Lecture 30 (12/4/20)

•Reading: Ch7; 258-267
Ch10; 371-373

•Problems: Ch7 (text); 26,27,28
Ch7 (study-guide: applying); **2,5**
Ch7 (study-guide: facts); 6

NEXT

•Reading: Chs **4,6,8,10,14,16,17,18**; 128-129, 189,311,377-380,555-557, 561, 621-622,639,662-663,679, 691-694

•Problems: –

Carbohydrates

A. Definition
B. Roles
C. Monosaccharides
   1. Chirality
      a. One or more asymmetric carbons
      b. Linear and ring forms
   2. Derivatives: the chemistry of carbohydrates
      a. Oxidation
      b. Reduction
      c. Esterification
      d. amines
   3. Polymerization
      a. The Glycosidic Bond
      b. Non-covalent bonds in macro-molecular structure
D. Oligosaccharides
   1. Glycoproteins & glycolipids
   2. O-linked
   3. N-linked
   4. Sequence determination-ABO
E. Polysaccharides
   1. Polymers of glucose
   2. Polymers of disaccharides

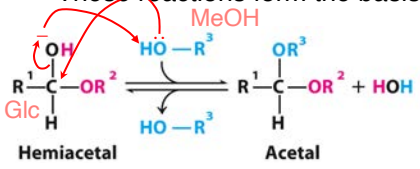
Carbohydrates

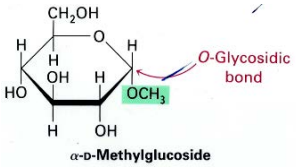
The Glycosidic Bond

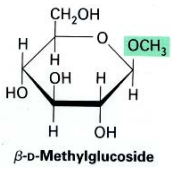
Carbohydrates

Hemiacetals and Hemiketals are reactive to alcohols in condensation reactions

- Hemiacetals condense with alcohols to form Acetals.
- Hemiketals condense with alcohols to form Ketals.
- These reactions form the basis of the **GLYCOSIDIC BOND**.

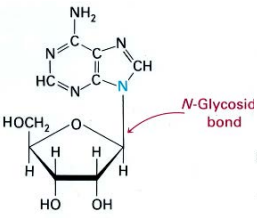


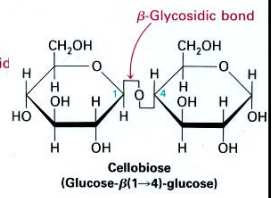




The Glycosidic Bond

- Two sugar molecules can be joined via a glycosidic bond between an anomeric carbon (the hemiacetal/hemiketal) and a hydroxyl carbon (the other sugar).
- The glycosidic bond between sugars is stable and does not readily hydrolyze.
  - The anomeric carbon involved in the glycosidic linkage is fixed in its chirality and is therefore nonreducing.
  - The second monomer, with its unreacted hemiacetal, is still reducing.





Carbohydrates

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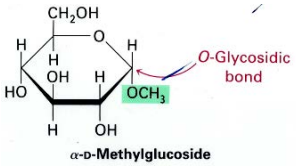

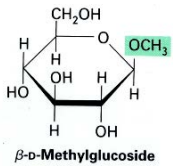

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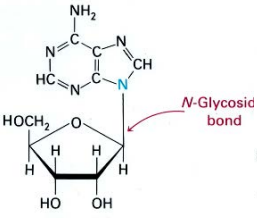
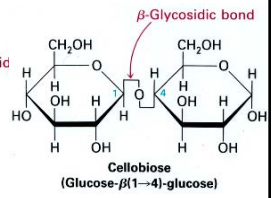




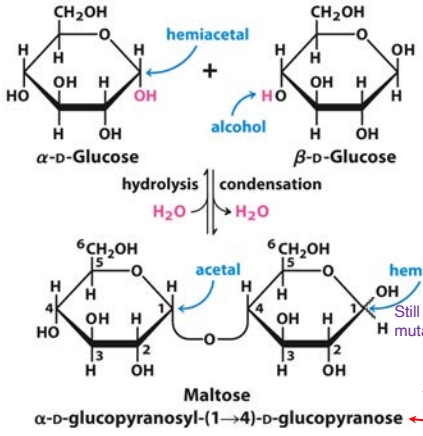
Carbohydrates

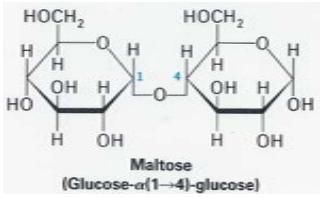
Disaccharides

Carbohydrates

Disaccharides:

- Disaccharides can be named by the organization and linkage or a common name.
  - The disaccharide formed upon condensation of two glucose molecules via a 1 → 4 bond is described as α-D-glucopyranosyl-(1→4)-D-glucopyranose.
  - The common name for this disaccharide is maltose.





If you only know the sugar and the linkage

Non-reducing → reducing

If you know the exact structure of the sugar

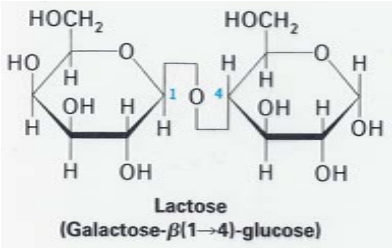
As we make sugar-polymers, the convention is to have the non-reducing sugar to the LEFT and the reducing end at the RIGHT.

Carbohydrates

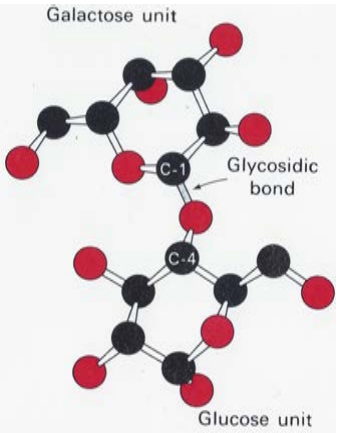
Disaccharides:

Here is likely the first disaccharide you encountered in your life:

Lactose.



Non-reducing → reducing

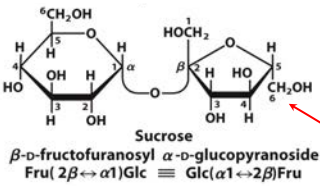


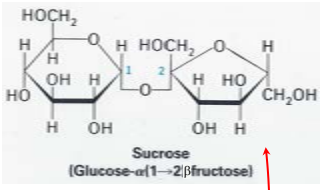
Carbohydrates

Disaccharides:

Here is likely the disaccharide you ingest the most:

Sucrose.





Notice that these are drawn upside down

Nonreducing Disaccharides

- Two sugar molecules can be also joined in a glycosidic bond between two anomeric carbons.
- The product has two acetal groups and no hemiacetals or hemiketals.
- There are no reducing ends; this is a nonreducing sugar.

Carbohydrates

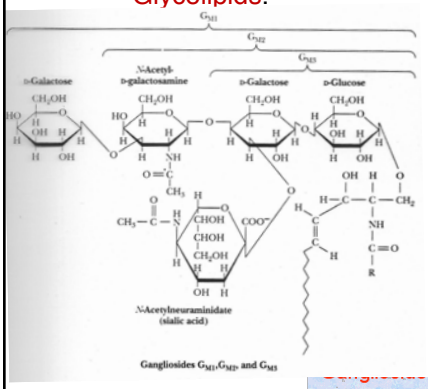
Oligosaccharides

Carbohydrates

Oligosaccharides:

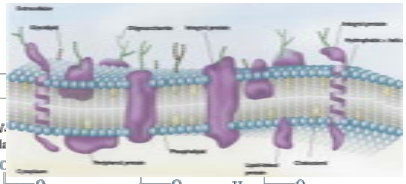
Here is an oligosaccharide that we encountered with sphingoglycolipids, or for short:

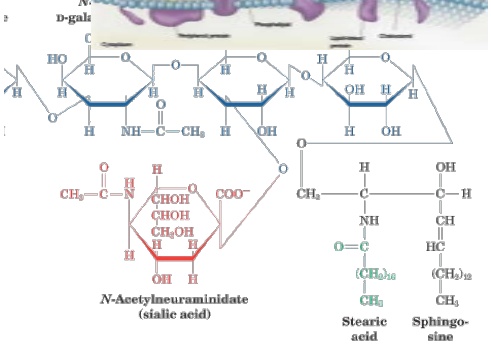
Glycolipids.



Gangliosides G_{M1}, G_{M2}, and G_{M3}

Glycoconjugates: Glycolipids





G_{M1}: structural formula with its sphingosine residue in Fischer projection (contain sialic acid)

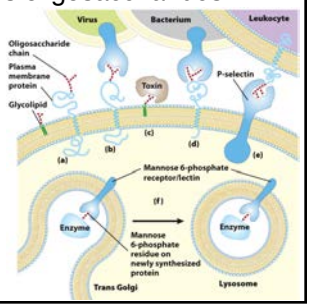
GM1 = Gal-β(1→3)GalNAc-β(1→4)Gal-β(1→4)Glc-β(1→Ceramide)
Sia-β(2→3)

Carbohydrates

Oligosaccharides: Glycoconjugates: Glycoprotein

When you attach an oligosaccharide to a protein: Glycoproteins.

- A protein with small oligosaccharides attached:
  - Carbohydrate is attached via its anomeric carbon to amino acids on the protein.
    - Common connections occur at Ser, Thr, and Asn.
  - About half of mammalian proteins are glycoproteins.
  - Generally, bacteria do not glycosylate their proteins.
  - Carbohydrates play role in protein-protein recognition.
  - Viral proteins are heavily glycosylated; this helps evade the immune system.
- Proteins whose role is to bind specific carbohydrates/oligosaccharides = Lectins (or selectins)
  - Lectins important for many biological functions
    - Recruitment of leukocytes to sites of inflammation
    - Sperm-egg recognition
    - Virus-target cell interaction
    - Attachment of flora (microbiome) in gut
    - Nervous system development
    - Serum-protein turnover (sialic acid)
    - Targeting proteins to lysosomes for degradation (Man)

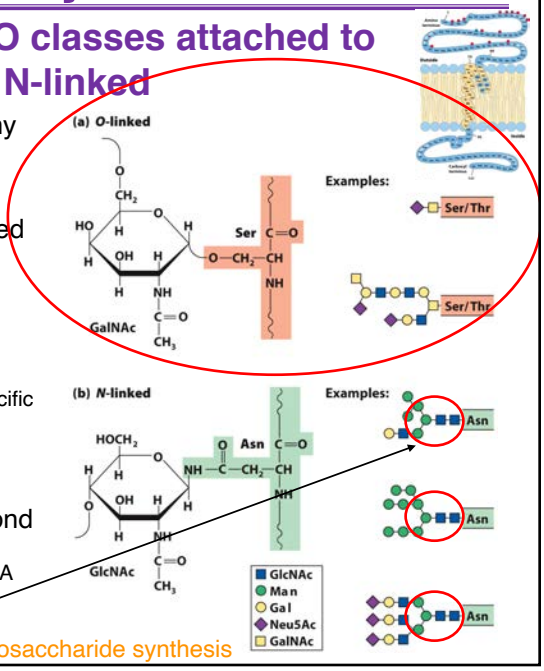


How are these sugars attached?

Carbohydrates

Oligosaccharides: TWO classes attached to proteins, O-linked and N-linked

- First, these polymers, unlike any others we have studies, are BRANCHED
- Second, like glycolipids, O-linked sugars are attached via an O-glycosidic bond
  - Use a Ser or Thr in the sequence (recall glycophorin)
  - Usually smaller than N-linked
  - Synthesized one at a time by specific glycosyltransferases (specific for sugar, linkage, and chirality)
- Third, N-linked sugars are attached via an N-glycosidic bond
  - Use an Asn
  - The Asn residues are within a 3 AA sequence context: $NX^{S}/_{T}$
  - All the same at the core



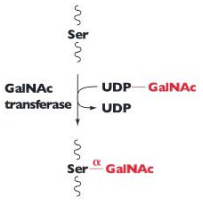
Let's look more closely at O-linked oligosaccharide synthesis

Carbohydrates

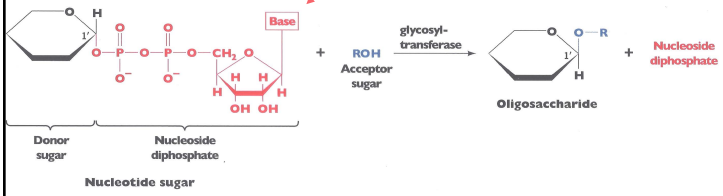
Oligosaccharides: O-linked

- Specific glycosyltransferases use activated sugars
- Activated by attaching to nucleotides: UDP, CDP, etc.

EXAMPLE:



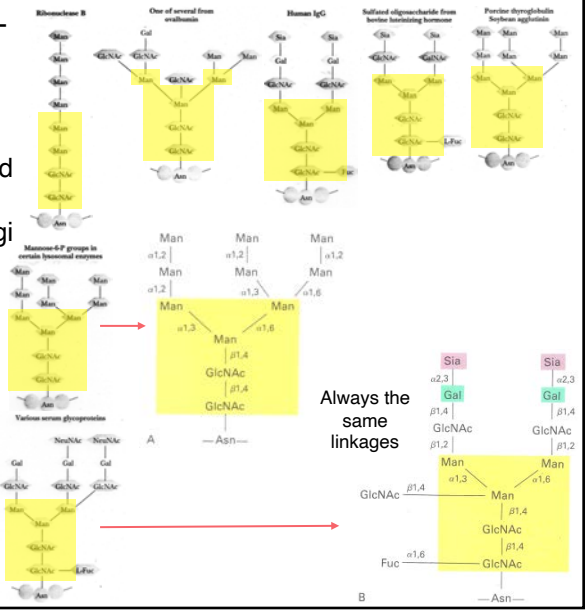
Sugar 1-P + UTP ⇌ UDP-sugar + PP_i



Carbohydrates

Oligosaccharides: N-linked

- Larger, more complex than O-linked
- All have a "Core" containing GlcNAc & Man
- Added as a unit, then modified before adding to Asn groups on proteins; all in the ER/Golgi
- Use an isoprene, called Dolichol, to build core
- Examples:

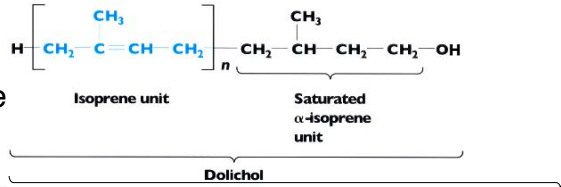


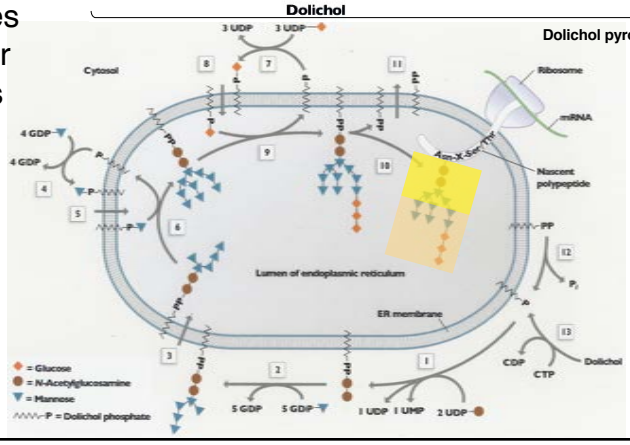
Let's look at this Dolichol & its use in biosynthesis more closely….

Carbohydrates

Oligosaccharides: N-linked

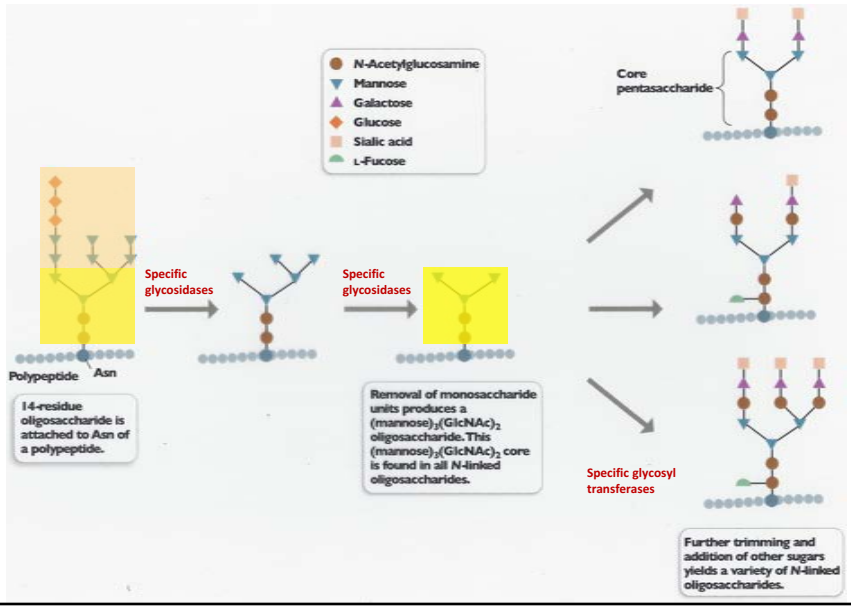
- Activated by attaching two phosphates, one using CTP (step 13)
- Again, uses NDP-sugar precursors





Carbohydrates

Oligosaccharides: N-linked



Carbohydrates

Oligosaccharides: Determination of Sequence

- Whole different problem compared to proteins and nucleic acids…. Its branched!!
- Moreover, a given residue can have several (and stereo-specific) ways of attaching to a neighboring residue.
- Need to use a combination of methods:
  - Chemical
    - Hydrolysis & chromatography to identify sugars
    - Exhaustive methylation & hydrolysis, then chromatography to identify what positions were not methylated
  - Biochemical
    - Use of enzymes that stereo-specifically hydrolyze glyosidic bonds (from the non-reducing end)

EXAMPLE: First, just like protein sequencing, you need to purify glyco-protein or lipid. Lets say we isolate the glycolipid from a person's RBC's who is O-positive. Treat it with a ceramidase to hydrolyze the lipid from the sugar.

Second, take an aliquot and just hydrolyze (like what was done for amino acid analysis). This gets the composition and stoichiometry.

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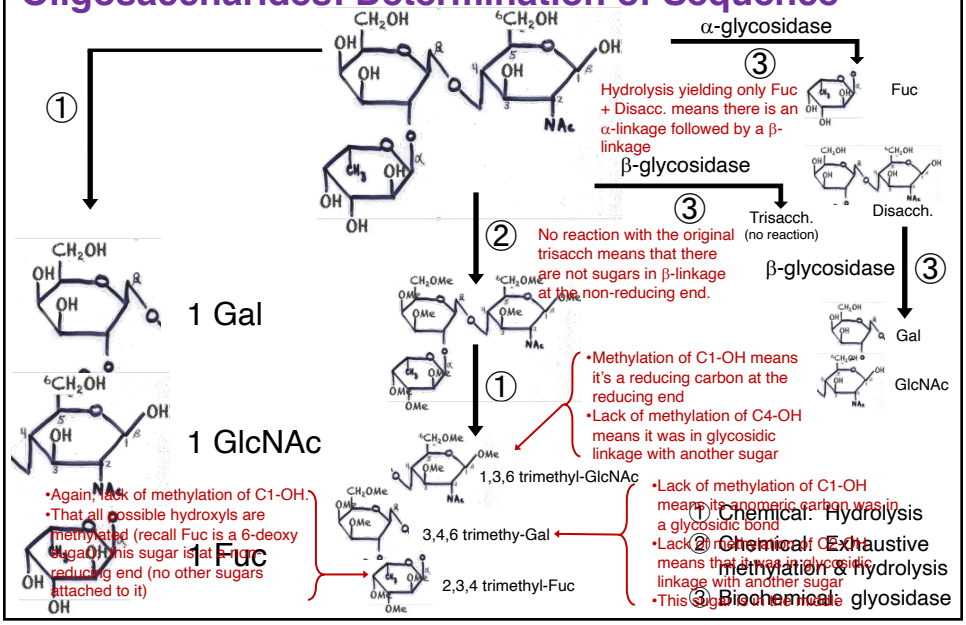
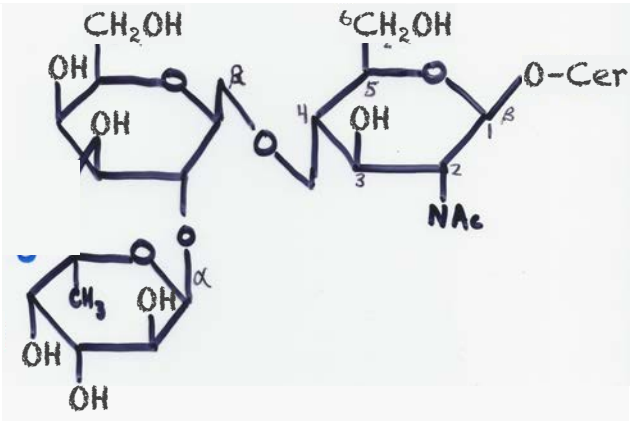


Carbohydrates

Oligosaccharides: Determination of Sequence

– In vertebrates, ganglioside carbohydrate composition determines blood groups.



L-Fuc α(1→2)-D-Gal β(1→4) D-GlcNAc β-Ceramide O blood group

D-Gal α(1→3) B blood group

D-GalNAc α(1→3) A blood group

Carbohydrates

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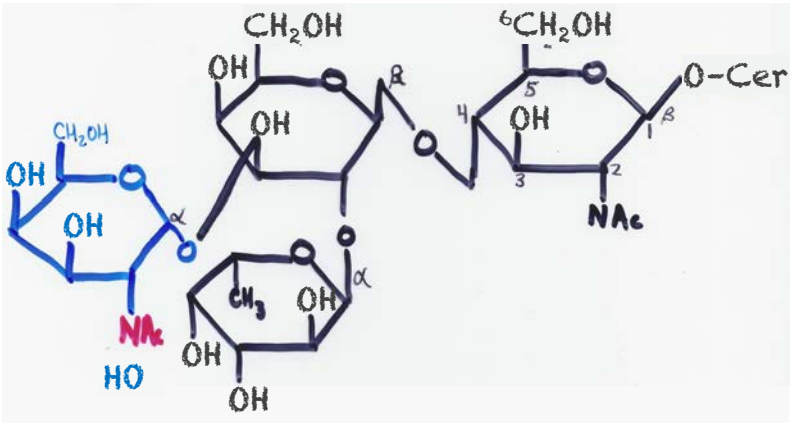

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Glycoconjugates: Analysis

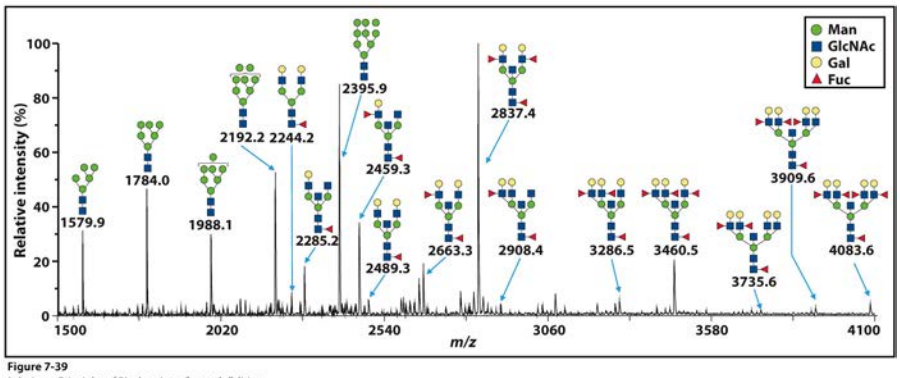

Figure 7-39
Lehninger Principles of Biochemistry, Seventh Edition