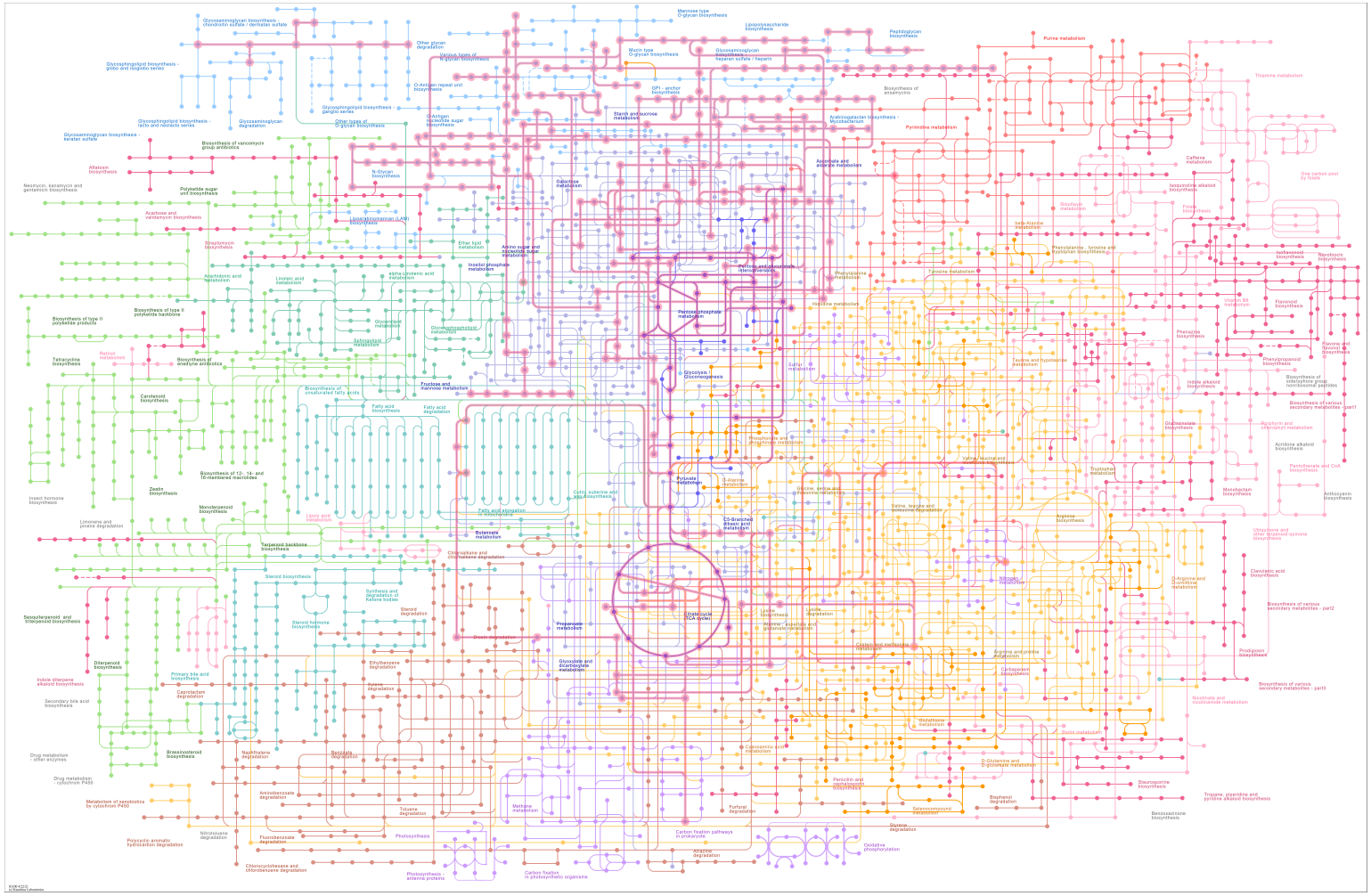
Carbohydrate Chemistry

Glycan synthesis and metabolyze are important pathways.

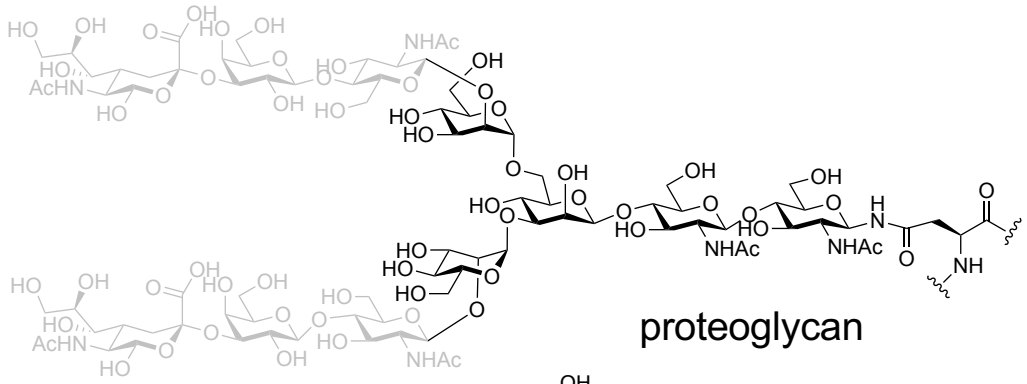


https://www.genome.jp/kegg-bin/show_pathway?map01100

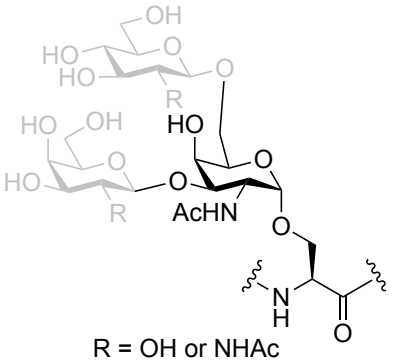
Structure

Glycans in human

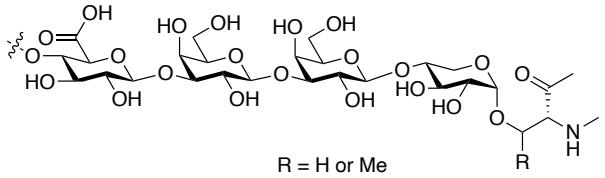
Protein *N*-glycan



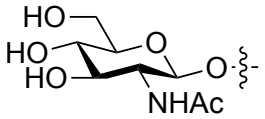
Protein *O*-glycan



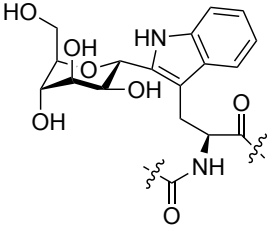
proteoglycan



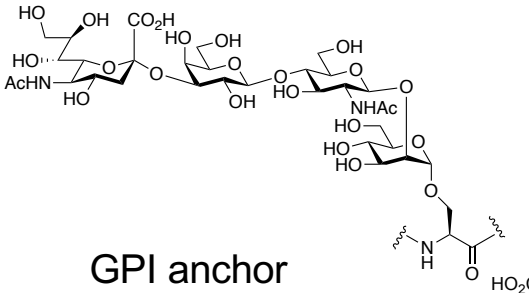
Protein *O*-GlcNAc



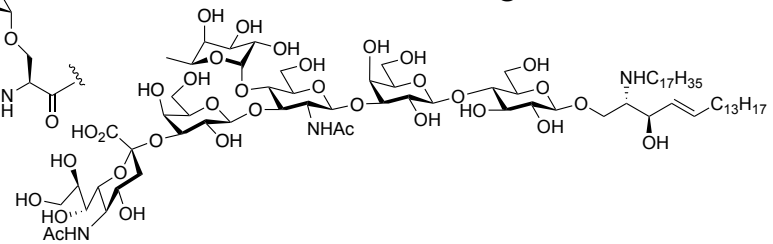
Protein *C*-Mannpsylation



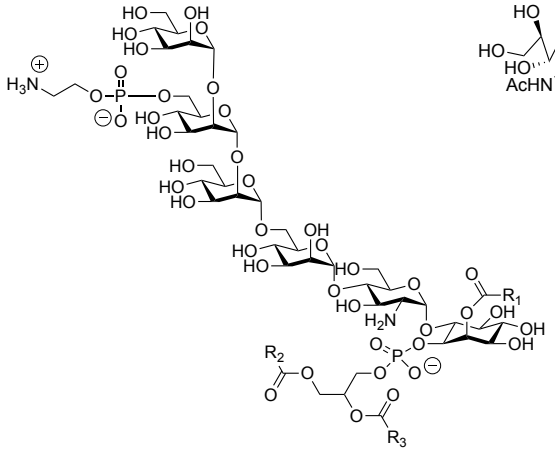
Protein *O*-Man



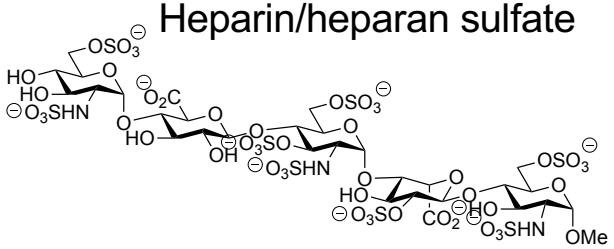
CA19-9 antigen



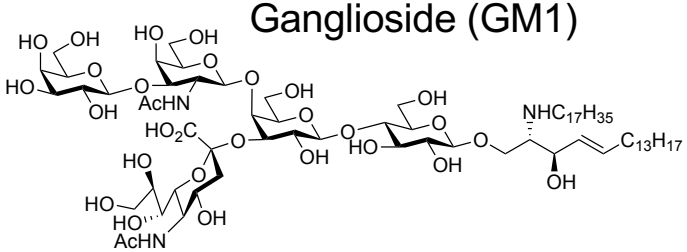
GPI anchor



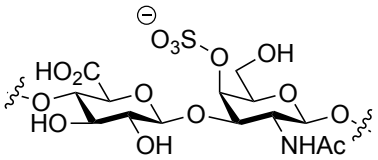
Heparin/heparan sulfate



Ganglioside (GM1)



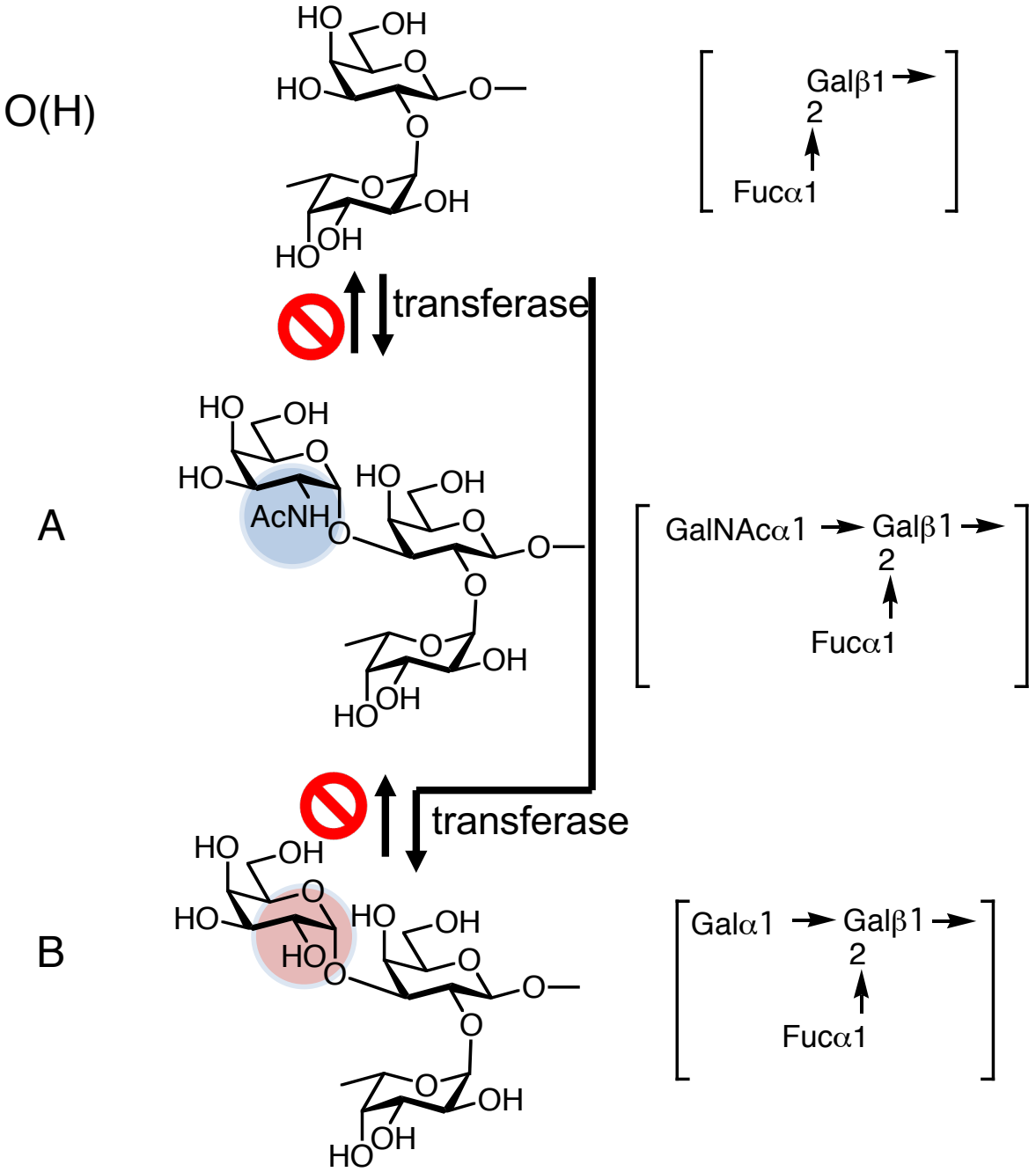
chondroitin sulfate



Glycan structure is complex and diverse.
Heterogeneity is caused by complex biosynthetic pathway.

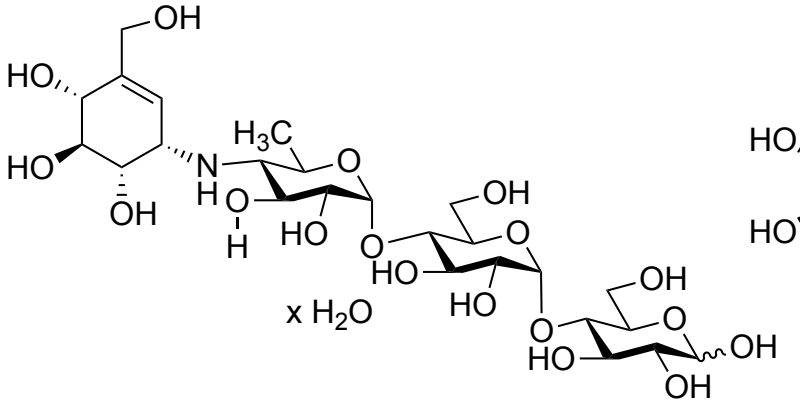
Structure

Blood type and glycan structure

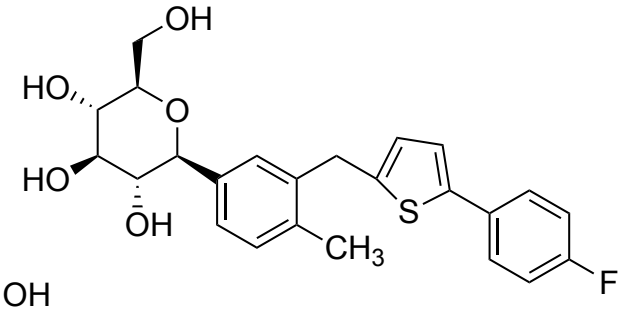


Structure

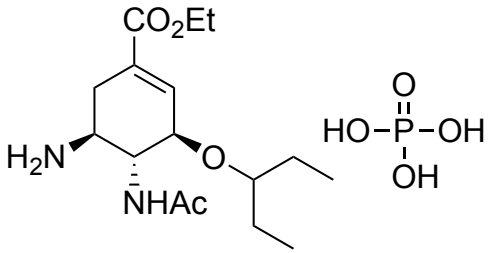
Medicine based on glycan structure



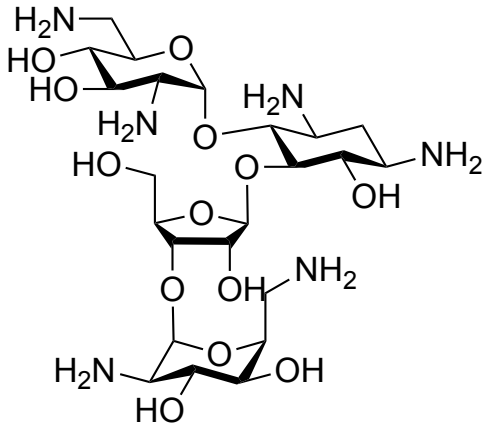
Acarbose
Type 2 diabetes



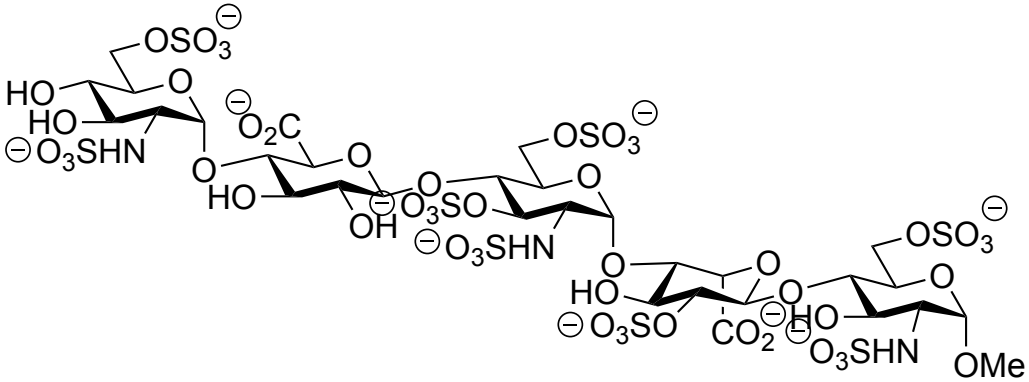
Canagliflozin
diabetes
sodium glucose cotransporter 2



Oseltamivir
Anti-influenza drug



Neomycin
antibiotics



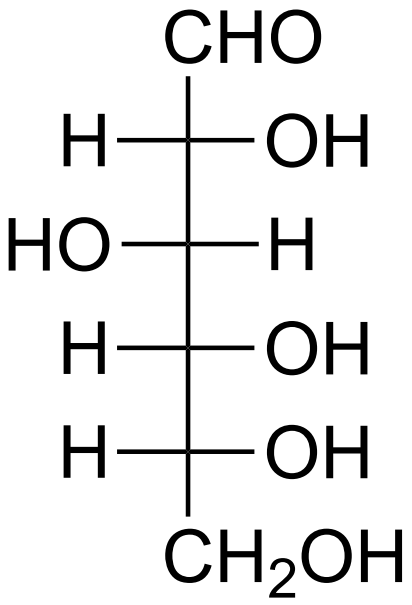
heparin
Fondaparinux
(chemically synthesized heparin)

Structure representation

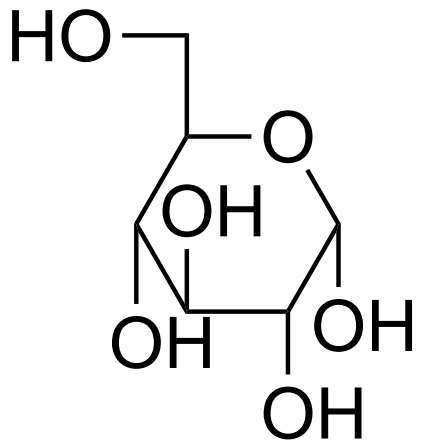
Carbohydrate = Polyhydroxylated aldehydes and ketones

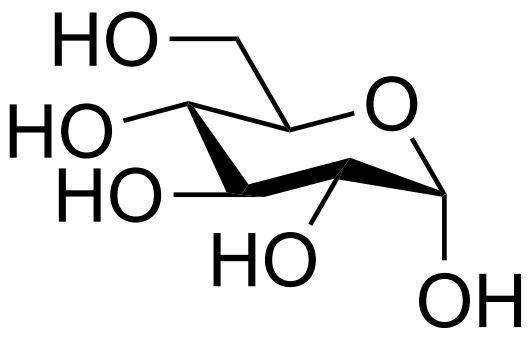
Carbohydrates have multiple hydroxyl groups and (hidden) carbonyl groups.

α-D-glucose



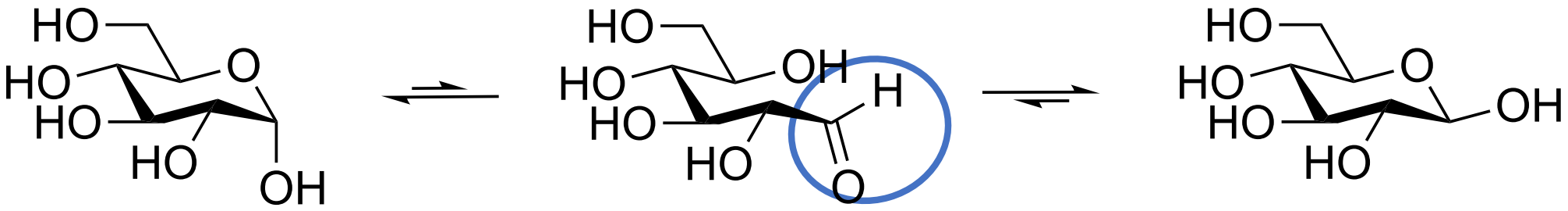
Fischer projection





Haworth projection

Cyclic structure : hemiacetal



α-D-glucopyranoside
$[\alpha]_D$ +112
36%

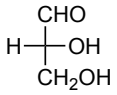
Linear D-glucose
Less than 0.1%

β-D-glucopyranoside
$[\alpha]_D$ +19
64%

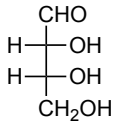
Structure

Nomenclature of stereochemistry of monosaccharides

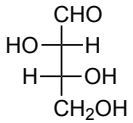
D-sugar and L-sugar



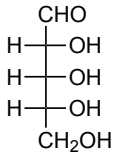
D-glyceraldehyde



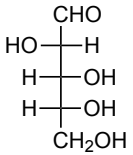
D-erythrose



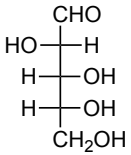
D-threose



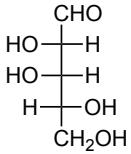
D-ribose



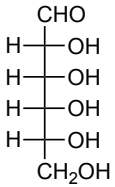
D-arabinose



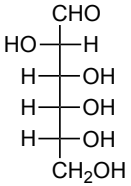
D-xylose



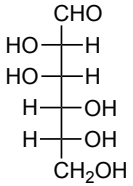
D-lyxose



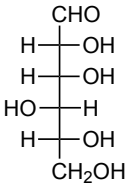
D-allose



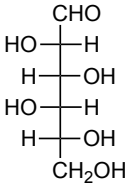
D-altrose



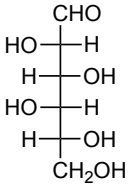
D-glucose



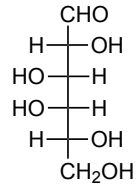
D-mannose



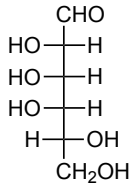
D-gulose



D-idose



D-galactose



D-talose

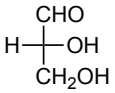
The chiral center farthest from the carbonyl group is the same configuration as D-glyceraldehyde →D-sugar, namely OH is on the right in Fischer projection.

The chiral center farthest from the carbonyl group is the same configuration as L-glyceraldehyde →L-sugar, namely OH is on the left in Fischer projection.

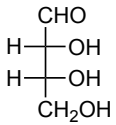
Structure

Nomenclature of stereochemistry of monosaccharides

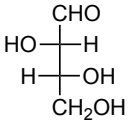
α–form and β-form



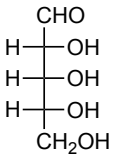
D-glyceraldehyde



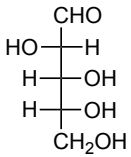
D-erythrose



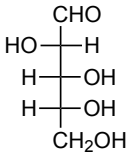
D-threose



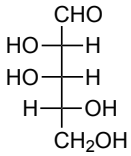
D-ribose



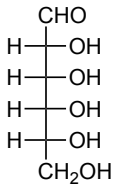
D-arabinose



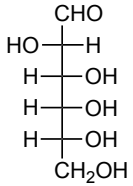
D-xylose



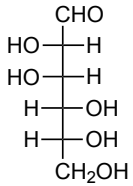
D-lyxose



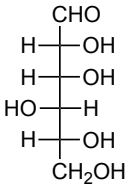
D-allose



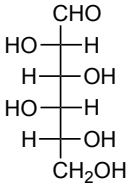
D-altrose



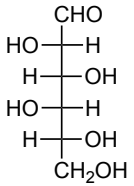
D-glucose



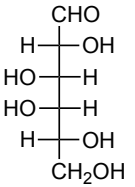
D-mannose



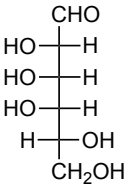
D-gulose



D-idose



D-galactose

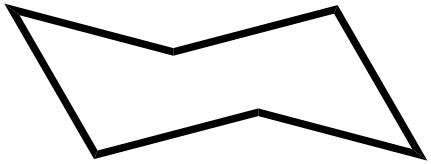


D-talose

In α-glycosides, heteroatom (oxygen atom) bonded anomeric carbon and the heteroatom (oxygen atom) bonded to the anomeric reference atom are in the *cis,* when described by the Fischer projection formula.
In the Fischer projection formula, the aldehyde is placed on top.

Features of sugar structure

Conformation of cyclohexane
(symmetric)



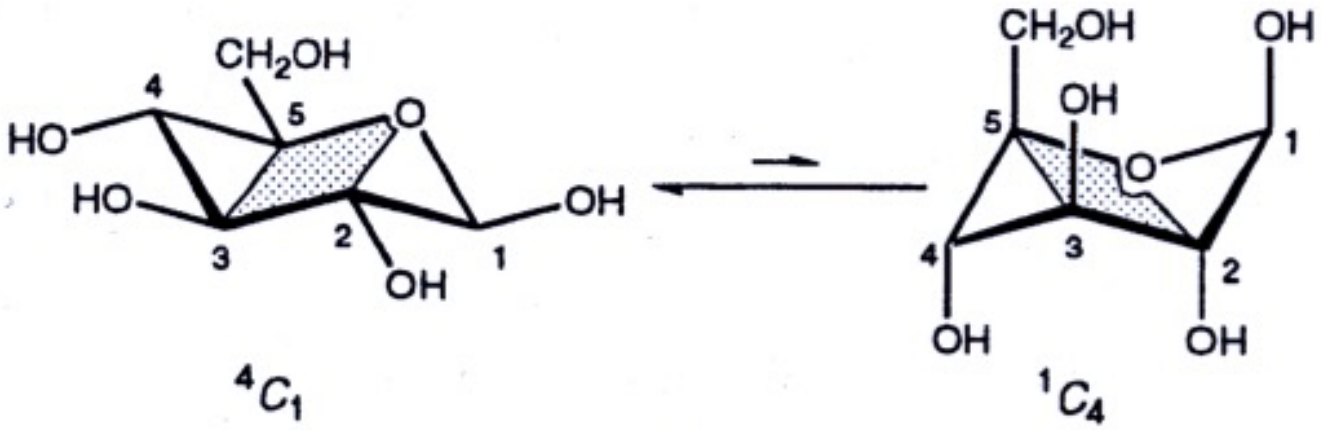
One *Chair*

Sugars do not have symmetry like cyclohexane due to oxygen atom.

Two *Chairs*

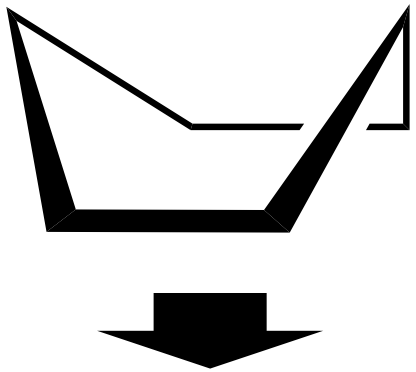
$^4C_1$ and $^1C_4$

$^4C_1$ is more stable than $^1C_4$.

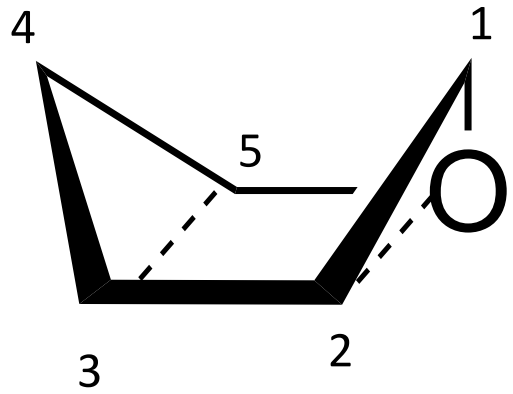


Features of sugar structure

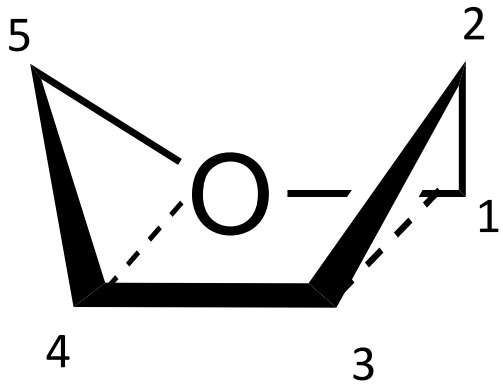
One “*boat” in cyclohexane*



There are two “boat”s in sugar.

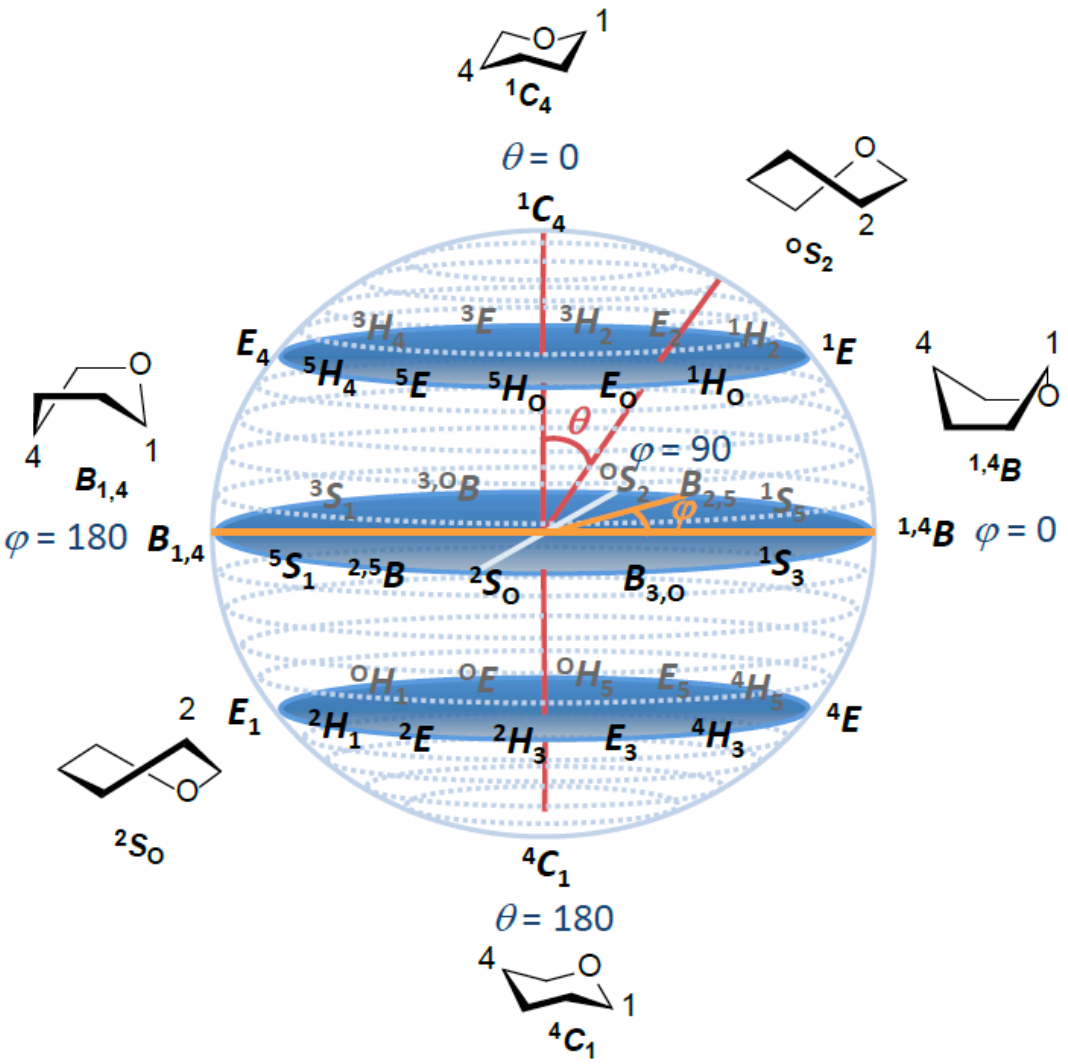


$^{1}B^{4}$ *or* $^{1,4}B$



$^{5}B^{2}$ *or* $^{2,5}B$

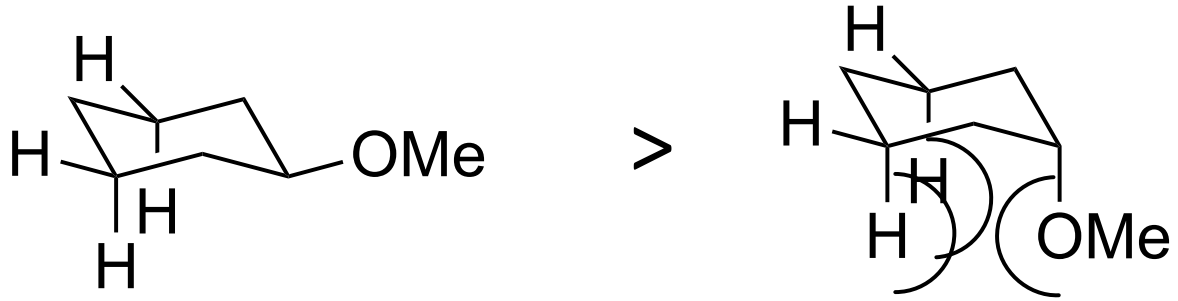
Cremer-Pople parameter



Cremer-Pople parameter allows the conformation of a pyranoside to be determined in an unambiguously using numerical parameters.
It is used to track conformational changes in enzymatic and chemical reactions.

Features of sugar structure

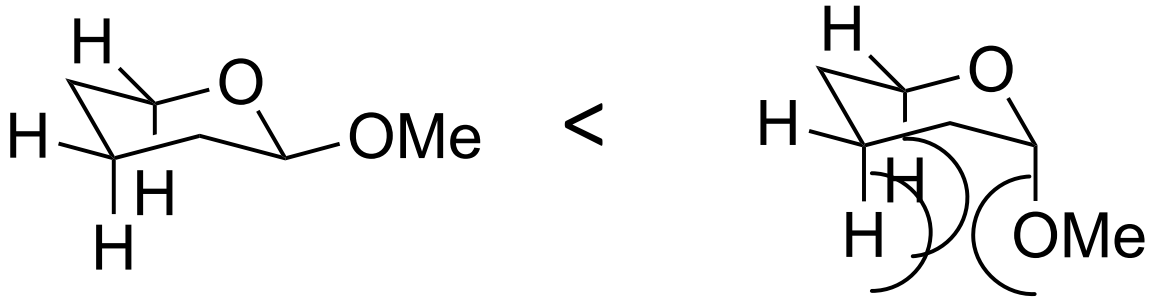
cyclohexane



Substituent orients in equatorial position because 1,3-diaxial repulsion.

Anomeric effect

pyranoside

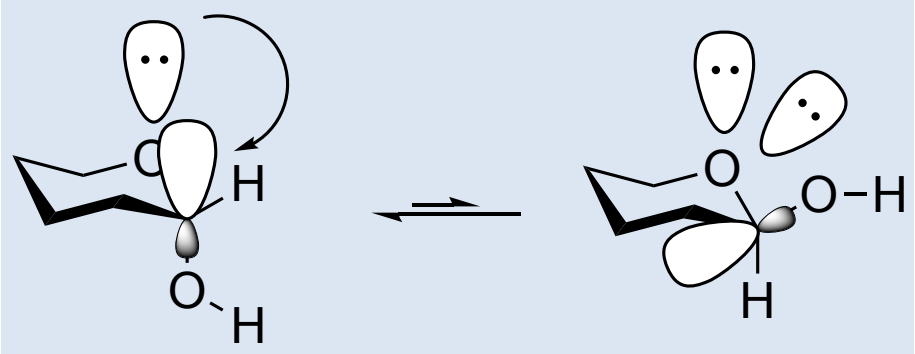


Electronegative substituent adjust to anomeric position favors in axial position.

″Stereoelectronic Effects” A. J. Kirby Oxford Science Publications

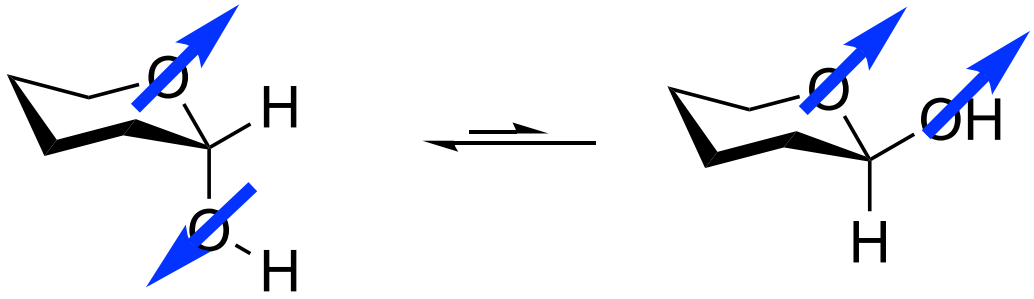
Anomeric effect

Stereoelectronic effect



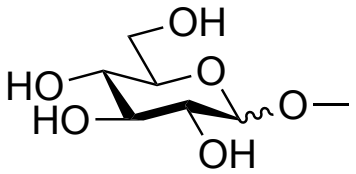
stabilizing interaction between the unshared electron pair on the endocyclic oxygen atom and the σ* orbital of the axial (exocyclic) C–X bond

Dipole moment

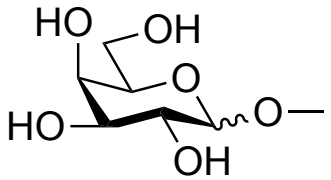


Structure

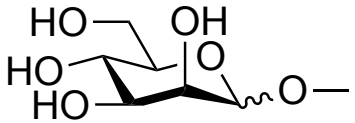
Pyranosides in human



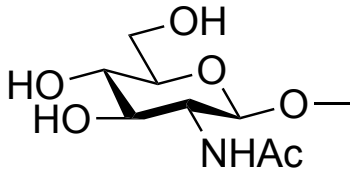
D-Glucose (Glu)
α,β



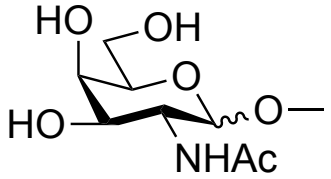
D-Galactose (Gal)
α,β



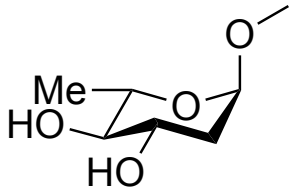
D-Mannose (Man)
α,β



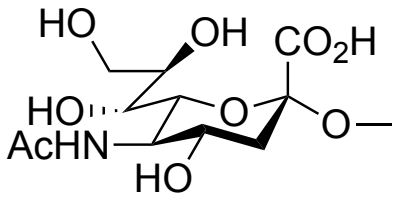
D-*N*-acetylglucosamine (Glu)
β



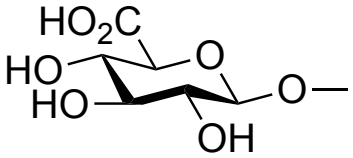
D-*N*-acetylgalactosamine (GalNAc)
α, β



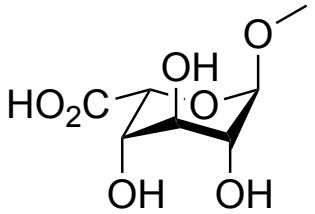
L-Fucose (Fuc)
α



N-acetylneuramic acid (Sialic acid)
α



D-Glucronic acid (GlcA)
β

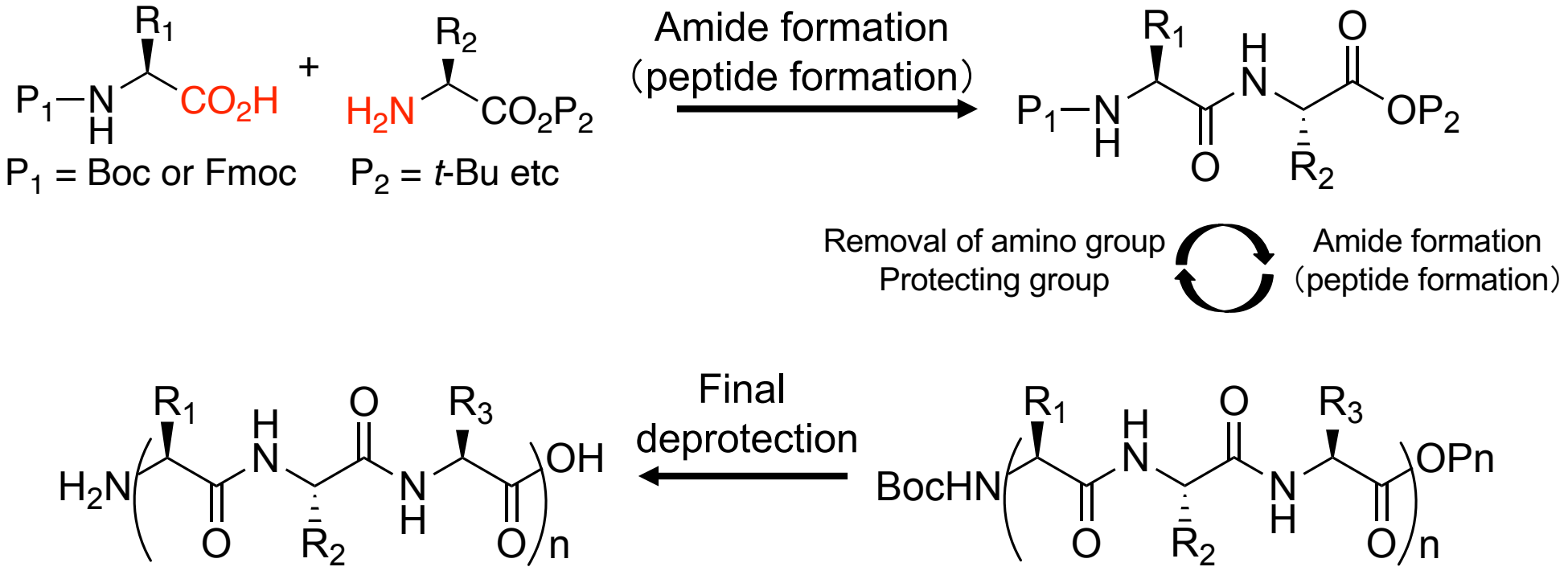


L-Iduronic acid (IdoA)
α

Difficulties in oligosaccharide synthesis

Peptide synthesis vs glycan synthesis

Peptide synthesis

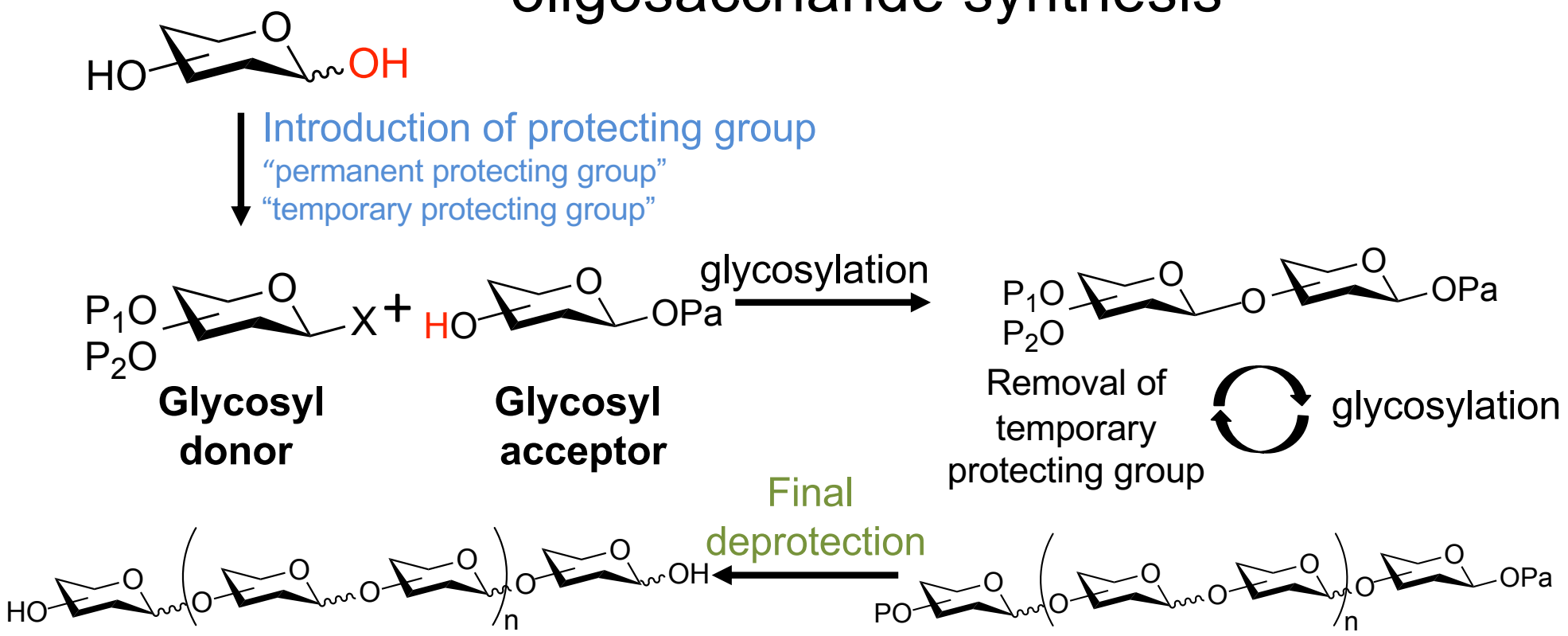


- Yields are generaly high.
- Stereocontrol is not necessary (racemization should be considered) .
- Side-chain functional groups are protected.
- Side-chain protecting groups are removed under strong acidic conditions.

Difficulties in oligosaccharide synthesis

Peptide synthesis vs glycan synthesis

oligosaccharide synthesis

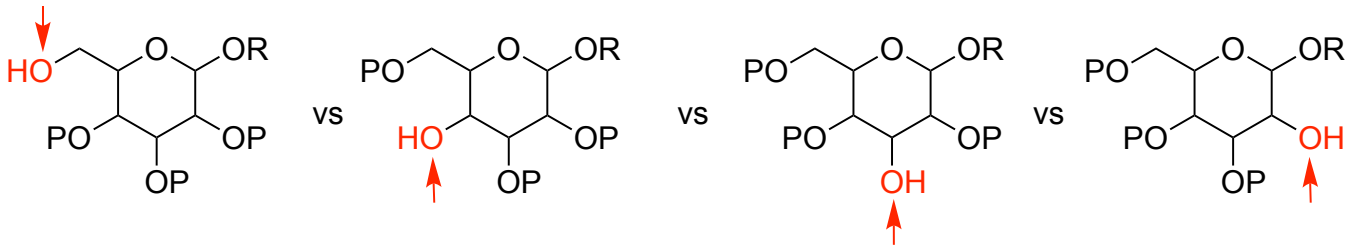


- Yields in glycosylation is not high.
- Stereochemistry control is necessary because asymmetric carbon is generated at anomeric center.
- There are multiple hydroxy groups in one sugar unit. Protection strategy is required.
- Both glycosylation and amide formation are dehydrative reaction.

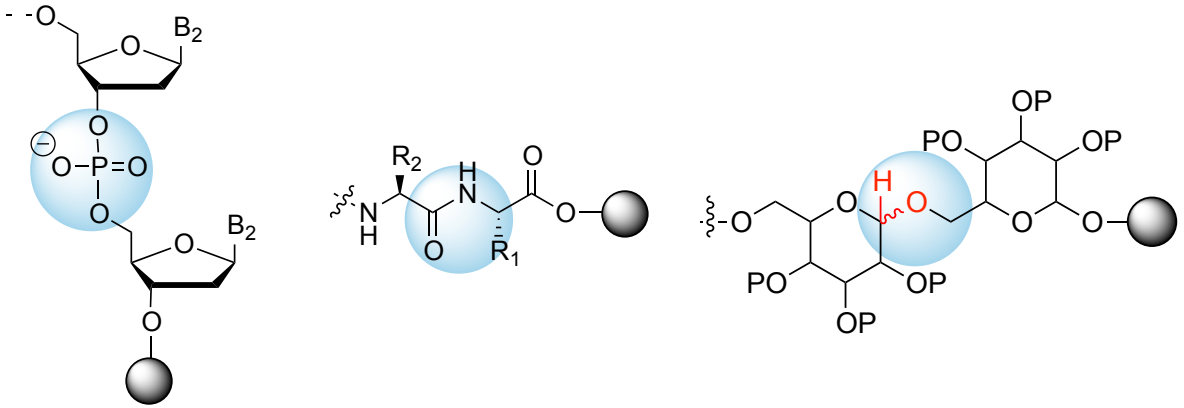
Difficulties in oligosaccharide synthesis

1. Yields in glycosylation are not high enough.

2. There are multiple hydroxy groups in one sugar unit. Protection strategy is required.

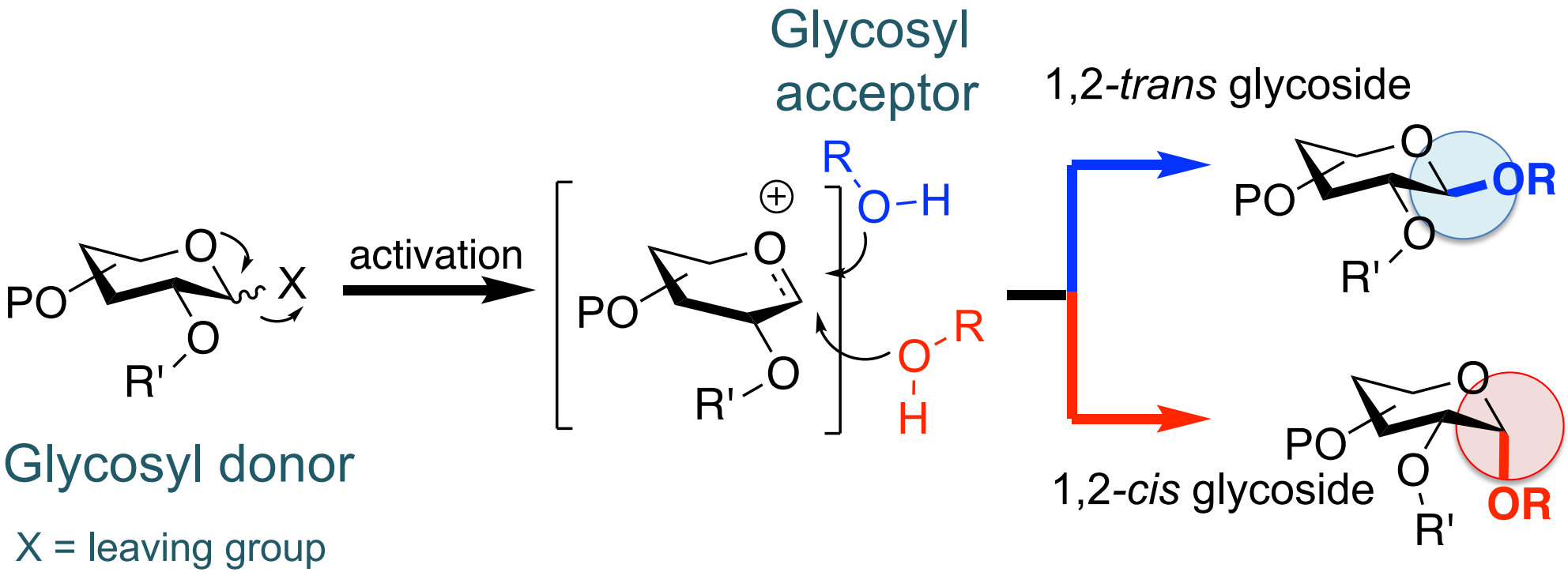


3. Stereochemistry control is necessary because asymmetric carbon is generated at anomeric center.



Glycosylation reaction

Improvement of yield and selectivity

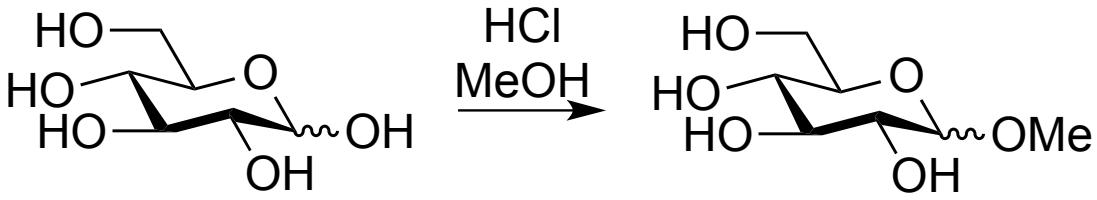


The first glycosylation

Hermann Emil Fischer

1852 Oct. 9 ～1919 July 15

@Berlin 2015



2400

Mittheilungen.

464. Emil Fischer: Ueber die Glucoside der Alkohole [1]).

[Aus dem I. Berliner Universitäts-Laboratorium.]

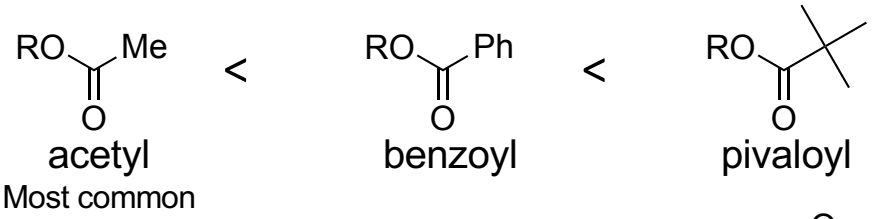
(Eingegangen am 9. October; vorgetragen in der Sitzung vom Verf.)

Für die künstliche Bereitung von Glucosiden ist zur Zeit nur die von A. Michael [2]) aufgefundene Methode bekannt. Dieselbe beruht auf der Wechselwirkung zwischen der sogenannten Acetochlorhydrose und den Alkalisalzen der Phenole. Sie ist nur für die letzteren anwendbar und wurde offenbar wegen des complexen Verlaufes der Reaction und der dadurch bedingten schlechteu Ausbeute bisher nur in wenigen Fällen mit Erfolg benutzt. Ich habe nun in der Salzsäure ein Mittel gefunden, die Zuckerarten mit den Alkoholen direct zu glucosidartigen Producten zu vereinigen. Leitet man in eine Auflösung von Traubenzucker in Methylalkohol unter Abkühlung gasförmige Salzsäure bis zur Sättigung ein, so verliert das Gemisch nach kurzer Zeit die Fähigkeit, Fehling'sche Lösung zu reduciren, und

Ber. Dtsch. Chem. Ges. **1853**, *26*, 2400.

Protecting group in glycoside synthesis

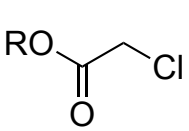
Acyl-type protecting group



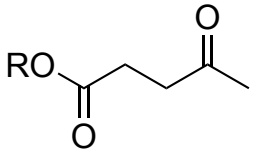
acetyl
Most common

benzoyl

pivaloyl

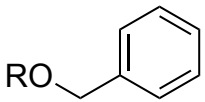


Chloroacetyl (ClAc)
Removal by thiourea, pyridine-AcOH-H_2O
in the presence of Ac

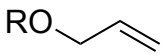


Levulinoate (Lev)
Removal of hydrazine
in the presence of Ac

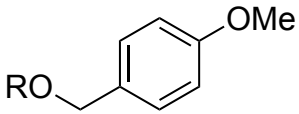
Ether-type protecting group



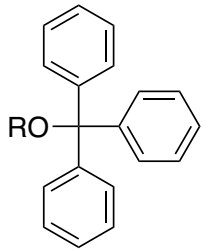
benzyl
"Permanent" protecting group



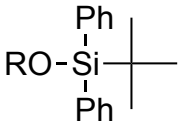
allyl
Removal by Pd(0)



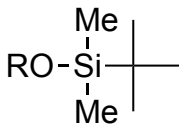
p-methoxy benzyl
(PMB)
Removal by DDQ



Trityl
(Tr)
For primary hydroxy group
Removal by acid

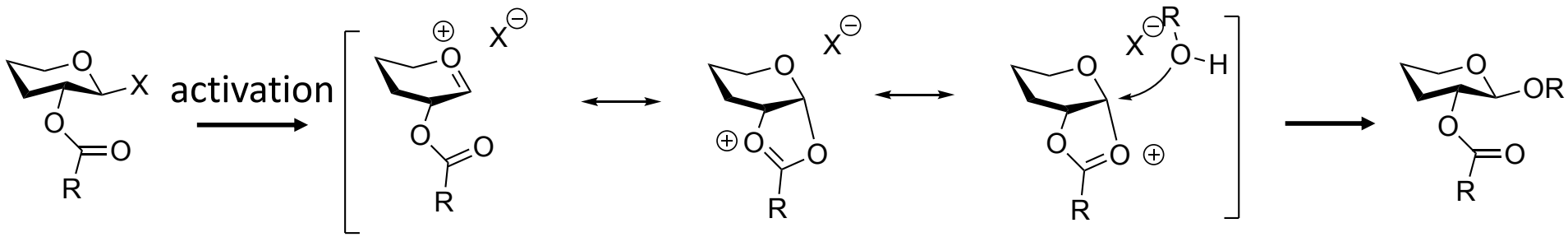


TBDPS
For primary hydroxy group
Removal by F^-



TBS
For primary hydroxy group
Removal by F^-

1,2-*trans* glycoside formation

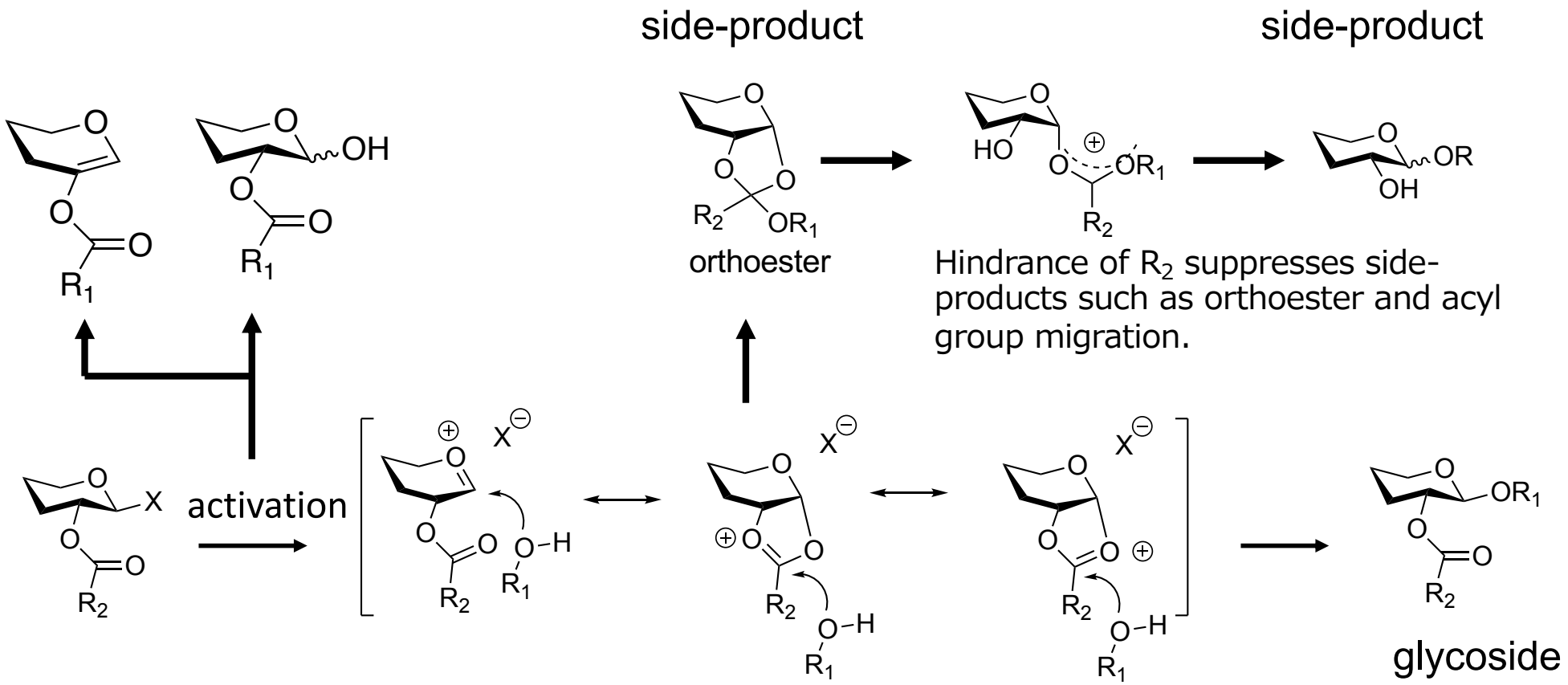


Acyl protecting group at 2-position

Acyl protecting group gives neighboring participation to oxacarbonium ion generated by activation of glycosyl donor. The acceptor attacks from the opposite side of five-membered ring.

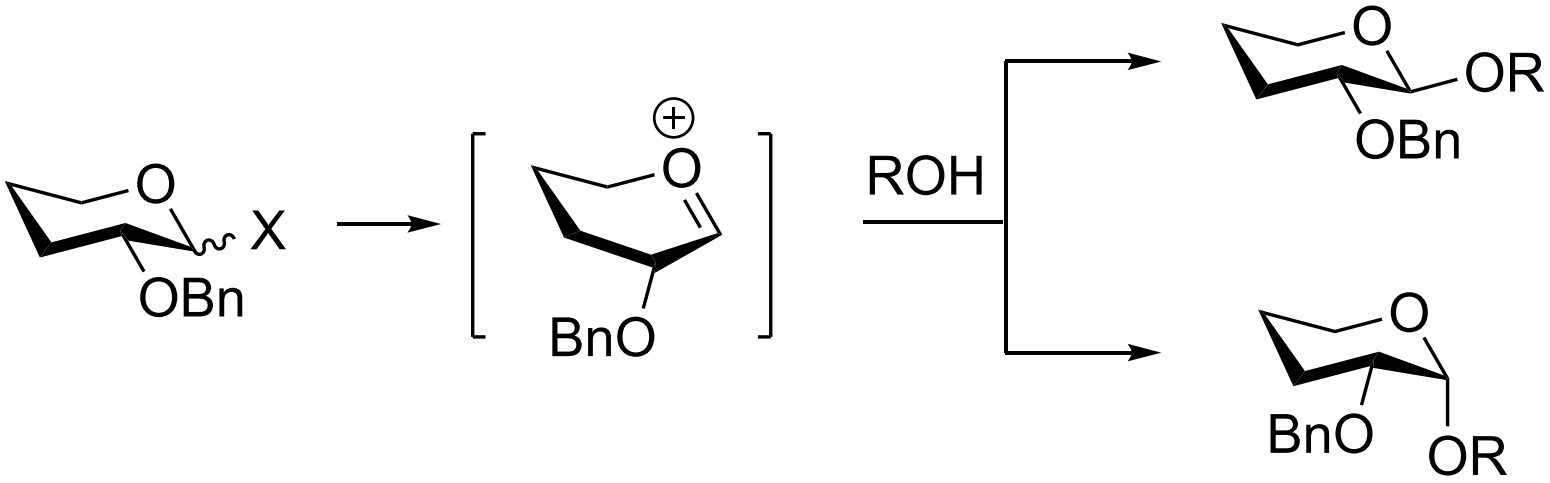
1,2-*trans* glycoside is formed in a stereoselective manner.

1,2-*trans* glycoside formation side-products



1,2-*cis* glycoside formation

- Stereoselective 1,2-*cis* glycosylation is difficult.
- Ether protecting group at 2-position
- Ether or dioxane as solvent

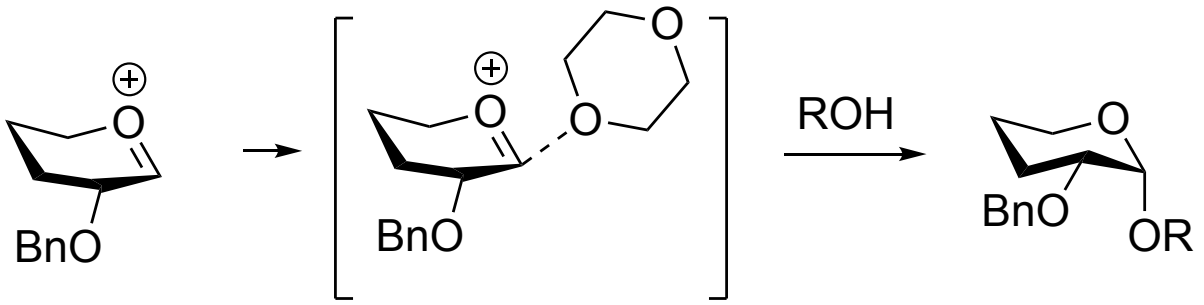


S. Manabe, *Heterocycles*, **2021**, *102,* 177.

Solvent effect

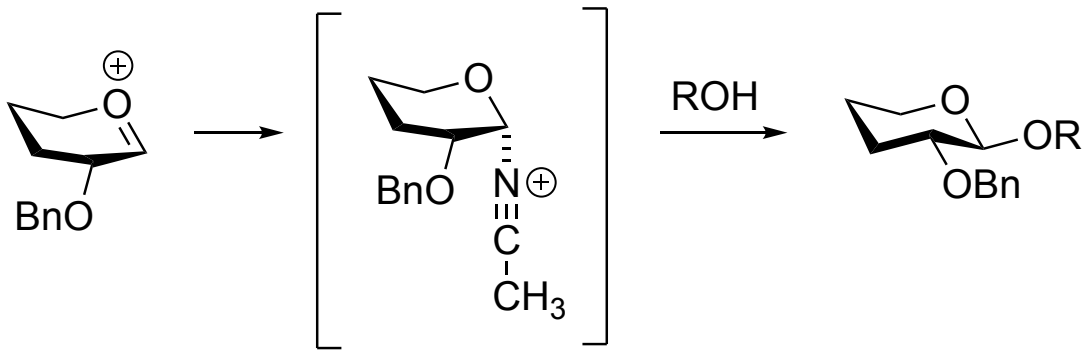
solvent coordination hypothesis

In ether type solvent (Et_2O, dioxane)



Predominant 1,2-*cis* glycoside

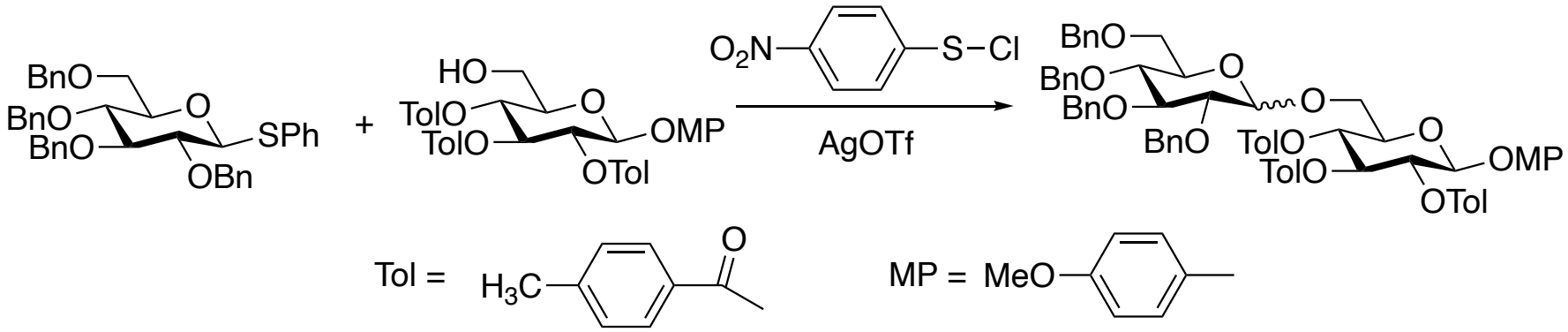
In CH_3CN



Predominant 1,2-*trans* glycoside

Glycosylation

Solvent effect

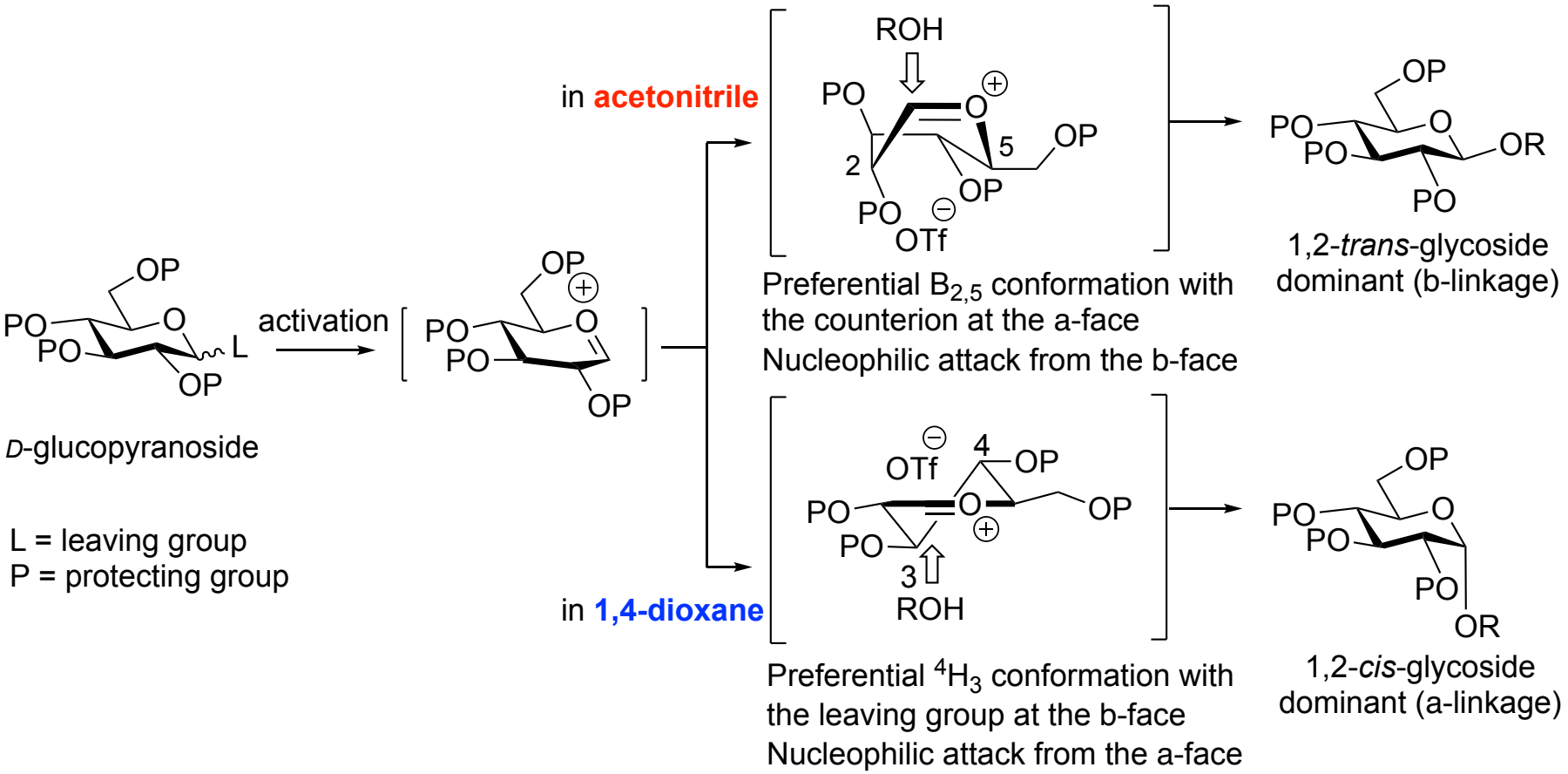


Solvent	1,2-*cis*	1,2-*trans*
Et_2O	50	50
CH_3CN	18	82
toluene-dioxane	53	47
toluene	24	76
dioxane-CH_3CN	24	76
CH_2Cl_2	44	56

H. Satoh *et al. J. Chem. Theory Comput*. **2010**, *6*, 1783.

Solvent effect

conformer and counterion distribution hypothesis

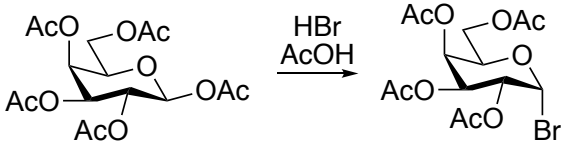


Satoh *et al. J. Chem. Theory Comput*. **2010**, *6*, 1783.

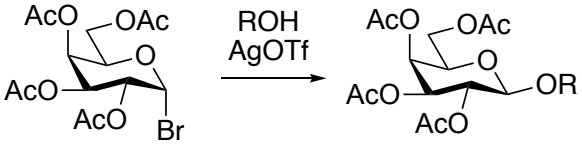
Typical glycosyl donor
Glycosyl bromide, chloride
Konig-Knorr method

- The traditional method.
- Soft Lewis acid ($HgBr_2$, $Hg(CN)_2$, Ag_2OTf, Ag_2CO_3) are used for activation.
- The donors are rather unstable.
- Preparation conditions of glycosyl donor are rather harsh. Many protecting groups are not stable under the conditions.

preparation



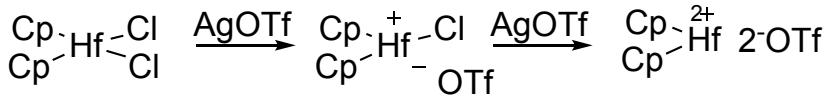
reaction

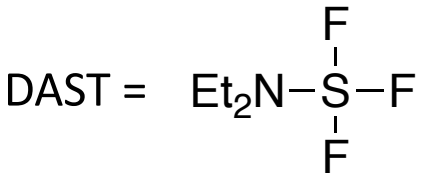


Typical glycosyl donor

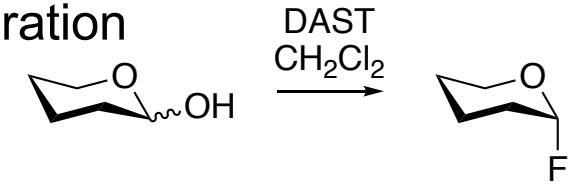
Glycosyl fluoride

- More stable than glycosyl bromide because C-F bond is strong.
- Activated by hard Lewis acid.
- Prepared by DAST (Diethylamino)sulfur trifluoride) from hemiacetal
- Activated by $SnCl_2$-$AgClO_4$, Cp_2HfCl_2-AgOTf, $Hf(OTF)_4$.
- AgOTf is recommended as an alternative of $AgClO_4$, because $AgClO_4$ is potentially explosive.
- Cp_2HfCl_2-AgOTf is more powerful than $SnCl_2$-$AgClO_4$, but activation ability is dramatically decreased in presence of base.
- $SnCl_2$-$AgClO_4$, Cp_2HfCl_2-AgOTf require pre-mix of reagent just before reaction. activation of molecular sieves are required. $Hf(OTf)_4$ does not need pre-activation of reagent.





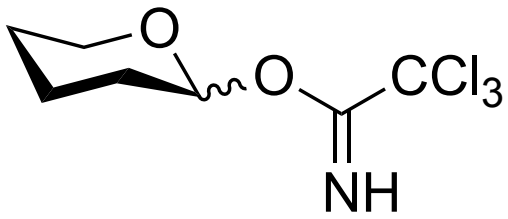
preparation



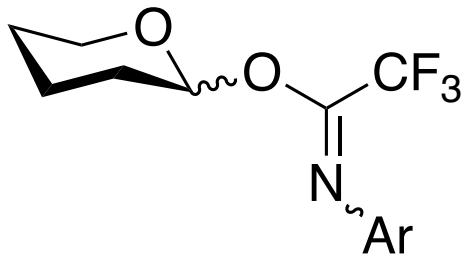
Typical glycosyl donor

Trichloroacetimidate

Most common

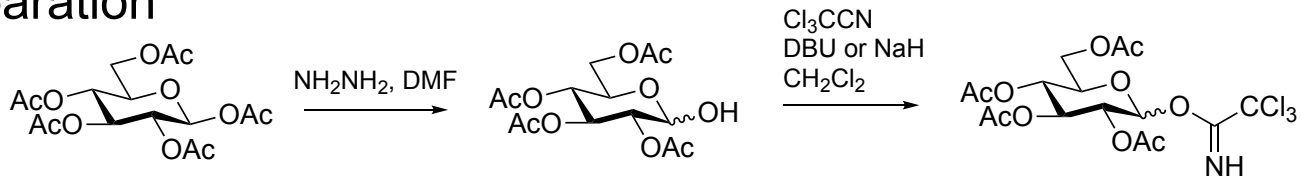


phenyltrifluoroacetimidate

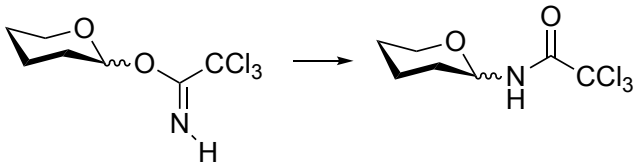


- Glycosyl imidates are activated by Lewis acid (BF_3•OEt_2, TMSOTf)
- The first choice of glycosylation reaction at present.

preparation



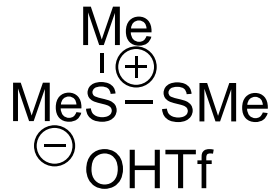
Amide product is generated as a side-product from trichloroacetimidate donor. This type of side-product is not generated from phenyltrifluoroacetimidate.



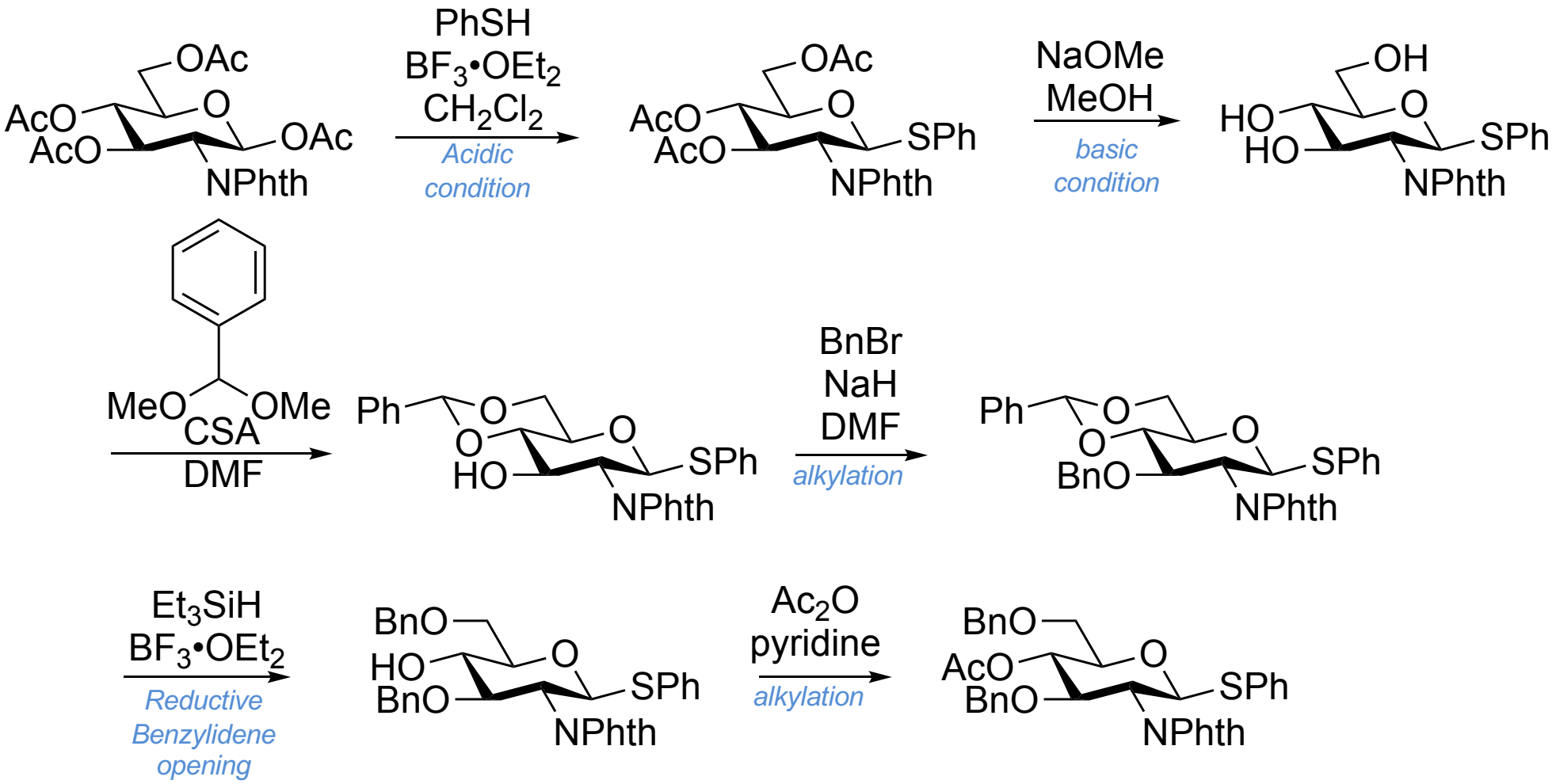
Typical glycosyl donor

thioglycoside

- Protecting group conversion is possible.
- Activation reagents
  MeOTf　(> room temperature)
  NIS-TMSOTf,
  DMTST（Dimethyl(methylthio)sulfonium trifluoromethanesulfonate),
  PhSCl-AgOTf (PhSOTf)
- Thioglycoside cannot be activated by Lewis acids.

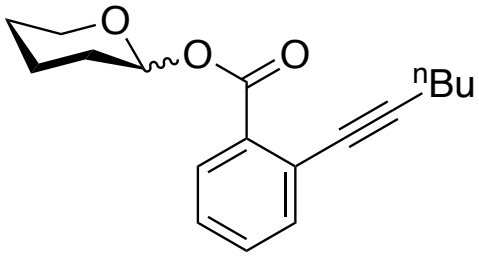
DMTST = 

Protecting group manipulation on thioglycoside



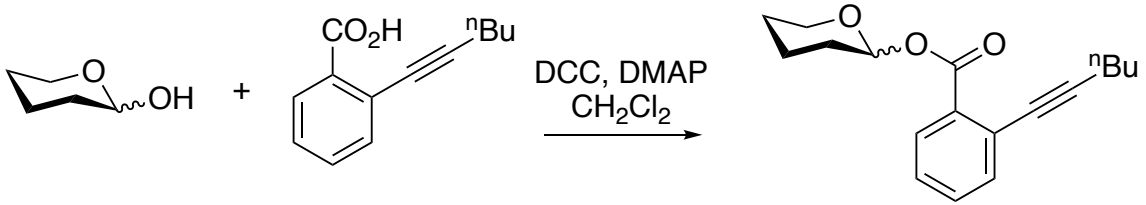
Typical glycosyl donor

alkyne

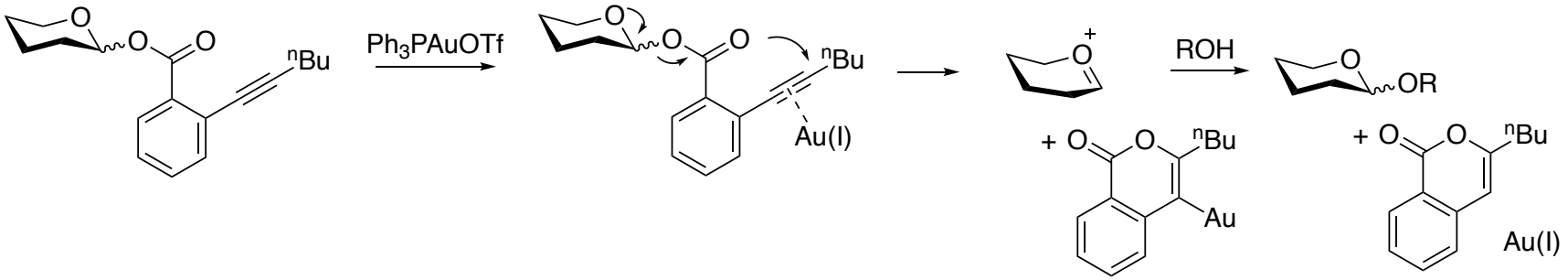


Au (I) coordination to alkyne is driving force.

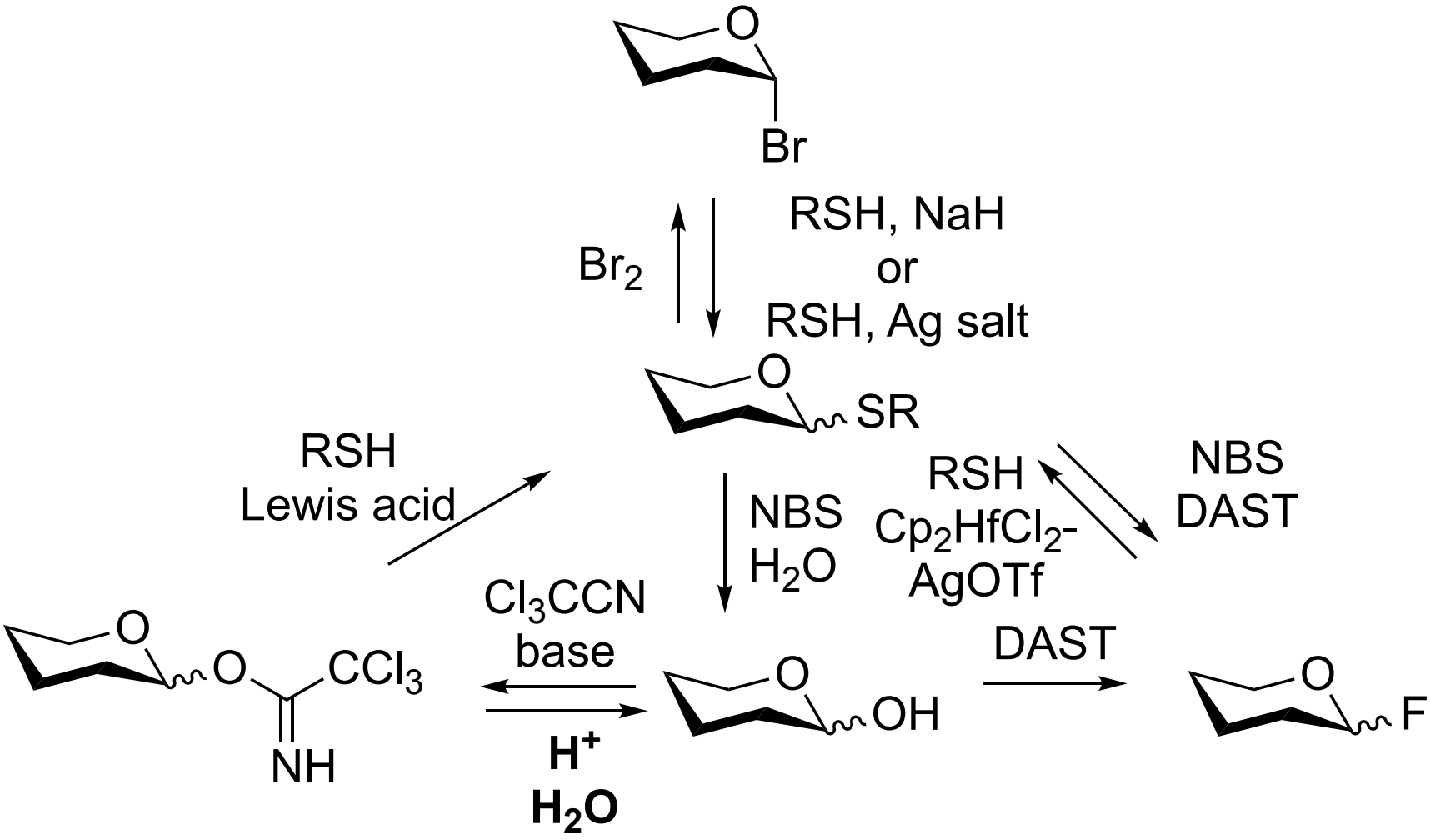
preparation



mechanism

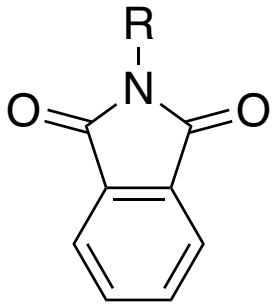


Conversion of glycosyl donors

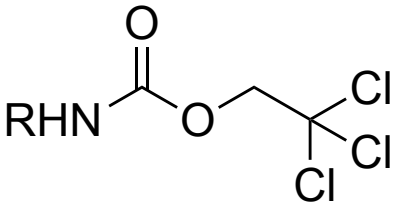


1,2-*trans* glycoside formation: aminoglycoside

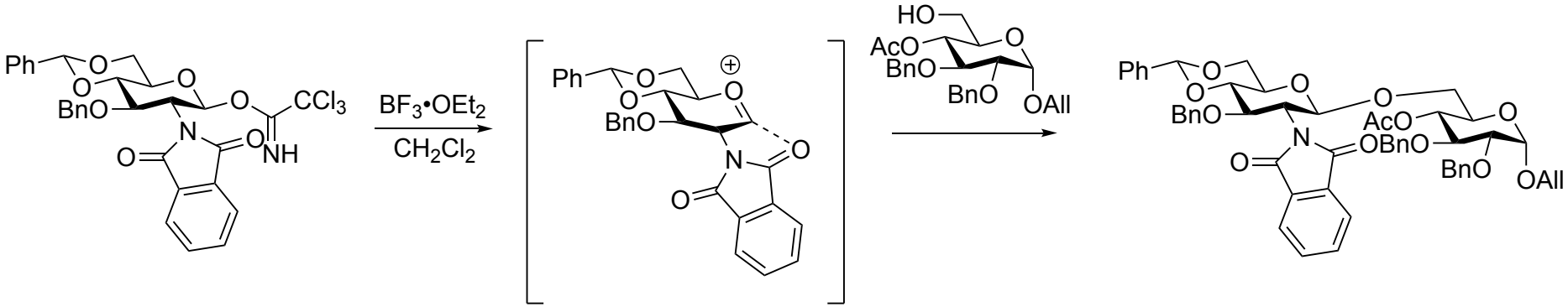
Neighboring participation group of amino group



Phthalimide (Phth)
Removable by hydrazine

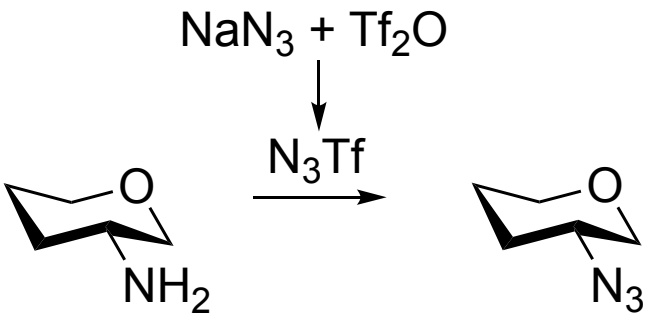


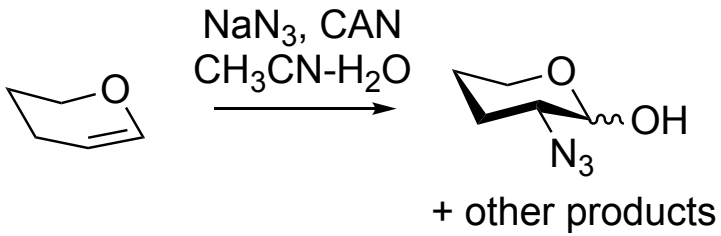
Trichloroethyl catrbamate (Troc)
Removable by Zn-AcOH

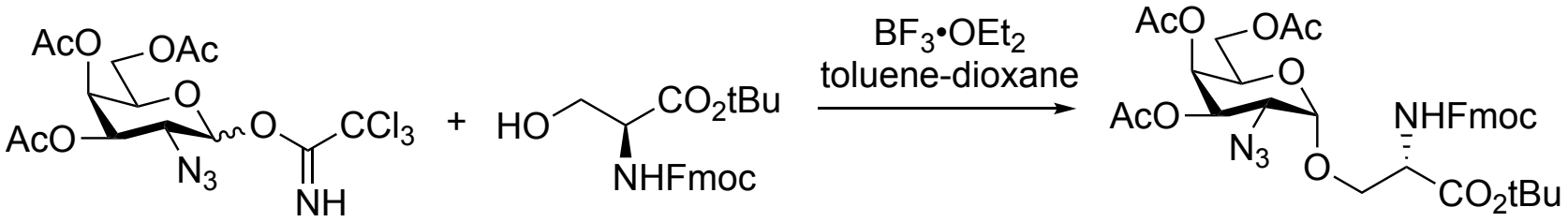


1,2-*cis* glycoside formation: aminoglycoside

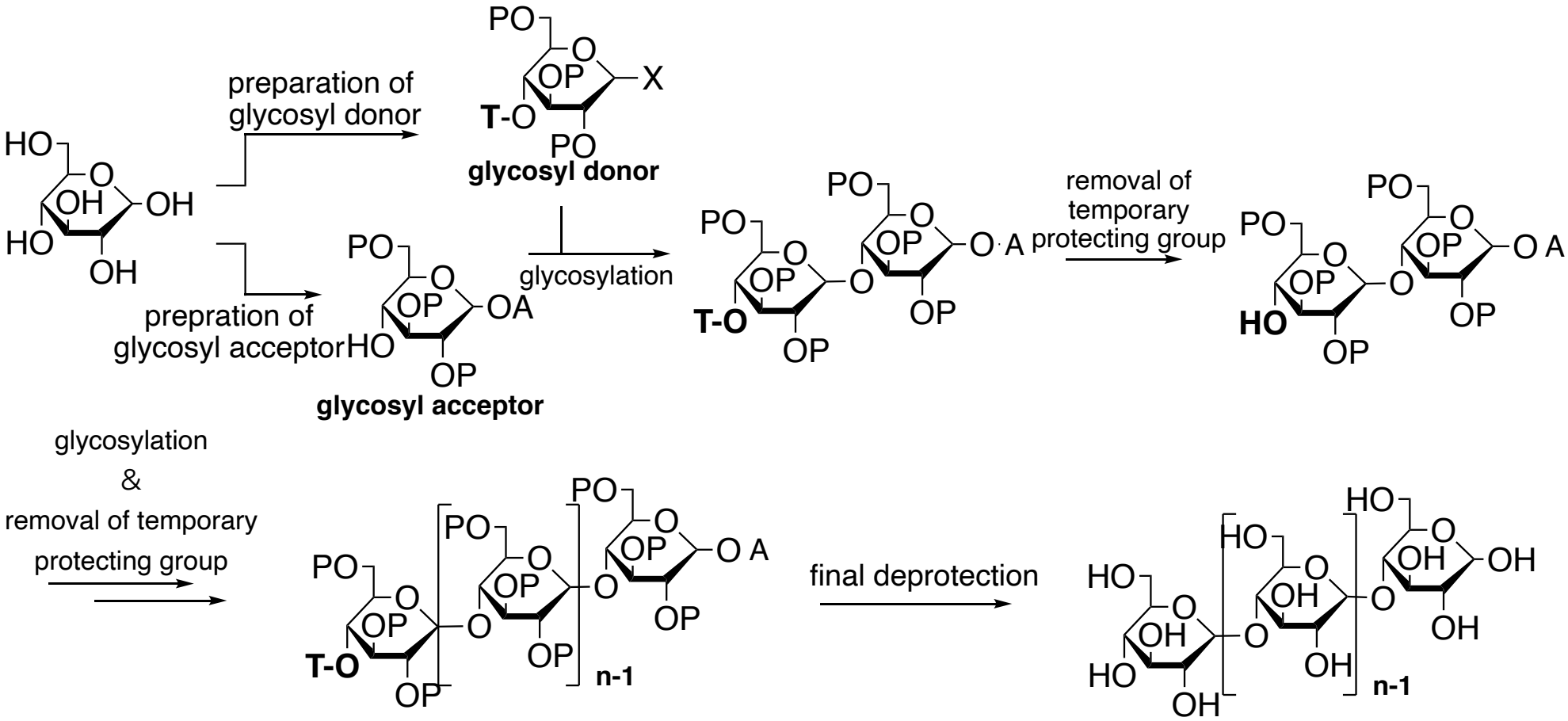
Azide group is used as a protecting group.
Azide can be reduced by various method such as PPh_3-H_2O.



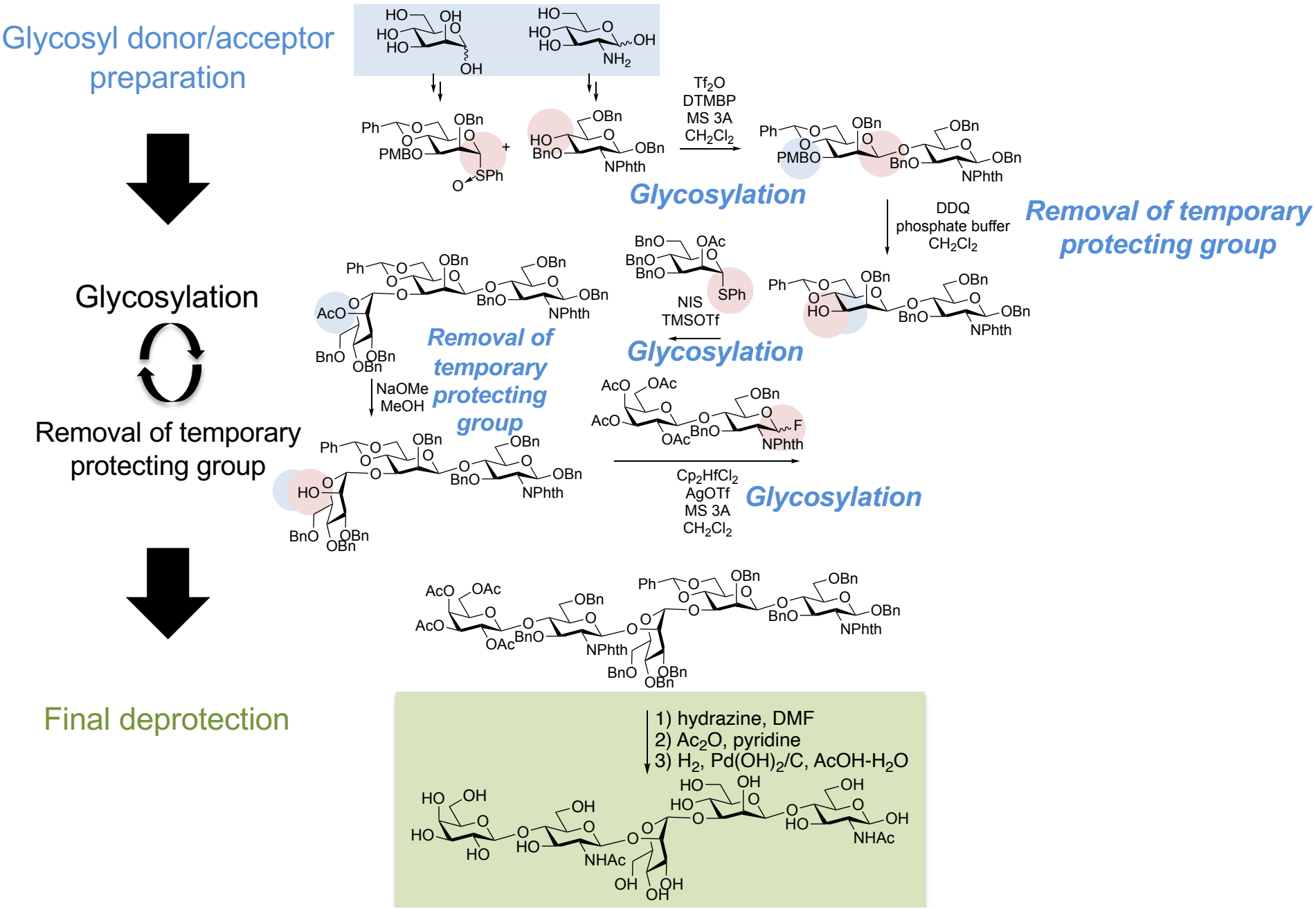




Strategy of oligosaccharide synthesis

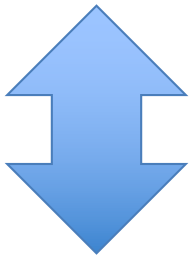


An example of oligosaccharide synthesis



Glycopeptide, glycoprotein synthesis

	Elongation	Final deprotection
Peptide synthesis	Almost neutral	Bn ether at side-functional group is removed under acidic conditions (such as TFA) Basic conditions are not employed.
Oligosaccharide synthesis	Lewis acid	Neutral or basic conditions are used. Acidic conditions are not employed.

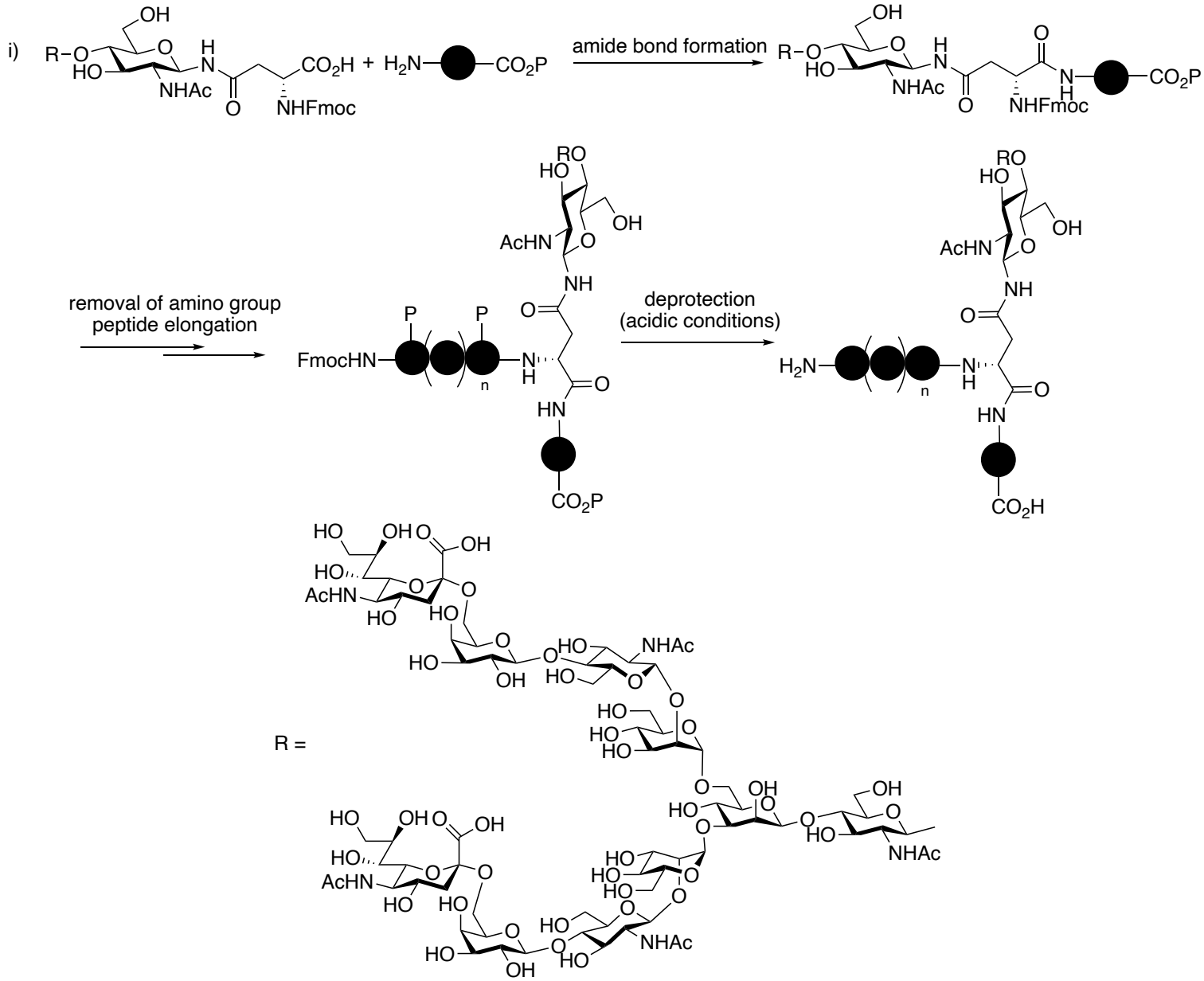


- Strong acidic conditions at final deprotection of peptide synthesis destroys glycan.
- Benzyl removal by Pd catalyst is difficult because amide group coordinates Pd catalyst.
- 50-mer peptide is length limitation on solid-phase peptide synthesis.
- Peptide/glycopeptide solubility is usually low.

Still under development

Glycoside synthesis

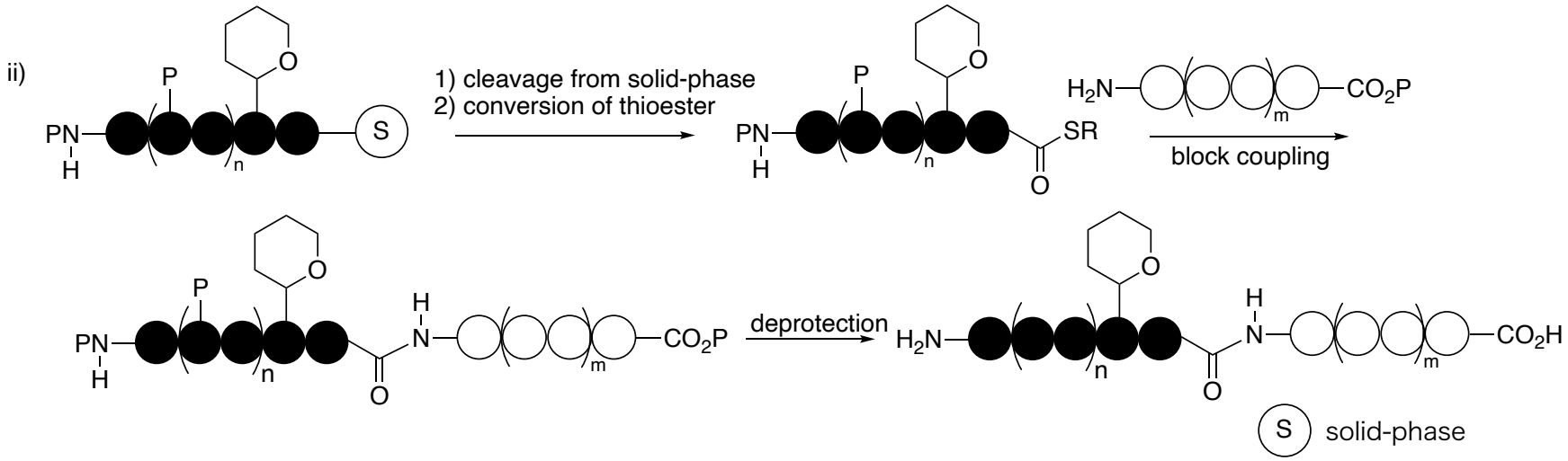
i) glyco-amino acid is used as special amino acid for peptide elongation.



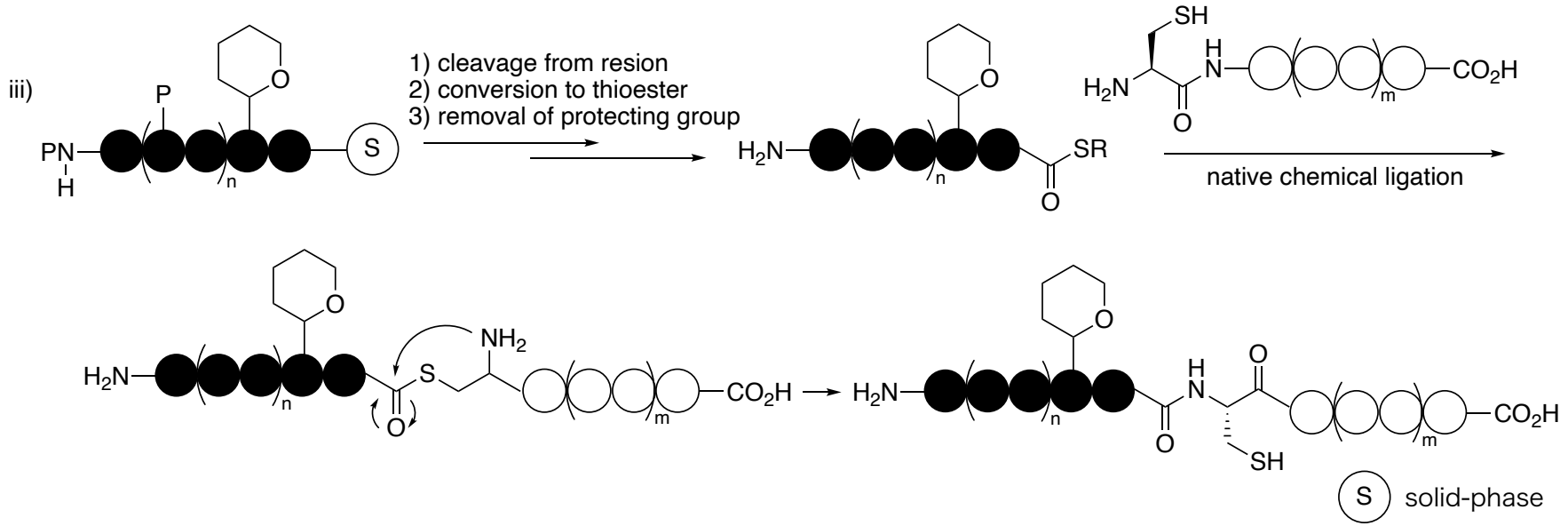
50-mer is limit for solid-phase peptide synthesis.

Glycoside synthesis

ii) Block synthesis



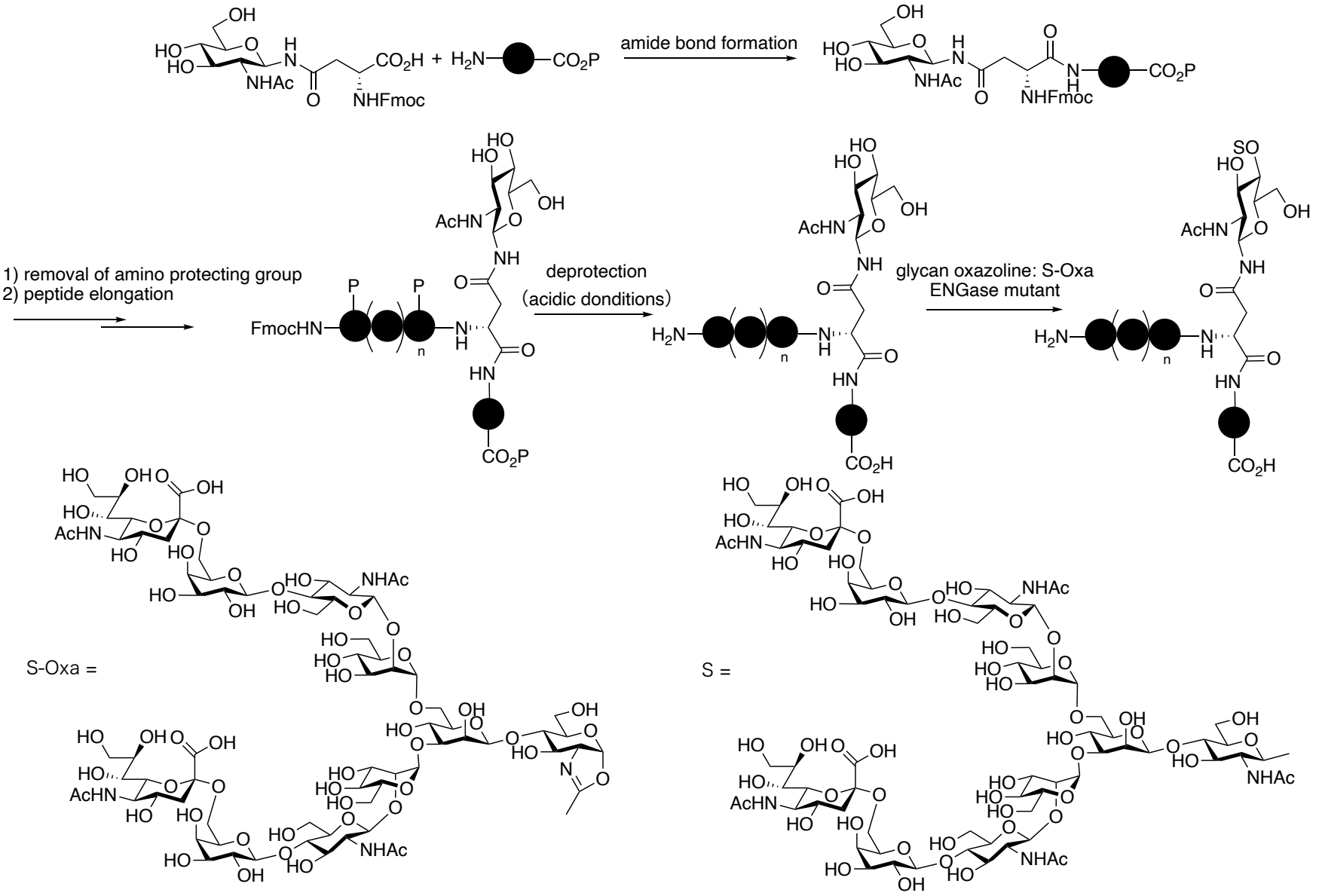
iii) Native Chemical Ligation



Glycoside synthesis

iv) chemo-enzymatic synthesis: ENGase mutant and glycan oxazoline

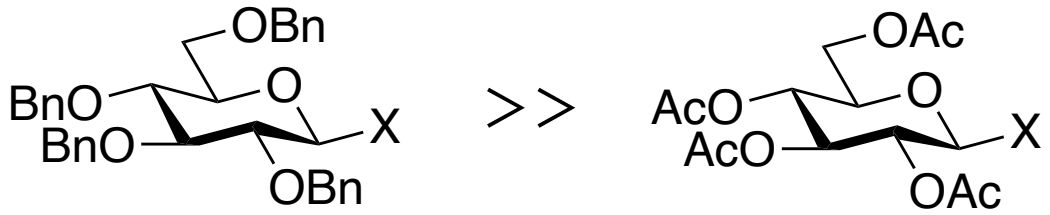
After removal of protecting group under acidic conditions, glycan is introduced by ENGase mutants



For efficient glycoside synthesis

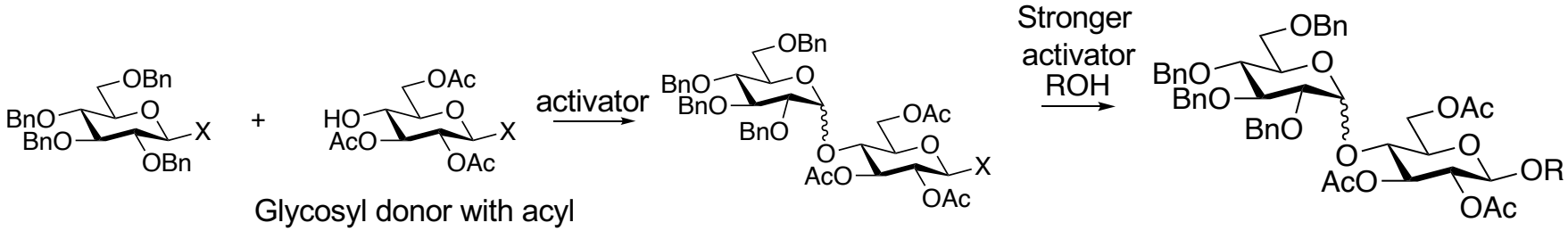
armed-disarmed concept

Reactivities of glycosyl donors are different upon protecting group.



Ether protected donor Acyl protected donor

B. Fraser-Reid *et al. J. Am. Chem. Soc*. **1988**, *110*, 5583.

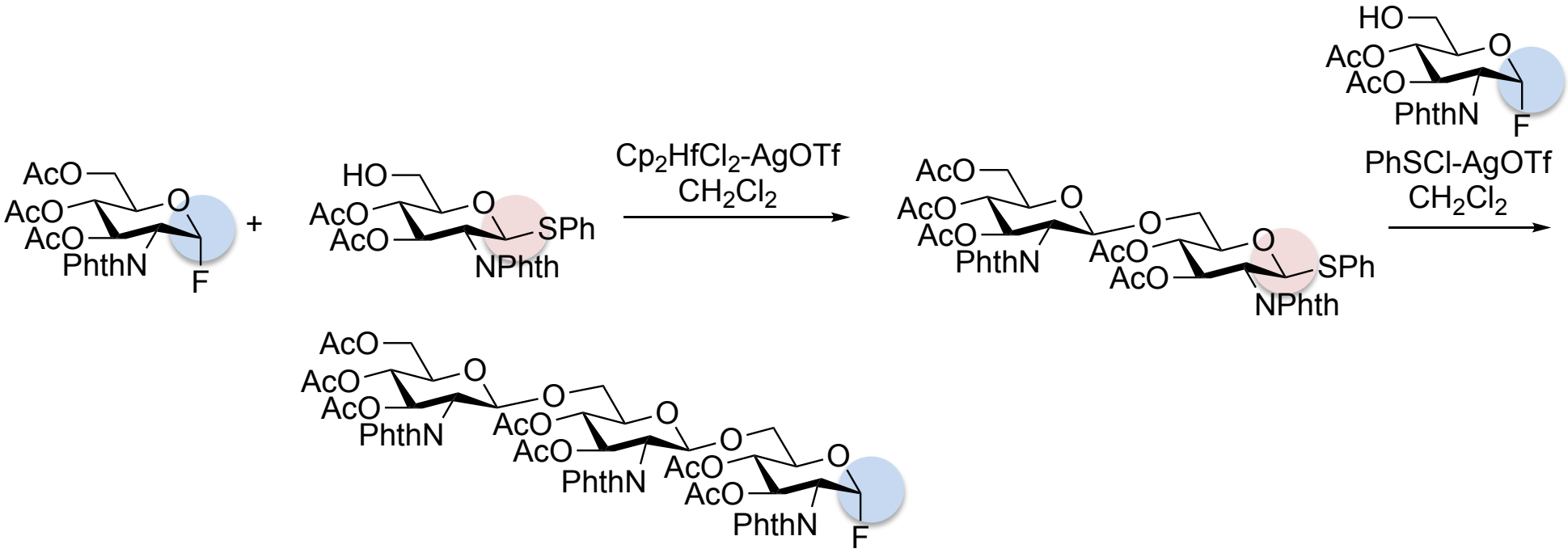


Glycosyl donor with acyl group is less reactive.

1. Protecting group manipulation steps can be omitted.
2. One-pot glycosylation can be possible.

For efficient glycoside synthesis

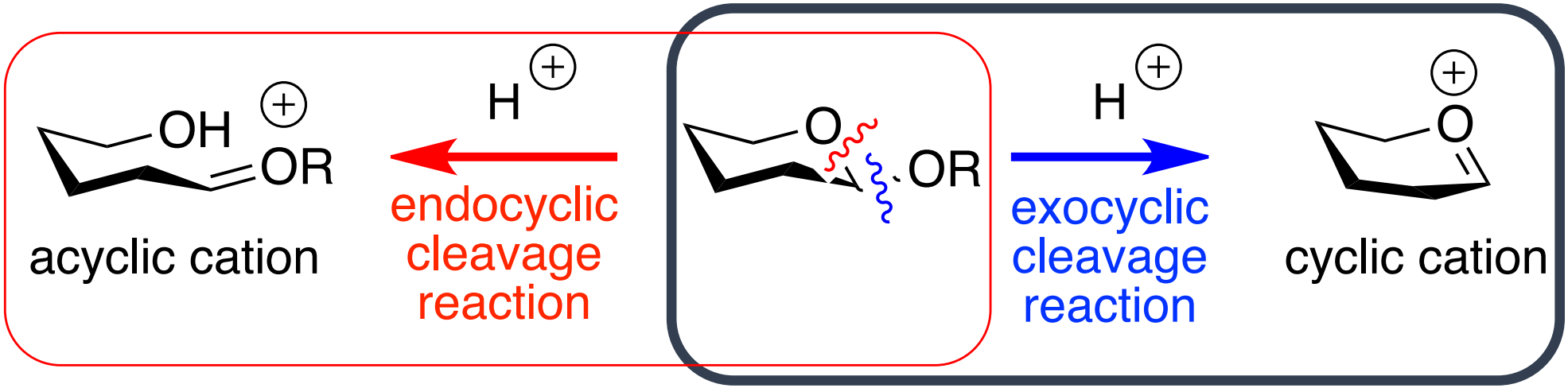
Orthogonal glycosylation



Glycosyl fluoride and thioglycoside are activated under orthogonal conditions. Orthogonal glycosylation shorten glycoside synthesis. Conversion of protecting group and introduction of leaving group at anomeric position can be omitted. By orthogonal glycosylation, glycan is elongated from non-reducing endo to reducing end.

O. Kanie *et al. J. Am. Chem. Soc*. **1994**, *116*, 12073.

Endocyclic cleavage vs exocyclic cleavage

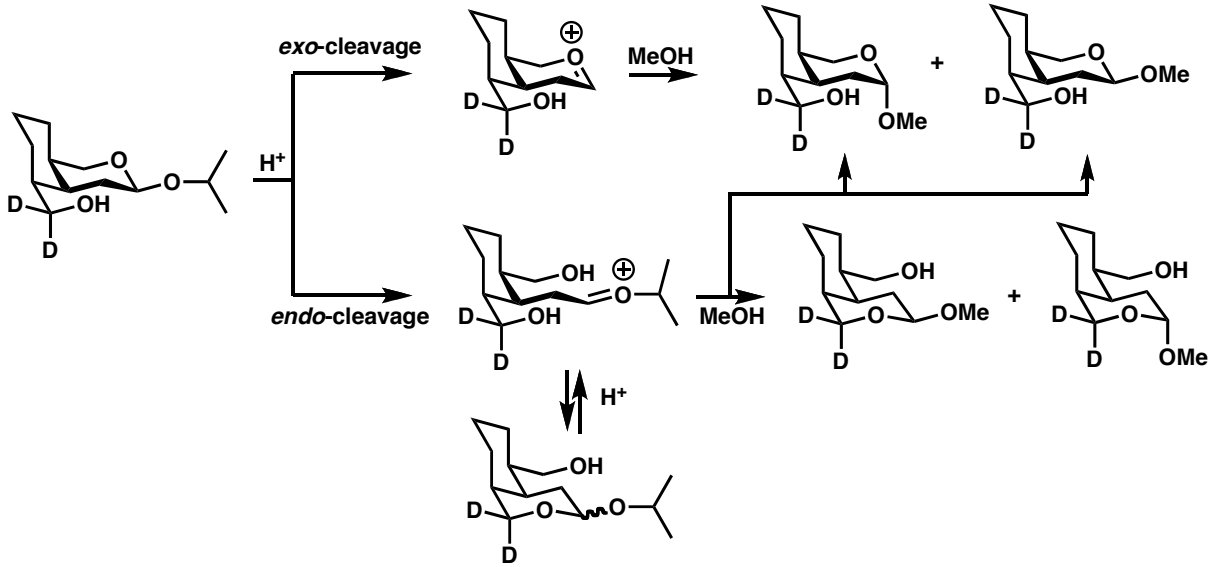


Our research

Attempts to prove endocyclic cleavage : 1980~1990's

By inspired calculations on lysozyme hydrolysis (*J. Am. Chem. Soc*. **1986**, *108*, 1317.), several groups tried to prove endocyclic cleavage reaction.

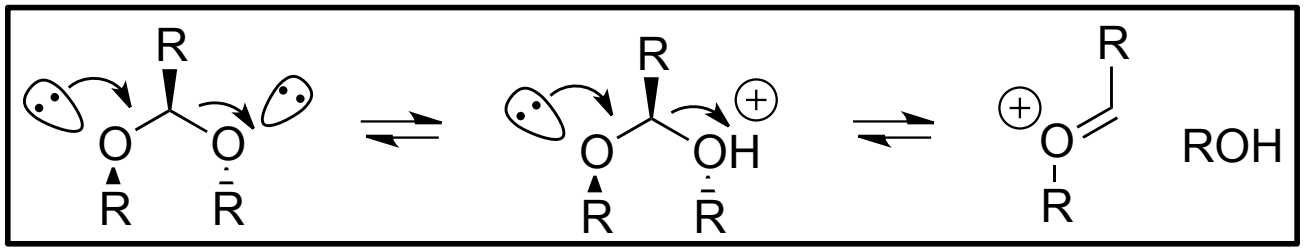
At that time, stereoelectronic effect is thought to be dominant factor. So conformationally locked substrates were used as substrates.



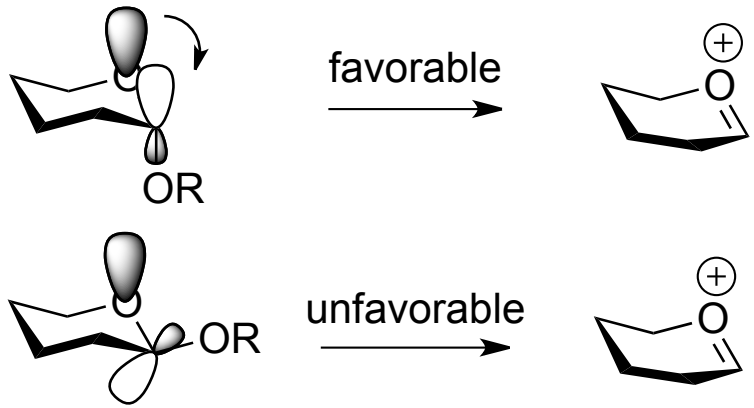
J. L. Liras, E. V. Anslyn, *J. Am. Chem. Soc*. **1994**, *116*, 2645.
R. B. Gupta, R. W. Franck, *J. Am. Chem. Soc*. **1987**, *109*, 6554.

- Endocyclic cleavage ratio is small compared to exocyclic cleavage reaction.
- Room temperature is required for endocyclic cleavage reaction.
- Only β-glycoside mimic compounds showed endocyclic cleavage reaction.

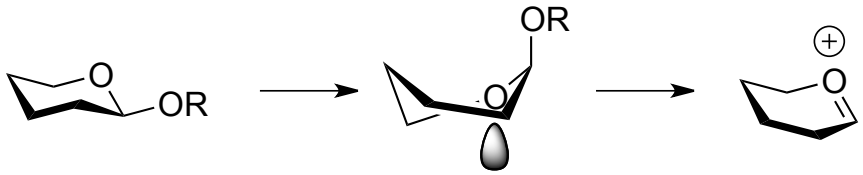
Acetal hydrolysis mechanism from stereoelectronic effect



The antiperiplanar geometry allows optimal overlap between non-bonding electron pair (HOMO) and vacant σ* orbital of the adjacent C-X bond.



Conformation change is important for hydrolysis for β-glycosides.

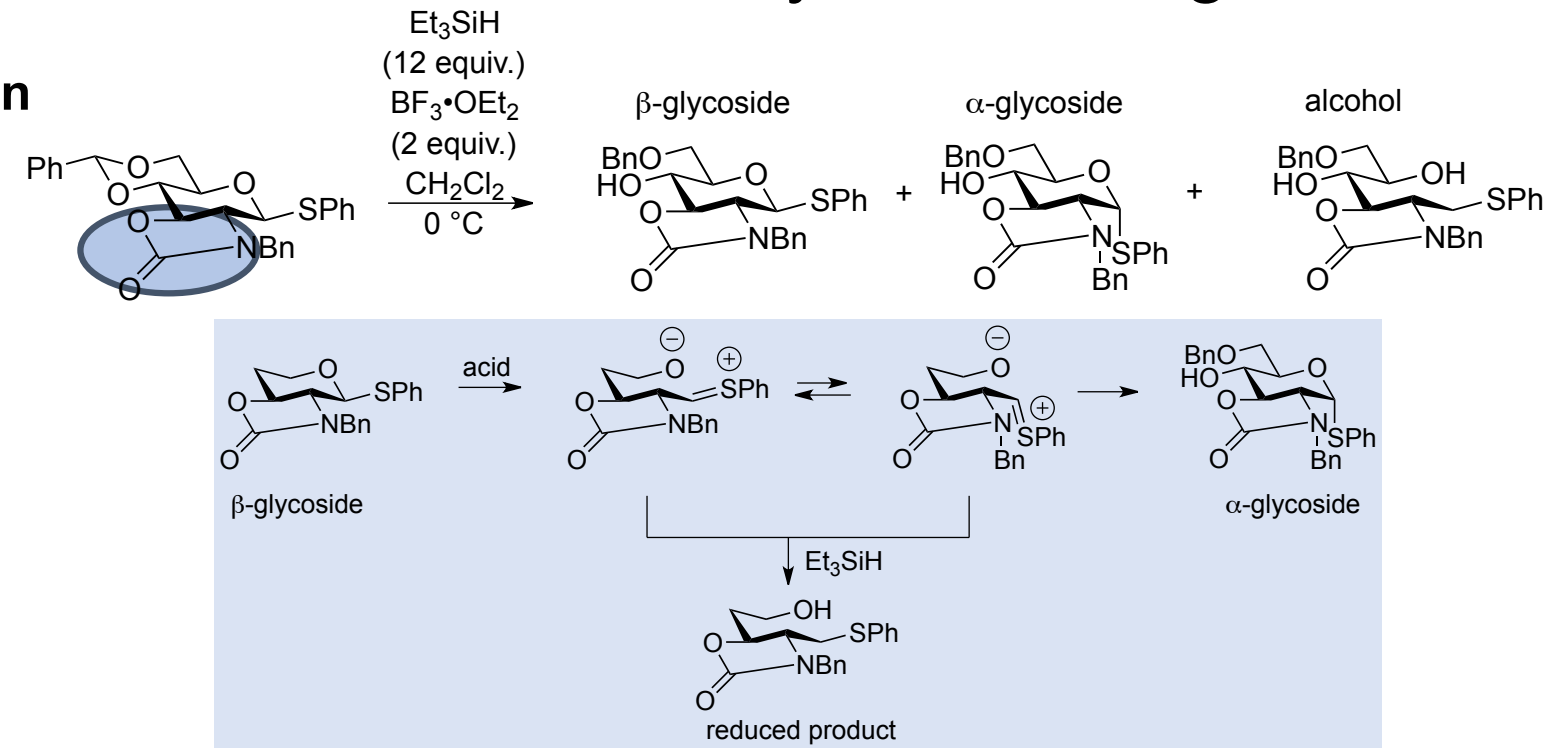


A. J. Kirby: Stereoelectronic effect Oxford Press

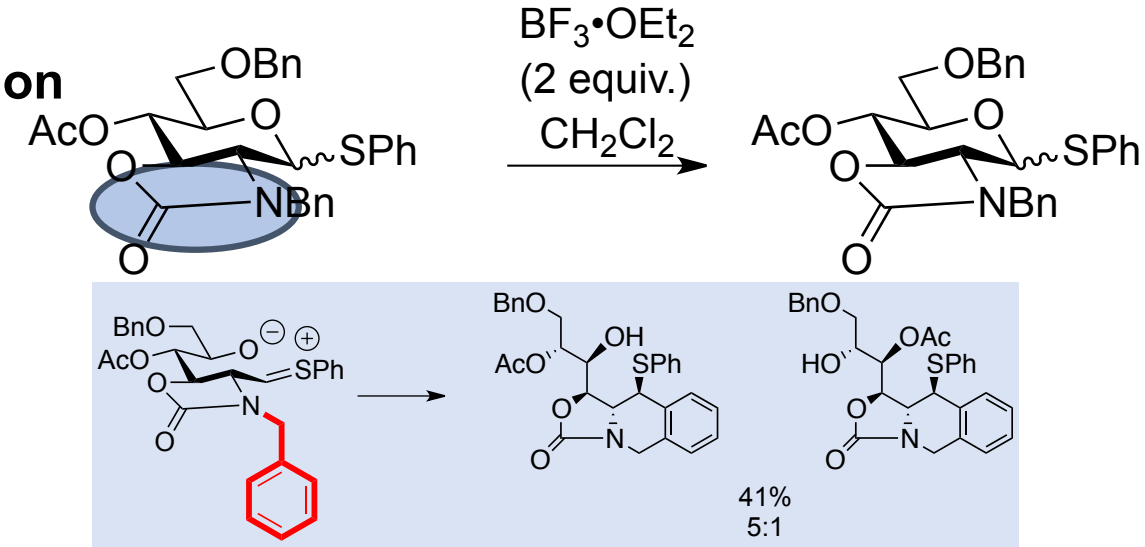
Our research

Evidence of endocyclic cleavage reaction

Reduction



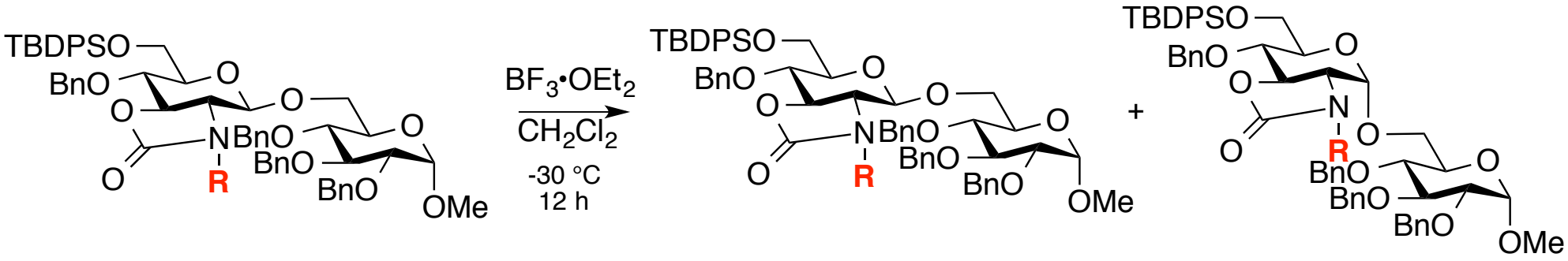
Intramolecular Friedel-Crafts reaction



S. Manabe *et al. Chem. Eur. J.* **2009**, *15*, 6874.

Our research

Substituent effect on anomerization via endocyclic cleavage



R	β	α
H	86	0
Bn	90	5
CO_2Me	21	73
Ac	0	88

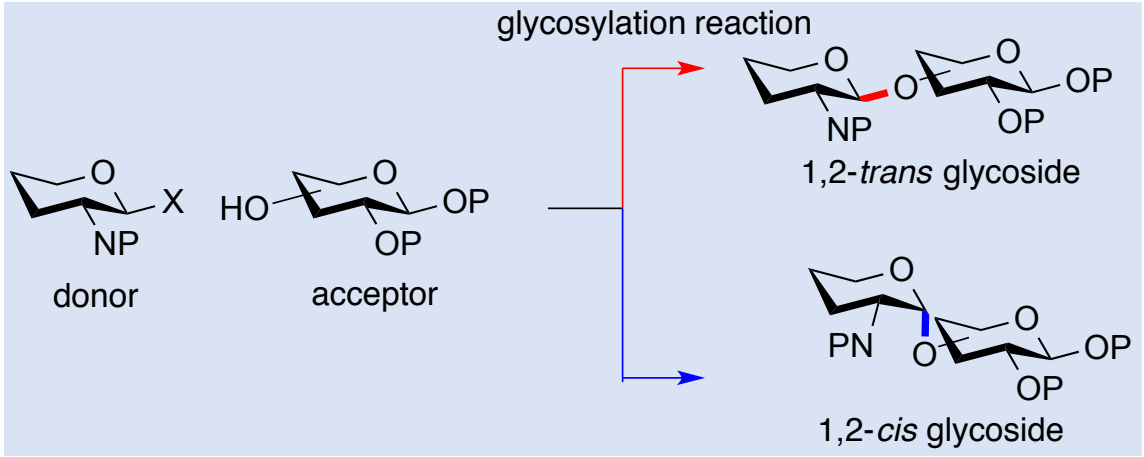
- Acetyl group is the best for anomerization reaction.
- Stereoselective 1,2-*cis* aminoglycoside formation is difficult by conventional glycosylation.
- Anomerization rection through endocyclic cleavage is useful for 1,2-cis aminoglycoside formation .

S. Manabe *et al. Tetrahedron,* **2011,** 67, 9966.
H. Satoh *et al. J. Am. Chem. Soc.* **2011**, 133, 5610.
S. Manabe *et al. Eur. J. Org. Chem*. **2011**, 497.
S. Manabe *et al. J. Am. Chem. Soc.* **2006,** 128, 10666.

Our research

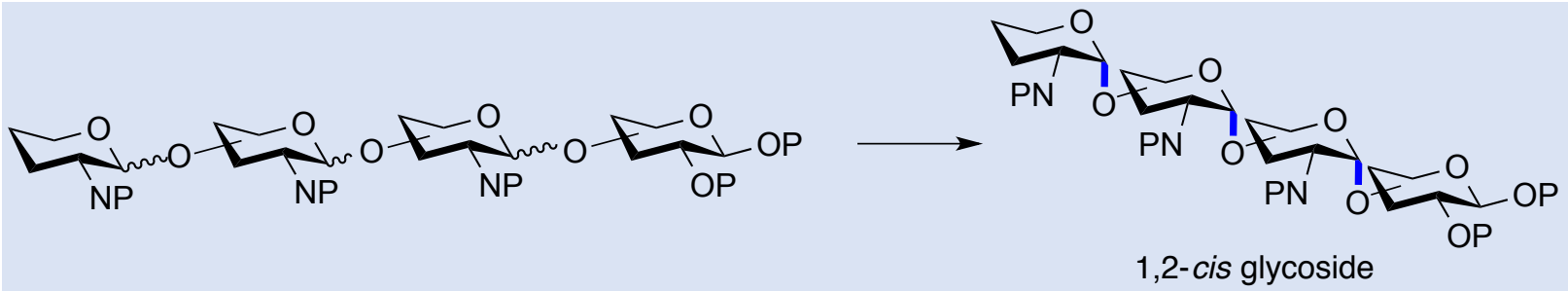
Stereocontrol of glycoside formation

Conventional glycosylation (since 1893, Fischer glycosylation)



- Stereochemistry at anomeric center is determined in glycosylation reaction.
- Stereochemistry is determined in each glycosylation reaction.

Anomerization of glycosides

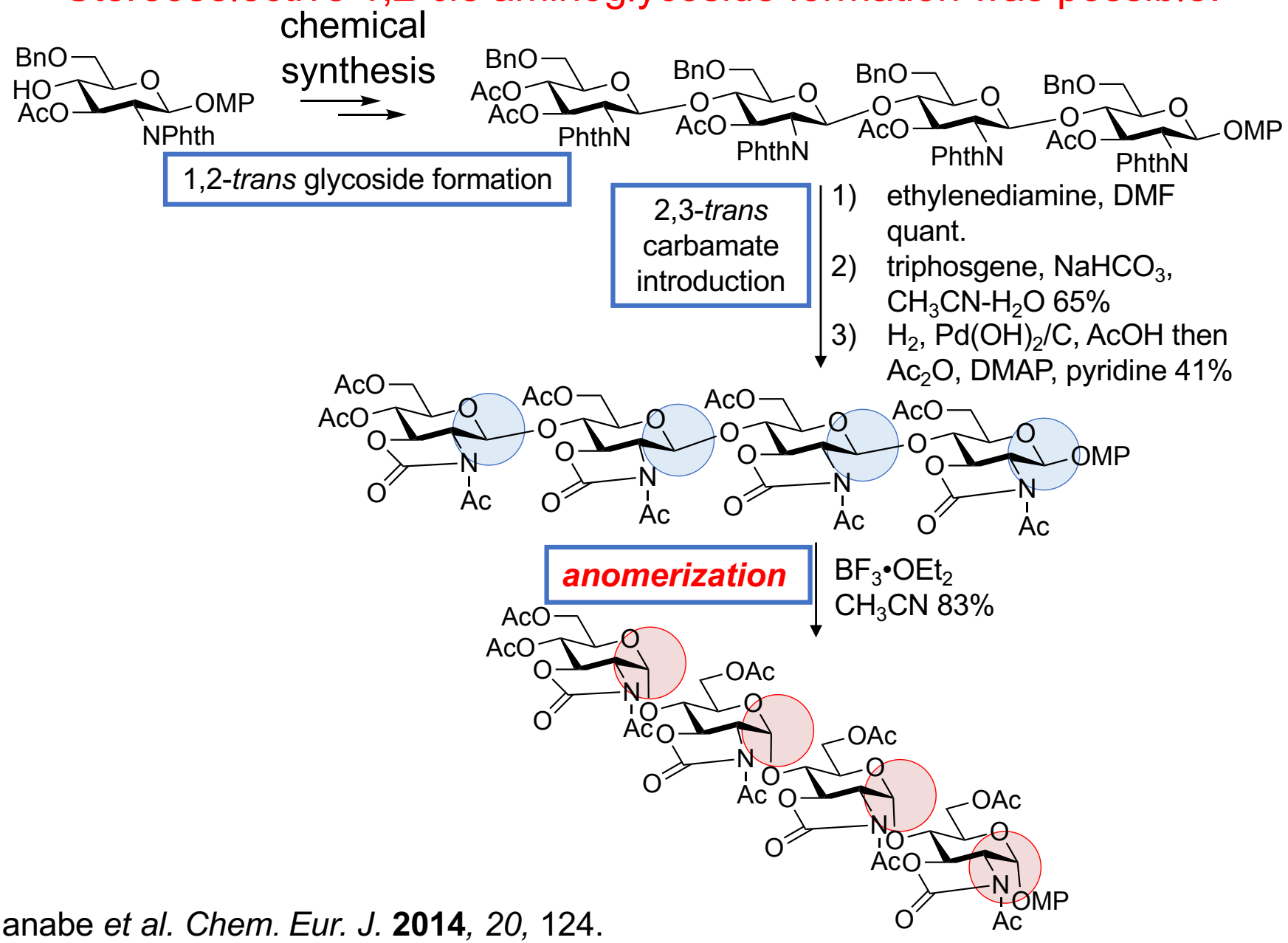


- Multiple glycoside stereochemistries can be changed at once.

Our research

Multiple stereochemistries at anomeric center was changed at once.

Stereoselective 1,2-*cis* aminoglycoside formation was possible.



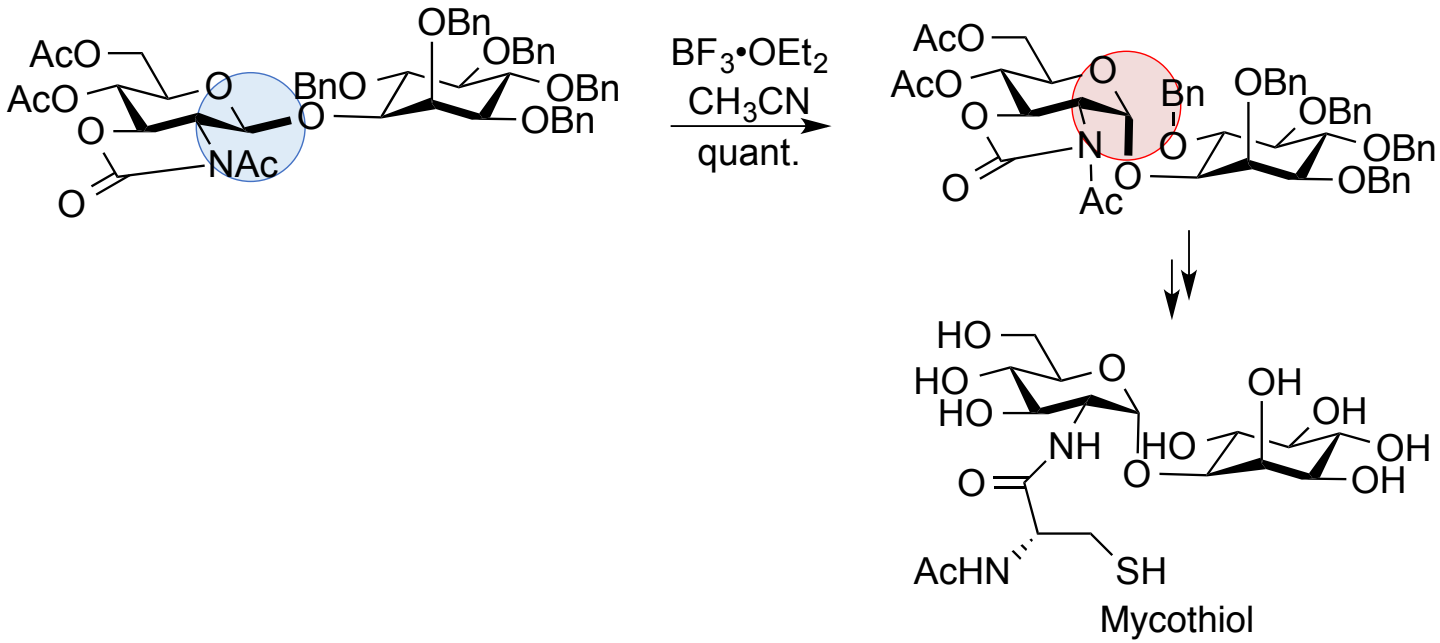
S. Manabe *et al. Chem. Eur. J.* **2014**, *20*, 124.

Our research

Synthesis of biologically active oligosaccharide by using anomerization reaction

Mycothiol synthesis

Key compound for anti-*tuberculosis* agent development



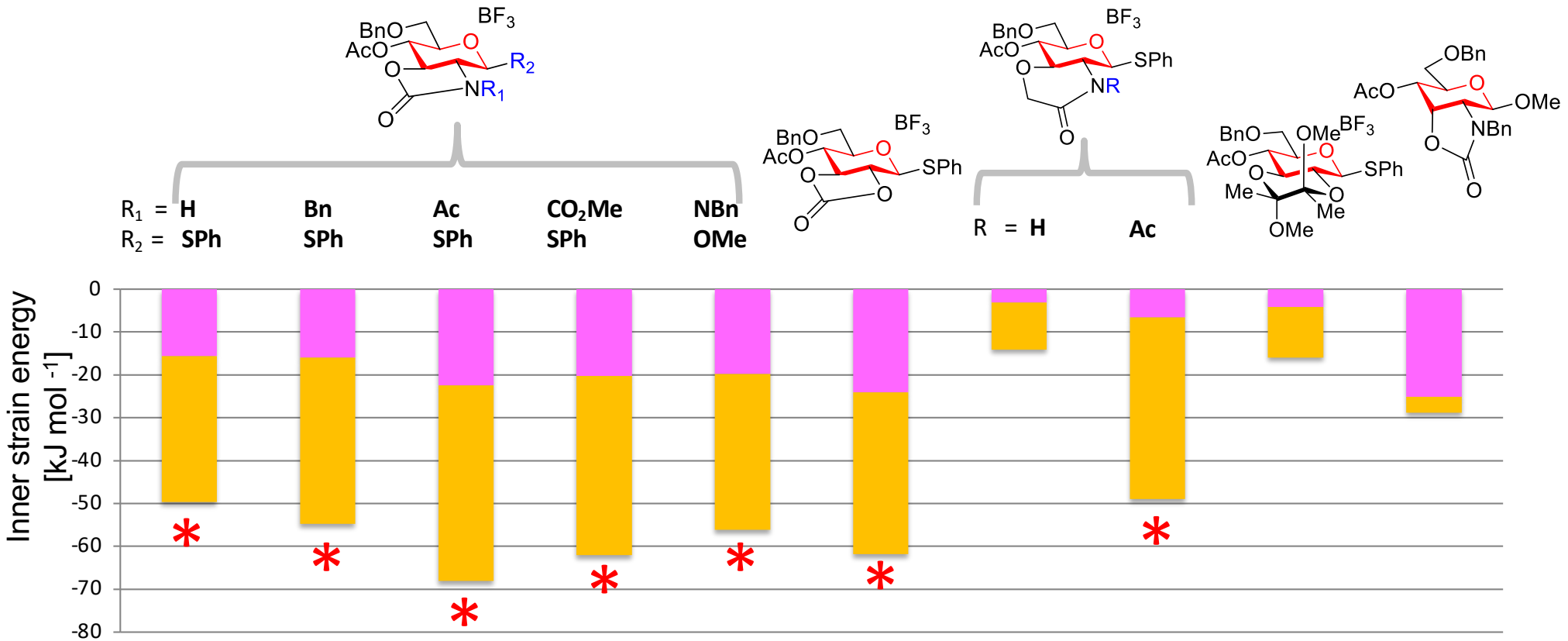
S. Manabe, Y. Ito, *Beilstein J. Org. Chem.* **2016**, *12*, 328.

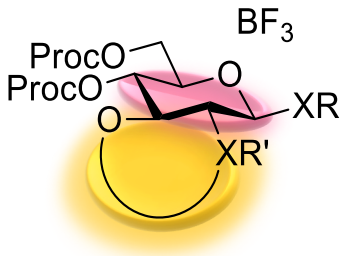
S. Manabe, Y. Ito, *Tetrahedron,* **2018,** 74*,* 2440.

S. Travis *et al. Med. Chem. Commun*. **2019**, *10*, 1948.

Our research

Inner Strain from Fused Ring is Predominant





E_I : Strain from pyranose ring

E_{II} : Strain from fused ring

∗ : Anomerization was observed (experiments)

Gaussian03
B3LYP/6-31G(d,p)

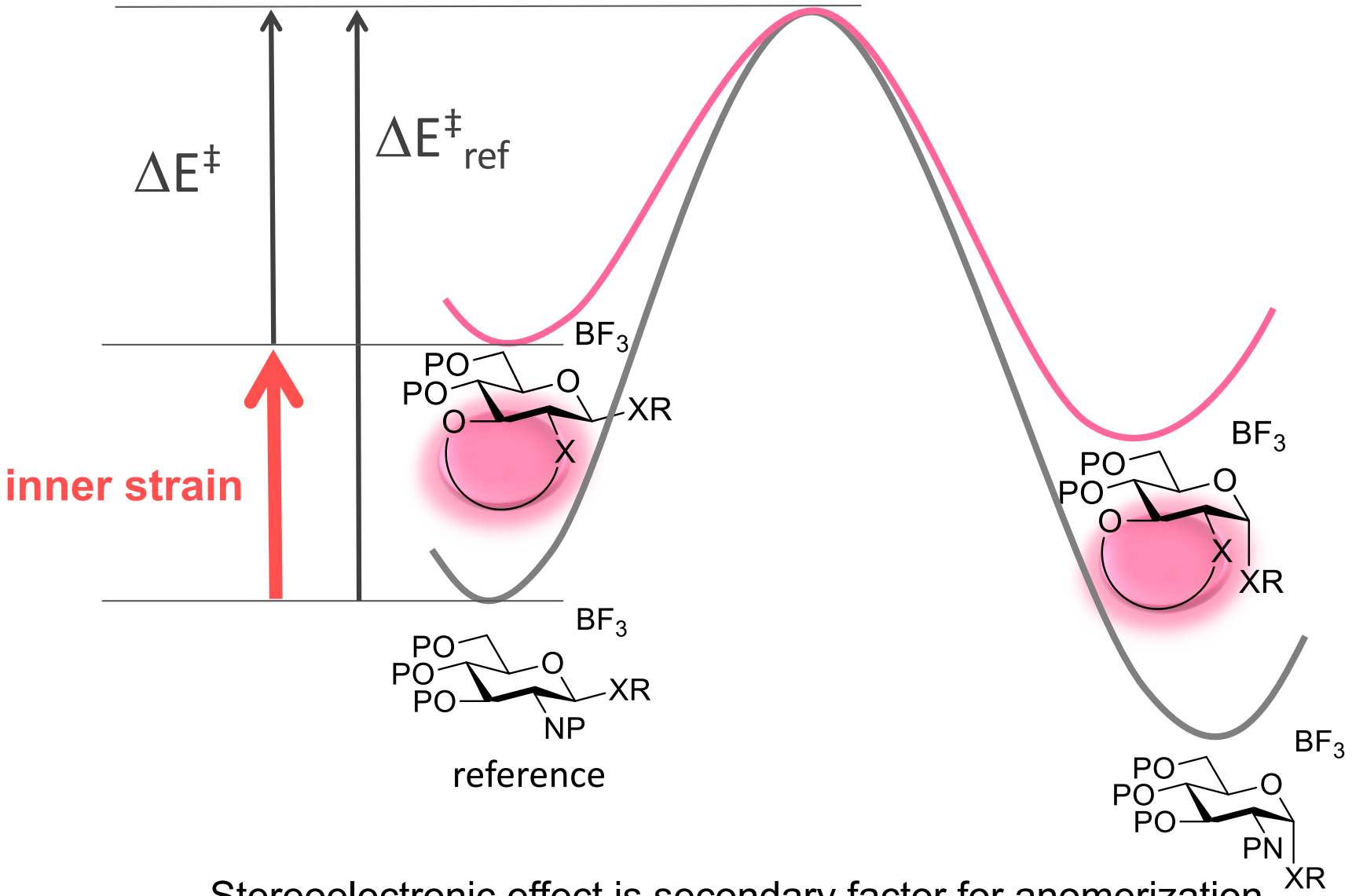
Calculations agrees with experiments well.

H. Satoh *et al. J. Am. Chem. Soc.* **2011**, *133*, 5610.

Our research

Theoretical Hypothesis

Inner strain pushes up the potential energy of reactants



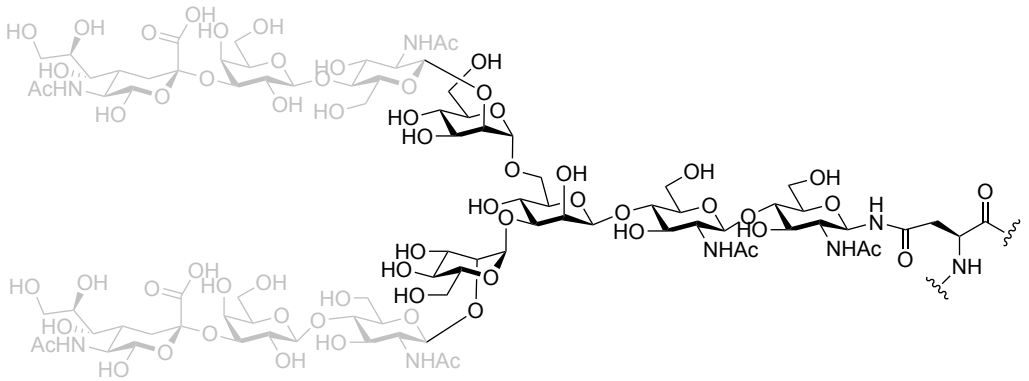
Stereoelectronic effect is secondary factor for anomerization.

H. Satoh *et al. J. Am. Chem. Soc.* **2011**, *133*, 5610.

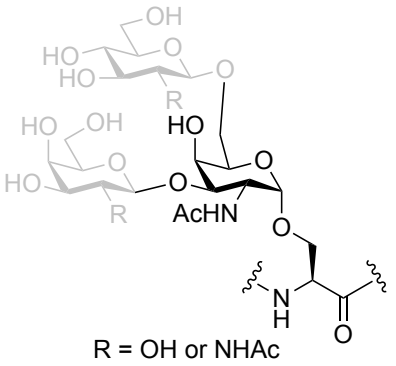
Our research

C-mannosyl tryptophan

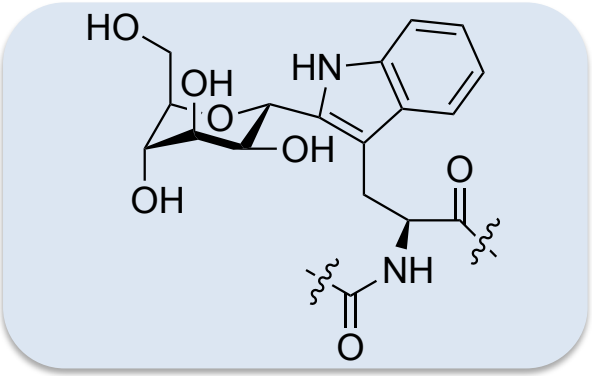
N-glycan on protein



O-glycan on protein



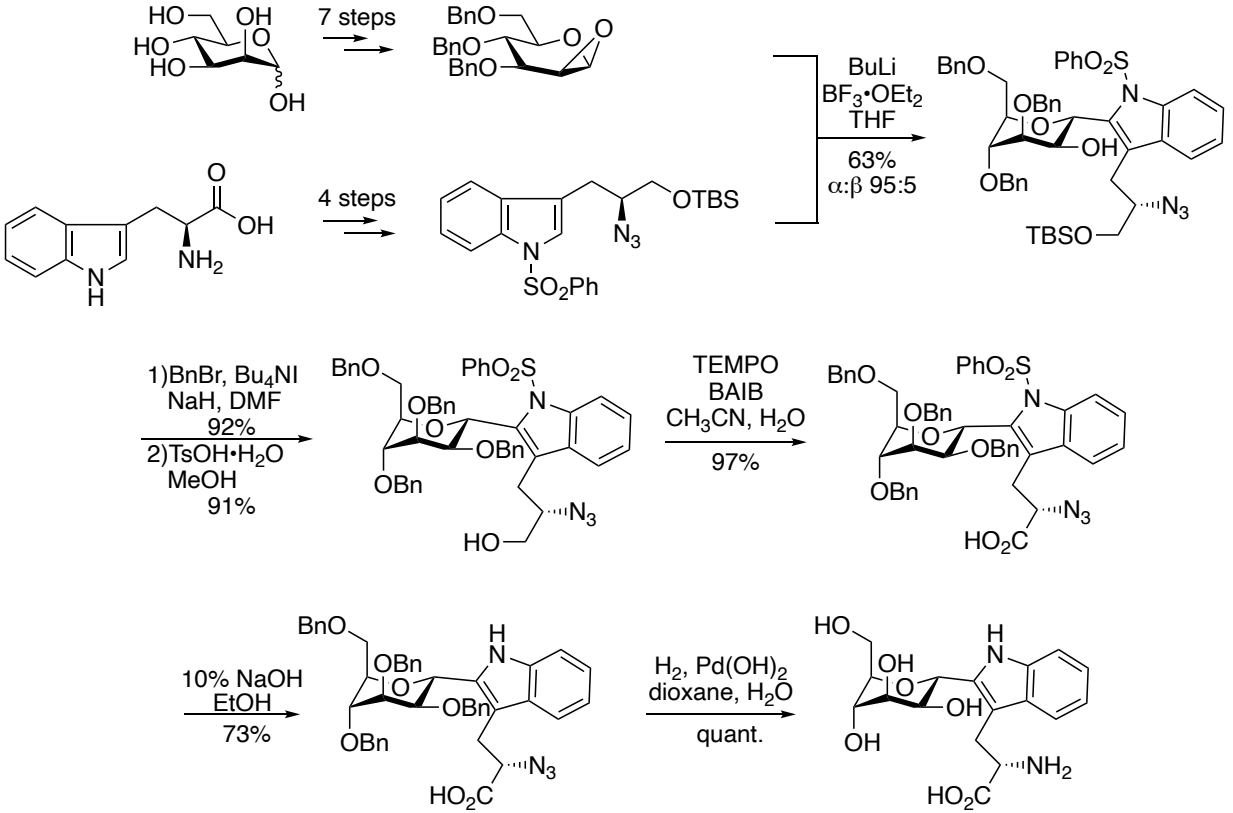
C-mannosyl tryptophan



- *C*-Man-Trp was found as post-translational modification in 1994.
- *C*-Man-Trp was found in various spieces from Ebora virus to human.
- Recognition sequence is Trp*-X-X-Trp（＊is mannosylated.）.
- *C*-Man-Trp can be a marker for renal function and Ovarian cancer.
- Biological function is not clear yet.

Our research

Synthesis of C-Man-Trp and diagnosis possibility



J. Am. Chem. Soc. **1999**, *121*, 9754.

Collaboration with Professor Ihara

Synthesis
High overall yield

- Antibody preparation
- Peptide synthesis

- Relationship to decreased renal function
- Increased in diabetic rat models
- Potential biomarker for ovarian cancer
  - Higher sensitivity (93.1%) than the current biomarker CA125 (sensitivity 72.4%)

N. Iwahashi *et al. Oncol. Lett.* **2020**, *19*, 908; S. Sakurai *et al*. *Sci. Rep*. **2019**, *9*, 4675;
Y. Ihara *et al*. *Glycobiology* **2010**, *20*, 1298; Y. Ihara *et al*. *Glycobiology* **2005**, *15*, 383.