Université de Paris-Sud, Orsay
Institut de Chimie Moléculaire
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UMR CNRS 8614

Recombinant Cells and Samarium Salts in the Carbohydrate Chemist's Toolbox

Outline for this lecture **:**

- **minimize chemical steps**
- **maximize efficiency (productive bond formation)**

Through the design o

- **new strategies**
- **new reactions**

Carbohydrates: Chemical and Biological Tools

Carbohydrates and synthetic chemistry

- **Starting material in stereoselective transformations**
- **Chiral auxiliaries**
- **Chiral ligands for catalysts**

Chemical tools for glycobiology

- **Enzyme substrates (inhibitors)**
- **Immobilized ligands (epitotes) or circulating biological signals**

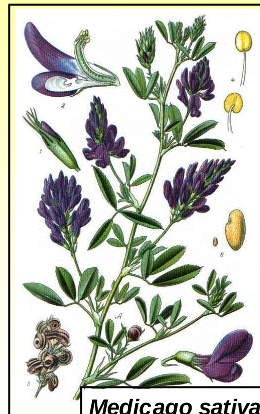
Fabaceae-Rhizobiaceae Symbiosis

• Atmospheric Nitrogen fixation (approx. 100 Mtons/year, equivalent to the industrial production of nitrogen-containing fertilizers)

• Symbiosis between
- a soil bacterium (rhizobium)
- a plant (Fabacea, or legume: pea, vetch, alfalfa, acacia, bean, soybean...)

• Traditionnally used, since the Roman Empire, for human and cattle feeding, and soil enrichment.

Rhizobia on a root hair



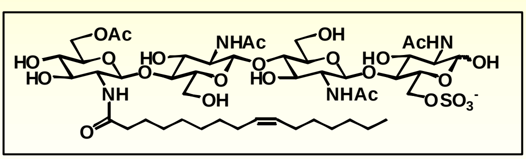
Medicago sativa
(Alfalfa)

Nod Factors

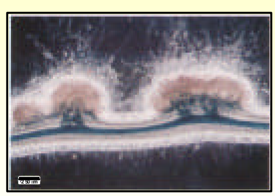
• Nod Factors are bacterial *LipoChitoOligosaccharidic* structures (LCOs) involved in the early stage of Rhizobium/Legume symbiosis.

• They induce the formation of new organs on the plant roots, called nodules, that will host the bacteria.

• The signal perception pathway is still unknown and is the subject of many biological and biochemical research.



NodRm-IV, a Nod Factor produced by *Sinorhizobium meliloti*



Synthetic Nod Factors induced nodule formation on alfalfa roots.

N. Demont-Caulet, F. Maillet, D. Tailler, J. –C. Jacquinet, J. –C. Promé, K. C. Nicolaou, G. Truchet, J. –M. Beau, J. Dénarié, ***Plant. Physiol.*, 1999, *120*, 83-92**

Chemical, Chemoenzymatic or Whole Cell Approaches to the Carbohydrate Core of the Nodulation Factors

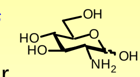
Chitin

from shellfish, squid bone,…

Controlled hydrolysis

Oligomers
(chitobiose, -triose, -tetraose,…)

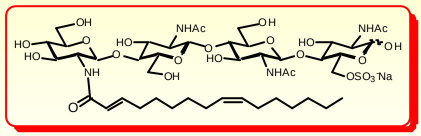
Hydrolysis



Monomer
(Glucosamine = $GlcNH_2$)

Enzymatic and chemical modification

Total synthesis



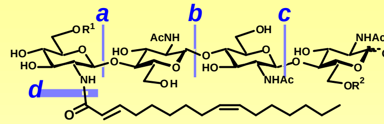
Chemical modification

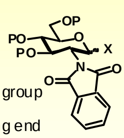
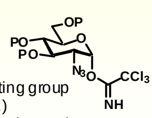
Overproducing strains of *Rhizobium*

Programmed recombinant cells (*E. coli*)

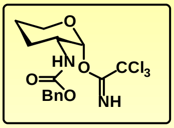
Total Synthesis: Building Blocks and Strategies

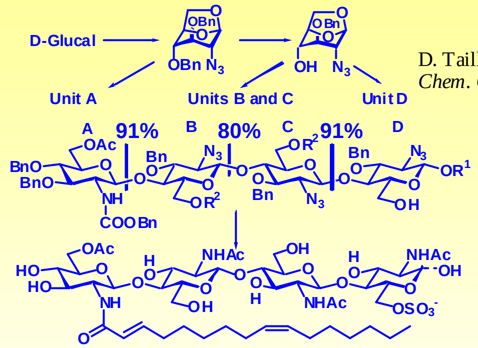
Synthetic problem: How to selectively differentiate hydroxyl and amino groups in a chitin type fragment?



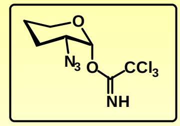
Glycosyl donor	*Key glycosylation reaction (% yield)* **a**	**b**	**c**	*Overall yield*	
Logical routes 	**50%** ⇐	**60%** ⇐	**56%**	**1%**	K. C. Nicolaou, **1992**
- Donor with a participating group at C-2 - Oligomer from the reducing end	**61%** ⇒	**72%** ⇐	**58%**	**2%**	L.-X. Wang, **1994**
Unusual route  - Donor with a *non*-participating group at C-2 (S_N2 reaction at C-1) - Oligomer from the *non*-reducing end	**91%** ⇒	**80%** ⇒	**91%**	**8%**	J.-M. Beau, **1994**

Nodule-Inducing Activity of Synthetic Nodulation Factors (NFs)





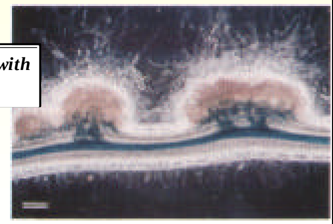
NodRm-IV (Ac, S)



D. Tailler *et al*, **1994**, *J. Chem. Soc., Chem. Commun.*, 1827-1828.

Plant treated with natural NFs

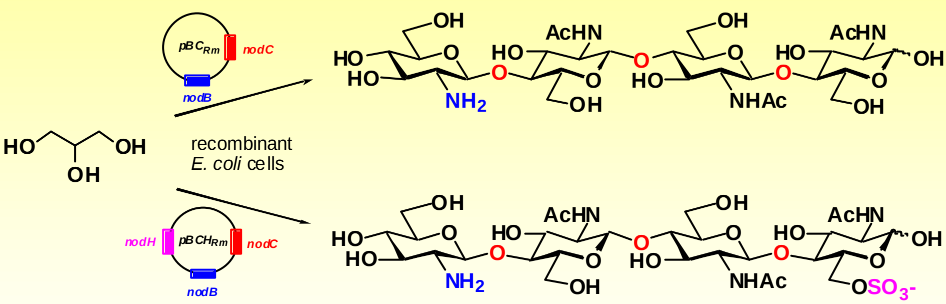
Plant treated with synthetic NFs



N. Demont-Caulet *et al*, **1999**, *Plant Physiol.*, *120* , 83-93.

Recombinant ChitoOligosaccharides

1. Plasmid construct containing the *nod* genes (and a "selection" gene - ampicillin resistant gene-)
2. High density *E. coli* cultures by selection of a good strain producer
   selection of the best carbon source

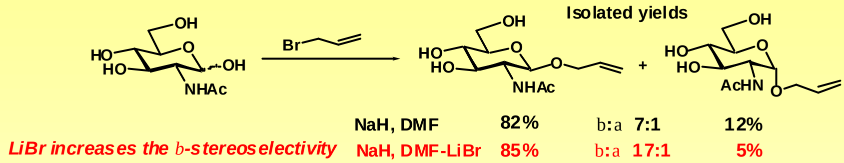


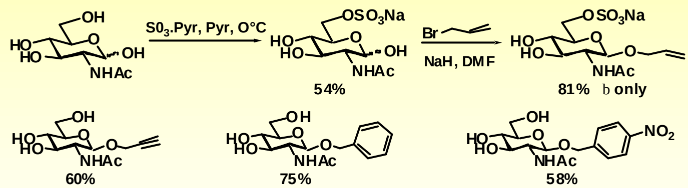
nodC: UDP-GlcNAc transferase
nodB: De-*N*-acetylase
nodH: Sulfotransferase

E. Samain *et al.*, ***Carbohydr. Res.*, 302, 1997, 35-42**
E. Samain *et al.*, ***J. Biotechnol.*, 72, 1999, 33-47**

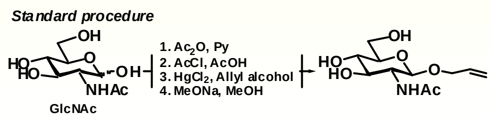
N. Riva, B. Vauzeilles

b-Selective Alkylation of Unprotected N-Acetyl Glucosamine and Chitooligosaccharides





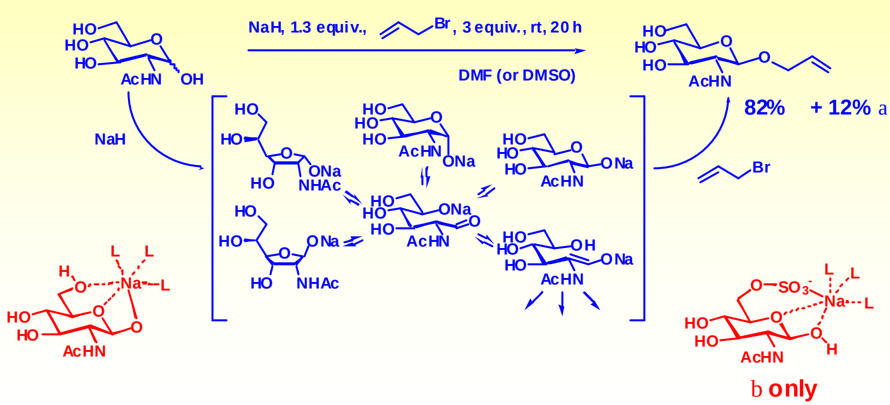
B. Vauzeilles, S. Palmier, B. Dausse, *Tetrahedron Lett.*, 2001, 42, 7567-7570



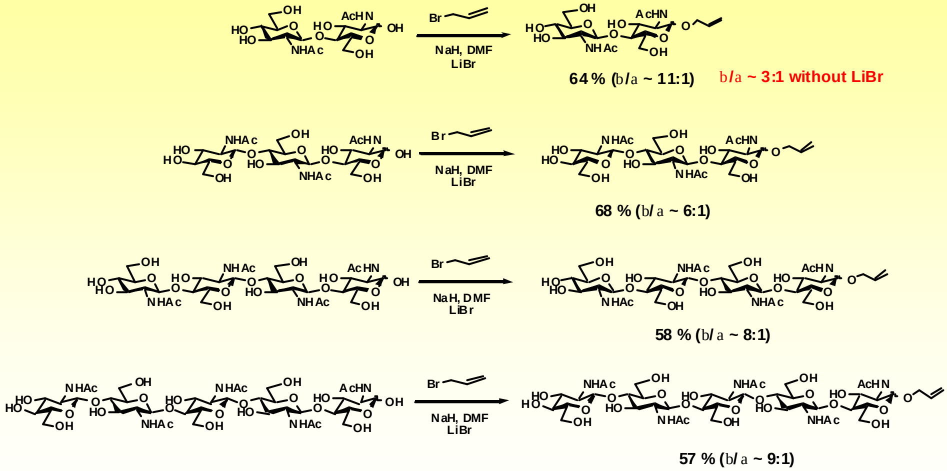
A surprisingly clean transformation (chemo-, regio- and stereoselective)
What happened to the side-reactions?

"Sugars are profoundly affected by alkalies even under very mild conditions...with isomerizations, fragmentations, and internal oxidations and reductions."

in *The Carbohydrates*, W. Pigman, Ed, Academic Press, New York, 1957, pp. 60-69
same comments in *Monosaccharides*, P. Collins and R. Ferrier, Wiley, 1995, pp. 139-144

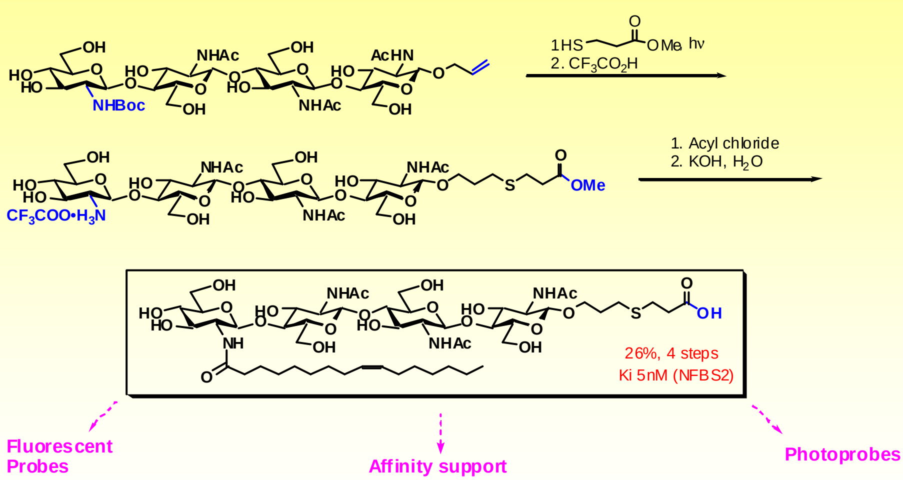


ChitoOligoSaccharides Allylation



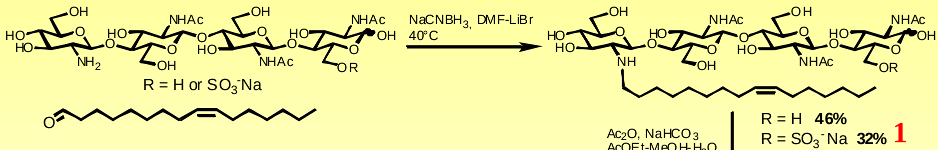
B. Vauzeilles, S. Palmier, B. Dausse, *Tetrahedron Lett.*, 2001, 42, 7567-7570

Construction of a Monofunctional Probe

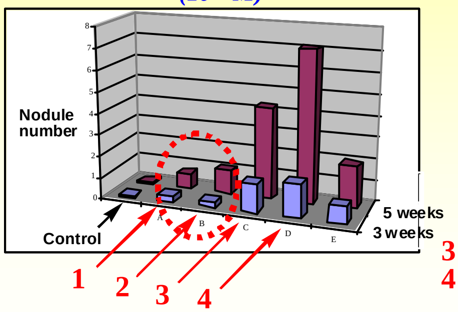


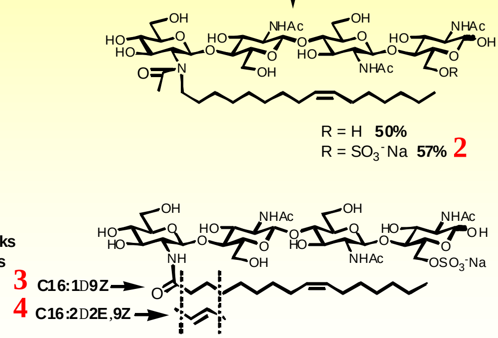
B. Vauzeilles, 2001

Synthesis of N-Alkyl Analogs



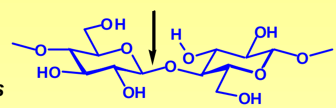
Morphogenic activity of the synthetic LCOs
(10^{-7} M)





The glycosidic bond is one of the most stable in Nature

Spontaneous cleavage of covalent bonds in biopolymers



	Half-life at 25 °C, pH 7
- Glycosidic bond in polysaccharides (cellulose)	4.7×10^6 years
- Phosphodiester bond in DNA	1.4×10^4 years
- Amide bond in proteins	4.6×10^2 years

R. Wolfenden et al, J. Am. Chem. Soc., 1998, 120, 6814.

...but is very sensitive to metabolisation

10^{17}-fold rate acceleration in the cleavage with glycosyl hydrolases

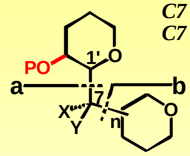
---

Drug development may be based rather on carbohydrate mimetics
- simpler synthesis
- increased chemical and enzymic stability
- more (orally) active

C1' electrophile / C7 nucleophile

C1' radical / C7 unsaturated

C1' nucleophile / C7 electrophile



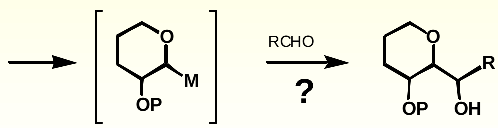
C7 electrophile / Cn nucleophile

C7 radical / Cn unsaturated

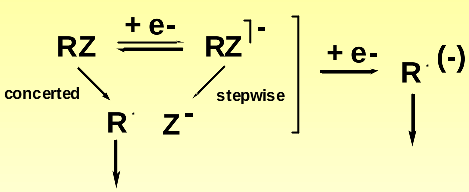
C7 nucleophile / Cn electrophile

T. Skrydstrup, B. Vauzeilles and J.-M. Beau: in *Oligosaccharides in Chemistry and Biology - A Comprehensive Handbook*, Vol. 1 B. Ernst, P. Sinaÿ, G. Hart, Eds., Wiley-VCH, Weinheim, 2000, pp. 495-530.

J.-M. Beau, B. Vauzeilles and T. Skrydstrup: in *Glycoscience: Chemistry and Chemical Biology*, Vol. 3B. Fraser-Reid, K. Tatsuta, J. Thiem, Eds., Springer Verlag, Heidelberg, 2001.



Electron Transfer and Reductive metallation

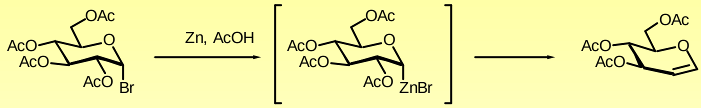


J.-M. Savéant,
Adv. Phys. Org. Chem. **2000**, *35*, 118

- electrochemistry
- photoinduced electron transfer
- radiolysis
- "homogeneous" electron donors ⟶ Organometallics by "reductive metallation"

(metals, metallic salts,...)

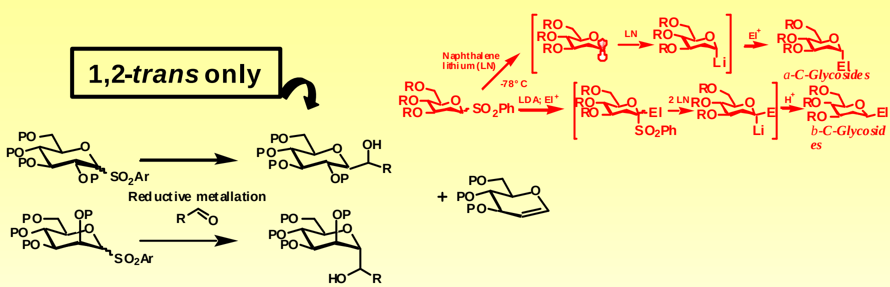
Reductive Metallation: A Long-known Process in Carbohydrate Chemistry



E. Fischer and K. Zach, *Sitzber. kgl. preuss. Akad. Wissen.* **1913**, *16*, 311

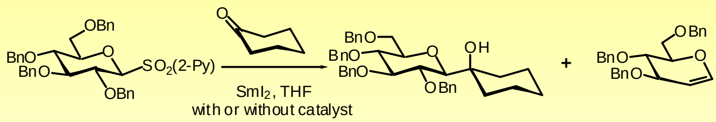
Na
K, K/graphite
Li/NH$_3$
Na and Li naphthalenide
Zn/Ag graphite
Al amalgam
Co^{II}
Sm^{II}
Cr^{II}
Ti^{III}, Ti^{IV}/Mn/TMSCl
electrochemistry

Formation of C-Glycosyl Compounds Under Barbier Conditions



SO$_2$Ph	Napththalene lithium, -78°C	0%	100%	*1985*	*Tetrahedron Lett.* **1985**, *26*, 6185; 6189; 6193;
SO$_2$Py	SmI$_2$, 25°C	50% *(gluco)* 70-90% *(manno)*	50% 0-10%	*1995*	*Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 909; *Chem. Eur. J.* **1998**, *4*, 655.
SO$_2$Py	SmI$_2$, 1% NiI$_2$, 0°C	70-95% *(gluco)* 70-90% *(manno)*	0-15% 0-10%	*2000*	Nicolas Miquel, Gilles Doisneau *Angew. Chem. Int. Ed. Engl.* ***2000***, *39*, 4111.

SmI_2-Induced C-Glycoside Formation with Catalytic Nickel at 0°C

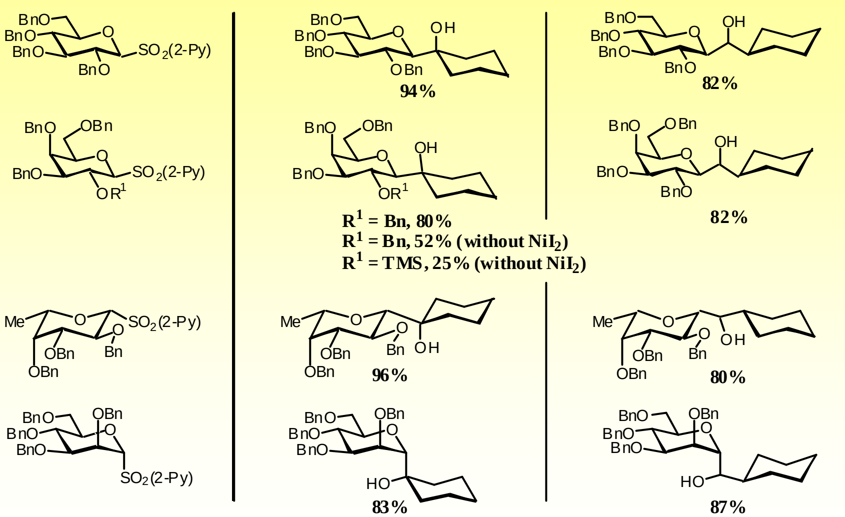


0°C	36%	22%
25°C	39%	51%
0°C, 1% NiI_2	*94%*	*not detected*
0°C, 1% $CuCl_2$	30%	64%
0°C, 1% $FeCl_3$	79%	17%

[F. Machrouhi *et al, Synlett* 1996, 633]

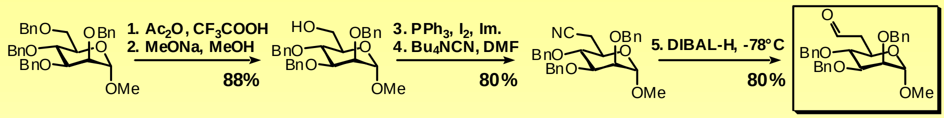
Nicolas Miquel, Gilles Doisneau
***Angew. Chem., Int. Ed. Engl.*, (39) 2000, 4111-4114.**

SmI_2-Induced C-Glycoside Formation with Catalytic Nickel at 0°C

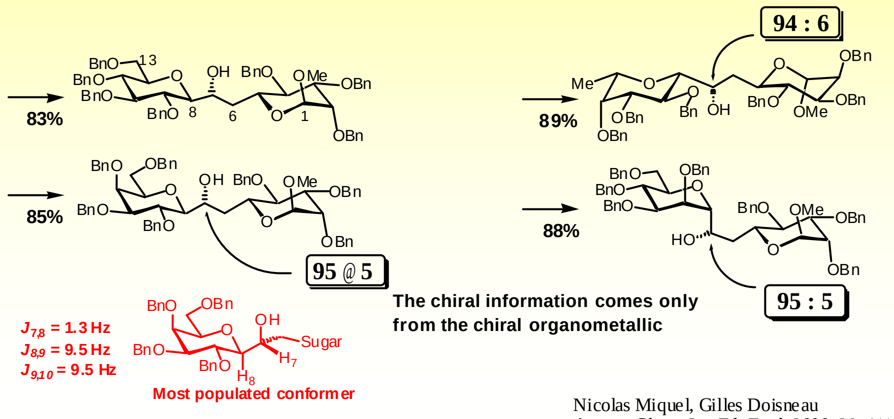


Nicolas Miquel, Gilles Doisneau
***Angew. Chem., Int. Ed. Engl.*, (39) 2000, 4111-4114.**

SmI_2-Induced C-Glycoside Formation with Catalytic Nickel at 0°C

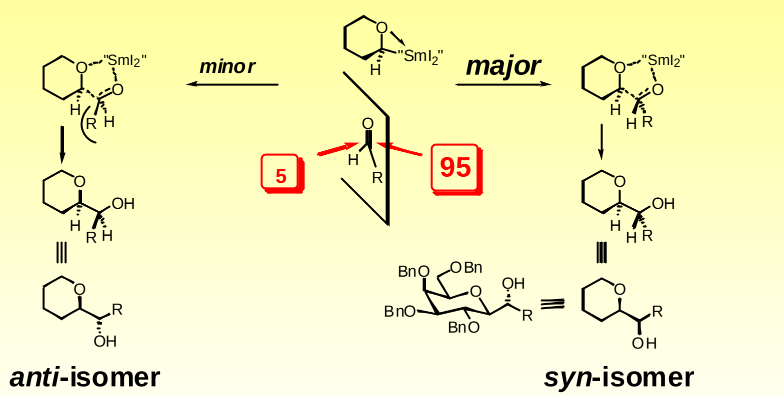


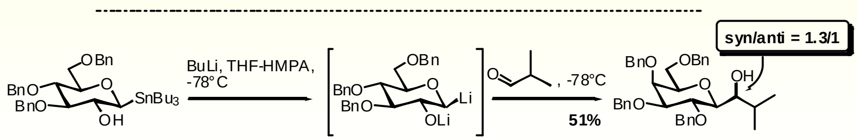
Procedure: Pyridyl sulfone (1 equiv.) with aldehyde (1.5 equiv.) in THF at 0°C under Ar;
Add SmI_2-1%NiI_2 in THF;
Workup after 0.5 h at 0°C and column chromatography.



Nicolas Miquel, Gilles Doisneau
Angew. Chem. Int. Ed. Engl. ***2000***, *39*, 4111

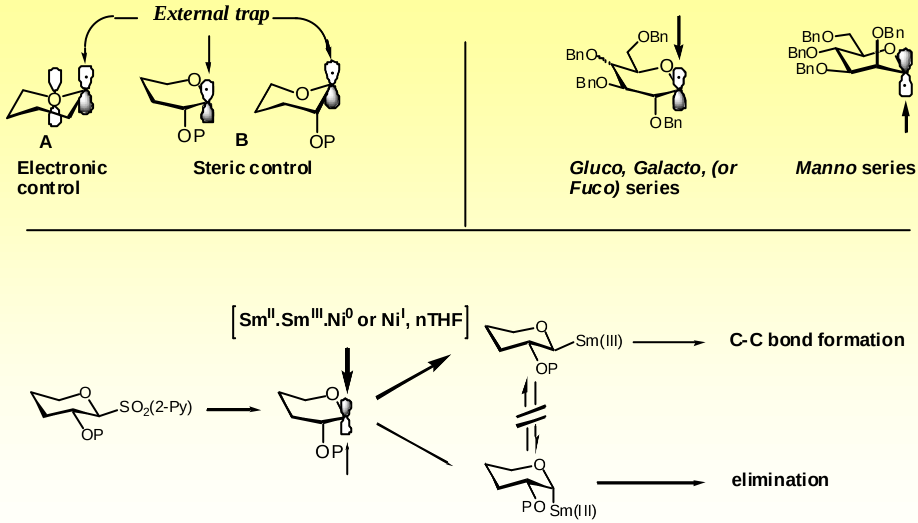
Stereochemical Consequences of the Organosamarium Asymmetry: High Diastereofacial Selectivity





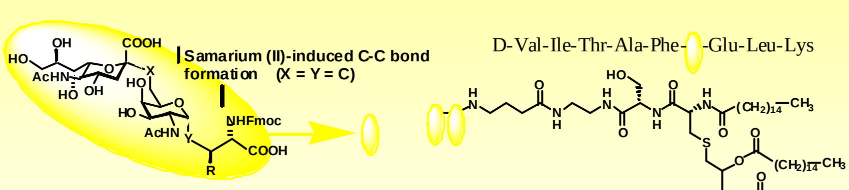
P. Lesimple, *Bioorg. Med. Chem.*, 1994, **2**, 1319

A Mechanistic Proposal for The Reductive Samariation with Catalytic Nickel



Trapping of the Intermediate Anomeric Radical Under Steric Control

Towards Building Blocks for SialylTn Mimics (for Synthetic Cancer Vaccines)



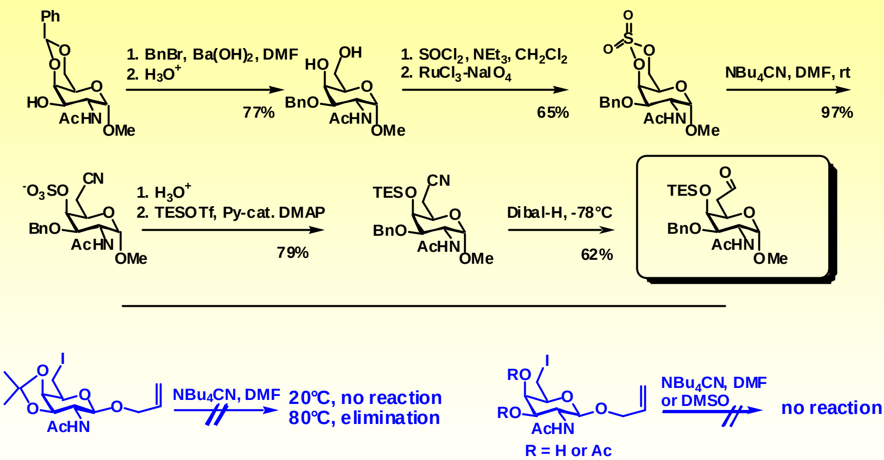
R = H ou CH_3; X = Y=O; SialylTn Antigen

R = H ou CH_3; X = CH_2 or fonctional C ; Y = CH_2

R = H ou CH_3; X = CH_2 or fonctional C ; Y = O

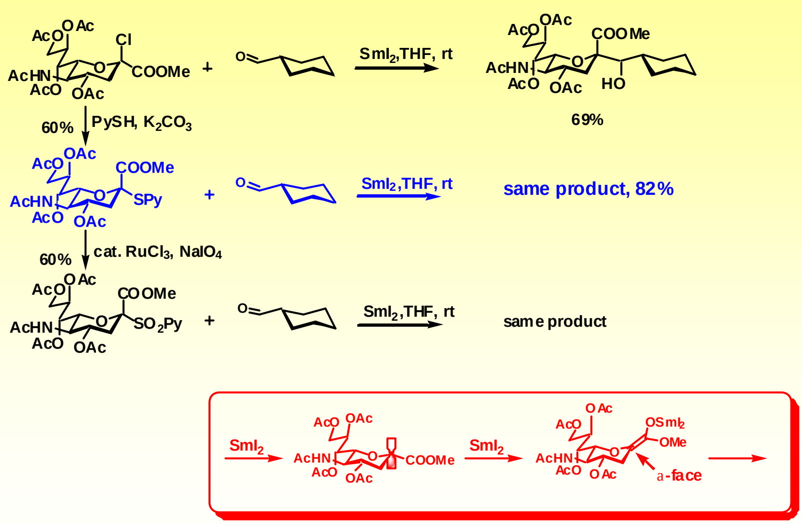
Z. Abdallah, G. Doisneau

Towards Building Blocks for SialylTn Mimics



Z. Abdallah, G. Doisneau, 2001

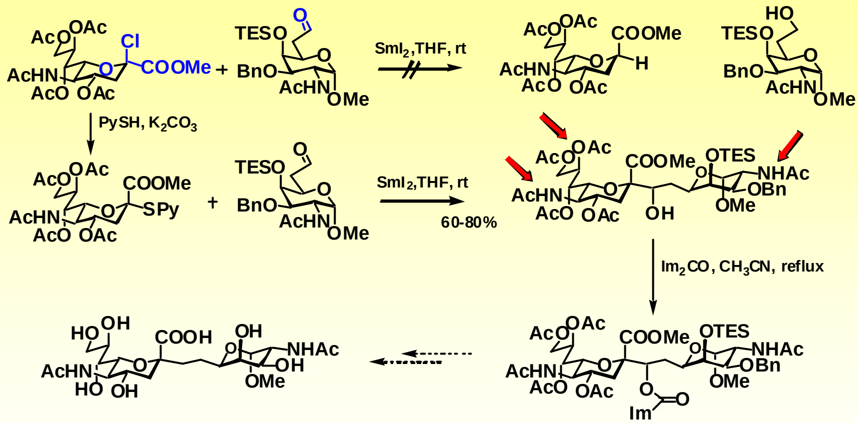
Towards Building Blocks for SialylTn Mimics



R. J. Linhardt et al, J. Am. Chem. Soc., 1997, 119, 1480
Carbohydr. Res., 1998, 308, 161

Z. Abdallah, G. Doisneau, 2002

Towards Building Blocks for SialylTn Mimics



Z. Abdallah, G. Doisneau, 2002

Synthesis of C-Glycosyl Compounds by Reductive Samariation of Anomeric 2-Pyridyl Sulfones

2-Pyridyl Sulfone derived from		*Stereoselectivity*		*Typical yield*
		a	b	
D-glucopyranose	**(with cat. NiI_2)**	**0**	**1**	**70-95 %**
D-galactopyranose	**(with cat. NiI_2)**	**0**	**1**	**80%**
D-mannopyranose		**1**	**0**	**70-90 %**
L-fucopyranose	**(with cat. NiI_2)**	**0**	**1**	**80-90%**
D-mannofuranose		**1**	**0**	**75-95 %**
2-acetamido-2-deoxy-D-glucopyranose		**4-3**	**1**	**50-80 %**
2-acetamido-2-deoxy-D-galactopyranose		**20-3**	**1**	**60-85 %**
N-acetylneuraminic acid		**1**	**0**	**80-90 %**
KDN		**1**	**0**	**85-96 %**

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UMR 8614

Bacterial Nodulation Signals
A. Burgard
B. Dausse
F.-Y. Dupradeau
S. Palmier
N. Riva-Grenouillat
B. Vauzeilles

C-Glycosyl Mimics (Sm^{III} chemistry)
Z. Abdallah
C. Bouvier
G. Doisneau
M. Elmouchir
O. Jarreton
D. Mazéas
N. Miquel
P. Riant
T. Skrydstrup
D. Urban

O-*Glycosides*
J.-M. Duffault
C. Spérandio-Leclercq
A. Valade

Aminyl Radicals
F. Garro-Hélion
M. Toffano

Recombinant E. coli
H. Driguez, E. Samain
CERMAV, Grenoble

Biological Studies
J.-J. Bono, J. Dénarié, J. Cullimore
LBMRPM, Castanet-Tolosan

Structural Studies
J. Jiménez-Barbero
Inst. de Quim.Organica, Madrid

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Aventis CropScience 1998-2001