Mass Spectrometry of Glycans and Glycoproteins

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Course Outline

Saturday Morning

7:00 am – 8:00 am	**Continental Breakfast**
8:00 am – 8:30 am	**Introduction to Glycans, Glycosylation, Glycobiology** – Ron Orlando
8:30 am – 9:00 am	**Derivatization and Classical Carbohydrate Characterization** – Ron Orlando
9:00 am – 10:00 am	**Issues related to Glycoproteins, and Glycan Biosynthesis** – Ron Orlando
10:00 am – 10:30 am	**Morning Break**
10:30 am - 12:00 pm	**Glycoproteins -- Release and Analyze, and Glycorproteomic Approaches** – Ron Orlando
12:00 pm – 1:00 pm	**Lunch**

Course Outline

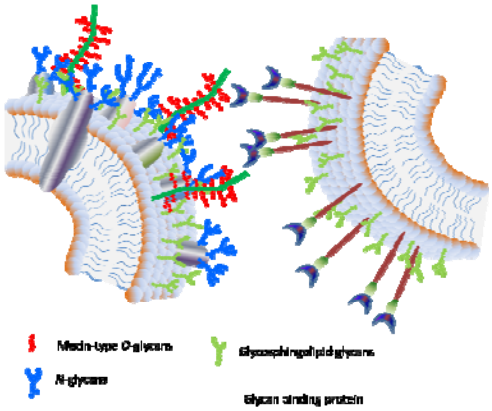
Saturday Afternoon

1:00 pm – 2:00 pm	**Glycopeptides and Methods for Site-specific Analysis** – Ron Orlando
2:00 pm – 3:00 pm	**Glycan Quantitation** – Ron Orlando
3:00 pm – 3:30 pm	**Afternoon Break**
3:30 pm – 4:30 pm	**Bioinformatics of Glycans and Glycoproteins** – Marshall Bern

Glycobiology: biology of sugars

Not just what we eat …

Sugar metabolism and nutrition are not a major focus most of the time



It really focuses on things like cell-surface sugar structures (glycans) in complex settings like cell-cell recognition

Glycobiology

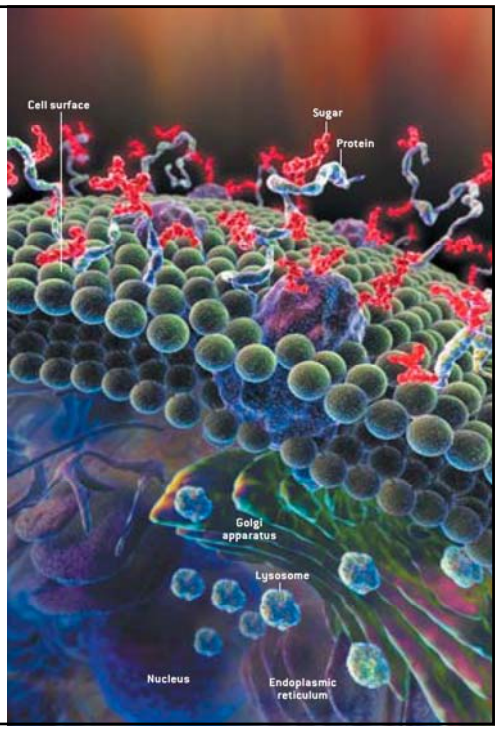
Carbohydrates in Biological Systems

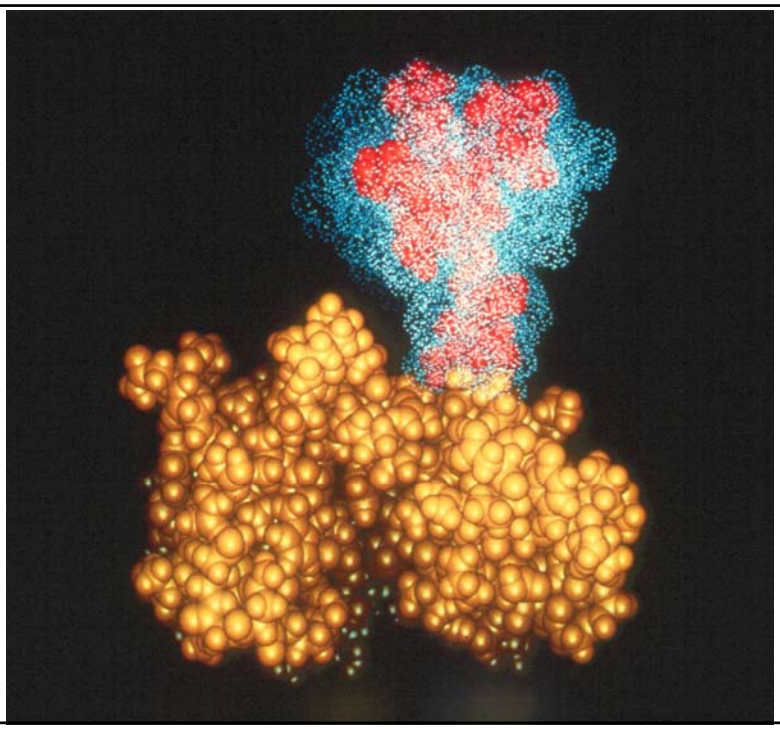
- Energy metabolism
- DNA, RNA
- Structural elements, e.g. chitin, cellulose, etc.
  (Cellulose the most abundant organic compound on Earth)
- Glycoconjugates
  - Glycolipids
  - GPI anchors
  - Proteoglycans
  - Protein N- and O-linked glycosylation

Roles of glycoconjugates

- Protein stability, folding, trafficking (intra & extracellular)
- Modulation of protein activity, circulatory half-life
- Signaling
- Development
- Infection & Immunity

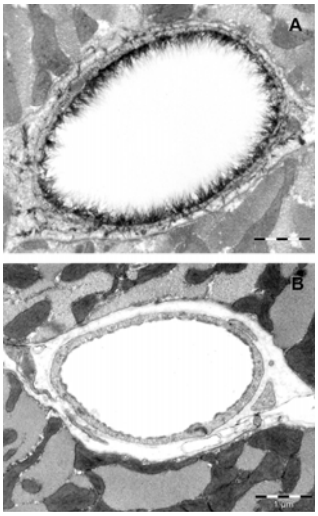
Glycoproteins constitute a large and heterogeneous class of glycoconjugates: most secreted or membrane bound/associate proteins are glycosylated

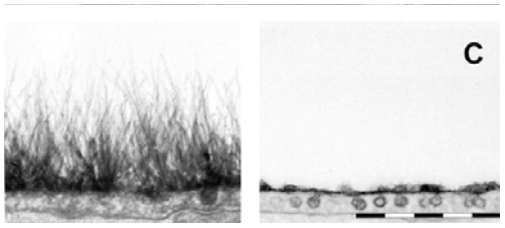




Cells are "sugar coated"

proteoglycans, glycolipids, glycoproteins

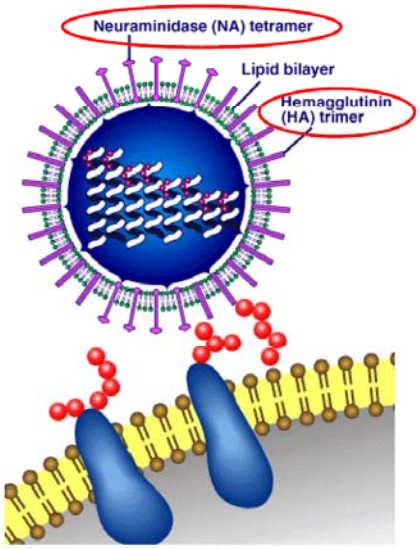




Electron microscopic overview of an Alcian blue 8GX–stained rat left ventricular myocardial capillary (bar=1 μm).

van den Berg B M et al. Circulation Research 2003;92:592-594

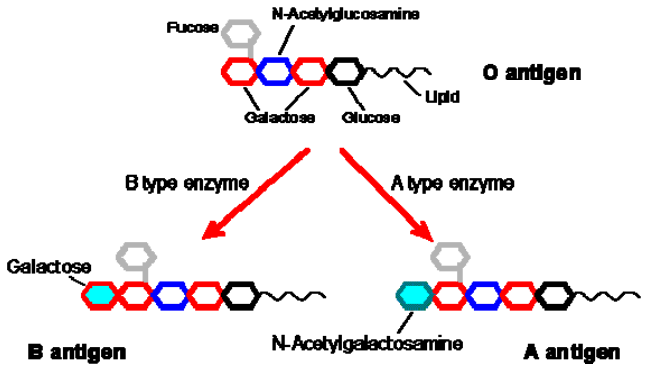
We catch the flu because of the sugars that coat our sinuses and bronchial airways



Influenza viruses recognize neuraminic acids

Tamiflu is a neuraminic acid analogue that inhibits this interaction

The ABO blood group antigens are carbohydrates



The difference between A/B and O is the addition of one additional sugar while the difference between A and B is the identity of this "extra" sugar.

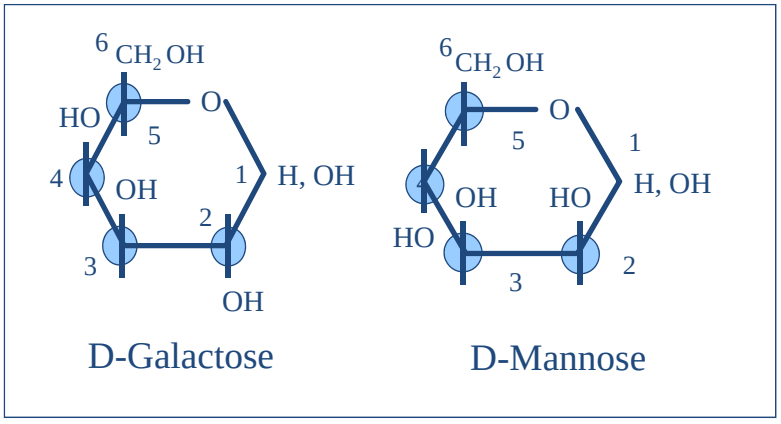
Glycomics: the sugar version of genomics and proteomics

"The cell surface landscape is richly decorated with oligosaccharides anchored to proteins or lipids within the plasma membrane. Cell surface oligosaccharides mediate the interactions of cells with each other …" Science 291:2337

Why is it so hard?
Glycosylation is the only protein modification requiring detailed structural characterization

Requires additional analytical and informatics tools, and requires a systems-based approach to understand properly.

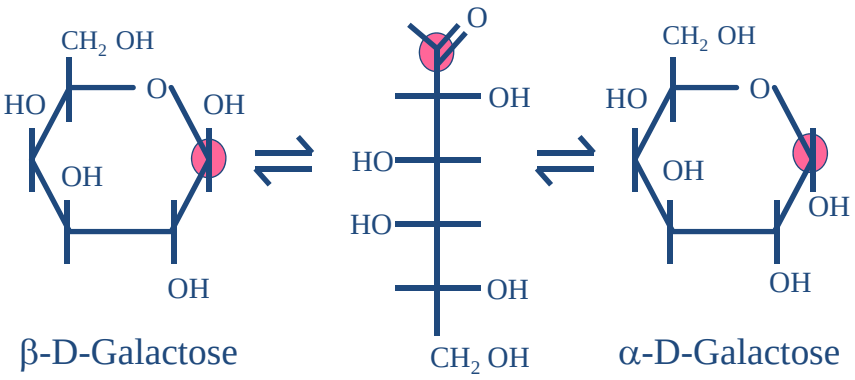
Glycosylation is Complex: Stereoisomers



Stereochemical Centers

Glycosylation is Complex: Anomers

Interconversion between the two anomers of galactose

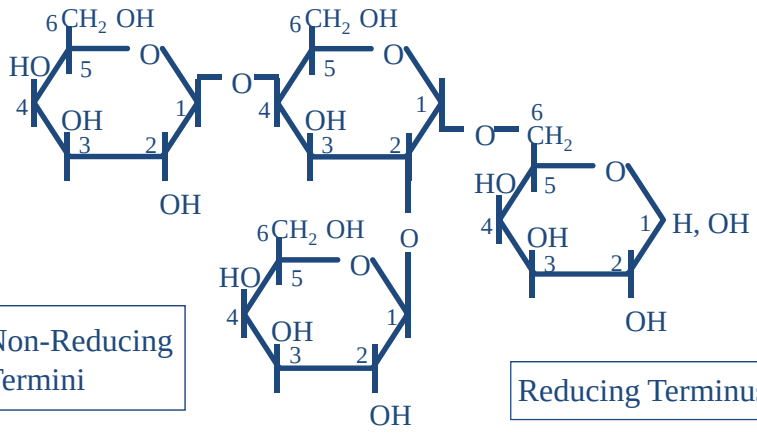


Anomeric Carbon

Branching & multiple linkages and termini

an example of different glycosidic linkage sites

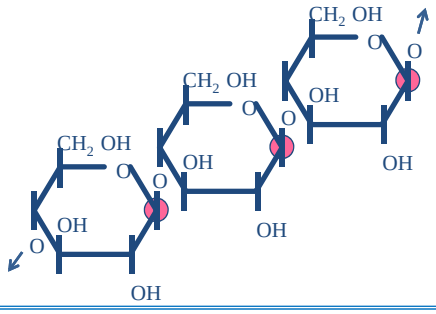
Galactose β 1-4 (Galactose β 1-2) Galactose α 1-6 Galactose



Non-Reducing Termini

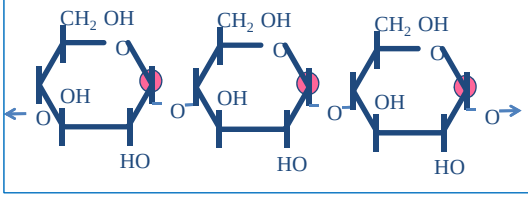
Reducing Terminus

Stereochemistry and Anomeric Configuration have a huge impact



poly β-1,4-D-Glucose

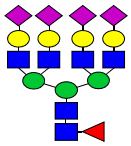
Cellulose



poly α-1,4-D-Glucose

Amylose, starch

Decoding Sugar symbols

What does  mean?

Shape designate type of sugar, i.e.,
- hexoses – circles
- HexNAcs – squares
- hexosamines – squares divided diagonally

Color denotes stereochemistry, i.e.,
- Galactose – Yellow
- Glucose – Blue
- Mannose – Green

Acidic sugars - Diamonds
- Neu5Ac – purple
- Neu5Gc – light blue

Detailed Glycan Characterization

Information needed to solve structures:
- Monomer ID
- Linkage positions (and branching points)
- Anomeric configurations
- Sequence

Two basic routes:
- Bottom up - break it apart, and characterize the pieces
- Top down – keep it together, take it apart systematically

Often both approaches are needed

Classical Methods

Composition Analysis – how much of which sugars are present

Problems with sialic acids – decomposed by strong acid

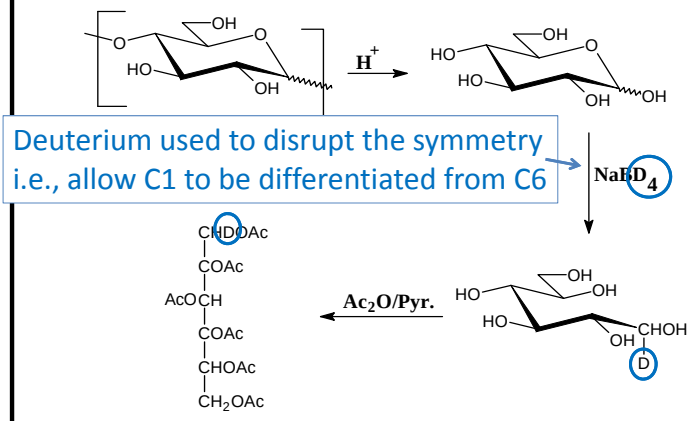
Linkage Analysis – Identify which hydroxyl on each glycan is involved in a glycosidic bond

Data is for monomers – no sequence –
only information on linkages to each sugars

Different experiments needed for neutral and amino sugars (HexNAc)

Composition Analysis

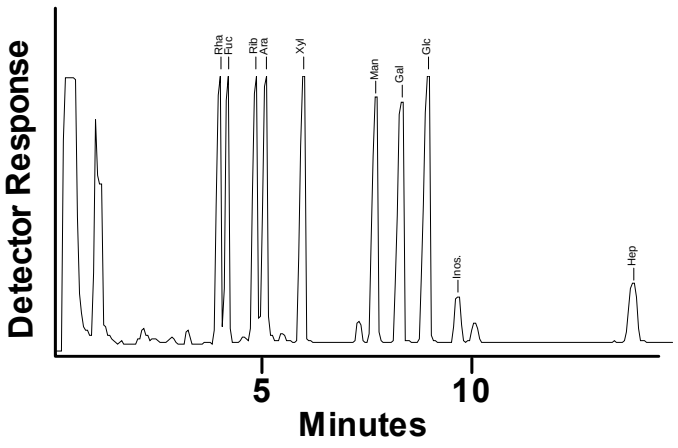
Preparation of Alditol Acetates



Composition Analysis

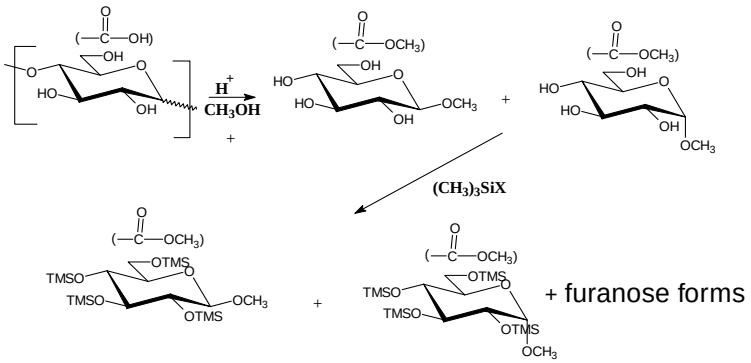
GLC Profile of Alditol Acetates

(Supelco SP2330 Column)



Composition Analysis – samples with acidic sugars

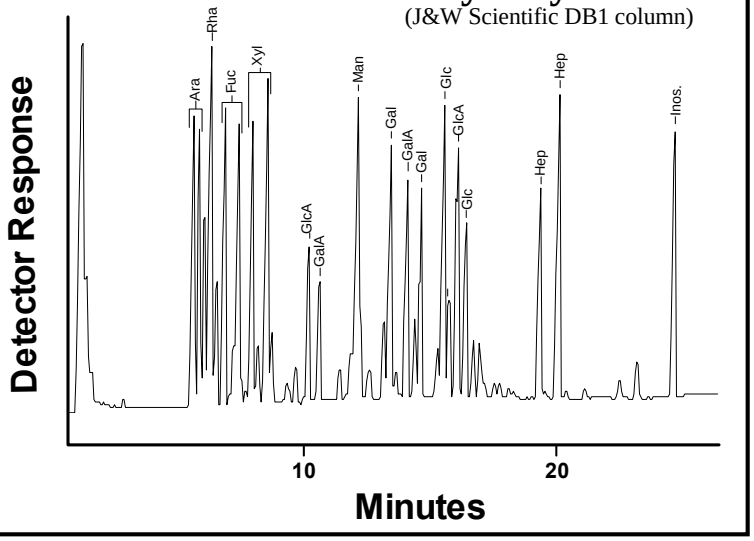
Preparation of Trimethylsilyl (TMS) Methyl Glycosides*



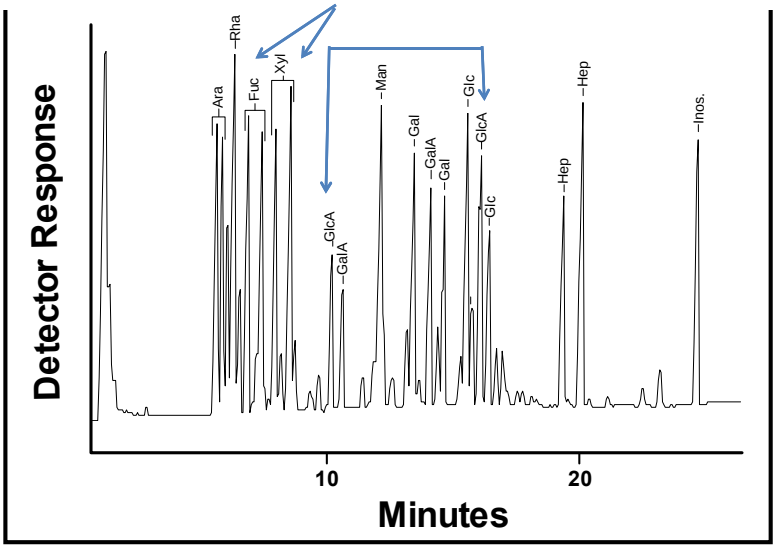
*allows detection of acidic sugars

GLC of TMS Methyl Glycosides

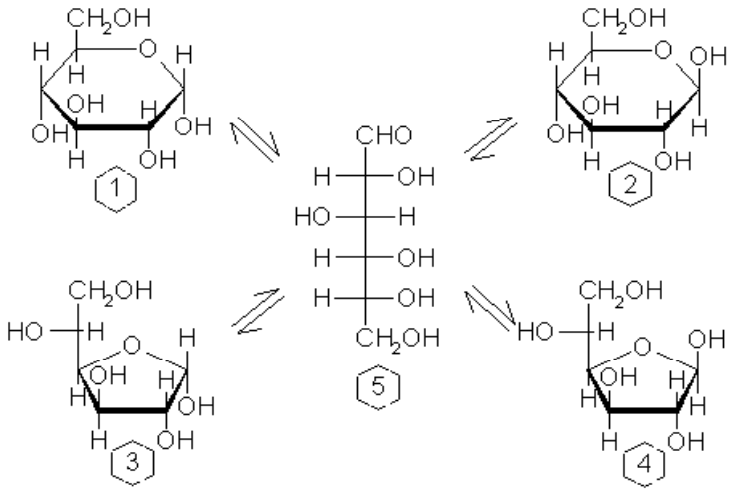
(J&W Scientific DB1 column)



Q: Why do the monosaccharides appear as multiple GC peaks?



A: There are multiple forms of each monosaccharide



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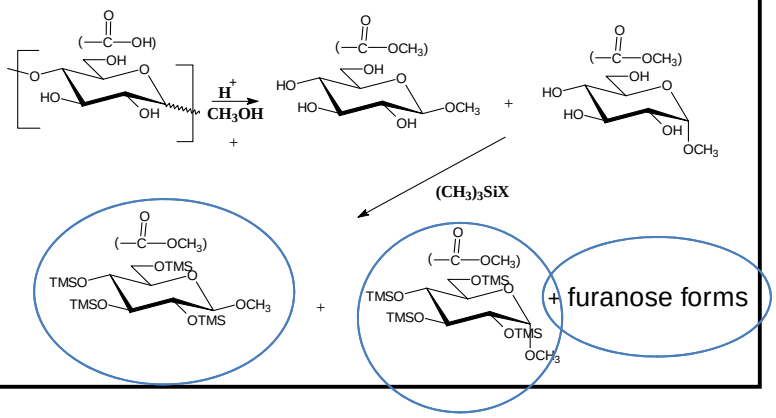
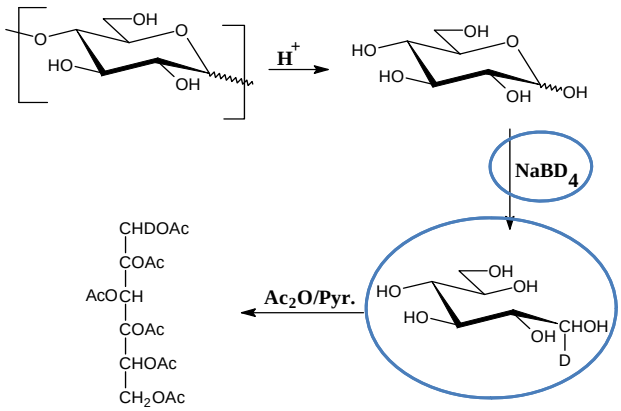
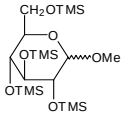


Composition Analysis

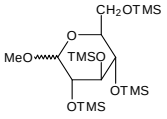
Preparation of Alditol Acetates



Determination of the Stereochemical Configuration of Glycosyl Residues

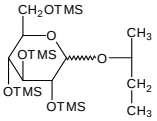


2,3,4,6-tetra- *O*-trimethylsilyl-methyl -D-glucoside

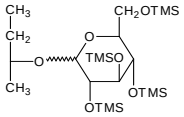


2,3,4,6-tetra- *O*-trimethylsilyl-methyl -L-glucoside

Enantiomers: Identical physical properties, unable to separate.



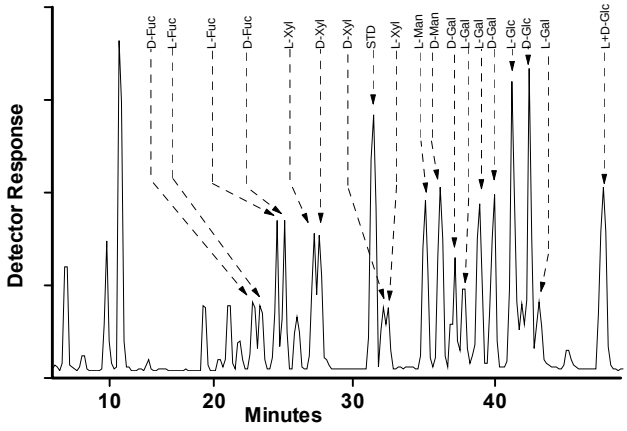
2,3,4,6-tetra- *O*-trimethylsilyl-2-(-)-butyl -D-glucoside



2,3,4,6-tetra- *O*-trimethylsilyl-2-(-)-butyl -L-glucoside

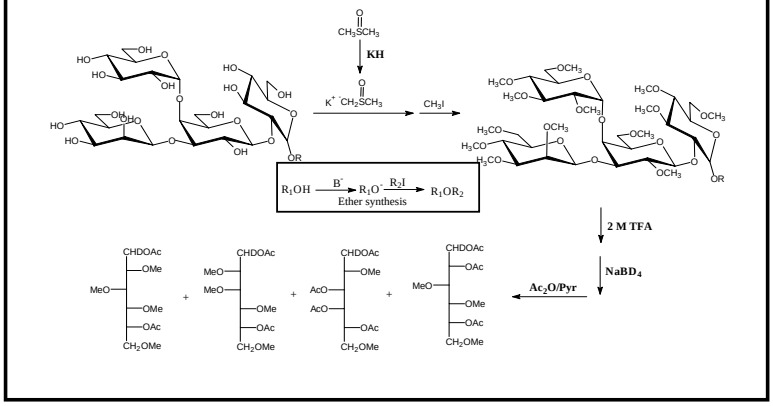
Diastereomers: Different physical properties, able to separate.

GC Separation of Trimethylsilyl (-)-2-Butyl Glycosides



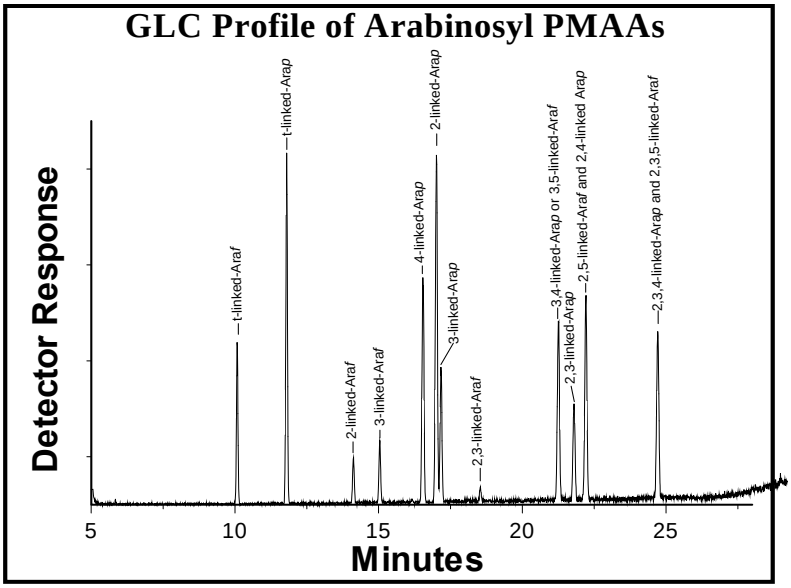
Gerwig, G.J., Kamerling,J.P., and Vliegenthart, J.F.G. 1978. *Carbohydr. Res.* **62**:349-357

Glycosyl Linkages by Preparing Partially Methylated Alditol Acetates (PMAAs)



Merkle and Poppe (1994) *Methods Enzymol*. 230: 1-15; York, et al. (1985) *Methods Enzymol.* 118:3-40.

GLC Profile of Arabinosyl PMAAs



Problems with Glycans/Glycoproteins

- Poor Ionization efficiency
  - large mass increase
  - no sites for protonation
  - sometimes has a negative charge
  - hydrophobicity
  - Transparent
- Heterogeneity
- Large size

Other useful Derivatizations

Depolarizing Derivatization Reactions for MS

Peracetylation
Pyridine/Acetic Anhydride, RT/overnight

Pertrimethylsilylation
Pyridine/BSTFA/TMCS, RT/half hour

Permethylation
Suspension of NaOH in dry DMSO
add to dry glycan(s)
Methyl Iodide
add after ~2 hrs
Chloroform/Water extraction, several washes, dry

Why Permethylate the Oligosaccharide?

The mass increase is not too much to shift the mass to higher mass range and decrease sensitivity.

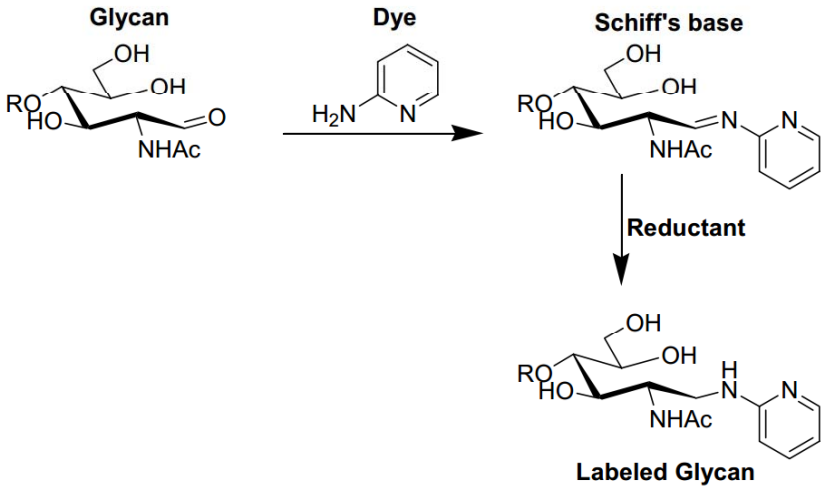
It increase the sensitivity of oligosaccharides for subsequent MS analysis. "Equalizes" the MS response for different glycans

It allows for diagnostic molecular ions which are easier to interpret than the native oligosaccharides.

Stabilizes negatively charged sugars (sialic acids for example)

Makes tandem mass spectra more interpretable

Other useful Derivatizations: Reductive Amination



Heterogeneity: the quality or state of being heterogeneous

Heterogeneous: consisting of dissimilar or diverse ingredients

No two cars are ever exactly the same, and the sequence in which they are produced must be carefully controlled to minimize resource utilization. For some of the larger options, the structure of the assembly must be adapted.

M. Dincbas, H. Simonis, and P. van Hentenryck. Solving the car-sequencing problem in constraint logic programming. In Y. Kodratoff, editor, Proceedings ECAI-88, pp. 290–295, 1988

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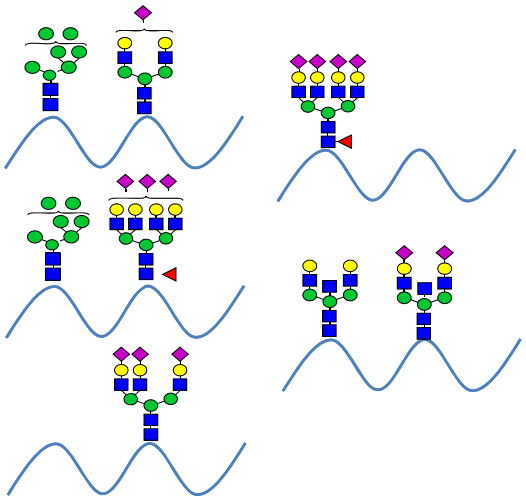
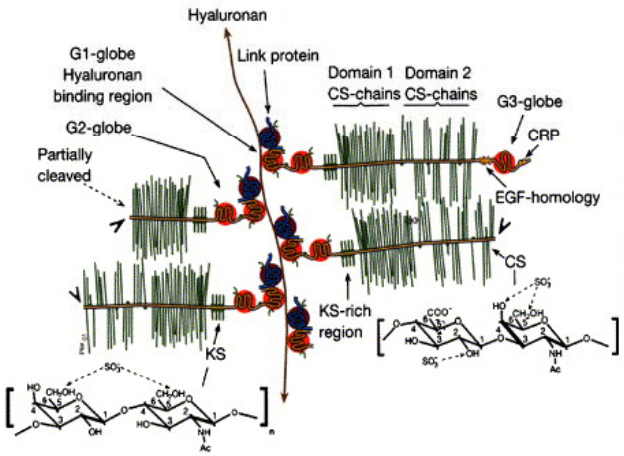


Large Size



Schematic illustration of a proteoglycan macromolecule aggregate in which multiple aggrecan molecules with glycosaminoglycans (GAG) side chains attach to a hyaluronan chain. X. Lux Lu, Chester Miller, Faye H. Chen, X. Edward Guo, Van C. Mow, J. Biomechanics, Vol 40 (11), 2007, Pg 2434–2441

Questions?