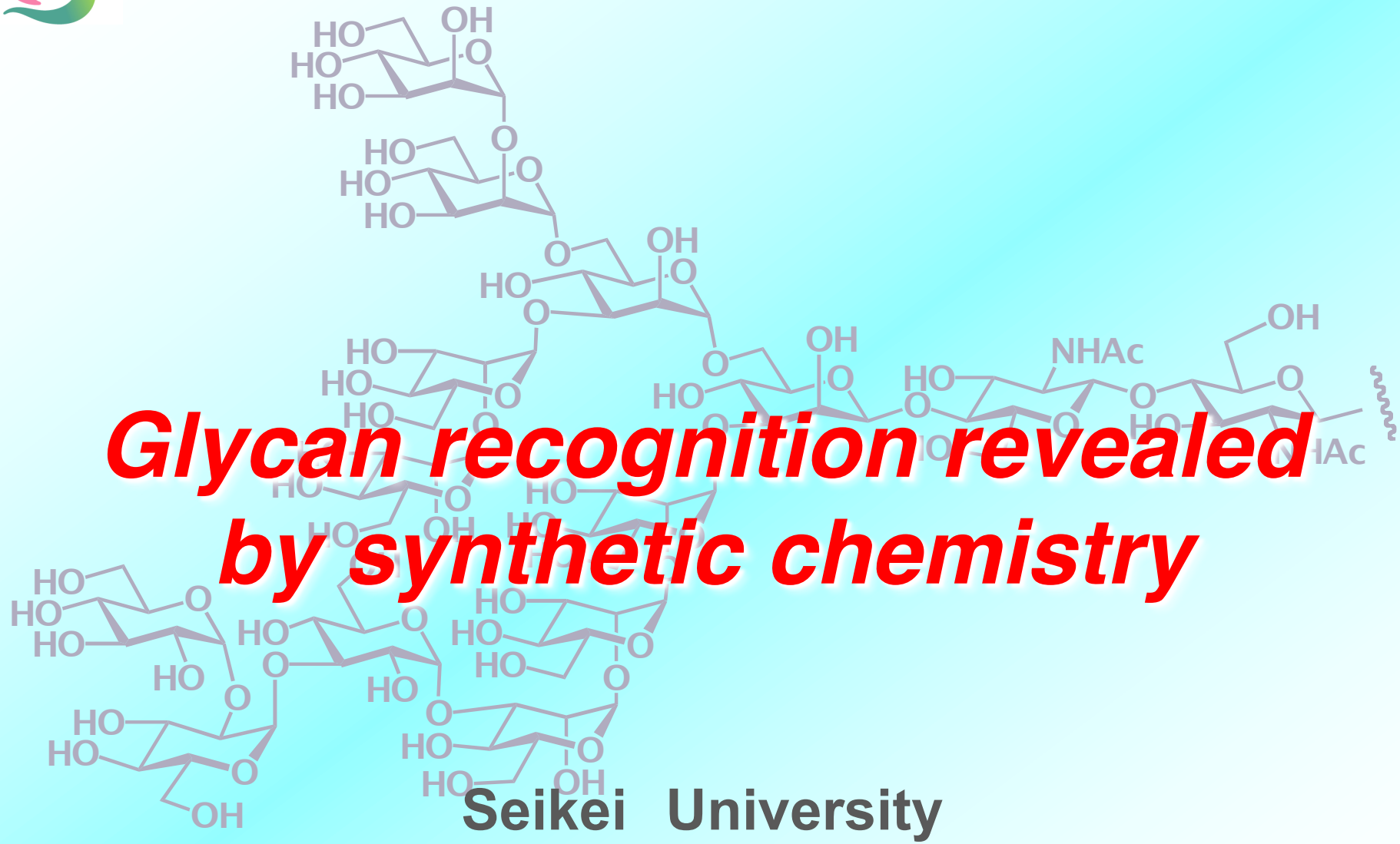
Glycan recognition revealed by synthetic chemistry



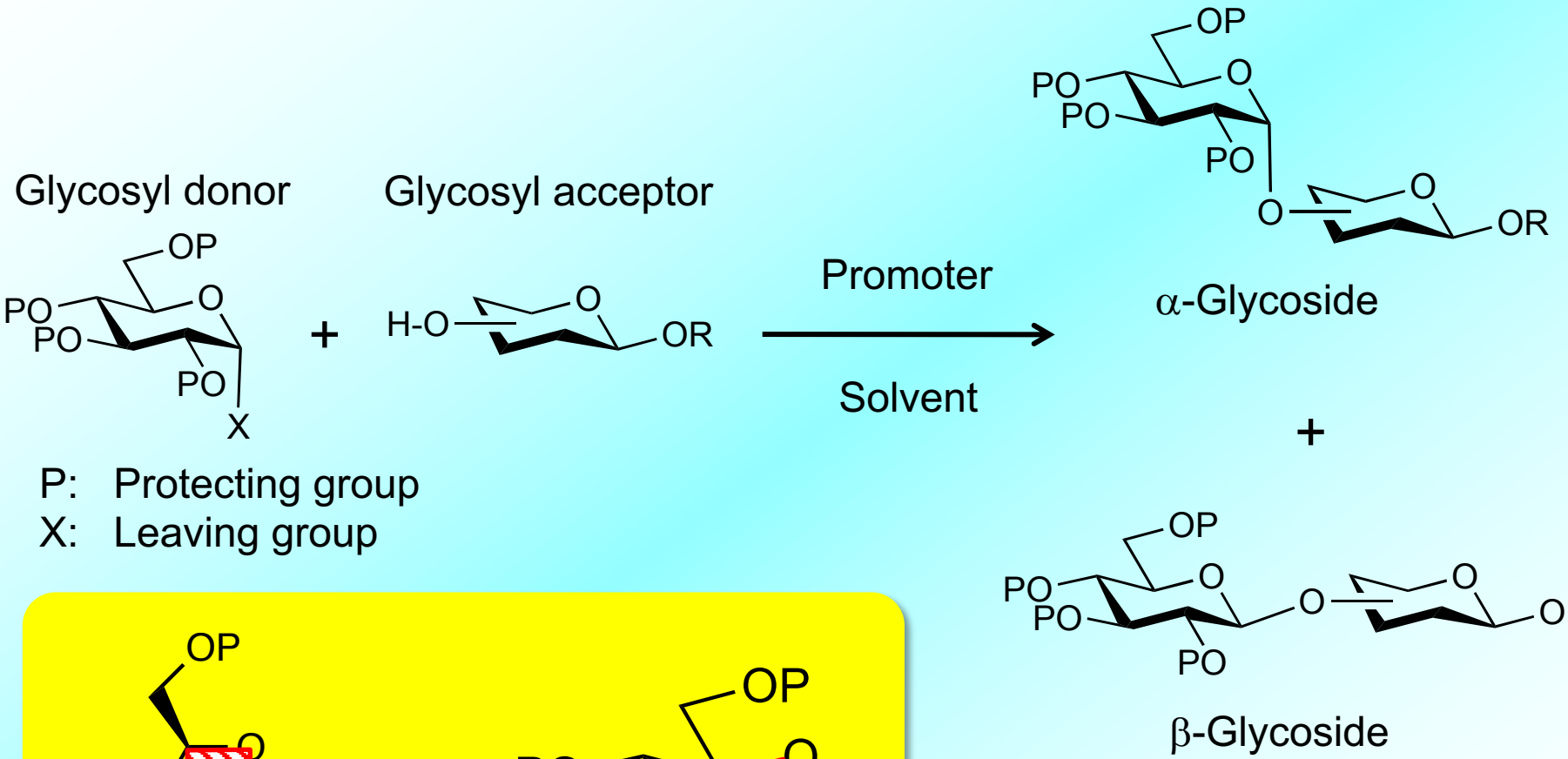
Seikei University

Kiichiro TOTANI

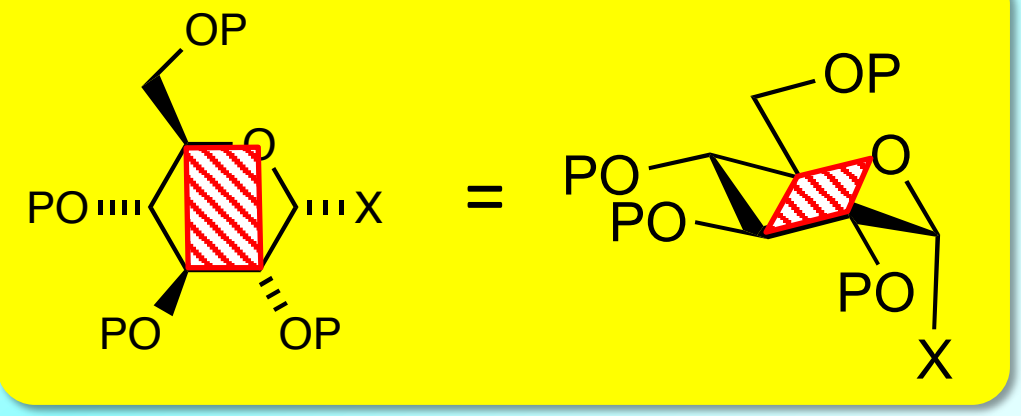
Contents

- Basics of glycosylation reaction
- Examples of oligosaccharide synthesis
- Development of stereoselective glycosylation reaction
- Analysis of glycoprotein quality control system using chemically synthesized oligosaccharides

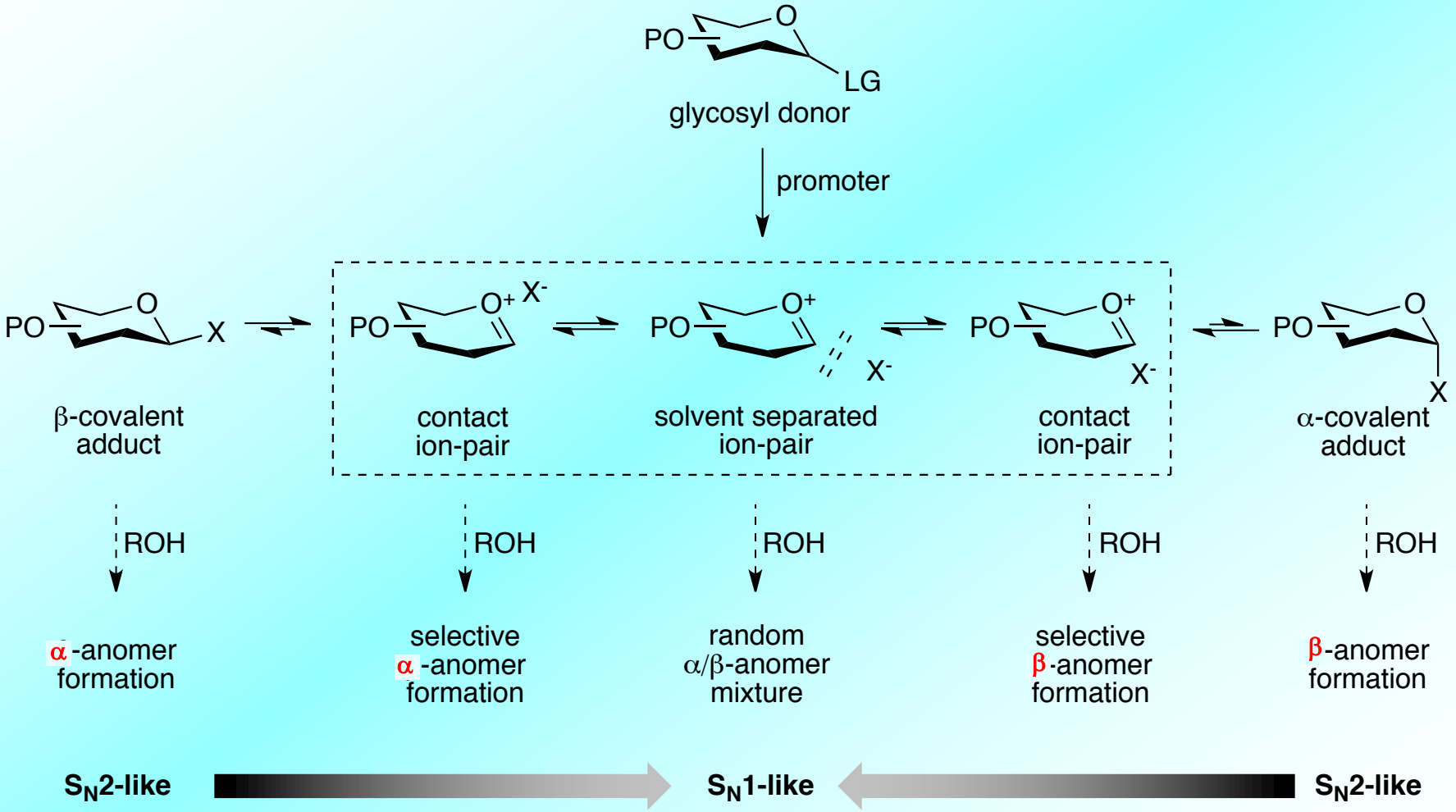
O-Glycosylation reaction



P: Protecting group
X: Leaving group

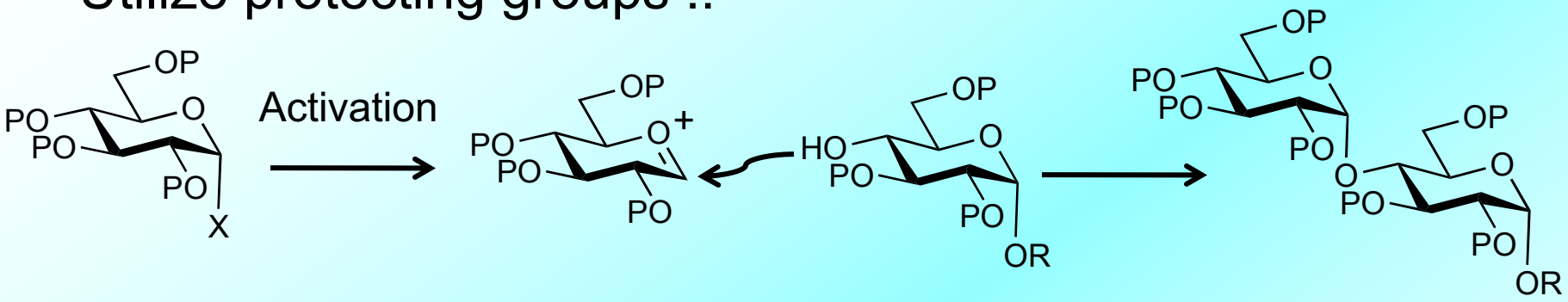


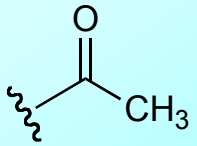
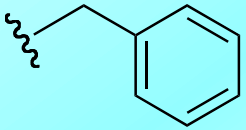
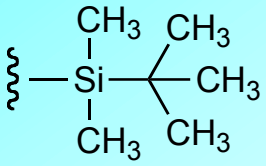
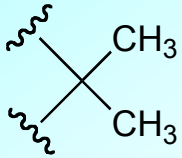
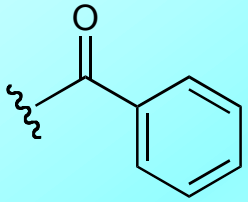
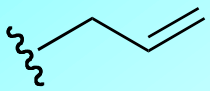
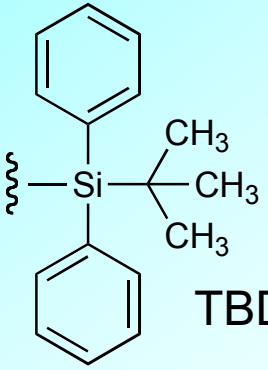
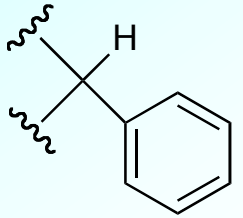
Reaction mechanism of O-glycosylation reaction



To control the regioselectivity of glycosylation

Utilize protecting groups !!



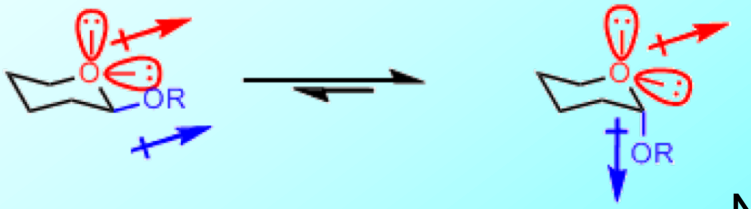
Acyl-Type	Ether-Type	Silyl Ether	Cyclic Acetal
 Ac	 Bn	 TBDMS	 isopropylidene
 Bz	 All	 TBDPS	 benzylidene

To control the stereoselectivity of glycosylation

Glycosyl donor	Preferential stereochemistry		Stereocontrol
	Anomeric effect	Neighboring group participation	
D-Glc D-Gal D-GlcNAc D-GalNAc	α	β	Possible
D-Man	α	α	Difficult
L-Fuc	α	β	Possible
NeuAc	β	none	Difficult

Anomeric effect

Dipole Minimization

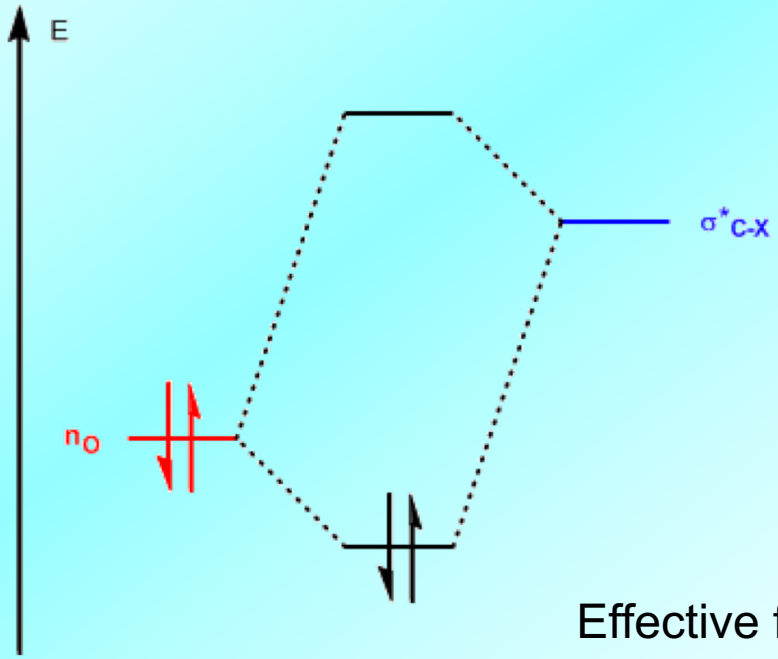


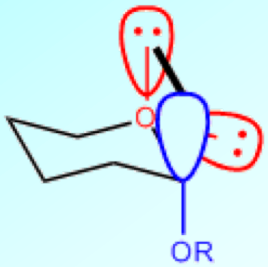
Non-coordinating solvent

React slowly at a relatively high temperature (RT to c.a. 50˚C)

Electron Delocalization

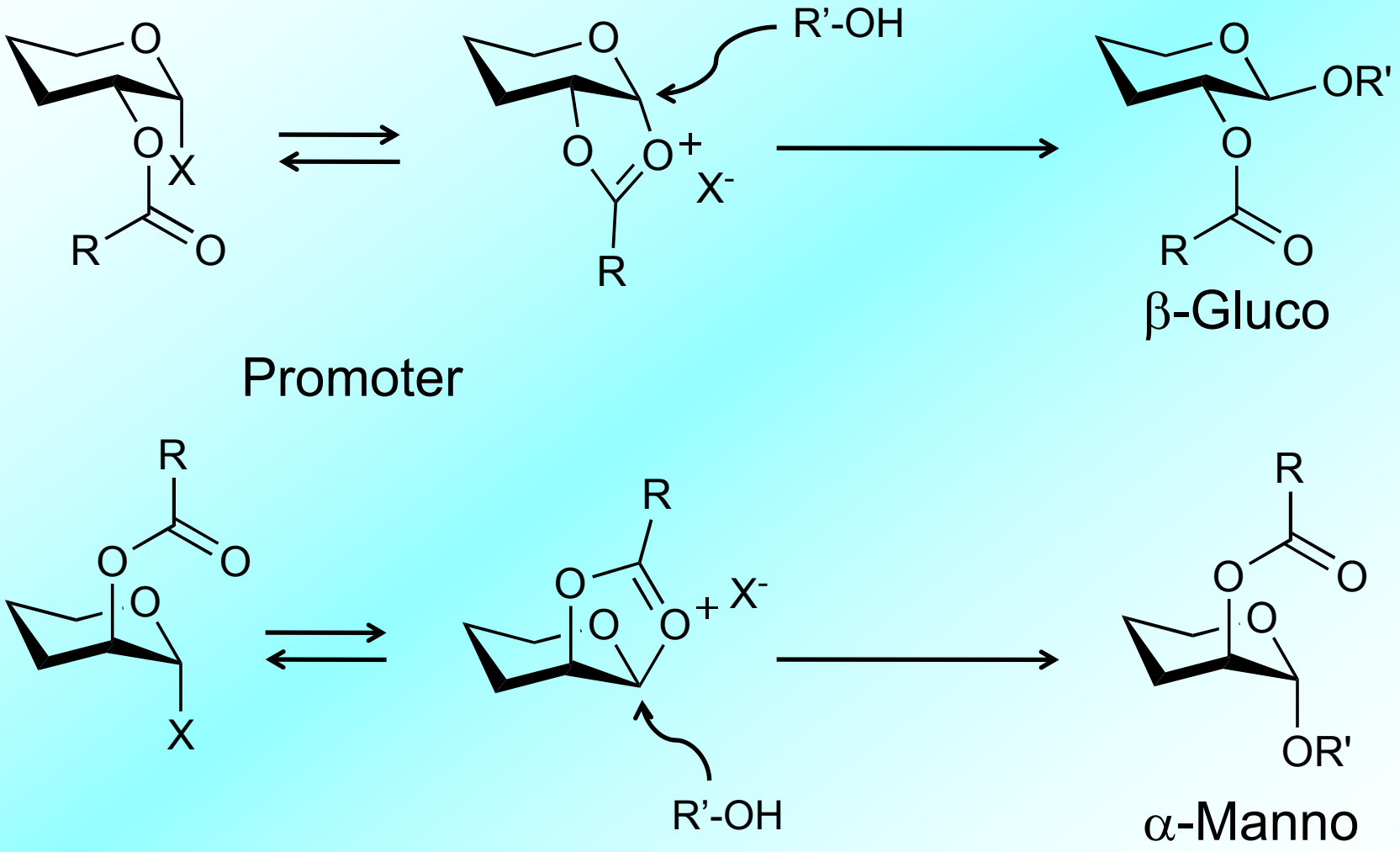
Non-acyl type protecting group at C-2 position



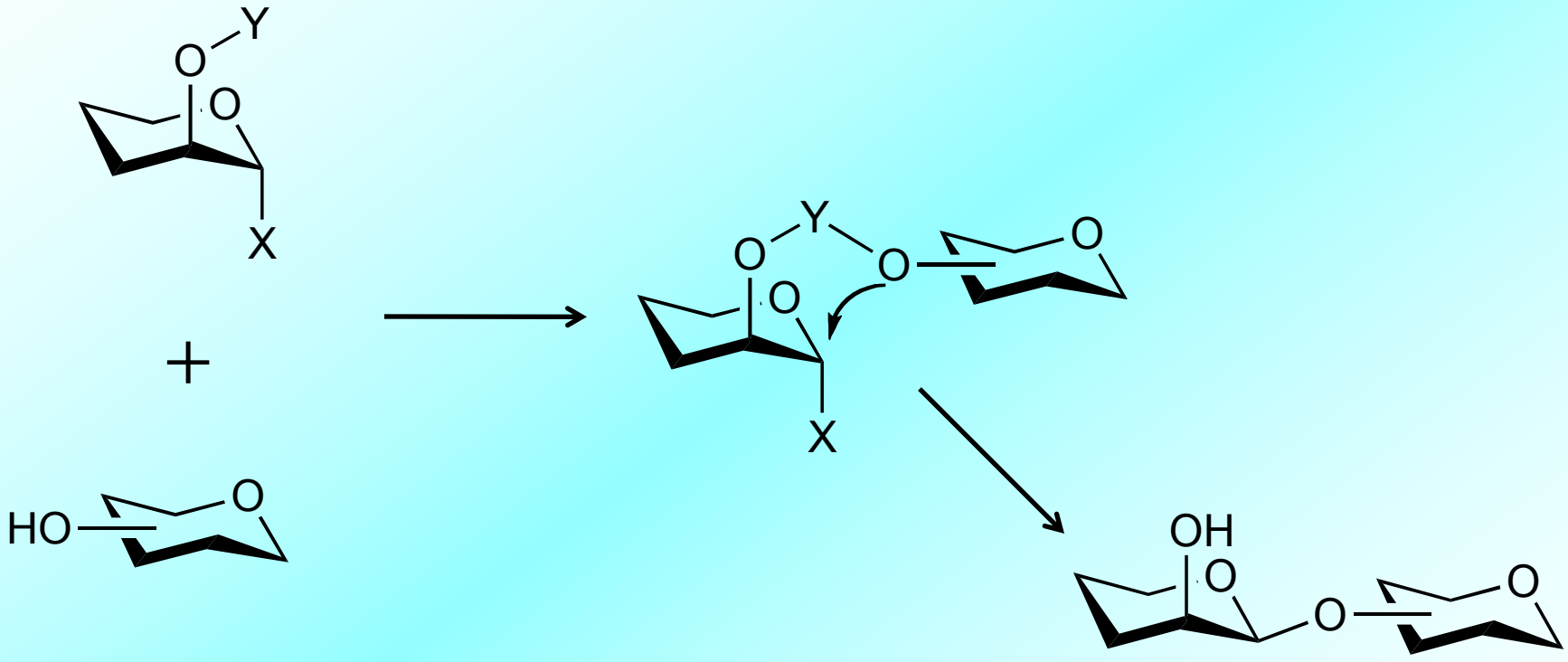


Effective for S_N1-like reaction mechanism

Neighboring group participation

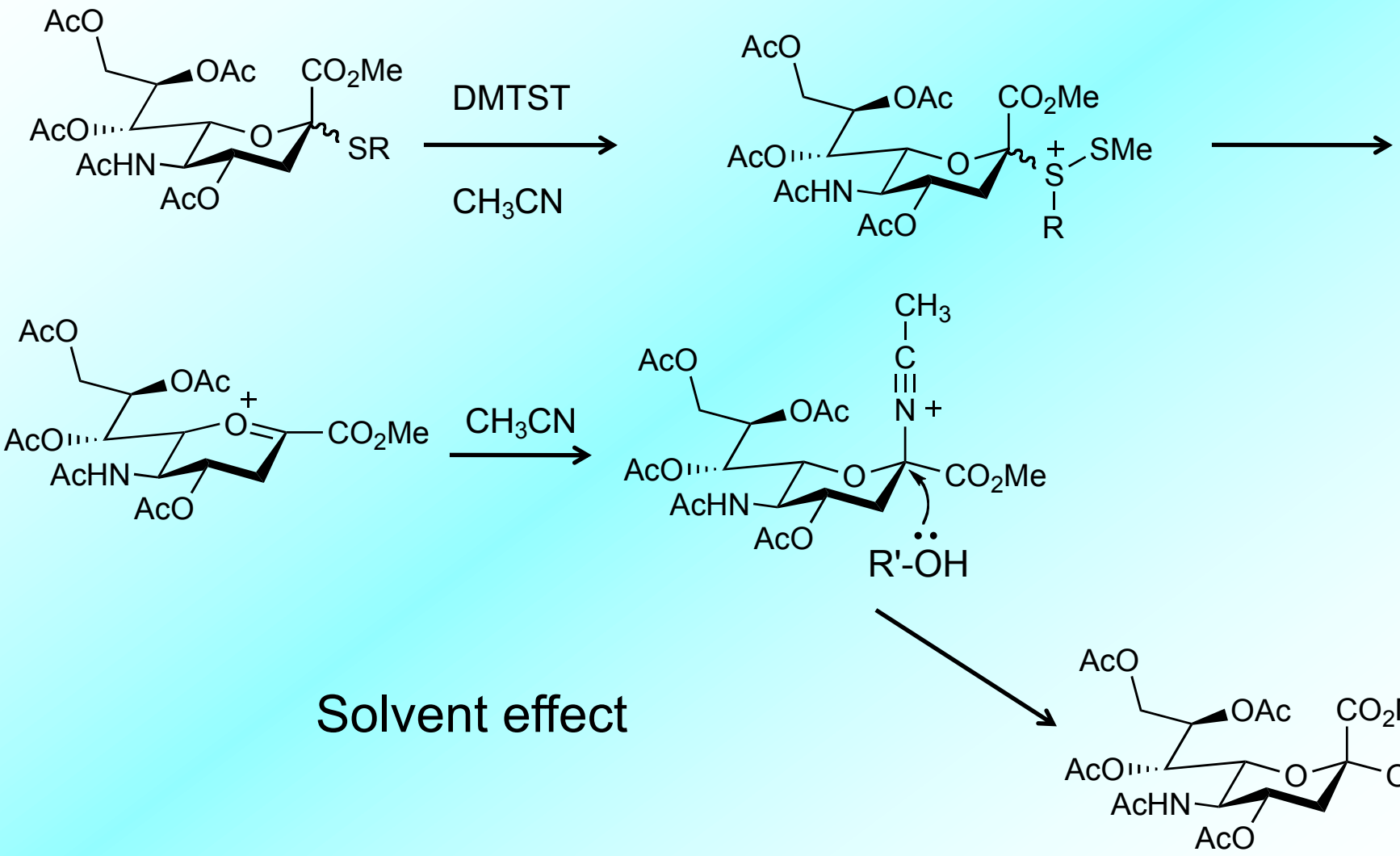


To prepare difficult-to-form β-mannoside

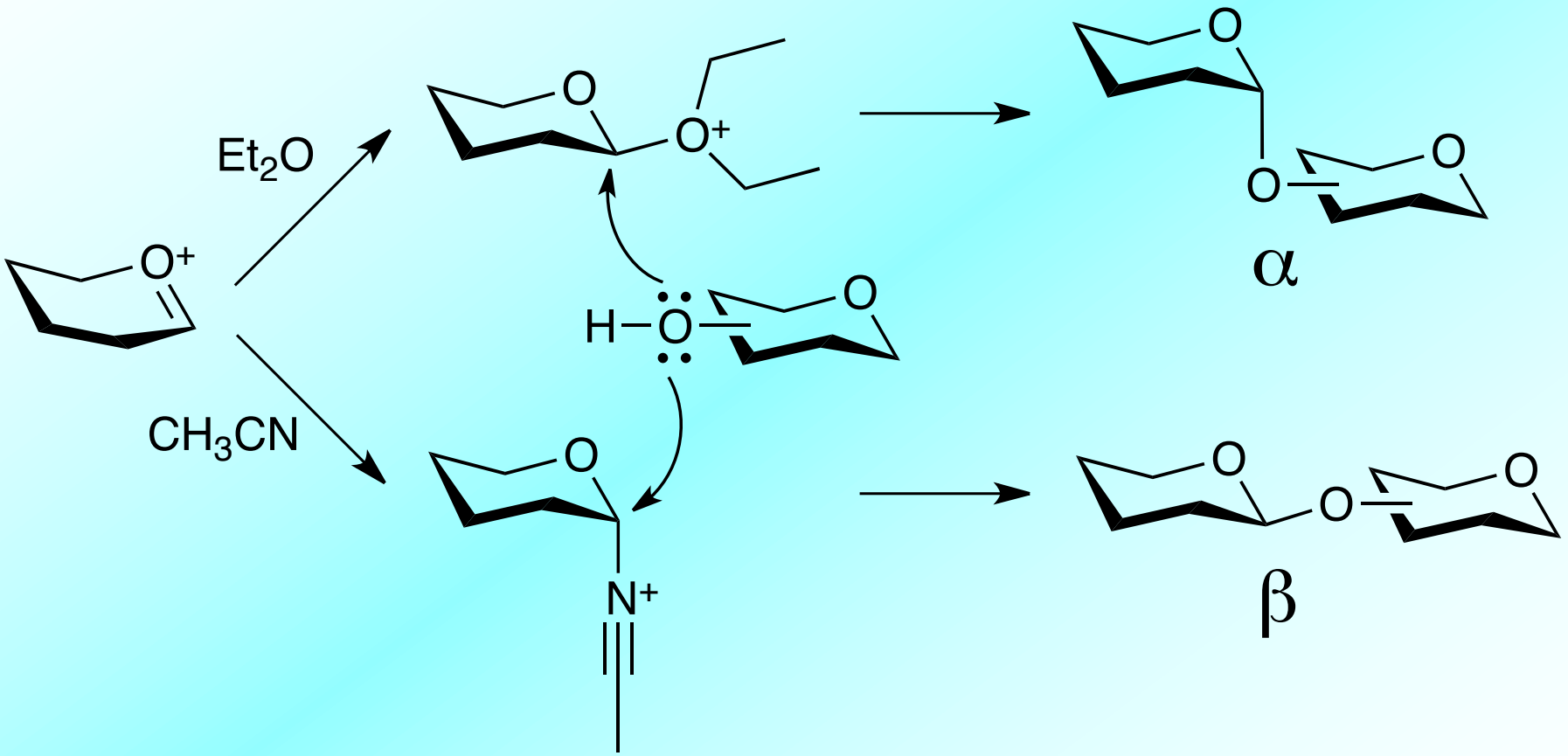


Intramolecular aglycone transfer

To prepare difficult-to-form α-sialoside

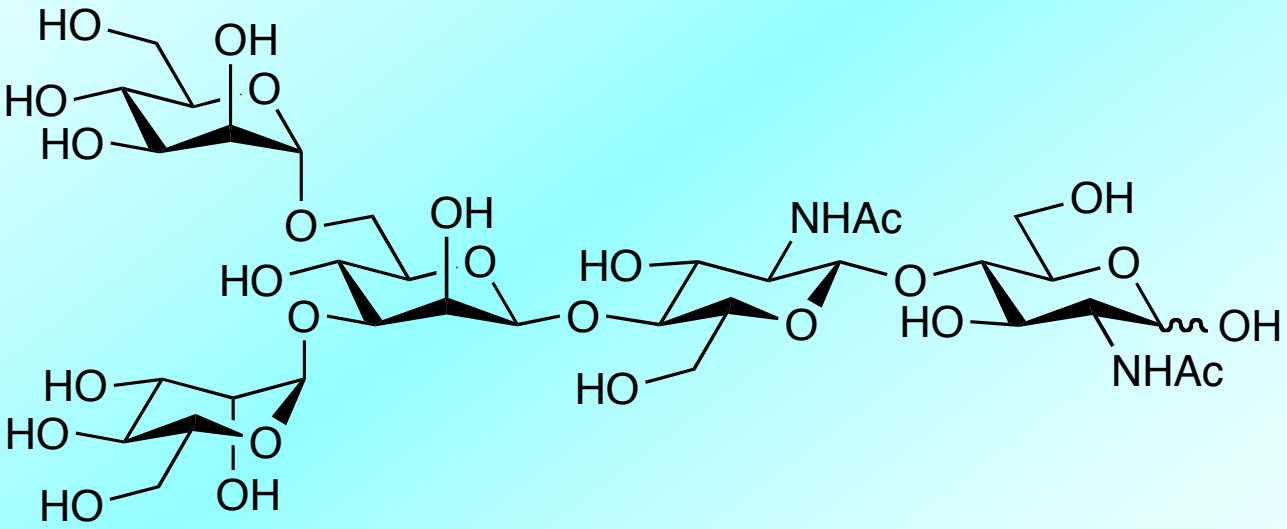


Stereoselective glycosylation by solvent effect



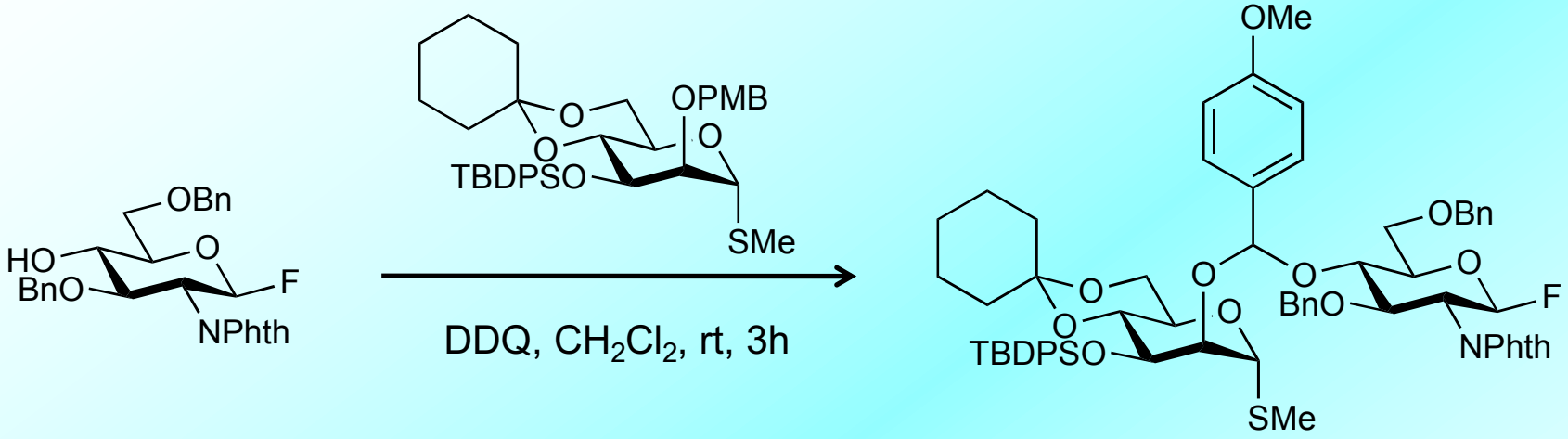
Synthesis of high-mannose-type core pentasaccharide

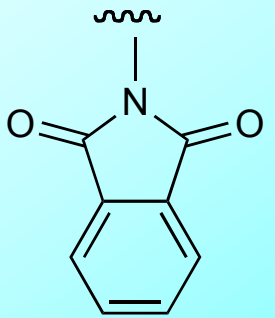
Totani, K.; Matsuo, I.; Ito, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2285.



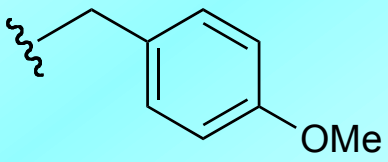
Chemical Formula: $C_{34}H_{58}N_2O_{26}$
Molecular Weight: 910.82

Synthesis of core pentasaccharide ~Part 1~



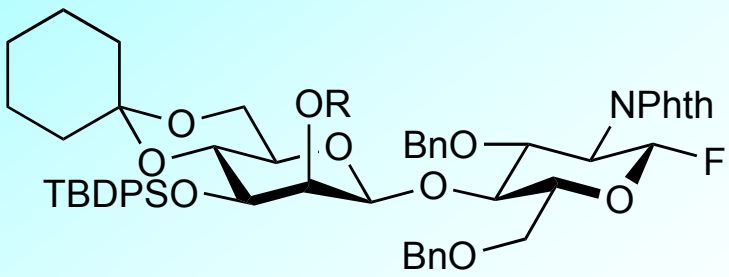


NPhth



PMB

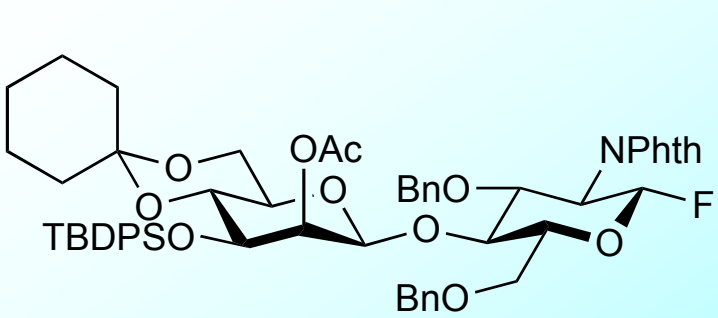
pyr. = pyridine
DMAP = dimethyl amino pyridine

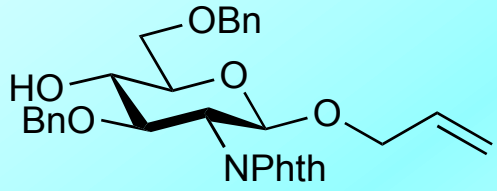


R = H
Ac

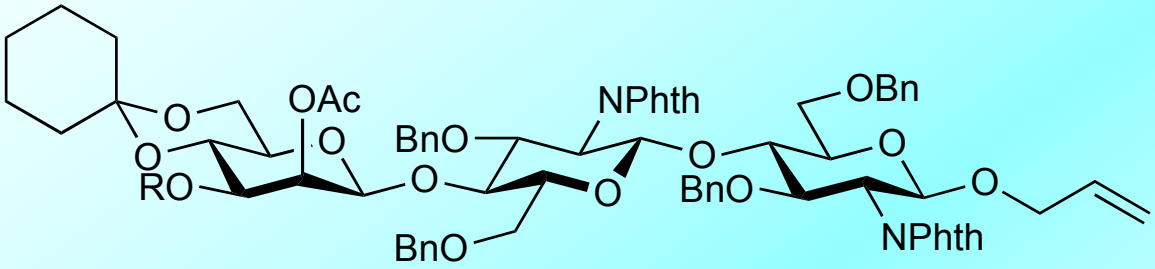
Ac_2O, DMAP, pyr.,
40° C, 8h (97%)

Synthesis of core pentasaccharide ~Part 2~



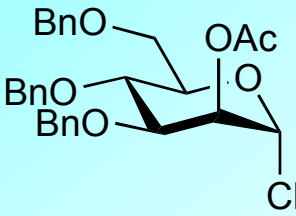


Cp_2HfCl, AgOTf, CH_2Cl_2, -10 ° C, 4h (78%)



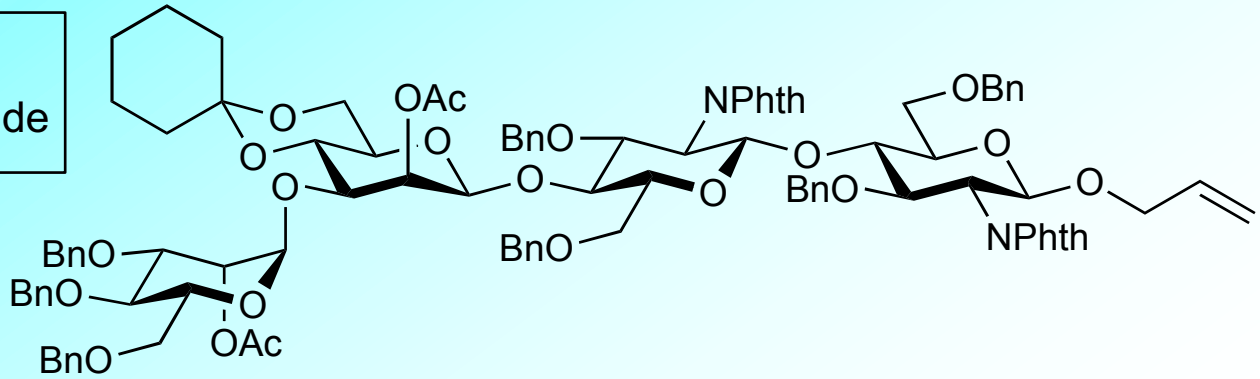
R = TBDPS
H

10% HF-Pyr., DMF, 1 Gpa,
30 ° C, 12h (87%)

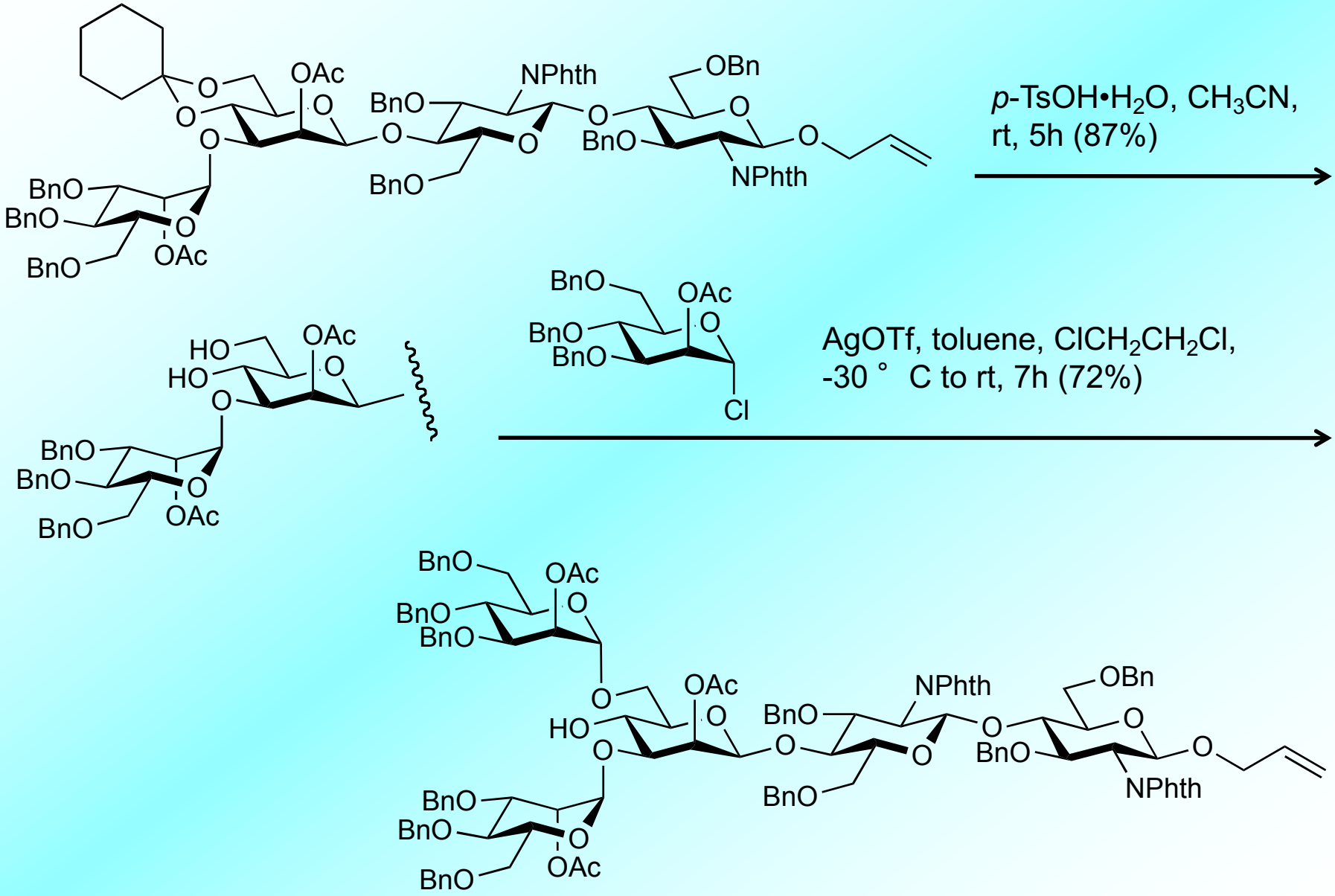


AgOTf, toluene, $ClCH_2CH_2Cl$,
-30 ° C to rt, 5h (87%)

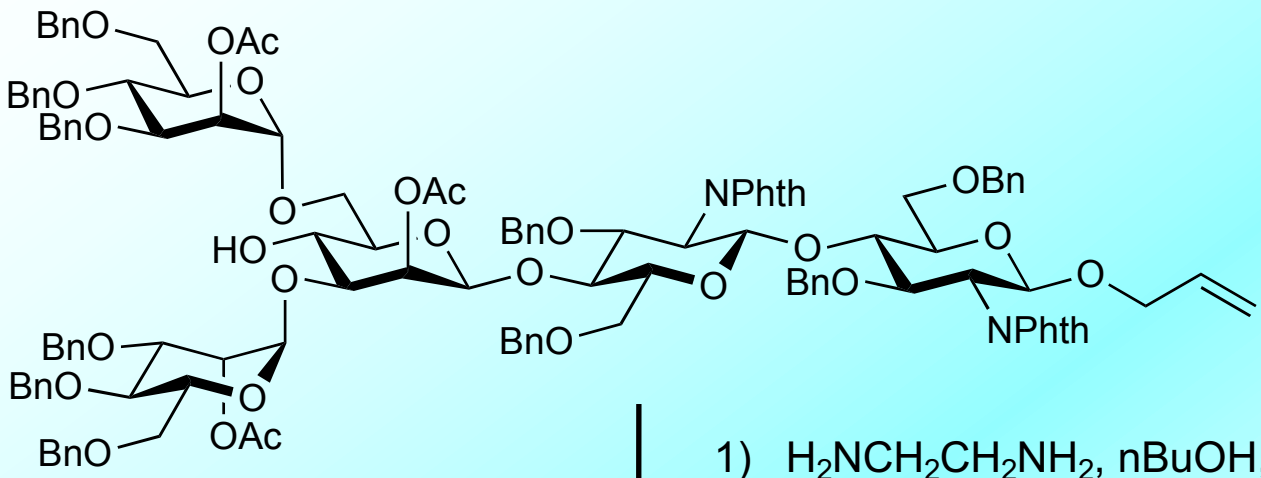
Cp = cyclopentadieny
DMF = N,N-dimethylformamide



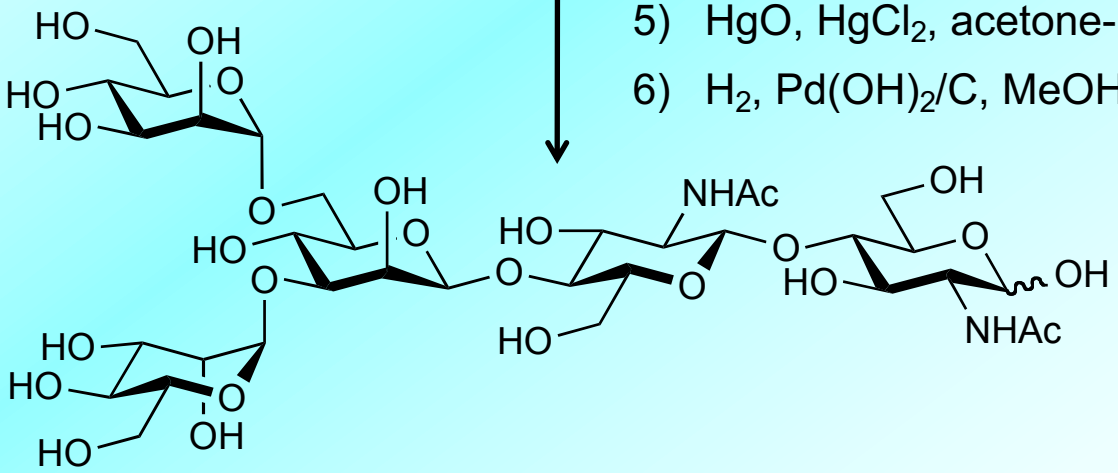
Synthesis of core pentasaccharide ~Part 3~



Synthesis of core pentasaccharide ~Part 4~

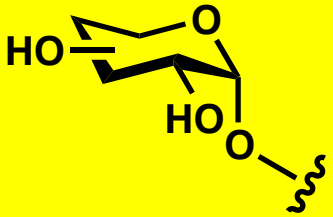


1) $H_2NCH_2CH_2NH_2$, nBuOH, 80 ° C, 18h
2) Ac_2O, pyr., DMAP, rt, 2.5h
3) 1M NaOMe, MeOH, rt, 3.5h (89%, 3 steps)
4) $[Ir(COD)(PMePh_2)_2]PF_6$, H_2, THF, rt, 2h
5) HgO, $HgCl_2$, acetone-H_2O (10:1), rt, 5h
6) H_2, $Pd(OH)_2/C$, MeOH, rt, 2.5 h (85%, 3 steps)



Anomeric effect is less potent in 1,2-cis-α-glucoside formation

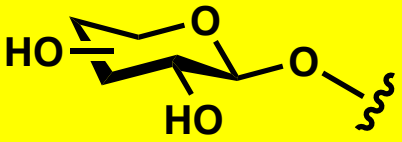
1,2-*cis*-α



Anomeric effect

vs

1,2-*trans*-β



Neighboring group participation

☒ **Solvent Effect**

- **Yield: 0 ~ 100%**
- **α/β: 1:1 ~ 20:1**

A. Ishiwata and Y. Ito *Tetrahedron Lett.* **2005**, *46*, 3521.

☒ **High-mannose-glycan synthesis**

I. Matsuo, K. Totani, A. Tatami and Y. Ito *Tetrahedron* **2006**, *62*, 8262.

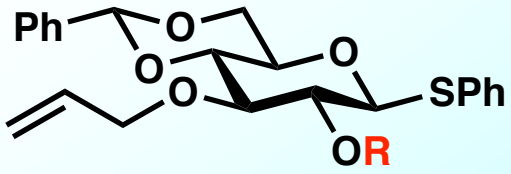
Manα1-2Manα1-6
Manα1-6
Manα1-2Manα1-3
Manβ1-4GlcNAcβ1-4GlcNAcβ1-**protein**

(90%) (45%) 3Manα1-2Manα1-2Manα1-3

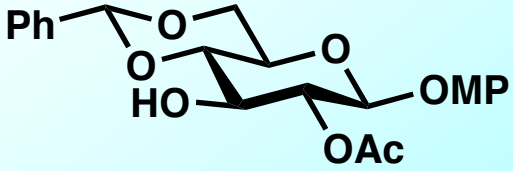
Glcα1-2Glcα1-3Glcα1

Development of 1,2-cis-α-glycoside formation reaction using stereoelectronic effect

K. Totani *et al. RSC Advances* **2015**, *5*, 75918.

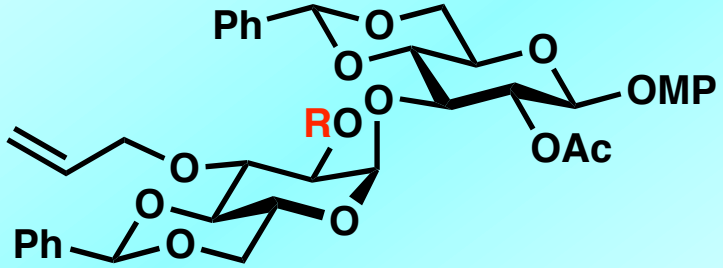


Glycosyl donor

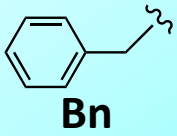


Glycosyl acceptor

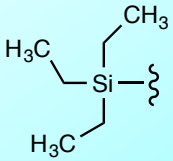
MeOTf, MS4A,
CH_2Cl_2



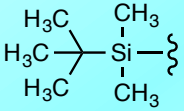
Entry	R	Temp. (° C)	Yield (%)	α / β
1	Bn	10	85	82 : 18
2	TES	0	-	-
3	TBS	0	96	>95 : 5
4	TIPS	0	50	>95 : 5



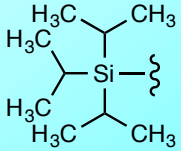
Bn



TES



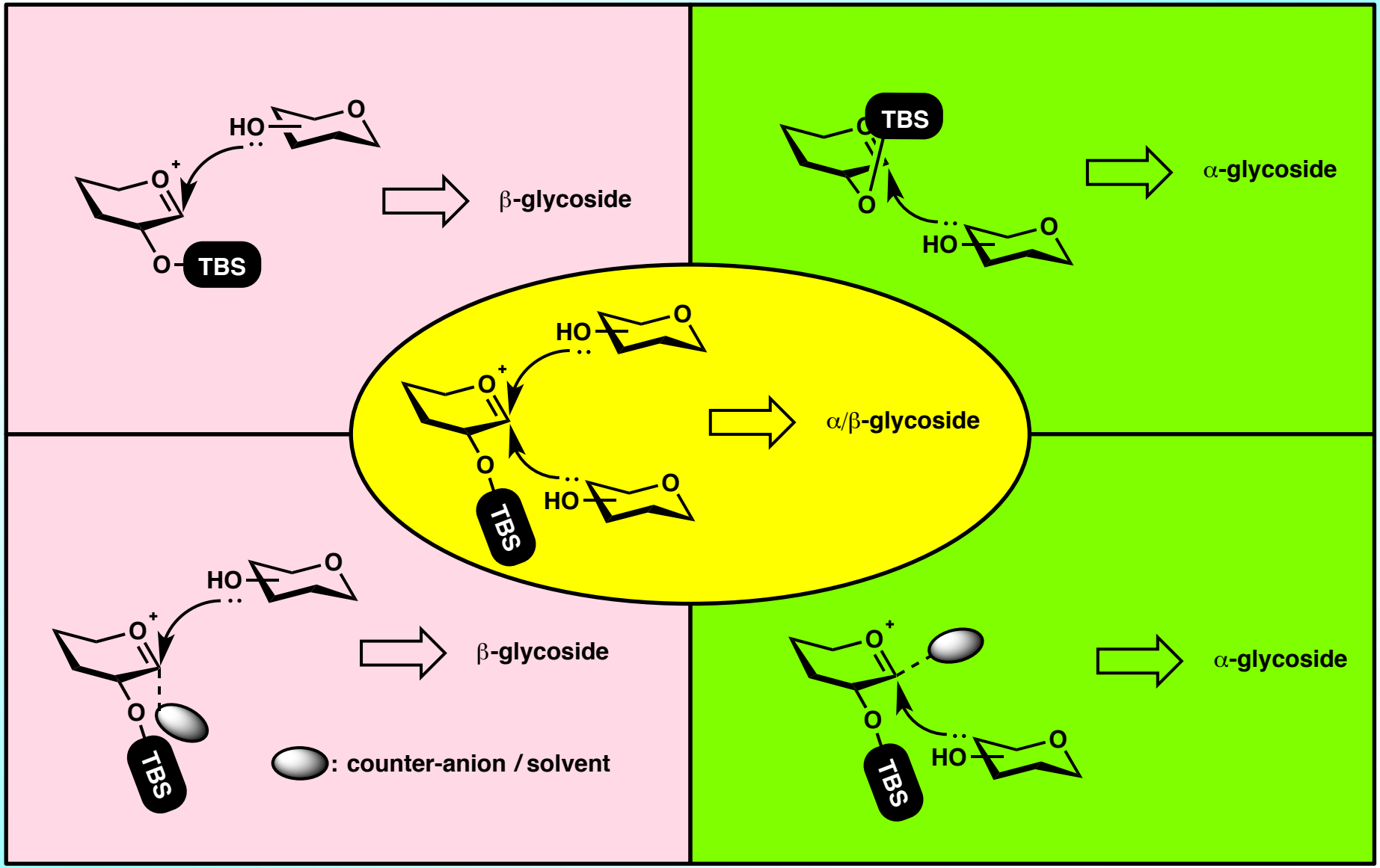
TBS



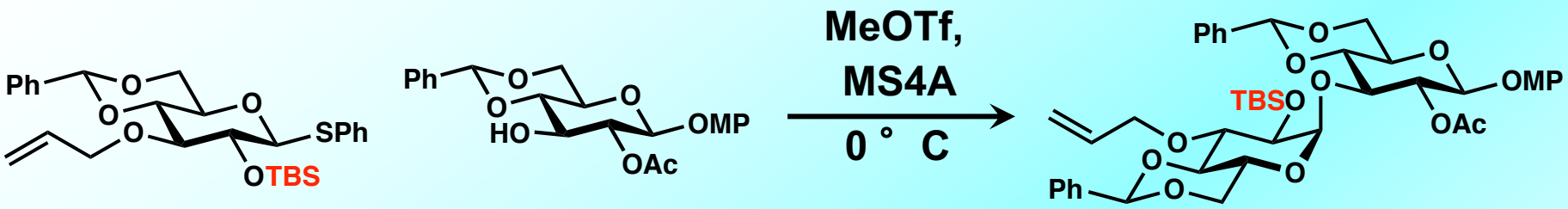
TIPS

- *TBS group is effective for high yield and α-selectivity*
- *TBS effect : Steric effect or Electronic effect?*

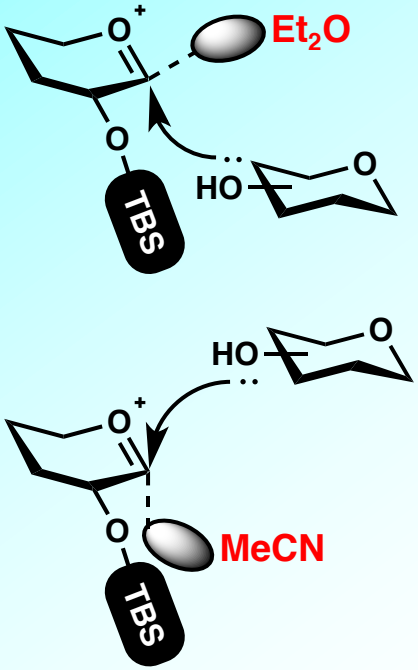
Possible stereocontol modes



Estimation of stereocontrol mode based on the solvent effects

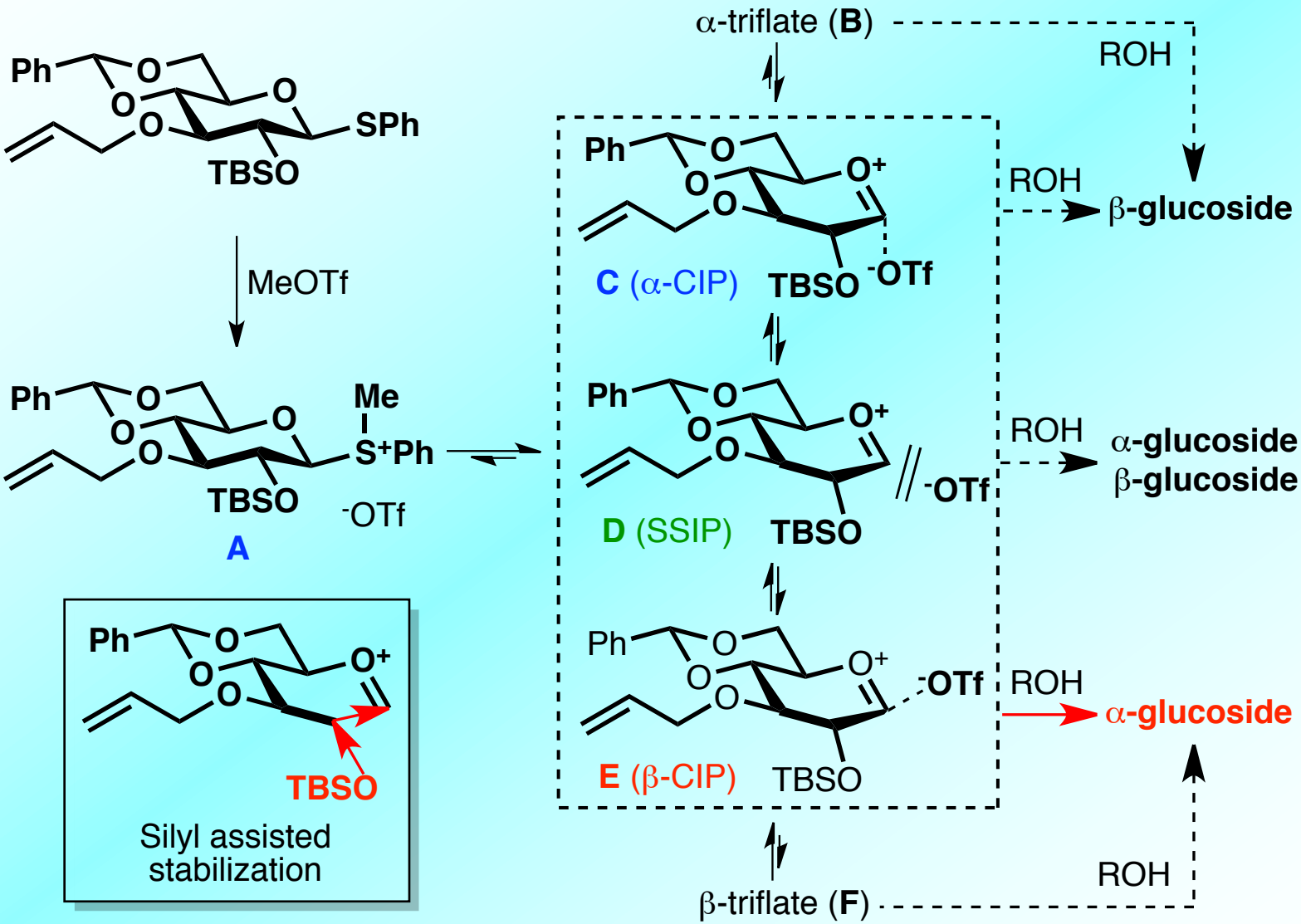


Entry	Solvent	Participation	Yield (%)	α / β
1	CH_2Cl_2	None	96	>95 : 5
2	Toluene	None	41	>95 : 5
3	Et_2O	β	20	>95 : 5
4	MeCN	α	21	48 : 52

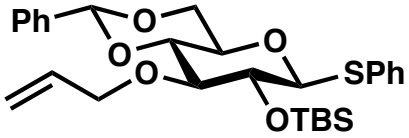
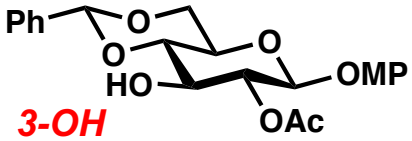
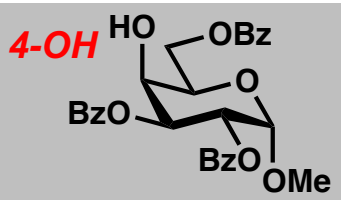
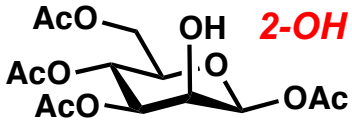
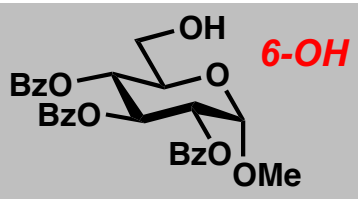
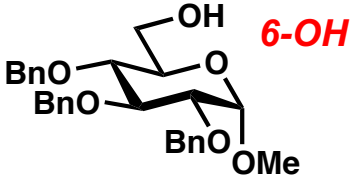


☒ *The steric bulkiness of TBS group does not affect the stereoselectivity*

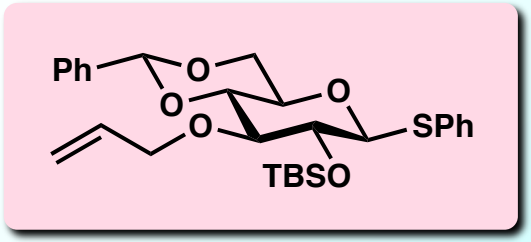
Stereocontrol mechanism of the α-glucosylation

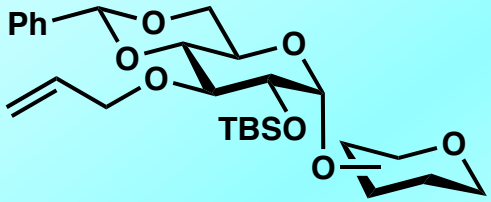


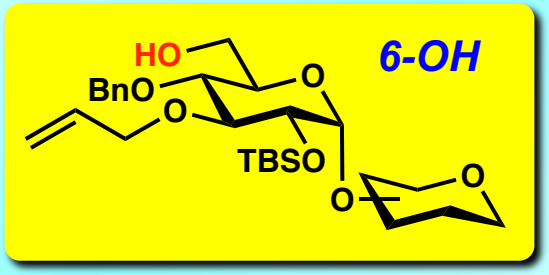
Versatility for glycosyl acceptors

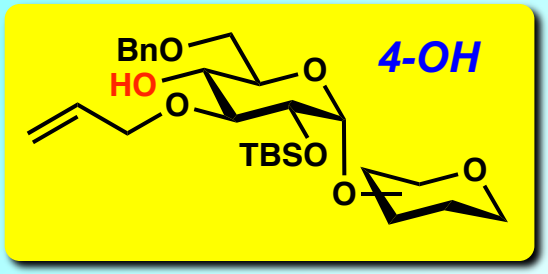
Entry	Donor	Acceptor	Yield (%)	α / β
1			96	>95 : 5
2			77	93 : 7
3			43	92 : 8
4	*Disarmed*		82	>95 : 5
5	*Armed*		96	>95 : 5

Advantages of the proposed glycosyl donor

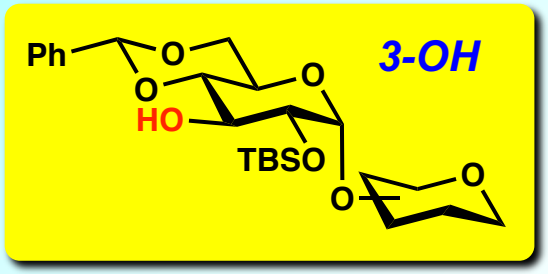


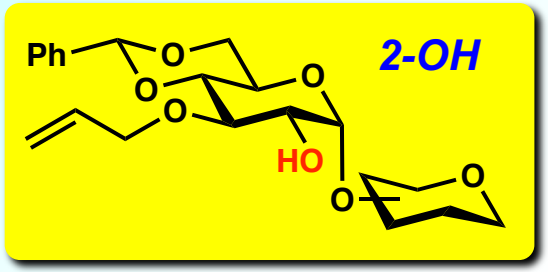






Regioselective deprotection





☒ *Further glycosylation is possible for all OH-groups*

☒ *Effective for oligosaccharide synthesis*

Trisaccharide synthesis ~Existing approach~

1,2-*cis*-α-linkage

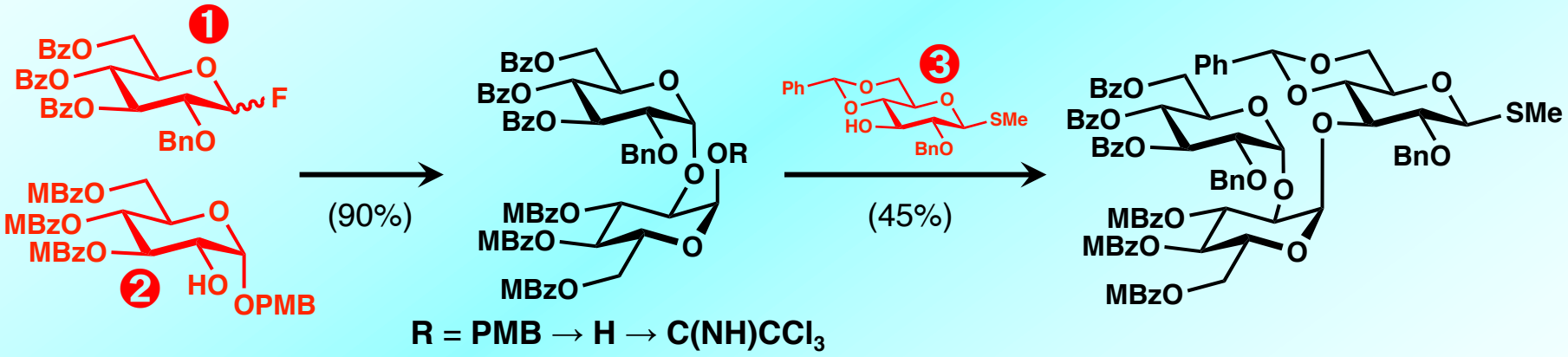
Glcα1-2Glcα1-3Glcα1

Manα1-2Manα1-6
Manα1-2Manα1-3
3Manα1-2Manα1-2Manα1-3

Manα1-6

Manβ1-4GlcNAcβ1-4GlcNAcβ1-**protein**

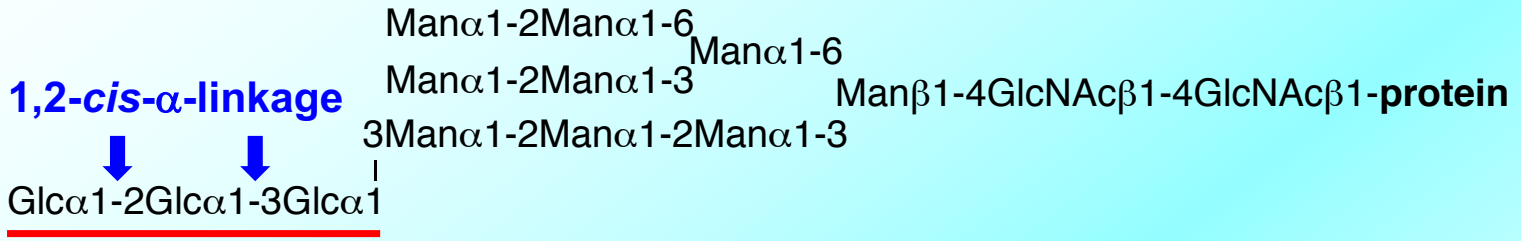
I. Matsuo, K. Totani, A. Tatami and Y. Ito *Tetrahedron* **2006**, *62*, 8262.

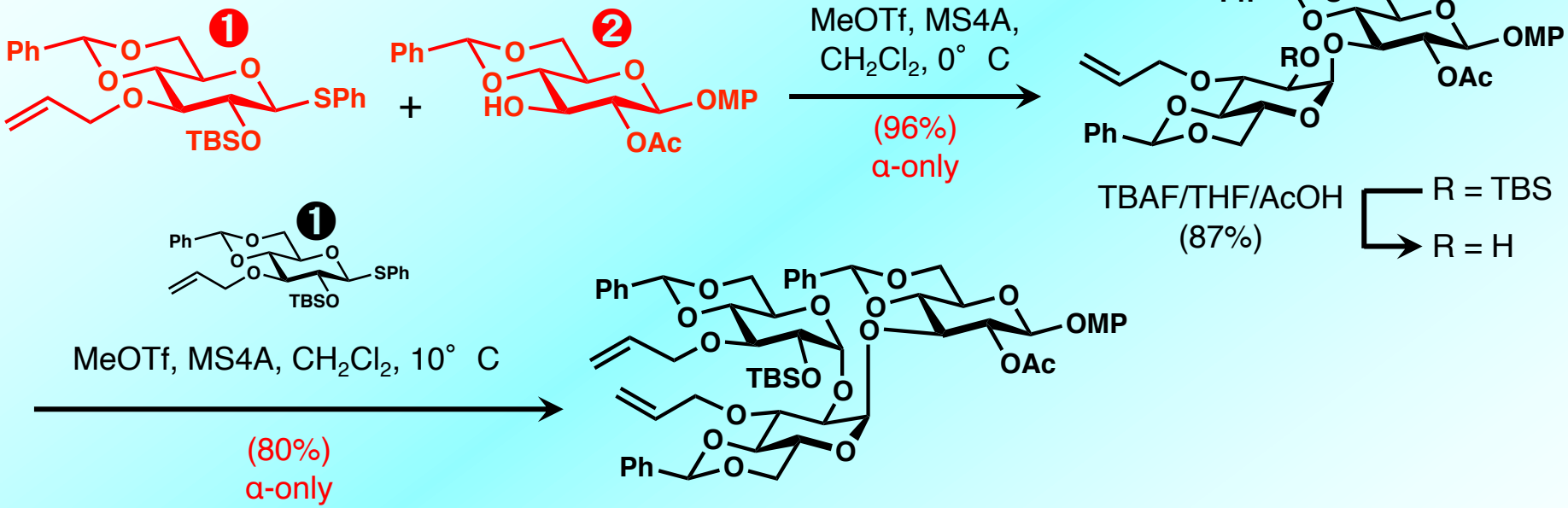


R = PMB → H → C(NH)CCl$_3$

- ***Three types of monosaccharide units are required***
- ***Four steps are required for conversion to target trisaccharide***
- ***Low yield for the second glycosylation***

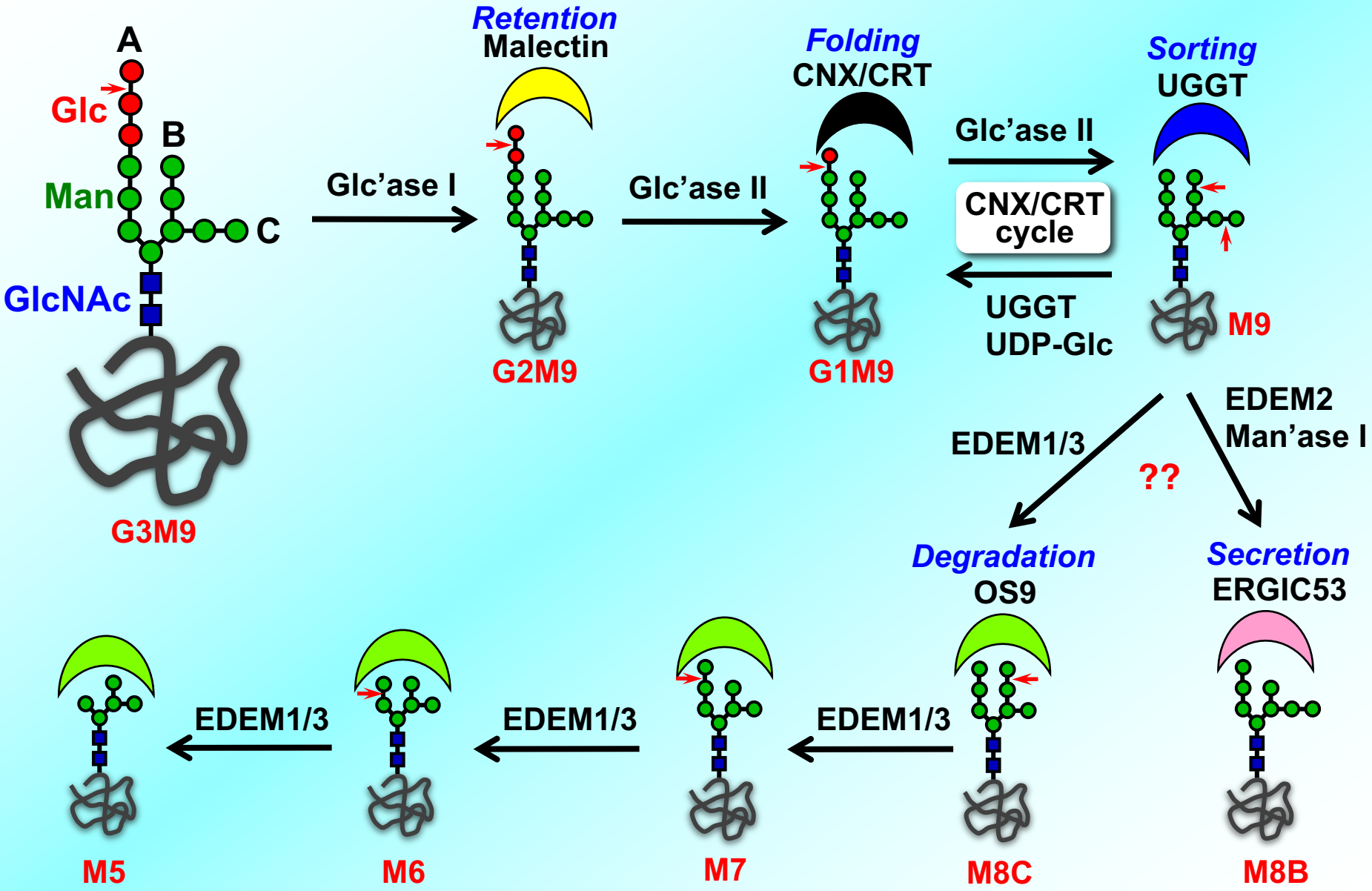
Trisaccharide synthesis ~Proposed approach~





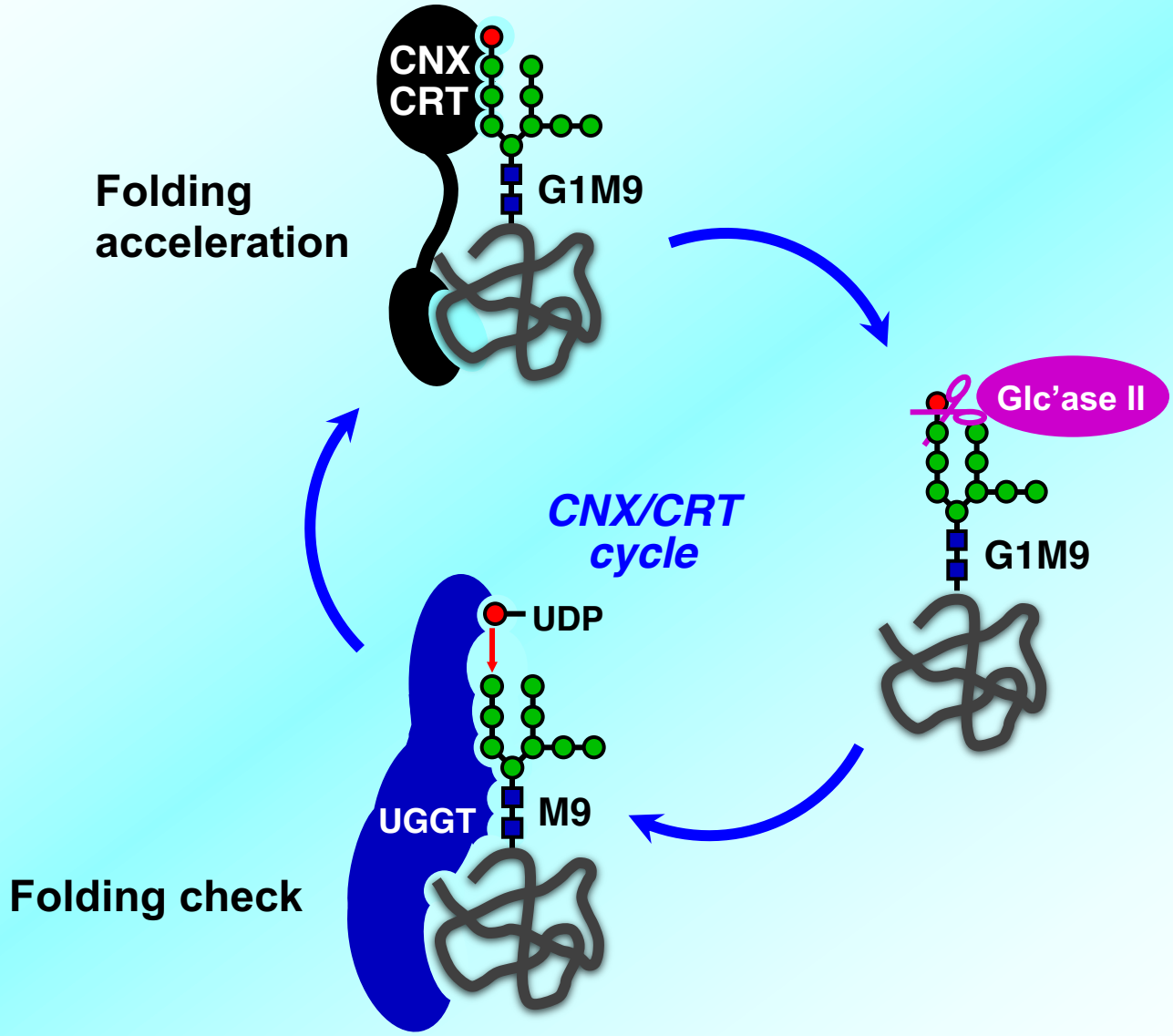
- ***Monosaccharide units (3 → 2)***
- ***Synthetic steps (4 → 3)***
- ***Yield for the secong glycosylation (Low → High)***

Glycoprotein quality control



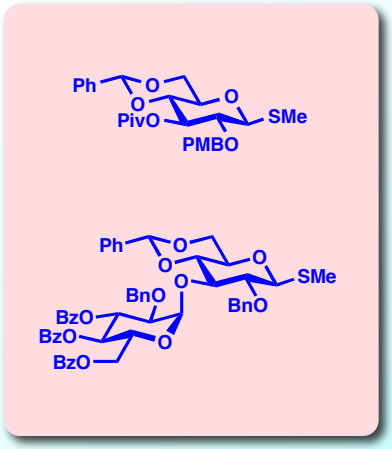
R. Benyair *et al. Int. Rev. Cell Mol. Biol.* **2011**, *292*, 197–280.

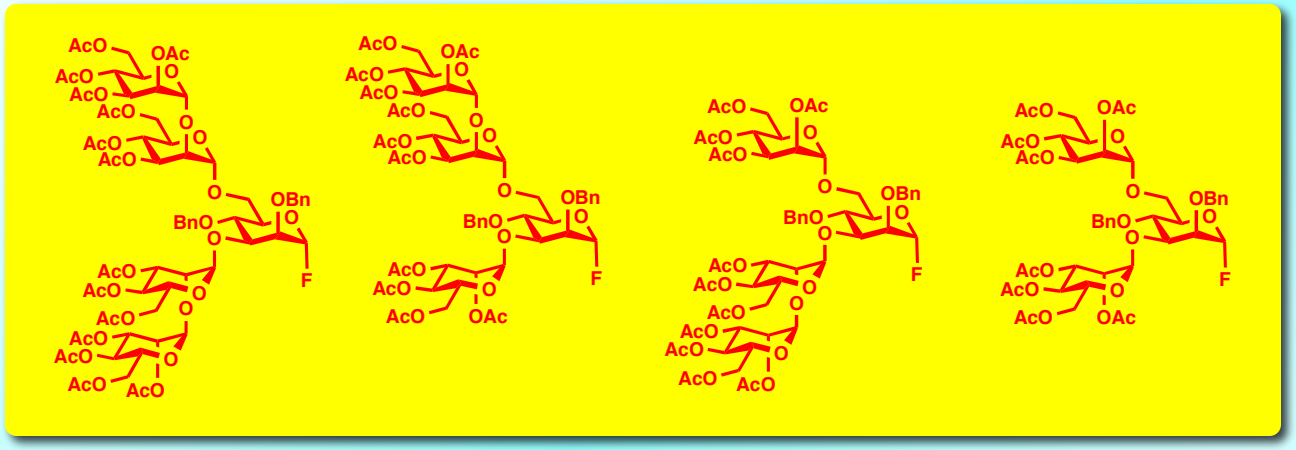
Central mechanism of glycoprotein quality control ~CNX/CRT cycle~

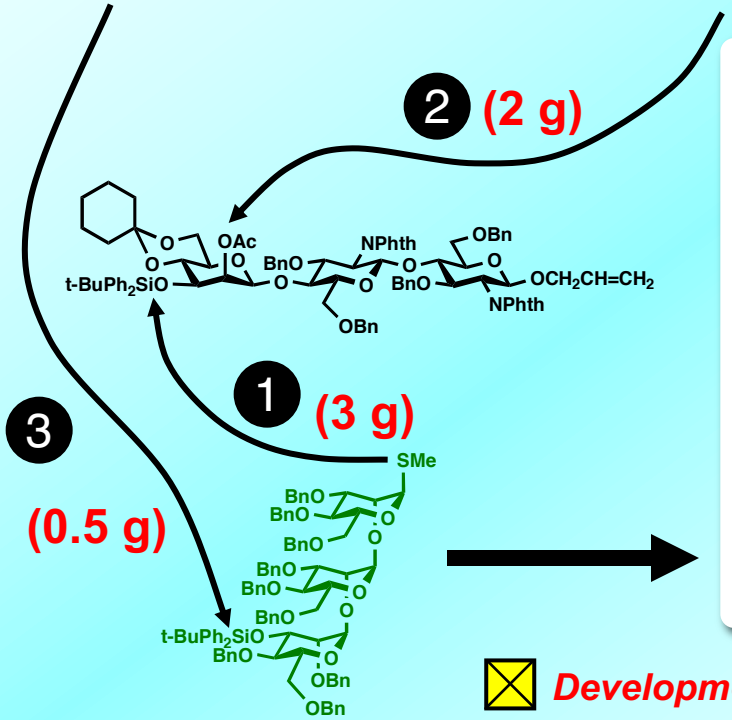


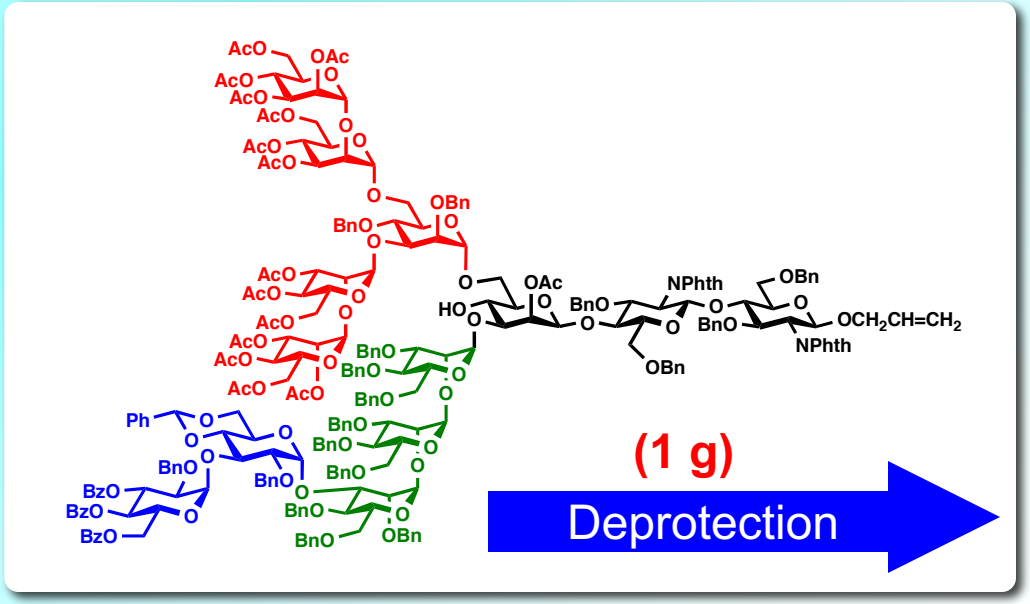
Synthesis of substrate glycans

I. Matsuo et al. *Tetrahedron* 2006, *62*, 8262.





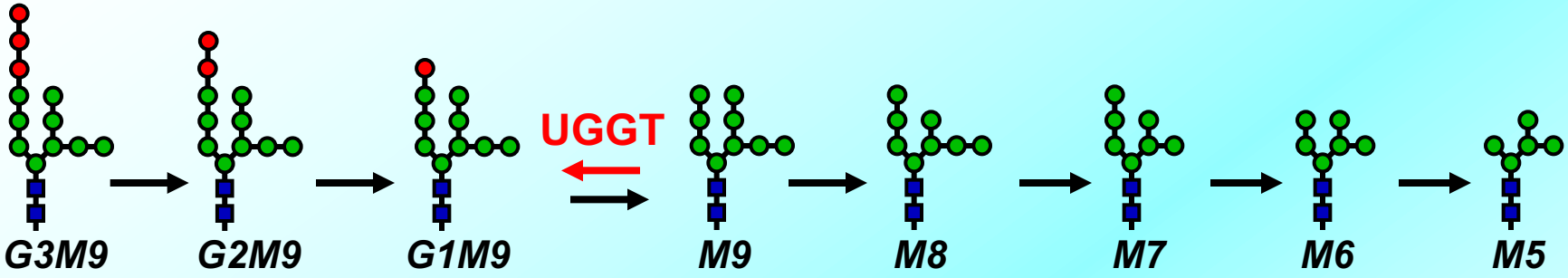




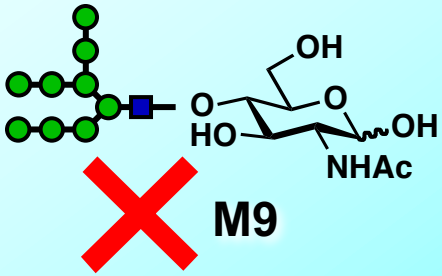
Development of optimal synthetic route for large scale glycan supply

What is the best substrate for glycan processing analysis?

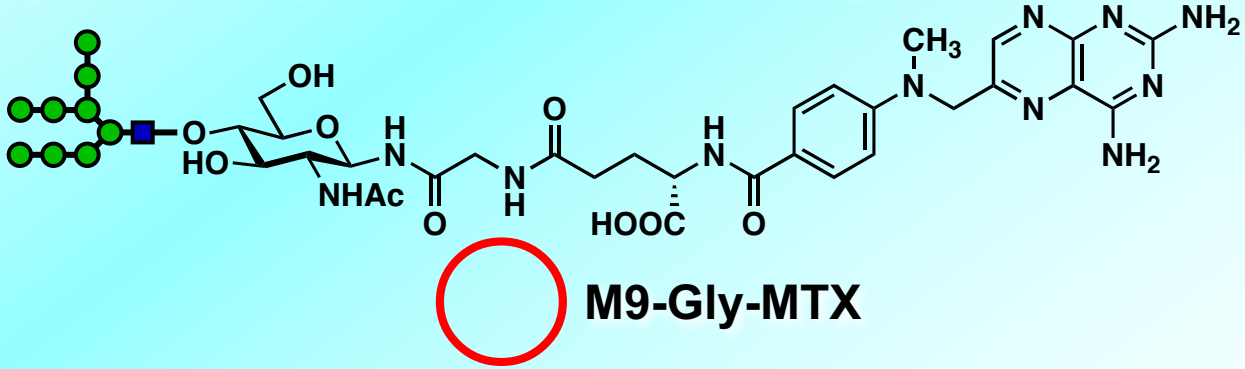
K. Totani et al. *Angew. Chem. Int. Ed.* 2005, *44*, 7950. K. Totani et al. *Biochemistry* 2009, *48*, 2933.



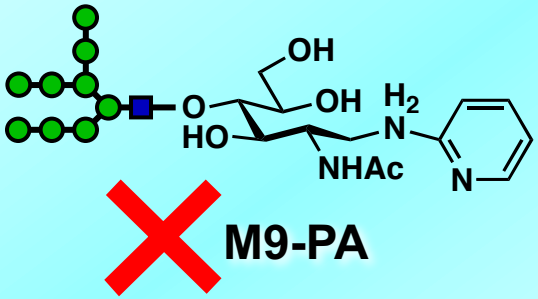
☒ ***Molten globule-like aglycone is required for substrate recognition of UGGT***



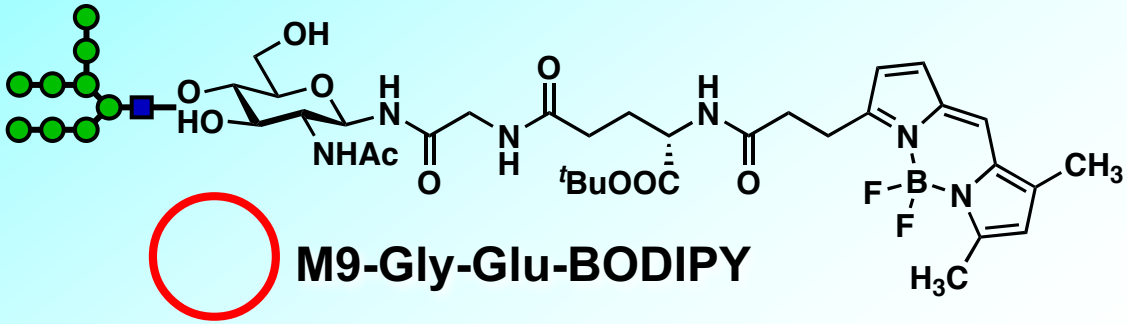
M9



M9-Gly-MTX



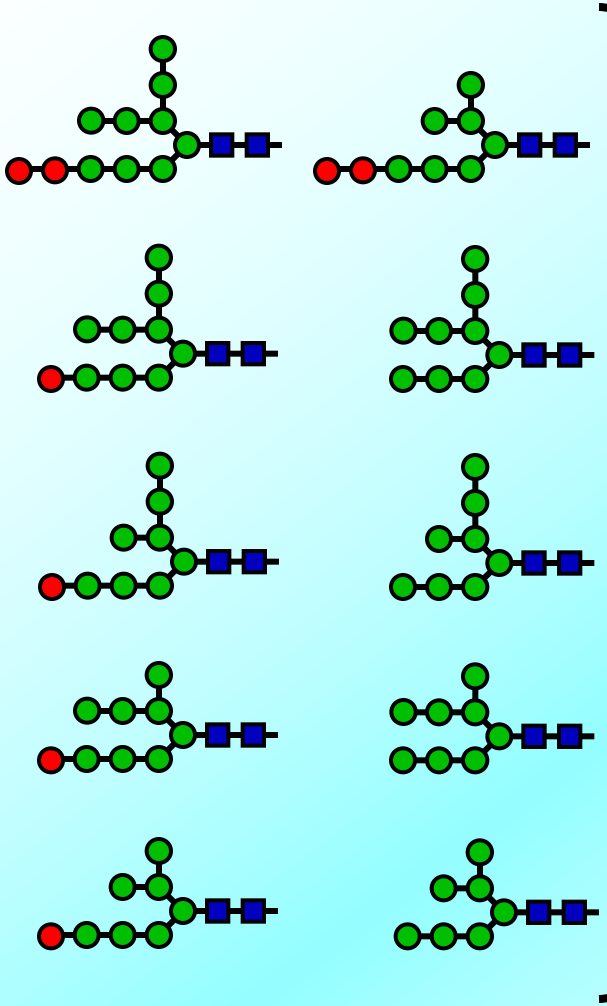
M9-PA



M9-Gly-Glu-BODIPY

Examples of synthetic substrates

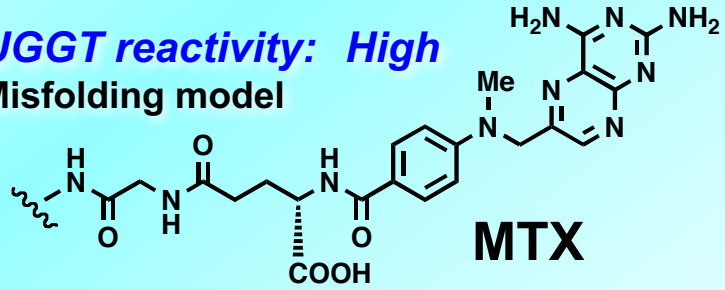
K. Totani et al. *Angew. Chem. Int. Ed.* 2005, *44*, 7950.
K. Totani et al. *Bioorg. Med. Chem.* 2006, *14*, 5220.



UGGT reactivity: none

UGGT reactivity: High

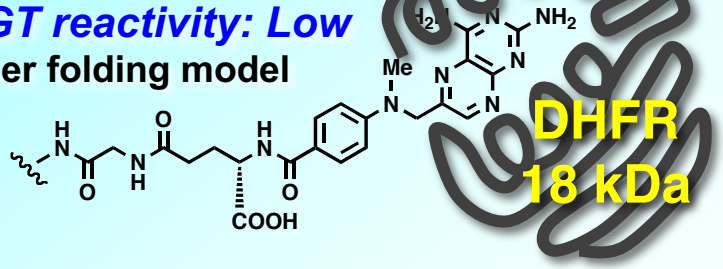
Misfolding model



MTX

UGGT reactivity: Low

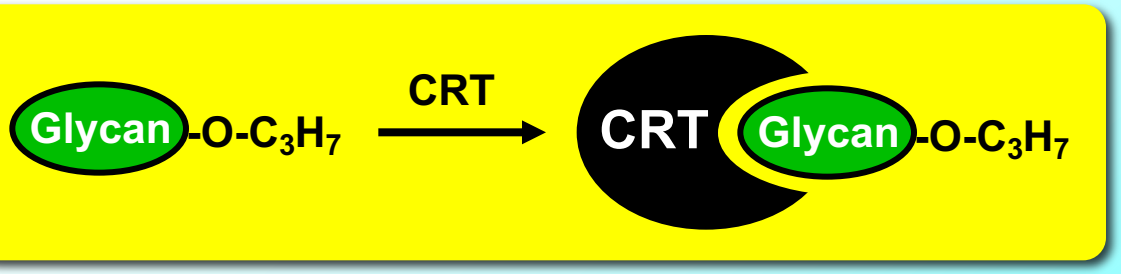
Proper folding model



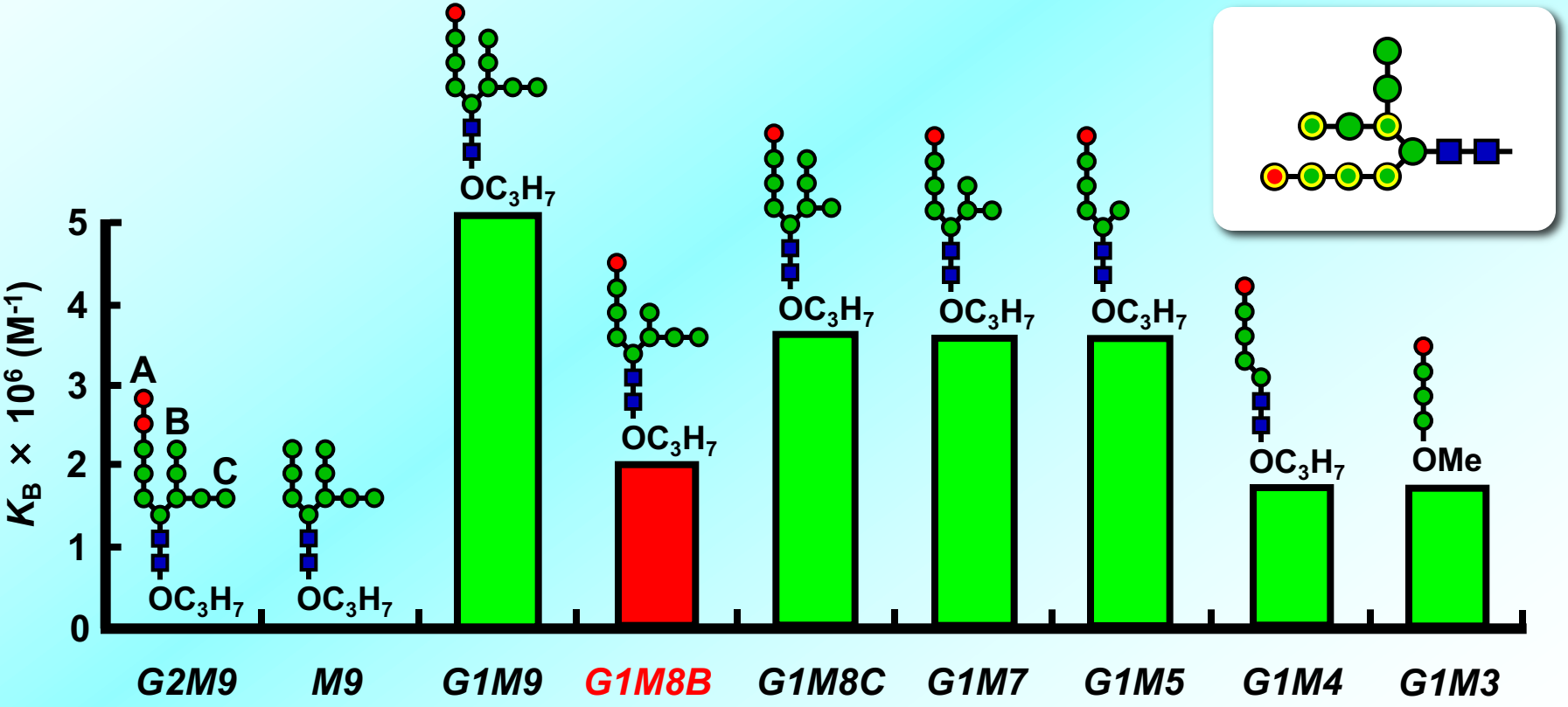
10 glycans × 3 functions = 30 substrate

Glycan specificity of CRT

Y. Ito et al. *Curr. Opin. Struct. Biol.* 2005, *15*, 481.

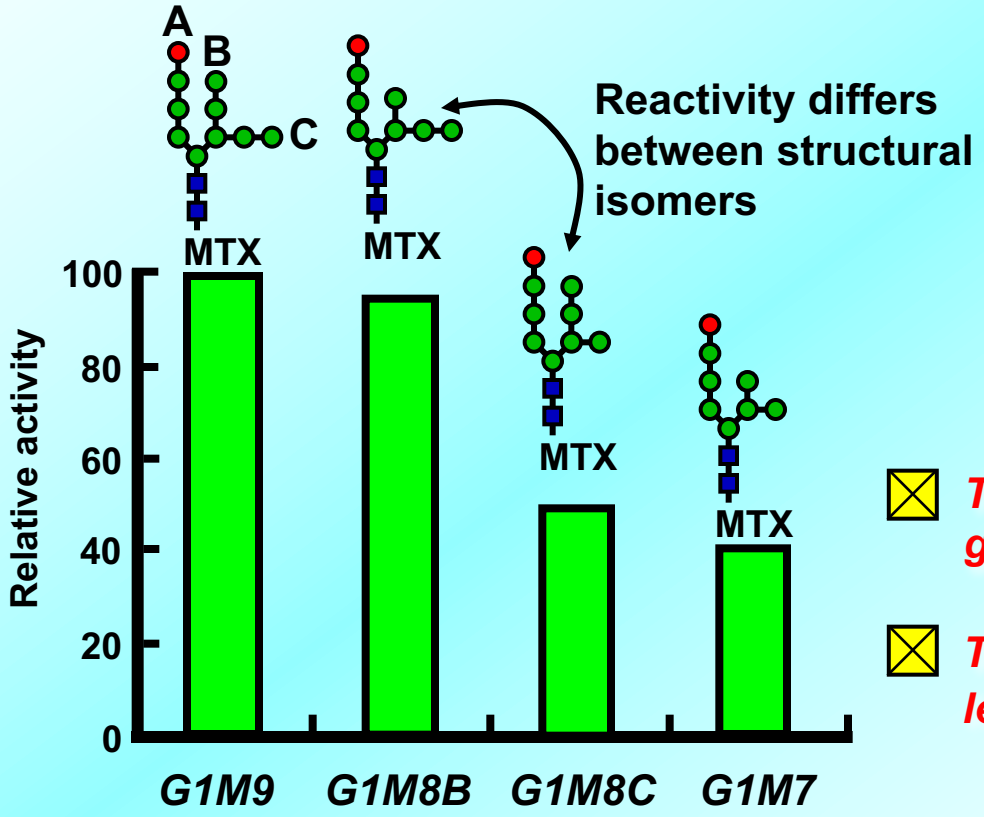


- Specific recognition of G1 glycans
- Low affinity for G1M8B
- Branch Man for B-, C-arm is essential



Glycan specificity of Glc'ase II

K. Totani et al. *J. Biol. Chem.* 2006, *281*, 31502.

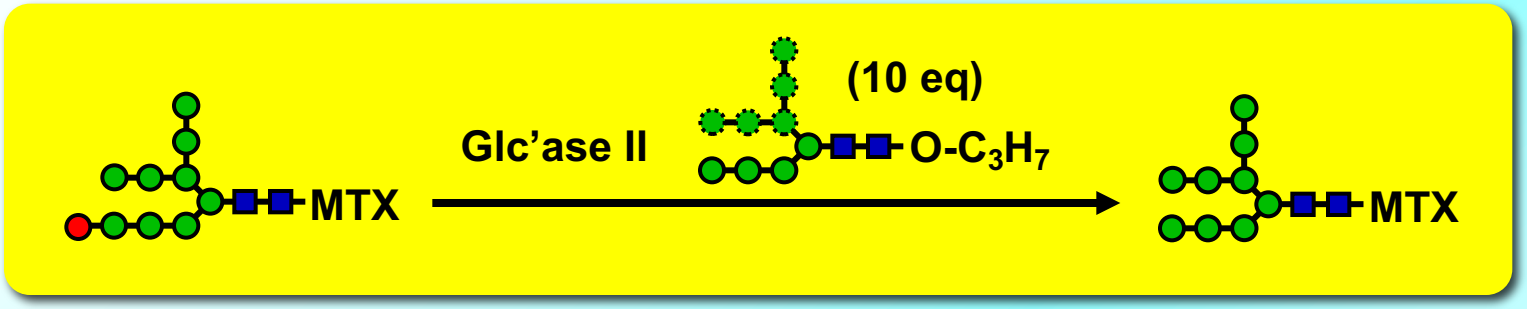


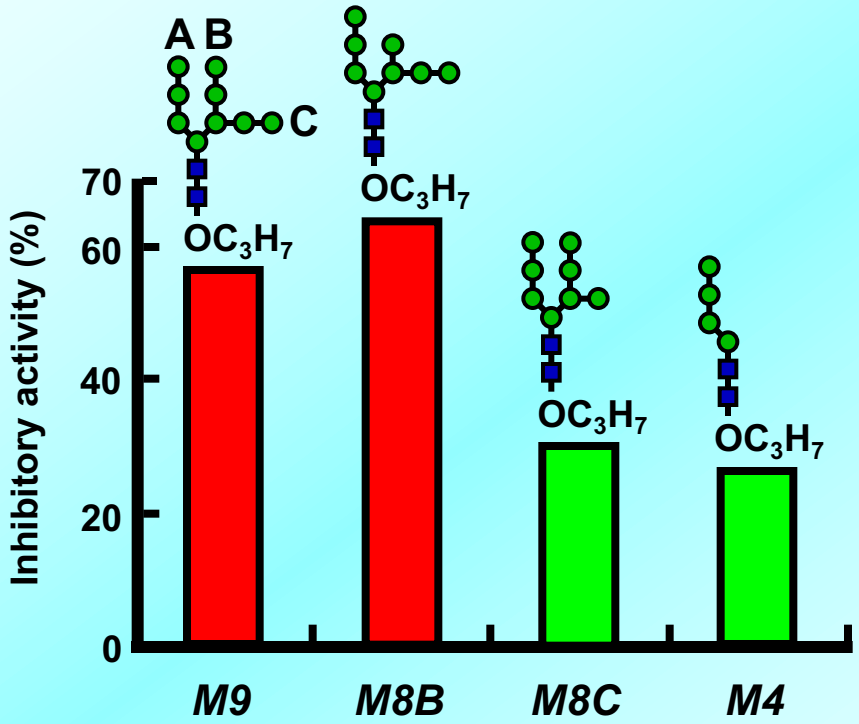
Substrate	K_m (μM)	V_{max} ($\mu mol/h/mg$)
G1M9-MTX	78	7.87
G1M8B-MTX	56	6.78
G1M8C-MTX	93	5.26
G1M7-MTX	102	5.25

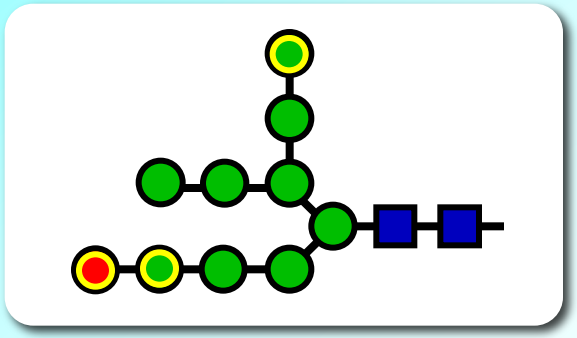
- *Terminal Man trimming at the B-arm gives has little influence on the activity*
- *Terminal Man trimming at the C-arm leads to reduced reaction efficiency*

Glycan residues recognized by Glc'ase II

K. Totani et al. *J. Biol. Chem.* 2006, *281*, 31502.



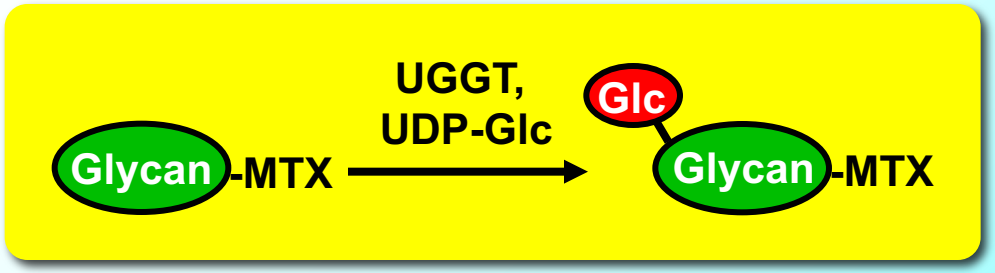




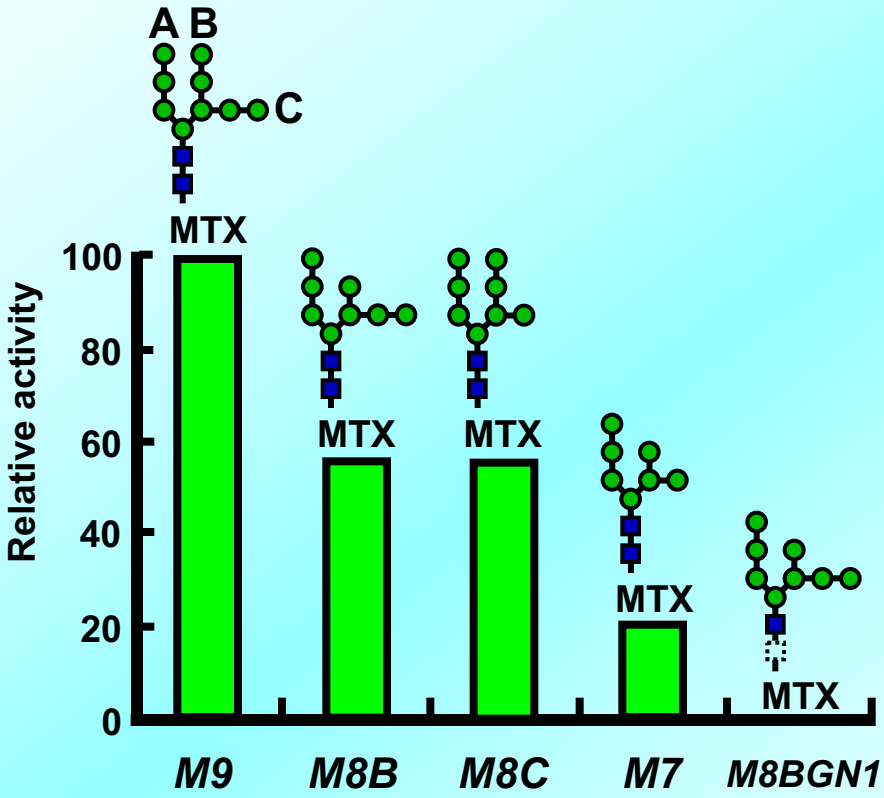
The terminal Man at the C-arm is important for the recognition

Glycan specificity of UGGT

K. Totani et al. *Angew. Chem. Int. Ed.* 2005, *44*, 7950.



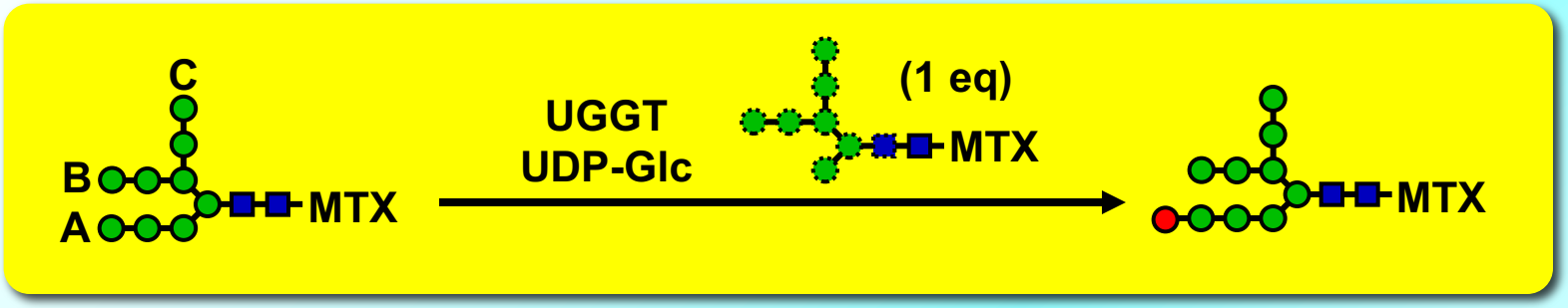
Substrate	K_m (μM)	V_{max} (nmol/h/mg)
M9-MTX	207	32.3
M8B-MTX	197	15.4
M8C-MTX	448	23.8
M7-MTX	46	4.8

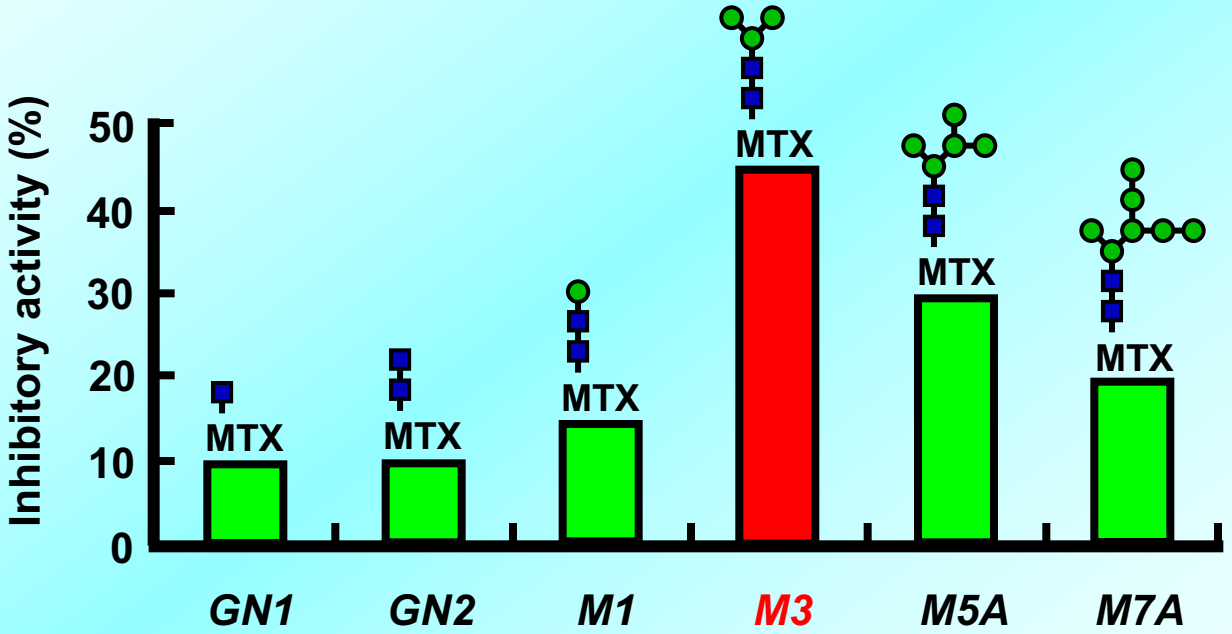


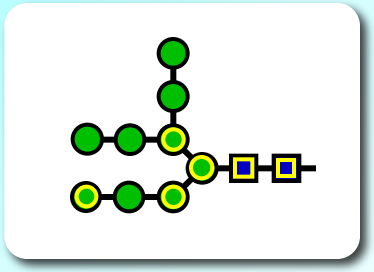
- ***Terminal Man trimmings at the B- and C-arm lead to reduced reaction efficiency***
- ***GlcNAc2 is required for Glc-transfer***

Glycan residues recognized by UGGT

K. Totani et al. *Biochemistry* 2009, *48*, 2933.



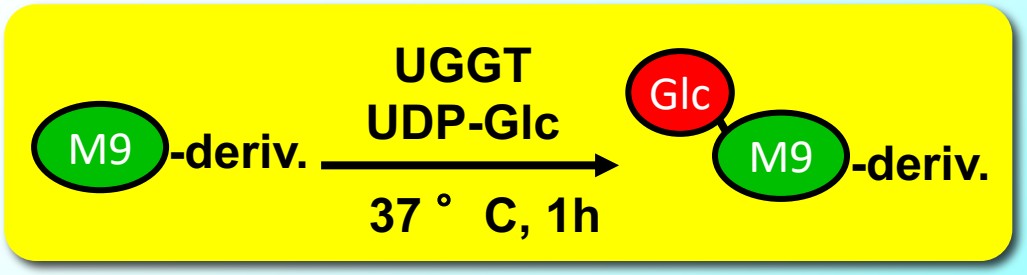


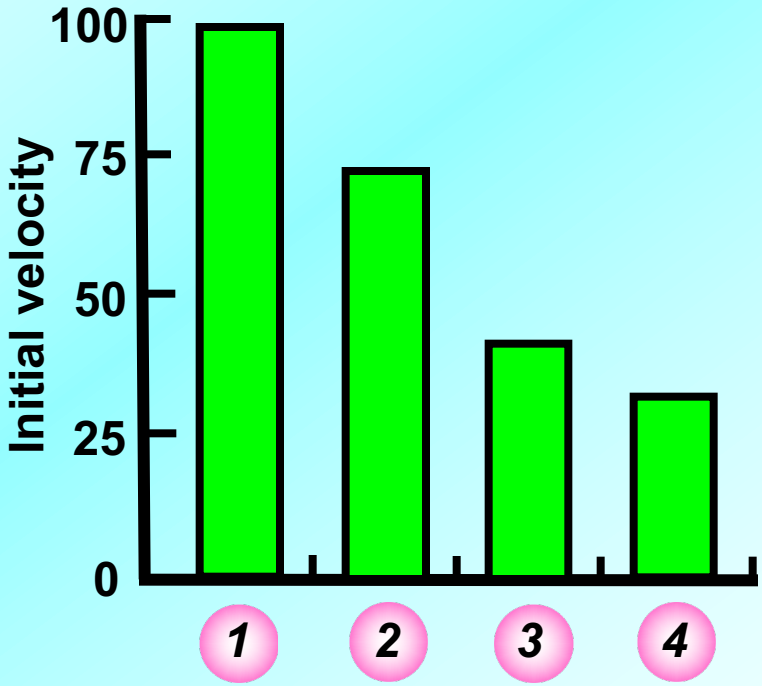


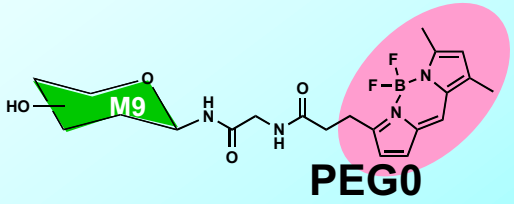
☒ *Man3GlcNAc2 structure is mainly recognized*

Aglycone specificity of UGGT

K. Totani et al. *Biochemistry* 2009, *48*, 2933.

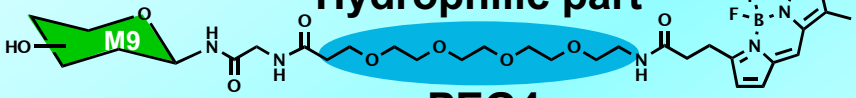




1 

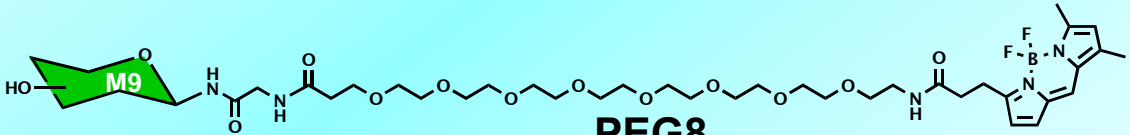
PEG0

Hydrophobic part (BODIPY)

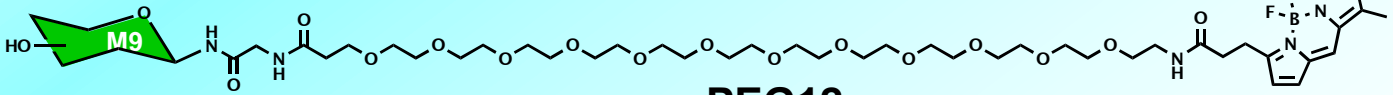
2 

Hydrophilic part

PEG4

3 

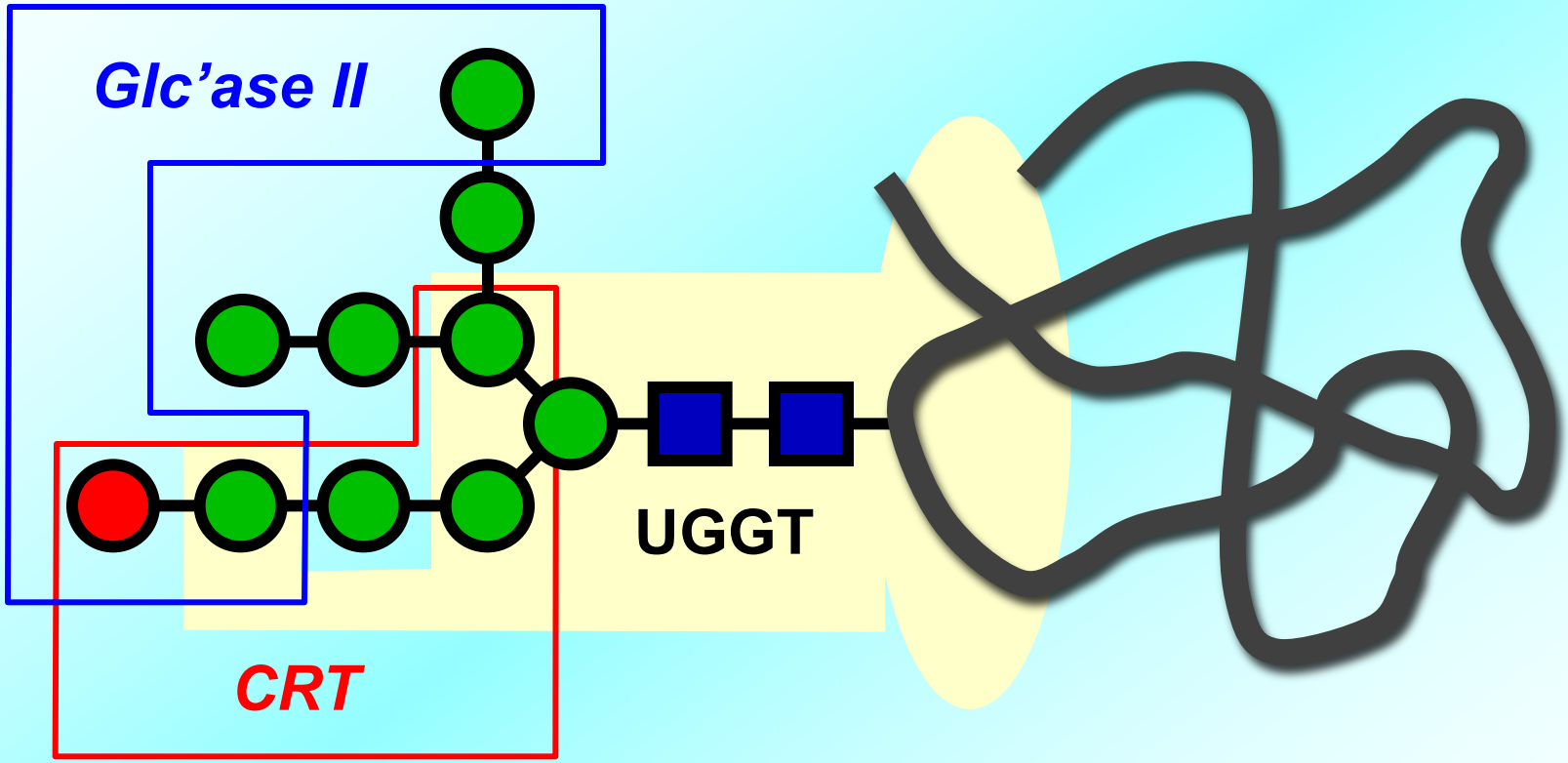
PEG8

4 

PEG12

Substrate recognition ability decreases as increasing hydrophilicity near the reducing end

Substrate recognition of CRT, UGGT and Glc'ase II



☒ Quality control that skilfully uses the recognition part