Reconsidering Glycosylations at High Temperature: Precise Microwave Heating

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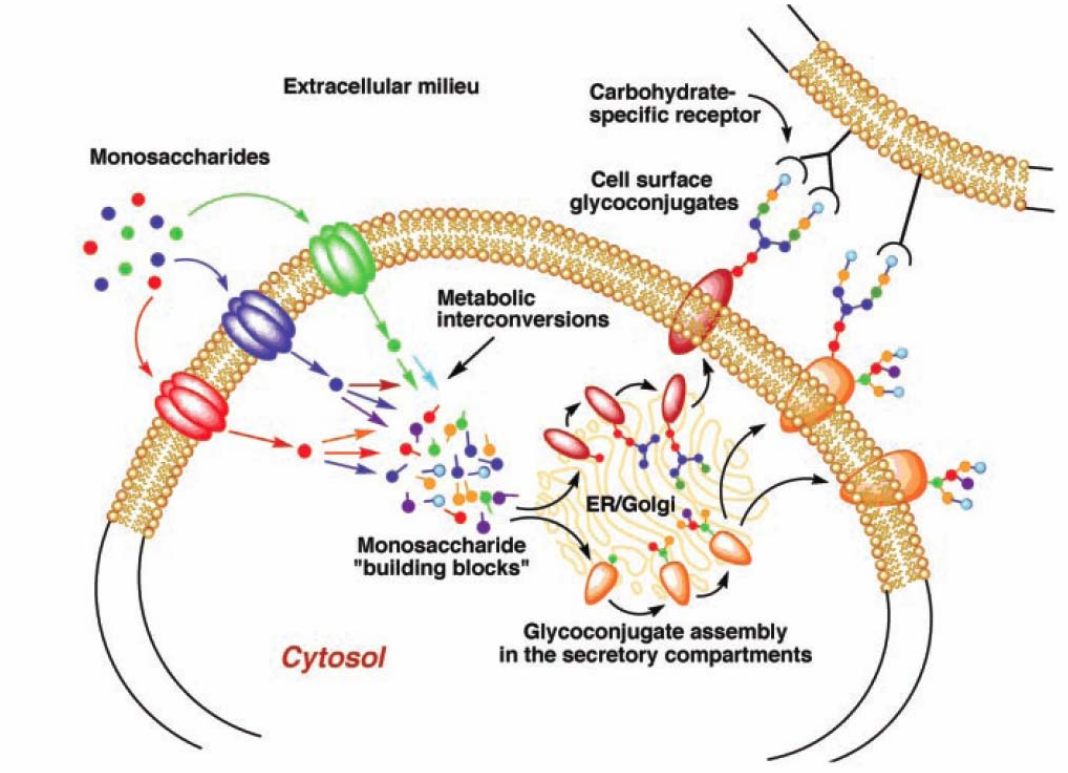
Glycoconjugates: Complex structures



Synthetic oligosaccharides and glycoconjugates provide materials for correlating structure with function.

Synthetic mimics of the complex assemblies found on cell surfaces can modulate cellular interactions and are under development as therapeutic agents.

C.R. Bertozzi & L.L. Kiessling *Science* 2001 **291**, 2357

Glycoconjugates; N- & *O*-linked saccharides

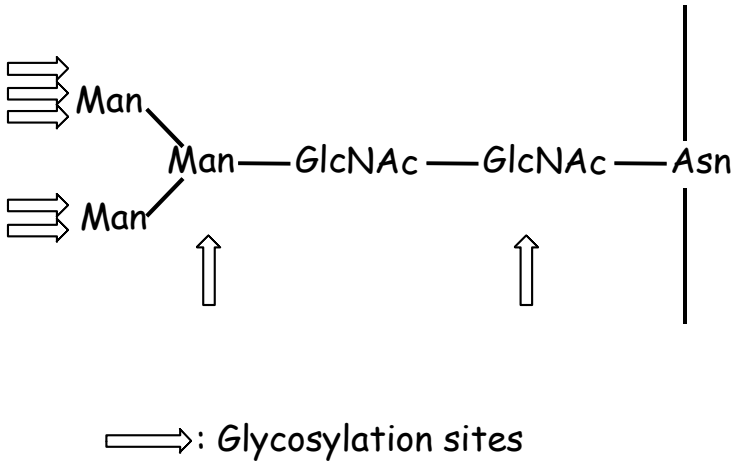
Glycoproteins

Glycosylation is the most abundant form of post-translational modification of proteins.
In eukaryotic systems more than 50% of all proteins are glycosylated.
Most noncytosolic proteins are glycosylated where the patterns of glycosylation are very diverse

A. Lohse *et al Angew. Chem. Int. Ed.* 2006, □**45**, 4167
R. Apweiler *et al Biochim. Biophys. Acta 1999, **1473**, 4*

N-linked saccharides

N-linked oligosaccharides of glycoproteins are mostly complex and branched molecules.
First *N*-acetylglucosamine is linked to a protein via the side-chain N of an Asn residue in a 3-amino acid sequence (Asn-X-Ser/Thr).
3 subgroups of *N*-linked all contains a pentasaccharide core.

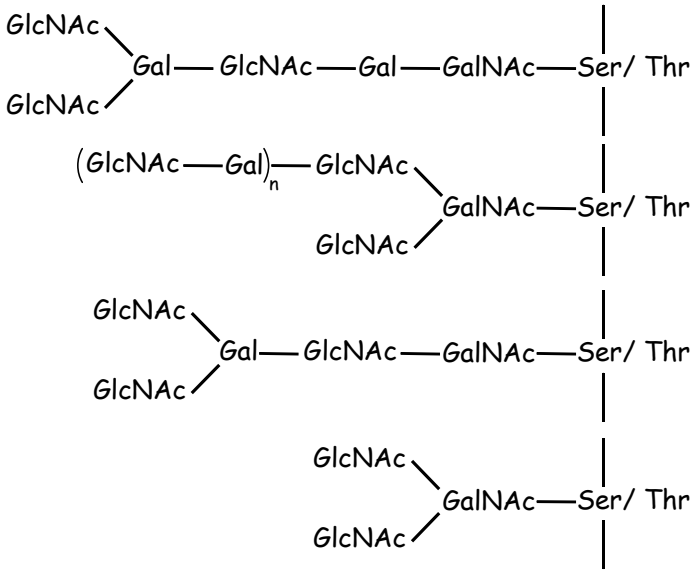


⟹: Glycosylation sites

O-linked saccharides

O-linked oligosaccharide chains of glycoproteins vary in complexity.

They link to a protein via a glycosidic bond between a sugar residue & a Ser or Thr OH. Their functions covers recognition, interaction, and enzyme regulation.



4 typical core structures found in *O*-linked glycans

D.F. Wyss & G. Wagner, *Curr. Opin. Biotech.* 1996, **7**, 409

Biological role of oligosaccharides

Structure

- Proteoglycans & collagens (tissue structure, integrity & porosity)
- Plant cell walls (cellulose, pectins)
- Mucopolysaccharides (bacterial cell walls)
- Chitin (skeletons of arthropods)

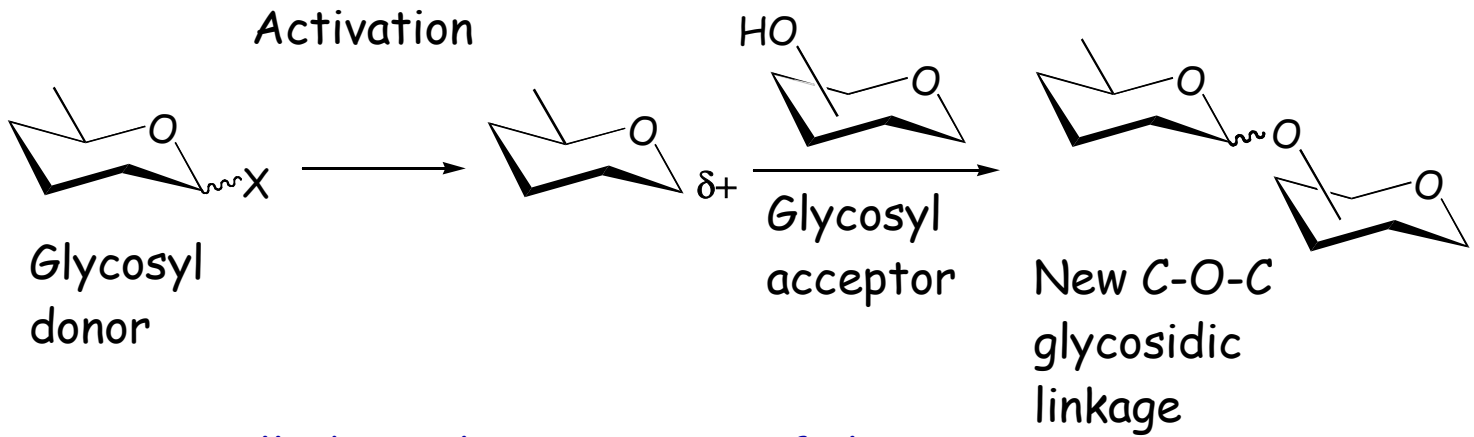
Energy storage

- Glycogen
- Starch

Other functions

- Involvement in protein folding
- Protein/substance solubility
- Transport

O-Glycosylations



The rate is controlled by the reactivity of the carbocation

Reactivity

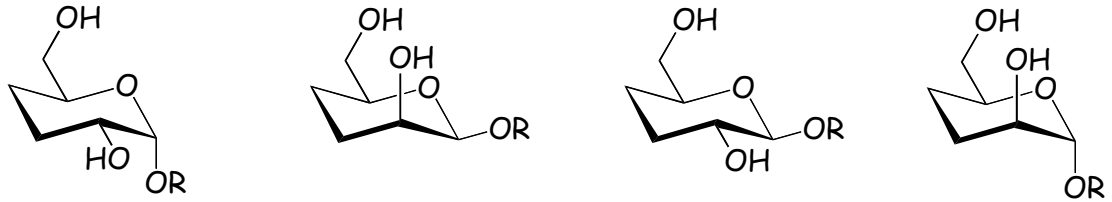
- Temperature
- Activating / deactivating protecting groups
- Nucleophilicity of acceptor OH

Stereoselectivity

- Neighbouring group participation (1,2-*trans*)
- Solvent - solvation of carbocation
  - participation in glycosylation
- Torsionally deactivated donors

Difficult glycosidic bonds

4 typical glycosidic bonds seen here for D-pyranoses



α-D-Gluco, galacto β-D-Manno β-D-Gluco, galacto α-D-Manno

DIFFICULT (1,2-cis) EASY (1,2-trans)

1,2-*trans* products can be achieved via anchimeric assistance (ester in 2-pos.)

1,2-*cis* products can be achieved using the anomeric effect (reduced overlap of orbital backlobes)
Difficult partly because of interference from pos. 2

Glycosylation under mild conditions and compatibility with microwave promotion?

Control of stereochemistry :

- Avoid use of Lewis acid promotors
  - Towards S_N2 type mechanisms
  - Diminish side-reactions and cleaner reaction mixture
  - Possibility to carry glycosylations out at r.t or higher
  - Better options for combinatorial approaches
- Compatibility with microwaves to accelerate the rate of glycosylation at high temperatures
- Universal glycosylation conditions?

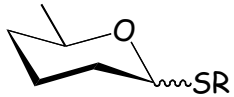
Review: K. J. Jensen, *J. Chem. Soc., Perkin Trans. 1*, 2002, 2219

Different types of glycosyl donors

X
F
Cl
Br
I

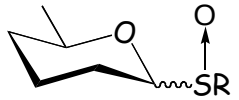
Glycosyl Halides

Trichloro-acetimidates



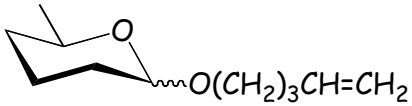
R = alkyl, (hetero)aryl

Thioglycosides

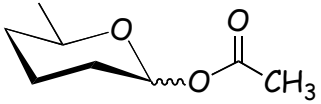


R = alkyl, aryl

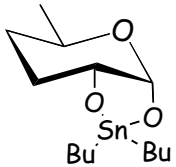
Glycosyl sulfoxides



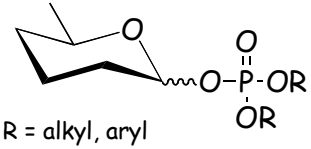
Pentenyl glycosides



Glycosyl acetates

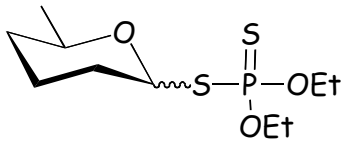


Stannylene acetals

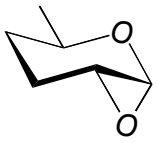


R = alkyl, aryl

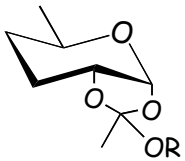
Glycosyl phosphates



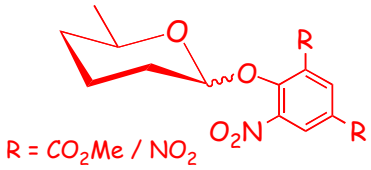
Phospho-dithioates



Glycal epoxides



Orthoesters

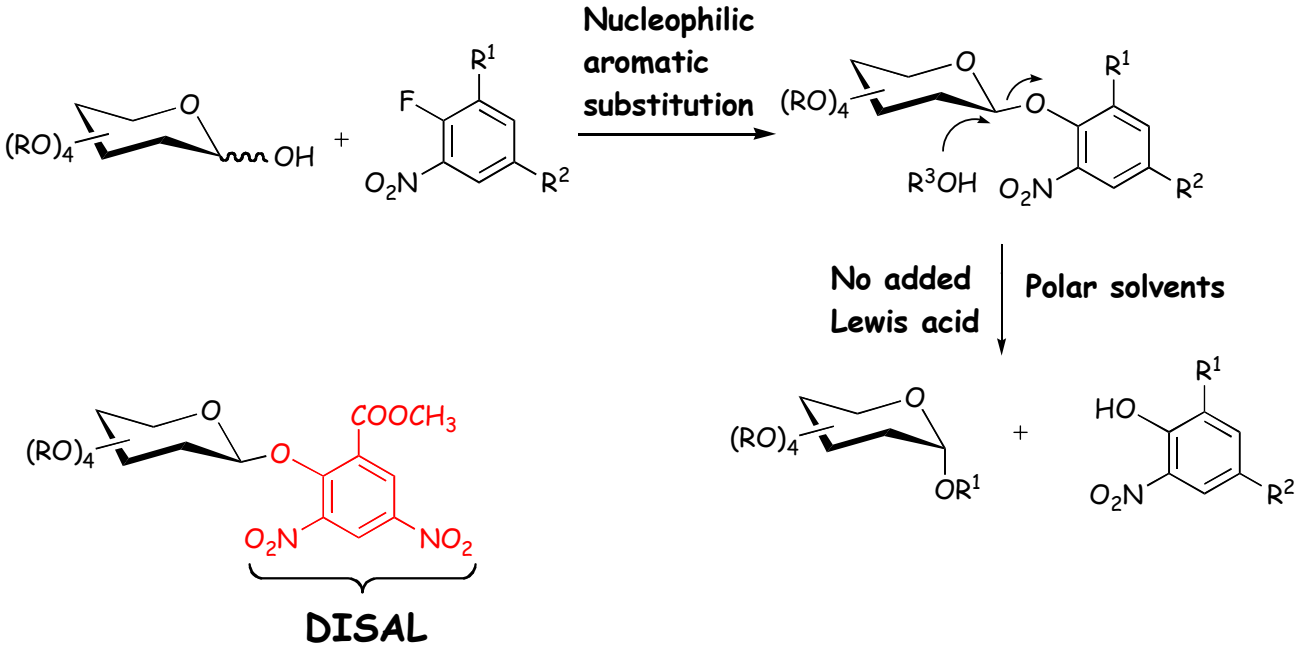


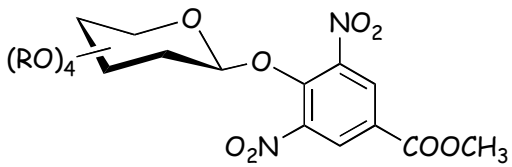
DISAL glycosides

DISAL Chemistry

DISAL: DInitroSALicylate

Concept



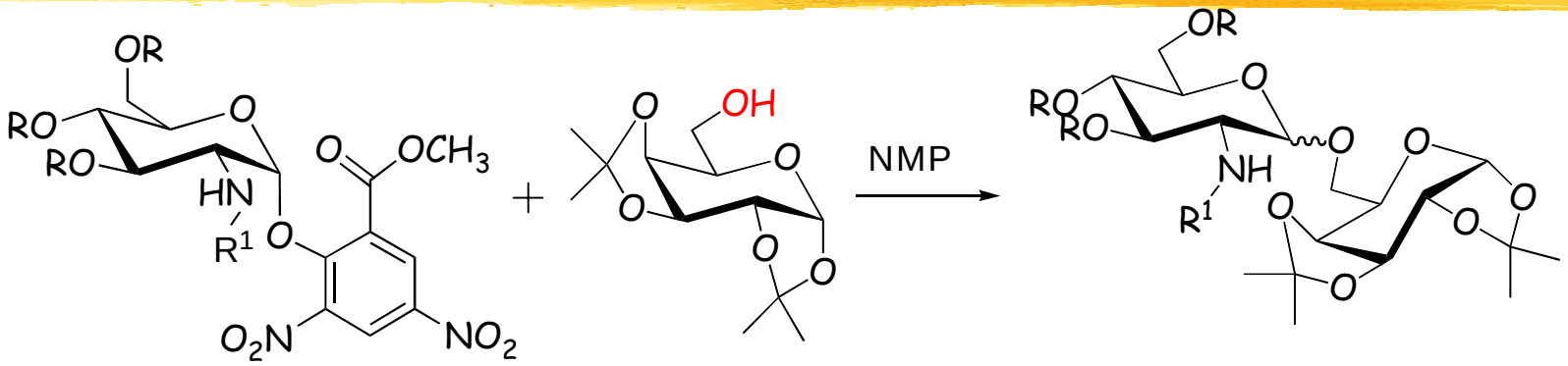


Properties of DISAL chemistry

- No or only weak activation is required
- Proceeds at 40°C or higher
- Stereospecific glycosylation of simple alcohols such as MeOH

L. Petersen & K.J. Jensen, *J.Org.Chem.* 2001, **66**, 6268

Glycosylation with GlcN DISAL



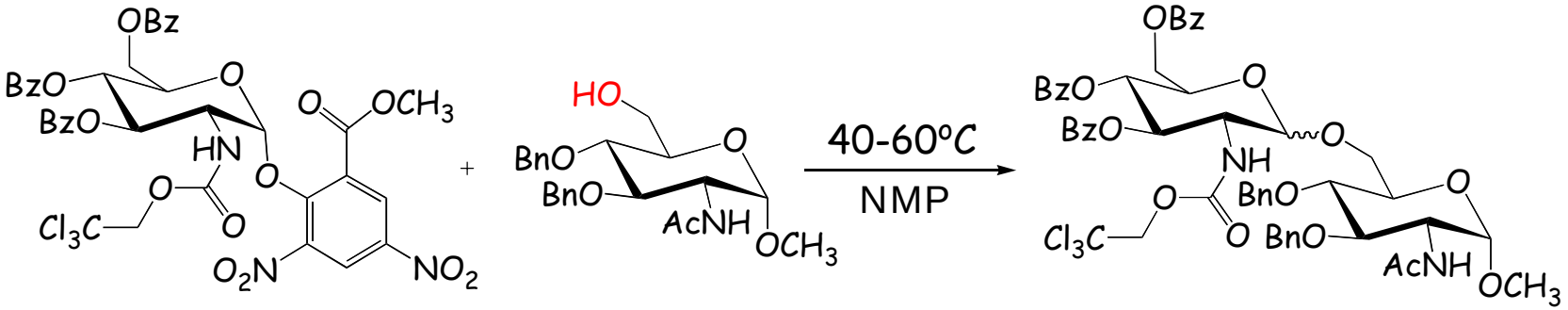
10 R: Ac; R^1: Troc
11 R: Bz; R^1: Troc

12 R: Ac; R^1: Troc
13 R: Bz; R^1: Troc

DISAL donor	Temp/ time	Eq. Donor	Glycoside	α/β ratio	Yield [%]
10	40°C/ 18 h	1.5	**12**	1 : 1	71
11	40°C/ 18 h	1.5	**13**	1 : 7	63
11	40°C/ 24 h then 60°C / 2h	1.5 1.5	**13**	1 : 6	85

S. Grathe *et al.*, *Tetrahedron: Asymm.* 2005, **16**, 1439

Glycosylation with Bz_3GlcN DISAL



2 x 1.5 equiv. **11** **14** **15**

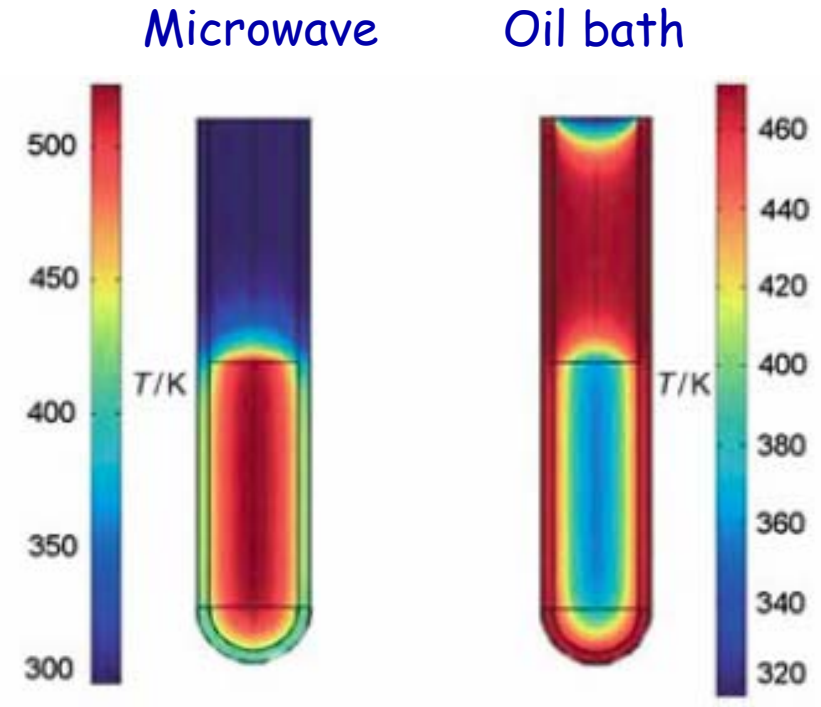
60%; α/β 1 : 4

But ... glycosylation of *sec.* OH only gave sluggish reactions

Therefore microwave heating was used to accelerate glycosylations

S. Grathe *et al.*, *Tetrahedron: Asymm.* 2005, **16**, 1439

Microwave Acceleration



O.Kappe, *Angew. Chem. Int. Ed.* 2004, **43**, 6250

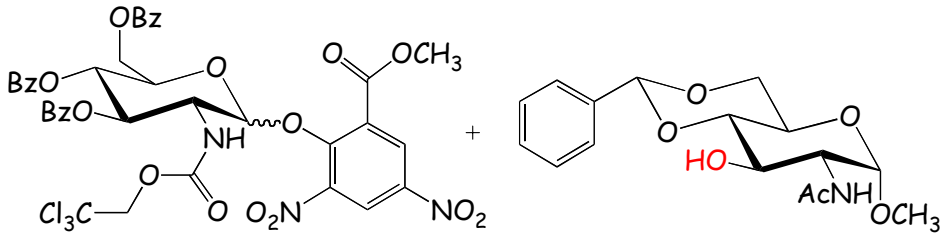
Oil bath :
(or other external heat source)
Temperature of the reaction vessel is higher than the reaction mixture

Thus a heterogeneous form of heating

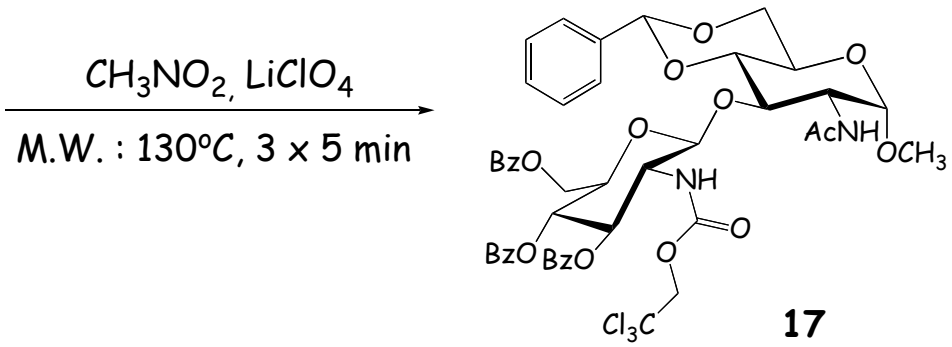
Microwave :
Produces internal heating (solvents, reagents, catalysts)
Reaction vessel is microwave transparent
Inverted temperature gradient

- reduces chemical reaction times
- increase yields
- reduces side reactions

Bz_3GlcNTroc DISAL glycosylation at sterically hindered *sec.* OH



3 x 1.5 equiv **11** **16**



17

38%

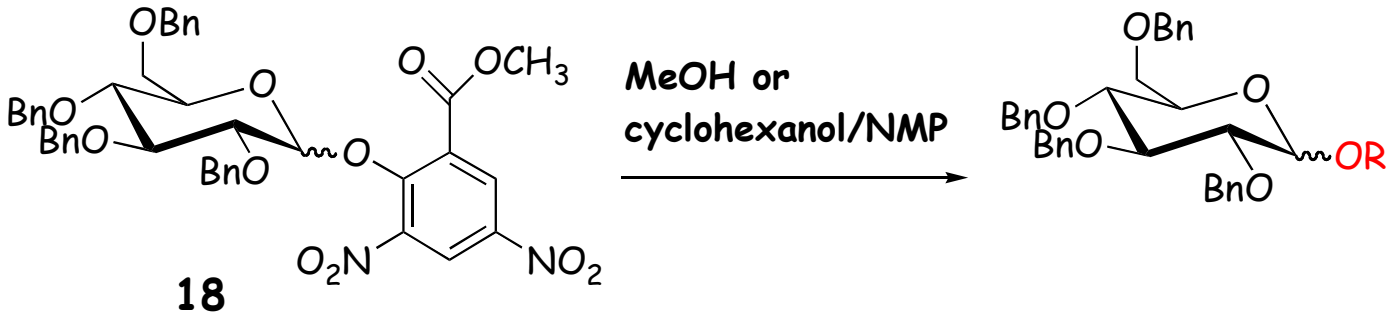
Reaction conditions :

- $LiClO_4$ added to absorb microwave heating & promote the reaction
- Reaction vial is closed during reaction
- Triple coupling with donor

S. Grathe *et al.*, *Tetrahedron: Asymm.* 2005, **16**, 1439

General glycosylation at high temperature

Initial studies using Bn_4Glc DISAL :

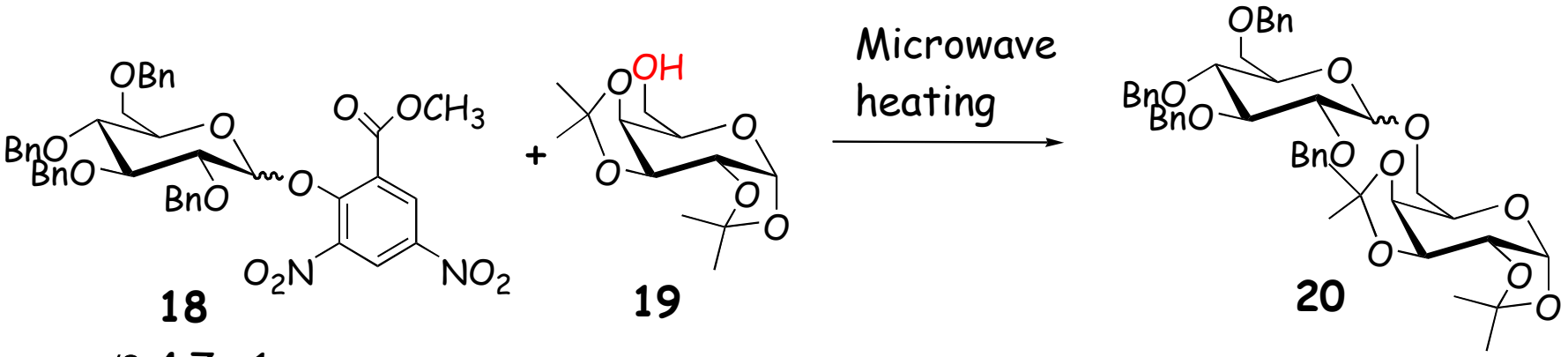


18

Donor α/β	Acceptor	Microwave	Yield (%) α/β
18 4.7 : 1	MeOH	100°C; 5 min	91 1 : 4.4
18 4.7 : 1	Cyclohexanol	100°C; 5 min	71 2.1 : 1

K. Larsen *et al.*, *Org. Biomol. Chem.* 2005, **3**, 3966

Microwave glycosylation of primary OH

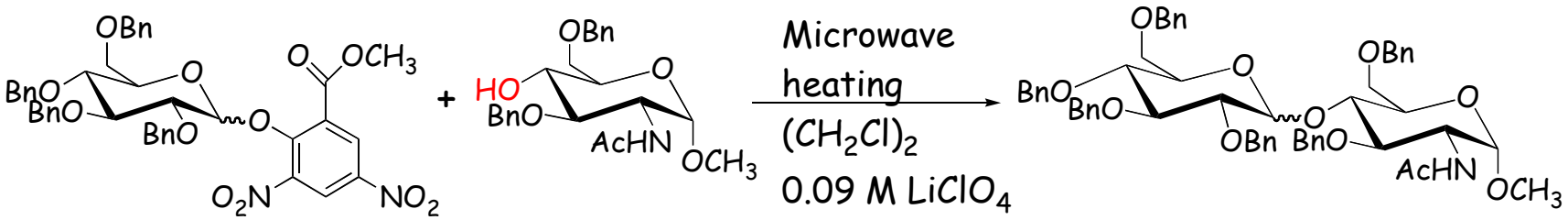


Activator, Solvent	Microwave	Yield; (α/β)
NMP	130°C, 40 min	60% (1.9:1)
0.09 M $LiClO_4$, $(CH_2Cl)_2$	100°C, 30 min	97% (2:1)

K. Larsen *et al.*, *Org. Biomol. Chem.* 2005, **3**, 3966

Microwave glycosylation of secondary OH

Glycosylation of the very poor nucleophile (acceptor) **21**



General conditions for activated donor systems :

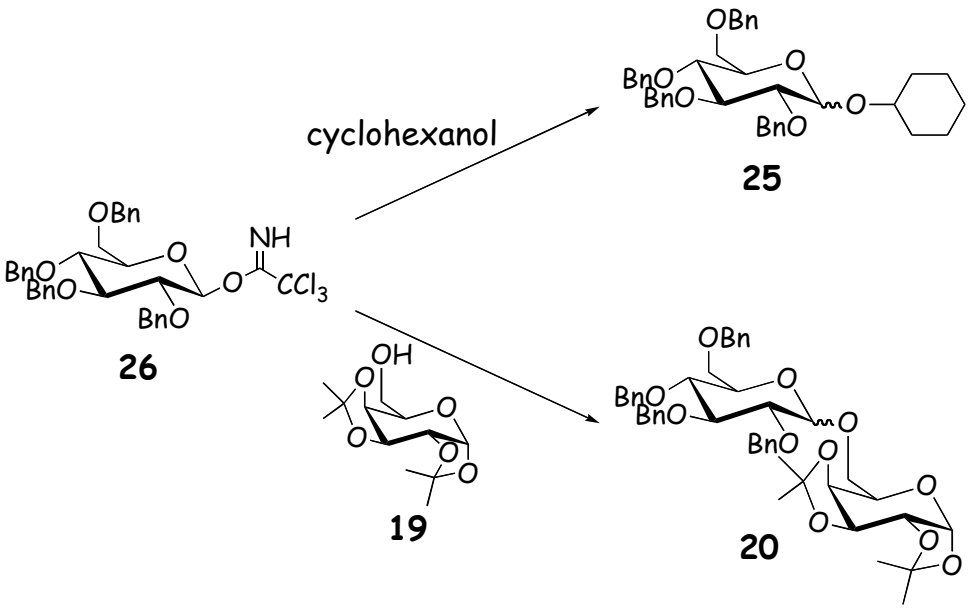
- NMP; no activator added; M.W. 130°C
- $(CH_2Cl)_2$ or CH_3NO_2;
  0.09 M $LiClO_4$; M.W. 100°C

Conditions & results :

Microwave	Yield;	(α/β)
100°C, 40 min	72%	(4:1)

K. Larsen *et al.*, *Org. Biomol. Chem.* 2005, **3**, 3966

Trichloroacetimidate & high temperature glycosylations



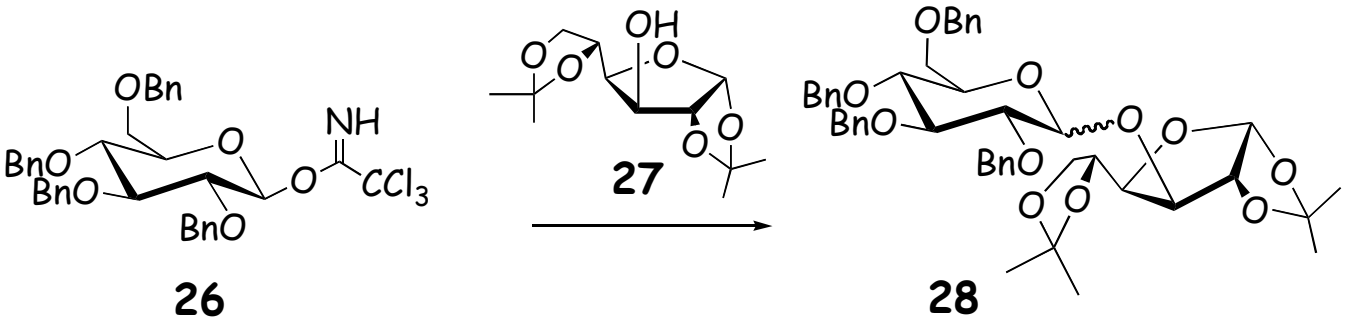
Prod.	Activator; Solvent	Heating; Time	Yield (α/β)
25*	1 M $LiClO_4$; Et_2O	r.t.; 24 h	50% (2:1)
25	0.09M $LiClO_4$; $(CH_2Cl)_2$	M.W. @ 100°C; 5 min	80% (2:1)
20*	1 M $LiClO_4$; Et_2O	r.t.; 24 h	47% (2:1)
20	0.09M $LiClO_4$; $(CH_2Cl)_2$	M.W. @ 100°C; 25 min	80% (1:1)

*G. Böhm & H. Waldmann, *Liebigs Ann.* 1996, 613.

K. Larsen *et al.*, *Org. Biomol. Chem.* 2005, **3**, 3966

Trichloroacetimidate & high temperature glycosylations

Glycosylation of Sec. OH :



26 **28**

Prod.	Activator; Solvent	Heating; Time	Yield (α/β)
28*	0.5 eq. LiOTf; AcCN	r.t.; 86 h	77% (1.2:1)
28	0.09M $LiClO_4$; $(CH_2Cl)_2$	M.W. @ 100°C; 30 min	72% (1.5:1)

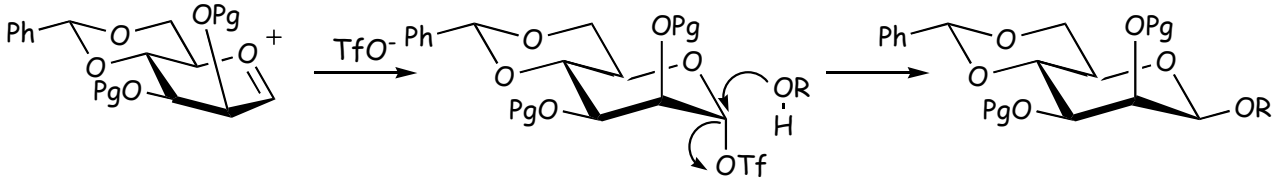
*A. Lubineau & B. Drouillat, *J. Carbohydr. Chem. 1997, **16**, 1179*

K. Larsen *et al., Org. Biomol. Chem.* 2005, **3**, 3966

Glycosylation with mannose

4,6-*O*-benzylidene protected α-mannosyl DISAL donors provide 1,2-*cis* (β-anomer) coupling products

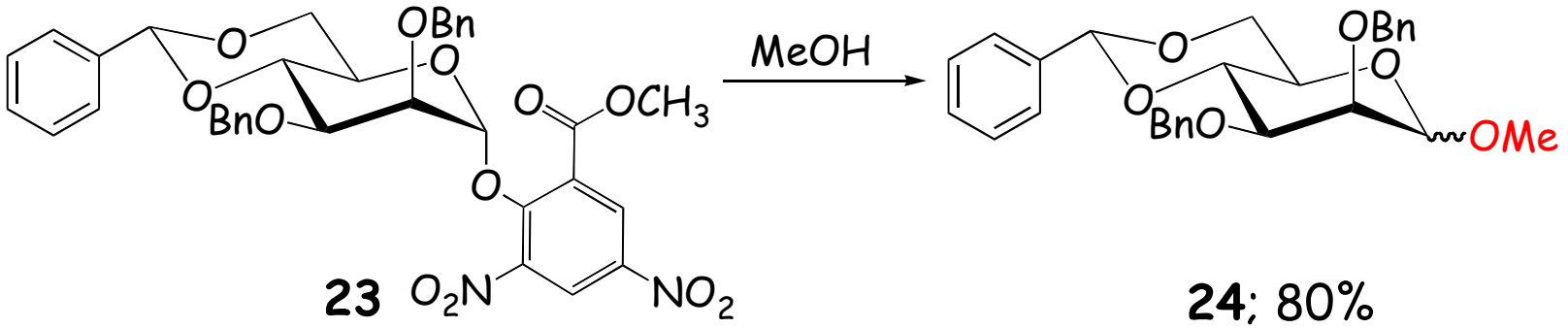
Torsional deactivation: Formation of the glycosyl cation disorts the pyranose ring. This becomes disfavoured if the glycosyl ring is fused to another ring such as a benzylidene protection group.
A possible twist-boat type intermediate could direct the incoming ROH from the β-face.



D. Crich & S. Sun, *Tetrahedron* 1998, **54**, 8321

Crich & Sun hypothesized a triflate-carbocation contact-pair directed the incoming ROH from the β-face.

Initial glycosylations with mannose

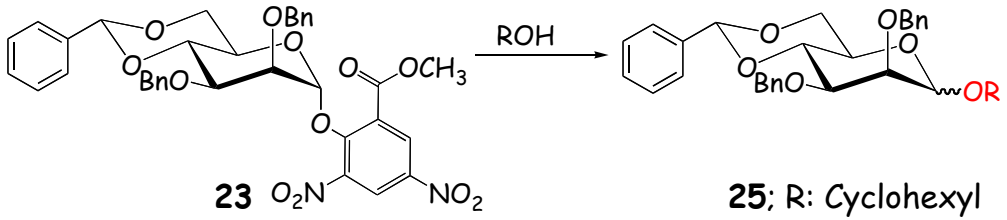


23

24; 80%
α/β (1:5)

K. Worm-Leonhard *et al.*, *J. Carb. Chem.* 2006, in press

Initial glycosylations with mannose II

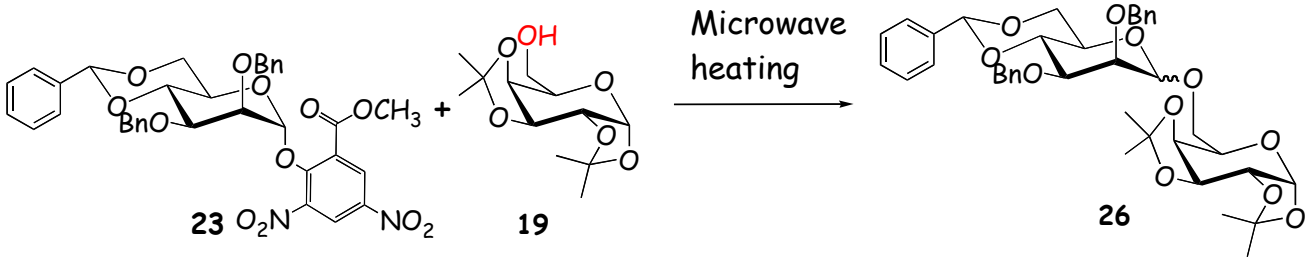


23 O_2N NO_2 **25**; R: Cyclohexyl

Product	Activator; Solvent	Temp.; Time	Heating	Yield (α/β)
25	$LiClO_4$; CH_3NO_2	70°C; 42 h	Convent.	50% (1:1)
25	$LiClO_4$; CH_3NO_2	130°C; 2 x 5 min	Microwave	50% (1:6)
25	$LiClO_4$; CH_3NO_2	130°C; 60 min	Microwave	67% (1:1)
25	$LiClO_4$; $(CH_2Cl)_2$	150°C; 15 min	Microwave	44% (1:2)

K. Worm-Leonhard *et al.*, *J. Carb. Chem.* 2006, in press

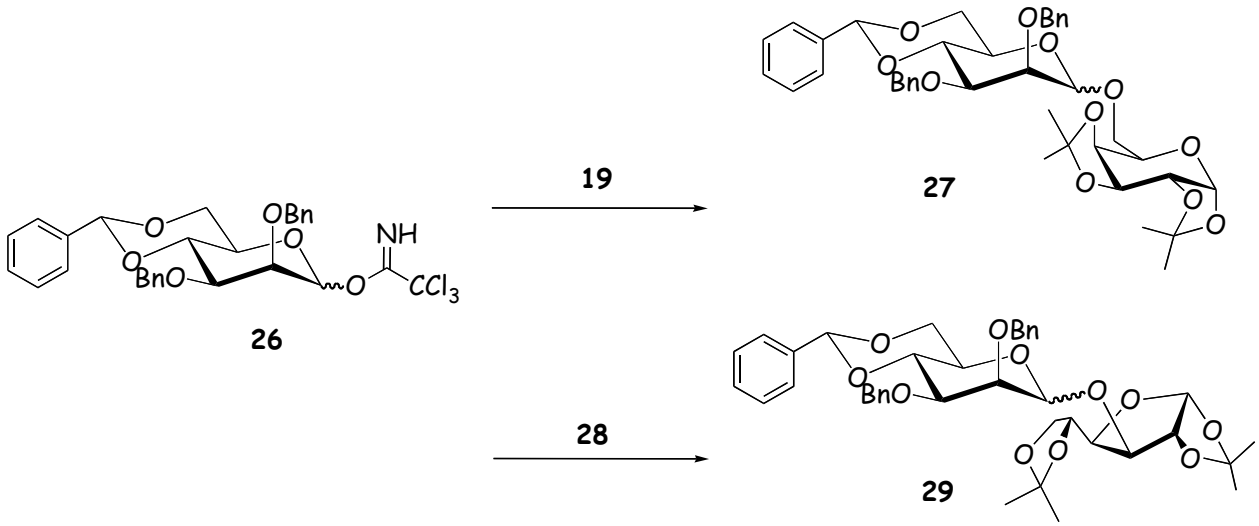
Mannosylation of a primary OH



Product	Activator; Solvent	Temp.; Time	Yield (α/β)
26	$LiClO_4$; $(CH_2Cl)_2$	150°C; 2 x 30 min	74% (2:1)
26	LiOTf; $(CH_2Cl)_2$	150°C; 30 min	38% (<1:99)
26	LiOTf; $(CH_2Cl)_2$	150°C; 2 x 30 min	35% (1:3)
26	TMSOTf (0.3 eq. to **23**); $(CH_2Cl)_2$	100°C; 10 min	26% (1:1)

K. Worm-Leonhard *et al.*, *J. Carb. Chem.* 2006, in press

Mannosylation using trichloroacetimidate



Product	Activator; Solvent	Temp.; Time	Yield (α/β)
27	$LiClO_4$; $(CH_2Cl)_2$	130°C; 25 min	88% (1:1)
29	LiOTf; $(CH_2Cl)_2$	130°C; 25 min	44% (1.3:1)

K. Worm-Leonhard *et al.*, *J. Carb. Chem.* 2006, in press

Conclusion & Outlook

- Microwave promoted glycosylations possible
- Bz_3GlcNTroc DISAL donor
- Bn_4Glc DISAL donor
- Works for 4BnGlc trichloroacetimidates as well
- Not a general glycosylation method yet;
- Standard conditions are (in microwave vials)
  - NMP at 130 °C, no activation
  - $(CH_2Cl)_2$, or CH_3NO_2 with $LiClO_4$ at 100-130 °C
  - Microwave 5-30 min.

- Benzylidene protected Man donors needs more optimerization
- Glycosylation with glycosyl bromides and thioglycosides needs investigation

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