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- Not included in this group meeting: Biomass to feedstock, Enzymatic glycosylation, Synthetic carbohydrate polymers, Carbohydrates as chirons (Martinez, 2014), Nucleoside Chemistry (O’Hara, 2012; Gianatassio, 2013)

**History:**
- **-10,000 BC:** Primitive sugarcane processing in New Guinea
- **-1500 BC:** Cotton cloth from India spreads to Persia and China
- **1891:** Fischer reports the structure of glucose, mannose, fructose, and arabinose
- **1929:** Haworth proposes ring structures for pyranose/furanose
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- **1956-1963:**
  - Bacterial peptidoglycan backbone structure
  - Major natural polysaccharides (chitin, cellulose, peptidoglycan) are β(1→4) linked
  - Mechanism of peptidoglycan biosynthesis and penicillin action
- **1972:** High field NMR for glycan structure analysis

**What is a Carb?** - Carbohydrates are defined as "hydrates of carbon" polyhydroxylaldehydes, polyhydroxyketones and their derivatives

**Alpha vs. Beta (Starch vs. Cellulose):**
- **Starch** - Most common carbohydrate in human diets (potatoes, wheat, maize (corn), rice, and cassava)
  - Water soluble polysaccharide
  - Highly branched polymer of glucose
  - Linear glucose with α(1→4) glycosidic bonds.
  - Branching α(1→6) bonds occurring every 24 to 30 glucose units

- **Cellulose** - Most abundant biomass on Earth. Main component of the primary cell wall of green plants, algae and some bacteria (biofilms)
  - Cotton, wood, hemp, etc.
  - Linear glucose with β(1→4)-glycosidic bonds.
  - No coiling or branching

**Other definitions:**
- Monosaccharides = carbs that cannot be hydrolyzed into a simpler form
- Polysaccharide = repeating oligosaccharides
- Glycan = general term for any of these...
- Often encountered conjugated, >50% of all proteins are glycosylated
- Glycoconjugate = mono/oligosaccharide attached to a non-carbohydrate moiety (agalcyone)

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Carbs

Monomeric Building Blocks
Letters of the alphabet

DNA
4 canonical nucleobases

H2NCOOH
Peptides
20 proteogenic AA's

OH
Carbohydrates

Only 36 building blocks are needed to construct 75% of the 3299 mammalian oligosaccharides.

Mammalian glycan structures comprise 78% of all the eukaryotic glycans

Oligomeric Biomolecules
Written Novel

5' -displace X
-oxidation

Linear
Modification results in similar stereochimical complexity

Simple PG strategy
3'→5' or C→N

Achiral link

Linear and Branched

OH

5 potential sites for reaction

Chiral link

Central Dogma of Molecular Biology:
Transcription → Translation → Proteins

DNA → RNA → Proteins

"DNA Chemistry" (Peters, 2017)

Replication
DNA Duplication

RNA Information

Translation
RNA Synthesis

Protein Information

Translation
Protein Synthesis

"Highlights in Peptide and Protein Synthesis" (Mallins, 2016)

Cell Surface
Signaling
Mobility/membrane movement (charge distribution)

Glycans are abundantly expressed on the outer-surface of prokaryote and eukaryote cells, creating a landscape of recognition sites, barriers and transporters

Diversity: Hexamer

Nucleotide* = 4^6 = 4096
Peptide* = 20^6 = 64 million

Carbohydrate** = 192 billion


*Assuming natural phosphodiester/amide linkages
**based on the "ten common" mammal monosaccharides

Carbs

Glycosyl Donors:

Y = potential leaving group

X = Leaving Group
R = Protecting Group
Z = participating or non-participating group

Glycosyl Acceptors:

Promotor (P)
Solvent (Ignored)

Donor (D)
Acceptor (A)
Disaccharide (D-A)

Two ways to set up reaction:

1) Normal Procedure (disarmed donors)
2) Inverse Procedure (armed donors)

PG Strategies:

Selective 1° protection
(Bulky, TBS, Trt, 0 °C, limiting reagents, etc.)

Selective 2° protection,
Bu₂SnO, R-X

Highly dependent on stereochem
Hemiacetal (H⁺, ROH)

2° equatorial. > 2° axial.

cis/trans vicinal diols (acetal formation)

Two step monomer:

Selective 2° protection,
Bu₂SnO, R-X

Carbonylation

100 °C, 1 hr.

Ac₂O

Esters induce slight positive charge, oxonium formation is slower
Not always the case!

1. Acetone, H₂SO₄
2. TsCl, pyridine
3. NaBH₄, DMSO
4. H⁺, DMSO
5. Bu₂SnH, NaH
6. TBSCI, Pyridine
7. H₂, Pd/C

PG Migration:

Epimerizations:

Interesting transformations:

Kilian Fischer Synthesis:

Wohl Degradation:

Modern Approach: Total Synthesis of the L-Hexoses - K. B. Sharpless

*Since the mirror image of every compound in Fig. 2 can be prepared by simple exchange of the chiral ligand in the AE reaction, the formal synthesis of the D-hexoses has also been achieved*

Works well for:

Selective 1° protection
(Bulky, TBS, Trt, 0 °C, limiting reagents, etc.)

Highly dependent on stereochem

Hemiacetal (H⁺, ROH)

Proposed glucose
H-bonding in CHCl₃

### Generalizations made independent of substitution and stereochem @ C-(3 to 6)

<table>
<thead>
<tr>
<th>1,2-cis</th>
<th>1,2-trans</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-D-glycero: allo-, gluco-, gulo-, and galactopyranosides&lt;br&gt;2-deoxy-glycosides</td>
<td>1,2-cis</td>
</tr>
<tr>
<td>2-L-glycero: altro-, manno-, ido-, and talopyranosides</td>
<td>2-keto-3-deoxy-ulosonic acids</td>
</tr>
</tbody>
</table>

### Participating Group at C-2? 1,2-trans rule!

- **Participating group** (Ester or Amide):<br>\[
\begin{align*}
&\text{X} \\
&\text{R-Cl} \\
&\text{ROH} \\
\end{align*}
\]
  - Blocks bottom face
  - \(\text{R-OH}\) blocks top face
- **Non-Participating Group** (Ether or Azide):<br>\[
\begin{align*}
&\text{X} \\
&\text{OBn} \\
\end{align*}
\]
  - \(\text{X} = \text{H}, \text{OTBS}, \text{OH}, \text{OTf} \)

### Non-Participating Group at C-2? 1,2-cis rule? (try again)

- **Participating group** (Ester or Amide):<br>\[
\begin{align*}
&\text{X} \\
&\text{R-Cl} \\
&\text{ROH} \\
\end{align*}
\]
  - Blocks top face
  - \(\text{R-OH}\) blocks bottom face
- **Non-Participating Group** (Ether or Azide):<br>\[
\begin{align*}
&\text{X} \\
&\text{OBn} \\
\end{align*}
\]
  - \(\text{X} = \text{H}, \text{OTBS}, \text{OH}, \text{OTf} \)
  - In the absence of a participating group, you must fight the anomeric effect.

### Glycosyl Halides - \(\alpha\)-selective

<table>
<thead>
<tr>
<th>Glycosyl Halides</th>
<th>(\beta)-Mannosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Lemieux]</td>
<td>[Paulsen]</td>
</tr>
<tr>
<td>[\text{Br} \rightarrow \text{R-OH}]</td>
<td>[\text{Br} \rightarrow \text{R-OH}]</td>
</tr>
<tr>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td>(\beta)-Mannosides:</td>
<td>(\beta)-Mannosides:</td>
</tr>
<tr>
<td>i. (\text{R-X}), insoluble Ag&lt;br&gt;ii. Privileged donors&lt;br&gt;iii. Epimerization&lt;br&gt;iv. IAD</td>
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</tr>
<tr>
<td>(good for fucose, galactose, fructose)</td>
<td>(mainly (\beta)-Mannosides)</td>
</tr>
</tbody>
</table>

### Solvent Effects:

<table>
<thead>
<tr>
<th>Solvent Effects</th>
<th>(\alpha)-glycosidation</th>
<th>(\beta)-glycosidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Reverse Anomeric Effect”</td>
<td>(\text{Et}_2\text{O})</td>
<td>(\text{MeCN})</td>
</tr>
<tr>
<td>“The Nitrile Effect”</td>
<td>3 (\text{MeCN}) molecules form a (\beta)-nitrilium-nitrile</td>
<td></td>
</tr>
</tbody>
</table>

### Non-participating group at C-2:

- **Insoluble silver salts, silver silicate or silver oxide work**


- J. Am. Chem. Soc. 1992, 114, 1087

β1-4-glycosyltransferase
aqueous buffer, M²⁺

Uses DMT for colorimetric loading analysis
Cleavage: (Cross-Metathesis)

Limited by number of available glycosyltransferases (GT)

Free hydroxyl generated
(Capping = double rxn time)

Long times / cold temperatures

Enzymatic:
- uses unprotected donor/acceptors
- high d.r, 10⁵-10⁸ rate accelerations
- No linkers, immobilized enzymes

Standard SPPS
Cleavage: (O₂)

Solid-Phase:
Based on pioneering work of Merrifield
General Requirements:
-C-1 reactive LG
-temporary vs. persistent PG’s

BnO
ONBz

1) 0.2 M NaOEt, EtOH, 30 min
2) 1:4 resin/monomer, PhH, 60 hr in the dark
75%

BnO
ONBz

1) DMDO, Acetone, 0°C
90 min, repeat 40 min
2) A, ZnCl₂, THF, 0°C - rt
8 hrs

5% yield, 102 steps - average 97.1% each

100 Benzyl Ethers - several treatments (72-96 hr) H₂ Pd/C

8 Solvents
16 reagents
-40 to +80°C

Homemade Synthesizer
(Max Planck)

The Glyconeer®
(Glyconiverse)

Nature, 2001, 1523
Science, 2002, 785

“ There are no universal reaction conditions for oligosaccharides synthesis” - Hans Paulsen


There are no universal reaction conditions for oligosaccharides synthesis” - Hans Paulsen

Carbs

Solid-Phase (contd):

Science 2001, 291, 1523

O\(\text{Bn}\)O\(\text{Bn}\)Piv

OLev

O

Carbs

Baran Group Meeting

2/10/18

Solution-Phase One Pot:


P(\text{OBu})_2

O

P(\text{OBu})_2

O

Armed/Disarmed Theory:

Fraser-Reid, B. J. Am. Chem. Soc. 1988, 110, 2662

Protecting Group Control (Constant L)

Anomeric Control (L_1>L_2)

Relative Reactivity Value (RRV)

Estimate OptiMer database would only need ~500 building blocks analyzed to prepare most bioactive saccharides.

Suitable for 3-6 length saccharides per one-pot reaction

Automated (Seeberger)

10 machine hours 80% yield Science 2001, 291, 1523

Manual (Nicolaou)

122 reaction hours 20% yield J. Am. Chem. Soc. 1997, 119, 449


Minimizes PG manipulations

1) Mix 1 + 2 + 3

2) Deprotect

63% for couplings
20% overall

OptiMer

1) Mix a + b + c

2) PG Manipulations

67% for couplings

RRV = 0

RRV = 13

RRV = 72,000

RRV = 4000

RRV = 850

RRV = 6500

RRV = 6,000

RRV = 32,000

RRV = 13

RRV = 4000

RRV = 850

RRV = 6,000

RRV = 32,000

RRV = 13

RRV = 4000

RRV = 850

RRV = 6,000

RRV = 32,000

RRV = 13

2nd gen route 83% yield

OptiMer

Minimizes PG manipulations

1) Mix 1 + 2 + 3

2) Deprotect

63% for couplings
20% overall

OptiMer

1) Mix a + b + c

2) PG Manipulations

67% for couplings

Suitable for 3-6 length saccharides per one-pot reaction

RRV = 6

RRV = 0

RRV = 6500

RRV = 302

RRV = 23

RRV = 302

RRV = 23

RRV = 302

RRV = 23
Carbs

Negative Free Energy of Binding
i. Hydrogen Bonding
ii. Association with Metals
iii. Hydrophobic packing
iv. Ionic interactions

Hydrogen Bonding:
- H-Bond residues: Aspartic acid, Asparagine, Glutamate, Glutamine, Arginine

Hydrophobic packing

Glycan Code:
- Increases diversity in the proteome
  i. Glycosidic Bond (site of linkage, stereo)
  ii. Sugar Type
  iii. Branched/Linear
  iv. Length

β-cyclodextrin
- Entraps hydrophilic compounds = Febreze
- Solubilizes hydrophobic compounds

Glycoconjugates:
- i. N-Linked - asparagine, arginine
- ii. O-Linked - serine, threonine, tyrosine

Blood types:
- Determined by GT's = present,
- Creates a barrier to zoonotic transmission of viruses

Advanced Glycation End Product (AGE):
- Lysine
- Arginine
- Glucosepane

Glucose Metabolism:
- After digestion of food (poly to monosaccharides):
  Normal glucose level =
  i. Glycolysis
  (I) Glucose conversion to oxalate, NADH, ATP.
  ii. Glycogen-isis/lysis
  (II) Saturates glycolysis pathway, glucose stored as Glycogen
  (III) Activates Polyol Pathway, (rapidly consuming NADPH) leads to cell damage.

High glucose levels (poor diet, diabetes)=
- Saturates glycolysis pathway, glucose stored as Glycogen
- Activates Polyol Pathway, (rapidly consuming NADPH) leads to cell damage.

Other monosaccharides:
- (Fructose, Galactose) go to liver;
- Converted to glyceraldehyde
- Converted to glucose, then metabolized

Immune System:
- Cell to cell adhesion
- Monoclonal antibodies

Antigen:
- i. peptide
  ii. oligosaccharide
  iii. lipid

Antibody:

Other monosaccharides:

Over 40 glycosylation disorders identified in humans
- No treatment for any
- 80% in CNS

TABLE 2. Abundance of monosaccharides and terminal monosaccharide moieties in mammalian carbohydrates

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>Abundance (%)</th>
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<tbody>
<tr>
<td>α-GlcNAc</td>
<td>31.8</td>
<td>N-Acetylneuraminic acid (Neu5Ac)</td>
<td>8.3</td>
</tr>
<tr>
<td>α-Gal</td>
<td>24.8</td>
<td>N-Acetylneuraminic acid (Neu5Ac)</td>
<td>7.2</td>
</tr>
<tr>
<td>α-Man</td>
<td>18.9</td>
<td>N-Acetylglactosamine (GalNAc)</td>
<td>4.8</td>
</tr>
<tr>
<td>α-Sia*</td>
<td>8.3</td>
<td>α-GalNAc</td>
<td>2.5</td>
</tr>
<tr>
<td>l-Fuc*</td>
<td>7.2</td>
<td>α-Glc</td>
<td>0.3</td>
</tr>
<tr>
<td>l-Glc</td>
<td>2.5</td>
<td>α-Gal</td>
<td>0.1</td>
</tr>
<tr>
<td>α-Rha</td>
<td>0.1</td>
<td>α-Man</td>
<td>0.1</td>
</tr>
<tr>
<td>Others</td>
<td>1.2</td>
<td>α-Sia</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*derivatives = sialic acids (α-Sia)

"All Altruists Gladly Make Gum in Gallon Tanks"; allose, altrose, glucose, mannose, gulose, idose, galactose, talose.