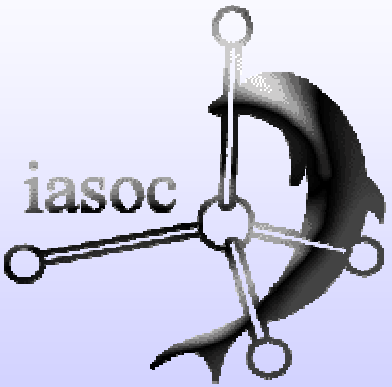
UNIVERSITÀ DEGLI STUDI DI MILANO
Dipartimento di Chimica Organica e Industriale

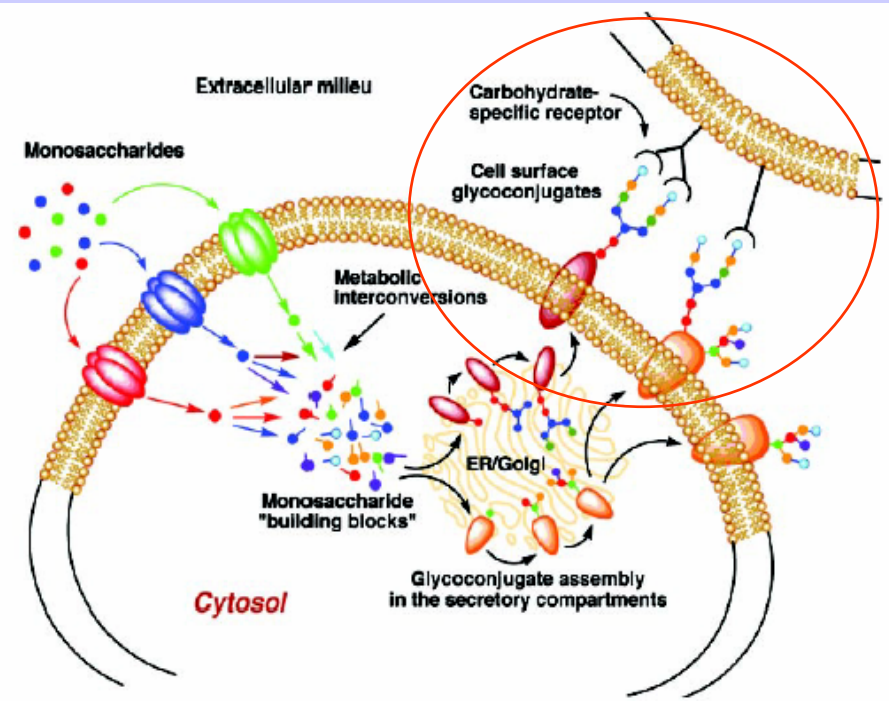
Carbohydrate Mimics: Design, Synthesis, and Biological Activity

Anna Bernardi



IASOC 2006
Ischia, September 16 - 21, 2006

Organic Chemistry from Synthesis to the Interfaces of Life Sciences



Glycoconjugate and cell surface recognition

from Science 291, 2357 (2001)

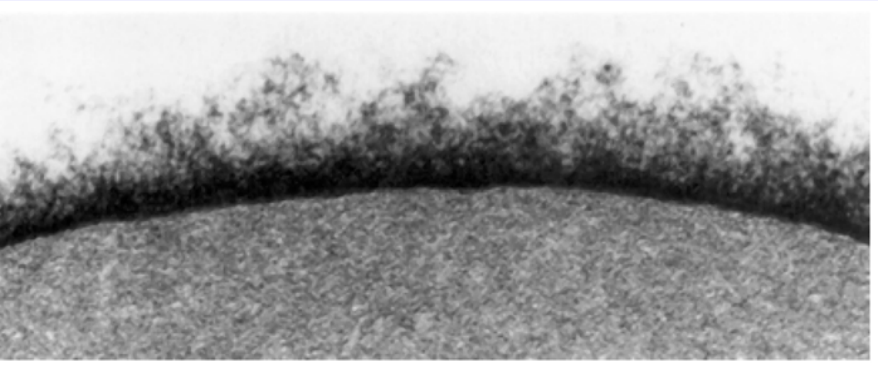
Functions resulting from glycan-lectin interactions

Directing trafficking of glycoconjugates (intra- and extra-cellular)

Mediating and modulating cell adhesion (cell-cell, cell-matrix)

Mediating and modulating signalling (intra- and extra-cellular)

www/sciencemag.org/Science Supplemental Material -- Carbohydrates Web Resources.htm



The glycocalix

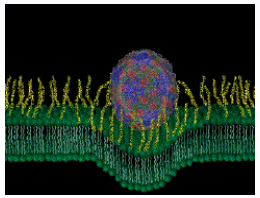
Electron micrograph of the glycocalix at the surface of a red blood cell

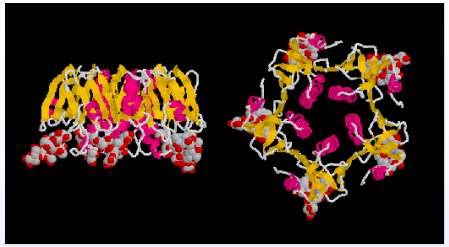
Cell surface carbohydrate-protein interactions play important roles in cell recognition processes.

Cell-cell interaction (inflammation, fertilization)

Bacterial adhesion and biofilm formation (E.coli, H. pylori)

Cell-virus interaction (e.g. flu virus)





Cell-molecules interaction (e.g. hormones, toxin)

Sugar/protein interactions as target
Development of probes, diagnostics and drugs

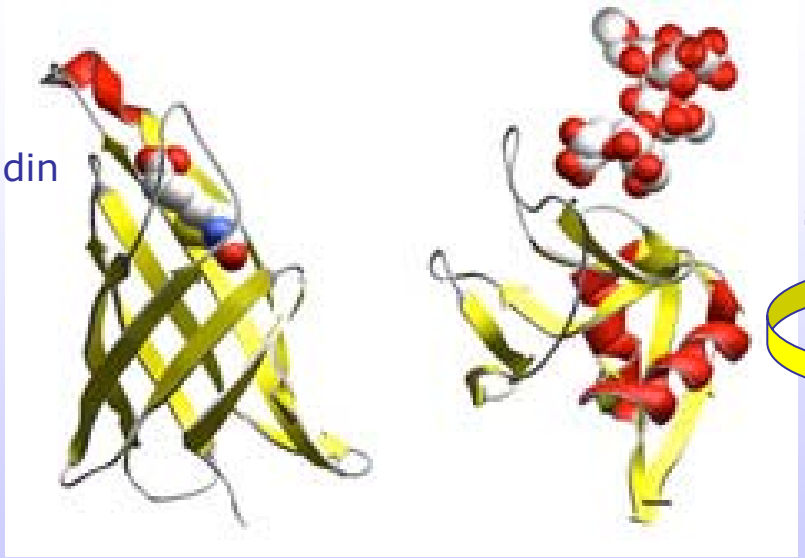
Sear, P.; Wong, C. H.; *Angew. Chem. Int. Ed.* **1999**, 38, 230

Carbohydrate - Protein interactions are involved in the early stages of many cell communication events, hence they are interesting targets for drug development

- **Oligosaccharides' drawbacks as drugs**
  - ✓ **metabolically unstable**
  - ✓ **low bioavailability**
  - ✓ **difficult to synthesize**
  - ✓ **low affinity ligands**

- **Oligosaccharide mimics could be made:**
  - ✓ **metabolically stable**
  - ✓ **more bioavailable**
  - ✓ **easier to synthesize**
  - ✓ **high affinity ligands ?**

Biotin – avidin complex



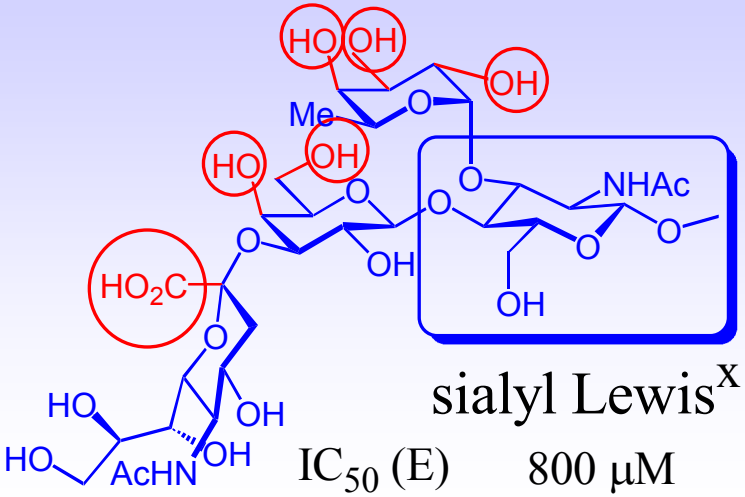
Oligosaccharide – MBP complex

small epitopes on complex scaffolds

REPLACE THE SCAFFOLD
KEEP EPITOPE ORIENTATION

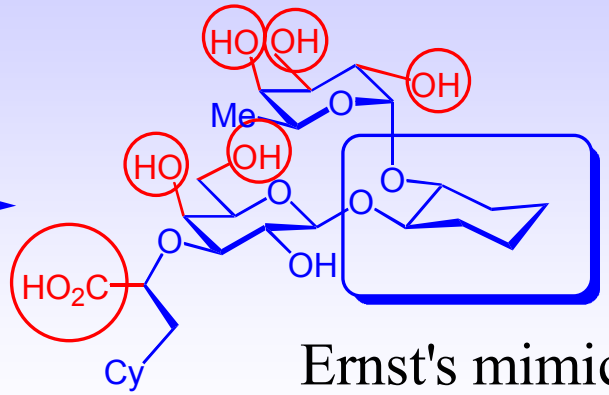
H. C. Kolb, B. Ernst *Chem. Eur. J.* 1997, *3*, 1571

The sialyl Lewis^x case



sialyl Lewis^x

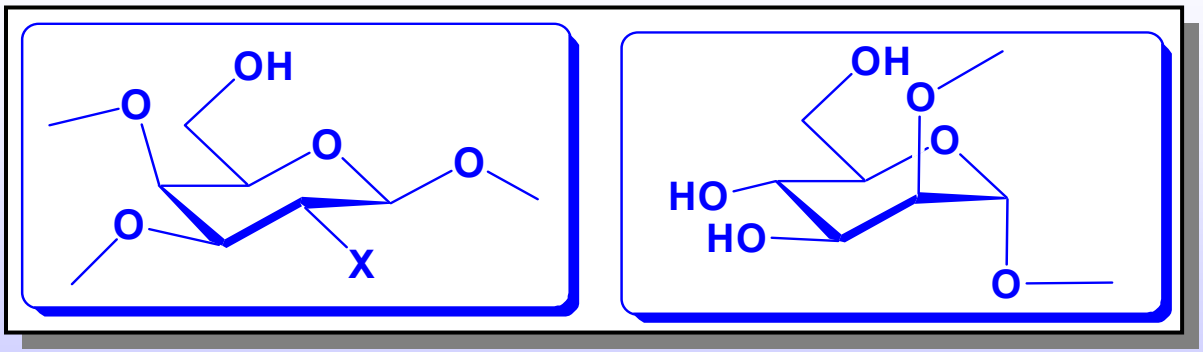
IC_{50} (E) 800 μM



Ernst's mimic

80 μM

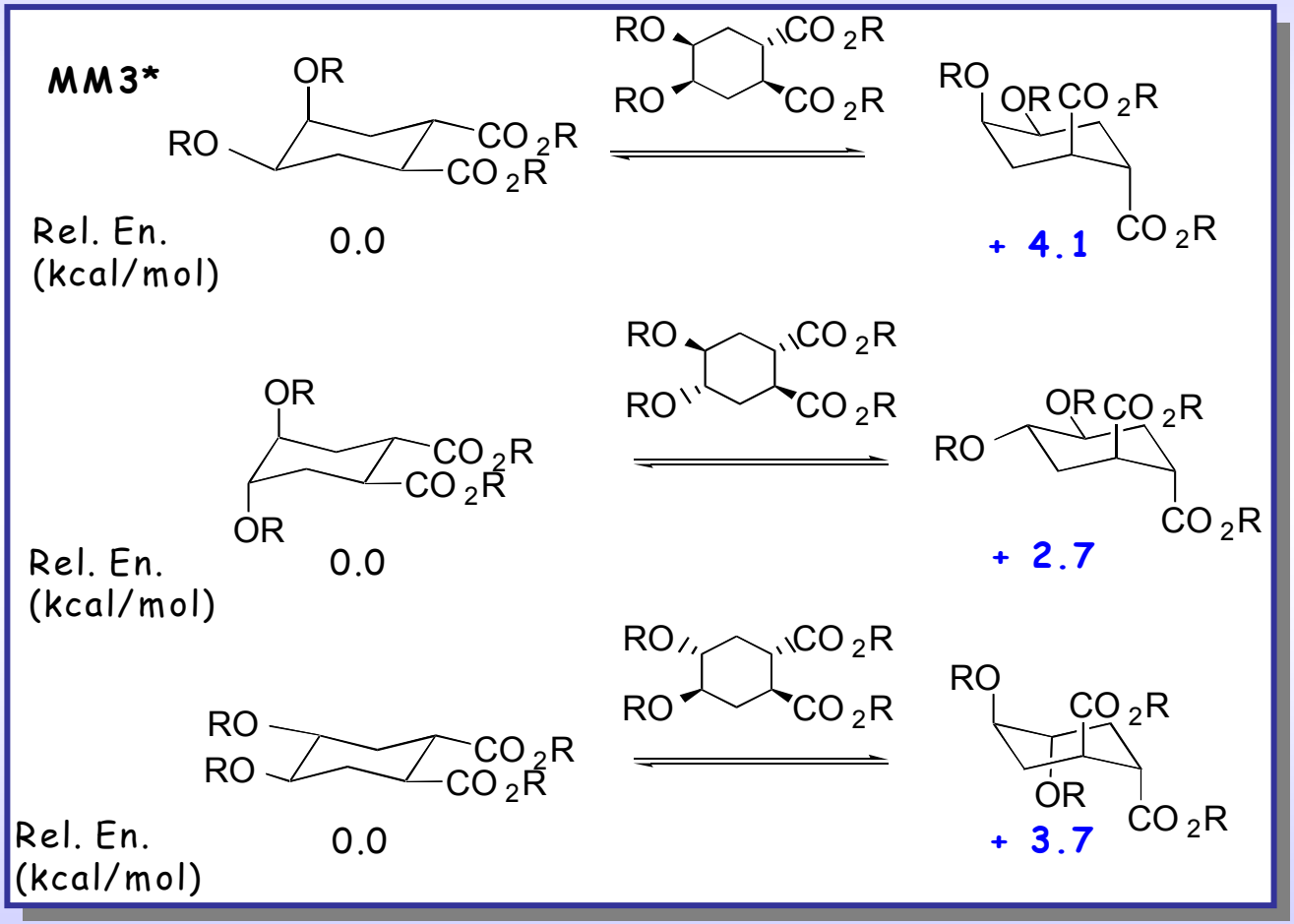
HOW DO WE REPLACE AXIALLY SUBSTITUTED RESIDUES ?



X=OH 3,4-Gal
X= NHAc 3,4-GalNAc

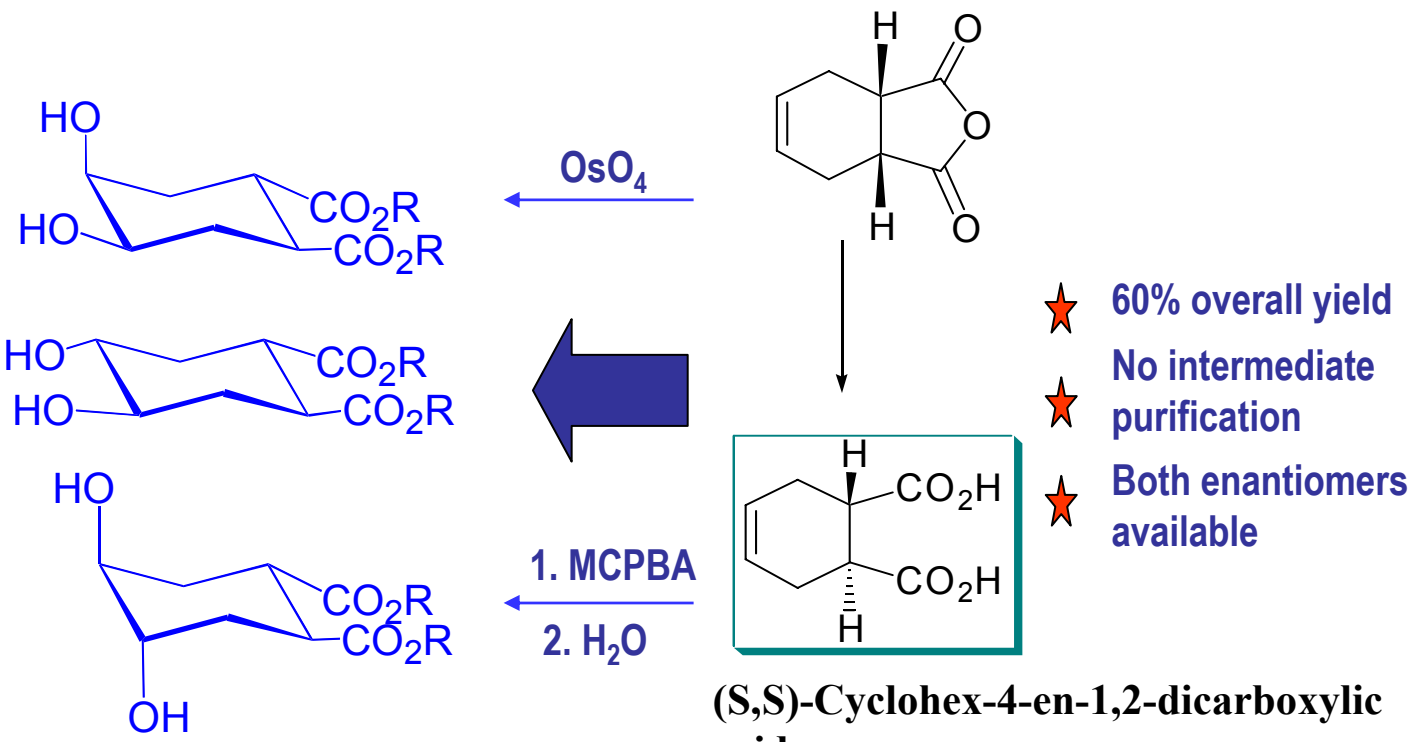
2 Manα1-

DCCHD
A Group of Conformationally Stable Cyclohexanediols



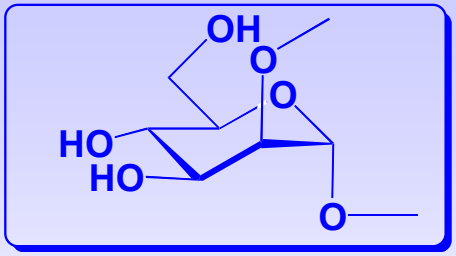
Bernardi, Arosio, Manzoni, Micheli, Pasquarello, Seneci *J.Org.Chem. 66*, 6209 (2001)

DCCHD:
A Class of Pseudo-Monosaccharide Scaffolds



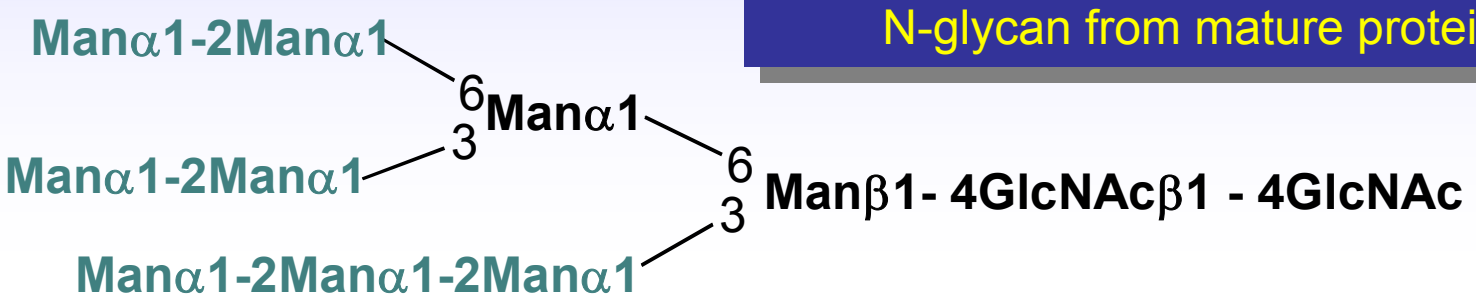
Bernardi, Arosio, Dellavecchia, Micheli ***Tetrahedron:Asymmetry 40*, 3403 (1999)**

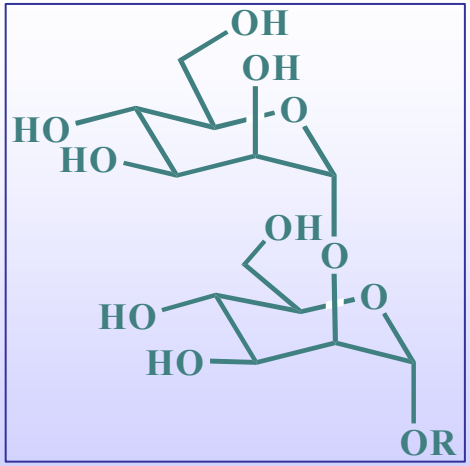
Bernardi, Arosio, Manzoni, Micheli, Pasquarello, Seneci ***J.Org.Chem. 66*, 6209 (2001)**



The 1,2-disusbtituted α Mannose Case: the oligomannose oligosaccharide

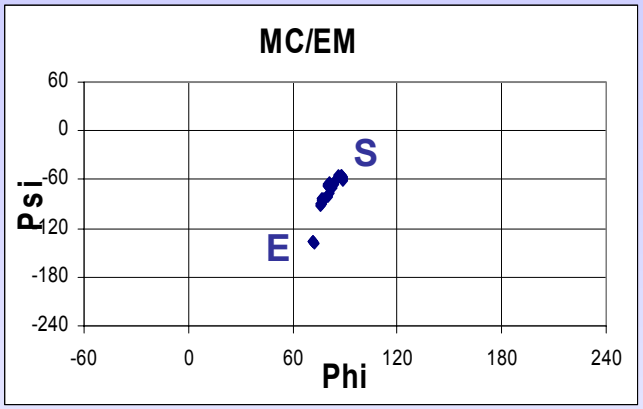
$Man_9GlcNAc_2$, the least processed N-glycan from mature proteins

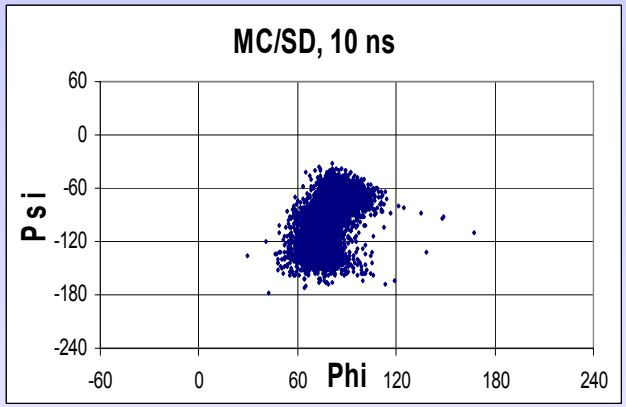




The Manα1-2Man fragment is recognized by DC-SIGN, the dendritic cell receptor that recognizes HIV-gp120 and other pathogens. Polyvalent mannobiosides have potential as antiviral compounds.

α–(1,2)-Mannobioside



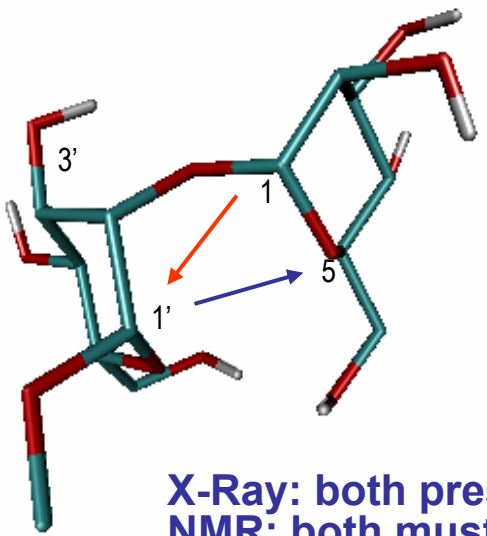


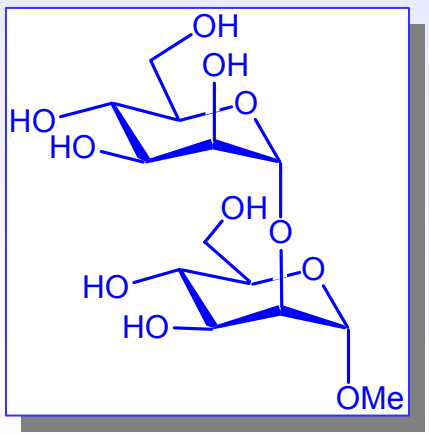
AMBER*
GB/SA water
MacroModel 5.5

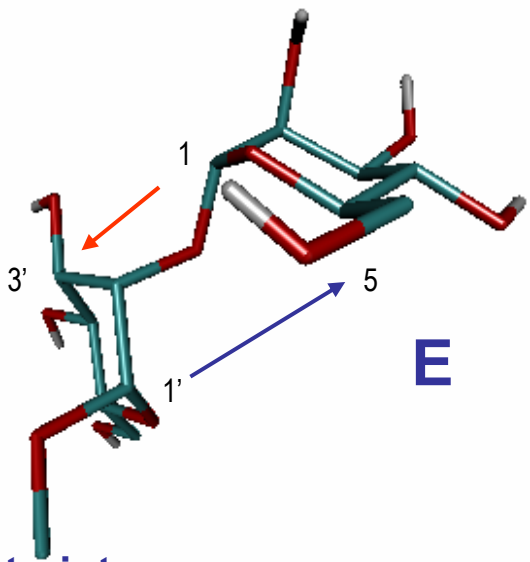
Phi: O5-C1-O1-C2'
Psi: C1-O1-C2'-C1'

ΔE S-E= 6 kJ/mol

S







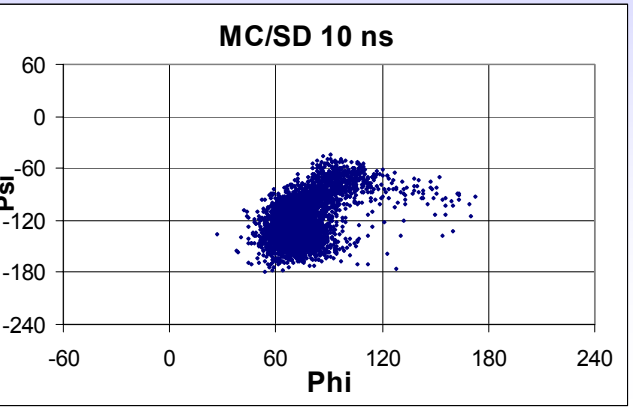
E

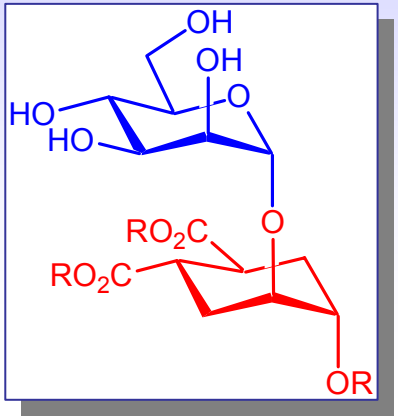
X-Ray: both present
NMR: both must be present to satisfy NOE constraints

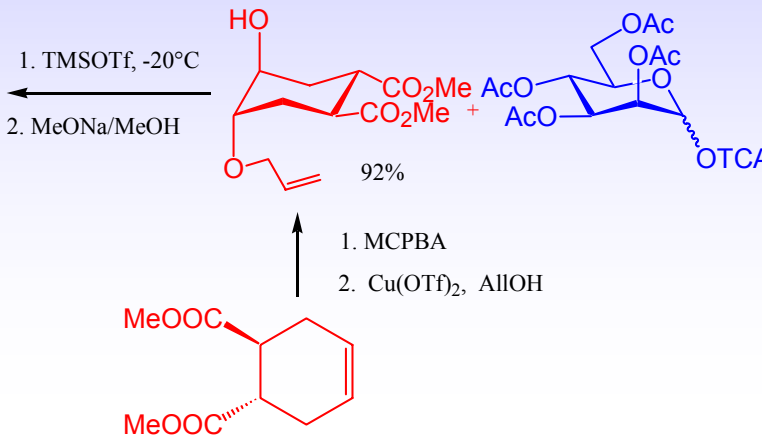
Dwek et al *Chem Rev.* **2002**, *102*, 371-386

Pseudo-1,2-Mannobioside

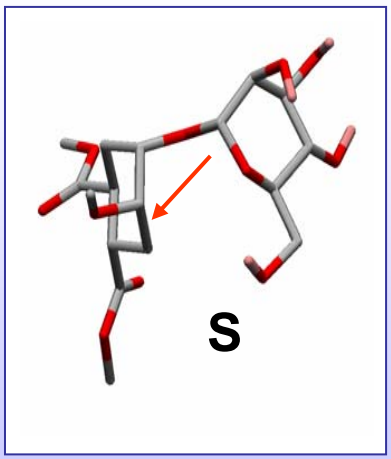
AMBER* , GB/SA water

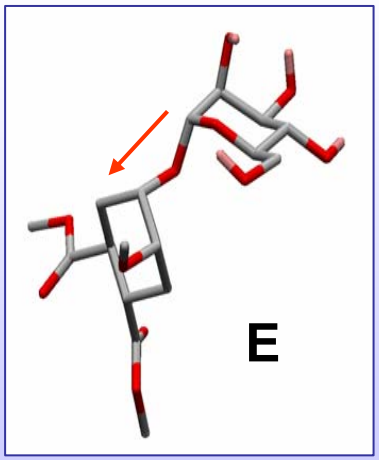






Manα1,2-Manα mimic

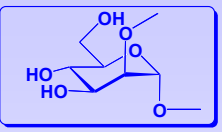




The NOE data closely parallel those found for the natural disaccharide and are in agreement with the two limit conformations predicted

The compound is more stable than mannobioside to mannosidase-catalyzed hydrolysis

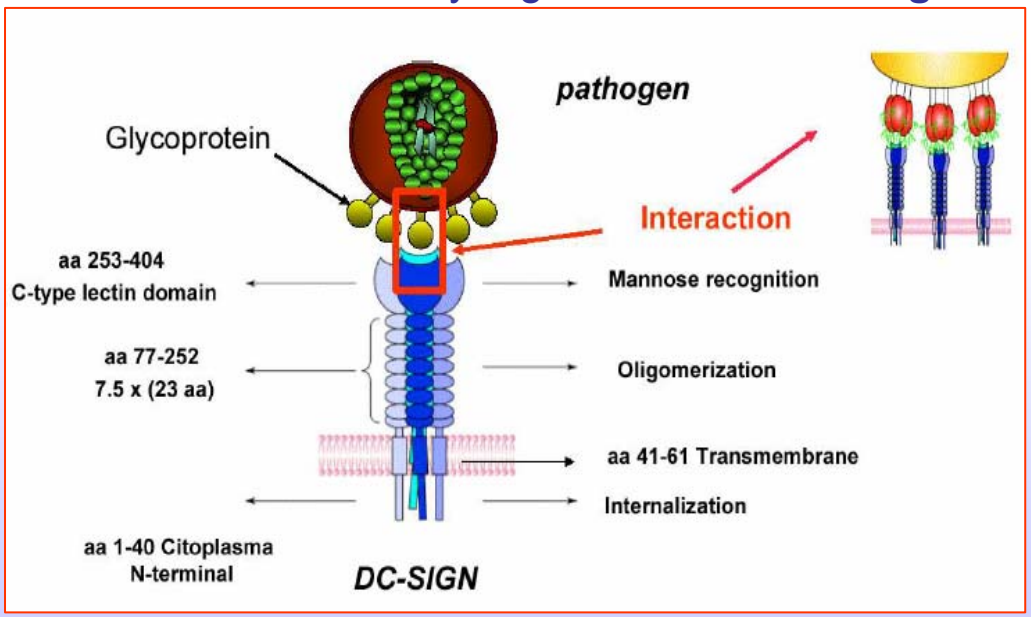
Eur. J. Org. Chem. **2004**,5119



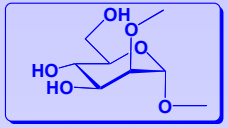
DC-SIGN

Dendritic Cell-Specific ICAM-3 Grabbing Nonintegrin

- Tetrameric, type II transmembrane protein (C-lectin), expressed by immature dendritic cells
- Binds to a broad spectrum of pathogens (including HIV, Ebola, Dengue, Leishmania etc) mainly by targeting highly mannosylated glycoproteins
- Some pathogens, including HIV, use DC-SIGN to infiltrate the immune system
- DC-SIGN is currently regarded as a **new target in infectious diseases**

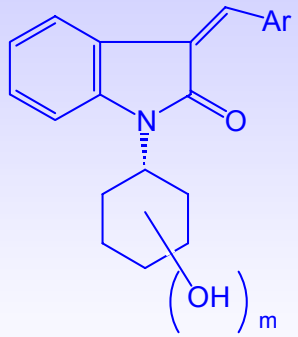


- ✓ The interaction is multivalent and Ca dependent
- ✓ Mannose-based multivalent inhibitors of DC-SIGN have been reported (J. Rojo *et al*)
- ✓ **At mM concentration ps-1,2-mannobioside inhibits DC-SIGN mediated viral entry in an Ebola model**



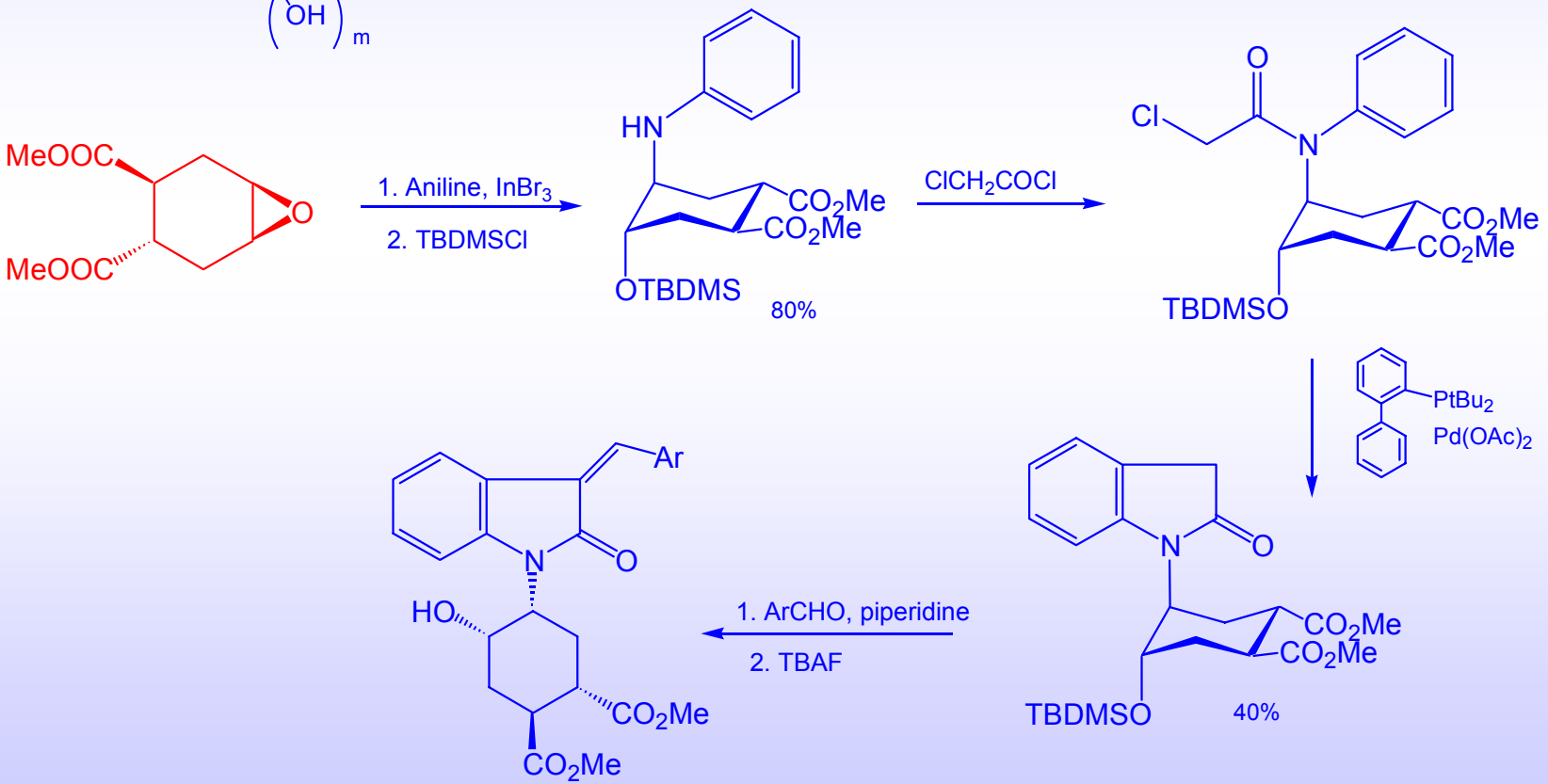
Synthesis of potential kinase inhibitors

Collaboration with Pierfausto Seneci - Unimi



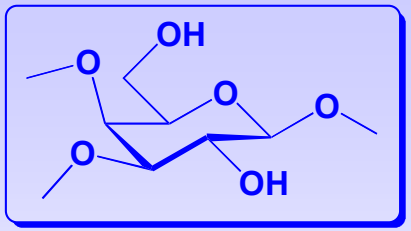
Indolidinones are known kinase inhibitors

N-substitution with polyhydroxylated rings known to improve activity



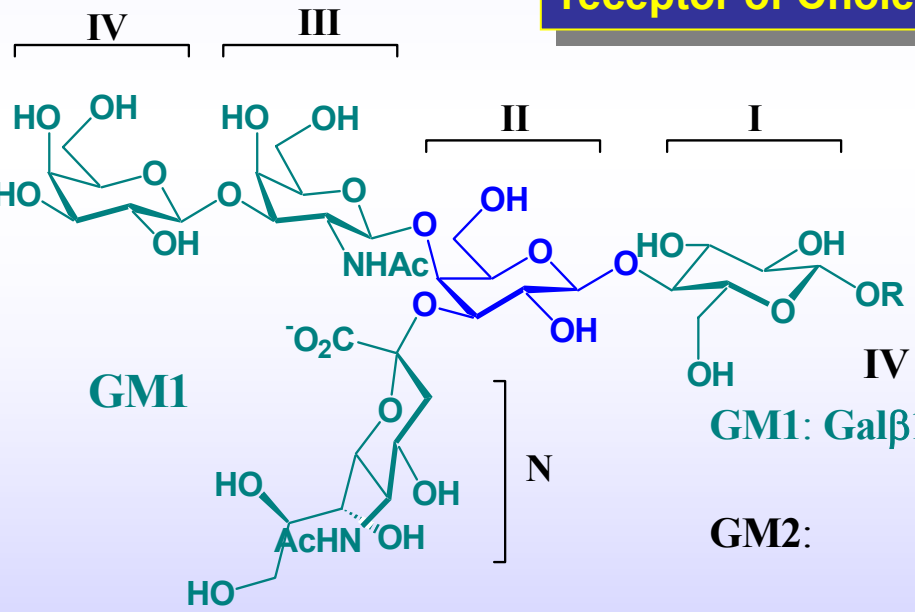
Marco Re

Buchwald *et al JACS* **2003**, xxxx



The 3,4 disusbtituted Galactose Case: Monosialo Gangliosides

Gangliosides are important constituents of biological membranes, particularly in the nervous system, where they participate in various recognition processes. Ganglioside GM1 is also the specific membrane receptor of Cholera Toxin (CT).



R= Ceramide

IV III N II I

GM1: Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ1-OR

GM2: GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ1-OR

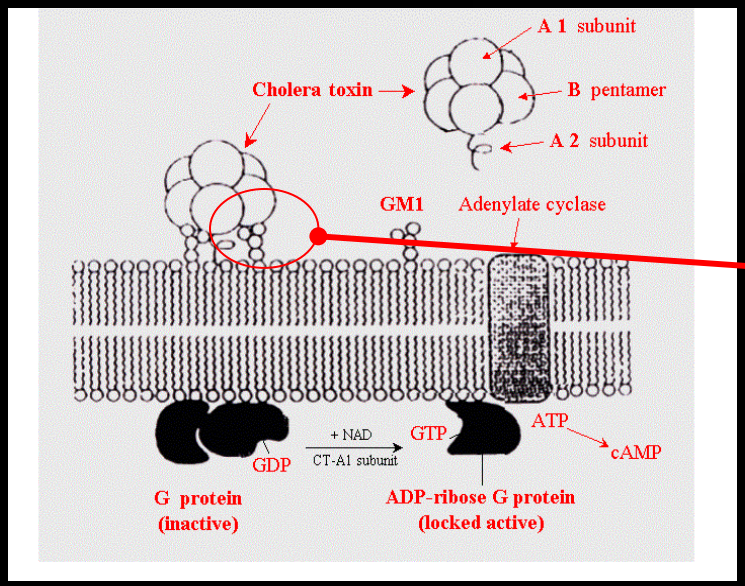
GM3 Neu5Acα2-3Galβ1-4Glcβ1-OR

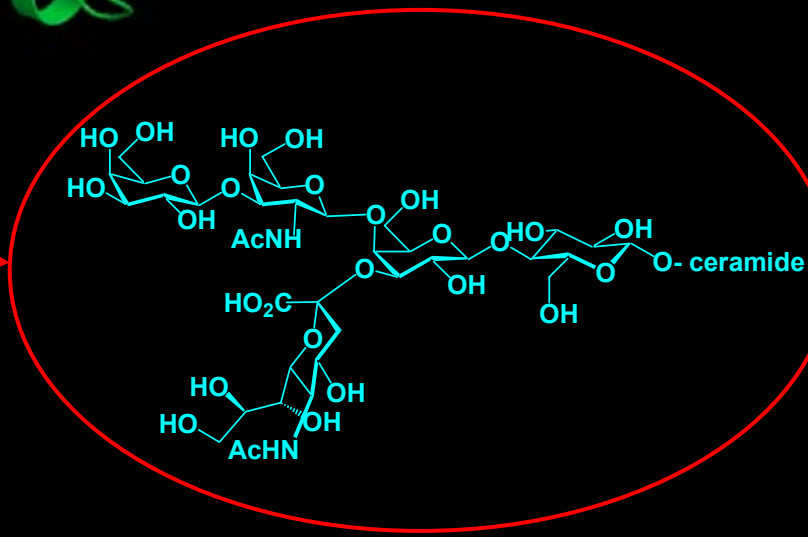
GM4: Neu5Acα2-3Galβ1-OR

The cholera toxin

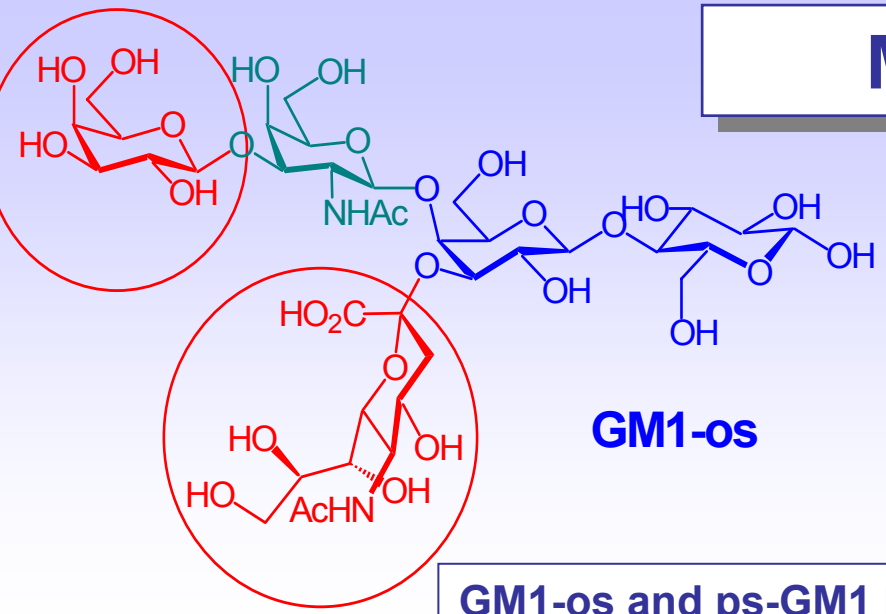
A SUBUNIT

B_5 PENTAMER



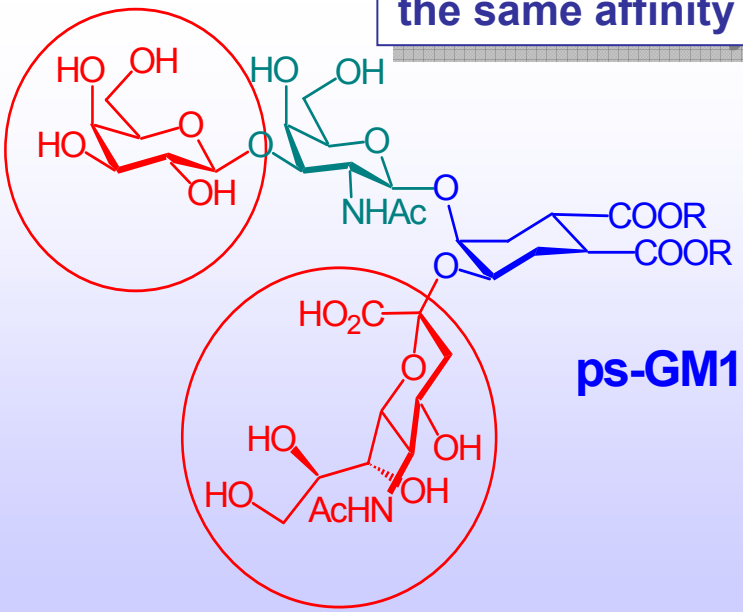


Mimicking the GM1-os

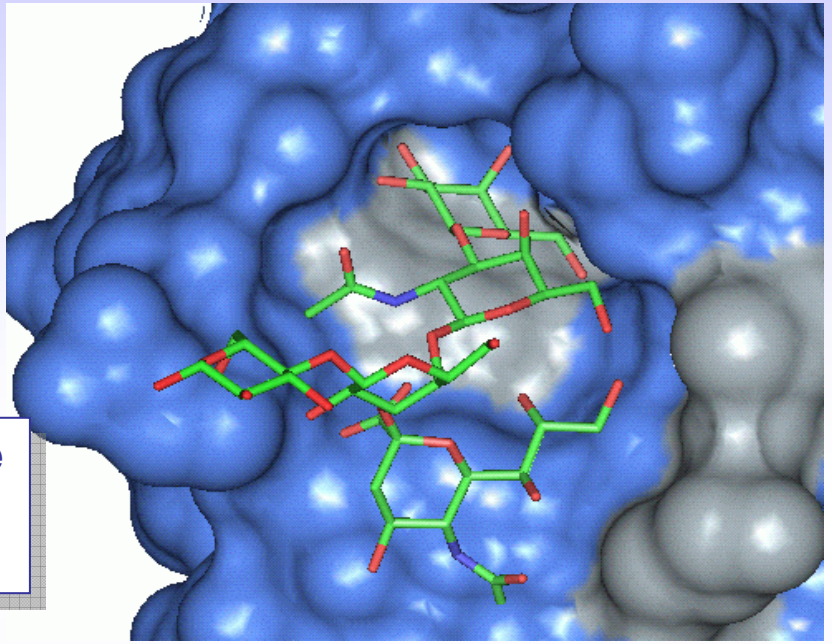


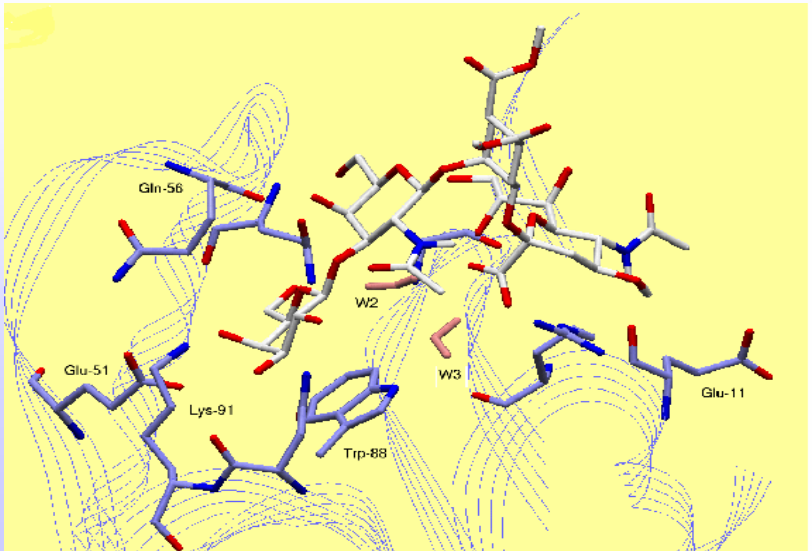
GM1-os

GM1-os and ps-GM1 have the same structure and the same affinity for CT

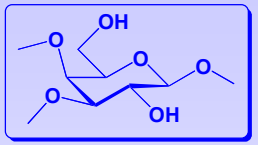


ps-GM1

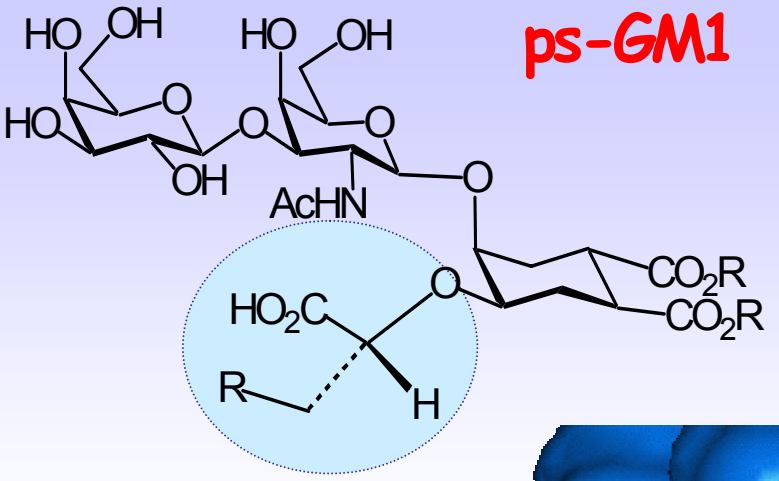


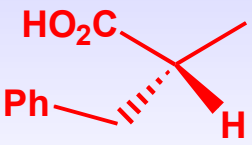


J. Am. Chem. Soc. 121, 2032 (1999)



K_D (μM)





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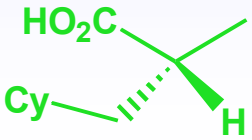

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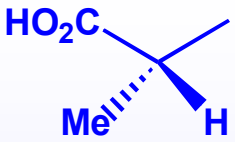

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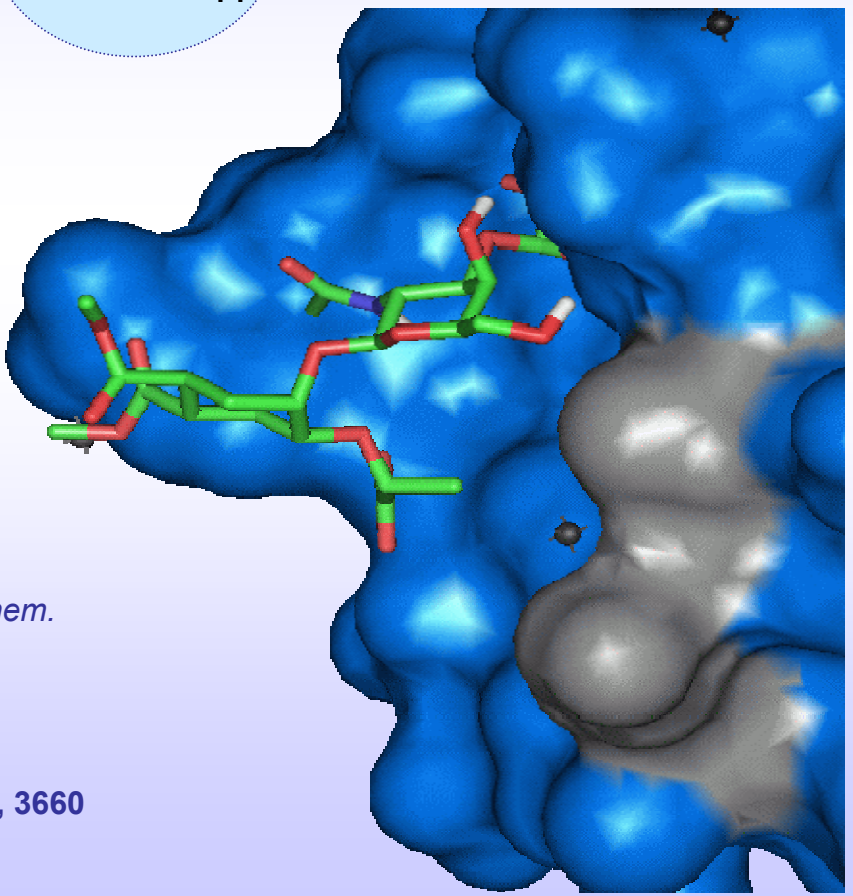
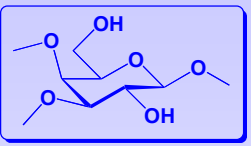


NMR studies have allowed rational design of the mimics

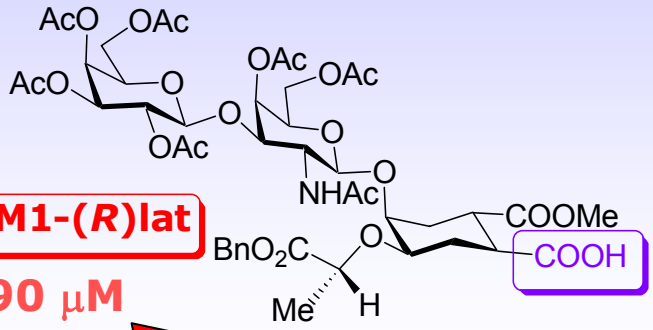
(*Org. Biomol. Chem.* **2004**,*1*, 785; *Chem. Eur. J.* **2002**, 4597; *Chem. Eur. J.* **2004**, 4395)

Polyvalency improves the affinity to the nM range

(*Org. Biomol. Chem.* **2004**, 2113; *J. Am. Chem. Soc.* **2005,*127*, 3660**
)

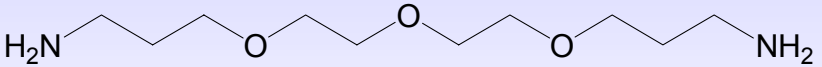


POLYVALENT psGM1 LIGANDS



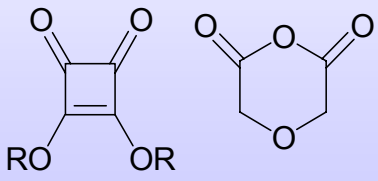
psGM1-(*R*)lat

190 μM

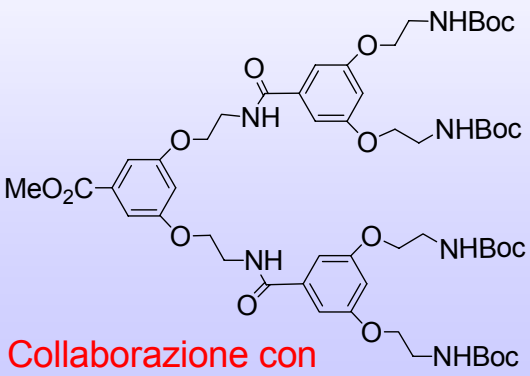


Spacer

SP



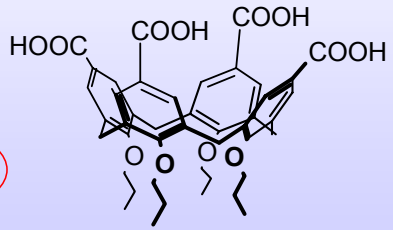
R = Me, Et



Collaborazione con Università di Utrecht

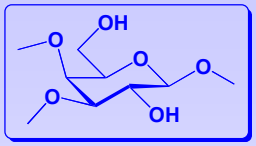
Org. Biomol. Chem. **2004**, 2113

SCAFFOLD

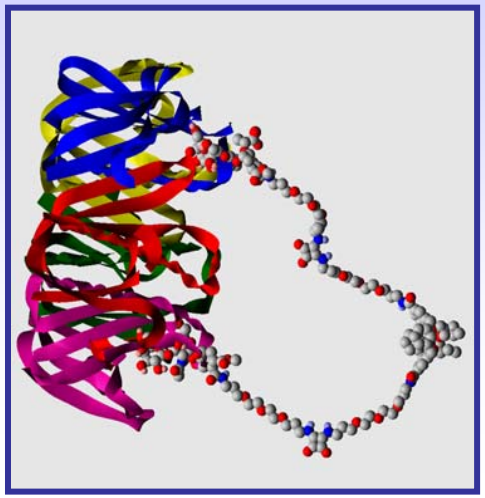


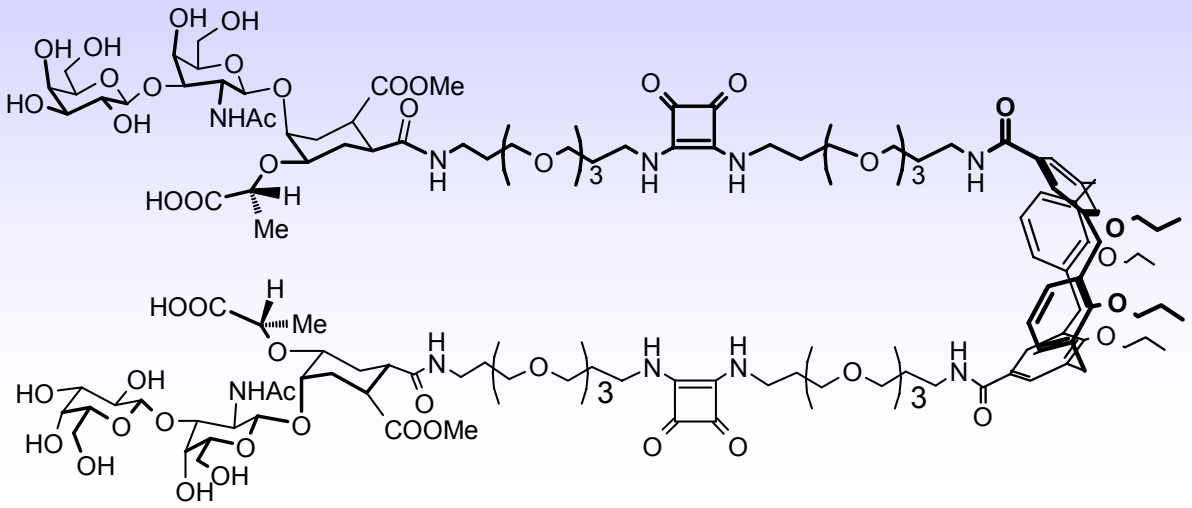
Collaborazione con Università di Parma

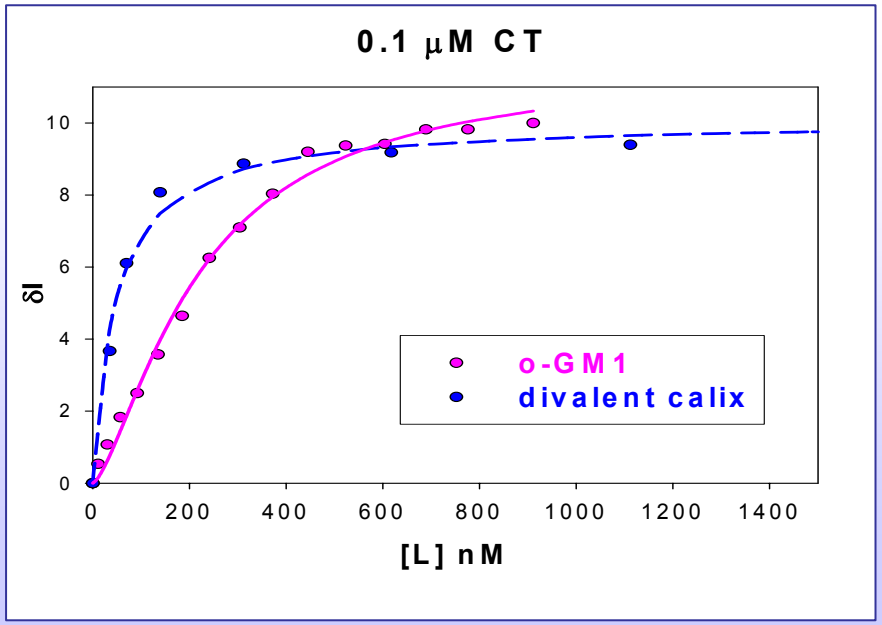
J. Am. Chem. Soc. **2005**, *127*, 3660



Divalent Calixarene: Fluorescence titrations







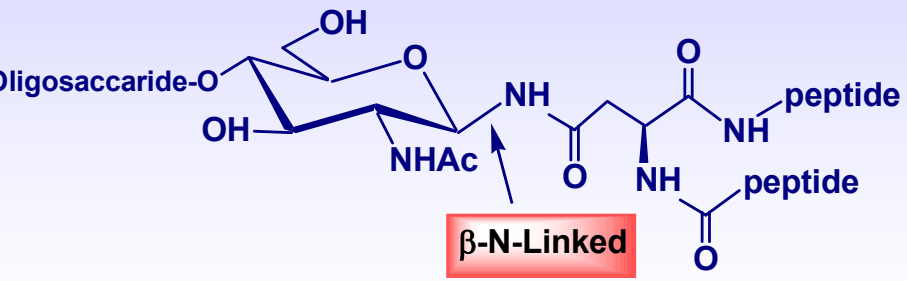
In this assay, the divalent calixarene appears more active than the natural ligand o-GM1

IC_{50}= 48 nM

β = 3950

J. Am. Chem. Soc. **2005**,*127*, 3660

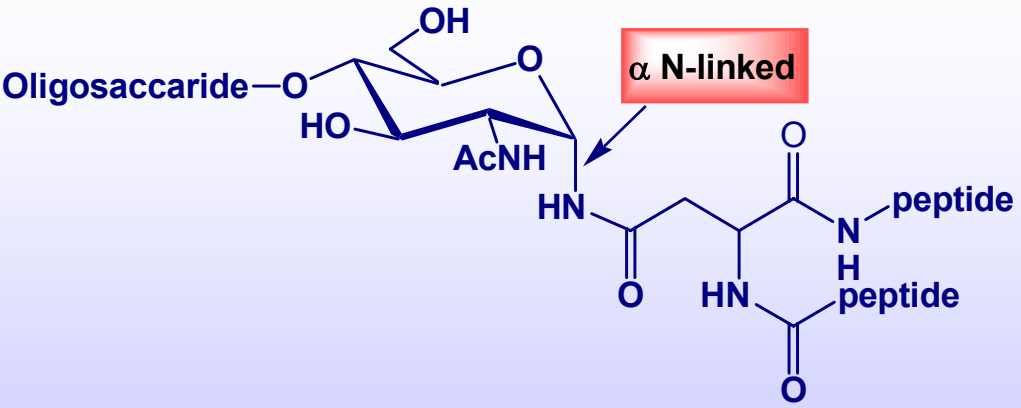
α-N-glycosylamides, a new class of glycoconjugates



In N-linked glycopeptides and glycoproteins the oligosaccharides are β linked to the Asn side chain of a Asn-Xaa-Thr/Ser consensus sequence

Kunz, H. *et al Chem. Rev*. 2000, 100, 4495-4537

α-N-linked glycosylamides and glycopeptides are likely to be metabolically stable analogues

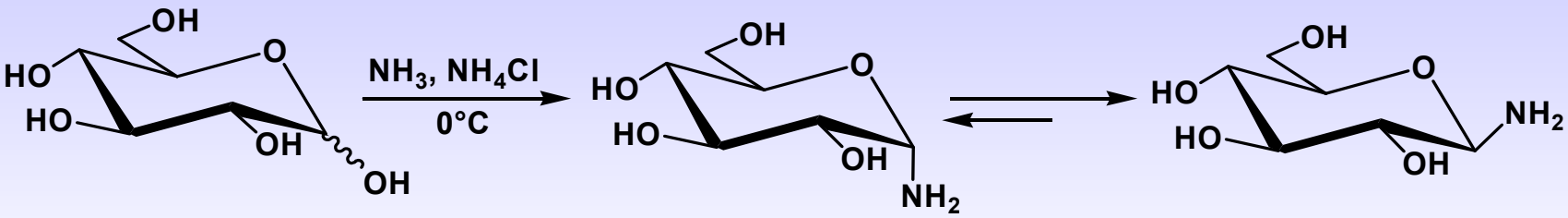


Their chemical properties and biological activity are virtually unexplored:

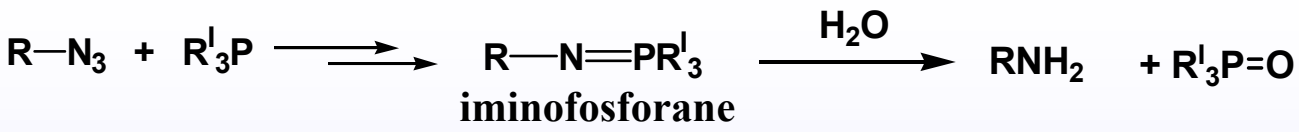
•one natural α-N-linked glycopeptide (nephritogenoside) isolated from natural sources (Shibata *et al J.Biol.Chem.* **1998,** *263*, 12843)

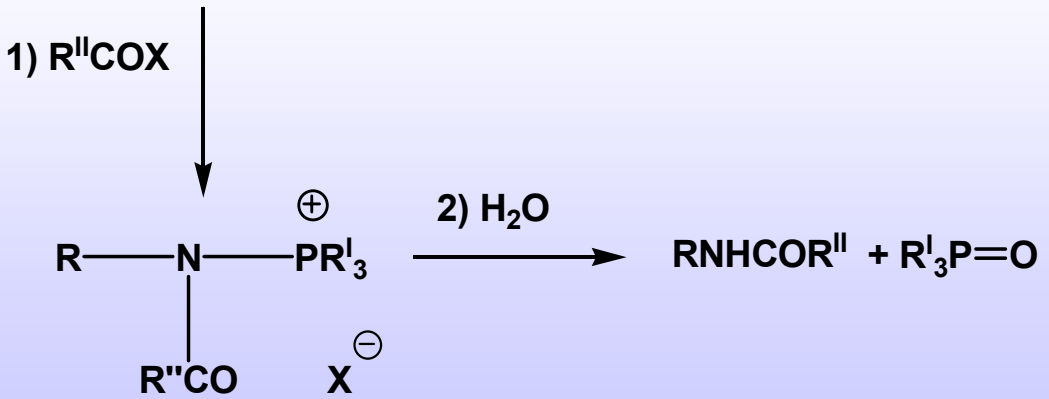
• one synthetic α-N-linked chitobiosyl peptide: the configuration of the anomeric carbon controls the conformation of the peptide (Imperiali *et al*

The stereoselective synthesis of α glycosylamides is not trivial



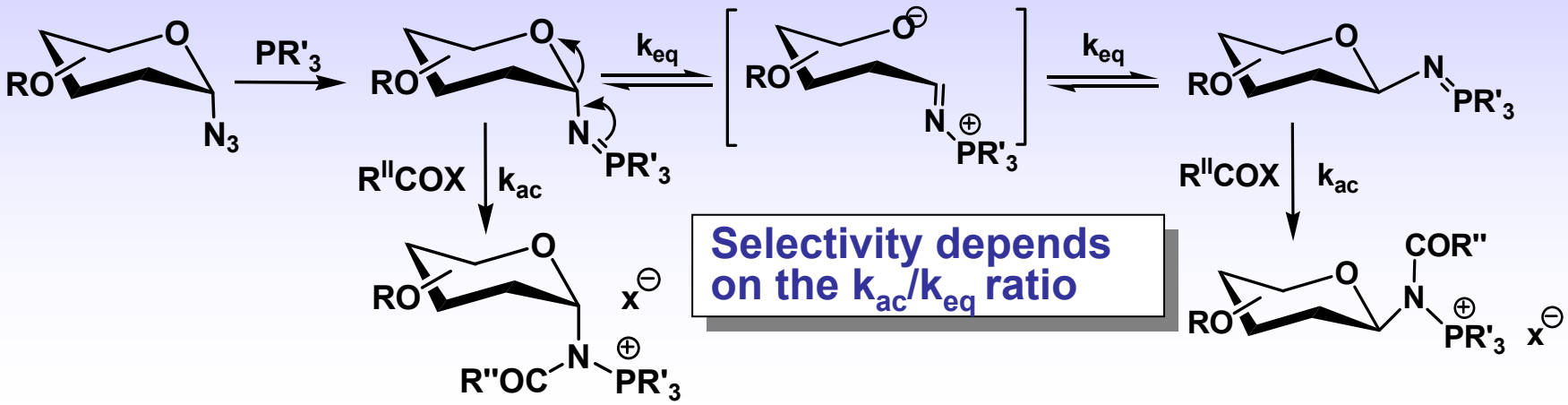
Staudinger reduction/acylation can be used to avoid amine anomerization

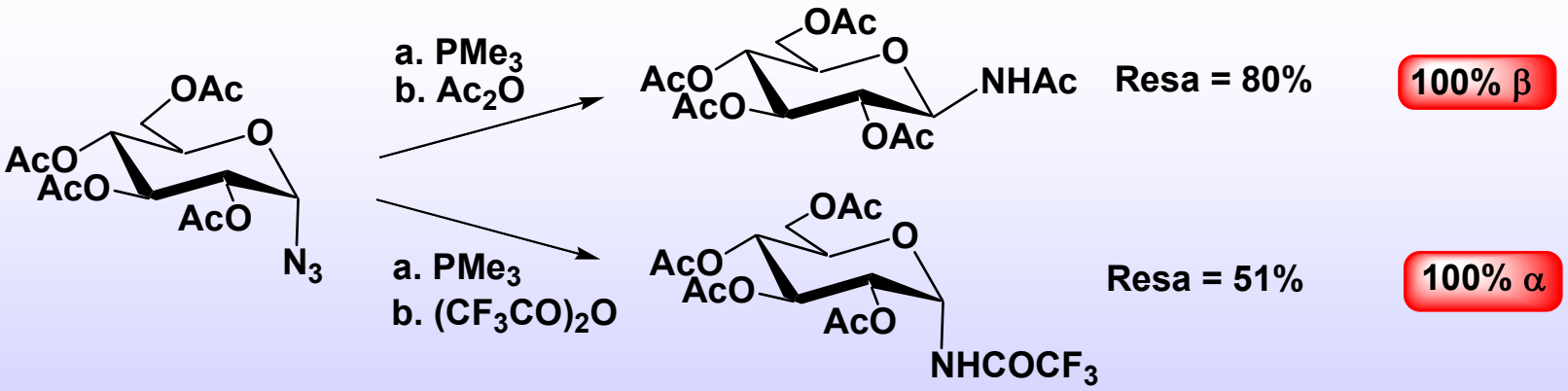




R = Pyranose

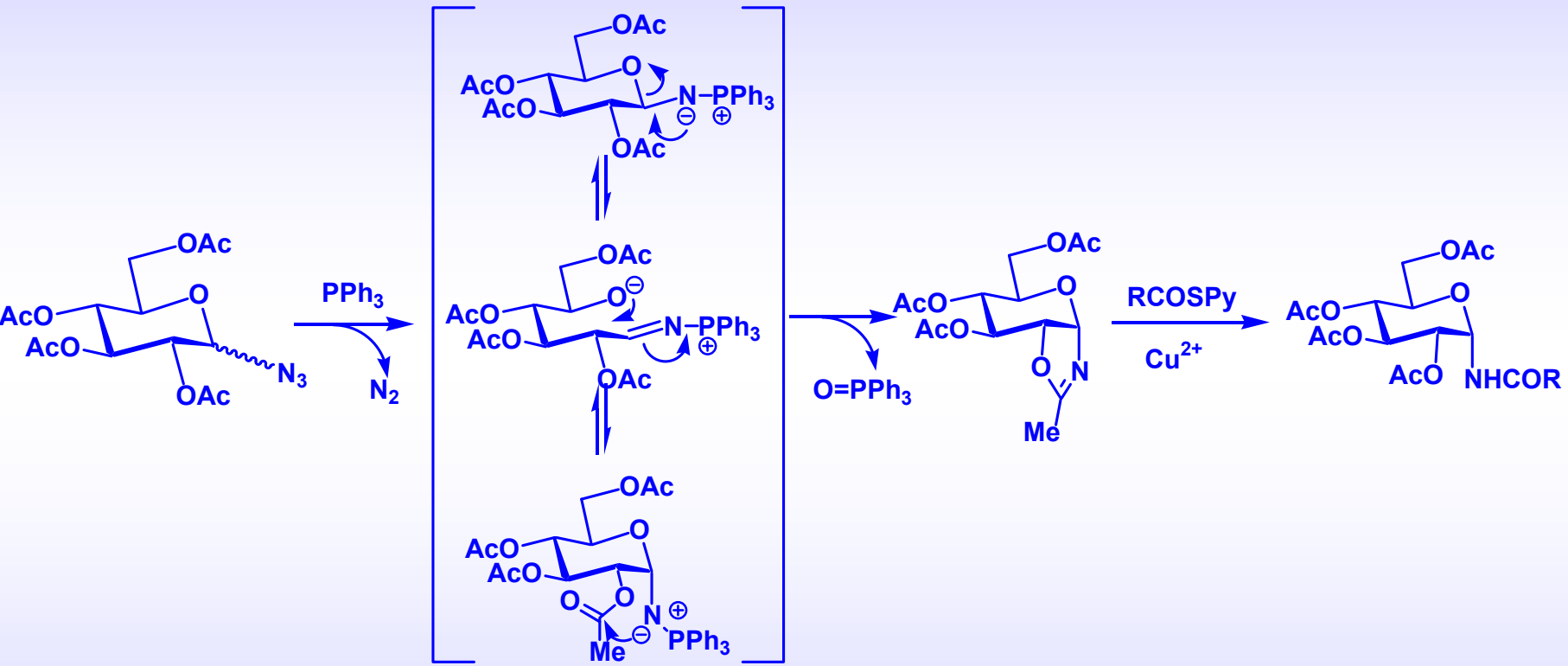
However, the iminofosforane is also prone to isomerization





Kovács, L.; Ösz, E.; Domokos, V.; Holzer, W.; Györgydeák, Z. Tetrahedron **2001**, 57, 4609-4621.

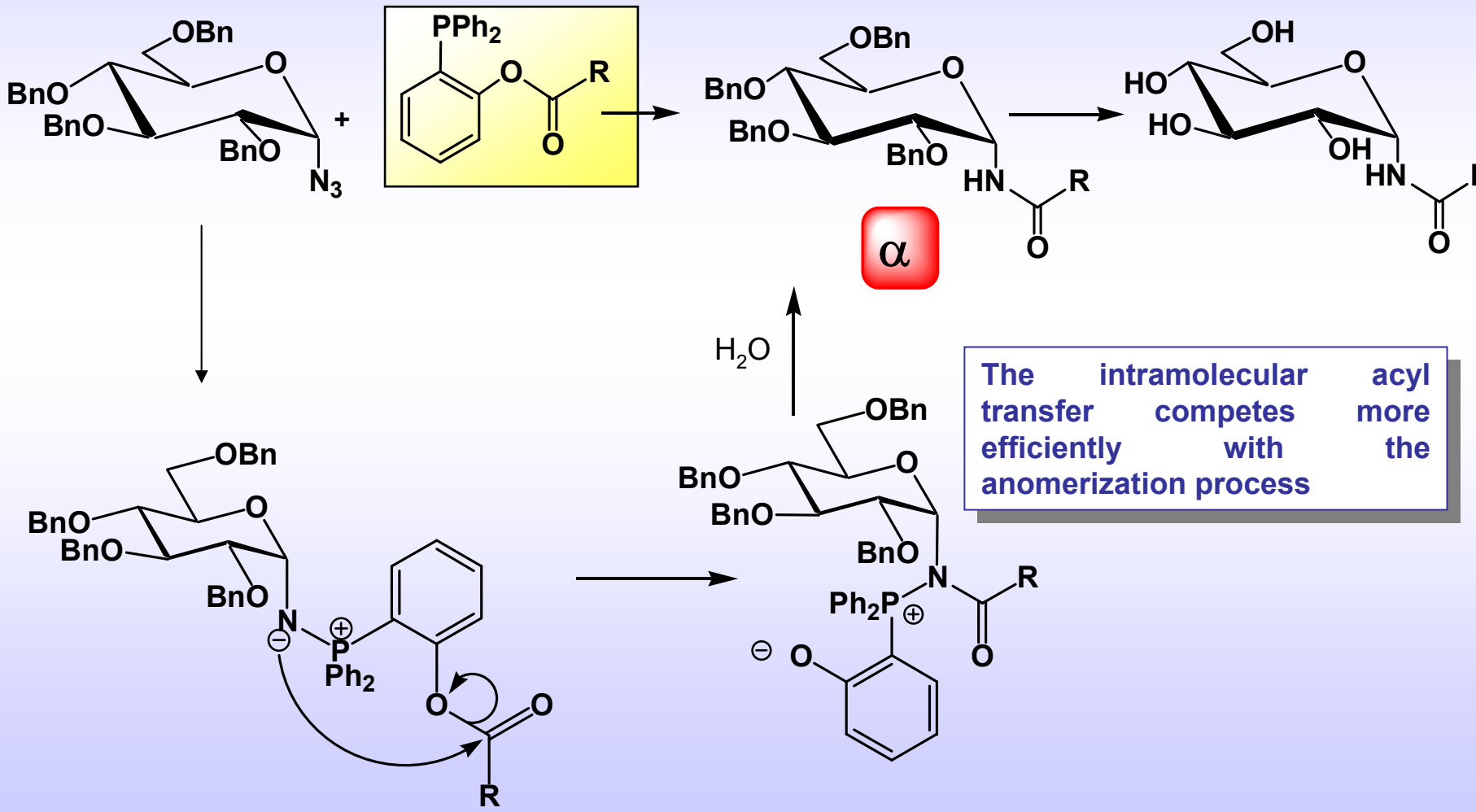
DeShong Isoxazoline Method



Damkaci, F.; DeShong, P. *J. Am. Chem. Soc.* **2003**, *125*, 4408

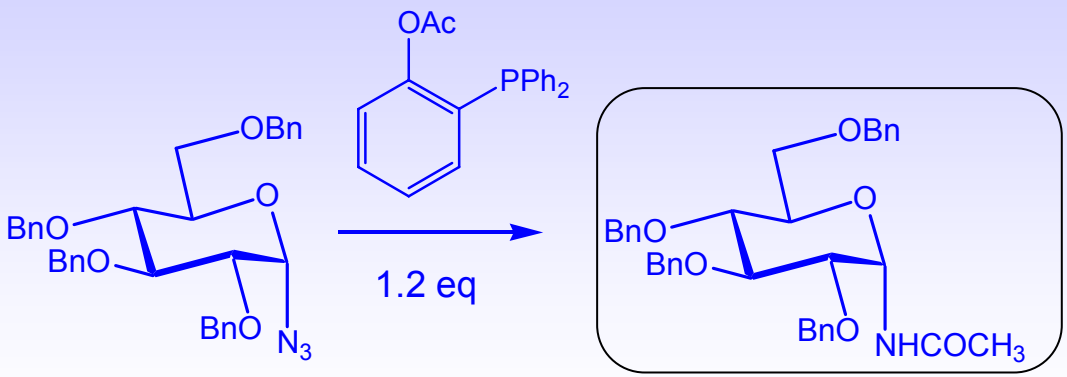
The Staudinger ligation: a general method for the synthesis of α-glycosylamides

Bianchi A., Bernardi A. *Tetrahedron Letters* **2004**, 2231; *J. Org. Chem.* **2006**, 4565



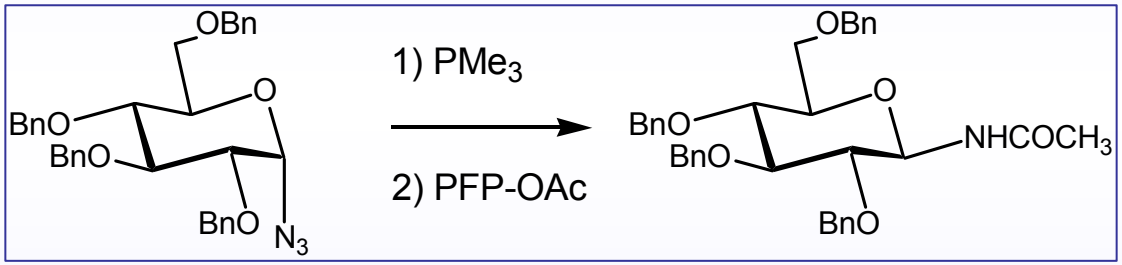
The intramolecular acyl transfer competes more efficiently with the anomerization process

Staudinger ligation: acetylation of α-glycosyl azides



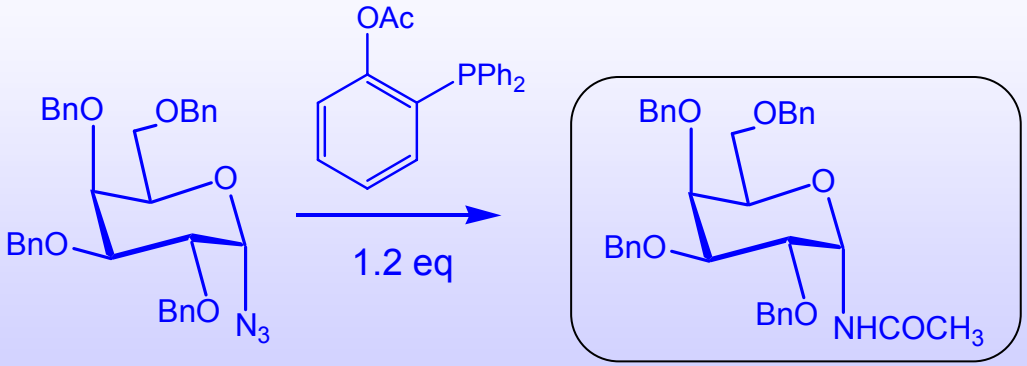
y = 77%

α only



y = 77%

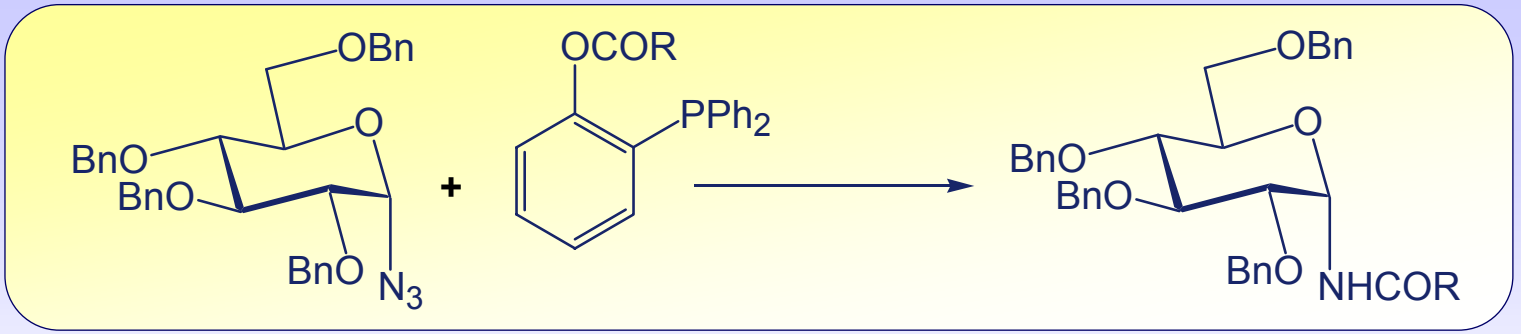
β only



y = 73%

α only

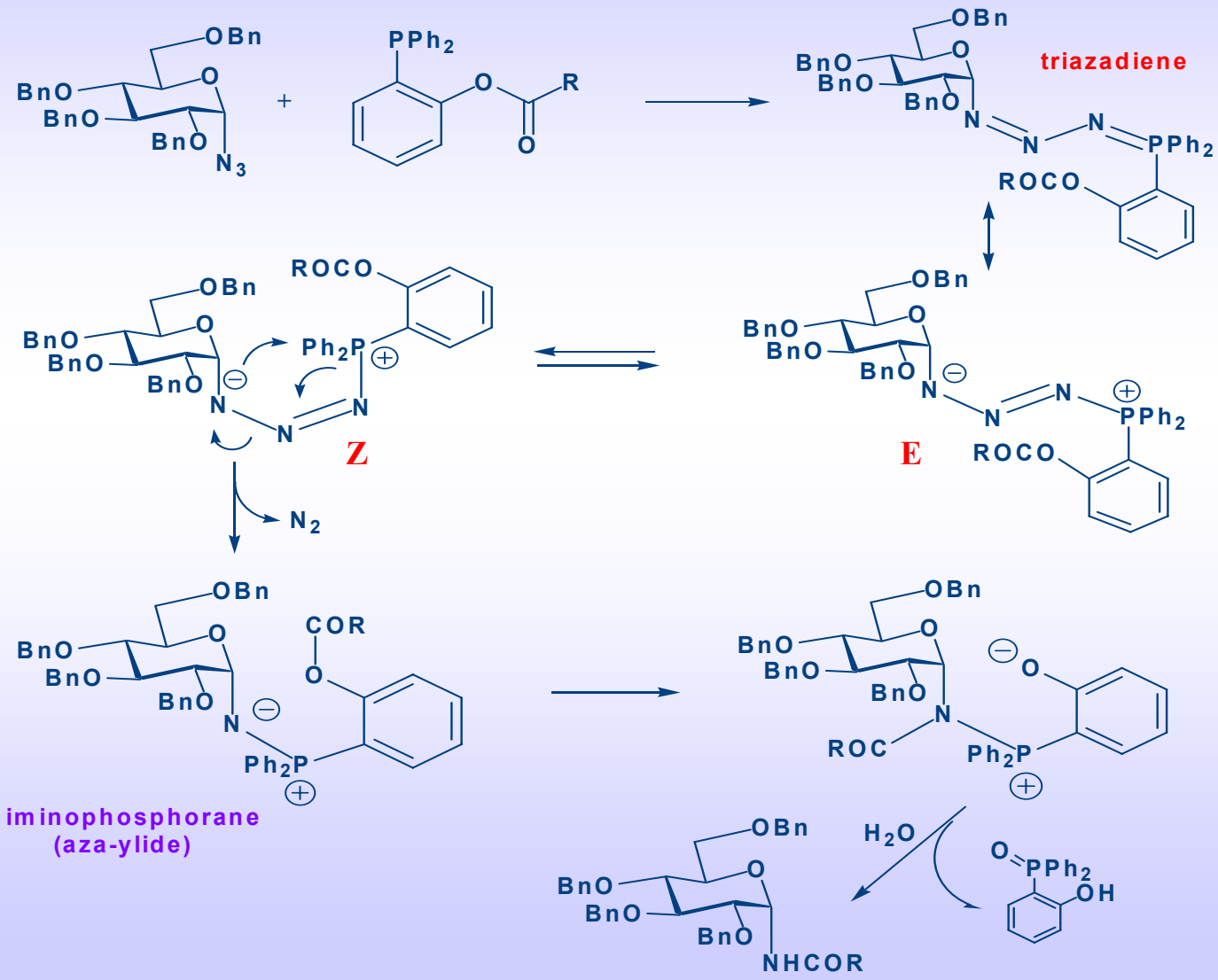
Bianchi, A.; Bernardi, A. *Tetrahedron Lett.* **2004** 45, 10, 2231-2234

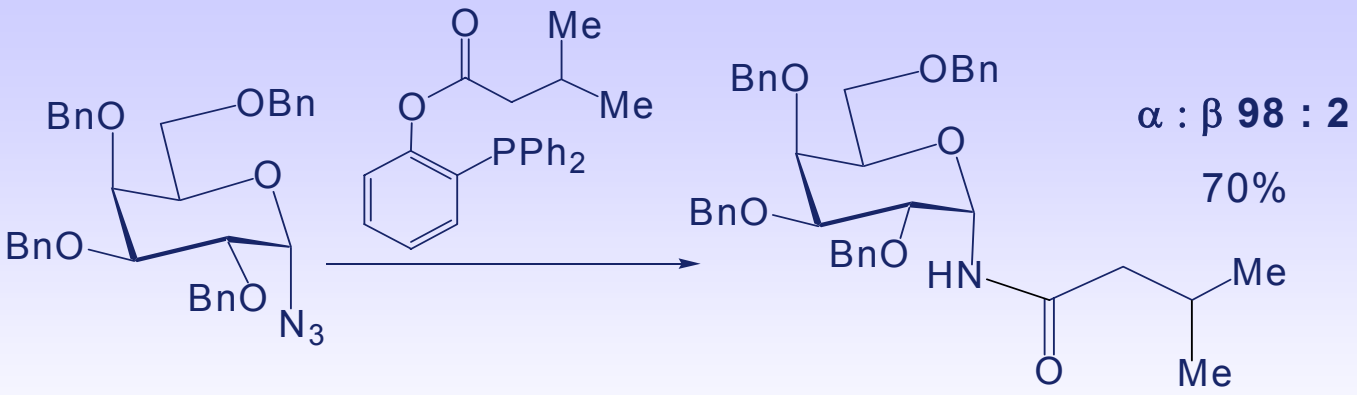


	Method A ($CHCl_3$, hν)		**Method B** (DMF)	
R =	Yield (%)	α/β ratio	Yield (%)	α/β ratio
-CH_3	**77**	**100:0**	-	-
-$(CH_2)_3CH_3$	60	84:16	**83**	**93:7**
-$CH_2CH(CH_3)_2$	70	83:17	**81**	**95:5**
-$CH(CH_3)_2$	40	84:16	**76**	**>97:3**
-$CH=C(CH_3)_2$	65	85:15	**56**	**>97:3**
-$(CH_2)_3COOMe$	-	-	**65**	**93:7**

Mechanism of the Staudinger ligation of glycosyl azides

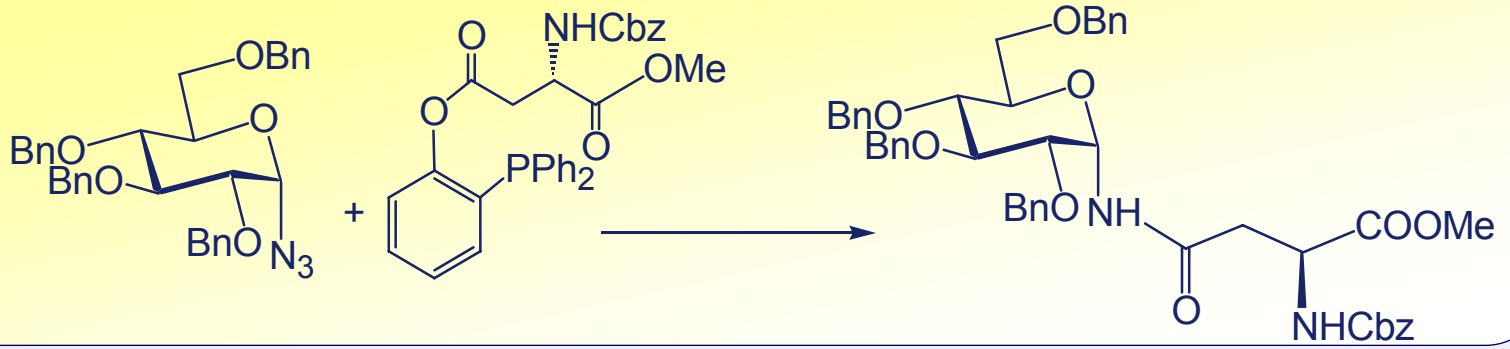
A. Bianchi, A. Russo, A. Bernardi *Tetrahedron: Asymmetry* **2005**, *16,* 381-386





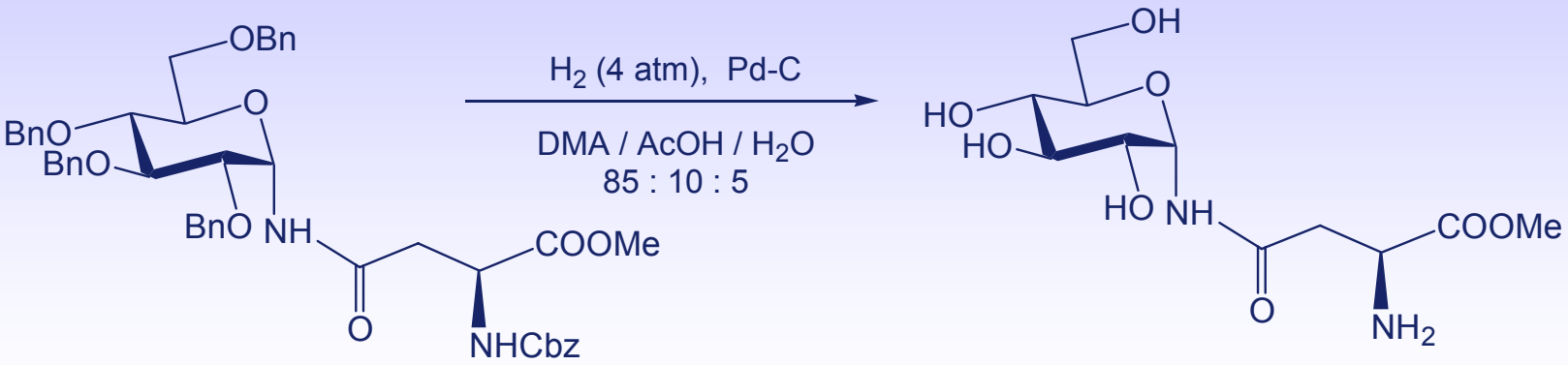
α : β **98 : 2**

70%

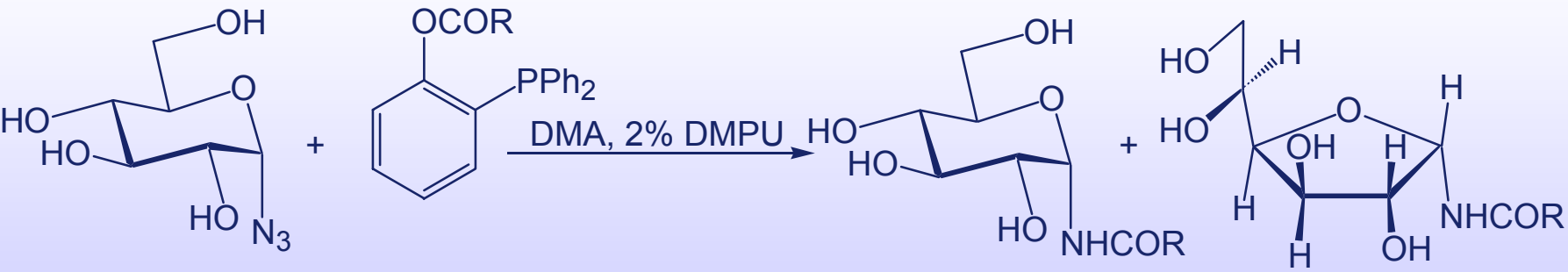


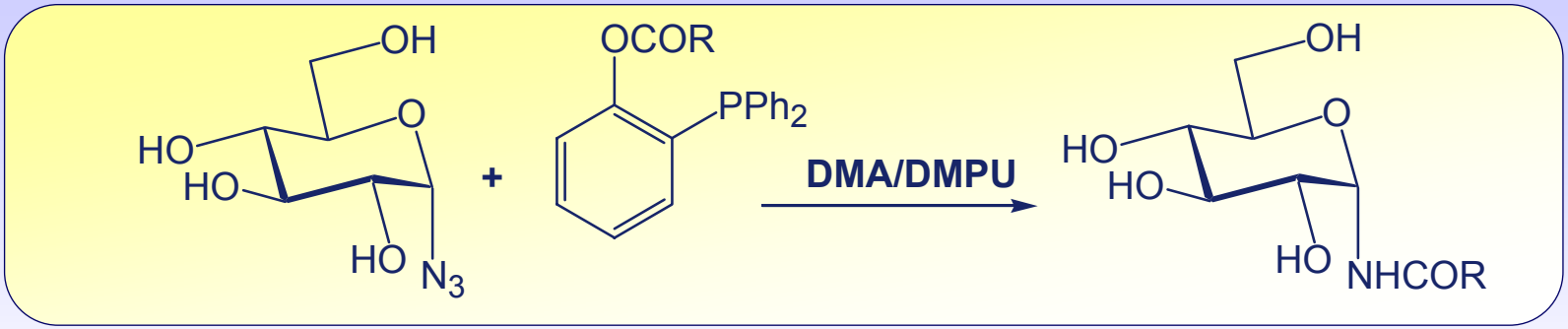
solvent	**α/β ratio**	**Yield (%)**
Toluene	**86:14**	**75***
1:3 DMA:Tol	**75:25**	**66**

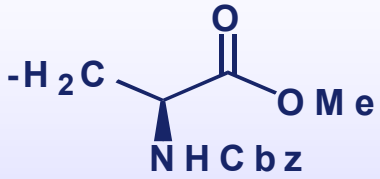
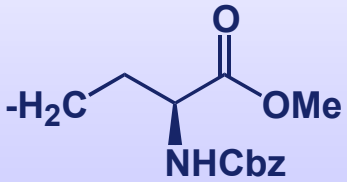
Deprotection



..or no protection

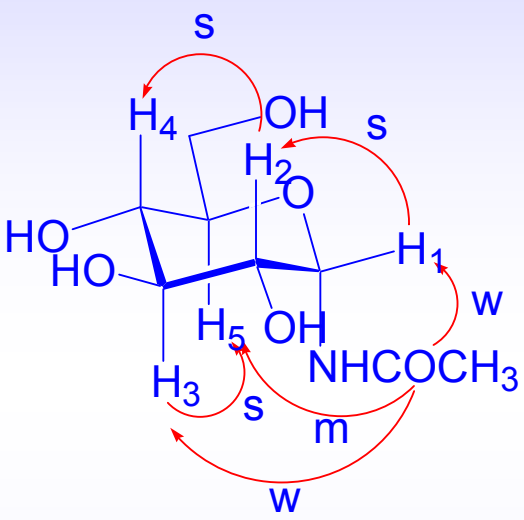


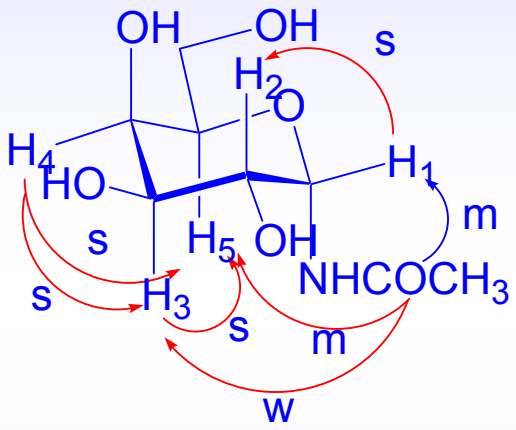


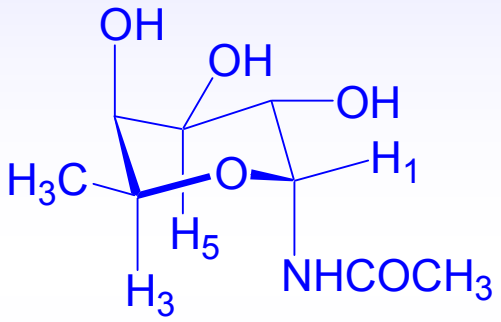
R =	Yield (%)	α/β ratio
-$(CH_2)_3CH_3$	50	≥ 98:2
-$(CH_2)_3COOMe$	56	98:2
	20	98:2
	40	≥ 98:2

Filippo Nisic

The α-glycosylamides examined so far conserve the usual chair conformation of the pyranose ring







$^4C_1$

$^1C_4$

Good mimicry of the sugar !

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