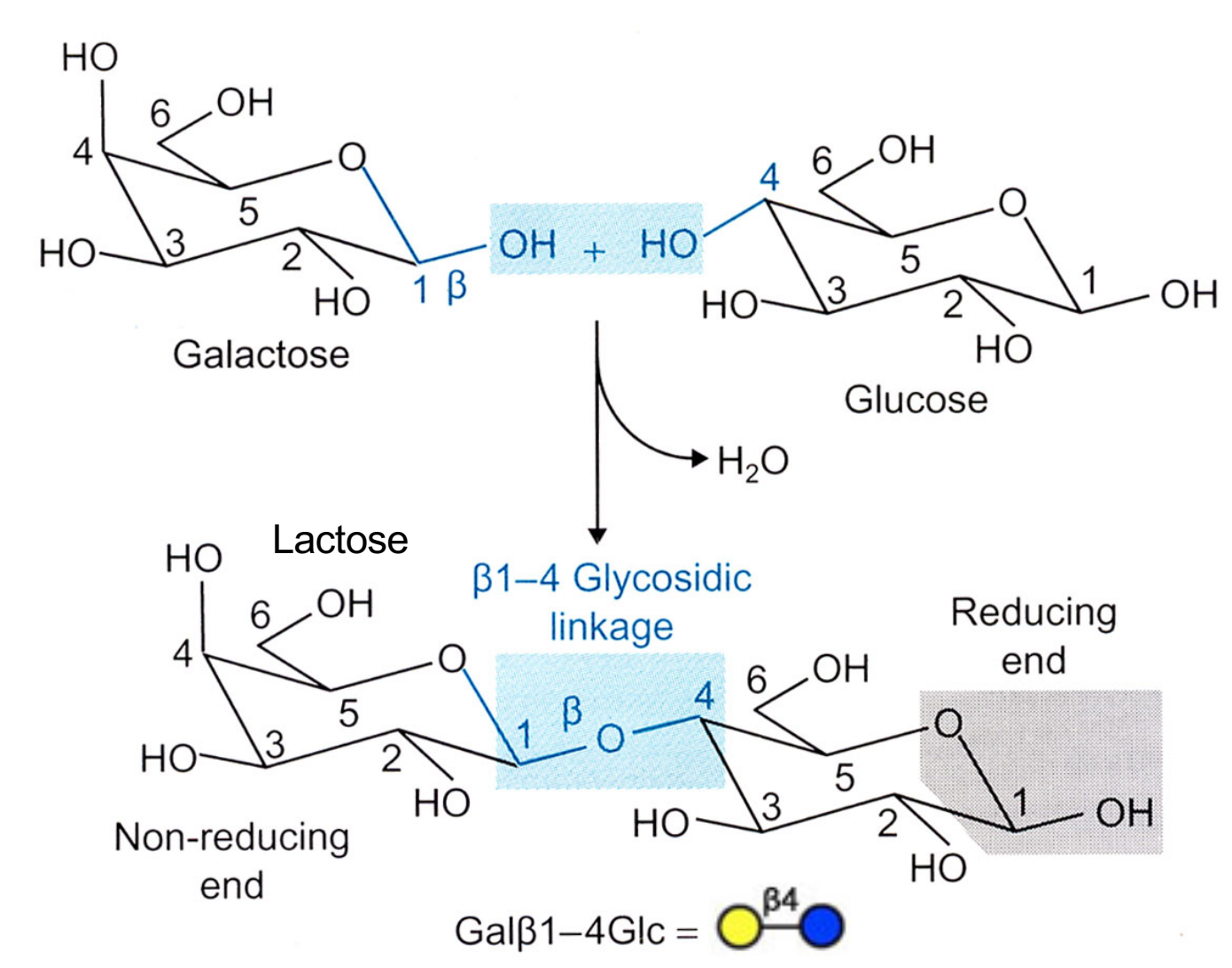
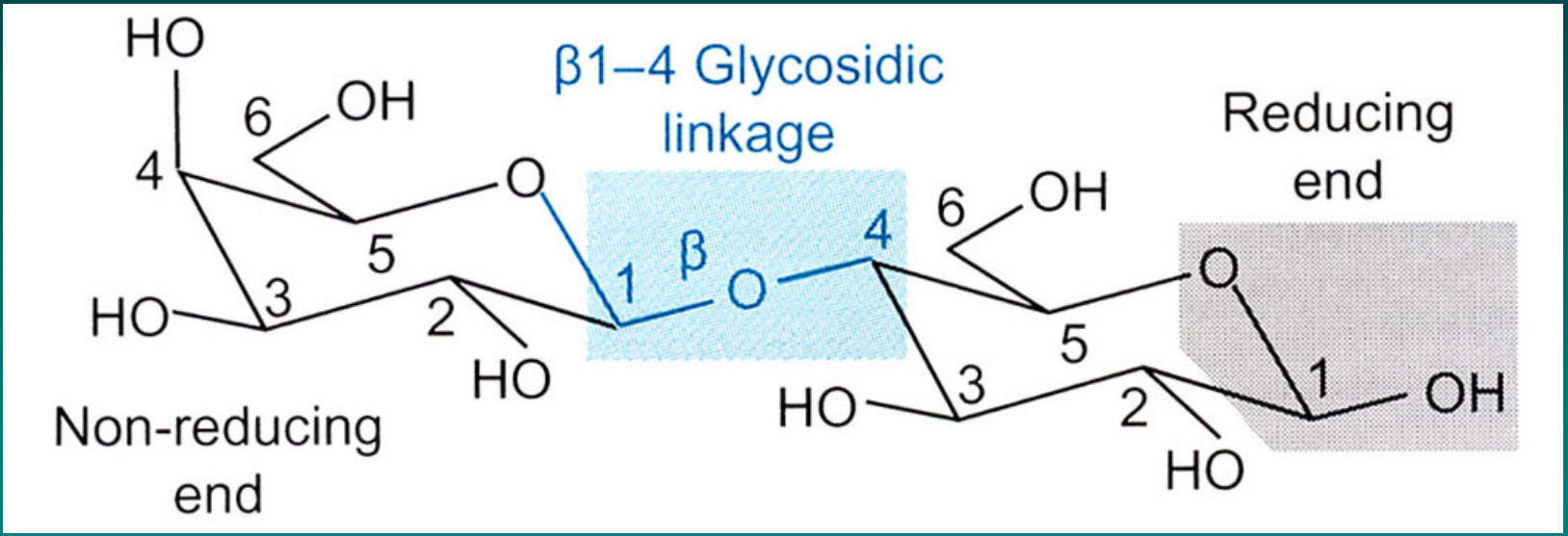
Glycosidic Bonds Link Sugars Together



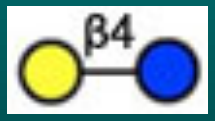
When glycosidic bond is formed the anomeric configuration is “locked”



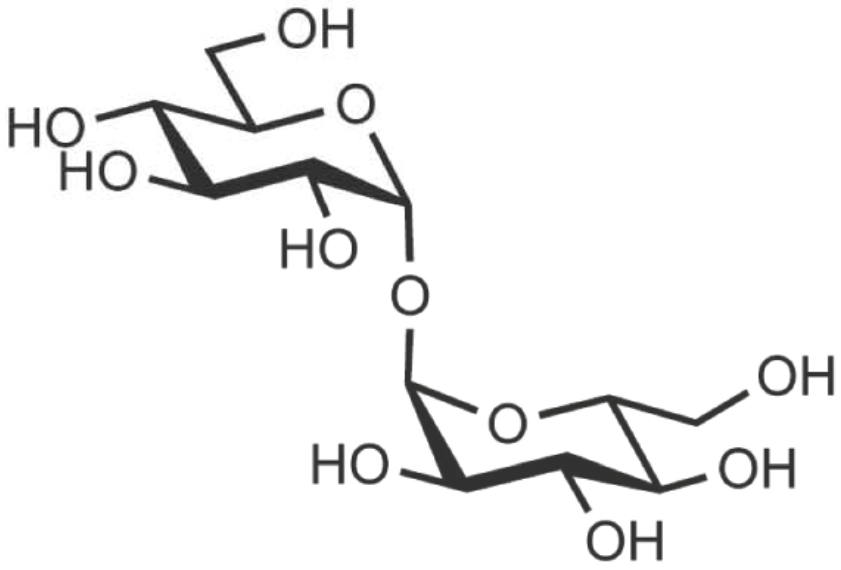
Nomenclature:

-Name the non-reducing (left-most) sugar (Gal)
-Name the anomeric configuration (β)
-Name the anomeric carbon number (1)
-Name the substituted carbon number (4)
-Name the substituted sugar (Glc)

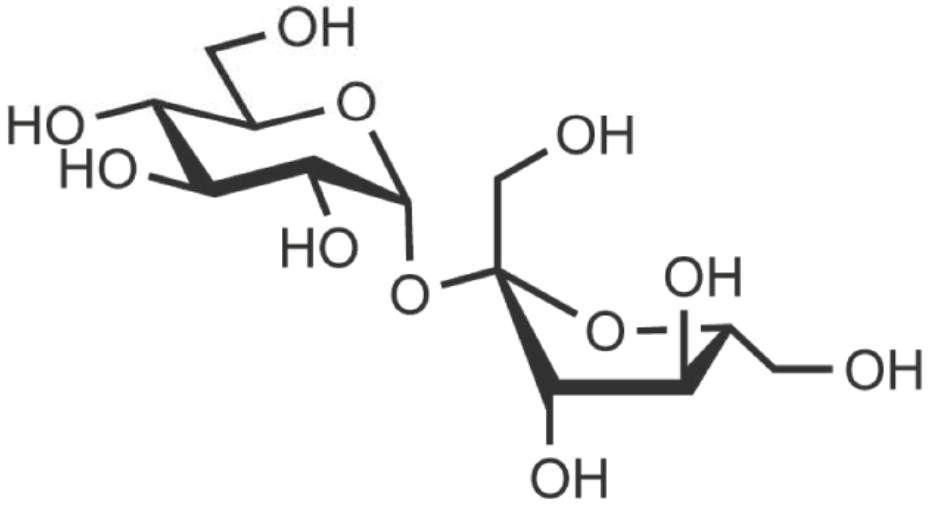
RESULT: Gal β1-4 Glc



Two Common Non-Reducing Disaccharides



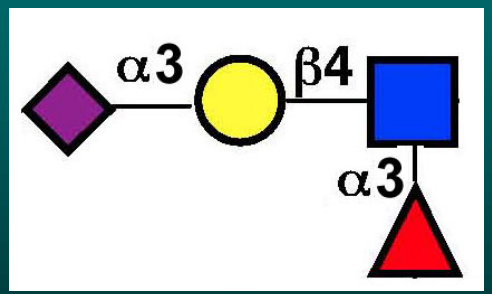
Glcα1Glcα1
(trehalose)



Glcα2Fruβ
(sucrose)

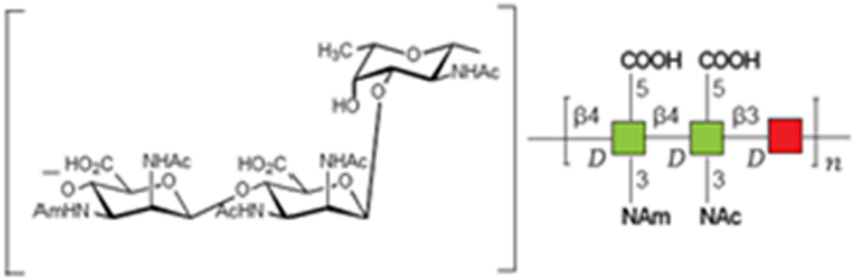
Nomenclature: Branches are Placed in Parentheses

NeuAc α2-3 Gal β1-4 (Fuc α1-3) GlcNAc



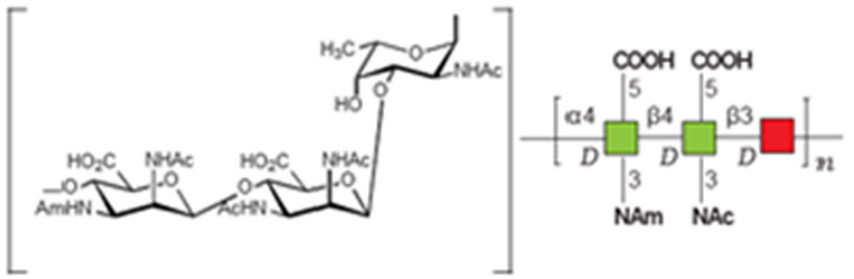
Examples of Complex Bacterial Polysaccharides

Pseudomonas aeruginosa O2a, O2b (IATS 16)



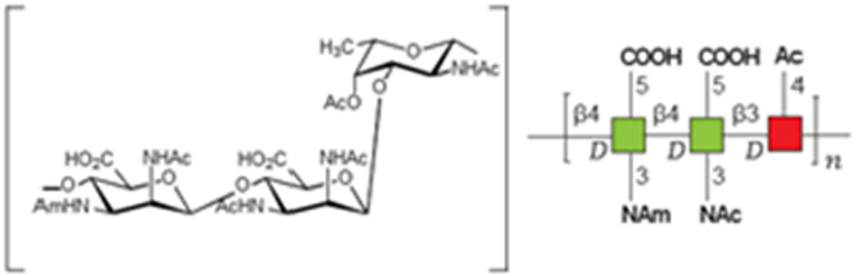
4)-β-D-ManpNAcA3NAm-(1→4)-β-D-ManpNAcA3NAc-(1→3)-β–D-FucpNAc-(1→]n

Pseudomonas aeruginosa O2a, O2d (IATS 5)



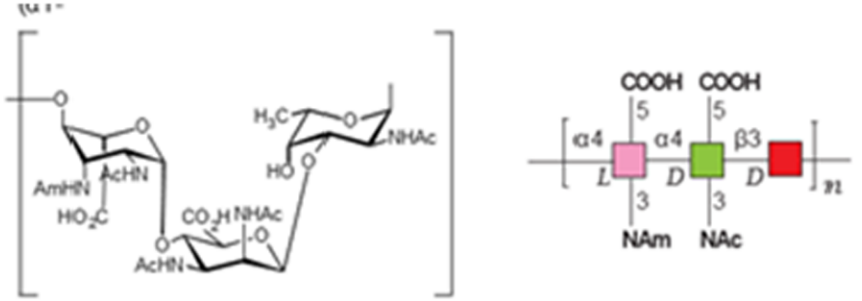
[4)-β-D-ManpNAcA3NAm-(1→4)-β-D-ManpNAcA3NAc-(1→3)-α-D-FucpNAc-(1→]n

Pseudomonas aeruginosa O2a, O2b, O2e



4)-β-D-ManpNAcA3NAm-(1→4)-β-D-ManpNAcA3NAc-(1→3)-β-D-FucpNAc4Ac-(1→]n

Pseudomonas aeruginosa O2 (IATS 18, FI 7)



[4)-α-L-GulpNAcA3NAm-(1→4)-β–D-ManpNAcA3NAc-(1→3)-α–D-FucpNAc-(1→]n

Glycan Symbol Nomenclature

Neutral Hexoses - Circles; N-Acetylhexosamines - Squares

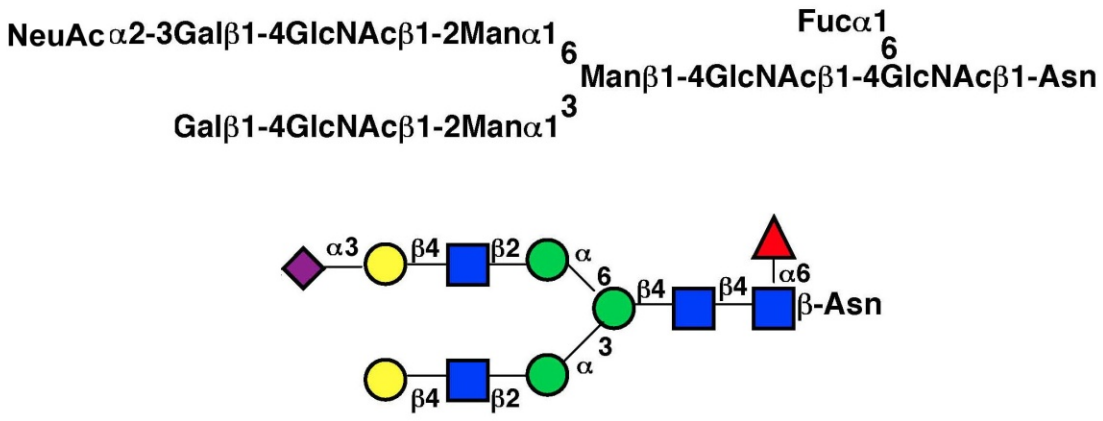
- Galactose stereochemistry: Yellow (255,255,0)
- Glucose stereochemistry: BLUE (0,0,250)
- Mannose stereochemistry: GREEN (0,200,50)

Deoxysugar - Triangle; Penose - Star

- Fucose: RED (250,0,0)
- Xylose: (5-pointed star) ORANGE (250,100,0)

Acidic Sugars (Diamonds)

- Neu5Ac: PURPLE (125,0,125)
- GlcA: BLUE (0,0,250)/Upper segment



Varki et al. (2009) *Proteomics*. 9:5398

Various Representations of an N-Glycan

FULL REPRESENTATION

9-O-Ac-Neu5Ac*p*α2-3Gal*p*β1-4GlcNAc*p*β1-2Man*p*α1
6
Man*p*β1-4GlcNAc*p*β1-4GlcNAc*p*β1-Asn
3
3-O-SO_3Gal*p*β1-4GlcNAc*p*β1-2Man*p*α1

Fuc*p*α1
6

MODIFIED REPRESENTATION

9Ac-Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1
6
Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn
3
3S-Galβ1-4GlcNAcβ1-2Manα1

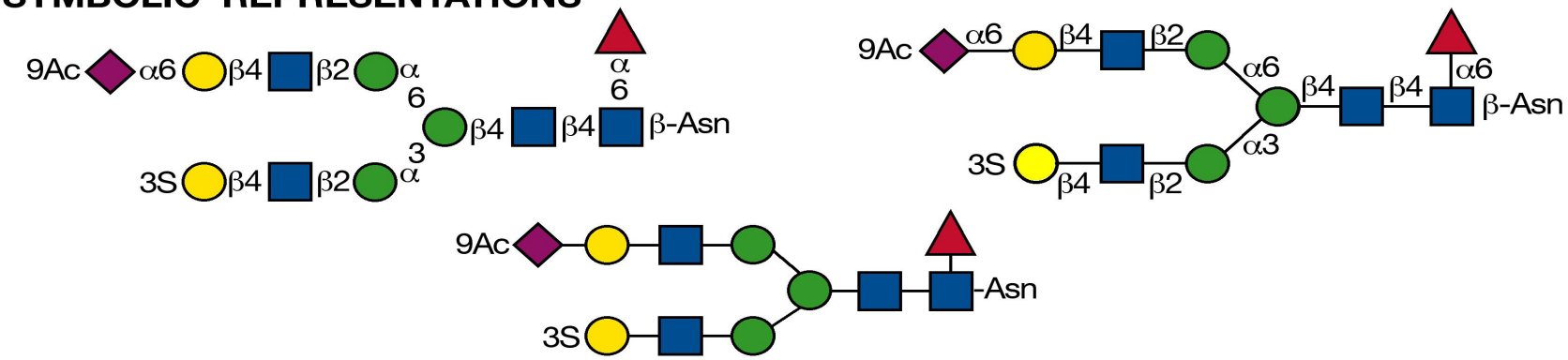
Fucα1
6

SIMPLIFIED REPRESENTATION

9-O-Ac-Neu5Acα3Galβ4GlcNAcβ2Manα
6
Manβ4GlcNAcβ4GlcNAcβ-Asn
3
3-O-SO_3Galβ4GlcNAcβ2Manα

Fucα
6

SYMBOLIC REPRESENTATIONS

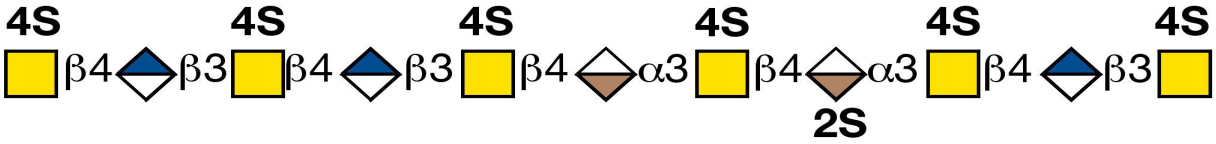


Symbolic Representation of Glycosaminoglycans

GlcNAcβ4GlcAβ3GlcNAcβ4GlcAβ3GlcNAcβ4GlcAβ3GlcNAcβ4GlcAβ3GlcNAc

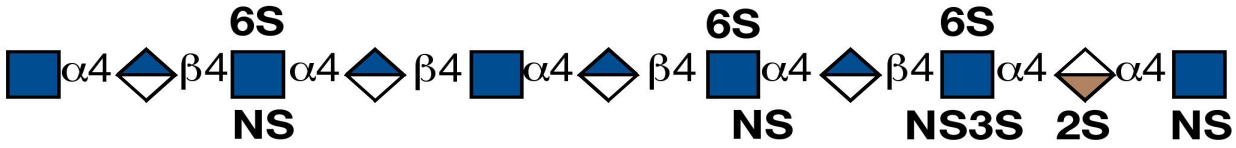
Hyaluronan

GalNAc4Sβ4GlcAβ3GalNAc4Sβ4IdoAα3GalNAc4Sβ4IdoAα3GalNAc4Sβ4IdoA2Sα3GalNAc4Sβ4GlcAβ3GalNAc4S



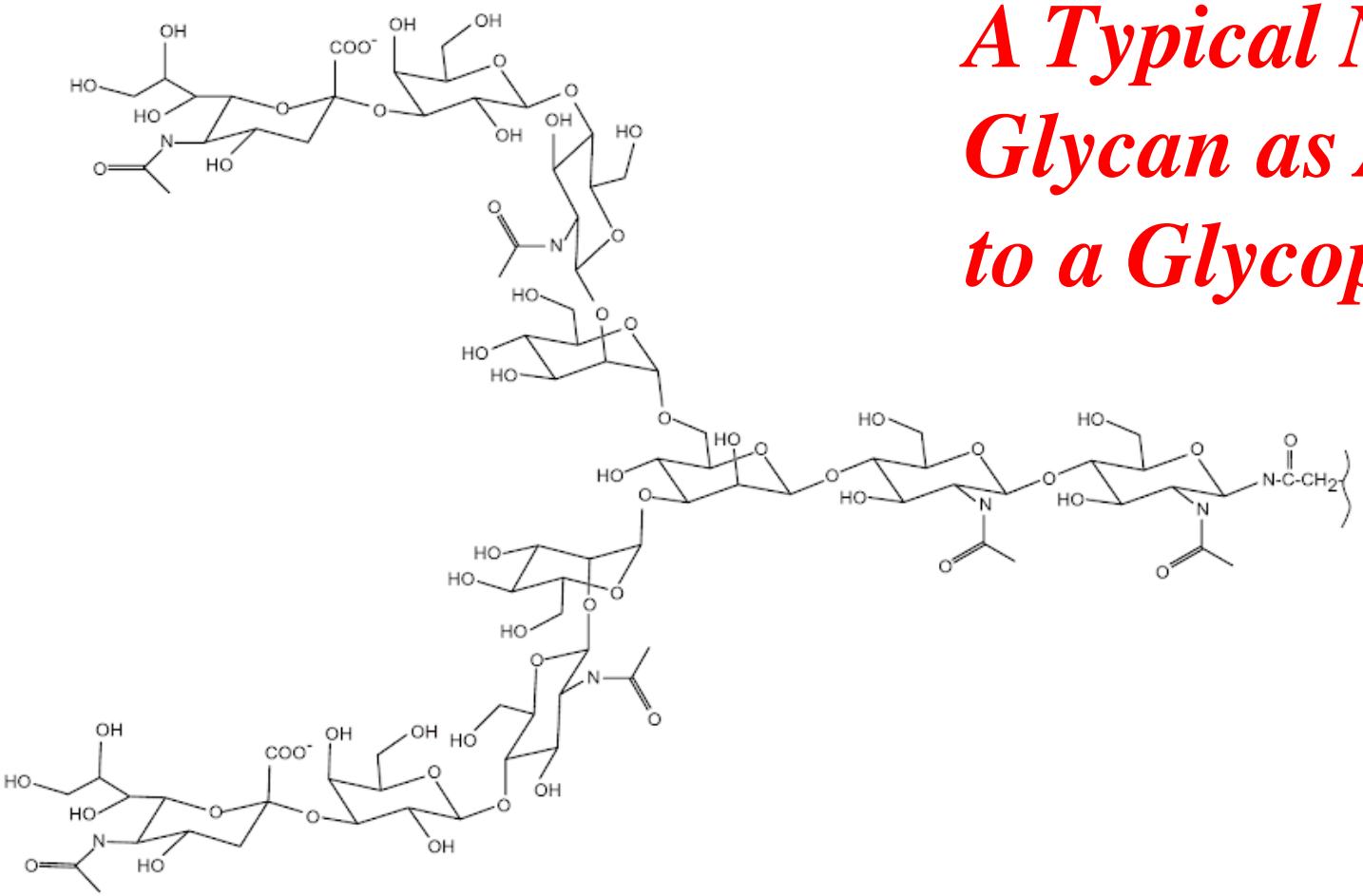
Chondroitin/Dermatan Sulfate

GlcNAcα4GlcAβ4GlcNAcNS6Sα4GlcAβ4GlcNAcα4GlcAβ4GlcNS6Sα4GlcAβ4GlcNS3S6Sα4IdoA2Sα4GlcNS



Heparan Sulfate/Heparin

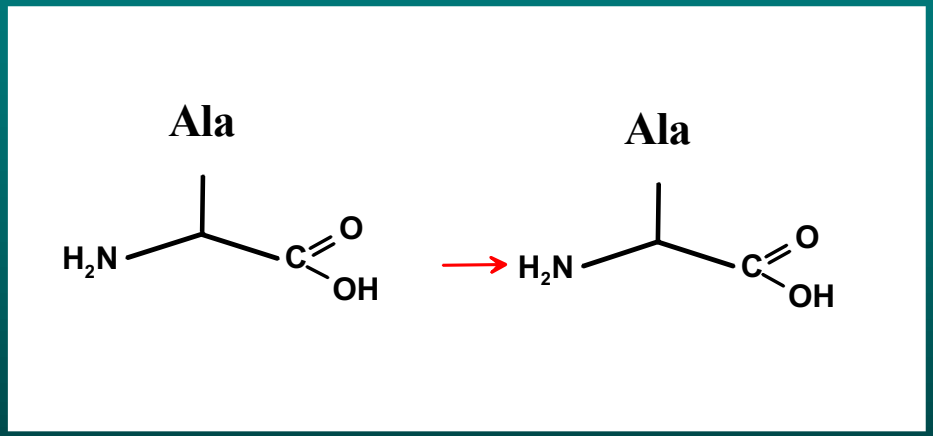
A Typical N-Linked Glycan as Attached to a Glycoprotein

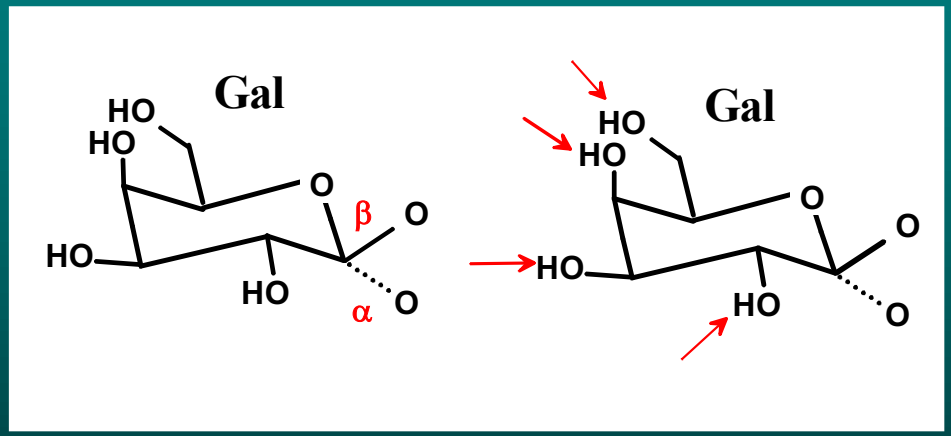


NeuAcα2-3Galβ1-4GlcNAcβ1-2Manα1 \
6 \
3 Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn \
NeuAcα2-3Galβ1-4GlcNAcβ1-2Manα1 /

Oligosaccharides: Molecular Diversity of Glycans

	Polypeptides	Glycans
Building blocks	amino acids	monosaccharides
Number of different monomers	20 common	9 common
Linkage sites per monomer	1	3-4
Possible linkage configurations	1	2
Possible homodimer structures	1	6-8
Linkage modes	linear	linear or branched





Man a1-4 Gal a1-3 Glc b1
Man a1-6 Gal a1-3 Glc b1
Man b1-2 Gal a1-3 Glc b1
Man b1-3 Gal a1-3 Glc b1
Man b1-4 Gal a1-3 Glc b1
Man b1-6 Gal a1-3 Glc b1
Man a1-2 Gal a1-4 Glc b1
Man a1-3 Gal a1-4 Glc b1
Man a1-4 Gal a1-4 Glc b1
Man a1-6 Gal a1-4 Glc b1
Man b1-2 Gal a1-4 Glc b1
Man b1-3 Gal a1-4 Glc b1
Man b1-4 Gal a1-4 Glc b1
Man b1-6 Gal a1-4 Glc b1
Man a1-2 Gal a1-6 Glc b1
Man a1-3 Gal a1-6 Glc b1

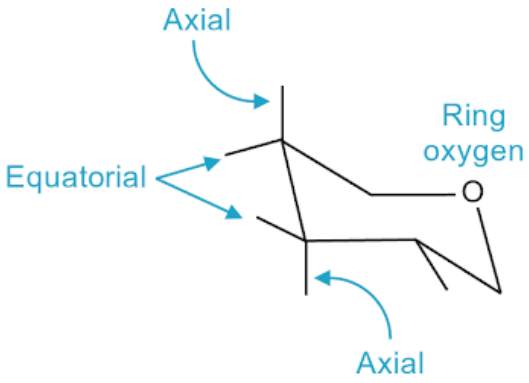
*Glycoproteins and glycolipids may contain ~3000 glycan determinants with an additional ~4000 theoretical pentasaccharide sequences in glycosaminoglycans

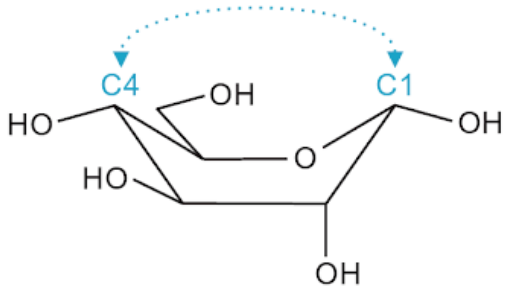
Cummings RD (2009) *Molecular BioSystems* 5, 1087

Types of Biomacromolecules

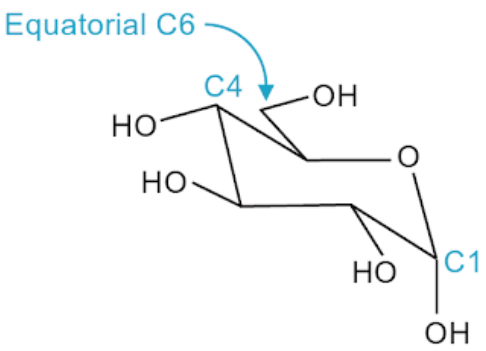
Macromolecule	Building Block	Aproximate Mass	Possible Variations in a Trimer
Protein	Amino acids	125 → 10^4-10^5	6
Nucleic Acid	Nucleotides	330 → 10^3-10^9	6
Lipid	Fatty acids	250 → 10^3	NA
Carbohydrate	Monosaccharides	200 → 10^2-10^6	1,056 to 27,648!

Monosaccharide Conformations

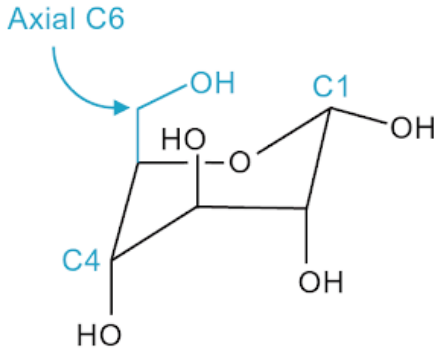




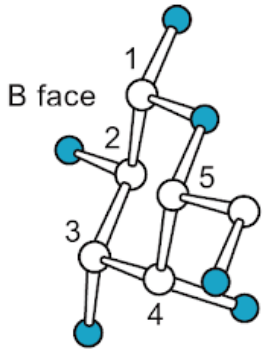
Boat conformation



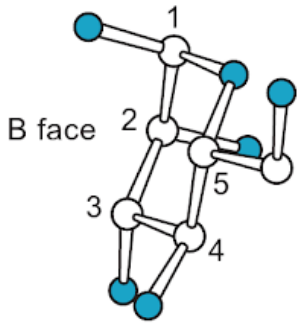
$^4C_1$ chair conformation



$^1C_4$ chair conformation



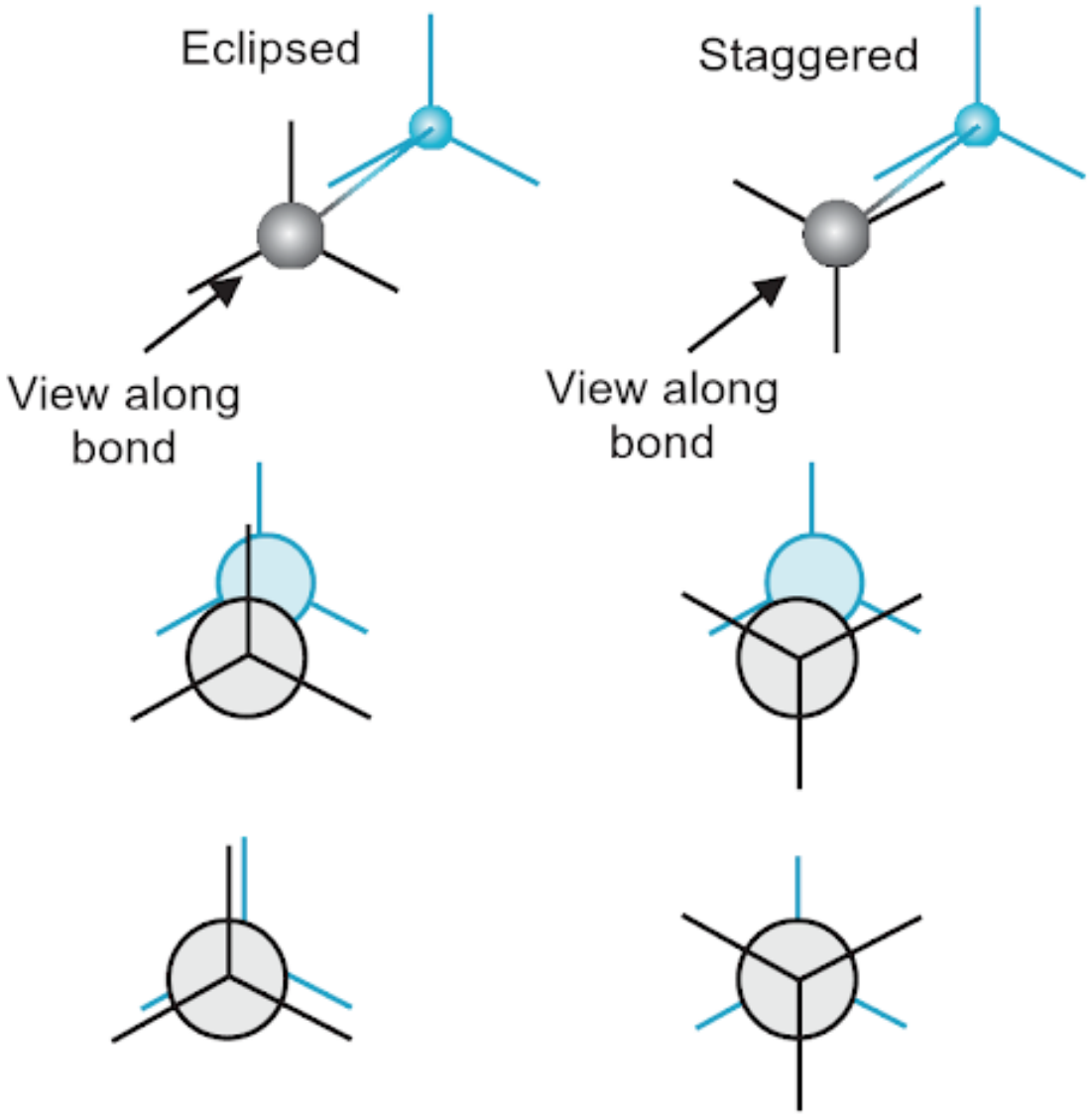
β-Gal

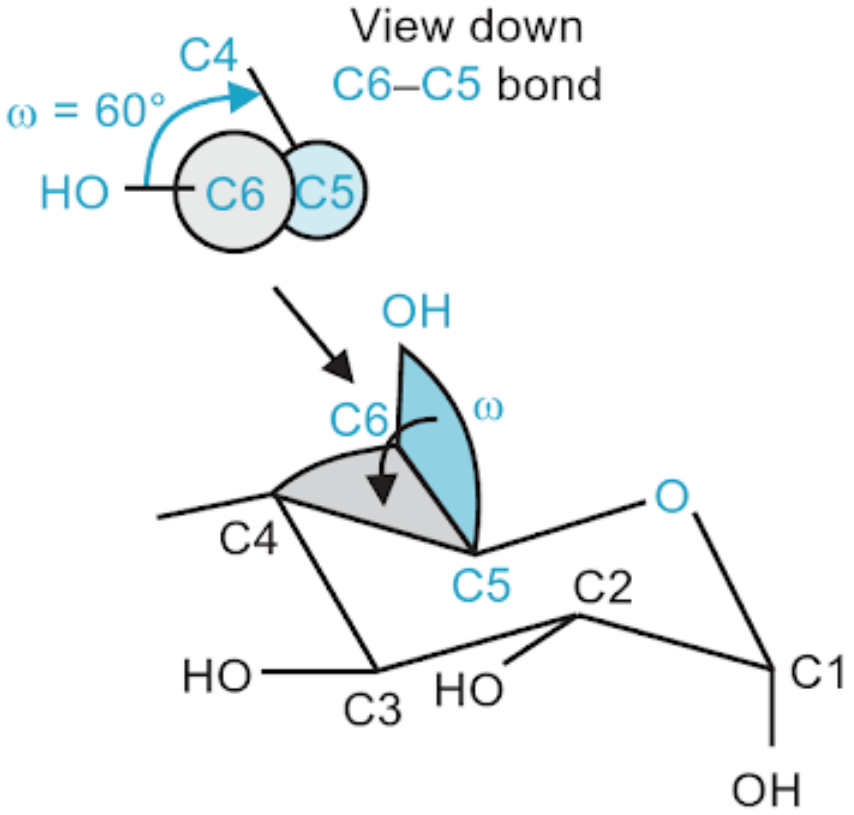


α-Man

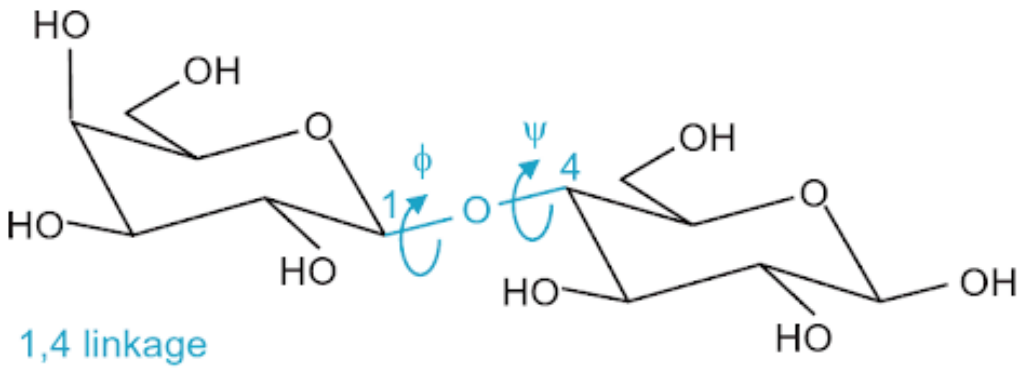
- **crowding between H's at C1 and C4 destabilize the boat**
- **hydroxyls at C2, C3 and C5 prefer to be equatorial**
- **the β face is the one where carbons are numbered in an anticlockwise arrangement**

Newman Projections & Glycan Torsional Angles

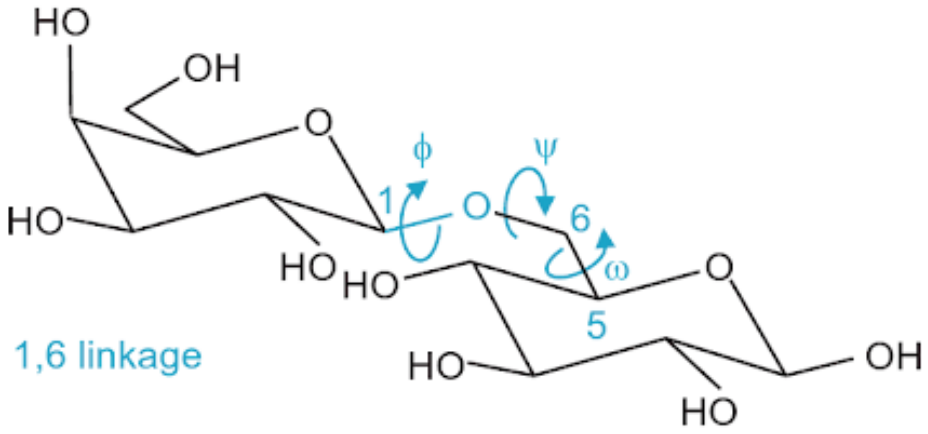




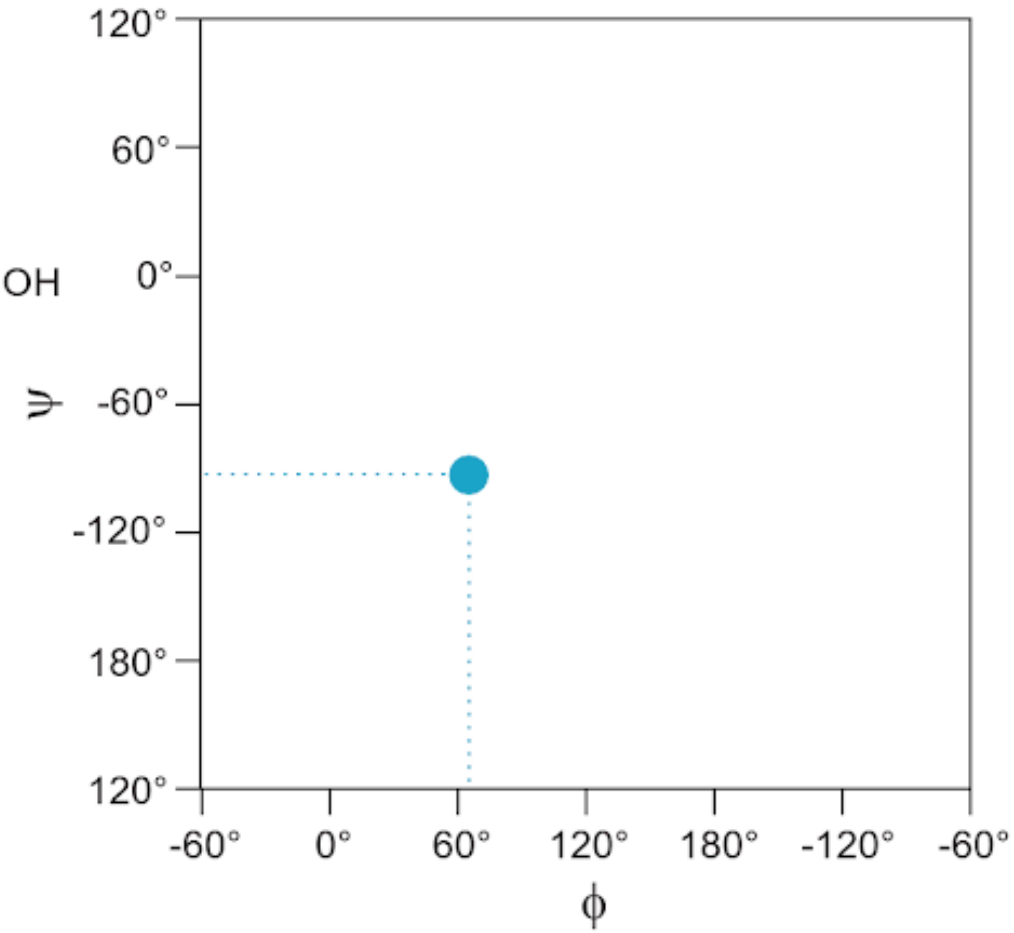
Glycosidic Linkages & Phi-Psi Plots



1,4 linkage

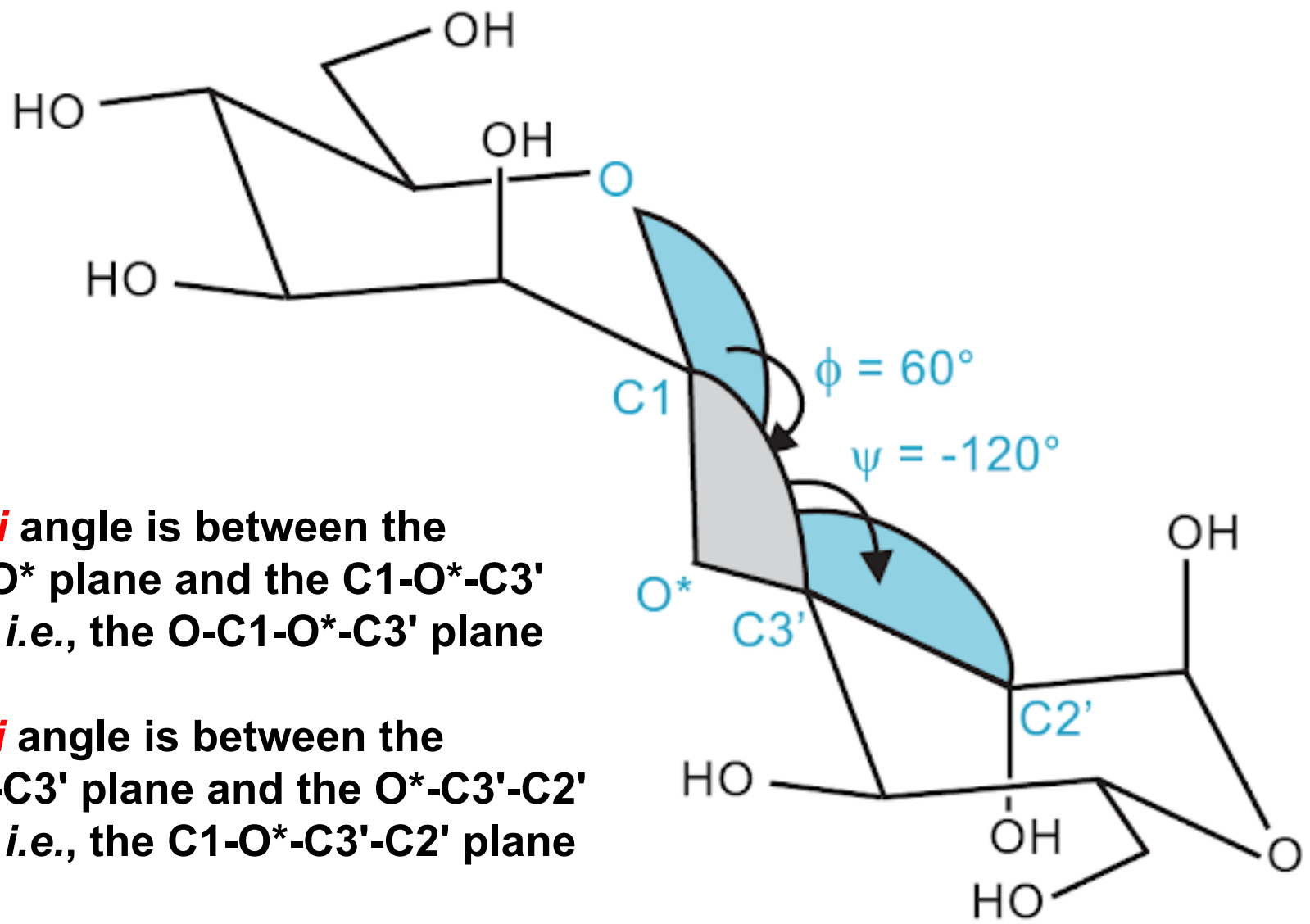


1,6 linkage



- an additional ω angle is required to describe a 1-6 linkage at the reducing monosaccharide

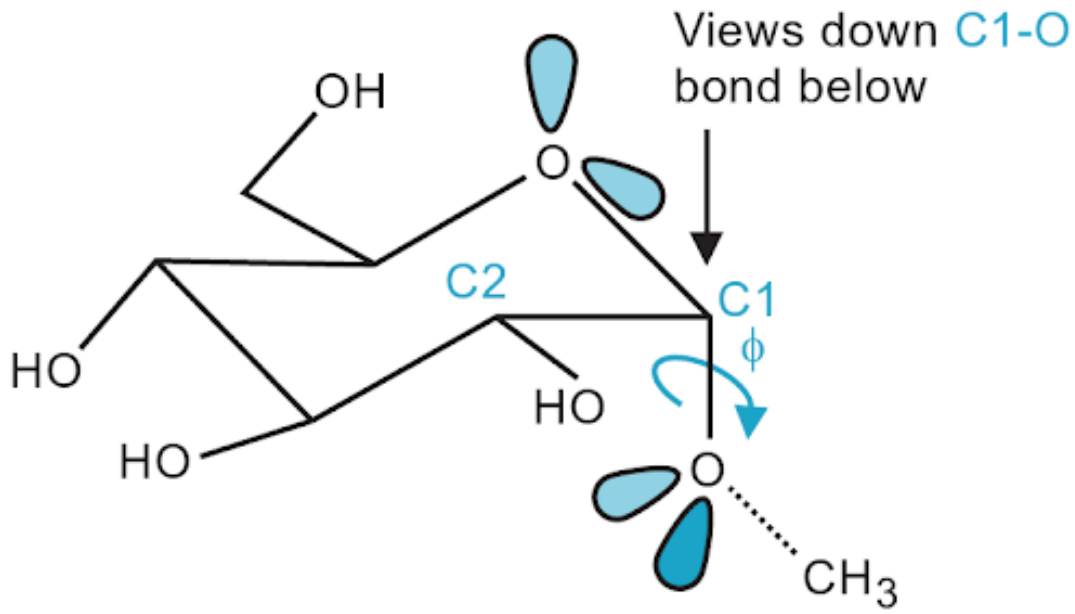
Definition of Linkage Torsion Angles

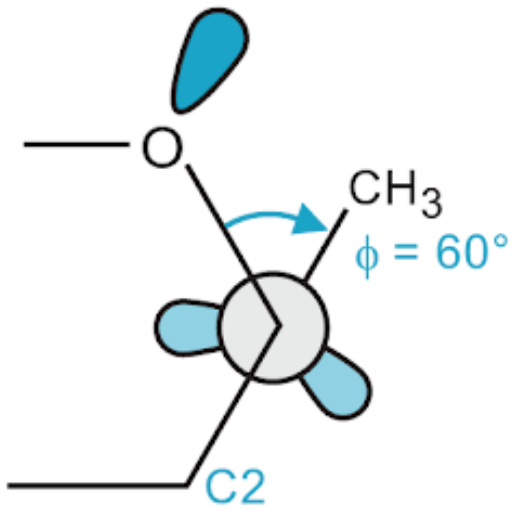


- the ***phi*** angle is between the O-C1-O* plane and the C1-O*-C3' plane, *i.e.*, the O-C1-O*-C3' plane

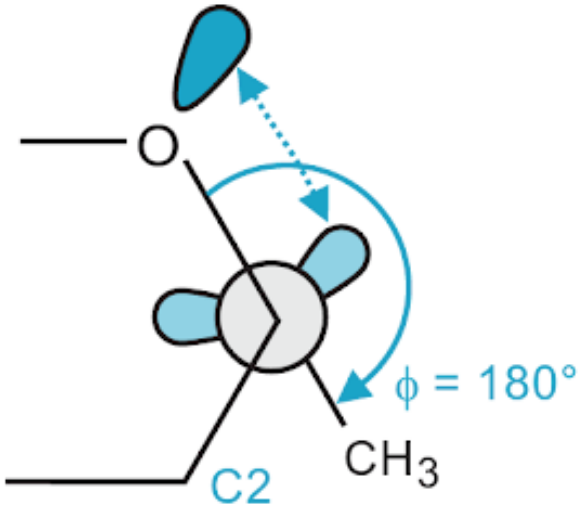
- the ***psi*** angle is between the C1-O*-C3' plane and the O*-C3'-C2' plane, *i.e.*, the C1-O*-C3'-C2' plane

Anomeric & Steric Effects for an α-Linkage

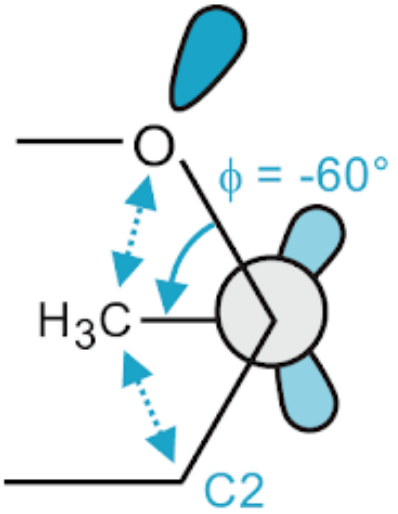




Favoured conformation

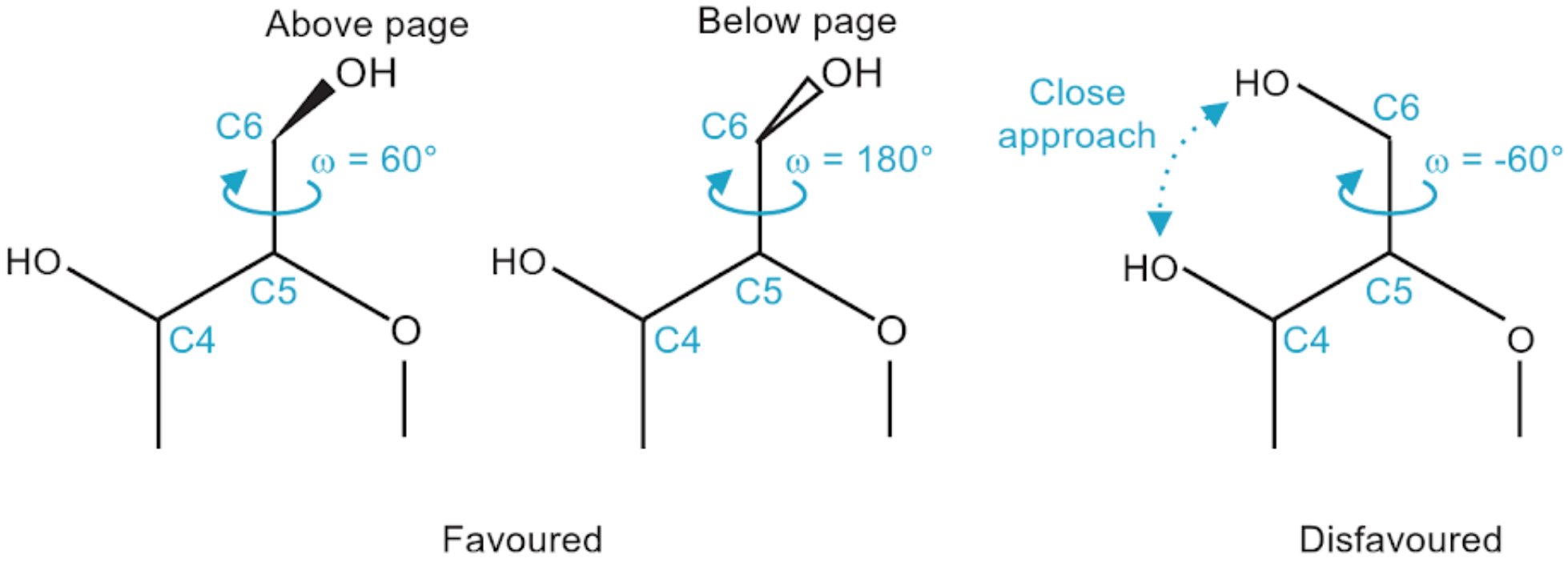


Close approach of orbitals



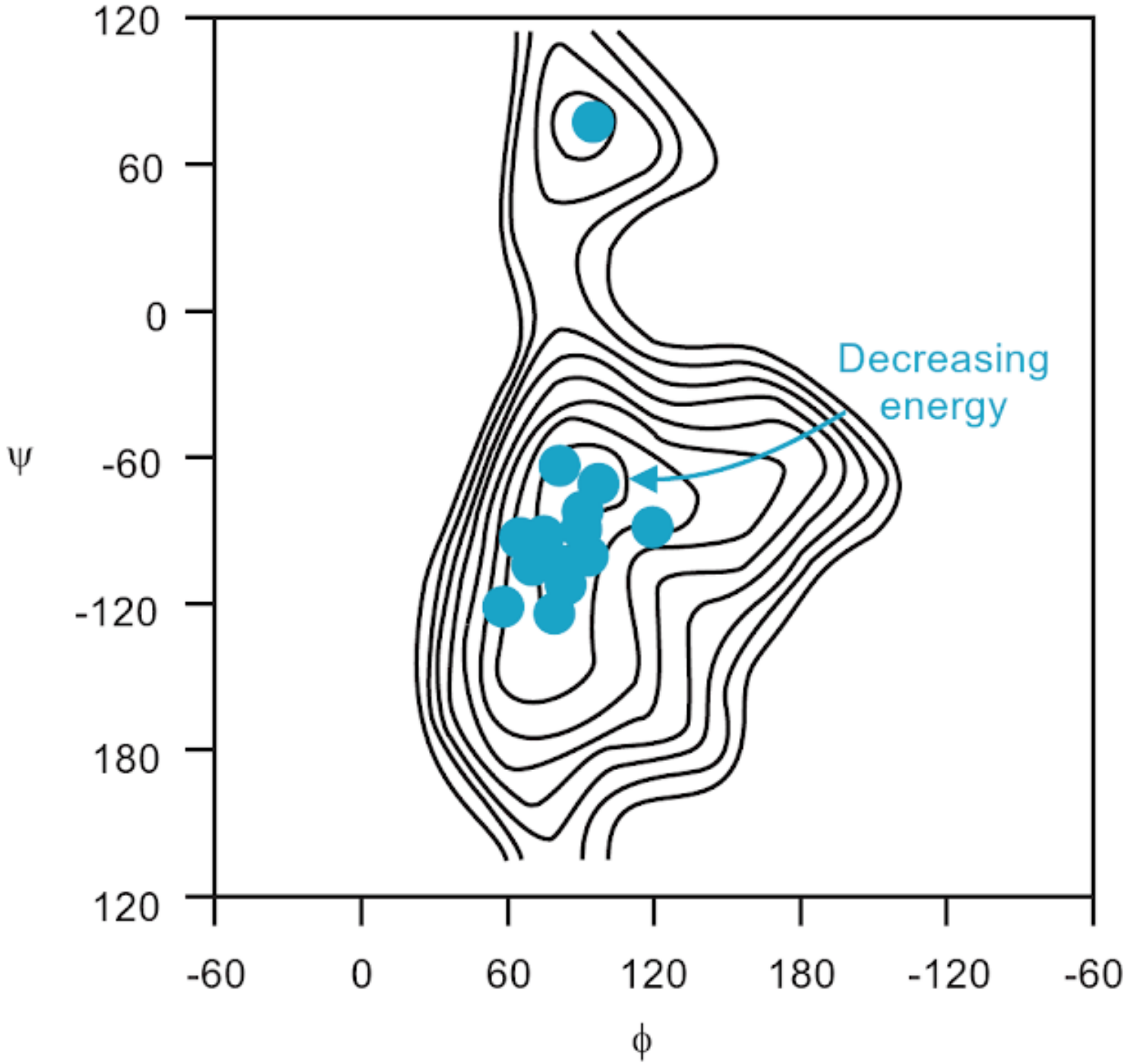
Steric crowding

Conformational Constraint on the ω Angle

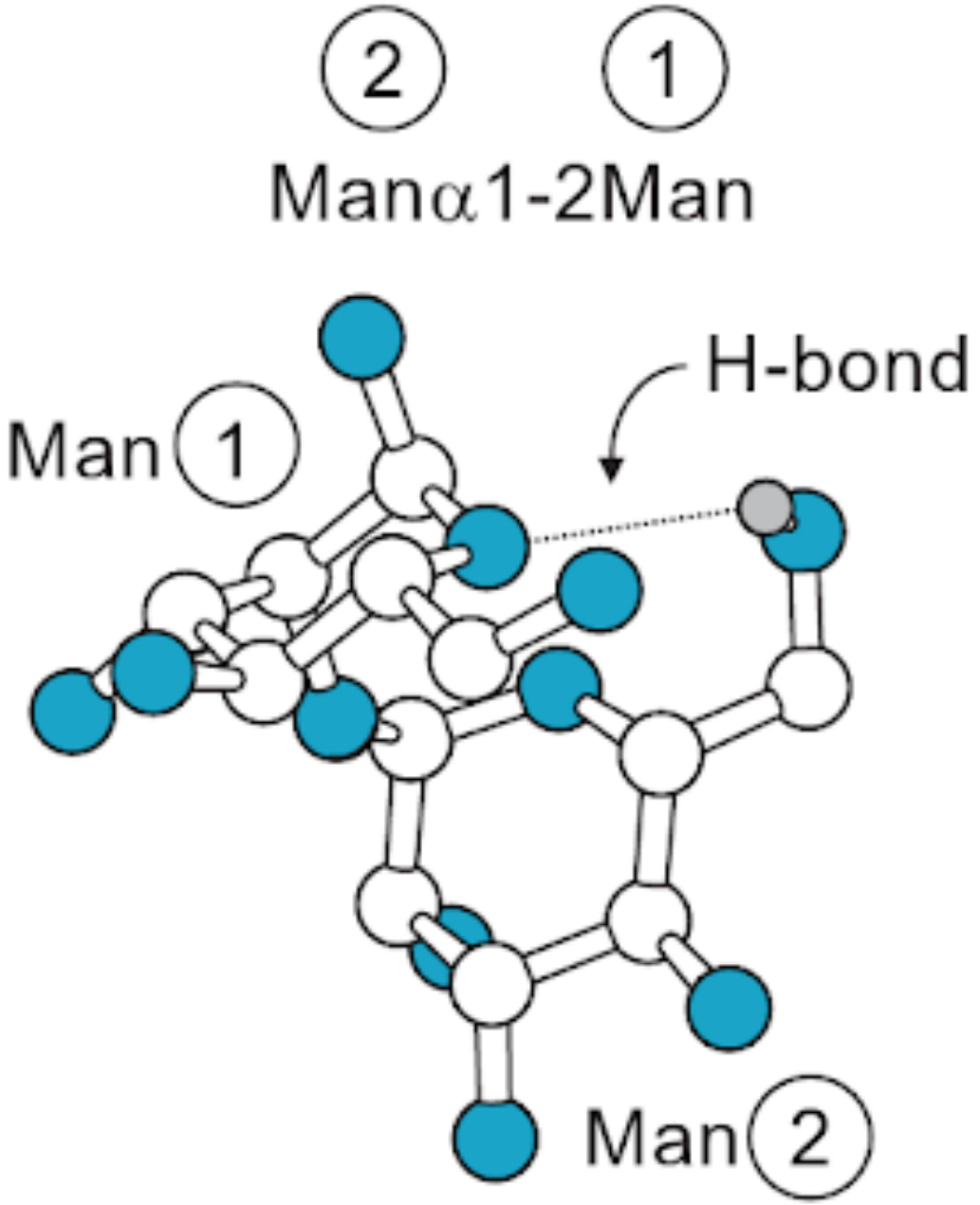


- **the ω = -60 angle is disfavored due to steric clash between hydroxyl groups at C4 and C6**

Energy Contour Map for Manα1-3Man



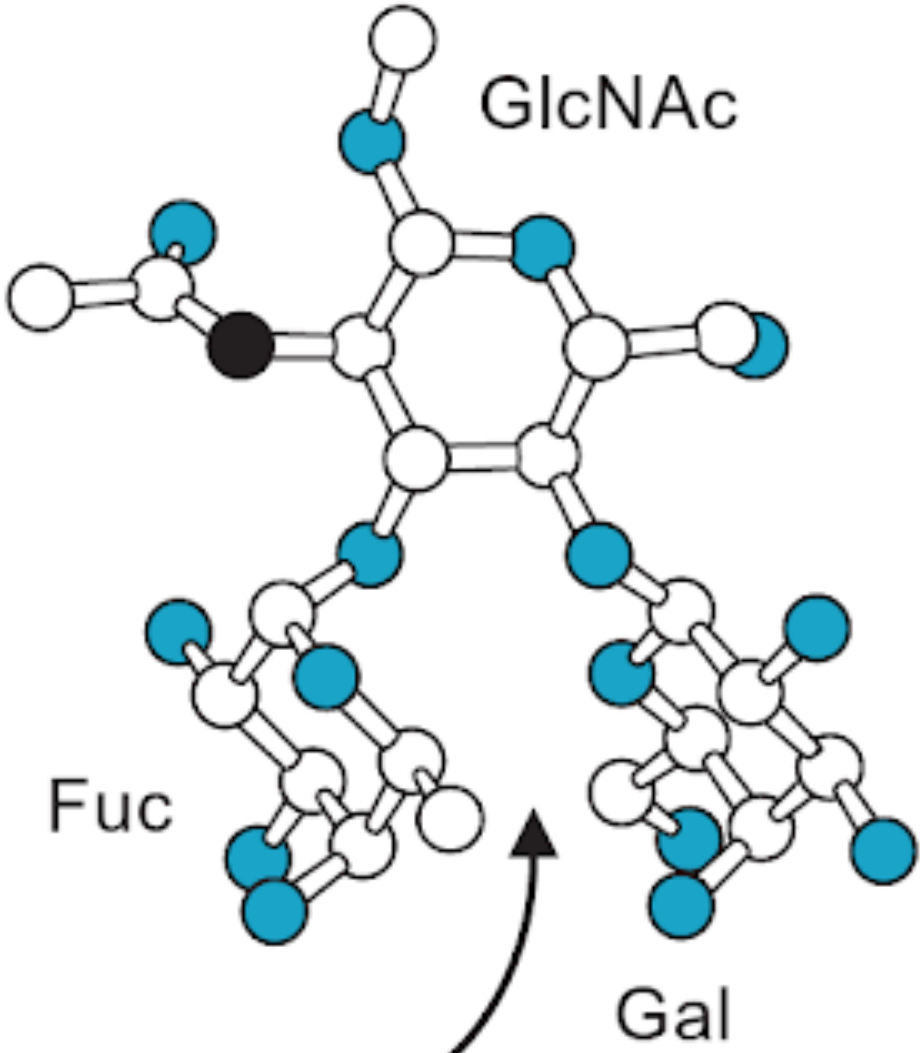
H-Bond Stabilization of a Manα1-2Man Structure



Hydrophobic Packing between Apolar Faces

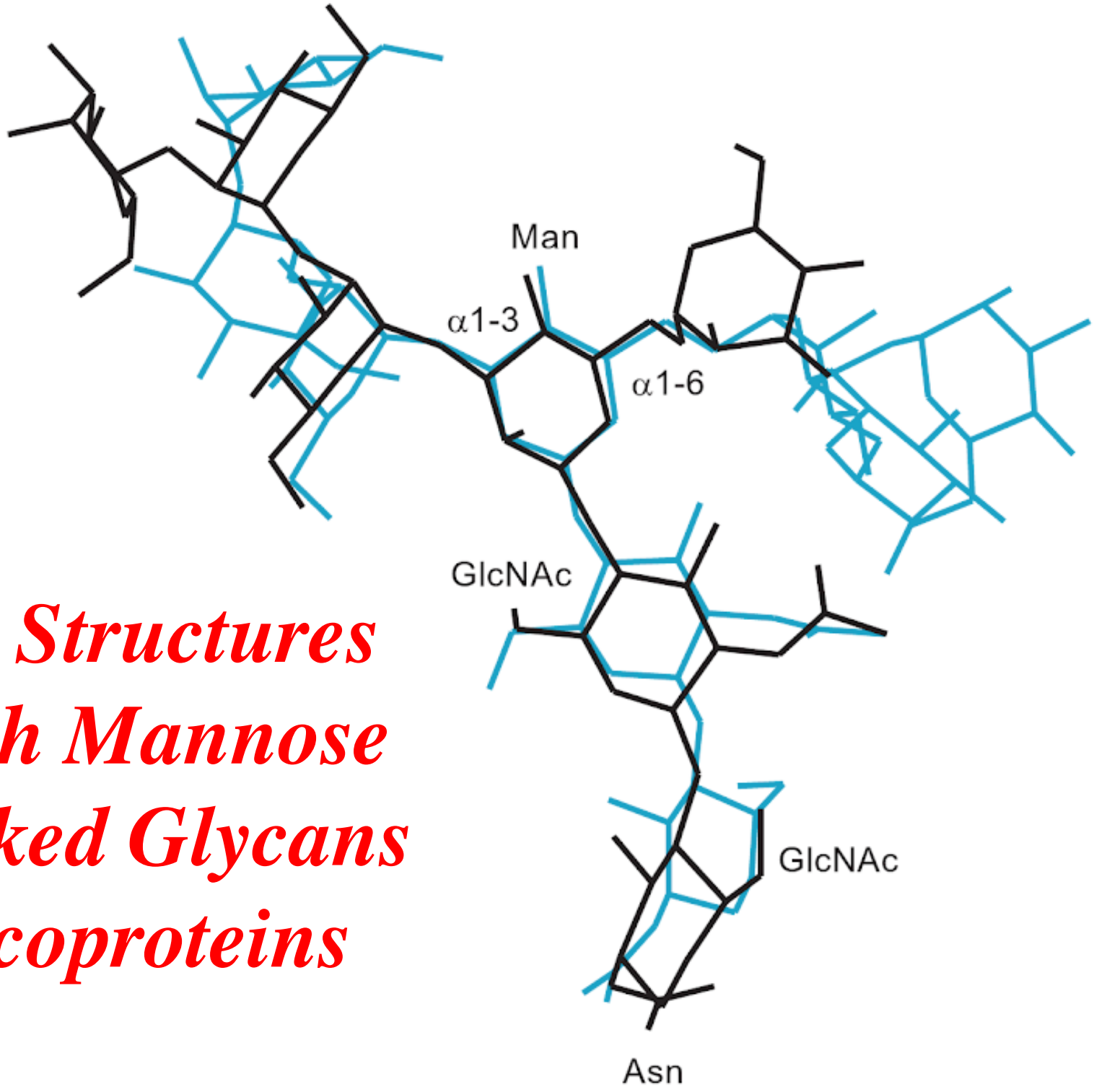
Lewis^{x} trisaccharide

Galβ1 — 4 GlcNAc
Fucα1 — 3 GlcNAc



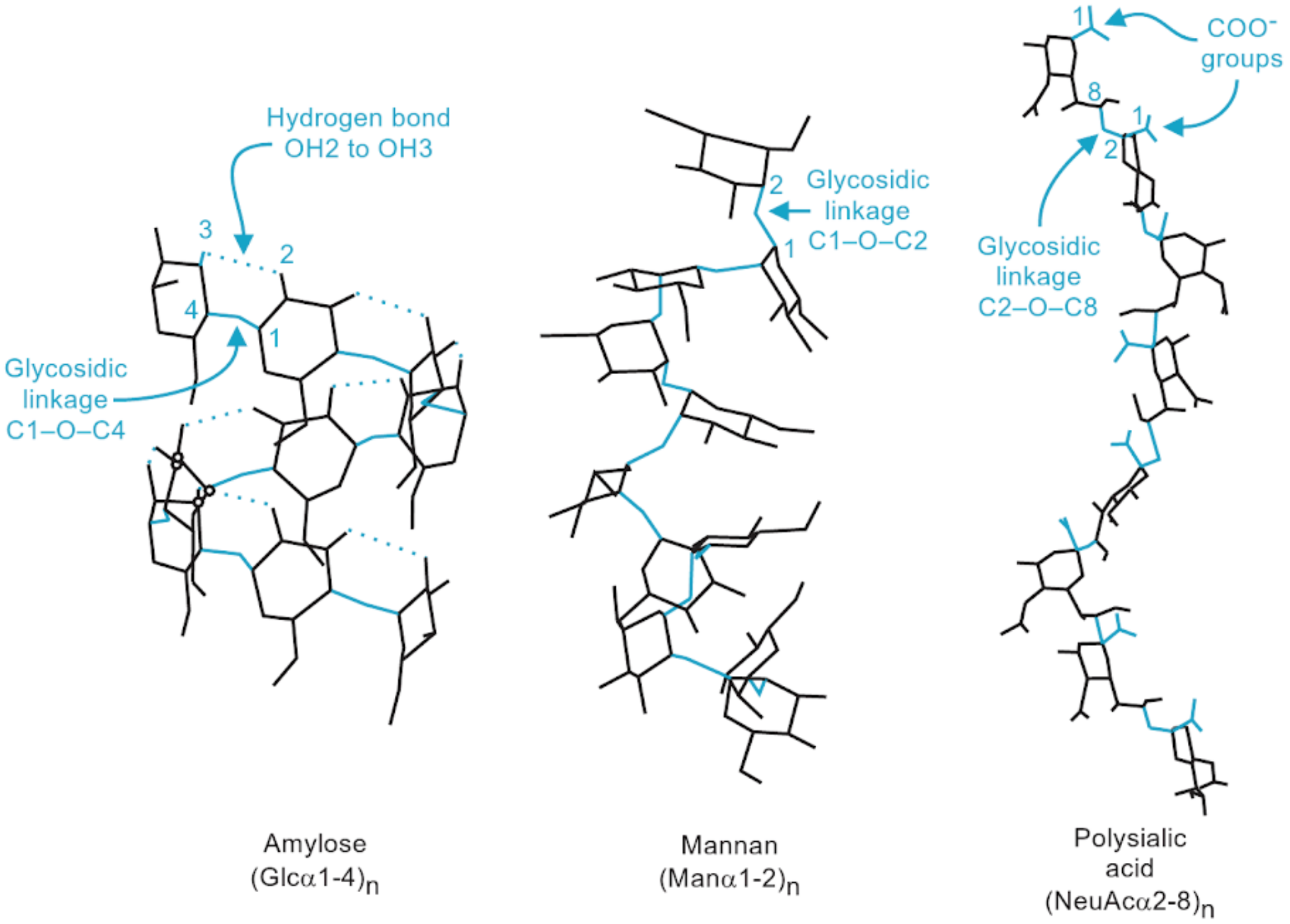
van der Waals
packing of planar faces

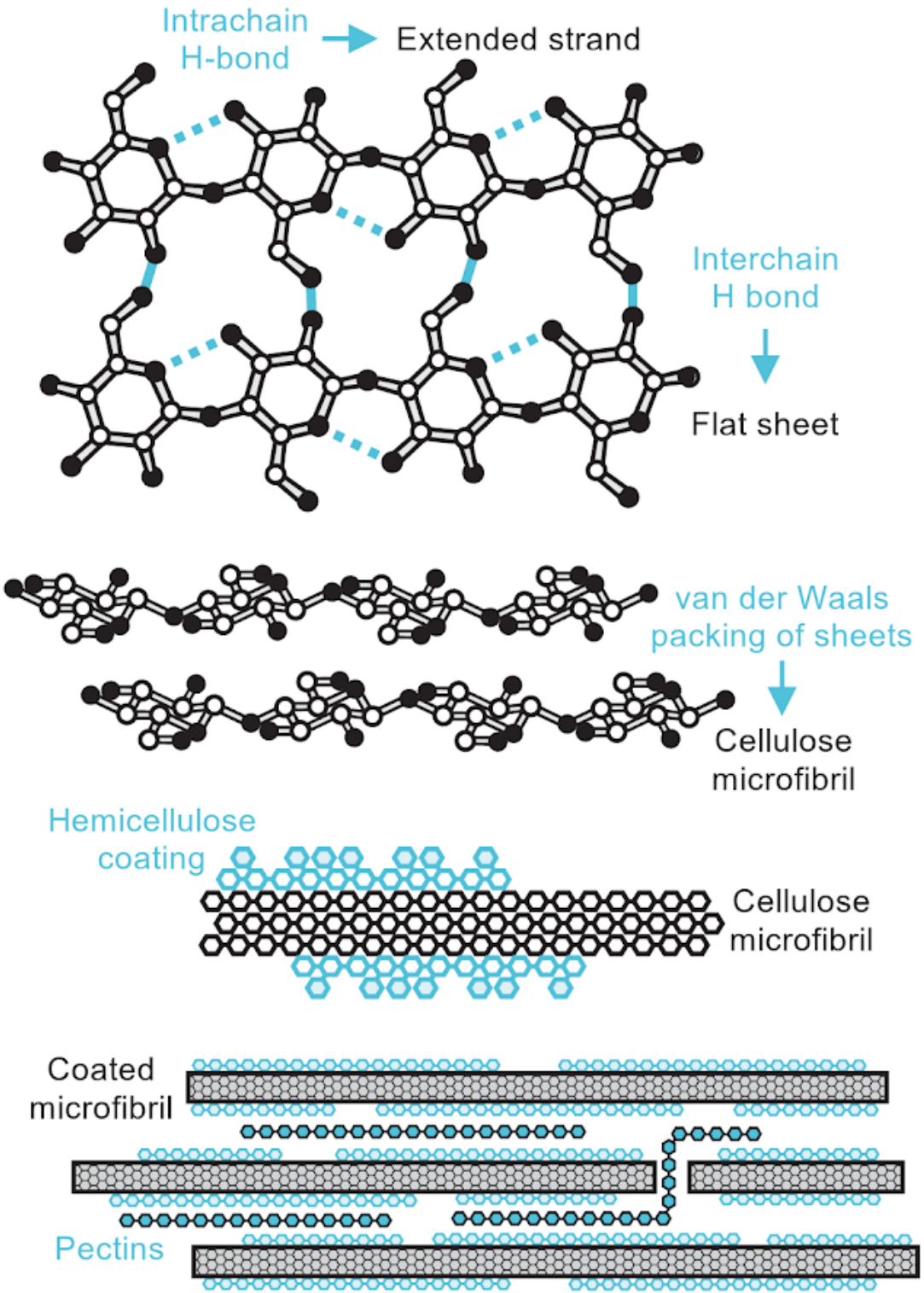
PDB: 2KMB



X-Ray Structures of High Mannose N-Linked Glycans in Glycoproteins

Helices in Polysaccharide Homopolymers

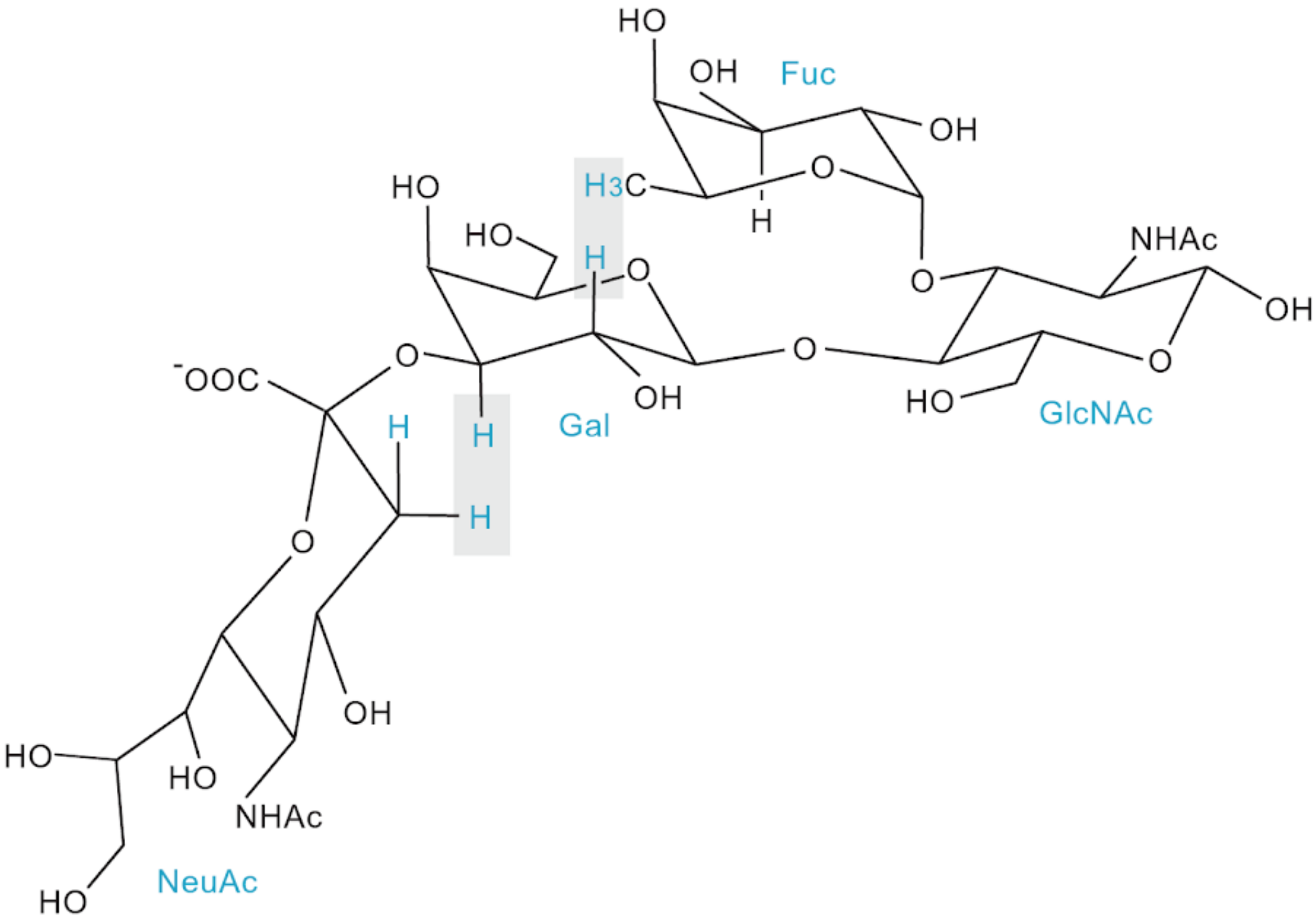




Extended Conformations of Polysaccharides with β-Linked Residues found in many Plant Cell Walls

- examples include Cellulose, Pectins & Hemicellulose

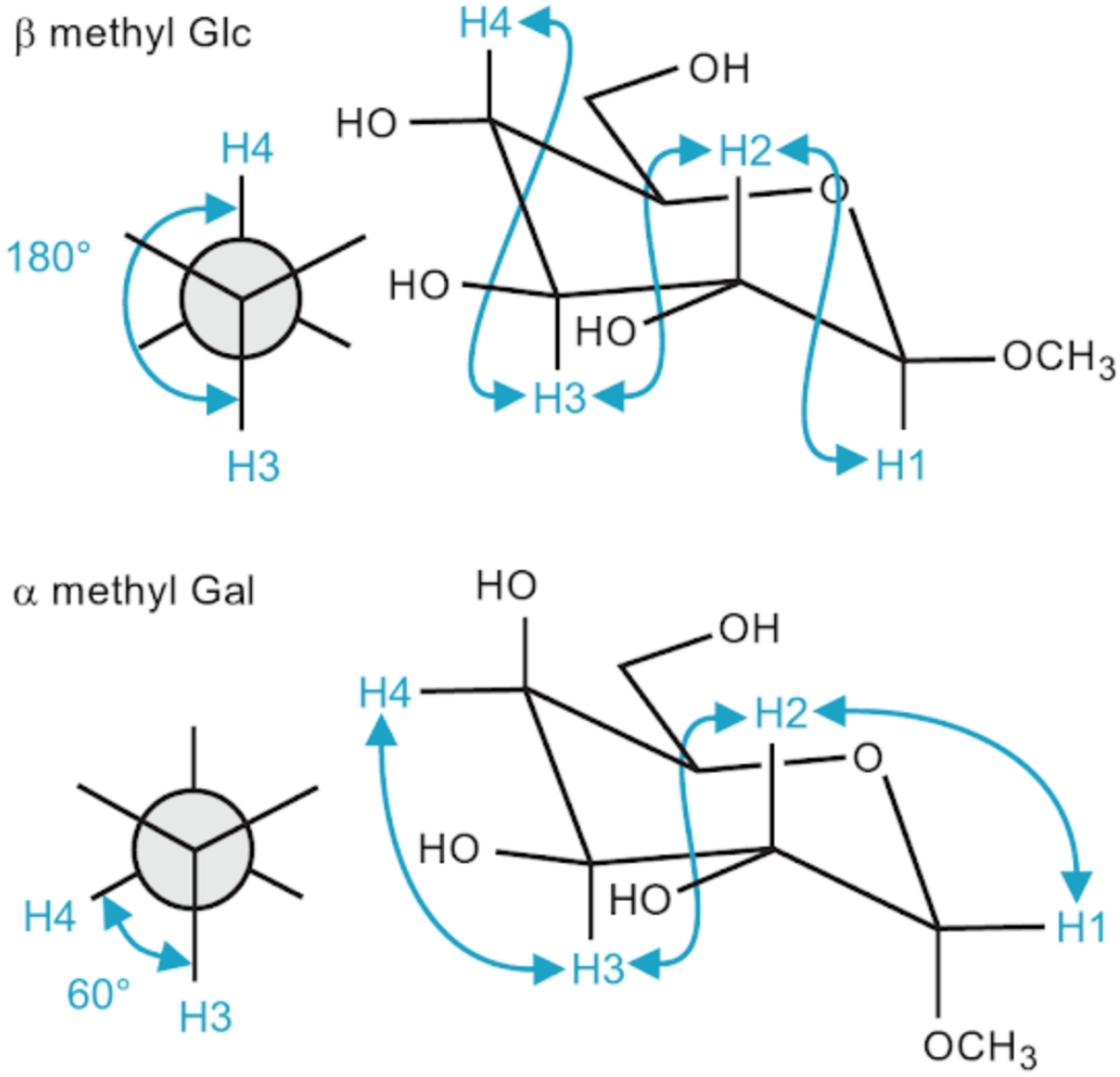
NMR NOEs Establish Inter-Proton Distances



NMR Coupling Constants Set Torsional Values

***trans* = large coupling constant**

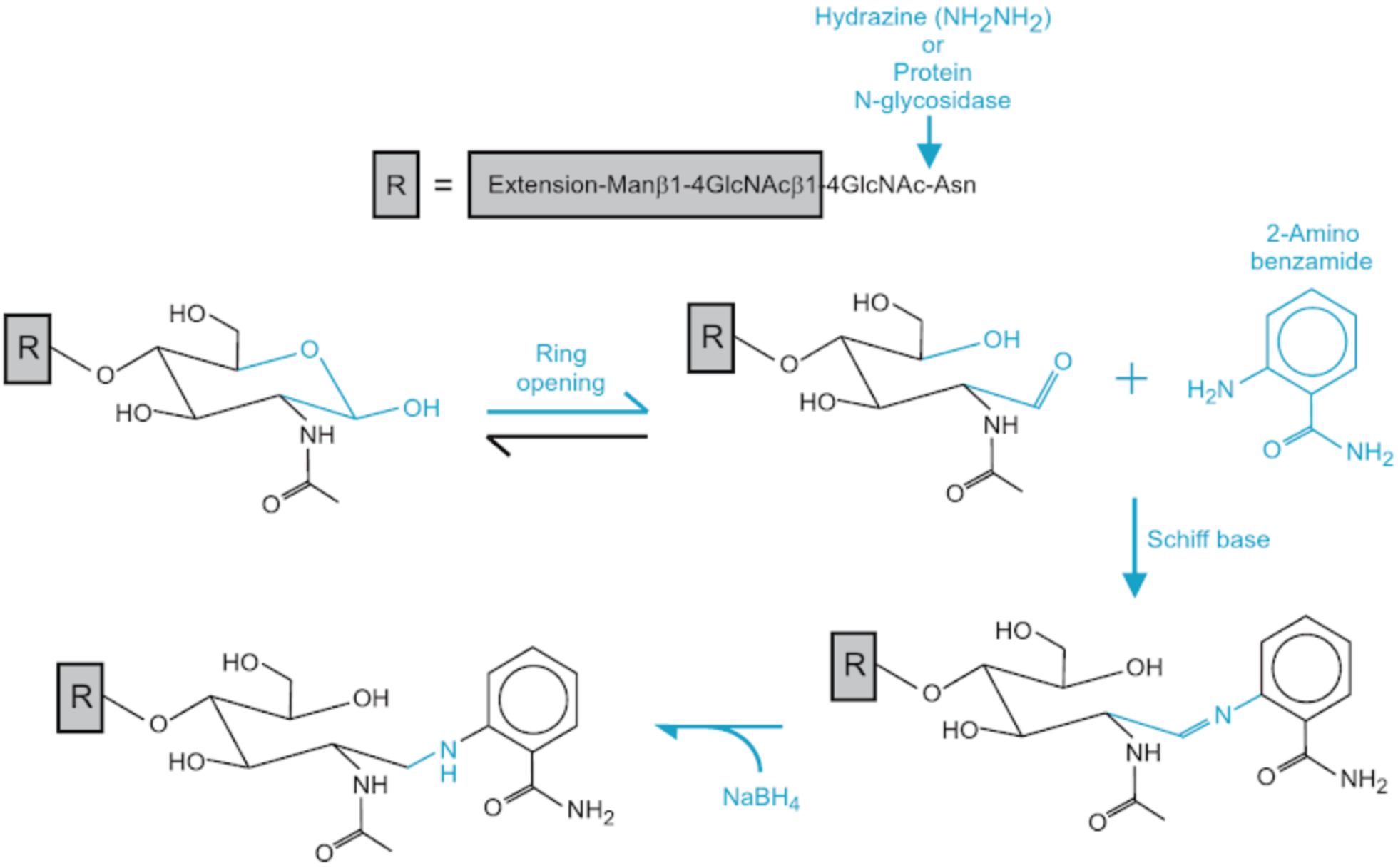
***gauche* = small coupling constant**



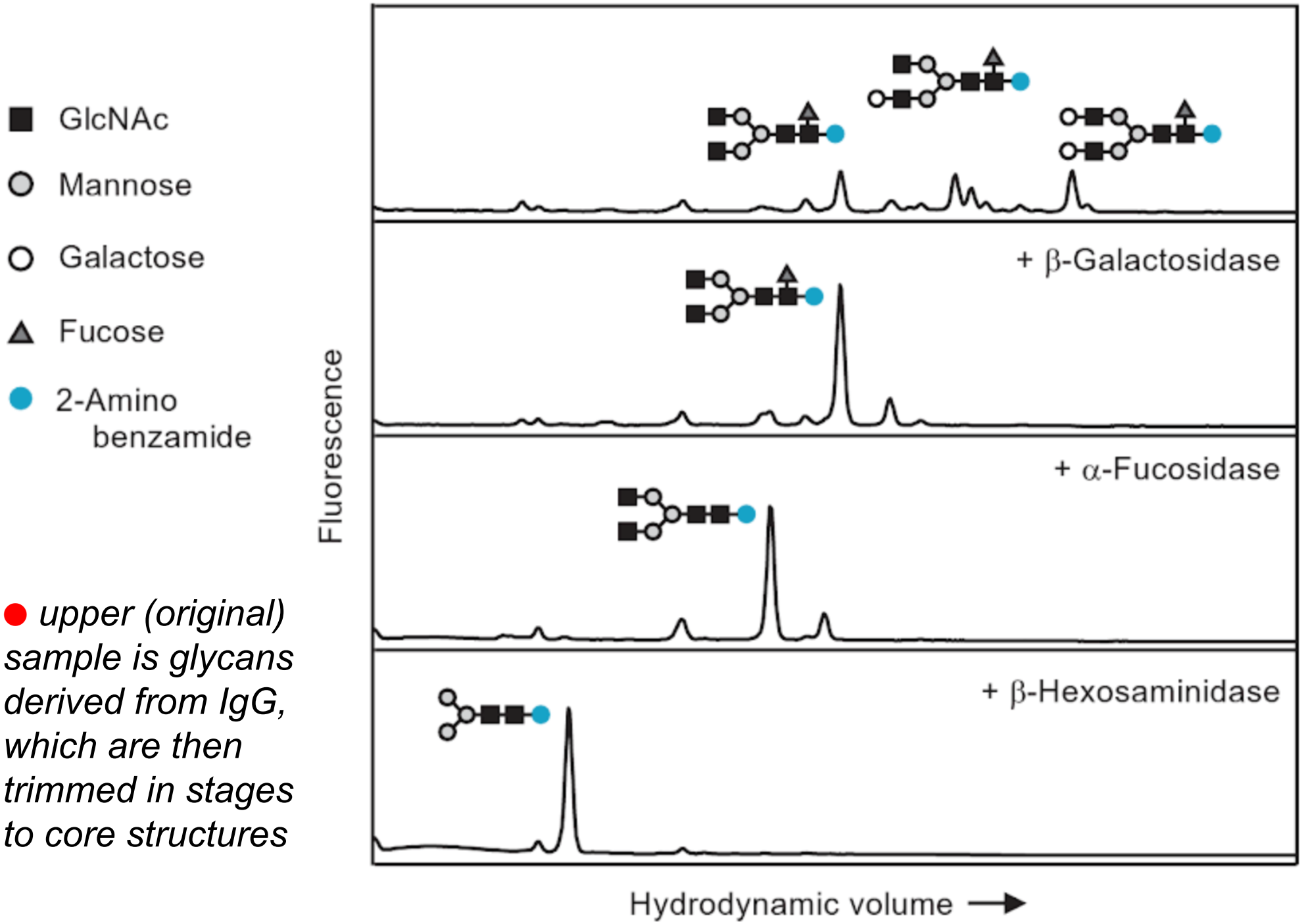
H2-H3-H4-H5 coupling identifies monosaccharide stereochemistry

H1-H2 coupling defines anomeric configuration

Release & Labelling of N-Linked Glycans



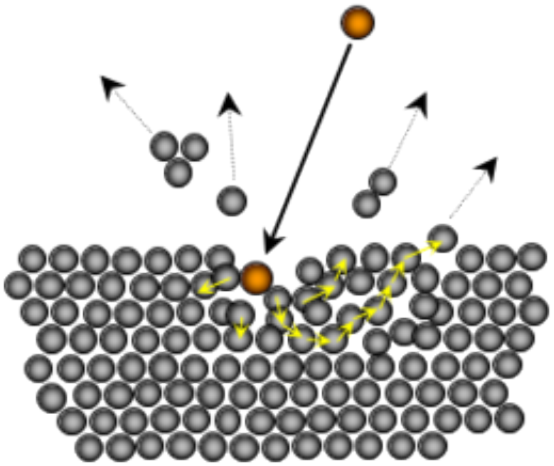
Sequential Enzyme Digestion of End-Labelled Glycan



● *upper (original) sample is glycans derived from IgG, which are then trimmed in stages to core structures*

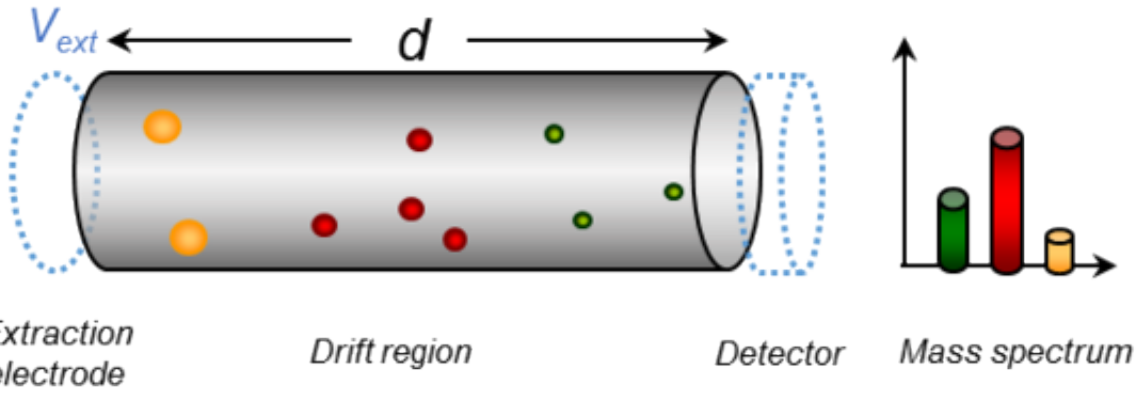
How Mass Spectrometry Works...

Collision Cascade



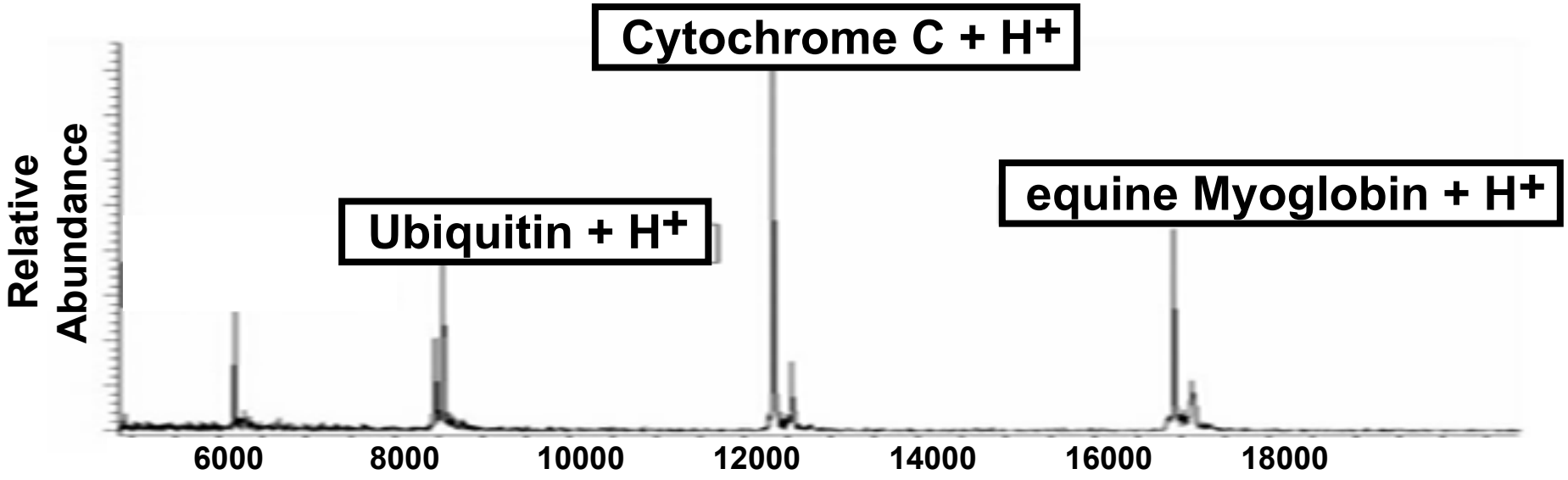
MALDI

Time-of-Flight Mass Analyzer

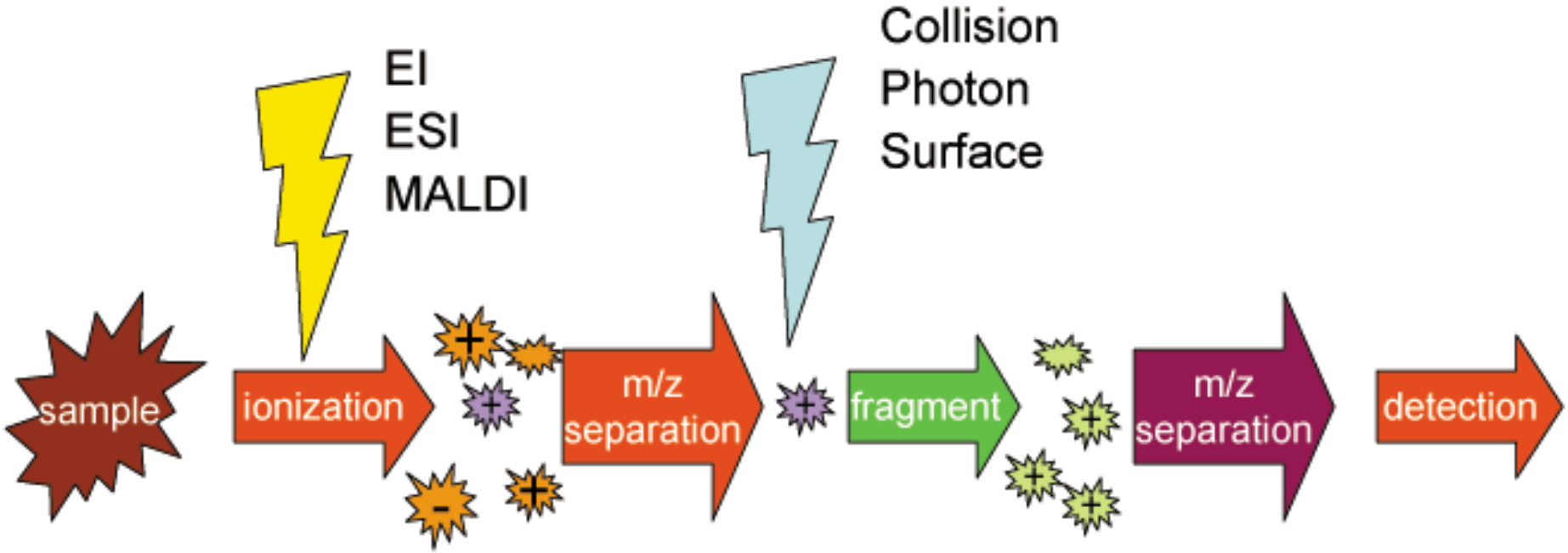


$$E = qV_{ext} = \frac{1}{2}mv^2$$

$$t = d\left(\frac{m}{2qV}\right)^{1/2} \propto m^{1/2}$$



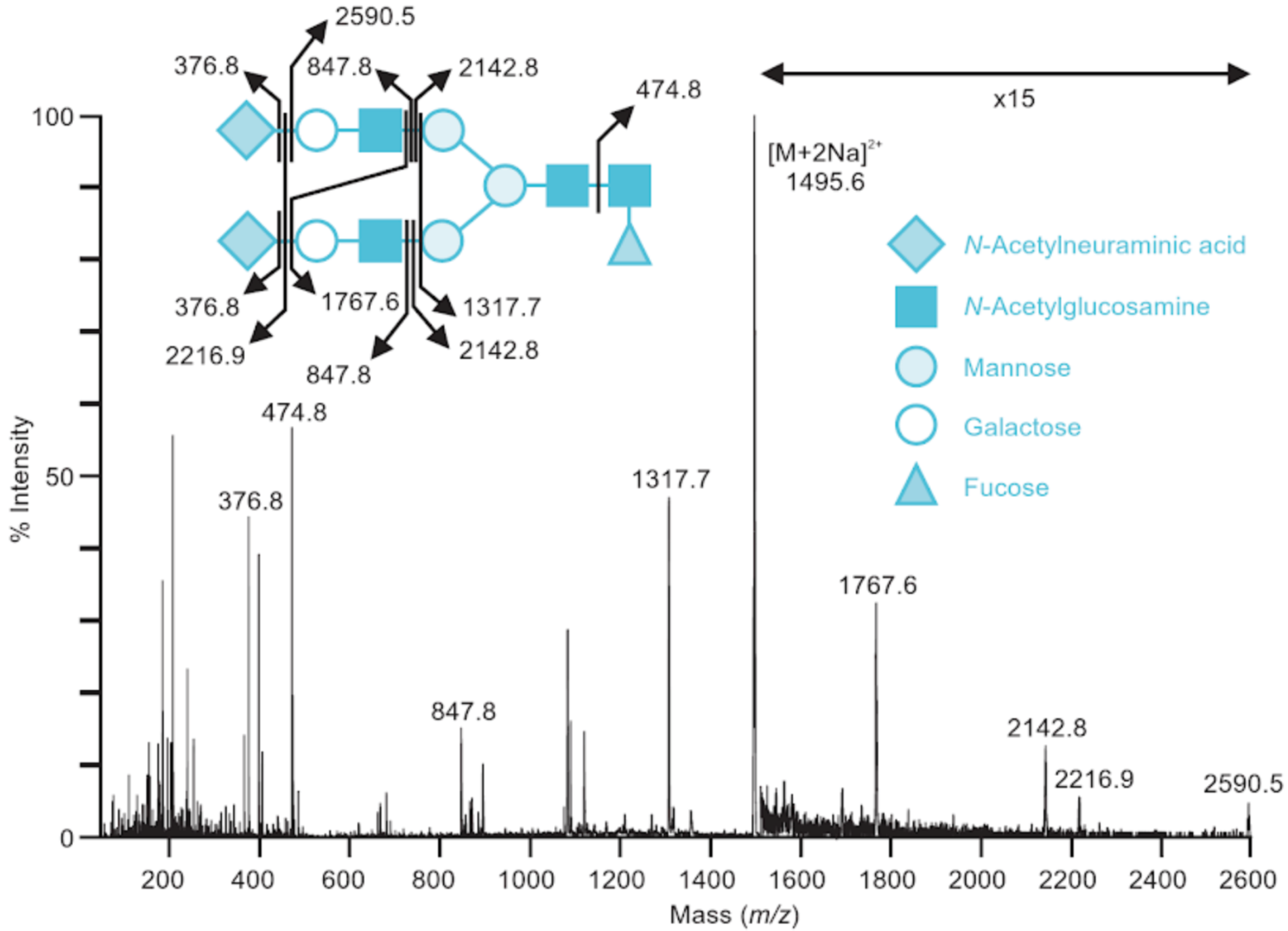
Tandem Mass Spectrometry



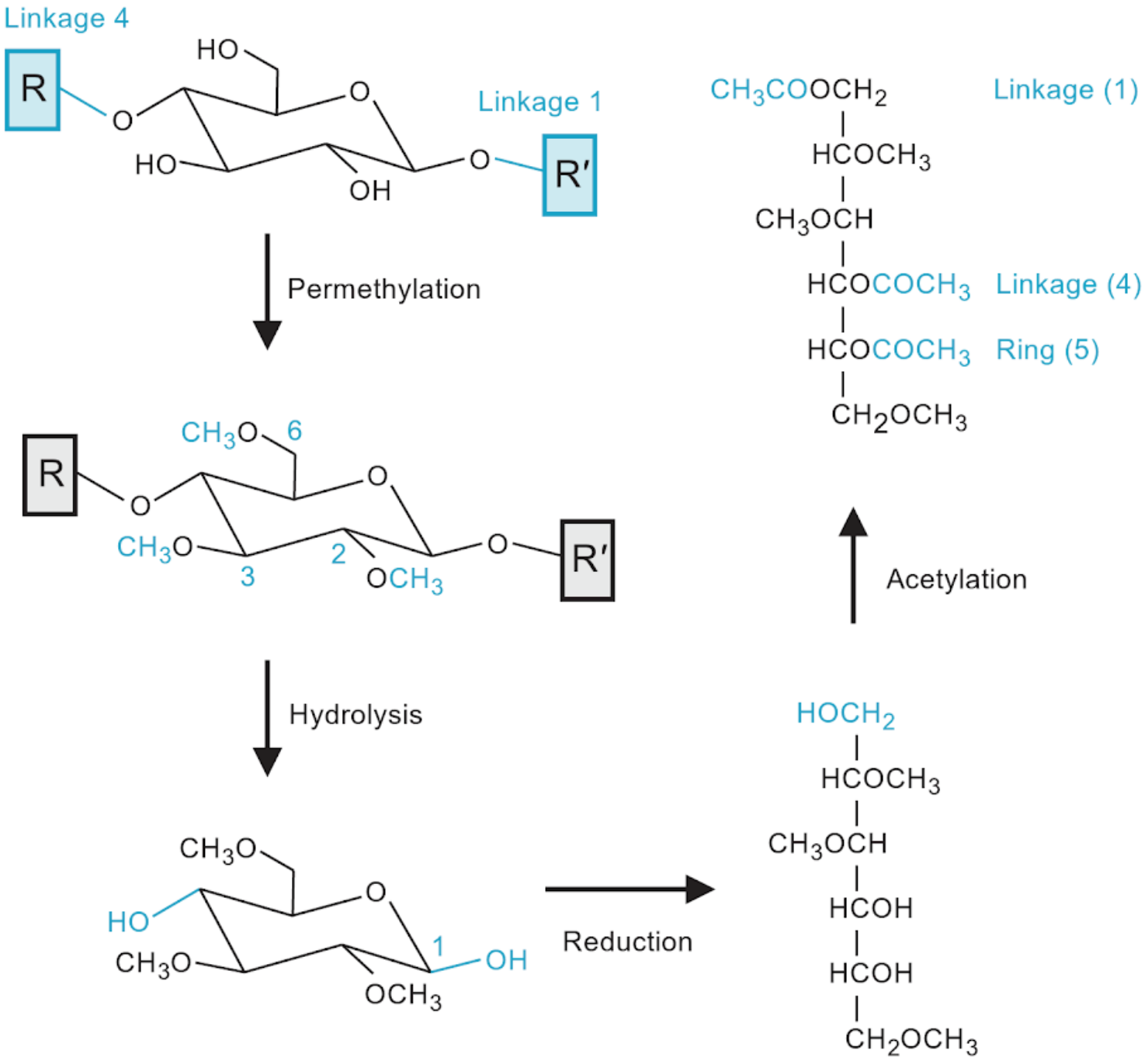
quadrupole time-of-flight hybrid tandem MS

- **the two mass spec stages can be separated in space (triple quad) or in time (*via* an ion trap)**

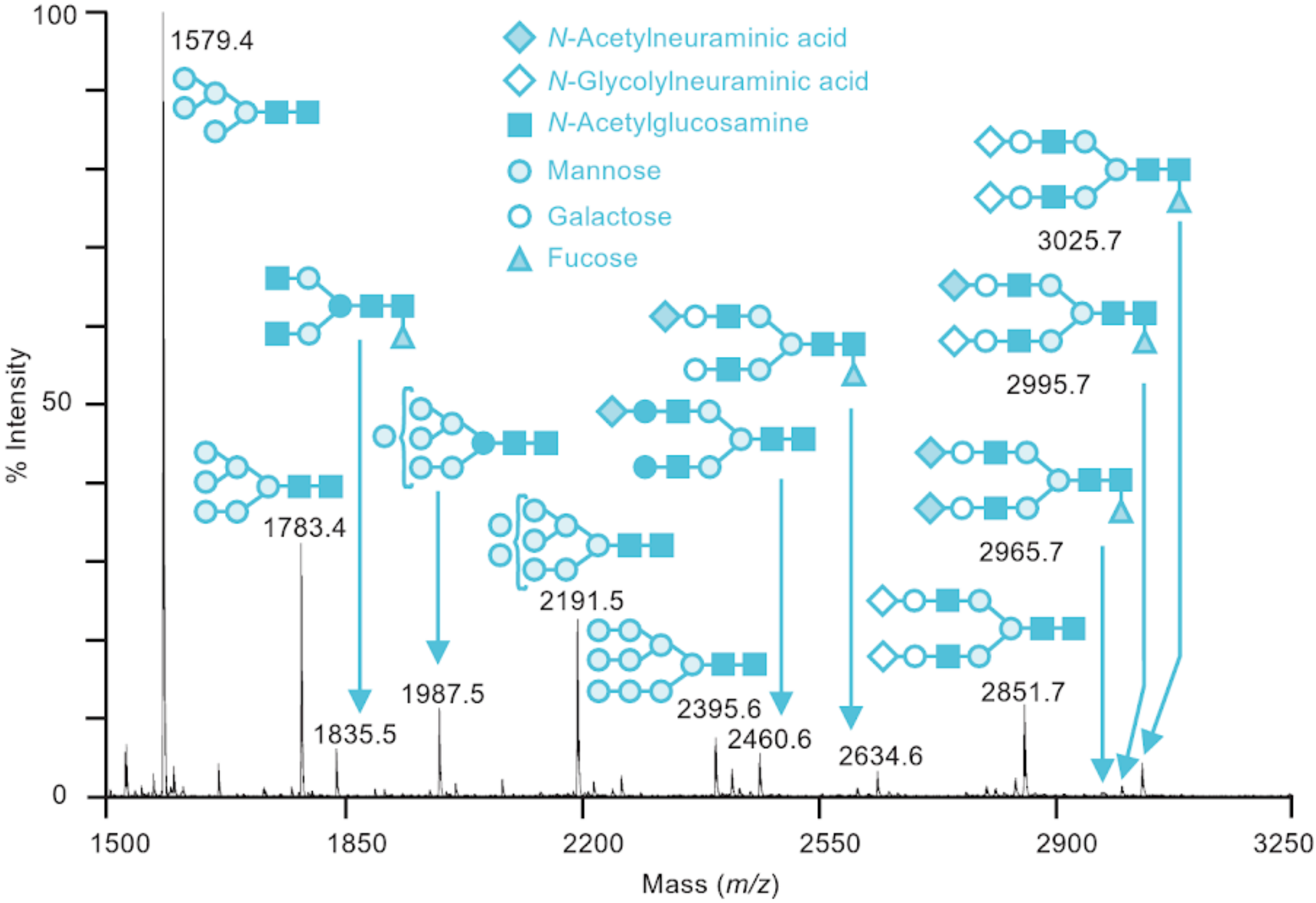
(Re)fragmentation of a Glycan by MS/MS Analysis



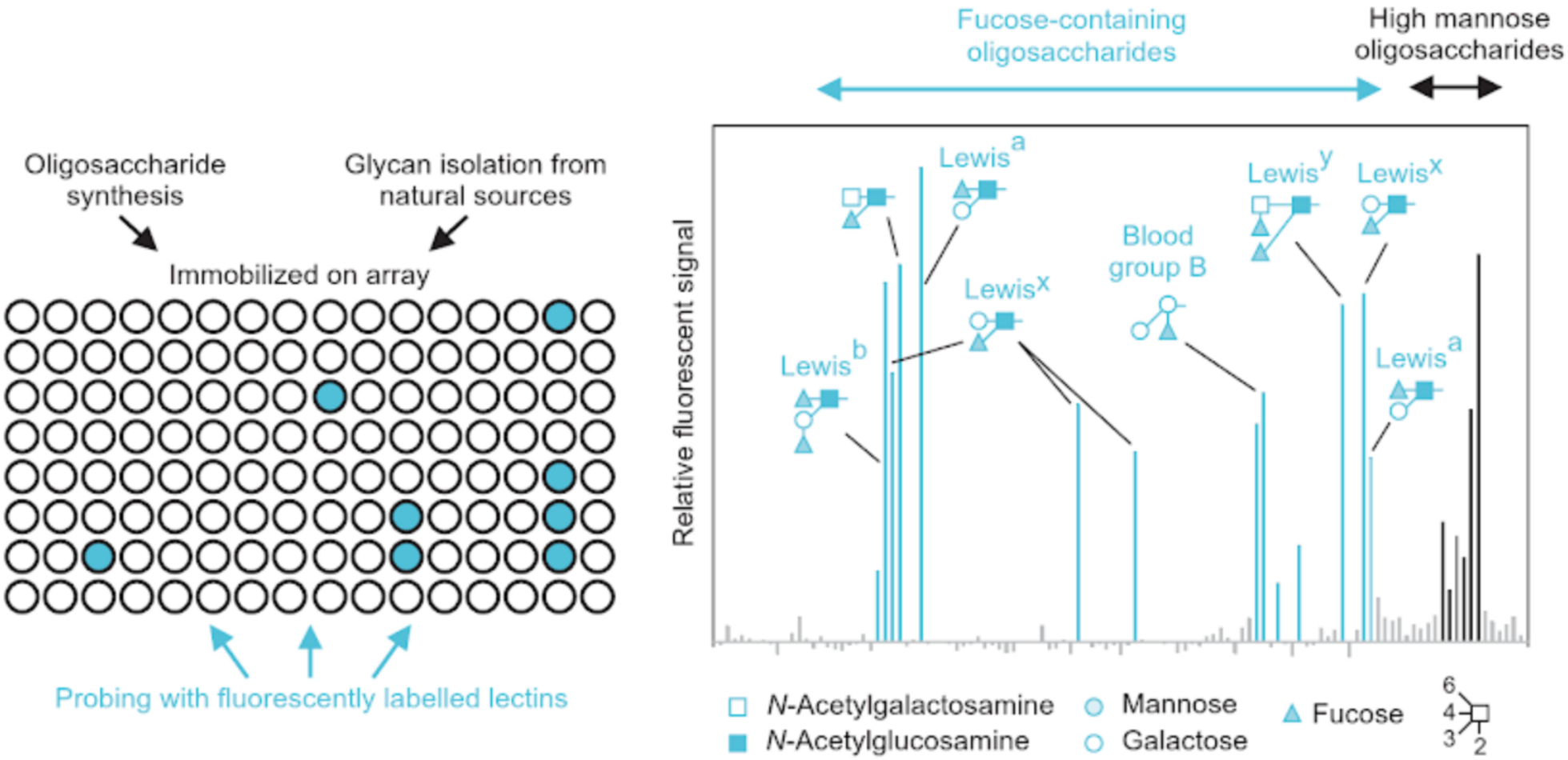
Permethylation/MS for Linkage Analysis



MS Profiling of N-Glycans from Mouse Liver

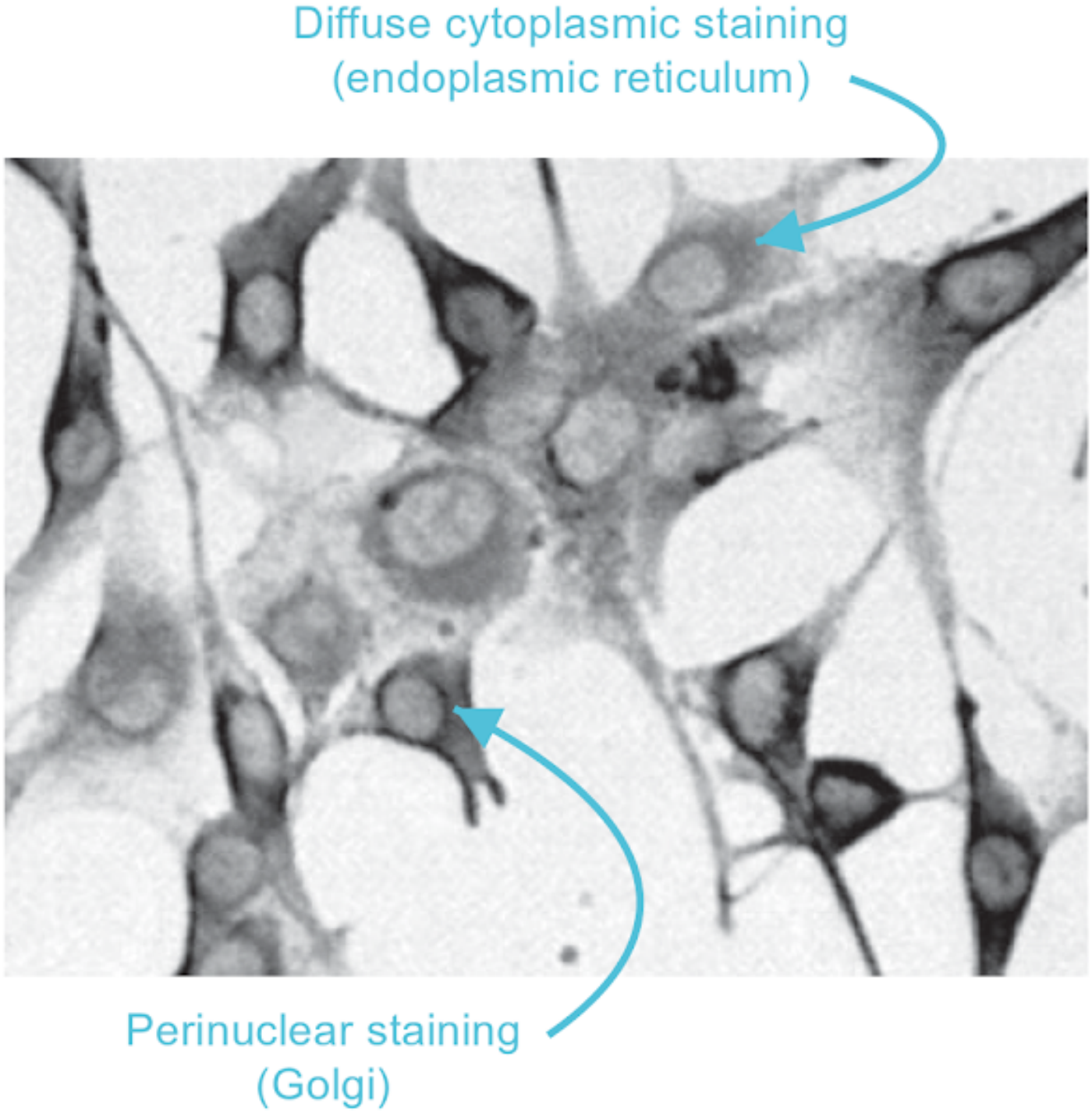


Binding Selectivity from a Glycan Array

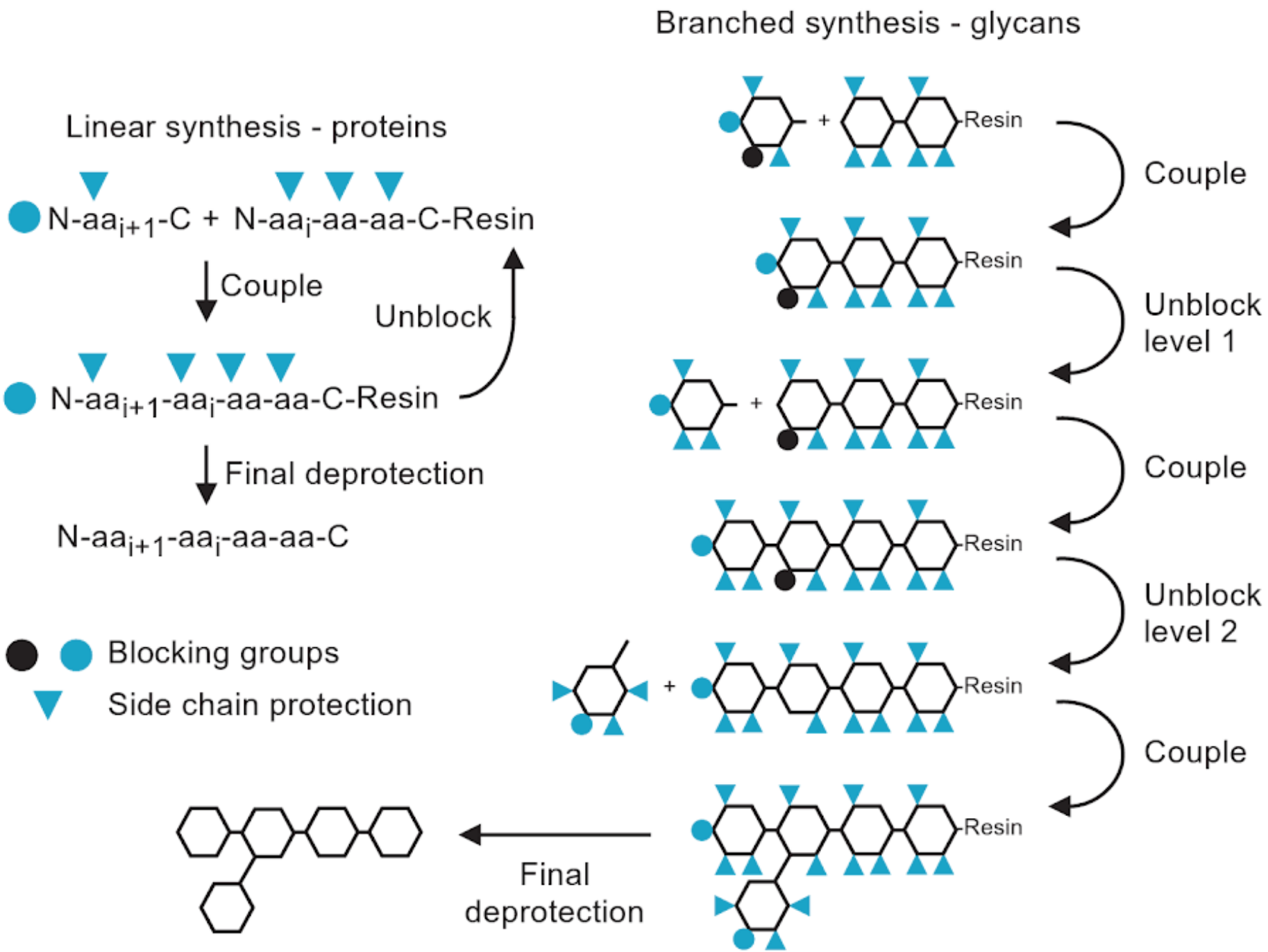


Fluorescently labelled DC-SIGN (a Lectin) binds to glycans immobilized in individual wells. Binding to high mannose glycans, and those with a terminal fucose & galactose or GalNAc is evident.

N-Linked Glycan Staining with Concanavalin A



Chemical Synthesis: Peptides vs. Glycans



Enzymatic Addition of Sialic Acid to a Glycan