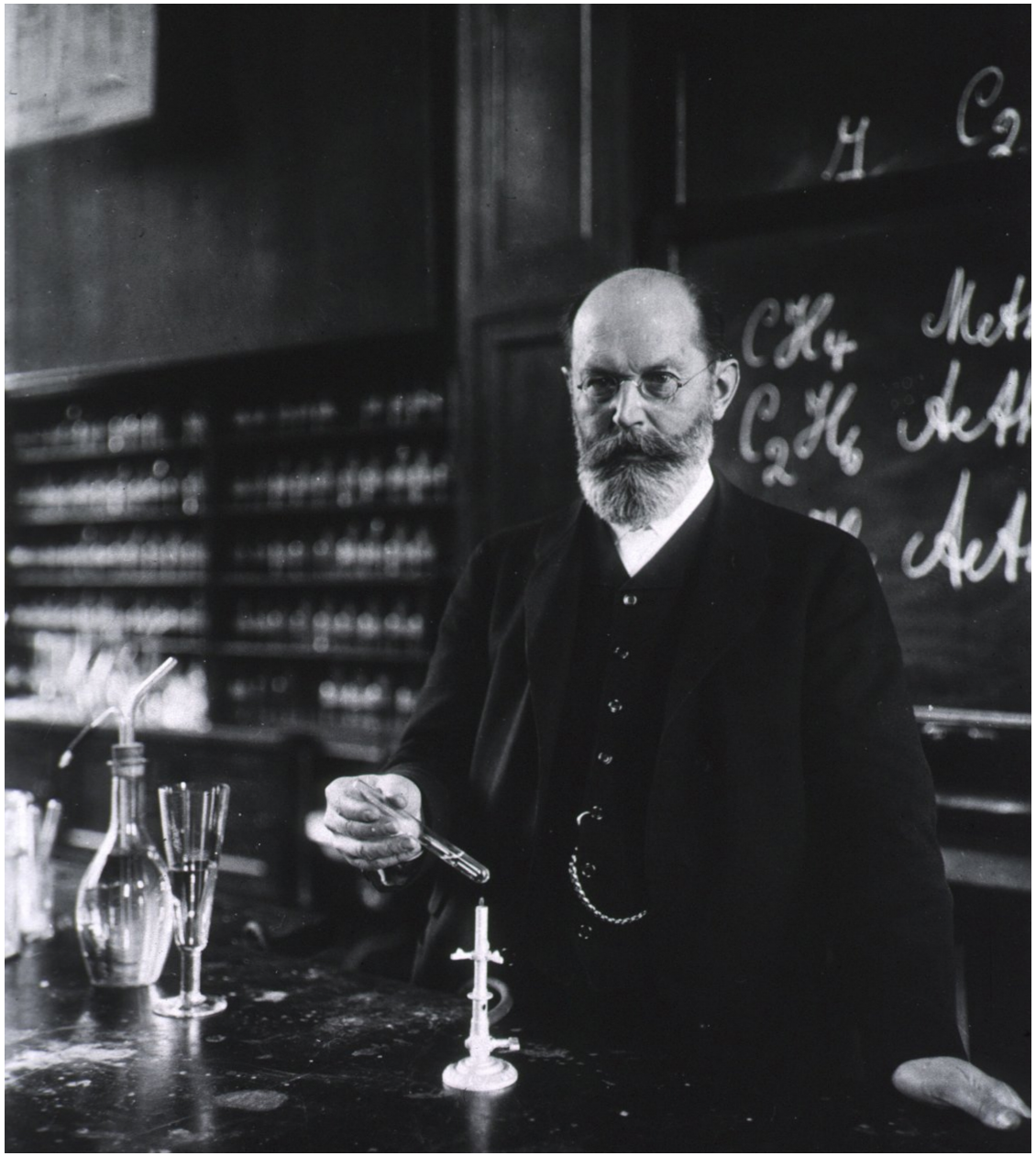


Carbohydrate chemistry – *the formation of glycosidic bonds*

“Their study also dates back to the early beginnings of organic chemistry and has persistently been conducted with such lively enthusiasm that the present state of knowledge in this group may even be used as a yardstick for the discipline as a whole.”
- Emil Fischer, Nobel Lecture (1902)

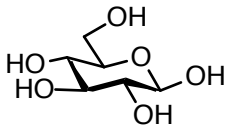
Sophie Shevick

Glycosidation Chemistry

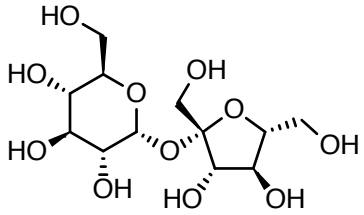
Helpful references:
Boons, G-J. and Hale, K.J. (2000). *Organic Synthesis with Carbohydrates*
Hanessian, S. (1997). *Preparative Carbohydrate Chemistry*.
Zhu, X. and Schmidt, R. *ACIE*, 2009, *48*, 1900-2934.

What I will not cover:
Chemoenzymatic transformations
Synthesis of unnatural sugars
Bioconjugation chemistry
N- or C- glycosylation

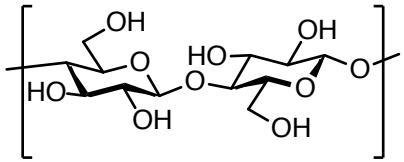
Carbohydrates…
as fundamental building blocks in nature



D-glucose
energy storage

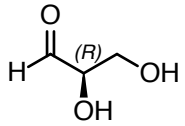


sucrose
sugar cane/beets

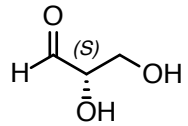


cellulose
300 - 10,000 units
green plant cell walls

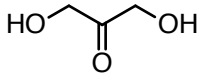
Carbohydrates…
are polyhydroxylated aldehydes and ketones



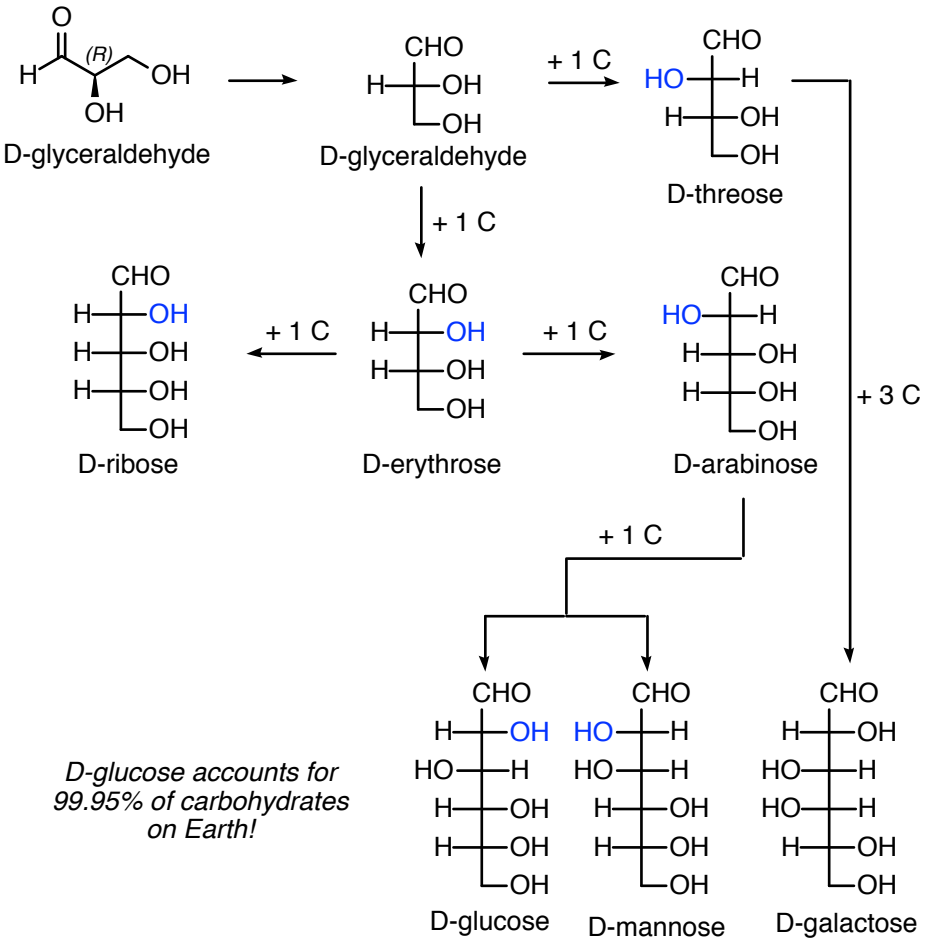
D-glyceraldehyde



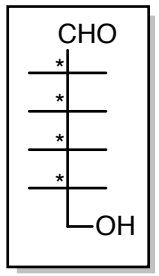
L-glyceraldehyde



dihydroxyacetone

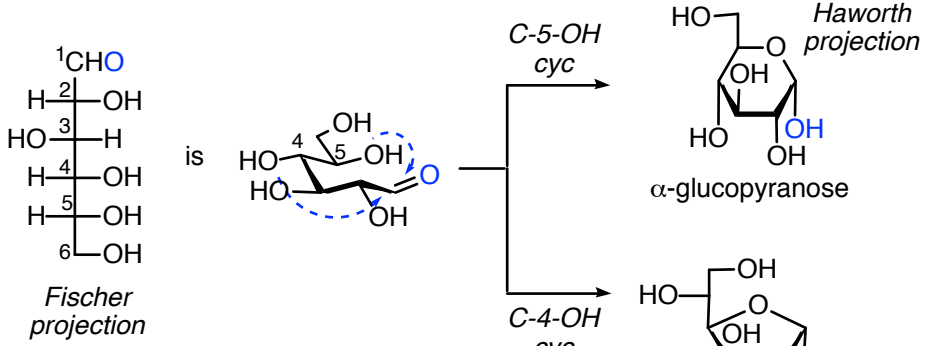


D-glucose accounts for 99.95% of carbohydrates on Earth!

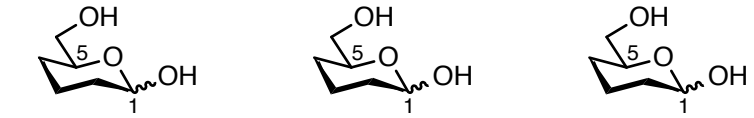


2^4 possible isomers = 16 D-/L-isomers
8 hexoses: glucose, mannose, galactose, allose, altrose, gulose, idose, talose.

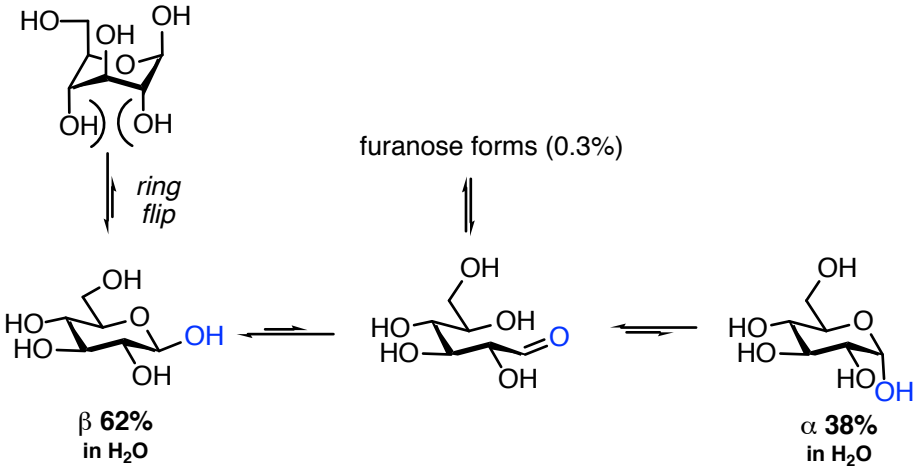
Glycosidation Chemistry



You fill it in!



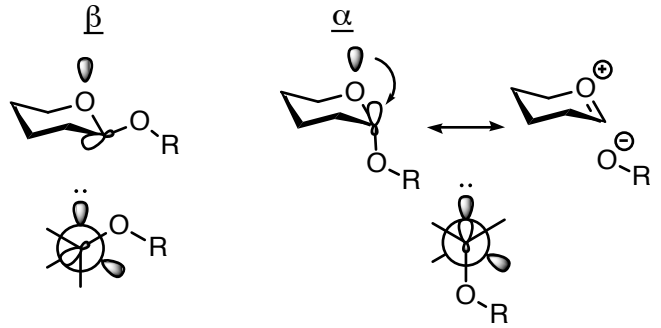
Mutarotation - interconversion of anomers



Anomeric Effect

α > β
stable

1) hyperconjugation stabilized α anomer

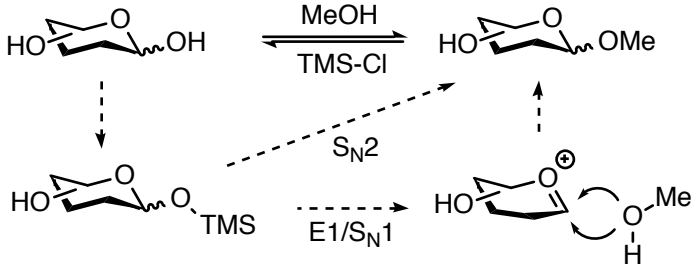


2) lone-pair repulsion destabilizes β anomer

3) dependent on solvent/temperature

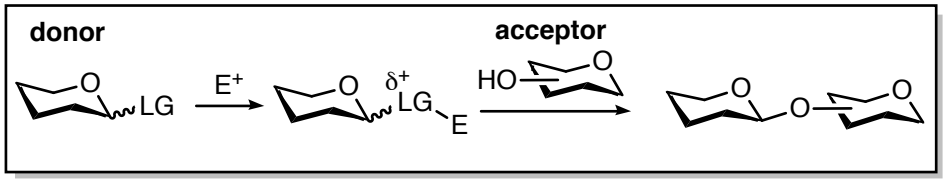
"half of carbohydrate chemistry is situated at the anomeric carbon," Sir Derek Barton (*indirect quote*, Hanessian, p. 263)

Fischer-Helferich esterification (1893-1895)

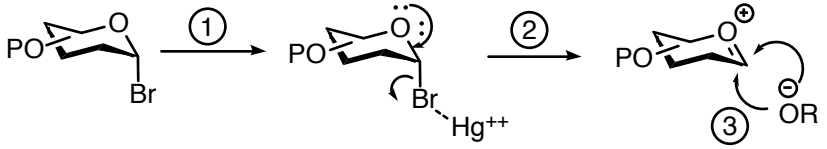


Sophie Shevick

Glycosidation Chemistry



Koenigs and Knörr (1901)



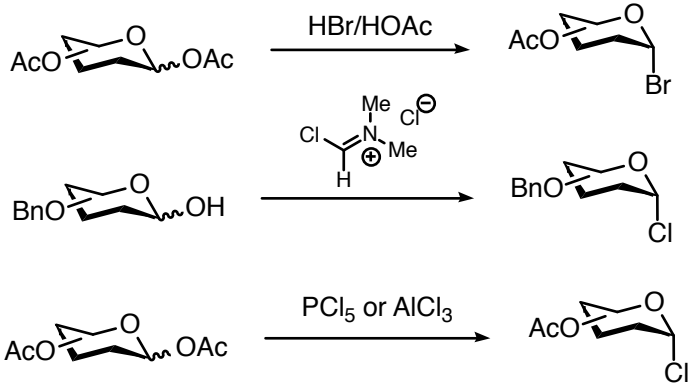
(1) activation by Ag^+, Hg^+
(2) irreversible loss of activated halide
(3) nucleophilic attack by glycosyl acceptor

react via contact ion pair, solv-separated ion pair, or free ion (solv dependent)

Factors that influence α/β ratios include: C-2 substituent, substituents at donor and acceptor, leaving group, promoter, solvent, temperature

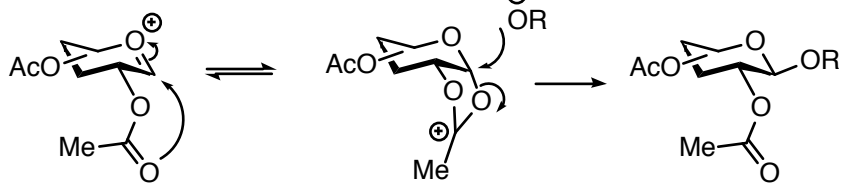
typically yields are low and α-linked glycosides cannot be formed exclusively (max ~50%) *ACIE*, 1974, *13*, 157-216

Synthesis of glycosyl halides:



Controlling anomeric selectivity

Participating protecting group - 1,2-*trans* glycoside



acyloxonium ion

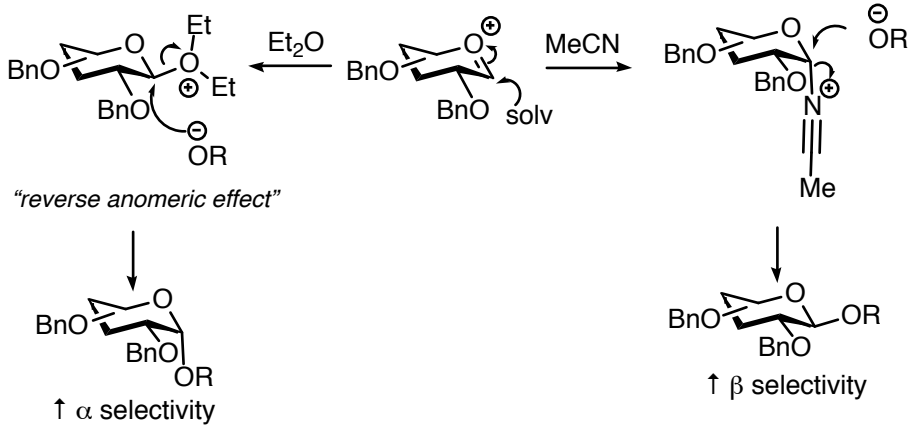
1,2-*trans*-linkage

Non-participating protecting group - 1,2-*cis*-glycoside

$E1/S_N1$

S_N2

Solvent participation -



"reverse anomeric effect"

↑ α selectivity

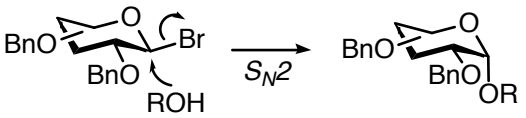
↑ β selectivity

Actual ratios depend on solvent, temperature, activating reagent, and protecting groups used

Glycosyl Halides

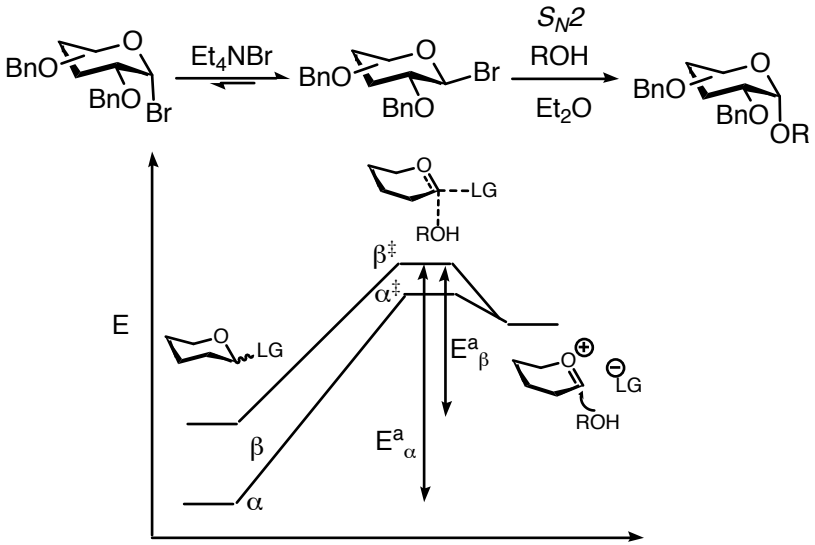
***In situ* anomerization** (Lemieux, 1974)
JACS, 1974, *97*, 4056-4062

*Enabled reliable α-glycosidic bond (1,2-*cis*) synthesis via kinetic control*

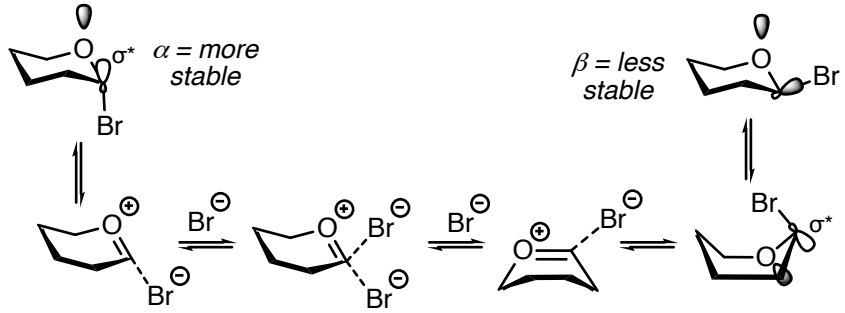


theoretically, α >> β
experimentally, α ~ β

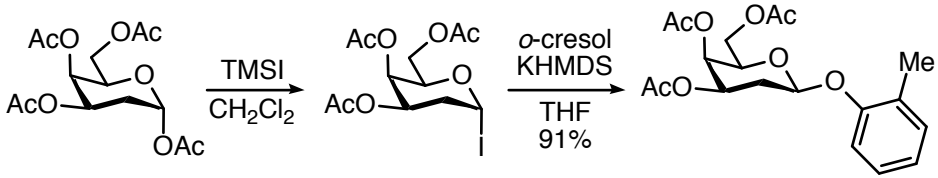
Curtin-Hammett principle: product ratio depends on relative activation energies



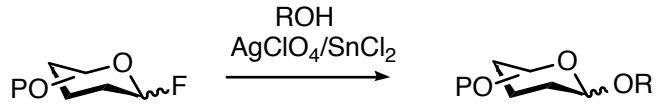
Non-polar solvents necessary to insure S_N2-like pathway



Glycosyl iodides
OL, 2003, *5*, 4219-4222

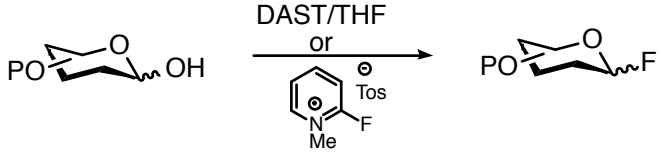


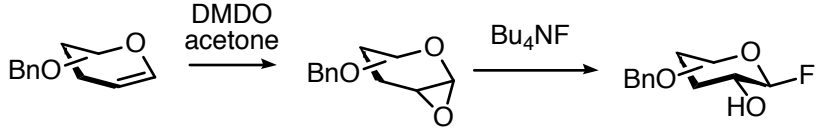
Glycosyl Fluorides (Mukaiyama, *Chem Lett*, 1981, 431-432)
Tet Lett. 1984, *25*, 1379 (Noyori)
Chem. Commun. 1984, 1155 (Nicolau)
Tet Lett. 1989, *30*, 4853 (Suzuki)



Other activators: $AgClO_4/Cp_2HfCl_2$, $AgClO_4/CpZrCl_2$, BF_3*OEt_2

Synthesis of glycosyl fluorides -





Glycosyl halide overview:

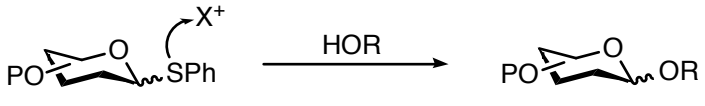
<u>bromide/chloride</u>
- Harsh conditions
- Easily hydrolized
- Thermally unstable, typically generated *in situ*

<u>iodide</u>
- Easily hydrolized
- Thermally unstable, typically generated *in situ*
+ no activating group required

<u>fluoride</u>
+ Mild preparation
+ Shelf/SGC stable
+ many activators available

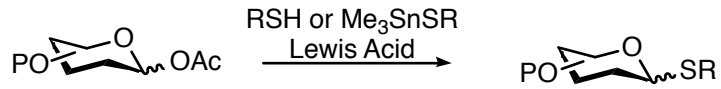
Thioglycosides

Thioglycosides (Fischer, 1909)
Ber Chem, 1909, 42, 1476 – 1482

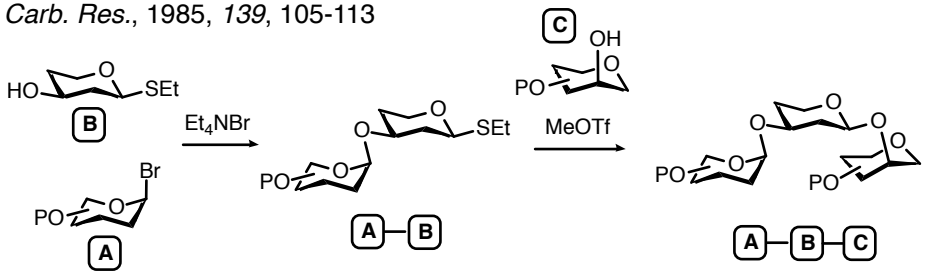


X^+ = MeOTf, DMTST, NIS-TfOH, IDCP, PhSeOTf, TBPA‡

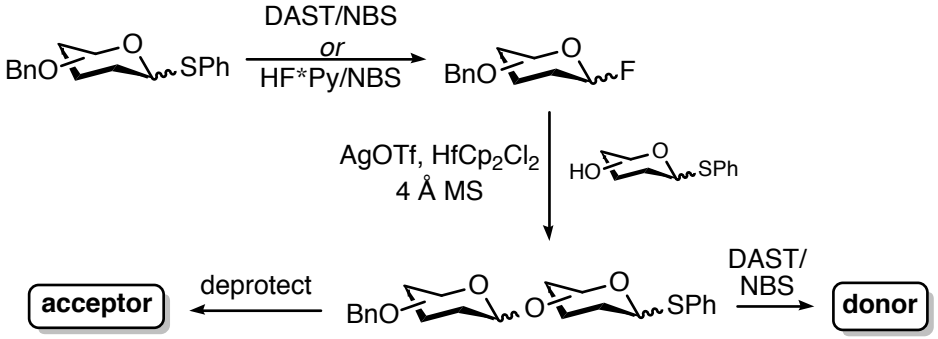
Synthesis of thioglycosides



Convergent block synthesis:
Carb. Res., 1985, *139*, 105-113

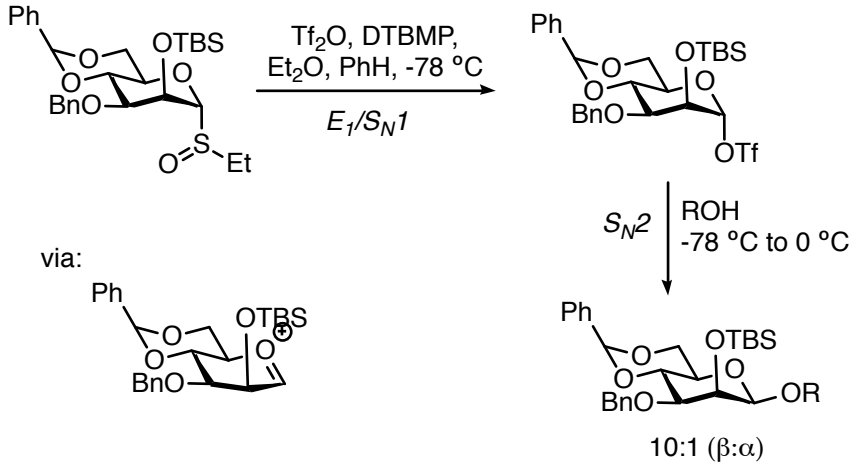


"Two-stage activation" (Nicolau, 1991)
Pure Appl. Chem., 1991, *63*, 555-560



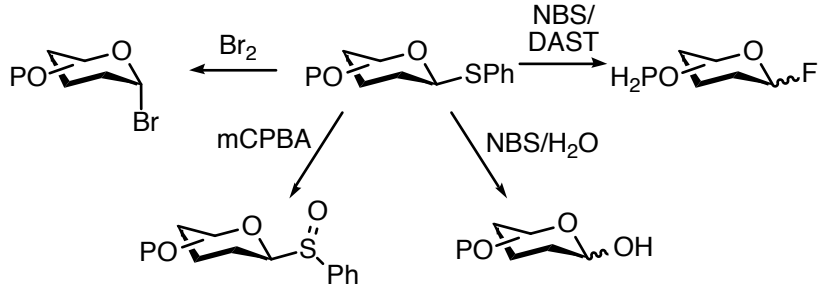
See: Rynchosporosides (*JACS*, 1985, *107*, 5556-5558)

Kahne glycosidation (1989)
JACS, 1989, *111*, 6881-6882
Crich β-mannosylation (*JOC,* 1996, *61*, 4506-4507)



PMB group locks conformation to ensure α-triflate formation

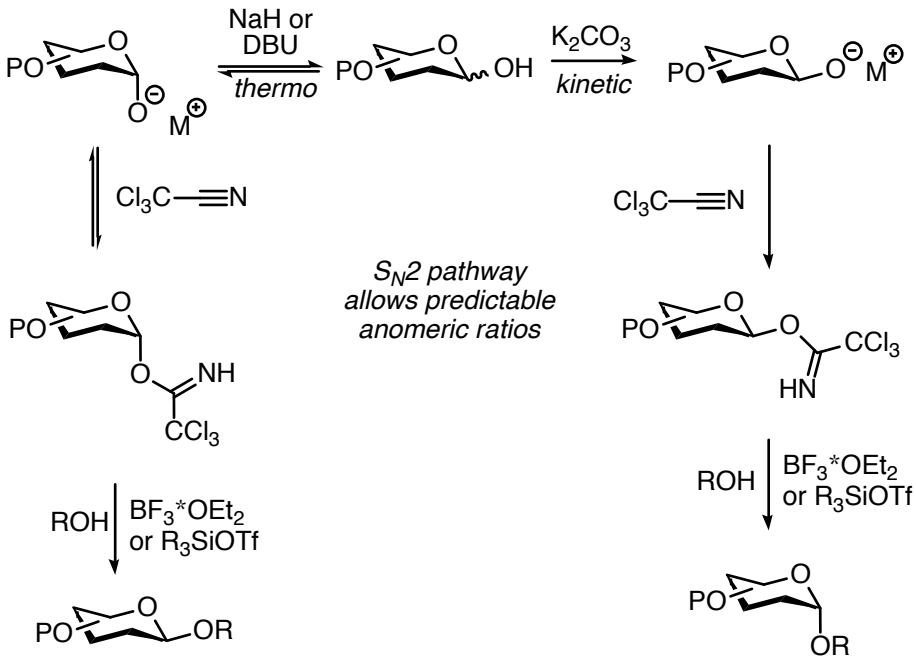
Thioglycoside overview:
+ can be converted into other donors depending on goal



+ stable to many chemical transformations
+ easy to synthesize/purify
+ many activators available

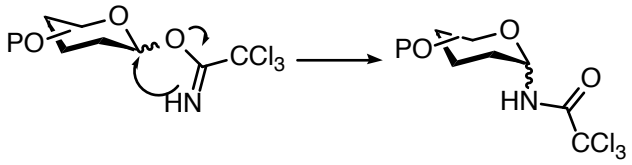
Trichloroacetimidates

Trichloroacetimide donors (Schmidt, 1980)
ACIE, 1980, *92*, 763-764
ACIE, 1986, *25*, 212-235



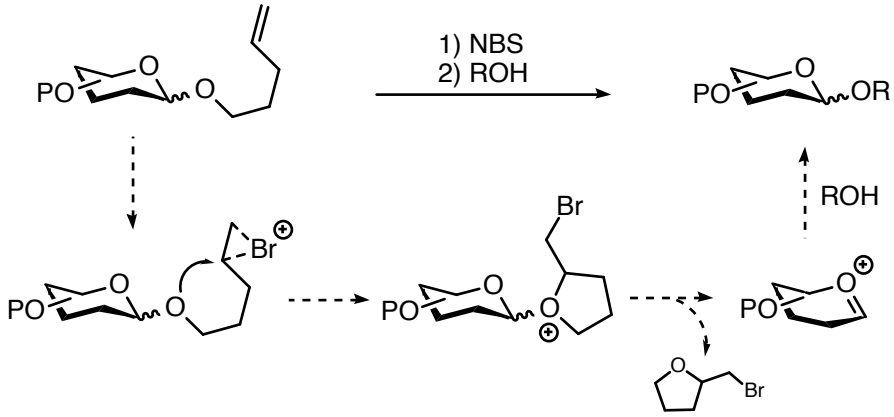
*Participating C-2 PGs can enhance 1,2-*trans *linkage*

Trichloroacetamide overview:
+ predictable anomeric outcomes via kinetic/thermo control
- short shelf life
- not stable to PG manipulations
- for less reactive acceptors, rearrangement is possible (Chapman rearrangement)

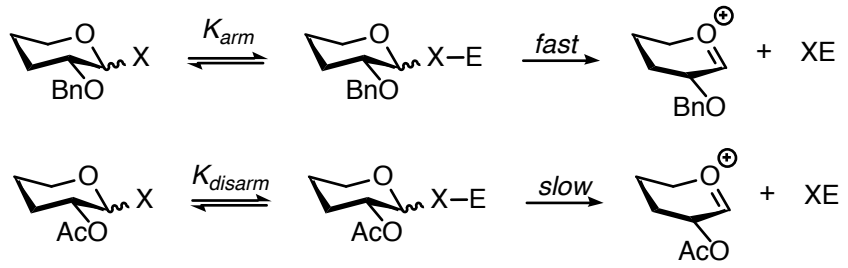


Armed-Disarmed Approach (Fraser-Reid)
JACS, 1988, *110*, 5583-5584
JOC, 1990, *55*, 6068-6070

n-Pentenyl Glycosides (NPG)



Stability of oxonium is influenced by C-2 protecting group -

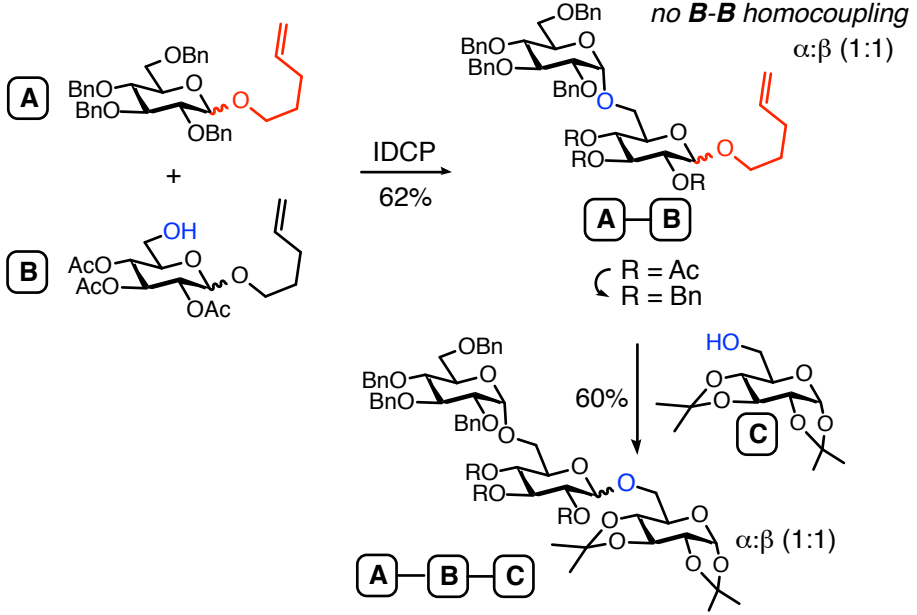


C-2 ethers (ENG, EDG) are *arming* (donors)

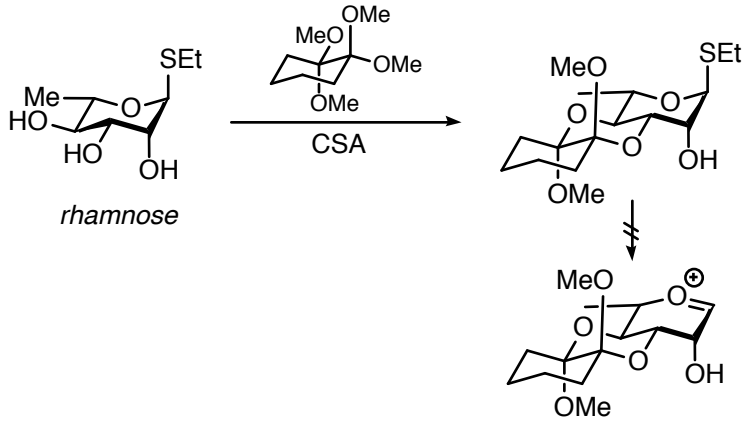
C-2 esters (EWG) are *disarming* (acceptors) and slow formation of oxonium

Oligosaccharide Synthesis

JACS, 1988, 110, 5583-5584



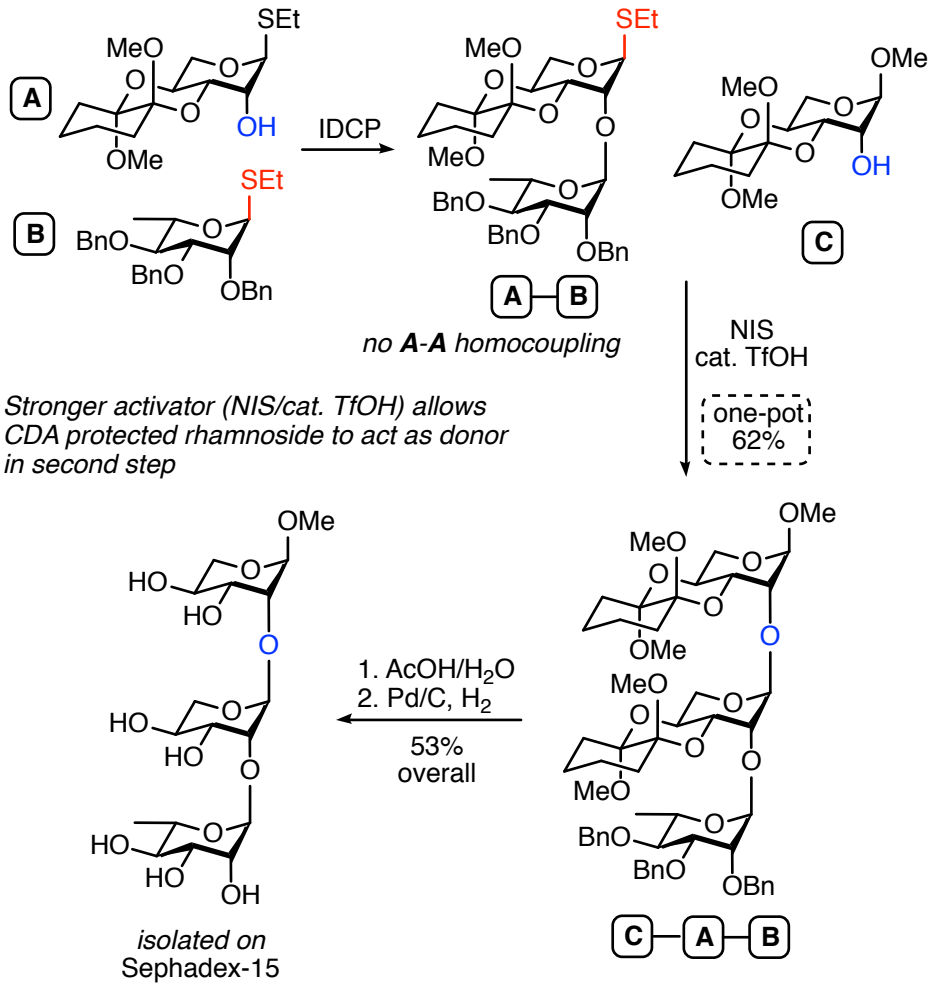
Semi-disarmed Cyclohexane 1,2-diacetal (CDA) Protecing Group (Ley, 1993)
Tetrahedron Lett., 1993, *34*, 8523-8526
JCS Perkins Trans 1, 1998, *1*, 51-66



rhamnose

flattening of CDA ring disfavors oxonium formation

Synthesis of Group B *Streptococci* Polysaccharide Antigen
ACIE, 1994, *33*, 2292-2294

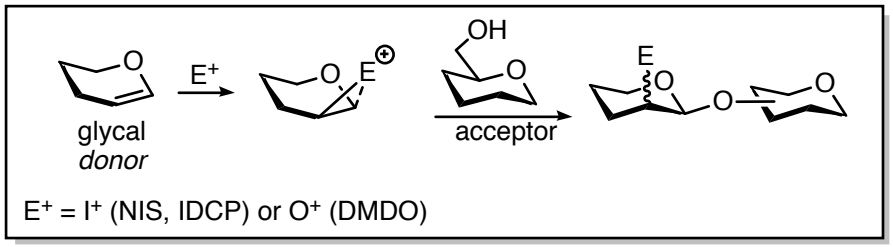


Stronger activator (NIS/cat. TfOH) allows CDA protected rhamnoside to act as donor in second step

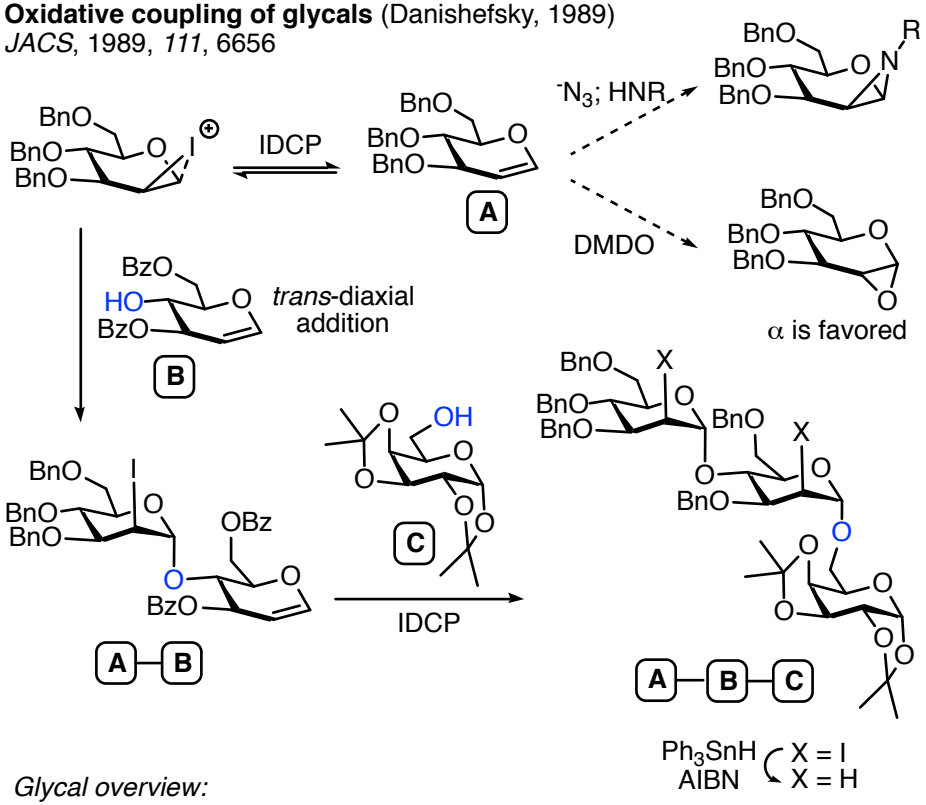
isolated on Sephadex-15

Arming-Disarming approach demonstrated ability to predict glyosidic bond formation in one-pot multiple donors/acceptors

Oligosaccharide Synthesis

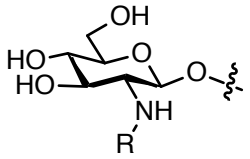


Oxidative coupling of glycals (Danishefsky, 1989)
JACS, 1989, *111*, 6656



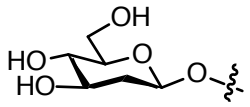
Glycal overview:
+ choice of activator can lead to C-2-OH, -NHR or -H
+ fewer oxygen atoms to protect/differentiate
+ derivatives can be synthesized from tri-O-acetyl-D-glucal (Sigma, $3/g)

Amino sugars



Glucosamine
typically N-Ac and β linkage
R = Ac, CO_2R, phthal, N_3

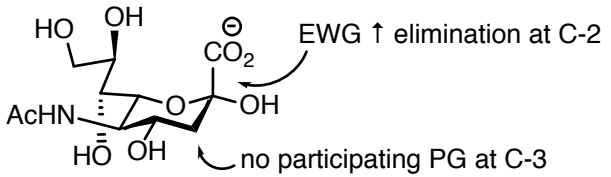
2-deoxysugars



Lack of C-2 functionality
indirect glycosylation

***N*-acetyl-neuraminic acid**

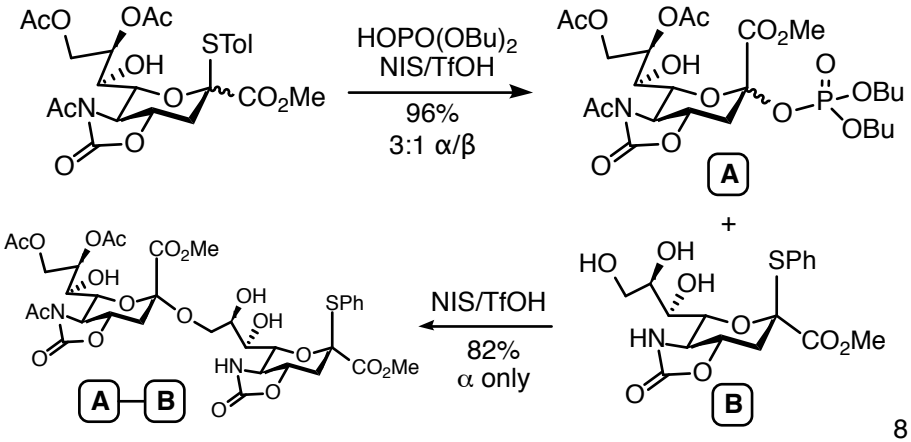
Sialosides are sugars containing a sialic acid sugar



Neu5Ac

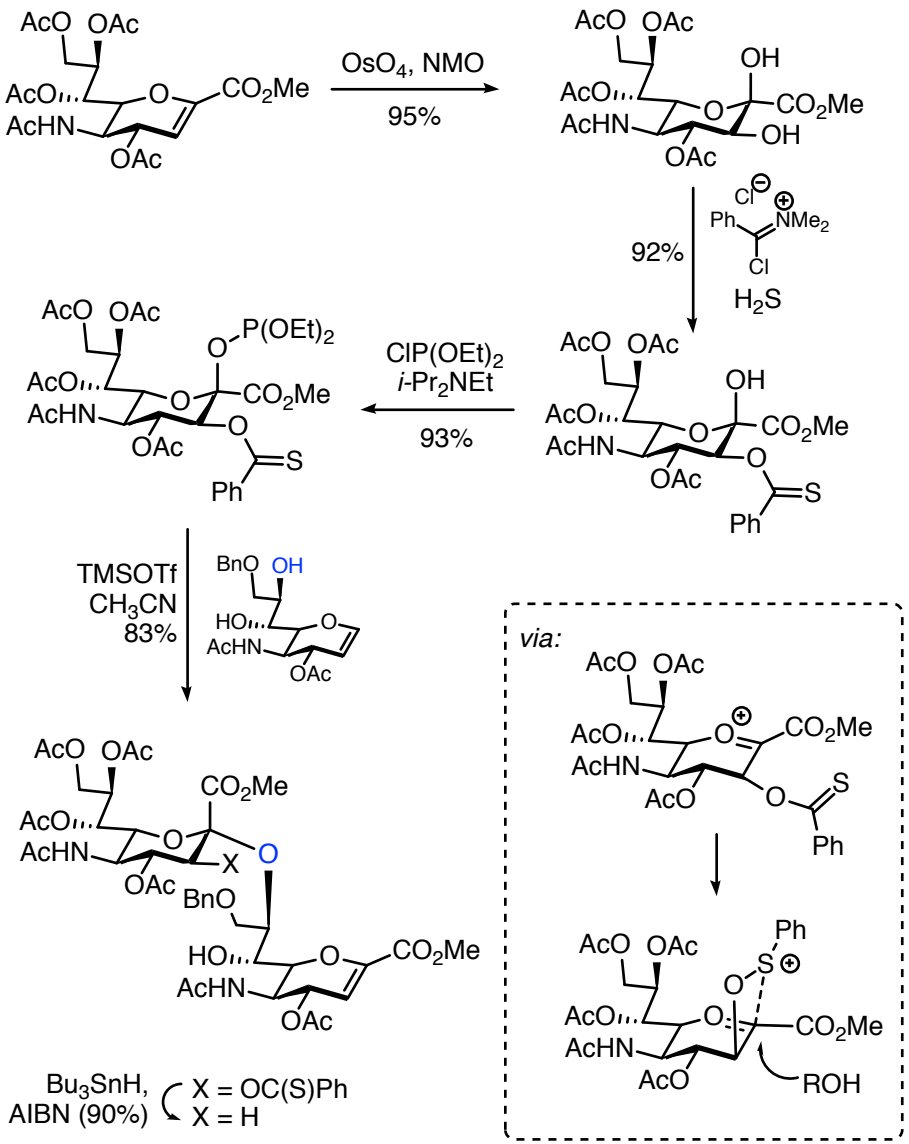
α-linkage is most common in biologically relevant compounds

Direct Glycosylation Approach -
Chem Eur J, 2010, *16*, 1754-1760

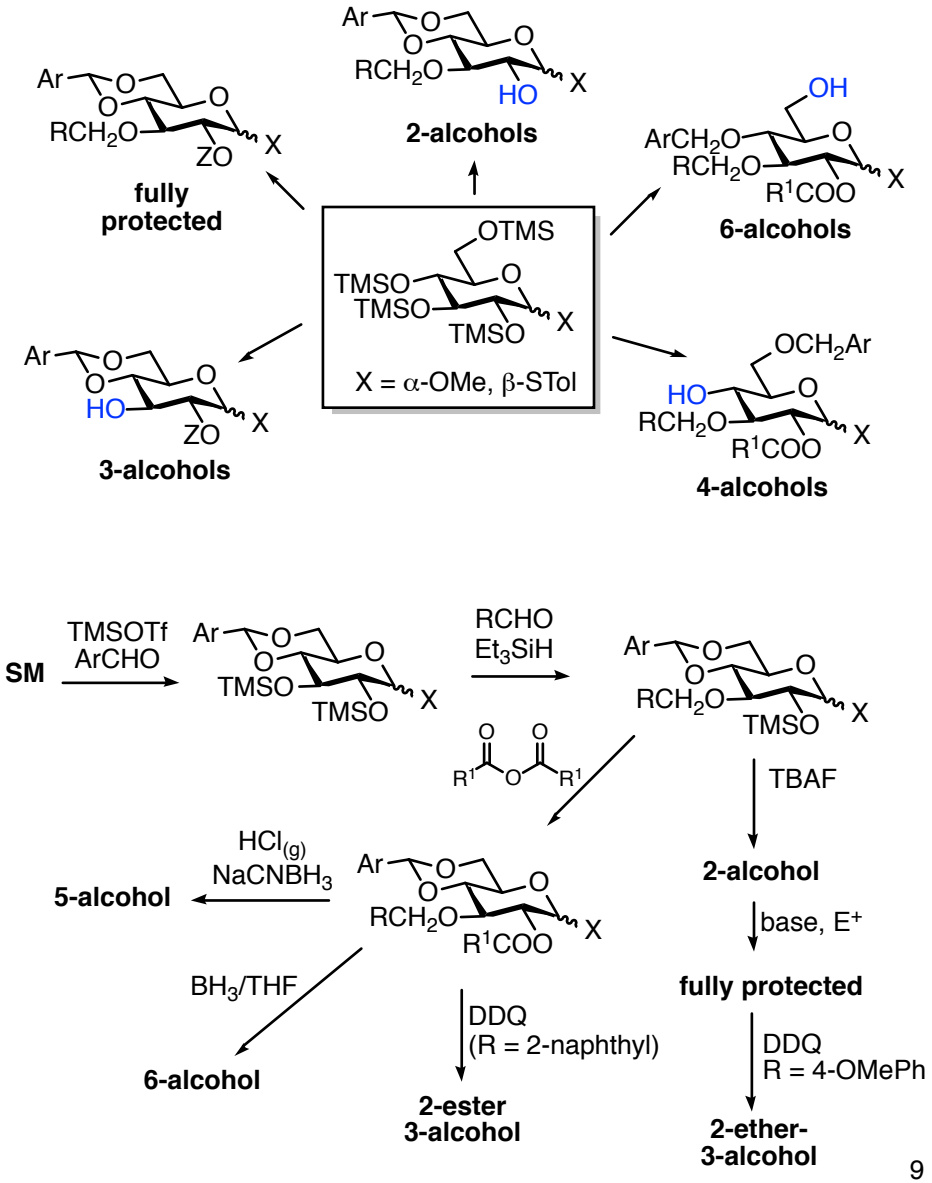


Oligosaccharide Synthesis

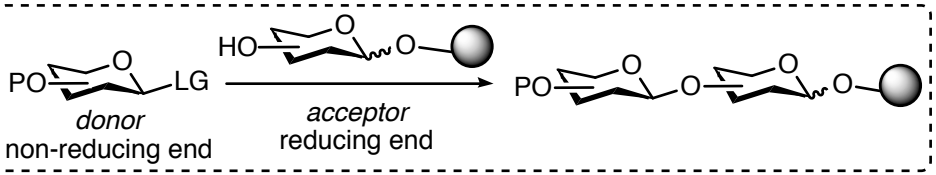
Indirect Glycosylation Approach -
JACS, 1998, *120*, 5434-5440



One-pot regioselective protection strategy (Hung)
Nature, 2007, *446*, 896–899

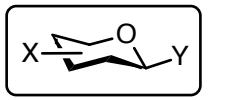


Oligosaccharide Synthesis

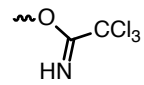
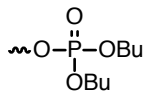


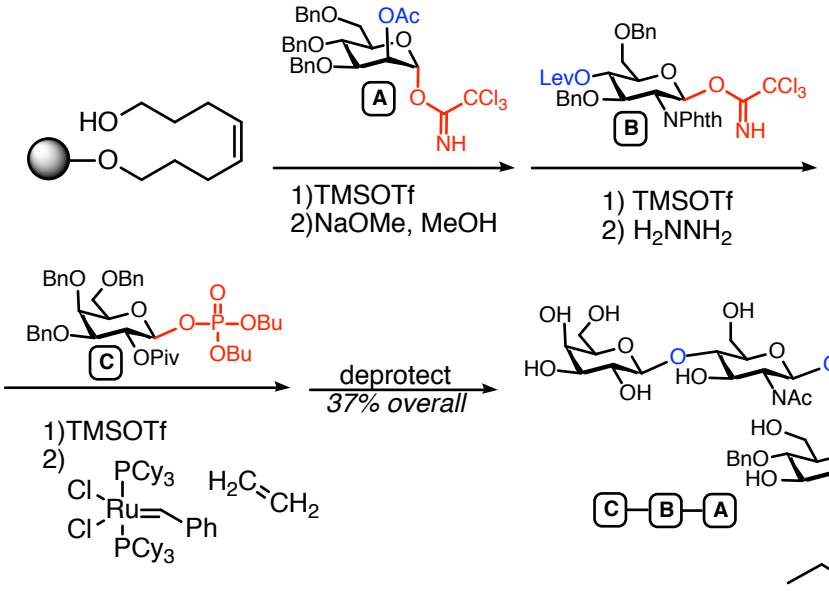
SPOS w/glycals (Danishefsky, Science, 1993, 260, 1307–1309)
SPOS w/thioglyosides (Nicolau, *JACS*, 1997, *119*, 449-450)
SPOS w/soluble polymer (Krepinsky, *JACS,* 1991*, 113*, 5095-5097)
SPOS w/trichloroacetamides (Schmidt, OL, 2000, *2*, 3043-3046)

Automated SPOS (Seeberger, 2001)
Science, 2001, *291*, 1523-1527

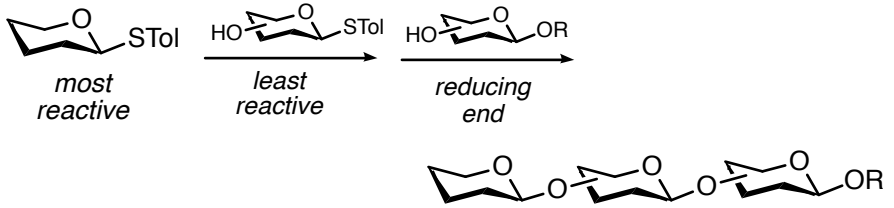


X =	*deprotect?*
NPhth	H_2NNH_2
OPiv	LiOH
OBn	reduction
OAc	NaOMe/MeOH
OLev	H_2NNH_2

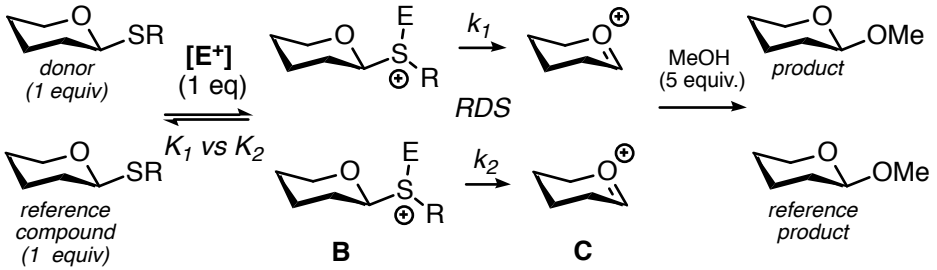
Y = α or β	*activate?*
	TMSOTf
	TMSOTf



Programmable one-pot oligosaccharide synthesis (Chi-Huey Wong,1998)
JACS, 1999, *121*, 734-753



Relative reactivity values (RRV) assigned to a large library of monomers



Assume **B** to **C** is RDS, so $k_{obs} = [E^+][\mathbf{A}]$
$= k[\mathbf{C}]$
and $k_{obs} = k/K$

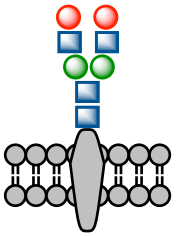
Synthesized tri- and tetra-saccharides with ~60% yield overall.
Main drawbacks
1) difficult to predict deactivating effect of glycosylation on donor
2) N-succinimide (from NIS/TfOH activation) can compete as a nucleophile with poor acceptors

"we have developed a general computer program compatible with Macintosh computers which can search the database to identify optimal combinations of glycosyl building blocks."

"This strategy enables the automated design of a rapid, one-pot sythetic protocol for the synthesis of linear and branched oligosaccharides"

Glycobiology

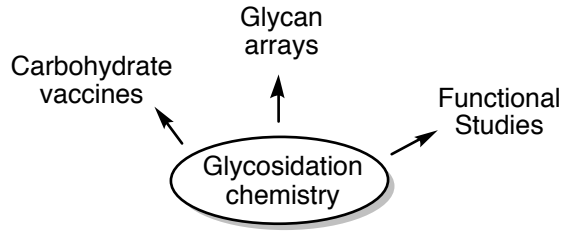
Glycobiology — *biological study enabled by chemistry*
Review: *ACIE*, 2000, *39*, 836-863



Over 90% of human proteins are predicted to be glycosylated

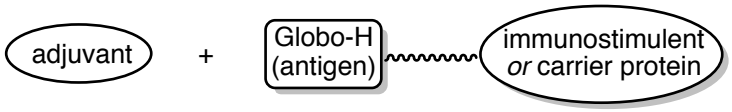
Involved in many biological functions (i.e. folding, trafficking, immunogenicity, and function)

Purification is difficult, particularly for functional study



"The goal in the development of anticancer vaccines is to break the tolerance which the immune system has for antigens expressed mainly or exclusively by the tumor."

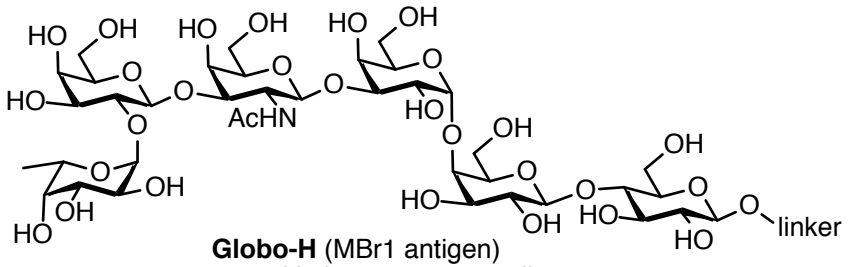
"eradication of circulating tumor cells (in blood stream), and micrometastases."



QS-21
non-immunologenic but
increases
immune response

KLH (keyhole limpet protein)
incites immune response

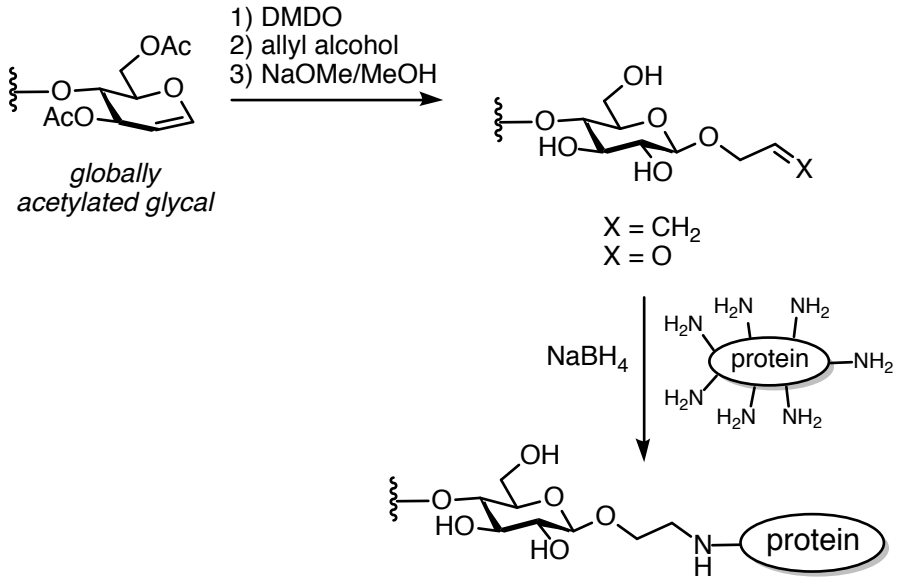
Potential anti-cancer vaccine antigens: Globo-H (MBr1 antigen), Lewis^y, fucosyl GM, KH-1, Tn and Tf antigens



Globo-H (MBr1 antigen)
expressed in breast cancer cells

Danishefsky: *JACS,* 1996, *118*, 11488–11500 (glycal)
Schmidt: *Liebigs Ann.*, 1996, 1417-1423 (glycosyl imidates)
Boons: *ACIE*, 1999, *38*, 3495-3497 (thioglycosides, glycosylfluorides)
C-H Wong: *ACIE*, 2001, *40*, 1274-1277 (one-pot)
Seeberger: *JOC*, 2002, *67*, 6659-6670 (SPOS)

Danishefsky completion of vaccine synthesis:

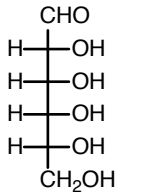


globally acetylated glycal

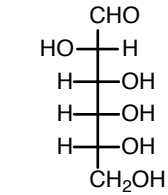
Appendix

Hexoses

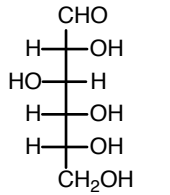
D-allose



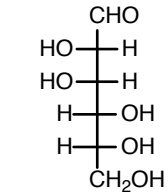
D-altrose



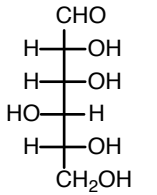
D-glucose



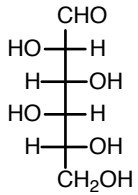
D-mannose



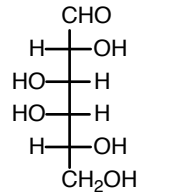
D-gulose



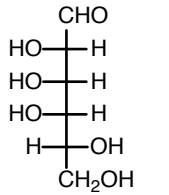
D-idose



D-galactose

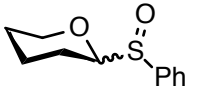


D-talose

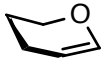


Glycosyl donors

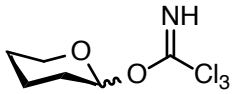
glycosyl halides
X = Cl, Br (AgOTf)
X = F ($SnCl_2$/$AgClO_4$)



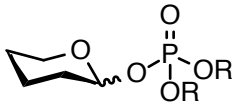
glycosyl sulfoxides
(Tf_2O)



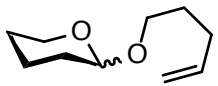
glycals
(1. DMSO 2. $ZnCl_2$)



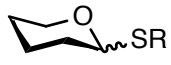
trichloroacetimidates
(TMSOTf)



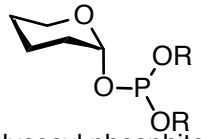
glycosyl phosphates
(TMSOTf)



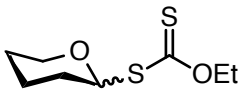
pentenyl glycosides
(NIS/TESOTf)



thioglycosides
(NIS/TfOH or DMTST)



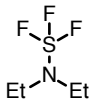
glycosyl phosphites
(TMSOTf)



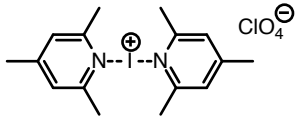
xanthates
(DMTST, $Cu(OTf)_2$)

Activators/Reagents:

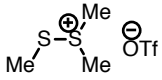
DAST = (diethylamino)sulfur trifluoride



IDCP = *sym*-collidine iodonium perchlorate



DMTST = dimethyl(methylthio)sulfonium triflate

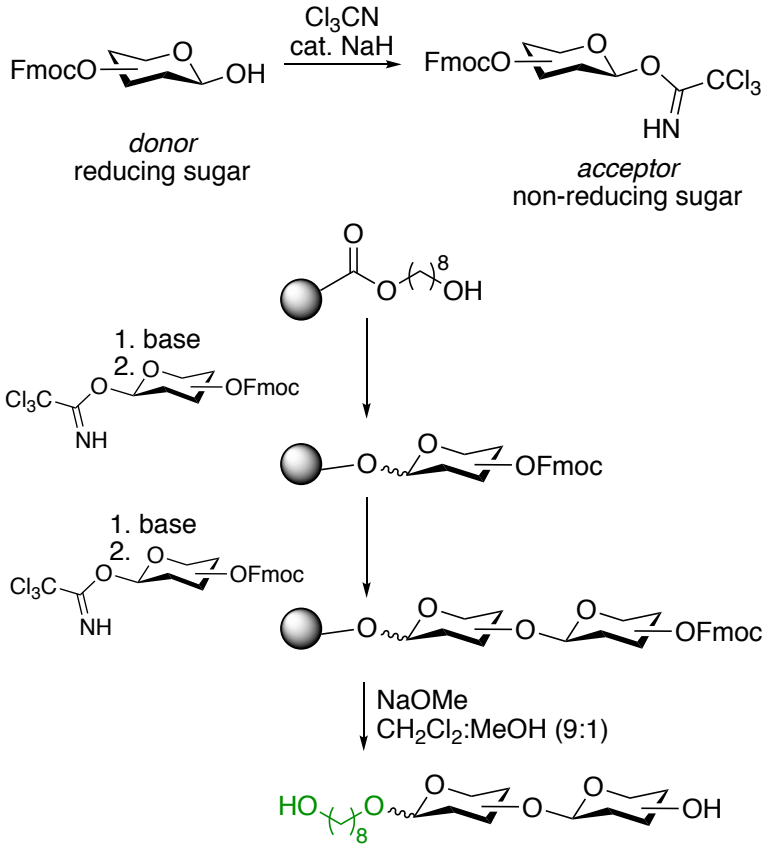


DTPMB = 2,6-Di-*tert*-butyl-4-methylpyridine

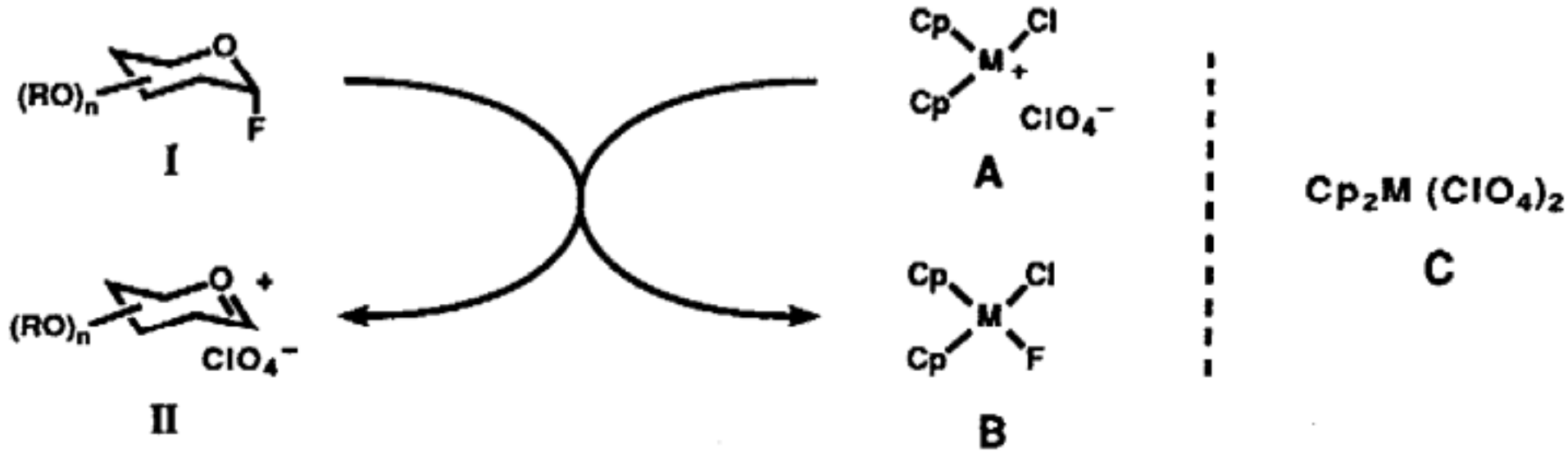
Back-up

Solid-phase oligopeptide and oligonucleotide synthesis is fairly routine and generalizeable, but oligosaccharide synthesis (SPOS) is more challenging due to branching and stereospecificity.

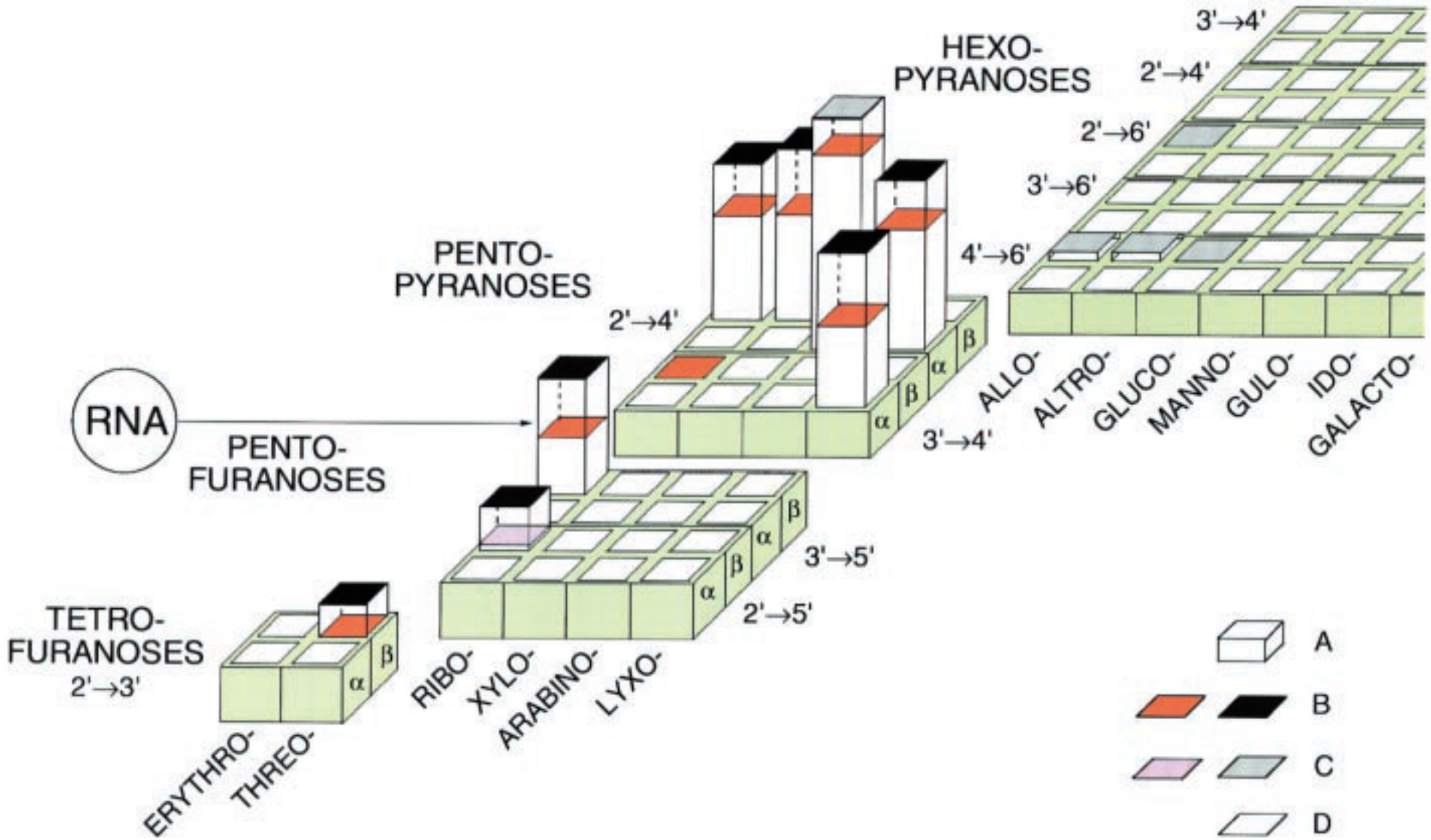
SPOS w/trichloroacetamides (Schmidt)
OL, 2000, *2*, 3043-3046



Mechanism of glycosyl fluoride activation



Why ribose?


RNA
PENTO-
FURANOSES
TETRO-
FURANOSES
2'→3'
ERYTHRO-
THREO-
PENTO-
PYRANOSES
2'→4'
3'→5'
2'→5'
RIBO-
XYLO-
ARABINO-
LYXO-
HEXO-
PYRANOSES
3'→4'
2'→4'
2'→6'
3'→6'
4'→6'
3'→4'
ALLO-
ALTRO-
GLUCO-
MANNO-
GULO-
IDO-
GALACTO-
α
β
A
B
C
D