CHEM 537
Carbohydrate Biochemistry and Glycobiology
Part II: Oligosaccharides & Polysaccharides

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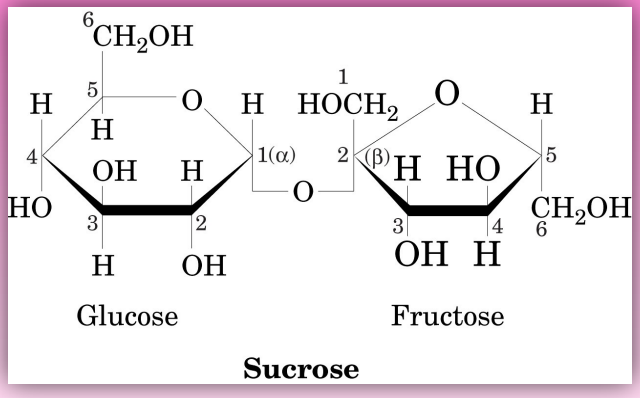
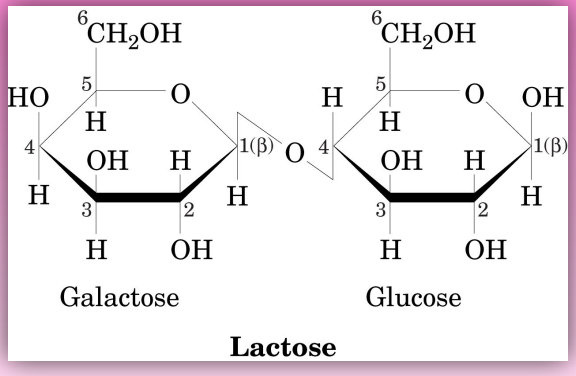
Slide Set 2b

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

Other common biologically important disaccharides



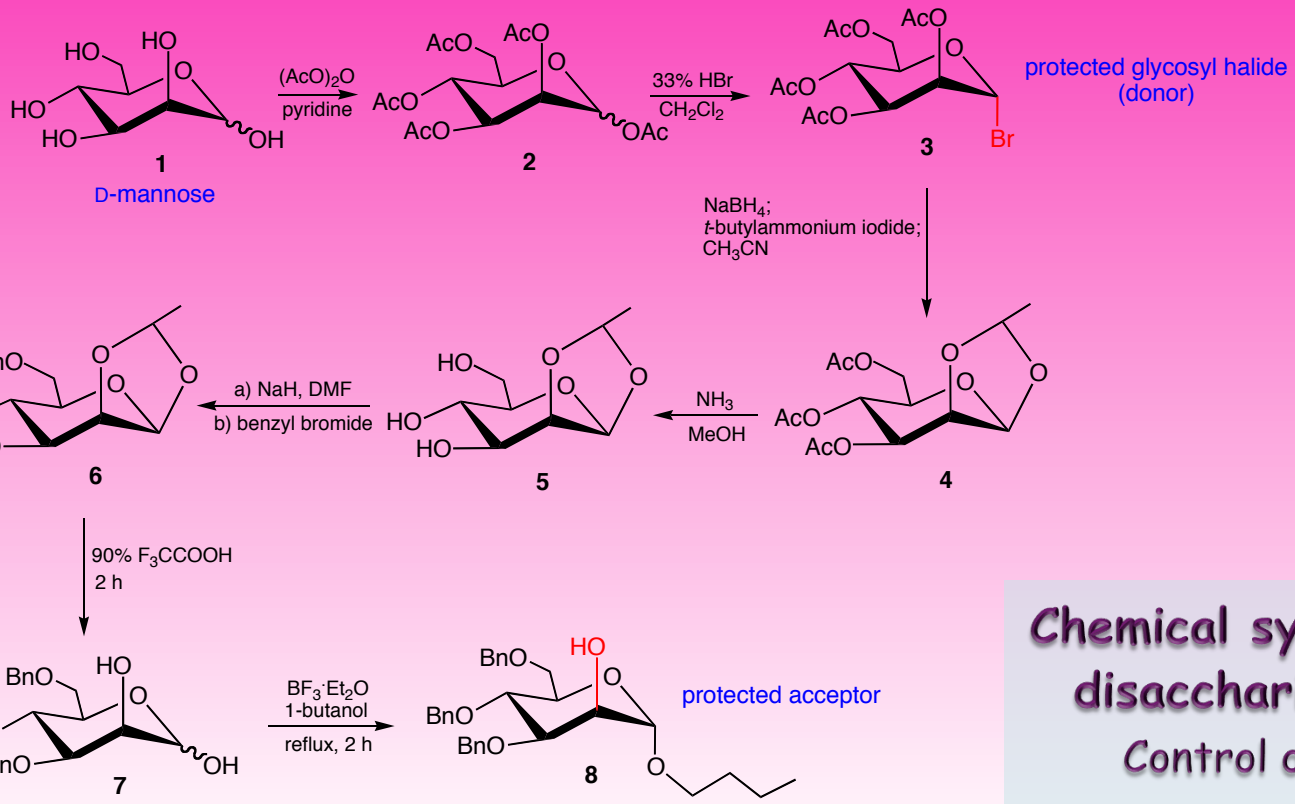


Distinguishing structural features of disaccharides

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)

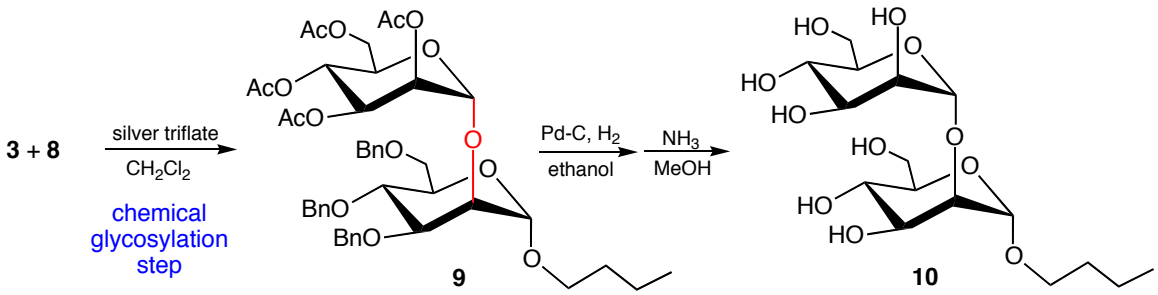
Functions of some common biologically important disaccharides

Disaccharide	Structure	Occurrence	Physiological Role
sucrose	Glcα(1→2)Fruβ	fruits, seeds, roots, honey	final product of photosynthesis; used as primary energy source in many organisms; most abundant disaccharide
lactose	Galβ(1→4)Glc	milk, plants	energy source
α,α-trehalose	Glcα(1→1)Glcα	yeast, fungi, insect hemolymph	insect energy source
maltose	Glcα(1→4)Glc	starch and glycogen	energy storage in animals
cellobiose	Glcβ(1→4)Glc	plants (cellulose)	structural stability
chitobiose	NAGβ(1→4)NAG	fungi, Insects, arthropods	exoskeleton structure



Chemical synthesis of a disaccharide: Man_2

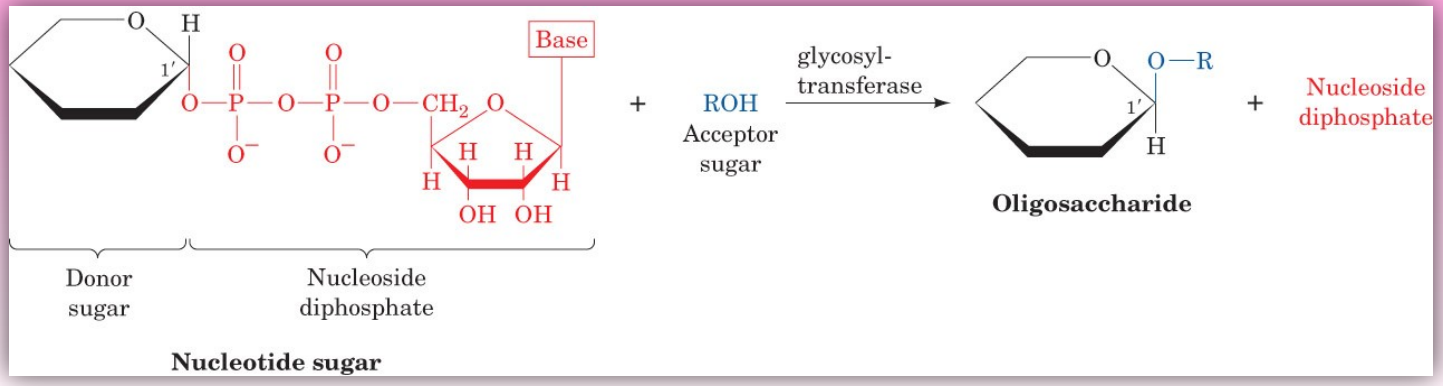
Control of regio- and stereochemistry



n-butyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside

Enzyme-catalyzed synthesis of glycosidic linkages: *in vivo* and *in vitro*

Glycosyltransferases



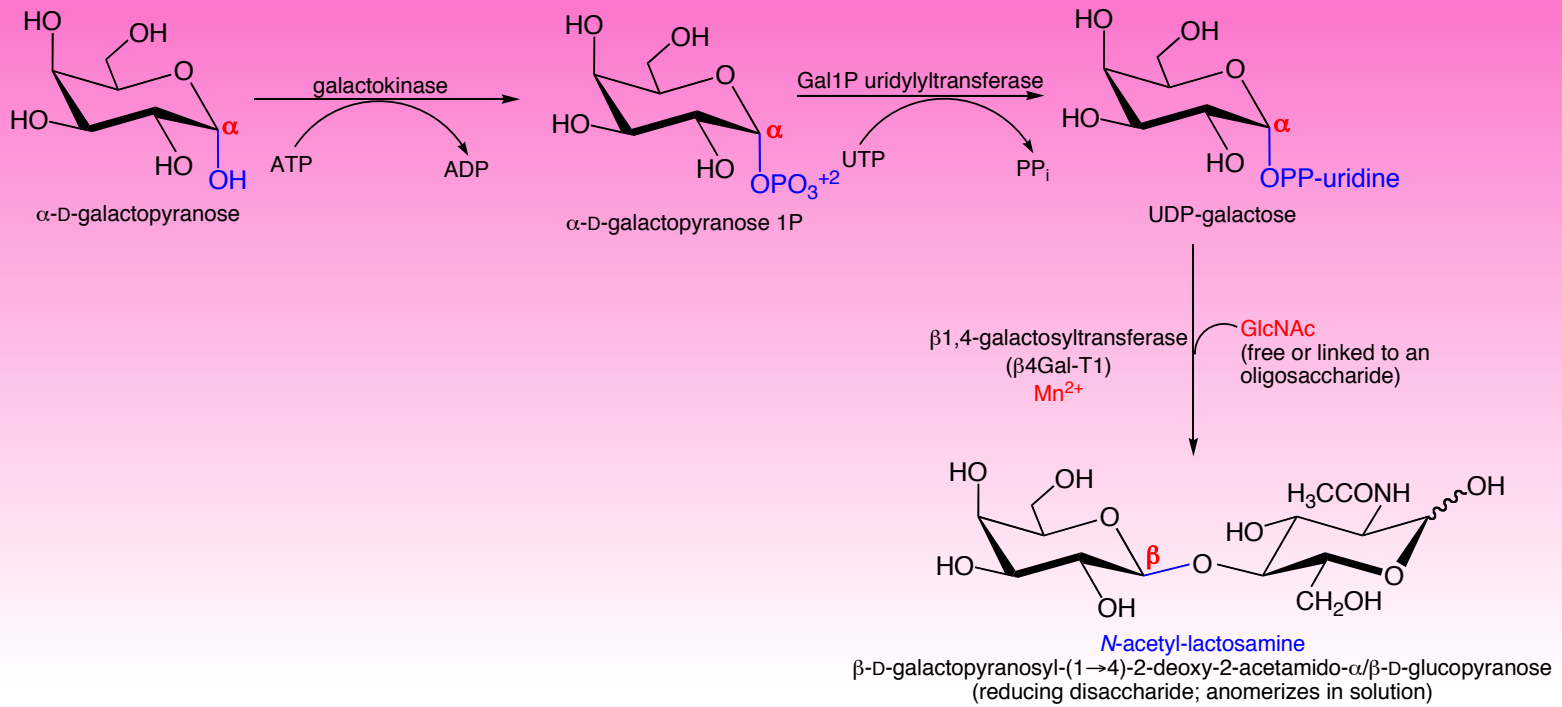
Examples of nucleotide sugars: UDP-Glc, UDP-Gal, UDP-GlcNAc, UDP-GalNAc, GDP-Man, GDP-fucose

TABLE 6.9 Glycosyltransferases in Eukaryotic Cells

Sugar Transferred	*Abbreviation*	*Donor*	*Glycosyltransferase*
Mannose	Man	GDP-Man Dolichol-Man	Mannosyltransferase
Galactose	Gal	UDP-Gal	Galactosyltransferase
Glucose	Glc	UDP-Glc Dolichol-Glc	Glucosyltransferase
Fucose	Fuc	GDP-Fuc	Fucosyltransferase
N-Acetylgalactosamine	GalNAc	UDP-GalNac	*N*-acetylgalactosaminyltransferase
N-Acetylglucosamine	GlcNAc	UDP-GlcNAc	*N*-acetylglucosaminyltransferase
N-Acetylneuraminic acid (or sialic acid)	NANA or NeuNAc SA	CMP-NANA CMP-SA	*N*-Acetylneuraminyltransferase (sialyltransferase)

Man-T
Gal-T
Glc-T
Fuc-T
GalNAc-T
GlcNAc -T
ST

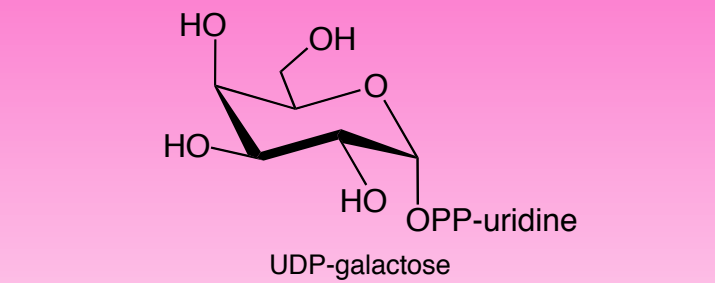
Biosynthesis of *N*-acetyl-lactosamine *in vivo*



Key characteristics:

- Galactokinase exhibits anomeric specificity (binds only α-Gal*p*)
- The β4Gal-T1 reaction proceeds with inversion of configuration of the α-Gal in UDP-Gal (an inverting transferase)
- β4Gal-T1 is a widely distributed, Golgi resident type-II membrane protein (~45 kDa)

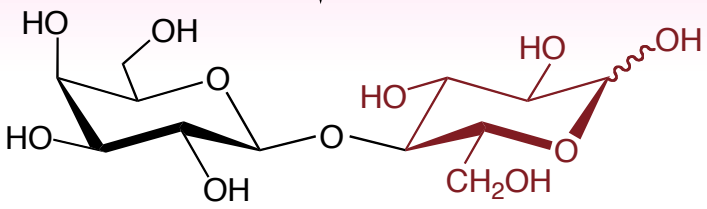
Protein-protein interactions modulate β4Gal-T1 substrate specificity



UDP-galactose

β4Gal-T1/
α-lactalbumin
Mn^{2+}

free Glc



lactose

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

In the presence of a specifier protein, α-lactalbumin (LA), the K_m for glucose is reduced from ~2 *M* to 2 m*M* (affinity increased by ~1000-fold), thus promoting the formation of lactose. The β4Gal-T1/α-lactalbumin complex is referred to as lactose synthetase.

α-Lactalbumin and lysozyme show considerable sequence and structural homologies, but α-lactalbumin has no glycosidase activity. LA does not bind oligosaccharide, and lysozyme does not bind β4Gal-T1.

α-Lactalbumin is a mammary gland-specific Ca^{2+}-binding protein (~14 kDa) expressed only during lactation. The synthetase complex is active only when the soluble lactalbumin protein binds to the membrane-bound transferase (GalT is localized in the internal membranes of mammary cells (Golgi/ER membranes, not plasma membranes).

Figure 1.10 Energetics of formation for a glycosidic bond

Overall energetics of glycosidic bond formation

Gal + Glc	→	Galβ1-4Glc	ΔG = +3.4 kcal/mole
2ATP	→	2ADP + 2Pi	ΔG = -14.6 kcal/mole

Gal + Glc + 2ATP → Galβ1-4Glc + 2ADP + $2P_i$ ΔG = -11.2 kcal/mole

Synthesis of nucleotide sugar donor

ATP + Gal	→	ADP + Gal-1P
ATP + UDP	→	ADP + UTP
UDP-Glc + Gal-1P	→	UDP-Gal + Glc-1P
Glc-1P + UTP	→	UDP-Glc + PP_i
PP_i	→	$2P_i$

Gal + UDP + 2ATP → UDP-Gal + 2ADP + $2P_i$

Creation of glycosidic bond

UDP-Gal + Glc → Galβ1-4Glc + UDP

Taylor and Drickamer
Introduction to Glycobiology

Hydrolysis of glycosidic linkages

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

Enzymatic methods: use of glycosidases (glycoside hydrolyzing enzymes)

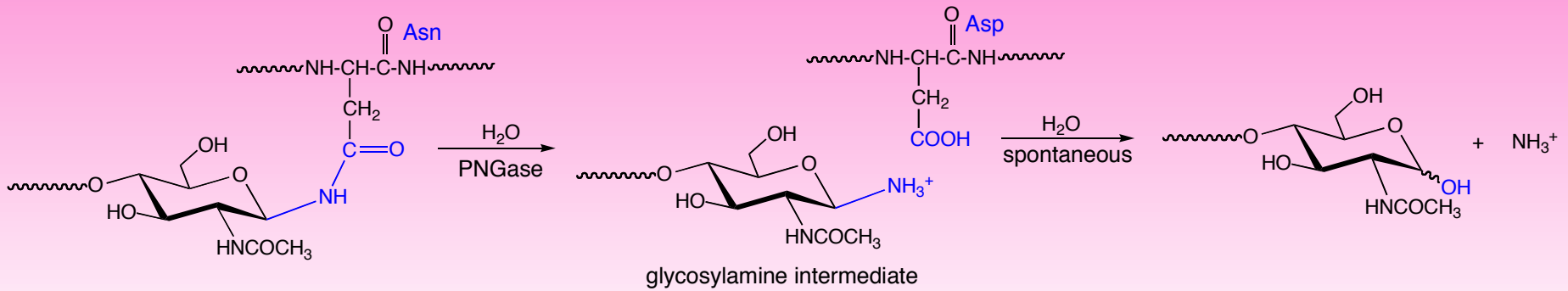
- Exoglycosidases: Hydrolyze glycosidic linkages involving terminal residues
- Endoglycosidases: Hydrolyze glycosidic linkages involving internal residues

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 specificities

- *endo* β-*N*-acetylglucosaminidases (Endo D, H, F): cleave internal GlcNAc-GlcNAc linkages (Endo F has broad specificity)
- *endo* β-galactosidases: cleave internal β-Gal*p* linkages
- peptide: *N*-glycanase: cleaves at the *N*-glycoside joining *N*-glycan to Asn
- 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

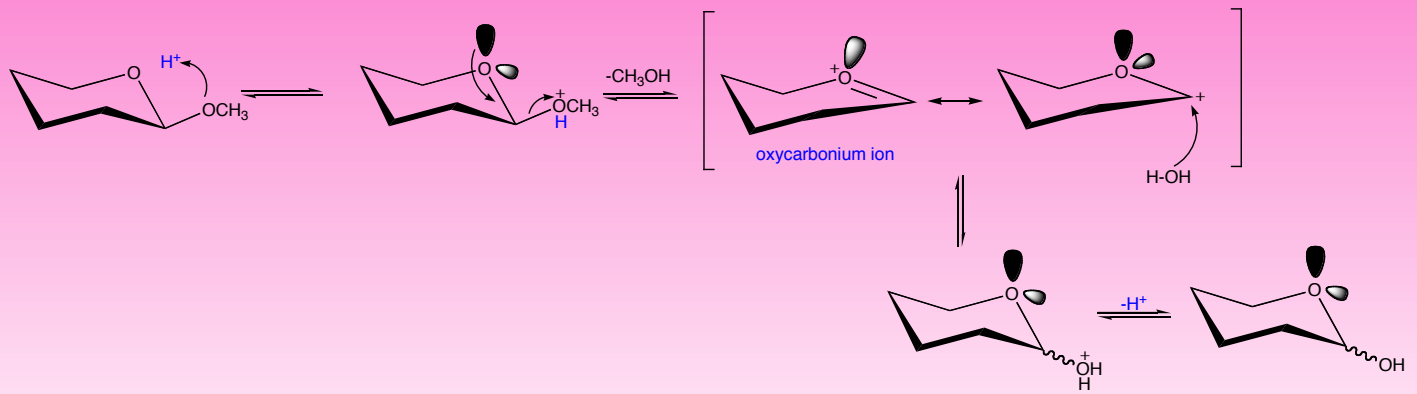
Hydrolysis of the *N*-glycoside bond of *N*-glycans by peptide *N*-glycanase (PNGase)



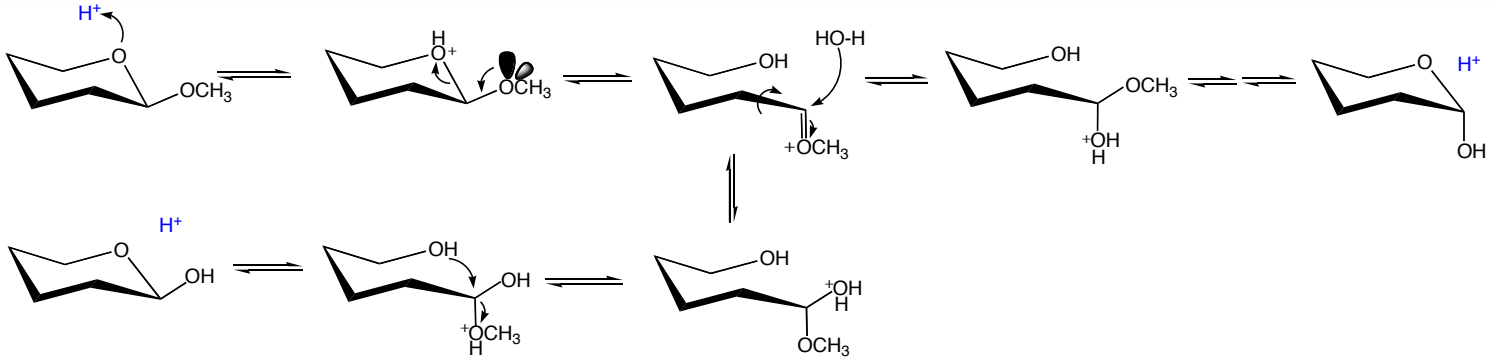
Results in the release of the intact *N*-glycan from the protein. The released *N*-glycan has a free reducing end available for derivatization.

Chemical mechanism of H^+-catalyzed hydrolysis of a glycosidic bond

Exocyclic mechanism: oxycarbonium ion intermediate

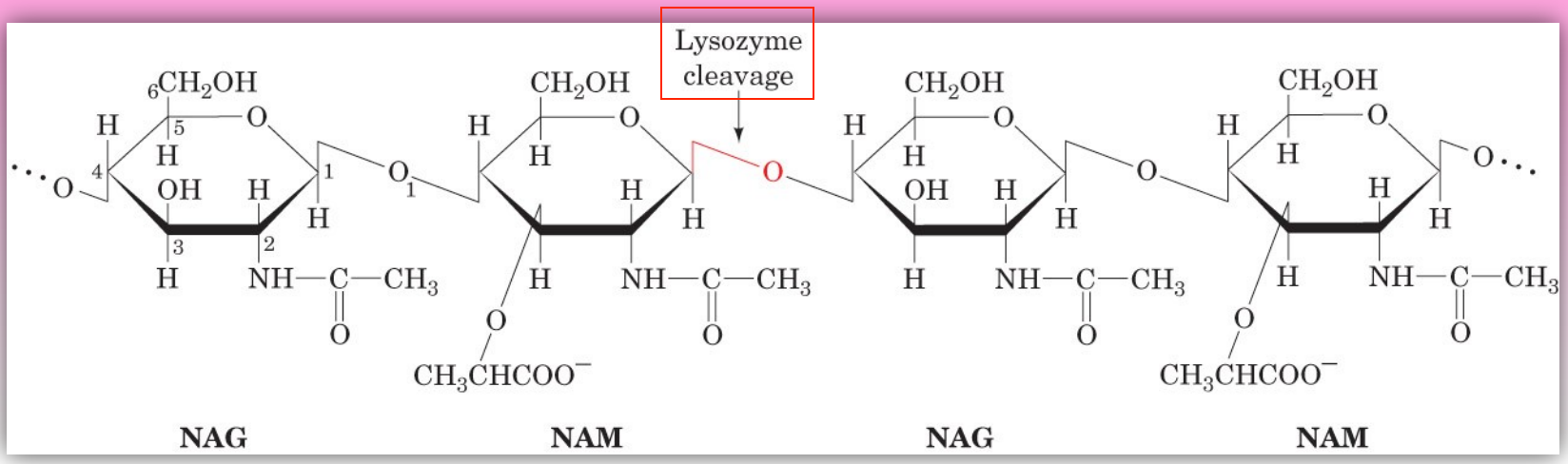


Endocyclic mechanism: acyclic hemiacetal intermediate



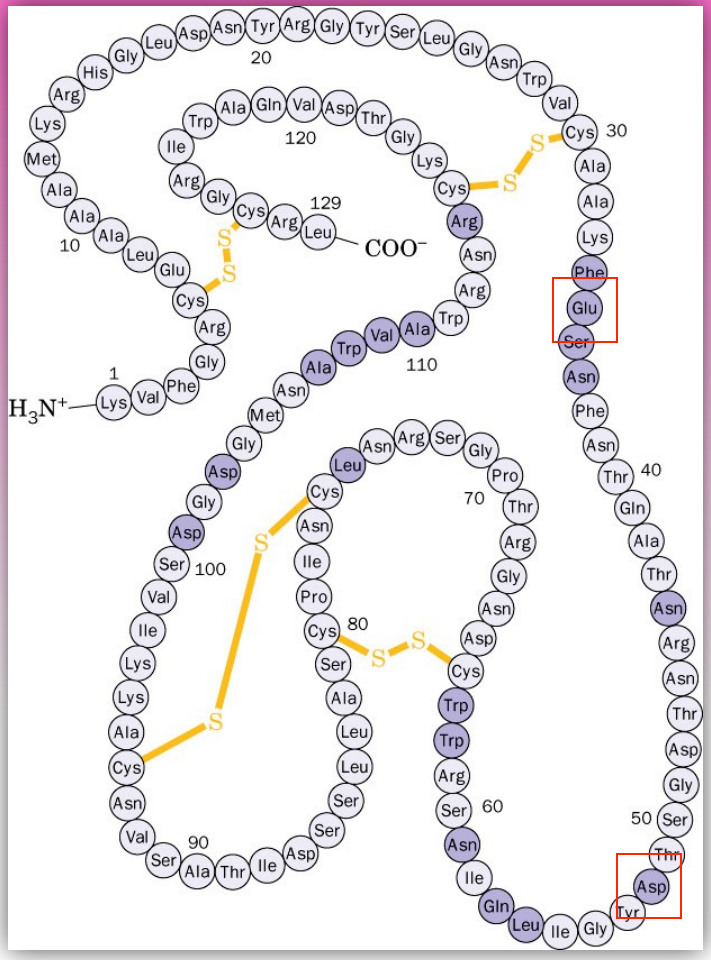
Mechanism of action of glycosidases: lysozyme

The substrate: The NAG-NAM polysaccharide of bacterial cell peptidoglycans (also hydrolyzes chitin in fungal cell walls)



NAM = *N*-acetylmuramic acid (a GlcNAc residue to which has been attached L-lactic acid in ether linkage at O3)

Note that lysozyme hydrolyzes the β-NAM (1 → 4)-β-NAG glycosidic linkage.



Primary structure of hen egg white (HEW) lysozyme (129 residues). Residues that comprise the substrate binding site are shown in dark purple. Protein is stabilized by four disulfide bonds (common for secreted proteins). Note the location of the two catalytic residues, Glu 35 and Asp 52 (surrounded by red boxes).