Overview

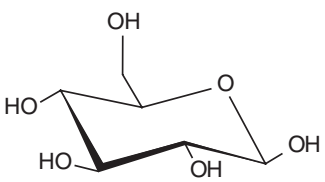
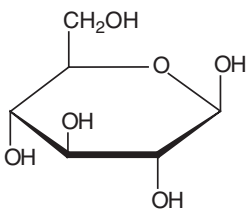
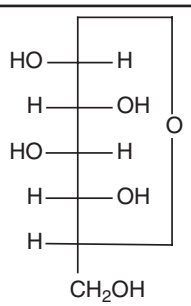
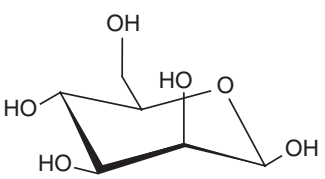
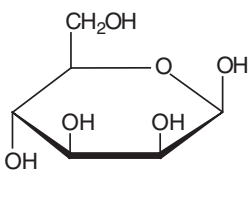
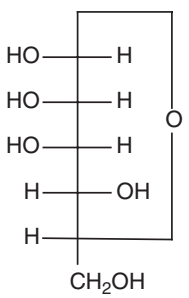
One of the distinguishing features of the proteome in eukaryotic cells is that most proteins are subject to post-translational modification, of which glycosylation is the most common form. It is estimated that more than half of all proteins that have been characterized are glycoproteins. The carbohydrate components of glycoproteins perform critical biological functions in protein sorting, immune and receptor recognition, inflammation, pathogenicity, metastasis, and other cellular processes.

Mammalian glycoproteins contain three major types of oligosaccharides (glycans): N-linked, O-linked, and glycosylphosphatidylinositol (GPI) lipid anchors. N-Linked glycans are linked to the protein backbone via an amide bond to asparagine residues in an Asn-X-Ser/Thr motif, where X can be any amino acid, except Pro. O-Linked glycans are linked to the hydroxyl group of serine or threonine. GPI-anchored proteins are attached at their carboxy-terminus through a phosphodiester linkage of phosphoethanolamine to a trimannosyl glucosamine core structure. The reducing end of the latter moiety is bound to the hydrophobic region of the membrane via a phosphatidylinositol group.

Variations in structure and degree of glycosylation site saturation can contribute to overall mass heterogeneity. The terminal residues of these glycans are commonly *N*-acetylneuraminic acid (sialic acids). The degree of sialylation affects both the mass and charge of a glycoprotein. Other modifications to the protein such as sulfation or phosphorylation also affect charge. O-Linked glycans often have lower mass than N-linked structures, but can be more abundant and heterogeneous.

Key to Monosaccharide Symbols, Abbreviations, and Projections

Symbols, Structure Projections, and Abbreviations for Monosaccharides

Monosaccharide	3-D Chair projection	Haworth projection	Fischer projection	Symbol
β-D-Glucose (Glc)				
β-D-Mannose (Man)				

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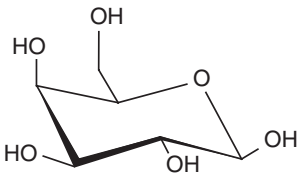
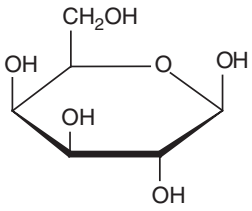
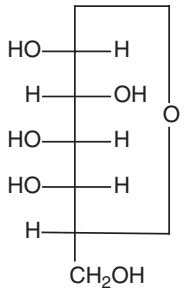
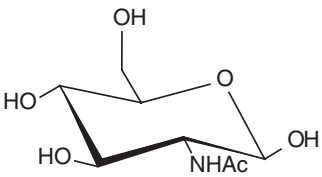
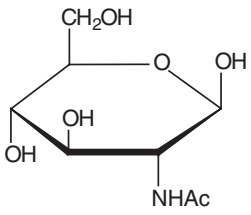
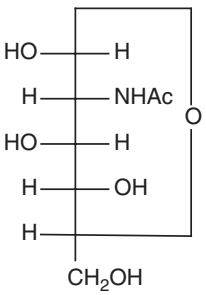
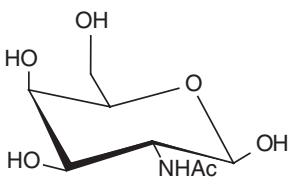
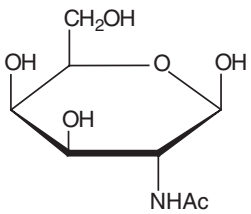
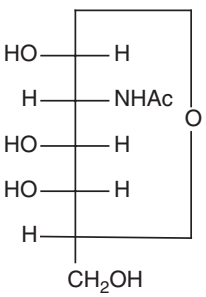
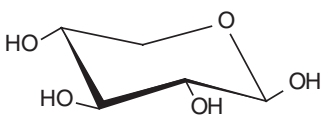
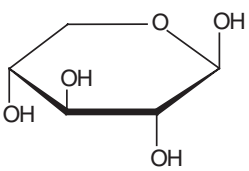
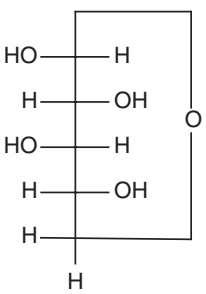
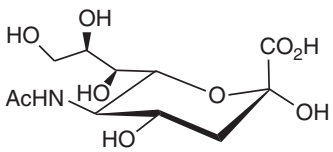
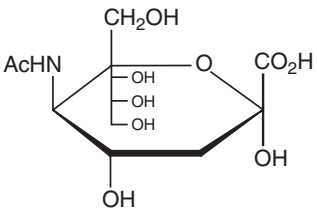
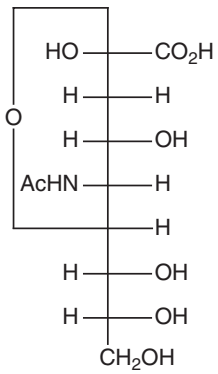
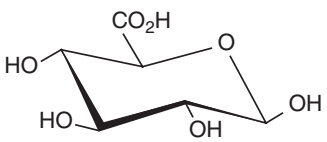
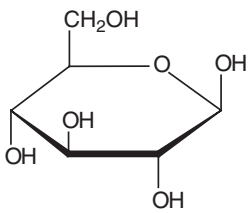
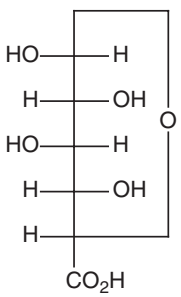
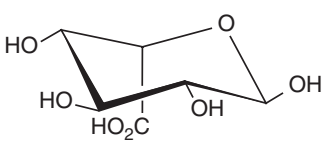
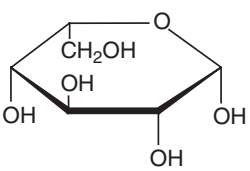
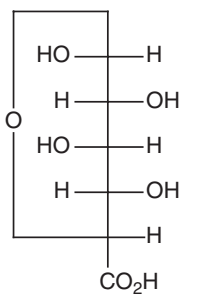
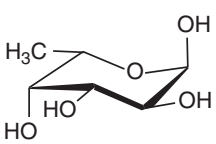
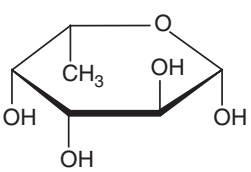
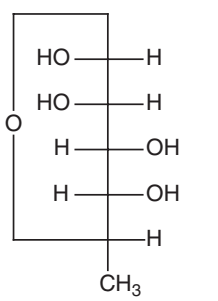
Symbols, Structure Projections, and Abbreviations for Monosaccharides

Monosaccharide	3-D Chair projection	Haworth projection	Fischer projection	Symbol
β-D-Galactose (Gal)				
β-D-*N*-Acetylglucosamine (GlcNAc)				
β-D-*N*-Acetylgalactosamine (GalNAc)				
β-D-Xylose (Xyl)				

Key to Monosaccharide Symbols, Abbreviations, and Projections

Symbols, Structure Projections, and Abbreviations for Monosaccharides

Monosaccharide	3-D Chair projection	Haworth projection	Fischer projection	Symbol
α-*N*-Acetylneuraminic acid Sialic Acid (NeuNAc)				
β-D-Glucuronic acid (GlcA)				
α-L-Iduronic acid (IdoA)				
α-L-Fucose (Fuc)				

Classification and Structure of Glycan Components

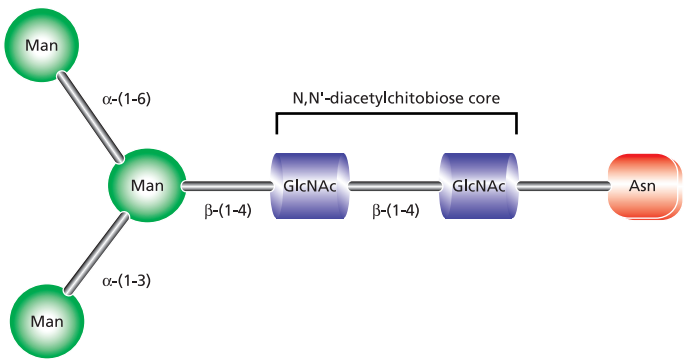
N-Linked Glycans

All eukaryotic cells express N-linked glycoproteins. Protein glycosylation of N-linked glycans is actually a co-translational event, occurring during protein synthesis. N-linked glycosylation requires the consensus sequence Asn-X-Ser/Thr. Glycosylation occurs most often when this consensus sequence occurs in a loop in the peptide. Oligosaccharide intermediates destined for protein incorporation are synthesized by a series of transferases on the cytoplasmic side of the endoplasmic recticulum (ER) while linked to the dolichol lipid. Following the addition of a specific number of mannose and glucose molecules, the orientation of the dolichol precursor and its attached glycan shifts to the lumen of the ER where further enzymatic modification occurs. The completed oligosaccharide is then transferred from the dolichol precursor to the Asn of the target glycoprotein by oligosaccharyltransferase (OST). Further processing includes trimming of residues such as glucose and mannose, and addition of new residues via transferases in the ER and, to a great extent, in the Golgi. In the Golgi, high mannose N-glycans can be converted to a variety of complex and hydrid forms which are unique to vertebrates.

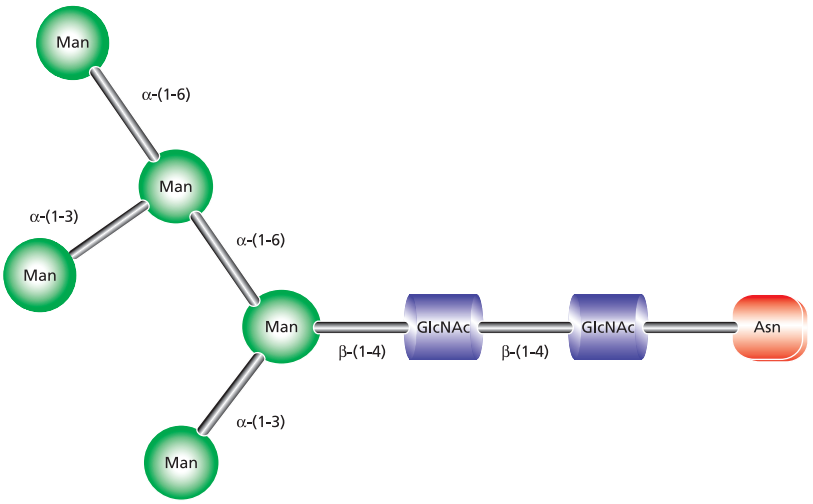
Inhibition or elimination of glycosylation in the study of N-linked glycans can be brought about by a number of compounds. In the presence of compactin, coenzyme Q, and exogenous cholesterol, N-glycosylation is greatly inhibited. Treatment with tunicamycin completely blocks deglycosylation in that it inhibits GlcNAc C-1-phosphotransferase, which is critical in the formation of the dolichol precursor necessary for synthesizing of N-glycans.

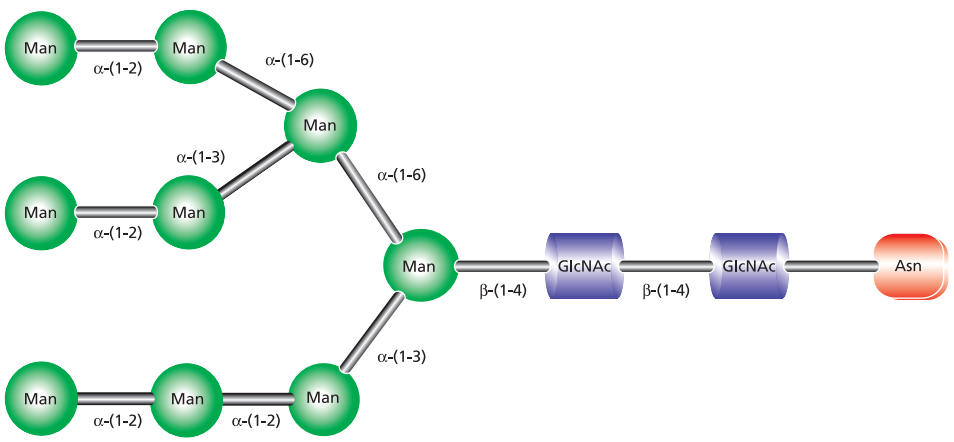
The diverse assortment of N-linked glycans are based on the common core pentasaccharide, $Man_3GlcNAc_2$. Further processing in the Golgi results in three main classes of N-linked glycan sub-types; High-mannose, Hybrid, and Complex. Complex glycans contain the common trimannosyl core. Additional monosaccharides may occur in repeating lactosamine units. Additional modifications may include a bisecting GlcNAc at the mannosyl core and/or a fucosyl residue on the innermost GlcNAc. Complex glycans exist in bi-, tri- and tetraantennary forms.

Basic N-linked Structure

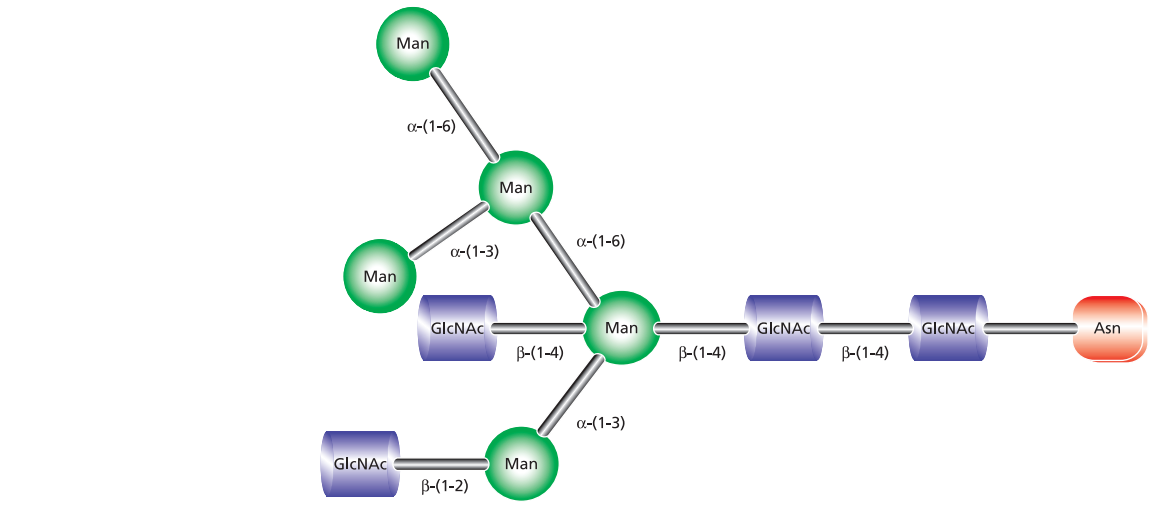


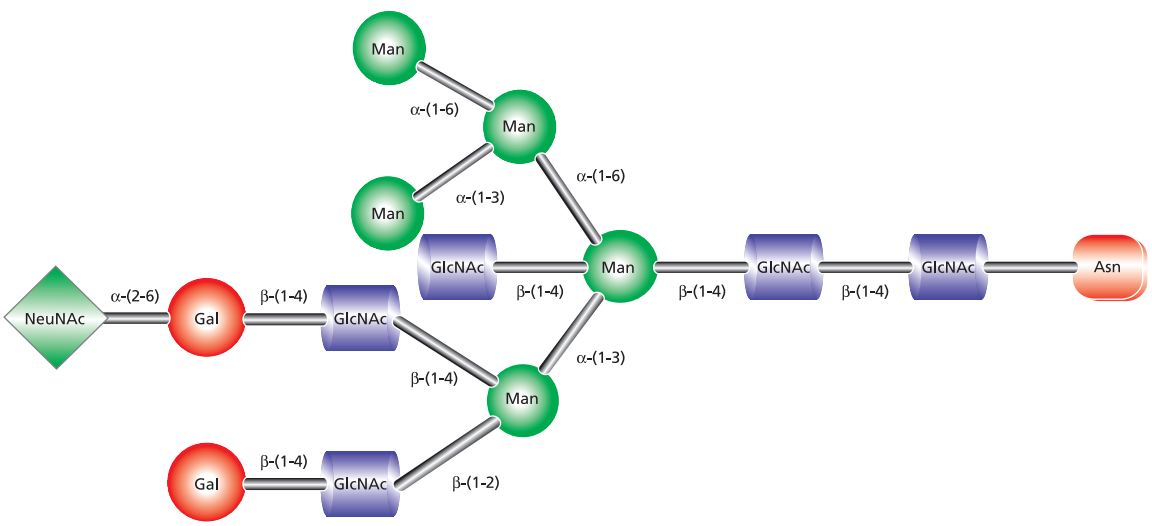
High-Mannose Structure



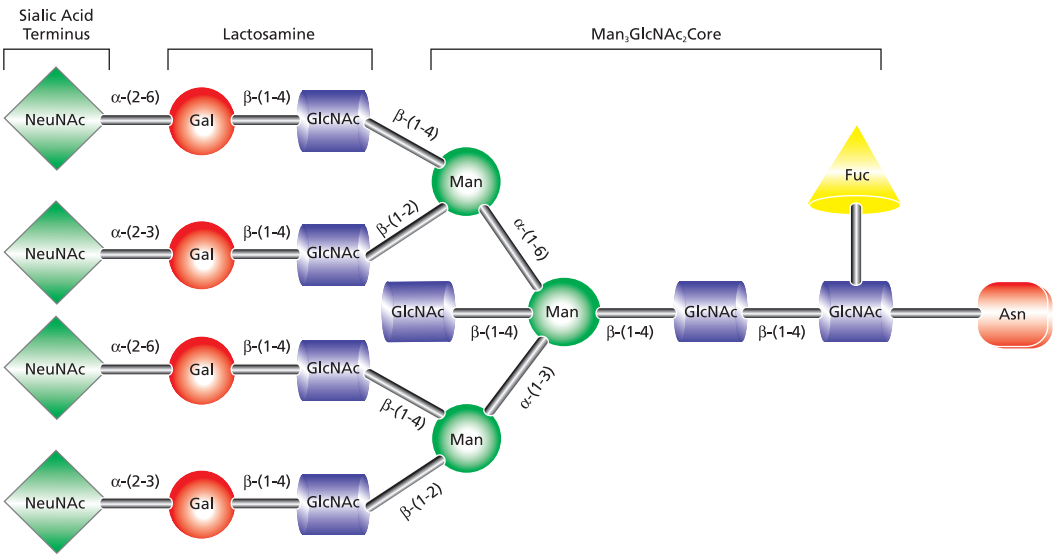


Hybrid Structure





Complex Structure (tetraantennary)

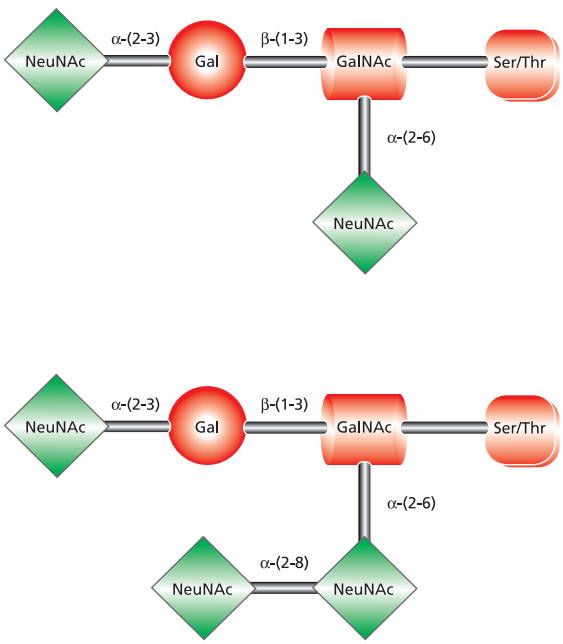


O-Linked Glycans

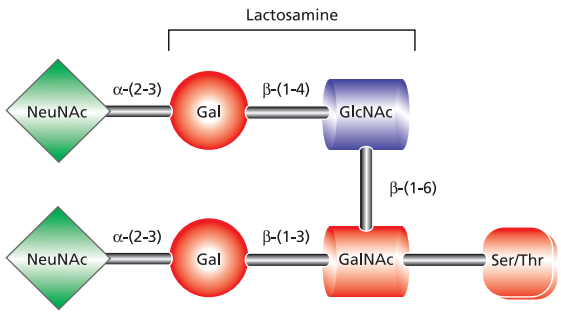
O-Linked glycans are usually attached to the peptide chain through serine or threonine residues. O-Linked glycosylation is a true post-translational event and does not require a consensus sequence and no oligosaccharide precursor is required for protein transfer. The most common type of O-linked glycans contain an initial GalNAc residue (or Tn epitope), these are commonly referred to as mucin-type glycans. Other O-linked glycans include glucosamine, xylose, galactose, fucose, or manose as the initial sugar bound to the Ser/Thr residues. O-Linked glycoproteins are usually large proteins (>200 kDa) that are commonly bianttennary with comparatively less branching than N-glycans. Glycosylation generally occurs in high-density clusters and may contribute as much as 50-80% to the overall mass. O-Linked glycans tend to be very heterogeneous, hence they are generally classified by their core structure. Non-elongated O-GlcNAc groups have been recently shown to be related to phosphorylation states and dynamic processing related to cell signaling events in the cell. O-Linked glycans are prevalent in most secretory cells and tissues. They are present in high concentrations in the zona pelucida surrounding mammalian eggs and may funtion as sperm receptors (ZP3 glycoprotein). O-Linked glycans are also involved in hematopoiesis, inflammation response mechanisms, and the formation of ABO blood antigens.

Elongation and termination of O-linked glycans is carried out by several glycosyltransferases. One notable core structure is the Gal-β(1-3)GalNAc (core 1) sequence that has antigenic properties. Termination of O-linked glycans usually includes Gal, GlcNAc, GalNAc, Fuc, or sialic acid. By far the most common modification of the core Gal-β(1-3)-GalNAc is mono-, di-, or trisialylation. A less common, but widely distributed O-linked hexasaccharide structure contains β(1-4)-linked Gal and β(1-6)-linked GlcNAc as well as sialic acid.

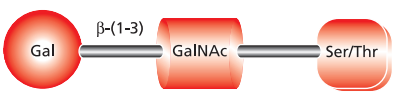
Di- and Trisialated O-Linked Core



O-Linked Core 2 Hexasaccharide



Core 1



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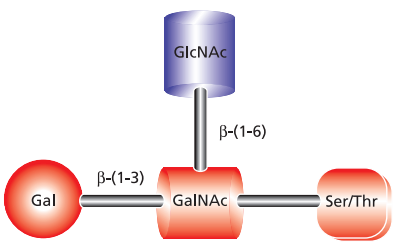
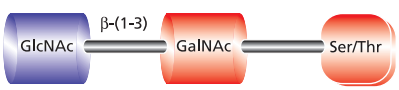


Core 3



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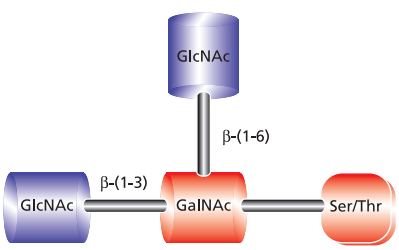
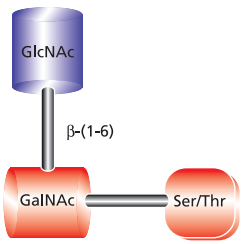


Core 5

Core 6



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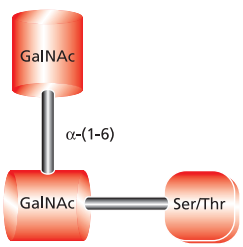
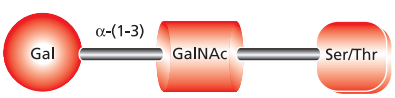


Core 8



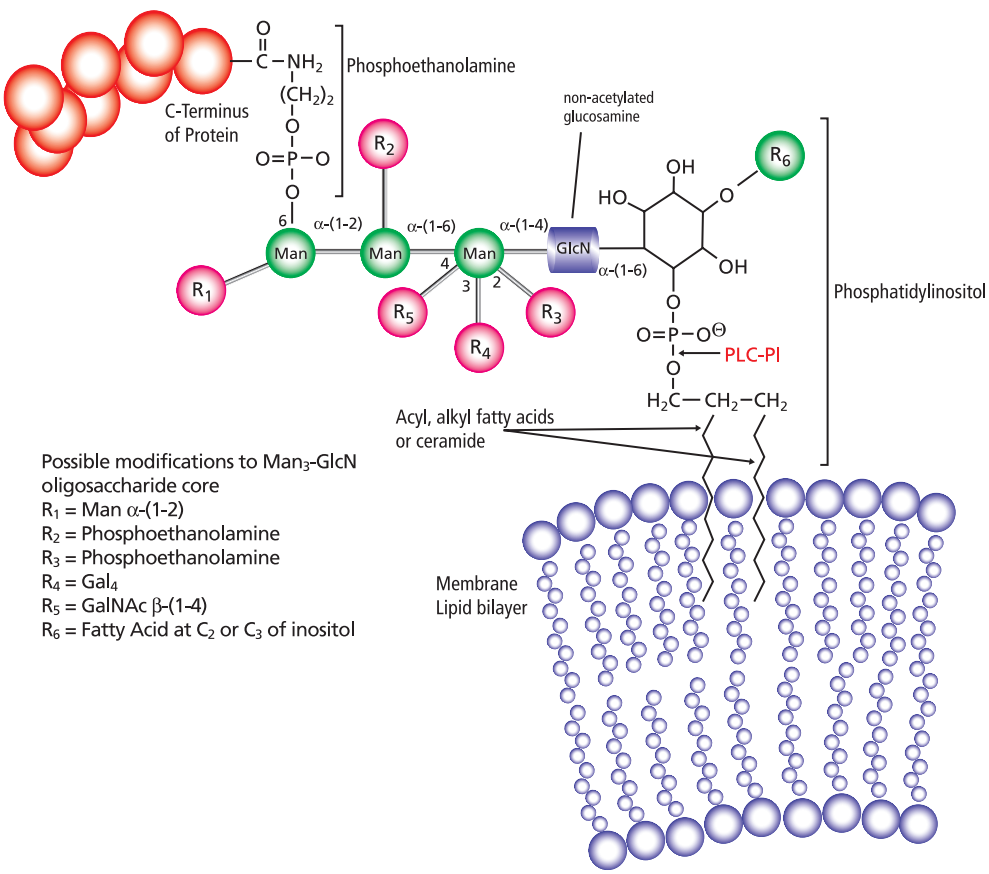
GPI Anchor Glycoproteins

Glycosylphosphatidylinisotol (GPI) anchored proteins are membrane bound proteins found throughout the animal kingdom. GPI anchored proteins are linked at their carboxy-terminus through a phosphodiester linkage of phospho-ethanolamine to a trimannosyl-non-acetylated glucosamine (Man3-GlcN) core. The reducing end of GlcN is linked to phosphatidylinositol (PI). PI is then anchored through another phosphodiester linkage to the cell membrane through its hydrophobic region. Intermediate forms are also present in high concentrations in microsomal preparations. The Man_3-GlcN oligosaccharide core may undergo various modifications during secretion from the cell.

Their functionality ranges from enzymatic to antigenic and adhesion. They contribute to the overall organization of membrane bound proteins and are important in apical protein postioning. GPI-anchored proteins also play a critical role in a variety of receptor-mediated signal transduction pathways.

Release of GPI anchored proteins can be accomplished by treatment with Phospholipase C, Phosphatidylinositol-specific (PLC-PI) (Product Codes P 5542 and P 8804). The enzyme specifically hydrolyzes the phosphodiester bond of phosphatidylinositol to form a free 1,2-diacylglycerol and glycopeptide-bound inositol cyclic-1,2-phosphate.

GPI Anchor



Possible modifications to Man_3-GlcN oligosaccharide core
R_1 = Man α-(1-2)
R_2 = Phosphoethanolamine
R_3 = Phosphoethanolamine
R_4 = Gal_4
R_5 = GalNAc β-(1-4)
R_6 = Fatty Acid at C_2 or C_3 of inositol