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FACOLTA' DI SCIENZE MATEMATICHE, FISICHE E NATURALI
DOTTORATO DI RICERCA IN SCIENZE CHIMICHE
XX CICLO

SYNTHESIS OF RHAMNANIC OLIGOSACCHARIDES

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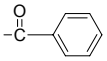
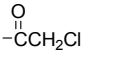
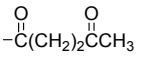
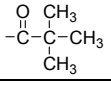
List of Abbreviations

)))	Sonication	Lev	Levulinoyl
		$Le^{x/y/a/b}$	$Lewis^{x/y/a/b}$ antigen
^{13}CNMR	Carbon nuclear magnetic resonance	MALDI	Matrix assisted laser desorption ionization
^{1}HNMR	Proton nuclear magnetic resonance	NBS	*N*-bromo succinimide
Ac	Acetyl	NIS	*N*-iodo succinimide
All	Allyl	Ph	Phenyl
Alloc	Allyloxycarbonyl	PhH	Benzene
AW 300 MS	Acid washed 4Å molecular sieves	Phth	Phtaloyl
Bn	Benzyl	Piv	Pivaloyl
Bz	Benzoyl	*p*MBn	*para*-Methoxybenzyl
Bzd	Benzylidene	ppm	Parts per million
Cbz	Benzyloxycarbonyl	Py	Pyridine
CIP	Contact ion pair	RT	Room temperature
CSA	Camphor-10-sulfonic acid	S_N1	Unimolecular nucleophilic substitution
DAST	(Diethylamino)sulphur trifluoride	S_N2	Bimolecular nucleophilic substitution
DBU	Diazabicyclo[5.4.0]undec-7-ene	SSIP	Solvent separed ion pair
DCC	*N,N'*-Dicyclohexylcarbodiimide	TBAB	Tetrabutylammonium bromide
DCE	1,2-Dichloroethane	TBDMS	*tert*-Butyldimethylsilyl
DCM	Dichloromethane	TBDPS	*tert*-Butyldiphenylsilyl
DIPEA	*N*-Ethyldiisopropylamine	TCA	Trichloroacetyl
DMAP	4-Dimethylaminopyridine	TLC	Thin layer chromatography
DMDO	Dimethyldioxirane	Tf	Trifluoromethansulfonyl (= Triflyl)
DME	1,2-Dimethoxyethane	TFA	Trifluoroacetic acid
DMF	*N,N*-Dimethylformamide	THF	Tetrahydrofuran
Fmoc	9-Fluorenylmethyl-carbonate	TMEDA	*N,N,N',N'*-Trimethylethylendiamine
GSLs	Glycosphingolipids	TMS	Trimethylsilyl
IAD	Intramolecular aglycon delivery	TMSOTf	Trimethylsilyl triflate
IDCP	Iodonium dicollidine perchlorate	TOF	Time of flight
IDCT	Iodonium dicollidine triflate	Tr	Triphenylmethyl (= Trityl)
ImH	Imidazole	Troc	Trichloroethoxycarbonyl

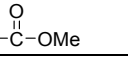
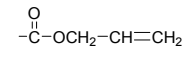
Table of Protecting Groups

HYDROXYL PROTECTING GROUPS

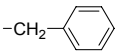
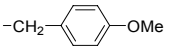
Esters

ACETYL		Ac
BENZOYL		Bz
CHLORO-ACETYL		ClAc
LEVULINOYL		Lev
PIVALOYL		Piv

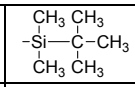
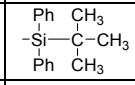
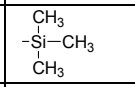
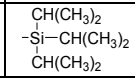
Carbonates

METHOXY-CARBONYL		
ALLYLOXY-CARBONYL		Alloc

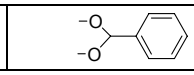
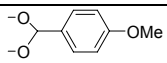
Ethers

BENZYL		Bn
*P*METHOXY -BENZYL		*p*MBn
ALLYL		All
TRITYL		Tr

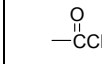
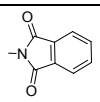
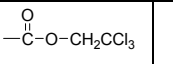
Silyl ethers

*TERT*BUTYL DIMETYLSILYL		TBDMS
*TERT*BUTYL DIPHENYLSILYL		TBDPS
TRIMETHYLSILYL		TMS
TRIISOPROPYL SILYL		TIPS

Acetals

BENZYLIDENE		Bzd
*P*METHOXY-BENZYLIDENE		
ISO-PROPYLIDENE		

AMINO PROTECTING GROUPS

ACETAMIDO		Ac
TRICHLORO-ACETAMIDO		TCA
PHTALIMIDO		NPhth
TRICHLORO-ETHYL-CARBAMATE		Troc

AMINO MASKING GROUPS

AZIDO	N_3

Chapter 1

Introduction

General Introduction

1.1 Carbohydrates in nature

Carbohydrates represent the biggest class of molecules widespread in nature. They can occur alone, as mono- oligo- or polysaccharides, or integrated in glycoconjugates as glycolipids, proteoglycans or glycoproteins. The ever growing interest of the scientific community in structural determination and synthesis of carbohydrates is due to the very great variety of biological phenomena in which they are involved. Carbohydrates cover the surface of many different cell types and they play an important role in cell-cell communication, signalling, recognition and catalysis by interacting with proteins. They can stimulate cell-growth and differentiation in immune response and cell-mobilization in inflammation. In this regard, it's known how the homing of lymphocytes to peripheral nodes is initiated by an adhesive interaction between L-selectins on lymphocytes and a set of sialomucins displayed on high endothelial venules (HEVs) of lymph nodes. This interaction allows the rolling of leucocytes through the blood vessels. Selectin ligands are glycoproteins.[1]

[1] Uchimura K. and Rosen S. D. *TRENDS in Immunology*, **2006**, *27*, 12.

Erythrocyte membranes are also characterised by the presence of genetically determined oligosaccharides called blood group antigens belonging to Lewis and AB0 systems.

A representative example of the carbohydrates eclecticism in nature is heparin which can bind an extraordinary range of proteins. This polysaccharide is a glycosaminoglycan chain belonging to proteoglycans, group of macromolecules inhabiting the extracellular space of animals from marine invertebrates to mammals. Heparin is commonly used in medicine as anti-clotting agent for its ability to activate serine protease inhibitors (serpins), such as antithrombin and annexin V, but it has also been studied in complex with fibroblast growth factors and viral proteins. All these proteins, whether involved in structure, growth, communication or adhesion, have evolved in the presence of this polysaccharide.[2] Moreover, carbohydrates are essential bricks of bacterial, fungal and algae cell wall and exert toxic actions.

Since the biosynthetic pathways of the oligosaccharidic moieties of pathogen cell envelopes are unique and differ from those of mammals, their glycosyltransferases are a promising targets for high selective carbohydrate-based drugs. Also glycosidases inhibitors are compounds of clinical relevance targeting influenza neuraminidases or used for control of blood sugar levels in several forms of diabete (*Figure 1.1*).[3]

[2] Mulloy B. and Linhardt R. J. *Current Opinion in Structural Biology* **2001**, *11*:623-628.
[3] Williams S. J. and Davies G. J. *TRENDS in Biotechnology* **2001**, *19*, 9.

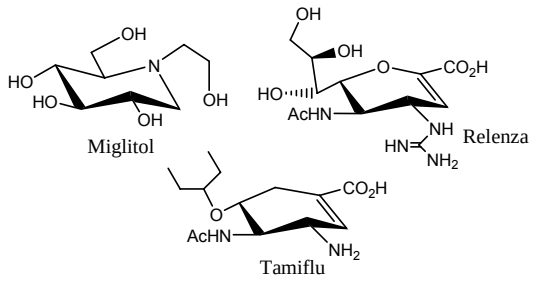


Figure 1.1 Example of carbohydrate-based therapeutics in clinical use. Miglitol is used in treatment of diabetes, Relenza and Tamiflu are flu virus neuraminidase inhibitors.

This small picture of carbohydrates roles in biological systems shows off that protein-carbohydrate interactions can be in many cases an useful tool for the development of novel therapeutics which grows together with the development of novel and ever simpler strategies for carbohydrates synthesis.

An emerging concept is the use of glycoconjugates as anticancer vaccines since cancerous cells express peculiar oligosaccharide motifs, such as the tumor associated antigens Globo-H or KH-1, that stimulate a cytotoxic response.[4] In the same way, glycoconjugate vaccines provide effective prophylaxis against bacterial infections. To date, however, just one commercial vaccine has been available in which the key carbohydrate antigen is synthetically produced, i.e. a conjugate vaccine composed of a synthetic capsular polysaccharide antigen of *Haemophilus influenzae* type b (Hib) (*Figure 1.2*).[5]

[4] Danishefsky, S. J.; Allen, J. R. *Angew. Chem. Int. Ed.* **2000**, *39*, 836.

[5] Verez-Bencomo, V.; Fernández-Santana, V.; Hardy, E.; Toledo, M. E.; Rodríguez, M. C.; Heynngnezz, L.; Rodriguez, A.; Baly A.; Herrera, L.; Izquierdo, M; Villar, A.; Valdés, Y.; Cosme, K.; Deler, M. L.; Montane, M.; Garcia, E.; Ramos, A.; Aguilar, A.; Medina, E.; Toraňo, G.; Sosa, I.; Hernandez, I.; Martínez, R.; Muzachio, A.; Carmenates, A.; Costa, L.; Cardoso F.; Campa, C.; Diaz, M. and Roy, R. *Science* **2004**, *305*, 522-525.

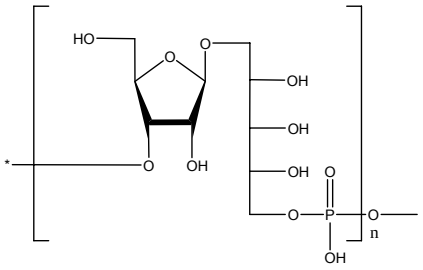


Figure 1.2 *H. influenzae* type b capsular polysaccharide repeating unit.

The vaccine was evaluated in clinical trials and showed comparable effects to licensed products prepared from the natural polysaccharide. Its extensive use generates long-lasting immunity achieved by covalently coupling the polysaccharide to carrier proteins. Furthermore, the large-scale synthesis allows the price cutting of pharmacological treatments. This demonstrates that access to synthetic complex carbohydrate-based vaccines is a feasible task that provides a basis for further development of similar approaches for other human, animal or plant pathogens.

1.2 Oligo-rhamnosides of biological interest

Among carbohydrates, rhamnose is a relatively rare sugar although an essential component of cell surface glyconjugates in pathogenic bacteria. It may be found either as a monomer or in homooligomers called rhamnans. Fragments of lipopolysaccarides (LPSs)[6] and exopolysaccarides (EPSs) from many human and plant-pathogenic species are based on oligorhamnosidic motifs, such as *Burkholderia cepacia* complex,[7]

[6] For a deeper treatment of LPSs see *Chapter 2*.
[7] a) Cérantola, S.; Montrozier, H. *Eur J. Biochem.* **1997**, *246*, 360-366; b) Silipo, A.; Molinaro, A.; Comegna, D.; Sturiale, L.; Cescutti, P.; Garozzo, D.; Lanzetta, R. and Parrilli, M. *Eur. J. Org. Chem.* **2006**, 4874-4883.

Pseudomonas aeruginosa,[8] *Helicobacter pylori*,[9] *Shigella dysenteriae*,[10] *Citrobacter freundii*,[11] *Strenotrophomonas maltophilia*,[12] *Xanthomonas campestris*,[13]and *Brucella*.[14] Each of them is implicated in virulence and persistence of bacterial infection.[15] However, simple isolation and characterization of these carbohydrate antigens is not sufficient to achieve full elucidation of their contribution to the molecular mechanism of pathogenesis and to their employment in drugs. Furthermore, D-rhamnose is only encountered in microorganisms and not in humans or animals, rarely in plants.[16] All these reasons make this sugar a promising target in the synthesis of novel anti-infective agents, including vaccines.[17]

The diffusion of rhamnans is an incredible evidence in phytopathogenic bacterial LPSs.[18] Actually, also many Gram-positive bacteria possess these types of structure: an example is the glycopeptidolipid (GLP) present in cell wall of *Mycobacteriun avium*,[19] or the major glycoprotein from the surface of *Bacillus anthracis* spores (*Figure 1.3*).[20]

---

[8] a) Yokota, S.-I.; Kaya, S.; Sawada, S.; Kawamura, T.; Araki, Y.; Ito, E. *Eur. J. Biochem.* **1987**, *167*, 203-209; b) Rivera, M.; Chivers, T. R.; Lam, J. S.; McGroarty, E. *J. Bacteriol.* **1992**, *174*, 2407-2411.

[9] Kocharova, N. A.; Knirel, Y. A.; Widmalm, G.; Jansson, P.-E.; Moran, A. P. *Biochemistry*, **2000**, *39*, 4755-4760.

[10] Pozsgay V. *J. Am. Chem. Soc.* **1995**, *117*, 6679-6681.

[11] Kocharova, N. A.; Borisova, S. A.; Zatonsky, G. V.; Shashkov, A. S.; Knirel, Y. A.; Kholodkova, E. V.; Staniskavsky, E. S. *Carbohydr. Res.* **1998**, *306*, 331-333.

[12] Winn, A. M.; Wilkinson, S. G. *Carbohydr. Res.* **1996**, *294*, 109-115.

[13] Molinaro, A.; Silipo, A.; Lanzetta, R.; Newmann, M.-A.; Dow, M. J. and Parrilli, M. *Carbohydr Res.* **2003**, *338*, 277-281.

[14] Kihlberg, J.; Bundle, D. R. *Carbohydr. Res.* **1991**, *216*, 67-78.

[15] Cuha, M. V.; Sousa, S. A.; Leitão, J. H.; Moreira, L. m.; Videira, P. A.; Sá-Correia, I. *J. Clin. Microbiol.* **2004**, *42*, 3052-3058.

[16] Hu, J.-F.; Garo, E.; Hough, G. W.; Matt, G.G.; O'Neil-Johnson, M. and Eldridge, G. R. *J. Nat. Prod.* **2006**, *69*, 585-590.

[17] Fauré, R.; Shiao, T. C.; Lagnoux, D. and Roy, R. *Org. Biomol. Chem.* **2007**, *5*, 2704-2708.

[18] this object is discussed in *Chapter 2*.

[19]Heidelbreg, T. and Martin, O. R. *J. Org. Chem.* **2004**, *69*, 2290-2301.

[20] Daubenspeck, J. M.; Zeng, H., Chen, P.; Dong, S.; Steichen, C. T.; Krishna, N. R., Pritchard, D. G.;Turnbough, C. L. *J. Biol. Chem.* **2004**, *279*, 30945-30953.

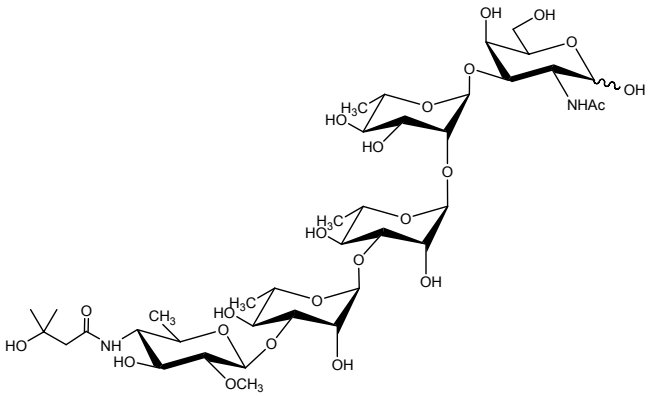


Figure 1.3 Structure of pentasaccharide from *Bacillus anthracis.*

Very recently, the first synthesis of a library of rhamnosylated lipids was performed. These bacterial molecules possess strong immunostimulatory properties triggering the excretion of cytokines and antibacterial peptides in human cells.[21]

Synthetic Aspects

1.3 Glycosylation reaction

A glycosylation reaction is the formation of a glycosidic linkage and represents the key step in oligosaccharide synthesis.

In solution carbohydrates exist in equilibrium between cyclic forms and open chains. The formers, five- (furanose) or six-membered (pyranose) rings (*Figure 1.4*), are hemiacetals formed by an intramolecular nucleophilic attack at the carbonyl carbon by a hydroxyl group.

[21] Bauer J.; Brandenburg, K.; Zähringer, U.; Rademann J. *Chem . Eur. J.* **2006**, *12*, 7116-7124.

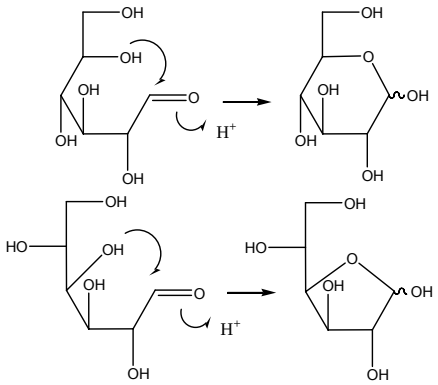


Figure 1.4 Anellation in D-glucose: pyranoid and furanoid forms.

Hemiacetal ring formation generates a new asymmetric carbon atom at C1 called *anomeric centre*, thereby giving rise to diastereoisomeric hemiacetals (*anomers*) labelled α and β and distinguishable for the relative correlation between the configuration at the anomeric centre with those at the highest-numbered asymmetric centre of the sugar. For all compounds of D-series, α-anomers have the hydroxyl group at the anomeric centre projecting downwards in Haworth formulae; α-L-compounds have this group projecting upwards. The β-anomers have the opposite configurations (*Figure 1.5*).

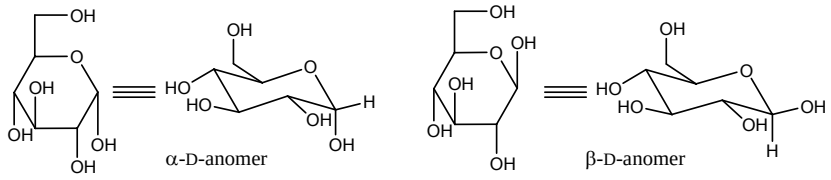


Figure 1.5 Haworth formula (left) and chair conformation (right) of pyranoid ring.

Normally, the more stable conformation is the chair allowing the highest number of equatorially-oriented hydroxyls, and in particular the 6-hydroxymethylene group.

The replacement of the 1-OH function with a nucleophile generates a full acetal called *glycoside* (*Figure 1.6*). When the nucleophile is not a sugar the resulting non-carbohydrate moiety is called *aglycon*.

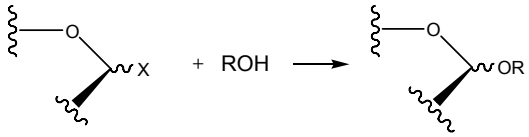


Figure 1.6 Glycoside formation.

Glycosides which carry a leaving group at the anomeric carbon are used as glycosylating agents in oligosaccharide synthesis and they are called *glycosyl donors*. The nucleophilic species in glycosylations are called *glycosyl acceptors.* Normally both molecules carry a pattern of protecting groups (PG) on all the functionalities except for those involved in the reaction. Glycosylation occurs through a nucleophilic substitution where the free nucleophilic group of the glycosyl acceptor attacks the electron-deficient anomeric carbon of the activated glycosyl donor (*Figure 1.7*).

Optimal results in glycosylation reactions entail strictly anhydrous conditions achieved by working under argon atmosphere and in presence of molecular sieves that capture traces of water.

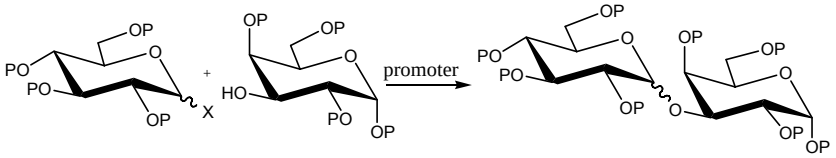


Figure 1.7 Glycosylation reaction scheme.

1.4 Glycosyl donors

Glycosyl donors are classified on the nature of the leaving group at the anomeric carbon. The glycosylation reaction is activated by a promoter that allows the departure of the leaving group weakening the aglycon-sugar linkage. Each type of donor has a specific set of promoters. The different kinds of glycosyl donors and their relative most commonly employed promoters are summarized in *Table 1.1.*

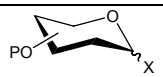
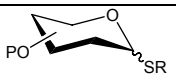
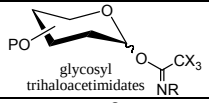
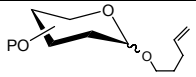
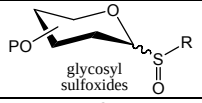
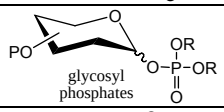
Glycosyl Donor	*Promoter*
 glycosyl halides	silver salts (Ag_2O, Ag_2CO_3); Lewis acids ($SnCl_4$, $BF_3{\cdot}Et_2O$, $ZnCl_2$)
 thioglycosides	NBS, IDCP, NIS/ TfOH (or TMSOTf, AgOTf and $BF_3{\cdot}Et_2O$)
 glycosyl trihaloacetimidates	$BF_3{\cdot}Et_2O$, TMSOTf; $Yb(OTf)_3$, $Bi(OTf)_3$
 *n*-pentenyl glycosides	NBS, IDCP, NIS/TfOH
 glycosyl sulfoxides	TfOH, TfO_2
 glycosyl phosphates	TMSOTf or other Lewis acids
 1,2 anidro sugars	$ZnCl_2$

Table 1.1 Glycosyl donors and relative activating systems.

Glycosyl halides are commonly used mainly as **bromides** and **chlorides**. Generally these types of halides are only moderately stable, so they are synthesized in the last

step by treatment of the 1-*O*-acetylated-sugar with the appropriate hydrogen halide in acetic acid. They are activated by stoichiometric amounts of silver salts (Ag_2O, Ag_2CO_3) in Koenings-Knorr method,[22] heavy metal salts such as $Hg(CN)_2$ and $HgBr_2$, or Lewis acids such as $SnCl_4$, $BF_3{\cdot}Et_2O$, $ZnCl_2$. However silver triflate is the most frequently used promoter. **Fluorides**[23] are more stable but also less reactive than bromide and chloride so that they can be synthesized partially unprotected functioning to serve first as an acceptor and then as a donor (*latent glycosylating agent*). They are commonly prepared from a protected lactol with diethylaminosulfur trifluoride (DAST). In contrast **iodides**[24] are very reactive and consequently unstable. So they are generally used as *in situ* generated intermediates.

Thioglycosides[25] are the most used kind of donors in oligosaccharide synthesis for their stability and their effective activation using chemoselective thiophilic promoters. Because of their stability in several glycosylation conditions they are often used as latent glycosylating agents. The most common synthetic routes to thioglycosides are two: the reaction of peracetylated glycosides with a thiol in presence of a Lewis acid and the alkylation of an anomeric thiolate generated from a glycosyl halide. In this latter approach the treatment of anomeric halides with thiourea generates a thiouronium intermediate from which a thiolate can be readiky obtained with a mild base and trapped by a suitable alkylating agent.. The resulting thiol can be alkylated to give a thioglycoside (*Figure 1.8*).

[22] a) Koenings, W.; Knorr, E. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 957; b) Wulff, G.; Röhle, G. *Angew. Chem. Int. Ed. Engl.* **1974**, *13*, 157.
[23] Mukayama, t.; Murai, Y.; Shoda, S.-Y. *Chem. Lett.* **1981**, 431.
[24] Fisher, E.; Fisher, H. *Ber. Dtsch. Chem. Ges.* **1910**, *43*, 2521.
[25] Fisher, E.; Delbrück, K. *Ber.* **1909**, *42*, 1476.

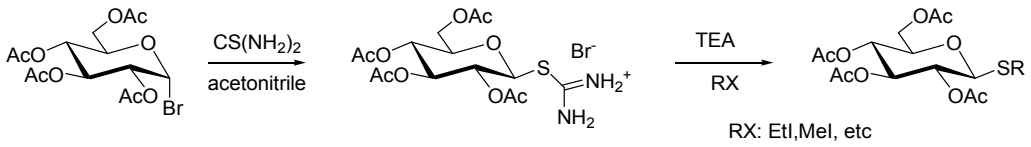


Figure 1.8 Thioglycoside preparation from glycosyl halides.

Thioglycosides are sensible to soft electrophilic reagents, so their promoters are halonium sources such as *N*-bromo succinimide (NBS), iodonium dicollidine perchlorate (IDCP), *N*-iodo succinimide (NIS) in combination with a catalytic amount of triflic acid (TfOH). The latter is frequently replaced by different Lewis acids such as TMSOTf, AgOTf or $BF_3{\cdot}Et_2O$. The activation mechanism proceeds through the formation of a sulfonium ion which evolves to an oxycarbenium ion species (*Figure 1.9*).

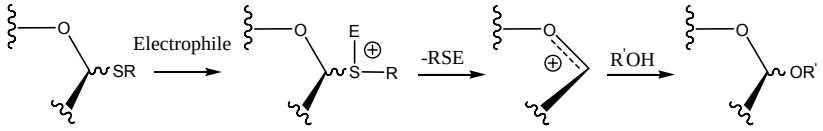


Figure 1.9 Activation mechanism of thioglycosides.

Thioglycosides can also be directly converted into other kinds of donors.

Trihaloacetimidates are another very useful class of donors with the advantage to require only catalytic amounts of promoter. **Trichloroacetimidates**[26] are the most commonly employed but they can be substituted in many cases with ***N*-phenyl trifluoroacetimidates**[27] which offer some advantages.[28] Trichloroacetimidates are

[26] Schmidt, R. R.; Michel, J. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 731.
[27] a) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405; b) Yu, B.; Tao, H. *J. Org. Chem.* **2002**, *67*, 9099.
[28] Trihaloacetimidates are object of *Chapter 3*.

synthesized starting from the protected hemiacetal by treatment with trichloroacetonitrile under catalytic basic conditions.

N-phenyl trifluoroacetimidates are synthesized from the hemiacetal by treatment with *N*-phenyl trifluoroacetimidoyl chloride in the presence of a stoichiometric base, in some applications Cs_2CO_3 has been found preferable to K_2CO_3 or NaH. When inorganic carbonates are used non-anhydrous solvents are required.

Both kinds of trihaloacetimidates are activated by Lewis or Brønsted acids, most frequently $BF_3{\cdot}Et_2O$ or TMSOTf are used even though in the last years several mild methods have been developed based on shelf-stable triflates such as $Sm(OTf)_3$[29] and $Yb(OTf)_3$[30] or $Bi(OTf)_3$.[31] These systems allow the employment of acid-labile protective groups.

More stable donors are ***n*-pentenyl glycosides**[32] which can endure the installation of many protective groups. Glycosylations with this type of glycosyl donors are catalyzed by the same promoters of thioglycosides (NBS, IDCP, NIS/TfOH) and proceed through electrophilic halogenation of the double bound and subsequent closure of a five-membered ring (*Figure 1.10*).

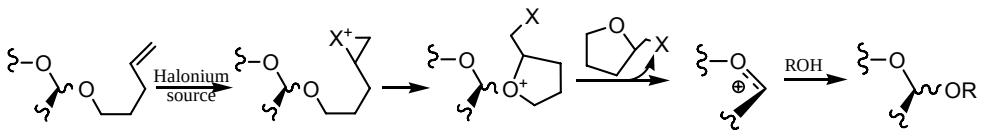


Figure 1.10 Activation mechanism of *n*-pentenyl glycosides.

[29] Adinolfi, M.; Barone, G.; Guariniello, L.; Iadonisi, A. *Tetrahedron Lett.* **2000**, 41, 9005.
[30] Adinolfi, M.; Barone; Iadonisi, A.; Mangoni, L.; Schiattarella, M. *Tetrahedron Lett.* **2001**, 42, 5967.
[31] Adinolfi, M; Iadonisi, A.; Ravidà, A; Valerio, S. *Tetrahedron Lett.* **2006**, 47, 2595.
[32] Fraser-Reid, B.; Konradsson, P.; Mootoo, D. R.; Udodong, U. *J. Chem. Soc. Chem. Commun.* **1988**, 823.

Glycosyl sulfoxides[33] are exploited for the synthesis of β-mannopyranosides since their activation with triflic anhydride or a catalytic amount of triflic acid produces an *α*-triflate.[34]

Glycosyl phosphates[35] can be synthesized from hemiacetals by treatment with a suitable chlorophosphonate in basic conditions and activated by stoichiometric amounts of TMSOTf or other Lewis acids.

Another family of glycosyl donors are **1,2-anidro sugars**. They are obtained *via* oxidation with dimethyldioxirane (DMDO) from glycals.[36] The epoxide is activated by $ZnCl_2$ (*Figure 1.11*).

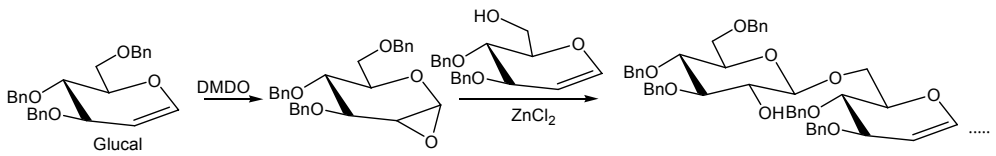


Figure 1.11 Glycosylation with glycals.

In this way a new latent donor is ready to be activated in an iterative glycosylation.

1.5 Stereoselectivity in glycosylation reactions

The mechanism of a glycosylation is almost never a S_N2. Thus, the nucleophilic attack to the anomeric carbon of glycosyl donor can occur from the two different faces of the ring. Therefore, due to the stereogenic nature of the anomeric carbon, the control of the stereoselectivity is necessary.

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[33] Kahne, D.; Walzer, S.; Cheng, Y.; van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881.
[34] see the next paragraph.
[35] Hashimoto, S.; Honda, T.; Ikegami, S. *J. Chem. Soc. Chem. Commun.* **1989**, 685.
[36] Halcomb, R. L.; Danishefsky, S. *J. Am. Chem. Soc.* **1989**, *11*, 6661.

The new bond is usually classified on the relative configuration at C-1 and C-2 on the ring of the sugar as 1,2-*trans* and 1,2-*cis* and on the orientation of substituent at C-1 in the space (anomeric configuration) as α or β (*Figure 1.12*).

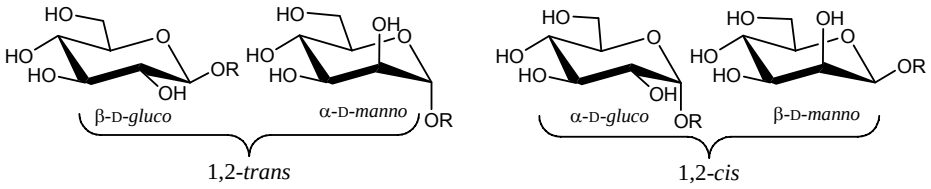


Figure 1.12 Classification of glycosidic linkages.

Formation of 1,2-*trans* linkages can be easily achieved taking advantage of the **neighbouring group participation**. Activation of the anomeric centre of the glycosyl donor results in the formation of an oxonium ion, then it can be attacked by an acyl group at *O*-2 position leading to a more stable acyloxonium ion. The nucleophilic attack at C-1 of acyloxonium ion occurs *in anti* to the exocyclic oxygen regenerating the acyl group while providing the 1,2-*trans* glycoside (*Figure 1.13*).

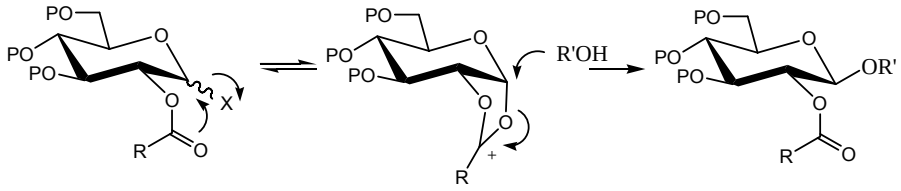


Figure 1.13 Anchimeric assistance of acyl group.

Thus, in the case of D-glucosyl-type donors, β-linked product can be obtained and D-mannosides will give α-linkages. Sometimes the acceptor attacks at the electrophilic carbon atom of the dioxolane ring resulting in the formation of an indesidered orthoester (*Figure 1.14*).

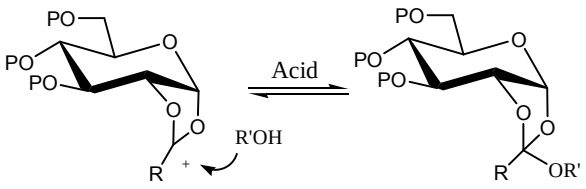


Figure 1.14 1,2-orthoster formation.

In acid conditions this side-reaction is reversible but with mild activation methods tricks are necessary to minimize it. The employment of acyl groups bearing bulky or electron-withdrawing ester moiety is very useful. Hindered pivaloyl groups are often used, orthoester formation being disfavoured by the presence of the bulky *tert*-butyl group adjacent to the electrophilic carbon atom. However, their cleavage requires harsher condition than those used for the common acetyl or benzoyl groups. Furthermore, a high strong steric hindrance between acceptor and donor in the transition state might be disfavouring the acyloxonium closure and the reaction proceed via oxonium ion loosing stereoselectivity like in the example showed in *Figure 1.15*.

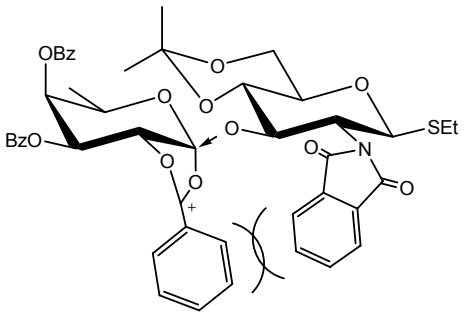


Figure 1.15 Mismatched pair.

Instead, methoxycarbonyl group guarantees a lower amount of orthoester-like coupling products with complete stereoselectivity, together with simpler conditions of installation and cleavage.[29,30]

However the use of acyl or carbonyl groups is not advisable in all cases. The nature of the protective group pattern also influences the reactivity of glycosides where are installed on. Glycosyl donors equipped with electron-withdrawing protective groups (*e.g.* acyl groups) are called *disarmed* and they are less reactive then their ether-equipped counterparts which are called *armed*.[37] The presence of electron-withdrawing substituents depresses reactivity destabilizing the oxonium ion formation. Depending on specific synthetic demands, this effect might be undesidered and 1,2-*trans*-selectivity has to be achieved in a different fashion.

When a non-assisting functionality at C-2 is present then the reaction conditions can influence the anomeric selectivity. The major factor is the **solvent effect**. The character of the nucleophilic substitution is due to the nature of the solvent. In non-coordinating solvent the ion pair formed after the leaving group departure is tight and is termed *Contact Ion Pair* (CIP), thus the reaction proceeds with a S_N2-like mechanism. However, mixtures of products are also obtained by the formation of the so termed *Solvent Sepatated Ion Pair* (SSIP) which gives a considerable S_N1 contribution to the reaction (*Figure 1.16*). With glycosyl halides inversion of anomeric configuration can also occur.[38]

[37] Fraser-Reid, B.; Wu, A.; Webster, A.C.; Skowronski, E. *J. Am. Chem. Soc.* **1991**, *113*, 1434.
[38] Lemieux, R. U.; Hendricks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056.

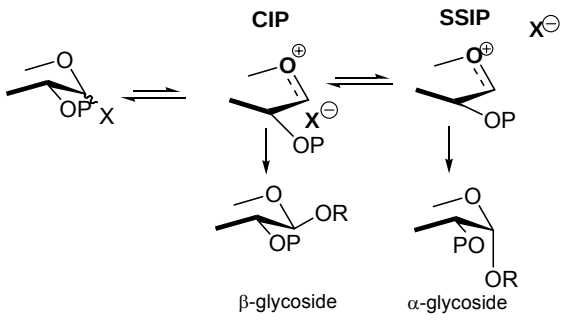


Figure 1.16 Glycosylation mechanism in non-coordinating solvent.

Instead, coordinating solvents are directly involved in the mechanism and their nature determines the α/β ratio of glycosylation. It is commonly known that ether-type solvents are α-directing, while nitrile-type solvents favour β-linkage formation. To understand the reasons which lead to these results kinetic and thermodynamically effects have to be examined. Glycosides are generally subject to the **anomeric effect** for which the α-anomer is the thermodynamic product. In stereoelectronic terms this is due to a stabilizing overlap of orbitals which allows delocalization of the lone-pair electrons of endocyclic oxygen. The interaction involves an occupied *p*-type orbital on oxygen and the antibonding σ* orbital of anomeric carbon-exocyclic heteroatom and is possible only when the aglycon is in axial position. This effect is supported by the evidence that C_1-O intracyclic bond shows a reduced length[39] (*Figure 1.17*).

[39] Carey F. and Sundberg R. J. *Advanced Organic Chemistry Part A* Kluwer Academy/Plenum Publishers **1977**, New York.

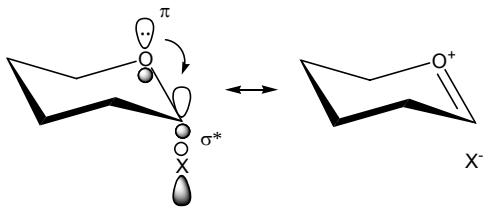


Figure 1.17 Orbitalic interaction in anomeric effect.

In terms of localized bonds, when the aglycon is equatorially oriented, a stronger dipole-dipole repulsion between the polar bonds at the anomeric carbon is present than in axial conformation.

In glycosylations occurring under S_N1 conditions, an oxocarbenium ion is generated. This intermediate evolves to a kinetic α-adduct with a nucleophile present in the reaction medium. Such a preference can be interpreted assuming a cyclo-hexene-like conformation of the cationic intermediate. The axial addition providing the α-adduct is favoured because the half-chair of oxonium ion evolves to a complete chair conformation while a *pseudoequatorial* attack brings to a twist conformation (*Figure 1.18*).

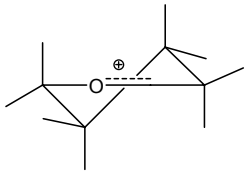


Figure 1.18 Half-chair conformation of oxocarbenium ion.

In glycosylations with nucleophilic solvents the solvated oxonium ion exhibits a positive charge on the heteroatom of the coordinating solvent. Thus, **reverse anomeric effect** occurs: one dipole changes direction and the kinetic product become the α-adduct while the β-adduct is thermodynamically favoured (*Figure 1.19*).

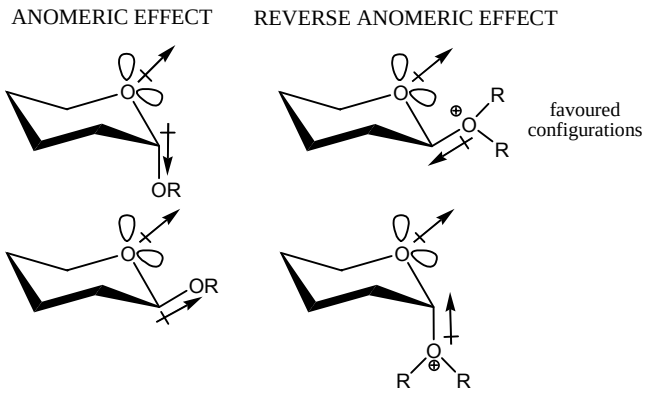


Figure 1.19 Dipole-dipole interaction in direct and reverse anomeric effects.

In ether-type solvents the conversion of the α-kinetic to the thermodynamic β-adduct proceeds very fast. So, the nucleophilic attack of the acceptor occurs more probably from the free axial side. In nitrile-type solvents the kinetic α-adduct is actually consisting of polynitrilium species where the positive charge is polydispersed. This results in an enhancement of the activation energy for the interconversion to the thermodynamic β-adduct which therefore proceeds more slowly. In this case the nucleophile attacks more probably in an equatorial orientation leading to β-glycosides (*Figure 1.20*).

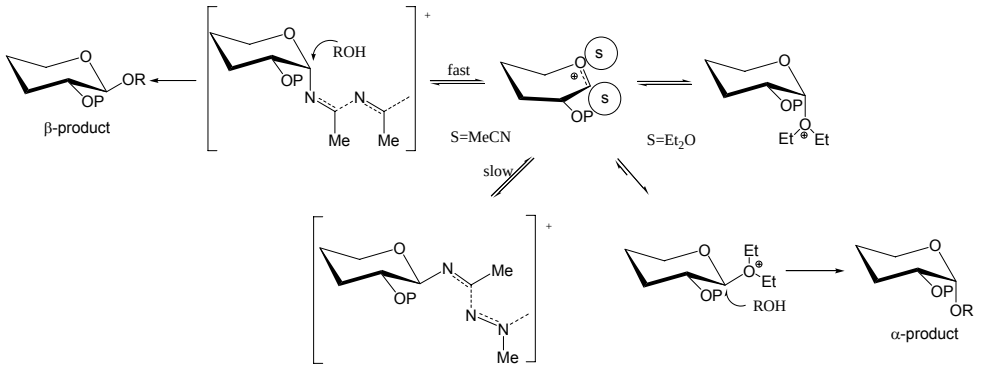


Figure 1.20 Solvent partecipation in glycosylations.

An improvement in α-selectivity has been achieved with the use of 1,2-dimethoxyethane (DME) as a component of the solvent system. This effect is believed to be due to a kinetically favoured anomerization which can occur by an intramolecular rearrangement of the bidentate ether[40] (*Figure 1.21*).

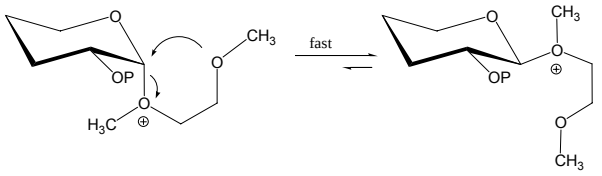
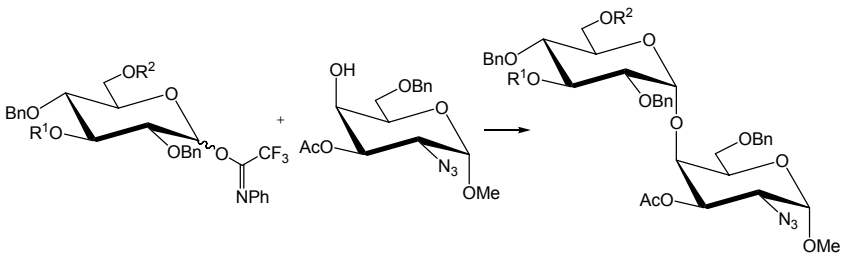


Figure 1.21 Hypothesis of anomerization in DME adducts.

The difficult task of 1,2-*cis* glycosidic linkage formation can be tackled with different approaches including the solvent effect. However, none of these is as efficient as the neighbouring participation effect used for stereocontrolled synthesis of 1,2-*trans* glycosides. Therefore, combinations of different effects have to be applied. One of these is the **remote group participation**. When an acyl group is installed on O-4 of galactose[41] or fucose[42] donors an increase of α-selectivity occurs. The same effect happens when 3-*O*-acyl group or 6-*O*-acyl group are installed on glucose donors.[43] In this case the single and the cooperative effects have been investigated (*Figure 1.22*).

[40] Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *Tetrahedron Lett.* **2004**, *45*, 4485-4488.
[41] Demchenko, A. V.; Rousson, E.; Boons, G. J. *Tetrahedron Lett.* **1999**, *40*, 6523-6526.
[42] Gerbst, A. G. *J. Carbohydr. Chem.* **2001**, *20*, 821-831.
[43] Komarova B. S.; Tsvetkov, Y., E.; Knirel, Y. A.; Zähringer, U.; Pier, G. B.; Nifantiev, N. E. *Tetrahedron Lett.* **2006**, *47*, 3583-3587.



R^1	R^2	*Yield (%)*	*α:β ratio*
Bn	Ac	90	5:1
Ac	Bn	90	4:1
Ac	Ac	96	8:1

Figure 1.22 Long-range effects on stereoselectivity in glycosylation.

The long-range effect exherted by the 3-*O*-acyl group is less efficient than the combined effect of a 3,6-di-acetylated donor. Bulky groups on the primary hydroxyl such as a dimethoxytrityl can also address α-selectivity.[44]

However one of the most challenging aspects in oligosaccharide synthesis is 1,2-*cis*-β-linkage formation. The anomeric effect that disfavours β-orientation of substituents in non-coordinating solvents joins the unfeasible use of neighbouring participating groups. On this regard, most of the strategies employ 4,6-*O*-benzylidene protection. It has been established that 2,3-*O*-alkylated-4,6-*O*-benzylidene protected sulfoxides[45] and trichloroacetimidates[46] donors undertaken respectively with triflic anhydride or trimethylsilyl triflate give α-mannosyl triflate. This is due to the torsional effect that

[44] Adinolfi, M.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 6479.

[45] a) Crich, D.; Sun, S. *J. Org. Chem.* **1996**, *61*, 4506-4507; b) Crich, D.; Sun, S. *J. Org. Chem.* **1997**, *62*, 1198-1199; c) Crich, D.; Sun, S. *J. Am. Soc.* **1997**, *119*, 11217-11223; d) Crich, D.; Sun, S. *J.Am.Chem.Soc.* **1998**, 120, 435-436; e) Crich, D.; Sun, S. *Tetrahedron* **1998**, *54*, 8321-8348; f) Crich, D.; Cai, W.; Dai, Z. *J. Org. Chem.* **2000**, *65*, 1291-1297; g) Crich, D.; Smith, M. *J.Am.Chem.Soc.* **2001**, *123*, 9015-9020.

[46] Weingart, R.; Schmidt, R. R. *Tetrahedron Lett.* **2000**, *41*, 8753-8758.

the cyclic acetal exerts on the oxocarbenium intermediate which is forced to assume a half-chair (*H*) conformation (*Figure 1.23*).

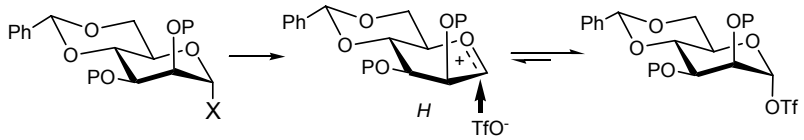


Figure 1.23 Mechanistic proposal for preferred β-linkage formation with 4,6-*O*-benzylidene equipped mannopyranosides. *H*=half-chair conformation.

The collapse of oxonium ion leads to a trapped axially triflate. Then, a subsequent addiction of the acceptor to premixed sulfoxide donor and Tf_2O in apolar solvents gives mainly the β-mannoside through an S_N2-like reaction as already discussed.

This mechanistic hypotesis also explained the poor β-selectivity obtained when less reactive secondary acceptors are used. The retardation of the S_N2 reaction by steric hindrance leads to a dissociative mechanism of the contact ion pair (CIP) via a Curtin-Hammett kinetic scheme.[47]

To exploit the torsional effect of 4,6-*O*-benzylidene protection also for β-rhamnosylation reductive cleavage methods have been developed. The most recently employed are 4,6-*O*-[1-ciano-2-(2-iodophenyl)ethylidene] protected glycosyl donors. Tin-mediated radical fragmentation of the protecting group provides a 6-deoxy function[48] (*Figure 1.24*).

[47] Crich D. *J. Carbohydr. Chem.* **2002**, *21*, 667-690.

[48] Crich, D.; Bowers, A. A. *J. Org. Chem.* **2006**, *71*, 3452-3463.

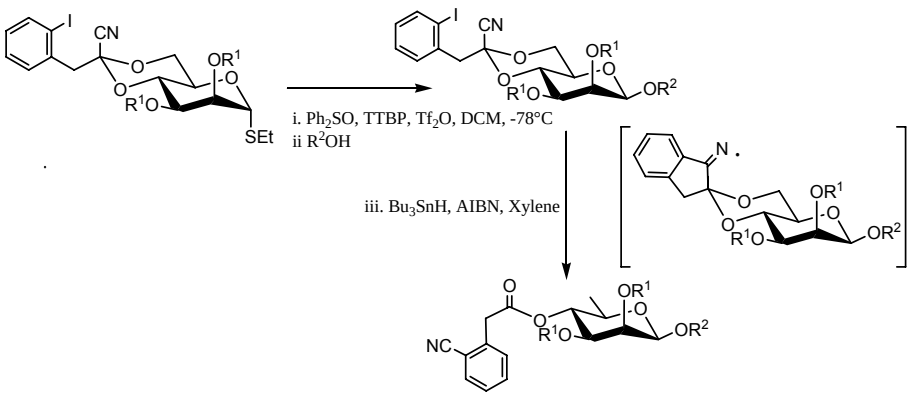


Figure 1.24 Tin-mediated radical fragmentation of 4,6-*O*-[1-ciano-2-(2-iodophenyl)ethylidene] protected glycosyl donor.

However, there are also several methods indipendent of the benzylidene and employing strong electron-withdrawing substituents at C-2 of the glycosyl donor. In this case a different mechanism of the anomeric stereocontrol is invoked. The new proposal involves a flattened twist-boat (*TB*) conformation as the intermediate.

For stereoelectronic and steric reasons the twist-boat will be preferentially attacked from the β side and subsequentially will equilibrate to the chair conformer (*Figure 1.25*).

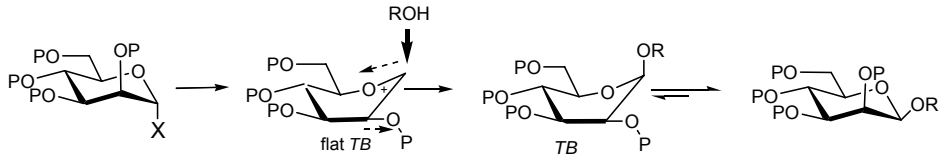


Figure 1.25 Mechanistic proposal for preferred β-linkage formation with 2-*O*-electrowithdrawing substituent equipped mannopyranosides. *TB*=twist-boat conformation.

The twist-boat conformation is induced by a strong dipole effect in the oxocarbenium ion which increases with the electron-withdrawing power of 2-*O*-protecting group.[49]

[49] Abdel-Rahman, A. A.-H.; Jonke, S.; El Ashry, E. S. H.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2002**, *41*, 2972-2974.

A very different approach for stereocontrol of glycosylations is the so called **locked anomeric conformation**. This method involves 1,2-*O*-*cis*-stannylene acetals of sugars as powerful nucleophiles capable of displacing, via S_N2 process, good leaving groups. This type of glycosylation does not involve oxacarbenum ion. The more nuclephilic equatorial oxygen involved in stannylene formation reacts with the electrophilic site of a carbohydrate bearing a triflyl group giving an 1,2-*cis*-oligosaccharide (*Figure 1.26*).[50]

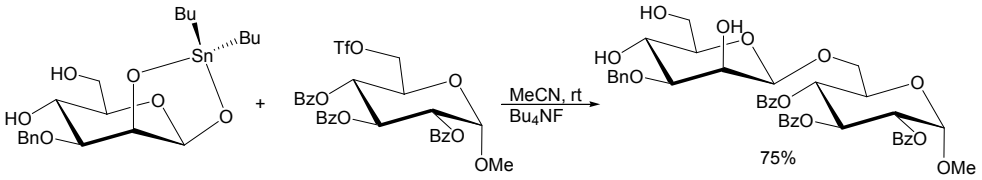


Figure 1.26 Locked anomeric conformation.

Intramolecular aglycon delivery is another protocol to achieve *O*-linked β-manno- and β-rhamnosides. It is based on an intermolecular glycosylation occurring in a β-manno-configurated donor bearing the acceptor already linked to an acetal at C-2. Ensuing activation of the donor releases the aglycon which is suitably disposed for a direct S_N2-like substitution at the anomeric centre (*Figure 1.27*).[51]

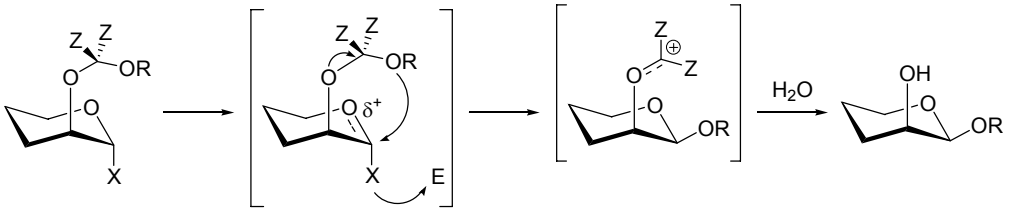


Figure 1.27 Intramolecolar aglycon delivery (IAD).

[50] Hodosi, G.; Kováč, P. *J. Am. Chem. Soc.* **1997**, *119*, 2335-2336.
[51] Lindhorst, T. K. *Essentials of Carbohydrate Chemistry and Biochemistry*, **2000**, Wiley-VCH, Weinheim, Germany.

Another strategy used takes advantage of the ready formation of a 1,2-*trans*-glucoside by neighbouring group participation which successively generates the 1,2-*cis*-mannoside by nucleophilic displacement or an oxidation-reduction sequence proceeding with inversion at the C-2 (*Figure 1.28*).

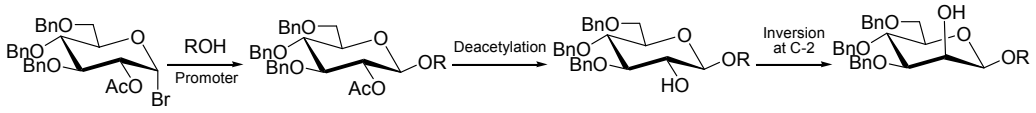


Figure 1.28 Synthesis of β-D-mannopyranosides by configurational inversion at C-2.

1.7 Regioselectivity in carbohydrate chemistry

Regioselectivity is an essential issue in glycosylations since sugars possess more than one free hydroxyl groups. To achieve this purpose a suitable protective group pattern has to be installed on both reactants. Generally, in the coupling step the acceptor exhibits a single hydroxyl group as free nucleophile, the others being protected. Additionally, glycosyl donors have to be reactive only at the anomeric position. A synthetic strategy aimed at an oligosaccharidic target needs two types of protecting groups: **permanent protecting groups** are installed on those positions which are not involved in the synthesis and are removed in the last steps. Functions involved in one reaction in the course of the total synthesis have to be derivatized by **temporary protecting groups** selectively removable in the presence of the others. Generally, this is necessary to obtain a new acceptor (*Figure 1.29*).

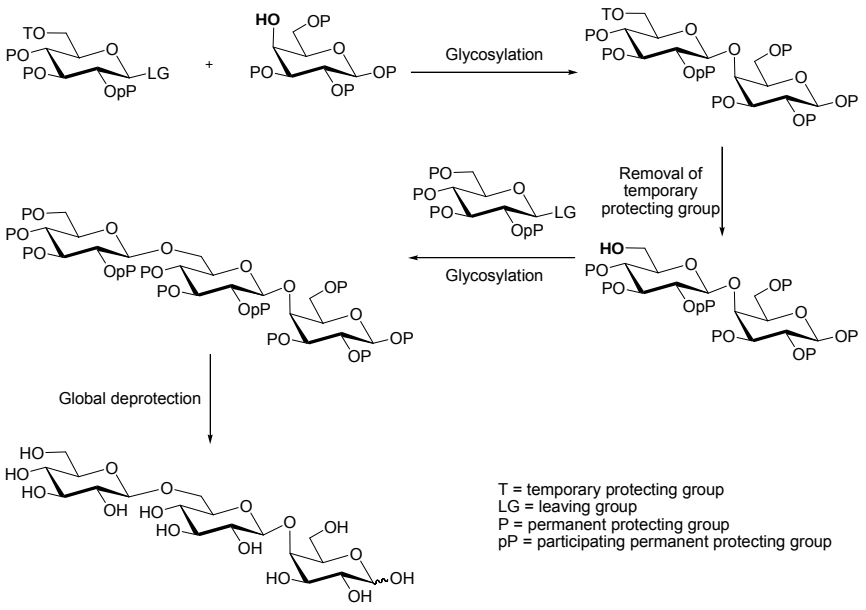


Figure 1.29 Schematic synthesis of a trisaccharide.

In all cases the choice of protecting groups is restricted to those withstanding the glycosidation conditions.

In more complex syntheses, a well varied range of protecting groups has to be employed. This is especially required in branched structures to differentiate positions that have to be glycosylated at different stages.

In this case the temporary groups adopted have to be in **orthogonal relationship**,[52] namely every group is removable in the presence of the others and in every order.

The protective groups are not only useful for regioselectivity but they are also critical in tuning the reactivity of molecules on which they are installed. This effect has already been briefly touched for glycosyl donors but its applications have to be discussed. Firstly, electron-withdrawing groups are very useful to enhance the stability of deoxy sugars donors. The absence of one or more hydroxyls amplifies the

[52] Barany, G.; Merrifield, R. B.; *J. Am. Chem. Soc.* **1977**, *116*, 7363.

reactivity and can accelerate the moisture-promoted degradation of the glycosyl donor. Furthermore, the armed-disarmed concept can be employed directly in glycosylations. It is possible to have two donors belonging to the same class but with different reactivity, and the disarmed can act as an acceptor if it bears a free hydroxyl. The first application was proofed with *n*-pentenyl glycosides[53] and then extended to other classes of donors (*Figure 1.30*).

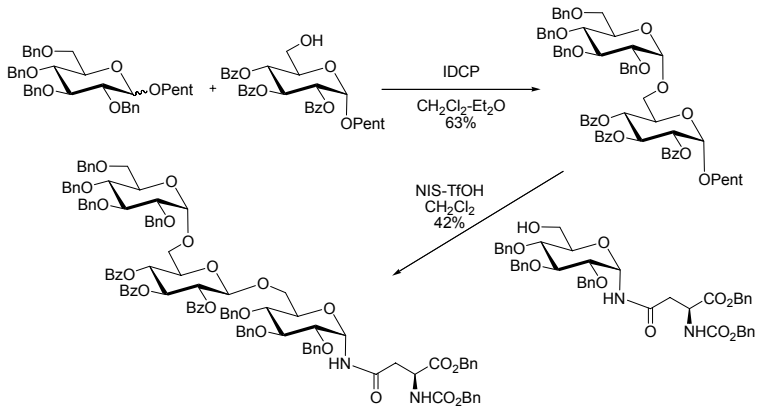


Figure 1.30 Oligosaccharide synthesis by armed-disarmed glycosylations.

In the example above depicted the armed benzylated glycoside, under activating conditions, shows a higher reaction rate which impedes an appreciable self-condensation of the disarmed acylated glycoside.

Only donors reactivity has been discussed so far, but the protective group pattern influences also acceptors behaviour. It is well documented that protecting groups influence the efficiency of glycosylations by modulating free hydroxyl reactivity. By exploiting this effect it is possible to simplify protecting group manipulation. In fact, diols can be used in some cases as acceptor in regioselective glycosylations. The

[53] Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottoson, H. *J. Org. Chem.* **1990**, *55*, 6068.

selective reaction of sterically less hindered primary hydroxyl groups in the presence of secondary ones is a routine protective-group strategy, which has found widespread use throughout organic synthesis.[54] The regioselectivity of glycosylation of *manno* and *gluco* 4,6 diols with donors equipped with 2-*O* participating group has been explored and it was found to be strongly dependent on the functionalization of the acceptor 3-OH. When 3-position is glycosylated good regioselectivity in (1→6)-linked product is achieved as expected. If the acceptor 3-OH is protected as benzyl ether reversed regioselectivity is observed (*Figure 1.31*).[55]

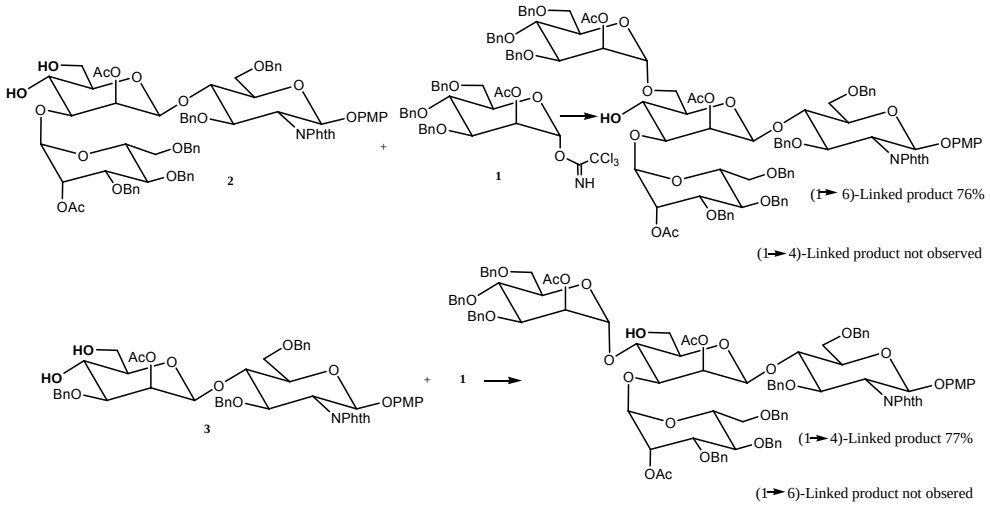


Figure 1.31 Regioselectivity decourse with 4,6-diols.

The proposed explanation of this unusual result is that electron-donating 3-*O*-benzyl protecting group increases the nucleophilicity of the neighbouring 4-hydroxyl; besides the mechanism may proceed via an initial reaction of the less hindered 6-hydroxyl

[54] Wuts, P. G. M.; Greene, T. W. *Protective Groups in Organic Synthesis*, 4th ed; Wiley: New York, **2007**.

[55] Rising, T. W. D. F. ; Heidecke, C. D. ; Fairbanks, A. J. *Synlett* **2007**, *9*, 1421-1425.

which, rather than forming a glycosydic linkage, might form an orthoester, which then rearranges to glycoside by meeting the remaining free hydroxyl at the 4-position (*Figure 1.32*).

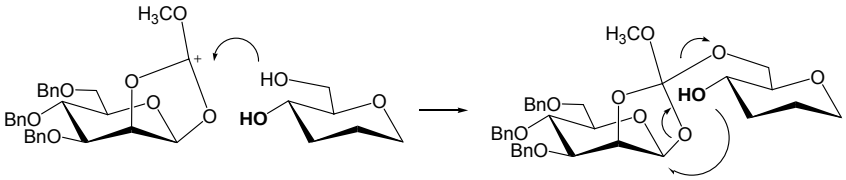


Figure 1.32 Hypothesis of orthoester rearrangement.

The presence of an adjacent hindered group can also depress the reactivity of hydroxyl otherwise more prone to glycosylations,[56] as it happens in the following example with a 3,4-diol 2-*O*-phtalimido protected glucosyl acceptor:[57]

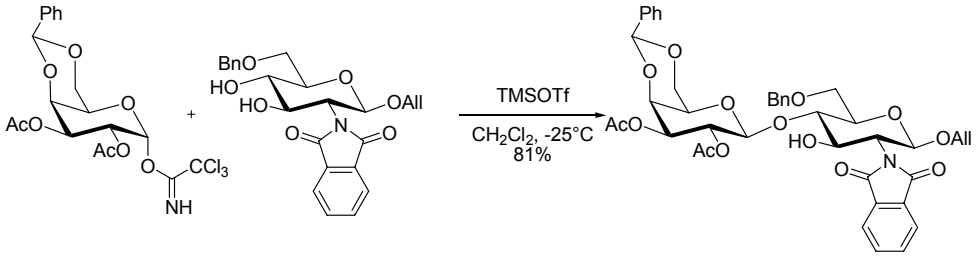


Figure 1.33 Influence of phtalimidoyl group on adjacent hydroxyl.

Finally, the assemblage of the desired protective pattern on each saccharidic residue requires the comparison of the relative reactivity of the hydroxyls present on each position. In aldo-hexo-pyranoses three types of hydroxyls can be recognized: the hemiacetalic at C-1, the carbinolics at C-2, C-3, C-4 and a primary alcohol at C-6. The

[56] Qui, D.; Ganghi, S. S. ; Koganty, R. R. *Tetrahedron Lett.* **1997**, *37*, 595.
[57] Figueroa-Pérez, S.; Verez-Bencomo, V. *Tetrahedron Lett.* **1998**, *39*, 9143.

hemiacetalic function can be easily transformed in a complete acetal under Fisher conditions (*Figure 1.34*).

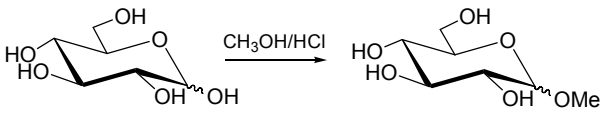


Figure 1.34 Protection of hemicetal position in Fisher conditions.

Generally the hemiacetal protection is the first reaction to be performed, mainly because of its instability under basic conditions.

The less hindered primary function is easily differentiated in the presence of secondary free positions with bulky protecting groups such as trityl (Tr), dimethoxytrityl (DMT) or *tert*-butyldiphenylsilyl (TBDPS).

Secondary axial hydroxyls generally exhibit a lower nucleophilic attitude than the equatorial ones (*Figure 1.35*).

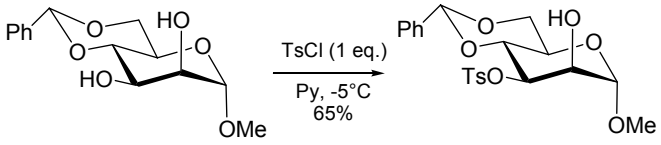


Figure 1.35 Relative nucleophilic behaviour of equatorial and axial hydroxyls.

Among these, in *gluco*-configurated sugars, 2-*O*-hydroxyl is the most reactive and 4-*O*-hydroxyl is the least one.

The 3-position is frequently regioselectively functionalized via stannylidene formation on vicinal diols enhancing the nucleophilicity of the equatorial oxygen.[58] The tin chemistry is used for the regioselective protection of an equatorial 3-hydroxyl group

[58] David, S.; Hanessian, S. *Tetrahedron Lett.* **1985**, *41*, 643.

cis-related with a vicinal 2-position in *manno*-configurated sugars or with the 4-position in *galacto*-configurated ones (*Figure 1.36*).

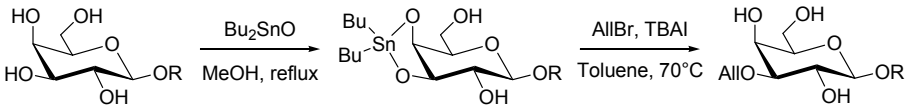


Figure 1.36 Regioselective 3-*O*-allylation *via* stannylidene acetal intermediate.

Cyclic protecting groups are very useful to lock two hydroxyls at the same time: benzylidene acetals usually involve the positions on C-4 and C-6 closing a six-membered ring; isopropylidene acetals, orthoesters and orthocarbonates require a *cis* relationship between the two groups to be protected (*Figure 1.37*).

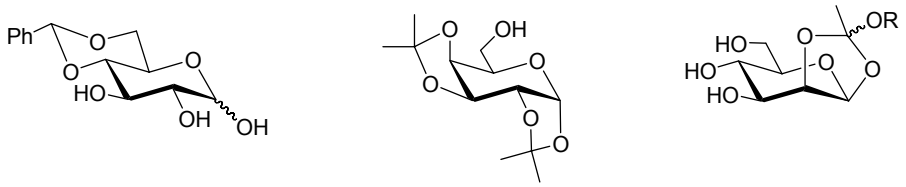


Figure 1.37 4,6-*O*-benzylidene-D-glucopyranose, 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside and β-D-mannopyranose 1,2-orthoacetate.

Regioselective opening of benzylidene acetals and orthoesters restores a free hydroxyl function and an already protected one.

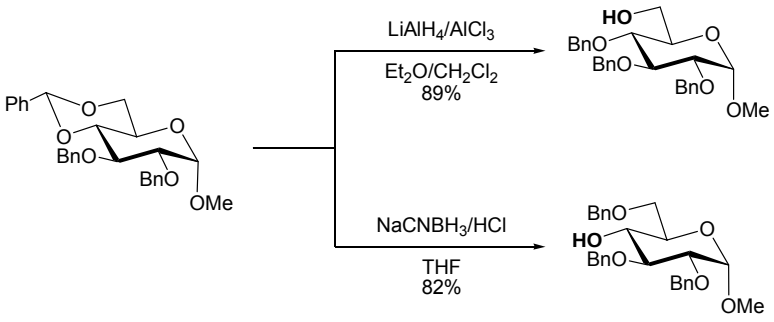


Figure 1.38 Reductive cleavage of benzylidene acetals.

Regioselectivity of benzylidene acetals reductive opening is strictly dependent on both the reducing system and the acid used (*Figure 1.38*).[59] Mild acidic hydrolytic opening of five-membered orthoesters affords the corresponding ester derivative on the axial position (*Figure 1.39*).[60]

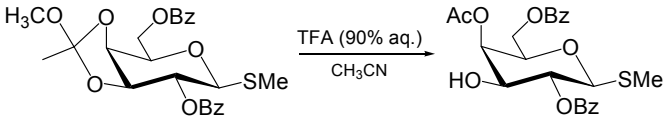


Figure 1.39 Regioselective cleavage of orthoesters.

1.8 Gold glyconanoparticles

As already discussed in the first part of this thesis, molecular recognition events involving carbohydrates occur in a wide variety of important biological processes including the functioning of the immune system. Involved in these phenomena are cell-cell communication events, which are driven by highly specific carbohydrate-lectin interaction on opposing cell surface.[61] The proteins involved in these molecular recognition processes are typically found in aggregated structures thereby presenting multiple binding or recognition sites for carbohydrate ligand. Such aggregates enhance the low binding affinity of the monomeric carbohydrate-protein interaction, an observation referred to as the cluster glycoside effect.[62] With consideration of the importance of such interactions, considerable research effort has focused on the development of new methodologies to study and quantify the formation of multivalent carbohydrate ligands. Previously, carbohydrate structures have been formulated: as self-assembled monolayers (SAMs) on 2D surfaces, both as single

[59] a) Gelas, J. *Adv. Carbohydr. Chem. Biochem.* **1981**, *39*, 71; b) Garegg, P. J. in *Preparative Carbohydrate Chemistry*, Hanessian, S., Ed., Marcel Dekker, New York, **1997**, pp.53-68.
[60] Lemieux, P. U.; Driguez, H. *J. Am. Chem. Soc.* **1975**, *15*, 4069.
[61] Sacchettini, J. C.; Baum, L. G.; Brewer, C. F. *Biochemistry* **2001**, *40*, 3009-2015.
[62] Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321-327.

ligands[63] and within arrays;[64] as dendrimers;[65] within liposome;[66] and on the backbone of polymers.[67] Recently, metal nanoparticles have been used to tether a variety of carbohydrate ligands.[68] Such 3D multivalent ligands provide a globular structure on which clustering and orientation effects may be studied.

In the formation of gold glyconanoparticles, a methanolic solution of the thiol-functionalized glycoconiugate is combined with an acqueous solution of tetrachloroauric acid ($HAuCl_4$) and an excess of $NaBH_4$ as reducing agent (*Figure 1.40*). The functionalized Au nanoparticles are purified by centrifugal filtration.

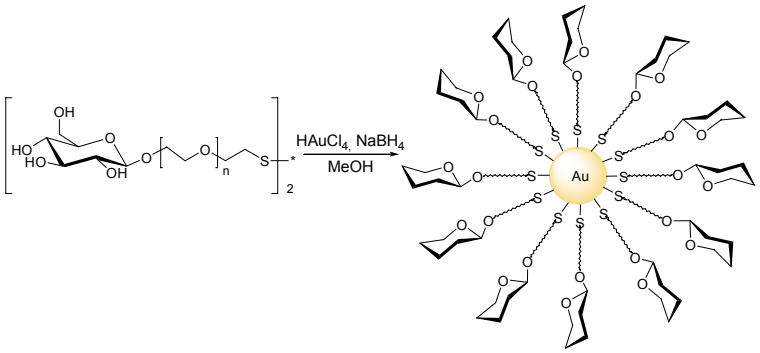


Figure 1.40 Formation of gold glyconanoparticles.

[63] a) Revell, D. J.; Knight, J. R.; Blyth, D. J.; Haines, A. H.; Russell, D. A. *Langmuir* **1998**, *14*, 4517-4524; b) Kitov, P. I.; Railton, C.; Bundle, D. R. *Carbohydr. Res.* **1998**, *307*, 361-369; c) Svedhem, S.; Ohberg, L.; Borreli, S.; Valiokas, R.; Andersson, M.; Oscarson, S.; Svensson, S. C. T.; Liedberg, B.; Konradsson, P. *Langmuir* **2002**, *18*, 2848-2858.

[64] a) Houseman, B. T.; Mrksich, M. *Chem. Biol.* **2002**, *9*, 443-454; b) Houseman, B. T.; Gawalt, E. S.; Mrksich, M. . *Langmuir* **2003**, *19*, 1522.1531; c) Wang, D.; Liu, S.; Trummer, B. J.; Deng, C.; Wang, A. *Nat. Biotechnol.* **2002**, *20*, 275-281; d) Love, K. R., Seeberger, P. H. *Angew. Chem. Int. Ed.* **2002**, *41*, 3583-3586.

[65] Turnbull, W. B.; Kalovidouris, S. A.; Stoddart, J. F. *Chem. Eur. J.* **2002**, *8*, 2988-3000.

[66] Tagawa, K.; Sendai, N.; Ohno, K.; Kawaguchi, T.; Kitano, H. *Bioconjugate Chem.* **1999**, *10*, 354-360.

[67] Choi, S.-K.; Mammen, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1997**, *119*, 4103-4111.

[68] a) Yoshizumi, A.; Kanayama, N.; Maehara, Y.; Ide, M; Kitano, H. *Langmuir* **1999**, *15*, 482-288; b) de la Fuente, J. M.; Barrientos, A. G.; Rojas, T. C.; Fernandez, A.; Penades, S. *Angew. Chem. Int. Ed.* **2001**, *40*, 2258-2261; c) Lin C.-C.; Yeh, Y.-C.; Chen, C.-L.; Chen, G.-F.; Chen, C.-C.; Wu, Y.-C. *J. Am. Soc.* **2002**, *124*, 3508-3509.

The properties of the metal core can be applied to develop methods for the study of molecular recognition between carbohydrates and their respective binding proteins. A recent approach makes use of nanoprobe-based affinity mass spectroscopy (NBAMS) for the identification of target-proteins and mapping binding-epitotes.[69] Once target proteins have been captured from a mixture by the nanoprobe, on-probe digestion followed by removal of unbound peptides allows rapid mapping of carbohydrate-recognition peptide sequences in the proteins as shown in *Figure 1.41*.

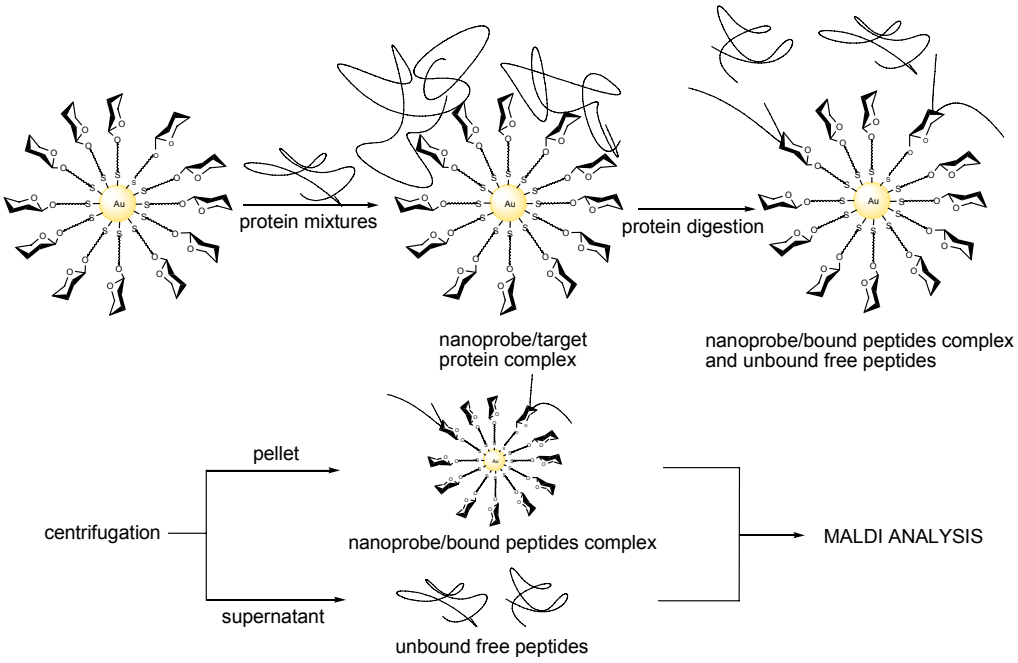


Figure 1.41 Analytical scheme of the NBAMS technique.

[69] Chen, Y.-J.; Chen, S.-H.; Chien, Y.-Y.; Chang, Y.-W.; Liao, H.-K.; Chang, C.-Y.; Jan, M.-D.; Wang, K.-T.; Lin, C.-C. *ChemBioChem* **2005**, *6*, 1169-1173.

Chapter 2

Synthesis of oligorhamnanic fragments from phytopathogenic bacteria

Introduction

2.1 Lipopolysaccharides

Gram-negative bacteria cell membrane is enveloped by a cell wall composed of a thin layer of peptidoglycan surrounded by an outer lipid membrane. Almost 80% of the cell surface is covered by lipopolysaccharides (LPSs) (*Figure 2.1*).

Lipopolysaccharides (*Figure 2.2*) are amphiphilic macromolecules consisting of a lipophilic moiety termed lipid A covalently linked to a hydrophilic heteropolysaccharide. **Lipid A** is composed of a bisphosphorylated β-D-(1→6)-linked glucosamine disaccharide backbone decorated with *N*- and *O*- linked fatty acids that anchor the molecule to the outer membrane via hydrophobic and electrostatic interactions. Some possible structural variations among bacterial serotypes are related to acyl and phosphate groups. The saccharidic portion can be composed of two different domains termed core and O-specific chain (or O-chain).

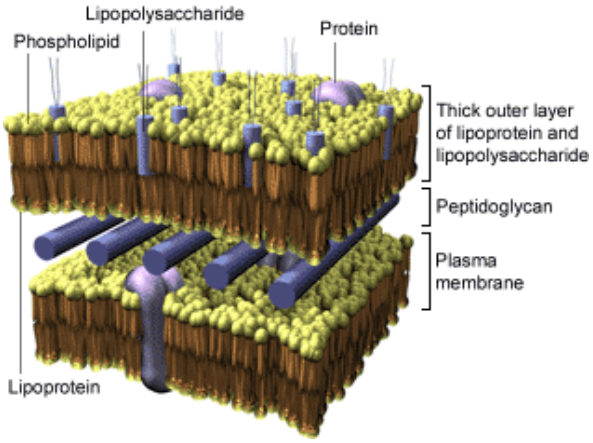


Figure 2.1 Gram-negative bacterial cell envelope.

The **core** is a complex oligosaccharide made up of two distinguishing regions: the inner core, which binds the entire saccharidic chain to lipid A by the means of a peculiar sugar, 3-deoxy-oct-2-ulosonic acid (Kdo), and the outer core consisting of more common sugars. The **O-chain** is the polysaccharide component structured in repeating units and lacking in rough-type lipopolysaccharides called lipooligosaccharides (LOSs).

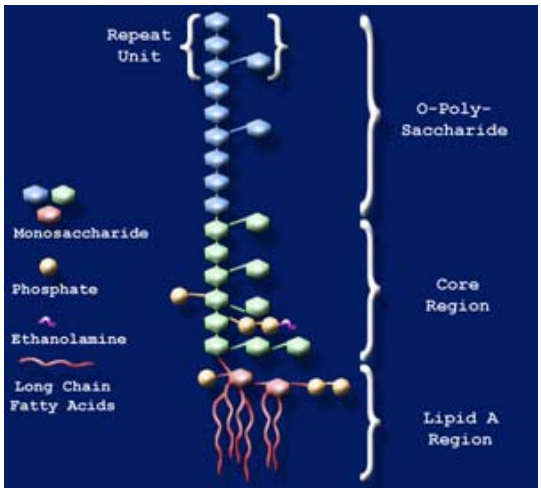


Figure 2.2 Schematic portrait of a lipopolysaccharide.

In animal and insect cells, innate immune defences are triggered by the perception of pathogen-associated molecular patterns (PAMPs), generally conserved and essential microbial structures including LPSs. The recognition of PAMPs by the receptors of the innate immune system (pathogen recognition receptors, PRRs) is often mediated by leucine-rich repeating proteins such as Toll in *Drosophila* and Toll-like receptors in mammals.[70] Recognition of LPSs occurs through the lipid A moiety, which is responsible for the most of biological effects of LPS in animals. Lipid A toxicity in animals strongly depends on its structure and is also modulated by the close core region, which possesses immunogenic properties.[71]

Instead, the O-chain possesses antigenic activity. Its variable structure among the different bacteria, in length, sugar residues, type of linkages, presence of non-stoichiometric functional groups and shape (linear or branched), allows a specific recognition stimulating the development of an acquired immunity in the host. So the O-chain is a serologically determinant which extends towards the outside of the cell.

2.2 O-specific polysaccharides of plant pathogenic bacteria

Most phytopathogenic bacteria belong to *Pseudomonas* (some species of the former are now settled in the *Burkolderia* and *Ralstonia* genera), *Xanthomonas* and *Erwinia* genera.[72] The polysaccharidic components arising from all phytopathogenic bacteria so far investigated are characterised by a relatively limited number of monoses. Generally the O-chain is composed of a linear backbone, mainly consisting of L-rhamnose, D-

[70] a) Miyake,K. *Trends Microbiol.* **2004**, *12*, 186-192; b) Medzhitov, R. *Nat. Rev. Immunol.* **2001**, *1*, 135-145; c) Triantafilou, M. and Triantafilou, K. *Trends Immunol*. **2002**, *23*, 301-304.

[71] a) Raetz, C. R. H. and Whitfield, C. *Annu. Rev. Biochem.* **2002**, *71*, 635-700; b) Alexander, C. and Rietschel, E. T. *J. Endotoxin Res.* **2001**, *7*, 167-202.

[72] For a review on phytopathogenic bacterial polysaccharides *O*-antigens, see: Corsaro, M. M.; de Castro, C.; Molinaro, A; Parrilli, M. *Recent Res. Devel. Phytochem.* **2001**, *5*, 119-138.

rhamnose and, sometimes, in 2-acetamido-2-deoxy-D-glucose, branched with a single sugar residue belonging to the following narrow range of monoses: L-xylose, D-xylose, D-fucose, 3-acetamido-3,6-dideoxy-D-galactose (*Figure 2.3*).

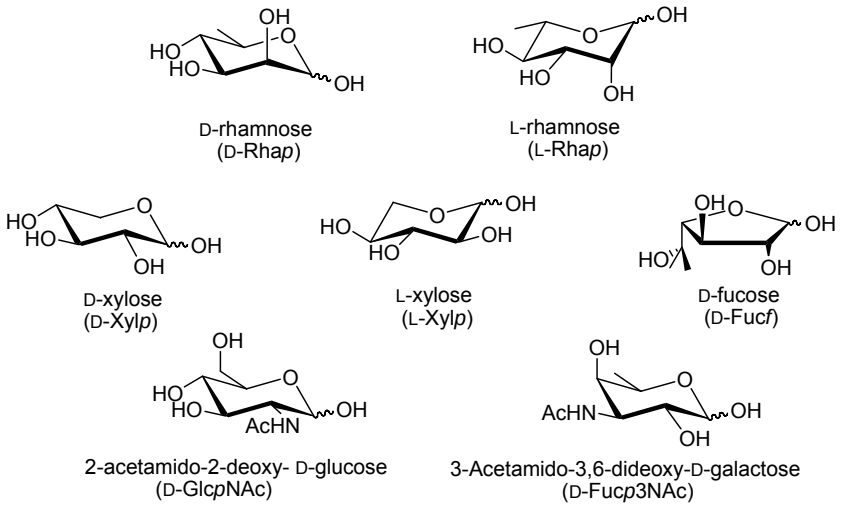


Figure 2.3 Common Sugars in Phytopathogenic *O*-antigens.

In the linear backbone residues are very frequently connected by α-(1→2) and α-(1→3) linkages and less frequently by β-(1→2), β-(1→3) and β-(1→4) ones. So, these structural restrictions lead to a small set of different backbones for the species of *Pseudomonas* and *Xanthomonas* so far studied, almost 80% of them presenting one of the following structures:

A	[→3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→]$_n$
B	[→3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→2)-α-L-Rha-(1→]$_n$
C	[→3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→]$_n$

Table 2.1 Common rhamnanic backbones of *O*-antigen polysaccharides from phytopathogenic bacteria.

In some cases there is a relationship between serological classification (serotype) based on monoclonal antibodies and monosaccharide composition of the O-specific polysaccharide (chemotype).

Then, LPSs are highly involved in bacterial pathogenesis both in animals and in plants; mechanisms of interaction between bacteria and eukaryotic host cells have been addressed by several studies on animal and human pathogens,[73] but very little is still known about LPS-plant interactions to date.

Apparently, LPSs play different roles in bacterial pathogenesis of plants. As major components of the outer membrane, LPSs are involved in the protection of bacterial cell, contributing to reduce the membrane permeability and thus allowing the growth in the unfavorable conditions of plant enviroment.[74] In contrast, LPSs can be recognized by plants to elicit or potentiate plant defence-related responses as part of a group of general elicitors that include flagellin and periplasmic oligosaccharides.[75] One of the most widely studied effects of LPSs on plant cells is their ability to prevent the **hypersensitive response** (HR) induced in plants by avirulent bacteria. HR is a programmed cell death response, triggered by live bacteria, that is often associated with plant host resistance. The mechanism of this effect, usually named as **localized induced resistance** (LIR),[76] is far from being completely elucidated but it seems to inhibit the diffusion of pathogen cells in the host. A recent study shows that lipid A and core region isolated from lipooligosaccharide of *Xanthomonas campestris* are able to trigger LIR response and the elicitation of transcription responses of specific genes

[73] *Endotoxins in health and desease* Brade, H.; Opal, S. M.; Vogel, S.; Morrison, D. C.; Eds. Marcel Dekker: New York 1999.

[74] Dow, J. M., Osbourn, A. E., Wilson, T. J. G., and Daniels, M. J. *Mol. Plant-Microbe. Interact.* **1995**, *8*, 768-777.

[75] a) Conrath, U., Thulke, O., Katz, V., Schwindling, S., and Kolher, A. *Eur. J. Plant Pathol.* **2001**, *107*, 113-119 b) Zeidler, D., Zahringer, U., Gerber, I., Dubery, I., Hartung, T., Bors, W., Hutzler, P., and Durner, J. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 15811-15816.

[76] Erbs, G., Newman, M.-A. *Mol Plant Pathol.* **2003**, *4*, 421-425.

(PR1 and PR2) associated with defence in the model plant *Arabidopsis thaliana* and this occurs by two independent ways.[77] Nevertheless, synthetic oligorhamnans mimicking the general structure named **A** in *Table 1* have also been proved to prevent hypersensitive response and to induce PR1 gene expression. This ability is believed to be based on structural features associated with the growing chain-length and the possibility to adopt a secondary structure defined by the presence of coils.[78]

An understanding of the effects that LPSs have on plants and how these are triggered, has implication both for the control of bacterial diseases through general non-specific mechanisms and for biocontrol of bacterial and fungal diseases by beneficial bacteria, which in some cases is believed to occur as a consequence of LPS recognition. The challenge ahead is to identify the plant components involved in LPS recognition and subsequent signal transduction. The appreciation that structurally distinct components within LPS may trigger the same plant responses, possibly via different receptors, has substantial implications for the design of genetic or biochemical screens to identify such receptors.

Since it is not possible to abstract pure O-antigens from natural LPS because of the presence of the inseparable core region, their synthesis is necessary for the purpose.

[77] Silipo, A.; Molinaro, A.; Sturiale, L.; Dow, J. M.; Erbs, G.; Lanzetta, R.; Newman, M-A.; Parrilli, M. *J. Biol. Chem.* **2005**, *280*, 33660-33668.

[78] Bedini, E.; De Castro, C.; Erbs, G.; Mangoni, L.; Dow, J. M.; Newman, M-A.; Parrilli, M. and Unverzagt, C. *J. Am. Chem. Soc.* **2005**, *127*, 2414-2416.

Results and discussion

2.3 Synthesis of oligorhamnans related to the most common *O*-chain backbone from phytopathogenic bacteria.

As it can be observed in *Table 2.1* all the depicted antigen structures are characterized by the presence of α-linked L-rhamnose units. Trisaccharide **A** and some of its oligomers have already been object of synthetic efforts in our laboratory,[79] but only a few bacterial strains present this structure as repeating unit of their O-chain backbone. Most frequently found are motifs **B** and **C**, above all the former that differs from **A** by the addition of a 2-linked rhamnose unit.

The synthesis of α-linked oligorhamnans was the target of several reports in the last two decades;[80] recently, a methyl and an octyl glycoside bearing a tetrasaccharide corresponding to motif **B** was also synthesized,[81] nevertheless their oligomerization was not attempted.

With this intent a tetrasaccharide building-block that would be easily functionalized both as a glycosyl donor and a glycosyl acceptor for stepwise condensation to higher oligosaccharides was assembled. Therefore, the protection pattern of this tetrasaccharide should have two orthogonal temporary protecting groups at position *O*-1A and *O*-3D and a permanent protecting group for all the remaining positions.

Thus, an allyl group was chosen for the anomeric position, a chloroacetyl for position *O*-3D and benzoyls for the other positions.

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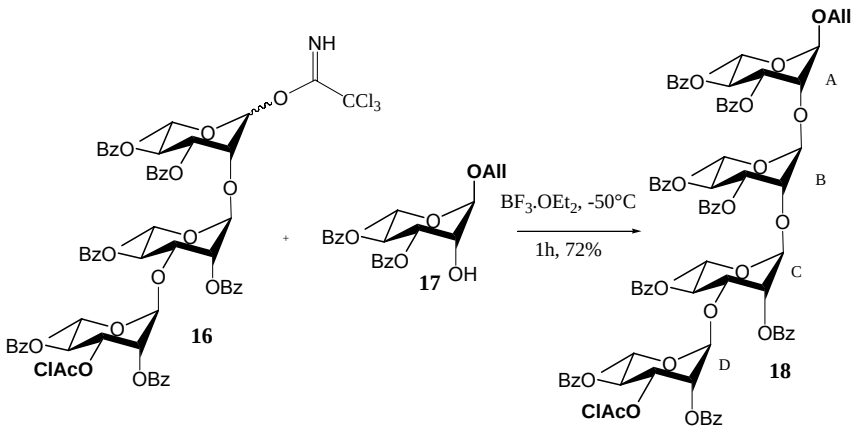
[79] Bedini, E.; Unverzagt, C.; Parrilli, M. *Tetrahedron Lett.* **2002**, *43*, 8879-8882.

[80] a) Zhu, Y.; Kong, F. *Synlett* **2000**, 1783-1787; b) Zou, W.; Sen, A. K.; Szarek, W. A.; MacLean, D. B. *Can. J. Chem.* **1993,** *71*, 2194-2200; c) Pozsgay, V.; Jennings, H. J. *J. Org. Chem.* **1988**, *53*, 4042-4052; d) Pozsgay, V.; Brisson, J.-R.; Jennings, H. J. *Can. J. Chem.* **1987**, *65*, 2764-2769; e) Pozsgay, V.; Nánási, P.; Neszmélyi, A. *Chem. Comm.* **1979**, 828-831.

[81] Zhang, J.; Zhu, Y.; Kong, F. *Carbohydr. Res.* **2001**, *336*, 229-235.

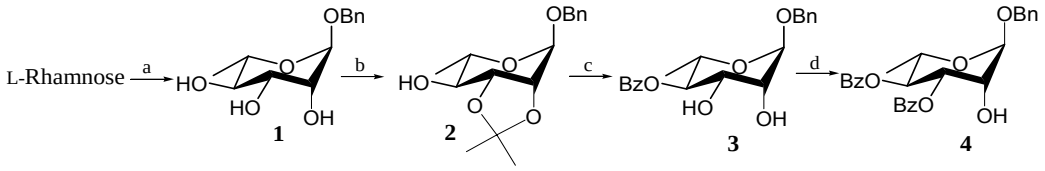


Scheme 2.1 Tetrasaccharide Building-Block with Orthogonal Protecting Set Assembly.

Tetrasaccharide **18** with this protecting pattern was obtained by glycosylation between trisaccharide donor **16** and allyl rhamnoside acceptor **17**. The reaction was performed in CH_2Cl_2 at -50°C with $BF_3.OEt_2$ as activator affording **18** in good yield and with the expected stereochemistry as illustrated in *Scheme 2.1*.

Trisaccharide **16** had already been synthesized in a previous work of our laboratory through the synthetic pathway reported in *Scheme 2.4*. It has been assembled from two different building-blocks: the thioglycoside donor **11** and the acceptor **4**.

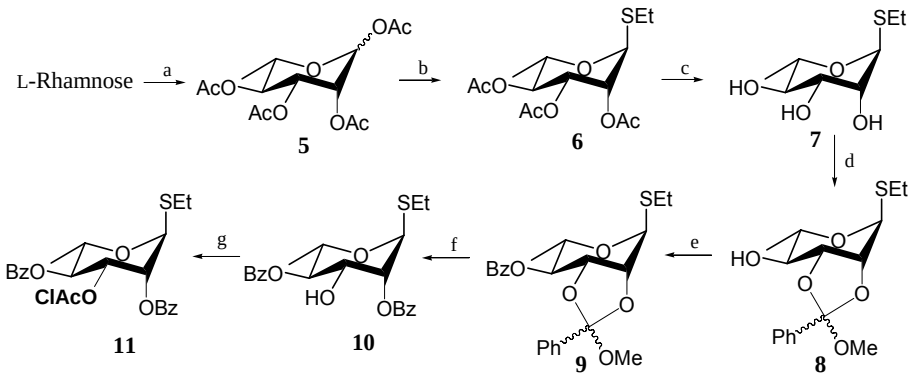
Acceptor **4** was synthesized from L-rhamnose as depicted in *Scheme 2.2*. Firstly, L-rhamnose was protected at the anomeric position by a Fisher glycosylation; regioselective benzoylations at positions *O*-3 and *O*-4 were achieved by isopropylidene temporary protection of the hydroxyls in *cis* relashionship, *O*-4 benzoylation, hydrolysis of the acetonide and regioselective benzoylation of equatorial *O*-3.



(a) Amberlist 15-H^+, BnOH, 45°C, 16 h, 54%; (b) pTsOH, DMP, rt, 1 h, 98%; (c) i. BzCl, Py, 0°C, 1 h; ii. TFA/water 4:1, rt, 45 min, 90%; (d) BzCl, Py/DCM 1:2, -25°C, 100 min, 57%.

Scheme 2.2 Synthesis of acceptor **4**.

Donor **11** was obtained as showed in *Scheme 2.3*.Thioglycoside **6** can be synthesized from the peracetylated rhamnose with ethanthiol in the presence of a Lewis acid. Deacetylation and closure of a cyclic orthoester at *O*-2 and *O*-3 positions allows the benzoylation of the only free position at *O*-4. A mild acidic hydrolytic opening of the orthoester gives the corresponding benzoic ester of the axial position and 3-OH free hydroxyl which is subsequentially chloroacetylated.

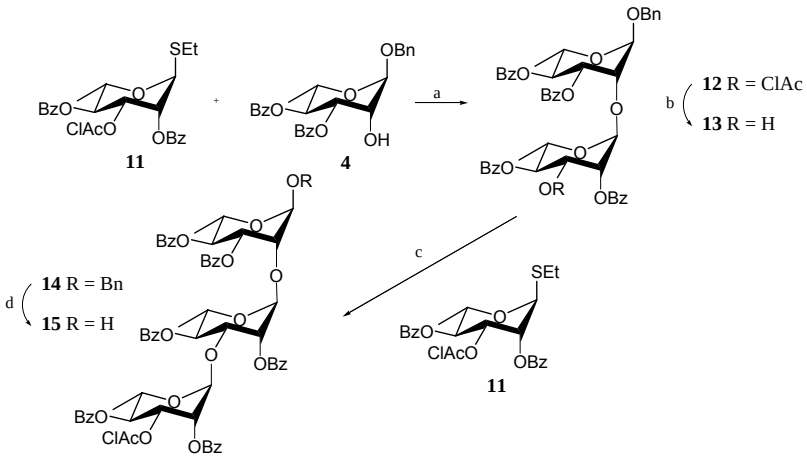


(a) 2:1 Ac_2O/Py, rt, overnight, 94%; (b) EtSH, $SnCl_4$, CH_2Cl_2, 0°C, 1h, 91%; (c) Na, MeOH, rt, 2h, 66%; (d) $PhC(OMe)_3$, CSA, DMF, 100 mBar, 50°C, 30 min; (e) BzCl, Py, 0°C, 30 min; (f) AcOH 80%, 0°C, 30 min, 52% over 3 steps; (g) $ClCH_2COCl$, Py/DMF 1:1, -45°C, 3 h, 71%

Scheme 2.3 Synthesis of thioglycoside donor **11**.

Thus, the coupling of donor **11** with acceptor **4** affords disaccharide **12** with NIS/TfOH, a typical activation system used for disarmed thioglycosides.

The benzoyls chosen as permanent protecting groups guarantee the complete α-stereoselectivity in glycosylations through neighbouring participation[82] and a single final deprotection step.



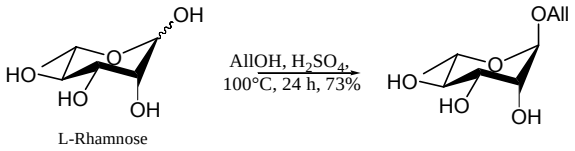
(a) NIS/TfOH, CH_2Cl_2, -20°C, 3h, 83%; (b) $(NH_2)_2CS$, EtOH, rt, 20h, 80%; (c) NIS/TfOH, CH_2Cl_2, -20°C, 40 min, 96%; (d) $FeCl_3$, CH_2Cl_2, rt, 66%.

Scheme 2.4 Trisaccharide acceptor assembly.

Selective removal of chloroacetyl group allows an iterative glycosylation with the same donor **11** furnishing trisaccharide **14**. Finally selective deprotection of the anomeric position with $FeCl_3$ gives the lactol **15** of the desired trisaccharide. Trichloroacetimidate was thus installed as a Fisher leaving group producing the starting point of the new work.

Acceptor **17** was obtained by anomeric allylation as shown below and regioselective benzoylation of *O*-3 and *O*-4 positions was achieved under the same conditions described in *Scheme 2.2*.

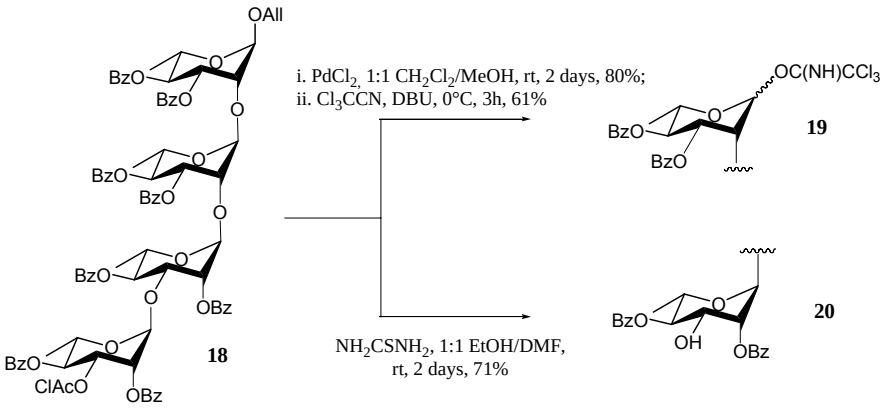
[82] See *Chapter 1* p.14.



Scheme 2.5 Anomeric Allylation

With the tetrasaccharide building-block **18** in hand the orthogonal set of protecting groups was selectively removed to afford directly acceptor **20,** by dechloroacetylation, or the lactol *via* Pd(II) catalyzed deallylation (*Scheme 2.6*).

The tetrasaccharide acceptor **20** was easily obtained in 71% yield by treating **18** with thiourea. Another aliquot of **18** was treated with catalytic $PdCl_2$ in 1:1 CH_2Cl_2/MeOH to afford the hemiacetal in 80% yield which was subsequently activated by treatment with Cl_3CCN in the presence of a base to give the trichloroacetimidate glycosyl donor **19** in 61% yield.



Scheme 2.6 Selective cleavage of orthogonal temporary protecting groups.

Unfortunately, glycosylation of **19** with **20** was unsuccessful: the resulting octasaccharide was obtained in a very low yield (<15%) by using $BF_3.OEt_2$ the

promoter. No improved yield was observed also by changing several reaction conditions.

However, the outcome of glycosylations is not completely predictable. We hypothesized that a too steric hindrance was responsible for the [4+4] glycosylation failure. So, the first plan was replaced by another one entailing the generation of requisite donor and acceptor from a single tetrasaccharidic building-block with a new approach involving the assemblage of shorter fragments.

The new designed strategy was based on a dimerization of the repeating unit by stepwise condensation of tetrasaccharide acceptor **20** with two different disaccharide donors, that would have a temporary and orthogonal protecting groups at position *O*-2_B and *O*-3_B respectively. Firstly, in analogy with the [4+4] strategy, a chloroacetyl was chosen as temporary protecting group; compounds **27** and **33** were therefore designed as suitable disaccharide donors (*Figure 2.4*).

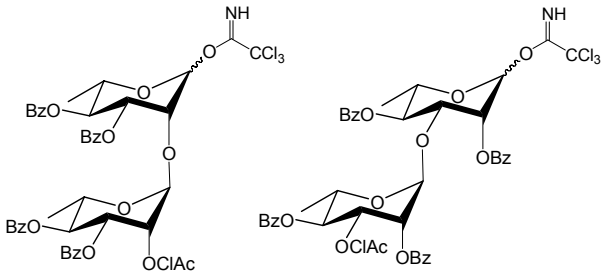
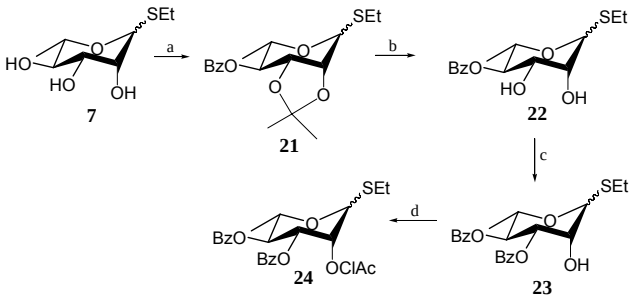


Figure 2.4 Suitable disaccharide donors.

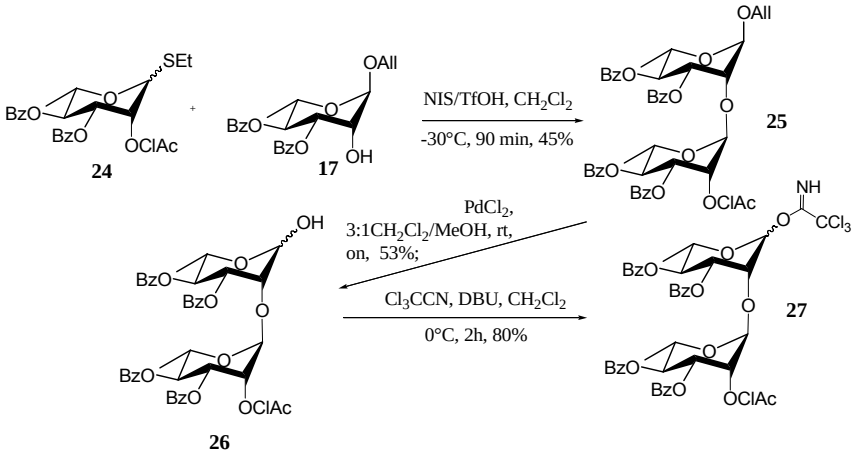
Compound **27** was obtained starting from ethyl thiorhamnopyranoside by regioselective *O*-4 benzoylation *via* an isopropylidene intermediate. The diol resulting from a mild hydrolysis was benzoylated at the equatorial position at low temperature to give selectively the 3,4-di-*O*-benzoylated alcohol **23** in 88% yield and chloroacetylation proceeded with modest yield affording **24** as reported in *Scheme 2.7*.



(a) i.CSA, DMP, rt, 45 min, 99%; ii. BzCl, Py, 0°C, 1h; (b) TFA/water 4:1, rt, 1h, 84% over 2 steps; (c) BzCl, 2:1 CH_2Cl_2/Py, -30°C, 2.5h, 88%; (d) $ClCH_2COCl$, 1:1 Py/DMF, rt, 4h, 56%.

Scheme 2.7

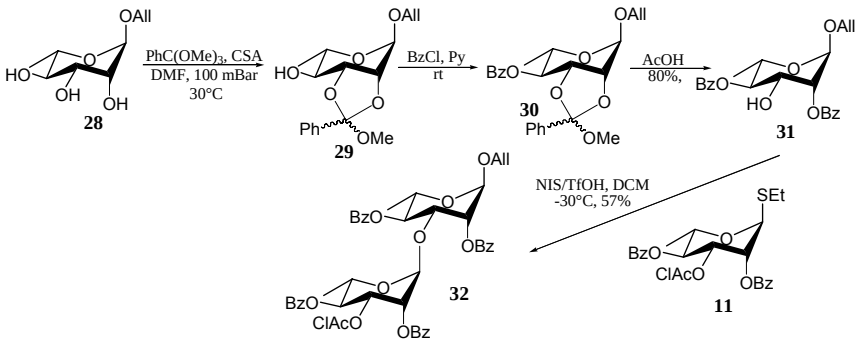
The resulting fully-protected thioglycoside was coupled with acceptor **17** by activation with NIS/TfOH at -30°C. Disaccharide **25** was obtained in not satisfactory yield (45%): this result was consistent with a recent report on the poor outcome of a condensation reaction involving a 2-chloroacetylated trichloroacetimidate as rhamnosyl donor.[83] In spite of the limited yield of the coupling, compound **25** was de-*O*-allylated with $PdCl_2$ to give hemi-acetal **26**, that in turn was converted to trichloroacetimidate **27** in 80% yield.



Scheme 2.8

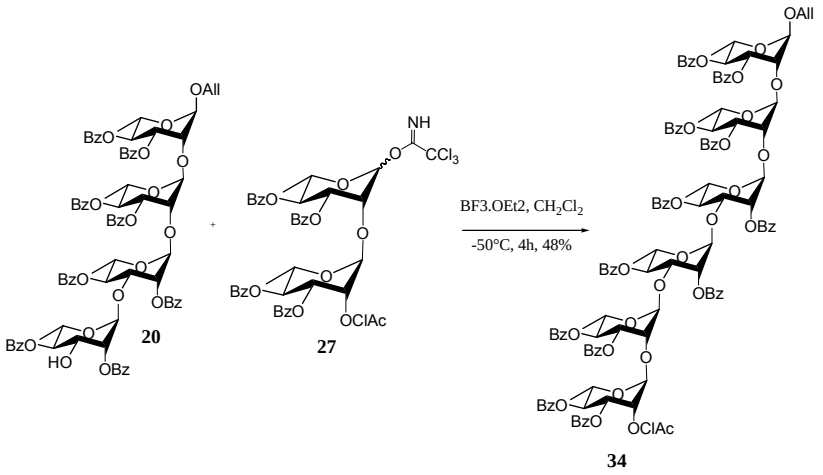
[83] Mulard, L. A.; Guerriero, C. *Tetrahedron* **2004**, *60*, 2475-2488.

Compound **33** was prepared from the lactol of the disaccharide **32** resulting from the coupling of thioglycosyde **11** with the acceptor **31** through the pathway depicted in *Scheme 2.9*.



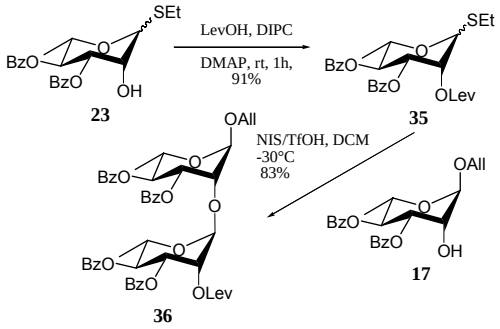
Scheme 2.9

Condensation of **27** with tetrasaccharide acceptor **20** at -50°C with $BF_3.OEt_2$ as activator gave hexasaccharide **34** in moderatly satisfying yield (*Scheme 2.10*).



Scheme 2.10

The low global yield of the synthetic path so designed was unsatisfactory. Thus, we decided to remodulate the temporary protecting group pattern of the new glycosyl donors replacing the chloroacetyl with a levulinoyl group. Levulinate esters are simply installed treating the alcohol with levulinic acid (LevOH) in the presence of an activating system composed by *N*,*N*'-diisopropylcarbodiimide (DIPC) and 4-dimethylaminopyridine (DMAP). Furthermore, a levulinate is cleaved in preference to a benzoate, generally under mild basic conditions as will be shown later. Thus, alcohol **23** was treated with levulinic acid, DIPC and DMAP to give the 2-*O*-levulinoylated thioglycoside **35** in very high yield, that was coupled with **17** by activation with NIS/TfOH at -30°C to afford disaccharide **36** in 83% yield (*Scheme 2.11*).

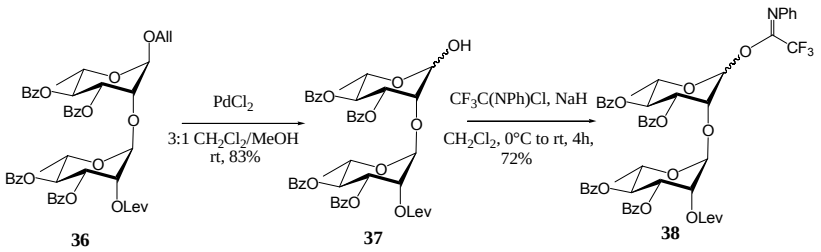


Scheme 2.11

In addition, the emergent use of *N*-phenyl trifluoroacetimidate donors recently shown to be very effective in glycosylation reactions involving deoxysugars,[84] urged us to replace the trichloroacetimidate leaving group, too.

Thus, hemiacetal **37** was obtained from **36** with Pd(II) catalysed deallylation and then converted into *N*-phenyltrifluoroacetimidate **38** by treatment with *N*-phenyl trifluoroacetimidoyl chloride in the presence of a base as shown in *Scheme 2.12*.

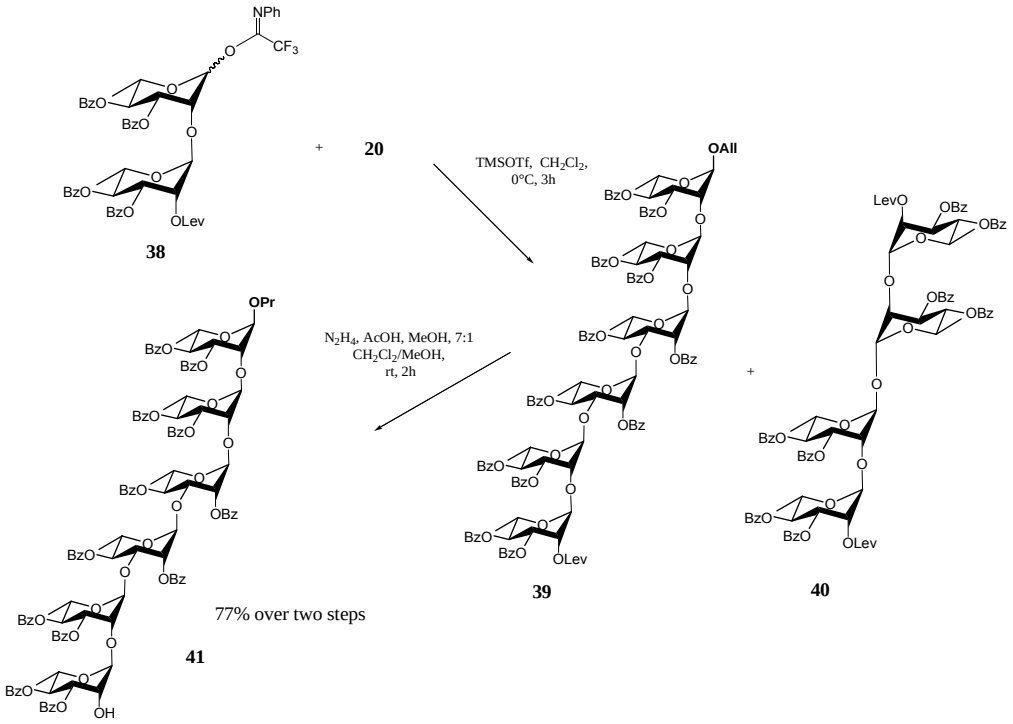
[84] A comparative study between the two trihaloacetimidate families is object of *Chapter 3*.



36 **37** **38**

Scheme 2.12

Coupling of disaccharide donor **38** with tetrasaccharide acceptor **20** by activation with TMSOTf in CH_2Cl_2 at 0°C proceeded very satisfyingly. Actually, an exact yield of this condensation was not obtained, since, after column chromatography, the resulting hexasaccharide **39** was contaminated by traces of tetrasaccharide **40**, a side product due to self-condensation of **38** (*Scheme 2.13*).



38 + **20** → **39** + **40**

39 → **41**

Scheme 2.13

The cleavage of levulinic group was performed with hydrazinium acetate in 7:1 CH_2Cl_2/MeOH affording pure hexasaccharide acceptor **41** in 77% yield (calculated from **20**) but an unexpected side-reaction reduced the allyl aglycon to propyl by a diimide-like species *in situ* generated.

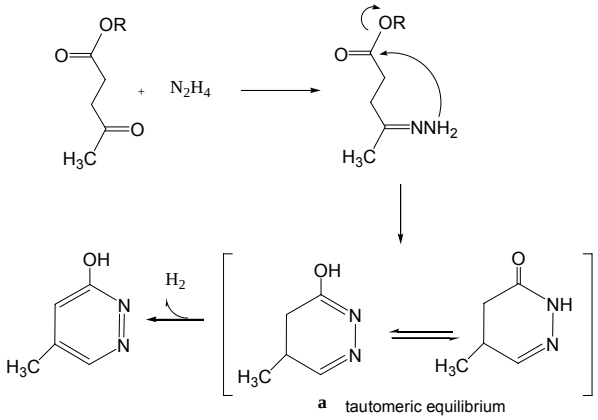


Figure 2.5 Hypothesized mechanism of riducent agent *in situ* generation.

Hydrazine reacts with the ketone to give an hydrazone that closes a six-membered ring though an addition-elimination mechanism at the acylic carbon atom. Compound **a** in equilibrium with its tautomer would transfer hydrogen like a diimide converting into an aromatic species.

The known mechanism with diimide is pictured as a transfer of hydrogen via a nonpolar cyclic transition state (*Figure 2.6*).[85]

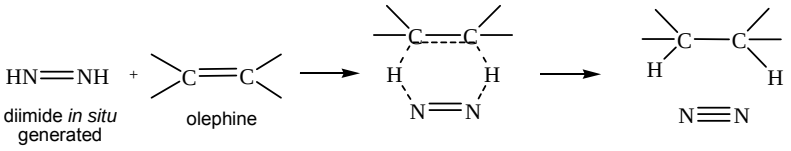
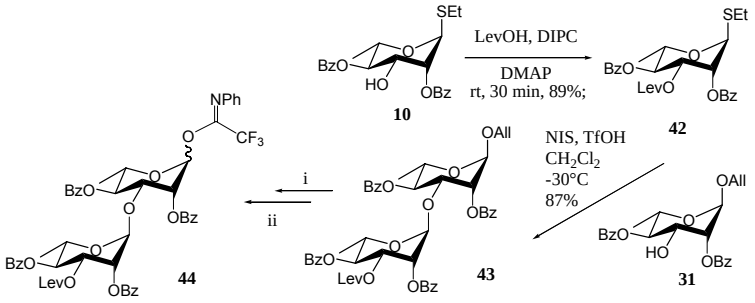


Figure 2.6 Reduction of the allyl group by diimide.

[85] Corey, E. J.; Pasto, D. J.; Mock, W. L. *J. Am. Chem. Soc.* **1961**, *83*, 2957-2958.

A *J*-coupled HSQC experiment on **41** confirmed the α-configuration of the newly formed glycosidic linkage ($^1J_{C,H}$=172 Hz). The increase of the global yield obtained with a levulinoyl in place of a chloroacetyl as *O*-2 temporary protecting group prompted us to re-design the synthesis of the second disaccharide donor by using the same protective pattern. Thus, the synthesis of donor **44** to replace **17** was undertaken. The alcohol **10** was converted to 3-*O*-levulinoylated thioglycoside **42** in 89% yield and also the coupling with acceptor **31** proceeded in high yield by activation with NIS/TfOH at -30°C.



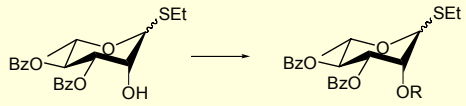
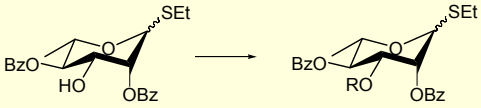
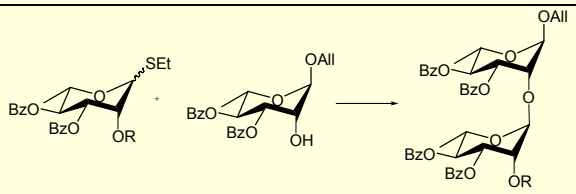
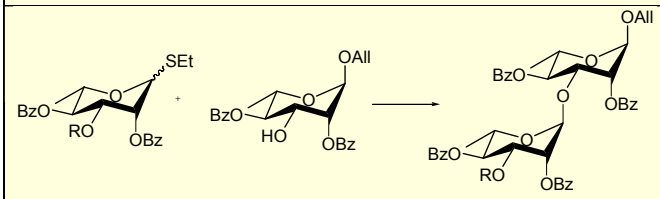
i. $PdCl_2$, 3:1 CH_2Cl_2/MeOH, rt, 60%; ii. $CF_3C(NPh)Cl$, NaH, CH_2Cl_2, 0°C to rt, 4h, 58%

Scheme 2.14

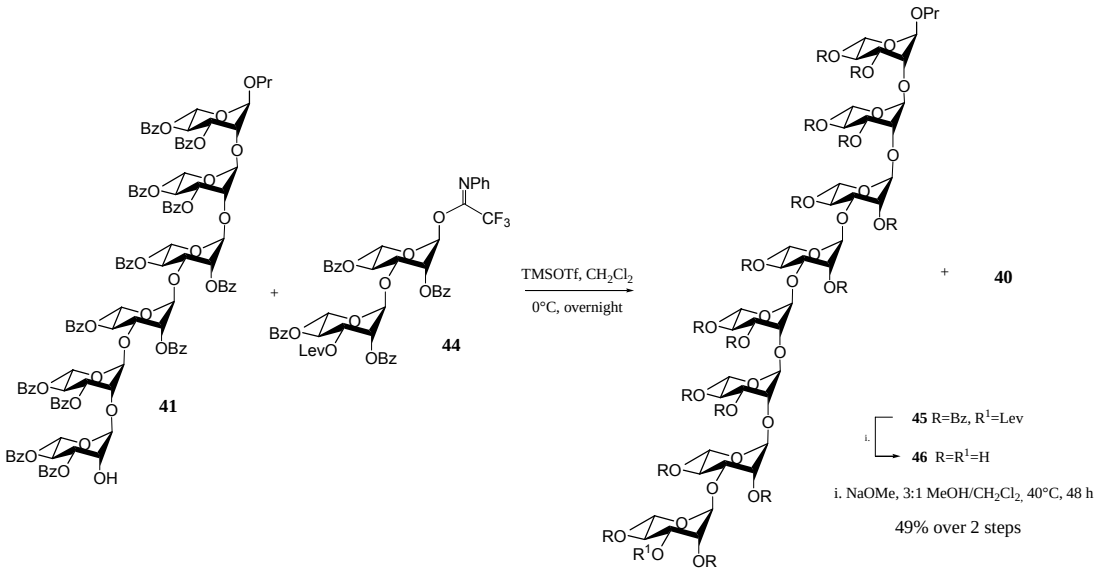
Disaccharide **43** was de-*O*-allylated and the resulting hemiacetal converted into *N*-phenyltrifluoroacetimidate **44** (*Scheme 2.14*).

The improvement of the global strategy obtained replacing a chloroacetyl with levulinoyl protecting group is summarized in T*able 2..2*. The increase of the yield regards both installation and glycosylation steps.

Table 2.2

	R	*Yield (%)*
	Lev	91
	ClAc	56
	Lev	89
	ClAc	71
	Lev	83
	ClAc	45
	Lev	87
	ClAc	57

Elongation of hexasaccharide **41** by condensation with disaccharide donor **44** gave, after column chromatography, an octasaccharide contaminated again by tetrasaccharide **40**. The use of levulinoyl group, furthermore, doesn't increase the synthetic steps number since the cleavage conditions are those used for benzoyls. Thus a unique final step by Zemplén transesterification on the mixture afforded pure propyl octasaccharide **46** (49% calculated from **41**) as depicted in *Scheme 2.15*.



Scheme 2.15 Octasaccharide assembly and global deprotection of the target

The deprotected target was then purified by size exclusion chromatography; its ^{1}H NMR spectrum is reported in *Figure 2.6*.

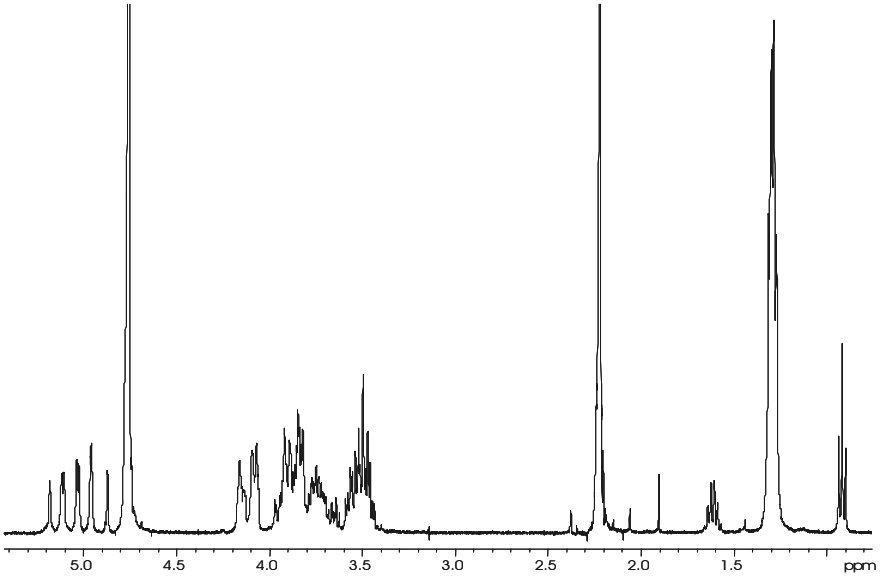
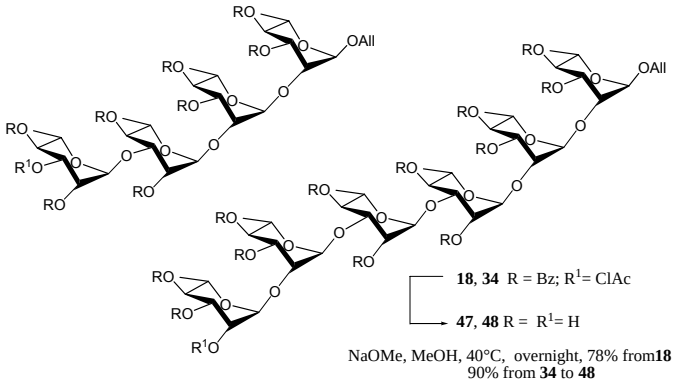


Figure 2.6 ^{1}H NMR spectrum (D_2O, 400 MHz; acetone as internal standard) of **46**.

Similarly, ester deprotection on **18** and **34** gave tetrasaccharide **47** (78%) and hexasaccharide **48** (90%) (*Scheme 2.17*). According to preliminary phytopathogenic tests compounds **46**, **47** and **48** resulted active in triggering plant defence response but more thorough investigations are currently in course.



Scheme 2.17 Global deprotection of the targets.

Conclusions

The synthesis of the tetrasaccharide rhamnanic motif α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→2)-α-L-Rha and its dimerization to octasaccharide has been developed. Three different pathways towards the dimerization have been investigated; the best one was based on a [4+2]+2 stepwise condensation of a rhamnose tetrasaccharide with two rhamnosyl *N*-phenyl trifluoroacetimidates as glycosyl donors and on an orthogonal set of protecting groups such as benzoyl, levulinoyl and allyl. Tetrasaccharidic, esasaccharidic and octasaccharidic synthetic moieties have also been isolated for phytopathogenic tests, which are currently underway.

2.4 Synthesis of the trisaccharidic repeating unit of the *O*-chain from *Xanthomonas campestris* pv. *campestris* 8004.

Xanthomonas campestris pv. *campestris* (*Xcc*) is the causative agent of black rot, a disease of cruciferous crops that is of worldwide importance. *Xcc* can also infect non-crop crucifers, such as the model plant *A. thaliana* on which leaves are routinely performed biological tests. A recent study has demonstrated the effectiveness of both lipid A and oligosaccharide extracted from this bacterium in LIR triggering and, moreover, with two independent mechanisms.[77] Investigation of biological activity of the *O*-chain moiety would implies its isolation in a pure form inaccessible by selective chemical degradation. Thus, synthetic procurement of *O*-chain fragments offers the only pratical approach.

The trisaccharide repeating unit of the *O*-chain from *Xcc* 8004 consists of a D-rhamnose disaccharide backbone decorated by a 3-acetamido-3,6-dideoxy-D-galactopyranose (D-Fuc*p*3NAc) unit as depicted under in *Figure 2.8*.

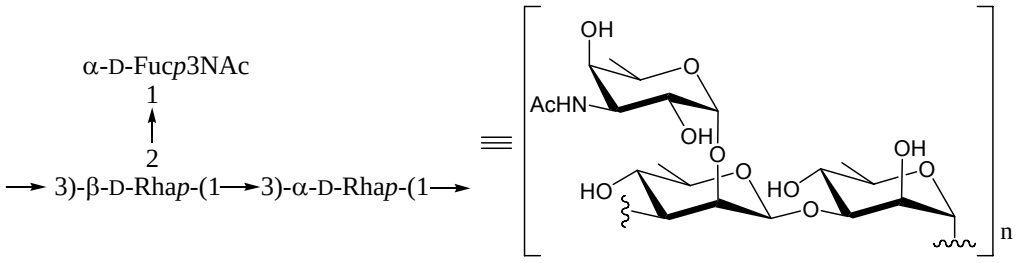


Figure 2.8 Trisaccharide repeating unit of *Xcc* 8004.

Very few syntheses of D-rhamnose oligosaccharides have been hitherto reported,[86] mainly because of the lack of direct access to D-rhamnose and protected derivatives thereof in large quantities. In addition, there is no commercial supply also for D-

[86] a) Kihlberg, J.; Bundle, D. R. *Carbohydr. Res.* **1991**, *216*, 67-78; b) Tsvetkov, Y. E.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1989**, *193*, 75-90; c) Fauré, R.; Shiao, T.C.; Lagnoux, D.; Giguère, D.; Roy, R. *Org. Biomol. Chem.* **2007**, *5*, 2704-2708.

Fuc*p*3NAc. Thus, the first difficulty of this synthesis is the non-commercial availability of both the unusual constituents monosaccharides, which had to be synthesized from other precursors.

Furthermore, the presence of two *cis*-configured sugars renders the synthesis very challenging. The stereoselective construction of α-fucosidic and β-rhamnosidic linkages can not be guaranteed by an approach as efficient as the neighbouring participation effect exerted by acyl groups on C-2 position in the stereo-controlled synthesis of 1,2-*trans* glycosides.[87]

The synthesis of this trisaccharide repeating unit as methyl glycoside was already reported in a previous work performed in our laboratory[88] but the overall yield of the synthesis was not high enough to allow further manipulation towards higher oligomers.

An efficient D-Fuc*p*3NAc donor requires essential features.

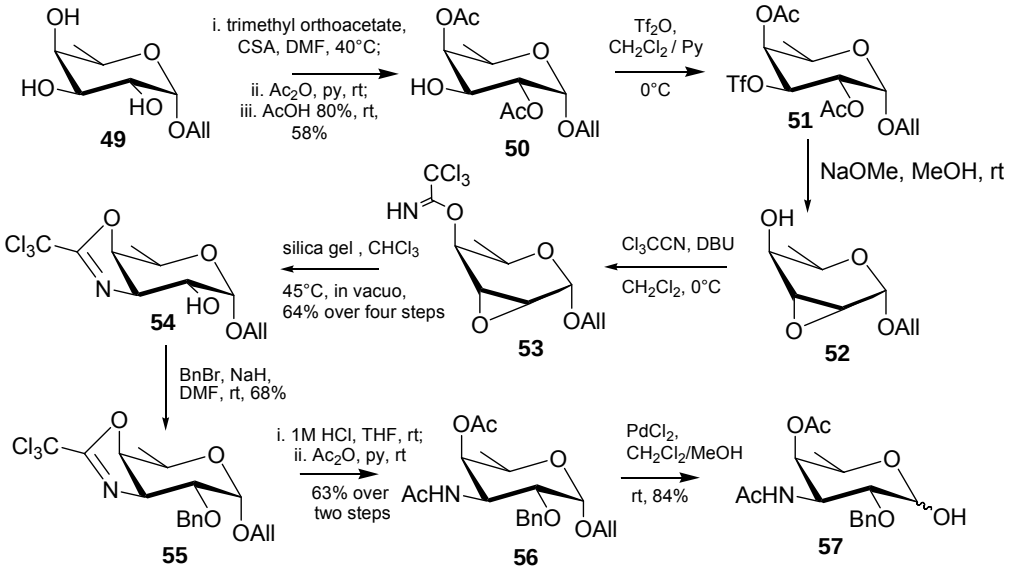
Firstly, since it occurs in the natural product as α-glycoside, a non-participating protecting group pattern, such as an ether-type one, has to be chosen. This restriction further increase the instability of the deoxy-sugar donor which is by itself acid-labile. In this regard, an high-yielding synthetic strategy is necessary to achieve the excess amount required in the glycosylation step.

Furthermore, introduction of a nitrogen moiety at *C*-3 on the commercially available D-fucose is the key point of the synthesis.

A previous but too long synthetic strategy[89] was performed on an allyl glycoside involving an intramolecular cyclization of the α,β-epoxytrichloroacetimidate **53** for the introduction of the nitrogen moiety. This synthetic pathway is reported in *Scheme 2.17*. The final lactol **57** thus obtained was derivatized either as trichloroacetimidate,

[87] For a discussion about the problem of 1,2-*cis* glycoside synthesis see *Chapter 1* pp.18-22.
[88] Bedini, E.; Carabellese, A.; Barone, G.; Parrilli, M. *J. Org. Chem.* **2005**, *70*, 8064-8070.
[89] Bedini, E.; Iadonisi, A.; Carabellese, A. and Parrilli, M. *Tetrahedron Lett.* **2004**, *45*, 4445-4448.

and or as the more stable *N*-phenyl trifluoroacetimidate (in 3.5% overall yield from D-Fucose). However, the glycosylation step with the sterically crowded rhamnose disaccharide acceptor proceeded in very poor yields under a wide range of activation conditions and donor amounts.



Scheme 2.17 First synthesis of D-fuc*p*3NAc donor

In the course of a previous investigation aimed at a more expedient donor, a thioalkyl group was installed on the lactol **57**, but an inseparable anomeric mixture was obtained and the glycosylation afforded only traces of the desired trisaccharide. To overcome the known inhibitory effect of NHAc in glycosylations,[90] a *N*,*N*-diacetylated thioglycoside was synthesized as an easily separable anomeric mixture.

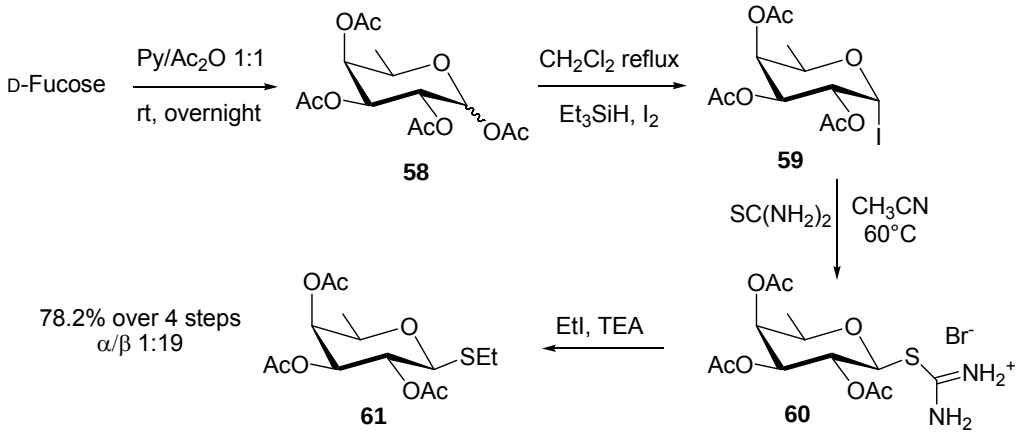
The two anomers showed different reactivity in glycosylation with the disaccharide acceptor: while the α-thioglycoside gave only traces of the product, the β-

[90] a) Schmidt, R.R., Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21-123; b) Lucas, R.; Hamza, D.; Lubineau, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **2004**, 2107-2117; c) Liao, L.; Auzanneau, F.-I. *Org. Lett.* **2003**, *5*, 2607-2610.

thioglycoside afforded the coupling in higher and satisfying yield. Unfortunately the α/β ratio of the thioalkyl group installation was 1:1 with a global yield of 3% over 15 steps from D-fucose.

Having established the β-thioglycoside as a suitable D-Fuc*p*3NAc donor, we tried to plan a more efficient synthesis by installing the thioalkyl group at the beginning with the intent to increase the global yield.

Thus, β-thioglycoside **61** was obtained in high yield and stereoselectivity from D-fucose via a thiouronium intermediate generated from fucosyl iodide[91] as showed in *Scheme 2.18*.

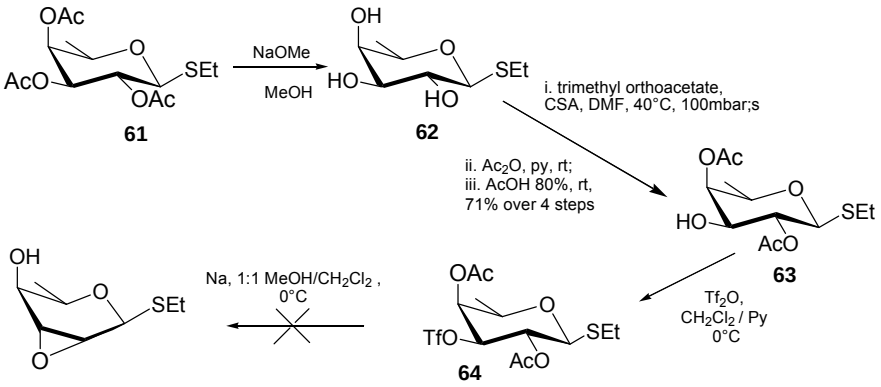


Scheme 2.18 Preparation of β-thiofucosylpyranoside by iodide intermediate.

The first intent was to follow the analogous route used for the installation of the nitrogen by an α,β-epoxytrichloroacetimidate intramolecular cyclization applied on the β-thioglycoside. Compound **61** was then deacetylated and regioselectively protected at positions *O*-2 and *O*-4 in a one-pot sequence of three steps (orthoesterification, acetylation, orthoester hydrolysis) to afford alcohol **63** (71% over four steps) (*Scheme 19*). Unfortunately, treatment of triflate derivative **64** with sodium

[91] Valerio, S.; Iadonisi, A.; Adinolfi, M.; Ravidà, A. *J. Org. Chem.* **2007**, *72*, 6097-6106.

methoxide did not afford the desired 2,3-epoxide derivative. Instead a complex mixture was obtained probably due to the *trans*-configuration of a good leaving group at position *O*-2 and the adjacent thioalkyl moiety, that was prone to give internal displacement of the epoxide.[92] This generated a glycosyl oxonium ion, whose uncontrollable reactivity could explain the complex mixture of products obtained in this reaction.



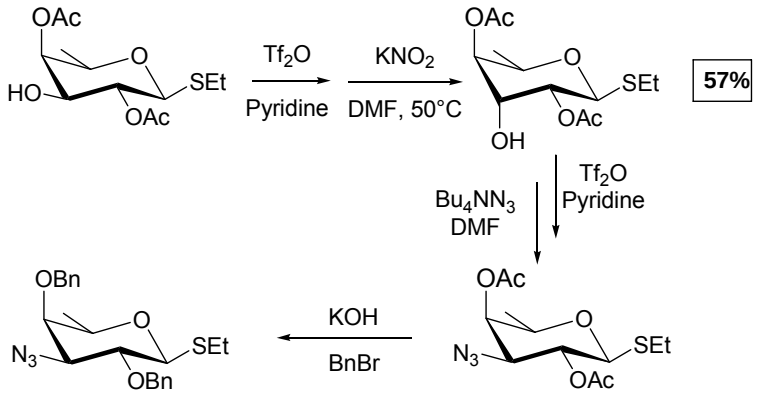
Scheme 2.19

Alternatively, the introduction of a nitrogen function at position C-3 was planned via a Lattrell-Dax[93] epimerization followed by a S_N2 azide displacement. Furthermore, we renounced to the α-directing long-range effect exerted by acetyl group on position *O*-4 introducing a benzyl group. This expedient reduces the protective pattern manipulation and would increase the reactivity of the donor towards the glycosylation. However, the treatment of triflate **64** with KNO_2 in strictly anhydrous

[92] a) Ryan, K.J.; Acton, E.M.; Goodman, L. *J. Org. Chem.* **1971**, *36*, 2646-2657 ; b) Hou, D. ; Lowary, T. L. Org. Lett. **2007**, *9*, 4487-4490.

[93] a) Lattrell, R.; Lohaus, G. *Justus Liebigs Ann. Chem.* **1974**, 901-920; b) Albert, R.; Dax, K.; Link, R.W.; Stuetz, A.E. *Carbohydr. Res.* **1983**, *118*, C5-C6.

DMF[94] afforded the desired 6-deoxy-gulose (antiarose) derivative in only moderate yield (57%) as showed in *Scheme 2.20*.



Scheme 2.20 Epimerization and S_N2 azide displacement.

Therefore, this route was not further developed. Inspired by the recent synthesis of ravidosamine (L-Fuc*p*3NMe2)[95] and by the synthesis of the unnatural 3-acetamido-3,6-dideoxy-L-galactopyranose (L-Fuc*p*3NAc)[96] (*Figure 2.9*), we planned the insertion of the nitrogen function at position C-3 by oxime reduction.

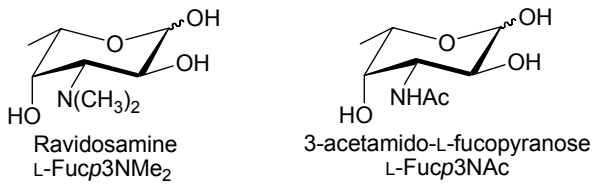
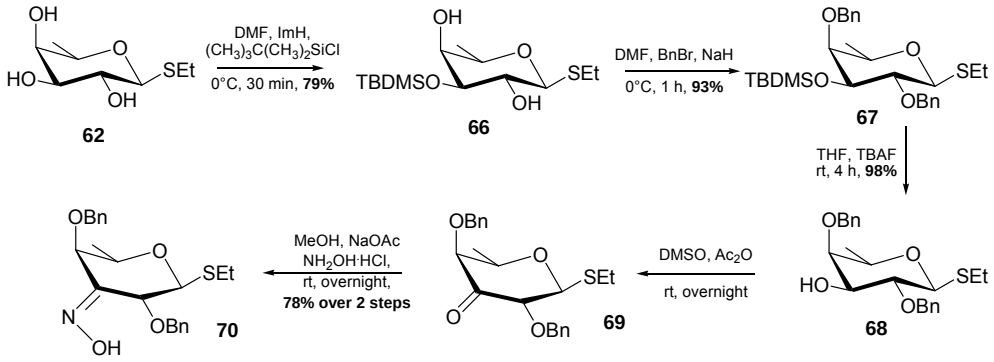


Figure 2.9

[94] Dong, H.; Pei, Z.; Ramström, O. *J. Org. Chem.* **2006**, *71*, 3306-3309.
[95] Hsu, D.-S.; Matsumoto, T.; Suzuki, K. *Synlett* **2005**, 801-804.
[96] Adinolfi, M.; Barone, G.; Corsaro, M.M.; Lanzetta, R.; Monaco, P. *J. Carbohydr. Chem.* **1995**, *14*, 913-928.

Thus, thioglycoside **61** was deacetylated and the resulting triol was regioselectively silylated at the most reactive position *O*-3[97] to give diol **66** (*Scheme 2.21)*. Benzylation and subsequent cleavage of TBDMS group afforded **68**, that gave in turn compound **70** after oxidation of the alcohol and oximation of the resulting ketone.



Scheme 2.21 Oxime formation by ketone intermediate

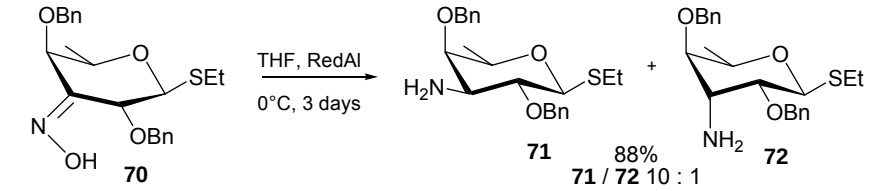
Several reduction conditions screened on oxime **70** are reported in *Table 2.3*.

Reducing system	*Solvent*	*Yield*
SmI_2	THF	no product
$LiAlH_4$	THF	no product
$LiAlH_4$	Et_2O	traces
Red-Al®	THF	88%

Table 2.3 Reduction conditions screened on the oxime.

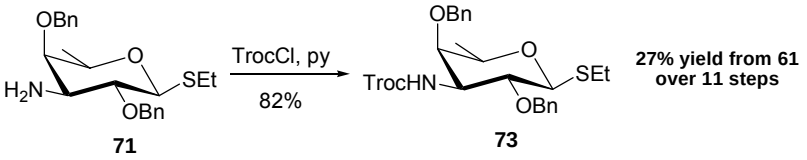
Red-Al® in THF at 0°C was found to be the suitable reducing agent giving good yield and stereoselectivity. The desired *galacto*-configured amine **71** was afforded together with the *gulo*-configured compound **72** in 88% yield (**71/72**=10:1) (*Scheme 2.22*).

[97] Hua, Y.; Gu, G.; Du, Y. *Carbohydr. Res.* **2004**, *339*, 867-872.



Scheme 2.22 Oxime reduction with Red-Al®

After separation of the two epimers by column chromatography, the amino moiety of **71** was protected with a trichloroethoxycarbonyl group (Troc) to give the D-Fuc*p*3NAc donor **73**. The overall yield of the new synthetic path from peracetylated D-Fucose is thus strongly improved giving the D-Fuc*p*3NAc donor **73** in 27% over 11 steps *versus* the 3% yield obtained from the previous way (*Scheme 2.17*).



Scheme 2.23 Protection of the amino group

The 1,2-*cis*-diglycosylated moiety present on the β-D-rhamnose unit clearly suggested a synthetic approach, in which the β-D-rhamnosidic linkage is firstly built up to give a rhamnose disaccharide with an orthogonal protecting-group pattern, that allows the selective deprotection on *O*-2$_B$ position, for the α-coupling with D-Fuc*p*3NAc donor **73**, and on *O*-1$_A$ and *O*-3$_B$ for the elongation towards oligosaccharides.

The *manno* configuration of D-rhamnose makes its β-stereoselective coupling part of a synthetic challenge on which several research groups focused their attention on last

two decades.[98] Nevertheless, many protocols for β-mannosylation require the use of 4,6-benzylidene-protected donor and therefore are not applicable to the rhamnose series. Thus we try to explore the possibility to apply a "non-benzylidene requiring" method.[99] Among such protocols, the attention was focused on the use of a 2-*O*-sulfonate group, whose electron-withdrawing effect was already demonstrated to be β-directing in glycosylation with L-rhamno-chlorides[100] and more recently extended to thioglycosides.[101] Among the several different sulfonate groups already reported to this purpose, the benzylsulfonyl has been recently exploited on a β-glycosylation of trichloroacetimidate mannosyl donors.[49] Since a benzylsulfonyl group can be very easily installed on a hydroxyl function and selectively cleaved in the presence of ether-based protecting groups,[102] its use both as β-directing and temporary protecting group could be advantageous. In orthogonal relationship methyl and allyl groups were chosen for the temporary protection respectively of *O*-1$_A$ and *O*-3$_B$ positions (*Figure 2.10*).

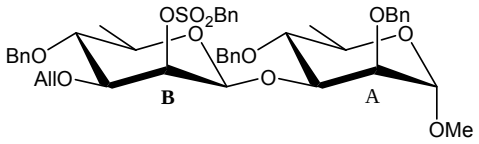
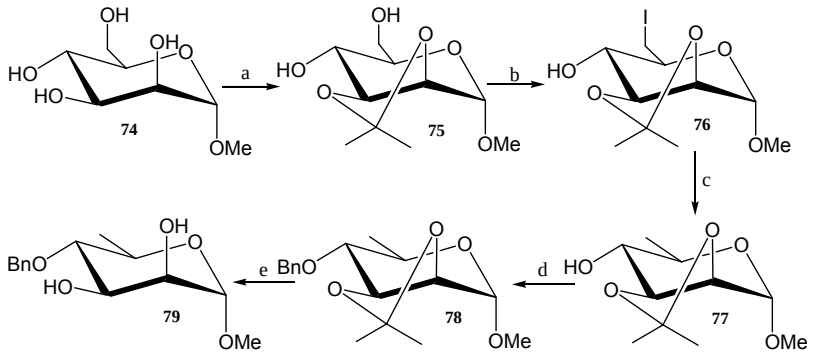


Figure 2.10 Suitable dirhamnose moiety.

The starting point of the synthesis is the commercially available methyl-α-D-mannoside that is converted to a 6-deoxy sugar by regioselective iodination of the primary function and subsequent reduction with tributyltin hydride in the presence of

[98] For a review see: Gridley, J.J.; Osborn, H.M.I. *J. Chem. Soc. Perkin Trans 1* **2000**, 1471-1491
[99] β-mannosylations have been discussed in *Chapter 1* pp.21-25.
[100] Srivastava, V.K.; Schuerch, C. *Carbohydr. Res.* **1982**, *100*, 411-417.
[101] Crich, D.; Picione, J. *Org. Lett.* **2003**, *5*, 781-784.
[102] Awad, L.F.; El Ashry, E.S.; Schuerch, C. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1587-1592.

azoisobutyronitrile (AIBN).[103] At the same time, position *O*-4 was regioselectively benzylated by isopropylidene lockage of *O*-2 and *O*-3 positions to afford the key intermediate **79** as shown in *Scheme 2.24*.[104]

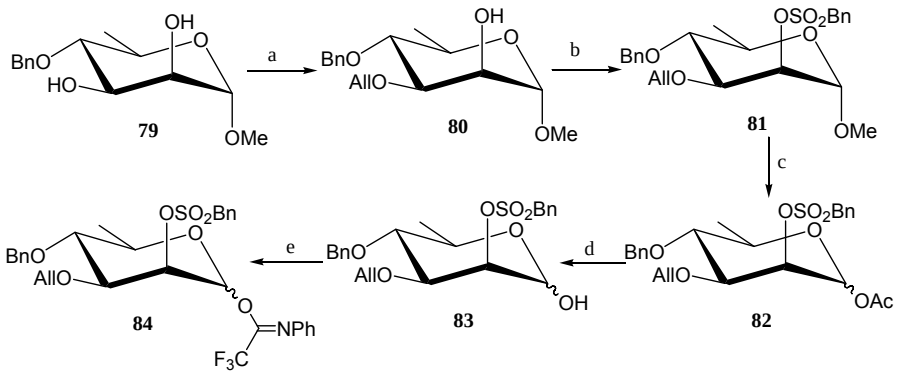


(a) i. 2,2'-dimethoxypropane/acetone, CSA cat, rt, 3 h; ii. H_2O, 1 h, 42%; (b) Ph_3P, ImH, I_2, toluene reflux,1 h 72%; (c) Bu_3Sn, AIBN, toluene, 80°C, 1 h, 95%; (d) BnBr, NaH, DMF, 0°C to rt , 1 h; (e) TFA/H_2O 4:1, 98% over 2 steps.

Scheme 2.24 Key intermediate formation from α-D-methylmannoside reduction.

From compound **79** both rhamnosyl donor and rhamnosyl acceptor are achievable. Thus, in order to prepare a suitable 2-*O*-benzylsulfonylated D-rhamnosyl donor, methyl 4-*O*-benzyl-α-D-rhamnopyranoside **79** was regioselectively allylated at position *O*-3 via stannylidene formation on 1,2-*cis* diol giving **80** in 82% yield (*Scheme 2.25*). This alcohol was then subjected to benzylsulfonylation with $BnSO_2Cl$ in pyridine; without any intermediate chromatography, subsequent acetolysis and cleavage of the anomeric acetate gave the hemiacetal **83** in 57% yield in three steps.

[103] Fauré, R.; Shiao, T. C.; Damerval, S. and Roy, R. *Tetrahedron Lett.* **2007**, *48*, 2385-2388.
[104] Zou, W.; Sen, A.K.; Szarek, W.A.; MacLean, D.B. *Can. J. Chem.* **1993**, *71*, 2194-2200.

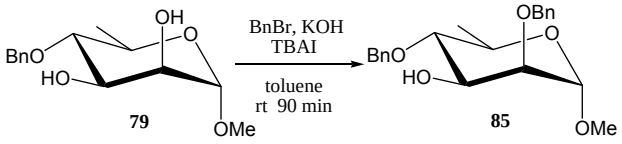


(a) i. Benzene/MeOH 10:1, BuSnO, 60°C 90 min; ii. TBAB, AllBr, toluene,65°C,60 min, 82% over 2 steps; (b) Py, $BnSO_2Cl$, rt, 30 min; (c)Ac_2O/ AcOH/ H_2SO_4 2,5:2:0,1 rt, 1 h; (d) DMF, N_2H_4,/AcOH, rt, 2 h, 57% over 3 steps; (e) CH_2Cl_2, $CF_3C(NPh)Cl$, NaH, 0°C, 1 h, 81%.

Scheme 2.25

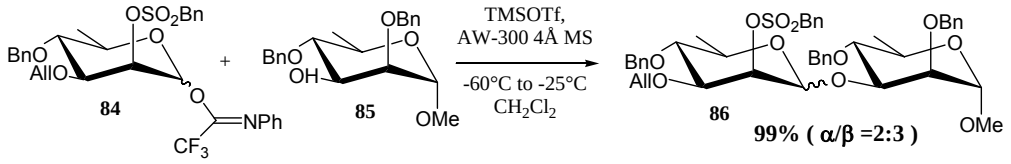
In order to enhance the stability of the donor a *N*-phenyltrifluoroacetimidate was chosen as leaving group on the anomeric position.

The acceptor was simply obtained by regioselective benzylation of *O*-2 positon as described in *Scheme 2.26*.[104,105]



Scheme 2.26 α-D-methylrhamnopyranoside acceptor

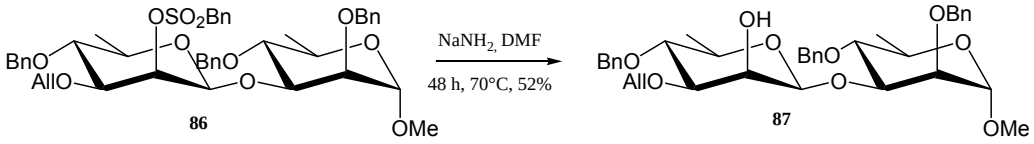
The coupling with donor **84** proceeded in very high yield (99%) affording the β-rhamnoside as the main product (*Scheme 2.27*).



Scheme 2.27 β-dirhamnoside assembly.

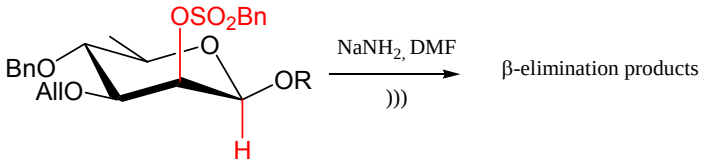
[105] a) Fang, Y.; Kong, F.; Wang, Q. *J. Carbohydr. Chem.* **1987**, *6*, 169-179.

Cleavage of the benzylsulfonyl group on **86β** proceeded in 52% yield with sodium amide in DMF.



Scheme 2.28 Cleavage of the benzylsulfonyl group

The reaction time is reduced by sonication or microwave irradiation[106] that, in this case, lowered considerably the yield. In fact, the 1,2-*cis* configuration allows β-elimination of $PhCH_2SO_2H$ whose rate increases with sonication and competes with benzylsulfonyl cleavage, as observed by MALDI mass spectrum of the crude reaction mixture.

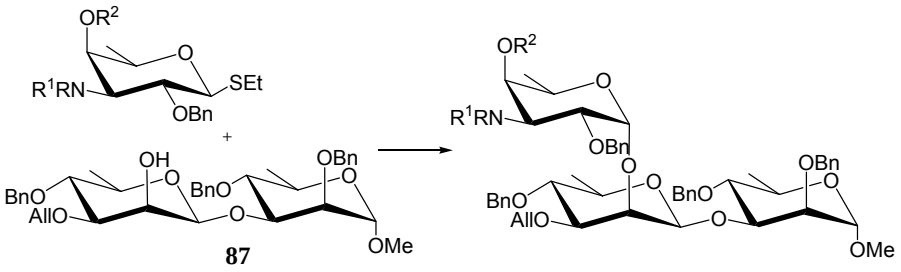


Scheme 2.29 Competitive β-elimination in benzylsulfonyl cleavage conditions with microwave.

Thus, the debenzensulfonylation have to be performed by modulating only the time reaction and the temperature.

The coupling of the new donor **73** with the rhamnose disaccharide **87** proceeded smoothly and in better yield (entry 1) than the prior donors (entries 2-3) as summarized in *Table 2.4*.

[106] Tanaka, H.; Yoshizawa, A.; Takahashi, T. *Angew. Chem. Int. Ed.* **2007**, *46*, 2505-2507.



Scheme 2.30 Trisaccharide assembly

Entry	*R*	*R¹*	*R²*	*Glycosylation conditions*	*Yield (%)*
1	H	Ac	Ac	1:1 CH_2Cl_2/Et_2O NIS/TfOH -20°C	traces
2	Ac	Ac	Ac	1:1 CH_2Cl_2/Et_2O NIS/TfOH -20°C	40
3	Troc	H	Bn	1:1 CH_2Cl_2/Et_2O NIS/AgOTf -20°C	77

Table 2.4 Comparative data

Difficulties in introducing an amino-fucose residue on a sterically crowded acceptor was already reported in literature.[107] Nevertheless the coupling between the new D-Fuc*p*3NAc donor **73** and disaccharide acceptor **87** under NIS/AgOTf activation at -20°C in 1:1 v/v CH_2Cl_2/Et_2O proceeded in good yield and the trisaccharidic target was obtained pure simply by column chromatography. The α-configuration of the new glycosidic bond was ascertained by a $^1J_{H\text{-}1,H\text{-}2}$ value of 3.3 Hz, typical of a 1,2-*cis*-configuration in the case of a *galacto*-configured residue. In conclusion, a new, high-yielding synthetic path to a protected form of the trisaccharide repeating unit of the *Xcc* O-antigen was accomplished.

Further goal was the opening of a synthetic way towards O-antigen higher oligomers. For this purpose, we had to test if the trisaccharide could be transformed into both a glycosyl donor and a glycosyl acceptor. The first step towards a glycosyl donor was planned to be the conversion of the anomeric methoxy group into an acetate by

[107] van den Bos, L.J.; Boltje, T.J. ; Provoost, T. ; Mazurek, J.; Overkleeft, H.S. ; van der Marel, G.A. *Tetrahedron Lett.* **2007**, *48*, 2697-2700.

acetolysis. Unfortunately, even in mild conditions recently reported on a disaccharide case,[108] a complex mixture of mono-, di- and trisaccharides was obtained, due to the simultaneous cleavage of the interglycosidic bonds (*Figure 2.11*). The explanation is that in this case both the two glycosidic linkages and the methoxy group are rather activated towards acid-mediated cleavage due to the electron-donating protecting groups at *O*-2 positions that stabilize the oxocarbenium ion.

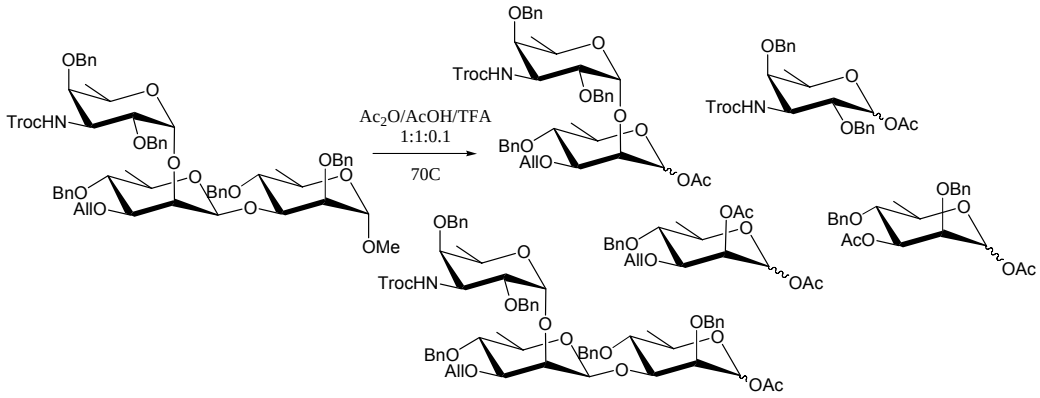
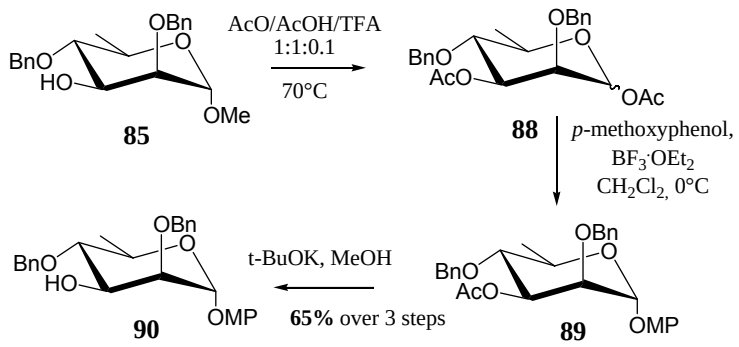


Figure 2.11 Product and byproducts in acetolysis treatment.

At this point, the synthesis of a new trisaccharide building block, possessing an orthogonal protecting group at the anomeric position, was mandatory.

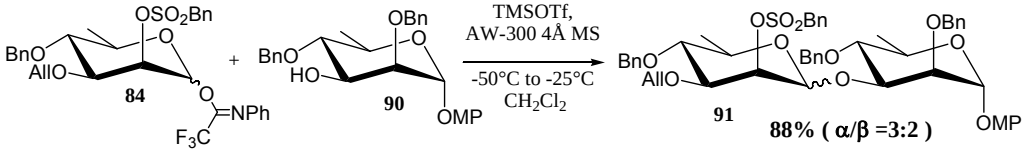
We chose a *p*-methoxyphenyl (MP) as new anomeric protecting group: its use as effective anomeric protecting group in D-rhamnose oligosaccharide synthesis was very recently reported.[86c, 103] Thus, the new D-rhamnose acceptor **90** was obtained from the former one **85** by acetolysis, *p*-methoxyphenylation and Zemplén deacetylation as reported in *Scheme 2.31*.

[108] Fekete, A.; Gyergyói, K.; Kövér, K.E. ; Bajza, I. ; Lipták, A. *Carbohydr. Res.* **2006**, *341*, 1312-1321.



Scheme 2.31 *p*-Methoxyphenyl group installation.

The coupling between **84** and **90** afforded disaccharide **91** in high yield but with inverted stereoselectivity affording α-disaccharide as main product.

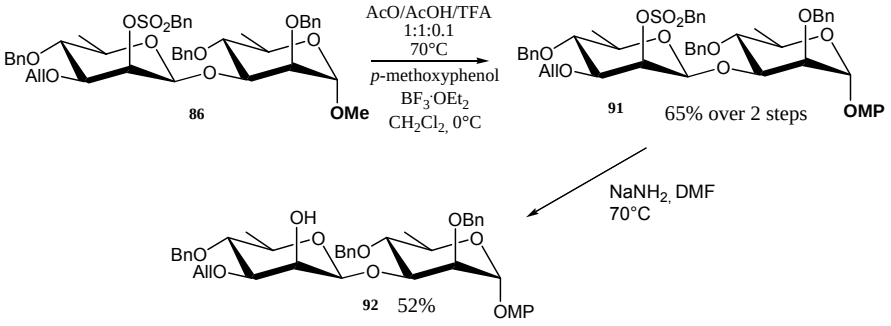


Scheme 2.32 Glycosylation with *p*-methoxyphenyl glycoside.

Since the coupling between **84** and the former acceptor **85** gave disaccharide **86** in quantitative yield and higher β-stereoselectivity as reported in *Scheme 2.27*, we attempted to insert the MP group at disaccharide level.

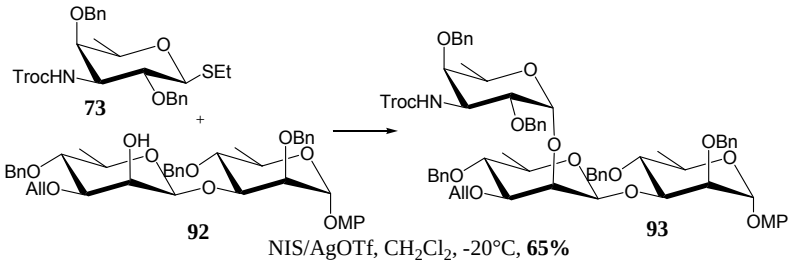
In this case, mild acetolysis of **86** proceeded smoothly to give the disaccharide with an acetyl group at the anomeric position. This result was explained with the strong electron-withdrawing character of the benzylsulfonyl group which disfavoured the acid-catalyzed cleavage of the interglycosidic bond; on the contrary the electron-donating benzyl group at position *O*-2A activated the anomeric methoxy group

towards acetolysis.[109] After subsequent *p*-methoxyphenylation, disaccharide **91β** was obtained in good yield.



Scheme 2.33

Disaccharide acceptor **91β** was debenzylsulfonylated to give acceptor **92** which was coupled with donor **73**. Glycosylation proceeded uneventfully to give trisaccharide **93** in 65% yield.

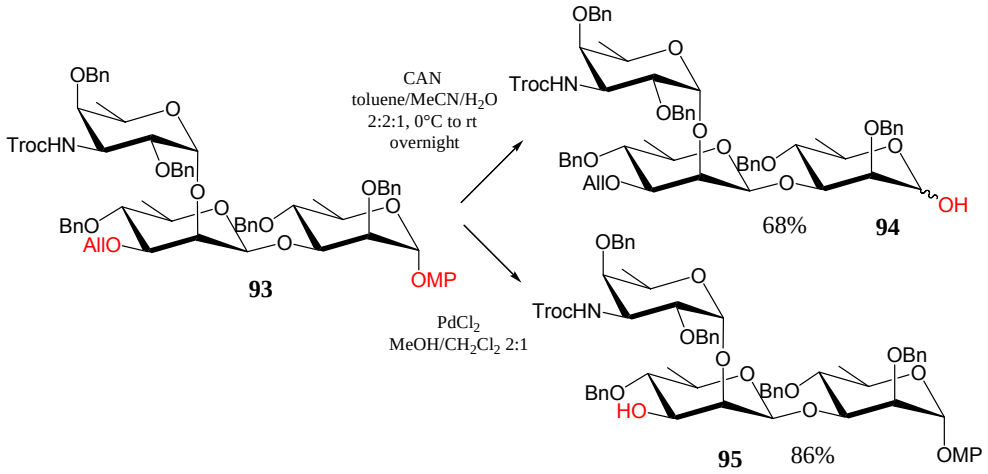


Scheme 2.34

The α-configuration of the new glycosidic bond was ascertained by a $^{1}J_{H\text{-}1,H\text{-}2}$ value of 3.2 Hz, typical of a 1,2-*cis*-configuration in the case of a *galacto*-configured residue. The protecting group pattern on **93** was demonstrated to be orthogonal enough to open a path towards higher oligomers of the repeating unit of *Xcc* O-antigen.

[109] Selective acetolysis are object of *Chapter 4*.

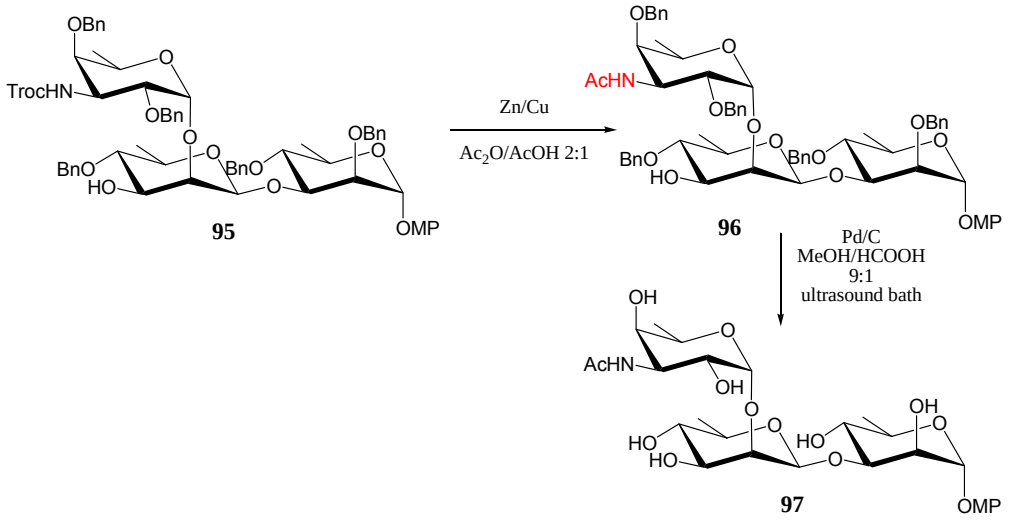
Actually, compound **93** was smoothly transformed into trisaccharide acceptor **95** (86%) by chemoselective de-*O*-allylation with $PdCl_2$. Selective deprotection of anomeric MP group was accomplished with cerium ammonium nitrate (CAN) in a ternary solvent mixture: hemiacetal **94** was obtained in 68% yield and can be activated as trisaccharide donor.



Scheme 2.35 Orthogonal cleavages of temporary protecting groups.

Finally, global deprotection of trisaccharide **95** was accomplished in 2 steps: firstly, the NHTroc group was converted into an acetamido group by treatment with Zn/Cu in 2:1 AcOH/Ac_2O, then de-*O*-benzylation was performed by palladium-catalysed transfer-hydrogenolysis under Perlin conditions[110] to give deprotected trisaccharide **97**.

[110] Rao, V.S.; Perlin, A.S. *Carbohydr. Res.* **1980**, *83*, 175-177.

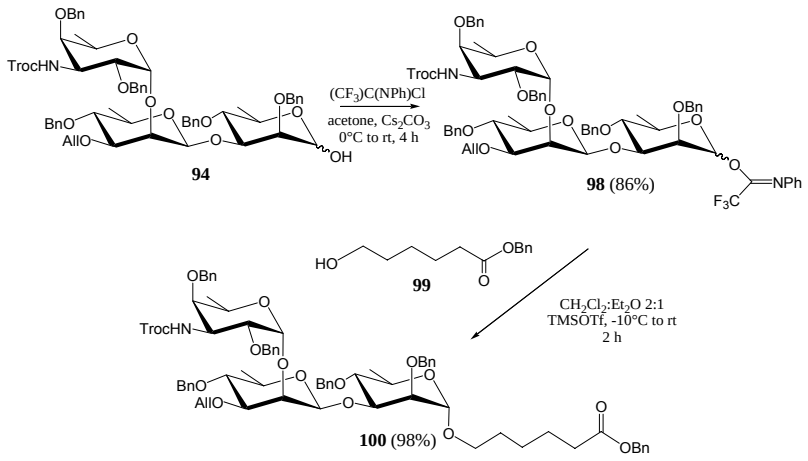


Scheme 2.36 Global deprotection

2.6 Towards the fabrication of gold glyconanoparticles.

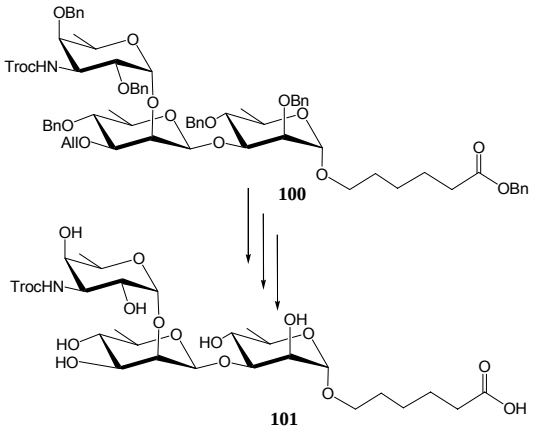
As already reported in *Chapter 1*, the synthesis of glycoconjugates adorned with appropriate linkers allows the preparation of metal nanoparticles as useful tool for glycobiological studies. With this intent, hemiacetal **94** has been converted in *N*-phenyl trifluoroacetimidate donor **98** and glycosylated with the benzylated linker **99** (*Scheme 2.37*) that was obtained from ε-caprolactone.[111] The global deprotection of glycoconjugate **100** has been achieved in three sequential steps: deallylation, Zn/Cu promoted detrichloroethoxycarbonylation, and debenzylation under Perlin conditions (*Scheme 2.38*).

[111] Weber, A.; Halgren, T. A.; Doyle, J. J.; Lynch, R. J.; Siegl, P. K. S.; Parsons, W. H.; Greenler, W. J.; Patchett, A. A. *J. Med. Chem.* **1991**, *34*, 2692-2701.



Scheme 2.37

The deprotected glycoconjugate **101** shows a carboxylic function that may be further derivatised through an amide linkage with a linker bearing a terminal thiol. Thus, it is possible to obtain a suitable tethered trisaccharide for attachment on a gold surface.



i. $PdCl_2$, MeOH/CH_2Cl_2; ii. Zn/Cu,AcOH/Ac_2O 2:1; iii. Pd(C),:MeOH:HCOOH 9:1,))).

Scheme 2.38

Conclusions

A new, improved synthesis of the *Xcc* O-antigen repeating unit have been developed. The main improvements in the synthesis are: 1) a shorter, high-yielding preparation of an efficient glycosyl donor of the rare sugar 3-acetamido-3,6-dideoxy-D-galactopyranose (3-acetamido-D-fucose, D-Fuc*p*3NAc); 2) a new protecting group pattern, which is demonstrated to open a path to the future syntheses of higher oligomers and golden nanoparticles.

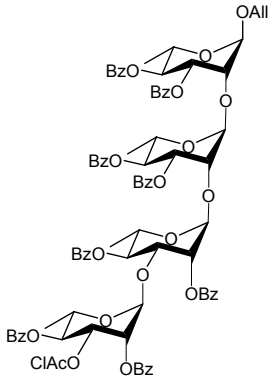
Experimental section

General methods:

^{1}H and ^{13}C NMR spectra were recorded on Varian XL-200 (^{1}H: 200 MHz, ^{13}C: 50 MHz), Varian Gemini-300 (^{1}H: 300 MHz, ^{13}C: 75 MHz) or Bruker DRX-400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) instruments in $CDCl_3$ ($CHCl_3$ as internal standard, ^{1}H: C*H*Cl_3 at δ 7.26; ^{13}C: *C*DCl_3 at δ 77.0) and in D_2O (acetone as internal standard, ^{1}H: (C*H*$_3$)$_2$CO at δ 2.22; ^{13}C: (*C*H_3)$_2$CO at δ 31.5). Assignment of proton and carbon chemical shifts of the deprotected oligosaccharides was based on 2D NMR experiments such as COSY, TOCSY, NOESY, HSQC. Heteronuclear C_1-H_1 coupling constants were measured with *J*-coupled HSQC experiments. Positive ESI-MS spectra were recorded on a Finnigan LCQ-DECA ion trap mass spectrometer. Positive MALDI-MS spectra were recorded on a Applied Biosystem Voyager DE-PRO MALDI-TOF mass spectrometer in the positive mode: compounds were dissolved in the appropriate solvent at a concentration of 1 mg/mL and one microliter of these solutions were mixed with one microliter of a 20 mg/mL solution of 2,5-dihydroxybenzoic acid in 7:3 CH_3CN/0.1 M aqueous TFA. IR spectra were recorded on a JASCO-FT/IR-430 spectrometer. Optical rotations were measured on a JASCO P-1010 polarimeter. Analytical thin layer chromatography (TLC) was performed on aluminium plates precoated with Merck

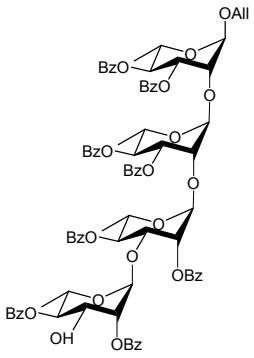
Silica Gel 60 F_{254} as the adsorbent. The plates were developed with 5% H_2SO_4 ethanolic solution and then heating to 130°C. Column chromatography was performed on Merck Kieselgel 60 (63-200 mesh), except where differently specified. Gel-filtration chromatographies were performed on a Sephadex G-10 column (2.0x90 cm) with water as eluant.

Oligorhamnans related to the most common *O*-chain backbone from phytopathogenic bacteria

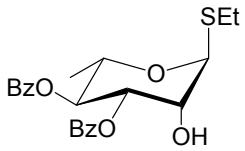


18. A mixture of **17** (179 mg, 0.43 mmol) and **16** (734 mg, 0.56 mmol) was coevaporated three times with toluene, the residue was dried and then mixed with freshly activated AW-300 4Å molecular sieves, suspended under argon in CH_2Cl_2 (15 mL) and stirred at –50°C. $BF_3.OEt_2$ (35 μL, 0.28 mmol) was then added. After 75 min the reaction was quenched with some drops of Et_3N. After filtration over a Celite pad, the mixture was concentrated to give a residue, that, after column chromatography (5:1 to 3:1 petroleum ether/ethyl acetate), afforded **18** (480 mg, 72%) as a white foam. $[\alpha]_D$= +95.8 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3030, 2913, 1720, 1458, 1275 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.15-7.09 (m, 40H), 5.98 (m, 1H), 5.83 (dd, *J*=9.9 Hz, *J*=3.1 Hz, 1H), 5.68-5.57 (m, 3H), 5.54 (bs, 1H), 5.43-5.27 (m, 5H), 5.18 (bs, 1H), 5.17 (bs, 1H), 5.12 (bs, 1H), 5.01 (bs, 1H), 4.80 (bs, 1H), 4.50 (dd, *J*=10.0 Hz, *J*=3.4 Hz, 1H), 4.40 (bs, 1H), 4.36 (bs, 1H), 4.30 (dd, *J*=9.7 Hz, *J*=3.6 Hz, 1H), 4.23 (dq, *J*=9.6 Hz, *J*=6.1 Hz, 1H), 4.13 (m, 3H), 4.00 (dq, *J*=10.0 Hz, *J*=6.1 Hz, 1H), 3.72 (AB d, *J*=14.9 Hz, 1H), 3.68 (AB d, *J*=14.9 Hz, 1H), 1.41 (d, *J*=6.1 Hz, 3H), 1.35 (d, *J*=6.1 Hz, 3H), 1.20 (d, *J*=6.1 Hz, 3H), 1.01 (d, *J*=6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.8-165.3 (CO), 133.8-133.3 (C_{ipso}, $OCH_2CH=CH_2$), 129.8-128.3 (C-Ar), 118.1

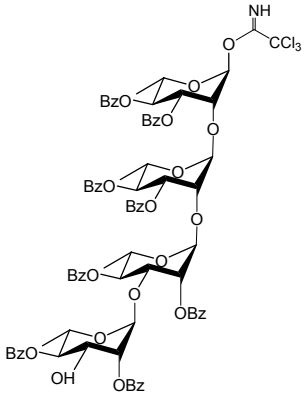
($OCH_2CH=CH_2$), 100.8, 99.3, 99.0, 97.9 (C_1^A, C_1^B, C_1^C, C_1^D), 78.0, 77.5, 75.1 (C_2^A, C_2^B, C_3^C), 72.8, 71.8, 71.7, 71.6, 71.5, 71.3, 70.6, 70.5, 70.3, 68.1, 67.5, 67.4, 67.3, 66.9 (C_2^C, C_2^D, C_3^A, C_3^B, C_3^D, C_4^A, C_4^B, C_4^C, C_4^D, C_5^A, C_5^B, C_5^C, C_5^D, $OCH_2CH=CH_2$), 40.3 (CH_2Cl), 17.6-17.2 (C_6^A, C_6^B, C_6^C, C_6^D). MALDI-MS for $C_{85}H_{79}ClO_{26}$ (*m*/*z*): M_r (calcd) 1550.45, M_r (found) 1573.27 $(M+Na)^+$. Anal. calcd.: C 65.78, H 5.13. Found: C 65.97, H 5.10.



20. A solution of **18** (189 mg, 0.12 mmol) was dissolved in 1:1 EtOH/DMF (12 mL) and then thiourea was added (93 mg, 1.29 mmol). After 2 days stirring at rt, the solution was diluted with CH_2Cl_2, washed with 1 M HCl, 1 M $NaHCO_3$ and water. The organic layer was collected, dried and concentrated to give a residue that, after column chromatography (4:1 petroleum ether/ethyl acetate), afforded **20** (128 mg, 71%) as a white foam. $[\alpha]_D$= +116.9 (c=1.7, CH_2Cl_2). IR (thin film, NaCl) 3053, 3031, 2925, 1723, 1460 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.17-7.07 (m, 40H), 5.98 (m, 1H), 5.85 (dd, *J*=9.9 Hz, *J*=3.1 Hz, 1H), 5.64 (m, 2H), 5.58 (t, *J*=9.8 Hz, 1H), 5.53 (bs, 1H), 5.39 (m, 2H), 5.29 (d, *J*=10.8 Hz, 1H), 5.21 (bs, 1H), 5.13-5.05 (m, 4H), 5.02 (bs, 1H), 4.80 (bs, 1H), 4.48 (dd, *J*=9.6 Hz, *J*=3.1 Hz 1H), 4.41 (bs, 1H), 4.37 (bs, 1H), 4.30 (dd, *J*=9.7 Hz, *J*=3.6 Hz, 1H), 4.23 (dq, *J*=9.6 Hz, *J*=6.1 Hz, 1H), 4.13 (m, 2H), 4.03 (m, 2H), 2.17 (bs, 1H), 1.39 (d, *J*=6.1 Hz, 3H), 1.34 (d, *J*=6.1 Hz, 3H), 1.16 (d, *J*=6.1 Hz, 3H), 1.01 (d, *J*=6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.8-165.3 (CO), 133.9-133.3 (C_{ipso}, $OCH_2CH=CH_2$), 130.0-128.2 (C-Ar), 118.1 ($OCH_2CH=CH_2$), 100.8, 99.2, 99.0, 98.3 (C_1^A, C_1^B, C_1^C, C_1^D), 77.9, 77.0, 75.3, 75.1, 73.3-71.7, 70.6, 68.4, 68.3, 68.0, 67.4, 66.9 (C_2^A, C_2^B, C_2^C, C_2^D, C_3^A, C_3^B, C_3^C, C_3^D, C_4^A, C_4^B, C_4^C, C_4^D, C_5^A, C_5^B, C_5^C, C_5^D, $OCH_2CH=CH_2$), 17.6-17.3 (C_6^A, C_6^B, C_6^C, C_6^D). MALDI-MS for $C_{63}H_{78}O_{25}$ (*m*/*z*): M_r (calcd) 1474.48, M_r (found) 1497.42 $(M+Na)^+$. Anal. calcd.: C 67.56, H 5.33. Found: C 67.67, H 5.30.

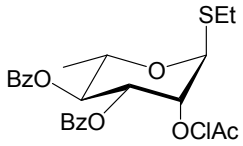


23. A solution of **22** (2.935 g, 9.40 mmol) in 2:1 CH_2Cl_2/py (12 mL) was cooled to -30°C and then treated with a 1.1 M solution (2.25 mL) of BzCl in 2:1 CH_2Cl_2/py. After stirring for 2.5 h, the mixture was treated with some drops of water, heated to rt and then diluted with CH_2Cl_2. The mixture was washed with water, 1 M HCl and water again. The organic layer was collected, dried and concentrated to give a residue, that, after column chromatography (7:1 petroleum ether/ethyl acetate) afforded **23** (3.446 g, 88%) as a white foam. $[\alpha]_D$=+8 (c=0.5, CH_2Cl_2). IR (thin film, NaCl) 3063, 3025, 2926, 1707, 1605 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.99-7.30 (m, 10H), 5.62 (t, *J*=9.8 Hz, 1H), 5.53 (dd, *J*=9.8 Hz, *J*=2.6 Hz, 1H), 5.37 (bs, 1H), 4.48 (dq, *J*=9.8 Hz, *J*=6.2 Hz, 1H), 4.39 (bs, 1H), 2.71 (app oct, *J*=8.4 Hz, 2H), 2.04 (bs, 1H), 1.35 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.6 (CO), 132.9, 132.8 (C_{ipso}), 129.4-128.0 (C-Ar), 84.1 (C_1), 72.7, 71.6, 70.5, 66.7 (C_2, C_3, C_4, C_5), 24.8 (SCH_2CH_3), 17.1 (C_6), 14.5 (SCH_2CH_3). ESI-MS for $C_{22}H_{24}O_6S$ (*m*/*z*): M_r (calcd) 416.13, M_r (found) 439.33 $(M+Na)^+$. Anal. calcd.: C 63.44, H 5.81. Found: C 63.66, H 5.88.



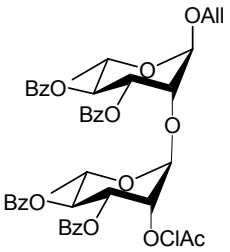
19. Compound **18** (299 mg, 0.19 mmol) was dissolved in 1:1 MeOH/CH_2Cl_2 (8.0 mL), $PdCl_2$ (8.6 mg, 95 μmol) was then added and the mixture was vigorously stirred at rt for two days, after that it was filtered over a Celite pad, diluted with CH_2Cl_2 and washed with 5 M NaCl. The organic layer was collected, dried and concentrated, and then dissolved in CH_2Cl_2 (15 mL) under Ar atmosphere. The solution was cooled to 0°C and then treated with Cl_3CCN (76 μL, 0.76 mmol) and DBU (6.7 μL, 45 μmol). After three hours the solution was concentrated at 30°C. The residue was subjected to neutral alumina (Brockman

grade 1) column chromatography (9:2 petroleum ether/ethyl acetate) to give **19** (152 mg, 61%) as a white foam. $[\alpha]_D$= +101.5 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3023, 2970, 1741, 1650 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.39 (s, 1H), 8.15-7.11 (m, 40H), 6.47 (bs, 1H), 5.84 (dd, *J*=9.8 Hz, *J*=3.2 Hz 1H), 5.72 (t, *J*=9.8 Hz, 1H), 5.68 (dd, *J*=9.6 Hz, *J*=3.2 Hz 1H), 5.60 (t, *J*=9.8 Hz, 1H), 5.55 (bs, 1H), 5.42 (m, 2H), 5.31 (t, *J*=9.5 Hz, 1H), 5.19 (bs, 2H), 5.17 (bs, 1H), 4.88 (bs, 1H), 4.63 (bs, 1H), 4.51 (dd, *J*=9.5 Hz, *J*=3.3 Hz 1H), 4.40 (bs, 1H), 4.33 (m, 2H), 4.13 (dq, *J*=9.5 Hz, *J*=6.1 Hz, 1H), 4.01 (dq, *J*=9.5 Hz, *J*=6.1 Hz, 1H), 3.72 (AB d, *J*=14.9 Hz, 1H), 3.68 (AB d, *J*=14.9 Hz, 1H), 1.43 (d, *J*=6.1 Hz, 3H), 1.39 (d, *J*=6.1 Hz, 3H), 1.30 (d, *J*=6.1 Hz, 3H), 0.93 (d, *J*=6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.0-165.4 (CO), 161.4 (Cl_3CC=NH), 133.8-133.4 (C_{ipso}), 130.0-128.3 (C-Ar), 100.9, 100.2, 100.1, 97.2 (C_1^A, C_1^B, C_1^C, C_1^D), 78.1, 75.1, 74.9, 72.9-71.1, 70.5, 70.2, 69.8, 69.7, 67.7-67.5 (C_2^A, C_2^B, C_2^C, C_2^D, C_3^A, C_3^B, C_3^C, C_3^D, C_4^A, C_4^B, C_4^C, C_4^D, C_5^A, C_5^B, C_5^C, C_5^D), 40.3 (CH_2Cl), 17.7-17.3 (C_6^A, C_6^B, C_6^C, C_6^D). Anal. calcd.: C 60.91, H 4.56, N 0.85. Found: C 61.09, H 4.45, N 0.88.

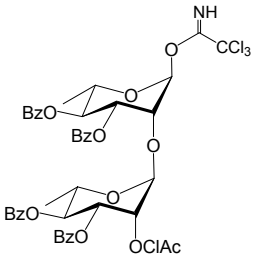


24. A solution of **23** (100 mg, 0.24 mmol) 1:1 pyridine/DMF (2.0 mL) was treated with $ClCH_2COCl$ (86 μL, 1.08 mmol) and then stirred at rt for 4 h. The mixture was coevaporated several times with toluene, then diluted with CH_2Cl_2 and washed with water. The organic layer was dried and concentrated to give a residue, that was subjected to column chromatography (7:1 petroleum ether/ethyl acetate) to afford **24** (66 mg, 56%) as a yellowish oil. $[\alpha]_D$=-17.4 (c=2.1, CH_2Cl_2). IR (thin film, NaCl) 3060, 3025, 2959, 1721, 1596 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.99-7.30 (m, 10H), 5.68-5.52 (m, 3H), 5.36 (bs, 1H), 4.50 (dq, *J*=9.8 Hz, *J*=6.2 Hz, 1H), 4.18 (s, 2H), 2.70 (m, 2H), 1.34 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 166.5, 165.6, 165.3 (CO), 133.3 (2 C_{ipso}), 129.7-128.3 (C-Ar), 81.7 (C_1), 73.7, 71.5, 70.0, 67.3 (C_2, C_3, C_4, C_5), 40.6 (CH_2Cl), 25.6 (SCH_2CH_3), 17.5 (C_6), 14.9

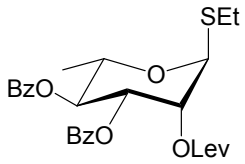
(SCH_2CH_3). ESI-MS for $C_{24}H_{25}ClO_7S$ (*m*/*z*): M_r (calcd) 492.10, M_r (found) 513.39 $(M+Na)^+$. Anal. calcd.: C 58.47, H 5.11. Found: C 58.77, H 5.02.



25. A mixture of **17** (50 mg, 0.12 mmol) and **24** (79 mg, 0.16 mmol) was coevaporated three times with toluene, the residue was dried and then mixed with freshly activated AW-300 4Å molecular sieves and NIS (45 mg, 0.20 mmol). The mixture was suspended in CH_2Cl_2 (4.0 mL) under an Ar atmosphere and rapidly cooled to -30°C. A 115 mg/mL solution of TfOH in CH_2Cl_2 (54 μL, 40 μmol) was then added. After 90 min the reaction mixture was rapidly filtered over a Celite pad, diluted with CH_2Cl_2 and washed with 10% $Na_2S_2O_3$ and 1 M $NaHCO_3$. The organic layer was collected, dried and concentrated to give a foamy residue. After column chromatography (7:1 petroleum ether/ethyl acetate), **25** (46 mg, 45%) was recovered as a white foam. $[\alpha]_D$=+45.1 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3055, 3026, 2948, 1726, 1600, 1255 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.02-7.30 (m, 20H, H-Ar), 5.98 (m, 1H), 5.87 (dd, *J*=10.0 Hz, *J*=3.2 Hz, 1H), 5.80 (dd, *J*=10.0 Hz, *J*=3.2 Hz, 1H), 5.70 (dd, *J*=3.2 Hz, *J*=1.8 Hz, 1H), 5.62 (t, *J*=10.0 Hz, 1H), 5.50 (t, *J*=10.0 Hz, 1H), 5.39 (d, *J*=17.0 Hz, 1H), 5.28 (d, *J*=10.4 Hz, 1H), 5.00 (bs, 2H), 4.34-4.12 (m, 5H), 4.08 (AB d, *J*=14.9 Hz, 1H), 3.98 (AB d, *J*=14.9 Hz, 1H), 1.39 (d, *J*=6.1 Hz, 3H), 1.30 (d, *J*=6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 166.0, 165.7, 165.6, 165.4, 165.1 (CO), 133.4-133.1 (C_{ipso}, $OCH_2CH=CH_2$), 129.8-128.4 (C-Ar), 117.9 ($OCH_2CH=CH_2$), 99.0, 97.6 (C_1^A, C_1^B), 76.7, 71.7, 71.6, 71.4, 71.1, 69.3, 68.2, 67.5, 67.0 (C_2^A, C_2^B, C_3^A, C_3^B, C_4^A, C_4^B, C_5^A, C_5^B, $OCH_2CH=CH_2$), 40.5 (CH_2Cl), 17.6 (C_6^A, C_6^B). ESI-MS for $C_{45}H_{43}ClO_{14}$ (*m*/*z*): M_r (calcd) 842.23, M_r (found) 865.49 $(M+Na)^+$. Anal. calcd.: C 64.09, H 5.14. Found: C 64.18, H 5.07.

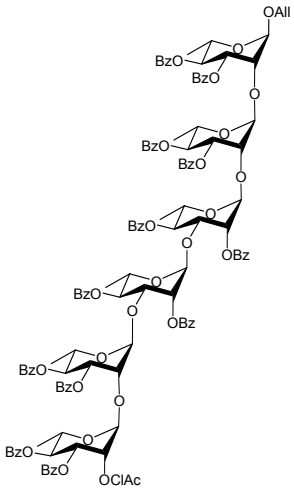


27. Compound **25** (130 mg, 0.15 mmol) was dissolved in 3:1 CH_2Cl_2/MeOH (4.0 mL), $PdCl_2$ (5.4 mg, 60 μmol) was then added and the mixture was vigorously stirred overnight. It was then filtered over a Celite pad, diluted with CH_2Cl_2 and washed with 5 M NaCl. The organic layer was dried and concentrated and then was dissolved in CH_2Cl_2 (3.0 mL) under Ar atmosphere. The solution was cooled to 0°C and then treated with Cl_3CCN (33 μL, 0.33 mmol) and DBU (4.3 μL, 29 μmol). After two hours the solution was concentrated at 30°C. The residue was subjected to neutral alumina (Brockman grade 1) column chromatography (6:1 petroleum ether/ethyl acetate) to give **27** (61 mg, 80%; α/β=6:1) as a white foam. $[\alpha]_D$=+34.5 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3048, 3020, 2970, 1738, 1655 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) (α -anomer) δ 8.76 (s, 1H), 8.02-7.33 (m, 20H, H-Ar), 6.46 (d, *J*=1.8 Hz, 1H), 5.86 (dd, *J*=10.0 Hz, *J*=3.3 Hz, 1H), 5.81 (dd, *J*=10.0 Hz, *J*=3.3 Hz, 1H), 5.73 (t, *J*=10.0 Hz, 1H), 5.70 (dd, *J*=3.3 Hz, *J*=1.5 Hz, 1H), 5.53 (t, *J*=10.0 Hz, 1H), 5.07 (d, *J*=1.5 Hz, 1H), 4.55 (dd, *J*=3.3 Hz, *J*=1.8 Hz 1H), 4.35 (m, 2H), 4.09 (AB d, *J*=15.0 Hz, 1H), 4.00 (AB d, *J*=15.0 Hz, 1H), 1.45 (d, *J*=6.2 Hz, 3H), 1.37 (d, *J*=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) (α-anomer) δ 165.9, 165.7, 165.4, 165.3 (CO), 161.4 (Cl_3CC=NH), 133.4-133.1 (C_{ipso}), 129.9-128.4 (C-Ar), 99.9, 95.8 (C_1^A, C_1^B), 77.2, 74.3, 72.5, 71.8, 71.6, 71.5, 71.0, 70.6, 69.3, 68.6 (C_2^A, C_2^B, C_3^A, C_3^B, C_4^A, C_4^B, C_5^A, C_5^B), 40.3 (CH_2Cl), 17.7, 17.3 (C_6^A, C_6^B).. Anal. calcd.: C 55.77, H 4.15, N 1.48. Found: C 56.01, H 4.11, N 1.45.



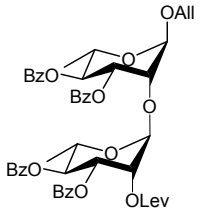
35. Compound **23** (1.541 g, 3.70 mmol) was dissolved in CH_2Cl_2 (15 mL) and levulinic acid (1.89 mL, 18.5 mmol), DMAP (271 mg, 2.20 mmol) and DIPC (2.90 mL, 18.5 mmol) were added in succession. The mixture was stirred for 1 h at rt, then filtered over a Celite pad, diluted with CH_2Cl_2 and washed with water. The organic layer was dried and concentrated to afford a residue that, after column chromatography (4:1 petroleum

ether/ethyl acetate), gave **35** (1.735 g, 91%) as a yellowish oil. $[\alpha]_D$=-7 (c=0.5, CH_2Cl_2). IR (thin film, NaCl) 3060, 3020, 2985, 2930, 1726, 1601, 1452 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.00-7.30 (m, 10H), 5.61-5.51 (m, 3H), 5.30 (bs, 1H), 4.46 (dq, *J*=8.8 Hz, *J*=6.4 Hz, 1H), 2.71-2.63 (m, 6H), 2.03 (s, 3H), 1.26 (t, *J*=6.2 Hz, 3H), 1.21 (d, *J*=6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.8 (CH_2COCH_3), 171.3 ($OCOCH_2CH_2$), 165.9-165.6 (PhCO), 133.6, 133.4 (C_{ipso}), 130.0-128.4 (C-Ar), 81.6 (C_1), 71.5, 71.4, 69.9, 66.6 (C_2, C_3, C_4, C_5), 37.2, 29.1, 27.5, 25.1 ($COCH_2CH_2COCH_3$), 17.0 (C_6), 14.4 (SCH_2CH_3). ESI-MS for $C_{27}H_{30}O_8S$ (*m*/*z*): M_r (calcd) 514.17, M_r (found) 537.41 $(M+Na)^+$. Anal. calcd.: C 63.02, H 5.88. Found: C 63.26, H 5.65.



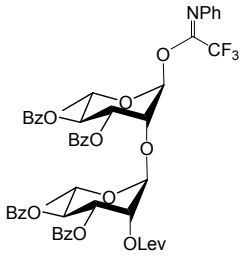
34. A mixture of **20** (30 mg, 20 μmol) and **27** (57 mg, 60 μmol) was coevaporated three times with toluene, the residue was dried and then mixed with freshly activated AW-300 4Å molecular sieves, suspended under argon in CH_2Cl_2 (2.0 mL) and stirred at -50°C. A 85 mg/mL solution of $BF_3.OEt_2$ in CH_2Cl_2 (50 μL, 30 μmol) was then added. After 3 h the reaction was quenched with some drops of Et_3N. After filtration over a Celite pad, the mixture was concentrated. The residue was subjected to column chromatography (11:1 to 6:1 toluene/ethyl acetate), to give **34** (22 mg, 48%) as a white foam. $[\alpha]_D$=+92 (c=0.5, CH_2Cl_2). IR (thin film, NaCl) 3024, 2927, 2857, 1722, 1604, 1449, 1272 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.15-7.24 (m, 60H, H-Ar), 6.00 (m, 1H), 5.85 (dd, *J*=9.9 Hz, *J*=3.1 Hz, 1H), 5.74 (dd, *J*=9.9 Hz, *J*=3.4 Hz, 1H), 5.65-5.51 (m, 4H), 5.44-5.30 (m, 7H), 5.23 (bs, 1H), 5.19 (bs, 1H), 5.14 (bs, 1H), 5.03 (bs, 1H), 4.94 (bs, 1H), 4.81 (bs, 1H), 4.71 (bs, 1H), 4.59 (bs, 1H), 4.49 (dd, *J*=9.6 Hz, *J*=3.2 Hz, 1H), 4.38-4.32 (m, 3H), 4.22-4.14 (m, 3H), 4.03-3.91 (m, 3H), 3.82 (dq, *J*=9.8 Hz, *J*=6.2 Hz,

1H), 3.73 (dq, *J*=9.9 Hz, *J*=6.2 Hz, 1H), 3.70 (AB d, *J*=14.9 Hz, 1H), 3.65 (AB d, *J*=14.9 Hz, 1H), 3.61 (dq, *J*=9.9 Hz, *J*=6.2 Hz, 1H), 1.42 (d, *J*=6.2 Hz, 3H), 1.37 (d, *J*=6.2 Hz, 3H), 1.15 (d, *J*=6.2 Hz, 3H), 1.00 (d, *J*=6.2 Hz, 3H), 0.85 (d, *J*=6.2 Hz, 3H), 0.79 (d, *J*=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.8-165.0 (Ph*C*O), 133.8-133.3 (C_{ipso}, $OCH_2CH=CH_2$), 130.3-128.2 (C-Ar), 117.9 ($OCH_2CH=CH_2$), 100.9, 100.8, 100.4, 99.6, 99.5, 98.9 (C_1^A, C_1^B, C_1^C, C_1^D, C_1^E, C_1^F), 77.8, 76.9, 76.2, 75.4, 73.2, 73.1, 73.0, 72.2-71.5, 70.5, 70.0, 69.9, 68.2-67.0 (C_2^A, C_2^B, C_2^C, C_2^D, C_2^E, C_2^F, C_3^A, C_3^B, C_3^C, C_3^D, C_3^E, C_3^F, C_4^A, C_4^B, C_4^C, C_4^D, C_4^E, C_4^F, C_5^A, C_5^B, C_5^C C_5^D C_5^E C_5^F, $OCH_2CH_2=CH_2$), 40.3 (CH_2Cl), 17.5-17.0 (C_6^A, C_6^B, C_6^C, C_6^D, C_6^E, C_6^F). MALDI-MS for $C_{125}H_{115}ClO_{38}$ (*m*/*z*): M_r (calcd) 2258.68, M_r (found) 2281.40 $(M+Na)^+$. Anal. calcd.: C 66.41, H 5.13. Found: C 66.66, H 5.00.



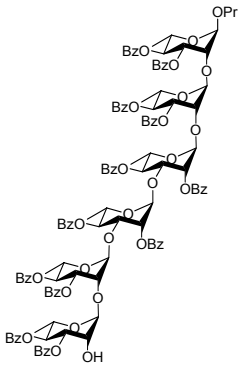
36. A mixture of **17** (544 mg, 1.32 mmol) and **35** (1.018 g, 1.98 mmol) was coevaporated three times with toluene, the residue was dried and then mixed with freshly activated AW-300 4Å molecular sieves and NIS (282 mg, 2.43 mmol). The mixture was suspended in CH_2Cl_2 (4.0 mL) under an Ar atmosphere, rapidly cooled to -30°C and treated with TfOH (43 μL, 0.49 mmol). After 90 min the reaction mixture was rapidly filtered over a Celite pad, diluted with CH_2Cl_2 and washed with 10% $Na_2S_2O_3$ and 1M $NaHCO_3$. The organic layer was collected, dried and concentrated to give a residue, that after column chromatography (7:2 petroleum ether/ethyl acetate) afforded **36** (946 mg, 83%) as a white foam. $[\alpha]_D$=+60.9 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3050, 3026, 1731, 1604, 1263 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.03-7.28 (m, 20H), 5.97 (m, 1H), 5.82 (dd, *J*=10.0 Hz, *J*=3.4 Hz, 1H), 5.78 (dd, *J*=10.0 Hz, *J*=3.0 Hz, 1H), 5.63 (m, 2H), 5.50 (t, *J*=10.0 Hz, 1H), 5.38 (d, *J*=17.2 Hz, 1H), 5.27 (d, *J*=10.6 Hz, 1H), 5.02 (d, *J*=1.6 Hz, 1H), 4.96 (d, *J*=1.4 Hz, 1H), 4.34-4.06 (m, 5H), 2.60 (s, 4H), 2.08 (s, 3H), 1.38 (d, *J*=6.2 Hz, 3H), 1.30 (d, *J*=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.8 (CH_2COCH_3), 171.3 ($OCOCH_2$), 165.9, 165.6, 165.5, 165.3 (Ph*C*O), 133.6-133.2 (C_{ipso}, $OCH_2CH=CH_2$), 130.1-128.4 (C-Ar), 117.9

($OCH_2CH{=}CH_2$), 99.6, 97.8 (C_1^A, C_1^B), 76.9, 72.1, 71.8, 71.2, 70.3, 69.7, 68.4, 67.6, 67.1 (C_2^A, C_2^B, C_3^A, C_3^B, C_4^A, C_4^B, C_5^A, C_5^B, $OCH_2CH{=}CH_2$), 37.9, 29.8, 28.0 ($COCH_2CH_2COCH_3$), 17.7, 17.6 (C_6^A, C_6^B). ESI-MS for $C_{48}H_{48}O_{15}$ (*m*/*z*): M_r (calcd) 864.30, M_r (found) 887.41 $(M+Na)^+$. Anal. calcd.: C 66.66, H 5.59. Found: C 66.86, H 5.50.



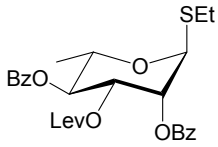
38. Compound **36** (838 mg, 0.97 mmol) was dissolved in 3:1 CH_2Cl_2/MeOH (4.0 mL), $PdCl_2$ (103 mg, 0.58 mmol) was then added and the mixture was vigorously stirred overnight. It was then filtered over a Celite pad, diluted with CH_2Cl_2 and washed with 5 M NaCl. The organic layer was dried and concentrated to and then was mixed with freshly powdered 4Å MS and suspended in CH_2Cl_2 (12 mL) under Ar atmosphere. The mixture was cooled to 0°C and then treated with $CF_3C(NPh)Cl$ (148 μL, 1.26 mmol) and NaH (60% dispersion in mineral oil; 58 mg, 1.45 mmol). After four hours the solution was concentrated at 30°C. The residue was subjected to neutral alumina (Brockman grade 1) column chromatography (6:1 petroleum ether/ethyl acetate) to give **38** (577 mg, 72%; α/β=1:1) as a white foam. IR (thin film, NaCl) 3026, 3012, 2917, 1732, 1600, 1452 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 8.08-6.84 (m, 50H), 6.40 (bs, 1H), 5.99 (bs, 1H), 5.88-5.43 (m, 11H), 5.13 (bs, 1H), 5.07 (bs, 1H), 5.04 (bs, 1H), 4.60-4.52 (m, 2H), 4.22-4.13 (m, 2H), 2.57 (s, 4H), 2.56 (s, 4H), 2.01 (s, 6H), 1.45 (d, *J*=6.0 Hz, 3H), 1.22 (d, *J*=6.0 Hz, 3H), 1.18 (d, *J*=6.0 Hz, 3H), 1.15 (d, *J*=6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.8 (CH_2COCH_3), 171.2 ($OCOCH_2$), 165.8, 165.7, 165.4, 165.3 (PhCO), 143.2 (C=N), 133.4-133.1 (C_{ipso}), 130.0-115.3 (C-Ar), 99.3, 98.3, 95.5, 93.9 (2 C_1^A, 2 C_1^B), 76.9, 76.8, 74.1, 73.3, 72.5, 72.0, 71.5, 71.4, 71.0, 70.5, 69.9, 69.8, 69.7, 69.5, 67.8, 67.1 (2 C_2^A, 2 C_2^B, 2 C_3^A, 2 C_3^B, 2 C_4^A, 2 C_4^B, 2 C_5^A, 2 C_5^B), 37.8, 29.7, 27.9 ($COCH_2CH_2COCH_3$), 17.7, 17.3 (2 C_6^A, 2 C_6^B). ESI-MS for $C_{53}H_{48}F_3NO_{15}$ (*m*/*z*): M_r

(calcd) 995.30, M_r (found) 1018.40 $(M+Na)^+$. Anal. calcd.: C 63.92, H 4.86, N 1.41. Found: C 63.67, H 4.72, N 1.38.

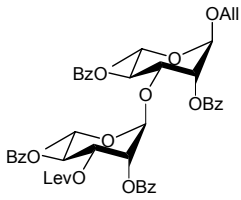


41. A mixture of **20** (73 mg, 49 μmol) and **16** (195 mg, 0.20 mmol) was coevaporated three times with toluene, the residue was dried and then mixed with freshly activated AW-300 4Å molecular sieves, suspended under argon in CH_2Cl_2 (6.0 mL) and stirred at 0°C. A 10 mg/mL solution of TMSOTf in CH_2Cl_2 (50 μL, 2.2 μmol) was then added. After 3 h the reaction was quenched with some drops of Et_3N. After filtration over a Celite pad, the mixture was concentrated. The residue was subjected to column chromatography (5:1 to 3:2 petroleum ether/ethyl acetate), to give a foamy residue, that was dissolved in CH_2Cl_2 (7.0 mL) and then treated with a 26 mg/mL solution of hydrazinium acetate in MeOH (1.0 mL, 0.28 mmol). After 2 h stirring at rt, the mixture was concentrated; a column chromatography (7:1 toluene/ethyl acetate) on the residue afforded **41** (82 mg, 77%) as a white foam. $[\alpha]_D$=+100.8 (c=2.0, CH_2Cl_2). IR (thin film, NaCl) 3019, 2924, 1729, 1600, 1269 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.11-7.25 (m, 60H, H-Ar), 5.82 (dd, *J*=10.0 Hz, *J*=3.2 Hz, 1H), 5.61-5.52 (m, 5H), 5.41-5.34 (m, 4H), 5.29 (t, *J*=9.6 Hz, 1H), 5.26 (bs, 1H), 5.15 (bs, 1H), 5.10 (bs, 1H), 5.00 (bs, 1H), 4.93 (bs, 1H), 4.91 (bs, 1H), 4.76 (bs, 1H), 4.57 (bs, 1H), 4.48 (dd, *J*=9.6 Hz, *J*=3.2 Hz, 1H), 4.36 (m, 2H), 4.22 (m, 3H), 4.11 (m, 1H), 3.99-3.88 (m, 3H), 3.73 (m, 2H), 3.48 (dq, *J*=9.4 Hz, *J*=6.2 Hz, 1H), 1.70 (app sextet, *J*=7.4 Hz, 2H), 1.37 (d, *J*=6.2 Hz, 3H), 1.33 (d, *J*=6.0 Hz, 3H), 1.11 (d, *J*=6.1 Hz, 3H), 0.99 (t, *J*=7.4 Hz, 3H), 0.96 (d, *J*=6.1 Hz, 3H), 0.84 (d, *J*=6.2 Hz, 3H), 0.75 (d, *J*=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.8-165.0 (Ph*C*O), 133.9-133.3 (C_{ipso}), 130.0-128.0 (C-Ar), 100.8, 100.7, 100.4, 99.3, 99.2, 98.9 (C_1^A, C_1^B, C_1^C, C_1^D, C_1^E, C_1^F), 77.6 76.9, 76.3 75.4, 74.8, 73.3 73.0, 72.0-71.4, 70.6, 70.0, 69.8, 68.4-67.3 (C_2^A, C_2^B, C_2^C, C_2^D, C_2^E, C_2^F, C_3^A, C_3^B, C_3^C, C_3^D, C_3^E, C_3^F, C_4^A, C_4^B, C_4^C, C_4^D, C_4^E, C_4^F, C_5^A, C_5^B, C_5^C C_5^D C_5^E C_5^F,

$OCH_2CH_2CH_3$), 22.7 ($OCH_2CH_2CH_3$), 17.4-16.9 (C_6^A, C_6^B, C_6^C, C_6^D, C_6^E, C_6^F), 10.5 ($OCH_2CH_2CH_3$). MALDI-MS for $C_{123}H_{116}O_{37}$ (*m*/*z*): M_r (calcd) 2184.72, M_r (found) 2207.49 $(M+Na)^+$. Anal. calcd.: C 67.57, H 5.35. Found: C 67.80, H 5.22.

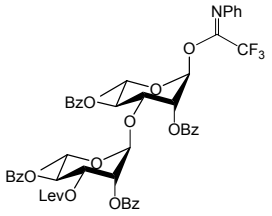


42. Compound **10** (86 mg, 0.21 mmol) was dissolved in CH_2Cl_2 (1.0 mL) and levulinic acid (172 μL, 2.07 mmol), DMAP (13 mg, 0.10 mmol) and DIPC (326 μL, 2.07 mmol) were added in succession. The mixture was stirred for 30 min at rt, then diluted with CH_2Cl_2 and washed with water. The organic layer was dried and concentrated to afford a residue that, after column chromatography (4:1 petroleum ether/ethyl acetate), gave **42** (96 mg, 89%) as a colourless oil. $[\alpha]_D$=+11.5 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3058, 3020, 2928, 1726, 1599, 1452 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.12-7.41 (m, 10H), 5.62 (bs, 1H), 5.51 (m, 2H), 5.39 (bs, 1H), 4.43 (dq, *J*=9.0 Hz, *J*=6.4 Hz, 1H), 2.71 (dt, *J*=7.6 Hz, *J*=3.8 Hz, 2H), 2.63 (dt, *J*=7.6 Hz, *J*=3.8 Hz, 2H), 2.48 (dq, *J*=7.2 Hz, *J*=3.2 Hz, 1H), 2.33 (dq, *J*=7.2 Hz, *J*=3.2 Hz, 1H), 2.00 (s, 3H), 1.30 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.7 (CH_2COCH_3), 171.5 ($OCOCH_2CH_2$), 165.6, 165.4 (Ph*C*O), 133.4, 133.3 (C_{ipso}), 129.8-128.4 (C-Ar), 82.0 (C_1), 72.1, 71.8, 69.7, 67.1 (C_2, C_3, C_4, C_5), 37.5, 29.2, 27.7, 25.4 ($COCH_2CH_2COCH_3$, SCH_2CH_3), 17.4 (C_6), 14.7 (SCH_2CH_3). ESI-MS for $C_{27}H_{30}O_8S$ (*m*/*z*): M_r (calcd) 514.17, M_r (found) 537.29 $(M+Na)^+$. Anal. calcd.: C 63.02, H 5.88. Found: C 62.95, H 5.96.



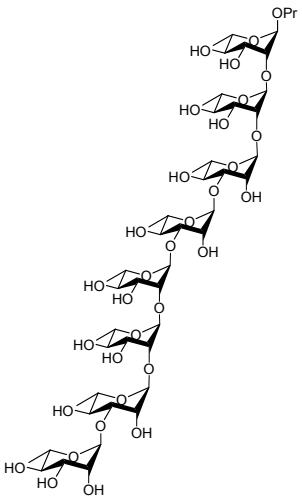
43. A mixture of **31** (53 mg, 0.13 mmol) and **42** (86 mg, 0.17 mmol) was coevaporated three times with toluene, the residue was dried and then mixed with freshly activated AW-300 4Å molecular sieves and NIS (47 mg, 0.21 mmol). The mixture was suspended in CH_2Cl_2 (4.0 mL) under an Ar atmosphere, rapidly cooled to -30°C and

treated with TfOH (5.5 μL, 63 μmol). After 3 h the reaction mixture was rapidly filtered over a Celite pad, diluted with CH_2Cl_2 and washed with 10% $Na_2S_2O_3$ and 1M $NaHCO_3$. The organic layer was collected, dried and concentrated to give a residue, that after column chromatography (10:1 to 8:1 toluene/ethyl acetate) afforded **43** (97 mg, 87%) as a white foam. $[\alpha]_D$=+84.4 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3052, 3026, 1733, 1604, 1263 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.23-7.32 (m, 20H), 5.92 (m, 1H), 5.57 (t, *J*=9.8 Hz, 1H), 5.52 (m, 2H), 5.42-5.21 (m, 3H), 5.16 (bs, 2H), 5.05 (bs, 1H), 4.48 (dd, *J*=10.0 Hz, *J*=3.4 Hz, 1H), 4.24 (ddt, *J*=12.8, *J*=5.0 Hz, *J*=1.2 Hz, 1H), 4.14-4.00 (m, 3H), 2.37 (dt, *J*=7.4 Hz, *J*=3.8 Hz, 2H), 2.23 (dt, *J*=7.4 Hz, *J*=3.8 Hz, 2H), 1.86 (s, 3H), 1.35 (d, *J*=6.2 Hz, 3H), 1.16 (d, *J*=6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.6 (CH_2COCH_3), 170.6 ($OCOCH_2CH_2$), 166.0, 165.6, 165.4, 164.8 (Ph*C*O), 133.4, 133.1 (C_{ipso}, $OCH_2CH=CH_2$), 129.8-128.3 (C-Ar), 117.8 ($OCH_2CH=CH_2$), 99.1, 96.3 (C_1^A, C_1^B), 75.9, 73.0, 72.2, 71.3, 70.2, 68.7, 68.4, 67.2, 66.7 (C_2^A, C_2^B, C_3^A, C_3^B, C_4^A, C_4^B, C_5^A, C_5^B, $OCH_2CH=CH_2$), 37.5, 29.2, 27.6 ($COCH_2CH_2COCH_3$), 17.5, 17.2 (C_6^A, C_6^B). ESI-MS for $C_{48}H_{48}O_{15}$ (*m*/*z*): M_r (calcd) 864.30, M_r (found) 887.48 $(M+Na)^+$. Anal. calcd.: C 66.66, H 5.59. Found: C 66.95, H 5.65.



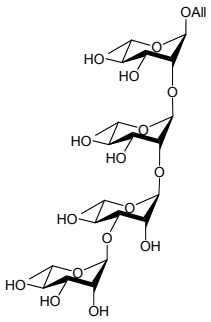
44. Compound **43** (178 mg, 0.21 mmol) was dissolved in 3:1 CH_2Cl_2/MeOH (4.0 mL), $PdCl_2$ (19 mg, 0.11 mmol) was then added and the mixture was vigorously stirred overnight. It was then filtered over a Celite pad, diluted with CH_2Cl_2 and washed with 5 M NaCl. The organic layer was dried and concentrated and then was mixed with freshly powdered 4Å MS and suspended in CH_2Cl_2 (6.0 mL) under Ar atmosphere. The mixture was cooled to 0°C and then treated with $CF_3C(NPh)Cl$ (19 μL, 0.16 mmol) and NaH (60% dispersion in mineral oil; 7.6 mg, 0.19 mmol). After 4 h the solution was concentrated at 30°C. The residue was subjected to neutral alumina (Brockman grade 1) column chromatography (10:1 to 5:1 petroleum ether/ethyl

acetate) to give **44** (73 mg, 58%; α/β=1:1) as a white foam. IR (thin film, NaCl) 3028, 3012, 2925, 1728, 1595, 1452 cm^{-}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25-6.80 (m, 50H), 6.43 (bs, 1H), 6.08 (bs, 1H), 5.70-5.57 (m, 3H), 5.40-5.12 (m, 11H), 4.52 (dd, *J*=10.0 Hz, *J*=3.4 Hz, 1H), 4.16-4.07 (m, 3H), 2.38 (m, 4H), 2.25 (m, 4H), 1.88 (s, 3H), 1.40 (d, *J*=6.0 Hz, 3H), 1.27 (d, *J*=6.0 Hz, 3H), 1.20 (d, *J*=6.0 Hz, 3H), 1.18 (d, *J*=6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 206.1 (CH_2COCH_3), 170.9 ($OCOCH_2$), 165.8, 165.6, 165.5, 164.8 (Ph*C*O), 143.0 (C=N), 133.7-133.3 (C_{ipso}), 130.0-115.3 (C-Ar), 99.3, 94.1, 93.6 (2 C_1^A, 2 C_1^B), 75.1, 72.4, 72.0, 71.3, 70.6, 70.1, 69.4, 68.9, 68.7, 67.6 (2 C_2^A, 2 C_2^B, 2 C_3^A, 2 C_3^B, 2 C_4^A, 2 C_4^B, 2 C_5^A, 2 C_5^B), 37.6, 29.3, 27.7 ($COCH_2CH_2COCH_3$), 17.7, 17.3 (2 C_6^A, 2 C_6^B). ESI-MS for $C_{53}H_{48}F_3NO_{15}$ (*m*/*z*): M_r (calcd) 995.30, M_r (found) 1018.29 $(M+Na)^+$. Anal. calcd.: C 63.92, H 4.86, N 1.41. Found: C 63.59, H 4.74, N 1.38.



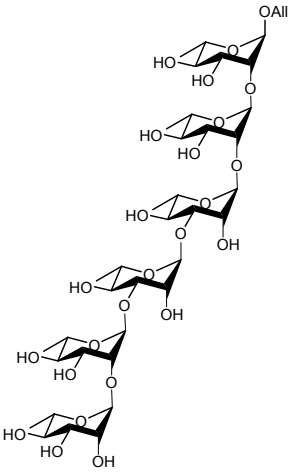
46. A mixture of **41** (40 mg, 18 μmol) and **44** (72 mg, 72 μmol) was coevaporated three times with toluene, the residue was dried and then mixed with freshly activated AW-300 4Å molecular sieves, suspended under argon in CH_2Cl_2 (3.0 mL) and stirred at 0°C. A 8.2 mg/mL solution of TMSOTf in CH_2Cl_2 (50 μL, 1.8 μmol) was then added. After stirring the reaction mixture at 0°C overnight, some drops of Et_3N were added. The mixture was filtered over a Celite pad and concentrated. The residue was subjected to column chromatography (10:1 to 8:1 toluene/ethyl acetate), to give a foamy residue, that was dissolved in 3:1 MeOH/CH_2Cl_2 (2.0 mL) and then treated with a 3.5 M methanolic solution of NaOMe (90 μL, 0.31 mmol). The solution was heated to 40°C and stirred at this temperature over two days; it was then neutralized with Amberlist-15 H^+, filtered and concentrated. The residue was

subjected to a gel-filtration chromatography to give **46** (10.8 mg, 49%) as a white wax. $[\alpha]_D$= +81 (c=0.6, H_2O). ^1H NMR (D_2O, 400 MHz) δ 5.19 (bs, 1H, H_1^E), 5.12 (bs, 1H, H_1^F), 5.11 (bs, 1H, H_1^B), 5.04 (bs, 1H, H_1^H), 5.02 (bs, 1H, H_1^D), 4.96 (bs, 2H, H_1^C, H_1^G), 4.87 (bs, 1H, H_1^A), 4.15 (m, 3H, H_2^C, H_2^D, H_2^G), 4.08 (m, 4H, H_2^B, H_2^E, H_2^F, H_2^H), 3.96 (dd, 1H, $J_{3,4}$=9.8 Hz, $J_{3,2}$=3.2 Hz, H_3^E), 3.92-3.82 (m, 9H, H_2^A, H_3^A, H_3^B, H_3^C, H_3^D, H_3^F, H_3^G, H_3^H, H_5^E), 3.77-3.72 (m, 8H, H_5^A, H_5^B, H_5^C, H_5^D, H_5^F, H_5^G, H_5^H), 3.65 (dt, 1H, J_{gem}=13.9 Hz, J_{vic}=6.5 Hz, OC*H*HCH$_2$CH$_3$), 3.56-3.46 (m, 9H, H_4^A, H_4^B, H_4^C, H_4^D, H_4^E, H_4^F, H_4^G, H_4^H, OC*H*HCH$_2$CH$_3$), 1.61 (app sixtet, 2H, *J*=7.0 Hz, OCH$_2$C*H*$_2$CH$_3$), 1.29 (m, 24H, H_6^A, H_6^B, H_6^C, H_6^D, H_6^E, H_6^F, H_6^G, H_6^H), 0.91 (t, 3H, *J*=7.0 Hz, OCH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (D_2O, 100 MHz) δ 103.0 (C_1^D, C_1^H), 102.7 (C_1^C, C_1^G), 101.6 (C_1^E), 101.5 (C_1^B, C_1^F), 98.9 (C_1^A), 79.0 (C_2^A), 78.9 (C_2^B, C_2^E, C_2^F), 78.8 (C_3^C, C_3^G), 78.3 (C_3^D), 72.9 (C_4^A, C_4^B, C_4^E, C_4^F, C_4^H), 72.0 (C_4^C, C_4^D, C_4^G), 70.6 (C_3^E), 70.4 (C_3^A, C_3^H, C_5^E), 70.3 (C_3^B, C_3^H, OCH$_2$CH$_2$CH$_3$), 70.1 (C_5^B, C_5^C, C_5^D, C_5^F, C_5^G, C_5^H), 69.8 (C_5^A), 22.5 (OCH$_2$CH$_2$CH$_3$), 17.4 (C_6^A, C_6^B, C_6^C, C_6^D, C_6^E, C_6^F, C_6^G, C_6^H), 10.4 (OCH$_2$CH$_2$CH$_3$). MALDI-MS for $C_{51}H_{88}O_{33}$ (*m*/z): M_r (calcd) 1228.52, M_r (found) 1251.15 $(M+Na)^+$. Anal. calcd.: C 49.83, H 7.22. Found: C 49.59, H 7.44.



47. A solution of **18** (24 mg, 15.5 μmol) in MeOH (1.0 mL) was treated with a 0.7 M methanolic solution of NaOMe (500 μL, 0.35 mmol). The solution was heated to 40°C and stirred at this temperature overnight; it was then neutralized with Amberlist-15 H^+, filtered and concentrated. The residue was subjected to a gel-filtration chromatography to give **47** (7.8 mg, 78%) as a white wax. $[\alpha]_D$= +25 (c=0.5, H_2O). ^1H NMR (D_2O, 400 MHz) δ 6.12 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.52 (d, *J*=17.2 Hz, 1H, *trans* OCH$_2$CH=C*H*H), 5.47 (d, *J*=10.4, 1H, *cis* OCH$_2$CH=C*H*H), 5.25 (bs, 1H, H-1$_B$), 5.20 (bs, 1H, H-1$_D$), 5.12 (bs, 1H, H-1$_C$), 5.08 (bs, 1H, H-1$_A$), 4.40 (bd, *J*=5.1, 1H, OC*H*HCH=CH$_2$), 4.37 (bd, *J*=5.1 Hz, 1H, OC*H*HCH=CH$_2$), 4.31 (bs, 1H, H_2^C), 4.25 (bs, 1H, H_2^B), 4.22 (bs, 1H, H_2^D), 4.09 (bs, 1H, H_2^A), 4.07-3.96 (m, 5H, H_3^A, H_3^B, H_3^C,

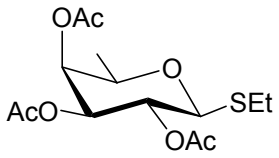
H_{3}^{D}, H_{5}^{D}), 3.93-3.86 (m, 3H, H_{5}^{A}, H_{5}^{B}, H_{5}^{C}), 3.70 (t, *J*=9.8 Hz, 1H, H_{4}^{C}), 3.63 (m, 3H, H_{4}^{A}, H_{4}^{B}, H_{4}^{D}), 1.45 (m, 12H, H_{6}^{A} H_{6}^{B}, H_{6}^{C}, H_{6}^{D}); ^{13}C NMR (D_2O, 100 MHz) δ 133.5 ($OCH_2CH=CH_2$), 119.1 ($OCH_2CH=CH_2$), 102.8 (C_{1}^{D}), 102.4 (C_{1}^{C}), 101.4 (C_{1}^{B}), 97.7 (C_{1}^{A}), 78.9 (C_{2}^{A}), 78.5 (C_{2}^{B}), 78.4 (C_{2}^{C}), 72.5-72.4 (C_{4}^{A}, C_{4}^{B}, C_{4}^{D}), 71.6 (C_{4}^{C}), 70.5-70.1 (C_{2}^{C}, C_{2}^{D}, C_{3}^{A}, C_{3}^{B}, C_{3}^{D}, C_{5}^{D}), 69.7 (C_{5}^{C}), 69.6 (C_{5}^{A}), 69.5 (C_{5}^{B}), 68.6 ($OCH_2CH=CH_2$), 17.0-16.9 (C_{6}^{A}, C_{6}^{B}, C_{6}^{C}, C_{6}^{D}). MALDI-MS for $C_{27}H_{46}O_{17}$ (*m*/*z*): M_r (calcd) 642.27, M_r (found) 643.49 $(M+Na)^+$. Anal. calcd.: C 50.46, H 7.21. Found: C 50.20, H 7.36.



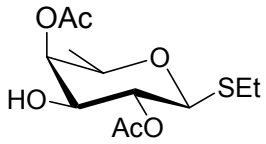
48. A solution of **34** (22 mg, 9.7 μmol) in 3:1 MeOH/CH_2Cl_2 (2.0 mL) was treated with a 3.5 M methanolic solution of NaOMe (90 μL, 0.31 mmol). The solution was heated to 40°C and stirred at this temperature overnight; it was then neutralized with Amberlist-15 H^+, filtered and concentrated. The residue was subjected to a gel-filtration chromatography to give **48** (7.0 mg, 77%) as a white wax. $[\alpha]_D$=+47 (c=0.5, H_2O). ^{1}H NMR (D_2O, 400 MHz) δ 5.96 (m, 1H, $OCH_2C\mathit{H}=CH_2$), 5.36 (d, *J*=17.2, 1H, *trans* $OCH_2CH=C\mathit{H}H$), 5.31 (d, *J*=10.4, 1H, *cis* $OCH_2CH=C\mathit{H}H$), 5.22 (bs, 1H, H_{1}^{E}), 5.10 (bs, 1H, H_{1}^{B}), 5.02 (bs, 1H, H_{1}^{D}), 4.96 (bs, 2H, H_{1}^{C}, H_{1}^{F}), 4.92 (bs, 1H, H_{1}^{A}), 4.24 (bd, *J*=5.1 Hz, 1H, $OC\mathit{H}HCH=CH_2$), 4.21 (bd, *J*=5.1 Hz, 1H, $OC\mathit{H}HCH=CH_2$), 4.17 (bs, 1H, H_{2}^{C}), 4.14 (bs, 1H, H_{2}^{D}), 4.11 (bs, 1H, H_{2}^{B}), 4.08 (bs, 2H, H_{2}^{E}, H_{2}^{F}), 4.01-3.77 (m, 13H, H_{2}^{A}, H_{3}^{A}, H_{3}^{B}, H_{3}^{C}, H_{3}^{D}, H_{3}^{E}, H_{3}^{F}, H_{5}^{A}, H_{5}^{B}, H_{5}^{C}, H_{5}^{D}, H_{5}^{E}, H_{5}^{F}), 3.58 (m, 2H, H_{4}^{C}, H_{4}^{D}), 3.51-3.44 (m, 4H, H_{4}^{A}, H_{4}^{B}, H_{4}^{E}, H_{4}^{F}), 1.29 (m, 18H, H_{6}^{A}, H_{6}^{B}, H_{6}^{C}, H_{6}^{D}, H_{6}^{E}, H_{6}^{F}); ^{13}C NMR (D_2O, 100 MHz) δ 133.9 ($OCH_2CH=CH_2$), 119.5 ($OCH_2CH=CH_2$), 103.0 (C_{1}^{D}), 102.9 (C_{1}^{C}, C_{1}^{F}), 101.7 (C_{1}^{B}, C_{1}^{E}), 98.0 (C_{1}^{A}), 79.3 (C_{2}^{A}), 79.0 (C_{2}^{E}), 78.9 (C_{2}^{B}), 78.8 (C_{3}^{C}), 78.5 (C_{3}^{D}), 72.8-72.2 (C_{4}^{A}, C_{4}^{B}, C_{4}^{C}, C_{4}^{D}, C_{4}^{E}, C_{4}^{F}), 70.7-70.5 (C_{2}^{C}, C_{2}^{D}, C_{2}^{F}, C_{3}^{A}, C_{3}^{B}, C_{3}^{E} C_{3}^{F}, C_{5}^{C}), 70.0-69.8 (C_{5}^{A}, C_{5}^{B},

C_5^D, C_5^E, C_5^F), 68.9 ($OCH_2CH{=}CH_2$), 17.4-17.3 (C_6^A, C_6^B, C_6^C, C_6^D, C_6^E, C_6^F). MALDI-MS for $C_{39}H_{66}O_{25}$ (*m*/*z*): M_r (calcd) 934.39, M_r (found) 935.41 $(M+H)^+$. Anal. calcd.: C 50.10, H 7.12. Found: C 49.91, H 7.29.

Trisaccharidic repeating unit of the *O*-chain from *Xanthomonas campestris* pv. *campestris* 8004

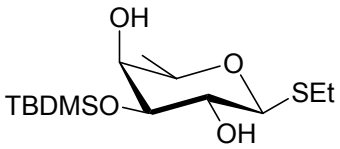


61. A solution of D-Fucose (2.5 g, 15.3 mmol) in 1:1 v/v Ac_2O/py (20 mL) was stirred overnight, after that it was diluted with CH_2Cl_2 (200 mL) and washed with 1M HCl (200 mL), 1M $NaHCO_3$ (200 mL) and water (200 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated. The obtained residue was dissolved in CH_2Cl_2 (20 mL) and treated with iodine (5.5 g, 21.7 mmol) and Et_3SiH (3.47 mL, 21.7 mmol). The mixture was refluxed for few minutes, then diluted with CH_2Cl_2 (200 mL) and washed with 1:1 v/v 1M $NaHCO_3$/10% $Na_2S_2O_3$ (200 mL) and water (200 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was mixed with thiourea (1.75 g, 23.0 mmol), suspended in CH_3CN (20 mL) and then heated to 60°C. After 20 min the reaction mixture was cooled to rt and EtI (2.5 mL, 31.3 mmol) and Et_3N (8.5 mL, 61.0 mmol) were added. The mixture was stirred for additional 20 min and then concentrated. The residue was subjected to column chromatography (15% to 30% ethyl acetate in petroleum ether) to give **61** (3.99 g, 74%).



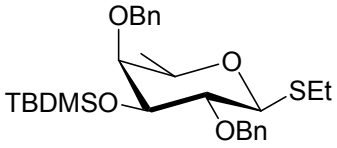
63. A solution of **61** (0.512 g, 1.75 mmol) in MeOH (5.0 mL) was treated at 0°C with *t*-BuOK until it was strongly basic. After 30 min stirring at rt, it was neutralized with Amberlist-15 (H^+), then filtered and concentrated to give a residue that was dissolved in 7:2 v/v $MeC(OMe)_3$/DMF (9.0 mL). CSA (80 mg, 0.34 mmol) was then added and the solution

was evacuated at 100 mbar for 20 min, after that pyridine (7.0 mL) and Ac_2O (7.0 mL) were sequentially added. The solution was stirred overnight at rt, then coevaporated four times with toluene (10 mL each). The residue was dissolved in 80% AcOH (10 mL) and the solution was stirred at rt for 10 min, after that it was coevaporated two times with toluene (5 mL each). The residue was subjected to column chromatography (40% ethyl acetate in petroleum ether) to give **63** (0.893 g, 82%) as a white solid. $[\alpha]_D$= -2.4 (c=1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.17 (dd, $J_{4,3}$=3.2 Hz, $J_{4,5}$=0.8 Hz, 1H, H-4), 4.97 (t, $J_{2,3}$= $J_{2,1}$=9.6 Hz, 1H, H-2), 4.37 (d, $J_{1,2}$=10.0 Hz, 1H, H-1), 3.79 (dd, $J_{3,2}$=9.6 Hz, $J_{3,4}$=3.2 Hz, 1H, H-3), 3.71 (dq, $J_{5,6}$=6.4 Hz, $J_{5,4}$=0.8 Hz, 1H, H-5), 2.67 (m, 2H, S$CH_2$$CH_3$), 2.14, 2.07 (2s, 6H, 2 Ac), 1.23 (t, J_{vic}=7.2 Hz, 3H, SCH_2CH_3), 1.16 (d, $J_{6,5}$=6.4 Hz, 3H, H-6); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.3, 171.0 (2 CO), 83.1 (C_1), 73.4, 73.1, 72.4, 71.0 (C_2, C_3, C_4, C_5), 24.1 (SCH_2CH_3), 20.9, 20.8 (2 $COCH_3$), 16.6 (C_6), 14.7 (SCH_2CH_3). ESI-MS for $C_{12}H_{20}O_6S$ (*m*/*z*): M_r (calcd) 292.10, M_r (found) 315.21 $(M+Na)^+$. Anal. calcd.: C 49.30, H 6.90. Found: C 49.35, H 6.85.

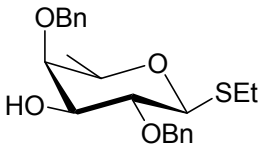


66. To a solution of **61** (3.800 g, 11.4 mmol) in MeOH (25 mL) at 0°C, *t*-BuOK was added until the solution was strongly basic. After 30 min stirring at rt, the mixture was neutralized with Amberlist-15 (H^+), then filtered and concentrated to give a residue that was dissolved in DMF (7.0 mL), cooled to 0°C and then treated with ImH (2.04 g, 29.6 mmol) and TBSCl (2.25 g, 14.8 mmol). The mixture was stirred at 0°C for 30 min and then diluted with ethyl acetate (200 mL) and washed with water (200 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to afford a residue that was subjected to a column chromatography (10% to 20% ethyl acetate in petroleum ether) to give **68** (2.888 g, 79%) as a yellowish oil. $[\alpha]_D$=-13 (c=1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.21 (d, $J_{1,2}$=8.4 Hz, 1H, H-1),

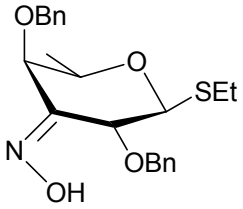
3.66-3.50 (m, 4H, H-2, H-3, H-4, H-5); 2.81-2.64 (m, 2H, SC*H*$_2$CH$_3$), 2.55 (bs, 1H, OH), 2.36 (bs, 1H, OH), 1.32 (d, $J_{6,5}$=6.6 Hz, 3H, H-6), 1.26 (t, J_{vic}=6.8 Hz, 3H, SCH$_2$C*H*$_3$), 0.87 (s, 9H, SiC(C*H*$_3$)$_3$), 0.12 (s, 3H, SiC*H*$_3$), 0.10 (s, 3H, SiC*H*$_3$); ^{13}C NMR (CDCl$_3$, 50 MHz) δ 85.8 (C$_1$), 75.9, 74.2, 72.3, 69.7 (C$_2$, C$_3$, C$_4$, C$_5$), 25.6 (Si*C*(CH$_3$)$_3$), 23.9 (S*C*H$_2$CH$_3$), 18.0 (C$_6$), 15.1 (SCH$_2$*C*H$_3$), -4.5, -5.1 (Si(*C*H$_3$)$_2$). ESI-MS for C$_{14}$H$_{30}$O$_4$SSi (*m*/*z*): *M*$_r$ (calcd) 322.16, *M*$_r$ (found) 345.33 (M+Na)$^+$. Anal. calcd.: C 52.13, H 9.38. Found: C 52.26, H 9.28.



67. A solution of **66** (2.888 g, 8.97 mmol) in DMF (10 mL) was cooled to 0°C and then treated with BnBr (1.97 mL, 19.6 mmol) and NaH (60% oil suspension; 1.808 g, 53.8 mmol). The mixture was stirred at 0°C for 1 hour, after that water (10 mL) was carefully added. The mixture was diluted with ethyl acetate (200 mL) and washed with water (200 mL). The organic layer was collected, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated to afford a residue, that was chromatographed (4% ethyl acetate in petroleum ether) to give **67** (4.188 g, 93%) as white amorphous crystals. [α]$_D$=-14 (c=0.8, CH$_2$Cl$_2$). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.47-7.24 (m, 10H, H-Ar), 5.15 (d, J_{gem}=11.7, 1H, OC*H*HPh), 4.94 (d, J_{gem}=10.5, 1H, OC*H*HPh), 4.80 (d, J_{gem}=10.5, 1H, OC*H*HPh), 4.68 (d, J_{gem}=11.7, 1H, OC*H*HPh), 4.44 (d, $J_{1,2}$=9.0 Hz, 1H, H-1), 3.81 (dd, $J_{3,2}$=9.3 Hz, $J_{3,4}$=3.0 Hz, 1H, H-3), 3.73 (t, $J_{2,3}$= $J_{2,1}$=9.3 Hz, 1H, H-2), 3.61 (q, $J_{5,6}$=6.6 Hz, 1H, H-5), 3.51 (d, $J_{4,3}$=3.0 Hz, 1H, H-4), 2.86-2.66 (m, 2H, SC*H*$_2$CH$_3$), 1.34 (t, J_{vic}=7.5 Hz, 3H, SCH$_2$C*H*$_3$), 1.29 (d, $J_{6,5}$=6.6 Hz, 3H, H-6), 1.02 (s, 9H, SiC(C*H*$_3$)$_3$), 0.19 (s, 3H, SiC*H*$_3$), 0.14 (s, 3H, SiC*H*$_3$); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 138.8, 138.3 (2 C$_{ipso}$), 128.3-127.2 (C-Ar), 84.8 (C$_1$), 80.6, 78.4 (C$_2$, C$_4$), 77.7, 75.3, 75.1, 74.2 (C$_3$, C$_5$, 2 OCH$_2$Ph), 26.0 (Si*C*(CH$_3$)$_3$), 24.5 (S*C*H$_2$CH$_3$), 18.0 (C$_6$), 14.9 (SCH$_2$*C*H$_3$), -4.1, -4.6 (Si(*C*H$_3$)$_2$). ESI-MS for C$_{28}$H$_{42}$O$_4$SSi (*m*/*z*): *M*$_r$ (calcd) 502.26, *M*$_r$ (found) 525.41 (M+Na)$^+$. Anal. calcd.: C 66.89, H 8.42. Found: C 66.76, H 8.68.

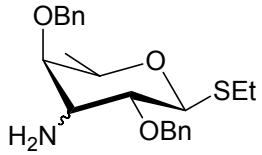


68. A 1M solution of TBAF in THF (20 mL, 20 mmol) was added to a solution of **67** (3.500 g, 6.97 mmol) in THF (20 mL), and the resulting solution was stirred at rt for 3 hours. After concentration, a column chromatography (10% to 30% ethyl acetate in petroleum ether) afforded **68** (2.650 g, 98%) as a yellowish oil. $[\alpha]_D$=+7 (c=0.7, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.49-7.25 (m, 10H, H-Ar), 5.01 (d, J_{gem}=10.8, 1H, OC*H*HPh), 4.88-4.76 (m, 3H, 3 OC*H*HPh), 4.44 (d, $J_{1,2}$=9.0 Hz, 1H, H-1), 3.75 (dd, $J_{3,2}$=9.3 Hz, $J_{3,4}$=3.0 Hz, 1H, H-3), 3.63 (t, $J_{2,3}$= $J_{2,1}$=9.3 Hz, 1H, H-2), 3.57 (d, $J_{4,3}$=3.0 Hz, 1H, H-4), 3.54 (q, $J_{5,6}$=6.6 Hz, 1H, H-5), 2.91-2.78 (m, 3H, SC*H*$_2$CH$_3$, OH), 1.39 (t, J_{vic}=7.5 Hz, 3H, SCH$_2$C*H*$_3$), 1.33 (d, $J_{6,5}$=6.6 Hz, 3H, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.1, 138.0 (2 C_{ipso}), 128.0-127.4 (C-Ar), 84.3 (C_1), 79.0, 78.9 (C_2, C_4), 75.5, 75.1, 74.9, 74.3 (C_3, C_5, 2 OCH$_2$Ph), 24.5 (SCH$_2$CH$_3$), 16.9 (C_6), 14.7 (SCH$_2$CH$_3$). ESI-MS for $C_{22}H_{28}O_4S$ (*m*/*z*): M_r (calcd) 388.17, M_r (found) 411.38 $(M+Na)^+$. Anal. calcd.: C 68.01, H 7.26. Found: C 68.36, H 7.20.



70. Alcohol **68** (2.796 g, 7.20 mmol) was dissolved in 2:1 v/v DMSO/Ac_2O (15 mL) and stirred at rt for 3 hours. The solution was then diluted with ethyl acetate (300 mL) and washed with water (300 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to afford a residue that was dissolved in MeOH (25 mL) and treated with NaOAc (847 mg, 10.1 mmol) and NH_2OH·HCl (750 mg, 10.1 mmol). After stirring at rt for 2 hours, the solution was concentrated and the obtained residue was subjected to column chromatography (10% to 30% ethyl acetate in petroleum ether) to afford **70** (2.252 g, 78%) as a yellowish oil. $[\alpha]_D$=-38.8 (c=1.1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36-7.20 (m, 10H, H-Ar), 4.83 (d, $J_{4,5}$=1.8 Hz, 1H, H-4), 4.81 (d, J_{gem}=11.2 Hz, 1H, OC*H*HPh), 4.71 (d, J_{gem}=11.2 Hz, 1H, OC*H*HPh), 4.54 (d, $J_{1,2}$=9.8 Hz, 1H, H-1), 4.51 (d, J_{gem}=12.2 Hz, 1H, OC*H*HPh), 4.34 (d, J_{gem}=12.2 Hz,

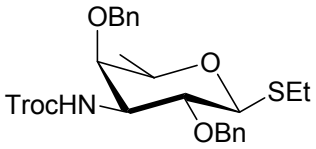
1H, OC*H*HPh), 4.27 (d, $J_{2,1}$=9.8 Hz, 1H, H-2), 3.58 (dq, $J_{5,6}$=6.6 Hz, $J_{5,4}$=1.8 Hz, 1H, H-5), 2.89-2.58 (m, 2H, SCH_2CH$_3$), 1.31 (d, $J_{6,5}$=6.6 Hz, 3H, H-6), 1.29 (t, J_{vic}=7.5 Hz, 3H, SCH$_2$CH_3); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 154.8 (CN), 137.6, 137.1 (2 C$_{ipso}$), 128.7-127.7 (C-Ar), 86.7 (C$_1$), 76.0, 74.7, 73.9, 71.2, 70.2 (C$_2$, C$_4$, C$_5$, 2 OCH$_2$Ph), 24.4 (SCH$_2$CH$_3$), 16.4 (C$_6$), 14.8 (SCH$_2$CH$_3$). ESI-MS for $C_{22}H_{27}NO_4S$ (*m*/*z*): M_r (calcd) 401.17, M_r (found) 424.42 (M+Na)$^+$. Anal. calcd.: C 65.81, H 6.78, N 3.49. Found: C 65.95, H 6.59, N 3.43.



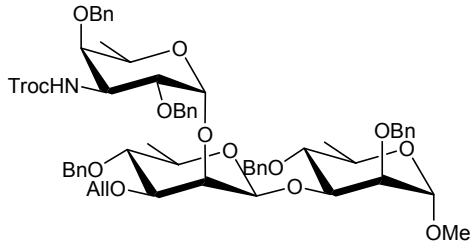
71/72. A solution of **70** (2.231 g, 5.56 mmol) in THF (25 mL) was cooled to 0°C and then treated with a 70% solution of Red-Al® in toluene (7.16 mL, 27.8 mmol). The solution was stirred at 0°C for 5 hours, then water (10 mL) was added dropwise. The mixture was diluted with ethyl acetate (200 mL) and washed with water (200 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was subjected to column chromatography (1% methanol in dichloromethane) to afford **72 (**168 mg, 8%) as a yellowish oil. [α]$_D$=-12 (c=0.4, CH_2Cl_2). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.36-7.25 (m, 10H, H-Ar), 4.90 (d, $J_{1,2}$=9.6 Hz, 1H, H-1), 4.71 (d, J_{gem}=11.6 Hz, 1H, OC*H*HPh), 4.55 (d, J_{gem}=11.6 Hz, 1H, OC*H*HPh), 4.53 (d, J_{gem}=12.0 Hz, 1H, OC*H*HPh), 4.49 (d, J_{gem}=12.0 Hz, 1H, OC*H*HPh), 4.57 (dq, $J_{5,6}$=6.4 Hz, $J_{5,4}$=2.0 Hz, 1H, H-5), 3.66 (dd, $J_{2,1}$=9.6 Hz, $J_{2,3}$=3.6 Hz, 1H, H-2), 3.64 (dd, $J_{4,3}$=3.6 Hz, $J_{4,5}$= 2.0 Hz, 1H, H-4), 3.47 (t, $J_{3,4}$= $J_{3,2}$= 3.6 Hz, 1H, H-3), 2.77-2.66 (m, 2H, SCH_2CH$_3$), 1.28 (t, J_{vic}=8.0 Hz, 3H, SCH$_2$CH_3), 1.20 (d, $J_{6,5}$=6.4 Hz, 3H, H-6); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 138.3, 138.1 (2 C$_{ipso}$), 128.4-127.7 (C-Ar), 80.6, 80.0, 75.6, 72.6, 70.6, 62.7 (C$_1$, C$_2$, C$_4$, C$_5$, 2 OCH$_2$Ph), 49.3 (C$_3$), 24.4 (SCH$_2$CH$_3$), 16.5 (C$_6$), 14.9 (SCH$_2$CH$_3$).. ESI-MS for $C_{22}H_{29}NO_3S$ (*m*/*z*): M_r (calcd) 387.19, M_r (found) 410.41 (M+Na)$^+$. Anal. calcd.: C 68.18, H 7.54, N 3.61. Found: C 68.06, H 7.66, N 3.57.

Second eluted compound **71** (1.726 g, 80%) was recovered as a yellowish oil. [α]$_D$=+18 (c=0.3, CH_2Cl_2). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.40-7.28 (m, 10H, H-Ar), 5.04 (d,

J_{gem}=10.8 Hz, 1H, OC*H*HPh), 4.76 (d, J_{gem}=11.4 Hz, 1H, OC*H*HPh), 4.65 (d, J_{gem}=11.4 Hz, 1H, OC*H*HPh), 4.43 (d, J_{gem}=10.8 Hz, 1H, OC*H*HPh), 4.34 (d, $J_{1,2}$=9.6 Hz, 1H, H-1), 3.61-3.57 (m, 2H, H-4, H-5), 3.30 (t, $J_{2,3}$=$J_{2,1}$= 9.6 Hz, 1H, H-2), 2.84-2.69 (m, 3H, H-3, SC*H*$_2$CH$_3$), 1.33 (t, J_{vic}=7.5 Hz, 3H, SCH$_2$C*H*$_3$), 1.31 (d, $J_{6,5}$=6.3 Hz, 3H, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.2, 138.0 (2 C_{ipso}), 128.5-127.7 (C-Ar), 85.5 (C_1), 81.0, 80.5 (C_2, C_4), 76.1, 76.0, 75.3 (C_5, 2 OCH$_2$Ph), 58.0 (C_3), 24.9 (SCH$_2$CH$_3$), 17.2 (C_6), 14.9 (SCH$_2$CH$_3$). ESI-MS for $C_{22}H_{29}NO_3S$ (*m*/*z*): M_r (calcd) 387.19, M_r (found) 410.29 $(M+Na)^+$. Anal. calcd.: C 68.18, H 7.54, N 3.61. Found: C 68.00, H 7.58, N 3.55.

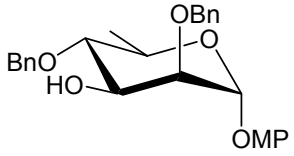


73. A solution of **71** (138 mg, 0.356 mmol) in pyridine (2.0 mL) was treated with 2,2,2-trichloroethyl chloroformate (122 μL, 0.890 mmol) and stirred at rt for 2 hours, after that MeOH (10 mL) was added and the solution was concentrated. The residue was subjected to a column chromatography (1% methanol in dichloromethane) to afford **6** (164 mg, 82%) as a yellowish powder. $[\alpha]_D$=+25 (c=0.8, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.28 (m, 10H, H-Ar), 4.86 (d, J_{gem}=10.8 Hz, 1H, OC*H*HPh), 4.76 (d, J_{gem}=11.3 Hz, 1H, OC*H*HPh), 4.63 (s, 2H, OCH$_2$CCl$_3$), 4.58 (d, J_{gem}=11.3 Hz, 1H, OC*H*HPh), 4.48 (d, $J_{1,2}$=9.3 Hz, 1H, H-1), 4.44 (d, J_{gem}=10.8 Hz, 1H, OC*H*HPh), 3.84 (dt, $J_{3,2}$=$J_{3,NH}$=9.9 Hz, $J_{3,4}$=3.0 Hz, 1H, H-3), 3.65 (m, 2H, H-4, H-5), 3.42 (t, $J_{2,3}$=$J_{2,1}$=9.9 Hz, 1H, H-2), 2.84-2.71 (m, 2H, SC*H*$_2$CH$_3$), 1.32 (m, 6H, H-6, SCH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.0 (CO), 137.7, 137.5 (2 C_{ipso}), 128.6-127.9 (C-Ar), 95.4 (CCl_3), 85.6 (C_1), 79.4, 76.3, 75.9, 75.0, 74.6, 74.3 (C_2, C_4, C_5, 2 OCH$_2$Ph, OCH$_2$CCl$_3$), 57.1 (C_3), 25.0 (SCH$_2$CH$_3$), 17.1 (C_6), 14.9 (SCH$_2$CH$_3$). ESI-MS for $C_{25}H_{30}Cl_3NO_5S$ (*m*/*z*): M_r (calcd) 561.09, M_r (found) 584.29 $(M+Na)^+$. Anal. calcd.: C 53.34, H 5.37, N 2.49. Found: C 53.38, H 5.35, N 2.48.



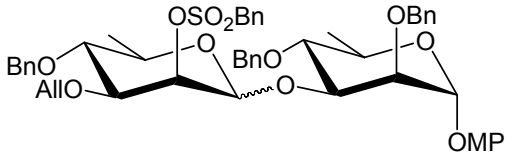
A mixture of acceptor **87** (34 mg, 0.054 mmol) and donor **73** (60 mg, 0.107 mmol) was coevaporated three times with toluene (2 mL). The residue was mixed with freshly activated AW-300 4Å molecular sieves, cooled to -40°C, suspended in 1:1 Et_2O/CH_2Cl_2 (2.0 mL) under Ar atmosphere and then treated with NIS (30 mg, 0.134 mmol) and AgOTf (12 mg, 0.047 mmol). The temperature was allowed to rise gradually to -20°C. After 1 hour stirring at -20°C, the mixture was diluted with CH_2Cl_2 (30 mL) and washed with 10% $Na_2S_2O_3$ (30 mL) and then with 1M $NaHCO_3$ (30 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue that, after column chromatography (10% to 30% ethyl acetate in petroleum ether), afforded trisaccharide (47 mg, 77%) as a yellowish oil. $[\alpha]_D$=+145 (c=0.7, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.49-7.18 (m, 25H, H-Ar), 5.95 (m, 1H, OCH_2CH=CH_2), 5.73 (d, $J_{1,2}$=3.3 Hz 1H, H-1_C), 5.34 (d, *J*=16.8 Hz, 1H, *trans* OCH_2CH=C*H*H), 5.24 (d, *J*=10.3 Hz, 1H, *cis* OCH_2CH=C*H*H), 5.14 (d, *J* =10.3 Hz, 1H, OC*H*HPh), 5.07 (d, *J* =12.1 Hz, 1H, OC*H*HPh), 5.01 (d, $J_{H,NH}$=5.1 Hz, 1H, NH), 4.81-4.46 (m, 12H, H-1_A, H-5_C, OCH_2CCl_3, 8 OC*H*HPh), 4.38 (bs, 1H, H-1_B), 4.26-4.20 (m, 3H, H-2_B, H-3_C, OC*H*HCH=CH_2), 4.17-4.11 (m, 3H, H-3_A, H-4_C, OC*H*HCH=CH_2), 3.78 (dd, $J_{2,3}$=11.1 Hz, $J_{2,1}$=3.3 Hz, 1H, H-2_C), 3.74-3.67 (m, 2H, H-2_A, H-5_A), 3.56 (t, $J_{4,5}$=$J_{4,3}$=8.8 Hz, 1H, H-4_A), 3.50 (t, $J_{4,5}$=$J_{4,3}$=9.3 Hz, 1H, H-4_B), 3.35 (s, 3H, OCH_3), 3.29 (dd, $J_{3,4}$=9.3 Hz, $J_{3,2}$=2.3 Hz, 1H, H-3_B), 3.21 (dq, $J_{5,4}$=9.3 Hz, $J_{5,6}$=6.0 Hz, 1H, H-5_B), 1.41 (d, $J_{6,5}$=6.2 Hz, 3H, H-6_A), 1.24 (m, 6H, H-6_B, H-6_C); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 153.9 (CO), 138.9, 138.6, 138.5, 138.1, 138.0 (5 C_{ipso}-Bn), 134.6 (OCH_2CH=CH_2), 128.5-126.8 (C-Ar), 117.2 (OCH_2CH=CH_2), 99.0, 97.8, 95.7, 95.1 (C_{1A}, C_{1B}, C_{1C}, CCl_3), 83.6, 80.4, 80.2, 78.9, 76.5, 76.3, 75.1, 74.8, 74.1, 73.5, 73.4, 72.4, 72.0, 71.7, 71.1, 69.9, 67.2, 66.0 (C_{2A}, C_{2B}, C_{2C}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{4C}, C_{5A}, C_{5B}, C_{5C}, 5 OCH_2Ph, OCH_2CH=CH_2, OCH_2CCl_3), 54.6, 52.0 (C_{3C}, OCH_3), 18.5, 17.8, 16.8 (C_{6A}, C_{6B}, C_{6C}).

MALDI-MS for $C_{60}H_{70}Cl_3NO_{14}$ (*m*/*z*): M_r (calcd) 1133.39, M_r (found) 1156.42 $(M+Na)^+$. Anal. calcd.: C 63.46, H 6.21, N 1.23. Found: C 63.30, H 6.30, N 1.21.



90. Alcohol **85** (90 mg, 0.254 mmol) was dissolved in 1:1:0.1 v/v/v $Ac_2OH/Ac_2O/TFA$ (2.2 mL). The yellow solution was stirred at 70°C overnight, after that the solution was cooled to 0°C and water (10 mL) was very carefully added. The solution was diluted with CH_2Cl_2 (50 mL) and washed twice with water (50 mL) and then with 0.1 M $NaHCO_3$ (50 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue that was mixed with *p*-methoxyphenol (63 mg, 0.508 mmol) and then dissolved at 0°C in CH_2Cl_2 (2.5 mL) under Ar atmosphere. The solution was then treated with $BF_3{\cdot}OEt_2$ (9.4 μl, 76.2 μmol), stirred at 0°C for 2 hours, then diluted with CH_2Cl_2 (40 mL) and washed with 1M $NaHCO_3$ (40 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue that was dissolved in MeOH (3.0 mL) and *t*-BuOK was added until the solution was strongly basic. The solution was stirred at rt for 40 min, then neutralized with Amberlist-15 (H^+), filtered and concentrated. The residue was subjected to column chromatography (8% to 16% ethyl acetate in petroleum ether) to give **8** (56 mg, 62%) as a colourless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42-7.29 (m, 10H, H-Ar), 6.97 (d, J_{ortho}=9.0 Hz, 2H, $C_6H_2H_2OCH_3$), 6.84 (d, J_{ortho}=9.0 Hz, 2H, $C_6H_2H_2OCH_3$), 5.45 (bs, 1H, H-1), 4.95 (d, J_{gem}=11.1 Hz, 1H, OC*H*HPh), 4.82 (d, J_{gem}=11.4 Hz, 1H, OC*H*HPh), 4.70 (d, J_{gem}=11.1 Hz, 1H, OC*H*HPh), 4.68 (d, J_{gem}=11.4 Hz, 1H, OC*H*HPh), 4.16 (m, 1H, H-3), 3.94 (dd, $J_{2,3}$=3.9 Hz, $J_{2,1}$=1.2 Hz, 1H, H-2), 3.84 (dq, $J_{5,4}$=9.3 Hz, $J_{5,6}$=6.3 Hz, 1H, H-5), 3.78 (s, 3H, $C_6H_4OCH_3$), 3.44 (t, $J_{4,5}$=$J_{4,3}$=9.3 Hz, 1H, H-4), 2.45 (d, $J_{H,OH}$=7.0 Hz, 1H, OH), 1.34 (d, $J_{6,5}$=6.3 Hz, 3H, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.7, 150.2 (2 C_{ipso}-MP), 138.3, 137.5 (2 C_{ipso}-Bn), 128.5-127.6 (C-Ar), 117.4, 114.5 (C-Ar MP), 95.9 (C_1),

82.1, 78.3 (C_2, C_4), 75.0, 73.1, 71.4, 67.8 (C_3, C_5, 2 OCH_2Ph), 55.6 (OCH_3), 18.0 (C_6). ESI-MS for $C_{21}H_{26}O_5$ (*m*/*z*): M_r (calcd) 358.18, M_r (found) 381.37 $(M+Na)^+$. Anal. calcd.: C 70.37, H 7.31. Found: C 70.58, H 7.19.



91. (From compounds **84** and **90**) A mixture of acceptor **84** (40 mg, 89.7 μmol) and donor **90** (89 mg, 143.5 μmol) was coevaporated three times with toluene (2 mL). The residue was mixed with freshly activated AW-300 4Å molecular sieves, cooled to -50°C and suspended in CH_2Cl_2 (3.0 mL) under Ar atmosphere. A 144 μM solution of TMSOTf in CH_2Cl_2 (70 μL, 9.9 μmol) was added. The temperature was allowed to rise gradually to -25°C. After 2 hours stirring at -25°C, some drops of Et_3N were added. The mixture was filtered over a Celite pad, then concentrated to give a residue that was subjected to column chromatography (1% to 3% ethyl acetate in toluene) to afford **91α** (42 mg, 53%) as a yellowish oil. As second eluted compound **91β** (28 mg, 35%) was recovered as a yellowish oil.

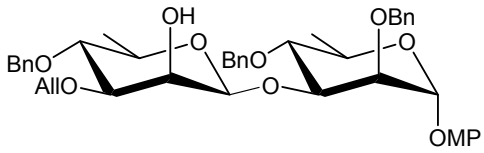
(From compound **86β**) Compound **86β** (1.066 g, 1.35 mmol) was dissolved in 1:1:0.1 v/v/v AcOH/Ac_2O/TFA (42 mL). The yellow solution was heated to 70°C and stirred overnight, after that it was cooled to 0°C and water (40 mL) was very carefully added. The solution was diluted with CH_2Cl_2 (500 mL) and washed twice with water (500 mL) and then with 0.1 M $NaHCO_3$ (500 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue that was mixed with *p*-methoxyphenol (336 mg, 2.71 mmol) and then dissolved at 0°C in CH_2Cl_2 (26 mL) under Ar atmosphere. The solution was then treated with $BF_3{\cdot}OEt_2$ (50 μl, 0.410 μmol), stirred at 0°C for 2 hours, then diluted with CH_2Cl_2 (200 mL) and washed with 1M $NaHCO_3$ (200 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue that was subjected to a column

chromatography (1% to 3% ethyl acetate in toluene) to afford **91α** (772 mg, 65%) as a yellowish oil.

91α: $[\alpha]_D$=+30 (c=0.7, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.55-7.15 (m, 20H, H-Ar), 6.93 (d, J_{ortho}=9.2 Hz, 2H, $C_6H_2H_2OCH_3$), 6.80 (d, J_{ortho}=9.2 Hz, 2H, $C_6H_2H_2OCH_3$), 5.92 (m, 1H, OCH_2CH=CH_2), 5.35 (d, $J_{2,3}$=3.0 Hz, 1H, H-2$_B$), 5.28 (d, J_{vic}=17.2 Hz, 1H, *trans* OCH_2CH=C*H*H), 5.18-5.08 (m, 3H, H-1$_A$, H-1$_B$, *cis* OCH_2CH=C*H*H), 4.93 (d, J_{gem}=11.0 Hz, 1H, OC*H*HPh), 4.82 (d, J_{gem}=10.8 Hz, 1H, OC*H*HPh), 4.72 (s, 2H, 2 OC*H*HPh), 4.64 (d, J_{gem}=11.0 Hz, 1H, OC*H*HPh), 4.60 (d, J_{gem}=10.8 Hz, 1H, OC*H*HPh), 4.46 (d, J_{gem}=14.0 Hz, 1H, OSO_2CHHPh), 4.37 (d, J_{gem}=14.0 Hz, 1H, OSO_2CHHPh), 4.25-4.14 (m, 2H, H-3$_A$, OC*H*HCH=CH_2), 4.06 (dd, 1H, J_{gem}=12.6 Hz, J_{vic}=5.4 Hz, OC*H*HCH=CH_2), 3.91-3.76 (m, 7H, H-2$_A$, H-3$_B$, H-5$_A$, H-5$_B$, $C_6H_4OCH_3$), 3.63 (t, $J_{4,5}$=$J_{4,3}$=9.2 Hz, 1H, H-4$_A$), 3.39 (t, $J_{4,5}$=$J_{4,3}$=9.4 Hz, 1H, H-4$_B$), 1.26 (m, 6H, H-6$_A$, H-6$_B$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.9, 150.3 (2 C_{ipso}-MP), 138.4, 137.9, 137.8 (3 C_{ipso}-Bn), 134.3 (OCH_2CH=CH_2), 130.9-126.3 (C-Ar), 120.5, 117.8, 114.6 (C-Ar MP, OCH_2CH=CH_2), 99.2, 96.4 (C_{1A}, C_{1B}), 84.6, 80.4, 79.7, 78.2, 77.5, 76.3, 76.2, 75.3, 72.8, 71.4, 68.7, 68.6 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph, OCH_2CH=CH_2), 57.6, 55.6 (OCH_3, OSO_2CH_2Ph), 18.0, 17.9 (C_{6A}, C_{6B}). MALDI-MS for $C_{50}H_{56}O_{12}S$ (*m*/*z*): M_r (calcd) 880.35, M_r (found) 903.41 $(M+Na)^+$. Anal. calcd.: C 68.16, H 6.41. Found: C 68.04, H 6.38.

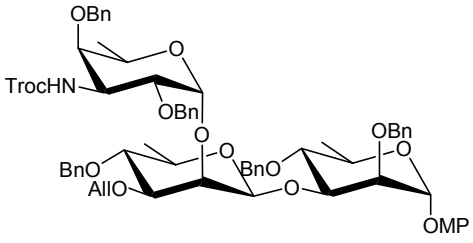
91β: $[\alpha]_D$=+2.8 (c=1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39-7.10 (m, 20H, H-Ar), 6.91 (d, J_{ortho}=9.0 Hz, 2H, $C_6H_2H_2OCH_3$), 6.76 (d, J_{ortho}=9.0 Hz, 2H, $C_6H_2H_2OCH_3$), 5.95 (m, 1H, OCH_2CH=CH_2), 5.44 (d, $J_{2,3}$=2.9 Hz, 1H, H-2$_B$), 5.35 (d, J_{vic}=17.3 Hz, 1H, *trans* OCH_2CH=C*H*H), 5.19 (d, J_{vic}=11.2 Hz, 1H, *cis* OCH_2CH=C*H*H), 5.08 (d, $J_{1,2}$=2.2 Hz 1H, H-1$_A$), 4.93 (d, J_{gem}=11.0 Hz, 1H, OC*H*HPh), 4.89 (d, J_{gem}=10.9 Hz, 1H, OC*H*HPh), 4.79 (d, J_{gem}=12.1 Hz, 1H, OC*H*HPh), 4.68 (d, J_{gem}=12.1 Hz, 1H, OC*H*HPh), 4.58 (d, J_{gem}=10.9 Hz, 1H, OC*H*HPh), 4.52-4.47 (m, 4H, H-1$_B$, OC*H*HPh, OSO_2CH_2Ph), 4.35-4.25 (m, 2H, H-3$_A$, OC*H*HCH=CH_2), 4.05 (dd, J_{gem}=12.4 Hz, J_{vic}=5.6 Hz, 1H, OC*H*HCH=CH_2), 3.95 (t,

$J_{2,1}$=$J_{2,3}$=2.2 Hz, 1H, H-2$_A$), 3.82 (dq, $J_{5,4}$=9.0 Hz, $J_{5,6}$=6.3 Hz, 1H, H-5$_A$), 3.70 (s, 3H, $C_6H_4OCH_3$), 3.60 (t, $J_{4,5}$=$J_{4,3}$=9.0 Hz, 1H, H-4$_A$), 3.39 (dd, $J_{3,4}$=9.0 Hz, $J_{3,2}$=2.9 Hz, 1H, H-3$_B$), 3.35 (t, $J_{4,5}$=$J_{4,3}$=9.0 Hz, 1H, H-4$_B$), 3.28 (dq, $J_{5,4}$=9.0 Hz, $J_{5,6}$=6.0 Hz, 1H, H-5$_B$), 1.27 (d, $J_{6,5}$=6.0 Hz, 3H, H-6$_B$), 1.19 (d, $J_{6,5}$=6.3 Hz, 3H, H-6$_A$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.4, 152.8 (2 C_{ipso}-MP), 137.8, 137.5, 137.3 (3 C_{ipso}-Bn), 133.6 ($OCH_2CH=CH_2$), 130.4-127.1 (C-Ar), 117.5, 115.5, 114.3 (C-Ar MP, $OCH_2CH=CH_2$), 97.1, 96.6 (C_{1A}, C_{1B}), 79.8, 78.9, 77.2, 76.6, 76.0, 75.3, 75.1, 73.3, 72.3, 71.4, 70.5, 67.8 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph, $OCH_2CH=CH_2$), 57.2, 55.3 (OCH_3, SO_2CH_2Ph), 17.8, 17.4 (C_{6A}, C_{6B}). MALDI-MS for $C_{50}H_{56}O_{12}S$ (*m*/*z*): M_r (calcd) 880.35, M_r (found) 903.54 $(M+Na)^+$. Anal. calcd.: C 68.16, H 6.41. Found: C 67.98, H 6.36.



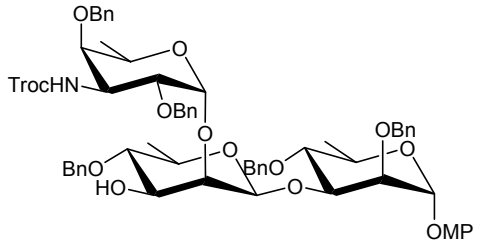
92. Disaccharide **91β** (776 mg, 0.882 mmol) was dissolved in dry DMF (15 mL) under Ar atmosphere and then $NaNH_2$ (690 mg, 17.6 mmol) was added. The mixture was stirred at rt for 48 hours, after that we added MeOH (60 mL) and then, dropwise, AcOH to neutralize the solution, that was then concentrated. The residue was dissolved in CH_2Cl_2 (300 mL) and washed with 1M $NaHCO_3$ (300 mL) and brine (300 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue that, after column chromatography (10% to 50% ethyl acetate in petroleum ether), afforded **92** (333 mg, 52%) as a yellowish oil. $[\alpha]_D$=+24.0 (c=1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44-7.26 (m, 15H, H-Ar), 6.98 (d, J_{ortho}=9.0 Hz, 2H, $C_6H_2H_2OCH_3$), 6.80 (d, J_{ortho}=9.0 Hz, 2H, $C_6H_2H_2OCH_3$), 6.08 (m, 1H, $OCH_2CH=CH_2$), 5.46 (d, $J_{1,2}$=2.4 Hz, 1H, H-1$_A$), 5.39 (d, J_{vic}=17.3 Hz, 1H, *trans* $OCH_2CH=CHH$), 5.27 (d, J_{vic}=11.2 Hz, 1H, *cis* $OCH_2CH=CHH$), 5.00 (d, J_{gem}=12.0 Hz, 1H, OC*H*HPh), 4.97 (d, J_{gem}=11.7 Hz, 1H, OC*H*HPh), 4.87 (d, J_{gem}=12.6 Hz, 1H, OC*H*HPh), 4.73-4.64 (m, 3H, OC*H*HPh), 4.46-4.43 (m, 2H, H-3$_A$, H-1$_B$), 4.28 (dd, J_{gem}=12.0 Hz, J_{vic}=6.0 Hz, 1H, OC*H*HCH=CH_2), 4.15 (dd, J_{gem}=12.0 Hz,

J_{vic}=6.0 Hz, 1H, OC*H*HCH=CH_2), 3.98-3.95 (m, 2H, H-2_A, H-2_B), 3.88 (dq, $J_{5,4}$=9.0 Hz, $J_{5,6}$=6.0 Hz, 1H, H-5_A), 3.68 (t, $J_{4,5}$=$J_{4,3}$=9.0 Hz, 1H, H-4_A), 3.53 (t, $J_{4,5}$=$J_{4,3}$=9.0 Hz, 1H, H-4_B), 3.38 (dd, $J_{3,4}$=9.0 Hz, $J_{3,2}$=2.7 Hz, 1H, H-3_B), 3.34 (dq, $J_{5,4}$=9.0 Hz, $J_{5,6}$=6.0 Hz, 1H, H-5_B), 1.36, 1.33 (6H, H-6_B, H-6_A); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 154.4, 149.7 (2 C_{ipso}-MP), 137.8, 137.7, 137.3 (3 C_{ipso}-Bn), 134.3 ($OCH_2CH=CH_2$), 127.9-127.1 (C-Ar), 117.2, 116.3, 114.0 (C-Ar MP, $OCH_2CH=CH_2$), 96.9, 96.3 (C_{1A}, C_{1B}), 80.8, 79.3, 79.0, 75.6, 74.9, 74.3, 74.1, 72.1, 71.1, 70.0, 68.2, 67.8 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph, $OCH_2CH=CH_2$), 55.1 (OCH_3), 17.6, 17.4 (C_{6A}, C_{6B}). ESI-MS for $C_{43}H_{50}O_{10}$ (*m*/z): M_r (calcd) 726.34, M_r (found) 749.57 $(M+Na)^+$. Anal. calcd.: C 71.05, H 6.93. Found: C 71.10, H 6.95.



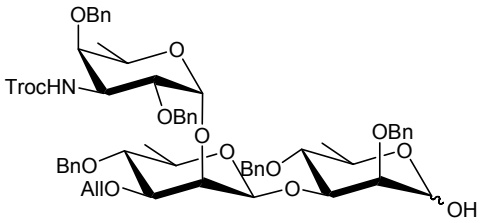
93. Acceptor **92** (179 mg, 0.246 mmol) was mixed with donor **73** (281 mg, 0.499 mmol). The mixture was coevaporated three times with toluene (5 mL), then mixed with freshly activated AW-300 4Å molecular sieves, cooled to -40°C and suspended in 1:1 v/v Et_2O/CH_2Cl_2 (4.0 mL) under Ar atmosphere. The mixture was treated with NIS (141 mg, 0.627 mmol) and AgOTf (54 mg, 0.209 mmol). The temperature was allowed to rise gradually to -20°C. After 90 min stirring at -20°C, the mixture was diluted with CH_2Cl_2 (100 mL) and washed with 10% $Na_2S_2O_3$ (100 mL) and then with 1M $NaHCO_3$ (100 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give an oily residue. After column chromatography (10% to 30% ethyl acetate in petroleum ether), **93** (196 mg, 65%) was obtained as a yellowish oil. $[\alpha]_D$=+130 (c=0.5, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.54-7.33 (m, 25H, H-Ar), 7.02 (d, J_{ortho}=9.0 Hz, 2H, $C_6H_2H_2OCH_3$), 6.88 (d, J_{ortho}=9.0 Hz, 2H, $C_6H_2H_2OCH_3$), 6.02 (m, 1H, $OCH_2CH=CH_2$), 5.78 (d, $J_{1,2}$=2.8 Hz 1H, H-1_C), 5.43 (d, $J_{1,2}$=3.2 Hz 1H, H-1_A), 5.39 (d, J_{vic}=17.5 Hz, 1H, *trans*

$OCH_2CH=CHH$), 5.29 (d, J_{vic}=10.0 Hz, 1H, *cis* $OCH_2CH=CHH$), 5.15 (d, J_{gem}=12.4 Hz, 1H, $OCHHPh$), 5.12 (d, J_{gem}=12.5 Hz, 1H, $OCHHPh$), 5.05 (d, $J_{H,NH}$=4.5 Hz, 1H, NH), 4.87 (d, J_{gem}=12.5 Hz, 1H, $OCHHPh$), 4.79-4.53 (m, 11H, H-1_B, H-5_C, OCH_2CCl_3, 7 $OCHHPh$), 4.36-4.18 (m, 6H, H-2_B, H-3_A, H-3_C, H-4_C, $OCH_2CH=CH_2$), 3.99 (bs, 1H, H-2_A), 3.95 (dq, $J_{5,4}$=9.9 Hz, $J_{5,6}$=6.0 Hz, 1H, H-5_A), 3.84 (m, 4H, H-2_C, OCH_3), 3.68 (t, $J_{4,5}$=$J_{4,3}$=9.9 Hz, 1H, H-4_A), 3.59 (t, $J_{4,5}$=$J_{4,3}$=9.0 Hz, 1H, H-4_B), 3.39 (dd, $J_{3,4}$=9.0 Hz, $J_{3,2}$=2.4 Hz, 1H, H-3_B), 3.33 (dq, $J_{5,4}$=9.0 Hz, $J_{5,6}$=6.0 Hz, 1H, H-5_B), 1.41 (d, $J_{6,5}$=6.0 Hz, 3H, H-6_C), 1.33 (d, $J_{6,5}$=6.0 Hz, 3H, H-6_B), 1.27 (d, $J_{6,5}$=6.0 Hz, 3H, H-6_A); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 154.9, 153.9, 150.6 (CO, 2 C_{ipso}-MP), 138.8, 138.6, 138.5, 138.0, 137.9 (5 C_{ipso}-Bn), 134.6 ($OCH_2CH=CH_2$), 129.0-125.3 (C-Ar), 118.2, 117.2, 114.5 ($OCH_2CH=CH_2$, C-Ar MP), 98.4, 98.1, 95.7, 95.1 (C_{1A}, C_{1B}, C_{1C}, CCl_3), 83.6, 80.8, 80.4, 79.0, 77.1, 76.2, 75.4, 75.1, 74.2, 73.5, 73.2, 72.6, 72.1, 71.6, 71.0, 70.0, 68.0, 66.0 (C_{2A}, C_{2B}, C_{2C}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{4C}, C_{5A}, C_{5B}, C_{5C}, 5 OCH_2Ph, $OCH_2CH=CH_2$, OCH_2CCl_3), 55.6, 52.0 (C_{3C}, OCH_3), 18.7, 17.8, 16.8 (C_{6A}, C_{6B}, C_{6C}). MALDI-MS for $C_{66}H_{74}Cl_3NO_{15}$ (*m*/*z*): M_r (calcd) 1225.41, M_r (found) 1248.64 $(M+Na)^+$. Anal. calcd.: C 64.57, H 6.08, N 1.14. Found: C 64.45, H 6.03, N 1.12.



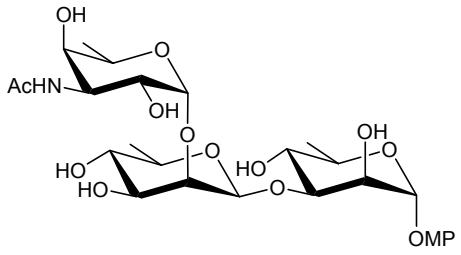
95. A solution of **93** (39 mg, 31.7 μmol) in 2:1 v/v MeOH/CH_2Cl_2 (2.1 mL) was treated with $PdCl_2$ (1.7 mg, 9.5 μmol). The mixture was vigorously stirred at rt overnight and then filtered over a Celite pad, diluted with CH_2Cl_2 (30 mL) and washed with water (30 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was subjected to column chromatography (10% ethyl acetate in toluene) to give **95** (32 mg, 86%) as a colourless oil. $[\alpha]_D$=+54.4 (c=1.5, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39-7.12 (m, 25H, H-Ar), 6.95 (d, J_{ortho}=9.0 Hz, 2H, $C_6H_2H_2OCH_3$), 6.81 (d, J_{ortho}=9.0 Hz, 2H, $C_6H_2H_2OCH_3$), 5.37 (d, $J_{1,2}$=2.7 Hz, 1H, H-1_A), 5.23 (d, $J_{1,2}$=3.2 Hz, 1H, H-1_C), 4.92 (d,

J_{gem}=10.4 Hz, 1H, OC*H*HPh), 4.84-4.24 (m, 14H, 9 OC*H*HPh, H-1$_B$, H-3$_C$, CH_2CCl$_3$, NH), 3.92-3.86 (m, 3H, H-2$_A$, H-3$_A$, H-5$_C$), 3.77 (s, 3H, OCH$_3$), 3.73 (dd, $J_{2,3}$=10.9 Hz, $J_{2,1}$=3.2 Hz, 1H, H-2$_C$), 3.65-3.55 (m, 2H, H-4$_C$, H-5$_A$), 3.47 (t, $J_{4,5}$=$J_{4,3}$=8.8 Hz, 1H, H-4$_A$), 3.34 (bs, 1H, H-2$_B$), 3.27-3.20 (m, 3H, H-3$_B$, H-4$_B$, H-5$_B$), 1.34 (d, $J_{6,5}$=6.2 Hz, 3H, H-6$_C$), 1.27 (m, 6H, H-6$_A$, H-6$_B$); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 155.0, 153.9, 150.5 (CO, 2 C$_{ipso}$-MP), 138.7, 138.6, 138.0, 137.7, 136.7 (5 C$_{ipso}$-Bn), 129.1-125.3 (C-Ar), 117.9, 114.6 (C-Ar MP), 99.8, 98.8, 97.8, 95.6 (C$_{1A}$, C$_{1B}$, C$_{1C}$, CCl$_3$), 82.1, 80.8, 80.6, 80.4, 76.6, 76.5, 76.0, 75.7, 75.6, 75.2, 74.4, 74.3, 73.4, 72.8, 71.6, 68.0, 66.8 (C$_{2A}$, C$_{2B}$, C$_{2C}$, C$_{3A}$, C$_{3B}$, C$_{4A}$, C$_{4B}$, C$_{4C}$, C$_{5A}$, C$_{5B}$, C$_{5C}$, 5 OCH$_2$Ph, OCH$_2$CCl$_3$), 55.6, 52.2 (C$_{3C}$, OCH$_3$), 18.4, 18.0, 16.7 (C$_{6A}$, C$_{6B}$, C$_{6C}$). MALDI-MS for C$_{63}$H$_{70}$Cl$_3$NO$_{15}$ (*m*/*z*): M_r (calcd) 1185.38, M_r (found) 1208.43 (M+Na)$^+$. Anal. calcd.: C 63.72, H 5.94, N 1.18. Found: C 63.63, H 6.02, N 1.16.



94. A solution of **93** (130 mg, 0.106 mmol) in 2:2:1 v/v/v toluene/CH$_3$CN/water (15 mL) was treated with CAN (290 mg, 0.530 mmol) and stirred at rt overnight. The solution was then diluted with ethyl acetate (100 mL) and washed with 1M NaHCO$_3$ (100 mL) and then with water (100 mL). The organic layer was collected, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated to give a residue that, after column chromatography (10% to 50% ethyl acetate in petroleum ether), afforded **94** (81 mg, 68%; α/β=4.5:1) as a yellowish oil. α-anomer: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.42-7.18 (m, 25H, H-Ar), 5.96 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.73 (d, $J_{1,2}$=2.8 Hz, 1H, H-1$_C$), 5.34 (d, J_{vic}=17.2 Hz, 1H, *trans* OCH$_2$CH=C*H*H), 5.24 (d, J_{vic}=10.3 Hz, 1H, *cis* OCH$_2$CH=C*H*H), 5.18 (d, $J_{1,2}$=2.3 Hz, 1H, H-1$_A$), 5.12 (d, J_{gem}=10.5 Hz, 1H, OC*H*HPh), 5.07 (d, J_{gem}=12.0 Hz, 1H, OC*H*HPh), 5.01 (d, $J_{H,NH}$=6.3 Hz, 1H, NH), 4.78-4.13 (m, 18H, H-1$_B$, H-2$_B$, H-3$_A$, H-3$_C$, H-4$_C$, H-5$_C$, 8

OC*H*HPh, C*H*$_2$CCl$_3$, OC*H*$_2$CH=CH$_2$), 3.97 (dq, $J_{5,4}$=7.9 Hz, $J_{5,6}$=6.2 Hz, 1H, H-5$_A$), 3.77 (m, 2H, H-2$_A$, H-2$_C$), 3.59 (t, $J_{4,5}$=$J_{4,3}$=7.9 Hz, 1H, H-4$_A$), 3.53 (t, $J_{4,5}$=$J_{4,3}$=9.4 Hz, 1H, H-4$_B$), 3.31 (dd, $J_{3,4}$=9.4 Hz, $J_{3,2}$=1.8 Hz, 1H, H-3$_B$), 3.23 (dq, $J_{5,4}$=9.4 Hz, $J_{5,6}$=6.2 Hz, 1H, H-5$_B$), 1.39 (d, $J_{6,5}$=6.1 Hz, 3H, H-6$_C$), 1.27 (m, 6H, H-6$_A$, H-6$_B$); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 153.9 (CO), 138.8, 138.5, 138.4, 138.0, 137.9 (5 C$_{ipso}$), 134.5 (OCH$_2$*C*H=CH$_2$), 128.7-126.8 (C-Ar), 117.2 (OCH$_2$CH=*C*H$_2$), 97.9, 95.6, 95.0, 92.6 (C$_{1A}$, C$_{1B}$, C$_{1C}$, *C*Cl$_3$), 83.5, 80.3, 78.9, 77.6, 76.3, 76.1, 75.3, 75.0, 74.1, 73.5, 73.3, 72.3, 71.9, 71.6, 71.1, 69.9, 67.5, 65.9 (C$_{2A}$, C$_{2B}$, C$_{2C}$, C$_{3A}$, C$_{3B}$, C$_{4A}$, C$_{4B}$, C$_{4C}$, C$_{5A}$, C$_{5B}$, C$_{5C}$, 5 OCH$_2$Ph, OCH$_2$CCl$_3$, OCH$_2$CH=CH$_2$), 51.9 (C$_{3C}$), 18.5, 17.7, 16.7 (C$_{6A}$, C$_{6B}$, C$_{6C}$). MALDI-MS for C$_{63}$H$_{70}$Cl$_3$NO$_{15}$ (*m*/*z*): *M*$_r$ (calcd) 1119.37, *M*$_r$ (found) 1142.73 (M+Na)$^+$. Anal. calcd.: C 63.18, H 6.11, N 1.25. Found: C 63.04, H 6.18, N 1.24.



97. A solution of **95** (9.0 mg, 7.6 μmol) in 2:1 v/v AcOH/Ac$_2$O (900 μL) was treated with Zn/Cu couple (23 mg) and vigorously stirred for three days. The mixture was filtered over a Celite pad, then diluted with ethyl acetate (15 mL) and washed with 1M NaHCO$_3$ (15 mL) and water (15 mL). The organic layer was collected, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated to give a residue that was dissolved in 9:1 v/v MeOH/HCOOH (1.0 mL). The solution was treated with a catalyst amount of 10% Pd/C under argon atmosphere. After 2 hours in an ultrasound bath, the mixture was filtered over a Celite pad and concentrated. The residue was subjected to a Sephadex G-10 column chromatography with water as eluant. After lyophilization, compound **97** (2.5 mg, 55%) was recovered as a white waxy solid. [α]$_D$=-224 (c=0.2, water). ^{1}H NMR (D$_2$O, 400 MHz) δ 7.20 (d, J_{ortho}=9.0 Hz, 2H, C$_6$*H*$_2$H$_2$OCH$_3$), 7.08 (d, J_{ortho}=9.0 Hz, 2H, C$_6$*H*$_2$H$_2$OCH$_3$), 3.89 (s, 3H, OCH$_3$), 1.98 (s, 3H, Ac); ^{13}C NMR (D$_2$O, 100 MHz) δ 176.0 (CO), 150.0, 147.5 (2 C$_{ipso}$-MP), 120.3, 116.4

(C-Ar MP), 56.8 (OCH_3), 24.1 ($COCH_3$). MALDI-MS for $C_{27}H_{41}NO_{14}$ (*m*/z): M_r (calcd) 603.25, M_r (found) 626.26 $(M+Na)^+$. Anal. calcd.: C 53.72, H 6.85, N 2.32. Found: C 53.50, H 6.99, N 2.29.

Chapter 3

Trihaloacetimidates behaviour in selected glycosylations involving deoxy sugars

Introduction

Trihaloacetimidates are glycosyl donors commonly employed in oligosaccharide synthesis. By comparison with other classes of donors they require only catalytic amounts of promoter to be activated. The mechanism of the activation with Lewis acids or Brønsted acids occurs through a weakening of the bond between the anomeric carbon atom and the exocyclic oxygen due to an electronic inducement towards the nitrogen atom of the leaving group. The driving force of the reaction is the formation of an amide which is a stable molecule (*Figure 3.1*).

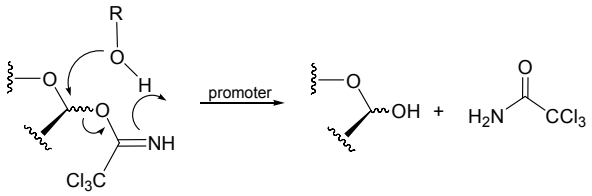


Figure 3.1 Activation of trichloroacetimidates.

The oldest promoters working for the activation of trihaloacetimidates are TfOH or $BF_3{\cdot}Et_2O$,[112] the latter is still employed as well as other Lewis acids such as TMSOTf and lanthanide salts. In the last method are counted $Sm(OTf)_3$[29] and $Yb(OTf)_3$[30], the last one allow the survival of acid-labile protective groups.

Trichloroacetimidates are synthesized starting from the protected hemiacetal by treatment with trichloroacetonitrile under basic conditions. Since the reaction is reversible the control of the stereoselectivity is sometimes possible. Using a weak base such as a carbonate, the β-imidate can be isolated as the kinetic product, whereas with a strong base, such as sodium hydride or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), a mainly formation of the α-imidate as the thermodynamic product can be achieved (*Figure 3.2*).

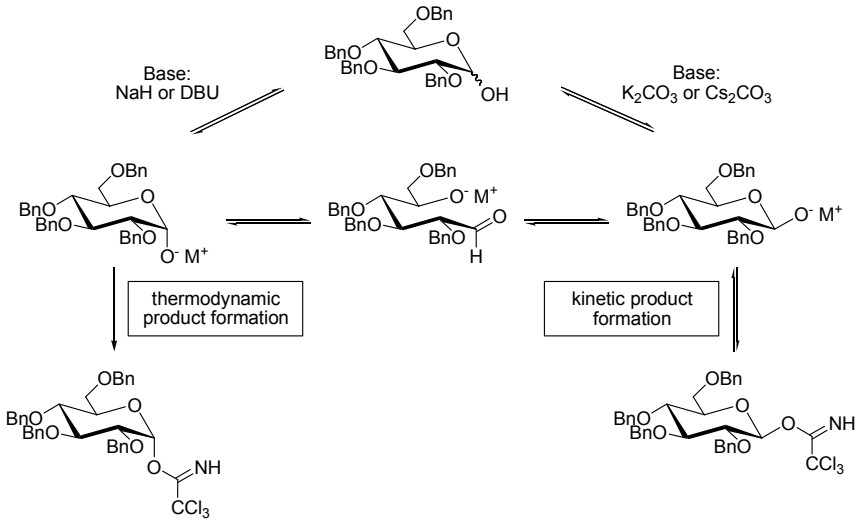


Figure 3.2 Anomeric control in trichloroacetimidate synthesis.

The isolation of the two anomers can be useful in some cases. The β-imidate is generally the most reactive, so a lower amount of promoter is required to activate it,

[112] Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.

furthermore if glycosylation proceeds through a concerted stadium the stereochemistry of products is given by the anomeric configuration.

N-phenyl trifluoroacetimidates are synthesized by treatment of the desired lactol with *N*-phenyl trifluoroacetimidoyl chloride in the presence of a base; in some applications Cs_2CO_3 has been found preferable to K_2CO_3 or NaH (*Figure 3.3*).

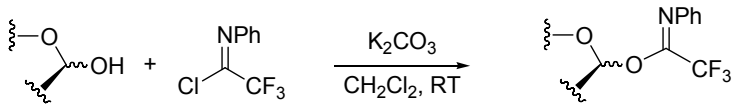


Figure 3.3 Synthesis of *N*-Phenyl Trifluoroacetimidates.

When carbonates are used non-anhydrous solvents are required for the dissolution of the base. Unfortunately *N*-phenyl trifluoroacetimidoyl chloride is not commercially available; it might be prepared from aniline and trifluoroacetic acid (*Figure 3.4*).

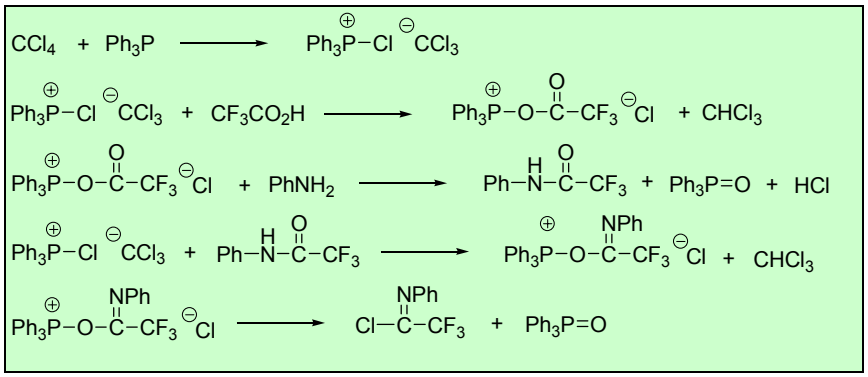


Figure 3.4 Synthesis of *N*-phenyl Trifluoroacetimidoyl Chloride.

The two families of trihaloacetimidates show a different reactivity. Trichloroacetimidates are less stable, usually their activation starts at lower

temperature and with smaller amounts of promoters. This aspect can be an useful tool for one-pot glycosylations with partially protected *N*-phenyl trifluoroacetimidates as latent donors (*Figure 3.5*). So, the latters can work first as acceptors in the activation conditions of trichloroacetimidates and then as donors toward another acceptor later added by modulation of promoter amount or temperature.[113]

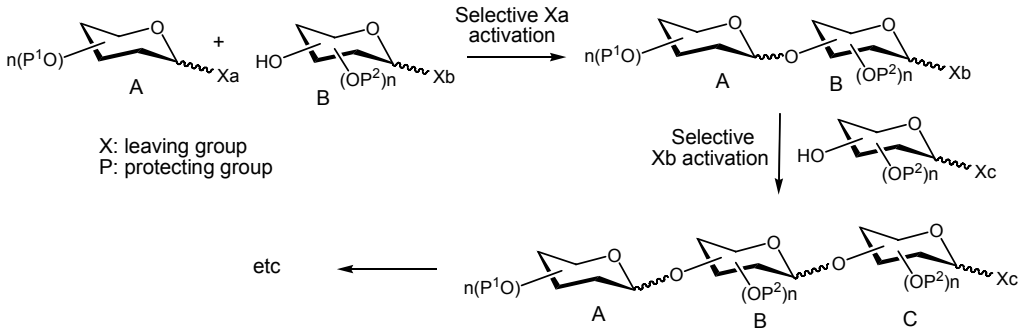


Figure 3.5 A General Scheme of One-Pot Sequential Multi-Glycosylation

The relative stability of *N*-phenyl trifluoroacetimidate donors play a very important role for the success of the reaction, expecially with more reactive molecules like deoxy sugars. Actually a too reactive glycosyl donor can degrade very quickly reacting with competitive nucleophiles such as traces of water or the nitrogen atom of the imidate function by an intra- or inter-molecular reaction,[114] or through β-elimination processes.[112] So, the yield of the reaction with trichloroacetimidates is often invalidated by the formation of hemiacetal, rearrangement byproducts (*Figure 3.6*) and glycans.

[113] Adinolfi, M.; Iadonisi, A.; Ravidà, A. *Synlett* (**2006**) 583-586.
[114] a) Cramer, F.; Hennrich, N. *Chem. Ber.* **1961**, *94*, 976; b) Zhu, T.; Boons, G.-J. *Carbohydr. Res.* **2000**, *329*, 709.

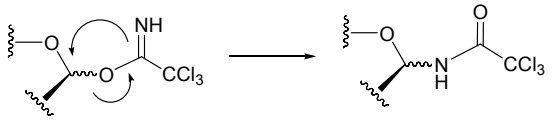


Figure 3.6 Trichloroacetimidate Rearrangement.

Side reactions decrease when *N*-phenyl trifluoroacetimidates are employed because of two factors: the presence of the three fluorine atoms and the bulky phenylic substituent. The electron-withdrawing attitude of fluorine reduces the basicity and the nucleophilicity of nitrogen atom at the same time. The former is correlated with the catalysis stadium: the lone-pair electrons on nitrogen are less prone to be activated by the promoter, besides together with the presence of the bulky phenyl group contributes to reduce the nucleophilicity of the imidate.

Despite these matters, trichloroacetimidates remain still the most commonly employed glycosyl donors of the two families. In our work we want to bring in the limelight the advantages offered in some cases by the use of *N*-phenyl trifluoroacetimidates instead of their chlorine analogues.

Results and Discussion

Glycosyl *N*-phenyl trifluoroacetimidates were already used with success as novel glycosyl donors in the synthesis of biologically relevant oligosaccharide sequences, such as Lewis X,[115] Globo-H moiety[116], saponins glycolipids,[117,27b] glycosylated antibiotics[118] and fragments of several lipopolysaccharides.[119] In some cases the

[115] Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *Synlett* **2004**, 275-278.
[116] Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *J. Org. Chem.* **2005**, *70*, 5316-5319.
[117] a) Peng, W.; Sun, J.; Lin, F.; Han, X.; Yu, B. *Synlett* **2004**, 259-262; b) Sun, J.; Han, X.; Yu, B. *Synlett* **2005**, 437-440.
[118] Doi, T.; Kinbara, A.; Inoue, H.; Takahashi, T. *Chem. Asian J.* **2007**, *2*, 188-198.

installation of the *N*-phenyl trifluoroacetimidate leaving group at the anomeric position was also demonstrated to be preferable: for example, the *N*-glycosylation of the amide group of some asparagine building blocks was firstly performed in high yields with glycosyl *N*-phenyl trifluoroacetimidates, whereas analogue trichloroacetimidate donors gave unsatisfying yields.[120] Besides, the observation that also some *O*- and *C*-glycosylations proceed with better yields when conducted with glycosyl *N*-phenyl trifluoroacetimidates in place of glycosyl trichloroacetimidates[121] prompted us to explore this feature in glycosylations involving deoxyhexose sugars because of their spread in nature. As already reported in *Chapter 2*, deoxyhexoses are commonly found as constituents of O-antigenic lipopolysaccharides, as well as components of various glycoconjugates such as cardioglycosides, natural antibiotics and anticancer agents.[122] Since their biological role in oligosaccharides seems important, the synthesis of deoxyhexose-containing oligosaccharides is a major target in carbohydrate chemistry.

The absence of one or more hydroxyls in glycosyl donors can sometimes increase too much the rate of leaving group release at the anomeric position. This is due to a loss of electron-withdrawing effect that increases the stabilization of the oxocarbenium ion positive charge. The emerging quite reactive glycosyl donor can give more quickly side-reactions with a consequent decrease of the desired glycosylation yield.

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[119] a) Bedini, E.; Carabellese, A.; Schiattarella, M.; Parrilli, M. *Tetrahedron* **2005**, *61*, 5439-5448; b) Bedini, E.; Carabellese, A.; Barone, G.; Parrilli, M. *J. Org. Chem.* **2005**, *70*, 8064-8070; c) Bedini, E.; Carabellese, A.; Comegna, D.; De Castro, C.; Parrilli, M. *Tetrahedron* **2006**, *62*, 8474-8483; d) Komarova, B.S.; Tsvetkov, Y.E.; Knirel, Y.A.; Zähringer, U.; Pier, G.B.; Nifantiev, N.E. *Tetrahedron Lett.* **2006**, *47*, 3583-3587.

[120] Tanaka, H.; Iwata, Y.; Takahashi, D.; Adachi, M.; Takahashi, T. *J. Am. Chem. Soc.* **2005**, *127*, 1630-1631.

[121] a) Tanaka, S.; Takashina, M.; Tokimoto, H.; Fujimoto, Y.; Tanaka, K.; Fukase, K. *Synlett* **2005**, 2325-2328; b) Li, Y.; Wei, G.; Yu, B. *Carbohydr. Res.* **2006**, *341*, 2717-2722; c) Ding, N.; Wang, P.; Zhang, Z.; Liu, Y.; Li, Y. *Carbohydr. Res.* **2006**, *341*, 2769-2776.

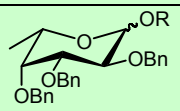
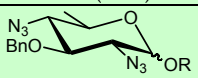
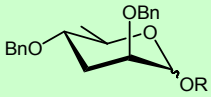
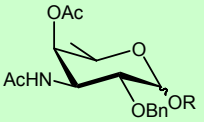
[122] He, X.M.; Liu, H.-W. *Annu. Rev. Biochem.* **2002**, *71*, 701-754.

Additionally, we decided to reconcile this methodological investigation with the possibility to prepare useful disaccharide building blocks to be elaborated into oligosaccharide antigenic fragments. Therefore L-fucose glycosyl donors were prepared, since it is the most relevant natural deoxyhexose and L-fucose-containing oligosaccharides have potential application in many medical fields.[123] Other deoxyhexoses treated in this study were D-rhamnose, D-bacillosamine (2,4-diamino-2,4,6-trideoxy-D-glucose), 3-fucosamine and D-tyvelose (D-*arabino*-hexose), which are peculiar constituents of bacterial O-antigens.[124] Their trihaloacetimidates donors were synthesized from the corresponding lactols functionalized with arming protecting groups. The choice of screening armed donors was dictated by the fact that biologically relevant oligosaccharides contain mainly α-linked deoxyhexoses: therefore, in the case of *galacto*- or *gluco*-configurated deoxyhexoses such as fucose, 3-fucosamine and bacillosamine, a non-participating protecting group at position *C*-2 was necessary. On the other hand, electron-withdrawing substituents equipped glycosyl donors are faster activated in glycosylation reactions and often show a too uncontrollably reactivity. This could be unfavourable for glycosylation yields, especially when quite unreactive glycosyl acceptors are involved. To solve this problem partially acylated 2-*O*-benzylated donors are routinely adopted,[125] but their preparation entails longer synthetic sequences as compared with the synthesis of fully benzylated donors.[126] Glycosyl *N*-phenyl trifluoroacetimidates were readily synthesized from the hemiacetals with $CF_3C(NPh)Cl$ and a stoichiometric amount of NaH in CH_2Cl_2,[127] whereas trichloroacetimidate donors were obtained by applying

[123] Vanhooren, P.T.; Vandamme, E.J. *J. Chem. Technol. Biotechnol.* **1999**, *74*, 479-497.
[124] Kochetkov, N.K. *Russ. Chem. Rev.* **1996**, *65*, 735-768.
[125] Manzoni, L.; Lay, L.; Schmidt, R.R. *J. Carbohydr. Chem.* **1998**, *17*, 739-758.
[126] Smid, P.; de Ruiter, G.A. ; van der Marel, G.A.; Rombouts, F.M.; van Boom, J.H. *J. Carbohydr. Chem.* **1991**, *10*, 833-849.
[127] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2002**, *43*, 5573-5577.

Schmidt protocol with Cl_3CCN and catalytic DBU in CH_2Cl_2. Interestingly, for every deoxyhexose *N*-phenyl trifluoroacetimidate donors were obtained in higher or at least similar yields as compared with the trichloro- counterparts (*Table 3.1*).

Table 3.1 Synthesis of Trihaloacetimidate Deoxyhexose Donors from Hemiacetals.

Entry	*Deoxy-hexose*	*Donor*	*Trichloroacetimidate Yield[a] (α / β ratio)[c]*	*N-phenyl trifluoroacetimidate Yield[b] (α/β ratio)[c]*
1	L-fucose	 **102**:R=C(NH)CCl_3 **103**: R=C(NPh)CF_3	65% (only α)	99% (only β)
2	D-bacillosamine	 **104**: R=C(NH)CCl_3 **105**[128]: R=C(NPh)CF_3	75% (only α)	75% (1:2)[d]
3	D-tyvelose	 **106**: R=C(NH)CCl_3 **107**: R=C(NPh)CF3	64% (only α)	77% (only α)
4	D-fucos-3-amine	 **108**: R=C(NH)CCl_3 **109**: R=C(NPh)CF_3	53% (only α)	74% (3:1)

[a]Reaction conditions: Cl_3CCN, DBU, CH_2Cl_2, rt; [b]Reaction conditions: $CF_3C(NPh)Cl$, NaH, CH_2Cl_2, 0°C to rt; [c]Measured by ^1H NMR; [d]Measured after separation of the two anomers.

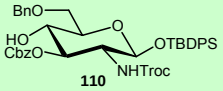
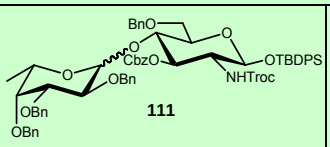
No tests with other bases where conducted to increase trichloroacetimidate yields, since such bases (NaH, K_2CO_3) were already employed in the synthesis of compound **102** with modest results. Actually, one of the most relevant cause of this difference in yields was the higher lability of glycosyl trichloroacetimidate to the chromatographical process even when conducted on a partially deactivated support

[128] Bedini, E.; Esposito, D.; Parrilli, M. *Synlett* **2006**, 825-830.

such as Brockman grade 2 alumina immediately after the work-up of the reaction. The anomeric configuration of the *N*-phenyl trifluoroacetimidate group was strictly dependent on the nature of the reacting hemiacetal, whereas the trichloroacetimidate function was always α-configurated.[112]

Glycosylation experiments were conducted, unless otherwise stated, by adding the promoter to a CH_2Cl_2 solution of donor and acceptor at -70°C and then allowing the temperature to rise spontaneously. Preliminary screenings evidenced that less reactive *N*-phenyl trifluoroacetimidate donors were best activated by TMSOTf than the more reactive armed trichloroacetimedate which instead worked better with the milder $BF_3{\cdot}OEt_2$ promoter. The results are summarized in tables *3.2*, *3.3*, *3.4* and *3.5*.

Table 3.2 Glycosylation reactions of deoxyhexosyl trihaloacetimidate donors

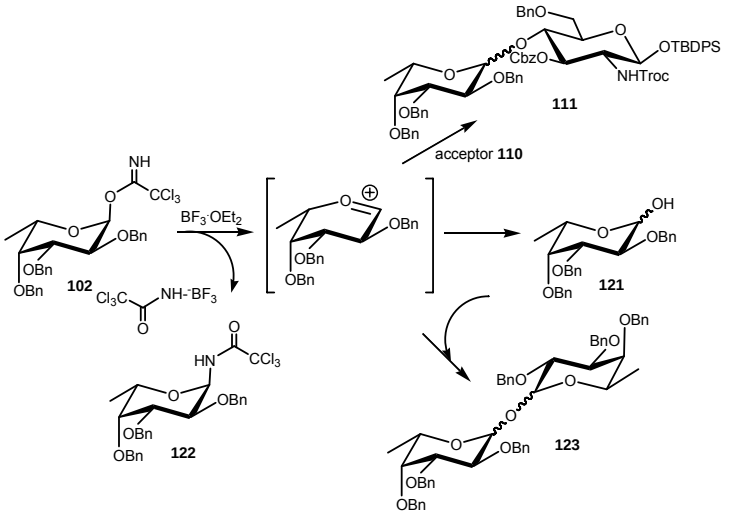
Entry	*Acceptor*	*Donor*[a]	*Procedure*	*Product (Natural source)*	*Yield*[b] *(α/β ratio)*[c]
1	 **110**	**102**	$BF_3{\cdot}OEt_2$, CH_2Cl_2, -70°C to -30°C	 **111** (Lewis A)	28 (4:3)[b]
2	**110**	**103**	TMSOTf, CH_2Cl_2, -50°C to -30°C	**111α**	71 (only α)
3	**110**	**102**	TMSOTf, CH_2Cl_2, -70°C to rt, inverse procedure	–	No product
4	**110**	**102**	$BF_3{\cdot}OEt_2$, CH_2Cl_2, -70°C to rt, inverse procedure	**111**	17 (>10:1)[b]

5	**110**	**102**	TMSOTf, Et_2O, -70°C to rt inverse procedure	**111α**	30 (only α)

[a]1.4 equivalents in respect to the acceptor; [b]Isolated yield; [c]Measured after separation of the two anomers; [d]Measured by ^1H NMR.

In the case of L-fucosylations, *N*-phenyl trifluoroacetimidate donor **103** provided better or at least comparable yields in respect to the trichloro- analogue **102**.[129] The difference in yield was particularly marked with an unreactive glycosyl acceptor such as glucosamine derivative **110** (entries 1,2), whose hydroxyl function at *C*-4, well-known to be scarcely nucleophilic,[130,90b,c] was even more unreactive in this case due to the presence of an adjacent electron-withdrawing Cbz group. The low reactivity of **110** did not match with the rapid activation of the fucosyl trichloroacetimidate donor; disaccharide **111** was thus obtained in low yield, together with perbenzylated fucose hemiacetal **121**, trichloroacetamide **122** and perbenzylated L-Fuc-(1→1)-L-Fuc disaccharide **123**. These byproducts resulted from the trapping of the fucosyl oxocarbenium ion with nucleophilic species, more reactive than **110**, which were *in situ* generated by the activation of the trichloroacetimidate donor as depicted in *Scheme 3.1*.

[129] Wegmann, B.; Schmidt, R.R. *Carbohydr. Res.* **1988**, *184*, 254-261.
[130] a) Paulsen, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155-173 ; b) Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2001**, *123*, 6819-6825.

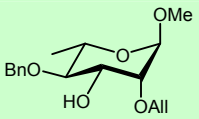
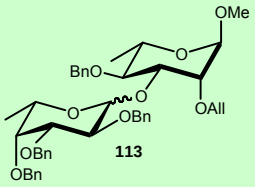
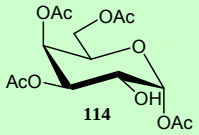
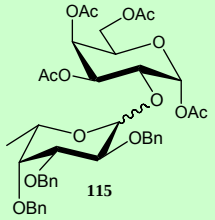


Scheme 3.1 Disaccharide product and byproducts formation in glycosylation involving L-fucose trichloroacetimidate.

Some yield improvements could be usually achieved with fucosyl trichloroacetimidate donors by applying the "inverse procedure",[131] which allows a slower oxocarbenium ion formation in the presence of an excess of the acceptor; the intermediate is so trapped prior to be degraded by other nucleophiles. We tested such protocol to ascertain if an improvement in yield was possible in glycosylations with donor **102** (*Table 3.2*). The best result was obtained by using TMSOTf as activating agent in Et_2O (entry 5), even though no significant yield improvement was observed, whereas the α-stereoselectivity was strongly enhanced in Et_2O as well as in CH_2Cl_2.

[131] Schmidt, R.R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353

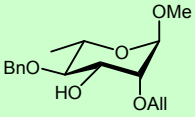
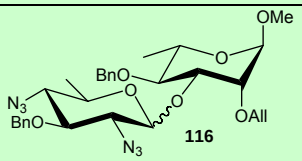
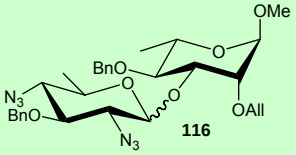
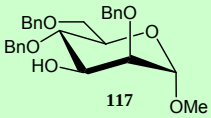
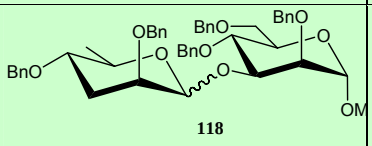
Table 3.3

Entry	*Acceptor*	*Donor*[a]	*Procedure*	*Product (Natural source)*	*Yield*[b] *(α/βratio)*[c]
1	 **112**	**102**	$BF_3{\cdot}OEt_2$, CH_2Cl_2, -70°C to -50°C	 **113** (O-Antigen from *Pseudomonas fluorescens* IMV472)	69 (2:3)
2	**112**	**103**	TMSOTf, CH_2Cl_2, -50°C to -30°C	**113** (O-Antigen from *Pseudomonas fluorescens* IMV472)	85 (3:2)
3	 **114**	**102**	$BF_3{\cdot}OEt_2$, CH_2Cl_2, -70°C to -50°C	 **115** (Lewis A, B)	68 (6:1)[d]
4	**114**	**103**	TMSOTf, CH_2Cl_2, -70°C to rt	–	No product

[a]1.4 equivalents in respect to the acceptor; [b]Isolated yield; [c]Measured after separation of the two anomers; [d]Measured by ^1H NMR.

Minor differences in efficiency between trichloro- and *N*-phenyl trifluoroacetimidate donors were observed with more nucleophilic acceptors, such as **112** and **114** (*Table 3.3*). Interestingly, the attempt to couple **103** and **114** by TMSOTf activation surprisingly afforded no disaccharide **115**; actually, acceptor **114** gave acyl migration from the anomeric position to *O*-2 as confirmed by treating **114** alone with TMSOTf. The undesided intramolecular acetyl transfer was instead minimized with the more reactive trichloroacetimidate **102** affording the expected disaccharide **115** in satisfying yield.

Table 3.4

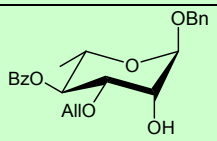
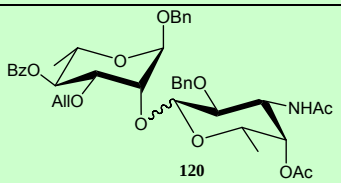
Entry	*Acceptor*	*Donor*[a]	*Procedure*	*Product (Natural source)*	*Yield*[c] *(α/βratio)*[d]
1	 **112**	**104**	$BF_3{\cdot}OEt_2$, CH_2Cl_2, -70°C to -50°C	 **116** (O-Antigen from *Pseudomonas aeruginosa* O1, O3, O13, O14, NCTC 8505; *Shewanella algae* BrY)	85 (1:1)[e]
2	**112**	**105α**	TMSOTf, CH_2Cl_2, 0°C	 **116** (O-Antigen from *Pseudomonas aeruginosa* O1, O3, O13, O14, NCTC 8505; *Shewanella algae* BrY)	75 (only α)
3	**112**	**105β**	TMSOTf, CH_2Cl_2, 0°C	**116α**	86 (only α)
4	 **117**	**106**[b]	$BF_3{\cdot}OEt_2$, CH_2Cl_2, -70°C -20°C	 **118** (O-Antigen from *Salmonella enterica* sv. *enteritidis*)	89 (3:2)
5	**117**	**107**[b]	TMSOTf, CH_2Cl_2, -70°C to rt	**118**	79 (5:2)

[a]1.4 equivalents in respect to the acceptor; [b]1.8 equivalents in respect to **117**; [c]Isolated yield; [d]Measured after separation of the two anomers; [d]Measured by ^1H NMR

Little differences in yields were obtained also for glycosylations involving bacillosamine and tyvelose trichloro- or *N*-phenyl trifluoroacetimidate donors **104**-**107** (*Table 3.4*). The good outcome in tyvelose glycosylations is remarkable, since in the

past the use of 3,6-dideoxyhexosyl thioglycosides was preferred to the use of trihaloacetimidate donors,[132] in spite of a more laborious preparation. The last two entries (15-16) in *Table 3.5* report previously published results concerning 3-fucosamine donors **108**, **109**: in this case only *N*-phenyl trifluoroacetimidate donor was efficient in glycosylation.

Table 3.5

Entry	*Acceptor*	*Donor*[a]	*Procedure*	*Product (Natural source)*	*Yield*[c] *(α/βratio)*[d]
1	 **119**	**108**[b]	$BF_3{\cdot}OEt_2$, CH_2Cl_2, -70°C to rt	–	No product
2	**119**	**109**[b]	TMSOTf, CH_2Cl_2, 0°C to rt	 **120** (O-Antigen from *Pseudomonas fluorescens* ATCC49271; *Pseudomonas syringae* pv. *tabaci* MV 223; *Pseudomonas syringae* pv. *coriandricola* GFPB2028	65 (62:38)

[a]1.4 equivalents in respect to the acceptor; [b]1.1 equivalents in respect to **119**; [c]Isolated yield; [d]Measured by 1H NMR.

The major α-stereoselectivity generally observed in glycosylations with glycosyl *N*-phenyl trifluoroacetimidates might be explained by taking into account that such donors require harsher conditions for their activation with respect to trichloroacetimidates; this would favour a S_N1-type mechanism and therefore α-stereoselectivity, whichever glycosyl acceptor was chosen. Instead, the mild conditions ($BF_3{\cdot}OEt_2$ in CH_2Cl_2 at low temperature) necessary for the activation of

[132] a) van Dorst, J.A.L.M.; van Heusden, C.J.; Voskamp, A.F.; Kamerling, J.P.; Vliegenthart, J.F.G. *Carbohydr. Res.* **1996**, *291*, 63-83; b) van Dorst, J.A.L.M.; van Heusden, C.J.; Tikkanen, J.M.; Kamerling, J.P.; Vliegenthart, J.F.G. *Carbohydr. Res.* **1997**, *297*, 209-227.

armed deoxyhexose α-trichloroacetimidate donors would render the mechanism more dependent on the reactivity of glycosyl acceptor. Indeed, with an armed acceptor such as **112,** β-disaccharide was predominantly obtained in agreement with a S_N2-type mechanism via a tight ion pair. On the contrary, less reactive acceptors such as **110** or **114** favour a S_N1 type mechanism, affording predominantly α-products.

Conclusions

In conclusion, we have reported some selected glycosylations involving deoxyhexoses. Good yields were obtained with *N*-phenyl trifluoroacetimidate glycosyl donors, which gave in some cases even better results in respect to trichloroacetimidate donors. This could be ascribed to a slower formation of the oxocarbenium ion, which can be therefore trapped with more efficiency even by a low reactive glycosyl acceptor. Actually, the conditions (temperature, acidity of the activator) necessary for the activation of *N*-phenyl trifluoroacetimidate donors were relatively harsher than for trichloro- ones. This would favour the formation of the thermodynamic α-configurated disaccharide, which is very often required in biological-targeted glycosylations involving deoxyhexoses, what might be the glycosyl acceptor. Although generalization of these results would still require a larger panel of examples, we feel that they could be of interest in selected glycosylations involving deoxyhexose donors and unreactive glycosyl acceptors.

Experimental Section

General methods

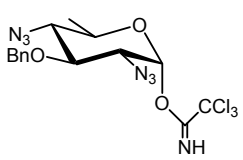
^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Gemini-300 NMR, in $CDCl_3$ (internal standard, for ^{1}H: $CHCl_3$ at δ 7.26; for ^{13}C: $CDCl_3$ at δ 77.0). Positive MALDI-MS spectra were recorded on a Applied Biosystem Voyager DE-PRO MALDI-TOF mass spectrometer in the positive mode: compounds were dissolved in CH_3CN at a concentration of 0.1 mg/mL and one microliter of these solutions were mixed with one microliter of a 20 mg/mL solution of 2,5-dihydroxybenzoic acid in 7:3 CH_3CN/H_2O. Optical rotations were measured on a JASCO P-1010 polarimeter. Elementar analysis were performed on a Carlo Erba 1108 instrument. Analytical thin layer chromatography (TLC) was performed on aluminium plates precoated with Merck Silica Gel 60 F_{254} as the adsorbent. The plates were developed with 5% H_2SO_4 ethanolic solution and then heating to 130°C. Column chromatography was performed on Kieselgel 60 (63-200 mesh) . Solvents used were purchased from Fluka and not further purified before use.

General procedure for trichloroacetimidate formation — Hemiacetal (0.50 mmol) was dissolved in CH_2Cl_2 (2.0 mL) and to the 0°C cooled solution Cl_3CCN (2.50 mmol) and DBU (0.10 mmol) were added. The solution was stirred at rt until the reaction finished (TLC analysis) and then concentrated. The resulting residue was then immediately chromatographed over neutral alumina (Brockman grade 2) gel.

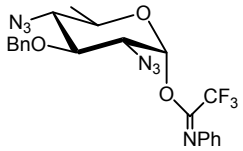
General procedure for *N*-phenyl trifluoroacetimidate formation — Hemiacetal (0.50 mmol) was dissolved in CH_2Cl_2 (2.0 mL) under an argon atmosphere and then cooled to 0°C under stirring. $CF_3C(NPh)Cl$ (0.65 mmol) and NaH (60% oil suspension; 0.75 mmol) were added and stirring was continued at 0°C and eventually at rt until the

reaction finished (TLC analysis), after that the solution was concentrated. Neutral alumina (Brockman grade 2) column chromatography afforded the pure *N*-phenyl trifluoroacetimidate donor.

General procedure for glycosylation — A mixture of acceptor (0.050 mmol) and donor (0.055-0.100 mmol: see Tables 3.2-3.5 for details) was coevaporated three times with toluene, the residue was then mixed with freshly activated AW-300 4Å molecular sieves, cooled to -70°C and then suspended under argon in the solvent (1.0 mL). Upon stirring, a solution of $BF_3{\cdot}OEt_2$ or TMSOTf in CH_2Cl_2 (0.02 equivalents of the activator in respect to the donor) was added and the temperature was allowed to rise spontaneously. After completion of the reaction (TLC analysis), the mixture was neutralized by adding Et_3N. The mixture was then filtered over Celite and concentrated to give a residue, that was purified by chromatography.

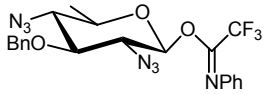


104. $[\alpha]_D$ +55.3 (*c* 1.4, CH_2Cl_2).; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.76 (s, 1H), 7.50-7.33 (m, 5H), 6.36 (d, *J*=3.4 Hz, 1H), 4.92 (s, 2H), 3.85 (m, 2H), 3.66 (dd, *J*=10.0, 3.4 Hz, 1H), 3.24 (t, *J*=10.0 Hz, 1H), 1.36 (d, *J*=6.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.7 (C=N), 136.9 (C_{ipso}), 128.6-128.2 (C-Ar), 94.5 (C-1), 78.3, 75.5, 69.4, 68.0, 63.2 (C-2, C-3, C-4, C-5, OCH_2Ph), 18.4 (C-6). Anal. calcd: C. 40.15; H. 3.59; N. 21.85. Found: C. 40.29; H. 3.56; N. 21.68.

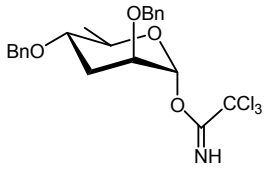


105α. $[\alpha]_D$ +126.2 (*c* 1.4, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46-6.84 (m, 10H, H-Ar), 6.34 (bs, 1H, H-1), 4.92 (s, 2H, C*H*$_2$Ph), 3.84-3.58 (m, 3H, H-2, H-3, H-5), 3.22 (t, 1H, 1H, $J_{4,3}$=$J_{4,5}$=9.6 Hz, H-4), 1.37 (d, 3H, $J_{6,5}$=6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.1, 136.8 (2 C_{ipso}),

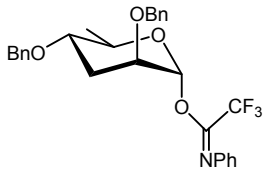
128.7-118.2 (C-Ar), 93.4 (C-1), 78.5 (C-3), 75.7, 69.3, 67.9, 63.1 (C-2, C-4, C-5, OCH_2Ph), 18.4 (C-6). MALDI-MS for $C_{21}H_{20}F_3N_7O_3$ (*m*/*z*): M_r (calcd) 475.16, M_r (found) 498.03 $(M+Na)^+$. Anal. calcd.: C. 53.05; H. 4.24; N. 20.62; Found: C. 53.00, H. 4.26, N. 20.74.



105β. $[\alpha]_D$ +98.9 (*c* 1.4, CH_2Cl_2). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.45-6.87 (m, 10H), 5.49 (bm, 1H), 4.93 (d, J_{gem}=10.8 Hz, 1H), 4.87 (d, J_{gem}=10.8 Hz, 1H), 3.65 (t, $J_{3,2}$=$J_{3,4}$=9.0 Hz, 1H), 3.34-3.15 (m, 3H), 1.39 (d, $J_{6,5}$=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.0, 136.9 (2 C_{ipso}), 128.7-119.2 (C-Ar), 95.3 (C-1), 81.2 (C-3), 75.6, 71.8, 67.2, 65.4 (C-2, C-4, C-5, CH_2Ph), 18.2 (C-6). MALDI-MS for $C_{21}H_{20}F_3N_7O_3$ (*m*/*z*): M_r (calcd) 475.16, M_r (found) 497.94 $(M+Na)^+$. Anal. calcd.: C. 53.05; H. 4.24; N. 20.62; Found: C. 52.89, H. 4.30, N. 20.86.

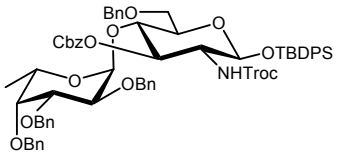


106. $[\alpha]_D$= +58.0 (*c* 0.9, CH_2Cl_2). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.24 (m, 10H), 6.19 (bs, 1H), 4.74-4.41 (m, 4H), 3.92 (m, 1H), 3.78 (bs, 1H), 3.53 (dq, *J*=9.8, 6.0 Hz, 1H), 2.28 (dt, *J*=13.2, 3.0 Hz, 1H), 1.82 (ddd, *J*=13.2, 10.2, 3.0 Hz, 1H), 1.19 (d, *J*=6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.5 (C=N), 138.0, 137.7 (2 C_{ipso}), 94.9 (C-1), 74.6, 73.4, 71.2, 71.1, 70.8 (C-2, C-4, C-5, 2 OCH_2Ph), 29.6 (C-3), 17.9 (C-6). Anal. calcd: C. 55.89; H. 5.12; N. 2.96. Found: C. 55.99; H. 5.10; N. 2.88.

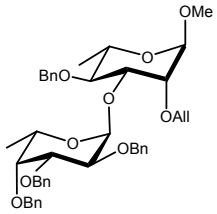


107α. $[\alpha]_D$ +15.6 (*c* 0.8, CH_2Cl_2). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.44-6.73 (m, 15H), 6.00 (bs, 1H), 4.70 (d, J_{gem}=12.6 Hz, 1H), 4.63 (d, J_{gem}=12.6 Hz, 1H), 4.53 (d, J_{gem}=11.7 Hz, 1H), 4.48 (d, J_{gem}=11.7 Hz, 1H), 3.91 (bs, 1H), 3.58 (m, 2H), 2.67 (bs, 1H), 2.32 (m, 1H), 1.37 (d, $J_{6,5}$=6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.1, 135.8 (2 C_{ipso}), 128.5-119.3 (C-Ar), 95.9 (C-1), 75.0, 74.9, 71.8, 71.3 (C-2, C-4, C-5, CH_2Ph), 29.9 (C-3),

18.6 (C-6). MALDI-MS for $C_{28}H_{28}F_3NO_4$ (*m*/*z*): M_r (calcd) 499.20, M_r (found) 522.26 $(M+Na)^+$. Anal. calcd.: C. 67.32; H. 5.65; N. 2.80; Found: C. 67.45, H. 5.75, N. 2.86.

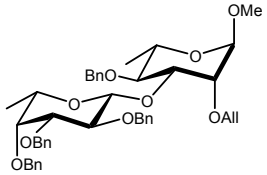


111. $[\alpha]_D$ -35.1 (*c* 1.0, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 7.73-7.12 (m, 35H), 5.13 (d, *J*=12.0 Hz, 1H), 5.06-5.00 (m, 3H), 4.88 (d, *J*=11.6 Hz, 1H), 4.75 (d, *J*=11.6 Hz, 1H), 4.72-4.62 (m, 4H), 4.59 (d, *J*=11.6 Hz, 1H), 4.56 (d, *J*=11.6 Hz, 1H), 4.51 (d, *J*=12.0 Hz, 1H), 4.45 (d, *J*=8.0 Hz, 1H), 4.32 (d, *J*=12.3 Hz, 1H), 4.25 (d, *J*=12.3 Hz, 1H), 3.97 (dd, *J*=9.9, 3.6 Hz, 1H), 3.92-3.83 (m, 2H), 3.72-3.65 (m, 3H), 3.43 (bd, *J*=6.4, 1H), 3.37 (bs, 1H), 3.12 (dd, *J*=9.6, 2.0 Hz, 1H), 1.01 (s, 9H), 0.96 (d, *J*=6.4, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.2, 153.8 (2 C=O), 138.1-127.3 (C-Ar), 98.7, 96.1 ($C\text{-}1_A$, $C\text{-}1_B$), 95.4 ($C(CH_3)_3$) 79.5, 78.0, 75.9, 75.2, 74.6, 74.5, 74.2, 74.1, 73.4, 72.7, 69.8, 67.7, 67.2, 60.4, 58.5 ($C\text{-}2_A$, $C\text{-}2_B$, $C\text{-}3_A$, $C\text{-}3_B$, $C\text{-}4_A$, $C\text{-}4_B$, $C\text{-}5_A$, $C\text{-}5_B$, $C\text{-}6_A$, 5 OCH_2Ph, OCH_2CCl_3), 26.7 ($C(CH_3)_3$), 16.4 ($C\text{-}6_B$). MALDI-MS for $C_{67}H_{72}Cl_3NO_{13}Si$ (*m*/*z*): M_r (calcd) 1231.38, M_r (found) 1254.58 $(M+Na)^+$. Anal. calcd: C. 65.23; H. 5.88; N. 1.14. Found: C. 65.35; H. 5.68; N. 1.16.

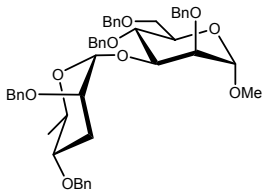


113*α*. $[\alpha]_D$ -38.6 (*c* 0.7, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 7.39-7.10 (m, 20H), 5.91 (m, 1H), 5.29 (dd, *J*=17.0, 2.1 Hz, 1H), 5.17-5.08 (m, 2H), 5.00 (d, *J*=11.6 Hz, 1H), 4.85 (d, *J*=12.2 Hz, 1H), 4.77-4.63 (m, 7H), 4.53 (d, *J*=11.8 Hz, 1H), 4.14-3.97 (m, 4H), 3.72-3.63 (m, 3H), 3.52 (t, *J*=9.6 Hz, 1H), 3.34 (m, 4H), 1.27 (d, *J*=6.2 Hz, 3H), 1.13 (d, *J*=6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.0, 138.9, 138.8, 138.6 (4 C_{ipso}), 135.1 ($OCH_2CH{=}CH_2$), 128.3-127.2 (C-Ar), 116.7 ($OCH_2CH{=}CH_2$), 99.7, 98.0 ($C\text{-}1_A$, $C\text{-}1_B$), 79.9, 79.3, 78.9, 78.3, 78.0, 76.2, 74.8, 74.7, 73.0, 72.9, 71.5, 67.8, 66.9 ($C\text{-}2_A$, $C\text{-}2_B$, $C\text{-}3_A$, $C\text{-}3_B$, $C\text{-}4_A$, $C\text{-}4_B$, $C\text{-}5_A$, $C\text{-}5_B$, 4 OCH_2Ph, $OCH_2CH{=}CH_2$), 54.7 (OCH_3), 18.0, 16.8 ($C\text{-}6_A$, $C\text{-}6_B$). MALDI-MS for

$C_{44}H_{52}O_9$ (*m*/*z*): M_r (calcd) 724.36, M_r (found) 747.50 $(M+Na)^+$. Anal. calcd: C. 72.90; H. 7.23. Found: C. 73.15; H. 7.07.

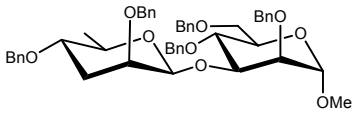


113β. $[\alpha]_D$ -26.2 (*c* 0.7, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 7.41-7.10 (m, 20H), 5.78 (m, 1H), 5.16 (dd, *J*=17.0, 2.1 Hz, 1H), 5.09-4.99 (m, 4H), 4.86 (d, *J*=11.8 Hz, 1H), 4.76 (s, 2H), 4.66 (d, *J*=2.0 Hz, 1H), 4.64 (d, *J*=11.6 Hz, 1H), 4.54 (d, *J*=7.6 Hz, 1H), 4.53 (d, *J*=12.0 Hz, 1H), 4.27 (dd, *J*=9.6, 3.2 Hz, 1H), 4.09 (m, 2H), 3.86 (dd, *J*=9.4, 7.6 Hz, 1H), 3.72 (dd, *J*=3.2, 2.0 Hz, 1H), 3.67-3.55 (m, 4H), 3.47 (q, *J*=6.6 Hz, 1H), 3.32 (s, 3H), 1.33 (d, *J*=6.2 Hz, 3H), 1.17 (d, *J*=6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.0, 138.8, 138.7, 138.5 (4 C_{ipso}), 135.1 ($OCH_2CH=CH_2$), 128.5-127.0 (C-Ar), 116.9 ($OCH_2CH=CH_2$), 100.8, 99.0 ($C\text{-}1_A$, $C\text{-}1_B$), 82.9, 79.6, 79.5, 76.8, 76.6, 75.0, 74.8, 74.7, 72.9, 72.1, 72.0, 70.4, 67.6 ($C\text{-}2_A$, $C\text{-}2_B$, $C\text{-}3_A$, $C\text{-}3_B$, $C\text{-}4_A$, $C\text{-}4_B$, $C\text{-}5_A$, $C\text{-}5_B$, 4 OCH_2Ph, $OCH_2CH=CH_2$), 54.5 (OCH_3), 18.0, 16.8 ($C\text{-}6_A$, $C\text{-}6_B$). MALDI-MS for $C_{44}H_{52}O_9$ (*m*/*z*): M_r (calcd) 724.36, M_r (found) 747.38 $(M+Na)^+$. Anal. calcd: C. 72.90; H. 7.23. Found: C. 73.09; H. 7.16.



118α. $[\alpha]_D$ +3.1 (*c* 0.6, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 7.41-7.09 (m, 25H), 5.07 (bs, 1H), 4.75 (m, 3H), 4.67 (d, *J*=12.3 Hz, 1H), 4.65 (d, *J*=11.4 Hz, 1H), 4.58 (d, *J*=12.0 Hz, 1H), 4.51 (d, *J*=12.0 Hz, 1H), 4.48 (d, *J*=11.1 Hz, 1H), 4.46 (d, *J*=11.4 Hz, 1H), 4.34 (d, *J*=12.0 Hz, 1H), 4.23 (d, *J*=12.0 Hz, 1H), 4.17 (dd, *J*=9.3, 3.0 Hz, 1H), 3.98 (t, *J*=9.3 Hz, 1H), 3.86 (dq, *J*=9.0, 6.3 Hz, 1H), 3.78-3.60 (m, 4H), 3.45 (td, *J*=8.2, 3.6 Hz, 1H), 3.34 (m, 4H), 2.22 (dt, *J*=13.2, 3.0 Hz, 1H), 1.78 (ddd, *J*=13.2, 10.2, 3.0 Hz, 1H), 1.26 (d, *J*=6.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.5-138.2 (5 C_{ipso}), 129.3-127.5 (C-Ar), 98.7 ($C\text{-}1_A$, $C\text{-}1_B$), 77.8, 77.1, 75.5, 75.3, 75.2, 74.4, 73.3, 72.6, 71.8, 70.9, 70.8, 69.1, 68.7 ($C\text{-}2_A$, $C\text{-}2_B$, $C\text{-}3_A$, $C\text{-}4_A$, $C\text{-}4_B$, $C\text{-}5_A$, $C\text{-}5_B$, $C\text{-}6_A$, 5 OCH_2Ph), 54.7 (OCH_3), 29.6 ($C\text{-}3_B$), 18.1 ($C\text{-}6_B$).

MALDI-MS for $C_{48}H_{54}O_9$ (*m*/*z*): M_r (calcd) 774.38, M_r (found) 797.49 $(M+Na)^+$. Anal. calcd: C. 74.39; H. 7.02. Found: C. 74.25; H. 7.09.



118β. $[\alpha]_D$ +4.1 (*c* 0.6, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 7.39-7.09 (m, 25H), 5.05 (d, *J*=10.2, 1H), 4.81 (m, 2H), 4.71 (d, *J*=12.3 Hz, 1H), 4.65-4.52 (m, 5H), 4.47 (bs, 1H), 4.43 (d, *J*=11.2 Hz, 1H), 4.38 (d, *J*=10.5 Hz, 1H), 4.21 (dd, *J*=8.1, 3.3 Hz, 1H), 3.86 (t, *J*=8.1 Hz, 1H), 3.76-3.54 (m, 5H), 3.47 (td, *J*=10.2, 3.9 Hz, 1H), 3.36 (m, 4H), 2.29 (dt, *J*=13.5, 3.9 Hz, 1H), 1.36 (ddd, *J*=13.5, 9.9, 3.0 Hz, 1H), 1.30 (d, *J*=6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.1, 138.8, 138.5, 138.4, 138.2 (5 C_{ipso}), 129.7-127.2 (C-Ar), 99.4, 98.9 (C-1_A, C-1_B), 76.9, 76.6, 75.5, 75.1, 74.9, 74.3, 74.2, 73.3, 72.7, 72.6, 71.7, 71.3, 69.6 (C-2_A, C-2_B, C-3_A, C-4_A, C-4_B, C-5_A, C-5_B, C-6_A, 5 OCH_2Ph), 54.7 (OCH_3), 33.7 (C-3_B), 18.4 (C-6_B). MALDI-MS for $C_{48}H_{54}O_9$ (*m*/*z*): M_r (calcd) 774.38, M_r (found) 797.45 $(M+Na)^+$. Anal. calcd: C. 74.39; H. 7.02. Found: C. 74.28; H. 7.10.

Chapter 4

Selective acetolysis of deoxysugar disaccharides

Introduction

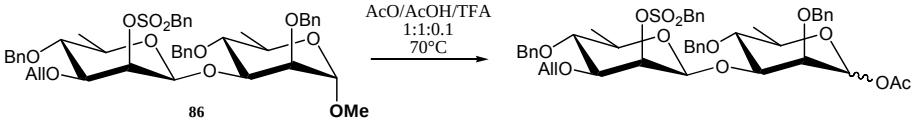
Acetolysis represents an important chemical tool in carbohydrate chemistry. It consists in exposing the saccharidic substrate to acetic anhydride under acidic conditions, determinig the *in situ* generation of active electrophilic acetylating species along with an acetate anion. This system may result in the cleavage of glycosidic linkages with the installation of an acetyl to both the sites committed in the initial glycosidic bond. This approach is frequently adopted for the selective degradation of polysaccharides containing (1→6)-linkages, which are normally less prone to acidic alcoholic cleavage.[133] On the other hand, acetolysis may also represent an important tool in the chemical elaboration of mono-, and oligosaccharides. Indeed, the reaction allows the introduction of a versatile acetyl protecting group at the anomeric position even starting from methyl glycosides, which require usually more harsh hydrolytic

[133] Aspinall, G. O. "*The Polisaccharides*", Vol. 1, Academic Press, New York, **1982**, 35-131.

conditions for their cleavage. In addition, acetolysis can be usefully exploited for the selective replacement of 6-O-benzyl and -silyl protecting groups with acetyls.[134]

Result and discussion

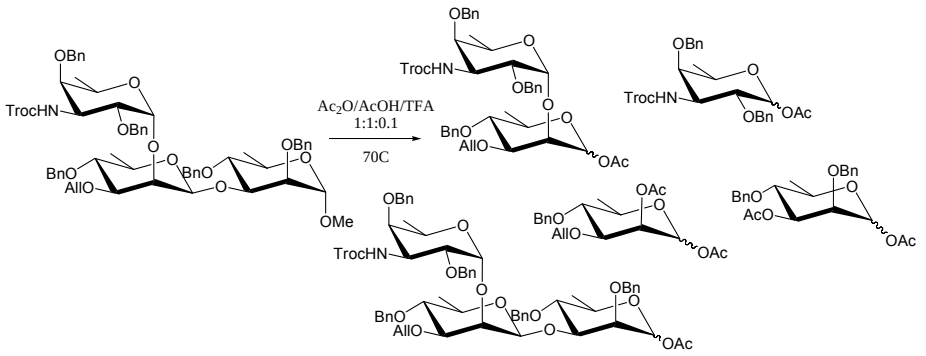
In the course of the syntesis of *Xanthomonas campestris* trisaccharide (see *Chapter 2*), a mild acetolysis[108] was successfully exploited for converting disaccharide methyl glycoside **86** into the corresponding 1-*O*-acetylated derivative (*Scheme 4.1*) which served well as a donor for the subsequent installation of a *p*-methoxyphenyl group.



Scheme 4.1

In contrast, when the trisaccharide methyl glycoside shown below was exposed to analogous conditions, the reaction resulted in a complex mixture of products predominantly derived from the cleavage of intersaccharidic linkages, including the glycosidic bond connecting the two rhamnose moieties which was unaffected starting from disaccharide **86** (*Scheme 4.2*).

[134] a) Yang, G.; Ding, X.; Kong, F . *Tetrahedron Lett.* **1997**, *38*, 6725-6728; b) Angibeaud, P.; Utille, J. P. *Synthesis*, **1991**, 737; c) Klemer, A.; Bieber, M.; Wilburs, H. *Liebigs Ann. Chem.* **1983**, 1416; d) Brar, A.; Vankar, Y. D. *Tetrahedron Lett.* **2006**, *47*, 5207-5210.



Scheme 4.2

This different stability prompted us to examine more thoroughly the synthetic potential of acetolysis in carbohydrate chemistry. We reasoned that the stability of the intersaccharidic linkage in disaccharide **86** might be ascribed to the presence of a strong electron-withdrawing 2B-*O*-benzylsulfonyl group which would destabilize the generation of the putative oxonium intermediate, whereas the fucosamine residue at the same position would act as an electron-donating substituent. The effect of substituents has been well rationalized in the field of glycosylation chemistry, as discussed in *Chapter 1* with the *armed-disarmed* concept,[37] but there are no systematic applications of this concept in selective degradations of oligosaccharides. To pursue this investigation, six model disaccharides (shown in *Figure 4.1*) were synthesized.

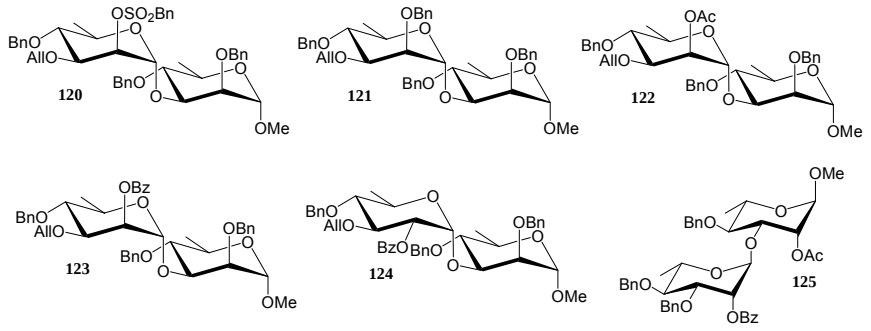
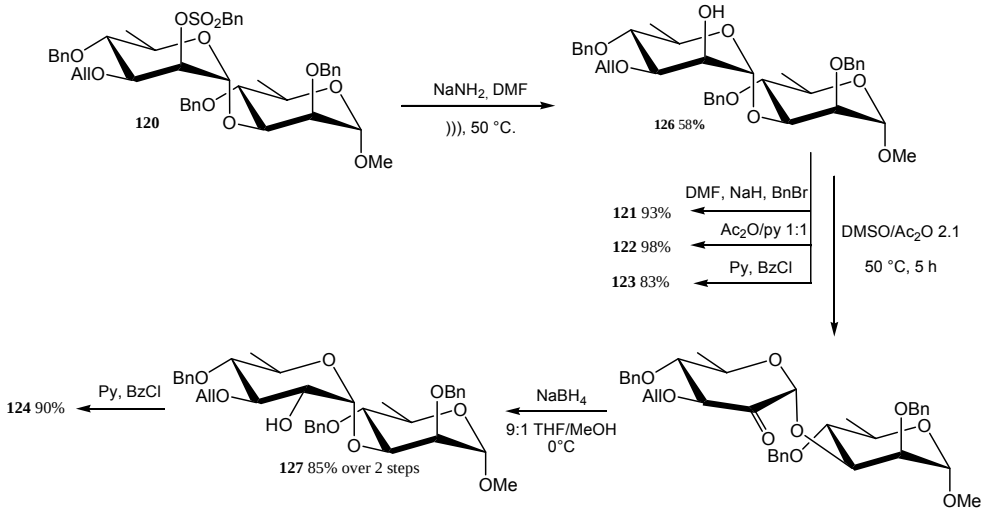


Figure 4.1

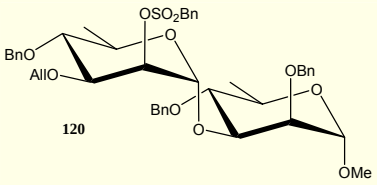
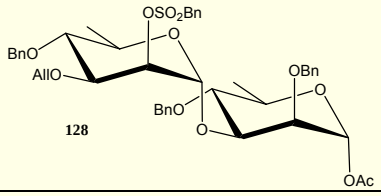
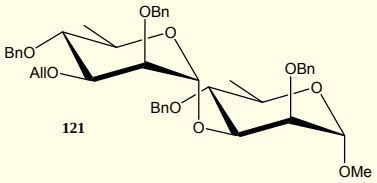
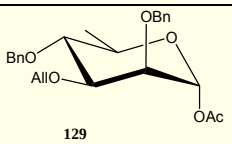
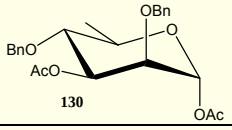
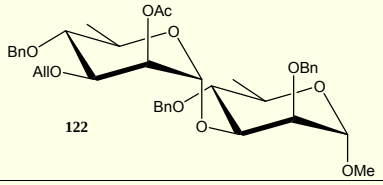
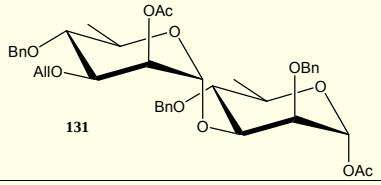
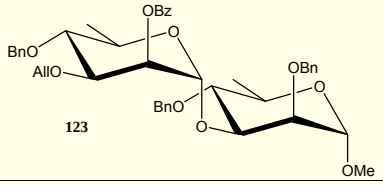
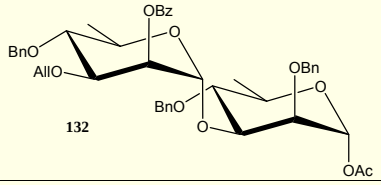
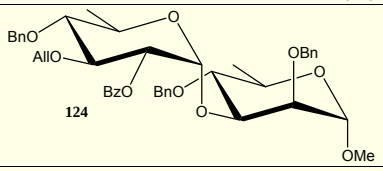
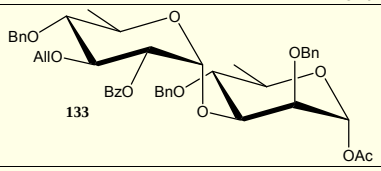
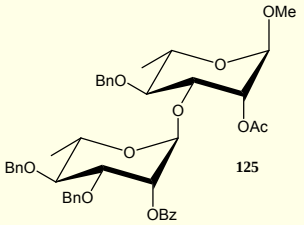
Compound **120** was prepared by a known procedure[88] and was utilized as precursor of compounds **121**-**124** as described in *Scheme 4.3*. Debenzylsulfonylation of **120** gave alcohol **126** that was respectively benzylated, acetylated, and benzoylated affording compounds **121**, **122**, and **123**. The epimer of disaccharide **123** was obtained though the invertion of configuration at $C2_B$ by a Moffat oxidation and a subsequent reduction with NaH_4.



Scheme 4.3

Disaccharides **120**-**125** were then submitted to mild acetolysis conditions previously adopted for disaccharide **86**. The results of such experiments are summarized on *Table 4.1*.

Table 4.1

Entry	*Substrate*	*Product*	*Yield (%)*
1	 **120**	 **128**	51
2	 **121**	 **129**  **130**	42 57
3	 **122**	 **131**	71
4	 **123**	 **132**	62
5	 **124**	 **133**	66
6	 **125**	Mixture of debenzylated/acetylated compounds and 58% recovered **125**	

Inspection of results reveals that the nature of the 2_B-OH substitution is crucial for the outcome of the reactions. Indeed, installation of a less electron-withdrawing benzyl group rendered disaccharide **121** more prone to the cleavage of the inter-rhamnosidic glycosidic bond, as indicated by the predominant recovery of acetylated monosaccharides **129** and **130** (*Table 4.1*, entry 2). In contrast, electron-withdrawing benzylsulfonyl, acetyl and benzoyl groups (entries 1, 3, 4) stabilized the inter-rhamonsidic linkage, and disaccharides acetylated at the anomeric extremity were recovered in good yield. In all cases the yield was slighty affected by some level of acetolytic cleavage of benzyl groups. To ascertain whether the configuration of the non reducing sugar was influencing the outcome of the process, benzoylated disaccharide **124** was also submitted to the same conditions (entry 5), and similar yields were obtained as compared to the corresponding epimer **123** (entry 4), indicating that the nature of the 2_B-O-protecting group had a predominant role in controlling the selectivity of the process.

Interestingly, disaccharide **125**, protected at both 2-*O*- positions with acyl groups was quite stable towards the acetolysis conditions utilized (entry 6).

All these results are consistent with the anticipated arming-disarming effect of substituents, and will be an useful tool in predicting the outcome of acetolysis reactions.

Conclusions

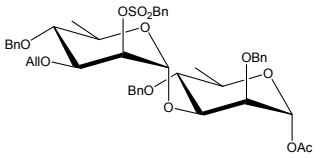
In this chapter it has been examined the scope of acetolysis processes in the selective manipulation of oligosaccharides. Consistently with the *armed-disarmed* theory, previously applied in glycosylation chemistry, the nature of 2-O- protecting groups was found decisive for the outcome of the reactions, the stability of the interglycosidic bonds being increased by adjacent electron-withdrawing groups.

Experimental section

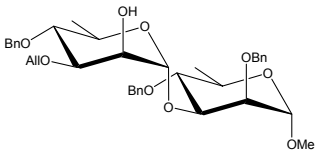
General methods:

^{1}H and ^{13}C NMR spectra were recorded on Varian XL-200 (^{1}H: 200 MHz, ^{13}C: 50 MHz), Varian Gemini-300 (^{1}H: 300 MHz, ^{13}C: 75 MHz) or Bruker DRX-400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) instruments in $CDCl_3$ ($CHCl_3$ as internal standard, ^{1}H: C*H*Cl_3 at δ 7.26; ^{13}C: *C*DCl_3 at δ 77.0). Positive MALDI-MS spectra were recorded on a Applied Biosystem Voyager DE-PRO MALDI-TOF mass spectrometer in the positive mode: compounds were dissolved in CH_3CN at a concentration of 0.1 mg/mL and one microliter of these solutions were mixed with one microliter of a 20 mg/mL solution of 2,5-dihydroxybenzoic acid in 7:3 CH_3CN/H_2O. Optical rotations were measured on a JASCO P-1010 polarimeter. Elementar analysis were performed on a Carlo Erba 1108 instrument. Analytical thin layer chromatography (TLC) was performed on aluminium plates precoated with Merck Silica Gel 60 F_{254} as the adsorbent. The plates were developed with 5% H_2SO_4 ethanolic solution and then heating to 130°C. Column chromatography was performed on Kieselgel 60 (63-200 mesh). Solvents used were purchased from Fluka and not further purified before use.

General procedure for selective anomeric acetolysis — The sugar (66 μmol) was dissolved in a mixture of 1:1:0.1 Ac_2O/AcOH/TFA (2.1 ml). The solution was stirred at 70°C and checked by TLC or MALDI analysis. The reaction was quenched by dilution with CH_2Cl_2, the organic layer was washed with brine and 1 M $NaHCO_3$ until neutralization then collected, dried and concentrated. The residue was subject to column chromatography.

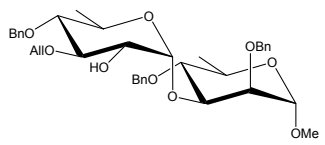


128. $[\alpha]_D$ +3.7 (*c* 1.0, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 7.39-7.26 (m, 20H), 6.13 (d, 1H, $J_{1,2}$=1.8, H_{1A}), 5.93 (m, 1H, OCH_2CH=CH_2), 5.29 (dd, 1H, J_{vic}=17.1 Hz, J_{gem}=1.5, *trans* OCH_2CH=C*H*H), 5.17-5.07 (m, 2H, H-1_B, *cis* OCH_2CH=C*H*H), 4.92 (d, 1H, J_{gem}=10.8 Hz, OC*H*HPh), 4.81 (d, 1H, J_{gem}=11.1 Hz, OC*H*HPh), 4.74 (d, 1H, J_{gem}=11.7 Hz, OC*H*HPh), 4.67-4.56 (3H, OC*H*HPh), 4.46 (d, 1H, J_{gem}=13.5 Hz, OSO_2CHHPh), 4.40 (d, 1H, J_{gem}=13.5 Hz, OSO_2CHHPh), 4.18 (dd, 1H, J_{gem}=11.7 Hz, J_{vic}=5.4 Hz, OC*H*HCH=CH_2), 4.09 (dd, 1H, J_{gem}=11.7 Hz, J_{vic}=5.4 Hz, OC*H*HCH=CH_2), 4.03 (dd, 1H, $J_{3,4}$=9.3, $J_{3,2}$=3.6, H-3), 3.85 (dd, 1H, $J_{3,4}$=9.6, $J_{3,2}$=3.0, H-3), 3.13-3.73 (m, 2H, H-2_A, H-5), 3.62 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.3 Hz, H-4_A), 3.69 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.3 Hz, H-4_B), 1.30 (d, 3H, $J_{6,5}$=6.3 Hz, H-6), 1.22 (d, 3H, $J_{6,5}$=6.0 Hz H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.1 (CO), 138.2-137.4 (3 C_{ipso}-Bn, C_{ipso}-OSO_2Bn), 134.2 (OCH_2CH=CH_2), 130.8-127.6 (C-Ar), 117.8 (OCH_2CH=CH_2), 99.4, 90.9 (C_{1A}, C_{1B}), 79.7, 79.6, 78.1, 77.6, 77.3, 76.6, 76.4, 75.3, 72.4, 71.5, 70.5, 68.7 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph, OCH_2CH=CH_2), 57.5 (OSO_2CH_2Ph), 21.0 (CH_3CO), 18.1, 17.9 (C_{6A}, C_{6B}). MALDI-MS for $C_{45}H_{52}O_{12}S$ (*m*/*z*): M_r (calcd) 816.32, M_r (found) 839.39 $(M+Na)^+$. Anal. calcd: C. 66.16; H. 6.42. Found: C. 66.28; H. 6.89.



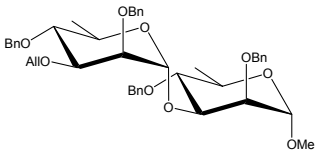
126. Compound **120** (250 mg, 317 μmol) was dissolved in dry DMF (5.0 mL) under Ar atmosphere and then $NaNH_2$ (542 mg, 138 mmol) was added. The mixture was stirred at 70°C in ultrasound bath for 48 hours. The mixture was diluted with MeOH, then AcOH was added dropwise to neutralization. The solution was concentrated and the residue was dissolved in CH_2Cl_2 and washed with 1M $NaHCO_3$ and brine. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue that, after column chromatography (8% to 16% ethyl acetate in toluene), afforded **126** (129 mg, 64%) as a yellowish oil. $[\alpha]_D$ +26.5 (*c* 2.0, CH_2Cl_2); ^1H

NMR (400 MHz, $CDCl_3$): δ 7.37-7.19 (m, 15), 5.91 (m, 1H, OCH_2CH=CH_2), 5.26 (dd, 1H, J_{vic}=17.2 Hz J_{gem}=1.6, *trans* OCH_2CH=C*H*H), 5.16-5.13 (m, 2H, *cis* OCH_2CH=C*H*H, H-1_B), 4.87 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.78 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.70 (s, 2H, OC*H*$_2$Ph), 4.64 (s, 1H, H-1_A), 4.63 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.62 (d, 1H, J_{gem}=11.6 Hz, OC*H*HPh), 4.07 (dd, 1H, J_{gem}=11.8 Hz, J_{vic}=6.6 Hz, OC*H*HCH=CH_2), 4.05 (dd, 1H, J_{gem}=11.8 Hz, J_{vic}=6.6 Hz, OC*H*HCH=CH_2), 4.00 (d, 1H, $J_{2,1}$=1.3 Hz, H-2), 3.80 (m, 1H, H-5), 3.75-3.61 (m, 3H, H-3_A, H-3_B, H-2), 3.66 (m, 1H, H-5), 3.58 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.3 Hz, H-4), 3.42 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.3 Hz, H-4), 3.31 (s, 3H, OCH_3), 1.32 (d, 3H, $J_{6,5}$=5.8 Hz, H-6), 1.25 (d, 3H, $J_{6,5}$=6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 138.6, 138.1, 138.0 (3 C_{ipso}-Bn), 134.6 (OCH_2CH=CH_2), 128.4-127.6 (C-Ar), 117.2 (OCH_2CH=CH_2), 100.8, 98.5 (C_{1A}, C_{1B}), 81.4, 80.2, 79.7, 77.9, 77.3, 76.6, 75.4, 75.3, 73.0, 71.2, 69.2, 68.2 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph, OCH_2CH=CH_2), 54.9 (OCH_3), 18.2 (C_{6A}, C_{6B}). MALDI-MS for $C_{37}H_{46}O_9$ (*m*/*z*): M_r (calcd) 634.31, M_r (found) 657.33 $(M+Na)^+$. Anal. calcd: C. 70.01; H. 7.30. Found: C. 70.18; H. 7.65.



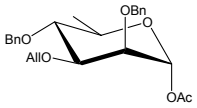
127. Alcohol **126** (165.0 mg, 260 μmol) was dissolved in 2:1 v/v DMSO/Ac_2O (4.5 mL) and stirred at 50°C for 45 minutes. The solution was then diluted with ethyl acetate (100 mL) and washed with water (100 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to afford a residue that was dissolved in 9:1 TFA/MeOH (4.0 mL), cooled at 0°C and then treated with $NaBH_4$ (55.0 mg, 1.44 mmol). After stirring for 1.5 hours, the solution was diluted with CH_2Cl_2 (100 ml) and the organic layer was washed with brine (100 ml) and concentrated. The residue was subjected to column chromatography (10% to 20% ethyl acetate in toluene) to afford **127** (140.8 g, 85% over 2 steps) as a yellowish oil. $[\alpha]_D$ +38.3 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.19 (m, 15H), 5.10 (m, 1H,

OCH_2CH=CH_2), 5.23 (dd, 1H, J_{gem}=1.8 Hz, J_{vic}=16.8 Hz, *trans* OCH_2CH=C*H*H), 5.13-5.10 (m, 2H, *cis* OCH_2CH=C*H*H, H-1_B), 4.91 (d, 1H, J_{gem}=10.9 Hz, OC*H*HPh), 4.89 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.73 (s, 2H, OCH_2Ph), 4.64 (d, 1H, J_{gem}=10.8 Hz, OC*H*HPh), 4.63 (bs, 1H, H-1_A), 4.62 (d, 1H, J_{gem}=12.3 Hz, OC*H*HPh), 4.24 (dd, 1H, J_{gem}=11.2 Hz, J_{vic}=6.6 Hz OC*H*HCH=CH_2), 4.21 (dd, 1H, J_{gem}=11.2 Hz, J_{vic}=6.6 Hz, OC*H*HCH=CH_2), 4.05 (dd, 1H, $J_{3,2}$=3.1 Hz, $J_{3,4}$=9.1 Hz, H-3_A), 3.87(m, 1H, H-5_B), 3.75 (dd, 1H, $J_{2,1}$=2.0 Hz, $J_{2,3}$=3.0 Hz, H-2_A), 3.70 (m, 1H, H-5_A), 3.64 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.2 Hz, H-4_A), 3.58 (s, 1H, H-2_B), 3.56 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.4 Hz, H-3_B), 3.31 (s, 3H, OCH_3), 3.07 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.1 Hz, H-4_B), 1.34 (d, 3H, $J_{6,5}$=6.0 Hz, H-6_A), 1.20 (d, 3H, $J_{6,5}$=5.2 Hz, H-6_B); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.5, 138.1, 138.0 (3 C_{ipso}-Bn), 135.3 (OCH_2CH=CH_2), 129.0-127.6 (C-Ar), 116.7 (OCH_2CH=CH_2), 99.8, 98.7 (C_{1A}, C_{1B}), 83.2, 82.8, 80.9, 78.0, 77.6, 75.1, 75.0, 74.1, 73.5, 72.9, 68.1, 67.7 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph, OCH_2CH=CH_2), 54.7 (OCH_3), 18.0, 17.9 (C_{6A}, C_{6B}). MALDI-MS for $C_{37}H_{46}O_9$ (*m*/*z*): M_r (calcd) 634.31, M_r (found) 657.30 $(M+Na)^+$. Anal. calcd: C. 70.01; H. 7.30. Found: C. 70.12; H. 7.54.

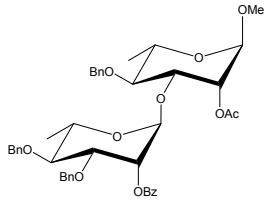


121. A solution of **126** (71.0 mg, 112 μmol) and DMF (1.0 ml) was treated with BnBr (16 μl, 160 μmol) and NaH (60% dispersion in mineral oil; 23 mg, 580 μmol). The mixture was stirred at room temperature for 30 minutes and then diluted with ethyl acetate and washed with brine. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to afford a residue, that was chromatographed (4% to 7% ethyl acetate in toluene) to give **121** (75.0 mg, 93%) as colourless oil. $[\alpha]_D$ +9.2 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.40-7.27 (m, 20), 5.92 (m, 1H, OCH_2CH=CH_2), 5.03 (dd, 1H, J_{vic}=17.4 Hz J_{gem}=1.4, *trans* OCH_2CH=C*H*H), 5.20 (bs, 1H, H-1_B), 5.15 (dd, 1H, J_{vic}=12.0 Hz J_{gem}=1.4, *cis* OCH_2CH=C*H*H), 4.92 (d, 1H, J_{gem}=11.2 Hz, OC*H*HPh), 4.79-4.41 (m, 4H, OC*H*HPh, H-1_A), 4.52 (s, 2H, OCH_2Ph), 4.14-4.06 (m, 2H, OCH_2CH=CH_2), 3.90-3.56 (m, 8H, H-2_A, H-2_B, H-3_A, H-3_B, H-4_A, H-4_B, H-5_A, H-5_B), 3.34

(s, 3H, OCH_3), 1.32 (d, 6H, $J_{6,5}$=5.8 Hz, H-6_A, H-6_B); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 139.0, 138.5, 138.4, 138.1 (4 C_{ipso}-Bn), 135.0 ($OCH_2CH=CH_2$), 128.4-126.9 (C-Ar), 116.4 ($OCH_2CH=CH_2$), 99.8, 98.6 (C_{1A}, C_{1B}), 80.9, 80.4, 79.6, 77.7, 76.4, 75.6, 75.0, 74.6, 72.7, 72.4, 70.9, 68.6, 67.9 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 4 OCH_2Ph, $OCH_2CH=CH_2$), 54.6 (OCH_3), 18.0, 17.9 (C_{6A}, C_{6B}). MALDI-MS for $C_{44}H_{50}O_{10}$ (*m*/*z*): M_r (calcd) 738.34, M_r (found) 761.33 $(M+Na)^+$. Anal. calcd: C. 71.52; H. 6.82. Found: C. 71.08; H. 6.91.

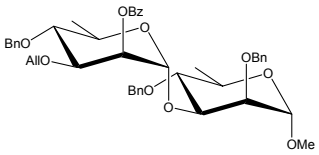


129. $[\alpha]_D$ +16.5 (*c* 0.9, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 7.41-7.26 (m, 10H), 6.11 (bs, 1H, H_1), 5.91 (m, 1H, $OCH_2CH=CH_2$), 5.31 (dd, 1H, J_{vic}=17.0 Hz, J_{gem}=1.8, *trans* $OCH_2CH=CHH$), 5.18 (dd, 1H, J_{vic}=10.2 Hz, J_{gem}=1.8, *cis* $OCH_2CH=CHH$), 4.95 (d, 1H, J_{gem}=10.5 Hz, $OCHHPh$), 4.80 (d, 1H, J_{gem}=12.6 Hz, $OCHHPh$), 4.75 (d, 1H, J_{gem}=12.6 Hz, $OCHHPh$), 4.64 (d, 1H, J_{gem}=10.5 Hz, $OCHHPh$), 4.07 (m, 2H, $OCH_2CH=CH_2$), 3.80-3.70 (m, 3H, H-2, H-3, H-5), 3.62 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.3 Hz, H-4), 2.04 (s, 3H, CH_3CO), 1.34 (d, 3H, $J_{6,5}$=6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.1 (CO), 138.4, 137.8 (2 C_{ipso}), 134.8 ($OCH_2CH=CH_2$), 128.4-127.8 (C-Ar), 116.8 ($OCH_2CH=CH_2$), 91.8 (C_1), 79.8, 79.1, 75.6, 73.5, 72.6, 71.1, 70.4 (C_2, C_3, C_4, C_5, 2 OCH_2Ph, $OCH_2CH=CH_2$), 21.0 (CH_3CO), 18.0 (C_6). MALDI-MS for $C_{25}H_{30}O_6$ (*m*/*z*): M_r (calcd) 426.20, M_r (found) 449.28 $(M+Na)^+$. Anal. calcd: C. 70.40; H. 7.09. Found: C. 70.68; H. 7.15.

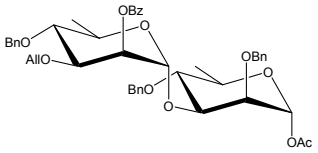


125. $[\alpha]_D$ +12.3 (*c* 2.0, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 8.06 (d, 2H, J_{ortho}=12.0, OBz), 7.38-7.21 (m, 20H), 5.68 (bs, 1H, H-2_B), 5.16-5.15 (m, 2H, H-1_B, H-2_A), 4.92 (d, 1H, J_{gem}=11.1 Hz, $OCHHPh$), 4.88 (d, 1H, J_{gem}=11.1 Hz, $OCHHPh$), 4.71 (d, 1H, J_{gem}=11.4 Hz, $OCHHPh$), 4.68 (d, 1H, J_{gem}=10.8 Hz, $OCHHPh$), 4.64 (s, 1H, H-1_A), 4.63 (d, 1H, J_{gem}=10.8 Hz, $OCHHPh$), 4.50 (d, 1H, J_{gem}=11.7 Hz, $OCHHPh$), 4.16 (dd, 1H, $J_{3,4}$=9.3, $J_{3,2}$=3.3, H-3), 4.00 (dd, 1H, $J_{3,4}$=9.3, $J_{3,2}$=3.3, H-3), 3.86 (dd, 1H, $J_{5,4}$=9.3, $J_{5,6}$=6.0,

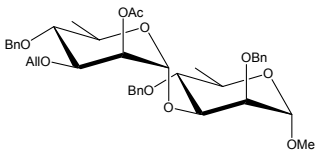
H-5), 3.73 (dd, 1H, $J_{5,4}$=9.6, $J_{5,6}$=6.3, H-5), 3.56 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.3 Hz, H-4), 3.49 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.6 Hz, H-4), 3.34 (s, 3H, CH_3CO), 1.33 (m, 6H, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.2, 169.5 (2 CO), 138.2, 137.8, 137.6 (4 C_{ipso}-Bn), 133.1 (C_{ipso}-Bz), 129.8-127.5 (C-Ar), 99.6, 97.9 (C_{1A}, C_{1B}), 80.2, 79.7, 77.7, 75.4, 75.1, 72.1, 71.4, 69.6, 69.5, 68.6, 67.5 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph), 54.8 (OCH_3), 21.1 (CH_3CO), 18.0, 17.9 (C_{6A}, C_{6B}). MALDI-MS for $C_{43}H_{48}O_{11}$ (*m*/*z*): M_r (calcd) 740.32, M_r (found) 763.41 $(M+Na)^+$. Anal. calcd: C. 69.71; H. 6.53 Found: C. 70.03; H. 6.48.



123. Compound **126** (86.3 mg, 136 μmol) was dissolved in pyridine (1.0 ml) an treated with BzCl (40 μl, 345 μmol) and stirred at rt for 19 hours. The mixture was diluted with CH_2Cl_2 and washed with 1 M HCl and water. The organic layer was concentrated and cromatographed (6% to 9% ethyl acetate in toluene) to give **16** (83.0 mg, 83%) as colourless oil. $[\alpha]_D$ -0.8 (*c* 0.9, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.06 (d, 2H, J_{ortho}=8.4, OBz), 7.52-7.24 (m, 18H), 5.88 (m, 1H, OCH_2CH=CH_2), 5.71 (dd, 1H, $J_{2,1}$=1.6 Hz, $J_{2,3}$=3.0 Hz, H-2_B), 5.24 (dd, 1H, J_{vic}=15.6 Hz, J_{gem}=1.8 Hz, *trans* OCH_2CH=C*H*H), 5.26 (bs, 1H, H-2_B), 5.09 (dd, 1H, J_{vic}=10.2 Hz, J_{gem}=1.8, *cis* OCH_2CH=C*H*H), 4.98 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.92 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.77 (s, 1H, OC*H*HPh), 4.07-4.62 (m, 3H, 2 OC*H*HPh, H-1_A), 4.22 (dd, 1H, J_{gem}=11.5 Hz, J_{vic}=5.2 Hz, OC*H*HCH=CH_2), 4.16-3.88 (m, 4H, OC*H*HCH=CH_2, H-3, H-5_A, H-5_B), 3.77 (dd, 1H, $J_{2,1}$=2.0 Hz, $J_{2,3}$=3.0 Hz, H-2_A), 3.71-3.67 (m, 2H, H-3, H-4_A), 3.54 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.2 Hz, H-4_B), 3.33 (s, 3H, OCH_3), 1.36-1.30 (m, 6H, H-6_A, H-6_B); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.4 (CO), 138.7, 138.1, 138.0 (3 C_{ipso}-Bn), 134.7 (OCH_2CH=CH_2), 133.0 (C_{ipso}-Bz), 129.8-127.5 (C-Ar), 99.2, 98.5 (C_{1A}, C_{1B}), 80.9, 80.0, 77.7, 76.4, 75.3, 75.1, 72.8, 70.5, 69.6, 68.3, 67.9 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph), 54.6 (OCH_3), 18.2, 17.9 (C_{6A}, C_{6B}). MALDI-MS for $C_{44}H_{50}O_{10}$ (*m*/*z*): M_r (calcd) 738.34, M_r (found) 761.39 $(M+Na)^+$. Anal. calcd: C. 71.52; H. 6.82. Found: C. 71.45; H. 6.77.

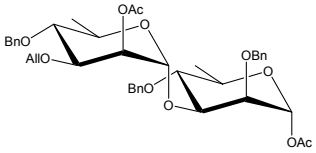


132. $[\alpha]_D$ -2.5 (*c* 1.2, CH_2Cl_2); ^1H NMR (200 MHz, $CDCl_3$): δ 8.05 (d, 2H, J_{ortho}=7.0, OBz), 7.51-7.16 (m, 18H), 6.14 (d, 1H, $J_{1,2}$=2.0 Hz, H-1_A), 5.84 (m, 1H, OCH_2CH=CH_2), 5.68 (dd, 1H, $J_{2,1}$=1.6 Hz, $J_{2,3}$=3.4 Hz, H-2_B), 5.23 (dd, 1H, J_{vic}=17.0 Hz, J_{gem}=1.8 Hz, *trans* OCH_2CH=C*H*H), 5.23 (bs, 1H, H-1_B), 5.08 (dd, 1H, J_{vic}=10.2 Hz, J_{gem}=1.8, *cis* OCH_2CH=C*H*H), 4.95 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.90 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.79 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.72-4.62 (m, 3H, 3 OC*H*HPh), 4.19 (dd, 1H, J_{gem}=12.0 Hz, J_{vic}=5.0 Hz, OC*H*HCH=CH_2), 4.12-4.5 (m, 2H, OC*H*HCH=CH_2, H-3), 3.98 (dd, 1H, $J_{3,2}$=3.2 Hz, $J_{3,4}$=9.2 Hz, H-3), 3.91-3.65 (m, 4H, H-2, H-4_A, H-5_A, H-5_B), 3.51 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.6 Hz, H-4_B), 2.06 (s, 3H, CH_3CO), 1.32-1.27 (m, 6H, H-6_A, H-6_B); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.2, 165.5 (2 CO), 138.6, 137.8, 137.5 (3 C_{ipso}-Bn), 134.6 (OCH_2CH=CH_2), 133.1 (C_{ipso}-Bz), 129.8-125.3 (C-Ar), 117.1 (OCH_2CH=CH_2), 99.5, 91.2 (C_{1A}, C_{1B}), 80.1, 79.9, 77.6, 77.4, 76.7, 76.4, 75.5, 75.2, 72.6, 70.6, 69.6, 68.4 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph, OCH_2CH=CH_2), 21.0 (CH_3CO), 18.2, 18.1 (C_{6A}, C_{6B}). MALDI-MS for $C_{45}H_{50}O_{11}$ (*m*/*z*): M_r (calcd) 766.34, M_r (found) 789.42 $(M+Na)^+$. Anal. calcd: C. 70.48; H. 6.57. Found: C. 70.28; H. 6.34.



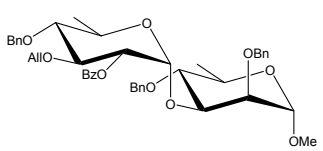
122. Compound **126** (67.0 mg, 106 μmol) was dissolved in 1:1 Ac_2O/Py (1.5 ml). The solution was stirred overnight at rt, then coevaporated four times with toluene. The residue was dissolved in CH_2Cl_2 and washed with water. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to afford **6** (70.3 mg, 98%). $[\alpha]_D$ +28.2 (*c* 0.9, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 7.41-7.26 (m, 15H), 5.87 (m, 1H, OCH_2CH=CH_2), 5.44 (bs, 1H, H-2_B), 5.25 (d, 1H, J_{vic}=17.4 Hz, *trans* OCH_2CH=C*H*H), 5.13-5.10 (m, 2H, *cis* OCH_2CH=C*H*H, H-1_A), 4.94 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.83 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.72 (s, 2H, OCH_2Ph), 4.66-4.61 (m,

3H, 2 OC*H*HPh, H-1B), 4.16-4.06 (m, 2H, OC*H*HCH=CH2, H-3), 3.98 (dd, 1H, J_{gem}=12.0 Hz, J_{vic}=5.4 Hz, OC*H*HCH=CH2), 3.88 (dd, 1H, $J_{3,2}$=3.3 Hz, $J_{3,4}$=9.3 Hz, H-3), 3.85 (m, 1H, H-5), 3.71 (bs, 1H, H-2), 3.65 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.0 Hz, H-4), 3.41 (m, 1H, H-5), 3.42 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.6 Hz, H-4), 3.31 (s, 3H, OCH3), 1.32 (d, 3H, $J_{6,5}$=5.4 Hz, H-6), 1.29 (d, 3H, $J_{6,5}$=5.7 Hz, H-6); ^{13}C NMR (CDCl3, 75 MHz) δ 169.9 (CO), 138.8, 138.1, 138.0 (3 C_{ipso}-Bn), 134.6 (OCH2*C*H=CH2), 128.4-127.5 (C-Ar), 117.1 (OCH2CH=*C*H2), 99.2, 98.5 (C_{1A}, C_{1B}), 81.0, 80.9, 79.9, 77.7, 77.4, 77.0, 75.2, 72.8, 70.6, 69.1, 68.2, 67.9 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 O*C*H2Ph, O*C*H2CH=*C*H2), 54.6 (OCH3), 20.9 (CH3CO), 18.0, 17.9 (C_{6A}, C_{6B}). MALDI-MS for $C_{39}H_{48}O_{10}$ (*m*/*z*): M_r (calcd) 676.32, M_r (found) 799.29 $(M+Na)^+$. Anal. calcd: C. 69.21; H. 7.15. Found: C. 69.15; H. 7.09.

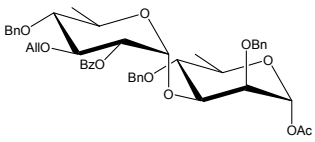


131. $[\alpha]_D$ +69.6 (*c* 1.0, CH2Cl2); ^{1}H NMR (400 MHz, CDCl3): δ 7.38-7.26 (m, 15H), 6.13 (d, 1H, $J_{1,2}$=2.0 Hz, H-1A), 5.86 (m, 1H, OCH2C*H*=CH2), 5.43 (dd, 1H, $J_{2,1}$=1.8 Hz, $J_{2,3}$=3.4 Hz, H-2B), 5.25 (dd, 1H, J_{vic}=17.0 Hz, J_{gem}=1.6 Hz, *trans* OCH2CH=C*H*H), 5.12 (dd, 1H, J_{vic}=10.0 Hz, J_{gem}=1.6, *cis* OCH2CH=C*H*H), 5.08 (d, 1H, $J_{1,2}$=1.6 Hz H-1B), 4.93 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.84 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.76 (d, 1H, J_{gem}=11.8 Hz, OC*H*HPh), 4.67 (d, 1H, J_{gem}=11.8 Hz, OC*H*HPh), 4.63 (d, 1H, J_{gem}=10.8 Hz, OC*H*HPh), 4.61 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.09 (dd, 1H, J_{gem}=12.8 Hz, J_{vic}=5.5 Hz, OC*H*HCH=CH2), 4.07 (dd, 1H, $J_{3,2}$=3.2 Hz, $J_{3,4}$=9.4 Hz, H-3), 4.03 (dd, 1H, J_{gem}=12.8 Hz, J_{vic}=5.5 Hz, OC*H*HCH=CH2), 3.85 (dd, 1H, $J_{3,2}$=3.4 Hz, $J_{3,4}$=9.3 Hz, H-3), 3.82-3.76 (m, 2H, H-5A, H-5B), 3.72 (dd, 1H, $J_{2,1}$=1.6 Hz, $J_{2,3}$=2.2 Hz, H-2A), 3.70 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.4 Hz, H-4), 3.39 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.4 Hz, H-4), 2.11 (s, 3H, CH3CO), 2.07 (s, 3H, CH3CO), 1.32 (d, 3H, $J_{6,5}$=6.1 Hz, H-6), 1.24 (d, 3H, $J_{6,5}$=6.2 Hz, H-6) ; ^{13}C NMR (CDCl3, 100 MHz) δ 170.0, 169.1 (2 CO), 138.6, 137.8, 137.5 (3 C_{ipso}-Bn), 134.6 (OCH2*C*H=CH2), 129.0-127.6 (C-Ar), 117.1 (OCH2CH=*C*H2), 99.4 (C_{1B}), 91.2 (C_{1A}), 80.1, 79.8, 77.4, 77.3, 76.7, 75.4, 75.1, 72.6, 70.7, 70.6, 69.2, 69.1 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 O*C*H2Ph, O*C*H2CH=*C*H2), 21.0

(CH_3CO), 18.1, 17.9 (C_{6A}, C_{6B}). MALDI-MS for $C_{40}H_{48}O_{11}$ (*m*/*z*): M_r (calcd) 704.32, M_r (found) 727.39 $(M+Na)^+$. Anal. calcd: C. 68.16; H. 6.86. Found: C. 68.23; H. 6.97.



124. A solution of **G** (46.6 mg, 73.5 μmol) in pyridine (500 μl) was undertaken with BzCl (41.4 μl, 294 μmol) and stirred at rt for 4 hours. The mixture was diluted with CH_2Cl_2 and washed with 1 M HCl and brine. The organic layer was concentrated and cromatographed (6% ethyl acetate in toluene) to give **124** (49.4 mg, 90%) as colourless oil. $[\alpha]_D$ +75.3 (*c* 2.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, 2H, J_{ortho}=8.2, OBz), 7.50-7.01 (m, 18H), 5.80 (m, 1H, OCH_2CH=CH_2), 5.40 (d, 1H, $J_{1,2}$=3.6 Hz, H-1_B), 5.22 (dd, 1H, $J_{2,1}$=3.6 Hz, $J_{2,3}$=10.0 Hz, H-2_B), 5.10 (dd, 1H, J_{gem}=1.6 Hz, J_{vic}=17.2 Hz, *trans* OCH_2CH=C*H*H), 4.99 (dd, 1H, J_{gem}=1.6 Hz, J_{vic}=10.2 Hz, *cis* OCH_2CH=C*H*H), 4.94 (d, 1H, J_{gem}=11.5 Hz, OC*H*HPh), 4.82 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.80 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.71 (d, 1H, J_{gem}=11.5 Hz, OC*H*HPh), 4.70 (bs, 1H, H-1_A), 4.69 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.42 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.26 (dd, 1H, J_{gem}=12.5 Hz, J_{vic}=6.6 Hz OC*H*HCH=CH_2), 4.22 (dd, 1H, J_{gem}=12.5 Hz, J_{vic}=6.6 Hz, OC*H*HCH=CH_2), 4.13 (dd, 1H, $J_{3,2}$=3.0 Hz, $J_{3,4}$=8.6 Hz, H-3_A), 4.10 (t, 1H, $J_{4,3}$=$J_{4,5}$=10.0 Hz, H-3_B), 4.01 (m, 1H, H-5_B), 3.80 (dd, 1H, $J_{2,1}$=2.1 Hz, $J_{2,3}$=2.9 Hz, H-2_A), 3.64 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.1 Hz, H-4_A), 3.63 (m, 1H, H-5_A), 3.31 (s, 3H, OCH_3), 3.28 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.3 Hz, H-4_B), 1.27 (d, 3H, $J_{6,5}$=6.2 Hz, H-6_B), 1.22 (d, 3H, $J_{6,5}$=5.8 Hz, H-6_A); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.6 (CO), 138.4, 138.3, 138.1 (3 C_{ipso}-Bn), 134.8 (OCH_2CH=CH_2), 133.0 (C_{ipso}-Bz), 129.8-127.0 (C-Ar), 116.9 (OCH_2CH=CH_2), 98.6, 97.3 (C_{1A}, C_{1B}), 83.9, 81.0, 79.4, 77.9, 77.3, 75.2, 74.5, 74.1, 73.4, 72.8, 67.9, 67.7 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph, OCH_2CH=CH_2), 54.6 (OCH_3), 17.9 (C_{6A}, C_{6B}). MALDI-MS for $C_{44}H_{50}O_{10}$ (*m*/*z*): M_r (calcd) 738.34, M_r (found) 761.43 $(M+Na)^+$. Anal. calcd: C. 71.52; H. 6.82. Found: C. 71.67; H. 6.45.



133. $[\alpha]_D$ +86.9 (*c* 1.3, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, 2H, J_{ortho}=8.2, OBz), 7.45-7.02 (m, 18H), 6.15 (d, 1H, $J_{1,2}$=2.0 Hz, H-1_A), 5.78 (m, 1H, OCH_2CH=CH_2), 5.40 (d, 1H, $J_{1,2}$=3.5 Hz, H-1_B), 5.22 (dd, 1H, $J_{2,1}$=3.6 Hz, $J_{2,3}$=10.0 Hz, H-2_B), 5.12 (d, 1H, J_{vic}=17.0 Hz, *trans* OCH_2CH=C*H*H), 5.00 (d, 1H, J_{vic}=10.3 Hz, *cis* OCH_2CH=C*H*H), 4.93 (d, 1H, J_{gem}=11.0 Hz, OC*H*HPh), 4.82 (d, 1H, J_{gem}=11.2 Hz, OC*H*HPh), 4.77 (d, 1H, J_{gem}=11.2 Hz, OC*H*HPh), 4.72-4.66 (m, 2H, 2 OC*H*HPh), 4.42 (d, 1H, J_{gem}=11.4 Hz, OC*H*HPh), 4.25 (dd, 1H, J_{gem}=12.2 Hz, J_{vic}=6.3 Hz OC*H*HCH=CH_2), 4.19 (dd, 1H, J_{gem}=12.2 Hz, J_{vic}=6.3 Hz, OC*H*HCH=CH_2), 4.07 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.4 Hz, H-3_B), 4.78 (dd, 1H, $J_{3,2}$=2.7 Hz, $J_{3,4}$=8.9 Hz, H-3_A), 4.00 (m, 1H, H-5_B), 3.82 (bs, 1H, H-2_A), 3.72 (m, 1H, H-5_A), 3.62 (t, 1H, $J_{4,3}$=$J_{4,5}$=8.9 Hz, H-4_A), 3.27 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.3 Hz, H-4_B), 2.07 (s, 3H, CH_3CO), 1.23 (d, 3H, $J_{6,5}$=6.1 Hz, H-6_B), 1.18 (d, 3H, $J_{6,5}$=5.9 Hz, H-6_A); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.2, 165.7 (2 CO), 138.2, 137.9, 137.8 (3 C_{ipso}-Bn), 134.8 (OCH_2CH=CH_2), 133.1 (C_{ipso}-Bz), 129.7-127.1 (C-Ar), 116.9 (OCH_2CH=CH_2), 97.3, 91.4 (C_{1A}, C_{1B}), 83.7, 80.2, 79.3, 76.8, 76.7, 75.2, 74.6, 74.2, 73.6, 72.6, 70.5, 67.7 (C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A}, C_{4B}, C_{5A}, C_{5B}, 3 OCH_2Ph, OCH_2CH=CH_2), 21.0 (CH_3CO), 17.9, 17.8 (C_{6A}, C_{6B}). MALDI-MS for $C_{45}H_{50}O_{11}$ (*m*/*z*): M_r (calcd) 766.34, M_r (found) 789.33 $(M+Na)^+$. Anal. calcd: C. 70.48; H. 6.57. Found: C. 70.32; H. 6.49.