

For GalNAc: Free D-galactosamine is cytotoxic; in mammalian systems, GalNAc residues are available via enzyme-catalyzed epimerization of UDP-GlcNAc to UDP-GalNAc.

A biologically-important C_9 *N*-acetylated sugar

N-Acetyl-neuraminic acid (Neu5Ac)

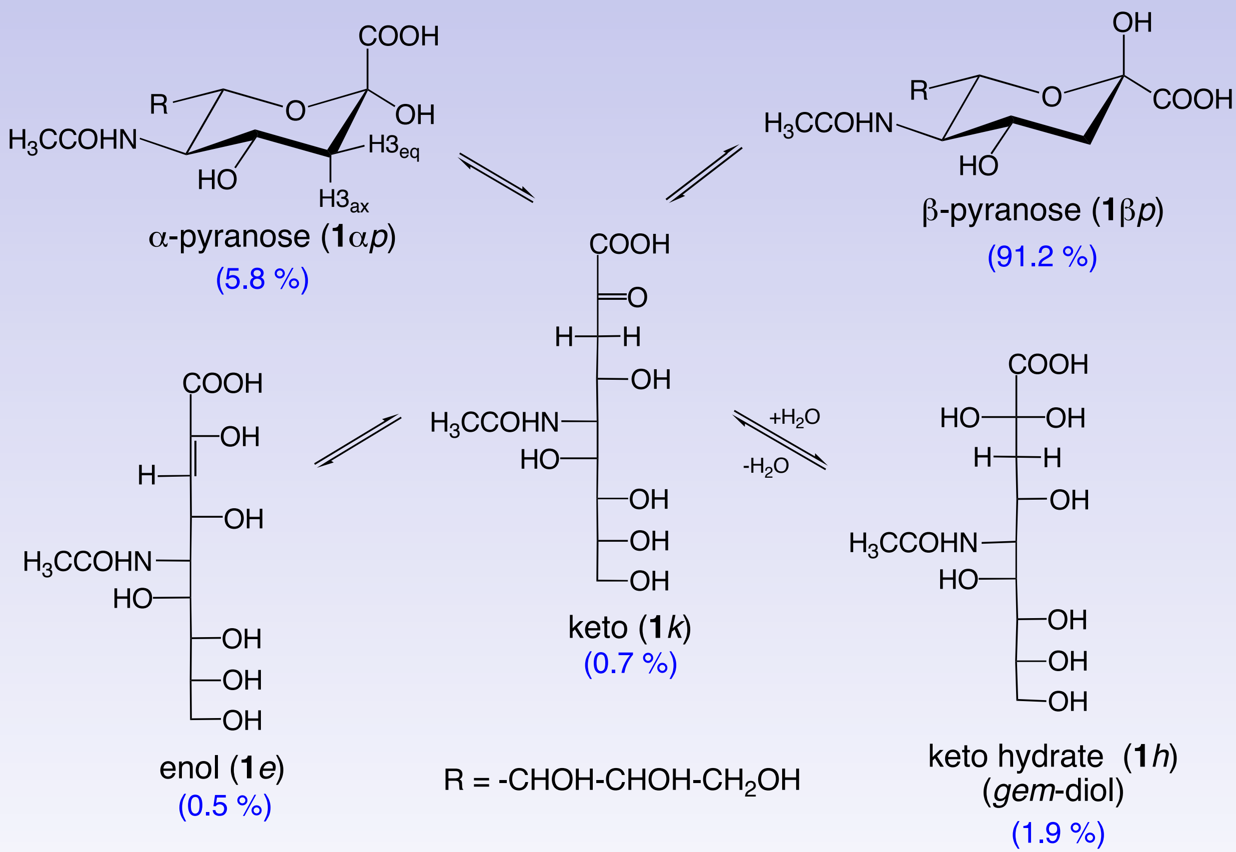


N-Acetylneuraminic acid (linear form)

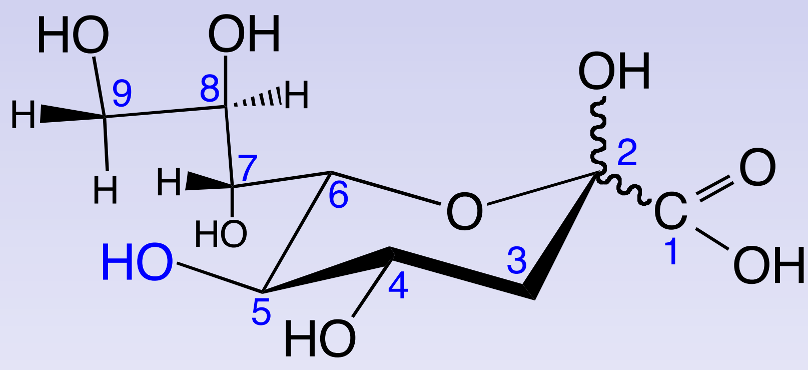
N-Acetylneuraminic acid (pyranose form)

Neu5Ac is a C9 <u>α-ketoacid</u> derived biosynthetically from C_6 (ManNAc) and C_3 (PEP or pyruvate) precursors.

Anomerization of *N*-acetyl-neuraminic acid and abundances of forms in aqueous solution at pH 2.0



Another biologically important C_9 α-ketoacid



KDN

(2-keto-3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid)

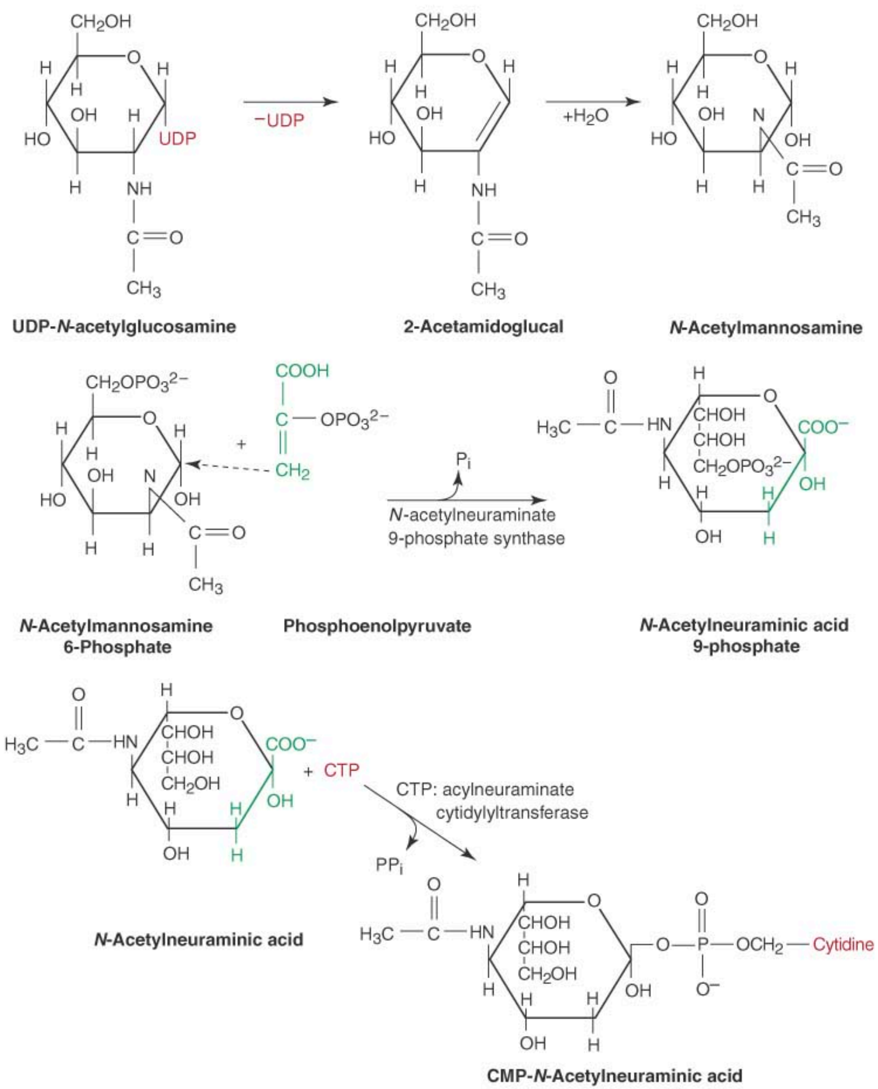
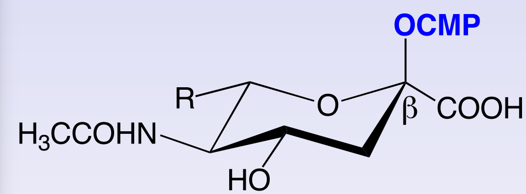
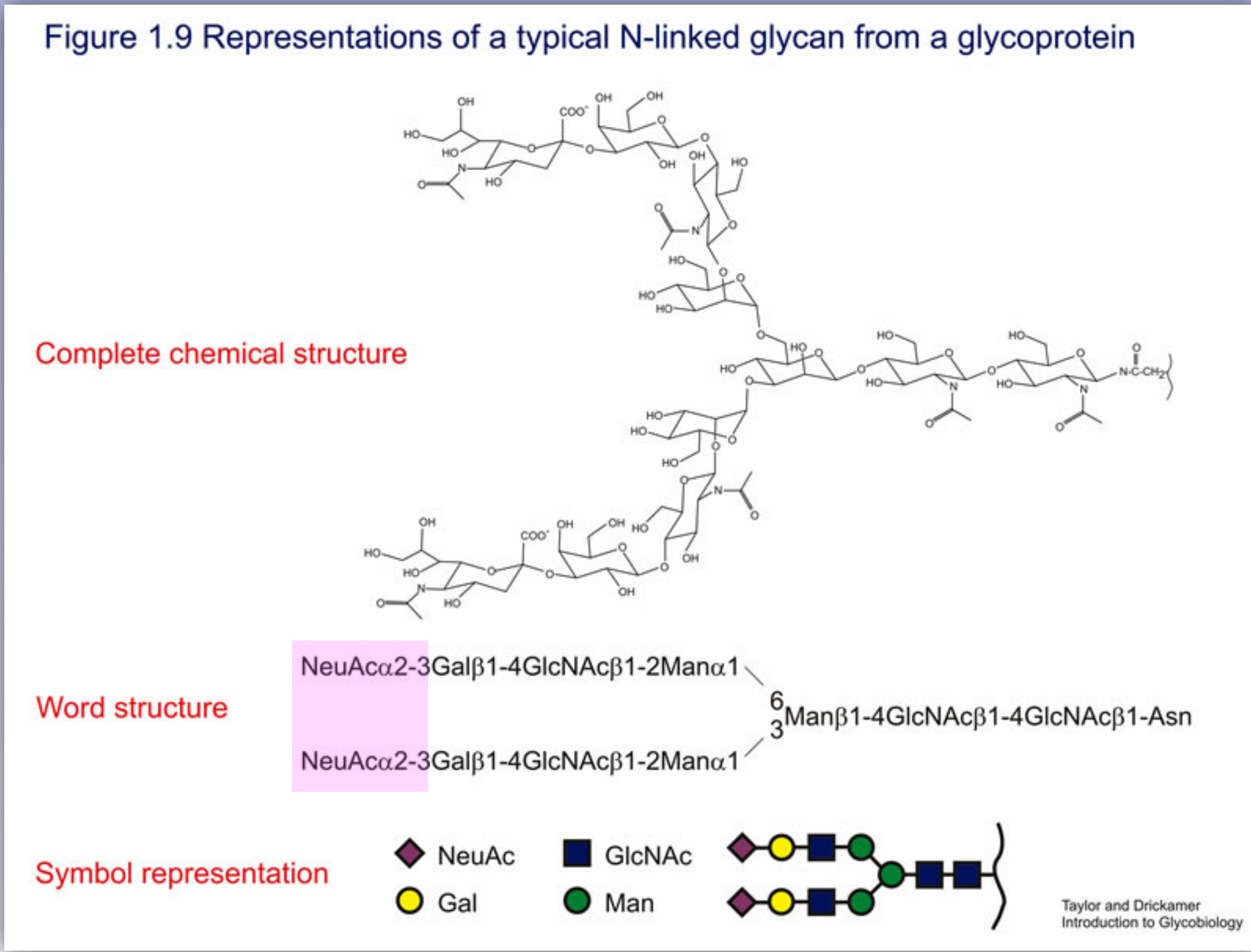


Figure 16.9. Biosynthesis of CMP-*N*-acetylneuraminic acid.

N-Acetyl-neuraminic acid is activated as the sugar nucleotide, CMP-*N*-acetyl-neuraminic acid. NeuAc is linked to CMP in the β-configuration.





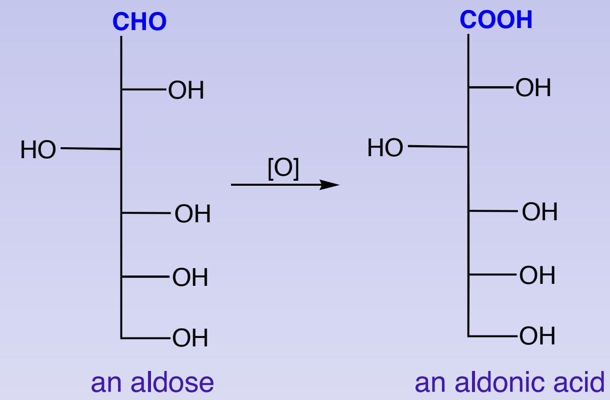
Neu5Ac is commonly found as the terminal sugar of *N*-glycans of glycoproteins. Note symbol representation of complex glycans as a convenient way to describe structure.

Sialyltransferases in the mammalian genome

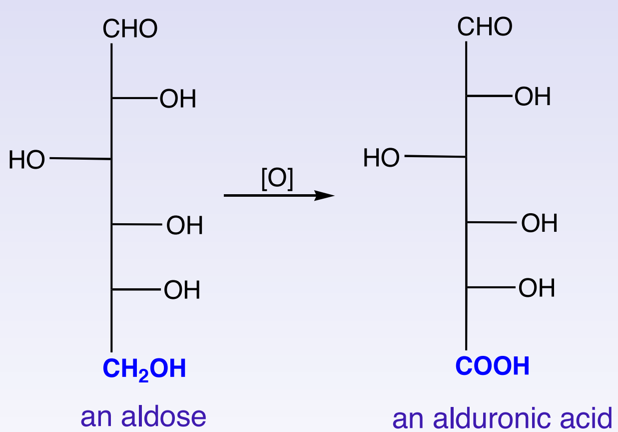
ST6Gal I-II: α-1,6 to β-Gal*p*
ST3Gal I-VI: α-2,3 to β-Gal*p*
ST8Sia I-VI: α-2,8 to α-Neu5Ac
ST6GalNAc I-VI: α-2,6 to GalNAc

Total sialyltransferases: 20

Oxidized Monosaccharide Derivatives

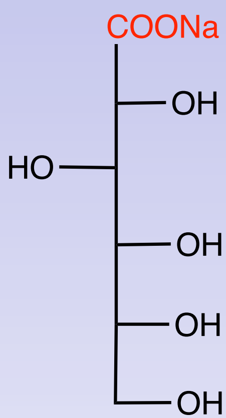


Aldonic acids: produced when C1 of an aldose is oxidized to the carboxylic acid; *e.g.,* D-glucose to D-gluc<u>onic</u> acid; D-mannose to D-mann<u>onic</u> acid. Since the carbonyl (aldehydic) carbon is destroyed, aldonic acids are not reducing sugars (aldonic acids do not undergo anomerization).

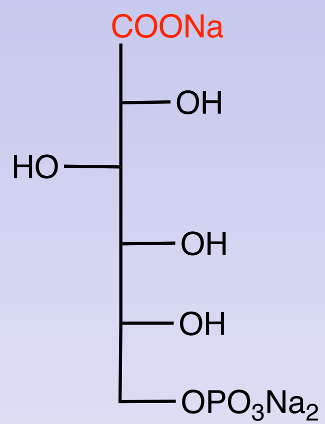


Alduronic acids: produced when the terminal primary alcohol (hydroxymethyl group) of an aldose is oxidized to the carboxylic acid; *e.g.,* D-glucose to D-gluc<u>uronic</u> acid; D-mannose to D-mann<u>uronic</u> acid. Since the carbonyl (aldehydic) carbon is <u>not</u> destroyed, alduronic acids are reducing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

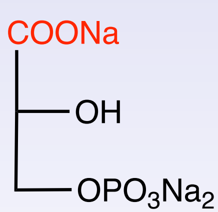
Some biologically important aldonic acids



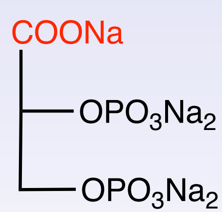
sodium D-gluconate



sodium D-gluconate
6-phosphate



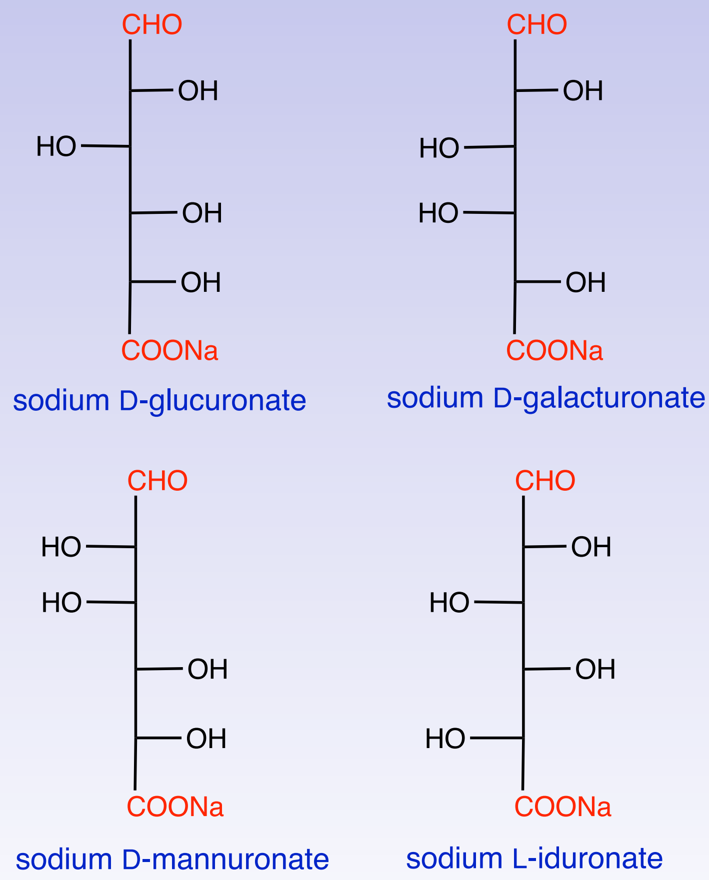
sodium D-glycerate
3-phosphate



sodium D-glycerate
2,3-bisphosphate

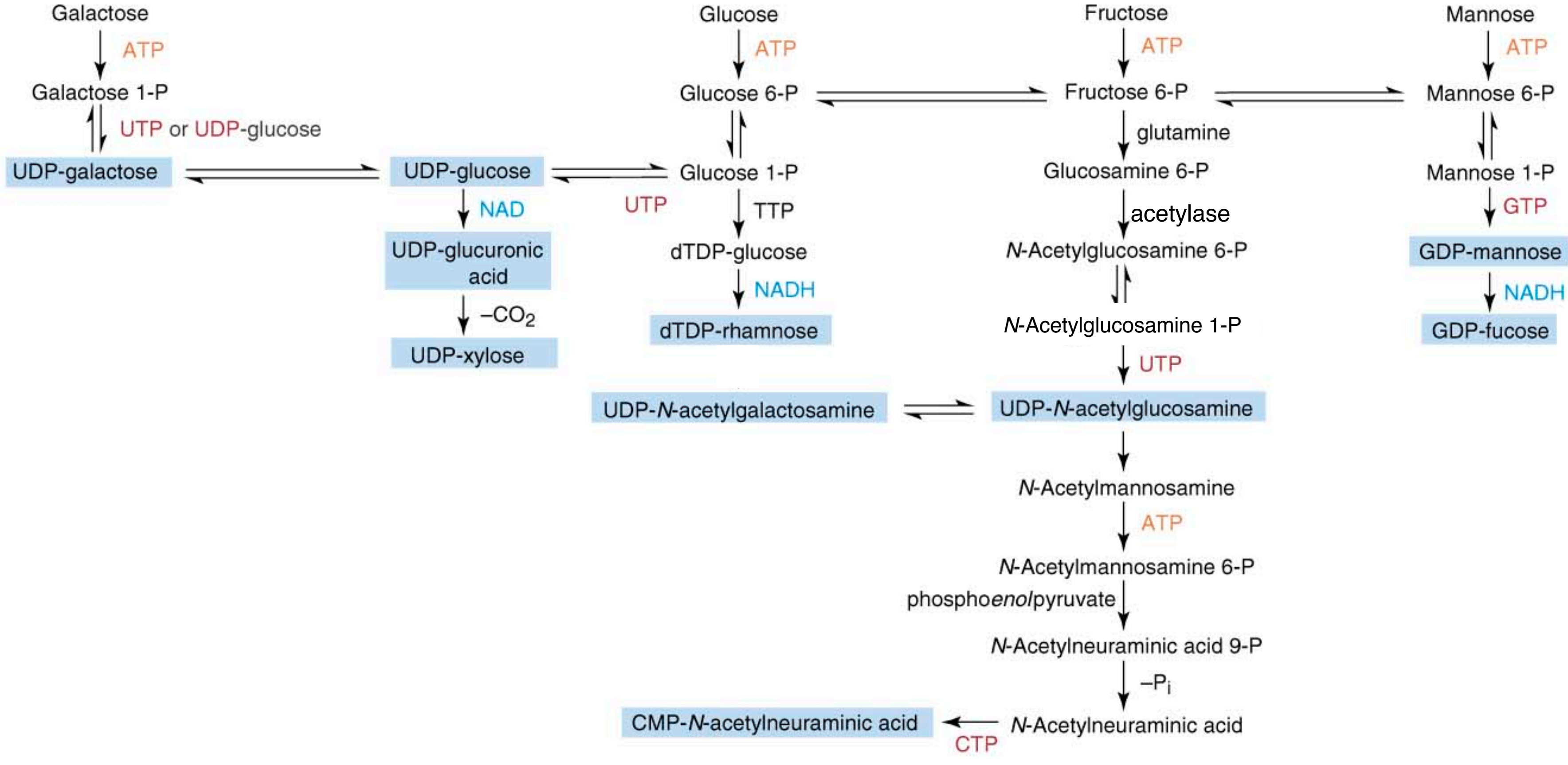
Produced *in vivo* from the corresponding aldose precursor via enzyme-catalyzed oxidation (dehydrogenases) using NAD^+ or $NADP^+$ as a coenzyme

Some biologically important uronic acids



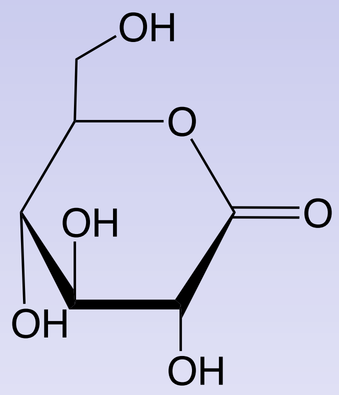
Produced *in vivo* from a sugar nucleotide precursor via enzyme-catalyzed oxidation (dehydrogenases) using NAD^+ or $NADP^+$ as a coenzyme

Biosynthesis of nucleotide sugars and interconversion of hexoses

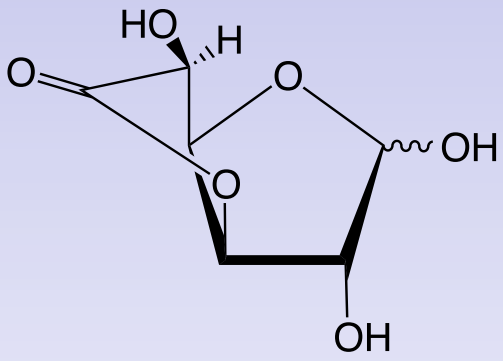


Textbook of Biochemistry With Clinical Correlations, Sixth Edition, Edited by Thomas M. Devlin.

Aldonic and alduronic acids can undergo lactonization to produce cyclic structures.

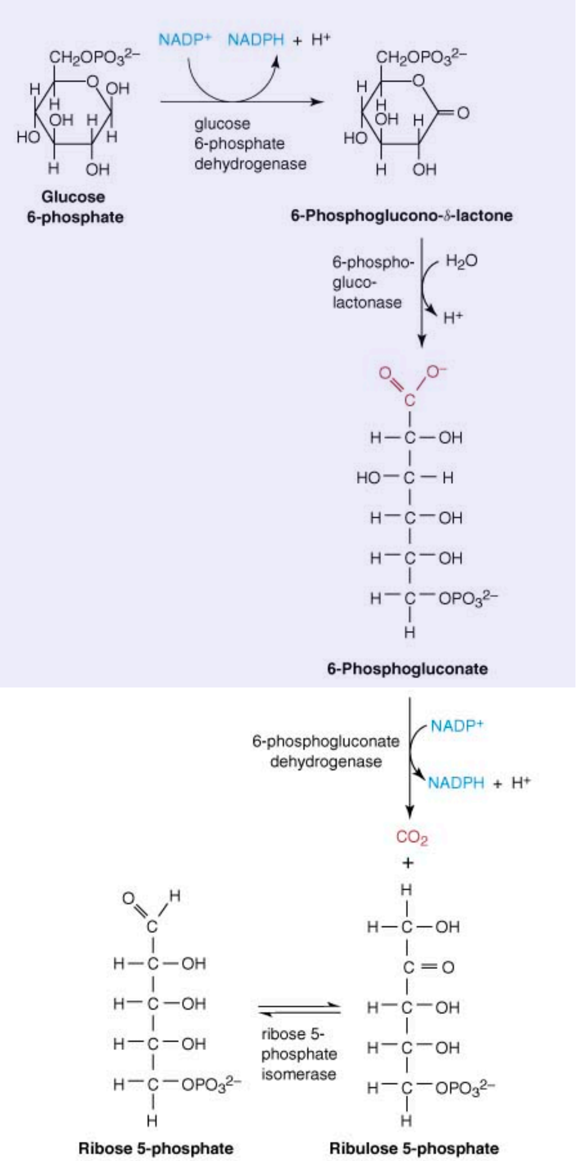


D-glucono-1,5-lactone
(D-glucono-δ-lactone)



D-glucurono-3,6-lactone

Lactonization is favored under acidic solution conditions. In basic solution, lactones hydrolyze to give acyclic acid salts. In vivo, hydrolysis of lactones is enzyme-catalyzed.



Formation of aldonolactones and aldonates in vivo: Oxidation of D-glucose 6P to its corresponding δ-lactone by G6P dehydrogenase, followed by hydrolysis of the δ-lactone by G6P lactonase.

Figure 16.1. Oxidative phase of the pentose phosphate pathway: Formation of pentose phosphate and NADPH.

Glucose can be converted to D-glucono-δ-lactone by the enzyme, glucose oxidase (GO)

$$\text{D-glucose} + O_2 \xrightarrow{\text{GO}} \text{D-glucono-}\delta\text{-lactone} + H_2O_2$$

The reaction can be driven to completion with the addition of catalase, which degrades the H_2O_2 by-product. This reaction is commonly used to determine glucose concentration in blood and tissue.

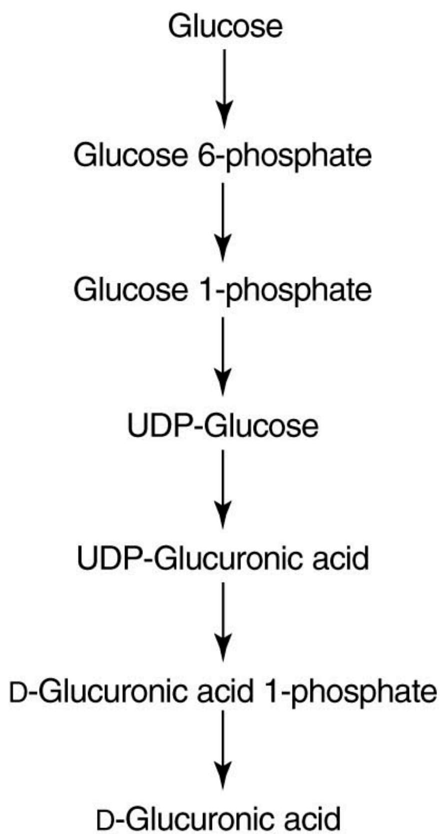


Figure 16.7. Biosynthesis of D-glucuronic acid from glucose.

Formation of alduronic acids in vivo: Biosynthesis of D-glucuronic acid from D-glucose. Note the involvement of sugar nucleotides in this transformation. In addition to its role as a sugar donor in oligo- and polysaccharide biosynthesis, UDP-GlcUA plays a key role in toxic heavy metal detoxification *in vivo*.

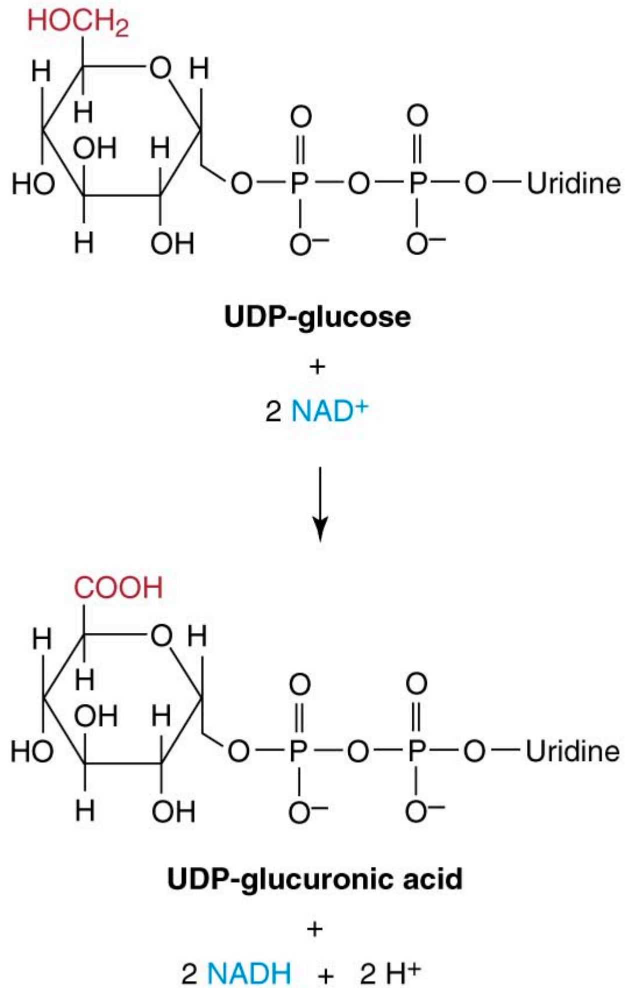


Figure 16.6. Formation of UDP-glucuronic acid from UDP-glucose.

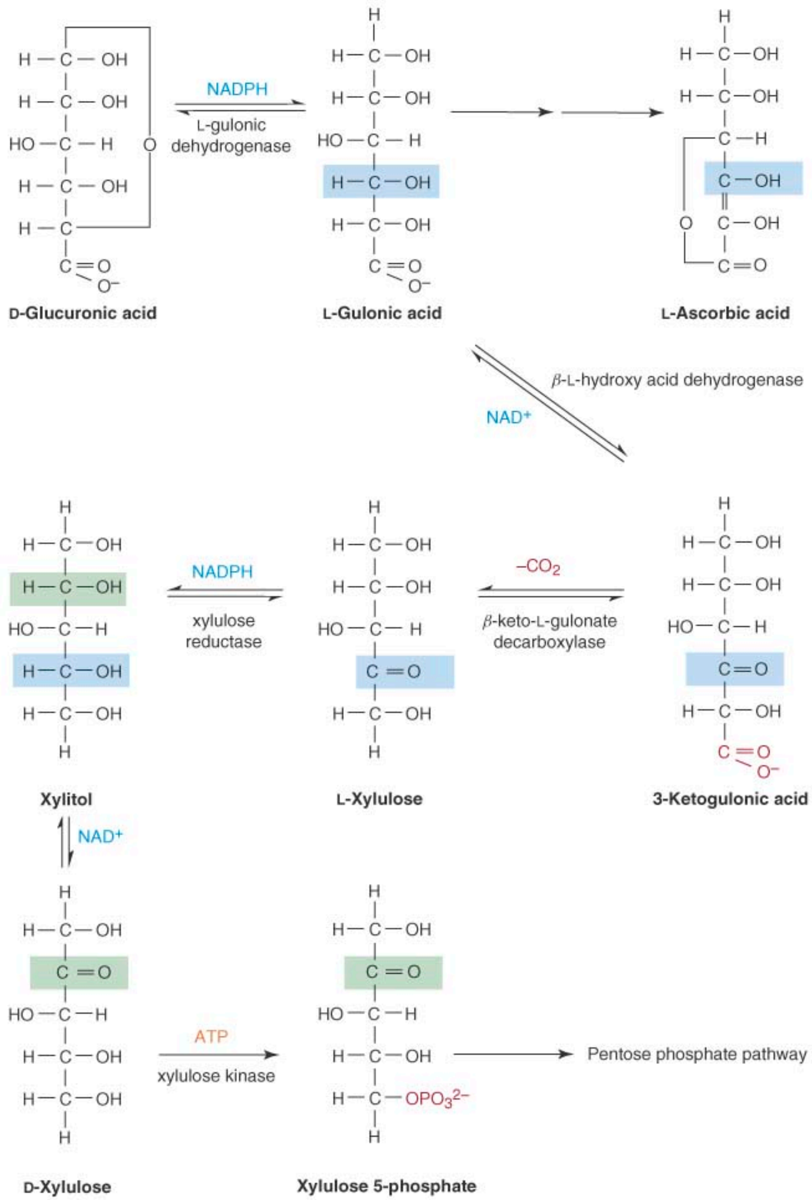
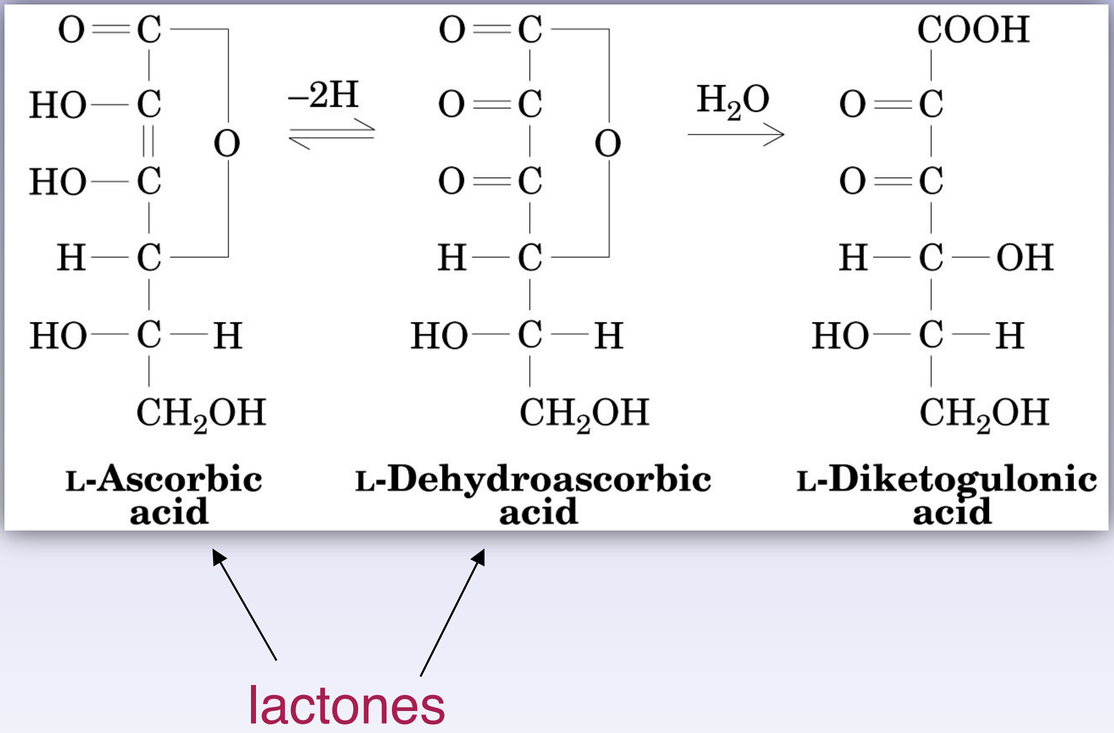


Figure 16.8. Glucuronic acid oxidation pathway.

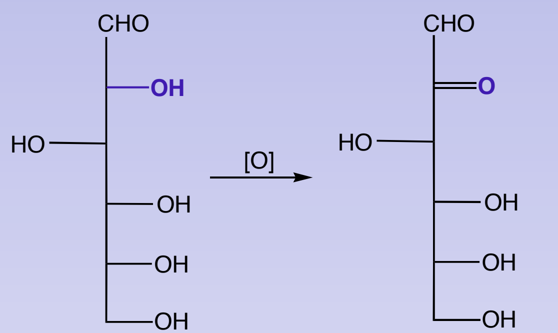
Textbook of Biochemistry With Clinical Correlations, Sixth Edition, Edited by Thomas M. Devlin.

Role of GlcUA in the biosynthesis of vitamin C (ascorbic acid) (not in humans)

The reversible oxidation of L-ascorbic acid to L-dehydroascorbic acid *in vivo*



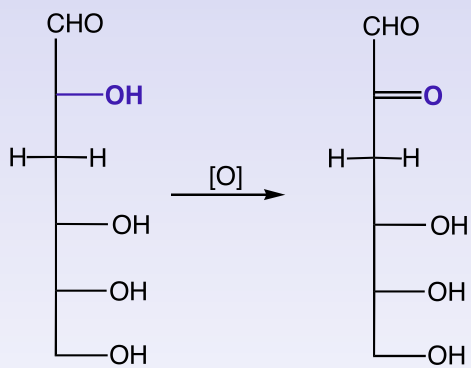
Oxidation of aldoses to osones



an aldose (D-glucose)

an osone (D-glucosone; 1,2-dicarbonyl)

Other types of dicarbonyl species are also possible (*e.g.*, 2,3-dicarbonyl species generated from 2-ketoses)

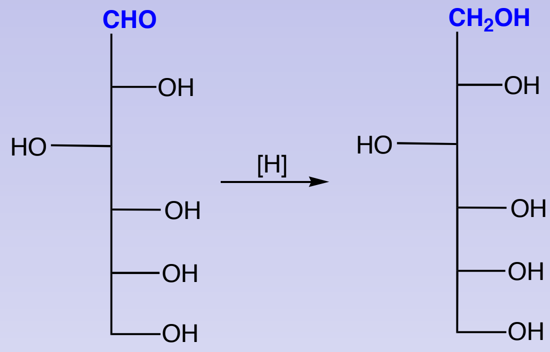


3-deoxy-D-glucose

3-deoxy-D-glucosone; 1,2-dicarbonyl)

A metabolite in diabetic patients; a by-product of protein glycation; reactive glycating agent (reacts mainly with Arg residues); may play a role in diabetic complications and aging.

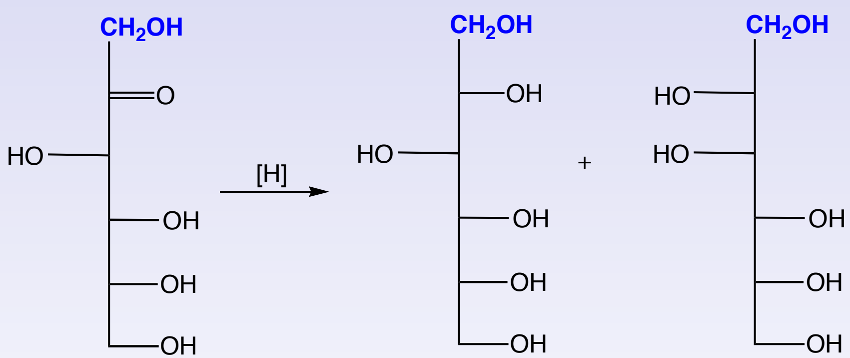
Reduction of aldoses and ketoses to alditols



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

A common chemical derivative used to simplify the analysis of monosaccharide mixtures generated from the hydrolysis of complex oligo- and polysaccharides (see below).

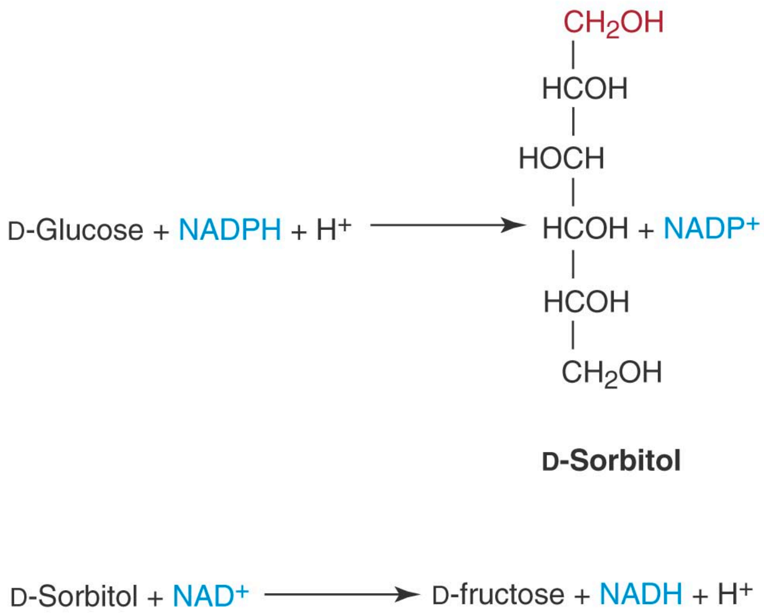
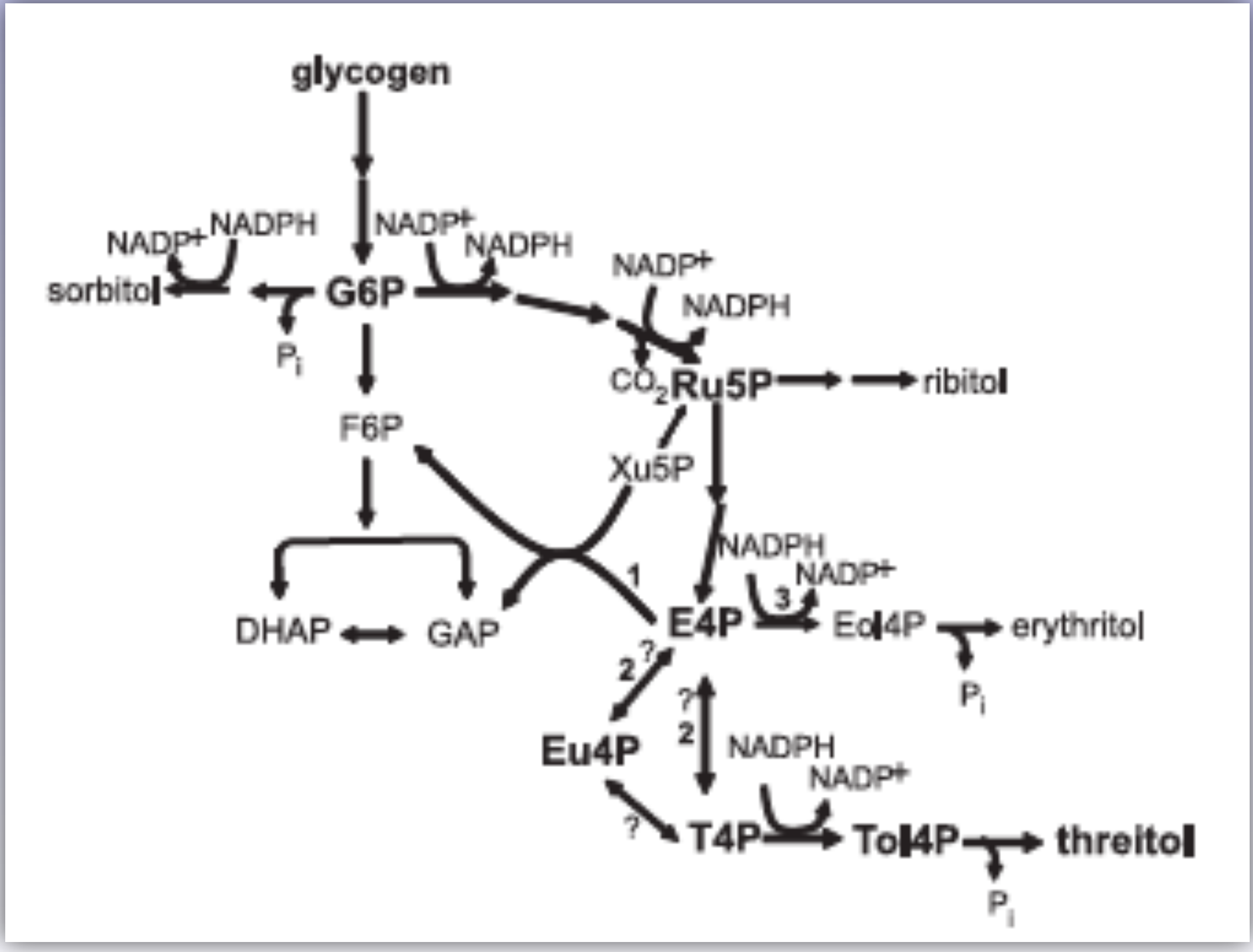


Figure 15.43. Pathway responsible for the formation of sorbitol and fructose from glucose.

Biosynthesis of D-glucitol (D-sorbitol). Note the involvement of NADPH as the cofactor, implying reaction in the cytosol. D-Glucitol accummulation in the eye lens is responsible for cataract formation in diabetic patients.

Alditol production in vivo as a cryoprotectant



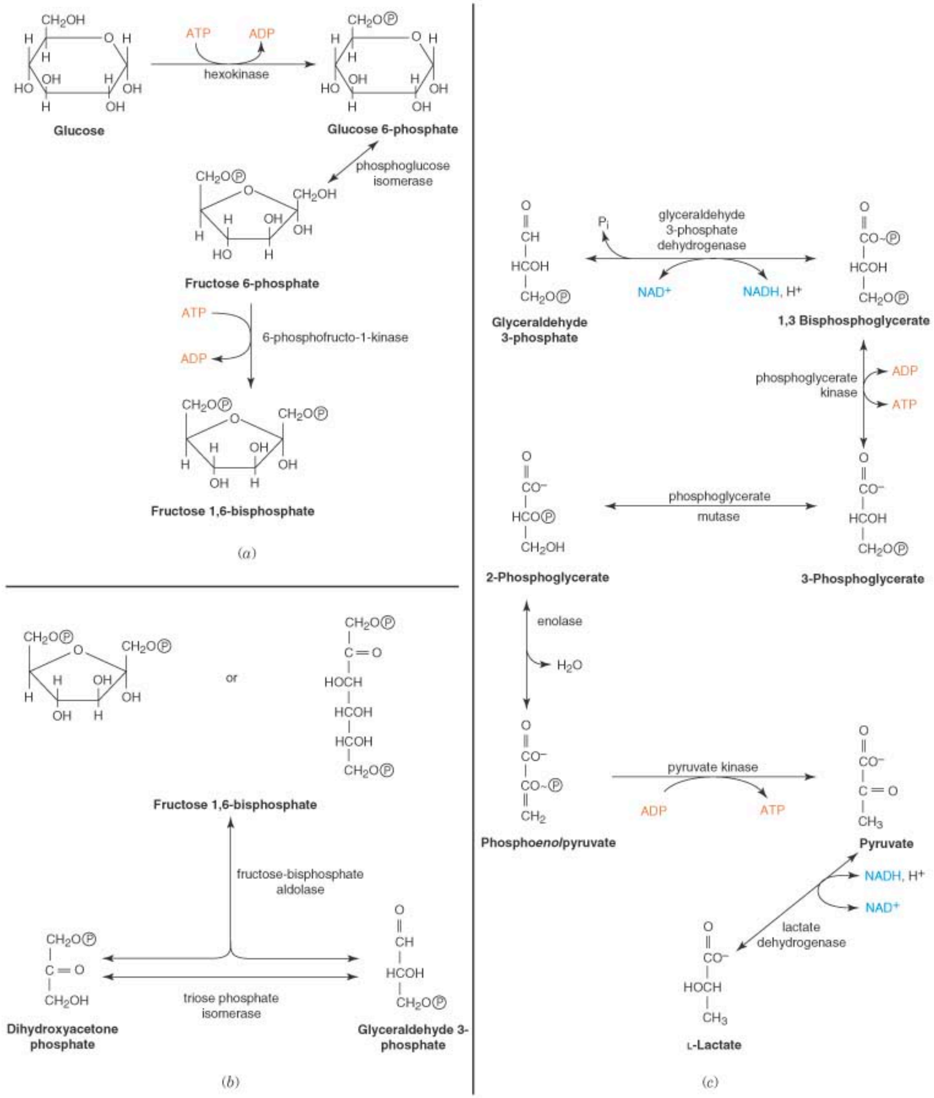
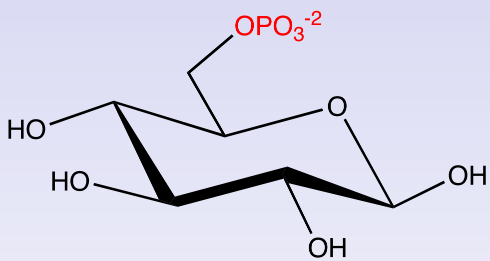


Figure 15.7. The glycolytic pathway, divided into three stages.

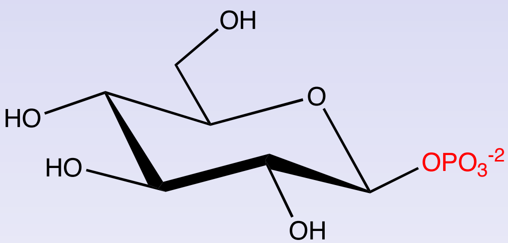
Phosphorylation:
The presence of phosphomonoesters is common in saccharide metabolites. Phosphorylation inhibits diffusion of metabolites through the plasma membrane and affects chemical and biological activities. Phosphate source is usually ATP.

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

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



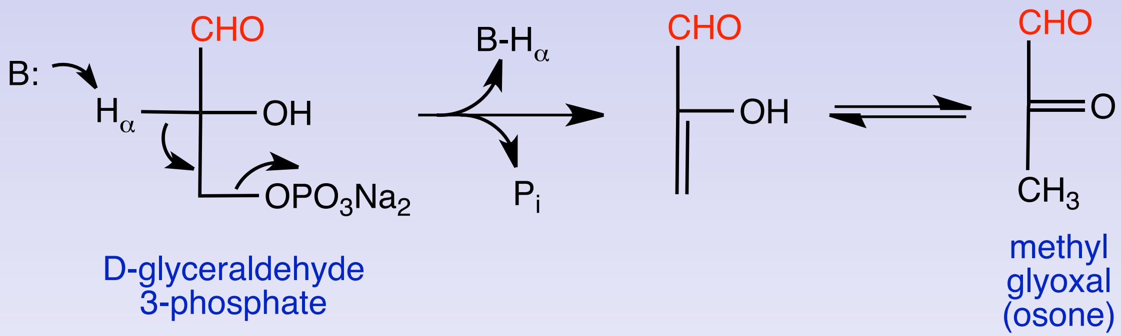
β-D-glucose 6P
(phosphomonoester)



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

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

β-Elimination mechanism in sugar phosphates



Saccharide sulfation is achieved via the sulfate donor, PAPS

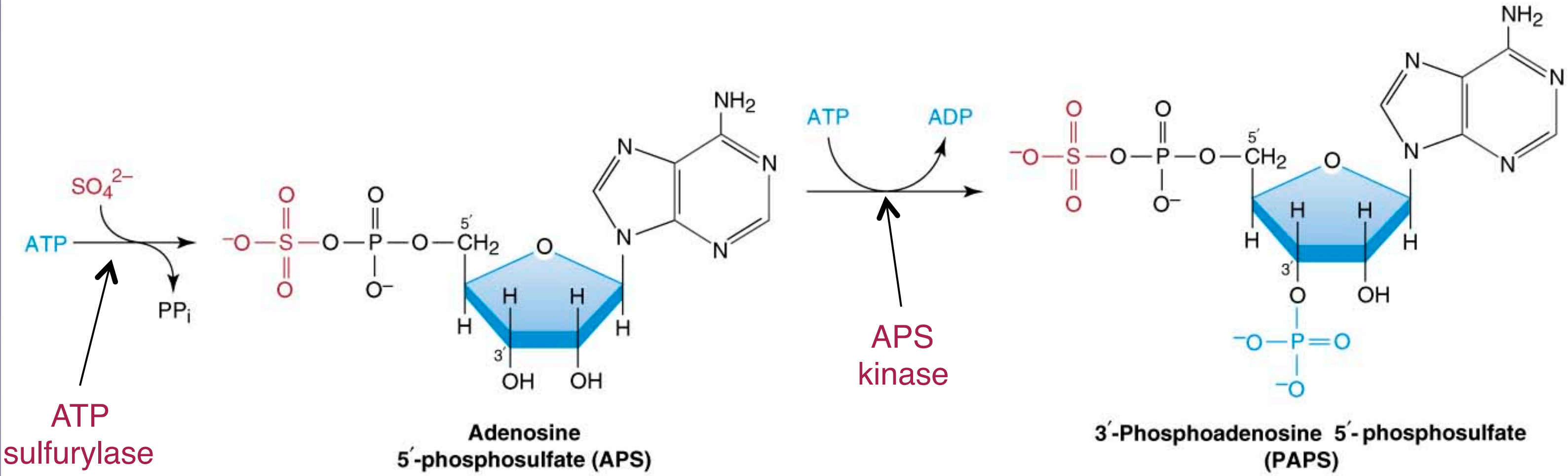


Figure 16.14. Biosynthesis of 3′-phosphoadenosine 5′-phosphosulfate (PAPS).

APS and PAPS are mixed anhydrides.

Enzyme-catalyzed saccharide sulfation reactions