Glycobiology

Jeffery Kelly for
Peter G. Schultz
“A remarkable universal finding in nature is that all cells of all species that have ever been studied are coated with a dense and complex array of glycans. Thus the evolution of life has resulted in repeated selection for the expression of glycans at cell surfaces and in extracellular spaces.”

-Ajit Varki

“Essentials of Glycobiology”
Edited by Ajit Varki, Richard D Cummings, Jeffrey D Esko, Hudson H Freeze, Pamela Stanley, Carolyn R Bertozzi, Gerald W Hart, and Marilynn E Etzler

Anomeric Effect: Hyperconjugation

- Antiperiplaner Lone Pair Hypothesis (ALPH)
- Axial: electron denisty from endocylic HOMO to excyclic LUMO
- Evidence for hyperconjugation
  - bond length (C1-O is shorter, C1-O’ is longer)
exo-Anomeric Effect

- Preferred conformation of the exocyclic oxygen R group (\(\phi\))
- Same principles as endo-anomeric effect (not as strong)
  - Dipole stabilization and hyperconjugation with exocyclic oxygen possible for axial and equatorial bonds
  - Most dominant for \(\beta\)-linked R groups
- Many more consequences with R is another sugar!!
Polysaccharides Generally Adopt an Extended Conformation
Intrinsic Properties

Carbohydrates interact directly with conjugate to modify its properties

- Solubility
- Cellular half-life
- Folding
- Stability
- Immunogenicity
- Structure & Activity
  - Dimerization
  - Enzymatic activity
- Recognition by other molecules -- extrinsic

Glycoprotein
General structure of GPI anchors

H₂N — Protein — NH₂

O

CH₂

CH₂

O

O — P — O

Ethanolamine

Manα1-2

Manα1-6

Manα1-4

GlcNα1-6

R₁ = fatty acid or OH
R₂ = fatty acid or alkyl or alkenyl chain
(Note, in some cases, the lipid may also
be a ceramide rather than a glycerolipid)
R₃ = fatty acid or OH
R₄, R₉ = ethanolamine phosphate or OH
R₅,₆,₇,₈,₁₀ = carbohydrate substituents or OH

Chapter 11, Figure 1
Extrinsic Properties

Carbohydrates interact with carbohydrate-binding proteins

- Molecular recognition
- Secretion
- Cell trafficking
- Cell adhesion
- Cell entry (pathogens)
- Cell signaling
  - Receptor binding
  - Modulation of ternary ligand-receptor complexes
  - Spatial & temporal gradients
Trivial Names for Common Monosaccharides in Vertebrates**

D-Glucose (Glc)  N-Acetyl-d-glucosamine (GlcNAc)  D-Galactose (Gal)  N-Acetyl-d-galactosamine (GalNAc)

D-Mannose (Man)  D-Xylose (Xyl)  D-Glucuronic acid (GlcA)

L-Fucose (Fuc)  N-Acetylneuraminic acid (NeuAc)

*Neu5Ac the most common form of sialic acid.
• Glycosidic bond is configurationally stable once formed (unlike hemi-acetal)
  • Glycoside: $R = \text{glycone (not a carbohydrate)}$
    • Replace sugar “$-e$” with “$-side$”
      • glucose $\rightarrow R\ \text{glucoside}$
  • Glycosyl Glycose (-side): $R = \text{reducing sugar/glycoside}$
    • Replace sugar “$-e$” with “$-syl$”
      • galactose $\rightarrow \text{galactosyl } R\text{-ose/R-osite)$}
Naming of Oligosaccharides

- Name from reducing to non-reducing (free- or aglycone-attached-) end
- Indicate: sugar-(CO\textsubscript{nonreducing} \rightarrow OC\textsubscript{reducing})-sugar
- Many abbreviated forms are used
  - \textit{p/f} to for pyranose/furanose, dashes
  - Trivial three letter abbreviations & anomeric numbering omitted
- Many disaccharides & oligosaccharides also have common names
  - Lactose a common name
Naturally Occurring Disaccharides

- Milk sugar (lactose) is a disaccharide with free reducing end
- Other naturally occurring disaccharides & free reducing sugars are rare!
- Sucrose and trehalose are anomerically linked to prevent reactivity
Disaccharide Building Blocks

- Cellulose: \((\text{Glc}\beta_1-4\text{Glc})_n\)-Cellbiose

- Starch: amylopectin/glycogen: \((\text{Glc}\alpha-4[\text{Glc}\alpha-6]\text{Glc})_m\)-(\text{Glc}\alpha-4\text{Glc})_n\)-Maltose

- Chitan: \((\text{Glc}\text{NAc}\beta_1-4\text{Glc}\text{Nac})_n\)-chitobiose

- Most commonly known disaccharides are derivatives of polysaccharides, released by partial chemical or enzymatic degradation.
Symbolic Representation (CFG)

- Consortium for Functional Genomics (CFG):
  glycobiology standards & resources

http://www.functionalglycomics.org/static/consortium/consortium.shtml
Glycosyltransferases Build Oligosaccharides

<table>
<thead>
<tr>
<th>Glycosyl donors</th>
<th>Glycosylated acceptor + Nucleotide or isoprenoid-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leloir* Donors:</td>
<td></td>
</tr>
<tr>
<td>nucleotide sugars</td>
<td></td>
</tr>
<tr>
<td>CMP-sialic acid</td>
<td></td>
</tr>
<tr>
<td>GDP-fucose</td>
<td></td>
</tr>
<tr>
<td>GDP-mannose</td>
<td></td>
</tr>
<tr>
<td>UDP-glucose</td>
<td></td>
</tr>
<tr>
<td>UDP-galactose</td>
<td></td>
</tr>
<tr>
<td>UDP-xylose</td>
<td></td>
</tr>
<tr>
<td>UDP-N-acetylglucosamine</td>
<td></td>
</tr>
<tr>
<td>UDP-N-acetylgalactosamine</td>
<td></td>
</tr>
<tr>
<td>UDP-glucuronic acid</td>
<td></td>
</tr>
<tr>
<td>Non-Leloir Donors:</td>
<td></td>
</tr>
<tr>
<td>Lipid phosphates</td>
<td></td>
</tr>
<tr>
<td>Dolichol-P-glucose</td>
<td></td>
</tr>
<tr>
<td>Dolichol-P-mannose</td>
<td></td>
</tr>
<tr>
<td>Dolichol-P-(glucose$_3$-mannose$_9$-GlcNAc$_2$)</td>
<td></td>
</tr>
<tr>
<td>Undecaprenyl-P-P-N-acetylMuramic acid-pentapeptide-GlcNAc</td>
<td></td>
</tr>
</tbody>
</table>

*1970 Nobel Prize for sugar metabolism

- There are not glycosyltransferases that catalyze the formation of every possible carbohydrate linkage!
- Multiple glycosyltransferases can use the same donor (nucleotide sugars) & have many potential acceptors (oligosaccharides, proteins, lipids, etc.)
• Diverse glycan structures can be linked to diverse biomolecules (glycosylation)
  → alter activity of glycan & aglycone
Glycosylation & Glycan Biosynthesis

Glycosylation in Secretory Pathway (> 250 enzymes)

Biosynthesis
- Many different aglycones
- Glycosylation: addition of core glycan to aglycone
- Build: glycosyltransferases
- Cleave: glycosidases
- Modify: sulfation, epimerization, acetylation, phosphorylation

No genetic code for structures
- Enzyme expression, activation & localization
- Competing/overlapping substrate specificity
- Substrate variability
- Tissue, metabolic state, etc.

*Starred positions are points of diversification/variation
Heterogeneity

- Glycan structures & glycosylation cannot be predicted from genome
- Even when glycosylation is known:
  - Glycoforms—glycosylated aglycone characterized by the attachment of multiple glycan structures
    - Microheterogeneity—multiple glycan structures at same attachment site
    - Macroheterogeneity—multiple sites of attachment and/or types of glycans (N-, O-, etc)
  - Occupancy—glycosylated vs. not
- Glycoforms will vary by cell line

CD59
- 1 N-glycan > 150 glycoforms
- 2 O-glycans variable occupancy > 5 glycoforms
- GPI anchor > 37 forms
Glycocode: Glycan Structures are Functionally Relevant

- Glycans built in a non-templated fashion
- Glycan interact with glycan-binding proteins for function
- How to define glycan structures & understand their functions at the molecular level?
Peptidoglycan

- Bacterial cell wall is made of a glycan backbone bearing crosslinked peptides
- Cell-wall enzymes are good targets for antibiotics (β-lactams, vancomycin, etc.)
  - Conserved across bacteria (thicker & exposed in gram-positive bacteria)
  - Critical to function
  - Found on the cell membrane
Lipopolysaccharides (LPS)

- In cell-envelope of gram-negative bacteria
- Structures diverse & specific to bacterial strains
  - Lipid A core is conserved (membrane embedding)
  - O-antigens are typically long polysaccharides (smooth appearance vs. rough when absent)
- Endotoxins (pyrogenic & immunogenic; sepsis)
- O-antigens promising for immunization strategies
- Biosynthetic enzymes, many of which have only recently been elucidated, can be targets for antibiotics
Synthesis of Nucleotide Sugar Donors

GTs requires activation of monosaccharides to nucleotide sugars. Monosaccharides are imported into the cell, salvaged from degraded glycans, or derived from other sugars within the cell. Although most glycosylation reactions occur in the Golgi, precursor activation and interconversions occur mostly in the cytoplasm. Nucleotide sugar–specific transporters carry the activated donors into the Golgi.
Glycosphingolipids (GSLs)

- GSLs = glycosylated ceramides
- Found in nearly all cell membranes (prokaryotes & eukaryotes)
- Major glycans of the vertebrate brain:
  - Cerebrosides: β-GalCer & sulfatide (3-sulfo β-GalCer)
  - Comprise 80% of myelin → essential to the structure & function of nerve cells
- Several classes of GSLs with complicated glycans (e.g., ganglio-, globo-)
  - Core β-GlcCer linkage with extended structural diversity
  - Displayed on many cell surfaces, in addition to brain function

Ceramide lipid: long-chain amino alcohol (sphingosine) in amide linkage to a fatty acid
Biosynthesis of Diverse GSLs in Brain

FIGURE 10.2.
Biosynthetic pathways for brain GSLs: synthesis by the stepwise addition of sugars first to ceramide, then to the growing glycan.

Cer is the acceptor for UDP-Gal:ceramide β-galactosyltransferase or UDP-Glc:ceramide β-glucosyltransferase in the major pathways to glycosphingolipid biosynthesis in oligodendrocytes and nerve cells, respectively.

In mammals and birds, the major gangliosides in brain are GM1, GD1a, GD1b, and GT1b.
Glycoproteins have diverse sugar-peptide bonds

Spiro (2002) Glycobiol 12, 43R

process by which a glycosylphosphatidylinositol (GPI) anchor is added to a protein.

Unusual sugars (FucNAc) N-acetylfucosamine (2-acetamido-2,6-dideoxy-d-galactose); (Bac) bacillosamine (2,4-diamino-2,4,6-trideoxy-d-glucose); (Pse) pseudaminic acid (5,7-diacetamido-3,5,7,9-tetradeoxy-1-glycero-1-manno-nonulosonic acid); (Hyl) hydroxylysine; (Hyp) hydroxyproline; (C-term) carboxy-terminal amino acid residue.
- \( \beta \)-GlcNAc in amide linkage to asparagine
- Consensus sequon: N-X-S/T motif (X not Pro)
- Conserved pentose core: \( \text{Man}_3 \text{Glc}_2 \)
- Three general types: oligomannose (5-9 Man), complex (terminal NeuAc, core Fuc, bi-, tri-, tetra-antenary) & hybrid
  - Complexity increases with sophistication of organism
- N-linked glycoproteins can have 100’s of glycoforms but they all start out the same: \( \text{Glc}_3 \text{Man}_9 \text{GlcNAc}_2 \)

N-Glycosylation by OST

- N-glycan precursor Glc₃Man₉GlcNAc₂ dolichol phosphate is built & transferred *en block* to Asn sequons on nascent peptides by the oligosaccharyltransferase (OST) during co-translational translocation into the ER lumen
  - OST is heterocomplex awaiting full characterization
- Even the first step of N-glycosylation at sequons cannot be predicted
- About 2/3 of secretory proteins have sequons & about 2/3 of these are occupied
The building and transfer of Glc$_3$Man$_9$GlcNAc$_2$ dolichol phosphate highly conserved process in eukaryotic secretory pathway
N-glycan Maturation:

Entire process:
- Build Glc$_3$Man$_9$GlcNAc$_2$-P-Dol
- Transfer by OST
- Fold or Degradation (ERAD)
- Processing (trimming)
- Diversification
- Trafficking/Secretion → Function
Maturation of N-Glycans Differs

- N-glycans vary based on organism, cell-type, metabolic status, etc.
  - Why is the core always conserved?
O-Glycans (Mucin-type)

Mucin Proteoglycan:

The VNTR (variable number of tandem repeat) region is highly O-glycosylated & extended. Hundreds of O-GalNAc glycans and glycoforms

\[ \text{Mucin linkage:} \]

<table>
<thead>
<tr>
<th>Core</th>
<th>X</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β₁ -Gal</td>
<td>H</td>
</tr>
<tr>
<td>2</td>
<td>β₁ -Gal</td>
<td>β₁ -GlcNAc</td>
</tr>
<tr>
<td>3</td>
<td>β₁ -GlcNAc</td>
<td>H</td>
</tr>
<tr>
<td>4</td>
<td>β₁ -GlcNAc</td>
<td>β₁ -GlcNAc</td>
</tr>
<tr>
<td>5</td>
<td>α₁ -GalNAc</td>
<td>H</td>
</tr>
<tr>
<td>6</td>
<td>H</td>
<td>β₁ -GlcNAc</td>
</tr>
<tr>
<td>7</td>
<td>H</td>
<td>α₁ -GalNAc</td>
</tr>
<tr>
<td>8</td>
<td>α₁ -Gal</td>
<td>H</td>
</tr>
</tbody>
</table>

→ Mucin linkage
- α-GalNAc glycosidic linkage to Ser or Thr
- 20 > polypeptide-N-acetyl-galactosaminyltransferases (ppGalNAcTs)
- No consensus sequon known & complex control mechanisms
  - Prediction tools: O-Glycbase and NetOGLc
- 8 mucin core structures from which more complex structures extend
- Many glycoproteins bear mucin O-linked glycans
  - Mucin-like glycoproteins (GlyCam1, PSGL-1, CD34, CD59)
  - Mucins are heavily O-glycosylated
Mucins are heavily O-glycosylated proteins (50-80% MW)
  - VNTRs (variable tandem repeat): rich in S, T, P (devoid of R)
  - Domains for extended oligimerization
  - Peptide conformation is extended with glycan projections ("bottle-brush")
  - Variable glycoforms & occupancy on same protein structure

Mucins are a large component of the GI tract (mucus membranes)
  - Hydrate & protect epithelial cells (non-penetratable barrier)
  - Display carbohydrate binding epitopes for adhesion & signaling
  - Symbiotic (2-lbs of bacteria) & pathogenic microorganism binding
  - Aberrant glycan structures related to cancer (MUC-1) & disease
Glycosaminoglycans (GAGs)

<table>
<thead>
<tr>
<th>GAG</th>
<th>Structure</th>
<th>Uronic Acid</th>
<th>Hexosamine</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronates</td>
<td><img src="image1" alt="Hyaluronan structure" /></td>
<td>GlcA</td>
<td>GlcNac</td>
<td>synovial fluid, vitreous humor, ECM of connective tissue</td>
</tr>
<tr>
<td>Chondroitin sulfate (CS)</td>
<td><img src="image2" alt="Chondroitin sulfate structure" /></td>
<td>GlcA, GlcA&lt;sub&gt;2S&lt;/sub&gt;</td>
<td>GalNAc&lt;sub&gt;4S&lt;/sub&gt;, GalNAc&lt;sub&gt;6S&lt;/sub&gt;, GalNAc&lt;sub&gt;6S,6S&lt;/sub&gt;</td>
<td>cartilage, bone, aorta, neural cells, basement membranes, connective tissue cells, endothelial cells, lymphocytes, brain, myeloid cells</td>
</tr>
<tr>
<td>Dermatan sulfate (DS)</td>
<td><img src="image3" alt="Dermatan sulfate structure" /></td>
<td>GlcA, IdoA, IdoA&lt;sub&gt;2S&lt;/sub&gt;</td>
<td>GalNAc&lt;sub&gt;4S&lt;/sub&gt;, GalNAc&lt;sub&gt;6S&lt;/sub&gt;, GalNAc&lt;sub&gt;6S,6S&lt;/sub&gt;</td>
<td>skin, blood vessels, heart valves</td>
</tr>
<tr>
<td>Keratan sulfate (KS)</td>
<td><img src="image4" alt="Keratan sulfate structure" /></td>
<td>Gal, Gal&lt;sub&gt;6S&lt;/sub&gt;</td>
<td>GlcNac, GlcNac&lt;sub&gt;6S&lt;/sub&gt;</td>
<td>cornea, bone, cartilage, CNS, synaptic vesicles</td>
</tr>
<tr>
<td>Heparan sulfate/heparin (HS)</td>
<td><img src="image5" alt="Heparan sulfate/heparin structure" /></td>
<td>GlcA, IdoA, IdoA&lt;sub&gt;2S&lt;/sub&gt;</td>
<td>GlcNac&lt;sub&gt;NS&lt;/sub&gt;, GlcNac&lt;sub&gt;NS,6S&lt;/sub&gt;, GlcNac&lt;sub&gt;NS,3S,6S&lt;/sub&gt;</td>
<td>basement membranes, neuromuscular junctions, epithelial cells, fibroblasts, mast cells</td>
</tr>
</tbody>
</table>

- GAGs are anionic polymers of repeating disaccharide units—comprised of a uronic acid (U) and a hexose (H)—made heterogeneous by varying linkage, length, epimerization, N-acetylation, and N- and O-sulfation (no sulfation for Hyaluronates). 5 classes
- Heparin is a highly sulfated form HS found in mast cells
- GAGs are also commonly known as the mucopolysaccharides
Proteoglycans: GAGs linked to proteins

Families of ECM proteoglycans

- Protein core mainly displays GAGs (brush-like appearance)
- Many families of Proteoglycans which are in the ECM or membrane-bound
  - diverse protein sequence & GAG occupancy, identity, and sequence
- GAGs chains are the business-end with highly charged linear structures
  - Important structural components of connective tissues (ECM)
  - hydrated gels, non-compressive
  - Provide binding sites which regulate cell proliferation & regeneration
GAGs Bind Proteins in ECM

- Cell-ECM & cell-cell attachment/interactions
- ECM complexes present many binding epitopes
  - Sequester proteins from receptors
  - Present proteins to receptors
  - GAG co-receptor signaling complexes
  - Spatial & temporal protein gradients
- Anti-thrombin HS epitope prevents blood clotting
  - HS is a pharmaceutical product
  - Deaths resulting from CS contamination!
Sialylation

- Key binding determinant & masking element → regulation of cell activity
  - Selectins, bacterial adhesions, viral hemagglutinins (influenza)
  - Siglecs—a large class of animal Sialic acid binding protiens
- Hyper & aberrant sialylation of glycoconjugates is a hallmark of cancer
- Family of over ~20 different enzymes to catalyze various linkages
  - $\alpha_2\text{-}3$ NueAc (> six different $\alpha_2\text{-}3$ sialylTs)—sialylate Gal residues
  - $\alpha_2\text{-}6$ NueAc (> six different $\alpha_2\text{-}6$ sialylTs)—sialylate Gal, GalNAc, & GlcNAc
  - $\alpha_2\text{-}8$ NueAc (> 5 different $\alpha_2\text{-}8$ sialylTs)—polysialic acid (PSA) linear polymer
Figure 5. Sialidase regulation of signal transduction. a) EGFR signaling is reduced in the presence of ganglioside GM3. NEU3-catalyzed conversion of GM3 to LacCer relieves this inhibition. b) NEU1 forms a cell surface receptor complex with PPCA and EBP. In the presence of elastin peptides, the receptor complex is activated leading to downstream ERK1/2 activation. In addition, the active complex enhances the catalytic activity of NEU1, leading to desialylation of microfibrillar glycoproteins and local release of elastin peptides. Liberated sialic acid may act as a second messenger that activates other signaling events. c) Sialylated TRPV5 is rapidly endocytosed from the cell membrane. Desialylation of TRPV5 by KL exposes underlying galactose residues that bind galectin-1, thereby retaining TRPV5 on the cell surface where it functions in Ca^{2+} transport.
Sulfate: Sulfotransferases & Sulfatases

Complementary enzymes that control sulfation state

Sulfate is another key binding determinant
- unique geometric & ionic binding characteristics for recognition
Sulfate-regulated GAG Signaling

(a) HSPG → SULF → signaling

(b) HSPG → SULF → signaling
Glycan Binding Proteins (GBPs)
GBP Characteristics

- GBP s recognize & bind glycans to modulate cellular activities
  - originally found in plants (lectins) \( \rightarrow \) now known in all organisms
  - distinguished from antibodies (agglutinating behavior)
  - distinguished from catalytic glycomodifying units
- Carbohydrate-recognition domains (CRDs)
  - interact with 1-4 sugar residues
  - may include aglycone components
  - high specificity but low affinity (\( K_d \sim \mu M-mM \) range)

→ Increase binding & modulate activity through multivalent mechanisms

Animal Lectin CRDs:
- GL = galectins (S-type)—Gal & LN
- CL = C-type—calcium dependent (selectins)
- MP = P-type (Man-6-P receptors)
- IL = I-type (siglecs)
General Constellation of Recognition

→ Common three-residue constellation for the recognition of a common hexose
  • OH recognition: frequently H-bonds to Asp & Asn
  • Aromatic stacking with hydrophobic underbelly of sugar (Phe, Tyr, Trp)
  • Recognition for different structures by placement of residues (specificity)

Sharon & Lis in “Lectins”
Kluwer Academic Publishers

Figure 2. Superposition of β-galactose in the combining site of ECorL on α-glucose in the combining site of LOL-1. Note that the galactose is hydrogen-bonded to the conserved combining site residues via its 3- and 4-OH, whereas the glucose is bonded to the same residues via its 4- and 6-OH. The stacking of the ligands on the combining site phenylalanine of the respective lectins is also evident.
GAG-binding Proteins

GAG-binding proteins are different

- Epitopes ≥ five residues
- Binding driven more by electrostatic interactions between negatively charged sulfate & carboxylate groups on GAG with extended Lys/Arg regions on protein
- Affinity can be high
  - $K_d$ values = nM to µM range
- Linear sequence specificity is more relaxed

**FIGURE 35.2**

Anti-thrombin-thrombin binding. (Solid lines) Electrostatic interactions between positively charged residues and sulfate groups; (broken lines) hydrogen bonds; (alternately broken and solid line) bridging water molecule.

**FIGURE 34.2**

HS-binding domain on dengue virus envelope protein
Chemical Glycosylation

- Donor: fully protected sugar selectively activated for attack at anomeric center
- Acceptor: sugar with a free OH to perform nucleophilic attack
  - Regioselective control via protecting groups (P)—only on free OH
  - Note: strict requirement for anhydrous conditions or water will attack!
- Stereocontrol of glycosidic bond (if any!)
  - C2 armed—no participation, quick, little stereocontrol (SN1-like)
  - C2 disarmed—destabilize glycosyl cation via electron withdrawal (SN2-like)
  - C2 assistance—captures glycosyl cation & blocks one face (SN2-like)
  - *Complete control: only by enzymes!*
Thioglycoside Method

Development by (Garegg '87, Fraser-Reid, van Boom, Sinay):

- very stable, often used as anomic protecting groups
- selective activation by soft electrophiles (NIS, MeOTf, IDCP, DMST)
- readily prepared from corresponding glycosyl acetate, thiol & Lewis Acid
- readily converted into other donors (e.g., DAST to fluoride or oxidation to sulfoxide)
- orthogonal to glycosyl fluorides & glycosyl sulfoxides
- To favor the 1,2-trans product: use catalytic triflic anhydride or run in AcCN (Sinay)
  - also, activation with BF$_3$•Et$_2$O

 Thioglycosyide

sulfonium
leaving group

favors SN1
Sufoxide Method

Developed by Kahne (‘89):

- oxidation of thioglycoside with mCPBA to yield sulfoxide
- hydrolytically stable and can be stored
- activate with triflic anhydride
- very reactive under mildest conditions
- orthogonal to thioglycosides
Trichloacetimidate Method

Developed by Schmidt (‘94):

- glycosyl imidate
- favor SN2
- inversion

- reaction is reliable & high yielding
- good leaving group but much more stable than the halides
- donors easy to prepare starting from a fully protected reducing sugar
  - α-imidate using Cl₃AcN & NaH
  - β-imidate using Cl₃AcN & K₂CO₃
- manipulate conditions to favor SN2
- form transient imidates by running glycosylation in AcCN (Sinay)—favor β
  - again see Crich for insitu triflate aguments
Enzymatic Synthesis of Oligosaccharides

Benefits of enzymatic oligosaccharide synthesis
1. Precise control of stereo- & regiochemistry
2. No need for protecting groups

Present limitations of enzymatic oligosaccharide synthesis
1. The nucleotide sugars for GTs are expensive
2. Not all enzymes are available in recombinant form (many are difficult)
3. Unnatural sugars are not accepted, making access to modified oligosaccharides troublesome (e.g., for SARs)
4. Most GTs are inhibited by high concentrations of the nucleotide product; thus, large scale synthesis is difficult
   - One solution: co-factor recycling schemes—see review

For a comprehensive review of enzymes in oligosaccharide synthesis:
Glycoprotein Remodeling

Remodel a glycoforms using enzymes glycosidase
- Many enzymes are commercially available for N-glycans
- Removal of the core fucose is not possible!

Also truncated & enzymatically re-elaborated to a new structure